SYNTHETIC STUDIES ON PROTEIN KINASE D, BOTULINUM NEUROTOXIN A1 LIGHT CHAIN, AND NUCLEAR ANDROGEN RECEPTOR MODULATORS, AND EFFORTS TOWARDS THE TOTAL SYNTHESIS OF 9-DESMETHYLPLEUROTIN

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The first chapter outlines the synthesis of pyrazolo[3,4-d]pyrimidines as inhibitors of Protein Kinase D, a potential target for cancer therapy. The second chapter describes the synthesis of novel inhibitors of Botulinum Neurotoxin A1. First, the synthesis of a constrained analog that contains a dibenzo[*b*,*g*][1,5]thiazocine is discussed. Second, we explore the use of a Staudinger *aza*-Wittig reaction to produce 1,2,4-triazines and report the progress towards an inhibitor of BoNT/A with increased metabolic stability.

The third chapter describes the development of antagonists of the androgen receptor, a potential target for the treatment of castration-resistant prostate cancer. A small library of analogs was synthesized to assess a structure-activity relationship of a hit compound. The new lead compound was tested *in vivo* and further optimized to improve its metabolic stability. This chapter also describes the synthesis of small molecule affinity labeled conjugates to identify the mechanism of action of the antagonists. The final chapter of this dissertation describes the progress towards the total synthesis of 9-desmethylpleurotin.

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LIST OF SCHEMES

LIST OF ABBREVIATIONS

Ac .... acetyl

AIBN .... azobisisobutyronitrile

Am .... amidine

AR .... androgen receptor

Bn .... benzyl

Boc .... *tert*-butyloxycarbonyl

BoNT .... botulinum neurotoxin

n-BuLi .... *n*-butyllithium

CAN .... ceric ammonium nitrate

cat .... catalytic

COSY .... correlation spectroscopy

CRPC .... castration-resistant prostate cancer

DBU .... 1,8-diazabicyclo[5.4.0]undec-7-ene

DCE .... dichloroethane

DIBAL-H .... diisobutylaluminium hydride

DIPEA .... *N,N*-diisopropylethylamine

DMAP 4-dimethylaminopyridine

DMA .... *N,N*-dimethylacetamide

DMF ....*N,N*-dimethylformamide

DMSO ....dimethylsulfoxide

DEPC ....diethyl cyanophosphonate

DMP.................Dess-Martin periodinane

DPPA ....diphenylphosphoryl azide

EDCI ....1-ethyl-3(3-dimethylaminopropyl)carbodiimide

EC_{50}...................effective concentration of 50% of maximal response

ee ....enantiomeric excess

ESI.....................electrospray ionization

EtOAc ....ethyl acetate

equiv..................equivalent(s)

er ....enantiomeric ratio

GFP ....green fluorescent protein

GI_{50}....................growth inhibition of 50%

h........................hour(s)

HATU ....2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium

hexafluorophosphate

HOAt.................1-hydroxy-7-azabenzotriazole

HPLC ....high performance liquid chromatography

HRMS ....high-resolution mass spectroscopy

HSP ....heat shock protein

HTD ....high throughput docking

HTS...................high throughput screen

Im 2-imidazoline

IMDA ....intramolecular Diels-Alder

ICP-OES ....inductively coupled plasma optical emission spectroscopy

IR......................infrared spectroscopy

K_i......................inhibitor binding affinity

LAH ....lithium aluminum hydride

LCMS................liquid chromatography mass spectroscopy

LC ....light chain

LDA ....lithium diisopropylamide

LD_{50}...................lethal dose at 50% survival

MBTS................2-mercaptobenzothiazole disulfide

Mn(dpm)$_3$ ....manganese (III) tris dipivaloylmethane

MMPP ....manganese monoperphthalate

MTBE ....methyl *tert*-butyl ether

MW ....microwave irradiation

NMR ....nuclear magnetic resonance

Ns (nosyl)..........4-nitrobenzenesulfonyl

PBu$_3$ ....*tri-n*-butylphosphine

PDT ....product

PCa....................prostate cancer

P-gp ....p-glycoprotein

PIFA..................phenyliodine bis(trifluoroacetate)

PK ....pharmacokinetic

PKD ....protein kinase D

PMB ....*p*-methoxybenzyl

PMe$_3$..................trimethylphosphine

PMePh$_2$ ....methyl diphenylphosphine

PPh$_3$..................triphenylphosphine

Py ....pyridine

PyBroP ....bromotripyrrolidiniophophonium hexafluorophosphate

rt ....room temperature

sc ....subcutaneous

SCID ....severe combined immunodeficiency

SFC ....supercritical fluid chromatography

SM.....................starting material

STAB ....sodium triacetoxyborohydride

T3P....................1-propanephosphonic anhydride

TBDPS ....*tert*-butyldiphenylsilyl

TBHP ....*tert*-butyl hydroperoxide

Tf.......................trifluoromethanesulfonyl

TFA ....trifluoroacetic acid

TFAA ....trifluoroacetic anhydride

THF ....tetrahydrofuran

TLC...................thin layer chromatography

TMDS ....tetramethyldisiloxane

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1.0   SYNTHESIS OF SMALL MOLECULE INHIBITORS OF PROTEIN KINASE D

1.1   INTRODUCTION

Since the early 2000s, protein kinase D (PKD) has been a target for cancer therapy[1-10] due to its association with a number of tumors, including prostate,[2,10-15] breast,[16-19] pancreatic,[9,20-25] lymphoma,[26-28] and colon[29-30] cancer. PKD forms a subgroup of the Ca^{+2}/calmodulin-dependent kinase (CAMK) family and consists of three isoforms, 1, 2, and 3 (Figure 1.1). Initially, PKD1 and PKD3 were thought to be a subclass of PKC and were named as PKC μ,[31] and ν,[32] respectively. The three isoforms each have specialized roles in signaling pathways, which stem from their varying expression in different tissues.[30,33]
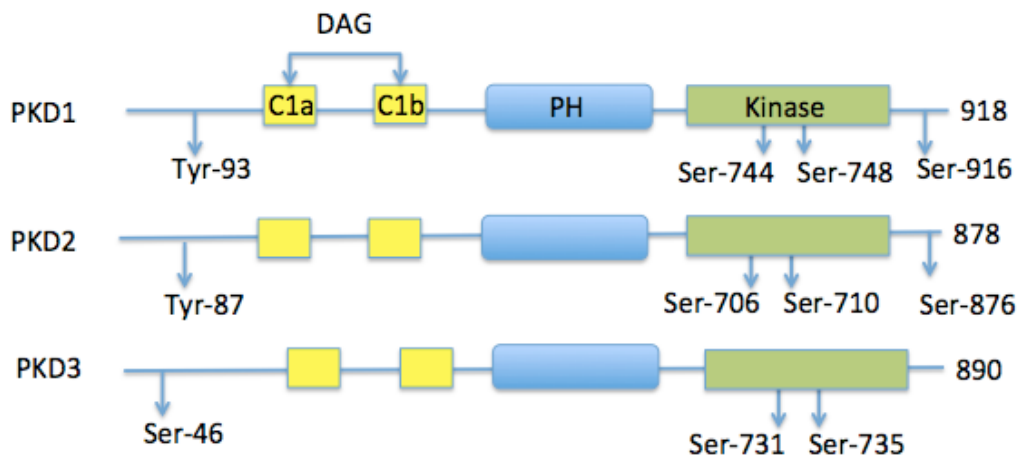
Figure 1.1. The PKD family.

Structurally, the three PKD isoforms show greater than 70% similarity (Figure 1.1). The cysteine rich domains (CRD) C1a and C1b, and the catalytic domain show high homology for

PKD1–3. The order of the domains is also conserved for the three isoforms and contains the CRD, a Pleckstrin Homology (PH) domain, and the catalytic kinase domain. The CRD are cysteine-rich zinc fingers that bind DAG (diacylglycerol) and PMA (phorbol ester).[34] The PH domain is involved in the autoinhibition of PKD.[35-36] Upon activation by PKC, PKD1 is phosphorylated at Ser 744, 748, and 916.[37] Once PKD is phosphorylated, it can maintain its activity through auto-phosphorylation in the presence of an agonist.[22,36-37]

PKD has been shown to play important roles in a number of cellular pathways including cell migration and invasion through protein exocytosis from the trans Golgi network (TGN), activation of the Ras-Raf-MEK-ERK pathway, inhibition of the JNK pathway, response to oxidative stress via NF-κB activation,[33,38-39] and nuclear export of histone deacetylases (HDAC's).[37,40-44] In hypoxic tumors, PKD2 overexpression is important for tumor vascularization through stabilization of hypoxia inducible factor 1α (HIF1α), and subsequent NF-κB activation. Heat shock protein 90 (HSP90) plays an important role in regulating the stability of PKD2 and could be used for synergistic therapy to treat hypoxic tumors.[45-46]

PKD activation begins with extracellular signaling via G-protein coupled receptors (GPCR) or a receptor tyrosine kinase (RTK). This signaling activates phospholipase C, which hydrolyzes $PI4,5P_2$ (phosphatidylinositol-4,5-bisphosphate) into the corresponding DAG and IP3 (inositol-1,4,5-triphosphate). DAG can bind to the CRD of nPKC, which in turn phosphorylates PKD, or directly binds to the CRD of PKD.[36,42,47-48] Once PKD has been phosphorylated, it circulates through the cytosol where it can phosphorylate Ras and Rab interactor 1 (RIN1).[49] The phosphorylated RIN1 releases Ras, which triggers the extracellular signal-regulated kinase (ERK) pathway involved in cell proliferation.[49-50] Overexpression of PKD is involved in the

upregulation of ERK and has been shown to be present in a number cell types including prostate cancer cells,[51] mouse embryonic fibroblasts (Swiss 3T3),[52-53] and endothelial cells.[54]

PKD can also downregulate the c-Jun *N*-terminal kinase (JNK) pathways through phosphorylation of c-Jun.[40,55-56] JNK is a subgroup of the mitogen activated protein kinases (MAPK) and is responsible for inflammatory/immune response, apoptosis, cell proliferation, and cell motility.[57-60] Dysregulation of these pathways has been implicated with tumorigenesis.[61]

The catalytically active PKD can also enter the nucleus, phosphorylate class II HDAC's (5 and 7)[62-67] and trigger their nuclear exocytosis.[41] HDAC's have been shown to be important in transcription and cardiac muscle contraction. Moreover, class II HDAC's can silence protein expression through DNA methylation.[68] In certain tumor types PKD is downregulated as a result of this gene silencing.[21-22,69-70] Dysregulation of PKD can cause cardiac hypertrophy and heart failure, and is one reason why PKD is being targeted as an upstream effector of HDAC export.[67,71-74]

PKD has been demonstrated to be important in cell migration and invasion events that lead to the metastasis of cancer. PKD plays a key role in the regulation of protein transport from the trans Golgi network (TGN) to the cell surface.[75-77] PKD has been shown to mitigate cell motility through phosphorylation of proteins involved in cell migration/invasion, including slingshot-1-like (SSH1L),[25] cortactin,[78-81] and Par-1.[82] Phosphorylated SSH1L can further bind to the 14-3-3 protein, which results in the release of F-actin into the cytosol. The release of F-actin then feeds into the cofilin remodeling of membranes of migrating cells.[17,83-87] PKD also inhibits F-actin remodeling by phosphorylation of RIN1, which activates c-Abl. c-Abl then alters the conformation of the adaptor protein Cullin-RING ubiquitin ligases (CRL), preventing activation of the F-actin remodeling proteins and disrupting cell motility.[50] PKD has also been associated

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with the regulation of spatial memory through the stabilization of F-actin in dendritic spines.[88] PKD also regulates matrix-metalloproteinases (MMP's) that are responsible for the degradation of the extracellular matrix (ECM).[89-90] These MMP's have been shown to play an important role in the metastasis of multiple cancers.[25,91-95] Recently, PKD1 has been shown to regulate focal adhesions, attachment sites connecting cells to the ECM, through the localization of $\alpha v\beta 3$ integrin and phosphatidylinositol-4-phosphate 5-kinase type-l gamma (PIP5Klγ).[96] PKD isoforms 1 and 3 have been found to regulate the cell cycle through microtubule modulation during mitosis.[97]

PKD can also be activated as a response to oxidative stress through a PKC dependent Src-Abl pathway.[98-100] PKD is phosphorylated at the PH domain, which allows for further phosphorylation on the CRD followed by PKCδ phosphorylation of the activation loop.[38,98,101-104] Phosphorylated PKD can phosphorylate the IKK complex that signals Nf-κB to induce expression for manganese superoxide dismutase (MnSOD) from the mitochondria, which then quenches ROS in the cytosol.[100,102,105]

Each of these pathways plays an important role in either cell survival or cell death and for this reason PKD represents a complex but viable target for cancer therapy.

1.1.1   Small Molecule Inhibitors of PKD

The ATP binding domain of PKD was the target of early PKD inhibitors.[106] Unfortunately, the ATP binding domain is highly conserved within the kinome and was the main downfall of early PKD inhibitors because they were non-selective and inhibited other kinases, resulting in off target effects. Over the past 15 years, potent PKD-selective inhibitors have been developed

targeting both the ATP binding domain and allosteric sites. In general, most ATP-competitive inhibitors are less kinase-selective due to the highly conserved nature of ATP binding sites.[106]

One of the earliest known PKD inhibitors, staurosporine (Figure 1.2), has low nanomolar PKD inhibition but also inhibits numerous other kinases, including protein kinase C (PKC), protein kinase A (PKA), and CAMK II.[107] Recently, fluorescein-staurosporine conjugates have been synthesized for their use as tools to monitor binding of other ATP competitive inhibitors.[108] The staurosporine type inhibitors including Gö 6976 (Figure 1.2) have been well studied for their kinase activity and exhibit low nanomolar inhibition of both PKC and PKD.[33,107,109-111]

The stilbene resveratrol was found to inhibit many proteins including PKD and required high micromolar concentrations for any observable inhibition effect (Figure 1.2). Resveratrol has been extensively studied as a cancer preventative and therapeutic but is limited due to its poor solubility, low bioavailability, and photochemical stability.[112] Despite these limiting factors, several formulations and analogs of resveratrol have been developed for the treatment of cancer and various diseases.[112] The flavonol, quercetin, was found to have low micromolar inhibition of PKD and PKC in addition to a number of other proteins and has been well studied for its use in the prevention and treatment of cancer (Figure 1.2).[113] The polysulfonylated naphthylurea, Suramin, was initially used for the treatment of sleeping sickness and parasitic diseases and later found be an effective activator of PKD (Figure 1.2).[114] Based on its ability to decrease tumor growth, Suramin has been advanced into a number of clinical trials including a combination treatment with paclitaxel in a phase I/II clinical trial for metastatic breast cancer (NCT00054028), a phase II clinical trial in combination with docetaxel for the treatment of non-small cell lung cancer (NCT01671332), a phase I clinical trial for bladder cancer (NCT00006476), and a phase III clinical trial for the treatment of castration resistant prostate

cancer (NCT00002723) (Figure 1.2). The marine-derived macrolactone bryostatin 1 was shown to activate PKD and PKC leading to alterations in cell aggregation and proliferation through localization of β-catenin.[115] Bryostatin 1 has been used in numerous (>40) clinical trials for the treatment of cancer, HIV, and Alzheimer's disease.[116-117]
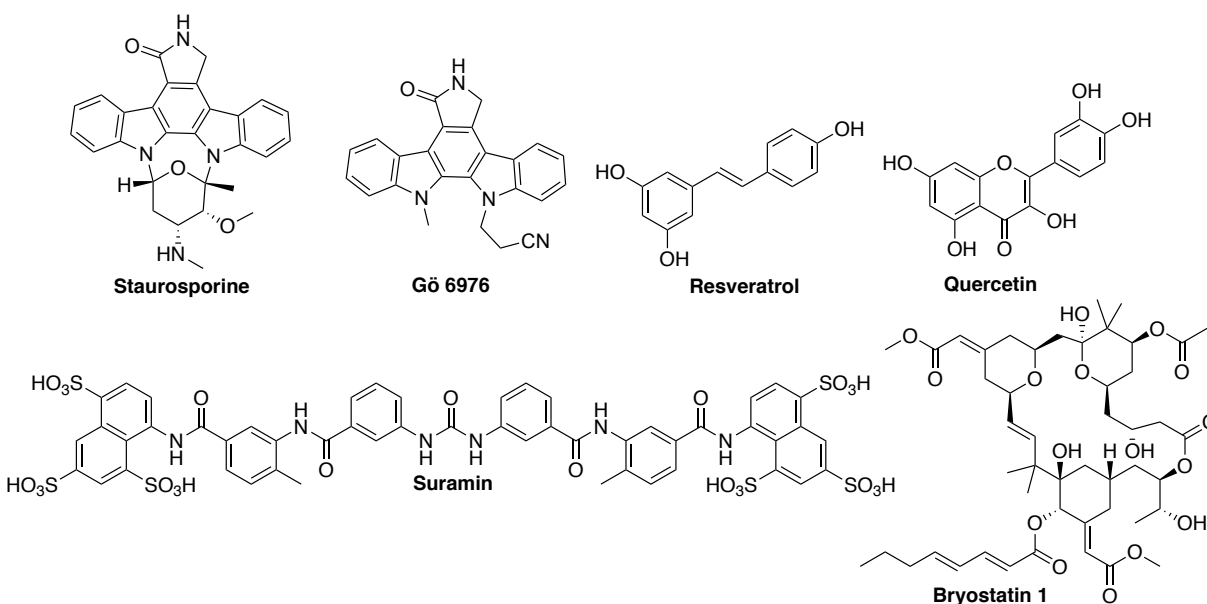
Figure 1.2. Early PKD modulators.

The novel 3,5-diarylazole **1.1** was identified from a HTS against PKD1 and had moderate kinase inhibition (PKD1 IC_{50} = 2.3 µM).[118] Subsequent structural optimization of **1.1** afforded compound **1.2** a potent, selective, and orally bioavailable inhibitor of PKD. Analog **1.2** exhibited modest isoform selectivity with a 9-fold and 3-fold selectivity for PKD1 over PKD2 and PKD3 respectively along with >1000-fold selectivity against a panel of 230 kinases.[118] PK data for **1.2** indicates that there is moderate oral bioavailability (38%) with a short plasma half-life (1.2 h). Combined, the selectivity, activity, and PK data for **1.2** make it a viable target for further optimization.

The 2,6-naphthyridines, developed by Novartis, have emerged as novel PKD inhibitors (Figure 1.3).[119-120] The 2,6-naphthyridine inhibitor **1.3** was identified in a HTS and shown to be

ATP-competitive PKC/PKD inhibitor. A series of modified 2,6-naphthyridines was used to investigate PKD as a possible therapeutic target for cardiac hypertrophy, since PKD is a downstream effector of nuclear export of class II HDAC's.[41,67,73-74,121-122] Subsequent SAR studies led to the orally available **1.4**, which was shown to block phosphorylation of PKD and nuclear export of class II HDAC's (Figure 1.3).[119] Additionally, when **1.4** was administered in 2 rat cardiac hypertrophy disease models it was found to have no effect. Unfortunately, it was unclear whether this was the result of PKD inhibition by **1.4** or the ability of **1.4** to also inhibit glycogen synthase kinase 3 β (GSK3β), which is known for its role in antihypertrophy.[123] An Using molecular modeling and conformational analysis, the 2,6-naphthyridine core was truncated to a bipyridyl.[119-120] Further optimization produced a new lead, BPKDi, a potent pan-PKD inhibitor that inhibits autophosphorylation of PKD and provides increased retention of HDAC4 and HDAC5 in cardiomyocytes (Figure 1.3).[120] In a PK panel in rats, BPKDi performed poorly due to high clearance, low solubility, and poor bioavailability. Attempts to improve the PK parameters were met with compounds less active or selective against PKD. To assess the *in vivo* activity, BPKDi was administered sc daily at 5 mg/kg in two rat models with no reduction in cardiac hypertrophy was observed. When higher doses of BPKDi were administered, a significant increase in blood pressure was observed, however, adequate plasma concentrations were reached at this dosing concentration. The lack of *in vivo* activity for these compounds ultimately led to their discontinuation as targets for the treatment of hypertrophy.

Cancer Research Technologies (CRT) produced the potent, orally available pan-PKD inhibitor CRT0066101 (Figure 1.3).[21] CRT0066101 was shown to have single-digit nanomolar pan-PKD activity *in vitro*. In a human pancreatic cancer tumor xenograft model, CRT0066101 was administered orally daily at 80 mg/kg for 28 days halting tumor growth completely.[21] In rat

pancreatic acinar cells, CRT0066101 promoted apoptosis and decreased necrosis in pancreatitic cells.[23,124] In malignant brain tumor cells, glioblastoma multiforme, CRT0066101 decreased cell migration and invasion events through inhibition of PKD2.[125] CRT0066101 was antiproliferative *in vitro* in low micromolar concentrations against multiple colorectal cancer cell lines.[126] Subsequent administration of CRT0066101 in HCT116 xenograft nude mice displayed significant inhibition of tumor growth.[126] When used in combination with Regorafenib, CRT0066101 demonstrated a synergistic effect on the inhibition and cell proliferation in metastatic colorectal cancer.[127] Additionally, CRT0066101 has also been shown to inhibit estrogen receptor negative breast cancer both *in vitro* and *in vivo*.[19] CRT0066101 remains a promising inhibitor of PKD for the treatment of cancer. In addition to CRT0066101, CRT also developed the pyrazine benzamide CRT5, which was a potent selective pan-PKD inhibitor (IC$_{50}$ = 1.5 nM) and decreased VEGF-induced endothelial migration, cell proliferation, and tubulogenesis (Figure 1.3).[128]

The pyrazolo[3,4-d]pyrimidine 3-IN-PP1 was identified from a recent SAR of 1-NM-PP1, a previously known inhibitor of Src and calcium dependent kinases,[15,129-130] as a potent (*in vitro* IC$_{50}$ = 33 nM) pan-PKD inhibitor (Figure 1.3).[131] Based on the interesting trends with the SAR, 3-IN-PP1 was proposed to have a alternate binding mode in the ATP binding pocket of PKD with the structure flipped 180° compared to other classically modeled kinase inhibitors.[131] 3-IN-PP1 is a promising new inhibitor that has potential for further structural development. Recently, the pyrazolo[3,4-d]pyrimidine **1.5** was identified as a low nanomolar pan-PKD inhibitor from a novel LC-MS based kinase screening technique (Figure 1.3).[132] This HTS technique allows for the rapid proteome profiling of new inhibitors of protein kinases and other purine-binding proteins.[133-134]
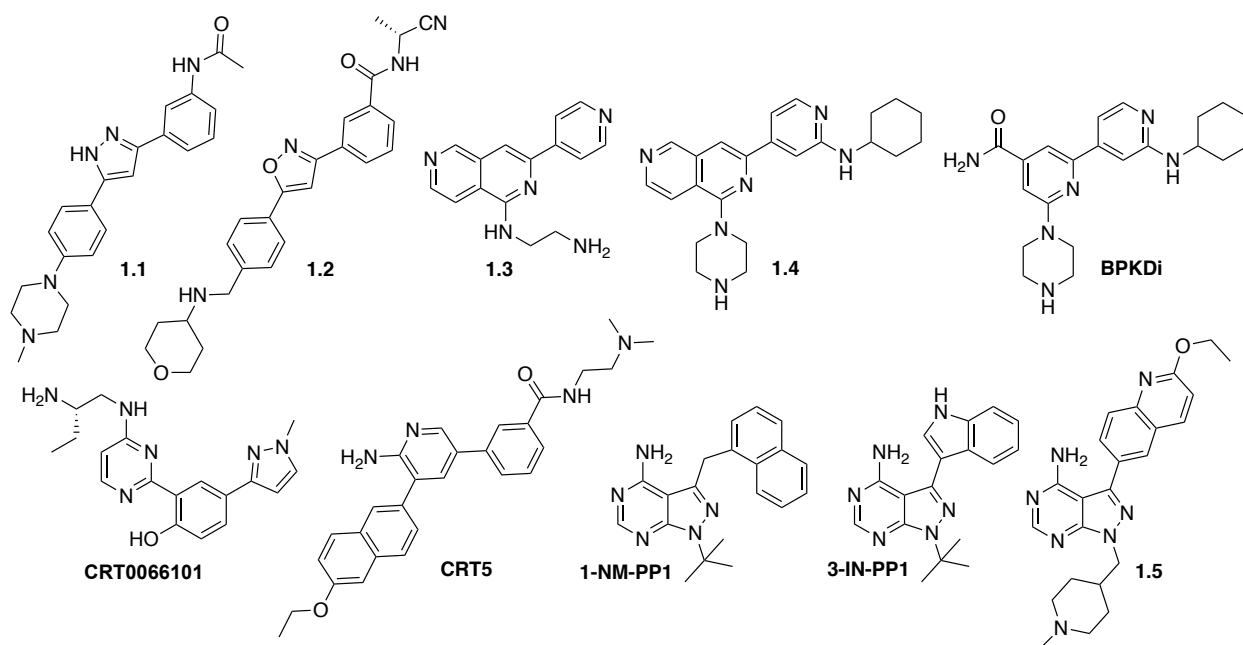
Figure 1.3. Recent inhibitors of PKD.

1.1.2 Small Molecule Inhibitors of PKD: Previous Work in the Wipf Group

Over the past 10 years, the Wipf group has been attracted to developing small molecule inhibitors of PKD as a potential target for cancer therapy. A library screen of the NIH SMRL produced the first potent PKD specific inhibitor CID 755673 with an *in vitro* IC$_{50}$ of 180 nM (Figure 1.4).[135] Previous work in the Wipf group aimed to improve potency of CID755673 which led to the synthesis of the more potent kb-NB142-70 with an *in vitro* IC$_{50}$ of 28 nM.[135-137] CID755673 and kb-NB142-70 were effective in reducing prostate and colorectal cancer cell proliferation and migration *in vitro*.[126,138] Unfortunately, no therapeutic effect was observed when kb-NB142-70 was administered to mice bearing PC-3 xenografts.[138] Subsequent PK analysis of kb-NB142-70 revealed that it had a plasma half-life of 6 min that can correlate directly to poor *in vivo* efficacy. Attempts to improve the metabolic stability of kb-NB142-70 resulted in kmg-NB4-23 that was slightly less potent with an *in vitro* IC$_{50}$ of 124 nM (Figure

1.4). Unfortunately, the poor solubility of kmg-NB4-23 (<0.4 mg/mL) prevented the *in vivo* biological characterization of this compound. The benzoxoloazepinolones were found to bind non-competitively with ATP indicating a potential alternative binding mode or location. An allosteric inhibitor may exhibit increased kinase selectivity making this scaffold a viable lead for further development.

SD-208 was identified from a targeted kinase inhibitor library screen and was found to be a potent, selective, orally bioavailable ATP-competitive inhibitor of PKD *in vitro* and *in vivo* (Figure 1.4).[139] A subsequent SAR of SD-208 revealed key binding interactions were required for potency in PKD and did not result in any analogs with increased potency. Finally, Ro139 was identified from a HTS campaign and was found to have low nanomolar activity against PKD *in vitro* (Figure 1.4).[140] Subsequent analog synthesis and biological characterization by WST-1 assay[141] revealed that analogs of Ro139 were selective towards rectal carcinoma RKO cells with low micromolar inhibition.
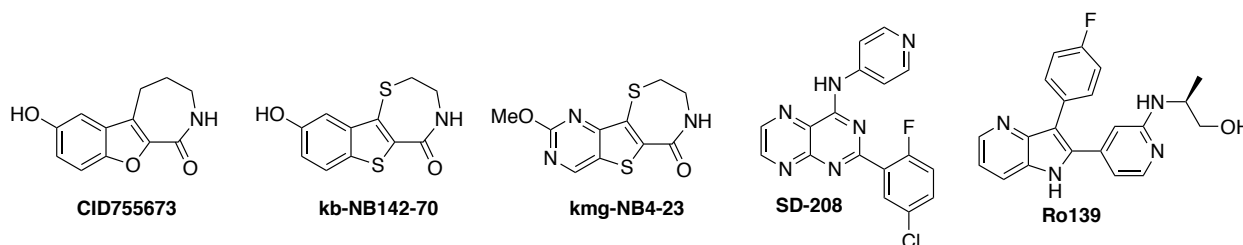
Figure 1.4. PKD inhibitors developed in the Wipf Group.

1.2   RESULTS AND DISCUSSION

The pyrazolopyrimidine 1-naphthyl-PP1 (PP1) was identified as a novel ATP competitive pan-PKD inhibitor (Figure 1.5).[15] Initially, PP1 was developed for analog specific mutants of src

kinases.[129] This scaffold was used to probe the ATP binding sites of gatekeeper mutant proteins that provide a more accessible binding pocket for new inhibitors.[129,142] PP1 has been shown to be highly selective for PKD over PKC and other members of the CAMK family.[15] To validate the analog specific mutant approach, PP1 was tested against the gatekeeper mutant of PKD that had been transfected and overexpressed in human embryonic kidney (HEK293) cells. **PP1** showed a 12-fold increase in activity from the wild type to the mutant. This suggests that the mutant PKD could be used to assess structural modifications of PP1 to develop a more potent PKD inhibitor as shown by Bishop and coworkers.[142] Additionally, **PP1** derivatives are active in mutants of ERK1/2 containing a single amino acid alteration.[143] PP1 is a potent pan-PKD inhibitor that has an IC_{50} of 155 nM and has been shown to inhibit growth and cell proliferation in prostate cancer (PC3 cells).[15] We envisioned 4 zones of modification around the **PP1** core to identify the substituent effect on the biological activity (Figure 1.5).
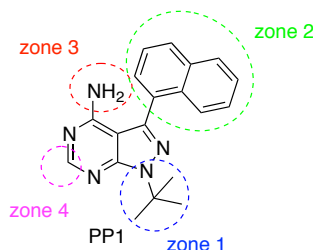
Figure 1.5. Zones of structural modification around PP1 core.

1.2.1   Synthesis of Pyrazolo[3,4-d]pyrimidine-Based Analogs

Our synthetic approach to the PP1 analogs utilized a late stage Suzuki cross coupling at halogenated C-3 with a variety of aryl boronic acids. We proposed that intermediate **1.7** could be formed by nucleophilic aromatic substitution with an amine at C-4 and subsequent halogenation

at C-3. Precursor **1.8** could be readily formed by a Vilsmeier-Haack reaction and hydrazine cyclization of commercially available barbituric acid **1.9** (Scheme 1.1).
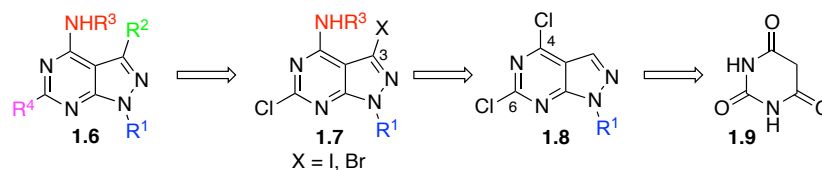
Scheme 1.1. Retrosynthetic approach to PP1 analogs.

The synthesis of PP1 and its analogs began with the tandem Vilsmeier-Haack chlorination and formylation reaction on commercially available barbituric acid (**1.9**) to give the trichloropyrimidine **1.10** in moderate yields.[144] Condensation and cyclization with methyl or *t*-butyl hydrazine afforded the pyrazolo[3,4-d]pyrimidines **1.8** in good yields. Initially, the cyclization with *t*-butyl hydrazine hydrochloride was low yielding due to poor solubility of the *t*-butyl hydrazine hydrochloride salt in THF at -78 °C. Attempts to freebase the hydrazine to improve the reaction were unsuccessful. After screening several solvents and solvent combinations, it was determined that addition of a solution of the hydrazine hydrochloride in acetonitrile to a THF solution of pyrimidine affords the desired product **1.8** in good yields. Regioselective aminolysis of **1.8** at the C4-position with ammonia or methylamine gave amines **1.11** in great yields. Initial attempts to iodinate at C-3 to form the 3-iodo intermediate with iodine, Barluenga's reagent, or *N*-iodosuccinimide were unsuccessful.[15] Gratifyingly, treatment with bromine afforded 3-bromo-pyrazolopyrimidines **1.7** in good yields. The C-3 bromide **1.7** was functionalized via a Suzuki cross coupling using a variety of aryl boronic acids to give the arylated compounds **1.12**. The low yields in some cases are the result of the arylation at both C-3 and C-6 to afford diarylated products. Finally, the 6-chloro substituent was reduced via a catalytic hydrogen transfer reaction to give the desired products **1.13** in poor to excellent yields

(Scheme 1.2). Overall PP1 and 17 analogs were prepared with varying substitution at the pyrazole nitrogen R^1, aryl ring at R^2, amine R^3, and C-6 position.
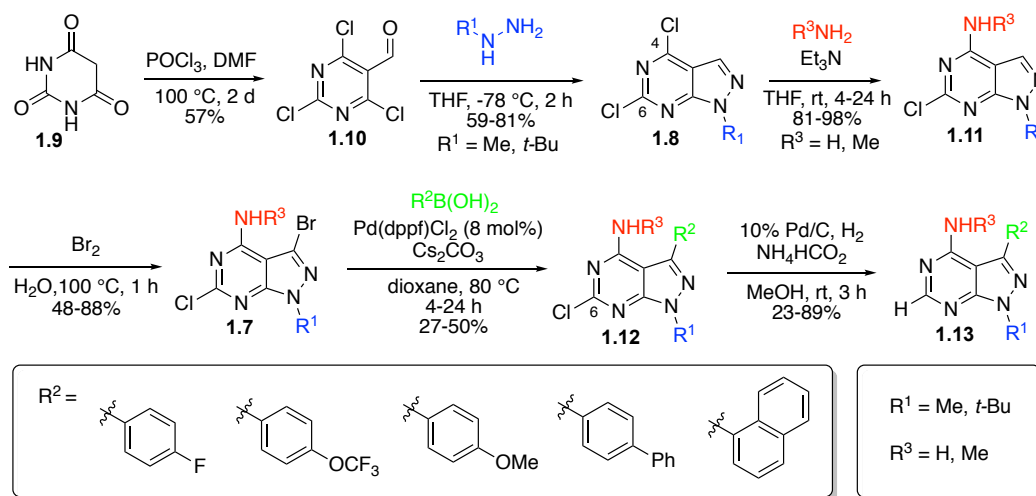
Scheme 1.2. Synthesis of PP1 analogs.

1.2.2 Biological Activity of PP1 Analogs

The *in vitro* PKD1 inhibition for PP1 and analogs was determined at 1 µM using a radiometric kinase assay.[15] The 17 analogs of PP1 showed that the PKD binding pocket was very resistant to structural modifications of the parent structure (Table 1.1). Modifying R_1 from a bulky *t*-butyl to a smaller methyl group resulted in a 50% loss in activity (entry 7). Replacement of the naphthyl group at R_2 with other aryl substituents also eliminated PKD activity. Analogs with methylation at R_3 were completely inactive and further confirmed the importance of the hydrogen bonding interactions within the binding pocket.[129] Electrophilic analogs (R_4=Cl) were also completely inactive. Unfortunately, the PP1 analog with a 6-chloro substitution was never synthesized and therefore no direct comparison can be made about the chloro substitution on activity of PP1. Based on the steep SAR observed, this series was deprioritized in favor of new cores.

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Table 1.1. *In vitro* radiometric kinase inhibition assay for PP1 analogs against PKD1.[15]
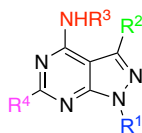
Entry	Compound	Structure R[1]	R[2]	R[3]	R[4]	PKD1 % inhibition[a]
1	**PP1**	*t*-butyl	1-naphthyl	H	H	80% (PP1)
2	**1.13a**	*t*-butyl	1-naphthyl	methyl	H	<10%
3	**1.13b**	*t*-butyl	(4-MeO)phenyl	H	H	<10%
4	**1.13c**	*t*-butyl	(4-MeO)phenyl	methyl	H	<10%
5	**1.13d**	*t*-butyl	(4-F)phenyl	H	H	<10%
6	**1.13e**	*t*-butyl	(4-Ph)phenyl	H	H	<10%
7	**1.13f**	methyl	1-naphthyl	H	H	32%
8	**1.13g**	methyl	1-naphthyl	methyl	H	<10%
9	**1.13h**	methyl	(4-MeO)phenyl	H	H	<10%
10	**1.13i**	methyl	(4-MeO)phenyl	methyl	H	<10%
11	**1.12h**	methyl	(4-MeO)phenyl	H	Cl	<10%
12	**1.12i**	methyl	(4-MeO)phenyl	methyl	Cl	<10%
13	**1.13j**	methyl	(4-CF$_3$O)phenyl	methyl	H	<10%
14	**1.12j**	methyl	(4-CF$_3$O)phenyl	methyl	Cl	<10%
15	**1.13k**	methyl	(4-F)phenyl	methyl	H	<10%
16	**1.12k**	methyl	(4-F)phenyl	methyl	Cl	<10%
17	**1.13l**	methyl	(4-Ph)phenyl	methyl	H	<10%
18	**1.12l**	methyl	(4-Ph)phenyl	methyl	Cl	<10%

[a]PKD1 %inhibition was determined at 1 μM concentration as previously described.[15,140]

1.3 CONCLUSIONS

PP1 was identified in an initial library screen and was found to be a potent PKD inhibitor with selectivity against similar kinases.[140] To validate the initial results, PP1 was resynthesized and 17 analogs were prepared to probe the effect of substitution around the PP1 core (Figure 1.3). Unfortunately, we discovered that the PKD binding pocket was very resistant to our structural modifications. The limited SAR produced compounds with no activity or poor activity, with PP1 remaining the most active analog (Table 1.1). The parent PP1 blocked 50% of PKD activity at

100 nM. A subsequent assay of PP1 against closely related kinases PKCα, PKCδ, and CAMKIIα had shown no inhibition even at concentrations as high as 10 μM. Although all new analogs of PP1 were inactive, PP1 was established as a potent pan-PKD inhibitor having antitumor activity in prostate cancer cells.[15]

2.0   SYNTHESIS OF BOTULINUM NEUROTOXIN A METALLOPROTEASE INHIBITORS

2.1   INTRODUCTION

The *Clostridium* genus of gram-positive bacteria includes several human pathogens that are well known for their toxicity, including *C. botulinum* (botulism), *C. tetani* (tetanus),[145-147] and *C. perfringens* (clostridial necrotizing enteritis).[148-149] The toxicity of *C. botulinum* is due to botulinum neurotoxins (BoNT's), which are a family of proteins consisting of eight serotypes (A-H).[150-154] The resulting disease, botulism, is characterized by flaccid muscle paralysis, leading to respiratory failure and death. Of the eight serotypes, BoNT/A is the most potent neurotoxin with an LD_{50} of less than 1 ng/kg.[155] There are five subtypes of BoNT/A (1-5) that contain unique characteristics.[156-157] In 1942, methods to effectively purify crystalline BoNT/A were developed leading to its use in the treatment of muscle disorders and increasing its potential for use in biological warfare.[158-164] Today, BoNT's are classified by the CDC as a class A biothreat.[165] Even with early detection, effective treatments of BoNT intoxication are limited and prophylactic. Several low-dose injectable forms of BoNT/A (Botox, Dysport, Xeomin) and BoNT/B (Myobloc) have been approved by the FDA for the treatment of a number of neuromuscular, urological, pain, and cosmetic disorders.[146-147,155,166-195]

BoNT's are metalloproteases[196] that consist of a 100 kDa heavy-chain (HC) and a 50 kDa light-chain (LC), both linked by a single disulfide bond (Figure 2.1).[197-200] The HC contains two 50 kDa domains, the *C*-terminal binding domain (HC$_C$) and the *N*-terminal translocation domain (HC$_N$). The LC is the catalytic domain containing zinc in the active site. Toxicity arises after disulfide cleavage, which enables release of the LC into the intracellular membrane.[201]
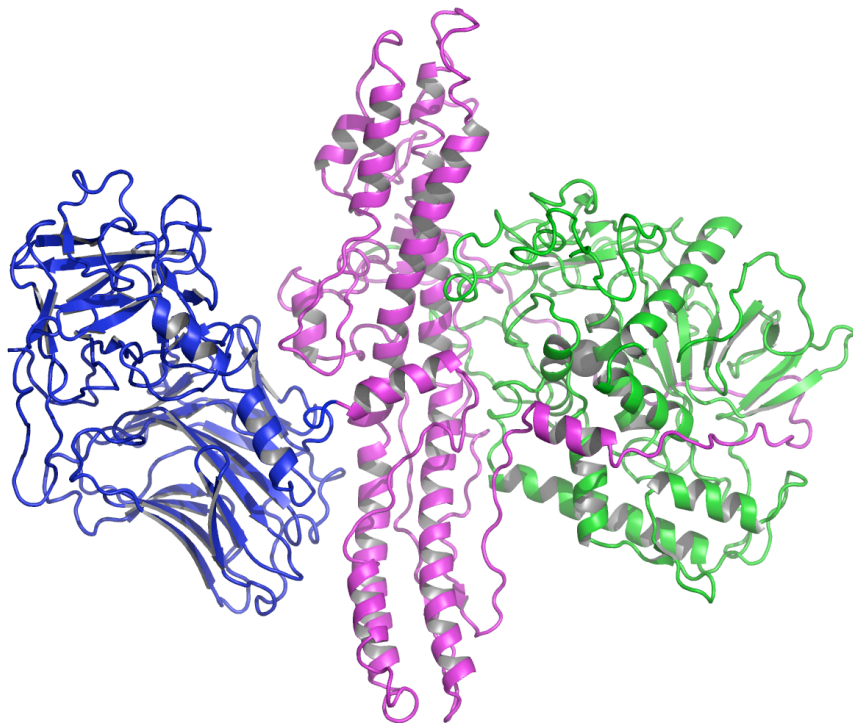
Figure 2.1. Structure of BoNTA (PDB: 3BTA) rendered in PyMol, HC$_C$ (Blue), HC$_N$ (Pink), LC (green), catalytic Zn (grey).

There are four main methods of BoNT intoxication including ingestion, wound, intentional intoxication (bioterrorism), or unintentional intoxication (side effect of therapeutic treatment).[202-203] Ingestion is the most common form of intoxication and occurs only with a preformed complex BoNT that consists of three components: BoNT, hemagglutinins (HAs; three HA-70, three HA-17, and six HA-33), and "non-toxic" non-hemagglutinin (NTNHA) also referred to as "non-toxic" neurotoxin associated proteins (NAP's). The complex provides heat

and hydrolytic stability as well as aid in intestinal absorption.[204-208] The HA and NTNH proteins are shed as BoNT circulates through the blood stream.[203] Homogeneous BoNT shows remarkable stability and rapid distribution in the bloodstream.[203] BoNT localizes at neuromuscular junctions (NMJ) where the heavy chain (HC_c) binds to extracellular membrane gangliosides (Figure 2.2).[209-211] Once bound to the membrane, BoNT triggers a recycling of synaptic vesicle membrane proteins (SV2) that cause endocytosis of the BoNT.[212] The newly formed vesicle is acidified by an ATP activated proton pump, which causes the translocation domain HC_n to insert into the vesicle membrane.[213-214] The translocation domain HC_n opens a pore within the vesicle that facilitates the extension of the LC out of the vesicle and into the cytosol.[145,214] Recently, it has been shown that the stability of the HC/LC complex under acidic pH increases and that under an acidic pH the LC interacts and permeates the anionic phospholipid bilayer.[215] Once exposed to the cytosol, the disulfide bond is reduced,[214] releasing the free LC into the cytosol. The LC of BoNT then facilitates hydrolysis of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins that facilitate pore formation along the synaptic membrane and are crucial in releasing the neurotransmitter acetylcholine into the synaptic cleft. There are three SNARE proteins that are targeted by the different serotypes of BoNT's: vesicle associated membrane proteins (VAMP also known as synaptobrevin) are targeted by BoNT B,[216] D,[217] F,[218] and G,[197] synaptosomal associated protein of 25 kDa (SNAP-25) is targeted by BoNT A, C, and E,[219-220] and syntaxin is targeted by BoNT C.[202,221] BoNT/A cleaves SNAP-25 at Gln[197] and Arg[198] to produce a very stable truncated peptide that will not form the SNARE complex responsible for membrane fusion, thus inhibiting the release of acetylcholine into the NMJ (Figure 2.2).[222-223]
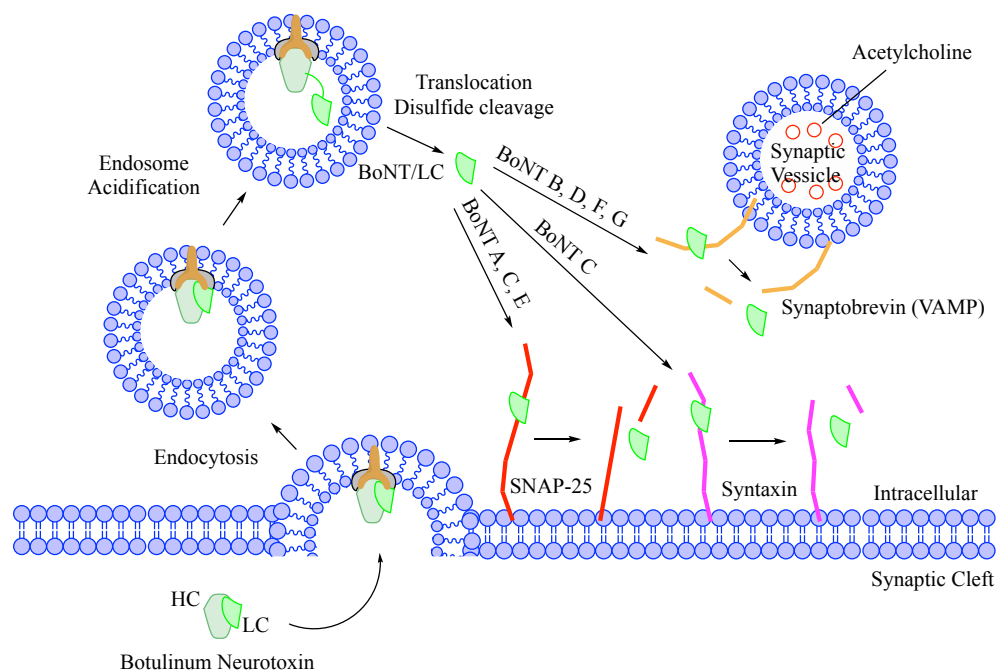
Figure 2.2. Mechanism of BoNT intoxication.[191,202]

The mechanism of zinc-mediated hydrolysis is exemplified by the reaction of SNAP-25 with the LC of BoNT/A. Cleavage begins with coordination of the catalytic zinc, to the carbonyl of the amide, thereby activating the amide toward hydrolysis. Water then adds into the amide to form a tetrahedral intermediate that is stabilized by the catalytic zinc and adjacent Tyr[366] residue. Subsequent elimination of the amine generates Gln[197] acid (Scheme 2.1).[222]
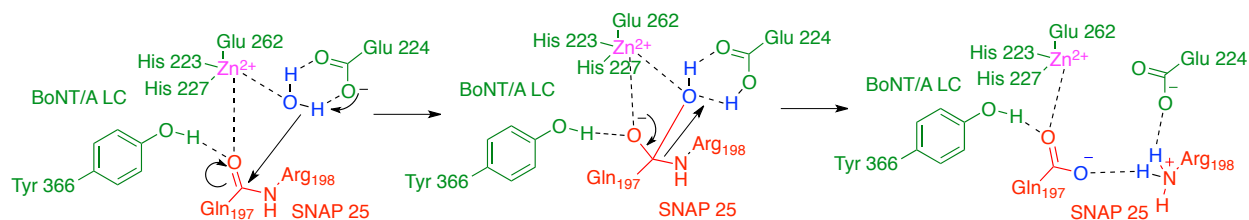
Scheme 2.1. Mechanism of SNAP-25 hydrolysis.[223]

To date, the only approved treatments of BoNT's are vaccines (antitoxins). In 2013, the Botulism Antitoxin Heptavalent (BAT) was approved by the FDA and is the current standard of care for BoNT intoxication of adults.[191] These vaccines work as prophylactic treatments and only remain effective for a few months to one year.[224] In addition, the vaccines are expensive to

produce (ca. $2100/dose), and are made from equine botulinum antitoxin that has been shown to have adverse effects in ~10% of the population.[191,225] The monoclonal antibodies produced by vaccines are only effective if applied before exposure to the toxin as the toxins have been found in the motor neurons within minutes to hours after exposure.[226] Due to the lack of a readily available treatment of BoNT's and the possibility of a widespread bioterrorism event, the development of a small molecule inhibitor of BoNT/A LC is highly desirable.

2.1.1 Peptide Based Inhibitors of BoNT's

Initial inhibitors of the LC were peptides and peptidomimetics that mimic the truncated SNAP-25 (amino acids 187-206) and have been primarily used as chemical probes and in assay development for the measurement of inhibition of BoNT's.[227-234] Early work by Schmidt and Bostian used a truncated SNAP-25 (amino acids 187-206) to monitor the proteolytic activity of BoNT/A LC. After screening a number of truncated peptides it was discovered that the relative size and sequence of the truncated peptide was important and that the 17-mer had the highest k_{cat}.[227,235] Schmidt and Stafford developed a HTS FRET (fluorescence resonance energy transfer) assay that uses the modified SNAP-25 fragment SNRTRIDEAN[dnpK]RA[daciaC]RML as a FRET substrate.[236] The FRET substrate for this assay tends to suffer from photodegradation or fluorescence quenching and is usually run along with HPLC analysis to eliminate false positives.[236-239] Janda and coworkers developed another HTS that relies on a modified SNAP-25 fragment (FITC)-TRIDEANQRATK(DABCYL)M known as SNAPtide, which is more resistant to photodegredation and has higher bench stability.[240-243] When using SNAPtide as a FRET substrate the N-terminal fluorophore, fluorescein isothiocyanate (FITC), is quenched by the C-terminal 4-((4-dimethylamino)phenyl)azo) benzoic acid (DABCYL).[243] These substrates are also

used in HPLC assays to measure the extent of proteolysis of the truncated peptide that can be directly correlated to a ligand binding affinity or IC_{50}.

In the search for more potent and specific small molecule non-peptidic inhibitors (SMNPI's) of BoNT/A, extensive work has focused on using computational models of the binding site and known SAR to probe for a more potent or specific structure. Based on the previous work on modified SNAP-25 fragments by Schmidt and coworkers, the pseudo-peptide mpp-RATKM (Figure 2.3) was developed. It was initially identified through a cysteine scan of a SNAP-25 (187-203) fragment and further optimized through SAR to provide a mpp-RATKM that had a K_i of 300 nM.[228-229,231,244] Burnett and coworkers used this pseudo-peptide along with conformational and docking studies of a library of previously known active compounds to elucidate the pharmacophore of BoNT/A LC (Figure 2.3).[202,238,244-247]

Compound I1 was derived from the SNAP-25 fragment QRATKML and is the most potent non-zinc chelating peptide mimetic to date.[222] In the Wipf lab, several peptidomimetics have also been previously synthesized as a hybrid of RRGC[248] and I1 resulting in 3 new peptides with K_i's ranging from 0.3 μM to 1 μM.[233] Further development of these peptides has led to cyclic peptides analogs.[249] Unfortunately, even shorter peptides suffer from high polar surface areas (>300), which could cause limited cell permeability that may lead to a poor therapeutic profile. The shorter tetrapeptide (RRGC) could be a potential lead compound for further backbone modifications as it has the lowest MW of all truncated peptides and a promising K_i of 160 nM. Compound RRGC was shown to be protective and non-toxic to chick motor neurons at 600 μM along with an intracellular stability over 24 hours (Figure 2.3).
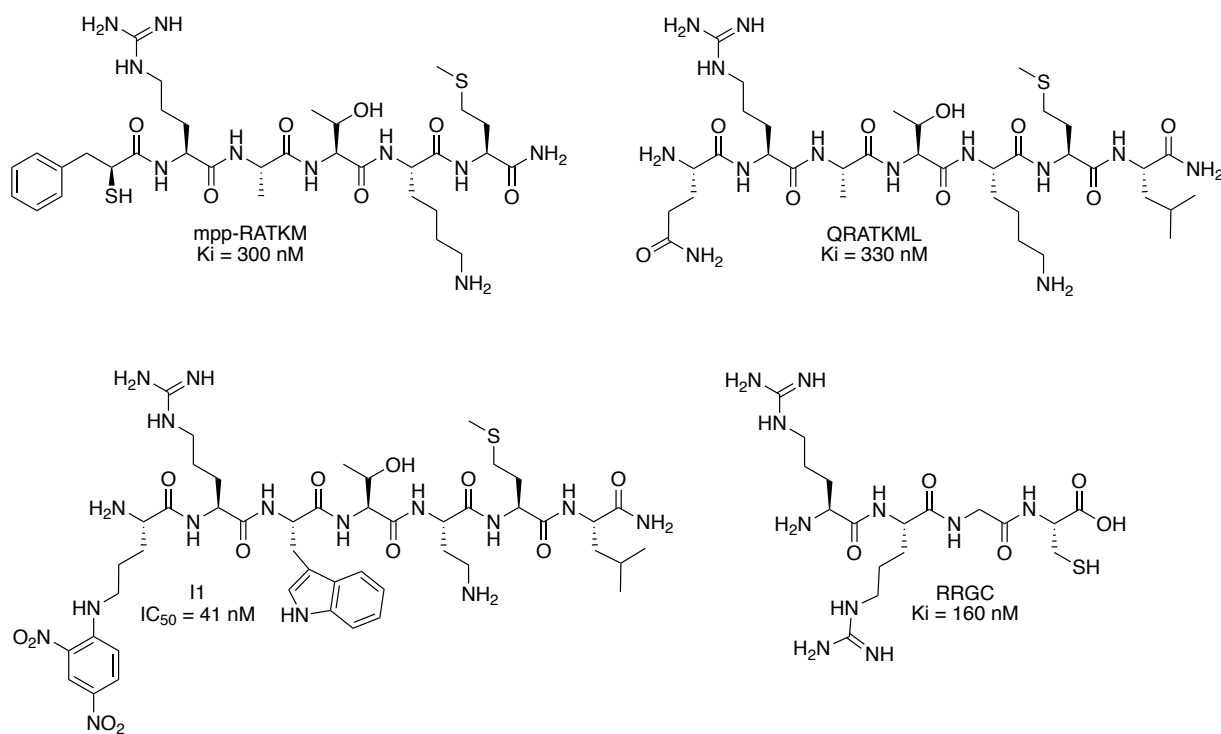
Figure 2.3. Structures of peptide-like inhibitors of BoNT/A.

2.1.2   Small Molecule Inhibitors of BoNT's

As peptides often suffer from high metabolism and low absorption, significant efforts have been made toward pinpointing a small molecule non-peptidic inhibitor of BoNT. As a result a variety of small molecule scaffolds have emerged in the literature for the treatment of BoNT over the past 30 years.

Hydroxamic acids have emerged as very potent inhibitors of BoNT LC. The catalytic domain has been cocrystalized to elucidate their binding mode, showing that the hydroxamic acids chelate zinc in the active site.[250-252] The earliest potent (< 1 μM) nonpeptidic hydroxamic acid inhibitor **2.1** was identified from a screen of a small library of hydroxamic acids (Figure 2.4).[239,252-253] Hydroxamic acid **2.1** had a promising *in vitro* IC_{50} of 0.4 μM but was found to be toxic in neuroblastoma N2a cells at 5 μM. Subsequently, in two separate mouse studies, **2.1** did

not show significant protection in BoNT intoxicated mice versus the controls.[253-256] *In silico* screening of 2.5 million hydroxamic acid derivatives led to the discovery of **2.2** that demonstrated low μM *in vitro* activity and promising *in vivo* survival with 60% survival compared to the control after 48 h.[257-258] Despite concerns due to hydroxamic acids being flagged as potentially concerning functionalities, several examples of FDA approved drugs contain this moiety.[259-261]

Covalent inhibitors of BoNT/A LC have also been investigated, which act by binding to the free Cys[165] produced by disulfide bond reduction during the release of the LC into the cytosol.[262-263] These compounds are noncompetitive inhibitors of BoNT and covalently bind through the Michael acceptor acrylonitrile. Compound **2.3** was identified from a 70,000 compound library screen and was further optimized to **2.4** that had shown increased selectivity for BoNT/A LC in a chick neuronal cell-based assay (Figure 2.4). IC_{50} values for **2.3** and **2.4** were calculated using a HTS FRET assay and verified using an HPLC assay.[235-236] Advantages of these selective covalent inhibitors include increased biochemical efficiency since their binding is not in equilibrium and can outcompete endogenous ligand binding at lower concentrations as exhibited by numerous FDA approved covalent drugs. Unfortunately, the possible off target effects of these covalent inhibitors include alkylation or reaction with glutathione, which could lead to potential toxicity.[264]
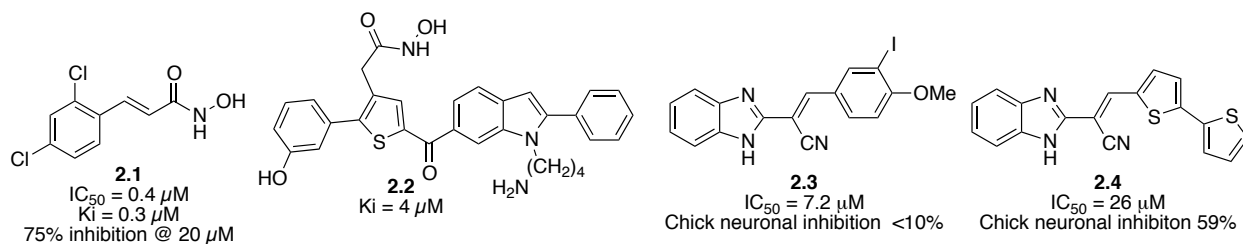
Figure 2.4. Hydroxamic acid and covalent inhibitors of BoNT.

The antimalarial aminochloroquinolines (ACQ) have also been explored as inhibitors of BoNT's (Figure 2.5). Compound **2.5** (also known as Q2-15) was discovered from a HTS of the NCI diversity set of a variety of ACQ containing substrates.[238] In particular, **2.5** was shown to inhibit BoNTA with an IC_{50} of 11 μM. More recently a series of hybrid structures such as **2.6** were synthesized and found to have submicromolar IC_{50} of 0.3 μM in an *in vitro* assay. Although compounds such as **2.6** show submicromolar *in vitro* inhibition of BoNT they suffer from high MW and LogP, which raises concerns regarding aqueous solubility and potentially short half-lives *in vivo*.

The quinolinol **2.7** was discovered as a result of several iterations of virtual screening, hit validation, and safety evaluations (Figure 2.5).[265] Compound **2.7** was shown to be efficacious both in *in vitro* with 97% inhibition of BoNTA/LC at 20 μM and *ex vivo* in a mouse phrenic nerve diaphragm assay (MPNHDA) by significantly slowing the onset of muscle paralysis at 10 μM. Recently, two independent efforts to optimize this scaffold yielded **2.8**[266] and **2.9**.[267] A small SAR of the quinolinol **2.7** afforded compound **2.8** with an improved *in vitro* IC_{50} of 0.6 μM and slowed the onset of muscle paralysis at 20 μM in the MPNHDA assay.[266] A crude library screen of sulfonylated quinolinols, hit validation, and SAR optimization yielded sulfonamide **2.9** with a similar in vitro efficacy to **2.7** (1.6 μM) and slowed the onset of muscle paralysis at 20 μM in the MPNHDA assay.[267] Interestingly, inhibition kinetics of quinolinol **2.9** have shown that the inhibition is uncompetitive with respect to SNAP-25 binding.[267] These compounds containing the quinolinol moiety remain interesting scaffolds in the pursuit of inhibitors of BoNT as they display promising physiochemical properties and show low μM activity in both *in vitro* and *ex vivo* assays (Figure 2.5).

Finally, NSC 240898 displayed a dose-dependent protection of SNAP-25 in chick spinal motor neurons with low cytotoxic effects even at 40 μM (Figure 2.5).[244] Based on its biological activity and low cytotoxicity, NSC 240898 is an attractive target for further development.
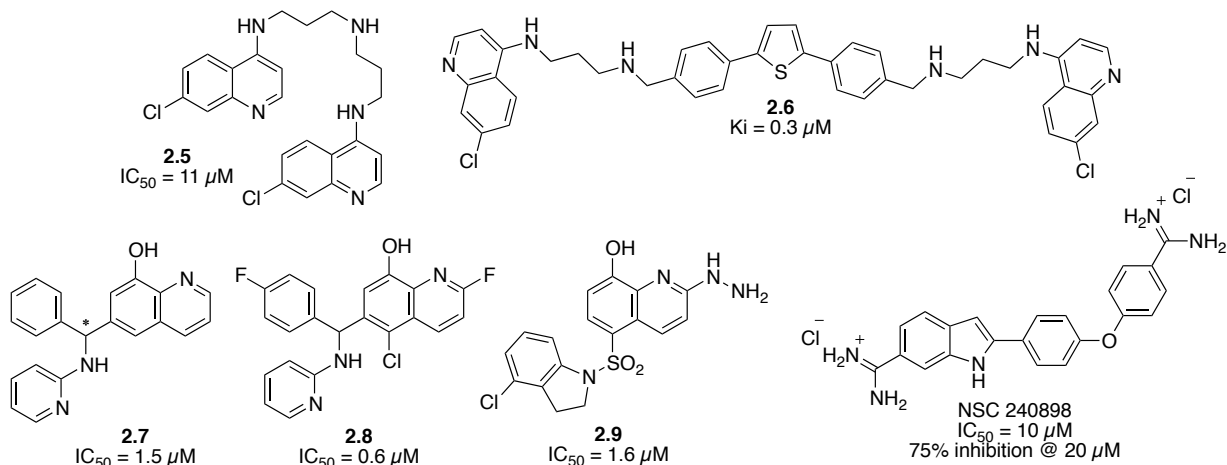
Figure 2.5. Other small molecule non-peptidic inhibitors of BoNT.

2.1.3   Structure-Activity Relationship of NSC 240898: Previous Work in the Wipf Group

Despite the dose-dependent protection of SNAP-25 and low cytotoxicity of NSC 240898, its activity against BoNT was relatively poor (IC_{50} = 10 μM). In a effort to enhance the potency and learn more about the BoNT binding pocket, Wipf and coworkers investigated the SAR around NSC 240898 with structural modifications on the indole, diaryl ether, and polar end groups.[268] The structure was divided into 5 major structural zones for modification (Figure 2.6).
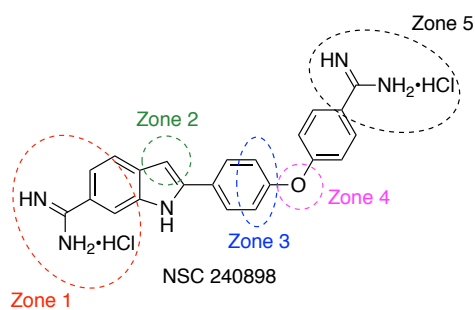
Figure 2.6. Zones for structural modifications of NSC 240898.

The SAR of the NSC 240898 scaffold showed a number of modifications that could be tolerated within the binding site (Table 2.1). Position R^1 in zone 3 tolerated functionalization with -OMe and –Cl, with the resulting analogs achieving similar potency to the parent scaffold NSC 240898. This indicates that there is an available pocket in the binding site that allows minor variations in steric bulk without a loss in potency. The diaryl thioether linkage was found to increase potency, which is most likely due to the smaller bond angle leading to closer proximity of the terminal imidazolines for hydrogen bonding with Glu^{56}. Interestingly, NSC 377363 (Table 2.1, entry 15) with a NH in place of the diaryl ether was equipotent to NSC 240898 demonstrating that both a hydrogen bond donor and hydrogen bond acceptor are tolerated in the binding site. Alternating R^2 and R^3 with amidine (Am) and imidazoline (Im) substituents showed some flexibility in the binding site, but substitution at R_3 was more potent. The terminal substituents R^3 and R^4 could tolerate either the bis-Am or bis-Im, however, neutral (nitriles, CF_3) or acidic functionalities (tetrazoles) were inactive (Table 2.1).[268-269] The basic Am or Im functionality likely interacts through hydrogen bonding within the binding site. Finally, shortening the distance between the two polar contacts or removing a polar contact led to a decrease in potency. Not only did this SAR reveal new electrostatic and steric information about the BoNTA/LC binding site it also led to the identification of compound **2.10** (Table 2.1, entry 13), as a new more potent lead structure.

Table 2.1. Structure-activity relationship of NSC 240898.[268]
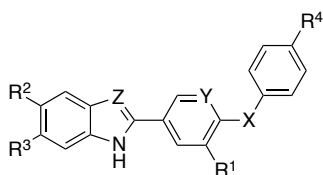
Entry	Structure[b]							% BoNT/A LC inhibition[a]
	X	Y	Z	R^1	R^2	R^3	R^4	
1	O	CH	N	H	H	Am	Am	24.7 (±1.5)
2	O	CH	N	H	H	Im	Im	34.8 (±1.4)
3	O	CH	CH	H	Am	H	Am	58.8 (± 3.3)
4	O	CH	CH	H	Im	H	Im	51.4 (± 4.6)
5	O	CH	CH	H	H	Am	CF_3	25.2 (± 3.4)
6	O	CH	CH	H	H	Im	CF_3	43.3 (± 7.2)
7	O	CH	CH	Cl	H	Am	Am	64.4 (± 2.1)
8	O	CH	CH	Cl	H	Im	Im	64.8 (± 4.4)
9	O	CH	CH	OMe	H	Am	Am	58.0 (± 6.7)
10	O	CH	CH	OMe	H	Im	Im	69.8 (± 1.8)
11	O	N	CH	H	H	Am	Am	52.7 (± 5.5)
12	O	N	CH	H	H	Im	Im	46.1 (± 1.2)
13	S	CH	CH	H	H	Am	Am	80.3 (± 6.7)
14	S	CH	CH	H	H	Im	Im	67.3 (± 3.6)
15	NH	CH	CH	H	H	Am	Am	70
15	O	CH	CH	H	H	CN	CN	NI[c]
16	O	CH	CH	H	H	Tet	Tet	NI[c]
17	O	CH	CH	H	H	Am	Am	73.5 (± 7.5)[d]

[a]inhibition of BoNTA/LC at 20 µM using the assay as previously described.[247,268] [b]Tet, tetrazole; Am, Amidine; Im, Imidazoline; [c]NI, no inhibition; [d]NSC 240898.

2.2   RESULTS AND DISCUSSION

With only a modest improvement in efficacy from NSC 240898, compound **2.10** remained a viable target for the further optimization of efficacy, solubility, and metabolic stability. To further optimize **2.10** we constrained the diaryl thioether into an eight membered ring in an attempt to increase solubility and potency. Additionally we also investigated the 1,2,4-triazine as

a substitution for the imidazoline rings of **2.10** as imidazolines pose a potential metabolic risk through hydrolysis or oxidation.

2.2.1 Synthesis of 3-(4,5-Dihydro-1*H*-imidazol-2-yl)-9-(6-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-indol-2-yl)-6,7-dihydro-5*H*-dibenzo[*b,g*][1,5]thiazocine

The previous SAR of NSC 240898[268] revealed that the diaryl thioether increased activity versus the parent compound due to the decreased bond angle of the aryl rings. It was also noted that steric bulk could be tolerated around the diaryl ether (Table 2.1). Conformationally constrained analogs of **2.10** were proposed to further increase binding affinity, solubility, and drug-likeness.[270] The thiazocinone and thiazocine inhibitors take advantage of the tight thioether bond angle, and further constraint into the 8-membered ring greatly reduces the entropic penalty of binding and potentially enhancing binding site interactions of the polar end caps. These analogs also have additional hydrogen bond donor and acceptor functionality that may lend to increased binding interactions in the active site. The thiazocinone analogs **2.11-2.14** were previously synthesized by Drs. Igor Opsenica and Jared Hammill (Figure 2.7).
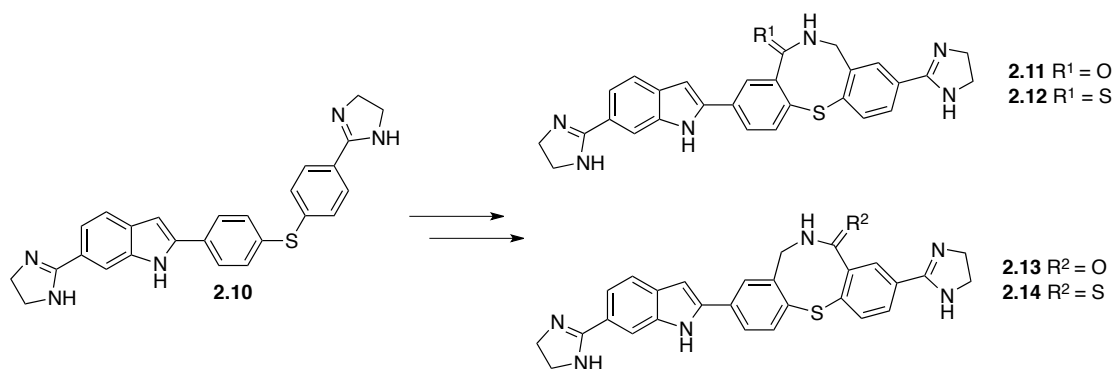
Figure 2.7. Structures of **2.10**, lactam, and thiolactam analogs.

The diaryl bond angle and conformation of the eight-membered ring mimic the binding mode of NSC 240898 (Figure 2.8). Smaller rings produce relatively planar conformations,[271] while larger rings increase steric bulk and are conformationally more labile.

X= O, 117°
X= S, 102°
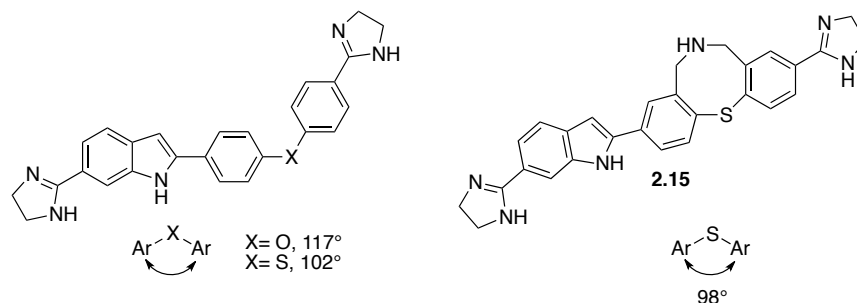
Figure 2.8. Bond angle of diaryl (thio)ether linkage.[272]

The thiazocine variant of **2.10** was the final member of this constrained series to be synthesized. Initially, we envisioned that we could access the thiazocine through a late stage reduction of the lactam **2.17** a common building block in the synthesis of the previous thiazocinone inhibitors **2.11-2.14** (Scheme 2.2).
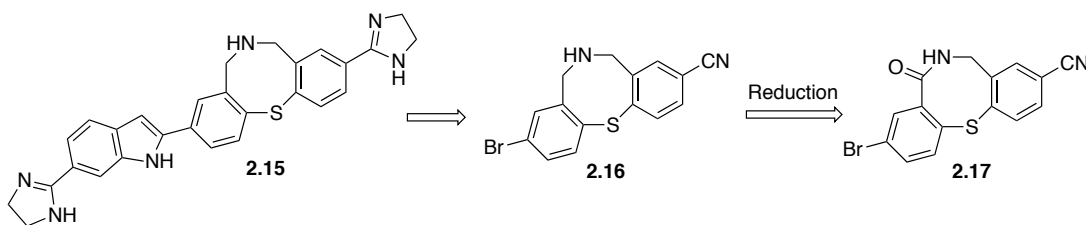
Scheme 2.2. Retrosynthetic approach to common intermediate lactam **2.17**.

The synthesis of the key lactam intermediate **2.17** began with the condensation of thiol **2.19** with fluorobenzonitrile **2.20** to form **2.22**. Thiol **2.19** can easily be prepared by diazotization of aniline **2.18** followed by displacement of the diazo intermediate using potassium *O*-ethyl xanthate.[273] The resulting xanthate ester is hydrolyzed to form the thiol. The carboxylic acid of **2.19** was esterified to afford the methyl ester **2.19**.[274] The aldehyde **2.21** was reduced using sodium borohydride and the resulting alcohol **2.22** was mesylated and displaced with sodium azide. The methyl ester **2.23** was saponified then subjected to a Steglich esterification with

pentafluorophenol (Pfp) to deliver Pfp ester **2.24**. An intramolecular Staudinger ligation was used to prepare the corresponding lactam **2.17** in good yield (Scheme 2.3). Attempts to reduce the lactam using transition metal catalysis with silanes, Hantzsch ester hydride reductions, borohydride reductions, and borane methyl sulfide reductions were unsuccessful and yielded complex mixtures of products, dehalogenation, or overreduction.[275-277] Using zinc triflate and TMDS afforded the product, but reaction mixtures were very difficult to purify due to the solubility and polarity of the free amine **2.16**.[278]
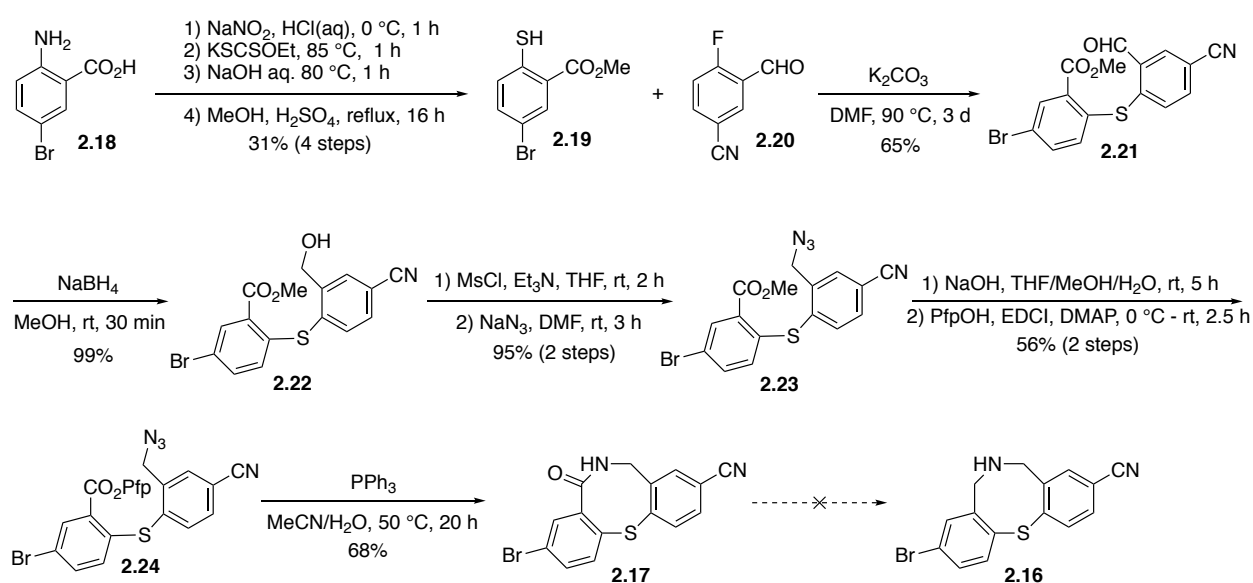
Scheme 2.3. Synthesis of thiazocinone **2.17**.

Due to the difficulty of reduction of the amide **2.17**, the thioamide **2.25** was pursued as a synthetic target (Scheme 2.4). Thioamides can be reduced with the use of mild reducing agents like Raney-Ni,[279] or via *S*-alkylation and reduction using metal borohydrides.[280] Unfortunately, forming the thioamide was unsuccessful using a number of precedented methods, including Lawesson's and Belleau's reagents or P_4S_{10}.[281-282]
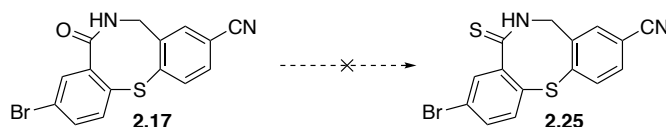
Scheme 2.4. Attempted synthesis of thiolactam **2.25**.

Dibenzo[*b*,*g*][1,5]thiazocines such as the desired intermediate **2.16** have been previously constructed by two main routes: the reduction of the corresponding lactam using LAH[283-284] or via an alkylation approach.[285-288] Since LAH reductions were not tolerated on this system due to the presence of the nitrile, an alkylation approach was explored. It has been shown that medium sized rings could be formed through a Mitsunobu reaction using an *N*-4-nitrophenylsulfonyl (nosyl, Ns) protected amino alcohol.[288]

Initially, we synthesized the *N*-nosyl thiazocine precursor **2.27** to determine if the Mitsunobu cyclization would be successful on our substrate. The synthesis of **2.28** began from aldehyde **2.21** (Scheme 2.5). Attempts at a classical reductive amination using STAB were unsuccessful due to slow imine formation.[289] Preforming the imine by $CeCl_3$,[290] $TiCl_4$,[291] or Bronsted acid catalysis[292] was also unsuccessful. Gratifyingly, the formation of *N*-nosyl protected amine **2.26** proved successful when aldehyde **2.21** was subjected to a titanium(IV)isopropoxide mediated condensation with *p*-nitrobenzenesulfonamide, followed by sodium borohydride reduction. The ester **2.26** was then further reduced to the amino alcohol **2.27**. Unfortunately, neither the Mitsunobu or PBr_3 cyclization of compound **2.27** were successful in obtaining the *N*-nosyl thiazocine **2.28**. This was most likely due to the fact that our system is more strained than what had been previously used by Kan et al. for the Mitsunobu cyclization[288] and the sulfonamide was not nucleophilic enough to displace the benzylic bromide formed under the PBr_3 conditions.
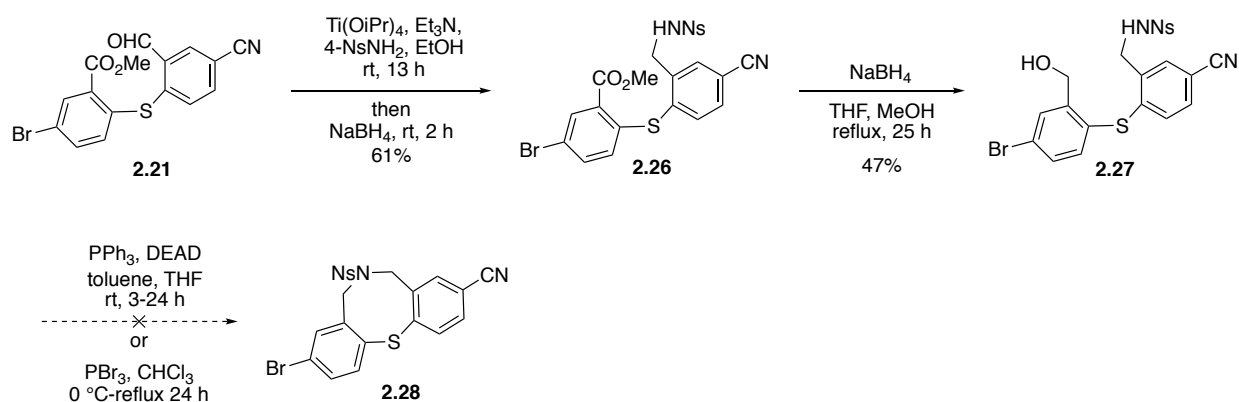
Scheme 2.5. Synthetic approach to *N*-nosyl thiazocine **2.28**.

To affect a cyclization, the protected amine was modified to a *p*-methoxybenzylamine, which is more nucleophilic and should be readily removed at the end of the synthesis. Combining the aldehyde **2.21** and *p*-methoxybenzylamine with titanium(IV)isopropoxide and an excess of sodium borohydride, delivered amino alcohol **2.29** in a one-pot reaction, albeit in modest yield (Scheme 2.6). Sequential reductions of the unreacted ester of **2.29** to the amino alcohol **2.29** were low yielding. In attempts to form the thiazocine ring **2.30**, only PBr$_3$[287] was successful. The literature PBr$_3$ conditions[287] using chloroform as a solvent afforded clean product on small scale, however, our large scale reactions were plagued by a chloroform adduct side product. Gratifyingly, when the solvent was changed to THF at a concentration of 10 mM, the cyclization proceeded in good yield. The thiazocine core **2.30** was coupled to indole stannane **2.31** via a Stille cross coupling to afford **2.32**.[293] The temperature of the coupling proved critical to the reaction yield as Boc removal could be observed by NMR analysis at 100 °C. Indole stannane **2.31** was prepared by a Boc-protection of 6-cyanoindole followed by metalation of the 2-position and quenching with trimethyltin chloride.
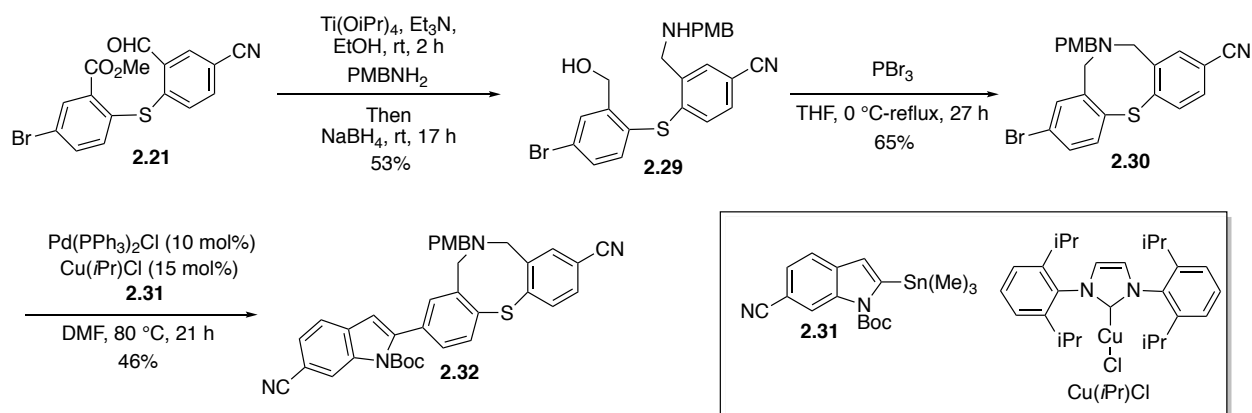
Scheme 2.6. Synthesis of coupling product **2.32**.

Following the successful synthesis of the coupling product **2.32**, PMB removal conditions were explored. The PMB protecting group removal by conventional methods proved unsuccessful. Catalytic hydrogenation[294] only returned starting material, while acidic conditions such as TFA or HCl led to decomposition and facilitated hydrolysis of the nitriles. Oxidative conditions using DDQ led to PMB deprotection with undesired oxidation of the thioether. TMSI[295] and TBSOTf provided an excellent method for Boc deprotection; however, the PMB group remained intact. Chlorosulfonyl isocyanate (CSI) provided the bis-deprotection product along with a number of other undesired products. Instead, the PMB protecting group was removed using α-chloroethylchloroformate (ACECl) to afford the PMB-deprotected thiazocine in good yield.[296] Boc deprotection of the indole was accomplished by heating in 4 M HCl in dioxane at 80 °C due to the insolubility of the compound in THF or 1,4-dioxane at rt. The HCl salt **2.33** was used for the formation of the imidazolines **2.15** using elemental sulfur, ethylene diamine, and microwave irradiation.[297] Thiazocine **2.15** was obtained as a bis TFA salt, and was submitted to the USAMRIID for biological testing (Scheme 2.7).
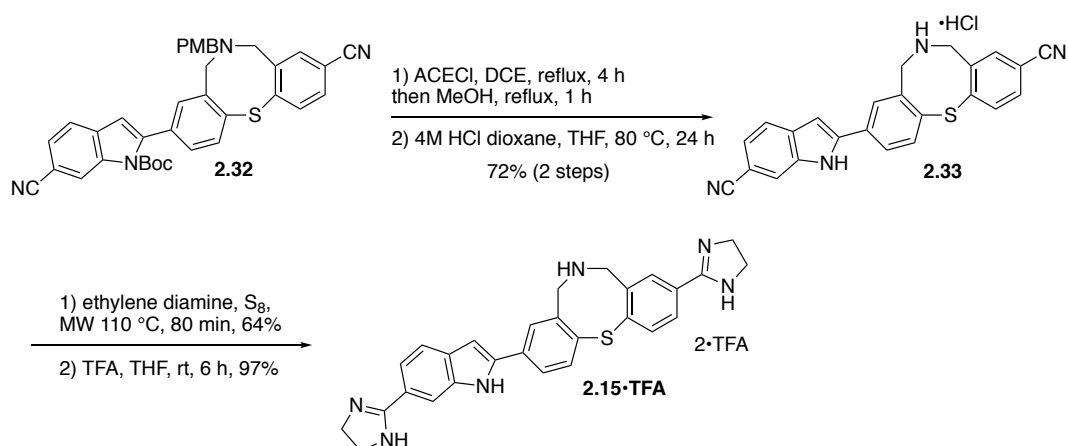
Scheme 2.7. Completion of the synthesis of **2.15•TFA**.

2.2.2 Biological Activity of Thiazocine Analogs

Following the completion of the synthesis of **2.15•TFA**, a sample was tested for BoNT inhibition using a variant of the SNAPtide HPLC assay at the USAMRIID. We discovered that the activity of **2.15•TFA** was very similar to **2.10**, further confirming that we are able to introduce steric bulk without significant penalties to the activity (Table 2.2).[268] However, this also indicates that **2.15•TFA** has not gained any favorable binding interactions within the large binding pocket of BoNT/LC. Additionally, it is possible that introduction of the thiazocine may have perturbed the imidazoline orientation in the binding site leading to a less favorable interactions that cause a slight decrease in activity.

Table 2.2. Biological activity of NSC 240898 analogs.

Entry	Compound	BoNT IC$_{50}$[a] (μM)
1	**2.10**	5.2 ± 0.1
2	**2.15•TFA**	6.3 ± 2.9
3	**NSC 240898**	10.7 ± 0.5

[a]IC$_{50}$'s were determined using the previously described SNAPtide HPLC assay.[242] Data were collected using duplicate data points over two independent assays.

2.2.3   Synthesis of 1,2,5,6-Tetrahydro-1,2,4-triazines

In addition to replacing the diarylthioether in **2.10** with a thiazocine ring to generate compound **2.15** we wanted to substitute the imidazoline rings as they are prone to hydrolysis and metabolic oxidation (Figure 2.9). Based on a previous SAR study, the bis-imidazole analogs were found to be inactive because they tend to have other binding modes to chelate Zn.[246] 1,2,5,6-Tetrahydro-1,2,4-triazines (1,2,4-triazines) represented an attractive alternative to the imidazoline functionality as they maintain a similar pKa while displaying increased hydrolytic stability.[298-300] Thus, a new analog containing terminal bis-1,2,4-triazine moieties should retain the potency of the parent compound without being rendered inactive by metabolic oxidation, as even the oxidation product has a similar pKa to the parent compound.(Figure 2.9).
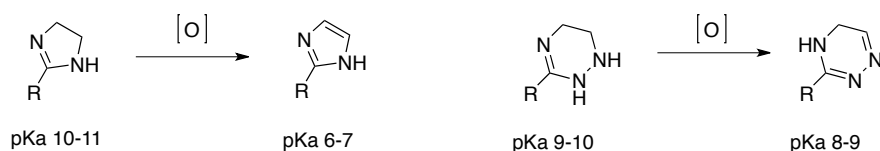
Figure 2.9. Effect of oxidation on pKa of imidazolines vs 1,2,4-triazines.

The Wipf group has been interested in constructing 1,2,4-triazines for many years as they are a key structural component of the furanosteroid natural product noelaquinone (Figure 2.10).[301-302] Wipf and coworkers' previous synthesis the DEF fragment of noelaquinone resulted in the development of a new method to form 1,2,4-triazines through an intramolecular Staudinger *aza*-Wittig (SAW) reaction.[303] The SAW reaction is a useful tool for the synthesis of heterocyclic compounds and natural products.[304] It combines the Staudinger phosphazine ylide formation with the *aza*-Wittig addition to a carbonyl group.[305-309]
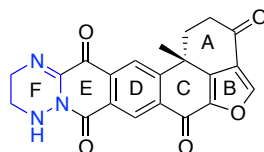
Figure 2.10. Structure of noelaquinone.

Initial routes to form the 1,2,4-triazines in the Wipf group were pioneered by former students Dhezi Fu and David Amantini. These linear approaches began with a reductive amination of aldehyde **2.35** on various aromatic hydrazides **2.34a-2.34d**. Benzylation and TBS deprotection afforded alcohols **2.38a-2.38d**. A subsequent mesylation, azidation, and SAW cyclization afforded the triazines **2.40a-2.40d** in good yields (Scheme 2.8).
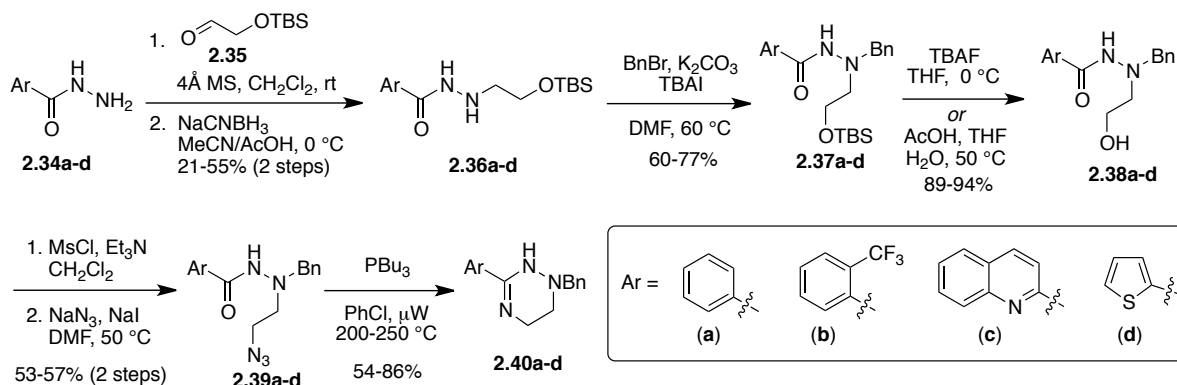
Scheme 2.8. Linear synthesis of 1,2,4-triazines.

Fu and Amantini also explored the deprotection of the benzyl group on **2.40a** and found that catalytic hydrogen conditions over 4 days the product was obtained in modest yields without reduction of the C=N bond (Scheme 2.9).
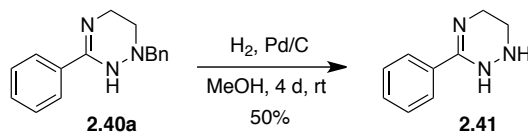
Scheme 2.9. Debenzylation of triazine **2.40a**.

A more convergent approach was developed by converting the hydrazine to the stable hydrazone, which can be unmasked prior to coupling with carboxylic acid or acid chloride

moieties.[303] The synthesis of hydrazones **2.45a** and **2.45b** began with the condensation of

hydrazinoethanol (**2.43**) onto ethyl pyruvate (**2.42**) to provide the hydrazone **2.44** (Scheme 2.10).

The alcohol **2.44** was mesylated and displaced using sodium azide. The corresponding

azidohydrazone **2.45** was protected with BnBr or PMBBr to afford the shelf-stable

azidohydrazine precursors **2.46a** and **2.46b** respectively. The pyruvate hydrazone **2.46b** was

previously prepared on 45 g scale and found to be bench stable for months with no noticeable

decomposition according to ^{1}H NMR.
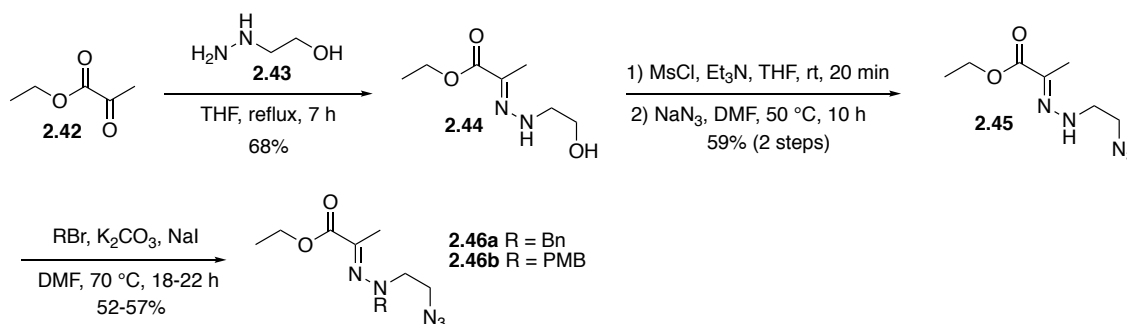
Scheme 2.10. Synthesis of hydrazones **2.46a** and **2.46b**.

Hydrazine exchange of hydrazones **2.46a-2.46b** afforded the desired *N*(2)-protected

hydrazines **2.47a-2.47b** in good yields. Using peptide coupling conditions or the acyl chloride,

hydrazides can be formed in varying yields. The corresponding hydrazide can then undergo

cyclization via a SAW reaction to form the 1,2,4-triazine (Scheme 2.11).
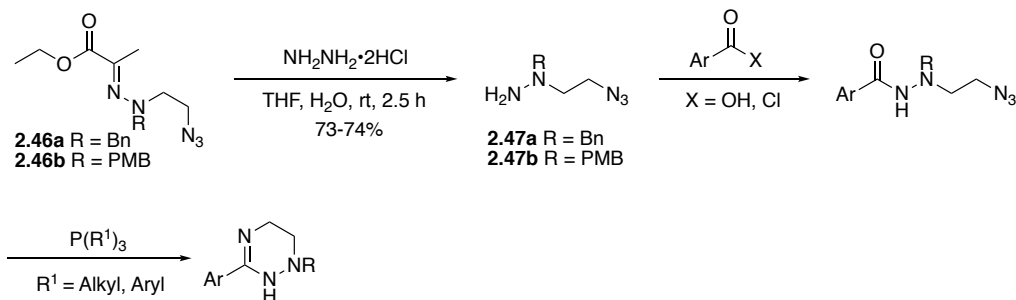
Scheme 2.11. Synthetic route to 1,2,4-triazines.

2.2.3.1 Screening and Optimization of 1,2,4-Triazines

To simulate the formation of the 1,2,4-triazine on a more complex system and to further expand the substrate scope we synthesized several new triazine systems. In addition to expanding the substrate scope we also developed a new hydrazine with a more labile protecting group to allow for milder deprotection conditions.

We synthesized a novel 3,4-dimethoxybenzyl protected hydrazine **2.47c** that should be more acid-labile than the corresponding PMB-protected derivative. In a similar synthetic sequence to previous hydrazines we arrived at azide intermediate **2.45**. Alkylation with 3,4-dimethoxybenzyl chloride afforded hydrazone **2.46c** in modest yields. Deprotection using hydrazine afforded our desired hydrazine **2.47c** in good yields (Scheme 2.12).
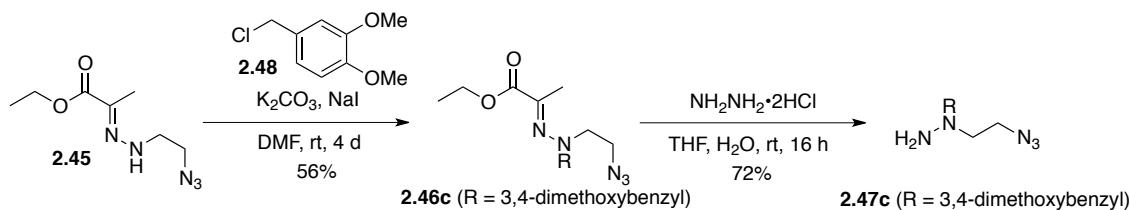
Scheme 2.12. Synthesis of 3,4-dimethoxybenzyl protected hydrazine **2.47c**.

Since low molecular weight compounds with a high N to C ratio are potentially explosive,[310] we also determined the thermal stability of azidohydrazines **2.47b** and **2.47c** by differential scanning calorimetry (DSC) to establish the safety profile of these compounds. Gratifyingly, we found that compounds **2.47b** and **2.47c** were stable up to 150 °C and did not undergo complete decomposition until >200 °C.

Azidohydrazine **2.47b** was acylated directly via the acyl chloride or by peptide coupling conditions with the aromatic carboxylic acid. The corresponding hydrazides **2.50a-2.50c** were subjected to the SAW conditions to afford the *N*-protected 1,2,4-triazines **2.51a-2.51c** in this convergent synthesis (Table 2.3). While the SAW reaction generally proceeded with acceptable

to good yields using *tri-n*-butyl phosphine, we used the conversion of **2.50a** to **2.51a** to explore

the scope of the variation of phosphines, solvents, temperature, and reaction times (Table 2.3). A

solvent switch to 1,2-dichlorobenzene provided similar yields to chlorobenzene. Using aromatic

phosphines such as P(Ph)$_3$ or PMe(Ph)$_2$ afforded the product in decreased yield and were difficult

to separate from the corresponding phosphine oxides via chromatography. Finally, using P(Me)$_3$,

the SAW reaction provided modest yields but the corresponding water soluble phosphine oxide

was readily separable from the triazine product.

Table 2.3. Screening of conditions for SAW cyclization.
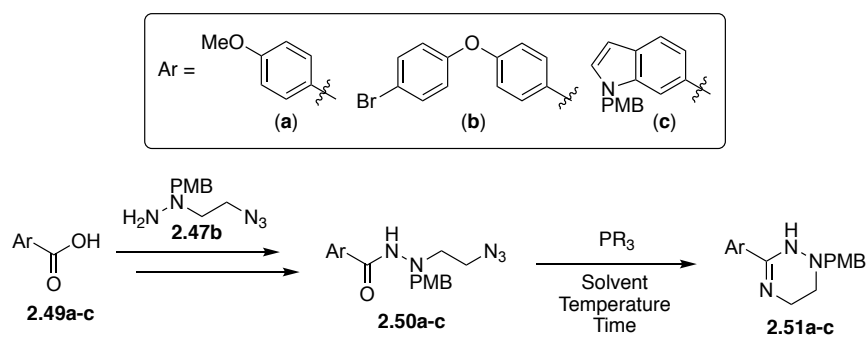
Entry	PR$_3$	Solvent	Temperature/time[c]	Yield
1	P(*n*-Bu)$_3$	*o*-1,2-dichlorobenzene	MW, 180 °C, 1 h	47%
2	P(*n*-Bu)$_3$	PhCl	MW, 180 °C, 1.5 h	41%
3	P(*n*-Bu)$_3$	PhCl	MW, 180 °C, 2 h	50%
4	P(Ph)$_3$	PhCl	MW, 180 °C, 1 h	42%[a]
5	P(Ph)$_3$	PhCl	MW, 180 °C, 1.5 h	38%[a]
6	P(Ph)$_3$	PhCl	150 °C, 12 h	dec.
7	PMe(Ph)$_2$	PhCl	MW, 180 °C, 1.5 h	37%[b]
8	PMe(Ph)$_2$	PhCl	MW, 180 °C, 2 h	42%[b]
9	P(Me)$_3$	PhCl	MW, 180 °C, 1 h	38%
10	P(Me)$_3$	PhCl	MW, 180 °C, 1.5 h	29%; dec.

[a]Product contains P(Ph)$_3$O; [b]product contains PMe(Ph)$_2$O; [c]all reactions used a 50 mg scale of **2.50a**

With the triazine substrates in hand, we investigated the removal of the PMB and 3,4-

DMB protecting group. The PMB proved difficult to remove. The use of reductive conditions

(H$_2$, Pd/C) returned only starting material, oxidative conditions (CAN) led to deprotection and partial oxidation of the triazine, and strongly acidic conditions (TfOH/TFA/anisole) led to decomposition. Finally, the PMB deprotection of **2.51b** was accomplished using neat TFA at 55 °C for 30 min, followed by a basic aqueous work up, to afford the deprotected triazine **2.52•TFA** in good yields (Scheme 2.13). For the deprotection of the 3,4-dimethoxybenzyl analog **2.53**, we discovered that 4 M HCl in dioxane at 50 °C for 2.5 days afforded the product **2.52** in modest yields after column chromatography.
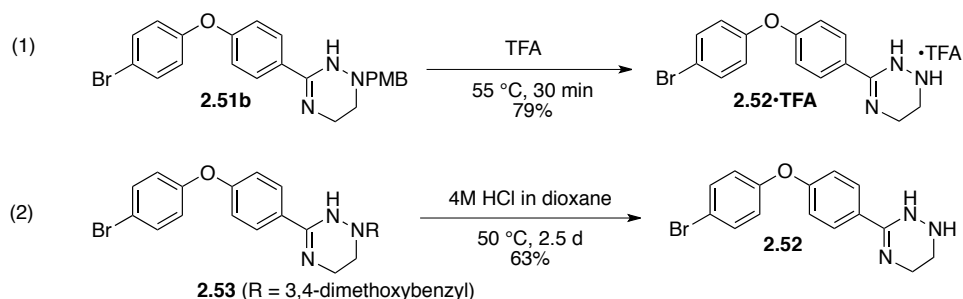
Scheme 2.13. Deprotection of PMB and 3,4-dimethoxybenzyl protecting groups.

2.2.3.2 Efforts Towards the Synthesis of 6-(1,2,5,6-Tetrahydro-1,2,4-triazin-3-yl)-2-(4-(4-(1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)phenoxy)phenyl)-1*H*-indole

To test our hypothesis that these 1,2,4-triazines could be used as isosteres of the metabolically labile imidazoline moiety, we proposed to synthesize an analog of **2.10** that contained the 1,2,4-triazines in place of the imidazolines. Our retrosynthetic approach to compound **2.54** features a double SAW cyclization of diazide **2.55** to form the bis-triazine rings of **2.54** (Scheme 2.14). We proposed that amide coupling of the diacid to our hydrazine building blocks **2.47a-2.47c** would give our hydrazide **2.55**. The diacid precursor can be formed via a Stille coupling of stannane **2.31** and aryl bromide **2.57**.
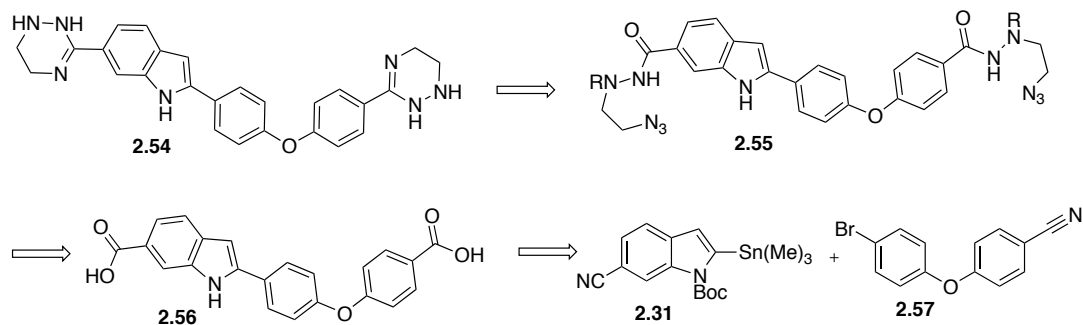
Scheme 2.14. Retrosynthetic approach to **2.54**.

The synthesis of the bis-1,2,4-triazine analog **2.54** began with the condensation of 4-bromophenol (**2.58**) and 4-fluorobenzonitrile (**2.59**) to form the diarylether **2.57**. The indole stannane **2.31** was installed via a Stille cross coupling to give nitrile **2.60** that was subsequently hydrolyzed over 11 days to afford the Boc-deprotected diacid **2.56** in good yields (Scheme 2.15).
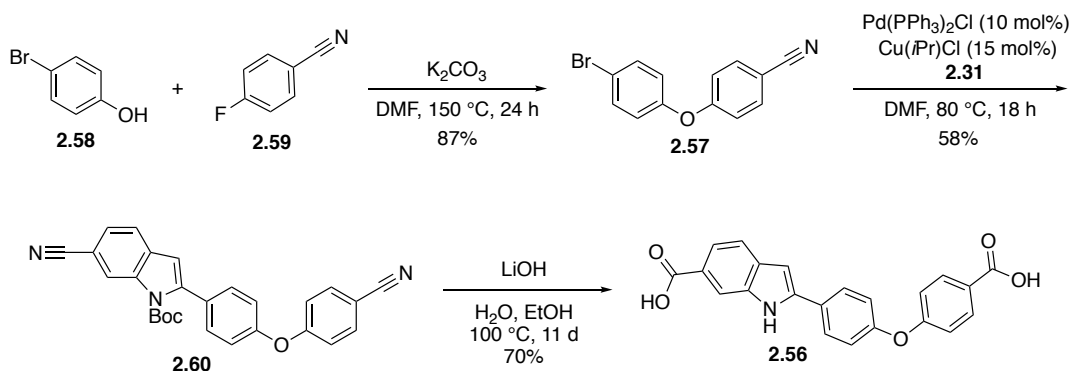
Scheme 2.15. Synthesis of diacid **2.56**.

Diacid **2.56** was coupled to the freshly deprotected hydrazines **2.47a-2.47c** using HATU/HOAt to provide the bis-hydrazides **2.61a-2.61c** in modest yields (Scheme 2.16).[311] Previous attempts to couple the hydrazine via the acid chloride, or by activation with EDCI/HOBt, DEPC, DPPA, or PyBrOP were unsuccessful, very low yielding, or gave complex mixtures of products. The bishydrazides **2.61a-2.61c** were subjected to the original SAW reaction conditions (PhCl, PBu$_3$), but the reaction was unsuccessful. Although in the model system it was found that either PMe$_3$ or PBu$_3$ gave the best yield and, in the case of PMe$_3$, the

phosphine oxide was easier to remove (Table 2.3), the conditions didn't translate to the synthesis of **2.54**. When PMe$_3$ (1 M in toluene) or P(Bu)$_3$ were used in PhCl, the reaction only afforded trace product. It was found that the solubility of the starting material/bisphosphazine ylide in PhCl/toluene was poor and the compound would precipitate out of solution during the reaction. We found that by using PMe$_3$ as a 1 M solution in THF, the bis-1,2,4-triazines **2.62a-2.62c** were formed in low yields. PMe$_3$/PhCl/THF provided the best results for the SAW cyclization as it kept the phosphazine ylide in solution during the reaction. At this point, the remaining step involved the removal of the protecting groups. Attempts at removing the Bn protecting groups on **2.62a** through hydrogenation were unsuccessful even after 1 week with 50 mol% Pd/C. Unfortunately, the deprotection conditions previously identified for the removal of the PMB protecting group used on **2.62b** afforded the deprotected product **2.54** as a 1:1:1 mixture with the monodeprotected products (Scheme 2.16). Attempts to resubject the mixtures or drive the reactions to completion were unsuccessful, as the reactions seem to stop at a mixture of partial deprotection or lead to decomposition. We propose that, based on the lack of reactivity and product mass distribution, the indole was scavenging the cation intermediates formed in the deprotection. Using cation scavengers (Et$_3$SiH, anisole, and thioanisole) did not appear to improve the ratio of products. The 3,4-dimethoxybenzyl protected compound **2.62c** also proved resistant to deprotection conditions.
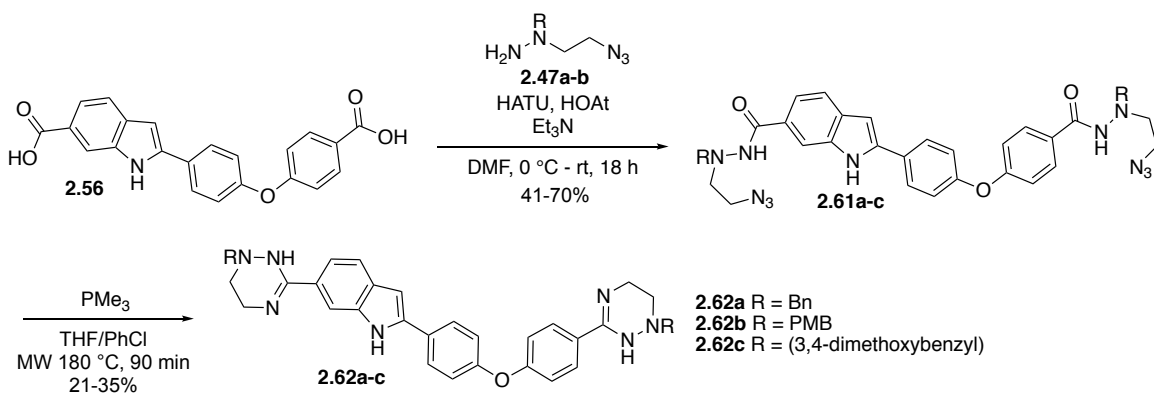
Scheme 2.16. Synthesis of protected bis-1,2,4-triazines **2.62a-2.62c**.

Although the deprotection of the 1,2,4-triazines was unsuccessful, possible alternatives to the benzyl protecting groups include the 2-(trimethylsilyl)ethoxymethyl (SEM) or the 2,3,6-trimethyl-4-methoxybenzenesulfonamide (Mtr) protecting groups. The SEM protecting group is a viable option as it can be deprotected using TBAF[312-315] or Lewis and Bronsted acids,[316-318] and is easily installed using SEMCl. The Mtr protecting group has been used in peptide synthesis for the protection of the ε-amino function of lysine using Mtr-Cl and is readily cleaved using either (0.15 M) methane sulfonic acid in a mixture of TFA and thioanisole[319] at room temperature in 2 h or 1 M HBF$_4$ in TFA at room temperature for 20 min.[320-321] The Mtr functionality has also been shown to be stable to Staudinger ligation conditions and is lipophilic, and therefore may aid to increase solubility during the SAW cyclization.[322]

2.3   CONCLUSIONS

In summary, we have successfully synthesized the final analog (**2.15•TFA**) of the thiazocine-based inhibitor series (Figure 2.7). Initial attempts to use the common thiazocinone intermediate **2.17** from the synthesis of the previous analogs were unsuccessful (Scheme 2.2). Therefore, we

developed a novel procedure to form the thiazocine through an activated ring closure using a PMB protected amine (Scheme 2.6). This analog will provide additional evidence for the hypothesis that constraining the diarylthioether will provide more favorable hydrogen bonding interactions within the binding site of BoNT/A LC.

Additionally, the first bis-triazine was synthesized in an attempt to use 1,2,4-triazine as isosteres of cyclic amidines. To date we have been unsuccessful in the deprotection of **2.62** after many attempts to remove the Bn, PMB, and 3,4-dimethoxybenzyl protecting groups. In addition to our work on the bis-triazine analog, we have further developed the scope of a convergent method for the synthesis of 1,2,4-triazines using newly developed azidohydrazine reagents. Hydrazines **2.47b** and **2.47c** were also shown to be thermally stable up to 150 °C by DSC analysis.

3.0 SYNTHESIS OF ANTAGONISTS OF THE NUCLEAR ANDROGEN RECEPTOR FOR THE TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER

3.1 INTRODUCTION

The androgen receptor (AR) is a 110-kDa ligand-inducible transcription factor that is a member of the nuclear steroid receptor family.[323] AR is responsible for normal prostate function, and plays a key role in the progression of prostate cancer (PCa). In humans, the AR gene is located on the q-arm of the X-chromosome and is comprised of eight coding exons (Figure 3.1).[324] Genetic disorders associated with AR include androgen-insensitivity syndrome, spinal and bulbar muscular atrophy (SBMA), hypogonadism, benign prostatic hyperplasia (BPH), and PCa.[325] PCa is the third deadliest cancer in men with 161,000 new cases and 27,000 deaths expected in 2017, and is typically diagnosed through measurement of prostate-specific antigen (PSA) in the blood serum, rectal examination, or biopsy.[326] PSA in blood serum is an indication that the prostatic epithelium has been disrupted as a result of hyperplasia of the prostate. Unfortunately, PSA levels in serum can also be the result of BPH, infection, or chronic inflammation. New PCa specific biomarkers are under investigation to reduce false positives associated with current detection techniques.[327-331]

The AR structure consists of four functional domains: the N-terminal domain (NTD), DNA binding domain (DBD), hinge region, and ligand-binding domain (LBD).[332-333] The NTD

is the largest and least conserved domain in AR that contains activation function-1 (AF-1), transcription activation units (TAU) 1 and 5, and contains polyglutamine and polyglycine repeats that are involved in regulating AR transcriptional activity by influencing the interaction with other receptor regions and altering the tertiary structure of the receptor.[334-335] The DBD is highly conserved and contains two zinc fingers that are responsible for DNA recognition, binding, and stabilization.[333,336] The nuclear localizing sequence (NLS) is a short overlapping segment between the DBD and the hinge region that functions in nuclear translocation of the androgen-activated AR.[337] The hinge region modulates the conformational changes associated with androgen activation and contains a site for phosphorylation.[338] The LBD binds to androgens, heat shock proteins (HSP's), and transcriptional co-activators and also contains the activation function-2 (AF-2) that stabilizes AR allowing the NTD to interact with co-regulators (Figure 3.1).[333,339]
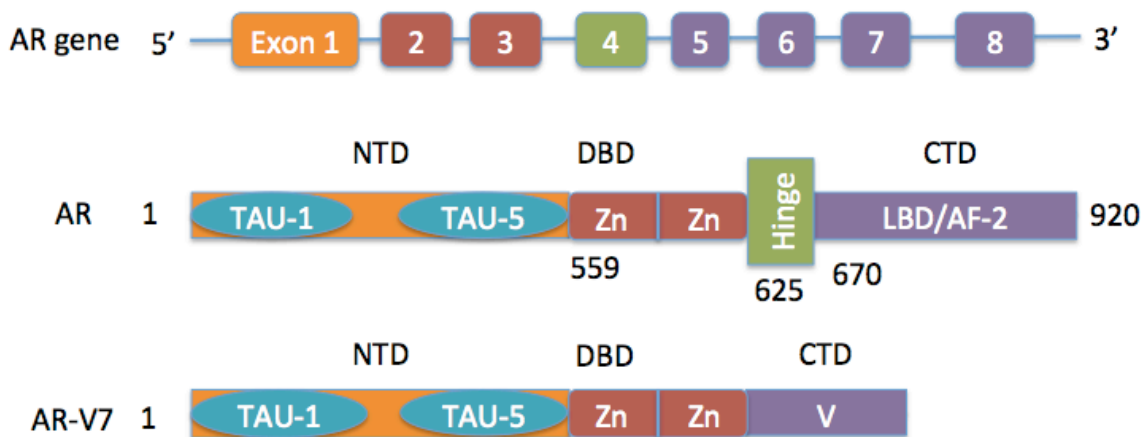
Figure 3.1. AR gene and functional domains.[323,325,340-341]

Normal transcriptional activity of AR involves the binding of androgens to the LBD and translocation of AR to the nucleus for transcription. In the absence of ligands, the LBD localizes AR to the cytosol through binding to HSPs (HSP 90, HSP 70, HSP 56, and p23) that maintain its inactive form.[323,342-343] The androgen testosterone is produced by the testes (ca. 90-95%) with a

46

small portion produced by the adrenal gland (ca. 5-10%), which is transported to AR-positive cells by sex hormone binding globulin (SHBG) and is subsequently reduced to DHT by 5α-reductase. When DHT binds to the LBD, the HSPs are displaced. This ligand-bound AR undergoes homo-dimerization and phosphorylation that results in a conformational change, exposing the NLS, which then undergoes translocation to the nucleus.[343] The nuclear translocated AR-dimer then binds to androgen response elements (AREs), short DNA sequences in the promoter or enhancer regions of target genes, as well as non-ARE associated upstream promoters of genes.[344-345] The AR-ARE complex then binds to co-activators and co-repressors to form the co-regulator complex that can either up- or downregulate transcription (Figure 3.1).[346] Dysregulation of these signaling pathways has been tied to the progression of PCa.
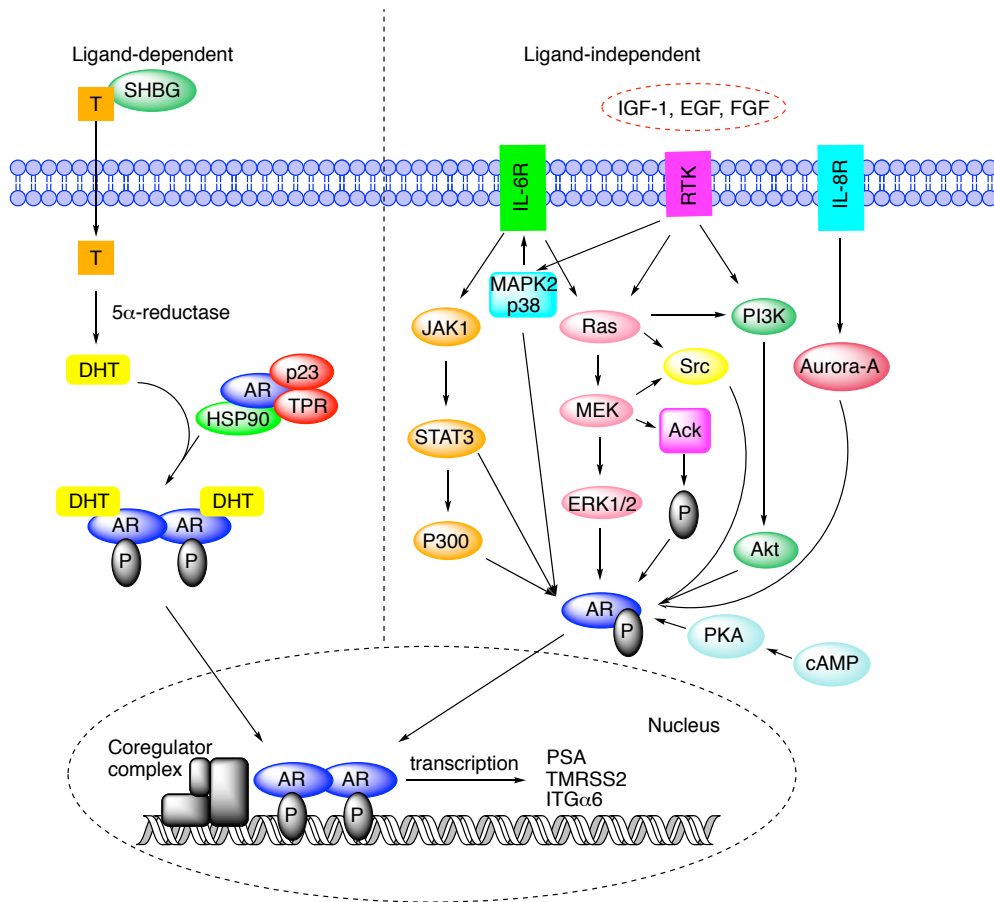
Figure 3.2. Signaling pathways of AR.[323,325,340,343]

At prognosis, 80-90% of PCa are androgen dependent therefore initial therapies of PCa focus on androgen deprivation therapy (ADT) a treatment that was first developed in 1941 by Huggins and Hodges.[347] ADT is achieved using luteinizing hormone-releasing hormone (LHRH) agonists in combination with antiandrogens and is accompanied by a number of side effects.[348] Unfortunately, AR mutations occur causing androgen insensitivity and independence that result in PCa advancing to its lethal stage of castration-resistant prostate cancer, CRPC (formerly known as hormone-refractory prostate cancer).[323,349] The mechanism of CRPC progression is associated with several androgen-dependent and androgen-independent signaling pathways of the AR.[350] Therefore, blocking AR signaling is an attractive therapeutic target for the treatment of CRPC.

AR overexpression has been observed in CRPC cell lines as a result of increased mRNA transcription or AR gene amplification.[351-352] Androgen insensitivity is thought to develop as a result of ADT, where mutations in the AR LBD will allow for more promiscuous ligand binding resulting in activation by other endogenous hormones (estrogen, progesterone, cortisone) and even antiandrogens such as flutamide.[333,353] Mutations in the NTD and DBD could influence nuclear localization, co-regulator binding affinity, and transcription.[354] Co-regulators of AR are proteins that activate (co-activators) or repress (co-repressors) the transcriptional activity of AR. Overexpression of the co-activators SRC-1 and TIF-2 have been associated with the progression of CRPC as they facilitate ligand binding to AR, resulting in increased nuclear concentrations of AR.[355]

De novo androgen synthesis is another mechanism of CRPC progression. Interestingly, testosterone levels in CRPC tumor cells are four times higher compared to primary tumors even with low serum testosterone levels caused by ADT.[323] This is a result of gene upregulation in the

testosterone biosynthetic pathway that leads to testosterone synthesis from cholesterol, and the adrenal androgen dehydroepiandrosterone (DHEA).[356-360]

AR splice variants (AR-V's) emerge from insertions or deletions of exons and more than 20 AR-V's have been characterized in prostate cancer cell lines.[323] Usually, exons 5 and 7 are deleted by RNA splicing factors producing a transcriptionally active AR that lacks the LBD (Figure 3.1).[361-362] In particular, AR-V7 is the most common AR-V found in CRPC cell lines and in place of a LBD or hinge region contains a short variable amino acid sequence (Figure 3.1).[363] Typically found localized in the nucleus, AR-V7 is important in the regulation of target genes involved in cancer cell proliferation.[363-365] Another splice variant, AR-V3 undergoes SRC phosphorylation of the Y^{534} on the NTD resulting in nuclear translocation.[365] Overexpression of AR-V7 in patients was correlated to poor clinical outcome, as patients encountered rapid resistance to current antiandrogens such as enzalutamide.[365-366] Interestingly, AR-V's are found in CRPC cell lines that also express full length AR, and typical treatments of AR were ineffective suggesting that AR-V's can maintain AR transcriptional activity in low androgen environments.[323,362] Additionally, AR-V's can also undergo homodimerization and even heterodimerization with full-length AR, and that dimerization was required for transcription.[367] AR-V's have been identified as markers for hormonal resistance and in combination with monitoring PSA levels can allow for early detection of the progression of CRPC.[368-369]

Ligand independent signaling of AR is also responsible for downstream genomic regulation. Growth factor dysregulation can lead to alterations in downstream pathways such as the PI3K/AKT pathway that leads to phosphorylation of AR, resulting in increased nuclear translocation and transcriptional activity.[12,370-372] Mutations in the receptor tyrosine kinase pathway (HER2) promote cell growth and survival, and have been associated with progression of

CRPC through the activation of Akt.[373-375] In PCa, AR can also activate NF-κB in the absence of androgens, which leads to cell proliferation.[376-377]

Unfortunately, all PCa patients will eventually progress to the incurable lethal CRPC stage in which current treatments are only effective in extending patient life by several months. Therefore, there is an urgent need for new therapeutics for the treatment of CRPC.

3.1.1 Treatments of PCa and CRPC

While ADT is used as a treatment for PCa, it is ineffective towards CRPC. New treatments have been developed mainly focused on using antiandrogen antagonists of AR. These antiandrogens bind to AR and halt its transcriptional activity. Several FDA approved antiandrogens are now available with additional treatments currently in phase II and phase III clinical trials. Structurally, antiandrogens can be divided into two classes: steroidal and nonsteroidal. In addition to antiandrogens, taxanes and radiopharmaceuticals have also been approved for the treatment of CRPC.

Steroidal antiandrogens are competitive inhibitors of androgens (testosterone and DHT) that also reduce plasma levels of androgens and the release of luteinizing hormone.[378] Discovered in 1967, cyproterone acetate (CPA) was one of the first reported steroidal antiandrogens (Figure 3.3).[379] In patients, CPA was less effective (13% increased risk of death) than the nonsteroidal antiandrogen flutamide (8% increased risk of death). In addition to the common side effects of ADT, CPA has also been shown to reduce hormonal flares associated with LHRH agonists when used as a combination therapy.[380-381] Unfortunately, CPA has a very limited effect against CRPC.[382]

Flutamide was the first widely used nonsteroidal antiandrogen. The biologically active form hydroxyl-flutamide is a result of metabolic oxidation and has a half-life of ~5 hours that requires three daily doses (Figure 3.3).[382] Apart from the common side effects of antiandrogens therapies, flutamide has been found to cause severe diarrhea in 10-20% of the patients that ultimately results in the discontinuation of treatment.[383] In addition, flutamide has also been shown to disrupt liver function in a small subset of patients that was resolved with discontinuing treatment.[383] Although flutamide is effective in the treatment of PCa, large doses are required (750 mg/day) and several undesired side effects make it less desirable than current treatments.

Nilutamide was the second most widely used antiandrogen for clinical use and has a half-life of ~38 hours enabling its administration via a once daily dose (Figure 3.3).[382] Similar to flutamide, nilutamide is also heavily metabolized in the liver due to the presence of the nitroaniline functionality, but does not require metabolism to get to its active form as observed with flutamide. It has been used in combination with LHRH agonists to prevent hormonal flares. Several side effects have been noted in patients that have used nilutamide including delayed adaption to dark, alcohol intolerance, pneumonitis, and nausea.[383]

The orally available bicalutamide (Casodex®) was approved by the FDA in 1995 and is one of the current standards of care for the treatment of both non-metastatic and metastatic CRPC (Figure 3.3). Interestingly, bicalutamide is marketed as the racemic drug even though the *S*-enantiomer is rapidly metabolized and cleared while the *R*-enantiomer is significantly more active and metabolically stable in plasma for up to a week.[382] Bicalutamide dosed at 150 mg/day is well tolerated compared to nilutamide and flutamide as common side effects include breast pain, gynaecomastia, loss of libido, and hot flashes.[384]
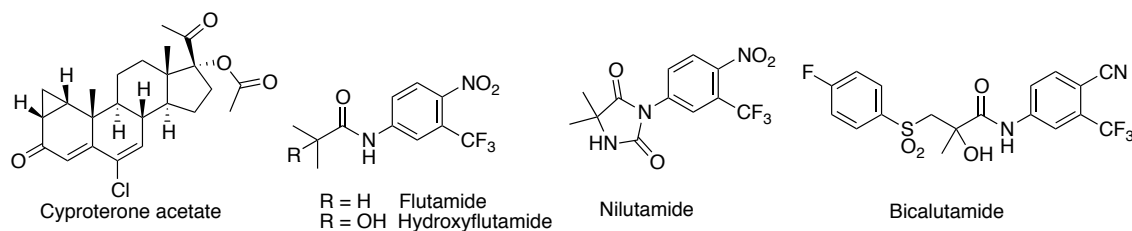
Figure 3.3. 1st generation antiandrogens.

The thiohydantoin enzalutamide (Xtandi®; formerly known as MDV3100) was approved by the FDA in 2012. It is an orally available antagonist of AR with a half-life of 1 week that acts by inhibiting AR nuclear translocation and by reducing the transcriptional activity of AR (Figure 3.4).[385] Patients with CRPC treated with enzalutamide displayed decreased levels of PSA. A recent double-blind study comparing the efficacy and safety of enzalutamide and bicalutamide in both metastatic and non-metastatic CRPC revealed that patients treated with enzalutamide had 19.4 months progression-free survival whereas patients treated with bicalutamide had progression-free survival of only 5.7 months.[386] Side effects of enzalutamide usually include fatigue, nausea, diarrhea, musculoskeletal pain, hot flashes, and headache.[387-388] Enzalutamide also presents a small risk of seizure, as it was shown to inhibit GABA-A currents *in vitro*.[389] Unfortunately, patients with CRPC develop resistance to enzalutamide after 3-5 months.[390]

A more recent thiohydantoin, apalutamide (formerly known as ARN-509) is a new investigational drug currently in phase II and phase III clinical trials for its use in combination with bicalutamide (NCT02531516), abiraterone acetate (NCT02789878), or radiation (NCT02531516) (Figure 3.4). Similar to enzalutamide, apalutamide also inhibits nuclear translocation of AR and reduces transcriptional activity, but has been shown to be more effective *in vivo*.[391] Additionally, apalutamide has shown low CNS penetration and therefore poses less of a risk for seizures than enzalutamide.[389]

Abiraterone acetate inhibits the enzyme CYP17, which blocks the production of androgens, reducing levels to less than 1 ng/dL,[392] and when used in combination with prednisone has been shown to improve progression-free and overall survival by an average of 16 months *vs*. 11 months with only prednisone (Figure 3.4).[393] Side effects of abiraterone acetate include fluid retention, hypokalemia, and hypertension.[394-395] When used in combination with glucocorticoids, cardiac disorders, and abnormal liver function were also observed.[393,395]

The taxanes, docetaxel and carbazitaxel[396] are also FDA approved treatments of CRPC and act through tubulin stabilization and cell cycle arrest (Figure 3.3). Unfortunately, docetaxel suffers from a high affinity to P-glycoprotein (P-gp), which leads to a decrease in intracellular docetaxel concentrations and promotes resistance. Further structural optimization led to carbazitaxel, the dimethoxy analog of docetaxel, which has decreased P-gp affinity and is effective towards docetaxel-resistant tumors.[397] In a recent study, taxanes were also found to be effective in patients with CRPC with detectable AR-V. Levels of PSA in these patients was reduced ~41%.[398]

Radium-223 dichloride (Xofigo®; formerly known as Alpharadin[TM]) was approved by the FDA in 2013 for the treatment of CRPC that has metastasized to the bone by acting as a calcium mimetic.[399-400] Other radiotherapy previously used was external beam radiotherapy for the management of localized PCa.[401]
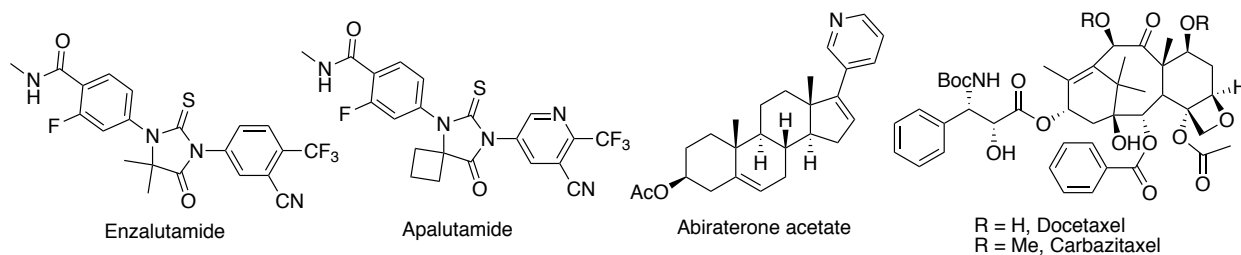
Figure 3.4. 2nd generation treatments of CRPC.

Several new scaffolds have emerged in the literature over the past few years as potential treatments of CRPC. The tropinol **3.1** was found to have stronger antagonistic behavior than bicalutamide with an 87% reduction in luciferase activity at 1 μM (Figure 3.5). In addition to the activity against wild-type AR, compound **3.1** also showed promising antiandrogen activity in the two most common AR mutants.[402] The oxabicyclic compound BMS-641988 was identified from a recent SAR and was found to have high binding affinity to AR (K_i = 1.7 nM) acting as a potent antagonist *in vitro* (IC_{50} = 16 nM). In addition to *in vitro* activity, BMS-641988 was also efficacious in the CWR22-BMSLD1 xenograft model that has shown resistance to bicaluatmide and flutamide. Based on its promising PK/PD profile, BMS-641988 was advanced into clinical development.[403] Efforts to improve the chemical stability of BMS-641988 led to the discovery of the bicyclic sultam **3.2**, which was also found to have high affinity for AR (K_i = 5 nM) and potent antagonist activity *in vitro* (IC_{50} = 57 nM). With a promising PK/PD profile, **3.2** was tested in human tumor xenograft studies where poor oral exposure was observed and was possibly due to high metabolism.[404] Ultimately, this result led to the discontinuation of **3.2**. Physachenolide C was identified from a HTS of a library of natural products and further structural optimization of the 17β-hydroxywithanolides for stability and potency. This analog is capable of reducing PSA expression, has nanomolar potency to both AR-positive and AR-negative cell lines, and was shown to be active in human tumor xenograft models. It is proposed that physachenolide C acts via sensitizing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to induce apoptosis.[405] The chlorohydrin EPI-002 and its analogs have been shown to inhibit AR-transcriptional activity through covalently binding to the AR-NTD (Figure 3.5).[335,406] Using high resolution solution NMR spectroscopy, Salvatella and coworkers have also shown that EPI-002 analogs interact with TAU-5 of the AR-NTD (Figure 3.5).[407] In a VCaP

human tumor xenograft model, EPI-002 was effective in reducing tumor volume.[335] A prodrug of EPI-002, EPI-506 (not shown), is currently in phase (I/II) clinical trials (NCT02606123) for the treatment of patients with metastatic CRPC.[408] The cyanobacteria metabolite sintokamide A (SINT1) has also been found to inhibit AR through binding to the AF-1 region of the NTD. Additionally, SINT1 has been shown to have low cytotoxicity and also inhibit AR-Vs.[409-410] Binding to the AR-NTD represents a novel mechanism of action to treat CRPC that potentially avoids the mechanisms of resistance commonly found with other antiandrogens, such as point mutations in the LBD and splice variants.
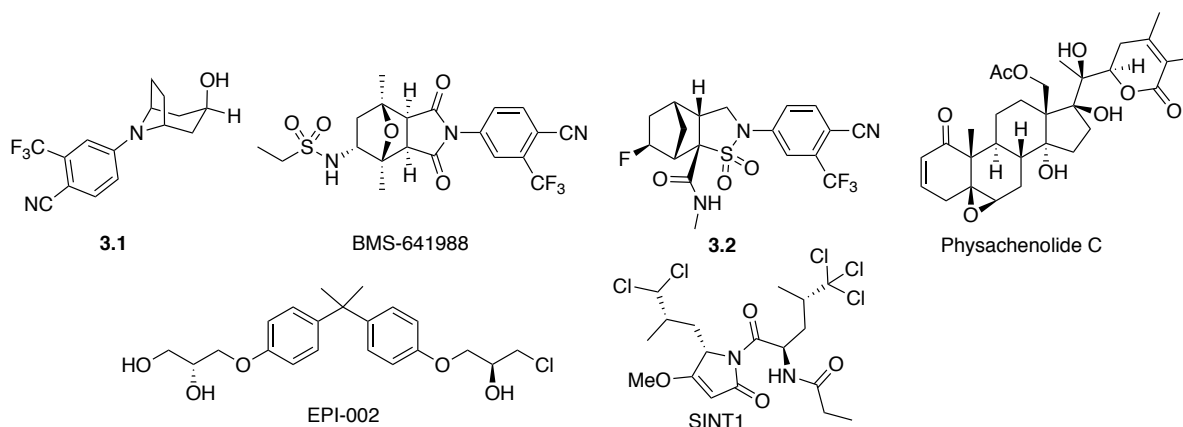
Figure 3.5. Recent inhibitors of the AR.

3.1.2 High Throughput Screen and Structure Activity Relationship: Previous Work in the Wipf Group

Although a number of treatments available treatments for CRPC, they eventually become ineffective as the cancer develops resistance. One therapeutic avenue that has not been developed is to block the nuclear localization of AR, therefore removing transcriptional activity of AR and blocking the progression of the cancer. In an effort to identify a viable treatment of CRPC, Wang and coworkers developed a high throughput screen to identify small molecules that

selectively reduce the nuclear localization of AR in CRPC cells.[411] Using a GFP labeled AR (GFP-AR) transfected into C4-2 CRPC cells and fluorescence visualization, 219,000 compounds from the National Institutes of Health (NIH) molecular libraries screening centers network (MLSCN) were screened at a concentration of 20 μM. After screening and hit validation, 3 compounds were identified that were selective towards GFP-AR with no inhibition of nuclear localization of GFP labeled glucocorticoid receptor (GFP-GR) or GFP labeled estrogen receptor (GFP-ER) and low micromolar potency in C4-2 CRPC cells (Figure 3.6). The structurally similar compounds **3.3** and **3.4** inhibited nuclear localization of GFP-AR in C4-2 cells and localized GFP-AR to the cytosol. Interestingly, compound **3.5** decreased nuclear GFP-AR without increasing cytoplasmic levels of GFP-AR. This indicates an overall downregulation of AR expression and potential inhibition of the activity of AR. Western blot analysis of C4-2 cells treated with **3.5** indicated that AR and PSA protein levels were reduced even at concentrations as low as 2 μM, suggesting that **3.5** can inhibit AR signaling. The three compounds identified from this HTS have structures that are very unique compared to androgens and known AR antagonists and as a result may have a different mechanism of action. These mechanisms could involve direct inhibition of AR or an indirect inhibition targeting AR cofactors.[411] All three HTS hits demonstrated low micromolar potency with low cytotoxicity in AR negative cell lines. However, structural analogs of **3.3** and **3.4** were found to have antifungal activity, thus raising concerns about potential off-target effects.[412] The scaffold of **3.5** did not show any precedence for biological activity and thus was a more attractive target for further investigations. Cell proliferation was inhibited in the AR-positive prostate cancer cell lines LNCAP, C4-2, LAPC4, 22Rv1, and CWR-R1 that were treated with **3.5** at 1 μM and no cell death was observed at 10 mM in AR-negative cell lines. A C4-2 mouse xenograft tumor model treated with **3.5** showed

tumor growth inhibition with no decrease in the body weight in the mice further indicating little to no cytotoxicity.

3.3
IC$_{50}$ = 2.34 μM

3.4
IC$_{50}$ = 4.31 μM

3.5
IC$_{50}$ = 1.79 μM
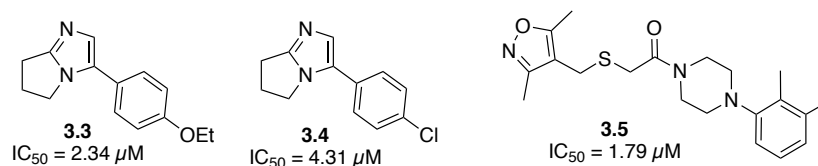
Figure 3.6. Hits identified from HTS.

Based on the promising biological activity and low cytotoxicity of **3.5**, Wipf and coworkers probed the structure-activity relationship of **3.5** by dividing the structure into 5-zones for structural modification: the aryl substitution pattern (zone 1), piperazine modifications (zone 2), carbonyl replacements (zone 3), thioether substitution (zone 4), and variations at the 3,5-dimethylisoxazole (3,5-DMI) ring (zone 5) (Figure 3.7).[349]
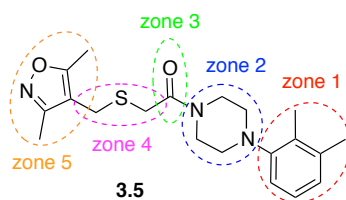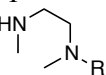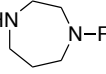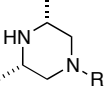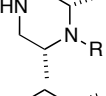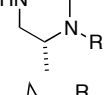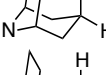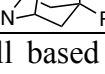
Figure 3.7. Zone of structural modification.

The analogs were biologically characterized by a Dual-Glo luciferase system in the presence of 1 nM of the synthetic androgen R1881 in C4-2-PSA-rl cells and were compared to the initial HTS hit **3.5** (EC$_{50}$ 7.3 μM) and enzalutamide (EC$_{50}$ 1.1 μM)(Table 3.1). Initially, amides **3.6a-p** were synthesized to probe structural changes in zones 1 and 2 to determine the effect of substitution around the aryl and piperazine rings. Small modifications on the aryl ring in zone 1 revealed that monomethyl substitution in the 3- and 4-positions resulted in a loss of activity. The 2-methyl analog **3.6b** had 2-fold loss in activity compared to **3.5**. Removal of the methyl groups resulted in a complete loss in activity. Additional steric bulk was also tolerated where the bulky naphthyl substitution of **3.6g** was also active (EC$_{50}$ 11.1 μM). Electron

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withdrawing aryl substituents (2-CN, **3.6e**, and 2-F, **3.6f**) were also moderately active (EC$_{50}$ ~12 µM), however, the analog with the electron-donating 2-OMe, **3.6h**, was completely inactive, possibly due to an increase in the aniline-nitrogen pKa. As multiple benzylic methyl groups are a potential metabolic liability, the 2-methyl substitution was used for further SAR investigations. Several analogs with more flexible or constrained amine linkers were prepared to probe the SAR around the piperazine in zone 2. The flexible *N,N'*-dimethylethylenediamine linker in **3.6i** and the homopiperazine **3.6j** had decreased activity. The 2,6-dimethylpiperazines **3.6l** and **3.6m** were also 2-fold less active than **3.5**. The more constrained 2,6-dimethylpiperazine analog **3.4k** resulted in increased activity, with a 2-fold improvement on **3.5**. The bridged bicyclic [3.2.1] systems **3.6o** and **3.6p** improved the activity slightly from **3.6b** with EC$_{50}$'s around 8 µM (Table 3.1).

Table 3.1. Structural modifications in zones 1 and 2.
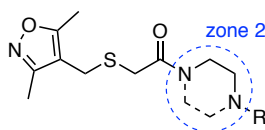
Entry	Compound	Structure Zone 2	R	EC_{50} $(\mu M)^a$
1	**3.5**	Piperazine	(2,3-Me)Ph	7.3 ± 2.5^d
2	**enzalutamide**	-	-	1.1 ± 0.5^e
3	**3.6a**	Piperazine	Ph	$>25^b$
4	**3.6b**	Piperazine	(2-Me)Ph	14.5 ± 3.2^c
5	**3.6c**	Piperazine	(3-Me)Ph	$>25^b$
6	**3.6d**	Piperazine	(4-Me)Ph	$>25^b$
7	**3.6e**	Piperazine	(2-CN)Ph	12.0 ± 1.6^c
8	**3.6f**	Piperazine	(2-F)Ph	12.6 ± 7.7^c
9	**3.6g**	Piperazine	1-naphthyl	11.1 ± 5.3^c
10	**3.6h**	Piperazine	(2-MeO)Ph	$>25^b$
11	**3.6i**		(2-Me)Ph	18.4 ± 9.2^c
12	**3.6j**		(2-Me)Ph	11.1 ± 3.3^b
13	**3.6k**		(2-Me)Ph	3.1 ± 1.1^b
14	**3.6l**		Ph	14.7 ± 4.4^b
15	**3.6m**		(3-Me)Ph	16.6 ± 4.8^c
16	**3.6o**		(2-Me)Ph	7.7 ± 1.6^c
17	**3.6p**		(2-Me)Ph	7.9 ± 2.8^b

^{a}EC$_{50}$'s were determined using a cell based luciferase assay as previously described.[349] EC$_{50}$ refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC$_{50}$ was calculated using GraphPad Prism and is the average ± stdev of at least two independent experiments using triplicate determinations at each concentration for each experiment; n = number of assay repeats: $^b n = 2$, $^c n = 3$, $^d n = 4$, $^e n = 6$.

For the investigation of zone 3, the piperazine and 2-Me-Ph substitution were retained for comparison. Interestingly, the amide was found to not be necessary for activity, as the reduced

analog **3.7b** maintained activity (Table 3.2). The sulfonamide **3.7a** was also as active as the initial hit **3.5** whereas the urea **3.7c** and carbamate **3.7d** were completely inactive.

Table 3.2. Modifications to amide of zone 3.
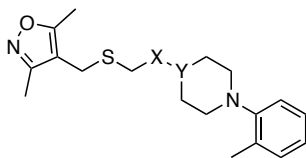
Entry	Compound	Structure		EC$_{50}$ (μM)[a]
		X	Y	
1	**3.7a**	SO$_2$	N	7.2 ± 2.7^d
2	**3.7b**	CH$_2$	N	10.8 ± 5.7^c
3	**3.7c**	(amide structure)	N	$>25^b$
4	**3.7d**	(carbamate structure)	CH	$>25^d$

[a]EC$_{50}$'s were determined using a cell based luciferase assay as previously described.[349] EC$_{50}$ refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC$_{50}$ was calculated using GraphPad Prism and are the average $\pm$ stdev of at least two independent experiments using triplicate determinations at each concentration for each experiment; n = number of assay repeats: [b]$n = 2$, [c]$n = 3$, [d]$n = 4$.

Finally zones 4 and 5 were investigated. The thioether linker was oxidized to sulfoxide **3.8a** and sulfone **3.8b** resulting in analogs with very poor or decreased activity, confirming that the biological activity was not a result of *S*-oxidation (Table 3.3). Substitution of the thioether with oxygen and *N*-Me resulted in a complete loss in activity. It was found that a phenyl ring could be used in place of the 3,5-DMI (**3.8d**) with no significant decrease in activity. Additionally, activity was sustained when using an all-carbon linker in place of the thioether (**3.8f**). Removing a carbon from the thioether linker **3.8g** deleted all activity. The *E*-alkene **3.8h**, *trans*-cyclopropane **3.8i**, and alkyne **3.8j** were also inactive. In contrast, the *Z*-alkene **3.8k** was found to have similar activity to **3.6b** (EC$_{50}$ ~13 μM). Gratifyingly, the corresponding *cis*-cyclopropane **3.8l** had greatly improved activity over the initial hit **3.5** with an EC$_{50}$ of 2.9 μM.

Table 3.3. Structural modifications in zones 4 and 5.
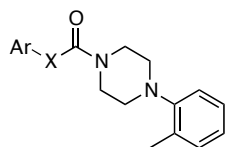
Entry	Compound	Structure		EC$_{50}$ (μM)^a
		X	Ar	
1	**3.5**^{e}	⟶S⟶	3,5-DMI^f	7.3 ± 2.5^d
2	**3.6b**	⟶S⟶	3,5-DMI^f	14.5 ± 3.2^c
3	**3.8a**	⟶S(O)⟶	3,5-DMI^f	$>25^c$
4	**3.8b**	⟶S(O)(O)⟶	3,5-DMI^f	16.1 ± 3.3^c
5	**3.8c**	⟶O⟶	3,5-DMI^f	$>25^c$
6	**3.8d**	⟶S⟶	Ph	13.7 ± 0.8^c
7	**3.8e**	⟶N(Me)⟶	Ph	$>25^c$
8	**3.8f**	⟶CH₂⟶	Ph	14.4 ± 3.7^c
9	**3.8g**	S	Ph	$>25^b$
10	**3.8h**	alkene	Ph	$>25^b$
11	**3.8i**	trans-cyclopropane	Ph	$>25^c$
12	**3.8j**	alkyne	Ph	20.3 ± 11.6^b
13	**3.8k**	alkene	Ph	12.7 ± 0.8^b
14	**3.8l**	cis-cyclopropane	Ph	2.9 ± 1.0^c

^{a}EC$_{50}$'s were determined using a cell based luciferase assay as previously described.[349] EC$_{50}$ refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC$_{50}$ was calculated using GraphPad Prism and are the average $\pm$ stdev of at least two independent experiments using triplicate determinations at each concentration for each experiment; n = number of assay repeats: $^bn = 2$, $^cn = 3$, $^dn = 4$. ^{e}piperazine N-aryl group is 2,3-Me-Ph. ^{f}3,5-DMI, 3,5-dimethylisoxazole.

3.2   RESULTS AND DISCUSSION

After identifying the *cis*-cyclopropane **3.8l** as a key component of our pharmacophore, we sought

to further develop the scaffold in order to increase its potency. We had previously discovered

that the *N*-aryl substitutions with 2-F and 5-Cl-2-Me showed a modest increase in biological activity. Our plan was to combine these substitutions with the cyclopropyl amide side chain to see if a similar trend in activity was observed. Initially, we attempted to generate the *cis*-cyclopropane carboxylic acid **3.11** as building block for a convergent synthesis of new analogs (Scheme 3.1). The synthesis began with a hydrogenation of phenylpropiolic acid **3.9** using a Lindlar catalyst to afford the *cis*-alkene **3.10**. A Simmons-Smith cyclopropanation afforded the *cis*-cyclopropane carboxylic acid **3.11** in poor yield after recrystallization.[413] The carboxylic acid was then used in a HATU-mediated amide coupling with commercially available piperazines **3.12a** and **3.12b** to afford amides **3.13a** and **3.13b**.
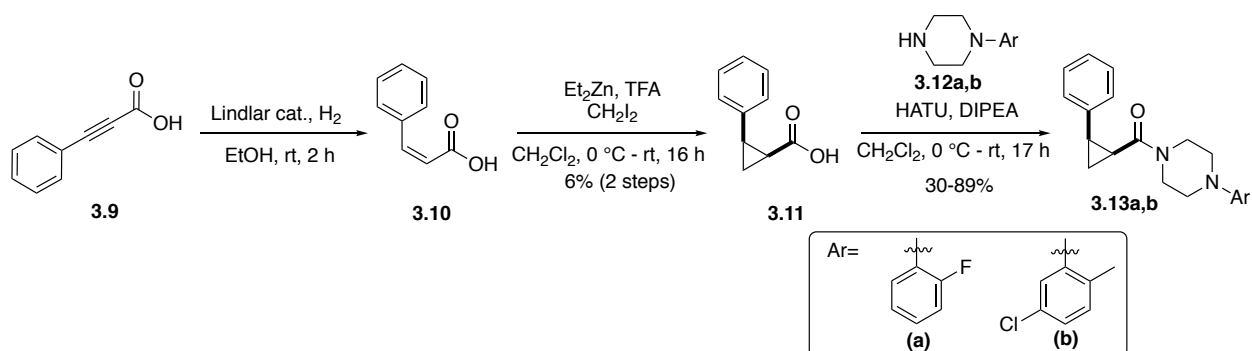
Scheme 3.1. Synthesis of cyclopropane analogs **3.13a** and **3.13b**.

To further improve these analogs, we decided to introduce a 4-fluoro substitution on the unsubstituted aryl ring to reduce the potential for metabolic oxidation (Figure 3.8).[414]
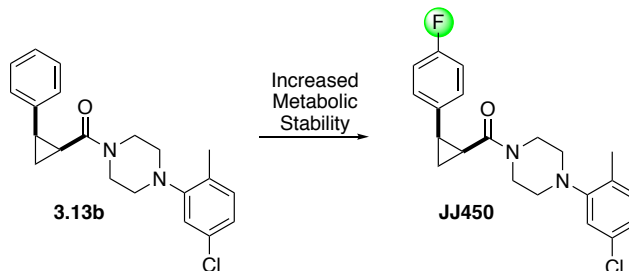
Figure 3.8. Proposed fluorine substitution for increased metabolic stability.

We first needed to synthesize 4-fluorophenylpropiolic acid (**3.16a**), as it wasn't readily available from commercial sources (Scheme 3.2). Initial attempts to directly couple propiolic acid with 4-bromofluorobenzene were messy, difficult to purify, and low yielding. Exploring an alternative stepwise approach previously described by Kondo et al., we first coupled TMS-acetylene to the aryl bromide **3.14a**. Concomitant desilylation and decarboxylation mediated by CsF under an atmosphere of CO_2 provided the carboxylic acid **3.15a** in excellent yields.[415] A T3P mediated amide coupling gave amide **3.17a** that was subsequently reduced under Lindlar hydrogenation conditions to the *cis*-alkene **3.18a** in excellent yields. This reaction is typically stopped prematurely as the alkene is readily separated by chromatography from the alkyne starting material but not the over-reduced alkane, which can be observed with long reaction times. We have also found the $Pd/BaSO_4$ quinoline catalyst system works well for the reduction of the alkyne to the *cis*-alkene.[416] Finally, the *cis*-alkene **3.18** was subjected to chromium mediated Simmons-Smith cyclopropanation conditions to give the cyclopropane **JJ450** in good yield. Other attempts to form the cyclopropane using samarium and diiodomethane were very low yielding with incomplete conversion of the starting material.[417] This route has been optimized for multigram-scale synthesis of **JJ450** and used to prepare more than 20 g of **JJ450**. Analysis of **JJ450** by inductively coupled plasma optical emission spectroscopy (ICP-OES) and chromium levels were measured at 1.3 ppm (1.3 mg/L) with no detectable amount of lead. Chromium (II) is readily oxidized to chromium (III), which has been found to be an essential nutrient with low toxicity by oral administration by the EPA and FDA. According to USP<232> the permissible daily exposure (PDE) is 11,000 µg/day and 1,100 µg/day for oral and parenteral exposure respectively. The environmental protection agency (EPA) set the maximum contaminant level of chromium in drinking water to 100 µg/L (100 ppb).[418]
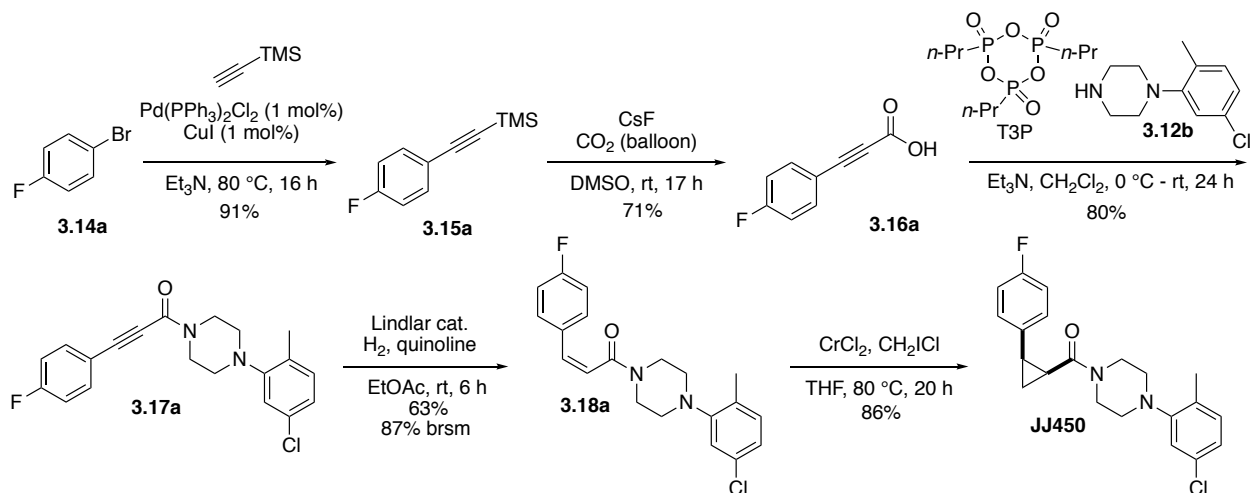
Scheme 3.2. Synthesis of **JJ450**.

In order to determine if a specific binding site is being engaged by our analogs, we separated racemic **JJ450** by semi-preparative chiral SFC using a Chiralpak IC column (25 x 1 cm) and 20% methanol as the modifier. The absolute configuration of the enantiomers were initially assigned based on the specific rotation of similar compounds in the literature,[419] and were later confirmed by single crystal X-ray diffraction of (1*R*,2*S*)-**JJ450** (Figure 3.9).
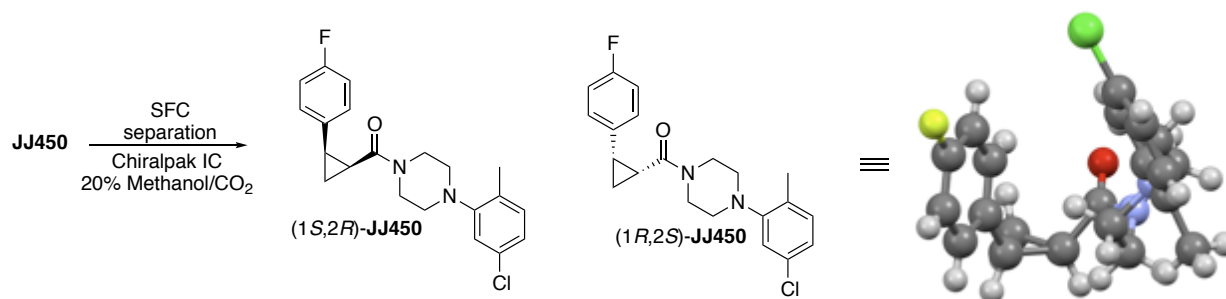
Figure 3.9. Resolution of racemic **JJ450** and crystal structure of (1*R*,2*S*)-**JJ450**.

3.2.1 Biological Data

Our therapeutic goal was to discover a potent, orally bioavailable modulator of AR with preclinical efficacy in CRPC in both *in vitro* and *in vivo* tumor models. Following the synthesis

of analogs **3.13a**, **3.13b**, and **JJ450**, the Wang lab characterized their biological activity in a PSA luciferase assay (Table 3.4). The assay utilizes a Dual-Glo luciferase system in the presence of 1 nM of the synthetic androgen R1881 in C4-2-PSA-rl cells. In this Dual-Glo luciferase system, the relative luciferase activity is calculated as the quotient of androgen-induced PSA-firefly/Renilla luciferase and as the PSA promoter activity correlates to AR transcriptional activity, inhibition of AR will result in decrease PSA-luciferase activity.[349] The EC_{50}'s were calculated using GraphPad Prism and are highly dependent on the type of curve fit used for analysis. The biological activities were compared to the cyclopropane **3.8l** and enzalutamide (EC_{50} 1.1 µM, Table 3.4, entry 7). The cyclopropane derivative **3.13a** with a 2-F-Ph ring was found to be equipotent to the 2-Me-Ph analog **3.8l**. Interestingly, compound **3.13b** (R_2=Cl) was three-fold more potent than **3.8l** and **JJ450** was equipotent to **3.8l**. Gratifyingly, chiral resolution of **JJ450** (EC_{50} 2.7 µM, entry 4) provided a more potent enantiomer (1*S*,2*R*)-**JJ450** (EC_{50} 1.7 µM, entry 5) and the ca. 10-fold less potent (1*R*,2*S*)-**JJ450** (EC_{50} 15.2 µM, entry 6), which supports a specific binding mode at a still to be defined AR binding site.

Table 3.4. Biological activity of *cis*-cyclopropane analogs.
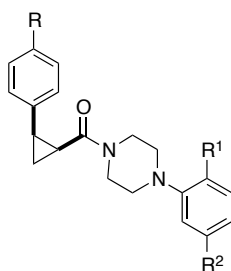
| Entry | Compound | Structure | | | $EC_{50}\,(\mu M)^a$ |
		R	R^1	R^2	
1	**3.8l**	H	Me	H	2.9 ± 1.0^c
2	**3.13a**	H	F	H	2.6 ± 1.6^b
3	**3.13b**	H	Me	Cl	0.9 ± 0.9^b
4	**JJ450**	F	Me	Cl	2.7 ± 1.1^d
5	(1*S*,2*R*)-**JJ450**	F	Me	Cl	1.7 ± 0.2^c
6	(1*R*,2*S*)-**JJ450**	F	Me	Cl	15.2 ± 3.3^c
7	**enzalutamide**	-	-	-	1.1 ± 0.5^e

[a]EC_{50}'s were determined using a cell based luciferase assay as previously described.[349] EC_{50} refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC_{50} was calculated using GraphPad Prism, and are the average ± stdev of at least two independent assays using triplicate determinations at each concentration for each experiment; *n* = number of assay repeats: [b]*n* = 2, [c]*n* = 3, [d]*n* = 4, [e]*n* = 5.

An initial mouse PK panel of **JJ450** proved promising as **JJ450** showed some oral bioavailability (23%), $t_{1/2}$ (IV) of 1.3 h, clearance of 68 mL/min/kg, and steady state volume of distribution (Vss) of 0.7 L/kg. The plasma concentrations were quantifiable until 8 h after dosing C57BL/6 mice with 10 mg/kg **JJ450** by IV and OG method of administration (Table 3.5). Comparison of **JJ450** with data for the FDA approved enzalutamide shows that there is room to improve the PK profile of **JJ450**.[391]

Table 3.5. PK data for **JJ450**.

Entry	Compound[a]	T_{max} [h]	C_0/C_{max} [ng/mL]	AUC_{last} [h•ng/ mL]	AUC_{inf} [h•ng/ mL]	$t_{1/2}$ [h]	CL [mL/min /kg]	V_{SS} [L/kg]	%F
1	**JJ450 (i.v)**[a]	-	6,822.2	2423	2430	1.26	68	2.79	-
2	**JJ450 (o.g)**[a]	0.25	582.3	559.7	569.2	-	-	-	23.1
3	**enzalutamide (o.g) (CD-1 mice)**[b]	4.0	19,800	-	212,000	15.8	0.675* (IV)	0.824* (IV)	94.3

[a]Pharmacokinetic Data was obtained from C57BL/6 mice administered with **JJ450** at 10 mg/kg by o.g or i.v as denoted. [b]Data for enzalutamide was obtained from ref[391].

The metabolic stability in mouse liver microsomes (MLM), rat liver microsomes (RLM), and human liver microsomes (HLM) was determined at 30 and 60 min (Table 3.6). It appears that mouse and rats have rapid metabolism of **JJ450** ($t_{1/2}$ = 10 min) while HLM metabolism is much slower. The microsome stability was also determined for both enantiomers. These are relatively similar with half-lives in MLM of 6.21 min and 7.48 min for the (1*S*,2*R*)-**JJ450** and (1*R*,2*S*)-**JJ450** enantiomers respectively. In comparison, for enzaluatamide (Table 3.6, entry 5) almost no metabolism is present in RLM after 30 min.[420]

Table 3.6. Liver microsome data for **JJ450** and enzalutamide.

Entry	Compound[a]	MLM[b]	RLM[b]	HLM[b]
1	**JJ450 (30 min)**	8%	1%	85%
2	**JJ450 (60 min)**	3%	0%	59%
3	(1*S*,2*R*)-**JJ450 (60 min)**	0.3%	-	27%
4	(1*R*,2*S*)-**JJ450 (60 min)**	0.4%	-	8%
5	**enzalutamide (30 min)**[420]	-	98%	-

[a]Initial compound concentrations were 1 µM. [b]Liver microsome data is reported as the % compound remaining a time point (X) and were measured in the presence of NADPH. MLM, mouse liver microsomes; RLM, rat liver microsomes, HLM, human liver microsomes.

From these studies, **JJ450** emerged as a potent lead analog from our SAR. Despite having decreased activity compared to **3.13b**, we proposed that **JJ450** would have an increased metabolic stability *in vivo* due to the presence of the 4-fluoro substitution and was taken forward as a promising lead for further biological testing. The initial xenograft studies were performed in

the Wang lab using racemic **JJ450** (Figure 3.10). **JJ450** was screened in three human tumor mouse xenograft models LNCaP, VCaP, and 22Rv1. Nude SCID mice bearing LNCaP xenografts were administered **JJ450** once daily by oral gavage (o.g) or intraperitoneal (i.p) at two concentrations (10 mg/kg o.g, 10 mg/kg i.p, and 75 mg/kg o.g) (Figure 3.10, (A)). Lower concentrations (10 mg/kg) administered by o.g had begun to show increased tumor growth around 30 days while the i.p (10 mg/kg) had shown almost no tumor growth until day 40. Interestingly, the highest dose of **JJ450** (75 mg/kg) o.g had no tumor growth until day 50 (Figure 3.10, A). **JJ450** was administered to nude SCID mice bearing the more aggressive 22Rv1 tumors, which are resistant towards enzalutamide, at 10 mg/kg and 75 mg/kg i.p (Figure 3.10, B). Although **JJ450** was able to slow the progression of the 22Rv1 tumor, the rapid tumor growth combined with the poor metabolism may have contributed to its ineffectiveness in this tumor model. Finally, a comparison study of **JJ450** and enzalutamide (MDV3100) in a VCaP xenograft model shows **JJ450** to be more effective than enzalutamide with only a small increase in tumor volume (Figure 3.10, C). In all of these xenograft models, mice administered **JJ450** showed no sign of weight loss indicating low cytotoxicity. Additionally, no adverse effects were observed in mice administered with a single dose of either 225 mg/kg or 375 mg/kg. Interestingly, a subsequent *in vivo* experiment using the separated enantiomers of **JJ450** revealed that (1*R*,2*S*)-**JJ450** was more efficacious, directly contrasting the *in vitro* result. We are still unsure as to the reason for this activity difference from the *in vitro* to *in vivo*.
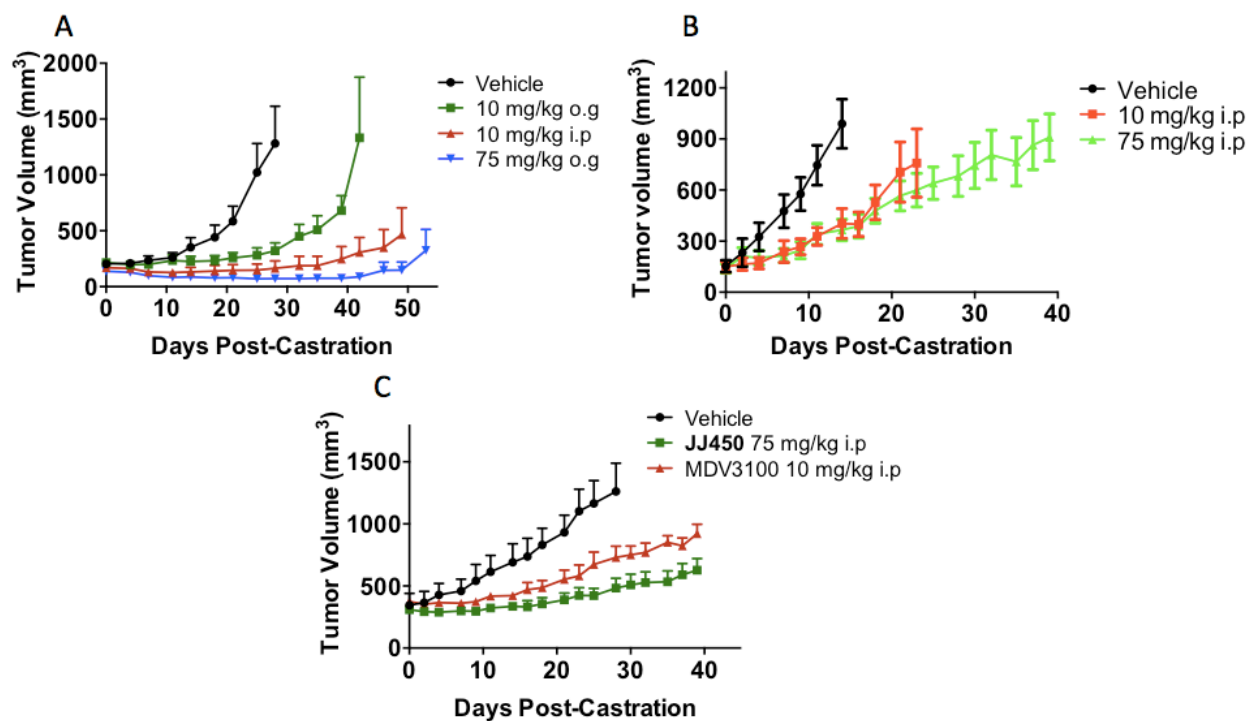
Figure 3.10. Effect of **JJ450** on LNCaP, 22Rv1, and VCaP s.c. tumor xenografts in nude SCID mice.[421]

To further understand the metabolism of **JJ450**, HLM were incubated with **JJ450** and LCMS analysis of the microsome fractions indicated two oxidation events were occurring. Our initial hypothesis was first *N*-oxidation of the aniline nitrogen to give *N*-oxide **3.20** (Scheme 3.3). We proposed that the second oxidation could occur in several other locations including the fluorinated aryl ring (A), next to the amide (B), the benzylic methyl group (C), and the 4-position of the aniline (D).
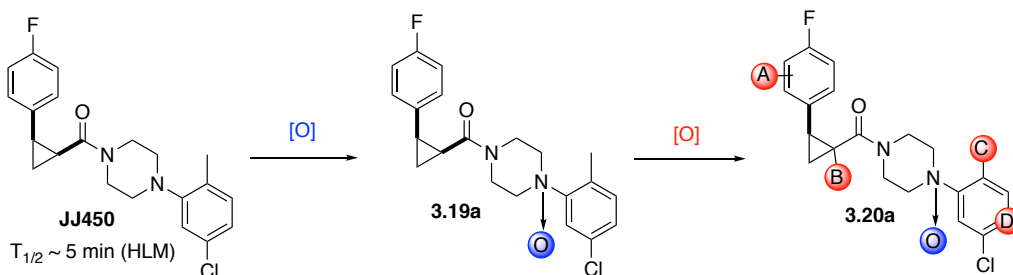
Scheme 3.3. Proposed metabolic oxidation and hotspots on **JJ450**.

3.2.2 Improving the Metabolic Stability of JJ450

With **JJ450** as our new starting point, we wanted to further improve its metabolic stability. Drug metabolism is an important factor to consider for further development of lead compounds as it plays a key role in toxicity, oral bioavailability, dosing. Several strategies have been developed to block or replace metabolically labile sites that include decreasing lipophilicity, introducing conformational constraint, introducing steric hindrance, tuning electronics, and altering stereochemistry.[422] Cytochrome P450s (CYP's) are responsible for most oxidative metabolism, which increases the polarity facilitating faster elimination. Liver microsomes serve as an *in vitro* model to predict CYP metabolism. Fluorine and fluorinated functional groups (CF_3, and SF_5) are commonly employed to block metabolically labile C-H bonds, decrease electron density of aromatic rings, increase lipophilicity, and lower the pKa of adjacent heteroatoms. In particular, we aimed to block several potentially metabolically labile sites on **JJ450** that included the benzylic methyl and aniline nitrogen (zone 1), the cyclopropane (zone 2), and the aryl ring (zone 3) (Figure 3.11). Additionally, as lipophilic compounds are more prone to CYP metabolism, we were also interested in decreasing the lipophilicity of **JJ450** through incorporation of heterocycles in zones 1 and 3 (Figure 3.11).
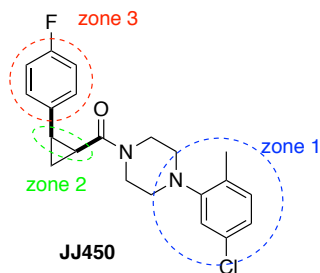
Figure 3.11. Proposed zones of structural modification.

The synthesis of new analogs were carried out in a similar fashion to **JJ450** and began with a Sonogashira coupling of commercially available aryl bromides **3.14a-3.14g** with TMS

acetylene followed by treatment with CsF under an atmosphere of CO_2 to give the corresponding carboxylic acids **3.16a-3.16g** in good yields (Scheme 3.3). A subsequent T3P coupling of carboxylic acids **3.16b-3.16r** with *N*-arylated piperazines **3.12b-3.12f** afforded amides **3.17b-3.17r**. Hydrogenation was accomplished using either Lindlar catalyst or Pd/BaSO$_4$ with quinoline to afford the *cis*-alkenes **3.18b-3.18r** that were subjected to the chromium cyclopropanation conditions to afford *cis*-cyclopropanes **3.21a-3.21r**.
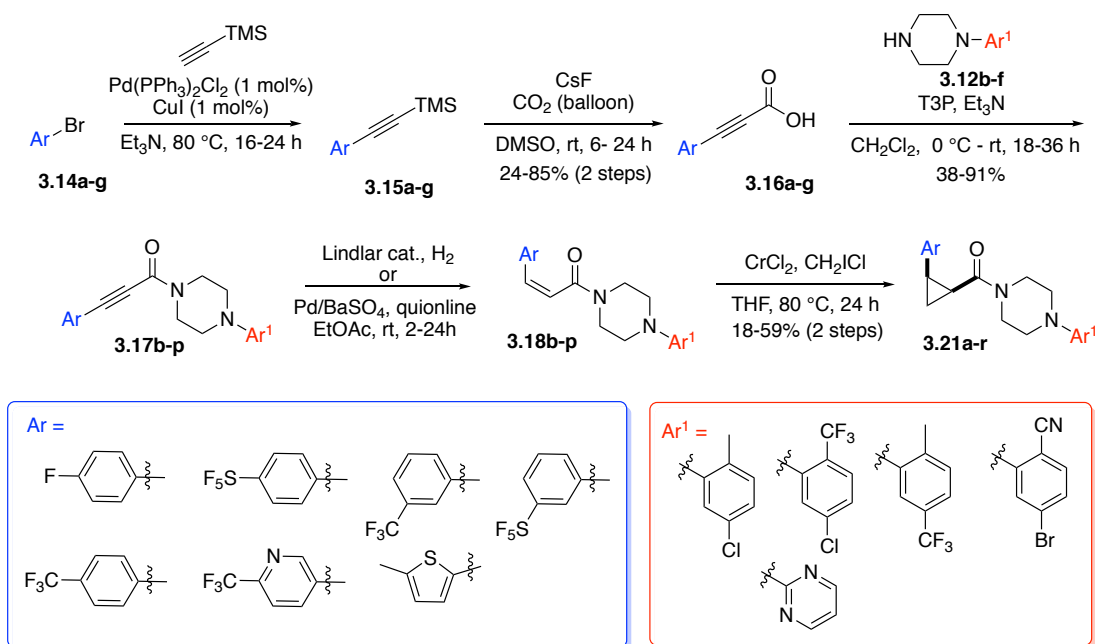
Scheme 3.4. Synthetic route to analogs of **JJ450**.

In addition to analogs with varying aryl substitutions, we prepared an analog containing a deuterium substituted cyclopropane ring. If the metabolic oxidation is a result of oxidation on the cyclopropane we should see a decrease in metabolic rate due to the primary kinetic isotope effect. The synthesis began with a Lindlar reduction of **3.17a** under an atmosphere of D_2 to afford the deuterated-*cis*-alkene **3.22** in excellent yields that was subsequently subjected to the Simmons-Smith cyclopropanation conditions to afford the desired deutero-cyclopropane **3.23** (Scheme 3.4). Interestingly, we had also attempted to form the deutero-*cis*-alkene using D_2O and

gold nanoparticle catalysis under a high pressure atmosphere of CO as previously described by Cao et al.,[423] but, were unable to reproduce the reported pressures (400 psi) and therefore had low conversions and deuterium incorporation.
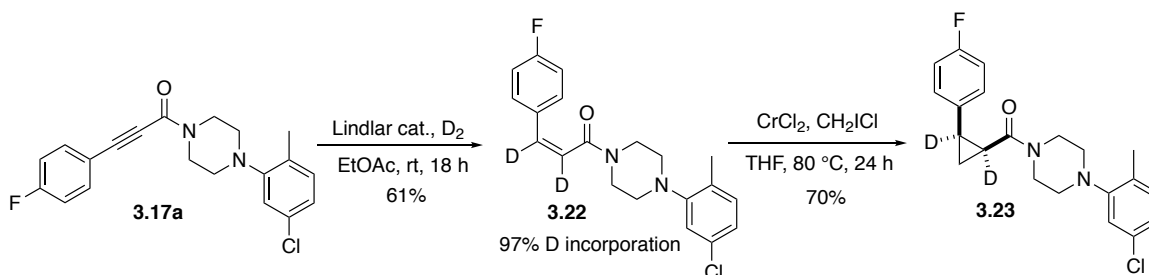
Scheme 3.5. Synthesis of deuterocyclopropane **3.23**.

In total 19 analogs of **JJ450** were synthesized. Several of these racemic analogues were further resolved into their component enantiomers by semi-preparative chiral SFC chromatography. The absolute configurations of the separated enantiomers were assigned through a combination of circular dichroism (CD) and specific rotation analysis. Since the separated enantiomers retain the same *cis*-cyclopropane substitution pattern of the parent compound (1*R*,2*S*)-**JJ450**, the absolute configurations of the separated enantiomers were assigned by comparison to the specific rotation of (1*R*,2*S*)-**JJ450**, whose absolute configuration had been previously assigned by X-ray crystallography. Additionally, these analogs displayed consistent cotton effects in the circular dichroism (CD) spectra of enantiomers with matching specific rotation signs to (1*S*,2*R*)-**JJ450** and (1*R*,2*S*)-**JJ450** (See Appendix B).

3.2.3 Biological Data of JJ450 Analogs: Cell Data and Metabolism

Following the synthesis of the 43 analogs of **JJ450**, their biological activity was determined in a PSA luciferase assay using a Dual-Glo luciferase system in the presence of 1 nM of the synthetic androgen R1881 in C4-2-PSA-rl cells.[349] The EC_{50}'s were calculated using GraphPad Prism and

are preliminary. The biological activities were compared to **JJ450** (EC$_{50}$ 2.7 µM, Table 3.7, entry 1). Deuteration of the cyclopropane did not have any significant effect on the activity or the metabolism, indicating that the oxidation is possibly not on the cyclopropane but on one of the aryl rings. Substituents on the piperazines with aryl groups replacing the benzylic methyl or chloro with a trifluoromethyl were met with slight loss in activity with no effect on the metabolism. Unfortunately, the pyrimidine aryl functionality was met with a complete loss in activity as it has one of the most promising cLogD values of 2.25.

Table 3.7. Biological activity and liver microsome data for 4-fluoro substituted analogs.
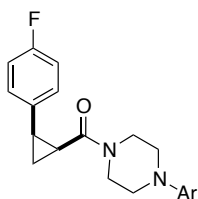
Entry	Compound	Structure Ar	EC$_{50}$ (µM)[a]	cLogD[e]	MLM[f]	HLM[f]
1	**JJ450**	2-Me-5-Cl-Ph	2.7 ± 1.1[d]	4.6	-	-
2	(1*S*,2*R*)-**JJ450**	2-Me-5-Cl-Ph	1.7 ± 0.2[c]	4.6	0.3%	27%
3	(1*R*,2*S*)-**JJ450**	2-Me-5-Cl-Ph	15.2 ± 3.3[c]	4.6	0.4%	8%
4	**3.23**	2-Me-5-Cl-Ph	2.2 ± 0.3[b]	4.6	-	-
5	(1*S*,2*R*)-**3.23**	2-Me-5-Cl-Ph	1.9 ± 0.5[b]	4.6	0.2%	28%
6	(1*R*,2*S*)-**3.23**	2-Me-5-Cl-Ph	14.3 ± 0.2[b]	4.6	0.5%	9%
7	**3.21a**[g]	2-Me-5-CF$_3$	6.3 ± 3.0[b]	4.9	-	-
8	(1*S*,2*R*)-**3.21a**[g]	2-Me-5-CF$_3$	2.5 ± 1.9[b]	4.9	0.8%	31%
9	(1*R*,2*S*)-**3.21a**[g]	2-Me-5-CF$_3$-Ph	9.4 ± 1.4[b]	4.9	7%	26%
10	**3.21b**	2-CF$_3$-5-Cl-Ph	4.9 ± 0.9[b]	5.0	-	-
11	**3.21c**		>25[b]	2.3	-	-
12	(1*S*,2*R*)-**3.21c**		>25[b]	2.3	-	-
13	(1*R*,2*S*)-**3.21c**		>25[b]	2.3	-	-

[a]EC$_{50}$'s were determined using a cell based luciferase assay as previously described.[349] EC$_{50}$ refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC$_{50}$ was calculated using GraphPad Prism, and are the average of at least one independent assays using triplicate determinations at each concentration for each experiment where noted; n = number of assay repeats: [b]n = 2, [c]n = 3, [d]n = 4. [e]cLogD were calculated using InstantJchem. [f]Liver microsome data is reported as the % compound remaining at 60 min and were measured in

The 4-trifluoromethyl substitution of the arylcyclopropane resulted in an overall 2 to 3-fold decrease in activity and an overall increase in microsome stability, possibly due to an increased in steric bulk and decreased electron density on the aryl ring respectively. The parent **3.21d** (2-Me-5-Cl-Ph) appears to have poor metabolism (Table 3.8, entries 1-3) while the trifluormethyl substitutions afford a 20-fold increase in microsome stability over **JJ450**. In particular, analog (1*S*,2*R*)-**3.21f** (Table 3.8, entry 8) had a promising half-life of 1.5 h with and EC_{50} of ~12 µM. Interestingly, compound **3.21g** with the 2-CN-5-Br-Ph substitution led to a significant decrease in activity contrary to what we had previously observed for compound **3.6e** in our original SAR (Table 3.8, entries 10-12). Additionally, these trifluoromethyl substituted analogs also are significantly lipophilic (cLogD ~5-6), possibly limiting their utility in animal and human studies.

Table 3.8. Biological activity and liver microsome data for 4-trifluoromethyl substituted analogs.

Entry	Compound	Structure Ar	EC_{50} (µM)[a]	cLogD[d]	MLM[e]	HLM[e]
1	**3.21d**[f]	2-Me-5-Cl-Ph	6.6 ± 1.7[c]	5.4	-	-
2	(1*S*,2*R*)-**3.21d**[f]	2-Me-5-Cl-Ph	8.4 ± 3.6[c]	5.4	11%	45%
3	(1*R*,2*S*)-**3.21d**[f]	2-Me-5-Cl-Ph	23.1 ± 2.6[c]	5.4	14%	5%
4	**3.21e**[f]	2-Me-5-CF$_3$-Ph	11.9 ± 2.1[c]	5.6	-	-
5	(1*S*,2*R*)-**3.21e**[f]	2-Me-5-CF$_3$-Ph	12.1 ± 4.5[c]	5.6	49%	58%
6	(1*R*,2*S*)-**3.21e**[f]	2-Me-5-CF$_3$-Ph	15.5 ± 5.7[c]	5.6	48%	23%
7	**3.21f**	2-CF$_3$-5-Cl-Ph	9.1 ± 0.8[c]	5.7	-	-
8	(1*S*,2*R*)-**3.21f**	2-CF$_3$-5-Cl-Ph	12.0[b]	5.7	60% ($t_{1/2}$ = 1.5 h)	65%
9	(1*R*,2*S*)-**3.21f**	2-CF$_3$-5-Cl-Ph	>25[b]	5.7	34%	51%
10	**3.21g**	2-CN-5-Br-Ph	>25[c]	4.9	-	-
11	(1*S*,2*R*)-**3.21g**	2-CN-5-Br-Ph	23.4 ± 3.5[c]	4.9	-	-
12	(1*R*,2*S*)-**3.21g**	2-CN-5-Br-Ph	>25[c]	4.9	-	-

[a]EC_{50}'s were determined using a cell based luciferase assay as previously described.[349] EC_{50} refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC_{50} was calculated using GraphPad Prism, and are the average ± stdev of at least one independent assay using triplicate determinations at each concentration for each experiment where noted; n = number of assay repeats: [b]n = 1, [c]n = 2. [d]cLogD were calculated using InstantJchem. [e]Liver microsome data is reported as the % compound remaining at 60 min and were measured in the presence of NADPH. MLM, mouse liver microsomes; HLM, human liver microsomes. [f]Compound was synthesized by Dr. Keita Takubo.

Analogs containing the 3-trifluoromethyl substitution also showed a slight decrease in activity (ca. 2-fold) compared to the 4-F analogs, exhibiting a similar trend to analogs containing the 4-trifluoromethyl substitution (Table 3.9). In contrast, the 3-substituted analogs were not as metabolically stable as the 4-substituted analogs with a ca. 5-fold decrease in stability.

Table 3.9. Biological activity and liver microsome data for 3-trifluoromethyl substituted analogs.

Entry	Compound	Structure Ar	EC_{50} (μM)[a]	cLogD[d]	MLM[e]	HLM[e]
1	**3.21h**	2-Me-5-Cl-Ph	3.3 ± 0.8^{c}	5.4	-	-
2	(1S,2R)-**3.21h**	2-Me-5-Cl-Ph	3.6 ± 0.2^{b}	5.4	0.2%	1%
3	(1R,2S)-**3.21h**	2-Me-5-Cl-Ph	6.3 ± 1.8^{b}	5.4	0.9%	4%
4	**3.21i**	2-Me-5-CF$_3$-Ph	7.7 ± 2.1^{b}	5.6	-	-
5	**3.21j**	2-CF$_3$-5-Cl-Ph	3.4 ± 2.3^{b}	5.7	12%	34%

[a]EC_{50}'s were determined using a cell based luciferase assay as previously described.[349] EC_{50} refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC_{50} was calculated using GraphPad Prism, and are the average $\pm$ stdev of two independent assay using triplicate determinations at each concentration for each experiment where noted; n = number of assay repeats: [b]$n = 2$, [c]$n = 3$. [d]cLogD were calculated using InstantJchem. MLM, mouse liver microsomes; HLM, human liver microsomes. [e]Liver microsome data is reported as the % compound remaining at 60 min and were measured in the presence of NADPH.

Similar to its trifluoromethyl counterpart, the 4-pentafluorosulfanyl substitution led to a global 3-fold decrease in activity but an overall increase in microsome stability (Table 3.10). In particular, analog (1S,2R)-**3.21l** (Table 3.10, entry 5) had a half-life in liver microsomes ~3 h with an EC_{50} of 11 μM. Unfortunately, these analogs are even more lipophilic than the trifluoromethyl analogs with cLogDs ~6-7 that could also limit their utility in animal and human studies.

Table 3.10. Biological activity and liver microsome data for 4-pentafluorosulfanyl substituted analogs.
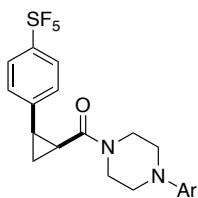
Entry	Compound	Structure Ar	EC$_{50}$ (µM)[a]	cLogD[d]	MLM[e]	HLM[e]
1	**3.21k**	2-Me-5-Cl-Ph	11.4 ± 1.6^{c}	6.5	-	-
2	(1*S*,2*R*)-**3.21k**	2-Me-5-Cl-Ph	8.2 ± 4.0^{b}	6.5	24%	3%
3	(1*R*,2*S*)-**3.21k**	2-Me-5-Cl-Ph	18.9 ± 4.4^{b}	6.5	40%	53%
4	**3.21l**	2-Me-5-CF$_3$-Ph	7.8 ± 4.3^{b}	6.9	-	-
5	(1*S*,2*R*)-**3.21l**	2-Me-5-CF$_3$-Ph	9.1 ± 2.7^{b}	6.9	80%	77% (t$_{1/2}$ = 3 h)
6	(1*R*,2*S*)-**3.21l**	2-Me-5-CF$_3$-Ph	21.3 ± 2.8^{b}	6.9	66%	30%

[a]EC$_{50}$'s were determined using a cell based luciferase assay as previously described.[349] EC$_{50}$ refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC$_{50}$ was calculated using GraphPad Prism, and are the average ± stdev of two independent assay using triplicate determinations at each concentration for each experiment where noted; n = number of assay repeats: [b]n = 2, [c]n = 3. [d]cLogD were calculated using InstantJchem. [e]Liver microsome data is reported as the % compound remaining at 60 min and were measured in the presence of NADPH. MLM, mouse liver microsomes; HLM, human liver microsomes.

The 3-pentafluorosulfanyl analogs were ca. 2-fold more potent compared to the 4-pentafluorosulfanyl analogs, but did not perform as well in the liver microsomes (Table 3.11). Even with the modest recovery of activity, the aqueous solubility will also be a concern for these analogs.

Table 3.11. Biological activity and liver microsome data for 3-pentafluorosulfanyl substituted analogs.

Entry	Compound	Structure Ar	EC_{50} (μM)[a]	cLogD[c]	MLM[d]	HLM[d]
1	**3.21m**	2-Me-5-Cl-Ph	4.6 ± 0.8^{b}	5.4	-	-
2	**3.21n**	2-Me-5-CF$_3$-Ph	5.7 ± 0.3^{b}	6.9	-	-
3	(1*S*,2*R*)-**3.21n**	2-Me-5-CF$_3$-Ph	9.9 ± 1.4^{b}	6.9	6%	4%
4	(1*R*,2*S*)-**3.21n**	2-Me-5-CF$_3$-Ph	7.1 ± 1.8^{b}	6.9	35%	11%

[a]EC_{50}'s were determined using a cell based luciferase assay as previously described.[349] EC_{50} refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC_{50} was calculated using GraphPad Prism, and are the average ± stdev of at least two independent assays using triplicate determinations at each concentration for each experiment where noted; *n* = number of assay repeats: [b]*n* = 2. [c]cLogD were calculated using InstantJchem. [d]Liver microsome data is reported as the % compound remaining at 60 min and were measured in the presence of NADPH. MLM, mouse liver microsomes; HLM, human liver microsomes.

Interestingly, the trifluoromethyl pyridine **3.21o** (Table 3.12, entries 1-3) was found to be less active and less stable in microsomes. Additionally, it appears that (1*R*,2*S*)-**3.21o** is slightly more potent than the (1*S*,2*R*)-**3.21o**, which is opposite from what is observed with other **JJ450** analogs. In contrast, the thiophene **3.21p-3.21r** was similar potency to **JJ450** with all substitutions on the piperazine. This is promising as smaller 5-membered heterocycles seem to recover the activity lost with introduction of bulky para-substituents as previously observed with the SF$_5$ and CF$_3$ substitutions.

Table 3.12. Biological activity and liver microsome data for heteroaryl substituted analogs.

Entry	Compound	Structure Ar	Structure Ar[1]	$EC_{50}\,(\mu M)^{a}$	$cLogD^{d}$	MLM^{e}	HLM^{e}
1	**3.21o**	F_3C–(pyridyl)	2-Me-5-CF_3-Ph	20.8 ± 6.0^{c}	4.8	-	-
2	(1*S*,2*R*)-**3.21o**	F_3C–(pyridyl)	2-Me-5-CF_3-Ph	18.0 ± 1.7^{b}	4.8	35%	39%
3	(1*R*,2*S*)-**3.21o**	F_3C–(pyridyl)	2-Me-5-CF_3-Ph	13.6 ± 1.8^{b}	4.8	24%	6%
4	**3.21p**	(thienyl)	2-Me-5-Cl-Ph	2.2 ± 0.5^{c}	5.0	-	-
5	**3.21q**	(thienyl)	2-Me-5-CF_3-Ph	4.1 ± 0.3^{c}	5.3	-	-
6	**3.21r**	(thienyl)	2-CF_3-5-Cl-Ph	2.0 ± 0.1^{b}	5.4	-	-

[a]EC_{50}'s were determined using a cell based luciferase assay as previously described.[349] EC_{50} refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC_{50} was calculated using GraphPad Prism, and are the average ± stdev of at least two independent assays using triplicate determinations at each concentration for each experiment where noted; n = number of assay repeats: [b]$n = 2$, [c]$n = 3$. [d]cLogD were calculated using InstantJchem. [e]Liver microsome data is reported as the % compound remaining at 60 min and were measured in the presence of NADPH.

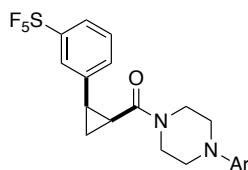In summary, 43 analogs were synthesized, and the SAR evaluated 3 zones of modifications of our lead compound **JJ450** (Figure 3.12). We gained further understanding about the environment that these compounds bind. Zone 1 modifications showed that the 2,5-aryl substitutions with Cl or CF_3 were well tolerated, while other modifications appeared to cause significant loss in activity. Limited substitutions were performed in zone 2 and deutero-**JJ450** was found to have similar activity to the parent compound. In zone 4, increasing the steric bulk of the *para* or *meta*-substituent reduced activity, and only the thiophene maintained activity comparable to **JJ450**.
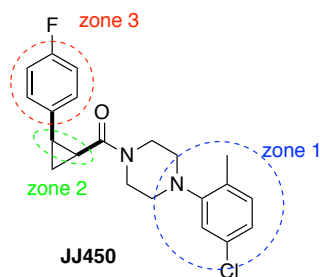
Figure 3.12. Zones of structural modification on **JJ450**.

Our primary aim of these 3 zones of structural modifications was to increase the stability of the parent compound **JJ450** in liver microsomes, a predictive model for phase 1 metabolism (Scheme 3.6). In zone 1, the 2-CF$_3$-5-Cl and 2-Me-5-CF$_3$ substitution only afforded modest increase in stability. The deuterium labeling in zone 2 resulted in no overall effect on the metabolism indicating that this is not a major or initial site of metabolism. Finally, modifications in zone 3 were shown to be the most influential in increasing the microsomal stability indicating that this is our primary site of metabolism. The most significant increase in microsomal stability was noted in compounds containing a *para*-CF$_3$ or SF$_5$. Gratifyingly, compound **3.21l** was found to have a half-life of ~3 h in liver microsomes, a ~40-fold increase in stability from the parent **JJ450**. Additionally, given this data we must reconsider our initial hypothesis of the metabolic oxidation of **JJ450**. The major metabolic liability appears to be the fluorinated aryl ring (Scheme 3.6). The second metabolic liability is found at the aniline ring where fluorinated substituents were found to slightly improve the microsomal stability.
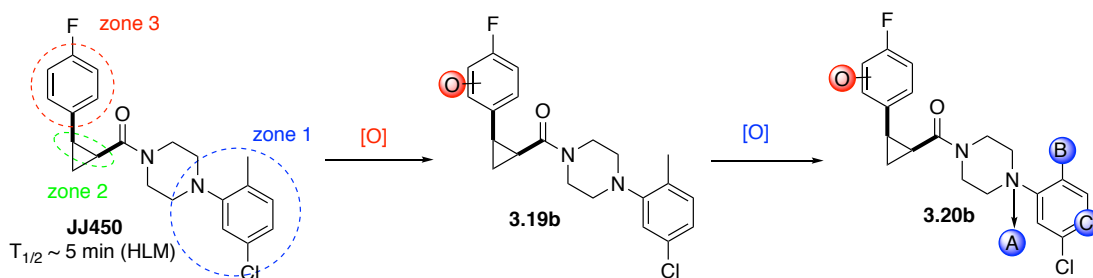
Scheme 3.6. Revised sites of metabolism on **JJ450**.

3.2.4 Synthesis of Chemical Probes to Elucidate the Mechanism of Action

In addition to developing new and more potent inhibitors of CRPC with increased metabolic stability, we also wanted to develop small molecules to probe the mechanism of action of our lead compounds. Several methods have been developed using small-molecule-based affinity reagents to identify cellular targets.[424-425] They require an effective conjugation protocol as well as methods for affinity purification. There are three main methods for target pull-down using small molecules that include immobilization onto agarose beads, conjugation to biotin, or photoaffinity labeling. In particular, many agarose beads are commercially available or readily tuned to have a variety of functionalized surfaces (containing carboxylic acids, amines, alkynes, or azides). There are many methods used for the conjugation step such as peptide coupling of amines and carboxylic acids, reductive amination, or Cu-(I)-catalyzed [3+2] cycloaddition. Agarose beads conjugated to active compounds are incubated with whole cell lysates to capture potential target proteins. Schreiber and coworkers developed a method to conjugate alcohol-containing small molecules to agarose beads that uses CDI to form an activated carbamate that can then undergo conjugation to an agarose bead functionalized with amine linkers (Scheme 3.7).[426]
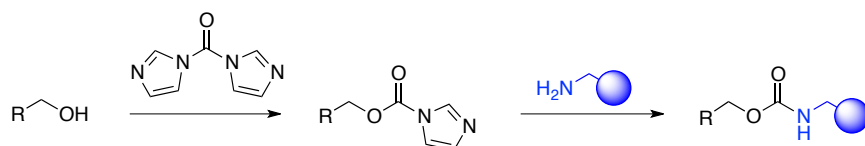
Scheme 3.7. Bead conjugation of alcohol-containing molecules.

Previously, we identified **3.24** as a new analog that was slightly more potent than the original HTS hit **3.5** and contained a propargyl alcohol handle that could be coupled to an agarose bead to form a derivative for pull-down studies. The bridged bicyclic [3.2.1] analogs

such as **3.6p** were of interest as both diastereomers were equipotent indicating that structural bulk could be tolerated around the piperazine as well as a 2-fold increase in potency from the 2-MePh analog **3.6b**. We proposed that a hybrid structure of **3.24** and the bridged bicyclic [3.2.1] analog **3.6p** would yield a 2-fold increase in binding affinity compared to **3.24**. This more potent analog **3.25** would be better suited for conjugation to agarose beads and a subsequent pull-down study (Scheme 3.8).
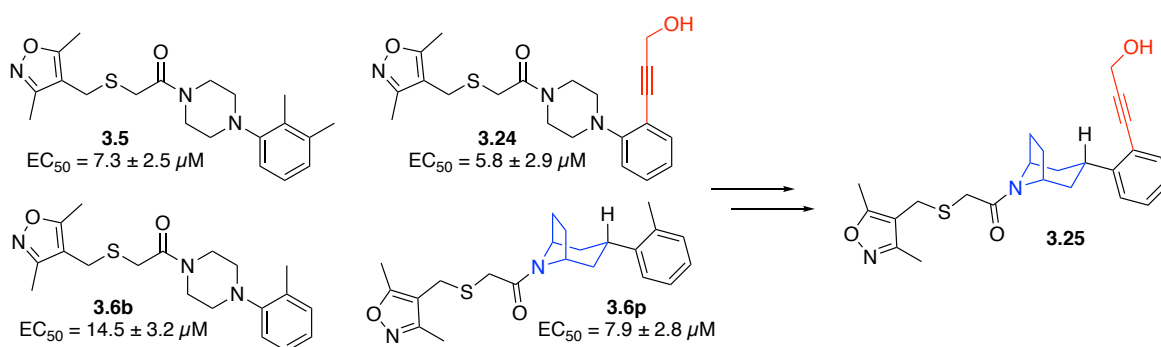
Scheme 3.8. Initial proposed analog **3.25** for affinity labeling.

Our retrosynthetic approach to **3.25** features a late stage coupling of amine **3.27** to commercially available carboxylic acid **3.26** (Scheme 3.9). We proposed hydrogenation of the styrene **3.28** followed by a Sonogashira coupling to install the key propargyl alcohol side chain. We envisioned that styrene **3.28** could be constructed from commercially available nortropinone hydrochloride **3.29** via a Boc-protection, enolization and vinyl triflate formation, and a Suzuki cross coupling.
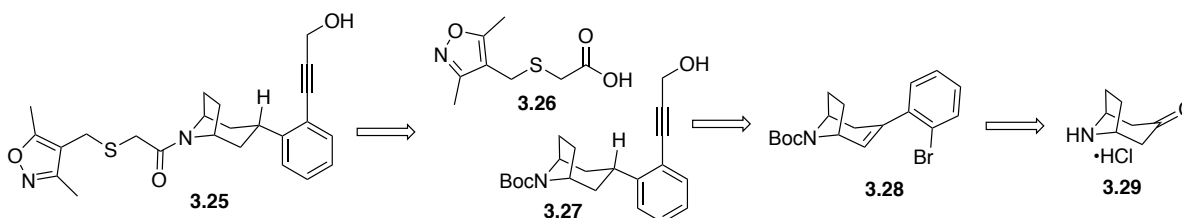
Scheme 3.9. Retrosynthetic approach to analog **3.25**.

The synthesis of the styrene intermediate **3.28** began with Boc-protection of commercially available nortropinone hydrochloride (**3.29**) followed by enolization with NaHMDS and trapping of the enolate with Comins' reagent to afford the vinyl triflate **3.31** (Scheme 3.10). A Suzuki coupling of vinyl triflate **3.31** and boronic acid **3.32** afforded the 2-bromostyrene **3.28** in good yields.
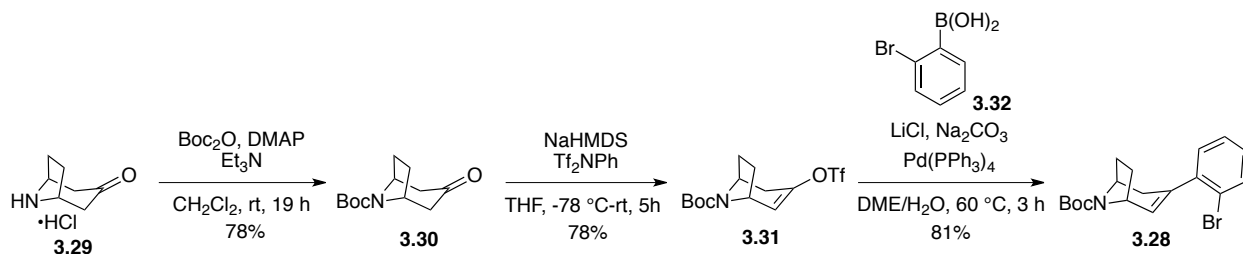
Scheme 3.10. Synthesis of styrene **3.28**.

Our next focus was to reduce the alkene in the presence of the aryl bromide. We screened many reaction conditions for this reduction including PtO_2, and diimide reductions; however, all attempts were unsuccessful due to overreduction of the alkene and aryl bromide or lack of reactivity.[427-429] In the case of the diimide reductions, a large excess of reagents was required to see even trace reduction. Gratifyingly, we discovered that the hydrogen atom transfer hydrogenation conditions, previously developed by Shenvi, were successful on our substrate to afford the product **3.33** in modest yields as a 3:1 mixture of diastereomers **3.33a** and **3.33b** (Scheme 3.11).[430-431] Using the mixture of diastereomers, we moved forward with the Sonogashira cross coupling. Using CuI, other sources of Pd, or a protected propargyl alcohol afforded only trace product. Ultimately, we found that we could use (iPr)CuCl[293] (*vide infra*) to give a single diastereomer of propargyl alcohol **3.27** in manageable yields with only trace amounts (<5%) of the minor diastereomer as separable products by chromatography. Additionally, using the styrene for the Sonogashira coupling was also unsuccessful and protodemetalation was the major pathway observed. Finally, Boc-deprotection of **3.27** and

coupling to commercially available carboxylic acid **3.26** afforded amide **3.25** in modest yields. The poor yield in the coupling is the result of over acylation of the propargyl alcohol.
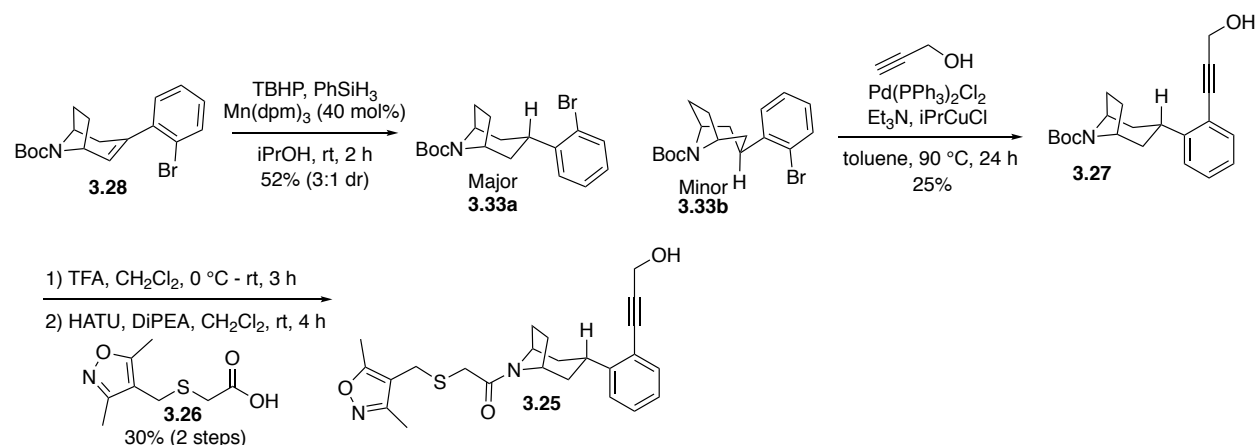
Scheme 3.11. Synthesis of **3.25**.

For the conjugation of **3.25** to the agarose beads, we had initially tried using CDI to form the activated carbamate but found it difficult to quantify by LCMS and IR. We modified our procedure for the use of 4-nitrophenylchloroformate to form the activated carbonate that releases the highly colored 4-nitrophenolate anion **3.35** upon amine conjugation in DMF at rt for 5 days with gentle inversion (Scheme 3.12).
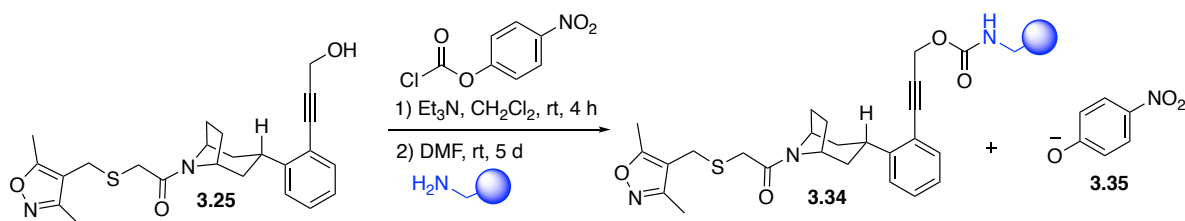
Scheme 3.12. Conjugation of **3.25** to agarose beads.

After washing of the beads, we were able to see several characteristic IR shifts compared to a blank bead sample. In particular, we can see a small alkyne stretch around 2050 cm^{-1} and significant changes in the fingerprint region (Figure 3.13).
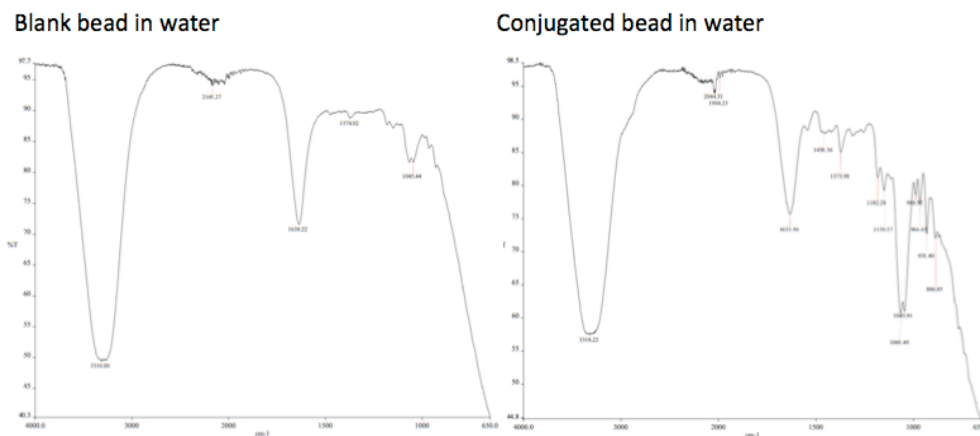
Figure 3.13. Comparison of the IR spectra of the unconjugated blank bead to the conjugated bead.

In addition to **3.25**, we also wanted to synthesize an analog **3.36** with a longer flexible linker to prevent any potential interference of the agarose bead with binding of the inhibitor and to avoid any nonspecific binding interactions (Figure 3.14). Initial attempts at the Sonogashira coupling with a PEG-alkyne were unsuccessful and instead we used a Heck coupling to install an ester as a more flexible handle for conjugation. We proposed that the benzyl ester could be selectively cleaved in the presence of the internal ester and the corresponding carboxylic acid can be used in an amide coupling for conjugation.
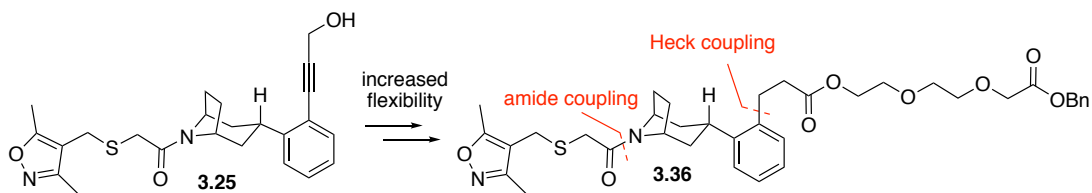
Figure 3.14. Proposed flexible linker modifications to **3.25**.

For a convergent approach we synthesized the acrylate PEG linker **3.29** for the Heck coupling (Scheme 3.13). The synthesis of **3.29** began from the monoalkylation of diethylene glycol (DEG, **3.37**) to afford benzyl ester **3.38** in poor yields. The terminal alcohol of **3.38** was acylated with acryoyl chloride to afford acrylate **3.39** in poor yields.
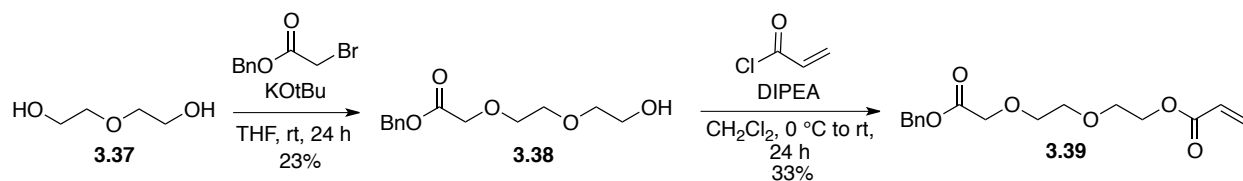
Scheme 3.13. Synthesis of acrylate linker **3.39**.

The acrylate side chain was coupled to the previously prepared bromide diastereomers **3.33** (enriched to a 10:1 mixture of **3.33a**:**3.33b** by chromatography) via a Heck coupling (Scheme 3.14). In contrast to the Sonogashira coupling, both diastereomers participate in the Heck coupling to give the alkene **3.40** as a similar mixture of diastereomers. Selective reduction of the αβ-unsaturated ester **3.40** in the presence of the benzyl ether was accomplished using nickel mediated conjugate reduction. Other attempts using Pd/C also resulted in competitive cleavage of the OBn ester. Boc-deprotection and coupling to carboxylic acid **3.26** afforded **3.36** as a mixture of diastereomers that could be separated by preparative HPLC to afford poor yields of **3.26** as a single diastereomer. In preparation for conjugation, we looked to selectively cleave the benzyl ester in the presence of the internal ester. Unfortunately, the benzyl ester could not readily cleaved by hydrogenation using Pd/C due to the presence of the thioether with no reactivity observed. Saponification in alcoholic solvents only afforded a mixture of transesterified products. We were delighted to find that tetrabutyl ammonium hydroxide (TBAH) selectively cleaved the benzyl ester in modest yields. Unfortunately, on large scale we encountered problems with the stability of these compounds during the conjugate reduction of **3.40** to **3.41**, where we observed reduction of the internal ester and loss of the PEG linker. As a result of this we wanted to modify the linker to have increased stability.

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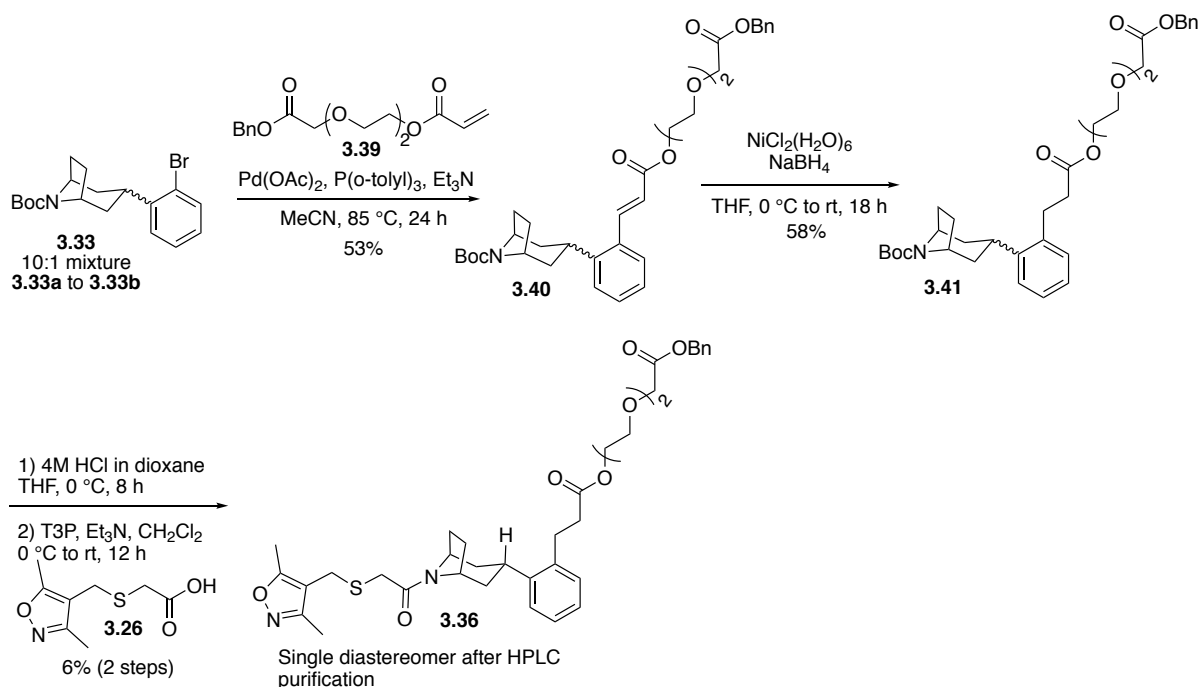
Scheme 3.14. Synthesis of **3.36**.

We hypothesized that a major source of instability was the presence of the internal ester. Therefore, we proposed analog **3.42**, which would contain an ether in place of the ester (Figure 3.15). We anticipated that this analog could be synthesized using a late stage amide coupling of carboxylic acid **3.26** and an amine precursor that can presumably be constructed by a Heck coupling with ethyl acrylate and then reduced to the propyl alcohol that can be alkylated with a PEG linker.
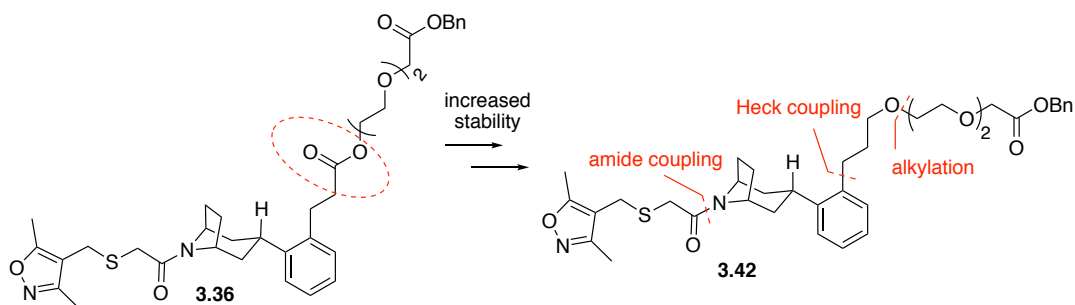
Figure 3.15. Proposed structural modifications to improve chemical stability.

Given the difficulty encountered in separating the diastereomers at the final step, we optimized the chromatographic separation after the reduction of alkene **3.28** to yield pure diastereomer **3.33a**, which, under Heck coupling conditions with ethyl acrylate, gave ester **3.43** in excellent yields (Scheme 3.15). Hydrogenation of the alkene **3.43** followed by reduction of the ester afforded alcohol **3.45**. Unfortunately, displacement of the activated mesylate and brosylate only afforded the transesterified product **3.48** with only trace amounts of the ether **3.47**.
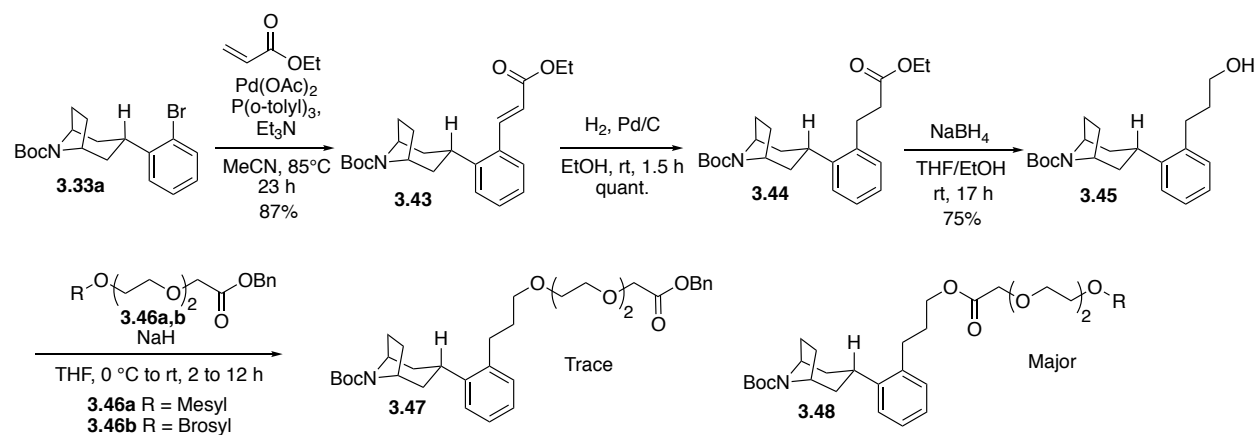
Scheme 3.15. Synthetic approach to ether intermediate **3.47**.

To circumvent issues of reactivity we decided to synthesize an analog of **3.36** containing an amide in place of the internal ester (Figure 3.16). This more stable variant containing a readily saponified aromatic ester will reduce problems of chemical intractability encountered with previous substrates. We envisioned the synthesis of this analog could be through a late stage amide coupling of an amine linker and the carboxylic acid of **3.44**.

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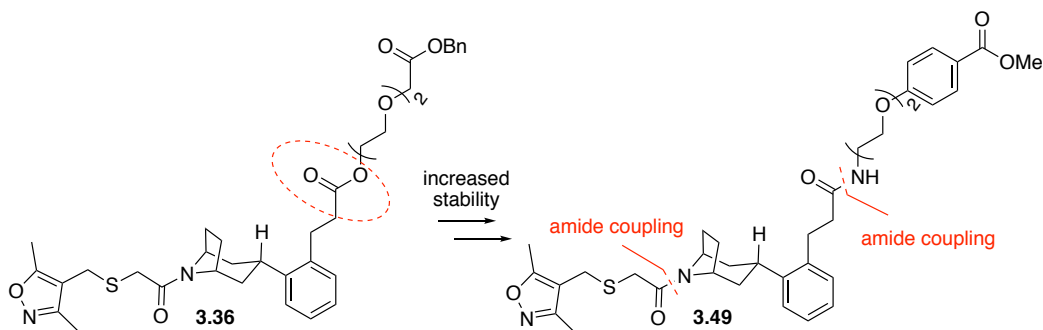
Figure 3.16. Proposed amide bead precursor **3.49**.

The synthesis of the amine linker **3.53** began with an Appel bromination of DEG (**3.37**) and displacement of the bromide with methyl paraben (**3.51**) to afford ester **3.52** (Scheme 3.16). The terminal alcohol of **3.52** was activated as the mesylate, displaced with sodium azide, and reduced under Staudinger reduction conditions to afford amine **3.53**.
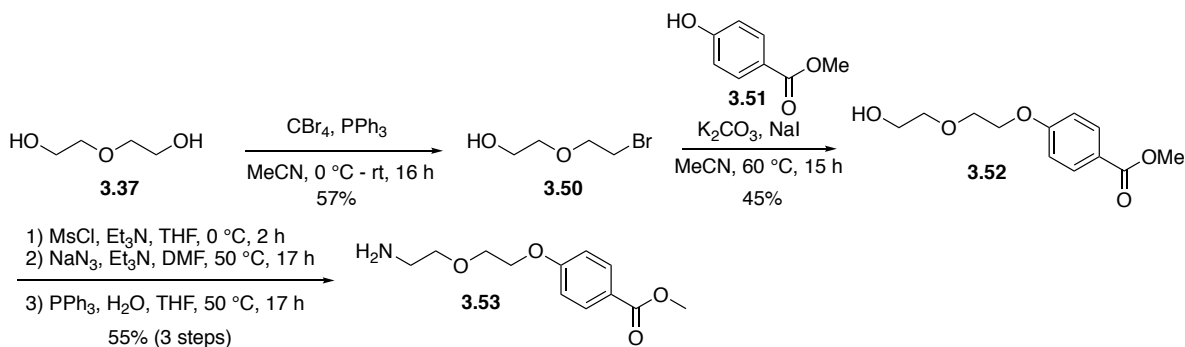
Scheme 3.16. Synthesis of amine linker **3.53**.

The previously synthesized ester **3.45** was hydrolyzed and coupled to the amine linker **3.53** to afford the amide **3.54** (Scheme 3.17). Deprotection of the Boc group and coupling with the commercially available carboxylic acid **3.26** gave the amide **3.55** in modest yields. Saponification of the methyl ester of **3.55** gave the carboxylic acid that was activated as the *N*-hydroxysuccinimide (NHS) ester and conjugated to the Carboxylink® agarose beads to afford the affinity labeled **3.57**.
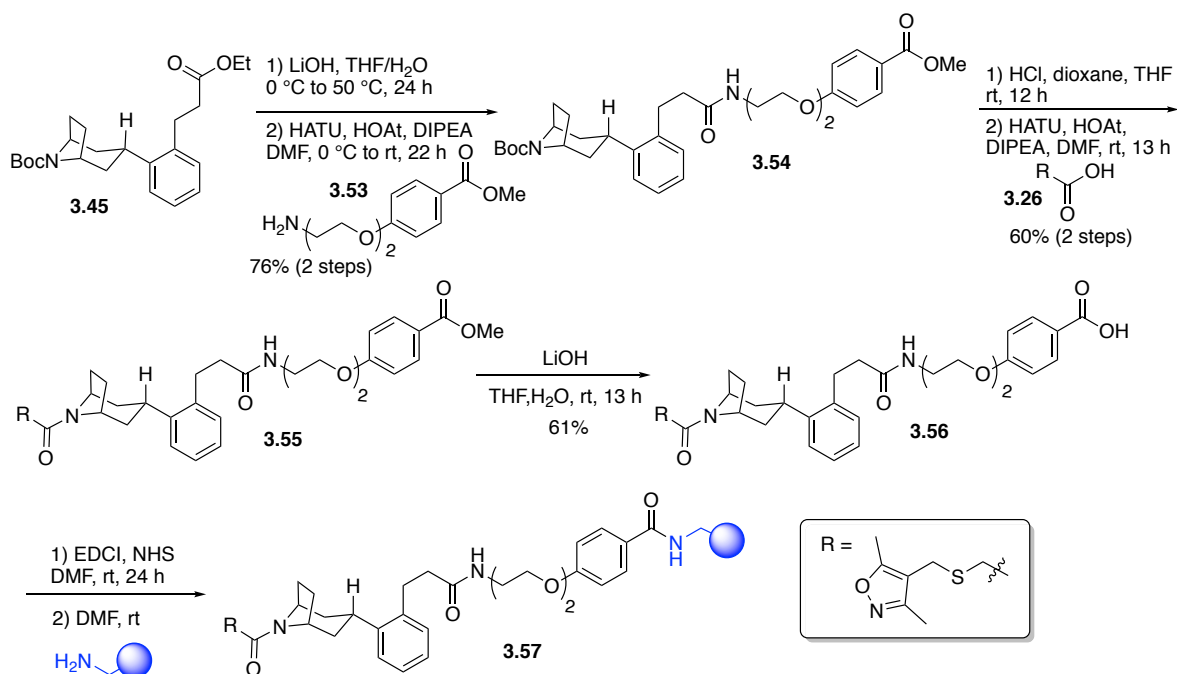
Scheme 3.17. Synthesis and conjugation of analog **3.55**.

In addition to the bridged bicyclic [3.2.1] analogs we also wanted to append a cyclopropane side chain in place of the thioether of **3.24** to test if we will see a similar increase in efficacy when attached to a piperazine containing a propargyl alcohol linker (Figure 3.17).
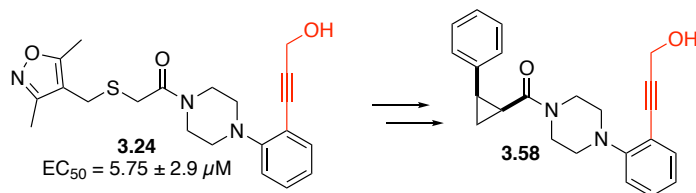
Figure 3.17. Proposed cyclopropane analog **3.58**.

The synthesis of the cyclopropane analog **3.58** began with a T3P coupling of cyclopropane carboxylic acid **3.11** and piperazine **3.59** that was previously prepared by Dr. Mustafa Kazancioglu to afford the amide **3.60** in poor yields (Scheme 3.18). The low yield in the coupling is possibly due to some decomposition of piperazine **3.59** after storage as the free base. A final TBS deprotection of **3.60** afforded the desired propargyl alcohol **3.58** in modest yields.
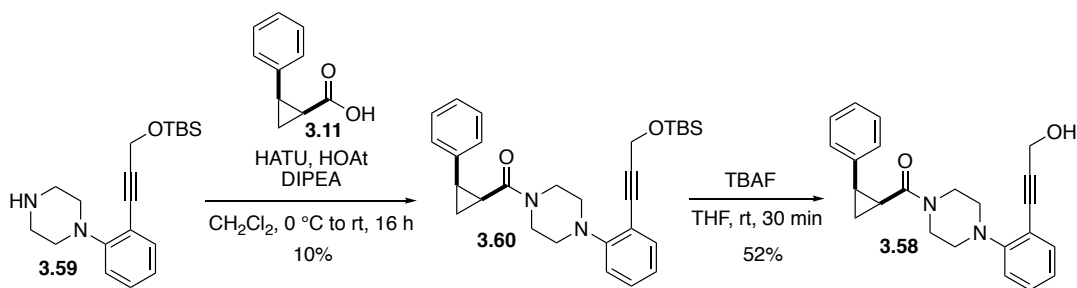
Scheme 3.18. Synthesis of cyclopropane analog affinity labeling precursor **3.58**.

3.2.5 Biological Activity of Analogs for Affinity Labeling

Following the synthesis of the analogs for affinity labeling, their biological activity was determined using a PSA luciferase assay using a Dual-Glo luciferase system in the presence of 1 nM of the synthetic androgen R1881 in C4-2-PSA-rl cells. The preliminary EC_{50}'s were calculated using GraphPad Prism. Gratifyingly, analog **3.27** was 2-fold more active compared to the alkyne **3.24** further supporting our hypothesis that the bridged bicyclic [3.2.1] system results in a 2-fold increase in activity (Table 3.13). The analog with the longer linker **3.55** was significantly less active than hybrid **3.25**. We are not sure if this is the result of a longer linker or the more flexible propyl side chain compared to the propargyl linker. Surprisingly, the cyclopropyl analog of **3.58** was 3-fold less active than **3.24** with the thioether side chain. With the promising activity of **3.25**, the conjugated beads were carried forward into whole cell pull-down assay.

Table 3.13. Biological activity of affinity precursors.

Entry	Compound	EC_{50} $(\mu M)^a$
1	**3.24**	5.8 ± 2.9^b
2	**3.25**	2.8 ± 1.0^b
3	**3.55**	11.1 ± 4.3^b
4	**3.58**	14.9 ± 9.1^b

^{a}EC_{50}'s were determined using a cell based luciferase assay as previously described.[349] EC_{50} refers to the concentration that results in 50% inhibition of PSA-luciferase activity. Each EC_{50}

was calculated using GraphPad Prism, and are the average ± stdev of two independent assays using triplicate determinations at each concentration for each experiment.

A subsequent pull-down experiment conducted in the Wang lab using C4-2 whole cell lysates. Cell lysates were precleared with acetylated control beads and incubated with or without 50-fold **3.5**. Levels of AR were detected using electrophoresis followed by western immunoblotting experiments with an anti-AR antibody. Using the control beads in the presence or absence of **3.5** there is no observed pull-down of AR. In contrast, using **3.34** beads resulted in AR pull-down. Additionally, compound **3.5** was found to competitively block the AR pull-down of the **3.34** beads.
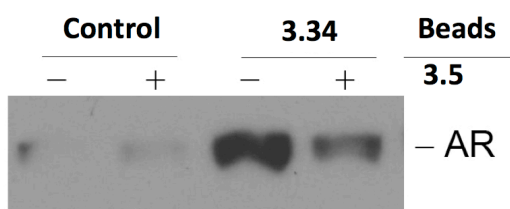
Figure 3.18. Affinity purification of AR from C4-2 whole cell lysate using immobilized **3.34**.

3.3   CONCLUSIONS

In conclusion, compound **3.5** was discovered in a HTS to reduce endogenous levels of AR in both the cytoplasm and the nucleus. An SAR of **3.5** was established using 5 zones of structural diversification that resulted in the synthesis of **JJ450** a new, more potent analog than the initial HTS hit **3.5** that contained a *cis*-cyclopropane in place of the potentially metabolically labile thioether side chain. **JJ450** was administered to nude mice bearing s.c. prostate cancer (LnCaP, VCaP, 22Rv1) xenografts via o.g and i.p to determine the *in vivo* efficacy. Gratifyingly, **JJ450** was found to have modest therapeutic efficacy with no toxicity in the xenograft models and was

even more efficacious than enzalutamide in a comparison study. A resolution of **JJ450** afforded the separated enantiomers, which were unambiguously assigned by X-ray crystallography. A preliminary xenograft study using the separated enantiomers indicated that the (1*R*,2*S*)-**JJ450** was more efficacious *in vivo*, contrary to the trend observed with the *in vitro* data. It was determined that **JJ450** had a poor metabolic half-life in liver microsomes and in a effort to improve metabolic stability 43 analogs were synthesized with varying substituents to decrease metabolism and increase half-life. Although increased potency was not achieved, we identified compound **3.21l** that was 40-fold more stable in liver microsomes compared to the parent **JJ450** with only a 3-fold decrease in activity. A subsequent xenograft study is currently underway using the resolved enantiomers of **3.21l**.
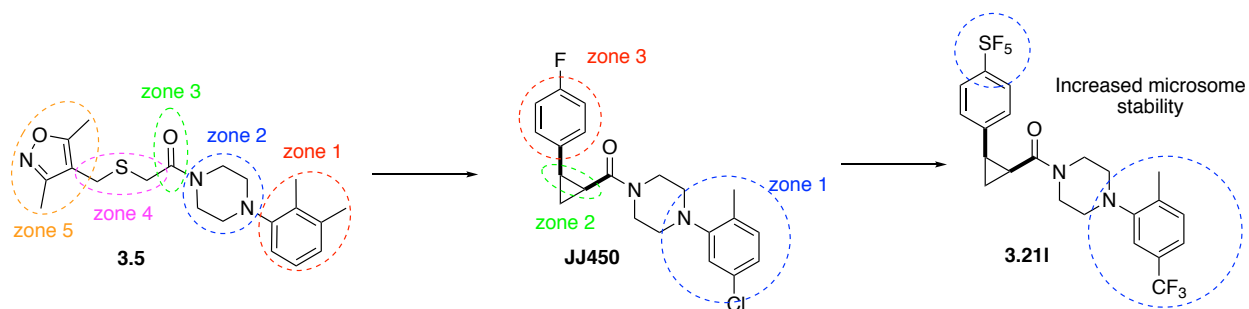
Figure 3.19. Structural improvements leading to compound **3.21l**.

Several analogs were synthesized as probes for the determination of mechanism of action. In particular, we discovered that **3.25**, a structural hybrid of **3.6p** and **3.24** had an EC_{50} of 2.8 μM that was conjugated to an agarose bead through a carbamate linker. Additionally, an analog of **3.55** containing a longer linker was synthesized and was found to have a 3-fold reduction in activity, and was also conjugated to the agarose beads for comparison to **3.25**. Interestingly, we found that the cyclopropyl side chain performed poorly on the analogs containing the propargyl alcohol linker when compared to those containing the thioether side chain. This indicated that the more flexible thioether allows for better binding with the more

rigid alkyne handle. A preliminary pull-down assay using bead conjugate **3.34** revealed that AR

was retained by the affinity reagent **3.34** and was competitively blocked by **3.5**.

4.0   EFFORTS TOWARDS THE TOTAL SYNTHESIS OF 9-

DESMETHYLPLEUROTIN

4.1   INTRODUCTION

The natural product pleurotin was first isolated from the fungus *Pleurotus griseus* in 1947. In preliminary biological evaluations it was found to inhibit *Staphylococcus aureus* along with several other gram-positive bacteria.[432] Although the structure of pleurotin was unknown at the time of isolation, Kavanagh and coworkers were able to determine several structural characteristics by chemical degradation studies.[432] The full structure was eventually elucidated in 1974 by NMR and later confirmed in 1981 by X-Ray crystallography.[433-434] Pleurotin has also been isolated from the fungus *Hohenbuehelia geogenius* and has demonstrated moderate antitumor activity against Ehrlich ascites carcinoma, L-1210 lymphoid leukemia and a spontaneous mammary tumor in mice.[435-436] More recently, pleurotin has been shown to inhibit bacterial peptidoglycan biosynthesis, a potential target for resistant infections.[437] Synthetically challenging structural features of the complex hexacyclic framework of pleurotin includes a redox labile *para*-quinone moiety with two adjacent β-leaving groups, and the presence of 8 contiguous stereocenters (Figure 4.1).
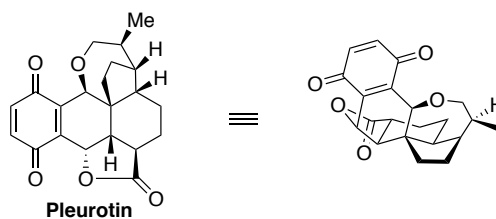
Figure 4.1. Structure of pleurotin.

Although pleurotin performed poorly in the National Cancer Institute (NCI) 60 cancer cell line panel having a GI_{50} of 20 µM and no selectivity toward tissue type, it has been a target of interest for many years as it is a potent irreversible inhibitor of the thioredoxin-thioreductase (Trx/TrxR) system (IC_{50} = 170 nM).[438-440] The Trx/TrxR system is important for the regulation of reactive oxygen species (ROS) and various redox processes, synthesis of deoxyribonucleotides, growth factor signaling, cytokine function, and protein biosynthesis.[441] The TrxR's contain a selenocysteine, which is essential for their redox activity.[441] In hypoxic tumors, Trx-1 is over-expressed leading to increasing levels of hypoxia-inducible factor-1α (HIF-1α). The hypoxia-activated transcription factor HIF-1 is the heterodimer of HIF-1α and HIF-1β and is responsible for the transcription of genes leading to angiogenesis, cell proliferation and survival, vasculogenisis, glucose metabolism, and iron homeostasis.[442-443] The activity of the HIF-1 complex is directly correlated to levels of HIF-1α, and pleurotin has been shown to reduce levels of HIF-1α and decrease HIF-1 transactivation both *in vitro* and *in vivo* through inhibition of TrxR.[439] Since hypoxia is a common feature that promotes the survival and metastasis of most cancerous tumors, a therapeutic treatment that targets hypoxic tumors is highly desirable.

In 2006, the NCI expressed renewed interest in pleurotin as a cancer therapeutic; however, the short supply of pleurotin largely limited further development. Previous isolations from *Hohenbuehelia atrocaerulea* were extremely low yielding (1-2 mg/L) and took over 2

months. Shipley and coworkers optimized a fermentation process of *H. atrocaerulea* to afford ~300 mg/L over 5 weeks resulting in the isolation of more than 10 g of pleurotin. Due to the long fermentation times, this optimization was accomplished over 5 years.[440]

Like many of the redox active natural products, the proposed mechanism of action of pleurotin is through bioreductive alkylation[444] and cross-linking of TrxR. In hypoxic cells, pleurotin can undergo a 2-electron reduction to the hydroquinone leukopleurotin **4.1** that can β-eliminate to the intermediate quinone methide **4.2**, a highly reactive Michael acceptor (Scheme 4.1). Quinone methide **4.2** can either tautomerise to dihydropleurotin acid, a proposed intermediate in the biosynthetic pathway, or be trapped with a nucleophile to afford the intermediate hydroquinone **4.3**. A subsequent β-elimination of the oxacycloheptane ether of **4.3** unveils a second quinone methide **4.4** that can undergo nucleophilic trapping to afford the hydroquinone **4.5**. Interestingly, bioreductive alkylating agents have been found to be selective for hypoxic tumor cells, and this is a mechanism of action observed with a number of FDA approved cancer treatments, including mitomycin C, which is used in combination therapies for the treatment of various solid tumors.[445]
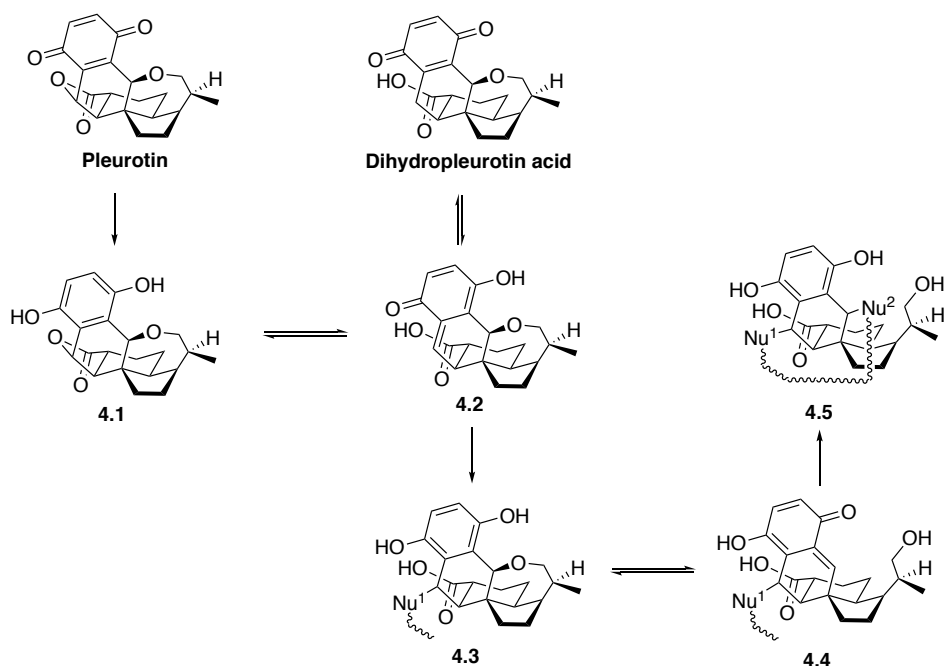
Scheme 4.1. Proposed bioreductive alkylation mechanism of pleurotin.

4.1.1 Previous Syntheses: Hart

The first total synthesis of (±)-pleurotin was accomplished by Hart and coworkers in 1989 and was inspired by a novel radical cyclization methodology developed by their group to form perhydroindans (Scheme 4.2). In their initial report, the radical cyclization precursor **4.9** was prepared from a Birch reduction of *m*-toluic acid, alkylation with 4-bromo-1-butene (**4.7**), and a subsequent iodolactonization of intermediate **4.8**.[446-447] Treating intermediate **4.9** with azobisisobutyronitrile (AIBN) and tributyltin hydride delivered a mixture of products with perhydroindan **4.12** as the major component of the mixture.
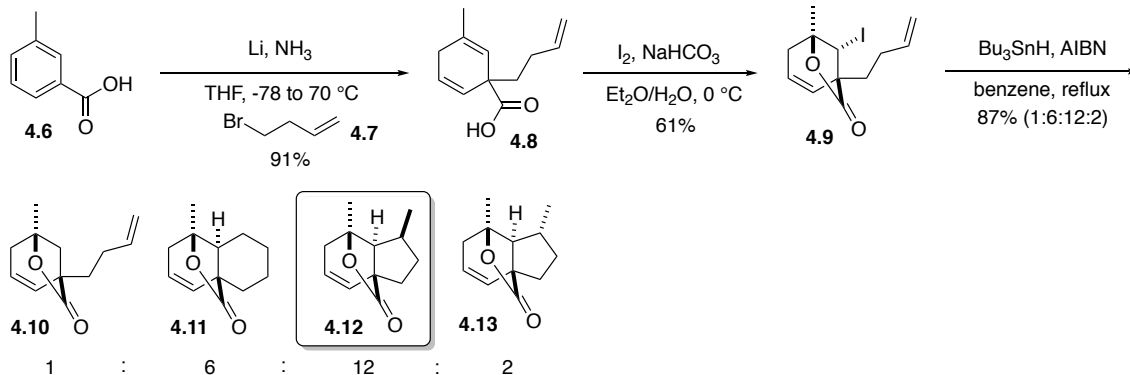
Scheme 4.2. Hart's initial radical cyclization discovery.

To showcase their chemistry, Hart and coworkers identified pleurotin as an appropriate synthetic target as its core contains the perhydroindan skeleton (Figure 4.2).
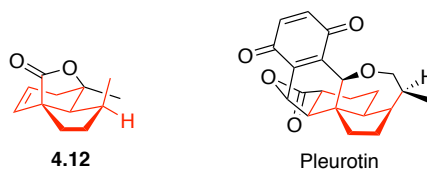
Figure 4.2. Perhydroindan scaffold **4.12** and pleurotin.

The first portion of their synthesis focused on the preparation of the key radical cyclization precursor **4.21**. The synthesis of **4.21** began with a Birch reduction of benzoic acid (**4.14**) and alkylation with bromide **4.15** (Scheme 4.3). Iodolactonization of carboxylic acid **4.16** resulted in decomposition and, instead, this transformation was accomplished using the amide **4.17**. The acetal **4.18** was deprotected with aqueous formic acid and then subjected to a Wittig olefination with ylide **4.20** to deliver the radical cyclization precursor **4.21** in excellent yields as a single diastereomer.[446-449]
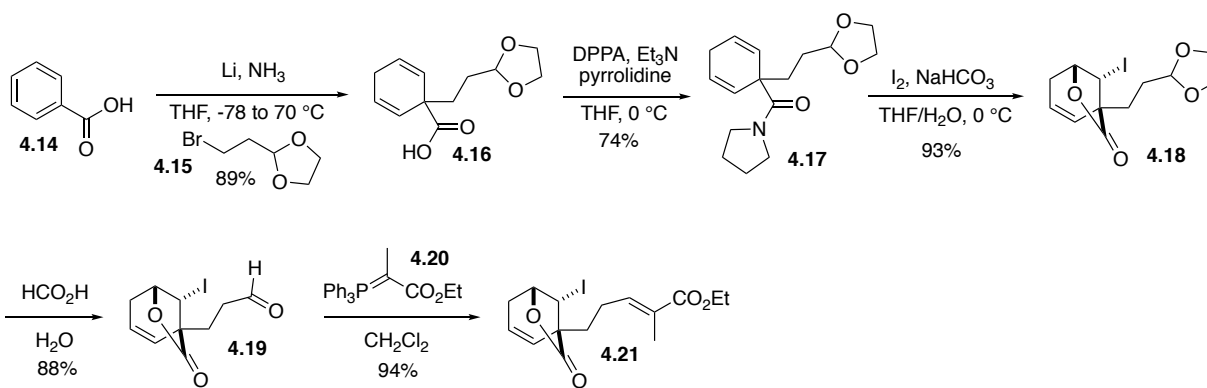
Scheme 4.3. Synthesis of radical cyclization precursor **4.21**.

Gratifyingly, the radical cyclization of **4.21** proceeded with high diastereoselectivity giving almost complete conversion to **4.23** (Scheme 4.4). To test whether the olefin geometry was responsible for the observed selectivity, the olefin with opposite geometry **4.22** was synthesized and subjected to the same reaction conditions. Interestingly, a similar ratio of **4.23** to **4.24** was obtained albeit in diminished yields. This indicated that while the alkene geometry influences the stereochemistry at C10, it is not responsible for the selectivity at C9. After conducting subsequent experiments where the alkene substituents were varied, they concluded that the stereochemistry at C9 is the result of a dipolar interaction between the two carbonyl groups.[449]
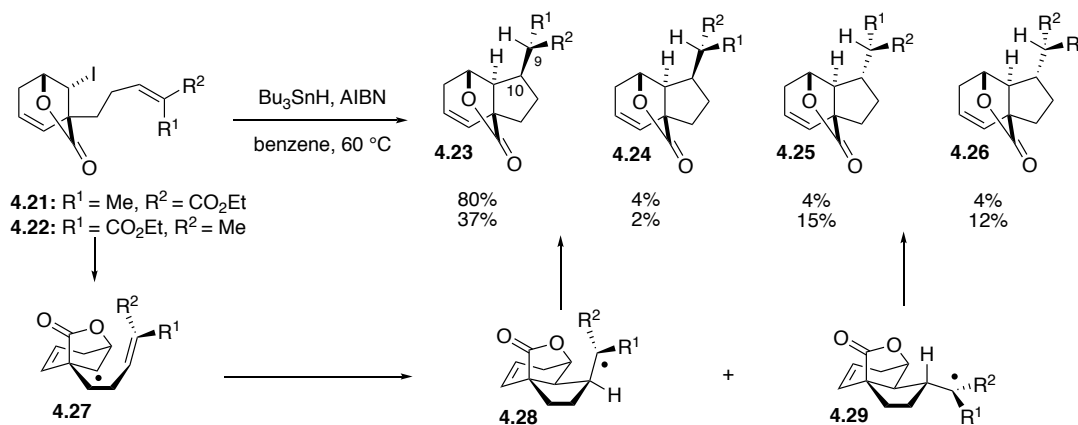
Scheme 4.4. Hart's radical cyclization.

With ample amounts of the radical cyclization product in hand, they turned their attention to modifying the oxidation states at C8 and C12 (Scheme 4.5). As the lactone is also susceptible to reduction, the ester of **4.23** was reduced over 2 steps. First, **4.23** was hydrolyzed using aqueous lithium hydroxide to form the diacid. Subsequent treatment with acid regenerated the lactone in **4.30**. The carboxylic acid was reduced by treatment with oxalyl chloride to form the acid chloride and then sodium borohydride to give an intermediate alcohol that was TBS protected to give **4.31** in excellent yields. Interestingly, the reduction of ester **4.23** to the alcohol can also be achieved in a single step using LAH in 95% yield, which is most likely due to the steric crowding of the lactone. Epoxidation of alkene **4.31** followed by lithium diethylamide mediated rearrangement of the epoxide gave the corresponding allylic alcohol **4.33**. Birch reduction of the allylic ester **4.33** resulted in opening of the lactone and a subsequent methylation with diazomethane gave the methyl ester **4.34**. Finally, Swern oxidation of the alcohol **4.34** afforded the ketone **4.35** in good yields. It was also noted that starting from **4.31**, the epoxide opening, reduction, and esterification could give **4.34** in a one-pot procedure with 59% overall yield.
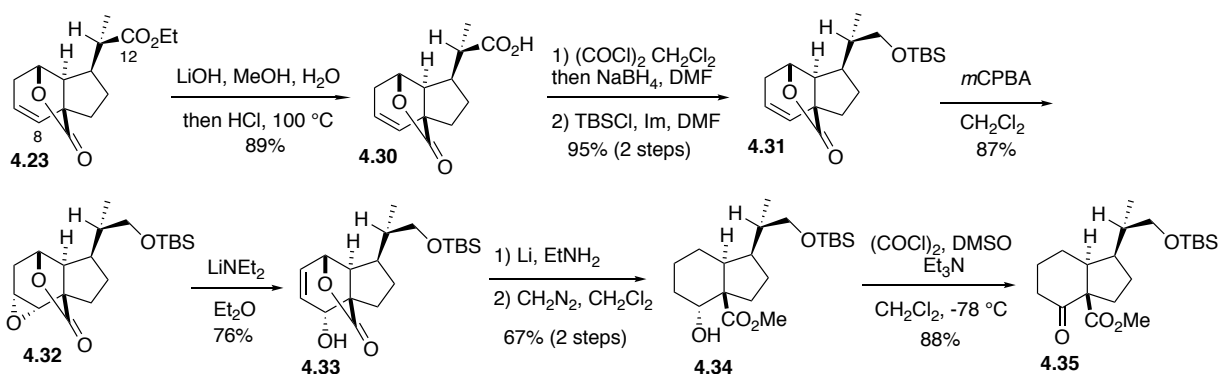
Scheme 4.5. Synthesis of ketone **4.35**.

With the ketone **4.35** in hand, Hart then focused on the formation of the oxacycloheptane ring and the pentacyclic core. Transmetalation of 2,5-dimethoxybenzyl magnesium chloride **4.36**

with cerium(III) followed by addition of the resulting organocerium reagent to ketone **4.35** afforded the alcohol **4.37** as a single diastereomer (Scheme 4.6). Subsequent elimination of the tertiary alcohol **4.37** afforded alkene isomers as an inseparable 1:1 mixture. The isomers were directly subjected to LAH reduction to give the separable alcohols **4.38** and **4.39**. Oxidation of alcohol **4.39** to aldehyde **4.40** followed by a mild acid catalyzed TBS deprotection of **4.40** using Dowex-50 in methanol provided acetal **4.41**. Treatment of acetal **4.41** with boron trifluoride etherate at low temperature initiated an intramolecular electrophilic aromatic substitution reaction to yield pentacyclic **4.42** in modest yields. Hart noted that performing the cyclization in other solvents at room temperature resulted in the formation of several rearranged products. Furthermore, it was also noted that product **4.42** was susceptible to rearrangement after prolonged exposure to acid.
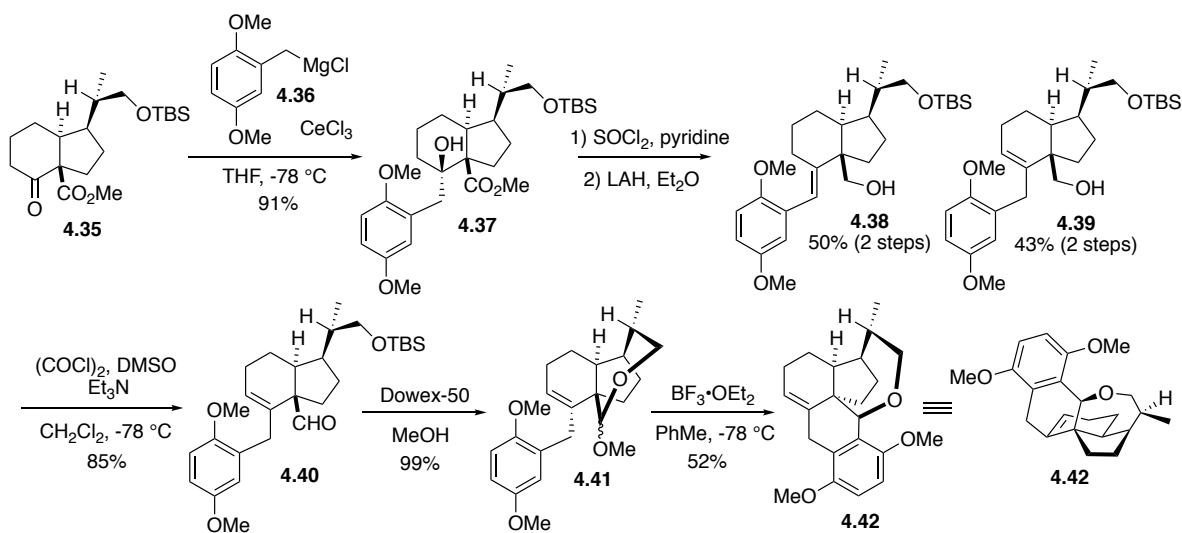
Scheme 4.6. Synthesis of pentacyclic core **4.42**.

Following the synthesis of the pentacyclic core **4.42**, Hart shifted his attention to the final installation of the lactone ring and the completion of the synthesis of pleurotin. A hydroboration of the alkene **4.42** followed by oxidation afforded an intermediate alcohol that was further oxidized to the ketone **4.43** using a Swern oxidation (Scheme 4.7). A Van Leusen reaction of

ketone **4.43** with tosylmethyl isocyanide (TosMIC) and KOtBu afforded the homologated nitrile

4.44. While direct hydrolysis of the nitrile to the carboxylic acid proved problematic, they found

that a two step protocol was effective whereby the nitrile was reduced to the corresponding

aldehyde with DIBAL-H then oxidized to the carboxylic acid **4.45** with silver (I) oxide.

Oxidation of **4.45** with ceric ammonium nitrate (CAN) in aqueous acetonitrile gave

dihydropleurotin acid in excellent yields. Initial attempts to oxidize dihydropleurotin acid using

amine bases in the presence or absence of oxygen were unsuccessful. Instead, the final oxidation

was accomplished by treating dihydropleurotin with an excess of manganese dioxide over 2 days

to give pleurotin in 32% yield along with 33% of recovered dihydropleurotin acid. Thus, the first

total synthesis of pleurotin was completed in 26 steps and 0.3% overall yield.
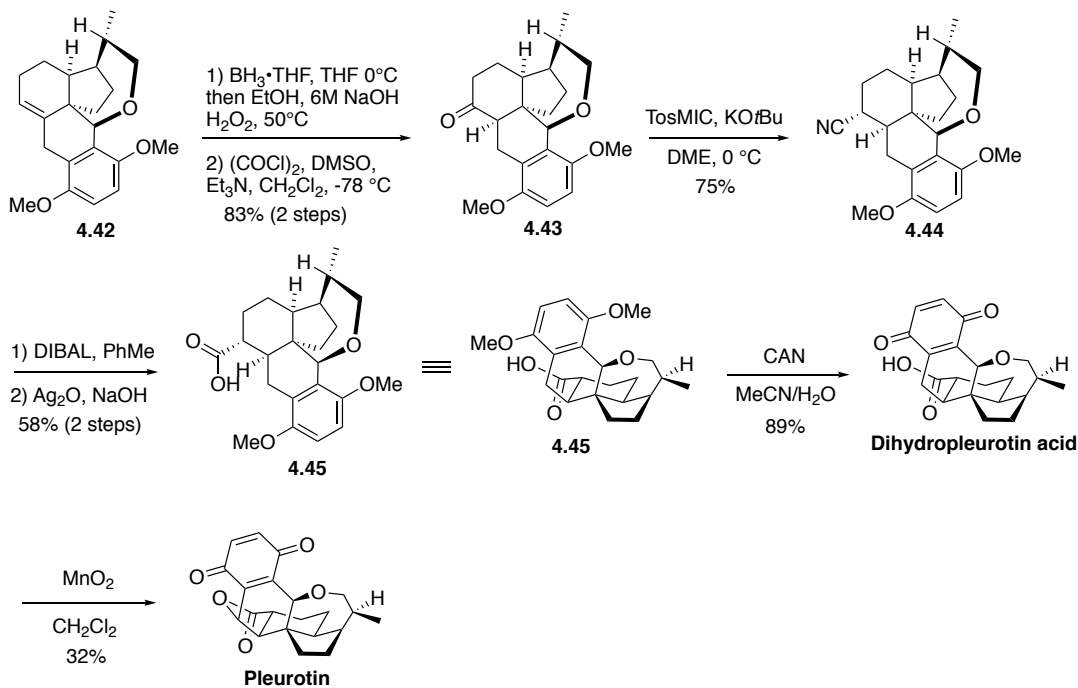
Scheme 4.7. Completion of the synthesis of pleurotin.

4.1.2   Synthesis of a Pleurotin Analog: Kraus

In 1993 Kraus and coworkers developed a photoenolization/Diels-Alder approach to an analog of pleurotin.[450-451] The synthesis began with the formation of the dianion of **4.46**, which underwent formylation with DMF followed by mild hydrolysis to afford lactol **4.47** (Scheme 4.8). Unfortunately, direct acylation of **4.47** with dihydrobenzoic acid (**4.49**) proved unsuccessful and an alternative approach was pursued where thioacetal **4.48** was prepared and readily acylated with dihydrobenzoic acid (**4.49**). Subsequent thioacetal deprotection using MeI in aqueous acetonitrile yielded aldehyde **4.50** in modest yields. With their photoenolization substrate **4.50** in hand, aldehyde **4.50** was subjected to their previously optimized photoenolization conditions to deliver intermediate **4.51**.[450-452] Gratifyingly, they found that heating the enolate intermediate **4.51** neat at 165 °C promoted the key intramolecular Diels-Alder (IMDA) reaction to afford the tetracyclic core **4.52** as a single diastereomer in modest yield over two steps. The relative configuration of **4.52** was supported by 2D-NMR analysis and molecular mechanics calculations revealed that *endo* adduct **4.52** was favored over the *exo* adduct by 1.6 kcal/mol. Finally, oxidation of **4.52** to the quinone **4.53** was accomplished using silver (II) oxide and nitric acid in THF.[451]
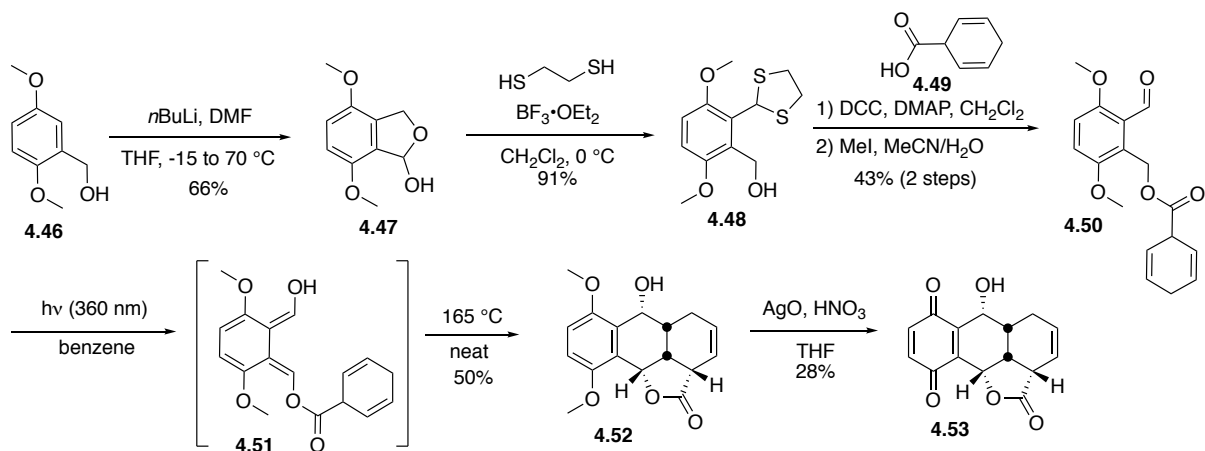
Scheme 4.8. Synthesis of pleurotin analog **4.53**.

Biological testing revealed that quinone **4.53** was active against a SR leukemia cell line (GI_{50} = 4.67 μM) and most colon cancer cell lines (GI_{50} = 22.4 μM to 17.0 μM). These activities are comparable to pleurotin, which has a mean value of GI_{50} of 3.09 μM against SR leukemia and 6.76 μM against colon cancer cell lines.[451]

4.1.3 Previous Synthetic Work: Wipf Group

The Wipf group has ongoing interest in the synthesis and biological evaluation of pleurotin and other quinoid inhibitors of the Trx/TrxR system.[453-457] Early synthetic efforts toward the total synthesis of pleurotin within the Wipf group were pioneered by Drs. Sonia Rodriguez, Shinya Immura, and Stephan Elzner. Retrosynthetically, the original approach to pleurotin featured a late stage reductive etherification to form pleurotin from intermediate **4.54** (Scheme 4.9). Intermediate **4.54** would then be formed by a key IMDA reaction of **4.55**. Diels-Alder precursor **4.55** could be constructed via a Horner Wadsworth Emmons reaction with aldehyde **4.56**, which in turn cold be formed from an Ireland-Claisen rearrangement of **4.57**. Lastly, it was envisaged

that intermediate **4.57** could be formed by a hydrozirconation-transmetalation-addition sequence of propargyl alcohol **4.58** to aldehyde **4.59**.
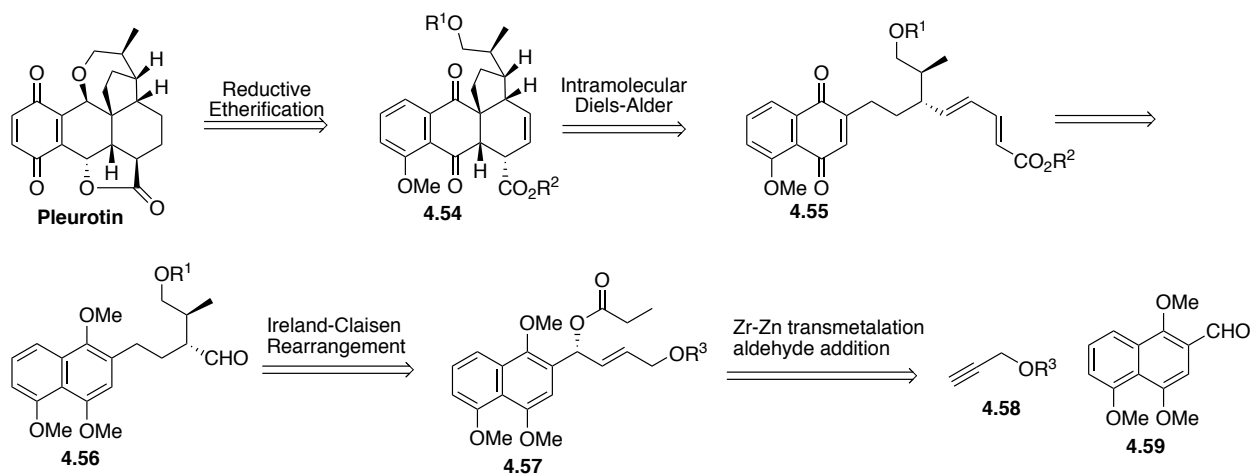
Scheme 4.9. Initial retrosynthetic approach to pleurotin.

The initial synthetic route used the known bromo-naphthylene **4.60** prepared from 1,5-naphthalenediol in 5 steps.[458] Lithium-halogen exchange of bromide **4.60** followed by formylation with *N,N'*-dimethylformamide gave the aldehyde **4.59 (**Scheme 4.10). Propargyl alcohol **4.61** was subjected to hydrozirconation with Cp_2ZrHCl followed by transmetalation to the corresponding organozinc species. Addition of the resulting alkenylzinc reagent to aldehyde **4.59** afforded the allylic alcohol **4.62** in excellent yields. Acylation of alcohol **4.62** and enolization set the stage for a subsequent Ireland-Claisen rearrangement to produce carboxylic acid **4.63**. Hydrogenation of the alkene **4.63**, followed by reduction of the carboxylic acid, benzylation of the corresponding alcohol, and removal of the TBDPS protecting group gave alcohol **4.64** in excellent yield over 4 steps. Alcohol **4.64** was oxidized to the corresponding aldehyde and treated with phosphonate **4.65** in a HWE reaction to install the diene fragment of **4.66**. The naphthalene was oxidized to the naphthoquinone **4.66** using CAN to deliver the IMDA precursor **4.66**. Gratifyingly, treatment of **4.66** with boron trifluoride etherate yielded the IMDA adduct that was subsequently reduced using sodium borohydride with an acidic workup to give

lactone **4.67** with unknown configuration at C10. To elucidate the configuration at C10, **4.67** was hydrogenated giving both reduction of the alkene and debenzylation in a single step. Epimerization of the intermediate alcohol at C14 under basic conditions afforded the thermodynamically favored *trans* lactone. The free alcohol was then acylated with *p*-bromobenzoyl chloride yielding the crystalline product **4.68** whose structure was confirmed by X-ray crystallographic analysis. Unfortunately, the configuration at C10 was the opposite of the natural product and thus, a route to invert this stereogenic center was pursued.
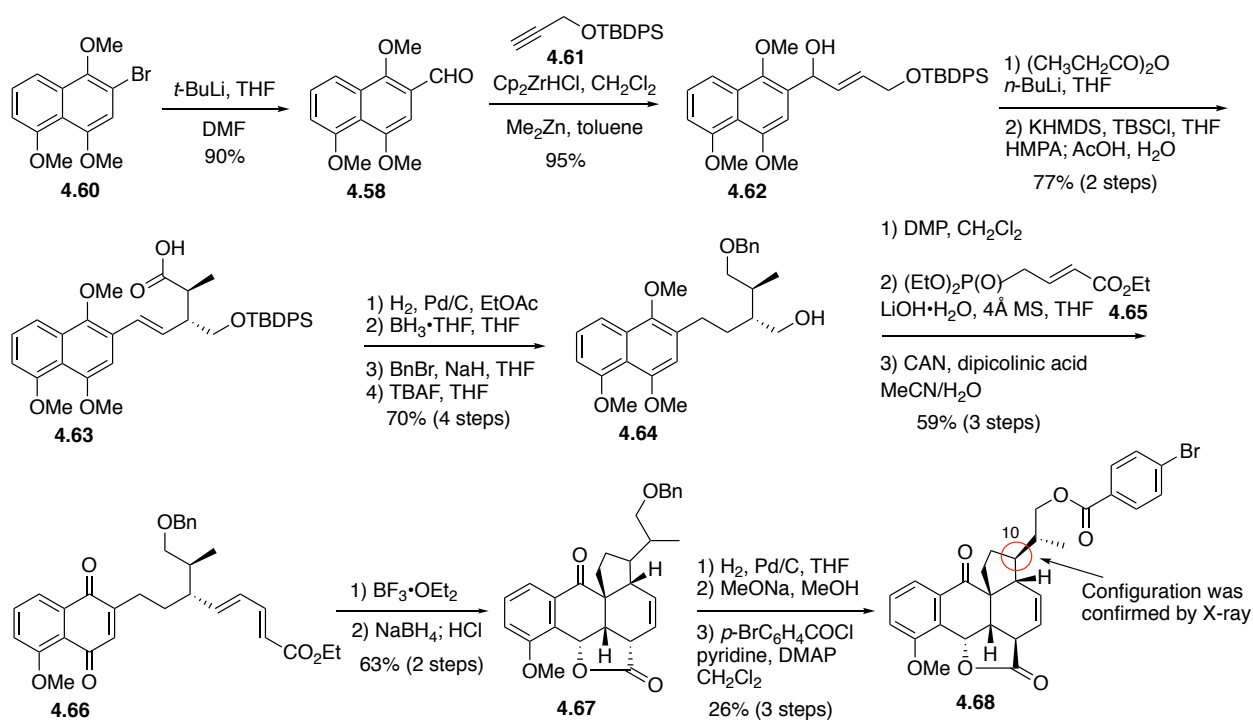
Scheme 4.10. Early synthetic studies towards pleurotin.

The strategy to invert the configuration was to first oxidize the side chain to the α,β-unsaturated aldehyde **4.70** (Scheme 4.11). Based on the caged nature of the structure, it was proposed that a reduction from the least hindered face would yield the desired stereochemistry at C10.
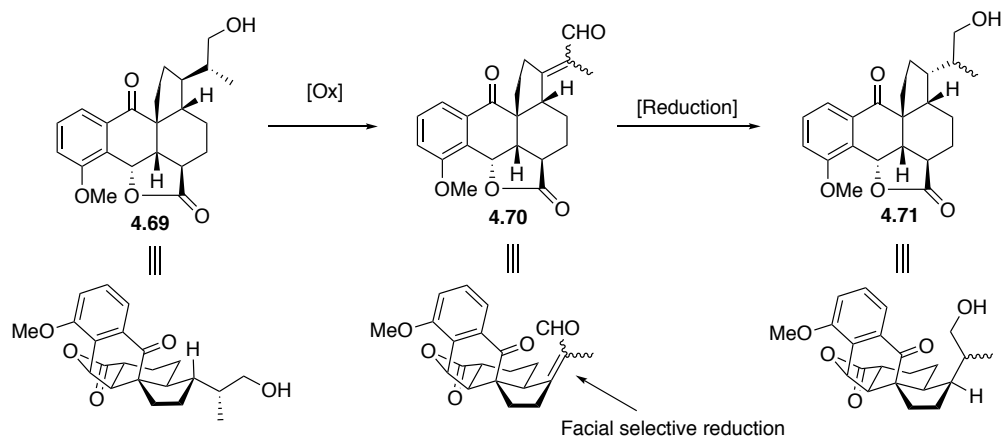
Scheme 4.11. Synthetic approach to side chain inversion.

Unfortunately, direct one step oxidations of **4.69** to the α,β-unsaturated aldehyde **4.70** were unsuccessful (Scheme 4.12). Instead a stepwise approach was developed beginning with Dess-Martin periodinane (DMP) oxidation to the aldehyde **4.72**. α-Selenation of **4.72** and oxidative elimination afforded the undesired α,β-unsaturated aldehyde isomer **4.73**. Unfortunately, attempts to isomerize **4.73** to **4.70** did not yield the desired product.
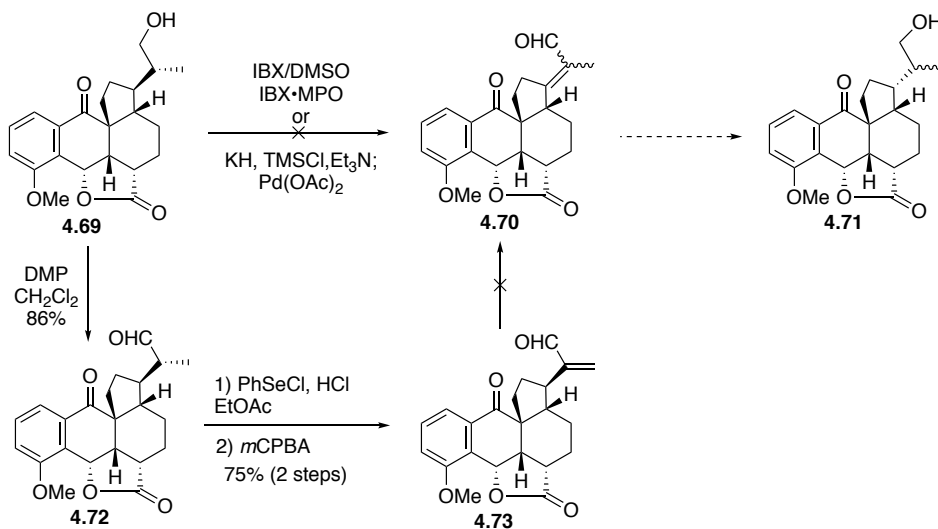
Scheme 4.12. Attempts to invert C10 stereochemistry.

An alternative alcohol elimination/alkene isomerization approach was also investigated (Scheme 4.13). Unfortunately, while the elimination of the alcohol **4.69** proceeded smoothly to afford alkene **4.74** in 75% yield over two steps, all attempts to isomerize the alkene to form the

tetrasubstituted exocyclic olefin failed. Acid mediated conditions resulted in isomerization of the alkene into the ring to give **4.75** and no reaction was observed when **4.74** was subjected to an isomerization with Grubbs catalyst. Given the numerous difficulties encountered in attempts to invert the configuration of the C10 stereocenter, the first-generation route was deprioritized and a second-generation approach was developed.
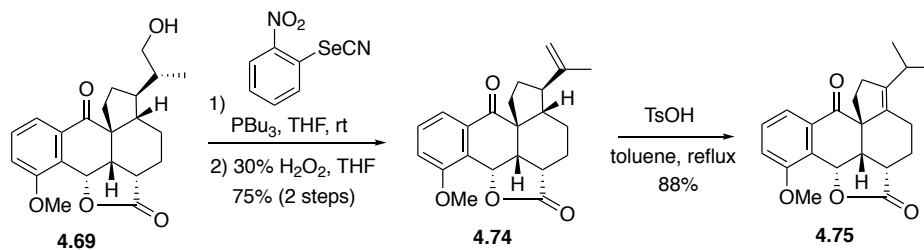
Scheme 4.13. Attempted alkene isomerization.

The second-generation retrosynthetic approach retains the late stage reductive etherification reaction to form pleurotin from intermediate **4.54** (Scheme 4.14). To circumvent the problematic inversion of the C10 stereocenter in the first approach, a key displacement of alcohol **4.76** could be implemented to install the inverted side chain. The tetracyclic core **4.76** would be formed via an IMDA reaction in an analogous manner to the previous route. It was envisioned that the Diels-Alder precursor **4.77** could be formed via a hydrozirconation/transmetalation of alkyne **4.79** and addition to aldehyde **4.78**.
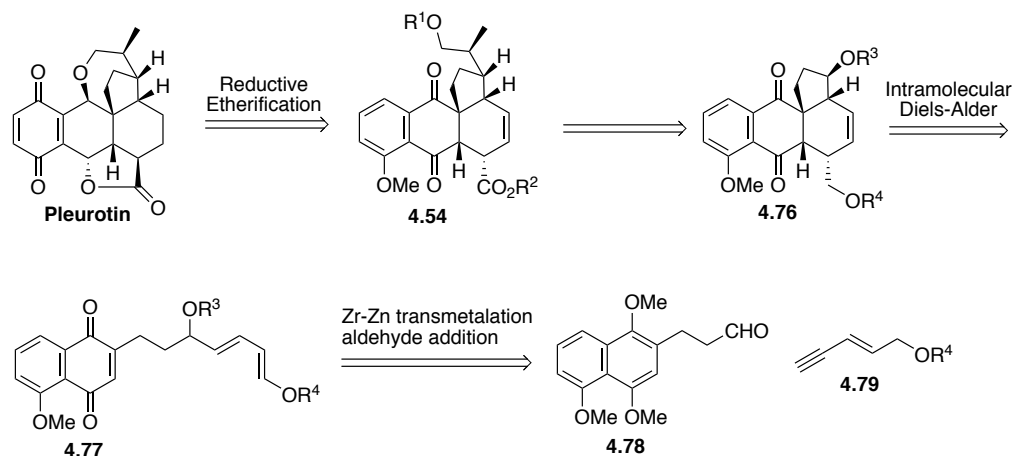
Scheme 4.14. 2nd generation retrosynthetic approach to pleurotin.

The 4-step synthesis of aldehyde **4.78** began with a Dötz benzannulation of triisopropylsilyl (TIPS) protected 1-pentynol **4.81** and the chromium Fischer carbene **4.80** followed by methylation of the naphthol intermediate to produce **4.82** (Scheme 4.15). TBAF desilylation of **4.82** and Swern oxidation of the intermediate alcohol gave aldehyde **4.78** in excellent yields.
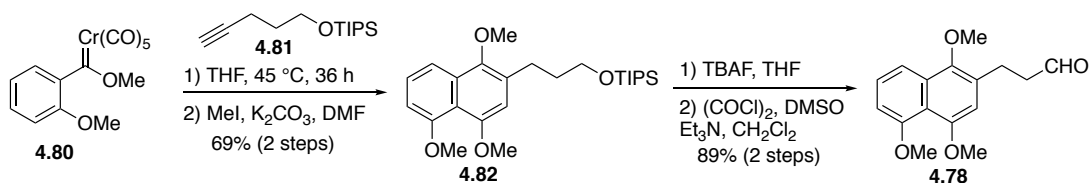
Scheme 4.15. Synthesis of naphthalene **4.78**.

The synthesis of alkyne fragment **4.86** was accomplished in 4 steps and began with the silylation of propargyl alcohol (**4.83**). Hydrozirconation/iodination of alkyne **4.61** afforded the *E*-vinyl iodide that was coupled to TMS-acetylene using Sonogashira coupling conditions to give alkyne **4.85**. Desilylation of alkyne **4.85** afforded the desired enyne **4.86**.
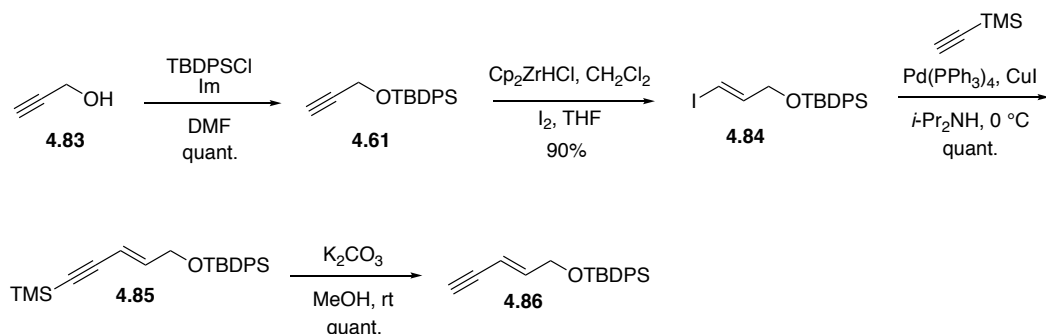
Scheme 4.16. Synthesis of enyne fragment **4.86**.

Hydrozirconation of alkyne **4.86** followed by transmetalation, and addition to aldehyde **4.78** went smoothly in 89% to give the diene **4.87** (Scheme 4.17). Benzylation of the allylic alcohol **4.87** and oxidation using CAN gave key napthoquinone **4.88** setting the stage for pivotal IMDA reaction. In contrast to the IMDA of the previous route, the Lewis acid catalyzed reaction was unsuccessful. Instead, the IMDA was accomplished thermally by heating **4.88** at 200 °C for 3 h to afford the product **4.90** as a single diastereomer in excellent yields. One-pot hydrogenation of the alkene and debenzylation gave alcohol **4.91**. Acylation of **4.91** produced the crystalline ester **4.92** that was unambiguously assigned by X-ray analysis.
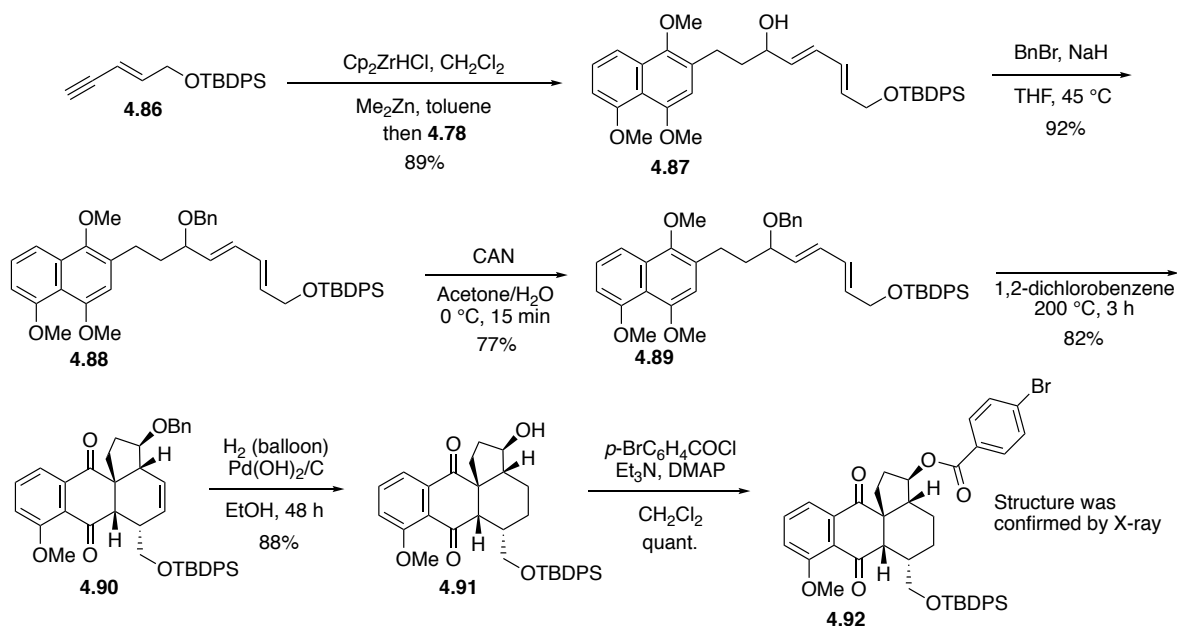
Scheme 4.17. Synthesis of alcohol **4.91**.

Unfortunately, all attempts to install the side chain on **4.76** or cyclize the oxacycloheptane ring were unsuccessful, forcing alteration of the synthetic strategy to install and invert the C10 side chain in a third generation approach (Scheme 4.18).
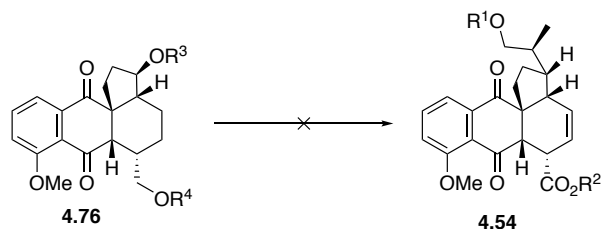
Scheme 4.18. Attempts at installation of the side chain at C10.

4.2   RESULTS AND DISCUSSION

4.2.1   Retrosynthetic analysis

Given the difficulties encountered in inverting the C10 configuration in the presence of the C9 methyl group, we envisioned that we could initially develop a synthetic route to 9-desmethylpleurotin and subsequently develop a late stage introduction of the methyl group at C9 (Figure 4.3).
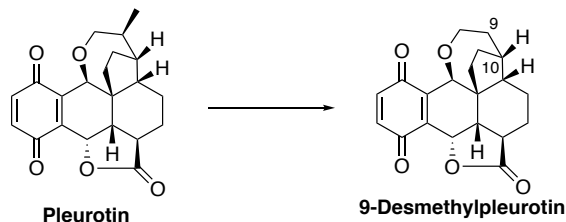
Figure 4.3. Proposed model system of pleurotin, 9-desmethylpleurotin.

Our third generation retrosynthesis of 9-desmethylpleurotin is outlined in Scheme 4.19. Our approach retains the late stage reductive etherification to close the oxacycloheptane ring. We

expect lactone epimerization will deliver the desired *trans* lactone from **4.93**. Inversion of the

C10 stereocenter leads retrosynthetically to Diels-Alder precursor **4.94**. In the absence of the C9

methyl group, we envisioned a more facile inversion of the C10 configuration in **4.94**.

Analogous to previous routes, we propose that **4.94** can be formed via an IMDA reaction from

the diene naphthaquinone **4.95**. The naphthalene **4.95** would be constructed using a Dötz

benzannulation of the chromium Fischer carbene **4.80** and lactone **4.97**. We envisioned that

butyrolactone **4.97** would serve as an easy handle for the construction of the diene fragment in

4.95.
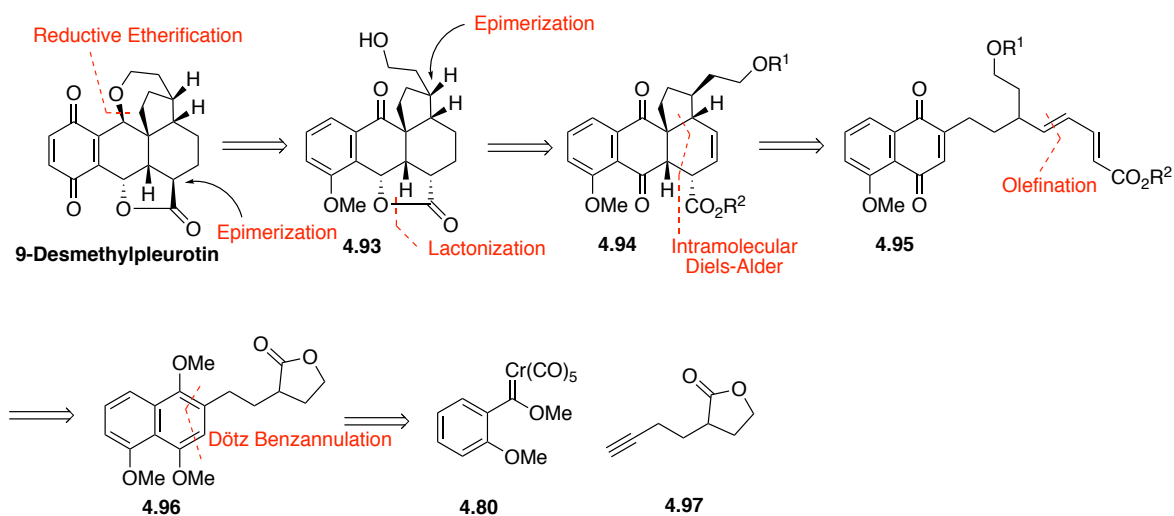
Scheme 4.19. Retrosynthetic approach to 9-desmethylpleurotin.

4.2.2 Approach 1: Synthesis of γ-Butyrolactone 4.97

Our initial approach to synthesize lactone fragment **4.97** was based on a direct α-alkylation of

the γ-butyrolactone (**4.100**) (Scheme 4.20). Synthesis of bromide **4.99** began with the TMS

protection of the dianaion of 1-butynol (**4.98**), mild hydrolysis of the TMS ether, and a

subsequent Appel bromination.[459-461] Many reaction conditions (solvents, temperature, times,

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bases, additives) were screened for the alkylation of **4.100** including the use of the iodide as the coupling partner. Unfortunately, all conditions resulted in little or no formation of the desired product. Enyne **4.102** was identified as the main byproduct in many cases. At best, the desired product **4.101** was formed in 20% yield along with elimination byproduct **4.102** using LDA/HMPA enolization conditions. However, attempts to scale up this reaction were met with even lower yields of **4.101**.
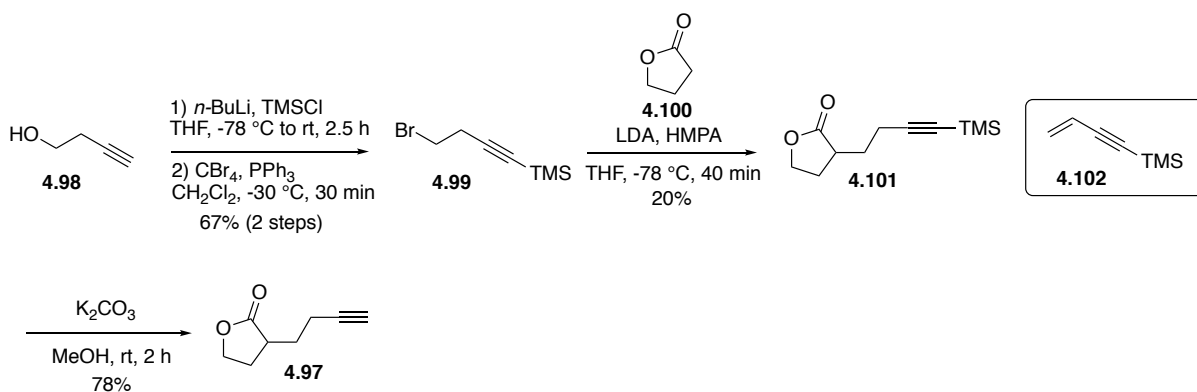
Scheme 4.20. Initial synthetic route to alkyne **4.97**.

Given the difficulty in obtaining meaningful quantities of lactone **4.101**, we investigated alternate approaches to form lactone **4.97**. Hoping to circumvent the issue of base mediated elimination that dominated the alkylation of **4.100**, we designed a new approach using activated lactone **4.103** (Scheme 4.21). We reasoned that alkylation of **4.103** should be favored using a mild base, hopefully avoiding the undesired elimination pathway that predominated under strongly basic conditions. The activated lactone **4.103** was rapidly synthesized by acylation of **4.100** with dimethyl carbonate.[462] With facile access to **4.103** we began to investigate alkylation conditions. After screening a variety of alkylating agents, we determined that the tosylate **4.104** was superior over the corresponding mesylate, bromide, or iodide in both synthetic tractability as well as performance in the reaction. Additionally, after screening a number of bases, solvents, and additives, it was determined that K_2CO_3, NaI in DMF at 60 °C proved optimal; however, on

scale the maximum yield was 30%. Even with a low yield we had enough of this material to test subsequent transformations in the synthesis. The alkylated product **4.105** was subjected to Krapcho decarboxylation conditions to give our desired lactone **4.97**.
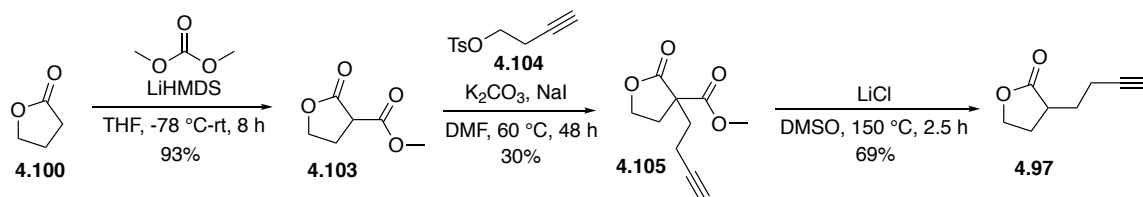
Scheme 4.21. Synthesis of alkyne **4.97**.

4.2.3   Approach 1: Investigation of the Key Dötz Benzannulation and IMDA Sequence Using Lactone 4.97

The chromium Fischer carbene **4.80** was prepared using a one-pot literature protocol starting with lithium-halogen exchange of 2-bromoanisole (**4.106**), addition of the resulting anion to chromium hexacarbonyl, and methylation using Meerwein's reagent (Scheme 4.22).[463] Subjecting lactone **4.97** to the Dötz benzannulation yielded naphthalene **4.96** in a modest yield of 41%. Attempts to optimize this reaction were unsuccessful in increasing the overall yield past 41% and the reaction profile was generally complex. Other methylating reagents such as dimethyl sulfate resulted in multiple methylations of the naphthalene substrate **4.96**. Reduction of the lactone **4.96** to the corresponding lactol worked well using DIBAL-H;[464] however, the subsequent HWE reaction with phosphonate **4.65** proved low yielding, forming diene **4.107** in 30% over 2 steps.[465]

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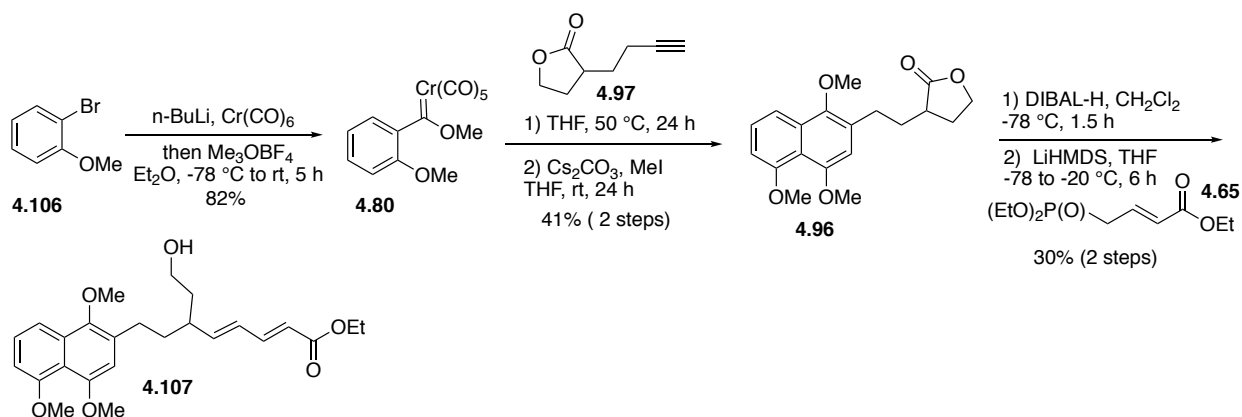
Scheme 4.22. Synthesis of diene **4.107**.

4.2.4 Approach 2: Synthesis of γ-Butyrolactone 4.110.

We reasoned that the presence of the free primary alcohol could be interfering in the diene formation sequence, contributing to the low isolated yield of **4.107**. Therefore, we decided to protect it prior to installation of the diene fragment. In order to introduce a protecting group, we needed to redesign the lactone precursor. In this second-generation approach, we envisioned that **4.95** could be synthesized from the nitrile **4.108** (Scheme 4.23). We reasoned that using a smaller activating substituent on the γ-butyrolactone would increase yields by decreasing steric hindrance in the enolate formation and alkylation. The nitrile was an ideal choice since it is smaller in size compared to the methyl ester, electron-withdrawing, and can be converted to the necessary aldehyde by treatment with DIBAL-H. Analogous to the previous routes, naphthalene **4.108** could be synthesized via a Dötz benzannulation of carbene **4.80** and alkyne **4.109**. Alkyne **4.109** can be prepared from the lactone **4.110**.
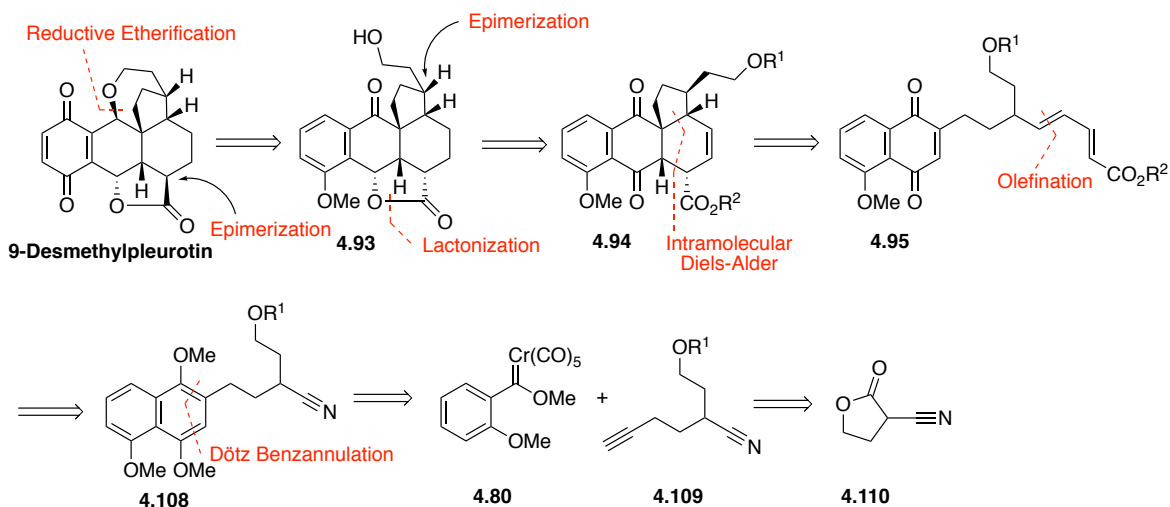
Scheme 4.23. 2nd Generation retrosynthesis of 9-desmethylpleurotin.

An extensive search of the literature yielded only three methods for the synthesis of α-cyanosubstituted γ-butyrolactones.[466-468] Unfortunately, these literature protocols did not appear amenable to a scalable synthesis of this building block due to low yields or safety concerns. Instead, we discovered a method developed by Rao *et al.* for the rearrangement of cyclopropane-1,1-dicarboxylic acid **4.111** with triethylammonium bromide to deliver 2-carboxy-γ-butyrolactone **4.112** (Scheme 4.24).[469] Encouraged by Rao's work we wanted to see if this transformation could be applied to a cyano-substituted cyclopropane **4.113**.
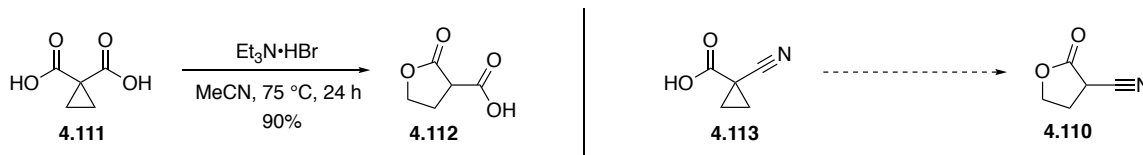
Scheme 4.24. Proposed synthesis of cyano-substituted lactone.

The synthesis of cyclopropane **4.113** began with alkylation of ethylcyanoacetate (**4.114**) with 1,2-dibromoethane to give cyclopropane ester **4.115** (Scheme 4.25). Saponification of ester **4.115** afforded carboxylic acid **4.113** that, gratifyingly, rearranged upon treatment with triethylammonium bromide to give the desired lactone **4.110**. This reaction sequence is highly scalable and has been carried out on upwards of 370 g scale with no incident. This sequence is

also appealing as it does not require any chromatography, and the only purification step is a distillation to purify lactone **4.110**. Additionally, we demonstrated that the triethylammonium bromide can be recovered after lactonization and recycled up to 7 times with no detectable loss in yield. We were also interested in determining if this rearrangement could be carried out catalytically in the presence of a metal ion or Lewis acid. After screening numerous additives, it was found that 5 mol% of either sodium iodide or magnesium iodide effectively promoted the rearrangement. A 10 g scale test reaction with NaI gave 77% isolated yield of the desired lactone **4.110**.
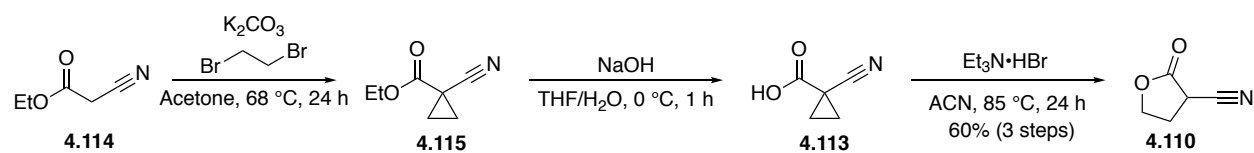
Scheme 4.25. Synthesis of lactone **4.110**.

With ample amounts of lactone **4.110** in hand, alkylation of **4.110** with tosylate **4.104** proceeded smoothly (Scheme 4.26). On large scale (100 g), mechanical stirring in the lactone alkylation greatly improved the overall yield of the sequence. Lactone hydrolysis and decarboxylation were screened using a number of bases solvents and temperatures. When lithium or sodium hydroxide were used for the hydrolysis of lactone **4.116**, the corresponding decarboxylation required high temperatures (~150 °C) and resulted in low yields and mixtures of products. We were delighted to find that when cesium hydroxide was used in the hydrolysis, the corresponding decarboxylation occurred under milder conditions in a mixture of THF and toluene to afford highly pure alcohol **4.118**. Lastly, the benzylation of the primary alcohol afforded the desired intermediate **4.119**, which was purified using the first chromatographic purification of the synthesis.
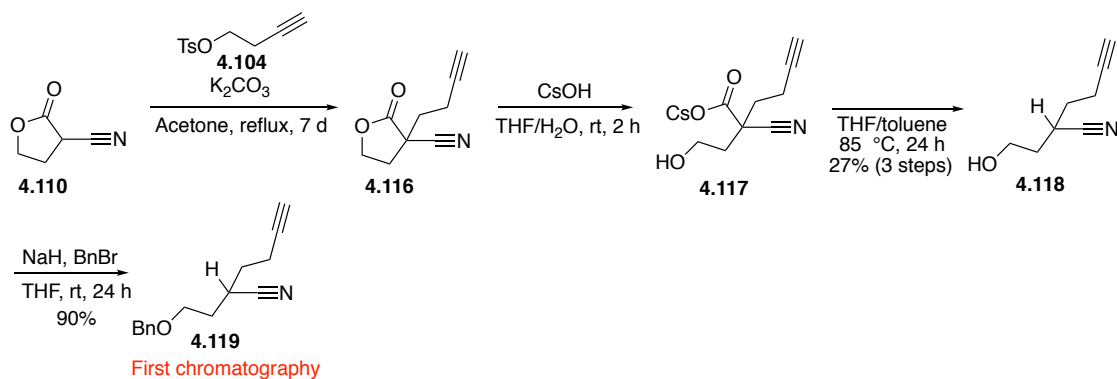
Scheme 4.26. Synthesis of alkyne **4.119**.

### 4.2.5 Approach 2: Investigation of the Key Dötz Benzannulation and IMDA Sequence Using Nitrile 4.119

After successfully preparing the alkyne **4.119**, we shifted our attention to the Dötz benzannulation and the IMDA (Scheme 4.27). Carbene **4.80** was combined with the previously prepared alkyne **4.119** and heated at 50 °C for 14 h to give a naphthol intermediate that was subsequently methylated with methyl iodide in DMF to give **4.120**. Performing the methylation in THF or acetone led to incomplete conversion and lower yields of the methylation product. The DIBAL-H reduction of nitrile **4.120** to the corresponding aldehyde was sluggish at low temperatures and required warming to room temperature to give even modest conversion. Interestingly, the reduction proved highly sensitive to the solvent in the commercial DIBAL solution. A 1.2 M solution of DIBAL in toluene was far superior to a 1.0 M solution of DIBAL in hexanes. The intermediate aldehyde **4.121** was treated with phosphorus ylide **4.122** in a Wittig olefination to give diene **4.123** in modest yields.[470] Additionally, this transformation has also been accomplished under HWE conditions using phosphonate **4.65** with LiOH in THF to give the diene product in comparable yields. Oxidation of the naphthalene **4.123** with CAN to the

naphthoquinone yielded the Diels-Alder precursor **4.124**.[471] Unfortunately, the yields for the CAN oxidation dropped significantly on scales larger than 1 g. Encouraged by the initial work on this transformation (*vide infra*), **4.124** was treated with boron trifluoride etherate at low temperature to yield Diels-Alder product **4.126** as a single diastereomer in modest yields. Using the TBS or TBDPS protected alcohol in the IMDA reaction resulted in low yields (~20%) of only the desilylated product. Additionally, the yields were much lower in other solvents such as THF or toluene.
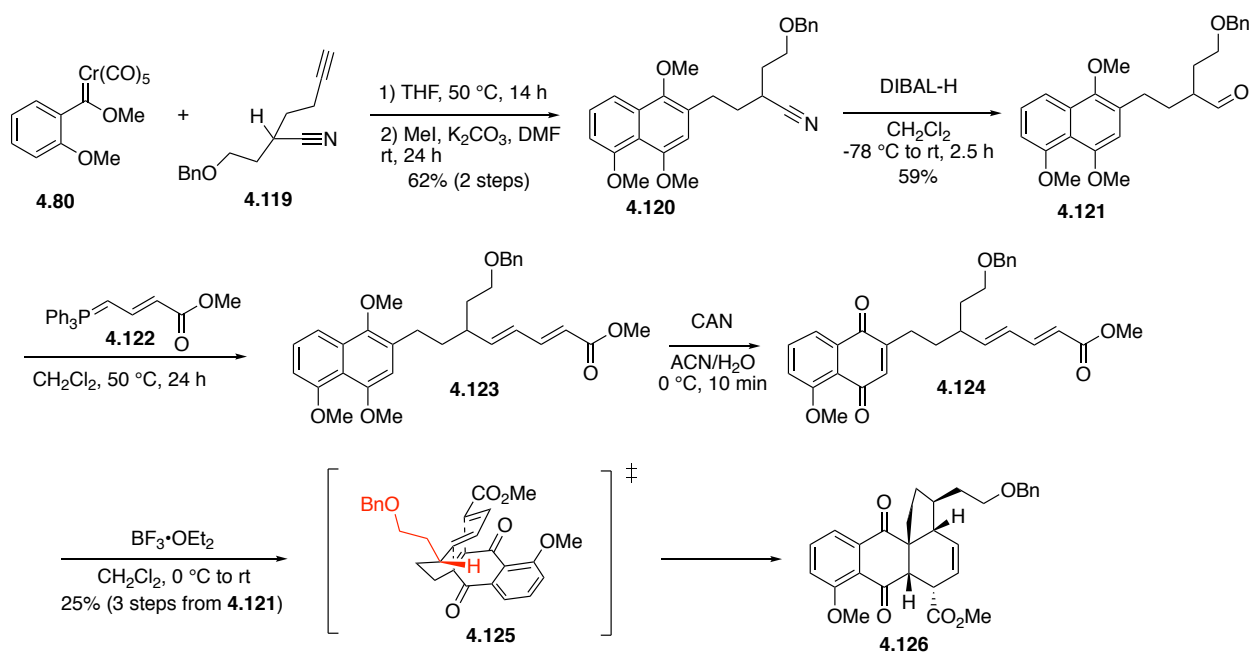
Scheme 4.27. Dötz and IMDA reaction sequence.

Given the stereochemical outcome of the Diels-Alder reaction to **4.126** we propose that the IMDA proceeds via the *endo*-transition state **4.125** with the side chain placed away from the plane of the dienophile, thus minimizing any destabilizing $A^{1,3}$ interactions (Figure 4.4). This result is similar to previous results on related systems such as **4.67**. The alternate *endo*-transition state **4.127** is destabilized by an $A^{1,3}$ strain between the side chain R and the plane of the diene, resulting in a 2-3 kcal/mol destabilization versus **4.125**.[472] Houk and coworkers used

DFT calculations to study the effect of BF$_3$ on the rate and stereoselectivity of IMDA reactions of cycloalkenones, and have shown, in their system, that the *endo* pathway is favored over the *exo* pathway by ~2.8 kcal/mol.[473] Furthermore, both *exo*-transition states **4.128** and **4.129** contain a combination of destabilizing A^{1,3} interactions. Therefore, we propose that all alternative transition states are higher in energy than **4.125**.
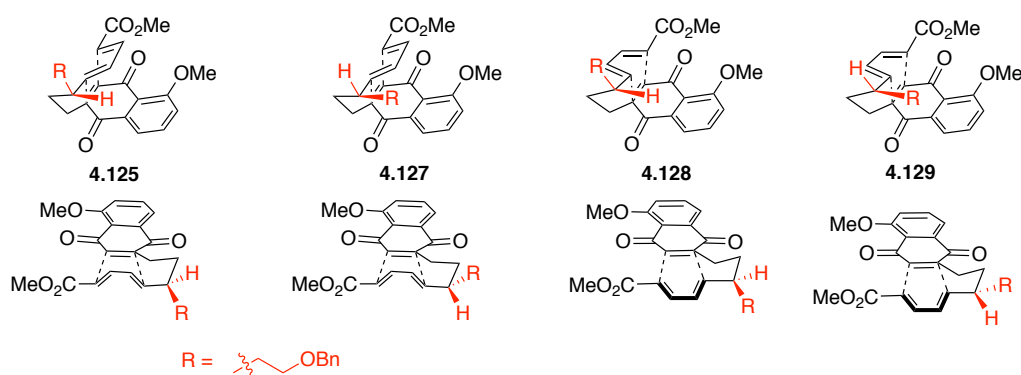
Figure 4.4. Diels-Alder transition state models.

The Diels-Alder product **4.126** was subjected to one-pot hydrogenation conditions to reduce the alkene and cleave the benzyl ether to give the alcohol **4.130** (Scheme 4.28). A subsequent selective reduction of the ketone and acidic workup yielded lactone **4.131**. The selectivity for *cis*-lactone formation is governed by hydride addition to the more accessible convex face. Interestingly, if this sequence is used in the reverse order, the alkene is prone to a base mediated conjugation with the ester. A conjugate reduction of the resulting α,β-unsaturated ester can be accomplished using NiBH$_4$ and affords the thermodynamically favored trans-fused lactone.
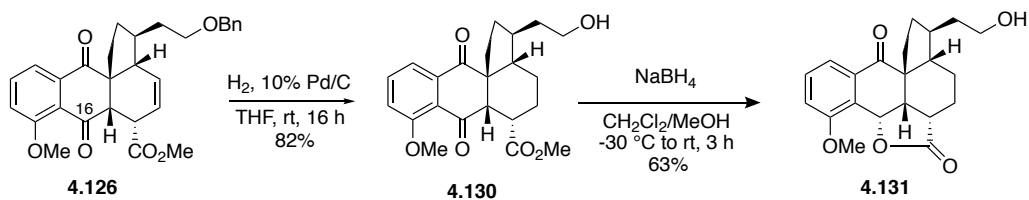
Scheme 4.28. Synthesis of lactone **4.131**.

4.2.6 End Game: Progress Toward the C10 Inversion Sequence and Preliminary Investigation Into the Reductive Etherification to Deliver the Hexacyclic Core of 9-Desmethylpleurotin

With the tetracyclic core successfully synthesized, we turned our attention to the challenging C10 inversion sequence. We intended to employ the groups' first-generation strategy to accomplish the inversion by converting the alcohol **4.132** to the enal **4.133** followed by a selective reduction to give **4.134** (Scheme 4.29).
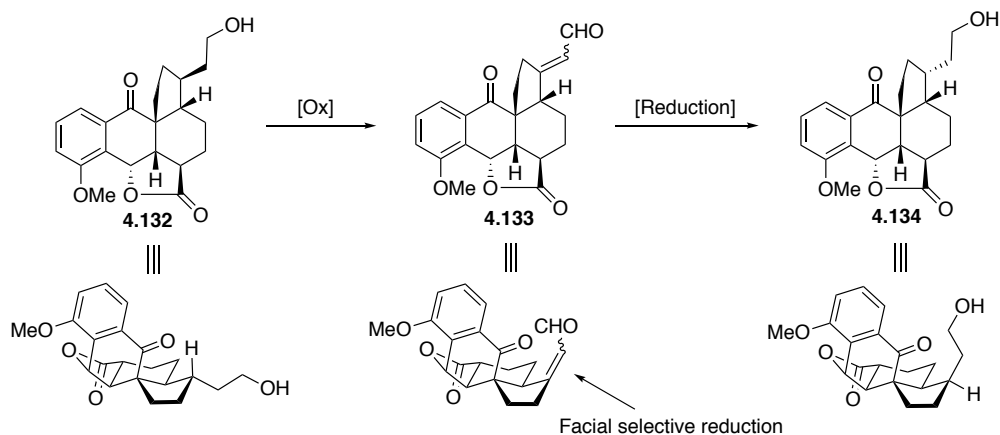
Scheme 4.29. Proposed inversion of side chain configuration.

Our initial studies were performed on the undesired C14 epimer, which we reasoned could be epimerized at a later stage to the desired *trans*-configuration. The inversion of the C10 side chain on the undesired C14 epimer began with a Dess-Martin oxidation of the alcohol **4.131** to give the aldehyde **4.135** in excellent yields (Scheme 4.30). Selenation under standard

conditions using PhSeCl and acid resulted in a complex mixture of products. However, we discovered a mild enamine catalyzed selenation using L-prolinamide and *N*-(phenylseleno)phthalimide (PhthSePh) to give selenide **4.136** in 72% yield as a 4:1 mixture of diastereomers.[474] Using DL-proline affords the same mixture of diastereomers in similar yields indicating that this transformation is highly substrate controlled. Next, several oxidants were screened for the elimination of selenide **4.136**, including sodium periodate, tetrabutyl ammonium periodate (TBAIO$_4$), and manganese monoperphthalate (MMPP).[475] The sodium periodate reaction proved optimal, proceeding with the cleanest reaction profile. The intermediate enal **4.137** was highly prone to isomerization; therefore, this product was directly subjected to a Luche reduction to give allylic alcohol **4.138**.
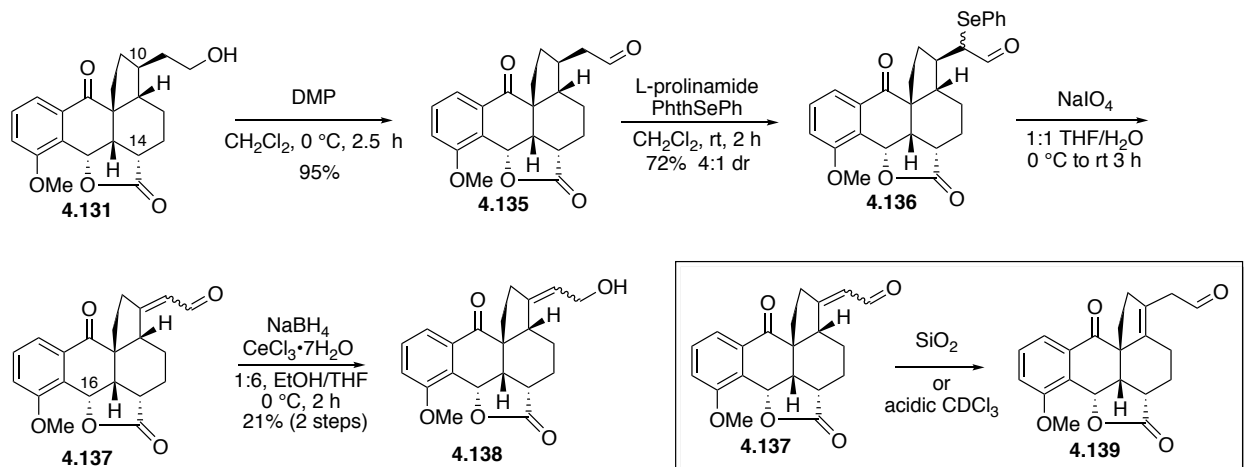
Scheme 4.30. Progress towards the inversion of the C10 configuration.

While attempts to reduce **4.138** using Pd/C resulted in a mixture of C16 benzylic cleavage products, we found that hydrogenation using Crabtree's catalyst gave the desired saturated alcohol **4.140** by crude ^{1}HNMR and LCMS. The crude alcohol products were directly subjected to reductive etherification conditions using TMSOTf and triethylsilane and a HRMS match by LCMS indicated formation of the desired product **4.141** (Scheme 4.31).[476]
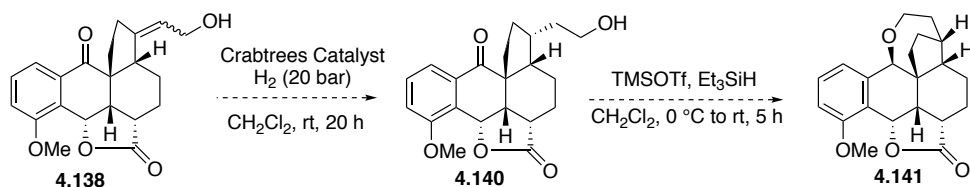
Scheme 4.31. Reduction of allylic alcohol **4.139** and reductive etherification to **4.142**.

4.2.7   Future Directions

Following the reductive etherification, we plan to epimerize at C14, demethylate the phenol, and oxidize to the quinone to give 9-desmethylpleurotin (Scheme 4.32).
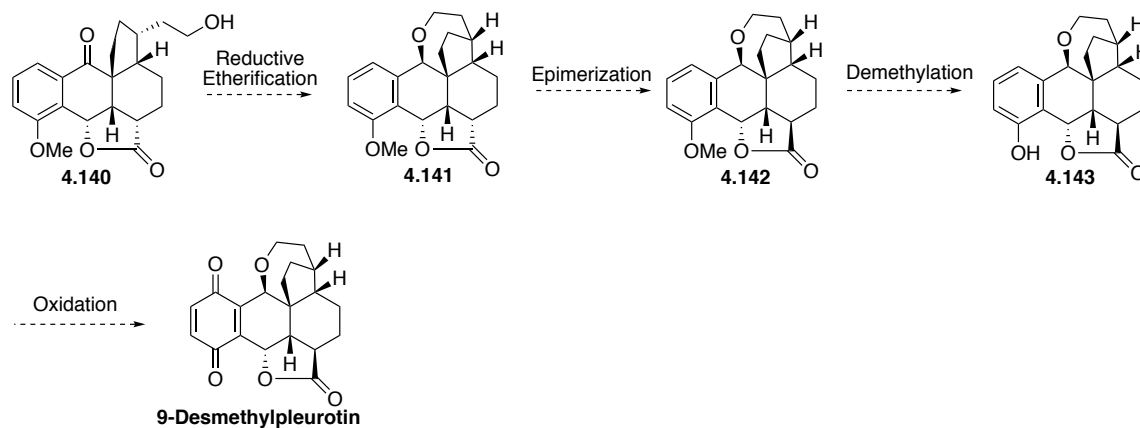
Scheme 4.32. Proposed endgame for the synthesis of 9-desmethylpleurotin.

After the completion of 9-desmethylpleurotin, we propose that we can perform a late stage methylation of aldehyde **4.144** (Scheme 4.33). Using the proposed reductive etherification/demethylation/oxidation sequence outlined in Scheme 4.32 we can arrive at the desired pleurotin.
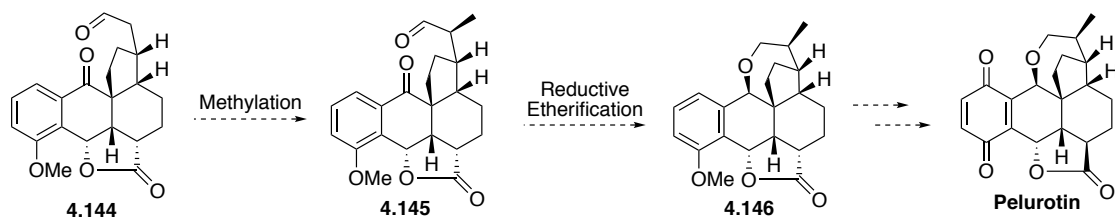
Scheme 4.33. Proposed synthesis of pleurotin by late stage methylation.

4.3 CONCLUSIONS

We have developed a third generation approach to pleurotin and a first generation approach to 9-desmethylpleurotin. This strategy utilizes a key IMDA reaction to afford the tetracyclic core of pleurotin and sets three of the required seven stereocenters. Progress has been made toward completing the challenging inversion sequence to correct the C10 side chain configuration that proved a significant synthetic challenge in previous routes. We have demonstrated that the saturated alcohol side chain with the incorrect C10 configuration can be oxidized to the enal and then subsequently reduced to the allylic alcohol. Synthetic investigations to reduce the allylic alcohol **4.138** to the saturated alcohol **4.140** and complete the inversion sequence were promising. Upon completion of 9-desmethylpleurotin, we can potentially access pleurotin via a late stage methylation.

5.0   EXPERIMENTAL PART

5.1   GENERAL EXPERIMENTAL

Moisture and air-sensitive reactions were performed under N_2 or Ar atmosphere and glassware used for these reactions was flamed dried and cooled under N_2 or Ar prior to use. All moisture sensitive reactions were performed using syringe-septum cap techniques under an atmosphere of dry N_2 or Ar. Reactions carried out below 0 °C employed a dry ice/acetone bath or a cryocool and an isopropanol bath. THF and Et_2O were distilled from sodium/benzophenone ketyl. DMF and CH_2Cl_2 were distilled from CaH_2. Anhydrous 1,4-dioxane, dimethylsulfoxide, and dimethylformamide were 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. ^{1}H and ^{13}C NMR spectra were obtained on Bruker Advance 300 MHz, 400 MHz, 500 MHz, 600 MHz, or 700 MHz instruments. Chemical shifts (δ) were reported in parts per million with the residual solvent peak used as an internal standard, δ ^{1}H/^{13}C (Solvent): 7.26/77.00 (CDCl$_3$); 2.05/29.84 (acetone-d6); 2.50/39.52 (DMSO-d6), 3.31/49.00 (CD$_3$OD); and are tabulated as follows: chemical shift,

multiplicity (s = singlet, bs = broad singlet, d = doublet, bd = 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, 125 MHz, 150 MHz, or 175 MHz using a proton-decoupled pulse sequence and are tabulated by observed peak. ^{19}F NMR spectra were obtained at 376 MHz or 470 MHz using a proton-decoupled pulse sequence, are tabulated by observed peak, and are uncorrected. CDCl$_3$ was filtered through dried basic alumina prior to use. Thin-layer chromatography was performed using pre-coated silica gel 60 F254 plates (EMD, 250 μm thickness) and visualization was accomplished with a 254 nm UV light and by staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), Vaughn's reagent (4.8 g of (NH$_4$)6Mo$_7$O$_{24}$•4H$_2$O and 0.2 g of Ce(SO$_4$)$_2$ in 100 mL of a 3.5 N H$_2$SO$_4$ solution) or a KMnO$_4$ solution (1.5 g of KMnO$_4$ and 1.5 g of K$_2$CO$_3$ 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. Automated column chromatography was done using an Isco Combiflash Rf. Concentrating under reduced pressure refers to the use of rotary evaporator connected to a PIAB Lab Vac H40 to remove solvent. Final products were of >95% purity as analyzed by RP HPLC (Alltech Prevail C-18, 100 × 4.6 mm, 1 mL/min, CH$_3$CN, H$_2$O and 0.1% TFA) with UV (210, 220 and 254 nm), ELS (nebulizer 45 °C, evaporator 45 °C, N$_2$ flow 1.25 SLM), and MS detection using a Thermo Scientific Exactive Orbitrap LC-MS (ESI positive). All other materials were obtained from commercial sources and used as received.

Safety Note: All organic azides and azide waste products should be considered toxic as well as potentially explosive and must be handled and stored with care. Avoid using halogenated solvents when performing reactions involving sodium azide, in addition to using halogenated solvents in reaction workup. Avoid quenching/manipulating/treating sodium azide reactions with

acid, as generation of trace amounts of hydrazoic acid (HN$_3$) may result in an explosion. In general, a safety shield must be used when conducting reactions involving either sodium azide or organic azide derivatives.

5.2 CHAPTER 1 EXPERIMENTAL PART
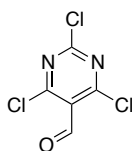
1.10

2,4,6-Trichloropyrimidine-5-carbaldehyde (**1.10**).[144] *N,N*-Dimethylformamide (4.85 mL, 62.5 mmol) was added dropwise to phosphorus oxychloride (25.1 mL, 269 mmol) at 0 ºC. Barbituric acid (**1.9**, 5.00 g, 62.5 mmol) was then added portion-wise over 1 h, and the resulting suspension was stirred in a pre-equilibrated oil bath at 90 ºC for 2 d. The reaction mixture was concentrated under reduced pressure and a H$_2$O-ice mixture (50 mL) was slowly added to the residue in an ice bath. The yellow precipitate that formed was filtered, washed with a H$_2$O-ice mixture (3 x 10 mL), dissolved in CH$_2$Cl$_2$ (100 mL) and washed with brine (30 mL) and satd. aqueous NaHCO$_3$ (30 mL). The organic extract was dried (MgSO$_4$) and concentrated under reduced pressure to give **1.10** (4.70 g, 22.3 mmol, 57%) as a pale yellow solid: ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.40 (s, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 184.6, 164.0, 161.5, 160.9, 159.9, 122.9.
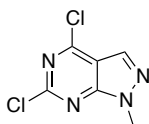
1.8a

4,6-Dichloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (1.8a).[144] A solution of 2,4,6-trichloropyrimidine-5-carbaldehyde (**1.10**, 1.00 g, 4.73 mmol) in dry THF (10 mL) at -78 ºC was treated with a solution of methyl hydrazine (0.26 mL, 4.73 mmol) in dry THF (2 mL) dropwise followed by Et$_3$N (2.0 mL, 14.2 mmol). The resulting mixture was stirred for 1.5 h at -78 ºC, warmed to rt, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 8:2) to give **1.8a** (0.570 g, 2.81 mmol, 59 %) as a colorless solid: Mp 88.9-90.9 °C; IR (neat) 3110, 1588, 1545, 1371, 1299, 1127 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.12 (s, 1 H), 4.10 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.5, 155.4, 154.2, 132.3, 112.6, 34.6.
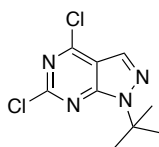
1.8b

1-(*t*-Butyl)-4,6-dichloro-1*H*-pyrazolo[3,4-*d*]pyrimidine (1.8b).[144] A solution of 2,4,6-trichloropyrimidine-5-carbaldehyde (**1.10**, 1.00 g, 4.73 mmol) in dry CH$_2$Cl$_2$ (20 mL) at -78 ºC under Ar was treated with a solution of *t*-butyl hydrazine hydrochloride (0.631 g, 4.73 mmol) and Et$_3$N (2.0 mL, 14.2 mmol) in dry CH$_3$CN (30 mL) dropwise. The mixture was stirred for 2 h at -78 °C where analysis by TLC (hexanes/EtOAc, 4:1) indicated the SM was consumed. SiO$_2$ was added and the reaction was concentrated under reduced pressure and was purified by chromatography on SiO$_2$ (solid load, hexanes/EtOAc, 8:1) to give **1.8b** (0.937 g, 3.82 mmol, 81%) as a colorless solid: IR (neat) 3118, 2984, 2934, 1582, 1525, 1320, 1230, 1208, 1152 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.08 (s, 1 H), 1.80 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 155.1,

155.0, 153.6, 130.8, 113.7, 61.9, 29.0; HRMS (ESI) *m/z* calcd for $C_9H_{10}Cl_2N_4$ ([M+H]$^+$) 245.0361, found 245.0357.
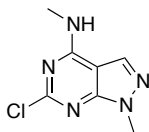
1.11a

6-Chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.11a**). To a solution of 4,6-dichloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1.8a**, 0.300 g, 1.48 mmol) in dry THF (15 mL) was added methylamine hydrochloride (0.110 g, 1.55 mmol), followed by freshly distilled Et₃N (1.0 mL, 7.38 mmol). The reaction mixture was stirred at rt for 2 d, concentrated under reduced pressure, and the residue was dissolved in EtOAc (100 mL), washed with H_2O (3 x 75 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by chromatography on SiO₂ (hexanes/EtOAc, 2:1) to give **1.11a** (0.267 g, 1.35 mmol, 91%) as a colorless solid: Mp 239.3-240.4 °C; IR (neat) 3230, 3105, 1614, 1579, 1316, 1128, 930 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d₆) δ 8.70 (bs, 1 H), 8.03 (s, 1 H), 3.83 (s, 3 H), 2.95 (d, *J* = 4.4 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d₆) δ 157.4, 157.2, 153.3, 131.6, 99.3, 33.7, 27.1; HRMS (ESI) *m/z* calcd for $C_7H_8ClN_5$ ([M+H]$^+$) 198.0546, found 198.0551.
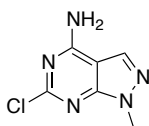
1.11b

6-Chloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.11b**). Ammonia gas was bubbled for 15 min through a suspension of 4,6-dichloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1.8a**, 0.300 g, 1.48 mmol) in dry THF (48 mL). The mixture was stirred at rt for 8 h, concentrated under reduced pressure, and the residue was dissolved in EtOAc (30 mL),

washed with H_2O (3 x 15 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:1 to EtOAc) to give **1.11b** (0.220 g, 1.20 mmol, 81%) as a colorless solid: Mp 265-275 °C; IR (neat) 3308, 3099, 2920, 1657, 1599, 1561, 1187 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.24 (bs, 1 H), 8.11 (bs, 1 H), 8.05 (s, 1 H), 3.83 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 158.6, 157.3, 153.9, 132.1, 98.9, 33.6; HRMS (ESI) *m/z* calcd for $C_6H_6ClN_5$ ([M+H]$^+$) 184.0390, found 184.0381.
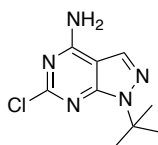
1.11c

1-(*t*-Butyl)-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1.11c). Ammonia gas was bubbled through a suspension of 1-(*t*-butyl)-4,6-dichloro-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1.8b**, 0.800 g, 3.26 mmol) in THF (100 mL) for 20 min. A white precipitate started to form after several minutes and the resulting mixture was stirred at rt for 24 h, concentrated under reduced pressure, and the residue was dissolved in EtOAc (250 mL), washed with H_2O (3 x 75 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give **1.11c** (0.740 g, 3.19 mmol, 98%) as a colorless solid which was used in the next step without further purification: Mp 272-274 °C; IR (neat) 3297, 3135, 2971, 2932, 1644, 1592, 1469, 1366, 1236, 1171 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.17 (bs, 1 H), 8.03 (s, 2 H), 1.65 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 158.7, 156.0, 153.5, 130.6, 100.3, 59.6, 28.7; HRMS (ESI) *m/z* calcd for $C_9H_{12}ClN_5$ ([M+H]$^+$) 226.0859, found 226.0882.
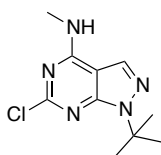
1.11d

1-(*t*-Butyl)-6-chloro-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **(1.11d)**. A solution of 1-(*t*-butyl)-4,6-dichloro-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1.8b**, 0.800 g, 3.26 mmol) in THF (24 mL) at rt under Ar was treated with methylamine hydrochloride (0.230 g, 3.43 mmol) and Et_3N (2.30 mL, 16.3 mmol). The reaction mixture was stirred at rt for 24 h, concentrated under reduced pressure, dissolved in EtOAc (75 mL), washed with H_2O (3 x 30 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 2:1) to give **1.11d** (0.700 g, 2.93 mmol, 90%) as a colorless solid: Mp 160.7-161.9 °C; IR (neat) 3236, 3133, 2947, 1579, 1428, 1361, 1310, 1282, 1124, 1075 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.63 (bd, J = 4.4 Hz, 1 H), 8.02 (s, 1 H), 2.93 (d, J = 4.4 Hz, 3 H), 1.65 (s, 9 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.3, 156.1, 152.8, 130.0, 100.7, 59.7, 28.7, 27.0; HRMS (ESI) *m/z* calcd for $C_{10}H_{14}ClN_5$ ([M+H]$^+$) 240.1016, found 240.1006.
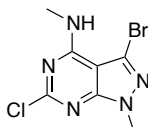
1.7a

3-Bromo-6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **(1.7a)**. A mixture of 6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.11a**, 0.260 g, 1.31 mmol) and Br_2 (0.21 mL, 3.92 mmol) in H_2O (90 mL) was stirred at rt for 1 h and heated at 100 °C for 2 h. The reaction mixture was cooled to rt and extracted with EtOAc (3 x 75 ml), washed with 5% aqueous $NaHSO_3$ solution (100 mL) and brine (75 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give **1.7a** (0.318 g, 1.15 mmol, 88%) as a colorless solid, which was used in the next step without further purification: Mp 246.9-248.0 °C; IR (neat) 3407, 2939, 1606, 1567, 1325, 1200 cm^{-1}; ^1H NMR (400 MHz, CDCl$_3$) δ 6.14 (bs, 1 H), 3.94 (s, 3 H),

3.20 (d, J = 4.8 Hz, 3 H); ^{13}C NMR δ 159.3, 157.4, 154.8, 116.8, 99.3, 34.4, 28.1; HRMS (ESI) m/z calcd for $C_7H_7BrClN_5$ ([M+H]$^+$) 275.9652, found 275.9652.
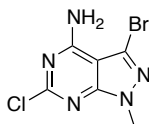
1.7b

3-Bromo-6-chloro-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1.7b**). A mixture of 6-chloro-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1.11b**, 0.196 g, 1.07 mmol) and Br$_2$ (0.23 mL, 4.27 mmol) in H$_2$O (5 mL) was stirred at rt for 1 h and at 100 °C for an additional 1 h. The reaction mixture was cooled to rt, extracted with EtOAc (3 x 15 mL), and washed with 5% aqueous NaHSO$_3$ solution (10 mL) and brine (15 mL). The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure. The residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1) to give **1.7b** (0.113 g, 0.431 mmol, 40%) as a colorless solid: Mp 285-295 °C; IR (neat) 3451, 3282, 3125, 1586, 1552, 1248, 1189 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.53 (bs, 1 H), 7.28 (bs, 1 H), 3.81 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 157.9 (2 C), 154.8, 117.3, 98.5, 34.0; HRMS (ESI) m/z calcd for $C_6H_6ClN_5$ ([M+H]$^+$) 262.9573, found 262.9593.
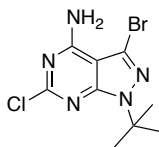
1.7c

3-Bromo-1-(t-butyl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1.7c). A mixture of 1-(t-butyl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1.11c**, 0.100 g, 0.440 mmol) and Br$_2$ (50 μL, 0.890 mmol) in H$_2$O (5 mL) was stirred at rt for 1 h and then at 100 °C for 30 min. In

order to increase the solubilizing power of the reaction mixture, EtOAc (3 mL) was added, and the resulting solution was heated at reflux for an additional 15 min. The two layers were separated and the aqueous phase was extracted with EtOAc (3 x 7.5 mL). The combined organic extracts were washed with 5% aqueous $NaHSO_3$ solution (10 mL), brine (15 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (CH_2Cl_2 to CH_2Cl_2:EtOAc, 1:1) to give **1.7c** (0.110 g, 0.371 mmol, 84%) as a light yellow solid: Mp 225-226°C; IR (neat) 3450, 3282, 2971, 2919, 2718, 1651, 1634, 1584, 1543, 1461, 1234, 1174, 1131 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.05 (bs, 2 H), 1.74 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 157.1, 154.7, 115.8, 100.1, 61.9, 29.1; HRMS (ESI+) *m/z* calcd for $C_9H_{11}BrClN_5$ ([M+H]$^+$) 303.9965, found 303.9983.
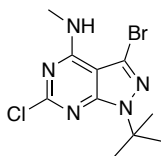
1.7d

3-Bromo-1-(*t*-butyl)-6-chloro-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine

(1.7d). A mixture of 1-(*t*-butyl)-6-chloro-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.11d**, 0.120 g, 0.500 mmol) and Br$_2$ (50 µL, 1.00 mmol) in H$_2$O (3 mL) was stirred at rt for 1 h, treated with EtOAc (1 mL), and heated at reflux for an additional 1 h. Additional EtOAc (3 mL) was added and the reaction mixture was heated at reflux for 15 min. Analysis by TLC (hexanes/EtOAc, 2:1) indicated the SM was consumed, the layers were separated and the aqueous phase was extracted with EtOAc (3 x 8 mL). The combined organic layers were washed with 5% aqueous NaHSO$_3$ solution (10 mL), brine (15 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (12 g column, liquid load CH$_2$Cl$_2$, stepwise gradient, 100% hexanes to 100% EtOAc over 15

min) to give **1.7d** (0.130 g, 0.355 mmol, 71%) as a colorless solid: Mp 106.8-108.4 °C; IR (neat) 3413, 2982, 2934, 1609, 1553, 1364, 1322, 1217, 1133 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.12 (bs, 1 H), 3.18 (d, J = 4.8 Hz, 3 H), 1.73 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 157.3, 154.2, 114.9, 100.2, 61.7, 29.1, 27.9; HRMS (ESI) m/z calcd for C$_{10}$H$_{13}$BrClN$_5$ ([M+H]$^+$) 318.0121, found 318.0110.
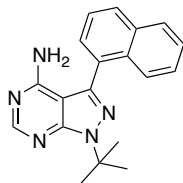
PP1

1-(*tert*-Butyl)-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (PP1).[1,2,3] A solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II)-CH$_2$Cl$_2$ adduct (0.0182 g, 0.0200 mmol) in freshly distilled and degassed 1,4-dioxane (2 mL) was treated with 3-bromo-1-(*t*-butyl)-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7c**, 0.0750 g, 0.250 mmol), Cs$_2$CO$_3$ (0.122 g, 0.370 mmol) and 1-naphthylboronic acid (0.0500 g, 0.300 mmol). The flask was sealed and the reaction mixture was stirred at 100 °C for 2 d, filtered through Celite, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give a mixture of 1-(*t*-butyl)-6-chloro-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and starting material (3:1, 0.0436 g) as an off-white solid.

A solution of this mixture (0.0400 g, 0.0825 mmol), 5% Pd/C (1.08 g, 0.460 mmol) and NH$_4$HCO$_2$ (0.0430 g, 0.680 mmol) in MeOH (8 mL) was stirred at rt for 10 min, purged with H$_2$ for 30 min and stirred under and atmosphere of H$_2$ (balloon) for 26 h. The reaction mixture was filtered through Celite, washed (CH$_2$Cl$_2$ (10 mL)), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 4:1) to give **PP1**

(0.00594 g, 0.0187 mmol, 7.6% (2 steps), 95% purity by LC-MS) as a colorless solid: ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.39 (bs, 1 H), 7.98 (d, J = 8.0 Hz, 1 H), 7.94 (t, J = 6.6 Hz, 1 H), 7.67 (d, J = 6.6 Hz, 1 H), 7.61 (d, J = 8.0 Hz, 1 H), 7.85-7.49 (m, 2 H), 5.00 (bs, 2 H), 1.89 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 154.7, 153.9, 134.0, 131.8, 130.6, 129.6, 128.4, 128.4, 127.1, 126.5, 125.6, 125.5, 60.6, 29.3; HRMS (ESI) m/z calcd for C$_{19}$H$_{19}$N$_5$ ([M+H]$^+$) 318.1719, found 318.1698.
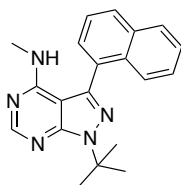
1.13a

1-(*tert*-Butyl)-*N*-methyl-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine

(1.13a).[129] To a solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II)-CH$_2$Cl$_2$ adduct (0.0174 g, 0.0200 mmol) in freshly distilled and degassed 1,4-dioxane (2 mL) was added 3-bromo-1-(*t*-butyl)-6-chloro-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7d**, 0.0750 g, 0.240 mmol) and Cs$_2$CO$_3$ (0.116 g, 0.350 mmol) and the resulting mixture was stirred for 10 min, and treated with 1-naphthylboronic acid (0.0500 g, 0.280 mmol). The flask was sealed, heated to 100 °C for 42 h, and the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure. The residue was purified by automated chromatography on SiO$_2$ (12 g column, liquid load CH$_2$Cl$_2$, stepwise gradient, 100% hexanes to 100% EtOAc over 15 min) to give a mixture of 1-(*t*-butyl)-6-chloro-*N*-methyl-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and starting material (3:1, 0.0318 g) as an off-white solid.

A solution of this mixture (0.0318 g, 0.0652 mmol), 10% Pd/C (0.0154 g, 0.130 mmol) and NH$_4$HCO$_2$ (0.0328 g, 0.521 mmol) in MeOH (5 mL) was stirred at rt for 30 min, purged with

H$_2$ for 15 min and stirred under an atmosphere of H$_2$ (balloon) for 22 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC on SiO$_2$ (hexanes/EtOAc, 5:1) to give **1.13a** (0.00485 g, 0.0146 mmol, 6.2% (2 steps), 100% purity by LC-MS) as a clear oil which crystallized upon standing at rt for ~1 h: Mp 152.4-156.3 °C; IR (neat) 3437, 3044, 2975, 2926, 1597, 1558, 1312, 1243, 1079, cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.47 (s, 1 H), 7.97 (dd, J = 14.4, 8.0 Hz, 2 H), 7.90 (d, J = 8.0 Hz, 1 H), 7.65-7.50 (m, 4 H), 4.71 (bs, 1 H), 2.86 (d, J = 4.8 Hz, 3 H), 1.88 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 154.7, 153.3, 139.7, 134.1, 131.9, 129.5, 128.4, 128.3, 127.0, 126.5, 125.7, 125.5, 101.7, 60.4, 29.3, 27.6; HRMS (ESI) *m/z* calcd for C$_{20}$H$_{21}$N$_5$ ([M+H]$^+$) 332.1875, found 332.1863.
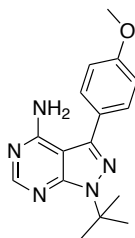
1.13b

1-(*t*-Butyl)-3-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine   (1.13b). A solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II)-CH$_2$Cl$_2$ adduct (0.0180 g, 0.0200 mmol) in freshly distilled and degassed 1,4-dioxane (2 mL) was treated with 3-bromo-1-(*t*-butyl)-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7c**, 0.0750 g, 0.250 mmol) and Cs$_2$CO$_3$ (0.122 g, 0.370 mmol). The reaction mixture was stirred for 10 min and 4-methoxybenzeneboronic acid (0.0450 g, 0.300 mmol) was added. The flask was sealed and the solution was stirred at 100 °C for 20 h, filtered through Celite and concentrated under reduced pressure. The residue was purified by automated chromatography on SiO$_2$ (4 g column, liquid load CH$_2$Cl$_2$, stepwise gradient, 100% hexanes to 100% EtOAc over 15 min) to give a mixture of

1-(*t*-butyl)-6-chloro-3-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and starting material (20:1, 0.0480 g) as an off-white solid.

A solution of this mixture (0.0200 g, 0.0571 mmol), 10% Pd/C (0.0857 g, 0.0700 mmol) and NH$_4$HCO$_2$ (0.0228 g, 0.360 mmol) in MeOH (2 mL) was stirred at rt for 30 min, purged with H$_2$ for 15 min and stirred under H$_2$ for 28 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give **1.13b** (0.0140 g, 0.0486 mmol, 48% (2 steps), 96% purity by LC-MS) as a colorless solid: ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.34 (s, 1 H), 7.61 (d, *J* = 8.6 Hz, 1 H), 7.04 (d, *J* = 8.6 Hz, 1 H), 5.51 (bs, 2 H), 3.87 (s, 3 H), 1.83 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.1, 157.8, 154.4, 154.1, 141.9, 129.9, 126.1, 114.7, 99.7, 60.3, 55.4, 29.2; HRMS (ESI) *m/z* calcd for C$_{16}$H$_{19}$N$_5$O ([M+H]$^+$) 298.1668, found 298.1649.

1.13c

1-(*t*-Butyl)-3-(4-methoxyphenyl)-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1.13c). A solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II)-CH$_2$Cl$_2$ adduct (0.0016 g, 0.0157 mmol) in freshly distilled and degassed 1,4-dioxane (2 mL) was treated with 3-bromo-1-(*t*-butyl)-6-chloro-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7d**, 0.0500 g, 0.157 mmol), and Cs$_2$CO$_3$ (0.0774 g, 0.235 mmol). The resulting mixture was stirred for 10 min and 4-methoxybenzeneboronic acid (0.0286 g, 0.188 mmol) was added. The flask was sealed and heated at 80 °C for 11 h and at 100 °C for an additional 4 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was

purified by automated chromatography on SiO_2 (4 g column, liquid load CH_2Cl_2, stepwise gradient, 100% hexanes to 100% EtOAc over 15 min) to give a mixture of 1-(*t*-butyl)-6-chloro-3-(4-methoxyphenyl)-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and starting material (20:1, 0.0275 g) as an off-white solid.

A solution of this mixture (0.0145 g, 0.0381 mmol), 10% Pd/C (0.0074 g, 0.0629 mmol) and NH_4HCO_2 (0.0159 g, 0.250 mmol) in MeOH (5 mL) was stirred at rt for 30 min, purged with H_2 for 15 min and stirred under H_2 for 25 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 4:1) to give **1.13c** (0.00760 g, 0.0245 mmol, 30% (2 steps), 100% purity by LC-MS) as a clear oil which crystallized to a colorless solid after ~1 h: Mp 89.3-91.2 °C; IR (neat) 3439, 2977, 2930, 1596, 1560, 1517, 1314, 1245, 1033 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.43 (s, 1 H), 7.57 (d, *J* = 8.8 Hz, 2 H), 7.05 (d, *J* = 8.8 Hz, 2 H), 5.29 (bd, *J* = 4.6 Hz, 1 H), 3.88 (s, 3 H), 3.21 (d, *J* = 4.6 Hz, 3 H), 1.82 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.0, 157.8, 154.5, 153.5, 141.3, 129.9, 126.4, 114.7, 100.0, 60.1, 55.4, 29.2, 27.7; HRMS (ESI) *m/z* calcd for $C_{20}H_{21}N_5$ ([M+H]$^+$) 312.1824, found 312.1814.
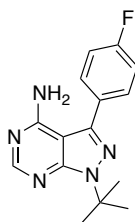
1.13d

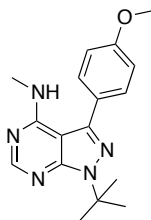**1-(*tert*-Butyl)-3-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1.13d).** A solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II)-CH$_2$Cl$_2$ adduct (0.0182 g, 0.0200 mmol) in freshly distilled and degassed 1,4-dioxane (2 mL) was treated with 3-bromo-1-(*t*-butyl)-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7c**, 0.0750 g, 0.250 mmol) and

Cs$_2$CO$_3$ (0.0122 g, 0.370 mmol). The resulting mixture was stirred for 10 min and 4-fluorobenzeneboronic acid (0.0400 g, 0.290 mmol) was added. The reaction was heated at 100 °C for 20 h, filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4 g column, liquid load CH$_2$Cl$_2$, stepwise gradient, 100% hexanes to 100% EtOAc over 15 min) to give a mixture of 1-(*t*-butyl)-6-chloro-3-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and starting material (10:1, 0.0540 g) as an off-white solid.

A solution of this mixture (0.0180 g, 0.0512 mmol), 10% Pd/C (0.0800 g, 0.0700 mmol) and NH$_4$HCO$_2$ (0.0200 g, 0.340 mmol) in MeOH (2 mL) was stirred at rt for 15 min, purged with H$_2$ for 15 min and stirred under H$_2$ for 28 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give **1.13d** (0.00410 g, 0.0144 mmol, 18% (2 steps), 93% purity by LC-MS) as a colorless solid: ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.36 (s, 1 H), 7.68 (dd, *J* = 8.2, 5.2 Hz, 2 H), 7.23 (t, *J* = 8.2 Hz, 2 H), 5.37 (bs, 2 H), 1.83 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.1 (d, *J$_{CF}$* = 247 Hz), 157.7, 154.6, 154.3, 141.0, 130.4 (d, *J$_{CF}$* = 8.3 Hz), 129.9 (d, *J$_{CF}$* = 3.1 Hz), 129.2, 128.6, 116.3 (d, *J$_{CF}$* = 22 Hz), 99.7, 60.5, 29.2; HRMS (ESI) *m/z* calcd for C$_{15}$H$_{16}$FN$_5$ ([M+H]$^+$) 286.1468, found 286.1452.

1.13e

3-([1,1'-Biphenyl]-4-yl)-1-(*tert*-butyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine   (1.13e).

To a solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II)-CH$_2$Cl$_2$ adduct (0.0182 g, 0.0200 mmol) in freshly distilled and degassed 1,4-dioxane (2 mL) was added 3-bromo-1-(*t*-butyl)-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7c**, 0.0750 g, 0.250 mmol), and Cs$_2$CO$_3$ (0.122 g, 0.370 mmol). The resulting mixture was stirred for 10 min and 4-phenylbenzeneboronic acid (0.0585 g, 0.300 mmol) was added. The flask was sealed and heated to 100 °C for 21 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by automated chromatography on SiO$_2$ (4 g column, liquid load CH$_2$Cl$_2$, stepwise gradient, hexanes to EtOAc over 15 min) to give a mixture of 3-([1,1'-biphenyl]-4-yl)-1-(*t*-butyl)-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine   and   starting material (9:1, 0.0350 g) as an off-white solid.

A solution of this mixture (0.0200 g, 0.0477 mmol), 10% Pd/C (0.0752 g, 0.0600 mmol), and NH$_4$CO$_2$ (0.0200 g, 0.320 mmol) in MeOH (2 mL) was stirred at rt for 30 min, purged with H$_2$ for 15 min and stirred under an atmosphere of H$_2$ (balloon) for 28 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 4:1) to give **1.13e** (0.00890 g, 0.0260 mmol, 19% (2 steps), 94% purity by LC-MS) as a colorless solid: ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.38 (s, 1 H), 7.78 (d, *J* = 8.4 Hz, 2 H), 7.75 (d, *J* = 8.4 Hz, 2 H), 7.65 (d, *J* = 7.6 Hz, 2 H), 7.48 (t, *J* = 7.6 Hz, 2 H), 7.39 (t, *J* = 7.6 Hz, 1 H), 5.84 (bs, 3 H), 1.86 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 154.5, 154.3, 141.6 (2 C), 140.3, 132.6, 128.9, 128.9, 127.9, 127.7, 127.0, 99.7, 60.5, 29.2; HRMS (ESI) *m/z* calcd for C$_{21}$H$_{21}$N$_5$ ([M+H]$^+$) 344.1875, found 344.1858.

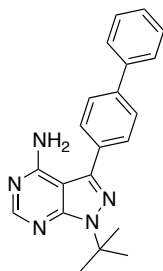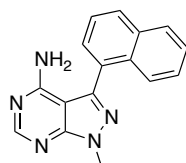**1.13f**

1-Methyl-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **(1.13f)**. A solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II)-CH$_2$Cl$_2$ adduct (0.0098 g, 0.0100 mmol) in freshly distilled and degassed 1,4-dioxane was treated with 3-bromo-6-chloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7b**, 0.0500 g, 0.190 mmol) and Cs$_2$CO$_3$ (0.0900 g, 0.0300 mmol). The reaction mixture was stirred for 10 min and 1-naphthylboronic acid (0.0393 g, 0.230 mmol) was added. The solution was continuously degassed by purging with N$_2$ over the course of the addition of reagent. The vial was capped and the solution was stirred at 80 °C for 34 h and at 100 °C for an additional 12 h, and filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:1) to give a mixture of 6-chloro-1-methyl-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and starting material (6:1, 0.0180 g) as an off-white solid.

A solution of this mixture (0.00420 g, 0.00857 mmol), 5% Pd/C (0.0819 g, 0.0300 mmol) and NH$_4$HCO$_2$ (0.0087 g, 0.140 mmol) in MeOH (1 mL) was stirred at rt for 10 min, purged with H$_2$ for 30 min and stirred under H$_2$ for 22 h. The reaction mixture was filtered through Celite, which was rinsed with CH$_2$Cl$_2$ (4 mL), and the combined filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give **1.13f** (0.00370 g, 0.0133 mmol, 17 % (2 steps), 96% purity by LC-MS) as a colorless solid: Mp 210-212 °C; IR (neat) 3465, 3299, 3220, 2937, 1631, 1565, 1513, 1312, 1269 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.43 (s, 1 H), 8.01-7.92 (m, 3 H), 7.67-7.52 (m, 4 H), 5.11 (bs, 2 H), 4.16 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 156.2, 154.3, 142.4, 133.9, 131.5, 130.0, 129.8,

128.6, 128.4, 127.3, 126.6, 125.5, 125.3, 100.3, 34.0; HRMS (ESI) *m/z* calcd for $C_{16}H_{13}N_5$ ([M+H]$^+$) 276.1249, found 276.1242.
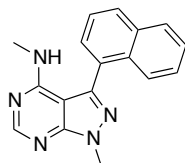
1.13g

N,1-Dimethyl-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.13g**). A solution of $Pd_2(dba)_3$ (0.0176 g 0.0200 mmol) and PA-Ph (0.0087 g, 0.0300 mmol) in degassed 1,4-dioxane (1.7 mL) was stirred at rt for 10 min, treated with 3-bromo-6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7a**, 0.0750 g, 0.270 mmol), Cs_2CO_3 (0.133 g, 0.410 mmol) and 1-naphthylboronic acid (0.0560 g, 0.330 mmol) and stirred in a pre-equilibrated oil bath at 100 °C for 25 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 4:1) to give a mixture of 6-chloro-*N*,1-dimethyl-3-(naphthalen-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and starting material (10:1, 0.0340 g) as an off-white solid.

A solution of this mixture (0.0080 g, 0.0182 mmol), 10% Pd/C (0.0400 g, 0.0400 mmol) and NH_4HCO_2 (0.0093 g, 0.150 mmol) in MeOH (1 mL) was stirred at rt for 30 min, purged with H_2 for 15 min and stirred under H_2 for 27 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 6:1) to give **1.13g** (0.00330 g, 0.0113 mmol, 18% (2 steps)) as a colorless solid: Mp 154.2-155.6 °C; IR (neat) 3438, 3045, 2939, 1599, 1569, 1312, 1269 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.51 (s, 1 H), 7.99 (dd, *J* = 16.0, 8.0 Hz, 2 H), 7.90 (d, *J* = 8.0 Hz, 2 H), 7.70-7.50 (m, 4 H), 4.76 (bs, 1 H), 4.15 (s, 3 H), 2.89 (d, *J* = 4.8 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 156.3, 153.7, 141.8, 134.0, 131.6, 130.3, 129.8, 128.5, 128.3,

127.2, 126.6, 125.5, 125.4, 100.5, 34.0, 27.7; HRMS (ESI) *m/z* calcd for C$_{17}$H$_{14}$ClN$_5$ ([M+H]$^+$) 290.1406, found 290.1385.
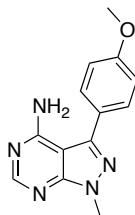
1.13h

3-(4-Methoxyphenyl)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **(1.13h)**. A mixture of 6-chloro-3-(4-methoxyphenyl)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.12h**, 0.0132 g, 0.0500 mmol), 5% Pd/C (0.162 g, 0.0700 mmol) and NH$_4$HCO$_2$ (0.0172 g, 0.270 mmol) in MeOH (3 mL) was stirred at rt for 10 min, purged with H$_2$ for 10 min and stirred under H$_2$ for 22 h. The reaction mixture was filtered through Celite, which was washed with CH$_2$Cl$_2$ (4 mL), and the combined filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give **1.13h** (0.00435 g, 0.0170 mmol, 37 %, 100% purity by LC-MS) as a colorless solid: Mp 185-188 °C IR (neat) 3468, 3071, 2926, 2851, 1647, 1581, 1568, 1322, 1288, 1249, 1172 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.40 (s, 1 H), 7.62 (d, *J* = 8.8 Hz, 2 H), 7.06 (d, *J* = 8.8 Hz, 2 H), 5.48 (bs, 2 H), 4.07 (s, 3 H), 3.88 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.3, 157.8, 156.0, 154.5, 144.0, 129.7, 125.5, 114.8, 98.4, 55.4, 33.8; HRMS (ESI) *m/z* calcd for C$_{13}$H$_{13}$N$_5$O ([M+H]$^+$) 256.1198, found 256.1181.
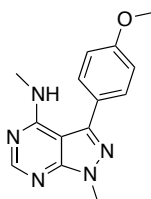
1.13i

3-(4-Methoxyphenyl)-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1.13i). A mixture of 6-chloro-3-(4-methoxyphenyl)-*N*-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-amine (**1.12i**, 0.0080 g, 0.0300 mmol), 10% Pd/C (0.0500 g, 0.100 mmol) and NH_4HCO_2 (0.0010 g, 0.160 mmol) in MeOH (0.8 mL) was stirred at rt for 30 min, purged with H_2 for 15 min and stirred under H_2 for 24 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (1 mL), washed with H_2O (2 x 1 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:2) to give **1.13i** (0.0053 g, 0.0196 mmol, 74%) as a colorless solid: Mp 110.2-111.6 °C; IR (neat) 3442, 2935, 1591, 1567,1526, 1496, 1246 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.46 (s, 1 H) 7.56 (d, *J* = 8.4 Hz, 2 H), 7.06 (d, *J* = 8.4 Hz, 2 H), 5.37 (bs, 1 H), 4.05 (s, 3 H), 3.89 (s, 3 H), 3.09 (d, *J* = 4.8 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.2, 157.8, 156.0, 153.9, 143.5, 129.6, 125.8, 114.8, 98.6, 55.4, 33.7, 27.8; HRMS (ESI) *m/z* calcd for $C_{14}H_{14}ClN_5O$ ([M+H]$^+$) 270.1355, found 270.1351.
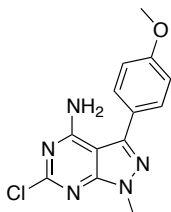
1.12h

6-Chloro-3-(4-methoxyphenyl)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1.12h). A solution of dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II)-CH$_2$Cl$_2$ adduct (0.0099 g, 0.0100 mmol) in freshly distilled and degassed 1,4-dioxane (2 mL) was stirred for 10 min, treated with 3-bromo-6-chloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7b**, 0.0500 g, 0.190 mmol), Cs$_2$CO$_3$ (0.0900 g, 0.290 mmol), and 4-methoxybenzeneboronic acid (0.0350 g, 0.230 mmol) and stirred at 80 °C for 20 h and at 100 °C for an additional 4 h.

The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1) to give **1.12h** (0.0137 g, 0.0473 mmol, 25%) as a colorless solid: Mp >250 °C; IR (neat) 3465, 3288, 3070, 3001, 2835, 1648, 1586, 1504, 1450, 1251, 1038 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.59 (d, J = 8.4 Hz, 2 H), 7.06 (d, J = 8.4 Hz, 2 H), 5.72 (bs, 2 H), 4.03 (s, 3 H), 3.88 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.5, 158.2, 157.9, 155.6, 144.4, 129.5, 124.9, 114.9, 97.1, 55.4, 34.1; HRMS (ESI) m/z calcd for C$_{13}$H$_{12}$ClN$_5$O ([M+H]$^+$) 290.0809, found 290.0799.
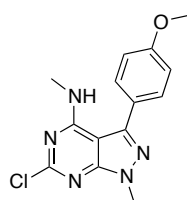
1.12i

6-Chloro-3-(4-methoxyphenyl)-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine

(**1.12i**). A solution of Pd$_2$(dba)$_3$ (0.0176 g 0.0200 mmol) and PA-Ph (0.0079 g, 0.0300 mmol) in degassed 1,4-dioxane (1 mL) was stirred for 10 min, treated with 3-bromo-6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7a**, 0.0748 g, 0.270 mmol), Cs$_2$CO$_3$ (0.133 g, 0.410 mmol) and 4-methoxybenzeneboronic acid (0.0390 g 0.270 mmol), and stirred at 50 °C for 24 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by preparative TLC on SiO$_2$ (hexanes/EtOAc, 3:7) to give **1.12i** (0.0284 g, 0.0935 mmol, 35%, 100% purity by LC-MS) as a colorless solid: Mp 159.3-160.9 °C; IR (neat) 3349, 2957, 1601, 1569, 1496, 1246, 1209, 1172 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.54 (d, J = 8.8 Hz, 2 H), 7.06 (d, J = 8.8 Hz, 2 H), 5.51 (bs, 1 H), 4.01 (s, 3 H), 3.89 (s, 3 H), 3.09 (d, J = 4.8 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.3, 158.2, 157.9,

154.8, 143.6, 129.4, 125.1, 114.8, 97.2, 55.3, 33.8, 27.9; HRMS (ESI) *m/z* calcd for C$_{14}$H$_{14}$ClN$_5$O ([M+H]$^+$) 304.0965, found 304.0974.
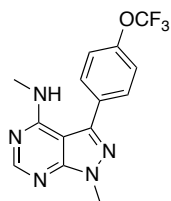
1.13j

_N_,1-Dimethyl-3-(4-(trifluoromethoxy)phenyl)-1_H_-pyrazolo[3,4-_d_]pyrimidin-4-amine (1l). A solution of 6-chloro-*N*,1-dimethyl-3-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.12j**, 0.0081 g, 0.0200 mmol), 5% Pd/C (0.0800 g, 0.0300 mmol) and NH$_4$HCO$_2$ (0.0085 g, 0.140 mmol) in MeOH (2 mL) was stirred at rt for 30 min, purged with H$_2$ for 15 min and stirred under H$_2$ for 19 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (1 mL), washed with H$_2$O (2 x 1 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give **1.13j** (0.00651 g, 0.0201 mmol, 89%, 91% purity by LC-MS) as a colorless solid: Mp 101.5-103.3 °C; IR (neat) 3452, 2943, 1592, 1568, 1249, 1219, 1204, 1161, 1103 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.49 (s, 1 H), 7.69 (d, *J* = 8.4 Hz, 2 H), 7.39 (d, *J* = 8.4 Hz, 2 H), 5.25 (bd, *J* = 2.4 Hz, 1 H), 4.10 (s, 3 H), 3.12 (d, *J* = 5.2 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 156.1, 154.1, 149.7, 142.1, 132.6, 129.9, 129.4, 128.4, 121.8, 120.4 (q, *J$_{CF}$* = 257 Hz), 98.6, 33.9, 28.0; HRMS (ESI) *m/z* calcd for C$_{14}$H$_{11}$ClF$_3$N$_5$O ([M-H]$^-$) 324.1072, found 324.1078.
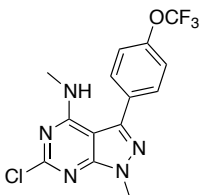
1.12j

6-Chloro-*N*,1-dimethyl-3-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazolo[3,4-

d]pyrimidin-4-amine (1.12j). A solution of Pd$_2$(dba)$_3$ (0.0176 g, 0.0200 mmol) and dppf

(0.0090 g, 0.0160 mmol) in freshly distilled and degassed 1,4-dioxane (1.7 mL) was stirred for

10 min, treated with 3-bromo-6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine

(**1.7a**, 0.0750 g, 0.270 mmol), Cs$_2$CO$_3$ (0.133 g, 0.410 mmol), and 4-

trifluoromethoxybenzeneboronic acid (0.0670 g, 0.330 mmol), and stirred at 100 °C for 2 d,

filtered through Celite, and the filtrate was concentrated under reduced pressure. The crude

residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 4:1) to give **1.12j** (0.0270 g,

0.0753 mmol, 28%, 100% purity by LC-MS) as a colorless solid: Mp 115.3-119.1 °C; IR (neat)

3444, 2944, 1593, 1567, 1246, 1202, 1155 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.66 (d, *J* = 8.4

Hz, 2 H), 7.38 (d, *J* = 8.4 Hz, 2 H), 5.40 (bs, 1 H), 4.00 (s, 3 H), 3.11 (d, *J* = 4.8 Hz, 3 H); ^{13}C

NMR (100 MHz, CDCl$_3$) δ 158.5, 157.9, 155.2, 149.9, 142.4, 131.7, 129.8, 121.7, 120.4 (q, *J*$_{CF}$

= 257 Hz), 97.3, 34.1, 28.2; HRMS (ESI) *m/z* calcd for C$_{14}$H$_{11}$ClF$_3$N$_5$O ([M-H]$^-$) 356.0526,

found 356.0521.
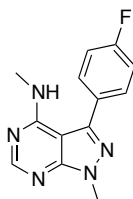
1.13k

3-(4-Fluorophenyl)-*N*,1-dimethyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.13k**). A

solution of 6-chloro-3-(4-fluorophenyl)-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.12k**,

0.0120 g, 0.0400 mmol), 10% Pd/C (0.0580 g, 0.0490 mmol) and NH$_4$HCO$_2$ (0.0053 g, 0.0800

mmol) in MeOH (1 mL) was stirred at rt for 30 min, purged with H$_2$ for 15 min and stirred under

an atmosphere of H_2 (balloon) for 24 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (1 mL), washed with H_2O (2 x 1 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1 to 1:4) to give **1n** (0.0080 g, 0.0311 mmol, 76%, 96% purity by LC-MS) as a colorless solid: Mp 111.8-114.5 °C; IR (neat) 3446, 2941, 1588, 1567, 1522, 1494, 1312, 1265, 1220 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.47 (s, 1 H), 7.62 (dd, J = 8.4, 5.2 Hz, 2 H), 7.23 (t, J = 8.4 Hz, 2 H), 5.26 (bs, 1 H), 4.06 (s, 3 H), 3.10 (d, J = 4.8 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.2 (d, J_{CF} = 248 Hz), 157.6, 156.1, 154.0, 142.6, 130.2 (d, J_{CF} = 8.2 Hz), 129.2 (d, J_{CF} = 3.2 Hz), 116.4 (d, J_{CF} = 22 Hz), 98.6, 33.8, 27.9; HRMS (ESI) m/z calcd for C$_{13}$H$_{12}$FN$_5$ ([M+H]$^+$) 258.1155, found 258.1150.
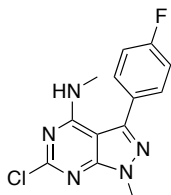

1.12k

6-Chloro-3-(4-fluorophenyl)-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine
(**1.12k**). A solution of Pd$_2$(dba)$_3$ (0.0176 g, 0.0200 mmol) and PA-Ph (0.0079 g, 0.0300 mmol) in 1,4-dioxane (1 mL) was stirred for 10 min, treated with 3-bromo-6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7a**, 0.0748 g, 0.270 mmol), Cs$_2$CO$_3$ (0.133 g, 0.410 mmol) and 4-fluorobenzeneboronic acid (0.0420 g 0.300 mmol), and stirred at 60 °C for 22 h and at 100 °C for an additional 4 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 5:1) to give **1.12k** (0.0210 g, 0.0734 mmol, 27%, 100% purity by LC-MS) as a

colorless solid: Mp 189.9-192.3 °C; IR (neat) 3438, 2943, 1597, 1569, 1526, 1493, 1347, 1260 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.60 (dd, J = 8.4, 5.6 Hz, 2 H), 7.20-7.30 (m, 2 H), 5.40 (bs, 1 H), 4.02 (s, 3 H), 3.11 (d, J = 4.8 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.4 (d, J_{CF} = 248 Hz), 158.5, 158.0, 155.1, 142.9, 130.2 (d, J_{CF} = 8.2 Hz), 129.2 (d, J_{CF} = 3.4 Hz), 116.7 (d, J_{CF} = 21.4 Hz), 97.4, 34.1, 28.2; HRMS (ESI) m/z calcd for C$_{13}$H$_{11}$ClFN$_5$ ([M+H]$^+$) 292.0765, found 292.0788.

1.13l
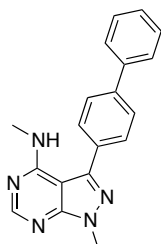
3-([1,1'-Biphenyl]-4-yl)-N,1-dimethyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1.13l). A solution of 3-([1,1'-biphenyl]-4-yl)-6-chloro-N,1-dimethyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1.12l**, 0.0200 g, 0.0500 mmol), 10% Pd/C (0.0850 g, 0.0725 mmol) and NH$_4$HCO$_2$ (0.0183 g, 0.290 mmol) in MeOH (2 mL) was stirred at rt for 30 min, purged with H$_2$ for 30 min and stirred under H$_2$ for 22 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give **1.13l** (0.0128 g, 0.0404 mmol, 84%, 100% purity by LC-MS) as a colorless solid: Mp 190.8-192.3 °C; IR (neat) 3439, 3027, 2937, 1590, 1566, 1487, 1264 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.49 (s, 1 H), 7.78-7.71 (m, 4 H), 7.66 (d, J = 7.6 Hz, 2 H), 7.49 (t, J = 7.6 Hz, 2 H), 7.40 (t, J = 7.2 Hz, 1 H), 5.46 (bd, J = 4.0 Hz, 1 H), 4.08 (s, 3 H), 3.12 (d, J = 4.8 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 156.0, 154.0, 143.3, 141.8, 140.1, 132.4, 128.9, 128.7, 128.0, 127.8, 127.1, 98.7, 33.8, 27.9; HRMS (ESI) m/z calcd for C$_6$H$_6$ClN$_5$ ([M+H]$^+$) 316.1562, found 316.1571.
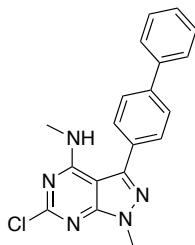
1.12l

3-([1,1'-Biphenyl]-4-yl)-6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1.12l). A solution of Pd$_2$(dba)$_3$ (0.0176 mg, 0.0200 mmol) and dppf (0.0098 g, 0.0160 mmol) in distilled and degassed 1,4-dioxane (1.7 mL) was stirred for 10 min, treated with 3-bromo-6-chloro-*N*,1-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**1.7a**, 0.0750 g, 0.270 mmol), Cs$_2$CO$_3$ (0.133 g, 0.410 mmol) and 4-phenylbenzeneboronic acid (0.0640 g, 0.330 mmol), and stirred at 100 °C for 21 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 4:1) to give **1.12l** (0.0470 g, 0.136 mmol, 50%) as a colorless solid: Mp 211.9-213.8 °C; IR (neat) 3435, 3058, 1593, 1569, 1485, 1347, 1260 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.77-7.66 (m, 4 H), 7.65 (d, *J* = 7.6 Hz, 2 H), 7.48 (t, *J* = 7.2 Hz, 2 H), 7.40 (t, *J* = 7.2 Hz, 1 H), 5.61 (bs, 1 H), 4.02 (s, 3 H), 3.11 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 158.4, 158.0, 155.1, 143.6, 142.0, 139.9, 131.8, 128.9, 128.6, 128.1, 127.8, 127.0, 97.3, 34.0, 28.1; HRMS (ESI) *m/z* calcd for C$_{19}$H$_{16}$ClN$_5$ (M-H)$^-$ 350.1172, found 350.1165.

5.3   CHAPTER 2 EXPERIMENTAL PART

5.3.1   Synthesis of Thiazocine Inhibitor 2.15
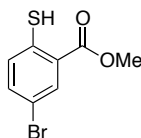
2.19

Methyl 5-bromo-2-mercaptobenzoate (2.19).[273-274] To a cooled (0-5 °C) stirring

solution of 2-amino-5-bromobenzoic acid (**2.18**, 1.00 g, 4.49 mmol), NaOH (0.180 g, 4.49

mmol), and NaNO$_2$ (0.310 g, 4.49 mmol) in H$_2$O (6 mL) was slowly treated with a solution of

concentrated HCl (12.4 mL) in H$_2$O (21.5 mL). The resulting mixture was stirred at 0 °C for 1 h,

and then neutralized with potassium acetate (1.47 g). This solution was added to a solution of

potassium O-ethylxanthate (2.23 g, 13.6 mmol) in H$_2$O (6 mL) which was preheated to 90 °C.

The mixture was stirred at the same temperature for 30 min, cooled to 0 °C and acidified with

conc. HCl. The aqueous phase was decanted from the resulting semisolid sludge. This was

dissolved in 10% aqueous NaOH (5 mL), and heated to 85 °C for 2 h. To this mixture was added

portionwise NaHSO$_3$ (0.42 g), and the mixture was heated to 85 °C for 10 min. The mixture was

filtered, cooled to 0 °C, and acidified with conc. HCl. The precipitate was collected by filtration

and washed with H$_2$O. The moist cake was dissolved in THF/EtOAc, washed with brine, dried

(Na$_2$SO$_4$), and concentrated under reduced pressure to give crude product as a yellow solid. This

material was used for the next reaction without further purification.

A mixture of crude 5-bromo-2-mercaptobenzoic acid (1.05 g, 4.49 mmol) and conc.

H$_2$SO$_4$ (0.45 mL) in MeOH (10 mL) was heated to reflux for 16 h, concentrated under reduced

pressure, diluted with ice-H$_2$O, and extracted with EtOAc. The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes:EtOAc, 10:1) to give **2.19** (0.338 g, 1.37 mmol, 31%) as a yellow solid: ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.12 (d, J = 2.1 Hz, 1 H), 7.41 (dd, J = 8.6, 2.1 Hz, 1 H), 7.18 (d, J = 8.6 Hz, 1 H), 4.80 (s, 1 H), 3.93 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 166.0, 137.5, 135.2, 134.3, 132.1, 127.2, 117.7, 52.5.
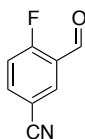
2.20

4-Fluoro-3-formylbenzonitrile (2.20).[477] A solution of 5-bromo-2-fluorobenzaldehyde (4.00 g, 19.3 mmol) in DMF (4 mL) was treated with CuCN (2.10 g, 23.2 mmol) and the mixture was heated at 100 °C for 2 h, and at 130 °C for 18 h. The mixture was cooled to rt, treated with a solution of FeCl$_3$ (13.0 g) in conc. HCl (4 mL) and H$_2$O (16 mL). The mixture was heated at 70-80 °C for 20 min. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 80 mL). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give **2.20** (1.52 g, 10.2 mmol, 53%) as a colorless solid: ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.34 (s, 1 H), 8.19 (d, J = 6.0 Hz, 1 H), 7.90 (m, 1 H), 7.35 (t, J = 9.0 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 184.7 (d, J_{CF} = 6.0 Hz), 167.5 (d, J_{CF} = 266.0 Hz), 139.4 (d, J_{CF} = 11.0 Hz), 133.4 (d, J_{CF} = 3.0 Hz), 124.8 (d, J_{CF} = 9.0 Hz), 118.3 (d, J_{CF} = 22.0 Hz), 116.7, 109.7 (d, J_{CF} = 4.0 Hz).
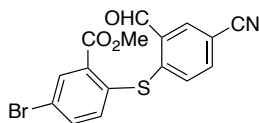
2.21

Methyl 5-bromo-2-((4-cyano-2-formylphenyl)thio)benzoate (**2.21**) A solution of methyl 5-bromo-2-mercaptobenzoate (**2.19**, 1.68 g, 6.80 mmol) and 5-cyano-2-fluorobenzaldehyde (**2.20**, 0.940 g, 6.18 mmol) in DMF (24 mL) was treated with K_2CO_3 (1.28 g, 9.27 mmol) and the reaction was heated to 90 °C for 3 d, cooled to rt, poured onto a mixture of EtOAc (150 mL) and H_2O (150 mL) and the mixture was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 9:1 to hexanes/EtOAc, 4:1) to give **2.21** (1.51 g, 4.01 mmol, 65%) as a light yellow solid: Mp 108-110 °C; IR (neat) 3115, 3083, 3070, 3059, 2945, 2837, 2227, 1739, 1672, 1596, 1569, 1534, 1458, 1430, 1282, 1221 cm-1; ^1H NMR (300 MHz, CDCl$_3$) δ 10.31 (s, 1 H), 8.18 (d, J = 1.8 Hz, 1 H), 8.13 (d, J = 2.2 Hz, 1 H), 7.68 (dd, J = 8.4, 1.8 Hz, 1 H), 7.57 (dd, J = 8.4, 2.1 Hz, 1 H), 7.27 (d, J = 8.4 Hz, 1 H), 7.06 (d, J = 8.4 Hz, 1 H), 3.88 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 189.2, 165.2, 145.6, 136.2, 135.9, 135.0, 134.9, 134.5, 134.3, 133.9, 133.5, 132.8, 122.4, 111.4, 52.8; HRMS (ESI) *m/z* calcd for $C_{16}H_{11}BrNO_3S$ ([M+H]$^+$) 375.9643, found 375.9668. Note: characterization was taken from Dr. Igor Opsenica's final postdoctoral research report.
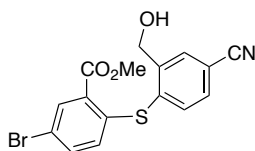
2.22

Methyl 5-bromo-2-((4-cyano-2-(hydroxymethyl)phenyl)thio)benzoate (2.22). To a stirred solution of methyl 5-bromo-2-((4-cyano-2-formylphenyl)thio)benzoate (**2.21**, 1.40 g, 3.72 mmol) in dry MeOH (60 mL) was added NaBH$_4$ (0.154 g, 4.09 mmol) and the mixture was stirred at rt for 30 min where analysis by TLC (hexanes/EtOAc, 7:3) indicated the starting

material had been consumed. The reaction was concentrated under reduced pressure, and the residue was diluted with EtOAc (80 mL) and 10% HCl aq. (15 mL), and the aqueous layer was extracted with EtOAc (80 mL). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was triturated with Et$_2$O to give **2.22** (1.39 g, 3.67 mmol, 99%) as a colorless solid: Mp 76-77 °C; IR (neat) 3515, 3088, 2945, 2235, 1711, 1456, 1446, 1301, 1236 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.18 (d, J = 2.2 Hz, 1 H), 7.94 (s, 1 H), 7.56 (dd, J = 8.0, 1.6 Hz, 1 H), 7.47 (d, J = 8.0 Hz, 1 H), 7.41 (dd, J = 8.6, 2.2 Hz, 1 H), 6.64 (d, J = 8.6 Hz, 1 H), 4.77 (s, 2 H), 3.92 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 165.4, 145.1, 138.0, 136.8, 135.6, 135.5, 134.2, 131.6, 131.3, 130.1, 129.8, 119.7, 118.2, 113.1, 62.1, 52.7; HRMS (ESI) *m/z* calcd for C$_{16}$H$_{13}$BrNO$_3$S ([M+H]$^+$) 377.9800, found 377.9820. Note: characterization was taken from Dr. Igor Opsenica's final postdoctoral research report.
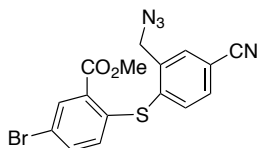
2.23

Methyl 2-((2-(azidomethyl)-4-cyanophenyl)thio)-5-bromobenzoate (2.23). To a solution of methyl 5-bromo-2-((4-cyano-2-(hydroxymethyl)phenyl)thio)benzoate (**2.22**, 1.00 g, 2.64 mmol) in CH$_2$Cl$_2$ (30 mL) under Ar at 0 °C was added Et$_3$N (0.56 mL, 3.97 mmol) and MsCl (0.23 mL, 2.91 mmol). The cooling bath was removed and the solution was stirred at rt for 2 h. The reaction mixture was poured into CH$_2$Cl$_2$ (40 mL) washed with H$_2$O (10 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure to give the mesylate as a crude intermediate.

A mixture of the above crude mesylate and sodium azide (0.868 g, 13.2 mmol) in DMF (10 mL) under an Ar atmosphere was stirred at rt for 3 h, poured onto EtOAc (100 mL), washed

with H$_2$O (30 mL) and brine, dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give

2.23 (1.01 g, 2.51 mmol, 95%) as a tan solid: Mp 121-122 °C; IR (neat) 3096, 3070, 3001, 2950, 2240, 2127, 2089, 1704, 1450, 1437, 1295, 1243 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.15 (d, *J* = 2.4 Hz, 1 H), 7.82 (s, 1 H), 7.61 (dd, *J* = 8.0, 2.0 Hz, 1 H), 7.53 (d, *J* = 8.0 Hz, 1 H), 7.44 (dd, *J* = 8.4, 2.4 Hz, 1 H), 6.64 (d, *J* = 8.4 Hz, 1 H), 4.58 (s, 2 H), 3.94 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 165.3, 140.1, 138.4, 137.5, 135.8, 135.7, 134.3, 132.4, 132.3, 130.2, 130.1, 120.0, 117.8, 113.2, 52.7, 52.0; HRMS (ESI) *m/z* calcd for C$_{16}$H$_{12}$BrN$_4$O$_2$S ([M+H]$^+$) 402.9864, found 402.9897. Note: characterization was taken from Dr. Igor Opsenica's final postdoctoral research report.
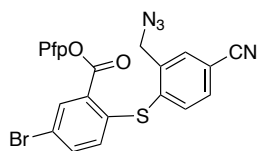
2.24

Perfluorophenyl 2-((2-(azidomethyl)-4-cyanophenyl)thio)-5-bromobenzoate (2.24) A solution of methyl 2-((2-(azidomethyl)-4-cyanophenyl)thio)-5-bromobenzoate (**2.23**, 0.800 g, 1.98 mmol) in MeOH (20 mL) and THF (20 mL) was treated with 1 M aqueous NaOH (18 mL). The solution was stirred for 5 h at rt, concentrated under reduced pressure and acidified to pH 2 with 1 M aqueous HCl, extracted with EtOAc (100 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure to give crude 2-((2-(azidomethyl)-4-cyanophenyl)thio)-5-bromobenzoic acid that was used in next reaction without purification.

A solution of 2-((2-(azidomethyl)-4-cyanophenyl)thio)-5-bromobenzoic acid (0.700 g, 1.80 mmol) in CH$_2$Cl$_2$ (60 mL) was treated with Pfp-OH (0.331 g, 1.80 mmol), EDCI (0.518 g, 2.70 mmol) and DMAP (0.0220 g, 0.180 mmol) at 0 °C. The mixture was warmed at rt for 2.5 h, poured onto H$_2$O (50 mL), and extracted with CH$_2$Cl$_2$ (2 x 100 mL). The combined organic

layers were washed with Brine, dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give **2.24** (0.619 g, 1.12 mmol, 56% (2 steps)) as a colorless solid: Mp 160-163 °C; IR (neat) 3089, 2938, 2235, 2103, 1750, 1517, 1454, 1214 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.41 (d, J = 2.0 Hz, 1 H), 7.85 (s, 1 H), 7.66 (dd, J = 8.0, 2.0 Hz, 1 H), 7.59-7.56 (m, 2 H), 6.71 (d, J = 8.4 Hz, 1 H), 4.59 (s, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.5, 142.2 (m, C-F coupling), 140.9 (m, C-F coupling), 140.6, 140.2 (m, C-F coupling), 140.1, 139.0 (C-F coupling), 137.4, 137.2, 137.0 (m, C-F coupling), 136.2, 135.3, 132.6, 132.5, 130.3, 126.2, 124.7 (m, C-F coupling), 120.1, 117.6, 113.9, 52.0; HRMS (ESI) *m/z* calcd for C$_{21}$H$_9$BrF$_5$N$_4$O$_2$S ([M+H]$^+$) 554.9550, found 554.9534. Note: characterization was taken from Dr. Igor Opsenica's final postdoctoral research report.
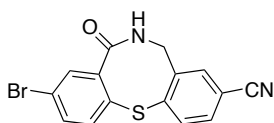
2.17

9-Bromo-7-oxo-6,7-dihydro-5H-dibenzo[*b,g*][1,5]thiazocine-3-carbonitrile (2.17). A solution of perfluorophenyl 2-((2-(azidomethyl)-4-cyanophenyl)thio)-5-bromobenzoate (**2.24**, 0.500 g, 0.900 mmol) in MeCN (48 mL) and H$_2$O (12 mL) was treated with PPh$_3$ (0.286 g, 1.08 mmol) at rt. The mixture was stirred at 50 °C for 20 h. During reaction a precipitate formed. The reaction mixture was concentrated under reduced pressure and filtered. The solid was washed with CH$_2$Cl$_2$ and acetone. The solid by NMR still contained trace PPh$_3$ oxide and was sonicated in acetone and decanted several times to give **2.17** (0.212 g, 0.614 mmol, 68%) as a colorless solid: Mp >250 °C; IR (neat) 3309, 3227, 3102, 2228, 1661, 1469, 1447, 1404, 1342 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.46 (t, J = 6.8 Hz, 1 H), 7.81-7.72 (m, 4 H), 7.68-7.63 (m, 2 H), 4.20-4.10 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 169.7, 144.3, 139.4, 138.0, 137.2, 134.5,

134.1, 131.7, 130.7, 130.1, 125.3, 123.6, 118.2, 109.6, 45.0; HRMS (ESI) *m/z* calcd for C$_{15}$H$_{10}$BrN$_2$OS ([M+H]$^+$) 344.9697, found 344.9669. Note: characterization was taken from Dr. Igor Opsenica's final postdoctoral research report.
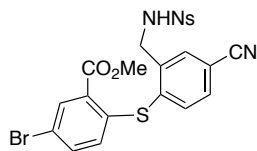
2.26

**Methyl** **5-bromo-2-((4-cyano-2-((4-nitrophenylsulfonamido)methyl)phenyl)thio)benzoate (2.26).** A solution of methyl 5-bromo-2-((4-cyano-2-formylphenyl)thio)benzoate (**2.21**, 0.500 g, 1.33 mmol) in absolute EtOH (10 mL) was treated titanium(IV)isopropoxide (2.45 mL, 7.97 mmol), 4-nitrobenzenesulfonamide (0.537 g, 2.66 mmol), Et$_3$N (0.37 mL, 2.66 mmol) and stirred at rt for 13 h. NaBH$_4$ (0.101 g, 2.66 mmol) was added and the mixture stirred for additional 2 h at rt, diluted with aqueous ammonium hydroxide (5 mL). The resulting inorganic precipitate was removed by filtration, washed with EtOAc (50 mL) and H$_2$O (10 mL). The organic layer was separated washed with 1 M aqueous HCl, brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:1) to give **2.26** (0.455 g, 0.808 mmol, 61%) as a colorless solid: Mp 138.7-141.5 °C; IR (neat) 3312, 3103, 2952, 2229, 1709, 1527, 1340, 1303, 1247, 1171, 1159, 818, 734 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.29 (dd, *J* = 2.2, 9.2 Hz, 2 H), 8.12 (d, *J* = 2.4 Hz, 1 H), 7.95 (dd, *J* = 2.2, 9.2 Hz, 2 H), 7.73 (d, *J* = 1.8 Hz, 1 H), 7.52 (dd, *J* = 1.8, 8.4 Hz, 1 H), 7.49 (dd, *J* = 1.8, 8.4 Hz, 1 H), 7.34 (d, *J* = 7.8 Hz, 1 H), 6.73 (d, *J* = 7.8 Hz, 1 H), 5.52 (bt, *J* = 6.2 Hz, 1 H), 4.28 (d, *J* = 6.2 Hz, 2 H), 3.91 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 165.2, 150.1, 145.5, 139.4, 139.3, 136.2, 135.9, 134.8, 134.6,

133.5, 132.5, 131.6, 130.4, 128.3, 124.3, 120.9, 117.5, 112.9, 52.9, 45.1; HRMS (ESI) *m/z* calcd for $C_{22}H_{16}N_3O_6BrS_2$ ([M+Na]$^+$) 583.9562, found 583.9558.
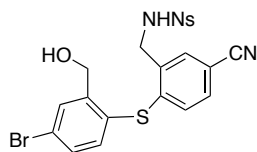
2.27

***N*-(2-((4-Bromo-2-(hydroxymethyl)phenyl)thio)-5-cyanobenzyl)-4-nitrobenzenesulfonamide (2.27)** A solution of methyl 5-bromo-2-((4-cyano-2-((4-nitrophenylsulfonamido)methyl)phenyl)thio)benzoate (**2.26**, 0.250 g, 0.445 mmol) in dry THF (16 mL) was treated with NaBH$_4$ (0.168 g, 4.45 mmol) and the mixture was refluxed for 30 min followed by the addition of MeOH (8 mL) and the solution was refluxed for 25 h, cooled to rt, and concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL) and washed with 10% HCl aq. (10 mL). The aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:1) to give **2.27** (0.110 g, 0.207 mmol, 47%) as a colorless amorphous solid: IR (neat) 3525, 3320, 3142, 2928, 2229, 1685, 1596, 1528, 1411, 1348, 1142, 1090, 1033, 736 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.31 (d, *J* = 8.8 Hz, 2 H), 8.01 (d, *J* = 8.8 Hz, 2 H), 7.74 (d, *J* = 1.8 Hz, 1 H), 7.56 (d, *J* = 1.8 Hz, 1 H), 7.46 (dd, *J* = 1.8, 8.4 Hz, 1 H), 7.49 (dd, *J* = 1.8, 8.4 Hz, 1 H), 7.32 (dd, *J* = 1.8, 8.4 Hz, 1 H), 7.19 (d, *J* = 8.2 Hz, 1 H), 6.77 (d, *J* = 8.2 Hz, 1 H), 5.30 (bt, *J* = 6.2 Hz, 1 H), 4.60 (s, 2 H), 4.30 (d, *J* = 6.2 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 150.1, 145.5, 145.4, 142.4, 136.9, 135.0, 132.7, 132.3 (2 C), 132.1, 128.5, 128.3, 127.0, 125.1, 124.4, 118.0, 109.7, 62.5, 44.7; HRMS (ESI) *m/z* calcd for $C_{21}H_{16}N_3O_5BrS_2$ ([M]$^+$) 534.9694, found 534.9688.
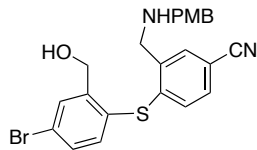
2.29

4-((4-Bromo-2-(hydroxymethyl)phenyl)thio)-3-(((4-

methoxybenzyl)amino)methyl)benzonitrile (2.29) A solution of methyl 5-bromo-2-((4-cyano-

2-formylphenyl)thio)benzoate (**2.21**, 0.300 g, 0.797 mmol) in absolute EtOH (8 mL) was treated

with titanium(IV)isopropoxide (0.612 mL, 1.99 mmol), 4-methoxybenzylamine (0.219 g, 1.59

mmol), Et$_3$N (0.22 mL, 1.59 mmol) and stirred at rt for 2 h. Analysis by ^{1}H NMR (300 MHz,

CDCl$_3$) indicated that the aldehyde had been consumed, NaBH$_4$ (0.0603 g, 1.59 mmol) was

added and the mixture stirred for 17 h where analysis by TLC (hexanes/EtOAc, 1:1) indicated

the imine intermediate had been consumed. The reaction was diluted with aqueous ammonium

hydroxide (5 mL). The resulting inorganic precipitate was filtered off washed with EtOAc (30

mL) and H$_2$O (10 mL), and the organic layer separated. The aqueous layer was extracted with

EtOAc (2 x 20 mL) and the combined organic layers were washed with satd. aqueous NaHCO$_3$,

brine, and dried (Na$_2$SO$_4$). The crude residue was purified by chromatography on SiO$_2$

(hexanes/EtOAc, 2:1 to hexanes/EtOAc, 1:2 w/ 3% Et$_3$N) to give **2.29** (0.196 g, 0.418 mmol,

53%) as a colorless solid: Mp 125.7-128.6 °C; IR (neat) 3262, 3006, 2870, 2833, 2220, 1592,

1510, 1454, 1238, 1174, 1070, 1031, 822, 736 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.76 (s, 1 H),

7.66 (s, 1 H), 7.48 (d, J = 8.0 Hz, 1 H), 7.32 (d, J = 8.2 Hz, 2 H), 7.28-7.24 (m, 3 H), 6.90 (d, J =

8.2 Hz, 2 H), 6.80 (d, J = 8.0 Hz, 1 H), 4.61(s, 2 H), 3.89 (s, 2 H), 3.82 (s, 3 H), 3.80 (s, 2 H);

^{13}C NMR (100 MHz, CDCl$_3$) δ158.9, 146.1, 143.0, 139.1, 137.5, 132.2, 132.0, 131.8, 131.5,

131.1, 129.4, 128.0, 127.9, 124.7, 118.6, 113.9, 113.8, 62.6, 55.3, 52.9, 50.2; HRMS (ESI) *m/z*

calcd for C$_{23}$H$_{22}$N$_2$O$_2$Br ([M+H]$^+$) 469.0580, found 469.0575.
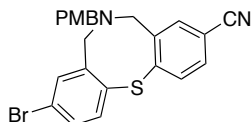
2.30

9-Bromo-6-(4-methoxybenzyl)-6,7-dihydro-5*H*-dibenzo[*b*,*g*][1,5]thiazocine-3-carbonitrile (2.30). A solution of 4-((4-bromo-2-(hydroxymethyl)phenyl)thio)-3-(((4-methoxybenzyl)amino)methyl)benzonitrile (**2.29**, 0.100 g, 0.213 mmol) in dry THF (20 mL) at 0 °C, was treated with phosphorus tribromide (13.5 μL, 0.142 mmol), stirred at 0 °C for 2 h, heated at reflux for 27 h, cooled to rt, diluted with H_2O (5 mL), and made basic with solid K_2CO_3. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 3:1 w/ 5% Et_3N) to give **2.30** (0.0624 g, 0.138 mmol, 65%) as a colorless solid: Mp 149.6-152.8 °C; IR (neat) 3064, 3003, 2949, 2833, 2803, 2227, 1614, 1514, 1463, 1253, 1174, 1092, 1046, 815, 746 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$, 100 °C) δ 7.75 (d, *J* = 8.0 Hz, 1 H), 7.65 (d, *J* = 8.0 Hz, 1 H), 7.58 (d, *J* = 7.6 Hz, 1 H), 7.47 (d, *J* = 7.6 Hz, 1 H) 7.41 (s, 1 H), 7.21 (d, *J* = 7.6 Hz, 1 H), 6.93 (d, *J* = 7.6 Hz, 1 H), 4.13 (s, 4 H), 4.79 (s, 3 H), 3.42 (s, 2 H); ^{13}C NMR (100 MHz, DMSO-d$_6$, 100 °C) δ 165.2, 150.1, 145.5, 139.4, 139.3, 136.2, 135.9, 134.8, 134.6, 133.5, 132.5, 131.6, 130.4, 128.3, 124.3, 120.9, 117.5, 112.9, 52.9, 45.1; HRMS (ESI) *m/z* calcd for $C_{23}H_{20}N_2OBrS$ ([M+H]$^+$) 451.0474, found 451.0482.
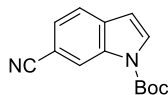
1-(*tert*-Butoxycarbonyl)-1*H*-indole-6-carbonitrile[478] A solution of 6-cyanoindole (4.00 g, 28.1 mmol) and Boc-anhydride (7.68 g, 35.2 mmol) in anhydrous CH_2Cl_2 (45 mL) at rt was

treated with DMAP (0.0530 g, 0.422 mmol) stirred for 3 h, diluted with ice H_2O (200 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (200 mL). The combined organic layers were washed with 0.5M aqueous HCl (50 mL), brine (100 mL) and H_2O (200 mL), and dried ($MgSO_4$). The solution was filtered through a pad of SiO_2 (CH_2Cl_2) and the combined filtrates were concentrated under reduced pressure. The product was crystallized from cold EtOH/hexanes (1:1) to give 1-(*tert*-butoxycarbonyl)-1*H*-indole-6-carbonitrile (6.15 g, 25.4 mmol, 90%) as a colorless solid: ^1H NMR (500 MHz, $CDCl_3$) δ 8.51 (bs, 1 H), 7.77 (bs, 1 H), 7.64 (d, $J = 8.0$ Hz, 1 H), 7.48 (d, $J = 8.0$ Hz, 1 H), 6.64 (d, $J = 3.5$ Hz, 1 H), 1.70 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.9, 134.2, 133.8, 129.2, 125.7, 121.7, 119.7, 107.2, 107.1, 85.0, 28.1.
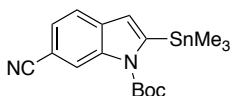
2.31

1-(*tert*-Butoxycarbonyl)-2-(trimethylstannyl)-1H-indole-6-carbonitrile[478] **(2.31).** A solution of distilled iPr$_2$NH (2.21 mL, 15.5 mmol) in anhydrous THF (15 mL) at -78 °C was treated with *n*-BuLi (2.5 M in hexanes)(5.40 mL, 13.4 mmol) dropwise. The solution was allowed to warm to 0 °C briefly then cooled back down to -78 °C. The solution was then cannulated dropwise into a solution, cooled to -78 °C, of 1-(tert-butoxycarbonyl)-1H-indole-6-carbonitrile (2.50 g, 10.3 mmol) and trimethyltin chloride (2.50 g, 12.4 mmol) in THF (30 mL) while keeping the temperature below -40 °C. The reaction mixture was stirred at -78 °C for 30 min then warmed to rt and left to stir for 22 h. After quenching the reaction with H_2O (5 mL), the reaction mixture was concentrated under reduced pressure. The crude residue was dissolved in Et$_2$O (250 mL), washed with 10% KF solution (50 mL) and H_2O, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure to give **2.31** (3.82 g, 9.43 mmol, 91%) as a colorless solid,

which was used immediately in the next reaction: ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.25 (bs, 1 H), 7.59 (d, J = 8.1 Hz, 1 H), 7.44 (dd, J = 8.1, 0.9 Hz, 1 H), 6.79 (t, J = 8.6 Hz, 1 H), 1.74 (s, 9 H), 0.35 (t, J_{Sn-H} = 28.8 Hz, 9 H).
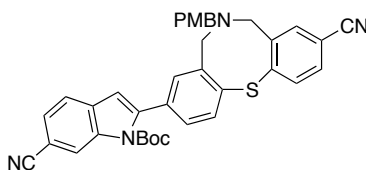
2.32

tert-**Butyl 6-cyano-2-(9-cyano-6-(4-methoxybenzyl)-6,7-dihydro-5*H*-dibenzo[*b,g*][1,5]thiazocin-3-yl)-1*H*-indole-1-carboxylate (2.32)** A solution of 9-bromo-6-(4-methoxybenzyl)-6,7-dihydro-5H-dibenzo[b,g][1,5]thiazocine-3-carbonitrile (**2.30**, 0.500 g, 1.11 mmol) and tert-butyl 6-cyano-2-(trimethylstannyl)-1H-indole-1-carboxylate (**2.31**, 0.769 g, 1.33 mmol) in dry degassed DMF (10 mL) was treated with Pd(PPh$_3$)$_2$Cl$_2$ (0.0778 g, 0.111 mmol) and (*i*Pr)CuCl[293] (0.0810 g, 0.166 mmol) at rt. The reaction mixture was heated to 80 °C for 21 h where analysis by LCMS indicated that the SM was consumed. The reaction mixture was poured onto H$_2$O (20 mL) and extracted EtOAc (3 x 75 mL). The combined organic layers were washed with satd. aqueous NaHCO$_3$ (10 mL), brine (10 mL), dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes to hexanes/EtOAc, 3:1 w/ 5% Et$_3$N). The product was sonicated with methanol and pelleted by centrifugation. The supernatant was removed and this process was repeated twice, and the product was dried under high vacuum to give **2.32** (0.312 g, 0.509 mmol, 46%) as a colorless solid. Due to the removal of the Boc group during high temperature NMR analysis this product was carried onto the Boc-deprotection: Characteristic ^{1}H NMR peaks, ^{1}H NMR (400 MHz, DMSO-d$_6$, 82 °C) δ 8.44 (s, 1 H), 7.82 (t, J = 8.2 Hz, 2 H), 7.76 (d, J = 8.0 Hz, 1 H), 7.70 (d, J = 8.0 Hz, 1 H), 7.64 (d, J = 8.2 Hz, 1 H), 7.45-7.43 (m, 2 H), 7.24-7.22 (m, 3 H), 6.92 (d, J = 8.2

Hz, 2 H), 6.88 (s, 1 H), 4.18 (s, 4 H), 3.77 (s, 3 H), 3.47 (s, 2 H), 1.30 (s, 9 H); HRMS (ESI) *m/z* calcd for $C_{37}H_{33}N_4O_3S$ ([M+H]$^+$) 613.2268, found 613.2267.
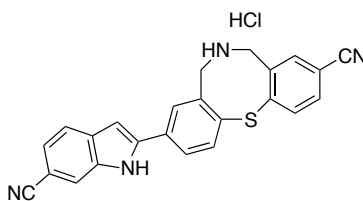
2.33

9-(6-Cyano-1*H*-indol-2-yl)-6,7-dihydro-5*H*-dibenzo[*b*,*g*][1,5]thiazocine-3-carbonitrile hydrochloride (2.33). A solution of tert-butyl 6-cyano-2-(9-cyano-6-(4-methoxybenzyl)-6,7-dihydro-5H-dibenzo[b,g][1,5]thiazocin-3-yl)-1H-indole-1-carboxylate (**2.33**, 0.150 g, 0.245 mmol) in dry 1,2-dichloroethane (4 mL) cooled to 0 °C was treated with α-chloroethylchloroformate (54 uL, 0.201 mmol) and stirred for 30 min at 0 °C. The reaction was heated to 90 °C for 4 h at which point analysis by LCMS indicated the starting material had been consumed. The reaction was concentrated under reduced pressure, dissolved in dry MeOH (2 mL), and heated to reflux for 1 h. The resulting precipitate was collected by vacuum filtration and washed with Et₂O to give *tert*-butyl 6-cyano-2-(9-cyano-6,7-dihydro-5*H*-dibenzo[*b*,*g*][1,5]thiazocin-3-yl)-1*H*-indole-1-carboxylate hydrochloride (0.106 g, 0.201 mmol) as a colorless solid. The product was taken on to the next deprotection with no further purification.

A solution of tert-butyl 6-cyano-2-(9-cyano-6,7-dihydro-5H-dibenzo[b,g][1,5]thiazocin-3-yl)-1H-indole-1-carboxylate hydrochloride (0.0200 g, 0.0378 mmol) in 4M HCl in dioxane (1 mL) was stirred in a sealed tube at 0 °C for 20 min, warmed to rt for 4 h, and heated at 80 °C for 20 h. The reaction had turned green and was diluted with Et₂O (10 mL). The resulting precipitate was collected by vacuum filtration and washed with Et₂O (20 mL), and dried under high vacuum

to give **2.33** (0.0130 g, 0.0331 mmol, 72% (2 steps)) as a green solid: Mp 290 °C (decomp.); IR (neat) 3541, 3458, 2937, 2729, 2608, 2216, 1735, 1596, 1458, 1314, 1254, 1221, 1117, 1055, 870, 818 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$, 100 °C) δ 12.16 (s, 1 H), 8.20 (s, 1 H), 8.00 (m, 2 H), 7.85-7.79 (m, 5 H), 7.72 (d, J = 8.0 Hz, 1 H), 7.31 (d, J = 8.0 Hz, 1 H), 7.09 (s, 1 H), 4.51 (s, 2 H), 4.43 (s, 2 H); ^{13}C NMR (100 MHz, DMSO-d$_6$, 100 °C) δ 143.7, 139.6, 137.3, 135.8, 134.7, 132.6, 132.0, 131.2, 130.9, 126.7, 121.7, 120.7, 119.6, 117.0, 115.5, 110.3, 103.0, 100.2; HRMS (ESI) m/z calcd for C$_{24}$H$_{17}$N$_4$S ([M+H]$^+$) 393.1168, found 393.1172.
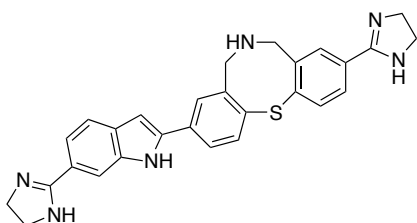
2.15

3-(4,5-Dihydro-1*H*-imidazol-2-yl)-9-(6-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-indol-2-yl)-6,7-dihydro-5*H*-dibenzo[*b,g*][1,5]thiazocine (2.15). A solution of 9-(6-cyano-1H-indol-2-yl)-6,7-dihydro-5H-dibenzo[b,g][1,5]thiazocine-3-carbonitrile (**30**, 0.0200 g, 0.0466 mmol) in ethylenediamine (1.0 mL) was treated with sulfur (0.0056 g, 0.0932 mmol). The reaction was irradiated in the microwave for 80 min at 110 °C, poured onto H$_2$O, filtered, and the resulting solid was washed with H$_2$O, Et$_2$O, and dried under high vacuum to give **2.15** (0.0142 g, 0.0297 mmol, 64%) as a brown orange solid: Mp 233 °C (decomp.); IR (neat) 3217, 2926, 2875, 1599, 1551, 1445, 1396, 1370, 1342, 1281, 1113, 1090, 1034, 984, 784, 708 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$, 100 °C) δ 11.45 (bs, 1 H), 7.91 (s, 1 H), 7.78 (s, 1 H), 7.72-7.63 (m, 5 H), 7.55 (s, 2 H), 6.92 (s, 1 H), 4.22 (s, 2 H), 4.18 (s, 2 H), 3.69 (s, 4 H), 3.63 (s, 4 H); ^{13}C NMR (100 MHz, DMSO-d$_6$, 100 °C) δ 164.4, 162.4, 143.5, 142.5, 138.6, 138.2, 136.3, 135.0, 132.3, 131.9, 131.3,

130.6, 130.0, 129.4, 127.5, 125.8, 124.1, 118.9, 118.5, 110.2, 99.1, 51.8, 49.2, 48.4; HRMS (ESI) *m/z* calcd for C$_{28}$H$_{27}$N$_6$S ([M+H]$^+$) 479.2012, found 479.2007.
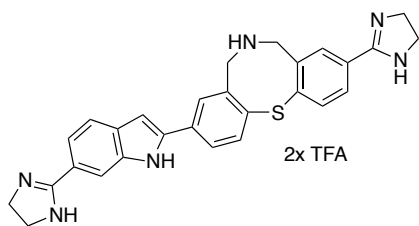
2.15•TFA

3-(4,5-Dihydro-1*H*-imidazol-2-yl)-9-(6-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-indol-2-yl)-6,7-dihydro-5*H*-dibenzo[*b,g*][1,5]thiazocine bis(2,2,2-trifluoroacetate) (2.15•TFA). A suspension of 3-(4,5-dihydro-1*H*-imidazol-2-yl)-9-(6-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-indol-2-yl)-6,7-dihydro-5*H*-dibenzo[*b,g*][1,5]thiazocine (**2.15**, 0.0400 g, 0.0836 mmol) in THF (0.5 mL) was treated with a solution of TFA in THF (100 µL, 4 M) and the reaction mixture was vigorously stirred at rt for 18 h, concentrated under reduced pressure, and co-evaporated with hexanes. The resulting solid was suspended in Et$_2$O and pelleted by centrifugation. The supernatant was removed and the solid was dried under high vacuum to give **2.15•TFA** (0.0572 g, 0.0809 mmol, 97%, 98.5% pure by ELSD) as a tan solid: Mp 272 °C (decomp.); IR (neat) 3210, 3094, 2753, 1664, 1609, 1482, 1426, 1370, 1289, 1197, 1174, 1130, 1034, 824, 798, 719 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$, 100 °C) δ 12.06 (bs, 1 H), 10.14 (bs, 1 H), 8.05-8.03 (m, 2 H), 7.96-7.79 (m, 6 H), 7.58 (dd, *J* = 8.4, 1.8 Hz, 1 H), 7.12 (d, *J* = 1.8 Hz, 1 H), 4.40 (s, 2 H), 4.28 (s, 2 H), 4.07 (s, 4 H), 4.05 (s, 4 H); ^{13}C NMR (100 MHz, DMSO-d$_6$, 100 °C) δ 166.0, 163.9, 158.3, 157.9, 150.3, 150.1, 147.8, 144.7, 140.7, 136.0, 134.7, 132.6, 132.1, 128.6, 126.6, 121.2, 120.2, 118.7, 114.4, 112.2 100.3, 44.2, 43.9; HRMS (ESI) *m/z* calcd for C$_{28}$H$_{27}$N$_6$S ([M+H]$^+$) 479.2012, found 479.2007.

5.3.2 Synthesis of 1,2,4-Triazines
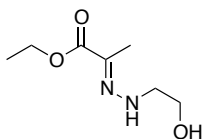
2.44

(*E*)-Ethyl 2-(2-(2-hydroxyethyl)hydrazono)propanoate (2.44).[303] A solution of ethyl pyruvate (**2.42**, 3.64 mL, 32.7 mmol) in THF (40 mL) was treated with a solution of 2-hydroxyethylhydrazine (**2.43**, 3.00 mL, 44.7 mmol) in EtOH (0.5 mL). This mixture was heated at reflux for 7 h, concentrated under reduced pressure, diluted with H_2O (30 mL) and EtOAc (30 mL), partitioned, and the aqueous layer was extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (100% EtOAc) to give **2.44** (3.90 g, 22.4 mmol, 68%) as a pale yellow oil: ^1H NMR (300 MHz, $CDCl_3$) δ 4.25 (q, *J* = 7.2 Hz, 2 H), 3.84 (dd, *J* = 5.1, 4.5 Hz, 2 H), 3.56 (dd, *J* = 5.1, 4.5 Hz, 2 H), 1.93 (s, 3 H), 1.31 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.9, 132.6, 62.3, 61.0, 52.1, 14.3, 10.0.
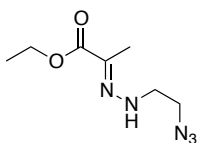
2.45

(*E*)-Ethyl 2-(2-(2-azidoethyl)hydrazono)propanoate (2.45).[303] A solution of (*E*)-Ethyl 2-(2-(2-hydroxyethyl)hydrazono)propanoate (**2.44**, 6.35 g, 36.5 mmol) and Et_3N (7.87 mL, 56.0 mmol) in THF (60 mL) was treated with MsCl (3.54 mL, 45.7 mmol). This mixture was stirred at rt for 20 min, diluted with satd. aqueous $NaHCO_3$ (80 mL) and H_2O (50 mL), and extracted with Et_2O (3 x 125 mL). The combined organic layers were dried (Na_2SO_4), and concentrated

under reduced pressure to give the crude mesylate as a yellow oil that was used without further purification.

The crude oil was dissolved in DMF (30 mL), treated with NaN_3 (7.14 g, 110. mmol) and heated to 50 °C behind a blast shield for 10 h where analysis by TLC (hexanes/EtOAc, 1:1) indicated that the mesylate intermediate was completely consumed. The reaction was cooled to rt, diluted with H_2O (60 mL) and brine (40 mL) and extracted with EtOAc (4 x 100 mL). The combined organic layers were washed with H_2O (50 mL), brine (2 x 100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes to hexanes/EtOAc, 2:1 gradient with 1% Et_3N) to give **2.45** (4.25 g, 21.4 mmol, 59% (2 steps)) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.83 (bs, 1 H), 4.29 (q, J = 7.2 Hz, 2 H), 3.84 (dd, J = 9.9, 5.1 Hz, 2 H), 3.56 (dd, J = 9.9, 5.1 Hz, 2 H), 1.96 (s, 3 H), 1.33 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.8, 113.7, 61.1, 51.2, 49.7, 14.3, 10.3.
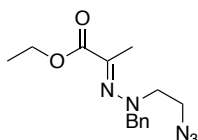
2.46a

Ethyl (E)-2-(2-(2-azidoethyl)-2-benzylhydrazineylidene)propanoate (2.46a).[303] A solution of (E)-ethyl 2-(2-(2-azidoethyl)hydrazono)propanoate (**2.45**, 1.00 g, 5.02 mmol) in dry DMF (28 mL) was treated with K_2CO_3 (1.39 g, 10.0 mmol), NaI (0.752 g, 5.02 mmol), and benzyl bromide (1.80 mL, 15.1 mmol), heated at 70 °C behind a blast shield for 18 h, cooled to rt, diluted with H_2O (15 mL), and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with H_2O (20 mL), brine (20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes to

hexanes/EtOAc, 1:1 with 1% NEt₃) to give **2.46a** (0.832 g, 2.88 mmol, 57%) as a golden oil: [1]H

NMR (300 MHz, CDCl₃) δ 7.37-7.29 (m, 5 H), 4.33 (q, *J* = 7.2 Hz, 2 H), 4.08 (s, 3 H), 3.34 (t, *J*

= 5.5 Hz, 2 H), 3.23 (t, *J* = 5.5 Hz, 2 H), 2.22 (s, 3 H), 1.36 (t, *J* = 7.2 Hz, 3 H);
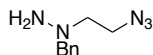
2.47a

1-(2-azidoethyl)-1-benzylhydrazine (2.47a).[303] A solution of (*E*)-ethyl 2-(2-(2-

azidoethyl)-2-benzylhydrazono)propanoate (**2.46a**, 1.30 g, 4.49 mmol) in THF (20 mL) and H₂O

(6 mL) at rt was treated with hydrazine dihydrochloride (1.41 g, 13.5 mmol). This mixture was

stirred at rt for 5 h, treated with solid Na₂CO₃, filtered through a cotton plug, washed (EtOAc),

and concentrated under reduced pressure. The resulting oil was immediately purified by

chromatography on SiO₂ (hexanes/EtOAc, 1:1 with 1% Et₃N) to give **2.47a** (0.634 g, 3.31 mmol,

74%) as an unstable colorless oil: ^{1}H NMR (300 MHz, CDCl₃) δ 7.38-7.29 (m, 5 H), 3.76 (s, 2

H), 3.49 (t, *J* = 5.7 Hz, 2 H), 2.77 (t, *J* = 5.7 Hz, 2 H).

2.46b

(*E*)-Ethyl 2-(2-(2-azidoethyl)-2-(4-methoxy benzyl)hydrazono)propanoate (2.46b).[303]

A solution of (*E*)-ethyl 2-(2-(2-azidoethyl)hydrazono)propanoate (**2.45**, 4.20 g, 21.1 mmol) in

DMF (50 mL) was treated with K₂CO₃ (5.83 g, 42.2 mmol), NaI (3.16 g, 21.1 mmol), and

freshly prepared paramethoxybenzyl bromide (9.2 mL, 12.7 g, 63.3 mmol). The mixture was

heated at 70 °C behind a blast shield for 22 h, cooled to rt, diluted with H₂O (100 mL) and

extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H₂O (100

mL), brine (100 mL), dried (Na₂SO₄), and concentrated under reduced pressure. The crude

residue was purified by chromatography on SiO$_2$ (hexanes to hexanes/EtOAc, 2:1 gradient with 1% Et$_3$N) to give **2.46b** (3.49 g, 10.9 mmol, 52%) as a golden oil: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.27 (d, J = 8.4 Hz, 2 H), 6.89 (d, J = 8.4 Hz, 2 H), 4.29 (q, J = 7.2 Hz, 2 H), 4.00 (s, 2 H), 3.79 (s, 3 H), 3.31 (t, J = 5.5 Hz, 2 H), 3.20 (t, J = 5.5 Hz, 2 H), 2.22 (s, 3 H), 1.35 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 164.6, 158.9, 152.6, 129.4, 128.3, 113.8, 61.4, 60.0, 55.5, 55.0, 49.4, 15.7, 14.0.
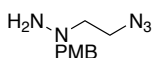
2.47b

1-(2-Azidoethyl)-1-(4-methoxybenzyl)hydrazine (2.47b).[303] A solution of (*E*)-ethyl 2-(2-(2-azidoethyl)-2-(4-methoxy benzyl)hydrazono)propanoate (**2.46b**, 0.150 g, 0.470 mmol) in THF (2 mL) and H$_2$O (1 mL) at rt was treated with hydrazine dihydrochloride (0.148 g, 1.41 mmol), stirred at rt for 2 h, treated with solid Na$_2$CO$_3$, and concentrated under reduced pressure. The crude residue was immediately purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:2 with 1% Et$_3$N) to give **2.47b** (0.0761 g, 0.344 mmol, 73%) as an unstable, colorless oil: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.26 (d, J = 8.6 Hz, 2 H), 6.89 (d, J = 8.6 Hz, 2 H), 3.81 (s, 3 H), 3.67 (s, 2 H), 3.48 (t, J = 5.6 Hz, 2 H), 2.74 (t, J = 5.6 Hz, 2 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 130.3, 114.0, 66.9, 58.5, 55.3, 44.7.
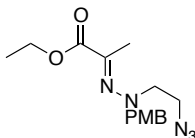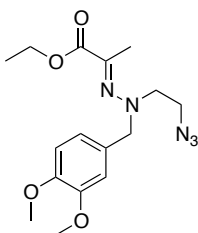

2.46c

(*E*)-Ethyl 2-(2-(2-azidoethyl)-2-(3,4-dimethoxybenzyl)hydrazono)propanoate (2.46c).

A solution of (*E*)-ethyl 2-(2-(2-azidoethyl)hydrazono)propanoate (**2.45**, 13.7 g, 68.8 mmol) in

DMF (138 mL) was treated 4-(chloromethyl)-1,2-dimethoxybenzene (32.1 g, 138 mmol), KI

(34.3 g, 206 mmol), and K_2CO_3 (28.5 g, 206 mmol). The reaction mixture was stirred at rt for 2

d, diluted with H_2O (500 mL) and extracted with EtOAc (3 x 250 mL). The combined organic

layers were washed with H_2O (300 mL), brine (300 mL), dried (Na_2SO_4), filtered, and

concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2

(hexanes/EtOAc, 5:1 with 1% Et_3N) to give **2.46c** (22.5 g, 57.9 mmol, 84%) as a yellow oil: IR

($CHCl_3$) 2921, 2848, 2097, 1708, 1590, 1512, 1462, 1258, 1234, 1135, 1025, 803 cm^{-1}; ^{1}H NMR

(300 MHz, $CDCl_3$) δ 6.94-6.81 (m, 3 H), 4.31 (q, *J* = 7.2 Hz, 2 H), 3.99 (s, 2 H), 3.87 (m, 6 H),

3.31 (t, *J* = 5.4 Hz, 2 H), 3.22 (t, *J* = 5.4 Hz, 2 H), 2.20 (s, 3 H), 1.34 (t, *J* = 7.2 Hz, 3 H); ^{13}C

NMR (75 MHz, $CDCl_3$) δ 164.6, 152.9, 149.0, 148.4, 128.9, 120.4, 111.1, 110.8, 61.5, 60.4,

55.7, 55.6, 49.4, 15.8, 14.0; HRMS (ESI) *m/z* calcd for $C_{16}H_{24}N_5O_2$ ([M+H]$^+$) 350.1823, found

350.1832.
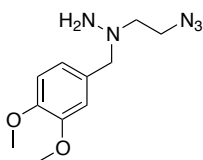
2.47c

1-(2-Azidoethyl)-1-(3,4-dimethoxybenzyl)hydrazine (2.47c). A solution of (*E*)-ethyl 2-

(2-(2-azidoethyl)-2-((3,4-methoxy benzyl)hydrazono)propanoate (**2.46c**, 1.00 g, 2.86 mmol) in

THF (40 mL) and H_2O (2 mL) at 25 °C was treated with hydrazine dihydrochloride (0.901 g,

8.59 mmol). The reaction mixture was stirred at rt for 24 h, treated with solid Na_2CO_3, filtered

and washed with EtOAc, concentrated under reduced pressure. The crude residue was purified

by chromatography on SiO_2 (EtOAc/hexanes, 3:2 with 1% Et_3N) to give **2.47c** (0.515 g, 2.05

mmol, 72%) as yellow oil: IR (CH₂Cl₂) 3426, 2934, 2831, 2097, 1696, 1590, 1512, 1450, 1260, 1232, 1135, 1025, 764 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.99 (s, 1 H), 6.82 (bd, J = 0.9 Hz, 2 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.72 (s, 2 H), 3.49 (t, J = 5.6 Hz, 2 H), 2.79 (t, J = 5.6 Hz, 2 H); ¹³C NMR (75 MHz, CDCl₃) δ 149.3, 148.6, 121.1, 111.8, 110.9, 67.1, 58.5, 55.9, 48.4; HRMS (ESI) m/z calcd for C₁₁H₁₈N₅O₂ ([M+H]⁺) 252.1455, found 252.1458.

5.3.3 Synthesis of Triazine Analogs 2.51a-c
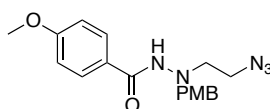
2.50a

***N*'-(2-Azidoethyl)-4-methoxy-*N*'-(4-methoxybenzyl)benzohydrazide (2.50a).** A solution of 4-methoxybenzoic acid (**2.49a**, 0.250 g, 1.64 mmol) in CH₂Cl₂ (10 mL) was cooled to 0 °C and treated with oxalyl chloride (0.16 mL, 1.81 mmol) and DMF (2 drops). The reaction mixture was stirred at rt for 2 h, concentrated under reduced pressure and used without further purification.

A solution of 4-methoxybenzoyl chloride, prepared above, in CH₂Cl₂ (8 mL) was cooled to 0 °C and treated with a solution of 1-(2-azidoethyl)-1-(4-methoxybenzyl)hydrazine (**2.47b**, 0.400 g, 1.81 mmol) in CH₂Cl₂ (1.0 mL), and Et₃N (0.69 mL, 4.93 mmol). The reaction mixture was allowed to warm to rt for 20 h, diluted with satd. aqueous NaHCO₃ (2 mL), and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The crude residue was washed with hexanes and filtered to give **2.50a** (0.436 g, 1.23 mmol, 75%) as a colorless solid that was used without further purification: Mp 90.4-91.8 °C; IR (neat) 3251, 2833, 2114, 2089, 1640, 1605, 1504, 1459, 1252,

172

1171, 1023, 820, 768 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.54 (d, J = 8.4 Hz, 2 H), 7.27 (d, J = 8.4 Hz, 2 H), 7.01 (bs, 1 H), 6.88 (d, J = 6.8 Hz, 2 H), 6.85 (d, J = 6.8 Hz, 2 H), 4.22 (s, 2 H), 3.83 (s, 3 H), 3.79 (s, 3 H), 3.42 (t, J = 5.6 Hz, 2 H); 3.34 (t, J = 5.6 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.9, 162.4, 159.2, 130.6, 128.6, 125.7, 113.9, 59.6, 55.4, 55.2, 54.3, 49.0; HRMS (ESI) m/z calcd for C$_{18}$H$_{22}$N$_5$O$_3$ ([M+H]$^+$) 356.1717, found 356.1715.
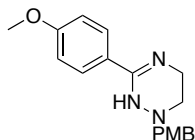
2.51a

1-(4-Methoxybenzyl)-3-(4-methoxyphenyl)-1,2,5,6-tetrahydro-1,2,4-triazine (2.51a).
A flame-dried microwave vial containing a solution of N'-(2-azidoethyl)-4-methoxy-N'-(4-methoxybenzyl)benzohydrazide (**2.50a**, 0.0500 g, 0.141 mmol) in dry o-1,2-dichlorobenzene (4 mL) was treated with P(n-Bu)$_3$ (0.139 mL, 0.563 mmol). The reaction mixture was irradiated in the microwave at 180 °C for 1 h, treated with Et$_3$N (0.1 mL) and then applied directly to a 4g silica cartridge and purified by automated chromatography on SiO$_2$ (gradient, 100% hexanes to 100% EtOAc over 15 min) to give **2.51a** (0.0206 g, 0.0662 mmol, 47%) as a tan solid: Mp 115.5-119.8 °C; IR (hexanes) 3410, 1609, 1508, 1491, 1446, 1351, 1238, 1172, 1025, 988, 843, 762 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.56 (d, J = 8.8 Hz, 2 H), 7.37 (d, J = 8.8 Hz, 2 H), 6.87 (dd, J = 8.8, 2.0 Hz, 4 H), 4.13 (s, 2 H), 3.81 (s, 6 H), 3.54 (bs, 2 H), 2.70 (t, J = 5.0 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.2, 158.8, 144.8, 130.6, 130.1, 128.1, 126.8, 113.7, 113.5, 63.3, 55.3, 55.2, 45.0, 41.7; HRMS (ESI) m/z calcd for C$_{18}$H$_{21}$N$_3$O$_2$ ([M+H]$^+$) 312.1707, found 312.1695.
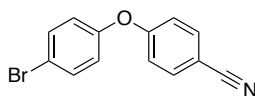
2.57

4-(4-Bromophenoxy)benzonitrile (2.57).[479] A solution of 4-bromophenol (**2.58**, 4.00 g, 22.7 mmol) and 4-fluorobenzonitrile (**2.59**, 3.05 g, 24.9 mmol) in DMF (22 mL) was treated with K_2CO_3 (3.46 g, 23.8 mmol) and the mixture was stirred for 10 min at rt, then heated at 150 °C under nitrogen for 24 h, cooled to rt, poured onto ice and stirred for 10 min. A tan solid formed and was collected by vacuum filtration and washed with H_2O. The solid was dissolved in CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was recrystallized with EtOH to give **2.57** (5.41 g, 19.8 mmol, 87%) as a tan crystalline solid: ^1H NMR (500 MHz, CDCl$_3$) δ 7.61 (d, J = 8.0 Hz, 2 H), 7.52 (d, J = 8.0 Hz, 2 H), 7.01 (d, J = 8.5 Hz, 2 H), 6.95 (d, J = 8.5 Hz, 2 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 161.0, 154.0, 134.1, 133.2, 121.9, 118.5, 118.0, 117.8, 106.3.
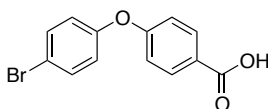
2.49b

4-(4-Bromophenoxy)benzoic acid (2.49b). A solution of 4-(4-bromophenoxy)benzonitrile (**2.57**, 0.300 g, 1.09 mmol) in EtOH (4 mL) and H_2O (1 mL) was treated with anhydrous LiOH (0.786 g, 32.8 mmol). The reaction was heated to reflux for 3 d, cooled to rt, acidified to pH 2 with 1 M aqueous HCl and the resulting precipitate was collected by vacuum filtration and washed with H_2O. The solid was then suspended in toluene and methanol and concentrated under reduced pressure to give **2.49b** (0.297 g, 1.01 mmol, 93%) as a colorless solid: ^1H NMR (500 MHz, DMSO-d$_6$) δ 7.93 (d, J = 8.0 Hz, 2 H), 7.59 (d, J = 8.0 Hz, 2 H), 7.05 (d, J = 7.8 Hz, 2 H), 7.00 (d, J = 7.8 Hz, 2 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 166.6, 160.4, 154.6, 133.1, 131.7, 125.7, 122.0, 117.5, 116.4; HRMS (ESI) *m/z* calcd for $C_{13}H_{10}O_3Br$ ([M+H]$^+$) 292.9808, found 292.9806.
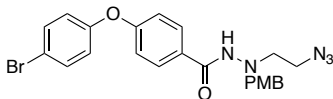
2.50b

***N'*-(Azidoethyl)-4-(4-bromophenoxy)-*N'*-(4-methoxybenzyl)benzohydrazide (2.50b).**

According to the procedure used to prepare compound **2.50a**, **2.49b** was converted to crude **2.50b**, which was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1) to give pure **2.50b** (0.328 g, 0.661 mmol, 55%) as a viscous oil that solidified in the freezer at -20 °C to a pale yellow solid: Mp 98.2-101.8 °C; IR (neat) 3310, 2958, 2101, 2076, 1653, 1603, 1512, 1495, 1478, 1232, 1174, 1010, 943, 869, 777 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.55 (d, *J* = 8.4 Hz, 2 H), 7.45 (d, *J* = 8.7 Hz, 2 H), 7.28 (d, *J* = 8.4 Hz, 1 H), 7.13 (bs, 1 H), 6.94-6.83 (m, 6 H), 4.21 (s, 2 H), 3.78 (s, 3 H), 3.42 (bd, *J* = 5.3 Hz, 2 H), 3.35 (bd, *J* = 5.3 Hz, 2 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 166.6, 160.1, 159.2, 155.1, 132.9, 130.5, 128.9, 128.5, 128.3, 121.3, 117.9, 116.9, 113.9, 59.6, 55.2, 54.3, 49.1; HRMS (ESI) *m/z* calcd for C$_{23}$H$_{23}$N$_5$O$_3$Br ([M+H]$^+$) 496.0979, found 469.0969.
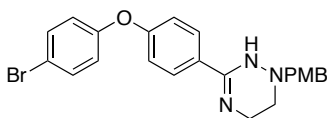
2.51b

3-(4-(4-Bromophenoxy)phenyl)-1-(4-methoxybenzyl)-1,2,5,6-tetrahydro-1,2,4-

triazine (2.51b). According to the procedure used to prepare compound **2.51a**, **2.50b** was converted to crude **2.51b**, which was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:1 with 1% Et$_3$N) to give **2.51b** (0.0288 g, 0.0637 mmol, 63%) as a tan solid: Mp 180.3-184.1 °C; IR (neat) 3450, 3040, 2939, 2814, 1610, 1579, 1510, 1482, 1247, 1238, 1167, 1033, 839, 818, 733 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.62 (d, *J* = 8.8 Hz, 2 H), 7.43 (d, *J* = 8.8 Hz, 2 H), 7.37

(d, J = 8.8 Hz, 1 H), 6.97 (d, J = 8.4 Hz, 2 H), 6.89-6.86 (overlapping doublets, J = 8.8, 8.4 Hz, 4

H), 4.14 (s, 2 H), 3.81 (s, 3 H), 3.56 (bd, J = 2.4 Hz, 2 H), 2.73 (t, J = 5.0 Hz, 2 H); ^{13}C NMR

(100 MHz, CDCl$_3$) δ 158.8, 157.4, 156.2, 132.7, 130.6, 129.8, 127.2, 120.5, 118.7, 115.9, 113.6,

63.2. 55.2, 44.9, 41.6; HRMS (ESI) m/z calcd for C$_{23}$H$_{23}$N$_3$O$_2$Br ([M+H]$^+$) 452.0968, found

452.0953.
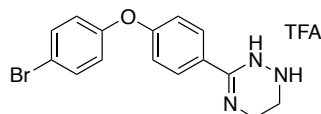
2.52•TFA

3-(4-(4-Bromophenoxy)phenyl)-1,2,5,6-tetrahydro-1,2,4-triazine **(2.52•TFA)** A

solution of 3-(4-(4-bromophenoxy)phenyl)-1-(4-methoxybenzyl)-1,2,5,6-tetrahydro-1,2,4-

triazine (**2.51b**, 0.0080 g, 0.0177 mmol) in TFA (0.2 mL) was warmed to 55 °C for 30 min. The

reaction was diluted with satd. aqueous NaHCO$_3$ (2.0 mL) and extracted with EtOAc (3 x 5 mL).

The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced

pressure to give **2.52•TFA** (0.00621 g, 0.0139 mmol, 79%) as a pale yellow amorphous solid: IR

(CH$_2$Cl$_2$) 3290, 2923, 2848, 1609, 1495, 1478, 1336, 1236, 1109, 1081, 1033, 736 cm^{-1}; ^{1}H

NMR (400 MHz, MeOD-d$_4$) δ 7.60 (d, J = 8.8 Hz, 2 H), 7.50 (d, J = 8.8 Hz, 2 H), 7.00 (d, J =

8.8 Hz, 1 H), 6.95 (d, J = 8.8 Hz, 2 H), 3.55 (t, J = 5.0 Hz, 2 H), 3.09 (t, J = 5.0 Hz, 2 H); ^{13}C

NMR (100 MHz, MeOD-d$_4$) δ 159.6, 157.5, 150.7, 134.0, 131.3, 129.0, 122.0, 119.4, 117.2,

42.1, 41.2; HRMS (ESI) m/z calcd for C$_{15}$H$_{15}$N$_3$OBr ([M+H]$^+$) 332.0393, found 332.0383.
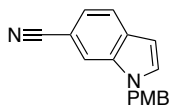
1-(4-Methoxybenzyl)-1*H*-indole-6-carbonitrile[480] A solution of 6-cyanoindole (0.800 g,

5.63 mmol) in THF (10 mL) at 0 °C was treated with NaH (60% dispersion in mineral oil) (0.472

g, 11.8 mmol) and stirred for 20 min at 0 °C followed by the addition of a solution of 4-methoxybenzyl bromide (1.25 g, 6.19 mmol) in THF (2 mL) dropwise. The reaction was stirred at 0 °C for 1 h, rt for 1 h, cooled to 0 °C, diluted with H_2O (1.0 mL), and concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), washed with H_2O (5 mL), brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 2:1 w/ 2% Et_3N) to give 1-(4-methoxybenzyl)-1H-indole-6-carbonitrile (0.575 g, 2.19 mmol, 39%) as a colorless solid: Mp 94.1-96.6 °C; IR (neat) 3128, 2958, 2932, 2218, 1610, 1510, 1465, 1446, 1349, 1314, 1247, 1174, 1025, 813, 738 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.67 (d, J = 8.0 Hz, 2 H), 7.62 (s, 1 H), 7.31 (bs, 2 H), 7.07 (d, J = 7.8 Hz, 2 H), 6.86 (d, J = 7.8 Hz, 2 H), 6.60 (s, 1 H), 5.25 (s, 2 H), 3.78 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 159.3, 134.8, 131.8, 128.1 (2 C), 122.2, 121.6, 120.6, 114.6, 114.2, 103.9, 102.3, 55.2, 49.9; HRMS (ESI) m/z calcd for C$_{17}$H$_{15}$N$_2$O ([M+H]$^+$) 263.1179, found 263.1181.
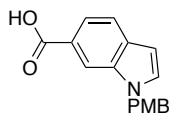
2.49c

1-(4-Methoxybenzyl)-1H-indole-6-carboxylic acid (2.49c) A solution of 1-(4-methoxybenzyl)-1H-indole-6-carbonitrile (0.100 g, 0.381 mmol) in EtOH (3.0 mL) and H_2O (3.0 mL) was treated with anhydrous LiOH (0.183 g, 7.62 mmol), and heated at reflux for 4 d, concentrated, suspended in H_2O (5.0 mL), and acidified to pH 2 using conc. HCl. The resulting precipitate was collected by vacuum filtration and dried under high vacuum to give **2.49c** (0.0980 g, 0.348 mmol, 91%) as an off-white solid: Mp 157.6-159.7 °C; IR (neat) 3107, 2962, 2636, 1672, 1612, 1508, 1463, 1286, 1234, 1174, 1019, 826, 809, 768, 736 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.25 (s, 1 H), 7.93 (dd, J = 8.4, 1.2 Hz, 1 H), 7.72 (d, J = 8.4 Hz, 1 H), 7.32 (d, J

= 3.2 Hz, 1 H), 7.14 (d, J = 8.7 Hz, 2 H), 6.89 (d, J = 8.7 Hz, 2 H), 6.63 (d, J = 3.2 Hz, 1 H), 5.36 (s, 2 H), 3.81 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.3, 159.2, 153.5, 133.0, 131.6, 128.8, 128.3, 122.3, 121.0, 120.5, 114.2, 112.7, 102.1, 55.2 (2 C), 49.6; HRMS (ESI) m/z calcd for C$_{17}$H$_{15}$NO$_3$ ([M+H]$^+$) 282.1125, found 282.1126.
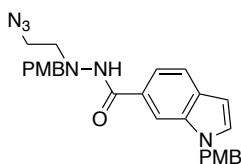
2.50c

N'-(2-Azidoethyl)-N',1-bis(4-methoxybenzyl)-1H-indole-6-carbohydrazide (2.50c). A solution of 1-(4-methoxybenzyl)-1H-indole-6-carboxylic acid (**2.49c**, 0.0750 g, 0.267 mmol) in CH$_2$Cl$_2$ (1 mL) was treated with 1-(2-azidoethyl)-1-(4-methoxybenzyl)hydrazine (**2.47b**, 0.0590 g, 0.267 mmol), DIPEA (0.14 mL, 0.800 mmol), hydroxybenzotriazole (0.0735 g, 0.533 mmol), and EDCI (0.0767 g, 0.400 mmol) and stirred at rt 2 d, diluted with CH$_2$Cl$_2$ (10 mL) and washed with a mixture of H$_2$O (2.0 mL) and satd. aqueous NaHCO$_3$ (5 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1 w/ 2% Et$_3$N) to give **2.50c** (0.0692 g, 0.143 mmol, 54%) as a colorless solid: Mp 110.6-114.4 °C; IR (neat) 3174, 3061, 2829, 2099, 1642, 1610, 1549, 1510, 1301, 1243, 1228, 1176, 1034, 822, 725 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.81 (s, 1 H), 7.64 (d, J = 8.4 Hz, 1 H), 7.36 (d, J = 7.6 Hz, 2 H), 7.28 (d, J = 11.6 Hz, 2 H), 7.11 (d, J = 7.6 Hz, 2 H), 6.90 (t, J = 7.6 Hz, 4 H), 6.59 (s, 1 H), 5.28 (s, 2 H), 4.30 (s, 2 H), 3.81 (s, 6 H), 3.47 (bs, 2 H), 3.42 (bs, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.3, 159.2, 159.1, 137.7, 131.3, 130.6 (2 C), 128.8, 128.7, 128.3, 126.5, 120.7, 117.2, 114.2, 113.8, 109.6, 101.8, 59.6, 55.2, 55.1, 54.3, 49.5, 49.0; HRMS (ESI) m/z calcd for C$_{27}$H$_{29}$N$_6$O$_3$ ([M+H]$^+$) 485.2296, found 485.2292.
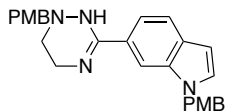
2.51c

1-(4-Methoxybenzyl)-6-(1-(4-methoxybenzyl)-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)-1*H*-indole (2.51c). A solution of N'-(2-azidoethyl)-N',1-bis(4-methoxybenzyl)-1H-indole-6-carbohydrazide (**2.50c**, 0.0400 g, 0.0826 mmol) in PhCl (4.0 mL) (sparged with Ar for 20 min) was treated with a solution of P(Me)$_3$ (1 M solution in toluene, 0.25 mL, 0.248 mmol) and irradiated in the microwave at 180 °C for 1 h. The reaction was applied directly to a column prequilibrated with hexanes and purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:3 w/ 5% Et$_3$N) to give **2.51c** (0.0198 g, 0.0449 mmol, 55%) as a yellow oil: IR (neat) 3383, 2951, 2833, 1609, 1510, 1461, 1355, 1241, 1174, 1023, 1007, 818, 759, 701 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.66 (s, 1 H), 7.59 (d, *J* = 8.0 Hz, 1 H), 7.39-7.36 (m, 3 H), 7.11 (d, *J* = 3.6 Hz, 1 H), 7.06 (d, *J* = 8.8 Hz, 2 H), 6.87 (d, *J* = 8.6 Hz, 2 H), 6.83 (d, *J* = 8.6 Hz, 2 H), 6.50 (d, *J* = 3.6 Hz, 1 H), 5.27 (s, 2 H), 4.16 (s, 2 H), 3.80 (s, 3 H), 3.77 (s, 3 H), 3.56 (bs, 2 H), 2.72 (t, *J* = 5.0 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 159.1, 158.8, 136.2, 130.6, 130.2, 129.4 (2 C), 129.1, 128.2, 120.7, 117.3, 114.1, 113.5, 107.2, 101.6, 63.3, 55.2 (2 C), 49.4, 45.1, 41.9; HRMS (ESI) *m/z* calcd for C$_{27}$H$_{29}$N$_4$O$_2$ ([M+H]$^+$) 441.2285, found 441.2283.
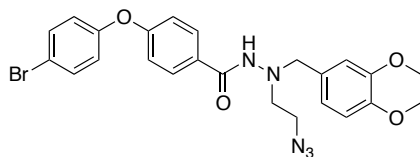
***N*'-(2-Azidoethyl)-4-(4-bromophenoxy)-*N*'-(3,4-dimethoxybenzyl)benzohydrazide.** A solution of 4-(4-bromophenoxy)benzoic acid (**2.49b**, 0.600 g, 2.05 mmol), HATU (1.17 g, 3.07 mmol), and HOAt (0.418 g, 3.07 mmol) in DMF (18 mL) cooled to 0 °C was treated with DIPEA (1.4 mL, 8.19 mmol) and stirred at 0 °C for 1 h. The reaction was treated, at 0 °C, with a

solution of 1-(2-azidoethyl)-1-(3,4-dimethoxybenzyl)hydrazine (**2.47c**, 0.955 g, 2.66 mmol) in DMF (2 mL). The reaction was allowed to warm to rt for 24 h, diluted with EtOAc (200 mL) and satd. aqueous NaHCO$_3$ (200 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 150 mL). The combined organic layers were washed with brine (2 x 150 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The residue was purified twice by automated chromatography on SiO$_2$ (12 g column, liquid load with CH$_2$Cl$_2$, hexanes to hexanes/EtOAc, 1:1) to give *N'*-(2-azidoethyl)-4-(4-bromophenoxy)-*N'*-(3,4-dimethoxybenzyl)benzohydrazide (1.01 g, 1.91 mmol, 93%) as a pale yellow oil: IR (CH$_2$Cl$_2$) 3303, 2952, 2097, 1650, 1512, 1478, 1260, 1230, 1156, 1025, 837 cm^{-1}; ^{1}H NMR (400 MHz, Acetone-d$_6$) δ 8.76 (s, 1 H), 7.78 (dt, *J* = 9.4, 2.4 Hz, 1 H), 7.57 (dt, *J* = 9.4, 2.8 Hz, 2 H), 7.16 (d, *J* = 1.6 Hz, 1 H), 7.03-6.98 (m, 4 H), 6.87-6.81 (m, 2 H), 4.21 (s, 2 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.44 (dd, *J* = 6.5, 4.7 Hz, 2 H), 3.33 (dd, *J* = 6.5, 4.7 Hz, 2 H); ^{13}C NMR (100 MHz, Acetone-d$_6$) δ 166.4, 160.3, 156.6, 150.2, 149.6, 133.8, 131.4 (2 C), 130.2, 122.2, 121.8, 118.7, 117.0, 113.6, 112.1, 60.9, 56.0 (2 C), 55.9, 49.5; HRMS (ESI) *m/z* calcd for C$_{24}$H$_{25}$BrN$_5$O$_4$ ([M+H]$^+$) 526.1084, found 526.1085.
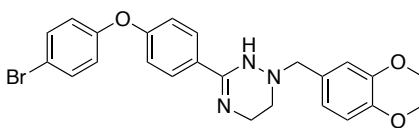
2.53

3-(4-(4-Bromophenoxy)phenyl)-1-(3,4-dimethoxybenzyl)-1,2,5,6-tetrahydro-1,2,4-triazine (2.53). To two flame-dried 20 mL microwave vials each containing a solution of N'-(2-azidoethyl)-4-(4-bromophenoxy)-N'-(3,4-dimethoxybenzyl)benzohydrazide (0.400 g, 0.760 mmol) in freshly distilled and degassed PhCl (16 mL) was added PMe$_3$ (1 M in THF) (1.5 mL, 1.52 mmol). The mixture was first warmed in an oil bath (preheated to 60 °C) for 30 min until

the evolution of gas had ceased. The microwave vial was sealed and heated at 180 °C for 2 h in the microwave. TLC analysis (hexanes/EtOAc, 1:2) showed the starting material had been consumed. The reactions were combined and the pale yellow solution was loaded directly onto a SiO₂ column preequilibrated with hexanes w/ 2 % Et₃N followed by chromatography on SiO₂ (hexanes/EtOAc, 1:1) to give **2.53** (0.409 g, 0.848 mmol, 56%) as a colorless solid: Mp 88.3-92.1 °C; IR (CH₂Cl₂) 3323, 2831, 1607, 1512, 1476, 1240, 1165, 1030, 834, 734 cm⁻¹; ¹H NMR (400 MHz, MeOD-d4) δ 7.65 (dt, J = 9.2, 2.5 Hz, 2 H), 7.50 (dt, J = 9.6, 2.8 Hz, 2 H), 7.08 (d, J = 1.8 Hz, 1 H), 7.00 (dt, J = 9.2, 2.5 Hz, 2 H), 6.97-6.90 (m, 4 H), 4.05 (s, 2 H), 3.84 (s, 3 H), 3.82 (s, 3 H), 3.49 (t, J = 5.0 Hz, 2 H), 2.69 (t, J = 5.0 Hz, 2 H); ¹³C NMR (100 MHz, MeOD-d4) δ 159.3, 157.6, 150.3, 149.9, 149.4, 134.0, 131.8, 131.2, 129.1, 123.2, 122.0, 119.4, 117.1, 114.3, 112.6, 64.0, 56.5, 56.4, 46.4, 42.0; HRMS (ESI) m/z calcd for $C_{24}H_{25}BrN_3O_3$ ([M+H]⁺) 482.1074, found 482.1071.
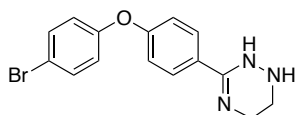
2.52

3-(4-(4-Bromophenoxy)phenyl)-1,2,5,6-tetrahydro-1,2,4-triazine (2.52). (deprotection of 3,4-dimethoxybenzyl protected triazine) A solution of 3-(4-(4-bromophenoxy)phenyl)-1-(3,4-dimethoxybenzyl)-1,2,5,6-tetrahydro-1,2,4-triazine (**2.53**, 0.0500 g, 0.104 mmol) in 4 M HCl in dioxane (2.5 mL) was heated at 50 °C for 2.5 d, diluted with Et₂O (20 mL) and the resulting precipitate was pelleted in the centrifuge and the supernatant removed and repeated 2 times. The resulting pellet was suspended in EtOAc (20 mL) and basified with 1 M NaOH (3 mL). The layers were separated and the aqueous layer was further extracted with EtOAc (2 x 10 mL). The combined organic layers were dried (Na₂SO₄), filtered and concentrated under reduced pressure.

The crude residue was purified by automated chromatography on SiO_2 (4 g column, liquid load CH_2Cl_2, gradient 100% CH_2Cl_2 to 10% $MeOH/CH_2Cl_2$; product eluted at 10% $MeOH:CH_2Cl_2$) to give **2.52** (0.0217 g, 0.0653 mmol, 63%) as a tan/orange waxy solid: IR (CH_2Cl_2) 3293, 2951, 1622, 1579, 1478, 1336, 1234, 1165, 1068, 749 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.55 (dt, J = 9.2, 2.4 Hz, 2 H), 7.43 (dt, J = 9.4, 2.7 Hz, 2 H), 6.96 (dt, J = 9.2, 2.4 Hz, 2 H), 6.88 (dt, J = 9.4, 2.7 Hz, 2 H), 4.53 (bs, 1 H), 3.64 (t, J = 4.9 Hz, 2 H), 3.17 (t, J = 4.9 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 156.0, 145.3, 132.7, 130.6, 127.0, 120.7, 118.6, 116.0, 41.9, 40.4; HRMS (ESI) *m/z* calcd for $C_{15}H_{15}BrN_3O$ ([M+H]$^+$) 332.0393, found 332.0388.

5.3.4 Synthesis of 6-(1,2,5,6-Tetrahydro-1,2,4-triazin-3-yl)-2-(4-(4-(1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)phenoxy)phenyl)-1*H*-indole
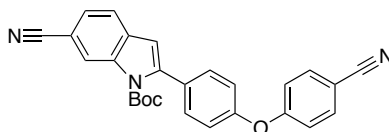
2.60

tert-**Butyl 6-cyano-2-(4-(4-cyanophenoxy)phenyl)-1*H*-indole-1-carboxylate (2.60).** A solution of 4-(4-bromophenoxy)benzonitrile (**2.57**, 0.750 g, 2.74 mmol) and tert-butyl 6-cyano-2-(trimethylstannyl)-1H-indole-1-carboxylate (**2.31**, 1.77 g, 3.28 mmol) in dry degassed DMF (15 mL) was treated with Pd(PPh$_3$)$_2$Cl$_2$ (0.192 g, 0.274 mmol) and (*i*Pr)CuCl^{293} (0.200 g, 0.410 mmol) at rt, heated to 80 °C for 18 h, cooled to rt, poured into H$_2$O (30 mL) and extracted with Et$_2$O (3 x 80 mL). The combined organic layers were washed with H$_2$O, brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography SiO$_2$ (gradient hexanes to hexanes/EtOAc, 5:1). The product was triturated/sonicated with methanol then decanted to give **2.60** (0.694 g, 1.59 mmol, 58%) as a colorless solid: ^{1}H NMR

(300 MHz, DMSO-d$_6$) δ 8.44 (bs, 1 H), 7.89 (d, J = 8.4 Hz, 2 H), 7.82 (d, J = 8.3 Hz, 1 H), 7.67

(d, J = 8.3 Hz, 1 H), 7.61 (d, J = 8.4 Hz, 2 H), 7.26 (d, J = 8.4 Hz, 2 H), 7.16 (d, J = 8.4 Hz, 2 H),

6.90 (s, 1 H), 1.33 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 160.6, 154.7, 148.8, 142.8, 135.7,

134.6, 132.2, 130.8, 129.8, 126.1, 121.8, 119.7, 119.6, 118.8, 118.5, 118.3, 109.7, 105.9, 105.5,

84.8, 27.0; HRMS (ESI) m/z calcd for C$_{23}$H$_{14}$N$_3$O$_3$ ([M-tBu+H]$^+$) 380.1030, found 380.1024.
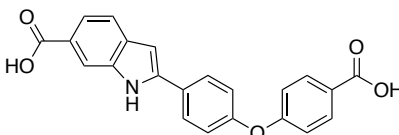
2.56

2-(4-(4-Carboxyphenoxy)phenyl)-1H-indole-6-carboxylic acid (2.56). A solution of

$tert$-butyl 6-cyano-2-(4-(4-cyanophenoxy)phenyl)-1H-indole-1-carboxylate (**2.60**, 0.500 g, 1.15

mmol) in a mixture of EtOH (125 mL) and H$_2$O (125 mL) was treated with LiOH monohydrate

(1.45 g, 34.5 mmol). The reaction was mixture was heated to reflux for 11 d, cooled to rt and

filtered. The filtrate was acidified with 1 M HCl to pH 2 and the resulting precipitate was

collected by vacuum filtration, and washed with a small amount of H$_2$O. Toluene was added to

the solid and concentrated under reduced pressure to give **2.56** (0.298 g, 0.799 mmol, 70%) as an

off-white solid: Mp >350 °C; IR (MeOH) 3437, 3070, 2665, 1681, 1599, 1491, 1417, 1291,

1260, 1230, 1200, 837, 790, 771 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.72 (bs, 2 H), 11.89

(s, 1 H), 8.02-7.96 (m, 5 H), 7.60 (dd, J = 10.4, 76.0 Hz, 2 H), 7.25 (d, J = 6.6 Hz, 2 H), 7.10 (d,

J = 6.6 Hz, 2 H), 6.99 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 168.1, 166.6, 160.6, 155.1,

140.3, 136.3, 132.0, 131.7, 128.0, 127.3, 123.4, 120.3, 119.4, 117.5, 113.1, 98.9; HRMS (ESI)

m/z calcd for C$_{22}$H$_{16}$NO$_5$ ([M+H]$^+$) 374.1023, found 374.1008.
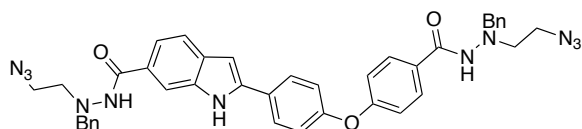
2.61a

N'-(2-Azidoethyl)-2-(4-(4-(2-(2-azidoethyl)-2-

benzylhydrazinecarbonyl)phenoxy)phenyl)-*N*'-benzyl-1*H*-indole-6-carbohydrazide (2.61a).

A solution of 2-(4-(4-carboxyphenoxy)phenyl)-1*H*-indole-6-carboxylic acid (**2.56**, 0.200 g,

0.536 mmol), HATU (0.713 g, 1.87 mmol), and HOAt (0.219 g, 1.61 mmol) in DMF (4.0 mL)

cooled to 0 °C was treated with DIPEA (0.60 mL, 3.21 mmol) and stirred at 0 °C for 2 h where

the color had changed from a brown yellow to a brown orange. The reaction mixture was treated

with a solution of 1-(2-azidoethyl)-1-benzylhydrazine (**2.47a**, 0.615 g, 3.21 mmol) in DMF (1.5

mL), warmed to rt, and stirred for 2 d, diluted with EtOAc (75 mL) and satd. aqueous NaHCO$_3$

(100 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 75

mL). The combined organic layers were washed with satd. aqueous NaHCO$_3$ (50 mL), brine (2 x

50 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude residue was

purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:4 w/ 2% Et$_3$N) followed by filtration

through basic alumina (hexanes/EtOAc, 1:2 to hexanes/EtOAc, 1:4) to give **2.61a** (0.158 g,

0.220 mmol, 41%) as a pale yellow foam: IR (CH$_2$Cl$_2$) 3259, 2925, 2854, 2096, 1644, 1599,

1489, 1458, 1238, 1171, 752 cm^{-1}; ^{1}H NMR (500 MHz, Acetone-d$_6$) δ 10.92 (bs, 1 H), 8.81 (bs,

1 H), 8.71 (bs, 1 H), 7.93-7.90 (m, 2 H), 7.87 (s, 1 H), 7.80-7.78 (m, 2 H), 7.53 (d, *J* = 8.0 Hz, 1

H), 7.47-7.43 (m, 5 H), 7.29 (td, *J* = 7.4, 2.0 Hz, 5 H), 7.25-7.21 (m, 2 H), 7.16-7.13 (m, 2 H),

7.05-7.02 (m, 2 H), 6.91 (dd, *J* = 2.0, 0.7 Hz, 1 H), 4.34 (s, 2 H), 4.30 (s, 2 H), 3.50-3.46 (m, 4

H), 3.37 (td, *J* = 10.4, 4.4 Hz, 4 H) ; ^{13}C NMR (125 MHz, Acetone-d$_6$) δ 168.1, 166.4, 163.3,

160.7, 157.1, 140.8, 139.3, 139.1, 137.7, 132.5, 130.2, 129.9 (2 C), 129.2, 129.0, 128.7, 128.0,

120.8, 120.4, 119.5, 118.7, 111.9, 99.9 (2 C), 61.2, 61.1, 55.8 (2 C), 49.8, 49.7; HRMS (ESI) *m/z*

calcd for C$_{40}$H$_{38}$N$_{11}$O$_3$ ([M+H]$^+$) 720.3154, found 720.3151.
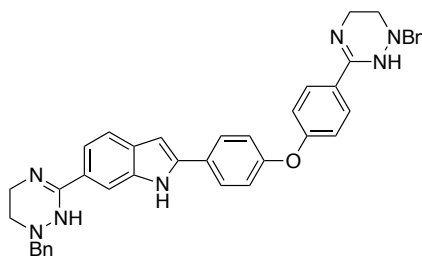
2.62a

6-(1-Benzyl-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)-2-(4-(4-(1-benzyl-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)phenoxy)phenyl)-1*H*-indole (2.62a). A solution of **2.61a** (0.130 g, 0.181 mmol) in degassed PhCl (14 mL) and THF (1 mL) was treated with a solution of PMe₃ (1 M solution in THF, 1.08 mL, 1.08 mmol) and the reaction was warmed to 50 °C for 2 h under an atmosphere of nitrogen. The reaction vessel was sealed and heated to 180 °C for 2 h. The resulting yellow solution was applied directly to a column of SiO₂ (equilibrated with hexanes w/ 3% Et₃N) and purified by chromatography on SiO₂ (hexanes to 4:1 EtOAc/Acetone w/ 3% Et₃N), and chromatographed on basic Al₂O₃ (EtOAc/Acetone, 2:1) to give **2.62a** (0.0239 g, 0.0378 mmol, 21%) as a pale yellow foam: IR (Acetone) 3657, 2990, 2889, 1611, 1489, 1382, 1242, 1153, 1072, 956, 821 cm⁻¹; ¹H NMR (400 MHz, Acetone-d₆) δ 10.71 (bs, 1 H), 7.88-7.85 (m, 2 H), 7.81 (s, 1 H), 7.78-7.74 (m, 2 H), 7.50-7.44 (m, 6 H), 7.32 (t, *J* = 7.6 Hz, 4 H), 7.26-7.23 (m, 2 H), 7.11-7.08 (m, 2 H), 7.04-7.00 (m, 2 H), 6.83 (bs, 1 H), 5.78 (bs, 1 H), 5.72 (bs, 1 H), 4.14 (s, 2 H), 4.13 (s, 2 H), 3.58-3.53 (m, 4 H), 2.73-2.70 (m, 4 H); ¹³C NMR (100 MHz, Acetone-d₆) δ 158.1, 157.6, 145.5, 143.7, 139.8, 139.6, 139.4, 138.1, 132.1, 130.6, 130.5, 130.0 (2 C), 128.9, 128.8, 127.7, 127.6, 127.5, 120.2, 120.0, 119.1, 118.4, 108.9, 99.6, 64.3, 64.2, 46.4, 46.2, 42.4, 42.3; HRMS (ESI) *m/z* calcd for C₄₀H₃₈N₇O ([M+H]⁺) 632.3132, found 632.3136.

2.61b

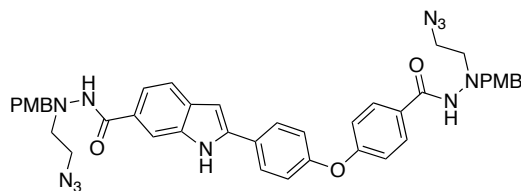*N'*-(2-azidoethyl)-2-(4-(4-(2-(2-azidoethyl)-2-(4-methoxybenzyl)hydrazinecarbonyl)phenoxy)phenyl)-*N'*-(4-methoxybenzyl)-1*H*-indole-6-carbohydrazide (2.61b). A solution of 2-(4-(4-carboxyphenoxy)phenyl)-1*H*-indole-6-carboxylic acid (**2.56**, 0.100 g, 0.268 mmol) in CH_2Cl_2 (5 mL) was treated with PyBrOP (0.375 g, 0.804 mmol), 1-(2-azidoethyl)-1-(4-methoxybenzyl)hydrazine (**2.47b**, 0.148 g, 0.670 mmol) and DIPEA (0.37 mL, 2.14 mmol). The solution was stirred at rt for 17 h, poured onto H_2O (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic layers were and washed with 5% $NaHCO_3$, brine, dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:1 with 5% Et_3N) to give **2.61b** (0.0512 g, 0.0657 mmol, 25%) as a light yellow solid: Mp 73.6-77.9 °C; IR (neat) 3241, 2930, 2093, 1642, 1610, 1510, 1487, 1454, 1301, 1234, 1171, 1031, 822 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.96 (bs, 1 H), 8.00 (s, 1 H), 7.77 (d, *J* = 8.4 Hz, 2 H), 7.57-7.53 (overlapping doublets, *J* = 8.4, 7.6 Hz, 3 H), 7.30-7.20 (m, 6 H), 7.02 (d, *J* = 8.8 Hz, 2 H), 6.95 (d, *J* = 8.8 Hz, 2 H), 6.84-6.77 (m, 5 H), 4.20 (s, 2 H), 4.18 (s, 2 H), 3.76 (s, 3 H), 3.73 (s, 3 H), 3.44-3.35 (m, 4 H), 3.32 (dd, *J* = 6.0, 5.2 Hz, 2 H), 3.27 (dd, *J* = 6.0, 5.2 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.3, 166.7, 160.2, 159.1 (2 C), 155.8, 140.4, 136.6, 132.0, 130.6, 130.5, 128.9, 128.4, 128.3, 128.1 (2 C), 127.3, 126.4, 120.1, 120.0, 118.0, 117.7, 113.8, 111.4, 99.4, 59.9, 59.7, 55.2 (2 C), 54.4, 49.0 (2 C); HRMS (ESI) *m/z* calcd for $C_{42}H_{42}N_{11}O_5$ ([M+H]$^+$) 780.3365, found 780.3350.
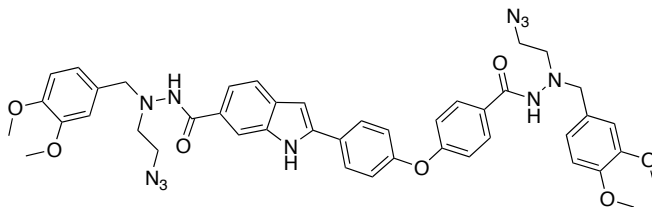
2.61c

***N*'-(2-Azidoethyl)-2-(4-(4-(2-(2-azidoethyl)-2-(3,4-dimethoxybenzyl)hydrazinecarbonyl)phenoxy)phenyl)-*N*'-(3,4-dimethoxybenzyl)-1*H*-indole-6-carbohydrazide (2.61c).** A solution of 2-(4-(4-carboxyphenoxy)phenyl)-1*H*-indole-6-carboxylic acid (**2.56**, 0.0500 g, 0.134 mmol) in DMF (1.5 mL) was treated with HATU (0.204 g, 0.536 mmol), 1-(2-azidoethyl)-1-(3,4-dimethoxybenzyl)hydrazine (**2.47c**, 0.135 g, 0.536 mmol) and DIPEA (0.240 mL, 1.34 mmol). The reaction mixture was stirred at rt for 17 h, diluted with H_2O, and stirred at rt for 5 min, extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with H_2O, satd. aqueous $NaHCO_3$, brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:6 to EtOAc with 2% Et_3N) to give **2.61c** (0.0429 g, 0.0511 mmol, 38%) as an off-white foam: IR (neat) 3277, 2930, 2833, 2095, 1646, 1596, 1512, 1487, 1452, 1232, 1156, 1025, 727 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.86 (s, 1 H), 7.93 (s, 1 H), 7.75 (d, *J* = 8.4 Hz, 2 H), 7.57-7.50 (m, 3 H), 7.37 (s, 1 H), 7.27 (s, 1 H), 7.20 (d, *J* = 8.1 Hz, 1 H), 7.03-6.93 (m, 6 H), 6.86-6.71 (m, 5 H), 4.21 (bd, *J* = 4.2 Hz, 4 H), 3.82 (s, 3 H), 3.79 (s, 6 H), 3.74 (s, 3 H), 3.42-3.34 (m, 8 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 168.4, 166.8, 160.2, 155.9, 149.0, 148.5, 140.4, 136.6, 132.0, 129.2, 128.9, 128.2, 128.0, 127.3, 126.5, 121.6, 121.4, 120.0, 118.1, 117.8, 112.4, 112.3, 111.3, 111.0, 99.4, 60.0, 55.8, 54.6, 48.9; HRMS (ESI) *m/z* calcd for $C_{44}H_{46}N_{11}O_7$ ([M+H]$^+$) 840.3576, found 840.3574.

2.62c

6-(1-(3,4-Dimethoxybenzyl)-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)-2-(4-(4-(1-(3,4-dimethoxybenzyl)-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)phenoxy)phenyl)-1*H*-indole (2.62c).

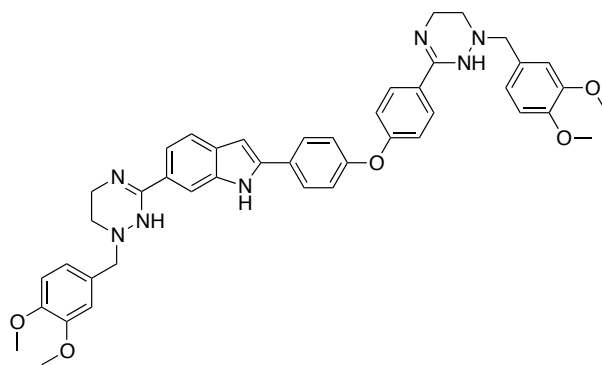Two flame-dried microwave vials each containing a solution of *N'*-(2-azidoethyl)-2-(4-(4-(2-(2-azidoethyl)-2-(3,4-dimethoxybenzyl)hydrazinecarbonyl)phenoxy)phenyl)-*N'*-(3,4-dimethoxybenzyl)-1*H*-indole-6-carbohydrazide (**2.61c**, 0.200 g, 0.238 mmol) in THF (6 mL) and chlorobenzene (12 mL) were sparged with Ar for 20 min, treated with PMe$_3$ (1 M solution in THF, 1.43 mL, 1.43 mmol) and heated at 180 °C in the microwave for 1 h. The two solutions were combined and concentrated under reduced pressure. The residue was dissolved in CH$_2$Cl$_2$ (100 mL) and washed with H$_2$O, brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (100% hexanes to MeOH/EtOAc, 1:9 with 2% Et$_3$N) to give **2.62c** (0.0947 g, 0.126 mmol, 27%) as a pale yellow foam: IR (CH$_2$Cl$_2$) 3419, 3268, 2992, 2947, 2880, 1668, 1603, 1512, 1488, 1462, 1352, 1236, 1156, 1025, 843, 735 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.50 (s, 1 H), 7.89 (s, 1 H), 7.61 (d, *J* = 8.5 Hz, 2 H), 7.56 (d, *J* = 8.5 Hz, 2 H), 7.49 (d, *J* = 8.5 Hz, 1 H), 7.30 (dd, *J* = 8.0, 1.0 Hz, 1 H), 7.04 (d, *J* = 1.5 Hz, 1 H), 6.98 (dd, *J* = 9.0, 2.0 Hz, 4 H), 6.94-6.89 (m, 2 H), 6.82 (d, *J* = 8.0 Hz, 1 H), 6.77 (d, *J* = 8.0 Hz, 1 H), 6.66 (d, *J* = 1.0 Hz, 1 H), 4.70 (bs, 1 H), 4.47 (s, 1 H), 4.14 (s, 4 H), 3.87 (s, 6 H), 3.83 (s, 3 H), 3.77 (s, 3 H), 3.56-3.52 (m, 4 H), 2.77 (t, *J* = 4.8 Hz, 2 H),

2.73 (t, J = 4.8 Hz, 2 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 157.6, 156.7, 148.8, 148.3, 148.2, 147.0, 144.5, 138.9, 136.9, 130.8, 130.6 (2 C), 130.1, 128.8, 127.7, 127.1, 126.9, 121.6 (2 C), 120.0, 119.1, 118.7, 117.4, 112.5, 112.4, 110.8, 109.1, 99.1, 63.8, 63.7, 55.9, 55.8, 45.5, 45.1, 41.7, 46.6; HRMS (ESI) m/z calcd for C$_{44}$H$_{46}$N$_7$O$_5$ ([M+H]$^+$) 752.3555, found 752.3537.
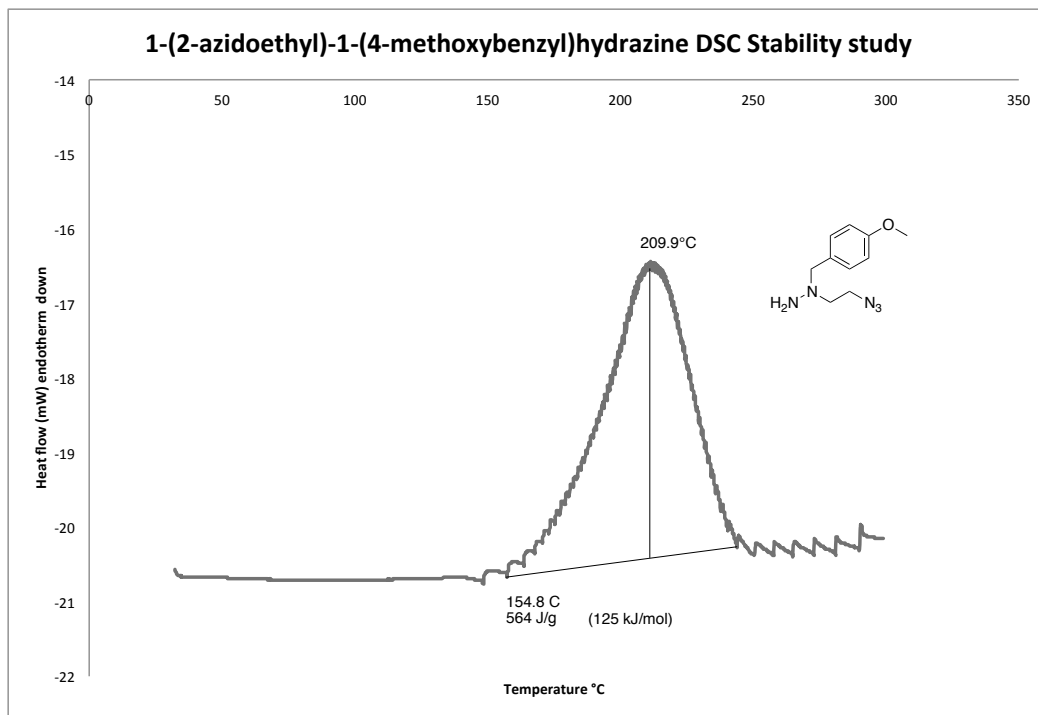
Figure 5.1. DSC thermogram for **2.47b**.

Table 5.1. DSC data for hydrazines **2.47b** and **2.47c**.

Compound	Onset (°C)	Peak (°C)	Energy J/g	Energy kJ/mol
2.47b	154.8	209.9	564.4	124.8
2.47c	185.3	238.5	490.7	123.7
Trityl azide	167.9	198.0	612.5	174.6

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5.4   CHAPTER 3 EXPERIMENTAL PART
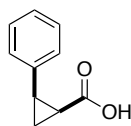
3.11

(1*RS*,2*SR*)-2-Phenylcyclopropanecarboxylic acid (3.11).[413] A solution of 3-phenylpropiolic acid (**3.9**, 1.60 g, 10.9 mmol) and quinoline (4.1 mL, 34.1 mmol) in EtOH (80 mL) at rt was treated with Lindlar catalyst (5% Pd on $CaCO_3$, lead poisoned, 1.17 g, equivalent to 5 mol% Pd). The flask was evacuated and back-filled with H_2 (3x) using a balloon. The resulting suspension was stirred for 20 h, filtered through a short pad of Celite, washed (EtOAc), and concentrated under reduced pressure. The residue was dissolved in ether and washed with 1 M aqueous HCl. The organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a mixture of *cis*-cinnamic acid (**3.10**) and *trans*-cinnamic acid (ratio 5:1, 0.527 g, 3.56 mmol) as a brown liquid.

A solution of Et_2Zn (1 M in CH_2Cl_2, 1.1 mL Et_2Zn in 10.5 mL CH_2Cl_2, 10.5 mmol) was diluted with CH_2Cl_2 (8.0 mL) and treated with a solution of TFA (0.78 mL, 10.5 mmol) in CH_2Cl_2 (6.0 mL) at 0 °C under an Ar atmosphere, stirred for 20 min, treated with a solution of CH_2I_2 (0.85 mL, 10.5 mmol) in CH_2Cl_2 (6.0 mL) stirred for 20 min and treated with a solution of cis/trans-cinamic acid (0.520 g, 3.51 mmol) in CH_2Cl_2 (10 mL) was added to the mixture. The reaction was stirred at rt for 16 h, diluted with satd. aqueous NH_4Cl, extracted with Et_2O, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (12 g column, liquid load CH_2Cl_2, gradient 100% hexanes to 30% EtOAc/hexanes) and, following recrystallization (toluene), to give **3.11** (0.0690 g, 0.591

mmol, 6% (2 steps)) as colorless needles: ^{1}H NMR (500 MHz, CDCl$_3$) δ 11.35 (bs, 1 H), 7.23-7.16 (m, 5 H), 2.58 (q, *J* = 8.3 Hz, 1 H), 1.99 (dd, *J* = 8.3, 6.3 Hz, 1 H), 1.62 (q, *J* = 6.3 Hz, 1 H), 1.34-1.29 (m, 1 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 177.4, 135.9, 129.2, 127.9, 126.7, 26.6, 21.4, 12.0.
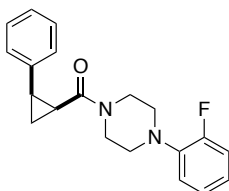
3.13a

(4-(2-fluorophenyl)piperazin-1-yl)((1*SR*,2*RS*)-2-phenylcyclopropyl)methanone

(3.13a). A solution of (1*SR*,2*RS*)-2-phenylcyclopropanecarboxylic acid (**3.11**, 0.0260 g, 0.160 mmol) and HATU (0.0914 g, 0.240 mmol) in CH$_2$Cl$_2$ (1 mL) was cooled to 0 °C and treated with DIPEA (83 µL, 0.481 mmol) warmed to rt over 45 min, cooled to 0 °C and treated with a solution of 1-2-fluorophenyl piperazine (**3.12a**, 0.0318 g, 0.176 mmol) in CH$_2$Cl$_2$ (0.5 mL) and stirred at rt for 17 h, diluted with H$_2$O and washed with EtOAc (2 × 10 mL). The combined organic layers were washed with 1 M aqueous NaHSO$_4$, satd. aqueous NaHCO$_3$, dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 100% EtOAc) to give **3.13a** (0.0463 g, 0.0143 mmol, 89%) a light orange oil: IR (CH$_2$Cl$_2$) 3005, 2895, 1635, 1489, 1458, 1431, 1223, 1206, 1033, 749, 695 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.26-7.22 (m, 2 H), 7.15-7.12 (m, 3 H), 7.04-6.98 (m, 2 H), 6.97-6.92 (m, 1 H), 6.70 (dt, *J* = 8.8, 1.6 Hz, 1 H), 3.88 (app dt, *J* = 13.2, 3.6 Hz, 1 H), 3.72 (app dt, *J* = 13.2, 4.0 Hz, 1 H), 3.60 (ddd, *J* = 12.0, 8.8, 3.2 Hz, 1 H), 3.32 (ddd, *J* = 12.0, 8.4, 2.8 Hz, 1 H), 2.97-2.94 (m, 2 H), 2.48-2.36 (m, 2 H), 2.21-2.12 (m, 2 H), 1.86 (dd, *J* = 12.8, 5.6 Hz, 1 H), 1.34 (ddd, *J* = 8.4, 8.4, 5.2 Hz, 1 H); [13]C

NMR (100 MHz, CDCl$_3$) δ 167.1, 155.6 (d, J_{CF} = 244.0 Hz), 139.6 (d, J_{CF} = 9.0 Hz), 137.5, 128.1, 127.3, 126.3, 124.4 (d, J_{CF} = 4.0 Hz), 122.9 (d, J_{CF} = 32.0 Hz), 119.0 (d, J_{CF} = 3.0 Hz), 116.1 (d, J_{CF} = 21 Hz), 50.7, 50.3, 45.2, 41.8, 24.3, 24.0, 10.5; HRMS (ESI) *m/z* calcd for C$_{20}$H$_{22}$FN$_2$O ([M+H]$^+$) 325.1711, found 325.1711.

3.13b

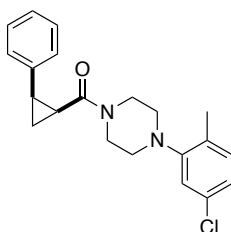**(4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1*SR*,2*RS*)-2-phenylcyclopropyl)methanone** **(3.13b).** A solution of (1*SR*,2*RS*)-2-phenylcyclopropanecarboxylic acid (**3.11**, 0.0260 g, 0.160 mmol) and HATU (0.0914 g, 0.240 mmol) in CH$_2$Cl$_2$ (1 mL) was cooled to 0 °C and treated with DIPEA (83 µL, 0.481 mmol) and warmed to rt over 45 min. The solution was cooled to 0 °C again and added a solution of 1-(5-chloro-2-methylphenyl)piperazine (**3.12b**, 0.0372 g, 0.176 mmol) in CH$_2$Cl$_2$ (0.5 mL) and stirred at rt for 17 h, diluted with H$_2$O and washed with EtOAc (2 × 10 mL). The combined organic layers were washed with 1 M aqueous NaHSO$_4$, satd. aqueous NaHCO$_3$, dried (Na$_2$SO$_4$), and concentrated. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 100% EtOAc) to give the **3.13b** (0.0169 g, 0.0476 mmol, 30%) a light orange oil: IR (CH$_2$Cl$_2$) 3018, 2913, 1635, 1487, 1458, 1431, 1223, 1206, 1033, 697 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.31-7.28 (m, 2 H), 7.22 (tt, *J* = 7.2, 1.6 Hz, 1 H), 7.18-7.15 (m, 2 H), 7.05 (d, *J* = 8.2 Hz, 1 H), 6.94 (dd, *J* = 8.2, 2.0 Hz, 1 H), 6.63 (d, *J* = 2.0 Hz, 1 H), 3.96 (app d, *J* = 12.8 Hz, 1 H), 3.76 (app d, *J* = 12.8 Hz, 1 H), 3.54-3.48 (m, 1 H), 3.22-3.16 (m, 1 H), 2.70-2.66 (m, 2 H), 2.45 (ddd, *J* = 9.2, 9.2, 7.2 Hz, 1 H), 2.21-2.13 (m, 5 H), 1.95-

1.89 (m, 1 H), 1.86 (dd, J = 12.8, 6.0 Hz, 1 H), 1.36 (ddd, J = 8.4, 8.4, 5.6 Hz, 1 H); ^{13}C NMR

(100 MHz, CDCl$_3$) δ 167.3, 152.0, 137.7, 131.9, 131.7, 131.1, 128.3, 127.4, 126.6, 123.5, 119.9,

51.6, 45.6, 42.2, 24.6, 24.1, 17.3, 10.7; HRMS (ESI) *m/z* calcd for $C_{21}H_{24}ClN_2O$ ([M+H]$^+$)

355.1572, found 355.1570.
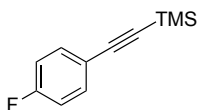
3.15a

((4-Fluorophenyl)ethynyl)trimethylsilane (3.15a).[415,481] A solution of Pd(PPh$_3$)$_2$Cl$_2$

(0.361 g, 0.514 mmol), CuI (0.0979 g, 0.514 mmol), and 4-fluorobromobenzene (**3.14a**, 5.66

mL, 51.4 mmol) in Et$_3$N (110 mL) was sparged with Ar for 15 min and treated with

(trimethylsilyl)acetylene (10.9 mL, 77.1 mmol) and the solution was sparged with Ar for 5 min.

The reaction mixture was heated to 80 °C for 16 h, cooled to rt, filtered through Celite, washed

(Et$_2$O) until the washes appeared colorless, and the combined filtrates were concentrated under

reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes) to give

3.15a (9.03 g, 47.0 mmol, 91%) as a pale orange oil: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.47-7.42

(m, 2 H), 6.99 (t, J = 8.7 Hz, 2 H), 0.25 (s, 9 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 162.6 (d, J_{CF} =

244.5 Hz), 133.9 (d, J_{CF} = 8.3 Hz), 119.3 (d, J_{CF} = 4.0 Hz), 115.5 (d, J_{CF} = 21.8 Hz), 104.0, 93.8,

-0.07.
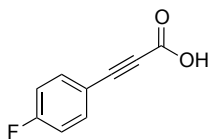
3.16a

3-(4-Fluorophenyl)propiolic acid (3.16a).[415] CsF (4.74 g, 31.2 mmol) was loaded into

an oven-dried 250-mL round bottom flask in a glovebox. The flask was removed from the

glovebox, attached to a CO_2 balloon, equipped with a magnetic stirrer and a septum, and filled

with anhydrous DMSO (60 mL). Neat ((4-fluorophenyl)ethynyl)trimethylsilane (**3.15a**, 5.00 g,

26.0 mmol) was added dropwise. The reaction mixture was stirred under and atmosphere of CO_2

at rt for 17 h, diluted with H_2O (600 mL) and washed with CH_2Cl_2 (2 × 150 mL). The aqueous

layer was acidified at 0 °C to pH 1 with 6 M aqueous HCl and then extracted with Et_2O (3 × 200

mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced

pressure to give **3.16a** (3.02 g, 18.4 mmol, 71%) as an orange solid: ^1H NMR (400 MHz,

Acetone-d_6) δ 11.74 (bs, 1 H), 7.71 (dd, J = 8.6, 5.6 Hz, 2 H), 7.26 (t, J = 8.6 Hz, 2 H); ^{13}C NMR

(100 MHz, Acetone-d_6) δ 164.8 (d, J_{CF} = 249.0 Hz), 154.7, 136.1 (d, J_{CF} = 9.0 Hz), 117.1 (d, J_{CF}

= 22.5 Hz), 84.6, 81.8.
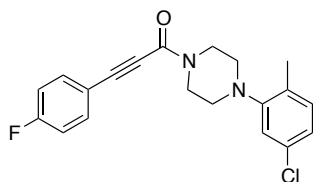
3.17a

1-(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-yn-1-one

(3.17a). A solution of 3-(4-fluorophenyl)propiolic acid (**3.16a**, 3.00 g, 18.3 mmol) and 1-(5-

chloro-2-methylphenyl)piperazine (**3.12b**, 4.62 g, 21.9 mmol) in anhydrous CH_2Cl_2 (180 mL) at

0 °C was treated with Et_3N (6.35 mL, 45.7 mmol), followed by dropwise addition of T3P (50

wt.% solution in EtOAc, 19.4 mL, 27.4 mmol). The reaction mixture was stirred at 0 °C for 30

min, warmed to rt for 24 h, diluted with CH_2Cl_2 (200 mL), washed with 1 M aqueous HCl (150

mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified

by chromatography on SiO_2 (hexanes/EtOAc, 2:1) to give **3.17a** (5.22 g, 14.6 mmol, 80%) as an

off white solid: Mp 138.7-140.4 °C; IR (neat) 2924, 2216, 1625, 1596, 1504, 1443, 1431, 1219,

1038, 837 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.55 (dd, J = 7.5, 5.4 Hz, 2 H), 7.12-6.94 (m, 5 H), 3.96 (app t, J = 4.8 Hz, 2 H), 3.82 (app t, J = 4.8 Hz, 2 H), 2.95 (app t, J = 4.8 Hz, 2 H), 2.87 (app t, J = 4.8 Hz, 2 H,), 2.28 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 163.5 (d, J_{CF} = 250.5 Hz), 153.0, 151.7, 134.5 (d, J_{CF} = 9.0 Hz), 132.1, 131.8, 130.9, 123.7, 119.8, 116.4 (d, J_{CF} = 3.8 Hz), 116.0 (d, J_{CF} = 22.5 Hz), 89.9, 80.9, 51.9, 51.3, 47.4, 41.8, 17.3; HRMS (ESI) m/z calcd for C$_{20}$H$_{19}$ClFON$_2$ ([M+H]$^+$) 357.1164, found 357.1165.
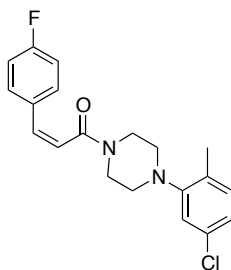
3.18a

(Z)-1-(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-en-1-one (3.18a). A solution of 1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-yn-1-one (5.00 g, 14.0 mmol) in dry EtOAc (140 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.298 g, equivalent to 1 mol% Pd) and quinoline (0.83 mL, 7.01 mmol). The reaction vessel was placed under vacuum, backfilled with H$_2$ (balloon, 2x) and allowed to stir under and atmosphere of H$_2$ for 6 h. Analysis by TLC (2:1, hexanes/EtOAc) indicated that **3.17a** had been mostly consumed. The reaction mixture was filtered through Celite, washed (EtOAc), and the combined filtrates were washed with 1 M aqueous HCl, dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1) to give **3.18a** (3.15 g, 8.78 mmol, 63%, 87% brsm) as a colorless solid: IR (neat) 2913, 2239, 1616, 1506, 1437, 1223, 837, 725 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.41-7.36 (m, 2 H), 7.08-7.02 (m, 3 H), 6.96 (dd, J

= 8.1, 2.1 Hz, 1 H), 6.80 (d, J = 2.1 Hz, 1 H), 6.66 (d, J = 12.5 Hz, 1 H), 6.05 (d, J = 12.5 Hz, 1 H), 3.80 (t, J = 5.0 Hz, 2 H), 3.49 (t, J = 5.0 Hz, 2 H), 2.80 (t, J = 5.0 Hz, 2 H), 2.53 (t, J = 5.0 Hz, 2 H), 2.21 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.3, 162.7 (d, J_{CF} = 247.0 Hz), 151.7, 132.6, 132.0, 131.8, 131.5 (d, J_{CF} = 4.0 Hz), 132.1, 131.8, 130.9, 130.2 (d, J_{CF} = 9.0 Hz), 123.6, 122.7, 119.6, 115.6 (d, J_{CF} = 22 Hz), 51.4, 51.2, 46.5, 41.5, 17.3; HRMS (ESI) *m/z* calcd for C$_{20}$H$_{21}$ClFON$_2$ ([M+H]$^+$) 359.1321, found 359.1329.
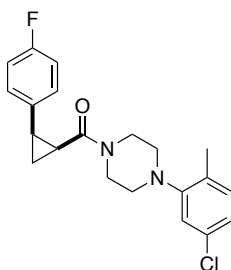
JJ450

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-fluorophenyl)cyclopropyl)methanone (JJ450). THF (90 mL) was degassed by sparging with Ar for 60 min and treated at rt under Ar atmosphere with anhydrous CrCl$_2$ (6.43 g, 51.8 mmol) followed by (*Z*)-1-(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-en-1-one (**3.18a**, 3.10 g, 8.64 mmol) and CH$_2$ICl (3.36 mL, 43.2 mmol). The reaction mixture was stirred for 20 h at 80 °C, cooled to rt, diluted with 1 M aqueous HCl (300 mL) and extracted with EtOAc (3 x 300 mL). The combined organic layers were filtered through a plug of basic Al$_2$O$_3$, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (1:1, hexanes/EtOAc) to give an oil that was further purified twice by chromatography on basic Al$_2$O$_3$ (hexanes/EtOAc, 1:1) to give **JJ450** (2.76 g, 7.41 mmol, 86%) as a clear oil that solidified after storage on high vacuum: Mp 78.2-80.4 °C (hexanes); IR (CH$_2$Cl$_2$) 2936, 1637, 1592, 1510, 1487, 1435, 1223, 1033, 837, 815 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.16-7.11 (m,

2 H), 7.07 (dd, J = 8.1, 0.5 Hz, 1 H), 7.00-6.94 (m, 3 H), 6.73 (d, J = 2.1 Hz, 1 H), 3.81-3.76 (m, 1 H), 3.71-3.60 (m, 2 H), 3.36 (ddd, J = 12.4, 8.8, 3.1 Hz, 1 H), 2.79-2.71 (m, 2 H), 2.45 (td, J = 8.8, 7.0 Hz, 1 H), 2.35-2.29 (m, 1 H), 2.26-2.16 (m, 5 H), 1.83 (dt, J = 7.0, 5.6 Hz, 1 H), 1.35 (td, J = 8.8, 5.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.2, 161.7 (d, J_{CF} = 244.0 Hz), 151.9, 133.1 (d, J_{CF} = 3.0 Hz), 132.0, 131.8, 130.9, 129.1 (d, J_{CF} = 8.0 Hz), 123.6, 119.7, 115.0 (d, J_{CF} = 21.0 Hz), 51.8, 51.6, 45.6, 42.2, 23.8, 23.5, 17.3, 10.7; HRMS (ESI) m/z calcd for C$_{21}$H$_{23}$ClFON$_2$ ([M+H]$^+$) 373.1477, found 373.1478.
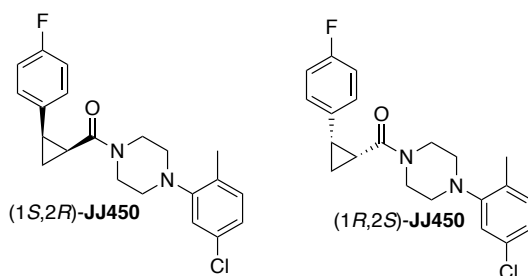
(1*S*,2*R*)-**JJ450**   (1*R*,2*S*)-**JJ450**

Racemic   (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-fluorophenyl)cyclopropyl)methanone (**JJ450**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (20% MeOH, 6 mL/min, 220 nM, P=100) to give (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1*S*,2*R*)-2-(4-fluorophenyl)cyclopropyl)methanone   ((1*S*,2*R*)-**JJ450**) (retention time 13.1 min) as a colorless viscous oil (100% purity by ELSD): [α]^{20}$_D$ -164.3 (*c* 1.34, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.17-7.10 (m, 2 H), 7.07 (d, J = 8.1 Hz, 1 H), 7.02-6.94 (m, 3 H), 6.72 (d, J = 2.1 Hz, 1 H), 3.83-3.75 (m, 1 H), 3.72-3.58 (m, 2 H), 3.39-3.31 (m, 1 H), 2.81-2.69 (m, 2 H), 2.45 (td, J = 8.6, 6.9 Hz, 1 H), 2.36-2.25 (m, 1 H), 2.25-2.15 (m, 5 H), 1.83 (dt, J = 6.9, 5.6 Hz, 1 H), 1.35 (td, J = 8.6, 5.6 Hz, 1 H); HRMS (ESI) m/z calcd for C$_{21}$H$_{23}$ClFON$_2$ ([M+H]$^+$) 373.1477, found 373.1476. The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 4.6 mm); 20% MeOH, 220 nM, 2 mL/min; retention time: 9.8 min).

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1*R*,2*S*)-2-(4-

fluorophenyl)cyclopropyl)methanone ((1*S*,2*R*)-**JJ450**) (retention time 16.5 min) was obtained as

a colorless viscous oil (100% purity by ELSD): $[\alpha]^{20}_D$ +167.1 (*c* 1.27, MeOH); ^{1}H NMR (300

MHz, CDCl$_3$) δ 7.17-7.10 (m, 2 H), 7.07 (d, *J* = 8.1 Hz, 1 H), 7.01-6.94 (m, 3 H), 6.72 (d, *J* = 2.1

Hz, 1 H), 3.82-3.74 (m, 1 H), 3.71-3.60 (m, 2 H), 3.39-3.30 (m, 1 H), 2.81-2.68 (m, 2 H), 2.45

(td, *J* = 8.6, 7.0 Hz, 1 H), 2.35-2.26 (m, 1 H), 2.25-2.15 (m, 5 H), 1.83 (dt, *J* = 7.0, 5.6 Hz, 1 H),

1.35 (td, *J* = 8.6, 5.6 Hz, 1 H); HRMS (ESI) *m/z* calcd for C$_{21}$H$_{23}$ClFON$_2$ ([M+H]$^+$) 373.1477,

found 373.1476. The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 4.6 mm);

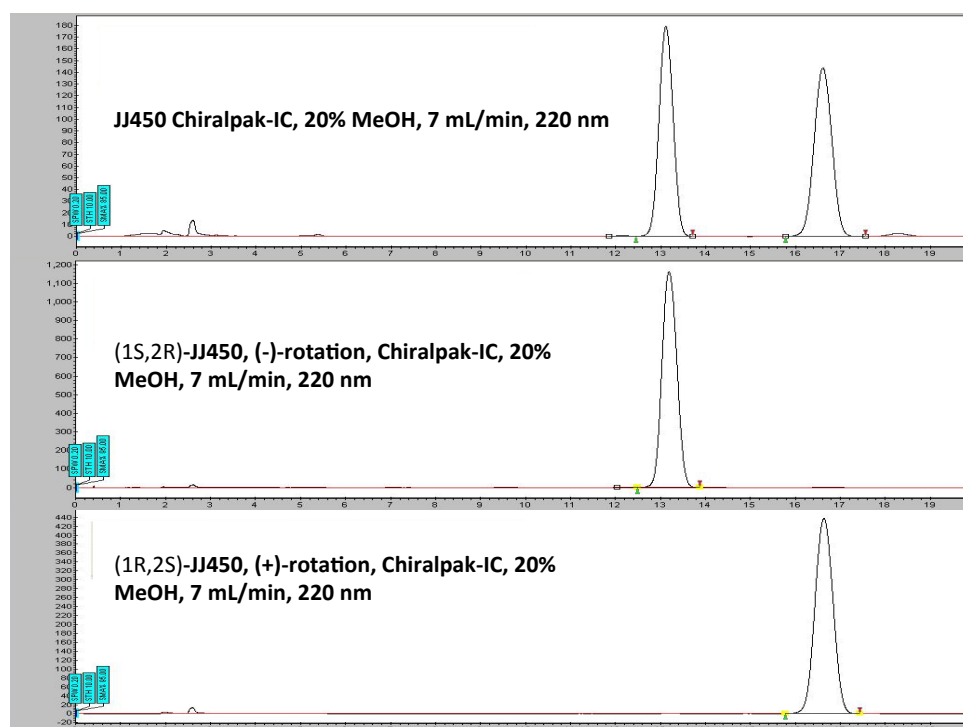20% MeOH, 220 nM, 2 mL/min; retention time: 12 min).

Figure 5.2. SFC chromatograms for **JJ450** and separated enantiomers.

5.4.1 Synthesis of JJ450 Analogs
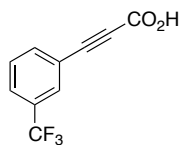
3.16b

3-(3-(Trifluoromethyl)phenyl)propiolic acid (3.16b).[415,482-483] A solution of Pd(PPh$_3$)$_2$Cl$_2$ (0.0306 g, 0.0436 mmol), CuI (0.00829 g, 0.0436 mmol), and 3-bromobenzotriflouride **3.14b** (0.62 mL, 4.36 mmol) in Et$_3$N (8.7 mL) was sparged with Ar for 15 min and treated with (trimethylsilyl)acetylene (0.93 mL, 6.53 mmol) and sparged for an additional 2 min, heated to 80 °C for 16 h, cooled to rt, filtered through Celite, washed (Et$_2$O) until the washes appeared colorless and the filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes) to give the trimethyl((3-(trifluoromethyl)phenyl)ethynyl)silane (**3.15b**, 1.03 g, 4.24 mmol) as a pale yellow oil.

A solution of CsF (0.775 g, 5.10 mmol) in dry DMSO (6.5 mL) under an atmosphere of CO$_2$ (balloon) at rt was treated with a solution of trimethyl((3-(trifluoromethyl)phenyl)ethynyl)silane (**3.15b**, 1.03 g, 4.25 mmol) dropwise and the reaction was stirred under CO$_2$ at rt for 17 h. The reaction mixture was diluted with H$_2$O (80 mL) and extracted with CH$_2$Cl$_2$ (2 × 25 mL). The aqueous layer was acidified (> pH 1) with 6 M aqueous HCl at 0 °C and extracted with Et$_2$O (3 × 25 mL). The combined organic layers were dried (MgSO$_4$), concentrated under reduced pressure, and dried under high vacuum to give **3.16b** (0.774 g, 3.62 mmol, 83% (2 steps)) as an pale tan orange waxy solid: ^{1}H NMR (400 MHz, Acetone-d$_6$) δ 11.85 (bs, 1 H), 7.95-7.87 (m, 3 H), 7.36 (t, J = 7.4 Hz, 1 H); ^{13}C NMR (100 MHz,

Acetone-d_6) δ 154.3, 137.1, 131.7 (q, J_{CF} = 33.0 Hz), 130.0 (q, J_{CF} = 3.8 Hz), 128.1 (q, J_{CF} = 3.4 Hz), 124.5 (q, J_{CF} = 272.0 Hz), 121.7, 83.6, 82.9.
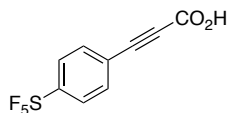
3.16c

3-(4-(Pentafluoro-λ6-sulfaneyl)phenyl)propiolic acid (3.16c). A solution of Pd(PPh$_3$)$_2$Cl$_2$ (0.0365 g, 0.0519 mmol), CuI (0.0100 g, 0.0519 mmol), and (4-bromophenyl)pentafluoro-λ6-sulfane (**3.14c**, 1.50 g, 5.19 mmol) in Et$_3$N (11 mL) was sparged with Ar for 10 min, treated with (trimethylsilyl)acetylene (1.10 mL, 7.79 mmol), sparged with Ar for 5 min, heated to 80 °C for 22 h, cooled to rt, filtered through Celite, washed (Et$_2$O) until the washes appeared colorless, and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes) to give **3.15c** (1.32 g, 4.40 mmol) as a pale yellow oil.

A solution of CsF (0.801 g, 5.27 mmol) in dry DMSO (3 mL) under CO$_2$ (balloon) at rt was treated with a solution of trimethyl((4-(pentafluoro-λ6-sulfaneyl)phenyl)ethynyl)silane (**3.15c**, 1.32 g, 4.40 mmol) in DMSO (5.8 mL) dropwise and the reaction was stirred under CO$_2$ at rt for 25 h, diluted with H$_2$O (90 mL) and extracted with CH$_2$Cl$_2$ (2 × 50 mL). The aqueous layer was acidified (> pH 1) with 6 M aqueous HCl at 0 °C and extracted with Et$_2$O (3 × 50 mL). The combined organic layers were washed with H$_2$O (50 mL), dried (MgSO$_4$), concentrated under reduced pressure, and dried under high vacuum to give **3.16c** (0.338 g, 1.24 mmol, 24% (2 steps)) as light brown solid: Mp 156.5–159.6 °C; IR (CHCl$_3$) 2979, 2876, 2577, 2235, 1677, 1416, 1298, 1217, 886, 829, 751 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.00 (bs, 1 H), 7.82 (dt, J = 9.0, 2.0 Hz, 2 H), 7.73 (d, J = 9.0 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 155.2 (quint,

J_{CF} = 19.0 Hz), 133.3, 126.5 (quint, J_{CF} = 4.0 Hz), 122.7, 85.9, 81.7; ^{19}F NMR (376 MHz, CDCl$_3$) δ 82.6 (quint, J = 150.5 Hz, 1 F), 62.3 (d, J = 150.3 Hz, 4 F); HRMS (ESI) *m/z* calcd for C$_9$H$_4$O$_2$F$_5$S ([M-H]$^-$) 270.9858, found 270.9858.
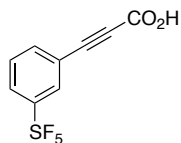

3.16d

3-(3-(Pentafluoro-λ6-sulfaneyl)phenyl)propiolic acid (3.16d). A solution of Pd(PPh$_3$)$_2$Cl$_2$ (0.0365 g, 0.0519 mmol), CuI (0.0100 g, 0.0519 mmol), and (3-bromophenyl)pentafluoro-λ6-sulfane (**3.14d**, 1.50 g, 5.19 mmol) in Et$_3$N (11 mL) was sparged with Ar for 10 min, treated with (trimethylsilyl)acetylene (1.10 mL, 7.79 mmol), sparged with Ar for 5 min heated to 80 °C for 22 h, cooled to rt, filtered through Celite, washed (Et$_2$O) until the washes appeared colorless. The combined filtrates were concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes) to give **3.15d** (1.29 g, 4.29 mmol) as a yellow oil.

A solution of CsF (0.783 g, 5.15 mmol) in dry DMSO (3 mL) under CO$_2$ (balloon) at rt was treated a solution of trimethyl((3-(pentafluoro-λ6-sulfaneyl)phenyl)ethynyl)silane (**3.15d**, 1.29 g, 4.30 mmol) in DMSO (5.6 mL) dropwise and the reaction was stirred under CO$_2$ at rt for 25 h. The reaction mixture was diluted with H$_2$O (90 mL) and extracted with CH$_2$Cl$_2$ (2 × 50 mL). The aqueous layer was acidified (> pH 1) with 6 M aqueous HCl at 0 °C and then extracted with Et$_2$O (3 × 50 mL). The combined organic layers were washed with H$_2$O (50 mL), dried (MgSO$_4$), concentrated under reduced pressure, and further dried under high vacuum to give **3.16d** (0.935 g, 3.43 mmol, 66% (2 steps)) as tan solid: Mp 121.1-124.4 °C; IR (CHCl$_3$) 2831, 2218, 1688, 1479, 1426, 1214, 831, 791, 768, 680 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.92 (s,

1 H), 8.01 (t, J = 2.0 Hz, 1 H), 7.87 (ddd, J = 8.0, 2.0, 0.9 Hz, 1 H), 7.75 (d, J = 8.0 Hz, 1 H), 7.54 (t, J = 8.0 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 158.1, 153.8 (quint, J_{CF} = 19.0 Hz), 135.9, 130.7 (quint, J_{CF} = 5.0 Hz), 129.3, 128.4 (quint, J_{CF} = 4.0 Hz), 120.2, 86.2, 81.0; ^{19}F NMR (376 MHz, CDCl$_3$) δ 82.5 (quint, J = 150.4 Hz, 1 F), 62.5 (d, J = 150.4 Hz, 4 F); HRMS (ESI) m/z calcd for C$_9$H$_4$O$_2$F$_5$S ([M-H]⁻) 270.9858, found 270.9856.
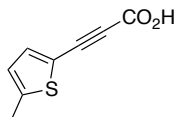
3.16e

3-(5-Methylthiophen-2-yl)propiolic acid (3.16e).[484-485, 486] A solution of Pd(PPh$_3$)$_2$Cl$_2$ (0.0615 g, 0.0877 mmol), CuI (0.0167 g, 0.0877 mmol), and 2-bromo-5-methyl thiophene (**3.14e**, 1.00 mL, 8.77 mmol) in Et$_3$N (17.5 mL) sparged with Ar for 15 min and treated with (trimethylsilyl)acetylene (1.9 mL, 13.2 mmol) and the mixture was further sparged for 2 min, heated to 80 °C for 17 h, cooled to rt, filtered through Celite, washed (Et$_2$O) until the washes appeared colorless and the filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes) to give the **3.15e** (1.06 g, 5.47 mmol) as a pale yellow oil.

To solution of CsF (0.994 g, 6.54 mmol) in dry DMSO (8 mL) under an atmosphere of CO$_2$ (balloon) at rt was added a solution of trimethyl((5-methylthiophen-2-yl)ethynyl)silane (**3.15e**, 1.06 g, 5.45 mmol) dropwise and the reaction was stirred under CO$_2$ at rt for 17 h. The reaction mixture was diluted with H$_2$O (100 mL) and extracted with CH$_2$Cl$_2$ (2 × 25 mL). The aqueous layer was acidified (> pH 1) with 6 M aqueous HCl at 0 °C and extracted with Et$_2$O (3 × 25 mL). The combined organic layers were washed with brine (50 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure to give **3.16e** (0.571 g, 3.44 mmol, 39% (2 steps)) as a

brown solid: ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.14 (bs, 1 H), 7.36 (d, J = 3.3 Hz, 1 H), 6.73 (dd, J = 3.3 Hz, 1 H), 2.52 (s, 3 H).
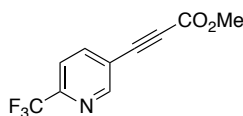
Methyl 3-(6-(trifluoromethyl)pyridin-3-yl)propiolate. To two flasks each containing a solution of Pd(PPh$_3$)$_2$Cl$_2$ (0.0466 g, 0.0664 mmol), CuI (0.0126 g, 0.0664 mmol), and 5-bromo-2-trifluoromethyl pyridine (**3.14f**, 1.50 g, 6.64 mmol) in Et$_3$N (13 mL) sparged with Ar for 10 min followed by addition of (trimethylsilyl)acetylene (1.4 mL, 9.96 mmol) and sparged with Ar for 2 min. The resulting mixtures were heated to 80 °C for 16 h where analysis by TLC (hexanes/EtOAc, 4:1) indicated that the SM had been consumed. After cooling the reaction to rt, the reactions were combined, the solution was filtered through Celite, which was washed with Et$_2$O (100 mL) until the washes appeared colorless. The filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give **3.15f** (3.37 g, 13.9 mmol) as orange/brown waxy solid.

A solution of CsF (2.52 g, 16.6 mmol) in DMSO (20 mL) under CO$_2$ at rt was treated with a solution of 2-(trifluoromethyl)-5-((trimethylsilyl)ethynyl)pyridine (**3.15f**, 3.37 g, 13.9 mmol) in DMSO (7 mL) dropwise and the reaction was stirred under CO$_2$ (balloon) at rt for 5 h, treated with MeI (0.95 mL, 15.2 mmol) was added and the solution was stirred for 1 h at rt. The reaction mixture was diluted with H$_2$O (200 mL), brine (100 mL) and extracted with Et$_2$O (3 × 150 mL). The combined organic layers were washed with H$_2$O (100 mL), dried (MgSO$_4$), and concentrated under reduced pressure. The crude product was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 4:1) to give methyl 3-(6-(trifluoromethyl)pyridin-3-yl)propiolate (1.87 g, 8.17 mmol, 59% (2 steps)) as tan solid: Mp 98.2-99.7 °C; IR (neat) 2962, 2233, 1712, 1433,

1337, 1242, 1127, 1085, 864, 745 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.87 (s, 1 H), 8.04 (ddd, J = 8.0, 1.2, 0.6 Hz, 1 H), 7.71 (d, J = 8.0 Hz, 1 H), 3.86 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.4, 153.1, 148.6 (q, J_{CF} = 35.5 Hz), 141.2, 121.0 (q, J_{CF} = 274.4 Hz), 120.1 (q, J_{CF} = 2.8 Hz), 120.0, 84.7, 80.7, 53.2; ^{19}F NMR (376 MHz, CDCl$_3$) δ -68.3 (s, 3 F); HRMS (ESI) m/z calcd for C$_{10}$H$_7$F$_3$NO$_2$ ([M+H]$^+$) 230.0423, found 230.0422.
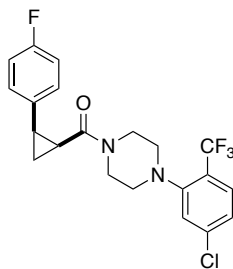
3.21b

(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-fluorophenyl)cyclopropyl)methanone (3.21b). A solution of 3-(4-fluorophenyl)propiolic acid (**3.16a**, 0.0800 g, 0.487 mmol) and 1-(5-chloro-2-(trifluoromethyl)phenyl)piperazinehydrochloride (**3.12b**, 0.142 g, 0.536 mmol) in CH$_2$Cl$_2$ (4.9 mL) at 0 °C was treated Et$_3$N (0.27 mL, 1.95 mmol). The cooled solution was treated with T3P (50% solution in EtOAc) (0.52 mL, 0.73 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 24 h, diluted with CH$_2$Cl$_2$ (30 mL), washed with H$_2$O (20 mL), satd. aqueous NaHCO$_3$ (20 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc/hexanes) to give **3.17b** (0.140 g, 0.341 mmol) as a colorless solid.

A solution of 1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-yn-1-one (**3.17b**, 0.140 g, 0.341 mmol) in EtOAc (3.4 mL) was treated with

Lindlar catalyst (5% Pd on $CaCO_3$, lead poisoned, 0.0363 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of H_2 (3 vacuum/backfill cycles) and stirred at rt for 2 d. TLC (hexanes/EtOAc, 2:1) indicated that the SM had been mostly consumed. The reaction was filtered through Celite (eluting with EtOAc (10 mL)) and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, 100% hexanes to 40% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes) to give **3.18b** (0.0510 g, 0.124 mmol) as a colorless solid.

A solution of $CrCl_2$ (0.0911 g, 0.741 mmol) and (*Z*)-1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-en-1-one (**3.18b**, 0.0510 g, 0.124 mmol) in dry degassed THF (1.2 mL) was sparged with Ar for 5 min and treated with CH_2ICl (0.071 mL, 0.618 mmol) at rt, stirred for 2 d at 80 °C, cooled to rt, diluted with EtOAc (50 mL) and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, 100% hexanes to 30% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes), filtered through basic Al_2O_3 (CH_2Cl_2/EtOAc, 1:1) and concentrated under reduced pressure. The resulting oil was recrystallized from a mixture of hexanes/cyclohexane (1:1), the crystals were washed with hexanes and dried under high vacuum to give **3.21b** (0.0238 g, 0.0558 mmol, 11% (3 steps) (100% purity by ELSD)) as a colorless solid: Mp 101.0-103.2 °C; IR (CH_2Cl_2) 2917, 1639, 1513, 1418, 1308, 1225, 1126, 1085, 1031, 838 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.45 (dd, *J* = 8.4, 0.6 Hz, 1 H), 7.25-7.22 (m, 1 H), 7.16-7.11 (m, 2 H), 7.00 (d, *J* = 1.8 Hz, 1 H), 6.99-6.93 (m, 2 H), 3.92-3.86 (m, 1 H), 3.79-3.73 (m, 1 H), 3.67 (ddd, *J* = 12.6, 8.6, 3.2 Hz, 1 H), 3.37 (ddd, *J* = 12.6, 9.0, 3.2 Hz, 1 H), 3.01-2.95 (m, 2 H), 2.49-2.39 (m, 2 H), 2.31-2.25 (m, 1 H), 2.19 (ddd, *J* = 9.0, 8.6, 5.8 Hz, 1 H), 1.83 (dt, *J*

= 6.8, 5.8 Hz, 1 H), 1.36 (td, J = 8.6, 5.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.2, 161.6

(d, J_{CF} = 245.1 Hz), 149.1, 132.5, 131.1, 130.1 (q, J_{CF} = 32.7 Hz), 129.1, 129.0, 123.6 (q, J_{CF} =

272.2 Hz), 120.8 (q, J_{CF} = 3.8 Hz), 117.2 (q, J_{CF} = 3.8 Hz), 115.0 (d, J_{CF} = 21.3 Hz), 51.3, 51.0,

45.3, 41.9, 23.9, 23.5 10.7; ^{19}F NMR (376 MHz, CDCl$_3$) δ -62.6 (s, 3 F), -116.4 (s, 1 F); HRMS

(ESI) m/z calcd for C$_{21}$H$_{20}$ClF$_4$N$_2$O ([M+H]$^+$) 427.1195, found 427.1192.
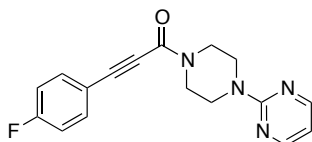
3.17c

3-(4-Fluorophenyl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)prop-2-yn-1-one (**3.17c**). A

solution of 3-(4-fluorolphenyl)propionic acid (**3.16a**, 0.150 g, 0.914 mmol) and 2-(1-

piperazinyl)pyrimidine (**3.12f**, 0.188 g, 0.841 mmol) in CH$_2$Cl$_2$ (9.1 mL) at 0 °C was treated

Et$_3$N (0.51 mL, 3.66 mmol). The cooled solution was treated with T3P (50% solution in EtOAc)

(1.0 mL, 1.37 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for

16 h, diluted with CH$_2$Cl$_2$ (30 mL), washed with H$_2$O (20 mL), dried (MgSO$_4$), filtered, and

concentrated under reduced pressure. The crude material was purified by automated

chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 100%

EtOAc) to give **3.17c** (0.223 g, 0.719 mmol, 79%) as a colorless solid: Mp 168.4-170.8 °C; IR

(CH$_2$Cl$_2$) 2859, 2217, 1618, 1585, 1506, 1435, 1355, 1261, 1227, 980, 838, 732 cm^{-1}; ^{1}H NMR

(400 MHz, CDCl$_3$) δ 8.34 (d, J = 4.6 Hz, 2 H), 7.56 (dd, J = 8.8, 5.3 Hz, 2 H), 7.08 (t, J = 8.8

Hz, 2 H), 6.56 (t, J = 4.6 Hz, 1 H), 3.95-3.93 (m, 2 H), 3.88 (app dd, J = 6.6, 3.4 Hz, 4 H), 3.76

(app dd, J = 6.6, 4.1 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.6 (d, J_{CF} = 253.0 Hz), 161.4,

157.8, 153.1, 134.6 (d, J_{CF} = 8.7 Hz), 116.4 (d, J_{CF} = 3.6 Hz), 116.1 (d, J_{CF} = 22.1 Hz), 110.6,

90.0, 80.8, 46.8, 44.0, 43.3, 41.5; ^{19}F NMR (376 MHz, CDCl$_3$) δ -107.3 (s, 1 F); HRMS (ESI) m/z calcd for C$_{17}$H$_{16}$FN$_4$O ([M+H]$^+$) 311.1303, found 311.1301.
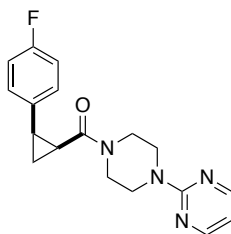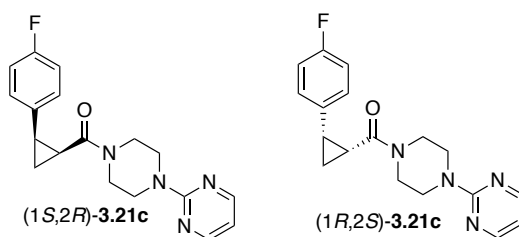

3.21c

((1RS,2SR)-2-(4-Fluorophenyl)cyclopropyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methanone (3.21c). A solution of 3-(4-fluorophenyl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)prop-2-yn-1-one (**3.17c**, 0.200 g, 0.644 mmol) in EtOAc (6.4 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0685 g, equivalent to 5 mol% Pd). The reaction vessel was placed under vacuum and backfilled with H$_2$ (balloon, 4x) and stirred for 18 h at rt, filtered through Celite, washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, gradient 100% hexanes to 40% EtOAc/hexanes) to give **3.18c** (0.211 g, 0.676 mmol) as a colorless solid.

A solution of CrCl$_2$ (0.472 g, 3.84 mmol) and (Z)-3-(4-fluorophenyl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)prop-2-en-1-one (**3.18c**, 0.200 g, 0.640 mmol) in dry degassed THF (6.4 mL) (previously sparged with Ar for 15 min) was treated with CH$_2$ICl (0.37 mL, 3.20 mmol) and further sparged with Ar for 2 min. The reaction mixture was stirred for 20 h at 80 °C, cooled to rt, diluted with EtOAc (10 mL), filtered through a plug of basic Al$_2$O$_3$ (EtOAc), and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 60% EtOAc/hexanes) to give **3.21c** (0.128 g, 0.392 mmol, 61% (2 steps) (100% purity by ELSD)) as a colorless solid: Mp 141.9-

145.3 °C; IR (CH$_2$Cl$_2$) 2922, 1636, 1583, 1510, 1432, 1359, 1224, 1027, 981, 837, 797 cm^{-1}; ^{1}H

NMR (400 MHz, CDCl$_3$) δ 8.28 (d, J = 4.6 Hz, 2 H), 7.12 (dd, J = 8.7, 5.3 Hz, 2 H), 6.92 (t, J =

8.7 Hz, 2 H), 6.50 (t, J = 4.6 Hz, 1 H), 4.00 (dt, J = 13.3, 4.4 Hz, 1 H), 3.93 (dt, J = 13.0, 4.4 Hz,

1 H), 3.76 (ddd, J = 13.0, 5.1, 3.6 Hz, 1 H), 3.66 (dt, J = 13.0, 4.4 Hz, 1 H), 3.53 (ddd, J = 13.0,

8.8, 3.6 Hz, 1 H), 3.26 (ddd, J = 13.0, 8.8, 3.4 Hz, 1 H), 3.13 (ddd, J = 13.0, 8.8, 3.6 Hz, 1 H),

3.00 (ddd, J = 13.0, 8.8, 3.6 Hz, 1 H), 2.49-2.43 (m, 1 H), 2.18 (ddd, J = 9.2, 8.4, 5.8 Hz, 1 H),

1.83 (q, J = 5.8 Hz, 1 H), 1.34 (td, J = 8.4, 5.8 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.3,

161.7 (d, J_{CF} = 244.9 Hz), 161.3, 157.7, 133.0, 129.0 (d, J_{CF} = 8.0 Hz), 115.0 (d, J_{CF} = 21.3 Hz),

110.3, 45.0, 43.8, 43.4, 41.6, 23.9, 23.5, 10.6; ^{19}F NMR (470 MHz, CDCl$_3$) δ -116.4 (s, 1 F);

HRMS (ESI) m/z calcd for C$_{18}$H$_{20}$FN$_4$O ([M+H]$^+$) 327.1616, found 327.1616.

Racemic ((1RS,2SR)-2-(4-fluorophenyl)cyclopropyl)(4-(pyrimidin-2-yl)piperazin-1-

yl)methanone (**3.21c**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column

(30% Methanol/CO$_2$, 7 mL/min, p = 100 bar, 90μL injection, 20 mg/mL in MeOH) to give

((1S,2R)-2-(4-fluorophenyl)cyclopropyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methanone ((1S,2R)**-**

3.21c) (retention time 7.63 min) as a colorless solid (100% purity by ELSD): [a]^{17}$_D$ -207.0 (c

0.53, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.28 (d, J = 4.6 Hz, 1 H), 7.12 (dd, J = 8.6, 5.3 Hz,

1 H), 6.92 (t, J = 8.6 Hz, 1 H), 6.50 (t, J = 4.6 Hz, 1 H), 4.02-3.91 (m, 2 H), 3.79-3.73 (m, 1 H),

3.69-3.64 (m, 1 H), 3.56-3.48 (m, 2 H), 3.29-3.21 (m, 1 H), 3.16-3.08 (m, 1 H), 2.98 (ddd, J =

12.6, 9.0, 3.7 Hz, 1 H), 2.51-2.42 (m, 1 H), 2.18 (ddd, J = 9.0, 8.4, 6.0, 1 H), 1.83 (q, J = 5.6 Hz,

1 H), 1.35 (td, J = 8.4, 5.6 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm; retention time: 8.0 min).

((1R,2S)-2-(4-Fluorophenyl)cyclopropyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methanone ((1R,2S)-**3.21c**) (retention time 8.61 min) was obtained as a colorless solid (100% purity by ELSD): $[a]^{17}_{D}$ +213.2 (c 0.54, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.28 (d, J = 4.6 Hz, 2 H), 7.12 (dd, J = 8.6, 5.3 Hz, 2 H), 6.92 (t, J = 8.6 Hz, 2 H), 6.50 (t, J = 4.6 Hz, 1 H), 4.02-3.90 (m, 2 H), 3.79-3.73 (m, 1 H), 3.69-3.62 (m, 1 H), 3.57-3.48 (m, 2 H), 3.30-3.21 (m, 1 H), 3.16-3.08 (m, 1 H), 2.98 (ddd, J = 13.0, 9.0, 3.5 Hz, 1 H), 2.47 (td, J = 9.0, 6.0 Hz, 1 H), 2.18 (ddd, J = 9.0, 8.4, 6.0 Hz, 1 H), 1.83 (q, J = 5.6 Hz, 1 H), 1.35 (td, J = 8.4, 5.6 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm; retention time: 9.0 min).
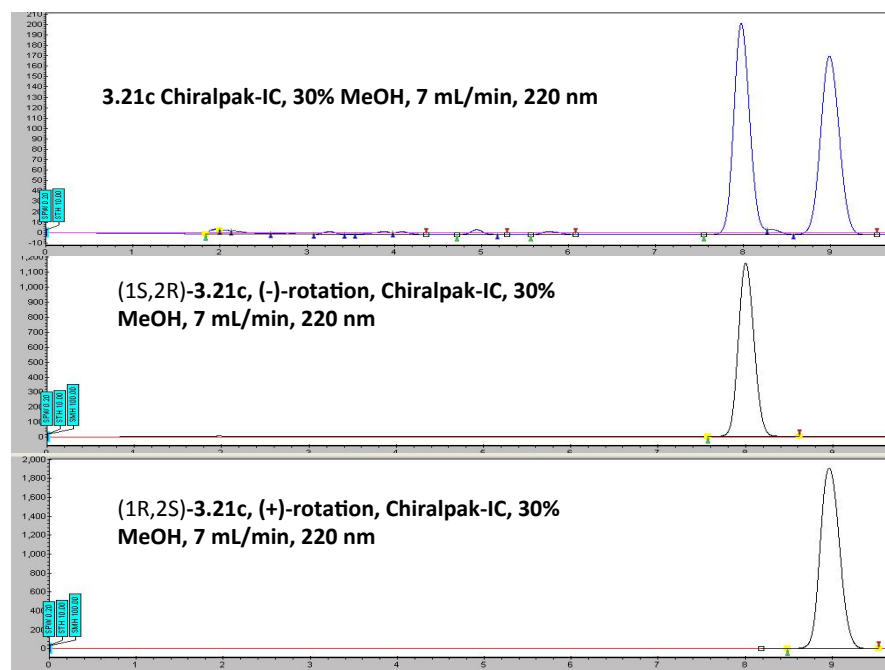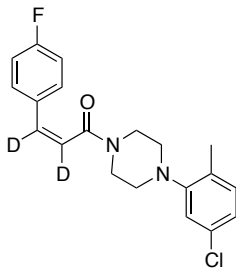

**Figure 5.3.** SFC chromatograms for **3.21c** and separated enantiomers.

3.22

(*Z*)-1-(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-en-1-one-2,3-*d2* (3.22). A solution of 1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(4-fluorophenyl)prop-2-yn-1-one (**3.17a**, 0.150 g, 0.140 mmol) in anhydrous EtOAc (4.2 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0447 g, 0.0210 mmol, equivalent to 5 mol% Pd). The reaction was placed under a balloon of D$_2$ (3 vacuum/backfill cycles) and stirred vigorously at rt for 24 h, filtered through Celite, washed (EtOAc), and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4 g column, liquid load with CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc:hexanes, product eluted at 25% EtOAc:hexanes) to give **3.22** (0.0919 g, 0.255 mmol, 61%, 97% deuterium incorporation) as a colorless solid: Mp 122.1-124.8 °C; IR (CH$_2$Cl$_2$) 2918, 2819, 1628, 1595, 1505, 1432, 1222, 1022, 852, 817, 735 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39 (dd, *J* = 8.6, 5.4 Hz, 2 H), 7.07-7.01 (m, 3 H), 6.95 (dd, *J* = 8.2, 2.0 Hz, 1 H), 6.79 (d, *J* = 2.0 Hz, 1 H), 3.79 (bs, 2 H), 3.48 (t, *J* = 5.0 Hz, 2 H), 2.80 (t, *J* = 5.0 Hz, 2 H), 2.52 (t, *J* = 5.0 Hz, 2 H), 2.20 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.2, 162.6 (d, *J* = 249.3 Hz), 151.7, 132.1 (t, *J$_{CD}$* = 23.6 Hz), 132.0, 131.7, 131.4 (d, *J$_{CF}$* = 3.2 Hz), 130.8, 130.2 (d, *J$_{CF}$* = 8.1 Hz), 123.6, 122.2 (t, *J$_{CD}$* = 24.2 Hz), 119.5, 115.6 (d, *J$_{CF}$* = 21.4 Hz), 51.4, 51.1, 46.5, 41.4 17.3; ^{19}F NMR (376 MHz, CDCl$_3$) δ -112.0 (s, 1 F); HRMS (ESI) *m/z* calcd for C$_{20}$H$_{19}$D$_2$ClFN$_2$O ([M+H]$^+$) 361.1446, found 361.1446
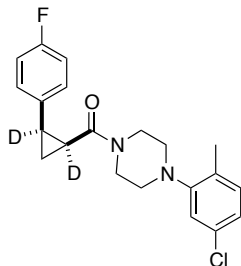
3.23

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-fluorophenyl)cyclopropyl-1,2-*d2*)methanone (3.23). A solution of $CrCl_2$ (0.225 g, 1.83 mmol) and (4-(5-chloro-2-methylphenyl)piperazin-1-yl)(2-(4-fluorophenyl)cyclopropyl-1,2-d2)methadone (**3.22**, 0.110 g, 0.305 mmol) in dry degassed THF (3 mL) was treated with CH_2ICl (0.18 mL, 1.52 mmol) and sparged with Ar for 2 min. The reaction mixture was stirred for 24 h at 80 °C, cooled to rt, diluted with EtOAc (50 mL), and washed with 1 M HCl (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 40% EtOAc/hexanes) to give a yellow oil that was filtered through basic Al_2O_3 (CH_2Cl_2/EtOAc, 1:1) concentrated under reduced pressure to a clear oil that was triturated in minimal cyclohexane to give a colorless solid that was dried under high vacuum to give **3.23** (0.0799 g, 0.213 mmol, 70% (100% purity by ELSD)) as a colorless solid: Mp 100.2-102.7 °C; IR (CH_2Cl_2) 2917, 1632, 1512, 1431, 1221, 1032, 818, 729 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.16-7.11 (m, 2 H), 7.06 (d, J = 8.2 Hz, 1 H), 7.00-6.94 (m, 3 H), 6.71 (d, J = 2.0 Hz, 1 H), 3.81-3.77 (m, 1 H), 3.72-3.59 (m, 2 H), 3.37-3.31 (m, 1 H), 2.79-2.69 (m, 2 H), 2.33-2.28 (m, 1 H), 2.24-2.20 (m, 4 H), 1.81 (d, J = 5.4 Hz, 1 H), 1.32 (d, J = 5.4 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.2, 161.5 (d, J_{CF} = 244.7 Hz), 151.8, 133.0 (d, J_{CF} = 3.1 Hz), 131.9, 131.7, 130.9, 129.0 (d, J_{CF} = 7.7 Hz), 123.5, 119.6, 114.9 (d, J_{CF} = 21.3 Hz), 51.7, 51.5, 45.5, 42.1, 23.4

(t, J_{CD} = 25.4 Hz), 23.0 (t, J_{CD} = 24.6 Hz) 17.3, 10.4; ^{19}F NMR (376 MHz, CDCl$_3$) δ -116.3 (s, 1

F); HRMS (ESI) *m/z* calcd for $C_{21}H_{21}D_2ClFN_2O$ ([M+H]$^+$) 375.1603, found 375.1602.
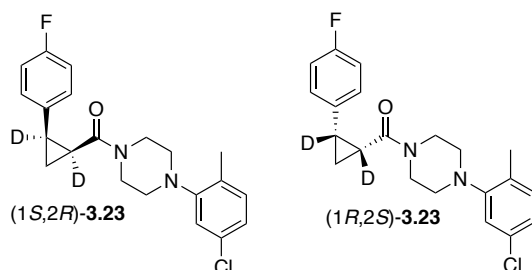
Racemic (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-fluorophenyl)cyclopropyl-1,2-*d*2)methanone (**3.23**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm) injection volume 90 μL, 20 mg/mL) to give (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1*S*,2*R*)-2-(4-fluorophenyl)cyclopropyl-1,2-*d*2)methanone ((1*S*,2*R*)-**3.23**) (retention time 7.58 min) as a colorless viscous oil (100% purity by ELSD): [a]$^{18}_D$ -152.0 (*c* 0.70, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.16-7.12 (m, 2 H), 7.07 (d, *J* = 8.1 Hz, 1 H), 7.01-6.94 (m, 3 H), 6.72 (d, *J* = 1.8 Hz, 1 H), 3.82-3.76 (m, 1 H), 3.74-3.58 (m, 2 H), 3.39-3.31 (m, 1 H), 2.80-2.69 (m, 2 H), 2.80-2.69 (m, 2 H), 2.35-2.17 (m, 5 H), 1.82 (d, *J* = 5.4 Hz, 1 H), 1.33 (d, *J* = 5.4 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 7.8 min).

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1*R*,2*S*)-2-(4-fluorophenyl)cyclopropyl-1,2-*d*2)methanone ((1*S*,2*R*)-**3.23**) (retention time 9.28 min) was obtained as a colorless viscous oil (100% purity by ELSD): [a]$^{19}_D$ +154.0 (*c* 0.66, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.17-7.10 (m, 2 H), 7.07 (d, *J* = 8.2 Hz, 1 H), 7.02-6.94 (m, 3 H), 6.72 (d, *J* = 2.0 Hz, 1 H), 3.83-3.74 (m, 1 H), 3.73-3.58 (m, 2 H), 3.39-3.31 (m, 1 H), 2.80-2.69 (m, 2 H), 2.36-2.18 (m, 5 H), 1.82 (d, *J* = 5.4 Hz, 1 H), 1.33 (d, *J* = 5.4 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC

Chiralpak-IC (250 x 10 mm); 30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm; retention time: 9.6 min).
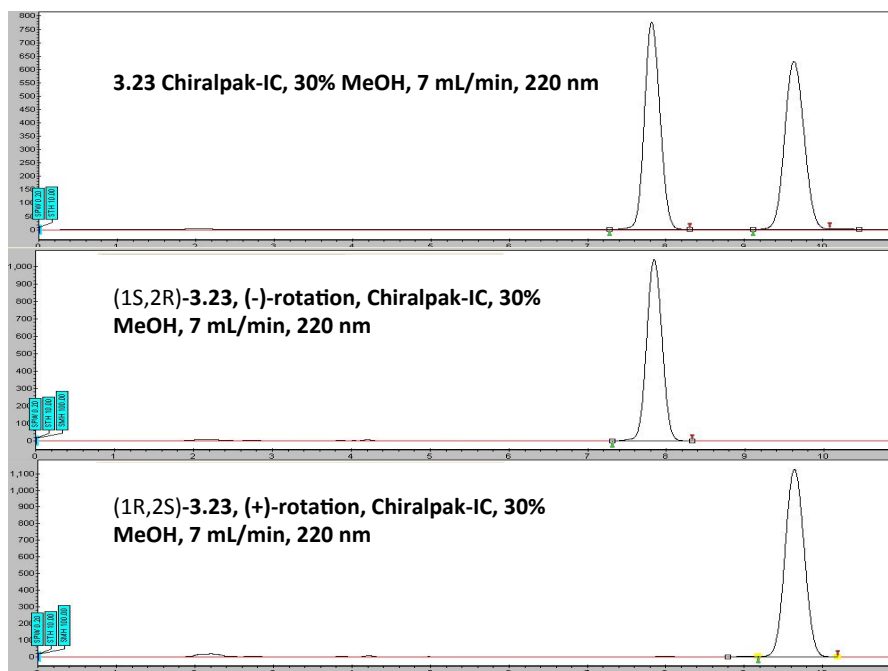
Figure 5.4. SFC chromatograms for **3.23** and separated enantiomers.
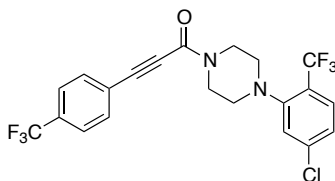
3.17d

1-(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-yn-1-one **(3.17d).** A solution of 3-(4-(trifluoromethyl)phenyl)propiolic acid (**3.16h**, 0.100 g, 0.467 mmol) and 1-(5-chloro-2-(trifluoromethyl)phenyl)piperazinehydrochloride (**3.12b**, 0.155 g, 0.514 mmol) in CH_2Cl_2 (4.7 mL) cooled to 0 °C was treated with Et_3N (0.26 mL, 1.87 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.49 mL, 0.701 mmol) dropwise and the reaction

was stirred at 0 °C for 30 min and allowed to warm to rt for 16 h, diluted with CH_2Cl_2 (30 mL) and washed with H_2O (20 mL), satd. aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, hexanes to 30% EtOAc/hexanes), to give **3.17d** (0.161 g, 0.350 mmol, 75%) as a colorless solid: Mp 145.8-148.8 °C; IR (CH_2Cl_2) 2928, 2827, 2223, 1634, 1596, 1432, 1322, 1310, 1122, 1107, 1033, 844 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.65 (app q, J = 7.3 Hz, 4 H), 7.58 (d, J = 8.4 Hz, 1 H), 7.28-7.24 (m, 2 H), 3.96 (app t, J = 5.0 Hz, 2 H), 3.84 (app t, J = 5.0 Hz, 2 H), 3.00 (app t, J = 5.0 Hz, 2 H), 2.94 (app t, J = 5.0 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 152.5, 138.8, 132.5, 131.6 (q, J_{CF} = 33.0 Hz), 128.5 (q, J_{CF} = 5.4 Hz), 125.7 (q, J_{CF} = 29.4 Hz), 125.7, 125.4 (q, J_{CF} = 3.7 Hz), 124.7, 124.1, 123.5 (q, J_{CF} = 270.8 Hz, 2 C), 89.0, 82.7, 53.6, 52.7, 47.3, 41.8; ^{19}F NMR (376 MHz, CDCl$_3$) δ -60.3 (s, 3 F), -63.1 (s, 3 F); HRMS (ESI) m/z calcd for $C_{21}H_{16}ClF_6N_2O$ ([M+H]$^+$) 461.0850, found 461.0847.
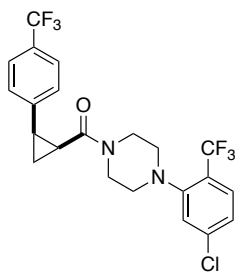
3.21f

(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)((1RS,2SR)-2-(4-(trifluoromethyl)phenyl)cyclopropyl)methanone (3.21f). A solution of 1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-yn-1-one **(3.17d**, 0.127 g, 0.276 mmol) in EtOAc (2.8 mL) was treated with quinoline (0.16 mL, 1.38 mmol) and 5% Pd/BaSO$_4$ (0.0059 g, equivalent to 1 mol% Pd). The reaction was placed under and

atmosphere of H$_2$ (balloon) (3 vacuum/backfill cycles) and stirred at rt for 1.5 h, filtered through Celite, washed (EtOAc), and the combined filtrates were washed with 1 M aqueous HCl (10 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4 g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc/hexanes; product eluted at 20% EtOAc) to give **3.18d** (0.114 g, 0.246 mmol) as a colorless solid.

A solution of CrCl$_2$ (0.182 g, 0.148 mmol) and (Z)-1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**3.18d**, 0.114 g, 0.246 mmol) in dry degassed THF (2.5 mL) was sparged with Ar for 15 min, treated with CH$_2$ICl (0.14 mL, 1.23 mmol), heated for 2 d at 80 °C. The reaction was cooled to rt, diluted with EtOAc (50 mL) and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried (MgSO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes), filtered through basic Al$_2$O$_3$ (CH$_2$Cl$_2$/EtOAc, 1:1), concentrated, and dried under high vacuum to give **3.21f** (0.0681 g, 0.143 mmol, 52% (2 steps) (100% purity by ELSD)) as a colorless solid: Mp 138.0-139.9 °C; IR (CH$_2$Cl$_2$) 3014, 2825, 1641, 1596, 1326, 1309, 1116, 1031, 844 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.56 (d, J = 8.2 Hz, 2 H), 7.51 (d, J = 8.5 Hz, 1 H), 7.29 (d, J = 8.2 Hz, 2 H), 7.19 (dd, J = 8.5, 1.4 Hz, 1 H), 6.89 (d, J = 1.4 Hz, 1 H), 3.95 (bd, J = 12.4 Hz, 1 H), 3.73 (bd, J = 12.4 Hz, 1 H), 3.54 (bt, J = 10.0 Hz, 1 H), 3.21 (bt, J = 10.0 Hz, 1 H), 2.74 (bt, J = 10.0 Hz, 2 H), 2.50 (td, J = 8.9, 7.1 Hz, 1 H), 2.29-2.19 (m, 2 H), 1.98 (bt, J = 8.9 Hz, 1 H), 1.92 (q, J = 6.2 Hz, 1 H), 1.43 (td, J = 8.4, 5.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.5, 152.7, 142.1, 138.9, 128.8 (q, J_{CF} = 32.6 Hz), 128.4 (q, J_{CF} = 5.8 Hz), 127.9, 125.7 (q, J_{CF} = 30.2 Hz), 125.6, 125.0 (q, J_{CF} =

3.7 Hz), 124.6 (q, J_{CF} = 273.0 Hz), 124.4, 123.6 (q, J_{CF} = 272.9 Hz), 53.7, 52.9, 45.5, 42.1, 24.7, 23.9, 11.2; ^{19}F NMR (376 MHz, CDCl$_3$) δ -60.4 (s, 3 F), -62.2 (s, 3 F); HRMS (ESI) *m/z* calcd for C$_{22}$H$_{20}$ClF$_6$N$_2$O ([M+H]$^+$) 477.1163, found 477.1160.

(1*S*,2*R*)-**3.21f**   (1*R*,2*S*)-**3.21f**
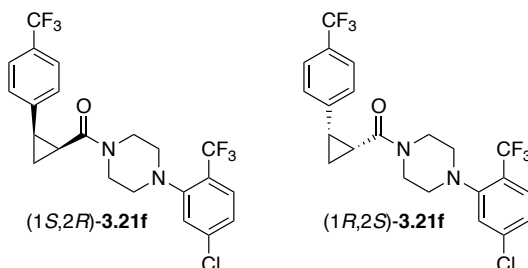
Racemic (4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-(trifluoromethyl)phenyl)cyclopropyl)methanone (**3.21f**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (25% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm) injection volume 90 μL, 20 mg/mL) to give (4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)((1*S*,2*R*)-2-(4-(trifluoromethyl)phenyl)cyclopropyl)methanone ((1*S*,2*R*)-**3.21f**) (retention time 3.57 min) as a colorless viscous oil (100% purity by ELSD): [a]^{19}$_D$ -106.6 (*c* 0.68, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.57 (d, *J* = 8.1 Hz, 2 H), 7.52 (d, *J* = 8.4 Hz, 1 H), 7.29 (d, *J* = 8.1 Hz, 2 H), 7.20 (d, *J* = 8.4 Hz, 1 H), 6.88 (d, *J* = 0.9 Hz, 1 H), 3.98-3.93 (m, 1 H), 3.76-3.70 (m, 1 H), 3.54 (ddd, *J* = 12.3, 9.3, 3.0 Hz, 2 H), 3.21 (ddd, *J* = 12.3, 9.3, 3.0 Hz, 2 H), 2.76-2.71 (m, 2 H), 2.51 (td, *J* = 9.3, 7.2 Hz, 1 H), 2.30-2.18 (m, 2 H), 2.00-1.89 (m, 2 H), 1.44 (td, *J* = 8.4, 5.7 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 25% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 3.8 min).

(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)((1*R*,2*S*)-2-(4-(trifluoromethyl)phenyl)cyclopropyl)methanone ((1*S*,2*R*)-**3.21f**) (retention time 4.20 min) was obtained as a colorless viscous oil (100% purity by ELSD): [a]^{19}$_D$ +111.1 (*c* 0.71, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.57 (d, *J* = 8.1 Hz, 2 H), 7.52 (d, *J* = 8.4 Hz, 1 H), 7.29 (d, *J* = 8.1

Hz, 2 H), 7.20 (d, *J* = 8.4 Hz, 1 H), 6.88 (s, 1 H), 3.99-3.93 (m, 1 H), 3.76-3.70 (m, 1 H), 3.58-

3.49 (m, 1 H), 3.25-3.16 (m, 1 H), 2.76-2.71 (m, 2 H), 2.51 (q, *J* = 9.6 Hz, 1 H), 2.30-2.18 (m, 2

H), 2.00-1.89 (m, 2 H), 1.44 (td, *J* = 8.4, 5.6 Hz, 1 H). The enantiomeric excess was >99.9% ee

(SFC Chiralpak-IC (250 x 10 mm); 25% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm;

retention time: 4.1 min).
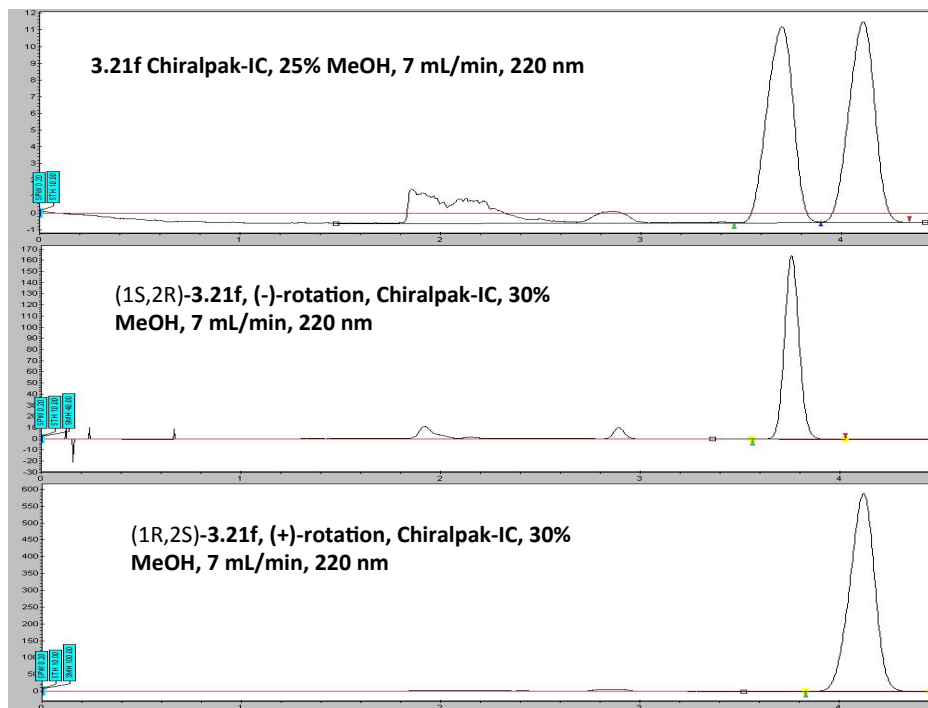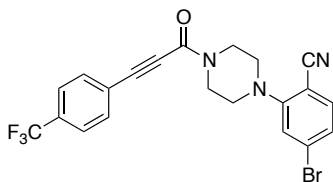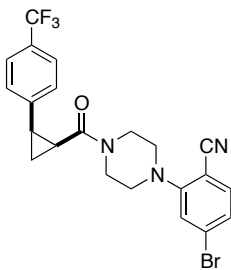

Figure 5.5. SFC chromatograms for **3.21f** and separated enantiomers.

3.17e

4-Bromo-2-(4-(3-(4-(trifluoromethyl)phenyl)propioloyl)piperazin-1-yl)benzonitrile

(3.17e). A solution of 3-(4-trifluoromethylphenyl)propiolic acid (**3.16h**, 0.400 g, 1.87 mmol) and

4-bromo-2-(piperazin-1-yl)benzonitrile hydrochloride (**3.12e**, 0.735 g, 2.43 mmol) in CH$_2$Cl$_2$ (19

mL) at 0 °C was treated Et$_3$N (1.0 mL, 7.47 mmol). The cooled solution was treated with T3P

(50% solution in EtOAc) (2.0 mL, 2.80 mmol) dropwise and the reaction was stirred at 0 °C for

30 min, warmed to rt for 16 h, diluted with EtOAc (80 mL) and washed with H$_2$O (20 mL), satd.

aqueous NaHCO$_3$ (20 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure.

The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1), to give **3.17e**

(0.784 g, 1.70 mmol, 91%) as a pale yellow solid: Mp 176.3-179.1 °C; IR (CH$_2$Cl$_2$) 2824, 2222,

1626, 1583, 1432, 1324, 1163, 1129, 1107, 1035, 949, 928, 846, 816 cm^{-1}; ^{1}H NMR (500 MHz,

CDCl$_3$) δ 7.66 (dd, J = 7.8 Hz, 4 H), 7.45 (d, J = 8.0 Hz, 1 H), 7.22 (dd, J = 8.0, 1.6 Hz, 1 H),

7.15 (d, J = 1.6 Hz, 1 H), 4.05 (t, J = 5.0 Hz, 2 H), 3.91 (t, J = 5.0 Hz, 2 H), 3.31 (t, J = 5.0 Hz, 2

H), 3.22 (t, J = 5.0 Hz, 2 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 155.6, 152.6, 135.1, 132.6, 131.8 (q,

J_{CF} = 32.8 Hz), 128.8, 125.9, 125.5 (q, J_{CF} = 3.8 Hz), 124.0, 123.5 (q, J_{CF} = 272.5 Hz), 122.7,

117.4, 105.1, 89.3, 82.5, 52.0, 50.7, 47.0, 41.5; ^{19}F NMR (470 MHz, CDCl$_3$) δ -63.1 (s, 3 F);

HRMS (ESI) m/z calcd for C$_{21}$H$_{16}$BrF$_3$N$_3$O ([M+H]$^+$) 462.0423, found 462.0423.

3.21g

4-Bromo-2-(4-((1*RS*,2*SR*)-2-(4-(trifluoromethyl)phenyl)cyclopropane-1-

carbonyl)piperazin-1-yl)benzonitrile **(3.21g).** A solution of 4-bromo-2-(4-(3-(4-

(trifluoromethyl)phenyl)propioloyl)piperazin-1-yl)benzonitrile (**3.17e**, 0.750 g, 1.62 mmol) in

EtOAc (16 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.691 g,

equivalent to 20 mol% Pd). The reaction was placed under a balloon of H$_2$ (3 vacuum/backfill

cycles) and stirred at rt for 3 d, filtered through Celite, washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:4) to give **3.18e** (0.318 g, 0.685 mmol) as a tan foam.

A solution of $CrCl_2$ (0.477 g, 0.899 mmol) and (Z)-4-bromo-2-(4-(3-(4-(trifluoromethyl)phenyl)acryloyl)piperazin-1-yl)benzonitrile (**3.18e**, 0.300 g, 0.646 mmol) in dry degassed THF (6.5 mL) was sparged with Ar for 5 min and treated with CH_2ICl (0.37 mL, 3.23 mmol) at rt, stirred for 2 d at 80 °C, cooled to rt, diluted with EtOAc (150 mL) and washed with 1 M aqueous HCl (3 x 50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:9) to give a colorless oil that was filtered through basic Al_2O_3 (EtOAc) concentrated and further dried under high vacuum to give **3.21g** (0.148 g, 0.309 mmol, 20% (2 steps) (100% purity by ELSD)) as a colorless oil: IR (CH_2Cl_2) 2833, 2222, 1642, 1583, 1484, 1436, 1326, 1228, 1116, 1069, 844 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 8.5 Hz, 2 H), 7.38 (d, J = 8.0 Hz, 1 H), 7.27 (d, J = 8.5 Hz, 2 H), 7.17 (dd, J = 8.0, 1.5 Hz, 1 H), 6.90 (d, J = 1.5 Hz, 1 H), 3.93 (dt, J = 13.0, 3.5 Hz, 1 H), 3.78 (dt, J = 13.0, 3.5 Hz, 1 H), 3.67 (ddd, J = 13.0, 9.0, 3.0 Hz, 1 H), 3.35 (ddd, J = 13.0, 9.0, 3.0 Hz, 1 H), 3.18-3.15 (m, 1 H), 3.08-3.06 (m, 1 H), 2.55-2.45 (m, 2 H), 2.29-2.24 (m, 2 H), 1.92 (q, J = 6.0 Hz, 1 H), 1.43 (td, J = 8.0, 6.0 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8, 155.7, 141.8, 135.0, 128.8, 128.8 (q, J_{CF} = 32.6 Hz), 127.9, 125.7, 125.1 (q, J_{CF} = 3.6 Hz), 124.1 (q, J_{CF} = 271.8 Hz), 122.4, 117.5, 105.0, 52.1, 50.8, 45.2, 41.7, 24.5, 24.1, 11.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.3 (s, 3 F); HRMS (ESI) m/z calcd for $C_{22}H_{20}BrF_3N_3O$ ([M+H]$^+$) 478.0736, found 478.0734.

(1S,2R)-**3.21g**   (1R,2S)-**3.21g**
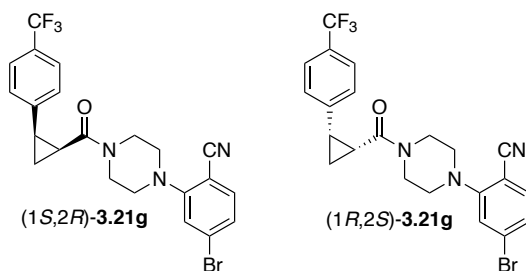
Racemic   4-bromo-2-(4-((1*RS*,2*SR*)-2-(4-(trifluoromethyl)phenyl)cyclopropane-1-carbonyl)piperazin-1-yl)benzonitrile (**3.21g**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (25% Methanol/CO_2, 7 mL/min, 220nm, p =100 bar, 20 mg/mL in MeOH) to give 4-bromo-2-(4-((1*S*,2*R*)-2-(4-(trifluoromethyl)phenyl)cyclopropane-1-carbonyl)piperazin-1-yl)benzonitrile ((1*S*,2*R*)-**3.21g**) (retention time 10.5 min) as a colorless solid (100% purity by ELSD): $[a]^{19}_D$ -144.1 (*c* 1.31, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.1 Hz, 2 H), 7.39 (d, *J* = 8.1 Hz, 1 H), 7.27 (d, *J* = 8.1 Hz, 2 H), 7.17 (dd, *J* = 8.1, 1.5 Hz, 1 H), 6.90 (d, *J* = 1.5 Hz, 1 H), 3.95-3.89 (m, 1 H), 3.76-3.67 (m, 2 H), 3.38-3.33 (m, 1 H), 3.16-3.04 (m, 2 H), 2.56-2.45 (m, 2 H), 2.27 (td, *J* = 8.7, 6.0 Hz, 2 H), 1.92 (q, *J* = 6.0 Hz, 1 H), 1.44 (td, *J* = 8.4, 5.5 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 25% MeOH, 7 mL/min, 220nm, p = 100 bar; retention time: 10.8 min).

4-Bromo-2-(4-((1*R*,2*S*)-2-(4-(trifluoromethyl)phenyl)cyclopropane-1-carbonyl)piperazin-1-yl)benzonitrile ((1*R*,2*S*)-**3.21g**) (retention time 11.6 min) was obtained as a colorless solid (100% purity by ELSD): $[a]^{19}_D$ +135.8 (*c* 1.43, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.1 Hz, 2 H), 7.39 (d, *J* = 8.1 Hz, 1 H), 7.29 (s, 2 H), 7.17 (dd, *J* = 8.1, 1.5 Hz, 1 H), 6.90 (d, *J* = 1.5 Hz, 1 H), 3.93-3.90 (m, 1 H), 3.78-3.67 (m, 2 H), 3.38-3.33 (m, 1 H), 3.17-3.04 (m, 2 H), 2.56-2.43 (m, 2 H), 2.27 (td, *J* = 8.7, 6.0 Hz, 2 H), 1.92 (q, *J* = 6.0 Hz, 1 H), 1.44 (td, *J* = 8.4, 5.6 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 25% MeOH, 7 mL/min, 220nm, p = 100 bar; retention time: 11.8 min).
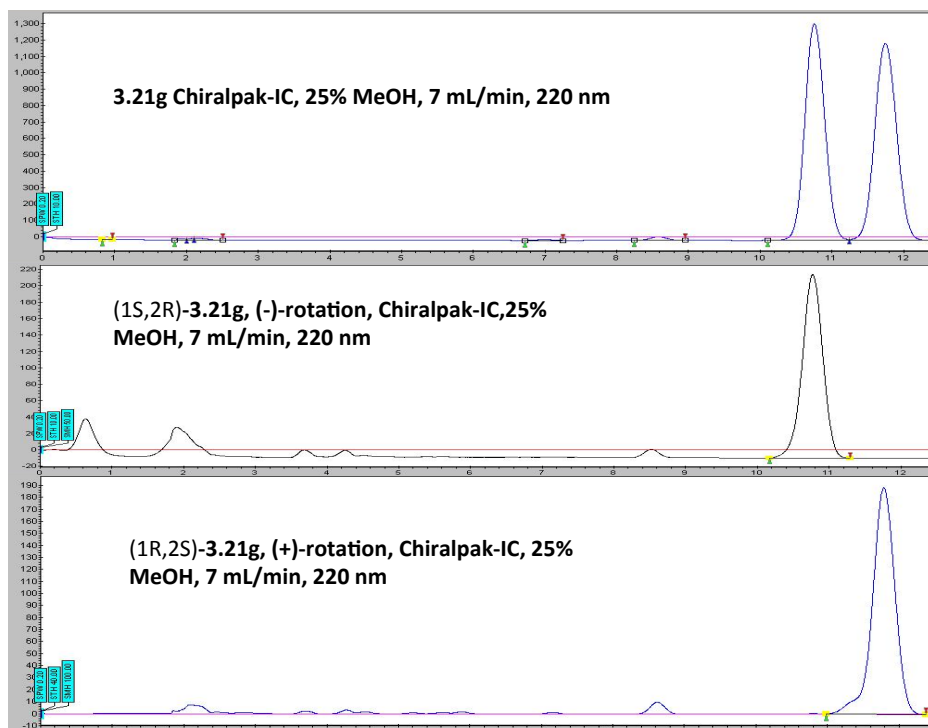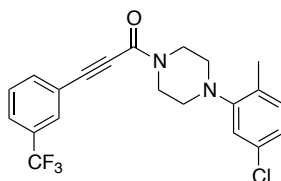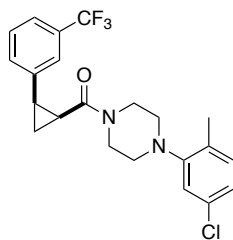
Figure 5.6. SFC chromatograms for **3.21g** and separated enantiomers.

3.17f

1-(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-yn-1-one (3.17f). A solution of 3-(3-(trifluoromethyl)phenyl)propionic acid (**3.16b**, 0.0750 g, 0.350 mmol) and 1-(5-chloro-2-methylphenyl)piperazine hydrochloride (**3.12b**, 0.104 g, 0.420 mmol) in CH$_2$Cl$_2$ (3.5 mL) cooled to 0 °C was treated with Et$_3$N (0.15 mL, 1.05 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc 0.37 mL, 0.525 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 16 h, diluted with CH$_2$Cl$_2$ (30 mL), washed with 1 M aqueous HCl (20 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude material was purified by automated

chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes) to give **3.17f** (0.106 g, 0.261 mmol, 75%) as a colorless solid: Mp 130.3-132.8 °C; IR (CH$_2$Cl$_2$) 2820, 2218, 2161, 1631, 1489, 1435, 1201, 1128, 1041, 805, 695 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.81 (s, 1 H), 7.74 (d, J = 7.8 Hz, 1 H), 7.68 (d, J = 7.8 Hz, 1 H), 7.52 (t, J = 7.8 Hz, 1 H), 7.12 (d, J = 8.1 Hz, 1 H), 7.00 (dd, J = 8.1, 2.1 Hz, 1 H), 6.96 (d, J = 2.1 Hz, 1 H), 3.98 (app t, J = 5.0 Hz, 2 H), 3.84 (app t, J = 5.0 Hz, 2 H), 2.98 (app t, J = 5.0 Hz, 2 H), 2.90 (app t, J = 5.0 Hz, 2 H), 2.29 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 152.6, 151.6, 135.4, 132.1, 131.8, 131.2 (q, J_{CF} = 33.0 Hz), 130.9, 129.17, 129.0 (q, J_{CF} = 4.1 Hz), 126.6 (q, J_{CF} = 3.6 Hz), 123.8, 123.5 (q, J_{CF} = 272.5 Hz), 121.4, 119.8, 88.9, 82.1, 51.9, 51.3, 47.4, 41.9; HRMS (ESI) *m/z* calcd for C$_{21}$H$_{19}$ClF$_3$N$_2$O ([M+H]$^+$) 407.1133, found 407.1130.
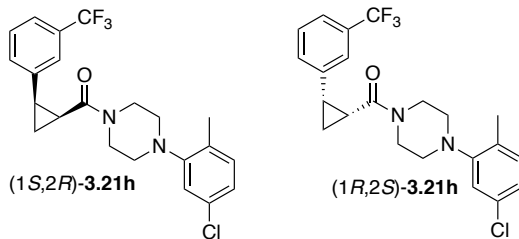

3.21h

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(3-(trifluoromethyl)phenyl)cyclopropyl)methanone (3.21h). A solution of 1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-yn-1-one (**3.17f**, 0.100 g, 0.246 mmol) in EtOAc (2.5 mL) was added Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0105 g, equivalent to 2 mol% Pd) and quinoline (0.015 mL, 0.123 mmol). The reaction vessel was placed under vacuum and backfilled with H$_2$ (balloon, 4x) stirred for 3.5 h at rt, filtered through Celite, washed (EtOAc), and the combined filtrates were washed with 1 M aqueous HCl, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, 10%

EtOAc/hexanes to 40% EtOAc/hexanes) to give **3.18f** (0.0932 g, 0.228 mmol, 93%) as a colorless solid.

A solution of $CrCl_2$ (0.105 g, 0.851 mmol) and (*Z*)-1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3.18f**, 0.0580 g, 0.142 mmol) in dry degassed THF (1.4 mL) was treated with CH_2ICl (82 uL, 0.709 mmol) and the mixture was sparged with Ar for 2 min, heated for 20 h at 80 °C, cooled to rt, diluted with Et_2O (50 mL) and washed with 1 M HCl (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, 100% hexanes to 40% EtOAc/hexanes) to give a clear oil that filtered through basic Al_2O_3 (CH_2Cl_2/EtOAc, 1:1), recrystallized from hot cyclohexane the mother liquor was decanted and the crystals were washed with hexanes (2 x 1 mL) and dried under high vacuum to give **3.21h** (0.0356 g, 0.0842 mmol, 55% (2 steps) (100% purity by ELSD)) as colorless needles: Mp 115.6-117.4 °C; IR (CH_2Cl_2) 2914, 2820, 1639, 1593, 1490, 1437, 1225, 1161, 1123, 1074, 806 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.48 (m, 2 H), 7.40 (t, *J* = 8.0 Hz, 1 H), 7.32 (d, *J* = 8.0 Hz, 1 H), 7.06 (d, *J* = 8.0 Hz, 1 H), 6.95 (dd, *J* = 8.0, 2.1 Hz, 1 H), 6.67 (d, *J* = 2.1 Hz, 1 H), 3.86 (dt, *J* = 13.0, 2.9 Hz, 1 H), 3.74 (dt, *J* = 13.0, 3.2 Hz, 1 H), 3.62-3.56 (m, 1 H), 3.26 (ddd, *J* = 12.4, 9.0, 3.2 Hz, 1 H), 2.79-2.70 (m, 2 H), 2.53 (td, *J* = 9.0, 6.9 Hz, 1 H), 2.26 (ddd, *J* = 9.0, 8.4, 5.9 Hz, 1 H), 2.20 (s, 3 H), 2.10 (ddd, *J* = 11.2, 9.0, 2.4 Hz, 1 H), 1.91 (app q, *J* = 5.8 Hz, 1 H), 1.41 (td, *J* = 8.4, 5.8 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 151.7, 138.7, 131.9, 131.8, 130.9, 130.5, 130.5 (d, J_{CF} = 32.2 Hz) 128.7, 125.1 (d, J_{CF} = 4.0 Hz), 124.2 (d, J_{CF} = 272.2 Hz), 123.6, 123.3 (d, J_{CF} = 3.7 Hz), 119.6, 51.7, 51.6, 45.5, 42.2, 24.1, 23.9, 17.3, 10.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.4 (s, 3 F); HRMS (ESI) *m/z* calcd for $C_{22}H_{23}ClF_3N_2O$ ([M+H]$^+$) 423.1446, found 423.1443.

(1*S*,2*R*)-**3.21h**   (1*R*,2*S*)-**3.21h**

Racemic (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(3-(trifluoromethyl)phenyl)cyclopropyl)methanone (**3.21h**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm) injection volume 90 µL, 20 mg/mL) to give (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1*S*,2*R*)-2-(3-(trifluoromethyl)phenyl)cyclopropyl)methanone ((1*S*,2*R*)-**3.21h**) (retention time 4.82 min) as a colorless viscous oil (100% purity by ELSD): $[a]^{19}_D$ -133.5 (*c* 0.70, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.48 (bs, 2 H), 7.41 (t, *J* = 8.0 Hz, 1 H), 7.33 (d, *J* = 7.4 Hz, 1 H), 7.06 (d, *J* = 8.1 Hz, 1 H), 6.95 (dd, *J* = 8.1, 2.0 Hz, 1 H), 6.67 (d, *J* = 2.0 Hz, 1 H), 3.89-3.82 (m, 1 H), 3.78-3.71 (m, 1 H), 3.63-3.55 (m, 1 H), 3.31-3.23 (m, 1 H), 2.79-2.69 (m, 2 H), 2.53 (td, *J* = 8.4, 7.2 Hz, 1 H), 2.30-2.07 (m, 6 H), 1.92 (q, *J* = 5.4 Hz, 1 H), 1.41 (td, *J* = 8.4, 5.4 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm; retention time: 5.0 min).

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1*R*,2*S*)-2-(3-(trifluoromethyl)phenyl)cyclopropyl)methanone ((1*R*,2*S*)-**3.21h**) (retention time 5.57 min) was obtained as a colorless viscous oil (100% purity by ELSD): $[a]^{19}_D$ +133.1 (*c* 0.65, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.48 (bs, 2 H), 7.41 (t, *J* = 8.0 Hz, 1 H), 7.33 (d, *J* = 8.0 Hz, 1 H), 7.06 (d, *J* = 8.0 Hz, 1 H), 6.95 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.67 (d, *J* = 2.0 Hz, 1 H), 3.90-3.82 (m, 1 H), 3.78-3.70 (m, 1 H), 3.64-3.55 (m, 1 H), 3.31-3.23 (m, 1 H), 2.80-2.69 (m, 2 H), 2.53 (td, *J* = 8.4, 6.8 Hz, 1 H), 2.30-2.07 (m, 6 H), 1.92 (q, *J* = 5.4 Hz, 1 H), 1.41 (td, *J* = 8.4, 5.4 Hz, 1 H).

The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 5.7 min).
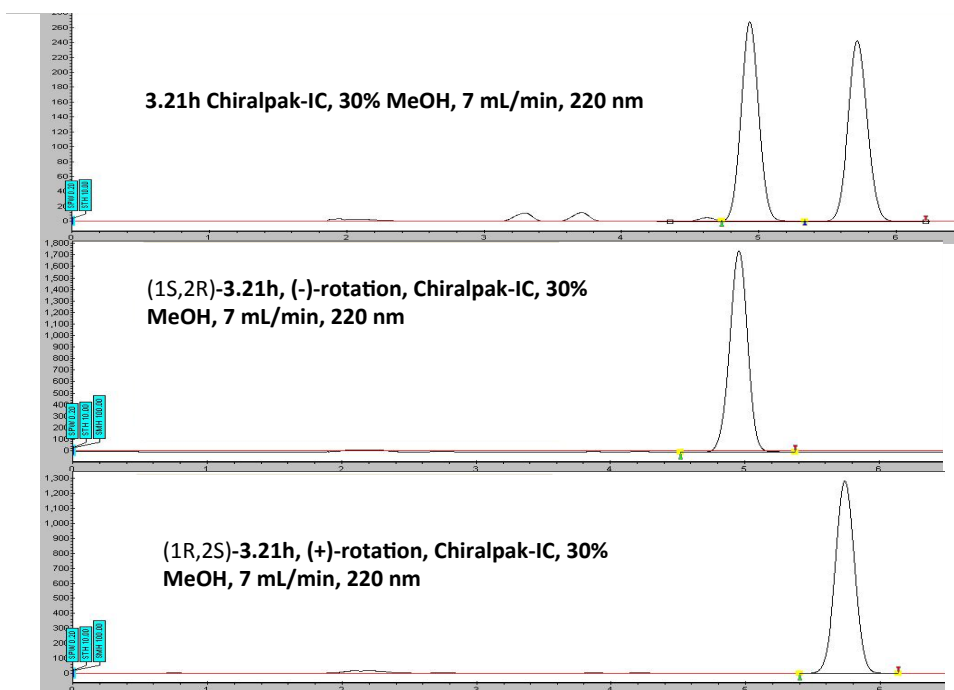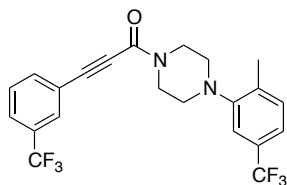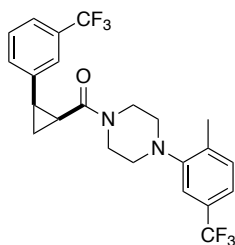

Figure 5.7. SFC chromatograms for **3.21h** and separated enantiomers.

3.17g

1-(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-yn-1-one (3.17g). A solution of 3-(3-(trifluoromethyl)phenyl)propionic acid (**3.16b**, 0.0763 g, 0.356 mmol) and 1-(2-methyl-5-(trifluoromethyl)phenyl)piperazine hydrochloride (**3.12c**, 0.100 g, 0.356 mmol) in CH$_2$Cl$_2$ (3.6 mL) cooled to 0 °C was treated with Et$_3$N (0.20 mL, 1.42 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.38 mL, 0.534 mmol) dropwise and the reaction

was stirred at 0 °C for 30 min, warmed to rt for 16 h, diluted with CH$_2$Cl$_2$ (30 mL), washed with 1 M aqueous HCl (20 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes), to give **3.17g** (0.0819 g, 0.186 mmol, 52%) as a pink solid: Mp 121.8-125.2 °C; IR (CH$_2$Cl$_2$) 2924, 2217, 1633, 1436, 1333, 1310, 1166, 1076, 1042, 695 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.81 (s, 1 H), 7.74 (d, J = 7.8 Hz, 1 H), 7.67 (d, J = 7.8 Hz, 1 H), 7.52 (t, J = 7.8 Hz, 1 H), 7.30 (d, J = 8.1 Hz, 1 H), 7.26 (d, J = 8.1 Hz, 1 H), 7.22 (s, 1 H), 4.00 (app t, J = 5.0 Hz, 2 H), 3.86 (app t, J = 4.8 Hz, 2 H), 3.03 (app t, J = 5.0 Hz, 2 H), 2.93 (app t, J = 5.0 Hz, 2 H), 2.39 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 152.6, 150.9, 136.8, 135.4, 135.4, 131.5, 131.2 (q, J_{CF} = 33.0 Hz), 129.2 (s), 129.0 (q, J_{CF} = 32.2 Hz), 129.0 (q, J_{CF} = 3.9 Hz), 126.6 (q, J_{CF} = 3.7 Hz), 124.0 (q, J_{CF} = 272.0 Hz), 123.4 (q, J_{CF} = 272.3 Hz), 121.3, 120.5 (q, J_{CF} = 3.8 Hz), 88.9, 82.0, 51.8, 51.3, 47.4, 41.9, 17.9; ^{19}F NMR (376 MHz, CDCl$_3$) δ -62.3 (s, 3 F), -63.0 (s, 3 F); HRMS (ESI) m/z calcd for C$_{22}$H$_{19}$F$_6$N$_2$O ([M+H]$^+$) 441.1396, found 441.1391.


3.21i

(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(3-(trifluoromethyl)phenyl)cyclopropyl)methanone (3.21i). A solution of 1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-yn-1-one **(3.17g,** 0.0800 g, 0.182 mmol) in EtOAc (1.8 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0193 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of

H₂ (3 vacuum/backfill cycles) and stirred at rt for 2 d, filtered through Celite, washed (EtOAc), and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 40% EtOAc/hexanes, product eluted at 10% EtOAc/hexanes) to give the **3.18g** (0.0572 g, 0.129 mmol) as clear/tan viscous oil.

A solution of $CrCl_2$ (0.0955 g, 0.777 mmol) and (*Z*)-1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3.18g**, 0.0573 g, 0.130 mmol) in dry degassed THF (1.3 mL) was sparged with Ar for 5 min and treated with CH_2ICl (75 uL, 0.648 mmol) at rt and under Ar atmosphere, heated for 20 h at 80 °C, cooled to rt, diluted with Et_2O (50 mL), and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 40% EtOAc/hexanes) to give a clear oil that was filtered through basic Al_2O_3 (CH_2Cl_2/EtOAc, 1:1), recrystallized from CH_2Cl_2/hexanes (ca. 1:5) the mother liquor was decanted and the clear colorless cubes were washed with hexanes (2 x 1 mL) and dried under high vacuum to give **3.21i** (0.0115 g, 0.0252 mmol, 14% (2 steps) (100% purity by ELSD)) as a colorless solid: Mp 113.4-116.8 °C; IR (CH_2Cl_2) 2914, 2824, 1641, 1418, 1328, 1309, 1163, 1121, 1073 cm⁻¹; ¹H NMR (400 MHz, $CDCl_3$) δ 7.47 (bd, *J* = 7.1 Hz, 2 H), 7.41 (t, *J* = 7.8 Hz, 1 H), 7.33 (d, *J* = 7.8 Hz, 1 H), 7.24 (s, 2 H), 6.92 (s, 1 H), 3.94 (d, *J* = 12.2 Hz, 1 H), 3.79 (d, *J* = 12.2 Hz, 1 H), 3.58 (ddd, *J* = 12.2, 9.2, 3.0 Hz, 1 H), 3.24 (ddd, *J* = 12.2, 9.2, 2.6 Hz, 1 H), 2.77 (td, *J* = 12.2, 3.7 Hz, 2 H), 2.53 (td, *J* = 9.2, 7.0 Hz, 1 H), 2.29 (s, 3 H), 2.28-2.18 (m, 2 H), 2.11-2.05 (m, 1 H), 1.92 (q, *J* = 6.0 Hz, 1 H), 1.42 (td, *J* = 8.5, 6.0 Hz, 1 H); ¹³C NMR (100 MHz, $CDCl_3$) δ 166.7, 151.1, 138.8, 136.9, 131.4, 130.6 (q, J_{CF} = 31.2 Hz), 130.5, 129.1 (q, J_{CF} = 32.1

Hz), 128.7, 125.1 (q, J_{CF} = 3.7 Hz), 124.2 (q, J_{CF} = 272.3 Hz, 2 C), 123.2 (q, J_{CF} = 3.8 Hz), 120.4

(q, J_{CF} = 3.8 Hz), 116.1 (q, J_{CF} = 3.7 Hz), 51.7 (2 C), 45.6, 42.2, 24.2, 24.0, 17.8, 10.9; ^{19}F NMR

(376 MHz, CDCl$_3$) δ -62.4 (s, 3 H), -62.6 (s, 3 H); HRMS (ESI) *m/z* calcd for $C_{22}H_{23}F_6N_2O$

([M+H]$^+$) 457.1709, found 457.1704.
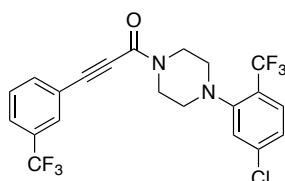
3.17h

1-(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(3-

(trifluoromethyl)phenyl)prop-2-yn-1-one **(3.17h).** A solution of 3-(3-

(trifluoromethyl)phenyl)propiolic acid (**3.16b**, 0.100 g, 0.467 mmol) and 1-(5-chloro-2-

(trifluoromethyl)phenyl)piperazinehydrochloride (**3.12d**, 0.155 g, 0.514 mmol) in CH$_2$Cl$_2$ (4.7

mL) cooled to 0 °C was treated with Et$_3$N (0.26 mL, 1.87 mmol). The cooled solution was

treated with T3P (50 wt. % solution in EtOAc, 0.49 mL, 0.701 mmol) dropwise and the reaction

was stirred at 0 °C for 30 min, warmed to rt for 16 h, diluted with CH$_2$Cl$_2$ (30 mL), washed with

H$_2$O (20 mL), satd. aqueous NaHCO$_3$ (20 mL), dried (MgSO$_4$), filtered, and concentrated under

reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g

column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc/hexanes) to give the crude

product that was recrystallized from hot EtOH. The supernatant was removed and the crystals

were washed with hexanes and then dried under high vacuum to give **3.17h** (0.168 g, 0.365

mmol, 78%) as a colorless solid: Mp 87.6-91.2 °C; IR (CH$_2$Cl$_2$) 2921, 2827, 2222, 1633, 1596,

1436, 1332, 1309, 1121, 1034, 695 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.78 (s, 1 H), 7.71 (d, *J*

= 7.8 Hz, 1 H), 7.65 (d, *J* = 8.5 Hz, 1 H), 7.56 (d, *J* = 8.5 Hz, 1 H), 7.50 (t, *J* = 7.8 Hz, 1 H),

7.26-7.22 (m, 2 H), 3.95 (app t, J = 5.0 Hz, 2 H), 3.81 (app t, J = 5.0 Hz, 2 H), 2.99 (app t, J = 5.0 Hz, 2 H), 2.92 (app t, J = 5.0 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 152.6, 138.8, 135.4, 131.2 (q, J_{CF} = 33.0 Hz), 129.2, 129.0 (q, J_{CF} = 3.7 Hz), 128.6 (q, J_{CF} = 5.4 Hz), 126.6 (q, J_{CF} = 3.7 Hz), 125.8 (q, J_{CF} = 29.5 Hz), 125.7, 124.7, 123.5 (q, J_{CF} = 273.0 Hz), 123.2 (q, J_{CF} = 272.5 Hz), 121.3, 88.9, 82.0, 53.6, 52.7, 47.4, 41.8; ^{19}F NMR (376 MHz, CDCl$_3$) δ -60.3 (s, 3 F), -63.0 (s, 3 F); HRMS (ESI) m/z calcd for C$_{21}$H$_{16}$ClF$_6$N$_2$O ([M+H]$^+$) 461.0850, found 461.0849.
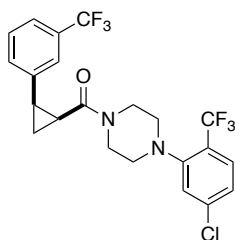
(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazine-1-yl)(2-(3-(trifluoromethyl)phenyl)cyclopropyl)methanone (3.21j). A solution of 1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazine-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-yn-1-one (**3.17h** 0.0684 g, 0.148 mmol) in EtOAc (1.5 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0158 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of H$_2$ (3 vacuum/backfill cycles) and stirred at rt for 3 d, filtered through Celite, washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes) to give **3.18h** (0.0512 g, 0.111 mmol) as a colorless solid.

A solution of CrCl$_2$ (0.816 g, 0.940 mmol) and (*Z*)-1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazine-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3.18h**, 0.0512 g, 0.111 mmol) in dry degassed THF (1.6 mL) was sparged with Ar for 5 min and treated with CH$_2$ICl (0.064 mL, 0.553 mmol) at rt, heated for 2 d at 80 °C, cooled to rt, diluted with EtOAc (50 mL), and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried

(MgSO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes) to give a clear oil that was filtered through basic Al$_2$O$_3$ (eluting with 1:1 CH$_2$Cl$_2$/EtOAc), concentrated under reduced pressure, and dried under high vacuum to give **3.21j** (0.0409 g, 0.0858 mmol, 59% (2 steps) (100% purity by ELSD)) as a clear colorless oil: IR (CH$_2$Cl$_2$) 2918, 1641, 1596, 1437, 1327, 1120, 1032, 809, 702 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.54-7.50 (m, 3 H), 7.44 (t, J = 8.0 Hz, 1 H), 7.34 (d, J = 8.0 Hz, 1 H), 7.20 (dd, J = 8.5, 1.4 Hz, 1 H), 6.87 (d, J = 1.6 Hz, 1 H), 4.02 (d, J = 13.0 Hz, 1 H), 3.80 (d, J = 13.0 Hz, 1 H), 3.51 (ddd, J = 12.8, 9.8, 3.0 Hz, 1 H), 3.13 (ddd, J = 12.5, 9.8, 2.7 Hz, 1 H), 2.73 (td, J = 7.7, 3.6 Hz, 2 H), 2.52 (td, J = 9.0, 7.0 Hz, 1 H), 2.25 (ddd, J = 9.0, 8.4, 6.0 Hz, 1 H), 2.21-2.15 (m, 1 H), 1.97-1.90 (m, 2 H), 1.42 (td, J = 8.4, 6.0 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.6, 152.7, 138.9, 138.7, 130.6 (q, J_{CF} = 32.1 Hz), 130.5, 128.7, 128.3 (q, J_{CF} = 5.4 Hz), 125.8 (q, J_{CF} = 29.4 Hz), 125.6, 125.1 (q, J_{CF} = 3.8 Hz), 124.6, 124.2 (q, J_{CF} = 272.4 Hz), 123.5 (q, J_{CF} = 272.9 Hz), 123.2 (q, J_{CF} = 3.7 Hz), 53.5, 53.0, 45.5, 42.1, 24.3, 23.9, 10.9; ^{19}F NMR (376 MHz, CDCl$_3$) δ -60.4 (s, 3 F), -62.4 (s, 3 F); HRMS (ESI) m/z calcd for C$_{22}$H$_{20}$ClF$_6$N$_2$O ([M+H]$^+$) 477.1163, found 477.1166.
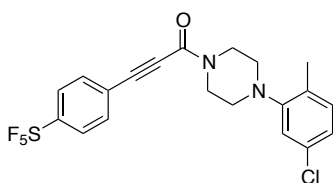
1-(4-(5-Chloro-2-methylphenyl)piperazine-1-yl)-3-(4-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-yn-1-one (3.17i). A solution of 3-(4-(pentafluoro-λ6-sulfaneyl)phenyl)propiolic acid (**3.16c**, 0.100 g, 0.367 mmol) and 1-(1-(5-chloro-2-methylphenyl)piperazine hydrochloride (**3.12b**, 0.0999 g, 0.404 mmol) in CH$_2$Cl$_2$ (3.7 mL)

cooled to 0 °C was treated with Et₃N (0.20 mL, 1.47 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.39 mL, 0.551 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 16 h, diluted with EtOAc (30 mL), washed with H_2O (20 mL), satd. aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 30% EtOAc/hexanes), to give **3.17i** (0.127 g, 0.272 mmol, 74%) as a pale yellow foam: IR (CHCl₃) 2981, 2222, 1627, 1490, 1432, 1275, 832, 792, 727 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.76 (d, J = 8.8 Hz, 2 H), 7.64 (d, J = 8.8 Hz, 2 H), 7.11 (d, J = 8.1 Hz, 1 H), 6.99 (dd, J = 8.1, 2.1 Hz, 1 H), 6.95 (d, J = 2.1 Hz, 1 H), 3.96 (app t, J = 5.0 Hz, 2 H), 3.84 (app t, J = 5.0 Hz, 2 H), 2.97 (app t, J = 5.0 Hz, 2 H), 2.89 (app t, J = 5.0 Hz, 2 H), 2.29 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 154.4 (quint, J_{CF} = 18.3 Hz), 152.4, 151.6, 132.4, 132.1, 131.9, 131.0, 126.2 (quint, J_{CF} = 4.6 Hz), 124.1, 123.8, 119.8, 88.2, 83.2, 51.9, 51.3, 47.4, 42.0, 17.3; ¹⁹F NMR (376 MHz, CDCl₃) δ 83.0 (quint, J = 150.3 Hz, 1 F), 62.4 (d, J = 150.3 Hz, 4 F); HRMS (ESI) m/z calcd for $C_{20}H_{19}ClF_5N_2OS$ ([M+H]⁺) 465.0821, found 465.0819.
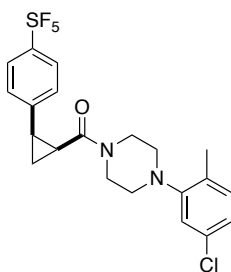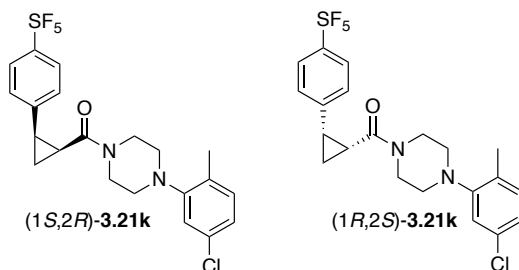

3.21k

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone (3.21k). A solution of 1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(4-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-yn-1-one **(3.17i,**

0.0850 g, 0.183 mmol) in EtOAc (1.8 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0195 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of H$_2$ (3 vacuum/backfill cycles) and stirred at rt for 2 d, filtered through Celite, washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes, product eluted at 35% EtOAc/hexanes) to give **3.18i** (0.0811 g, 0.174 mmol) as a colorless solid.

A solution of CrCl$_2$ (0.126 g, 1.03 mmol) and (*Z*)-1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(4-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-en-1-one (**3.18i**, 0.0800 g, 0.171 mmol) in dry degassed THF (1.7 mL) was sparged with Ar for 5 min and added CH$_2$ICl (0.10 mL, 0.857 mmol) at rt, heated for 2 d at 80 °C, cooled to rt, diluted with EtOAc (50 mL) and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried (MgSO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc/hexanes, product eluted at 30% EtOAc/hexanes) to give a clear oil that was filtered through basic Al$_2$O$_3$ (CH$_2$Cl$_2$/EtOAc, 1:1), concentrated under reduced pressure, and dried under high vacuum to give **3.21k** (0.0451 g, 0.0938 mmol, 52% (2 steps) (100% purity by ELSD)) as a colorless solid: Mp 169.8-172.0 °C; IR (CH$_2$Cl$_2$) 2919, 1639, 1490, 1466, 1438, 1225, 1035, 835, 750 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.66 (d, *J* = 8.6 Hz, 2 H), 7.25 (d, *J* = 8.6 Hz, 2 H), 7.06 (dd, *J* = 8.1, 0.4 Hz, 1 H), 6.95 (dd, *J* = 8.1, 2.1 Hz, 1 H), 6.69 (d, *J* = 2.1 Hz, 1 H), 3.82 (bd, *J* = 14.0 Hz, 1 H), 3.70-3.58 (m, 2 H), 3.33 (ddd, *J* = 12.4, 8.9, 3.0 Hz, 1 H), 2.75 (tdd, *J* = 14.0, 7.7, 3.4 Hz, 2 H), 2.50 (td, *J* = 8.9, 7.0 Hz, 1 H), 2.32-2.23 (m, 2 H), 2.20 (s, 3 H), 2.10 (ddd, *J* = 11.1, 8.2, 3.0 Hz, 1 H), 1.91 (q, *J* = 6.3 Hz, 1 H), 1.44 (td, *J* = 8.4, 5.6 Hz, 1 H); ^{13}C

NMR (100 MHz, CDCl$_3$) δ 166.5, 152.1 (quint, J_{CF} = 17.3 Hz), 151.6, 141.9, 131.9 (2 C), 130.9, 127.7, 125.7 (quint, J_{CF} = 4.6 Hz), 123.7, 119.5, 51.9, 51.5, 45.5, 42.2, 24.6, 23.7, 17.3, 11.3; ^{19}F NMR (376 MHz, CDCl$_3$) δ 84.8 (quint, J = 150.4 Hz, 1 F), 63.2 (d, J = 150.4 Hz, 4 F); HRMS (ESI) m/z calcd for C$_{21}$H$_{23}$ClF$_5$N$_2$OS ([M+H]$^+$) 481.1134, found 481.1134.

(1S,2R)-3.21k   (1R,2S)-3.21k

Racemic (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1RS,2SR)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone (**3.21k**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm) injection volume 90 µL, 20 mg/mL) to give (4-(5-chloro-2-methylphenyl)piperazin-1-yl)((1S,2R)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone ((1S,2R)-**3.21k)**) (retention time 5.14 min) as a colorless viscous oil (100% purity by ELSD): [a]^{17}$_D$ -134.2 (c 0.60, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.66 (d, J = 8.7 Hz, 2 H), 7.26 (overlap, 2 H), 7.06 (d, J = 8.1 Hz, 1 H), 6.96 (dd, J = 8.1, 1.8 Hz, 1 H), 6.70 (d, J = 1.8 Hz, 1 H), 3.87-3.79 (m, 1 H), 3.73-3.58 (m, 3 H), 3.38-3.31 (m, 1 H), 2.81-2.70 (m, 2 H), 2.50 (q, J = 8.7 Hz, 1 H), 2.33-2.24 (m, 2 H), 2.20 (s, 3 H), 2.16-2.08 (m, 1 H), 1.91 (q, J = 5.7 Hz, 1 H), 1.44 (td, J = 8.1, 5.7 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 5.1 min).
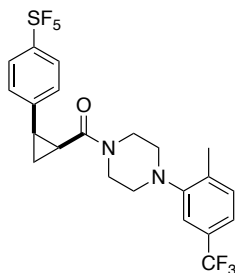
(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1R,2S)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone ((1R,2S)-**3.21k)**) (retention time 6.57 min) was obtained as a colorless viscous oil (100% purity by ELSD): [a]^{17}$_D$ +136.3 (c 0.59, MeOH); ^{1}H

NMR (300 MHz, CDCl$_3$) δ 7.66 (d, J = 8.7 Hz, 2 H), 7.26-7.24 (m, 2 H), 7.06 (d, J = 8.4 Hz, 1 H), 6.96 (dd, J = 8.4, 1.8 Hz, 1 H), 6.70 (d, J = 1.8 Hz, 1 H), 3.85-3.78 (m, 1 H), 3.67-3.61 (m, 2 H), 3.39-3.30 (m, 1 H), 2.81-2.70 (m, 2 H), 2.50 (q, J = 8.7 Hz, 1 H), 2.33-2.24 (m, 2 H), 2.20 (s, 3 H), 2.16-2.10 (m, 1 H), 1.91 (q, J = 5.7 Hz, 1 H), 1.44 (td, J = 8.4, 5.7 Hz, 2 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 6.5 min).
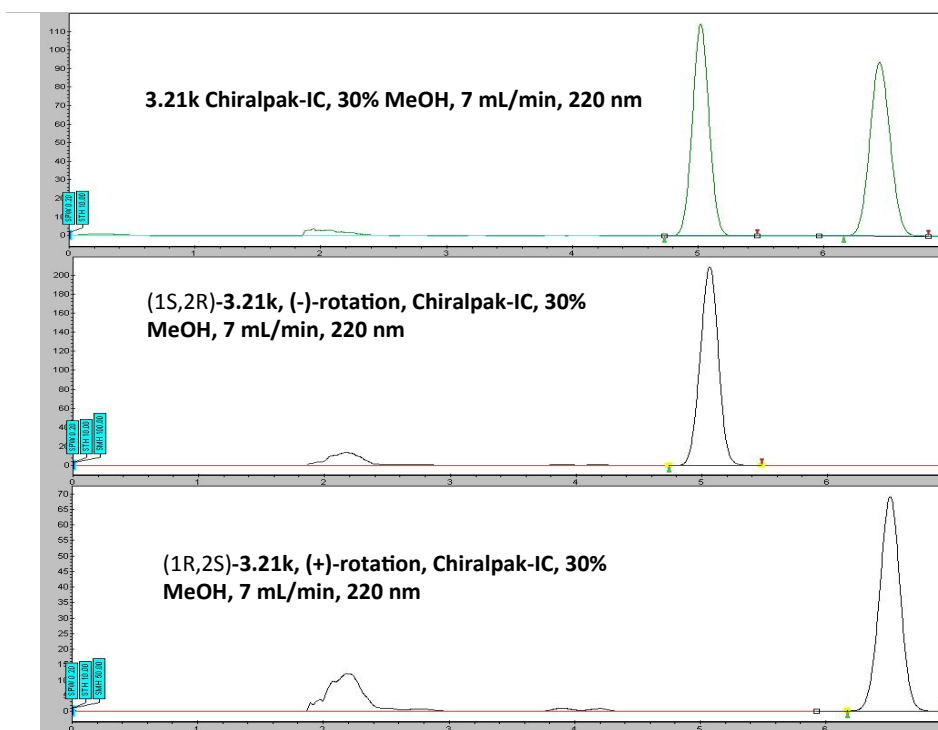
Figure 5.8. SFC chromatograms for **3.21k** and separated enantiomers.
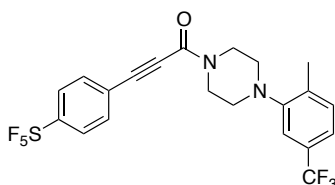
3.17j

1-(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-yn-1-one (3.17j). A solution of 3-(4-(pentafluoro-λ6-

sulfaneyl)phenyl)propiolic acid (**3.16c**, 0.100 g, 0.367 mmol) and 1-(2-methyl-5-(trifluoromethyl)phenyl)piperazine hydrochloride (**3.12b**, 0.113 g, 0.404 mmol) in CH_2Cl_2 (3.7 mL) cooled to 0 °C was treated with Et_3N (0.20 mL, 1.47 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.39 mL, 0.551 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 16 h, diluted with EtOAc (30 mL), washed with H_2O (20 mL), satd. aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 40% EtOAc/hexanes), to give **3.17j** (0.139 g, 0.278 mmol, 76%) as a tan foam: IR (CH_2Cl_2) 2920, 2222, 1632, 1418, 1340, 1310, 1279, 1122, 839, 793 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.75 (d, J = 8.8 Hz, 2 H), 7.63 (d, J = 8.8 Hz, 2 H), 7.31-7.25 (m, 2 H), 7.21 (s, 1 H), 3.98 (t, J = 5.0 Hz, 2 H), 3.85 (app t, J = 5.0 Hz, 2 H), 3.02 (app t, J = 5.0 Hz, 2 H), 2.92 (app t, J = 5.0 Hz, 2 H), 2.38 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 154.3 (quint, J_{CF} = 18.1 Hz), 152.4, 150.9, 136.8, 132.4, 131.5, 129.0 (q, J_{CF} = 32.1 Hz), 126.2 (quint, J_{CF} = 4.6 Hz), 124.1 (q, J_{CF} = 272.1 Hz), 124.0, 120.5 (q, J_{CF} = 3.8 Hz), 116.0 (q, J_{CF} = 3.6 Hz), 88.2, 83.1, 51.8, 51.3, 47.4, 41.9, 17.8; ^{19}F NMR (376 MHz, CDCl$_3$) δ 83.0 (quint, J = 150.4 Hz, 1 F), 62.4 (d, J = 150.2 Hz, 4 F), -62.3 (s, 3 F); HRMS (ESI) *m/z* calcd for $C_{21}H_{19}F_8N_2OS$ ([M+H]$^+$) 499.1085, found 499.1086.
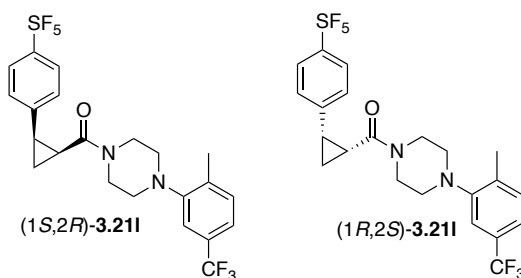

3.21l

(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone (3.21l). A solution of 1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-(pentafluorosulfanyl)phenyl)prop-2-yn-1-one (**3.17j**, 0.100 g, 0.201 mmol) in EtOAc (2 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0214 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of H$_2$ (3 vacuum/backfill cycles) and stirred at rt for 2 d, filtered through Celite, washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, gradient 100% hexanes to 40% EtOAc/hexanes, product eluted at 30% EtOAc/hexanes) to give **3.18j** (0.0765 g, 0.153 mmol) as a colorless foam.

A solution of CrCl$_2$ (0.111 g, 0.899 mmol) and (*Z*)-1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(4-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-en-1-one (**3.18j**, 0.0750 g, 0.150 mmol) in dry degassed THF (1.5 mL) was sparged with Ar for 5 min, treated with CH$_2$ICl (0.087 mL, 0.749 mmol) sparged with Ar for 2 min, heated for 2 d at 80 °C, cooled to rt, diluted with EtOAc (50 mL), washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried (MgSO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 30% EtOAc/hexanes, product eluted at 30% EtOAc/hexanes) to give the product as a clear oil that was filtered through basic Al$_2$O$_3$ (CH$_2$Cl$_2$/EtOAc, 1:1) concentrated to a colorless solid. The solid was recrystallized from hot cyclohexane (2x). The supernatant was removed and the crystals were washed with cyclohexane (2 x 1 mL) and hexanes (2 x 1 mL) and dried under high vacuum to give **3.21l** (0.0176 g, 0.0342 mmol, 18% (2 steps) (100% purity by ELSD)) as a colorless solid: Mp 122.1-125.8 °C; IR (CH$_2$Cl$_2$) 2919, 1638, 1417, 1338, 1308,

1120, 824, 749 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.65 (d, J = 8.8 Hz, 2 H), 7.26-7.24 (m, 5 H), 6.96 (s, 1 H), 3.85 (bd, J = 13.0 Hz, 1 H), 3.72-3.60 (m, 2 H), 3.38-3.32 (m, 1 H), 2.86-2.75 (m, 2 H), 2.51 (td, J = 8.9, 7.0 Hz, 1 H), 2.32-2.26 (m, 5 H), 2.14 (ddd, J = 11.0, 8.6, 2.6 Hz, 1 H), 1.92 (q, J = 6.2 Hz, 1 H), 1.45 (td, J = 8.4, 5.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.5, 152.2 (quint, J_{CF} = 17.4 Hz), 150.9, 141.8, 136.7, 131.4, 129.1 (q, J_{CF} = 32.1 Hz), 127.7, 125.7, 125.7 (quint, J_{CF} = 4.6 Hz), 124.0 (q, J_{CF} = 271.9 Hz), 120.4 (q, J_{CF} = 3.9 Hz), 115.7 (q, J_{CF} = 3.8 Hz), 51.9, 51.4, 45.5, 42.2, 24.6, 23.7, 17.9, 11.3; ^{19}F NMR (376 MHz, CDCl$_3$) δ 84.6 (quint, J = 149.9 Hz, 1 F), 63.0 (d, J = 149.8 Hz, 4 F), -62.4 (s, 3 F); HRMS (ESI) m/z calcd for C$_{22}$H$_{23}$F$_8$N$_2$OS ([M+H]$^+$) 515.1398, found 515.1378.

(1S,2R)-**3.21l**   (1R,2S)-**3.21l**

Racemic   (4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1RS,2SR)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone (**3.21l**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm) injection volume (90 µL, 20 mg/mL) to give (4-(2-methyl-5-(trifluoromethyl)phenyl)piperazine-1-yl)((1S,2R)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone ((1S,2R)-**3.21l**) (retention time 3.40 min) as a colorless viscous oil (100% purity by ELSD): [a]^{17}$_D$ -119.1 (c 0.79, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.66 (d, J = 8.7 Hz, 2 H), 7.25 (d, J = 8.7 Hz, 3 H), 6.97 (s, 1 H), 3.89-3.80 (m, 1 H), 3.72-3.60 (m, 2 H), 3.40-3.31 (m, 1 H), 2.86-2.74 (m, 2 H), 2.50 (q, J = 8.4 Hz, 1 H), 2.33-2.25 (m, 5 H), 2.18-2.12 (m, 1 H), 1.92 (q, J = 6.0 Hz, 1 H), 1.44 (td, J = 8.4, 6.0 Hz, 1 H). The enantiomeric

excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm; retention time: 3.3 min).
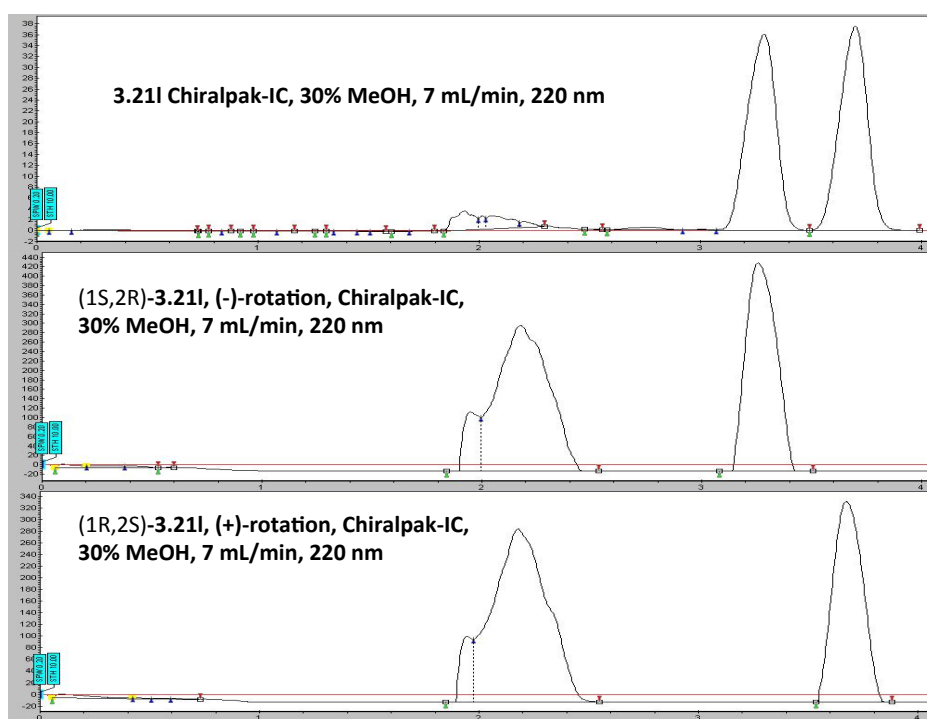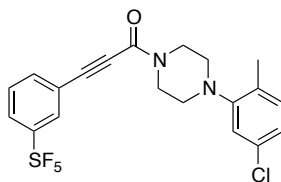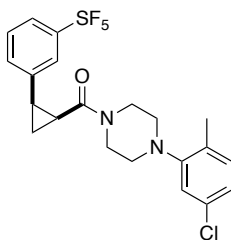
(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazine-1-yl)((1*R*,2*S*)-2-(4-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone ((1*R*,2*S*)-**3.21l**) (retention time 3.65 min) was obtained as a colorless viscous oil (100% purity by ELSD): [a]$^{17}_D$ +117.0 (*c* 0.87, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.66 (d, *J* = 8.7 Hz, 2 H), 7.26 (d, *J* = 8.7 Hz, 3 H), 6.97 (s, 1 H), 3.88-3.82 (m, 2 H), 3.69-3.63 (m, 3 H), 3.40-3.31 (m, 2 H), 2.89-2.70 (m, 3 H), 2.50 (q, *J* = 8.4 Hz, 2 H), 2.34-2.25 (m, 5 H), 2.20-2.12 (m, 1 H), 1.92 (q, *J* = 6.0 Hz, 1 H), 1.44 (td, *J* = 8.4, 6.0 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm; retention time: 3.7 min).

Figure 5.9. SFC chromatograms for **3.21l** and separated enantiomers.

1-(4-(5-Chloro-2-methylphenyl)piperazine-1-yl)-3-(3-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-yn-1-one (3.17k). A solution of 3-(3-(pentafluoro-λ6-sulfaneyl)phenyl)propiolic acid (**3.16d**, 0.100 g, 0.367 mmol) and 1-(1-(5-chloro-2-methylphenyl)piperazine hydrochloride (**3.12b**, 0.0999 g, 0.404 mmol) in CH_2Cl_2 (3.7 mL) cooled to 0 °C was treated with Et_3N (0.20 mL, 1.47 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.39 mL, 0.551 mmol) dropwise and the reaction was stirred at 0 °C for 30 min and allowed to warm to rt for 16 h. The reaction was diluted with EtOAc (30 mL) and washed with H_2O (20 mL), satd. aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 30% EtOAc/hexanes), to give **3.17k** (0.113 g, 0.244 mmol, 66%) as a pale yellow solid: Mp 127.9-133.0 °C; IR (CHCl₃) 2921, 2219, 1627, 1432, 1288, 1223, 1041, 838, 797, 728 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.94 (t, J = 2.0 Hz, 1 H), 7.80 (ddd, J = 8.4, 2.0, 0.8 Hz, 1 H), 7.69 (d, J = 7.7 Hz, 1 H), 7.49 (t, J = 8.1 Hz, 1 H), 7.11 (d, J = 8.1 Hz, 1 H), 6.99 (dd, J = 8.1, 2.1 Hz, 1 H), 6.96 (d, J = 2.1 Hz, 1 H), 3.97 (app t, J = 5.0 Hz, 2 H), 3.84 (app t, J = 5.0 Hz, 2 H), 2.98 (app t, J = 5.0 Hz, 2 H), 2.89 (app t, J = 5.0 Hz, 2 H), 2.29 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 153.8 (quint, J_{CF} = 18.5 Hz), 152.4, 151.6, 135.1, 132.1, 131.9, 131.0, 129.7 (quint, J_{CF} = 7.1 Hz), 129.0, 127.3 (quint, J_{CF} = 4.5 Hz), 123.8, 121.6, 119.8, 88.4, 82.3, 51.9, 51.3, 47.4, 42.0, 17.3; ¹⁹F NMR (376 MHz, CDCl₃) δ 82.9 (quint, J = 150.6 Hz, 1 F), 62.6 (d, J = 150.5 Hz, 4 F); HRMS (ESI) *m/z* calcd for $C_{20}H_{19}ClF_5N_2OS$ ([M+H]⁺) 465.0821, found 465.0821.

(4-(5-Chloro-2-methylphenyl)piperazine-1-yl)((1*RS*,2*SR*)-2-(3-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone (3.21m). A solution of 1-(4-(5-chloro-2-methylphenyl)piperazine-1-yl)-3-(4-(pentafluorosulfanyl)phenyl)prop-2-yn-1-one (**3.17k**, 0.0930 g, 0.200 mmol) in EtOAc (2 mL) was treated with Lindlar catalyst (5% Pd on CaCO₃, lead poisoned, 0.0213 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of H_2 (3 vacuum/backfill cycles) and stirred at rt for 3 d, filtered through Celite, washed (EtOAc) and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 30% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes) to give **3.18k** (0.0470 g, 0.101 mmol) as a colorless solid.

A solution of $CrCl_2$ (0.0742 g, 0.604 mmol) and (*Z*)-1-(4-(5-chloro-2-methylphenyl)piperazine-1-yl)-3-(3-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-en-1-one (**3.18k**, 0.0470 g, 0.101 mmol) in dry degassed THF (1 mL) was treated with CH_2ICl (0.058 mL, 0.503 mmol) at rt, heated for 2 d at 80 °C. The reaction was cooled to rt, diluted with EtOAc (50 mL) and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 30% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes), filtered through basic Al_2O_3 (CH_2Cl_2/EtOAc, 1:1) concentrated and dried under high vacuum to give **3.21m** (0.0182 g, 0.0378 mmol, 19% (2 steps) (100% purity by ELSD)) as a colorless solid: Mp 112.9-116.1 °C; IR

(CH$_2$Cl$_2$) 2854, 1640, 1490, 1439, 1225, 1036, 841 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.62-

7.59 (m, 2 H), 7.38 (t, J = 8.1 Hz, 1 H), 7.29 (d, J = 7.8 Hz, 1 H), 7.06 (d, J = 8.5 Hz, 1 H), 6.95

(dd, J = 8.1, 2.1 Hz, 1 H), 6.70 (d, J = 2.1 Hz, 1 H), 3.84 (bd, J = 12.8 Hz, 1 H), 3.76-3.71 (m, 1

H), 3.61 (ddd, J = 12.8, 8.9, 3.2 Hz, 1 H), 3.29 (ddd, J = 12.8, 9.0, 3.1 Hz, 1 H), 2.80-2.71 (m, 2

H), 2.53 (td, J = 8.9, 6.9 Hz, 1 H), 2.31-2.23 (m, 2 H), 2.20 (s, 3 H), 2.15-2.09 (m, 1 H), 1.91 (q,

J = 6.2 Hz, 1 H), 1.43 (td, J = 8.4, 5.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.6, 153.9

(quint, J_{CF} = 16.8 Hz), 151.8, 139.0, 132.0, 131.8, 131.0, 130.2, 128.6, 125.9 (quint, J_{CF} = 4.5

Hz), 124.0 (quint, J_{CF} = 4.6 Hz), 123.7, 119.7, 51.8, 51.6, 45.6, 42.2, 24.1, 24.0, 17.3, 11.0; ^{19}F

NMR (376 MHz, CDCl$_3$) δ 84.7 (quint, J = 150.1 Hz, 1 F), 62.9 (d, J = 149.9 Hz, 4 F); HRMS

(ESI) m/z calcd for C$_{21}$H$_{23}$ClF$_5$N$_2$OS ([M+H]$^+$) 481.1134, found 481.1133.
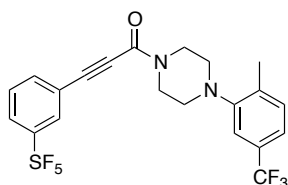
3.17l

1-(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazine-1-yl)-3-(3-(pentafluoro-λ6-

sulfaneyl)phenyl)prop-2-yn-1-one (3.17l). A solution of 3-(3-(pentafluoro-λ6-

sulfaneyl)phenyl)propiolic acid (**3.16d**, 0.100 g, 0.367 mmol) and 1-(2-methyl-5-

(trifluoromethyl)phenyl)piperazine hydrochloride (**3.12c**, 0.113 g, 0.404 mmol) in CH$_2$Cl$_2$ (3.7

mL) cooled to 0 °C was treated with Et$_3$N (0.20 mL, 1.47 mmol). The cooled solution was

treated with T3P (50 wt. % solution in EtOAc, 0.39 mL, 0.551 mmol) dropwise and the reaction

was stirred at 0 °C for 30 min, warmed to rt for 18 h, diluted with EtOAc (30 mL), washed with

H$_2$O (20 mL), satd. aqueous NaHCO$_3$ (20 mL), dried (MgSO$_4$), filtered, and concentrated under

reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g

column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes) to give **3.17l** (0.150

g, 0.300 mmol, 82%) as a tan foam: IR (CH$_2$Cl$_2$) 2919, 2825, 2219, 1629, 1418, 1309, 1152,

1119, 840, 797, 730 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.94 (t, J = 1.8 Hz, 1 H), 7.81 (dt, J =

8.2, 1.1 Hz, 1 H), 7.70 (d, J = 7.8 Hz, 1 H), 7.50 (t, J = 8.2 Hz, 1 H), 7.29 (q, J = 7.8 Hz, 2 H),

7.22 (s, 1 H), 4.00 (app t, J = 5.0 Hz, 2 H), 3.87 (app t, J = 5.0 Hz, 2 H), 3.04 (app t, J = 5.0 Hz,

2 H), 2.94 (app t, J = 5.0 Hz, 2 H), 2.39 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.8 (quint,

J_{CF} = 18.2 Hz), 152.5, 150.9, 136.8, 135.1, 131.6, 129.7 (quint, J_{CF} = 4.7 Hz), 129.1 (q, J_{CF} =

32.3 Hz), 129.1, 127.4 (quint, J_{CF} = 4.7 Hz), 124.1 (q, J_{CF} = 271.8 Hz), 121.5, 120.6 (q, J_{CF} = 3.9

Hz), 116.1 (q, J_{CF} = 3.6 Hz), 88.4, 82.3, 51.9, 51.3, 47.4, 42.0, 17.9; ^{19}F NMR (376 MHz,

CDCl$_3$) δ 82.9 (quint, J = 150.6 Hz, 1 F), 62.6 (d, J = 150.4 Hz, 4 F), -62.3 (s, 3 F); HRMS

(ESI) m/z calcd for C$_{21}$H$_{19}$F$_8$N$_2$OS ([M+H]$^+$) 499.1085, found 499.1086.
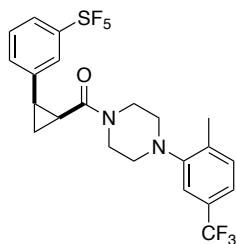
3.21n

(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazine-1-yl)((1*RS*,2*SR*)-2-(3-

(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone (3.21n). A solution of 1-(4-(2-

methyl-5-(trifluoromethyl)phenyl)piperazine-1-yl)-3-(3-(pentafluoro-λ6-sulfaneyl)phenyl)prop-

2-yn-1-one (**3.17l**, 0.100 g, 0.201 mmol) in EtOAc (2 mL) was treated with Lindlar catalyst (5%

Pd on CaCO$_3$, lead poisoned, 0.0214 g, equivalent to 5 mol% Pd). The reaction was placed under

a balloon of H$_2$ (3 vacuum/backfill cycles) and stirred at rt for 2 d, filtered through Celite,

washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The

crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load

CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes, product eluted at 30% EtOAc/hexanes) to give **3.18l** (0.0938 g, 0.187 mmol) as a colorless foam.

A solution of CrCl$_2$ (0.138 g, 1.12 mmol) and (*Z*)-1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazine-1-yl)-3-(3-(pentafluoro-λ6-sulfaneyl)phenyl)prop-2-en-1-one (**3.18l**, 0.0938 g, 0.187 mmol) in dry degassed THF (1.9 mL) was treated with CH$_2$ICl (0.11 mL, 0.937 mmol) at rt, sparged for 2 min, heated for 2 d at 80 °C, cooled to rt, diluted with EtOAc (50 mL), and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried (MgSO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes, product eluted at 30% EtOAc/hexanes), filtered through basic Al$_2$O$_3$ (CH$_2$Cl$_2$/EtOAc, 1:1), concentrated under reduced pressure, and dried under high vacuum to give **3.21n** (0.0641 g, 0.125 mmol, 62% (2 steps) (100% purity by ELSD)) as a pale yellow oil: IR (CH$_2$Cl$_2$) 2917, 1638, 1438, 1417, 1337, 1307, 1120, 835, 758 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.62-7.58 (m, 2 H), 7.38 (t, *J* = 7.8 Hz, 1 H), 7.30 (d, *J* = 7.8 Hz, 1 H), 7.24-7.21 (m, 2 H), 6.96 (s, 1 H), 3.93 (d, *J* = 13.0 Hz, 1 H), 3.79 (dt, *J* = 13.0, 3.8 Hz, 1 H), 3.60 (ddd, *J* = 12.6, 9.2, 3.1 Hz, 1 H), 3.25 (ddd, *J* = 12.6, 9.2, 3.0 Hz, 1 H), 2.79 (ddt, *J* = 12.7, 11.9, 3.8 Hz, 2 H), 2.53 (td, *J* = 8.9, 6.9 Hz, 1 H), 2.31-2.20 (m, 5 H), 2.16-2.10 (m, 1 H), 1.92 (q, *J* = 6.2 Hz, 1 H), 1.43 (td, *J* = 8.4, 5.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.5, 153.9 (quint, J_{CF} = 16.9 Hz), 151.0, 139.0, 136.8, 131.3, 130.3, 129.0 (q, J_{CF} = 33.1 Hz), 128.5, 125.7 (quint, J_{CF} = 4.6 Hz), 124.2 (q, J_{CF} = 272.0 Hz), 123.9 (quint, J_{CF} = 4.6 Hz), 120.3 (q, J_{CF} = 3.9 Hz), 115.9 (q, J_{CF} = 3.6 Hz), 51.7, 51.6, 45.5, 42.2, 24.0 (2 C), 24.0, 17.8, 10.9; ^{19}F NMR (376 MHz, CDCl$_3$) δ 84.5 (quint, *J* = 150.0 Hz, 1 F), 62.7 (d, *J* = 149.8 Hz, 4 F), -62.4 (s, 3 F); HRMS (ESI) *m/z* calcd for C$_{22}$H$_{23}$F$_8$N$_2$OS ([M+H]$^+$) 515.1398, found 515.1380.
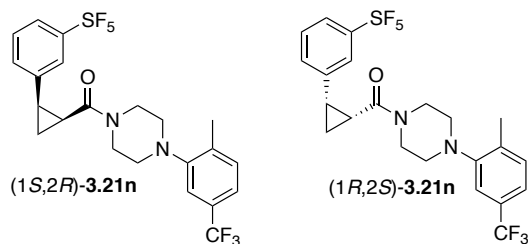
(1*S*,2*R*)-**3.21n**   (1*R*,2*S*)-**3.21n**

Racemic (4-(2-methyl-5-(trifluoromethyl)phenyl)piperazine-1-yl)((1*RS*,2*SR*)-2-(3-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone (**3.21n**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm) injection volume 90 μL, 20 mg/mL) to give (4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1*S*,2*R*)-2-(3-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone ((1*S*,2*R*)-**3.21n**) (retention time 3.25 min) as a colorless viscous oil (100% purity by ELSD): $[a]^{18}_D$ -104.7 (*c* 0.84, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.62-7.58 (m, 2 H), 7.38 (t, *J* = 7.7 Hz, 1 H), 7.31-7.24 (m, 3 H), 6.96 (s, 1 H), 3.94-3.89 (m, 1 H), 3.81-3.75 (m, 1 H), 3.65-3.56 (m, 1 H), 3.31-3.22 (m, 1 H), 2.84-2.74 (m, 2 H), 2.53 (td, *J* = 9.3, 6.9 Hz, 1 H), 2.32-2.21 (m, 5 H), 2.19-2.11 (m, 1 H), 1.93 (q, *J* = 5.7 Hz, 1 H), 1.43 (td, *J* = 8.4, 5.7 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO_2, 7 mL/min, p = 100 bar, 220 nm; retention time: 3.3 min).

(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1*R*,2*S*)-2-(3-(pentafluoro-λ6-sulfaneyl)phenyl)cyclopropyl)methanone ((1*R*,2*S*)-**3.21n**) (retention time 3.60 min) was obtained as a colorless viscous oil (100% purity by ELSD): $[a]^{18}_D$ +103.4 (*c* 0.87, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.54-7.81 (m, 2 H), 7.62-7.59 (m, 2 H), 7.38 (t, *J* = 7.8 Hz, 1 H), 7.31-7.24 (m, 3 H), 6.96 (s, 1 H), 3.94-3.88 (m, 1 H), 3.81-3.75 (m, 1 H), 3.65-3.56 (m, 1 H), 3.31-3.22 (m, 1 H), 2.84-2.74 (m, 2 H), 2.53 (td, *J* = 9.0, 7.2 Hz, 1 H), 2.32-2.22 (m, 5 H), 2.18-2.11 (m, 1 H), 1.93 (q, *J* = 5.7 Hz, 1 H), 1.43 (td, *J* = 8.4, 5.7 Hz, 1 H); The enantiomeric excess was >99.9% ee

(SFC Chiralpak-IC (250 x 10 mm); 30% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 3.6 min).
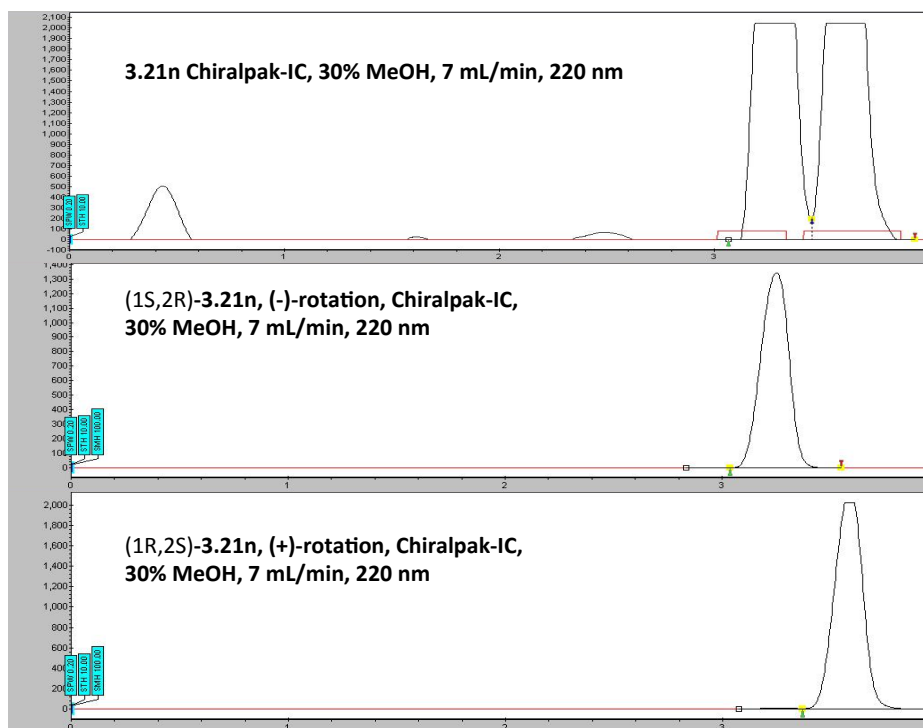
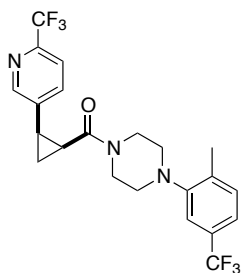**Figure 5.10.** SFC chromatograms for **3.21n** and separated enantiomers.
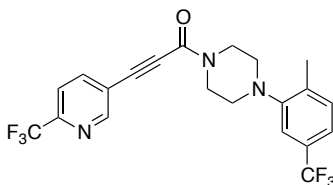
3.17m

1-(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(6-(trifluoromethyl)pyridin-3-yl)prop-2-yn-1-one (3.17m). A solution of methyl 3-(5-chloropyridin-2-yl)propionate (0.500 g, 2.18 mmol) in THF (4 mL) was treated with 2 M NaOH (1.2 mL, 2.40 mmol) and the mixture was stirred at rt for 3 h, acidified with 1 M aqueous HCl, and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried (MgSO$_4$),

filtered and concentrated under reduced pressure to give **3.16f** (0.422 g, 1.96 mmol) as a colorless solid. This solid was taken on to the coupling reaction with no further purification.

A solution of 3-(6-(trifluoromethyl)pyridin-3-yl)propionic acid (**3.16f**, 0.422 g, 1.96 mmol) and 1-(2-methyl-5-(trifluoromethyl)phenyl)piperazine hydrochloride (**3.12c**, 0.661 g, 2.35 mmol) in CH_2Cl_2 (19 mL) cooled to 0 °C was treated Et_3N (0.83 mL, 5.88 mmol). The cooled solution was treated with T3P (50% solution in EtOAc) (2.1 mL, 2.94 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 18 h, diluted with EtOAc (50 mL), washed with H_2O (20 mL), satd. aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude material was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:1) to give **3.17m** (0.781 g, 1.77 mmol, 81% (2 steps)) as a pale yellow solid: Mp 110.7-113.5 °C; IR (CH_2Cl_2) 3652, 2981, 2890, 1635, 1382, 1337, 1240, 1150, 1087, 956 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.85 (dd, J = 1.6, 0.6 Hz, 1 H), 8.04 (ddd, J = 8.2, 1.6, 0.6 Hz, 1 H), 7.72 (dd, J = 8.2, 0.6 Hz, 1 H), 7.32-7.26 (m, 2 H), 7.21 (s, 1 H), 3.99 (t, J = 5.0 Hz, 2 H), 3.87 (t, J = 5.0 Hz, 2 H), 3.04 (t, J = 5.0 Hz, 2 H), 2.95 (t, J = 5.0 Hz, 2 H), 2.39 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 152.5, 152.0, 150.8, 148.2 (q, J_{CF} = 35.3 Hz), 140.7, 136.8, 131.6, 129.1 (q, J_{CF} = 32.3 Hz), 124.1 (q, J_{CF} = 272.1 Hz), 121.0 (q, J_{CF} = 274.4 Hz), 120.7, 120.6 (q, J_{CF} = 3.9 Hz), 120.0 (q, J_{CF} = 2.8 Hz), 116.1 (q, J_{CF} = 3.6 Hz), 85.6, 51.8, 51.3, 47.4, 42.0, 17.9; ^{19}F NMR (376 MHz, CDCl$_3$) δ -62.3 (s, 3 F). -68.2 (s, 3 F); HRMS (ESI) *m/z* calcd for $C_{21}H_{18}F_6N_3O$ ([M+H]$^+$) 442.1349, found 442.1345.

3.21o

(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-(6-(trifluoromethyl)pyridin-3-yl)cyclopropyl)methanone (3.21o). A solution of 1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(6-(trifluoromethyl)pyridin-3-yl)prop-2-yn-1-one (**3.17m**, 0.720 g, 1.63 mmol) in EtOAc (16 mL) at rt was treated with quinoline (1.0 mL, 8.16 mmol) and 5% Pd/BaSO$_4$ (0.0347 g, 0.0163 mmol, equivalent to 1 mol% Pd) and the reaction was stirred under an atmosphere of H$_2$ (3 x backfill cycles) for 4 h. Analysis by TLC (hexanes/EtOAc, 1:1) indicated that the starting material had been mostly consumed. The reaction was filtered (eluting with EtOAc), and the filtrate was washed with 1 M aqueous HCl (3 x 30 mL), dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1 to EtOAc) to give **3.18m** (0.640 g, 1.44 mmol) as a yellow/orange oil.

A solution of CrCl$_2$ (1.06 g, 8.66 mmol) and (Z)-1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(6-(trifluoromethyl)pyridin-3-yl)prop-2-en-1-one (**3.18m**, 0.640 g, 1.44 mmol) in dry degassed THF (14 mL) was sparged with Ar for 5 min and treated with CH$_2$ICl (0.83 mL, 7.22 mmol) at rt and under Ar atmosphere. The reaction mixture was stirred for 2 d at 80 °C, cooled to rt, diluted with EtOAc (50 mL), and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried (MgSO$_4$), filtered and concentrated under reduced pressure. The crude material was purified by chromatography on SiO$_2$ (4:1, EtOAc:hexanes), filtered through basic Al$_2$O$_3$ (eluting with EtOAc) concentrated under reduced pressure, and dried under high vacuum to give **3.21o** (0.280 g, 0.612 mmol, 37% (2 steps) (100% purity by ELSD)) as an orange oil: IR (CH$_2$Cl$_2$) 2981, 1640, 1418, 1339, 1309, 1166, 1126, 1035, 828 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.64 (s, 1 H), 7.60 (t, *J* = 2.0 Hz, 2 H), 7.24 (t, *J* = 2.0

Hz, 2 H), 7.02 (s, 1 H), 3.78-3.65 (m, 3 H), 3.53-3.46 (m, 1 H), 2.92-2.87 (m, 1 H), 2.80-2.75 (m, 1 H), 2.59-2.53 (m, 1 H), 2.47-2.35 (m, 4 H), 2.30 (s, 3 H), 1.95 (q, J = 5.6 Hz, 1 H), 1.49 (td, J = 8.4, 5.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.4, 150.8, 150.5, 146.3 (q, J_{CF} = 34.8 Hz), 136.9, 136.7, 135.5, 131.4, 129.1 (q, J_{CF} = 32.2 Hz), 124.1 (q, J_{CF} = 272.1 Hz), 121.6 (q, J_{CF} = 273.7 Hz), 120.4 (q, J_{CF} = 3.9 Hz), 119.8 (q, J_{CF} = 2.6 Hz), 115.9 (q, J_{CF} = 3.7 Hz), 52.0, 51.5, 45.6, 42.3, 23.7, 21.5, 17.9, 11.0; ^{19}F NMR (376 MHz, CDCl$_3$) δ -62.4 (s, 3 F), -67.8 (s, 3 F); HRMS (ESI) m/z calcd for C$_{22}$H$_{22}$F$_6$N$_3$O ([M+H]$^+$) 458.1662, found 458.1660.

(1S,2R)-**3.21o**   (1R,2S)-**3.21o**

Racemic   (4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1RS,2SR)-2-(6-(trifluoromethyl)pyridin-3-yl)cyclopropyl)methanone (**3.21o**) was separated on a SFC Chiralpak-IC semiprep (250 x 10 mm) column (15% Methanol/CO$_2$, 7 mL/min, 220nm, p = 100 bar, 20 mg/mL in MeOH) to give (4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1S,2R)-2-(6-(trifluoromethyl)pyridin-3-yl)cyclopropyl)methanone ((1S,2R)-**3.21o**) (retention time 7.65 min) as a colorless solid (100% purity by ELSD): [a]^{19}$_D$ -134.8 (*c* 0.90, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.64 (s, 1 H), 7.60 (s, 2 H), 7.26 (s, 4 H), 7.04 (s, 1 H), 3.75-3.64 (m, 3 H), 3.55-3.47 (m, 1 H), 2.94-2.87 (m, 1 H), 2.82-2.74 (m, 1 H), 2.56 (q, J = 8.4 Hz, 1 H), 2.49-2.34 (m, 3 H), 2.31 (s, 3 H), 1.96 (q, J = 5.8 Hz, 1 H), 1.50 (td, J = 8.4, 5.8 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 15% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 7.8 min).
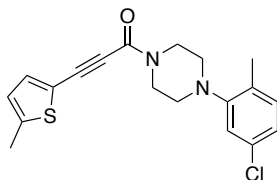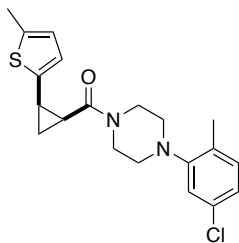
(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1R,2S)-2-(6-

(trifluoromethyl)pyridin-3-yl)cyclopropyl)methanone ((1*R*,2*S*)-**3.21o**) (retention time 8.38 min) was obtained as a colorless solid (100% purity by ELSD): $[a]^{19}_D$ +128.2 (*c* 1.08, MeOH); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.64 (s, 1 H), 7.60 (s, 2 H), 7.26 (s, 2 H), 7.04 (s, 1 H), 3.77-3.63 (m, 3 H), 3.55-3.47 (m, 1 H), 2.93-2.87 (m, 1 H), 2.81-2.75 (m, 1 H), 2.61-2.52 (m, 1 H), 2.50-2.35 (m, 2 H), 2.31 (s, 3 H), 1.96 (q, *J* = 5.8 Hz, 1 H), 1.50 (td, *J* = 8.4, 5.8 Hz, 1 H). The enantiomeric excess was >99.9% ee (SFC Chiralpak-IC (250 x 10 mm); 15% Methanol:CO$_2$, 7 mL/min, p = 100 bar, 220 nm; retention time: 8.4 min).
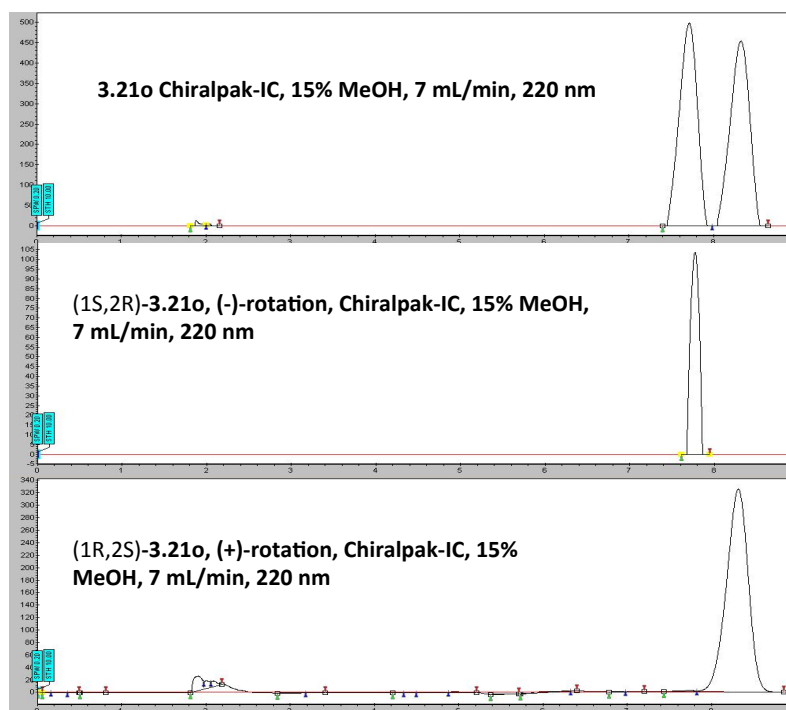
Figure 5.11. SFC chromatograms for **3.21o** and separated enantiomers.

3.17n

1-(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)-3-(5-methylthiophen-2-yl)prop-2-yn-1-one (3.17n). A solution of 3-(5-methylthiophen-2-yl)propiolic acid (**3.16e**, 0.0750 g, 0.451 mmol) and 1-(5-chloro-2-methylphenyl)piperazine hydrochloride (**3.12b**, 0.134 g, 0.542 mmol) in CH_2Cl_2 (4.5 mL) cooled to 0 °C was treated with Et_3N (0.19 mL, 1.35 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.48 mL, 0.677 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 16 h, diluted with CH_2Cl_2 (30 mL), washed with 1 M aqueous HCl (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 40% EtOAc/hexanes), to give **3.17n** (0.0620 g, 0.173 mmol, 38%) as a colorless solid: Mp 114.4-116.0 °C; IR (CH_2Cl_2) 2919, 2197, 1643, 1593, 1489, 1426, 1224, 1025, 806 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 3.6 Hz, 1 H), 7.10 (d, J = 8.1 Hz, 1 H), 6.98 (dd, J = 8.1, 1.9 Hz, 1 H), 6.94 (d, J = 1.9 Hz, 1 H), 6.69 (dd, J = 3.6, 0.8 Hz, 1 H), 3.91 (app t, J = 5.0 Hz, 2 H), 3.81 (app t, J = 5.0 Hz, 2 H), 2.94 (app t, J = 5.0 Hz, 2 H), 2.86 (app t, J = 5.0 Hz, 2 H), 2.49 (s, 3 H), 2.28 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.1, 151.7, 145.5, 135.7, 132.0, 131.8, 130.9, 125.8, 123.6, 119.7, 117.4, 85.4, 84.5, 51.8, 51.3, 47.2, 41.8, 17.4, 15.5; HRMS (ESI) m/z calcd for $C_{19}H_{20}ClN_2OS$ ($[M+H]^+$) 359.0979, found 359.0978.

3.21p

(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)((1RS,2SR)-2-(5-methylthiophen-2-yl)cyclopropyl)methanone (3.21p). A solution of 1-(4-(5-chloro-2-methylphenyl)piperazin-1-

yl)-3-(5-methylthiophen-2-yl)prop-2-yn-1-one (**3.17n**, 0.0620 g, 0.173 mmol) in EtOAc (1.7 mL) was treated with Lindlar catalyst (5% Pd on $CaCO_3$, lead poisoned, 0.00735 g, equivalent to 2 mol% Pd) and quinoline (10 μL, 0.172 mmol). The reaction was placed under a balloon of H_2 (4 vacuum/backfill cycles) and stirred at rt for 6 h, filtered through Celite, washed (EtOAc), and the combined filtrates were washed with 1 M aqueous HCl, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 10% EtOAc/hexanes to 40% EtOAc/hexanes) to give **3.18n** (0.0263 g, 0.0729 mmol) as a colorless solid.

A solution of $CrCl_2$ (0.0511 g, 0.416 mmol) and (*Z*)-1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one (**3.18n**, 0.0250 g, 0.0693 mmol) in dry degassed THF (0.7 mL) was sparged with Ar for 5 min and treated with CH_2ICl (40 uL, 0.346 mmol) at rt, heated for 20 h at 80 °C, cooled to rt, combined, diluted with Et_2O (50 mL), and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 40% EtOAc/hexanes), filtered through basic Al_2O_3 (CH_2Cl_2/EtOAc, 1:1), concentrated under reduced pressure and dried under high vacuum to give **3.21p** (0.0154 g, 0.0411 mmol, 25% (2 steps) (100% purity by ELSD)) as a pale yellow oil: IR (CH_2Cl_2) 2918, 1643, 1593, 1490, 1462, 1436, 1226, 1029, 802 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (dd, *J* = 8.1, 0.4 Hz, 1 H), 6.97 (dd, *J* = 8.1, 2.1 Hz, 1 H), 6.79 (d, *J* = 2.1 Hz, 1 H), 6.57-6.55 (m, 2 H), 3.96 (d, *J* = 13.0 Hz, 1 H), 3.85 (dt, *J* = 13.0, 3.5 Hz, 1 H), 3.63 (ddd, *J* = 13.0, 8.9, 3.5 Hz, 1 H), 3.34 (ddd, *J* = 13.0, 9.0, 3.2 Hz, 1 H), 2.79 (ddt, *J* = 19.3, 11.2, 4.1 Hz, 2 H), 2.52 (td, *J* = 8.9, 6.8 Hz, 1 H), 2.45-2.36 (m, 5 H), 2.24 (s, 3 H), 2.16 (ddd, *J* = 8.9, 8.2, 6.3 Hz, 1 H), 1.77 (td, *J* = 6.3, 5.6 Hz, 1 H), 1.38

(ddd, J = 8.9, 8.2, 5.6 Hz, 1 H).^{13}C NMR (100 MHz, CDCl$_3$) δ 167.2, 152.1, 138.2, 137.5, 131.9, 131.7, 131.1, 125.0, 124.1, 123.5, 119.7, 51.8, 51.7, 45.7, 42.4, 24.1, 19.3, 17.3, 15.3, 12.2; HRMS (ESI) m/z calcd for C$_{19}$H$_{24}$ClN$_2$OS ([M+H]$^+$) 375.1292, found 375.1292.
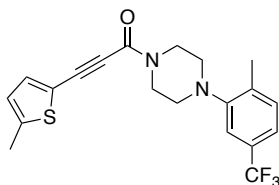
3.17o

1-(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(5-methylthiophen-2-yl)prop-2-yn-1-one (**3.17o**). A solution of 3-(5-methylthiophen-2-yl)propiolic acid (**3.15e**, 0.0592 g, 0.356 mmol) and 1-(2-methyl-5-(trifluoromethyl)phenyl)piperazine hydrochloride (**3.12c**, 0.100 g, 0.356 mmol) in CH$_2$Cl$_2$ (3.6 mL) cooled to 0 °C was treated with Et$_3$N (0.20 mL, 1.42 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.38 mL, 0.534 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 18h, diluted with CH$_2$Cl$_2$ (30 mL), washed with 1 M aqueous HCl (20 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes), to give **3.17o** (0.0685, 0.175 mmol, 49%) as a yellow solid. Mp 145.7-149.3 °C; IR (CH$_2$Cl$_2$) 2919, 2199, 1635, 1420, 1340, 1309, 1120, 1029, 955, 732 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.31-7.24 (m, 3 H), 7.21 (s, 1 H), 6.69 (dd, J = 3.6, 0.8 Hz, 1 H), 3.94 (app t, J = 5.0 Hz, 2 H), 3.83 (app t, J = 5.0 Hz, 2 H), 3.01 (app t, J = 5.0 Hz, 2 H), 2.91 (app t, J = 5.0 Hz, 2 H), 2.49 (d, J = 0.8 Hz, 3 H), 2.38 (s, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.2, 151.0, 145.5, 136.8, 135.7, 131.5, 129.0 (q, J_{CF} = 32.2 Hz), 125.9, 124.1 (q, J_{CF} = 272.1 Hz), 120.4 (q, J_{CF} = 3.8 Hz), 117.4, 116.0 (q, J_{CF} = 3.6 Hz), 85.4, 84.5, 51.8, 51.3, 47.3, 41.8, 17.9, 15.5; ^{19}F NMR

(376 MHz, CDCl$_3$) δ -62.2 (s, 3 F); HRMS (ESI) *m/z* calcd for C$_{20}$H$_{20}$F$_3$N$_2$OS ([M+H]$^+$) 393.1243, found 393.1241.
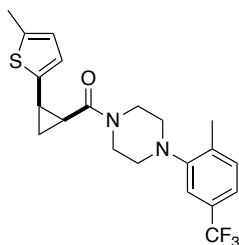
3.21q

(4-(2-Methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)((1R*S*,2S*R*)-2-(5-methylthiophen-2-yl)cyclopropyl)methanone (3.21q). A solution of 1-(4-(2-methyl-5-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(5-methylthiophen-2-yl)prop-2-yn-1-one (**3.17o**, 0.0650 g, 0.166 mmol) in EtOAc (1.7 mL) was treated with Lindlar catalyst (5% Pd on CaCO$_3$, lead poisoned, 0.0176 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of H$_2$ (3 vacuum/backfill cycles) and stirred at rt for 3 d, filtered through Celite, washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes) to give **3.18o** (0.0443 g, 0.112 mmol) as a colorless solid.

A solution of CrCl$_2$ (0.105 g, 0.851 mmol) and (Z)-1-(4-(5-chloro-2-methylphenyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3.18o**, 0.0420 g, 0.142 mmol) in dry degassed THF (1.1 mL) was sparged with Ar for 5 min and treated with CH$_2$ICl (62uL, 0.532 mmol) at rt, heated for 22 h at 80 °C, cooled to rt, diluted with Et$_2$O (50 mL), and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried (MgSO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, 100% hexanes to 40%

EtOAc/hexanes), filtered through basic Al$_2$O$_3$ (CH$_2$Cl$_2$/EtOAc, 1:1), concentrated under reduced pressure, and dried under high vacuum to **3.21q** (0.0187 g, 0.0458 mmol, 29% (2 steps) (100% purity by ELSD)) as pale yellow viscous oil: IR (CH$_2$Cl$_2$) 2921, 2822, 1639, 1464, 1434, 1417, 1337, 1306, 1116, 1079, 1031, 801 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.26 (d, J = 8.4 Hz, 1 H), 7.24 (dd, J = 8.4, 1.4 Hz, 1 H), 7.04 (s, 1 H), 6.58 (t, J = 4.7 Hz, 1 H), 6.55 (dt, J = 3.5, 1.0 Hz, 1 H), 4.00 (bd, J = 13.0 Hz, 1 H), 3.88 (dt, J = 13.0, 3.5 Hz, 1 H), 3.64 (ddd, J = 12.4, 9.0, 3.2 Hz, 1 H), 3.34 (ddd, J = 13.0, 9.2, 2.9 Hz, 1 H), 2.82 (ddt, J = 19.4, 11.6, 3.7 Hz, 2 H), 2.53 (td, J = 8.6, 6.6 Hz, 1 H), 2.48-2.40 (m, 1 H), 2.38 (d, J = 1.0 Hz, 3 H), 2.34 (s, 3 H), 2.17 (td, J = 8.6, 6.6 Hz, 1 H), 1.77 (q, J = 6.0 Hz, 1 H), 1.38 (td, J = 8.6, 5.3 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.2, 151.4, 138.3, 137.6, 136.9, 131.4, 128.9 (q, J_{CF} = 32.0 Hz), 124.9, 124.3 (q, J_{CF} = 272.1 Hz), 124.2, 120.3 (q, J_{CF} = 4.0 Hz), 116.0 (q, J_{CF} = 3.7 Hz), 51.8, 51.7, 45.7, 42.3, 24.1, 19.4 , 17.9, 15.1, 12.3; ^{19}F NMR (376 MHz, CDCl$_3$) δ -62.2 (s, 3 F); HRMS (ESI) m/z calcd for C$_{21}$H$_{24}$F$_3$N$_2$OS ([M+H]$^+$) 409.1556, found 409.1555.
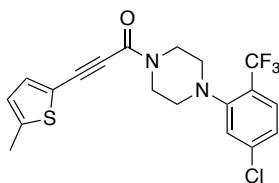
3.17p

1-(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(5-methylthiophen-2-yl)prop-2-yn-1-one (3.17p). A solution of 3-(5-methylthiophen-2-yl)propiolic acid (**3.16e**, 0.0770 g, 0.463 mmol) and 1-(5-chloro-2-(trifluoromethyl)phenyl)piperazine hydrochloride (**3.12d**, 0.153 g, 0.510 mmol) in CH$_2$Cl$_2$ (4.6 mL) cooled to 0 °C was treated with Et$_3$N (0.26 mL, 1.85 mmol). The cooled solution was treated with T3P (50 wt. % solution in EtOAc, 0.49 mL, 0.695 mmol) dropwise and the reaction was stirred at 0 °C for 30 min, warmed to rt for 18

h, diluted with CH_2Cl_2 (30 mL), washed with H_2O (20 mL), satd. aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, hexanes to 40% EtOAc/hexanes), to give **3.17p** (0.135 g, 0.326 mmol, 70%) as a light orange waxy solid: IR (CH_2Cl_2) 2918, 2200, 1629, 1596, 1425, 1309, 1224, 1144, 1120, 1027, 809 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.4 Hz, 1 H), 7.27-7.23 (m, 3 H), 6.69 (dd, J = 3.6, 0.8 Hz, 1 H), 3.91 (app t, J = 4.9 Hz, 2 H), 3.81 (app t, J = 5.0 Hz, 2 H), 2.98 (app t, J = 5.0 Hz, 2 H), 2.91 (app t, J = 5.0 Hz, 2 H), 2.49 (d, J = 0.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 152.7, 152.6, 145.5, 138.8, 135.7, 128.5 (q, J_{CF} = 5.4 Hz), 125.9, 125.8 (q, J_{CF} = 29.3 Hz), 125.6, 124.7, 123.6 (q, J_{CF} = 273.0 Hz), 117.4, 85.4, 84.5, 53.6, 52.8, 47.3, 41.7, 15.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -60.3 (s, 3 F); HRMS (ESI) m/z calcd for $C_{19}H_{17}ClF_3N_2OS$ ([M+H]$^+$) 413.0697, found 413.0693.
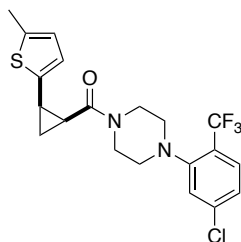
3.21r

(4-(5-Chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)((1_RS_,2_SR_)-2-(5-methylthiophen-2-yl)cyclopropyl)methanone (3.21r). A solution of 1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(5-methylthiophen-2-yl)prop-2-yn-1-one (**3.17p**, 0.112 g, 0.271 mmol) in EtOAc (2.7 mL) was treated with Lindlar catalyst (5% Pd on $CaCO_3$, lead poisoned, 0.0289 g, equivalent to 5 mol% Pd). The reaction was placed under a balloon of H_2 (3 vacuum/backfill cycles) and stirred at rt for 3 d, filtered through Celite, washed (EtOAc), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by

automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, hexanes to 40% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes) to give **3.18p** (0.0655 g, 0.158 mmol) as a colorless foam.

A solution of $CrCl_2$ (0.116 g, 0.947 mmol) and (Z)-1-(4-(5-chloro-2-(trifluoromethyl)phenyl)piperazin-1-yl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one (**3.18p**, 0.0655 g, 0.158 mmol) in dry degassed THF (1.6 mL) was sparged with Ar for 5 min and treated with CH_2ICl (0.091 mL, 0.789 mmol) at rt, heated for 2 days at 80 °C, cooled to rt, diluted with EtOAc (50 mL), and washed with 1 M aqueous HCl (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 30% EtOAc/hexanes, product eluted at 20% EtOAc/hexanes), filtered through basic Al_2O_3 (CH_2Cl_2/EtOAc, 1:1), and concentrated under reduced pressure. The resulting oil was crystalized from cyclohexane (~0.5 mL at rt), the crystals were washed with hexanes and dried under high vacuum to give **3.21r** (0.0313 g, 0.0730 mmol, 27% (2 steps) (100% purity by ELSD)) as a colorless solid: Mp 84.5-86.8 °C; IR (CH_2Cl_2) 3007, 2920, 1642, 1595, 1464, 1435, 1405, 1308, 1144, 1120, 1031, 825, 803 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 8.5 Hz, 1 H), 7.21 (dd, J = 8.5, 1.5 Hz, 1 H), 7.03 (d, J = 1.5 Hz, 1 H), 6.61 (dd, J = 3.4, 1.0 Hz, 1 H), 6.59 (d, J = 3.4 Hz, 1 H), 4.08 (d, J = 13.0 Hz, 1 H), 3.87 (d, J = 13.0 Hz, 1 H), 3.57 (ddd, J = 13.0, 9.4, 3.0 Hz, 1 H), 3.23 (ddd, J = 13.0, 9.6, 3.0 Hz, 1 H), 2.78 (tt, J = 9.6, 4.7 Hz, 2 H), 2.52 (td, J = 8.8, 6.8 Hz, 1 H), 2.46-2.40 (m, 4 H), 2.31-2.25 (m, 1 H), 2.16 (ddd, J = 8.8, 8.2, 6.0 Hz, 1 H), 1.77 (q, J = 6.0 Hz, 1 H), 1.39 (td, J = 8.6, 6.0 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 153.2, 138.6, 138.5, 137.4, 128.4 (q, J_{CF} = 5.4 Hz), 126.0 (q, J_{CF} = 28.2 Hz), 125.5, 125.1, 124.8, 124.0, 123.6 (q, J_{CF} = 273.0 Hz), 53.6, 53.2, 45.7, 42.4, 24.4, 19.4, 15.3, 12.4; ^{19}F NMR (376 MHz,

CDCl$_3$) δ -60.4 (s, 3 F); HRMS (ESI) *m/z* calcd for C$_{20}$H$_{21}$ClF$_3$N$_2$OS ([M+H]$^+$) 429.1010, found 429.1006.
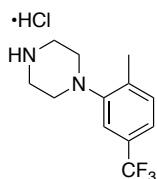
3.12c

1-(2-Methyl-5-(trifluoromethyl)phenyl)piperazine hydrochloride (3.12c). A solution of Boc-piperazine (2.14 g, 11.5 mmol), KOtBu (2.35 g, 20.9 mmol), (racemic)-BINAP (0.671 g, 1.05 mmol), Pd$_2$(dba)$_3$ (0.194 g, 0.209 mmol) in dry toluene (105 mL) was sparged with Ar for 20 min, treated with 2-bromo-1-methyl-4-(trifluoromethyl)benzene (2.50 g, 10.5 mmol), and the mixture was heated under Ar at 100 °C for 24 h, cooled to rt, diluted with Et$_2$O (125 mL), filtered through Celite, washed (Et$_2$O), and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give tert-butyl 4-(2-methyl-5-(trifluoromethyl)phenyl)piperazine-1-carboxylate (2.49 g, 7.23 mmol) as a orange oil.

A solution of tert-butyl 4-(2-methyl-5-(trifluoromethyl)phenyl)piperazine-1-carboxylate (2.49 g, 7.23 mmol) in THF (3.5 mL) was cooled to 0 °C and treated with 4M HCl in dioxane (9.0 mL, 36.2 mmol) was added and the reaction was stirred at 0 °C for 30 min and then rt for 4 h. The solution was concentrated under reduced pressure and the tan solid was precipitated in Et$_2$O, filtered off from the solution, washed with Et$_2$O, dried under vacuum to give the crude product. The crude material was recrystallized from EtOH/hexanes (1:1) to give **3.12c** (1.71 g, 6.10 mmol, 58% (2 steps)) as colorless needles: Mp 296 °C (decomp); IR (CH$_2$Cl$_2$) 2939, 2799, 2498, 1610, 1418, 1338, 1307, 1153, 1076, 950, 827 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.44 (bs, 2 H), 7.42 (d, *J* = 7.9 Hz, 1 H), 7.35 (dd, *J* = 7.9, 1.0 Hz, 1 H), 7.25 (d, *J* = 1.0 Hz, 1 H),

3.22 (dd, J = 6.2, 3.6 Hz, 4 H), 3.12 (dd, J = 6.2, 3.6 Hz, 4 H), 2.33 (s, 3 H); ^{13}C NMR (100

MHz, DMSO-d$_6$) δ 150.8, 137.2, 131.9, 127.5 (q, J_{CF} = 31.6 Hz), 124.2 (q, J_{CF} = 272.1 Hz),

120.1 (q, J_{CF} = 3.9 Hz), 115.3 (q, J_{CF} = 3.8 Hz), 47.9, 43.1, 17.7; ^{19}F NMR (376 MHz, CDCl$_3$) δ

-60.7 (s, 3 F); HRMS (ESI) m/z calcd for C$_{12}$H$_{16}$F$_3$N$_2$ ([M+H]$^+$) 245.1260, found 245.1261.
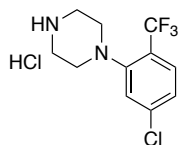
3.12d

1-(5-Chloro-2-(trifluoromethyl)phenyl)piperazine hydrochloride (3.12d). Two flasks

each containing a solution Boc-piperazine (1.58 g, 8.48 mmol), KOtBu (1.73 g, 15.4 mmol),

(racemic)-BINAP (0.480 g, 0.771 mmol), Pd2dba3 (0.142 g, 0.154 mmol) in toluene (8 mL) was

sparged with argon for 15 min and treated with 2-bromo-4-chloro-1-(trifluoromethyl)benzene

(2.00 g, 7.71 mmol), heated under N$_2$ at 80 °C for 24 h, cooled to rt, combined, diluted with Et$_2$O

(100 mL) and added Celite and filtered through Celite, washed (Et$_2$O), and the combined organic

layers were concentrated under reduced pressure. The crude residue was purified by

chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give 4-(5-chloro-2-

(trifluoromethyl)phenyl)piperazine-1-carboxylate (3.12 g, 8.55 mmol) as a orange oil.

A solution of tert-butyl 4-(5-chloro-2-(trifluoromethyl)phenyl)piperazine-1-carboxylate

(3.12 g, 8.55 mmol) in THF (8 mL) was treated with 4M HCl in dioxane (10.7 mL, 42.8 mmol),

stirred at rt for 18 h, diluted with hexanes (100 mL), and the resulting precipitate was filtered and

washed with additional hexanes and Et$_2$O. The crude material was recrystallized at rt

(EtOH/hexanes), crystals were collected by vacuum filtration, washed (hexanes) and dried under

high vacuum to give **3.12d** (1.88 g, 6.24 mmol, 41% (2 steps)) as tan solid: Mp 272 °C

(decomp); IR (neat) 2947, 2726, 2480, 1599, 1576, 1307, 1118, 1037, 946, 819 cm^{-1}; ^{1}H NMR

(400 MHz, DMSO-d_6) δ 9.58 (s, 2 H), 7.72 (d, J = 8.8 Hz, 1 H), 7.57 (d, J = 1.6 Hz, 1 H), 7.46 (dd, J = 8.8, 1.6 Hz, 1 H), 3.13 (bs, 8 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.2, 138.3, 129.0 (q, J = 5.4 Hz), 125.9, 124.7, 124.2 (q, J = 29.7 Hz), 123.7 (q, J = 273.0 Hz), 49.6, 43.1; ^{19}F NMR (376 MHz, CDCl$_3$) δ -59.0 (s, 3 F); HRMS (ESI) m/z calcd for C$_{11}$H$_{13}$N$_2$ClF$_3$ ([M+H]$^+$) 265.0714, found 265.0712.
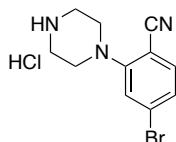
3.12e

4-Bromo-2-(piperazin-1-yl)benzonitrile hydrochloride (3.12e). A suspension of 4-bromo-2-fluorobenzonitrile (2.00 g, 10.0 mmol), 1-boc-piperazine (1.86 g, 10.0 mmol), and Et$_3$N (1.4 mL, 10.0 mmol) in anhydrous MeCN (5.0 mL) was heated to 110 °C for 21 h, cooled to rt, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give tert-butyl 4-(5-bromo-2-cyanophenyl)piperazine-1-carboxylate (3.03 g, 8.27 mmol) as a yellow oil.

A solution of tert-butyl 4-(5-bromo-2-cyanophenyl)piperazine-1-carboxylate (3.03 g, 8.27 mmol) in THF (4 mL) was cooled to 0 °C and treated with 4M HCl in dioxane (10.3 mL, 41.4 mmol), stirred at 0 °C for 30 min, rt for 16 h, diluted with hexanes (200 mL), and the resulting precipitate was collected by vacuum filtration, washed with hexanes, Et$_2$O, and dried under high vacuum to give **3.12e** (3.82 g, 13.6 mmol, 79% (2 steps)) as a colorless solid: Mp 288 °C (decomp); IR (neat) 2901, 2748, 1459, 1129, 1581, 1411, 1241, 1118, 949, 874 cm^{-1}; ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.59 (d, J = 8.0 Hz, 1 H), 7.44 (d, J = 1.6 Hz, 1 H), 7.37 (dd, J = 8.0, 1.6 Hz, 1 H), 3.49 (td, J = 4.0, 1.2 Hz, 4 H), 3.44 (td, J = 4.0, 1.2 Hz, 4 H); ^{13}C NMR (100

MHz, MeOD-d$_4$) δ 156.5, 136.4, 129.9, 127.8, 124.2, 118.2, 106.7, 49.6, 44.9; HRMS (ESI) *m/z* calcd for C$_{11}$H$_{13}$N$_3$Br ([M+H]$^+$) 266.0287, found 266.0286.

5.4.2 Synthesis of Analogs for Affinity Labeling
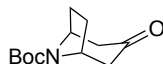
3.30

(*tert*-Butyl 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.30**).[349] A solution of nortropinone•HCl (**3.29**, 2.00 g, 12.4 mmol) in a minimum amount of H$_2$O (6.0 mL) was cooled to 0 °C, treated dropwise with 1 M NaOH (14.8 mL, 14.8 mmol), warmed to rt over 20 min, extracted with CH$_2$Cl$_2$ (3 x 40 mL), dried (MgSO$_4$), filtered, and concentrated under reduced pressure (bath at 23 °C) to give nortropinone **3.29** as the free base (1.54 g, quant.). The colorless oil was used without further purification.

A solution of nortropinone (**3.29**, 1.54 g, 12.3 mmol) in CH$_2$Cl$_2$ (50 mL) cooled to 0 °C was treated with Boc anhydride (4.26 mL, 18.6 mmol), DMAP (0.302 g, 2.47 mmol), and Et$_3$N (7.0 mL, 50.2 mmol). The reaction mixture was allowed to warm to rt and stirred for 19 h, concentrated under reduced pressure, and the residue was diluted with H$_2$O, extracted with EtOAc (3x), washed with brine, dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (CH$_2$Cl$_2$) to give **21a** (2.18 g, 9.68 mmol, 78% (2 steps)) as a pale yellow oil that solidified to an off-white solid upon standing at rt: ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 4.34-4.30 (m, 2 H), 2.55 (dt, *J* = 15.6, 4.2 Hz, 2 H), 2.23 (d, *J* = 15.6 Hz, 2 H), 2.20 (app s, 1 H), 2.03-1.94 (m, 2 H), 1.60-1.52 (m, 2 H), 1.44 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 207.4, 152.6, 79.2, 52.7, 48.1, 28.0 (2 C).
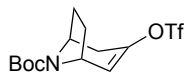
3.31

***tert*-Butyl 3-(((trifluoromethyl)sulfonyl)oxy)-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (3.31).**[349] A solution of NaHMDS (0.895 g, 4.88 mmol) in THF (12 mL) was added dropwise (over 10 min) at -78 °C to a solution of *tert*-butyl 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.30**, 1.00 g, 4.44 mmol) in THF (12 mL). The reaction mixture was stirred at -78 °C for 2 h, treated dropwise (over 20 min) with a solution of PhN(Tf)$_2$ (1.90 g, 5.33 mmol) in THF (12 mL), stirred for an additional 30 min at -78 °C and then allowed to warm to rt and stirred for 2 h, diluted with 10% aqueous Na$_2$CO$_3$ (50 mL), and extracted with Et$_2$O (2 x 75 mL). The combined organic layers were washed with 10% aqueous Na$_2$CO$_3$ solution, dried (MgSO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (1:19, EtOAc/hexanes w/ 1% Et$_3$N) to give **3.31** (1.24 g, 3.47 mmol, 78%) as a clear oil that solidified to a wax upon storage at -20 °C: ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.09 (bs, 1 H), 4.54-4.38 (m, 2 H), 3.07-3.02 (m, 1 H), 2.30-2.20 (m, 1 H), 2.11-1.99 (m, 3 H), 2.00-1.97 (m, 2 H), 1.79-1.70 (m, 1 H), 1.45 (s, 9 H).
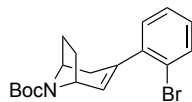
3.28

(1*SR*,5*RS*)-*tert*-Butyl 3-(2-bromophenyl)-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (3.28). A solution of Na$_2$CO$_3$ (0.860 g, 8.11 mmol), lithium chloride (0.156 g, 3.69 mmol), (1SR,5RS)-tert-butyl 3-(((trifluoromethyl)sulfonyl)oxy)-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (**3.31**, 1.20 g, 3.69 mmol), and 2-bromobenzeneboronic acid (**3.32**, 0.794 g, 3.87 mmol) in DME (36 mL) and H$_2$O (7 mL) was sparged with Ar for 20 min and treated with

Pd(PPh$_3$)$_4$ (0.128 g, 0.111 mmol), sparged with Ar for 2 min, heated to 60 °C for 6 h, cooled to rt, diluted with brine, and extracted with Et$_2$O (3x). The combined organic layers were dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 9:1 w/ 1% Et$_3$N) to give **3.28** (1.08 g, 2.97 mmol, 81%) as a clear oil that solidified to a colorless solid upon storage at -20 °C: Mp 50.2-52.6 °C, IR (neat) 3280, 2971, 1685, 1379, 1327, 1169, 1092, 755 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.53 (dd, J = 7.8, 1.2 Hz, 1 H), 7.09 (dt, J = 7.8, 1.2 Hz, 1 H), 7.12-7.06 (m, 2 H), 5.94-5.92 (m, 1 H), 4.45 (t, J = 5.0 Hz, 1 H), 4.38 (dd, J = 7.6, 4.4 Hz, 1 H), 2.95 (dt, J = 17.2, 2.0 Hz, 1 H), 2.26-2.19 (m, 1 H), 2.14 (s, 1 H), 2.10-1.91 (m, 3 H), 1.49 (s, 9 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 154.2, 142.5, 136.3, 132.7, 132.0, 129.8, 128.5, 127.2, 122.6, 79.3, 53.1, 52.3, 37.8, 34.5, 29.7, 28.4; HRMS (ESI) m/z calcd for C$_{15}$H$_{17}$BrNO$_2$ ([M+H]$^+$) 364.0915, found 364.0907.
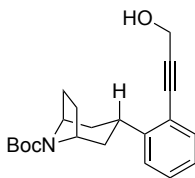
3.27

(1*SR*,3*RS*,5*RS*)-*tert*-Butyl 3-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (3.27). To four separate flasks each containing a solution of (1*SR*,5*RS*)-tert-butyl 3-(2-bromophenyl)-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (**3.28**, 0.150 g, 0.412 mmol) in anhydrous *i*PrOH (2 mL, 0.5 M) under argon and treated with phenylsilane (304 μL, 2.47 mmol) and TBHP (5.5 M solution in decane, 150 uL, 0.824 mmol) and the resulting mixture was sparged with Ar for 10 minutes. Mn(dpm)$_3$ (0.0249 g, 0.0412 mmol) was then added in one portion and the reaction was then degassed for an additional 30 seconds. The reaction was stirred for 2 h where analysis by LCMS indicated that the SM was completely consumed. The reactions were combined, concentrated under reduced pressure and

purified by automated chromatography on SiO$_2$ (12 g column, liquid load CH$_2$Cl$_2$, isocratic 10% EtOAc/hexanes) to give **3.33** (0.309 g, 0.844 mmol) as a 3:1 mixture of diastereomers as a colorless oil.

A solution of *tert*-butyl (1*SR*,5*RS*)-3-(2-bromophenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (0.300 g, 0.803 mmol) in dry toluene (6.0 mL) was sparged with Ar for 60 min and treated with propargyl alcohol (0.72 mL, 12.0 mmol), Et$_3$N (1.70 mL, 12.0 mmol), (*i*Pr)CuCl[293] (0.0587 g, 0.0587 mmol), and Pd(PPh$_3$)$_2$Cl$_2$ (0.0287 g, 0.0040 mmol) further degassed for 5 min and the mixture was stirred 24 h at 90 °C. The reaction was diluted with EtOAc (30 mL) and washed with H$_2$O (3 x 5 mL), dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (12g column, liquid load CH$_2$Cl$_2$, gradient 100% hexanes to 40% EtOAc/hexanes) to give a yellow brown oil that was contaminated with Cu. The oil was then dissolved in hexanes and decanted leaving behind solid Cu. This was repeated 3 times to give **3.27** (0.0695 g, 0.205 mmol, 12% (2 steps)) as a dark yellow oil as a single diastereomer: IR (neat) 3413, 2971, 2865, 1661, 1404, 1364, 1161, 1105, 1029, 757 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.36 (dd, *J* = 7.5, 0.9 Hz, 1 H), 7.29-7.24 (m, 1 H), 7.18-7.11 (m, 2 H), 4.47 (t, *J* = 4.5 Hz, 2 H), 4.31-4.21 (m, 3 H), 3.00 (app quint, *J* = 6.3 Hz, 1 H), 2.91-2.82 (m, 1 H), 2.38 (app pent, *J* = 4.5 Hz, 1 H), 2.25-2.14 (m, 1 H), 2.07-1.96 (m, 1 H), 1.76-1.54 (m, 4 H), 1.50 (s, 9 H), 1.01 (t, *J* = 12.9 Hz, 1 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 155.6, 145.7, 131.4, 128.3, 125.9, 125.6, 122.9, 93.2, 83.1, 79.7, 51.7, 51.3, 51.0, 38.6, 35.6, 32.5, 31.9, 28.5; HRMS (ESI) *m/z* calcd for C$_{21}$H$_{27}$NaNO$_3$ ([M+Na]$^+$) 364.1881, found 364.1883.
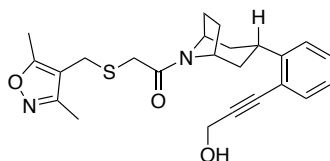
3.25

2-(((3,5-Dimethylisoxazol-4-yl)methyl)thio)-1-((1*SR*,3*RS*,5*RS*)-3-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-8-azabicyclo[3.2.1]octan-8-yl)ethan-1-one **(3.25).** A solution of (1*SR*,3*RS*,5*RS*)-*tert*-butyl 3-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.27** 0.0450 g, 0.0132 mmol) in CH_2Cl_2 (2.5 mL) was treated with TFA (0.2 mL, 2.64 mmol) at 0 °C. After 6 h, the solution was concentrated. The residual oil was dissolved in CH_2Cl_2 and satd. aqueous $NaHCO_3$, and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure to give 3-(2-((1*SR*,3*RS*,5*RS*)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)prop-2-yn-1-ol (0.0368 g, 0.152 mmol) as a dark red oil that was used without further purification.

A solution of 2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)acetic acid (**3.26**, 0.0584 g, 0.290 mmol), HATU (0.0121 g, 0.319 mmol) and DIPEA (101 µL, 0.580 mmol) in CH_2Cl_2 (1 mL) was stirred for 30 min at 0 °C and treated with a solution of 3-(2-((1*SR*,3*RS*,5*RS*)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)prop-2-yn-1-ol (0.0350 g, 0.145 mmol) and DIPEA (50 µL, 0.260 mmol) in CH_2Cl_2 (1 mL). The mixture is stirred at rt for 3 h, treated with 1 M NaOH (0.2 mL) and stirred at rt for 2 h. The reaction was diluted with EtOAc, and the layers separated. The organic layer was washed with 1 M aqueous $NaHSO_4$, satd. aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by automated chromatography SiO_2 (4g column, liquid load CH_2Cl_2, gradient 50% EtOAc/hexanes to 100% EtOAc) to give **3.25** (0.0182 g, 0.0429 mmol, 30% (2 steps)) as a yellow oil that partially solidified to a waxy off white solid: IR (CH_2Cl_2) 3387, 2924, 2865, 1616, 1448, 1031, 759 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35 (dd, *J* = 8.4, 1.6 Hz, 1 H), 7.26 (dt, *J* = 7.6, 1.2 Hz, 1 H), 7.16-7.13 (m, 2 H), 4.64 (t, *J* = 8.4 Hz, 1 H), 4.47 (ddd, *J* = 16.4, 16.4, 6.0 Hz, 2 H), 4.25 (t, *J* = 8.0 Hz, 1 H), 4.10 (app t, *J* = 5.6 Hz, 1 H),

3.70 (dd, J = 20.0, 13.6 Hz, 2 H), 3.23 (dd, J = 20.0, 14.0 Hz, 2 H), 3.09 (app quint, J = 6.0 Hz, 1 H), 2.91-2.83 (m, 1 H), 2.51-2.44 (m, 1 H), 2.37 (s, 3 H), 2.24 (s, 3 H), 2.23-2.17 (m, 1 H), 2.09-2.00 (m, 1 H), 1.83 (ddd, J = 9.2, 9.2, 4.4 Hz, 1 H), 1.74-1.65 (m, 2 H), 1.15 (dt, J = 13.2, 1.2 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.1, 166.9, 159.7, 145.0, 132.0, 131.4, 128.5, 126.1, 125.6, 122.7, 109.7, 93.4, 83.1, 53.5, 51.2, 50.2, 38.6, 36.6, 33.1, 32.7, 31.0, 24.2, 10.9, 10.0; HRMS (ESI) m/z calcd for C$_{24}$H$_{29}$N$_2$O$_3$S ([M+H]$^+$) 425.1893, found 425.1894.

Bead conjugation Conjugation of 3.25 to the Carboxylink Agarose beads

Activation of the alcohol with *p*-nitrophenylchloroformate: A solution of 2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)-1-((1R,3s,5S)-3-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-8-azabicyclo[3.2.1]octan-8-yl)ethanone (**3.25**, 0.00566 g, 0.0133 mmol) in CH$_2$Cl$_2$ (200 uL) was added p-nitrophenylchloroformate (0.00403 g, 0.0199 mmol) and Et$_3$N (3.7 uL, 0.0266 mmol) and the reaction was stirred at rt for 4 hours, diluted with H$_2$O (50 μL) and stirred for another 10 min where a deep yellow color was noted. The reaction was diluted with CH$_2$Cl$_2$ (1 mL) filtered through a small column of Na$_2$SO$_4$ and washed (CH$_2$Cl$_2$ (1 mL)). The solution was concentrated under reduced pressure to give the product as a clear yellow oil that was dissolved in DMF (400 μL) to give the "**activated alcohol solution**".

Conjugation reaction: A suspension of Carboxylink Coupling Gel (1.0 mL) was washed with DMF (1.0 mL). The tube was capped and the gel resuspended by inverting with gentle shaking. The beads were pelleted in the centrifuge (2 min), the supernatent was removed (pipet). This washing was repeated 3 times and the resulting pellet was treated with the **activated alcohol solution** in DMF, the eppendorf tube was capped and rotated for 5 d at 20 °C then added 5% MES buffer and warmed to 30 °C for 3 d. The gel was pelleted and washed with DMF (3 x 1.0 mL), PBS buffer (3 x 1.0 mL), and (HPLC grade) H$_2$O (3 x 1.0 mL). Similar to the initial

suspension/pelleting/and pipetting. Finally the gel was suspended with 1.0 mL of 0.05% azide solution to give **3.34** conjugated beads.

Quantification: The DMF, phosphate buffer and HPLC H_2O washes were combined and concentrated by centrifugal evaporation in the HT4 (24h@40 °C). The resulting solid was suspended in CH_2Cl_2 and filtered through a plug of cotton. The combined filtrates were concentrated under reduced pressure to give (4.74 mg of a 1:0.5:0.2:0.5 mixture of p-nitrophenol (M=139), activated carbonate (M=589): unknown pdt (M=508): unknown elimination pdt (M=443)). By mass the recovered material was 0.87 mg of p-nitrophenol with the rest comprised of 571 containing material (3.86 mg) was remaining resulting in 1.80 mg (4.24 μmol) of 571 conjugated to bead. (Mass component X/Mass p-nitrophenol)*number of equivalents in ^{1}HNMR. giving us the ratio by weight of the products as 1:2.1:0.73:1.6 respectively. Adding the values gives us 5.43 and 1/5.43 is the amount of 4.74 mg that contains pnitrophenol. Subtracting that from the mass and then an overall subtraction from the SM gives us 1.80 mg of SM consumed.

Control beads:
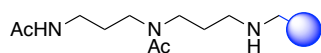
Carboxylink Coupling Gel was washed with DMF (1.0 mL). The eppendorf tube was capped and the gel resuspended by inverting with gentle shaking. The beads were pelleted in the centrifuge tube (2 min), the supernatent was removed (pipet). This washing was repeated 3 times. The pelleted beads were treated with a solution of *N*-acetoxysuccinimide (1 M in DMF, 400 μL) and the eppendorf tube was placed on the rotator for 4 h at rt. After 4 h the gel was washed with DMF (3 x 1 mL), phosphate buffer (pH 7.0) (3 x 1 mL), and H_2O (3 x 1.0 mL (HPLC grade)). The gel was suspended with 1.0 mL of 0.05% azide solution.
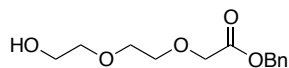
3.38

Benzyl 2-(2-(2-hydroxyethoxy)ethoxy)acetate (3.38).[487] A suspension of tBuOK (2.96 g, 26.4 mmol) in dry THF (70 mL) at 0 °C was treated with di(ethylene)glycol (**3.37**, 4.81 mL, 50.3 mmol) warmed to rt and stirred for 1 h, treated dropwise with a solution of bromobenzoacetate (6.00 g, 4.15 mL, 25.1 mmol) in dry THF (14 mL). The resulting mixture was allowed to stir at rt for 24 h. The mixture was diluted with THF (200 mL) and filtered through Celite, washed (THF), and the filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (100% EtOAc) to give **3.38** (1.49 g, 5.86 mmol, 23%) as a pale yellow liquid: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.35 (bs, 5 H), 5.19 (s, 2 H), 4.35-4.28 (m, 2 H), 4.18 (s, 2 H), 3.74-3.70 (m, 10 H), 3.63-3.56 (m, 2 H) 2.09 (bs, 1 H).
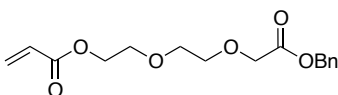
3.39

2-(2-(2-(Benzyloxy)-2-oxoethoxy)ethoxy)ethyl acrylate (3.39). A solution of benzyl 2-(2-(2-hydroxyethoxy)ethoxy)acetate (**3.38**, 1.49 g, 5.86 mmol) in dry THF (30 mL) cooled to 0 °C and treated with acryloyl chloride (0.5 mL, 6.15 mmol) and Et$_3$N (0.91 mL, 6.45 mmol) and stirred at rt for 24 h. The reaction was concentrated under reduced pressure and was dissolved in CH$_2$Cl$_2$, successively washed with 5% aqueous HCl, 5% aqueous NaHCO$_3$, and brine, dried (MgSO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:1) to give the **3.39** (0.589 g, 1.91 mmol, 33%) as a clear oil: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.37-7.35 (m, 5 H), 6.46 (m, 2 H), 6.39-6.10 (m, 1 H), 5.87-5.79 (m, 1 H), 5.19 (s, 2 H), 4.35-4.29 (m, 4 H), 4.20 (s, 2 H), 3.76-3.69 (m, 8 H); ^{13}C NMR

(100 MHz, CDCl$_3$) δ 170.3, 166.1, 135.4, 131.1, 131.0, 128.6, 128.5, 128.4 (2 C), 128.2, 128.1 (2 C), 73.3, 70.9, 70.7, 69.1, 69.0, 68.9, 68.7, 67.0, 66.5, 63.7, 63.6, 63.5.
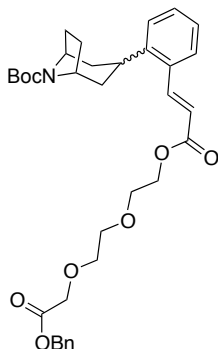
3.40

tert-**Butyl (1*SR*,5*RS*)-3-(2-((*E*)-3,12-dioxo-1-phenyl-2,5,8,11-tetraoxatetradec-13-en-14-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (3.40).** A solution of a 10:1 mixture of **3.33a** and **3.33b** (1SR,5RS)-tert-butyl 3-(2-bromophenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.33**, 0.215 g, 0.587 mmol) in dry degassed MeCN (3.0 mL) was treated with Pd(OAc)$_2$ (0.0132 g, 0.0587 mmol), tri(o-tolyl)phosphine (0.0295 g, 0.0939 mmol) and Et$_3$N (215 μL, 1.53 mmol), followed by 2-(2-(2-(benzyloxy)-2-oxoethoxy)ethoxy)ethyl acrylate (**3.39**, 0.181 g, 0.587 mmol). The reaction mixture was heated at 85 °C for 24 h, cooled to rt, concentrated under reduced pressure, diluted with EtOAc (50 mL), filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by automated chromatography on SiO$_2$ (12 g column, liquid load CH$_2$Cl$_2$, gradient, 100% hexanes to 70% EtOAc/hexanes product eluted at 50% EtOAc/hexanes) to give the **3.40** as a 10:1 mixture of diastereomers (0.183 g, 0.308 mmol, 53%) as a light brown/orange oil: IR (CH$_2$Cl$_2$) 2962, 2872, 1752, 1711, 1687, 1390, 1310, 1165, 1122, 980, 751 cm^{-1}; Characteristic NMR for the major diastereomer ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.04 (d, *J* = 15.7 Hz, 1 H), 7.47 (d, *J* = 7.7 Hz, 1 H), 7.34-7.17 (m, 9 H), 6.34 (d, *J* = 15.7 Hz, 1 H), 5.17 (s, 1 H), 4.62 (s, 1 H), 4.35 (t, *J* =

4.5 Hz, 4 H), 4.26 (s, 1 H), 4.21 (s, 2 H), 4.13 (s, 1 H), 3.75 (t, *J* = 4.8 Hz, 6 H), 2.99-2.89 (m, 1 H), 2.50-2.45 (m, 3 H), 2.07 (s, 2 H), 1.70-1.63 (m, 2 H), 1.53 (s, 9 H), 1.46-1.43 (m, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 170.1, 166.2, 154.5, 143.8, 142.4, 135.3, 133.4, 129.8, 128.4, 128.2, 127.9, 127.5, 127.1, 126.3, 120.2, 79.2, 73.2, 70.8, 70.5, 69.0, 68.8, 68.5, 66.9, 66.3, 63.6, 63.4, 63.3, 51.3, 50.5, 38.6, 37.7, 32.1, 31.4, 30.4, 28.3; HRMS (ESI) *m/z* calcd for C$_{34}$H$_{44}$NO$_8$ ([M+H]$^+$) 594.3061, found 594.3060.
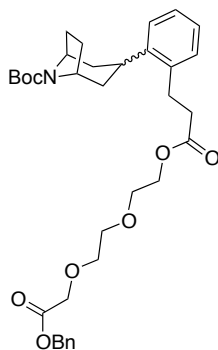
3.41

tert-**Butyl   (1*SR*,5*RS*)-3-(2-(3,12-dioxo-1-phenyl-2,5,8,11-tetraoxatetradecan-14-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (3.41).** A 10:1 diastereomer mixture of (1*SR*,5*RS*)-tert-butyl   3-(2-((*E*)-3,12-dioxo-1-phenyl-2,5,8,11-tetraoxatetradec-13-en-14-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.40**, 0.400 g, 0.674 mmol) and NiCl$_2$-6H$_2$O (0.320 g, 1.35 mmol) in THF (6.7 mL) was treated with NaBH$_4$ (0.153 g, 4.04 mmol) in portions at 0 °C. Within 5 min gas evolution started and the solution turned black. The reaction was stirred for 6 h where by LCMS it appeared that a small amount of the SM remained so an additional NiCl$_2$•6H$_2$O (1eq) and NaBH$_4$ (2 eq) was added and the reaction was left to stir for 12 h. The reaction was diluted with Et$_2$O (20 mL) and 1 M aqueous HCl (50 mL), extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with satd. aqueous NaHCO$_3$ (3 × 30 mL), dried (MgSO$_4$), and concentrated under reduced pressure. The crude residue was

purified by automated chromatography on SiO$_2$ (4g column, liquid load CH$_2$Cl$_2$, gradient 100%

hexanes to 30% hexanes/EtOAc) to give the product as a 10:1 mixture of diastereomers (0.233 g,

0.391 mmol, 58%) as a clear colorless oil: IR (CH$_2$Cl$_2$) 2958, 2895, 1733, 1685, 1390, 1163,

1120, 753 cm^{-1}; Characteristic NMR for the major diasteromer ^{1}H NMR (400 MHz, CDCl$_3$) δ

7.37-7.33 (m, 6 H), 7.20-7.07 (m, 4 H), 5.18 (s, 1 H), 4.62 (s, 1 H), 4.30 (dd, J = 5.4, 4.2 Hz, 2

H), 4.19 (t, J = 4.9 Hz, 4 H), 4.13 (s, 1 H), 3.73-3.63 (m, 7 H), 2.95 (t, J = 7.8 Hz, 2 H), 2.81-

2.74 (m, 1 H), 2.58 (t, J = 7.8 Hz, 2 H), 2.46-2.39 (m, 2 H), 2.07 (dd, J = 6.4, 4.2 Hz, 2 H), 1.69-

1.64 (m, 2 H), 1.49 (s, 9 H), 1.37 (t, J = 13.0 Hz, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.4,

170.2, 154.7, 142.5, 137.9, 137.0, 135.3, 129.3, 128.5, 128.4, 128.0, 126.0, 79.1, 73.2, 70.8, 70.5,

69.2, 68.6, 66.9, 66.5, 63.6, 63.5, 63.3, 35.5, 29.4, 28.4, 27.8; HRMS (ESI) m/z calcd for

C$_{34}$H$_{46}$NO$_8$ ([M+H]$^+$) 596.3218, found 596.3220.
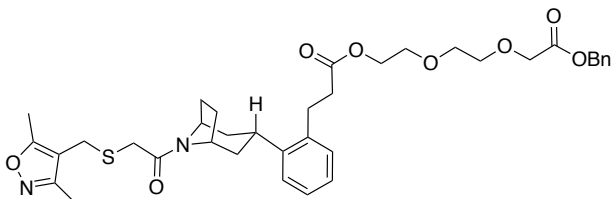
3.36

2-(2-(2-(Benzyloxy)-2-oxoethoxy)ethoxy)ethyl   3-(2-((1*SR*,3*RS*,5*RS*)-8-(2-(((3,5-

dimethylisoxazol-4-yl)methyl)thio)acetyl)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanoate

(3.36).   A   solution   of   (1SR,5RS)-tert-butyl   3-(2-(3,12-dioxo-1-phenyl-2,5,8,11-

tetraoxatetradecan-14-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.41**, 0.122 g, 0.205

mmol) in THF (2 mL) was cooled to 0 ºC, treated with 4M HCl in dioxane (1.5 mL, 30 eq) and

stirred at 0 ºC for 8 h. The solution was concentrated under reduced pressure and the residue was

sonnicated with Et$_2$O and hexanes and decanted (3x) leaving behind the product. The product

was   dried   under   high   vacuum   to   give   2-(2-(2-(benzyloxy)-2-oxoethoxy)ethoxy)ethyl   3-(2-

((1SR,3RS,5RS)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanoate hydrochloride (0.115 g, 0.216 mmol, quant) as a viscous oil that was taken onto the T3P coupling with no further purification.

A solution of 2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)acetic acid (**3.26**, 0.0495 g, 0.246 mmol) and 2-(2-(2-(benzyloxy)-2-oxoethoxy)ethoxy)ethyl 3-(2-((1SR,3RS,5RS)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanoate hydrochloride (0.109 g, 0.205 mmol) in CH_2Cl_2 (2 mL), was cooled to 0 °C and treated with Et_3N (86 µL, 0.0622 mmol). The cooled mixture was treated with T3P (50 wt. % solution in EtOAc, 217 µL, 0.307 mmol) and the reaction was warmed to rt, stirred for 12 h, diluted with CH_2Cl_2, and washed with satd. aqueous NH_4Cl, satd. aqueous $NaHCO_3$, brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude material was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% CH_2Cl_2 to 60% CH_2Cl_2/EtOAc, pdt eluted at 15% EtOAc CH_2Cl_2) to give the product as a 3:2 mixture of product:impurity. This mixture was then purified by preparative HPLC (isocratic 16% *i*PrOH/hexanes (Varian Dynamax 250 X 21.4 microsorb 60 - 8 Si 20 mL/ min)) and filtered through a short plug of SiO_2 (2:1, CH_2Cl_2/EtOAc) to give **3.36** as a single diastereomer (0.00850 g, 0.0125 mmol, 6%) as a clear oil: IR ($CHCl_3$) 2951, 1733, 1629, 1448, 1193, 1144 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37-7.32 (m, 5 H), 7.22-7.18 (m, 2 H), 7.14-7.10 (m, 2 H), 5.17 (s, 2 H), 4.75 (t, J = 7.9 Hz, 1 H), 4.18 (t, J = 4.7 Hz, 4 H), 3.82 (d, J = 14.4 Hz, 1 H), 3.71 (dd, J = 5.6, 3.3 Hz, 2 H), 3.64 (dd, J = 4.8, 4.2 Hz, 4 H), 3.62 (d, J = 14.4 Hz, 1 H), 3.17 (d, J = 13.8 Hz, 1 H), 3.03 (d, J = 13.8 Hz, 1 H), 2.99-2.95 (m, 3 H), 2.58 (t, J = 8.0 Hz, 2 H), 2.54-2.47 (m, 5 H), 2.31 (s, 3 H), 2.17-2.05 (m, 2 H), 1.82-1.73 (m, 2 H), 1.51 (t, J = 12.8 Hz, 1 H), 1.46 (t, J = 12.8 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.6, 170.2, 167.0, 166.4, 159.9, 141.9, 138.2, 135.4, 129.3, 128.6, 128.4 (2 C), 127.5, 126.7, 126.3, 110.0, 70.9,

70.5, 69.1, 68.6, 66.5, 63.5, 53.2, 49.2, 40.1, 38.8, 35.7, 32.9, 31.4, 31.1, 29.5, 27.7, 23.6, 11.1, 10.2; HRMS (ESI) m/z calcd for $C_{37}H_{47}O_8N_2S$ ([M+H]$^+$) 679.3048, found 679.3045.
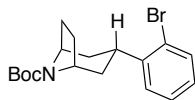
3.33a

***tert*-Butyl (1S*R*,3*RS*,5*RS*)-3-(2-bromophenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (3.33a).** A solution of (1R,5S)-tert-butyl 3-(2-bromophenyl)-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (1.75 g, 4.80 mmol) in anhydrous iPrOH (20 mL, 0.25 M) under argon was treated with phenylsilane (3.6 mL, 28.8 mmol) and TBHP (5.5 M in decane) (1.31 mL, 7.20 mmol) and the resulting mixture was degassed by bubbling argon through the solution for 10 minutes. Mn(dpm)$_3$[431] (0.290 g, 0.480 mmol) was added in one portion and the reaction was then degassed for an additional 30 seconds. After 1 h another portion of Mn(dpm)$_3$ (0.290 g, 0.480 mmol) was added and the solution was stirred for another hour. After 1 h another portion Mn(dpm)$_3$ (0.290 g, 0.480 mmol) was added and the solution was stirred for another hour. After stirring for 1 h another portion of Mn(dpm)$_3$ (0.290 g, 0.480 mmol) and the reaction was stirred for 16 at rt, quenched by SLOW ADDITION of 1 M NaOH (100 mL) at 0 °C and stirred for 15 min, filtered, washed (EtOAc) the layers separated and the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give the major diastereomer **3.33a** (0.470 g, 1.28 mmol, 27%) as a clear colorless oil. Additionally, a 1:1 mixture of diastereromers was also recovered as mixed fractions (0.327 g, 0.892 mmol, 19%) as a clear colorless oil: Characterization for **3.33a**: IR (CH$_2$Cl$_2$) 3280, 2971, 1685, 1379, 1327, 1169, 1092, 755 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.55 (dd, J = 7.8, 1.2 Hz, 1 H), 7.45 (dd, J = 7.8, 1.2 Hz, 1 H), 7.35

(td, J = 7.8, 1.2 Hz, 1 H), 7.13 (td, J = 7.8, 1.2 Hz, 1 H), 4.17-4.13 (m, 2 H), 2.90 (tt, J = 12.0, 6.6 Hz, 1 H), 2.33-2.32 (m, 2 H), 2.01-1.98 (m, 1 H), 1.96-1.92 (m, 1 H), 1.71-1.67 (m, 2 H), 1.45 (s, 9 H), 1.36 (t, J = 12.0 Hz, 2 H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 153.7, 143.0, 132.5, 129.3, 128.1, 128.1, 123.8, 78.4, 50.8, 50.1, 37.1, 36.4, 33.1, 31.7, 31.1, 28.2; HRMS (ESI) m/z calcd for C$_{18}$H$_{24}$BrNNaO$_2$ ([M+Na]$^+$) 388.0883, found 388.0880.
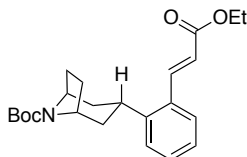
3.43

***tert*-Butyl (1*SR*,3*RS*,5*RS*)-3-(2-((*E*)-3-ethoxy-3-oxoprop-1-en-1-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (3.43).** A solution of (1SR,3RS,5RS)-tert-butyl 3-(2-bromophenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.33a**, 0.469 g, 1.28 mmol) in dry degassed MeCN (6.4 mL) was treated with Pd(OAc)$_2$ (0.0287 g, 0.128 mmol), tri(*o*-tolyl)phosphine (0.0643 g, 0.205 mmol), Et$_3$N (467 µL, 3.33 mmol), and ethyl acrylate (181 µL, 1.66 mmol) and heated 18 h at 85 °C, concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL), filtered through Celite, washed (EtOAc) and the combined filtrates were concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 3:1) to give **3.43** (0.432 g, 1.12 mmol, 87%) as a clear colorless oil: IR (CH$_2$Cl$_2$) 2973, 1701, 1691, 1392, 1310, 1172, 1102, 764 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.00 (d, J = 15.7 Hz, 1 H), 7.47 (dd, J = 7.7, 0.9 Hz, 1 H), 7.33 (td, J = 7.4, 1.2 Hz, 1 H), 7.28-7.26 (m, 1 H), 7.20 (td, J = 7.4, 1.2 Hz, 1 H), 6.28 (d, J = 15.7 Hz, 1 H), 4.37 (bs, 1 H), 4.25 (q, J = 7.1 Hz, 3 H), 2.94 (tt, J = 11.4, 7.3 Hz, 1 H), 2.53-2.39 (m, 2 H), 2.10-2.05 (m, 2 H), 1.67 (q, J = 7.3 Hz, 2 H), 1.52 (s, 9 H), 1.48-1.39 (m, 2 H), 1.34 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.5, 154.6, 143.8, 142.0, 133.6, 129.8, 127.7, 127.3, 126.4, 120.9, 79.3, 60.4, 51.4,

50.6, 38.7, 37.8, 32.3, 31.6, 30.6, 28.4, 14.3; HRMS (ESI) *m/z* calcd for $C_{23}H_{32}O_4N$ ([M+H]$^+$) 386.2326, found 386.2327.
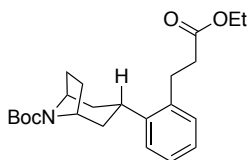
3.44

***tert*-Butyl** **(1*SR*,3*RS*,5*RS*)-3-(2-(3-ethoxy-3-oxopropyl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (3.44).** A solution of (1*SR*,3*RS*,5*RS*)-tert-butyl 3-(2-((*E*)-3-ethoxy-3-oxoprop-1-en-1-yl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.43**, 0.0500 g, 0.130 mmol) in absolute EtOH (1.3 mL) was treated with 10% Pd/C (0.0138 g, 0.0130 mmol, equivalent to 10 mol% Pd) and stirred under a balloon of H$_2$ for 1.5 h where analysis by ^{1}H NMR indicated that the alkene had been consumed. The reaction was filtered through basic Al$_2$O$_3$ (eluting with EtOAc) to give **3.44** (0.0517 g, 0.133 mmol, quant.) as a clear colorless oil: IR (CH$_2$Cl$_2$) 2971, 1732, 1687, 1390, 1564, 1163, 1100, 755 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.22-7.16 (m, 2 H), 7.14-7.08 (m, 2 H), 4.35 (bs, 1 H), 4.24 (bs, 1 H), 4.10 (q, *J* = 7.1 Hz, 2 H), 2.95 (t, *J* = 7.9 Hz, 2 H), 2.79 (tt, *J* = 11.9, 7.1 Hz, 1 H), 2.56-2.51 (m, 2 H), 2.09-2.07 (m, 2 H), 1.67 (q, *J* = 7.4 Hz, 2 H), 1.50 (s, 9 H), 1.41-1.34 (m, 2 H), 1.22 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.5, 154.7, 142.5, 138.0, 129.3, 127.6, 126.6, 126.0, 79.1, 60.3, 51.3, 50.6, 39.5 (2 C), 38.6, 35.8, 32.5, 31.8, 29.4, 28.4, 27.9, 14.1; HRMS (ESI) *m/z* calcd for $C_{23}H_{34}O_4N$ ([M+H]$^+$) 388.2482, found 388.2479.
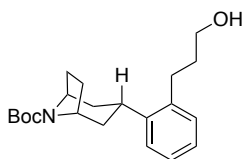
3.45

***tert*-Butyl (1*SR*,3*RS*,5*RS*)-3-(2-(3-hydroxypropyl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (3.45).** A solution of (1*SR*,3*RS*,5*RS*)-tert-butyl 3-(2-(3-ethoxy-3-oxopropyl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.44**, 0.400 g, 1.03 mmol) in THF (8 mL) was treated with NaBH$_4$ (0.390 g, 10.3 mmol) and stirred for 15 min at rt, treated with absolute ethanol (2 mL) and stirred at rt for 16 h where analysis by TLC (hexanes/EtOAc, 1:1) indicated there was still SM remaining. An additional 2 equiv. of NaBH$_4$ was added and the reaction was stirred for another 1.5 h where analysis by TLC (hexanes/EtOAc, 1:1) indicated the SM had been consumed. The reaction was cooled to 0 °C and diluted with 1 M aqueous HCl (40 mL), extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (MgSO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1) to give **3.45** (0.266 g, 0.771 mmol, 75%) as colorless solid: Mp 99.3-101.7 °C; IR (CH$_2$Cl$_2$) 3485, 2971, 1670, 1689, 1407, 1163, 1107, 753 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.21-7.08 (m, 4 H), 4.33 (bt, *J* = 7.4 Hz, 1 H), 4.24 (bt, *J* = 7.4 Hz, 1 H), 3.66 (t, *J* = 6.5 Hz, 2 H), 2.83 (tt, *J* = 12.5, 6.5 Hz, 1 H), 2.76-2.67 (m, 2 H), 2.49-2.36 (m, 2 H), 2.17-2.01 (m, 2 H), 1.98-1.86 (m, 1 H), 1.81 (quint, *J* = 7.1 Hz, 2 H), 1.51 (s, 9 H), 1.43-1.32 (m, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 154.9, 142.3, 139.5, 129.6, 127.5, 126.3, 125.9, 79.2, 62.1, 51.4, 50.7, 39.4, 38.9, 34.6, 32.4 (2 C), 32.0, 29.4, 29.3, 28.5; HRMS (ESI) *m/z* calcd for C$_{21}$H$_{32}$O$_3$N ([M+H]$^+$) 346.2377, found 346.2373.
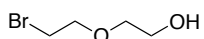
3.50

2-(2-Bromoethoxy)ethanol (3.50).[488] A solution of diethylene glycol (**3.37**, 1.79 mL, 18.9 mmol) in MeCN (30.0 mL) at rt was treated with carbon tetrabromide (6.88 g, 20.7 mmol), and triphenylphosphine (4.95 g, 18.9 mmol) and the resulting mixture was stirred at rt for 16 h,

diluted with 1 M aqueous NaOH (25 mL) and MeCN, and the mixture was concentrated under reduced pressure. The aqueous residue was extracted with EtOAc (3 × 30 mL), dried (Na$_2$SO$_4$), filtered, and concentrated. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1) to give **3.50** (2.16 g, 10.7 mmol, 57%) as yellow oil contaminated with 16% PPh$_3$O. This material was carried on with no further purification: ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.83 (t, J = 6.0 Hz, 2 H), 3.76 (dd, J = 5.3, 3.7 Hz, 2 H), 3.63 (dd, J = 5.3, 3.7 Hz, 2 H), 3.49 (t, J = 6.0 Hz, 2 H), 1.97 (s, 1 H).
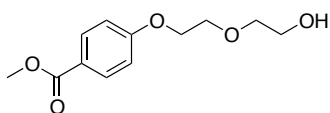
Methyl 4-(2-(2-hydroxyethoxy)ethoxy)benzoate (3.52).[488] A solution of methyl 4-hydroxybenzoate (**3.51**, 2.33 g, 15.3 mmol) in MeCN (80 mL) was treated with K$_2$CO$_3$ (4.41 g, 31.9 mmol), NaI (1.91 g, 12.8 mmol), and 2-(2-bromoethoxy)ethanol (**3.50**, 2.16 g, 12.8 mmol) and the resultant mixture was heated to 60 °C 15 h. Then the mixture was diluted with H$_2$O, extracted with EtOAc. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:3) to give **3.52** that was contaminated with PPh$_3$O (15%) (1.62 g, 5.73 mmol, 45%) as a clear/pale-yellow oil: ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.01-7.96 (m, 2 H), 6.96-6.91 (m, 2 H), 4.20 (t, J = 4.7 Hz, 2 H), 3.91-3.87 (m, 5 H), 3.79-3.75 (m, 2 H), 3.70-3.66 (m, 2 H), 1.80 (s, 1 H).
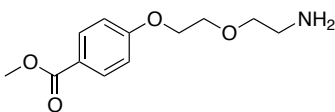
3.53

Methyl 4-(2-(2-aminoethoxy)ethoxy)benzoate (3.53). A solution of methyl 4-(2-(2-hydroxyethoxy)ethoxy)benzoate (**3.52**, 0.300 g, 1.25 mmol) in dry THF (5 mL) at 0 °C was treated Et$_3$N (193 uL, 1.37 mmol) followed by MsCl (106 μL, 1.37 mmol) and stirred at 0 °C for 2 h, diluted with satd. aqueous NaHCO$_3$ and extracted with Et$_2$O (3 x 20 mL). The combined organic layers were dried (MgSO$_4$), filtered, and concentrated under reduced pressure to give the crude mesylate (0.369 g, 1.16 mmol) as a colorless solid.

A solution of the crude mesylate (0.230 g, 0.723 mmol) in DMF (1.5 mL) was treated with Et$_3$N (0.16 mL, 1.54 mmol) and NaN$_3$ (0.142 g, 2.18 mmol). This mixture was heated to 50 °C behind a blast shield for 17 h where analysis by TLC (hexanes/EtOAc; 1:1) indicated that the mesylate intermediate was completely consumed. The reaction was cooled to rt, diluted with H$_2$O (5 mL) and brine (10 mL) and extracted with Et$_2$O (3 x 10 mL). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), concentrated under reduced pressure to give the crude methyl 4-(2-(2-azidoethoxy)ethoxy)benzoate (0.170 g, 0.449 mmol) as a colorless wax.

To a solution of crude methyl 4-(2-(2-azidoethoxy)ethoxy)benzoate (0.161 g, 1.70 mmol (contaminated with ~30% PPh$_3$O)) in THF (2 ml) was added triphenyl phosphine (0.223 g, 0.850 mmol) and H$_2$O (30 uL, 1.70 mmol) then heated to 50 °C for 17 h, and concentrated under reduced pressure. The residue was dissolved in CH$_2$Cl$_2$ and 1 M aqueous HCl (10 mL) and the aqueous layer was washed with CH$_2$Cl$_2$ (2x). The aqueous layer was neutralized with solid K$_2$CO$_3$ to pH 10. The aqueous layer was washed with CH$_2$Cl$_2$ (3 x 30 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give **3.53** (0.0893 g, 0.373 mmol, 55% (3 steps)) as a clear oil that solidified in the freezer: Mp ~10-20 °C; (CH$_2$Cl$_2$) 3368, 2945, 1707, 1603, 1249, 1169, 1103, 846 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.94 (dt, J = 9.0, 2.6 Hz, 2 H), 6.90 (dt, J = 9.0, 2.6 Hz, 2 H), 4.14 (dd, J = 5.4, 4.1 Hz,

2 H), 3.84 (s, 3 H), 3.80 (dd, J = 5.4, 4.1 Hz, 2 H), 3.54 (t, J = 5.4 Hz, 2 H), 2.85 (bs, 2 H); ^{13}C

NMR (100 MHz, CDCl$_3$) δ 166.7, 162.4, 131.4, 122.6, 114.1, 73.6, 69.2, 67.4, 51.7, 41.7;

HRMS (ESI) m/z calcd for C$_{12}$H$_{18}$O$_4$N ([M+H]$^+$) 240.1230, found 240.1229.
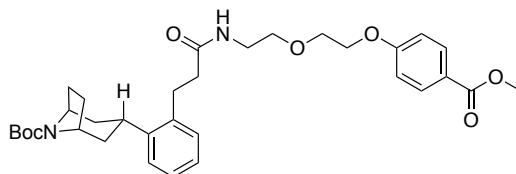
3.54

***tert*-Butyl** **(1*SR*,3*RS*,5R*S*)-3-(2-(3-((2-(2-(4-**
(methoxycarbonyl)phenoxy)ethoxy)ethyl)amino)-3-oxopropyl)phenyl)-8-
azabicyclo[3.2.1]octane-8-carboxylate (3.54). A solution of (1*SR*,3*RS*,5*RS*)-tert-butyl 3-(2-(3-

ethoxy-3-oxopropyl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.45**, 0.380 g, 0.981

mmol) in THF (4 mL) at 0 °C was treated with 2 M aqueous LiOH (0.98 mL, 1.96 mmol) and

the reaction was stirred at 0 °C for 30 min and warmed to rt. A precipitate was noted after

addition of the LiOH. The reaction was stirred at rt for 24 h where analysis by TLC (EtOAc)

indicated the SM remained. The reaction was heated to 50 °C for 5 h where analysis by TLC

(EtOAc) indicated the SM was consumed. The THF was removed under reduced pressure and

the aqueous layer was acidified with 1 M aqueous HCl. The aqueous layer was extracted with

EtOAc (3 x 20 mL). The combined organic layers were dried (MgSO$_4$), filtered and concentrated

under reduced pressure to give 3-(2-((1*SR*,3*RS*,5*RS*)-8-(tert-butoxycarbonyl)-8-

azabicyclo[3.2.1]octan-3-yl)phenyl)propanoic acid (0.289 g, 0.804 mmol) as a colorless solid.

A solution of 3-(2-((1*SR*,3*RS*,5*RS*)-8-(tert-butoxycarbonyl)-8-azabicyclo[3.2.1]octan-3-

yl)phenyl)propanoic acid (0.100 g, 0.278 mmol), HATU (0.127 g, 0.334 mmol), and HOAt

(0.0464 g, 0.334 mmol) in DMF (1 mL) at 0 °C was treated with DIPEA (0.19 mL, 1.11 mmol).

(The pale yellow solution became dark yellow). After 30 min at 0 °C, a solution of methyl 4-(2-

(2-aminoethoxy)ethoxy)benzoate (**3.53**, 0.0732 g, 0.306 mmol) in DMF (0.4 mL) was added and the reaction mixture was stirred at 0 °C for 1 h, rt for 21 h, diluted with EtOAc (50 mL) and satd. aqueous NaHCO$_3$ (2 x) and brine, dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (100% EtOAc) to give **3.54** (0.150 g, 0.259 mmol, 76% (2 steps)) as a colorless foam: IR (CH$_2$Cl$_2$) 2969, 1711, 1655, 1603, 1411, 1253, 1167, 1107, 841, 770 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 7.91-7.86 (m, 3 H), 7.27 (d, J = 7.8 Hz, 1 H), 7.14 (dt, J = 7.8, 1.8 Hz, 1 H), 7.08-7.04 (m, 4 H), 4.16 (t, J = 4.5 Hz, 2 H), 4.12-4.10 (m, 2 H), 3.81 (s, 3 H), 3.72 (t, J = 4.5 Hz, 2 H), 3.44 (t, J = 5.9 Hz, 2 H), 3.22-3.17 (m, 2 H), 2.79-2.76 (m, 2 H), 2.67 (tt, J = 12.6, 6.5 Hz, 1 H), 2.31-2.26 (m, 4 H), 2.00-1.93 (m, 2 H), 1.72-1.66 (m, 2 H), 1.42 (s, 9 H), 1.37-1.31 (m, 2 H); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 171.0, 165.8, 162.3, 153.9, 142.2, 138.5, 131.2, 128.8, 127.6, 126.3, 125.7, 121.9, 114.5, 78.3, 69.3, 68.5, 67.4, 51.8, 51.0, 50.3, 38.5, 37.8, 36.6, 31.9, 31.2, 30.7, 28.7, 28.2, 27.6; HRMS (ESI) m/z calcd for C$_{33}$H$_{45}$O$_7$N$_2$ ([M+H]$^+$) 581.3221, found 581.3222.
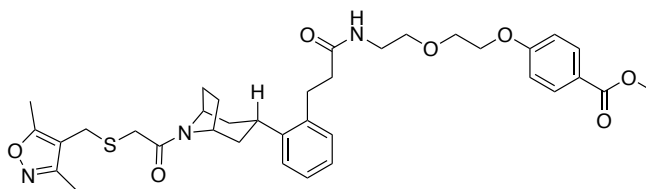
3.55

Methyl **4-(2-(2-(3-(2-((1*SR*,3*RS*,5*RS*)-8-(2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)acetyl)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanamido)ethoxy)ethoxy)benzoate (3.55).** A solution of (1*SR*,3*RS*,5*RS*)-tert-butyl 3-(2-(3-((2-(2-(4-(methoxycarbonyl)phenoxy)ethoxy)ethyl)amino)-3-oxopropyl)phenyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**3.54**, 0.230 g, 0.396 mmol) in THF (4 mL) was cooled to 0 ºC, treated with 4M HCl in dioxane (3.0 mL, 30 eq) and stirred at 0 ºC and slowly warmed to rt

for 12 h. The solution was concentrated under reduced pressure and the residue was sonicated with dry THF (4 x 10 mL) and concentrated under reduced pressure each time and dried under high vacuum to give methyl 4-(2-(2-(3-(2-((1SR,3RS,5RS)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanamido)ethoxy)ethoxy)benzoate hydrochloride (0.202 g, 0.419 mmol) as a colorless solid that was taken on to the coupling reaction with no further purification.

A solution of 2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)acetic acid (**3.26**, 0.0620 g, 0.308 mmol), HATU (0.141 g, 0.370 mmol), and HOAt (0.0513 g, 0.370 mmol) in DMF (1.5 mL) at 0 °C was treated with DIPEA (214 µL, 1.23 mmol) and stirred for 20 min at 0 °C where a deep orange solution developed, and treated with a solution of methyl 4-(2-(2-(3-(2-((1SR,3RS,5RS)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanamido)ethoxy)ethoxy)benzoate hydrochloride (0.191 g, 0.370 mmol) in DMF (1.6 mL) was added and the reaction was stirred at 0 °C for 30 min and warmed to rt for 12 h. The reaction was diluted with EtOAc (50 mL) and washed with satd. aqueous NaHCO$_3$ (2 x 20 mL), followed by brine (20 mL), dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude material was purified by chromatography on SiO$_2$ (2% MeOH/EtOAc w/ 2% Et$_3$N) to give **3.55** (0.122 g, 0.183 mmol, 60% (2 steps)) as a colorless foam: IR (CH$_2$Cl$_2$) 2947, 1709, 1603, 1433, 1253, 1167, 1113, 887 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.94 (dt, J = 8.8, 2.5 Hz, 2 H), 7.16-7.13 (m, 2 H), 7.10-7.06 (m, 2 H), 6.89 (dt, J = 8.8, 2.5 Hz, 2 H), 6.22 (t, J = 5.5 Hz, 1 H), 4.65 (t, J = 8.0 Hz, 1 H), 4.13 (t, J = 8.0 Hz, 1 H), 4.11 (t, J = 4.5 Hz, 2 H), 3.84 (s, 3 H), 3.77-3.72 (m, 3 H), 3.59-3.54 (m, 3 H), 3.46-3.35 (m, 2 H), 3.19 (d, J = 13.8 Hz, 1 H), 3.06 (d, J = 13.8 Hz, 1 H), 2.97-2.89 (m, 2 H), 2.86 (dt, J = 12.7, 6.1 Hz, 1 H), 2.46-2.39 (m, 5 H), 2.33 (td, J = 8.0, 2.7 Hz, 2 H), 2.26 (s, 3 H), 2.13 (ddt, J = 11.5, 6.9, 4.4 Hz, 1 H), 2.03 (ddt, J = 11.5, 7.6, 3.5 Hz, 1 H), 1.80-1.69 (m, 2 H), 1.44 (td, J = 13.0, 3.5 Hz, 2 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 172.0, 166.9, 166.8, 166.6,

162.3, 159.7, 141.4, 138.5, 131.5, 129.4, 127.3, 126.6, 126.2, 122.7, 114.0, 109.9, 69.9, 69.1, 67.3, 53.2, 51.7, 49.5, 40.0, 39.1, 38.9, 38.3, 32.6, 32.0, 31.0, 29.5, 28.9, 23.7, 10.9, 10.0; HRMS (ESI) m/z calcd for $C_{36}H_{46}O_7N_3S$ ([M+H]$^+$) 664.3051, found 664.3050.
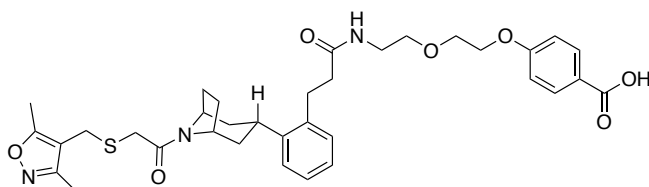
3.56

4-(2-(2-(3-(2-((1SR,3RS,5RS)-8-(2-(((3,5-Dimethylisoxazol-4-yl)methyl)thio)acetyl)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanamido)ethoxy)ethoxy)benzoic acid (3.56). To a solution of methyl 4-(2-(2-(3-(2-((1SR,3RS,5RS)-8-(2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)acetyl)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanamido)ethoxy)ethoxy)benzoate (**3.55**, 0.0616 g, 0.0928 mmol) in THF (0.5 mL) and MeOH (0.5 mL) was treated with 2 M aqueous LiOH 4(0.46 mL) and the solution was stirred at rt for 13 h where analysis by TLC (100% EtOAc) indicated the starting material still remained. By TLC there was still a small amount of remaining SM but the reaction was diluted with H$_2$O (1 mL), washed with Et$_2$O (2 x 5 mL), and the aqueous layer was acidified to pH 2 with 1 M aqueous HCl at 0 °C. The aqueous layer was extracted with EtOAc (3 x 6 mL). The combined organic layers were dried (MgSO$_4$), filtered and concentrated under reduced pressure to give **3.56** (0.0367 g, 0.0565 mmol, 61%) as a colorless foam: IR (CH$_2$Cl$_2$) 2949, 1702, 1603, 1452, 1249, 1167, 1122, 734 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.98 (d, J = 8.7 Hz, 2 H), 7.18-7.09 (m, 4 H), 6.86 (d, J = 8.7 Hz, 2 H), 6.44 (s, 1 H), 4.70 (t, J = 7.5 Hz, 1 H), 4.16 (t, J = 7.5 Hz, 1 H), 4.07 (t, J = 4.4 Hz, 2 H), 3.75 (t, J = 4.4 Hz, 2 H), 3.56 (t, J = 4.4 Hz, 3 H), 3.50-3.47

(m, 1 H), 3.37-3.34 (m, 1 H), 3.23-3.20 (m, 1 H), 3.04-2.89 (m, 3 H), 2.50-2.41 (m, 5 H), 2.37-2.32 (m, 2 H), 2.29 (s, 3 H), 2.18-2.06 (m, 2 H), 1.82-1.74 (m, 2 H), 1.51 (t, J = 12.8 Hz, 1 H), 1.45 (t, J = 12.8 Hz, 1 H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.3, 1 69.7, 167.2, 162.8, 141.5, 138.8, 132.1, 129.8, 127.4, 126.7, 126.4, 122.4, 114.2, 70.0, 69.3, 67.3, 40.3, 39.2, 39.1, 38.3, 32.7, 31.7, 31.2, 29.5, 29.1, 23.7, 11.1, 10.2; HRMS (ESI) m/z calcd for C$_{35}$H$_{44}$O$_7$N$_3$S ([M+H]$^+$) 650.2894, found 650.2888.

Bead conjugation Conjugation of 3.56 to the Carboxylink Agarose beads

Activation of the carboxylic acid as the NHS ester: A solution of 4-(2-(2-(3-(2-((1R,3s,5S)-8-(2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)acetyl)-8-azabicyclo[3.2.1]octan-3-yl)phenyl)propanamido)ethoxy)ethoxy)benzoic acid (**3.56**, 0.0120 g, 0.0185 mmol) in dry DMF (0.2 mL) was treated with *N*-hydroxysuccinimide (0.0657 g, 0.0554 mmol) followed by EDCI (0.0108 g, 0.0554 mmol). The resulting solution was stirred at rt under an argon atmosphere for 16 h. The reaction was diluted with dry DMF (0.5 mL) to give the **activated ester solution**.

Conjugation reaction: A short (1 cm diameter) column fitted with a magnetic stir bar and a drain cap was loaded suspension of Carboxylink Coupling Gel (1.0 mL) and washed with DMF (5 x 4 mL) allowing the solvent to drain after each addition of DMF. The washed gel was treated with the **activated ester solution** and gently stirred at rt under Ar for 48 h. The beads were transferred to a 1.5 mL eppendorf tube and placed on the little shot rotator for 2 d at rt. The suspension was centrifuged and the DMF was removed via pipet, the pellet was suspended in DMF (1 mL) and centrifuged (5 min in speed vac centrifuge). This washing was repeated 5 times with DMF. The gel was further washed with PBS buffer (3 x 1 mL), and (HPLC grade) water (3 x 1 mL), respectively. Similar method to the DMF washing. The gel was suspended with 1.0 mL

of 0.05% wt. sodium azide solution in HPLC water and stored at 4 °C to give the **3.57** conjugated beads.
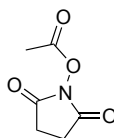
2,5-dioxopyrrolidin-1-yl acetate. (*N*-acetoxysuccinimide).[489] A solution of N-Hydroxysuccinimide (1.00 g, 8.69 mmol) in acetic anhydride (2.5 mL, 26.1 mmol) was stirred at rt for 18 h. A white precipitate had formed and the reaction was cooled to 0 °C and the colorless solid was collected by vacuum filtration and washed with an excess of hexanes to give *N*-acetoxysuccinimide (0.793 g, 5.04 mmol, 58%) as a colorless solid: ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.81 (s, 4 H), 2.32 (bd, *J* = 0.9 Hz, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 169.1, 165.5, 25.5, 17.5.
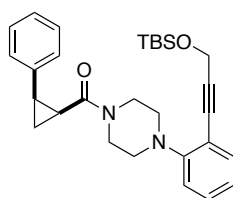
3.60

(4-(2-(3-(((*tert*-Butyldimethylsilyl)oxy)prop-1-yn-1-yl)phenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-phenylcyclopropyl)methanone (3.60). A solution of (1*SR*,2*RS*)-2-phenylcyclopropanecarboxylic acid (**3.11**, 0.0260 g, 0.160 mmol) and HATU (0.0914 g, 0.240 mmol) in CH$_2$Cl$_2$ (1 mL), was cooled to 0 °C and treated with DIPEA (83 µL, 0.481 mmol) and warmed to rt over 45 min. The solution was cooled to 0 °C and treated with a solution of 1-(2-(3-((tert-butyldimethylsilyl)oxy)prop-1-yn-1-yl)phenyl)piperazine (**3.59**, 0.0583 g, 0.176 mmol) in CH$_2$Cl$_2$ (0.5 mL) and stirred at rt 16 h, diluted with H$_2$O, washed with EtOAc (2 × 10 mL). The combined organic layers were washed with 1 M aqueous NaHSO$_4$, satd. aqueous NaHCO$_3$, dried

(Na$_2$SO$_4$), and concentrated under reduced pressure. The crude residue was purified twice by automated chromatography SiO$_2$ column (4g column, liquid load CH$_2$Cl$_2$, gradient hexanes/EtOAc, 6:1 to hexanes/EtOAc, 2.5:1) to give **3.60** (0.00763 g, 0.0161 mmol, 10%) as a pale yellow oil: IR (CH$_2$Cl$_2$) 2951, 2855, 1640, 1459, 1370, 1251, 1226, 1079, 1027, 833, 777 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.36 (dd, J = 15.0, 1.5 Hz, 1 H), 7.24-7.19 (m, 3 H), 7.15-7.13 (m, 1 H), 6.92 (dt, J = 7.5, 1.0 Hz, 1 H), 4.55 (s, 2 H), 3.93 (bd, J = 13.0 Hz, 1 H), 3.76 (app d, J = 13.0 Hz, 1 H), 3.60 (ddd, J = 12.3, 9.0, 2.5 Hz, 1 H), 3.30 (ddd, J = 12.3, 9.0, 3.0 Hz, 1 H), 3.12-3.09 (m, 2 H), 2.47-2.40 (m, 2 H), 2.20-2.13 (m, 2 H), 1.85 (dd, J = 12.5, 6 Hz, 1 H), 1.35 (ddd, J = 14.0, 8.5, 5.5 Hz, 1 H), 0.93 (s, 9 H), 0.14 (s, 6 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.3, 153.6, 137.6, 133.9, 129.3, 128.1, 127.4, 126.4, 122.1, 117.7, 116.7, 93.2, 82.9, 52.4, 51.4, 50.9, 45.4, 42.0, 25.9, 24.4, 24.1, 18.3, 10.6, -5.1; HRMS (ESI) m/z calcd for C$_{29}$H$_{39}$SiO$_2$N$_2$ ([M+H]$^+$) 475.2775, found 475.2769.
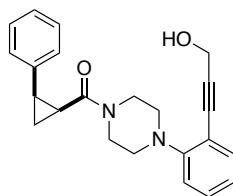
(4-(2-(3-Hydroxyprop-1-yn-1-yl)phenyl)piperazin-1-yl)((1*RS*,2*SR*)-2-phenylcyclopropyl)methanone (3.58). A solution of (4-(2-(3-((tert-butyldimethylsilyl)oxy)prop-1-yn-1-yl)phenyl)piperazin-1-yl)((1SR,2RS)-2-phenylcyclopropyl)methanone (0.0200 g, 0.0421 mmol) in TBAF (1 M solution in THF) (126 µL, 0.126 mmol) was stirred at rt for 30 min where analysis by TLC (1:1 hexanes/EtOAc) indicated the starting material had been consumed. The reaction was diluted with EtOAc and washed with satd. aqueous NH$_4$Cl, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (1:2, hexanes/EtOAc) to

give the product (0.00784 g, 0.0218 mmol, 52%) as a colorless solid: Mp 121.1-124.3 °C; IR (EtOAc) 3383, 2904, 2820, 1616, 1486, 1437, 1226, 1027, 751, 697 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.37 (dd, J = 7.6, 1.2 Hz, 1 H), 7.25-7.21 (m, 4 H), 7.16-7.13 (m, 3 H), 6.93 (dt, J = 7.6, 1.2 Hz, 1 H), 6.68 (d, J = 7.6 Hz, 1 H), 4.51 (d, J = 6.0 Hz, 2 H), 3.93 (app d, J = 13.0 Hz, 1 H), 3.74 (app d, J = 13.0 Hz, 1 H), 3.64-3.61 (m, 1 H), 3.33-3.30 (m, 1 H), 3.11-3.07 (m, 2 H), 2.48-2.41 (m, 2 H), 2.21-2.15 (m, 2 H), 1.84 (q, J = 6.2 Hz, 1 H), 1.78 (t, J = 6.2 Hz, 1 H), 1.35 (ddd, J = 14.0, 8.4, 5.2 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.4, 153.6, 137.6, 133.9, 129.6, 128.1, 127.3, 126.4, 122.2, 117.8, 116.3, 92.6, 83.9, 51.8, 51.4, 50.9, 45.4, 42.0, 24.5, 24.1, 10.6; HRMS (ESI) m/z calcd for C$_{23}$H$_{25}$O$_2$N$_2$ ([M+H]$^+$) 361.1911, found 361.1908.

5.5   CHAPTER 4 EXPERIMENTAL PART

5.5.1   First Approach

4.99

(4-Bromobut-1-yn-1-yl)trimethylsilane (4.99).[459-461] A solution of 3-butyn-1-ol (**4.98**, 5.4 mL, 71.3 mmol) in dry THF (200 mL) cooled to -78 °C was treated dropwise with n-BuLi (63.0 mL, 2.5M, 157 mmol). Upon the completion of addition the reaction mixture became cloudy. Stirring was continued at -78 °C for 1 h, when freshly distilled TMSCl (27.4 mL, 214 mmol) was added dropwise. The reaction was stirred at -78 for another 15 min. The reaction mixture was allowed to warm to rt and stirred for another 1.5 h when 2 M aqueous HCl (25 mL) was added. The aqueous layer was extracted with EtOAc. The organic layers were combined,

washed with satd. aqueous NaHCO$_3$, H$_2$O, brine, dried (Na$_2$SO$_4$), filtered and concentrated in vacuo to provide crude 4-(trimethylsilyl)but-3-yn-1-ol (10.2 g, 71.7 mmol) as a clear oil that was taken on to the next step with no further purification.

A solution of the crude 4-(trimethylsilyl)but-3-yn-1-ol (10.2 g, 71.7 mmol) in dry CH$_2$Cl$_2$ (210 mL) cooled to -30 °C was treated with CBr$_4$ (30.0 g, 90.3 mmol) in one portion. After stirring for 15 min a solution of PPh$_3$ (19.7 g, 75.3 mmol) in CH$_2$Cl$_2$ (75 mL) was added by cannula. The solution was stirred for 1.5 h at -30 °C and for another 1 h at 0 °C. The crude reaction mixture was filtered through small plug of silica gel, washing with hexanes, and was concentrated until 1/3 of the original volume was left in the flask. Cold hexanes were added to the solution and the solids were suspended, and filtered through Celite and the cake washed with cold hexanes. The filtrate was concentrated under reduced pressure (rotovap bath at 0 °C) and purified by chromatography on SiO$_2$ (100% hexanes) to give **4.99** (9.86 g, 48.1 mmol, 67% (2 steps)) as a colorless liquid: ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.42 (t, J = 7.5 Hz, 2 H), 2.77 (t, J = 7.5 Hz, 2 H), 0.16 (s, 9 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 103.2, 87.0, 29.1, 24.3, -0.1.
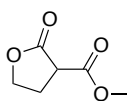
4.103

Methyl 2-oxotetrahydrofuran-3-carboxylate (4.103).[462] A solution of LHMDS (1 M in hexanes, 23.2 mL, 23.2 mmol) in THF (15 mL) was cooled to -78 °C and treated dropwise over 10 min with a solution of γ-butyrolactone (**4.100**, 1.00 g, 11.6 mmol) in THF (12 mL). After stirring for 10 min at -78 °C, the reaction was treated with dimethyl carbonate (1.04 ml, 12.2 mmol). The mixture was allowed to slowly warm to room temperature and stirring was continued for 8 h, poured onto a mixture of conc. HCl (5 ml) and ice (5 g), extracted with EtOAc. The organic layers were combined washed with brine, dried (Na$_2$SO$_4$), filtered and

concentrated under reduced pressure to give **4.103** (1.63 g, 10.8 mmol, 93%) as light brown oil. The residue was used in subsequent reactions without further purification: ^{1}H NMR (300 MHz, CDCl$_3$) δ 4.41-4.27 (m, 1 H), 4.25-4.19 (m, 1 H), 3.69 (s, 3 H), 3.50 (dt, J = 8.1, 2.4 Hz, 1 H) 2.60-2.38 (m, 2 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 172.2, 168.1, 67.2, 52.7, 45.5, 26.1.

TsO⌒⟍≡
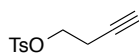
4.104

But-3-yn-1-yl 4-methylbenzenesulfonate (4.104).[490] A solution of 3-butyn-1-ol (5.00 g, 71.3 mmol) in CH$_2$Cl$_2$ (71 mL) cooled to 0 °C was treated with Et$_3$N (10.0 mL, 71.3 mmol) and TsCl (12.4 g, 64.9 mmol). The reaction was warmed to rt and stirred for 11 h, diluted with H$_2$O (100 mL), and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 100 mL). The combined organic layers were washed with brine (200 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give **4.104** (14.3 g, 63.9 mmol, 99%) as a light brown oil: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.76 (d, J = 8.4 Hz, 2 H), 7.31 (d, J = 8.4 Hz, 2 H), 4.05 (dt, J = 6.9, 1.2 Hz, 2 H), 2.53-2.48 (m, 2 H), 2.40 (s, 3 H), 1.95 (t, J = 2.4 Hz, 2 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 144.9, 132.6, 129.7, 127.7, 78.3, 70.6, 67.3, 21.4, 19.2.
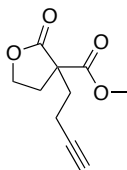
4.105

Methyl 3-(but-3-yn-1)-2-oxotetrahydrofuran-3-carboxylate (4.105). A solution of methyl 2-oxotetrahydrofuran-3-carboxylate (**4.103**, 0.100 g, 0.659 mmol) in dry DMF (1.5 mL) at rt was added K$_2$CO$_3$ (0.228 g, 1.64 mmol), and NaI (0.247 g, 1.65 mmol). The mixture was stirred for 15 min and a solution of but-3-yn-1-yl 4-methylbenzenesulfonate (**4.104**, 0.177 g,

0.791 mmol) in DMF (0.5 mL) was added. After the mixture was stirred at rt for 16 h, and heated to 60 °C for 24 h, cooled to rt, diluted with H_2O and extracted with Et_2O (3 x 20 mL) The organic layers were combined washed with H_2O, brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by automated chromatography on SiO_2 (4g column, liquid load CH_2Cl_2, gradient 100% hexanes to 60% EtOAc/hexanes) to give **4.105** (0.0383 g, 0.195 mmol, 30%) as a colorless oil: IR (CH_2Cl_2) 3286, 2924, 1772, 1732, 1436, 1378, 1213, 1167, 1029 cm^{-1} ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.37-4.33 (m, 2 H), 3.77 (s, 3 H), 2.81-2.75 (m, 1 H), 2.41-2.23 (m, 4 H), 2.08-1.98 (m 1 H), 1.98 (t, J = 1.6 Hz, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 174.1, 169.4, 82.4, 69.5, 66.2, 53.5, 53.2, 32.6, 31.8, 14.4; HRMS (ESI) m/z calcd for $C_{10}H_{13}O_4$ ([M+H]$^+$) 197.0808, found 197.0808.
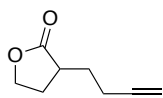
4.97

3-(But-3-yn-1-yl)dihydrofuran-2(3*H*)-one (4.97). A solution of methyl 3-(but-3-yn-1-yl)-2-oxotetrahydrofuran-3-carboxylate (**4.105**, 0.100 g, 0.510 mmol) in wet DMSO (100 uL) was treated with LiCl (0.0432 g, 1.02 mmol) and the mixture was heated to 150 °C in a sandbath for 2.5 h, cooled to rt, diluted with H_2O the extracted with EtOAc (3 x 20 mL). The organic layers were combined washed with H_2O (3 x 20 mL), brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:1) to give **4.97** (0.0484 g, 0.350 mmol, 69%) as a clear oil: IR (CH_2Cl_2) 3286, 2919, 1764, 1455, 1377, 1204, 1154, 1024 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.33 (td, J = 8.8, 2.5 Hz, 1 H), 4.17 (ddd, J = 10.0, 9.2, 6.4 Hz, 1 H), 2.70 (dtd, J = 10.8, 8.8, 5.2 Hz, 1 H), 2.48-2.37 (m, 2 H), 2.28 (m, J = 6.6, 2.6 Hz, 1 H), 2.08 (dddd, J = 13.6, 8.0, 6.8, 5.6 Hz, 1 H), 1.99-1.88 (m, 2 H); ^{13}C NMR (100

MHz, CDCl$_3$) δ 178.8, 82.6, 69.4, 66.4, 38.0, 28.9, 28.5, 16.4; HRMS (ESI) *m/z* calcd for C$_8$H$_{11}$O$_2$ ([M+H]$^+$) 139.0754, found 139.0754.
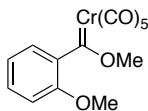
4.80

Pentacarbonyl(methoxy(2-methoxyphenyl)methylene)chromium (4.80).[491] A solution of 2-bromoanisole (**4.106**, 3.00 mL, 23.6 mmol) in Et$_2$O (60 mL) was treated at -50 °C with a solution of *n*-BuLi (2.5 M in hexanes, 10.4 mL, 25.9 mmol) and stirred at -50 °C for 30 min and then at 0 °C for another 30 min. The resulting solution was cannulated to a cold (-20 °C) suspension of chromium hexacarbonyl (6.29 g, 28.3 mmol) in Et$_2$O (60 mL). The reaction mixture (brown at first and then yellow suspension) was allowed to warm to rt and stirred at rt for 3 h and concentrated under reduced pressure. The residue was dissolved in H$_2$O, filtered through Celite that was washed (H$_2$O) and then washed with hexanes. The layers were separated and the aqueous layer was added CH$_2$Cl$_2$ (100 mL) (in the separatory funnel) and treated with trimethyloxonium tetrafluoroborate (6.18 g, 40.1 mmol) until acidic pH (the mixture turned red right away). The aqueous layer was further extracted with CH$_2$Cl$_2$ (3 x 100 mL) until it was no longer red. The combined organic layers were washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude residue was purified by column chromatography on SiO$_2$ (hexanes/EtOAc, 9:1) to give **4.80** (6.63 g, 19.4 mmol, 82%) as red crystals: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.29 (t, *J* = 7.5 Hz, 1 H), 7.04 (t, *J* = 7.5 Hz, 1 H), 6.92 (d, *J* = 8.4 Hz, 1 H), 6.79 (d, *J* = 7.5 Hz, 1 H), 4.16 (brs, 3 H), 3.83 (s, 3 H). Note: for best purification column must be preequilibrated with 9:1 hexanes/EtOAc.
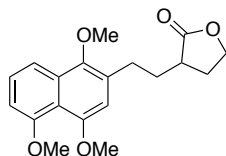
4.96

3-(2-(1,4,5-Trimethoxynaphthalen-2-yl)ethyl)dihydrofuran-2(3*H*)-one **(4.96).** A solution of the chromium carbene (**4.80**, 1.00 g, 2.78 mmol) and 3-(but-3-yn-1-yl)dihydrofuran-2(3H)-one (**4.97**, 0.460 g, 3.33 mmol) in dry deoxygenated THF (55 mL) and stirred at 50 °C for 36 h. After 36 h, Cs$_2$CO$_3$ (2.71 g, 8.33 mmol) and MeI (0.53 mL, 8.33 mmol) were added and the resulting suspension was stirred at rt for 24 h, filtered through Celite, washed (CH$_2$Cl$_2$), and the filtrate was concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:1) to give **4.96** (0.378 g, 1.14 mmol, 41%) as a red orange oil: IR (CH$_2$Cl$_2$) 2930, 2837, 1761, 1597, 1581, 1377, 1260, 1126, 1068, 1021, 755 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.64 (dd, *J* = 8.4, 0.8 Hz, 1 H), 7.40 (t, *J* = 8.0 Hz, 1 H), 6.85 (d, *J* = 7.2 Hz, 1 H), 6.67 (s, 1 H), 4.33 (dt, *J* = 8.8, 2.4 Hz, 1 H), 4.13 (dt, *J* = 9.6, 6.8 Hz, 1 H), 3.96 (s, 3 H), 3.94 (s, 3 H), 3.84 (s, 3 H), 2.94-2.89 (m, 2 H), 2.57-2.50 (m, 1 H), 2.47-2.40 (m, 1 H), 2.34-2.26 (m, 1 H), 2.03-1.93 (m, 1 H), 1.88-1.81 (m, 1 H); ^{13}C NMR (75 MHz) δ 179.4, 157.4, 153.5, 147.2, 131.4, 129.2, 126.7, 117.4, 114.6, 107.8, 106.1, 66.5, 61.8, 56.9, 56.4, 38.7, 31.1, 28.9, 27.4; HRMS (ESI) *m/z* calcd for C$_{19}$H$_{23}$O$_5$ ([M+H]$^+$) 331.1540, found 331.1538.

5.5.2   Second Approach
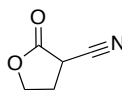
4.110

2-Oxotetrahydrofuran-3-carbonitrile (4.110).[492-493] A 3L 3-Necked flask was charged with ethyl 2-cyanoacetate (**4.114**, 370 g, 350 mL, 3.27 mol), 1,2-dibromoethane (509 mL, 5.88 mol) and K_2CO_3 (903 g, 6.54 mol), and acetone (1.3 L), the flask was equipped with a thermometer/thermometer adapter, a mechanical string rod with a teflon stir head and a stir rod condenser, and a reflux condenser. The mixture was mechanically stirred for 24 h at 68 °C (internal temp) reflux. The reaction was monitored by ^{1}HNMR for the disappearance of the SM singlet at 3.44 ppm ($CDCl_3$, 300 MHz). The reaction mixture was cooled to rt diluted with EtOAc (0.5 L), filtered, washed (EtOAc (3 L)) until the washes became colorless and the combined filtrates were concentrated in vacuo to afford a crude oil. The oil was dissolved in Et_2O (1 L) and H_2O (500 mL). The organic layer was separated and the aqueous layer was further extracted with Et_2O (2 x 500 mL). The organic layers were combined, dried ($MgSO_4$), filtered through a fritted filtered that was washed with Et_2O (200 mL), and concentrated by rotary evaporation (water bath at 50 °C) to give crude **4.115** (461 g, 2.85 mol, (86% pure contaminated with excess dibromoethane) as a orange oil that was taken on with no further purification to the saponification.

A 4 L Erlenmeyer flask containing a solution of ethyl 1-cyanocyclopropanecarboxylate (**4.115**, 300 g, 1.85 mol) in THF (0.9 L) cooled to 0 °C was treated with NaOH (2M aqueous) (1.9 L, 2 eq) in one portion and the solution was stirred for 1 h at 0 °C where analysis by TLC (1:1 hexanes:EtOAc, $KMnO_4$) indicated that the starting material was consumed. The reaction was acidified at 0 °C with portion-wise addition of 6 M aqueous HCl (620 mL over 10 min) to a pH of 2 and the aqueous layer was extracted portion-wise with EtOAc (2 x (3 x 500 mL)). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated in vacuo (water bath at 35 °C). The crude residue was concentrated from hexanes (1 L) to afford the product (191 g, 1.55

mol (90% pure)) as an off-white waxy solid. This solid was taken on to the lactone formation with no further purification.

To a 3L 3-necked flask equipped with a thermometer, mechanical stirrer, and condenser connected to a N_2 bubbler was added 1-cyanocyclopropanecarboxylic acid (**4.113**, 191 g, 1.55 mol), triethylammonium bromide (254 g, 1.39 mol) and dry MeCN (2.5 L) and was heated to 85 °C for 24 h where analysis by ^{1}H NMR indicated that the cyclopropane had been consumed. The reaction was cooled to rt, concentrated under reduced pressure, the residue was suspended in EtOAc (1 L) and filtered, washed with ethyl acetate (4 x 500 mL) and concentrated in vacuo. The crude residue was purified by short path distillation (165 °C) (internal temp 140 °C) to give **4.110** (138 g, 1.25 mol, 60% (3 steps)) as a pale yellow oil: ^{1}H NMR (300 MHz, CDCl$_3$) δ 4.54 (ddd, J = 9.3, 8.3, 3.3 Hz, 1 H), 4.35 (td, J = 9.3, 6.7 Hz, 1 H), 3.72 (t, J = 9.3 Hz, 1 H), 2.81-2.57 (m, 2 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 168.6, 114.8, 67.3, 31.4, 27.6.

Triethylammonium Hydrobromide. To a 1 L 1-neck flask containing a solution triethylamine (110 mL, 785 mmol) in acetone (0.6 L). The solution was cooled to 0 °C (ice bath) and added HBr (48% aqueous) (89 mL, 785 mmol) dropwise over 30 min. The solution was let stir for 1 h, diluted with Et$_2$O (400 mL) and the precipitated salt was filtered through a buchner funnel with filterpaper. The solid was washed Et$_2$O (1.4 L) and dried under vacuum (50 torr) at 45 °C to give the product (62.0 g, 340 mmol, 80%) as a colorless solid: Mp 256-258 (Lit 248)

General procedure for screening conditions in Table 5.2:

A solution 1-cyanocyclopropanecarboxylic acid (**4.110**, 0.0500 g, 0.450 mmol) in dry ACN (3 mL) was added additive (See Table 5.2) (0.0225 mmol, 5 mol%) and stirred at 75 °C for

24, 48, or 72 h and reaction progress was monitored by TLC and ^{1}HNMR using $CDCl_3$ as a solvent. Reactions with no product formation after 24 h were not analyzed further.

Table 5.2. Screening conditions for lactone formation.
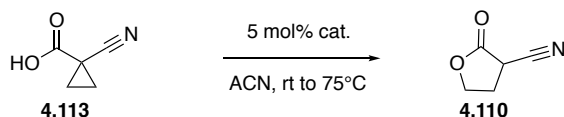
Entry	Cat. (5 mol%)	24h 75 °C	48h 75 °C	72h 75 °C
			Conditions and analysis	
1	NiBr$_2$[ab]	12%[d]	30%[d]	50%[d]
2	PtBr$_2$[ab]	NR[d]	ND	ND
3	Rh$_2$(OAc)$_4$[ab]	NR[d]	ND	ND
4	Pd(PPh$_3$)$_4$[ab]	NR[d]	ND	ND
5	I$_2$[a]	NR[d]	ND	ND
6	Yb(OTf)$_3$[a]	NR[d]	ND	ND
7	ZnCl$_2$[a]	NR[d]	ND	ND
8	Sn(OTf)$_2$[a]	NR[d]	ND	ND
9	MgI$_2$[a]	60%[d]	>95%[d]	ND
10	Ti(OiPr)$_4$[a]	NR[d]	ND	ND
11	NiCl$_2$•6H$_2$O[a]	NR[d]	NR[d]	ND
12	CaCl$_2$[a]	NR[d]	NR[d]	ND
13	CaI$_2$[a]	15%[d]	25%[d]	30%[d]
14	MnCl$_2$•4H$_2$O[a]	NR[d]	NR[d]	ND
15	MnBr$_2$[a]	NR[d]	NR[d]	ND
16	Bu$_4$NBr[a]	ND	12%	ND
17	NiI$_2$[a]	ND	76%[d]	ND
18	CuI[a]	ND	5%[d]	ND
19	NaI[a]	ND	94%[d]	ND
20	NaI[c]	ND	77%[e] (42 h)	ND

[a]50 mg scale; [b]reaction was degassed by sparging with Ar; [c]10 g scale; [d]conversion by ^{1}HNMR; [e]isolated yield; NR, no reaction; ND, not determined.

Lactonization using sodium iodide:
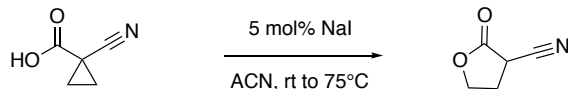
A solution of 1-cyanocyclopropanecarboxylic acid (**4.113**, 10.0 g, 81.0 mmol) in dry

MeCN (162 mL) was treated with NaI (0.607 g, 4.05 mmol) and the reaction was heated to 80 °C

for 42 h where analysis by ^{1}HNMR indicated that the cyclopropane starting material had been

consumed. The reaction was concentrated under reduced pressure and the residue was suspended

in EtOAc (150 mL), filtered, washed (EtOAc), the filtrates were combined, and concentrated

under reduced pressure. The crude residue was purified by short path distillation (150 °C at 0.25

mmHg) (internal temp 120 °C) to give **4.110** (6.97 g, 62.8 mmol, 77%) as an orange oil. NMR

data matches what was previously reported for the synthesis of this compound.
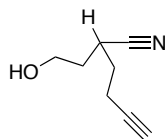
4.118

2-(2-Hydroxyethyl)hex-5-ynenitrile (4.118). A solution of 2-oxotetrahydrofuran-3-

carbonitrile (**4.110**, 22.0 g, 198 mmol) in acetone (570 mL) at rt was treated with K_2CO_3 (41.1 g,

297 mmol) and but-3-yn-1-yl 4-methylbenzenesulfonate (**4.104**, 44.4 g, 198 mmol) and the

mixture was stirred at 65 °C for 7 days. The reaction was concentrated under reduced pressure

and the solid was dissolved in CH_2Cl_2 (300 mL) and H_2O (300 mL) and the layers separated. The

aqueous layer was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were dried

(MgSO$_4$), filtered, and concentrated under reduced pressure to give the crude **4.116** (35.4, 86.8

mmol, (40% pure) as a brown oil. This oil was taken on with no further purification.

A solution of 3-(but-3-yn-1-yl)-2-oxotetrahydrofuran-3-carbonitrile (**4.116**, 35.4 g, 86.8 mmol) in THF (30 mL) at rt was treated with aqueous cesium hydroxide (52 mL, 104 mmol, 2 M, 1.2 eq) and stirred at rt for 2 h where analysis by TLC (1:1, hexanes/EtOAc, $KMnO_4$ Stain) indicated that the SM had been consumed. The reaction was concentrated under reduced pressure (rotovap bath ~25 °C) and washed with EtOAc (3 x 200 mL) and decanted where the washes became colorless. The aqueous layer was concentrated under reduced pressure (Azeotrope with toluene (3 x 100 mL) (rotovap bath at 50 °C) and the product was dried under high vacuum to give crude **4.117** (26.4 g, 84.4 mmol, 97% crude) as a waxy orange solid that was taken on to the decarboxylation with no further purification.

A solution of cesium 2-cyano-2-(2-hydroxyethyl)hex-5-ynoate (**4.117**, 26.4 g, 84.4 mmol) in THF (100 mL) and toluene (50 mL) was heated at 85 °C for 24 h, cooled to rt, and filtered. The solid/oil remaining in the flask was washed with EtOAc (3 x 100 mL) and filtered. The combined filtrates were concentrated under reduced pressure to give **4.118** (7.28 g, 53.1 mmol, 27% (3 steps) as a light orange oil: IR (CH_2Cl_2) 3452, 3288, 2952, 2887, 2239, 1433, 1053, 749 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79-3.69 (m, 2 H), 2.99 (quint, J = 7.5 Hz, 1 H), 2.57 (s, 1 H), 2.45-2.28 (m, 2 H), 2.01 (t, J = 2.4 Hz, 1 H), 1.88-1.74 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 121.2, 81.8, 70.0, 59.1, 34.2, 30.7, 27.1, 16.3; HRMS (ESI) *m/z* calcd for $C_8H_{12}NO$ ([M+H]$^+$) 138.0913, found 138.0914.
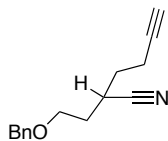
4.119

2-(2-(Benzyloxy)ethyl)hex-5-ynenitrile (4.119). A solution of 2-(2-hydroxyethyl)hex-5-ynenitrile (**4.118**, 7.28 g, 53.1 mmol) in THF (132 mL) at rt was treated with NaH (60%

dispersion in mineral oil) (4.67 g, 117 mmol) portion-wise over 5 min with vigorous stirring. After stirring for 20 min the reaction mixture was treated with BnBr (12.7 mL, 106 mmol), stirred at rt for 24 h, diluted with satd. aqueous NH$_4$Cl, and extracted with EtOAc (3 x 200 mL). The combined organic layers were dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 6:1) to give **4.119** (10.8 g, 47.6 mmol, 90%) as a yellow oil: IR (CHCl$_3$) 3290, 2889, 2242, 1495, 1452, 1094, 907, 731 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39-7.29 (m, 6 H), 4.54 (d, J = 8.0 Hz, 2 H), 3.65 (dd, J = 6.3, 5.5 Hz, 2 H), 3.06 (tt, J = 8.9, 6.1 Hz, 1 H), 3.06 (tt, J = 8.9, 6.1 Hz, 1 H), 2.50-2.35 (m, 2 H), 2.03 (t, J = 2.6 Hz, 1 H), 1.95-1.78 (m, 4 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 137.8, 128.3, 127.7, 127.6, 121.1, 81.8, 73.2, 69.9, 66.7, 32.1, 30.8, 27.5, 16.4; HRMS (ESI) *m/z* calcd for C$_{15}$H$_{18}$NO ([M+H]$^+$) 228.1383, found 228.1380.
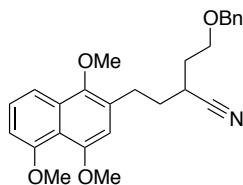
4.120

4-(Benzyloxy)-2-(2-(1,4,5-trimethoxynaphthalen-2-yl)ethyl)butanenitrile (4.120). A solution of pentacarbonyl[methoxy(methoxy-2-phenyl)carben]chromium(IV) (**4.80**, 8.01 g, 21.1 mmol) and 2-(2-(benzyloxy)ethyl)hex-5-ynenitrile (**4.119**, 7.18 g, 31.6 mmol) in dry deoxygenated THF (420 mL) was stirred at 50 °C for 14 h. The reaction was cooled to rt and added dry SiO$_2$ and concentrated under reduced pressure to a free flowing powder. The crude residue was purified by chromatography on SiO$_2$ solid load (hexanes/EtOAc, 2:1) to give the naphthol intermediate (8.1 g) that was taken on to the methylation.

A solution of the naphthol intermediate in dry DMF (42 mL) was treated with MeI (5.3 mL, 84.3 mmol) and K_2CO_3 (11.7 g, 84.3 mmol) and the reaction was stirred at rt 24 h. Analysis by LCMS indicated that the SM appeared to be consumed. The reaction was diluted with H_2O (250 mL) and brine (150 mL) and extracted with Et_2O (3 x 250 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 3:1) to give the product as a red oil. This oil was filtered through basic Al_2O_3 (hexanes/EtOAc, 1:1) to give **4.120** (5.49 g, 13.1 mmol, 62%) as a dark orange oil: IR (CH_2Cl_2) 2930, 2861, 1597, 1581, 1450, 1379, 1260, 1124, 1070, 1003, 734 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.65 (dd, J = 8.4, 1.0 Hz, 1 H), 7.42 (t, J = 8.1 Hz, 1 H), 7.35-7.28 (m, 5 H), 6.86 (dd, J = 7.5, 0.5 Hz, 1 H), 6.64 (s, 1 H), 4.50 (q, J = 10.2 Hz, 2 H), 3.97 (s, 3 H), 3.94 (s, 3 H), 3.86 (s, 3 H), 3.64 (t, J = 5.9 Hz, 2 H), 3.02 (ddd, J = 13.5, 9.2, 5.6 Hz, 1 H), 2.93-2.87 (m, 2 H), 2.02-1.91 (m, 4 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 157.4, 153.5, 147.2, 137.9, 131.5, 128.8, 128.4, 127.7 (2 C), 126.7, 121.9, 117.6, 114.6, 108.0, 106.2, 73.3, 67.0, 61.8, 56.9, 56.4, 33.1, 32.5, 28.5, 28.1; HRMS (ESI) m/z calcd for $C_{26}H_{30}NO_4$ ([M+H]$^+$) 420.2169, found 420.2167.
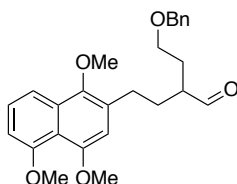
4.121

4-(Benzyloxy)-2-(2-(1,4,5-trimethoxynaphthalen-2-yl)ethyl)butanal **(4.121).** A solution of 4-(benzyloxy)-2-(2-(1,4,5-trimethoxynaphthalen-2-yl)ethyl)butanenitrile (**4.120**, 0.650 g, 1.55 mmol) in CH_2Cl_2 (7.8 mL) was cooled to -78 °C and treated with DIBAL (1.2 M in toluene, 1.4 mL, 1.70 mmol) over 5 min. The mixture was stirred at -78 °C for 30 min and

allowed to warm to rt over 2.5 h. The reaction was treated with satd. aqueous NH$_4$Cl solution (~ 1.5 mL) followed by Celite/florisil at 0 °C. The mixture was diluted with CH$_2$Cl$_2$ (20 mL), warmed slowly to room temperature, and stirred till all aluminum precipitated (20 min) and dried (MgSO$_4$). The solid was filtered through a pad of Celite/florisil and washed with CH$_2$Cl$_2$ (100 mL) and EtOAc (50 mL), and the combined filtrates were concentrated in vacuo. The crude residue was purified by chromatography on SiO$_2$ (CH$_2$Cl$_2$/EtOAc, 4:1) to give **4.121** (0.384 g, 0.909 mmol, 59%) as a red/brown oil: IR (CH$_2$Cl$_2$) 2934, 2841, 1720, 1600, 1583, 1454, 1381, 1263, 1071, 909, 729 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.64 (dd, J = 8.5, 0.9 Hz, 1 H), 7.40 (t, J = 8.1 Hz, 1 H), 7.34-7.27 (m, 5 H), 6.84 (d, J = 7.7 Hz, 1 H), 6.62 (s, 1 H), 4.46 (s, 2 H), 3.97 (s, 3 H), 3.93 (s, 3 H), 3.82 (s, 3 H), 3.56-3.48 (m, 2 H), 2.77 (ddd, J = 9.0, 6.9, 2.1 Hz, 2 H), 2.58-2.50 (m, 1 H), 2.07 (tt, J = 14.4, 7.1 Hz, 2 H), 1.94-1.76 (m, 2 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 204.5, 157.4, 153.4, 147.0, 138.1, 131.4, 129.9, 128.4, 127.6 (2 C), 126.6, 117.4, 114.6, 108.1, 106.0, 73.1, 67.7, 61.7, 56.9 (2 C), 56.4, 49.0, 29.6, 27.6; HRMS (ESI) m/z calcd for C$_{26}$H$_{31}$O$_5$ ([M+H]$^+$) 423.2166, found 423.2177.
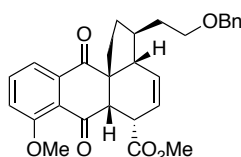
4.126

Methyl (3S,6S,6aS,12aR)-3-(2-(benzyloxy)ethyl)-8-methoxy-7,12-dioxo-1,2,3,3a,6,6a,7,12-octahydrocyclopenta[d]anthracene-6-carboxylate (4.126). A solution of 4-((benzyloxy)-2-(2-(1,4,5-trimethoxynaphthalen-2-yl)ethyl)butanal (**4.121**, 0.655 g, 1.55 mmol) in dry degassed CH$_2$Cl$_2$ (11 mL) at 0 °C was treated with (E)-methyl 4-(triphenylphosphoranylidene)but-2-enoate[470] (**4.122**, 1.55 g, 3.87 mmol) and the reaction was warmed to 50 °C for 24 h. The reaction was concentrated in vacuo and the crude residue was

purified by chromatography on SiO$_2$ (hexanes/EtOAc, 2:1) to give **4.123** (0.437 g, 0.867 mmol) as a light brown oil that was taken on with no further purification.

A solution of (2*E*,4*E*)-methyl 8-(benzyloxy)-6-(2-(1,4,5-trimethoxynaphthalen-2-yl)ethyl)octa-2,4-dienoate (**4.123**, 0.437 g, 0.866 mmol) in CH$_3$CN (44 ml) was treated with a solution of CAN (1.14 g, 2.08 mmol) in H$_2$O (14 ml) at 0 °C and stirred for 10 min at 0 °C. The orange solution was diluted with CH$_2$Cl$_2$ (50 ml). The layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (1 x 50 ml). The organic layers were combined, dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:1) to give **4.124** (0.387 g, 0.814 mmol) as a orange/brown oil.

A solution of quinone (**4.124**, 0.387 g, 0.816 mmol) in dry CH$_2$Cl$_2$ (27 mL) was treated with BF$_3$•OEt$_2$ (0.60 mL, 4.89 mmol) at 0 °C and stirred at rt for 4 h. Analysis by TLC (hexanes/EtOAc, 1:1) the starting material had been consumed. The reaction was diluted with satd. aqueous NaHCO$_3$ solution and the reaction mixture was extracted with CH$_2$Cl$_2$ (3 x 20 mL). The organic layers were combined, dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (hexanes/EtOAc, 1:2) to give **4.126** (0.181 g, 0.382 mmol, 25% (3 steps)) as a dark brown oil: IR (CH$_2$Cl$_2$) 2943, 1732, 1689, 1582, 1273, 1226, 1098, 995, 732, 697 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.63-7.56 (m, 2 H), 7.31-7.27 (m, 5 H), 7.21 (dd, *J* = 7.8, 1.7 Hz, 1 H), 6.26 (dt, *J* = 10.1, 2.3 Hz, 1 H), 5.58 (dt, *J* = 10.1, 3.0 Hz, 1 H), 4.51 (s, 2 H), 3.97 (s, 3 H), 3.79-3.74 (m, 1 H), 3.55 (t, *J* = 7.0 Hz, 2 H), 3.39 (d, *J* = 10.4 Hz, 1 H), 3.03 (s, 3 H), 2.78-2.68 (m, 1 H), 2.00-1.87 (m, 3 H), 1.78-1.66 (m, 3 H), 1.59-1.52 (m, 1 H), 1.50-1.42 (m, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 196.8, 195.4, 171.4, 158.6, 138.4, 136.5, 134.8, 131.1, 128.3, 127.6, 127.5, 122.7, 122.2, 119.1, 116.7, 77.3,

77.0, 76.7, 73.0, 69.4, 59.6, 59.4, 56.4, 52.3, 51.4, 43.5, 36.3, 33.8, 29.3; HRMS (ESI) *m/z* calcd for $C_{29}H_{31}O_6$ ([M+H]$^+$) 475.2115, found 475.2107.
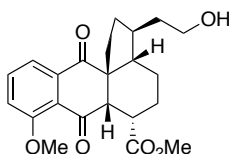
4.130

(3*S*,6*S*,6a*S*,12a*R*)-Methyl 3-(2-hydroxyethyl)-8-methoxy-7,12-dioxo-1,2,3,3a,4,5,6,6a,7,12-decahydrocyclopenta[*d*]anthracene-6-carboxylate (4.130). A solution of methyl (3*S*,6*S*,6a*S*,12a*R*)-3-(2-(benzyloxy)ethyl)-8-methoxy-7,12-dioxo-1,2,3,3a,6,6a,7,12-octahydrocyclopenta[*d*]anthracene-6-carboxylate (**4.126**, 0.311 g, 0.678 mmol) in THF (14 mL) was added 10% Pd/C (0.0722 g, 0.0678 mmol) and stirred at room temperature under hydrogen atmosphere for 2 d. The reaction mixture was filtered through Celite (eluting with EtOAc) and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (4:1, EtOAc/hexanes to 100% EtOAc) to give **4.130** (0.217 g, 0.562 mmol, 83%) as a tan foam: IR (CH_2Cl_2) 3543, 2928, 2869, 1732, 1679, 1584, 1465, 1433, 1275, 1228, 1199, 999, 729 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.64-7.61 (m, 2 H), 7.23 (quint, *J* = 4.6 Hz, 1 H), 3.98 (s, 3 H), 3.73-3.68 (m, 2 H), 3.22-3.19 (m, 1 H), 2.92 (s, 3 H), 2.89 (d, *J* = 6.6 Hz, 1 H), 2.65-2.55 (m, 1 H), 2.23-2.12 (m, 2 H), 1.97-1.89 (m, 1 H), 1.84-1.71 (m, 3 H), 1.60-1.46 (m, 3 H), 1.38-1.27 (m, 2 H), 1.06-1.00 (m, 1 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 197.0, 196.4, 173.3, 159.2, 137.7, 134.9, 122.0, 119.0, 116.7, 62.3, 60.4, 56.9, 56.5, 55.0, 50.8, 42.2, 39.0, 36.7, 36.2, 28.9, 28.1, 21.1; HRMS (ESI) *m/z* calcd for $C_{22}H_{27}O_6$ ([M+H]$^+$) 387.1802, found 357.1802.
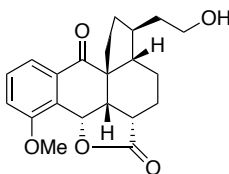
4.131

(2a*S*,2a^1*S*,5*S*,7a*R*,12b*S*)-5-(2-Hydroxyethyl)-12-methoxy-2a^1,3,4,4a,5,6,7,12b-octahydro-2*H*-cyclopenta[4,4a]anthra[9,1-*bc*]furan-2,8(2a*H*)-dione (4.131). A solution of ((3S,6S,6aS,12aR)-methyl 3-(2-hydroxyethyl)-8-methoxy-7,12-dioxo-1,2,3,3a,6,6a,7,12-octahydrocyclopenta[d]anthracene-6-carboxylate (**4.130**, 0.0630 g, 0.163 mmol) in CH$_2$Cl$_2$/MeOH (1:3, 9.3 mL) at -30 °C (cryocool) was treated with NaBH$_4$ (0.0154 g, 0.408 mmol) and warmed to rt over 3 h. Analysis by TLC (4:1, EtOAc/hexanes) and LCMS indicated that the starting material was consumed. The reaction was diluted with 1 M HCl at 0 °C, warmed to rt, and extracted with CH$_2$Cl$_2$ (3 x 20 mL). The combined organic layers were dried (MgSO$_4$), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (4:1, EtOAc/hexanes) to give **4.131** (0.0368 g, 0.103 mmol, 63%) as a colorless foam: IR (CHCl$_3$) 3536, 2934, 2865, 2252, 1754, 1679, 1588, 1471, 1273, 1182, 1061, 973, 731 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.52 (dd, *J* = 8.0, 1.1 Hz, 1 H), 7.46 (t, *J* = 8.0 Hz, 1 H), 7.14 (dd, *J* = 8.0, 1.1 Hz, 1 H), 6.16 (d, *J* = 10.0 Hz, 1 H), 3.92 (s, 3 H), 3.69 (t, *J* = 7.1 Hz, 2 H), 3.26 (t, *J* = 10.0 Hz, 1 H), 2.84 (ddd, *J* = 13.8, 11.3, 5.0 Hz, 1 H), 2.55 (qt, *J* = 10.0, 5.0 Hz, 1 H), 2.04-1.79 (m, 5 H), 1.71-1.66 (m, 1 H), 1.54-1.28 (m, 6 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 198.9, 178.3, 158.3, 132.5, 130.8, 125.6, 118.9, 115.6, 72.1, 62.1, 56.1, 53.6, 52.2, 45.9, 39.4, 38.7, 37.0, 30.4, 23.7, 21.9; HRMS (ESI) *m/z* calcd for C$_{21}$H$_{25}$O$_5$ ([M+H]$^+$) 357.1697, found 357.1693.
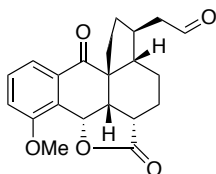
4.135

2-((2a*S*,2a^1*S*,5*S*,7a*R*,12b*S*)-12-Methoxy-2,8-dioxo-2a,2a^1,3,4,4a,5,6,7,8,12b-decahydro-2*H*-cyclopenta[4,4a]anthra[9,1-*bc*]furan-5-yl)acetaldehyde (4.135). A solution of

the (2aS,2a1S,5S,7aR,12bS)-5-(2-hydroxyethyl)-12-methoxy-2a1,3,4,4a,5,6,7,12b-octahydro-2H-cyclopenta[4,4a]anthra[9,1-bc]furan-2,8(2aH)-dione (**4.131**, 0.0667 g, 0.187 mmol) in CH$_2$Cl$_2$ (10.0 mL) at rt was treated with DMP (0.160 g, 0.374 mmol) and stirred at 0 °C for 2.5 h, diluted with CH$_2$Cl$_2$ (50 mL), 1 M Na$_2$S$_2$O$_3$ (25 mL) and satd. aqueous NaHCO$_3$ (25 mL). The organic layer was separated, and the aqueous layer was washed with CH$_2$Cl$_2$ (2 x 20 mL). The combined organic layers were washed with satd. aqueous NaHCO$_3$ (2 x 25 mL), 1 M Na$_2$S$_2$O$_3$ (25 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give **4.135** (0.0632 g, 0.178 mmol, 95%) as a yellow-orange amorphous solid: IR (CH$_2$Cl$_2$) 3059, 2937, 2870, 1760, 1719, 1681, 1588, 1273, 1077, 736 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.79 (t, J = 2.3 Hz, 1 H), 7.55 (dd, J = 8.0, 1.1 Hz, 1 H), 7.48 (t, J = 8.0 Hz, 1 H), 7.16 (dd, J = 8.0, 1.1 Hz, 1 H), 6.18 (d, J = 9.9 Hz, 1 H), 3.93 (s, 3 H), 3.27 (t, J = 10.5 Hz, 1 H), 3.05 (dtd, J = 14.9, 9.9, 5.3 Hz, 1 H), 2.84 (ddd, J = 13.8, 11.3, 5.1 Hz, 1 H), 2.63 (ddd, J = 15.8, 5.3, 2.3 Hz, 1 H), 2.35 (ddd, J = 15.8, 8.8, 2.3 Hz, 1 H), 2.12-1.99 (m, 2 H), 1.93-1.81 (m, 2 H), 1.77-1.70 (m, 1 H), 1.51-1.33 (m, 4 H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 202.2, 198.7, 178.1, 158.4, 132.4, 131.0, 125.6, 119.0, 115.8, 72.0, 56.1, 53.5, 51.6, 50.1, 45.8, 39.3, 37.0, 36.5, 30.2, 23.6, 21.5; HRMS (ESI) *m/z* calcd for C$_{21}$H$_{23}$O$_5$ ([M+H]$^+$) 355.1540, found 355.1539.
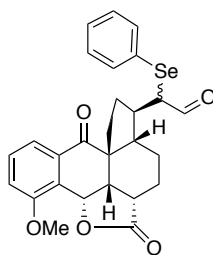
4.136

2-((2a*S*,2a^1*S*,5*R*,7a*R*,12b*S*)-12-Methoxy-2,8-dioxo-2a,2a^1,3,4,4a,5,6,7,8,12b-decahydro-2*H*-cyclopenta[4,4a]anthra[9,1-*bc*]furan-5-yl)-2-(phenylselanyl)acetaldehyde

(4.136). A solution of 2-((2aS,2a1S,4aS,5S,7aR,12bS)-12-methoxy-2,8-dioxo-2a,2a1,3,4,4a,5,6,7,8,12b-decahydro-2H-cyclopenta[4,4a]anthra[9,1-bc]furan-5-yl)acetaldehyde (**4.135**, 0.0632 g, 0.178 mmol) and *N*-(phenylseleno)phthalimide (0.0719 g, 0.214 mmol) in anhydrous CH_2Cl_2 (1.8 mL) at rt was treated with L-prolinamide (0.0010 g, 0.00892 mmol). After 2 h, analysis by TLC (2:1, EtOAc/hexanes) indicated the starting material was consumed. H_2O (5 mL) was added and the reaction was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 1:1) to give **4.136** (0.0650 g, 0.128 mmol, 72%) as a colorless foam as a 4:1 mixture of diastereomers (by ^{1}HNMR): IR (CH_2Cl_2) 3374, 3070, 2934, 2867, 1758, 1702, 1588, 1471, 1273, 1202, 1014, 979, 738 cm^{-1}; Characteristic ^{1}H NMR peaks for major diastereomer ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.44 (d, J = 5.2 Hz, 1 H), 7.56-7.53 (m, 3 H), 7.49 (t, J = 8.0 Hz, 1 H), 7.34-7.29 (m, 4 H), 7.16 (dd, J = 8.0, 1.0 Hz, 1 H), 6.19 (d, J = 9.9 Hz, 1 H), 3.94 (s, 3 H), 3.62 (dd, J = 8.3, 5.2 Hz, 1 H), 3.30 (t, J = 10.6 Hz, 1 H), 3.11-3.03 (m, 1 H), 2.81 (ddd, J = 14.1, 11.4, 5.2 Hz, 1 H), 2.16-1.94 (m, 3 H), 1.87-1.69 (m, 6 H), 1.62-1.56 (m, 2 H), 1.36-1.24 (m, 2 H); Characteristic peaks for major diastereomer ^{13}C NMR (100 MHz, CDCl$_3$) δ 198.4, 192.8, 178.0, 177.9, 158.4, 132.0, 131.0, 129.5, 129.4, 128.7, 127.2, 125.6, 119.0, 115.9, 72.0, 61.3, 54.6, 54.1, 45.6, 40.6, 39.1, 29.2, 23.5, 22.0; HRMS (ESI) *m/z* calcd for $C_{27}H_{27}O_5Se$ ([M+H]$^+$) 511.1018, found 511.1019.
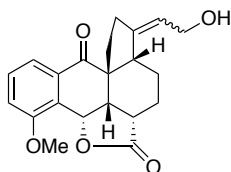
4.138

(2a*S*,2a^1*S*,7a*R*,12b*S*)-5-(2-Hydroxyethylidene)-12-methoxy-2a^1,3,4,4a,5,6,7,12b-octahydro-2*H*-cyclopenta[4,4a]anthra[9,1-*bc*]furan-2,8(2a*H*)-dione (4.138). A solution of 2-((2aS,2a1S,4aS,5R,7aR,12bS)-12-methoxy-2,8-dioxo-2a,2a1,3,4,4a,5,6,7,8,12b-decahydro-2H-cyclopenta[4,4a]anthra[9,1-bc]furan-5-yl)-2-(phenylselanyl)acetaldehyde (**4.136**, 0.0467 g, 0.0917 mmol) in THF (1.2 mL) at 0 °C was added a solution of NaIO$_4$ (0.0784 g, 0.367 mmol, 4 equiv.) in HPLC H$_2$O (1.2 mL) dropwise over 2 min and the mixture was stirred at 0 °C for 10 min and allowed to warm to rt over 3 h where by TLC the reaction looked complete. The reaction was cooled to 0 °C diluted with EtOAc (1 mL) and Satd. NaHCO$_3$ (1 mL) were added sequentially. The layers were separated; the aqueous layer was extracted with EtOAc (4 x 2 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude enal **4.137** (0.0247 g) was carried on to the Luche reduction with no further purification.

A solution of crude 2-((2aS,2a1S,7aR,12bS)-12-methoxy-2,8-dioxo-2a,3,4,4a,6,7-hexahydro-2H-cyclopenta[4,4a]anthra[9,1-bc]furan-5(2a1H,8H,12bH)-ylidene)acetaldehyde (**4.137**, 0.0247 g, 0.0701 mmol) in THF (1.2 mL) and EtOH (0.2 mL) was cooled to 0 °C and treated with cerium (III) chloride heptahydrate (0.0522 g, 0.140 mmol) and NaBH$_4$ (0.0053 g, 0.140 mmol) and stirred at 0 °C for 2 h. The reaction was diluted with Satd. NH$_4$Cl (1 mL) and allowed to warm to rt for 10 min where the reaction was extracted with EtOAc (4 x 3 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The crude residue was dissolved in MeOH and concentrated onto C18-SiO$_2$ and purified by automated reverse phase chromatography on C18 (solid load, 10% ACN:H$_2$O to 95% ACN:H$_2$O) to give **4.138** (0.0070 g, 0.0198 mmol, 21% (2 steps)) as a 2:1 mixture of isomers as a pale yellow oil: Characteristic NMR for major isomer ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.56 (d, *J* = 8.0 Hz, 1 H),

7.48 (t, J = 8.0 Hz, 1 H), 7.17 (d, J = 8.0 Hz, 1 H), 6.20 (d, J = 10.0 Hz, 1 H), 5.56 (dd, J = 7.2, 4.6 Hz, 1 H), 4.33 (dd, J = 12.0, 6.6 Hz, 1H), 4.22-4.19 (m, 1 H), 3.94 (s, 3 H), 3.36 (t, J = 10.5 Hz, 1 H), 2.89-2.83 (m, 1 H), 2.57-2.42 (m, 4 H), 2.29 (td, J = 16.0, 8.4 Hz, 1 H), 2.22-2.17 (m, 1 H), 2.07-2.03 (m, 2 H), 1.80 (ddd, J = 13.1, 6.0, 2.9 Hz, 2 H), 1.55 (dd, J = 11.6, 8.8 Hz, 1 H), 1.32-1.19 (m, 2 H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 197.5, 178.1, 158.5, 145.7, 131.6, 131.0, 125.7, 119.6, 118.9, 116.0, 72.2, 59.7, 56.2, 54.4, 47.7, 45.1, 39.0, 35.9, 32.1, 23.7, 23.4; HRMS (ESI) m/z calcd for C$_{21}$H$_{23}$O$_5$ ([M+H]$^+$) 355.1540, found 355.1539.
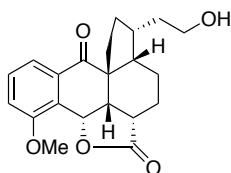
4.140

(2aS,2a1S,4aS,5R,7aR,12bS)-5-(2-Hydroxyethyl)-12-methoxy-2a1,3,4,4a,5,6,7,12b-octahydro-2H-cyclopenta[4,4a]anthra[9,1-bc]furan-2,8(2aH)-dione (4.140). A solution of (2aS,2a1S,7aR,12bS,Z)-5-(2-hydroxyethylidene)-12-methoxy-2a1,3,4,4a,5,6,7-octahydro-2H-cyclopenta[4,4a]anthra[9,1-bc]furan-2,8(2aH)-dione (**4.138**, 0.00400 g, 0.0113 mmol) in dry degassed CH$_2$Cl$_2$ (0.2 mL) in a 1 dram vial equipped with a magnetic stirbar was added Crabtree's catalyst (0.0010 g, 0.00113 mmol) under Ar. The vial was placed in a Parr bomb under Ar, sealed, purged with Ar, then purged with H$_2$ (3 x) and pressurized to 21 bar and stirred at rt for 4 h. Analysis by ^{1}H NMR and LCMS showed SM remaining. The reaction was treated with additional Crabtree's catalyst (0.0010 g, 0.00113 mmol) under Ar. The vial was placed in a Parr bomb under Ar, sealed, purged with Ar, then purged with H$_2$ (3 x), pressurized to 20 bar and stirred at rt for 16 h. The reaction was concentrated under reduced pressure. Crude ^{1}H NMR indicates consumption of the allylic alcohol SM and the product mass of **4.140** is present in the LCMS. Crude NMR peaks: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.55-7.44 (m, 2 H), 7.15 (d, J = 7.0

Hz, 1 H), 6.21-6.16 (m, 1 H), 3.99-3.91 (m, 3 H), 3.73-3.68 (m, 1 H), 3.36-3.22 (m, 1 H), 2.99-2.84 (m, 1 H), 2.33-2.17 (m, 2 H), 1.90-1.61 (m, 4 H), 1.51-1.16 (m, 4 H), 1.06-1.01 (m, 2 H), 0.89-0.85 (m, 2 H); HRMS (ESI) m/z calcd for $C_{21}H_{25}O_5$ ([M+H]$^+$) 357.1697, found 357.1695.
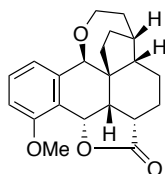
4.141

(2aS,2a1S,4aS,4a1R,5R,8aS,12bS)-12-methoxy-2a,2a1,3,4,4a,5,6,7,8a,12b-decahydro-2H-4a1,5-ethanofuro[4',3',2':4,10]anthra[9,1-bc]oxepin-2-one (4.141). A solution of crude (2aS,2a1S,4aS,5R,7aR,12bS)-5-(2-Hydroxyethyl)-12-methoxy-2a1,3,4,4a,5,6,7,12b-octahydro-2H-cyclopenta[4,4a]anthra[9,1-bc]furan-2,8(2aH)-dione (**4.140**, 0.0050 g, 0.0140 mmol) in dry CH$_2$Cl$_2$ (7.0 mL) cooled to 0 °C was treated with a freshly prepared solution of TMSOTf (1M in CH$_2$Cl$_2$) (28 μL, 0.0281 mmol) followed by Et$_3$SiH (6.7 μL, 0.0421 mmol) and stirred at 0 °C for 1 h. The reaction was warmed to rt for 4 h where analysis by TLC (EtOAc/hexanes, 4:1; CAM stain) indicated the formation of a new more non-polar spot. The reaction was cooled to 0 °C and diluted with Satd. NaHCO$_3$ (2 mL) and extracted with CH$_2$Cl$_2$ (3 x 2 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO$_2$ (gradient, hexanes/EtOAc, 1:1 to EtOAc) to afford mostly recovered SM along with a mixed fraction of two non-polar spots (>0.5 mg): HRMS (ESI) m/z calcd for $C_{21}H_{25}O_4$ ([M+H]$^+$) 341.1747, found 341.1746.

APPENDIX A

X-RAY DATA

A.1 X-RAY STRUCTURE AND DATA FOR (1R,2S)-JJ450
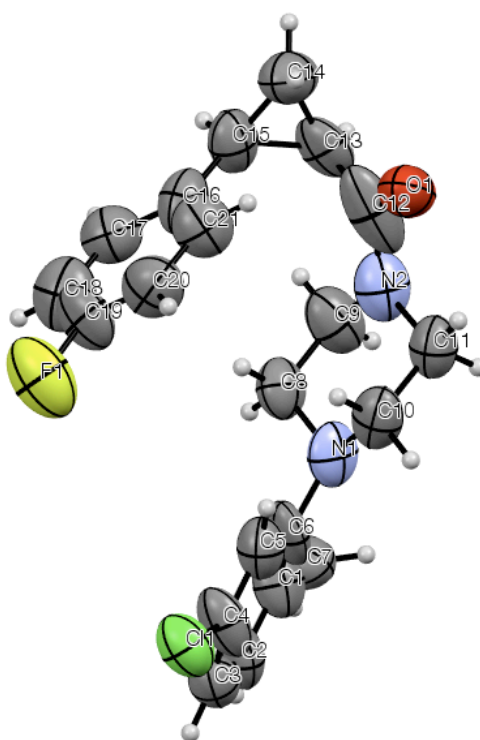
Figure A.1. X-ray crystal structure of (1*R*,2*S*)-**JJ450**.

Table A.1. Crystal data and structure refinement for (1*R*,2*S*)-**JJ450.**

Identification code	(1*R*,2*S*)-**JJ450**
Chemical formula	$C_{21}H_{22}ClFN_2O$
Formula weight	372.85 g/mol
Temperature	230(2) K
Wavelength	1.54178 Å
Crystal size	0.010 x 0.020 x 0.160 mm
Crystal system	Monoclinic
Space group	P 1 21 1
Unit cell dimensions	a = 8.9297(6) Å $\quad$ α = 90°
	b = 10.0143(7) Å $\quad$ β = 93.382(5)°
	c = 10.7194(8) Å $\quad$ γ = 90°
Volume	956.91(12) Å^3
Z	2
Density (calculated)	1.294 g/cm^3
Absorption coefficient	1.942 mm^{-1}
F(000)	392
Diffractometer	Bruker Apex II CCD
Radiation source	IMuS micro-focus source, Cu
Theta range for data collection	4.13 to 68.31°
Index ranges	-10<=h<=10, -11<=k<=12, -12<=l<=12
Reflections collected	13088
Independent reflections	3491 [R(int) = 0.1250]
Absorption correction	multi-scan
Max. and min. transmission	0.9800 and 0.7500
Structure solution technique	direct methods
Structure solution program	SHELXS-97 (Sheldrick, 2008)
Refinement method	Full-matrix least-squares on F^2
Refinement program	SHELXL-97 (Sheldrick, 2008)
Function minimized	$\Sigma \, w(F_o^2 - F_c^2)^2$
Data / restraints / parameters	3491 / 1 / 237
Goodness-of-fit on F^2	1.725
Δ/σ$_{max}$	0.004
Final R indices [I>2σ(I)]	R1 = 0.1107, wR2 = 0.2229
R indicies (all data)	R1 = 0.2637, wR2 = 0.2932
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0680P)^2]$ where $P=(F_o^2+2F_c^2)/3$
Absolute structure parameter	0.05(7)
Extinction coefficient	0.0760(100)
Largest diff. peak and hole	0.555 and -0.639 eÅ^{-3}
R.M.S. deviation from mean	0.143 eÅ^{-3}

Table A.2. Atomic coordinates and equivalent isotropic atomic displacement parameters (Å^2) for (1*R*,2*S*)-

JJ450.

	x/a	y/b	z/c	U(eq)
Cl1	0.8388(4)	0.4211(5)	0.6385(4)	0.0959(19)
F1	0.7333(11)	0.6332(12)	0.2209(12)	0.142(5)
O1	0.1000(13)	0.2703(13)	0.1361(11)	0.096(4)
N1	0.2734(15)	0.4723(13)	0.5135(14)	0.084(4)
N2	0.0834(15)	0.4113(18)	0.2985(18)	0.099(5)
C1	0.377(2)	0.5931(17)	0.692(2)	0.087(5)
C2	0.500(2)	0.6171(17)	0.7724(18)	0.088(5)
C3	0.6389(19)	0.5680(16)	0.7653(16)	0.080(5)
C4	0.6607(17)	0.4813(17)	0.660(2)	0.095(6)
C5	0.5424(19)	0.4540(14)	0.5773(16)	0.085(5)
C6	0.4026(18)	0.5086(19)	0.5993(19)	0.085(5)
C7	0.2290(17)	0.6514(18)	0.7079(14)	0.089(5)
C8	0.2331(17)	0.5738(15)	0.4151(16)	0.083(5)
C9	0.082(2)	0.5420(19)	0.3531(19)	0.104(6)
C10	0.2783(18)	0.3412(19)	0.4524(17)	0.090(6)
C11	0.1289(17)	0.3017(17)	0.3865(16)	0.084(5)
C12	0.074(2)	0.384(2)	0.173(3)	0.134(12)
C13	0.0245(17)	0.487(2)	0.0836(19)	0.094(6)
C14	0.071(2)	0.4722(19)	0.9512(17)	0.104(6)
C15	0.1354(19)	0.5830(16)	0.0329(17)	0.091(6)
C16	0.299(2)	0.588(2)	0.0775(16)	0.090(5)
C17	0.358(2)	0.717(2)	0.1101(15)	0.092(6)
C18	0.504(3)	0.731(2)	0.159(2)	0.117(7)
C19	0.591(2)	0.622(3)	0.1697(19)	0.100(6)
C20	0.536(2)	0.501(3)	0.1398(18)	0.108(7)
C21	0.388(2)	0.482(2)	0.0893(16)	0.097(6)

U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Table A.3. Bond lengths (Å) for (1*R*,2*S*)-**JJ450**.

Cl1-C4	1.728(16)	F1-C19	1.36(2)
O1-C12	1.23(2)	N1-C10	1.47(2)
N1-C6	1.478(19)	N1-C8	1.494(17)
N2-C12	1.37(3)	N2-C9	1.43(2)
N2-C11	1.49(2)	C1-C6	1.33(2)
C1-C2	1.38(2)	C1-C7	1.46(2)
C2-C3	1.34(2)	C2-H2A	0.94
C3-C4	1.45(2)	C3-H3A	0.94
C4-C5	1.365(19)	C5-C6	1.40(2)
C5-H5A	0.94	C7-H7A	0.97
C7-H7B	0.97	C7-H7C	0.97
C8-C9	1.50(2)	C8-H8A	0.98
C8-H8B	0.98	C9-H9A	0.98
C9-H9B	0.98	C10-C11	1.525(19)
C10-H10A	0.98	C10-H10B	0.98
C11-H11A	0.98	C11-H11B	0.98
C12-C13	1.46(3)	C13-C15	1.51(2)
C13-C14	1.51(2)	C13-H13A	0.99
C14-C15	1.51(2)	C14-H14A	0.98
C14-H14B	0.98	C15-C16	1.51(2)
C15-H15A	0.99	C16-C21	1.33(2)
C16-C17	1.43(2)	C17-C18	1.39(2)
C17-H17A	0.94	C18-C19	1.34(3)
C18-H18A	0.94	C19-C20	1.34(3)
C20-C21	1.41(2)	C20-H20A	0.94
C21-H21A	0.94		

Table A.4. Bond angles (°) for (1*R*,2*S*)-**JJ450**.

C10-N1-C6	117.0(14)	C10-N1-C8	107.8(13)
C6-N1-C8	114.7(13)	C12-N2-C9	125.7(19)
C12-N2-C11	118.2(19)	C9-N2-C11	115.0(16)
C6-C1-C2	114.3(16)	C6-C1-C7	122.5(18)
C2-C1-C7	123.1(18)	C3-C2-C1	127.3(19)
C3-C2-H2A	116.4	C1-C2-H2A	116.4
C2-C3-C4	115.6(16)	C2-C3-H3A	122.2
C4-C3-H3A	122.2	C5-C4-C3	119.4(15)
C5-C4-Cl1	121.8(17)	C3-C4-Cl1	118.7(14)
C4-C5-C6	118.6(17)	C4-C5-H5A	120.7
C6-C5-H5A	120.7	C1-C6-C5	124.7(16)

C1-C6-N1	117.2(17)	C5-C6-N1	118.2(19)
C1-C7-H7A	109.5	C1-C7-H7B	109.5
H7A-C7-H7B	109.5	C1-C7-H7C	109.5
H7A-C7-H7C	109.5	H7B-C7-H7C	109.5
N1-C8-C9	109.9(13)	N1-C8-H8A	109.7
C9-C8-H8A	109.7	N1-C8-H8B	109.7
C9-C8-H8B	109.7	H8A-C8-H8B	108.2
N2-C9-C8	110.3(15)	N2-C9-H9A	109.6
C8-C9-H9A	109.6	N2-C9-H9B	109.6
C8-C9-H9B	109.6	H9A-C9-H9B	108.1
N1-C10-C11	113.0(14)	N1-C10-H10A	109.0
C11-C10-H10A	109.0	N1-C10-H10B	109.0
C11-C10-H10B	109.0	H10A-C10-H10B	107.8
N2-C11-C10	107.5(14)	N2-C11-H11A	110.2
C10-C11-H11A	110.2	N2-C11-H11B	110.2
C10-C11-H11B	110.2	H11A-C11-H11B	108.5
O1-C12-N2	120.(3)	O1-C12-C13	120.(3)
N2-C12-C13	120.(2)	C12-C13-C15	120.7(14)
C12-C13-C14	117.4(19)	C15-C13-C14	59.8(12)
C12-C13-H13A	115.8	C15-C13-H13A	115.8
C14-C13-H13A	115.8	C15-C14-C13	60.0(12)
C15-C14-H14A	117.8	C13-C14-H14A	117.8
C15-C14-H14B	117.8	C13-C14-H14B	117.8
H14A-C14-H14B	114.9	C14-C15-C16	122.3(16)
C14-C15-C13	60.2(10)	C16-C15-C13	123.4(15)
C14-C15-H15A	113.6	C16-C15-H15A	113.6
C13-C15-H15A	113.6	C21-C16-C17	119.2(18)
C21-C16-C15	124.7(18)	C17-C16-C15	116.1(17)
C18-C17-C16	120.4(19)	C18-C17-H17A	119.8
C16-C17-H17A	119.8	C19-C18-C17	119.(2)
C19-C18-H18A	120.7	C17-C18-H18A	120.7
C20-C19-C18	121.(2)	C20-C19-F1	120.(2)
C18-C19-F1	119.(2)	C19-C20-C21	122.6(19)
C19-C20-H20A	118.7	C21-C20-H20A	118.7
C16-C21-C20	118.2(18)	C16-C21-H21A	120.9
C20-C21-H21A	120.9		

Table A.5. Anisotropic atomic displacement parameters (Å^2) for (1R,2S)-**JJ450**.

	U$_{11}$	U$_{22}$	U$_{33}$	U$_{23}$	U$_{13}$	U$_{12}$
Cl1	0.068(3)	0.090(3)	0.129(4)	-0.012(3)	-0.001(2)	0.004(2)
F1	0.073(8)	0.145(10)	0.209(13)	-0.035(9)	0.009(7)	0.001(6)
O1	0.102(10)	0.091(10)	0.092(8)	0.007(8)	-0.018(7)	-0.008(8)
N1	0.091(11)	0.040(8)	0.121(12)	0.009(9)	0.010(9)	0.012(7)
N2	0.081(10)	0.074(13)	0.142(15)	0.003(14)	0.014(10)	0.010(10)
C1	0.076(13)	0.056(11)	0.130(16)	-0.009(12)	0.020(12)	0.010(10)
C2	0.059(11)	0.082(12)	0.125(16)	0.013(11)	0.010(11)	-0.011(10)
C3	0.073(13)	0.074(12)	0.093(13)	0.010(11)	-0.008(10)	-0.001(9)
C4	0.043(10)	0.079(13)	0.161(18)	-0.013(12)	-0.003(11)	0.005(8)
C5	0.074(12)	0.069(12)	0.113(13)	0.013(10)	0.013(10)	-0.003(10)
C6	0.038(10)	0.071(12)	0.145(17)	0.017(12)	-0.012(10)	-0.013(9)
C7	0.082(12)	0.110(15)	0.076(12)	-0.003(10)	0.009(9)	-0.001(10)
C8	0.089(13)	0.059(12)	0.103(13)	-0.008(11)	0.015(10)	-0.004(10)
C9	0.092(15)	0.069(14)	0.149(19)	-0.006(13)	-0.017(12)	0.008(11)
C10	0.054(11)	0.092(16)	0.124(15)	0.033(13)	0.005(10)	0.010(10)
C11	0.073(13)	0.071(12)	0.111(14)	0.015(12)	0.015(10)	0.002(10)
C12	0.054(13)	0.061(15)	0.28(4)	-0.02(2)	-0.024(18)	0.017(10)
C13	0.040(10)	0.108(17)	0.132(18)	-0.008(13)	-0.015(10)	0.003(10)
C14	0.135(17)	0.096(15)	0.078(13)	0.002(11)	-0.008(12)	-0.012(11)
C15	0.078(13)	0.064(12)	0.129(16)	0.021(12)	-0.002(11)	0.003(10)
C16	0.102(15)	0.069(14)	0.103(13)	0.004(11)	0.036(11)	0.003(13)
C17	0.088(16)	0.099(17)	0.089(14)	-0.001(11)	0.010(12)	0.015(12)
C18	0.096(18)	0.115(17)	0.142(19)	0.004(14)	0.038(15)	0.019(15)
C19	0.039(11)	0.123(18)	0.139(17)	-0.014(14)	0.005(10)	-0.002(13)
C20	0.069(15)	0.13(2)	0.122(17)	0.019(15)	0.000(12)	0.012(13)
C21	0.088(14)	0.083(14)	0.119(16)	-0.005(11)	0.004(11)	0.016(11)

The anisotropic atomic displacement factor exponent takes the form: $-2\pi^2[\,h^2\,a^{*2}\,U_{11} + ... + 2\,h\,k\,a^*\,b^*\,U_{12}\,]$

Table A.6. Hydrogen atomic coordinates and isotropic atomic displacement parameters (Å^2) for (1R,2S)-**JJ450.**

	x/a	y/b	z/c	U(eq)
H2A	0.4847	0.6743	0.8400	0.106
H3A	0.7170	0.5885	0.8249	0.096
H5A	0.5548	0.3996	0.5072	0.102
H7A	0.2343	0.7099	0.7802	0.134
H7B	0.1974	0.7023	0.6340	0.134

	x/a	y/b	z/c	U(eq)
H7C	0.1572	0.5806	0.7204	0.134
H8A	0.3090	0.5741	0.3527	0.1
H8B	0.2308	0.6628	0.4530	0.1
H9A	0.0058	0.5460	0.4148	0.125
H9B	0.0569	0.6084	0.2881	0.125
H10A	0.3070	0.2735	0.5154	0.108
H10B	0.3556	0.3427	0.3913	0.108
H11A	0.1397	0.2179	0.3407	0.101
H11B	0.0528	0.2891	0.4477	0.101
H13A	-0.0778	0.5223	0.0925	0.113
H14A	0.1377	0.3976	-0.0671	0.124
H14B	-0.0017	0.4961	-0.1168	0.124
H15A	0.0921	0.6718	0.0119	0.109
H17A	0.2965	0.7933	0.0983	0.11
H18A	0.5417	0.8152	0.1841	0.14
H20A	0.5977	0.4259	0.1531	0.13
H21A	0.3538	0.3968	0.0647	0.116

APPENDIX B

CIRCULAR DICHROISM SPECTRA

All CD spectra were measured on a Jasco J-815 CD spectrometer using a quartz cuvette (0.1 cm)

in length. All samples were dissolved in HPLC grade methanol using a 2 mL volumetric flask.
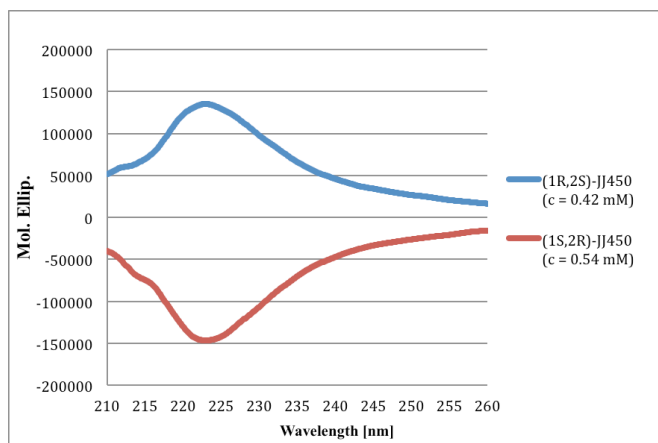
Figure B.1. CD spectra for enantiomers of **JJ450**.
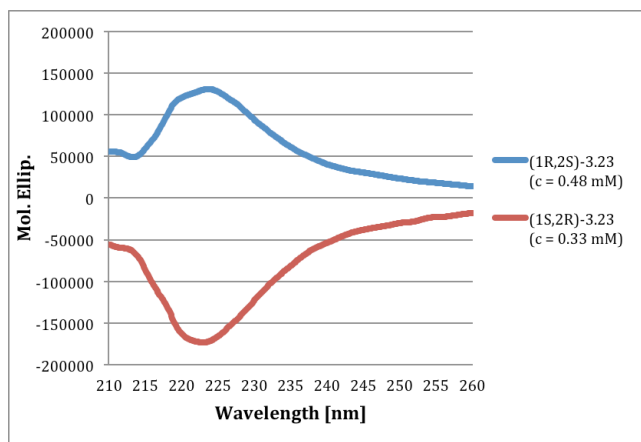
Figure B.2. CD spectra for enantiomers of **3.23**.
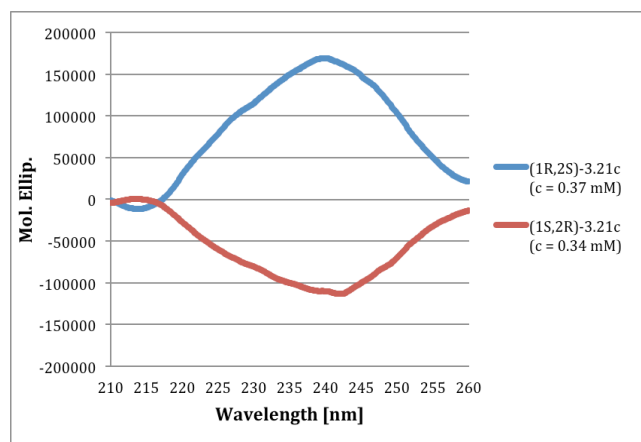
Figure B.3. CD spectra for enantiomers of **3.21c**.
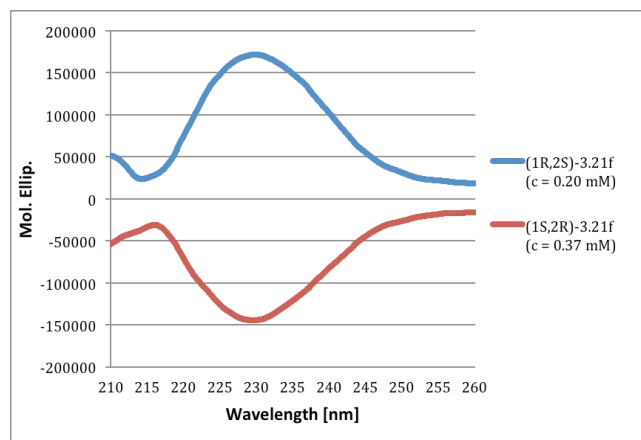
Figure B.4. CD spectra for enantiomers of **3.21f**.
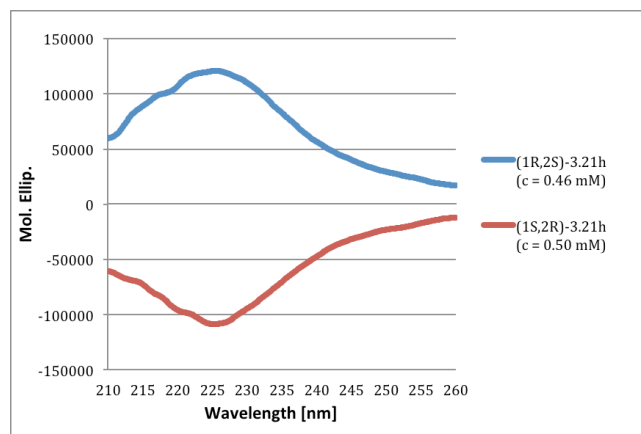
Figure B.5. CD spectra for enantiomers of **3.21h**.
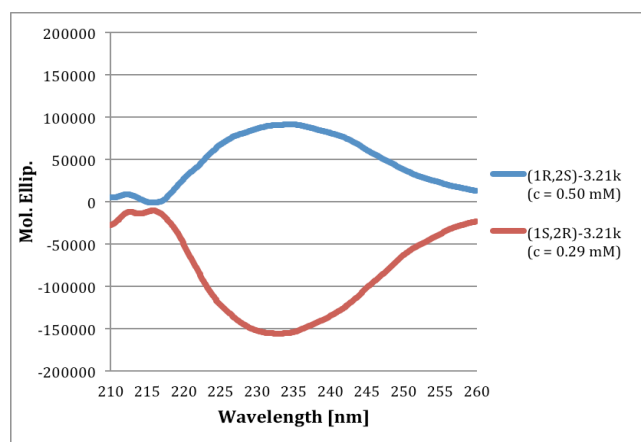
Figure B.6. CD spectra for enantiomers of **3.21k**.
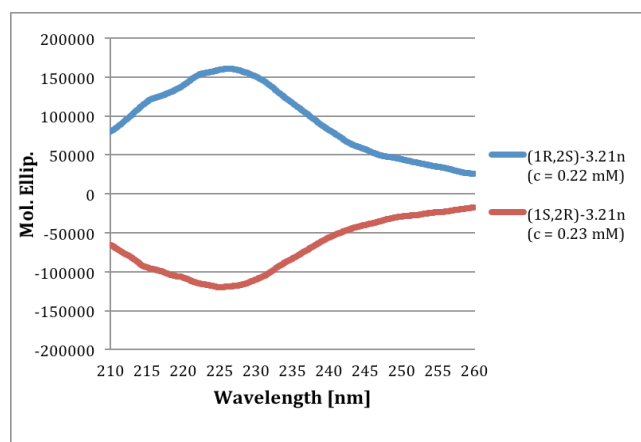
Figure B.7. CD spectra for enantiomers of **3.21n**.

EC50 CURVES

All EC50's are preliminary and were calculated using GraphPad prism using data obtained from the luciferase assay performed in the Wang lab. All data was normalized (0%, Y = 0; 100%, First value in data set). The normalized data was analyzed using a nonlinear regression (curve fit) (log(inhibitor) vs normalized response – Variable slope) with a least squares fit.
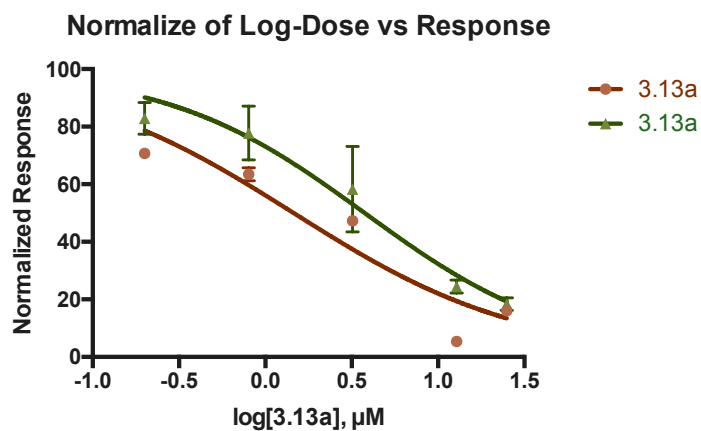
Figure C.1. EC$_{50}$ curves for **3.13a**.
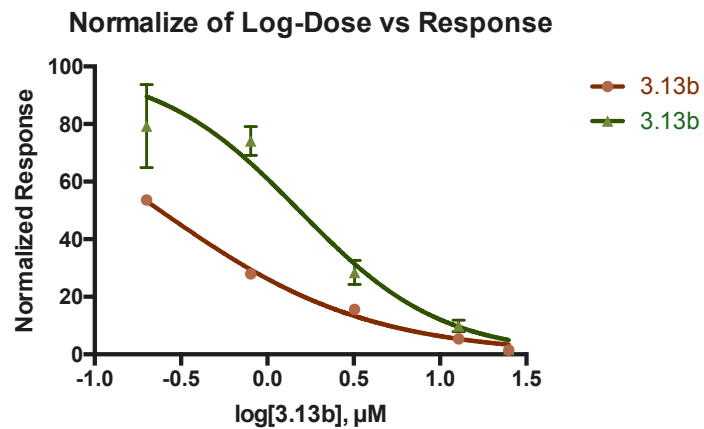
Figure C.2. EC$_{50}$ curves for **3.13b**.
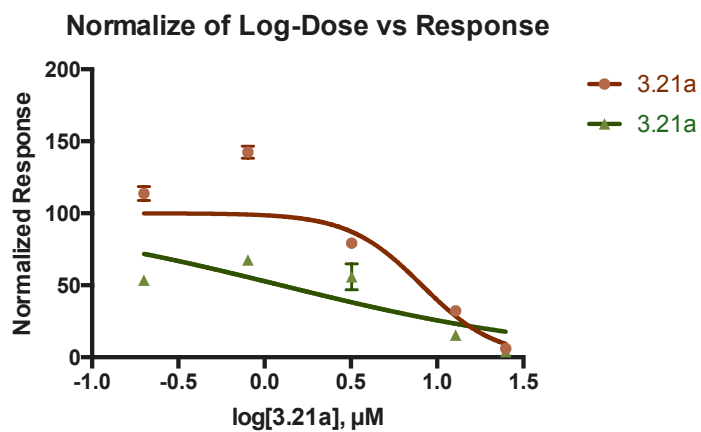
Figure C.3. EC$_{50}$ curves for **3.21a**.
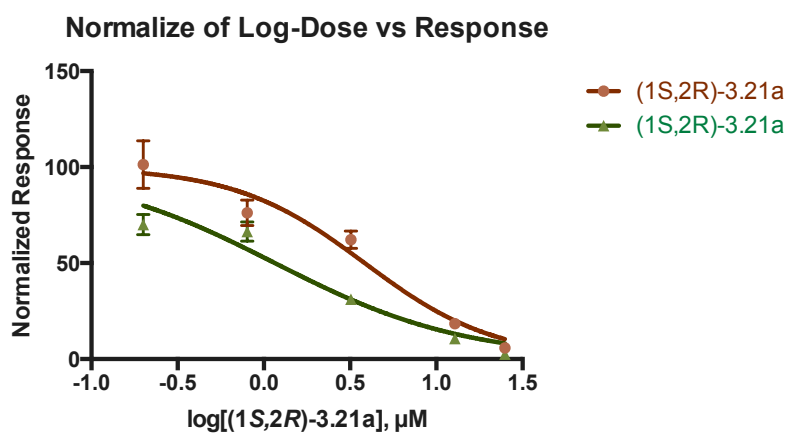
Figure C.4. EC$_{50}$ curves for (1*S*,2*R*)-**3.21a**.
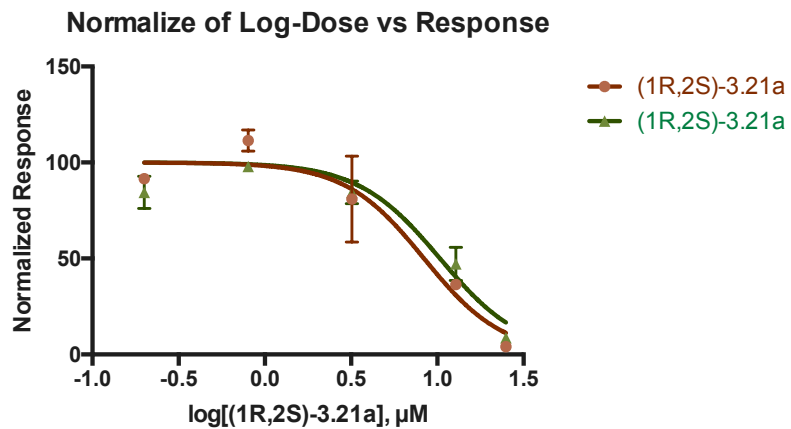
Figure C.5. EC$_{50}$ curves for (1*R*,2*S*)-**3.21a**.
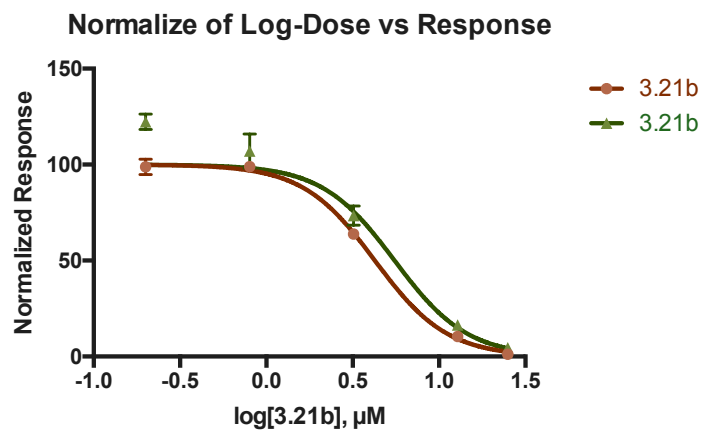
Figure C.6. EC$_{50}$ curves for **3.21b**.
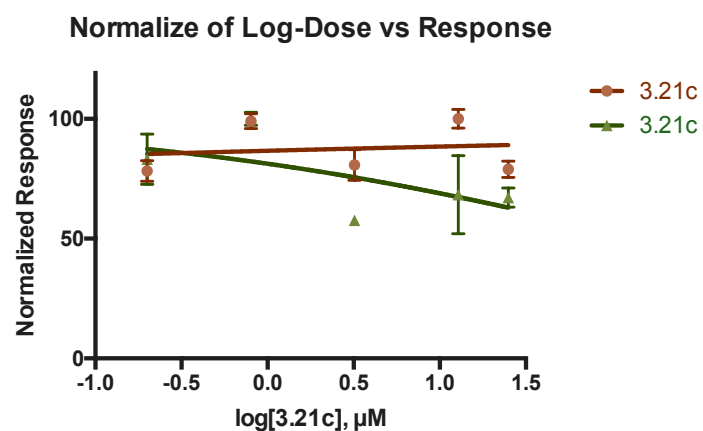
Figure C.7. EC$_{50}$ curves for **3.21c**.
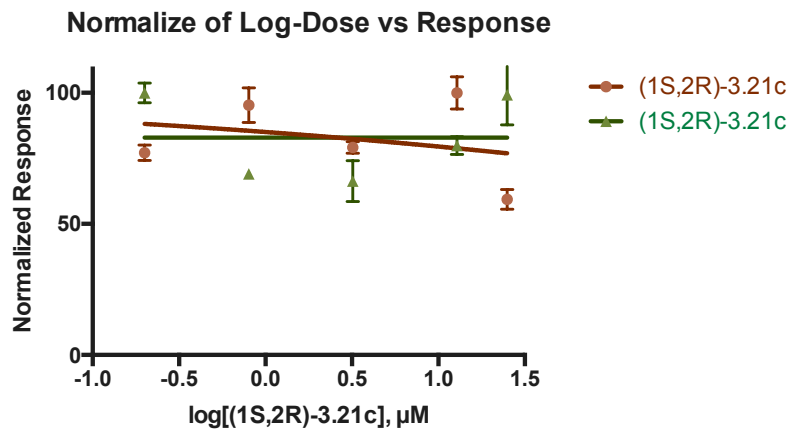
Figure C.8. EC$_{50}$ curves for (1*S*,2*R*)-**3.21c**.
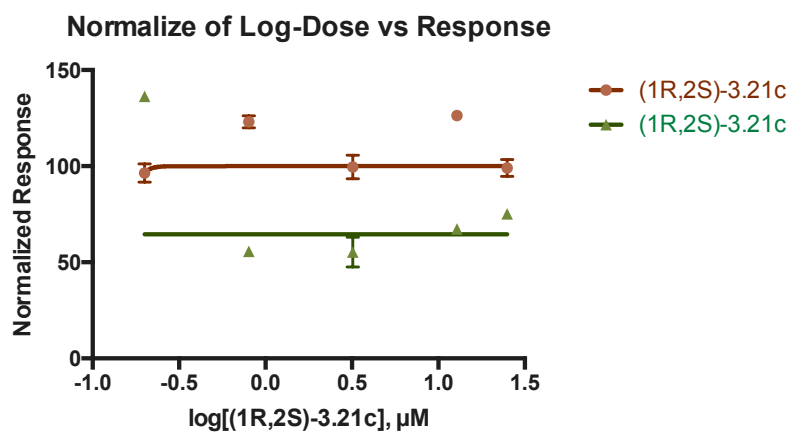
Figure C.9. EC$_{50}$ curves for (1*R*,2*S*)-**3.21c**.
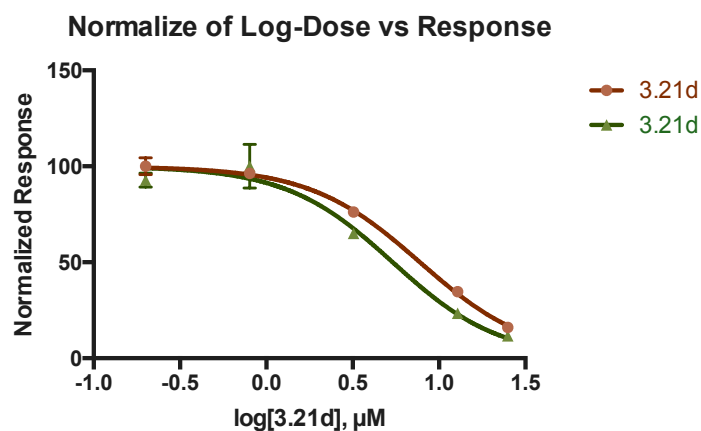
Figure C.10. EC$_{50}$ curves for **3.21d**.
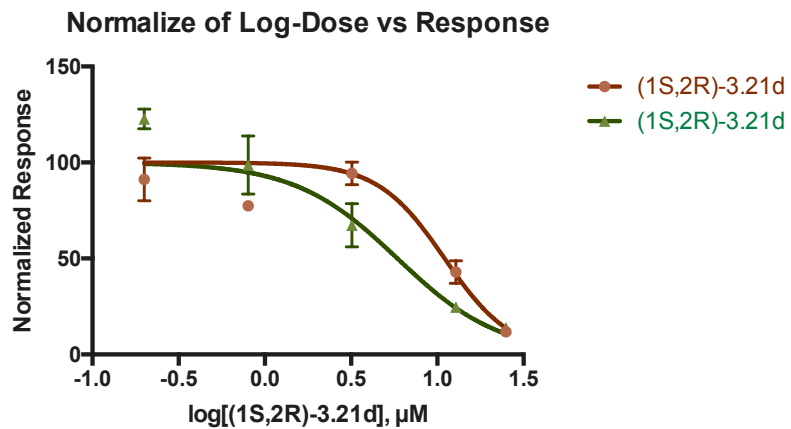
Figure C.11. EC$_{50}$ curves for (1*S*,2*R*)-**3.21d**.
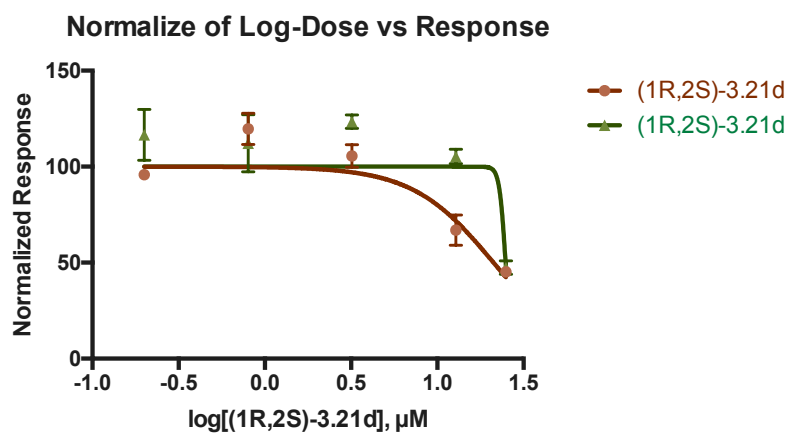
Figure C.12. EC$_{50}$ curves for (1*R*,2*S*)-**3.21d**.
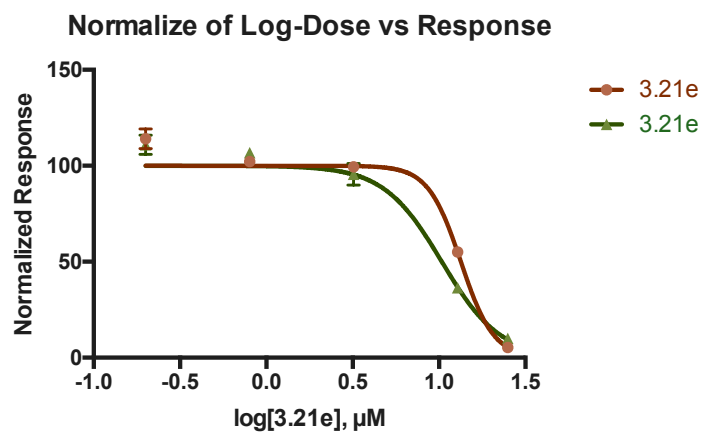
Figure C.13. EC$_{50}$ curves for **3.21e**.
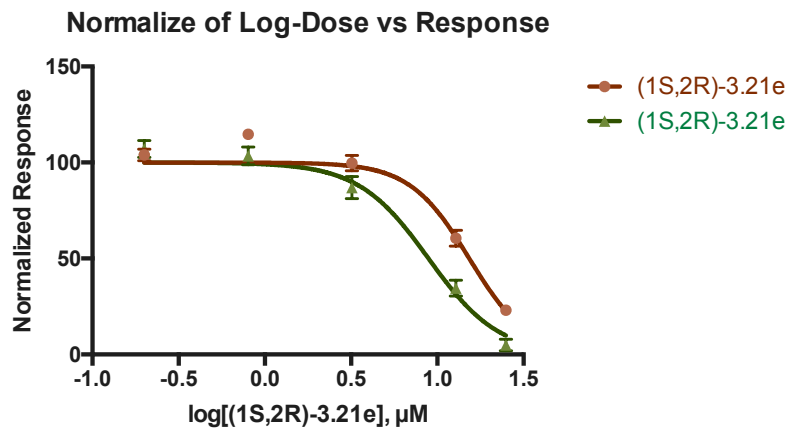
Figure C.14. EC$_{50}$ curves for (1*S*,2*R*)-**3.21e**.
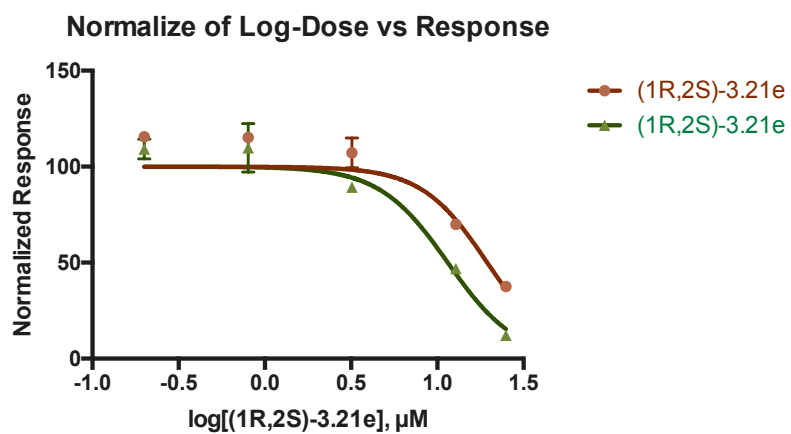
Figure C.15. EC$_{50}$ curves for (1*R*,2*S*)-**3.21e**.
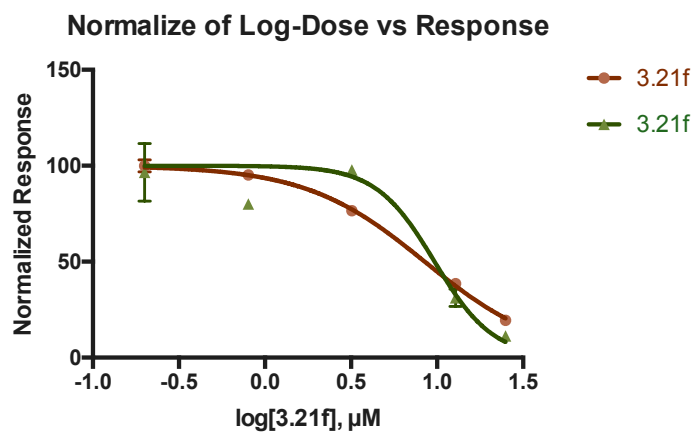
Figure C.16. EC$_{50}$ curves for **3.21f**.
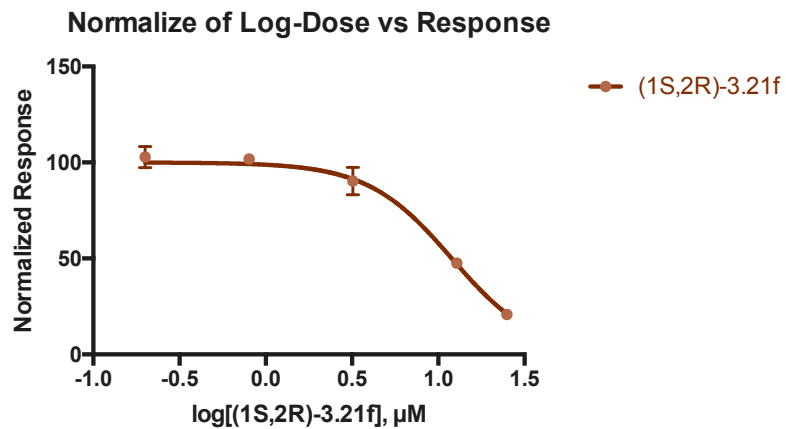
Figure C.17. EC$_{50}$ curve for (1*S*,2*R*)-**3.21f**.
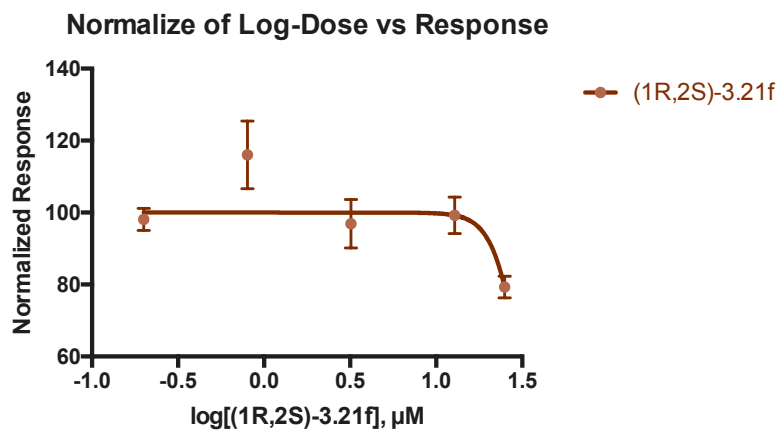
Figure C.18. EC$_{50}$ curve for (1*R*,2*S*)-**3.21f**.
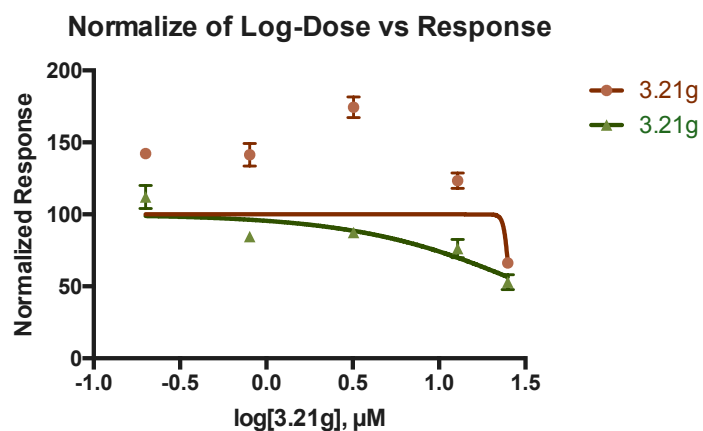
Figure C.19. EC$_{50}$ curves for **3.21g**.

Normalize of Log-Dose vs Response
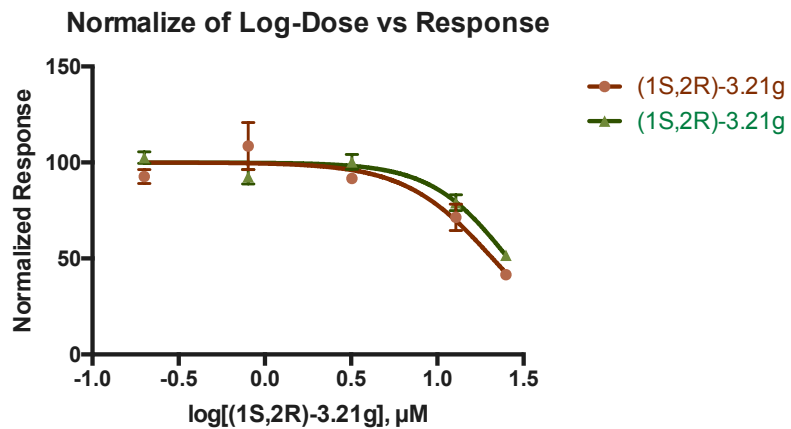
Figure C.20. EC$_{50}$ curves for (1*S*,2*R*)-**3.21g**.

Normalize of Log-Dose vs Response
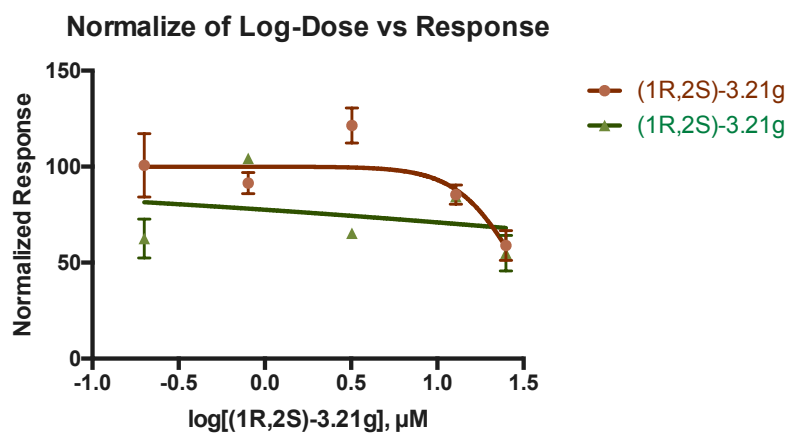
Figure C.21. EC$_{50}$ curves for (1*R*,2*S*)-**3.21g**.

Normalize of Log-Dose vs Response
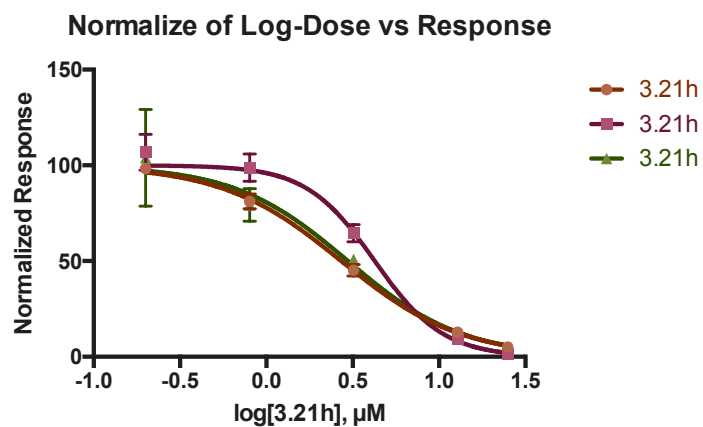
Figure C.22. EC$_{50}$ curves for **3.21h**.
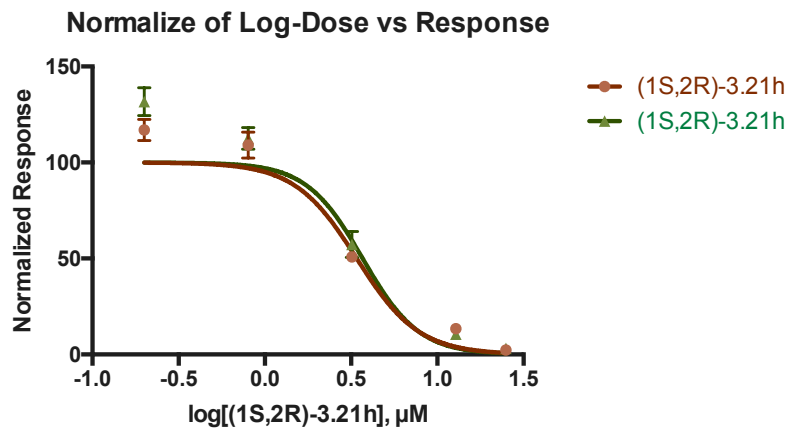
Figure C.23. EC$_{50}$ curves for (1*S*,2*R*)-**3.21h**.
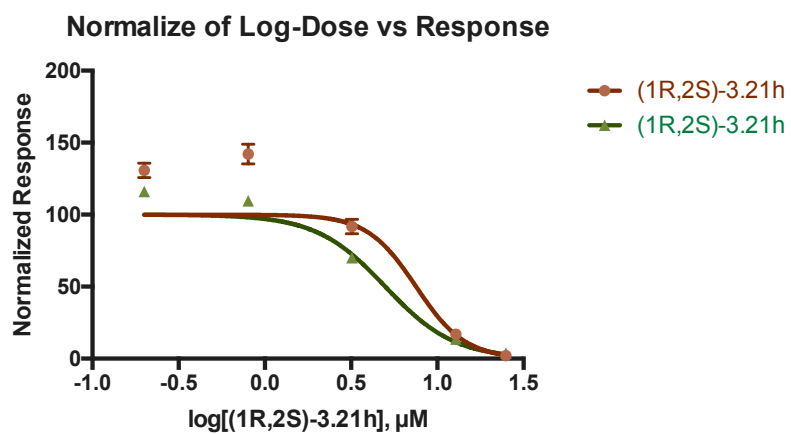
Figure C.24. EC$_{50}$ curves for (1*R*,2*S*)-**3.21h**.
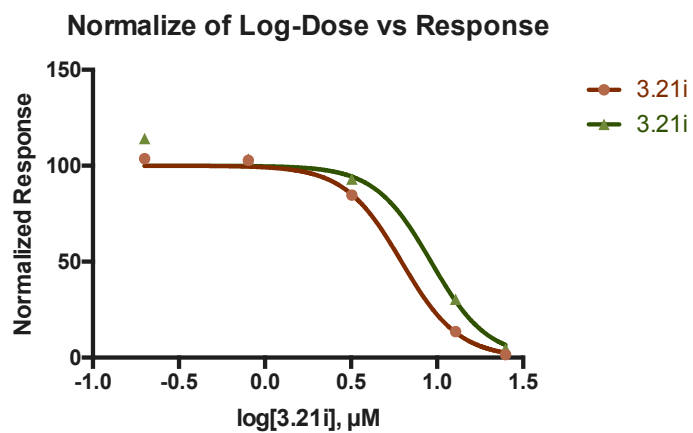
Figure C.25. EC$_{50}$ curves for **3.21i**.
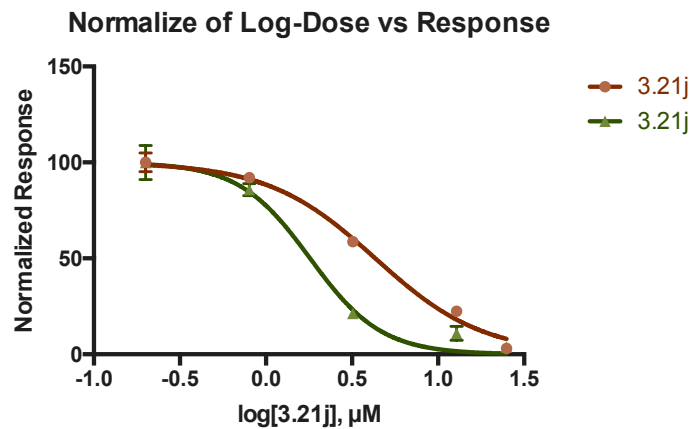
Figure C.26. EC$_{50}$ curves for **3.21j**.
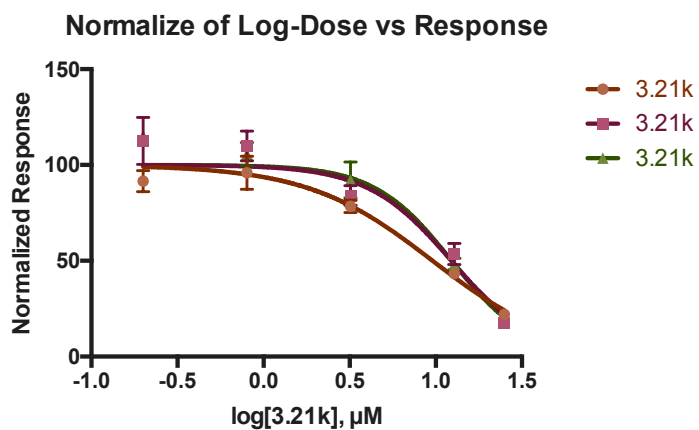
Figure C.27. EC$_{50}$ curves for **3.21k**.
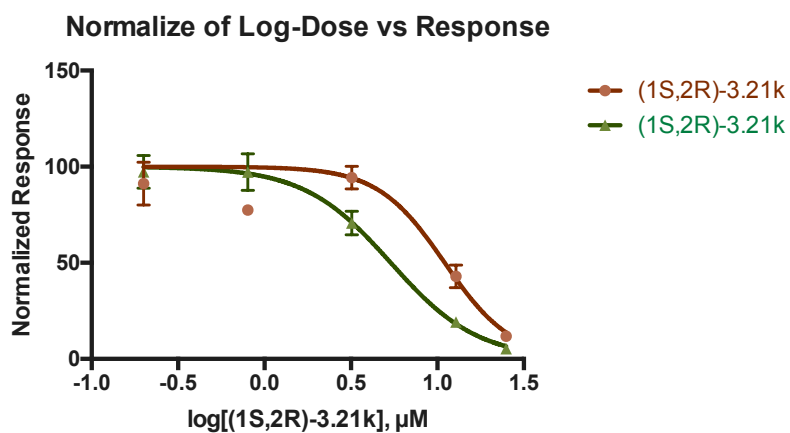
Figure C.28. EC$_{50}$ curves for (1*S*,2*R*)-**3.21k**.
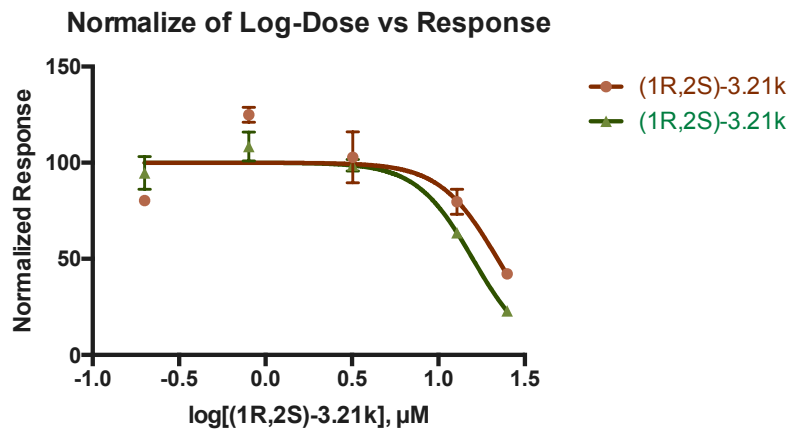
Figure C.29. EC$_{50}$ curves for (1*R*,2*S*)-**3.21k**.
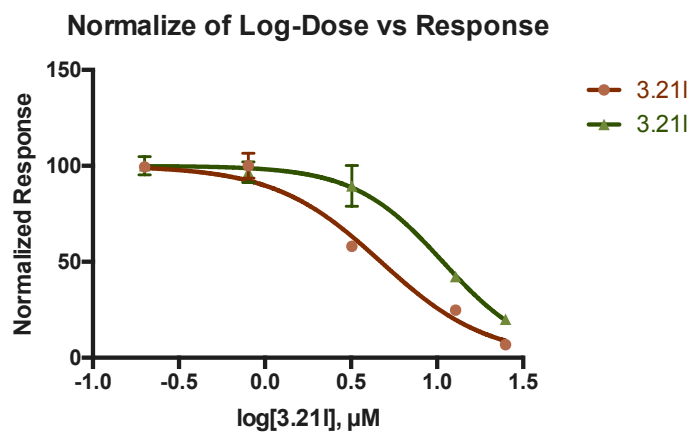
Figure C.30. EC$_{50}$ curves for **3.21l**.
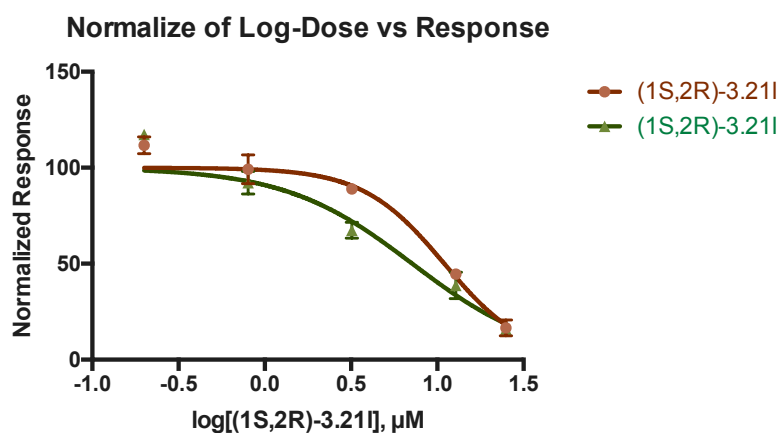
Figure C.31. EC$_{50}$ curves for (1*S*,2*R*)-**3.21l**.
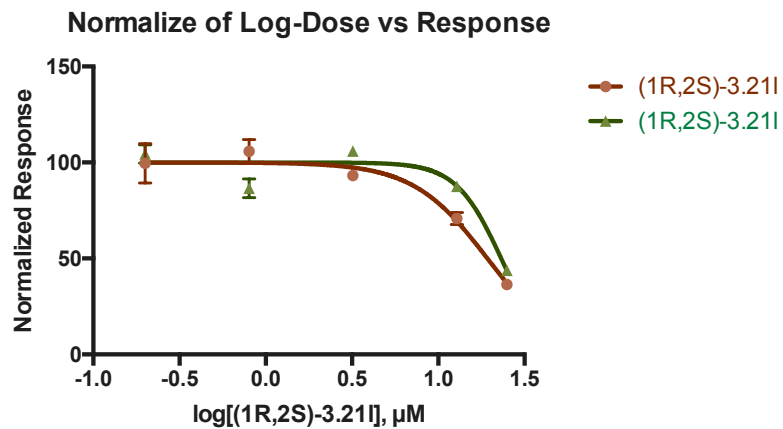
Figure C.32. EC$_{50}$ curves for (1*R*,2*S*)-**3.21l**.
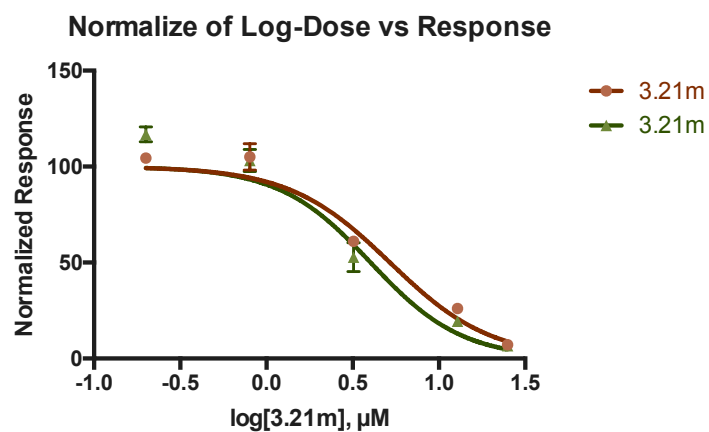
Figure C.33. EC$_{50}$ curves for **3.21m**.
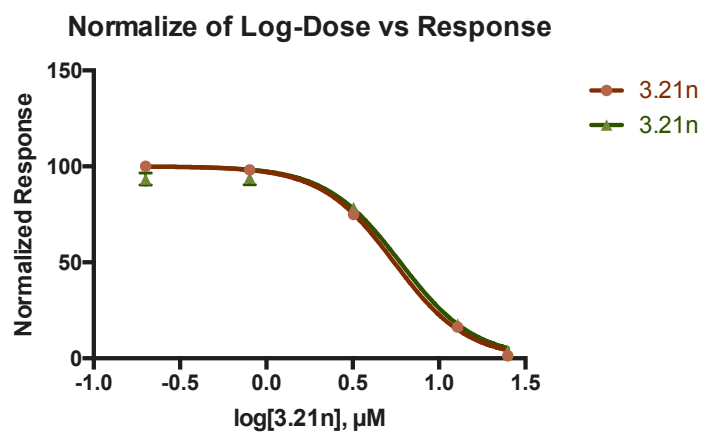
Figure C.34. EC$_{50}$ curves for **3.21n**.
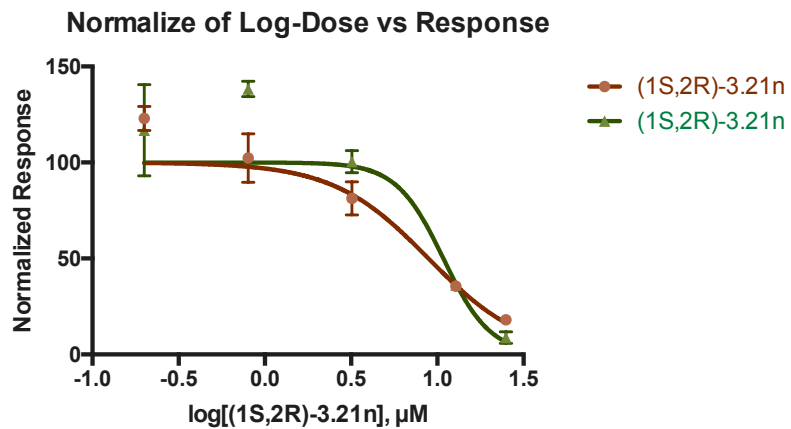
Figure C.35. EC$_{50}$ curves for (1*S*,2*R*)-**3.21n**.
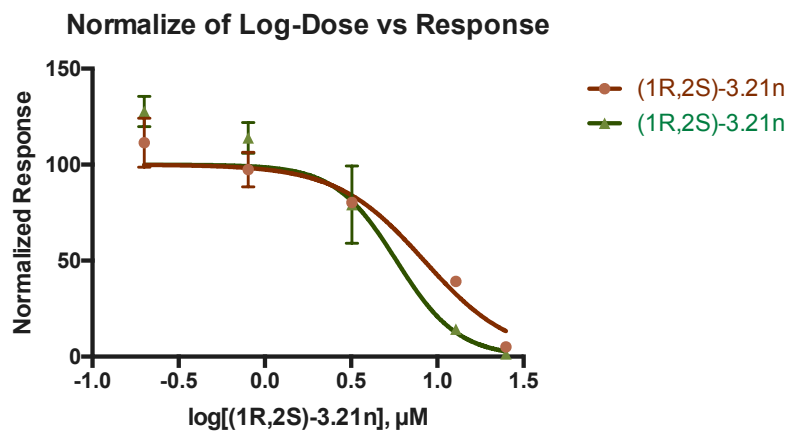
Figure C.36. EC$_{50}$ curves for (1*R*,2*S*)-**3.21n**.
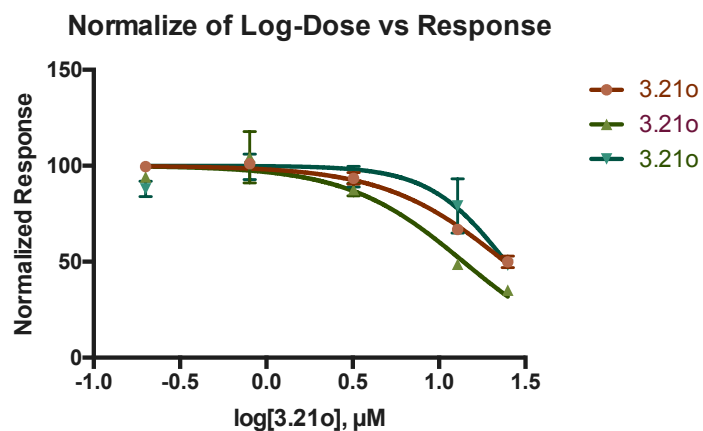
Figure C.37. EC$_{50}$ curves for **3.21o**.
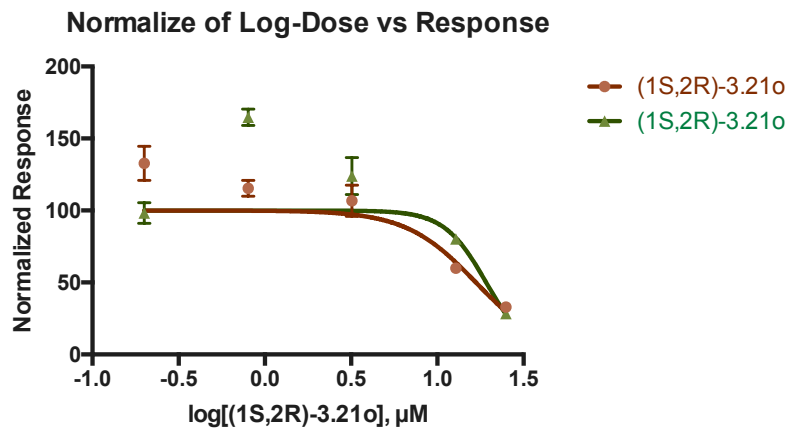
Figure C.38. EC$_{50}$ curves for (1*S*,2*R*)-**3.21o**.
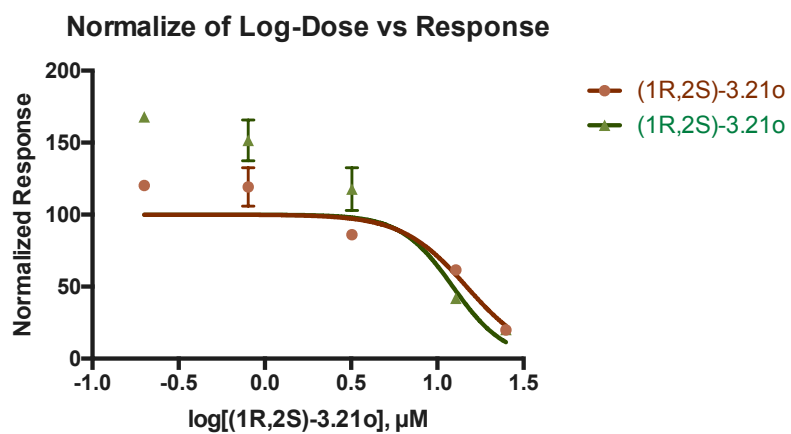
Figure C.39. EC$_{50}$ curves for (1*R*,2*S*)-**3.21o**.
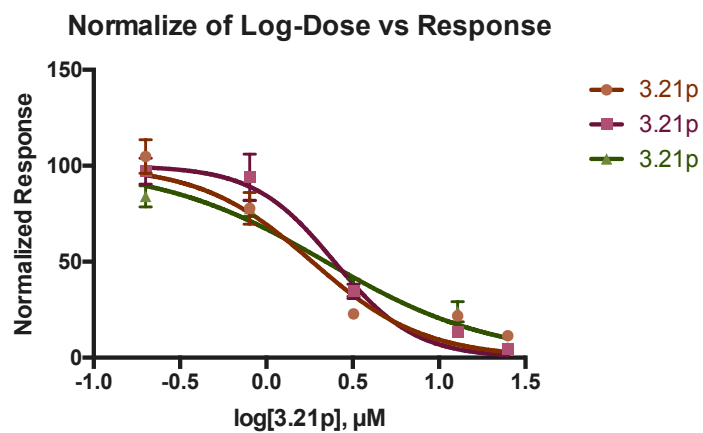
Figure C.40. EC$_{50}$ curves for **3.21p**.
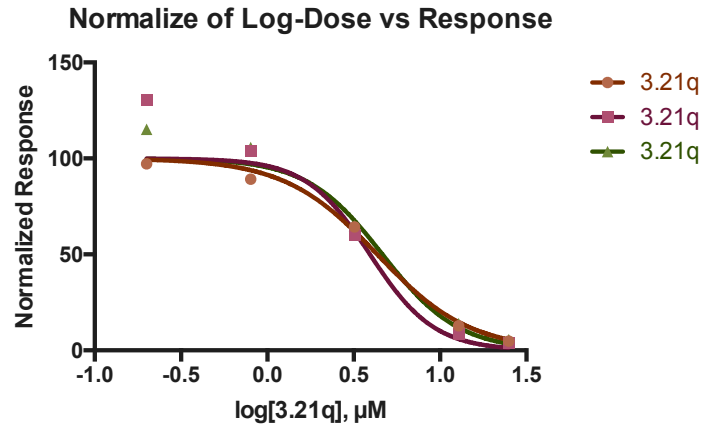
Figure C.41. EC$_{50}$ curves for **3.21q**.
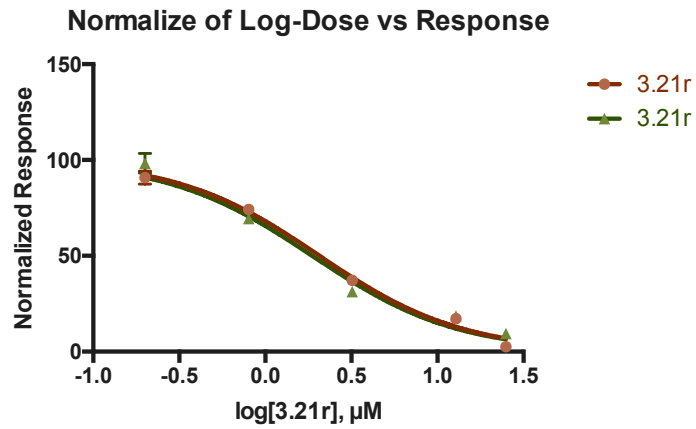
Figure C.42. EC$_{50}$ curves for **3.21r**.
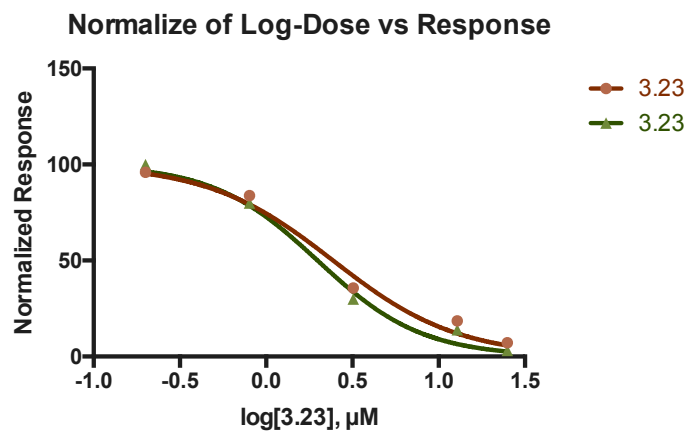
Figure C.43. EC$_{50}$ curves for **3.23**.
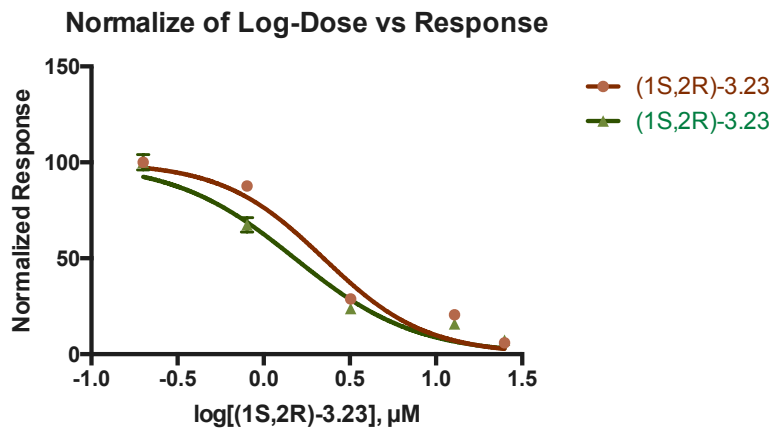
Figure C.44. EC$_{50}$ curves for (1*S*,2*R*)-**3.23**.
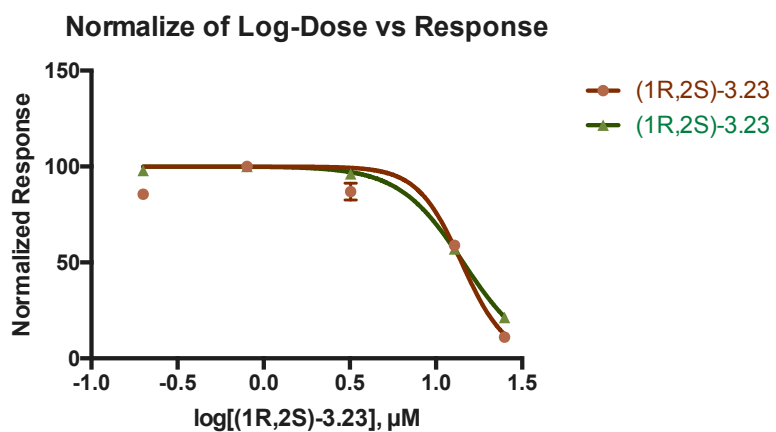
Figure C.45. EC$_{50}$ curves for (1*R*,2*S*)-**3.23**.
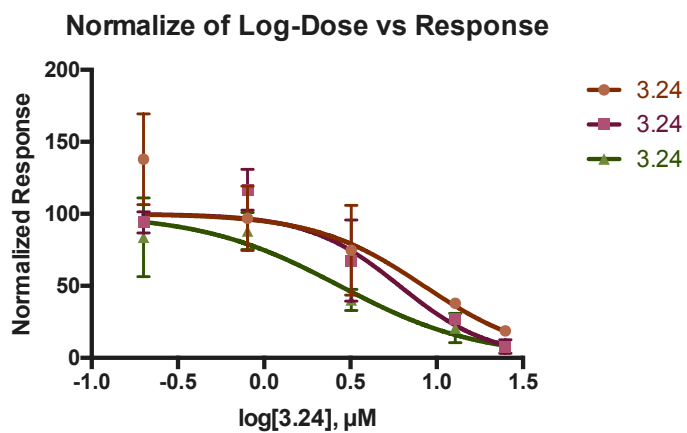
Figure C.46. EC$_{50}$ curves for **3.24**.
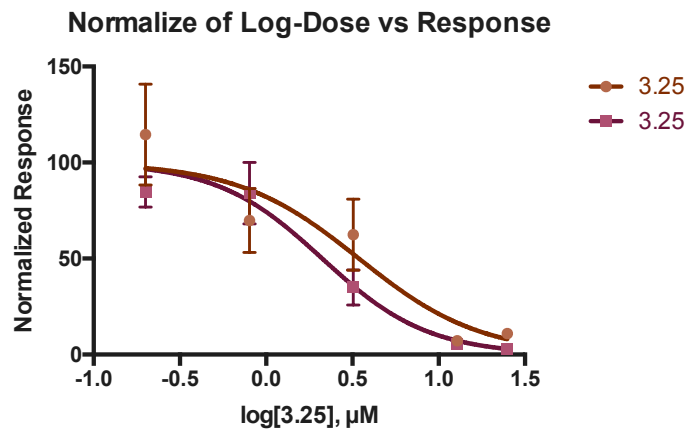
Figure C.47. EC$_{50}$ curves for **3.25**.
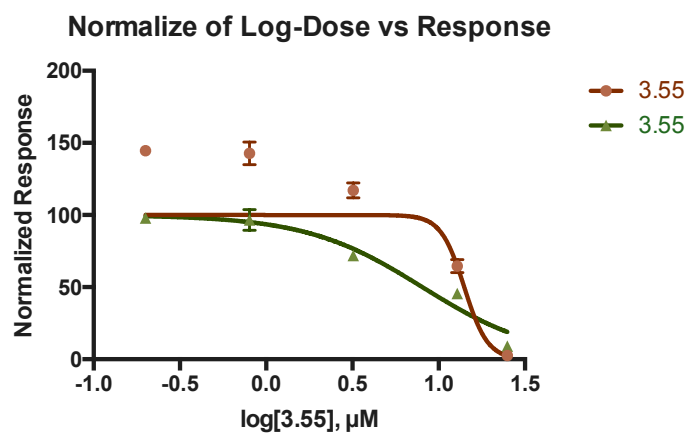
Figure C.48. EC$_{50}$ curves for **3.55**.
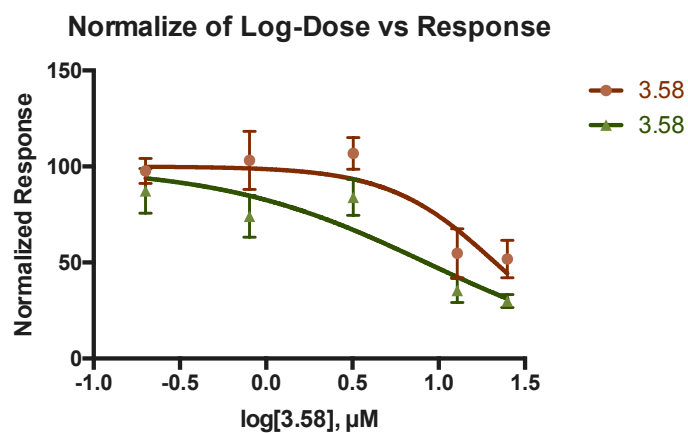
Figure C.49. EC$_{50}$ curves for **3.58**.

APPENDIX D

SELECTED NMR SPECTRA
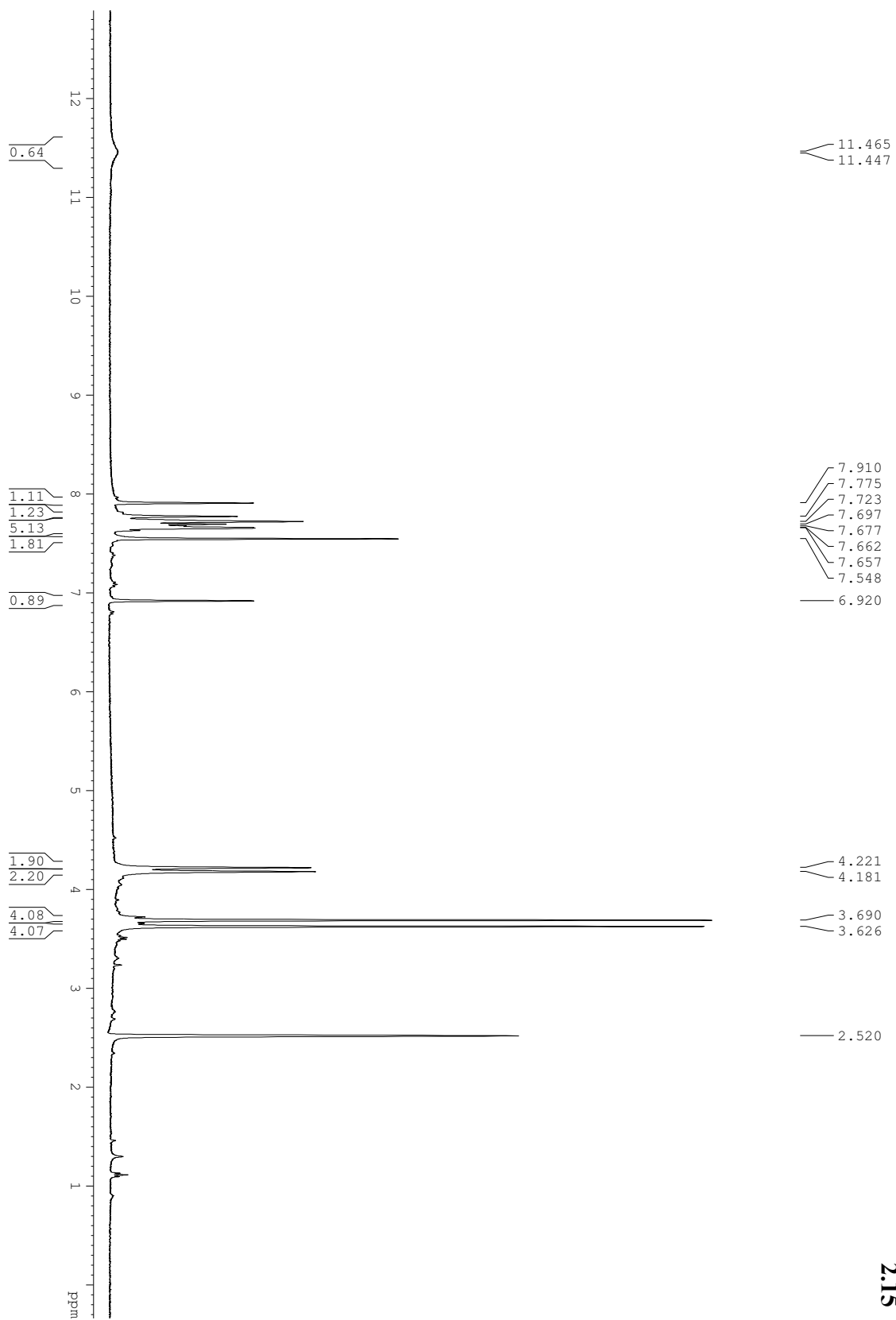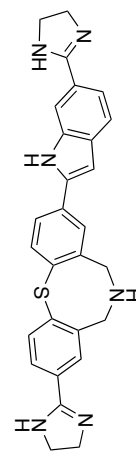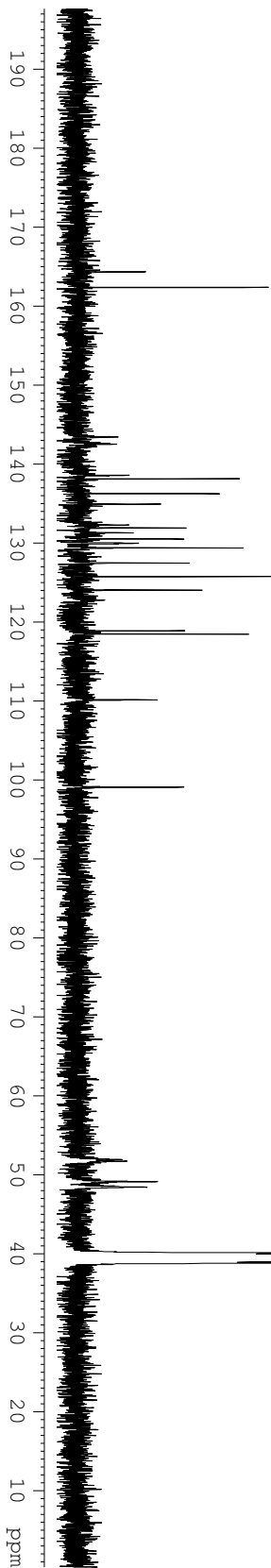
2.15
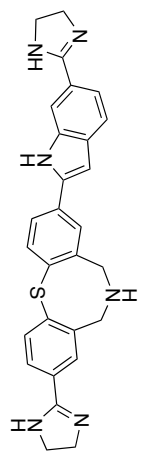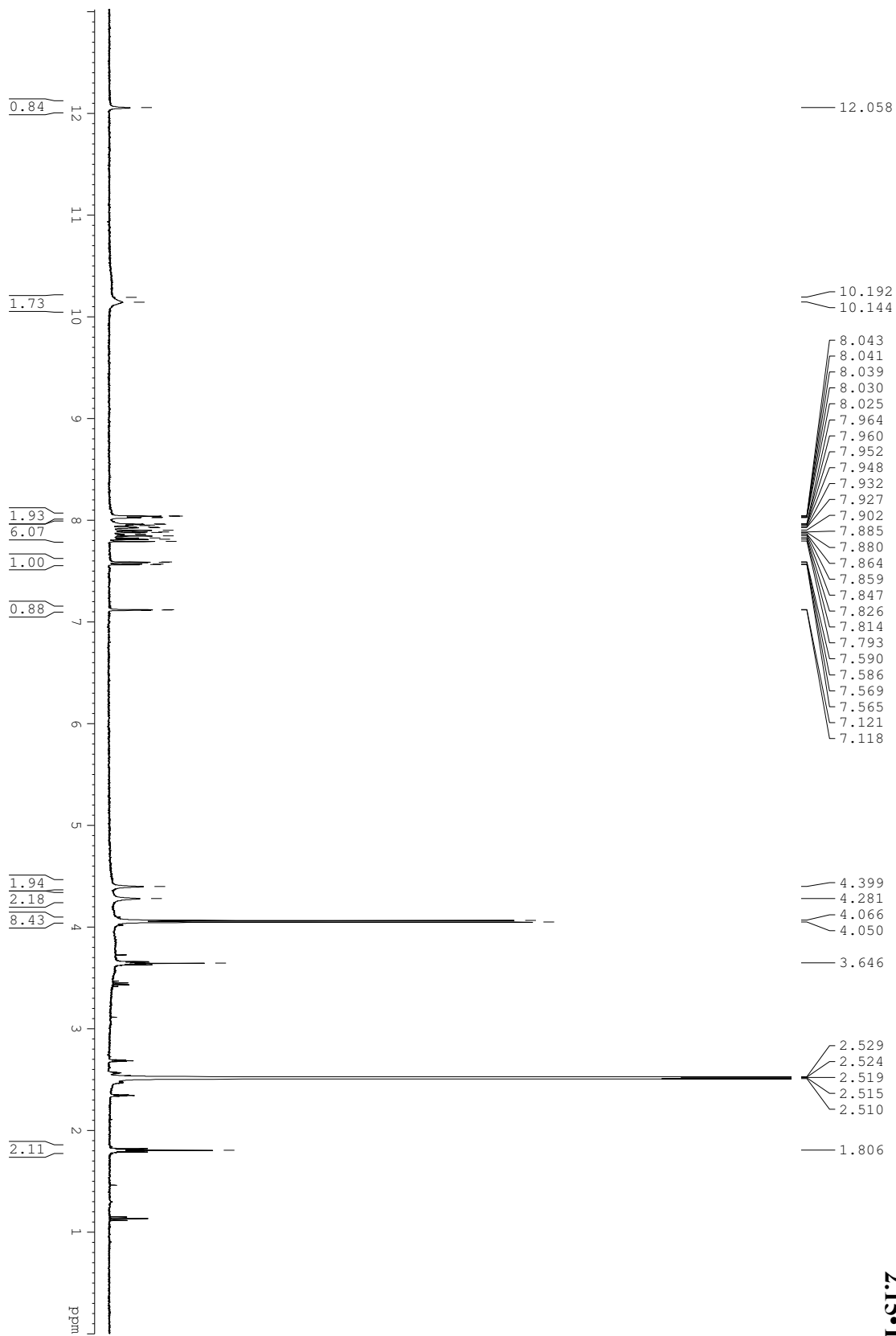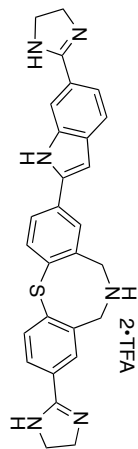
2.15•TFA

43-534-2-1-DMSO-400Mhz-100C

166.39

158.48
158.17
158.12
155.30

139.14
136.03
130.41
129.54
129.47
129.45
128.92
128.81
127.50
127.04
126.66
118.94
118.52
118.02
117.16
113.67
113.17
110.45

98.15

59.32
54.61
54.14
48.27
48.18
40.13
39.92
39.71
39.50
39.29
39.08
38.87

2.15•TFA

2•TFA

7.576
7.559
7.553
7.376
7.354
7.260

6.885
6.880
6.875
6.868
6.863
6.858

4.128
3.838
3.809
3.805
3.544

2.712
2.700
2.687

2.51a

339

160.21
158.79

144.76

130.57
130.14
128.09
126.75

113.68
113.54

77.32
77.21
77.00
76.69

63.30

55.29
55.23

45.02
41.73

2.51a

2.51b

7.627
7.622
7.609
7.605
7.438
7.433
7.421
7.416
7.370
7.348
7.259
6.978
6.974
6.961
6.957
6.886
6.883
6.878
6.865
6.861

4.136

3.806
3.567
3.561

2.738
2.725
2.713

2.00
2.00
2.03
2.07
4.06

1.99
3.06
1.99

1.98

158.83
157.41
156.21

132.70
130.58
129.82
127.17

120.52
118.74
115.87
113.55

77.31
77.00
76.68

63.18

55.23

44.86
41.58

2.51b

7.606
7.584
7.513
7.491

7.012
6.990
6.958
6.935

4.829

3.560
3.548
3.535
3.318
3.314
3.310
3.306
3.302
3.099
3.086
3.074

2.07
2.11

2.34
2.20

2.15

2.00

2.52

·TFA

159.58
157.49
150.65
134.00
131.25
129.01
122.03
119.43
117.19

49.63
49.41
49.20
48.99
48.78
48.56
48.35
42.07
41.24

14.44

2.52

·TFA

344

10.710

7.875
7.870
7.858
7.853
7.816
7.770
7.765
7.753
7.748
7.478
7.473
7.469
7.457
7.439
7.340
7.322
7.303
7.263
7.245
7.227
7.105
7.100
7.088
7.083
7.028
7.023
7.011
7.006
6.832
5.779
5.719

4.142
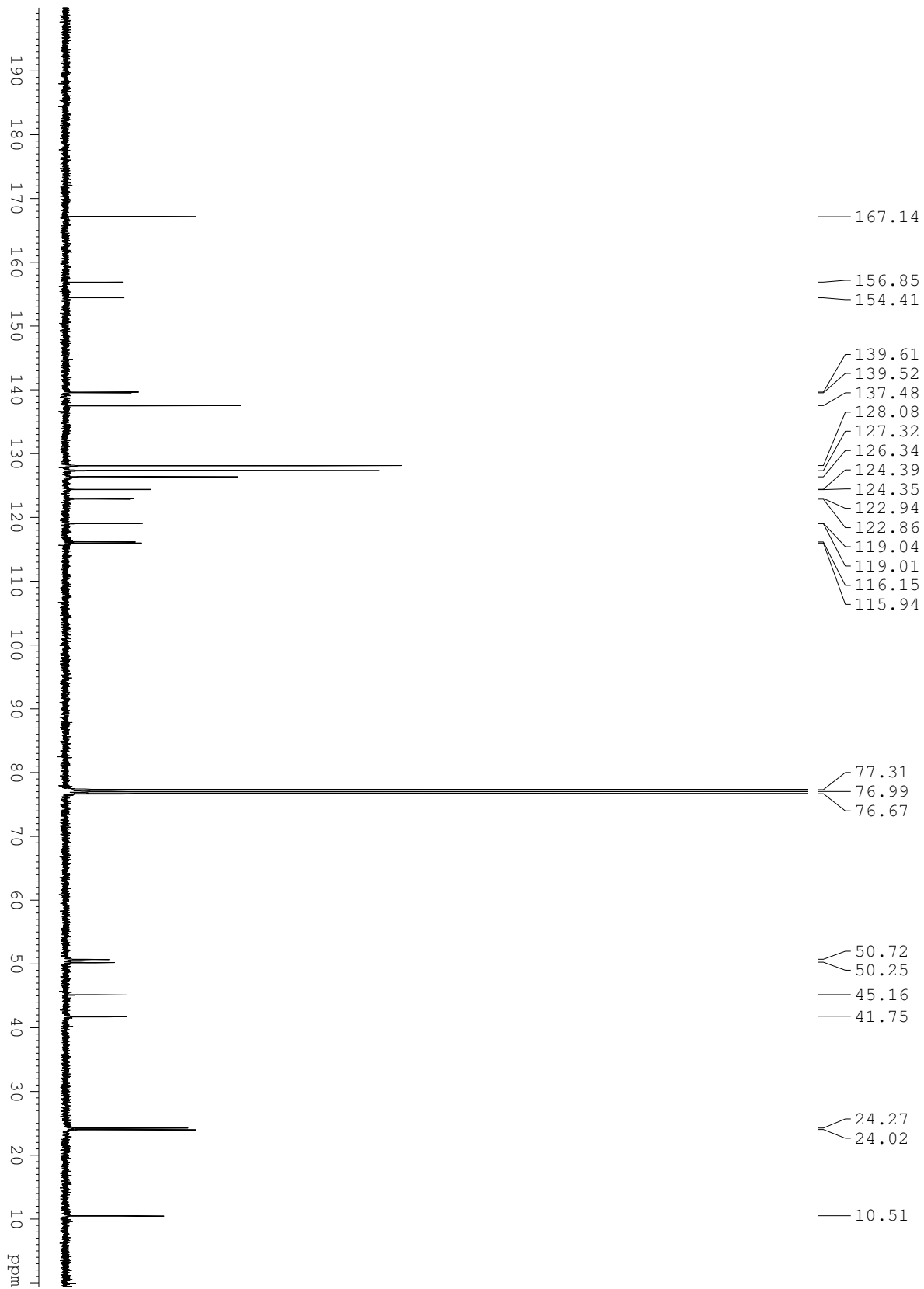4.130

3.562
3.552
3.540
3.528

2.730
2.724
2.714
2.700
2.061
2.056
2.050
2.045
2.039

2.62a

206.16

158.06
157.61
145.52
143.65
139.85
139.62
139.36
138.13
132.08
130.57
130.01
129.98
128.90
128.81
127.66
127.59
127.51
120.15
119.99
119.07
118.38
108.90

99.57

64.31
64.18

46.44
46.21
42.40
42.26
30.41
30.22
30.02
29.83
29.64
29.45
29.25

2.62a

157.57
156.65
148.83
148.26
148.22
146.99
144.45
138.94
136.94
130.77
130.59
130.57
130.09
128.78
127.70
127.05
126.89
121.63
121.56
119.96
119.14
118.73
117.38
112.52
112.44
110.75
109.14
99.05

77.25
76.99
76.74

63.76
63.68

55.87
55.80

45.45
45.05
41.72
41.64

2.62c

349

3.13a

3.13a

7.314
7.311
7.307
7.294
7.290
7.278
7.275
7.259
7.242
7.239
7.235
7.226
7.221
7.214
7.206
7.202
7.175
7.171
7.166
7.154
7.057
7.036
6.952
6.947
6.932
6.927
6.630
6.625
3.969
3.937
3.774
3.742
3.544
3.537
3.521
3.514
3.506
3.490
3.482
3.222
3.216
3.191
3.167
3.160
2.696
2.685
2.676
2.667
2.657
2.485
2.467
2.462
2.445
2.439
2.422
2.213
2.199
2.192
2.185
2.178
2.169
2.154
2.132
2.125
1.947
1.941
1.918
1.896
1.889
1.881
1.866
1.849
1.835
1.391
1.377
1.370
1.356
1.348
1.335
0.000

2.01
1.03
2.02
1.01
0.99
1.00

0.96
1.00
0.99
0.96
1.98
1.02
5.12
0.96
1.09
1.06

3.13b

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

—167.25

—151.99

—137.66
—131.89
—131.70
—131.05
—128.27
—127.42
—126.58
—123.54
—119.91

—77.34
—77.02
—76.70

—51.58

—45.56
—42.24

—24.57
—24.14

—17.26

—10.70

—0.00

3.13b

8.5
8.0
7.5
7.259
7.161
7.154
7.149
7.140
7.132
7.124
7.119
7.111
7.076
7.056
6.999
6.994
6.982
6.977
6.970
6.964
6.956
6.949
6.944
6.725
6.720
3.805
3.773
3.714
3.692
3.681
3.672
3.658
3.650
3.638
3.630
3.606
3.599
3.378
3.372
3.358
3.350
3.326
3.320
2.793
2.775
2.765
2.752
2.737
2.718
2.707
2.483
2.465
2.460
2.443
2.438
2.420
2.345
2.338
2.317
2.295
2.289
2.257
2.230
2.207
2.199
2.192
2.190
2.178
2.175
2.169
2.155
1.850
1.836
1.819
1.805
1.575
1.373
1.360
1.352
1.339
1.331
1.317

2.12
1.04
3.08
1.00
1.04
2.09
0.99
2.06
1.03
1.01
5.24
1.01
1.07

JJ540

353

190

180

170 ── 167.22

160 ── 162.88
── 160.44

150 ── 151.87

140

133.08
133.05
131.96
131.82
130.94
129.13
129.05
123.60
119.71
115.12
114.91

130

120

110

100

90

80 ── 77.32
── 77.00
── 76.69

70

60

50 ── 51.82
── 51.60

45.58
42.22

40

30

23.84
23.50

20 ── 17.31

10 ── 10.67

ppm

JJ540

354

3.21b

7.458
7.437
7.260
7.245
7.241
7.224
7.221
7.153
7.148
7.140
7.131
7.123
7.118
7.111
7.000
6.995
6.985
6.980
6.963
6.958
6.947
6.942
3.904
3.894
3.870
3.783
3.773
3.760
3.750
3.695
3.688
3.674
3.667
3.656
3.642
3.635
3.404
3.397
3.383
3.374
3.365
3.350
3.343
2.998
2.993
2.988
2.978
2.969
2.964
2.960
2.489
2.471
2.466
2.449
2.444
2.426
2.417
2.395
2.388
2.309
2.302
2.281
2.260
2.252
2.220
2.205
2.199
2.184
2.182
2.176
2.161
1.851
1.837
1.820
1.806
1.602
1.388
1.374
1.366
1.353
1.345
1.331

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

167.23

162.86
160.43

149.12
133.09
133.06
132.53
131.11
130.58
130.26
129.93
129.61
129.07
128.99
127.64
124.93
122.23
120.83
120.79
120.75
120.72
119.51
117.30
117.27
117.23
117.20
115.11
114.90

77.32
77.00
76.68

51.26
50.96

45.27

41.92

23.93
23.49

10.72

3.21b

-62.63

-116.38

10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 ppm

3.21b

8.281
8.269
7.255
7.128
7.122
7.114
7.106
7.098
7.093
6.935
6.930
6.913
6.908
6.896
6.891
6.503
6.491
6.479
4.007
3.997
3.985
3.974
3.963
3.955
3.943
3.933
3.922
3.909
3.900
3.778
3.768
3.756
3.744
3.735
3.668
3.646
3.635
3.624
3.552
3.544
3.531
3.522
3.281
3.273
3.260
3.251
3.227
3.219
3.153
3.146
3.132
3.122
3.113
3.021
3.013
3.000
2.990
2.981
2.967
2.959
2.487
2.464
2.447
2.442
2.424
2.204
2.189
2.183
2.169
2.160
2.146
1.843
1.829
1.812
1.798
1.610
1.364
1.350
1.343
1.329
1.322
1.308

3.21c

3.21c

167.35
162.78
161.31
160.35
157.72

132.97
132.93
129.00
128.92

115.09
114.88
110.29

77.31
76.99
76.68

44.97
43.79
43.44
41.61

23.88
23.47

10.62

−116.37

3.21c

360

7.404
7.390
7.382
7.369
7.259
7.071
7.056
7.051
7.035
7.013
6.959
6.955
6.939
6.934
6.796
6.791

3.794

3.494
3.482
3.470

2.810
2.798
2.785
2.535
2.523
2.511
2.204

3.22

167.19
163.84
161.37

151.66

132.34
132.10
131.94
131.87
131.67
131.41
131.38
130.82
130.23
130.15
123.55
122.42
122.18
121.94
119.54
115.67
115.45

77.30
76.99
76.67

51.36
51.13
46.46

41.39

17.25

3.22

10 0 −10 −20 −30 −40 −50 −60 −70 −80 −90 −100 −110 −120 −130 −140 −150 −160 −170 −180 −190 −200 ppm

—−112.04

363

3.22

10

0

-10

-20

-30

-40

-50

-60

-70

-80

-90

-100

-110

-120

-130

-140

-150

-160

-170

-180

-190

-200

ppm

-116.31

3.23

366

7.570
7.549
7.518
7.497
7.300
7.280
7.259
7.201
7.198
7.180
7.177
6.885
6.883

3.962
3.930
3.744
3.711
3.566
3.560
3.543
3.536
3.512
3.505
3.229
3.206
3.180
2.766
2.737
2.709
2.531
2.509
2.492
2.470
2.286
2.271
2.265
2.250
2.248
2.242
2.227
2.198
2.001
1.979
1.956
1.938
1.923
1.907
1.892
1.454
1.440
1.433
1.419
1.412
1.398
-0.007

3.21f

166.55

152.68
142.07
138.88
129.26
128.93
128.61
128.41
128.36
128.29
128.26
127.88
127.57
126.16
125.87
125.57
125.29
125.05
125.02
124.98
124.94
124.86
124.40
122.86
122.15
120.15
119.44

77.32
77.00
76.68

53.70
52.85

45.47
42.13

24.69
23.93

11.19

-0.07

3.21f

-60.39
-62.20

3.21f

7.523
7.506
7.391
7.374
7.279
7.262
7.260
7.177
7.174
7.161
7.158
6.896
6.893

3.944
3.937
3.917
3.789
3.782
3.763
3.696
3.690
3.678
3.672
3.665
3.653
3.647
3.373
3.367
3.356
3.348
3.341
3.329
3.324
3.177
3.153
3.081
3.072
3.065
3.057
2.545
2.527
2.513
2.495
2.476
2.459
2.454
2.288
2.275
2.271
2.258
2.253
2.240
1.935
1.923
1.910
1.898
1.457
1.445
1.440
1.429
1.423
1.412

3.21g

166.77

155.69

141.82
135.00
129.14
128.88
128.80
128.62
128.36
127.86
127.36
125.72
125.20
125.08
125.05
125.02
124.99
123.04
122.39
120.88
117.45
104.97

77.25
77.00
76.74

52.05
50.75

45.17

41.65

24.43
24.02

11.13

3.21g

371

-62.33

3.21g

7.493
7.478
7.425
7.405
7.385
7.332
7.313
7.259
7.068
7.067
7.048
7.047
6.965
6.960
6.945
6.939
6.673
6.667

3.879
3.847
3.759
3.750
3.727
3.622
3.615
3.600
3.592
3.583
3.568
3.561
3.297
3.291
3.267
3.243
3.237
2.785
2.764
2.748
2.730
2.709
2.557
2.539
2.534
2.517
2.512
2.495
2.290
2.275
2.269
2.267
2.254
2.252
2.246
2.231
2.207
2.197
2.127
2.121
2.099
2.077
1.936
1.922
1.905
1.890
1.574
1.441

3.21h

2.03
1.02
1.02
1.03
1.01
1.00

1.00
1.06
1.03
1.00
2.07
1.09
1.29
3.88
1.13
1.09
1.22

166.74

151.73
138.72
131.92
131.78
130.98
130.92
130.67
130.45
130.35
130.03
128.68
128.15
125.45
125.10
125.06
125.03
123.62
123.31
123.28
123.24
122.74
120.03
119.65

77.32
77.00
76.68

51.67
51.58

45.53
42.18

24.06
23.91

17.26

10.84

3.21h

CF$_3$

Cl

-62.44

3.21h

3.21i

166.73
151.10
138.77
136.87
131.36
130.80
130.51
129.56
129.24
128.92
128.69
128.62
128.18
125.48
125.44
125.12
125.07
125.04
125.00
123.31
123.27
123.23
123.20
122.78
122.73
120.42
120.39
120.02
116.07
116.04

77.32
77.00
76.68

51.70

45.58
42.23

24.14
23.96

17.79

10.88

3.21i

CF₃

O

N

N

CF₃

-62.41
-62.61

3.21i

7.570
7.549
7.518
7.497
7.300
7.280
7.259
7.201
7.198
7.180
7.177
6.885
6.883

3.962
3.930
3.744
3.711
3.566
3.560
3.543
3.536
3.512
3.505
3.229
3.206
3.180
2.766
2.737
2.709
2.531
2.509
2.492
2.470
2.286
2.271
2.265
2.250
2.248
2.242
2.227
2.198
2.001
1.979
1.956
1.938
1.923
1.907
1.892
1.454
1.440
1.433
1.419
1.412
1.398
-0.007

3.21i

166.55
152.68
142.07
138.88
129.26
128.93
128.61
128.41
128.36
128.29
128.26
127.88
127.57
126.16
125.87
125.57
125.29
125.05
125.02
124.98
124.94
124.86
124.40
122.86
122.15
120.15
119.44

77.32
77.00
76.68

53.70
52.85

45.47
42.13

24.69
23.93

11.19

-0.07

3.21i

10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
ppm

—-60.39
—-62.20

3.21i

85.57
85.16
84.77
84.37
83.97

63.42
63.02

110 100 90 80 70 60 50 40 30 20 10 0 −10 −20 −30 −40 −50 −60 −70 −80 −90 −100 ppm

3.21k

SF$_5$

O

N

N

Cl

7.665
7.643

7.260
7.242

6.962

3.870
3.837
3.713
3.701
3.689
3.679
3.670
3.656
3.649
3.636
3.628
3.617
3.603
3.596
3.371
3.349
3.324
2.848
2.828
2.819
2.785
2.774
2.765
2.756
2.536
2.514
2.497
2.474
2.321
2.305
2.295
2.286
2.277
2.262
2.169
2.162
2.141
2.122
1.940
1.925
1.909
1.894
1.579
1.474
1.460
1.453
1.438
1.432
1.418

3.211

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

166.54
152.53
152.36
152.19
152.02
151.84
150.92
141.81
136.68
131.41
129.60
129.28
128.96
128.64
128.11
127.69
125.78
125.73
125.68
125.64
125.59
125.41
122.71
120.46
120.42
120.38
120.34
120.03
115.81
115.77
115.74
115.70

77.32
77.00
76.68

51.89
51.45

45.48
42.20

24.61
23.67

17.86

11.29

3.211

SF₅

CF₃

85.37
84.98
84.97
84.57
84.17
83.78

63.24
62.84

-62.45

110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 ppm

3.211

387

3.21m

388

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

— 166.56
— 154.18
— 154.02
— 153.85
— 153.69
— 153.52
— 151.73
— 139.01
— 131.92
— 131.81
— 130.92
— 130.21
— 128.54
— 125.91
— 125.87
— 125.82
— 125.78
— 125.74
— 124.08
— 124.03
— 123.99
— 123.94
— 123.90
— 123.65
— 119.65

— 77.32
— 77.00
— 76.68

— 51.73
— 51.57

— 45.58

— 42.20

— 24.03
— 23.98

— 17.27

— 10.98

3.21m

85.47
85.09
85.07
84.68
84.28
83.88

63.11
63.06
63.03
62.71
62.67

3.21m

7.617
7.613
7.605
7.603
7.583
7.580
7.398
7.378
7.358
7.309
7.290
7.260
7.236
7.232
7.214
7.211
6.957

3.942
3.910
3.810
3.778
3.627
3.620
3.605
3.597
3.589
3.573
3.566
3.282
3.276
3.252
3.228
3.222
2.827
2.799
2.787
2.775
2.767
2.753
2.745
2.563
2.546
2.541
2.524
2.519
2.501
2.315
2.295
2.280
2.278
2.271
2.257
2.234
2.211
2.204
2.155
2.149
2.127
2.105
2.099
1.945
1.930
1.914
1.899
1.754
1.454
1.440
1.433
1.419
1.412
1.398

1.98
1.01
0.99
2.01
1.00

0.96
1.02
1.00
0.96
2.00
0.99
5.01
1.06
1.02
1.01

3.21n

SF$_5$

CF$_3$

166.51
154.20
154.03
153.87
153.70
153.53
151.01
138.99
136.81
131.34
130.32
129.47
129.15
128.83
128.50
128.15
125.78
125.73
125.68
125.64
125.59
125.45
124.03
123.99
123.94
123.90
123.85
122.74
120.40
120.36
120.32
120.29
120.04
116.00
115.96
115.92
115.89

77.32
77.00
76.68

51.75
51.57

45.54

42.15

24.03
23.94

17.76

10.90

3.21n

392

85.27
84.86
84.47
84.07
83.67

62.94
62.55

-62.42

3.21n

SF$_5$

CF$_3$

ppm

3.21o

166.40
150.85
150.50
146.79
146.44
146.10
145.75
136.86
136.72
135.48
131.45
129.58
129.26
128.94
128.62
128.12
125.67
125.41
122.95
122.71
120.50
120.47
120.43
120.39
120.23
120.01
119.85
119.83
119.80
119.77
117.51
115.92
115.89
115.85
115.81

77.32
77.00
76.68

51.96
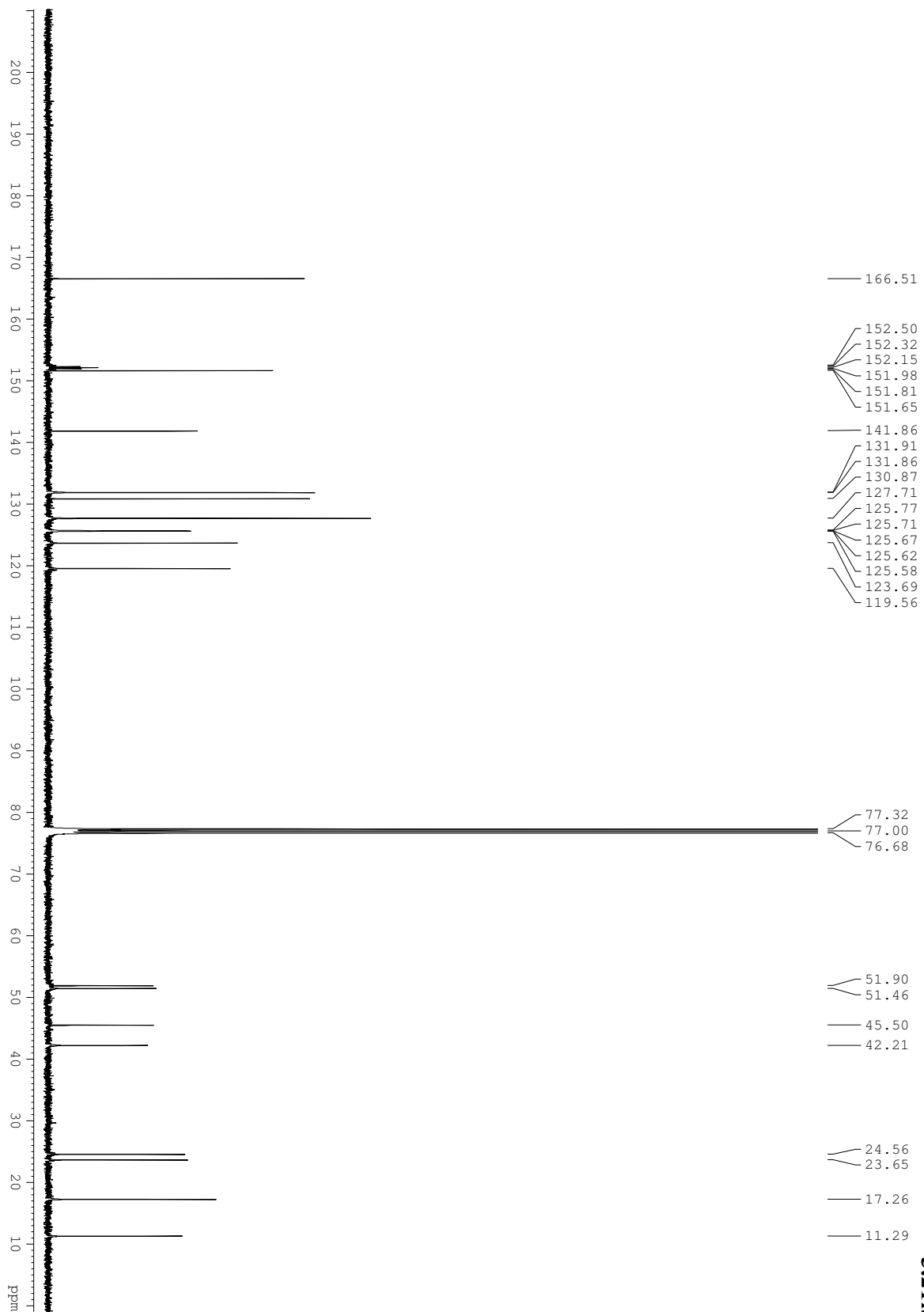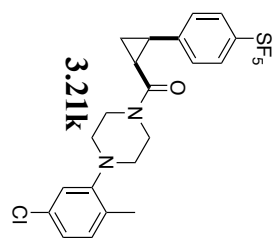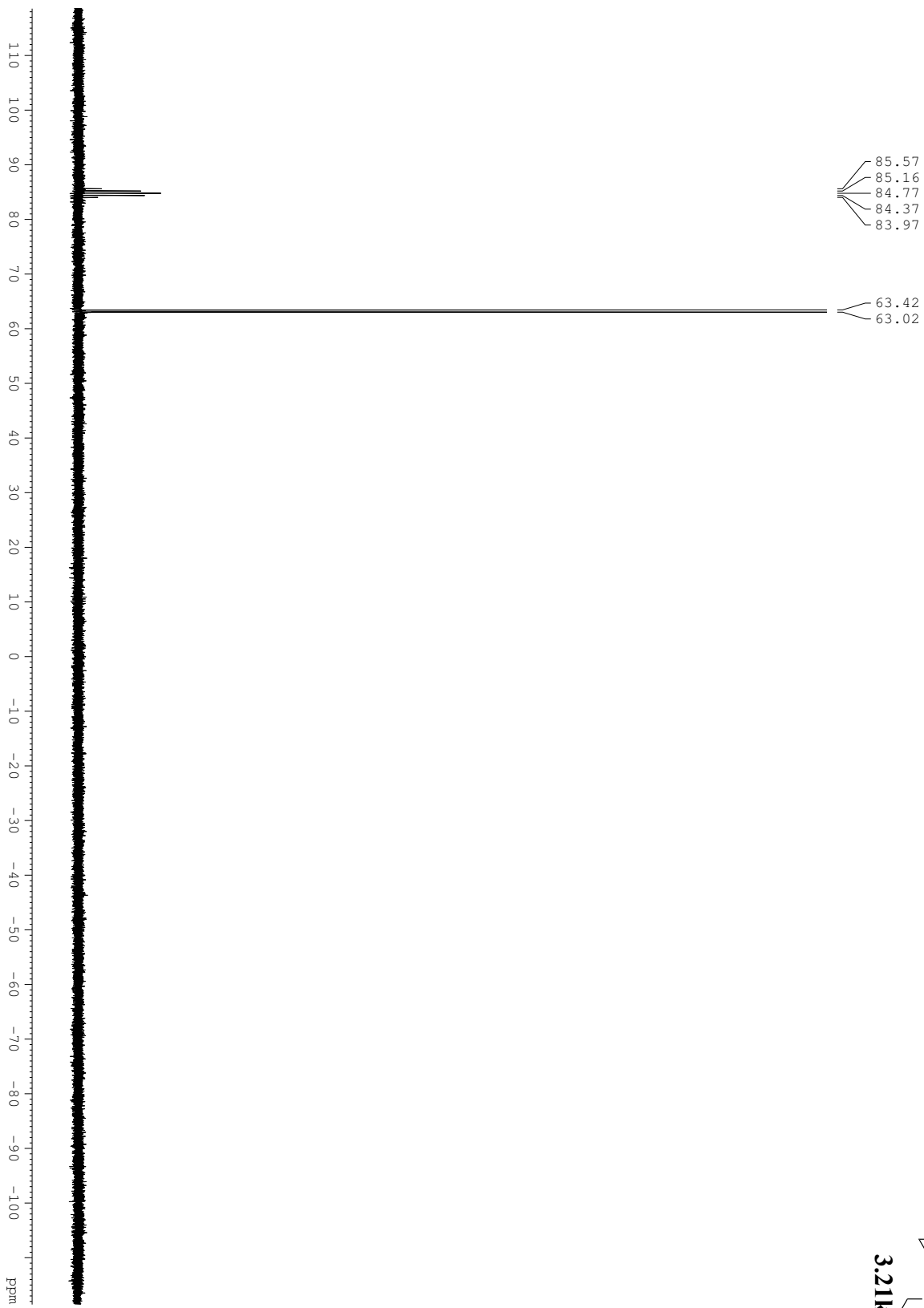51.49

45.59

42.28

23.67
21.46
17.87

10.96

3.210

10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200

—62.37

—67.77

3.210

CF₃

N

O

N

N

CF₃
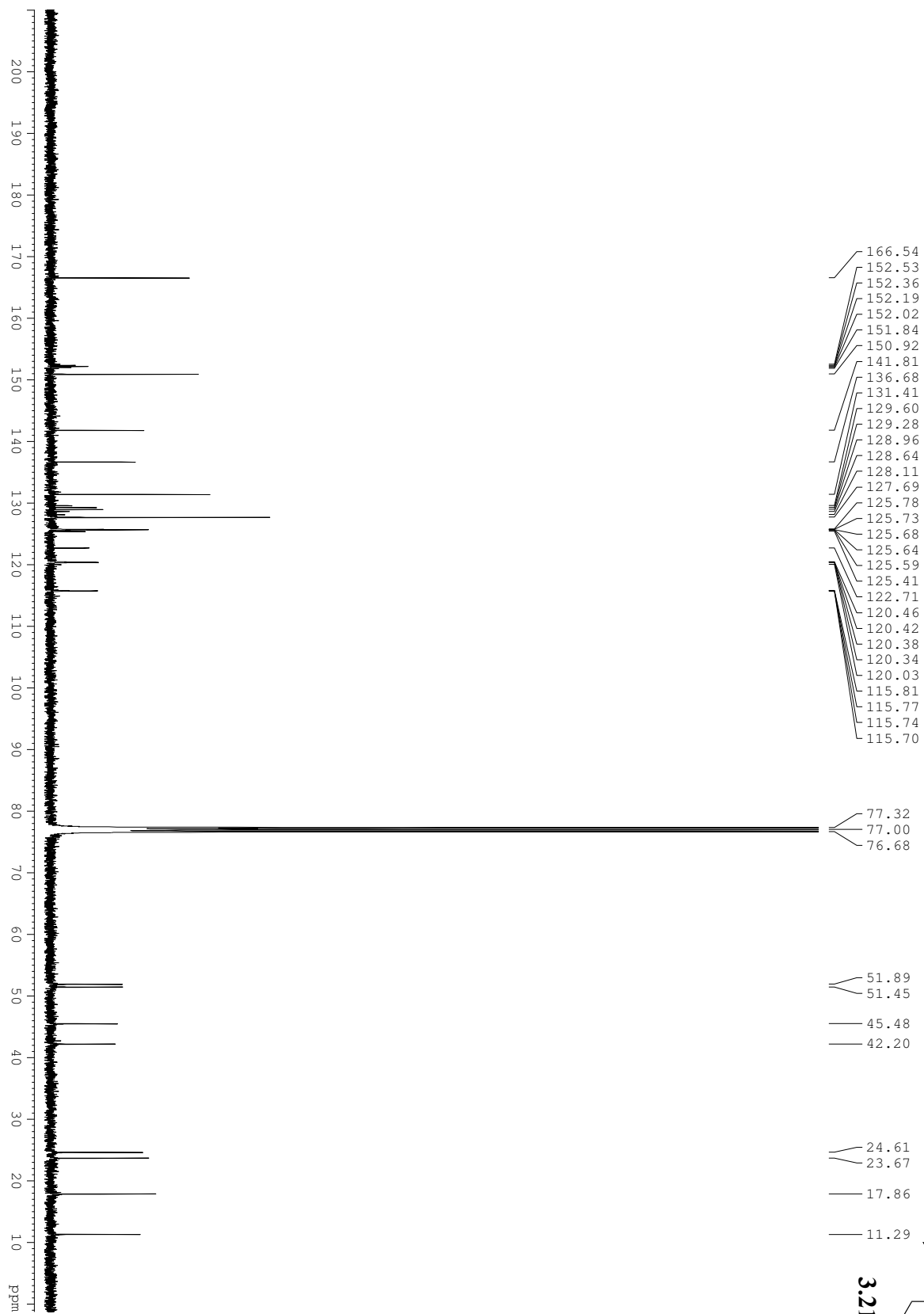
396

3.21p

167.19

152.06

138.23
137.49
131.94
131.68
131.07
125.00
124.10
123.53
119.74

77.31
76.99
76.67

51.81
51.69

45.66
42.34

24.11

19.30
17.29
15.26
12.21

3.21p

9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

2.50
1.02
1.97

1.00
1.03
1.05
0.98
2.03
1.09
1.63
3.34
3.09
1.05
1.03
1.03

7.278
7.257
7.248
7.245
7.224
7.041
6.583
6.575
6.557
6.554
6.548
6.546

4.018
3.986
3.893
3.860
3.671
3.664
3.649
3.641
3.632
3.617
3.610
3.366
3.342
3.318
2.857
2.846
2.837
2.828
2.819
2.809
2.800
2.779
2.561
2.539
2.522
2.499
2.474
2.468
2.446
2.439
2.433
2.424
2.416
2.404
2.377
2.334
2.193
2.177
2.172
2.156
2.150
2.135
1.787
1.772
1.757
1.742
1.409
1.396
1.388
1.374
1.367
1.353

3.21q

399

167.23

151.36
138.27
137.58
136.95
131.39
129.42
129.10
128.78
128.47
128.24
125.53
124.93
124.23
122.83
120.33
120.29
120.25
120.22
116.03
115.99
115.95
115.91

77.32
77.00
76.68

51.83
51.73

45.67

42.34

24.14

19.41
17.86
15.10
12.26

3.21q

-62.23

3.21q

7.551
7.530
7.260
7.227
7.224
7.206
7.202
7.036
7.033
6.614
6.612
6.606
6.603
6.589
6.580
4.094
4.061
3.892
3.859
3.599
3.592
3.576
3.568
3.560
3.544
3.537
3.264
3.258
3.234
3.209
3.203
2.802
2.776
2.751
2.549
2.531
2.527
2.510
2.505
2.488
2.458
2.456
2.430
2.408
2.401
2.312
2.305
2.283
2.261
2.255
2.189
2.173
2.168
2.152
2.146
2.131
1.789
1.774
1.759
1.744
1.660
1.423
1.418
1.405
1.397
1.383
1.376
1.362
-0.003

3.21r

— 167.14

— 153.18
— 138.63
— 138.52
— 137.38
— 128.46
— 128.41
— 128.36
— 128.30
— 127.57
— 126.40
— 126.11
— 125.82
— 125.51
— 125.08
— 124.86
— 124.81
— 124.03
— 122.14
— 119.43

— 77.32
— 77.00
— 76.68

— 53.60
— 53.19

— 45.65
— 42.36

— 24.36

— 19.43

— 15.29
— 12.44

— -0.04

3.21r

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

-60.39

3.21r

7.362
7.358
7.341
7.339
7.283
7.280
7.264
7.258
7.245
7.242
7.163
7.159
7.156
7.146
7.144
7.140
7.128
7.125
5.292
4.656
4.635
4.615
4.489
4.473
4.467
4.454
4.268
4.248
4.228
4.114
4.100
3.712
3.692
3.236
3.223
3.125
3.109
3.094
3.078
3.062
2.888
2.871
2.855
2.797
2.490
2.479
2.474
2.465
2.458
2.441
2.436
2.367
2.240
2.212
2.195
2.182
2.178
2.077
2.058
2.046
2.038
2.029
1.860
1.849
1.837
1.827
1.819
1.806
1.796
1.743
1.740
1.711
1.708
1.689
1.679
1.658
1.647
1.249
1.181
1.178
1.148
1.118
1.114

3.25

1.00
1.68
2.07
0.96
2.06
0.98
0.93
2.00
1.91
1.13
0.97
0.48
1.29
2.94
3.10
1.00
1.33
1.16
2.42
1.13

168.11
166.86

159.72

144.96

132.01
131.43
128.47
126.12
125.58
122.67

109.74

93.44

83.11
77.30
76.98
76.67

53.53
51.24
50.16

38.57
36.60
33.11
32.73
31.02
24.18

10.93
10.04

3.25

406

7.344
7.335
7.328
7.323
7.321
7.260
7.218
7.206
7.198
7.195
7.185
7.140
7.128
7.120
7.116

5.171
4.758
4.745
4.732
4.186
4.178
4.170
3.830
3.806
3.717
3.711
3.708
3.703
3.652
3.644
3.637
3.626
3.602
3.181
3.158
3.038
3.015
2.983
2.971
2.959
2.595
2.582
2.569
2.526
2.516
2.503
2.485
2.446
2.313
2.170
2.160
2.150
2.143
2.139
2.132
2.125
2.120
2.116
2.100
2.086
2.080
2.074
2.068
2.060
2.055
2.048
1.819
1.813
1.798
1.780
1.774
1.769
1.755
1.741
1.734
1.636
1.535
1.514
1.492
1.479
1.457
1.436

3.25

190
180
170
——172.60
——170.24
——167.05
——166.42
——159.88
150
——141.87
——138.16
——135.37
——129.30
——128.59
——128.44
——128.40
——127.45
——126.74
——126.31
——109.99
100
——77.21
——77.00
——76.79
——70.87
——70.53
——69.06
——68.63
——66.51
——63.55
——53.19
——49.15
——40.13
——38.83
——35.67
——32.88
——31.43
——31.11
——29.48
——27.68
——23.62
——11.07
——10.21

3.25

3.25

171.986
166.937
166.761
166.589
162.291
159.666

141.419
138.473
131.455
129.428
127.294
126.592
126.233
122.750

114.026
109.874

77.253
76.999
76.743
69.862
69.064
67.316

53.151
51.727
49.453
39.959
39.045
38.858
38.298
32.619
31.979
30.988
29.448
28.940
23.662

10.898
10.025

-0.130

3.25

3.25

7.386
7.383
7.367
7.364
7.260
7.252
7.248
7.242
7.240
7.234
7.229
7.228
7.223
7.213
7.209
7.160
7.155
7.152
7.148
7.143
7.138
7.134
7.133
7.126
6.954
6.951
6.935
6.932
6.916
6.914
6.676
6.675
6.656
4.517
4.502
3.937
3.904
3.766
3.743
3.734
3.725
3.640
3.633
3.618
3.610
3.332
3.317
3.309
3.299
3.113
3.102
3.097
3.085
3.074
2.481
2.458
2.440
2.436
2.430
2.419
2.408
2.213
2.198
2.192
2.190
2.177
2.175
2.169
2.154
1.865
1.850
1.834
1.819
1.797
1.781
1.766
1.595
1.382
1.369
1.361
1.347
1.340
1.326

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

167.36

153.64

137.59
133.91
129.61
128.14
127.33
126.37
122.22
117.77
116.27

92.63

83.88
77.31
76.99
76.67

51.75
51.44
50.86
45.35
41.96

24.47
24.06

10.62

3.25

412

4.110

7.259

4.585
4.572
4.557
4.553
4.545
4.541
4.526
4.514
4.398
4.376
4.367
4.345
4.337
4.314
3.700
3.668
3.636
2.805
2.793
2.783
2.775
2.770
2.762
2.750
2.739
2.731
2.727
2.719
2.709
2.696
2.690
2.686
2.676
2.663
2.659
2.656
2.647
2.643
2.629
2.619
2.616
2.586

1.02
1.00
0.93
1.98

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

168.57

114.82

77.42
77.00
76.57

67.30

31.40
27.63

4.110

4.118

7.260

5.163
5.140
5.118
5.095
5.072
4.786
4.776
4.765
4.754
4.744
3.791
3.774
3.771
3.755
3.749
3.736
3.728
3.713
3.691
3.043
3.019
2.993
2.968
2.943
2.569
2.451
2.447
2.442
2.438
2.427
2.415
2.406
2.398
2.390
2.386
2.382
2.371
2.367
2.362
2.349
2.340
2.317
2.306
2.293
2.283
2.021
2.013
2.004
1.877
1.869
1.854
1.833
1.825
1.812
1.792
1.768
1.751
1.746
1.741

0.04
0.08
2.00
0.93
1.00
2.06
0.86
3.94

121.21

81.75
77.43
77.01
76.58
69.97

59.12

34.21
30.69
27.05

16.28

4.118

7.389
7.375
7.370
7.368
7.354
7.340
7.336
7.327
7.323
7.320
7.314
7.306
7.297
7.290
7.260

4.575
4.546
4.533
4.503
3.666
3.652
3.637
3.101
3.086
3.079
3.071
3.064
3.057
3.049
3.041
3.026
2.454
2.447
2.439
2.433
2.430
2.427
2.421
2.414
2.408
2.406
2.395
2.388
2.370
2.365
2.364
2.037
2.031
2.024
1.954
1.934
1.925
1.918
1.912
1.902
1.890
1.887
1.877
1.869
1.853
1.847
1.837
1.832
1.818
1.814
1.800
1.677
0.019

4.119

137.79
128.35
127.68
127.59
121.08

81.80
77.32
77.00
76.68
73.16
69.91
66.69

32.05
30.78
27.50

16.35

-0.09

4.119

4.119

419

7.659
7.658
7.643
7.641
7.432
7.416
7.400
7.343
7.331
7.329
7.326
7.320
7.315
7.300
7.294
7.287
7.278
7.260
6.864
6.848
6.635

4.537
4.513
4.496
4.472
3.971
3.936
3.861
3.654
3.643
3.631
3.040
3.033
3.024
3.013
3.006
2.994
2.926
2.918
2.912
2.907
2.903
2.900
2.893
2.889
2.885
2.882
2.867
2.017
2.006
1.999
1.988
1.982
1.974
1.970
1.956
1.949
1.944
1.937
1.922
1.910

1.00
1.05
4.88
1.00
1.00
2.06
2.92
3.22
3.04
1.98
1.01
2.03
4.10

4.120

157.416
153.553
147.240
137.895
131.478
128.772
128.405
127.712
127.661
126.741
121.878
117.589
114.629
108.047
106.185
77.253
76.999
76.746
73.265
66.954
61.756
56.923
56.431
33.098
32.506
28.462
28.122

4.120

OMe OMe

OMe

N

OBn

Peak list:
9.702
9.696

7.647
7.627
7.422
7.403
7.382
7.325
7.308
7.289
7.272
7.264
7.259
6.851
6.832
6.619

4.456
3.966
3.947
3.928
3.856
3.814
3.539
3.531
3.524
3.516
3.509
3.500
2.795
2.790
2.778
2.772
2.755
2.751
2.559
2.552
2.545
2.539
2.532
2.526
2.520
2.120
2.100
2.084
2.064
2.048
2.027
2.014
1.935
1.917
1.901
1.886
1.872
1.865
1.857
1.850
1.836
1.826
1.819

4.121

Integration values:
0.94
1.12
1.23
6.68
1.11
1.04
2.00
6.40
3.33
2.04
1.87
0.99
2.08
2.42

204.54

157.36
153.43

147.04

138.10
131.42
129.90
128.43
128.36
127.69
127.62
127.60
126.65
117.37
114.62

108.07
105.98

77.32
77.00
76.68
73.06

67.67

61.74

56.92
56.42

49.04

29.62
29.47
27.58

OMe

OMe OMe

OMe

4.121

OBn

4.126

196.79
195.46

171.44

158.63

138.39
136.55
134.82
131.12
128.33
127.62
127.49
122.74
122.16
119.07
116.73

77.32
77.00
76.68
72.97
69.39

59.56
59.36
56.40
52.26
51.38

43.53

36.32
33.81

29.33

4.126

4.126

7.628
7.617
7.614
7.260
7.241
7.229
7.217
3.996
3.984
3.725
3.721
3.711
3.702
3.690
3.684
3.219
3.204
3.191
3.115
2.918
2.894
2.877
2.600
2.587
2.218
2.208
2.204
2.197
2.188
2.175
2.171
2.162
2.154
2.131
1.951
1.941
1.937
1.928
1.918
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1.836
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1.806
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1.773
1.760
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1.741
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1.726
1.716
1.587
1.580
1.573
1.565
1.557
1.548
1.538
1.521
1.515
1.509
1.501
1.487
1.382
1.366
1.356
1.351
1.342
1.331
1.325
1.312
1.298
1.292
1.283
1.280
1.063
1.055
1.031
1.026
1.002
0.995

4.130

4.130

429

7.529
7.527
7.509
7.507
7.475
7.455
7.435
7.259
7.146
7.144
7.126
7.123
6.176
6.151
3.918
3.705
3.687
3.670
3.281
3.254
3.229
2.881
2.868
2.853
2.846
2.840
2.834
2.818
2.806
2.558
2.545
2.533
2.023
2.012
2.000
1.988
1.976
1.960
1.935
1.927
1.911
1.905
1.903
1.897
1.888
1.875
1.865
1.852
1.837
1.832
1.823
1.817
1.805
1.711
1.704
1.682
1.678
1.671
1.655
1.616
1.540
1.516
1.507
1.495
1.482
1.462
1.445
1.438
1.434
1.428
1.421
1.412
1.405
1.394
1.389
1.371
1.358
1.347
1.335
1.323
1.313
1.300

4.131

4.131

9.797
9.791
9.785
7.558
7.555
7.538
7.536
7.499
7.479
7.460
7.260
7.167
7.165
7.147
7.144
6.193
6.168
3.935
3.296
3.268
3.243
2.883
2.870
2.848
2.842
2.836
2.820
2.807
2.661
2.655
2.647
2.642
2.621
2.616
2.608
2.602
2.382
2.376
2.360
2.354
2.343
2.337
2.321
2.315
2.102
2.074
2.062
2.042
2.025
2.006
1.920
1.899
1.894
1.889
1.872
1.865
1.840
1.759
1.736
1.732
1.725
1.710
1.705
1.589
1.556
1.544
1.531
1.511
1.497
1.491
1.466
1.458
1.451
1.442
1.430
1.425
1.403
1.399
1.388
1.377
1.363
1.342

4.135

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 ppm

202.20
198.66
178.06
158.37
132.40
130.96
125.58
119.04
115.79
77.32
77.00
76.68
72.03
56.13
53.50
51.64
50.10
45.83
39.31
36.99
36.50
30.62
30.19
23.57
21.51

4.135

OMe

434

4.135

435

4.136

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10 ppm

— 198.39

— 192.80

— 177.91

— 158.41

134.92
132.02
131.00
129.49
129.44
128.67
127.20
125.60
119.02
115.94

77.31
77.00
76.68
72.02

61.31
56.14
54.59
54.14
50.09
45.58
40.62
39.14
36.82
29.16
23.47
21.99

4.136

437

4.136

7.567
7.555
7.548
7.542
7.538
7.515
7.500
7.492
7.479
7.471
7.466
7.259
7.173
7.160
6.210
6.193
5.558
5.555
4.332
4.324
4.313
4.224
4.211
4.205
4.191
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2.461
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2.066
2.061
2.051
2.041
2.036
2.026
1.912
1.900
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1.808
1.804
1.798
1.792
1.787
1.781
1.777
1.760
1.751
1.568
1.551
1.547
1.532
1.528
1.513
1.298
1.284
1.280
1.266
1.255
1.250
1.214
1.202
1.190

3.93
1.56
1.59
1.00
0.42
1.09
1.61
0.94
0.77
5.82
1.78
1.86
5.29
4.33
2.31
10.05
3.30
0.29
0.69

4.138

OMe
H
O
H
O
OH

4.138

441

4.140

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