SYNTHETIC EFFORTS TOWARD P97 AAA+ ATPASE AND P75 NEUROTROPHIN RECEPTOR INHIBITORS

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The first chapter presented in this dissertation describes the synthesis of diverse 3-sulfanyl-1,2,4-triazole allosteric p97 AAA+ ATPase inhibitors for structure activity/property relationship (SAR/SPR) investigations. This work supported an iterative medicinal chemistry strategy that led to the systematic development of allosteric p97 inhibitors that demonstrated single-digit nanomolar biochemical potency. We were able to scale-up selected analogues to the gram-scale. Further, we synthesized inhibitors to covalently modify p97, as well as potential p97 protein degraders that utilize the hydrophobic tag (HyT) strategy. The second chapter describes a gram-scale synthesis of known p75 neurotrophin receptor inhibitors LM11A-31 and LM11A-24 without the requirement for chromatography. Subsequent investigation of LM11A-31 key pharmacophore features was evaluated through a molecular docking study and the synthesis of LM11A-31 derivatives for SAR investigations. The pursuit of novel $p75^{NTR}$ inhibitors led to the synthesis of the LM11A-31 and LM11A-24 hybrid analog series as well as the 5-aminooxazole series. The chapter is concluded with efforts toward the 5-aminooxazole series, leading to the development of novel methodology that enabled C-5 regioselective direct amination followed by C-2 direct (het)arylation of 4-cyanooxazole.

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AAA.....	ATPases Associated with various cellular Activities
AD……	Alzheimer's disease
ADME……..	Adsorption, distribution, metabolism, and excretion
ADP.....	Adenosine diphosphate
AMRI.....	Albany Molecular Research Inc.
ATP.....	Adenosine triphosphate
BDNF……...	Brain-derived neurotrophic factor
Boc……...	*Tert*-butyloxycarbonyl
CBC.....	Chemical Biology Consortium
CBS……..	Cerebrospinal fluid
CDI.....	Carbonyldiimidazole
Cl_{int}	Intrinsic clearance
CNS……..	Central nervous system
CRD…….	Cysteine-rich domains
CYP450.....	Cytochrome P450 enzymes
DIPEA……..	N,N-diisopropylethylamine
DMAP……..	4-Dimethylaminopyridine
DME…….	1,2-Dimethoxyethane
DMF…….	N,N-Dimethylformamide
DMP…….	Dess-Martin periodinane

DNA.....	Deoxyribonucleic acid
DoM…….	Directed *ortho* metallation
EDC……..	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide
ELSD.....	Evaporative light scattering detection
EMS.....	Electrospray mass spectroscopy
ER.....	Endoplasmic reticulum
ERAD.....	Endoplasmic reticulum associated degradation
ERK……..	Extracellular signal-regulated kinase
ESI……	Electrospray ionization
FDA.....	Food and Drug Administration
FDPP……	Pentafluorophenyl diphenylphosphinate
GFP.....	Green fluorescent protein
GI_{50}…….	Half maximal inhibition of cell proliferation
HB……	Hydrogen bond
HBA…….	Hydrogen bond acceptor
HBD…….	Hydrogen bond donor
HBTU……...	3-[Bis(dimethylamino)methyliumyl]-3H-benzotriazol-1-oxide hexafluorophosphate
HFIP…….	Hexafluoroisopropanol
HIF.....	Hypoxia inducible factor
HLM.....	Human liver microsome
HMBC…….....	Heteronuclear multiple bond correlation
HOBt……	Hydroxybenzotriazole

HyT.....	Hydrophobic tag
IC_{50}.....	Half maximal inhibitory concentration
JohnPhos……..	(2-Biphenyl)di-tert-butylphosphine
JNK……..	c-Jun N-terminal kinase
Kd.....	Dissociation constant
Luc.....	Luciferase
mAb……..	Monoclonal antibody
MAD.....	Mitochondrial associated degradation
MAGE……..	Melanoma-associated antigen
Mat……...	Mature
MDCK.....	Madin Darby Canine Kidney cells
MLM.....	Mouse liver microsome
NExT.....	National Cancer Institute Experimental Therapeutics
NGF……	Nerve growth factor
nM……	Nanomolar
NMR……	Nuclear magnetic resonance
NMP……	N-methyl-2-pyrrolidone
NRAGE……	Neurotrophin receptor p75 interacting MAGE homologue
NRIF……	Neurotrophin receptor-interacting factor
NSCLC…….	Non-small cell lung cancer
NT3……..	Neurotrophin 3
NT4……..	Neurotrophin 4

[O] or [Ox].....	Oxidation
ODC.....	Ornithine decarboxylase
ODD.....	Oxygen-dependent degradation domain
P_{app}.....	Apparent permeability
$p95^{NTR}$……..	p75 neurotrophin receptor
PDB……..	Protein data bank
PKC……..	Protein kinase C
PLC……..	Phospholipase C
pM……	Picomolar
PPI……	Protein-protein interaction
PPII……...	Protein-protein interaction inhibitors
PPTS…….	Pyridinium *p*-toluenesulfonate
p-TSA.....	*p*-Toluenesulfonic acid
rac…….	Racemic
RAD…….	Ribosomal associated degradation
rt……...	Room temperature
SAR……..	Structure-activity relationship
SBDD……...	Structure-based drug design
SPR.....	Structure-property relationship
T3P……...	Propylphosphonic anhydride solution
TBDPS.....	*Tert*-butyl diphenyl silyl
TFAA……...	Trifluoroacetic anhydride
TFNR……...	Tumor necrosis factor receptor

THF……..	Tetrahydrofuran
THP.....	Tetrahydro-2*H*-pyran
TLC……..	Thin layer chromatography
TMP…….	2,2,6,6-Tetramethylpiperidine
TRAF6…….	TNF receptor associated factor 6
Trk……	Tropomyosin receptor kinase
VCP.....	Valosine-containing protein
UFD.....	Ubiquitin fusion degradation
UPCDC.....	University of Pittsburgh Chemical Diversity Center
UPS.....	Ubiquitin-proteasome system
μW or MW……...	Microwave radiation

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1.0 Synthesis of Allosteric p97 AAA+ ATPase Inhibitors

1.1 Introduction

1.1.1 Cancer and the Proteotoxic Stress Hypothesis

Cancer is a complex collection of genetic diseases that develops through an evolutionary mutagenic process.[1, 2] Genes within a cancer genome are characterized with a diverse collection of alterations that include non-silent point mutations, deletions, amplifications, inversions, and/or translocations.[3, 4] The accumulation of specific alterations that result in both gain-of-function and/or overexpression of oncogenes and concomitant loss-of-function and/or silencing/elimination of key tumor suppressor genes can provide cancer with a selective growth advantage over normal, healthy cells.[3, 5, 6] The consequence of this genetic reprogramming leads to abnormal cellular phenotypes that are classically described as hallmarks of cancer.[7, 8]

Due to the haphazardous events associated with tumorgenesis, cancer cells can become dependent on a number of signaling events for their uncontrolled behavior. For example, through a process described as oncogenic addiction, cancer cells can become addicted to the continuous expression and activity of their oncogenic drivers.[9-11] Recent reports have introduced the concept of non-oncogenic addiction, which is based on the elevated burden of stress the cell endures to sustain its tumorigenic state.[12-14] These stress phenotypes include DNA damage/replication stress, proteotoxic stress, mitotic stress, metabolic stress, and oxidative stress.[14] To manage and mitigate the non-oncogenic traits incurred by the neoplastic transformation, cancer cells can

become heavily reliant on pathways that do not intrinsically drive cancer but support its progressive cell performance.

Protein homeostasis or proteostasis refers to a controlled balance of protein synthesis, folding, transport, and degradation. Due to genomic alterations and extensive genetic rewiring, cancer cells demonstrate abnormal protein synthesis and a heightened level of mutated proteins.[15, 16] The improper abundance of proteins within the cancer cell as well as the production of misfolded mutant protein challenges protein folding and degradation pathways, thereby creating proteotoxic stress.[17, 18] To manage this stress, cancer cells portray an elevated dependence on protein-quality control mechanisms, which include molecular chaperones to support proper protein folding and the ubiquitin-proteasome system (UPS) to control degradative processes.[19] It was hypothesized that agents that inhibit protein-quality control pathways heighten the proteotoxic stress within cancer cells inducing a proteotoxic crisis that ultimately results in cancer cell death.[13, 14, 20-22]

The proteotoxic stress hypothesis suggests protein-quality control inhibitors would demonstrate selective cytotoxicity toward cancer cells and be an effective therapeutic strategy against diverse cancer types.[22] Support for the proteotoxic stress hypothesis came from the clinical success of targeting the ubiquitin-proteasome system (UPS).[23-25] The UPS is a major protein quality control pathway that degrades target protein and is essential for conserving protein homeostasis within cells.[26] Proteins that require degradation by the UPS are bioconjugated with a ubiquitin protein tag from an ubiquitin-conjugating (E2) and ubiquitin ligase (E3) enzyme complex. The assembly of polyubiquitin on the target protein results in its proteolytic degradation by the 26S proteasome, which contain multiple protease enzyme domains.[27] The first clinical proteasome inhibitor, bortezomib, acquired FDA approval for the

treatment of multiple myeloma and mantle cell lymphoma.[28-30] Despite its cytotoxicity against hematological malignancies, bortezomib as well as other proteasome inhibitors have yet to demonstrate clinical utility in additional cancers, including solid tumors.[31-33] The lack of generality and broad applicability of proteasome inhibitors against diverse cancers challenges the robustness of the proteotoxic stress hypothesis. However, the clinical success of bortezomib proved that tumors can be sensitive to modulation of protein homeostasis, thereby encouraging the investigation of alternative targets influential in protein-quality control.

1.1.2 P97 AAA+ ATPase

p97, also known as valosine-containing protein (VCP), is an abundant and ubiquitous protein that plays an influential role in a variety of cellular processes that effect protein-quality control.[34-36] As a member of the *A*TPase *A*ssociated with various cellular *A*ctivities (AAA) protein family, p97 is assembled as a barrel-like homohexameric protein complex that is capable of performing mechanical processes on target proteins.[37, 38] This occurs by utilizing energy generated from the hydrolysis of ATP to undergo significant conformational changes.[39-41] Topologically, each protomer of p97 contains a regulatory N-terminal domain (N) and two tandem AAA domains (D1 and D2) (Figure 1, PDB: 5FTK).[38, 42] The N-terminal domain associates with various co-factors/adaptor proteins and these interacting partners govern p97's substrate recognition and subcellular recruitment, implicating p97's cellular function in different pathways.[43] The D1 domain has very low ATPase activity; however, it mediates the assembly of the hexameric structure and positively cooperates with the D2 domain, which is primarily responsible for p97's ATPase activity.[44, 45] The hydrolysis of ATP and the resulting dynamic conformational changes that occur allow p97 to exert a mechanical force on target proteins.[46]

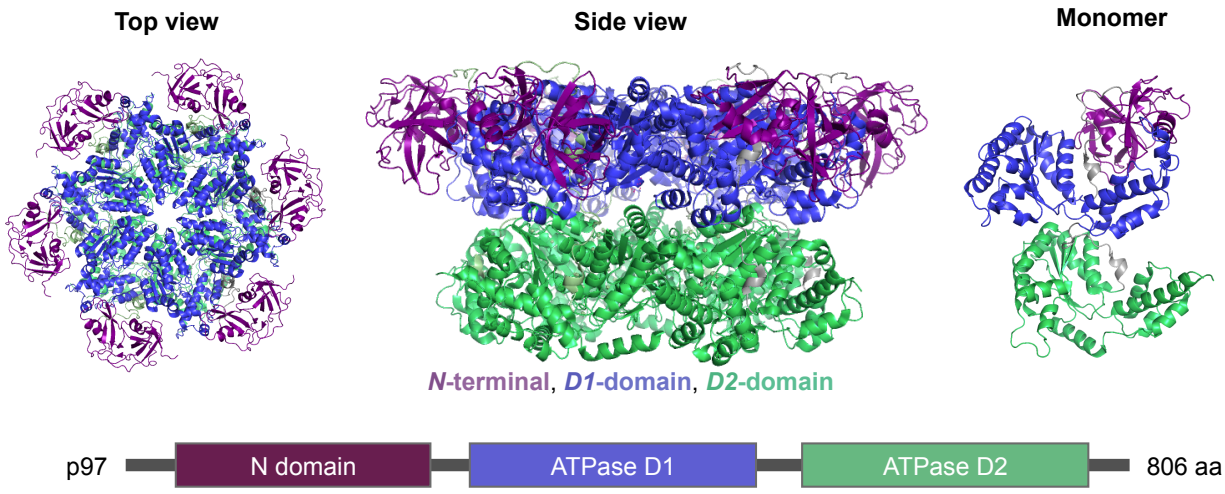

Figure 1. Structure of p97 AAA ATPase
N domain (purple), D1 domain, (blue), and D2 domain (green); PDB: 5FTK [41]

p97 is associated in several protein-quality control pathways and is characterized as a master regulator of protein homeostasis (Figure 2). In general, p97 facilitates the degradation of ubiquinated protein in the ubiquitin proteasome system by separating targets from protein complexes or cellular membranes and chaperoning them to the proteasome.[35, 47] Therefore, this segregase function has implicated p97 in a number of important cellular processes, including endoplasmic reticulum associated degradation (ERAD)[48-50], mitochondrial associated degradation (MAD)[51-53], ubiquitin fusion degradation (UFD)[54], ribosome associated degradation (RAD)[55-57], chromatin associated degradation (CAD)[58, 59], and autophagy[60-62].

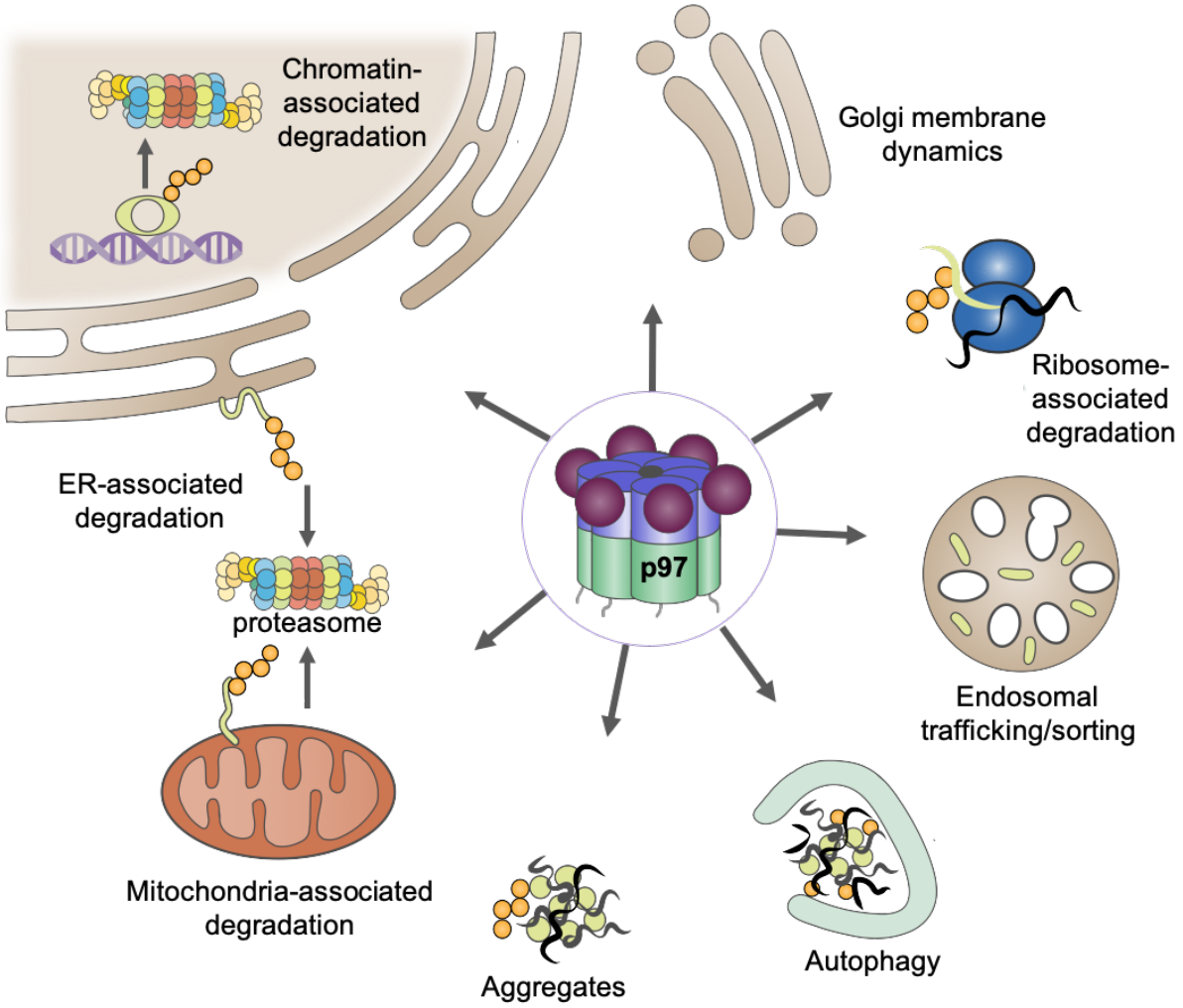

Figure 2. Summary of p97 Biological Function
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The significance of p97's physiological function is best characterized and demonstrated in the endoplasmic reticulum associated degradation pathway (ERAD) (Figure 3). The endoplasmic reticulum (ER) contains a complex network of interconnected pathways that are primarily responsible for proper protein assembly, folding, and glycosylation.[63] While folded protein are exported to the Golgi for further processing, misfolded protein are either resubjected to refolding machinery or cleared from the ER by the ERAD pathway.[64, 65] The ERAD pathway is essential for conserving ER protein homeostasis by retrotranslocating excess protein or

misfolded protein from the ER into the cytoplasm where it can be degraded by the ubiquitin proteasome system.[66-68] During the ERAD process, misfolded protein must be identified, transported across the ER membrane, and delivered to the proteasome. In this series of events p97 is recruited to the ER membrane through its association with mammalian ER resident proteins Derlins, Hrd1, and VIMP where it assists with protein retrotranslocation and subsequent delivery to the proteasome.[48, 50, 69-73] p97 recognizes the ubiquinated tag and extracts the misfolded protein from the ER membrane through mechanical force generated through its dynamic conformational changes upon ATP hydrolysis.[74] Dislocated ERAD substrates from the ER membrane are then presented to the proteasome for degradation.[75, 76] Burdening ER function and/or perturbing ERAD leads to an accumulation of toxic poly-ubiquitinated protein aggregates consisting of hydrophobic misfolded proteins, which results in significant ER proteotoxic stress.[77, 78] High level of misfolded protein, prolonged ER stress, and/or inhibition of ERAD activate the lethal unfolded protein response (UPR), leading to cell apoptosis.[79-81] The UPR is critical for stress management and the activation of the UPR is necessary to properly regulate secretory pathways by inversely increasing ER resident chaperones and decreasing overall protein synthesis.[82] Beyond the ER, p97 also manages various other aspects of proteotoxic stress primarily through its ability to disrupt and clear misfolded proteins in diverse quality control pathways.

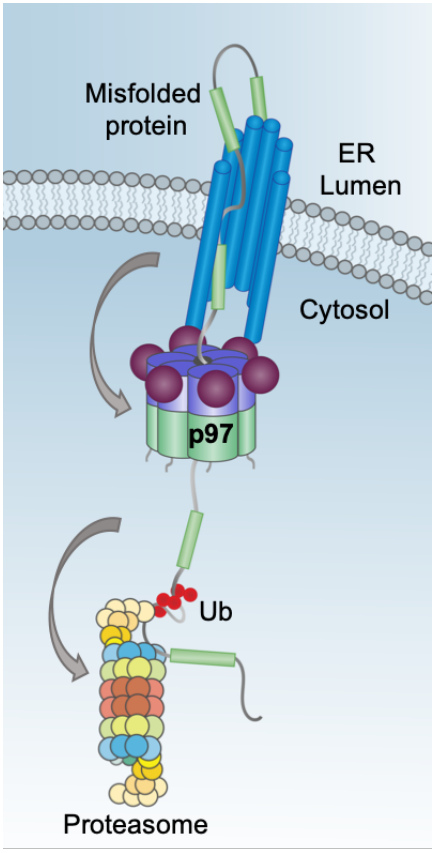


Figure 3. Biological Function of p97 in ERAD

1.1.3 P97 as a Cancer Target

p97's role in a diverse array of cellular processes associated in protein homeostasis and mitigating proteotoxic stress has led to its investigation in cancer.[34, 83] p97's expression is upregulated in a variety of cancer types (colorectal, pancreatic, thyroid, breast, squamous cell carcinoma, gastric carcinoma, osteosarcoma, and lung), suggesting a potential role for p97 in promoting or sustaining abnormal cellular behavior.[84-86] Additionally, elevated levels of p97 in cancer patients correlated with poor clinical outcomes.[87, 88] Genetic knockdown of p97 using siRNA technology led to an increase in polyubiquitinated proteins and the activation of the unfolded protein response.[89] Because p97 has a central role in the UPS and protecting cancer

cells from developing proteotoxic stress, it is conceivable that p97 inhibition would induce the accumulation of misfolded proteins and toxic protein aggregates, leading to cancer cell death. Further, due to elevated levels of proteotoxic stress that exist in cancer, it is possible that p97 inhibition would demonstrate heightened sensitivity and greater selectivity against cancer cells. Therefore, there is considerable interest in small molecules that modulate p97 activity to clinically substantiate p97 as therapeutic target.

1.1.4 Small Molecule Inhibitors of p97

There is growing evidence that p97 is an important regulator of proteotoxic stress and the conservation of protein homeostasis; thus, inhibition of p97 function provides an opportunity to target a key vulnerability of cancer. Due to its association in cancer, p97 has garnered attention as a possible novel anti-cancer target, which led to the identification and development of small molecule inhibitors that modulate p97 activity.[90-102] This includes natural products withaferin A[100], rheoemodin[99], and anticancer agent sorafenib[101] as well as reversible inhibitor 2-(cyclohexylmethylamino)pyrimidines[102] and irreversible inhibitors eeyarestatin[95] and NMS-859[93] (Figure 4). Many of the described inhibitors were evaluated using an *in vitro* ATPase assay measuring recombinant p97 ATPase inhibition through a chemoluminescent readout of ADP generated from ATP hydrolysis, thereby reporting their respective biochemical IC_{50}. Cellular degradation assays were also employed to identify compounds that distinguish p97-dependent and p97-independent degradation. Ub^{G76V}-GFP is a fluorescently labeled protein that is degraded in a p97-dependent manner. The cellular Ub^{G76V}-GFP reporter assay was utilized to evaluate the cellular inhibition of p97 by disrupting Ub^{G76V}-GFP degradation, leading to the accumulation/stabilization of the fluorescent reporter protein and concomitant elevation of

fluorescence signaling.[103, 104] Two additional luciferase assays that are associated with p97-independent degradation pathways were employed in parallel to assess p97 selectivity. Luciferase was co-expressed with Ub^{G76V}-GFP and the oxygen-dependent degradation domain of hypoxia inducible factor 1a (HIF1a) (ODD-Luc).[103, 105] Degradation of ODD-Luc requires ubiquitination and is p97 independent. The second luciferase assay used luciferase fused to Ub^{G76V}-GFP and the ubiquitin independent degradation domain of ornithine decarboxylase (Luc-ODC).[103, 105] Degradation of Luc-ODC requires the proteasome but is ubiquitin and p97 independent.[106] Selective p97 inhibitors would stabilize Ub^{G76V}-GFP protein but not ODD-Luc or Luc-ODC protein. Results from all assays provided insight into p97 inhibition and cellular selectivity over related AAA+ ATPases as well as other members of the ubiquitin proteasome system.

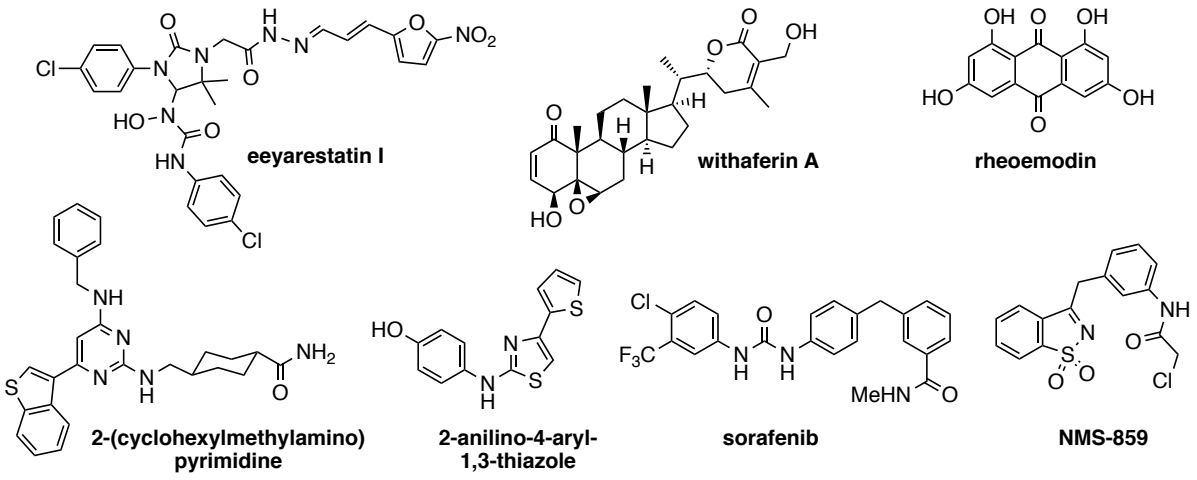


Figure 4. Select p97 Inhibitors

The earliest reported p97 inhibitors were the eeyarestatins, which primarily consisted of EerI and EerII (Figure 4).[95] This class of inhibitors was identified through a high-throughput screen using a developed class I major histocompatibility complex heavy chain linked to

enhanced green fluorescent protein assay to evaluate ERAD function.[107] The eeyarestatins demonstrated poor micromolar potency and utilizing surface-plasmon resonance, EerI was found to bind to p97 with a K_d of 5-10 μM and exhibit a cellular Ub^{G76V}-GFP IC_{50} of 3.7 μM. [95, 108] Further, EerI stabilized both ODD-Luc and Luc-ODC, suggesting additional targets in the UPS beyond p97. Biochemical investigations found the eeyarestatins are bifunctional compounds, whereby the aromatic motif is responsible for membrane binding and its nitrofuran motif targets p97 in an allosteric, irreversible fashion.[108] The eeyarestatin compounds were employed in a number of contexts as a pharmacological tool to elucidate p97 biological functions. Moreover, cancer cell treatment with a combination of Eer1 and proteasome inhibitor bortezomib disrupted ER secretory pathways and elevated ER stress-induced cell apoptosis.[109-111] EerI was established as suitable effector of the ERAD pathway and demonstrated synergistic effects with bortezomib in cancer cell death; however, due to its limited potency and undeveloped mode of action, the eeyarestatins have yet to advance beyond cellular investigations.

The 2-anilino-4-aryl-1,3-thiazole inhibitors (Figure 4) were the first p97 targeted agents reported.[92] This series was identified through a high-throughput screen using a glucokinase coupled enzyme assay. The synthetic strategy employed for this series utilized the Hantszsh thiazole synthesis to conduct parallel medicinal chemistry, resulting in a SAR investigation that generated a series of nanomolar potent inhibitors. The lead compound demonstrated a p97 ATPase IC_{50} of 110 nM and a cellular Ub^{G76V}-luciferase EC_{50} of 90 nM. Mechanistically, the thiazole chemotye demonstrated sensitivity to ATP concentration, indicating a competitive mechanism. The series provided proof-of-concept, concluding with potent p97 inhibitors with drug-like characteristics. However, continued investigations indicated a lack of target specificity with other members of the UPS, therefore, further developments have not occurred.[45]

The discovery and development of the diaminoquinazoline-based compounds led to the first reported reversible, selective small molecule p97 inhibitor series.[90] The hit compound, DBeQ (Figure 5), was identified through a high-throughput screen from the NIH Molecular Libraries Small Molecule Repository using an ATPase assay.[90] DBeQ showed a p97 ATPase IC_{50} of <10 μM and a cellular Ub^{G76V}-GFP EC_{50} of 2.3 μM. Mechanistically, DBeQ was found to be sensitive to ATP, suggesting an ATP competitive mode of action. Further, it was found to target both D1 and D2 ATPase domain. The selectivity of DBeQ was demonstrated by the degradation of p97-dependent substrate Ub^{G76V}-GFP and the limited effect on p97-indpendent substrates ODD-Luc and Luc-ODC. Further, DBeQ portrayed no significant inhibition towards related ATPases as well as a panel of 170 evaluated protein kinases. Cellular treatment of DBeQ compromised the ERAD and induced the UPR, resulting in cell death. This provided specific evidence that DBeQ-dependent inhibition of p97 leads to a cytotoxic ER stress-induced response. Medicinal chemistry hit-to-lead optimization of the quinazoline scaffold led to lead compound ML240 (Figure 5).[112] ML240 demonstrated improved p97 ATPase IC_{50} of 110 nM and cellular Ub^{G76V}-GFP IC_{50} of 2.3 μM. ML240 elicited cell apoptosis and anti-proliferative activity against a series of cancer cell lines found in the NCI-60 panel. Although potent, ML240 demonstrated poor solubility and high plasma protein binding activity, making its utility in *in vivo* studies limited.[112] Further lead optimization of the quinazoline series resulted in CB-5083 (Figure 5), which demonstrated an exceptional p97 ATPase IC_{50} of 11 nM.[113] Furthermore, CB-5083 exhibited excellent oral bioavailability and showcased anti-tumor effects in multiple animal xenograft models as well as patient-derived xenograft models of colorectal cancer.[83] Due to its promising pre-clinical performance, CB-5083 entered Phase I clinical trials for relapsed/refractory multiple myeloma (NCT02243917) and advanced solid tumors

(NCT02223598); however, these investigations were recently discontinued due to off-target PDE6 inhibition, resulting in retinal toxicity.[114] Nonetheless, the pre-clinical performance of CB-5083 against multiple myeloma and advanced solid tumors has provided credence to p97 inhibition; therefore, there is considerable interest to actively develop alternative small molecules that modulate p97 activity to clinically substantiate p97 as an anti-cancer target.

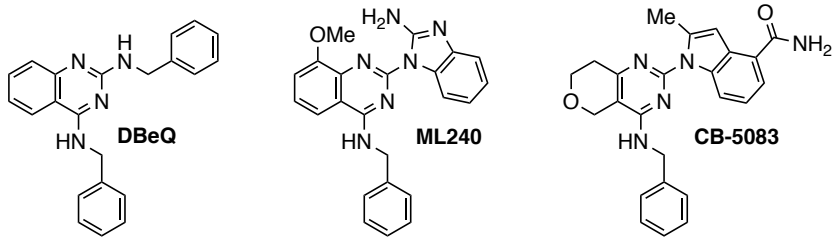


Figure 5. Diaminoquinazoline p97 Inhibitors

Small Molecule Inhibitors of p97: Previous Work from the UPCDC

The University of Pittsburgh Chemical Diversity Center (UPCDC) has been interested in the development of small molecule inhibitors of p97 as potential anti-cancer agents. Their first reports identified an indole amide series that was discovered through a high-throughput screen. The initial hit compound SMDC818909 demonstrated an *in vitro* IC_{50} of 11.5 μM in the presence of 20 μM ATP.[96] It was found that SMDC818909 is an uncompetitive inhibitor that presumably binds at an allosteric site on the D2 domain.[96] SMDC818909 demonstrated modest aqueous solubility of 56 μM; however, its low molecular weight and ease of synthesis provided an opportunity for rapid medicinal chemistry optimization.[96] This effort led to the development of indole amide (Figure 6), which demonstrated an *in vitro* IC_{50} of 0.5 μM using a p97 ADP-Glo biochemical ATPase assay.[96] This represented a roughly 7-fold improvement in potency

compared to SMDC818909. Moreover, the indole amide also had an improved solubility of 330 μM (PBS, pH 7.4).[96] Further investigation revealed indole amide had an apparent cell permeability values (P_{app}) in a MDCK bidirectional permeability assay of 71 x 10^{-6} and 45 x 10^{-6} cm/s, respectively, and an *in vitro* half-life in human microsomal stability of $t_{1/2}$ >60 min.[96] Therefore, indole amide displayed a favorable mechanism of inhibition as well as excellent pharmaceutical properties that included high potency, aqueous solubility, cell permeability, and human microsomal stability. However, advancement of indole amide in cellular models demonstrated limited efficacy, having an Ub^{G76V}-GFP EC_{50} > 40 μM.[96] Evaluation of indole amide in the NCI-60 panel revealed a modest anti-proliferative activity ($logGI_{50} \approx -4.5$) in leukemia (RPMI-8226), breast cancer (MDA-MB-468), and melanoma (LOX IMVI) cell lines.[96] Therefore, indole amide is a promising tool compound but requires further development in order to demonstrate cellular efficacy.

As a continued effort to develop small molecule allosteric inhibitors of p97, the UPCDC identified the 2-phenyl indole scaffold through a high-throughput screen.[114] The first-generation of the phenyl indole series demonstrated low micromolar activity in a biochemical p97 ATPase assay. It was found that this class of compounds had an uncompetitive binding mechanism and exhibited promising drug-like properties: low molecular weight, optimal calculated cell permeability (cLogP), and total polar surface area (tPSA). An iterative medicinal chemistry optimization program was pursued to advance the phenyl indole series. This led to the development and characterization of a number of potent inhibitors, including the fluoro-indole compound (Figure 6).[114] The fluoro-indole compound demonstrated an *in vitro* biochemical IC_{50} of 20 nM in ADP-Glo ATPase assay.[114] Further, fluoro-indole exhibited excellent physical properties including aqueous solubility (pH 7.4) of 330 μM.[114] The fluoro-indole compound also

proved to have great metabolic stability in both human and mouse liver microsome stability assays with a half-life ($t_{1/2}$) of 475 and 277 min, respectively.[114] However, advancement of fluoro-indole in a p97-dependent cell model demonstrated limited efficacy, resulting in an Ub^{G76V}-GFP EC_{50} of 15 μM at 1 h.[114] Further evaluation of fluoro-indole in the NCI-60 panel demonstrated broad anti-proliferative activity (mean $logGI_{50} \approx -5.7$) against a number of cancer cell lines, including leukemia, NSCLC, colon cancer, CNS tumor, melanoma, ovarian cancer, prostate cancer, and breast cancer.[114] The optimization of the phenyl indole series led to the development of biochemically potent inhibitors that demonstrate excellent physical properties. The utility of UPCDC30245 (Figure 6) facilitated the structural investigation of p97 utilizing cryo-electron microscopy, which produced a high-resolution image of UPCDC30245 bound between the D1 and D2 domains.[41] Although the cellular activity was limited against p97-dependent activities, the fluoro-indole compound demonstrated anti-proliferative effects in multiple cancer cell lines, suggesting an opportunity to further develop this series into a possible novel class of cancer therapeutics.

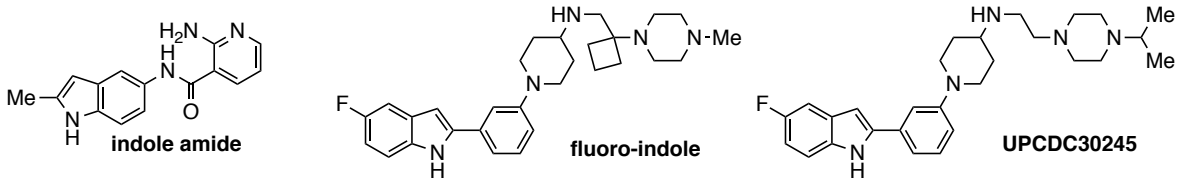


Figure 6. p97 Inhibitors Developed by the UPCDC

1.1.5 Discovery and Structure-Activity Relationships of Allosteric 3-Alkylsulfanyl-1,2,4-triazoles Inhibitors of p97: Previous Work from Nerviano/Genentech

A new class of p97 ATPase inhibitors was pursued in an effort to develop biochemically potent, reversible compounds that showcase cellular activity and target selectivity. A

biochemical high-throughput screen of an internal chemical library collection of more than one million compounds led to the identification of a 3-alkylsulfanyl-1,2,4-triazole compound, NMS-862 (Figure 7), which showcased an enzymatic p97 ATPase IC_{50} of 2.65 μM.[94] It was later revealed that NMS-862 is insensitive to ATP concentration and targets p97 ATPase through a reversible, noncompetitive allosteric mechanism.[94] This mechanism was further investigated through the performance of photo affinity labeling experiments. Inhibitor derivatives containing arylazides were UV cross-linked to p97. Subsequent peptidase digestion and mass spectroscopy analysis indicated modification of amino acid residues Lys615 and Asn616, which are positioned between the D1 and D2 domains.[93] Further, several independent mutations were generated within this general region, including K615V and N616F. Although these mutants were enzymatically active, inhibition with the alkylsulfanyl compounds were significantly reduced. Therefore, the reported putative binding site for this class of molecules was postulated to be located at the juncture between the D1 and D2 domain of each monomeric protomer of p97's homohexameric structure. It was recognized that this location undergoes significant conformational changes upon ATP binding and subsequent hydrolysis and therefore, it was hypothesized that the sulfanyltriazole compounds stabilized the D2-ADP bound domain and impairs the dynamic movement necessary for proper biological function. Advancement of the promising hit compound NMS-862 that demonstrated a favorable and novel mechanism of action into cells resulted in negligible anti-proliferative effects. Consequentially, this led to the initiation of a hit-to-lead medicinal chemistry optimization campaign with a focus on enhancing NMS-862 biochemical and biological performance.

Medicinal Chemistry Strategy

A SAR investigation of the hit 3-alkylsulfanyl-1,2,4-triazole compound NMS-862 surveyed structural derivation at the 1,2,4-triazole core.[94] This included the thioisopropyl motif at position 3 in zone I, the phenyl group at position 4 in zone II, and the phenoxy moiety at position 5 in zone III (Figure 7). Studying these three zones led to an improved understanding on the structural determinants that are responsible for p97 inhibition and the generation of biologically potent inhibitors.

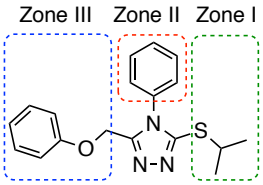
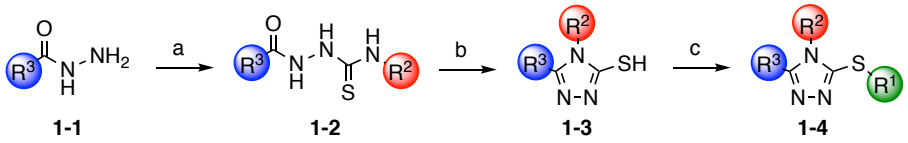


Figure 7. Structural Zones of NMS-862 Evaluated for SAR Analysis

The synthetic strategy employed for medicinal chemistry optimization utilized a modular approach that was amenable for rapid structural derivation at the 3-, 4-, and 5-position of the triazole core, leading to diverse analogues. Reacting pre-constructed hydrazide **1-1** with the appropriately prepared isothiocyanate afforded thiourea intermediate **1-2** that incorporates zone II derivation (Scheme 1). The construction of the 3-sulfanyl-1,2,4-triazole core framework occurred through a subsequent base-mediated cyclization reaction. Employing the advanced intermediate **1-3** enabled rapid zone I derivation by conducting *S*-alkylation reactions with various alkyl and cycloalkyl electrophiles to fashion a biologically relevant compound **1-4**.

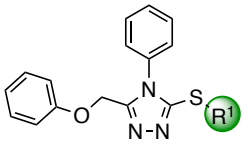
Scheme 1. General Synthesis of 3-Alkylsufanyl-1,2,4-triazoles [94]



Reagents and conditions: (a) R^2NCS, EtOH, reflux; (b) 1 M NaOH, reflux; (c) R^1Br or R^1I, K_2CO_3, DMF, rt.

Their initial SAR investigation evaluated the significance of the alkylsulfanyl motif at position 3 of the triazole core in zone I (Table 1).[94] It was reported that the presence of a nonpolar, lipophilic group at this position was necessary; however, the contrary polar analogues to support such a claim were not disclosed. It was shown that the *S*-alkyl group was necessary in zone I for biological efficacy; the absence of an alkyl group entirely, compound **1-5a**, resulted in negligible activity (>30 μM). Replacement of the *S*-isopropyl motif from hit compound NMS-862 with aliphatic straight chains, such *S*-methyl analog **1-5b** and *S*-ethyl analog **1-5c**, resulted in decreased activity, >30 μM and 9.92 μM respectively. However, the branched *S*-isobutyl motif from analog **1-5d** demonstrated improved activity of 1.29 μM. Moreover, select *S*-cycloalkyl groups proved beneficial, including *S*-cyclobutyl analog **1-5e**, *S*-cyclopentyl analog **1-5f**, and *S*-cyclohexyl analog **1-5g**. Contrarily, larger aromatic *S*-cycloalkyl groups, such as *S*-phenyl analog **1-5h** and *S*-benzyl analog **1-5i**, proved to be detrimental. These results indicate the size of the alkyl group plays a critical role for biological activity and indicated a narrow structural tolerance in zone I, whereby the *S*-cyclopentyl motif proved optimal. Further, it was hypothesized that these non-polar, lipophilic *S*-cycloalkyl groups occupy a hydrophobic pocket within p97.[94]

Table 1. SAR of Select S-alkyl Motifs at Position 3 in Zone I [94]

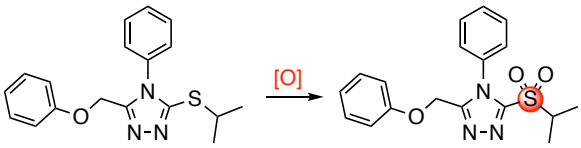


1-5

Entry	R^1	p97 IC_{50} (μM)[a]	Compound
1	*S*-isopropyl	2.69	**NMS-862**
2	*S*-H	>30	**1-5a**
3	*S*-methyl	>30	**1-5b**
4	*S*-ethyl	9.92	**1-5c**
5	*S*-isobutyl	1.29	**1-5d**
6	*S*-cyclobutyl	0.96	**1-5e**
7	*S*-cyclopentyl	0.72	**1-5f**
8	*S*-cyclohexyl	1.15	**1-5g**
9	*S*-Ph	3.79	**1-5h**
10	*S*-Bn	>30	**1-5i**

[a]Values are the means of two or more experiments.

Sulfur is recognized to readily undergo *in vivo* oxidative metabolism from cytochrome P450 and flavin-containing monooxygenase (FMO) enzymes in the liver, making the sulfur atom in zone I a metabolic liability.[115-117] The synthesis and biological evaluation of the *S*-oxidative metabolite of hit compound NMS-862, the isopropyl sulfone analog **1-6**, demonstrated an IC_{50} of >30 μM activity, indicating an inactive compound (Figure 8).



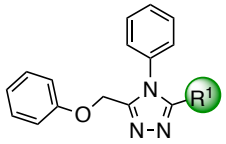
NMS-862 **1-6**

Entry	Motif	p97 IC_{50} (μM)	Compound
1	*S*-isopropyl	2.69	**NMS-862**
2	*SO_2*-isopropyl	>30	**1-6**

Figure 8. Proposed Sulfur Oxidation[94]

To mitigate *S*-oxidative metabolism, the SAR investigation pursued a heteroatom isostere replacement of sulfur (Table 2). Accordingly, *O*-cyclopentyl, *NH*-cyclopentyl, and *CH_2*-cyclopentyl analogues were initially synthesized and evaluated. The results from these analogues indicated no conservation or improvement in biological activity compared to the parental sulfanyl compound **1-5f**, providing the following trend in biological potency: *S*-cyclopentyl **1-5f** (IC_{50} of 0.72 μM) > *CH_2*-cyclopentyl **1-7c** (IC_{50} of 1.97 μM) > *O*-cyclopentyl **1-7a** (IC_{50} of 2.25 μM) > *NH*-cyclopentyl **1-7b** (IC_{50} of 2.85 μM). Perhaps due to its bond length and/or preferred dihedral angle, sulfur in zone I may play a critical role in optimally positioning the cyclopentyl group in the putative hydrophobic pocket and therefore, changing the heteroatom as well as the cycloalkyl group may be required to conserve biological potency. Thus, *S*-cyclohexyl, *O*-cyclohexyl, *NH*-cyclohexyl, and *CH_2*-cyclohexyl analogues were also evaluated. The tested isostere replacements universally demonstrated lower biological activity. However, compared to the cyclopentyl motif, the cyclohexyl derivatives provided a different trend in biological potency: *S*-cyclohexyl **1-5g** (IC_{50} of 1.15 μM) > *O*-cyclohexyl **1-7d** (IC_{50} of 1.96 μM) > *NH*-cyclohexyl **1-7e** (IC_{50} of 3.45 μM) > *CH_2*-cyclohexyl **1-7f** (IC_{50} of 4.30 μM). The medicinal chemistry optimization at position 3 in zone I was relatively limited, whereby the cyclopentylsulfanyl group proved to be most optimal.

Table 2. SAR of Heteroatom Isostere Replacement of Sulfur[94]



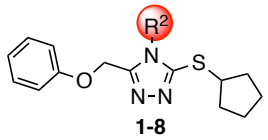
1-7

Entry	R^1	p97 IC_{50} (μM)[a]	Compound
1	*S*-cyclopentyl	0.72	**1-5f**
2	*O*-cyclopentyl	2.25	**1-7a**
3	*NH*-cyclopentyl	2.85	**1-7b**
4	CH_2-cyclopentyl	1.97	**1-7c**
5	*S*-cyclohexyl	1.15	**1-5g**
6	*O*-cyclohexyl	1.96	**1-7d**
7	*NH*-cyclohexyl	3.45	**1-7e**
8	CH_2-cyclohexyl	4.30	**1-7f**

[a]Values are the means of two or more experiments

Next, the aryl substituent at position 4 in zone II was evaluated (Table 3). It was reported that the phenyl ring, **1-5f**, portrayed the ideal size and shape for this region of the molecule. Assessing substituents on the phenyl ring did not prove beneficial.[94] *Ortho*- and *para*-substituents were detrimental to biological activity as well as most of the *meta*-substituents (**1-8c** to **1-8g**); however, *meta*-chloro or *meta*-bromo analogues (**1-8a** and **1-8b**) demonstrated conservation of potency. Replacement of the *meta*-halogen with nitrogen, resulting in the 3-pyridyl analog **1-8i**, enhanced biological activity. Additionally, the 3-pyridyl motif also improved aqueous solubility. SAR of zone II indicated substituents on the phenyl ring were not well tolerated and the 3-pyridyl motif was optimal.

Table 3. SAR of Zone II [94]



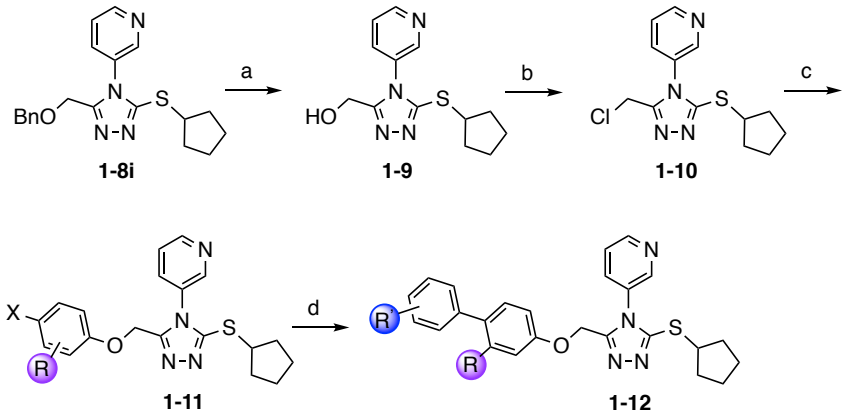
1-8

Entry	R^2	p97 IC_{50} (μM)[a]	Compound
1	Ph	0.72	**1-5f**
2	3-Cl-Ph	0.74	**1-8a**
3	3-Br-Ph	0.96	**1-8b**
4	3-Me-Ph	2.78	**1-8c**
5	3-NO_2-Ph	2.81	**1-8d**
6	3-CF_3-Ph	>30	**1-8e**
7	3-NH_2-Ph	1.29	**1-8f**
8	3-N_2-Ph	2.40	**1-8g**
9	2-pyridyl	>30	**1-8h**
10	3-pyridyl	0.47	**1-8i**

[a]Values are the means of two or more experiments

Investigation of zone III occurred through the manipulation of intermediate **1-8i**. Initial benzyl deprotection of **1-7i** using boron trichloride afforded the free hydroxide **1-9** (Scheme 2). Subsequent chlorination using thionyl chloride nicely provided the electrophilic intermediate **1-10** that was able to react with varying commercially available phenoxy-based nucleophiles to rapidly generate zone III derivatives **1-11** in a parallel sequence. Chemical expansion in the presence of the aryl bromide intermediate **1-11** (X = Br) enabled Suzuki coupling with diverse boronic acids at 100 ^{o}C under microwave irradiation to provide biphenyl analogues **1-12**.

Scheme 2. General Synthesis of Zone III Side Chain Variants [94]

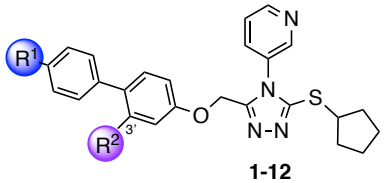


Reagents and conditions: (a) 1 M BCl_3, CH_2Cl_2, 0-5 ^oC; (b) $SOCl_2$, CH_2Cl_2, 0-5 ^oC; (c) Ar-OH, K_2CO_3, DMF, 80-100 ^oC; (d) when X = Br: Ar-$B(OH)_2$, Pd(dppf)Cl_2•CH_2Cl_2, Cs_2CO_3, MeCN, H_2O, 100 ^oC, microwave irradiation.

Evaluation of zone III enabled significant improvement in biological potency. Initially, over 200 phenoxy **1-11** derivatives were constructed and evaluated, providing no discernable trends in SAR. Progress was achieved for the biphenyl analogues **1-12**, which demonstrated both biochemical potency and significant anti-proliferative activity. It was initially postulated that the improved biological performance was due to their enhanced lipophilic character and elevated cell permeability.[94] To further understand the effects of the biphenyl system on biological activity a set of biphenyl analogues were surveyed. It was found that hydrogen-bond acceptor motifs at the *para* position were most effective, which included cyano, amides, hydroxamates, ketones, sulfones, and sulfonamide (**1-12a** to **1-12f**) (Table 4). Therefore, it was hypothesized that the biphenyl side chain functioned as a linker to optimally position a terminal motif with hydrogen bonding capabilities. Further developments found that functionalizing the 3' position of the proximal ring with a methyl substituent elicited enhanced anti-proliferative activity (Table 4). 2'-Substituents did not induce the same biological effect, perhaps suggesting the 3'-methyl group influences the planarity of the biphenyl system. The SAR study was concluded with the

identification of NMS-873, which exhibited the most potent biochemical (p97 IC_{50} = 0.024 μM) and cellular activity (HCT-116 IC_{50} = 0.38 μM).[94]

Table 4. SAR of Zone III [94]



1-12

Entry	R^1	R^2	p97 IC_{50} (μM)[a]	HCT-116 IC_{50} (μM)	Compound
1	H	H	2.031	>10	**1-12a**
2	CN	H	0.088	4.07	**1-12b**
3	CONHOMe	H	0.043	3.13	**1-12c**
4	SO_2NH_2	H	0.071	1.37	**1-12d**
5	CONHMe	H	0.042	6.44	**1-12e**
6	SO_2Me	H	0.063	3.03	**1-12f**
7	CN	Me	0.071	2.02	**1-12g**
8	CONHOMe	Me	0.058	4.60	**1-12h**
9	SO_2NH_2	Me	0.025	1.82	**1-12i**
10	CONHMe	Me	0.054	4.98	**1-12j**
11	SO_2Me	Me	0.024	0.38	**NMS-873**

[a]Values are the means of two or more experiments

Lead candidate - NMS-873

NMS-873 was the most potent compound in the 3-sulfanyl-1,2,4-triazole series. Molecular docking studies of NMS-873 by Segura-Cabrera *et al.* led to a proposed 2-D model of NMS-583 bound p97 (Figure 9).[118] This study employed a blind AutoDock[119] docking protocol to identify potential allosteric binding sites of NMS-873 on the p97 protein structure (PDB ID: 3CF1).[40] Evaluated sites included amino acid residues Lys615 and Asp616, which are located at the protomer interface and were experimentally shown to influence NMS-873 binding.[94] Their

selected putative binding site provided structural insights into NMS-873 inhibition. These postulated intermolecular interactions between NMS-873 and p97 were predominantly governed by a collection of positive charged, polar, and hydrophobic residues. It was suggested that in zone I, the cyclopentyl motif is positioned in a hydrophobic pocket that consists of Ile531, Ala532, Cys535, Ala537, Pro571, Cys572, and Val493. In zone II, the 3-pyridyl motif established a π-π interaction with Phe618. Lastly, in zone III, Lys614 was shown to form a strong hydrogen bond, electrostatic interaction with the terminal methylsulfone of NMS-873 as well as a cation-π interaction with the proximal phenyl ring.[118]

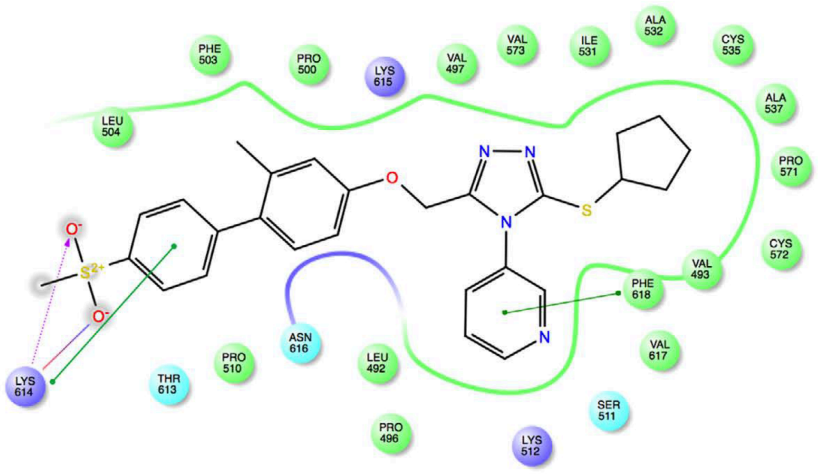


Figure 9. 2-D Molecular Model of NMS-873 Bound to p97

Blue ball – positive charged residue; green ball – hydrophobic residue, teal ball - polar residue, green line – π-π stacking or π-cation interaction, purple line – salt bridge. This figure was reproduced with permission from Springer Nature Creative Commons Attribution 4.0 International License in Scientific Reports article Scientific Reports volume 7, Article number: 44912 (2017)

NMS-873 was also advanced for further biological evaluation. NMS-873 induced a dose-dependent response on key p97 cellular biomarkers associated with ERAD and the UPR,

including α-Poly-Ub, α-Cyclin E, and α-CHOP.[94] NMS-873 also portrayed an excellent selectivity profile for p97 over other related AAA ATPases (NSF, SPATA5, VPS4B, and cdc6) as well as against a panel of 50 protein kinases tested at a concentration of 10 μM.[94] To assess the 3-sulfanyl-1,2,4-triazole series as a potential drug candidate, the physicochemical and pharmacokinetic properties of NMS-873 were also determined. This investigation found NMS-873 had good cell permeability (P_{app} of 21.6 cm 10^{-6}/s in Caco-2 cells), however, limited solubility (7 μM in aqueous ammonium acetate buffer at pH 7).[94] The *in vivo* performance of NMS-873 was subsequently evaluated in a mouse pharmacology study. Oral administration of NMS-873 in mice demonstrated poor systemic exposure due to high *in vivo* clearance (115 mL/min/kg), which was also in line with their reported high *in vitro* clearance in oxidative metabolism stability testing in human liver microsomes (HLM Cl_{int} = 400 mL/min/kg).[94] Importantly, NMS-873 demonstrated low exposure, having inadequate bioavailability (F = 16.4%), perhaps due to elevated first-pass metabolism and low aqueous solubility.[94] The poor physicochemical and pharmacokinetic properties of NMS-873 limited its *in vivo* efficacy and therefore, further optimization was required to improve its solubility and metabolic stability.

1.2 Results and Discussion

The National Cancer Institute Experimental Therapeutics (NExT) program supports promising new drug discovery and development projects that demonstrate potential clinical benefit. The Chemical Biology Consortium (CBC) serves as an experimental branch of the NExT program and members of this consortium utilize their scientific and medical expertise to advance drug discovery candidate molecules and progress them into clinical development. The CBC recognized the University of Pittsburgh Chemical Diversity Center (UPCDC) as a dedicated member responsible for facilitating the progression of NExT discovery projects. The UPCDC supports this effort through medicinal, synthetic, and computational chemistry. Because p97 is a promising anti-cancer target, the UPCDC team of chemists pursued a drug discovery program to develop p97 inhibitors with therapeutic potential. Our objective was to produce an optimal clinical candidate by performing a structure-activity/property relationship study (SAR/SPR) on the 3-sulfanyl-1,2,4-triazole series in order to optimize and enhance structural, physicochemical, biochemical, and pharmacokinetic properties. To achieve this objective, we applied hypothesis-driven drug design, developed synthetic routes, synthesized and characterized novel compounds, delivered biweekly submissions, and critically evaluated biological results.

1.2.1 Medicinal Chemistry Strategy

To further investigate and advance the 3-sulfanyl-1,2,4-triazole p97 allosteric inhibitor series, we initiated our SAR/SPR investigation by strategically and systematically analyzing five structural zones of NMS-873 in order to optimize each zone's binding capabilities. Specifically, each zone featured the following chemical motif: zone I included the cyclopentyl motif; zone II

included the 1,4-disubsituted benzene; zone III included the 3-methylphenyl motif; zone IV included the terminal methyl sulfone; and zone V included the 3-pyridyl motif (Figure 10).

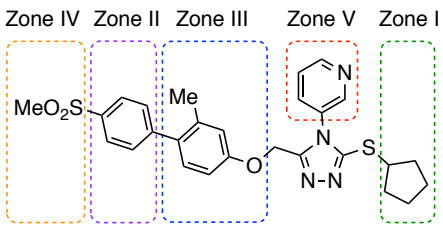


Figure 10. Structural Zones of NMS-873 for SAR/SPR Investigation

To pursue SAR/SPR, we employed a similar synthetic strategy reported by Polucci *et al.* (Scheme 1).[94] Specifically, we proposed to incorporate zone I derivation through a *S*-alkylation reaction (Figure 11). Zone II and zone III constitute the left arm of the molecule and was simplistically described as the side chain, which can be derivatized using diverse aryl and heteroaryl starting materials to pre-construct the side chain using transition metal-mediated coupling conditions. Subsequently, the pre-constructed side chain can be attached to the triazole core through Williamson etherification conditions. Zone IV can exploit an advantageous terminal functional group (*e.g.* -OH, -NH_2) to undergo a facile acylation reaction. Lastly, zone V derivation can occur early in the synthetic strategy by forming thiohydrazides using diverse heteroaryl isothiocyantes followed by cyclization conditions to afford the triazole core. The established medicinal chemistry discovery routes enabled rapid synthesis for structural derivation.

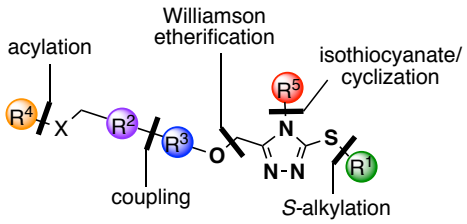


Figure 11. General Synthetic Strategy

1.2.2 Strategy to Improve Solubility

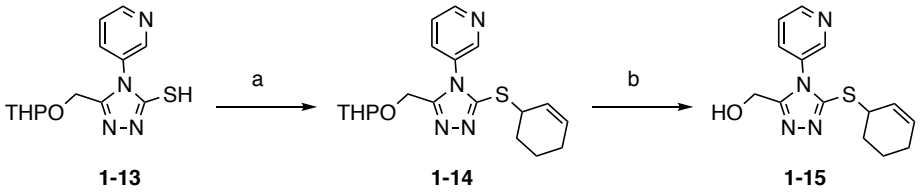
Aqueous solubility is an important physicochemical property that influences *in vivo* performance.[120, 121] Low aqueous solubility of a drug candidate molecule can result in poor absorption and cause limited oral bioavailability following oral dosing.[121, 122] Because NMS-873 has poor *in vivo* performance due, in part, to limited aqueous solubility, one objective of our investigation was to conserve or enhance biological potency while improving aqueous solubility. To achieve this objective, there are number of reported structural modification strategies to enhance small molecule solubility in aqueous media.[121, 123] Addition of ionizable functional groups at physiological pH is commonly employed and is an effective strategy to enhance solubility.[124] For example, incorporation of an amine or a carboxylic acid to the small molecule's structure has been reported to enhance solubility.[125, 126] Moreover, introducing polar groups, for example those that contain heteroatoms and have the capability to participate in hydrogen bonding may also enhance aqueous solubility.[127-129] Further, addition of out-of-plane substituents can reduce planarity and disrupt crystal packing and increase solubility.[130, 131] In our effort, we sought to apply a number of these reported strategies to improve the water solubility of NMS-873 analogues.

1.2.3 Zone II

The objective of a chemical bioisosteric replacement is to create a new molecule with similar biological properties to the parent compound but alter its physicochemical and pharmacokinetic properties.[132, 133] We hypothesized that the biaryl side chain of NMS-873 provided enhanced lipophilicity and contributed to its poor aqueous solubility. To conserve the appropriate geometrical characteristics of the *para*-substituted benzene ring in zone II but reduce planarity and enhance solubility, we hypothesized that zone II could be amenable to a bioisostere replacement. Therefore, we pursued classical and non-classical bioisosteres of the 1,4-disubstituted benzene ring.

Our synthetic efforts focused on the pre-construction of phenoxy-based side chains with zone II bioisostere replacements. The advanced intermediate **1-15** (Scheme 3) was constructed beginning with the 1,2,4-triazole-3-thiol **1-13**, which was supplied by Albany Molecular Research Inc. (AMRI). During our initial SAR investigation, it was found that the cyclohexene motif in zone I demonstrated improved biological performance and added novelty.[134] To initiate our SAR in zone II, intermediate **1-13** was amenable to *S*-alkylation with 3-bromocyclohex-1-ene in the presence of cesium carbonate base to provide the cyclohexenyl adduct **1-14**. Subsequent tetrahydro-2*H*-pyran (THP) deprotection using catalytic tosyl acid in methanol provided the advanced intermediate alcohol **1-15**. This enabled the attachment of the constructed phenoxy-based side chains containing zone II derivation to the triazole core by electrophilic activation of the hydroxyl group of **1-15** followed by a Williamson etherification to afford biologically relevant derivatives.

Scheme 3. Construction of the Advanced Intermediate 1-15

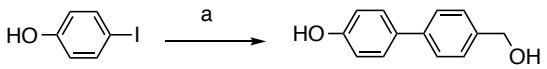


1-13 **1-14** **1-15**

Reagents and conditions: (a) 3-bromocyclohex-1-ene, Cs_2CO_3, DMF, 80 ^oC, 2.5 h, 87%; (b) *p*-TsOH, MeOH, rt, 24 h, 98%.

We initiated zone II replacements with the generation of a control compound, a 1,1'-biphenyl-4-ol side chain **1-17** (Scheme 4), that would serve as a basis for direct comparison of zone II analogues. Additionally, the terminal alcohol can participate in hydrogen bonding with the postulated Lys614 and synthetically provide a functional handle for further derivation in zone IV. The synthesis of **1-17** was constructed through a Suzuki coupling reaction using commercially available 4-iodophenol **1-16** and (4-(hydroxymethyl)phenyl)boronic acid in the presence of Pd/C in water heated at an elevated temperature.

Scheme 4. Synthesis of 1,1'-Biphenyl-4-ol Side Chain 1-17



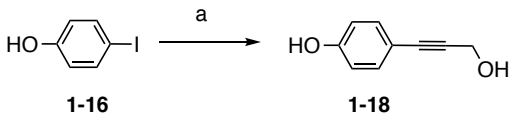
1-16 **1-17**

Reagents and conditions: (a) (4-(hydroxymethyl)phenyl)boronic acid, Pd/C, K_2CO_3, H_2O, 120 ^oC, 1.5 h, 79%.

Internal alkynes are recognized to be a potential bioisostere of 1,4-disubstituted phenyl rings due to the 180 degree dihedral angle, comparable distance between substituents, and conservation of π-character.[135] Therefore, we hypothesized it would be a suitable replacement in zone II. We prepared an alkynyl derivative through a Sonogashira coupling reaction with 4-

iodophenol **1-16** and propargyl alcohol to furnish the acetylenic phenoxy side chain **1-18** (Scheme 5).

Scheme 5. Synthesis of Acetylenic Phenol Side Chain 1-18



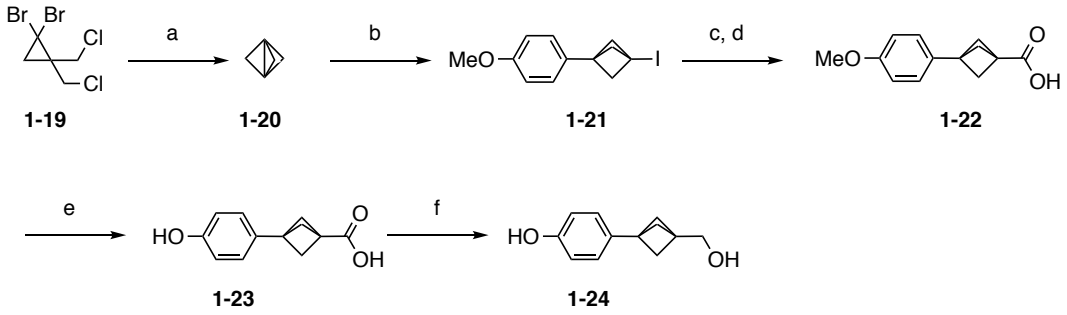
1-16 **1-18**

Reagents and conditions: (a) propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, rt, 17 h, 73%.

The bicyclo[1,1,1]pentane motif has also been reported to be a bioisostere of a 1,4-disubstituted benzene ring.[135, 136] Similar to the alkyne, the bicyclo[1,1,1]pentane motif can conserve the linear vector orientation of the *para*-substituted phenyl ring as well as demonstrate comparable distance between substituents. However, the bicyclo[1,1,1]pentane motif has increased three-dimensionality capable of disrupting planarity and escaping the flatness associated with aryl systems.[137] Replacement of a central, *para*-substituted phenyl ring with the bicyclo[1,1,1]pentane motif has been shown to conserve biological potency and enhance aqueous solubility and drug exposure.[136] Therefore, the bicyclo[1,1,1]pentane side chain **1-24** (Scheme 6) was a promising alternative in zone II. Synthesis of **1-24** began with the commercially available tetrahalide **1-19**, which underwent cyclization using two equivalents of phenyl lithium to form propellane **1-20** (Scheme 6). Propellane was isolated by distillation under reduced pressure under mild heating and due to chemical instability, it was stored as a solution in diethyl ether at -20 ^{o}C. A solution of propellane and 4-iodoanisole was irradiated at 254 nm using a 450-W Mercury lamp to afford the unstable intermediate **1-21**, which was subsequently treated with *t*-butyl lithium undergoing lithium-halogen exchange and quenched with an atmosphere of gaseous CO_2 to provide the corresponding carboxylic acid **1-22**. Demethylation of the anisole occurred with

the treatment of boron tribromide followed by borane-mediated reduction of the carboxylic acid to provide the [1.1.1] bicyclopentane phenol side chain **1-24**.

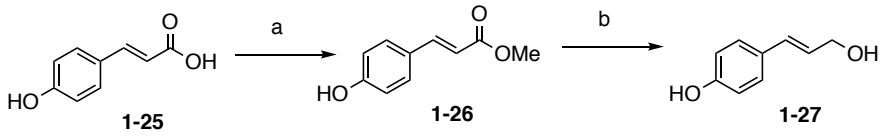
Scheme 6. Synthesis of the Bicyclo[1,1,1]pentane Phenol Side Chain 1-24



Reagents and conditions: (a) PhLi, Et_2O, -45-0 ^oC, 2 h, 95%; (b) 4-iodoanisole, hν, Et_2O, pentane, 6.5 h; (c) *t*-BuLi, Et_2O, -78 ^oC, 0.5 h; (d) CO_2 (g), -78 ^oC-rt, 0.5 h, 64%; (e) BBr_3, CH_2Cl_2, -10 ^oC-rt, 3 h; (f) BH_3 THF, -20 ^oC-rt, 14 h, 54% (two steps).

Next, we were interested in surveying non-classical bioisosteres of the 1,4-disubstituted benzene ring. This led us to construct a number of derivatives, including the *trans*-alkene and its derivatives. Beginning with commercially available *para*-hydroxyl cinnamic acid **1-25** (Scheme 7), Fischer esterification in the presence of sulfuric acid in methanol afforded the methyl ester **1-26**. Subsequent reduction of the methyl ester **1-26** using lithium aluminum hydride furnished the *para*-hydroxyl cinnamyl alcohol side chain **1-27**.

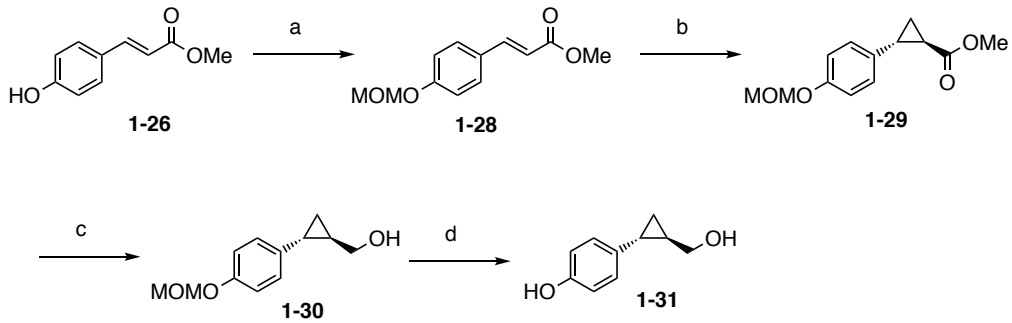
Scheme 7. Synthesis of *para*-Hydroxyl Cinnamyl Alcohol Side Chain 1-27



Reagents and conditions: (a) H_2SO_4, MeOH, reflux, 20 h, 86%; (b) $LiAlH_4$, THF, 0 ^oC, 2 h, 76%.

We were interested in further developing diverse ring systems with three-dimensionality, which led us to explore the *trans*-cyclopropane derivative **1-31** (Scheme 8). The *trans*-cyclopropane is a known bioisostere of the *trans*-alkene and therefore, we hypothesized the *trans*-cyclopropane would demonstrate comparable biological performance.[138] Utilizing the methyl ester intermediate **1-26**, the phenol hydroxyl functionality was temporarily protected using a methoxy methyl (MOM) ether protecting group to afford intermediate **1-28** (Scheme 8). Following, we conducted a Corey-Chaykovsky cyclopropanation reaction on the *α,β*-unsaturated methyl ester **1-26**. The dimethyloxosulfonium methylide Corey-Chaykovsky reagent was generated *in-situ* by reacting trimethylsulfoxonium iodide with sodium hydride. 1,4-Addition of the Corey-Chaykovsky reagent into the *trans*-enone **1-28** followed by ring closure provided the *trans*-cyclopropane **1-29**. Lithium aluminum hydride reduction of the methyl ester to the resulting alcohol **1-30** followed by acid-mediated methoxymethyl ether deprotection generated the *trans*-cyclopropyl phenol side chain **1-31**.

Scheme 8. Synthesis of the *trans*-Cyclopropyl Side Chain 1-31

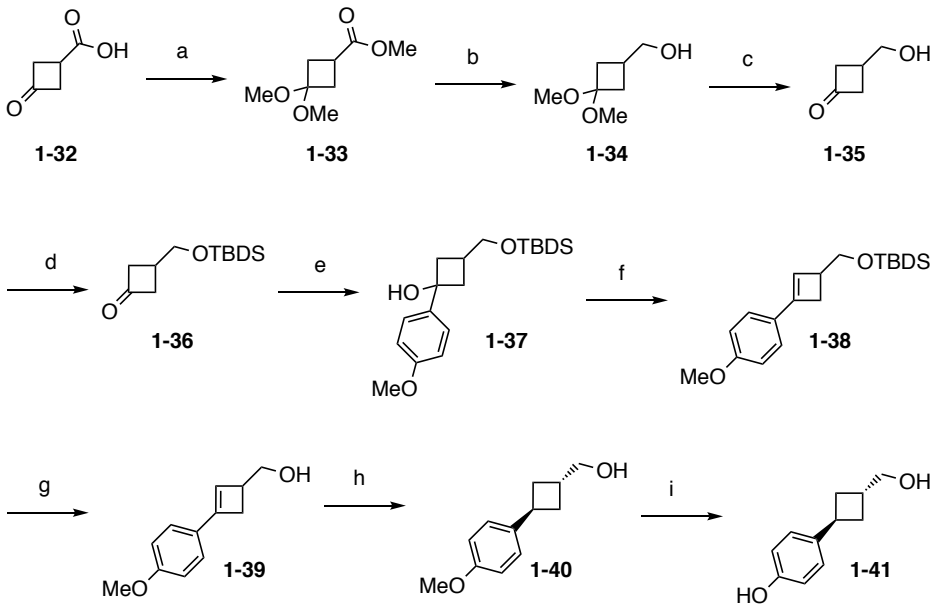


1-26 **1-28** **1-29** **1-30** **1-31**

Reagents and conditions: (a) chloro(methoxy)methane, DIPEA, CH_2Cl_2, rt, 18 h, 90%; (b) trimethyl sulfoxonium iodide, NaH, DMSO, rt, 12 h, 15%; (c) $LiAlH_4$, Et_2O, 0 ^oC, 1 h, 94%; (d) MeOH, AcCl, 0 ^oC-rt, 24 h, 34%.

Continuing our pursuit of non-classical bioisosteres in zone II, we were interested in the development of 1,3-disubstituted cyclobutane derivative **1-41** (Scheme 9). Synthesis began using commercially available 3-oxycyclobutane-1-carboxylic acid **1-32**. Methyl ester formation as well as ketone protection occurred using trimethyl orthoformate and catalytic amounts of *p*-TsOH to generate the dimethyl acetal methyl ester intermediate **1-33**. Subsequent lithium aluminum hydride reduction of the methyl ester afforded the primary alcohol **1-34** followed by acid-mediated dimethyl acetal deprotection and reformation of cyclobutanone **1-35**. Subsequently, the primary alcohol was then protected to form the *tert*-butyl diphenyl silyl (TBDPS) ether **1-36**. Freshly prepared 4-methoxyphenyl magnesium bromide Grignard reagent cleanly reacted with ketone **1-36** to afford the tertiary alcohol **1-37**. Tosyl acid-mediated dehydration resulted in cyclobutene **1-38** formation followed by silyl ether deprotection using tetra-butyl ammonium fluoride to regenerate the free primary alcohol **1-39**. Asymmetric catalytic hydrogenation of cyclobutene in the presence of hydrogen and Crabtree's catalyst resulted in the *trans*-1,3-disubstituted cyclobutane intermediate **1-40**. Lastly, demethylation of the anisole motif using aluminum trichloride and ethyl mercaptan afforded the *trans*-cyclobutyl phenol side chain **1-41**.

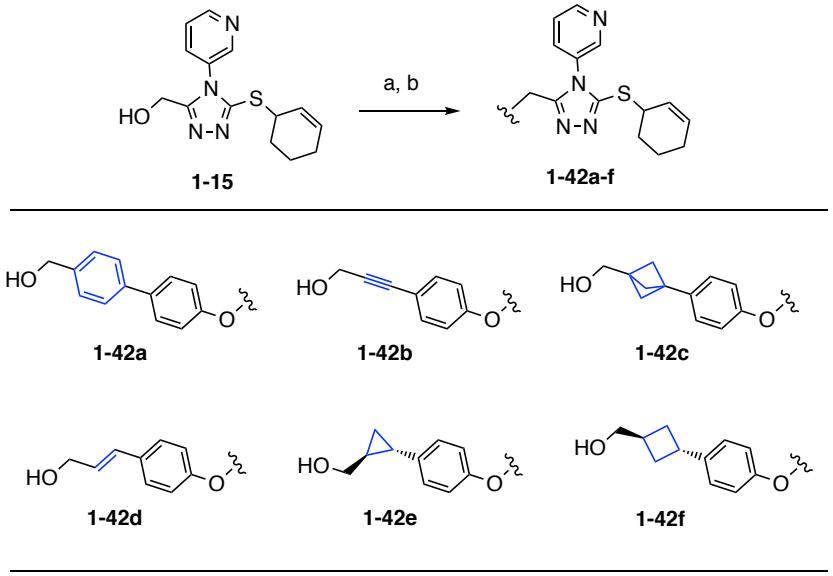
Scheme 9. Synthesis of *trans*-Cyclobutyl Phenol Side Chain 1-41



Reagents and conditions: (a) $HC(OMe)_3$, *p*-TsOH, MeOH, reflux, 19 h, 99%; (b) $LiAlH_4$, Et_2O, rt, 69%; (c) HCl, H_2O, acetone, rt, 73%; (d) TBDPSCl, imidazole, CH_2Cl_2, rt, 80%; (e) (4-methoxyphenyl)magnesium bromide, THF, -78-0 ^oC, 1 h, 65%; (f) *p*-TsOH, PhMe, rt, 24 h, 71%; (g) TBAF, THF, 0 ^oC-rt, 12 h, 53%; (h) H_2, $[Ir(cod)(PCy_3)(Py)]PF_6$, CH_2Cl_2, rt, 2 h, 72%; (i) $AlCl_3$, EtSH, 0 ^oC-rt, 2.5 h, 65%.

The independent assembly of the phenol derivatives to the triazole advanced intermediate was carried out as previously described. In brief, electrophilic activation of the free alcohol on the triazole intermediate **1-15** occurred using mesyl chloride (Scheme 10). Subsequently, Williamson etherification utilizing the respective pre-constructed phenol side chain in the presence of cesium carbonate enabled the completion of biologically relevant analogues **1-42a-f** with zone II derivation.

Scheme 10. Assembly of Zone II Analogues 1-42a-f

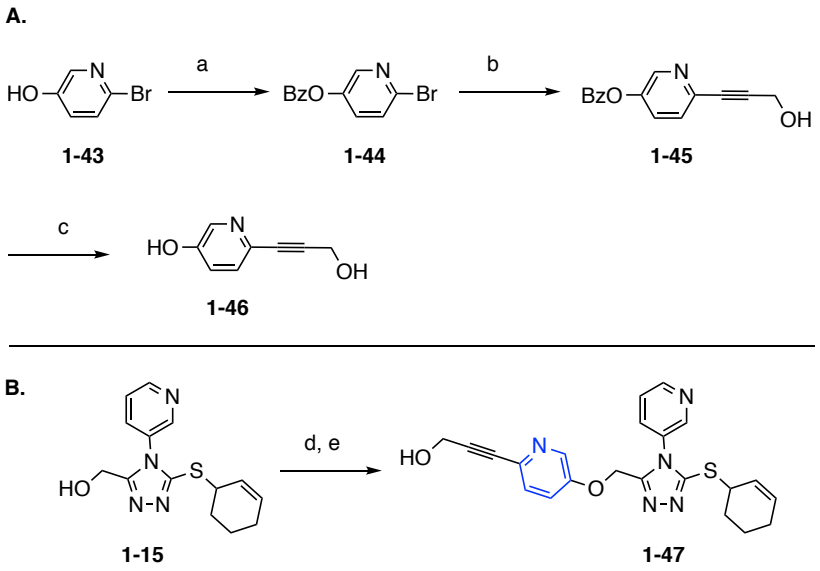


Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (b) **1-17** or **1-18** or **1-24** or **1-27** or **1-31** or **1-41**, Cs_2CO_3, DMF, rt, 34-68%.

1.2.4 Zone III

In zone III, we explored heterocycles and incorporation of polar substituents to introduce hydrogen-bonding capabilities that may enhance aqueous solubility. My initial contributions were focused on developing the pyridine derivative **1-47** (Scheme 11). Synthesis began with temporarily protecting the hydroxyl group by treating commercially available 6-bromopyridin-3-ol **1-43** with benzoic anhydride to provide the benzoyl protected intermediate **1-44** (Scheme 11). Subsequent Sonogashira coupling with propargyl alcohol occurred in modest yield to provide te coupled intermediate **1-45** followed by benzoyl deprotection to establish the 5-hydroxypyridine side chain **1-46**. Electrophilic activation of the triazole intermediate **1-15** with the treatment of mesyl chloride followed by subsequent Williamson etherification utilizing **1-46** in the presence of cesium carbonate completed zone III analog **1-47**.

Scheme 11. Synthesis of Zone III Pyridine Analog 1-47

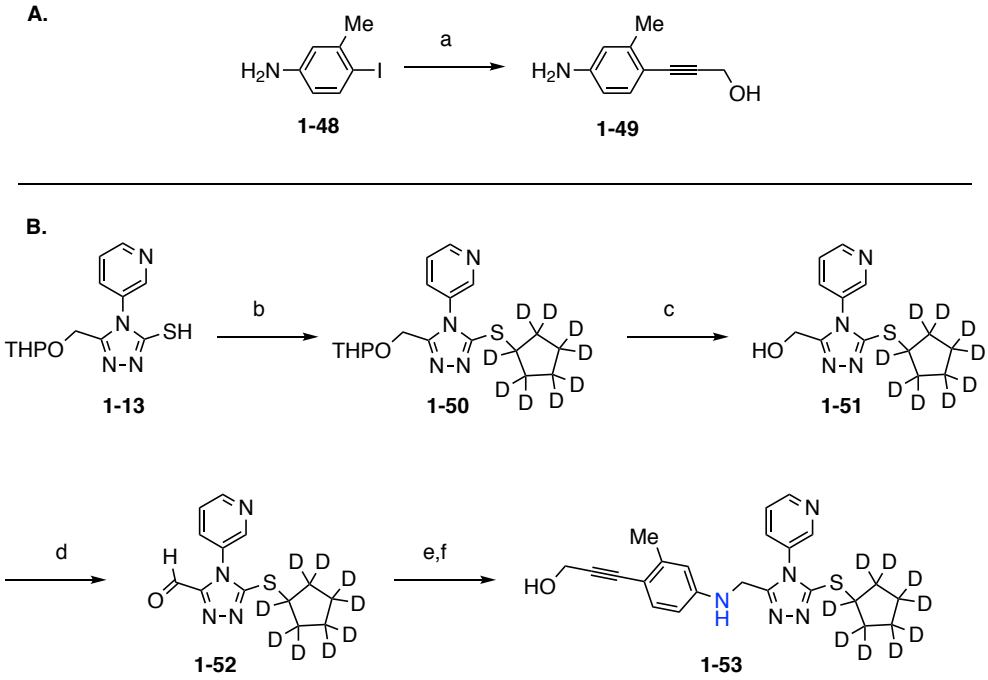


Reagents and conditions: **A.** (a) Bz_2O, Cs_2CO_3, DMF, rt, 14 h, 99%; (b) propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 60 ^oC, 13 h, 33%; (c) NaOH, MeOH, THF, H_2O, rt, 22 h, 74%; **B**. (d) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 1 h; (e) **1-46**, Cs_2CO_3, DMF, rt, 16 h, 68%.

We further explored the replacement of the oxymethylene linker between zone III and the triazole core with a nitrogen methylene isostere replacement. Our preliminary molecular model suggested an opportunity to establish a new hydrogen bond intermolecular interaction between zone III of the 3-sulfanyl-1,2,4-triazole and its putative p97 binding pocket. Therefore, we hypothesized that the introduction of a hydrogen bond donor in zone III would enhance potency and perhaps solubility. The synthesis utilized commercially available 3-methyl-4-iodoaniline **1-48** to perform a Sonogashira coupling reaction with propargyl alcohol to obtain the aniline side chain **1-49** (Scheme 12). Incorporation of the aniline side chain **1-49** onto the triazole core was initiated with the 1,2,4-triazole-3-thiol intermediate **1-13**. During our initial SAR investigation, it was found that the d_9-cylopentyl motif in zone I conserved biological activity and added structural novelty, and therefore, the d_9-cylopentyl motif was commonly employed.[134] *S*-alkylation occurred with **1-13** upon treatment with d_9-cylopentyl bromide and cesium carbonate

to furnish the cycloalkyl intermediate **1-50**. Subsequent THP-deprotection using catalytic tosyl acid afforded the free alcohol **1-51**. Dess-Martin periodinane (DMP) oxidation of the resulting alcohol **1-51** resulted in the aldehyde **1-52**. Lastly, reductive amination occurred with aldehyde **1-52** and the pre-constructed aniline side chain **1-49** upon treatment of with acetic acid followed by sodium cyanoborohydride to achieve the nitrogen methylene isostere analog **1-53**.

Scheme 12. Synthesis of Zone III Nitrogen Isostere 1-53



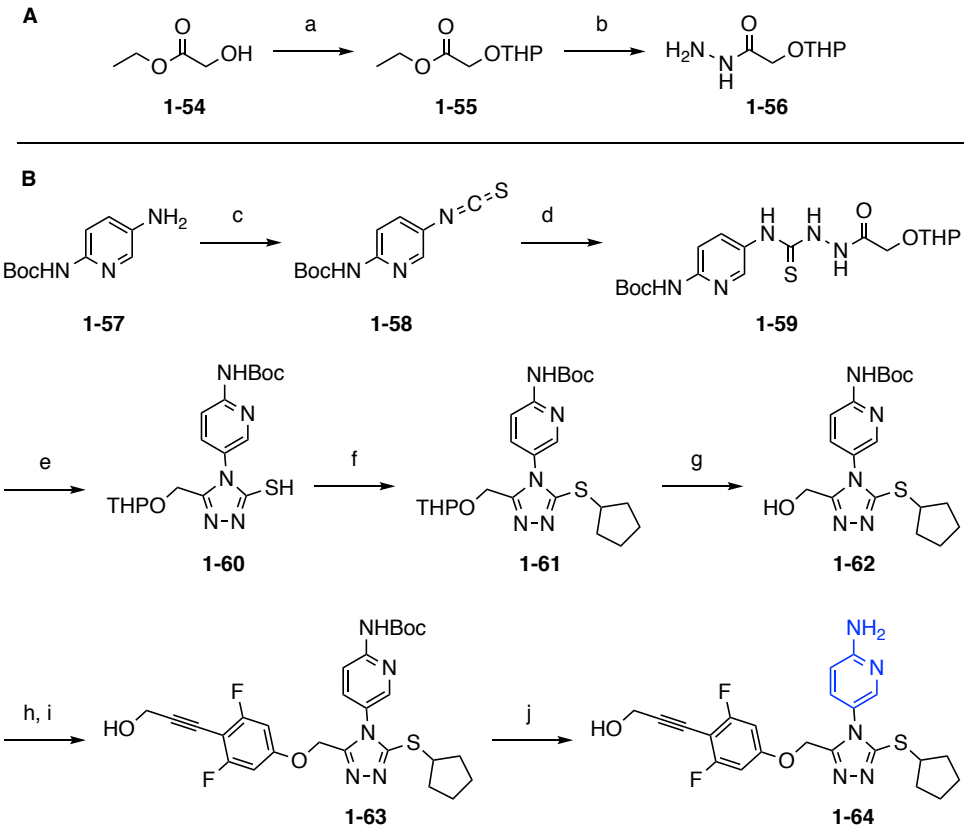
Reagents and conditions: **A.** (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, rt, 24 h, 78%; **B.** (b) bromocyclopentane-d_9; Cs_2CO_3, DMF, 80 ^oC, 2.5 h, 38%; (c) *p*-TsOH, MeOH, 40 ^oC, 3 h, 64%; (d) DMP, CH_2Cl_2, rt, 2 h, 80%; (e) **1-49**, AcOH, THF, rt, 19 h; (f) $NaBH(OAc)_3$, 24 h, 46% (two steps).

1.2.5 Zone V

It was previously reported that the introduction of the pyridyl motif in zone V conserved biological potency and enhanced solubility.[94] We explored alternative nitrogen heterocycles in

zone V to introduce additional hydrogen-bonding capabilities to enhance biological potency and aqueous solubility. Construction of zone V derivatives required a common acetohydrazide intermediate **1-56** (Scheme 13). Therefore, synthesis began with the tetrahydropyranyl (THP) protection of commercially available ethyl glycolate **1-54** (Scheme 13). Subsequent aminolysis of the ethyl ester of **1-55** occurred in the presence of hydrazine at reflux to afford the necessary acetohydrazide intermediated **1-56**. According to our preliminary molecular model, it was hypothesized that the 4-aminopyridyl motif in zone V could establish new intermolecular interactions with p97 and therefore, enhance biological potency. The synthesis of analog **1-64** was initiated with the prepared intermediate *tert*-butyl (5-aminopyridin-2-yl)carbamate **1-57**, which was provided by Kaylan Karrigan (Wipf group). Treatment of **1-57** with thiophosgene and sodium bicarbonate in a biphasic solution of methylene chloride and water provided the isothiocyanate **1-58**. Introducing the previously prepared acetohydrazide **1-56** with the established isothiocyanate **1-58** resulted in the formation of thiourea intermediate **1-59**. This enabled base-mediated cyclization of **1-59** to afford the 3-sulfanyl-1,2,4-triazole core **1-60** with zone V derivation. *S*-alkylation with bromocyclopentane yielded intermediate **1-61** followed by tetrahydropyranyl deprotection using pyridinium *p*-toluenesulfonate (PPTS) resulted in the hydroxyl intermediate **1-52**. Our SAR investigation of zone 3 found that the 3,5-difluorophenol motif resulted in enhanced biological performance.[134] Therefore, subsequent Williamson etherification by electrophilic activation of the benzyl alcohol using mesyl chloride followed by displacement with 3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol side chain (provided by AMRI) resulted in the formation of aryl ether intermediate **1-63**. Lastly, *tert*-butyloxycarbonyl (Boc) deprotection occurred in the presence of potassium carbonate in methanol at 100 ^{o}C to provide the amino pyridine analog **1-64**.

Scheme 13. Synthesis of Amino Pyridine Analog 1-64

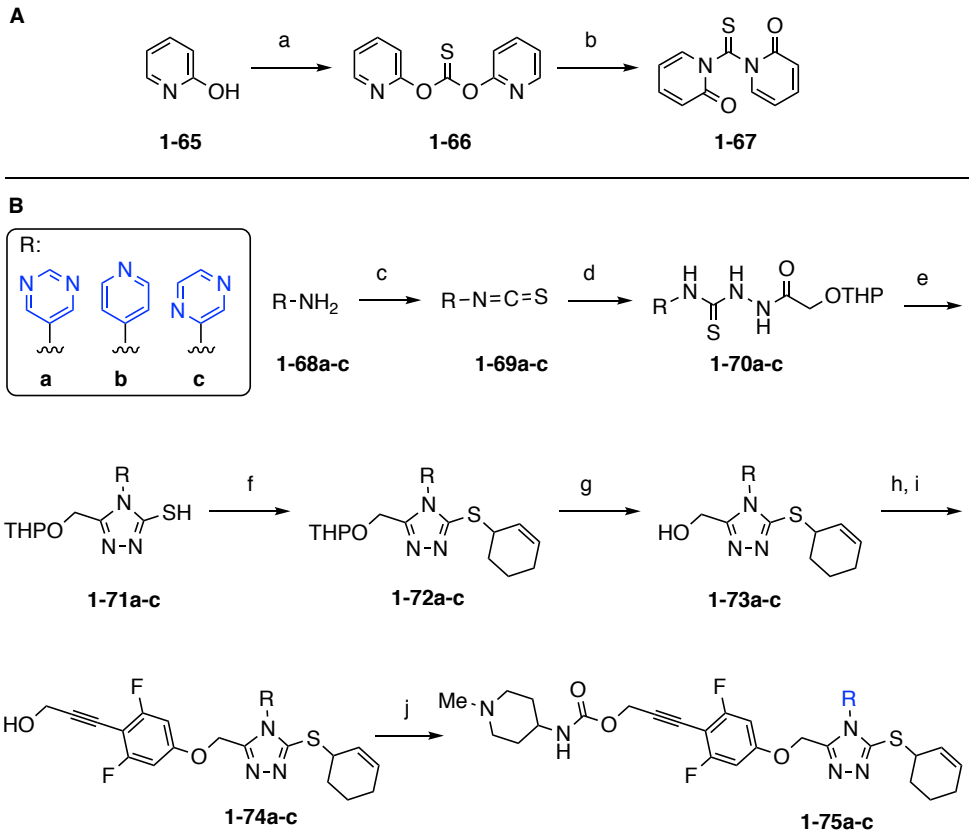


Reagents and conditions: **A.** (a) DHP, *p*-TSA, rt, 48 h, 92%; (b) hydrazine, EtOH, reflux, 21 h, 86%; **B.** (c) thiophosgene, $NaHCO_3$, $CHCl_3$, H_2O, 0 °C, 3 h, 86%; (d) **1-56**, EtOH, 80 °C, 2 h; (e) NaOH, H_2O, 50 °C, 15 h, 56%; (f) bromocyclopentane, Cs_2CO_3, DMF, rt, 22 h, 73% (g) PPTS, EtOH, 70 °C, 24 h, 98%; (h) MsCl, DIPEA, CH_2Cl_2, 0 °C, 3 h; (i) 3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol, Cs_2CO_3, DMF, rt, 24 h, 56%; (j) K_2CO_3, MeOH, 100 °C, 18 h, 63%.

We next developed a series of additional nitrogen-containing heterocycles in zone V. For this effort, we found it was necessary to construct and use the 1,1'-thiocarbonylbis(pyridin-2(1*H*)-one) reagent, **1-67** (Scheme 14), which enabled clean and facile preparation of isothiocyanate intermediates. To fashion **1-67**, treatment of commercially available 2-hydroxypyridine **1-65** with thiophosgene formed the di(pyridine-2-yl) carbonothioate **1-66**. Subsequent, DMAP-catalyzed rearrangement afforded the desired reagent **1-67** as a bright

orange solid. Similar to the synthesis of analog **1-64**, construction of zone V analogues began with nitrogen-containing heterocyclic amines **1-68a-c**. Isothiocyanation nicely occurred using the prepared thiocarbonylbis(pyridin-2(1*H*)-one) reagent **1-67** to form the respective substituted isothiocyantes **1-69a-c**. Introducing **1-69a-c** to the previously prepared hydrazide **1-56** provided the thiourea intermediate **1-70a-c**. Subsequent base-mediated cyclization furnished the 1,2,4-triazole-3-thiol core **1-71a-c**, which underwent *S*-alkylation with 3-bromocyclohex-1-ene to form **1-72a-c** followed by tetrahahydropyranyl deprotection using tosyl acid to provide the hydroxyl intermediate **1-73a-c**. Williamson etherification occurred through electrophilic activation of the established alcohol using mesyl chloride followed by nucleophilic displacement with the provided 3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol side chain to provide the terminal alcohol intermediate **1-74a-c**. Our SAR investigation also found that the functionalization of the propargylic alcohol to form diverse carbamates in zone IV improved biological performance.[134] Therefore, carbodiimidazole-mediated activation of the alcohol followed by treatment with 1-methylpiperidin-4-amine afforded the carbamates **1-75a-c**, completing the formation of the series of zone V N-heterocyclic analogues.

Scheme 14. Synthesis of Zone V N-heterocyclic Analogues 1-75a-c



Reagents and conditions: **A.** (a) thiophosgene, Et_3N, CH_2Cl_2, 0 ^oC, 1 h, 86%; (b) DMAP, CH_2Cl_2, rt, 13 h, 63%; **B.** (c) **1-67**, CH_2Cl_2, rt, 2 h; (d) **1-53**, EtOH, 80 ^oC, 1 h; (e) NaOH, H_2O, 120 ^oC, 4 h, 55-73%; (f) 3-bromocyclohex-1-ene, Cs_2CO_3, DMF, rt, 21 h, 38-91%; (g) *p*-TsOH, MeOH, rt, 18 h, 72-98%; (h) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (i) 3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol, Cs_2CO_3, DMF, rt, 15 h, 50-63%; (j) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 24 h, 62-92%.

1.2.6 Metabolic Stability

Drug metabolism plays a pivotal role dictating drug exposure and oral bioavailability.[121, 139] Cytochrome P450 are a class of enzymes mostly responsible for oxidative metabolism of drugs, leading to increased hydrophilicity and rapid elimination.[121, 140] Our collective SAR/SPR investigation resulted in improved biological activity and physicochemical properties; however,

no significant improvement was demonstrated in oxidative metabolic stability using an *in vitro* human and mouse liver microsome assay. Therefore, we sought to address potential metabolic sites that are susceptible to oxidative metabolism, which influence first pass metabolism and oral bioavailability.

Although the cyclohexenyl group in zone I improved the biochemical and cellular performance and provided a degree of novelty from NMS-873, we hypothesized the alkene motif **1-76** (Figure 12) within the carbocyclic framework was a metabolic liability. Reports have shown alkenes are susceptible to oxidative metabolism by cytochrome P450 enzymes.[141-145] Therefore, we envisioned the oxidation of the cycloalkene **1-76** could lead to the transient formation of epoxide **1-77** followed by ring opening hydrolysis to provide the polar 1,2-diol **1-78** metabolite.

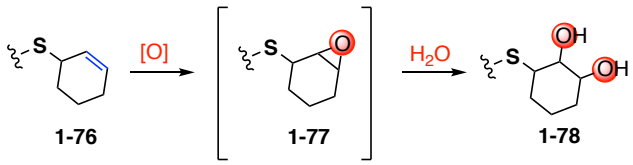
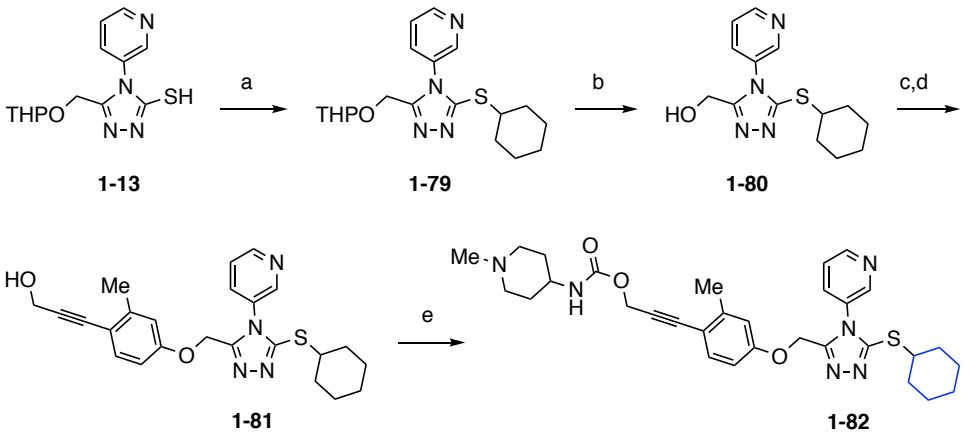


Figure 12. Postulated Oxidation of Cyclohexene

To initially affirm and test the metabolic stability of the cycloalkene motif, we constructed and evaluated the cyclohexane derivative **1-82** (Scheme 15) in zone I. Synthesis of this analog occurred as previously described. Synthesis began with the *S*-alkylation of 1,2,4-triazole-3-thiol **1-13** with bromocyclohexane to provide the cyclohexyl intermediate **1-79** (Scheme 15). Subsequent tetrahydropyranyl deprotection using tosyl acid afforded the free alcohol **1-80**. Williamson etherification occurred through the mesylation of the established hydroxyl group followed by nucleophilic displacement with the pre-constructed 4-(3-

hydroxyprop-1-yn-1-yl)-3-methylphenol side chain, furnishing the alcohol intermediate **1-81**. Carbodiimidazole-mediated activation of the alcohol followed by treatment with 1-methylpiperidin-4-amine afforded the carbamate **1-82.**

Scheme 15. Synthesis of Zone I Cyclohexyl Analog 1-82



Reagents and conditions: (a) bromocyclohexane, Cs_2CO_3, DMF, 80 ^oC, 3 h, 28%; (b) *p*-TsOH, MeOH, rt, 13 h, 98%; (c) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (d) 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol, Cs_2CO_3, DMF, rt, 12 h, 64%; (e) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 24 h, 85%.

Several reported strategies describe structural modifications that assist on mitigating phase I oxidative drug metabolism and enhancing metabolic stability.[121, 146, 147] One strategy included the removal or replacement of identified metabolically labile groups.[148-150] Moreover, blocking a metabolic site with fluorine or deuterium is also a common and recognized as an effective strategy.[151-153] Further, introduction of steric interference and electron-withdrawing groups can influence metabolic stability as well.[154] Interestingly, changing ring size or chirality has also been reported.[155, 156] We explored a number of these strategies in order to enhance metabolic stability of the 3-sulfanyl-1,2,4-triazole series.

We initially explored derivatives that replaced the cyclohexenyl motif in zone I. Previously reported SAR indicated the importance of a nonpolar, lipophilic cycloalkyl group in zone I.[94] Additionally, SAR of zone I demonstrated a ring size preference, indicating a narrow spatial requirement (Table 1; Figure 13).[94] Therefore, this led us to pursue a number of small aliphatic cyclic and spirocylic ring systems.

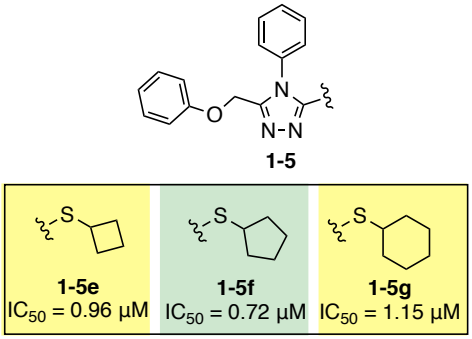
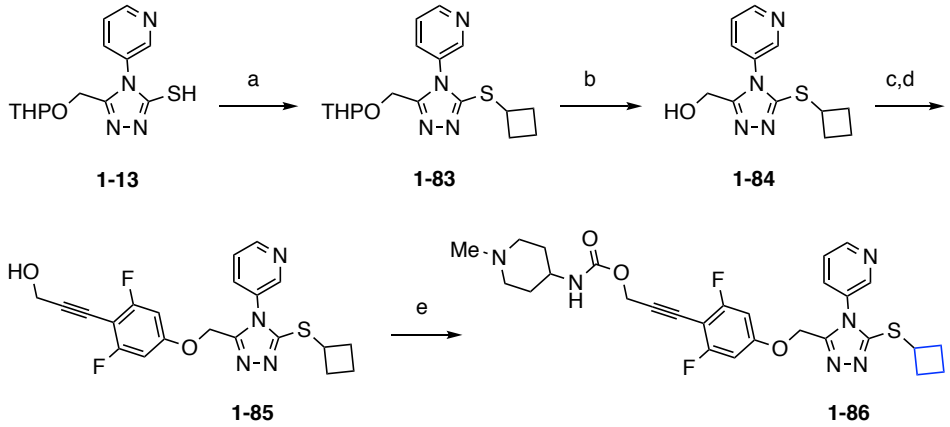


Figure 13. Zone I Ring Size Preference [94]

We sought to replace the cyclohexenyl group in zone I with small, substituted carbocycles. The unsubstituted cyclobutyl analog **1-86** (Scheme 16) was initially synthesized. Synthesis of this analog occurred as previously described. In brief, synthesis began with the *S*-alkylation of 1,2,4-triazole-3-thiol **1-13** with bromocyclobutane to provide the cyclobutyl intermediate **1-83**. Subsequent tetrahydropyranyl deprotection using tosyl acid afforded the free hydroxyl group **1-84**. Williamson etherification occurred through the mesylation of the established hydroxyl group followed by nucleophilic displacement with the pre-constructed 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol side chain, furnishing the alcohol intermediate **1-85**. Carbonyldiimidazole-mediated activation of the alcohol followed by treatment with 1-methylpiperidin-4-amine afforded the carbamate **1-86.**

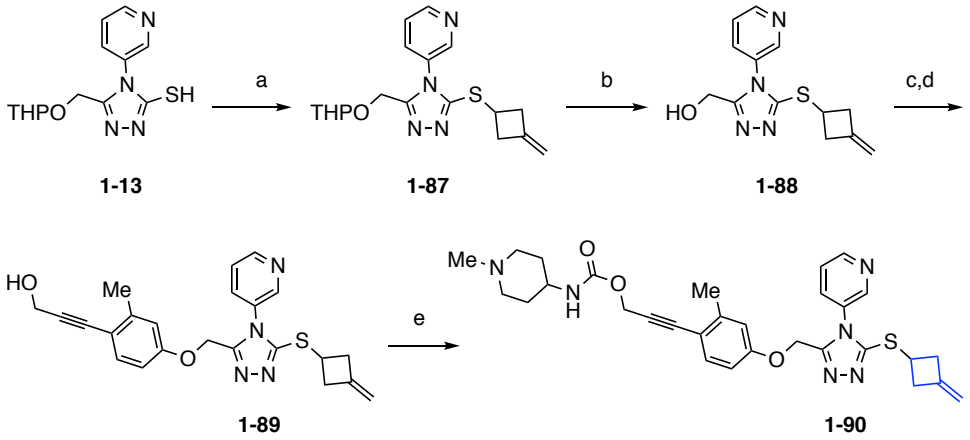
Scheme 16. Synthesis of Zone I Cyclobutyl Analog 1-86



Reagents and conditions: (a) bromocyclobutane, Cs_2CO_3, DMF, rt, 24 h, 63%; (b) *p*-TsOH, MeOH, rt, 24 h, 94%; (c) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (d) 3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol, Cs_2CO_3, DMF, rt, 21 h, 67%; (e) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 24 h, 86%.

To increase the steric bulk of the initial cyclobutyl motif, we investigated the incorporation of aliphatic substituents, which included the methylene cyclobutyl analog **1-90** (Scheme 17). Synthesis began with a radical mediated *S*-alkylation of 1,2,4-triazole-3-thiol **1-13** with the previously prepared propellane **1-20** in the presence of AIBN at 70 ^oC to provide the methylene cyclobutyl intermediate **1-87**. Subsequent tetrahydropyranyl deprotection using tosyl acid afforded the free hydroxyl group in **1-88**. Williamson etherification occurred through the mesylation of the established hydroxyl group followed by nucleophilic displacement with the pre-constructed 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol side chain, furnishing the alcohol intermediate **1-89**. Carbodiimidazole-mediated activation of the alcohol followed by treatment with 1-methylpiperidin-4-amine afforded the carbamate **1-90**.

Scheme 17. Synthesis of Zone I Methylenecyclobutyl Analog 1-90

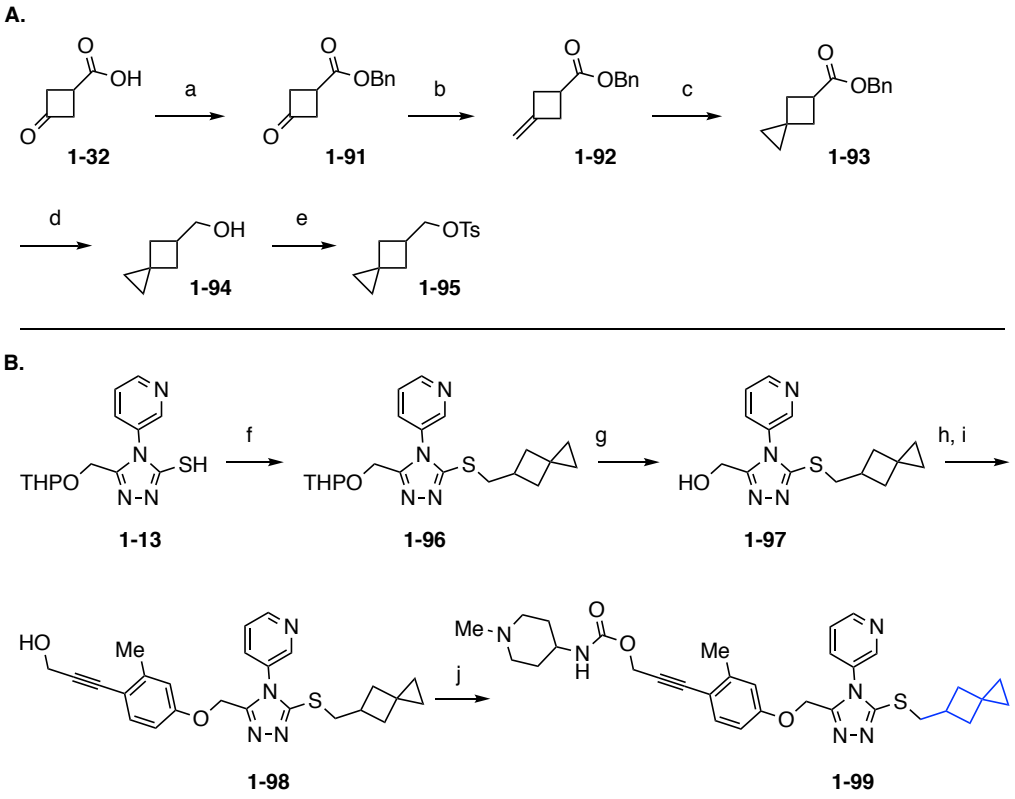


Reagents and conditions: (a) **1-20**, AIBN, THF, 0-70 ^{o}C, 13 h, 56%; (b) *p*-TsOH, MeOH, rt, 21 h, 96%; (c) MsCl, DIPEA, CH_2Cl_2, 0 ^{o}C, 3 h (d) 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol, Cs_2CO_3, DMF, rt, 12 h, 84%; (e) CDI, CH_2Cl_2, 0 ^{o}C-rt, 2 h; (f) 1-methylpiperidin-4-amine, rt, 22 h, 32%.

We hypothesized that the previous developed cyclobutyl derivatives did not have sufficient steric bulk to properly secure the aliphatic zone I motif in the postulated hydrophobic pocket. Therefore, we explored a spirocycle with relatively greater effective volume. Synthesis of analog **1-99** (Scheme 18) began with the construction of the zone I spirocyclic motif. Formation of benzyl ester **1-91** from commercially available 3-oxocyclobutane-1-carboxylic acid **1-32** occurred upon treatment with potassium carbonate and benzyl bromide. Olefination of the ketone occurred using freshly prepared Petasis reagent (dimethyltitanocene) to provide the methylene cyclobutane intermediate **1-92**. Subsequent application of Simmon-Smith cyclopropanation conditions afforded the spiro[2,3]hexane **1-93**. Lithium aluminum hydride-mediated reduction of the benzyl ester provided the resulting alcohol **1-94**, which was then tosylated to afford the activated spirocyclic motif **1-95**. Consequent *S*-alkylation of 1,2,4-triazole-3-thiol **1-13** with the prepared electrophile **1-95** established the spirocyclic intermediate **1-96**. Then as previously described, tetrahydropyranyl deprotecton to provide the free alcohol **1-**

97, which subsequently underwent Williamson etherification occurred through the mesylation of the established hydroxyl group followed by nucleophilic displacement with the pre-constructed 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol side chain to furnish the alcohol intermediate **1-98**. Carbodiimidazole-mediated activation of the alcohol followed by treatment with 1-methylpiperidin-4-amine afforded the carbamate **1-99**.

Scheme 18. Synthesis of Zone I Spirocyclic Analog 1-99



Reagents and conditions: **A.** (a) BnBr, K_2CO_3, CH_3CN, rt, 24 h, 76%; (b) Petasis reagent, THF, 60 °C, 5 d, 50%; (c) CH_2I_2, Et_2Zn, TFA, CH_2Cl_2, 0 °C-rt, 72 h, 81%; (d) $LiAlH_4$, THF, -78-0 °C, 1 h; (e) TsCl, pyridine, rt, 2.5 h, 66% (two steps); **B.** (f) **1-87**, Cs_2CO_3, DMF, 80 °C, 2.5 h, 54%; (g) TsOH, MeOH, rt, 17 h, 99%; (h) MsCl, DIPEA, CH_2Cl_2, 0 °C, 3 h; (i) 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol, Cs_2CO_3, DMF, rt, 15 h, 84%; (j) CDI, CH_2Cl_2, rt, 2 h, 1-methylpiperidin-4-amine, rt, 21 h, 48%.

1.2.7 Zone I Sulfur Metabolism

Sulfur is known to undergo oxidative-metabolism and therefore, we postulated sulfur in zone I could also represent a metabolic liability.[116, 157-159] It was previously demonstrated that the oxidized sulfur product, the isopropylsulfone **1-6**, resulted in an inactive metabolite (Figure 8).[94] One obvious strategy to reduce metabolic oxidation of sulfur would be to remove and replace it with a heteroatom isostere.[146] However, it was previously demonstrated that there is a heteroatom preference in zone I, whereby sulfur demonstrated greater biological potency compared to oxygen, nitrogen, and carbon (Table 2; Figure 14).[94] Therefore, we conserved the sulfur atom in zone I, but we investigated derivatives that introduced steric interference around the sulfur atom in aspiration to reduce/interfere with CYP450-mediated oxidation. Additionally, we further explored analogues that reduced sulfur electron density by incorporating an inductively electron-withdrawing group alpha to the sulfur atom to influence metabolic stability.

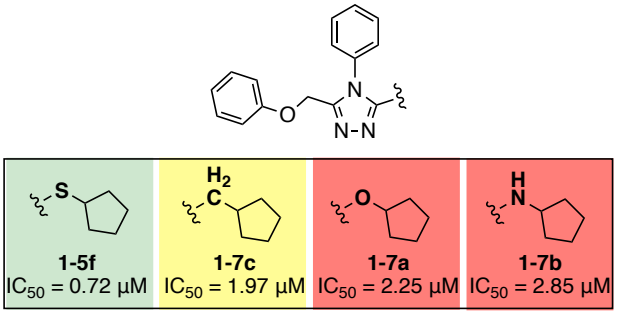
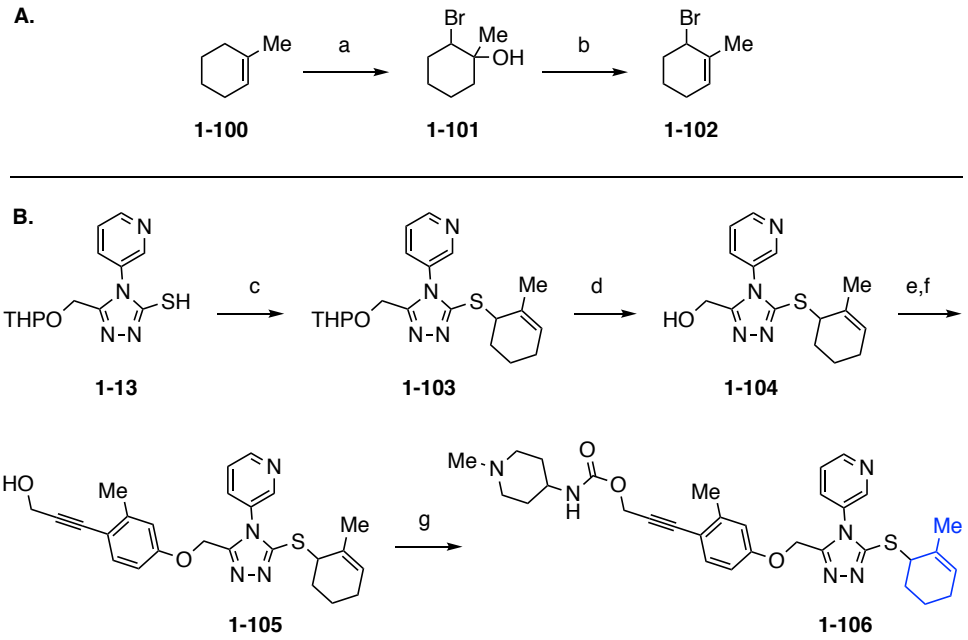


Figure 14. Zone I Heteroatom Preference [94]

Our initial pursuit incorporated a methyl group at position 2 of the cyclohexenyl motif to provide steric interference of sulfur. We initiated our synthesis of analog **1-106** (Scheme 19) with the formation of the methylcyclohexenyl motif in zone I. Electrophilic bromonation of 1-methylcyclohexene **1-100** using N-bromosuccimide (NBS) followed by hydrolysis with water

provided the tertiary alcohol intermediate **1-101**. Subsequently dehydration using strong lewis acid boron trifluoride etherate generated 6-bromo-1-methylcyclohexene, **1-102**. *S*-alkylation of 1,2,4-triazole-3-thiol **1-13** with the prepared electrophile **1-102** incorporated the methylcyclohexenyl motif and provided intermediate **1-103**. Tetrahydropyranyl deprotecton afforded the free alcohol **1-104**, which subsequently underwent Williamson etherification through mesylation of the established hydroxyl group followed by nucleophilic displacement with the pre-constructed 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol side chain to furnish the alcohol intermediate **1-105**. Carbonyldiimidazole-mediated activation of the alcohol followed by treatment with 1-methylpiperidin-4-amine afforded the carbamate **1-106**.

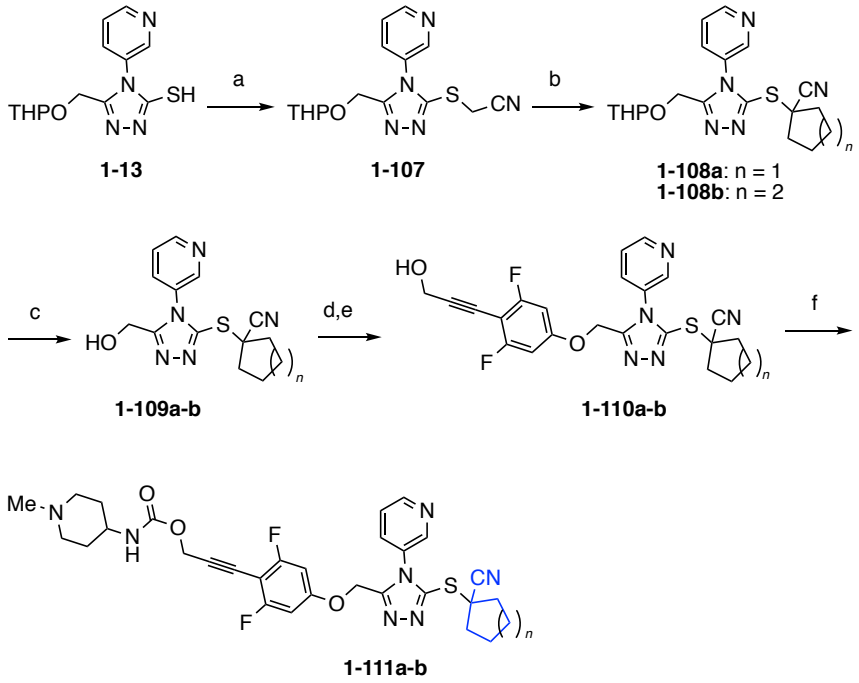
Scheme 19. Synthesis of Zone I Methylcyclohexenyl Analog 1-106



Reagents and conditions: (a) NBS, water, acetone, 0 ^{o}C - rt, 21 h, 70%; (b) BF_3 Et_2O, CH_2Cl_2, reflux, 2 h, 60%; (c) **1-102**, Cs_2CO_3, DMF, 80 ^{o}C, 2 h, 82%; (d) TsOH, MeOH, rt, 17 h, 98%; (e) MsCl, DIPEA, CH_2Cl_2, 0 ^{o}C, 1 h; (f) 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol, Cs_2CO_3, DMF, rt, 17 h, 27%; (g) CDI, CH_2Cl_2, rt, 3 h, 1-methylpiperidin-4-amine, rt, 11 h, 58%

Next, we were interested in developing analogues that reduced sulfur electron density by incorporating inductively electron-withdrawing groups alpha to the sulfur atom. This led to the pursuit of thiocyclic-1-carbonitrile analogues **1-111a** and **1-111b** (Scheme 20). Synthesis of these analogues began with the *S*-alkylation of 1,2,4-triazole-3-thiol **1-13** with 2-iodoacetonitrile to provide the alkyl intermediate **1-107**. Treatment of intermediate **1-107** with two equivalents of sodium hydride and the respective dibromoalkane established the thiocycloalkyl motif **1-108a-b** with the electron withdrawing cyano group alpha to the sulfur. Then, tetrahydropyranyl deprotecton to provide the free alcohol **1-109a-b**, which subsequently underwent Williamson etherification through the mesylation of the established hydroxyl group followed by nucleophilic displacement with the pre-constructed 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol side chain to furnish the alcohol intermediate **1-110a-b**. Carbonyldiimidazole-mediated activation of the alcohol followed by treatment with 1-methylpiperidin-4-amine afforded the carbamate **1-111a-b**.

Scheme 20. Synthesis of Zone I Cyanocycloalkyl Analogues 1-111a and 1-111b



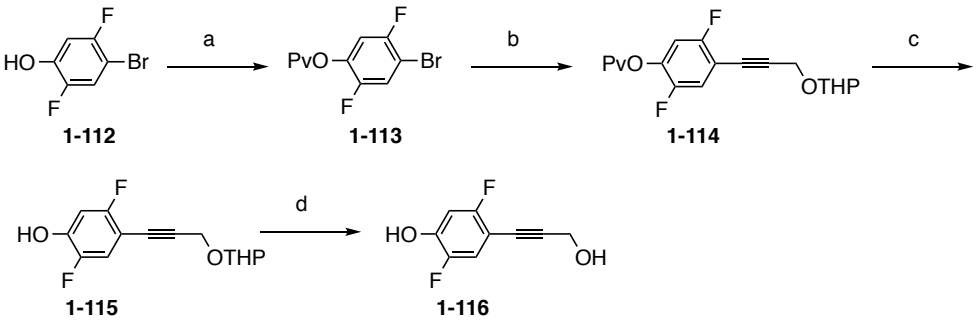
Reagents and conditions: (a) 2-iodoacetonitrile, Cs_2CO_3, DMF, 80 ^oC, 2 h, 97%; (b) 1,4-dibromobutane or 1,5-dibromobutane, NaH, DMF, rt, 4 h, 83%; (c) TsOH, MeOH, rt, 18 h, 85%; (d) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (e) 3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol, Cs_2CO_3, DMF, rt, 24 h, 76%; (f) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 17 h, 85%.

1.2.8 Scale-up

The UPCDC medicinal chemistry team performed an extensive SAR/SPR investigation, synthesizing over 400 analogues of NMS-873. This effort resulted molecules that demonstrated promising *in vitro* and cellular properties. Subsequently, the NIH Chemical Biology Concortium (CBC) was interested in pursuing animal pharmacology studies to characterize the pharmacokinetic and pharmacodynamic profile of this scaffold. Accordingly, we pursued a scale-up synthesis to access product on gram-scale in >98% purity by ^1H NMR and LC-MS ELSD.

The scale-up began with the construction of the 2,5-difluorophenol side chain **1-116** (Scheme 21). The commercially available 4-bromo-2,5-difluorophenol **1-112** was protected using pivaloyl chloride and triethylamine to provide the phenol **1-113**. Subsequent Sonogashira coupling occurred with the tetrahydropyranyl protected propargyl alcohol to obtain coupled intermediate **1-114**. Consecutive pivaloyl and tetrahydropyranyl deprotection afforded the requisite side chain **1-116**.

Scheme 21. Synthesis of 2,5-Difluorophenol Side Chain 1-116



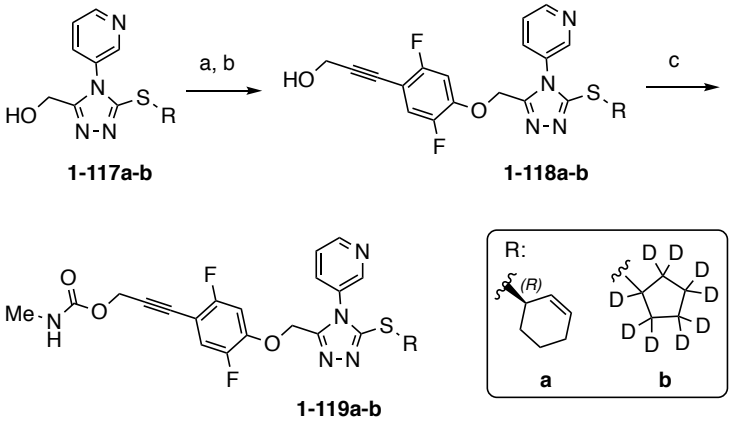
1-112 **1-113** **1-114** **1-115** **1-116**

Reagents and conditions: (a) PivCl, Et_3N, CH_2Cl_2, 0 ^oC-rt, 4 h, 99%; (b) 2-(prop-2-yn-1-yloxy)tetrahydro-2*H*-pyran, CuI, $Pd(PPh_3)_4$, Et_3N, 90 ^oC, 24 h, 74%, NaOH, MeOH, THF, 21 h, 83%; (d) PPTS, MeOH, rt, 24 h, 42%.

The 2,5-difluorophenol side chain **1-116** was fashioned onto two 3-sulfanyl-1,2,4-triazole intermediates that differ in their zone I motif: intermediate **1-117a** contained the (*R*)-cyclohexenyl motif (provided by AMRI) and intermediate **1-117b** contained the d_9-cyclopentyl group (provided by Celeste Alverez, Wipf group) (Scheme 22). Williamson etherification required the treatment of intermediate **1-117a** with mesyl chloride and treatment of intermediate **1-117b** with thionyl chloride. The electrophilic intermediate reacted with 2,5-difluorophenol **1-116** to furnish the alcohol **1-118a-b**. Subsequent, carbonyldiimidazole-mediated activation of the alcohol followed by treatment with methylamine hydrochloride afforded the

methylcarbamates **1-119a-b**. Both **1-119a** and **1-119b** demonstrated >98% purity by ^{1}H NMR and LC-MS ELSD.

Scheme 22. Scale-up of 1-119a and 1-119b

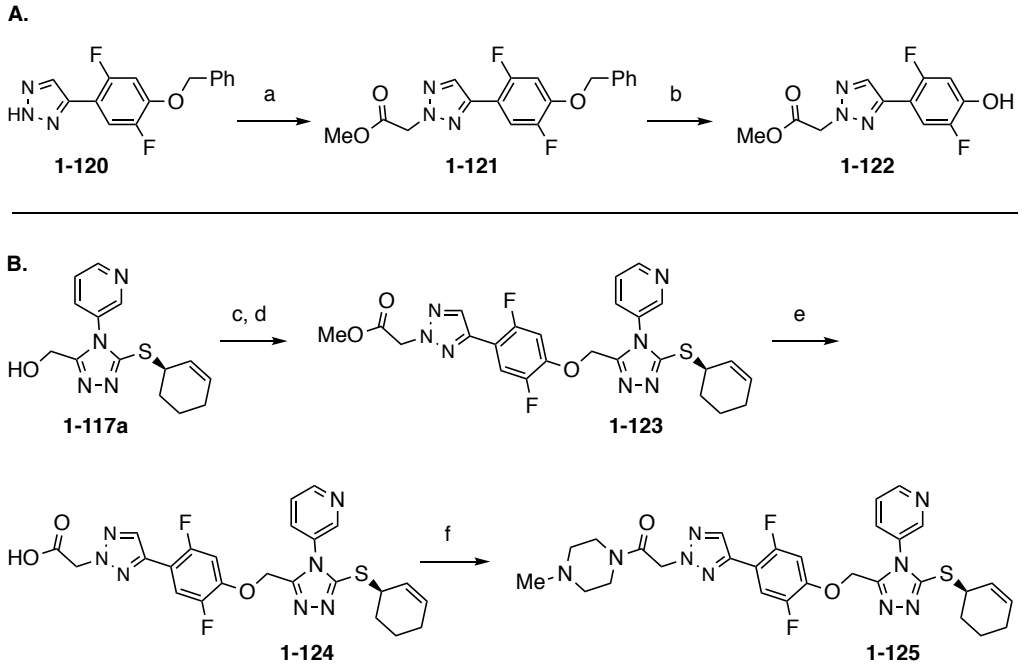


Reagents and conditions: (a) **1-117a**: MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 5 h or **1-117b**: $SOCl_2$, Et_3N, CH_2Cl_2, 0 ^oC, 2 h; (b) 2,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol, Cs_2CO_3, DMF, rt, 18 h, 91%; (c) methylamine hydrochloride, Et_3N, CDI, CH_2Cl_2, rt, 17 h, 98%.

We were also interested in the scale-up of triazole of **1-125** (Scheme 23). The synthesis started with 2,5-difluorophenyl-2*H*-1,2,3-triazole intermediate **1-120**, which was prepared by Dr. Joel Walker at Pittsburgh. *N*-alkylation of **1-120** occurred upon treatment with sodium hydride and methyl 2-bromoacetate in the presence of tetrabutylammonium iodide to provide the N-alkylated product **1-121** (Scheme 23). Subsequent benzyl deprotection using Pd/C under an atmosphere of hydrogen provided the phenol side chain **1-122**. The prepared phenol side chain **1-122** was then incorporated onto the sulfanyl-1,2,4-triazole intermediate **1-117a** (supplied by AMRI) under Williamson etherification conditions. The resulting methyl ester intermediate **1-123** was saponified using lithium hydroxide and the carboxylic acid was subsequently coupled with 1-methylpiperazine using propanephosphonic acid anhydride (T3P)-mediated conditions to

complete the preparation of **1-125**. Compound **1-125** demonstrated >98% purity by ^{1}H NMR and LC-MS ELSD.

Scheme 23. Scale-up of 1-125

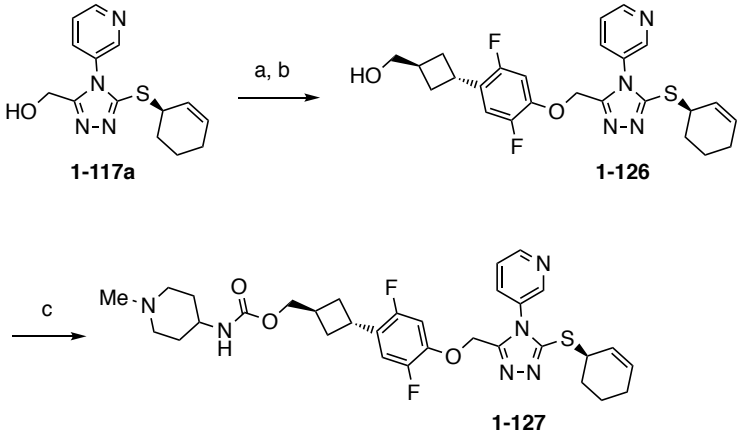


Reagents and conditions: **A.** (a) methyl 2-bromoacetate, NaH, TBAI, THF, rt, 6 h, 48%; (b) H_2, Pd/C, MeOH, rt, 24 h, 93%; **B.** (c) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (d) **1-122**, Cs_2CO_3, DMF, rt, 21 h, 57%; (e) LiOH, H_2O, THF, 0 ^oC-rt, 24 h, 97%; (f) 1-methylpiperazine, T3P, Et_3N, DMF, rt, 19 h, 65%.

Compound **1-127** (Schme 24) was the final target that was synthesized on gram-scale. The 2,5-difluoro-4-((1*R*,3*R*)-3-(hydroxymethyl)cyclobutyl)phenol side chain (supplied by AMRI) was fashioned onto the 3-sulfanyl-1,2,4-triazole intermediate **1-117a** (supplied by AMRI). Williamson etherification occurred by mesylation of the alcohol intermediate **1-117a** followed nucleophilic displacement with the provided phenol side chain to provide the alcohol intermediate **1-126** (Scheme 24). Subsequent carbamoylation occurred upon carbonyldiimide activation of the terminal alcohol **1-126** followed by treatment of 1-methylpiperidin-4-amine,

providing over one gram of compound **1-127**. Compound **1-127** demonstrated >98% purity by ^{1}H NMR and LC-MS ELSD.

Scheme 24. Scale-up of 1-127



Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^{o}C, 7 h; (b) 2,5-difluoro-4-((1*R*,3*R*)-3-(hydroxymethyl)cyclobutyl)phenol, Cs_2CO_3, DMF, rt, 21 h, 90%; (c) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 22 h, 45%.

1.2.9 P97 Covalent Modifiers

Targeted covalent inhibitors (TCI) are playing an important role in drug discovery.[160, 161] This is due, in part, to covalent inhibitors having a number of advantages over reversible inhibitors, which include sustained target engagement and prolonged pharmacodynamics effects.[161, 162] Accordingly, covalent modifiers can have similar pharmacological responses at lower concentrations compared to non-covalent inhibitors.[163] TCIs are best developed using structure-based design of a known, optimized reversible ligand whereby, the reversible ligand can be strategically modified to optimally position a reactive functional group in proximity to a nucleophilic amino acid side chain found within the protein's small-molecule binding site.[161] The

electrophilic warheads utilized by TCIs are well investigated and can be selected to differentiate between specific amino acid side chains, for example cysteine, lysine, or tyrosine.[164, 165] Cysteine is the most frequently targeted amino acid due to its nucleophilic thiol-containing side chain that is capable of reacting with diverse electrophilic functional groups.[166] Michael acceptors, α,β-unsaturated carbonyl functional groups, have been extensively employed to react with cysteine and are the most widely used electrophilic warhead for TCI development.[167-169] Mechanistically, irreversible covalent inhibitors may bind noncovalently to their respective target, forming a reversible complex (Figure 15). Then proper positioning of a Michael acceptor motif with the appropriate orientation to a cysteine thiol side chain found within the binding pocket enables covalent bond formation, thereby establishing an irreversible complex and inhibiting protein function.

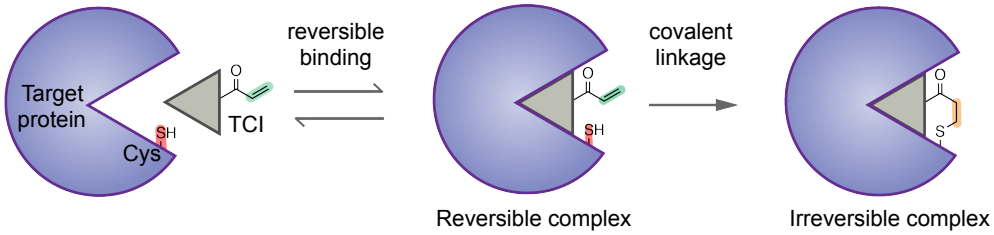
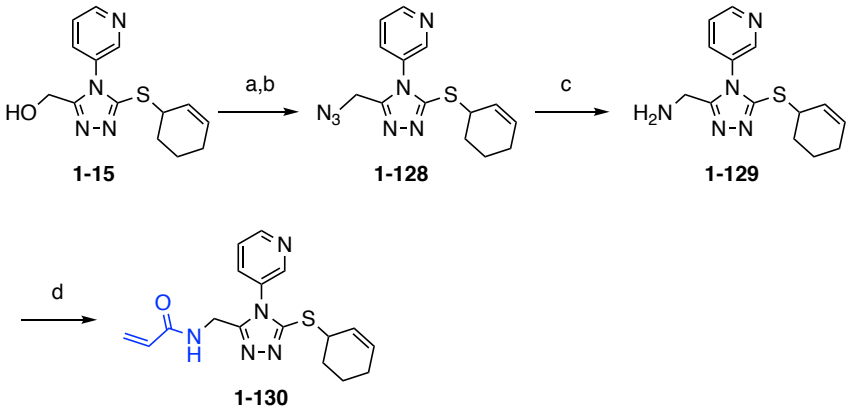


Figure 15. Mechanism of Targeted Irreversible Covalent Inhibitors

We were interested in developing targeted covalent p97 allosteric inhibitors. According to our preliminary molecular model, we hypothesized that an advantageous cysteine residue 535 (Cys535) within the 3-sulfanyl-1,2,4-triazole binding pocket could be exploited by covalent linkage. We further hypothesized that the incorporation of an electrophilic α,β-unsaturated Michael acceptor, specifically the α,β-unsaturated amide (acrylamide), in zone II and/or zone III would support proper positioning for optimal reactivity. Our initial design focused on

incorporating the electrophilic warhead off the 5 position of the 3-sulfanyl-1,2,4-triazole core in zone III. Our synthesis began with the mesylation of alcohol intermediate **1-15** followed by nucleophilic displacement with sodium azide to provide the organoazide intermediate **1-128** (Scheme 25). Subsequent Staudinger reduction using triphenylphosphine afforded the terminal amine **1-129**. Subsequent amide bond formation using T3P-mediated coupling conditions with acrylic acid established the acrylamide analog **1-130**.

Scheme 25. Synthesis of Acrylamide Analog 1-130

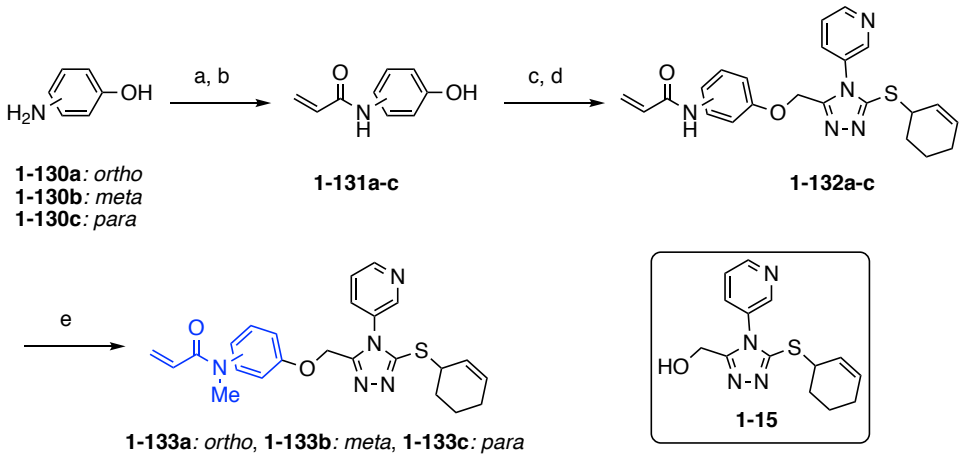


Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 °C, 1 h; (b) NaN_3, DMF, rt, 2 h, 87% (two steps); (c) PPh_3, THF, H_2O, rt, 72 h, 93%; (d) acrylic acid, T3P, Et_3N, EtOAc, 0 °C-rt, 21 h, 61%.

In order to survey greater physical space to optimally position the electrophilic acrylamide motif near the postulated Cys535, we sought to independently functionalize the *ortho*-, *meta*-, and *para*-positions of the phenyl ring in zone II. The respective synthesis of these analogues began with commercially available *ortho*-, *meta*-, and *para*-substituted aminophenol building blocks **1-130a-c** (Scheme 26). Acylation of the respective amine using acryloyl chloride furnished the acrylamide functionalized phenol side chains **1-131a-c** (Scheme 26). Subsequent mesylation of alcohol intermediate **1-15** followed by Williamson etherification with the phenol

side chain **1-131a-c** enabled the inclusion of the electrophilic warhead onto the sulfanyl-1,2,4-triazole core to provide **1-132a-c**. Further, compounds **1-132a-c** were treated with potassium hydride followed by methyl iodide to generate the N-methyl acrylamide derivatives **1-133a-c**.

Scheme 26. Synthesis of Acrylamide Analogues in Zone II



Reagents and conditions: (a) acryloyl chloride, DIPEA, CH_2Cl_2, 0 °C; (b) LiOH, MeOH, 0 °C-rt, 30-76% (two steps); (c) **1-15**, MsCl, DIPEA, CH_2Cl_2, 0 °C; (d) **1-131a** (*ortho)* or **1-131a** (*meta)* or **1-131a** (*para),* Cs_2CO_3, DMF, rt, 68-89% (two steps); (e) KH, THF, 0 °C, 0.5 h; MeI, 0 °C-rt, 2 h, 62-74%.

1.2.10 P97 Protein Degraders Using the Hydrophobic Tag Strategy

Small molecule inhibitors are a proven strategy to functionally modulate a respective target. This occupancy-driven model requires the small molecule inhibitor to stoichiometrically occupy a functional site within the protein target. Recently, an emerging strategy employs heterobifinctional small molecules that bind to their respective target and elicit protein degradation.[170-172] In contrast to small molecule inhibitors, the heterobifunctional small molecule degraders function through event-driven pharmacology, whereby the degrader catalytically binds

to the protein of interest and exploits the endogenous protein-quality control machinery to selectively degrade the protein target.[173] The hydrophobic tag (HyT) technology employs a targeted ligand that is strategically functionalized with a hydrophobic motif.[171, 172, 174] Ligation of the functionalized ligand tags the respective target protein with the hydrophobic motif, thereby mimicking an exposed hydrophobic surface and suggesting an unstable protein in a misfolded state.[175] Therefore, the hydrophobically tagged target protein would be recognized by protein-quality control machinery as terminally misfolded and subsequently eliminated by the proteasome (Figure 16).[176] The effectiveness of the HyT strategy has been demonstrated through the degradation of a number of endogenous proteins, including erythroblastosis oncogene B3 (Her3) receptor tyrosine kinase[177, 178] and androgen receptor (AR).[179] Therefore, we were interested in the development of allosteric p97 protein degraders utilizing the HyT technology on the 3-sulfanyl-1,2,4-triazole inhibitor series.

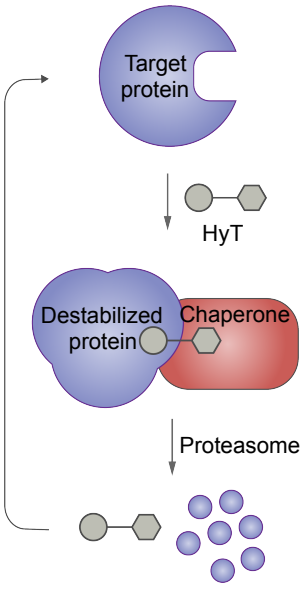
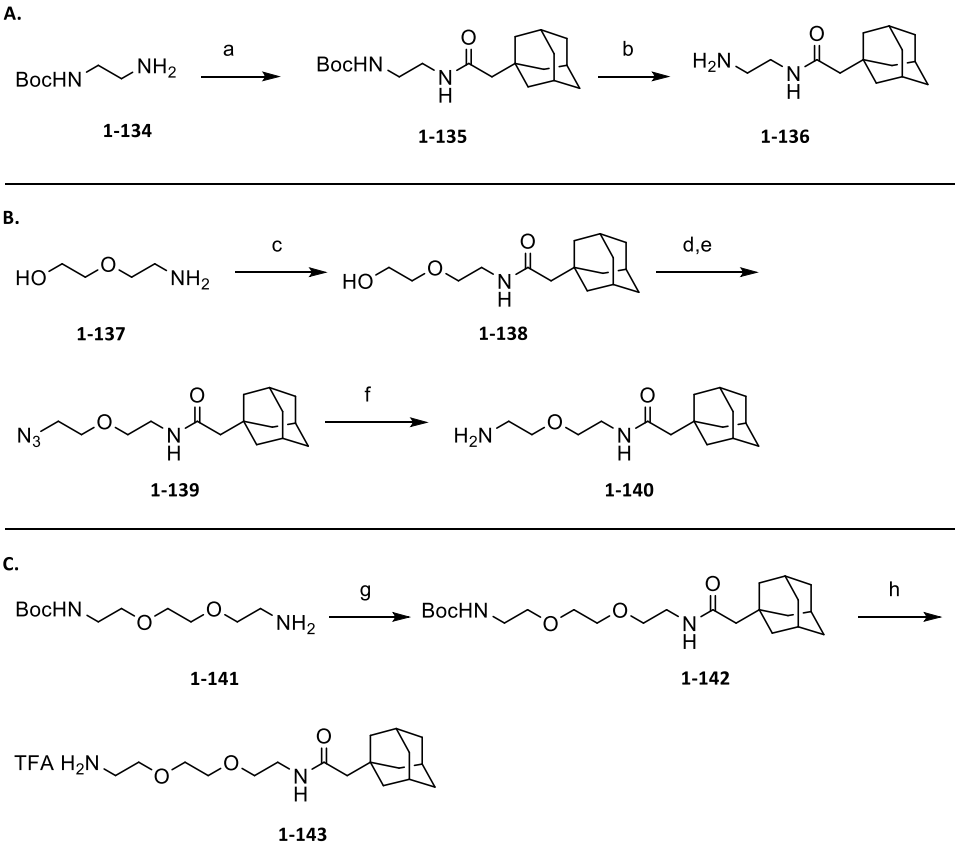


Figure 16. General Mechanism of HyT Technology

We designed a small series of HyT molecules by coupling a hydrophobic adamantyl-fused linker to the terminus of our p97 small molecule binder.[180] It has been demonstrated that linker length plays a critical role toward the successful function of protein degraders.[181-183] Therefore, we initially developed a series of adamantyl-hydrophobic tag linkers with varying lengths and composition. Synthesis of the four atom (n = 4) linker began with the commercially available *tert*-butyl (2-aminoethyl)carbamate **1-134** (Scheme 27). Amide bond formation occurred using EDC coupling conditions in the presence of 1-adamantanylacetic acid to provide the amide **1-135** (Scheme 27). Subsequent Boc-deprotection occurred with *in situ* generated hydrogen chloride followed by a basic work-up to provide the four atom adamantyl-fused linker **1-136**. Synthesis of the seven atom (n = 7) linker began with EDC-mediated coupling with 2-(2-aminoethoxy)ethan-1-ol (**1-137**) and 1-adamantanylacetic acid to provide amide **1-138**. Mesylation of the primary alcohol followed by displacement with sodium azide cleanly provided the alkyl azide intermediate **1-139**. Subsequent Staudinger reduction of the alkyl azide yielded the terminal amine, concluding the seven atom adamantyl-fused linker **1-140**. Lastly, the synthesis of the ten atom (n = 10) linker began with EDC-mediated coupling between commercially available (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (**1-141**) and 1-adamantanylacetic acid to provide amide **1-142**. Subsequent Boc-deprotection using trifluoroacetic acid (TFA) afforded the ten atom adamantyl-fused linker **1-143** as the TFA salt.

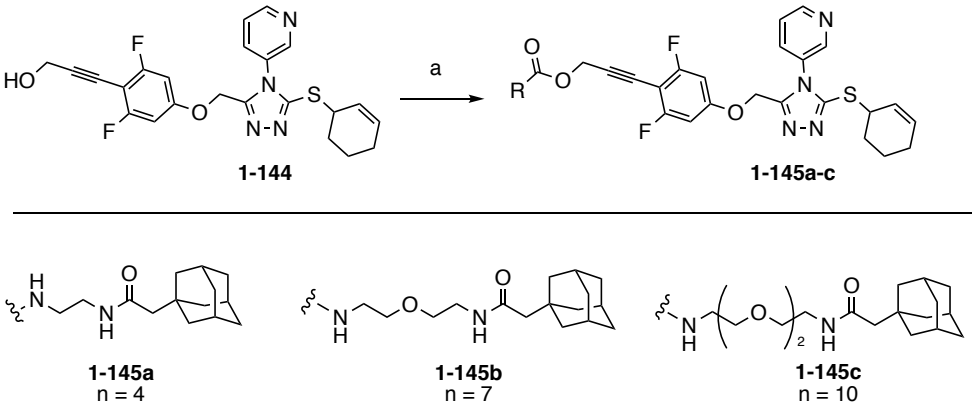
Scheme 27. Synthesis of HyT linkers



Reagents and conditions: **A**. (a) 1-adamantanylacetic acid, EDC HCl, HOBt, DIPEA, CH_2Cl_2, 0 ^oC-rt, 24 h, 90%; (b) AcCl, MeOH, 0 ^oC-rt, 24 h, 82%. **B**. (c) 1-adamantanylacetic acid, EDC HCl, HOBt, DIPEA, CH_2Cl_2, 0 ^oC-rt, 24 h, 93%; (d) MsCl, DIPEA, CH_2Cl_2, 0 ^oC-rt, 12 h, (e) NaN_3, DMF, 50 ^oC, 12 h, 75%, (two steps); (f) PPh_3, THF, H_2O, rt, 72 h, 94%. **C**. (g) 1-adamantanylacetic acid, EDC HCl, HOBt, DIPEA, CH_2Cl_2, 0 ^oC-rt, 13 h, 53%; (h) TFA, CH_2Cl_2, 50 ^oC, 2 h.

The adamantyl-based linkers were then incorporated onto the 3-sulfanyl-1,2,4-triazole p97 ligand. This occurred through a carbamoylation reaction utilizing carbonyldiimide-mediated activation of the previously prepared alcohol intermediate **1-144** followed by the independent treatment with the constructed amine linker (**1-136**, **1-140**, and **1-143**), providing the series of p97 3-sulfanyl-1,2,4-triazole-based HyT compounds **1-145a-c** (Scheme 28).

Scheme 28. Synthesis of p97 Sulfanyl-1,2,4-triazole Based HyT Compounds



Reagents and conditions: (a) **1-136** or **1-140** or **1-143**, CDI, Et_3N, CH_2Cl_2, rt, 14-24 h, 65-92%.

1.3 Conclusion

This work described the synthesis of diverse 3-sulfanyl-1,2,4-triazole allosteric p97 AAA+ ATPase inhibitors for structure activity/property relationship (SAR/SPR) investigations. We employed medicinal chemistry strategies to rapidly construct target molecules that enabled a systematic evalution of biochemical potencies. This effort supported an iterative medicinal chemistry campaign that led to the development of allosteric p97 inhibitors that demonstated single-digit nanomolar biochemical potency, increased aqueous solubility, and lower lipophilicity compared to NMS-873. We were able to scale-up selected analogues to the gram-scale. Further, we synthesized inhibitors to covalently modify p97, as well as potential p97 protein degraders that utilize the hydrophobic tag (HyT) strategy.

2.0 Synthesis of p75 Neurotrophin Receptor Inhibitors

2.1 Introduction

2.1.1 Neurotrophins and Neurotrophin Receptors

The neurotrophin family constitutes four secretory proteins that regulate neuronal cell differentiation, function, and survival. The four identified neurotrophins in the mammalian brain - nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and neurotrophin 4 (NT4) - are ubiquitously expressed throughout the central nervous system (CNS) and individually participate in both independent and redundant processes.[184, 185] Neurotrophins regulate these processes by targeting two distinct classes of transmembrane receptors – the p75 neurotrophin receptor ($p75^{NTR}$) and the Tropomyosin family of receptor tyrosine kinases (Trk), which includes TrkA, TrkB, and TrkC.[186, 187] Their respective selectivity and subsequent binding affinity is highly contingent upon the neurotrophin present at the cell surface and its respective maturity status.[188, 189]

Akin to other secreted proteins, neurotrophins exist in different maturation states and depending on their maturity status neurotrophins can demonstrate diverse functions in the CNS.[190] Neurotrophins are initially synthesized in the endoplasmic reticulum as precursors proteins called pro-neurotrophins (30-35 kDa). The pro-domain of proneurotrophins can be proteolytically cleaved by intracellular or extracellular proteases, resulting in mature proteins called mature-neurotrophins (12-13 kDa).[191, 192] The proteolytic processing of pro-neurotrophins governs neurotrophin action and regulates cell signaling. It is now recognized that pro-

neurotrophins have distinct biological effects on cell signaling that often oppose those of mature-neurotrophins.[193] This is due, in part, to proneurtrophin and mature-neurotrophin receptor selectivity.

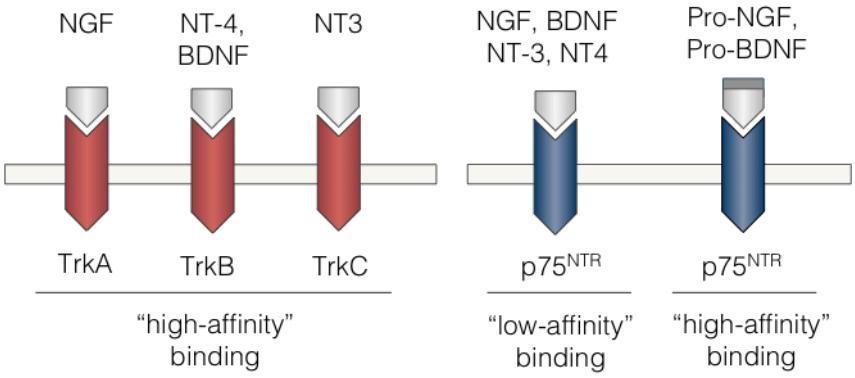


Figure 17. Neurotrophin Receptor Binding Selectivity and Respective Binding Affinity

The Trk family members are receptor tyrosine kinases that display picomolar-binding affinity to mature-neurotrophins and demonstrate distinct neurotrophin binding specificity (Figure 17).[189] At the cell surface, mature-NGF specifically binds to TrkA; whereas, mature-BDNF and mature-NT-4 selectively bind to Trk B, and mature-NT-3 explicitly targets TrkC. Upon neurotrophin-receptor ligation, the respective Trk receptor undergoes dimerization, which induces intracellular autophosphorylation and the subsequent activation of the cyctoplasmic kinase domain. This initiates downstream signaling by activating the Ras/Erk, PI3K/Akt, and PLC/PKC pathways, which promote neuronal cell differentiation or survival.[185]

Contrary to Trk receptors, mature neurotrophins indiscriminately bind to the extracellular domain of $p75^{NTR}$ with low, nanomolar-binding affinity (Figure 17).[194] $p75^{NTR}$ is associated with the tumor necrosis factor receptor (TFNR) superfamily, containing the characteristic intracellular death domain.[195, 196] $p75^{NTR}$ also resides at the cell surface; however, it notably lacks intrinsic

enzymatic activity. Consequently, $p75^{NTR}$ promotes the activation of various intracellular pathways by coordinating with other cell surface proteins - e.g. tropomyosin kinase receptor, sortilin, NogoR. This can lead to diverse and possibly divergent signaling events. For example, the ligation of mature-NGF to $p75^{NTR}$ alone can lead to survival or cell death (Figure 18).[197-199] However, the $p75^{NTR}$-TrkA complex binds to mature-NGF with enhanced affinity and commonly promotes neuronal cell differentiation or survival (Figure 18).[200, 201] Conversely, the $p75^{NTR}$-sortilin complex binds to pro-NGF with high affinity and promotes neuronal cell death (Figure 18).[202] The effective biological signaling event and the resulting physiological outcome is contingent upon the presence of specific cell surface proteins and the selective secretion of pro- or mature-neurotrophins.

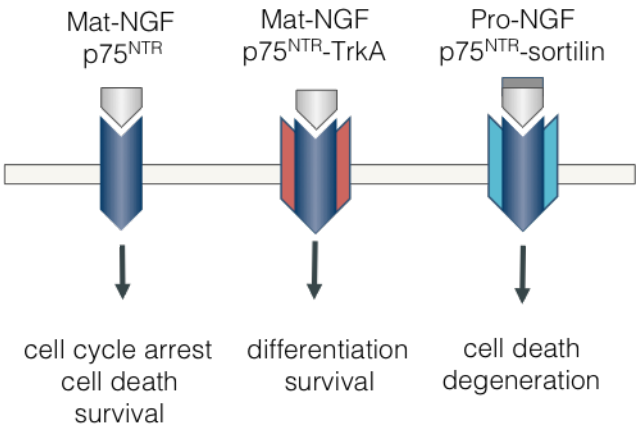


Figure 18. NGF-receptor Mediated Cellular Phenotypes

2.1.2 Cellular Signaling in Disease Implications

Emerging evidence has associated proneurotrophin secretion and $p75^{NTR}$-mediated signaling to a variety of adverse pathological conditions. In a recent report, proneurotrophins

were found to bind to $p75^{NTR}$ with high-affinity and elicit cell death.[203] This seminal discovery prompted a surge of new investigations, accelerating our understanding of proneurotrophin and $p75^{NTR}$ related events. Previous studies have demonstrated proneutrophin-mediated cell death in a variety of cell types;[204, 205] however, the mechanism by which cell death occurred was not well understood. It is now known that the sorting receptor, sortilin, binds to the pro-domain of pro-NGF or pro-BDNF and serves as a co-receptor with $p75^{NTR}$ to mediate cell death.[202] In $p75^{NTR}$ expressing neuronal cells, inhibiting sortilin suppressed pro-NGF-mediated cell death; conversely, overexpression of sortilin enhanced pro-NGF-mediated cell death. It was concluded that the presence of both $p75^{NTR}$ and sortilin is necessary for proneurotrophin-induced cellular apoptosis.

The specific intracellular signaling mechanism for proneurotrophin-$p75^{NTR}$-sortilin-mediated cell death is unclear. However, prior investigations of neurotrophin-$p75^{NTR}$ signaling provide preliminary insight. Following neurotrophin ligation to the extracellular domain of $p75^{NTR}$, several intracellular adaptor proteins are recruited to the cell surface, including Traf 6, neurotrophin receptor-interacting factor (NRIF), melanoma-associated antigen (MAGE), and neurotrophin receptor p75 interacting MAGE homologue (NRAGE).[185] These adaptor proteins are capable of modulating multiple signaling pathways. The Jun kinase-signaling cascade is one major pathway activated by neurotrophin binding that can lead to p53 activation and apoptosis. Activation of this pathway can induce the expression of the Fas ligand in neuronal cells and subsequent Fas ligation to its cognate Fas receptor also promotes apoptosis.[206] Alternatively, overexpressing NRAGE can promote NGF-$p75^{NTR}$-dependent cell cycle arrest and death through Jun kinase-signaling. Interestingly, NRAGE has been demonstrated to prevent the association of $p75^{NTR}$ with TrkA and reduced cell survive.[207] NADE has also been reported to bind to $p75^{NTR}$'s

intracellular death domain and induce caspase activation, resulting in the death of primary cortical neurons.[208] Although a number of adaptor proteins have been associated with neurotrophin and $p75^{NTR}$ signaling, continued efforts are required to fully characterize their complex mechanisms.

$p75^{NTR}$'s association with neuronal cell death has prompted the investigation of $p75^{NTR}$ in neural injury and disease. Traditionally, $p75^{NTR}$ is ubiquitously expressed during nervous system development; however, in adult neurons, $p75^{NTR}$ expression is largely suppressed or kept at basal levels.[204] After neural injury or cellular stress $p75^{NTR}$ expression is upregulated, which can result in cell death. For example, thoracic contusions of the spinal cord induce $p75^{NTR}$ expression in oligodendrocytes, leading to apoptosis.[209] Schwann cells from the distal sciatic nerve unregulated $p75^{NTR}$ as a result of axotomoy, which led to Schwann cell death.[210, 211] $p75^{NTR}$ expression is enhanced by neuronal activation following seizures in the hippocampus, which also results in neuronal cell death.[212, 213] In disease, elevated levels of $p75^{NTR}$ were observed in cholinergic neurons of the cortical and basal forebrain in patients with Alzheimer's disease (AD).[214] Additionally, in the brains of patients with multiple sclerosis, oligodendrocytes isolated from plaques contained high levels of $p75^{NTR}$.[215]

Proneurotrophin secretion is also elevated under similar conditions and is associated with adverse pathological conditions. Following spinal cord injury, secreted pro-NGF in cerebrospinal fluid (CBS) induced apoptosis in cultured oligodendrocytes.[216] Treatment with anti-pro-NGF antibody strongly attenuated this effect and apoptosis was not observed in $p75^{NTR}$ knockout cells. These results indicated proneurotrophin's role in initiating cell death by activating $p75^{NTR}$. Therefore, disrupting the pro-neurotrophin-$p75^{NTR}$ complex can reduce neuronal cell death. This constitutes a novel therapeutic opportunity; however, the development of targeted

pharmacological agents has remained largely undeveloped. In order to efficiently develop targeted agents that disrupt pro-neurotrophin-p75^{NTR}-mediated pathologies, it is important to have a clear understanding of the structural determinants necessary for ligand-receptor complexation.

2.1.3 Protein Structure and Complex Formation

2.1.3.1 Neurotrophin Structure

The neurotrophin family members have similar sequence homology and portray common structural features. In its monomeric state, the central hydrophobic core of neurotrophins displays an elongated saddle shape formed by two pairs of twisted, antiparallel beta-stands.[217] Additionally, they contain three variable beta-hairpin loops (L1-4), which encompass charged residues that are important for protein solubility and receptor binding specificity. In their respective X-ray co-crystal structure, beta-hairpin loops 1, 3, and 4 are important for p75^{NTR} binding; whereas, beta hairpin loops 2 and 4 coordinate with Trk family members.[218, 219] Under physiological conditions, neurotrophins exist as a tight, non-covalently bound dimers.[220] Dimerization is supported by key intermolecular interactions established by the central beta-strands and a cysteine-knot motif - formed by three disulfide bonds, which stabilizes the protein fold and locks the two monomers in their dimeric conformation.

2.1.3.2 p75^{NTR} Structure

p75^{NTR} is a 75-kDa type I transmembrane glycoprotein.[221] Its extracellular domain is structurally characterized by four tandem extracellular cysteine-rich domains (CRD1-4, Figure 19A), which contains three intrachain disulfide bridges.[222, 223] Structure-function studies

suggested CRD1-2 is primarily responsible for neurotrophin binding.[222, 224] Its intracellular domain lacks enzymatic function; however, it contains the characteristic TNFR superfamily death domain. The intracellular death domain can interact with adapter proteins and potentially initiate cell apoptosis.[225] The three-dimensional structures for both the extracellular domain and the intracellular death domain of $p75^{NTR}$ have been reported.[196, 226]

2.1.3.3 NGF – $p75^{NTR}$ Complex

Endogenous $p75^{NTR}$ complex formation with NGF, specifically, occurs between a dimer of glycosylated extracellular domains of $p75^{NTR}$ and a dimer of NGF (Figure 19A).[227] This 2:2 binding stoichiometry leads to high affinity coordination between $p75^{NTR}$ and NGF. The molecular features of the NGF-$p75^{NTR}$ complex are well defined and the binding of NGF to $p75^{NTR}$ is a result of key intermolecular interactions, which can be bifurcated into two major sites - Site 1 and Site 2 (Figure 19B).[227-229] Site 1 corresponds to the junction of NGF and $p75^{NTR}$ between CRD1 and CRD2 domains. This predominant site is governed by complementary surface charge, which forms a network of favorable hydrogen bonds and electrostatic interactions. Specifically, NGF's beta-hairpin loop 1 residues $Lys32^{NGF}$ and $Lys34^{NGF}$ establish a critical salt bridge and a water-mediated hydrogen bond, respectively, with the side chain of $Asp75^{p75NTR}$ (Figure 20). Additionally, $Lys88^{NGF}$ forms a salt bridge with the side chain of $Asp41^{p75NTR}$. Site 2 is less influential in NGF binding; however, the side chain of $Arg114^{NGF}$ forms a stabilizing hydrogen bond and an electrostatic salt bridge with the side chains of residues $Cys136^{p75NTR}$ and $Glu119^{p75NTR}$, respectively.

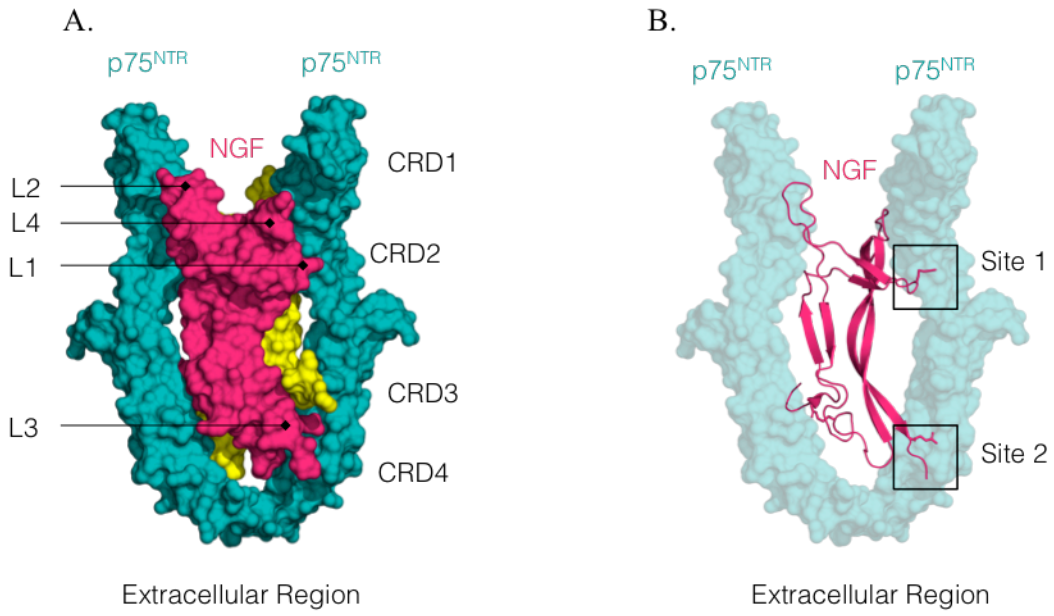


Figure 19. NGF-p75^{NTR} Complex

A. One NGF monomer is shown in red, one NGF monomer is shown in yellow, p75^{NTR} is shown in teal. **B.** Simplified view of NGF-p75^{NTR} complex that highlights two major sites of ligand-receptor binding. (PDB: 1SG1)[229]

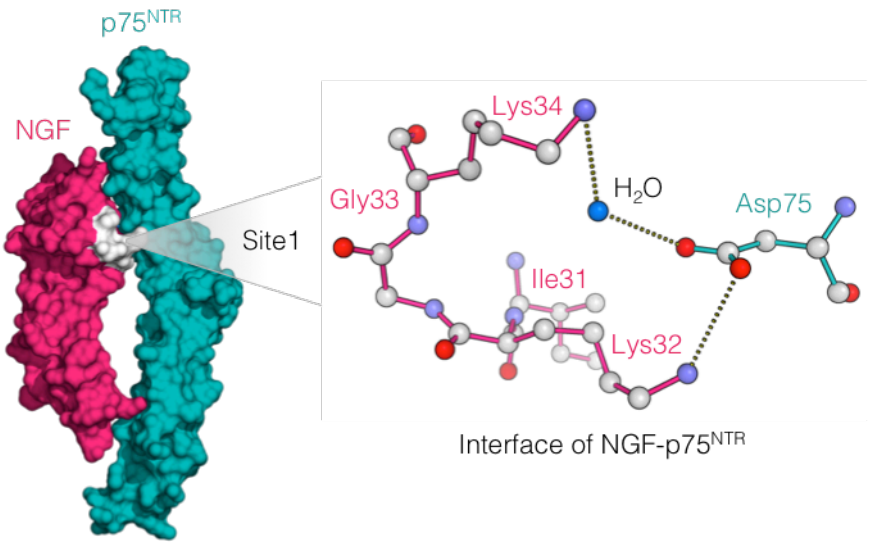


Figure 20. The Interface of NGF-p75^{NTR} at Site 1

The NGF beta-hairpin turn loop 1 amino acid at the interface of NGF-p75^{NTR} site 1 is highlighted. NGF beta-hairpin turn loop 1 coordinates with Asp75^{p75NTR} through a network of hydrogen bonding and electrostatic interactions with water, Lys34^{NGF}, and Lys32^{NGF}. NGF monomer is shown in red, p75^{NTR} monomer is shown in teal, NGF beta turn loop 1 is highlighted in white, water is shown as blue, and intermolecular interactions are shown as a black dotted line. (PDB: 1SG1)[229]

Efforts to understand the mechanism of p75^{NTR} activation by neurotrophin ligation have identified specific structural motifs responsible for activity. Prior to having crystallographic evidence, early investigations delineated specific NGF domains implicated in biological activity by conducting a peptide scanning campaign of mouse NGF.[230] This work identified NGF amino acid sequence Lys32-Gly33-Lys34-Glu35 (^{32}KGKE^{35}), which proved to be important for biological activity. Site-directed mutagenesis corroborated the functional relevance of Lys32^{NGF} and Lys34^{NGF} in the binding of NGF to p75^{NTR}.[230] In an alanine scanning mutagenesis campaign mutating Lys32^{NGF} or Lys34^{NGF} to alanine resulted in 16% and 50% receptor binding, respectively, in human melanoma A875 cells (Figure 21).[231] Triple NGF mutant with alanine

mutations of Lys32^{NGF}, Lys34^{NGF}, Glu35^{NGF} resulted in *complete* ablation in mutant NGF binding to p75^{NTR}.[231, 232] The X-ray crystal structure of the NGF-p75^{NTR} complex confirmed that the ^{32}KGKE^{35} amino acid sequence constituted the core of NGF's beta-hairpin loop 1 domain. Crystallographic evidence also supports the notion that positively charged residues at the surface of NGF is required to form critical stabilizing interactions with p75^{NTR} at site 1 and disrupting these interactions have significant impact on NGF binding.

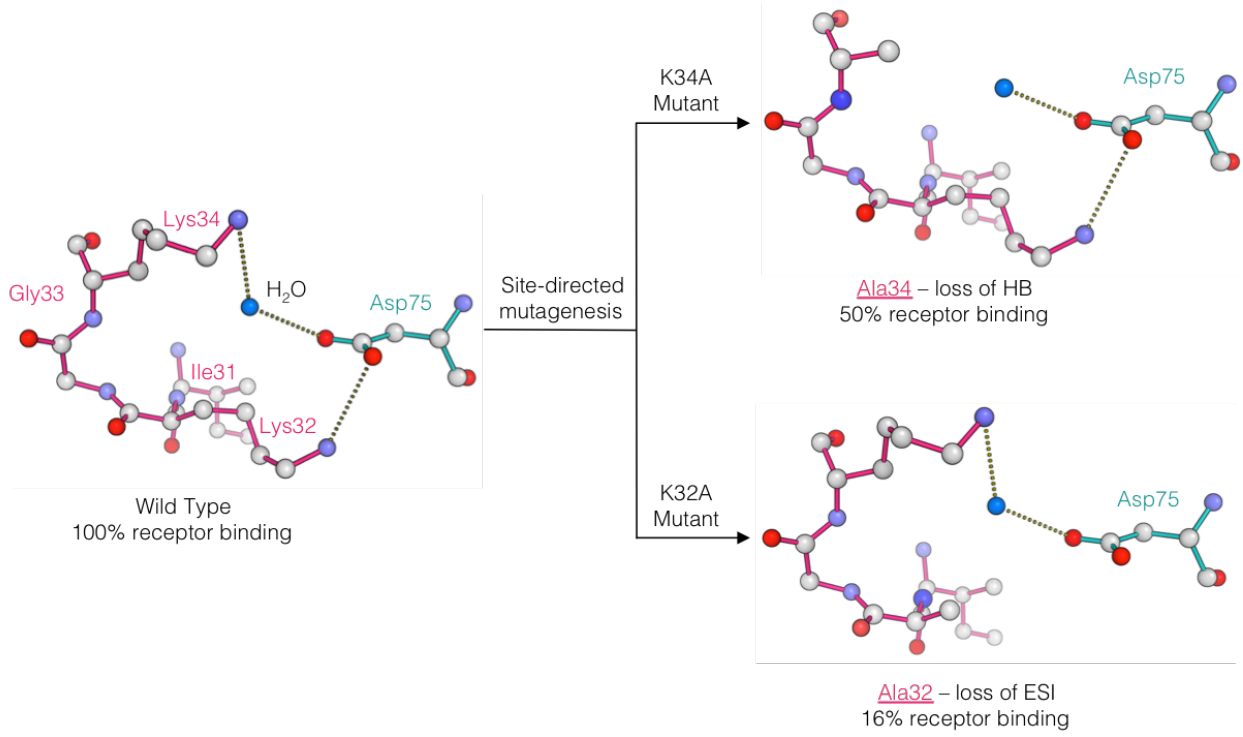


Figure 21. Site-directed Mutagenesis at the Interface of NGF-p75^{NTR} at Site 1

Wild-type NGF demonstrated 100% binding to p75^{NTR}. NGF-K34A eliminated a water-mediated hydrogen bond with Asp75^{p75NTR}, resulting in 50% NGF-K34A binding. NGF-K32A eliminated an electrostatic interaction with Asp75^{p75NTR}, resulting in 16% NGF-K32A binding. NGF residues are shown in red, p75^{NTR} residues are shown in teal, water is shown in blue, intermolecular interactions are shown as a black dotted line. (PDB: 1SG1)[229]

2.1.4 Targeted Agents

2.1.4.1 NGF Mimetic Peptides

The pursuit of neurotrophin mimetic antagonists led to the development of peptide-based agents that modulated neurotrophin-mediated signaling.[233] A peptide scanning campaign identified NGF's amino acid sequence ^{32}KGKE^{35} as an important feature for biological activity.[230] Linear synthetic NGF mimetic peptide **C5** (peptide sequence: KGKE) was able to reduce NGF-promoted neurite outgrowth; however, it had limited effect on the binding of radiolabelled ^{125}I-NGF.[230] The development of linear synthetic peptides derived from NGF's primary amino acid sequence demonstrated the capability of antagonizing NGF-mediated signaling. Later it was discovered through X-ray crystallography that the ^{32}KGKE^{35} constituted the core of NGF's beta-hairpin loop 1.[229] To address the initial deficiencies of linear synthetic peptides, cyclized peptide derivatives were pursued in order to stabilize the native three-dimensional characteristics of the beta-turn structural motif. This led to NGF mimetic cyclic peptide **C7** (peptide sequence: IPenKGKEVCT), which was shown to prevent neuronal cell death and inhibit NGF-mediated activity.[234] In a mechanistic evaluation, the antagonistic activity of **C7** was eliminated in the presence of a p75^{NTR} antibody and absent in p75^{NTR} knockout cells.[234] Additionally, cyclic peptides containing the KGKE amino acid sequence had limited off-target activity against NGF-TrkA ligation.[235] These results suggested that the cyclic KGKE mimetic peptide derivatives were able to selectively modulate NGF signaling through a p75^{NTR} mechanism. The pursuit of NGF mimetic peptides determined the functional significance of NGF's beta-hairpin loop 1 peptide sequence ^{32}KGKE^{35} for p75^{NTR} binding and highlighted NGF-p75^{NTR} antagonism in both biochemical- and cell-based assays; however, their therapeutic utility has not yet expanded beyond pre-clinical evaluation.

2.1.4.2 Anti-NGF Antibody

Application of therapeutic monoclonal antibody (mAb) drug development has led to multiple anti-NGF mAbs that potently diminished NGF-mediate signaling. Anti-NGF mAbs were designed to sequester secreted NGF and obstruct receptor binding. Several pharmaceutical and biotechnology companies [Pfizer (Tanezumab), Amgen (Fulranumab), Regeneron/Sanofi-Aventis (REGN475/SAR164877, Medimmune/AstraZeneca (Medi0578), Abbott Laboratories (ABT-110)] pursued anti-NGF mAbs in clinical trials.[236] Pfizer's ant-NGF humanized mAb, Tanezumab, is most notable - advancing to phase III clinical trials, demonstrating exceptional specificity (Kd > 10 pM) and showcasing prolonged antibody ligation time (Td > 100 h).[237] In a phase II proof-of-concept study, Tanezumab effectively treated pain from osteoarthritis of the knee with limited adverse effects.[238] After advancing to phase III clinical trials, a small cohort of patients receiving Tanezumab treatment experienced severe disease progression and developed bone necrosis; and therefore, due to these severe adverse side effects, the FDA suspended all non-cancer pain related clinical trials of anti-NGF mAbs.[239] It has been proposed that blocking NGF with anti-NGF antibodies impacts bone remodeling through a NGF-Trk mediated mechanism; however, further investigations are required.[236] Future efforts to address these observed adverse effects may lead to alternative targeted strategies that focus on specific inhibition of NGF-receptor complex formation. Nevertheless, Tanezumab clearly demonstrated the functional significance of anti-NGF mAbs as effective analgesic agents in humans and clinically validated NGF-mediated signaling as a therapeutic target.

2.1.4.3 Small Molecule Antagonists

NGF Inhibitors

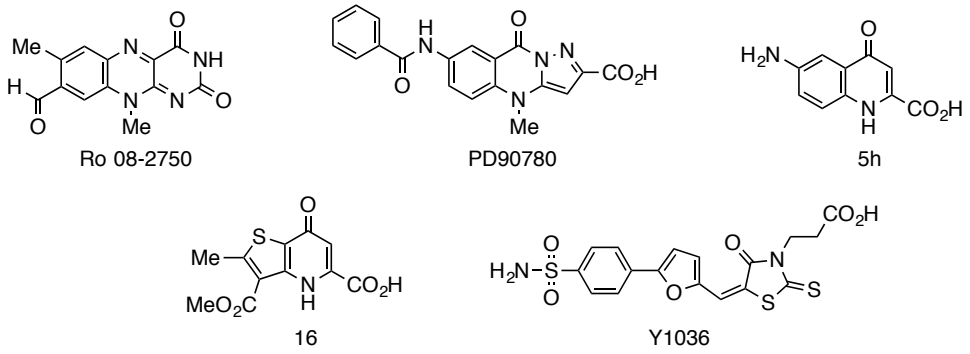


Figure 22. NGF Small Molecule Inhibitors

Pharmacological modulation of NGF-mediated signaling using small molecules has led to the development of protein-protein interaction inhibitors (PPII) that effectively target both NGF and $p75^{NTR}$. Initial library screens focused on modulators that disrupted radiolabelled ^{125}I-NGF ligation, resulting in several NGF-binding non-peptide small molecule inhibitors. The first reported anti-NGF non-peptide small molecule inhibitor was the pyrazoloquinazolinone PD90780 (Figure 22). PD90780 demonstrated NGF selectivity and inhibited ^{125}I-NGF binding to the extracellular domain of $p75^{NTR}$ with an IC_{50} of 220 nM in a microplate binding assay.[240] In an in-vitro receptor binding assay, PD90780 inhibited ^{125}I-NGF binding to Chinese Hamster Ovary cells (Q1-CHO) expressing $p75^{NTR}$ with an IC_{50} of 300 nM.[240] Moreover, PD90780 inhibition of ^{125}I-NGF binding using PC12 cells (expressing both $p75^{NTR}$ and TrkA) occurred at an IC_{50} of 23.1 μM.[241] However, PD90780 inhibited ^{125}I-NGF binding using $PC12^{nnr5}$ cells (expressing only $p75^{NTR}$) with an IC_{50} of 1.8 μM. A preliminary docking study suggested PD90780 binds to a region near NGF beta-hairpin loop I and IV, forming critical intermolecular

interactions between its acidic functionality with the basic amines of $Lys32^{NGF}$ and $Lys34^{NGF}$.[242] This postulated binding mode corroborated the SAR study and suggests PD90780 antagonized NGF-mediated signaling through an orthosteric mechanism.[242, 243]

Further exploration of NGF modulators identified 6-aminokynurenic acid, **5h** (Figure 22), and subsequent SAR generated thienopyridone derivative, **16** (Figure 22), that exhibited an IC_{50} of 3.1 and 2.3 μM, respectively, against ^{125}I-NGF-receptor ligation in PC12 cells.[244] Unlike PD90780, it is unclear whether the kynurenic acid derivatives, **5h** and **16**, specifically target NGF or its cognate receptors $p75^{NTR}$ and TrkA. Since PD90780 and the kynurenic acid derivatives have similar structural requirements for biological activity, it has been predicted that the kynurenic acid series target NGF instead of NGF-receptors; however, further studies are required to validate this hypothesis.

Work conducted at Hoffmann-LaRoche led to the development of a non-peptide small molecule, Ro 08-2750 (Figure 22), which demonstrated anti-NGF activity. In an equilibrium ultracentrifugation experiment, Ro 08-2750 molecule was found to bind to the NGF dimer with a K_D of approximately 1 μM.[245] It is presumed that Ro 08-2750 binds to the NGF's hydrophobic dimer interface; however, further studies are required to validate this hypothesis. Ro 08-2750 abrogated ^{125}I-NGF-receptor ligation in both PC12 cells and SK-N-MC cells at low micromolar concentrations. In a dose-escalation study, Ro 08-2750 treatment of SK-N-MC cells, which solely express $p75^{NTR}$ and not TrkA, reduced NGF-mediated apoptosis. Moreover, Ro 08-2750 treatment of PC12 cells did not affect neurite-outgrowth, suggesting the conservation of NGF-mediated Trk signalling. Addionally, in the absence of NGF, Ro 08-2750 showed little impact on TrkA intracellular phosphorylation in PC12 cells; however, at 10 μM, Ro 08-2750 treatment completely inhibited TrkA phosphorylation and the subsequent activation of downstream Trk

effector proteins.[245] Therefore, it was noted that higher concentrations of Ro 08-2750 showed enhanced Trk activity. This study highlighted the use of Ro 08-2750 as an anti-NGF agent that can prevent apoptosis and enhance neuronal cell survival in a context-specific, dose-dependent manner.

Pursuits toward a dual-specific inhibitor that pharmacologically perturbs both NGF- and BDNF-mediated cellular function led to the furyl-thioxothiazolidinone compound Y1036 (Figure 22). Y1036 was identified from an *in-silico* pharmacophore screen against a combinatorial panel of furan-based derivatives that showed similar structural features to known NGF antagonists ALE-0540, Ro 08-2750, and PD 90780. A molecular docking study of Y1036 with the crystal structures of NGF and BDNF established a putative molecular model for each respective neurotrophin. In an equilibrium ultracentrifugation experiment, Y1036 exhibited low micromolar affinity for both NGF and BDNF, having a K_D of 3.0 μM and 3.5 μM, respectively.[246] Moreover, Y1036 inhibited ^{125}I-NGF-ligation to $p75^{NTR}$ in PC12 cells at 5.7 μM; and unlike previously reported NGF small molecule modulators, Y1036 inhibited ^{125}I-BDNF-ligation to $p75^{NTR}$ at 6.2 μM.[246] Y1036 also demonstrated low-micromolar activity against NGF- and BDNF-binding to TrkA and TrkB, respectively. Functionally Y1036 was able to block NGF- and BDNF-mediated neurite outgrowth in rat embryonic dorsal-root ganglions in a dose-dependent manner.

p75^{NTR} Inhibitors

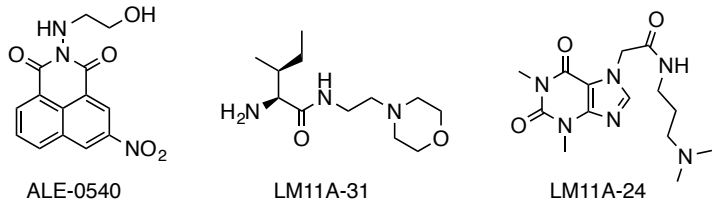


Figure 23. p75^{NTR} Small Molecule Inhibitors

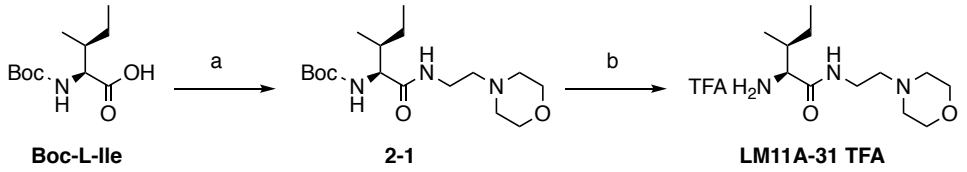
The pursuit of *in-vivo* active small molecule probes that antagonize NGF activity led to the discovery and development of the benzisoquinoline ALE-0540 (Figure 23). ALE-0540 was found to inhibit ^{125}I-NGF-ligation with low micromolar activity in PC12 cells.[247] Further analysis showed ALE-0540 inhibited ligand binding to both NGF-receptors p75^{NTR} and TrkA with similar micromolar potency, 3.7 and 5.8 μM, respectively.[247] Functional characterization of ALE-0540 showed dose-dependent reduction in neurite outgrowth of DRG neurons and NGF-mediated TrkA autophosphorylation in a PC12 cells.[247] Contrary to the previously described inhibitors, preliminary results from NGF-affinity chromatography suggested ALE-0540 does not bind to NGF; and therefore, ALE-0540 may exploit a common NGF-epitope recognition site on both p75^{NTR} and TrkA. Pre-clinical evaluation and *in-vivo* application of ALE-0540 demonstrated pharmacological perturbation of NGF induced antiallodynic effects in a rat pain model, which supports the association of NGF-mediated signaling in pain.

Peptide scanning studies, site-directed mutagenesis, and X-ray crystallography identified critical NGF-p75^{NTR} binding motifs at the protein-protein interface, providing strategic insight toward developing targeted pharmacological agents that can modulate NGF-p75^{NTR} activity. The structural interpretation of the NGF-p75^{NTR} complex enabled the rational design of non-peptide

small molecule protein-protein interaction inhibitors (PPII) that targeted $p75^{NTR}$. The electrostatic complementarity of the target region of $p75^{NTR}$ is enriched with acidic, anionic amino acids, which can be targeted by a small molecule to compete with NGF. A high-throughput virtual pharmacophore screen resembling the structural and chemical features of NGF beta-hairpin loop 1 domain sequence ^{32}KGKE^{35} led to the identification of LM11A-31 (Figure 23) and LM11A-24 (Figure 23).[248] LM11A-31, an isoleucine derivative, and LM11A-24, a theophylline derivative, are small molecule, non-peptide $p75^{NTR}$ ligands that potently inhibit NGF-$p75^{NTR}$ signaling. Also, LM11A-31 and LM11A-24 potently diminished NGF-induced apoptosis of hippocampal neurons in culture at picomolar concentrations.[248] It has been implied that LM11A-31 and LM11A-24 target $p75^{NTR}$ at the ^{32}KGKE^{35} recognition site and disrupt neurotropic ligation; however, there is currently no experimental evidence justifying their molecular mode of action.

A general synthesis of LM11A-31 was reported.[249] Synthesis of LM11A-31 began with amide bond formation of Boc-protected L-isoleucine with 2-morpholinoethan-1-amine using the coupling carbonyldiimide reagent EDC and hydroxybenzotriazole (Scheme 29). Subsequent Boc deprotection occurred in the presence of trifluoroacetic acid, leading to the synthesis of LM11A-31 as a TFA salt.

Scheme 29. General Synthesis of LM11A-31 [249]

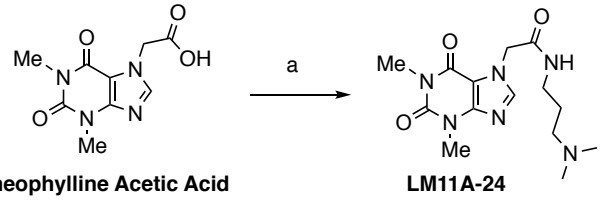


Boc-L-Ile **2-1** **LM11A-31 TFA**

Reagents and conditions: (a) 2-morpholinoethan-1-amine, EDC, HOBt, DIPEA, DMF, rt; (b) TFA, CH_2Cl_2, rt.

A general synthesis of LM11A-23 was also reported.[249] The synthesis of LM11A-24 began with commercially available theophylline acetic acid (Scheme 30). Amide bond formation occurred between theophylline acetic acid and N,N-dimethyl-1,3-propanediamine using known coupling reagent HBTU (Scheme 30).

Scheme 30. General Synthesis of LM11A-24 [249]



Theophylline Acetic Acid **LM11A-24**

Reagents and conditions: (a) N,N-dimethyl-1,3-propanediamine, HBTU, DIPEA, DMF, rt.

2.1.5 Pharmacological Profile of LM11A-31

LM11A-31 treatment has demonstrated efficacy in mitigating adverse phenotypes in various neuropathological conditions.[248, 250-253] In a spinal contusion injury mouse model, oral treatment with LM11A-31 was shown to improve motor coordination and functional recovery without noticeable toxicity.[254] This occurred, in part, by increasing survival of oligodendrocytes and enhancing myelinated axons in the spinal cord, which was a result of *in-vivo* inhibition of proNGF-p75^{NTR} signaling and subsequent attenuation of the JNK3-mediate apoptotic pathway.[254] In an early stage Alzheimer disease (AD) mouse model, oral treatment of LM11A-31 improved cognitive function and prevented further degeneration of cholinergic neurites.[253] Interestingly, in an advanced AD mouse model, LM11A-31 treatment prevented the progression and reversed

atrophy of basal forebrain cholinergic neurites and cortical dystrophic neurites.[251] These findings further suggest $p75^{NTR}$ as a promising therapeutic target and demonstrate LM11A-31 as important lead for further drug development.

Currently, the selectivity profile of LM11A-31 is not well established. It is known that LM11A-31 inhibits NGF and proNGF binding to $p75^{NTR}$ without effecting TrkA signaling.[248, 254] In $p75^{NTR}$ -/- knockout model LM11A-31 treatment did not promote neuronal cell survival. Additionally, anti-$p75^{NTR}$ antibodies that target the extracellular domain of 75^{NTR} completely abolished LM11A-31 functional activity.[254] Screening LM11A-31 against a CEREP panel of receptors did not indicate any off-target activity.[255] Additional studies are required to elucidate possible off-target mechanisms.

The *in-vivo* performance of LM11A-31 was evaluated and provided an initial pharmacokinetic and toxicological profile.[253] Oral administration of LM11A-31 in an $APP^{L/S}$ mouse model demonstrated favorable therapeutic brain concentrations and efficacy on cognitive impairment and neurodegeneration. After 50 mg/kg oral dosing, the maximum concentration of LM11A-31 in the brain of CD-1 mice was 1.08 μM, which significantly exceeds the reported therapeutic does of 100 nM (Figure 24).[253] Furthermore, the maximum brain concentration occurred after 0.5 h and the brain half-life was 3-4 hours.[253] Conversely, the maximum plasma concentration of LM11A-31 exceeded 600 ng/mL and demonstrated a significantly poor half-life, resulting in plasma levels less than 200 ng/mL in less than 1 h following oral administration (Figure 24).[253] In a toxicology study, mice treated with LM11A-31 at 50 mg/kg once daily for nine continuous days exhibited no behavior fluctuations (assessed according to total ambulatory events and total fine movements) and 2-week dosing indicated no weight change in treatment groups.[253]

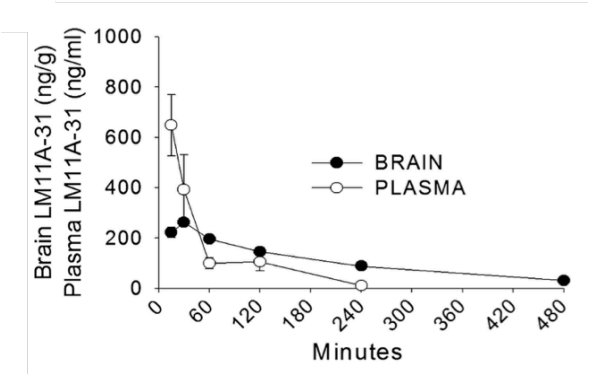


Figure 24. LM11A-31 *In-vivo* Performance
Concentration after a single 50-mg/kg oral dose in CD-1 mice [253]

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https://doi.org/10.1016/j.neurobiolaging.2013.02.015

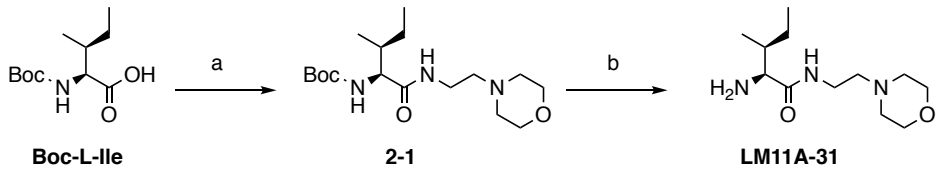
2.2 Results and Discussion

2.2.1 Gram-Scale Synthesis of LM11A-31 and LM11A-24 for Mouse Studies

In collaboration with Professor Anthony Kanai at the University of Pittsburgh Department of Medicine, we were interested in investigating $p97^{NTR}$ role in spinal cord injury (SCI) induced lower urinary tract dysfunction using a mouse model using $p97^{NTR}$ pharmacological agents. For this mouse study, adult female T_8-T_9 transected mice were independently treated with with daily doses (100 mg/kg) of LM11A-31 and LM11A-24 for up to six weeks.[256] Subsequently, mice were evaluated *in vivo* using urine spot analysis, cystometrograms, and external urethral sphincter electromyograms.

To support this investigation, we were interested in developing a gram-scale synthesis of both LM11A-31 and LM11A-24 without the requirement for chromatography. Previous reports introduced a general synthetic strategy; however, percent yields were not reported and both product as well as intermediates required chromatography.[249] Our gram-scale synthesis of LM11A-31 was efficiently conducted using peptide coupling conditions followed by protecting group removal. Amide bond formation occurred between commercially available 2-morpholinoethan-1-amine and Boc-L-isoleucine in the presence of propylphosphonic anhydride (T3P) (Scheme 31). Following dilution with ethyl acetate, impurities were readily removed through multiple aqueous washes, resulting in pure intermediate **2-1** in 84% yield as a white solid. Subsequent Boc-deprotection occurred in the presence of *in-situ* generated hydrogen chloride resulting from a pre-mixed solution of acetyl chloride in methanol. Acid was quenched with a saturated aqueous solution of potassium carbonate and the resulting aqueous mixture was concentrated under reduced pressure. The produced solid was triturated in methylene chloride and upon filtration, the isolated filtrate was concentrated under reduced pressure and dried to afford pure LM11A-31 in 80% yield as a white solid. LM11A-31 was produced on gram-scale in two steps in 67% overall yield with >95% purity by H^1 NMR and LC-MS ELSD without requiring chromatographic purification. In total, over six grams were produced in two batches for animal investigation.

Scheme 31. Gram-Scale Synthesis of LM11A-31

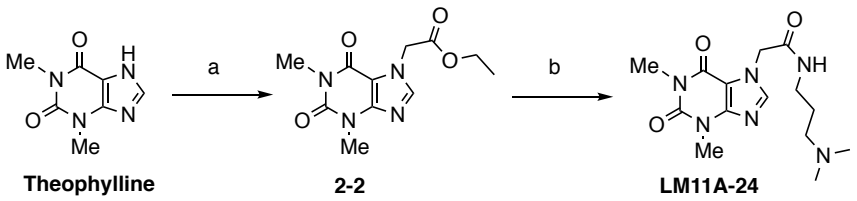


Boc-L-Ile **2-1** **LM11A-31**

Reagents and conditions: (a) 2-morpholinoethan-1-amine, T3P, Et_3N, EtOAc, 0 ^oC-rt, 84%; (b) AcCl, MeOH, 0 ^oC-rt, 3 h; K_2CO_3, H_2O, 80%.

The gram-scale synthesis for LM11A-24 was also efficiently constructed through a two-step process that included an N-alkylation reaction followed by ester aminolysis. Facile *N*-alkylation of commercially available theophylline with ethyl 2-chloroacetate occurred in the presence of potassium carbonate at elevated temperature (Scheme 32). Dilution in water followed by ethyl acetate extraction provided pure intermediate **2-2** in 79% yield as a white solid. Amide bond formation occurred through nucleophilic displacement of the ethyl ester **2-2** with N,N-dimethyl-propane-1,3-diamine in a high pressure vial contiaing ethanol and heated to 110 ^{o}C. The completed reaction mixture was concentrated under reduced pressure and the resulting solid was triturated with diethyl ether to remove impurities. The isolated solid was dried to provide pure LM11A-24 in 73% as a white solid. LM11A-24 was produced on gram-scale in two steps in 58% overall yield >95% purity by H^1 NMR and LC-MS ELSD without requiring chromatography. In total, approximately 4 grams of LM11A-24 were produced in two batches for animal studies.

Scheme 32. Gram-Scale Synthesis of LM11A-24



Reagents and conditions: (a) ethyl 2-chloroacetate, K_2CO_3, DMF, 90 ^{o}C, 12 h, 79%; (b) N,N-dimethyl-1,3-propanediamine EtOH, 110 ^{o}C, 2 h, 73%.

2.2.2 Molecular Modeling Studies of LM11A-31

The determination of a NGF-p75^{NTR} co-crystal structure provided an opportunity to investigate LM11A-31 binding mode and enabled the application of structure-based methodology. Prior to performing medicinal chemistry, we conducted a molecular docking study of LM11A-31 at site 1 of p75^{NTR}, giving priority to p75^{NTR} amino acid residues that significantly contribute to the binding of NGF's ^{32}KGKE^{35} peptide sequence. Twenty configurations of LM11A-31 were evaluated for complementarity to p75^{NTR}'s ^{32}KGKE^{35} recognition site using the software program Glide (Schrödinger, LLC.). Following, we manually examined each binding mode for features not explicitly recognized by the scoring function - specifically, the interaction with key polar residue Asp75. Ultimately, four binding modes were considered, providing a putative model of LM11A-31-mediated p75^{NTR} inhibition.

There is currently no co-crystal structure of the inhibitor-receptor complex of LM11A-31 bound to p75^{NTR}; however, molecular docking of LM11A-31 at the ^{32}KGKE^{35} recognition site of p75^{NTR} established a series of putative binding modes that suggest potential key interactions between LM11A-31 and p75^{NTR}. The target region of p75^{NTR} is enriched with acidic, anionic amino acids, which can be exploited by LM11A-31 and compete with NGF (Figure 25). Consistent with our structural predictions, the molecular model of LM11A-31 bound to p75^{NTR} highlighted electrostatic complementarity and recapitulated a number of notable ionic and hydrogen bonding interactions (Figure 25). The cationic primary amine of LM11A-31 formed hydrogen bonds with the amino acid backbone of Pro70^{p75NTR} and Asp76^{p75NTR}. Additional hydrogen bonding was also observed between the amide hydrogen of LM11A-31 and the side chain of Asp75^{p75NTR}. Furthermore, the flexible ethyl linker enabled the positioning of the

cationic tertiary amine, establishing an ion-pair, salt bridge with the amino acid side chain of Asp75^{p75NTR}.

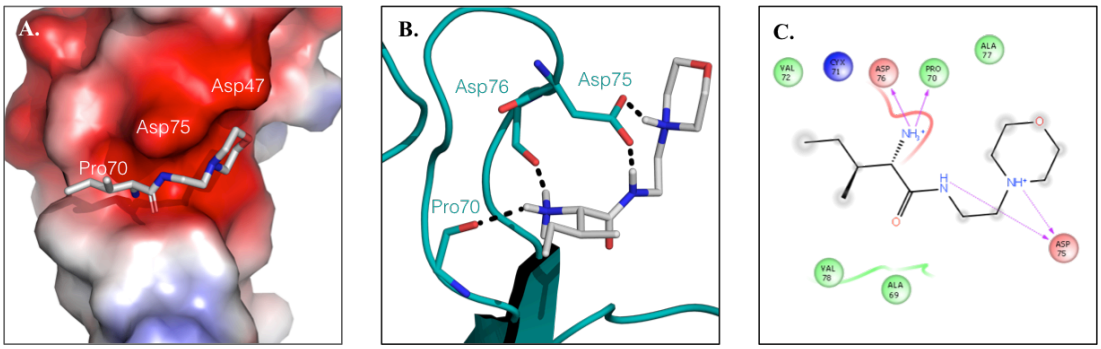


Figure 25. Putative Binding Mode of LM11A-31 at the NGF ^{32}KGKE^{35} Recognition Site of p75^{NTR}

A. LM11A-31 bound to an electrostatic potential map of p75^{NTR} at site 1. Red surface indicates an electron-rich environment; blue surface indicates an electron-poor environment; white indicates a neutral environment. **B.** three-dimensional putative binding mode of LM11A-31. P75^{NTR} is teal, LM11A-31 is colored according to its elemental composition, and intermolecular interactions are indicated by black dotted lines. **C.** two-dimensional putative binding mode of LM11A-31. Intermolecular interactions are indicated by arrows.

2.2.3 Focused Structure Activity Relationship Study of LM11A-31

There is currently no experimental evidence to support LM11A-31's mechanism of action. Furthermore, the structural determinants necessary for LM11A-31's potent and selective modulation of NGF-p75^{NTR}-mediated signaling are unknown. We conducted a focused SAR to further enhance our understanding of LM11A-31 and delineate the chemical features necessary for inhibition. To simplify our effort and guide our SAR, we partitioned LM11A-31 into five zones and each respective zone contained a key chemical feature that was independently evaluated. Zones are the following: the primary amine (Zone I), sec-butyl side chain (Zone II),

the internal amide (Zone III), the alkyl linker (Zone IV), and the morpholine ring (Zone V) (Figure 26).

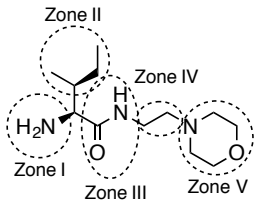
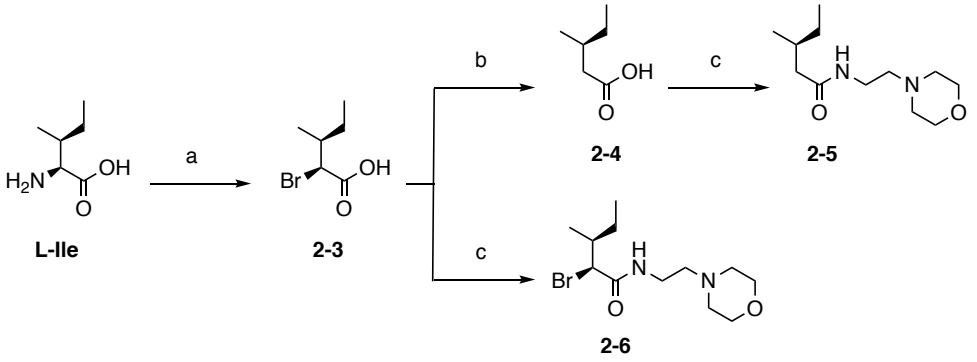


Figure 26. Structural Zones of LM11A-31 for SAR Investigation

Zone I analogues were pursued to determine the functional significance of LM11A-31's primary amine. Zone I analogues (**2-5**, **2-6**, **2-9**, **2-10**) were synthesized by independently performing peptide coupling reactions with 2-morpholinoethan-1-amine using amide bond formation conditions with propylphosphonic anhydride (T3P) (Schemes 33, 34, and 35).[257] Molecular modeling suggested the primary amine of LM11A-31 functioned as a hydrogen bond donor with the main chain amide-carbonyl of $Pro70^{p75NTR}$ and $Asp76^{p75NTR}$. The functional significance of the amine of LM11A-31 was evaluated by synthesizing the deaminated analog **2-5** (Scheme 33). Synthesis of **2-5** began with the diazotization of L-isoleucine using sodium nitrite ($NaNO_2$) in the presence of a 47% aqueous solution of hydrogen bromide (HBr) at 0 ^oC followed by the formation of the corresponding α-bromo carboxylic acid intermediate **2-3**.[258, 259] **2-3** was then reduced by elemental zinc and copper (I) chloride in the presence of ammonium chloride in methanol to generate (*S*)-3-methylpentanoic acid **2-4** in 44% yield. Subsequent peptide coupling with 2-morpholinoethan-1-amine resulted in analog **2-5** in 57% yield. The α-bromo analog **2-6** may be able to recapitulate binding affinity by forming a stabilizing halogen bond, suggesting the presence of an electron rich environment. Carboxylic acid **2-3** was coupled with 2-

morpholinoethan-1-amine using the previously described amide coupling conditions with T3P to afford α-bromo analog **2-6** in 63% yield.

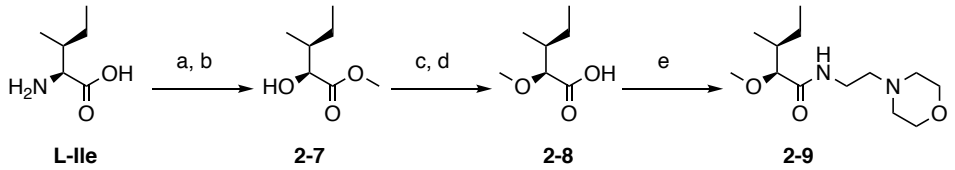
Scheme 33. Formation of Zone I Analogues 2-5 and 2-6



Reagents and conditions: (a) $NaNO_2$, HBr, H_2O, 0 ^oC, 2 h; (b) Zn, CuCl, NH_4Cl, MeOH, rt, 3 h, 44%; (c) 2-morpholinoethan-1-amine, T3P, Et_3N, EtOAc, 0 ^oC-rt, 57-63%.

Based on the performance of **2-5**, the primary amine may demonstrate functional significance; therefore, further investigation is required to delineate its potential role. Although unlikely, the primary amine of LM11A-31 may act as a hydrogen bond acceptor. To test this hypothesis, the α-methoxy derivative **2-9** was pursued – the presence of the methoxy substituent would reduce the HBA effect. Synthesis occurred by diazotization of L-isoleucine with the treatment of sodium nitrite ($NaNO_2$) and in the presence of aqueous sulfuric acid led to the α-hydroxyl carboxylic acid (Scheme 34). Subsequent Fisher esterification of the crude acid resulted in the methyl ester **2-7** in 24% yield over two steps. Deprotonation of the α-hydroxyl group of **2-7** using sodium hydride followed by treatment with methyl iodide resulted in the α-methoxy ester. Subsequent, saponification of the α-methoxy ester provided the carboxylic acid **2-8**, which enabled peptide coupling with 2-morpholinoethan-1-amine to afford the α-methoxy analog **2-9** in 23% yield over three steps.

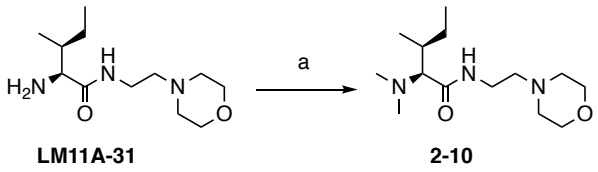
Scheme 34. Formation of Zone I Analog 2-9



[a]Reagents and conditions: (a) $NaNO_2$, H_2SO_4, H_2O, 0 ^oC-rt, 12.5 h; (b) AcCl, MeOH, reflux, 3 h, 24% (two steps); (c) NaH, MeI, THF, 0 ^oC-rt, 2 h; (d) LiOH, H_2O, rt, 17 h; (e) 2-morpholinoethan-1-amine, T3P, Et_3N, EtOAc, 0 ^oC-rt, 23% (three steps).

The methyl group of **2-9** may influence its performance through adverse steric events. To address this concern, we pursued the dimethyl amine analog **2-10** (Scheme 35), which should conserve a potential HBD effect and therefore, indicate if the methyl substituent is tolerated. The synthesis of **2-10** occurred by functionalizing the primary amine of **LM11A-31** under reductive amination conditions using formaldehyde in the presence of hydrogen gas at 8 bar of pressure and Pd/C (Scheme 35).

Scheme 35. Formation of Zone I Analog 2-10

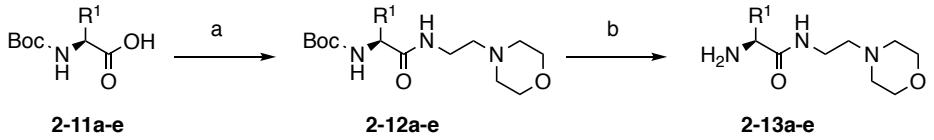


Reagents and conditions: (a) CH_2O, H_2, Pd/C, MeOH, 8 bar, rt, 15 h, 18%.

The evaluation of isoleucine's *sec*-butyl side chain and derivation of R^1 in Zone II led to analogues **2-13a-e** (Scheme 36). These amino acid derivatives - Gly, Ala, Val, Leu, Phe – contain hydrophobic, aliphatic side chains that exhibit increasing steric bulk. If the *sec*-butyl side chain of LM11A-31 is positioned in a hydrophobic pocket, these derivatives provide insight into the importance of the aliphatic side chain and suggest the relative size of the hydrophobic pocket.

Synthesis of Zone II analogues occurred through previously described peptide coupling conditions using commercially available Boc-protected L-amino acids **2-11a-e** and 2-morpholinoethan-1-amine followed by subsequent Boc-deprotection (Scheme 36).

Scheme 36. Formation of Zone II Analogues 2-13a-e

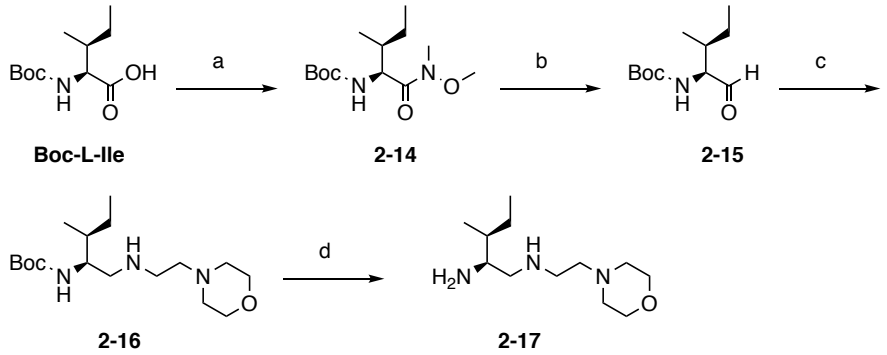


R^1: **a** = H; **b** = Me; **c** = *i*-Pr; **d** = *i*-Bu; **e** = Bn

[a]Reagents and conditions: (a) 2-morpholinoethan-1-amine, T3P, Et_3N, EtOAc, 0 ^oC-rt, 71-96%; (b) AcCl, MeOH, 0 ^oC-rt, 3 h; K_2CO_3, H_2O, 21-62%.

The functional significance of the internal amide moiety of LM11A-31, specifically the carbonyl oxygen and the N-H, led to evaluation of Zone III. The amide functional group has dual pharmacophoric features – the carbonyl may be acting as a hydrogen bond acceptor and the N-H may be acting as a hydrogen bond donor. Analog **2-17** (Scheme 37) was synthesized to assess the carbonyl. Synthesis began with the Weinreb amide intermediate **2-14**, which was formed through peptide coupling of Boc-protected L-isoleucine and N,O-dimethylhydroxylamine hydrochloride in the presence of EDC and HOBt (Scheme 37). Subsequent reduction of the Weinreb amide intermediate with lithium aluminum hydride afforded the aldehyde **2-15**. Then, reductive amination of the aldehyde with sodium triacetoxyborohydride in the presence of 2-morpholinoethan-1-amine provided **2-16** followed by Boc-deprotection afforded the desired analog **2-17** in 67% yield over two steps.

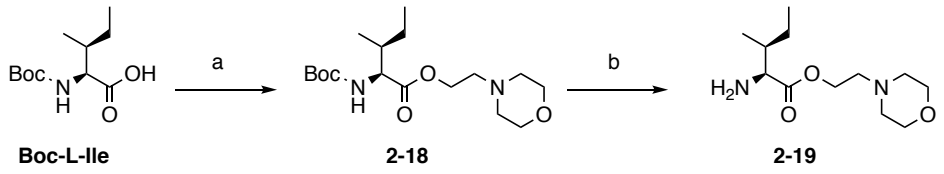
Scheme 37. Formation of Zone III Analog 2-17



[a]Reagents and conditions: (a) MeNH(OMe) HCl, EDC, HOBt, NMM, CH_2Cl_2, 0 ^oC-rt, 22 h, 88%; (b) $LiAlH_4$, THF, 0 ^oC-rt, 1 h, 60%; (c) 2-morpholinoethan-1-amine, $NaBH(OAc)_3$, DCE, rt, 23 h, 64%; (d) AcCl, MeOH, 0 ^oC-rt, 3 h; NaOH, H_2O, 67%.

Our putative molecular model suggested LM11A-31's amide N-H participates as an important HBD. To test this hypothesis, the amide N-H feature was evaluated by pursuing analog **2-19** (Scheme 38) – the presence of the ester functionality eliminates the possibility for hydrogen bonding. Also, the additional electron density from the lone pair electrons of oxygen may have a repulsive effect and deter **2-19** binding. Synthesis of **2-19** occurred through a Steglich esterification with Boc-protected L-isoleucine in the presence of 2-morpholinoethan-1-ol to form intermediate **2-18** followed by subsequent Boc-deprotection to generate ester **2-19** (Scheme 38).

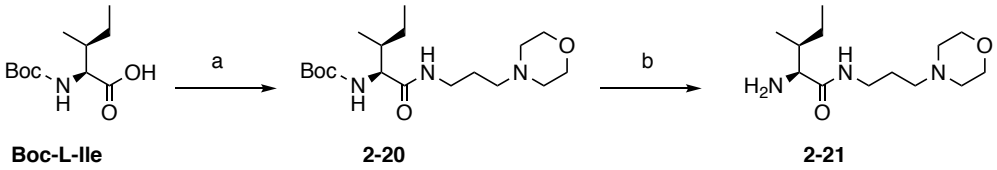
Scheme 38. Formation of Zone III analog 2-19



Reagents and conditions: (a) 2-morpholinoethan-1-ol, EDC HCl, DMAP, CH_2Cl_2, rt, 11 h, 87%; (b) AcCl, MeOH, 0 ^oC-rt, 3 h; K_2CO_3, H_2O, 69%.

The ethyl linker in Zone IV provides flexibility that may promote the optimal positioning of the morpholine ring. Additionally, the distance between the primary amine and tertiary amine may be critical for receptor binding. To assess the alkyl linker length on inhibitor binding, we pursued analog **2-21** (Scheme 39), which constitutes an extended three carbon chain in zone IV. Synthesis of **2-21** occurred through peptide coupling of Boc-protected L-isoleucine and 3-morpholinopropan-1-amine followed by subsequent Boc-deprotection to provide the desired product **2-21** (Scheme 39).

Scheme 39. Formation of Zone IV Analog 2-21



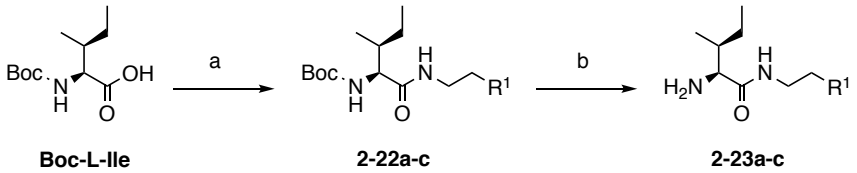
Boc-L-Ile **2-20** **2-21**

Reagents and conditions: (a) 3-morpholinopropan-1-amine, T3P, Et_3N, EtOAc, 0 ^oC-rt, 81% (b). AcCl, MeOH, 0 ^oC-rt, 3 h; K_2CO_3, H_2O, 58%.

The morpholine ring in Zone V may present critical functionality necessary for $p75^{NTR}$ inhibition. Therefore, Zone V analogues **2-23a-c** (Scheme 40) were generated to evaluate the morpholino moiety, specifically the tertiary amine and the cyclic ether functionality. Zone V analogs were synthesized using previously described peptide coupling conditions in the presence of Boc-protected L-isoleucine and the respective amine derivative (Scheme 40). Molecular modeling suggested the tertiary amine from the morpholine ring formed a critical electrostatic interaction with the side chain of $Asp75^{p75NTR}$. Removal of this feature may result in reduced inhibitor binding. To test this hypothesis, we replaced the morpholine ring with a methoxy group, resulting in analog **2-23a**. The cyclic ether functionality may also play a role in LM11A-

31 binding. The dimethyl amine analog **2-23b** was synthesized, which conserves the tertiary amine functionality but eliminates the cyclic ether. Both chemical features were eliminated by replacing the morpholine ring, forming the cyclohexyl analog **2-23c**.

Scheme 40. Formation of Zone V Analogues 2-23a-c



Boc-L-Ile **2-22a-c** **2-23a-c**

R^1: **a** = OMe; **b** = NMe_2; **c** = *c*-Hex

Reagents and conditions: (a) N,N-dimethylethylenediamine (1-26a) or 2-methoxyethylamine (1-26b) or 2-cyclohexylethylamine (1-26c), T3P, Et_3N, EtOAc, 0 ^oC-rt, 80-93% (b). AcCl, MeOH, 0 ^oC-rt, 3 h; K_2CO_3, H_2O, 42-58%.

2.2.4 Novel p75 Neurotrophin Receptor Inhibitors

2.2.4.1 Hybrid Series

LM11A-31 displayed potent inhibition of NGF-mediated $p75^{NTR}$ signaling in cellular assays; however, it exhibited poor bioavailability and rapid clearance in a mouse model, which limits its *in-vivo* utility.[253] Therefore, we were interested in developing a novel NGF-$p75^{NTR}$ modulator that demonstrates improved physiochemical and pharmacokinetic characteristics that would allow for *in-vivo* evaluation of NGF-$p75^{NTR}$-mediated events. Our discovery efforts began with the application of small molecule hybridization strategies.[260-263] By grafting chemical features of $p75^{NTR}$ inhibitors LM11A-31 and LM11A-24 (Figure 27), we were able to develop a series of novel $p75^{NTR}$ inhibitors.

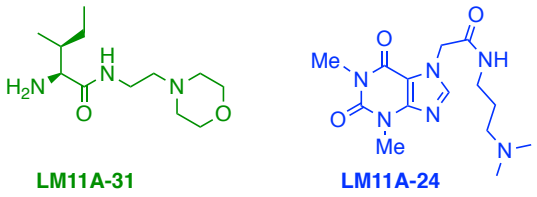
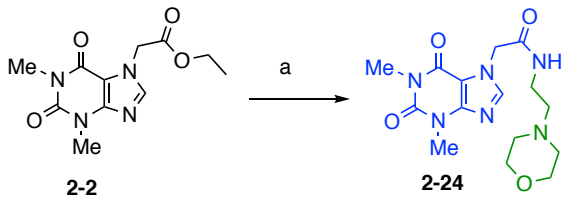


Figure 27. Structure of LM11A-31 (green) and LM11A-31 (blue)

The primary amine of LM11A-31 is a potential site for metabolism.[264] Therefore, our design focused on eliminating the potential biochemically labile primary amine by replacing it with LM11A-24's theophylline core. Amide bond formation occurred through nucleophilic displacement of **2-2** with the 2-morpholinoethan-1-amine of LM11A-31 to provide **2-24** in 76% yield (Scheme 41).

Scheme 41. Formation of Hybrid Analog 2-24

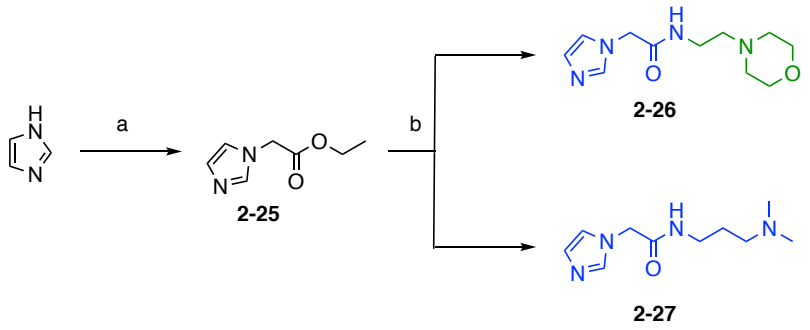


Reagents and conditions: (a) 2-morpholinoethylamine, EtOH, 110 ^{o}C, 2 h, 76%.

Simplification of the theophylline core by eliminating the pyrimidine-dione ring led to the imidazole-based analogues **2-26** and **2-27** (Scheme 42). Synthetic efforts followed the previous analogues. Base-mediated alkylation of imidazole with ethyl 2-chloroacetate provided intermediate **2-25** in 49% yield. Amide bond formation occurred through nucleophilic

displacement of **2-25** with the respective amine under thermal heating, resulting in analouges **2-26** and **2-27** in 77% and 100% yield, respectively.

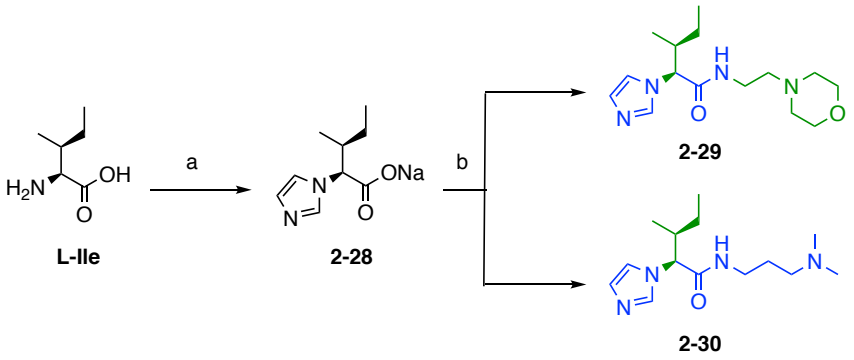
Scheme 42. Formation of Hybrid Analogues 2-26 and 2-27



Reagents and conditions: (a) ethyl 2-chloroacetate, K_2CO_3, MeCN, reflux, 3 h, 49%; (b) 2-morpholinoethylamine (2-26) or N,N-dimethyl-1,3-propanediamine (2-27), EtOH, 110 ^oC, 2 h, 77-100%.

Next, we were interested in incorporating the chiral aliphatic, *sec*-butyl side chain from LM11A-31 on the imidazole hybrid analog series. Using L-isoleucine, we performed a multi-component dehydration-cyclization reaction using glyoxal, formaldehyde, and ammonia under basic conditions to form the corresponding crude sodium salt **2-28** (Scheme 43).[265] Subsequent amide bond formation proved to be challenging, presumably due to the sterically encumbered chiral acid. However, the application of the peptide coupling reagent pentafluorophenyl diphenylphosphinate (FDPP) in the presence of the respective amine provided analogues **2-29** and **2-30** in modest yields of 34-45%.

Scheme 43. Formation of Hybrid Analogues 2-29 and 2-30



Reagents and conditions: (a) glyoxal, CH_2O, NH_3, NaOH, H_2O, 50 ^oC, 5 h; (b) 2-morpholinoethylamine (2-29) or N,N-dimethyl-1,3-propanediamine (2-30), FDPP, DIPEA, CH_2Cl_2, rt, 34-45%.

2.2.4.2 5-Aminooxazole Series

The peptide bond of LM11A-31 may be subjected to *in-vivo* proteolytic hydrolysis and contribute toward its rapid clearance.[264, 266] Additionally, the primary amine of LM11A-31 is also a potential site for metabolism.[264] Replacement of biochemically labile functionality may increase its plasma and metabolic stability and improve its pharmacokinetic profile.[148-150] We envisaged installing an isosteric surrogate that would demonstrate enhanced physiochemical properties and metabolic stability. Incorporation of heterocyclic moieties to replace sites of metabolism is an effective strategy toward improving a peptidomimetic's pharmacokinetic profile.[267-270] Our initial design unified the primary amine with the amide carbonyl, leading to a 5-aminooxazole core (Figure 28). According to our molecular model, we hypothesized that the 2-morpholinoethan-1-amine was critical for $p75^{NTR}$ binding and therefore, this feature was conserved. However, medicinal chemistry efforts were necessary to optimize the 5-aminooxazole series.

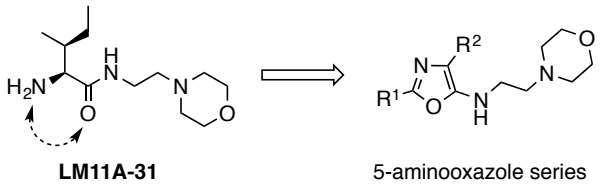
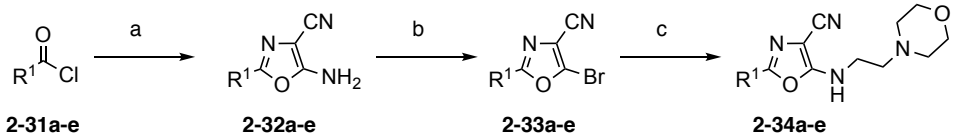


Figure 28. Design of the 5-Aminooxazole Series

Medicinal chemistry optimization began by evaluating substituents at C-2. Since LM11A-31 contained an aliphatic side-chain, we explored C-2 substituents with similar chemical properties. Synthesis of the 5-aminooxaozole analogues **2-34a-e** began with a microwave mediated cyclization of an acid chloride **2-31a-e** with aminomalononitrile *p*-toluenesulfonate at 120 ^{o}C in N-methyl-2-pyrrolidone (NMP), resulting in the 5-aminooxazole intermediate **2-32a-e** (Scheme 44).[271] Direct functionalization of the heteroaryl amine with an alkyl halide under thermal and microwave heating proved inconsequential, perhaps due to the lack of nucleophilicity of the heteroaryl amine. Pre-treatment of **2-32a-e** with NaH did not facilitate electrophilic alkylation as well. In order to circumvent this problem, a Sandmeyer reaction was performed by treating **2-32a-e** with *tert*-butyl nitrite in the presence of copper (II) bromide, generating the heteroaryl bromide **2-33a-e**.[272] This enabled nucleophilic aromatic substitution using 2-morpholinoethan-1-amine to furnish analogues **2-34a-e**.

Scheme 44. Formation of 5-Aminooxazole Analogues 2-34a-e



R^1: **a** = Et; **b** = *c*-Pr; **c** = *c*-Pent; **d** = *c*-Hex; **e** = Ph

Reagents and conditions: (a) aminomalononitrile *p*-toluenesulfonate, NMP, 120 ^{o}C, μW, 0.25 h, 18-72%; (b) *tert*-BuONO, $CuBr_2$, MeCN, rt, 3 h, 33-60%; (c) 2-morpholinoethan-1-amine, THF, 70 ^{o}C, 2 h, 45-80%.

Oxazoles are a well-established structural motif in biologically relevant molecules.[273] To further expand our SAR of the 5-aminooxazole series and mitigate the utility of a repetitive multistep synthesis, we were interested in pursuing a late stage, advanced intermediate that would enable rapid derivation. Direct functionalization of oxazoles provides an attractive approach toward this effort.[274] To date, there are multiple reports that independently demonstrate effective direct arylation and amination of oxazoles.[275-277] Therefore, we initially envisaged 4-cyanooxazole as a reasonable platform for sequential C-5 selective direct amination to afford the 5-aminooxazole intermediate followed by C-2 direct arylation to provide the polyfunctionalized product (Figure 29). However, there are a number of challenges that require investigation. Regioselective functionalization of an unsubstituted oxazole is well studied; whereby, C-2 activation is preferred over C-5.[278] Further, metalation of an oxazole can be challenging and requires strictly controlled reaction conditions due to the instability of the intermediary metal species and its tendency to favor the ring open isonitrile tautomer.[279-281]

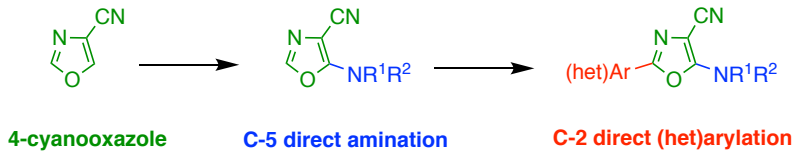


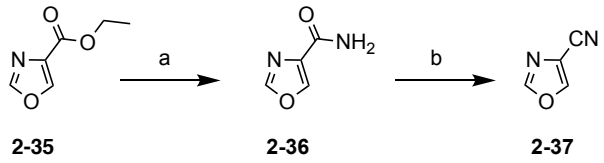
Figure 29. Sequential C-5 Direct Amination C-2 Direct (het)arylation of 4-Cyanooxazole

Our investigation demonstrated successive metalations initiated by regioselective C-5 zincation followed by copper-catalyzed electrophilic amination and subsequent palladium-catalyzed C-2 direct arylation to rapidly provide diversely functionalized aminooxazole derivatives. Selective C-H amination occurred utilizing a strong, non-nucleophilic zinc base, ZnTMP•LiCl (TMP=2,2,6,6-tetramethylpiperidyl). Treatment of 4-cyanooxazole underwent

ortho-metalation to regioselectively deprotonate the C-5 position. The resulting organozincate intermediate was then able to react with *O*-acylhydroxylamines in the presence of a copper catalyst, $Cu(OAc)_2$, to furnish the C-5 aminated product. Lastly, employing established palladium-catalyzed direct arylation conditions, C-2 functionalization occurred with diverse (hetero)arenes.

Our synthetic approach began with commercially available ethyl oxazole-4-carboxylate **2-35** (Scheme 45). Nucleophilic substitution with ammonia furnished the primary amide **2-36** in 99% yield followed by TFAA-mediated dehydration to generate 4-cyanooxazole **2-37** in 84% yield. 4-Cyanooxazole **2-37** was generated on gram-scale in 83% over two steps without requiring chromatography.

Scheme 45. Synthesis of 4-Cyanooxazole 2-37



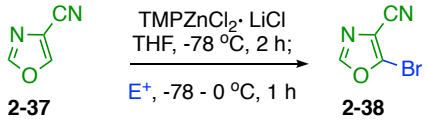
2-35 **2-36** **2-37**

Reagents and conditions: (a) NH_3, MeOH, 60 ^oC, 20 h, 99%; (b) TFAA, Et_3N, THF, 0 ^oC-rt, 1.5 h, 84%.

Previous reports have demonstrated the application of a cyano group to achieve regioselective alpha-metalation of pi-deficient heterocycles in the presence TMP-Zinc base.[282] Therefore, we hypothesized that the inductive and/or possibly complex-induced proximity effects of the 4-cyano substituent would lead to preferential directed *ortho* metalation (DoM) at the C-5 position.[283-285] To test this hypothesis, we utilized Knochel's mixed lithium and zinc base, $TMPZnCl_2$•LiCl, which has indefinite stability at room temperature in the absence of air.[278, 286] To evaluate regioselectivity, **2-37** was treated with $TMPZnCl_2$•LiCl at -78 ^oC for 2 h

and subsequently quenched with bromine. Gratifyingly, upon warming to 0 °C, this reaction resulted in the exclusive regioselective metalation and bromination of C-5 to provide **2-38** in 60% yield (Entry 1, Table 5). Subsequent evaluation of other electrophilic bromine sources, $(Cl_2BrC)_2$ and NBS, resulted in lower yields (Entry 2 and 3).

Table 5. Regioselective C-5 Zincation



2-37 **2-38**

Entry	E^+	Yield[a]
1	Br_2	60%
2	$(Cl_2BrC)_2$	32%
3	NBS	19%

$TMPZnCl_2$•LiCl (1.1 eq); E+ (1.1 eq); [a]Isolated yields

Regioselective metalation and subsequent bromonation of C-5 was affirmed using 1D and 2D nuclear magnetic resonance (NMR) spectroscopy. ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation (HMBC) analysis of **2-37** clearly demonstrated correlation between H-5 and the 4-cyano carbon. Following $TMPZnCl_2$•LiCl–mediated metalation and subsequent bromination, ^{1}H-^{13}C HMBC analysis of **2-38** no longer indicated the correlation between H-5 and the 4-cyano carbon (Figure 30). This suggested C-5 metalation occurred, resulting in the C-5 brominated product. Interestingly, the ^{13}C chemical shift of **2-38** for C-5 was 136.0 ppm – a difference of 10.4 ppm upfield compared to **2-37**. This observed ^{13}C chemical shift may be due to two possible phenomena: the heavy-atom α-effect or the mesomeric effect.[287] The heavy-atom α-effect is attributed to bromine's non-bonding lone-pair electrons that provide enhanced diamagnetic

shielding. Alternatively, the mesomeric effect is associated with bromine's non-bonding lone-pair electrons that interact with the conjugated pi-electrons of the oxazole ring, which would enhance the electron density of C-5.

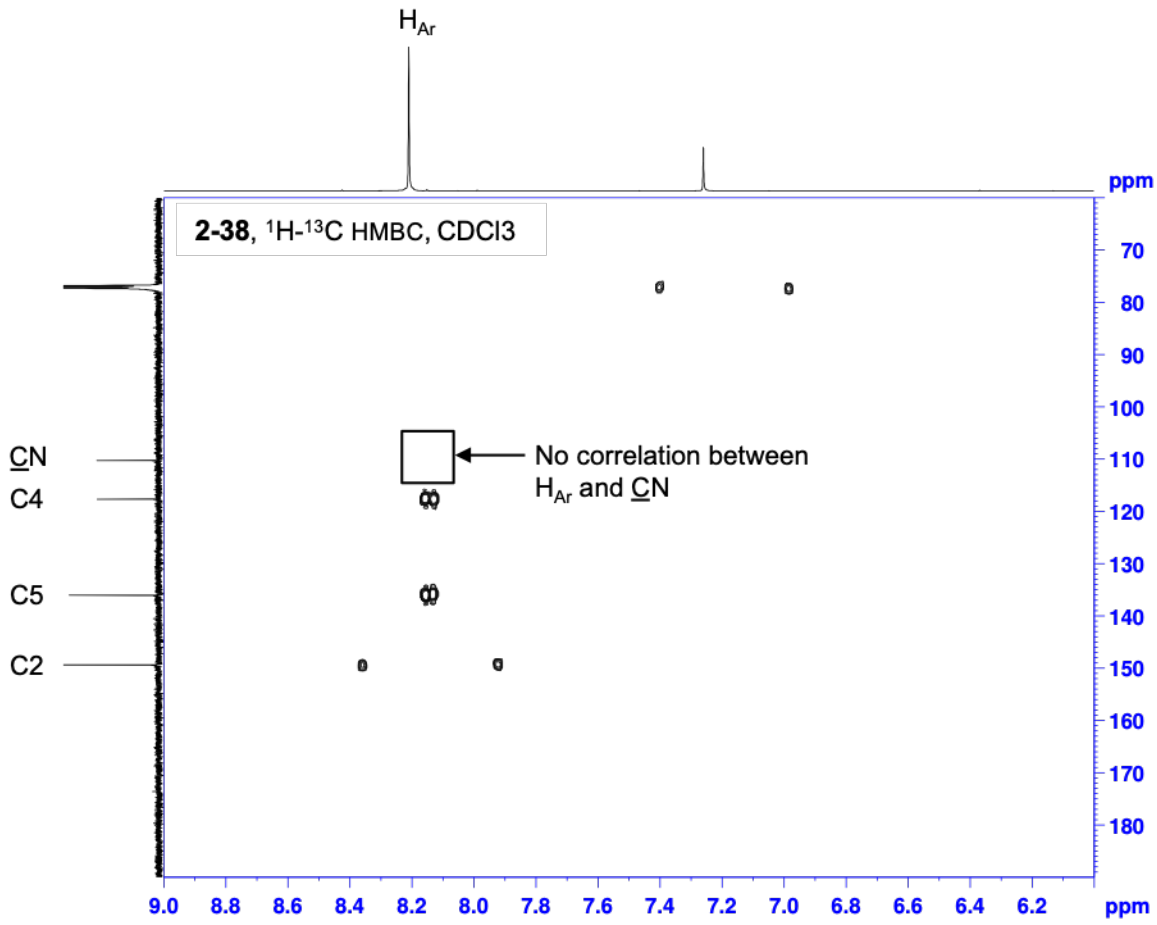
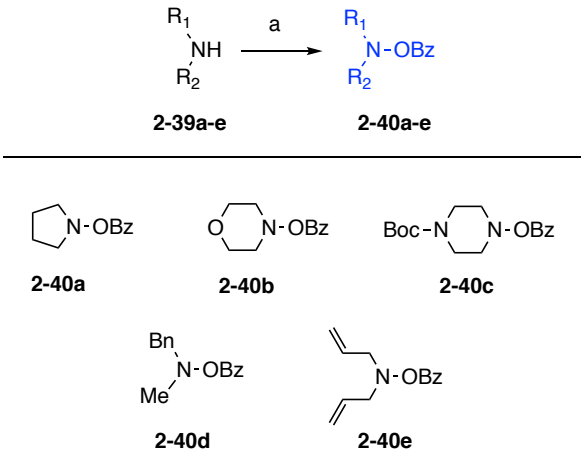


Figure 30. ^{1}H-^{13}C HMBC of 2-38

Encouraged by the regioselective C-5 deprotonation and metalation of 4-cyanooxazole, we next investigated the feasibility of C-H amination with electrophilic amines. Based on previous reports and ease of preparation, we utilized *O*-acylhydroxylamines as a suitable electrophilic nitrogen source.[288, 289] Treatment of diverse secondary amines with benzoic

anhydride and disodium hydrogen phosphate led to the facile construction of *O*-acylhydroxylamines **2-40a-e** in 49-89% yield (Scheme 46).

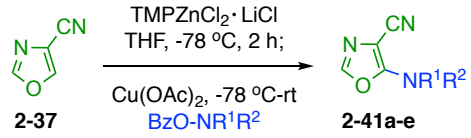
Scheme 46. Synthesis of *O*-Acylhydroxylamines



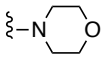
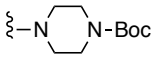
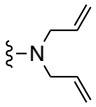
Reagents and conditions: (a) $(BzO)_2$, Na_2HPO_4, DMF, rt, 2 h, 49-89%

Treatment of the heteroaryl zincate intermediate with diverse *O*-acylhydroxylamines **2-40a-e** in the presence of $Cu(OAc)_2$ gratifyingly provided the respective aminated product in modest to good yields (58-67%) (Table 6). The focused scope of the amination reaction included cyclic (Entry 1-3) and acyclic (Entry 4 and 5) alkylamino groups. Notably, cleavage of the benzyl or allyl group under mild conditions can afford either a secondary or primary amino derivatives (e.g. Entry 4 and 5).

Table 6. C-5 Direct Amination



2-37 **2-41a-e**

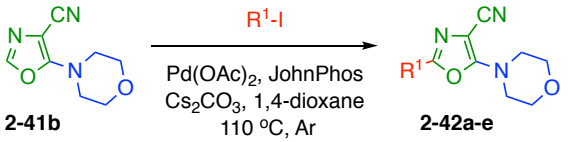
Entry	-NR^1R^2	Yield[a]	Compound
1		67%	**2-41a**
2		64%	**2-41b**
3		63%	**2-41c**
4		60%	**2-41d**
5		58%	**2-41e**

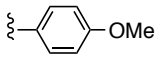
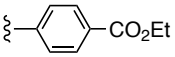
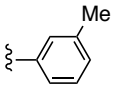
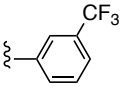
$TMPZnCl_2$•LiCl (1.1 eq); BzO-NR^1R^2 (1.2 eq), $Cu(OAc)_2$ (10 mol%); [a]Isolated yields

Next, we explored C-H arylation at C-2 utilizing the morphilino derivative **2-41b**. Multiple reports have demonstrated direct arylation of oxazole at C-2; however, to the best of our knowledge, there are no literature examples for the direct functionalization of 4-cyano and/or 5-aminooxazole. Due to the electron-withdrawing effect of the 4-cyano substituent, our initial efforts employed Hoarau's conditions, which effectively demonstrated palladium-catalyzed direct arylation of an electron-deficient ethyl oxazole-4-carboxylate using an electron-rich JohnPhos ligand with various iodo, bromo, and chloro (hetero)arenes.[290, 291] Gratifyingly, direct C-2 arylation of **2-41b** using Hoarau's conditions afforded the respective arylated product **2-42a-e** in a respectable 69-81% yield (Table 7). Both electron rich (Entry 1 and 3) and electron deficient (Entry 2 and 4) arenes at the *meta*- and *para*-position were surveyed as well as 2-

pyridyl derivative (Entry 5). This work established an effective platform for rapid and efficient C-5 direct amination followed by C-2 direct arylation of 4-cyanooxazole.

Table 7. C-2 Direct (het)Arylation



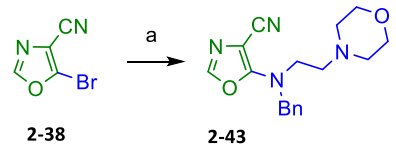
Entry	R^1	Yield[a]	Compound
1		81%	**2-42a**
2		70%	**2-42b**
3		71%	**2-42c**
4		75%	**2-42d**
5		69%	**2-42e**

R^1-I (2 eq.), $Pd(OAc)_2$ (5 mol %), JohnPhos (10 mol%), Cs_2CO_3 (2 eq.); [a]Isolated yields

The developed methodology was applied toward the pursuit of our medicinal chemistry optimization of the 5-aminooxazole series. The advanced 5-amino-4-cyanooxazole intermediate **2-43** was established in 76% yield through nucleophilic aromatic substitution of bromide **2-38** with pre-constructed *N*-benzyl-2-morpholinoethan-1-amine at 80 °C (Scheme 47).

.

Scheme 47. Synthesis of 4-Cyanooxazole Advanced Intermediate

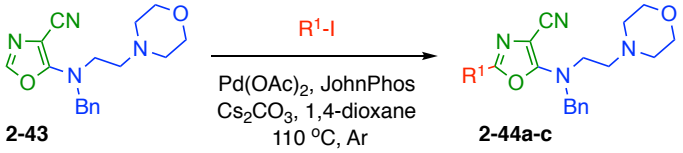


2-38 2-43

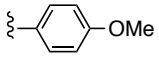
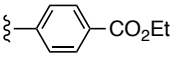
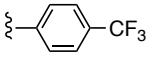
Reagents and conditions: (a) *N*-benzyl-2-morpholinoethan-1-amine, THF, 80 ^{o}C, 43 h, 76%.

With the advanced 5-amino-4-cyanooxazole intermediate **2-43** in hand, continuation of SAR of C-2 occurred in Table 8 through direct arylation with various electron rich (Entry 1) and electron deficient arenes (Entry 2 and 3) to provided the C-2 functionalized product **2-44a-c** in modest to good yields (55-82%).

Table 8. Direct C-2 Arylation of an Advanced Intermediate



2-43 2-44a-c

Entry	R^1	Yield[a]	Compound
1		55%	**2-44a**
2		77%	**2-44b**
3		82%	**2-44c**

R^1-I (2 eq.), $Pd(OAc)_2$ (5 mol %), JohnPhos (10 mol%), Cs_2CO_3 (2 eq.); [a]Isolated yields

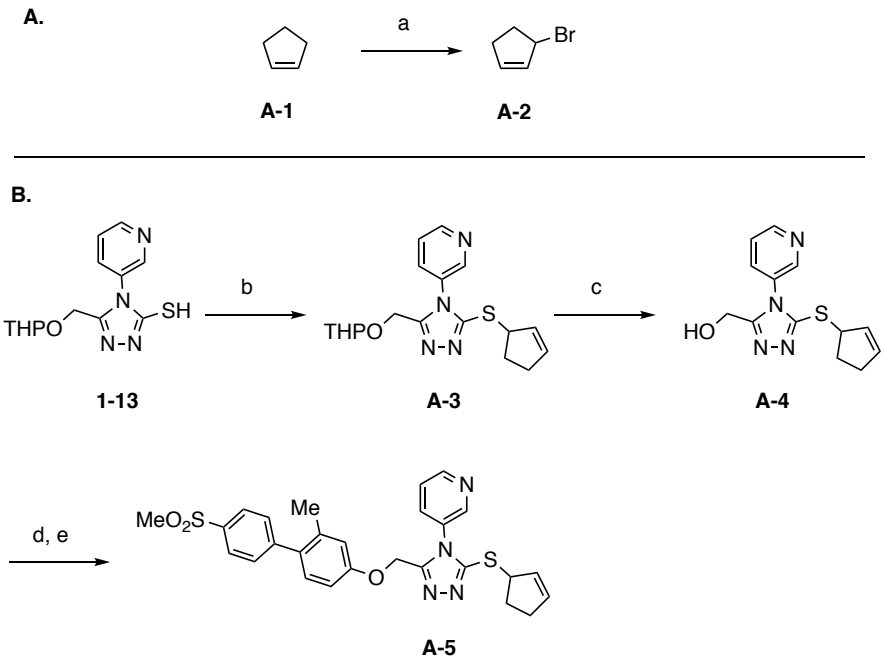
2.3 Conclusion

Our initial effort provided a gram-scale synthesis of known p75 neurotrophin receptor ($p75^{NTR}$) inhibitors LM11A-31 and LM11A-24 without the requirement for chromatography. Additionally, we investigated the experimental binding of LM11A-31 to $p75^{NTR}$ through the development of a computational model and the synthesis of LM11A-31 derivatives for SAR investigation. Furthermore, we constructed two $p75^{NTR}$ inhibitor series – the hybrid analog series and the 5-aminooxazole series. Due to inconsistent and irreproducible biological results, further studies evaluating the performance of the synthesized compounds will be necessary. Efforts toward the 5-aminooxazole series led to the development of novel methodology that enabled C-5 regioselective direct amination followed by C-2 direct (het)arylation of 4-cyanooxazole. This work supported the construction of a 5-aminooxazole advanced intermediate and rapid C-2 substitution using direct arylation conditions.

3.0 Appendix

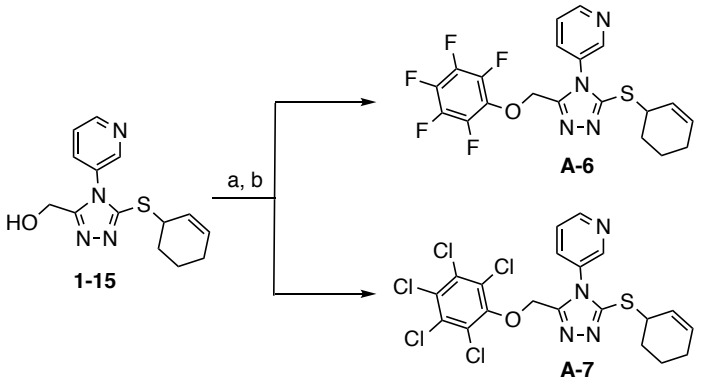
The following appendix describes the synthesis of additional chapter 1 NMS-873 analogues that do not fit within the framework and the context of the main text. These analogues include zone I-IV derivation as well as HyT derivatives. The experimental procedures associated with these intermediates and final products are found in the experimental section.

Scheme 48. Synthesis of A-5



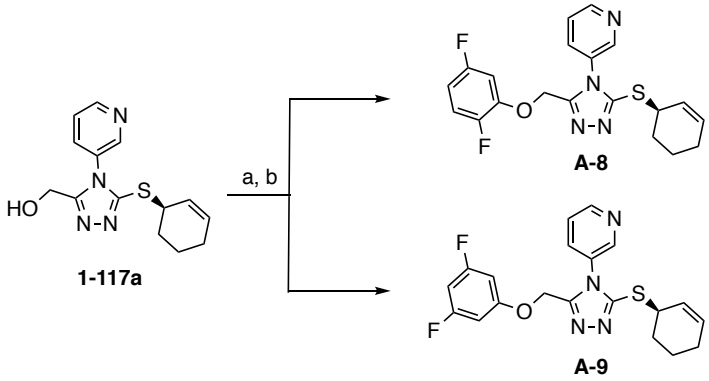
Reagents and conditions: **A.** (a) NBS, AIBN, CCl_4, 90 ^oC, 2 h; **B.** (b) **A2**, Cs_2CO_3, DMF, 80 ^oC, 2 h; (c) *p*-TsOH, MeOH, rt, 24 h, 33%; (d) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 1 h; (e) 2-methyl-4'-(methylsulfonyl)-[1,1'-biphenyl]-4-ol, Cs_2CO_3, DMF, rt, 21 h, 60%.

Scheme 49. Synthesis of A-6 and A-7



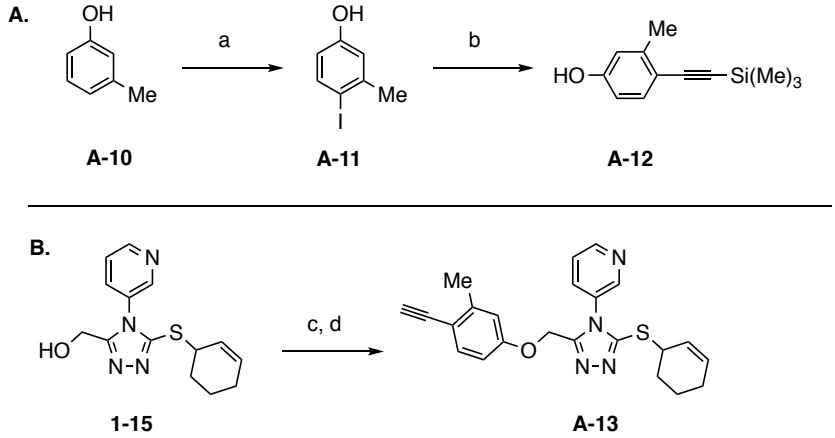
Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 1 h; (b) pentafluorophenol (A-6) or pentachlorophenol (A-7), Cs_2CO_3, DMF, rt, 16 h, 54-57%.

Scheme 50. Synthesis of A-8 and A-9



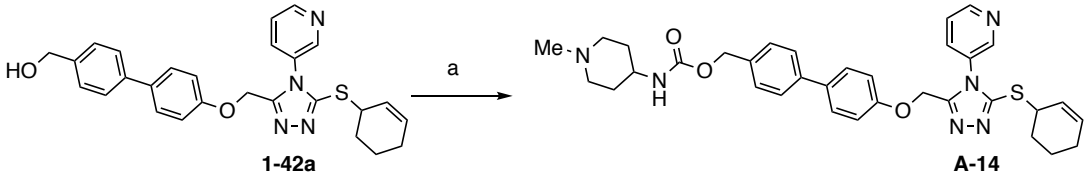
Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 2 h; (b) 2,5-difluorophenol (A-8) or 3,5-difluorophenol (A-9), Cs_2CO_3, DMF, rt, 20 h, 84-87%.

Scheme 51. Synthesis of A-13



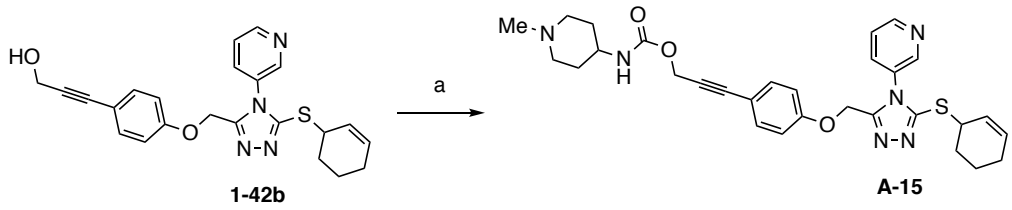
Reagents and conditions: **A.** (a) KI, KIO_3, HCl, MeOH, H_20, rt, 1 h; (b) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, rt, 17 h, 79%; **B.** (c) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 1 h; (d) **A-12**, Cs_2CO_3, DMF, rt, 13 h, 64%.

Scheme 52. Synthesis of A-14



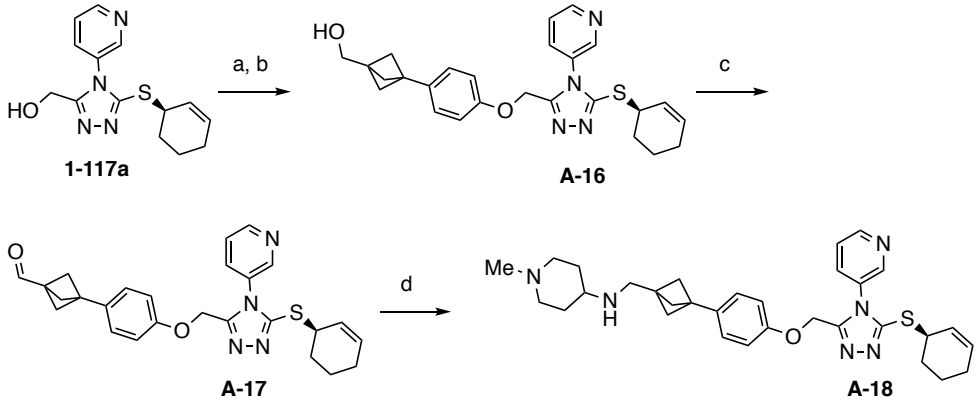
Reagents and conditions: (a) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 14 h, 70%

Scheme 53. Synthesis of A-15



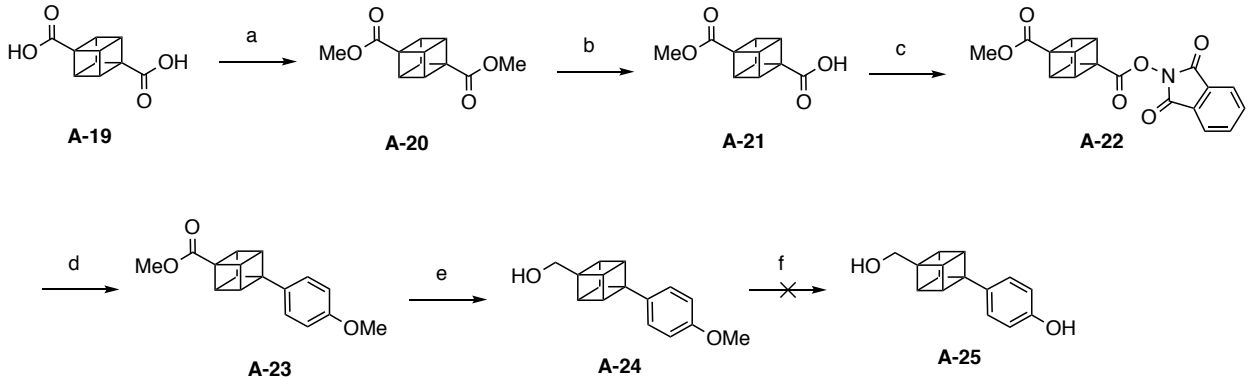
Reagents and conditions: (a) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 18 h, 68%

Scheme 54. Synthesis of A-18



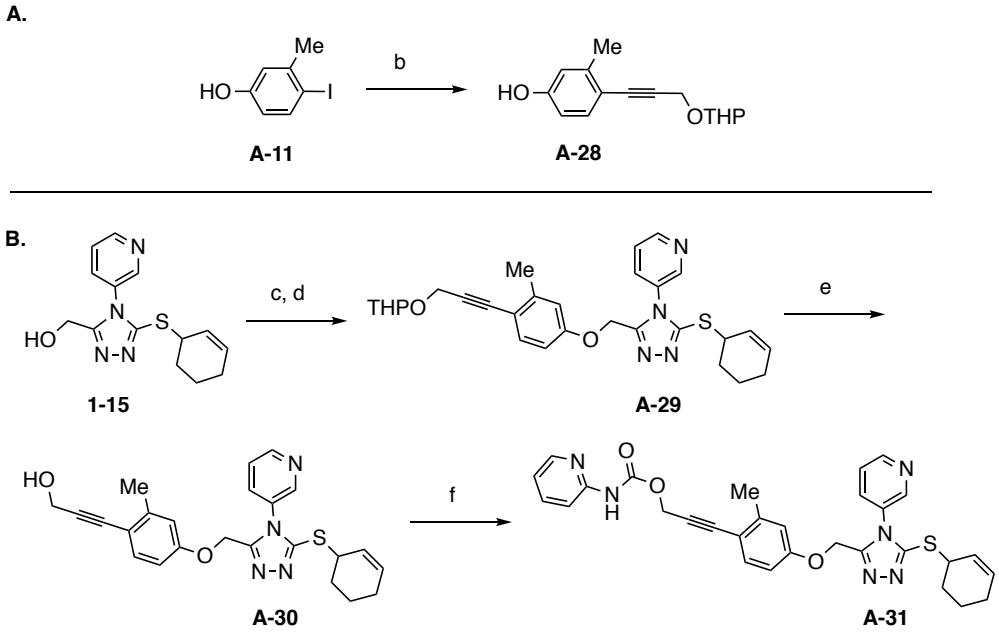
Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 2 h; (b) **34**, Cs_2CO_3, DMF, rt, 22 h, 61%; (c) DMP, CH_2Cl_2, 0 ^oC, 1.5 h, 87%; (d) 1-methylpiperidin-4-amine, $NaBH_4$, MeOH, rt, 16 h, 42%.

Scheme 55. Synthetic Efforts Toward A-25



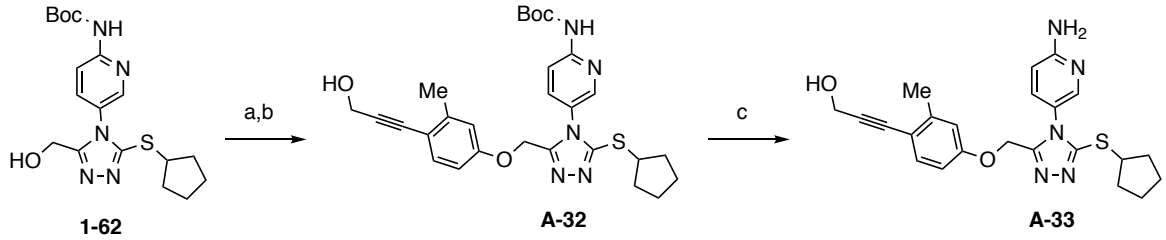
Reagents and conditions: (a) H_2SO_4, MeOH, 80 ^oC, 24 h, 100%; (b) NaOH, THF, MeOH, rt, 13 h, 86%; (c) 2-hydroxyisoindoline-1,3-dione, DIC, DMAP, CH_2Cl_2, rt, 18 h, 89%; *para*-anisole magnesium bromide, $NiCl_2$ glyme, dtbbpy, DMF/THF, rt, 2 h, 39%, $LiAlH_4$, Et_2O, CH_2Cl_2, -10 ^oC-rt, 18 h; (f) 1-dodecanethiol, $AlCl_3$, PhMe, 0 ^oC-rt, 14 h.

Scheme 56. Synthesis of A-31



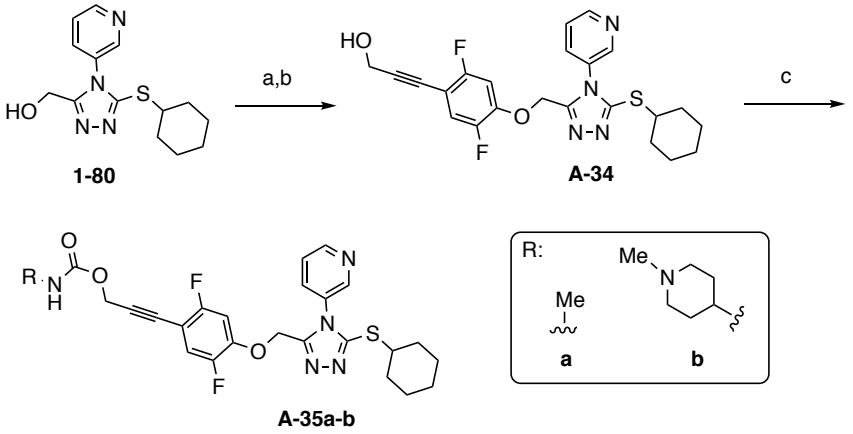
Reagents and conditions: **A**. (a) KI, KIO_3, HCl, MeOH, H_2O, 2 h; (b) 2-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, MeCN, 38 h, 41%; **B.** (c) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 2 h; (d) **A-28**, Cs_2CO_3, DMF, rt, 15 h, 49%; (e) *p*-TsOH, MeOH, rt, 48 h, 20%; (f) 4-nitrophenyl chloroformate, DMAP, 2-aminopyridine, CH_2Cl_2, rt, 0.5 h; 2-aminopyridine, rt – 70 ^oC, 1 h, 62%.

Scheme 57. Synthesis of A-33



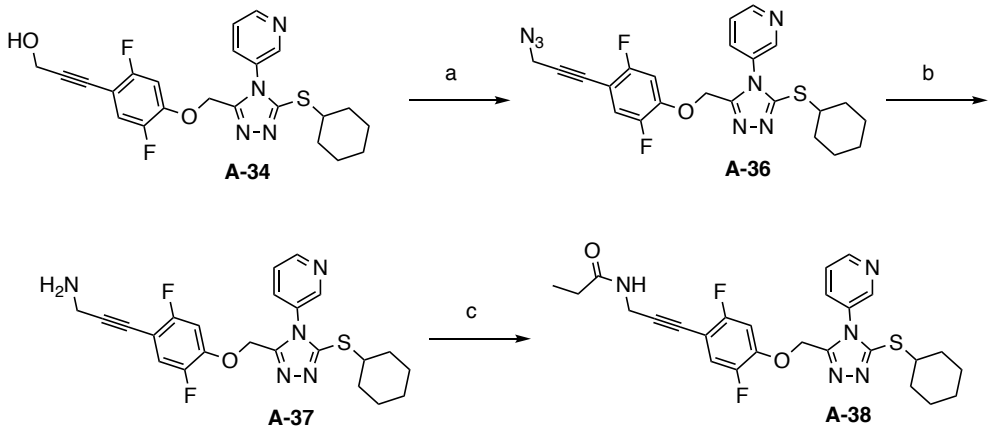
Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (b) 4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol, Cs_2CO_3, DMF, rt, 26 h, 79%, K_2CO_3, MeOH, 100 ^oC, 18 h, 68%.

Scheme 58. Synthesis of A-35a and A-35b



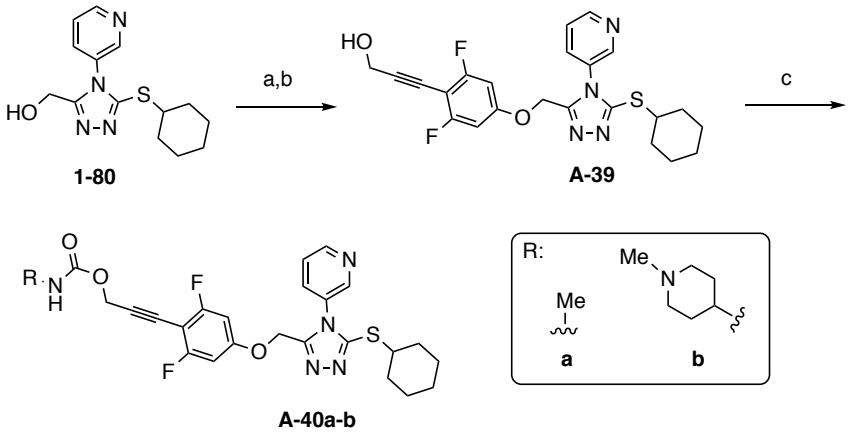
Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (b) 2,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol, Cs_2CO_3, DMF, rt, 15 h, 81%; (c) methylamine hydrochloride (A-35a), Et_3N, CDI, CH_2Cl_2, rt, 16 h, 97% or 1-methylpiperidin-4-amine, (A-35b), CDI, CH_2Cl_2, rt, 16 h, 91%.

Scheme 59. Synthesis of A-38



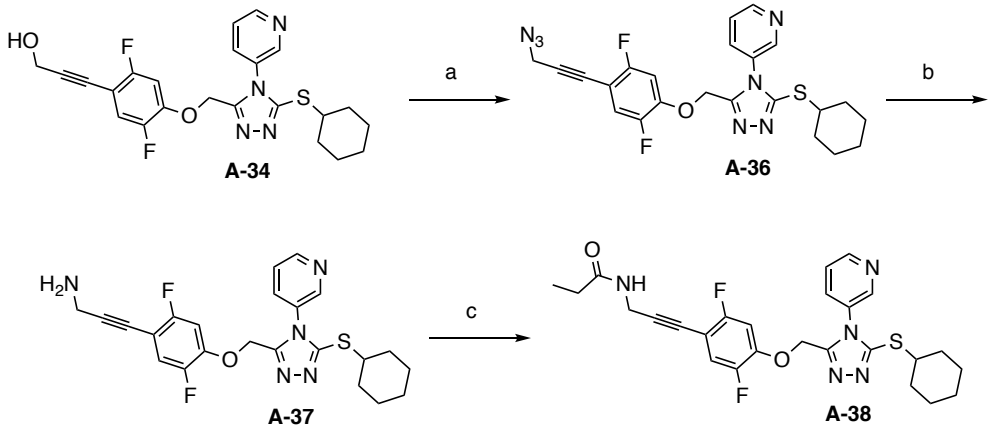
Reagents and conditions: (a) DPPA, DBU, THF, 0 ^oC – rt, 18 h, 65%; (b) PPh_3, THF, H_2O, rt, 20 h, 78%; (c) propionic acid, T3P Et_3N, DMF, rt, 15 h, 70%.

Scheme 60. Synthesis of A-40a and A-40b



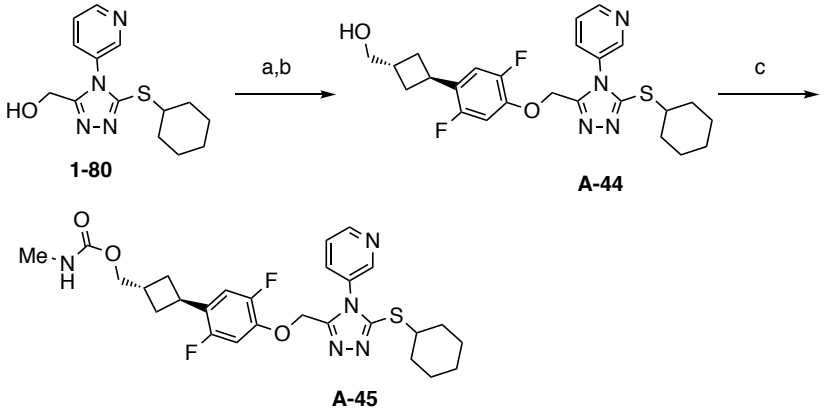
Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (b) 3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol, Cs_2CO_3, DMF, rt, 18 h, 74%; (c) methylamine hydrochloride (A-40a), Et_3N, CDI, CH_2Cl_2, rt, 22 h, 95% or 1-methylpiperidin-4-amine, (A-40b), CDI, CH_2Cl_2, rt, 7 h, 92%.

Scheme 61. Synthesis of A-38



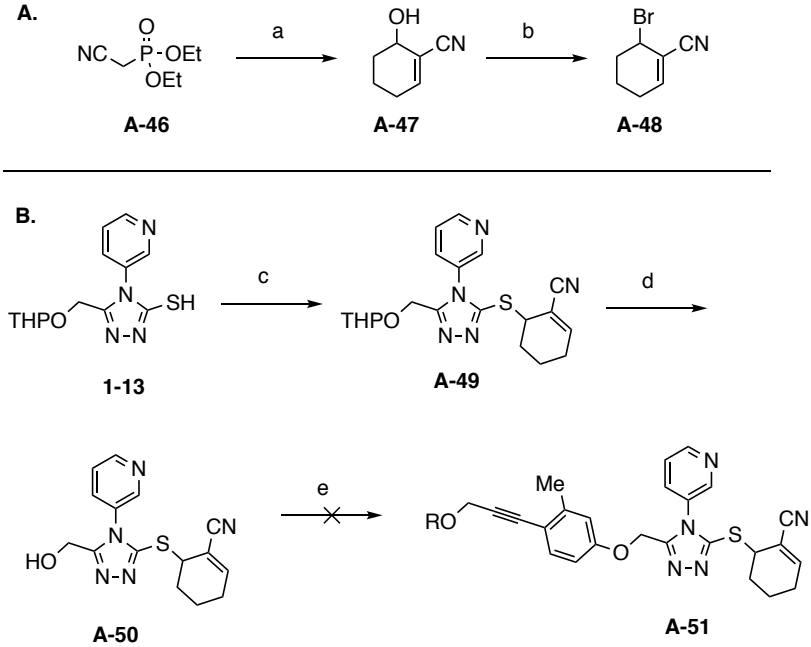
Reagents and conditions: (a) DPPA, DBU, THF, 0 ^oC – rt, 24 h, 70%; (b) PPh_3, THF, H_2O, rt, 22 h, 67%; (c) propionic acid, EDC, HOBt, DIPEA, DMF, rt, 18 h, 53%.

Scheme 62. Synthesis of A-45



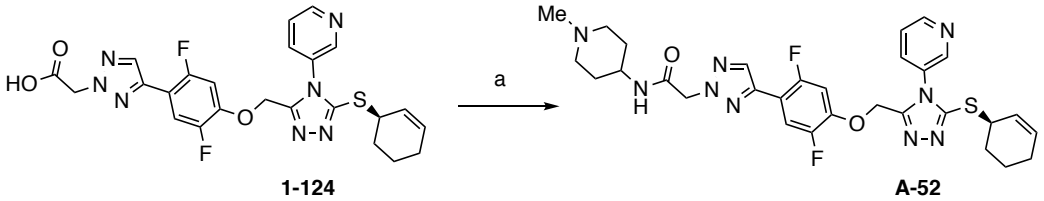
Reagents and conditions: (a) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; (b) 2,5-difluoro-4-((1*r*,3*r*)-3-(hydroxymethyl)cyclobutyl)phenol, Cs_2CO_3, DMF, rt, 14 h, 64%; (c) methylamine hydrochloride, Et_3N, CDI, CH_2Cl_2, rt, 16 h, 96%.

Scheme 63. Synthetic efforts toward A-51



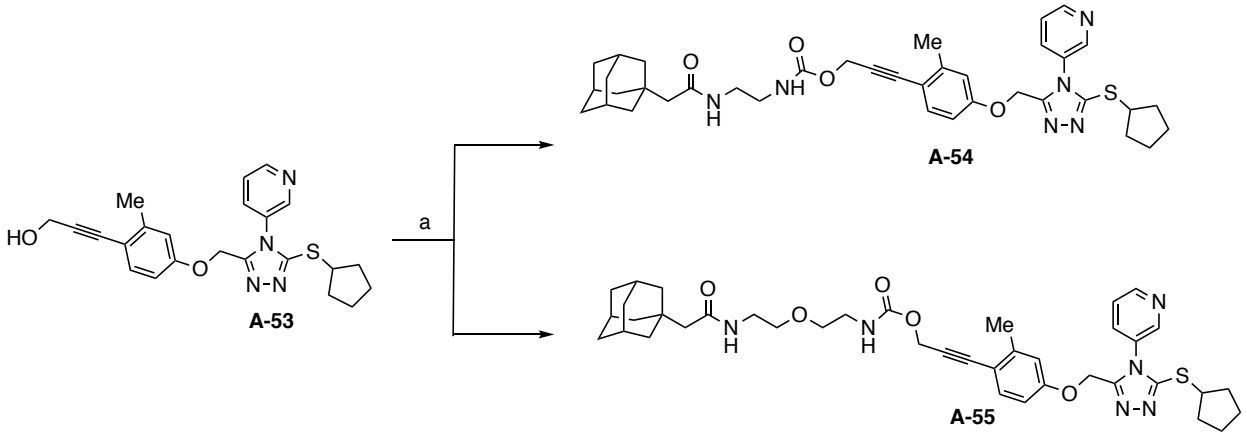
Reagents and conditions: **A**. (a) glutaraldehyde, K_2CO_3, H_2O, rt, 0.5 h, 70%; (b) PPh_3, CBr_4, Et_2O, rt, 24 h, 46%; **B**. (c) **A-48**, Cs_2CO_3, DMF, 80 ^oC, 2.5 h, 59%; (d) *p*-TsOH, MeOH, rt, 24 h, 89%; (e) MsCl, DIPEA, CH_2Cl_2, 0 ^oC, 3 h; Ar-OH, Cs_2CO_3, DMF, rt or Ar-OH, PPh_3, DIAD, THF, 0 ^oC-rt.

Scheme 64. Synthesis of A-52



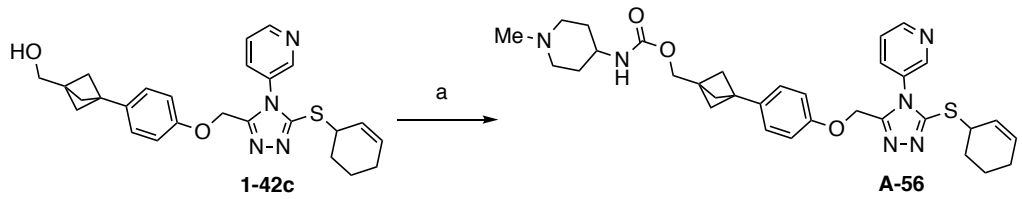
Reagents and conditions: (a) 1-methylpiperidin-4-amine, T3P, Et_3N, DMF, rt, 17 h, 43%.

Scheme 65. Synthesis of A-54 and A-55



Reagents and conditions: (a) **1-136** (A-53), CDI, CH_2Cl_2, rt, 24 h, 90% or **1-140** (A-54), CDI, CH_2Cl_2, rt, 24 h; 70%.

Scheme 66. Synthesis of A-56



Reagents and conditions: (a) 1-methylpiperidin-4-amine, CDI, CH_2Cl_2, rt, 24 h, 48%

4.0 Experimental Section

4.1 General

4.1.1 Molecular Modeling and *In-Silico* Docking

Molecular modeling and *in-silico* receptor-ligand docking studies of $p75^{NTR}$ inhibitors to $p75^{NTR}$ was performed using the Glide software program (Schrödinger, LLC). $p75^{NTR}$ structural coordinates were obtained from the X-ray co-crystal structure of the NGF-$p75^{NTR}$ complex (PDB: 1SG1). The NGF co-structure was deleted and the $p75^{NTR}$ structure was preprocessed and optimized using an Optimized Potentials for Liquid Simulations 3 (OPLS3) molecular mechanical force field prior to docking. $p75^{NTR}$ inhibitors were also prepared using an OPLS3 molecular mechanical force field prior to docking. Inhibitor ionization states were determined using Epik (pH range between 2-7) and specific chiralities were conserved. The inhibitor binding site was defined by a rectangular docking grid confined to site 1 – NGF ^{32}KGKE^{35} recognition site residues. Inhibitor binding was also constrained to interact with $Asp75^{p75NTR}$. The search grid was expanded to 20 Å from Asp75. Glide standard (SP) and extra-precision (XP) modes were employed for docking and the maximum number of confirmations per inhibitor was set to 20. The pose that demonstrated the most favorable binding to Asp75 was reported.

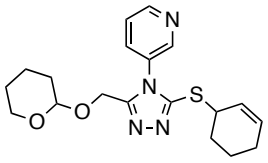
4.1.2 Chemistry

Moisture and air-sensitive reactions were performed under nitrogen or argon atmosphere and glassware used for these reactions was flamed dried and cooled under nitrogen or argon prior to use. THF and Et_2O were distilled from sodium/benzophenone and CH_2Cl_2 were distilled from CaH_2. 1,4-Dioxane was purchased from Acros (Sure/Seal bottle) and used as received. Et_3N was distilled from CaH_2 and stored over KOH. Toluene was purified by passage through an activated alumina filtration system. Melting points were determined using a Mel-Temp II instrument and are not corrected. Infrared spectra were determined using a Smiths Detection IdentifyIR FT-IR spectrometer. High-resolution mass spectra were obtained on a Micromass UK Limited, Q-TOF Ultima API, Thermo Scientific Exactive Orbitrap LC-MS. ^1H and ^{13}C NMR spectra were obtained on Bruker Advance 300 MHz, 400 MHz, or 500 MHz instruments. Chemical shifts (δ) were reported in parts per million with the residual solvent peak used as an internal standard, δ ^1H (Solvent): 7.26 ($CDCl_3$); 2.50 ($(CD_3)_2SO$), 3.31 (CD_3OD); and are tabulated as follows: chemical shift, multiplicity (s = singlet, br. s = broad singlet, d = doublet, br. d = broad doublet, t = triplet, app. t = apparent triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). ^{13}C NMR spectra were obtained at 75 MHz, 100 MHz, or 125 MHz using a proton-decoupled pulse sequence and are tabulated by observed peak. Thin-layer chromatography was performed using pre-coated silica gel 60 F_{254} plates (EMD, 250 μm thickness) and visualization was accomplished with a 254 nm UV light or by staining with a $KMnO_4$ solution (1.5 g of $KMnO_4$ and 1.5 g of K_2CO_3 in 100 mL of a 0.1% NaOH solution). Chromatography on SiO_2 (Silicycle, Silia-P Flash Silica Gel or SiliaFlash® P60, 40-63 μm) was used to purify crude reaction mixtures. Final products were of >95% purity as analyzed by reverse phase HPLC

(Alltech Prevail C-18, 100 × 4.6 mm, 1 mL/min, CH_3CN, H_2O and 0.1% TFA) with UV (210, 220 and 254 nm), ELS (nebulizer 45 ^oC, evaporator 45 ^oC, N_2 flow 1.25 SLM), and S7 MS detection using a Thermo Scientific Exactive Orbitrap LC-MS (ESI positive). All other materials were obtained from commercial sources and used as received.

4.2 Procedures

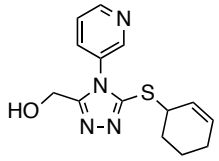
4.2.1 Chapter 1.0 Procedures



1-14

4-(Pyridin-3-yl)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-4*H*-1,2,4-triazole-3-thiol (**1-14**). 3-Bromocyclohexene (1.03 mL, 8.04 mmol) was added to a high pressure reaction vial containing a mixture of 4-(pyridin-3-yl)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-4*H*-1,2,4-triazole-3-thiol **1-13** (1.00 g, 3.42 mmol), cesium carbonate (1.39 g, 4.28 mmol) in dimethylformamide (7 mL). The reaction vial was sealed and heated to 80 ^oC for 2.5 h. The reaction mixture was cooled to room temperature, diluted with a saturated aqueous solution of sodium bicarbonate (25 mL), and extracted with ethyl acetate (50 mL, three times). The combined organic solutions were washed with water (50 mL), brine (50 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (ethyl acetate) to provide **1-14** (1.10 g, 2.96 mmol, 87%) as an off-white solid: **Rf** (ethyl acetate) 0.28;

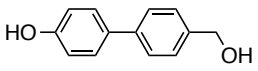
Mp (methylene chloride) 122-124 °C; **IR** (neat) 2934.1, 1575.0, 1481.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.65 (br. d, 1 H, *J* = 2.5 Hz), 7.73-7.71 (m, 1 H), 7.49 (ddd, 1 H, *J* = 8.1, 4.8, 0.7 Hz), 5.87 (dtd, 1 H, *J* = 9.8, 3.8, 1.5 Hz), 5.76-5.73 (m, 1 H), 4.68 (dd, 1 H, *J* = 12.6, 5.0 Hz), 4.57 (app. q, 1 H, *J* = 2.9 Hz), 4.55-5.53 (m, 1 H), 4.50 (dd, 1 H, *J* = 12.6, 4.8 Hz), 3.47-3.39 (m, 2 H), 2.11-1.96 (m, 4 H), 1.77-1.39 (m, 9 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 152.8, 152.7, 150.9, 148.4, 135.0, 132.2, 130.4, 125.5, 123.9, 98.2, 98.2, 61.6, 58.8, 44.0, 30.0, 29.2, 25.1, 24.8, 19.1, 18.7; **LC-MS** (ES) *m/z* calcd for $C_{19}H_{25}O_2N_4S$ $[M+H]^+$ 373.1693, found 373.1691.



1-15

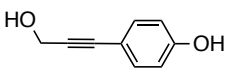
3-(3-(Cyclohex-2-en-1-ylthio)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-4*H*-1,2,4-triazol-4-yl)pyridine (**1-15**). A solution of **1-14** in methanol (18 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.258 g, 1.36 mmol) at room temperature and stirred for 24 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (10 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **1-15** (0.765 g, 2.65 mmol, 98%) as an off-white solid: **Rf** (EtOAc; neutral alumina) 0.14; **Mp** (methanol) 150-152 °C; **IR** (neat) 3152.2, 2921.7, 2856.7, 1482.9, 1425.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.80 (s, 1 H), 8.71 (s, 1 H), 7.88 (ddd, 1 H, *J* = 8.1, 2.1, 1.3 Hz), 7.56-7.53 (m, 1H), 5.89 (dtd, 1 H, *J* = 9.7, 3.9, 1.4 Hz), 5.76-5.72 (m, 1H), 4.66 (s, 2 H), 4.52-

4.51 (m, 1 H), 2.11-1.95 (m, 4 H) 1.79-1.62 (m, 2 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 150.9, 147.9, 135.1, 132.3, 130.1, 125.4, 124.2, 54.2, 44.1, 29.2, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{17}ON_4S$ $[M+H]^+$ 289.1118, found 289.1116.



1-17

4'-(Hydroxymethyl)-[1,1'-biphenyl]-4-ol (**1-17**). Potassium carbonate (1.26 g, 9.09 mmol) and 10% palladium on carbon (0.0484 g, 0.0455 mmol) was sequentially added to a high pressure reaction vial containing a mixture of 4-hydroxymethylphenylboronic acid (0.345 g, 2.27 mmol) and 4-iodophenol **1-16** (0.500 g, 2.27 mmol) in 11 mL of water. The reaction vial was sealed and heated at 120 °C for 1.5 h The reaction mixture was cooled to room temperature, neutralized with a 1M aqueous solution of potassium hydrogen sulfate, and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were passed through a plug of silica, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-17** (0.362 g, 1.81 mmol, 79%) as an-off white solid: **Rf** (50% ethyl acetate in hexane) 0.57; **^{1}H NMR** ($(CD_3)_2SO$, 400 MHz) δ 9.51 (s, 1H), 7.49 (dd, *J* = 21.5, 8.4 Hz, 4H), 7.34 (d, *J* = 8.1 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 5.17 (t, *J* = 5.7 Hz, 1H), 4.50 (d, *J* = 5.7 Hz, 2H).



1-18

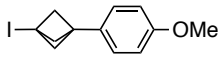
4-(3-Hydroxyprop-1-yn-1-yl)phenol (**1-18**). Propargyl alcohol (0.315 mL, 545 mmol) was added to a mixture of 4-iodophenol **1-16** (1.00 g, 4.55 mmol), bis(triphenylphosphine)palladium chloride (0.159 g, 0.227 mmol), copper iodide (0.0866 g,

0.455 mmol) in anhydrous, degassed triethylamine (2.8 mL) and tetrahydrofuran (2.8 mL) under an atmosphere of argon. The reaction mixture was stirred for 17 h, diluted with water (10 mL), and extracted methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (50-60 % ethyl acetate in hexane; dry loaded) to provide **1-18** (0.491 g, 3.32 mmol, 73%) as a brown/tan solid: **Rf** (50% ethyl acetate in hexane) 0.50; **^{1}H NMR** ($(CD_3)_2SO$, 300 MHz) δ 9.80 (s, 1 H), 7.23 (d, *J* = 8.6 Hz, 2 H), 6.74 (d, *J* = 8.6 Hz, 2 H), 5.24-5.20 (m, 1 H), 4.25 (d, *J* = 4.7 Hz, 2 H); Characterization: NB-00789.028.

1-20

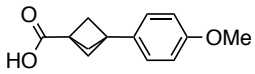
Propellane (**1-20**). A three-neck 1 L round bottom flask was equipped with a graduated addition funnel fitted with a jacket, inert gas valve, and an internal thermometer. The reaction flask was charged with 1,1-dibromo-2,2-bis(chloromethyl)cyclopropane **1-19** (50.0 g, 168 mmol) in anhydrous diethyl ether (105 mL) under an atmosphere of nitrogen and cooled to -45 ^{o}C (dry ice dissolved in isopropanol). A 1.9 M solution of phenyl lithium in n-butyl ether (177 mL, 337 mmol) was slowly cannulated to the graduated addition funnel. The 1.9 M solution of phenyl lithium in n-butyl ether was cooled to -50 ^{o}C (dry ice in acetone) and then slowly added dropwise to the reaction mixture over a span of 2 h under an atmosphere of nitrogen. The reaction mixture was warmed to 0 ^{o}C and stirred for an additional 2 h. The reaction mixture was purified using vacuum distillation (vacuum aspirator, -700 mmHg; water bath, 30 ^{o}C) with a collection flask cooled to -78 ^{o}C. The concentration of (**1-20**) in diethyl ether was determined by quantitative H NMR. A sample of (**1-20**) (200 uL) in diethyl ether was diluted with

dichloroethane (50 uL, 0.63 mmol) and $CDCl_3$ (ca. 0.5 mL). The ratio of dichloroethane:propellane based on H NMR peak integration indicated a 1.68 M solution of (**1-20**) in diethyl ether (10.5 g, 159 mmol, 95%) as a colorless solution: **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.94 (s, 6 H).



1-21

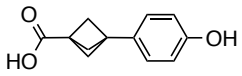
1-Iodo-3-(4-methoxyphenyl)bicyclo[1.1.1]pentane (**1-21**). A quartz vessel was charged with a mixture of 4-iodoanisole (42.5 g, 182 mmol) and **1-20** (4.00 g, 36.0 mL, 1.68 M in diethyl ether solution) in anhydrous diethyl ether (105 mL) and pentane (420 mL). The reaction mixture was irradiated at 254 nm using a 450-W Mercury lamp for 6.5 h and then concentrated under reduced pressure, and purified by chromatography (2.5% ethyl acetate in hexane; dry loaded, absorbed onto Celite; silica pre-washed with 1% Et_3N in hexane) to provide crude **1-21** (1.03 g, 1.86 mmol, 3%, ~60% purity by ^{1}H NMR) as a green solid. This material was carried forward without further purification: **Rf** (10% ethyl acetate in hexane) 0.43. This experimental procedure was redacted from NB-00789.007.



1-22

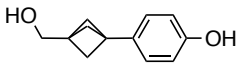
3-(4-Methoxyphenyl)bicyclo[1.1.1]pentane-1-carboxylic acid (**1-22**). A 1.7 M solution of tert-butyllithium in pentane (2.49 mL, 4.16 mmol) was added dropwise to a solution of crude **1-21** (0.500 g, 1.67 mmol) in anhydrous diethyl ether (6.7 mL) at -78 ^{o}C under an atmosphere of nitrogen. The reaction mixture was stirred for 0.5 h and then carbon dioxide gas was bubbled

through the solution. The reaction mixture was stirred for 10 minutes and then slowly warmed to room temperature and stirred for an additional 20 minutes. The reaction mixture was extracted with water (5 mL, three times) and the combined aqueous solutions were acidified with a 2 M aqueous solution of hydrogen chloride until a pH of 2 was reached and subsequently saturated with brine. The resulting aqueous mixture was extracted with diethyl ether (25 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Pivalic acid was removed by Kugelrohr distillation (100 ^oC, high vacuum) resulting in **1-22** (0.233 g, 1.07 mmol, 64%) as an off-white solid: **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.16-7.13 (m, 2 H), 6.87-6.84 (m, 2 H), 3.80 (s, 3 H), 2.33 (s, 6 H).



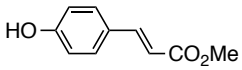
1-23

3-(4-Hydroxyphenyl)bicyclo[1.1.1]pentane-1-carboxylic acid (**1-23**). A 1 M solution of boron tribromide in methylene chloride (0.57 mL, 0.57 mmol) was added dropwise to a solution of **1-22** (0.0500 g, 0.229 mmol) in anhydrous methylene chloride (1.15 mL) at -10 ^oC under an atmosphere of argon. The reaction mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-23** (0.0581 g) as a black solid. This material was used in the subsequent reaction without further purification: **^{1}H NMR** (CD_3OD, 400 MHz) δ 7.04 (d, J = 8.5 Hz, 2 H), 6.71 (d, J = 8.5 Hz, 2 H), 2.23 (s, 6 H).



1-24

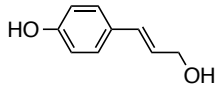
4-(3-(Hydroxymethyl)bicyclo[1.1.1]pentan-1-yl)phenol (**1-24**). Borane tetrahydrofuran (0.57 mL, 0.57 mmol) was added dropwise to solution of **1-23** (0.0468 g, 0.229 mmol) in anhydrous tetrahydrofuran (0.43 mL) at -20 ^{o}C under an atmosphere of nitrogen. The reaction mixture was slowly warmed to room temperature and stirred for 14 h. The reaction mixture was cooled to 0 ^{o}C, diluted with water (2 mL), treated with solid potassium carbonate (0.42 g), and stirred for 15 minutes. The resulting mixture was extracted with ethyl acetate (5 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (30% ethyl acetate in hexane, dry loaded) to provide **1-24** (0.0237 g, 0.124 mmol, 54%, two steps) as a white solid: **Rf** (ethyl acetate) = 0.65; **Mp** (methanol, decomposition) > 102 ^{o}C; **IR** (neat) 3420.7, 3137.7, 2972.9, 2908.3, 2868.8, 1610.9, 1521.5 cm^{-1}; **^{1}H NMR** (CD_3OD, 500 MHz) δ δ 7.02 (d, J = 8.5 Hz, 2 H), 6.69 (d, J = 8.5 Hz, 2 H), 3.58 (s, 2 H), 1.89 (s, 6 H); **^{13}C NMR** (CD_3OD, 100 MHz) δ 155.6, 132.3, 126.6, 114.4, 62.0, 50.3, 41.2, 38.4; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{15}O_2$ $[M+H]^+$ 191.1067, found 191.1066.



1-26

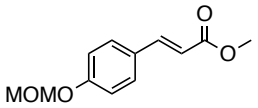
Methyl (*E*)-3-(4-hydroxyphenyl)acrylate (**1-26**) Three drops of concentrated sulfuric acid was added to a solution of (*E*)-3-(4-hydroxyphenyl)acrylic acid **1-25** (1.50 g, 9.14 mmol) in methanol (18 mL) and stirred at reflux for 24 h. The reaction mixture was concentrated under reduced pressure and resuspended in a saturated aqueous solution of sodium bicarbonate (15

mL). The aqueous solution was extract with ethyl acetate (25 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-26** (1.53 g, 8.59 mmol, 94%) as an off-white solid: **Rf** (20% ethyl acetate in hexane) 0.23; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.64 (d, *J* = 16.0 Hz, 1H), 7.44-7.41 (m, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.30 (d, *J* = 16.0 Hz, 1H), 5.70 (s, 1H), 3.80 (s, 3H).



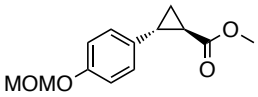
1-27

(*E*)-4-(3-hydroxyprop-1-en-1-yl)phenol (**1-27**). A 1M solution of lithium aluminum hydride in ether (4.21 mL, 4.21 mmol) was slowly added dropwise to a solution of **1-26** (0.500 g, 2.81 mmol) in anhydrous THF (11.2 mL) at 0 ^oC under an atmosphere of nitrogen and stirred for 2 h. The reaction mixture was slowly quenched with acetone (5 mL) and diluted with a 0.2 M aqueous solution of hydrogen chloride (20 mL). The resulting mixture was extracted with ethyl acetate (25 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (35% ethyl acetate in hexane) to provide **1-27** (0.321 g, 2.14 mmol, 76%) as white solid: **Rf** (50% ethyl acetate in hexane) 0.59; **^{1}H NMR** ($(CD_3)_2SO$, 300 MHz) δ 9.42 (s, 1H), 7.22 (d, *J* = 8.6 Hz, 2H), 6.71 (d, *J* = 8.6 Hz, 2H), 6.41 (d, *J* = 16.0 Hz, 1H), 6.12 (dt, *J* = 15.9, 5.4 Hz, 1H), 4.74 (t, *J* = 5.5 Hz, 1H), 4.06 (td, *J* = 5.4, 1.5 Hz, 2H).



1-28

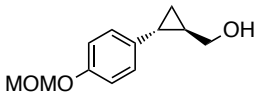
Methyl (*E*)-3-(4-(methoxymethoxy)phenyl)acrylate (**1-28**). Chloro(methoxy)methane (0.78 mL, 10 mmol) was added to a mixture of **1-26** (1.53 g, 8.59 mmol), N,N-diisopropylethylamine (2.24 mL, 12.9 mmol) in anhdrous methylene chloride (43 mL) at room temperature under an atmosphere of nitrogen and stirred for 18 h. The reaction mixture was washed with water (25 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-28** (1.71 g, 7.69 mmol, 90%) as a light-brown oil: **Rf** (20% ethyl acetate in hexane) 0.37; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.65 (d, J = 16.0 Hz, 1H), 7.48-7.46 (m, 2H), 7.06-7.03 (m, 2H), 6.33 (d, J = 16.0 Hz, 1H), 5.20 (s, 2H), 3.80 (s, 3H), 3.48 (s, 3H).



1-29

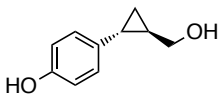
Methyl 2-(4-(methoxymethoxy)phenyl)cyclopropane-1-carboxylate (**1-29**). Sodium hydride (0.410 g, 15.4 mmol) was added to a solution of trimethylsulfoxonium iodide (3.39 g, 15.4 mmol) in anhydrous DMSO (40 mL) at room temperature under nitrogen and stirred for 0.5 h. A solution of **1-28** (0.250 g, 1.12 mmol) in anhydrous DMSO (10 mL) was added dropwise and the reaction mixture was stirred for 11.5 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (100 mL) and then diluted with ethyl acetate (100 mL). The isolated organic solution was washed with water (50 mL, three times). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (10% ethyl acetate in hexane) to provide **1-29** (0.282 g,

1.19 mmol, 15%) as a colorless oil: **Rf** (20% ethyl acetate in hexane) 0.32; **IR** 3001.4, 2952.6, 2901.1, 2827.1, 1612.2, 1515.3 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.04-7.01 (m, 2H), 6.97-6.94 (m, 2H), 5.15 (s, 2H), 3.71 (s, 3H), 3.46 (s, 3H), 2.52-2.47 (m, 1H), 1.83 (ddd, *J* = 8.4, 5.2, 4.2 Hz, 1H), 1.59-1.54 (m, 1H), 1.27 (ddd, *J* = 8.4, 6.6, 4.5 Hz, 1H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 173.9, 156.0, 133.3, 127.4, 116.4, 94.6, 55.9, 51.8, 25.7, 23.6, 16.7; **LC-MS** (ES) *m/z* calcd for $C_{13}H_{17}O_4$ $[M+H]^+$ 237.1121, found 237.1119.



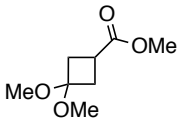
1-30

2-(4-(Methoxymethoxy)phenyl)cyclopropyl)methanol (**1-30**). A 1 M solution of lithium aluminum hydride in diethyl ether (1.79 mL, 1.79 mmol) was added dropwise to a solution of **1-29** (0.282 g, 1.19 mmol) in anhydrous diethyl ether (12 mL) at 0 ^oC under nitrogen and stirred for 1 h. The reaction mixture was slowly quenched with water (5 mL) and further diluted with a saturated aqueous solution of Rochelle salt (10 mL). The mixture was stirred for 0.5 h and the isolated aqueous solution was extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered and concentrated under reduced pressure to provide **1-30** (0.233 g, 1.12 mmol, 94%) as a colorless oil: **Rf** (20% ethyl acetate in hexane) 0.15; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.01 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 2H), 5.14 (s, 2H), 3.61 (quintet, *J* = 9.1 Hz, 2H), 3.47 (s, 3H), 1.79 (dt, *J* = 8.9, 4.6 Hz, 1H), 1.41-1.37 (m, 2H), 0.91-0.86 (m, 2H).



1-31

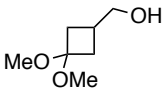
4-2-(Hydroxymethyl)cyclopropyl)phenol (**1-31**). **1-30** (0.233 g, 1.12 mmol) was added to a solution of acetyl chloride (0.48 mL, 6.7 mmol) in methano (11 mL) at 0 ^{o}C. The reaction mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was diluted with water 10 mL and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-31** (0.0622 g, 0.379 mmol, 34%) as a colorless residue: **Rf** (50% ethyl acetate in hexane) 0.2; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.99-6.97 (m, 2H), 6.76 (d, *J* = 8.6 Hz, 2H), 3.66-3.61 (m, 2H), 1.81-1.78 (m, 1H), 1.57 (d, *J* = 3.3 Hz, 1H), 1.41-1.37 (m, 2H), 0.92-0.88 (m, 2H).



1-33

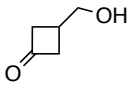
Methyl 3,3-dimethoxycyclobutane-1-carboxylate (**1-33**). *p*-Toluenesulfonic acid monohydrate (1.13 g, 6.57 mmol) was added to a mixture of trimethyl orthoformate (36.0 ml, 329 mmol), 3-oxo-cyclobutanecarboxylic acid **1-32** (7.5 g, 65.7 mmol) in methanol (94 ml) and stirred at reflux for 19 h. The reaction mixture was concentrated under reduced pressure, suspended in diethyl ether (100 mL), washed with a saturated aqueous solution of sodium bicarbonate (50 mL, twice), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-33** (11.4 g, 65.2 mmol, 99%) as a yellow oil: **Rf** (20% ethyl acetate in hexane) 0.36;

^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.69 (s, 3H), 3.15 (d, *J* = 10.3 Hz, 6H), 2.92-2.85 (m, 1H), 2.46-2.36 (m, 4H).



1-34

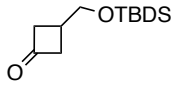
(3,3-Dimethoxycyclobutyl)methanol (**1-34**). A 4M solution of lithium aluminum hydride in diethyl ether (8.18 mL, 32.7 mmol) was added dropwise over a span of 1 h to a solution of **1-33** (11.4 g, 65.4 mmol) in anhydrous diethyl ether (65 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was cooled to 0 ^oC and quenched with water (50 mL) followed by 15% aqueous solution of sodium hydroxide (50 mL). The solid material was filtered through a Celite and the resulting filtrate was extracted with diethyl ether (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-34** (6.60 g, 45.2 mmol, 69%) as a colorless oil. **Rf** (50% ethyl acetate in hexane) 0.15; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 3.66-3.64 (m, 2H), 3.15 (d, *J* = 6.0 Hz, 6H), 2.31-2.25 (m, 3H), 1.91-1.88 (m, 2H).



1-35

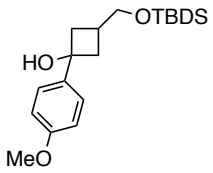
3-(Hydroxymethyl)cyclobutan-1-one (**1-35**). 1M aqueous hydrogen chloride (4.52 mL, 4.52 mmol) was added to a mixture of **1-34** (6.60 g, 45.2 mmol) and water (8.13 mL, 452 mmol) in acetone (45 mL) at room temperature and stirred for 18 h. The reaction mixture was cooled to

0 ^{o}C and quenched with a saturated aqueous solution of sodium bicarbonate (10 mL). The resulting aqueous solution was extracted with ethyl acetate (50 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **1-35** (3.29 g, 32.9 mmol, 73%) as a colorless oil: **Rf** (50% ethyl acetate in hexane) 0.15; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 3.80 (d, *J* = 6.4 Hz, 2H), 3.18-3.09 (m, 2H), 2.92-2.84 (m, 2H), 2.63 (tquintet, *J* = 8.8, 6.1 Hz, 1H), 1.62 (s, 2H).



1-36

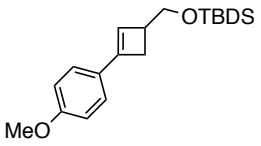
3-(((Tert-butyldiphenylsilyl)oxy)methyl)cyclobutan-1-one (**1-36**). *Tert*-butylphenylchlorosilane (10.3 mL, 39.4 mmol) was added to a solution of **1-35** (3.29 g, 32.9 mmol), imidazole (3.36 g, 49.3 mmol) in anhydrous methylene chloride (132 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 2.5 h. The reaction mixture was washed with water (50 mL), a saturated aqueous solution of sodium bicarbonate (50 mL, twice), brine (50 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The resulting crude material was purified by recrystallization using hexane to provide **1-36** (8.86 g, 26.2 mmol, 80%) as a white solid: **Rf** (20% ethyl acetate in hexane) 0.70; **Mp** (CH_2Cl_2) 82-84 ^{o}C; **IR** (neat) 2931.7, 2857.1, 1778.8, 1092.5cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.66-7.64 (m, 4H), 7.46-7.37 (m, 6H), 3.80 (d, *J* = 5.4 Hz, 2H), 3.09-3.01 (m, 2H), 2.98-2.91 (m, 2H), 2.64-2.57 (m, 1H), 1.06 (s, 9H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 207.6, 135.6, 133.4, 129.8, 127.8, 66.0, 49.3, 26.9, 25.7, 19.3; **LC-MS** (ES) *m/z* calcd for $C_{21}H_{27}O_2Si$ $[M+H]^+$ 339.1775; found 339.1999.



1-37

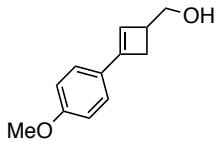
3-(((*Tert*-butyldiphenylsilyl)oxy)methyl)-1-(4-methoxyphenyl)cyclobutan-1-ol (**1-37**). A three-neck round bottom flask was equipped with a stopper, condenser, and septum and charged with metallic magnesium turnings (0.179 g, 7.39 mmol). Iodine (*ca*. 2 mg) was added and the mixture was briefly heated with a Bunsen burner and vigorously stirred under an atmosphere of nitrogen. Once cooled to room temperature, anhydrous THF (12 mL) was added followed by 4-bromoanisole (0.925 g, 7.39 mmol). The mixture spontaneously began to reflux and stirred for 0.5 h to provide freshly prepared Grignard reagent.

The Grignard reagent was added dropwise to a solution of **1-36** in anhydrous THF (12 mL) at -78 ^{o}C. The reaction mixture was warmed to 0 ^{o}C and stirred for 1 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (15 mL) and extracted with ethyl acetate (20 ml, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate) to provide **1-37** (0.854 g, 1.91 mmol, 65%) as colorless oil: **Rf** (20% ethyl acetate in hexane) 0.38; **IR** 3425.0, 3070.6, 2930.7, 2893.3, 1610.7, 1512.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.72-7.68 (m, 4H), 7.47-7.38 (m, 8H), 6.94-6.90 (m, 2H), 3.82 (s, 3H), 3.73 (d, *J* = 4.5 Hz, 2H), 2.73-2.66 (m, 2H), 2.32-2.25 (m, 2H), 1.10 (s, 9H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 158.7, 138.3, 135.7, 133.6, 129.7, 127.7, 126.5, 113.7, 73.2, 67.2, 55.3, 39.8, 27.8, 26.9, 19.3; **LC-MS** (ES) *m/z* calcd for $C_{28}H_{35}O_3Si$ $[M+H]^+$ 447.2350; found 447.2833.



1-38

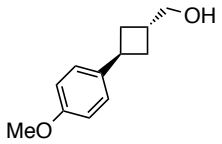
***Tert*-butyl((3-(4-methoxyphenyl)cyclobut-2-en-1-yl)methoxy)diphenylsilane** (**1-38**). *p*-Toluenesulfonic acid monohydrate (0.434 g, 2.28 mmol) was added to a solution of **1-37** (0.850 g, 1.90 mmol) in toluene (7.6 mL) at room temperature and stirred for 24 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (0-5% ethyl acetate in hexane) to provide **1-38** (0.580 g, 1.35 mmol, 71%) as a colorless oil: **Rf** (20% ethyl acetate in hexane) 0.56; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.69-7.67 (m, 4H), 7.42-7.35 (m, 9H), 7.28 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.16 (d, *J* = 1.1 Hz, 1H), 3.81 (s, 3H), 3.77 (ddd, *J* = 6.6, 4.7, 2.1 Hz, 2H), 3.02-2.98 (m, 1H), 2.83 (dd, *J* = 13.0, 4.5 Hz, 1H), 2.38 (dd, *J* = 13.0, 1.7 Hz, 1H), 1.06 (s, 9H).



1-39

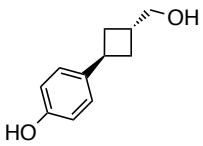
(3-(4-Methoxyphenyl)cyclobut-2-en-1-yl)methanol (**1-39**). A 1M solution of tetrabutylammonium fluoride in THF (2.03 mL, 2.03 mmol) was added dropwise to a solution of **1-38** (0.580 g, 1.35 mmol) in anhydrous THF (13.5 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was slowly warmed to room temperature and stirred for 12 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (15 mL) and

extracted with ethyl acetate (20 mL, three times). The combined organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20-30% ethyl acetate in hexane) to provide **1-39** (0.137 g, 0.721 mmol, 53%) as a white solid: **Rf** (20% ethyl acetate in hexane) 0.11; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.31 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.3 Hz, 2H), 6.18 (s, 1H), 3.81 (s, 3H), 3.76 (d, *J* = 5.9 Hz, 2H), 3.03 (d, *J* = 4.8 Hz, 1H), 2.88 (dd, *J* = 13.1, 4.2 Hz, 1H), 2.48 (d, *J* = 12.9 Hz, 1H).



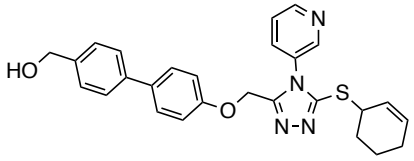
1-40

***trans*-3-(4-Methoxyphenyl)cyclobutyl)methanol** (**1-40**). A mixture of (1,5-cyclooctadiene)(pyridine)(tricyclohexylphosphine)-iridium(I) hexafluorophosphate (0.0174 g, 0.0216 mmol) and **1-39** (0.132 g, 0.720 mmol) in anhydrous methylene chloride (14 mL) was purged with hydrogen. The reaction mixture was stirred at room temperature under an atmosphere of hydrogen for 2 h. The reaction mixture was concentrated under reduced pressure, resuspended in a 50% solution of ethyl acetate in hexane (10 mL), filtered through a pad of silica, and concentrated under reduced pressure to provide **1-40** (0.0993 g, 0.516 mmol, 72%) as a yellow oil: **Rf** (20% ethyl acetate in hexane) 0.15; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.18-7.16 (m, 2H), 6.87-6.85 (m, 2H), 3.81 (s, 1H), 3.80 (s, 4H), 3.58-3.51 (m, 2H).



1-41

4-(*trans*-3-(Hydroxymethyl)cyclobutyl)phenol (**1-41**). A solution of **1-40** (0.0993 g, 0.517 mmol) in ethanethiol (0.5 mL) was added to a solution of anhydrous aluminum chloride (0.344 g, 2.58 mmol) in ethanethiol (1.5 mL) at 0 ^{o}C. The reaction mixture was slowly warmed to room temperature and stirred for 2.5 h. The reaction mixture was poured into a 0.2 M aqueous solution of hydrogen chloride (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-41** (0.0597 g, 0.335 mmol, 65%) as a light yellow solid: **Rf** (50% ethyl acetate in hexane) 0.38; **^{1}H NMR** (CD_3OD, 500 MHz) δ 7.07-7.05 (m, 2H), 6.71 (d, *J* = 8.6 Hz, 2H), 3.70 (dd, *J* = 7.3, 2.8 Hz, 2H), 3.48 (quintet, *J* = 8.5 Hz, 1H), 2.45-2.39 (m, 1H), 2.45-2.39 (m, 1H), 2.19-2.15 (m, 4H).

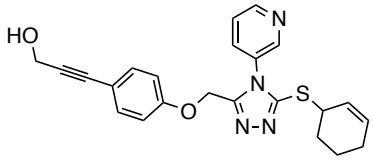


1-42a

(4'-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-[1,1'-biphenyl]-4-yl)methanol (**1-42a**). Methanesulfonyl chloride (0.024 mL, 0.31 mmol) was added dropwise to mixture of **1-15** (0.0750 g, 0.260 mmol) and N,N-diisopropylethylamine (0.068 mL, 0.39 mmol) in anhydrous methylene chloride (2.6 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 3 h. The reaction mixture was diluted with a saturated aqueous

solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

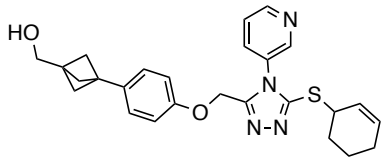
1-17 (0.0625 g, 0.312 mmol) was added to a mixture of crude mesylate and cesium carbonate (0.169 g, 0.520 mmol) in anhydrous DMF (1.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 20 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-42a** (0.0787 g, 0.167 mmol, 64%) as a white solid: **Rf** (ethyl acetate) 0.16; **Mp** (methylene chloride) 158-160 ^oC; **IR** 3339.5, 3029.2, 2925.1, 1604.7, 1583.9, 1498.6 1484.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76 (dd, *J* = 4.8, 1.2 Hz, 1H), 8.76 (dd, *J* = 4.8, 1.2 Hz, 1H), 8.65 (d, *J* = 2.4 Hz, 1H), 8.65 (d, *J* = 2.4 Hz, 1H), 7.71 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.71 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.51-7.45 (m, 4H), 7.51-7.45 (m, 4H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 6.94-6.92 (m, 2H), 6.94-6.92 (m, 2H), 5.89-5.86 (m, 1H), 5.89-5.86 (m, 1H), 5.77-5.73 (m, 1H), 5.77-5.73 (m, 1H), 5.15-5.10 (m, 2H), 5.15-5.10 (m, 2H), 4.73 (d, *J* = 4.8 Hz, 2H), 4.73 (d, *J* = 4.8 Hz, 2H), 4.58 (d, *J* = 2.3 Hz, 1H), 4.58 (d, *J* = 2.3 Hz, 1H), 2.13-2.06 (m, 1H), 2.13-2.06 (m, 1H), 2.06-1.98 (m, 3H), 2.06-1.98 (m, 3H), 1.86-1.84 (m, 1H), 1.86-1.84 (m, 1H), 1.75-1.64 (m, 2H), 1.75-1.64 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 156.7, 153.4, 151.6, 151.1, 148.1, 139.9, 139.6, 134.83, 134.70, 132.3, 130.1, 128.2, 127.4, 126.8, 125.5, 124.0, 115.1, 64.9, 60.1, 44.1, 29.2, 24.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{27}H_{27}O_2N_4S$ $[M+H]^+$ 471.1849, found 471.1664.



1-42b

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-ol (**1-42b**). Methanesulfonyl chloride (0.018 mL, 0.23 mmol) was added dropwise to mixture of **1-15** (0.0600 g, 0.208 mmol), N,N-diisopropylethylamine (0.045 mL, 0.26 mmol) in anhydrous methylene chloride (2 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred at room temperature for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with methylene chloride (15 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-18 (0.0339 g, 0.229 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.136 g, 0.416 mmol) in anhydrous DMF (1 mL) under an atmosphere of nitrogen and stirred at room temperature for 23 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-42b** (0.0578 g, 0.137 mmol, 66%) as a white solid: **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.67 (d, *J* = 8.4 Hz, 1 H), 7.47 (dt, *J* = 6.1, 3.1 Hz, 1 H), 7.31 (d, *J* = 8.7 Hz, 2 H), 6.80 (d, *J* = 8.7 Hz, 2 H), 5.89-5.86 (m, 1 H), 5.76-5.73 (m, 1 H), 5.12-5.06 (m, 2 H), 4.58-4.57 (m, 1 H), 4.47 (s, 2 H), 2.12-2.05 (m, 1 H), 2.03-1.98 (m, 3 H), 1.82-1.76 (m, 2 H), 1.77-1.64 (m, 3 H); **LC-MS** (ES) *m/z* calcd for $C_{23}H_{23}O_2N_4S$ $[M+H]^+$ 419.1536, found 419.1533.

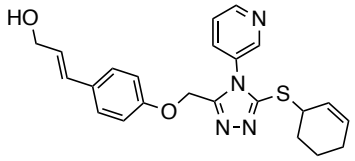


1-42c

(3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)bicyclo[1.1.1]pentan-1-yl)methanol (1-42c). Methanesulfonyl chloride (0.011 mL, 0.15 mmol) was added dropwise to mixture of 1-15 (0.0417 g, 0.145 mmol), N,N-diisopropylethylamine (0.034 mL, 0.19 mmol) in anhydrous methylene chloride (1.3 mL) under an atmosphere of nitrogen at 0 ^{o}C for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-24 (0.0250 g, 0.131 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.0856 g, 0.263 mmol) in anhydrous DMF (0.7 mL) under an atmosphere of nitrogen and stirred at room temperature for 22 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide 1-42c (0.0409 g, 0.0888 mmol, 68%) as a white solid: Rf (ethyl acetate) 0.40; Mp (methanol) 53-55 ^{o}C; IR 3334.4, 3032.3, 2957.5, 2905.9, 2905.9, 2865.7, 1484.1 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.73 (d, *J* = 3.6 Hz, 1 H), 8.61 (s, 1 H), 7.69 (ddd, *J* = 8.1, 1.9, 1.6 Hz, 1 H), 7.45 (dd, *J* = 8.0, 4.8 Hz, 1 H), 7.08 (d, *J* = 8.6 Hz, 2 H), 6.78 (d, *J* = 8.6 Hz, 2 H), 5.88-5.84 (m, 1 H), 5.73 (dt, *J* = 9.9, 2.0 Hz, 1 H), 5.06 (d, *J* = 1.3 Hz, 2 H), 4.55 (d, *J* = 2.3 Hz, 1 H), 3.67 (s, 2 H), 2.08-1.97 (m, 5 H), 1.89 (d, *J* = 3.8 Hz, 6 H), 1.74-1.63 (m, 3 H).; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.9, 153.4, 151.6, 151.0, 148.0, 134.84, 134.8, 132.3, 127.3,

125.5, 124.0, 114.5, 63.1, 60.1, 50.7, 44.1, 41.5, 39.0, 29.2, 24.8, 19.2; LC-MS (ES) *m/z* calcd for $C_{26}H_{29}O_2N_4S$ $[M+H]^+$ 461.2006, found 461.2004.

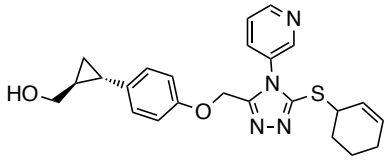


1-42d

(*E*)-3-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-en-1-ol (**1-42d**). Methanesulfonyl chloride (0.015 mL, 0.19 mmol) was added dropwise to mixture of **1-15** (0.0500 g, 0.173 mmol), N,N-diisopropylethylamine (0.045 mL, 0.26 mmol) in anhydrous methylene chloride (1.7 mL) under an atmosphere of nitrogen at 0 ^oC for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-27 (0.0286 g, 0.191 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.113 g, 0.347 mmol) in anhydrous DMF (0.85 mL) under an atmosphere of nitrogen and stirred at room temperature for 24 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-42d** (0.0250 g, 0.0594 mmol, 34%) as an off-white solid: **Rf** (ethyl acetate) 0.41; **Mp** (methylene chloride) ^oC; **IR** 3309.6, 3030.2, 2929.3, 2853.5, 1720.1, 1639.3, 1603.1, 1578.0, 1508.9 cm^{-1}; **^{1}H NMR** (CD_3OD, 600 MHz) δ 8.72 (d, J = 4.4 Hz, 1H), 8.65 (d, J = 1.8 Hz, 1H), 7.97 (d, J = 8.1 Hz, 1H), 7.63 (dd, J = 8.1, 4.9 Hz, 1H), 7.29 (d, J = 8.6

Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.51 (d, *J* = 15.9 Hz, 1H), 6.22 (dt, *J* = 15.9, 5.7 Hz, 1H), 5.90-5.88 (m, 1H), 5.68-5.66 (m, 1H), 5.15 (s, 2H), 4.33 (s, 1H), 4.18 (d, *J* = 5.4 Hz, 2H), 2.05-2.00 (m, 4H), 1.91 (ddt, *J* = 13.7, 6.5, 3.4 Hz, 1H), 1.71 (ddt, *J* = 13.8, 7.0, 3.4 Hz, 1H), 1.66-1.62 (m, 1H); **^{13}C NMR** (CD_3OD, 151 MHz) δ 156.8, 153.0, 152.4, 150.6, 147.7, 135.9, 132.2, 131.1, 130.4, 129.4, 127.28, 127.19, 124.8, 124.5, 114.5, 62.3, 59.7, 44.5, 28.8, 24.4, 18.6; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{25}O_2N_4S$ $[M+H]^+$ 421.1693, found 421.1689.

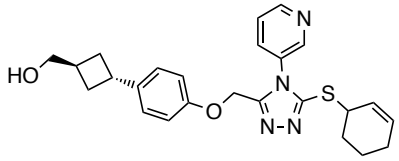


1-42e

2-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)cyclopropyl)methanol (**1-42e**). Methanesulfonyl chloride (0.032 mL, 0.42 mmol) was added dropwise to mixture of **1-15** (0.100 g, 0.327 mmol) and N,N-diisopropylethylamine (0.091 mL, 0.52 mmol) in anhydrous methylene chloride (3.5 mL) under an atmosphere of nitrogen at 0 ^oC and stirred for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-31 (0.0569 g, 0.347 mmol) was added to a mixture of crude mesylate and cesium carbonate (0.226 g, 0.694 mmol) in anhydrous DMF (1.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 19 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried

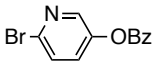
($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-42e** (0.0702 g, 0.162 mmol, 47%) as a white solid: **Rf** (ethyl acetate) 0.15; **Mp** (methylene chloride) 55-57 ^{o}C; **IR** 3344.4, 3029.2, 2932.2, 2862.4, 1484.9, 1445.4, 1393.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.61 (d, *J* = 2.4 Hz, 1H), 7.68 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1H), 7.45 (dd, *J* = 8.1, 4.8 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.76-6.74 (m, 2H), 5.89-5.85 (m, 1H), 5.76-5.73 (m, 1H), 5.05 (d, *J* = 1.4 Hz, 2H), 4.56 (s, 1H), 3.60 (s, 2H), 2.11-1.98 (m, 4H), 1.77-1.65 (m, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 155.4, 153.3, 151.7, 151.0, 148.0, 136.0, 134.8, 132.3, 130.1, 127.1, 125.5, 124.0, 114.8, 66.3, 60.2, 44.1, 29.2, 24.92, 24.81, 20.5, 19.2, 13.4; **LC-MS** (ES) *m/z* calcd for $C_{24}H_{27}O_2N_4S$ $[M+H]^+$ 435.1849, found 435.1854.



1-42f

(*trans*-3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)cyclobutyl)methanol (**1-42f**). Methanesulfonyl chloride (0.026 mL, 0.34 mmol) was added dropwise to mixture of **1-15** (0.0971 g, 0.337 mmol) and N,N-diisopropylethylamine (0.073 mL, 0.42 mmol) in anhydrous methylene chloride (3.3 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (5 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

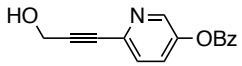
1-41 (0.0500 g, 0.281 mmol) was added to a mixture of crude mesylate and cesium carbonate (0.183 g, 0.561 mmol) in anhydrous DMF (1.6 mL) under an atmosphere of nitrogen and stirred at room temperature for 8.5 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded) to provide **1-42f** (0.0661 g, 0.147 mmol, 53%) as a white solid: **Rf** (ethyl acetate) 0.31; **Mp** (CH_2Cl_2) 98-100 ^oC; **IR** 3385.2, 3045.1, 2926.5, 2858.8, 1706.3, 1642.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.77 (s, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.47 (s, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 5.91-5.85 (m, 1H), 5.77-5.72 (m, 1H), 5.07 (s, 2H), 4.58-4.56 (m, 1H), 3.79 (d, *J* = 7.3 Hz, 2H), 3.57-3.45 (m, 1H), 2.50-2.43 (m, 1H), 2.23-2.15 (m, 4H), 2.10-1.99 (m, 4H), 1.75-1.55 (m, 4H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 155.4, 151.0, 148.0, 139.8, 135.6, 134.8, 132.3, 129.7, 127.67, 127.50, 126.5, 125.5, 114.6, 66.6, 60.1, 44.1, 36.0, 32.9, 31.1, 29.2, 24.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{25}H_{29}O_2N_4O_2S$ $[M+H]^+$ 449.2006, found 449.2007.



1-44

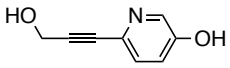
6-Bromopyridin-3-yl benzoate (**1-44**). Benzoic anhydride (3.90 g, 17.2 mmol) was added to a mixture of 6-bromopyridin-3-ol **1-43** (2.00 g, 11.5 mmol) and cesium carbonate (5.62 g, 17.2 mmol) in anhydrous DMF (57 mL) under an atmosphere of nitrogen. The reaction was stirred for 14 h, diluted with water (500 mL), and extracted with ethyl acetate (250 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-44** (3.15 g, 11.3 mmol, 99%) as a white solid: **Rf** (20% ethyl

acetate in hexane) = 0.46; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.35 (d, *J* = 2.7 Hz, 1 H), 8.19 (app. dd, *J* = 8.0, 0.9 Hz, 2 H), 7.68 (tt, *J* = 7.4, 1.5 Hz, 1 H), 7.58-7.49 (m, 4 H).



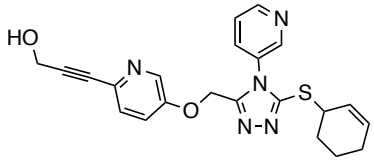
1-45

6-(3-Hydroxyprop-1-yn-1-yl)pyridin-3-yl benzoate (**1-45**). **1-44** (1.00 g, 3.59 mmol), propargyl alcohol (0.23 mL, 3.9 mmol), copper (I) Iodide (0.0685 g, 0.359 mmol), bis(triphenylphosphine)palladium(II) chloride (0.126 g, 0.179 mmol) was added to a reaction vial. The reaction vial was degassed under vacuum and refilled with argon; this process was conducted three times. Degassed, anhydrous tetrahydrofuran (4.5 mL) and triethylamine (2.00 mL, 14.4 mmol) was added to the reaction vial and the reaction vial was purged with argon for 5 minutes. The reaction vial was sealed and stirred at 60 ^{o}C for 13 h. The reaction mixture was diluted with methylene chloride (25 mL) and sequentially washed with a saturated aqueous solution of ammonium chloride (25 mL) and water (25 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (60% ethyl acetate in hexane, dry loaded) to provide **1-45** (0.300 g, 1.18 mmol, 33%) as a yellow solid: **Rf** (ethyl acetate) 0.55; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.17 (d, *J* = 2.7 Hz, 1 H), 8.03 (dd, *J* = 8.4, 1.3 Hz, 2 H), 7.58-7.54 (m, 1 H), 7.43 (t, *J* = 7.7 Hz, 2 H), 7.33 (d, *J* = 8.5 Hz, 1 H), 7.20 (dd, *J* = 8.6, 2.8 Hz, 1 H), 5.08 (s, 2 H).



1-46

6-(3-Hydroxyprop-1-yn-1-yl)pyridin-3-ol (**1-46**). A 2.2 M aqueous solution of sodium hydroxide (3 mL) was added dropwise to a mixture of **1-45** (0.300 g, 1.18 mmol) in tetrahydrofuran (6 mL) and methanol (6 mL) at room temperature. The reaction mixture was stirred for 22 h and then slowly quenched with a 1 M aqueous solution of potassium hydrogen sulfate at 0 ^{o}C until a pH 7 was reached. The resulting aqueous solution was extracted with ethyl acetate (25 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-45** (0.131 g, 0.880 mmol, 74 %) as a white solid: **Rf** (ethyl acetate) 0.40; **^{1}H NMR** ((CD_3)$_2$SO, 500 MHz) δ 10.30 (s, 1 H), 8.08 (d, *J* = 2.8 Hz, 1 H), 7.32 (d, *J* = 8.5 Hz, 1 H), 7.15-7.12 (m, 1 H), 5.33 (t, *J* = 5.7 Hz, 1 H), 4.27 (d, *J* = 5.3 Hz, 2 H).

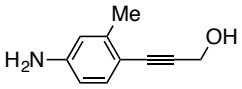


1-47

3-(5-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)pyridin-2-yl)prop-2-yn-1-ol (**1-47**). Methanesulfonyl chloride (0.015 mL, 0.19 mmol) was added dropwise to mixture of **1-15** (0.0500 g, 0.173 mmol), N,N-diisopropylethylamine (0.0378 mL, 0.22 mmol) in anhydrous methylene chloride (1.73 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred at room temperature for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and

extracted with methylene chloride (15 mL, three times). The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

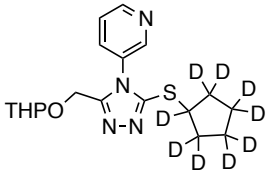
1-46 (0.0284 g, 0.191 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.113 g, 0.347 mmol) in anhydrous DMF (0.85 mL) under an atmosphere of nitrogen and stirred at room temperature for 16 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by reverse-phase chromatography on C18 silica (55% acetonitrile in water, dry loaded) to provide **1-47** (0.0498 g, 0.119 mmol, 68%) as a yellow solid: **Rf** (1% methanol in ethyl acetate) 0.07; **Mp** (methylene chloride) 151-153 ^oC; **IR** 3263.3, 2926.9, 1566.1, 1484.7 cm^{-1}; **^{1}H NMR** (CD_3OD, 300 MHz) δ 8.75-8.73 (m, 1 H), 8.69-8.68 (m, 1 H), 8.11 (d, J = 1.6 Hz, 1 H), 8.02-7.98 (m, 1 H), 7.67-7.63 (m, 1 H), 7.42 (t, J = 2.3 Hz, 1 H), 5.93-5.87 (m, 1 H), 5.71-5.65 (m, 1 H), 5.28 (s, 2 H), 4.39 (s, 2 H), 4.35 (s, 1 H), 2.06-2.00 (m, 3 H), 1.96-1.87 (m, 2 H), 1.75-1.62 (m, 3 H), 0.97-0.81 (m, 1 H); **^{13}C NMR** (CD_3OD, 125 MHz) δ 153.5, 153.3, 151.6, 150.9, 147.7, 137.8, 135.9, 135.7, 132.3, 127.9, 124.8, 124.6, 122.1, 87.4, 82.3, 60.1, 49.6, 44.5, 28.8, 24.4, 18.6; **LC-MS** (ES) *m/z* calcd for $C_{22}H_{22}O_2N_5S$ $[M+H]^+$ 420.1489, found 420.1487.



1-49

3-(4-Amino-2-methylphenyl)prop-2-yn-1-ol (**1-49**). 4-Iodo-3-methylaniline **1-48** (2.00 g, 8.58 mmol), propargyl alcohol (0.99 mL, 17 mmol), copper (I) Iodide (0.163 g, 0.858 mmol), bis(triphenylphosphine)palladium(II) chloride (0.301 g, 0.429 mmol) was added to a reaction

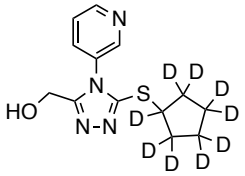
vial. The reaction vial was degassed under vacuum and refilled with argon; this process was conducted three times. The premixed degassed, anhydrous THF (9 mL) and anhydrous triethylamine (9 mL) was added to reaction vial. The reaction vial was purged with argon for 5 minutes. The reaction vial was sealed and stirred at room temperature for 24 h. The reaction mixture was diluted with a saturated aqueous solution of ammonium chloride (25 mL) and extracted with methylene chloride (25 mL, three times). The organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure, and purified by chromatography on SiO_2 (50% ethyl acetate in hexane, dry loaded) to provide **1-49** (1.08 g, 6.69 mmol, 78%) as a brown residue: **Rf** (50% ethyl acetate in hexane) 0.46; **IR** (neat) 3340.8, 3221.0, 2917.9, 2857.2, 2216.1, 1607.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.20 (d, 1 H, *J* = 8.2 Hz), 6.50 (d, 1 H, *J* = 2.0 Hz), 6.44 (dd, 1 H, *J* = 8.2, 2.3 Hz), 4.51 (d, 2 H, *J* = 6.1 Hz), 3.73 (s, 2 H), 3.49 (d, 1 H, *J* = 5.5 Hz), 2.34 (s, 3 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 146.7, 141.8, 133.5, 115.8, 112.3, 112.0, 88.9, 85.2, 51.8, 20.7; **LC-MS** (ES) *m/z* calcd for $C_{10}H_{12}ON$ $[M+H]^+$ 162.0913, found 162.0913.



1-50

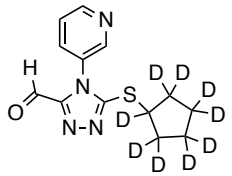
3-(3-((Cyclopentyl-d9)thio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**1-50**). Bromocyclopentane-d9 (0.23 mL, 2.1 mmol) was added to a high pressure reaction vial containing a mixture of **1-13** (0.500 g, 1.71 mmol), cesium carbonate (0.697 g, 2.14 mmol) in DMF (3.5 mL). The reaction vial was sealed and stirred at room temperature for 12 h. The reaction mixture was cooled to room temperature, diluted with a saturated solution of aqueous sodium bicarbonate (15 mL), and extracted with ethyl acetate (25

mL, three times). The combined organic solutions were washed with water (25 mL), brine (25 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (60% ethyl acetate in hexane) to provide **1-50** (0.242 g, 0.654 mmol, 38%) as an off-white solid: **Rf** (ethyl acetate) 0.52; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.74 (dd, 1 H, *J* = 4.8, 1.4 Hz), 8.70 (d, *J* = 2.2 Hz, 1H), 7.85 (ddd, 1 H, *J* = 8.1, 2.3, 1.6 Hz), 7.50 (dd, 1 H, *J* = 8.2, 4.7 Hz), 4.53 (t, 2 H, *J* = 10.1 Hz), 4.33 (d, 1 H, *J* = 12.6 Hz), 3.42-3.38 (m, 2 H), 1.63-1.39 (m, 6 H).



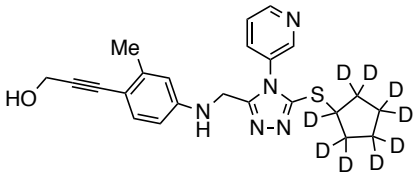
1-51

(5-((Cyclopentyl-d9)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-51**). *p*-Toluenesulfonic acid monohydrate (0.0623 g, 0.327 mmol) was added to a solution of **1-50** (0.242 g, 0.655 mmol) in methanol (6.5 mL). The reaction mixture was stirred at 40 ^{o}C for 3 h. The reaction mixture was quenched with a saturated solution of aqueous sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **1-51** (0.187 g, 0.422 mmol, 64%) as an off-white solid: **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.70 (d, 1 H, *J* = 4.2 Hz), 8.67 (d, 1 H, *J* = 1.7 Hz), 7.89 (ddd, 1 H, *J* = 8.1, 2.5, 1.5 Hz), 7.51 (dd, 1 H, *J* = 8.1, 4.9 Hz), 4.54 (d, 2 H, *J* = 1.5 Hz), 2.74 (d, 1 H, *J* = 4.8 Hz)..



1-52

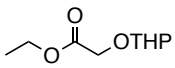
3-Methyl-4-((phenylsulfonyl)ethynyl)phenol5-((cyclopentyl-d9)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazole-3-carbaldehyde (**1-52**). Dess-Martin Periodinane (0.372 g, 0.876 mmol) was added to a solution of **1-51** (0.200 g, 0.700 mmol) in anhydrous methylene chloride (3.5 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction was diluted with methylene chloride (10 mL) and a saturated aqueous solution of sodium thiosulfate (10 mL). The mixture was stirred for 5 minutes and the isolated organic solution was sequentially washed with sodium bicarbonate (10 mL) and brine (10 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **1-52** (0.158 g, 0.558 mmol, 80%) as an off-white solid: **Rf** (ethyl acetate) 0.47; **IR** 3044.5, 2878.4, 2226,6, 1687.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 9.98 (s, 1 H), 8.79 (dd, 1 H, J = 4.8, 1.4 Hz), 8.55 (d, 1 H, J = 2.2 Hz), 7.63 (ddd, 1 H, J = 8.1, 2.5, 1.6 Hz), 7.52-7.47 (m, 1 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 179.4, 158.3, 151.7, 151.2, 147.3, 134.3, 130.0, 124.0; **LC-MS** (ES) *m/z* calcd for $C_{13}H_8O_2N_4SD_9$ $[M+H_3O]^+$ 302.1631, found 302.1494.



1-53

3-(4-(((5-((Cyclopentyl-d9)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methyl)amino)-2-methylphenyl)prop-2-yn-1-ol (1-53). Acetic acid (0.020 mL, 0.35 mmol)

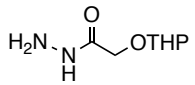
was added to a mixture of 1-52 (0.0500 g, 0.176 mmol), 1-49 (0.0341, 0.212 mmol) in anhydrous THF (0.9 mL) and stirred at room temperature under nitrogen for 19 h. Sodium triacetoxyborohydride (0.0935 g, 0.441 mmol) was added and the reaction mixture was stirred for an additional 24 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (7% methanol in methylene chloride) to provide 1-53 (0.0350 g, 0.0817, 46%) as a brown solid: Rf (ethyl acetate) 0.11; Mp (methylene chloride): 68-70 ^{o}C; IR 3277.8, 2923.2, 2222.2, 1608.0 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.77 (d, 1 H, *J* = 3.9 Hz), 8.56 (d, 1 H, *J* = 1.9 Hz), 7.61-7.59 (m, 1 H), 7.46 (dd, 1 H, *J* = 8.0, 4.8 Hz), 7.14 (d, 1 H, *J* = 8.3 Hz), 6.32 (s, 1H), 6.28 (dd, 1 H, *J* = 8.3, 2.1 Hz), 4.50 (s, 2 H), 4.30 (s, 2 H), 2.28-2.25 (m, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.2, 153.0, 151.2, 147.8, 146.7, 141.8, 134.7, 133.4, 130.1, 124.3, 113.9, 112.2, 110.4, 89.3, 84.9, 51.8, 39.1, 20.9; LC-MS (ES) *m/z* calcd for $C_{23}H_{17}ON_5SD_9$ $[M+H]^+$ 429.2417, found 429.2416.



1-55

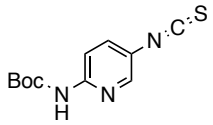
Ethyl 2-((tetrahydro-2*H*-pyran-2-yl)oxy)acetate (**1-55**). 3,4,-Dihydro-2H-pyran (4.39 mL, 48.0 mmol) was added dropwise to a mixture of ethyl glycolate **1-54** (4.55 mL, 48.0 mmol) and *p*-toluenesulfonic acid (0.0914 g, 0.480 mmol) at room temperature and stirred for 48 h. The reaction mixture was diluted with diethyl ether (250 mL), washed with a saturated aqueous solution of sodium bicarbonate (50 mL, two times), and brine (50 mL). The organic layer was then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-55** (8.32 g,

44.2 mmol, 92%) as a light-yellow oil: **Rf** (20% ethyl acetate in hexane) 0.38; **^{1}H NMR** (CD_3Cl, 400 MHz) δ 4.74 (t, *J* = 3.4 Hz, 1H), 4.24-4.18 (m, 4H), 3.86 (ddd, *J* = 11.4, 8.9, 2.9 Hz, 1H), 3.54-3.49 (m, 1H), 1.88-1.80 (m, 1H), 1.75 (dt, *J* = 9.2, 3.7 Hz, 2H), 1.62-1.52 (m, 4H), 1.28 (t, *J* = 7.1 Hz, 3H).



1-56

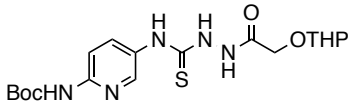
2-((Tetrahydro-2*H*-pyran-2-yl)oxy)acetohydrazide (**1-56**). Hydrazine monohydrate (4.29 mL, 88.4 mmol) was added to a solution of **1-55** (8.32 g, 44.2 mmol) in ethanol (35 mL) and heated to reflux for 21 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting oil was resuspended in water (25 mL) and extracted with methylene chloride (50 mL, three times). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to provide **1-56** (6.65 g, 38.2 mmol, 86%) as a light-yellow oil: **Rf** (ethyl acetate) 0.11; **^{1}H NMR** (CD_3Cl, 500 MHz) δ 4.61 (dd, *J* = 4.4, 2.9 Hz, 1 H), 4.28 (d, *J* = 15.5 Hz, 1 H), 4.09 (d, *J* = 15.5 Hz, 1 H), 3.82 (ddd, *J* = 11.3, 8.0, 3.3 Hz, 1 H), 3.56-3.51 (m, 1 H).



1-58

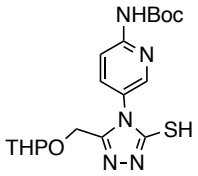
***Tert*-butyl (5-isothiocyanatopyridin-2-yl)carbamate** (**1-58**). Thiophosgene (0.431 mL, 4.78 mmol) was added dropwise to a mixture of **1-57** (1.00 g, 4.78 mmol) and sodium bicarbonate (1.20 g, 14.3 mmol) in chloroform (4.25 mL) and water (0.5 mL) at 0 °C. The

reaction mixture was stirred for 3 h, diluted with water (10 mL), and filtered through a pad of Celite. The pad of Celite was washed with methylene chloride and the isolated aqueous solution was extracted with methylene chloride (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-58** (1.03 g, 4.09 mmol, 86%) as a brown solid: **Rf** (ethyl acetate) 0.83; **^{1}H NMR** (CD_3Cl, 300 MHz) δ 8.33 (s, 1 H), 8.22 (dd, *J* = 2.6, 0.6 Hz, 1 H), 8.01 (d, *J* = 9.0 Hz, 1 H), 7.51 (ddd, *J* = 9.0, 2.6, 0.3 Hz, 1 H), 1.55 (s, 9 H).



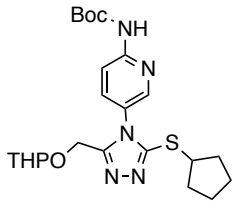
1-59

***Tert*-butyl (5-(2-(2-((tetrahydro-2H-pyran-2-yl)oxy)acetyl)hydrazine-1-carbothioamido)pyridin-2-yl)carbamate** (**1-59**). **1-58** (1.03 g, 4.09 mmol) was added to a high pressure vial containing a solution of **1-56** (0.714 g, 4.09 mmol) in ethanol (5.8 mL). The reaction vial was sealed and stirred at 80 ^oC for 2 h. The reaction mixture was concentrated under reduced pressure to provide crude **1-59** (1.71 g) as a brown solid. This material was carried forward without further purification: **LC-MS** (ES) *m/z* calcd for $C_{18}H_{28}O_5N5S$ $[M+H]^+$ 426.1806, found 426.1805.



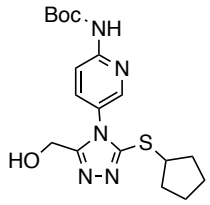
1-60

***Tert*-butyl (5-(3-mercapto-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridin-2-yl)carbamate** (**1-60**). Crude **1-59** (1.20 g, 2.82 mmol) was added to a solution of sodium hydroxide (0.113 g, 2.82 mmol) in water (9.4 mL) and stirred at 50 ^{o}C for 15 h. The reaction mixture was diluted with water (10 mL), acidified to a pH of 6 using a 1M aqueous solution of potassium hydrogen sulfate, and extracted with ethyl acetate (25 mL, six times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by column chromatography on silica (50% ethyl acetate in hexane) to provide **1-60** (0.639 g, 1.568 mmol, 56%) as a yellow solid: **Rf** (50% ethyl acetate in hexane) 0.37; **Mp** (methylene chloride) 198-200 ^{o}C; **IR** 2923.4, 2754.2, 1724.8, 1593.4, 1534.6; **^{1}H NMR** (CD_3Cl, 400 MHz) δ 11.85 (s, 1 H), 8.35 (d, *J* = 2.4 Hz, 1 H), 8.20 (d, *J* = 8.6 Hz, 2 H), 7.79 (dd, *J* = 9.0, 2.5 Hz, 1 H), 4.56-4.53 (m, 2 H), 4.34 (d, *J* = 12.8 Hz, 1 H), 3.56-3.43 (m, 2 H), 1.70-1.62 (m, 2 H), 1.54-1.48 (m, 14 H); **^{13}C NMR** (CD_3Cl, 125 MHz) δ 153.1, 152.2, 149.6, 146.8, 138.3, 124.8, 112.5, 98.3, 81.8, 61.8, 58.7, 29.9, 28.2, 25.0, 18.6; **LC-MS** (ES) *m/z* calcd for $C_{18}H_{26}O_4N_5S$ $[M+H]^+$ 408.1700, found 408.1698.



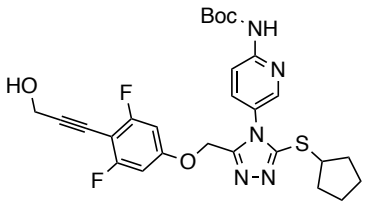
1-61

***Tert*-butyl (5-(3-(cyclopentylthio)-5-(hydroxymethyl)-4H-1,2,4-triazol-4-yl)pyridin-2-yl)carbamate** (**1-61**). Bromocyclopentane (0.20 mL, 0.19 mmol) was added to a mixture of **1-60** (0.639 g, 1.57 mmol) and cesium carbonate (0.295 g, 0.906 mmol) in DMF (1.2 mL) and stirred at room temperature for 22 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethylacetate in hexane) to provide **1-61** (0.541 g, 1.14 mmol, 73%) as a light-yellow solid: **Rf** (ethyl acetate) 0.48; **Mp** (methylene chloride) 190-192 ^{o}C; **IR** 3190.1, 2947.9, 2870.7, 1731.3, 1590.9, 1529.9; **^{1}H NMR** (CD_3Cl, 500 MHz) δ 8.23 (d, *J* = 2.3 Hz, 1 H), 8.14 (d, *J* = 8.9 Hz, 1 H), 7.75 (s, 1 H), 7.66 (dd, *J* = 8.9, 2.5 Hz, 1 H), 4.66 (d, *J* = 12.5 Hz, 1 H), 4.60 (d, *J* = 3.0 Hz, 1 H), 4.47 (d, *J* = 12.5 Hz, 1 H), 4.05-3.99 (m, 1 H), 3.54-3.49 (m, 1 H), 3.45-3.42 (m, 1 H), 2.23-2.15 (m, 2 H), 1.73-1.68 (m, 2 H), 1.67-1.59 (m, 6 H), 1.54 (s, 11 H), 1.51-1.41 (m, 5 H); **^{13}C NMR** (CD_3Cl, 125 MHz) δ 153.6, 153.1, 152.9, 152.1, 146.4, 137.3, 124.9, 112.2, 98.2, 81.9, 61.6, 58.7, 45.9, 33.8, 30.0, 28.2, 25.1, 24.6, 18.7; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{34}O_4N_5S$ $[M+H]^+$ 476.2326, found 476.2325.



1-62

***Tert*-butyl (5-(3-(cyclopentylthio)-5-(hydroxymethyl)-4H-1,2,4-triazol-4-yl)pyridin-2-yl)carbamate** (**1-62**). A high pressure vial containing solution of **1-61** (0.400 g, 0.841 mmol) in ethanol (6.50 mL) was treated with pyridinium *p*-toluenesulfonate (0.0634 g, 0.252 mmol). The reaction vial was sealed and the reaction mixture was stirred at 70 ^{o}C for 24 h. The reaction mixture was concentrated under reduced pressure and resuspended with a saturated aqueous solution of sodium bicarbonate (10 mL). The resulting aqueous solution was extracted with methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-62** (0.321 g, 0.820 mmol, 98%) as an off-white solid: **^{1}H NMR** (CD_3OD, 500 MHz) δ 8.28 (d, *J* = 2.2 Hz, 1 H), 8.08 (d, *J* = 8.9 Hz, 1 H), 7.80 (dd, *J* = 8.9, 2.7 Hz, 1 H), 4.56 (s, 2 H), 3.87-3.82 (m, 1 H), 2.14-2.07 (m, 2 H), 1.75-1.72 (m, 2 H), 1.66-1.58 (m, 4 H), 1.55 (s, 9 H). **LC-MS** (ES) *m/z* calcd for $C_{18}H_{26}O_3N_5S$ $[M+H]^+$ 392.1751, found 392.1749.

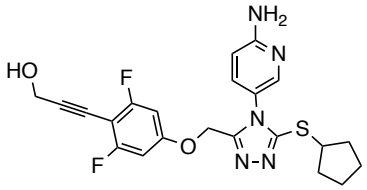


1-63

***Tert*-butyl (5-(3-(cyclopentylthio)-5-((3,5-difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenoxy)methyl)-4H-1,2,4-triazol-4-yl)pyridin-2-yl)carbamate (1-63).** Methanesulfonyl

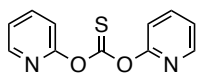
chloride (0.033 mL, 0.42 mmol) was added dropwise to mixture of 1-62 (0.150 g, 0.383 mmol), N,N-diisopropylethylamine (0.10 mL, 0.58 mmol) in anhydrous methylene chloride (3.8 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.0776 g, 0.421 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.249 g, 0.766 mmol) in anhydrous DMF (1.9 mL) under an atmosphere of nitrogen and stirred at room temperature for 21 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-63** (0.120 g, 0.215 mmol, 56%) as a white solid: **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.18 (d, *J* = 2.4 Hz, 1H), 8.15 (d, *J* = 9.0 Hz, 1H), 7.76 (s, 1H), 7.60 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.50 (d, *J* = 8.6 Hz, 2H), 5.03 (s, 2H), 4.52 (s, 2H), 4.10-4.01 (m, 1H), 2.26-2.17 (m, 2H), 1.73-1.60 (m, 6H), 1.53 (s, 9H); **LC-MS** (ES) *m/z* calcd for $C_{27}H_{30}O_4N_5S$ $[M+H]^+$ 558.1983, found 558.1979.



1-64

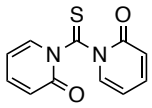
3-(4-((4-(6-Aminopyridin-3-yl)-5-(cyclopentylthio)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-ol (**1-64**). Potassium carbonate (0.0309 g, 0.224 mmol) was added to a high pressure reaction vial containing a solution of **1-63** (0.0500 g, 0.0897 mmol) in methanol (0.90 mL). The reaction vial was sealed and stirred at 100 ^{o}C for 18 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-64** (0.0259 g, 0.0566 mmol, 63%) as a white solid: **Rf** (5% methanol in ethyl acetate) 0.45; **Mp** (methanol) 103-105 ^{o}C; **IR** 3335.3, 3208.3, 2952.7, 2865.4, 1634.72, 1501.01 cm^{-1}; **^{1}H NMR** (CD_3OD, 500 MHz) δ 7.91 (d, *J* = 2.3 Hz, 1H), 7.46 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.67 (dd, *J* = 8.9, 4.2 Hz, 3H), 5.13 (s, 2H), 4.42 (s, 2H), 3.91 (t, *J* = 6.4 Hz, 1H), 2.17-2.12 (m, 2H), 1.76-1.74 (m, 2H), 1.67-1.59 (m, 5H); **^{13}C NMR** (CD_3OD, 126 MHz) δ 164.66, 164.59, 162.66, 162.60, 160.4, 159.07, 158.96, 158.85, 154.9, 151.8, 145.3, 136.8, 118.6, 108.9, 100.0, 98.72, 98.68, 98.55, 98.50, 96.6, 70.4, 60.2, 49.8, 45.9, 33.3, 24.1; **^{19}F NMR** (CD_3OD, 470 MHz) δ -108.4; **LC-MS** (ES) *m/z* calcd for $C_{22}H_{22}O_2N_5S$ $[M+H]^+$ 458.1457, found 458.1458.



1-66

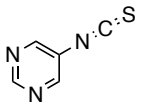
O,O-Di(pyridin-2-yl) carbonothioate (**1-66**). Thiophosgene (3.33 mL, 36.9 mmol) was slowly added dropwise to a solution of 2-hydroxypyridine **1-65** (7.03 g, 73.9 mmol),

triethylamine (10.8 mL, 77.6 mmol) in androus methylene chloride (185 mL) at 0 ^{o}C under argon and stirred for 1 h. The reaction mixture was diluted with a 5% aqueous solution of sodium bicarbonate (100 mL). The isolated aqueous solution was extracted with methylene chloride (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (50% ethyl acetate) to provide **1-66** (7.38 g, 31.8 mmol, 86%) as a yellow solid: **Rf** (ethyl acetate) 0.75; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.52-8.50 (m, 1H), 7.93-7.88 (m, 1H), 7.35 (ddd, *J* = 7.4, 4.9, 0.8 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 1H).



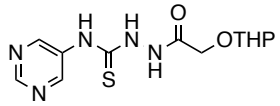
1-67

1,1'-Thiocarbonylbis(pyridin-2(1H)-one (**1-67**). 4-(Dimethylamino)pyridine (0.332 g, 2.72 mmol) was added to a solution of **1-66** (6.32 g, 27.2 mmol) in anhydrous methylene chloride (82 mL) and stirred at room temperature under an atmosphere of nitrogen for 18 h. The reaction mixture was sequentially washed with 5% aqueous solution of hydrogen chloride (50 mL) and brine (50 mL). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **1-67** (5.94 g, 25.6 mmol, 94%) as a bright-orange/red solid: **Rf** (ethyl acetate) 0.57; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.77 (dd, *J* = 7.3, 1.9 Hz, 1H), 7.32 (ddd, *J* = 9.1, 6.7, 2.2 Hz, 1H), 6.39 (d, *J* = 9.4 Hz, 1H), 6.29-6.26 (m, 1H).



1-69a

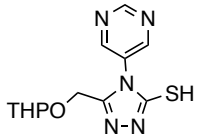
Pyrimidin-5-amine (**1-69a**). Pyrimidin-5-amine **1-68a** (0.409 g, 4.31 mmol) was added to a solution of **1-67** (1.00 g, 4.31 mmol) in anhydrous methylene chloride (11 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 2 h and then concentrated under reduced pressure. The crude material was carried forward without purification.



1-70a

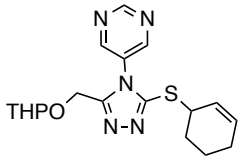
N-(Pyrimidin-5-yl)-2-(2-((tetrahydro-2H-pyran-2-yl)oxy)acetyl)hydrazine-1-carbothioamide (**1-70a**). Crude **1-69a** (0.591 g, 4.31 mmol) was added to a high pressure reaction vial containing a solution of **1-56** (0.750 g, 4.31 mmol) in ethanol (12 mL). The reaction vial was sealed and stirred at 80 ^{o}C for 1 h. The reaction mixture was concentrated under reduced pressure and resuspended in water (15 mL). The aqueous solution was extracted with ethyl acetate (25 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (85-100% ethyl acetate in hexane) to provide **1-70a** (1.26 g) as a tan solid: **^{1}H NMR** ((CD_3)$_2$SO, 500 MHz) δ 11.54 (bs, 1H), 10.07-10.05 (m, 2H), 9.82 (bs, 1H), 8.98 (s, 1H), 8.83 (s, 2H), 7.41 (ddd, *J* = 9.1, 6.7, 2.3 Hz, 1H), 7.35 (dd, *J* = 6.5, 1.6 Hz, 1H), 6.30 (d, *J* = 9.2 Hz, 1H), 6.16-6.13 (m, 1H), 4.71

(s, 1H), 4.12 (q, *J* = 19.0 Hz, 2H), 3.79-3.74 (m, 1H), 3.49-3.45 (m, 1H), 1.80-1.78 (m, 1H), 1.68-1.62 (m, 2H), 1.53-1.44 (m, 4H).



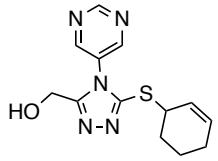
1-71a

4-(Pyrimidin-5-yl)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazole-3-thiol (**1-71a**). **1-70a** (0.900 g, 2.89 mmol) was added to a solution of sodium hydroxide (0.116 g, 2.89 mmol) in water (10 mL) and stirred at room temperature for 45 h. The reaction mixture was diluted with water (5 mL), acidified to a pH of 6 using a 1M aqueous solution of potassium hydrogen sulfate, and extracted with ethyl acetate (25 mL, six times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (45% ethyl acetate in hexane, dry loaded) to provide **1-71a** (0.465 g, 1.59 mmol, 55%, three steps) as a white solid: **Rf** (ethyl acetate) 0.57; **Mp** (methylene chloride) 134-136 ^{o}C; **IR** 3107.8, 3037.3, 2921.9, 1570.4, 1496.9, 1433.5 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 12.28 (s, 1H), 9.37 (s, 1H), 8.96 (s, 2H), 4.59 (t, *J* = 12.1 Hz, 2H), 4.42 (d, *J* = 13.0 Hz, 1H), 3.55-3.44 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 170.0, 159.0, 156.2, 149.1, 129.6, 98.8, 62.4, 58.8, 29.9, 24.9, 18.9; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{16}O_2N_5S$ $[M+H]^+$ 294.1019, found 294.1016.



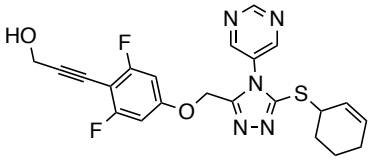
1-72a

5-(3-(Cyclohex-2-en-1-ylthio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyrimidine (**1-72a**). 3-Bromocyclohexene (0.21 mL, 1.8 mmol) was added to a mixture of **1-71a** (0.445 g, 1.52 mmol) and cesium carbonate (0.741 g, 2.28 mmol) in DMF (3 mL) and stirred at 80 ^{o}C for 2.5 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (30% ethyl acetate in hexane, dry loaded) to provide **1-72a** (0.216 g, 0.578 mmol, 38%) as a white solid: **Rf** (ethyl acetate) 0.52; **Mp** (methylene chloride) 86-88 ^{o}C; **IR** 3218.7, 3028.3, 2924.2, 2849.9, 1664.7, 1558.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 9.33 (s, 1H), 8.81 (s, 2H), 5.89 (dd, *J* = 10.0, 1.3 Hz, 1H), 5.76-5.73 (m, 1H), 4.72 (dd, *J* = 12.8, 3.7 Hz, 1H), 4.57-4.52 (m, 3H), 3.50-3.43 (m, 2H), 2.08-2.01 (m, 4H), 1.72-1.40 (m, 8H); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 159.1, 155.4, 152.7, 132.5, 129.5, 125.28, 125.27, 98.58, 98.56, 62.1, 58.9, 44.56, 44.53, 30.0, 29.20, 29.18, 25.00, 24.80, 19.10, 18.95; **LC-MS** (ES) *m/z* calcd for $C_{18}H_{24}O_2N_5S$ $[M+H]^+$ 374.1645, found 374.1644.



1-73a

(5-(Cyclohex-2-en-1-ylthio)-4-(pyrimidin-5-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-73a**). A solution of **10** (0.216 g, 0.578 mmol) in methanol (4 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0550 g, 0.289 mmol) at room temperature and stirred for 24 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-73a** (0.151 g, 0.521 mmol, 90%) as a white solid: **^{1}H NMR** (CD_3OD, 400 MHz) δ 9.34 (s, 1H), 8.98 (s, 2H), 5.91-5.89 (m, 1H), 5.67-5.66 (m, 1H), 4.64 (s, 2H), 4.28-4.27 (m, 1H), 2.05-1.98 (m, 3H), 1.93-1.86 (m, 1H), 1.74-1.61 (m, 2H); **LC-MS** (ES) *m/z* calcd for $C_{13}H_{16}ON_5S$ $[M+H]^+$ 290.1070, found 290.1066.

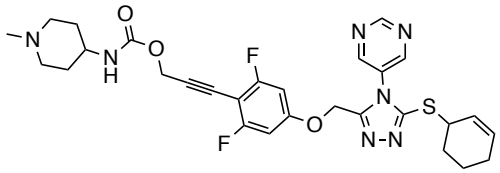


1-74a

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyrimidin-5-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-ol (**1-74a**). Methanesulfonyl chloride (0.040 mL, 0.52 mmol) was added dropwise to mixture of **1-73a** (0.100 g, 0.346 mmol), N,N-diisopropylethylamine (0.12 mL, 0.69 mmol) in anhydrous methylene chloride (3.5 mL) under an atmosphere of

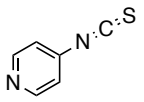
nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.0955 g, 0.518 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.225 g, 0.691 mmol) in anhydrous DMF (1.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 15 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (50% ethyl acetate in hexane, dry loaded) to provide **1-74a** (0.0995 g, 0.218 mmol, 63%) as a white solid: **Rf** (ethyl acetate) 0.76; **Mp** (methylene chloride) 132-134 ^{o}C; **IR** 3207.2, 2929.9, 2859.6, 1635.8, 1562.3 cm^{-1}; **^{1}H NMR** (CD_3OD, 400 MHz) δ 9.33 (s, 1H), 8.99 (s, 2H), 6.67 (d, J = 9.0 Hz, 2H), 5.93-5.89 (m, 1H), 5.25 (s, 2H), 4.42 (s, 2H), 4.35 (s, 1H), 2.05-2.02 (m, 2H), 1.98-1.91 (m, 1H), 1.76-1.63 (m, 2H); **^{13}C NMR** (CD_3OD, 126 MHz) δ 164.67, 164.60, 162.67, 162.60, 159.2, 158.6, 155.7, 153.3, 151.5, 132.4, 129.2, 124.7, 100.0, 98.70, 98.65, 98.52, 98.47, 96.79, 96.76, 95.1, 70.3, 60.3, 49.8, 45.0, 28.8, 24.3, 18.6; **^{19}F NMR** (CD_3OD, 376 MHz) δ -108.2; **LC-MS** (ES) *m/z* calcd for $C_{22}H_{20}O_2N_5F_2S$ $[M+H]^+$ 456.1300, found 456.1300.



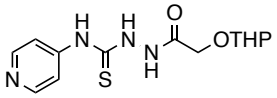
1-75a

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyrimidin-5-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (1-75a). Carbonyldiimidazole (0.0267 g, 0.165 mmol) was added to a solution of **1-74a** (0.0500 g, 0.109 mmol) in anhydrous methylene chloride (1 mL) and stirred for 2 h under nitrogen. 4-Amino-1-methylpiperidine (0.0413 mL, 0.329 mmol) was added and stirred for an additional 13 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (8% methanol in methylene chloride, dry loaded) to provide 1-75a (0.0603 g, 0.101 mmol, 92%) as a white solid: Rf (50% methanol in ethyl acetate) 0.13; Mp (methylene chloride) 154-156 ^{o}C; IR 3303.6, 2936.9, 2777.4, 1690.3, 1637.3 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 9.33 (s, 1H), 9.33 (s, 1H), 8.99 (s, 2H), 8.99 (s, 2H), 6.68 (d, *J* = 8.9 Hz, 2H), 6.68 (d, *J* = 8.9 Hz, 2H), 5.94-5.89 (m, 1H), 5.94-5.89 (m, 1H), 5.70-5.66 (m, 1H), 5.70-5.66 (m, 1H), 5.25 (s, 2H), 5.25 (s, 2H), 4.35 (s, 1H), 4.35 (s, 1H), 3.47-3.42 (m, 1H), 3.47-3.42 (m, 1H), 2.89-2.85 (m, 2H), 2.89-2.85 (m, 2H), 2.31 (s, 2H), 2.31 (s, 2H), 2.23-2.16 (m, 2H), 2.23-2.16 (m, 2H), 2.05-2.02 (m, 2H), 2.05-2.02 (m, 2H), 1.94-1.89 (m, 3H), 1.94-1.89 (m, 3H), 1.75-1.64 (m, 2H), 1.75-1.64 (m, 2H), 1.60-1.49 (m, 2H), 1.60-1.49 (m, 2H); ^{13}C NMR (CD_3OD, 126MHz) δ 164.74, 164.67, 162.74, 162.67, 159.2, 158.9, 155.86, 155.70, 153.3, 151.5, 132.4, 129.2, 124.7, 98.8, 98.5, 60.3, 53.86, 53.81, 53.4, 52.1, 45.0, 44.6, 31.3, 31.1, 28.8, 24.3, 22.3, 18.6, 13.0; ^{19}F NMR (CD_3OD, 376 MHz) δ -108.0; LC-MS (ES) *m/z* calcd for $C_{29}H_{32}O_3N_7F_2S$ $[M+H]^+$ 596.2250, found 596.2251.



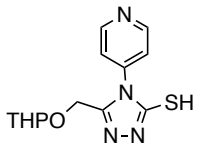
1-69b

4-Isothiocyanatopyridine (**1-69b**). 4-Aminopyridine (0.405 g, 4.31 mmol) was added to a high pressure reaction vial containing **1-67** (1.00 g, 4.31 mmol) in anhydrous THF (11 mL). The reaction vial was sealed and stirred at 60 ^{o}C for 2 h. The reaction mixture was concentrated under reduced pressure and carried forward without purification.



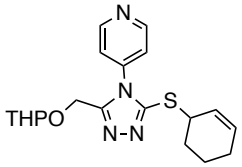
1-70b

N-(Pyridin-4-yl)-2-(2-((tetrahydro-2H-pyran-2-yl)oxy)acetyl)hydrazine-1-carbothioamide (**1-70b**). **1-56** (0.569 g, 3.27 mmol) was added to a solution of crude **1-69b** (0.405 g, 2.97 mmol) and N,N-diisopropylethylamine (0.777 mL, 4.46 mmol) in anhydrous THF (10 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 17 h and then concentrated under reduced pressure. The resulting residue was resuspended in water (10 mL) and concentrated with sodium chloride. The aqueous solution was extracted with 10% methanol in methylene chloride (15 mL, six times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (10% methanol in methylene chloride) to provide **1-70b** (0.475 g) as a yellow solid: **^{1}H NMR** (CD_3OD, 500 MHz) δ 8.19-8.17 (m, 2H), 8.01 (s, 2H), 4.79 (t, *J* = 3.1 Hz, 1H), 4.31 (d, *J* = 15.5 Hz, 1H), 4.15 (d, *J* = 15.5 Hz, 1H), 3.96-3.92 (m, 1H), 3.57 (dtd, *J* = 11.3, 4.1, 1.3 Hz, 1H), 2.08-2.04 (m, 1H), 1.84-1.81 (m, 2H), 1.65-1.55 (m, 3H), 1.37 (d, *J* = 6.6 Hz, 2H); **LC-MS** (ES) *m/z* calcd for $C_{13}H_{19}O_3N_4S$ $[M+H]^+$ 311.1172, found 311.1167.



1-71b

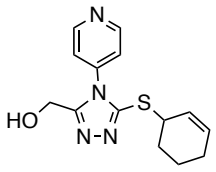
4-(Pyridin-4-yl)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazole-3-thiol (**1-71b**). Sodium hydroxide (0.0612 g, 0.1.53 mmol) was added to a solution of **1-70b** (0.475 g, 1.53 mmol) in water (5 mL) and stirred at 100 ^{o}C for 3 h. The reaction mixture was diluted with water (5 mL), acidified to a pH of 6 using a 1M aqueous solution of potassium hydrogen sulfate, and extracted with ethyl acetate (25 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (4% methanol in methylene chloride) to provide **1-71b** (0.266 g, 0.911 mmol, 60%) as a white residue: **Rf** (ethyl acetate in hexane) 0.67; **Mp** (methylene chloride) 188-190 ^{o}C; **IR** 3055.2, 2942.8, 2735.7, 1586.5, 1503.6 cm^{-1}; **^{1}H NMR** (CD_3OD, 500 MHz) δ 8.79 (dd, *J* = 4.7, 1.5 Hz, 2H), 7.64 (dd, *J* = 4.7, 1.5 Hz, 2H), 4.61 (d, *J* = 13.1 Hz, 1H), 4.51 (t, *J* = 3.2 Hz, 1H), 4.46 (d, *J* = 13.0 Hz, 1H), 3.47 (td, *J* = 10.2, 2.7 Hz, 1H), 3.41-3.38 (m, 1H), 1.63-1.34 (m, 6H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 151.3, 148.8, 141.3, 122.6, 98.4, 62.0, 58.7, 29.9, 25.0, 18.7; **LC-MS** (ES) *m/z* calcd for $C_{13}H_{17}O_2N_4S$ $[M+H]^+$ 293.1067, found 293.1064.



1-72b

4-(3-(Cyclohex-2-en-1-ylthio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**1-72b**). 3-Bromocyclohex-1-ene (0.12 mL, 1.1 mmol) was added to a

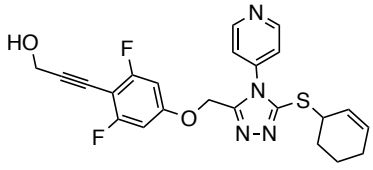
mixture of **1-71b** (0.255 g, 0.872 mmol) and cesium carbonate (0.426 g, 1.31 mmol) in DMF (1.75 mL) and stirred at 80 ^{o}C for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (85% ethyl acetate in hexane, dry loaded) to provide **1-72b** (0.297 g, 0.797 mmol, 91%) as an off-white solid: **Rf** (ethyl acetate) 0.37; **Mp** (methylene chloride) 92-94 ^{o}C; **IR** 3031.9, 2939.9, 2870.6, 1587.1, 1573.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.76 (d, *J* = 4.8 Hz, 2H), 7.34 (d, *J* = 5.6 Hz, 2H), 5.82 (d, *J* = 10.0 Hz, 1H), 5.71-5.67 (m, 1H), 4.64 (dd, *J* = 12.6, 3.5 Hz, 1H), 4.55-4.46 (m, 3H), 3.50-3.36 (m, 2H), 1.97 (d, *J* = 2.9 Hz, 4H), 1.68-1.36 (m, 8H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 151.5, 141.1, 132.3, 125.5, 121.6, 98.28, 98.25, 61.9, 58.7, 44.1, 30.0, 29.23, 29.21, 25.1, 24.8, 19.2, 18.8; **LC-MS** (ES) *m/z* calcd for $C_{19}H_{25}O_2N_4S$ $[M+H]^+$ 373.1693, found 373.1690.



1-73b

(5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-73b**). A solution of **1-72b** (0.280 g, 0.752 mmol) in methanol (5 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0715 g, 0.376 mmol) at room temperature and stirred for 18 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The

combined organic solutions were washed with a saturated aqueous solution of sodium bicarbonate (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-73b** (0.157 g, 0.544 mmol, 72%) as white solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.81 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.58 (dd, *J* = 4.5, 1.6 Hz, 2H), 5.86-5.84 (m, 1H), 5.67 (ddd, *J* = 7.9, 4.0, 2.0 Hz, 1H), 5.51 (t, *J* = 5.6 Hz, 1H), 4.49-4.48 (m, 2H), 4.27-4.26 (m, 1H), 1.97-1.94 (m, 2H), 1.93-1.89 (m, 1H), 1.84-1.78 (m, 1H), 1.62-1.55 (m, 2H).; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{17}ON_4S$ $[M+H]^+$ 289.1118, found 289.1113.

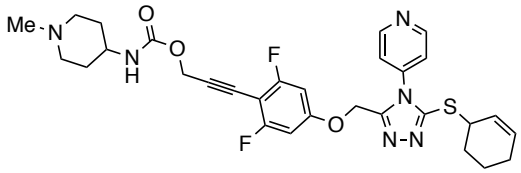


1-74b

(5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-74b**). Methanesulfonyl chloride (0.025 mL, 0.33 mmol) was added dropwise to mixture of **1-73b** (0.0450 g, 0.156 mmol), N,N-diisopropylethylamine (0.12 mL, 0.69 mmol) in anhydrous methylene chloride (1.6 mL) under an atmosphere of nitrogen at 0 ^oC for 5 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.0345 g, 0.187 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.107 g, 0.328 mmol) in anhydrous DMF (0.80 mL) under an atmosphere of nitrogen and stirred at room temperature for 24 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The

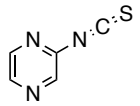
combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded) to provide **1-74b** (0.0425 g, 0.0935 mmol, 60%) as a white solid: **Rf** (ethyl acetate) 0.61; **Mp** (methylene chloride) 163-165 ^{o}C; **IR** 3308.1, 3037.2, 2928.5, 1635.7, 1587.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.83 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.32-7.31 (m, 2H), 6.52 (d, *J* = 8.5 Hz, 2H), 5.92-5.89 (m, 1H), 5.78-5.75 (m, 1H), 5.10-5.05 (m, 2H), 4.63-4.62 (m, 1H), 4.53 (s, 2H), 2.11-2.00 (m, 5H), 1.75-1.66 (m, 2H), 1.61-1.58 (m, 1H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.8, 162.7, 158.4, 153.1, 151.8, 149.7, 140.5, 132.6, 125.2, 121.2, 99.02, 98.97, 98.84, 98.79, 96.4, 72.2, 60.3, 51.6, 44.2, 29.2, 24.8, 19.2; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.4; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{21}O_2N_4F_2S$ $[M+H]^+$ 455.1348, found 455.1346.



1-75b

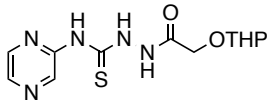
3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-75b**). Carbonyldiimidazole (0.0268 g, 0.165 mmol) was added to a solution of **1-74b** (0.0500 g, 0.110 mmol) in anhydrous methylene chloride (1.1 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.0414 mL, 0.330 mmol) was added and stirred for an additional 18 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18 silica (65% acetonitrile in water, dry loaded) to provide **1-75b** (0.0408 g, 0.0686 mmol, 62%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.22; **Mp** (methylene chloride) 73-75 ^{o}C; **IR** 2938.3, 2789.0, 1717.9, 1635.6, 1503.3 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400

MHz) δ 8.83 (dd, *J* = 4.5, 1.6 Hz, 2H), 7.30 (d, *J* = 6.1 Hz, 2H), 6.52 (d, *J* = 8.5 Hz, 2H), 5.93-5.88 (m, 1H), 5.78-5.74 (m, 1H), 5.07 (d, *J* = 0.2 Hz, 2H), 4.92 (s, 2H), 4.75-4.71 (m, 1H), 4.65-4.61 (m, 1H), 3.55-3.50 (m, 1H), 2.77-2.72 (m, 2H), 2.27 (s, 3H), 2.12-2.01 (m, 6H), 1.98-1.93 (m, 2H), 1.78-1.67 (m, 3H), 1.52-1.42 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.94, 164.88, 162.93, 162.86, 158.6, 153.1, 151.8, 149.7, 140.5, 132.6, 125.3, 121.1, 99.03, 98.98, 98.84, 98.80, 92.5, 72.9, 60.4, 54.26, 54.25, 53.1, 46.1, 44.2, 32.44, 32.43, 29.2, 24.8, 19.2; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -105.0; **LC-MS** (ES) *m/z* calcd for $C_{30}H_{33}O_3N_6F_2S$ $[M+H]^+$ 595.2297, found 595.2296.



1-69c

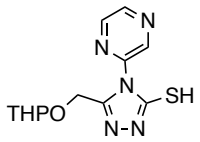
2-Isothiocyanatopyrazine (**1-69**). Pyrazin-2-amine **1-68c** (0.409 g, 4.31 mmol) was added to a solution of **1-67** (1.00 g, 4.31 mmol) in anhydrous methylene chloride (11 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 16 h and then concentrated under reduced pressure. The crude material was carried forward without purification.



1-70c

N-(pyrazin-2-yl)-2-(2-((tetrahydro-2H-pyran-2-yl)oxy)acetyl)hydrazine-1-carbothioamide (**1-70c**). Crude **1-69c** (0.591 g, 4.31 mmol) was added to a high pressure

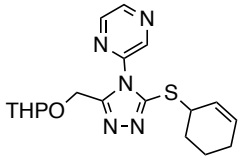
reaction vial containing a solution of **1-56** (0.750 g, 4.31 mmol) in ethanol (12 mL). The reaction vial was sealed and stirred at 80 ^{o}C for 2 h. The reaction mixture was concentrated under reduced pressure and the resulting solid material was washed with water. The solid material was dissolved in methylene chloride (50 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-70c** (1.06 g, 3.40 mmol, 79%) as a brown solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 12.76 (s, 1H), 11.37 (s, 1H), 10.48 (s, 1H), 8.62 (s, 1H), 8.31 (s, 2H), 4.74 (t, *J* = 3.3 Hz, 1H), 4.16 (q, *J* = 17.5 Hz, 2H), 3.78 (ddd, *J* = 12.5, 7.2, 2.9 Hz, 1H), 3.50-3.46 (m, 1H), 1.82-1.76 (m, 1H), 1.71-1.66 (m, 1H), 1.63-1.58 (m, 1H), 1.53-1.45 (m, 3H); **LC-MS** (ES) *m/z* calcd for $C_{12}H_{17}O_3N_5S$ $[M+H]^+$ 312.1052, found 312.0782.



1-71c

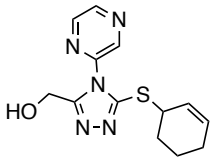
4-(Pyrazin-2-yl)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazole-3-thiol (**1-71c**). Sodium hydroxide (0.0384 g, 2.12 mmol) was added to a solution **1-70c** (0.660 g, 2.12 mmol) in water (7 mL) and stirred at 120 ^{o}C for 4 h. The reaction mixture was diluted with water (5 mL), acidified to a pH of 6 using a 1M aqueous solution of potassium hydrogen sulfate, and extracted with ethyl acetate (25 mL, four times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40-50% ethyl acetate in hexane) to provide **1-71c** (0.456 g, 1.56 mmol, 73%) as a white yellow solid: **Rf** (ethyl acetate) 0.71; **IR** 3127.1, 3057.4, 2941.9, 2869.4, 2756.9, 1577.5, 1472.5, 1427.2 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 11.50 (t, *J* = 0.6 Hz, 1H), 9.20 (s, 1H), 8.72 (d, *J* = 2.4 Hz, 1H), 8.61 (t, *J* = 0.9 Hz, 1H), 4.84 (d, *J* = 13.1 Hz, 1H), 4.68 (d, *J* = 13.1 Hz, 1H), 4.47

(d, J = 3.2 Hz, 1H), 3.55-3.49 (m, 1H), 3.45-3.42 (m, 1H), 1.50-1.43 (m, 3H), 1.43-1.38 (m, 2H), 1.26-1.15 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz)149.7, 144.7, 144.2, 143.2, 98.5, 62.0, 59.6, 29.8, 25.0, 18.6; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{16}O_2N5S$ $[M+H]^+$ 294.1019, found 294.1015.



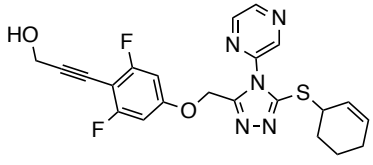
1-72c

2-(3-(Cyclohex-2-en-1-ylthio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyrazine (**1-72c**). 3-Bromocyclohexene (0.32 mL, 2.8 mmol) was added to a mixture of **1-71c** (0.681 g, 2.32 mmol) and cesium carbonate (1.13 g, 3.48 mmol) in DMF (4.6 mL) and stirred at room temperature for 21 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (30% ethyl acetate in hexane, dry loaded) to provide **1-72c** (0.594 g, 1.59 mmol, 68%) as a light-yellow residue: **Rf** (ethyl acetate) 0.52; **IR** 3028.7, 2940.0, 2867.5, 1473.3, 1418.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.84 (d, J = 1.1 Hz, 1H), 8.72 (d, J = 2.5 Hz, 1H), 8.62 (dd, J = 2.4, 1.4 Hz, 1H), 5.89-5.86 (m, 1H), 5.76-5.73 (m, 1H), 4.90 (dd, J = 12.8, 4.1 Hz, 1H), 4.73 (dd, J = 12.8, 3.0 Hz, 1H), 4.54 (d, J = 2.6 Hz, 2H), 3.58-3.54 (m, 1H), 3.45-3.41 (m, 1H), 2.07-1.97 (m, 4H), 1.76-1.70 (m, 1H), 1.69-1.62 (m, 3H), 1.55-1.47 (m, 3H), 1.43-1.37 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 152.6, 151.70, 151.68, 144.9, 144.3, 143.4, 142.6, 132.2, 125.5, 98.35, 98.31, 61.9, 59.39, 59.37, 44.6, 30.0, 29.1, 25.0, 24.8, 19.1, 18.8; **LC-MS** (ES) *m/z* calcd for $C_{18}H_{24}O_2N_5S$ $[M+H]^+$ 374.1645, found 374.1643.



1-73c

(5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-73c**). A solution of **1-72c** (0.0469 g, 0.247 mmol) in methanol (3.3 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0469 g, 0.247 mmol) at room temperature and stirred for 13 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-73c** (0.141 g, 0.486 mmol, 98%) as a white solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.3 Hz, 1H), 8.65 (d, *J* = 2.0 Hz, 1H), 7.95 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.64 (dd, *J* = 7.9, 5.0 Hz, 1H), 5.44 (s, 1H), 4.44 (d, *J* = 5.6 Hz, 2H), 1.92-1.90 (m, 2H), 1.64-1.61 (m, 2H), 1.53-1.50 (m, 1H), 1.36-1.25 (m, 4H).; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{19}ON_4S$ $[M+H]^+$ 291.1274, found 291.1271.

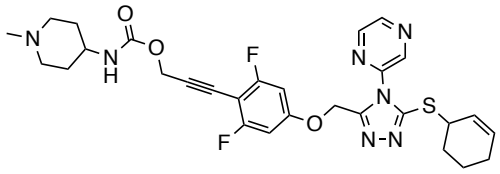


1-74c

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyrazin-2-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-ol (**1-74c**). Methanesulfonyl chloride (0.040 mL, 0.52 mmol) was added dropwise to mixture of **1-73c** (0.100 g, 0.346 mmol), N,N-diisopropylethylamine (0.12 mL, 0.69 mmol) in anhydrous methylene chloride (3.5 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.0955 g, 0.518 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.225 g, 0.691 mmol) in anhydrous DMF (1.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 20 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (50% ethyl acetate in hexane, dry loaded) to provide **1-74c** (0.0784 g, 0.172 mmol, 50%) as a white solid: **Rf** (ethyl acetate) 0.78; **Mp** (methylene chloride) 143-145 ^{o}C; **IR** 3329.1, 3059.4, 2925.0, 2856.7, 1634.8, 1579.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.82 (d, *J* = 1.0 Hz, 1H), 8.73 (d, *J* = 2.4 Hz, 1H), 8.56 (dd, *J* = 2.4, 1.4 Hz, 1H), 6.37-6.34 (m, 2H), 5.92-5.88 (m, 1H), 5.77-5.74 (m, 1H), 5.33 (s, 2H), 4.60 (dd, *J* = 4.1, 1.7 Hz, 1H), 4.52 (s, 2H), 2.10-2.00 (m, 4H), 1.78-1.65 (m, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.70, 164.63, 162.69, 162.62, 158.4, 152.5, 150.3, 145.3, 143.70, 143.65, 141.9, 132.6, 125.2,

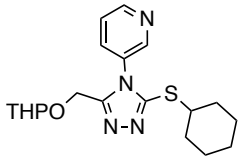
98.81, 98.77, 98.63, 98.59, 96.49, 96.47, 95.3, 72.0, 61.1, 51.5, 44.8, 29.2, 24.8, 19.1; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.5; **LC-MS** (ES) *m/z* calcd for $C_{22}H_{20}O_2N_5F_2S$ $[M+H]^+$ 456.1300, found 456.1298.



1-75c

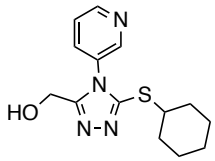
3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyrazin-2-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-75c**). Carbonyldiimidazole (0.0267 g, 0.165 mmol) was added to a solution of **1-74c** (0.0500 g, 0.109 mmol) in anhydrous methylene chloride (1.1 mL) and stirred for 2 h under nitrogen. 4-Amino-1-methylpiperidine (0.041 mL, 0.33 mmol) was added and stirred for an additional 24 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (11% methanol in methylene chloride, dry loaded) to provide **1-75c** (0.0602 g, 0.101 mmol, 92%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.14; **Mp** (methylene chloride) 144-146 ^{o}C; **IR** 3320.6, 2936.2, 2789.0, 1718.3, 1635.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.81 (d, *J* = 1.2 Hz, 1H), 8.73 (d, *J* = 2.4 Hz, 1H), 8.55 (dd, *J* = 2.2, 1.5 Hz, 1H), 6.36 (d, *J* = 8.4 Hz, 2H), 5.91-5.88 (m, 1H), 5.77-5.75 (m, 1H), 5.33 (s, 2H), 4.91 (s, 2H), 4.75-4.73 (m, 1H), 4.60 (t, *J* = 1.8 Hz, 1H), 3.55-3.51 (m, 1H), 2.77-2.75 (m, 2H), 2.27 (s, 3H), 2.12-2.00 (m, 7H), 1.97-1.94 (m, 2H), 1.76-1.65 (m, 3H), 1.52-1.45 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.85, 164.79, 162.83, 162.77, 158.78, 158.67, 158.57, 154.7, 152.4, 150.3, 145.3, 143.67, 143.64, 141.8, 132.6, 125.2, 98.86, 98.81, 98.68, 98.63, 95.23, 95.08, 92.44, 92.42, 72.9, 61.2,

54.2, 53.1, 46.1, 44.8, 32.4, 29.2, 24.8, 19.1; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.1; **LC-MS** (ES) *m/z* calcd for $C_{29}H_{32}O_3N_7F_2S$ $[M+H]^+$ 596.2250, found 596.2250.



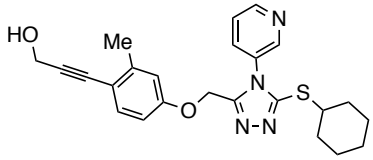
1-79

3-(3-(Cyclohexylthio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**1-79**). 3-Bromocyclohexene (0.25 mL, 2.1 mmol) was added to a mixture of **1-13** (0.500 g, 1.71 mmol) and cesium carbonate (0.836 g, 2.56 mmol) in DMF (3.5 mL) and stirred at 80 ^oC for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40-50% ethyl acetate in hexane, dry loaded) to provide **1-79** (0.182 g, 0.485 mmol, 28%) as a colorless oil: **Rf** (ethyl acetate) 0.43; **IR** 2930.7, 2852.8, 1484.2, 1444.8, 1392.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.77-8.76 (m, 1H), 8.64 (d, *J* = 1.4 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.49 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.67 (d, *J* = 12.6 Hz, 1H), 4.57 (s, 1H), 4.50 (d, *J* = 12.6 Hz, 1H), 3.80-3.75 (m, 1H), 3.46-3.39 (m, 2H), 2.11-2.08 (m, 2H), 1.73-1.70 (m, 3H), 1.61-1.51 (m, 4H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 152.8, 152.5, 150.8, 148.4, 135.0, 130.5, 123.8, 98.2, 61.6, 58.8, 46.9, 33.4, 30.0, 25.7, 25.5, 25.1, 18.7; **LC-MS** (ES) *m/z* calcd for $C_{19}H_{27}O_2N_4S$ $[M+H]^+$ 375.1849, found 375.1846.



1-80

(5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-80**). A solution of **1-79** (0.0469 g, 0.247 mmol) in methanol (3.3 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0469 g, 0.247 mmol) at room temperature and stirred for 13 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-80** (0.141 g, 0.486 mmol, 98%) as white solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.3 Hz, 1H), 8.74 (dd, *J* = 4.8, 1.3 Hz, 1H), 8.65 (d, *J* = 2.0 Hz, 1H), 8.65 (d, *J* = 2.0 Hz, 1H), 7.95 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.95 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.64 (dd, *J* = 7.9, 5.0 Hz, 1H), 7.64 (dd, *J* = 7.9, 5.0 Hz, 1H), 5.44 (s, 1H), 5.44 (s, 1H), 4.44 (d, *J* = 5.6 Hz, 2H), 4.44 (d, *J* = 5.6 Hz, 2H), 1.92-1.90 (m, 2H), 1.92-1.90 (m, 2H), 1.64-1.61 (m, 2H), 1.64-1.61 (m, 2H), 1.53-1.50 (m, 1H), 1.53-1.50 (m, 1H), 1.36-1.25 (m, 4H), 1.36-1.25 (m, 4H); **LC-MS** (ES) *m/z* calcd for $C_{14}H_{19}ON_4S$ $[M+H]^+$ 291.1274, found 291.1271.

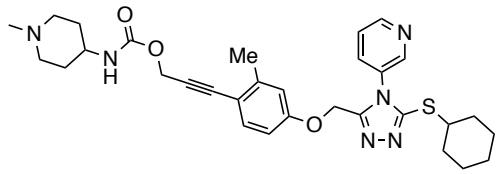


1-81

3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2-methylphenyl)prop-2-yn-1-ol (**1-81**). Methanesulfonyl chloride (0.029 mL, 0.39 mmol) was added dropwise to mixture of **1-80** (0.0750 g, 0.258 mmol), N,N-diisopropylethylamine (0.089 mL, 0.52 mmol) in anhydrous methylene chloride (2.6 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

4-(3-Hydroxyprop-1-yn-1-yl)-3-methylphenol (0.0628 g, 0.387 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.168 g, 0.517 mmol) in anhydrous DMF (1.3 mL) under an atmosphere of nitrogen and stirred at room temperature for 12 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (85% ethyl acetate in hexane, dry loaded) to provide **1-81** (0.0717 g, 0.165 mmol, 64%) as a white solid: **Rf** (ethyl acetate) 0.52; **Mp** (methylene chloride) 143-145 ^{o}C; **IR** 3288.9, 2928.3, 2856, 1604.5, 1486.1 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (d, *J* = 4.4 Hz, 1H), 8.60-8.60 (m, 1H), 7.66 (dt, *J* = 8.2, 1.8 Hz, 1H), 7.47 (dd, *J* = 8.1, 4.9 Hz, 1H), 7.28 (s, 1H), 6.68 (d, *J* = 2.2 Hz, 1H), 6.63 (dd, *J* = 8.5, 2.4 Hz, 1H), 5.06 (s, 2H), 4.51 (s, 2H), 3.83-3.79 (m, 1H), 2.35 (s, 3H), 2.12-2.09 (m, 2H), 1.73-1.70 (m, 3H), 1.62-1.58 (m, 1H), 1.47-1.37 (m, 5H), 1.27-1.24 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 157.1,

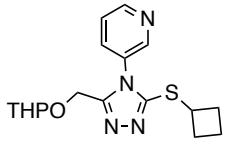
153.3, 151.3, 151.1, 148.0, 142.3, 134.9, 133.5, 130.1, 124.0, 116.1, 115.7, 111.9, 90.5, 83.8, 59.9, 51.6, 46.9, 33.4, 25.7, 25.4, 20.9; **LC-MS** (ES) *m/z* calcd for $C_{24}H_{27}O_2N_4S$ $[M+H]^+$ 435.1849, found 435.1847.



1-82

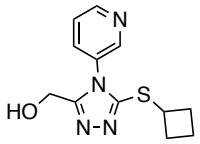
3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2-methylphenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-82**). Carbonyldiimidazole (0.0278 g, 0.172 mmol) was added to a solution of **1-81** (0.0500 g, 0.115 mmol) in anhydrous methylene chloride (1.1 mL) and stirred for 2 h under nitrogen. 4-Amino-1-methylpiperidine (0.043 mL, 0.35 mmol) was added and stirred for an additional 24 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (14% methanol in methylene chloride, dry loaded) to provide **1-82** (0.0561 g, 0.0976 mmol, 85%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.15; **Mp** (methylene chloride) 166-168 ^oC; **IR** 3291.8, 2937.8, 2852.4, 2792.4, 1717.1, 1604.1 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.59 (d, *J* = 2.4 Hz, 1H), 7.66-7.64 (m, 1H), 7.46 (dd, *J* = 8.1, 4.8 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 1H), 6.68 (d, *J* = 2.2 Hz, 1H), 6.63 (dd, *J* = 8.6, 2.4 Hz, 1H), 5.06 (s, 2H), 4.90 (s, 2H), 4.74-4.72 (m, 1H), 3.83-3.78 (m, 1H), 3.53 (s, 1H), 2.78 (s, 2H), 2.35 (s, 3H), 2.29 (s, 3H), 2.12-2.09 (m, 4H), 1.97-1.95 (m, 2H), 1.73-1.70 (m, 2H), 1.62-1.37 (m, 7H), 1.29-1.23 (m, 2H).; **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 157.3, 154.8, 153.3, 151.25, 151.11, 148.1, 142.7, 134.8, 133.7, 130.1, 124.0, 115.7, 111.9, 86.4, 84.7, 59.9, 54.2, 53.4, 46.9,

46.0, 33.4, 32.3, 25.7, 25.4, 20.8; **LC-MS** (ES) *m/z* calcd for $C_{31}H_{39}O_3N_6S$ $[M+H]^+$ 575.2799, found 575.2794.



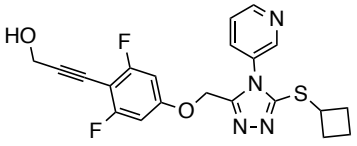
1-83

3-(3-(Cyclobutylthio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**1-83**). Bromocyclobutane (0.32 mL, 3.4 mmol) was added to a mixture of **1-13** (0.500 g, 1.71 mmol) and cesium carbonate (0.836 g, 2.56 mmol) in DMF (3.5 mL) and stirred at room temperature for 24 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40-50% ethyl acetate in hexane, dry loaded) to provide **1-83** (0.372 g, 1.07 mmol, 63%) as a colorless oil: **Rf** (ethyl acetate) 0.21; **IR** 2942.1, 2868.8, 1484.0, 1443.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76 (dd, *J* = 4.6, 1.0 Hz, 1H), 8.64 (d, *J* = 2.1 Hz, 1H), 7.71 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1H), 7.49 (dd, *J* = 8.0, 4.7 Hz, 1H), 4.67 (d, *J* = 12.6 Hz, 1H), 4.57 (t, *J* = 2.9 Hz, 1H), 4.49 (d, *J* = 12.6 Hz, 1H), 4.30 (t, *J* = 8.1 Hz, 1H), 3.46-3.38 (m, 2H), 2.56-2.49 (m, 2H), 2.14-2.07 (m, 2H), 2.04-1.95 (m, 2H), 1.64-1.50 (m, 3H), 1.49-1.39 (m, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 152.73, 152.61, 150.9, 148.3, 134.9, 130.4, 123.9, 98.2, 61.6, 58.7, 40.0, 31.0, 30.0, 25.1, 18.87, 18.68; **LC-MS** (ES) *m/z* calcd for $C_{17}H_{23}O_2N_4S$ $[M+H]^+$ 347.1536, found 347.1534.



1-84

(5-(Cyclobutylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-84**). A solution of **1-83** (0.372 g, 1.07 mmol) in methanol (7 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.102 g, 0.533 mmol) at room temperature and stirred for 24 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-84** (0.265 g, 1.01 mmol, 94%) as white solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.2 Hz, 1H), 8.66 (d, *J* = 2.4 Hz, 1H), 7.96 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.64 (dd, *J* = 8.1, 4.8 Hz, 1H), 5.45 (t, *J* = 5.6 Hz, 1H), 4.44 (d, *J* = 5.6 Hz, 2H), 4.04 (q, *J* = 8.0 Hz, 1H), 2.36-2.30 (m, 2H), 2.03-1.97 (m, 2H), 1.91-1.85 (m, 2H); **LC-MS** (ES) *m/z* calcd for $C_{12}H_{15}ON_4S$ $[M+H]^+$ 263.0961, found 263.0959.

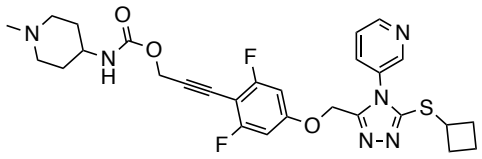


1-85

3-(4-((5-(Cyclobutylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-ol (**1-85**). Methanesulfonyl chloride (0.044 mL, 0.57 mmol) was

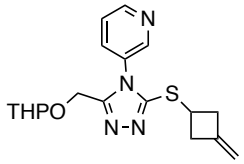
added dropwise to mixture of **1-84** (0.100 g, 0.381 mmol), N,N-diisopropylethylamine (0.13 mL, 0.76 mmol) in anhydrous methylene chloride (3.8 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.105 g, 0.572 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.248 g, 0.762 mmol) in anhydrous DMF (1.9 mL) under an atmosphere of nitrogen and stirred at room temperature for 21 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (85% ethyl acetate in hexane, dry loaded) to provide **1-85** (0.109 g, 0.256 mmol, 67%) as a white solid: **Rf** (ethyl acetate) 0.68; **Mp** (methylene chloride) 64-66 ^{o}C; **IR** 3269.5, 3053.3, 1984.8, 2948.4, 2862.1, 1635.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.79 (d, *J* = 4.5 Hz, 1H), 8.61 (d, *J* = 2.2 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.51 (dd, *J* = 8.1, 4.8 Hz, 1H), 6.47 (d, *J* = 8.5 Hz, 2H), 5.05 (s, 2H), 4.52 (s, 2H), 4.38-4.32 (m, 1H), 2.58-2.52 (m, 2H), 2.17-2.09 (m, 3H), 2.07-1.97 (m, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.69, 164.62, 162.68, 162.61, 158.39, 158.28, 158.18, 153.9, 151.51, 151.37, 150.3, 148.0, 147.8, 134.7, 129.8, 124.36, 124.25, 98.97, 98.94, 98.89, 98.76, 98.71, 96.7, 95.4, 71.8, 60.3, 51.4, 39.9, 31.0, 19.0; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.5; **LC-MS** (ES) *m/z* calcd for $C_{21}H_{19}O_2N_4F_2S$ $[M+H]^+$ 429.1191, found 429.1188.



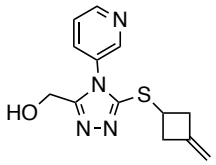
1-86

3-(4-((5-(Cyclobutylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-86**). Carbonyldiimidazole (0.0284 g, 0.175 mmol) was added to a solution of **1-85** (0.0500 g, 0.117 mmol) in anhydrous methylene chloride (1.2 mL) and stirred for 2 h under nitrogen. 4-Amino-1-methylpiperidine (0.044 mL, 0.35 mmol) was added and stirred for an additional 24 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (14% methanol in methylene chloride, dry loaded) to provide **1-86** (0.0570 g, 0.100 mmol, 86%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.12; **Mp** (methylene chloride) 68-70 ^{o}C; **IR** 2940.5, 2786.3, 1718.9, 1635.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.79 (d, *J* = 4.8 Hz, 1H), 8.60 (d, *J* = 2.3 Hz, 1H), 7.67-7.64 (m, 1H), 7.50 (dd, *J* = 8.1, 4.8 Hz, 1H), 6.49 (d, *J* = 8.5 Hz, 2H), 5.05 (s, 2H), 4.92 (s, 2H), 4.76-4.73 (m, 1H), 4.35 (quintet, *J* = 8.0 Hz, 1H), 3.54 (s, 1H), 2.78 (s, 2H), 2.59-2.52 (m, 2H), 2.29 (s, 3H), 2.16-2.08 (m, 4H), 2.05-1.95 (m, 4H), 1.55-1.47 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.90, 164.83, 162.88, 162.81, 158.68, 158.57, 158.46, 154.7, 153.9, 151.48, 151.43, 150.2, 147.8, 134.60, 134.56, 129.8, 124.23, 124.18, 98.99, 98.94, 98.81, 98.76, 95.1, 92.47, 92.44, 72.9, 60.4, 54.2, 53.1, 46.1, 39.9, 32.3, 31.6, 31.0, 22.6, 19.0, 14.1; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.0; **LC-MS** (ES) *m/z* calcd for $C_{28}H_{31}O_3N_6F_2S$ $[M+H]^+$ 569.2141, found 569.2137.



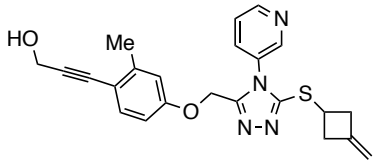
1-87

3-(3-((3-Methylenecyclobutyl)thio)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-4*H*-1,2,4-triazol-4-yl)pyridine (**1-87**). Azobisisobutyronitrile (0.00843 g, 0.0513 mmol) was added to a mixture of **1-13** (0.500 g, 1.71 mmol) and 1.58 M solution of propellane **1-20** (1.19 mL, 1.88 mmol) in anhydrous THF (5.7 mL) at 0 ^{o}C. The reaction vial was sealed and stirred at 70 ^{o}C for 13 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (1% methanol in ethyl acetate) to provide **1-87** (0.341 g, 0.949 mmol, 56%) as a tan solid: **Rf** (ethyl acetate) 0.11; **Mp** (methylene chloride) 99-101 ^{o}C; **IR** 2938.9, 2857.9, 1674.2, 1576.6, 1479.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.76 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.76 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.65 (dd, *J* = 2.5, 0.6 Hz, 1 H), 8.65 (dd, *J* = 2.5, 0.6 Hz, 1 H), 7.72 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1 H), 7.72 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1 H), 7.49 (ddd, *J* = 8.1, 4.8, 0.8 Hz, 1 H), 7.49 (ddd, *J* = 8.1, 4.8, 0.8 Hz, 1 H), 4.82 (quintet, *J* = 2.5 Hz, 2 H), 4.82 (quintet, *J* = 2.5 Hz, 2 H), 4.66 (d, *J* = 12.6 Hz, 1 H), 4.66 (d, *J* = 12.6 Hz, 1 H), 4.56 (t, *J* = 3.0 Hz, 1 H), 4.56 (t, *J* = 3.0 Hz, 1 H), 4.49 (d, *J* = 12.6 Hz, 1 H), 4.49 (d, *J* = 12.6 Hz, 1 H), 4.25 (tt, *J* = 8.1, 6.7 Hz, 1 H), 4.25 (tt, *J* = 8.1, 6.7 Hz, 1 H), 3.47-3.37 (m, 2 H), 3.47-3.37 (m, 2 H), 3.31-3.23 (m, 2 H), 3.31-3.23 (m, 2 H), 2.77 (dddtd, *J* = 15.6, 6.6, 3.9, 2.6, 1.2 Hz, 2 H), 2.81-2.73 (m, 2 H), 1.66-1.37 (m, 7 H), 1.66-1.37 (m, 7 H); **^{13}C NMR** ($CDCl_3$, 400 MHz) δ 152.9, 152.5, 151.0, 148.2, 143.2, 134.8, 130.3, 124.0, 107.6, 98.2, 61.6, 58.7, 40.69, 40.68, 34.9, 30.0, 25.1, 18.7; **LC-MS** (ES) *m/z* calcd for $C_{18}H_{23}O_2N_4S$ $[M+H]^+$ 359.1536, found 359.1535.



1-88

(5-((3-Methylenecyclobutyl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-88**). A solution of **1-87** (0.340 g, 0.949 mmol) in methanol (6.5 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0902 g, 0.474 mmol) at room temperature and stirred for 21 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (10 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **1-88** (0.249 g, 0.906 mmol, 96%) as a tan solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.75 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.68 (dd, *J* = 2.5, 0.7 Hz, 1 H), 7.98 (ddd, *J* = 8.1, 2.6, 1.5 Hz, 1 H), 7.64 (ddd, *J* = 8.1, 4.8, 0.8 Hz, 1 H), 5.46 (t, *J* = 5.6 Hz, 1 H), 4.81 (quintet, *J* = 2.5 Hz, 2 H), 4.44 (d, *J* = 5.6 Hz, 2 H), 4.02 (tt, *J* = 8.1, 6.6 Hz, 1 H), 3.17-3.09 (m, 2 H), 2.73-2.65 (m, 2 H); **^{13}C NMR** ($(CD_3)_2SO$, 400 MHz) δ 156.0, 151.1, 150.6, 148.4, 143.9, 135.7, 130.7, 124.8, 107.9, 100.0, 54.0, 35.4; **LC-MS** (ES) *m/z* calcd for $C_{13}H_{15}ON_4S$ $[M+H]^+$ 275.0961, found 275.0959.

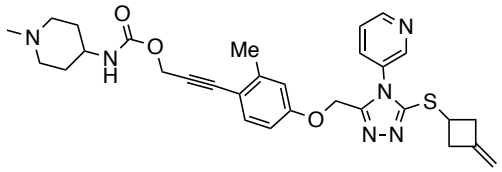


1-89

3-(2-Methyl-4-((5-((3-methylenecyclobutyl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-ol (**1-89**). Methanesulfonyl chloride (0.031 mL, 0.40 mmol) was added dropwise to mixture of **1-88** (0.100 g, 0.365 mmol), N,N-diisopropylethylamine (0.079 mL, 0.46 mmol) in anhydrous methylene chloride (3.6 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred at room temperature for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with methylene chloride (15 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

4-(3-Hydroxyprop-1-yn-1-yl)-3-methylphenol (0.0650 g, 0.401 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.238 g, 0.729 mmol) in anhydrous DMF (1.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 12 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (6% methanol in methylene chloride, dry loaded) to provide **1-89** (0.129 g, 0.307 mmol, 84%) as a tan solid: **Mp** (methylene chloride) 135-137 ^{o}C; **IR** 3242.5, 2920.2, 1669.9, 1604.2 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.76 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.62 (d, *J* = 2.5 Hz, 1 H), 7.69 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1 H), 7.48 (ddd, *J* = 8.1, 4.8, 0.6 Hz, 1 H), 7.26 (q, *J* = 2.9 Hz, 2 H), 6.72-6.61 (m, 2 H), 5.06 (s, 2 H), 4.84 (quintet, *J* = 2.5 Hz, 2 H), 4.51 (s, 2 H), 4.32-4.24 (m, 1 H), 3.30 (dddt, *J* = 17.3, 8.3, 4.3, 2.2 Hz, 2 H), 2.82-2.74 (m, 2 H), 2.35 (s, 3 H). ; **^{13}C NMR** ($CDCl_3$, 500 MHz) δ 157.3, 153.5, 151.5, 151.4, 148.0,

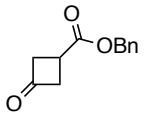
143.2, 142.5, 134.8, 133.8, 133.7, 130.1, 124.3, 116.7, 116.2, 115.9, 112.1, 107.9, 90.4, 84.2, 59.9, 51.9, 40.8, 35.1, 21.0; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{23}O_2N_4S$ $[M+H]^+$ 419.1536, found 419.1536.



1-90

3-(2-Methyl-4-((5-((3-methylenecyclobutyl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-90**). Carbonyldiimidazole (0.0285 g, 0.176 mmol) was added to a solution of **1-89** (0.0490 g, 0.117 mmol) in anhydrous methylene chloride (1.2 mL) and stirred for 2 h under nitrogen. 4-Amino-1-methylpiperidine (0.044 mL, 0.35 mmol) was added and stirred for an additional 22 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18 silica (65% acetonitrile in water, dry loaded) to provide **1-90** (0.0212 g, 0.0379 mmol, 32%) as an off-white solid: **Rf** (50% methanol in ethyl acetate) 0.11; **Mp** (methylene chloride) 143-145 ^oC; **IR** 3187.5, 2943.5, 2851.9, 2780.1, 1702.46 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76-8.75 (m, 1 H), 8.61 (d, *J* = 2.2 Hz, 1 H), 7.68-7.66 (m, 1 H), 7.46 (dd, *J* = 8.1, 4.8 Hz, 1 H), 7.28 (d, *J* = 8.5 Hz, 1 H), 6.67 (d, *J* = 1.9 Hz, 1 H), 6.62 (dd, *J* = 8.6, 2.3 Hz, 1 H), 5.06 (s, 2 H), 4.90 (s, 2 H), 4.83 (t, *J* = 2.3 Hz, 2 H), 4.75 (s, 1 H), 4.30-4.24 (m, 1 H), 3.53 (s, 1 H), 3.32-3.27 (m, 2 H), 2.80-2.75 (m, 4 H), 2.35 (s, 3 H), 2.28 (s, 3 H), 2.11 (t, *J* = 11.0 Hz, 3 H), 1.95 (d, *J* = 11.5 Hz, 2 H), 1.54-1.46 (m, 2 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 157.3, 154.8, 153.2, 151.4, 151.3, 147.9, 143.0, 142.7, 134.6, 133.7, 129.9, 124.1, 115.8, 115.7, 111.9,

107.6, 86.5, 84.7, 59.8, 54.2, 53.3, 46.0, 40.7, 35.0, 32.3, 20.8; **LC-MS** (ES) *m/z* calcd for $C_{30}H_{35}O3N_6S$ $[M+H]^+$ 559.2486, found 559.2486.



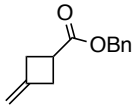
1-91

Benzyl 3-oxocyclobutane-1-carboxylate (**1-91**). Benzyl bromide (5.76 mL, 48.2 mmol) was added dropwise to a mixture of 3-oxocyclobutane-1-carboxylic acid **1-32** (5.00 g, 43.8 mmol) and potassium carbonate (18.2 g, 131.5 mmol) in acetonitrile (86 mL) at room temperature under an atmosphere of nitrogen and stirred for 24 h. The reaction mixture filtered through Celite and concentrated under reduced pressure. The resulting residue was purified by chromatography on silica (20-25% ethyl acetate in hexane) to provide **1-91** (6.81 g, 33.3 mmol, 76%) as a colorless liquid: **Rf** (20% ethyl acetate in hexane) 0.44; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.37 (s, 5 H), 5.20 (s, 2 H), 3.50-3.24 (m, 5 H).

Petasis Reagent

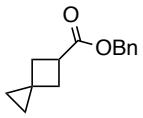
Petasis reagent. A 1.45 M solution of methyl lithium in ether (8.73 mL, 12.7 mmol) was added dropwise to a solution of titanocene dichloride (1.50 g, 6.03 mmol) in anhydrous diethyl ether (16 mL) under an atmosphere of nitrogen at 0 ^oC. The reaction mixture was stirred for 2 h and then carefully quenched with water (15 mL). The isolated aqueous solution was extracted with diethyl ether (25 mL, three times). The combined organic solutions were dried ($MgSO_4$),

filtered and concentrated under reduced pressure to provide the **Petasis reagent** (1.21 g, 5.21 mmol, 87%) as an orange solid. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.06 (s, 10 H), -0.15 (s, 6 H).



1-92

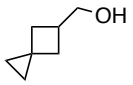
Benzyl 3-methylenecyclobutane-1-carboxylate (**1-92**). Freshly prepared Petasis reagent (1.02 g, 4.89 mmol) was added to a high pressure reaction vessel containing a solution of **1-91** (1.00 g, 4.89 mmol) in anhydrous THF (10 mL) under nitrogen. The reaction vessel was sealed, wrapped in aluminum foil, and heated to 60 ^{o}C in the dark for 5 d. The reaction mixture was diluted with diethyl ether (25 mL) and a saturated aqueous solution of sodium bicarbonate (15 mL). The resulting mixture was filtered through Celite and the resulting filtrate was extracted with diethyl ether (25 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (15-20% ethyl acetate in hexane) to provide **1-92** (0.492 g, 2.43 mmol, 50%) as a yellow oil: **Rf** (20% ethyl acetate in hexane) 0.73; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.38-7.32 (m, 5 H), 5.14 (s, 2 H), 4.81 (quintet, J = 2.4 Hz, 2 H), 3.22-3.14 (m, 1 H), 3.07-2.99 (m, 2 H), 2.95-2.87 (m, 2 H).



1-93

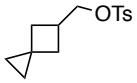
Benzyl spiro[2.3]hexane-5-carboxylate (**1-93**). A solution of trifluoroacetic acid (0.43 mL, 5.9 mmol) in anhydrous methylene chloride (6 mL) was added dropwise to a solution of

diethyl zinc (0.57 mL, 5.9 mmol) in anhydrous methylene chloride (25 ml) at 0 ^{o}C under an atmosphere of nitrogen. A solution of diiodomethane (0.45 mL, 5.9 mmol) in anhydrous methylene chloride (6 ml) was added dropwise and stirred for 1 h. A solution of **1-92** (0.492 g, 2.43 mmol) in anhydrous methylene chloride (2.5 mL) was added dropwise and the reaction mixture was warmed to room temperature and stirred for 24 h. H NMR of the reaction mixture indicated an approximate 1:1 mixture of starting material to product. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (15 mL) and extracted with methylene chloride (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide a crude mixture. A solution of trifluoroacetic acid (0.428 mL, 5.95 mmol) in anhydrous methylene chloride (6 mL) was added dropwise to a solution of diethyl zinc (0.57 mL, 5.9 mmol) in anhydrous methylene chloride (25 ml) at 0 ^{o}C under an atmosphere of nitrogen. A solution of diiodomethane (0.45 mL, 5.9 mmol) in anhydrous methylene chloride (6 ml) was added dropwise and stirred for 1 h. A solution of the crude mixture in anhydrous methylene chloride (2.5 mL) was added dropwise and the reaction mixture was warmed to room temperature and stirred for 48 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (15 mL) and extracted with methylene chloride (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **1-93** (0.427 g, 1.97 mmol, 81%) as a yellow oil. **Rf** (20% ethyl acetate in hexane) 0.64; **IR** 3067.9, 3033.6, 2933.1, 2859.5, 1731.2 cm^{-1} **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.39-7.35 (m, 4 H), 7.39-7.30 (m, 1 H), 5.15 (s, 2 H), 3.33 (tt, J = 9.2, 7.4 Hz, 1 H), 2.54-2.50 (m, 2 H), 2.27-2.23 (m, 2 H), 0.48-0.40 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 175.4, 136.3, 128.5, 128.1, 66.2, 33.78, 33.68, 16.7, 12.4, 11.1; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{17}O_2$ $[M+H]^+$ 217.1223, found 217.1222.



1-94

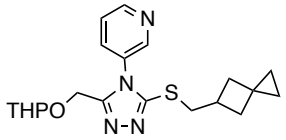
Spiro[2.3]hexan-5-ylmethanol (**1-94**). A 1M solution of lithium aluminum hydride in diethyl ether (0.93 mL, 0.93 mmol) was added to a solution of **1-93** (0.100 g, 0.462 mmol) in anhydrous THF (1.85 mL) at -78 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to 0 ^{o}C and stirred for 1. The reaction mixture was sequentially diluted with water (0.056 mL), 15% aqueous sodium hydroxide (0.056 mL), and water (0.14 mL). The mixture was warmed to room temperature and stirred for 15 minutes. Anhydrous magnesium sulfate was added and stirred for 15 minutes. The mixture was filtered through a pad of Celite and the pad of Celite was washed with diethyl ether (10 mL). The combined organic solution were concentrated under reduced pressure and carried forward without further purification. **Rf** (20% ethyl acetate in hexane) 0.26.



1-95

Spiro[2.3]hexan-5-ylmethyl 4-methylbenzenesulfonate (**1-95**). A solution of crude **1-94** (0.0519 g, 0.462) in anhydrous pyridine (1 mL) was treated with *p*-toluenesulfonyl chloride (0.220 g, 1.16 mmol) at 0 ^{o}C under nitrogen and stirred for 2.5 h. The reaction mixture was quenched with water (5 mL) and extracted with diethyl ether (10 mL, three times). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (10% ethyl acetate in hexane, dry loaded absorbed onto Celite) to provide **1-95** (0.0814 g, 0.306 mol, 66%, two steps) as a colorless oil: **Rf** (20% ethyl acetate in

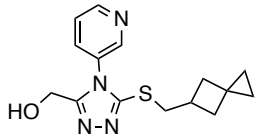
hexane) 0.52; **IR** 3067.1, 2956.4, 2927.1, 2855.9, 1598.5, 1358.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.82-7.79 (m, 2 H), 7.35 (dd, *J* = 8.6, 0.6 Hz, 2 H), 4.10 (d, *J* = 7.3 Hz, 2 H), 2.75-2.64 (m, 1 H), 2.45 (s, 3 H), 2.16-2.11 (m, 2 H), 1.81-1.77 (m, 2 H), 0.40-0.30 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 144.6, 133.4, 129.8, 127.9, 74.4, 32.8, 21.6, 16.5, 11.9, 11.6; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{19}O_3S$ $[M+H]^+$ 267.1049, found 267.1049.



1-96

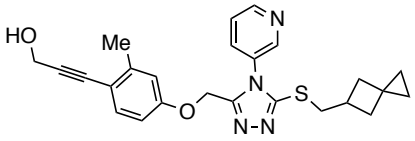
3-(3-((Spiro[2.3]hexan-5-ylmethyl)thio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**1-96**). **1-95** (0.50 mL, 1.7 mmol) was added to a high pressure reaction vial containing a mixture of **1-13** (0.500 g, 1.71 mmol), and cesium carbonate (0.697 g, 2.14 mmol) in DMF (3.42 mL). The reaction vial was sealed and heated to 80 ^oC for 2.5 h. The reaction mixture was cooled to room temperature, diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane) to provide **1-96** (0.359 g, 0.930 mmol, 54%) as a colorless oil: **Rf** (ethyl acetate) 0.33; **IR** 3414.5, 3066.5, 2944.9, 2850.8, 1484.4, 1445.5 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (d, *J* = 3.0 Hz, 1 H), 8.64 (s, 1 H), 7.72 (ddd, *J* = 8.1, 2.1, 1.6 Hz, 1 H), 7.48 (dd, *J* = 8.0, 4.8 Hz, 1 H), 4.65 (d, *J* = 12.6 Hz, 1 H), 4.55 (d, *J* = 3.3 Hz, 1 H), 4.48 (d, *J* = 12.6 Hz, 1 H), 3.45-3.38 (m, 4 H), 2.80-2.73 (m, 1 H), 2.19-2.15 (m, 2 H), 1.84 (dd, *J* = 12.4, 6.4 Hz, 2 H), 1.63-1.38 (m, 6 H), 0.39-0.33 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 153.1, 152.9, 150.9,

148.3, 130.4, 123.9, 98.2, 61.6, 58.7, 39.3, 36.0, 30.1, 29.9, 25.1, 18.7, 16.1, 11.72, 11.6; **LC-MS** (ES) *m/z* calcd for $C_{20}H_{27}O_2N_4S$ $[M+H]^+$ 387.1849, found 387.1848.



1-97

(4-(Pyridin-3-yl)-5-((spiro[2.3]hexan-5-ylmethyl)thio)-4H-1,2,4-triazol-3-yl)methanol (**1-97**). A solution of **1-96** (0.359 g, 0.929 mmol) in methanol (6.2 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0883 g, 0.464 mmol) at room temperature and stirred for 17 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (10 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-97** (0.278 g, 0.919 mmol, 99%) as white solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.67 (d, *J* = 2.3 Hz, 1 H), 7.97 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1 H), 7.64 (ddd, *J* = 8.1, 4.8, 0.5 Hz, 1 H), 5.44 (t, *J* = 5.6 Hz, 1 H), 4.43 (d, *J* = 5.6 Hz, 2 H), 3.32 (s, 1 H), 3.26-3.25 (m, 2 H), 2.64 (td, *J* = 11.3, 5.4 Hz, 1 H), 2.50 (t, *J* = 1.8 Hz, 4 H), 2.09-2.04 (m, 2 H), 1.83-1.79 (m, 2 H), 0.39-0.32 (m, 4 H); **LC-MS** (ES) *m/z* calcd for $C_{15}H_{19}ON_4S$ $[M+H]^+$ 303.1274, found 303.1006.

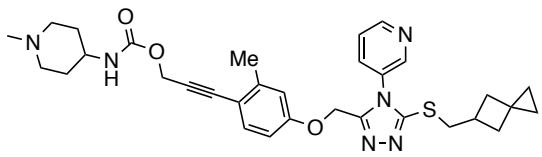


1-98

3-(2-Methyl-4-((4-(pyridin-3-yl)-5-((spiro[2.3]hexan-5-ylmethyl)thio)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-ol (**1-98**). Methanesulfonyl chloride (0.028 mL, 0.36 mmol) was added dropwise to a mixture of **1-97** (0.100 g, 0.331 mmol), N,N-diisopropylethylamine (0.086 mL, 0.49 mmol) in anhydrous methylene chloride (3.3 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

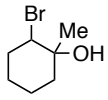
4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol (0.0590 g, 0.364 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.216 g, 0.661 mmol) in anhydrous DMF (1.7 mL) under an atmosphere of nitrogen and stirred at room temperature for 15 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-98** (0.123 g, 0.277 mmol, 84%) as a white solid: **Rf** (ethyl acetate) 0.52; **Mp** (methylene chloride) 128-130 ^{o}C; **IR** 3216.4, 2919.9, 2851.6, 1601.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76 (d, *J* = 3.6 Hz, 1 H), 8.62 (s, 1 H), 7.68 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1 H), 7.48-7.46 (m, 1 H), 6.68 (d, *J* = 2.5 Hz, 1 H), 6.63 (dd, *J* = 8.5, 2.6 Hz, 1 H), 5.07 (s, 2 H), 4.51 (s, 2 H), 3.47 (d, *J* = 7.8 Hz, 2 H), 2.82-2.76 (m, 1 H), 2.36 (s, 3 H), 2.21-2.17 (m, 2 H), 1.88-1.84 (m, 3 H), 0.41-0.35 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 157.1, 151.1, 147.9, 142.3, 134.8, 133.5, 124.1, 116.1, 115.7, 111.9,

100.0, 90.5, 83.8, 59.8, 51.6, 39.3, 36.0, 30.1, 20.9, 16.1, 11.7, 11.6; **LC-MS** (ES) *m/z* calcd for $C_{25}H_{27}O_2N_4S$ $[M+H]^+$ 447.1849, found 447.1848.



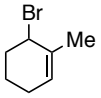
1-99

3-(2-Methyl-4-((4-(pyridin-3-yl)-5-((spiro[2.3]hexan-5-ylmethyl)thio)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-99**). Carbonyldiimidazole (0.0272 g, 0.168 mmol) was added to a solution of **1-98** (0.0490 g, 0.117 mmol) in anhydrous methylene chloride (1.1 mL) and stirred for 2 h under nitrogen. 4-Amino-1-methylpiperidine (0.042 mL, 0.34 mmol) was added and stirred for an additional 21 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18 silica (65% acetonitrile in water, dry loaded) to provide **1-99** (0.0315 g, 0.0537 mmol, 48%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.17; **Mp** (methylene chloride) 48-50 ^oC; **IR** 3270.2, 2944.2, 2850.1, 2792.1, 1714.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (dd, *J* = 4.8, 1.3 Hz, 1 H), 8.61 (d, *J* = 2.3 Hz, 1 H), 7.67 (ddd, *J* = 8.1, 2.3, 1.6 Hz, 1 H), 7.46 (dd, *J* = 8.1, 4.9 Hz, 1 H), 7.28 (d, *J* = 8.5 Hz, 1 H), 6.68 (d, *J* = 2.3 Hz, 1 H), 6.63 (dd, *J* = 8.5, 2.4 Hz, 1 H), 5.06 (s, 2 H), 4.90 (s, 2 H), 4.80 (s, 1 H), 3.60 (s, 1 H), 3.46 (d, *J* = 7.8 Hz, 2 H), 2.94 (s, 2 H), 2.83-2.74 (m, 1 H), 2.40 (s, 2 H), 2.35 (s, 3 H), 2.21-2.17 (m, 2 H), 2.02 (d, *J* = 11.1 Hz, 2 H), 1.86 (dd, *J* = 12.5, 6.4 Hz, 2 H), 0.39-0.36 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 157.3, 153.8, 151.4, 151.2, 148.0, 142.7, 134.6, 133.7, 130.0, 124.0, 115.7, 111.9, 86.4, 84.7, 60.4, 59.9, 54.1, 53.4, 45.7, 39.3, 36.0, 31.9, 31.6, 30.1, 22.6, 21.0, 20.8, 16.1, 14.2, 14.1, 11.7, 11.6; **LC-MS** (ES) *m/z* calcd for $C_{32}H_{39}O_3N_6S$ $[M+H]^+$ 587.2799 , found 587.2801.



1-101

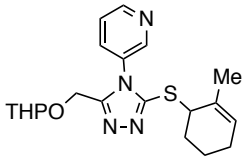
2-Bromo-1-methylcyclohexan-1-ol (**1-101**). N-Bromosuccinimide (5.09 g, 28.6 mmol) was added to a mixture of 1-methylcyclohex-1-ene **1-100** (3.08 mL, 25.9) in acetone (85 mL) and water (45 mL) at 0 ^{o}C. The reaction was warmed to room temperature and stirred for 21 h. Acetone was removed under reduced pressure and the resulting aqueous solution was extracted with methylene chloride (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40-50% ethyl acetate in hexane, dry loaded) to provide **1-101** (3.49 g, 18.1 mmol, 70%) as a light-yellow oil: **Rf** (50% ethyl acetate in hexane) 0.69; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 4.15 (dd, *J* = 11.6, 4.3 Hz, 1 H), 2.29-2.20 (m, 1 H), 2.11 (s, 1 H), 2.04-1.97 (m, 1 H), 1.89-1.83 (m, 1 H), 1.75-1.67 (m, 2 H), 1.56-1.37 (m, 3 H), 1.35 (s, 3 H).



1-102

6-Bromo-1-methylcyclohex-1-ene (1-102). Boron trifluoride diethyl etherate (2.13 mL, 36.2mmol) was added dropwise to a solution of **1-101** (3.49 g, 18.1 mmol) in anhydrous methylene chloride (90 mL) at room temperature under nitrogen. The reaction mixture was heated at reflux for 2 h and then cool to room temperature. The reaction mixture was quenched with a 50% aqueous solution of sodium bicarbonate (50 mL). The isolated aqueous solution was extracted with methylene chloride (50 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by kugelrohr

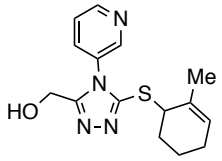
distillation (high vacuum, ca. 70-75 ^{o}C) to provide **1-101** (1.89 g, 10.8 mmol, 60 %) as a colorless oil: **Rf** (20% ethyl acetate in hexane) 0.87; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.61 (ddd, *J* = 5.0, 2.6, 1.3 Hz, 1 H), 4.67 (s, 1 H), 2.28-2.23 (m, 1 H), 2.21-2.07 (m, 3 H), 2.03-1.99 (m, 1 H), 1.96-1.89 (m, 1 H), 1.81-1.80 (m, 3 H), 1.70-1.65 (m, 1 H).



1-103

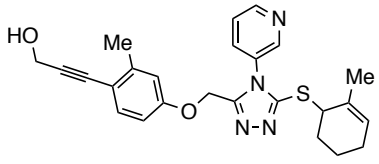
3-(3-((2-Methylcyclohex-2-en-1-yl)thio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**1-103**). **1-102** (0.374 g, 2.14 mmol) was added to a high pressure reaction vial containing a mixture of **1-13** (0.500 g, 1.71 mmol), cesium carbonate (0.697 g, 2.14 mmol) in anhydrous DMF (3.5 mL). The reaction vial was sealed and stirred at 80 ^{o}C for 2 h. The reaction mixture was cooled to room temperature, diluted with a saturated aqueous solution of sodium bicarbonate (15 mL), and extracted with ethyl acetate (25 mL, three times). The combined organic solutions were washed with water (25 mL), brine (25 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate) to provide **1-103** (0.542 g, 1.40 mmol, 82%) as white solid: **Rf** (ethyl acetate) 0.14; **Mp** (methanol): 121-123 ^{o}C**; IR** 3046.5, 2937.6, 1482.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (d, *J* = 4.1 Hz, 1 H), 8.66 (s, 1 H), 7.73 (dt, *J* = 8.1, 1.9 Hz, 1 H), 7.49 (dd, *J* = 8.1, 4.8 Hz, 1 H), 5.58 (s, 1 H), 4.67 (dd, *J* = 12.6, 1.3 Hz, 1 H), 4.56 (s, 1 H), 4.50 (dd, *J* = 12.6, 1.5 Hz, 1 H), 4.35 (s, 1 H), 3.46-3.38 (m, 2 H), 2.15 (ddq, *J* = 12.4, 5.2, 2.6 Hz, 1 H), 1.97-1.90 (m, 3 H), 1.70 (dd, *J* = 8.3, 2.3 Hz, 3 H), 1.67-1.39 (m, 8 H); **^{13}C NMR** ($CDCl_3$, 500 MHz) δ 153.3, 152.9, 150.8, 148.4, 135.0, 130.9, 130.9, 130.5, 128.2, 127.2, 123.9, 98.2, 61.6, 58.8, 49.3,

30.0, 29.7, 25.1, 25.0, 22.1, 18.7, 17.6; **LC-MS** (ES) *m/z* calcd for $C_{20}H_{27}O_2N_4S$ $[M+H]^+$ 387.1849, found 387.1846.



1-104

(5-((2-Methylcyclohex-2-en-1-yl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol (**1-104**). A solution of **1-103** (0.500 g, 1.29 mmol) in methanol (8.6 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.123 g, 0.647 mmol) at room temperature and stirred for 17 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (25 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (25 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-104** (0.383 g, 1.27 mmol, 98%) as white solid: **^{1}H NMR** ($(CD_3)_2SO$, 400 MHz) δ 8.75 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.70 (dd, *J* = 2.5, 0.6 Hz, 1 H), 8.00 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1 H), 7.64 (ddd, *J* = 8.1, 4.8, 0.8 Hz, 1 H), 5.56-5.55 (m, 1 H), 5.46 (t, *J* = 5.6 Hz, 1 H), 4.45 (d, *J* = 5.6 Hz, 2 H), 3.94 (d, *J* = 0.4 Hz, 1 H), 1.96-1.90 (m, 3 H), 1.83-1.74 (m, 1 H), 1.57-1.53 (m, 4 H); **LC-MS** (ES) *m/z* calcd for $C_{15}H_{19}ON_4S$ $[M+H]^+$ 303.1274, found 303.1272.

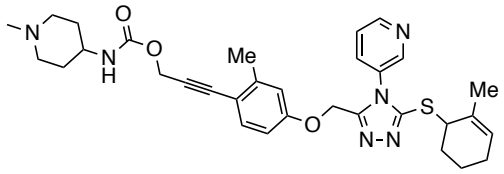


1-105

3-(2-Methyl-4-((5-((2-methylcyclohex-2-en-1-yl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-ol (**1-105**). Methanesulfonyl chloride (0.028 mL, 0.36 mmol) was added dropwise to mixture of **1-104** (0.100 g, 0.331 mmol), N,N-diisopropylethylamine (0.072 mL, 0.41 mmol) in anhydrous methylene chloride (3.3 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred at room temperature for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with methylene chloride (15 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

4-(3-Hydroxyprop-1-yn-1-yl)-3-methylphenol (0.0590 g, 0.364 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.216 g, 0.661 mmol) in anhydrous DMF (1.65 mL) under an atmosphere of nitrogen and stirred at room temperature for 17 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (50% ethyl acetate in hexane, dry loaded) to provide **1-105** (0.0392 g, 0.0878 mmol, 27%) as an off-white solid: **Rf** (5% methanol in ethyl acetate) 0.44; **Mp** (methylene chloride) 153-155 ^{o}C; **IR** 3290.9, 2918.4, 2856.6, 2232.6, 1603.8, 1485.5 cm^{-1}; ^{1}**H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (d, J = 4.1 Hz, 1 H), 8.63 (s, 1 H), 7.70-7.69 (m, 1 H), 7.48-7.45 (m, 1 H), 7.26 (d, J = 4.5 Hz, 1 H), 6.67 (s, 1 H), 6.64-6.62 (m, 1 H), 5.60 (s, 1H), 5.06 (s, 2 H), 4.51 (s, 2 H), 4.40 (s, 1 H), 2.35 (s, 3 H), 2.18-2.15 (m, 1 H), 1.98-1.93 (m, 3

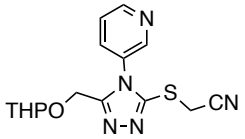
H), 1.72 (s, 3 H), 1.65 (s, 2 H); **^{13}C NMR** ($CDCl_3$, 500 MHz) δ 157.2, 154.1, 151.4, 151.1, 148.0, 142.4, 134.8, 133.5, 130.8, 130.1, 128.4, 124.1, 116.0, 115.7, 111.9, 90.3, 84.0, 59.8, 51.7, 49.3, 29.7, 25.0, 22.1, 20.9, 17.7; **LC-MS** (ES) *m/z* calcd for $C_{25}H_{27}O_2N_4S$ $[M+H]^+$ 447.1849, found 447.1848.



1-106

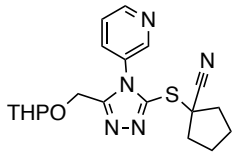
3-(2-Methyl-4-((5-((2-methylcyclohex-2-en-1-yl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-106**). Carbonyldiimidazole (0.0458 g, 0.282 mmol) was added to a solution of **1-105** (0.0840 g, 0.188 mmol) in anhydrous methylene chloride (1.88 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.0708 mL, 0.564 mmol) was added and stirred for 11 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (8-10% methanol in methylene chloride, dry loaded) to provide **1-106** (0.0641 g, 0.109 mmol, 58%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.14; **Mp** (methylene chloride) 53-55 ^{o}C; **IR** 3265.3, 2939.0, 2790.4, 1701.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (dd, *J* = 4.7, 1.1 Hz, 1 H), 8.63 (d, *J* = 2.5 Hz, 1 H), 7.68 (dt, *J* = 8.1, 1.8 Hz, 1 H), 7.46 (dd, *J* = 8.2, 4.8 Hz, 1 H), 7.29 (d, *J* = 8.5 Hz, 1 H), 6.69 (d, *J* = 2.3 Hz, 1 H), 6.64 (dd, *J* = 8.5, 2.5 Hz, 1 H), 5.60 (d, *J* = 0.2 Hz, 1 H), 5.07 (s, 2 H), 4.90 (s, 2 H), 4.74-4.72 (m, 1 H), 4.40 (d, *J* = 0.3 Hz, 1 H), 3.65 (s, 1 H), 3.53 (s, 1 H), 2.78 (s, 2 H), 2.36 (s, 3 H), 2.29 (s, 4 H), 2.19-2.08 (m, 4 H), 1.99-1.93 (m, 7 H), 1.72 (s, 3 H), 1.66-1.63 (m, 2 H), 1.55-1.46 (m, 3 H); **^{13}C NMR** ($CDCl_3$, 500 MHz) δ δ 157.3, 154.9, 154.0, 151.4, 151.1, 148.0, 142.7, 134.8, 133.7, 132.2, 130.8, 130.0, 128.4, 124.0, 115.7, 111.9,

86.4, 84.7, 59.8, 54.3, 53.3, 49.3, 46.1, 32.3, 29.7, 25.0, 22.1, 20.9, 17.7; **LC-MS** (ES) *m/z* calcd for $C_{32}H_{39}O_3N_6S$ $[M+H]^+$ 587.2799, found 587.2799.



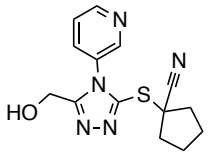
1-107

2-((4-(Pyridin-3-yl)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-3-yl)thio)acetonitrile (**1-107**). Iodoacetonitrile (0.19 mL, 2.7 mmol) was added to a mixture of **1-13** (0.650 g, 2.22 mmol) and cesium carbonate (1.09 g, 3.33 mmol) in DMF (4.4 mL) and stirred at 80 ^oC for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (85% ethyl acetate in hexane, dry loaded) to provide **1-107** (0.714 g, 2.15 mmol, 97%) as a brown tar: **Rf** (ethyl acetate) 0.21; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.79-8.77 (m, 2H), 8.08 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1H), 7.68 (ddd, *J* = 8.1, 4.8, 0.7 Hz, 1H), 4.63 (d, *J* = 12.8 Hz, 1H), 4.54 (d, *J* = 12.8 Hz, 1H), 4.48 (t, *J* = 3.0 Hz, 1H), 4.26 (d, *J* = 0.4 Hz, 2H), 3.29-3.26 (m, 1H), 3.22-3.16 (m, 1H), 1.47-1.34 (m, 4H), 1.30-1.22 (m, 2H).; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{18}O_2N_4S$ $[M+H]^+$ 332.1176, found 332.1143.



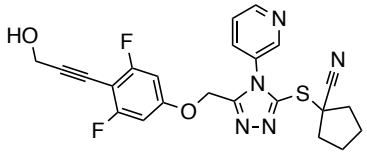
1-108a

1-((4-(Pyridin-3-yl)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-3-yl)thio)cyclopentane-1-carbonitrile (**1-108a**). Sodium hydride (0.0380 g, 1.58 mmol) was added to a solution of **1-107** (0.250 g, 0.754 mmol) in anhydrous DMF (15 mL) at 0 ^{o}C under an atmosphere of nitrogen and stirred for 5 minutes. 1,4-Dibromobutane (0.090 mL, 0.75 mmol) was added and the reaction mixture was warmed to room temperature and stirred for an additional 16 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (10 mL) the resulting aqueous solution was extracted with methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (60-80% ethyl acetate in hexane) to provide **1-108a** (0.240 g, 0.623 mmol, 83%) as a brown oil: **Rf** (ethyl acetate) 0.44; **Mp** (methylene chloride) ^{o}C; **IR** 2943.8, 2874.2, 1483.7, 1440.2 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.80 (s, 1H), 8.65 (s, 1H), 7.85 (d, *J* = 8.1 Hz, 1H), 7.55-7.51 (m, 1H), 4.73 (d, *J* = 12.7 Hz, 1H), 4.58-4.54 (m, 2H), 3.49 (s, 1H), 3.45-3.41 (m, 2H), 2.35-2.31 (m, 4H), 2.01-1.83 (m, 5H), 1.63-1.41 (m, 9H).; **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 154.5, 151.1, 148.8, 148.1, 135.8, 123.8, 121.2, 98.4, 61.7, 58.9, 48.9, 39.91, 39.85, 29.9, 25.0, 24.0, 18.7; **LC-MS** (ES) *m/z* calcd for $C_{19}H_{24}O_2N_5S$ $[M+H]^+$ 386.1645, found 386.1642.



1-109a

1-((5-(Hydroxymethyl)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)cyclopentane-1-carbonitrile (**1-109a**). A solution of **1-108a** (0.300 g, 0.778 mmol) in methanol (5.2 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0740 g, 0.389 mmol) at room temperature and stirred for 18 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were washed with a saturated aqueous solution of sodium bicarbonate (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-109a** (0.200 g, 0.664 mmol, 85%) as an off-white solid: **^{1}H NMR** ($(CD_3)_2SO_3$, 500 MHz) δ 8.76-8.73 (m, 2H), 8.03 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.65 (dd, *J* = 8.1, 4.8 Hz, 1H), 5.56 (t, *J* = 5.6 Hz, 1H), 4.50 (d, *J* = 5.6 Hz, 2H), 2.24 (dd, *J* = 7.5, 6.0 Hz, 2H), 2.10-2.07 (m, 2H), 1.74-1.69 (m, 4H).; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{16}ON_5S$ $[M+H]^+$ 302.1070, found 302.1068.

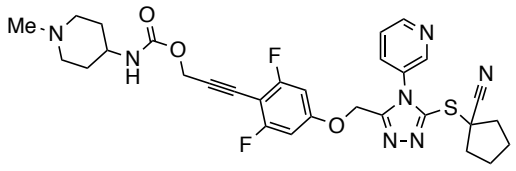


1-110a

1-((5-((3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenoxy)methyl)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)cyclopentane-1-carbonitrile (**1-110a**). Methanesulfonyl chloride

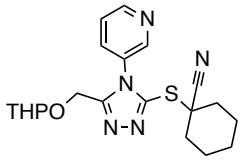
(0.031 mL, 0.39 mmol) was added dropwise to mixture of **1-1-109a** (0.100 g, 0.331 mmol), N,N-diisopropylethylamine (0.087 mL, 0.49 mmol) in anhydrous methylene chloride (3.3 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.733 g, 0.398 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.216 g, 0.664 mmol) in anhydrous DMF (1.6 mL) under an atmosphere of nitrogen and stirred at room temperature for 24 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded). The resulting material was purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water) to provide **1-110a** (0.118 g, 0.253 mmol, 76%) as a white solid: **Rf** (ethyl acetate) 0.57; **Mp** (methylene chloride) 185-187 ^{o}C; **IR** 3243.3, 1635.8, 1578.1, 1503.1 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.82 (d, *J* = 4.5 Hz, 1H), 8.62 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.53 (dd, *J* = 7.8, 4.9 Hz, 1H), 6.46 (d, *J* = 8.4 Hz, 2H), 5.11 (s, 2H), 4.52 (d, *J* = 5.9 Hz, 2H), 2.40-2.30 (m, 3H), 2.04-1.87 (m, 4H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.78, 164.72, 162.77, 162.71, 158.2, 151.9, 151.7, 149.2, 148.3, 135.4, 129.5, 124.1, 121.1, 98.97, 98.93, 98.79, 98.75, 96.37, 96.35, 72.2, 60.5, 51.6, 49.0, 40.0, 24.0; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -105.3; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{20}O_2N_5F_2S$ $[M+H]^+$ 468.1300, found 468.1298.



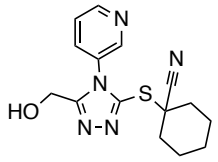
1-111a

3-(4-((5-((1-Cyanocyclopentyl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-111a**). Carbonyldiimidazole (0.0260 g, 0.160 mmol) was added to a solution of **1-110a** (0.0500 g, 0.107 mmol) in anhydrous methylene chloride (1.1 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.04 mL, 0.32 mmol) was added and stirred for an additional 14 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water, dry loaded) to provide **1-110a** (0.0554 g, 0.0912 mmol, 85%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.15; **Mp** (methylene chloride) 61-63 ^{o}C; **IR** 3328.1, 3054.3, 2944.9, 2785.3, 1718.2, 1635.8, 1574.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.82-8.81 (m, 1H), 8.62-8.61 (m, 1H), 7.80-7.77 (m, 1H), 7.55-7.51 (m, 1H), 6.47 (d, *J* = 8.4 Hz, 2H), 5.10 (s, 2H), 4.92 (s, 2H), 4.73-4.70 (m, 1H), 3.52 (s, 1H), 2.81-2.70 (m, 2H), 2.37-2.32 (m, 3H), 2.26 (s, 3H), 2.09-2.04 (m, 2H), 1.98-1.88 (m, 6H), 1.73 (s, 3H), 1.52-1.43 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.90, 164.84, 162.88, 162.82, 158.54, 158.43, 154.6, 151.9, 151.7, 149.2, 148.3, 135.4, 129.4, 124.1, 121.1, 98.97, 98.93, 98.79, 98.75, 95.3, 92.54, 92.51, 72.9, 60.6, 54.2, 53.1, 49.0, 46.1, 39.9, 32.4, 31.6, 24.0, 22.6, 14.1; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -104.9; **LC-MS** (ES) *m/z* calcd for $C_{30}H_{32}O_3N_7F_2S$ $[M+H]^+$ 608.2177, found 608.2244.



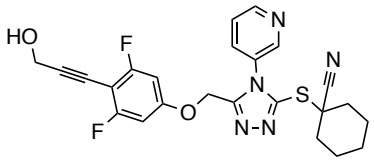
1-108b

1-((4-(Pyridin-3-yl)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-3-yl)thio)cyclohexane-1-carbonitrile (**1-108b**). Sodium hydride (0.0532 g, 2.22 mmol) was added to a solution of **1-107** (0.350 g, 0.754 mmol) in anhydrous DMF (21 mL) at 0 ^{o}C under an atmosphere of nitrogen and stirred for 5 minutes. 1,5-Dibromopentane (0.14 mL, 1.1 mmol) was added and the reaction mixture was warmed to room temperature and stirred for an additional 16 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (10 mL) the resulting aqueous solution was extracted with methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (60-80% ethyl acetate in hexane) to provide **1-108b** (0.329 g, 0.824 mmol, 88%) as a brown oil: **Rf** (ethyl acetate) 0.35; **IR** 2939.1, 2859.5, 1483.8, 1440.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.80-8.78 (m, 1H), 8.64 (dt, *J* = 1.0, 0.5 Hz, 1H), 7.84 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.54-7.50 (m, 1H), 4.73 (d, *J* = 12.7 Hz, 1H), 4.59-4.54 (m, 2H), 3.44-3.41 (m, 2H), 2.36-2.29 (m, 2H), 1.86-1.77 (m, 4H), 1.70-1.38 (m, 12H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 154.7, 151.1, 148.8, 146.9, 135.9, 130.1, 123.8, 119.9, 98.4, 61.7, 59.0, 47.7, 36.77, 36.72, 29.9, 25.0, 24.4, 23.3, 18.7; **LC-MS** (ES) *m/z* calcd for $C_{20}H_{26}O_2N_5S$ $[M+H]^+$ 400.1802, found 400.1796.



1-109b

1-((5-(Hydroxymethyl)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)cyclohexane-1-carbonitrile (**1-109b**). A solution of **1-108b** (0.300 g, 0.751 mmol) in methanol (5 mL) was treated with *p*-toluenesulfonic acid monohydrate (0.0714 g, 0.375 mmol) at room temperature and stirred for 18 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were washed with a saturated aqueous solution of sodium bicarbonate (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-109b** (0.228 g, 0.722 mmol, 96%) as a tan solid: **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.76-8.72 (m, 2H), 8.01 (ddd, *J* = 8.2, 2.5, 1.5 Hz, 1H), 7.65 (ddd, *J* = 8.2, 4.8, 0.6 Hz, 1H), 5.55 (t, *J* = 5.6 Hz, 1H), 4.50 (d, *J* = 5.6 Hz, 2H), 2.14-2.09 (m, 2H), 1.72-1.63 (m, 4H), 1.57-1.51 (m, 1H), 1.44-1.36 (m, 2H), 1.32-1.22 (m, 2H); **LC-MS** (ES) *m/z* calcd for $C_{15}H_{18}ON_5S$ $[M+H]^+$ 316.1227, found 316.1223.

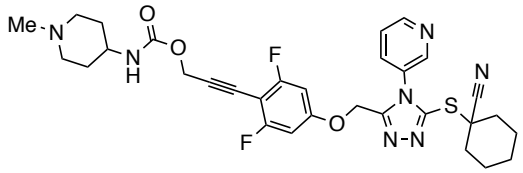


1-110b

1-((5-((3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenoxy)methyl)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)cyclohexane-1-carbonitrile (1-110b). Methanesulfonyl chloride (0.029

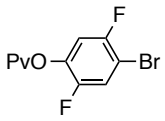
mL, 0.38 mmol) was added dropwise to mixture of 1-109b (0.100 g, 0.317 mmol), N,N-diisopropylethylamine (0.083 mL, 0.48 mmol) in anhydrous methylene chloride (3.2 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.0700 g, 0.380 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.207 g, 0.634 mmol) in anhydrous DMF (1.1 mL) under an atmosphere of nitrogen and stirred at room temperature for 24 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded). The resulting material was then purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water) to provide 1-110b (0.109 g, 0.228 mmol, 72%) as a white solid: Rf (ethyl acetate) 0.61; Mp (methylene chloride) 175-177 ^{o}C; IR 3203.6, 3095.0, 2942.9, 2859.2, 1633.3, 1577.8 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.81 (d, *J* = 4.4 Hz, 1H), 8.61 (t, *J* = 0.3 Hz, 1H), 7.79-7.78 (m, 1H), 7.54-7.51 (m, 1H), 6.46 (d, *J* = 8.4 Hz, 2H), 5.10 (s, 2H), 4.52 (d, *J* = 5.3 Hz, 2H), 2.37-2.32 (m, 2H), 2.03-2.01 (m, 1H), 1.85-1.78 (m, 4H), 1.70-1.66 (m, 1H), 1.64-1.55 (m, 4H), 1.33-1.30 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 164.78, 164.72, 162.77, 162.70, 158.2, 152.1, 151.6, 148.4, 148.0, 135.8, 135.5, 129.6, 124.1, 119.8, 98.98, 98.93, 98.80, 98.75, 96.40, 96.37, 96.35, 95.57, 95.41, 72.1, 60.6, 51.6, 48.0, 36.8, 24.4, 23.3; ^{19}F NMR ($CDCl_3$, 376 MHz) δ -105.4; LC-MS (ES) *m/z* calcd for $C_{24}H_{22}O_2N_5F_2S$ $[M+H]^+$ 482.1457, found 482.1456.



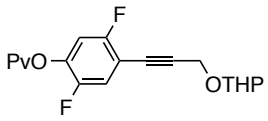
1-111b

3-(4-((5-((1-Cyanocyclohexyl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**1-111b**). Carbonyldiimidazole (0.0253 g, 0.156 mmol) was added to a solution of **1-110b** (0.0500 g, 0.104 mmol) in anhydrous methylene chloride (1.0 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.039 mL, 0.31 mmol) was added and stirred for an additional 14 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water, dry loaded) to provide **1-111b** (0.0372 g, 0.0598 mmol, 58%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.14; **Mp** (methylene chloride) 87-89 ^{o}C; **IR** 3045.9, 2939.5, 2858.4, 2785.9, 1717.4, 1635.3 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.81-8.80 (m, 1H), 8.60 (d, *J* = 2.2 Hz, 1H), 7.79-7.76 (m, 1H), 7.54-7.50 (m, 1H), 6.46 (d, *J* = 8.4 Hz, 2H), 5.10 (s, 2H), 4.92 (s, 2H), 4.76-4.73 (m, 1H), 3.52 (s, 1H), 2.76 (s, 2H), 2.36 (d, *J* = 12.2 Hz, 2H), 2.28 (s, 2H), 2.10 (t, *J* = 11.2 Hz, 2H), 1.97-1.93 (m, 2H), 1.86-1.79 (m, 4H), 1.71-1.44 (m, 6H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 152.1, 151.6, 148.4, 148.0, 135.5, 129.6, 124.0, 119.8, 98.98, 98.93, 98.75, 72.9, 60.6, 54.2, 53.09, 53.07, 48.0, 46.1, 36.8, 32.4, 31.6, 24.4, 23.3, 22.6, 14.1; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -104.9; **LC-MS** (ES) *m/z* calcd for $C_{31}H_{34}O_3N_7F_2S$ $[M+H]^+$ 622.2406, found 622.2397.



1-113

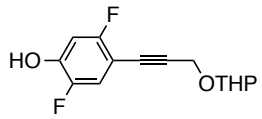
4-Bromo-2,5-difluorophenyl pivalate (**1-113**). Pivaloyl chloride (8.99 mL, 195 mmol) was slowly added to a solution of 4-bromo-2,5-difluorophenol **1-112** (20.0 g, 95.7 mmol) and triethylamine (14.8 mL) in methylene chloride (200 mL) at 0 ^{o}C under atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 4 h. The reaction mixture was diluted with ether (200 mL) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure and the resulting material was resuspended in diethyl ether (200 mL) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure to provide **1-113** (27.8 g, 94.9 mmol, 99%) as a tan solid: **Rf** (20% ethyl acetate in hexane) 0.76; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.38 (dd, *J* = 9.0, 6.2 Hz, 1H), 6.97 (dd, *J* = 8.1, 6.5 Hz, 1H), 1.36 (s, 9H); **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -110.5 (d, *J* = 14.4 Hz), -131.4 (d, *J* = 14.8 Hz).



1-114

2,5-Difluoro-4-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-yn-1-yl)phenyl pivalate (**1-114**). **1-113** (10.0 g, 34.1 mmol), 2-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran (6.24 mL, 44.4 mmol), copper (I) iodide (0.650 g, 3.41 mmol), tetrakis(triphenylphosphine)palladium(0) (1.97 g, 1.71 mmol) was added to a reaction vial. The reaction vial was degassed under vacuum and refilled with argon; this process was conducted three times. Degassed triethylamine (26.4 mL)

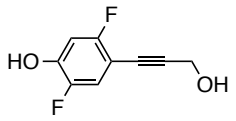
was added to reaction vial and the reaction mixture was purged with argon for 5 minutes. The reaction vial was sealed and stirred at 90 ^{o}C for 20 h. The reaction mixture was diluted with diethyl ether (100 mL) and filtered through a pad of Celite. The isolated filtrate was washed with a saturated aqueous solution of ammonium chloride (50 mL, twice), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (6% ethyl acetate in hexane) to provide **1-114** (8.85 g, 25.1 mmol, 74%) as a yellow oil: **Rf** (20% ethyl acetate in hexane) 0.56; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.22 (dd, *J* = 9.8, 6.3 Hz, 1H), 6.89 (dd, *J* = 8.8, 6.4 Hz, 1H), 4.88 (t, *J* = 3.3 Hz, 1H), 4.55-4.50 (m, 2H), 3.91-3.85 (m, 1H), 3.60-3.54 (m, 1H), 1.86-1.73 (m, 3H), 1.68-1.54 (m, 6H), 1.36 (s, 9H).



1-115

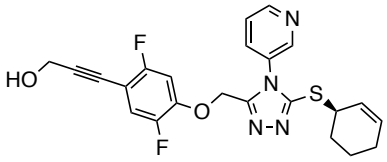
2,5-Difluoro-4-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-yn-1-yl)phenol (**1-115**). A 1 M aqueous solution of sodium hydroxide (164 mL, 164 mmol) was added dropwise to a solution of **1-114** (11.6 g, 32.8 mmol) in THF/MeOH (1:1, 320 mL) and stirred for 21 h. The organic solutions were removed under reduced pressure and the resulting aqueous solution was neutralized to pH 7 with a 1 M aqueous solution of potassium hydrogen sulfate. The aqueous solution was extracted with methylene chloride (150 mL, three times) and the combined organic solutions were dried ($MgSO_4$) and filtered through a pad of silica to provide **1-115** (7.31 g, 27.3 mmol, 83%) as an off-white solid: **Rf** (methylene chloride) 0.2; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.11 (dd, *J* = 10.4, 6.4 Hz, 1H), 6.72 (dd, *J* = 9.6, 7.4 Hz, 1H), 4.91 (t, *J* = 3.3 Hz, 1H), 4.54-4.44

(m, 2H), 3.92-3.86 (m, 1H), 3.60-3.56 (m, 1H), 1.87-1.69 (m, 3H), 1.69-1.52 (m, 5H); **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -112.7 (d, *J* = 14.8 Hz), -145.2 (d, *J* = 15.2 Hz).



1-116

2,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (**1-116**). Pyridinium *p*-toluenesulfonate (3.42 g, 13.6 mmol) was dded to a solution of **1-115** (7.31 g, 27.3 mmol) in methanol (182 mL) and stirred for 24 h at room temperature. The reaction mixture was water (100 mL) and extracted with methylene chloride (100 mL, thee times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40-50% ethyl acetate in hexane) to provide **1-116** (2.12 g, 11.5 mmol, 42%) as a light-yellow solid: **Rf** (25% ethyl acetate in hexane) 0.19; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 10.83 (d, *J* = 0.2 Hz, 1H), 7.30 (dd, *J* = 11.2, 6.9 Hz, 1H), 6.81 (dd, *J* = 10.6, 7.4 Hz, 1H), 5.34 (dd, *J* = 1.3, 0.7 Hz, 1H), 4.29 (s, 2H); **^{19}F NMR** ($(CD_3)_2SO$, 470 MHz) δ -109.6 (d, *J* = 14.5 Hz), -135.7 (d, *J* = 15 Hz).

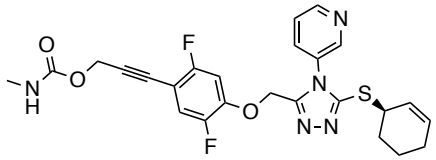


1-118a

(*R*)-3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-ol (**1-118a**). Methanesulfonyl chloride (1.29 mL, 16.6 mmol) was added dropwise to mixture of (*R*)-(5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-

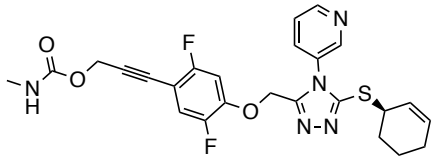
4H-1,2,4-triazol-3-yl)methanol **1-117a** (4.00 g, 13.9 mmol) and N,N-diisopropylethylamine (3.62 mL, 20.8 mmol) in anhydrous methylene chloride (140 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 5 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (50 mL) and extracted with methylene chloride (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-116 (3.07 g, 16.6 mmol) was added to a mixture of crude mesylate and cesium carbonate (9.04 g, 27.7 mmol) in anhydrous DMF (70 mL) under an atmosphere of nitrogen and stirred at room temperature for 18 h. The reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate (100 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (0-4% methanol in ethyl acetate, dry loaded) to provide **1-118a** (5.74 g, 12.6 mmol, 91%) as a white solid: **Rf** (ethyl acetate) 0.48; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78 (dd, *J* = 4.7, 1.0 Hz, 1H), 8.64 (d, *J* = 2.3 Hz, 1H), 7.75-7.73 (m, 1H), 7.50 (dd, *J* = 8.1, 4.8 Hz, 1H), 7.07 (dd, *J* = 10.8, 6.5 Hz, 1H), 6.86 (dd, *J* = 9.8, 7.0 Hz, 1H), 5.90-5.87 (m, 1H), 5.77-5.74 (m, 1H), 5.11 (s, 2H), 4.60 (s, 1H), 4.49 (s, 2H), 2.13-2.07 (m, 1H), 2.04-2.03 (m, 4H), 1.76-1.65 (m, 2H); **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -111.6 (d, *J* = 14.1 Hz), -138.5 (d, *J* = 14.1 Hz); **LC-MS** (ES) *m/z* calcd for $C_{23}H_{21}O_2N_4F_2S$ $[M+H]^+$ 455.1348, found 455.1349.



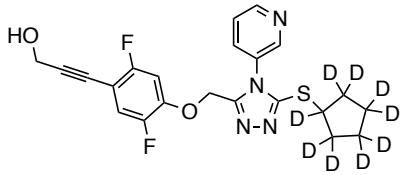
1-119a

(*R*)-3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-yl methylcarbamate (**1-119a**). Carbonyldiimidazole (2.22 g, 13.7 mmol) was added to a solution of **1-118a** (4.14 g, 9.11 mmol) in anhydrous methylene chloride (91 mL) and stirred for 4 h under nitrogen. Triethylamine (3.84 mL, 27.3 mmol) and methylamine hydrochloride (1.85 g, 27.3 mmol) were sequentially added and stirred for an additional 13 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (4-6% methanol in methylene chloride, dry loaded) to provide **1-119a** (4.57 g, 8.93 mmol, 98%) as a white solid: **Rf** (20% methanol in ethyl acetate) 0.64; **Mp** (methylene chloride) 138-140 ^{o}C; $[\alpha]_D$ = +107.5 (*c.* 0.272, CH_2Cl_2); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.77 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.63 (d, *J* = 2.4 Hz, 1H), 7.75-7.72 (m, 1H), 7.49 (dd, *J* = 8.1, 4.8 Hz, 1H), 7.10 (dd, *J* = 10.8, 6.5 Hz, 1H), 6.89-6.85 (m, 1H), 5.91-5.86 (m, 1H), 5.77-5.73 (m, 1H), 5.11 (s, 2H), 4.90 (s, 2H), 4.75 (s, 1H), 4.60-4.59 (m, 1H), 2.83 (d, *J* = 4.9 Hz, 3H), 2.13-1.98 (m, 4H), 1.77-1.64 (m, 2H); **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -111.1 (d, *J* = 14.2 Hz), -138.6 (d, *J* = 13.9 Hz); **LC-MS** (ES) *m/z* calcd for $C_{25}H_{24}O_3N_5F_2S$ $[M+H]^+$ 512.1562, found 512.1560.



1-119a composite

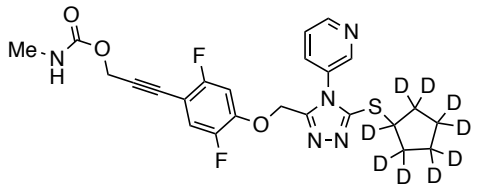
(*R*)-3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-yl methylcarbamate Composite (1-119a composite). **1-119a** from NB-00851.028, NB-00851.076, and NB-00851.081 were composited in a container and thoroughly homogenized to provide **1-119a composite** (NB-00851.083) (10,012.48 mg) as a uniform, white solid. All subsequent analytical testing were performed on NB-00851.083. **Rf** (20% methanol in ethyl acetate) 0.62; **Mp** 139-140 ^{o}C; $[\alpha]_D$ = +108.6 (*c.* 0.277, CH_2Cl_2); **IR** 3334.4, 3032.3, 2950.1, 1713.5, 1674.5 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78 (dd, *J* = 4.7, 1.1 Hz, 1H), 8.63 (d, *J* = 2.2 Hz, 1H), 7.74 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.50 (dd, *J* = 8.0, 4.7 Hz, 1H), 7.10 (dd, *J* = 10.7, 6.5 Hz, 1H), 6.87 (dd, *J* = 9.7, 7.0 Hz, 1H), 5.90-5.87 (m, 1H), 5.77-5.74 (m, 1H), 5.11 (s, 2H), 4.90 (s, 2H), 4.72 (s, 1H), 4.60 (s, 1H), 2.83 (d, *J* = 4.9 Hz, 3H), 2.13-2.07 (m, 1H), 2.05-2.00 (m, 3H), 1.77-1.65 (m, 2H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.20, 160.19, 158.2, 156.1, 154.0, 151.39, 151.34, 150.3, 149.07, 149.04, 147.9, 147.12, 147.10, 146.48, 146.40, 146.38, 146.30, 135.0, 132.4, 129.8, 125.4, 124.1, 120.11, 120.09, 119.94, 119.92, 104.22, 104.16, 104.08, 104.02, 103.8, 103.6, 88.90, 88.87, 78.11, 78.09, 61.3, 53.0, 44.2, 29.3, 24.8, 19.2; **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -111.1 (d, *J* = 14.6 Hz), -138.5 (d, *J* = 14.6 Hz); **LC-MS** (ES) *m/z* calcd for $C_{25}H_{24}O_3N_5F_2S$ $[M+H]^+$ 512.1562, found 512.1559.



1-118b

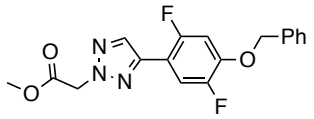
3-(4-((5-((Cyclopentyl-d9)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-ol (**1-118a**). Thionyl chloride (0.28 mL, 1.6 mmol) was added dropwise to mixture of **1-117b** (0.730 g, 2.56 mmol) in anhydrous methylene chloride (26 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-116 (0.518 g, 2.81 mmol) was added to a mixture of crude chloride and cesium carbonate (1.25 g, 3.84 mmol) in anhydrous DMF (13 mL) under an atmosphere of nitrogen and stirred at 50 ^{o}C for 2 h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-118b** (0.920 g, 2.04 mmol, 80%) as a white solid: **Rf** (1% methanol in ethyl acetate) 0.52; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.78 (dd, *J* = 4.8, 0.9 Hz, 1H), 8.64 (d, *J* = 2.2 Hz, 1H), 7.75 (ddd, *J* = 8.2, 2.4, 1.5 Hz, 1H), 7.50 (dd, *J* = 8.1, 4.9 Hz, 1H), 7.07 (dd, *J* = 10.8, 6.5 Hz, 1H), 6.85 (dd, *J* = 9.8, 7.0 Hz, 1H), 5.10-5.09 (m, 2H), 4.49-4.48 (m, 2H); **^{19}F NMR** ($CDCl_3$, 375 MHz) δ 111.6 (d, *J* = 14.6 Hz), 138.6 (d, *J* = 14.3 Hz).



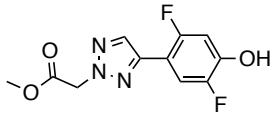
1-119b

3-(4-((5-((Cyclopentyl-d9)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-yl methylcarbamate (**1-119b**). Carbonyldiimidazole (0.496 g, 3.06 mmol) was added to a solution of **1-118b** (0.920 g, 2.04 mmol) in anhydrous methylene chloride (20 mL) and stirred for 3 h under nitrogen. Triethylamine (0.86 mL, 6.1 mmol) and methylamine hydrochloride (0.413 g, 6.11 mmol) were sequentially added and stirred for an additional 4 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (5% methanol in methylene chloride, dry loaded) to provide **1-119b** (1.03 g, 2.03 mmol, 99%) as a white solid: **Rf** (10% methanol in ethyl acetate) 0.59; **Mp** (CH_2Cl_2) 161-163 ^oC; **IR** (neat) 3296.5, 3057.9, 2932.4, 2231.2, 1711.5, 1510.3 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.63 (d, *J* = 2.4 Hz, 1H), 7.73 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1H), 7.50-7.48 (m, 1H), 7.10 (dd, *J* = 10.8, 6.5 Hz, 1H), 6.87 (dd, *J* = 9.8, 7.0 Hz, 1H), 5.10 (s, 2H), 4.90 (s, 2H), 2.83 (d, *J* = 4.9 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.20, 160.18, 158.2, 156.1, 154.6, 151.4, 150.2, 149.0, 147.89, 147.85, 147.07, 147.05, 146.50, 146.42, 146.39, 146.31, 134.9, 129.80, 129.76, 129.39, 129.33, 128.36, 128.31, 124.2, 120.07, 119.90, 103.7, 103.5, 88.86, 88.83, 78.1, 61.2, 53.0, 27.7; **^{19}F NMR** ($CDCl_3$, 375 MHz) δ 111.1 (d, *J* = 11.3 Hz), 138.6 (d, *J* = 11.3 Hz); **LC-MS** (ES) *m/z* calcd for $C_{24}H_{15}F_2O_3N_5SD_9$ $[M+H]^+$ 509.2127, found 509.2126.



1-121

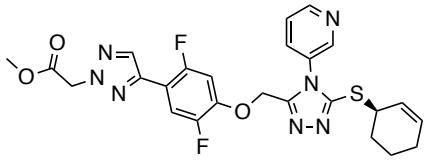
Methyl 2-(4-(4-(benzyloxy)-2,5-difluorophenyl)-2*H*-1,2,3-triazol-2-yl)acetate (**1-121**). Crude **1-120** (NB-00831.041, Dr. Joel Walker) was concentrated onto Celite and purified by chromatography on silica (30% ethyl acetate in hexane) to provide **1-121** (3.00 g, 8.35 mmol, 48%) as a white solid: **Rf** (50% ethyl acetate in hexane) 0.57; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.98 (d, *J* = 4.0 Hz, 1H), 7.72 (dd, *J* = 11.6, 6.9 Hz, 1H), 7.46-7.35 (m, 6H), 6.84-6.79 (m, 1H), 5.26 (s, 2H), 5.16 (s, 2H), 3.80 (s, 3H).



1-122

Methyl 2-(4-(2,5-difluoro-4-hydroxyphenyl)-2*H*-1,2,3-triazol-2-yl)acetate (**1-122**). A Parr hydrogenation vessel was charged with 10% palladium on carbon (0.296 g, 0.278 mmol) and **1-121** (1.00 g, 2.78 mmol) in methanol (28 mL). This process was repeated two additional times. The reaction vessel was purged with argon and then hydrogen three times each. The reaction mixture was then stirred under an atmosphere of hydrogen at 13-15 bar at room temperature for 24 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure to provide **1-122** (2.08 g, 7.73 mmol, 93%) as a white solid: **Rf** (ethyl acetate) 0.81; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 10.74 (s, 1H), 8.07 (d, *J* = 3.3 Hz, 1H), 7.60 (dd, *J* = 11.6, 7.0 Hz, 1H), 6.91 (dd, *J* = 11.8, 7.3 Hz, 1H), 5.52 (s, 2H); **^{19}F NMR** ($(CD_3)_2SO$, 470

MHz) δ -117.9 (d, *J* = 15.5 Hz), -140.3 (d, *J* = 15.0 Hz); **LC-MS** (ES) *m/z* calcd for $C_{11}H_{10}O_3N_3F_2$ $[M+H]^+$ 270.0685, found 270.0684.

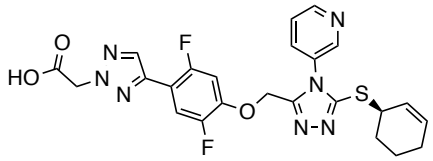


1-123

Methyl (*R*)-2-(4-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)-2*H*-1,2,3-triazol-2-yl)acetate (**1-123**). Methanesulfonyl chloride (0.64 mL, 8.3 mmol) was added dropwise to mixture of **1-117a** (2.00 g, 6.94 mmol) and N,N-diisopropylethylamine (1.81 mL, 10.4 mmol) in anhydrous methylene chloride (69 mL) under an atmosphere of nitrogen at 0 ^oC and stirred for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (50 mL) and extracted with methylene chloride (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-122 (2.05 g, 7.63 mmol) was added to a mixture of crude mesylate and cesium carbonate (4.52 g, 13.9 mmol) in anhydrous DMF (35 mL) under an atmosphere of nitrogen and stirred at room temperature for 21 h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80-100% ethyl acetate in hexane, dry loaded) to provide **1-123** (2.12 g, 3.93 mmol, 57%) as a white solid: **Rf** (ethyl acetate) 0.44; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.77 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.65 (d, *J* = 2.5 Hz, 1H), 8.01 (s, 1H), 7.97 (d, *J* = 4.0 Hz, 1H), 7.76 (ddd, *J* = 8.1, 2.5, 1.6

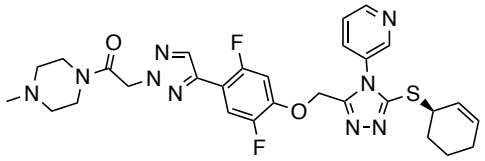
Hz, 1H), 7.67 (dd, *J* = 11.5, 6.7 Hz, 1H), 7.51-7.48 (m, 1H), 6.96 (dd, *J* = 11.3, 6.8 Hz, 1H), 5.90-5.87 (m, 1H), 5.77-5.74 (m, 1H), 5.25 (s, 2H), 5.15 (d, *J* = 0.8 Hz, 2H), 4.59 (dd, *J* = 4.0, 1.7 Hz, 1H), 3.79 (s, 3H), 2.95 (s, 2H), 2.88 (s, 2H), 2.13-2.07 (m, 1H), 2.05-2.00 (m, 3H), 1.76-1.63 (m, 4H); **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -116.2 (d, *J* = 15.0 Hz), -138.2 (d, *J* = 15.5 Hz); **LC-MS** (ES) *m/z* calcd for $C_{25}H_{24}O_3N_7F_2S$ $[M+H]^+$ 540.1624, found 540.1624.



1-124

(*R*)-2-(4-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)-2H-1,2,3-triazol-2-yl)acetic acid (**1-124**). A 1 M aqueous solution of lithium hydroxide (11.8 mL, 11.8 mmol) was added to a solution of **1-123** (2.12 g, 3.93 mmol) in THF (26 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was acidified to a pH of 5 with a 1 M aqueous solution of potassium hydrogen sulfate. The resulting solution was extracted with a 10% solution of methanol in chloroform (50 mL, five times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under pressure to provide **1-124** (2.01 g, 3.82 mmol, 97%) as a white solid: **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.73 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.71 (d, *J* = 2.3 Hz, 1H), 8.02-8.00 (m, 2H), 7.63-7.60 (m, 2H), 7.39 (dd, *J* = 12.0, 7.2 Hz, 1H), 5.87-5.83 (m, 1H), 5.68-5.65 (m, 1H), 5.35 (s, 2H), 5.06 (d, *J* = 0.3 Hz, 2H), 4.30-4.29 (m, 1H), 1.98-1.91 (m, 3H), 1.88-1.82 (m, 1H), 1.62-1.56 (m, 2H); **^{19}F NMR** ($(CD_3)_2SO$, 470 MHz) δ -116.6 (*J* = 15.0

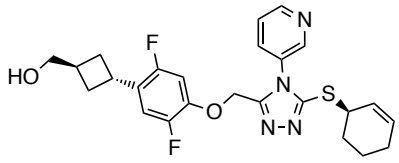
Hz), -138.6 (J = 14.6 Hz); **LC-MS** (ES) *m/z* calcd for $C_{24}H_{22}O_3N_7F_2S$ $[M+H]^+$ 526.1467, found 526.1465.



1-125

(*R*)-2-(4-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)-2H-1,2,3-triazol-2-yl)-1-(4-methylpiperazin-1-yl)ethan-1-one (**1-125**). A 50% solution of propylphosphonic anhydride in ethyl acetate (2.58 mL, 1.07 mmol) was added dropwise to a mixture of **1-124** (1.90 g, 3.62 mmol), 1-methylpiperazine (0.48 mL, 4.3 mmol), triethylamine (1.26 mL, 9.04 mmol) in anhydrous DMF (18 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 19 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (50 mL) and extracted with a 10% solution of methanol in chloroform (50 mL, four times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (10% methanol in methylene chloride) to provide **1-125** (1.42 g, 2.33 mmol, 65%) as a white solid. **Rf** (50% methanol in ethyl acetate) 0.19; $[\alpha]_D$ = +85.8 (*c.* 0.290 , CH_2Cl_2); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.77 (dd, J = 4.8, 1.5 Hz, 1H), 8.65 (d, J = 2.5 Hz, 1H), 7.97 (d, J = 4.0 Hz, 1H), 7.76 (ddd, J = 8.1, 2.5, 1.5 Hz, 1H), 7.68 (dd, J = 11.5, 6.7 Hz, 1H), 7.49 (ddd, J = 8.2, 4.8, 0.6 Hz, 1H), 6.94 (dd, J = 11.2, 6.8 Hz, 1H), 5.90-5.87 (m, 1H), 5.78-5.74 (m, 1H), 5.33 (s, 2H), 5.14 (d, J = 1.0 Hz, 2H), 4.60 (dd, J = 4.1, 1.8 Hz, 1H), 3.66 (t, J = 4.9 Hz, 2H), 3.52 (t, J = 5.0 Hz, 2H), 2.43-2.40 (m, 4H), 2.32 (s, 3H), 2.13-2.07 (m, 1H), 2.05-2.00 (m, 3H), 1.76-1.65 (m, 2H); **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -116.2 (J = 14.6 Hz),

-138.4 (J = 15.5 Hz); **LC-MS** (ES) *m/z* calcd for $C_{29}H_{32}O_2N_9F_2S$ $[M+H]^+$ 608.2362, found 608.2359.

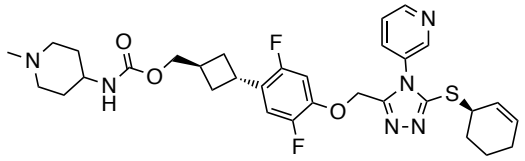


1-126

(*trans*-3-(4-((5-(((*R*)-cyclohex-2-en-1-yl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)cyclobutyl)methanol (**1-126**). Methanesulfonyl chloride (0.48 mL, 6.2 mmol) was added dropwise to mixture of (*R*)-(5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol **1-117a** (1.50 g, 5.20 mmol) and N,N-diisopropylethylamine (1.36 mL, 7.80 mmol) in anhydrous methylene chloride (52 mL) under an atmosphere of nitrogen at 0 ^oC and stirred for 7 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (25 mL) and extracted with methylene chloride (25 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

2,5-Difluoro-4-((1r,3r)-3-(hydroxymethyl)cyclobutyl)phenol (1.34 g, 6.24 mmol) was added to a mixture of crude mesylate and cesium carbonate (3.39 g, 10.4 mmol) in anhydrous DMF (26 mL) under an atmosphere of nitrogen and stirred at room temperature for 21 h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded) to provide **1-126** (2.27 g, 4.68 mmol, 90%) as a white solid: **Rf** (ethyl acetate) 0.31; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.77 (dd, J = 4.8, 1.5 Hz, 1H), 8.64 (d, J = 2.4 Hz, 1H), 7.77 (ddd, J

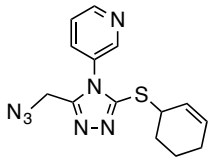
= 8.1, 2.5, 1.5 Hz, 1H), 7.51-7.48 (m, 1H), 6.96 (dd, *J* = 11.7, 7.0 Hz, 1H), 6.75 (dd, *J* = 10.5, 7.0 Hz, 1H), 5.88 (dtd, *J* = 9.8, 3.8, 1.4 Hz, 1H), 5.75 (ddd, *J* = 9.9, 4.0, 1.9 Hz, 1H), 5.07 (d, *J* = 0.8 Hz, 2H), 4.58 (dd, *J* = 4.0, 1.8 Hz, 1H), 3.79 (dd, *J* = 7.2, 5.4 Hz, 2H), 3.71-3.64 (m, 1H), 2.52-2.46 (m, 1H), 2.22-2.17 (m, 4H), 2.13-2.06 (m, 1H), 2.05-1.99 (m, 3H), 1.76-1.66 (m, 2H), 1.50 (t, *J* = 5.5 Hz, 1H); **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -119.7 (*J* = 15.5 Hz), -139.1 (*J* = 15.5 Hz); **LC-MS** (ES) *m/z* calcd for $C_{25}H_{27}O_2N_4F_2S$ $[M+H]^+$ 485.1817, found 485.1817.



1-127

(*trans*-3-(4-((5-(((*R*)-cyclohex-2-en-1-yl)thio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)cyclobutyl)methyl (1-methylpiperidin-4-yl)carbamate (**1-127**). Carbonyldiimidazole (1.14 g, 7.03 mmol) was added to a solution of **1-126** (2.27 g, 4.68 mmol) in anhydrous methylene chloride (47 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (1.76 mL, 14.1 mmol) was added and stirred for an additional 19 h. The reaction mixture was concentrated under reduced pressure, and purified by chromatography on silica (10-15% methanol in methylene chloride, dry loaded). The resulting material dissolved into chloroform (50 mL) and washed with a saturated aqueous solution of sodium bicarbonate (25 mL, three times). The resulting organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **1-127** (1.31 g, 2.09 mmol, 45%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.14; $[\alpha]_D$ = +75.5 (*c.* 0.290 , CH_2Cl_2); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.77 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.65-8.64 (m, 1H), 7.77 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1H), 7.49 (ddd, *J* = 8.1, 4.8, 0.7 Hz, 1H), 6.94 (dd, *J* = 11.7, 7.0 Hz, 1H), 6.75 (dd, *J* = 10.5, 7.0

Hz, 1H), 5.91-5.86 (m, 1H), 5.77-5.73 (m, 1H), 5.07 (s, 2H), 4.60-4.57 (m, 2H), 4.20 (d, J = 7.2 Hz, 2H), 3.73-3.64 (m, 1H), 3.54-3.47 (m, 1H), 2.78-2.73 (m, 2H), 2.59-2.54 (m, 1H), 2.27 (s, 3H), 2.22-2.18 (m, 4H), 2.12-2.07 (m, 3H), 2.06-2.01 (m, 4H), 1.98-1.93 (m, 2H), 1.76-1.64 (m, 3H), 1.52-1.42 (m, 3H); **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -119.6 (J = 15.0 Hz), -139.1 (J = 15.4 Hz); **LC-MS** (ES) *m/z* calcd for $C_{32}H_{39}O_3N_6F_2S$ $[M+H]^+$ 625.2767, found 625.2768.

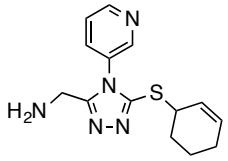


1-128

3-(3-(Azidomethyl)-5-(cyclohex-2-en-1-ylthio)-4*H*-1,2,4-triazol-4-yl)pyridine (**1-128**). Methanesulfonyl chloride (0.059 mL, 0.76 mmol) was added to a mixture of **1-15** (0.200 g, 0.694 mmol) and N,N-diisopropylethylamine (0.14 mL, 0.87 mmol) in anhydrous methylene chloride (7 mL) under and atmosphere of nitrogen at 0 ^oC. The reaction mixture stirred for 2 h and then concentrated under reduced pressure and dried under high vacuum. The resulting off-white solid (0.410 g) was carried forward as a crude mesylate without additional purification: **Rf** (ethyl acetate) 0.41; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.82 (dd, 1 H, J = 4.8, 1.4 Hz), 8.61 (d, 1 H, J = 2.5 Hz), 7.72 (ddd, 1 H, J = 8.1, 2.5, 1.6 Hz), 7.56-7.54 (m, 1H), 5.90 (dtd, 1 H, J = 9.8, 3.8, 1.4 Hz), 5.77-5.73 (m, 1 H), 5.20 (s, 2 H), 4.62-4.59 (m, 1 H), 3.02 (s, 3 H), 2.14-1.98 (m, 6 H), 1.78-1.65 (m, 3 H).

Sodium azide (0.146 g, 2.24 mmol) was added to a crude mesylate (0.410 g) in dimethylformamide (5.6 mL) and stirred at room temperature for 2 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were washed with brine (10 mL, three times), dried ($MgSO_4$), filtered,

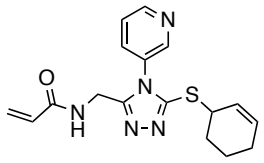
concentrated under reduced pressure, and dried under high vacuum to provide **1-128** (0.189 g, 0.601 mmol, 87%, two steps) as a light-brown solid: **Rf** (ethyl acetate) 0.46; **Mp** (ethyl acetate) 117-119 ^{o}C; **IR** 2924.8, 2095.3, 1483.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.81 (d, 1 H, *J* = 4.4 Hz), 8.62 (s, 1 H), 7.71 (dtd, 1 H, *J* = 8.1, 1.6, 0.7 Hz), 7.54 (dd, 1 H, *J* = 8.1, 4.8 Hz), 5.90-5.87 (m, 1H), 5.76-5.73 (m, 1H), 4.58-4.57 (m, 1 H), 4.38 (s, 2 H), 2.13-1.98 (m, 4H), 1.77-1.64 (m, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 153.5, 151.5, 150.8, 147.9. 134.8, 132.4, 129.7, 125.4, 124.4, 44.2, 44.1, 29.2, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{16}N_7S$ $[M+H]^+$ 314.1182, found 314.1184.



1-129

(5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methanamine (**1-129**). Triphenylphosphine (0.133 g, 0.508 mmol) was added to a high-pressure vial containing a mixture of **1-128** (0.139 g, 0.508 mmol), water (50 μL), and anhydrous, degassed THF (4.4 mL). The reaction vial was sealed under argon and stirred at room temperature for 24 h. TLC indicated the presence of starting material so additional triphenylphosphine (0.133 g, 0.508 mmol) and water (50 μL) were added to the reaction mixture and stirred for 24 h. This was repeated three additional times. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18 silica (40% acetonitrile in water, dry-loaded) to provide **1-129** (0.118 g, 0.412 mmol, 93%) as a white solid: **Rf** (10% methanol in ethyl acetate; neutral alumina) 0.15; **Mp** (methylene chloride) 96 – 98 ^{o}C; **IR** 3368.9, 3299.7, 3028.9, 1920.9, 2857.1, 1574.1, 1480.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.77 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.62 (d, 1 H,

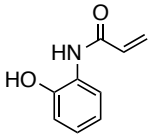
J = 2.5 Hz), 7.72 (ddd, 1 H, J = 8.1, 2.5, 1.6 Hz), 7.52-7.50 (m, 1 H), 5.86 (dtd, 1 H, J = 9.7, 3.8, 1.3 Hz), 5.73-5.71 (m, 1H), 4.49-4.48 (m, 1 H), 3.84 (br. s, 2 H), 2.09-1.95 (m, 5 H), 1.76-1.61 (m, 6 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 156.4, 151.9, 151.0, 148.0, 134.8, 132.2, 130.3, 125.5, 124.2, 44.1, 37.1, 29.2, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{18}N_5S$ $[M+H]^+$ 288.1277, found 288.1275.



1-130

***N*-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methyl)acrylamide** (**1-130**). A solution of 50% propylphosphonic anhydride in ethyl acetate (0.12 mL, 0.19 mmol) was added drop wise to a solution of **1-129** (0.0450 g, 0.157 mmol), acrylic acid (0.011 mL, 0.16 mmol) and triethylamine (0.065 mL, 0.46 mmol) in anhydrous ethyl acetate (2.5 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 21 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by reverse-phase chromatography on C18 silica (40% acetonitrile in water) to provide **1-130** (0.0325 g, 0.0952 mmol, 61%) as a white solid: **Rf** (10% methanol in ethyl acetate; neutral alumina) 0.33; **Mp** (methylene chloride) 172–174 ^oC; **IR** 3334.8, 3032.0, 2920.6, 2850.9, 1628.9, 1518.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.79 (dd, 1 H, J = 4.8, 1.3 Hz), 8.59 (d, 1 H, J = 2.4 Hz), 7.77 (ddd, 1 H, J = 8.1, 2.5, 1.5 Hz), 7.54-7.51 (m, 1H), 7.44 (br. s, 1 H), 6.25 (dd, 1 H, J = 17.0, 1.7 Hz), 6.17 (dd, 1 H, J = 17.0, 10.0 Hz), 5.89-5.85 (m, 1 H), 5.72-5.69

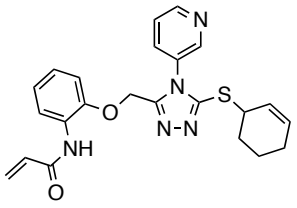
(m, 1 H), 5.64 (dd, 1 H, J = 10.0, 1.7 Hz), 4.47 (br. d, 3 H, J = 5.3 Hz), 2.05-1.96 (m, 5 H), 1.76-1.61 (m, 3 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 165.7, 153.2, 152.5, 151.4, 148.1, 135.0, 132.5, 130.2, 129.6, 127.2, 125.3, 124.4, 44.2, 34.3, 29.1, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{17}H_{20}ON_5S$ $[M+H]^+$ 342.1383, found 342.1381.



1-131a

N-(2-Hydroxyphenyl)acrylamide (**1-131a**). A solution of acryloyl chloride (0.41 mL, 5.0 mmol) in methylene chloride (10 mL) was added dropwise to a mixture of 2-aminophenol **1-130a** (0.500 g, 4.58 mmol), N,N-diisopropylethylamine (1.59 mL, 9.16 mmol) in anhydrous methylene chloride (46 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 1 h. Additional acryloyl chloride (0.407 mL, 5.04 mmol) was added and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was resuspended in methanol (10 mL). The solution was cooled to 0 ^{o}C and diluted with a 1 M aqueous solution of lithium hydroxide (10 mL). The mixture was warmed to room temperature and stirred for 3 h. The solution was cooled to ^{o}0 C and neutralized with a 1 M aqueous solution of potassium bisulfate. The aqueous solution was extracted with methylene chloride (20 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by column chromatography on SiO_2 (30-40% ethyl acetate in hexane) to provide **1-131a** (0.569 g, 3.48 mmol, 76%) as an off-white solid: **Rf** (50% ethyl acetate in hexane) 0.56; **Mp** (methylene chloride) 123-125 ^{o}C; **IR** 3404.8, 3093.7, 2873.6, 1659.5 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz)

δ 9.88 (s, 1 H), 9.50 (s, 1 H), 7.84 (dd, 1 H, *J* = 7.9, 1.0 Hz), 6.96 (td, 1 H, *J* = 7.7, 1.2 Hz), 6.88 (dd, 1 H, *J* = 8.0, 1.4 Hz), 6.80-6.76 (m, 1 H), 6.69 (dd, 1 H, *J* = 17.0, 10.2 Hz), 6.23 (dd, 1 H, *J* = 17.0, 2.0 Hz), 5.72 (dd, 1 H, *J* = 10.2, 1.9 Hz); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 163.9, 148.4, 132.3, 127.2, 126.6, 125.4, 122.8, 119.5, 116.2; **LC-MS** (ES) *m/z* calcd for $C_9H_{10}O_2N$ $[M+H]^+$ 164.0706, found 164.0705.

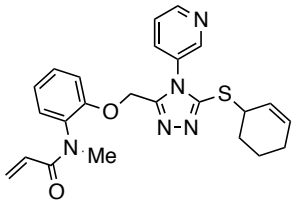


1-132a

N-(2-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)acrylamide (**1-132a**). Methanesulfonyl chloride (0.012 mL, 0.15 mmol) was added dropwise to mixture of **1-15** (0.0450 g, 0.139 mmol), N,N-diisopropylethylamine (0.030 mL, 0.15 mmol) in anhydrous methylene chloride (1.4 mL) under an atmosphere of nitrogen at 0 ^oC and stirred for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (5 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide crude mesylate. This material was carried forward without further purification.

1-131a (0.0249 g, 0.153 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.0904 g, 0.277 mmol) in anhydrous DMF (0.7 mL) under an atmosphere of nitrogen and stirred at room temperature for 19 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried

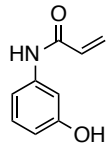
($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (EtOAc, dry loaded) to provide **1-132a** (0.0457 g, 0.105 mmol, 76%) as a white solid: **Rf** (ethyl acetate) 0.50; **Mp** (methylene chloride) 152-154 ^oC; **IR** 3258.7, 2919.8, 1654.0, 1522.3, 1487.5 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.74 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.58 (d, 1 H, *J* = 2.4 Hz), 8.37 (d, 1 H, *J* = 7.1 Hz), 7.65 (s, 1 H), 7.51 (ddd, 1 H, *J* = 8.1, 2.4, 1.6 Hz), 7.41 (ddd, 1 H, *J* = 8.1, 4.8, 0.6 Hz), 7.03-6.94 (m, 3 H), 6.42 (dd, 1 H, *J* = 16.8, 1.3 Hz), 6.23 (dd, 1 H, *J* = 16.8, 10.2 Hz), 5.88 (dtd, 1 H, *J* = 9.8, 3.8, 1.5 Hz), 5.79 (dd, 1 H, *J* = 10.2, 1.3 Hz), 5.74-5.71 (m, 1 H), 5.21 (s, 2 H), 4.57-4.54 (m, 1 H), 2.11-1.97 (m, 4 H), 1.76-1.65 (m, 3 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 163.3, 153.6, 151.4, 151.2, 147.5, 146.0, 134.6, 132.5, 131.4, 129.9, 127.9, 127.7, 125.3, 124.2, 124.2, 122.8, 121.1, 112.4, 61.1, 44.2, 29.2, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{24}O_2N_5S$ $[M+H]^+$ 434.1645, found 434.1644.



1-133a

N-(2-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)-N-methylacrylamide (**1-133a**). Potassium hydride (0.00407 g, 0.0761 mmol) was added to a solution of **1-132a** (0.0400 g, 0.0923 mmol) in anhydrous THF (0.7 mL) at 0 ^oC under an atmosphere of nitrogen and stirred for 0.5 h. Methyl iodide (0.01 mL, 0.10 mmol) was added and the reaction mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with water (5 mL) and the aqueous solution was extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered,

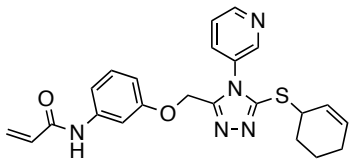
concentrated under reduced pressure, and purified by chromatography on SiO_2 (5% methanol in methylene chloride, dry loaded) to provide **1-133a** (0.0306 g, 0.0684 mmol, 74%) as an off-white solid: **Rf** (ethyl acetate) 0.23; **Mp** (methylene chloride) 47 ^{o}C, gel; **IR** 3449.5, 3029.1, 2926.3, 1653.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.72 (dd, 1 H, *J* = 4.8, 1.3 Hz), 8.56 (d, 1 H, *J* = 2.5 Hz), 7.65 (dq, 1 H, *J* = 8.1, 1.9 Hz), 7.46 (ddd, 1 H, *J* = 7.8, 5.0, 2.6 Hz), 7.34-7.30 (m, 1 H), 7.18 (d, 1 H, *J* = 8.4 Hz), 7.10 (dd, 1 H, *J* = 7.5, 0.9 Hz), 7.01 (td, 1 H, *J* = 7.6, 0.8 Hz), 6.31 (ddd, 1 H, *J* = 16.8, 5.2, 2.0 Hz), 5.90-5.81 (m, 2 H), 5.77-5.74 (m, 1 H), 5.46 (ddd, 1 H, *J* = 10.3, 5.4, 2.0 Hz), 5.14-5.06 (m, 2 H), 4.58-4.57 (m, 1 H), 3.09 (d, 3 H, *J* = 2.2 Hz), 2.12-2.02 (m, 4 H), 1.76-1.65 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 165.9, 153.8, 153.1, 151.3, 150.9, 147.5, 135.1, 132.4, 132.42, 131.8, 129.8, 129.8, 129.6, 127.9, 127.9, 127.6, 127.5, 125.4, 124.5, 122.4, 113.4, 60.2, 44.1, 44.1, 36.2, 29.2, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{24}H_{26}O_2N_5S$ $[M+H]^+$448.1802, found 448.1799.



1-131b

N-(3-Hydroxyphenyl)acrylamide (**1-131b**). A solution of acryloyl chloride (0.41 mL, 5.0 mmol) in methylene chloride (10 mL) was added dropwise to a mixture of 3-aminophenol **1-130b** (0.500 g, 4.58 mmol), N,N-diisopropylethylamine (1.59 mL, 9.16 mmol) in anhydrous methylene chloride (46 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was stirred for 1 h and then warmed to room temperature and stirred for 1 h. Additional acryloyl chloride (0.407 mL, 5.04 mmol) was added and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was resuspended in

methanol (10 mL). The solution was cooled to 0 °C and diluted with a 1 M aqueous solution of lithium hydroxide (10 mL). The mixture was warmed to room temperature and stirred for 3 h. The solution was cooled to 0 °C and neutralized with a 1 M aqueous solution of potassium bisulfate. The aqueous solution was extracted with methylene chloride (20 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by column chromatography on silica (60% ethyl acetate in hexane, dry loaded) to provide **1-131b** (0.222 g, 1.36 mmol, 30%) as a white solid: **Rf** (50% ethyl acetate in hexane) 0.32; **Mp** (CH_2Cl_2) 158-160 °C; **IR** 3306.3, 3088.1, 1738.6, 1656.1 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 9.99 (s, 1 H), 9.40 (s, 1 H), 7.25 (t, 1 H, *J* = 2.1 Hz), 7.08 (t, 1 H, *J* = 8.0 Hz), 7.02 (ddd, 1 H, *J* = 8.1, 1.7, 1.0 Hz), 6.46 (ddd, 1 H, *J* = 8.0, 2.4, 1.0 Hz), 6.42 (dd, 1 H, *J* = 17.0, 10.1 Hz), 6.23 (dd, 1 H, *J* = 17.0, 2.0 Hz), 5.73 (dd, 1 H, *J* = 10.1, 2.0 Hz); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 163.5, 158.1, 140.5, 132.4, 129.9, 127.1, 111.1, 110.6, 106.9; **LC-MS** (ES) *m/z* calcd for $C_9H_{10}O_2N$ $[M+H]^+$ 164.0706, found 164.0707. Full Characterization: NB-00746.019.

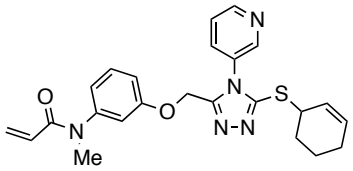


1-132b

N-(3-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)acrylamide (**1-132b**). Methanesulfonyl chloride (0.01 mL, 0.15 mmol) was added dropwise to mixture of **1-15** (0.0400 g, 0.139 mmol), N,N-diisopropylethylamine (0.03 mL, 0.15 mmol) in anhydrous methylene chloride (1.4 mL) under an atmosphere of nitrogen at 0 °C and stirred for 19 h. The reaction mixture was diluted with a saturated solution of aqueous

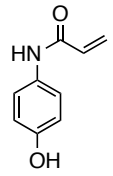
sodium bicarbonate (5 mL) and extracted with methylene chloride (5 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide crude mesylate. This material was carried forward without further purification.

1-131b (0.0249 g, 0.153 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.0904 g, 0.277 mmol) in anhydrous DMF (0.7 mL) under an atmosphere of nitrogen and stirred at room temperature for 20 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **1-132b** (0.0503 g, 0.116 mmol, 84%) as a white solid: **Rf** (ethyl acetate) 0.38; **Mp** (methylene chloride) 157-159 ^oC; **IR** 3268.4, 3083.1, 2927.9, 1682.7, 1604.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.74 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.62 (d, 1 H, *J* = 2.4 Hz), 7.71 (ddd, 1 H, *J* = 8.1, 2.5, 1.6 Hz), 7.56 (br. s, 1 H), 7.48 (dd, 1 H, *J* = 8.1, 4.9 Hz), 7.18 (br. d, 2 H, *J* = 5.6 Hz,), 6.63 (dt, 1 H, *J* = 6.0, 2.9 Hz), 6.42 (dd, 1 H, *J* = 16.8, 1.2 Hz), 6.25 (dd, 1 H, *J* = 16.8, 10.2 Hz), 5.87 (dtd, 1 H, *J* = 9.8, 3.8, 1.5 Hz), 5.77 (dd, 1 H, *J* = 10.2, 1.2 Hz), 5.75-5.72 (m, 1 H), 5.07 (d, 2 H, *J* = 1.5 Hz), 4.56-4.54 (m, 1 H), 2.11-1.98 (m, 5 H), 1.77-1.63 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 163.8, 157.6, 153.5, 151.6, 151.2, 148.0, 139.4, 134.9, 132.4, 131.2, 129.9, 129.9, 127.8, 125.3, 124.2, 115.1, 113.5, 110.8, 106.4, 59.9, 44.2, 29.2, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{24}O_2N_5S$ $[M+H]^+$ 434.1645, found 434.1646.



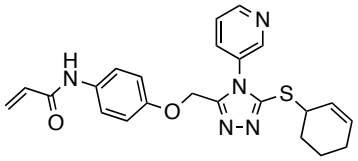
1-133b

N-(3-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)-N-methylacrylamide (**1-133b**). Potassium hydride (0.00407 g, 0.0761 mmol) was added to a solution of **1-132b** (0.0400 g, 0.0923 mmol) in anhydrous THF (0.9 mL) at 0 ^{o}C under an atmosphere of nitrogen and stirred for 0.5 h Methyl iodide (0.01 mL, 0.10 mmol) was added and the reaction mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with water (5 mL) and the aqueous solution was extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (5% methanol in methylene chloride, dry loaded) to provide **1-133b** (0.0258 g, 0.0576 mmol, 62%) as an off-white solid: **Rf** (ethyl acetate) 0.53; **Mp** (methanol) 56 ^{o}C, gel; **IR** 3427.8, 3028.6, 2925.7, 1652.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76 (dd, 1 H, *J* = 4.8, 1.4 Hz), 8.63 (d, 1 H, *J* = 2.4 Hz), 7.71 (ddd, 1 H, *J* = 8.1, 2.5, 1.6 Hz), 7.48 (dd, 1 H, *J* = 8.1, 4.8 Hz), 7.29-7.26 (m, 1 H), 6.89 (dd, 1 h, *J* = 8.3, 2.4 Hz), 6.79 (dd, 1 H, *J* = 7.5, 1.5 Hz), 6.66 (t, 1 H, *J* = 2.2 Hz), 6.35 (dd, 1 H, *J* = 16.8, 2.0 Hz), 6.04 (dd, 1 H, *J* = 16.7, 10.5 Hz), 5.89-5.86 (m, 1 H), 5.76-5.73 (m, 1 H), 5.52 (dd, 1 H, *J* = 10.3, 1.9 Hz), 5.08 (s, 2 H), 4.58 (dd, 1 H, *J* = 4.2, 1.8 Hz), 3.30 (s, 3 H), 2.12-2.06 (m, 1 H), 2.05-1.98 (m, 4 H), 1.77-1.63 (m, 3 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ; **LC-MS** (ES) *m/z* calcd for $C_{24}H_{26}O_2N_5S$ $[M+H]^+$448.1802, found 448.1800.



1-131c

N-(4-Hydroxyphenyl)acrylamide (**1-131c**). A solution of acryloyl chloride (0.41 mL, 5.0 mmol) in methylene chloride (10 mL) was added dropwise to a mixture of 4-aminophenol **1-130c** (0.500 g, 4.58 mmol), N,N-diisopropylethylamine (1.59 mL, 9.16 mmol) in anhydrous methylene chloride (46 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was stirred for 1 h and then warmed to room temperature and stirred for 1 h. Additional acryloyl chloride (0.41 mL, 5.0 mmol) was added and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was resuspended in methanol (10 mL). The solution was cooled to 0 ^{o}C and diluted with a 1 M aqueous solution of lithium hydroxide (10 mL). The mixture was warmed to room temperature and stirred for 3 h. The solution was cooled to 0 ^{o}C and neutralized with a 1 M aqueous solution of potassium bisulfate. The aqueous solution was extracted with methylene chloride (20 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by column chromatography on silica (60% EtOAc in hexane, dry loaded) to provide **1-131c** (0.478 g, 2.89, 63%) as an off-white solid: **Rf** (50% ethyl acetate in hexane) 0.28; **Mp** (methanol) 196-198 ^{o}C; **IR** 3297.2, 3096.6, 1654.5, 1439.3 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 9.89 (s, 1 H), 9.23 (s, 1 H), 7.46-7.43 (m, 2 H), 6.72-6.69 (m, 2 H), 6.38 (dd, 1 H, *J* = 17.0, 10.1 Hz), 6.19 (dd, 1 H, *J* = 17.0, 2.1 Hz), 5.68 (dd, 1 H, *J* = 10.1, 2.1 Hz); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 163.0, 154.0, 132.5, 131.1, 126.4, 121.5, 115.6; **LC-MS** (ES) *m/z* calcd for $C_9H_{10}O_2N$ $[M+H]^+$ 164.0706, found 164.0706.

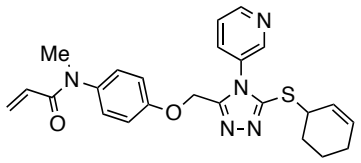


1-132c

N-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)acrylamide (**1-132c**). Methanesulfonyl chloride (0.024 mL, 0.31 mmol) was added dropwise to mixture of **1-15** (0.0800 g, 0.277 mmol), N,N-diisopropylethylamine (0.06 mL, 0.35 mmol) in anhydrous methylene chloride (2.8 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (5 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide crude mesylate. This material was carried forward without further purification.

1-131c (0.0498 g, 0.305 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.181 g, 0.555 mmol) in anhydrous DMF (1.4 mL) under an atmosphere of nitrogen and stirred at room temperature for 28 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (ethyl acetate, dry loaded) to provide **1-132c** (0.0821 g, 0.189 mmol, 68%) as a white solid. **Rf** (ethyl acetate) 0.21; **Mp** (methylene chloride) > 65 ^{o}C, gel; **IR** 3264.9, 3029.8, 2928.9, 1664.2, 1542.3 cm^{-1}; **^{1}H NMR** (CD_3Cl, 500 MHz) δ 8.75 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.62 (d, 1 H, *J* = 2.4 Hz), 7.69 (ddd, 1 H, *J* = 8.1, 2.4, 1.6 Hz), 7.57 (br. s, 1 H), 7.47 (dt, 3 H, *J* = 8.3, 4.3 Hz), 6.81 (d, 2 H, *J* = 9.0 Hz), 6.41 (dd, 1 H, *J* = 16.9, 1.0 Hz), 6.25 (dd, 1 H, *J* = 16.9, 10.2 Hz), 5.87 (dtd, 1 H, *J* = 9.8, 3.8, 1.4 Hz), 5.76-5.72 (m, 2 H), 5.09-5.03 (m, 2 H), 4.55 (t, 1 H, *J* = 1.9 Hz), 2.11-

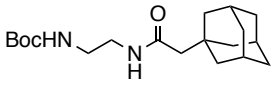
1.97 (m, 4 H), 1.76-1.63 (m, 3 H); **^{13}C NMR** (CD_3Cl, 125 MHz) δ 163.7, 153.8, 153.5, 151.7, 151.2, 147.9, 134.9, 132.6, 132.5, 131.3, 130.0, 127.3, 125.3, 124.2, 121.8, 115.1, 60.1, 44.1, 29.2, 24.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{24}O_2N_5S$ $[M+H]^+$ 434.1645, found 434.1644.



1-133c

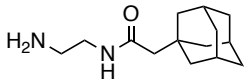
N-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)-N-methylacrylamide (**1-133c**). Potassium hydride (0.00457 g, 0.114 mmol) was added to a solution of **1-132c** (0.0450 g, 0.104 mmol) in anhydrous THF (1 mL) at 0 ^{o}C under an atmosphere of nitrogen and stirred for 0.5 h Methyl iodide (0.01 mL, 0.11 mmol) was added and the reaction mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with water (5 mL) and the aqueous solution was extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (5% methanol in methylene chloride, dry loaded) to **1-133c** (0.0328 g, 0.0733 mmol, 71%) as an off-white solid: **Rf** (ethyl acetate) 0.54; **Mp** (methanol) 53 ^{o}C, gel; **IR** (neat) 3252.2, 3029.4, 2926.9, 1650.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.65 (d, 1 H, *J* = 2.5 Hz), 7.72 (ddd, 1 H, *J* = 8.1, 2.5, 1.5 Hz), 7.50 (ddd, 1 H, *J* = 8.1, 4.8, 0.7 Hz), 7.07-7.04 (m, 2 H), 6.93-6.90 (m, 2 H), 6.34 (dd, 1 H, *J* = 16.8, 2.0 Hz), 6.02 (dd, 1 H, *J* = 16.8, 10.3 Hz, 1H), 5.91-5.87 (m, 1 H), 5.77-5.74 (m, 1 H), 5.50 (dd, 1 H, *J* = 10.3, 1.1 Hz, 1H), 5.10 (s, 2 H), 4.61-4.58 (m, 1 H), 3.29 (s, 3 H), 2.14-2.07 (m, 1 H), 2.06-1.99 (m, 3 H), 1.78-1.65 (m, 2 H), 1.63 (s, 1 H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 165.8, 156.4, 153.6, 151.23, 151.19, 148.0, 137.4, 134.8, 132.4,

130.0, 128.6, 128.4, 127.4, 125.4, 124.1, 115.6, 60.1, 44.1, 37.5, 29.2, 24.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{24}H_{26}O_2N_5S$ $[M+H]^+$448.1802, found 448.1799.



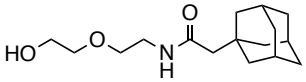
1-135

***Tert*-butyl (2-(2-((3*R*,5*R*,7*R*)-adamantan-1-yl)acetamido)ethyl)carbamate** (**1-135**). Hydroxybenzotriazole (0.527 g, 3.90 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.606 g, 3.12 mmol) were added to a mixture of tert-butyl (2-aminoethyl)carbamate **1-134** (0.494 g, 3.12 mmol), N,N-diisopropylethylamine (1.03 mL, 6.24 mmol), and 1-adamantaneacetic acid (0.606 g, 3.12 mmol) in anhydrous methylene chloride (16 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 24 h. The reaction was concentrated under reduced pressure and purified by chromatography on silica (70% ethyl acetate in hexane) to provide **1-135** (0.943 g, 2.80 mmol, 90%) as a white solid: **Rf** (ethyl acetate) 0.65; **Mp** (methylene chloride) 114-116 ^oC; **IR** (neat) 3297.1, 2901.2, 2847.2, 1684.5, 1546.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 6.02 (br. s, 1 H), 4.95 (br. s, 1 H), 3.35 (app. q, 2 H, *J* = 5.6 Hz), 3.27 (app. q, 2 H, *J* = 5.5 Hz), 1.96 (br. s, 3 H), 1.92 (s, 2 H), 1.70 (br. s, 1 H), 1.68 (br. s, 2 H), 1.64 (app. d, 2 H, *J* = 4.3 Hz), 1.60 (d, 7 H, *J* = 2.4 Hz), 1.43 (s, 9 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.8, 156.9, 79.5, 51.7, 42.6, 40.5, 40.4, 36.7, 32.7, 28.6, 28.4; **LC-MS** (ES) *m/z* calcd for $C_{19}H_{33}O_3N_2$ $[M+H]^+$ 337.2486, found 337.2484.



1-136

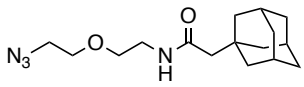
2-((3*R*,5*R*,7*R*)-adamantan-1-yl)-*N*-(2-aminoethyl)acetamide (**1-136**). **1-135** (0.500 g, 1.49 mmol) was added to a solution of acetyl chloride (1.69 mL, 23.8 mmol) in methanol (15 mL) at 0 ^{o}C. The reaction mixture was warmed to room temperature and stirred under nitrogen for 24 h. The reaction was cooled to 0 ^{o}C and slowly quenched with a 1M aqueous solution of sodium hydroxide until a pH of 12 was reached. The resulting mixture was concentrated under reduced pressure and resuspended in anhydrous methylene chloride (25 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **1-136** (0.287 g, 1.21 mmol, 82%) as an off-white solid: **Rf** (10% methanol in ethyl acetate) 0.26; **Mp** (methylene chloride) 55-57 ^{o}C; **IR** (neat) 3291.1, 2897.9, 2845.6, 1630.2, 1541.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.82 (s, 1 H), 3.48 (s, 1 H), 3.30 (q, 2 H, *J* = 5.9 Hz), 2.83 (s, 2 H), 1.97 (s, 3 H), 1.94 (s, 2 H), 1.71 (s, 1 H), 1.69 (s, 2 H), 1.63 (dd, 10 H, *J* = 9.4, 1.7 Hz, 1.36 (s, 2 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.2, 51.9, 42.7, 36.8, 32.7, 28.7; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{25}ON_2$ $[M+H]^+$ 237.1961, found 237.1960.



1-138

2-((3*R*,5*R*,7*R*)-adamantan-1-yl)-*N*-(2-(2-hydroxyethoxy)ethyl)acetamide (**1-138**). Hydroxybenzotriazole (0.562 g, 4.16 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.798 g, 4.16 mmol) were added to a mixture of 2-(2-

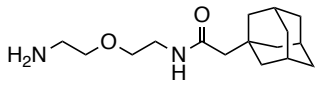
aminoethoxy)ethan-1-ol **1-137** (0.334 mL, 3.33 mmol), N,N-diisopropylethylamine (1.10 mL, 6.66 mmol), and 1-adamantaneacetic acid (0.647 g, 3.33 mmol) in anhydrous methylene chloride (17 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 24 h. The reaction was concentrated under reduced pressure and purified by chromatography on silica (ethyl acetate) to provide **1-138** (0.872 g, 3.09 mmol, 93%) as a colorless oil: **Rf** (ethyl acetate) 0.19; **IR** 3299.4, 2846.5, 1639.5, 1545.5, 1450.5 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ) 5.80 (br. s, 1 H), 3.75 (q, 2 H, *J* = 4.6 Hz), 3.59-3.55 (m, 4 H), 3.46 (q, 2 H, *J* = 5.2 Hz), 2.17 (t, 1 H, *J* = 5.9 Hz), 1.96 (br. s, 3 H), 1.94 (s, 2 H), 1.71 (br. s, 1 H), 1.68 (s, 3 H), 1.64 (s, 2 H), 1.62 (d, 7 H, *J* = 2.4 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.3, 72.3, 70.1, 61.7, 51.7, 42.6, 39.1, 36.8, 32.7, 28.6; **LC-MS** (ES) *m/z* calcd for $C_{16}H_{28}O_3N$ $[M+H]^+$ 282.2064, found 282.2062.



1-139

2-((3*R*,5*R*,7*R*)-adamantan-1-yl)-*N*-(2-(2-azidoethoxy)ethyl)acetamide (**1-139**). Methanesulfonyl chloride (0.26 mL, 3.4 mmol) was added to a mixture of **1-138** (0.872 g, 3.09 mmol) and N,N-diisopropylethylamine (0.64 mL, 3.9 mmol) in anhydrous methylene chloride (30 mL) under and atmosphere of nitrogen at 0 ^{o}C. The reaction mixture was warmed to room temperature, stirred for 12 h, concentrated under reduced pressure, and completely dried under high vacuum. The crude material was dissolved in anhydrous DMF (15 mL). Sodium azide (0.403 g, 6.19 mmol) was added and the reaction mixture was stirred at 50 ^{o}C for 12 h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL, three times). The combined organic solutions were washed with brine (10 mL, three times), dried

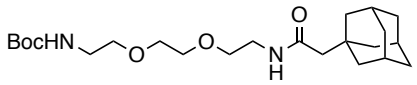
($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40% ethyl acetate in hexane) to provide **1-139** (0.711 g, 2.31 mmol, 75%, two steps) as a colorless oil: **Rf** (ethyl acetate) 0.44; **IR** 3300.2, 2899.7, 2846.8, 2098.4, 1640.2, 1543.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.78 (s, 1 H), 3.68-3.66 (m, 2 H), 3.56 (dd, 2 H, *J* = 5.5, 4.6 Hz), 3.48-3.45 (m, 2 H), 3.37 (t, 2 H, *J* = 4.9 Hz), 1.97 (s, 3 H), 1.93 (s, 2H), 1.71 (s, 1 H), 1.69 (s, 2 H), 1.65 (app. d, *J* = 1.3 Hz, 2 H), 1.62 (app. d, 7 H, *J* = 2.5 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.0, 70.2, 70.1, 51.7, 50.7, 42.6, 38.9, 36.8, 32.7, 28.7; **LC-MS** (ES) *m/z* calcd for $C_{16}H_{27}O_2N_4$ $[M]^+$ 307.2129, found 307.2126.



1-140

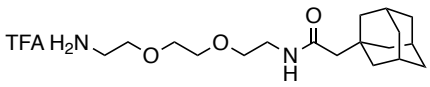
2-((3*R*,5*R*,7*R*)-adamantan-1-yl)-*N*-(2-(2-aminoethoxy)ethyl)acetamide (**1-140**). Triphenylphosphine (0.256 g, 0.976 mmol) was added to a solution of **1-139** (0.250 g, 0.813 mmol) in degassed, anhydrous THF (4 mL) at room temperature under an atmosphere of nitrogen. After 2 h, water (35 uL) was added and the reaction mixture was stirred for an additional 22 h. TLC indicated the presence of starting material so additional triphenylphosphine (0.256 g, 0.976 mmol) and water (35 uL) were added to the reaction mixture and stirred for 24 h. This was repeated an additional time. The reaction was concentrated under reduced pressure and the resulting residue was resuspended with a 1M aqueous solution of hydrogen chloride (5 mL) and washed with ethyl acetate (5 mL, two times). The aqueous solution was basified with a 3M aqueous solution of sodium hydroxide (20 mL) and extracted with a 10% solution of methanol in methylene chloride (25 mL, four times). The combined organic solutions were sequentially washed with water (10 mL), brine (10 mL), dried (MgSO4), filtered, and concentrated under

reduced pressure to provide **1-140** (0.214 g, 0.762 mmol, 94%) as a colorless residue: **Rf** (10% methanol in ethyl acetate; neutral alumina) 0.48; **IR** 3284.5, 2899.0, 2846.4, 1640.0, 1544.1, 1450.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.98 (br. s, 1 H), 3.52 (dt, 4 H, *J* = 12.0, 5.2 Hz), 3.45 (q, 3 H, *J* = 5.2 Hz), 2.90 (t, 2 H, *J* = 5.2 Hz), 2.26 (br. s, 2 H), 1.96 (br. s, 3 H), 1.94 (s, 3 H), 1.71 (s, 1 H), 1.68 (s, 2 H), 1.64-1.62 (m, 11 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.1, 72.4, 69.9, 51.7, 42.6, 41.5, 39.1, 36.8, 32.7, 28.7; **LC-MS** (ES) *m/z* calcd for $C_{16}H_{28}O_2N_2$ $[M]^+$ 281.2224, found 281.2221.



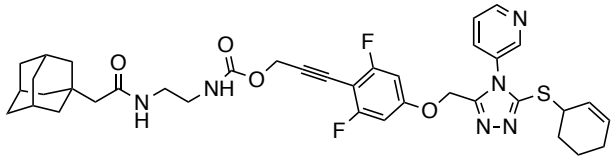
1-142

***Tert*-butyl (2-(2-(2-(2-((3*R*,5*R*,7*R*)-adamantan-1-yl)acetamido)ethoxy)ethoxy)ethyl)carbamate** (**1-142**). Hydroxybenzotriazole (0.340 g, 2.52 mmol), 1-adamanylacetic acid (0.391 g, 2.01 mmol), and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.218 g, 1.14 mmol) were added to a mixture of tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate **1-141** (0.48 mL, 2.0 mmol), N,N-diisopropylethylamine (0.67 mL, 4.0 mmol) in anhydrous methylene chloride (10 mL) at 0 °C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 13 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (ethyl acetate) to provide **1-142** (0.460 g, 1.08 mmol, 54%) as a colorless oil: **Rf** (ethyl acetate) 0.43; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.87-5.82 (m, 1H), 5.02-4.96 (m, 1H), 3.61 (s, 3H), 3.55 (t, *J* = 5.2 Hz, 4H), 3.45 (q, *J* = 5.0 Hz, 2H), 3.35-3.29 (m, 2H), 1.96-1.94 (m, 4H), 1.72-1.62 (m, 13H), 1.45 (s, 8H).



1-143

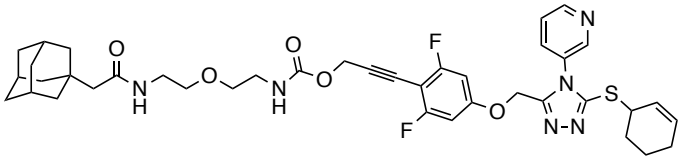
2-((3*R*,5*R*,7*R*)-adamantan-1-yl)-*N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)acetamide trifluoroacetate salt (**1-143**). Trifluoroacetic acid (1.21 mL, 16.3 mmol) was added to a high-pressure reaction vial containing a solution of **1-142** (0.460 g, 1.08 mmol) in anhydrous methylene chloride (9 mL). The reaction vial was sealed and the reaction mixture was stirred at 50 ^{o}C for 2 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in THF (5 mL) and concentrated under reduced pressure; this was repeated two additional times. The resulting material was dried under high vacuum to provide the **1-143** (0.496 g) as a colorless residue. This material was carried forward without purification: **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.09-7.99 (m, 2H), 6.61-6.55 (m, 1H), 5.16-5.05 (m, 2H), 3.79-3.76 (m, 2H), 3.69-3.58 (m, 5H), 3.46-3.43 (m, 2H), 3.21-3.17 (m, 2H), 1.99-1.95 (m, 4H), 1.73-1.56 (m, 10H).



1-145a

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (2-(2-((3*R*,5*R*,7*R*)-adamantan-1-yl)acetamido)ethyl)carbamate (**1-145a**). Carbonyldiimidazole (0.0268 g, 0.165 mmol) was added to a solution of **1-144** (0.0500 g, 0.110 mmol) in anhydrous methylene chloride (1.60 mL). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 3 h. **1-**

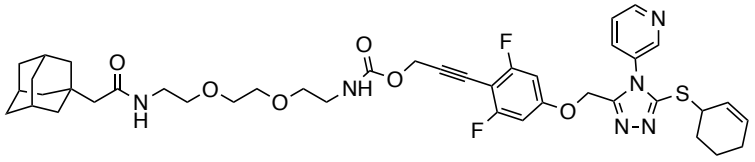
136 (0.0555 g, 0.220 mmol) was added and the reaction mixture was stirred for 21 h. The reaction mixture was diluted with methylene chloride (5 mL) and washed with water (2.5 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water) to provide **1-145a** (0.0742 g, 0.104 mmol, 94%) as a white solid: **Rf** (10% methanol in ethyl acetate) 0.59; **Mp** (methylene chloride) 81-83 ^{o}C; **IR** 3305.8, 2902.1, 2846.4, 1719.3, 1635.8, 1503.4, 1484.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.78 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.61 (d, *J* = 2.4 Hz, 1H), 7.67 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1H), 7.52-7.48 (m, 1H), 6.53-6.46 (m, 2H), 5.92-5.87 (m, 1H), 5.78-5.72 (m, 1H), 5.37-5.31 (m, 1H), 5.06 (s, 2H), 4.92 (s, 2H), 4.61-4.59 (m, 1H), 3.41-3.34 (m, 4H), 2.12-2.01 (m, 3H), 1.95-1.92 (m, 5H), 1.71-1.59 (m, 16H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 171.9, 164.91, 164.85, 162.89, 162.83, 158.6, 156.3, 154.0, 151.4, 150.3, 147.9, 134.6, 132.5, 129.8, 125.3, 124.2, 99.00, 98.96, 98.82, 98.78, 95.10, 94.94, 92.35, 92.33, 92.31, 73.0, 60.4, 53.3, 51.7, 44.1, 42.6, 41.3, 39.8, 36.7, 32.7, 31.6, 29.2, 28.6, 24.8, 22.6, 19.2, 14.1; **^{13}F NMR** ($CDCl_3$, 376 MHz) δ -105.0; **LC-MS** (ES) *m/z* calcd for $C_{38}H_{43}O_4N_6S$ $[M+H]^+$ 717.3029, found 717.3030.



1-145b

3-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (2-(2-(2-((3*R*,5*R*,7*R*)-adamantan-1-yl)acetamido)ethoxy)ethyl)carbamate (**1-145b**). Carbonyldiimidazole (0.0268 g, 0.165 mmol) was added to a solution of **1-144** (0.0500 g, 0.110 mmol) in anhydrous methylene chloride (1.60

mL). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 3 h. **1-140** (0.0652 g, 0.220 mmol) was added and the reaction mixture was stirred for 21 h. The reaction mixture was diluted with methylene chloride (5 mL) and washed with water (2.5 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water) to provide **1-145b** (0.0750 g, 0.0986 mmol, 90%) as a white solid: **Rf** (10% methanol in ethyl acetate) 0.52; **Mp** (methylene chloride) 64-66 ^{o}C; **IR** 3292.9, 2899.3, 1718.1, 1634.9, 1503.6, 1484.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.78 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.61 (d, *J* = 2.3 Hz, 1H), 7.67 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1H), 7.52-7.48 (m, 1H), 6.52-6.48 (m, 2H), 5.06 (s, 2H), 4.94 (s, 2H), 4.61-4.58 (m, 1H), 3.54-3.51 (m, 4H), 3.46-3.37 (m, 4H), 2.10-2.05 (m, 4H), 1.95 (d, *J* = 4.5 Hz, 6H), 1.72-1.59 (m, 18H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 171.1, 165.17, 165.09, 162.65, 162.57, 158.6, 155.6, 154.0, 151.4, 150.3, 147.9, 134.6, 132.6, 129.7, 125.3, 124.2, 99.03, 99.02, 98.7, 95.1, 92.36, 92.33, 73.0, 69.95, 69.80, 60.4, 51.7, 44.1, 42.6, 39.0, 36.8, 34.7, 32.7, 31.6, 29.2, 28.6, 25.3, 24.8, 22.7, 19.1, 14.1; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -105.1; **LC-MS** (ES) *m/z* calcd for $C_{40}H_{47}O_5N_6F_2S$ $[M+H]^+$ 761.3291, found 761.3288.

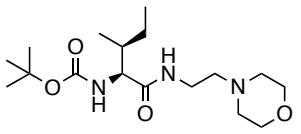


1-145c

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (2-(2-(2-(2-((3*R*,5*R*,7*R*)-adamantan-1-yl)acetamido)ethoxy)ethoxy)ethyl)carbamate (**1-145c**). Carbonyldiimidazole (0.0401 g, 0.248 mmol) was added to a solution of **1-144** (0.0750 g, 0.165 mmol) in anhydrous methylene

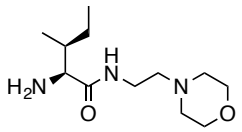
chloride (2.4 mL). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 3 h. **1-143** (0.145 g, 0.330 mmol) and triethylamine (0.115 mL, 0.825 mmol) were added and the reaction mixture was stirred for 11 h. The reaction mixture was diluted with methylene chloride (5 mL) and washed with water (2.5 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (5% methanol in methylene chloride) to provide **1-145c** (0.0865 g, 0.107 mmol, 65%) as a white solid: **Rf** (10% methanol in ethyl acetate) 0.52; **Mp** (methylene chloride, gel) 52 ^{o}C; **IR** 3313.5, 3062.3, 2902.7, 2847.6, 1719.5, 1636.1 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.79 (d, *J* = 4.5 Hz, 1H), 8.61 (s, 1H), 7.69-7.66 (m, 1H), 7.51 (dd, *J* = 8.3, 4.8 Hz, 1H), 6.50 (d, *J* = 8.5 Hz, 2H), 5.91-5.84 (m, 2H), 5.78-5.73 (m, 1H), 5.45-5.41 (m, 1H), 8.79-1.60 (m, 100H), 5.06 (s, 2H), 4.94 (s, 2H), 4.61-4.60 (m, 1H), 3.61-3.54 (m, 7H), 3.43 (dq, *J* = 16.9, 5.4 Hz, 4H), 2.14-2.01 (m, 3H), 1.95-1.92 (m, 5H), 1.76-1.66 (m, 6H), 1.61 (t, *J* = 4.2 Hz, 9H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 171.1, 165.16, 165.07, 162.64, 162.56, 158.75, 158.62, 158.48, 155.5, 154.0, 151.4, 150.3, 147.9, 134.7, 132.5, 129.7, 125.3, 124.2, 99.0, 98.8, 70.25, 70.15, 69.9, 60.4, 53.3, 51.7, 44.1, 42.6, 40.9, 39.1, 36.8, 32.7, 29.2, 28.7, 24.8, 19.1; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -105.0; **LC-MS** (ES) *m/z* calcd for $C_{42}H_{51}O_6N_6F_2S$ $[M+H]^+$ 805.3553, found 805.3557.

4.2.2 Chapter 2.0 Procedures



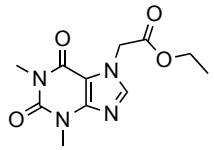
2-1

***Tert*-butyl-((2*S*,3*S*)-3-methyl-1-((2-morpholinoethyl)amino)-1-oxopentan-2-yl)carbamate (2-1)**. A solution of 50% propylphosphonic anhydride in ethyl acetate (14.5 mL, 48.6 mmol) was added drop wise to a solution of Boc-L-isoleucine (4.50 g, 19.5 mmol), 4-(2-aminoethyl)morpholine (2.55 mL, 19.5 mmol), and triethylamine (8.14 mL, 58.4 mmol) in anhydrous ethyl acetate (130 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction was diluted with ethyl acetate (100 mL) and washed with water (75 mL), saturated sodium bicarbonate (75 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to **2-1** (5.61 g, 16.3 mmol, 84%) as a white solid: **Rf** (5% methanol in ethyl acetate) 0.32; **Mp** (ethyl acetate) 113-115 ^{o}C; **IR** 3312.1, 2961.7, 2933.7, 2874.1, 2810.7, 2245.9, 1649.5, 1502.3 1166.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.35 (br. s, 1 H), 5.09 (d, 1 H, *J* = 7.8 Hz), 3.93 (dd, 1 H, *J* = 8.4, 6.3 Hz), 3.71 (t, 4 H, *J* = 4.2 Hz), 3.41-3.35 (m, 2 H), 2.51-2.46 (m, 6 H), 1.84 (br. S, 1 H), 1.54-1.37 (m, 10 H), 1.23-1.06 (m, 1 H), 0.94-0.89 (m, 6 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.4, 155.7, 79.7, 66.8, 59.2, 56.8, 53.3, 37.5, 35.5, 28.3, 24.9, 15.5, 11.5; **LC-MS** (ES) *m/z* calcd $C_{17}H_{34}O_4N_3$ for $[M+H]^+$ 344.2544, found 344.2539.



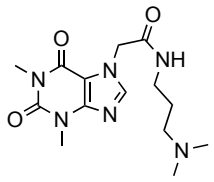
LM11A-31

(2*S*,3*S*)-2-Amino-3-methyl-*N*-(2-morpholinoethyl)pentanamide (LM11A-31). **2-1** (5.61 g, 16.3 mmol) was added to a solution of acetyl chloride (18.6 mL, 261 mmol) in anhydrous methanol (160 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 16 h. The reaction was slowly neutralized with a saturated solution of aqueous potassium carbonate. The resulting mixture was concentrated under reduced pressure and resuspended in anhydrous methylene chloride (100 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide LM11A-31 (3.19 g, 13.1 mmol, 80%) as a white solid. Rf (12:3:5 BuOH:$AcCO_2H$:H_2O) 0.15; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{17}}$ = -16.0 (*c*. 1.18 in $CHCl_3$); Mp (hexane) 52-53 ^{o}C; IR 3306.5, 2959.8, 2931.8, 2870.3, 1627.1, 1550.7, 1457.5, 1118.3 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.47 (br. s, 1H), 3.73 (t, 4 H, *J* = 4.5 Hz), 3.46-3.32 (m, 2 H), 3.29 (d, 1 H, *J* = 3.6 Hz), 2.54-2.48 (m, 6 H), 2.01-1.93 (m, 3 H), 1.48-1.35 (m, 1 H), 1.19-1.05 (m, 1 H), 0.96 (d, 3 H, *J* = 6.9 Hz), 0.90 (t, 3 H *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.1, 66.9, 59.9, 57.3, 53.4, 38.1, 35.4, 23.9, 16.0, 11.9; LC-MS (ES) *m/z* calcd $C_{12}H_{26}O_2N_3$ for $[M+H]^+$ 244.2020, found 244.2019.



2-2

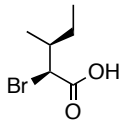
Ethyl 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)acetate (2-2). Ethyl chloroacetate (2.67 mL, 24.9 mmol) was added to a solution of theophylline (4.50 g, 24.9 mmol) and potassium carbonate (3.79 g, 27.5 mmol) in DMF (120 mL) and heated to 90 ^{o}C under an atmosphere of nitrogen. The reaction was stirred for 12 h and then cooled to room temperature. The mixture was diluted with water (250 mL) and extracted with ethyl acetate (150 mL, three times). The combined organic solutions were dried ($MgSO_4$) and concentrated under reduced pressure to provide **2-2** (5.27 g, 19.8 mmol, 79 %) as a white solid: **Rf** (1% methanol in ethyl acetate) 0.43; **Mp** (methylene chloride) 145-146 ^{o}C; **IR** 3116.4, 2991.5, 2952.4, 1750.2, 1698.0, 1643.9, 1547.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.59 (s, 1 H), 5.08 (s, 2 H), 4.27 (q, 2 H, *J* = 7.2 Hz), 3.59 (s, 3 H), 3.38 (s, 3 H), 1.31 (t, 3 H, *J* = 7.2 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 167.0, 155.3, 151.7, 148.5, 141.9, 107.1, 62.4, 47.3, 29.8, 27.9, 14.1; **LC-MS** *m/z* calcd for $C_{11}H_{15}N_4O_4$ $[M+H]^+$ 267.1088, found 267.1086.



LM11A-24

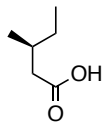
2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)-*N*-(3-(dimethylamino)propyl)acetamide (LM11A-24). **2-2** (0.800 g, 3.00 mmol) was added to a high pressure vial containing a solution of 3-(dimethylamino)-1-propylamine (0.42 mL, 3.3 mmol) in

ethanol (3.75 mL). This was repeated for four additional reactions. Each vial was sealed, heated to 110 ^{o}C, and stirred for 2 hours. All reaction mixtures were combined and the unified solution was concentrated under reduced pressure. The solid material was crushed into small particles, suspended in anhydrous diethyl ether (10 mL), sonicated for 30 min, and filtered. The resulting solid was dried under reduced pressure to provide **LM11A-24** (3.53 g, 10.95 mmol, 73%) as a white solid: **Rf** (1% methanol in ethyl acetate) 0.57; **Mp** (methylene chloride) 169-171 ^{o}C; **IR** 3340.0, 3105.2, 2946.8, 2777.1, 2245.9 1699.8, 1647.7, 1548.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.99 (s, br, 1 H), 7.68 (s, 1 H), 4.90 (s, 2 H), 3.60 (s, 3 H), 3.40 (s, 3 H), 3.35 (q, 2 H, *J* = 6 Hz), 2.33 (t, 2 H, *J* = 6.5 Hz), 2.10 (s, 6 H), 1.62 (quin, 2 H, *J* = 6 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 165.5, 155.4, 151.6, 148.8, 142.3, 106.8, 58.7, 49.7, 45.3, 40.0, 29.8, 27.9, 25.3; **LC-MS** (ES) *m/z* calcd $C_{14}H_{23}O_3N_6$ for $[M+H]^+$ 323.1826, found 323.1825.



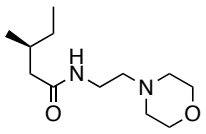
2-3

(2*S*,3*S*)-2-Bromo-3-methylpentanoic acid (**2-3**).[292] Sodium nitrite (1.68 g, 24.4 mmol) was added portion wise to a mixture of L-isoleucine (1.00 g, 7.62 mmol), 47% aqueous solution of hydrogen bromide (7.05 mL, 60.9 mmol) in water (30 mL) at 0 ^{o}C and stirred for 2 h. The reaction mixture was degassed under reduced pressure and extracted with diethyl ether (30 mL, three times). The combined organic solution was washed with water (30 mL), brine (30 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide crude **2-3** as a yellow liquid. This material was carried forward without further purification.



2-4

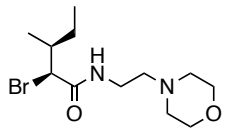
(*S*)-3-Methylpentanoic acid (**2-4**).[292] A solution of crude **2-3** (1.00 g, 5.13 mmol) in methanol (8 mL) was added dropwise to a mixture of zinc dust (3.35 g, 51.3 mmol), copper (I) chloride (0.976 g, 5.13 mmol) in methanol (10 mL) at room temperature. The reaction was allowed to stir for 17 h and then filtered through a pad of silica. The pad of silica was washed with ether (25 mL) and the combined organic solutions were concentrated under reduced pressure. The resulting residue was resuspended in a 1M aqueous solution of hydrogen chloride (10 mL) and extracted with diethyl ether (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by Kugelrohr distillation (ca. 75 ^oC, high vacuum) to provide **2-4** (0.261 g, 2.25 mmol, 44%) as a colorless oil: **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 2.36 (dd, 1 H, *J* = 15.0, 6.0 Hz), 2.15 (dd, 1 H, *J* = 15.0, 8.2 Hz), 1.93-1.86 (m, 1 H), 1.43-1.36 (m, 1 H), 1.30-1.21 (m, 1 H), 0.97 (d, 3 H, *J* = 6.7 Hz), 0.90 (t, 3 H, *J* = 7.4 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) 180.1, 41.2, 31.7, 29.3, 19.2, 11.2.



2-5

(*S*)-3-Methyl-*N*-(2-morpholinoethyl)pentanamide (**2-5**). A solution of 50% propylphosphonic anhydride in ethyl acetate (0.807 mL, 1.36 mmol) was added drop wise to a solution of **2-4** (0.126 g, 1.08 mmol), 2-morpholinoethan-1-amine (0.14 mL, 1.1 mmol), and triethylamine (0.45 mL, 3.23 mmol) in anhydrous ethyl acetate (7.25 mL) at 0 ^oC under an

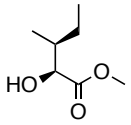
atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 11 h. The reaction was diluted with a saturated solution of aqueous sodium bicarbonate (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified on neutral alumina (5% methanol in ethyl acetate) to provide **2-5** (0.140 g, 0.613 mmol, 57%) as an off-white solid: $[\boldsymbol{\alpha}]_D^{17}$ = +3.0 (*c*. 1.09 in $CHCl_3$); **Rf** (10% methanol in ethyl acetate; neutral alumina) 0.44; **Mp** (CH_2Cl_2) 61-63°C; **IR** (neat) 3289.7, 2965.4, 2810.7, 1638.3, 1550.7, 1112.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.02 (br. s, 1 H), 3.73 (br. s, 4 H), 3.38 (app. q, 2 H, *J* = 5.6 Hz), 2.50 (br. s, 6 H), 2.20 (dd, 1 H, *J* = 13.2, 5.6 Hz), 1.99-1.87 (m, 2 H), 1.44-1.33 (m, 1 H), 1.27-1.14 (m, 1 H), 0.93-0.88 (m, 6 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 172.7, 66.7, 57.2, 53.3, 44.1, 35.4, 32.3, 29.4, 19.2, 11.3; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{25}O_2N_2$ $[M+H]^+$ 229.1911, found 229.1910.



2-6

(2*S*,3*S*)-2-Bromo-3-methyl-*N*-(2-morpholinoethyl)pentanamide (**2-6**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.91 mL, 3.20 mmol) was added drop wise to a solution of **2-3** (0.500 g, 2.56 mmol), (2-aminoethyl)morpholine (0.33 mL, 2.6 mmol) and triethylamine (1.07 mL, 7.69 mmol) in anhydrous ethyl acetate (17 mL) at 0 °C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by

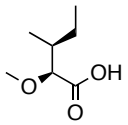
chromatography on silica (ethyl acetate) to provide **2-6** (0.494 g, 1.61 mmol, 63%) as an off-white solid: **Rf** (10% methanol in ethyl acetate) 0.40; $[\boldsymbol{\alpha}]_D^{17}$ = -20.1 (*c*. 1.08 in $CHCl_3$); **Mp** (methylene chloride) 83-85 ^oC; **IR** 3259.9, 3101.5, 2956.1, 1647.7, 1567.5, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.15 (br. s, 1 H), 4.32 (d, 1 H, *J* = 5.2 Hz), 3.73 (t, 4 H, *J* = 4.8 Hz), 3.46-3.32 (m, 2 H), 2.57-2.50 (m, 6 H), 2.20-2.07 (m, 1H), 1.60-1.50 (m, 1 H), 1.34-1.23 (m, 1 H), 1.04 (d, 3 H, *J* = 6.8 Hz), 0.91 (t, 3 H, 7.6 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 168.2, 66.9, 60.3, 56.5, 53.2 38.8, 36.2, 25.7, 17.1, 11.2; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{24}O_2N_2Br$ $[M+H]^+$ 307.1016, found 307.1016.



2-7

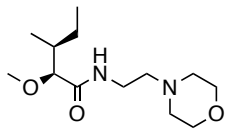
(2*S*,3*S*)-2-Hydroxy-3-methylpentanoic acid (**2-7**).[293] A solution of sodium nitrite (1.57 g, 22.9 mmol) in water (8 mL) was added drop wise to a solution of L-isoleucine (2.00 g, 15.2 mmol) in 2.5 N aqueous H_2SO_4 (10 mL) at 0 ^oC. The reaction was slowly warmed to room temperature and stirred for 12.5 h. The reaction mixture was saturated with sodium chloride and the resulting aqueous solution was extracted with ethyl acetate (25 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide the crude acid (0.810 g) as a colorless oil. The crude acid was added to a solution of acetyl chloride (0.5 mL) in methanol (40 mL) and heated to reflux for 3 h. The reaction mixture was concentrated under reduced pressure, resuspended in ethyl acetate (40 mL), and sequentially washed with a saturated aqueous solution of sodium bicarbonate (20 mL), brine (20 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **2-7** (0.532 g, 3.64 mmol,

24%; two steps) as a colorless liquid: **Rf** (50% ethyl acetate in hexane) 0.81; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 4.09 (d, 1 H, *J* = 3.6 Hz), 3.79 (s, 3 H), 2.66 (br. s. 1 H), 1.88-1.78 (m, 1 H), 1.42-1.33 (m, 2 H), 0.98 (d, *J* = 6.9 Hz), 0.89 (t, 3 H, *J* = 7.5 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 175.5, 74.8, 52.3, 39.1, 23.8, 15.4, 11.7.



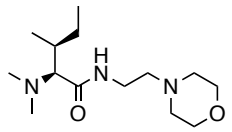
2-8

(2*S*,3*S*)-2-Methoxy-3-methylpentanoic acid (**2-8**). A solution of **2-7** (0.532 g 3.64 mmol) in anhydrous THF (2.5 mL) was added drop wise to a solution of sodium hydride (0.218 g, 5.46 mmol) in anhydrous THF (5 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was stirred for 15 minutes and then methyl iodide (0.34 mL, 5.5 mmol) was added drop wise. The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction was diluted with 1 M aqueous lithium hydroxide (5 mL) and stirred for 17 h. The organic solvent was removed under reduced pressure and the resulting aqueous solution was washed with ethyl acetate (5 mL). The aqueous solution was cooled to 0 ^{o}C and acidified with 1 M aqueous hydrogen chloride until pH 2 was reached. The resulting mixture was extracted with ethyl acetate (15 mL, three times). The combined organic solutions were washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide crude **2-8** as red liquid. This material was carried forward without further purification.



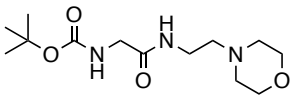
2-9

(2*S*,3*S*)-2-Methoxy-3-methyl-*N*-(2-morpholinoethyl)pentanamide (**2-9**). A solution of 50% propylphosphonic anhydride in ethyl acetate (2.71 mL, 4.55 mmol) was added drop wise to a solution of crude **2-8**, 2-morpholinoethan-1-amine (0.48 mL, 3.6 mmol), and triethylamine (1.52 mL, 10.9 mmol) in anhydrous ethyl acetate (24 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (ethyl acetate) to provide **2-9** (0.221 g, 0.855 mmol, 23%, three steps) as a light yellow solid: $[\boldsymbol{\alpha}]_D^{17}$ = -50.2 (*c.* 1.01 in $CHCl_3$); **Rf** (15% methanol in ethyl acetate) 0.17; **Mp** (methylene chloride) 60-62^{o}C; **IR** 3297.2, 2959.8, 1643.9, 1526.5, 1112.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.83 (br. s, 1 H), 3.69 (t, 4 H, *J* = 4.6 Hz), 3.46 (d, 1 H, *J* = 4.8 Hz), 3.42-3.37 (m, 5 H), 2.52-2.46 (m, 6 H), 1.87-1.76 (m, 1 H), 1.53-1.43 (m, 1 H), 1.27-1.14 (m, 1 H), 0.95 (d, 3 H, *J* = 6.8 Hz), 0.88 (t, 3 H, *J* = 7.6 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.9, 87.2, 66.9, 59.1, 57.2, 53.4, 38.1, 35.1, 24.2, 15.3, 11.7; **LC-MS** (ES) *m/z* calcd for $C_{13}H_{27}O_3N_2$ $[M+H]^+$ 259.2016, found 259.2015.



2-10

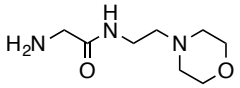
(2*S*,3*S*)-2-(Dimethylamino)-3-methyl-*N*-(2-morpholinoethyl)pentanamide (**2-10**). 10% Palladium on carbon (0.525 g, 0.493 mmol) was added to a mixture of **LM11A-31** (0.150 g, 0.616 mmol), 37% formaldehyde solution (0.577 mL, 10.5 mmol) in methanol (5 mL) in a Parr hydrogenation apparatus. The reaction mixture was hydrogenated under 8 bar of hydrogen for 15 h at room temperature. The reaction mixture was filtered through Celite and the resulting filtrated was extracted with ethyl acetate (5 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure, and purified by chromatography on neutral alumina (70% ethyl acetate in hexane) to provide **2-10** (0.0307 g, 0.113 mmol, 18%) as a colorless residue: $[\boldsymbol{\alpha}]_D^{17}$ = -5.1 (*c*. 1.08 in $CHCl_3$); **Rf** (ethyl acetate, neutral alumina) 0.48; **IR** 3321.4, 2957.9, 2866.6, 1638.3, 1116.5 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.68 (br. s, 1 H), 3.69 (t, 4 H, *J* = 4.6 Hz), 3.39 (q, 2 H, *J* = 5.4 Hz), 2.51-2.44 (m, 6 H), 2.29 (br. s, 5 H), 1.84-1.81 (m, 1 H), 1.60-1.56 (m, 1 H), 1.26-1.14 (m, 1 H), 0.96-0.89 (m, 6 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.0, 74.5, 66.9, 57.3, 53.3, 42.9, 35.2, 34.3, 26.7, 14.5, 11.9; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{30}O_2N_3$ $[M+H]^+$ 272.2333, found 272.2329.



2-12a

***Tert*-butyl (2-((2-morpholinoethyl)amino)-2-oxoethyl)carbamate** (**2-12a**). A solution of 50% propylphosphonic anhydride in ethyl acetate (2.12 mL, 3.57 mmol) was added drop wise

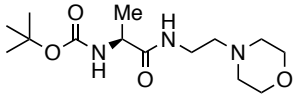
to a solution of Boc-glycine **2-11a** (0.500 g, 2.85 mmol), 4-(2-aminoethyl)morpholine (0.38 mL, 2.9 mmol), and triethylamine (1.19 mL, 8.56 mmol) in anhydrous ethyl acetate (19 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 13.5 h. The reaction was diluted with a saturated solution of aqueous sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **2-12a** (0.786 g, 2.74 mmol, 96%) as a light yellow residue: **Rf** (5% methanol in ethyl acetate) 0.10; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.58 (br. s, 1 H), 5.16 (br. s, 1 H), 3.80 (d, 2 H, *J* = 6.0 Hz), 3.73 (br. s, 4 H), 3.40 (app. q, 2 H, *J* = 5.4 Hz), 2.52 (br. s, 6 H), 1.46 (s, 9 H).



2-13a

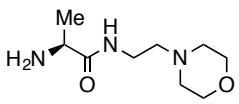
2-Amino-N-(2-morpholinoethyl)acetamide (**2-13a**). **2-12a** (0.786 g, 2.74 mmol) was added to a solution of acetyl chloride (3.11 mL, 43.8 mmol) in dry methanol (27 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until pH of 12 was reached. The solution was concentrated under reduced pressure and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure to provide the **2-13a** (0.106 g, 0.568 mmol, 21%) as a white solid: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.15; **Mp** (methylene chloiride) 38-40 ^{o}C; **IR** 3343.8, 2952.4, 2851.7, 1647.7, 1526.5, 1112.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.44 (br. s, 1 H), 3.71 (t, 4 H, *J* = 4.5 Hz), 3.41-3.36 (m, 4 H), 2.52-2.45 (m, 6 H), 1.58 (br. s, 2 H); **^{13}C NMR**

($CDCl_3$, 125 MHz) δ 172.7, 66.9, 57.3, 53.4, 44.9, 35.4; **LC-MS** (ES) *m/z* calcd for $C_8H_{18}O_2N_3$ $[M+H]^+$, 188.1394 found 188.1394.



2-12b

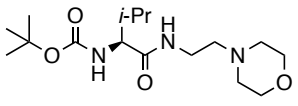
***Tert*-butyl (*S*)-(1-((2-morpholinoethyl)amino)-1-oxopropan-2-yl)carbamate** (**2-12b**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.97 mL, 3.30 mmol) was added drop wise to a solution of Boc-L-alanine **2-11b** (0.500 g, 2.64 mmol), 4-(2-aminoethyl)morpholine (0.35 mL, 2.6 mmol), and triethylamine (1.11 mL, 7.93 mmol) in anhydrous ethyl acetate (18 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 13.5 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **2-12b** (0.568 g, 1.88 mmol, 71 %) as a light yellow residue: **Rf** (5% methanol in ethyl acetate) 0.10; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.59 (br. s, 1 H), 5.03 (br. s, 1 H), 4.17-4.11 (m, 1 H), 3.75 (br. s, 4 H), 3.39 (br. s, 2 H), 2.53 (br. s, 6 H), 1.45 (s, 9 H), 1.36 (d, 3 H, J = 6.9 Hz).



2-13b

(*S*)-2-Amino-N-(2-morpholinoethyl)propanamide (**2-13b**). **2-12b** (0.568 g, 1.88 mmol) was added to a solution of acetyl chloride (2.14 mL, 30.1 mmol) in dry methanol (24 mL) at 0 ^oC

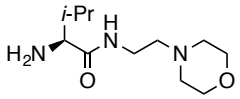
under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until a pH of 12 was reached. The solution was concentrated under reduced pressure and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure to provide **2-13b** (0.186 g, 0.926 mmol, 49%) as a white solid: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.30; $[\boldsymbol{\alpha}]_D^{17}$ = +2.9 (*c*. 1.30 in $CHCl_3$); **Mp** (methylene chloride) 52-54 ^oC; **IR** 3317.7, 2954.2, 2808.8, 1647.7, 1522.8, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.47 (br. s., 1 H), 3.71 (t, 4 H, *J* = 4.5 Hz), 3,50 (q, 1 H, *J* = 6.9 Hz), 3.37 (q, 2 H, *J* = 6 Hz), 2.53-2.47 (m, 6 Hz), 1.78 (br. s., 2 H), 1.34 (d, 3 H, *J* = 6.9 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 175.6, 66.9, 57.3, 53.4, 50.8, 35.5, 21.8; **LC-MS** (ES) *m/z* calcd for $C_9H_{20}O_2N_3$ $[M+H]^+$ 202.1550, found 202.1550.



2-12c

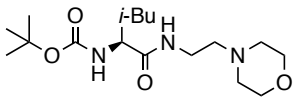
***Tert*-butyl (*S*)-(3-methyl-1-((2-morpholinoethyl)amino)-1-oxobutan-2-yl)carbamate** (**2-12c**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.71 mL, 2.88 mmol) was added drop wise to a solution of Boc-L-valine **2-11c** (0.500 g, 2.30 mmol), 4-(2-aminoethyl)morpholine (0.30 mL, 2.3 mmol), and triethylamine (0.96 mL, 6.9 mmol) in anhydrous ethyl acetate (15 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 11 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under

reduced pressure to provide **2-12c** (0.644 g, 1.96 mmol, 85%) as a white solid: **Rf** (5% methanol in ethyl acetate) 0.13; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.40 (br. s, 1 H), 5.09 (br. s, 1 H), 3.88 (app. q, 1 H, *J* = 6.3 Hz), 3.72 (app. t, 4 H, *J* = 4.2 Hz), 3.39 (br. s, 2 H), 2.50 (br. s, 6 H), 2.14-2.04 (m, 1 H), 1.44 (s, 9 H), 0.93 (app. q, 6 H, *J* = 7.2 Hz).



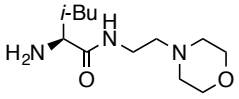
2-13c

(*S*)-2-Amino-3-methyl-N-(2-morpholinoethyl)butanamide (**2-13c**). **2-12c** (0.645 g, 1.96 mmol) was added to a solution of acetyl chloride (2.23 mL, 31.3 mmol) in dry methanol (20 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until a pH of 12 was reached. The mixture was concentrated and the resulting aqueous solution was washed with ethyl acetate (15 mL), concentrated under reduced pressure, and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-13c** (0.279 g, 1.22 mmol, 62%) as a colorless residue: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.33; $[\boldsymbol{\alpha}]_D^{17}$ = -19.7 (*c*. 1.13 in $CHCl_3$); **IR** 3310.2, 2954.2, 2887.1, 1645.8, 1517.2, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.41 (br. s, 1 H), 3.70 (t, 4 H, *J* = 4.8 Hz), 3.47-3.29 (m, 2 H), 3.22 (d, 1 H, *J* = 3.9 Hz), 2.52-2.46 (m, 6 H), 2.33-2.24 (m, 1 H), 1.58 (br. s, 2 H), 0.98 (d, 3 H, *J* = 6.9 Hz), 0.84 (d, 3 H, *J* = 6.9 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.4, 66.9, 60.4, 57.4, 53.4, 35.5, 31.0, 19.6, 16.2; **LC-MS** (ES) *m/z* calcd for $C_{11}H_{24}O_2N_3$ $[M+H]^+$ 230.1863, found 230.1863.



2-12d

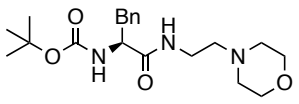
***Tert*-butyl (*S*)-(4-methyl-1-((2-morpholinoethyl)amino)-1-oxopentan-2-yl)carbamate** (**2-12d**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.61 mL, 2.70 mmol) was added drop wise to a solution of Boc-L-leucine **2-11d** (0.500 g, 2.16 mmol), 4-(2-aminoethyl)morpholine (0.28 mL, 2.2 mmol), and triethylamine (0.91 mL, 6.5 mmol) in anhydrous ethyl acetate (14 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 10.5 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **2-12d** (0.634 g, 1.85 mmol, 85%) as a colorless residue: **Rf** (1% methanol in ethyl acetate) 0.15; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.66 (br. s, 1 H), 4.97 (br. s, 1 H), 4.09 (br. s, 1 H), 3.74 (t, 4 H, *J* = 4 Hz), 3.37 (q, 2 H, *J* = 5.6 Hz), 2.53 (br. s, 6 H), 1.69-1.62 (m, 2 H), 1.44 (s, 9 H), 0.94 (dd, 6 H, *J* = 6.0, 3.6 Hz).



2-13d

(*S*)-2-Amino-4-methyl-N-(2-morpholinoethyl)pentanamide (**2-13d**). **2-12d** (0.634 g, 1.85 mmol) was added to a solution of acetyl chloride (2.10 mL, 29.6 mmol) in dry methanol (19 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 2 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate. The resulting solution was concentrated under reduced pressure and

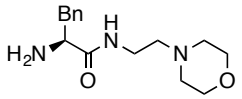
suspended in anhydrous methylene chloride (20 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-13d** (0.230 g, 0.945 mmol, 51%) as a colorless oil: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.77; $[\boldsymbol{\alpha}]_D^{17}$ = -7.4 (*c.* 1.14 in $CHCl_3$); **IR** 3340.0, 2950.5, 2864.8, 2857.3, 1645.8, 1519.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.47 (br. s, 1 H), 3.73 (t, 4 H, *J* = 4.5 Hz), 3.42-3.35 (m, 3 H), 2.53-2.48 (m, 6 H), 1.87 (br. s, 2 H), 1.75 (m, 2 H), 1.39-1.32 (m, 1 H), 0.95 (dd, 6 H, *J* = 8.1, 6.3 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 175.6, 66.9, 57.3, 53.7, 53.4, 44.3, 35.5, 24.9, 23.4, 21.5; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{26}O_2N_3$ $[M+H]^+$ 244.20195, found 244.20040.



2-12e

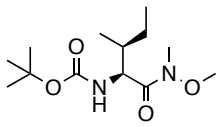
***Tert*-butyl (*S*)-(1-((2-morpholinoethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate** (**2-12e**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.40 mL, 2.36 mmol) was added drop wise to a solution of Boc-L-phenylalanine (0.500 g, 1.89 mmol), 4-(2-aminoethyl)morpholine (0.25 mL, 1.9 mmol), and triethylamine (0.79 mL, 5.7 mmol) in anhydrous ethyl acetate (13 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 10.5 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **2-12e** (0.707 g, 1.87 mmol, 99%) as a white solid: **Rf** (1% methanol in ethyl acetate) 0.14; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.34-7.29 (m, 3H), 7.26-7.22 (m, 2 H), 6.11 (br. s, 1 H), 5.22 (br. s, 1 H), 4.31 (q, 1 H, *J* = 6.6 Hz), 3.64 (br. s, 4 H), 3.26 (br. s, 2

H), 3.13 (dd, 1 H, *J* = 13.5, 6.3 Hz), 3.00 (dd, 1 H, *J* = 12.9, 8.1 Hz), 2.33 (br. s, 6 H), 1.45 (s, 9 H).



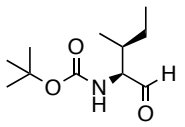
2-13e

(*S*)-2-Amino-N-(2-morpholinoethyl)-3-phenylpropanamide (**2-13e**). **2-12e** (0.566 g, 1.49 mmol) was added to a solution of acetyl chloride (1.71 mL, 23.9 mmol) in dry methanol (15 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until a pH of 12 was reached. The mixture was concentrated and the resulting aqueous solution was washed with ethyl acetate (15 mL), concentrated under reduced pressure, and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-13e** (0.135 g, 0.487 mmol, 32%) as a colorless oil: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.49; $[\boldsymbol{\alpha}]_D^{17}$ = -2.1 (*c*. 1.21 in $CHCl_3$); **IR** 3368.0, 2954.2, 1647.7, 1517.2, 1451.9, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.44 (br. s., 1 H), 7.37-7.24 (m, 5 H), 3.72 (t, 4 H, *J* = 4.5 Hz), 3.63 (dd, 1 H, *J* = 9, 4.5), 3.39 (q, 2 H, *J* = 6.0 Hz), 3.26 (dd, 1 H, *J* = 13.8, 4.5 Hz), 2.77 (dd, 1 H, *J* = 13.5, 9 Hz), 2.52-2.46 (m, 6 H), 1.75 (br. s., 2 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.2, 138.0, 129.3, 128.6, 126.7, 66.9, 57.2, 56.7, 53.4, 41.3, 35.5; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{24}O_2N_3$ $[M+H]^+$ 278.1863, found 278.1860.



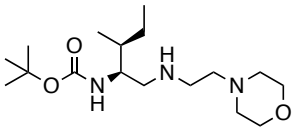
2-14

***Tert*-butyl-((2*S*,3*S*)-1-(methoxy(methyl)amino)-3-methyl-1-oxopentan-2-yl)carbamate** (**2-14**).[294] Hydroxybenzotriazole (0.643 g, 4.76 mmol and EDC HCl (0.995 g, 5.19 mmol) were added to a solution of Boc-L-isoleucine (1.00 g, 4.32 mmol) in anhydrous methylene chloride (18 mL) at 0 ^{o}C under an atmosphere of nitrogen. The mixture was allowed to stir for 15 minutes and then N,O-dimethylhydroxylamine hydrogen chloride (0.485 g, 4.97 mmol) and N-methylmorpholine (0.57 mL, 5.2 mmol) was added. The reaction was warmed to room temperature and stirred for 22 h. The reaction mixture was concentrated under reduced pressure and resuspended in ethyl acetate (20 mL). The organic solution was sequentially washed with 1N aqueous hydrogen chloride (10 mL), saturated aqueous solution of sodium bicarbonate (10 mL), and brine (10 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, purified by chromatography on silica (15% ethyl acetate in hexane) to provide **2-14** (1.05 g, 3.81 mmol, 88%) as a colorless oil: **Rf** (ethyl acetate) 0.77; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.09 (d, 1 H, *J* = 9.6 Hz), 4.82 (app. t, 1 H, *J* = 9.3 Hz), 3.77 (s, 3 H), 3.21 (s, 3 H), 1.76-1.67 (m, 1 H), 1.58-1.49 (m, 1 H), 1.43 (s, 9 H), 1.17-1.05 (m, 1 H), 0.93-0.86 (m, 6 H).



2-15

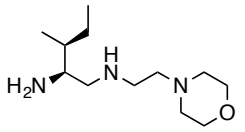
***Tert*-butyl ((2S,3*S*)-3-methyl-1-oxopentan-2-yl)carbamate** (**2-15**).[295] A 1 M solution of lithium aluminum hydride (2.28 mL, 2.28 mmol) was added drop wise to a solution of **2-14** (0.500 g, 1.82 mmol) in anhydrous THF (38 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 0.75 h. The reaction was quenched with a 1 M aqueous solution of potassium bisulfate (2.75 mL). The organic solution was sequentially washed with 1 N aqueous hydrogen chloride (15 mL, three times), saturated aqueous solution of sodium bicarbonate (15 mL, three times), and brine (15 mL, three times). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **2-15** (0.237 g, 1.10 mmol, 60%) as a colorless oil: **Rf** (30% ethyl acetate in hexane) 0.74; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 9.66 (s, 1 H), 5.11 (br. s, 1 H), 4.29 (app. q, J = 4.4 Hz), 2.02 (br. s, 1 H), 1.53-1.43 (m, 11 H), 1.32-1.19 (m, 1 H), 0.99-0.94 (m, 6 H).



2-16

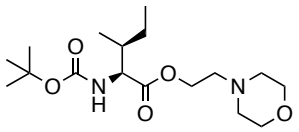
***Tert*-butyl ((3*S*)-3-methyl-1-((2-morpholinoethyl)amino)pentan-2-yl)carbamate** (**2-16**). 2-Morpholinoethan-1-amine (0.14 mL, 1.1 mmol) was added to a solution of **2-15** (0.237 g, 1.10 mmol) in dichloroethane (6.5 mL) and stirred under an atmosphere of nitrogen at room temperature for 1 h. Sodium triacetoxyborohydride (0.664 g, 2.13 mmol) was added the reaction mixture was stirred for an additional 23 h. The reaction mixture was concentrated under reduced

pressure and purified by chromatography on neutral alumina (8% methanol in ethyl acetate, dry loaded) to provide **2-16** (0.233 g, 0.706 mmol, 64%) as a white residue: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.42; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 4.98 (d, 1 H, *J* = 6.8 Hz), 3.71 (t, 5 H, *J* = 4.4 Hz), 3.01-2.91 (m, 3 H), 2.85-2.79 (m, 1 H), 2.69 (br. s, 2 H), 2.49 (br. s, 4 H), 1.62 (br. s, 1 H), 1.55-1.38 (m, 10 H), 1.22-1.10 (m, 1 H), 0.94-0.91 (m, 6 H).



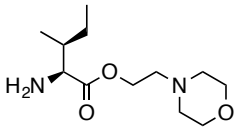
2-17

(2*S*,3*S*)-3-Methyl-*N*^{1}-(2-morpholinoethyl)pentane-1,2-diamine (**2-17**). **2-16** (0.230 g, 0.698 mmol) was added to a mixture of acetyl chloride (0.79 mL, 11 mmol) in anhydrous methanol (7 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction was then cooled to 0 ^{o}C and quenched with a 1M aqueous solution of sodium hydroxide until pH 12 was reached. The mixture was concentrated under reduced pressure and resuspended in methylene chloride (15 mL). The heterogeneous mixture was sonicated for 15 minutes, filtered, and the resulting filtrate was concentrated under reduced pressure to provide **2-17** (0.107 g, 0.467 mmol, 67%) as a tan residue: **Rf** (20% methanol in ethyl acetate, neutral alumina) 0.15; $[\boldsymbol{\alpha}]_D^{17}$ = +5.1 (*c.* 1.06 in $CHCl_3$); **IR** 3292.9, 2930.0, 2855.0, 1454.9, 1115.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 3.80 (br. s, 3 H), 3.70 (t, 4 H, *J* = 4.4 Hz), 2.99-2.92 (m, 2 H), 2.89-2.84 (m, 1 H), 2.81-2.75 (m, 1 H), 2.69-2.57 (m, 2 H), 2.50-2.48 (m, 5 H), 1.57-1.43 (m, 2 H), 1.25-1.16 (m, 1 H), 0.91 (app. q, 6 H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 66.9, 56.6, 54.4, 53.5, 51.0, 45.1, 38.7, 25.3, 14.9, 11.5; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{28}ON_3$ $[M+H]^+$ 230.2227, found 230.2226.



2-18

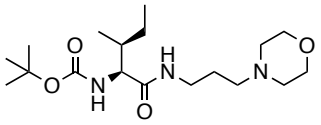
2-Morpholinoethyl (2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylpentanoate (**2-18)**. EDC HCl (0.497 g, 2.59 mmol) and DMAP (0.264 g, 2.16 mmol) were sequentially added to a solution of Boc-L-isoleucine (0.500 g, 2.16 mmol) and 2-morpholinoethan-1-ol (0.26 mL, 2.2 mmol) in anhydrous methylene chloride (6 mL) at room temperature under an atmosphere of nitrogen and stirred for 11 h. The reaction mixture was sequentially washed with water (5 mL) and brine (5 mL). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (50% ethyl acetate in hexane) to provide **2-18** (0.649 g, 1.88 mmol, 87%) as a colorless oil: **Rf** (ethyl acetate) 0.37; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.03 (d, 1 H, *J* = 8.4 Hz), 4.37-4.31 (m, 1 H), 4.27 (dd, 1 H, *J* = 8.8, 4.8 Hz), 4.22-4.15 (m, 1 H), 3.69 (t, 4 H, *J* = 4.8 Hz), 2.67-2.58 (m, 2 H), 2.49 (app. q, *J* = 4 Hz), 1.85 (br. s, 1 H), 1.50-1.40 (m, 10 H), 1.28-1.13 (m, 1 H), 0.95-0.90 (m, 6 H).



2-19

(*S*)-2-Morpholinoethyl (3*S*)-2-amino-3-methylpentanoate (**2-19**). **2-18** (0.649 g, 1.88 mmol) was added to a solution of acetyl chloride (2.14 mL, 30.2 mmol) in dry methanol (19 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until a pH of 12 was reached. The mixture was concentrated and the

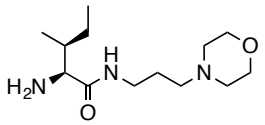
resulting aqueous solution was washed with ethyl acetate (15 mL), concentrated under reduced pressure, and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-19** (0.318 g, 1.30 mmol, 69%) as a colorless oil: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.60; **IR** (neat) 2957.9, 2870.3, 1727.8, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 4.34-4.28 (ddd, 1 H, *J* =11.6, 6.2, 5.6 Hz), 4.22-4.17 (m, 1 H), 3.69 (t, 4 H, *J* = 4.4 Hz), 3.37 (d, 1 H, *J* = 4.8 Hz), 2.62 (td, 2 H, *J* = 5.2, 2.0 Hz), 1.77-1.70 (m, 1 H), 1.65 (br. s, 2 H), 1.52-1.42 (m, 1 H), 1.29-1.14 (m, 1 H), 0.96 (d, 3 H, *J* = 6.8 Hz), 0.91 (3 H, *J* = 7.2 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 175.5, 66.9, 61.4, 59.1, 57.1, 53.7, 39.2, 24.6, 15.6, 11.7; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{25}O_3N_2$ $[M+H]^+$ 245.1860, found 245.1859.



2-20

***Tert*-butyl-((2*S*,3*S*)-3-methyl-1-((3-morpholinopropyl)amino)-1-oxopentan-2-yl)carbamate** (**2-20**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.61 mL, 2.70 mmol) was added drop wise to a solution of Boc-L-isoleucine (0.500 g, 2.16 mmol), N-(3-aminopropyl)morpholine (0.32 mL, 2.2 mmol), and triethylamine (0.91 mL, 6.5 mmol) in anhydrous ethyl acetate (14 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 19 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under

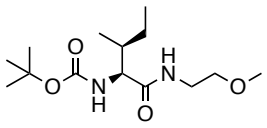
reduced pressure to provide **2-20** (0.628 g, 1.76 mmol, 81%) as a white solid: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.70; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.01 (br. s, 1 H), 5.06 (br. s, 1 H), 3.88 (t, 1 H *J* = 6.9 Hz), 3.74 (br. s, 4 H), 3.44-3.29 (m, 2 H), 2.47 (br. s, 5 H), 1.87-1.83 (m, 1 H), 1.70 (br. s, 2 H), 1.52-1.44 (m, 11 H), 1.18-1.04 (m, 1 H), 0.93-0.88 (m, 7 H).



2-21

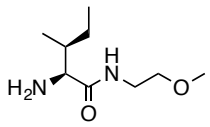
(2*S*,3*S*)-2-Amino-3-methyl-*N*-(3-morpholinopropyl)pentanamide (**2-21**). **2-20** (0.628 g, 1.76 mmol) was added to a solution of acetyl chloride (1.99 mL, 28.1 mmol) in dry methanol (17.5 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until a pH of 12 was reached. The mixture was concentrated and the resulting aqueous solution was washed with ethyl acetate (15 mL), concentrated under reduced pressure, and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-21** (0.452 g, 0.264 mmol, 58%) as a yellow oil: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.16; $[\boldsymbol{\alpha}]_D^{17}$ = -18.2 (*c* = 1.01 in $CHCl_3$); **IR** (neat) 3304.6, 2954.2, 2808.8, 1643.9, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.78 (br. s, 1 H), 3.73 (t, 4 H, *J* = 4.8 Hz), 3.41-3.29 (m, 2 H), 3.24 (d, 1 H, *J* = 3.6 Hz), 2.46-2.42 (m, 6 H), 2.02-1.92 (m, 1 H), 1.70 (quint, 2 H, *J* = 6.8 Hz), 1.57 (br. s, 1 H), 1.44-1.33 (m, 1 H), 1.18-1.04 (m, 1 H), 0.96 (d, 3 H, *J* = 7.2 Hz), 0.89 (t, 3 H, *J*

= 7.2 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.3, 66.9, 60.2, 57.4, 53.8, 38.2, 38.1, 25.8, 23.8, 16.2, 11.9; **LC-MS** (ES) *m/z* calcd for $C_{13}H_{28}O_2N_3$ $[M+H]^+$ 258.2176, found 258.2173.



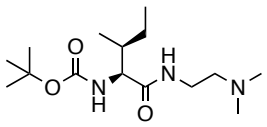
2-22a

***Tert*-butyl-((2*S*,3*S*)-1-((2-methoxyethyl)amino)-3-methyl-1-oxopentan-2-yl)carbamate** (**2-22a**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.61 mL, 2.70 mmol) was added drop wise to a solution of Boc-L-isoleucine (0.500 g, 2.16 mmol), 2-methoxyethylamine (0.19 mL, 2.2 mmol), and triethylamine (0.91 mL, 6.5 mmol) in anhydrous ethyl acetate (14 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 16 h. The reaction was diluted with a saturated aqueous solution of sodium carbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **2-22a** (0.579 g, 2.01 mmol, 93%) as a white solid: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.85; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.21 (br. s, 1 H), 5.04 (br. s, 1 H), 3.92 (t, 1 H, *J* = 8.1 Hz), 3.45-3.44 (m, 4 H), 3.34 (s, 3 H), 1.88 (br. s, 1 H), 1.52-1.44 (m, 11 H), 1.19-1.06 (m, 1 H), 0.93-0.88 (m, 6 H).



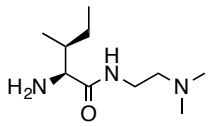
2-23a

(2*S*,3*S*)-2-Amino-N-(2-methoxyethyl)-3-methylpentanamide (**2-23a**). **2-22a** (0.623 g, 2.16 mmol) was added to a solution of acetyl chloride (2.46 mL, 34.6 mmol) in dry methanol (22 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonated until a pH of 12 was reached. The mixture was concentrated and the resulting aqueous solution was washed with ethyl acetate (15 mL), concentrated under reduced pressure, and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-22a** (0.279 g, 1.48 mmol, 69%) as a light yellow oil: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.25; $[\boldsymbol{\alpha}]_D^{17}$ = -28.8 (c = 1.17 in $CHCl_3$); **IR** 3306.5, 2957.9, 2890.8, 1647.7, 1520.9, 1122.0cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.49 (br. s, 1 H), 3.46-3.45 (m, 4 H), 3.36 (s, 3 H), 3.27 (d, 1 H, J = 3.6 Hz), 2.04-1.94 (m, 1 H), 1.47 (br. s, 2 H), 1.44-1.33 (m, 1 H), 1.16-1.04 (m, 1 H), 0.96 (d, 3 H, J = 6.8 Hz), 0.89 (t, 3 H, J = 7.6 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.4, 71.4, 59.9, 58.7, 38.7, 38.0, 23.7, 16.2, 11.9; **LC-MS** (ES) *m/z* calcd for $C_9H_{21}O_2N_2$ $[M+H]^+$ 189.1598, found 189.1598.



2-22b

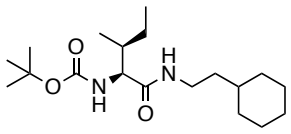
***Tert*-butyl-((2*S*,3*S*)-1-((2-(dimethylamino)ethyl)amino)-3-methyl-1-oxopentan-2-yl)carbamate** (**2-22b**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.61 mL, 2.70 mmol) was added drop wise to a solution of Boc-L-isoleucine (0.500 g, 2.16 mmol), 2-dimethylaminoethylamine (0.20 mL, 2.2 mmol), and triethylamine (0.91 mL, 6.5 mmol) in anhydrous ethyl acetate (14 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 15 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (10% methanol in ethyl acetate) to provide **2-22b** (0.498 g, 1.65 mmol, 76%) as a white solid: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.68; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.69 (br. s, 1 H), 5.14 (d, 1 H, *J* = 7.6 Hz), 3.94 (t, 1 H, *J* = 7.6 Hz), 3.37 (q, 2 H, *J* = 5.2 Hz), 2.55-2.44 (m, 2 H), 2.29 (br. s, 6 H), 1.85 (br. s, 1 H), 1.56-1.44 (m, 11 H), 1.17-1.04 (m, 1 H), 0.93-0.89 (m, 6 H).



2-23b

(2*S*,3*S*)-2-Amino-N-(2-(dimethylamino)ethyl)-3-methylpentanamide (**2-23b**). **2-22b** (0.498 g, 1.65 mmol) was added to a solution of acetyl chloride (1.9 mL, 26 mmol) in dry methanol (16.5 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room

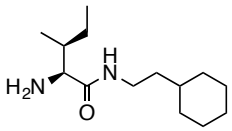
temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until a pH of 12 was reached. The mixture was concentrated and the resulting aqueous solution was washed with ethyl acetate (15 mL), concentrated under reduced pressure, and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-23b** (0.140 g, 0.697 mmol, 42%) as a colorless oil: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.62; $[\boldsymbol{\alpha}]_D^{17}$ = -19.3 (*c* = 1.09 in $CHCl_3$); **IR** 3306.5, 2956.1, 2872.2, 1645.8, 1520.9, 1457.5 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.42 (br. s, 1 H), 3.35 (q, 2 H, *J* = 6 Hz), 3.24 (d, 1 H, *J* = 4 Hz), 2.43 (t, 2 H, *J* = 6 Hz), 2.25 (br. s, 6 H), 1.99-1.89 (m, 1 H), 1.83 (br. s, 2 H), 1.44-1.34 (m, 1 H), 1.17-1.05 (m, 1 H), 0.95 (d, 3 H, *J* = 6.8 Hz), 0.89 (t, 3 H, *J* = 7.2 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.5, 60.1, 58.2, 45.2, 38.2, 36.5, 23.8, 16.1, 11.8; **LC-MS** (ES) *m/z* calcd for $C_{10}H_{24}ON_3$ $[M+H]^+$ 202.1914, found 202.1915.



2-22c

Tert-butyl-((2*S*,3*S*)-1-((2-cyclohexylethyl)amino)-3-methyl-1-oxopentan-2-yl)carbamate (**2-22c**). A solution of 50% propylphosphonic anhydride in ethyl acetate (1.61 mL, 2.70 mmol) was added drop wise to a solution of Boc-L-isoleucine (0.500 g, 2.16 mmol), 2-cyclohexylethan-1-amine (0.275 g, 2.16 mmol), and triethylamine (0.91 mL, 6.5 mmol) in anhydrous ethyl acetate (14 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 15 h. The reaction was diluted with a saturated

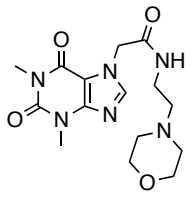
solution of aqueous sodium bicarbonate (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (30% ethyl acetate in hexane) to provide **2-22c** (0.587 g, 1.72 mmol, 80%) as a white solid: **Rf** (ethyl acetate, neutral alumina) 0.81; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.82 (br. s, 1 H), 5.02 (br. s, 1 H), 3.85 (dd, 1 H, J = 8.7 6.6 Hz), 3.37-3.19 (m, 2 H), 1.88 (br. s, 1 H), 1.71-1.63 (m, 6 H), 1.43-1.35 (m, 12 H), 1.29-1.05 (m, 6 H), 0.95-0.88 (m, 8 H).



2-23c

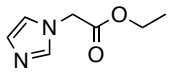
(2*S*,3*S*)-2-Amino-N-(2-cyclohexylethyl)-3-methylpentanamide (**2-23c**). **2-22c** (0.587 g, 1.72 mmol) was added to a solution of acetyl chloride (1.96 mL, 27.6 mmol) in dry methanol (17 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 3 h. The reaction mixture was slowly neutralized with a saturated aqueous solution of potassium carbonate until a pH of 12 was reached. The mixture was concentrated and the resulting aqueous solution was washed with ethyl acetate (15 mL), concentrated under reduced pressure, and resuspended in anhydrous methylene chloride (30 mL). The heterogeneous mixture was sonicated for roughly 30 minutes and filtered. The resulting filtrate was concentrated under reduced pressure and dried under high vacuum to provide **2-23c** (0.241 g, 1.00 mmol, 58%) as a white solid: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.38; $[\boldsymbol{\alpha}]_D^{17}$ = -26.6 (c = 1.13 in $CHCl_3$); **Mp** (methylene chloride): 56-58 ^{o}C; **IR** 3293.4, 2916.9, 2849.8, 1627.1, 1554.5, 1448.2 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.20 (br. s, 1 H), 3.36-3.21 (m, 3 H), 2.06-1.96 (m, 1 H),

1.73-1.62 (m, 5 H), 1.43-1.03 (m, 11 H), 0.96 (d, 3 H, J = 6.5 Hz), 0.922-0.88 (m, 5 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.0, 59.9, 37.9, 37.1, 36.8, 35.5, 33.2, 33.1, 26.5, 26.2, 23.7, 16.2, 11.9; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{29}ON_2$ $[M+H]^+$ 241.2274, found 241.2273.



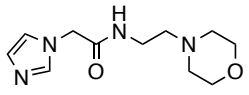
2-24

2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(2-morpholinoethyl)acetamide (**2-24**). **2-2** (0.200 g, 0.751 mmol) was added to a high pressure vial containing a solution of 4-(2-aminoethyl)morpholine (0.11 mL, 0.83 mmol) in ethanol (1 mL). The reaction vial was sealed, heated to 110 ^oC, and stirred for 2 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on SiO_2 (10% methanol in methylene chloride) to provide **2-24** (0.200 g, 0.571 mmol, 76%) as a white solid: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.62; **Mp** (methylene chloride): 203-204 ^oC; **IR** 3280.6, 2948.9, 2815.6, 1648.3, 1548.01, 1114.23 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.71 (s, 1 H), 7.13 (br. s, 1 H), 4.89 (s, 2 H), 3.69 (t, 4 H, J = 4.4 Hz), 3.60 (s, 3 H), 3.39-3.34 (m, 5 H), 2.51-2.46 (m, 6 H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 165.5, 155.7, 151.5, 148.9, 142.3, 106.7, 66.8, 56.7, 53.2, 49.9, 36.1, 29.9, 28.0; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{23}O_4N_6$ $[M+H]^+$ 351.1775, found 351.1772.



2-25

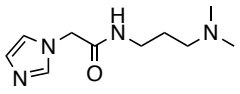
Ethyl 2-(1H-imidazol-1-yl)acetate (**2-25**).[296] Ethyl bromoacetate (0.81 mL, 7.3 mmol) was added to a mixture of imidazole (0.500 g, 7.34 mmol), potassium carbonate (2.13 g, 15.4 mmol) in acetonitrile (30 mL). The reaction mixture was heated to reflux and stirred for 2.5 h. The reaction mixture was cooled to room temperature and quenched with a saturated aqueous solution of ammonium chloride (20 mL) and extracted with ethyl acetate (30 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by column chromatography on SiO_2 (ethyl acetate) to provide **2-25** (0.558 g, 3.62 mmol, 49%) as a yellow oil. **Rf** (ethyl acetate) 0.13; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.58 (s, 1 H), 7.11 (s, 1 H), 6.96 (s, 1 H), 4.70 (s, 2 H), 4.24 (q, 2 H, *J* =7.2 Hz), 1.29 (t, 3 H, *J* = 7.2 Hz).



2-26

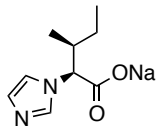
2-(1*H*-Imidazol-1-yl)-*N*-(2-morpholinoethyl)acetamide (**2-26**). **2-25** (0.100 g, 0.649 mmol) was added to a high pressure vial containing a solution of 4-(2-aminoethyl)morpholine (0.09 mL, 0.65 mmol) in ethanol (0.800 mL). The reaction vial was sealed, heated to 110 ^{o}C, and stirred for 3 hours. The reaction mixture was concentrated under reduced pressure and heated to 85 ^{o}C under high vacuum overnight to provide **2-26** (0.119 g, 0.499 mmol, 77%) as a brown residue: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.23; **IR** 3321.4, 2930.0, 1664.4, 1112.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.53 (s, 1 H), 7.18 (s, 1 H), 6.97 (s, 1 H), 6.18 (br.

s., 1 H), 4.66 (s, 2 H), 3.59 (t, 4 H, *J* = 4.5 Hz), 3.31 (q, 2 H, *J* = 5.7 Hz), 2.41 (t, 2 H, *J* = 6 Hz), 2.35 (t, 4 H, *J* = 4.5 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 166.7, 138.1, 130.6, 119.6, 66.8, 56.1, 53.1, 50.1, 35.6; **LC-MS** (ES) *m/z* calcd for $C_{11}H_{19}O_2N_2$ $[M+H]^+$ 239.1503, found 239.1502.



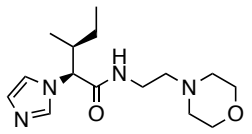
2-27

N-(3-(Dimethylamino)propyl)-2-(1H-imidazol-1-yl)acetamide (**2-27**). **2-25** (0.100 g, 0.649 mmol) was added to a high pressure vial containing a solution of 3-(dimethylamino)-1-propylamine (0.08 mL, 0.65 mmol) in ethanol (0.800 mL). The reaction vial was sealed, heated to 110 ^oC, and stirred for 13.5 hours. The reaction mixture was concentrated under reduced pressure and heated to 85 ^oC under high vacuum overnight to provide **2-27** (0.136 g, 0.647 mmol, 100%) as a light yellow residue: **Rf** (10% methanol in ethyl acetate, neutral alumina) 0.26; **IR** 3280.4, 2939.3, 2890.8, 1662.6, 1558.2, 1505.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.16 (br. s., 1 H), 7.48 (s, 1 H), 7.12 (s, 1 H), 6.92 (s, 1 H), 4.61 (s, 2 H), 3.36 (q, 2 H, *J* = 5.4 Hz), 2.31 (t, 2 H, *J* = 5.4 Hz), 1.99 (s, 6 H), 1.56 (quin, 2 H, *J* = 5.4 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 166.9, 137.9, 130.3, 119.8, 59.6, 50.2, 45.1, 40.9, 24.3; **LC-MS** (ES) *m/z* calcd for $C_{10}H_{19}ON_4$ $[M+H]^+$ 211.1553, found 211.1553.



2-28

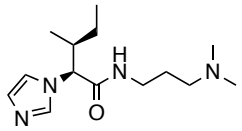
Sodium (2*S*,3*S*)-2-(1*H*-imidazol-1-yl)-3-methylpentanoate (2-28).[265] A mixture of (2*S*,3*S*)-isoleucine (2.50 g, 19.1 mmol), sodium hydroxide (0.762 g, 19.1 mmol), and 7 N aq. ammonia (2.99 mL, 20.9 mmol) in water (25 mL) was slowly added drop wise to a mixture of 37% formaldehyde solution (1.56 mL, 20.9 mmol) and 39% glyoxal solution (2.46 mL, 20.9 mmol) at 50 ^{o}C. The reaction mixture was allowed to stir for 4 h and then concentrated under reduced pressure. The resulting residue was diluted with methanol, sonicated, and concentrated under reduced pressure three times. The final material was dried on high vacuum overnight to provide **2-28** (3.94 g, 101% by mass) as a light red/brown solid: **^{1}H NMR** ($(CD_3)_2SO$, 400 MHz) δ 7.59 (s, 1 H), 7.17 (s, 1 H), 6.76 (s, 1 H), 4.05 (d, 1 H, *J* = 9.2 Hz), 2.01-1.94 (m, 1 H), 1.10-1.04 (m, 1 H), 0.85 (d, 3 H, *J* = 6.4 Hz), 0.83-0.77 (m, 1 H), 0.75-0.72 (m, 3 H); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 172.8, 137.5, 127.2, 119.8, 68.9, 38.4, 25.0, 16.5, 11.4; **LC-MS** (ES) *m/z* calcd for $C_9H_{15}N_2O_2$ $[M+H]^+$ 183.1128, found 183.1044.



2-29

(2*S*,3*S*)-2-(1H-imidazol-1-yl)-3-methyl-N-(2-morpholinoethyl)pentanamide (**2-29**). **2-28** (0.250 g, 0.1.22mmol), pentafluorophenyl diphenylphosphinate (0.517 g, 0.1.35 mmol, and N,N-diisopropylethylamine (0.32 mL, 0.74 mmol) were sequentially added to anhydrous methylene chloride (8.15 mL) under an atmosphere of nitrogen. 4-(2-aminoethyl)morpholine

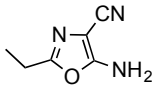
(0.16 mL, 1.2 mmol) was added and the reaction mixture and was allowed to stir for 23 h. The reaction mixture was concentrated, diluted with a 50% aqueous solution of sodium bicarbonate (5 mL), and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by RP-HPLC to provide **2-29** (0.163 g, 0.552 mmol, 45%) as a colorless oil: $[\boldsymbol{\alpha}]_D^{17}$ = +11.1 (*c.* 1.27 in $CHCl_3$); **IR** 3057.8, 2973.8, 2882.8, 1665.5, 1176.1 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 373 K, 400 MHz) δ 8.90 (s, 1 H), 8.59 (br. s, 1 H), 7.66 (s, 1 H), 7.53 (s, 1 H), 4.81 (d, 1 H, *J* = 9.2 Hz), 3.76 (t, 4 H, *J* = 4.4 Hz), 3.50-3.44 (m, 2 H), 3.04-2.99 (m, 6 H), 2.33-2.22 (m, 1 H), 1.28-1.19 (m, 1 H), 1.08-1.01 (m, 1 H), 0.97 (d, 3 H, *J* = 6.8 Hz), 0.84 (t, 3 H, *J* = 7.2 Hz); **^{13}C NMR** (CD_3OD, 125 MHz) δ 172.5, 139.2, 125.9, 123.3, 70.8, 67.4, 59.5, 55.9, 41.9, 37.6, 28.2, 18.1, 13.2; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{27}O_2N_4$ $[M+H]^+$ 295.2129, found 295.2126.



2-30

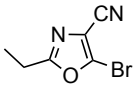
(2*S*,3*S*)-N-(3-(dimethylamino)propyl)-2-(1H-imidazol-1-yl)-3-methylpentanamide (**2-30**) **2-28** (0.250 g, 1.22 mmol), pentafluorophenyl diphenylphosphinate (0.517 g, 1.35 mmol, and N,N-diisopropylethylamine (0.32 mL, 1.8 mmol) were sequentially added to anhydrous methylene chloride (5 mL) under an atmosphere of nitrogen. N,N'-dimethyl-1,3-propanediamine (0.15 mL, 1.2 mmol) was added and the reaction mixture and was allowed to stir for 19.5 h. The reaction mixture was concentrated, diluted with a 50% aqueous solution of sodium bicarbonate (5 mL), and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by RP-HPLC to

provide **2-30** (0.111 g, 0.418 mmol, 34%) as a colorless oil: $[\alpha]_D^{17}$ = +10.7 (*c.* 1.07 in $CHCl_3$); **IR** 3063.5, 2947.4, 2728.2, 1666.5, 1176.1 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 373 K, 400 MHz) δ 8.79 (s, 1 H), 8.57 (br. s, 1 H), 7.62 (s, 1 H), 7.47 (s, 1 H), 4.78 (d, 1 H, *J* = 9.6 Hz), 3.77 (t, 1 H, *J* = 4.4 Hz), 3.28-3.16 (m, 2 H), 3.07-2.95 (m, 3 H), 2.77 (s, 6 H), 2.33-2.22 (m, 1 H), 1.92-1.79 (m, 2 H), 1.26-1.16 (m, 1 H), 1.07-0.89 (m, 5 H), 0.83 (t, 3 H, *J* = 7.6 Hz); **^{13}C NMR** (CD_3OD, 125 MHz) δ 168.2, 135.1, 121.9, 119.4, 67.1, 63.6, 55.3, 42.1, 37.6, 36.2, 24.3, 24.2, 14.1, 9.2; **LC-MS** (ES) *m/z* calcd for $C_{14}H_{27}ON_4$ $[M+H]^+$ 267.2179, found 267.2177.



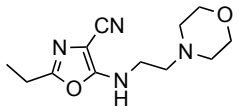
2-32a

5-Amino-2-ethyloxazole-4-carbonitrile (2-32a). Aminomalononitrile *p*-toluenesulfonate (0.500 g, 1.97 mmol) was added to a microwave vial containing a solution of propionyl chloride (0.21 mL, 2.4 mmol) in N-methyl-2-pyrrolidone (19 mL). The vessel was sealed and irradiated to 120 ^oC for 15 minutes. This reaction was repeated an additional time. The combined reaction mixtures were diluted with ethyl acetate (80 mL) and sequentially washed with water (60 mL, twice) and a saturated aqueous solution of sodium bicarbonate (60 mL, twice). Each aqueous solution was back extracted with ethyl acetate (60 mL). The organic solutions were combined, dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (15% ethyl acetate in hexane) to provide **2-32a** (0.209 g, 1.52 mmol, 39%) as an off-white solid: **Rf** (30% ethyl acetate in hexane) 0.30; **Mp** (methylene chloride) 149-151 ^oC; **IR** 3306.5, 3146.2, 2212.4, 1653.2, 1591.7, 1424.0, 1155.6 cm^{-1}; **^{1}H NMR** (CD_3OD, 400 MHz) δ 2.62 (q, 2 H, *J* = 7.6 Hz), 1.24 (t, 3 H, *J* = 7.6 Hz); **^{13}C NMR** (CD_3OD, 125 MHz) δ 162.5 155.2, 114.4, 82.7, 20.5, 9.6; **LC-MS** (ES) *m/z* calcd $C_6H_8ON_3$ for $[M+H]^+$ 138.0662, found 138.0662.



2-33a

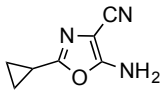
5-Bromo-2-ethyloxazole-4-carbonitrile (2-33a). *Tert*-butylnitrite (0.35 mL, 2.9 mmol) was added to a solution of copper (II) bromide (0.651 g, 2.92 mmol) in anhydrous acetonitrile (8.5 mL). **2-32a** (0.200 g, 1.46 mmol) was added to the mixture portion wise and stirred at room temperature for 3 h. The reaction mixture was diluted with a 1:1 solution of ethyl acetate and water (30 mL) and washed with a 5% aqueous solution of hydrogen chloride (10 mL). The aqueous layer was isolated and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, purified by chromatography on SiO_2 (10% ethyl acetate in hexane) to provide **2-33a** (0.171g, 0.848 mmol, 58%) as a white solid: **Rf** (30% ethyl acetate in hexane) 0.66; **Mp** (methylene chloride) 39-41 ^{o}C; **IR** 2987.8, 2238.5, 1582.4, 1138.8, 1051.2, 913.3 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 2.83 (q, 2 H, *J* = 7.8 Hz), 1.35 (t, 3 H, *J* = 7.5 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 168.5, 130.5, 115.7, 111.1, 21.9, 10.5; **LC-MS** (ES) *m/z* calcd $C_6H_6ON_2Br$ for $[M+H]^+$ 200.9658, found 200.9658.



2-34a

2-Ethyl-5-((2-morpholinoethyl)amino)oxazole-4-carbonitrile (2-34a). 2-33a (0.100 g, 0.497 mmol) was added to a microwave vial containing a solution of 4-(2-aminoethyl)morpholine (0.65 mL, 4.9 mmol) in anhydrous THF (1.25 mL). The microwave vial

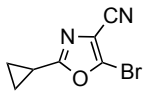
was sealed and heated to 70 °C for 1.5 h. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (5 mL). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, purified by chromatography on silica (5% methanol in ethyl acetate) to provide **2-34a** (0.0997 g, 0.398 mmol, 80%) as a white solid: **Rf** (ethyl acetate) 0.21; **Mp** (methylene chloride) 52-53 °C; **IR** 3185.3, 2974.7, 2816.3, 2197.5, 1638.3, 1597.3, 1455.7, 1349.4, 1110.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.39 (s, br, 1 H), 3.72 (t, 4 H, *J* = 4.2 Hz), 3.47 (app. q, 2 H, *J* = 5.1 Hz), 2.66-2.59 (m, 4 H), 2.49 (app. d, 4 H, *J* = 4.2 Hz), 1.27 (t, 3 H, *J* = 7.8 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.6, 155.3, 115.4, 84.6, 66.8, 56.5, 53.2, 39.4, 21.3, 10.7; **LC-MS** (ES) *m/z* calcd $C_{12}H_{19}O_2N_4$ for $[M+H]^+$ 251.1503, found 251.1501.



2-32b

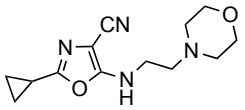
5-Amino-2-cyclopropyloxazole-4-carbonitrile (2-32b).[297] Aminomalononitrile *p*-toluenesulfonate (0.500 g, 1.97 mmol) was added to a microwave vial containing a solution of cyclopropanecarbonyl chloride (0.22 mL, 2.4 mmol) in N-methyl-2-pyrrolidone (19 mL). The vessel was sealed and irradiated to 120 °C for 15 minutes. This reaction was repeated an additional time. The combined reaction mixtures were diluted with ethyl acetate (80 mL) and sequentially washed with water (60 mL, twice) and a saturated aqueous solution of sodium bicarbonate (60 mL, twice). Each aqueous solution was back extracted with ethyl acetate (60 mL). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (30% ethyl acetate in hexane) to provide **2-32b** (0.1043 g, 0.699 mmol, 18%) as a yellow solid: **Rf** (30% ethyl acetate in hexane) 0.48; **^{1}H**

NMR ($CDCl_3$, 300 MHz) δ 4.85 (s, br, 2 H), 1.89 (p, 1 H, *J* = 6.9 Hz) 0.99 (app. d, 4 H, *J* = 6.6 Hz). **LC-MS** (ES) *m/z* calcd for $C_7H_8ON_3$ $[M+H]^+$ 150.0662, found 150.0662.



2-33b

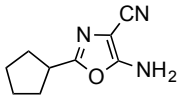
5-Bromo-2-cyclopropyloxazole-4-carbonitrile (2-33b). *Tert*-butylnitrite (0.17 mL, 1.4 mmol) was added to a solution of copper (II) bromide (0.312 g, 1.39 mmol) in anhydrous acetonitrile (4 mL). **2-32b** (0.104 g, 0.699 mmol) was added to the mixture portion wise and stirred at room temperature for 3 h. The reaction mixture was dilute with a 1:1 solution of ethyl acetate and water (10 mL) and washed with a 5% aqueous solution of hydrogen chloride (5 mL). The aqueous layer was isolated and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, purified by chromatography on SiO_2 (0-5% ethyl acetate in hexane) to provide **2-33b** (0.0894 g, 0.419 mmol, 60%) as a white solid: **Rf** (30% ethyl acetate in hexane) 0.81; **Mp** (methylene chloride) 57-58 ^oC; **IR** 2231.0, 1575.0, 1547.0, 1148.1, 1058.7, 1049.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 2.12-2.03 (m, 1 H), 1.15-1.13 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 168.9, 129.1, 115.7, 111.16, 9.21, 9.03; **LC-MS** (ES) *m/z* calcd for $C_7H_6ON_2Br$ $[M+H]^+$ 212.9658, found 212.9659.



2-34b

2-Cyclopropyl-5-((2-morpholinoethyl)amino)oxazole-4-carbonitrile (2-34b). **2-33b** (0.0250 g, 0.117 mmol) was added to a microwave vial containing a solution of 4-(2-

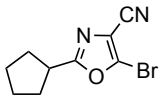
aminoethyl)morpholine (0.15 mL, 1.2 mmol) in anhydrous THF (0.3 mL). The microwave vial was sealed and heated to 70 ^{o}C for 1.5 h. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (5 mL). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (70% ethyl acetate in hexane) to provide **2-34b** (0.0182 g, 0.069 mmol, 59%) as a tan solid: **Rf** (ethyl acetate) 0.32; **Mp** (methylene chloride) 70-72 ^{o}C, **IR** 3293.4, 2952.4, 2855.4, 2204.9, 1642.1, 1597.3, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.42 (s, br., 1 H), 3.70 (t, 4 H, *J* = 4.5 Hz), 3.43 (app. q, 2 H, *J* = 5.1 Hz), 2.59 (t, 2 H, *J* = 6 Hz), 2.47 (t, 4 H, *J* = 4.5 Hz), 1.85 (p, 1 H, *J* = 6.9 Hz), 0.96 (app. d, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.2, 155.5, 115.4, 84.6, 66.8. 56.5, 53.2, 39.5, 8.32, 7.44; **LC-MS** (ES) *m/z* calcd $C_{13}H_{19}O_2N_4$ for $[M+H]^+$ 263.1503, found 263.1501.



2-32c

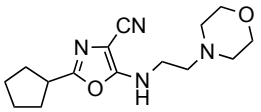
5-Amino-2-cyclopentyloxazole-4-carbonitrile (2-32c). Aminomalononitrile *p*-toluenesulfonate (0.500 g, 1.97 mmol) was added to a microwave vial containing a solution of cyclopentanecarbonyl chloride (0.26 mL, 2.2 mmol) in N-methyl-2-pyrrolidone (19 mL). The vessel was sealed and irradiated to 120 ^{o}C for 15 minutes. This reaction was repeated an additional time. The reaction mixtures were combined and diluted with ethyl acetate (80 mL) and sequentially washed with water (60 mL, twice) and sodium bicarbonate (60 mL, twice). Each aqueous solution was back extracted with ethyl acetate (60 mL). The organic solutions were combined, dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (40% ethyl acetate in hexane) to provide **2-32c** (0.484 g, 2.73 mmol,

69%) as a white solid: **Rf** (30% ethyl acetate in hexane) 0.2; **Mp** (methylene chloride) 171-173 ^{o}C; **IR** 3315.8, 3146.2, 2963.5, 2866.6, 2212.4, 1647.7, 1425.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 4.82 (s, br, 2 H), 3.10-2.99 (m, 1 H), 2.06-1.95 (m, 2 H), 1.87-1.75 (m, 6 H); **^{13}C NMR** (CD_3OD, 125 MHz) δ 162.4, 157.3, 114.5, 82.7, 37.8, 30.4, 24.8; **LC-MS** (ES) *m/z* calcd for $C_9H_{12}ON_3$ $[M+H]^+$ 178.0975, found 178.0974.



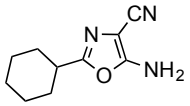
2-33c

5-Bromo-2-cyclopentyloxazole-4-carbonitrile (2-33c). *Tert*-butylnitrite (0.47 mL, 3.9 mmol) was added to a solution of copper (II) bromide (0.882 g, 3.95 mmol) in anhydrous acetonitrile (11.5 mL). **2-32c** (0.350 g, 1.98 mmol) was added to the mixture portion wise and stirred at room temperature for 3 h. The reaction mixture was dilute with a 1:1 solution of ethyl acetate and water (30 mL) and washed with a 5% aqueous solution of hydrogen chloride (10 mL). The aqueous layer was isolated and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (5% ethyl acetate in hexane) to provide **2-33c** (0.157 g, 0.651 mmol, 33%) as a yellow oil: **Rf** (30% ethyl acetate in hexane) 0.69; **IR** 2957.9, 2870.3, 2238.5, 1573.1, 1122.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 3.26-3.18 (m, 1 H), 2.12-2.04 (m, 2H), 1.93-1.66 (m, 6 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.1, 130.2, 115.5, 111.2, 38.6, 31.1, 25.4; **LC-MS** (ES) *m/z* calcd for $C_9H_{10}ON_2Br$ $[M+H]^+$ 240.9971, found 240.9969.



2-34c

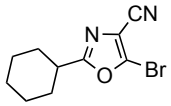
2-Cyclopentyl-5-((2-morpholinoethyl)amino)oxazole-4-carbonitrile (2-34c). **2-32c** (0.150 g, 0.622 mmol) was added to a microwave vial containing a solution of 4-(2-aminoethyl)morpholine (0.82 mL, 6.2 mmol) in anhydrous THF (1.55 mL). The microwave vial was sealed and heated to 70 ^{o}C for 1.5 h. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (5 mL). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate) to provide **2-34c** (0.103 g, 0.354 mmol, 57%) as a white solid: **Rf** (ethyl acetate) 0.29; **Mp** (methylene chloride) 111-113 ^{o}C; **IR** 3174.2, 2957.9, 2862.9, 2197.5, 1636.5, 1591.7, 1349.4, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.37 (s, br, 1 H), 3.73 (t, *J* = 4.5 Hz), 3.47 (app. q, 2 H, *J* = 5.1 Hz), 3.03 (quin, 1 H, *J* = 7.8 Hz), 2.62 (t, 2 H, *J* = 6 Hz), 2.49 (t, 4 H, *J* = 4.8 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.6, 157.6, 115.6, 84.4, 66.8, 56.5, 53.2, 39.4, 38.0, 30.9, 25.4; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{23}O_2N_4$ $[M+H]^+$ 291.1816, found 291.1814.



2-32d

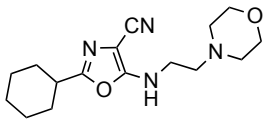
5-Amino-2-cyclohexyloxazole-4-carbonitrile (2-32d).[297] Aminomalononitrile *p*-toluenesulfonate (0.500 g, 1.97 mmol) was added to a microwave vial containing a solution of cyclohexanecarbonyl chloride (0.32 mL, 2.4 mmol) in N-methyl-2-pyrrolidone (19 mL). The vessel was sealed and irradiated to 120 ^{o}C for 15 minutes. This reaction was repeated an

additional time. The combined reaction mixtures were diluted with ethyl acetate (80 mL) and sequentially washed with water (60 mL, twice) and a saturated aqueous solution of sodium bicarbonate (60 mL, twice). The organic solutions were combined, dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (30% ethyl acetate in hexane). The resulting material was washed with water and dried under reduced pressure to provide **2-32d** (0.477 g, 2.49 mmol, 63%) as a white solid: **Rf** (30% ethyl acetate in hexane) 0.65; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 7.56 (s, 2 H), 2.64-2.58 (m, 1 H), 1.89-1.87 (m, 2 H), 1.71-1.67 (m, 2 H), 1.62-1.59 (m, 1 H), 1.43-1.18 (m, 5H).



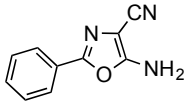
2-33d

5-Bromo-2-cyclohexyloxazole-4-carbonitrile (2-33d).[297] *Tert*-butylnitrite (0.19 mL, 1.6 mmol) was added to a solution of copper (II) bromide (0.347 g, 1.55 mmol) in anhydrous acetonitrile (4.5 mL). **2-32d** (0.149 g, 0.777 mmol) was added to the mixture portion wise and stirred at room temperature for 3 h. The reaction mixture was dilute with a 1:1 solution of ethyl acetate and water (10 mL) and washed with a 5% aqueous solution of hydrogen chloride (5 mL). The aqueous layer was isolated and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (5% ethyl acetate in hexane) to provide **2-33d** (0.1279 g, 0.466 mmol, 60%) as a colorless oil. **Rf** (30% ethyl acetate in hexane) 0.87; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 2.85-2.76 (m, 1 H), 2.06-2.02 (m, 2 H), 1.87-1.80 (m, 2 H), 1.73-1.32 (m, 6 H).



2-34d

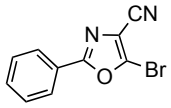
2-Cyclohexyl-5-((2-morpholinoethyl)amino)oxazole-4-carbonitrile (2-34d). **2-33d** (0.200 g, 0.784 mmol) was added to a microwave vial containing a solution of 4-(2-aminoethyl)morpholine (1.03 mL, 7.84 mmol) in anhydrous THF (2 mL). The microwave vial was sealed and heated to 70 ^{o}C for 10 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (10 mL). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (70% ethyl acetate in hexane) to provide **2-34d** (0.145 g, 0.475 mmol, 61%) as a white solid: **Rf** (ethyl acetate) 0.31; **Mp** (methylene chloride) 108-110 ^{o}C; **IR** 3181.6, 2928.1, 2848.0, 2199.4, 1634.6, 1589.9, 1444.5, 1349.4, 1114.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.44 (s, br, 1 H), 3.73 (t, 4 H, *J* = 4.5 Hz), 3.47 (app. q, 2 H, *J* = 5.1 Hz), 2.63 (t, 2 H, 6.3 Hz), 2.50 (t, 4 H, *J* = 4.5 Hz), 2.08 (s, br., 1 H), 2.00-1.28 (m, 10 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.4, 157.7, 115.6, 84.4, 66.7, 56.6, 53.2, 39.4, 37.0, 30.2, 25.6, 25.4; **LC-MS** (ES) *m/z* calcd for $C_{16}H_{25}O_2N_4$ $[M+H]^+$ 305.1972, found 305.1969.



2-32e

5-Amino-2-phenyloxazole-4-carbonitrile (2-32e). Aminomalononitrile *p*-toluenesulfonate (0.500 g, 1.97 mmol) was added to a microwave vial containing a solution of benzoyl chloride (0.25 mL, 2.2 mmol) in N-methyl-2-pyrrolidone (19 mL). The vessel was

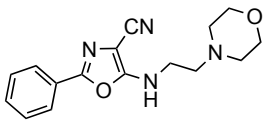
sealed and irradiated to 120 ^{o}C for 15 minutes. This reaction was repeated an additional time. The reaction mixtures were combined and diluted with ethyl acetate (80 mL) and sequentially washed with water (60 mL, twice) and a saturated aqueous solution of sodium bicarbonate (60 mL, twice). Each aqueous solution was back extracted with ethyl acetate (60 mL). The organic solutions were combined, dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (40% ethyl acetate in hexane) to provide **2-32e** (0.529 g, 2.86 mmol, 72%) as a white solid: **Rf** (30% ethyl acetate in hexane) 0.24; **Mp** (methylene chloride, decomp.) > 248 ^{o}C; **IR** 3306.5, 3161.1, 2204.9, 1647.7, 1599.2, 1051.2 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 300 MHz) δ 7.98 (s, br, 1 H), 7.78-7.75 (m, 2 H), 7.50-7.48 (m, 3 H); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 162.8, 149.9, 130.5, 129.6, 126.4, 125.4, 115.9, 84.7; **LC-MS** (ES) *m/z* calcd for $C_{10}H_8ON_3$ $[M+H]^+$ 186.0662, found 186.0659.



2-33e

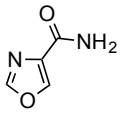
5-Bromo-2-phenyloxazole-4-carbonitrile (2-33e). *Tert*-butylnitrite (0.39 mL, 3.2 mmol) was added to a solution of copper (II) bromide (0.724 g, 3.24 mmol) in anhydrous acetonitrile (9.5 mL). **1-41e** (0.300 g, 1.62 mmol) was added to the mixture portion wise and stirred at room temperature for 4 h. The reaction mixture was dilute with a 1:1 solution of ethyl acetate and water (30 mL) and washed with a 5% aqueous solution of hydrogen chloride (10 mL). The aqueous layer was isolated and extracted with ethyl acetate (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, purified by chromatography on SiO_2 (5% ethyl acetate in hexane) to provide **2-33e** (0.206 g, 0.827 mmol,

51%) as a white solid: **Rf** (30% ethyl acetate in hexane) 0.69; **Mp** (methylene chloride) 139-140 ^{o}C; **IR** 2969.1, 2242.2, 2195.6, 1640.2, 1446.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.03-7.99 (m, 2 H), 7.59-7.47 (m, 3 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 163.9, 132.2, 130.8, 129.2, 126.7, 124.9, 117.1, 111.1; **LC-MS** (ES) *m/z* calcd for $C_{10}H_6ON_2Br$ $[M+H]^+$ 248.9658, found 248.9658.



2-34e

5-((2-Morpholinoethyl)amino)-2-phenyloxazole-4-carbonitrile (2-34e). **2-33e** (0.150 g, 0.602 mmol) was added to a microwave vial containing a solution of 4-(2-aminoethyl)morpholine (0.79 mL, 6.0 mmol) in anhydrous THF (1.50 mL). The microwave vial was sealed and heated to 70 ^{o}C for 2 h. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (5 mL). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (90% ethyl acetate in hexane) to provide **2-34e** (0.129 g, 0.433 mmol, 72%) as a white solid: **Rf** (ethyl acetate) 0.28; **Mp** (methylene chloride) 121-122 ^{o}C; **IR** 2974.7, 2816.3, 2201.2, 1629.0, 1450.1, 1347.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.88-7.85 (m, 2 H), 7.43 (t, 3 H, *J* = 3.6 Hz), 5.63 (s, br., 1 H), 3.75 (t, 4 H, *J* = 4.5 Hz), 3.58 (app. q, 2 H, *J* = 5.1 Hz), 2.67 (t, 2 H, *J* = 5.7 Hz), 2.53 (t, 4 H, *J* = 4.2 Hz); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.6, 151.3, 130.2, 128.8, 126.2, 125.6, 115.3, 86.5, 66.9, 56.5, 53.2, 39.5; **LC-MS** (ES) *m/z* calcd for $C_{16}H_{19}O_2N_4$ $[M+H]^+$ 299.1503, found 299.1499.



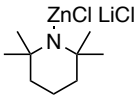
2-36

Oxazole-4-carboxamide (2-36). Ethyl oxazole-4-carboxylate **2-35** (1.05 g, 7.44 mmol) was added to a microwave vial containing a 7 N solution of ammonia in methanol (23.8 mL, 167 mmol). The microwave vial was sealed and heated to 60°C for 20 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to provide **2-36** (0.829 g, 7.46 mmol, 98%) as a brown solid: **Rf** (ethyl acetate) 0.37; **Mp** (methylene chloride, decomp.) > 162 ^{o}C; **IR** 3364.3, 3107.1, 3149.9, 1649.5, 1588.0, 1409.1 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 300 MHz) δ 8.58 (d, 1 H, *J* = 0.6 Hz), 8.48 (s, 1 H), 7.64 (s, 1 H), 7.49 (s, 1 H); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 162.2, 152.6, 142.4, 136.3; **LC-MS** (ES) *m/z* calcd for $C_4H_5O_2N_2$ $[M+H]^+$ 113.0346, found 113.0347.

2-37

Oxazole-4-carbonitrile (2-37). Trifluoroacetic anhydride (2.92 mL, 21.0 mmol) was added drop wise to a solution of **2-36** (2.14 g, 19.1 mmol) and triethylamine (5.37 mL, 38.2 mmol) in anhydrous THF (38 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction was warmed to room temperature and stirred for 20 h. The reaction mixture was diluted with water (30 mL) and extracted with ether (50 mL, three times). The combined organic solutions were sequentially washed with a 1N aqueous solution of hydrogen chloride (30 mL), a saturated aqueous solution of sodium bicarbonate (30 mL), and brine (30 mL). The resulting solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography

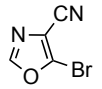
on SiO_2 (20% ethyl acetate in hexane) to provide **2-37** (1.51 g, 16.1 mmol, 84%) as a yellow liquid. **Rf** (ethyl acetate) 0.81; **IR** 3144.3, 3095.9, 2247.8 1511.6, 1110.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.22 (d, 1 H, *J* = 0.9 Hz), 7.99 (s, 1 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 151.9, 146.5, 115.0, 111.3.



TMPZnCl•LiCl

Preparation of a 0.5 M solution of $ZnCl_2$ in THF: Zinc (II) chloride (4.49 g, 33 mmol) was dried under vacuum at 160 ^oC for 3 hours. The flask was cooled to room temperature and charged with anhydrous THF (66 mL). The salts were vigorously stirred until it was completely dissolved.

Preparation of a 1.05 M solution of TMPZnCl•LiCl in THF. Dry 250 mL round bottom flask was charged with freshly distilled 2,2,6,6-tetramethylpiperidine (5.10 mL, 29.9 mmol) dissolved in anhydrous THF (30 mL) under at atmosphere of nitrogen. The solution was cooled to -40 C and a 1.82 M solution of *n*-butyl lithium in hexane (16.5 mL, 29.9 mmol) was added dropwise. The reaction mixture was slowly warmed to -10 ^oC and stirred for 1 h. A 0.5 M solution of zinc (II) chloride in anhydrous THF (66 mL, 33 mmol) was added dropwise and the reaction mixture was stirred for 0.5 h at -10 ^oC and then at room temperature for an additional 0.5 h. The reaction mixture was concentrated under reduced pressure and then resuspended in fresh anhydrous THF (30 mL). The mixture was vigorously stirred until salts were completely dissolved. The resulting solution was cannulated into a dry 100 mL Schlenk flask. The solution was titrated using benzoic acid (0.122 g, 1 mmol) and 4-(phenylazo)diphenylamine (2 mg) as a indicator in anhydrous THF (10 mL) to indicate a 1.05 M solution of TMPZnCl•LiCl in THF.



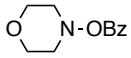
2-38

5-Bromooxazole-4-carbonitrile (2-38). A fresh 0.65 M solution of TMPZnCl•LiCl[286] in THF (8.99 mL, 5.85 mmol) was added drop wise to a solution of **2-37** (0.500 g, 5.31 mmol) in anhydrous THF (3 mL) at -78 ^{o}C under an atmosphere of nitrogen and stirred for 2 h. Bromine (0.301 mL, 5.85 mmol) was introduced drop wise and the reaction was warmed to 0 ^{o}C. After 1 h, the reaction was sequentially diluted with a saturated aqueous solution of ammonium chloride (10 mL) and a saturated aqueous solution of sodium thiosulfate (10 mL). The resulting mixture was extracted with ether (20 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane) to provide **2-38** (0.552 g, 3.19 mmol, 60%) as a yellow oil: **Rf** (20% ethyl acetate in hexane) 0.53; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.21 (s, 1 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 149.3, 136.0, 117.6, 110.2.

2-40a

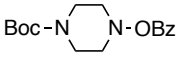
Pyrrolidin-1-yl benzoate (**2-40a**). Pyrrolidine (1.52 mL, 18.6 mmol) was added portionwise to a mixture of benzoyl peroxide (4.50 g, 18.6 mmol), disodium phosphate (2.96 g, 27.9 mmol) in anhydrous DMF (37 mL) at room temperature under an atmosphere of nitrogen. The reaction was stirred for 3 h, diluted with ethyl acetate (100 mL), and washed with water (40 mL, three times) and a saturated aqueous solution of sodium bicarbonate (40 mL, twice). The

resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography onsilica (20% ethyl acetate in hexane) to provide **1** (1.81 g, 9.47 mmol, 51%) as a tan solid: **Rf** (20% ethyl acetate in hexane) 0.17; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.98-7.96 (m, 2H), 7.55 (tt, *J* = 7.4, 1.5 Hz, 1H), 7.44-7.41 (m, 2H), 3.33-3.30 (m, 4H), 1.97 (s, 4H).



2-40b

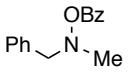
Morpholino benzoate (**2-40b**). Morpholine (1.60 mL, 18.6 mmol) was added portionwise to a mixture of benzoyl peroxide (4.50 g, 18.6 mmol), disodium phosphate (2.96 g, 27.9 mmol) in anhydrous DMF (37 mL) at room temperature under an atmosphere of nitrogen. The reaction was stirred for 3 h and then diluted with ethyl acetate (100 mL) and washed with water (40 mL, three times) and a saturated aqueous solution of sodium bicarbonate (40 mL, twice). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane) to provide **2-40b** (2.29 g, 11.1 mmol, 59%) as a white solid: **Rf** (20% ethyl acetate in hexane) 0.16; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.02-8.00 (m, 2H), 7.59-7.56 (m, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 3.99-3.85 (m, 4H), 3.45 (d, *J* = 9.6 Hz, 2H), 3.06-3.03 (m, 2H).



2-40c

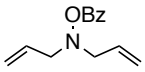
***Tert*-butyl 4-(benzoyloxy)piperazine-1-carboxylate** (**2-40c**). 1-Boc-piperazine (2.50 g, 13.4 mmol) was added portionwise to a mixture of benzoyl peroxide (3.25 g, 13.4 mmol),

disodium phosphate (2.86 g, 20.1 mmol) in anhydrous DMF (27 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 2.5 h, diluted with ethyl acetate (100 mL), and washed with water (30 mL, three times) and a saturated aqueous solution of sodium bicarbonate (30 mL, twice). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane) to provide **2-40c** (2.72 g, 8.88 mmol, 66%) as a white solid: **Rf** (20% ethyl acetate in hexane) 0.17; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.01-7.99 (m, 2H), 7.59-7.56 (m, 1H), 7.45 (t, *J* = 7.8 Hz, 2H), 4.03 (s, 2H), 3.44 (s, 2H), 3.32 (s, 2H), 2.92 (s, 2H), 1.48 (d, *J* = 0.6 Hz, 9H).



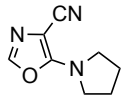
2-40d

***O*-benzoyl-N-benzyl-N-methylhydroxylamine** (**2-40d**). N-Benzylmethylamine (1.73 mL, 13.4 mmol) was added portionwise to a mixture of benzoyl peroxide (3.25 g, 13.4 mmol), disodium phosphate (2.86 g, 20.1 mmol) in anhydrous DMF (27 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 2 h, diluted with ethyl acetate (100 mL), and washed with water (30 mL, three times) and a saturated aqueous solution of sodium bicarbonate (30 mL, twice). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (25% ethyl acetate in hexane) to provide **2-40d** (2.88 g, 11.9 mmol, 89%) as a colorless oil: **Rf** (20% ethyl acetate in hexane) 0.34; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.90 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.54-7.51 (m, 1H), 7.43-7.38 (m, 4H), 7.33-7.30 (m, 2H), 7.28-7.25 (m, 1H), 4.17 (s, 2H), 2.93 (s, 3H).



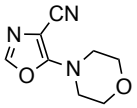
2-40e

N,N-diallyl-O-benzoylhydroxylamine (**2-40e**). Diallylamine (2.29 mL, 18.6 mmol) was added portionwise to a mixture of benzoyl peroxide (4.50 g, 18.6 mmol), disodium phosphate (2.96 g, 27.9 mmol) in anhydrous DMF (37 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 6 h, diluted with ethyl acetate (100 mL), and washed with water (40 mL, three times) and a saturated aqueous solution of sodium bicarbonate (40 mL, twice). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (20% ethyl acetate in hexane). Desired fractions were combined and washed with a saturated aqueous solution of sodium bicarbonate (50 mL, four times). The resulting organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The resulting material was dissolved into methanol (25 mL) and diluted with a saturated aqueous solution of sodium thiosulfate and stirred overnight. The mixture was extracted with methylene chloride (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **2-40e** (1.96 g, 9.01 mmol, 49%) as light-yellow oil: **Rf** (20% ethyl acetate in hexane) 0.36; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.98 (dd, J = 8.4, 1.4 Hz, 2H), 7.58-7.53 (m, 1H), 7.46-7.40 (m, 2H), 6.02 (ddt, J = 17.0, 10.3, 6.7 Hz, 2H), 5.30-5.16 (m, 4H), 3.67 (dt, J = 6.6, 1.1 Hz, 4H).



2-41a

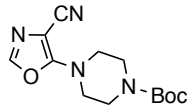
5-(Pyrrolidin-1-yl)oxazole-4-carbonitrile (**2-41a**). A 0.95 M solution of TMPZnCl•LiCl in THF (1.23 mL, 1.17 mmol) was added dropwise to a solution of **2-37** (0.100 g, 1.063 mmol) in anhydrous THF (5.32 mL) at -78 ^{o}C under an atmosphere of nitrogen and stirred for 2 h. A solution of **2-40a** (0.224 g, 1.28 mmol) and copper (II) acetate (0.0193 g, 0.106 mmol) in anhydrous THF (1 mL) was added and the reaction mixture was slowly warmed to room temperature and stirred for 22 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (10 mL), and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane) to provide **2-41a** (0.116 g, 0.713 mmol, 67%) as a tan solid: **Rf** (20% ethyl acetate in hexane) 0.21; **Mp** (CH_2Cl_2) 109-111 ^{o}C; **IR** 3160.2, 2969.0, 2883.1, 2235.2, 1643.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.67 (s, 1H), 3.53-3.50 (m, 4H), 2.02-1.99 (m, 4H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.3, 139.9, 114.7, 112.9, 47.5, 25.6; **LC-MS** (ES) *m/z* calcd for $C_8H_{10}ON_3$ $[M+H]^+$ 164.0818, found 164.0818.



2-41b

5-Morpholinooxazole-4-carbonitrile (**2-41b**). A 1.05 M solution of TMPZnCl•LiCl in THF (1.11 mL, 1.17 mmol) was added dropwise to a solution of **2-37** (0.100 g, 1.06 mmol) in anhydrous THF (5.3 mL) at -78 ^{o}C under an atmosphere of nitrogen and stirred for 2 h. A

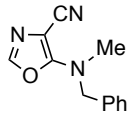
solution of **2-40b** (0.264 g, 1.28 mmol) and copper (II) acetate (0.0193 g, 0.106 mmol) in anhydrous THF (1 mL) was added and the reaction mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (15 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane). The selected fractions were combined, washed with a saturated aqueous solution of sodium bicarbonate (15 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **2-41b** (0.121 g, 0.675 mmol, 64%) as a white solid: **Rf** (20% ethyl acetate in hexane) 0.15; **Mp** (CH_2Cl_2) 107-109 ^oC; **IR** (neat) 3168.5, 2971.7, 2926.7, 2873.5, 2244.7, 1634.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.71 (s, 1H), 3.78 (t, *J* = 4.9 Hz, 4H), 3.52 (t, *J* = 4.9 Hz, 4H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 161.4, 140.3, 114.7, 112.6, 65.9, 45.6; **LC-MS** (ES) *m/z* calcd for $C_8H_{10}O_2N_3$ $[M+H]^+$ 180.0768, found 180.0767.



2-41c

***Tert*-butyl 4-(4-cyanooxazol-5-yl)piperazine-1-carboxylate** (**2-41c**). A 0.95 M solution of TMPZnCl•LiCl in THF (1.23 mL, 1.17 mmol) was added dropwise to a solution of **2-37** (0.100 g, 1.06 mmol) in anhydrous THF (5.32 mL) at -78 C under an atmosphere of nitrogen and stirred for 2 h. A solution of **2-40c** (0.391 g, 1.28 mmol) and copper (II) acetate (0.0193 g, 0.106 mmol) in anhydrous THF (1 mL) was added and the reaction mixture was slowly warmed to room temperature and stirred for 19 h. The reaction mixture was quenched with a saturated

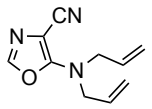
aqueous solution of ammonium chloride (10 mL), and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane) to provide the **2-41c** (0.187 g, 0.674 mmol, 63%) as a white solid: **Rf** (40% ethyl acetate in hexane) 0.41; **Mp** (CH_2Cl_2) 115-117 ^oC; **IR** 3161.8, 2980.2, 2887.2, 2236.7, 1688.7, 1636.2 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.70 (s, 1H), 3.51 (d, *J* = 2.1 Hz, 8H), 1.48 (s, 9H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 161.3, 154.4, 140.3, 114.7, 112.5, 80.5, 45.4, 28.4; **LC-MS** (ES) *m/z* calcd for $C_{13}H_{19}O_3N_4$ $[M+H]^+$ 279.1452, found 279.1449.



2-41d

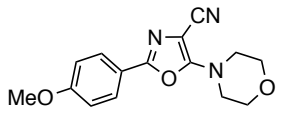
5-(Benzyl(methyl)amino)oxazole-4-carbonitrile (**2-41d**). A 0.95 M solution of TMPZnCl•LiCl in THF (1.23 mL, 1.17 mmol) was added dropwise to a solution of **2-37** (0.100 g, 1.063 mmol) in anhydrous THF (5.32 mL) at -78 ^oC under an atmosphere of nitrogen and stirred for 2 h. A solution of **2-40d** (0.308 g, 1.28 mmol) and copper (II) acetate (0.0193 g, 0.106 mmol) in anhydrous THF (1 mL) was added and the reaction mixture was slowly warmed to room temperature and stirred for 21 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (10 mL), and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (10% ethyl acetate in hexane) to provide **2-41d** (0.1371 g, 0.643 mmol, 60%) as a colorless oil: **Rf** (20% ethyl acetate in hexane) 0.38; **IR** 3065.1, 3031.7, 2926.4, 2241.1, 1635.4 cm^{-1}; **^{1}H NMR** (CD_2Cl_2, 500 MHz) δ 7.72 (s,

1H), 7.36-7.33 (m, 2H), 7.31-7.25 (m, 3H), 4.59 (s, 2H), 3.00 (s, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 164.1, 142.1, 138.3, 130.6, 129.69, 129.60, 116.5, 114.9, 37.0; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{12}ON_3$ $[M+H]^+$ 214.0975, found 214.0974.



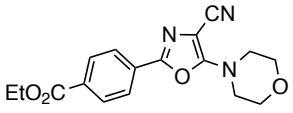
2-41e

5-(Diallylamino)oxazole-4-carbonitrile (**2-41e**). A 0.95 M solution of TMPZnCl•LiCl in THF (1.23 mL, 1.17 mmol) was added dropwise to a solution of **2-37** (0.100 g, 1.06 mmol) in anhydrous THF (5.3 mL) at -78 ^{o}C under an atmosphere of nitrogen and stirred for 2 h. A solution of **2-40e** (0.277 g, 1.28 mmol) and copper (II) acetate (0.0193 g, 0.106 mmol) in anhydrous THF (1 mL) was added and the reaction mixture was warmed to room temperature and stirred for 14 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (15 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (10% ethyl acetate in hexane) to provide **2-41e** (0.117 g, 0.616 mmol, 58%) as a light yellow oil: **Rf** (20% ethyl acetate in hexane) 0.41; **IR** (neat) 3164.0, 3085.6, 3014.3, 2987.0, 2926.6, 2238.3, 1624.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.66 (s, 1H), 5.79 (ddt, *J* = 16.8, 10.6, 6.0 Hz, 2H), 5.22-5.17 (m, 4H), 3.99 (d, *J* = 5.9 Hz, 4H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 161.4, 140.0, 132.1, 118.3, 114.8, 112.8, 50.1; **LC-MS** (ES) *m/z* calcd for $C_{10}H_{12}ON_3$ $[M+H]^+$ 190.0975, found 190.0973.



2-42a

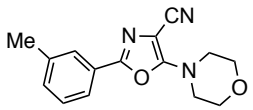
2-(4-Methoxyphenyl)-5-morpholinooxazole-4-carbonitrile (**2-42a**). **2-41b** (0.0700 g, 0.391 mmol), $Pd(OAc)_2$ (0.00438 g, 0.0195 mmol), JohnPhos (0.0117 g, 0.0391 mmol), and cesium carbonate (0.254 g, 0.781 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with argon; this was repeated three times. Degassed, anhydrous 1,4-dioxane (1.95 mL) and 4-iodoanisole (0.183 g, 0.781 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^{o}C for 15.5 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and purified by chromatography on silica (30% ethyl acetate in hexane) to provide **2-42a** (0.0907 g, 0.318 mmol, 81%) as an off-white solid: **Rf** (50% ethyl acetate in hexane) 0.58; **Mp** (CH_2Cl_2) 134-136 ^{o}C; **IR** 2229.8, 1636.2, 1609.8, 1508.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.74-7.71 (m, 2H), 6.97-6.94 (m, 2H), 3.85 (s, 3H), 3.80 (t, J = 4.9 Hz, 4H), 3.56 (t, J = 4.9 Hz, 4H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.7, 159.2, 152.8, 128.3, 126.2, 118.5, 114.6, 106.2, 66.0, 55.4, 45.7; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{16}O_3N_3$ $[M+H]^+$ 286.1186, found 286.1185.



2-42b

Ethyl 4-(4-cyano-5-morpholinooxazol-2-yl)benzoate (**2-42b**). **2-41b** (0.0600 g, 0.335 mmol), Pd(OAc)2 (0.00376 g, 0.0167 mmol), JohnPhos (0.00999 g, 0.0335 mmol), and cesium

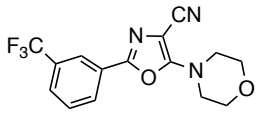
carbonate (0.218 g, 0.669 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with argon; this was repeated three times. Degassed, anhydrous dioxane (1.67 mL) and ethyl 4-iodobenzoate (0.11 mL, 0.67 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^{o}C for 15 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and purified by chromatography on silica (30% ethyl acetate in hexane) to provide **2-42b** (0.0765 g, 0.234 mmol, 70%) as a yellow solid. **Rf** (50% ethyl acetate in hexane) 0.48; **Mp** (CH_2Cl_2) 182-184 ^{o}C; **IR** 2972.5, 2917.6, 2864.3, 2228.1, 1705.1, 1637.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.12-8.10 (m, 2 H), 7.86-7.84 (m, 2 H), 4.40 (q, *J* = 7.1 Hz, 2 H), 3.82 (t, *J* = 4.9 Hz, 4 H), 3.62 (d, *J* = 5.1 Hz, 4 H), 1.41 (t, *J* = 7.1 Hz, 3 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 165.7, 160.0, 151.0, 131.0, 130.4, 129.4, 123.9, 114.1, 110.0, 65.9, 61.3, 34.6, 14.3; **LC-MS** (ES) *m/z* calcd for $C_{17}H_{18}O_4N_3$ $[M+H]^+$ 328.1292, found 328.1289.



2-42c

5-Morpholino-2-(m-tolyl)oxazole-4-carbonitrile (**2-42c**). **2-41b** (0.0600 g, 0.335 mmol), $Pd(OAc)_2$ (0.00376 g, 0.0167 mmol), JohnPhos (0.00999 g, 0.0335 mmol), and cesium carbonate (0.218 g, 0.669 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with argon; this was repeated three times. Degassed, anhydrous 1,4-dioxane (1.67 mL) and 3-iodotoluene (0.09 mL, 0.67 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^{o}C for 24 h. The reaction mixture was filtered through Celite, concentrated

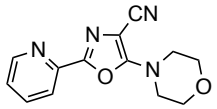
under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane) to provide **2-42c** (0.0641 g, 0.238 mmol, 71%) as an off-white solid: **Rf** (50% ethyl acetate in hexane) 0.48; **Mp** (CH_2Cl_2) 132-134 ^oC; **IR** 2971.6, 2857.6, 2222.9, 1629.4, 1578.0 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.62-7.58 (m, 2H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.21 (d, *J* = 7.5 Hz, 1H), 3.82 (t, *J* = 4.5 Hz, 4H), 3.59 (t, *J* = 4.6 Hz, 4H), 2.41 (s, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 159.6, 152.6, 138.9, 130.6, 129.0, 125.7, 124.9, 121.7, 114.5, 107.9, 66.0, 45.7, 21.5; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{16}O_2N_3$ $[M+H]^+$ 270.1237, found 270.1236.



2-42d

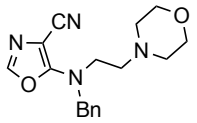
5-Morpholino-2-(3-(trifluoromethyl)phenyl)oxazole-4-carbonitrile (**2-42d**). **2-41b** (0.0600 g, 0.335 mmol), Pd(OAc)2 (0.00376 g, 0.0167 mmol), JohnPhos (0.00999 g, 0.0335 mmol), and cesium carbonate (0.218 g, 0.669 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with argon; this was repeated three times. Degassed, anhydrous 1,4-dioxane (1.67 mL) and 3-iodobenzotrifluoride (0.09 mL, 0.67 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^oC for 22 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and purified by chromatography on silica (20% ethyl acetate in hexane) to provide **2-42d** (0.0809 g, 0.250 mmol, 75%) as a white solid: **Rf** (20% ethyl acetate in hexane) 0.21; **Mp** (CH_2Cl_2) 178-180 ^oC; **IR** 2971.7, 2913.7, 2860.2, 2228.2, 1630.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.05 (d, *J* = 7.3 Hz, 1H), 7.93 (s, 1H), 7.66-7.57 (m, 2H), 3.83 (t, *J* = 4.9 Hz, 4H), 3.62 (dd, *J* = 5.6, 4.1 Hz, 4H); **^{13}C NMR**

($CDCl_3$, 125 MHz) δ 159.9, 150.5, 132.1, 131.9, 131.6, 131.4, 129.8, 127.3, 126.6, 126.04, 126.02, 125.99, 125.96, 124.7, 122.5, 120.94, 120.91, 120.88, 120.85, 113.9, 109.6, 65.9, 45.6; **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -63.0; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{13}F_3O_2N_3$ $[M+H]^+$ 324.0954, found 324.0952.



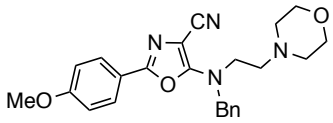
2-42e

5-Morpholino-2-(pyridin-2-yl)oxazole-4-carbonitrile (**2-42e**). **2-41b** (0.0600 g, 0.335 mmol), $Pd(OAc)_2$ (0.00376 g, 0.0167 mmol), JohnPhos (0.00999 g, 0.0335 mmol), and cesium carbonate (0.218 g, 0.669 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with argon; this was repeated three times. Degassed, anhydrous dioxane (1.67 mL) and 2-iodopyridine (0.07 mL, 0.67 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^oC for 13 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and purified by chromatography on silica (40-50% ethyl acetate in hexane) to provide **2-42b** (0.0592 g, 0.231 mmol, 69%) as a yellow solid: **Rf** (20% ethyl acetate in hexane) 0.16; **Mp** (CH_2Cl_2) 136-138 ^oC; **IR** 2926.1, 2865.7, 2233.9, 1624.5, 1601.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.70 (d, *J* = 4.7 Hz, 1H), 7.77 (td, *J* = 7.8, 1.6 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.28-7.26 (m, 1H), 3.81 (t, *J* = 4.9 Hz, 4H), 3.63 (t, *J* = 4.8 Hz, 4H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 160.2, 151.0, 150.3, 145.1, 136.9, 123.6, 119.5, 113.9, 111.5, 66.0, 45.6; **LC-MS** (ES) *m/z* calcd for $C_{13}H_{13}O_2N_4$ $[M+H]^+$ 257.1033, found 257.1031.



2-43

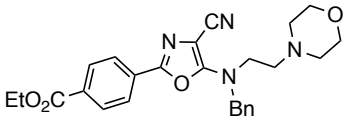
5-(Benzyl(2-morpholinoethyl)amino)oxazole-4-carbonitrile (**2-43**). **2-38** (0.451 g, 2.61 mmol) was added to a high pressure vial containing a solution of N-benzyl-2-morpholinoethan-1-amine (2.87 g, 13.0 mmol) in anhydrous THF (6.5 mL). The high pressure vial was sealed and heated to 80 ^{o}C for 43 h. The reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, purified by chromatography on silica (ethyl acetate) to provide **2-43** (0.620 g, 1.98 mmol, 76%) as a yellow oil. **Rf** (ethyl acetate) 0.36; **IR** 3154.7, 3030.3, 2954.4, 2854.3, 2809.0, 2237.3, 1629.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.68 (s, 1H), 7.36-7.29 (m, 3 H), 7.26 (d, *J* = 5.6 Hz, 2 H), 4.69 (s, 2 H), 3.65 (t, *J* = 4.6 Hz, 4 H), 3.49 (t, *J* = 6.6 Hz, 2 H), 2.51 (t, *J* = 6.6 Hz, 2H), 2.42 (t, *J* = 4.3 Hz, 4 H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 162.0, 139.8, 136.5, 128.8, 127.9, 127.6, 114.8, 112.9, 66.9, 56.2, 53.8, 52.4, 44.9; **LC-MS** (ES) *m/z* calcd for $C_{17}H_{21}O_2N_4$ $[M+H]^+$ 313.1659, found 313.1658.



2-44a

5-(Benzyl(2-morpholinoethyl)amino)-2-(4-methoxyphenyl)oxazole-4-carbonitrile (**2-44a**). **2-43** (0.0750 g, 0.240 mmol), Pd(OAc)2 (0.00269 g, 0.0120 mmol), JohnPhos (0.00716 g, 0.0240 mmol), and cesium carbonate (0.156 g, 0.480 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with

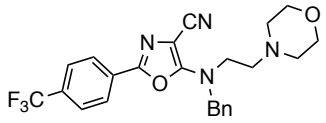
argon; this was repeated three times. Degassed, anhydrous 1,4-dioxane (1.60 mL) and 4-iodoanisole (0.112 g, 0.480 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^{o}C for 14 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate) to provide **2-44a** (0.0553 g, 0.132 mmol, 55%) as a yellow oil: **Rf** (ethyl acetate) 0.57; **IR** 2948.8, 2808.5, 2224.2, 1635.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.72-7.69 (m, 2 H), 7.37-7.34 (m, 2 H), 7.32-7.29 (m, 3 H), 6.97-6.94 (m, 2 H), 4.73 (s, 2 H), 3.85 (s, 3 H), 3.64 (t, *J* = 4.6 Hz, 5 H), 3.54 (t, *J* = 6.5 Hz, 2 H), 2.56 (t, *J* = 6.5 Hz, 2 H), 2.45 (s, 4 H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 160.5, 159.8, 152.2, 136.6, 128.8, 127.9, 127.7, 126.0, 118.8, 115.1, 114.6, 106.5, 66.9, 56.2, 55.4, 53.8, 52.4, 44.8; **LC-MS** (ES) *m/z* calcd for $C_{24}H_{27}O_3N_4$ $[M+H]^+$ 419.2078, found 419.2076.



2-44b

Ethyl 4-(5-(benzyl(2-morpholinoethyl)amino)-4-cyanooxazol-2-yl)benzoate (**2-44b**). **2-43** (0.0750 g, 0.240 mmol), Pd(OAc)2 (0.00269 g, 0.0120 mmol), JohnPhos (0.00716 g, 0.0240 mmol), and cesium carbonate (0.156 g, 0.480 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with argon; this was repeated three times. Degassed, anhydrous 1,4-dioxane (1.6 mL) and ethyl ethyl 4-iodobenzoate (0.0808 g, 0.480 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^{o}C for 14 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and purified by chromatography on

silica (ethyl acetate) to provide **2-44b** (0.0851 g, 0.185 mmol, 77%) as a yellow oil: **Rf** (ethyl acetate) 0.52; **IR** 2959.0, 2857.1, 2814.5, 2230.0, 1715.5, 1633.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.10-8.08 (m, 2 H), 7.81-7.79 (m, 2 H), 7.38-7.35 (m, 2 H), 7.32 (dd, *J* = 6.9, 4.3 Hz, 3 H), 4.77 (s, 2 H), 4.39 (q, *J* = 7.1 Hz, 2 H), 3.65 (t, *J* = 4.5 Hz, 4 H), 3.58 (t, *J* = 6.4 Hz, 2 H), 2.58 (t, *J* = 6.4 Hz, 2 H), 2.46 (s, 4 H), 1.40 (t, *J* = 7.1 Hz, 3 H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 165.7, 160.7, 150.5, 136.3, 130.7, 130.4, 129.6, 128.9, 128.1, 127.7, 126.4, 123.7, 114.4, 110.3, 66.9, 61.3, 56.1, 53.8, 52.5, 44.9, 27.5, 14.3; **LC-MS** (ES) *m/z* calcd for $C_{26}H_{29}O_4N_4$ $[M+H]^+$ 461.2183, found 461.2184.



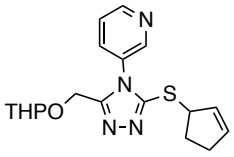
2-44c

5-(Benzyl(2-morpholinoethyl)amino)-2-(4-(trifluoromethyl)phenyl)oxazole-4-carbonitrile (**2-44c**). **2-43** (0.0750 g, 0.240 mmol), Pd(OAc)2 (0.00269 g, 0.0120 mmol), JohnPhos (0.00716 g, 0.0240 mmol), and cesium carbonate (0.156 g, 0.480 mmol) were added to a high pressure vial under an atmosphere of argon. The reaction vial was evacuated under vacuum and filled with argon; this was repeated three times. Degassed, anhydrous 1,4-dioxane (1.6 mL) and 4-iodobenzotrifluoride (0.07 mL, 0.48 mmol) were sequentially added and the reaction vessel was sealed under an atmosphere of argon and heated to 110 ^oC for 23 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and purified by chromatography on silica (50-100% ethyl acetate in hexane) to provide **2-44c** (0.094 g, 0.196 mmol, 82%) as a yellow oil: **Rf** (ethyl acetate) 0.57; **IR** 3060.0, 2962.4, 2857.4, 2812.5, 2231.6, 1632.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.85 (d, *J* = 8.3 Hz, 2 H), 7.68 (d, *J* = 8.4 Hz, 2H),

7.38-7.35 (m, 2H), 7.32 (t, J = 6.3 Hz, 3H), 4.77 (s, 2H), 3.65 (t, J = 4.5 Hz, 4 H), 3.58 (t, J = 6.4 Hz, 2 H), 2.58 (t, J = 6.4 Hz, 2 H), 2.46 (t, J = 4.0 Hz, 4 H); **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -62.9; **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 160.8, 150.0, 136.3, 131.1, 130.9, 130.6, 130.3, 129.1, 128.9, 128.17, 128.07, 127.6, 126.20, 126.17, 126.14, 126.11, 124.1, 114.2, 110.4, 66.9, 53.8, 52.5, 45.0; **LC-MS** (ES) *m/z* calcd for $C_{24}H_{24}O_2N_4F_3$ $[M+H]^+$ 457.1846, found 457.1845.

A-2

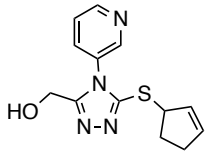
3-Bromocyclopent-1-ene (**A-2**). A high-pressure reaction vial was charged with a mixture of cyclopentene (3.97 mL, 44.9 mmol), N-bromosuccinimide (2.00 g, 11.4 mmol), and AIBN (0.0738 g, 0.449 mmol) in tetrachloromethane (7.5 mL). The reaction vial was sealed and heated at 90 ^{o}C for 1 h. The reaction mixture was cooled to 0 °C and filtered through a pad of Celite to provide a crude solution of **A-1** that was carried forward without further purification.



A-3

3-(3-(Cyclopent-2-en-1-ylthio)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**A-3**). A crude solution of **A-2** (0.825 g, 5.61 mmol) in carbon tetrachloride (3.25 mL) was added to a mixture of **1-13** (0.500, 1.71 mmol) and cesium carbonate (0.836 g, 2.56 mmol) in DMF (3.5 mL) and stirred at 80 ^{o}C for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL), and extracted with

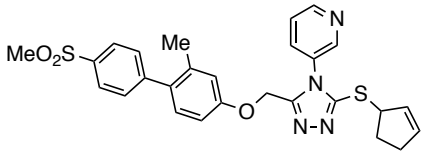
ethyl acetate (20 mL, three times). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (75% ethyl acetate in hexane, dry loaded) to provide crude tentative **A-3** (0.200 g) as a light-yellow solid. This material was carried forward without further purification: **Rf** (ethyl acetate) 0.52; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.75 (d, *J* = 4.8 Hz, 1H), 8.71 (d, *J* = 2.0 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.51 (dd, *J* = 7.9, 4.8 Hz, 1H), 6.22-6.20 (m, 1H), 6.13-6.10 (m, 1H), 5.86-5.84 (m, 1H), 4.57-4.52 (m, 1H), 4.48-4.45 (m, 1H), 4.34-4.27 (m, 1H), 4.15 (q, *J* = 7.1 Hz, 1H), 3.39-3.33 (m, 2H), 2.74-2.66 (m, 1H), 2.58-2.27 (m, 5H), 2.20-2.07 (m, 2H), 1.60-1.38 (m, 8H), 1.36-1.32 (m, 1H), 1.28-1.25 (m, 2H); **LC-MS** (ES) *m/z* calcd for $C_{18}H_{23}O_2N_4S$ $[M+H]^+$ 359.1536, 359.1535.



A-4

(5-(Cyclopent-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methanol (**A-4**). *p*-Toluenesulfonic acid monohydrate (0.0486 g, 0.279 mmol) was added to a solution of crude **A-3** (0.200 g, 0.558 mmol) in methanol (5.5 mL) at room temperature and stirred overnight. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate) to provide **A-4** (0.0509 g, 0.186 mmol, 33%) as a white solid: **Rf** (ethyl acetate) 0.25; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.73-8.70 (m, 2H), 7.91 (d, *J* = 8.3 Hz, 1H), 7.55-7.51 (m, 1H), 6.21 (dt, *J* = 3.6, 1.9 Hz, 1H), 6.12-6.09 (m, 1H),

5.84-5.82 (m, 1H), 4.53 (s, 2H), 2.73-2.65 (m, 1H), 2.57-2.39 (m, 3H), 2.14-2.03 (m, 1H); **LC-MS** (ES) *m/z* calcd for $C_{13}H_{15}ON_4S$ $[M+H]^+$ 275.0961, 275.0835.

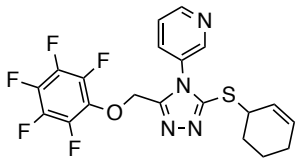


A-5

3-(3-(Cyclopent-2-en-1-ylthio)-5-(((2-methyl-4'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**A-5**). Methanesulfonyl chloride (0.02 mL, 0.22 mmol) was added dropwise to mixture of **A-4** (0.0509 g, 0.186 mmol) and N,N-diisopropylethylamine (0.05 mL, 0.04 mmol) in anhydrous methylene chloride (1.85 mL) under an atmosphere of nitrogen at 0 ^oC and stirred for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (5 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3-(3-(Cyclopent-2-en-1-ylthio)-5-(((2-methyl-4'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (0.0584 g, 0.223 mmol) was added to a mixture of crude mesylate and cesium carbonate (0.121 g, 0.371 mmol) in anhydrous DMF (0.9 mL) under an atmosphere of nitrogen and stirred at room temperature for 21 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (5 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (70% ethyl acetate in hexane, dry loaded) to provide **A-5** (0.0578 g, 0.111 mmol, 60%) as a white solid: **Rf** (ethyl acetate) 0.68; **Mp** (methylene chloride,

gel) 92-95 °C; **IR** 3054.0, 2924.0, 1604.6, 1576.6, 1483.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.77-8.72 (m, 2H), 7.99-7.95 (m, 2H), 7.87 (ddd, *J* = 8.2, 2.4, 1.5 Hz, 1H), 7.53 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.47-7.44 (m, 2H), 7.08 (d, *J* = 8.2 Hz, 1H), 6.73-6.68 (m, 2H), 6.26-6.22 (m, 1H), 6.16-6.10 (m, 1H), 5.88-5.84 (m, 1H), 4.96 (d, *J* = 2.4 Hz, 2H), 3.11 (s, 3H), 2.77-2.68 (m, 1H), 2.60-2.45 (m, 2H), 2.21-2.18 (m, 3H), 2.19-2.08 (m, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 168.4, 156.7, 150.9, 148.6, 146.9, 146.1, 138.9, 137.22, 137.08, 136.0, 134.1, 130.93, 130.86, 130.2, 128.4, 127.3, 124.0, 116.7, 112.2, 64.8, 59.9, 44.6, 29.3, 20.6; **LC-MS** (ES) *m/z* calcd for $C_{27}H_{27}O_3N_4S_2$ $[M+H]^+$ 519.1519, found 519.1521.

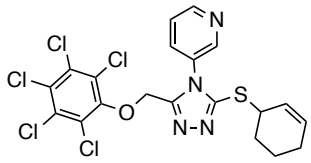


A-6

3-(3-(Cyclohex-2-en-1-ylthio)-5-((perfluorophenoxy)methyl)-4*H*-1,2,4-triazol-4-yl)pyridine (**A-6**). Methanesulfonyl chloride (0.01 mL, 0.19 mmol) was added dropwise to mixture of **1-15** (0.0500 g, 0.173 mmol), N,N-diisopropylethylamine (0.04 mL, 0.22 mmol) in anhydrous methylene chloride (1.7 mL) under an atmosphere of nitrogen at 0 °C and stirred at room temperature for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with methylene chloride (15 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

Pentafluorophenol (0.0351 g, 0.191 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.113 g, 0.347 mmol) in anhydrous DMF (0.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 16 h. The reaction mixture was diluted with water (5

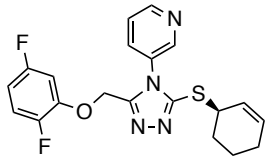
mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (60% ethyl acetate in hexane, dry loaded) to provide **A-6** (0.0424 g, 0.0933 mmol, 54%) as a white solid: **Rf** (ethyl acetate) 0.52; **Mp** (methylene chloride) 73-75 ^{o}C; **IR** 30131.3, 2938.2, 1512.0, 1484.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.83 (s, 1 H), 8.71 (s, 1 H), 7.83-7.81 (m, 1 H), 7.56-7.55 (m, 1 H), 5.90-5.88 (m, 1 H), 5.75-5.73 (m, 1 H), 4.57-4.56 (m, 1 H), 2.10-1.96 (m, 5 H), 1.76-1.66 (m, 3 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 154.1, 151.4, 150.3, 147.9, 143.1-142.9 (m, 1 C), 141.1-140.9 (m, 1 C), 139.1-138.8 (m, 1 C), 137.5-137.3 (m, 1 C), 137.1-136.9 (m, 1 C), 134.9, 132.5, 131.4-131.2 (m, 1 C), 65.2, 44.3, 29.2, 24.8, 19.2; **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -155.7, -160.3, -162.1**; LC-MS** (ES) *m/z* calcd for $C_{20}H_{16}ON_4F_5S$ $[M+H]^+$ 455.0959, found 455.0959.



A-7

3-(3-(Cyclohex-2-en-1-ylthio)-5-((perchlorophenoxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**A-7**). Methanesulfonyl chloride (0.01 mL, 0.19 mmol) was added dropwise to mixture of **1-15** (0.0500 g, 0.173 mmol), N,N-diisopropylethylamine (0.04 mL, 0.22 mmol) in anhydrous methylene chloride (1.7 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred at room temperature for 1 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with methylene chloride (15 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

Pentachlorophenol (0.0508 g, 0.191 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.113 g, 0.347 mmol) in anhydrous DMF (0.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 16 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (60% ethyl acetate in hexane, dry loaded) to provide **A-7** (0.0532 g, 0.0991 mmol, 57%) as a white solid: **Rf** (ethyl acetate) 0.63; **Mp** (methylene chloride) 72-74 ^{o}C; **IR** 3032.1, 2936.9, 1483.7, 1388.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.83 (s, 1 H), 8.76 (s, 1 H), 7.86 (d, *J* = 8.1 Hz, 1 H), 7.56-7.54 (m, 1 H), 5.90-5.87 (m, 1 H), 5.75 (dt, *J* = 10.0, 1.9 Hz, 1 H), 5.10-5.05 (m, 2 H), 4.58 (d, *J* = 2.2 Hz, 1 H), 2.13-1.97 (m, 4 H), 1.78-1.65 (m, 2 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 153.7, 151.3, 150.5, 149.9, 148.2, 135.1, 132.4, 132.1, 130.6, 130.0, 128.3, 125.4, 124.2, 63.9, 44.3, 29.2, 24.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{20}H_{15}Cl_5ON_4S$ $[M+H]^+$ 536.6770, found 536.9453.

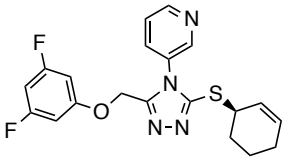


A-8

(*R*)-3-(3-(cyclohex-2-en-1-ylthio)-5-((2,5-difluorophenoxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**A-8**). Methanesulfonyl chloride (0.03 mL, 0.42 mmol) was added dropwise to mixture of **1-117A** (0.100 g, 0.347 mmol) and N,N-diisopropylethylamine (0.09 mL, 0.52 mmol) in anhydrous methylene chloride (3.5 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (5 mL, three times). The combined

organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

2,5-Difluorophenol (0.0541 g, 0.416 mmol) was added to a mixture of crude mesylate and cesium carbonate (0.226 g, 0.694 mmol) in anhydrous DMF (1.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 20 h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40% ethyl acetate in hexane, dry loaded) to provide **A-8** (0.117 g, 0.291 mmol, 84%) as a white solid: **Rf** (ethyl acetate) 0.70; $[\alpha]_D$ = +137.3 (*c.* 0.275, CH_2Cl_2); **Mp** (methylene chloride) 123-124 ^oC; **IR** 2896.2, 2875.9, 2845.5, 1719.3, 1648.5, 1608.1 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.78-8.77 (m, 1H), 8.65-8.65 (m, 1H), 7.77 (ddd, *J* = 8.2, 2.5, 1.6 Hz, 1H), 7.49 (dd, *J* = 8.0, 4.8 Hz, 1H), 6.98 (ddd, *J* = 10.5, 9.0, 5.2 Hz, 1H), 6.83 (ddd, *J* = 9.4, 6.6, 2.9 Hz, 1H), 6.62 (ddt, *J* = 9.0, 7.7, 3.1 Hz, 1H), 5.91-5.86 (m, 1H), 5.78-5.73 (m, 1H), 5.10 (s, 2H), 4.60-4.59 (m, 1H), 2.14-1.98 (m, 4H), 1.77-1.63 (m, 3H).; **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 159.71, 159.68, 157.28, 157.26, 153.9, 151.3, 150.7, 150.14, 150.10, 148.0, 147.73, 147.70, 145.73, 145.63, 145.60, 145.50, 135.1, 132.4, 129.8, 125.4, 124.1, 116.79, 116.69, 116.58, 116.48, 108.62, 108.55, 108.38, 108.31, 104.1, 103.8, 61.3, 44.1, 29.2, 24.8, 19.1; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -115.6 (d, *J* = 15.0 Hz), -139.3 (d, *J* = 15.4 Hz); **LC-MS** (ES) *m/z* calcd for $C_{20}H_{19}ON_4F_2S$ $[M+H]^+$ 401.1242, found 401.1237.

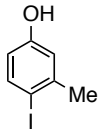


A-9

(*R*)-3-(3-(cyclohex-2-en-1-ylthio)-5-((3,5-difluorophenoxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**A-9**). Methanesulfonyl chloride (0.03 mL, 0.42 mmol) was added dropwise to mixture of **1-117A** (0.100 g, 0.347 mmol) and N,N-diisopropylethylamine (0.09 mL, 0.52 mmol) in anhydrous methylene chloride (3.5 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (5 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

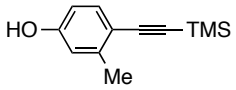
2,5-Difluorophenol (0.0541 g, 0.416 mmol) was added to a mixture of crude mesylate and cesium carbonate (0.226 g, 0.694 mmol) in anhydrous DMF (1.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 16 h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (40% ethyl acetate in hexane, dry loaded) to provide **A-9** (0.120 g, 0.300 mmol, 87%) as a white solid: **Rf** (ethyl acetate) 0.69 $[\alpha]_D$ = +136.0 (*c.* 0.264, CH_2Cl_2); **Mp** (methylene chloride) 138-139 ^{o}C; **IR** 3083.5, 3059.1, 2918.2, 1626.1, 1601.9, 1483.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.78 (s, 1H), 8.62 (s, 1H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 6.2 Hz, 1H), 6.43 (d, *J* = 7.7 Hz, 2H), 5.89 (d, *J* = 9.1 Hz, 1H), 5.76 (d, *J* = 7.8 Hz, 1H), 5.05 (s, 2H), 4.60 (s, 1H), 2.12-1.97 (m, 4H), 1.75-1.64 (m, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 164.87, 164.87, 164.71, 164.71, 162.40, 162.40, 162.25, 162.25, 159.10, 159.10, 158.96, 158.96, 158.80,

158.80, 153.77, 153.77, 151.34, 151.34, 150.69, 150.69, 150.66, 150.66, 147.93, 147.93, 134.70, 134.70, 132.47, 132.47, 129.86, 129.86, 125.32, 125.32, 124.14, 124.14, 98.83, 98.83, 98.55, 98.55, 97.92, 97.92, 97.68, 97.68, 97.42, 97.42, 60.30, 60.30, 44.06, 44.06, 29.21, 29.21, 24.82, 24.82, 19.13, 19.13; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -108.1; **LC-MS** (ES) *m/z* calcd for $C_{20}H_{19}ON_4F_2S$ $[M+H]^+$ 401.1242, found 401.1084.



A-11

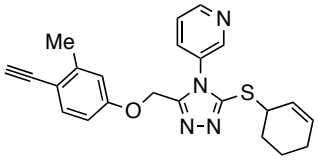
4-Iodo-3-methylphenol (**A-11**). A solution of potassium iodide (2.49 g, 15.0 mmol) and potassium iodate (0.441 mL, 8.09 mmol) in water (80 mL) was added to a mixture of m-cresol (2.43 mL, 23.1 mmol) in methanol (100 mL) and 12 N aqueous solution of hydrogen chloride (58 mL). After 2 h, additional water (50 mL) was added and the mixture was extracted with toluene (100 mL, three times). The combined organic solutions were washed with brine (100 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide crude **A-11** (5.43 g) as a purple/brown solid: **Rf** (10% ethyl acetate in hexane) 0.31; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.61 (d, 1 H, *J* = 8.5 Hz), 6.76 (d, 1 H, *J* = 2.9 Hz), 6.42 (dd, 1 H, *J* = 8.5, 3.0 Hz), 4.77 (br. s, 1 H), 2.37 (s, 3 H).



A-12

3-Methyl-4-((trimethylsilyl)ethynyl)phenol (**A-12**). **A-11** (1.00 g, 4.27 mmol),

trimethylsilylacetylene (1.18 mL, 8.55 mmol), copper (I) iodide (0.0814 g, 0.427 mmol), bis(triphenylphosphine)palladium(II) chloride (0.149 g, 0.214 mmol) was added to a reaction vial. The reaction vial was degassed under vacuum and refilled with argon; this process was conducted three times. The premixed degassed, anhydrous THF (4.25 mL) and anhydrous triethylamine (4.25 mL) was added to reaction vial. The reaction vial was purged with argon for 5 minutes. The reaction vial was sealed and stirred at room temperature for 19 h. The reaction mixture diluted with methylene chloride (25 mL) and sequentially washed with a saturated aqueous solution of ammonium chloride (25 mL) and water (25 mL). The organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure, and purified by chromatography on SiO_2 (20% ethyl acetate in hexane, dry loaded) to provide **A-12** (0.791 g, 3.87 mmol, 91%) as a light-brown solid: **Rf** (20% ethyl acetate in hexane) 0.50; **IR** 3260.1, 2957.5, 2152.7, 1604.1, 1492.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.31 (d, 1 H, *J* = 8.3 Hz), 6.66 (d, 1 H, *J* = 2.6 Hz), 6.58 (ddd, 1 H, *J* = 8.3, 2.6, 0.4 Hz), 2.38 (s, 3 H), 0.24 (s, 9 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 155.6, 142.9, 133.7, 116.4, 115.5, 112.7, 104.1, 96.5, 20.7, 0.1; **LC-MS** (ES) *m/z* calcd for $C_{12}H_{15}OSi$ $[M-H]^+$ 203.0887, found 203.0888.

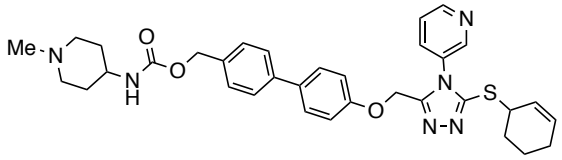


A-13

3-(3-(Cyclohex-2-en-1-ylthio)-5-((3-methyl-4-((trimethylsilyl)ethynyl)phenoxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**A-13**). Methanesulfonyl chloride (0.06 mL, 0.76 mmol) was added drop wise to mixture of **1-15** (0.200 g, 0.0.694 mmol), N,N-diisopropylethylamine (0.15 mL, 0.87 mmol) in anhydrous methylene

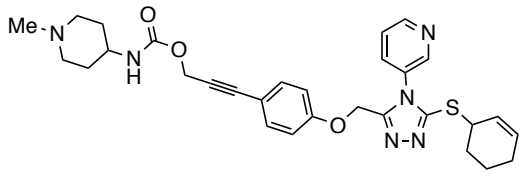
chloride (7 mL) under an atmosphere of nitrogen at 0 ^{o}C and stirred for 1 h. The reaction mixture was diluted with a saturated solution of aqueous sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

A-12 (0.156 g, 0.763 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.452 g, 1.39 mmol) in anhydrous DMF (3.5 mL) under an atmosphere of nitrogen and stirred at room temperature for 13 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (65% ethyl acetate in hexane, dry loaded) to provide **A-13** (0.179 g, 0.445 mmol, 64%) as an off-white solid: **Rf** (ethyl acetate) 0.52; **Mp** (methylene chloride) 116-118 ^{o}C; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.62 (dd, 1 H, *J* = 2.5, 0.5 Hz), 7.67 (ddd, 1 H, *J* = 8.1, 2.6, 1.5 Hz), 7.46 (ddd, 1 H, *J* = 8.1, 4.8, 0.7 Hz), 7.34 (d, 1 H, *J* = 8.5 Hz), 6.70 (d, 1 H, *J* = 2.6 Hz), 6.65 (dd, 1 H, *J* = 8.5, 2.6 Hz), 5.88 (dtd, 1 H, *J* = 9.8, 3.8, 1.5 Hz), 5.77-5.73 (m, 1 H), 5.07 (d, 2 H, *J* = 1.4 Hz), 4.59-4.56 (m, 1 H), 3.19 (s, 1 H), 2.39 (s, 3 H), 2.13-2.06 (m, 1 H), 2.05-1.99 (m, 3 H), 1.77-1.64 (m, 2 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 157.3, 153.5, 151.3, 151.2, 148.0, 142.8, 134.7, 133.9, 132.4, 130.0, 125.4, 124.0, 115.74, 115.60, 111.9, 82.1, 80.0, 59.9, 44.1, 29.2, 24.8, 20.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{23}ON_4S$ $[M+H]^+$ 403.1587, found 403.1587.



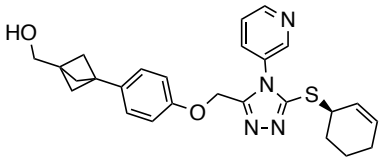
A-14

(4'-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-[1,1'-biphenyl]-4-yl)methyl (1-methylpiperidin-4-yl)carbamate (**A-14**). Carbonyldiimidazole (0.0258 g, 0.159 mmol) was added to a solution of **A-13** (0.0500 g, 0.106 mmol) in anhydrous methylene chloride (1 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.04 mL, 0.32 mmol) was added and stirred for an additional 11 h. The reaction mixture was concentrated under reduced pressure, and purified by chromatography on silica (15% methanol in methylene chloride, dry loaded) to provide **A-14** (0.0456 g, 0.0747 mmol, 70%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.08; **Mp** (methylene chloride) 73-75 ^{o}C; **IR** 3259.1, 3031.8, 2937.2, 2854.3, 2784.0, 1710.81, 1607.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.65 (d, *J* = 2.2 Hz, 1H), 7.71 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1H), 7.50-7.45 (m, 5H), 7.40 (d, *J* = 8.2 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 5.89-5.86 (m, 1H), 5.77-5.73 (m, 1H), 5.13 (d, *J* = 1.4 Hz, 2H), 5.11 (s, 1H), 4.68-4.66 (m, 1H), 4.58 (dd, *J* = 3.9, 1.6 Hz, 1H), 3.54 (s, 1H), 2.77 (s, 2H), 2.28 (s, 3H), 2.13-2.06 (m, 3H), 2.05-2.01 (m, 3H), 1.98-1.94 (m, 3H), 1.76-1.64 (m, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 156.9, 153.4, 151.5, 151.1, 148.1, 140.3, 135.2, 134.76, 134.58, 132.3, 130.1, 128.6, 128.3, 126.9, 125.5, 124.0, 115.1, 66.3, 60.1, 54.30, 54.29, 54.27, 46.1, 44.1, 34.7, 32.4, 31.6, 29.3, 24.8, 22.6, 19.2, 14.1; **LC-MS** (ES) *m/z* calcd for $C_{34}H_{39}O_3N_6S$ $[M+H]^+$ 611.2799, found 611.2796.



A-15

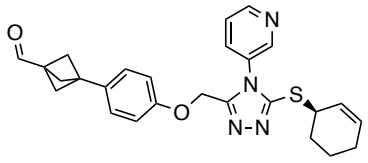
3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**A-15**). Carbonyldiimidazole (0.0337 g, 0.208 mmol) was added to a solution of **1-42b** (0.0578 g, 0.138 mmol) in anhydrous methylene chloride (1.4 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.05 mL, 0.42 mmol) was added and stirred for 15 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (10% methanol in methylene chloride, dry loaded) to provide **A-15** (0.0523 g, 0.0936 mmol, 68%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.14; **Mp** (methylene chloride) 68-70 ^{o}C; **IR** 3273.8, 3041.7, 2939.8, 2858.4, 2792.9, 1712.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.3 Hz, 1 H), 8.60 (d, *J* = 2.4 Hz, 1 H), 7.67-7.65 (m, 1 H), 7.45 (dd, *J* = 8.1, 4.8 Hz, 1 H), 7.32 (d, *J* = 8.7 Hz, 2 H), 6.79 (d, *J* = 8.8 Hz, 2 H), 5.88-5.85 (m, 1 H), 5.74 (dt, *J* = 9.9, 2.0 Hz, 1 H), 5.11-5.05 (m, 2 H), 4.86 (s, 3 H), 4.56 (t, *J* = 1.9 Hz, 1 H), 3.56 (s, 1 H), 2.85 (s, 2 H), 2.33 (s, 3 H), 2.20 (t, *J* = 8.3 Hz, 2 H), 2.11-2.05 (m, 1 H), 2.02-1.97 (m, 5 H), 1.75-1.64 (m, 2 H), 1.62-1.55 (m, 2 H); **^{13}C NMR** ($CDCl_3$, 500 MHz) δ 157.4, 154.8, 153.5, 151.2, 151.2, 148.0, 134.7, 133.5, 132.3, 130.0, 125.4, 124.0, 115.8, 114.7, 85.8, 82.7, 60.0, 54.1, 53.3, 47.5, 45.7, 44.1, 31.9, 31.86, 29.2, 24.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{30}H_{35}O_3N_6S$ $[M+H]^+$ 559.2486, found 559.2485.



A-16

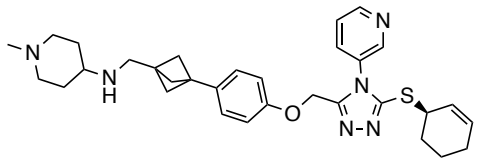
(*R*)-(3-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)bicyclo[1.1.1]pentan-1-yl)methanol (**A-16**). Methanesulfonyl chloride (0.05 mL, 0.19 mmol) was added dropwise to mixture of **1-117a** (0.0550 g, 0.191 mmol), N,N-diisopropylethylamine (0.05 mL, 0.26 mmol) in anhydrous methylene chloride (1.9 mL) under an atmosphere of nitrogen at 0 ^{o}C for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-24 (0.0330 g, 0.173 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.113 g, 0.37 mmol) in anhydrous DMF (1 mL) under an atmosphere of nitrogen and stirred at room temperature for 22 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **A-16** (0.0490 g, 0.106 mmol, 61%) as a white solid: **Rf** (ethyl acetate) 0.3; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.76 (s, 1 H), 8.63 (s, 1 H), 7.73-7.71 (m, 1 H), 7.50-7.47 (m, 1 H), 7.11-7.09 (m, 2 H), 6.80-6.78 (m, 2 H), 5.90-5.86 (m, 1 H), 5.76-5.72 (m, 1 H), 5.11-5.05 (m, 2 H), 4.59-4.58 (m, 1 H), 3.68 (s, 2 H), 2.12-2.07 (m, 1 H), 2.06-1.99 (m, 4 H), 1.93 (s, 6 H), 1.75-1.64 (m, 3 H); **LC-MS** (ES) *m/z* calcd for $C_{26}H_{29}O_2N_4S$ $[M+H]^+$ 461.2006, found 461.2004.



A-17

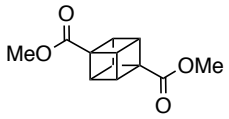
(*R*)-3-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)bicyclo[1.1.1]pentane-1-carbaldehyde (**A-17**). Dess–Martin periodinane (0.0677 g, 0.159 mmol) was added to a solution of **A-16** (0.0490 g, 0.106 mmol) in anhydrous methylene chloride (0.9 mL) at 0 ^{o}C under an atmosphere of nitrogen and stirred for 1.5 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were washed with a saturated aqueous solution of sodium thiosulfate (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **A-17** (0.0424 g, 0.0925 mmol, 87%) as a white solid: **Rf** (ethyl acetate) 0.46; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 9.66 (s, 1 H), 8.75 (d, *J* = 3.8 Hz, 1 H), 8.62 (d, *J* = 2.1 Hz, 1 H), 7.69 (ddd, *J* = 8.1, 2.4, 1.6 Hz, 1 H), 7.46 (dd, *J* = 8.1, 4.7 Hz, 1 H), 7.10 (d, *J* = 8.7 Hz, 2 H), 6.82 (d, *J* = 8.7 Hz, 2 H), 5.89-5.86 (m, 1 H), 5.76-5.73 (m, 1 H), 5.10-5.05 (m, 2 H), 4.56 (t, *J* = 1.9 Hz, 1 H), 2.25 (s, 6 H), 2.11-2.05 (m, 1 H), 2.04-1.97 (m, 3 H), 1.75-1.58 (m, 3 H).



A-18

(*R*)-N-((3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)bicyclo[1.1.1]pentan-1-yl)methyl)-1-methylpiperidin-4-amine (**A-18**). 1-

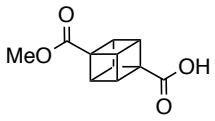
Methylpiperidin-4-amine (0.01 mL, 0.11 mmol) was added to a solution of **A-17** (0.0424 g, 0.0925 mmol) in anhydrous methanol under an atmosphere of nitrogen and stirred for 2 h. Sodium borohydride (0.005 g, 0.139 mmol) was added and the reaction mixture was stirred for an additional 14 h. The reaction mixture was quenched with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water) to provide **A-18** (0.0218 g, 0.0392 mmol, 42%) as a white solid: **Mp** (methylene chloride) 133-135 ^oC; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{22}}$ (*c*. 0.9, methanol) +72.2^o; **IR** 3386.3, 3033.8, 2934.4, 2864.2, 2795.7, 1447.1 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.74 (dd, *J* = 4.7, 1.2 Hz, 1 H), 8.61 (d, *J* = 2.2 Hz, 1 H), 7.69-7.67 (m, 1 H), 7.45 (dd, *J* = 8.0, 4.8 Hz, 1 H), 7.08 (d, *J* = 8.6 Hz, 2 H), 6.78 (d, *J* = 8.6 Hz, 2 H), 5.88-5.85 (m, 1 H), 5.75-5.72 (m, 1 H), 5.08-5.03 (m, 2 H), 4.56-4.55 (m, 1 H), 2.84 (d, *J* = 11.6 Hz, 2 H), 2.75 (s, 2 H), 2.47 (t, *J* = 10.3 Hz, 1 H), 2.27 (s, 3 H), 2.10-2.05 (m, 1 H), 2.01 (dd, *J* = 3.7, 1.9 Hz, 5 H), 1.90 (s, 8 H), 1.75-1.63 (m, 3 H), 1.46-1.38 (m, 2 H).; **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 155.9, 153.3, 151.6, 151.1, 148.1, 134.9, 134.8, 132.3, 130.1, 127.3, 125.5, 123.9, 114.5, 60.1, 54.7, 51.5, 50.7, 47.9, 46.2, 44.1, 41.3, 38.3, 32.7, 29.2, 24.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{32}H_{41}ON_6S$ $[M+H]^+$ 557.3057, found 557.3055.



A-20

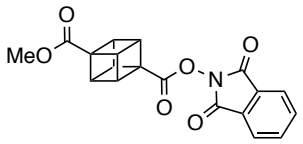
Dimethyl cubane-1, 4-dicarboxylate (**A-20**). Concentrated sulfuric acid (0.141 mL, 2.60 mmol) was added to a high pressure vial containing a solution of 1,4-cubanedicarboxylic acid (0.500 g, 2.60 mmol) in methanol (10 mL). The vial was sealed and the reaction mixture was

stirred at 80 ^{o}C for 21 h. The reaction mixture was cooled to room temperature, diluted with a saturated solution of sodium bicarbonate (15 mL), and extracted with methylene chloride (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide **A-20** (0.572 g, 2.59 mmol, 100%) as a white solid. **Rf** (10% ethyl acetate in hexane) 0.24; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 4.23 (s, 6H), 3.71 (s, 6H).



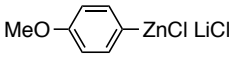
A-21

4-(Methoxycarbonyl)cubane-1-carboxylic acid (**A-21**). A solution sodium hydoxide (0.102 g, 2.55 mmol) in methanol (1.2 mL) was added to a solution of **A-20** (0.510 g, 2.32 mmol) in THF (23 mL) at room temperature and stirred for 13 h. The reaction mixture was concentrated under reduced pressure and resuspend in water (20 mL) and washed with methylene chloride (10 mL, three times). The aqueous solution was acidified with a 1M aqueous solution of sodium potassium sulfate to reach pH 1 and extracted with methylene chloride (20 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **A-21** (0.410 g, 1.99 mmol, 86%) as a white solid: **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 4.28-4.26 (m, 6H), 3.71 (s, 3H).



A-22

1-(1,3-Dioxoisoindolin-2-yl) 4-methyl-cubane-1,4-dicarboxylate (**A-22**). N,N'-Diisopropylcarbodiimide (0.38 mL, 2.4 mmol) was added to a mixture of **A-21** (0.451 g, 2.19 mmol), N-hydroxyphthalimide (0.393 g, 2.41 mmol), 4-dimethylaminopyridine (0.0267 g, 0.219 mmol) in anhydrous methylene chloride (11 mL) at room temperature under an atmosphere of nitrogen and stirred for 16 h. The reaction mixture was filtered through a pad of Celite and rinsed with methylene chloride. The filtrate was concentrated under reduced pressure purified by chromatography on silica (methylene chloride) to provide **A-22** (0.685 g, 1.95 mmol, 89%) as a white solid: **Rf** (50% ethyl acetate in hexane) 0.68; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.90 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.80 (dd, *J* = 5.5, 3.1 Hz, 2H), 4.50-4.48 (m, 3H), 4.38-4.36 (m, 3H), 3.73 (s, 3H).

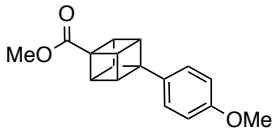


A-23a

(4-Methoxyphenyl)zinc(II) chloride lithium chloride (**A-23a**). A three-neck round bottom flask was equipped with a stopper, condenser, and septum and charged with metallic magnesium turnings (0.875 g, 36 mmol) and lithium chloride (1.27 g, 30 mL). Iodine (*ca*. 2 mg) was added and the mixture was briefly heated with a Bunsen burner and vigorously stirred under an atmosphere of nitrogen. Once cooled to room temperature, anhydrous THF (20 mL) was added followed by 4-bromoanisole (3.00 mL, 24 mmol). The mixture was stirred at reflux for 3 h then cooled to room temperature. Titration of the mixture with iodine (0.253 g, 0.1 mmol) in 0.5

M solution lithium chloride in THF (3 mL) indicated a 1.05 M solution of (4-methoxyphenyl)magnesium chloride in THF.

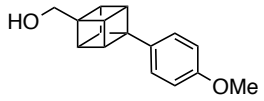
Zinc chloride was (1.50 g, 11.0 mmol) was added to a two-neck flask and heated with a heat gun for 5 minutes under vaccum. After cooling to room temperature, THF (11 mL) was added under nitrogen and stirred for 5 minutes. A 1.05 M solution of (4-methoxyphenyl)magnesium chloride in THF (10.5 mL, 11.0 mmol) was added dropwise to provide a dense-grey solution that was used without further titration (**A-23a**, *ca.* 0.50 M).



A-23

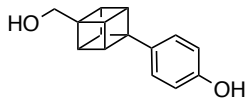
Methyl 4-(4-methoxyphenyl)cubane-1-carboxylate (**A-23**). **A-22** (0.0700 g, 0.199 mmol), dichloro(dimethoxyethane)nickel (0.004 g, 0.019 mmol), 4,4'-di-tert-butyl-2,2'-dipyridyl (0.011 g, 0.039 mmol) were added to the reaction vial. The reaction vial was pulled under vacuum and filled with nitrogen; this was repeated three times. Anhydrous DMF (2 mL) was added and the reaction mixture was stirred under nitrogen for 10 minutes. A *ca.* 0.50 M solution of **A-23a** in anhydrous THF (1.40 mL, 0.697 mmol) was added in one portion and the reaction mixture was stirred under nitrogen for 2 h. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (5 mL) and extracted with diethyl ether (5 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (0-5 % ethyl acetate in hexane) to provide **A-23** (0.0211 g, 0.0786 mmol, 39%) as a white solid: **Rf** (10% ethyl acetate in hexane)

0.41; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.12 (d, *J* = 6.9 Hz, 2H), 6.91-6.89 (m, 2H), 4.22 (d, *J* = 0.3 Hz, 3H), 4.11 (d, *J* = 0.4 Hz, 3H), 3.80 (s, 3H), 3.74 (s, 3H).



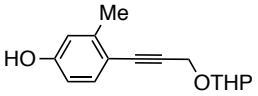
A-24

4-(4-Methoxyphenyl)cuban-1-yl)methanol (**A-24**). A 4M solution of lithium aluminum hydride in diethyl ether (0.18 mL, 0.73 mmol) was added dropwise to a solution of **A-23** (0.320 g, 0.728 mmol, 61% purity) in anhydrous diethyl ether (3.6 mL) and anhydrous methylene chloride (3.6 mL) at -10 ^oC under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was quenched with water (5 mL) followed by 15% aqueous solution of sodium hydroxide (5 mL) and stirred for 15 minutes. The solid material was filtered through a pad of Celite and the resulting filtrate was extracted with methylene chloride (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20-30% ethyl acetate in hexane) to provide **A-24** (0.109 g, 0.454 mmol, 62%) as a white solid: **Rf** (20% ethyl acetate in hexane) 0.17; **Mp** (CH_2Cl_2) 97-99 ^oC; **IR** 3191.8, 2973.7, 2834.9, 1609.0, 1511.6 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.14 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 4.02 (t, *J* = 4.9 Hz, 3H), 3.86 (t, *J* = 4.9 Hz, 3H), 3.84 (s, 2H), 3.81 (s, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 157.8, 135.4, 125.8, 113.9, 63.9, 60.5, 59.5, 55.3, 48.1, 43.2; **LC-MS** (ES) *m/z* calcd for $C_{16}H_{17}O_2$ $[M+H]^+$ 241.1223, found 241.1221.



A-25

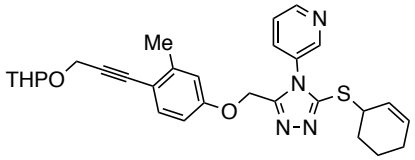
4-(4-(Hydroxymethyl)cuban-1-yl)phenol (**A-25**). A solution of **A-24** (0.0993 g, 0.517 mmol) in anhydrous toluene (1 mL) and 1-dodecanethiol (1.00 mL, 4.17 mmol) was added dropwise to a pre-mixed mixture of 1-dodecanethiol (1.00 mL, 4.17 mmol) and anhydrous aluminum chloride (0.302 g, 2.27 mmol) at 0 ^{o}C. The reaction mixture was slowly warmed to room temperature and stirred for 14 h. The reaction mixture was poured into a 0.2 M aqueous solution of hydrogen chloride (10 mL) and extracted with methylene chloride (15 mL, three times). The combined organic solutions were washed with brine (10 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (0-60% ethyl acetate in hexane). The desired product was not observed or isolated. This experiment was not pursued further.



A-28

3-methyl-4-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-yn-1-yl)phenol (**A-28**). 4-Iodo-3-methylphenol (1.00 g, 5.37 mmol), 2-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran (1.79 g, 12.8 mmol), copper (I) Iodide (0.102 g, 0.534 mmol), bis(triphenylphosphine)palladium(II) chloride (0.375 g, 0.534 mmol) was added to a reaction vial. The reaction vial was degassed under vacuum and refilled with argon; this process was conducted three times. Degassed acetonitrile (18 mL) and triethylamine (3.00 mL, 21.4 mmol) was added to reaction vial. The reaction vial was purged with argon for 5 minutes and stirred at room temperature for 38 h. The reaction

mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (15% ethyl acetate in hexane) to **A-28** (1.09 g) as a brown residue: **Rf** (25% ethyl acetate in hexane) 0.53; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.28 (s, 1H), 6.66 (d, *J* = 2.5 Hz, 1 H), 6.59 (dd, *J* = 8.3, 2.6 Hz, 1 H), 4.95 (t, *J* = 3.4 Hz, 1 H), 4.52 (t, *J* = 3.3 Hz, 2 H), 3.91 (ddd, *J* = 11.4, 8.7, 2.9 Hz, 1 H), 3.60-3.55 (m, 1 H), 2.36 (s, 3 H), 1.88-1.79 (m, 2 H), 1.78-1.74 (m, 1 H), 1.70-1.62 (m, 2 H), 1.61-1.51 (m, 4 H).

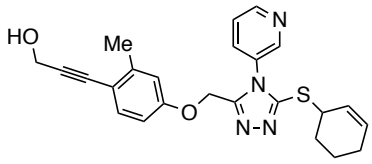


A-29

3-(3-(cyclohex-2-en-1-ylthio)-5-((3-methyl-4-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-yn-1-yl)phenoxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (**A-29**). Methanesulfonyl chloride (0.09 mL, 1.1 mmol) was added dropwise to mixture of **1-15** (0.300 g, 1.04 mmol), N,N-diisopropylethylamine (0.23 mL, 1.3 mmol) in anhydrous methylene chloride (10 mL) under an atmosphere of nitrogen at 0 C and stirred for 2 h. The reaction mixture was diluted with methylene chloride (15 mL) and sequentially washed with a saturated solution of aqueous sodium bicarbonate (10 mL) and ammonium chloride (10 mL). The isolated organic solution was dried (MgSO4), filtered, and concentrated under reduced pressure to provide crude methanesulfonate. This material was carried forward without further purification.

A-28 (0.282 g, 1.14 mmol) was added to a mixture of crude methanesulfonate, cesium carbonate (0.169 g, 1.14 mmol) in anhydrous DMF (5 mL) under an atmosphere of nitrogen and stirred at room temperature for 15 h. The reaction mixture was diluted with water (15 mL) and

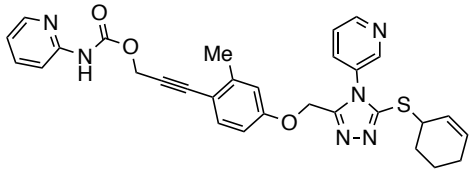
extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (70% EtOAc in hexane) to provide **A-29** (0.261g) as a yellow residue. This material was carried forward without additional purification: **Rf** (50% ethyl acetate in hexane) 0.71.



A-30

3-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2-methylphenyl)prop-2-yn-1-ol (**A-30**). *p*-Toluenesulfonic acid monohydrate (0.347 g, 2.02 mmol) was added to a solution of **A-29** (0.261 g, 0.504 mmol) in methanol and stirred under an atmosphere of nitrogen at room temperature for 48 h. The reaction was diluted with a saturated solution of aqueous sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica to provide **A-30** (0.043 g, 0.0994 mmol, 20%) as a white solid: **Rf** (ethyl acetate) 0.60; **Mp** (methylene chloride/methanol) 142-144 ^{o}C; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.61 (dd, *J* = 2.5, 0.5 Hz, 1 H), 7.67 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1 H), 7.46 (ddd, *J* = 8.1, 4.8, 0.7 Hz, 1 H), 7.26 (d, *J* = 5.9 Hz, 1H), 6.67 (d, *J* = 2.6 Hz, 1 H), 6.62 (dd, *J* = 8.4, 2.5 Hz, 1 H), 5.87 (dtd, *J* = 7.9, 3.9, 1.9 Hz, 1 H), 5.76-5.73 (m, 1 H), 5.09-5.03 (m, 2 H), 4.57 (dd, *J* = 4.1, 1.7 Hz, 1 H), 4.51 (d, *J* = 4.9 Hz, 2 H), 2.35 (s, 3 H), 2.09 (dddd, *J* = 14.0, 10.4, 4.7, 3.6 Hz, 2 H), 2.04-1.99 (m, 3 H), 1.74-1.65 (m, 2 H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 157.1, 153.5, 151.4, 151.1, 148.0, 142.3, 134.8, 133.5,

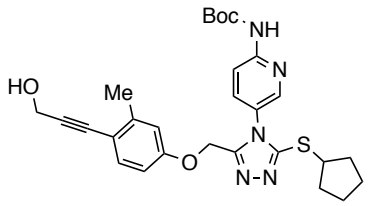
132.4, 130.0, 125.4, 124.0, 116.0, 115.7, 111.9, 90.3, 84.0, 59.8, 51.7, 44.1, 29.2, 24.8, 20.9, 19.1.



A-31

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2-methylphenyl)prop-2-yn-1-yl pyridin-2-ylcarbamate (**A-31**). In a high pressure vial was charged with a mixture of 4-nitrophenyl chloroformate (0.112 g, 0.555 mmol), 2-aminopyridine (0.0653 g, 0.694 mmol), 4-dimethylaminopyridine (0.0141 g, 0.116 mmol) in anhydrous tetrahydrofuran (4.5 mL) and stirred at room temperature under an atmosphere of argon for 0.5 h. **A-30** (0.0400 g, 0.0925 mmol) was added and the reaction vial was sealed under an atmosphere of argon and heated to 70 ^{o}C for 1.5 h. The reaction mixture was cooled to room temperature and quenched with a saturated aqueous solution of sodium bicarbonate (10 mL). The isolated aqueous solution was extracted with ethyl acetate (15 mL, three times) and the combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (85-100% ethyl acetate in hexane) to provide **A-31** (0.0317 g, 0.0574 mmol, 62%) as a white solid: **Rf** (ethyl acetate) 0.27; **Mp** (methylene chloride/methanol) 153-155 ^{o}C; **IR** 2921.3, 1729.8, 1684.1, 1543.4 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 10.34 (s, 1 H), 8.71 (dd, *J* = 4.8, 1.5 Hz, 1 H), 8.69 (d, *J* = 2.5 Hz, 1 H), 8.27 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1 H), 7.99 (ddd, *J* = 8.2, 2.5, 1.5 Hz, 1 H), 7.82 (dt, *J* = 8.4, 0.9 Hz, 1 H), 7.77 (td, *J* = 7.8, 1.8 Hz, 1 H), 7.61 (ddd, *J* = 8.1, 4.8, 0.6 Hz, 1 H), 7.29 (d, *J* = 8.6 Hz, 1 H), 7.06 (ddd, *J* = 7.1, 4.9, 1.1 Hz, 1 H), 6.78 (d, *J* = 2.4 Hz, 1 H), 6.69 (dd, *J* = 8.6, 2.6 Hz, 1 H), 5.85 (dtd, *J* = 9.8, 3.8, 1.5 Hz, 1

H), 5.68-5.64 (m, 1 H), 5.16 (s, 2 H), 5.03 (s, 2 H), 4.28 (dt, J = 4.0, 1.8 Hz, 1 H), 3.32 (s, 1 H), 2.29 (s, 3 H), 1.99-1.90 (m, 3 H), 1.86-1.81 (m, 1 H), 1.64-1.55 (m, 2 H); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 158.0, 153.3, 152.4, 152.1, 151.9, 151.4, 148.4, 148.3, 142.3, 138.6, 135.8, 133.7, 132.4, 130.4, 125.9, 124.8, 119.3, 116.4, 114.9, 112.9, 112.8, 87.6, 84.8, 60.4, 53.4, 44.4, 29.0, 24.7, 20.8, 19.1; **LC-MS** (ES) *m/z* calcd for $C_{30}H_{29}O_3N_6S$ $[M+H]^+$ 553.2016, found 553.2017.

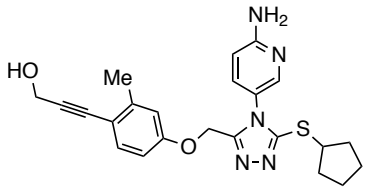


A-32

***Tert*-butyl (5-(3-(cyclopentylthio)-5-((4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenoxy)methyl)-4*H*-1,2,4-triazol-4-yl)pyridin-2-yl)carbamate** (**A-32**). Methanesulfonyl chloride (0.013 mL, 0.17 mmol) was added dropwise to mixture of tert-butyl **1-62** (0.0603 g, 0.154 mmol), N,N-diisopropylethylamine (0.04 mL, 0.23 mmol) in anhydrous methylene chloride (1.5 mL) under an atmosphere of nitrogen at 0 ^oC for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

4-(3-Hydroxyprop-1-yn-1-yl)-3-methylphenol (0.0275 g, 0.169 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.100 g, 0.308 mmol) in anhydrous DMF (0.75 mL) under an atmosphere of nitrogen and stirred at room temperature for 19 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The

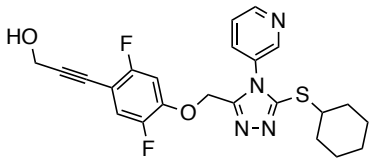
combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **A-33** (0.0650 g, 0.121 mmol, 79%) as a white solid: **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.19 (d, *J* = 2.4 Hz, 1 H), 8.12 (d, *J* = 8.9 Hz, 1 H), 7.82 (s, 1 H), 7.61 (dd, *J* = 8.9, 2.6 Hz, 1 H), 7.28-7.26 (m, 2 H), 6.71 (d, *J* = 2.6 Hz, 1 H), 6.66 (dd, *J* = 8.5, 2.7 Hz, 1 H), 5.04 (s, 2 H), 4.51 (s, 2 H), 4.07-4.01 (m, 1 H), 2.36 (s, 3 H), 2.23-2.18 (m, 2 H), 1.72 (dd, *J* = 4.5, 3.5 Hz, 2 H), 1.65-1.61 (m, 5 H), 1.53 (s, 9 H); **LC-MS** (ES) *m/z* calcd for $C_{28}H_{34}O_4N_5S$ $[M+H]^+$ 536.2326, found 536.2325.



A-33

3-(4-((4-(6-Aminopyridin-3-yl)-5-(cyclopentylthio)-4H-1,2,4-triazol-3-yl)methoxy)-2-methylphenyl)prop-2-yn-1-ol (**A-33**). Potassium carbonate (0.0497 g, 0.359 mmol) was added to a high pressure reaction vial containing a solution of **A-32** (0.0770 g, 0.143 mmol) in methanol (1.4 mL). The reaction vial was sealed and stirred at 100 ^oC for 18 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (ethyl acetate, dry loaded) to provide **A-33** (0.0425 g, 0.0976 mmol, 68%) as a white solid: **Rf** (ethyl acetate) 0.34; **Mp** (methanol) 173-175 ^oC; **IR** 3172.7, 2944.1, 1641.64, 1602.3, 1496.9 cm^{-1}; **^{1}H NMR** (1:1 $CDCl_3$:MeOD, 500 MHz) δ 7.58-7.57 (m, 1 H), 7.04 (dd, *J* = 8.9, 2.7 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.42 (d, *J* = 2.4 Hz, 1H), 6.35 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.31 (dd, *J* = 8.9, 0.5 Hz, 1H), 4.71 (s, 2H), 4.11 (s, 2H), 3.64-3.59 (m, 1H), 2.05 (s, 3H), 1.88-1.81 (m, 2H),

1.44-1.41 (m, 2H), 1.37-1.27 (m, 5H); **^{13}C NMR** (1:1 $CDCl_3$:MeOD, 126 MHz) δ 160.2, 157.3, 155.0, 152.1, 145.8, 142.1, 136.5, 133.2, 118.9, 116.3, 115.6, 111.9, 109.0, 90.5, 82.9, 59.5, 50.4, 45.8, 33.6, 24.4, 20.3; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{26}O_2N_5S$ $[M+H]^+$ 436.1802, found 436.1802.

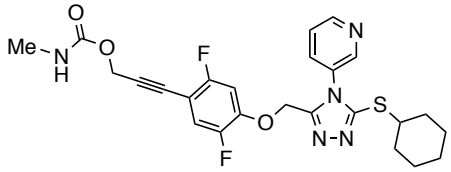


A-34

3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-ol (**A-34**). Methanesulfonyl chloride (0.06 mL, 0.83 mmol) was added dropwise to mixture of **1-80** (0.200 g, 0.689 mmol) and N,N-diisopropylethylamine (0.179 mL, 1.03 mmol) in anhydrous methylene chloride (6.9 mL) under an atmosphere of nitrogen at 0 ^oC and stirred for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

1-116 (0.152 g, 0.827 mmol) was added to a mixture of crude mesylate and cesium carbonate (0.449 g, 1.38 mmol) in anhydrous DMF (3.8 mL) under an atmosphere of nitrogen and stirred at room temperature for 15 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (15 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded) to provide **A-34** (0.254 g, 0.556 mmol, 81%) as a white solid: **Rf** (ethyl acetate) 0.50; **Mp** (methylene chloride) 173-175 ^oC; **IR** 3196.0, 3057.7,

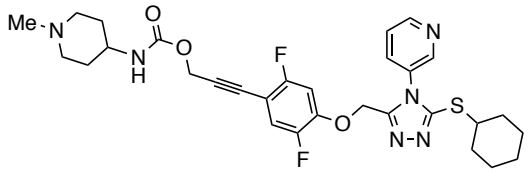
2932.9, 2853.8, 1629.5, 1483.7 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.62 (d, *J* = 2.5 Hz, 1H), 7.73 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1H), 7.50 (ddd, *J* = 8.1, 4.8, 0.6 Hz, 1H), 7.07 (dd, *J* = 10.8, 6.5 Hz, 1H), 6.87 (dd, *J* = 9.8, 7.0 Hz, 1H), 5.10 (s, 2H), 4.49 (d, *J* = 6.2 Hz, 2H), 3.86-3.81 (m, 1H), 2.14-2.11 (m, 2H), 1.95-1.92 (m, 1H), 1.74-1.71 (m, 2H), 1.63-1.58 (m, 1H), 1.51-1.38 (m, 4H), 1.31-1.23 (m, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 160.18, 160.16, 157.70, 157.68, 153.9, 151.3, 150.3, 149.22, 149.19, 147.9, 146.80, 146.76, 146.21, 146.11, 146.08, 145.98, 135.2, 129.8, 124.2, 119.89, 119.86, 119.68, 119.65, 104.44, 104.35, 104.25, 104.17, 103.6, 103.3, 92.97, 92.93, 61.1, 51.3, 46.9, 33.4, 25.7, 25.4; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -111.5 (d, *J* = 16 Hz), -138.5 (d, *J* = 15.5 Hz); **LC-MS** (ES) *m/z* calcd for $C_{23}H_{23}O_2N_4F_2S$ $[M+H]^+$ 457.1504, found 457.1504.



A-35a

3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-yl methylcarbamate (**A-35a**). Carbonyldiimidazole (0.0266 g, 0.164 mmol) was added to a solution of **A-34** (0.0500 g, 0.101 mmol) in anhydrous methylene chloride (1 mL) and stirred for 3 h under nitrogen. Triethylamine (0.05 mL, 0.33 mmol) and methylamine hydrochloride (0.0222 g, 0.329 mmol) were sequentially added and stirred for an additional 13 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (8% methanol in methylene chloride, dry loaded) to provide **A-35a** (0.0546 g, 0.106 mmol, 97%) as a white solid: **Rf** (20% methanol in ethyl acetate) 0.76; **Mp** (methylene chloride) ^oC; **IR** 3347.8, 2933.4, 2853.0, 1724.8, 1629.7, 1485.2 cm^{-1}; **^{1}H NMR**

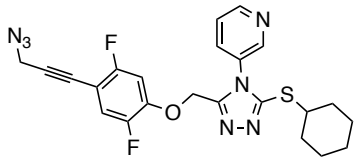
($CDCl_3$, 500 MHz) δ 8.78 (dd, *J* = 4.8, 1.3 Hz, 1H), 8.62 (d, *J* = 2.3 Hz, 1H), 7.72 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.51-7.48 (m, 1H), 7.10 (dd, *J* = 10.7, 6.5 Hz, 1H), 6.87 (dd, *J* = 9.8, 7.0 Hz, 1H), 5.10 (s, 2H), 4.90 (s, 2H), 4.72 (s, 1H), 3.86-3.81 (m, 1H), 2.83 (d, *J* = 4.9 Hz, 3H), 2.13-2.10 (m, 2H), 1.74-1.71 (m, 2H), 1.59 (d, *J* = 4.7 Hz, 2H), 1.49-1.40 (m, 4H), 1.28-1.25 (m, 2H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 160.45, 160.43, 157.96, 157.94, 156.1, 153.9, 151.4, 150.2, 149.24, 149.20, 148.0, 146.81, 146.77, 146.51, 146.42, 146.39, 146.29, 135.0, 129.8, 124.2, 120.12, 120.09, 119.90, 119.87, 104.0, 103.71, 103.69, 103.43, 103.42, 88.85, 88.81, 78.12, 78.11, 61.2, 53.0, 46.9, 33.4, 31.6, 27.7, 25.7, 25.4, 22.7, 14.1 ; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -111.1 (d, *J* = 15.5 Hz), -138.6 (d, *J* = 15 Hz); **LC-MS** (ES) *m/z* calcd for $C_{25}H_{26}O_3N_5F_2S$ $[M+H]^+$ 514.1719, found 514.1719.



A-35b

3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**A-35b**). Carbonyldiimidazole (0.0266 g, 0.164 mmol) was added to a solution of **A-34** (0.0500 g, 0.101 mmol) in anhydrous methylene chloride (1 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.04 mL, 0.33 mmol) was added and stirred for an additional 13 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water, dry loaded) to provide **A-35b** (0.0596 g, 0.0999 mmol, 91%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.15; **Mp** (methylene chloride) 67-69 ^{o}C; **IR** 2934.4, 2854.2, 2786.4, 1718.1, 1629.1, 1484.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$,

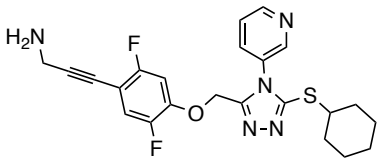
500 MHz) δ 8.78 (dd, *J* = 4.7, 1.1 Hz, 1H), 8.62 (d, *J* = 2.2 Hz, 1H), 7.73-7.71 (m, 1H), 7.49 (dd, *J* = 8.1, 4.9 Hz, 1H), 7.11-7.08 (m, 1H), 6.89-6.86 (m, 1H), 5.10 (s, 2H), 4.88 (s, 2H), 4.70 (s, 1H), 3.87-3.81 (m, 1H), 3.53 (s, 1H), 2.74 (s, 2H), 2.27 (s, 3H), 2.14-2.05 (m, 4H), 1.97-1.93 (m, 2H), 1.74-1.69 (m, 2H), 1.63-1.58 (m, 1H), 1.51-1.38 (m, 6H), 1.30-1.24 (m, 3H).; **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 160.5, 158.0, 154.6, 153.9, 151.4, 150.2, 149.2, 148.0, 146.8, 146.53, 146.43, 146.40, 146.30, 135.0, 129.8, 124.2, 120.08, 120.06, 119.87, 119.84, 103.81, 103.71, 103.4, 88.75, 88.71, 78.21, 78.19, 61.2, 54.3, 52.9, 46.9, 46.2, 34.7, 33.4, 32.4, 31.6, 25.7, 25.44, 25.27, 22.6, 14.1; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -111.1 (d, J = 15 Hz), -138.6 (d, J = 15 Hz); **LC-MS** (ES) *m/z* calcd for $C_{30}H_{35}O_3N_6F_2S$ $[M+H]^+$ 597.2454, found 597.2450.



A-36

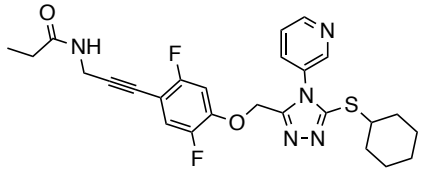
3-(3-((4-(3-Azidoprop-1-yn-1-yl)-2,5-difluorophenoxy)methyl)-5-(cyclohexylthio)-4H-1,2,4-triazol-4-yl)pyridine (**A-36**). Diphenylphosphoryl azide (0.06 mL, 0.26 mmol) and 1,8-diazabicyclo(5.4.0)undec-7-ene (0.039 mL, 0.26 mmol) was sequentially to a solution of **A-34** (0.100 g, 0.219 mmol) in anhydrous THF (1.5 mL) at 0 ^oC under an atmosphere of nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction mixture was diluted with a saturated aqueous solution of ammonium chloride (5 mL) and the resulting solution was extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (50% ethyl acetate in hexane) to provide **A-36** (0.0691 g, 0.143 mmol, 65%) as a white solid: **Rf** (ethyl acetate) 0.56; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78

(dd, *J* = 4.8, 1.4 Hz, 1H), 8.62 (d, *J* = 2.4 Hz, 1H), 7.72 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1H), 7.51-7.49 (m, 1H), 7.11 (dd, *J* = 10.7, 6.5 Hz, 1H), 6.91 (dd, *J* = 9.8, 7.0 Hz, 1H), 5.11 (s, 2H), 4.14 (s, 2H), 3.86-3.82 (m, 1H), 2.14-2.11 (m, 2H), 1.74-1.71 (m, 2H), 1.63-1.59 (m, 1H), 1.52-1.38 (m, 4H), 1.28-1.25 (m, 1H); **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -111.9 (d, J = 15 Hz), -138.4 (d, J = 14.5 Hz); **LC-MS** (ES) *m/z* calcd for $C_{23}H_{22}ON_7F_2S$ $[M+H]^+$ 482.1569, found 482.1565.



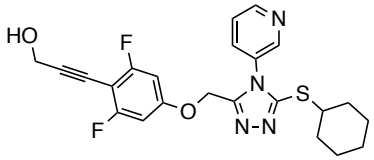
A-37

3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-amine (**A-37**). Triphenylphosphine (0.0563 g, 0.215 mmol) was added to a solution of **A-36** (0.0691 g, 0.143 mmol) in THF (1.4 mL) and stirred for 3 h. Water (0.13 mL, 7.2 mmol) was added and the reaction mixture was stirred for an additional 17 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (10% methanol in methylene chloride, dry loaded) to provide **A-37** (0.0562 g, 0.123 mmol, 86%) as a white solid: **Rf** (10% methanol in ethyl acetate) 0.11; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.62 (d, *J* = 2.3 Hz, 1H), 7.73 (ddd, *J* = 8.1, 2.4, 1.6 Hz, 1H), 7.51-7.48 (m, 1H), 7.05 (dd, *J* = 10.9, 6.6 Hz, 1H), 6.86 (dd, *J* = 9.8, 7.0 Hz, 1H), 5.09 (s, 2H), 3.86-3.82 (m, 1H), 3.64 (s, 2H), 2.13-2.10 (m, 2H), 1.74-1.71 (m, 2H), 1.62-1.59 (m, 1H), 1.51-1.38 (m, 7H), 1.30-1.23 (m, 2H); **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -112.2 (d, J = 14.5 Hz), -138.7 (d, J = 15 Hz).



A-38

N-(3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)prop-2-yn-1-yl)propionamide (**A-38**). A 50% solution of propylphosphonic anhydride (0.08 mL, 0.14 mmol) was added dropwise to a mixture of **A-37** (0.0507 g, 0.111 mmol), propionic acid (0.01 mL, 0.13 mmol), triethylamine (0.03 mL, 0.22 mL) in anhydrous DMF (1.1 mL) at room temperature under an atmosphere of nitrogen and stirred for 15 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (ethyl acetate) to provide **A-38** (0.0401 g, 0.0784 mmol, 70%) as a white solid: **Rf** (ethyl acetate) 0.13; **Mp** (methylene chloride) 149-151 ^{o}C; **IR** 3280.9, 3055.3, 2933.3, 2854.8, 1655.1, 1629.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78-8.77 (m, 1H), 8.62 (d, *J* = 2.1 Hz, 1H), 7.73-7.72 (m, 1H), 7.50 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.06 (dd, *J* = 10.7, 6.5 Hz, 1H), 6.89-6.86 (m, 1H), 5.70 (s, 1H), 5.10 (s, 2H), 4.28 (d, *J* = 5.2 Hz, 2H), 3.86-3.81 (m, 1H), 2.25 (q, *J* = 7.5 Hz, 2H), 2.13-2.10 (m, 2H), 1.74-1.71 (m, 2H), 1.61-1.59 (m, 2H), 1.49-1.37 (m, 4H), 1.30-1.24 (m, 1H), 1.18 (t, *J* = 7.6 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 173.3, 160.09, 160.08, 158.11, 158.09, 153.8, 151.3, 150.3, 149.09, 149.06, 148.0, 147.14, 147.12, 146.24, 146.16, 146.14, 146.06, 135.0, 129.8, 124.1, 119.96, 119.94, 119.79, 119.77, 104.45, 104.38, 104.30, 104.24, 103.8, 103.6, 90.31, 90.28, 75.3, 61.3, 47.0, 33.4, 29.9, 29.5, 25.7, 25.4, 9.6; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -111.7 (d, *J* = 15 Hz), -138.5 (d, *J* = 15 Hz; **LC-MS** (ES) *m/z* calcd for $C_{26}H_{28}O_2N_5F_2S$ $[M+H]^+$ 512.1854, found 512.1916.

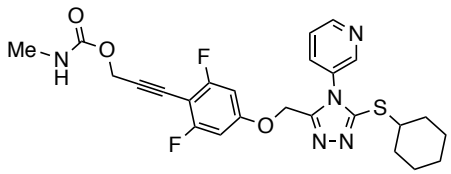


A-39

3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-ol (**A-39**). Methanesulfonyl chloride (0.05 mL, 0.62 mmol) was added dropwise to mixture of **1-80** (0.150 g, 0.517 mmol), N,N-diisopropylethylamine (0.14 mL, 0.78 mmol) in anhydrous methylene chloride (5.2 mL) under an atmosphere of nitrogen at 0 ^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

3,5-Difluoro-4-(3-hydroxyprop-1-yn-1-yl)phenol (0.114 g, 0.619 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.337 g, 1.03 mmol) in anhydrous DMF (1.6 mL) under an atmosphere of nitrogen and stirred at room temperature for 21 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded) to provide **A-39** (0.175 g, 0.383 mmol, 74%) as a white solid: **Rf** (ethyl acetate) 0.53; **Mp** (methylene chloride) 187-189 ^{o}C; **IR** 3193.9, 2932.5, 2853.0, 1634.3, 1578.3, 1501.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.79 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.60-8.60 (m, 1H), 7.66 (ddd, *J* = 8.1, 2.5, 1.6 Hz, 1H), 7.51 (ddd, *J* = 8.1, 4.8, 0.7 Hz, 1H), 6.51-6.46 (m, 2H), 5.05 (s, 2H), 4.52 (d, *J* = 6.2 Hz, 2H), 3.88-3.80 (m, 1H), 2.14-2.10 (m, 2H), 2.01 (t, *J* = 6.3 Hz, 1H), 1.74-1.69 (m, 2H), 1.63-1.57 (m, 2H), 1.52-1.36 (m, 4H), 1.30-1.23 (m, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.7,

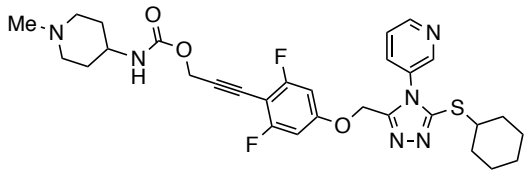
162.6, 158.3, 153.8, 151.3, 150.3, 147.9, 134.8, 129.9, 124.2, 98.94, 98.89, 98.76, 98.71, 96.61, 96.58, 71.9, 60.4, 51.5, 47.0, 33.4, 25.7, 25.4; **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -105.5; **LC-MS** (ES) *m/z* calcd for $C_{23}H_{23}O_2N_4F_2S$ $[M+H]^+$ 457.1504, found 457.1502.



A-40a

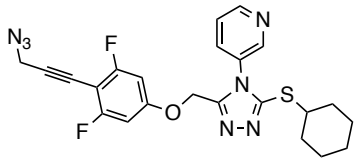
3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl methylcarbamate (**A-40a**). Carbonyldiimidazole (0.0266 g, 0.164 mmol) was added to a solution of **A-39** (0.0500 g, 0.101 mmol) in anhydrous methylene chloride (1 mL) and stirred for 3 h under nitrogen. Triethylamine (0.05 mL, 0.33 mmol) and methylamine hydrochloride (0.0222 g, 0.329 mmol) were sequentially added and stirred for an additional 19 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (8% methanol in methylene chloride, dry loaded) to provide **A-40a** (0.0533 g, 0.104 mmol, 95%) as a white solid: **Rf** (20% methanol in ethyl acetate) 0.76; **Mp** (methylene chloride) 61-63 ^{o}C; **IR** 3325.8, 2933.2, 2854.2, 1720.4, 1635.1, 1575.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.78 (dd, *J* = 4.8, 1.3 Hz, 1H), 8.59 (d, *J* = 2.4 Hz, 1H), 7.65 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.50 (dd, *J* = 8.0, 4.8 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 2H), 5.05 (s, 2H), 4.93 (s, 2H), 4.74 (s, 1H), 3.86-3.81 (m, 1H), 2.82 (d, *J* = 4.9 Hz, 3H), 2.13-2.10 (m, 2H), 1.74-1.70 (m, 2H), 1.63-1.58 (m, 1H), 1.49-1.38 (m, 4H), 1.30-1.24 (m, 2H).; **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 165.17, 165.08, 162.65, 162.56, 158.71, 158.57, 158.44, 156.1, 153.8, 151.4, 150.3, 147.9, 134.7, 129.8, 124.2, 100.1, 99.00, 98.99, 98.93, 98.79, 98.73, 98.72, 95.34, 95.14, 94.9, 92.54, 92.51,

92.49, 72.8, 60.4, 53.2, 46.9, 33.4, 31.6, 27.7, 25.7, 25.4, 22.7, 14.1; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.0; **LC-MS** (ES) *m/z* calcd for $C_{25}H_{26}O_3N_5F_2S$ $[M+H]^+$ 514.1719, found 514.1716.



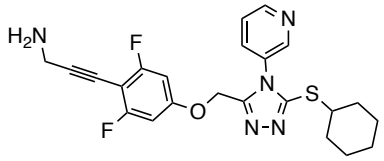
A-40b

3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl (1-methylpiperidin-4-yl)carbamate (**A-40b**). Carbonyldiimidazole (0.0159 g, 0.0.096 mmol) was added to a solution of **A-39** (0.0300 g, 0.0657 mmol) in anhydrous methylene chloride (0.66 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.025 mL, 0.19 mmol) was added and stirred for an additional 4 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water, dry loaded) to provide **A-40b** (0.0359 g, 0.0602 mmol, 92%) as an off-white solid: **Rf** (50% methanol in ethyl acetate) 0.14; **Mp** (methylene chloride) 91-93 ^{o}C; **IR** 2933.3, 2853.4, 2787.5, 1717.1, 1634.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 8.79-8.78 (m, 1H), 8.59 (d, *J* = 2.2 Hz, 1H), 7.66-7.64 (m, 1H), 7.50 (dd, *J* = 8.0, 4.9 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 2H), 5.05 (s, 2H), 4.92 (s, 2H), 4.72 (s, 1H), 3.84 (s, 1H), 3.53 (s, 1H), 2.75 (s, 2H), 2.27 (s, 3H), 2.13-2.08 (m, 4H), 1.95 (d, *J* = 11.9 Hz, 2H), 1.74-1.66 (m, 5H), 1.62-1.59 (m, 2H), 1.51-1.39 (m, 6H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 164.90, 164.83, 162.88, 162.82, 158.70, 158.63, 158.59, 154.7, 153.7, 151.43, 151.37, 150.28, 150.25, 147.9, 134.7, 129.9, 124.20, 124.14, 100.0, 98.99, 98.95, 98.85, 98.81, 98.77, 92.47, 92.45, 72.9, 60.4, 54.27, 54.25, 54.24, 53.1, 47.0, 46.1, 33.4, 32.39, 32.33, 31.6, 25.7, 25.4; **^{19}F NMR** ($CDCl_3$, 565 MHz) δ -105.1; **LC-MS** (ES) *m/z* calcd for $C_{30}H_{35}O_3N_6F_2S$ $[M+H]^+$ 597.2454, found 597.2451.



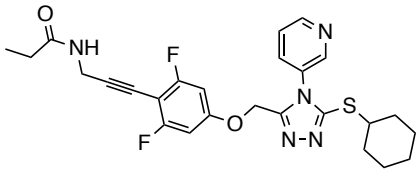
A-41

3-(3-((4-(3-Azidoprop-1-yn-1-yl)-3,5-difluorophenoxy)methyl)-5-(cyclohexylthio)-4H-1,2,4-triazol-4-yl)pyridine (**A-41**). Diphenylphosphoryl azide (0.07 mL, 0.33 mmol) and 1,8-Diazabicyclo(5.4.0)undec-7-ene (0.05 mL, 0.33 mmol) was sequentially to a solution of **A-39** (0.125 g, 0.274 mmol) in anhydrous THF (1.8 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was diluted with a saturated aqueous solution of ammonium chloride (5 mL) and the resulting solution was extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (60% ethyl acetate in hexane) to provide **A-41** (0.0924 g, 0.191 mmol, 70%) as a white solid: **Rf** (ethyl acetate) 0.64; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.79 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.60 (d, *J* = 2.5 Hz, 1H), 7.65 (ddd, *J* = 8.1, 2.4, 1.6 Hz, 1H), 7.50 (dd, *J* = 8.1, 4.8 Hz, 1H), 6.52 (d, *J* = 8.4 Hz, 2H), 5.06 (s, 2H), 4.17 (s, 2H), 3.87-3.82 (m, 1H), 2.14-2.11 (m, 2H), 1.74-1.70 (m, 2H), 1.63-1.59 (m, 1H), 1.52-1.39 (m, 4H); **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.0.



A-42

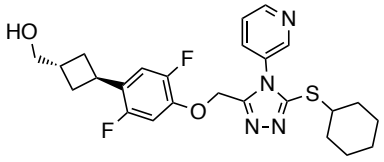
3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-amine (**A-42**). Triphenylphosphine (0.0690 g, 3.83 mmol) was added to a solution of **A-41** (0.0924 g, 0.191 mmol) in THF (1.9 mL) and stirred for 2 h. Water (0.173 mL, 9.57 mmol) was added and the reaction mixture was stirred for an additional 20 h. The reaction mixture was concentrated under reduced pressure and purified by chromatography on silica (9% methanol in methylene chloride, dry loaded) to provide **A-42** (0.0585 g, 0.128 mmol, 67%) as an off-white solid: **Rf** (10% methanol in ethyl acetate) 0.15; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.78 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.60 (d, *J* = 2.4 Hz, 1H), 7.66 (ddd, *J* = 8.1, 2.4, 1.6 Hz, 1H), 7.52-7.48 (m, 1H), 6.48 (d, *J* = 8.6 Hz, 2H), 5.04 (s, 2H), 3.87-3.81 (m, 1H), 3.68 (s, 2H), 2.14-2.10 (m, 2H), 1.74-1.68 (m, 3H), 1.64-1.58 (m, 2H), 1.49-1.39 (m, 4H), 1.28-1.24 (m, 2H); **^{19}F NMR** ($CDCl_3$, 376 MHz) δ -106.0.



A-43

N-(3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,6-difluorophenyl)prop-2-yn-1-yl)propionamide (**A-43**). Hydroxybenzotriazole (0.0226 g, 0.167 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.0320 g, 0.167 mmol) was sequentially added to a mixture of **A-42** (0.0585 g, 0.128 mmol), propionic acid (0.01

mL, 0.15 mmol), and N-diisopropylethylamine (0.04 mL, 0.26 mmol) in anhydrous DMF (1.3 mL) at room temperature under an atmosphere of nitrogen and stirred for 18 h. The reaction was diluted with methylene chloride (10 mL) and washed with a saturated aqueous solution of ammonium chloride (5 mL, two times). The resulting organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane) to provide **A-43** (0.0350 g, 0.0684 mmol, 53%) as a white solid: **Rf** (ethyl acetate) 0.26; **Mp** (methylene chloride) 185-187 ^{o}C; **IR** 3284.2, 3058.9, 2932.7, 2854.9, 1655.3, 1634.7, 1575.8 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.79-8.78 (m, 1H), 8.59 (t, *J* = 0.4 Hz, 1H), 7.65 (dd, *J* = 6.4, 1.5 Hz, 1H), 7.50 (dd, *J* = 8.0, 4.8 Hz, 1H), 6.49 (d, *J* = 8.5 Hz, 2H), 5.69 (s, 1H), 5.05 (s, 2H), 4.32 (d, *J* = 5.1 Hz, 2H), 3.86-3.82 (m, 1H), 2.25 (q, *J* = 7.6 Hz, 2H), 2.13-2.10 (m, 2H), 1.74-1.71 (m, 2H), 1.61-1.59 (m, 2H), 1.49-1.38 (m, 4H), 1.30-1.24 (m, 1H), 1.18 (t, *J* = 7.6 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 173.2, 164.82, 164.75, 162.81, 162.74, 158.3, 153.7, 151.4, 150.3, 147.9, 134.7, 129.9, 124.1, 98.99, 98.94, 98.81, 98.76, 95.52, 95.36, 93.97, 93.95, 70.0, 60.4, 47.0, 33.4, 30.1, 29.5, 25.7, 25.4, 9.6; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -105.6; **LC-MS** (ES) *m/z* calcd for $C_{26}H_{28}O_2N_5F_2S$ $[M+H]^+$ 512.1926, found 512.1924.

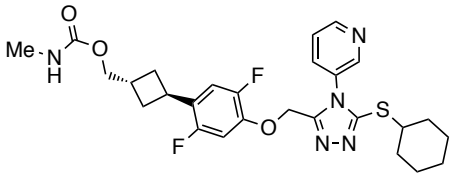


A-44

(*trans*-3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)cyclobutyl)methanol (**A-44**). Methanesulfonyl chloride (0.05 mL, 0.62 mmol) was added dropwise to mixture of **1-80** (0.150 g, 0.517 mmol), N,N-diisopropylethylamine (0.13 mL, 0.78 mmol) in anhydrous methylene chloride (5.2 mL) under an atmosphere of nitrogen at 0

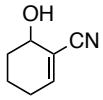
^{o}C for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This material was carried forward without further purification.

2,5-Difluoro-4-((1r,3r)-3-(hydroxymethyl)cyclobutyl)phenol (0.133 g, 0.619 mmol) was added to a mixture of crude mesylate, cesium carbonate (0.337 g, 1.03 mmol) in anhydrous DMF (2.6 mL) under an atmosphere of nitrogen and stirred at room temperature for 14 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (80% ethyl acetate in hexane, dry loaded) to provide **A-44** (0.161 g, 0.221 mmol, 64%) as a white solid: **Rf** (ethyl acetate) 0.37; **Mp** (methylene chloride) 123-125 ^{o}C; **IR** 3330.5, 2932.1, 2855.7, 1633.4, 1484.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.77 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.63-8.63 (m, 1H), 7.76 (ddd, *J* = 8.1, 2.5, 1.5 Hz, 1H), 7.50 (ddd, *J* = 8.1, 4.8, 0.7 Hz, 1H), 6.96 (dd, *J* = 11.6, 7.0 Hz, 1H), 6.75 (dd, *J* = 10.5, 7.0 Hz, 1H), 5.06 (s, 2H), 3.84-3.78 (m, 3H), 3.71-3.64 (m, 1H), 2.52-2.46 (m, 1H), 2.22-2.18 (m, 4H), 2.13-2.10 (m, 2H), 1.74-1.70 (m, 2H), 1.63-1.58 (m, 1H), 1.52-1.38 (m, 5H), 1.30-1.22 (m, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 157.05, 157.02, 154.63, 154.61, 153.6, 151.2, 150.8, 150.08, 150.05, 148.0, 147.67, 147.64, 143.35, 143.24, 143.22, 143.12, 135.1, 130.0, 126.88, 126.82, 126.70, 126.64, 124.1, 114.88, 114.82, 114.68, 114.61, 104.36, 104.35, 104.08, 104.07, 66.3, 61.6, 46.9, 33.4, 33.1, 30.2, 30.0, 25.8, 25.4; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -119.7 (d, *J* = 16.5 Hz), -139.1 (d, *J* = 16.5 Hz); **LC-MS** (ES) *m/z* calcd for $C_{25}H_{29}O_2N_4F_4S$ $[M+H]^+$ 487.1974, found 487.1972.



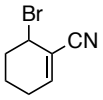
A-45

(*trans*-3-(4-((5-(Cyclohexylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)cyclobutyl)methyl methylcarbamate (**A-45**). Carbonyldiimidazole (0.0249 g, 0.154 mmol) was added to a solution of **A-44** (0.0500 g, 0.103 mmol) in anhydrous methylene chloride (1 mL) and stirred for 3 h under nitrogen. Triethylamine (0.04 mL, 0.31 mmol) and methylamine hydrochloride (0.02 mL, 0.31 mmol) were sequentially added and stirred for an additional 13 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18-silica (65% acetonitrile in water, dry loaded) to provide **A-45** (0.0534 g, 0.0982 mmol, 96%) as an off-white solid: **Rf** (20% methanol in ethyl acetate) 0.79; **Mp** (methylene chloride) 137-139 ^{o}C; **IR** 3337.1, 3058.4, 2934.2, 2855.2, 1712.3, 1633.9, 1484.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.77 (s, 1H), 8.62 (s, 1H), 7.75 (s, 1H), 7.49 (s, 1H), 6.93 (s, 1H), 6.74 (s, 1H), 5.05 (s, 2H), 4.65 (s, 1H), 4.21 (s, 2H), 3.81 (s, 1H), 3.68 (s, 1H), 2.80 (d, *J* = 0.4 Hz, 3H), 2.56 (s, 1H), 2.19 (s, 4H), 2.09 (s, 2H), 1.71 (s, 2H), 1.59 (s, 1H), 1.47-1.39 (m, 4H), 1.25 (s, 2H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 157.4, 156.84, 156.82, 154.91, 154.89, 153.5, 151.2, 150.8, 149.87, 149.85, 148.03, 147.95, 147.92, 143.40, 143.32, 143.30, 143.22, 135.1, 130.0, 126.68, 126.63, 126.53, 126.49, 124.1, 114.81, 114.76, 114.65, 114.60, 104.5, 104.2, 67.8, 61.7, 47.0, 33.4, 31.6, 30.30, 30.11, 30.05, 25.7, 25.4; **^{19}F NMR** ($CDCl_3$, 471 MHz) δ -119.6 (d, *J* = 16 Hz), -139.1 (d, *J* = 16 Hz); **LC-MS** (ES) *m/z* calcd for $C_{27}H_{32}O_3N_5F_2S$ $[M+H]^+$ 544.2188, found 544.2184.



A-47

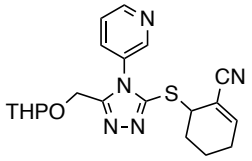
6-Hydroxycyclohex-1-ene-1-carbonitrile (**A-47**). Diethyl cyanomethylphosphate (1.83 mL, 11.3 mmol) was added to a solution potassium carbonate (3.12 g, 22.6 mmol) in water (3 mL) and stirred at room temperature for 5 minutes. A 24 wt% aqueous solution of glutaraldehyde (6.65 mL, 16.9 mmol) was added and the reaction mixture and stirred for an additional 0.5 h. The aqueous solution was extracted with ether (20 mL, three times) and the combined organic solutions were dried, filtered, concentrated under reduced pressure, and purified by chromatography on silica (40% ethyl acetate in hexane) to provide **A-47** (0.975 g, 7.92 mmol, 70%) as a colorless oil. **Rf** (50% ethyl acetate in hexane) 0.53; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.75 (td, *J* = 4.0, 0.7 Hz, 1 H), 4.33-4.29 (m, 1 H), 2.29-2.22 (m, 2 H), 2.20-2.10 (m, 1 H), 1.93-1.87 (m, 1 H), 1.84-1.71 (m, 2 H), 1.68-1.62 (m, 1 H).



A-48

6-Bromocyclohex-1-ene-1-carbonitrile (**23**). Tetrabromomethane (5.25 g, 15.8 mmol) was added to a solution of **A-47** (0.975 g, 7.92 mmol) in anhydrous diethyl ether (40 ml) at 0 ^oC under nitrogen and stirred for 5 minutes. Triphenylphosphine (5.25 g, 15.8 mmol) was added in one portion and the mixture was stirred at 0 ^oC for 1 h and then at room temperature for additional 23 hours. The reaction mixture was concentrated under reduced pressure and purified by chromatography on SiO_2 (20% ethyl acetate in hexane) to provide **A-48** (0.679 g, 3.65 mmol, 46%) as a colorless liquid: **Rf** (50% ethyl acetate in hexane) 0.73; **^{1}H NMR** ($(CD_3)_2SO$, 500

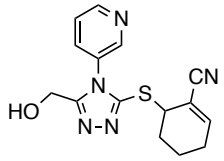
MHz) δ 6.72 (dd, 1 H, *J* = 4.8, 3.3 Hz), 4.76 (s, 1 H), 2.47-2.42 (m, 1 H), 2.36-2.24 (m, 2 H), 2.09-2.01 (m, 1 H), 2.00-1.96 (m, 1 H), 1.81-1.76 (m, 1 H).



A-49

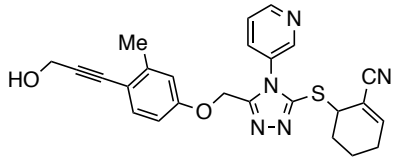
6-((4-(pyridin-3-yl)-5-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)-4H-1,2,4-triazol-3-yl)thio)cyclohex-1-ene-1-carbonitrile (**A-49**). **A-48** (0.398 g, 2.14 mmol) was added to a high pressure reaction vial containing a mixture of **1-13** (0.500 g, 1.71 mmol), cesium carbonate (0.697 g, 2.14 mmol) in DMF (3.5 mL). The reaction vial was sealed and heated to 80 ^{o}C for 2.5 h. The reaction mixture was cooled to room temperature, diluted with a saturated aqueous solution of sodium bicarbonate (15 mL), and extracted with ethyl acetate (25 mL, three times). The combined organic solutions were washed with water (25 mL), brine (25 mL), dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (80% ethyl acetate in hexane) to provide **A-49** (0.402 g, 1.01 mmol, 59%) as an off-white solid: **Rf** (ethyl acetate) 0.45; **Mp** (methylene chloride) > 127 ^{o}C, gel; **IR** 2944.2, 2871.9, 2216.5, 1581.9, 1439.0 cm^{-1}; **^{1}H NMR** (CD_3Cl, 500 MHz) δ 8.76 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.74-8.73 (m, 1 H), 7.90-7.86 (m, 1 H), 7.53-7.50 (m, 1 H), 6.99-6.97 (m, 1 H), 5.79-5.75 (m, 1 H), 4.58-4.49 (m, 2 H), 4.38 (dd, 1 H, *J* = 16.5, 12.9 Hz), 3.53-3.37 (m, 2 H), 2.46-2.39 (m, 1 H), 2.37-2.29 (m, 1 H), 2.23-2.09 (m, 2 H), 2.01-1.93 (m, 1 H), 1.84-1.75 (m, 1 H), 1.66-1.51 (m, 4 H), 1.50-1.39 (m, 4 H), 1.32-1.24 (m, 2 H); **^{13}C NMR** (CD_3Cl, 125 MHz) δ 169.6, 169.6, 150.8, 150.0, 149.9, 148.9, 148.9, 148.1, 148.0, 136.2, 136.1, 131.1, 123.8, 117.0, 112.1, 112.1, 98.4,

98.3, 62.1, 61.7, 58.7, 58.6, 53.9, 31.9, 29.9, 29.8, 27.0, 25.6, 25.0, 24.9, 22.7, 18.8, 18.5, 18.5; **LC-MS** (ES) *m/z* calcd for $C_{20}H_{24}O_2N_4S$ $[M+H]^+$ 398.1645, found 398.1648.



A-50

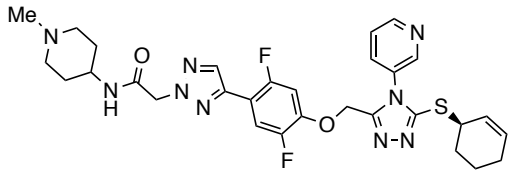
6-((5-(Hydroxymethyl)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)cyclohex-1-ene-1-carbonitrile (**A-50**). *p*-Toluenesulfonic acid monohydrate (0.0479 g, 0.252 mmol) was added to a solution of **A-49** (0.200 g, 0.503 mmol) in methanol (3.3 mL) at room temperature and stirred for 24 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate (5 mL). Organic solvent was removed under reduced pressure and the resulting aqueous solution was extracted with a 10% solution of methanol in methylene chloride (10 mL, three times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure to provide **A-50** (0.140 g, 0.448 mmol, 89%) as an off-white solid: **Rf** (ethyl acetate) 0.59; **Mp** (methanol) 203-204 ^oC; **IR** 3135.3, 2876.6, 222.1, 1634.1, 1581.7, 1411.5 cm^{-1}; **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz) δ 8.72 (dd,1 H, *J* = 4.8, 1.5 Hz), 8.68 (d, 1 H, *J* = 2.2 Hz), 7.97 (ddd, 1 H, *J* = 8.1, 2.5, 1.5 Hz), 7.64 (ddd, 1 H, *J* = 8.1, 4.8, 0.6 Hz), 7.18 (td, 1 H, *J* = 4.0, 1.8 Hz), 5.65 (ddd, 1 H, *J* = 8.8, 6.3, 2.6 Hz), 5.62 (dd, 1 H, *J* = 6.8, 4.5 Hz), 4.40 (dd, 2 H, *J* = 5.7, 1.4 Hz), 2.42-2.25 (m, 2 H), 2.01-1.95 (m 2 H), 1.88-1.68 (m, 2 H); **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz) δ 168.7, 152.0, 151.5, 150.8, 149.1, 136.5, 131.5, 124.6, 117.9, 111.0, 54.3, 53.5, 27.1, 25.7, 18.1; **LC-MS** (ES) *m/z* calcd for $C_{15}H_{16}ON_5S$ $[M+H]^+$ 314.1070, found 314.1068.



A-51

6-((5-((4-(3-Hydroxyprop-1-yn-1-yl)-3-methylphenoxy)methyl)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)cyclohex-1-ene-1-carbonitrile (**A-51**). Methanesulfonic anhydride (0.0306 g, 0.176 mmol) was added to a mixture of **A-50** (0.0500 g, 0.159 mmol), N,N-diisopropylethylamine (0.035 mL, 0.19 mmol) in anhydrous methylene chloride (1.6 mL) under an atmosphere of nitrogen at 0 C and stirred for 2 h. The reaction mixture was diluted with a saturated solution of aqueous sodium bicarbonate (5 mL) and extracted with methylene chloride (10 mL, three times) The isolated organic solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide crude methanesulfonate. This material was carried forward without further purification.

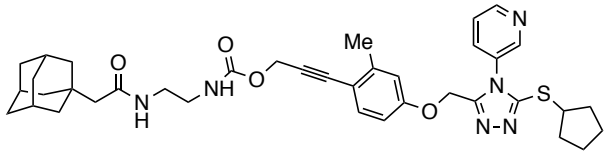
4-(3-hydroxyprop-1-yn-1-yl)-3-methylphenol (0.0518 g, 0.319 mmol) was added to a mixture of crude methanesulfonate, cesium carbonate (0.104 g, 0.319mmol) in anhydrous DMF (0.8 mL) under an atmosphere of nitrogen and stirred at room temperature for overnight. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL, three times). LC-MS of the crude material did not indicate the desired product. This experiment was not pursued further.



A-52

(*R*)-2-(4-(4-((5-(cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2,5-difluorophenyl)-2H-1,2,3-triazol-2-yl)-N-(1-methylpiperidin-4-yl)acetamide (**A-52**). A 50% solution of propylphosphonic anhydride in ethyl acetate (0.12mL, 0.19 mmol) was added dropwise to a mixture of **1-124** (0.0850 g, 0.162 mmol), 1-methylpiperidin-4-amine (0.02 mL, 0.19 mmol), triethylamine (0.06 mL, 0.40 mmol) in anhydrous DMF (0.8 mL) at 0 ^{o}C under an atmosphere of nitrogen. The reaction mixture was warmed to room temperature and stirred for 17 h. The reaction mixture was diluted with a saturated aqueous solution of sodium bicarbonate (5 mL) and extracted with a 10% solution of methanol in chloroform (10 mL, four times). The combined organic solutions were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on silica (20% methanol in methylene chloride) to provide **A-52** (0.0430 g, 0.0692 mmol, 43%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.08; $[\alpha]_D$ = +82.3 (*c.* 0.280, CH_2Cl_2); **Mp** (methylene chloride) 175-177 ^{o}C; **IR** 3275.9, 2937.8, 2783.5, 1659.7, 1571.0, 1493.4 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.79 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.66 (d, *J* = 2.5 Hz, 1H), 8.00 (d, *J* = 3.8 Hz, 1H), 7.77 (ddd, *J* = 8.1, 2.3, 1.6 Hz, 1H), 7.65 (dd, *J* = 11.3, 6.7 Hz, 1H), 7.51 (dd, *J* = 8.1, 4.8 Hz, 1H), 7.00 (dd, *J* = 11.3, 6.8 Hz, 1H), 5.96-5.94 (m, 1H), 5.91-5.87 (m, 1H), 5.77-5.74 (m, 1H), 5.16 (s, 2H), 5.14 (s, 2H), 4.61-4.59 (m, 1H), 3.85-3.79 (m, 1H), 2.70-2.67 (m, 1H), 2.25 (s, 3H), 2.14-2.07 (m, 3H), 2.04-2.02 (m, 3H), 1.91-1.88 (m, 2H), 1.76-1.65 (m, 2H), 1.47-1.39 (m, 2H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 164.5, 156.63, 156.61, 154.66, 154.64, 154.0, 151.4, 150.4, 150.01, 150.00, 148.08, 148.06, 147.93, 146.02, 145.93, 145.92, 145.83, 142.5,

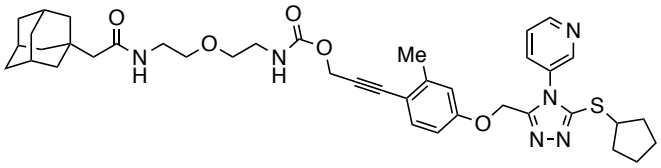
135.0, 134.63, 134.53, 132.4, 129.8, 125.4, 124.2, 114.99, 114.94, 114.81, 114.77, 111.53, 111.47, 111.41, 111.35, 104.4, 104.2, 61.3, 57.7, 54.0, 46.0, 44.1, 31.7, 29.3, 24.8, 19.2; **^{19}F NMR** ($CDCl_3$, 470 MHz) δ -115.9 (d, *J* = 15.0 Hz), -137.8 (*J* = 15.0 Hz); **LC-MS** (ES) *m/z* calcd for $C_{30}H_{34}O_2N_9F_2S$ $[M+H]^+$ 622.2519, found 622.2519.



A-54

3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2-methylphenyl)prop-2-yn-1-yl adamantane (**A-54**). Carbonyldiimidazole (0.0115 g, 0.0892 mmol) was added to a solution of (5-(cyclopentylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methanol (0.0250 g, 0.0594 mmol) in anhydrous methylene chloride (0.8 mL). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 3 h. **1-136** (0.0300 g, 0.119 mmol) was added and the reaction mixture was stirred for 21 h. The reaction mixture was diluted with methylene chloride (5 mL) and washed with water (2.5 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (5% methanol in methylene chloride) to provide **A-54** (0.0366 g, 0.0524 mmol, 90%) as a white solid: **Rf** (ethyl acetate, neutral alumina) 0.12; **Mp** (methylene chloride) 83-85 ^{o}C; **IR** 3306.5, 2902.2, 2847.6, 2236.2, 1717.0, 1649.7, 1529.3 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (dd, 1 H, *J* = 4.8, 1.5 Hz), 8.61 (d, 1 H, *J* = 2.5 Hz), 7.67 (ddd, 1 H, *J* = 8.1, 2.5, 1.5 Hz), 7.46 (ddd, 1 H, *J* = 8.1, 4.8, 0.7 Hz), 7.28 (d, 1 H, *J* = 8.5 Hz), 6.68 (d, 1 H, *J* = 2.5 Hz), 6.63 (dd, 1 H, *J* = 8.5, 2.6 Hz), 5.85 (br. s, 1 H), 5.29 (br. s, 1 H), 5.06 (s, 2 H), 4.90 (s, 2 H), 4.08-4.02 (m, 1 H), 3.41-3.33 (m, 4 H), 2.35 (s, 3 H), 2.25-2.18 (m, 2 H), 1.95 (br. s, 3

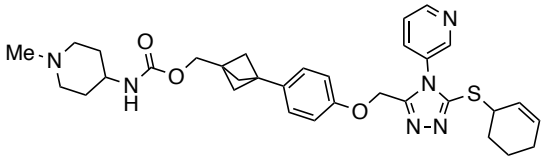
H), 1.91 (s, 2 H), 1.73-1.59 (m, 20 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.9, 157.4, 156.5, 154.1, 151.2, 148.0, 142.7, 134.7, 133.7, 130.1, 124.0, 115.71, 115.66, 111.9, 86.3, 84.8, 59.8, 53.6, 51.7, 46.1, 42.6, 41.3, 39.8, 36.7, 33.8, 32.7, 28.6, 24.6, 20.9; **LC-MS** (ES) *m/z* calcd for $C_{38}H_{47}O_4N_6S$ $[M+H]^+$ 683.3374, found 683.3372.



A-55

3-(4-((5-(Cyclopentylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2-methylphenyl)prop-2-yn-1-yl adamantine (**A-55**). Carbonyldiimidazole (0.0115g, 0.0892 mmol) was added to a solution of (5-(cyclopentylthio)-4-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)methanol (0.0250 g, 0.0594 mmol) in anhydrous methylene chloride (0.800 mL). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 3 h. **1-140** (0.0352 g, 0.119 mmol) was added and the reaction mixture was stirred for 21 h. The reaction mixture was diluted with methylene chloride (5 mL) and washed with water (2.5 mL). The organic solution was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by chromatography on SiO_2 (8% methanol in methylene chloride) to provide **A-55** (0.0304 g, 0.0418 mmol, 70%) as a colorless residue: **Rf** (10% methanol in ethyl acetate; neutral alumina) 0.73; **IR** 3312.9, 2901.4, 2847.6, 1721.6, 1651.9, 1534.9 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.75 (dd, 1 H, *J* = 4.8, 1.4 Hz), 8.61 (d, 1 H, *J* = 2.3 Hz), 7.67 (ddd, 1 H, *J* = 8.1, 2.5, 1.6 Hz), 7.48-7.45 (m, 1 H), 7.29 (d, 1 H, *J* = 8.5 Hz), 6.69 (d, 1 H, *J* = 2.5 Hz), 6.64 (dd, 1 H, *J* = 8.5, 2.5 Hz), 5.76 (br. s, 1 H), 5.14 (br. s, 1H), 5.06 (s, 2 H), 4.92 (s, 2 H), 4.08-4.02 (m, 1 H), 3.52 (q, 4 H, *J* = 5.1 Hz), 3.45-3.38 (m, 4 H), 2.36 (s, 3 H), 2.25-2.17 (m, 2 H), 1.96 (br. s, 2 H), 1.94 (s, 2

H), 1.73-1.61 (m, 18 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.1, 157.4, 154.1, 151.2, 148.0, 142.7, 134.7, 133.7, 130.1, 124.0, 115.7, 115.7, 111.9, 86.4, 84.8, 69.9, 69.8, 59.8, 53.5, 51.7, 46.1, 42.6, 40.9, 39.0, 36.8, 33.8, 32.7, 28.6, 24.6, 20.9; **LC-MS** (ES) *m/z* calcd for $C_{40}H_{51}O_5N_6S$ $[M+H]^+$ 727.3636, found 727.3636.



A-56

(3-(4-((5-(Cyclohex-2-en-1-ylthio)-4-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)phenyl)bicyclo[1.1.1]pentan-1-yl)methyl (1-methylpiperidin-4-yl)carbamate (**36**). Carbonyldiimidazole (0.0317 g, 0.195 mmol) was added to a solution of **1-42c** (0.0600 g, 0.130 mmol) in anhydrous methylene chloride (1.3 mL) and stirred for 3 h under nitrogen. 4-Amino-1-methylpiperidine (0.05 mL, 0.39 mmol) was added and stirred for an additional 21 h. The reaction mixture was concentrated under reduced pressure and purified by reverse-phase chromatography on C18 silica (65% acetonitrile in water, dry loaded) to provide **A-56** (0.0376 g, 0.0626 mmol, 48%) as a white solid: **Rf** (50% methanol in ethyl acetate) 0.12; **Mp** (methylene chloride) 164-166 ^{o}C; **IR** 3258.9, 2937.9, 2870.9, 2791.9, 2791.9, 1689.1 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 8.74 (dd, *J* = 4.8, 1.3 Hz, 1 H), 8.61 (d, *J* = 2.4 Hz, 1 H), 7.68 (ddd, *J* = 8.1, 2.3, 1.7 Hz, 1 H), 7.45 (dd, *J* = 8.1, 4.8 Hz, 1 H), 7.07 (d, *J* = 8.6 Hz, 2 H), 6.79 (d, *J* = 8.6 Hz, 2 H), 5.89-5.85 (m, 1 H), 5.76-5.73 (m, 1 H), 5.09-5.03 (m, 2 H), 4.66 (s, 1 H), 4.56-4.55 (m, 1 H), 4.10 (s, 2 H), 3.57 (s, 1 H), 2.95 (s, 1 H), 2.40 (s, 3 H), 2.27 (s, 2 H), 2.09-2.05 (m, 1 H), 2.04-1.97 (m, 5 H), 1.94 (s, 6 H), 1.77-1.61 (m, 4 H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 156.0, 153.3, 151.6, 151.1, 148.1, 134.8, 132.3, 130.1, 127.3, 125.5, 124.0, 114.5, 64.5, 60.1, 54.4, 51.5, 46.1,

44.1, 41.9, 36.7, 32.5, 29.2, 24.8, 19.2; **LC-MS** (ES) *m/z* calcd for $C_{33}H_{41}O_3N_6S$ $[M+H]^+$ 601.2955, found 601.2953.

Bibliography

1. Bishop, J. M. The molecular genetics of cancer. *Science* **1987,** *235*, 305–11.

2. Martincorena, I.; Raine, K. M.; Gerstung, M.; Dawson, K. J.; Haase, K.; Van Loo, P.; Davies, H.; Stratton, M. R.; Campbell, P. J. Universal patterns of selection in cancer and somatic tissues. *Cell* **2017,** *171*, 1029–1041 e21.

3. Vogelstein, B.; Papadopoulos, N.; Velculescu, V. E.; Zhou, S.; Diaz, L. A., Jr.; Kinzler, K. W. Cancer genome landscapes. *Science* **2013,** *339*, 1546–58.

4. Chakravarthi, B. V.; Nepal, S.; Varambally, S. Genomic and epigenomic alterations in cancer. *Am. J. Pathol.* **2016,** *186*, 1724–35.

5. Osada, H.; Takahashi, T. Genetic alterations of multiple tumor suppressors and oncogenes in the carcinogenesis and progression of lung cancer. *Oncogene* **2002,** *21*, 7421–34.

6. Vogelstein, B.; Kinzler, K. W. The Path to Cancer --Three Strikes and You're Out. *N. Engl. J. Med.* **2015,** *373*, 1895–8.

7. Hanahan, D.; Weinberg, R. A. The hallmarks of cancer. *Cell* **2000,** *100*, 57–70.

8. Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **2011,** *144*, 646–74.

9. Torti, D.; Trusolino, L. Oncogene addiction as a foundational rationale for targeted anti-cancer therapy: promises and perils. *EMBO Mol. Med.* **2011,** *3*, 623–36.

10. Weinstein, I. B.; Joe, A. K. Mechanisms of disease: Oncogene addiction--a rationale for molecular targeting in cancer therapy. *Nat. Clin. Pract. Oncol.* **2006,** *3*, 448–57.

11. Pagliarini, R.; Shao, W.; Sellers, W. R. Oncogene addiction: pathways of therapeutic response, resistance, and road maps toward a cure. *EMBO Rep.* **2015,** *16*, 280–96.

12. Luo, J.; Solimini, N. L.; Elledge, S. J. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* **2009,** *136*, 823–37.

13. Nagel, R.; Semenova, E. A.; Berns, A. Drugging the addict: non-oncogene addiction as a target for cancer therapy. *EMBO Rep.* **2016,** *17*, 1516–1531.

14. Solimini, N. L.; Luo, J.; Elledge, S. J. Non-oncogene addiction and the stress phenotype of cancer cells. *Cell* **2007,** *130*, 986–8.

15. Bastola, P.; Oien, D. B.; Cooley, M.; Chien, J. Emerging Cancer Therapeutic Targets in Protein Homeostasis. *AAPS J.* **2018,** *20*, 94.

16. Van Drie, J. H. Protein folding, protein homeostasis, and cancer. *Chin. J. Cancer* **2011,** *30*, 124–37.

17. Denoyelle, C.; Abou-Rjaily, G.; Bezrookove, V.; Verhaegen, M.; Johnson, T. M.; Fullen, D. R.; Pointer, J. N.; Gruber, S. B.; Su, L. D.; Nikiforov, M. A.; Kaufman, R. J.; Bastian, B. C.; Soengas, M. S. Anti-oncogenic role of the endoplasmic reticulum differentially activated by mutations in the MAPK pathway. *Nat. Cell Biol.* **2006,** *8*, 1053–63.

18. Donnelly, N.; Storchova, Z. Dynamic karyotype, dynamic proteome: buffering the effects of aneuploidy. *Biochim. Biophys. Acta* **2014,** *1843*, 473–81.

19. Dai, C.; Dai, S.; Cao, J. Proteotoxic stress of cancer: implication of the heat-shock response in oncogenesis. *J. Cell. Physiol.* **2012,** *227*, 2982–7.

20. Freije, J. M.; Fraile, J. M.; Lopez-Otin, C. Protease addiction and synthetic lethality in cancer. *Front. Oncol.* **2011,** *1*, 25.

21. LaFave, L. M.; Levine, R. L. Targeting a regulator of protein homeostasis in myeloproliferative neoplasms. *Nat. Med.* **2016,** *22*, 20–1.

22. Guang, M. H. Z.; Kavanagh, E. L.; Dunne, L. P.; Dowling, P.; Zhang, L.; Lindsay, S.; Bazou, D.; Goh, C. Y.; Hanley, C.; Bianchi, G.; Anderson, K. C.; O'Gorman, P.; McCann, A. Targeting Proteotoxic Stress in Cancer: A Review of the Role that Protein Quality Control Pathways Play in Oncogenesis. *Cancers* **2019,** *11*.

23. Shen, M.; Schmitt, S.; Buac, D.; Dou, Q. P. Targeting the ubiquitin-proteasome system for cancer therapy. *Expert Opin. Ther. Targets* **2013,** *17*, 1091–108.

24. Soave, C. L.; Guerin, T.; Liu, J.; Dou, Q. P. Targeting the ubiquitin-proteasome system for cancer treatment: discovering novel inhibitors from nature and drug repurposing. *Cancer Metastasis Rev.* **2017,** *36*, 717–736.

25. Yang, Y.; Kitagaki, J.; Wang, H.; Hou, D. X.; Perantoni, A. O. Targeting the ubiquitin-proteasome system for cancer therapy. *Cancer Sci.* **2009,** *100*, 24–8.

26. Kleiger, G.; Mayor, T. Perilous journey: a tour of the ubiquitin-proteasome system. *Trends Cell Biol.* **2014,** *24*, 352–9.

27. Ravid, T.; Hochstrasser, M. Diversity of degradation signals in the ubiquitin-proteasome system. *Nat. Rev. Mol. Cell Biol.* **2008,** *9*, 679–90.

28. Goy, A.; Younes, A.; McLaughlin, P.; Pro, B.; Romaguera, J. E.; Hagemeister, F.; Fayad, L.; Dang, N. H.; Samaniego, F.; Wang, M.; Broglio, K.; Samuels, B.; Gilles, F.; Sarris, A. H.; Hart, S.; Trehu, E.; Schenkein, D.; Cabanillas, F.; Rodriguez, A. M. Phase II study

of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. *J. Clin. Oncol.* **2005,** *23*, 667–75.

29. O'Connor, O. A.; Wright, J.; Moskowitz, C.; Muzzy, J.; MacGregor-Cortelli, B.; Stubblefield, M.; Straus, D.; Portlock, C.; Hamlin, P.; Choi, E.; Dumetrescu, O.; Esseltine, D.; Trehu, E.; Adams, J.; Schenkein, D.; Zelenetz, A. D. Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. *J. Clin. Oncol.* **2005,** *23*, 676–84.

30. Richardson, P. G.; Sonneveld, P.; Schuster, M. W.; Irwin, D.; Stadtmauer, E. A.; Facon, T.; Harousseau, J. L.; Ben-Yehuda, D.; Lonial, S.; Goldschmidt, H.; Reece, D.; San-Miguel, J. F.; Blade, J.; Boccadoro, M.; Cavenagh, J.; Dalton, W. S.; Boral, A. L.; Esseltine, D. L.; Porter, J. B.; Schenkein, D.; Anderson, K. C. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N. Engl. J. Med.* **2005,** *352*, 2487–98.

31. Caravita, T.; de Fabritiis, P.; Palumbo, A.; Amadori, S.; Boccadoro, M. Bortezomib: efficacy comparisons in solid tumors and hematologic malignancies. *Nat. Clin. Pract. Oncol.* **2006,** *3*, 374–87.

32. Roeten, M. S. F.; Cloos, J.; Jansen, G. Positioning of proteasome inhibitors in therapy of solid malignancies. *Cancer Chemother. Pharmacol.* **2018,** *81*, 227–243.

33. Rolfe, M. The Holy Grail: Solid Tumor Efficacy by Proteasome Inhibition. *Cell Chem. Biol.* **2017,** *24*, 125–126.

34. Vekaria, P. H.; Home, T.; Weir, S.; Schoenen, F. J.; Rao, R. Targeting p97 to Disrupt Protein Homeostasis in Cancer. *Front. Oncol.* **2016,** *6*, 181.

35. van den Boom, J.; Meyer, H. VCP/p97-Mediated Unfolding as a Principle in Protein Homeostasis and Signaling. *Mol. Cell* **2018,** *69*, 182–194.

36. Lan, B.; Chai, S.; Wang, P.; Wang, K. VCP/p97/Cdc48, A Linking of Protein Homeostasis and Cancer Therapy. *Curr. Mol. Med.* **2017,** *17*, 608–618.

37. Wang, Q.; Song, C.; Li, C. C. Molecular perspectives on p97-VCP: progress in understanding its structure and diverse biological functions. *J. Struct. Biol.* **2004,** *146*, 44–57.

38. Xia, D.; Tang, W. K.; Ye, Y. Structure and function of the AAA+ ATPase p97/Cdc48p. *Gene* **2016,** *583*, 64–77.

39. DeLaBarre, B.; Brunger, A. T. Nucleotide dependent motion and mechanism of action of p97/VCP. *J. Mol. Biol.* **2005,** *347*, 437–52.

40. Davies, J. M.; Brunger, A. T.; Weis, W. I. Improved structures of full-length p97, an AAA ATPase: implications for mechanisms of nucleotide-dependent conformational change. *Structure* **2008,** *16*, 715–26.

41. Banerjee, S.; Bartesaghi, A.; Merk, A.; Rao, P.; Bulfer, S. L.; Yan, Y.; Green, N.; Mroczkowski, B.; Neitz, R. J.; Wipf, P.; Falconieri, V.; Deshaies, R. J.; Milne, J. L.; Huryn, D.; Arkin, M.; Subramaniam, S. 2.3 A resolution cryo-EM structure of human p97 and mechanism of allosteric inhibition. *Science* **2016,** *351*, 871–5.

42. Meyer, H.; Bug, M.; Bremer, S. Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. *Nat. Cell Biol.* **2012,** *14*, 117–23.

43. Hanzelmann, P.; Schindelin, H. The structural and functional basis of the p97/valosin-containing protein (VCP)-interacting motif (VIM): mutually exclusive binding of cofactors to the N-terminal domain of p97. *J. Biol. Chem.* **2011,** *286*, 38679–90.

44. Song, C.; Wang, Q.; Li, C. C. ATPase activity of p97-valosin-containing protein (VCP). D2 mediates the major enzyme activity, and D1 contributes to the heat-induced activity. *J. Biol. Chem.* **2003,** *278*, 3648–55.

45. Chou, T. F.; Bulfer, S. L.; Weihl, C. C.; Li, K.; Lis, L. G.; Walters, M. A.; Schoenen, F. J.; Lin, H. J.; Deshaies, R. J.; Arkin, M. R. Specific inhibition of p97/VCP ATPase and kinetic analysis demonstrate interaction between D1 and D2 ATPase domains. *J. Mol. Biol.* **2014,** *426*, 2886–99.

46. Davies, J. M.; Tsuruta, H.; May, A. P.; Weis, W. I. Conformational changes of p97 during nucleotide hydrolysis determined by small-angle X-Ray scattering. *Structure* **2005,** *13*, 183–95.

47. Ye, Y.; Tang, W. K.; Zhang, T.; Xia, D. A Mighty "Protein Extractor" of the Cell: Structure and Function of the p97/CDC48 ATPase. *Front. Mol. Biosci.* **2017,** *4*, 39.

48. Ye, Y.; Shibata, Y.; Yun, C.; Ron, D.; Rapoport, T. A. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* **2004,** *429*, 841–7.

49. Ye, Y.; Meyer, H. H.; Rapoport, T. A. Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. *J. Cell Biol.* **2003,** *162*, 71–84.

50. Neuber, O.; Jarosch, E.; Volkwein, C.; Walter, J.; Sommer, T. Ubx2 links the Cdc48 complex to ER-associated protein degradation. *Nat. Cell Biol.* **2005,** *7*, 993–8.

51. Heo, J. M.; Livnat-Levanon, N.; Taylor, E. B.; Jones, K. T.; Dephoure, N.; Ring, J.; Xie, J.; Brodsky, J. L.; Madeo, F.; Gygi, S. P.; Ashrafi, K.; Glickman, M. H.; Rutter, J. A stress-responsive system for mitochondrial protein degradation. *Mol. Cell* **2010,** *40*, 465–80.

52. Xu, S.; Peng, G.; Wang, Y.; Fang, S.; Karbowski, M. The AAA-ATPase p97 is essential for outer mitochondrial membrane protein turnover. *Mol. Biol. Cell.* **2011,** *22*, 291–300.

53. Hemion, C.; Flammer, J.; Neutzner, A. Quality control of oxidatively damaged mitochondrial proteins is mediated by p97 and the proteasome. *Free Radic. Biol. Med.* **2014,** *75*, 121–8.

54. Blythe, E. E.; Olson, K. C.; Chau, V.; Deshaies, R. J. Ubiquitin- and ATP-dependent unfoldase activity of P97/VCP*NPLOC4*UFD1L is enhanced by a mutation that causes multisystem proteinopathy. *Proc. Natl. Acad. Sci. U.S.A* **2017,** *114*, E4380–E4388.

55. Brandman, O.; Stewart-Ornstein, J.; Wong, D.; Larson, A.; Williams, C. C.; Li, G. W.; Zhou, S.; King, D.; Shen, P. S.; Weibezahn, J.; Dunn, J. G.; Rouskin, S.; Inada, T.; Frost, A.; Weissman, J. S. A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell* **2012,** *151*, 1042–54.

56. Defenouillere, Q.; Yao, Y.; Mouaikel, J.; Namane, A.; Galopier, A.; Decourty, L.; Doyen, A.; Malabat, C.; Saveanu, C.; Jacquier, A.; Fromont-Racine, M. Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products. *Proc. Natl. Acad. Sci. U.S.A* **2013,** *110*, 5046–51.

57. Verma, R.; Oania, R. S.; Kolawa, N. J.; Deshaies, R. J. Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome. *Elife* **2013,** *2*, e00308.

58. Dantuma, N. P.; Acs, K.; Luijsterburg, M. S. Should I stay or should I go: VCP/p97-mediated chromatin extraction in the DNA damage response. *Exp. Cell Res.* **2014,** *329*, 9–17.

59. Dantuma, N. P.; Hoppe, T. Growing sphere of influence: Cdc48/p97 orchestrates ubiquitin-dependent extraction from chromatin. *Trends Cell Biol.* **2012,** *22*, 483–91.

60. Ju, J. S.; Fuentealba, R. A.; Miller, S. E.; Jackson, E.; Piwnica-Worms, D.; Baloh, R. H.; Weihl, C. C. Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J. Cell. Biol.* **2009,** *187*, 875–88.

61. Ju, J. S.; Weihl, C. C. p97/VCP at the intersection of the autophagy and the ubiquitin proteasome system. *Autophagy* **2010,** *6*, 283–5.

62. Bug, M.; Meyer, H. Expanding into new markets--VCP/p97 in endocytosis and autophagy. *J. Struct. Biol.* **2012,** *179*, 78–82.

63. Schwarz, D. S.; Blower, M. D. The endoplasmic reticulum: structure, function and response to cellular signaling. *Cell Mol. Life Sci.* **2016,** *73*, 79–94.

64. Kincaid, M. M.; Cooper, A. A. Misfolded proteins traffic from the endoplasmic reticulum (ER) due to ER export signals. *Mol. Biol. Cell* **2007,** *18*, 455–63.

65. Ruggiano, A.; Foresti, O.; Carvalho, P. Quality control: ER-associated degradation: protein quality control and beyond. *J. Cell. Biol.* **2014,** *204*, 869–79.

66. Matouschek, A. Recognizing misfolded proteins in the endoplasmic reticulum. *Nat. Struct. Biol.* **2000,** *7*, 265–6.

67. Smith, M. H.; Ploegh, H. L.; Weissman, J. S. Road to ruin: targeting proteins for degradation in the endoplasmic reticulum. *Science* **2011,** *334*, 1086–90.

68. Christianson, J. C.; Ye, Y. Cleaning up in the endoplasmic reticulum: ubiquitin in charge. *Nat. Struct. Mol. Biol.* **2014,** *21*, 325–35.

69. Baldridge, R. D.; Rapoport, T. A. Autoubiquitination of the Hrd1 Ligase Triggers Protein Retrotranslocation in ERAD. *Cell* **2016,** *166*, 394–407.

70. Lilley, B. N.; Ploegh, H. L. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* **2004,** *429*, 834–40.

71. Lilley, B. N.; Ploegh, H. L. Multiprotein complexes that link dislocation, ubiquitination, and extraction of misfolded proteins from the endoplasmic reticulum membrane. *Proc. Natl. Acad. Sci. U.S.A* **2005,** *102*, 14296–301.

72. Schuberth, C.; Buchberger, A. Membrane-bound Ubx2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation. *Nat. Cell. Biol.* **2005,** *7*, 999–1006.

73. Carvalho, P.; Stanley, A. M.; Rapoport, T. A. Retrotranslocation of a misfolded luminal ER protein by the ubiquitin-ligase Hrd1p. *Cell* **2010,** *143*, 579–91.

74. Flierman, D.; Ye, Y.; Dai, M.; Chau, V.; Rapoport, T. A. Polyubiquitin serves as a recognition signal, rather than a ratcheting molecule, during retrotranslocation of proteins across the endoplasmic reticulum membrane. *J. Biol. Chem.* **2003,** *278*, 34774–82.

75. Ye, Y.; Meyer, H. H.; Rapoport, T. A. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* **2001,** *414*, 652–6.

76. Zhang, T.; Ye, Y. The final moments of misfolded proteins en route to the proteasome. *DNA Cell Biol.* **2014,** *33*, 477–83.

77. Wilhelm, T.; Bick, F.; Peters, K.; Mohta, V.; Tirosh, B.; Patterson, J. B.; Kharabi-Masouleh, B.; Huber, M. Infliction of proteotoxic stresses by impairment of the unfolded protein response or proteasomal inhibition as a therapeutic strategy for mast cell leukemia. *Oncotarget* **2018,** *9*, 2984–3000.

78. Schonthal, A. H. Endoplasmic reticulum stress: its role in disease and novel prospects for therapy. *Scientifica* **2012,** *2012*, 857516.

79. Korennykh, A.; Walter, P. Structural basis of the unfolded protein response. *Annu. Rev. Cell Dev. Biol.* **2012,** *28*, 251–77.

80. McKibbin, C.; Mares, A.; Piacenti, M.; Williams, H.; Roboti, P.; Puumalainen, M.; Callan, A. C.; Lesiak-Mieczkowska, K.; Linder, S.; Harant, H.; High, S.; Flitsch, S. L.; Whitehead, R. C.; Swanton, E. Inhibition of protein translocation at the endoplasmic reticulum promotes activation of the unfolded protein response. *Biochem. J.* **2012,** *442*, 639–48.

81. Schonthal, A. H. Targeting endoplasmic reticulum stress for cancer therapy. *Front. Biosci.* **2012,** *4*, 412–31.

82. Walter, P.; Ron, D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **2011,** *334*, 1081–6.

83. Anderson, D. J.; Le Moigne, R.; Djakovic, S.; Kumar, B.; Rice, J.; Wong, S.; Wang, J.; Yao, B.; Valle, E.; Kiss von Soly, S.; Madriaga, A.; Soriano, F.; Menon, M. K.; Wu, Z. Y.; Kampmann, M.; Chen, Y.; Weissman, J. S.; Aftab, B. T.; Yakes, F. M.; Shawver, L.; Zhou, H. J.; Wustrow, D.; Rolfe, M. Targeting the AAA ATPase p97 as an Approach to Treat Cancer through Disruption of Protein Homeostasis. *Cancer cell* **2015,** *28*, 653–665.

84. Deshaies, R. J. Proteotoxic crisis, the ubiquitin-proteasome system, and cancer therapy. *BMC Biol.* **2014,** *12*, 94.

85. Haines, D. S. p97-containing complexes in proliferation control and cancer: emerging culprits or guilt by association? *Genes Cancer* **2010,** *1*, 753–763.

86. Valle, C. W.; Min, T.; Bodas, M.; Mazur, S.; Begum, S.; Tang, D.; Vij, N. Critical role of VCP/p97 in the pathogenesis and progression of non-small cell lung carcinoma. *PloS one* **2011,** *6*, e29073.

87. Yamamoto, M.; Takasaki, K.; Otsubo, T.; Katsuragawa, H.; Katagiri, S.; Yoshitoshi, K.; Ariizumi, S.; Saito, A.; Nakano, M. Favorable surgical outcomes in patients with early hepatocellular carcinoma. *Ann. Surg.* **2004,** *239*, 395–9.

88. Tsujimoto, Y.; Tomita, Y.; Hoshida, Y.; Kono, T.; Oka, T.; Yamamoto, S.; Nonomura, N.; Okuyama, A.; Aozasa, K. Elevated expression of valosin-containing protein (p97) is associated with poor prognosis of prostate cancer. *Clin. Cancer Res.* **2004,** *10*, 3007–12.

89. Wojcik, C.; Yano, M.; DeMartino, G. N. RNA interference of valosin-containing protein (VCP/p97) reveals multiple cellular roles linked to ubiquitin/proteasome-dependent proteolysis. *J. Cell Sci.* **2004,** *117*, 281–92.

90. Chou, T. F.; Brown, S. J.; Minond, D.; Nordin, B. E.; Li, K.; Jones, A. C.; Chase, P.; Porubsky, P. R.; Stoltz, B. M.; Schoenen, F. J.; Patricelli, M. P.; Hodder, P.; Rosen, H.; Deshaies, R. J. Reversible inhibitor of p97, DBeQ, impairs both ubiquitin-dependent and autophagic protein clearance pathways. *Proc. Natl. Acad. Sci. U.S.A* **2011,** *108*, 4834–9.

91. Fang, C. J.; Gui, L.; Zhang, X.; Moen, D. R.; Li, K.; Frankowski, K. J.; Lin, H. J.; Schoenen, F. J.; Chou, T. F. Evaluating p97 inhibitor analogues for their domain selectivity and potency against the p97-p47 complex. *ChemMedChem* **2015,** *10*, 52–6.

92. Bursavich, M. G.; Parker, D. P.; Willardsen, J. A.; Gao, Z. H.; Davis, T.; Ostanin, K.; Robinson, R.; Peterson, A.; Cimbora, D. M.; Zhu, J. F.; Richards, B. 2-Anilino-4-aryl-1,3-thiazole inhibitors of valosin-containing protein (VCP or p97). *Bioorg. Med. Chem. Lett.* **2010,** *20*, 1677–9.

93. Magnaghi, P.; D'Alessio, R.; Valsasina, B.; Avanzi, N.; Rizzi, S.; Asa, D.; Gasparri, F.; Cozzi, L.; Cucchi, U.; Orrenius, C.; Polucci, P.; Ballinari, D.; Perrera, C.; Leone, A.; Cervi, G.; Casale, E.; Xiao, Y.; Wong, C.; Anderson, D. J.; Galvani, A.; Donati, D.; O'Brien, T.; Jackson, P. K.; Isacchi, A. Covalent and allosteric inhibitors of the ATPase VCP/p97 induce cancer cell death. *Nat. Chem. Biol.* **2013,** *9*, 548–56.

94. Polucci, P.; Magnaghi, P.; Angiolini, M.; Asa, D.; Avanzi, N.; Badari, A.; Bertrand, J.; Casale, E.; Cauteruccio, S.; Cirla, A.; Cozzi, L.; Galvani, A.; Jackson, P. K.; Liu, Y.; Magnuson, S.; Malgesini, B.; Nuvoloni, S.; Orrenius, C.; Sirtori, F. R.; Riceputi, L.; Rizzi, S.; Trucchi, B.; O'Brien, T.; Isacchi, A.; Donati, D.; D'Alessio, R. Alkylsulfanyl-1,2,4-triazoles, a new class of allosteric valosine containing protein inhibitors. Synthesis and structure-activity relationships. *J. Med. Chem.* **2013,** *56*, 437–50.

95. Wang, Q.; Li, L.; Ye, Y. Inhibition of p97-dependent protein degradation by Eeyarestatin I. *J. Biol. Chem.* **2008,** *283*, 7445–54.

96. Alverez, C.; Bulfer, S. L.; Chakrasali, R.; Chimenti, M. S.; Deshaies, R. J.; Green, N.; Kelly, M.; LaPorte, M. G.; Lewis, T. S.; Liang, M.; Moore, W. J.; Neitz, R. J.; Peshkov, V. A.; Walters, M. A.; Zhang, F.; Arkin, M. R.; Wipf, P.; Huryn, D. M. Allosteric Indole Amide Inhibitors of p97: Identification of a Novel Probe of the Ubiquitin Pathway. *ACS Med Chem Lett* **2016,** *7*, 182–7.

97. Alverez, C.; Arkin, M. R.; Bulfer, S. L.; Colombo, R.; Kovaliov, M.; LaPorte, M. G.; Lim, C.; Liang, M.; Moore, W. J.; Neitz, R. J.; Yan, Y.; Yue, Z.; Huryn, D. M.; Wipf, P. Structure-Activity Study of Bioisosteric Trifluoromethyl and Pentafluorosulfanyl Indole Inhibitors of the AAA ATPase p97. *ACS Med. Chem. Lett.* **2015,** *6*, 1225–30.

98. Chapman, E.; Maksim, N.; de la Cruz, F.; La Clair, J. J. Inhibitors of the AAA+ chaperone p97. *Molecules* **2015,** *20*, 3027–49.

99. Kang, M. J.; Wu, T.; Wijeratne, E. M.; Lau, E. C.; Mason, D. J.; Mesa, C.; Tillotson, J.; Zhang, D. D.; Gunatilaka, A. A.; La Clair, J. J.; Chapman, E. Functional chromatography reveals three natural products that target the same protein with distinct mechanisms of action. *Chembiochem* **2014,** *15*, 2125–31.

100. Tao, S.; Tillotson, J.; Wijeratne, E. M. K.; Xu, Y. M.; Kang, M.; Wu, T.; Lau, E. C.; Mesa, C.; Mason, D. J.; Brown, R. V.; Clair, J. J.; Gunatilaka, A. A. L.; Zhang, D. D.; Chapman, E. Withaferin A Analogs That Target the AAA+ Chaperone p97. *ACS Chem. Biol.* **2015,** *10*, 1916–1924.

101. Yi, P.; Higa, A.; Taouji, S.; Bexiga, M. G.; Marza, E.; Arma, D.; Castain, C.; Le Bail, B.; Simpson, J. C.; Rosenbaum, J.; Balabaud, C.; Bioulac-Sage, P.; Blanc, J. F.; Chevet, E. Sorafenib-mediated targeting of the AAA(+) ATPase p97/VCP leads to disruption of the

secretory pathway, endoplasmic reticulum stress, and hepatocellular cancer cell death. *Mol. Cancer Ther.* **2012,** *11*, 2610–20.

102. Cervi, G.; Magnaghi, P.; Asa, D.; Avanzi, N.; Badari, A.; Borghi, D.; Caruso, M.; Cirla, A.; Cozzi, L.; Felder, E.; Galvani, A.; Gasparri, F.; Lomolino, A.; Magnuson, S.; Malgesini, B.; Motto, I.; Pasi, M.; Rizzi, S.; Salom, B.; Sorrentino, G.; Troiani, S.; Valsasina, B.; O'Brien, T.; Isacchi, A.; Donati, D.; D'Alessio, R. Discovery of 2-(cyclohexylmethylamino)pyrimidines as a new class of reversible valosine containing protein inhibitors. *J. Med. Chem.* **2014,** *57*, 10443–54.

103. Chou, T. F.; Deshaies, R. J. Quantitative cell-based protein degradation assays to identify and classify drugs that target the ubiquitin-proteasome system. *J. Biol. Chem.* **2011,** *286*, 16546–54.

104. Dantuma, N. P.; Lindsten, K.; Glas, R.; Jellne, M.; Masucci, M. G. Short-lived green fluorescent proteins for quantifying ubiquitin/proteasome-dependent proteolysis in living cells. *Nat. Biotechnol.* **2000,** *18*, 538–43.

105. Kimbrel, E. A.; Davis, T. N.; Bradner, J. E.; Kung, A. L. In vivo pharmacodynamic imaging of proteasome inhibition. *Mol. Imaging* **2009,** *8*, 140–7.

106. Zhang, M.; Pickart, C. M.; Coffino, P. Determinants of proteasome recognition of ornithine decarboxylase, a ubiquitin-independent substrate. *EMBO J.* **2003,** *22*, 1488–96.

107. Fiebiger, E.; Hirsch, C.; Vyas, J. M.; Gordon, E.; Ploegh, H. L.; Tortorella, D. Dissection of the dislocation pathway for type I membrane proteins with a new small molecule inhibitor, eeyarestatin. *Mol. Biol. Cell* **2004,** *15*, 1635–46.

108. Wang, Q.; Shinkre, B. A.; Lee, J. G.; Weniger, M. A.; Liu, Y.; Chen, W.; Wiestner, A.; Trenkle, W. C.; Ye, Y. The ERAD inhibitor Eeyarestatin I is a bifunctional compound with a membrane-binding domain and a p97/VCP inhibitory group. *PloS one* **2010,** *5*, e15479.

109. Wang, Q.; Mora-Jensen, H.; Weniger, M. A.; Perez-Galan, P.; Wolford, C.; Hai, T.; Ron, D.; Chen, W.; Trenkle, W.; Wiestner, A.; Ye, Y. ERAD inhibitors integrate ER stress with an epigenetic mechanism to activate BH3-only protein NOXA in cancer cells. *Proc. Natl. Acad. Sci. U.S.A* **2009,** *106*, 2200–5.

110. Auner, H. W.; Moody, A. M.; Ward, T. H.; Kraus, M.; Milan, E.; May, P.; Chaidos, A.; Driessen, C.; Cenci, S.; Dazzi, F.; Rahemtulla, A.; Apperley, J. F.; Karadimitris, A.; Dillon, N. Combined inhibition of p97 and the proteasome causes lethal disruption of the secretory apparatus in multiple myeloma cells. *PloS one* **2013,** *8*, e74415.

111. Brem, G. J.; Mylonas, I.; Bruning, A. Eeyarestatin causes cervical cancer cell sensitization to bortezomib treatment by augmenting ER stress and CHOP expression. *Gynecol. Oncol.* **2013,** *128*, 383–90.

112. Chou, T. F.; Li, K.; Frankowski, K. J.; Schoenen, F. J.; Deshaies, R. J. Structure-activity relationship study reveals ML240 and ML241 as potent and selective inhibitors of p97 ATPase. *ChemMedChem* **2013,** *8*, 297–312.

113. Zhou, H. J.; Wang, J.; Yao, B.; Wong, S.; Djakovic, S.; Kumar, B.; Rice, J.; Valle, E.; Soriano, F.; Menon, M. K.; Madriaga, A.; Kiss von Soly, S.; Kumar, A.; Parlati, F.; Yakes, F. M.; Shawver, L.; Le Moigne, R.; Anderson, D. J.; Rolfe, M.; Wustrow, D. Discovery of a First-in-Class, Potent, Selective, and Orally Bioavailable Inhibitor of the p97 AAA ATPase (CB-5083). *J. Med. Chem.* **2015,** *58*, 9480–97.

114. LaPorte, M. G.; Burnett, J. C.; Colombo, R.; Bulfer, S. L.; Alverez, C.; Chou, T. F.; Neitz, R. J.; Green, N.; Moore, W. J.; Yue, Z.; Li, S.; Arkin, M. R.; Wipf, P.; Huryn, D. M. Optimization of Phenyl Indole Inhibitors of the AAA+ ATPase p97. *ACS Med. Chem. Lett.* **2018,** *9*, 1075–1081.

115. Rydberg, P.; Ryde, U.; Olsen, L. Sulfoxide, Sulfur, and Nitrogen Oxidation and Dealkylation by Cytochrome P450. *J. Chem. Theory Comput.* **2008,** *4*, 1369–77.

116. Foti, R. S.; Dalvie, D. K. Cytochrome P450 and Non-Cytochrome P450 Oxidative Metabolism: Contributions to the Pharmacokinetics, Safety, and Efficacy of Xenobiotics. *Drug Metab. Dispos.* **2016,** *44*, 1229–45.

117. Zuniga, F. I.; Loi, D.; Ling, K. H.; Tang-Liu, D. D. Idiosyncratic reactions and metabolism of sulfur-containing drugs. *Expert Opin. Drug Metab. Toxicol.* **2012,** *8*, 467–85.

118. Segura-Cabrera, A.; Tripathi, R.; Zhang, X.; Gui, L.; Chou, T. F.; Komurov, K. A structure- and chemical genomics-based approach for repositioning of drugs against VCP/p97 ATPase. *Sci. Rep.* **2017,** *7*, 44912.

119. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009,** *30*, 2785–91.

120. Savjani, K. T.; Gajjar, A. K.; Savjani, J. K. Drug solubility: importance and enhancement techniques. *ISRN Pharm* **2012,** *2012*, 195727.

121. Di, L.; Kerns, E. H.; Carter, G. T. Drug-like property concepts in pharmaceutical design. *Curr. Pharm. Des.* **2009,** *15*, 2184–94.

122. Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **2002,** *45*, 2615–23.

123. Walker, M. A. Novel tactics for designing water-soluble molecules in drug discovery. *Expert Opin. Drug Discov.* **2014,** *9*, 1421–33.

124. Li, Y.; Zhu, Y. M.; Jiang, H. J.; Pan, J. P.; Wu, G. S.; Wu, J. M.; Shi, Y. L.; Yang, J. D.; Wu, B. A. Synthesis and antimalarial activity of artemisinin derivatives containing an amino group. *J. Med. Chem.* **2000,** *43*, 1635–40.

125. Milbank, J. B.; Tercel, M.; Atwell, G. J.; Wilson, W. R.; Hogg, A.; Denny, W. A. Synthesis of 1-substituted 3-(chloromethyl)-6-aminoindoline (6-amino-seco-CI) DNA minor groove alkylating agents and structure-activity relationships for their cytotoxicity. *J .Med. Chem.* **1999,** *42*, 649–58.

126. Al-Awar, R. S.; Ray, J. E.; Schultz, R. M.; Andis, S. L.; Kennedy, J. H.; Moore, R. E.; Liang, J.; Golakoti, T.; Subbaraju, G. V.; Corbett, T. H. A convergent approach to cryptophycin 52 analogues: synthesis and biological evaluation of a novel series of fragment a epoxides and chlorohydrins. *J. Med. Chem.* **2003,** *46*, 2985–3007.

127. Xie, L.; Yu, D.; Wild, C.; Allaway, G.; Turpin, J.; Smith, P. C.; Lee, K. H. Anti-AIDS agents. 52. Synthesis and anti-HIV activity of hydroxymethyl (3'R,4'R)-3',4'-di-O-(S)-camphanoyl-(+)-cis-khellactone derivatives. *J. Med. Chem.* **2004,** *47*, 756–60.

128. Borgos, S. E.; Tsan, P.; Sletta, H.; Ellingsen, T. E.; Lancelin, J. M.; Zotchev, S. B. Probing the structure-function relationship of polyene macrolides: engineered biosynthesis of soluble nystatin analogues. *J. Med. Chem.* **2006,** *49*, 2431–9.

129. Kim, I. H.; Morisseau, C.; Watanabe, T.; Hammock, B. D. Design, synthesis, and biological activity of 1,3-disubstituted ureas as potent inhibitors of the soluble epoxide hydrolase of increased water solubility. *J. Med. Chem.* **2004,** *47*, 2110–22.

130. Ishikawa, M.; Hashimoto, Y. Improvement in aqueous solubility in small molecule drug discovery programs by disruption of molecular planarity and symmetry. *J. Med. Chem.* **2011,** *54*, 1539–54.

131. Fray, M. J.; Bull, D. J.; Carr, C. L.; Gautier, E. C.; Mowbray, C. E.; Stobie, A. Structure-activity relationships of 1,4-dihydro-(1H,4H)-quinoxaline-2,3-diones as N-methyl-D-aspartate (glycine site) receptor antagonists. 1. Heterocyclic substituted 5-alkyl derivatives. *J. Med. Chem.* **2001,** *44*, 1951–62.

132. Patani, G. A.; LaVoie, E. J. Bioisosterism: A Rational Approach in Drug Design. *Chem. Rev.* **1996,** *96*, 3147–3176.

133. Brown, N. Bioisosteres and Scaffold Hopping in Medicinal Chemistry. *Mol. Inform* **2014,** *33*, 458–62.

134. Huryn, D. M.; Wipf, P.; LaPorte, M. G. Preparation of alkynyl substituted triazoles as modulators of p97 AAA ATPase activity. WO2017197080A1, 2017.

135. Makarov, I. S.; Brocklehurst, C. E.; Karaghiosoff, K.; Koch, G.; Knochel, P. Synthesis of Bicyclo[1.1.1]pentane Bioisosteres of Internal Alkynes and para-Disubstituted Benzenes from [1.1.1]Propellane. *Angew. Chem.* **2017,** *56*, 12774–12777.

136. Stepan, A. F.; Subramanyam, C.; Efremov, I. V.; Dutra, J. K.; O'Sullivan, T. J.; DiRico, K. J.; McDonald, W. S.; Won, A.; Dorff, P. H.; Nolan, C. E.; Becker, S. L.; Pustilnik, L. R.; Riddell, D. R.; Kauffman, G. W.; Kormos, B. L.; Zhang, L.; Lu, Y.; Capetta, S. H.; Green, M. E.; Karki, K.; Sibley, E.; Atchison, K. P.; Hallgren, A. J.; Oborski, C. E.; Robshaw, A. E.; Sneed, B.; O'Donnell, C. J. Application of the bicyclo[1.1.1]pentane motif as a nonclassical phenyl ring bioisostere in the design of a potent and orally active gamma-secretase inhibitor. *J. Med. Chem.* **2012,** *55*, 3414–24.

137. Lovering, F.; Bikker, J.; Humblet, C. Escape from flatland: increasing saturation as an approach to improving clinical success. *J. Med. Chem.* **2009,** *52*, 6752–6.

138. Wipf, P.; Xiao, J. Convergent approach to (E)-alkene and cyclopropane peptide isosteres. *Org. Lett.* **2005,** *7*, 103–6.

139. Stepan, A. F.; Kauffman, G. W.; Keefer, C. E.; Verhoest, P. R.; Edwards, M. Evaluating the differences in cycloalkyl ether metabolism using the design parameter "lipophilic metabolism efficiency" (LipMetE) and a matched molecular pairs analysis. *J. Med. Chem.* **2013,** *56*, 6985–90.

140. McDonnell, A. M.; Dang, C. H. Basic review of the cytochrome p450 system. *J. Adv. Pract. Oncol.* **2013,** *4*, 263–8.

141. Obach, R. S. Cytochrome P450-catalyzed metabolism of ezlopitant alkene (CJ-12,458), a pharmacologically active metabolite of ezlopitant: enzyme kinetics and mechanism of an alkene hydration reaction. *Drug Metab. Dispos.* **2001,** *29*, 1057–67.

142. Wu, X.; Fu, Q.; Gan, J. Metabolism of pharmaceutical and personal care products by carrot cell cultures. *Environ. Pollut.* **2016,** *211*, 141–7.

143. Gorycki, P. D.; Macdonald, T. L. The oxidation of tetrasubstituted alkenes by cytochrome P450. *Chem. Res. Toxicol.* **1994,** *7*, 745–51.

144. Vaz, A. D.; McGinnity, D. F.; Coon, M. J. Epoxidation of olefins by cytochrome P450: evidence from site-specific mutagenesis for hydroperoxo-iron as an electrophilic oxidant. *Proc. Natl. Acad. Sci. U.S.A* **1998,** *95*, 3555–60.

145. Guengerich, F. P.; Munro, A. W. Unusual cytochrome p450 enzymes and reactions. *J. Biol. Chem.* **2013,** *288*, 17065–73.

146. Wehn, P. M.; Harrington, P. E.; Carlson, T. J.; Davis, J.; Deprez, P.; Fotsch, C. H.; Grillo, M. P.; Lu, J. Y.; Morony, S.; Pattabiraman, K.; Poon, S. F.; Reagan, J. D.; St Jean, D. J., Jr.; Temal, T.; Wang, M.; Yang, Y.; Henley, C., 3rd; Lively, S. E. Metabolism-guided discovery of a potent and orally bioavailable urea-based calcimimetic for the treatment of secondary hyperparathyroidism. *Bioorg. Med. Chem. Lett.* **2013,** *23*, 6625–8.

147. Stepan, A. F.; Mascitti, V.; Beaumont, K.; Kalgutkar, A. S. Metabolism-guided drug design. *MedChemComm* **2013,** *4*, 631–652.

148. Plobeck, N.; Delorme, D.; Wei, Z. Y.; Yang, H.; Zhou, F.; Schwarz, P.; Gawell, L.; Gagnon, H.; Pelcman, B.; Schmidt, R.; Yue, S. Y.; Walpole, C.; Brown, W.; Zhou, E.; Labarre, M.; Payza, K.; St-Onge, S.; Kamassah, A.; Morin, P. E.; Projean, D.; Ducharme, J.; Roberts, E. New diarylmethylpiperazines as potent and selective nonpeptidic delta opioid receptor agonists with increased In vitro metabolic stability. *J. Med. Chem.* **2000,** *43*, 3878–94.

149. Mano, T.; Okumura, Y.; Sakakibara, M.; Okumura, T.; Tamura, T.; Miyamoto, K.; Stevens, R. W. 4-[5-Fluoro-3-[4-(2-methyl-1H-imidazol-1-yl)benzyloxy]phenyl]-3,4,5,6-tetrahydro-2H-pyran-4-carboxamide, an orally active inhibitor of 5-lipoxygenase with improved pharmacokinetic and toxicology characteristics. *J. Med. Chem.* **2004,** *47*, 720–5.

150. Peglion, J. L.; Goument, B.; Despaux, N.; Charlot, V.; Giraud, H.; Nisole, C.; Newman-Tancredi, A.; Dekeyne, A.; Bertrand, M.; Genissel, P.; Millan, M. J. Improvement in the selectivity and metabolic stability of the serotonin 5-HT(1A) ligand, S 15535: a series of cis- and trans-2-(arylcycloalkylamine) 1-indanols. *J. Med. Chem.* **2002,** *45*, 165–76.

151. Tandon, M.; O'Donnell, M. M.; Porte, A.; Vensel, D.; Yang, D.; Palma, R.; Beresford, A.; Ashwell, M. A. The design and preparation of metabolically protected new arylpiperazine 5-HT1A ligands. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 1709–12.

152. Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2015,** *58*, 8315–59.

153. Pirali, T.; Serafini, M.; Cargnin, S.; Genazzani, A. A. Applications of Deuterium in Medicinal Chemistry. *J. Med. Chem.* **2019,** *62*, 5276–5297.

154. Madsen, P.; Ling, A.; Plewe, M.; Sams, C. K.; Knudsen, L. B.; Sidelmann, U. G.; Ynddal, L.; Brand, C. L.; Andersen, B.; Murphy, D.; Teng, M.; Truesdale, L.; Kiel, D.; May, J.; Kuki, A.; Shi, S.; Johnson, M. D.; Teston, K. A.; Feng, J.; Lakis, J.; Anderes, K.; Gregor, V.; Lau, J. Optimization of alkylidene hydrazide based human glucagon receptor antagonists. Discovery of the highly potent and orally available 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-tetramethylbenzyl)-1H-indol-4-ylmethylene]hydrazide. *J. Med. Chem.* **2002,** *45*, 5755–75.

155. MacKenzie, A. R.; Marchington, A. P.; Middleton, D. S.; Newman, S. D.; Jones, B. C. Structure-activity relationships of 1-alkyl-5-(3,4-dichlorophenyl)- 5-[2-[(3-substituted)-1-azetidinyl]ethyl]-2-piperidones. 1. Selective antagonists of the neurokinin-2 receptor. *J. Med. Chem.* **2002,** *45*, 5365–77.

156. Sanins, S. M.; Adams, W. J.; Kaiser, D. G.; Halstead, G. W.; Baillie, T. A. Studies on the metabolism and chiral inversion of ibuprofen in isolated rat hepatocytes. *Drug Metab. Dispos.* **1990,** *18*, 527–33.

157. Kashiyama, E.; Yokoi, T.; Odomi, M.; Funae, Y.; Inoue, K.; Kamataki, T. Cytochrome P450 responsible for the stereoselective S-oxidation of flosequinan in hepatic microsomes from rats and humans. *Drug Metab. Dispos.* **1997,** *25*, 716–24.

158. Southers, J. A.; Bauman, J. N.; Price, D. A.; Humphries, P. S.; Balan, G.; Sagal, J. F.; Maurer, T. S.; Zhang, Y.; Oliver, R.; Herr, M.; Healy, D. R.; Li, M.; Kapinos, B.; Fate, G. D.; Riccardi, K. A.; Paralkar, V. M.; Brown, T. A.; Kalgutkar, A. S. Metabolism-guided design of short-acting calcium-sensing receptor antagonists. *ACS Med. Chem. Lett.* **2010,** *1*, 219–23.

159. Benoit, E.; Buronfosse, T.; Moroni, P.; Delatour, P.; Riviere, J. L. Stereoselective S-oxygenation of an aryl-trifluoromethyl sulfoxide to the corresponding sulfone by rat liver cytochromes P450. *Biochem. Pharmacol.* **1993,** *46*, 2337–41.

160. Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A. The resurgence of covalent drugs. *Nature reviews. Drug discovery* **2011,** *10*, 307–17.

161. Potashman, M. H.; Duggan, M. E. Covalent modifiers: an orthogonal approach to drug design. *J. Med. Chem.* **2009,** *52*, 1231–46.

162. Johnson, D. S.; Weerapana, E.; Cravatt, B. F. Strategies for discovering and derisking covalent, irreversible enzyme inhibitors. *Future Med. Chem.* **2010,** *2*, 949–64.

163. Smith, A. J.; Zhang, X.; Leach, A. G.; Houk, K. N. Beyond picomolar affinities: quantitative aspects of noncovalent and covalent binding of drugs to proteins. *J. Med. Chem.* **2009,** *52*, 225–33.

164. Abranyi-Balogh, P.; Petri, L.; Imre, T.; Szijj, P.; Scarpino, A.; Hrast, M.; Mitrovic, A.; Fonovic, U. P.; Nemeth, K.; Barreteau, H.; Roper, D. I.; Horvati, K.; Ferenczy, G. G.; Kos, J.; Ilas, J.; Gobec, S.; Keseru, G. M. A road map for prioritizing warheads for cysteine targeting covalent inhibitors. *Eur. J. Med. Chem.* **2018,** *160*, 94–107.

165. Gehringer, M.; Laufer, S. A. Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: Applications in Medicinal Chemistry and Chemical Biology. *J. Med. Chem.* **2019**.

166. Leproult, E.; Barluenga, S.; Moras, D.; Wurtz, J. M.; Winssinger, N. Cysteine mapping in conformationally distinct kinase nucleotide binding sites: application to the design of selective covalent inhibitors. *J. Med. Chem.* **2011,** *54*, 1347–55.

167. Jackson, P. A.; Widen, J. C.; Harki, D. A.; Brummond, K. M. Covalent Modifiers: A Chemical Perspective on the Reactivity of alpha,beta-Unsaturated Carbonyls with Thiols via Hetero-Michael Addition Reactions. *J. Med. Chem.* **2017,** *60*, 839–885.

168. Liu, Q.; Sabnis, Y.; Zhao, Z.; Zhang, T.; Buhrlage, S. J.; Jones, L. H.; Gray, N. S. Developing irreversible inhibitors of the protein kinase cysteinome. *Chem. Biol.* **2013,** *20*, 146–59.

169. Zhao, Z.; Bourne, P. E. Progress with covalent small-molecule kinase inhibitors. *Drug Discov. Today* **2018,** *23*, 727–735.

170. Toure, M.; Crews, C. M. Small-Molecule PROTACS: New Approaches to Protein Degradation. *Angew. Chem.* **2016,** *55*, 1966–73.

171. Bondeson, D. P.; Crews, C. M. Targeted Protein Degradation by Small Molecules. *Annu. Rev. Pharmacol. Toxicol.* **2017,** *57*, 107–123.

172. Lai, A. C.; Crews, C. M. Induced protein degradation: an emerging drug discovery paradigm. *Nature reviews. Drug discovery* **2017,** *16*, 101–114.

173. Burslem, G. M.; Smith, B. E.; Lai, A. C.; Jaime-Figueroa, S.; McQuaid, D. C.; Bondeson, D. P.; Toure, M.; Dong, H.; Qian, Y.; Wang, J.; Crew, A. P.; Hines, J.; Crews, C. M. The Advantages of Targeted Protein Degradation Over Inhibition: An RTK Case Study. *Cell Chem. Biol.* **2018,** *25*, 67–77 e3.

174. Neklesa, T. K.; Tae, H. S.; Schneekloth, A. R.; Stulberg, M. J.; Corson, T. W.; Sundberg, T. B.; Raina, K.; Holley, S. A.; Crews, C. M. Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. *Nat. Chem. Biol.* **2011,** *7*, 538–43.

175. Neklesa, T. K.; Crews, C. M. Chemical biology: Greasy tags for protein removal. *Nature* **2012,** *487*, 308–9.

176. Kubota, H. Quality control against misfolded proteins in the cytosol: a network for cell survival. *J. Biochem.* **2009,** *146*, 609–16.

177. Xie, T.; Lim, S. M.; Westover, K. D.; Dodge, M. E.; Ercan, D.; Ficarro, S. B.; Udayakumar, D.; Gurbani, D.; Tae, H. S.; Riddle, S. M.; Sim, T.; Marto, J. A.; Janne, P. A.; Crews, C. M.; Gray, N. S. Pharmacological targeting of the pseudokinase Her3. *Nat. Chem. Biol.* **2014,** *10*, 1006–12.

178. Lim, S. M.; Xie, T.; Westover, K. D.; Ficarro, S. B.; Tae, H. S.; Gurbani, D.; Sim, T.; Marto, J. A.; Janne, P. A.; Crews, C. M.; Gray, N. S. Development of small molecules targeting the pseudokinase Her3. *Bioorg. Med. Chem. Lett.* **2015,** *25*, 3382–9.

179. Gustafson, J. L.; Neklesa, T. K.; Cox, C. S.; Roth, A. G.; Buckley, D. L.; Tae, H. S.; Sundberg, T. B.; Stagg, D. B.; Hines, J.; McDonnell, D. P.; Norris, J. D.; Crews, C. M. Small-Molecule-Mediated Degradation of the Androgen Receptor through Hydrophobic Tagging. *Angew. Chem.* **2015,** *54*, 9659–62.

180. Tae, H. S.; Sundberg, T. B.; Neklesa, T. K.; Noblin, D. J.; Gustafson, J. L.; Roth, A. G.; Raina, K.; Crews, C. M. Identification of hydrophobic tags for the degradation of stabilized proteins. *Chembiochem* **2012,** *13*, 538–41.

181. Cyrus, K.; Wehenkel, M.; Choi, E. Y.; Han, H. J.; Lee, H.; Swanson, H.; Kim, K. B. Impact of linker length on the activity of PROTACs. *Mol. Biosyst.* **2011,** *7*, 359–64.

182. Smith, B. E.; Wang, S. L.; Jaime-Figueroa, S.; Harbin, A.; Wang, J.; Hamman, B. D.; Crews, C. M. Differential PROTAC substrate specificity dictated by orientation of recruited E3 ligase. *Nat. Commun.* **2019,** *10*, 131.

183. Bondeson, D. P.; Smith, B. E.; Burslem, G. M.; Buhimschi, A. D.; Hines, J.; Jaime-Figueroa, S.; Wang, J.; Hamman, B. D.; Ishchenko, A.; Crews, C. M. Lessons in PROTAC Design from Selective Degradation with a Promiscuous Warhead. *Cell Chem. Biol.* **2018,** *25*, 78–87 e5.

184. Lewin, G. R.; Barde, Y. A. Physiology of the neurotrophins. *Annu. Rev. Neurosci.* **1996,** *19*, 289–317.

185. Huang, E. J.; Reichardt, L. F. Neurotrophins: roles in neuronal development and function. *Annu. Rev. Neurosci.* **2001,** *24*, 677–736.

186. Dechant, G.; Barde, Y. A. The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat. Neurosci.* **2002,** *5*, 1131–6.

187. Huang, E. J.; Reichardt, L. F. Trk receptors: roles in neuronal signal transduction. *Annu. Rev. Biochem.* **2003,** *72*, 609–42.

188. Kaplan, D. R.; Miller, F. D. Neurotrophin signal transduction in the nervous system. *Curr. Opin. Neurobiol.* **2000,** *10*, 381–91.

189. Chao, M. V. Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat. Rev. Neurosci.* **2003,** *4*, 299–309.

190. Chao, M. V.; Bothwell, M. Neurotrophins: to cleave or not to cleave. *Neuron* **2002,** *33*, 9–12.

191. Mowla, S. J.; Farhadi, H. F.; Pareek, S.; Atwal, J. K.; Morris, S. J.; Seidah, N. G.; Murphy, R. A. Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J. Biol. Chem.* **2001,** *276*, 12660–6.

192. Seidah, N. G.; Benjannet, S.; Pareek, S.; Chretien, M.; Murphy, R. A. Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. *FEBS Lett.* **1996,** *379*, 247–50.

193. Lu, B.; Pang, P. T.; Woo, N. H. The yin and yang of neurotrophin action. *Nat. Rev. Neurosci.* **2005,** *6*, 603–14.

194. Rodriguez-Tebar, A.; Dechant, G.; Barde, Y. A. Neurotrophins: structural relatedness and receptor interactions. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **1991,** *331*, 255–8.

195. Chao, M. V. The p75 neurotrophin receptor. *J. Neurobiol.* **1994,** *25*, 1373–85.

196. Liepinsh, E.; Ilag, L. L.; Otting, G.; Ibanez, C. F. NMR structure of the death domain of the p75 neurotrophin receptor. *EMBO J.* **1997,** *16*, 4999–5005.

197. Barrett, G. L.; Bartlett, P. F. The p75 nerve growth factor receptor mediates survival or death depending on the stage of sensory neuron development. *Proc. Natl. Acad. Sci. U.S.A* **1994,** *91*, 6501–5.

198. Rabizadeh, S.; Oh, J.; Zhong, L. T.; Yang, J.; Bitler, C. M.; Butcher, L. L.; Bredesen, D. E. Induction of apoptosis by the low-affinity NGF receptor. *Science* **1993,** *261*, 345–8.

199. Hamanoue, M.; Middleton, G.; Wyatt, S.; Jaffray, E.; Hay, R. T.; Davies, A. M. p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. *Mol. Cell Neurosci.* **1999,** *14*, 28–40.

200. Esposito, D.; Patel, P.; Stephens, R. M.; Perez, P.; Chao, M. V.; Kaplan, D. R.; Hempstead, B. L. The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. *J. Biol. Chem.* **2001,** *276*, 32687–95.

201. Hempstead, B. L.; Martin-Zanca, D.; Kaplan, D. R.; Parada, L. F.; Chao, M. V. High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature* **1991,** *350*, 678–83.

202. Nykjaer, A.; Lee, R.; Teng, K. K.; Jansen, P.; Madsen, P.; Nielsen, M. S.; Jacobsen, C.; Kliemannel, M.; Schwarz, E.; Willnow, T. E.; Hempstead, B. L.; Petersen, C. M. Sortilin is essential for proNGF-induced neuronal cell death. *Nature* **2004,** *427*, 843–8.

203. Lee, R.; Kermani, P.; Teng, K. K.; Hempstead, B. L. Regulation of cell survival by secreted proneurotrophins. *Science* **2001,** *294*, 1945–8.

204. Ibanez, C. F.; Simi, A. p75 neurotrophin receptor signaling in nervous system injury and degeneration: paradox and opportunity. *Trends Neurosci.* **2012,** *35*, 431–40.

205. Longo, F. M.; Massa, S. M. Small molecule modulation of p75 neurotrophin receptor functions. *CNS Neurol. Disord. Drug Targets* **2008,** *7*, 63–70.

206. Reichardt, L. F. Neurotrophin-regulated signalling pathways. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **2006,** *361*, 1545–64.

207. Salehi, A. H.; Roux, P. P.; Kubu, C. J.; Zeindler, C.; Bhakar, A.; Tannis, L. L.; Verdi, J. M.; Barker, P. A. NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. *Neuron* **2000,** *27*, 279–88.

208. Mukai, J.; Hachiya, T.; Shoji-Hoshino, S.; Kimura, M. T.; Nadano, D.; Suvanto, P.; Hanaoka, T.; Li, Y.; Irie, S.; Greene, L. A.; Sato, T. A. NADE, a p75NTR-associated cell death executor, is involved in signal transduction mediated by the common neurotrophin receptor p75NTR. *J. Biol. Chem.* **2000,** *275*, 17566–70.

209. Beattie, M. S.; Harrington, A. W.; Lee, R.; Kim, J. Y.; Boyce, S. L.; Longo, F. M.; Bresnahan, J. C.; Hempstead, B. L.; Yoon, S. O. ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron* **2002,** *36*, 375–86.

210. Syroid, D. E.; Maycox, P. J.; Soilu-Hanninen, M.; Petratos, S.; Bucci, T.; Burrola, P.; Murray, S.; Cheema, S.; Lee, K. F.; Lemke, G.; Kilpatrick, T. J. Induction of postnatal schwann cell death by the low-affinity neurotrophin receptor in vitro and after axotomy. *J. Neurosci.* **2000,** *20*, 5741–7.

211. Petratos, S.; Butzkueven, H.; Shipham, K.; Cooper, H.; Bucci, T.; Reid, K.; Lopes, E.; Emery, B.; Cheema, S. S.; Kilpatrick, T. J. Schwann cell apoptosis in the postnatal axotomized sciatic nerve is mediated via NGF through the low-affinity neurotrophin receptor. *J. Neuropathol. Exp. Neurol.* **2003,** *62*, 398–411.

212. Troy, C. M.; Friedman, J. E.; Friedman, W. J. Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *J. Biol. Chem.* **2002,** *277*, 34295–302.

213. Volosin, M.; Trotter, C.; Cragnolini, A.; Kenchappa, R. S.; Light, M.; Hempstead, B. L.; Carter, B. D.; Friedman, W. J. Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. *J. Neurosci.* **2008,** *28*, 9870–9.

214. Mufson, E. J.; Kordower, J. H. Cortical neurons express nerve growth factor receptors in advanced age and Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A* **1992,** *89*, 569–73.

215. Dowling, P.; Ming, X.; Raval, S.; Husar, W.; Casaccia-Bonnefil, P.; Chao, M.; Cook, S.; Blumberg, B. Up-regulated p75NTR neurotrophin receptor on glial cells in MS plaques. *Neurology* **1999,** *53*, 1676–82.

216. Harrington, A. W.; Leiner, B.; Blechschmitt, C.; Arevalo, J. C.; Lee, R.; Morl, K.; Meyer, M.; Hempstead, B. L.; Yoon, S. O.; Giehl, K. M. Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. *Proc. Natl. Acad. Sci. U.S.A* **2004,** *101*, 6226–30.

217. McDonald, N. Q.; Chao, M. V. Structural determinants of neurotrophin action. *J. Biol. Chem.* **1995,** *270*, 19669–72.

218. Wehrman, T.; He, X.; Raab, B.; Dukipatti, A.; Blau, H.; Garcia, K. C. Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. *Neuron* **2007,** *53*, 25–38.

219. Covaceuszach, S.; Cassetta, A.; Konarev, P. V.; Gonfloni, S.; Rudolph, R.; Svergun, D. I.; Lamba, D.; Cattaneo, A. Dissecting NGF interactions with TrkA and p75 receptors by structural and functional studies of an anti-NGF neutralizing antibody. *J. Mol. Biol.* **2008,** *381*, 881–96.

220. Robinson, R. C.; Radziejewski, C.; Stuart, D. I.; Jones, E. Y. Structure of the brain-derived neurotrophic factor/neurotrophin 3 heterodimer. *Biochemistry* **1995,** *34*, 4139–46.

221. Large, T. H.; Weskamp, G.; Helder, J. C.; Radeke, M. J.; Misko, T. P.; Shooter, E. M.; Reichardt, L. F. Structure and developmental expression of the nerve growth factor receptor in the chicken central nervous system. *Neuron* **1989,** *2*, 1123–34.

222. Yan, H.; Chao, M. V. Disruption of cysteine-rich repeats of the p75 nerve growth factor receptor leads to loss of ligand binding. *J. Biol. Chem.* **1991,** *266*, 12099–104.

223. Smith, C. A.; Farrah, T.; Goodwin, R. G. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell* **1994,** *76*, 959–62.

224. Baldwin, A. N.; Bitler, C. M.; Welcher, A. A.; Shooter, E. M. Studies on the structure and binding properties of the cysteine-rich domain of rat low affinity nerve growth factor receptor (p75NGFR). *J. Biol. Chem.* **1992,** *267*, 8352–9.

225. Feinstein, E.; Kimchi, A.; Wallach, D.; Boldin, M.; Varfolomeev, E. The death domain: a module shared by proteins with diverse cellular functions. *Trends Biochem. Sci.* **1995,** *20*, 342–4.

226. Huang, B.; Eberstadt, M.; Olejniczak, E. T.; Meadows, R. P.; Fesik, S. W. NMR structure and mutagenesis of the Fas (APO-1/CD95) death domain. *Nature* **1996,** *384*, 638–41.

227. Gong, Y.; Cao, P.; Yu, H. J.; Jiang, T. Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex. *Nature* **2008,** *454*, 789–93.

228. Feng, D.; Kim, T.; Ozkan, E.; Light, M.; Torkin, R.; Teng, K. K.; Hempstead, B. L.; Garcia, K. C. Molecular and structural insight into proNGF engagement of p75NTR and sortilin. *J. Mol. Biol.* **2010,** *396*, 967–84.

229. He, X. L.; Garcia, K. C. Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. *Science* **2004,** *304*, 870–5.

230. Longo, F. M.; Vu, T. K.; Mobley, W. C. The in vitro biological effect of nerve growth factor is inhibited by synthetic peptides. *Cell Regul.* **1990,** *1*, 189–95.

231. Ibanez, C. F.; Ebendal, T.; Barbany, G.; Murray-Rust, J.; Blundell, T. L.; Persson, H. Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product. *Cell* **1992,** *69*, 329–41.

232. Kahle, P.; Barker, P. A.; Shooter, E. M.; Hertel, C. p75 nerve growth factor receptor modulates p140trkA kinase activity, but not ligand internalization, in PC12 cells. *J. Neurosci. Res.* **1994,** *38*, 599–606.

233. Josephy-Hernandez, S.; Jmaeff, S.; Pirvulescu, I.; Aboulkassim, T.; Saragovi, H. U. Neurotrophin receptor agonists and antagonists as therapeutic agents: An evolving paradigm. *Neurobiol. Dis.* **2017,** *97*, 139–155.

234. Longo, F. M.; Manthorpe, M.; Xie, Y. M.; Varon, S. Synthetic NGF peptide derivatives prevent neuronal death via a p75 receptor-dependent mechanism. *J. Neurosci. Res.* **1997,** *48*, 1–17.

235. LeSauteur, L.; Wei, L.; Gibbs, B. F.; Saragovi, H. U. Small peptide mimics of nerve growth factor bind TrkA receptors and affect biological responses. *J. Biol. Chem.* **1995,** *270*, 6564–9.

236. Garber, K. Fate of novel painkiller mAbs hangs in balance. *Nat. Biotechnol.* **2011,** *29*, 173–4.

237. Abdiche, Y. N.; Malashock, D. S.; Pons, J. Probing the binding mechanism and affinity of tanezumab, a recombinant humanized anti-NGF monoclonal antibody, using a repertoire of biosensors. *Protein Sci.* **2008,** *17*, 1326–35.

238. Lane, N. E.; Schnitzer, T. J.; Birbara, C. A.; Mokhtarani, M.; Shelton, D. L.; Smith, M. D.; Brown, M. T. Tanezumab for the treatment of pain from osteoarthritis of the knee. *N. Engl. J. Med.* **2010,** *363*, 1521–31.

239. Kumar, V.; Mahal, B. A. NGF - the TrkA to successful pain treatment. *J. Pain Res.* **2012,** *5*, 279–87.

240. Spiegel, K.; Agrafiotis, D.; Caprathe, B.; Davis, R. E.; Dickerson, M. R.; Fergus, J. H.; Hepburn, T. W.; Marks, J. S.; Van Dorf, M.; Wieland, D. M.; et al. PD 90780, a non peptide inhibitor of nerve growth factor's binding to the P75 NGF receptor. *Biochem. Biophys. Res. Commun.* **1995,** *217*, 488–94.

241. Colquhoun, A.; Lawrance, G. M.; Shamovsky, I. L.; Riopelle, R. J.; Ross, G. M. Differential activity of the nerve growth factor (NGF) antagonist PD90780 [7-(benzolylamino)-4,9-dihydro-4-methyl-9-oxo-pyrazolo[5,1-b]quinazoline-2-carboxylic acid] suggests altered NGF-p75(NTR) interactions in the presence of TrkA. *J Pharmacol Exp Ther* **2004,** *310*, 505–511.

242. Colquhoun, A.; Lawrance, G. M.; Shamovsky, I. L.; Riopelle, R. J.; Ross, G. M. Differential activity of the nerve growth factor (NGF) antagonist PD90780 [7-(benzolylamino)-4,9-dihydro-4-methyl-9-oxo-pyrazolo[5,1-b]quinazoline-2-carbox ylic acid] suggests altered NGF-p75NTR interactions in the presence of TrkA. *The Journal of pharmacology and experimental therapeutics* **2004,** *310*, 505-11.

243. Jaen, J. C.; Laborde, E.; Bucsh, R. A.; Caprathe, B. W.; Sorenson, R. J.; Fergus, J.; Spiegel, K.; Marks, J.; Dickerson, M. R.; Davis, R. E. Kynurenic Acid-Derivatives Inhibit the Binding of Nerve Growth-Factor (Ngf) to the Low-Affinity P75 Ngf Receptor. *J. Med. Chem.* **1995,** *38*, 4439–4445.

244. Jaen, J. C.; Laborde, E.; Bucsh, R. A.; Caprathe, B. W.; Sorenson, R. J.; Fergus, J.; Spiegel, K.; Marks, J.; Dickerson, M. R.; Davis, R. E. Kynurenic acid derivatives inhibit the binding of nerve growth factor (NGF) to the low-affinity p75 NGF receptor. *J. Med. Chem.* **1995,** *38*, 4439–45.

245. Niederhauser, O.; Mangold, M.; Schubenel, R.; Kusznir, E. A.; Schmidt, D.; Hertel, C. NGF ligand alters NGF signaling via p75(NTR) and trkA. *J. Neurosci. Res.* **2000,** *61*, 263–72.

246. Eibl, J. K.; Chapelsky, S. A.; Ross, G. M. Multipotent neurotrophin antagonist targets brain-derived neurotrophic factor and nerve growth factor. *The Journal of pharmacology and experimental therapeutics* **2010,** *332*, 446–54.

247. Owolabi, J. B.; Rizkalla, G.; Tehim, A.; Ross, G. M.; Riopelle, R. J.; Kamboj, R.; Ossipov, M.; Bian, D.; Wegert, S.; Porreca, F.; Lee, D. K. Characterization of antiallodynic actions of ALE-0540, a novel nerve growth factor receptor antagonist, in the rat. *The Journal of pharmacology and experimental therapeutics* **1999,** *289*, 1271–6.

248. Massa, S. M.; Xie, Y.; Yang, T.; Harrington, A. W.; Kim, M. L.; Yoon, S. O.; Kraemer, R.; Moore, L. A.; Hempstead, B. L.; Longo, F. M. Small, nonpeptide p75NTR ligands induce survival signaling and inhibit proNGF-induced death. *J. Neurosci.* **2006,** *26*, 5288–300.

249. Longo, F. M. Preparation of peptide amide, amino acid amide, and 2-heterocyclylacetamide derivatives as neurotrophin mimetics and uses thereof screening. WO2010102212A2, 2010.

250. Shi, J.; Longo, F. M.; Massa, S. M. A small molecule p75(NTR) ligand protects neurogenesis after traumatic brain injury. *Stem Cells* **2013,** *31*, 2561–74.

251. Simmons, D. A.; Knowles, J. K.; Belichenko, N. P.; Banerjee, G.; Finkle, C.; Massa, S. M.; Longo, F. M. A small molecule p75NTR ligand, LM11A-31, reverses cholinergic neurite dystrophy in Alzheimer's disease mouse models with mid- to late-stage disease progression. *PloS one* **2014,** *9*, e102136.

252. Hara, Y.; McKeehan, N.; Fillit, H. M. Translating the biology of aging into novel therapeutics for Alzheimer disease. *Neurology* **2019,** *92*, 84–93.

253. Knowles, J. K.; Simmons, D. A.; Nguyen, T. V.; Vander Griend, L.; Xie, Y.; Zhang, H.; Yang, T.; Pollak, J.; Chang, T.; Arancio, O.; Buckwalter, M. S.; Wyss-Coray, T.; Massa, S. M.; Longo, F. M. Small molecule p75NTR ligand prevents cognitive deficits and neurite degeneration in an Alzheimer's mouse model. *Neurobiol. Aging* **2013,** *34*, 2052–63.

254. Tep, C.; Lim, T. H.; Ko, P. O.; Getahun, S.; Ryu, J. C.; Goettl, V. M.; Massa, S. M.; Basso, M.; Longo, F. M.; Yoon, S. O. Oral administration of a small molecule targeted to block proNGF binding to p75 promotes myelin sparing and functional recovery after spinal cord injury. *J. Neurosci.* **2013,** *33*, 397–410.

255. Yang, T.; Knowles, J. K.; Lu, Q.; Zhang, H.; Arancio, O.; Moore, L. A.; Chang, T.; Wang, Q.; Andreasson, K.; Rajadas, J.; Fuller, G. G.; Xie, Y.; Massa, S. M.; Longo, F. M. Small molecule, non-peptide p75 ligands inhibit Abeta-induced neurodegeneration and synaptic impairment. *PloS one* **2008,** *3*, e3604.

256. Zabbarova, I. V.; Ikeda, Y.; Carder, E. J.; Wipf, P.; Wolf-Johnston, A. S.; Birder, L. A.; Yoshimura, N.; Getchell, S. E.; Almansoori, K.; Tyagi, P.; Fry, C. H.; Drake, M. J.; Kanai, A. J. Targeting p75 neurotrophin receptors ameliorates spinal cord injury-induced detrusor sphincter dyssynergia in mice. *Neurourol Urodyn.* **2018,** *37*, 2452–2461.

257. Basavaprabhu; Vishwanatha, T. M.; Panguluri, N. R.; Sureshbabu, V. V. Propanephosphonic Acid Anhydride (T3P (R)) - A Benign Reagent for Diverse Applications Inclusive of Large-Scale Synthesis. *Synthesis-Stuttgart* **2013,** *45*, 1569–1601.

258. Koppenhoefer, B.; Schurig, V. (S)-2-Chloroalkanoic Acids of High Enantiomeric Purity from (S)-2-Amino Acids - (S)-2-Chloropropanoic Acid. *Org Synth* **1988,** *66*, 151–159.

259. Fu, S. C. J.; Birnbaum, S. M.; Greenstein, J. P. Influence of Optically Active Acyl Groups on the Enzymatic Hydrolysis of N-Acylated-L-Amino Acids. *J. Am. Chem. Soc.* **1954,** *76*, 6054–6058.

260. Lazar, C.; Kluczyk, A.; Kiyota, T.; Konishi, Y. Drug evolution concept in drug design: 1. Hybridization method. *J. Med. Chem.* **2004,** *47*, 6973–6982.

261. Pierce, A. C.; Rao, G.; Bemis, G. W. BREED: Generating novel inhibitors through hybridization of known ligands. Application to CDK2, P38, and HIV protease. *J. Med. Chem.* **2004,** *47*, 2768–2775.

262. Anderson, M.; Beattie, J. F.; Breault, G. A.; Breed, J.; Byth, K. F.; Culshaw, J. D.; Ellston, R. P. A.; Green, S.; Minshull, C. A.; Norman, R. A.; Pauptit, R. A.; Stanway, J.; Thomas, A. P.; Jewsbury, P. J. Imidazo[1,2-a]pyridines: A potent and selective class of cyclin-dependent kinase inhibitors identified through structure-based hybridisation. *Bioorg. Med. Chem. Lett.* **2003,** *13*, 3021–3026.

263. Farmer, L. J.; Ledeboer, M. W.; Hoock, T.; Arnost, M. J.; Bethiel, R. S.; Bennani, Y. L.; Black, J. J.; Brummel, C. L.; Chakilam, A.; Dorsch, W. A.; Fan, B.; Cochran, J. E.; Halas, S.; Harrington, E. M.; Hogan, J. K.; Howe, D.; Huang, H.; Jacobs, D. H.; Laitinen, L. M.; Liao, S. K.; Mahajan, S.; Marone, V.; Martinez-Botella, G.; McCarthy, P.; Messersmith, D.; Namchuk, M.; Oh, L.; Penney, M. S.; Pierce, A. C.; Raybuck, S. A.; Rugg, A.; Salituro, F. G.; Saxena, K.; Shannon, D.; Shlyakter, D.; Swenson, L.; Tian, S. K.; Town, C.; Wang, J.; Wang, T. S.; Wannamaker, M. W.; Winquist, R. J.; Zuccola, H. J. Discovery of VX-509 (Decernotinib): A Potent and Selective Janus Kinase 3 Inhibitor for the Treatment of Autoimmune Diseases. *J. Med. Chem.* **2015,** *58*, 7195–7216.

264. Uetrecht, J. P.; Trager, W. *Drug metabolism : chemical and enzymatic aspects*. Informa Healthcare: New York, **2007**; p v, 173 p.

265. Meyer, A.; Taige, M. A.; Strassner, T. Chiral bisimidazolium salts derived from amino acids and their palladium(II)- and platinum(II)-biscarbene complexes. *J Organomet Chem* **2009,** *694*, 1861–1868.

266. Di, L. Strategic Approaches to Optimizing Peptide ADME Properties. *AAPS J.* **2015,** *17*, 134–143.

267. Borg, S.; Estennebouhtou, G.; Luthman, K.; Csoregh, I.; Hesselink, W.; Hacksell, U. Synthesis of 1,2,4-Oxadiazole-Derived, 1,3,4-Oxadiazole-Derived, and 1,2,4-Triazole-Derived Dipeptidomimetics. *J. Org. Chem.* **1995,** *60*, 3112–3120.

268. Thompson, S. K.; Murthy, K. H. M.; Zhao, B.; Winborne, E.; Green, D. W.; Fisher, S. M.; Desjarlais, R. L.; Tomaszek, T. A.; Meek, T. D.; Gleason, J. G.; Abdelmeguid, S. S. Rational Design, Synthesis, and Crystallographic Analysis of a Hydroxyethylene-Based Hiv-1 Protease Inhibitor Containing a Heterocyclic P-1' P-2' Amide Bond Isostere. *J. Med. Chem.* **1994,** *37*, 3100–3107.

269. Gordon, T. D.; Singh, J.; Hansen, P. E.; Morgan, B. A. Synthetic Approaches to the Azole Peptide Mimetics. *Tetrahedron Lett.* **1993,** *34*, 1901–1904.

270. Gordon, T.; Hansen, P.; Morgan, B.; Singh, J.; Baizman, E.; Ward, S. Peptide Azoles - a New Class of Biologically-Active Dipeptide Mimetics. *Bioorg. Med. Chem. Lett.* **1993,** *3*, 915–920.

271. Freeman, F.; Kim, D. S. H. L. Preparation of 2-Alkyl-5-Amino-4-Cyano-1,3-Oxazoles and 2-Aryl-5-Amino-4-Cyano-1,3-Oxazoles. *Tetrahedron Lett.* **1989,** *30*, 2631–2632.

272. Rai, G.; Joshi, N.; Jung, J. E.; Liu, Y.; Schultz, L.; Yasgar, A.; Perry, S.; Diaz, G.; Zhang, Q. L.; Kenyon, V.; Jadhav, A.; Simeonov, A.; Lo, E. H.; van Leyen, K.; Maloney, D. J.; Holman, T. R. Potent and Selective Inhibitors of Human Reticulocyte 12/15-Lipoxygenase as Anti-Stroke Therapies. *J. Med. Chem.* **2014,** *57*, 4035–4048.

273. Jin, Z. Muscarine, imidaozle, oxazole and thiazole alkaloids. *Nat. Prod. Rep.* **2013,** *30*, 869–915.

274. Verrier, C.; Lassalas, P.; Theveau, L.; Queguiner, G.; Trecourt, F.; Marsais, F.; Hoarau, C. Recent advances in direct C-H arylation: Methodology, selectivity and mechanism in oxazole series. *Beilstein J. Org. Chem.* **2011,** *7*, 1584–1601.

275. Verrier, C.; Lassalas, P.; Theveau, L.; Queguiner, G.; Trecourt, F.; Marsais, F.; Hoarau, C. Recent advances in direct C-H arylation: Methodology, selectivity and mechanism in oxazole series. *Beilstein J Org Chem* **2011,** *7*, 1584-601.

276. Matsuda, N.; Hirano, K.; Satoh, T.; Miura, M. Copper-catalyzed direct amination of electron-deficient arenes with hydroxylamines. *Org. Lett.* **2011,** *13*, 2860–3.

277. Kuang, J.; Xia, Y.; Yang, A.; Zhang, H.; Su, C.; Lee, D. Copper-catalyzed aminothiolation of terminal alkynes with tunable regioselectivity. *Chem. Commun.* **2019,** *55*, 1813–1816.

278. Haas, D.; Mosrin, M.; Knochel, P. Regioselective functionalization of the oxazole scaffold using TMP-bases of Mg and Zn. *Org. Lett.* **2013,** *15*, 6162–5.

279. Pirrung, M. C.; Ghorai, S. Versatile, fragrant, convertible isonitriles. *J. Am. Chem. Soc.* **2006,** *128*, 11772–11773.

280. Pirrung, M. C.; Ghorai, S.; Ibarra-Rivera, T. R. Multicomponent Reactions of Convertible Isonitriles. *J. Org. Chem.* **2009,** *74*, 4110–4117.

281. Whitney, S. E.; Rickborn, B. Substituted Oxazoles - Syntheses Via Lithio Intermediates. *J. Org. Chem.* **1991,** *56*, 3058–3063.

282. Kondo, Y.; Shilai, M.; Uchiyama, M.; Sakamoto, T. TMP−Zincate as Highly Chemoselective Base for Directed Ortho Metalation. *J. Am. Chem. Soc.* **1999,** *121*, 3539–3540.

283. Snieckus, V. Directed Ortho Metalation - Tertiary Amide and O-Carbamate Directors in Synthetic Strategies for Polysubstituted Aromatics. *Chemical Reviews* **1990,** *90*, 879–933.

284. Beak, P.; Meyers, A. I. Stereocontrol and Regiocontrol by Complex Induced Proximity Effects - Reactions of Organolithium Compounds. *Acc. Chem. Res.* **1986,** *19*, 356–363.

285. Schlosser, M. The 2x3 toolbox of organometallic methods for regiochemically exhaustive functionalization. *Angew. Chem.* **2005,** *44*, 376–393.

286. Mosrin, M.; Knochel, P. TMPZnCl center dot LiCl: A New Active Selective Base for the Directed Zincation of Sensitive Aromatics and Heteroaromatics. *Org. Lett.* **2009,** *11*, 1837–1840.

287. Balc*, M. *Basic p1 sH- and p13 sC-NMR spectroscopy*. 1st ed.; Elsevier: Amsterdam ; Boston, **2005**; p xii, 427 p.

288. Seko, S.; Kawamura, N. Copper-Catalyzed Direct Amination of Nitrobenzenes with O-Alkylhydroxylamines. *J. Org. Chem.* **1996,** *61*, 442–443.

289. Yan, X.; Yang, X.; Xi, C. Recent progress in copper-catalyzed electrophilic amination. *Catal. Sci. Technol.* **2014,** *4*, 4169–4177.

290. Verrier, C.; Martin, T.; Hoarau, C.; Marsais, F. Palladium-catalyzed direct (hetero)arylation of ethyl oxazole-4-carboxylate: An efficient access to (hetero)aryloxazoles. *J. Org. Chem.* **2008,** *73*, 7383–7386.

291. Hoarau, C.; de Kerdaniel, A. D. F.; Bracq, N.; Grandclaudon, P.; Couture, A.; Marsais, F. Regioselective palladium-catalyzed phenylation of ethyl 4-oxazolecarboxylate. *Tetrahedron Lett.* **2005,** *46*, 8573–8577.

292. Beneteau, R.; Despiau, C. F.; Rouaud, J. C.; Boussonniere, A.; Silvestre, V.; Lebreton, J.; Denes, F. Synthesis of Polysubstituted -Butenolides via a Radical Pathway: Cyclization of -Bromo Aluminium Acetals and Comparison with the Cyclization of -Bromoesters at High Temperature. *Chem.: Eur. J.* **2015,** *21*, 11378–11386.

293. Elgersma, R. C.; Meijneke, T.; Posthuma, G.; Rijkers, D. T. S.; Liskamp, R. M. J. Self-assembly of amylin(20-29) amide-bond derivatives into helical ribbons and peptide nanotubes rather than fibrils. *Chem.: Eur. J.* **2006,** *12*, 3714–3725.

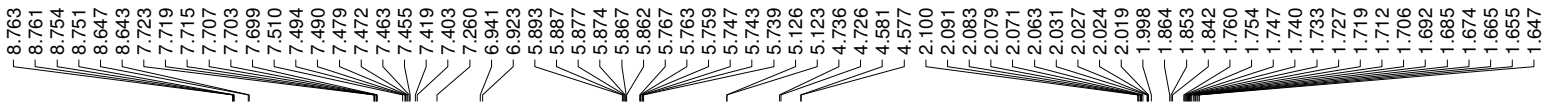
294. Ko, E. W.; Burgess, K. Pyrrole-Based Scaffolds for Turn Mimics. *Org. Lett.* **2011,** *13*, 980–983.

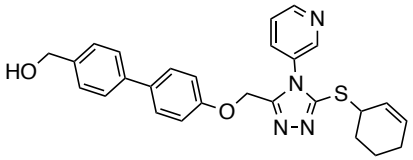
295. Moore, C. L.; Leatherwood, D. D.; Diehl, T. S.; Selkoe, D. J.; Wolfe, M. S. Difluoro ketone peptidomimetics suggest a large S1 pocket for Alzheimer's gamma-secretase: Implications for inhibitor design. *J. Med. Chem.* **2000,** *43*, 3434–3442.

296. Hernandez-Fernandez, E.; Sanchez-Lara, P. P.; Ordonez, M.; Ramirez-Marroquin, O. A.; Avalos-Alanis, F. G.; Lopez-Cortina, S.; Jimenez-Perez, V. M.; Ibarra-Rivera, T. R. Synthesis of beta-hydroxyacetamides from unactivated ethyl acetates under base-free conditions and microwave irradiation. *Tetrahedron-Asymmetry* **2015,** *26*, 73–78.

297. Spencer, J.; Patel, H.; Amin, J.; Callear, S. K.; Coles, S. J.; Deadman, J. J.; Furman, C.; Mansouri, R.; Chavatte, P.; Millet, R. Microwave-mediated synthesis and manipulation of a 2-substituted-5-aminooxazole-4-carbonitrile library. *Tetrahedron Lett.* **2012,** *53*, 1656–1659.

Spectra





1-42a

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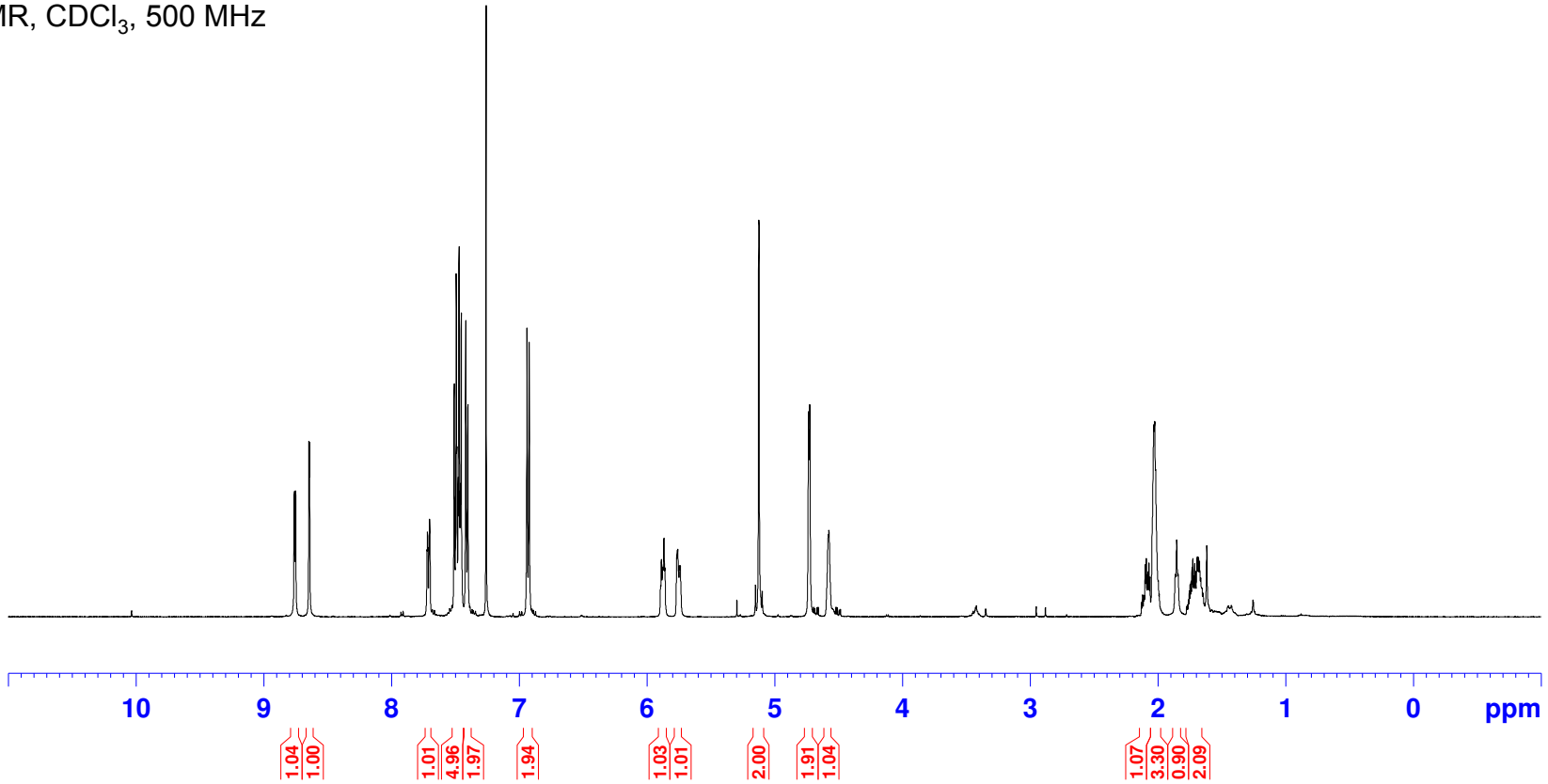

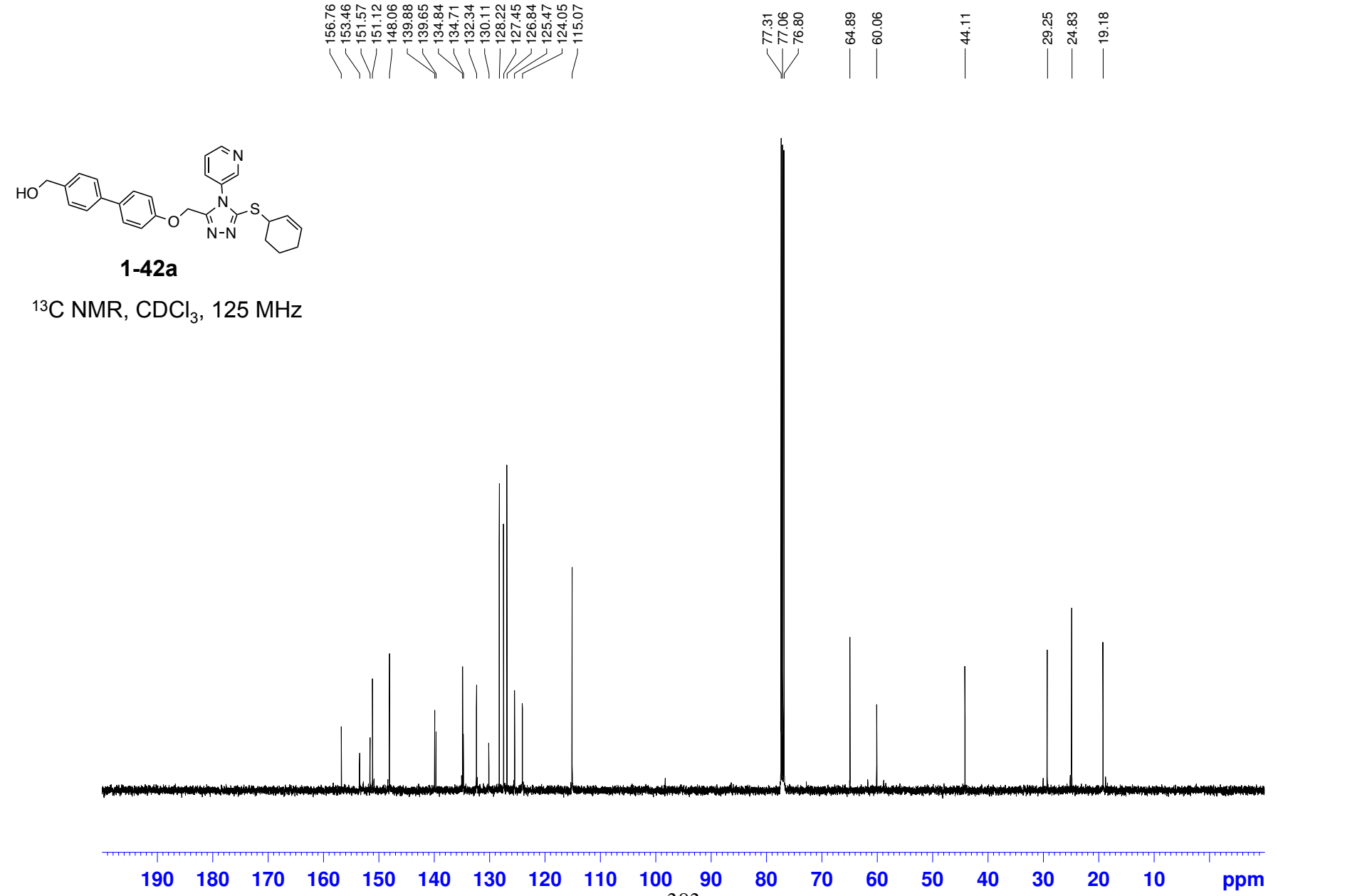
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124.05
115.07
77.31
77.06
76.80
64.89
60.06
44.11
29.25
24.83
19.18
HO
O
N
N
N-N
S
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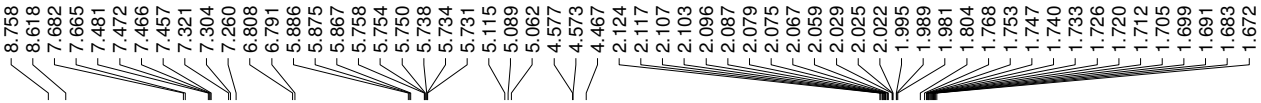

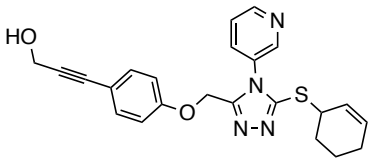

1-42b

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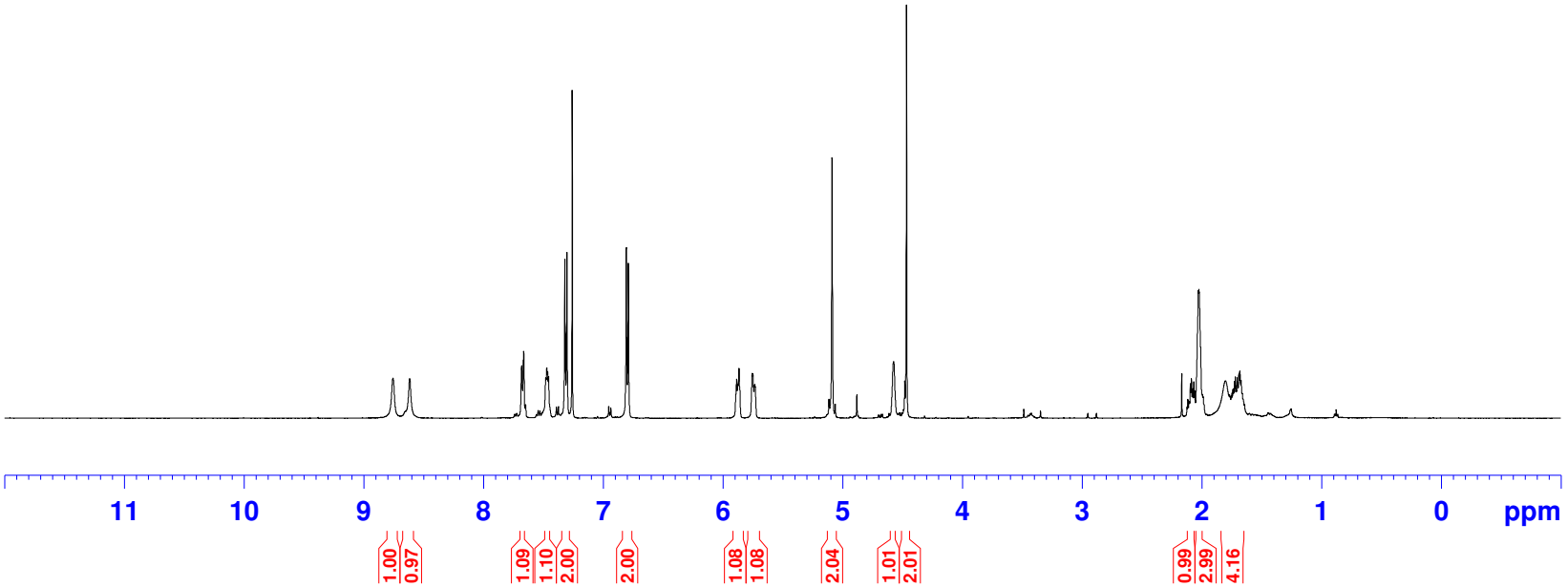

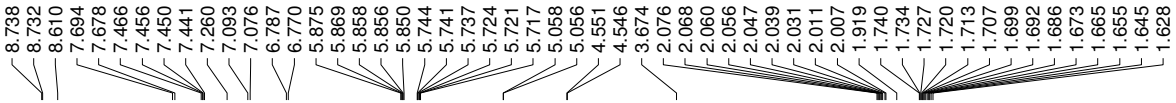

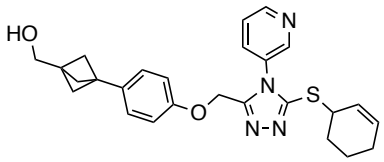

1-42c

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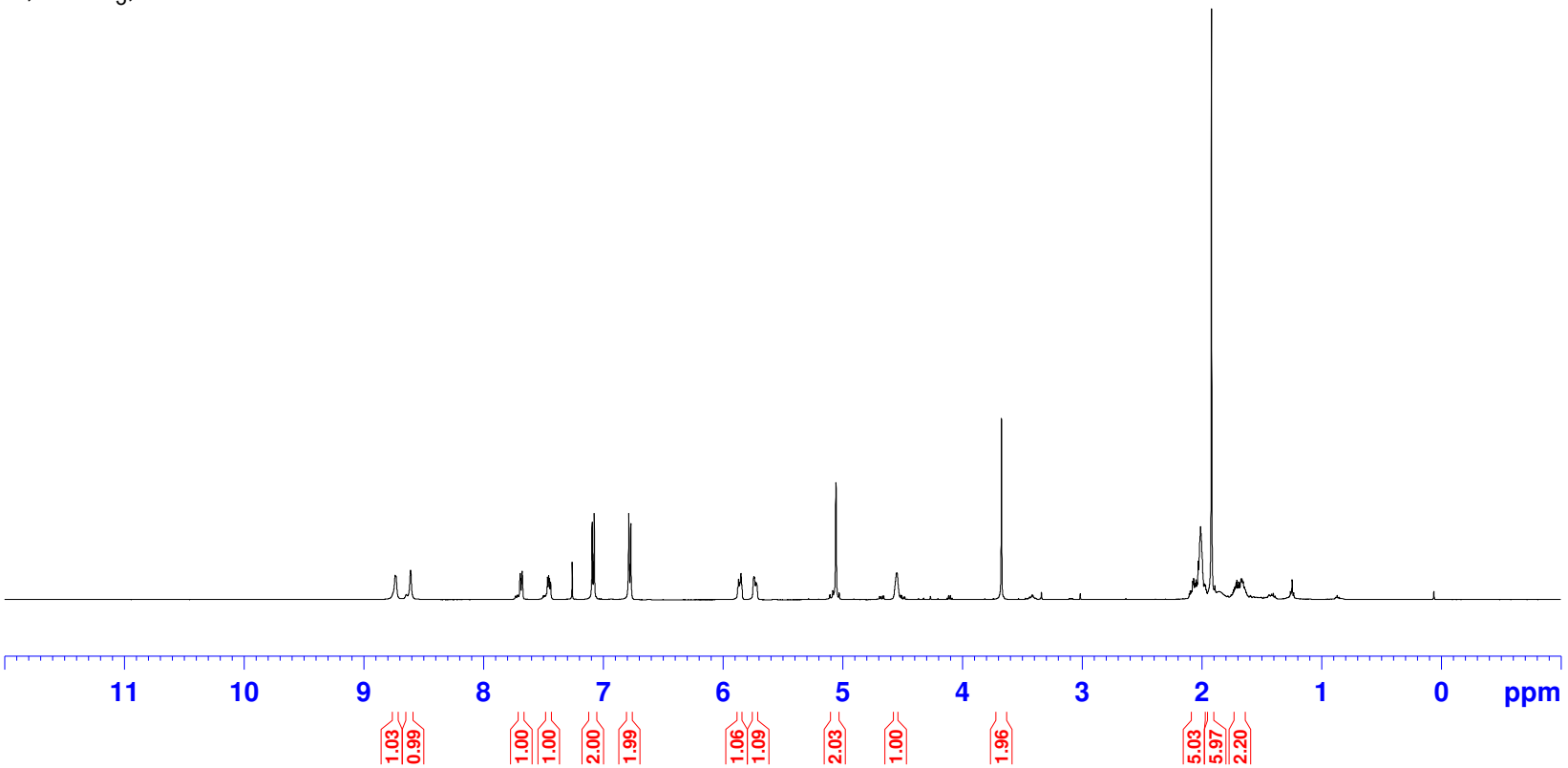

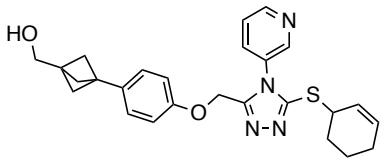

1-42c

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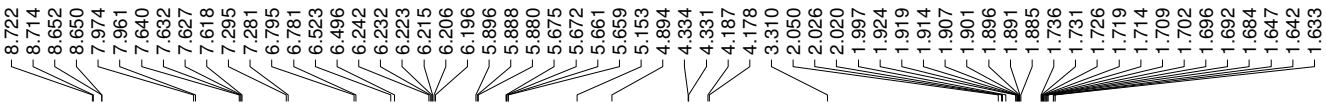

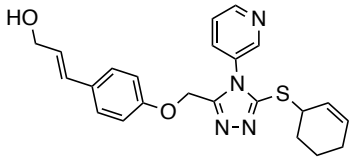

1-42D

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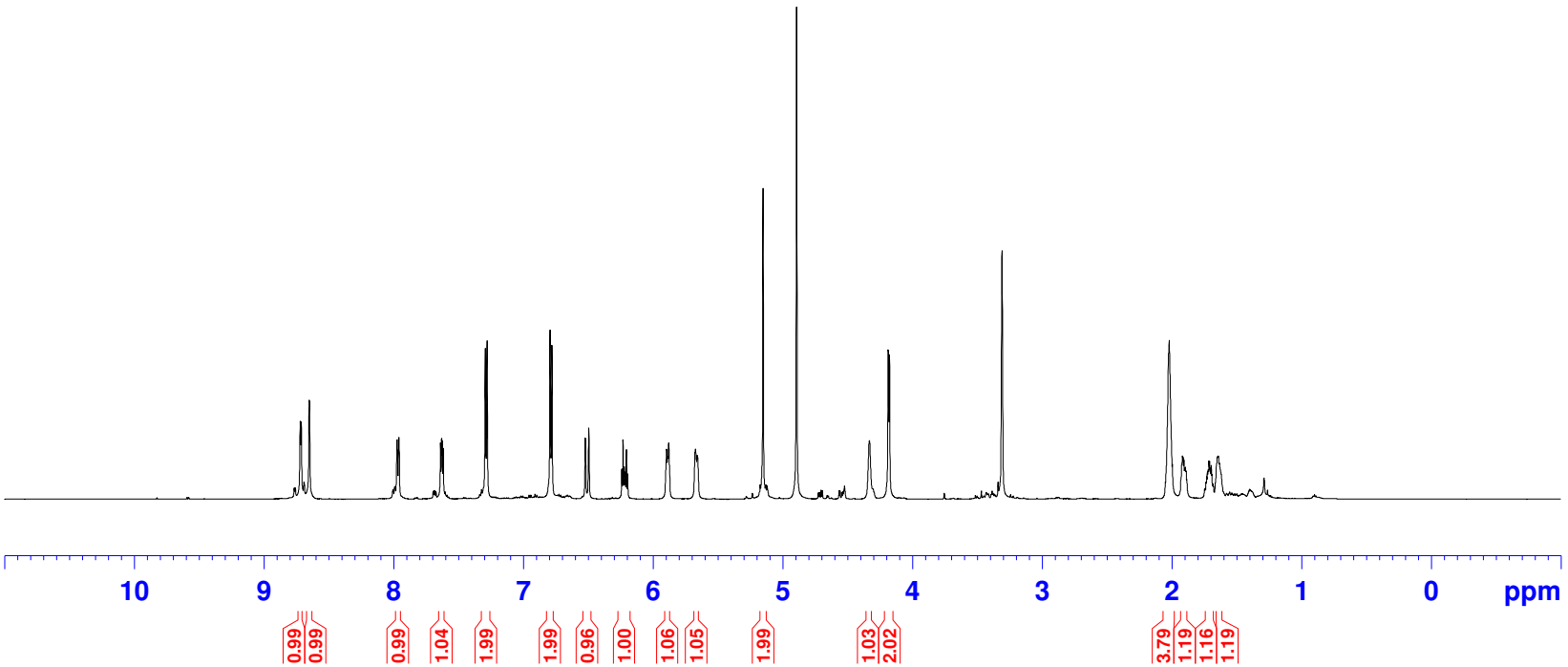

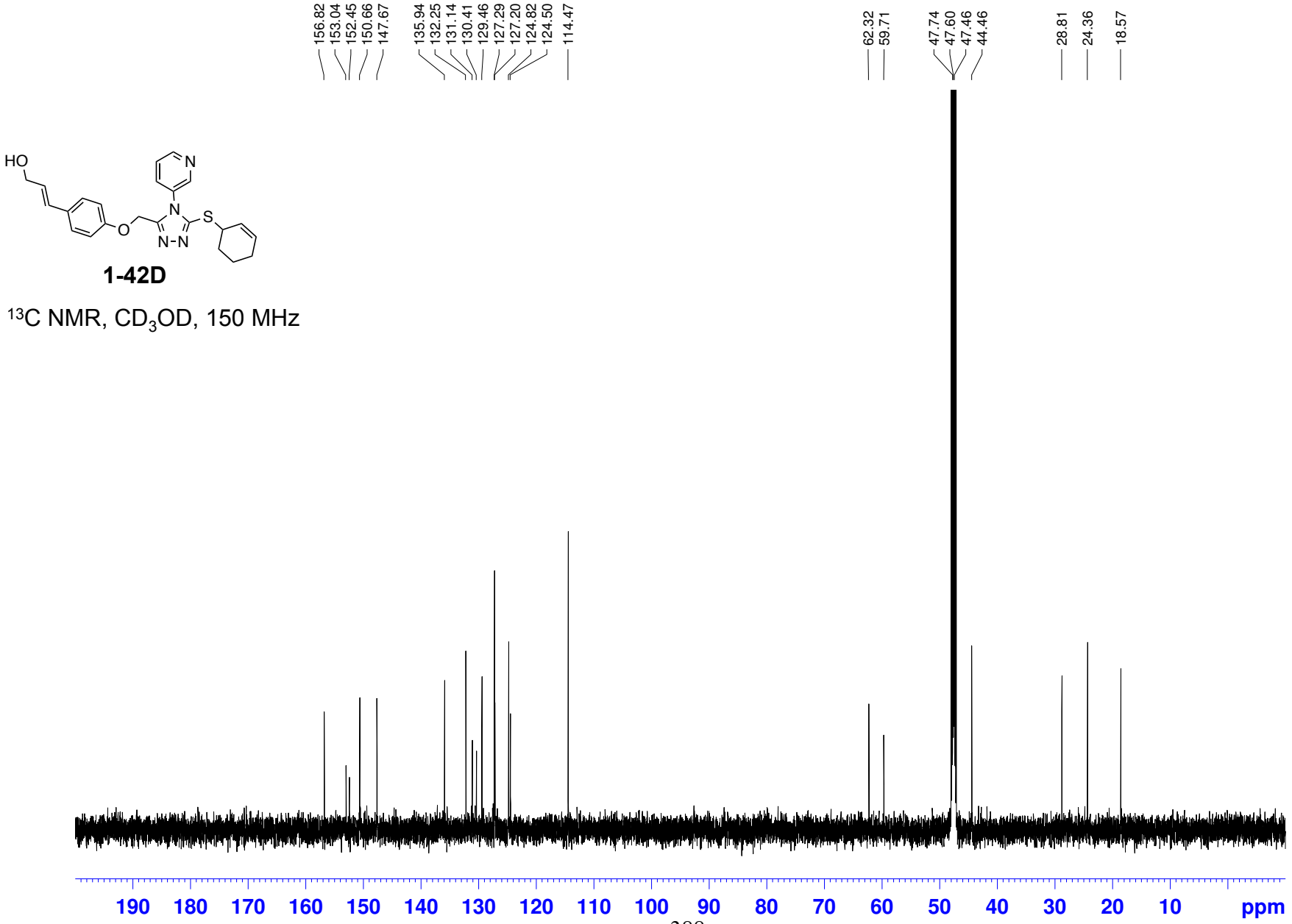

1-42D

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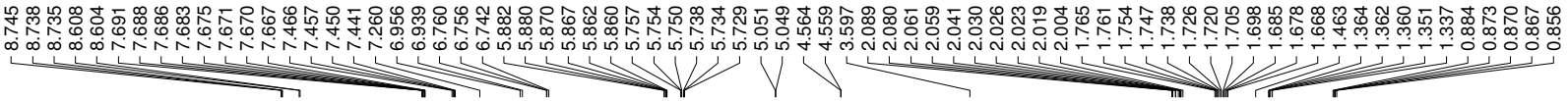
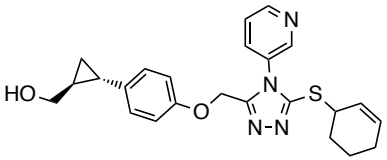
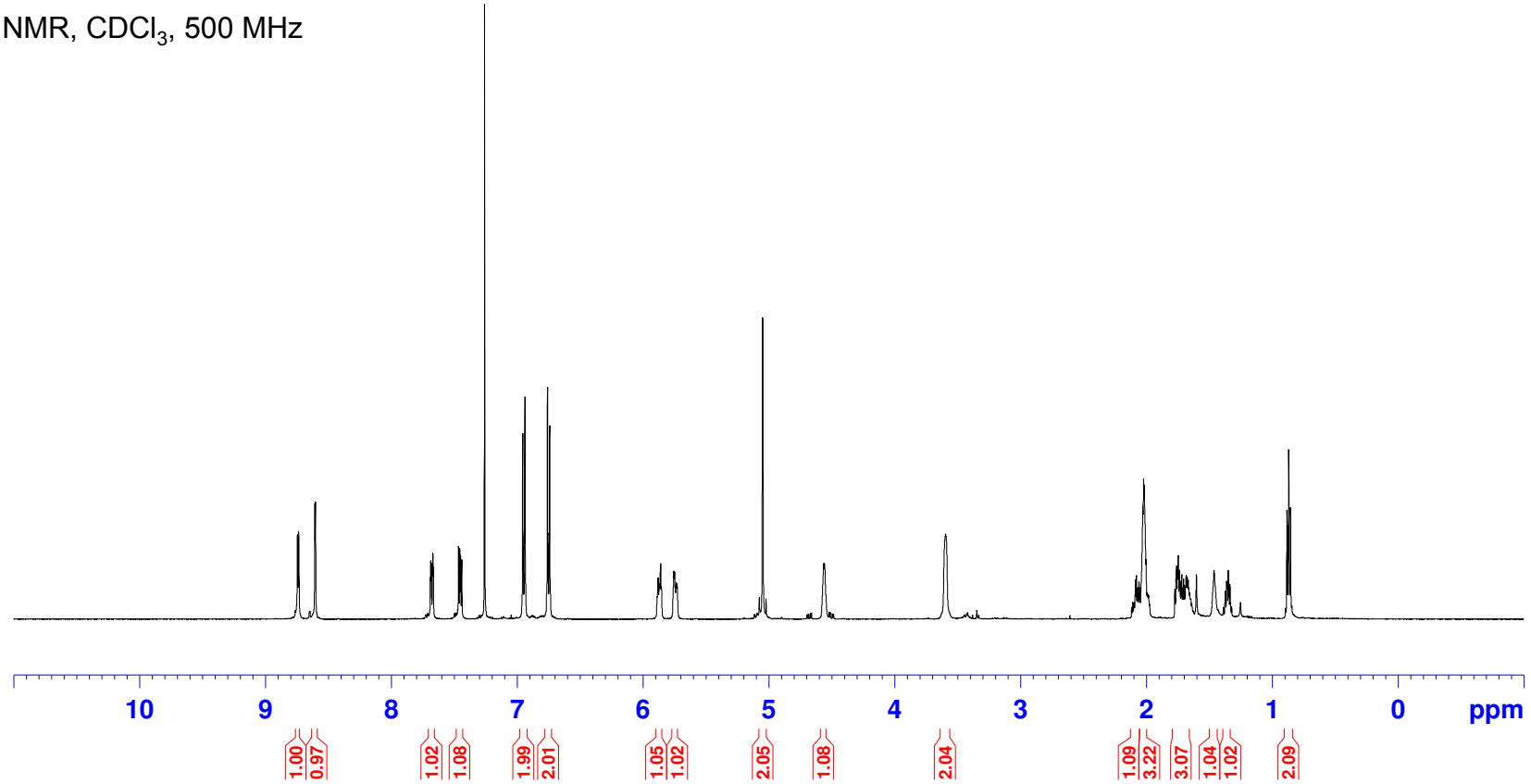
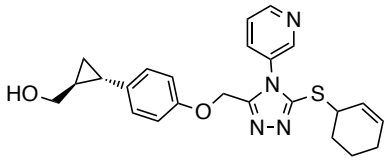




1-42E

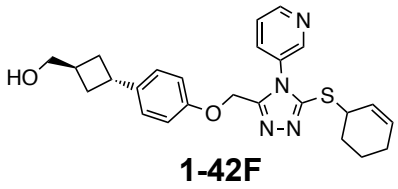
^{1}H NMR, $CDCl_3$, 500 MHz





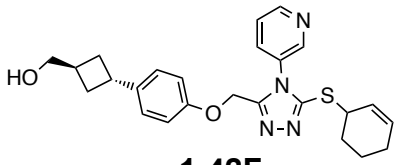
1-42E

^{13}C NMR, $CDCl_3$, 125 MHz



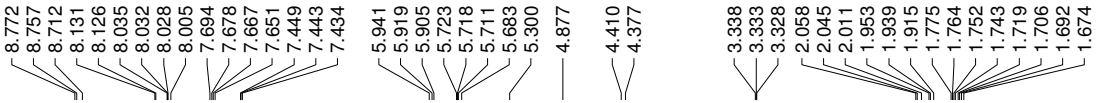
1-42F

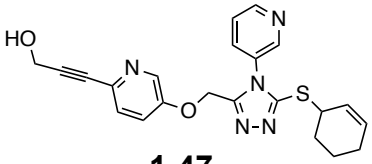
^{1}H NMR, $CDCl_3$, 500 MHz



1-42F

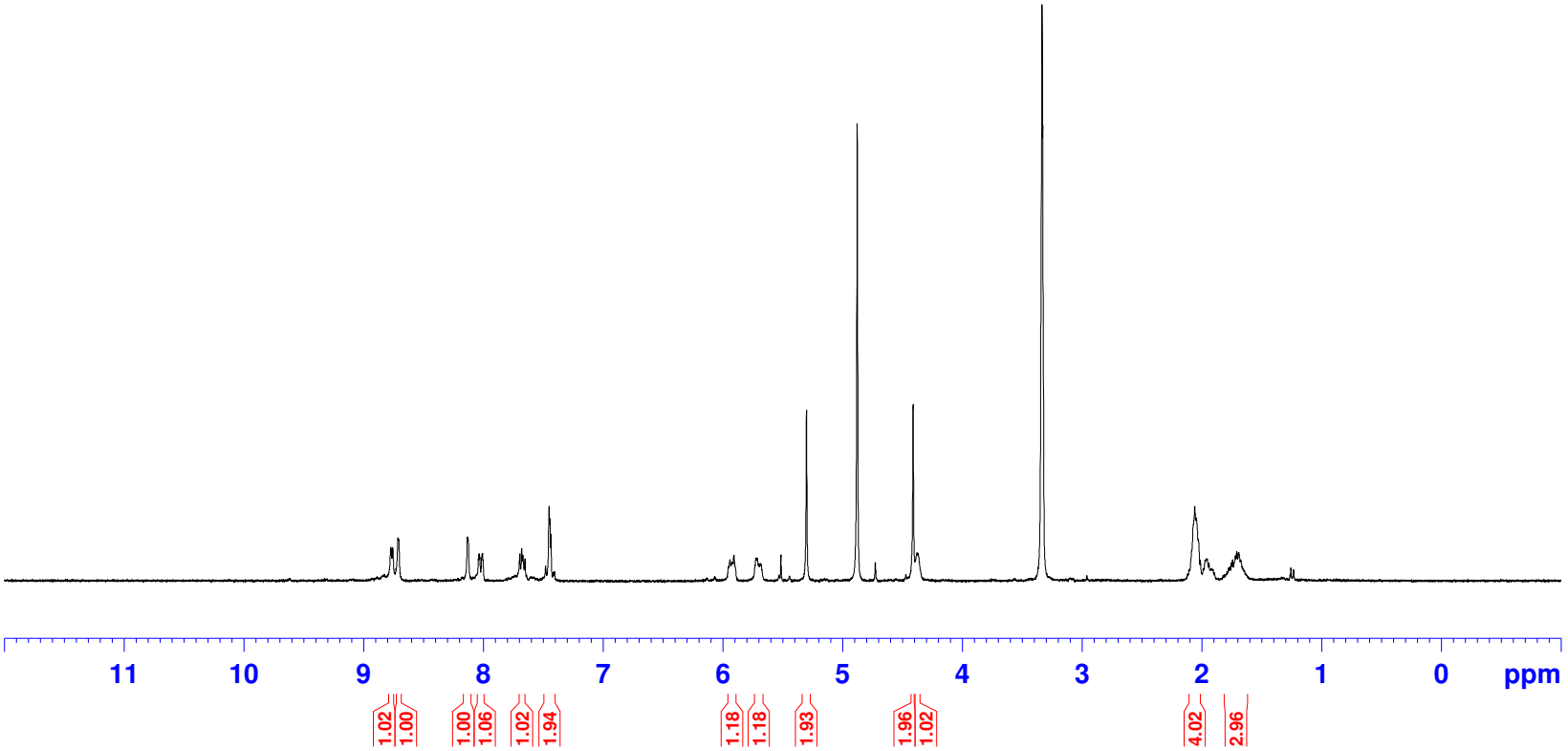
^{13}C NMR, $CDCl_3$, 125 MHz

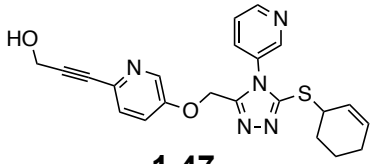




1-47

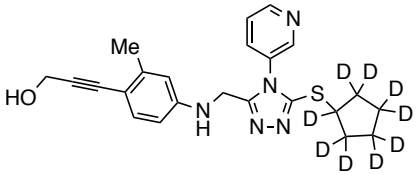
^{1}H NMR, CD_3OD, 300 MHz





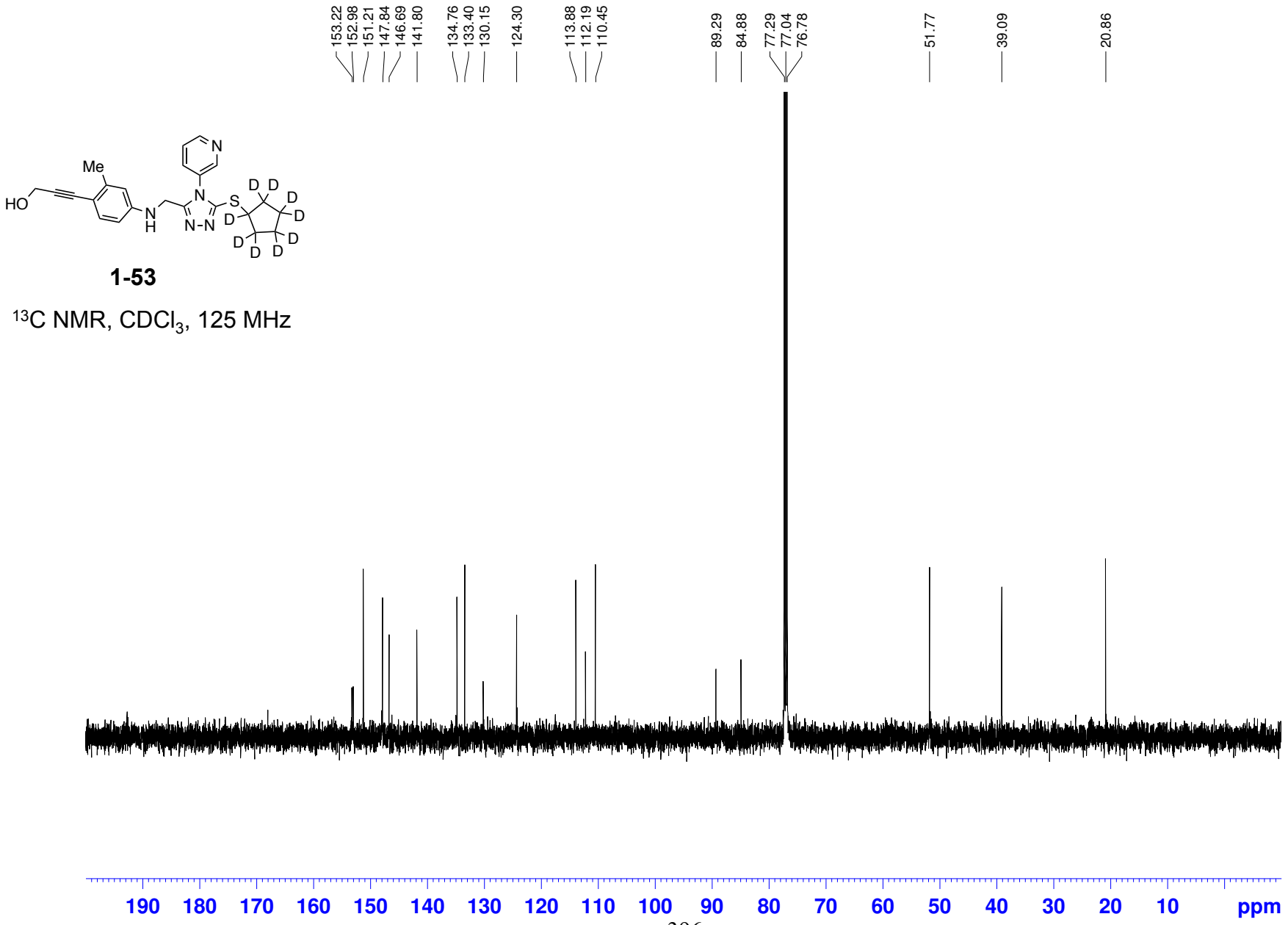
1-47

^{13}C NMR, CD_3OD, 125 MHz



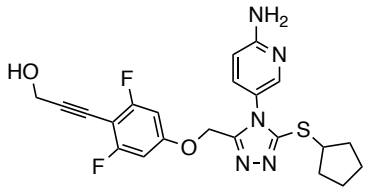
1-53

^{1}H NMR, $CDCl_3$, 500 MHz



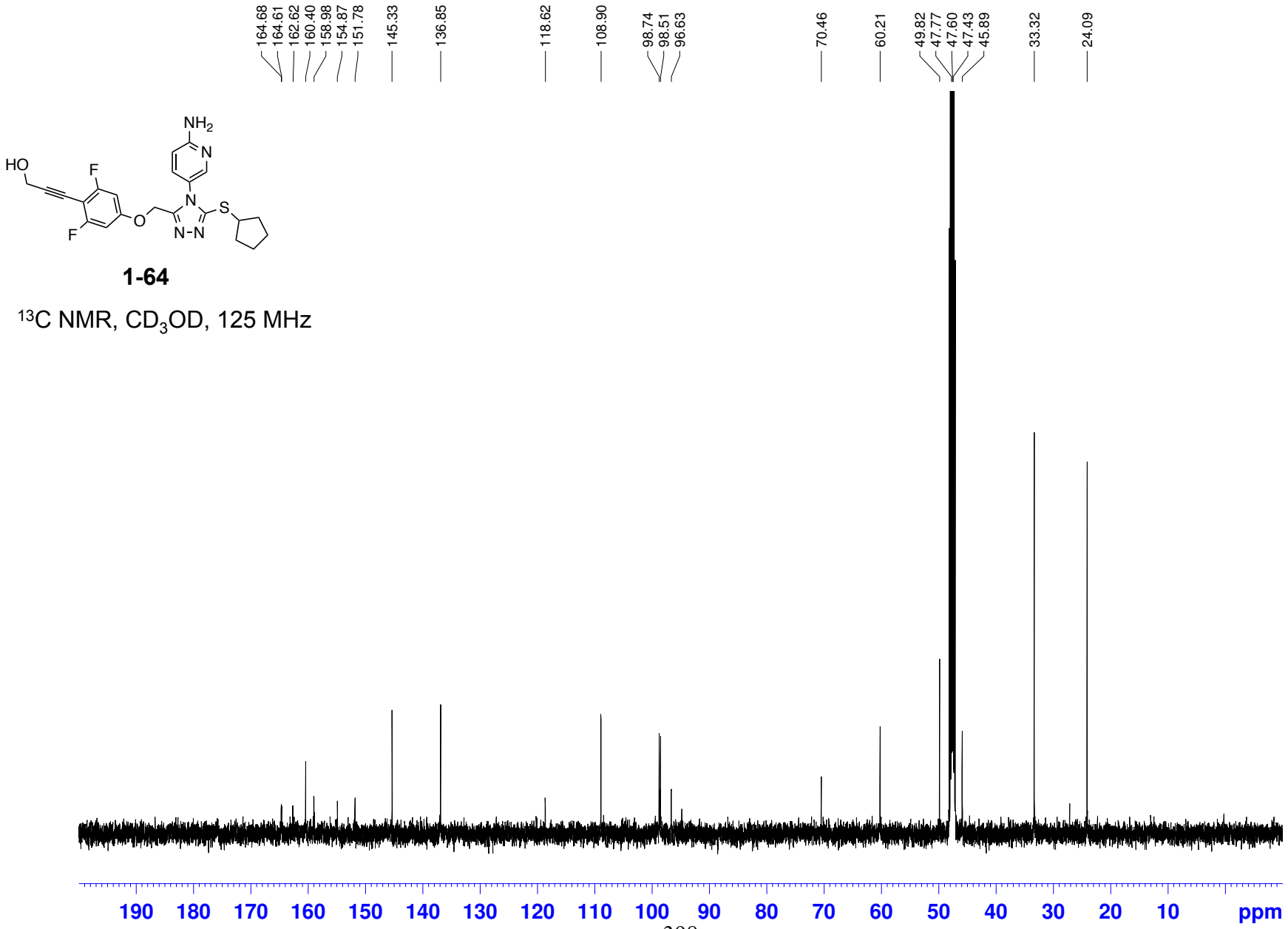
1-53

^{13}C NMR, $CDCl_3$, 125 MHz



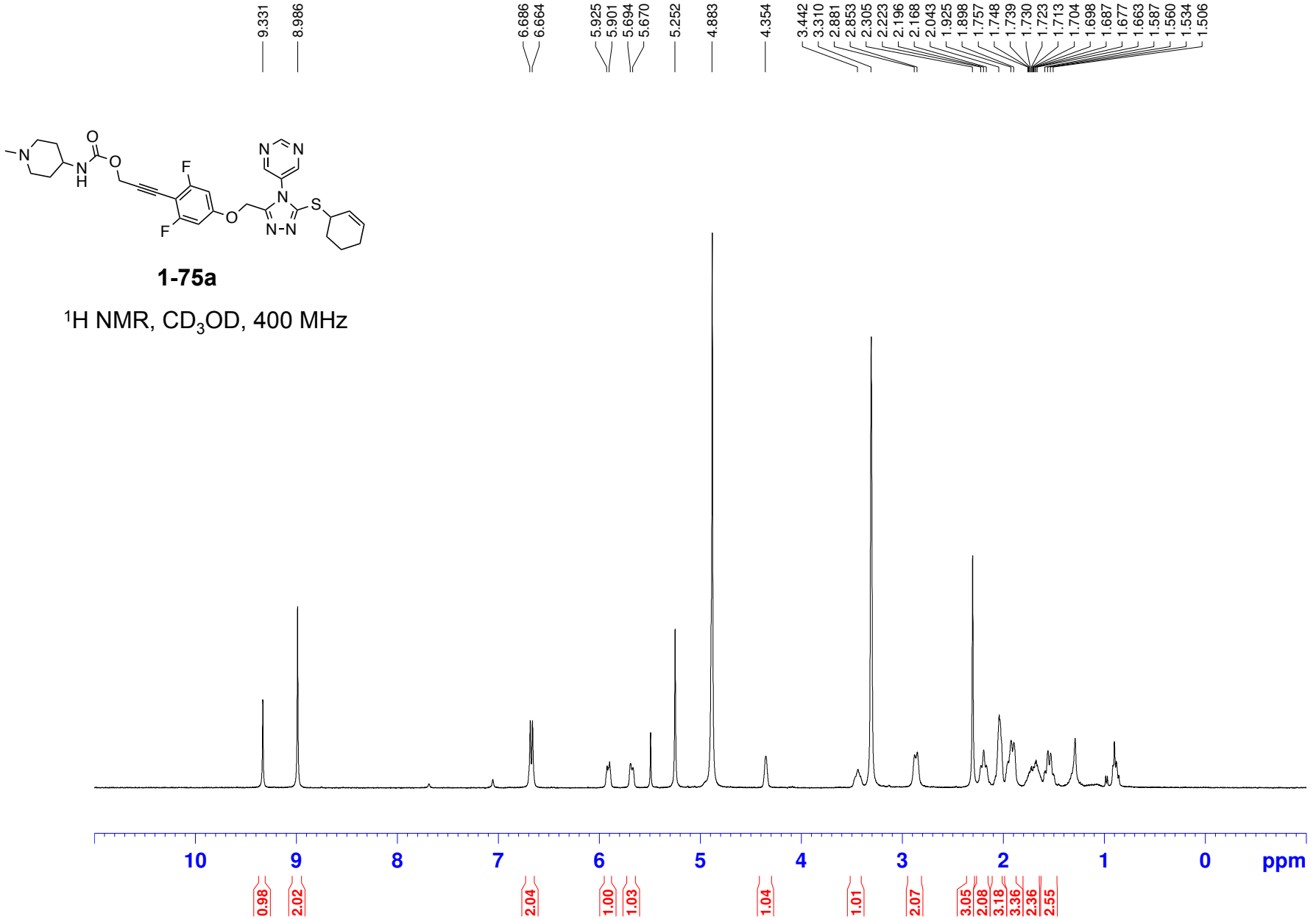
1-64

^{1}H NMR, CD_3OD, 500 MHz



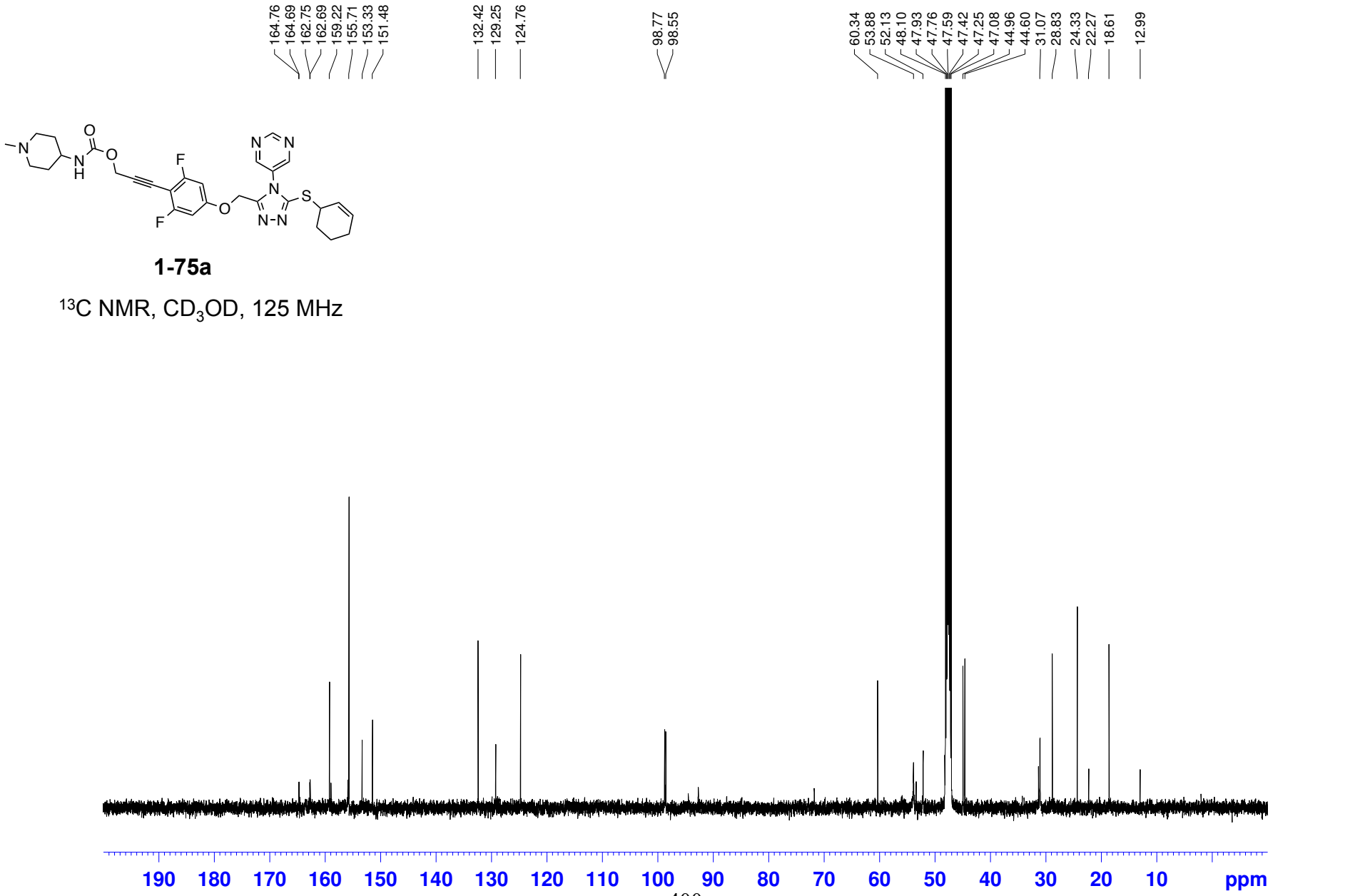
1-64

^{13}C NMR, CD_3OD, 125 MHz



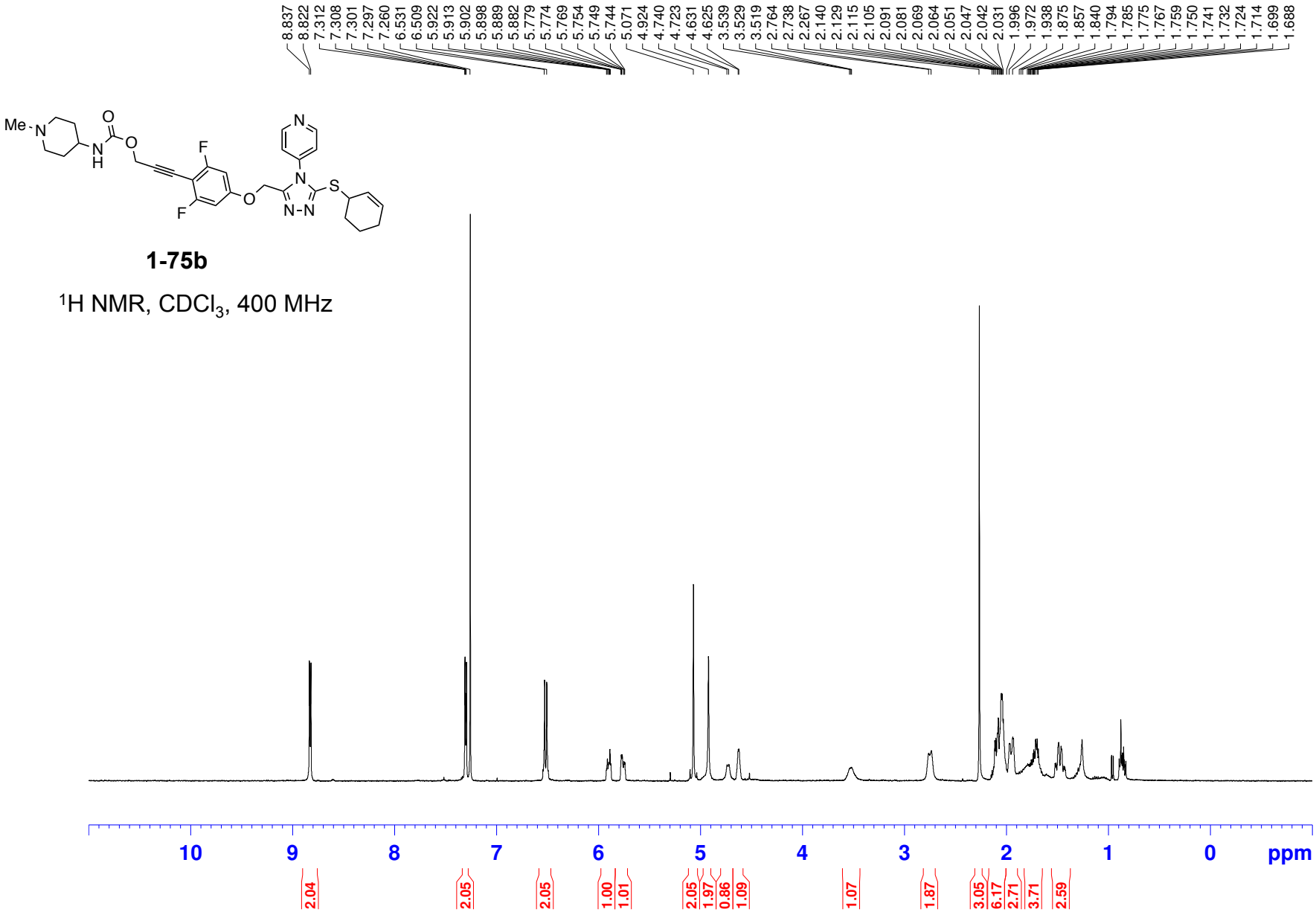
1-75a

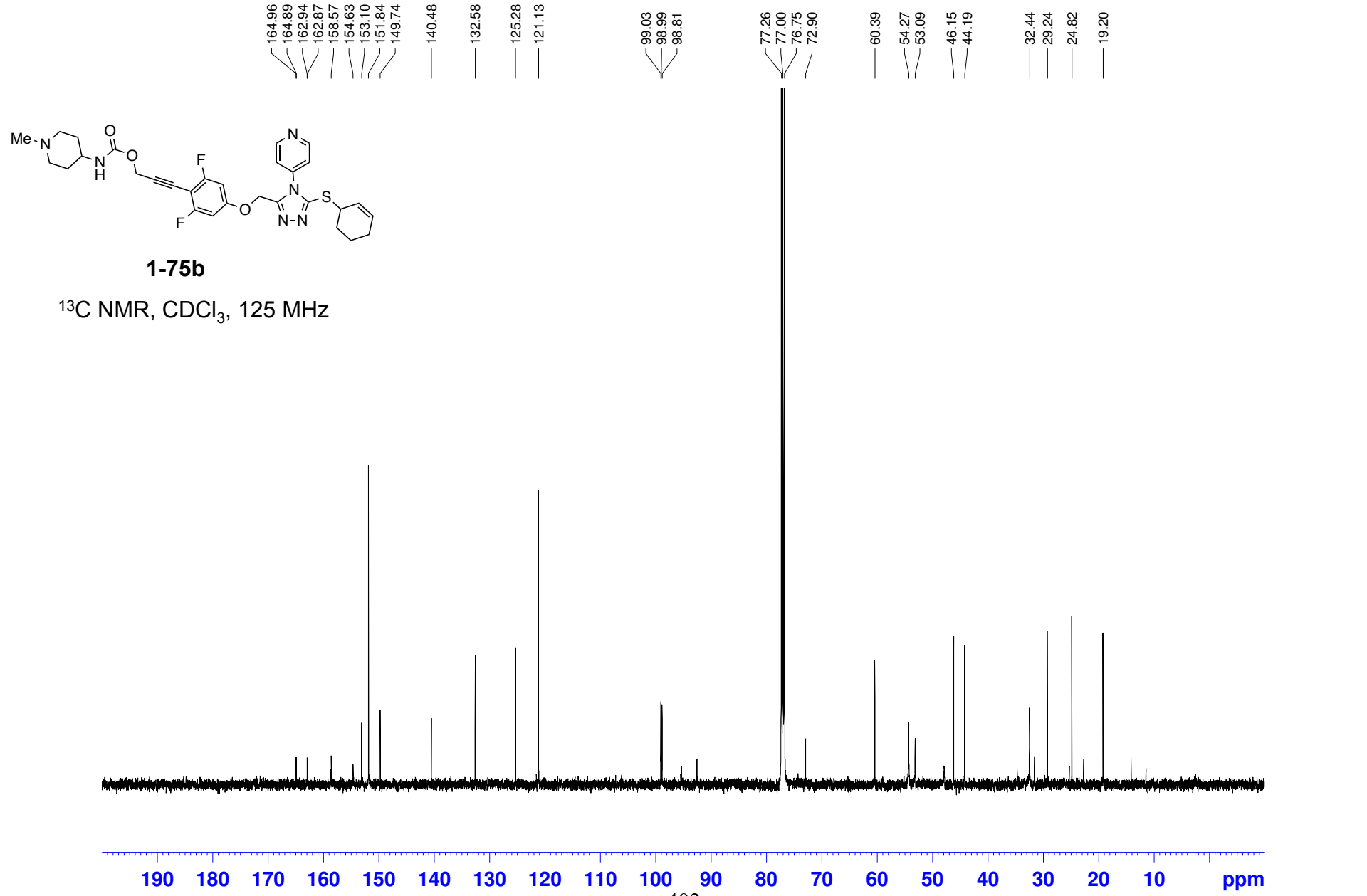
^{1}H NMR, CD_3OD, 400 MHz



1-75a

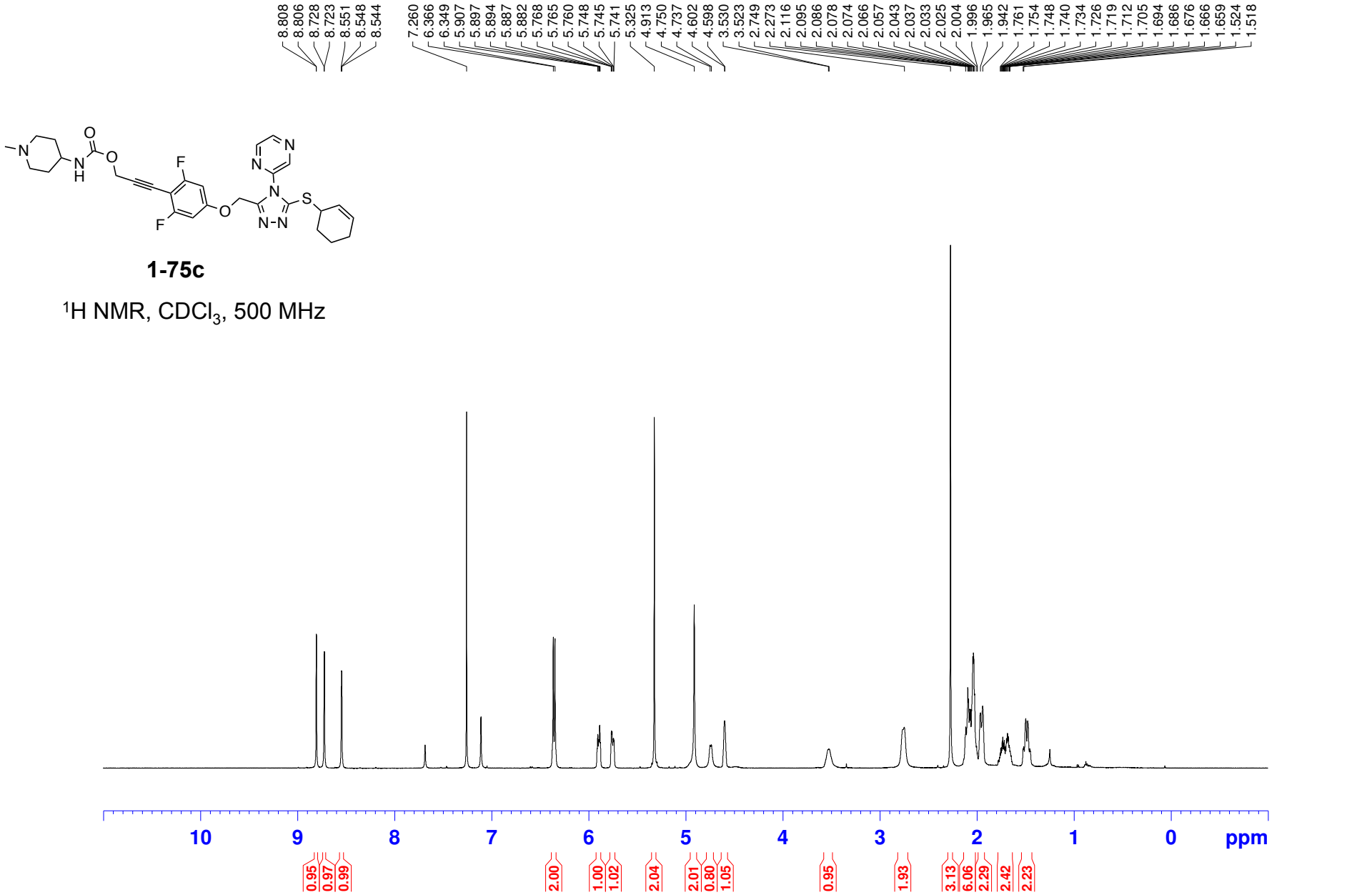
^{13}C NMR, CD_3OD, 125 MHz


8.837
8.822
7.312
7.308
7.301
7.297
7.260
6.531
6.509
5.922
5.913
5.902
5.898
5.889
5.882
5.779
5.774
5.769
5.754
5.749
5.744
5.071
4.924
4.740
4.723
4.631
4.625
3.539
3.529
3.519
2.764
2.738
2.267
2.140
2.129
2.115
2.105
2.091
2.081
2.069
2.064
2.051
2.047
2.042
2.031
1.996
1.972
1.938
1.875
1.857
1.840
1.794
1.785
1.775
1.767
1.759
1.750
1.741
1.732
1.724
1.714
1.699
1.688
Me
N
O
N
H
O
F
F
O
N
N
N
N
S
1-75b
1H NMR, CDCl3, 400 MHz
10
9
8
7
6
5
4
3
2
1
0
ppm
2.04
2.05
2.05
1.00
1.01
2.05
1.97
0.86
1.09
1.07
1.87
3.05
6.17
2.71
3.71
2.59



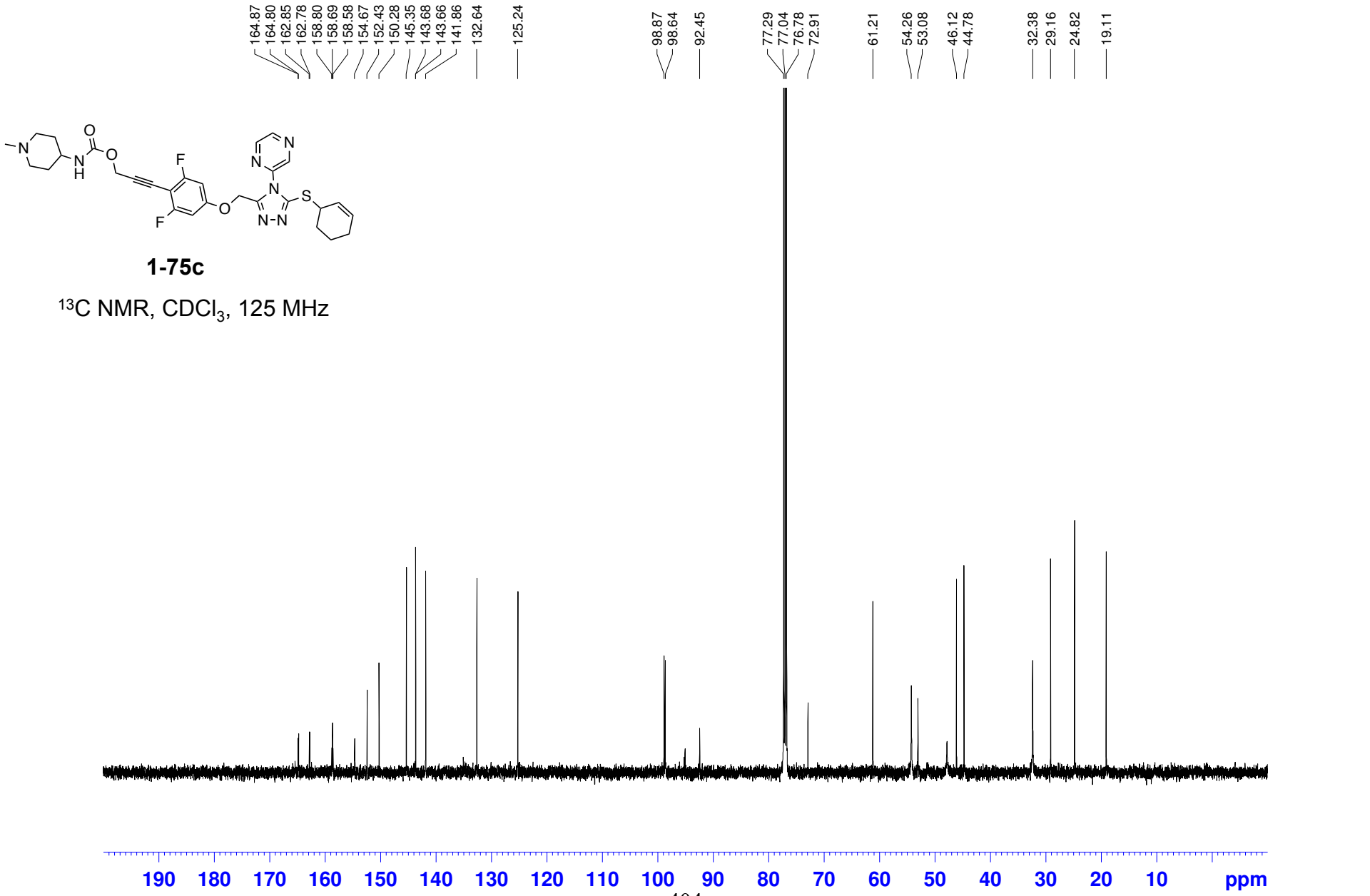
1-75b

^{13}C NMR, $CDCl_3$, 125 MHz



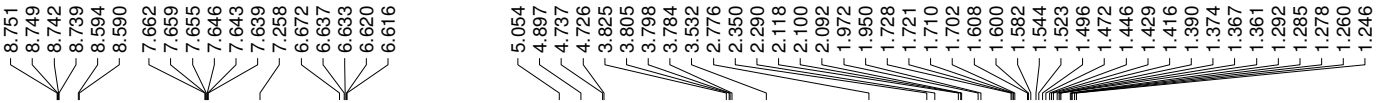
1-75c

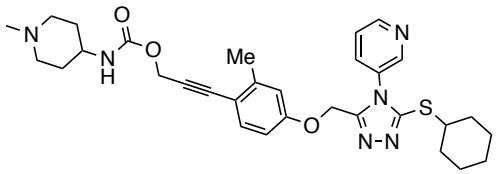
^{1}H NMR, $CDCl_3$, 500 MHz



1-75c

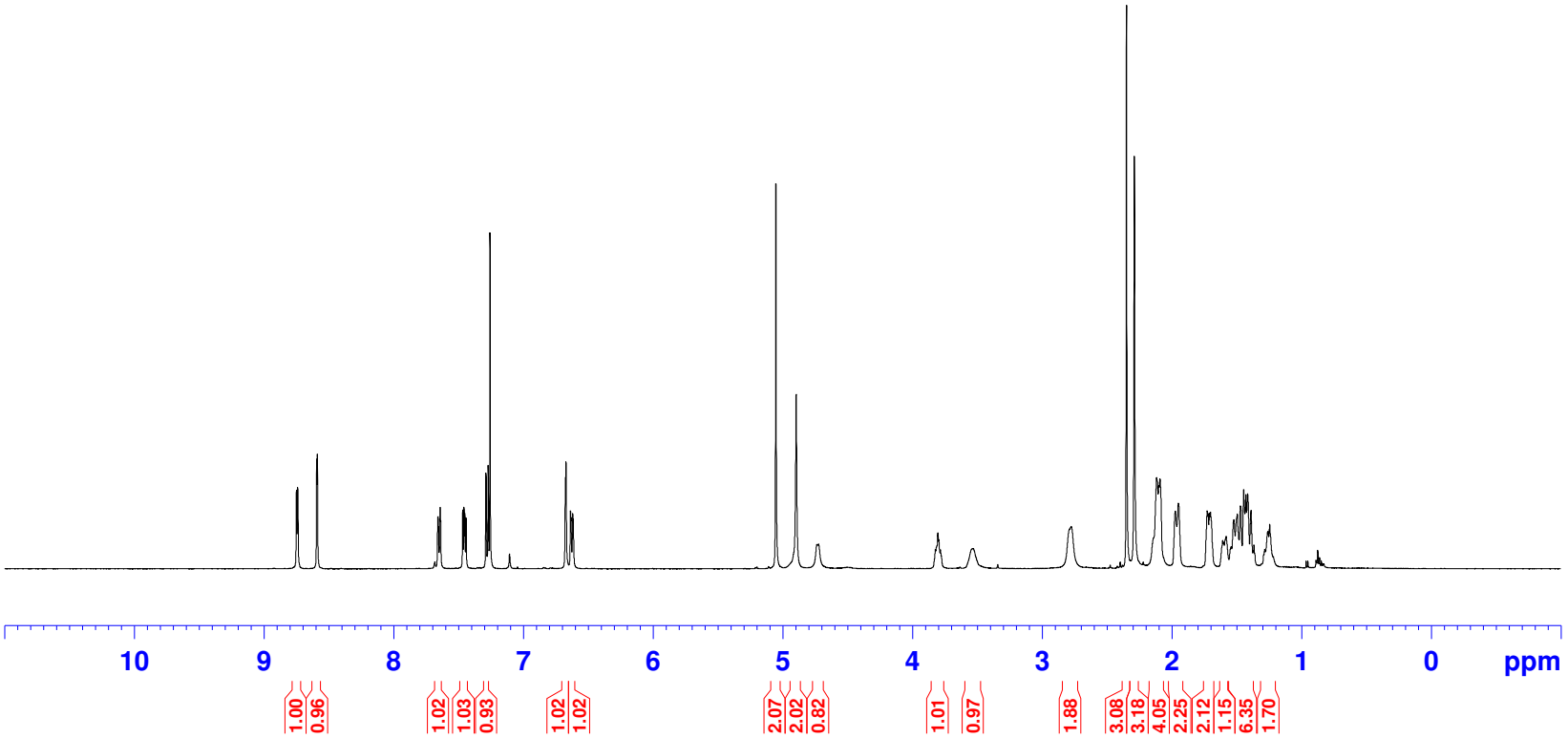
^{13}C NMR, $CDCl_3$, 125 MHz

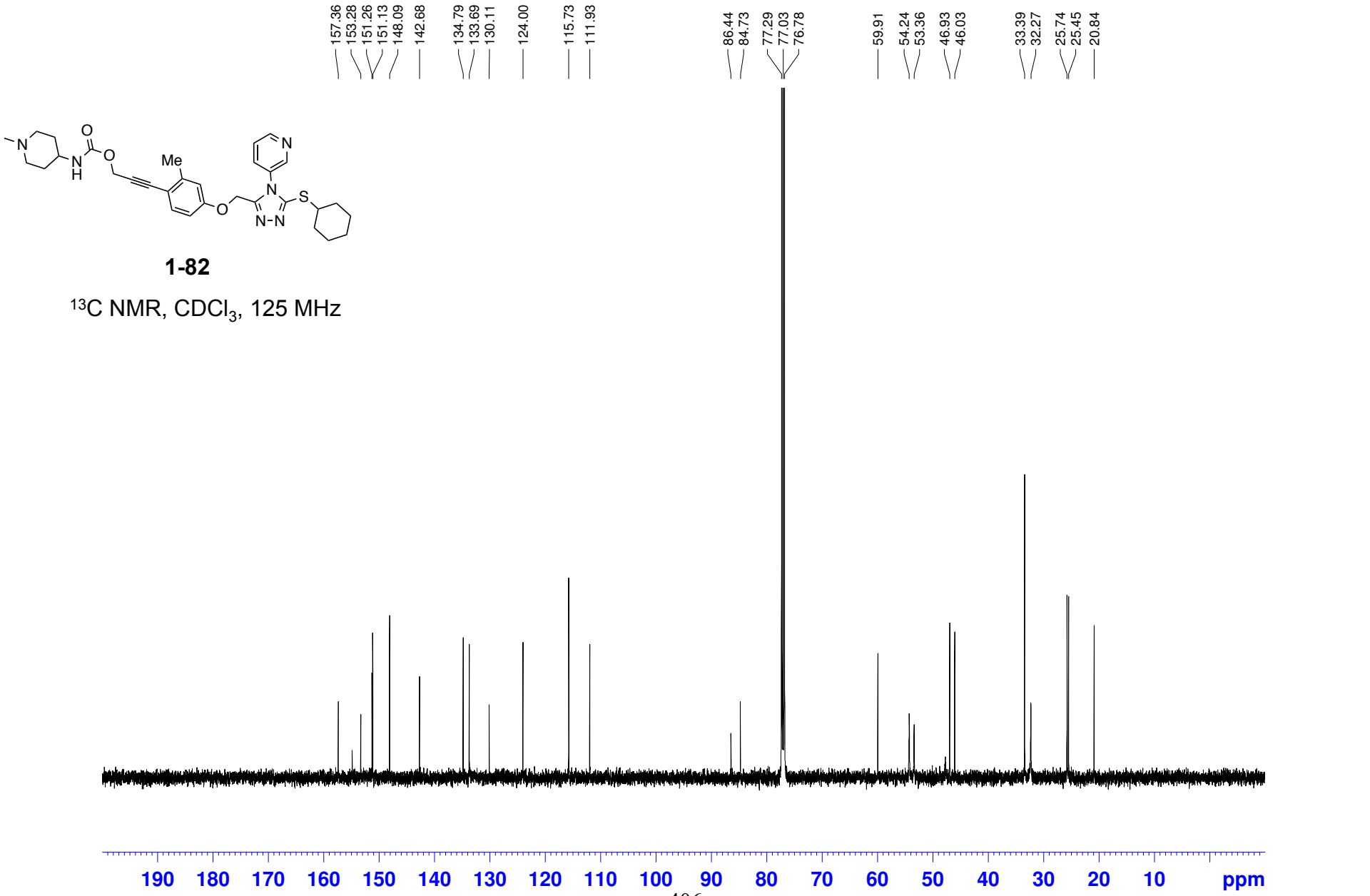




1-82

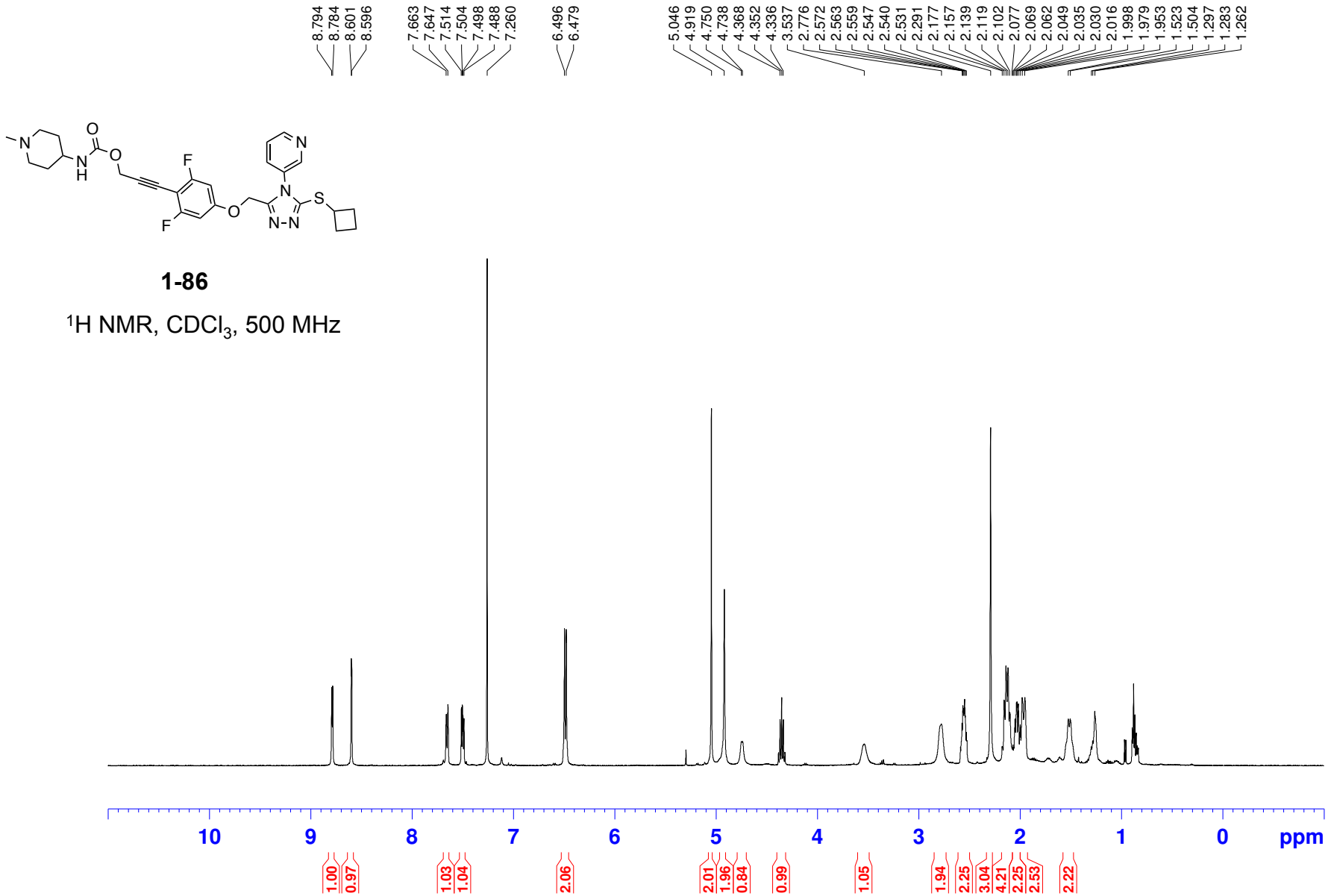
^{1}H NMR, $CDCl_3$, 500 MHz





1-82

^{13}C NMR, $CDCl_3$, 125 MHz


8.794
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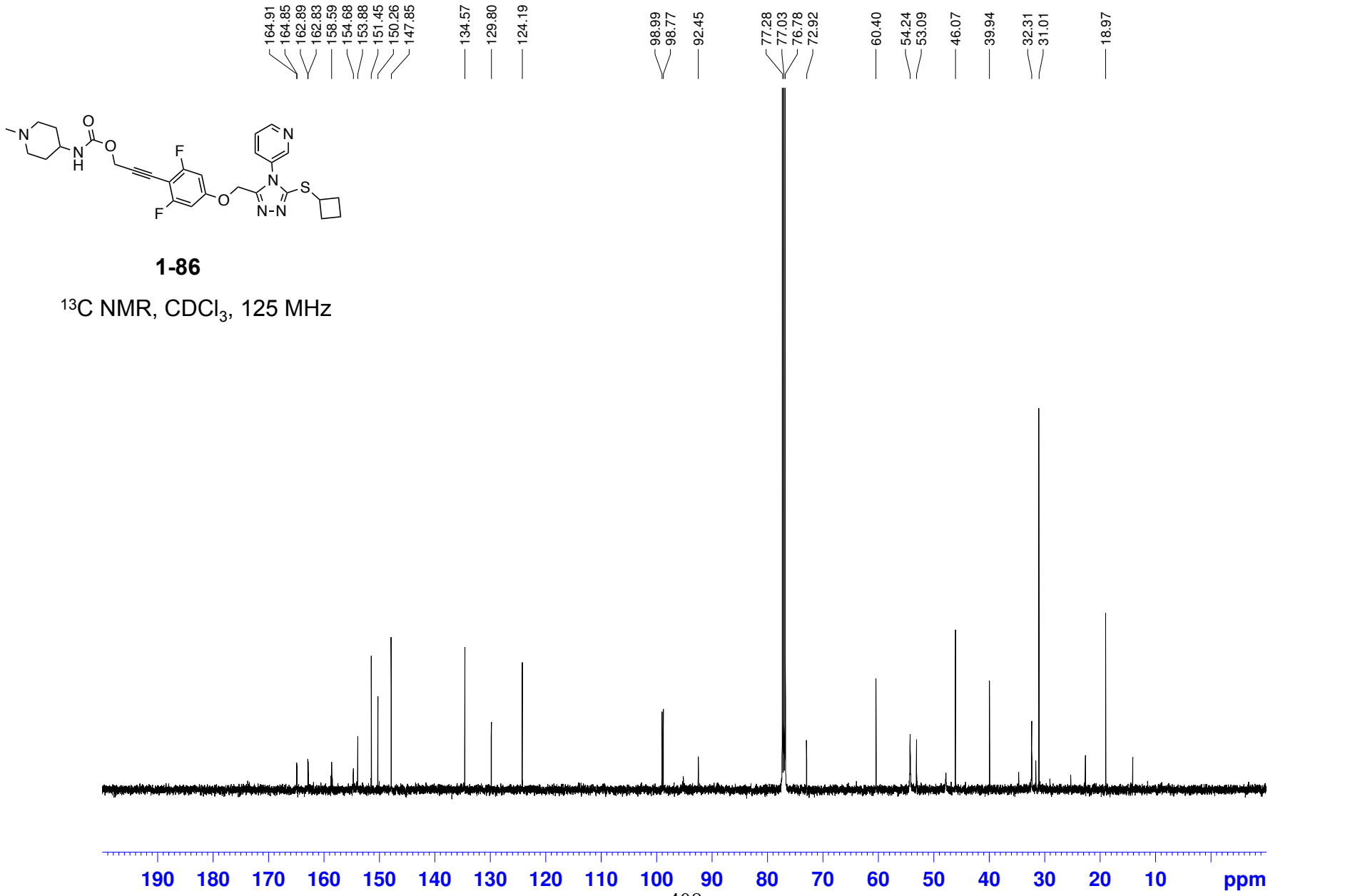
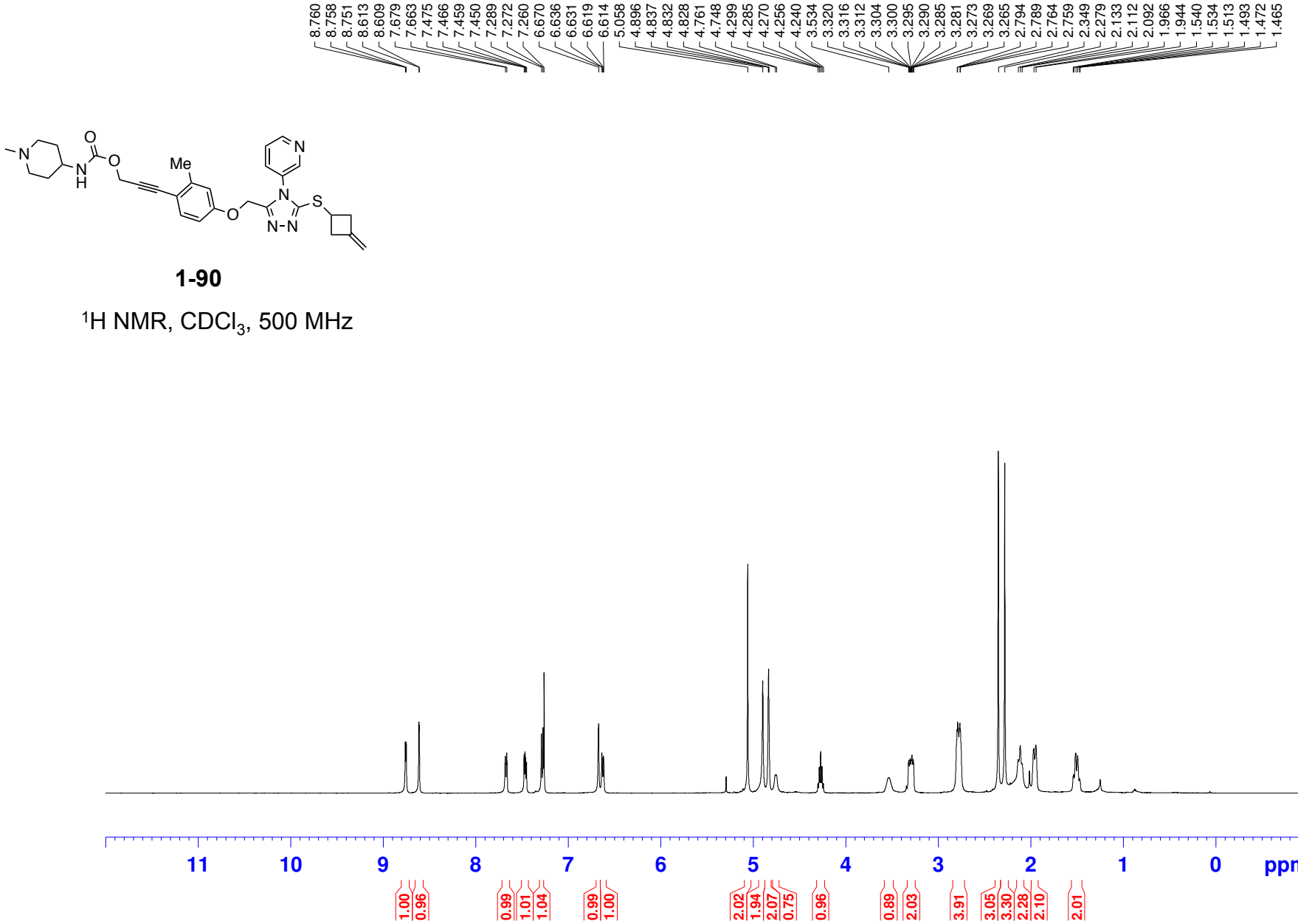
8.601
8.596
7.663
7.647
7.514
7.504
7.498
7.488
7.260
6.496
6.479
5.046
4.919
4.750
4.738
4.368
4.352
4.336
3.537
2.776
2.572
2.563
2.559
2.547
2.540
2.531
2.291
2.177
2.157
2.139
2.119
2.102
2.077
2.069
2.062
2.049
2.035
2.030
2.016
1.998
1.979
1.953
1.523
1.504
1.297
1.283
1.262
1-86
1H NMR, CDCl3, 500 MHz
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.97
1.03
1.04
2.06
2.01
1.96
0.84
0.99
1.05
1.94
2.25
3.04
4.21
2.25
2.53
2.22



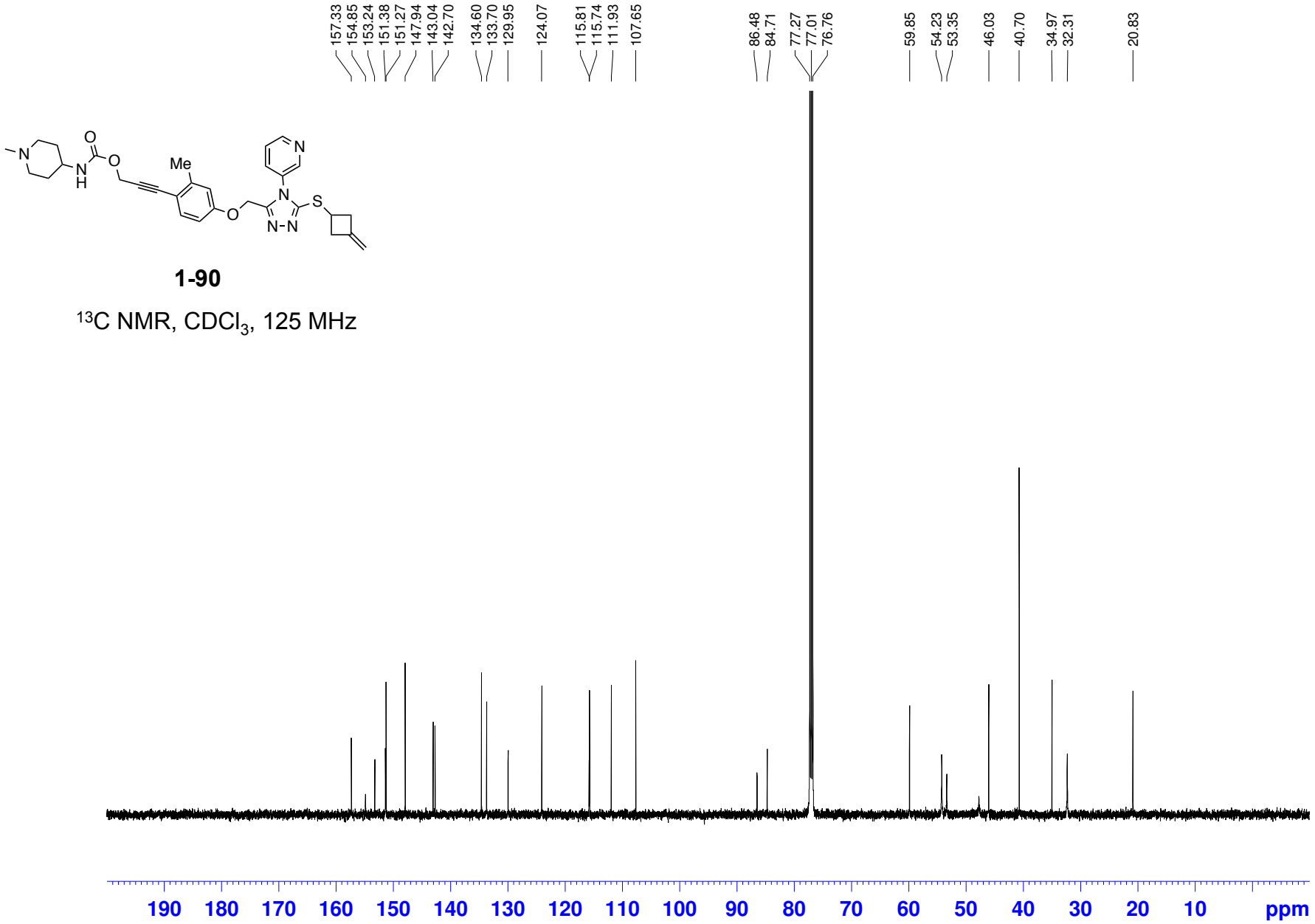
1-86

^{13}C NMR, $CDCl_3$, 125 MHz



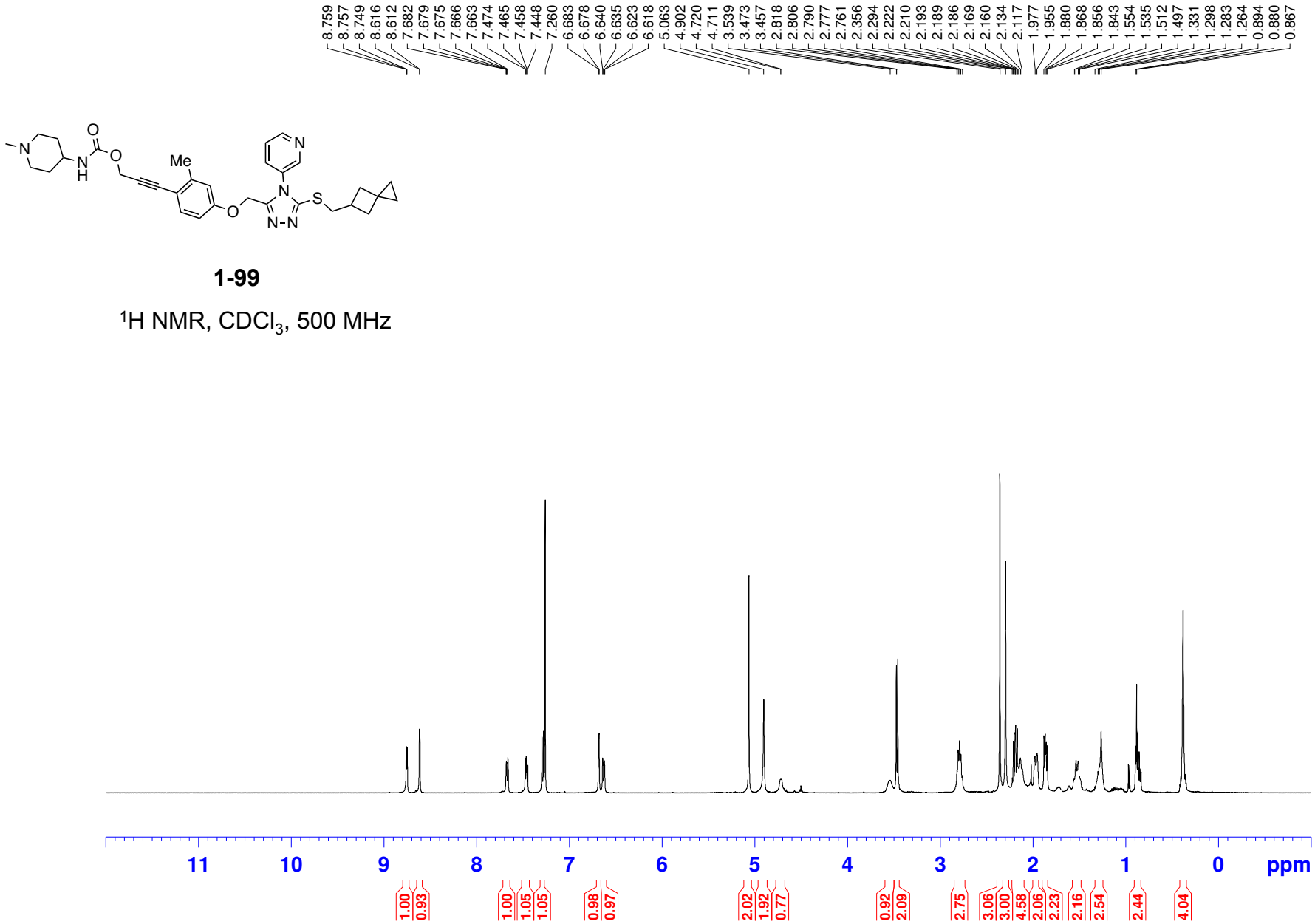
1-90

^{1}H NMR, $CDCl_3$, 500 MHz



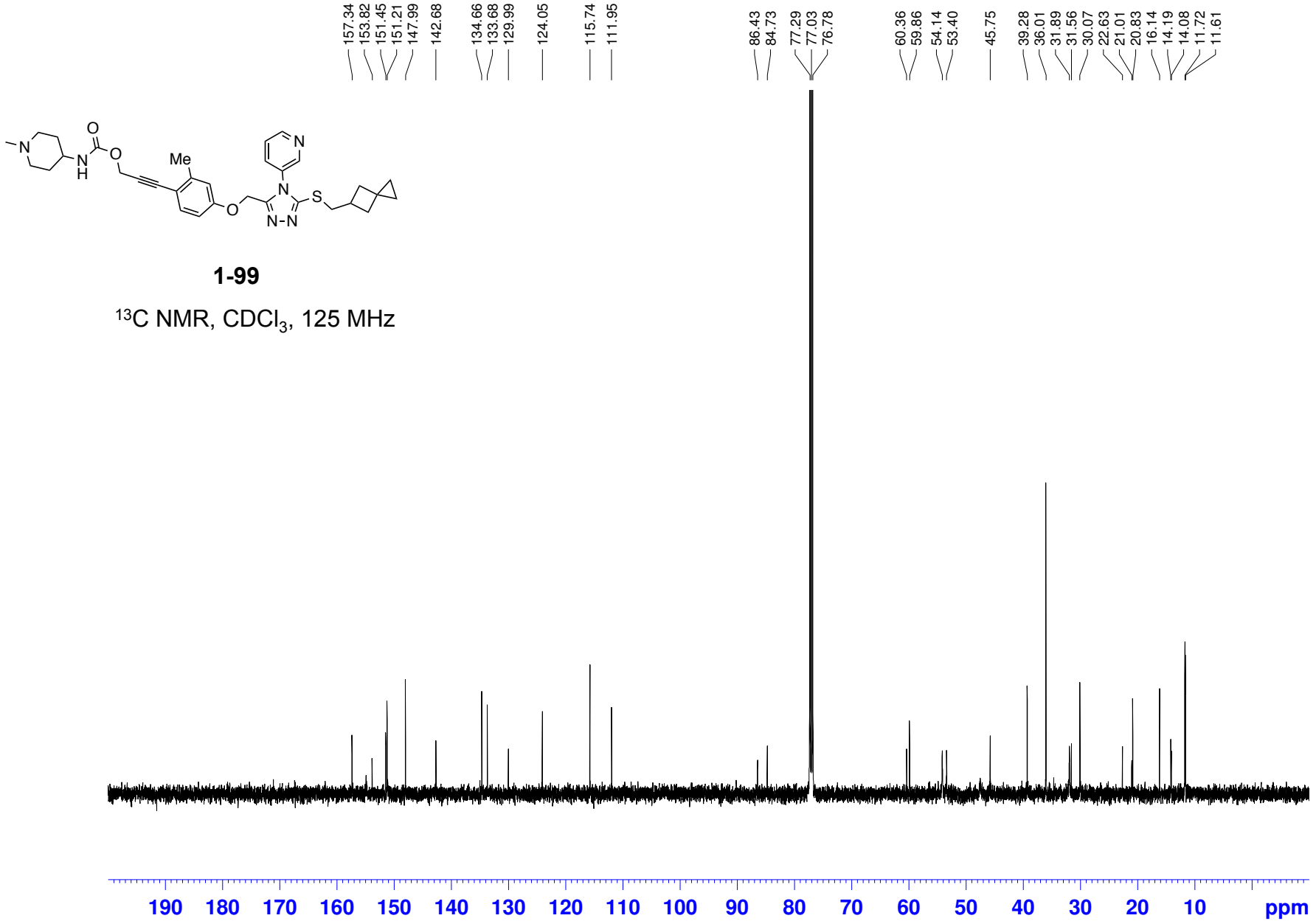
1-90

^{13}C NMR, $CDCl_3$, 125 MHz



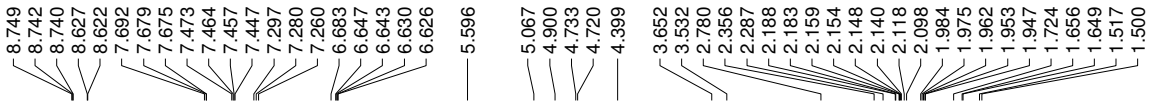
1-99

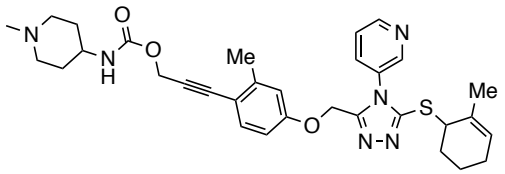
^{1}H NMR, $CDCl_3$, 500 MHz



1-99

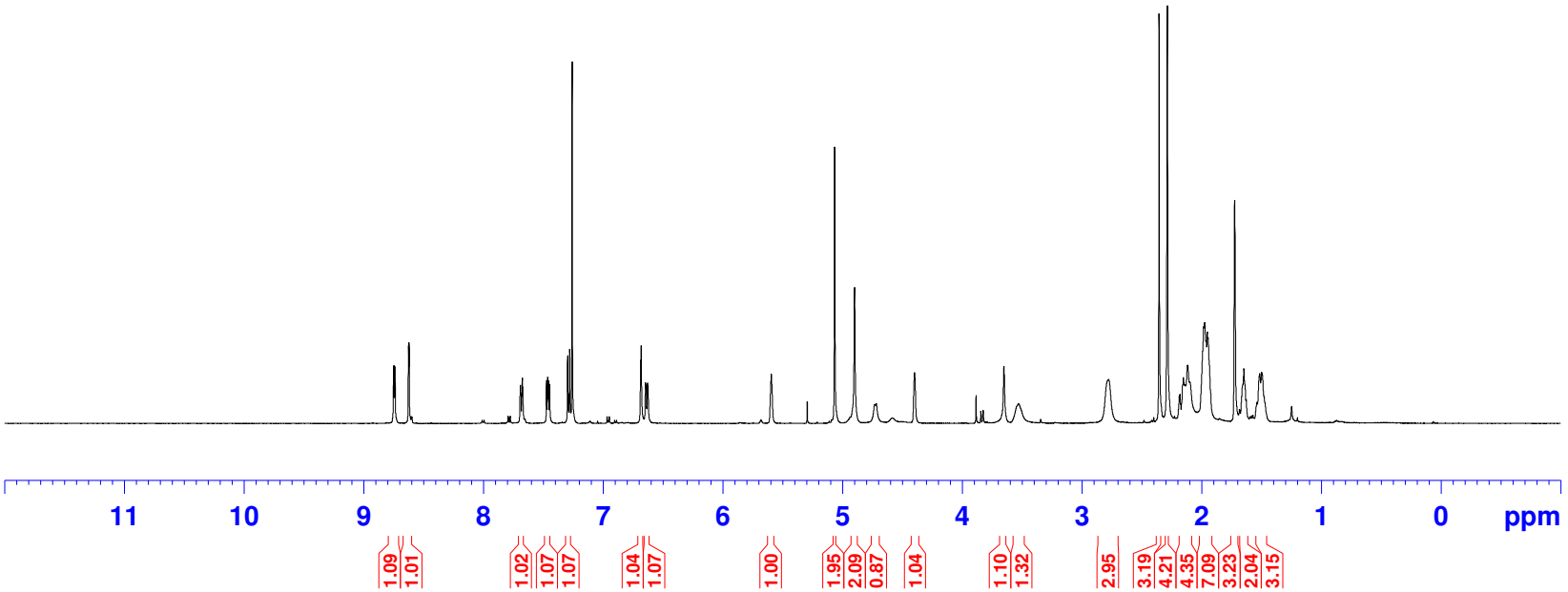
^{13}C NMR, $CDCl_3$, 125 MHz

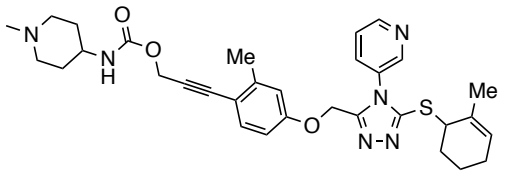




1-106

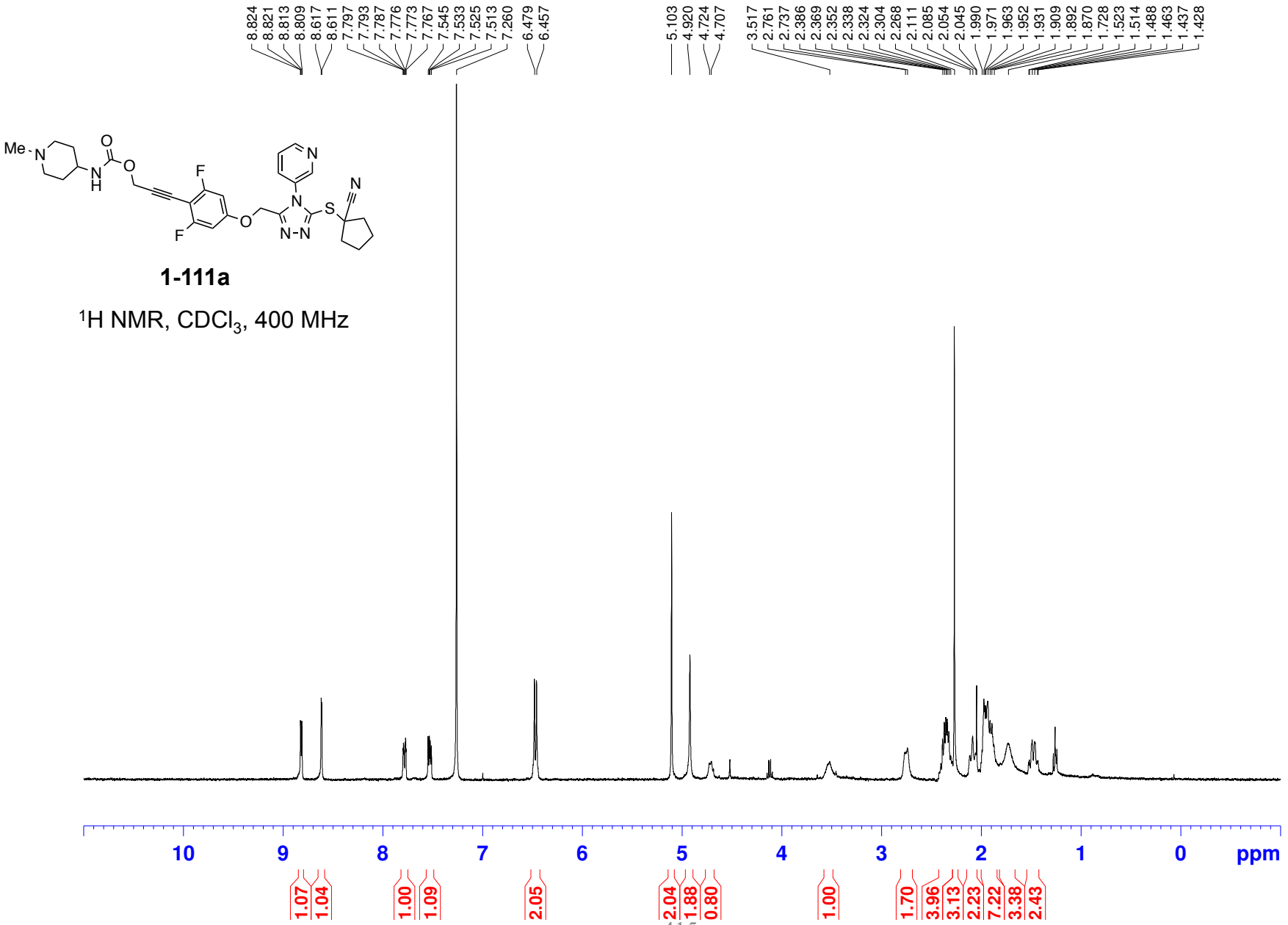
^{1}H NMR, $CDCl_3$, 500 MHz





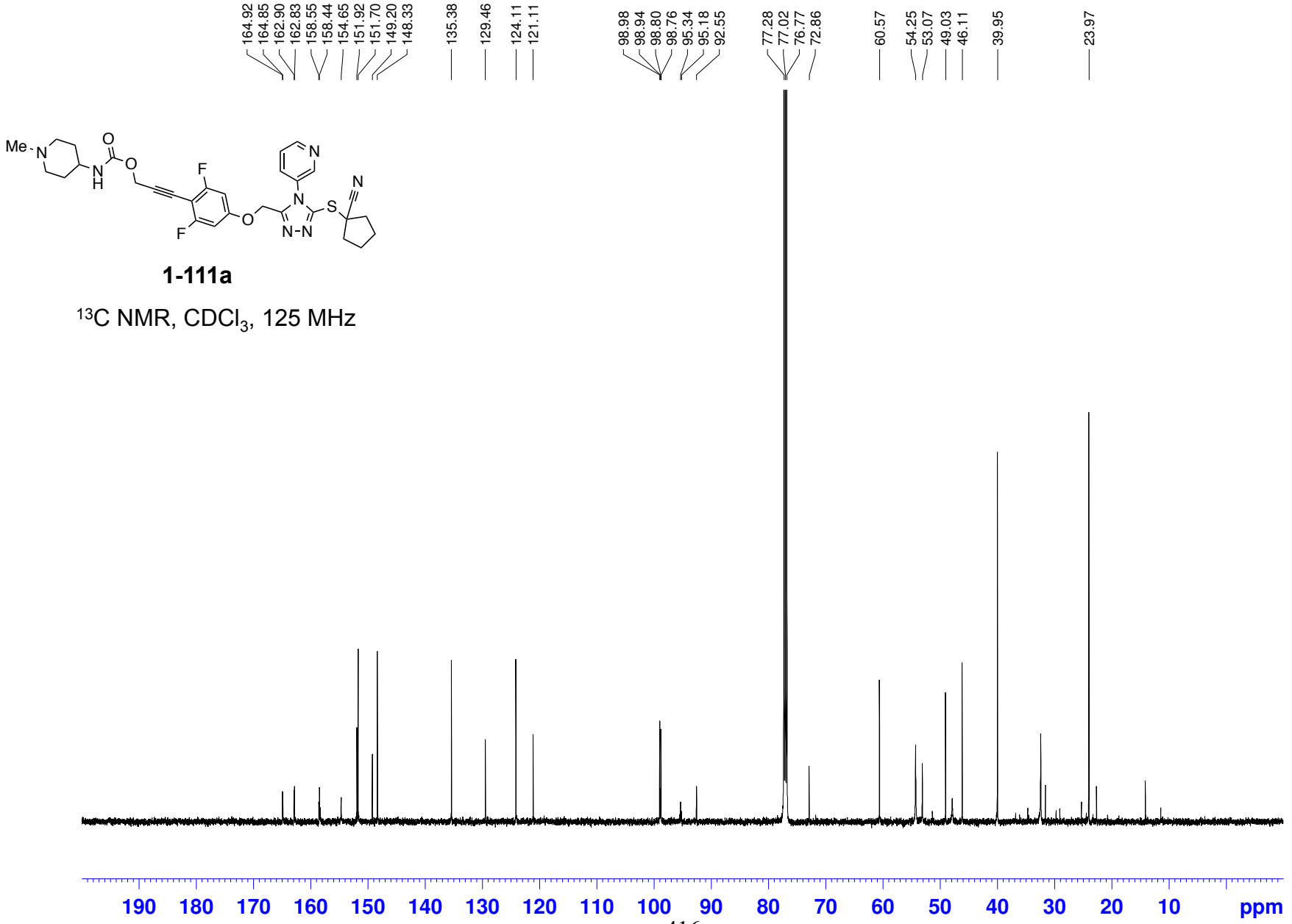
1-106

^{13}C NMR, $CDCl_3$, 125 MHz



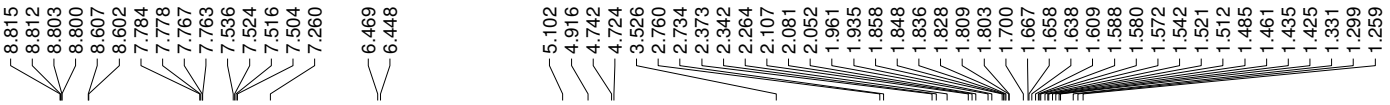
1-111a

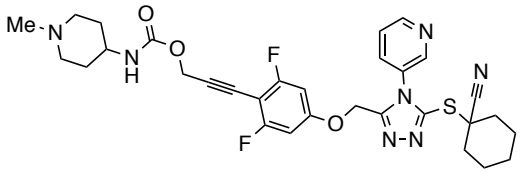
^{1}H NMR, $CDCl_3$, 400 MHz



1-111a

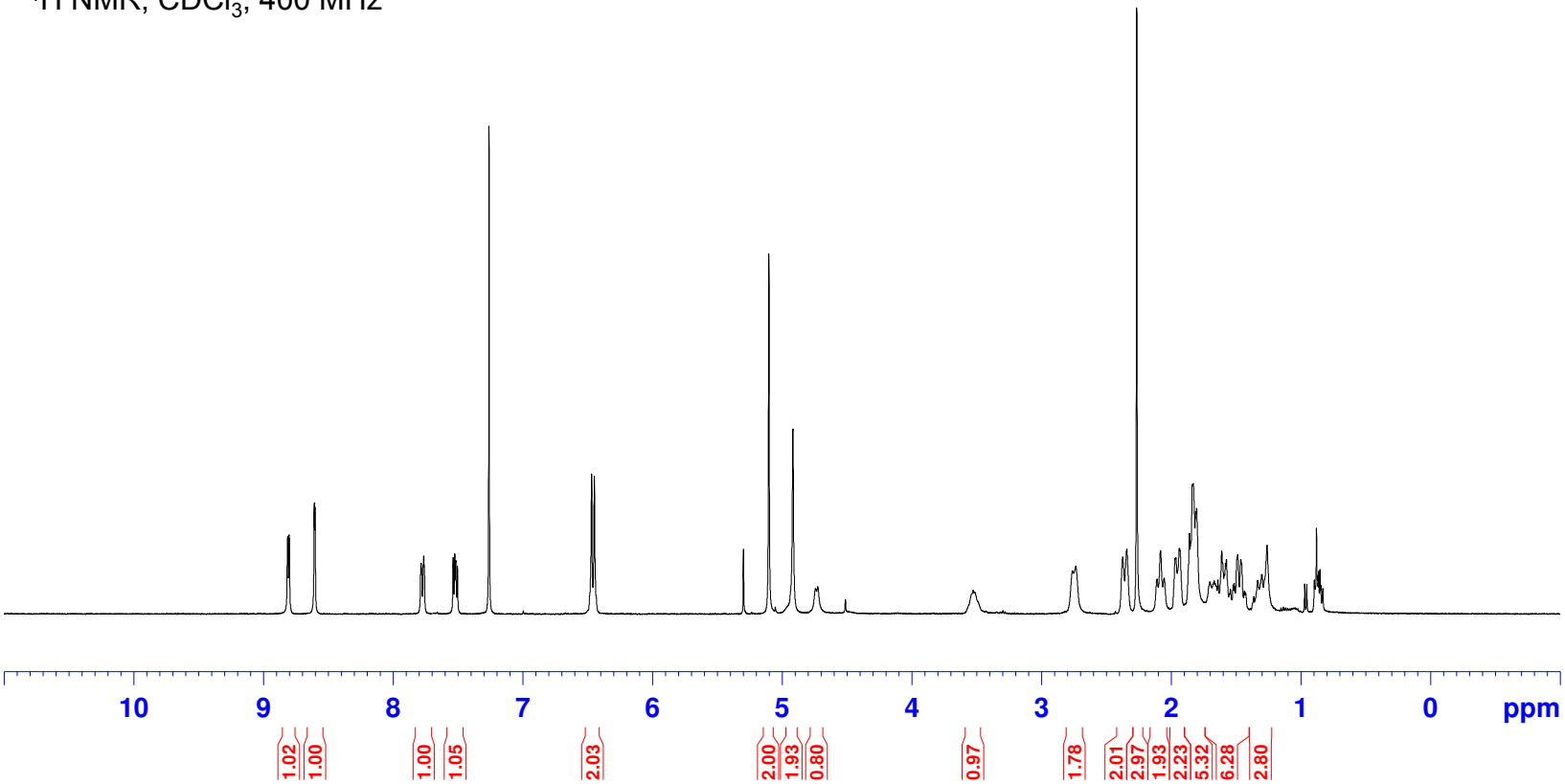
^{13}C NMR, $CDCl_3$, 125 MHz

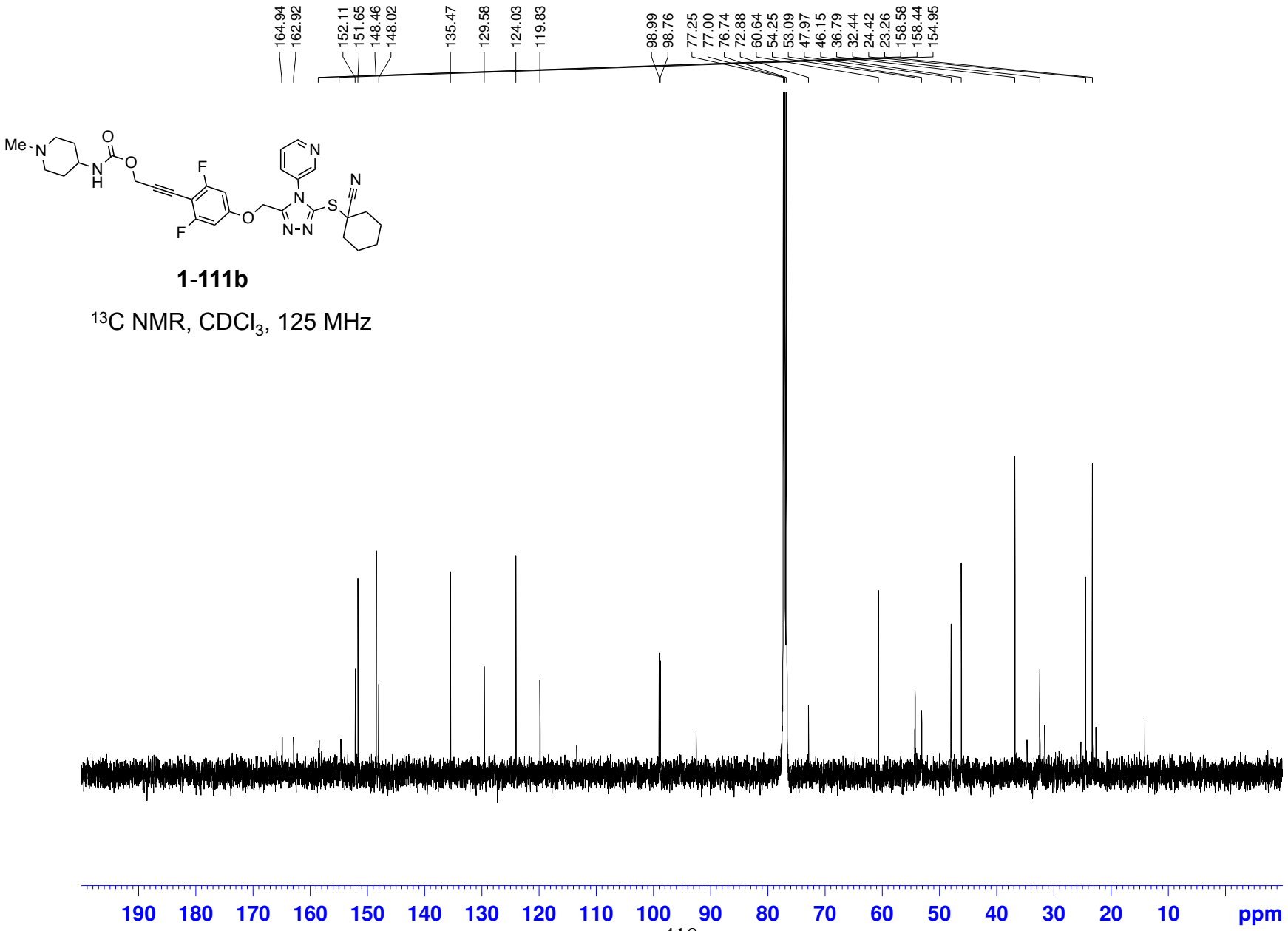




1-111b

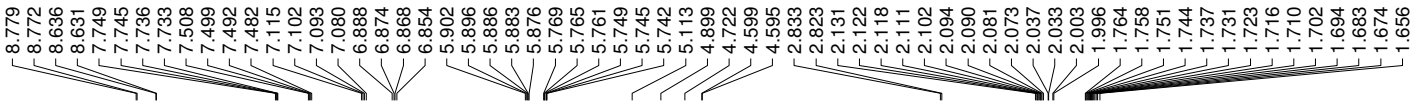
^{1}H NMR, $CDCl_3$, 400 MHz

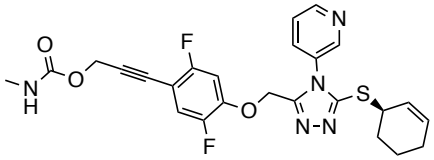




1-111b

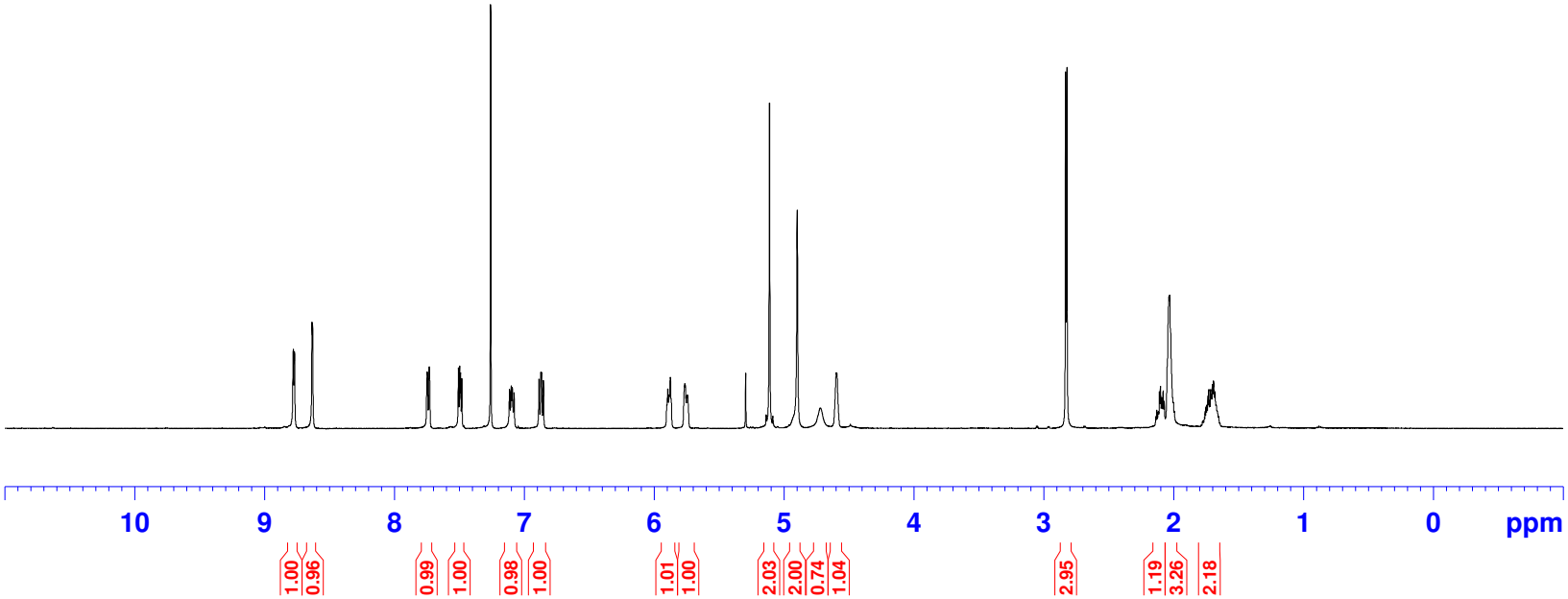
^{13}C NMR, $CDCl_3$, 125 MHz

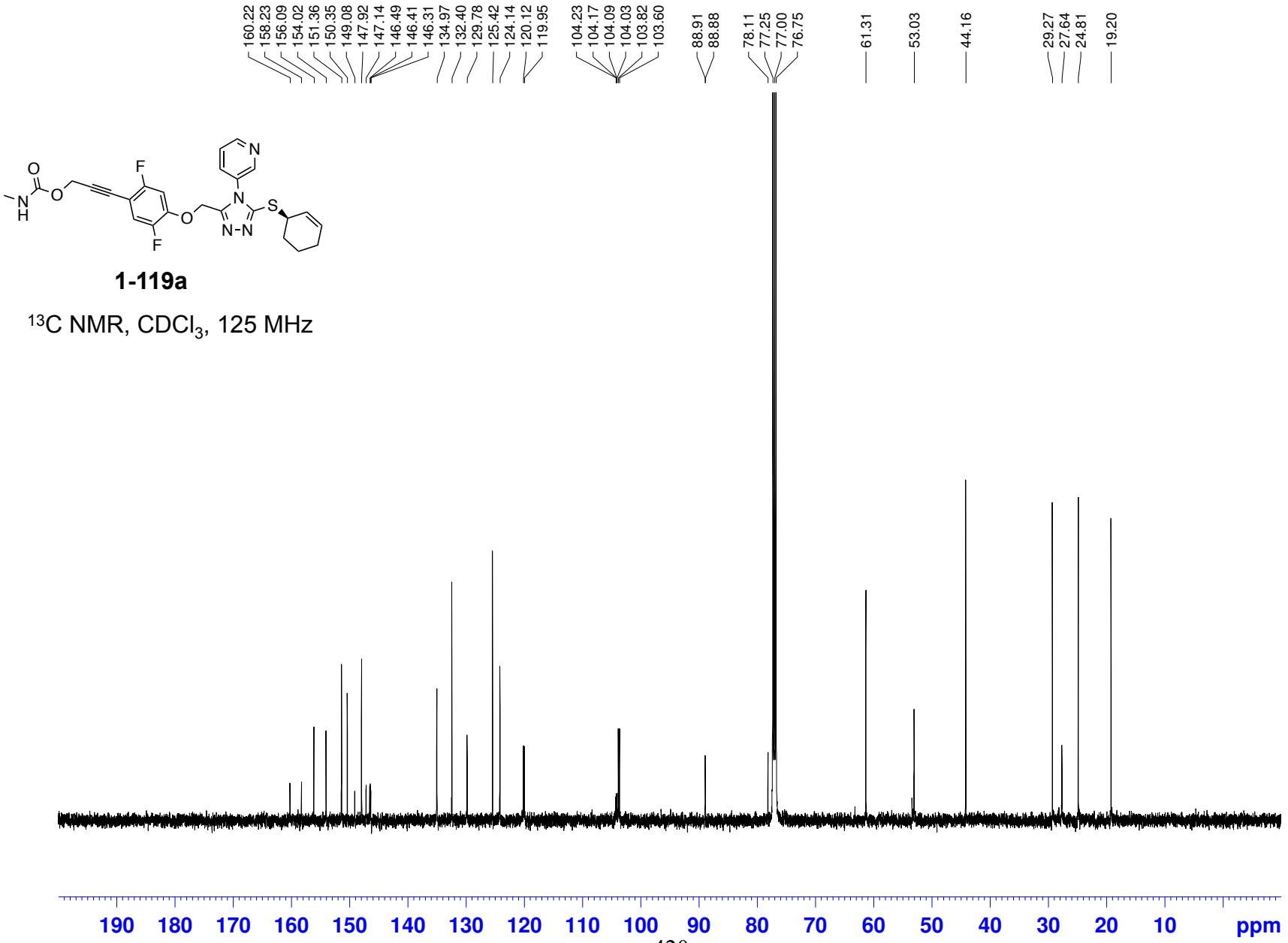




1-119a

^{1}H NMR, $CDCl_3$, 500 MHz

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158.23
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154.02
151.36
150.35
149.08
147.92
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146.41
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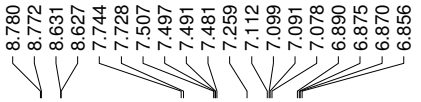
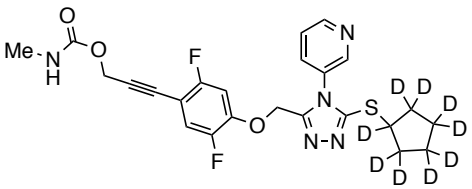
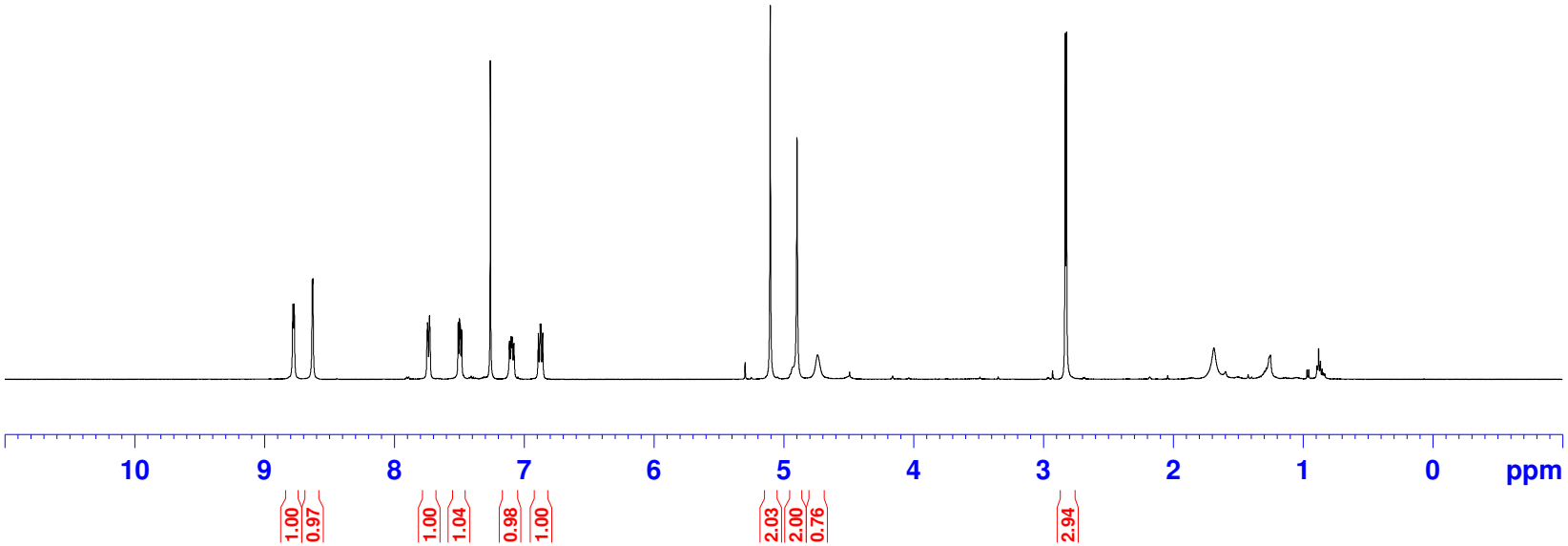
132.40
129.78
125.42
124.14
120.12
119.95
104.23
104.17
104.09
104.03
103.82
103.60
88.91
88.88
78.11
77.25
77.00
76.75
61.31
53.03
44.16
29.27
27.64
24.81
19.20
O
N
H
O
F
F
O
N
N-N
N
S
1-119a
13C NMR, CDCl3, 125 MHz
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

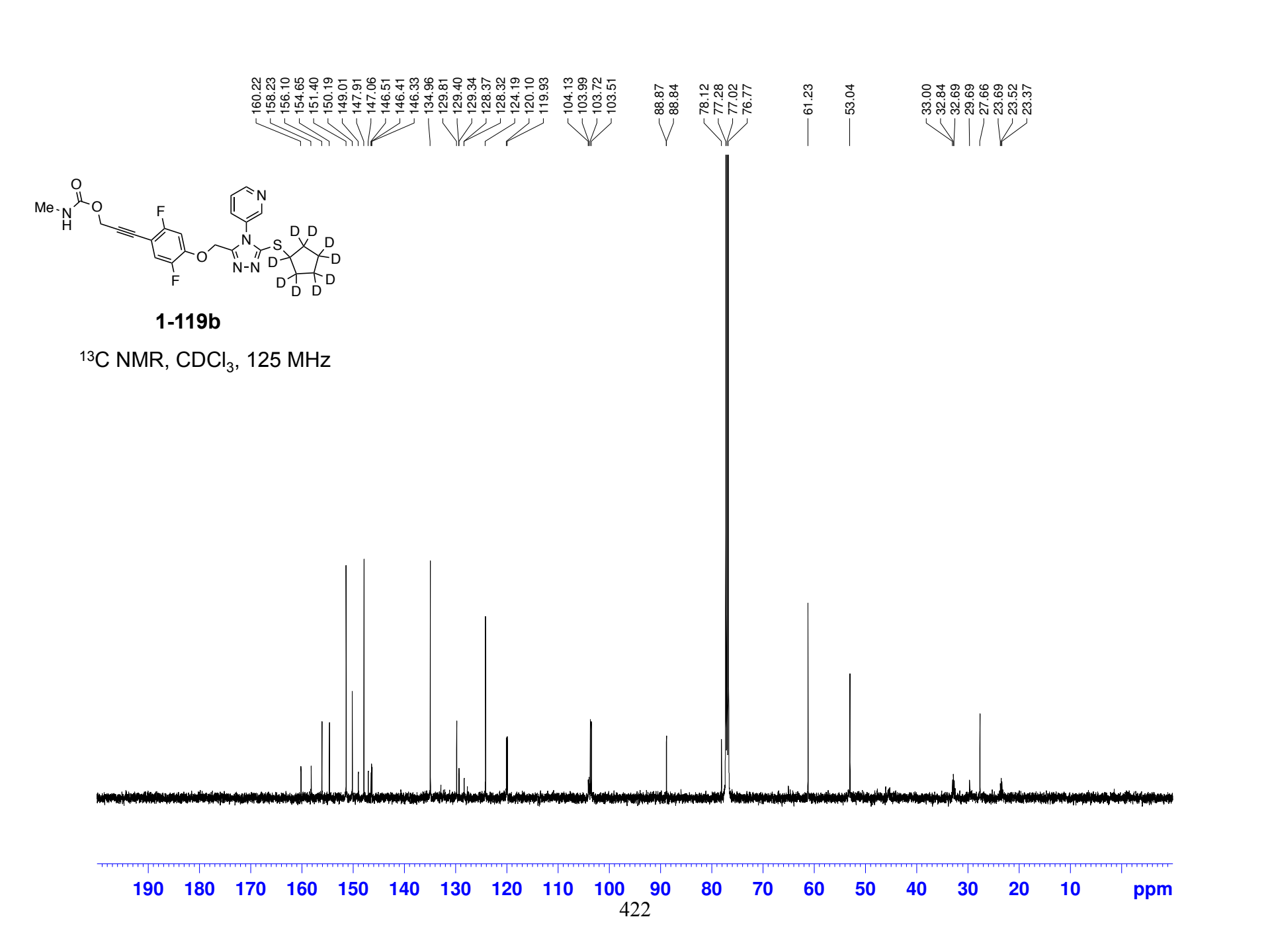




1-119b

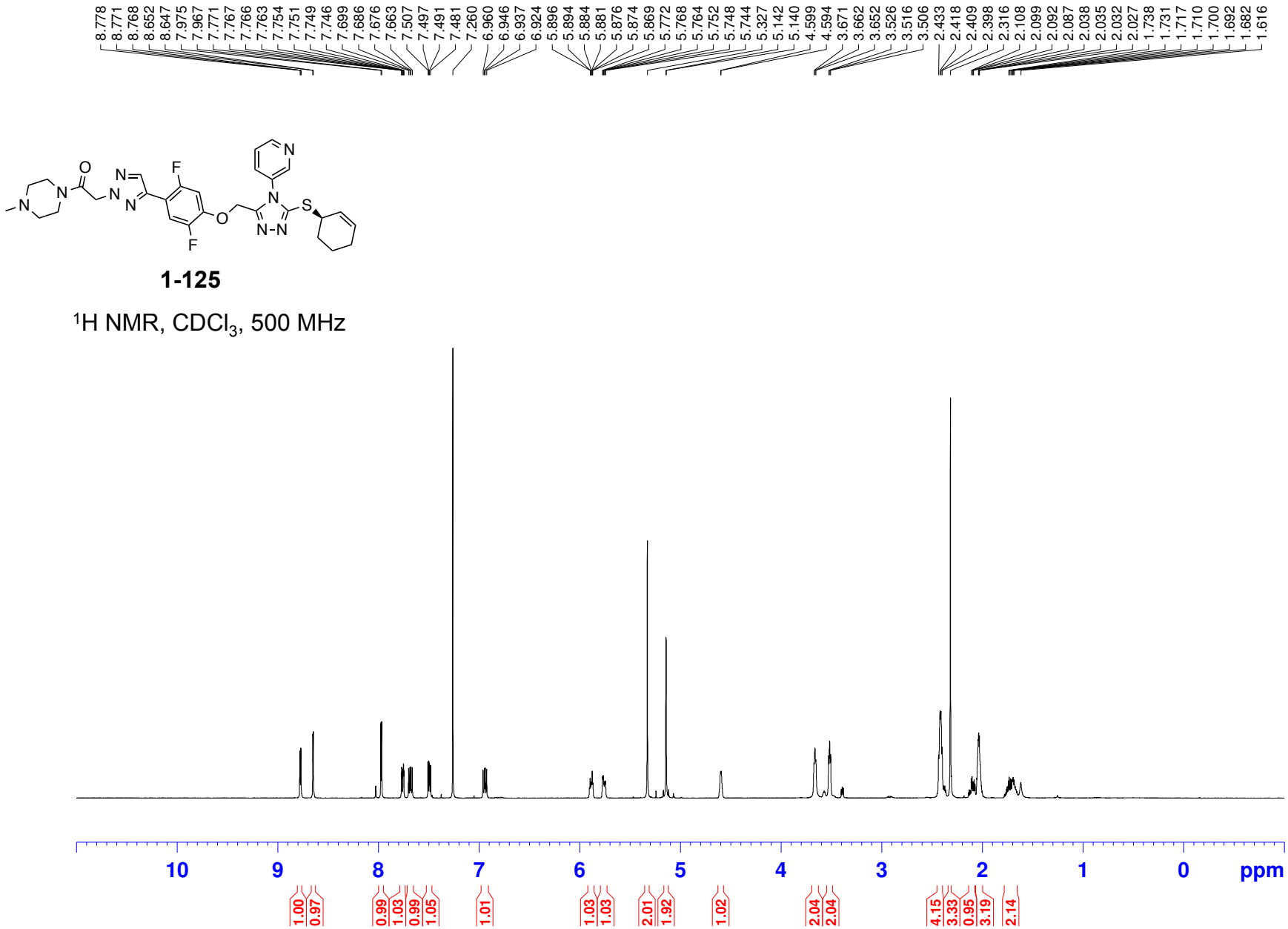
^{1}H NMR, $CDCl_3$, 500 MHz

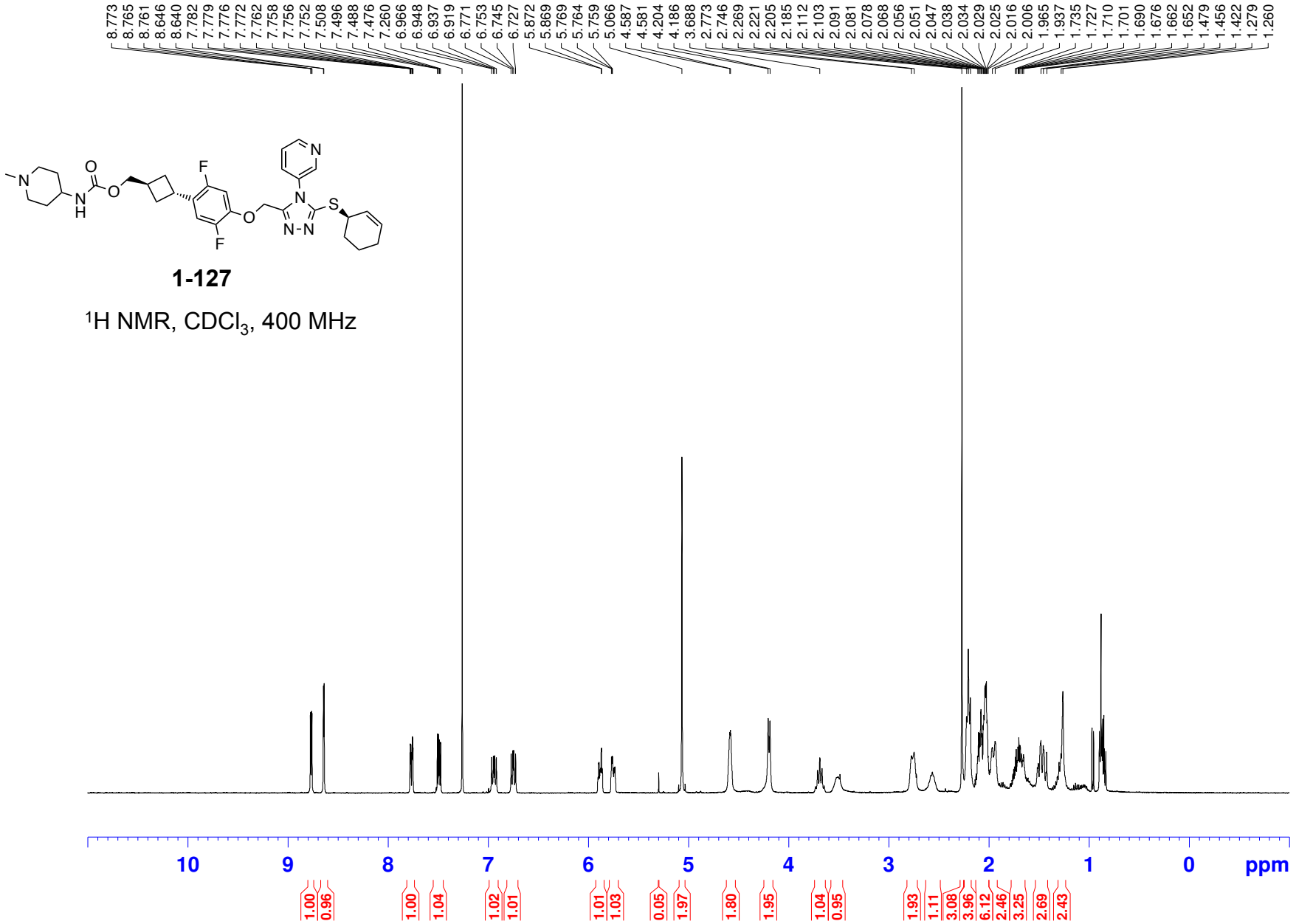


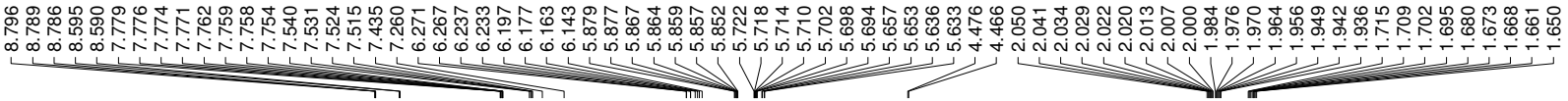


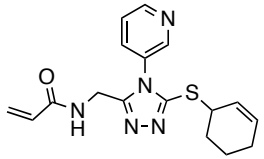
1-119b

^{13}C NMR, $CDCl_3$, 125 MHz


8.778
8.771
8.768
8.652
8.647
7.975
7.967
7.771
7.767
7.766
7.763
7.754
7.751
7.749
7.746
7.699
7.686
7.676
7.663
7.507
7.497
7.491
7.481
7.260
6.960
6.946
6.937
6.924
5.896
5.894
5.884
5.881
5.876
5.874
5.869
5.772
5.768
5.764
5.752
5.748
5.744
5.327
5.142
5.140
4.599
4.594
3.671
3.662
3.652
3.526
3.516
3.506
2.433
2.418
2.409
2.398
2.316
2.108
2.099
2.092
2.087
2.038
2.035
2.032
2.027
1.738
1.731
1.717
1.710
1.700
1.692
1.682
1.616
O
N
N
N
N
F
F
O
N
N
N
S
N-N
1-125
1H NMR, CDCl3, 500 MHz
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.97
0.99
1.03
0.99
1.05
1.01
1.03
1.03
2.01
1.92
1.02
2.04
2.04
4.15
3.33
0.95
3.19
2.14

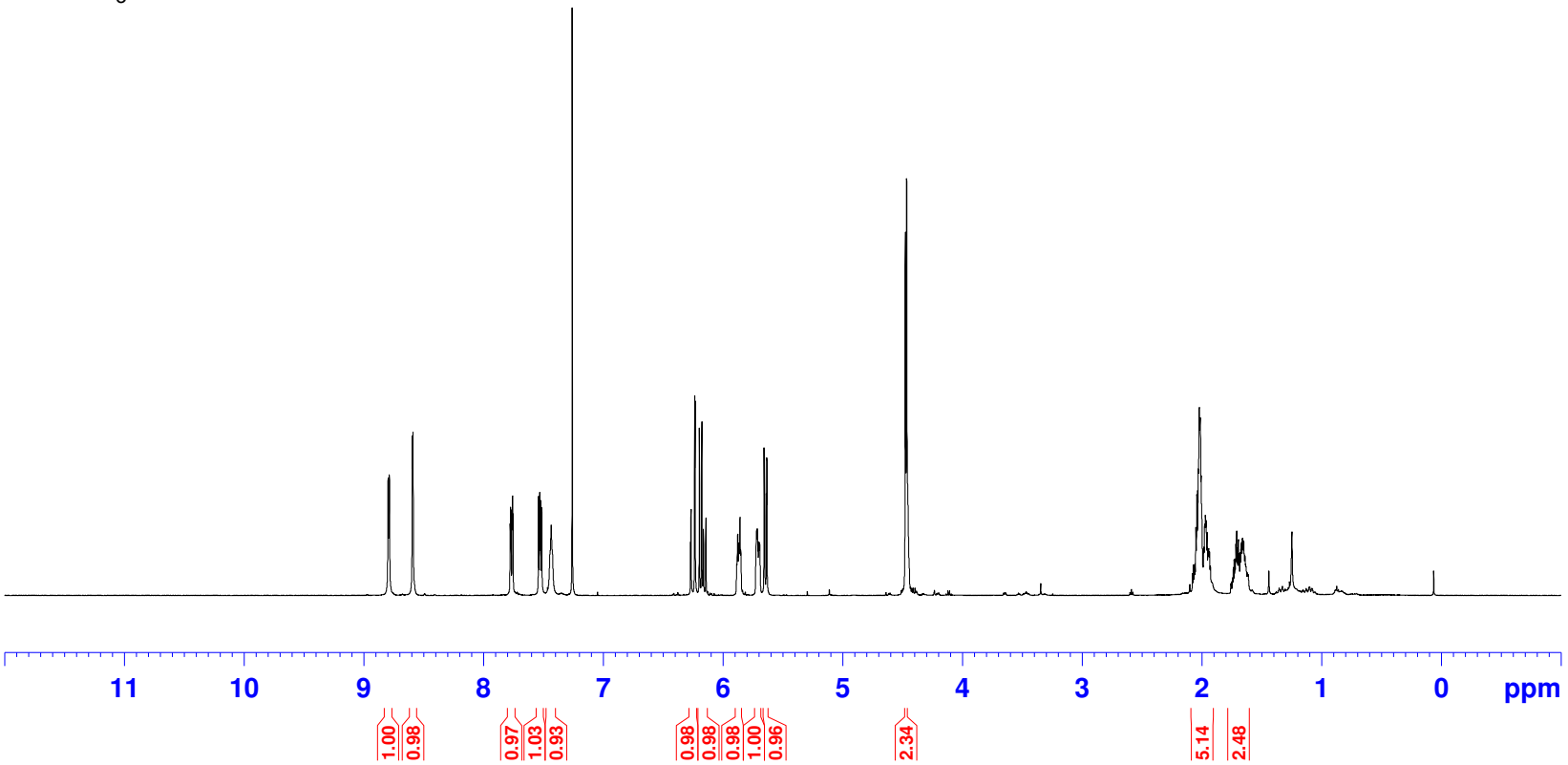

8.773
8.765
8.761
8.646
8.640
7.782
7.779
7.776
7.772
7.762
7.758
7.756
7.752
7.508
7.496
7.488
7.476
7.260
6.966
6.948
6.937
6.919
6.771
6.753
6.745
6.727
5.872
5.869
5.769
5.764
5.759
5.066
4.587
4.581
4.204
4.186
3.688
2.773
2.746
2.269
2.221
2.205
2.185
2.112
2.103
2.091
2.081
2.078
2.068
2.056
2.051
2.047
2.038
2.034
2.029
2.025
2.016
2.006
1.965
1.937
1.735
1.727
1.710
1.701
1.690
1.676
1.662
1.652
1.479
1.456
1.422
1.279
1.260
N
O
N
H
O
F
F
O
N
N-N
N
S
1-127
1H NMR, CDCl3, 400 MHz
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.96
1.00
1.04
1.02
1.01
1.01
1.03
0.05
1.97
1.80
1.95
1.04
0.95
1.93
1.11
3.08
3.96
6.12
2.46
3.25
2.69
2.43

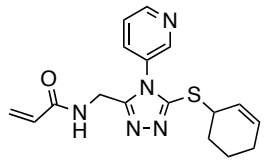




1-130

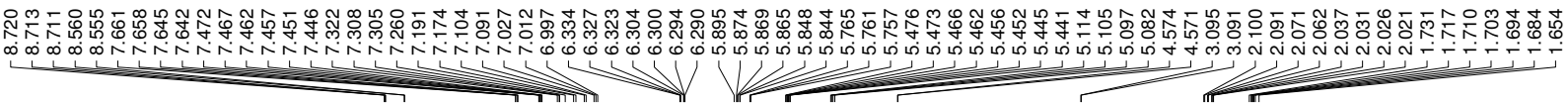
^{1}H NMR, $CDCl_3$, 500 MHz

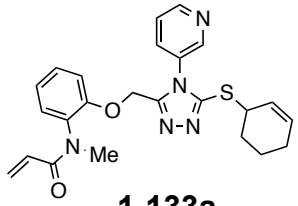




1-130

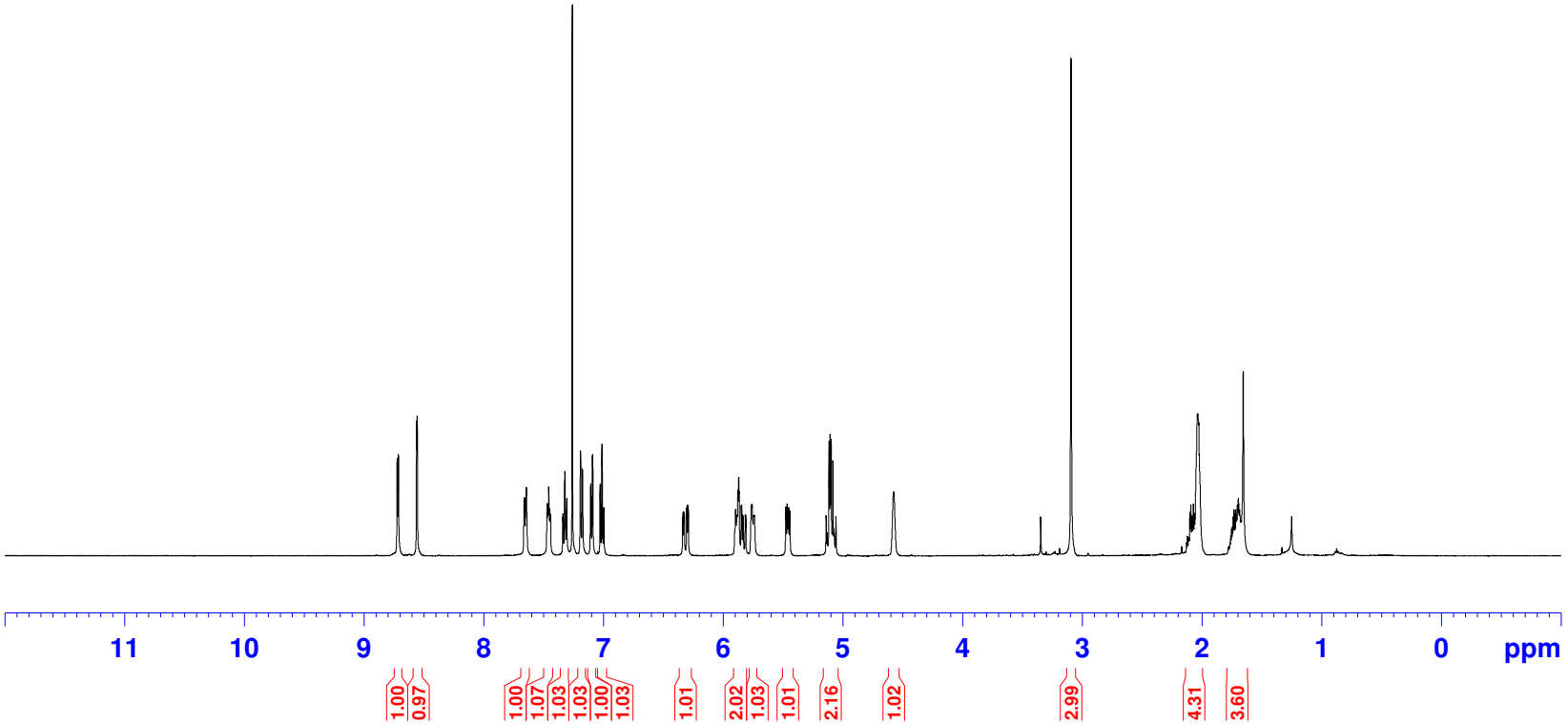
^{13}C NMR, $CDCl_3$, 125 MHz

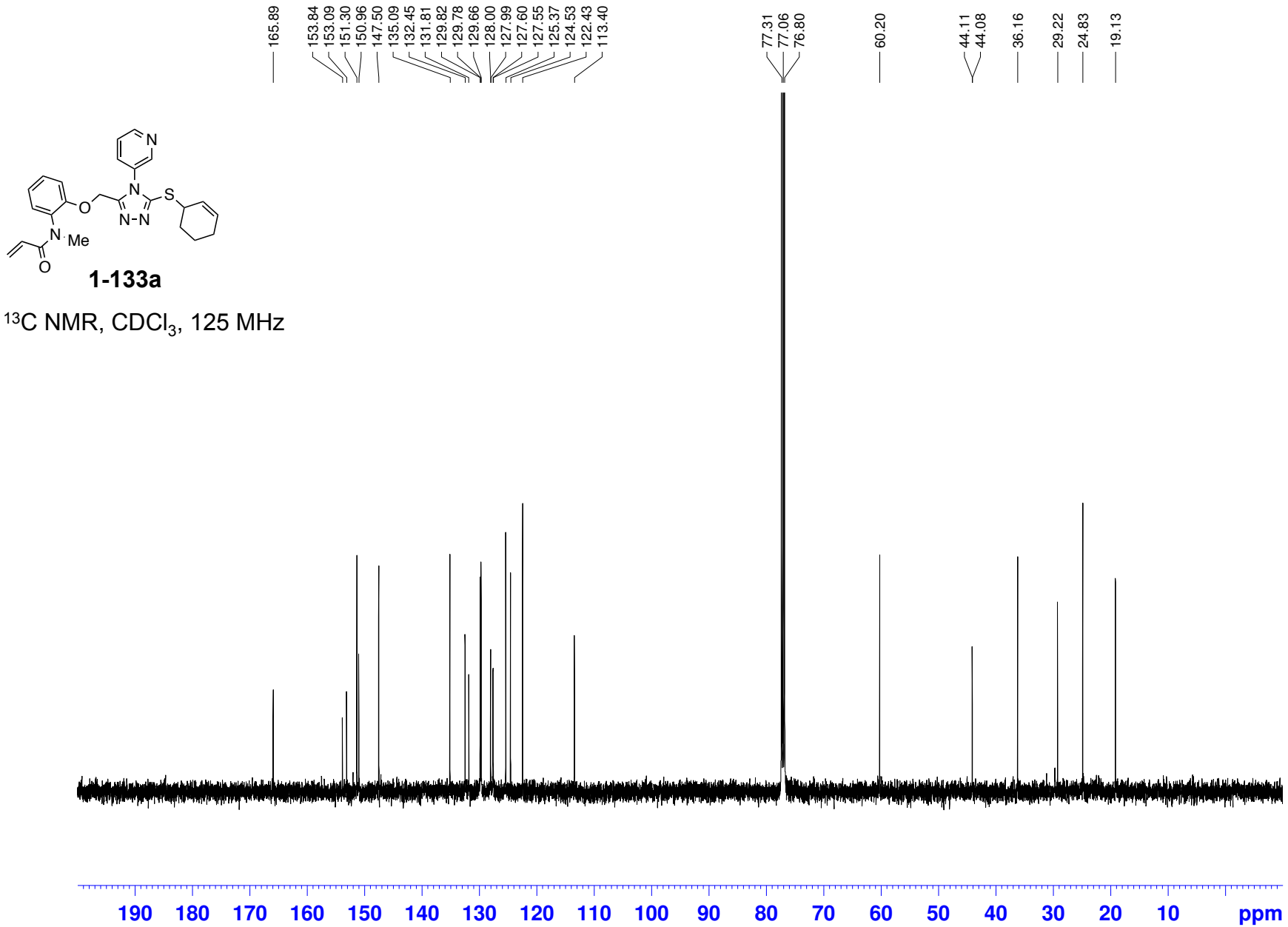




1-133a

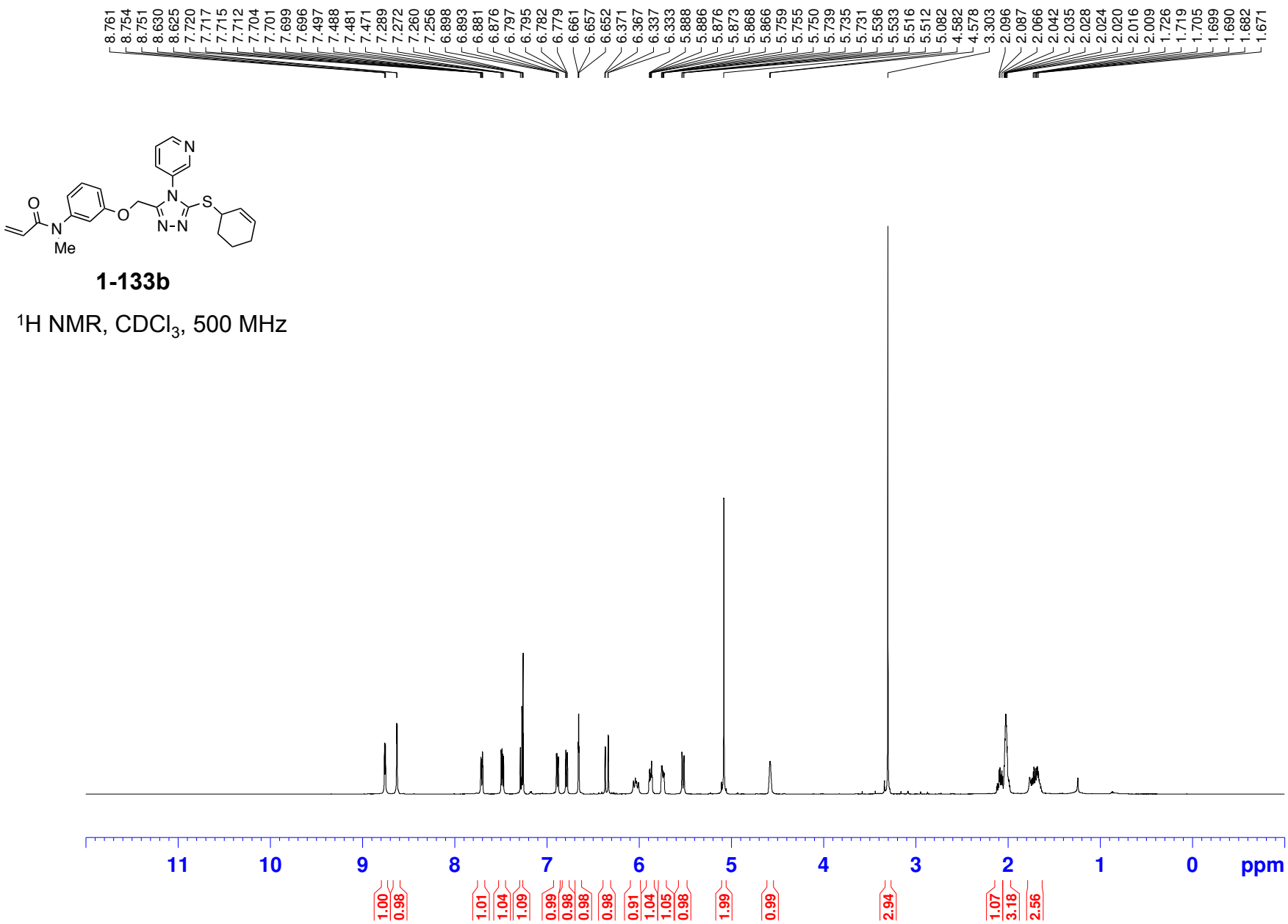
^{1}H NMR, $CDCl_3$, 500 MHz





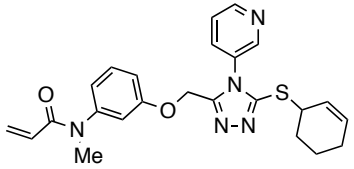
1-133a

^{13}C NMR, $CDCl_3$, 125 MHz



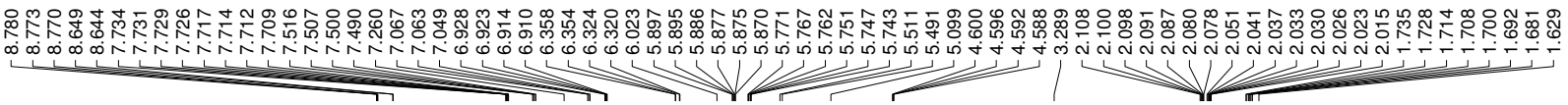
1-133b

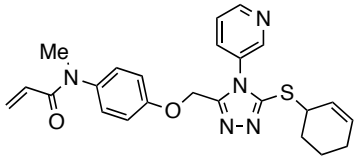
^{1}H NMR, $CDCl_3$, 500 MHz



1-133b

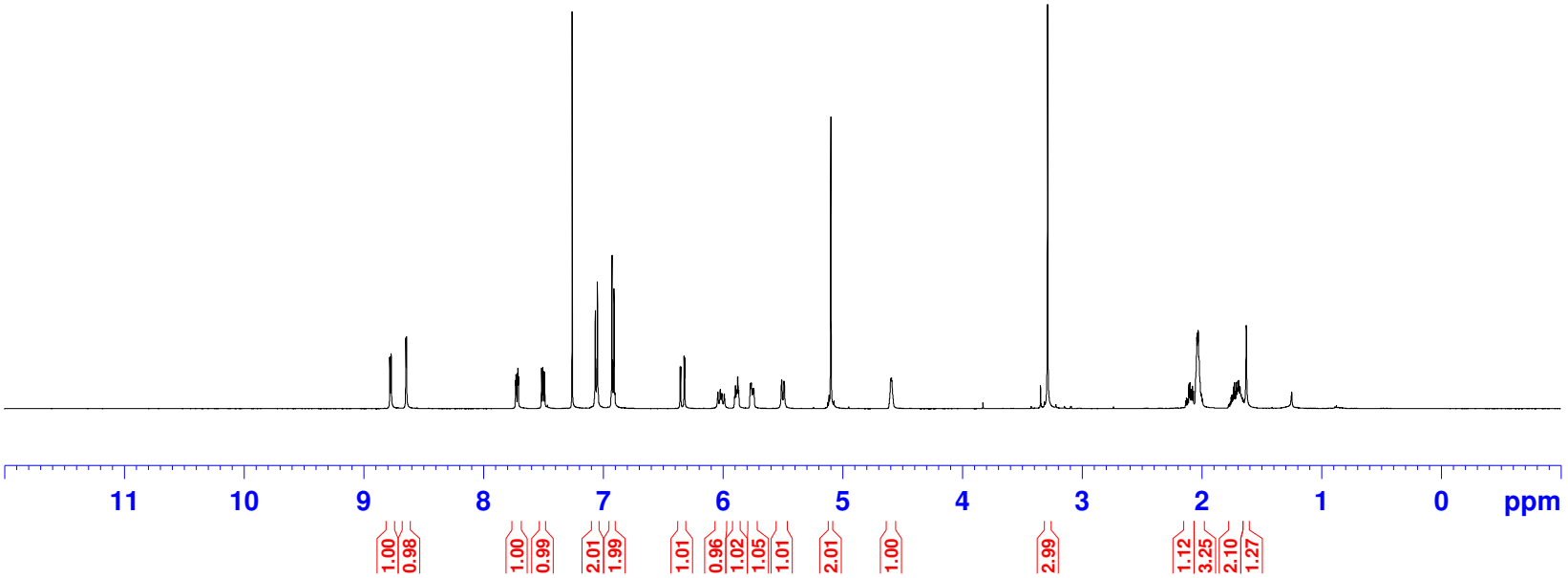
^{13}C NMR, $CDCl_3$, 125 MHz

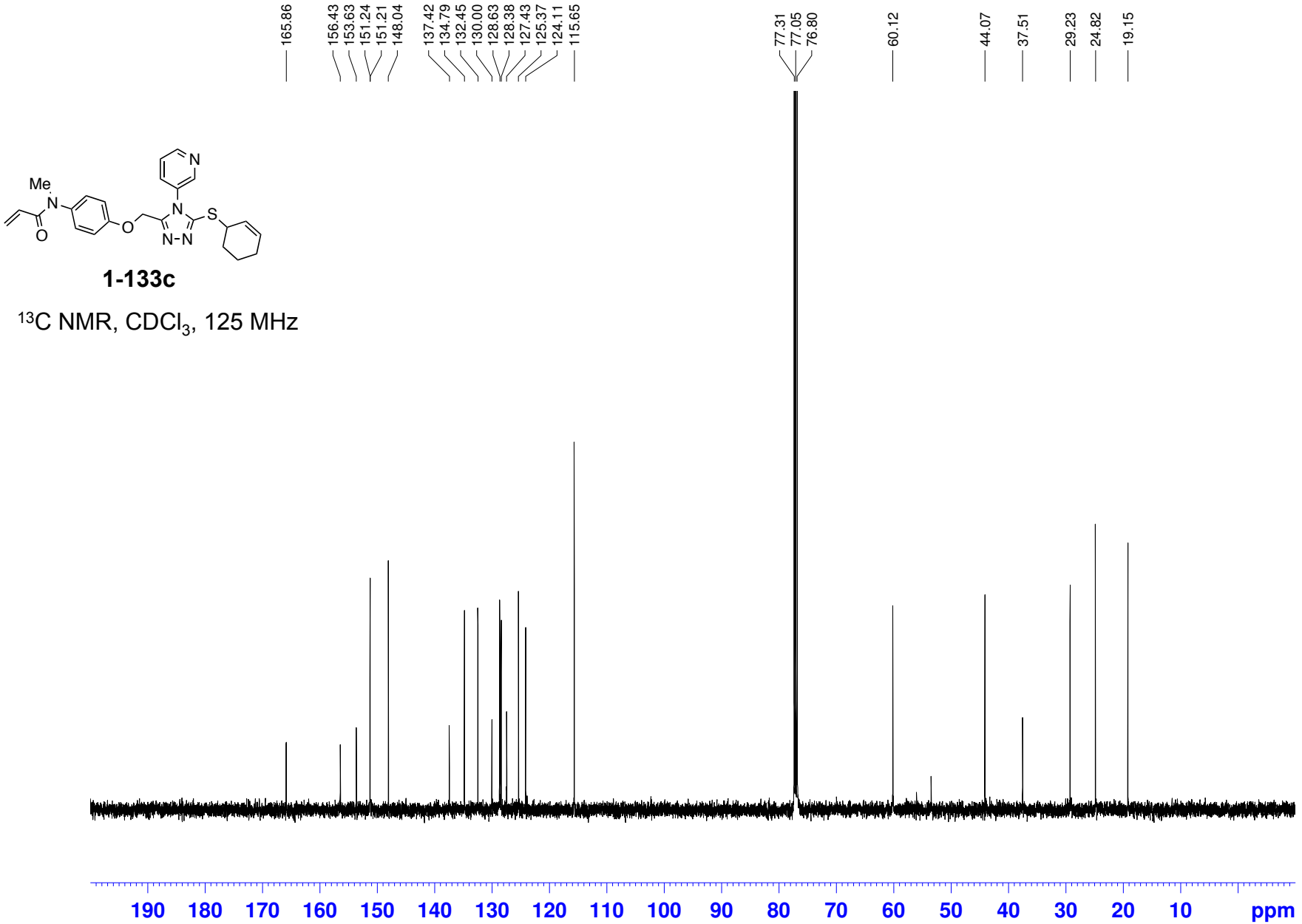


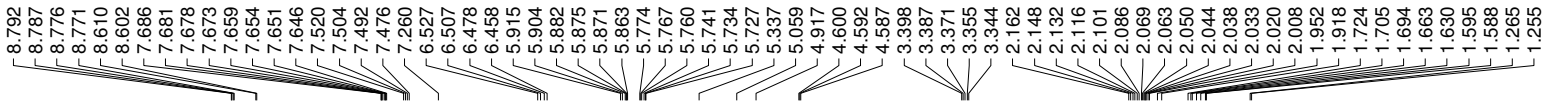


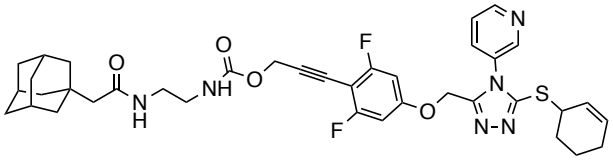
1-133c

^{1}H NMR, $CDCl_3$, 500 MHz



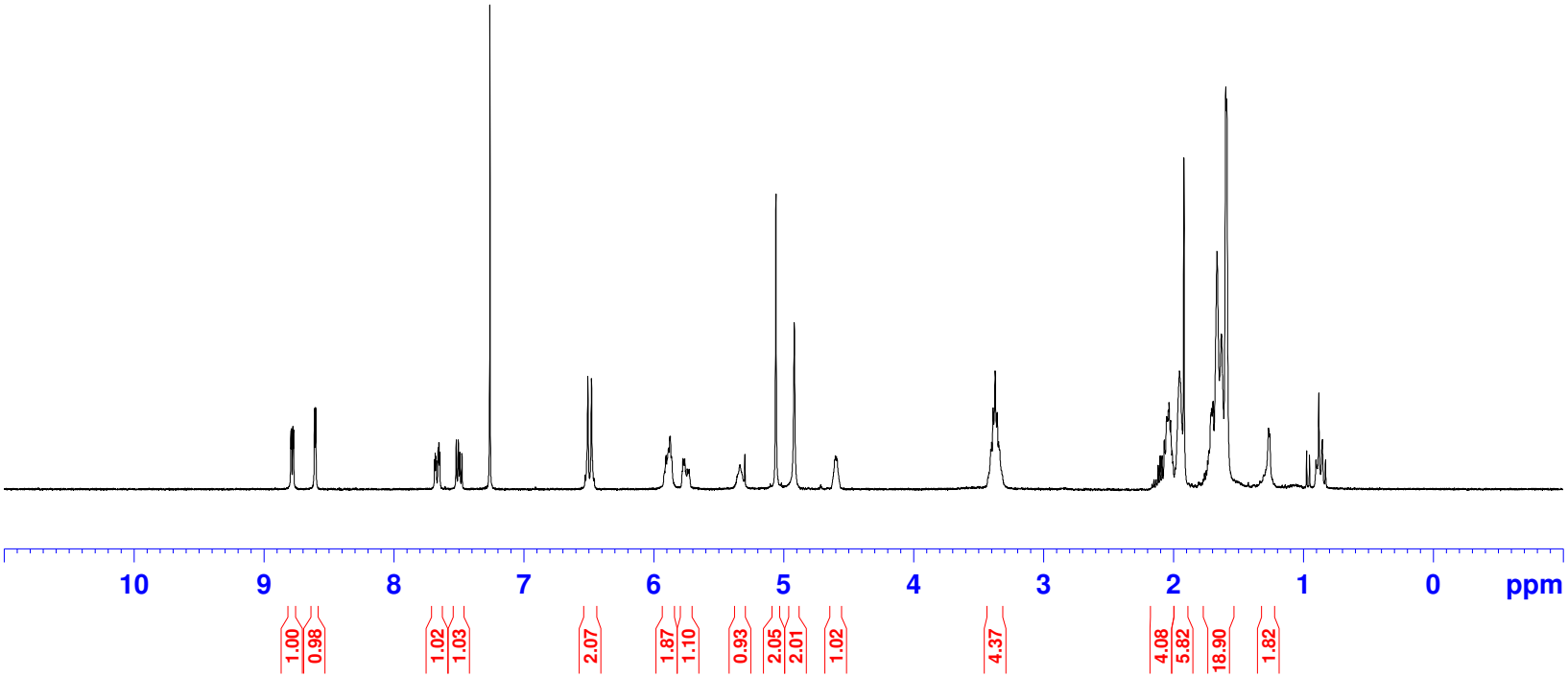

165.86
156.43
153.63
151.24
151.21
148.04
137.42
134.79
132.45
130.00
128.63
128.38
127.43
125.37
124.11
115.65
77.31
77.05
76.80
60.12
44.07
37.51
29.23
24.82
19.15
Me
N
O
O
N
N
N-N
S
N
1-133c
13C NMR, CDCl3, 125 MHz
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

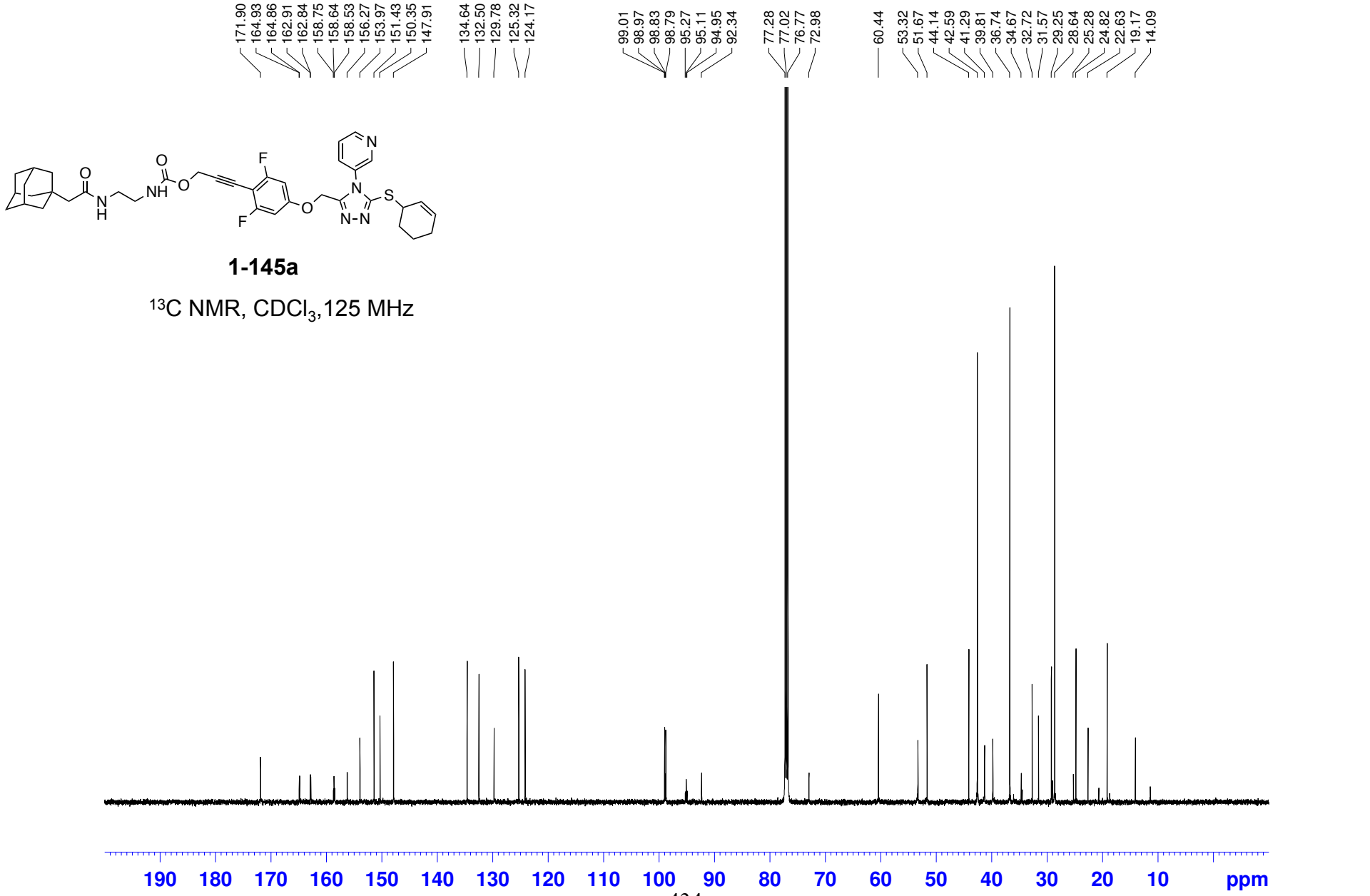




1-145a

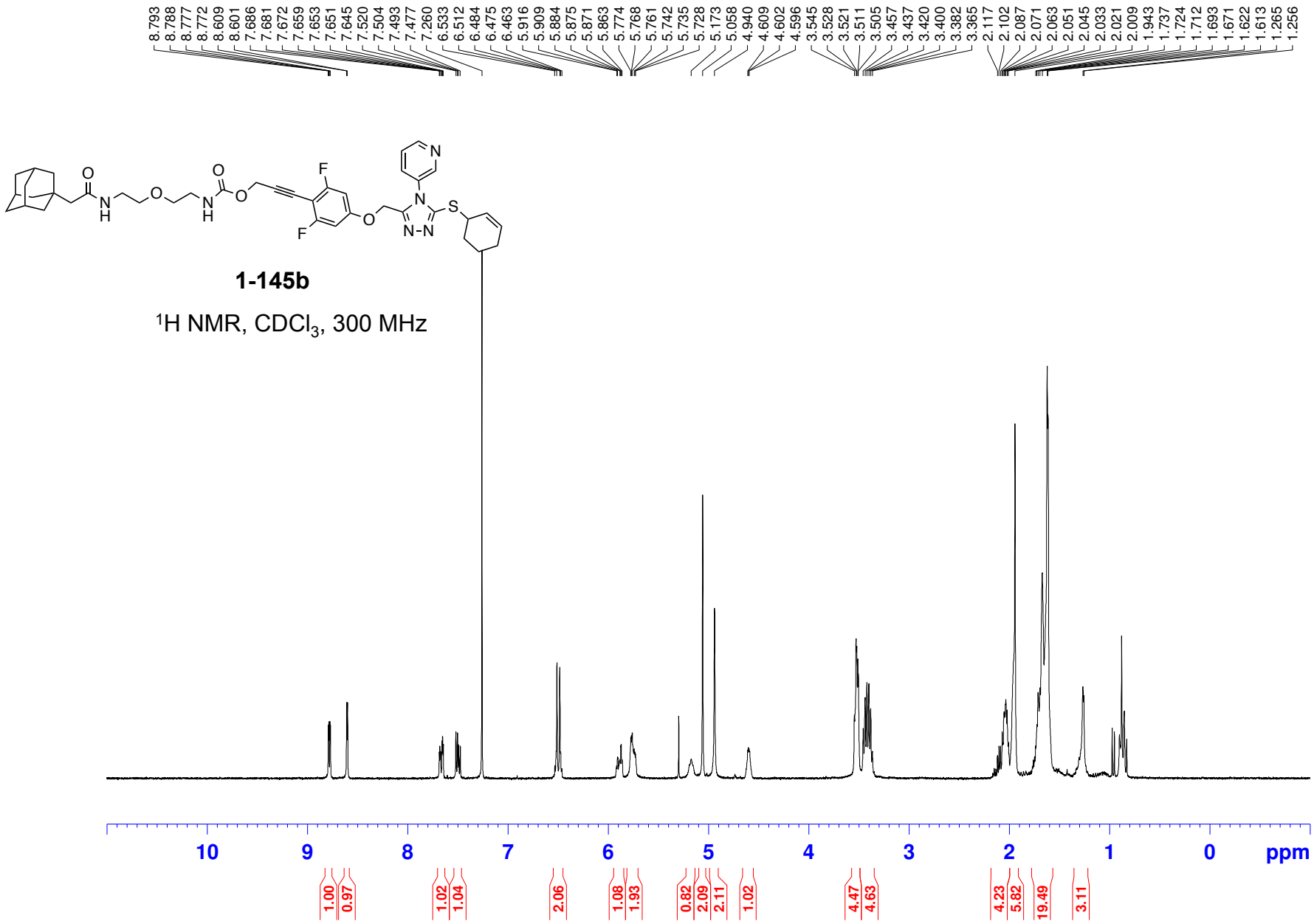
^{1}H NMR, $CDCl_3$, 300 MHz

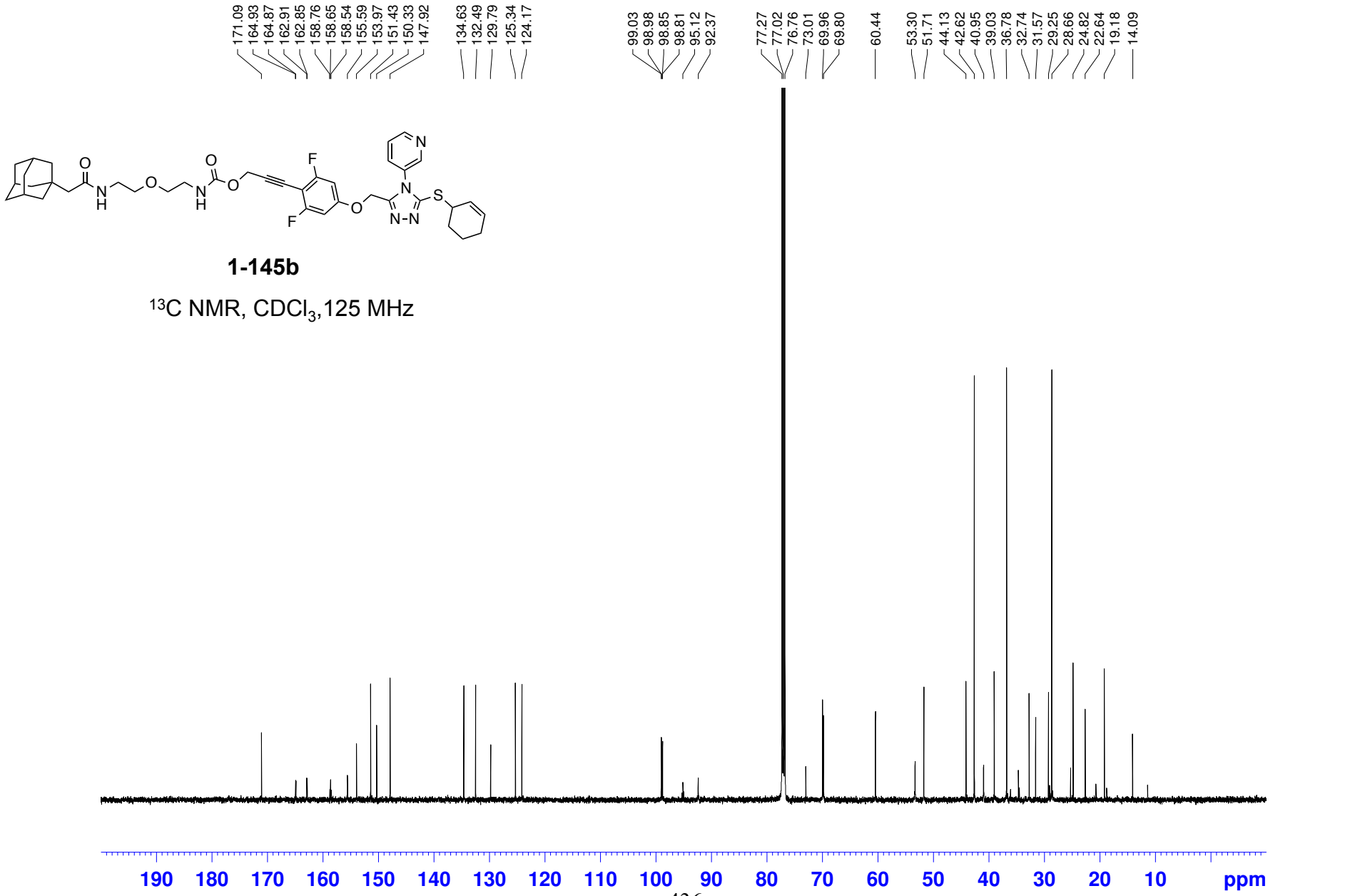




1-145a

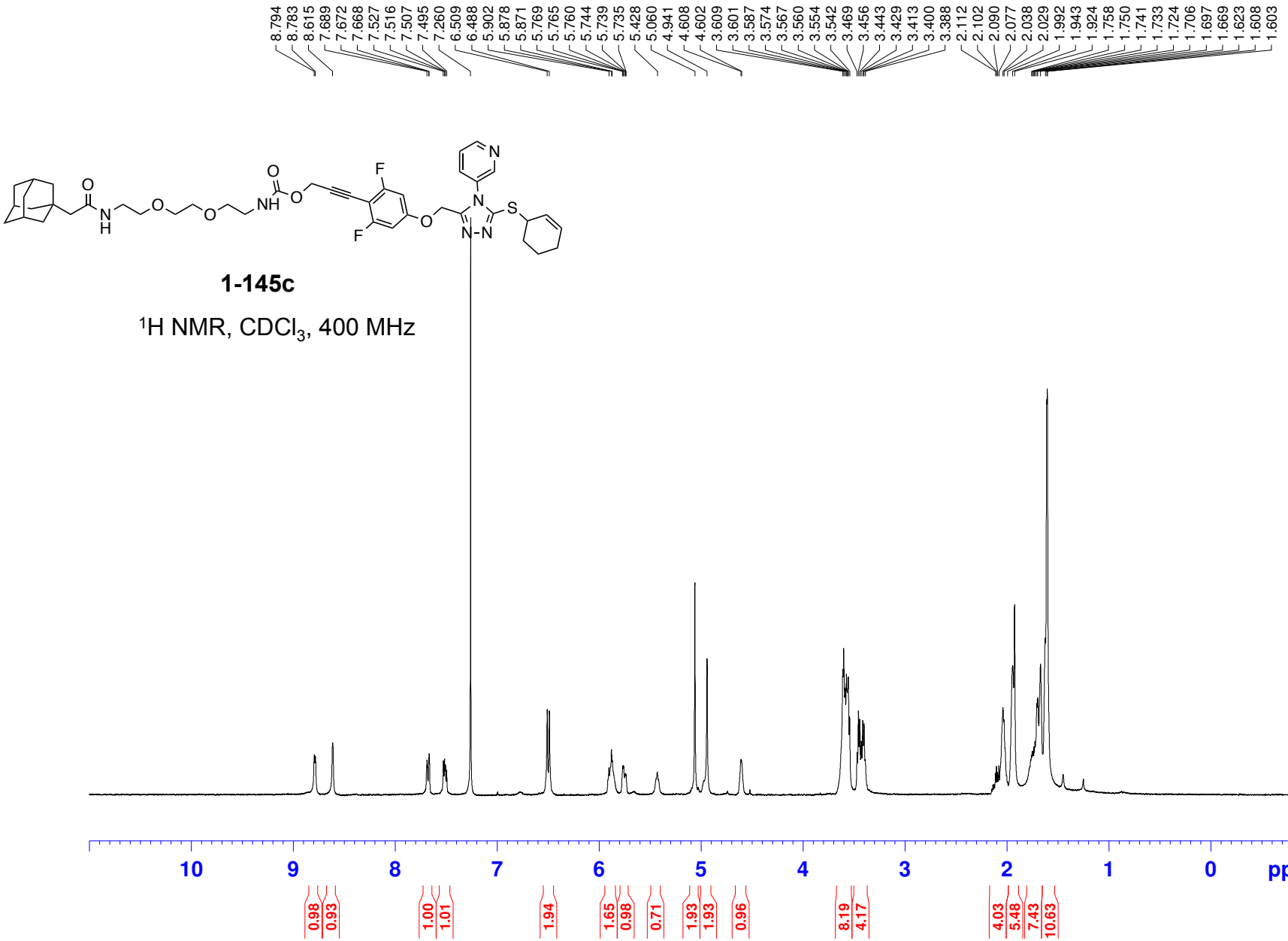
^{13}C NMR, $CDCl_3$,125 MHz

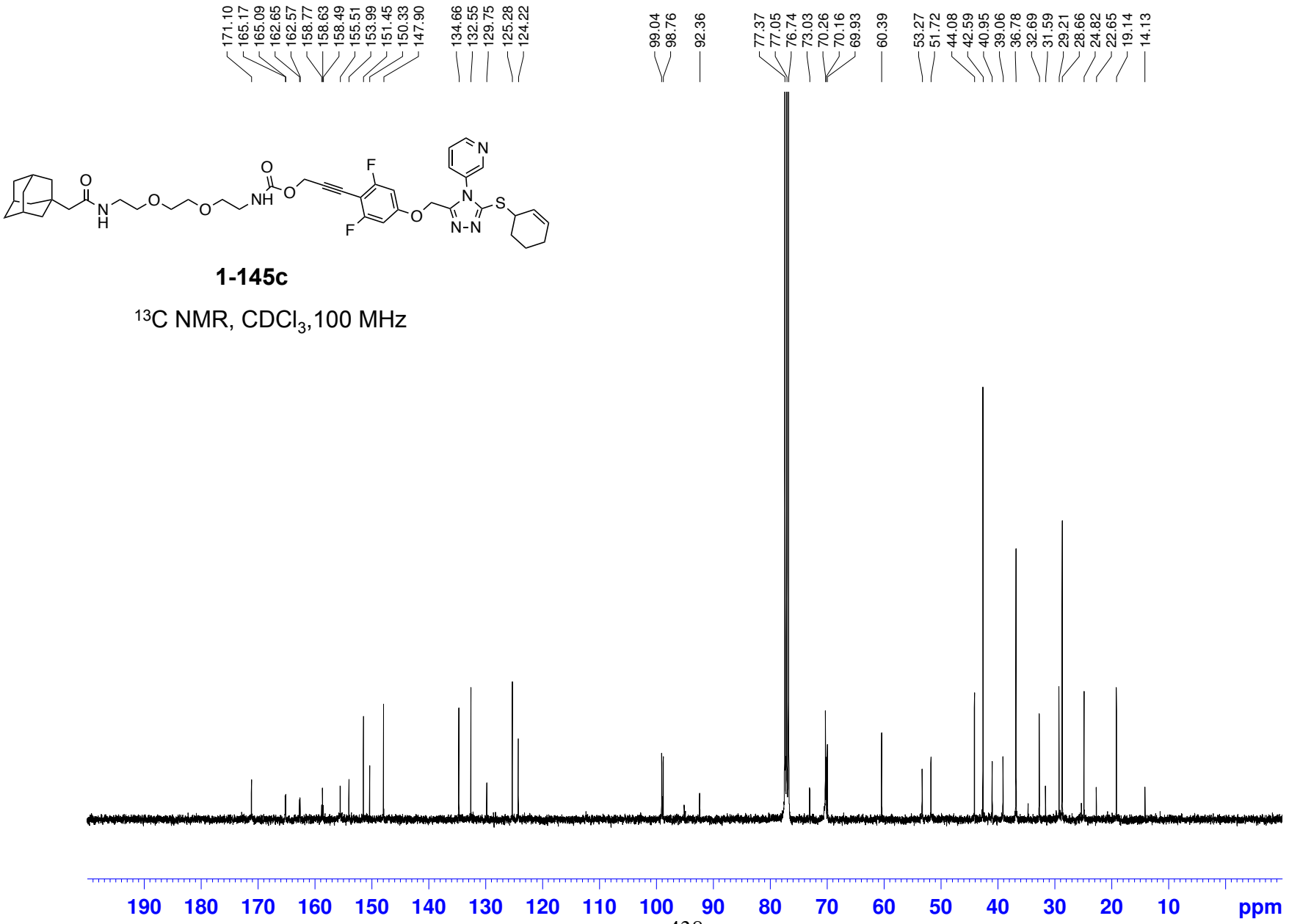

8.793
8.788
8.777
8.772
8.609
8.601
7.686
7.681
7.672
7.659
7.653
7.651
7.645
7.520
7.504
7.493
7.477
7.260
6.533
6.512
6.484
6.475
6.463
5.916
5.909
5.884
5.875
5.871
5.863
5.774
5.768
5.761
5.742
5.735
5.728
5.173
5.058
4.940
4.609
4.602
4.596
3.545
3.528
3.521
3.511
3.505
3.457
3.437
3.420
3.400
3.382
3.365
2.117
2.102
2.087
2.071
2.063
2.051
2.045
2.033
2.021
2.009
1.943
1.737
1.724
1.712
1.693
1.671
1.622
1.613
1.265
1.256
O
N
H
O
N
H
O
O
F
F
O
N
N
N-N
S
1-145b
1H NMR, CDCl3, 300 MHz
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.97
1.02
1.04
2.06
1.08
1.93
0.82
2.09
2.11
1.02
4.47
4.63
4.23
5.82
19.49
3.11

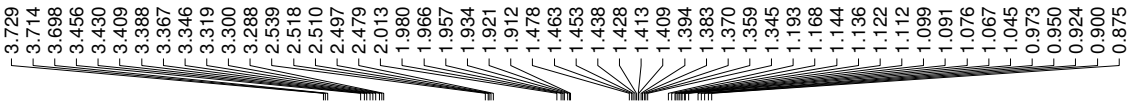


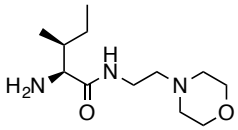
1-145b

^{13}C NMR, $CDCl_3$,125 MHz


8.794
8.783
8.615
7.689
7.672
7.668
7.527
7.516
7.507
7.495
7.260
6.509
6.488
5.902
5.878
5.871
5.769
5.765
5.760
5.744
5.739
5.735
5.428
5.060
4.941
4.608
4.602
3.609
3.601
3.587
3.574
3.567
3.560
3.554
3.542
3.469
3.456
3.443
3.429
3.413
3.400
3.388
2.112
2.102
2.090
2.077
2.038
2.029
1.992
1.943
1.924
1.758
1.750
1.741
1.733
1.724
1.706
1.697
1.669
1.623
1.608
1.603
1-145c
1H NMR, CDCl3, 400 MHz
10
9
8
7
6
5
4
3
2
1
0
ppm
0.98
0.93
1.00
1.01
1.94
1.65
0.98
0.71
1.93
1.93
0.96
8.19
4.17
4.03
5.48
7.43
10.63

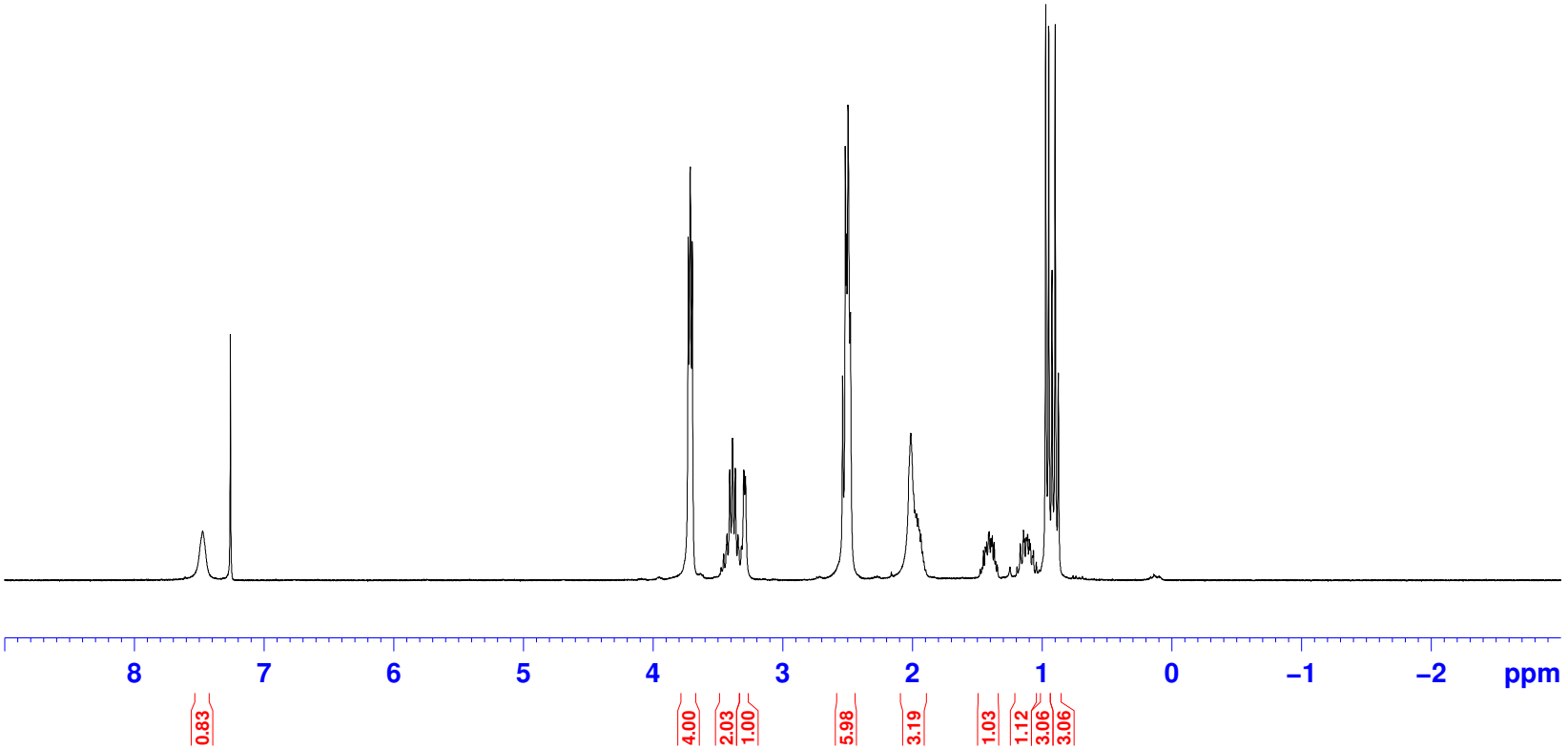

171.10
165.17
165.09
162.65
162.57
158.77
158.63
158.49
155.51
153.99
151.45
150.33
147.90
134.66
132.55
129.75
125.28
124.22
99.04
98.76
92.36
77.37
77.05
76.74
73.03
70.26
70.16
69.93
60.39
53.27
51.72
44.08
42.59
40.95
39.06
36.78
32.69
31.59
29.21
28.66
24.82
22.65
19.14
14.13
O
N
H
O
O
O
NH
O
F
F
O
N
N
N-N
S
1-145c
^{13}C NMR, $CDCl_3$,100 MHz
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

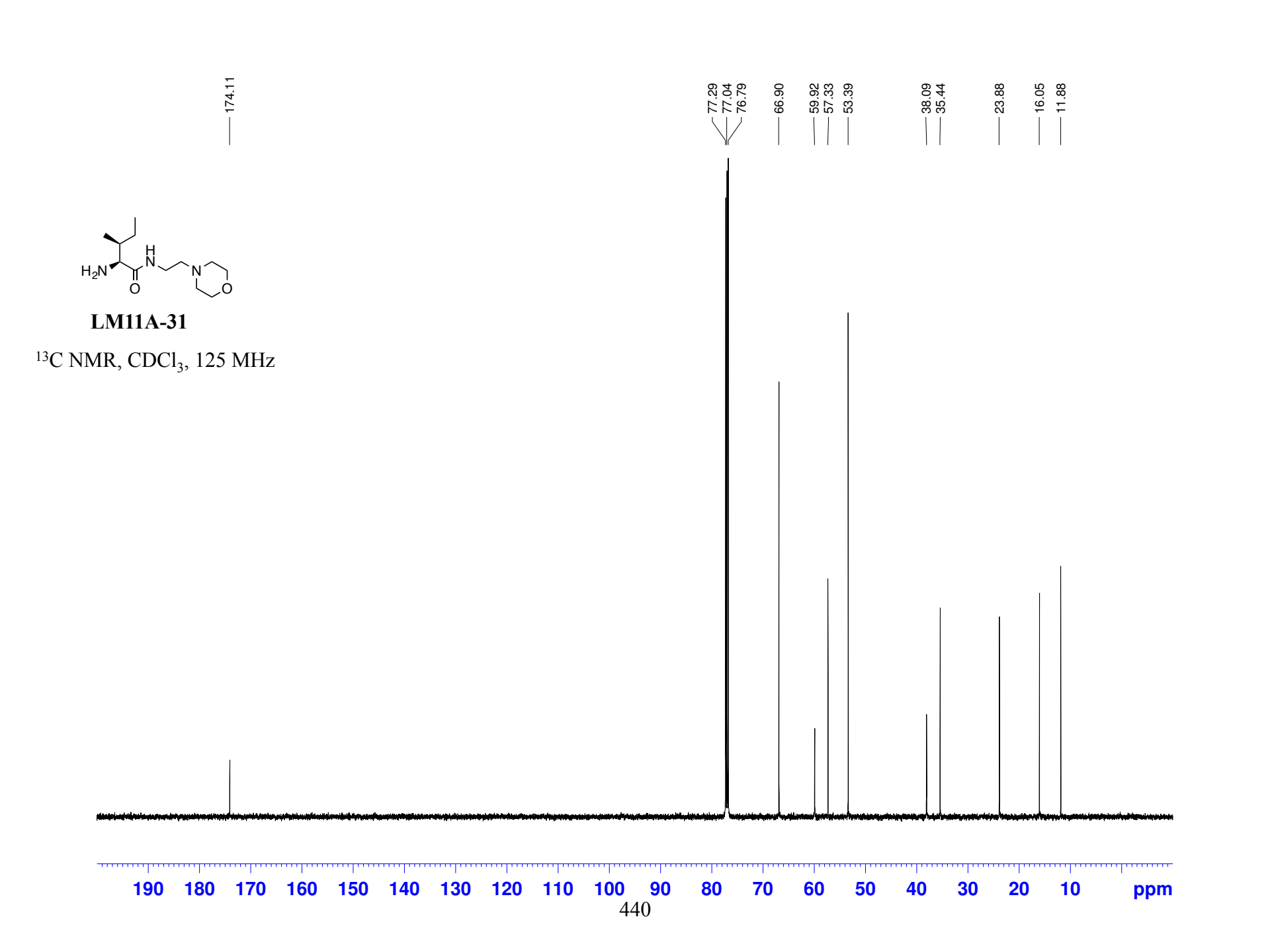


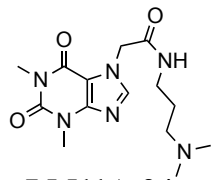


LM11A-31

^{1}H NMR, $CDCl_3$, 300 MHz

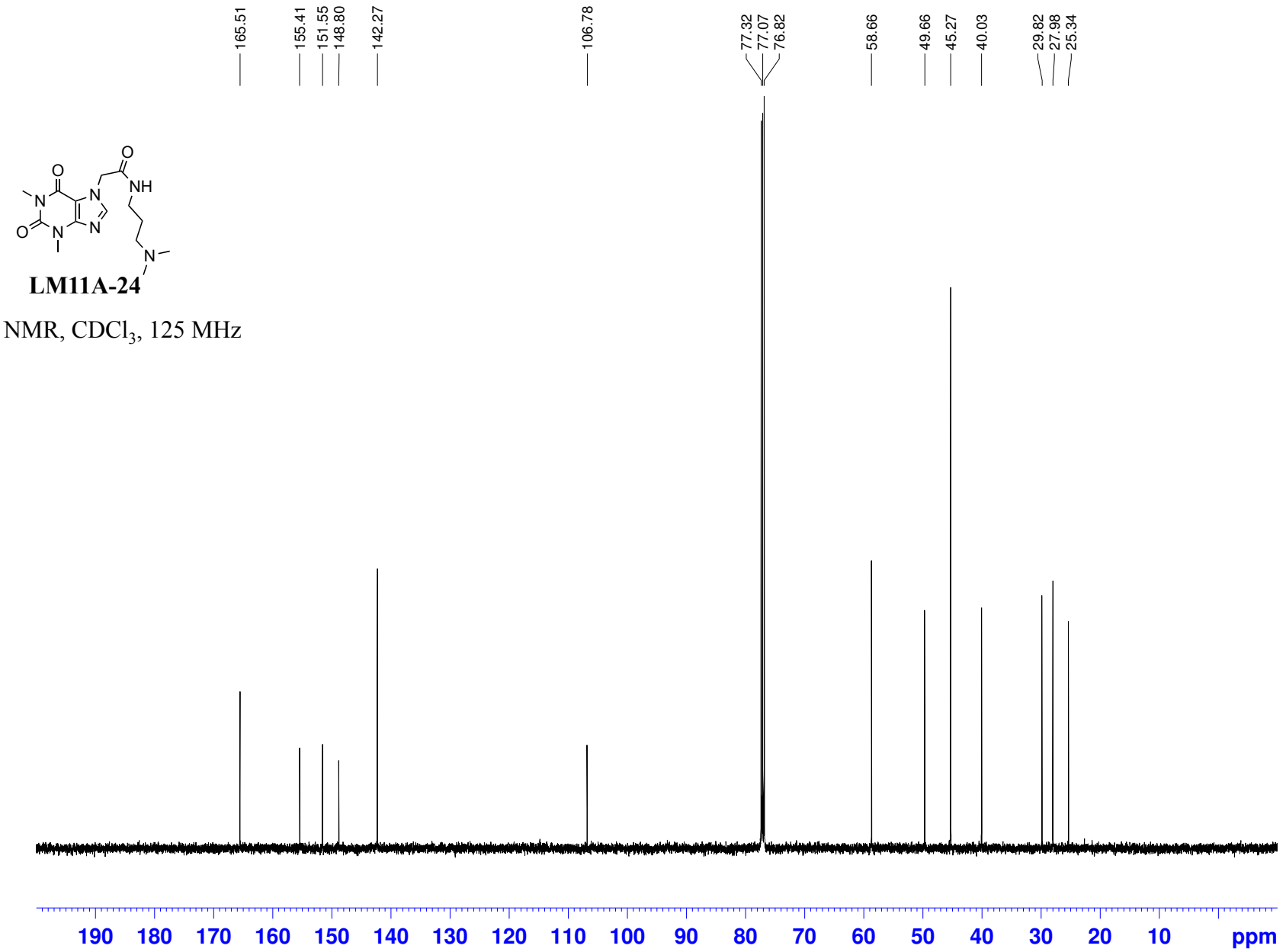



174.11
77.29
77.04
76.79
66.90
59.92
57.33
53.39
38.09
35.44
23.88
16.05
11.88
H2N
H
N
O
N
O
LM11A-31
13C NMR, CDCl3, 125 MHz
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm



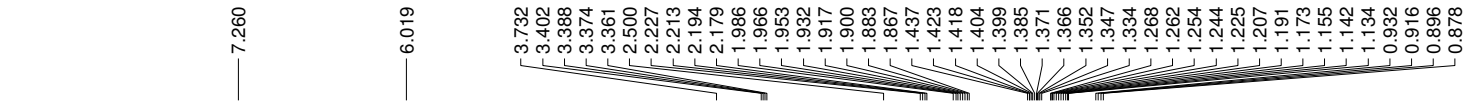
LM11A-24

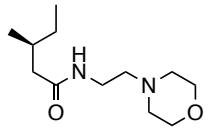
^{1}H NMR, $CDCl_3$, 500 MHz



LM11A-24

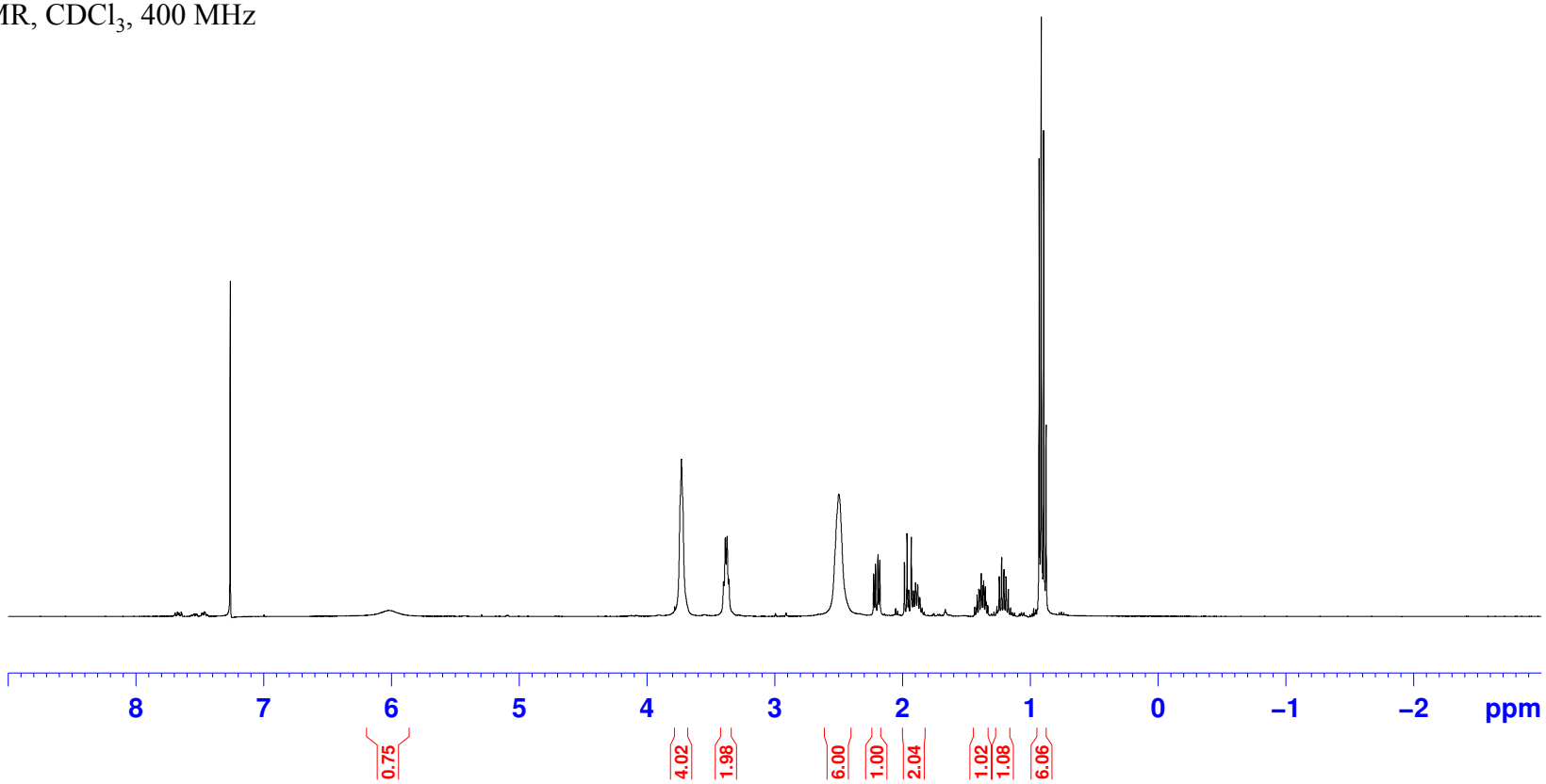
^{13}C NMR, $CDCl_3$, 125 MHz

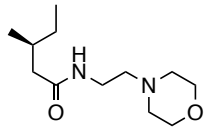




2-5

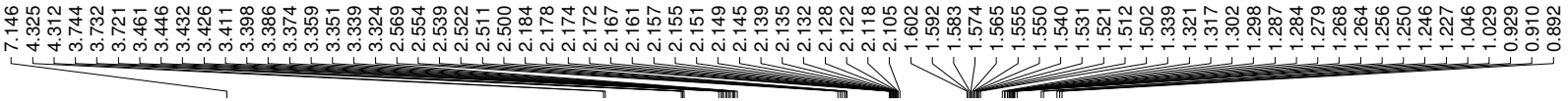
^{1}H NMR, $CDCl_3$, 400 MHz

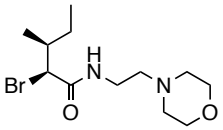




2-5

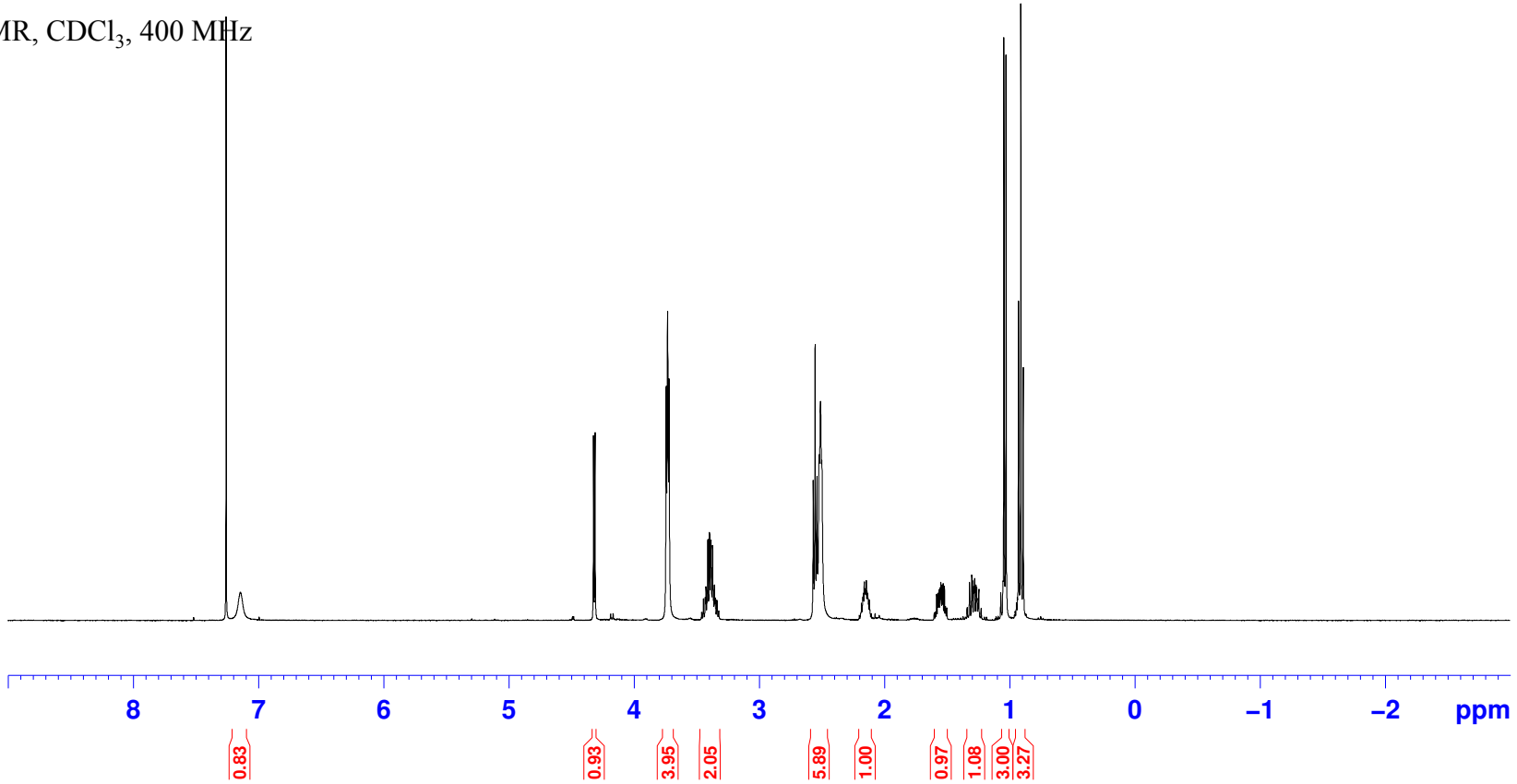
^{13}C NMR, $CDCl_3$, 125 MHz

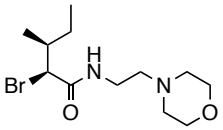




2-6

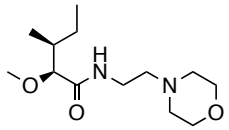
^{1}H NMR, $CDCl_3$, 400 MHz





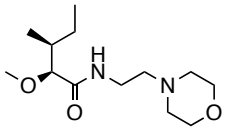
2-6

^{13}C NMR, $CDCl_3$, 125 MHz



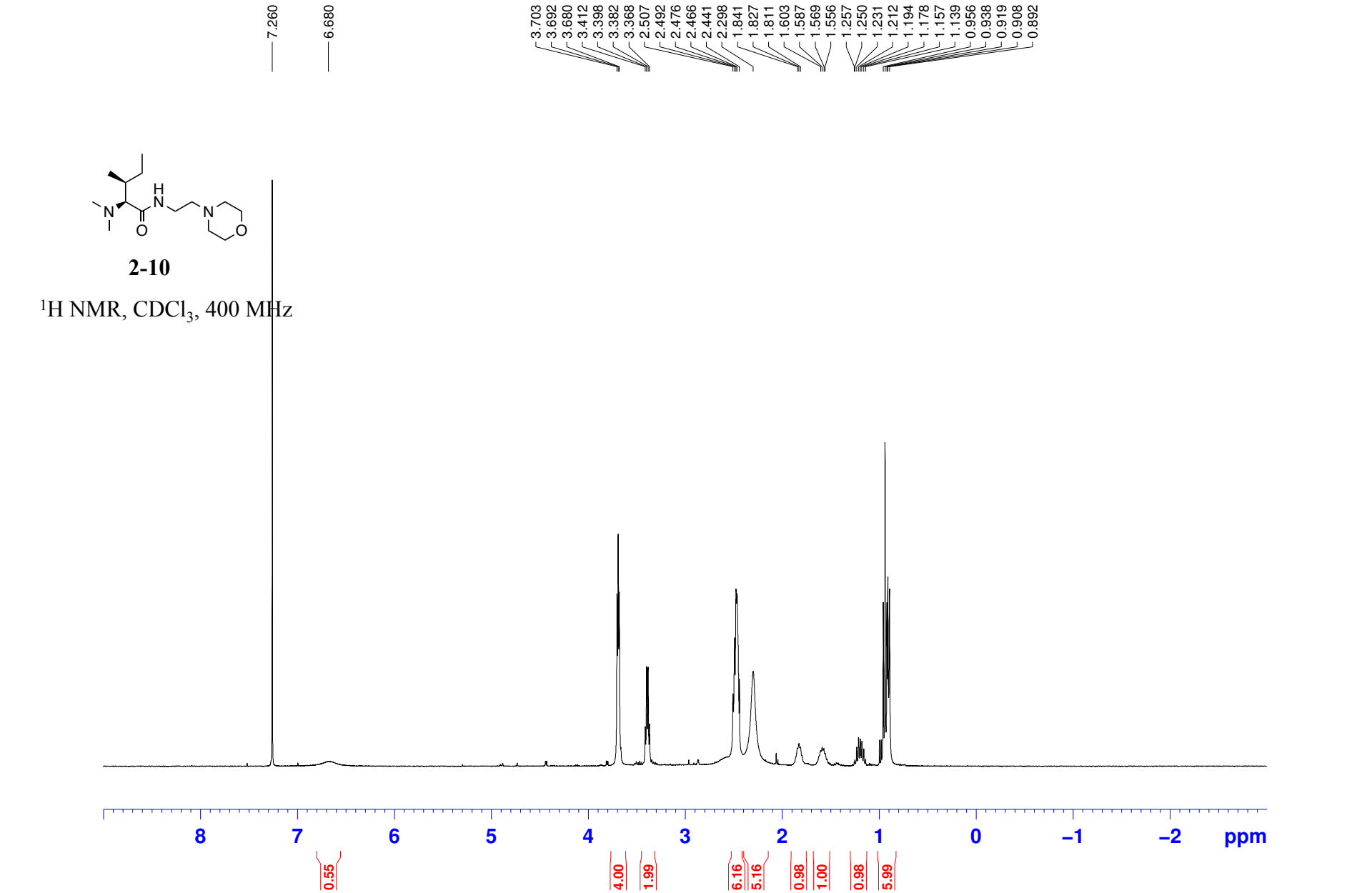
2-9

^{1}H NMR, $CDCl_3$, 400 MHz



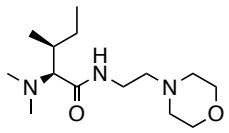
2-9

^{13}C NMR, $CDCl_3$, 3125 MHz



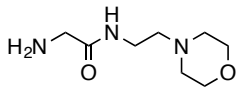
2-10

^{1}H NMR, $CDCl_3$, 400 MHz



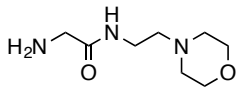
2-10

^{13}C NMR, $CDCl_3$, 125 MHz



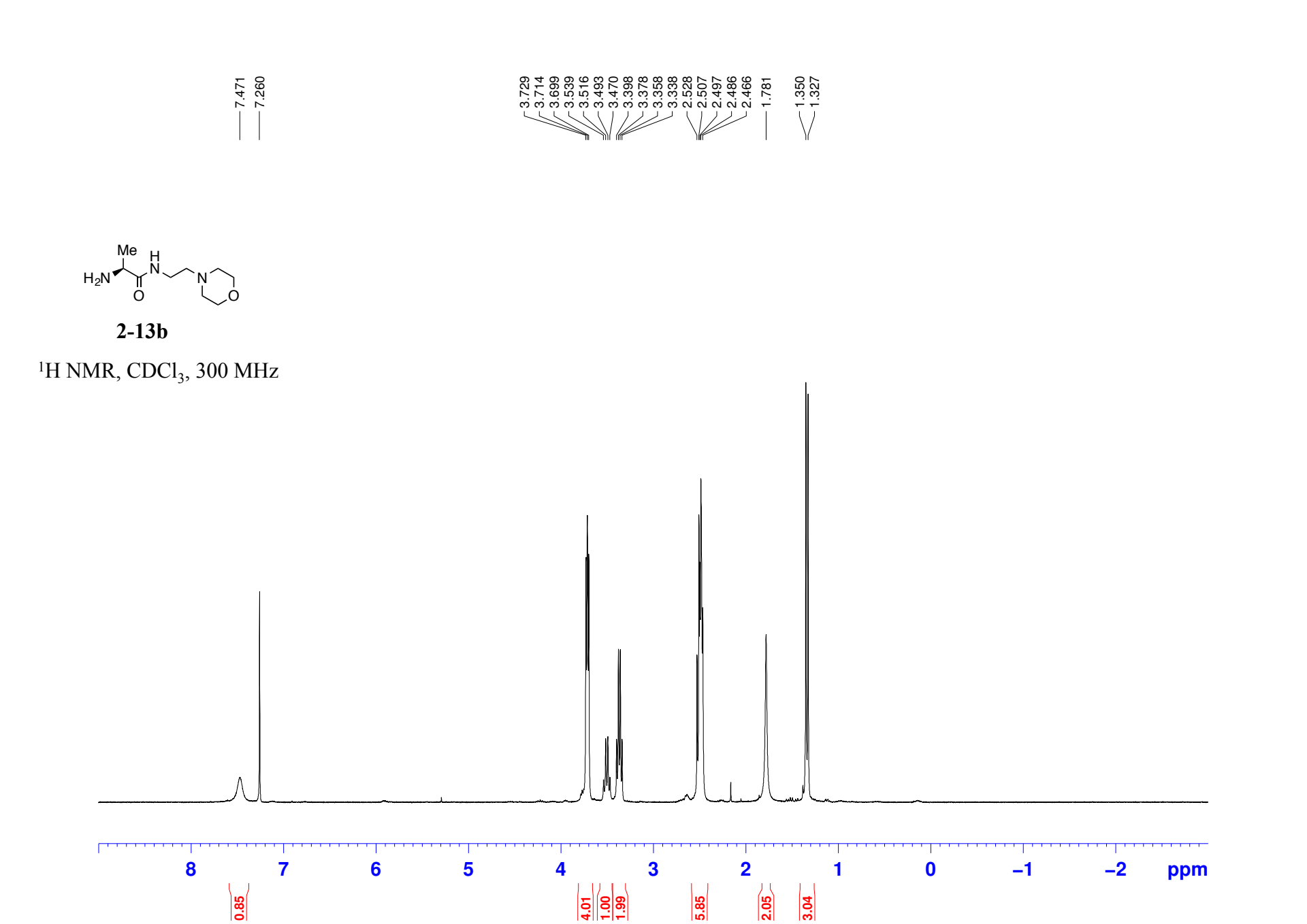
2-13a

^{1}H NMR, $CDCl_3$, 300 MHz



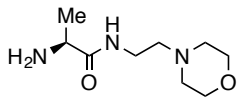
2-13a

^{13}C NMR, $CDCl_3$, 125 MHz



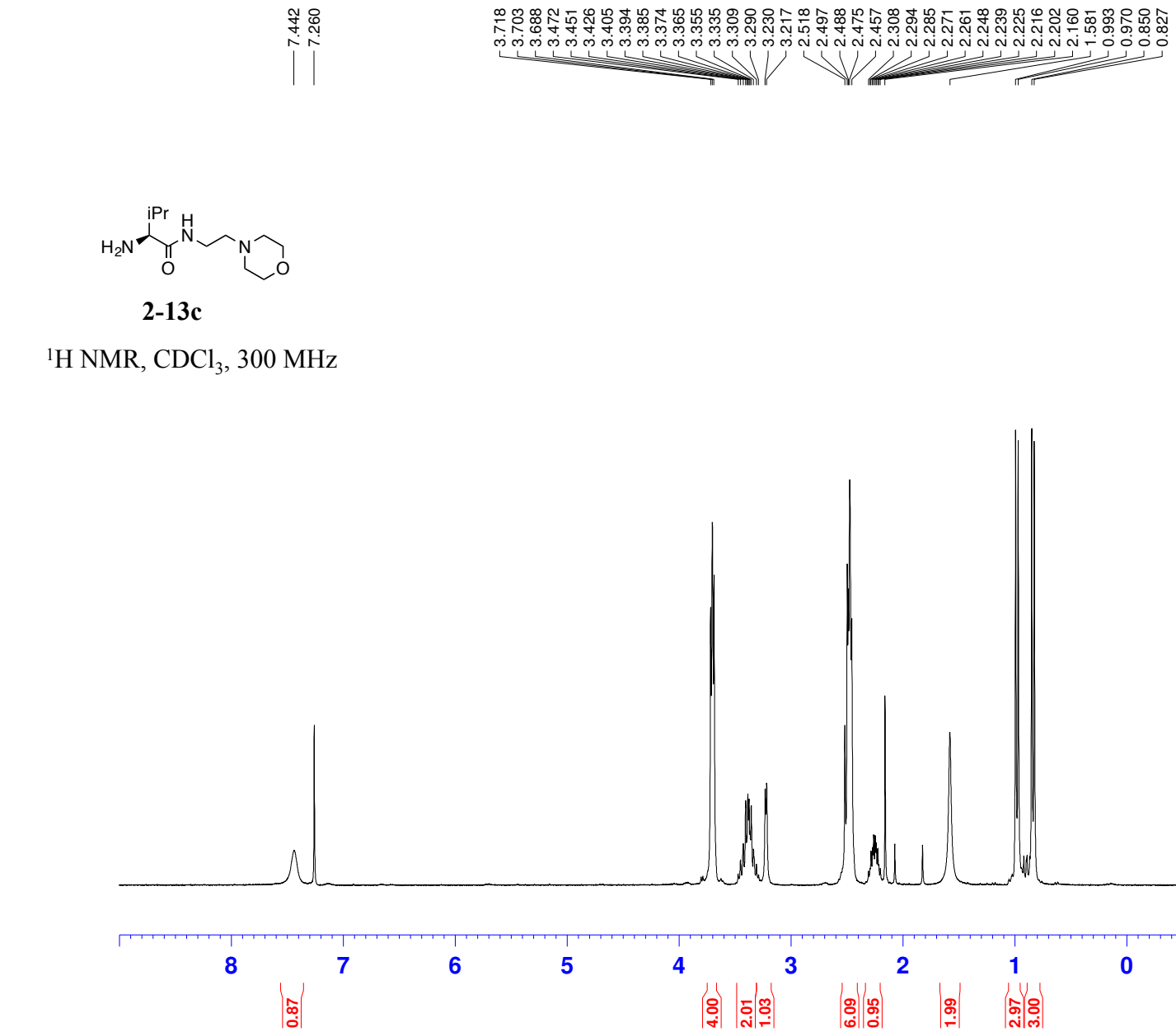
2-13b

^{1}H NMR, $CDCl_3$, 300 MHz



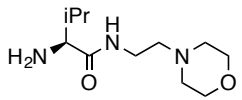
2-13b

^{13}C NMR, $CDCl_3$, 125 MHz



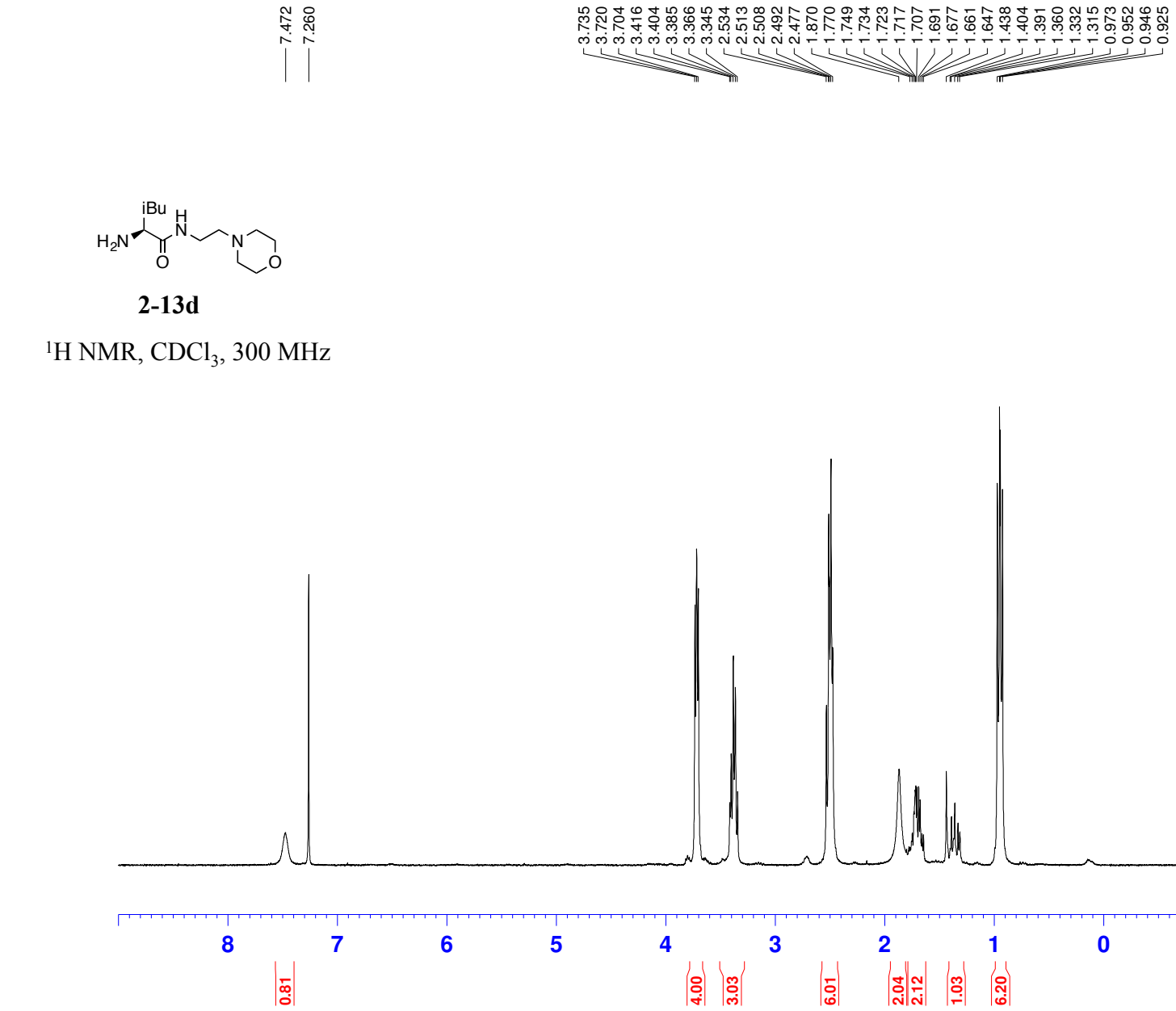
2-13c

^{1}H NMR, $CDCl_3$, 300 MHz



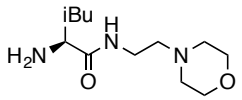
2-13c

^{13}C NMR, $CDCl_3$, 125 MHz



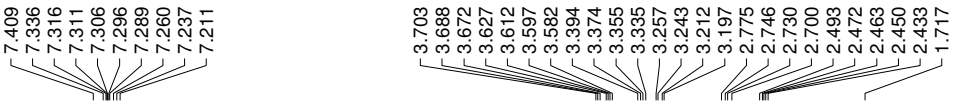
2-13d

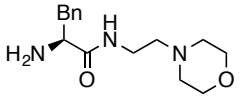
^{1}H NMR, $CDCl_3$, 300 MHz



2-13d

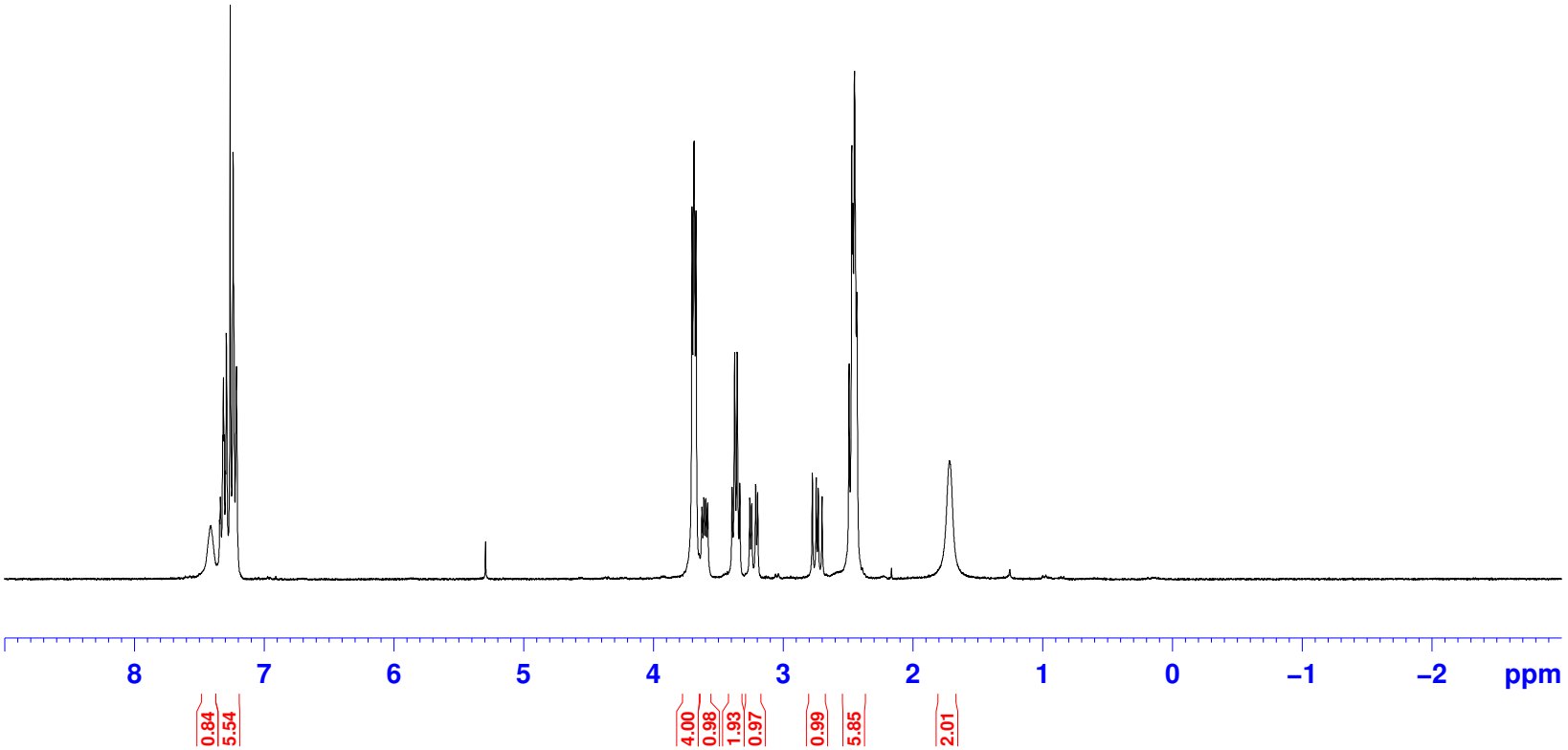
^{13}C NMR, $CDCl_3$, 125 MHz

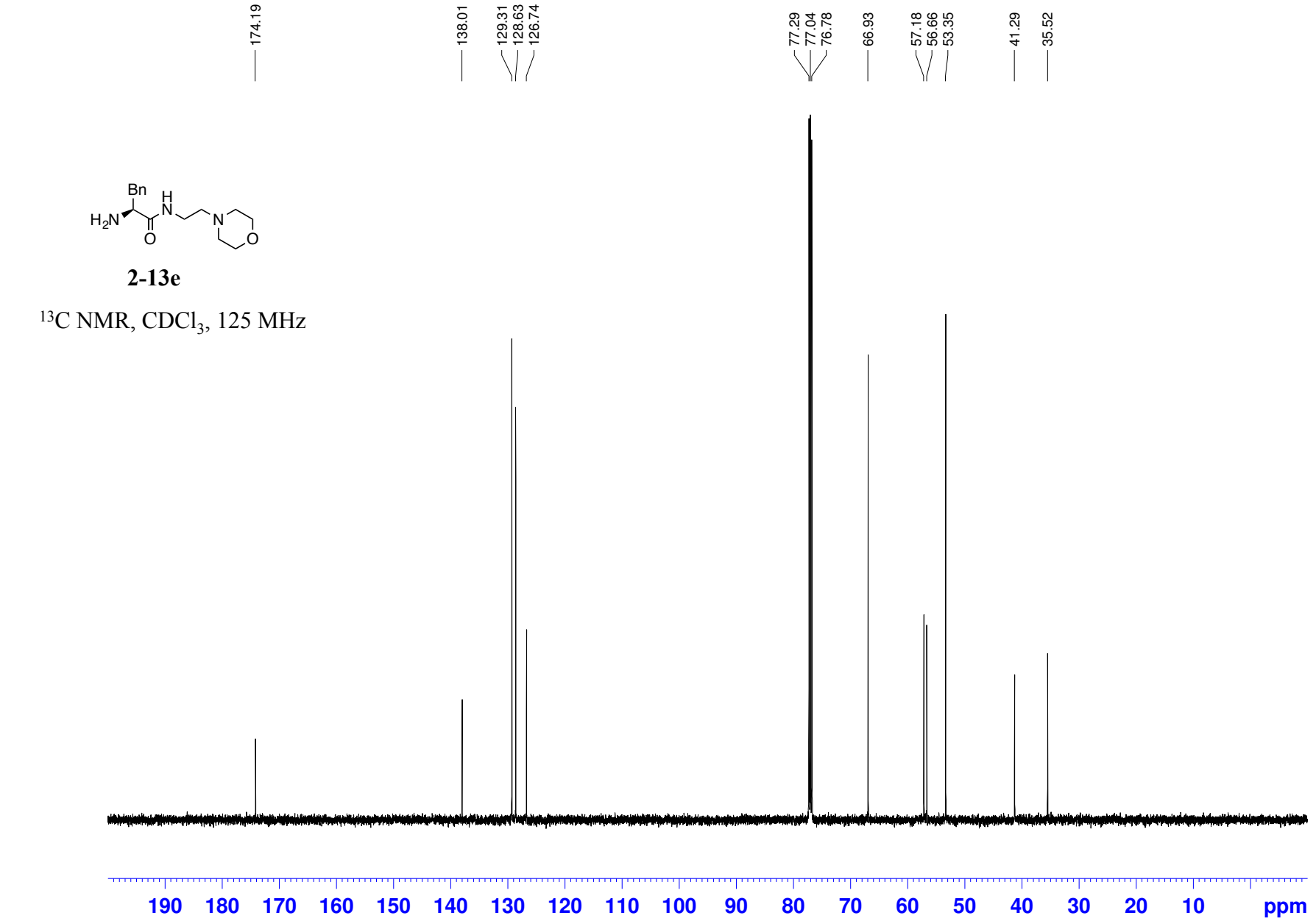


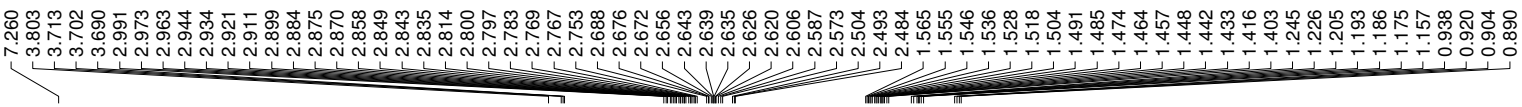


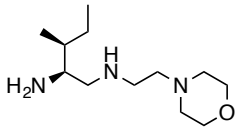
2-13e

^{1}H NMR, $CDCl_3$, 300 MHz



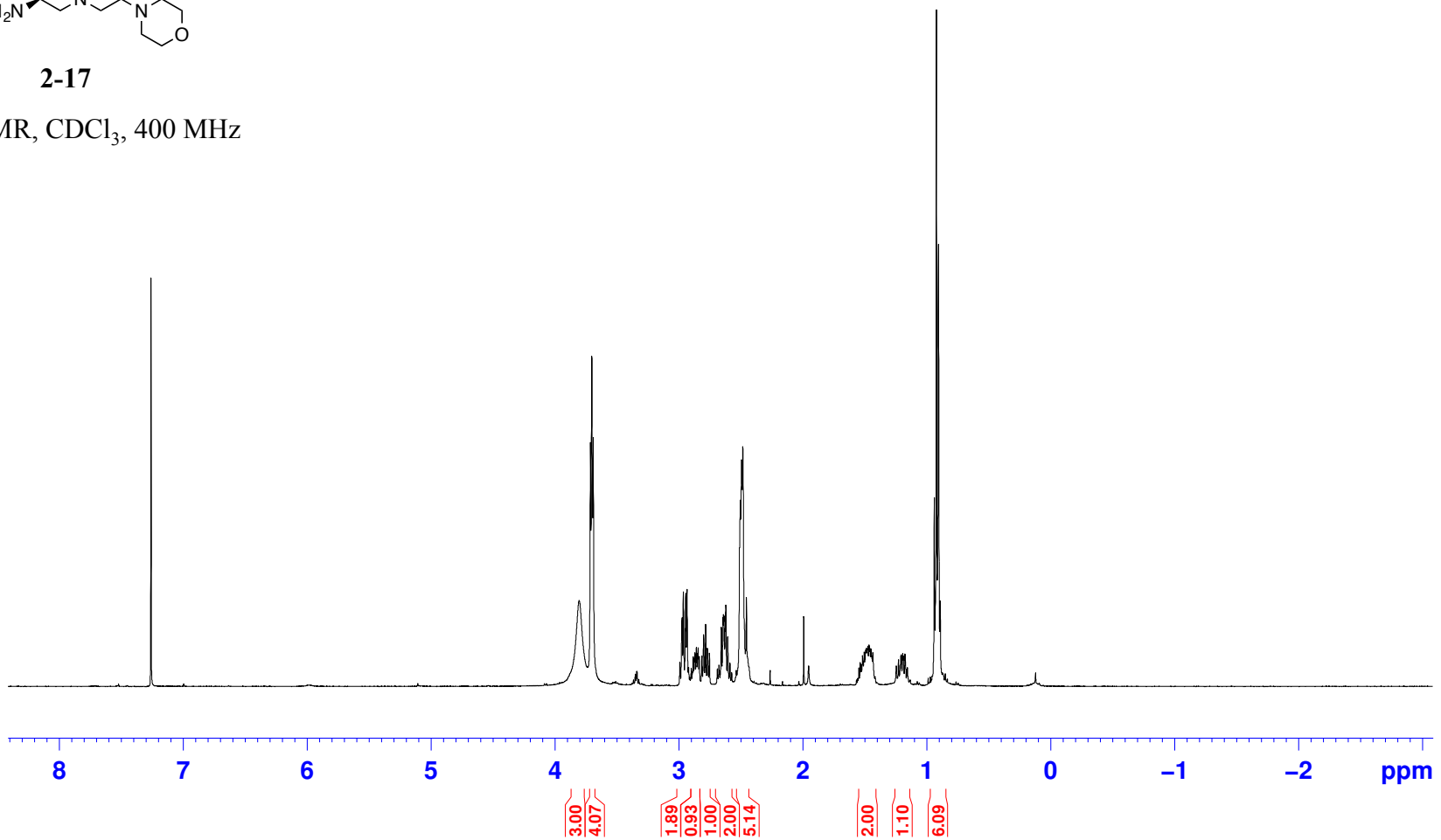

174.19
138.01
129.31
128.63
126.74
77.29
77.04
76.78
66.93
57.18
56.66
53.35
41.29
35.52
Bn
H
N
H2N
O
N
O
2-13e
13C NMR, CDCl3, 125 MHz
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

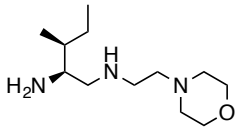




2-17

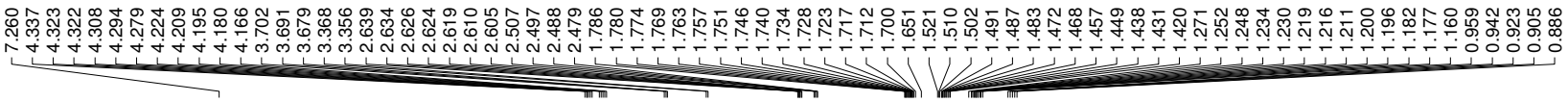
^{1}H NMR, $CDCl_3$, 400 MHz

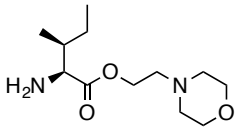




2-17

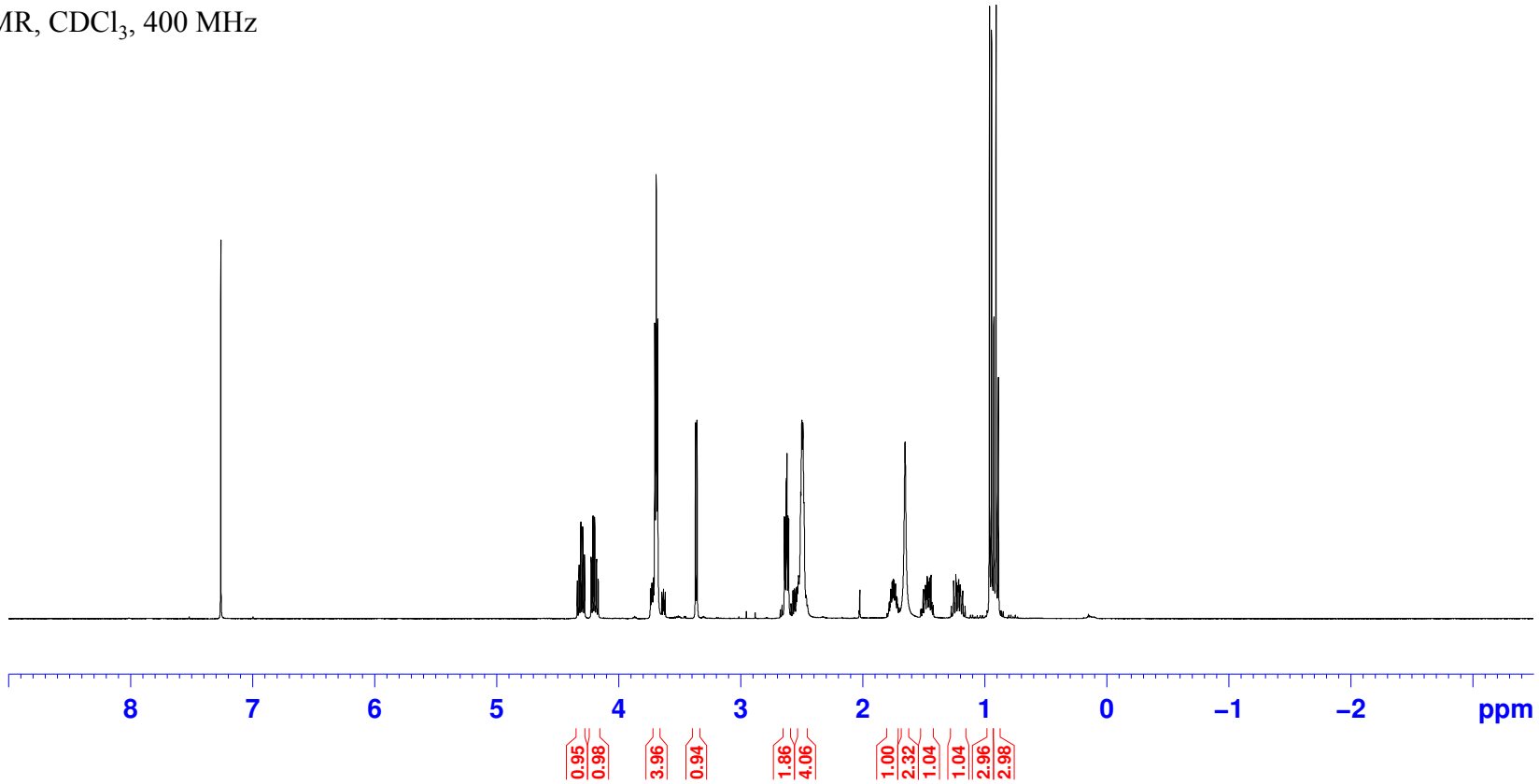
^{13}C NMR, $CDCl_3$, 100 MHz

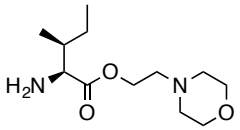




2-19

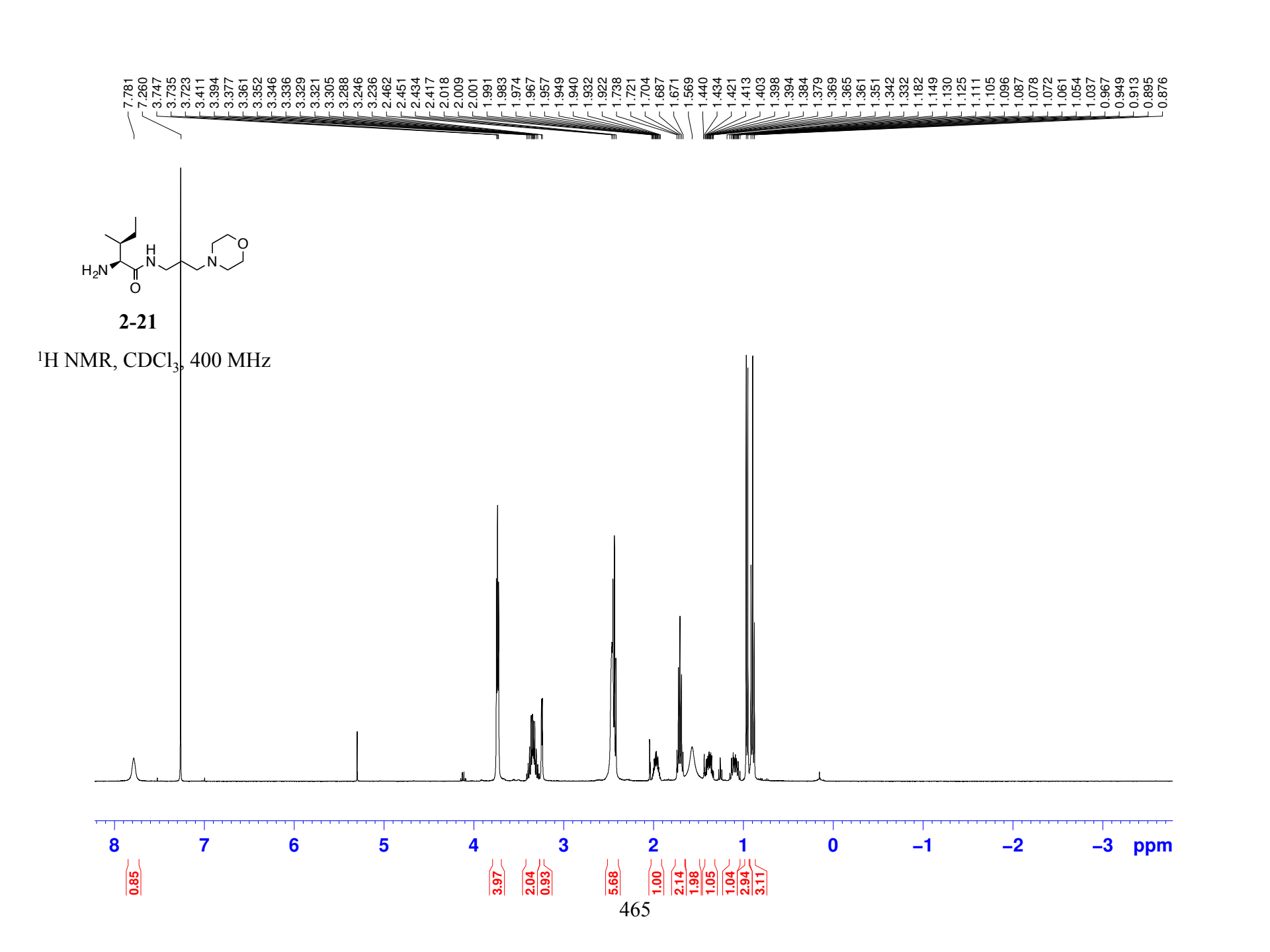
^{1}H NMR, $CDCl_3$, 400 MHz

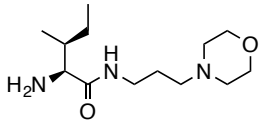




2-19

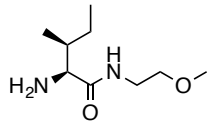
^{13}C NMR, $CDCl_3$, 125 MHz


7.781
7.260
3.747
3.735
3.723
3.411
3.394
3.377
3.361
3.352
3.346
3.336
3.329
3.321
3.305
3.288
3.246
3.236
2.462
2.451
2.434
2.417
2.018
2.009
2.001
1.991
1.983
1.974
1.967
1.957
1.949
1.940
1.932
1.922
1.738
1.721
1.704
1.687
1.671
1.569
1.440
1.434
1.421
1.413
1.403
1.398
1.394
1.384
1.379
1.369
1.365
1.361
1.351
1.342
1.332
1.182
1.149
1.130
1.125
1.111
1.105
1.096
1.087
1.078
1.072
1.061
1.054
1.037
0.967
0.949
0.913
0.895
0.876
H2N
H
N
O
N
O
2-21
1H NMR, CDCl3, 400 MHz
8
7
6
5
4
3
2
1
0
–1
–2
–3
ppm
0.85
3.97
2.04
0.93
5.68
1.00
2.14
1.98
1.05
1.04
2.94
3.11



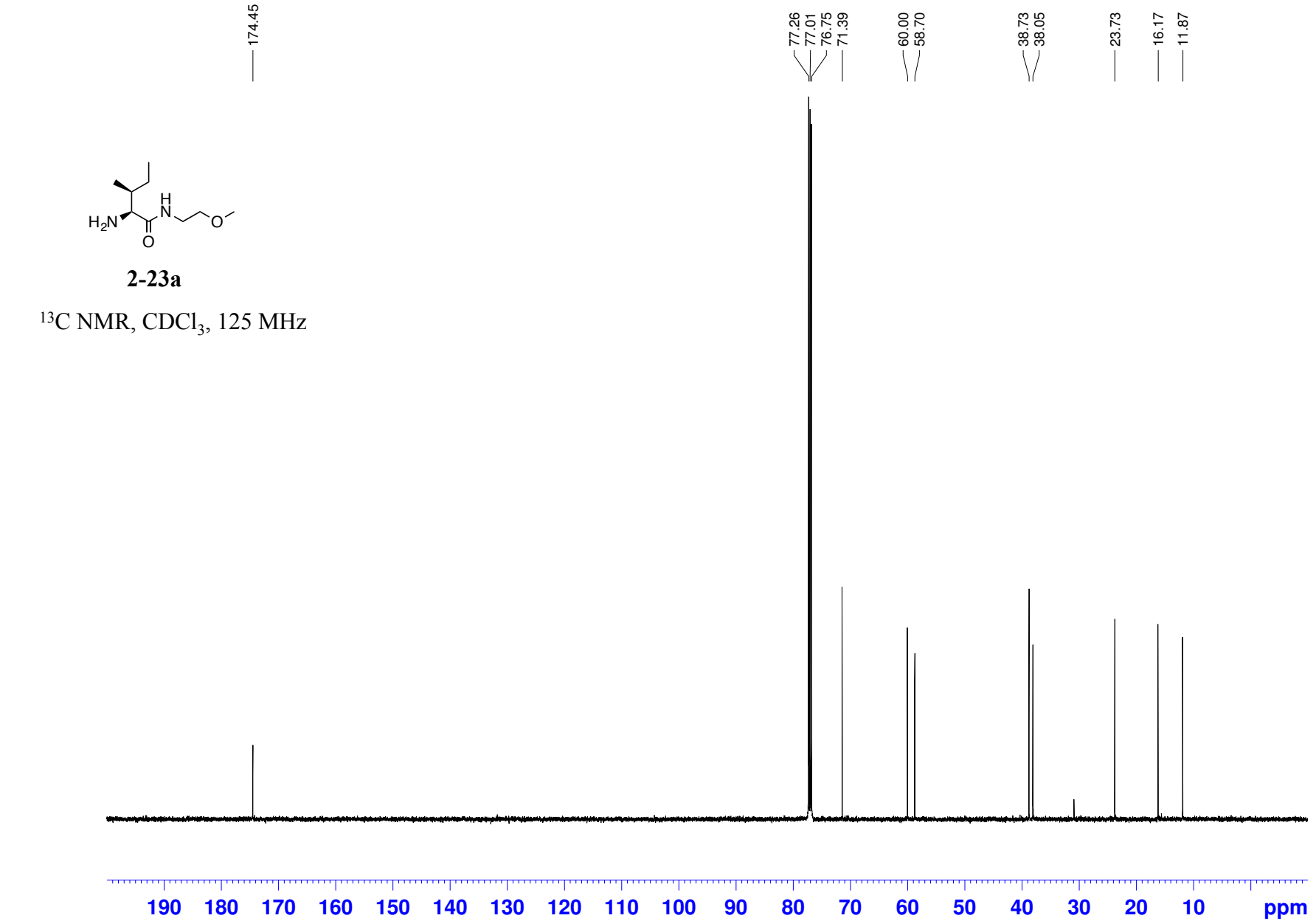
2-21

^{13}C NMR, $CDCl_3$, 125 MHz



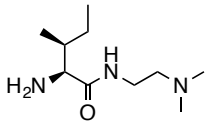
2-23a

^{1}H NMR, $CDCl_3$, 400 MHz



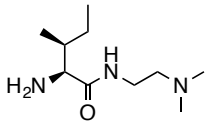
2-23a

^{13}C NMR, $CDCl_3$, 125 MHz



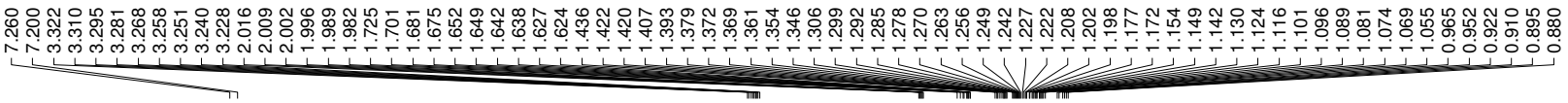
2-23b

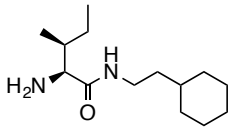
^{1}H NMR, $CDCl_3$, 400 MHz



2-23b

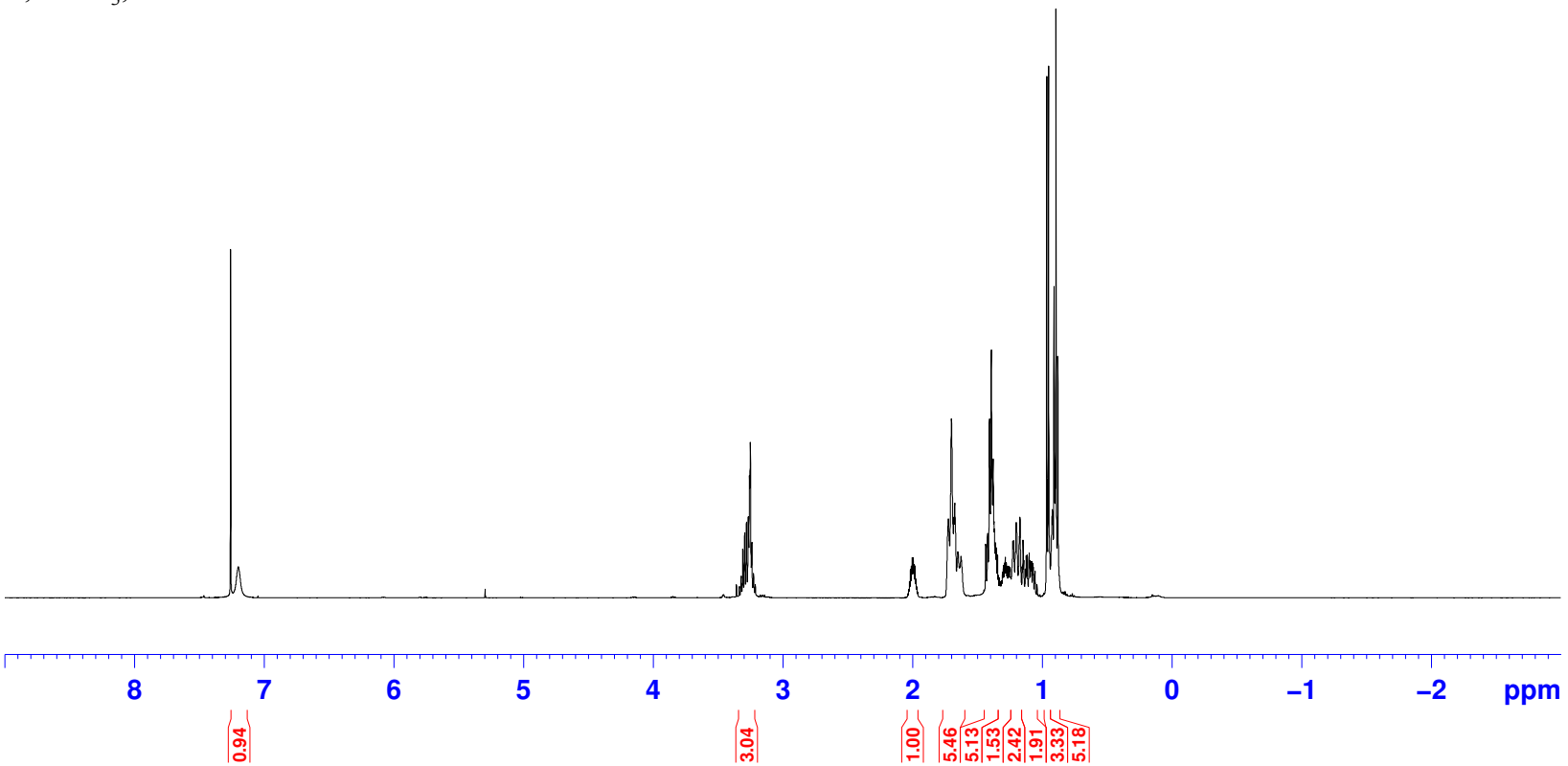
^{13}C NMR, $CDCl_3$, 125 MHz

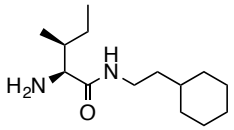




2-23c

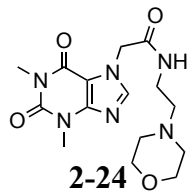
^{1}H NMR, $CDCl_3$, 500 MHz





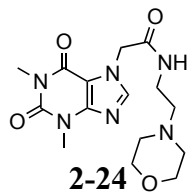
2-23c

^{13}C NMR, $CDCl_3$, 125 MHz



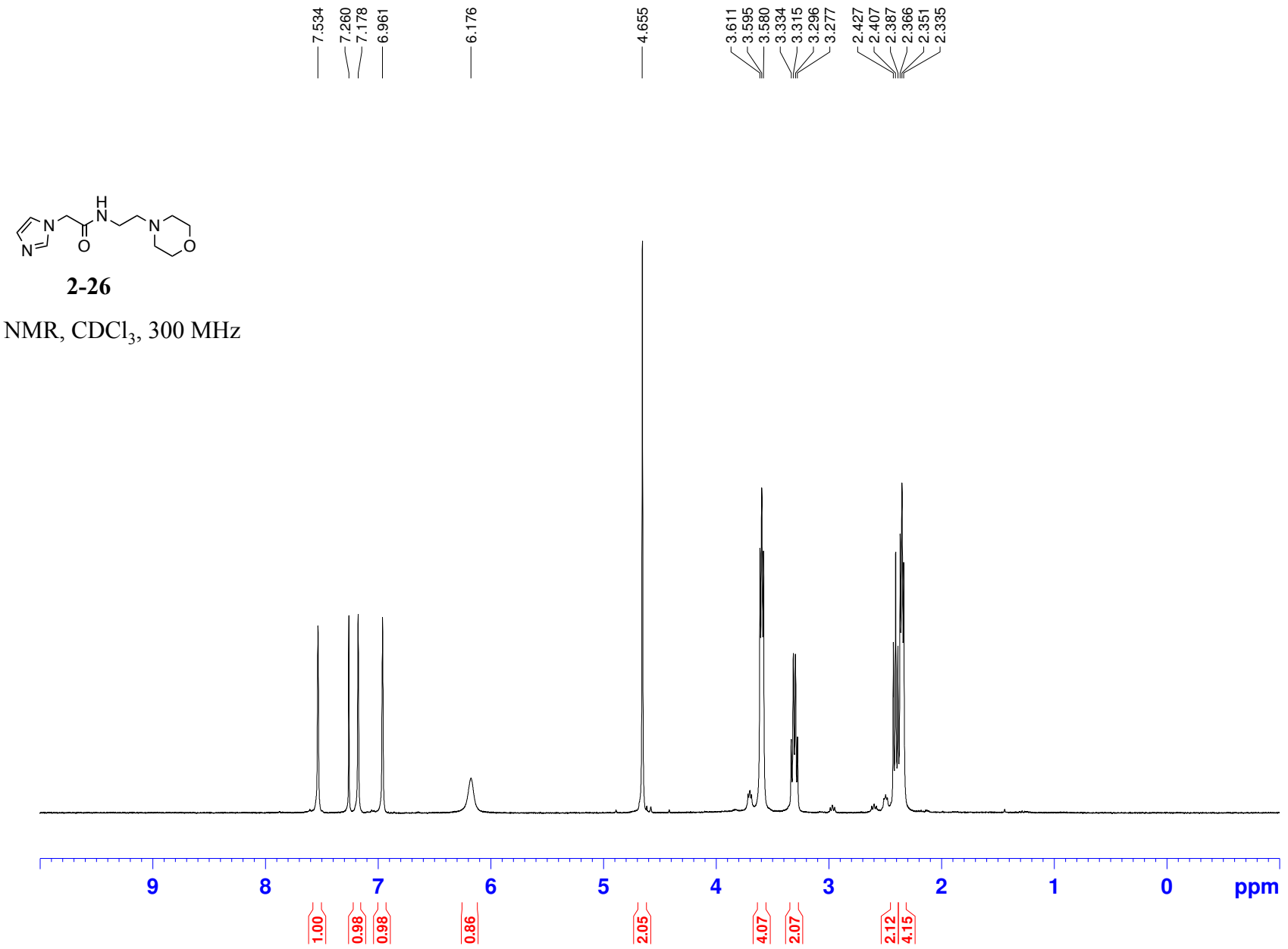
2-24

^{1}H NMR, $CDCl_3$, 400 MHz



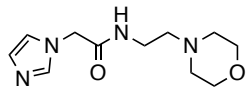
2-24

^{13}C NMR, $CDCl_3$, 100 MHz



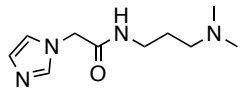
2-26

^{1}H NMR, $CDCl_3$, 300 MHz



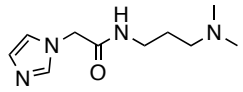
2-26

^{13}C NMR, $CDCl_3$, 125 MHz



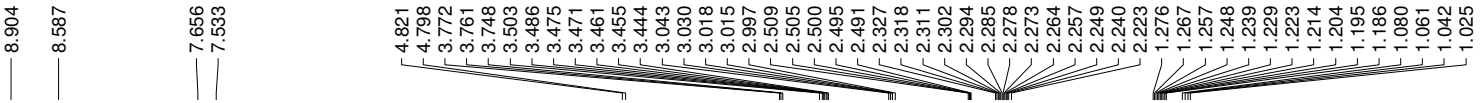
2-27

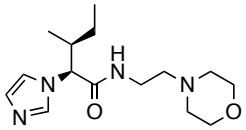
^{1}H NMR, $CDCl_3$, 300 MHz



2-27

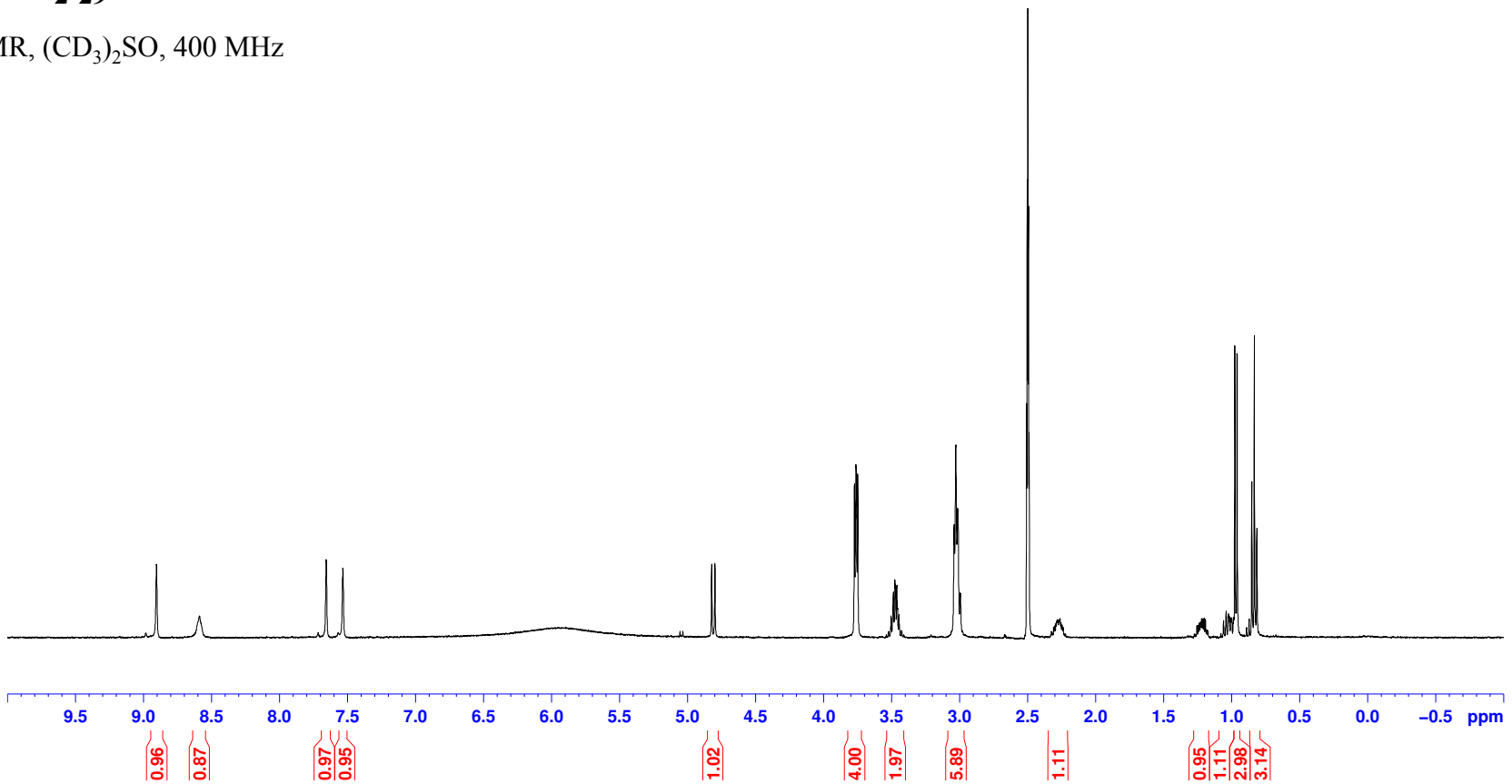
^{13}C NMR, $CDCl_3$, 125 MHz

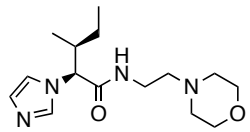




2-29

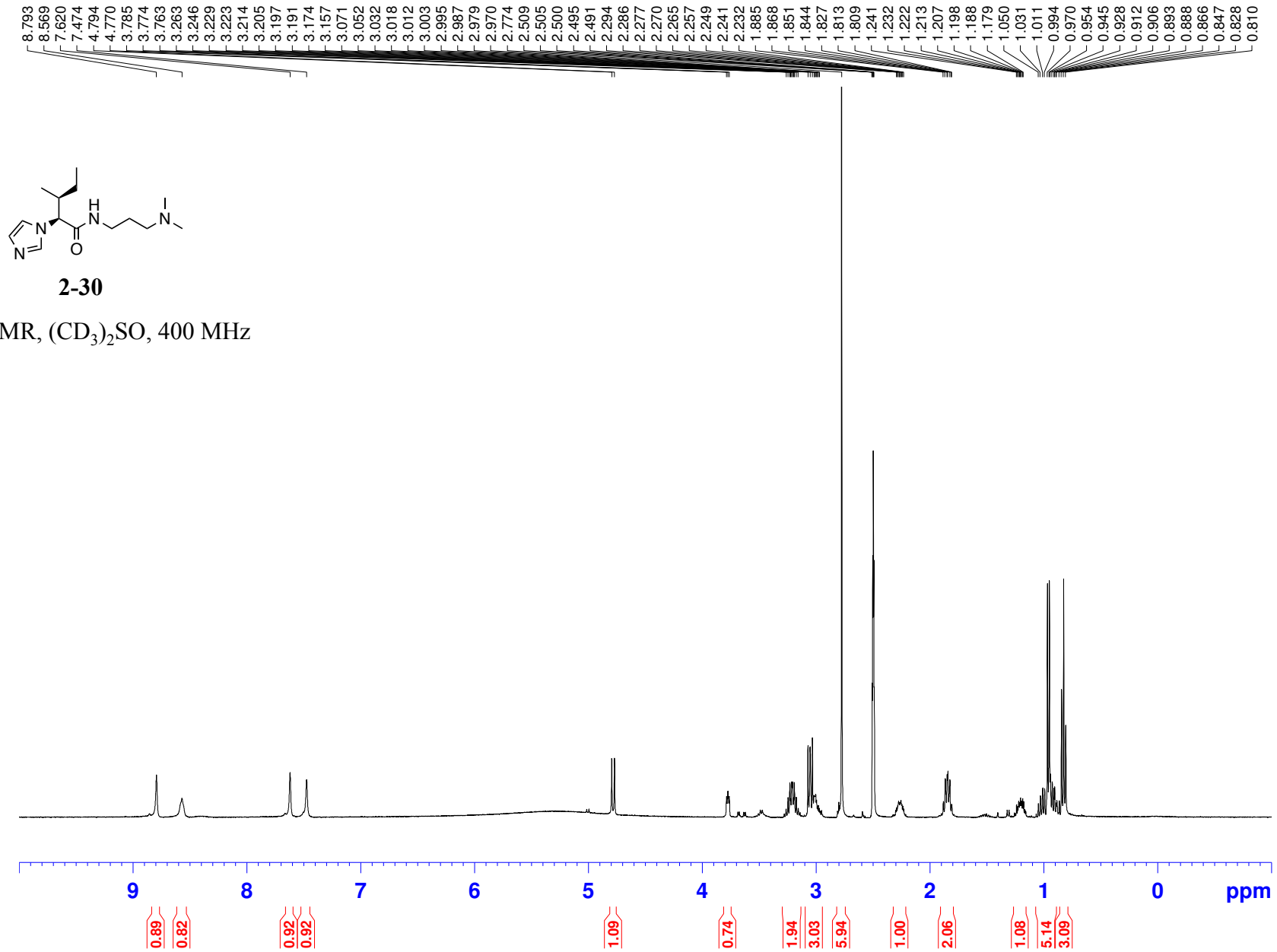
^{1}H NMR, $(CD_3)_2SO$, 400 MHz





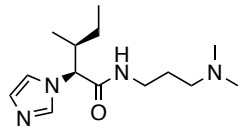
2-29

^{13}C NMR, CD_3OD, 125 MHz



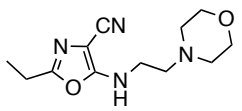
2-30

^{1}H NMR, $(CD_3)_2SO$, 400 MHz



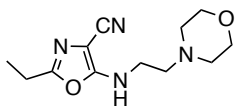
2-30

^{13}C NMR, CD_3OD, 125 MHz



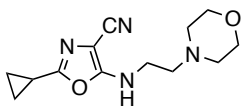
2-34a

^{1}H NMR, $CDCl_3$, 300 MHz



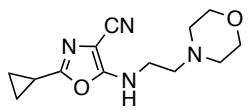
2-34a

^{13}C NMR, $CDCl_3$, 125 MHz



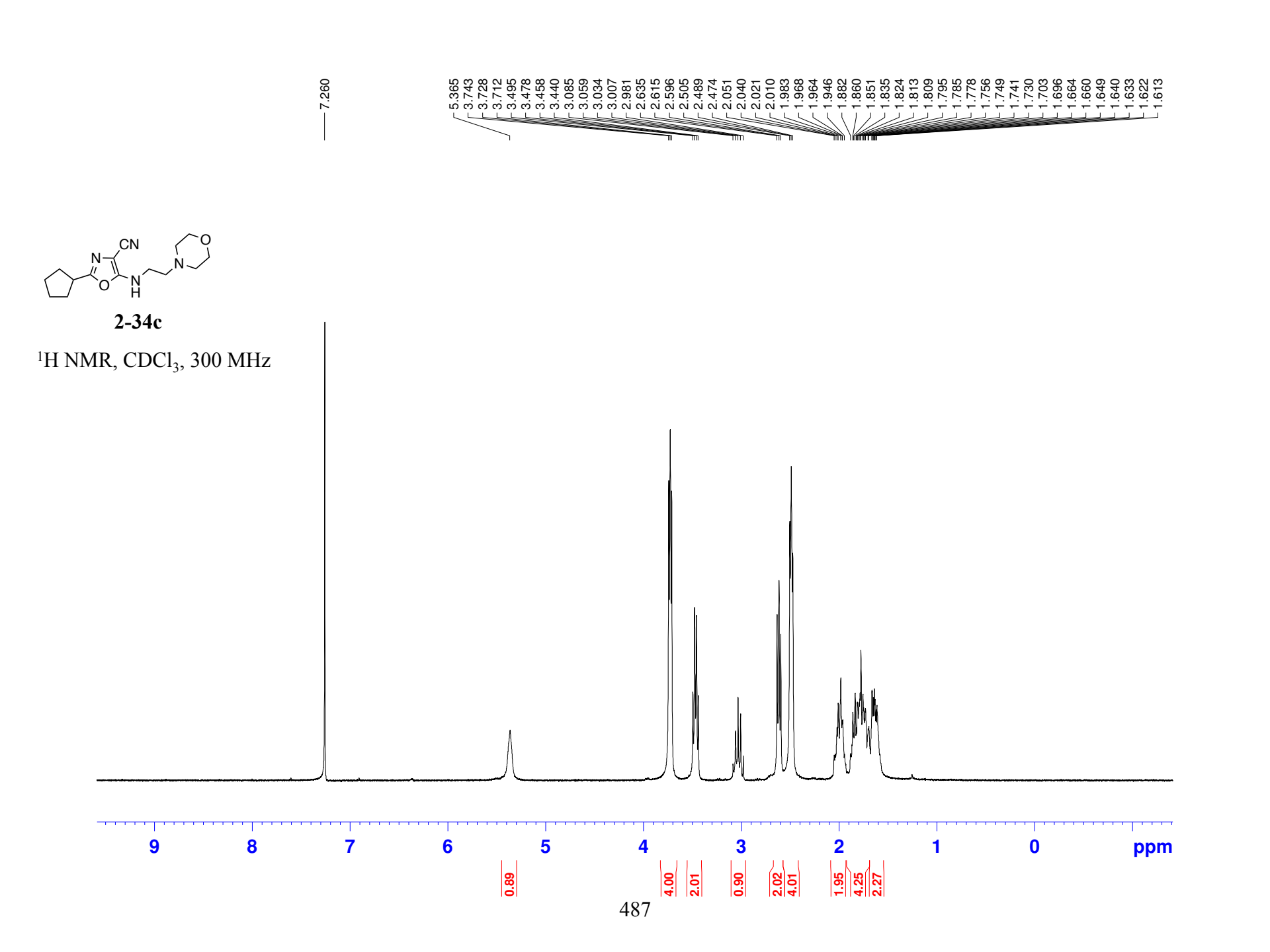
2-34b

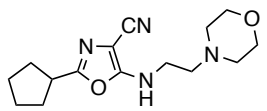
^{1}H NMR, $CDCl_3$, 300 MHz



2-34b

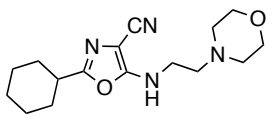
^{13}C NMR, $CDCl_3$, 125 MHz


7.260
5.365
3.743
3.728
3.712
3.495
3.478
3.458
3.440
3.085
3.059
3.034
3.007
2.981
2.635
2.615
2.596
2.505
2.489
2.474
2.051
2.040
2.021
2.010
1.983
1.968
1.964
1.946
1.882
1.860
1.851
1.835
1.824
1.813
1.809
1.795
1.785
1.778
1.756
1.749
1.741
1.730
1.703
1.696
1.664
1.660
1.649
1.640
1.633
1.622
1.613
CN
N
O
N
H
O
N
2-34c
1H NMR, CDCl3, 300 MHz
9
8
7
6
5
4
3
2
1
0
ppm
0.89
4.00
2.01
0.90
2.02
4.01
1.95
4.25
2.27



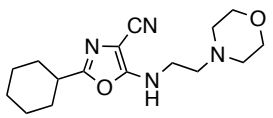
2-34c

^{13}C NMR, $CDCl_3$, 125 MHz



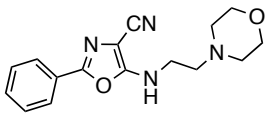
2-34d

^{1}H NMR, $CDCl_3$, 300 MHz



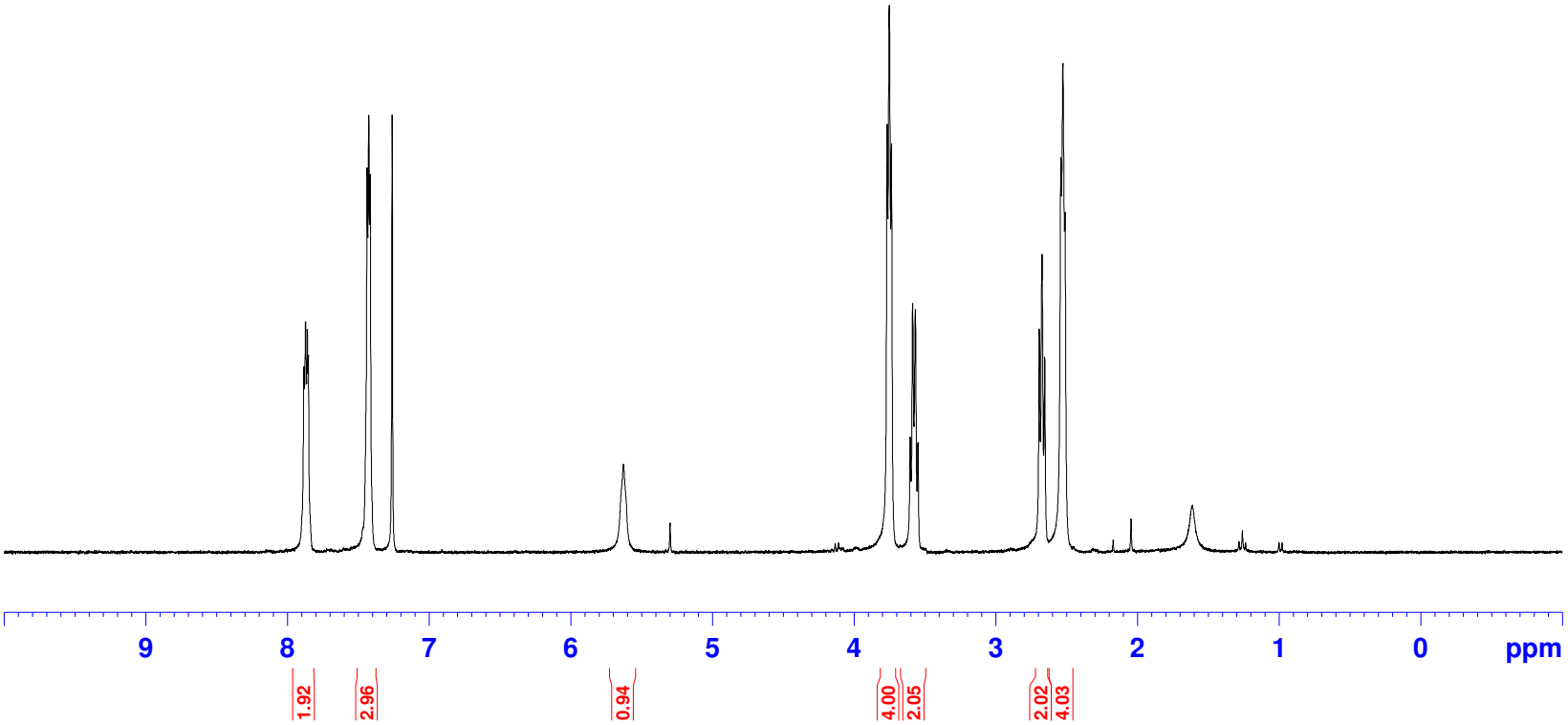
2-34d

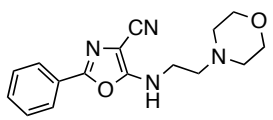
^{13}C NMR, $CDCl_3$, 125 MHz



2-34e

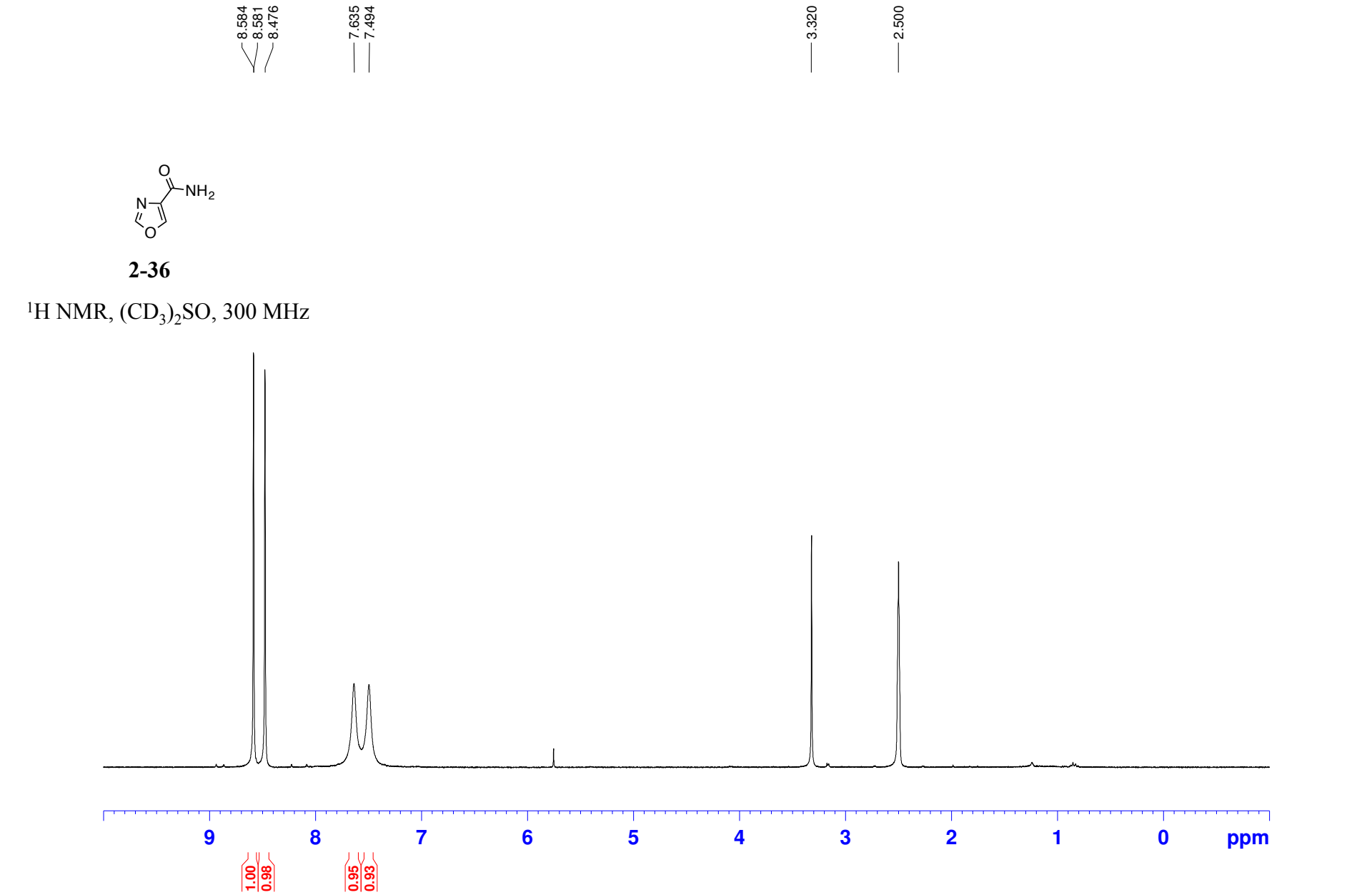
^{1}H NMR, $CDCl_3$, 300 MHz





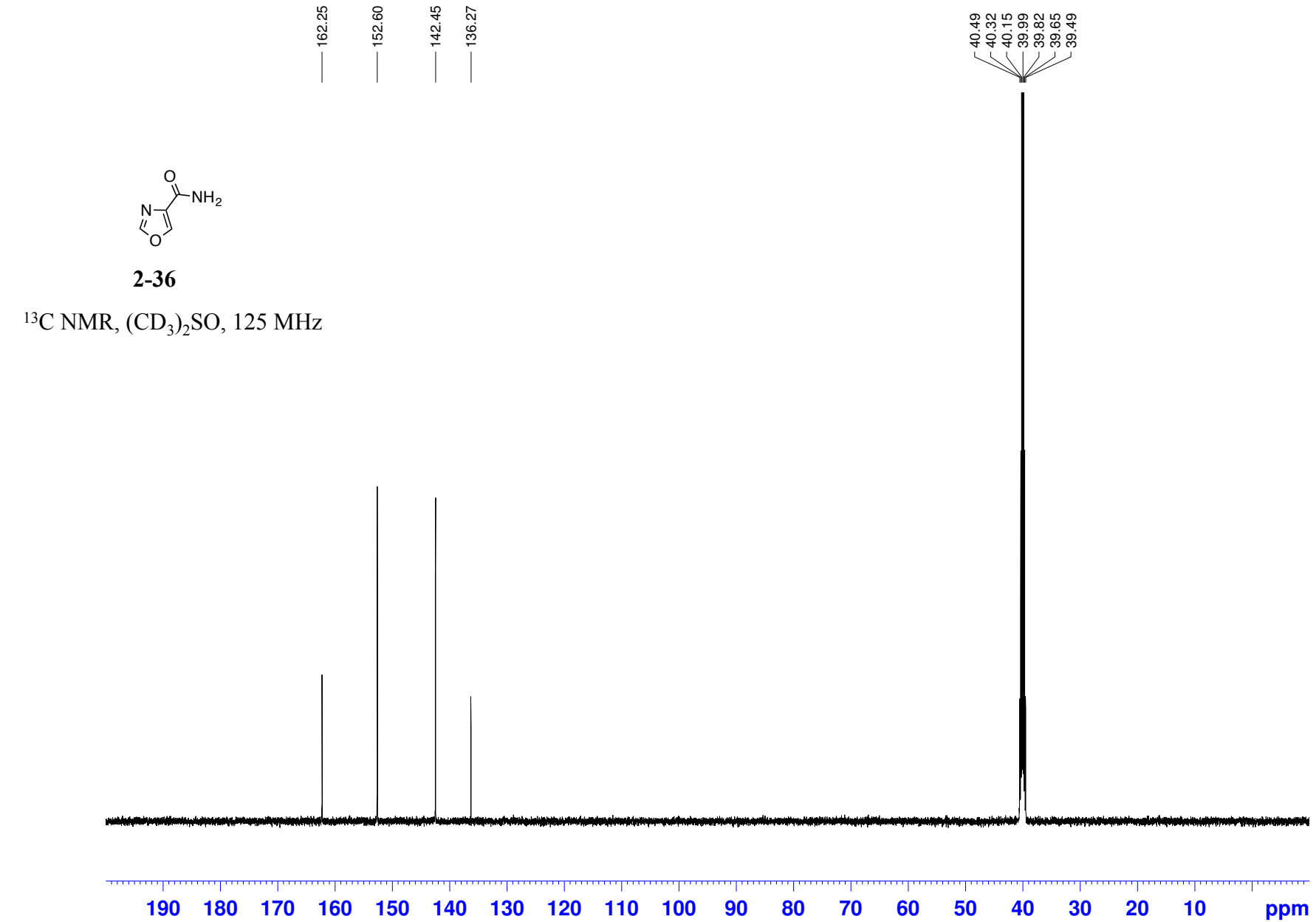
2-34e

^{13}C NMR, $CDCl_3$, 125 MHz



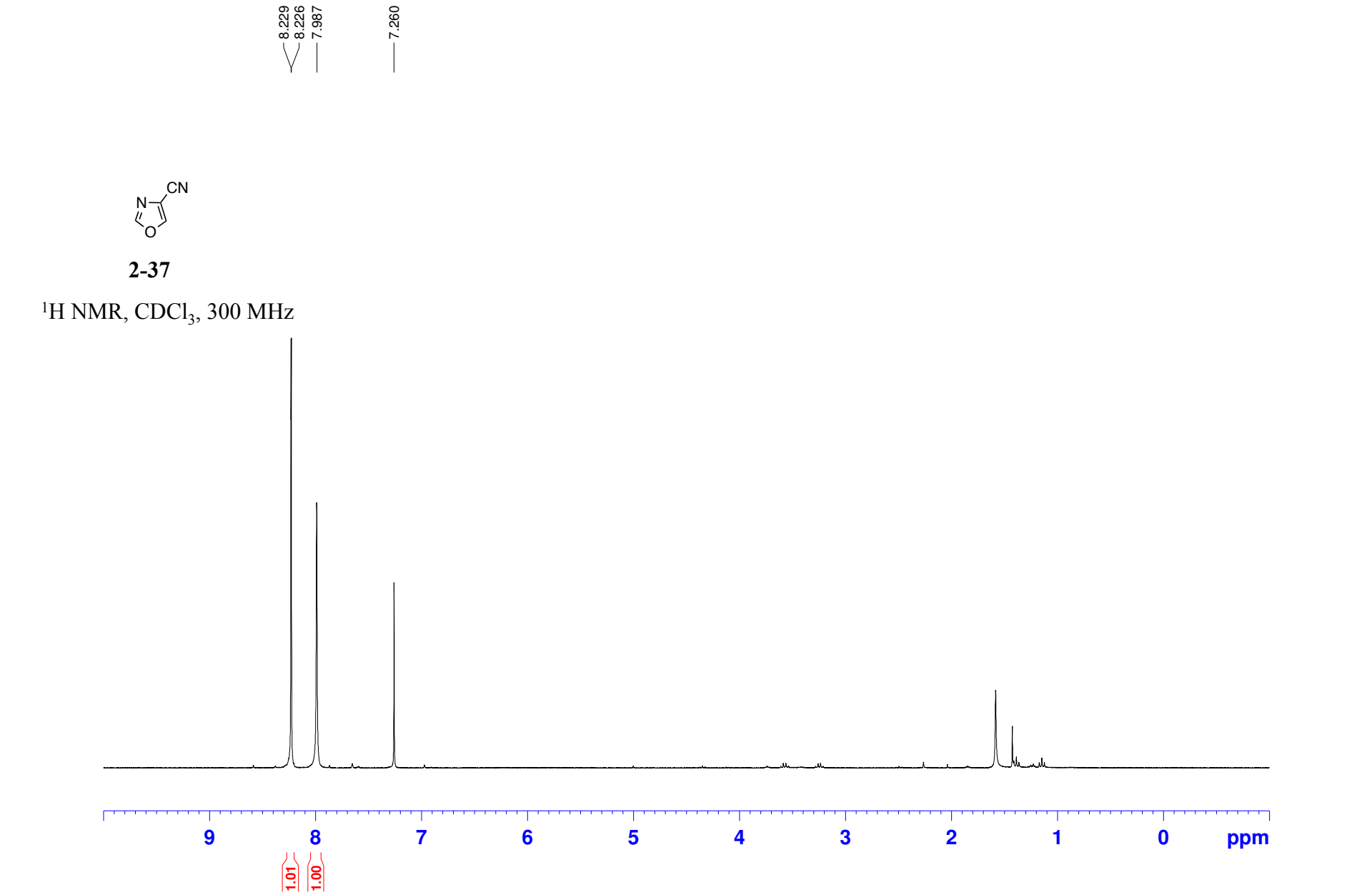
2-36

^{1}H NMR, $(CD_3)_2SO$, 300 MHz



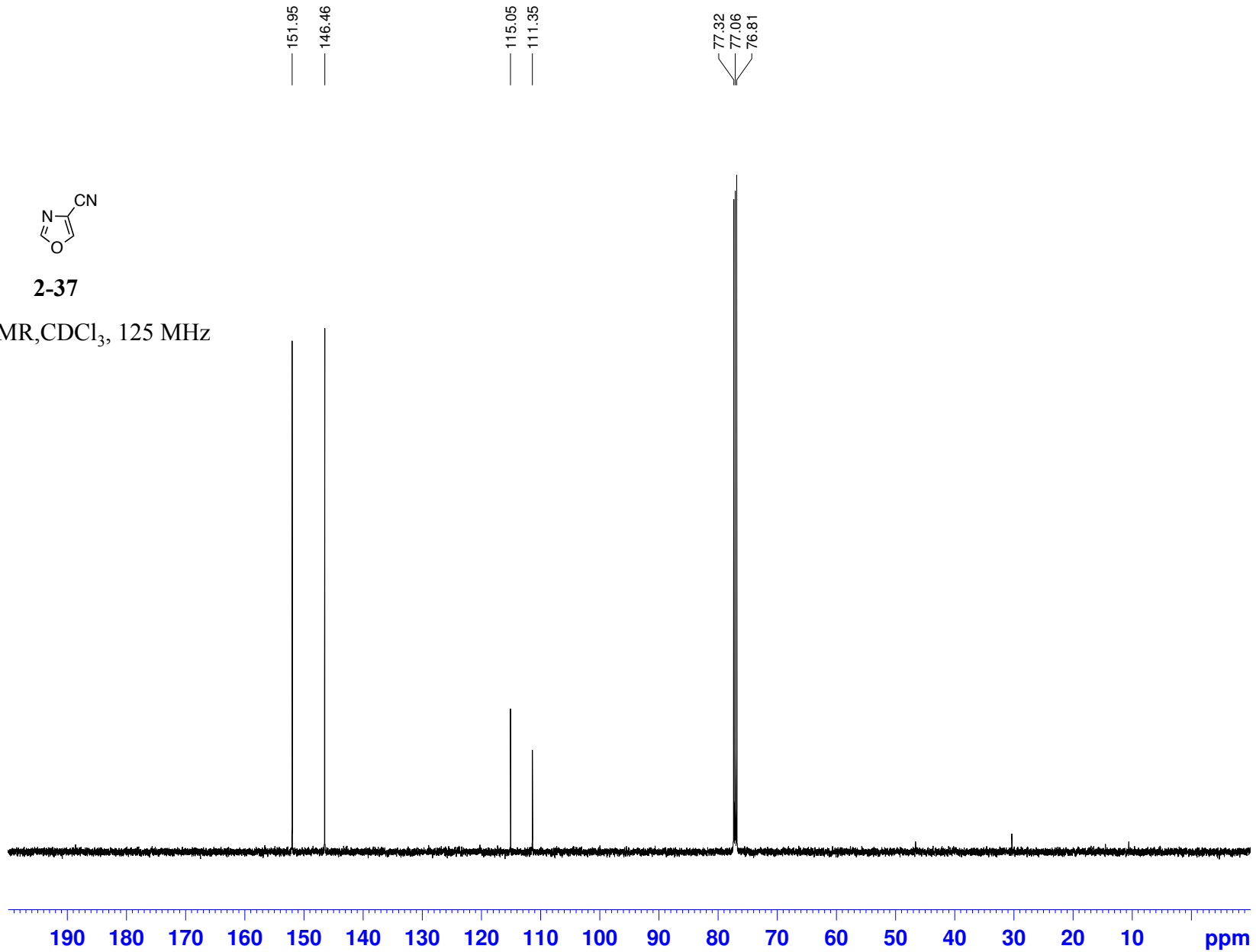
2-36

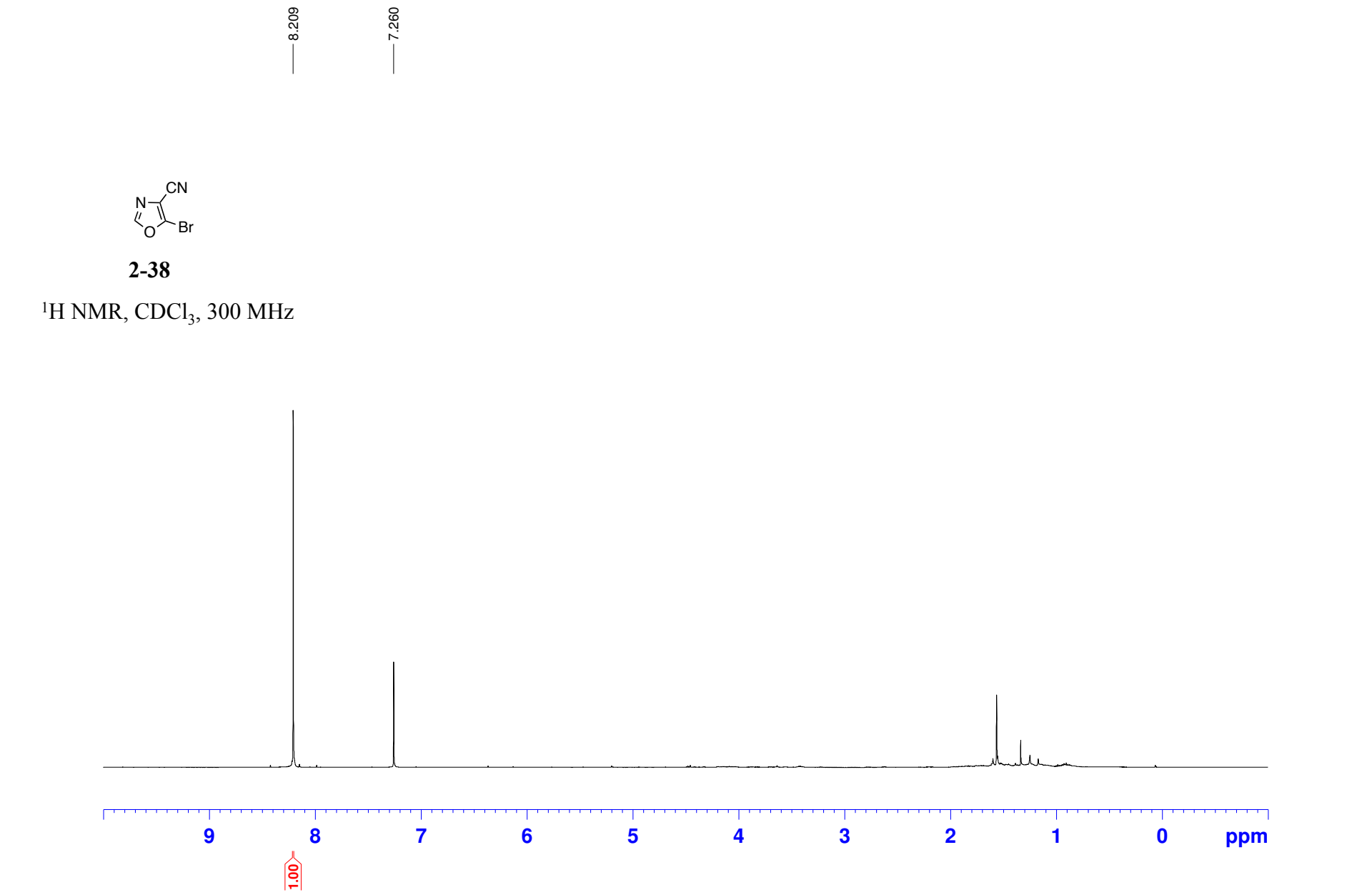
^{13}C NMR, $(CD_3)_2SO$, 125 MHz

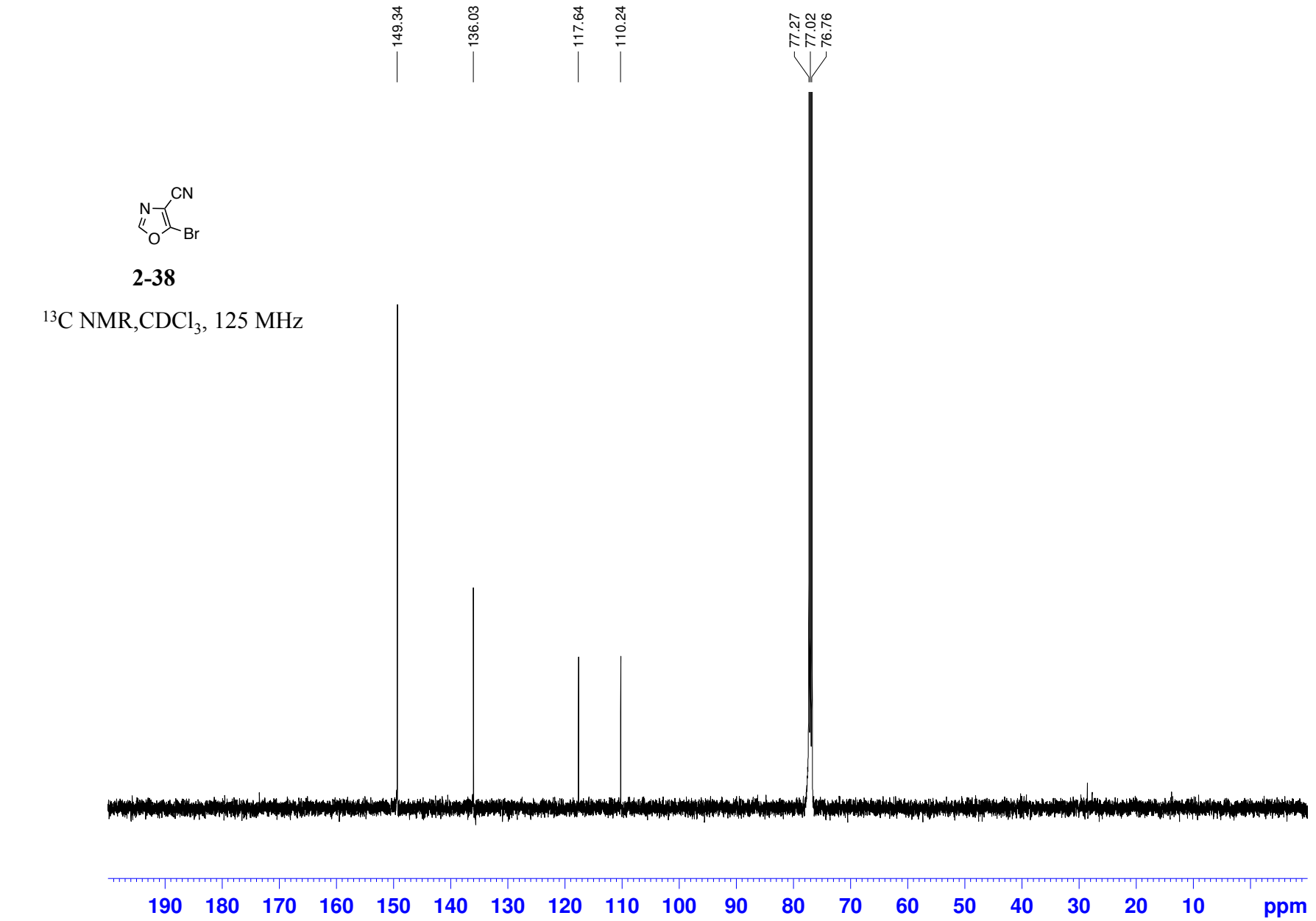


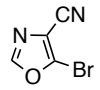
2-37

^{1}H NMR, $CDCl_3$, 300 MHz


151.95
146.46
115.05
111.35
77.32
77.06
76.81
CN
N
O
2-37
13C NMR,CDCl3, 125 MHz
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

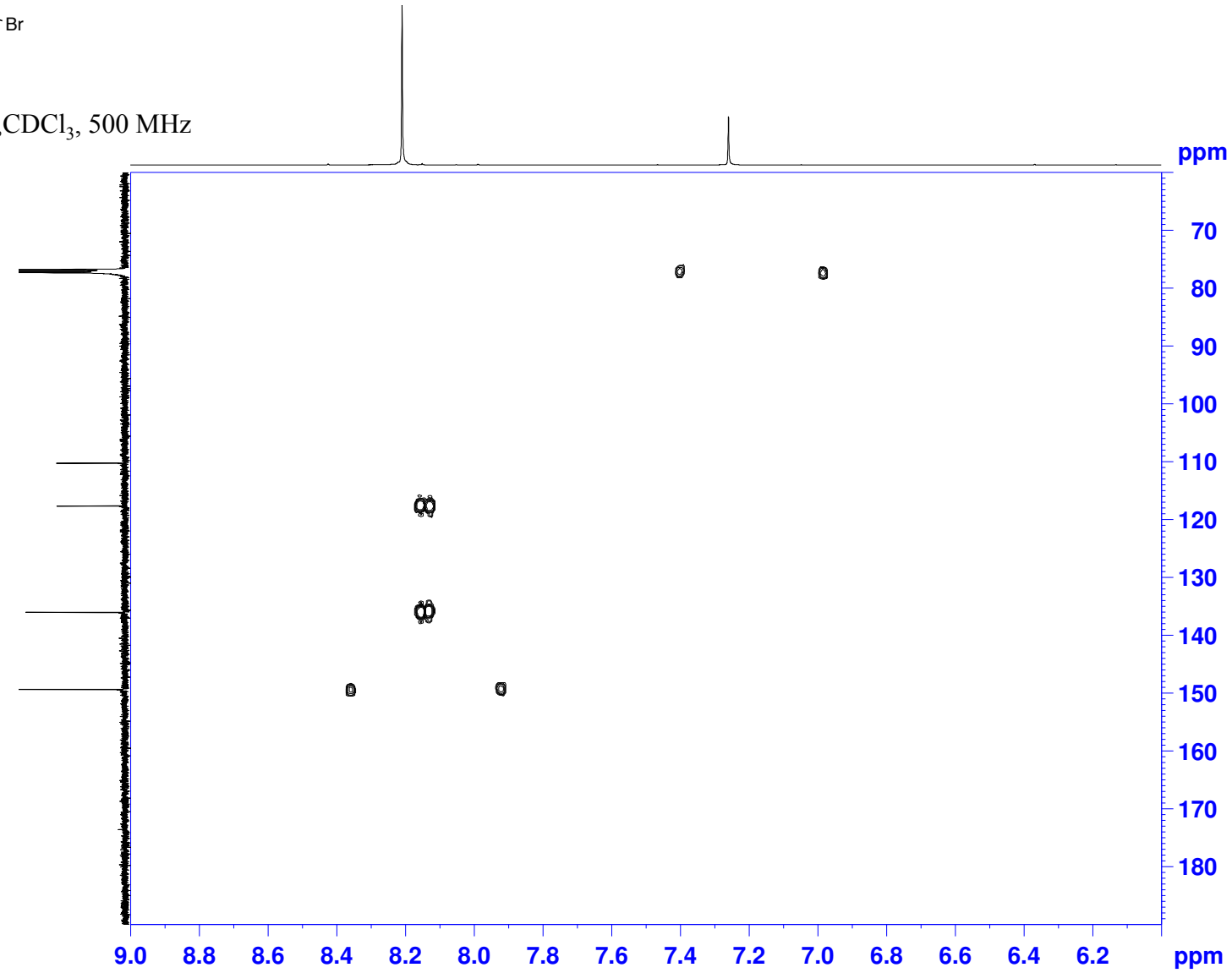

8.209
7.260
CN
N
O
Br
2-38
^{1}H NMR, $CDCl_3$, 300 MHz
9
8
7
6
5
4
3
2
1
0
ppm
1.00

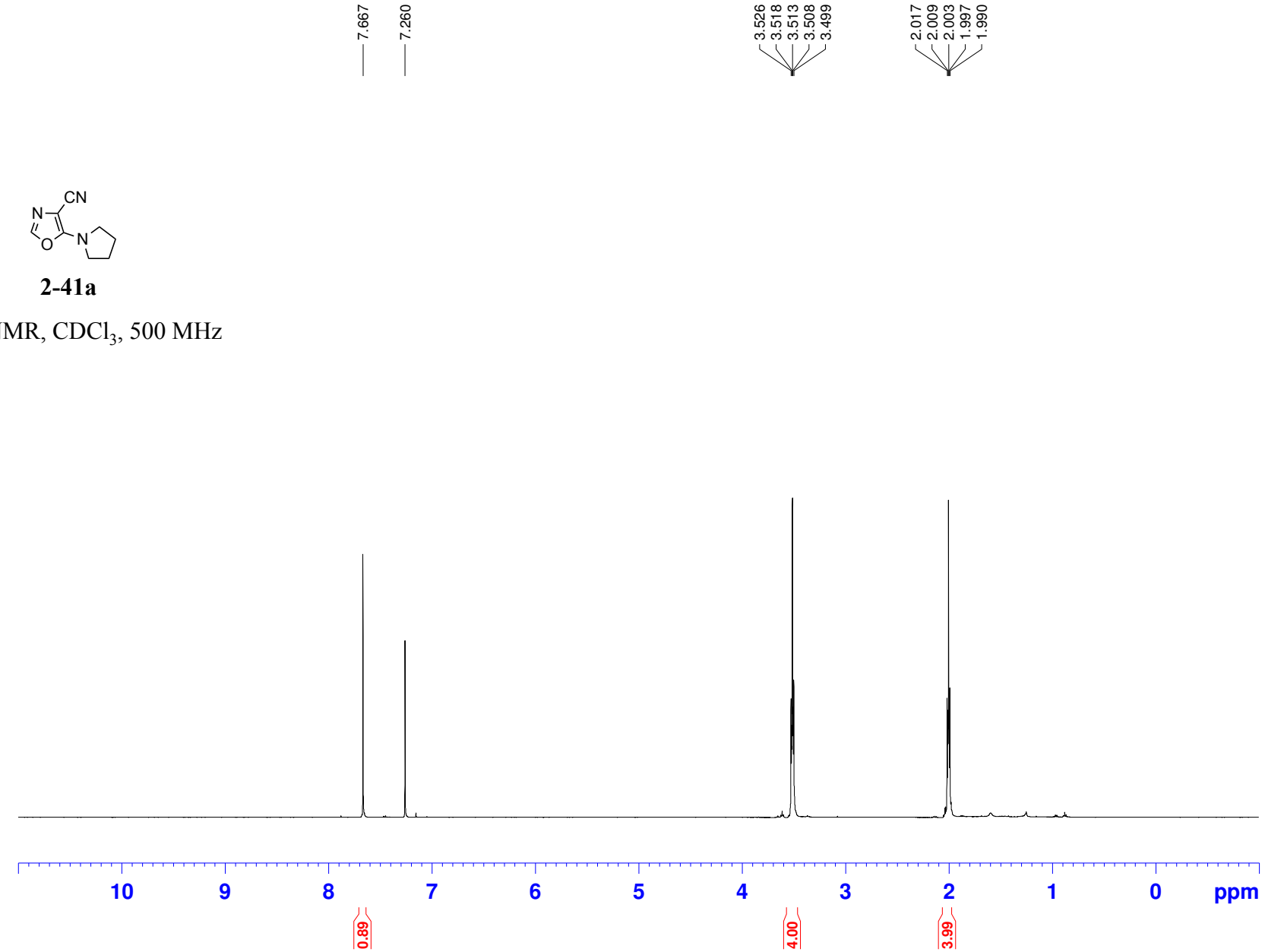

149.34
136.03
117.64
110.24
77.27
77.02
76.76
CN
N
O
Br
2-38
13C NMR,CDCl3, 125 MHz
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm



2-38

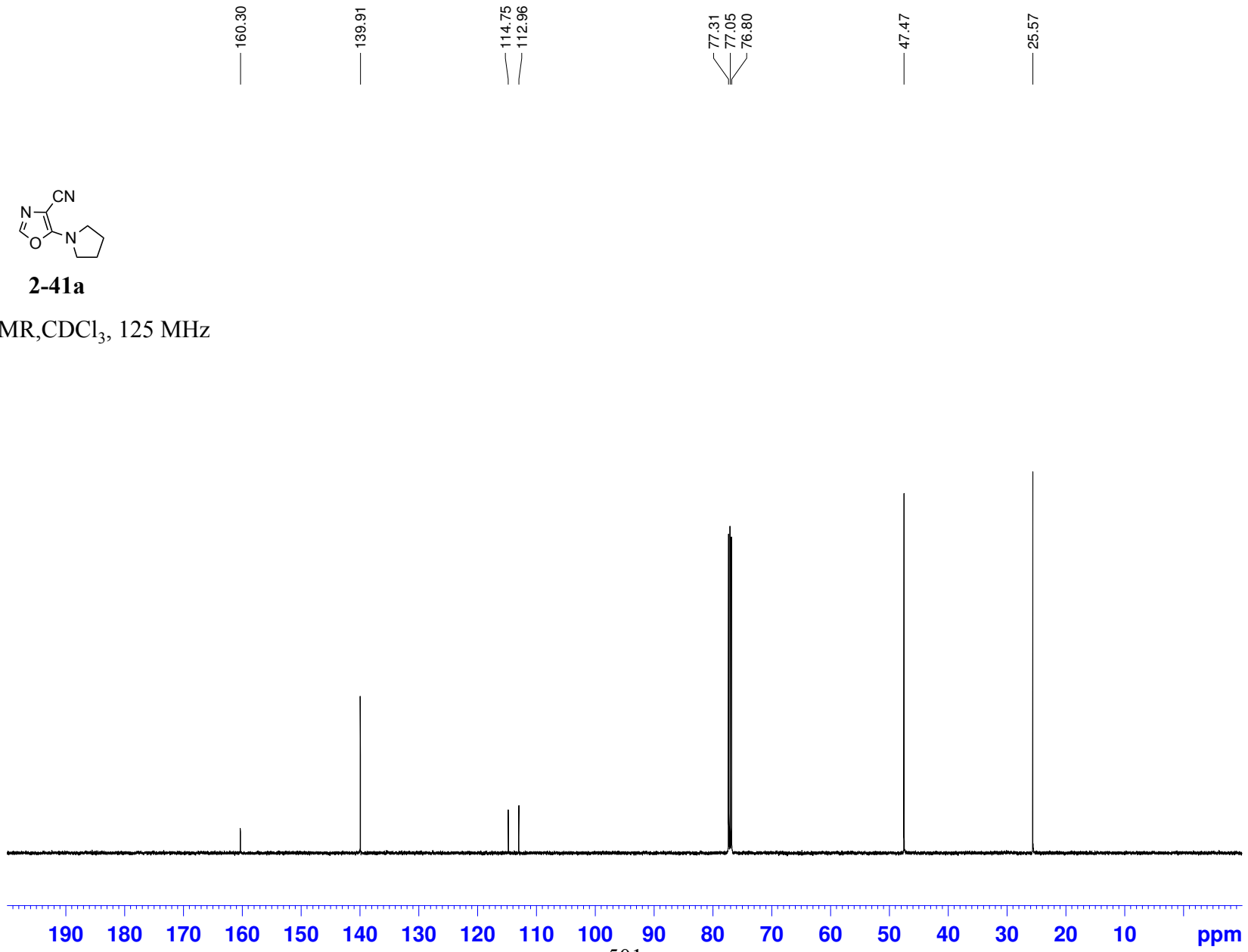
^{1}H-^{13}C HMBC,$CDCl_3$, 500 MHz





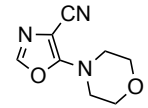
2-41a

^{1}H NMR, $CDCl_3$, 500 MHz



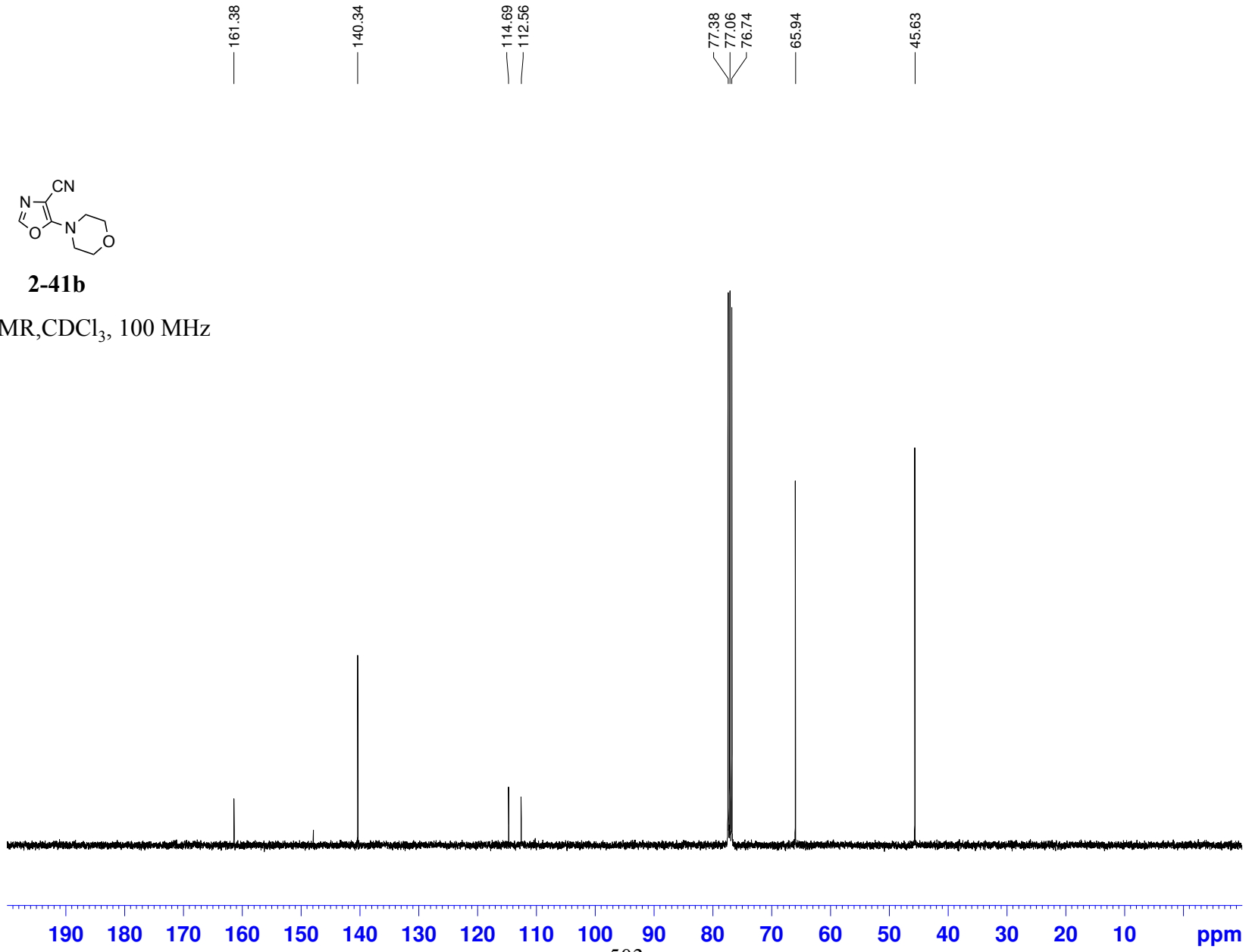
2-41a

^{13}C NMR,$CDCl_3$, 125 MHz



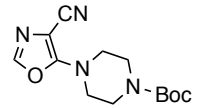
2-41b

^{1}H NMR, $CDCl_3$, 400 MHz



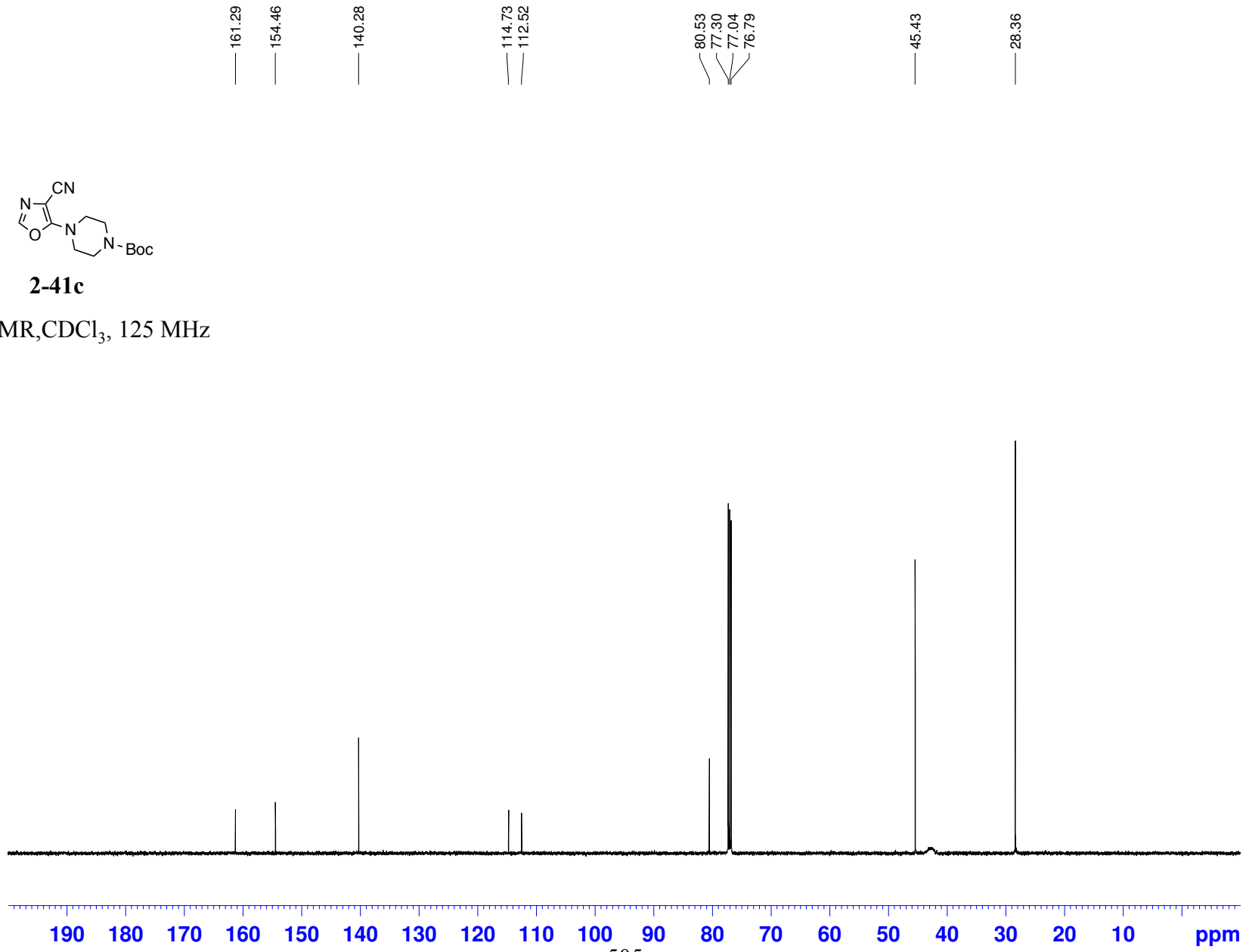
2-41b

^{13}C NMR,$CDCl_3$, 100 MHz



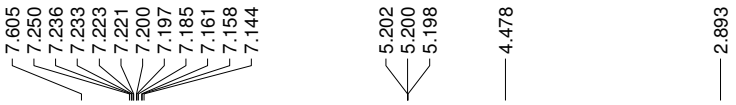
2-41c

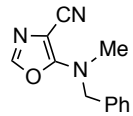
^{1}H NMR, $CDCl_3$, 400 MHz



2-41c

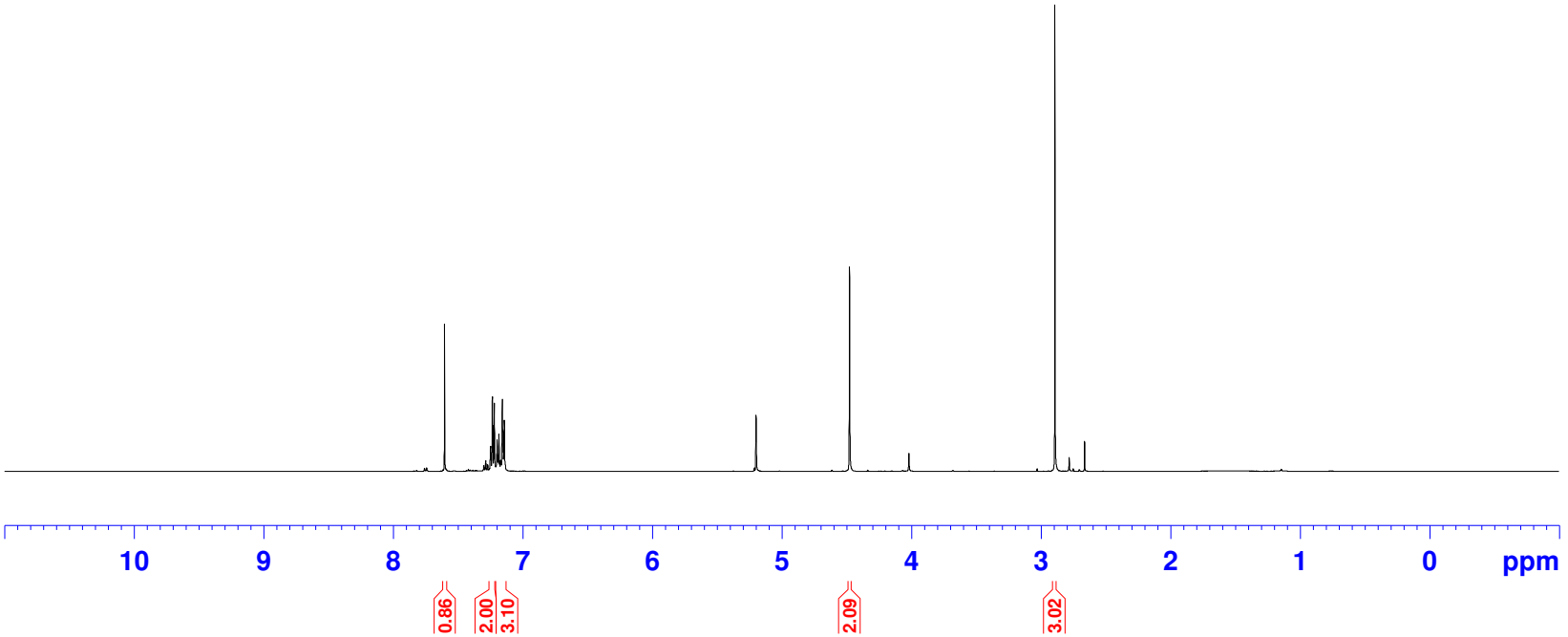
^{13}C NMR,$CDCl_3$, 125 MHz

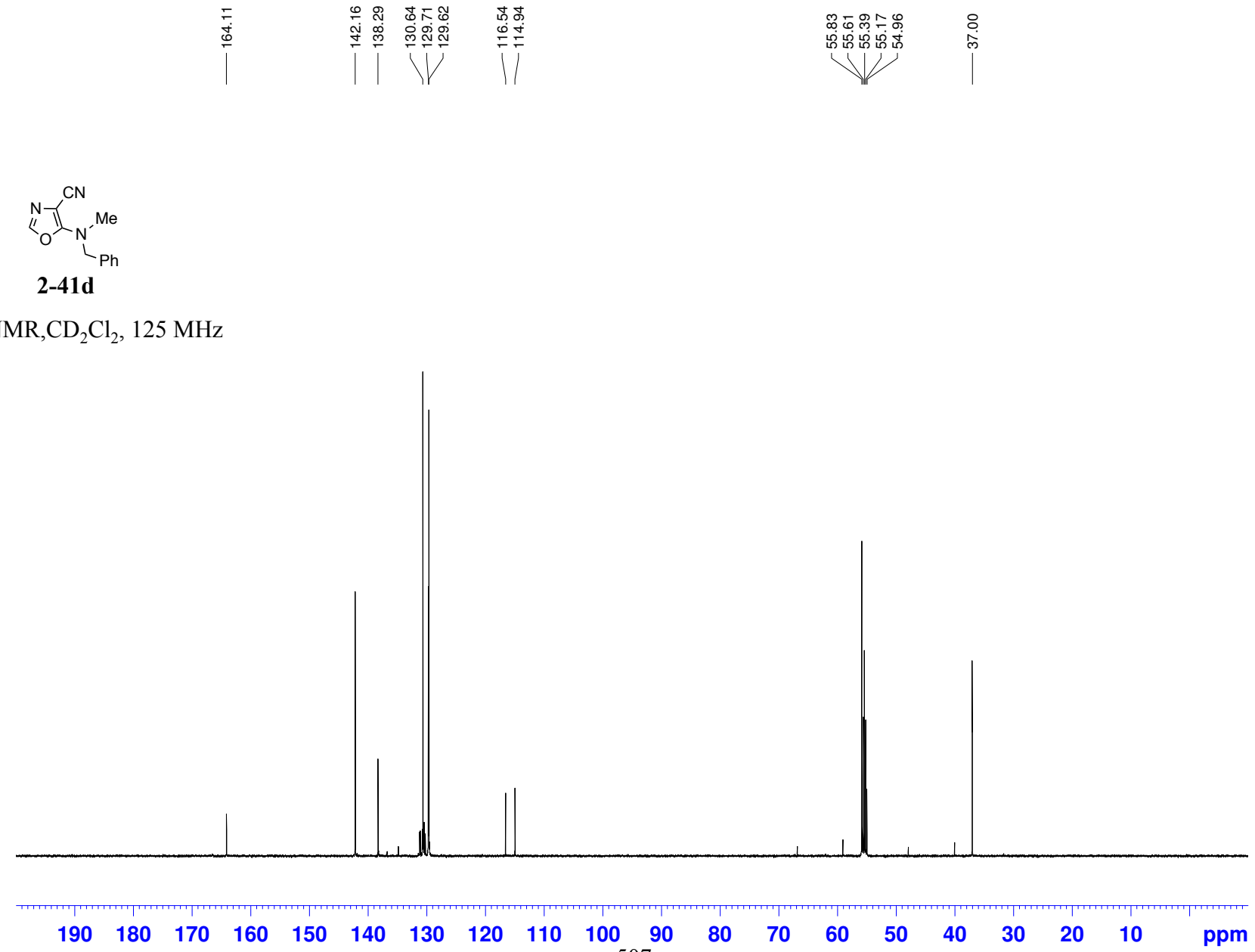




2-41d

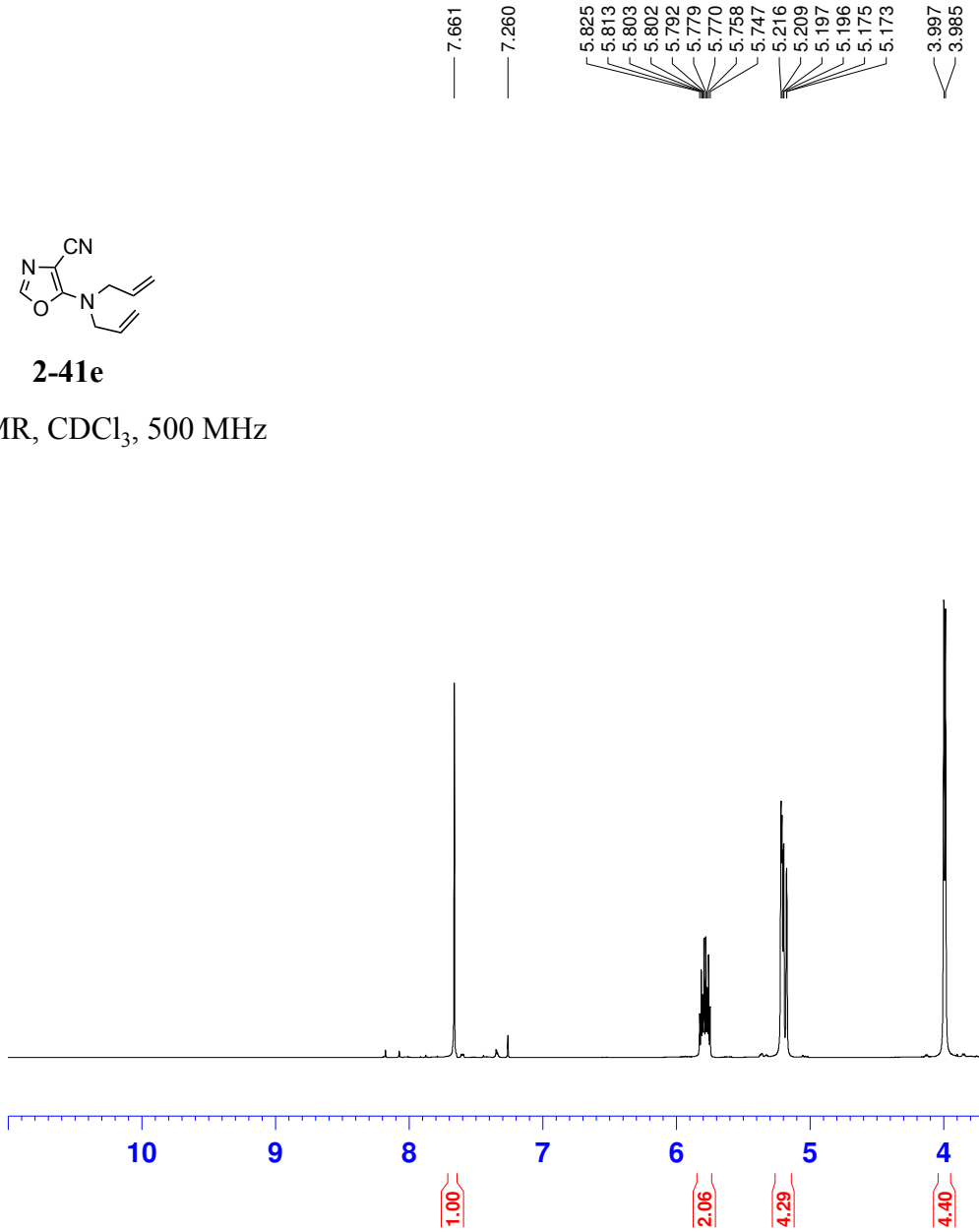
^{1}H NMR, $CDCl_3$, 500 MHz





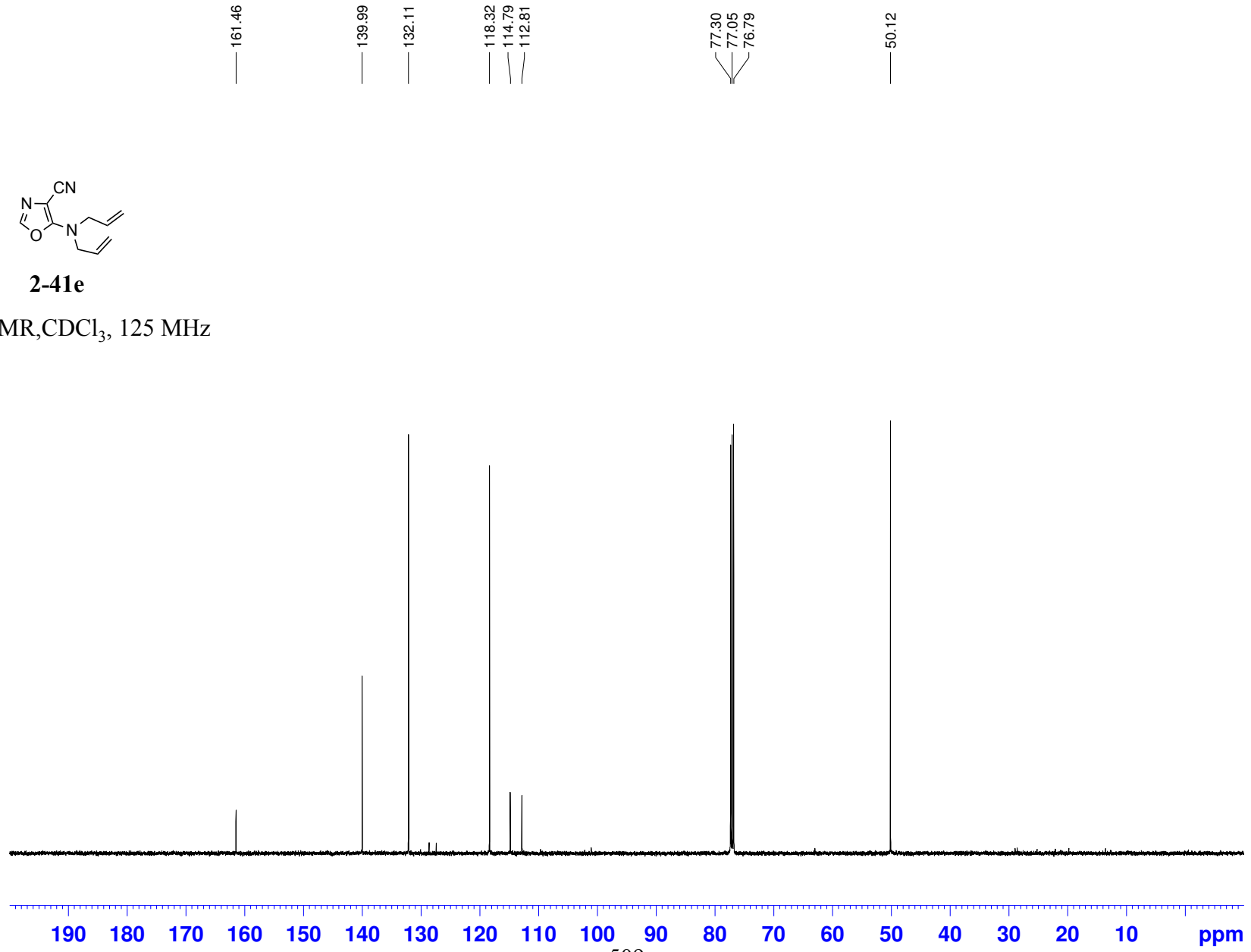
2-41d

^{13}C NMR,CD_2Cl_2, 125 MHz



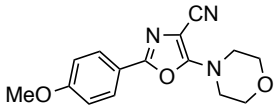
2-41e

^{1}H NMR, $CDCl_3$, 500 MHz



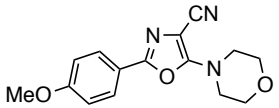
2-41e

^{13}C NMR,$CDCl_3$, 125 MHz



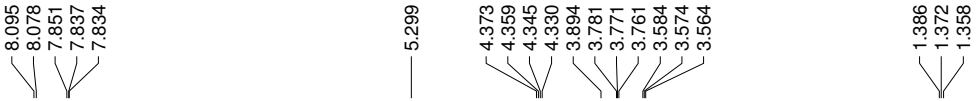
2-42a

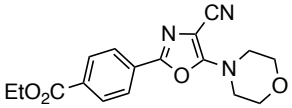
^{1}H NMR, $CDCl_3$, 500 MHz



2-42a

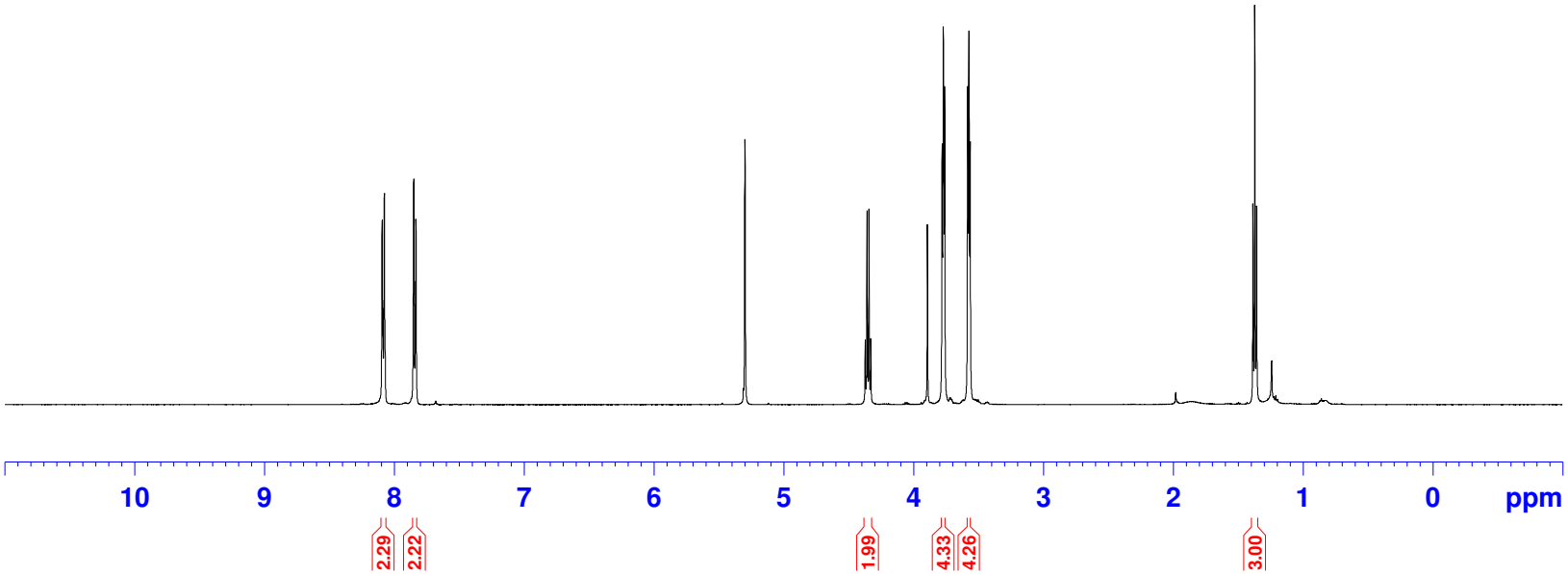
^{13}C NMR,$CDCl_3$, 125 MHz

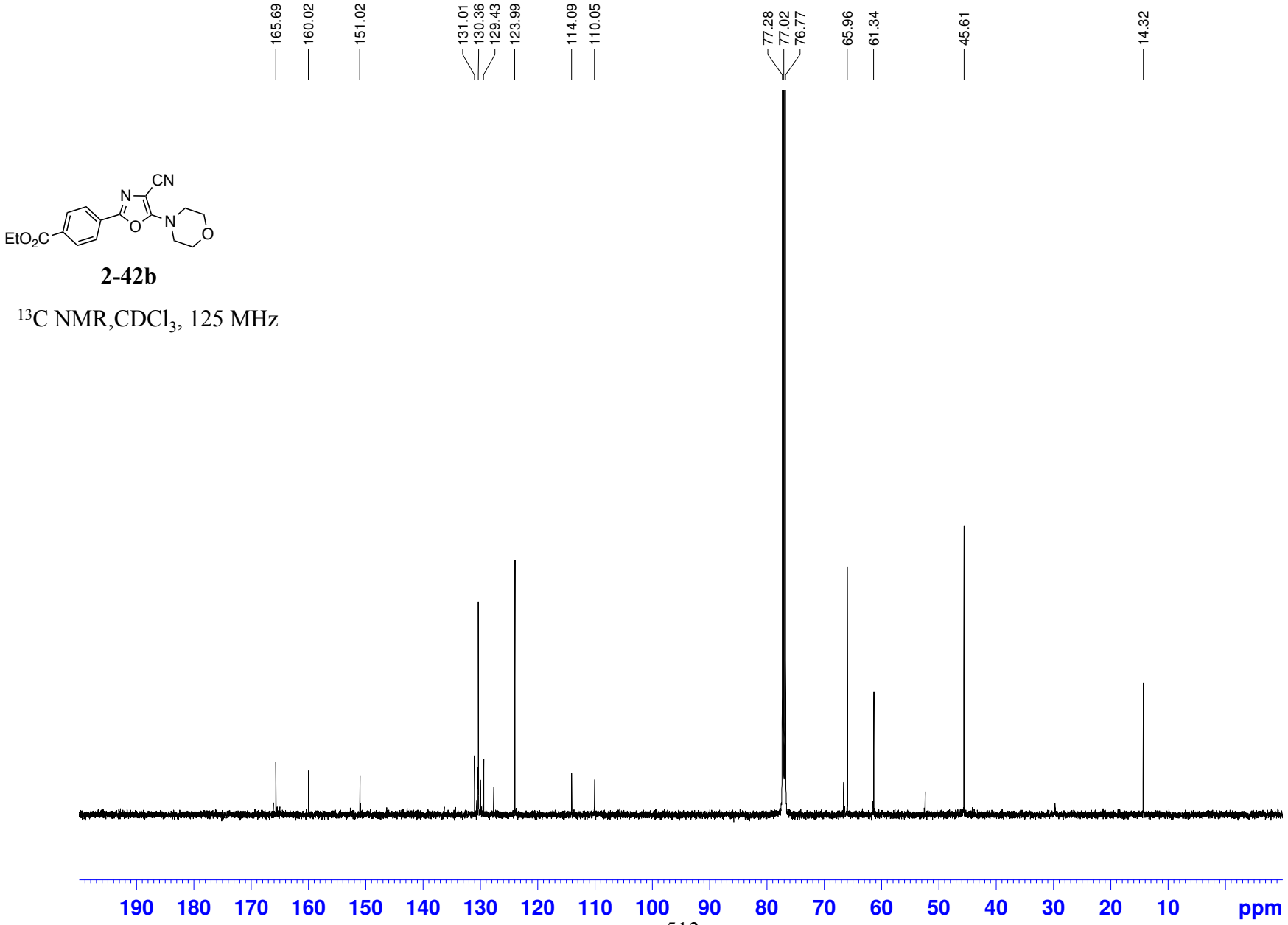




2-42b

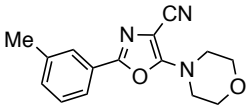
^{1}H NMR, CD_2Cl_2, 500 MHz





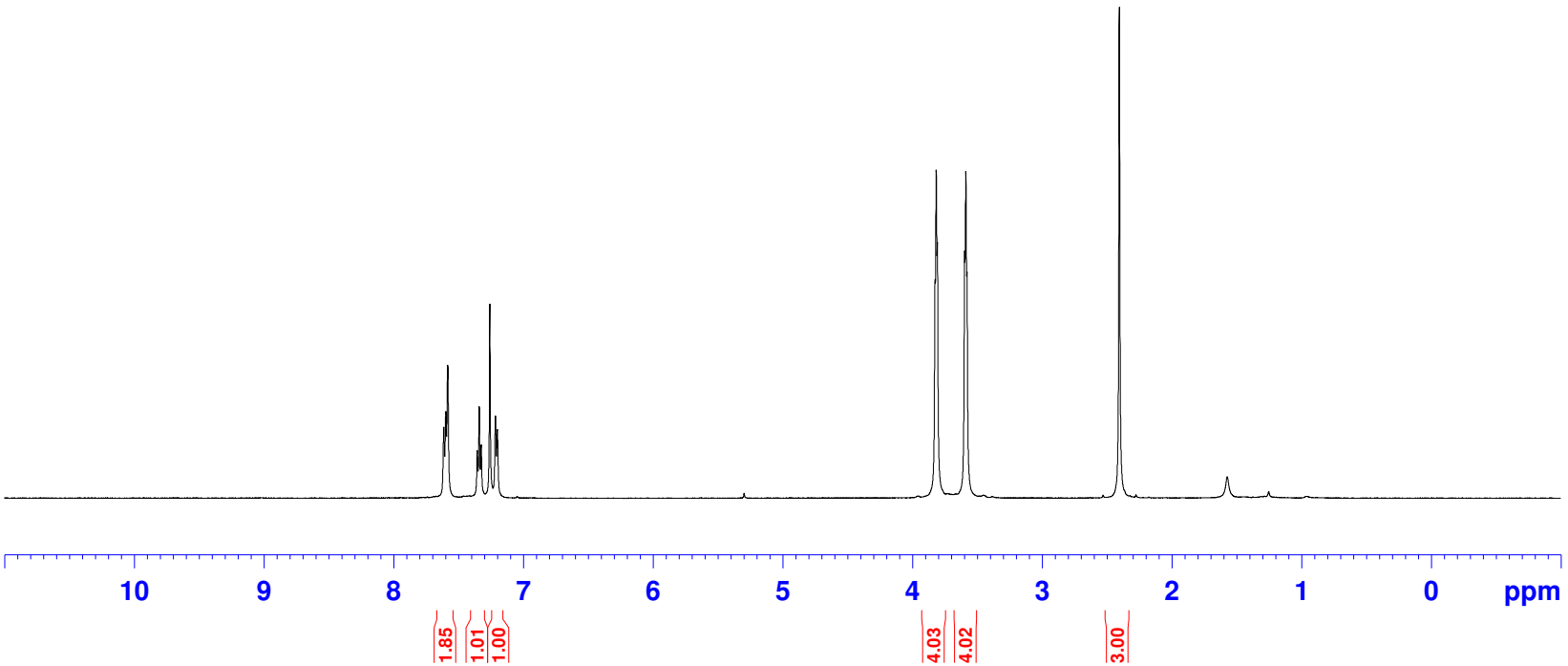
2-42b

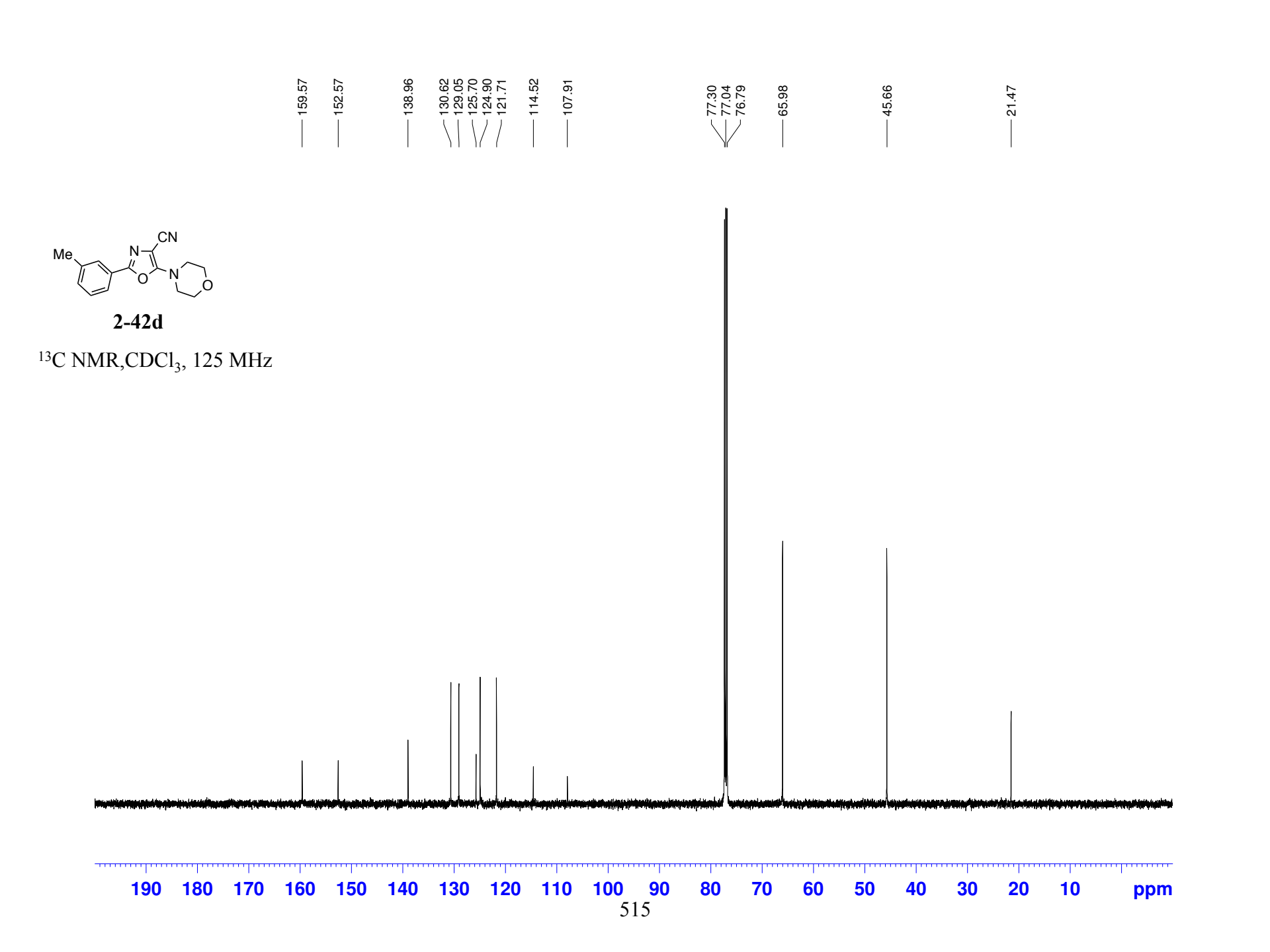
^{13}C NMR,$CDCl_3$, 125 MHz



2-42c

^{1}H NMR, $CDCl_3$, 500 MHz

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129.05
125.70
124.90
121.71
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107.91
77.30
77.04
76.79
65.98
45.66
21.47
CN
N
Me
O
N
O
2-42d
13C NMR,CDCl3, 125 MHz
190
180
170
160
150
140
130
120
110
100
90
80
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60
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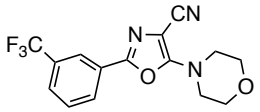
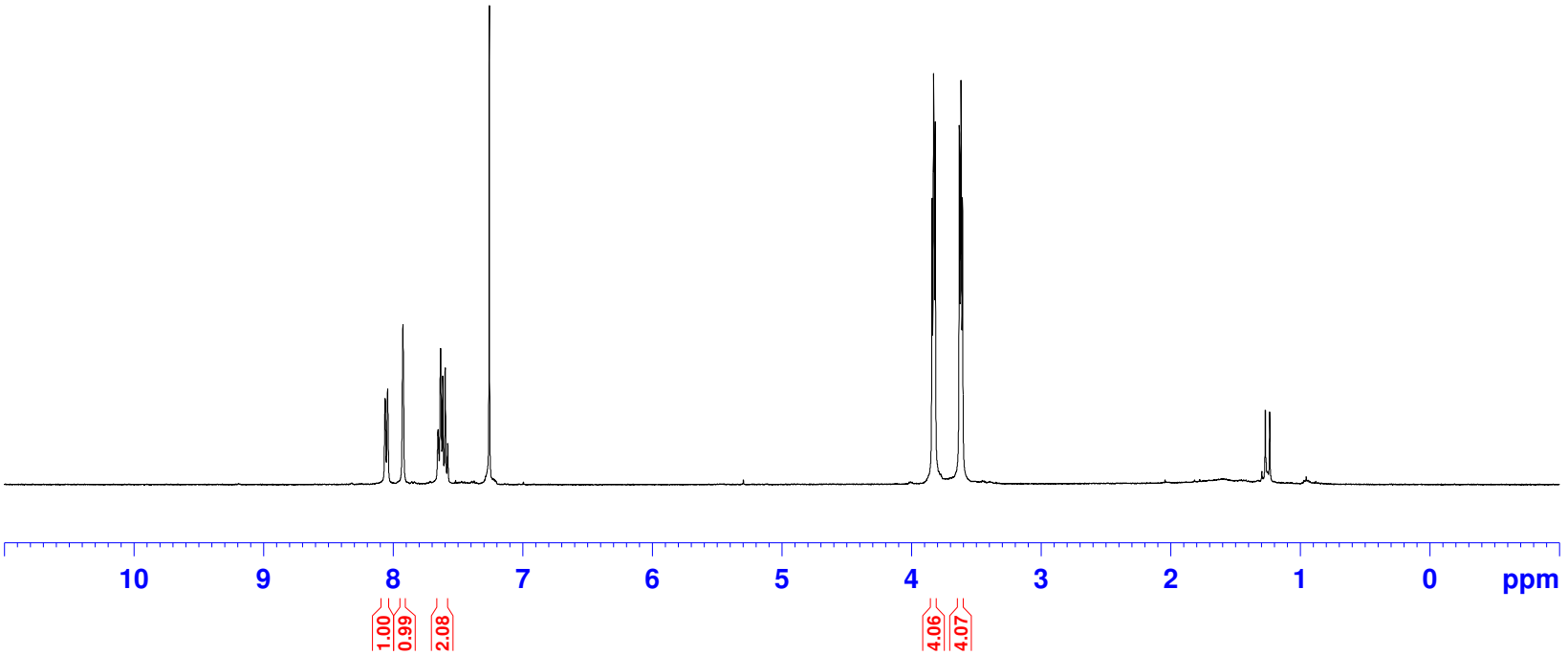
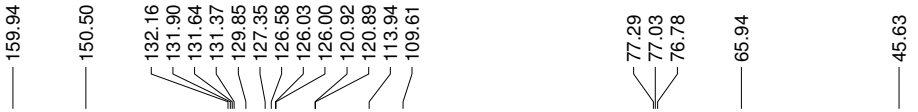
40
30
20
10
ppm

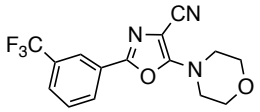


2-42d

^{1}H NMR, $CDCl_3$, 500 MHz

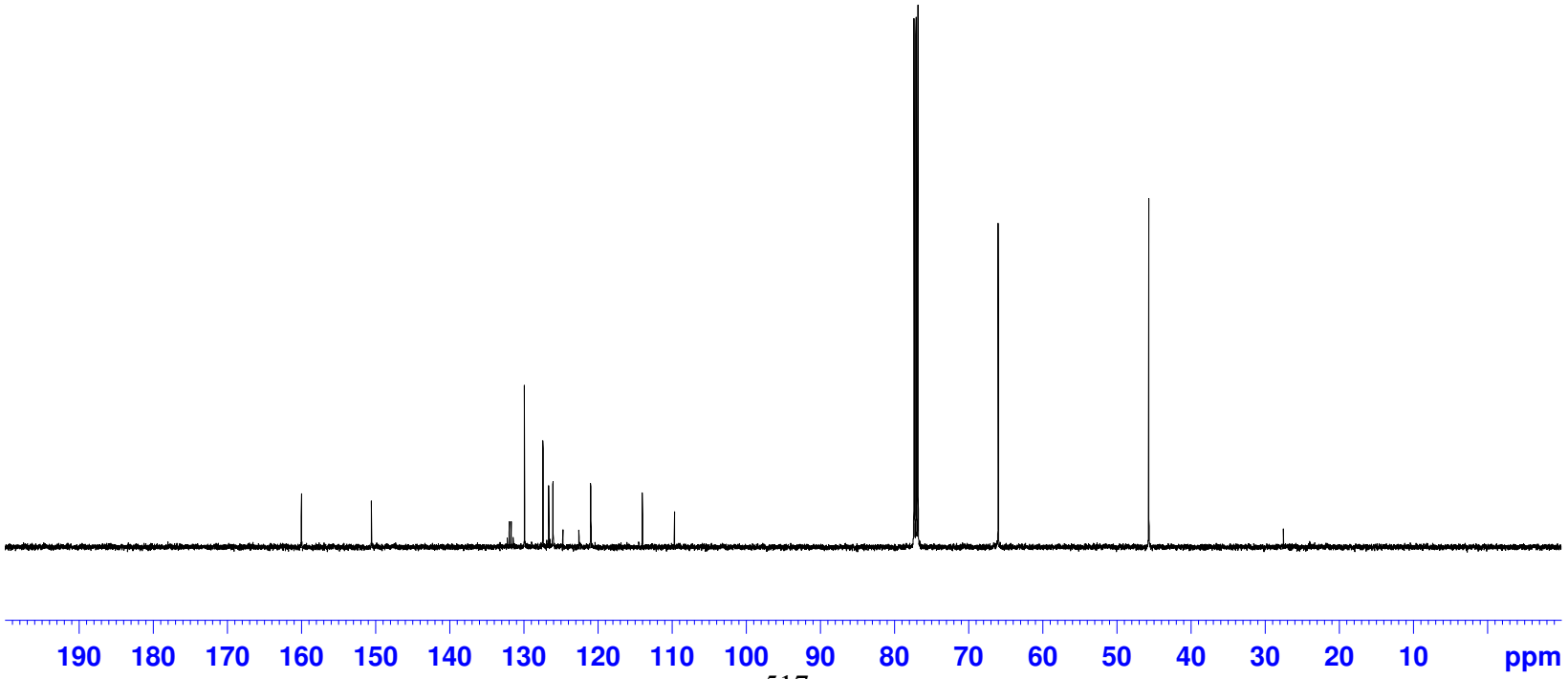


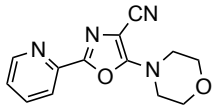




2-42d

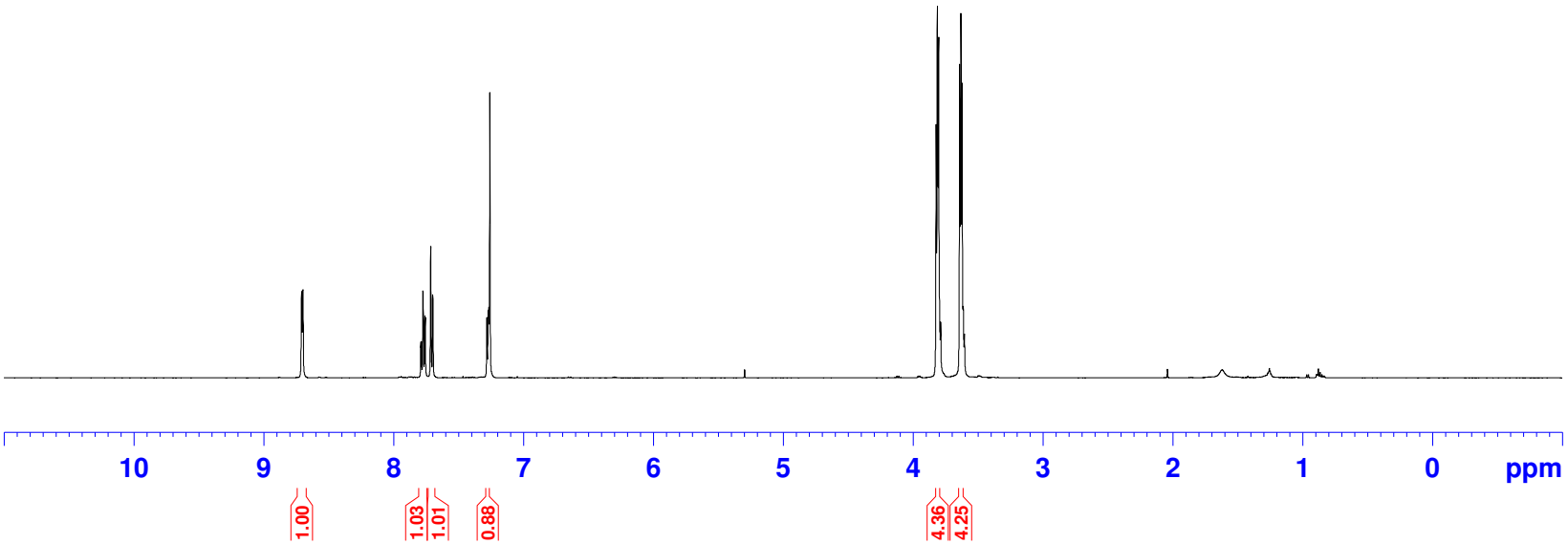
^{13}C NMR,$CDCl_3$, 125 MHz

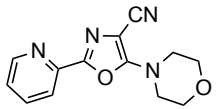




2-42e

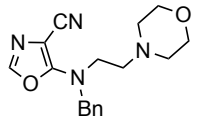
^{1}H NMR, $CDCl_3$, 500 MHz





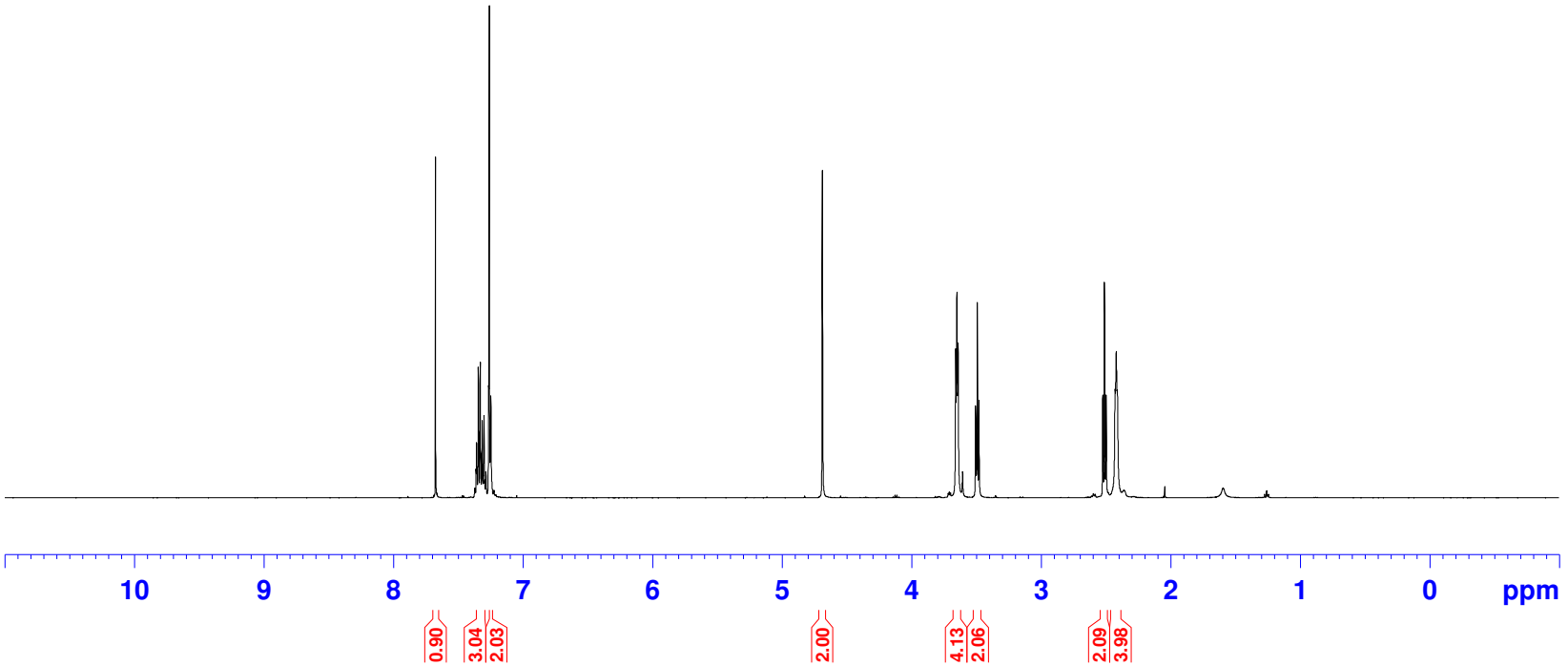
2-42e

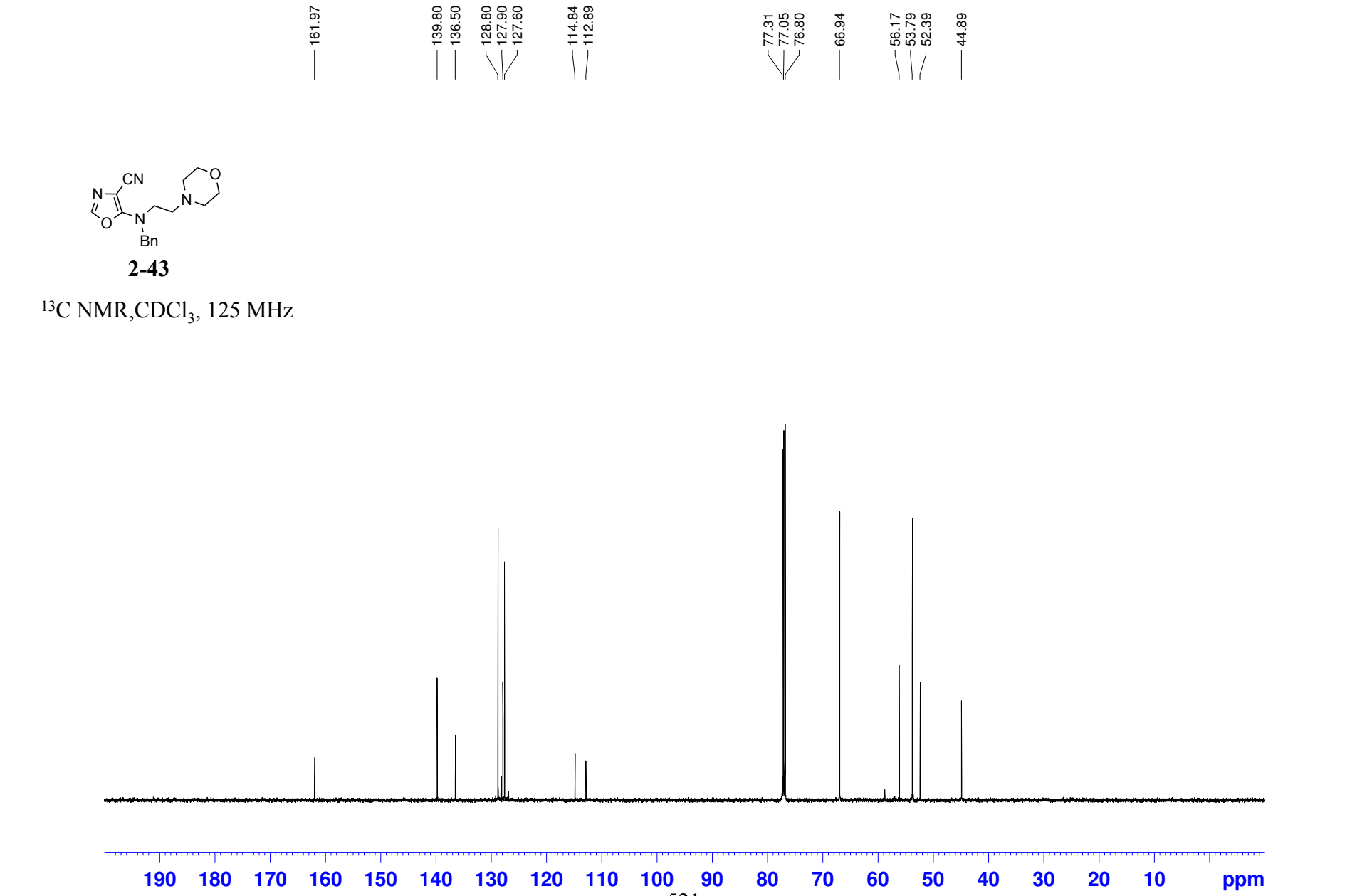
^{13}C NMR,$CDCl_3$, 125 MHz



2-43

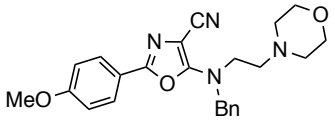
^{1}H NMR, $CDCl_3$, 500 MHz





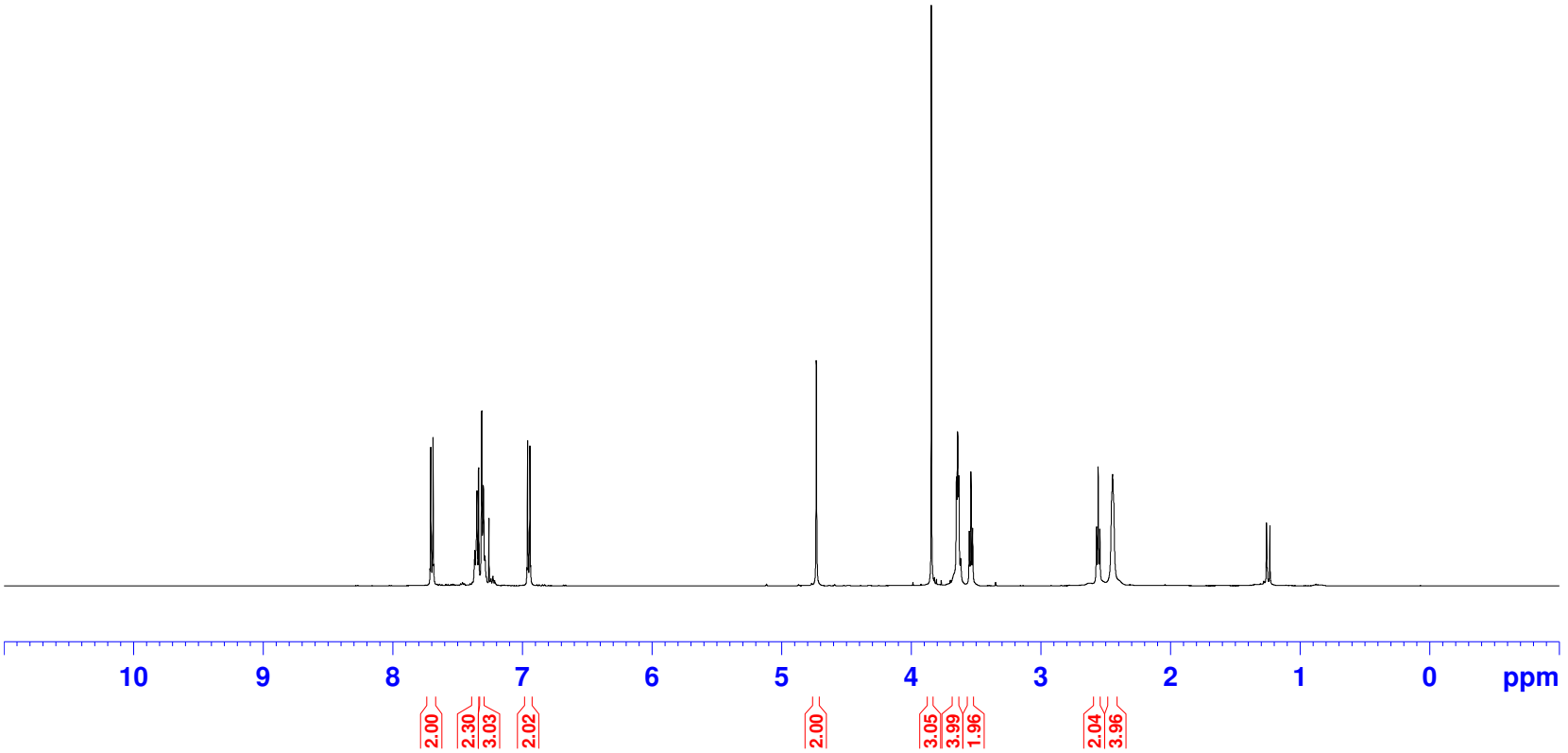
2-43

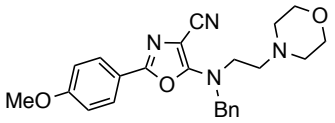
^{13}C NMR,$CDCl_3$, 125 MHz



2-44a

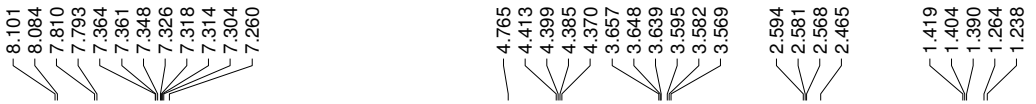
^{1}H NMR, $CDCl_3$, 500 MHz

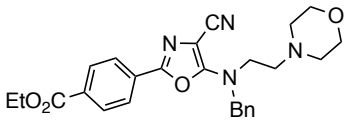




2-44a

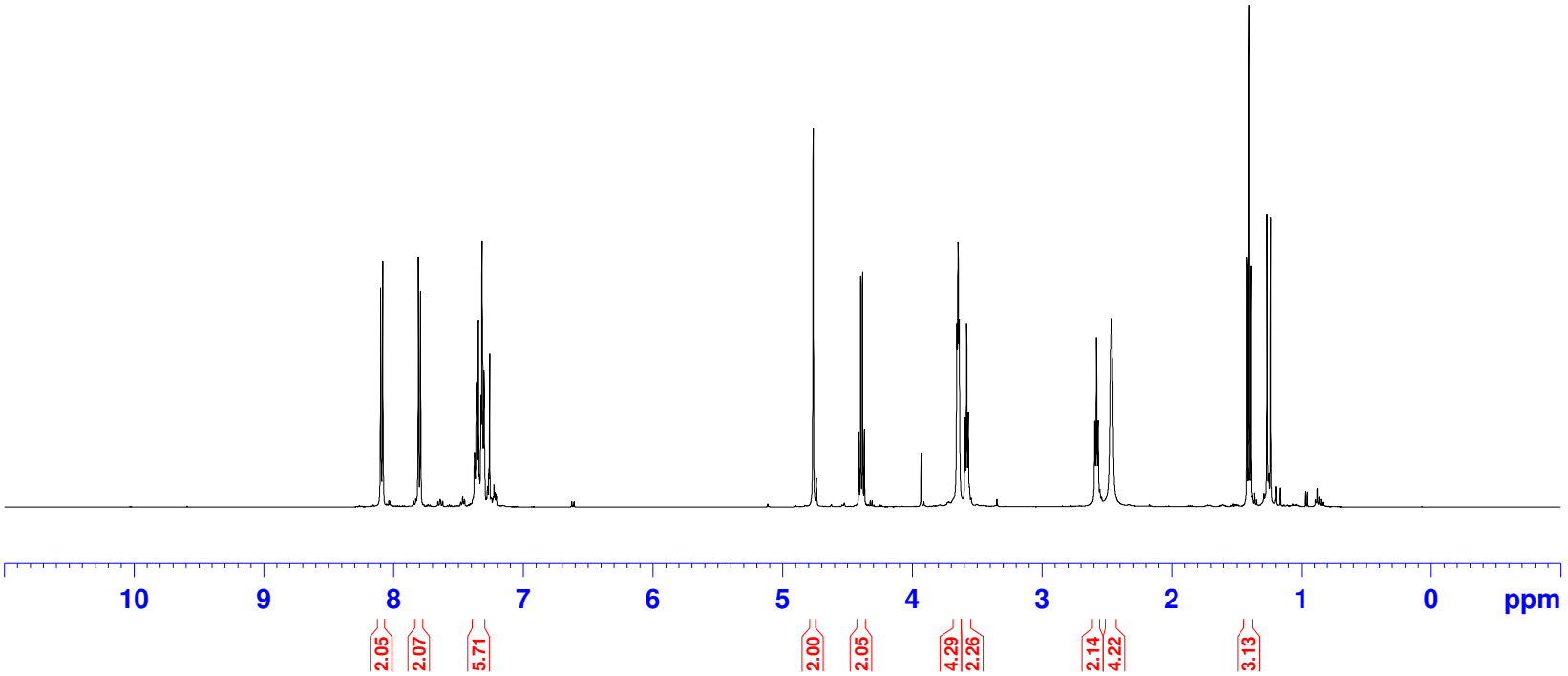
^{13}C NMR,$CDCl_3$, 125 MHz

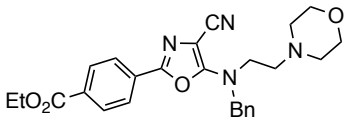




2-44b

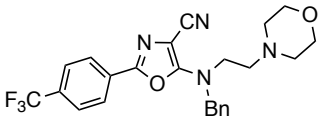
^{1}H NMR, $CDCl_3$, 500 MHz





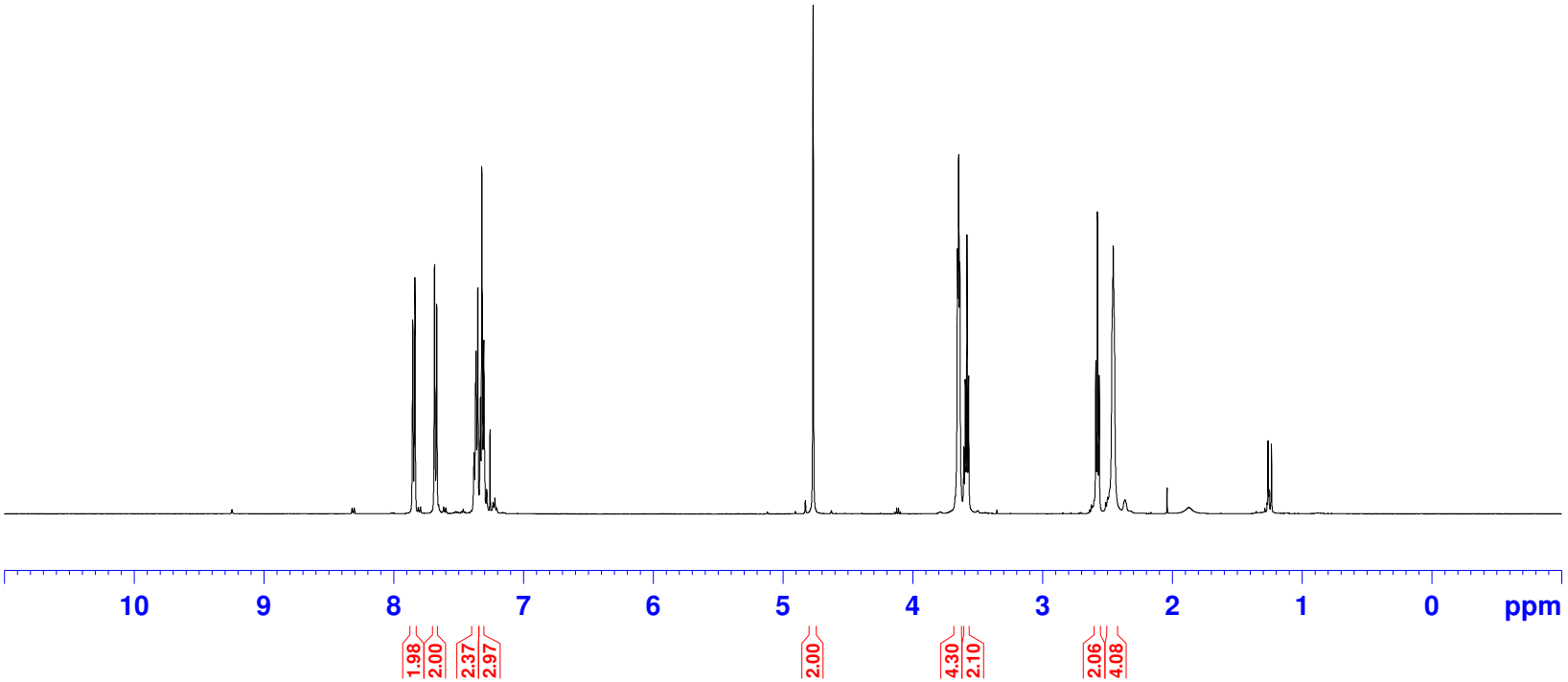
2-44b

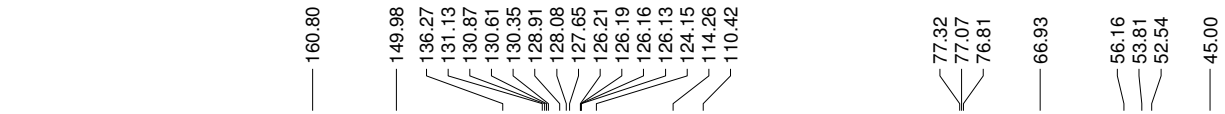
^{13}C NMR,$CDCl_3$, 125 MHz

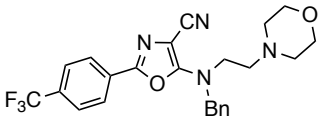


2-44c

^{1}H NMR, $CDCl_3$, 500 MHz







2-44c

^{13}C NMR,$CDCl_3$, 125 MHz