Synthesis of Biologically Active Heterocycles and Development of New Organometallic Methodologies

by

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David M. Arnold, M.S.

University of Pittsburgh, 2010

The study of the synthesis of highly functionalized heterocyclic compounds represents an important subset of synthetic organic transformations leading to target compounds with a wide set of applications in medicinal chemistry, biological chemistry, materials sciences and natural product synthesis. Through this work, synthetic strategies leading to a novel set of ester linked substituted quinoline – uracil scaffolds have been developed and the resulting products were found to be inhibitors of MPK1, an important target in cancer research. This work has also led to the development and implementation of novel synthetic strategies toward highly functionalized, traditionally pharmacologically important 6-amino-, 6-hydroxy- and 6-oxo-uracil and 4,6-dihydroxypiperidone scaffolds. A novel Plk1-PBD inhibitor from this series would be important to probe the mechanism of this enzyme during mitosis and to develop a clinical candidate for this validated cancer target. The Plk1-PBD research endeavor also documents a case for a tandem synthetic/analytical structure determination study. The structure of an initially elusive compound from a high throughput screening of 97,090 compounds was determined by this approach.

Finally, the utility of novel heterocyclic sulfonyl and sulfinyl nitrogen protecting groups has been demonstrated through the addition of organometallic reagents to 2-methylthiadiazole-, 2-benzothiazolesulfonylbenzaldimines and 2-pyridylsulfinylbenzaldimines. It was found that these addition reactions proceeded with a variety of organometallic

nucleophiles including Gringnard reagents, organozinc and organocuprates. The heterocyclic sulfonyl protecting groups were easily cleaved from the α-branched amines, affording a useful protecting group strategy for the synthesis of this important class of compounds.

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I would like to thank my advisor, Professor Peter Wipf, for his guidance, encouragement and patience during my studies at the University of Pittsburgh. His inspiration has allowed me to develop as a scientist and has greatly deepened my appreciation for the art and application of synthetic organic chemistry. I would like to thank Professors Dennis Curran and Kazunori Koide for their useful discussions and helpful suggestions over the last five years. I would like to further thank Damodaran Krishnan and Sage Bowser for NMR assistance, Dr. John Williams for mass spectrometry assistance and Dr. Steven Geib for his valuable X-ray crystallography contributions. I would like to thank Dr. John S. Lazo and Dr. Paul A. Johnston for their biological contributions to the MKP-1 and Plk1-PBD projects and Dr. Donna M. Huryn for her valuable discussions on these projects. I would also like to thank Peter Chambers for his analytical LC/MS assistance that was instrumental in the Plk1-PBD project.

ABBREVIATIONS

ACN: Acetonitrile

Ac_2O: Acetic anhydride

AcOH: Acetic acid

ATP: Adenosine triphosphate

ATR: Attenuated total reflectance

BnBr: Benzyl bromide

Boc_2O: Di-*tert*-butyl dicarbonate

BozPHOS: (2*R*,5*R*)-1-{2-[(2*R*,5*R*)-2,5-Dimethylphospholan-1-yl]phenyl}-2,5-dimethylphospholane 1-oxide

Bt: Benzothiazole

Bts: Benzothiazole-2-sulfonyl

Cdc25B: Cell division cycle 25B

CDI: *N*,*N*'-Carbonyldiimidazole

CID: Chemical identification number

DBU: Diazabicyclo[5.4.0]undec-7-ene

DCM: Dichloromethane

DIBAL-H: Diisobutylaluminium hydride

DIPEA: *N*,*N*-Diisopropylethylamine

DMA: Dimethyl acetal

DMAP: 4-Dimethylaminopyridine

DMF: Dimethyl formamide

DMPU: 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone

DMSO: Dimethyl sulfoxide

DPI: Discovery Partners International

ELS: Evaporative light scattering detector

EtOAc: Ethyl acetate

EtOH: Ethanol

ESI: Electrospray ionization

HMDS: Hexamethyldisilazane

HOAc: Acetic acid

HPLC: High-performance liquid chromatography

HTS: High throughput screen

IR: Infrared spectroscopy

LiHMDS: Lithium hexamethyldisilazide

MAPKs: Mitogen-activated protein kinases

m-CPBA: *meta*-Chloroperoxybenzoic acid

MeCN: Acetonitrile

MeI: Methyl iodide

MeOH: Methanol

MKP-1: Mitogen-activated protein kinase phosphatase-1

MKP-3: Mitogen-activated protein kinase phosphatase-3

MLSCN: Molecular libraries screening centers network

MPLC: Medium pressure liquid chromatography

MS: Mass spectrometry

NaHMDS: Sodium hexamethyldisilazide

NBS: N-Bromo succinimide

NIH SMR: National Institutes of Health small molecule repository

NMR: Nuclear magnetic resonance spectroscopy

PBD: Polo Box Domain

Plk1: Polo Like Kinase 1

PMLSC: Pittsburgh molecular libraries screening center

PPA: Polyphosphoric acid

PTP1B: Protein tyrosine phosphatase-1B

PTSA: *p*-Toluenesulfonic acid

Py: Pyridyl

RNA: Ribonucleic acid

R,R-MeDUPHOS: 1,2-Bis[(2*R*,5*R*)-2,5-dimethyl-phospholano]benzene

SAR: Structure activity relationship

SFC: Supercritical fluid chromatography

SID: Substance identification number

TFA: Trifluoroacetic acid

THF: Tetrahydrofuran

Ths: 5-Methyl-1,3,4-thiadiazole-2-sulfonyl

TMSCl: Trimethylsilyl chloride

TOF: Time of flight

TLC: Thin layer chromatography

UPMLSC: University of Pittsburgh molecular libraries screening center

UV: Ultraviolet spectroscopy

VHR: Vaccinia virus related dual-specific protein phosphatase

1.0 SYNTHESIS AND BIOLOGICAL ACTIVITY OF A FOCUSED LIBRARY OF MITOGEN-ACTIVATED PROTEIN KINASE PHOSPHATASE INHIBITORS[1]

1.1 INTRODUCTION

The synthesis of a small library of mitogen-activated protein kinase phosphatase-1 (MKP-1) inhibitors and their resultant biological activity will be discussed. After a brief biological discussion, the background for the project is presented and the library design is introduced. The discussion focuses on the routes used for the synthesis of the library and the resulting biological data. The work in this chapter has appeared in reference 1.

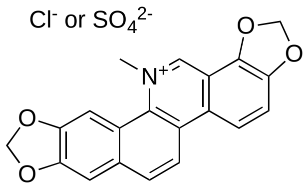
1.1.1 Brief biological background

The family of mitogen-activated protein kinases (MAPKs) represents a class of evolutionary conserved enzymes which are involved in highly regulated signaling pathways within the cell.[2] Mitogen-activated protein kinases play a crucial role in the regulation of cellular processes such as gene expression, cell proliferation, cell survival and cell death.[2] In the MAPK signaling pathway, MKP-1 has been identified as a crucial regulatory enzyme whose function affects the outcome of many of these cellular processes, a result which has supported the role of MKP-1 as an important target for biological research.[3] Resent studies have also shown MKP-1 to be a potentially significant target for cancer therapy since the gene is overexpressed in human lung, prostate, gastric, breast and pancreatic cancers.[4,5]

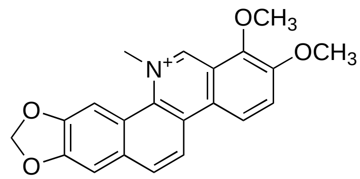
1.1.2 Project background

Over the last two decades, the increasing biological importance of MKP-1 as a crucial regulatory enzyme in MAPK signaling cascades and as a potentially important therapeutic target has spurred the search for a potent and selective MKP-1 inhibitor. Unfortunately, to this date, there is no crystal structure of the MKP-1 enzyme available to guide rational inhibitor design. Therefore, the identification of MKP-1 inhibitors has been the result of high throughput screening (HTS) efforts in conjunction with secondary MKP-1 cellular assays.[6,7]

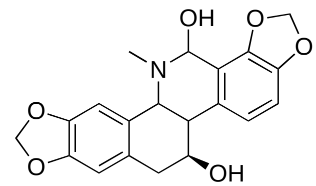
In 2005, the University of Pittsburgh Department of Pharmacology reported sanguinarine as the first selective inhibitor of MKP-1, (**1**, Figure 1). Sanguinarine was identified via a high-content analysis of 720 natural products and was shown to selectively inhibit MKP-1 *in vitro* with an IC_{50} = 17.3 μM in comparison with other related dual-specificity phosphatases such as MKP-3 (IC_{50} >>100 μM), Cdc25B (IC_{50} = 57.8 μM), VHR (IC_{50} = 74.0 μM) and PTP1B (IC_{50} = 67.9 μM).[7] Sanguinarine was also found to preferentially inhibit MKP-1 over MKP-3 in intact HeLa cells, showing **1** to be active at the cellular level.[7] Selective inhibition of MKP-1 over MKP-3 is a good criteria for general MKP-1 selectivity, given the structural homology between the catalytic domains of these two dual-specificity phosphatases in which the amino acid sequence is 82% identical.[6] Utilizing the scaffold of sanguinarine, the authors investigated the inhibitory effect on MKP-1 of five other structurally similar compounds: chelerythrine **2**, hydroxychelidonine **3**, berberine **4**, tetrahydroberberine **5** and protopine **6** (Figure 1), but only **2** was found to inhibit MKP-1 with comparable potency (IC_{50} = 16.2 μM).[7]



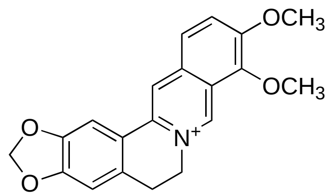
1
IC_{50}: 17.3 μM



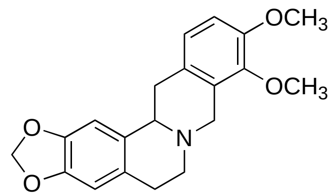
2
IC_{50}: 16.2 μM



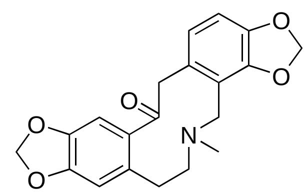
3
IC_{50}: >>100 μM



4
IC_{50}: >>100 μM



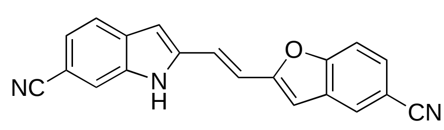
5
IC_{50}: >>100 μM



6
IC_{50}: >>100 μM

Figure 1. Structure of sanguinarine and related analogues tested for MKP-1 inhibition.[7]

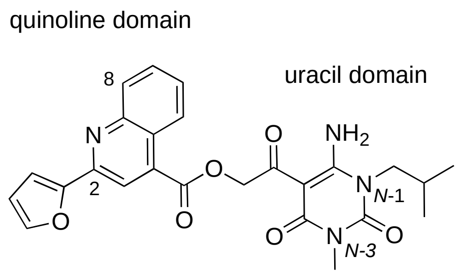
In 2006, a second paper demonstrated the potency of novel benzofuran based inhibitors of MKP-1, such as NU-126 (**7**, Figure 2).[6] The potency of **7** was similar to that of **1**, inhibiting MKP-1 with an *in vitro* IC_{50} value of 28.2 μM.[6] The selectivity of **7** for MKP-1 versus other dual-specificity phosphatases was enhanced over that of **1**, as demonstrated by the *in vitro* IC_{50} values of **7** for MKP-3 (IC_{50} > 400 μM), Cdc25B (IC_{50} > 400 μM), VHR (IC_{50} = 38.1 μM) and PTP1B (IC_{50} > 100 μM).[6]



7
IC_{50}: 28.2 μM

Figure 2. Structure of NU-126 (**7**).[6]

In 2007, a high throughput screen of 13,309 compounds was conducted by the Pittsburgh Molecular Libraries Screening Center (PMLSC) to identify small molecule inhibitors of MKP-1.[1] Among the hits, a compound containing a linked quinoline-uracil scaffold was identified as an inhibitor of MKP-1 with an average IC_{50} value of 19.2 $\pm$ 5.6 μM (**8**, Figure 3).[1] The structure of **8** was unique among the HTS hits since it was the only quinoline-uracil based scaffold that was tested in this screen.[1] This fact, along with the low μM *in vitro* potency of **8** and its relatively easily accessible structure encouraged the synthesis of analogues designed to systematically probe favorable binding interactions with MKP-1.

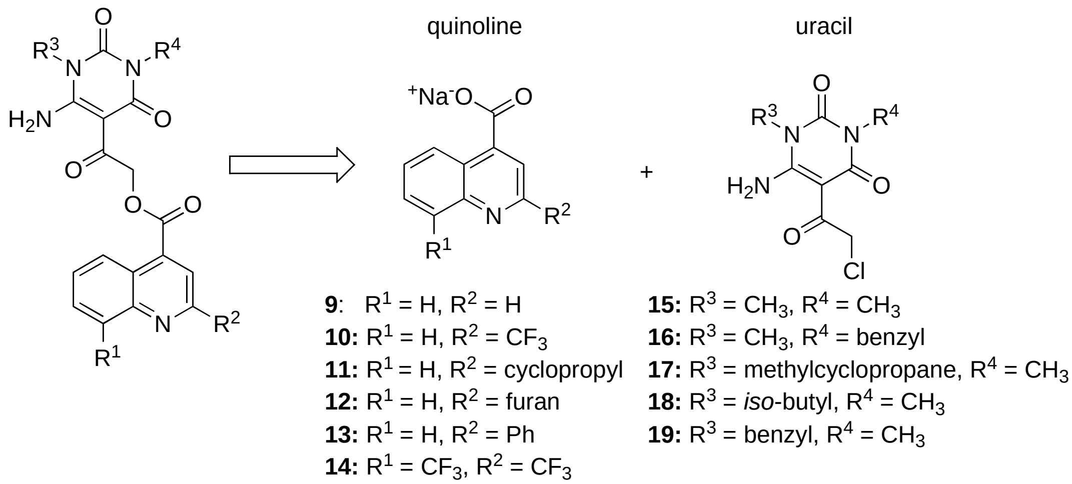


8

Figure 3. Structure of MKP-1 inhibitor **8**.

1.1.3 Library design

It was envisioned that the dual-domain nature of scaffold **8** could be used to generate a small library of compounds in which the points of derivatization would be the uracil *N*-1 and *N*-3 positions and the quinoline 2 and 8 positions. Augmentation of alkyl substituent size at the uracil *N*-1 and *N*-3 positions was chosen to probe steric effects and possible hydrophobic interactions within the MKP-1 inhibitor binding domain. Variation at the uracil *N*-1 position included methyl, methylcyclopropyl, *iso*-butyl and benzyl substituents. Variation at the uracil *N*-3 position included methyl and benzyl substituents. Derivatization at the quinoline 2 and 8 positions was intended to probe electronic and steric requirements. Variations at the 2-quinoline position included hydrogen, trifluormethyl, cyclopropyl, furanyl and phenyl substituents, while variation at the 8-quinoline position was chosen to include hydrogen and trifluoromethyl groups. This choice of substituents leads to a library of 26 compounds, including the original HTS hit **8**.

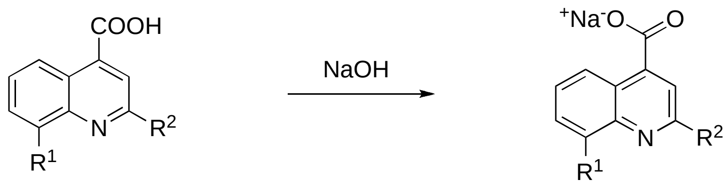


Scheme 1. Main retrosynthetic disconnection for the library analogues.

1.2 LIBRARY SYNTHESIS

1.2.1 Synthesis of 2,8-substituted quinolinecarboxylic acid sodium salts

The 2,8-substituted quinolinecarboxylic acid sodium salts **9**, **10**, **11**, **12**, **13**, and **14** were synthesized from their precursor quinolinecarboxylic acids **20**, **21**, **22**, **23**, **24** and **25** by reaction with 1 equiv. of a 10% NaOH solution according to Scheme 2.[8] The resulting 2,8-substituted quinolinecarboxylic acid sodium salts were isolated in yields ranging from 97 – 100%.

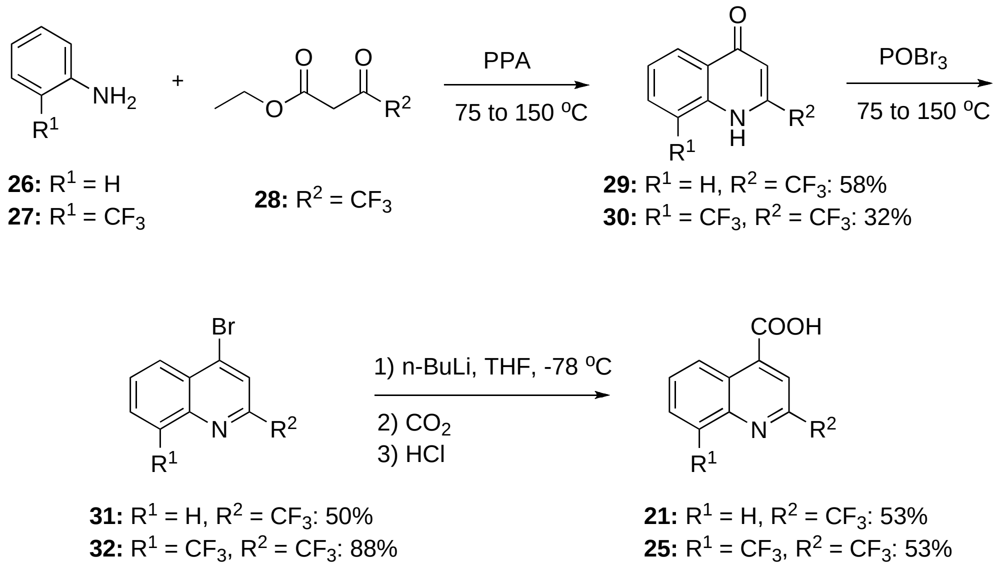


20: R^1 = H, R^2 = H
21: R^1 = H, R^2 = CF_3
22: R^1 = H, R^2 = cyclopropyl
23: R^1 = H, R^2 = furan
24: R^1 = H, R^2 = Ph
25: R^1 = CF_3, R^2 = CF_3

9: R^1 = H, R^2 = H: 100%
10: R^1 = H, R^2 = CF_3: 99%
11: R^1 = H, R^2 = cyclopropyl: 100%
12: R^1 = H, R^2 = furan: 100%
13: R^1 = H, R^2 = Ph: 99%
14: R^1 = CF_3, R^2 = CF_3: 97%

Scheme 2. Synthesis of the quinolinecarboxylic acid sodium salts **9**, **10**, **11**, **12**, **13**, and **14**.

The quinoline-4-carboxylic acid **20** and the 2-phenylquinoline-4-carboxylic acid **24** were purchased from commercial sources. The 2-(trifluoromethyl)quinoline-4-carboxylic acid **21** and the 2,8-bis(trifluoromethyl)quinoline-4-carboxylic acid **25** were synthesized according to the route shown in Scheme 3.

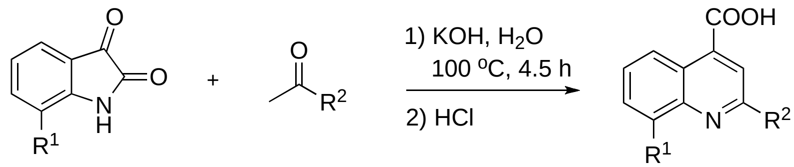


Scheme 3. Synthesis of the mono- and bis-(trifluoromethyl)-4-quinoline carboxylic acids **21** and **25**.

The synthesis of **21** and **25** began with the PPA condensation and subsequent cyclization of ethyl 4,4,4-trifluoro-3-oxobutanoate **28** with either aniline **26** or 2-(trifluoromethyl)aniline **27** to generate **29** and **30** in 58% and 32% yield, respectively.[9] Quinolines **29** and **30** were then reacted with $POBr_3$ at 150 °C, forming the corresponding 4-bromo-(trifluoromethyl)quinolines **31** and **32** in 50% and 88% yield, respectively.[9] In the last step, a lithium-halogen exchange reaction was performed on the 4-bromo-(trifluoromethyl)quinoline compounds **31** and **32** by reacting them with a solution of 1.34 M butyllithium in THF at -78 °C. This reaction generated the corresponding 4-lithium-(trifluoromethyl)quinoline reagents, which were then carboxylated with CO_2 (dry ice) and protonated with HCl to generate the final mono- and bis-(trifluoromethyl)-4-quinolinecarboxylic acids **21** and **25** in 53% yield.[9]

The remaining two quinolinecarboxylic acids, 2-cyclopropylquinoline-4-carboxylic acid **22** and 2-(furan-2-yl)quinoline-4-carboxylic acid **23**, were synthesized by using the

Pfitzinger reaction.[10] Reaction of isatin **33** with either 1-cyclopropylethanone **34** or 2-acetylfuran **35** in an 8.75 M solution of KOH at reflux, followed by subsequent acidification with concentrated HCl, generated **22** and **23** in 78% and 34% yield, respectively.[11]



33: R^1 = H

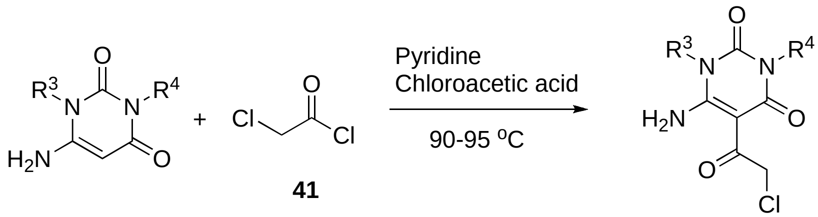
34: R^2 = cyclopropyl
35: R^2 = furan

22: R^1 = H, R^2 = cyclopropyl: 78%
23: R^1 = H, R^2 = furan: 34%

Scheme 4. Synthesis of quinolinecarboxylic acids **22** and **23**.

1.2.2 Synthesis of 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils

The 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils **15**, **16**, **17**, **18** and **19** were synthesized from their precursor 1-*N*-alkyl-3-*N*-alkyl-6-aminouracils **36**, **37**, **38**, **39** and **40** by reaction with 2-chloroacetyl chloride **41** in a mixture of pyridine and chloroacetic acid heated at 90–95 °C (Scheme 5).[12] 1-*N*-Alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils were precipitated from the reaction mixtures at 0 °C with deionized water and recrystallized from ethyl acetate and hexanes, affording the products **15**, **16**, **17**, **18** and **19** in 53%, 67%, 47%, 35% and 72% yield, respectively.

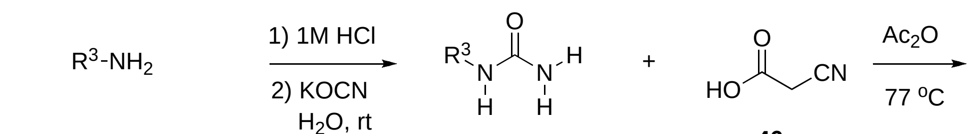


36: $R^3 = CH_3$, $R^4 = CH_3$
37: $R^3 = CH_3$, R^4 = benzyl
38: R^3 = methylcyclopropane, $R^4 = CH_3$
39: R^3 = *iso*-butyl, $R^4 = CH_3$
40: R^3 = benzyl, $R^4 = CH_3$

15: $R^3 = CH_3$, $R^4 = CH_3$: 53%
16: $R^3 = CH_3$, R^4 = benzyl: 67%
17: R^3 = methylcyclopropane, $R^4 = CH_3$: 47%
18: R^3 = *iso*-butyl, $R^4 = CH_3$: 35%
19: R^3 = benzyl, $R^4 = CH_3$: 72%

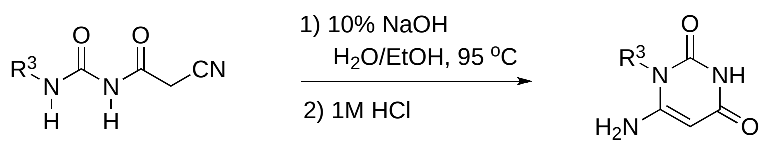
Scheme 5. Synthesis of 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils **15**, **16**, **17**, **18** and **19**.

The 1,3-*N*,*N*-dimethyl-6-aminouracil **36** and the 1-*N*-*iso*-butyl-3-*N*-methy-6-aminouracil **39** were purchased from commercial sources. The remaining three 1-*N*-alkyl-3-*N*-alkyl-6-aminouracils **37**, **38** and **40** were synthesized according to the reaction sequence shown in Schemes 6 and 7, which was chosen to control the positioning of the alkyl substituents around the uracil ring.



42: R^3 = benzyl
43: R^3 = methylcyclopropane

44: R^3 = benzyl: 58%
45: R^3 = methylcyclopropane: 76%

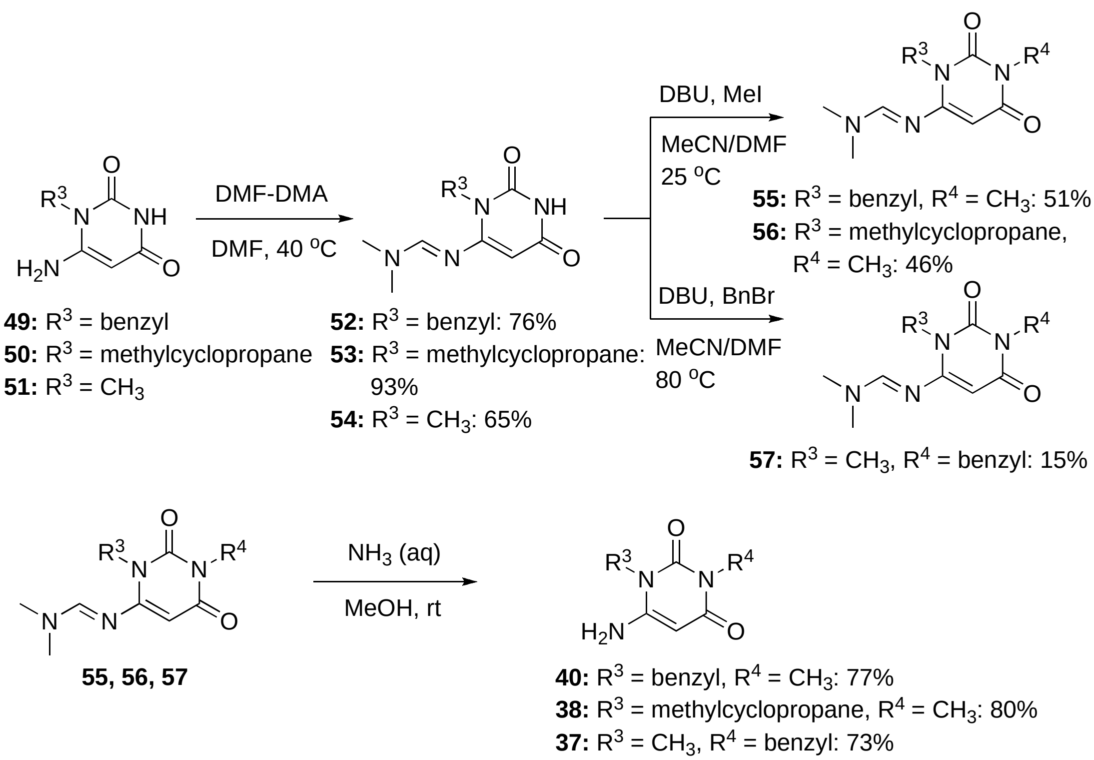


47: R^3 = benzyl: 75%
48: R^3 = methylcyclopropane: 56%

49: R^3 = benzyl: 50%
50: R^3 = methylcyclopropane: 36%

Scheme 6. Synthesis of 1-*N*-alkyl-6-aminouracils **49** and **50**.

For the synthesis of the 1-*N*-alkyl-6-aminouracils **49** and **50**, benzylamine hydrochloride **42** and methylcyclopropylamine hydrochloride **43** were reacted with KOCN in aqueous media to afford 1-benzylurea **44** and 1-(cyclopropylmethyl)urea **45** in 58% and 76% yield, respectively.[13] The urea products **44** and **45** were then condensed with 2-cyanoacetic acid **46** at 77 ^{o}C in acetic anhydride to produce the corresponding cyanoacetylureas **47** and **48** in 75% and 56% yields.[14] The products **47** and **48** cyclized by treatment with 10% NaOH in ethanol and water at 95 ^{o}C, affording the 1-*N*-alkyl-6-aminouracils **49** and **50** in 50% and 36% yield upon acidification.[14]

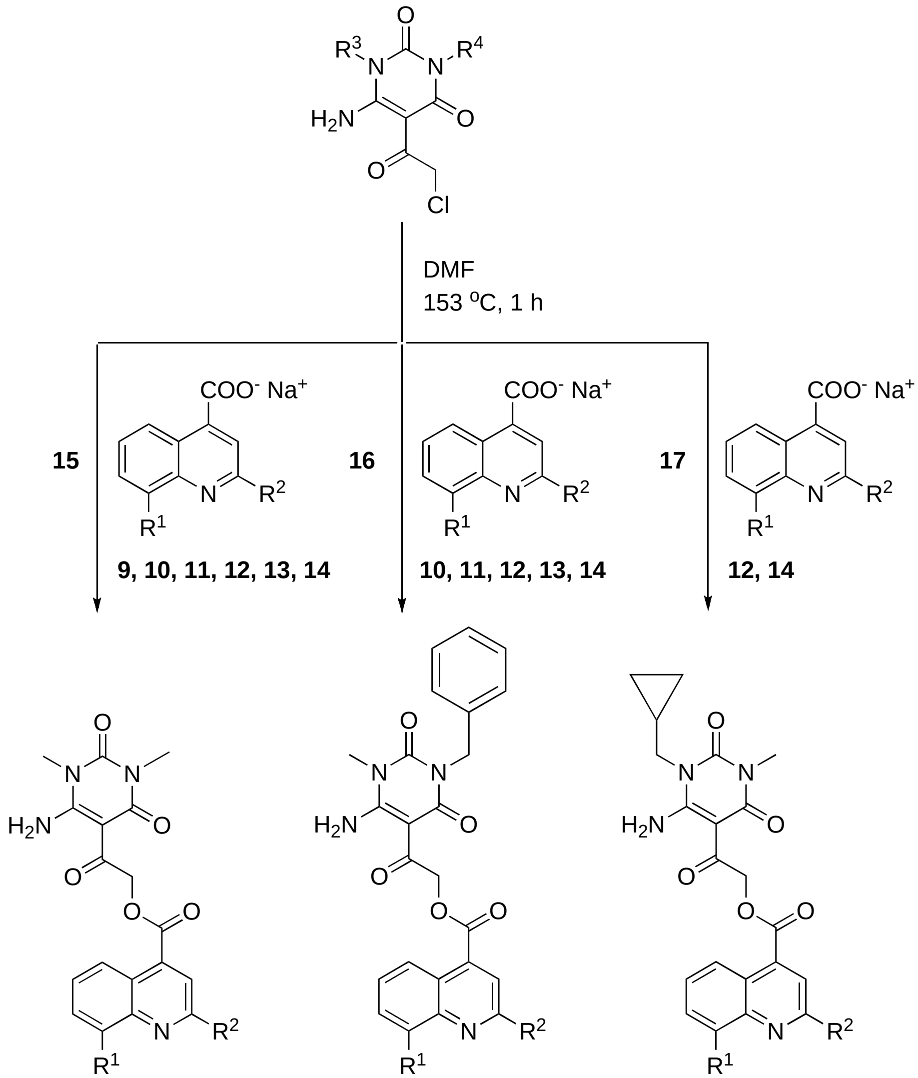


Scheme 7. Synthesis of 1-*N*-alkyl-3-*N*-alkyl-6-aminouracils **37**, **38** and **40**.

The route for the synthesis of the 1-*N*-alkyl-3-*N*-alkyl-6-aminouracils **37**, **38** and **40** is shown in Scheme 7. The 1-*N*-methyl-6-aminouracil **51** was purchased from a commercial source. The amino functionality of 1-*N*-alkyl-6-aminouracils **49**, **50** and **51** was protected by the way of DMF-DMA in DMF at 40 °C, generating the 1-*N*-alkyl-6-[(dimethylamino)methylene]uracils **52**, **53** and **54** in 76%, 93% and 65% yield, respectively.[15] The 1-*N*-alkyl-6-[(dimethylamino)methylene]uracils **52** and **53** were alkylated at the *N*-3 position by reaction with DBU and MeI in a mixture of MeCN and DMF at room temperature over a 2 d period, affording the 1-*N*-alkyl-3-*N*-methyl-6-[(dimethylamino)methylene] uracils **55** and **56** in 51% and 46% yield.[15] The 1-*N*-methyl-6-[(dimethylamino)methylene]uracil **54** was alkylated at the *N*-3 position by reaction with DBU and BnBr in a mixture of MeCN and DMF at 80 °C over a 5 h period, affording the 1-*N*-methyl-3-*N*-benzyl-6-[(dimethylamino)methylene]uracil **57** in 15% yield. Following the synthesis of **55**, **56** and **57**, the (dimethylamino)methylene group was removed by a solution of aqueous ammonia in methanol at room temperature over several days, affording the final 1-*N*-alkyl-3-*N*-alkyl-6-aminouracils **40**, **38** and **37** in 77%, 80% and 73% yield, respectively.[15]

1.2.3 Synthesis of the final library compounds

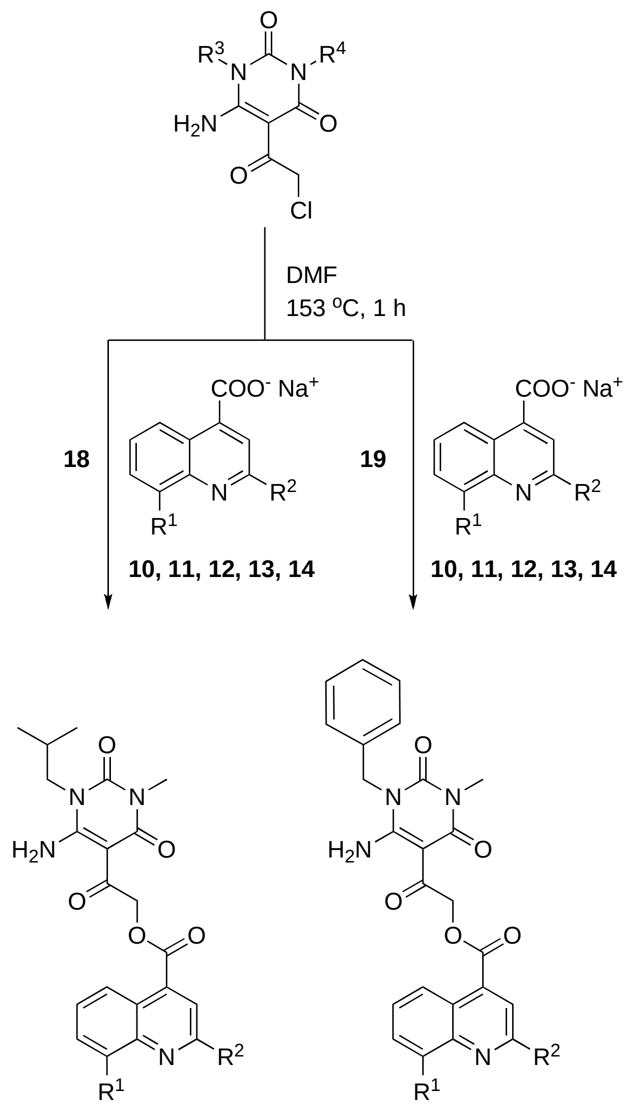
Segment assembly followed the route shown in Scheme 8. A convergent strategy for the final library of analogues was based on reacting six 2,8-substituted-4-quinolinecarboxylic acid sodium salts **9**, **10**, **11**, **12**, **13** and **14** with five 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils **15**, **16**, **17**, **18** and **19** through an S_N2 reaction in DMF at reflux.[8] The products were precipitated from the reaction mixture by the slow addition of deionized water while cooling to 0 °C over a 1 h period. The resulting library analogues were then dried in a Genevac solvent evaporator to remove residual traces of DMF. The 23 library components **58 – 80** were synthesized in yields ranging from 31 – 74% (Scheme 8). All of the final products were analyzed by ^{1}H NMR and RP LC/MS and were found to be >85% pure by UV detection at 210 nm or >90% pure by ELSD with the exception of **63** which was found to be >95% pure by ^{1}H NMR analysis.[1] A total of 12 compounds were fully characterized by Mp, IR, ^{1}H NMR, ^{13}C NMR and HRMS. At least one compound containing each unique quinoline or uracil subunit was chosen for full characterization.



58: R^1 = H, 74%
R^2 = H
59: R^1 = H, 45%
R^2 = CF_3
60: R^1 = H, 59%
R^2 = cyclopropyl
61: R^1 = H, 70%
R^2 = furan
62: R^1 = H, 66%
R^2 = Ph
63: R^1 = CF_3, 56%
R^2 = CF_3

64: R^1 = H, 62%
R^2 = CF_3
65: R^1 = H, 50%
R^2 = cyclopropyl
66: R^1 = H, 59%
R^2 = furan
67: R^1 = H, 47%
R^2 = Ph
68: R^1 = CF_3, 53%
R^2 = CF_3

69: R^1 = H, 56%
R^2 = furan
70: R^1 = CF_3, 55%
R^2 = CF_3



71: R^1 = H, 58%
R^2 = CF_3
72: R^1 = H, 31%
R^2 = cyclopropyl
73: R^1 = H, 61%
R^2 = furan
74: R^1 = H, 60%
R^2 = Ph
75: R^1 = CF_3, 55%
R^2 = CF_3

76: R^1 = H, 51%
R^2 = CF_3
77: R^1 = H, 48%
R^2 = cyclopropyl
78: R^1 = H, 54%
R^2 = furan
79: R^1 = H, 39%
R^2 = Ph
80: R^1 = CF_3, 58%
R^2 = CF_3

Scheme 8. Synthesis of the final MKP-1 inhibitor library.

Among the five 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils, only the 1,3-*N*,*N*-dimethyl-5-chloroacetyl-6-aminouracil **15** was reacted with quinoline-4-carboxylic acid sodium salt **9**. The resulting derivative **58** represents the simplest analogue used to probe the MKP-1 inhibitor binding domain. Analogue **58** was also readily constructed from commercially available starting materials (**20** and **51**) as a way to test the methodology proposed for synthesizing the 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils and the final library analogues. Only four of the 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils **15**, **16**, **18** and **19** were reacted with the five 2,8-(substituted)quinoline-4-carboxylic acid sodium salts **10**, **11**, **12**, **13** and **14**. This was the result of a low overall yield for the synthesis of **17** (2% yield over 7 steps), which generated only enough material for the synthesis of two final library analogues. Therefore, **17** was reacted with **12** to allow for a direct biological activity comparison to the original hit **8**, and was also reacted with **14** due to the known biological activity of the 2,8-bis(trifluoromethy)quinoline substructure found in mefloquine, an antimalarial agent.[16]

1.3 BIOLOGICAL RESULTS FOR MKP-1 INHIBITORS

A total library of 47 compounds comprised of 23 fully-assembled analogues and 24 intermediate quinolines or uracils was submitted to the University of Pittsburgh Molecular Libraries Screening Center (UPMLSC) for testing against MKP-1. The 47-compound library was also registered on PubChem and the PubChem DMA-xyz compound names have been retained in the experimental section of this document.[17] None of the quinoline- or uracil-based precursors were found to be active inhibitors of MKP-1, suggesting that both the quinoline and uracil subunits are necessary for activity. The results of the enzyme assays have been published and are summarized in Table 1.[1]

Table 1. Biological Results[a] Against MKP-1 and MKP-3.[1]

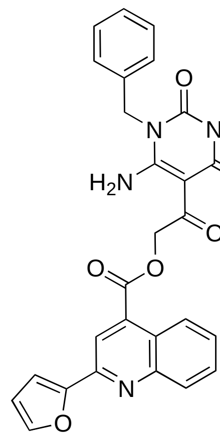
Compound	PubChem CID[a]	R^1	R^2	R^3	R^4	MKP-1 IC_{50} (μM)	MKP-3 IC_{50} (μM)
58	9547710	H	H	CH_3	CH_3	>50	>50
59	9547724	H	CF_3	CH_3	CH_3	>50	>50
60	9547721	H	cyclopropyl	CH_3	CH_3	>50	>50
61	2364091	H	furan	CH_3	CH_3	20.6	>50
62	2358568	H	Ph	CH_3	CH_3	>50	>50
63	9547725	CF_3	CF_3	CH_3	CH_3	>50	>50
64	9547733	H	CF_3	CH_3	Bn	>50	>50
65	9547736	H	cyclopropyl	CH_3	Bn	28.9	>50
66	9547735	H	furan	CH_3	Bn	16.9	>50
67	9547737	H	Ph	CH_3	Bn	>50	>50
68	9547734	CF_3	CF_3	CH_3	Bn	>50	>50
69	9547740	H	furan	methylcyclopropyl	CH_3	24.6	>50
70	9547741	CF_3	CF_3	methylcyclopropyl	CH_3	>50	>50
71	9547738	H	CF_3	isobutyl	CH_3	>50	>50
72	9547722	H	cyclopropyl	isobutyl	CH_3	>50	>50
73	2094474	H	furan	isobutyl	CH_3	50	>50
74	2098087	H	Ph	isobutyl	CH_3	>50	>50
75	9547728	CF_3	CF_3	isobutyl	CH_3	>50	>50
76	9547729	H	CF_3	Bn	CH_3	>50	>50
77	9547732	H	cyclopropyl	Bn	CH_3	>50	>50
78	9547731	H	furan	Bn	CH_3	13.4	>50
79	9547726	H	Ph	Bn	CH_3	50	>50
80	9547730	CF_3	CF_3	Bn	CH_3	>50	>50

[a]Pubchem compound identification number

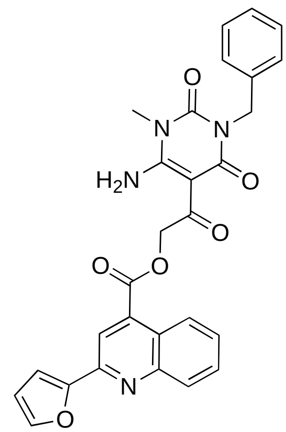
Curiously, the resynthesized **73** suffered from an almost three-fold loss of potency with an IC_{50} value of 50 μM, versus 19.2 μM for the original hit **8**. The reasons for this loss of potency are unclear. The analogues **61**, **65**, **66**, **69** and **78** showed an IC_{50} value comparable to

[a] Assays performed in the laboratory of professor John S. Lazo.

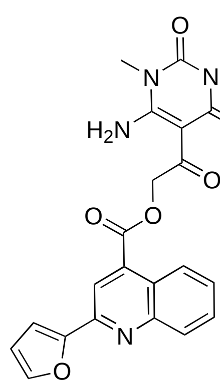
the original hit **8** (Figure 4). These five analogues also demonstrated selectivity for MKP-1 when compared to MKP-3. Analogues **61**, **65**, **66**, **69** and **78** all inhibited MKP-3 with IC_{50} values >50 μM.



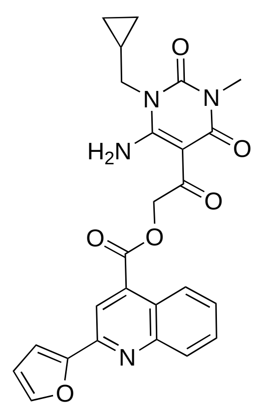
78
IC_{50} = 13.4 μM



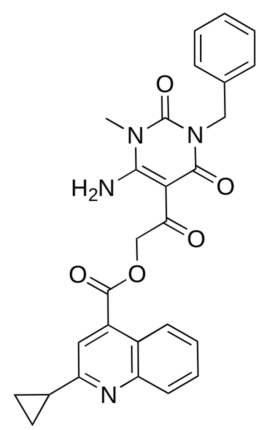
66
IC_{50} = 16.9 μM



61
IC_{50} = 20.6 μM



69
IC_{50} = 24.6 μM



65
IC_{50} = 28.9 μM

Figure 4. Library analogues found to inhibit MKP-1 with a similar potency as to the original hit.

A comparison of the structures for the five library analogues found to selectively inhibit MKP-1 shows some interesting trends leading to valuable structure activity relationship (SAR) information about the binding of this novel class of quinoline-uracil based MKP-1 inhibitors. The alkyl substituents on the uracil *N*-1 and *N*-3 positions have only a modest effect on binding, with bulkier substituents, such as benzyl, being favored. Interestingly, both isomeric forms **78** and **66** of the benzyl-substituted aminouracil demonstrated tight inhibitor binding. This result suggests that a maximization of hydrophobic interactions at the position where the *N*-1 and *N*-3 alkyl substituents on the aminouracil sit in the inhibitor binding domain of MKP-1 may lead to a second generation library of more potent inhibitors.

The nature of the substituents on the quinoline ring system plays an important role in a tight binding of the quinoline-uracil analogues to MKP-1. Substitution at the 4-quinoline position was found to greatly decrease the IC_{50}, as shown for analogues **63**, **68**, **70**, **75** and **80**. As seen in Figure 4, four of the five most potent inhibitors of MKP-1 retained the furan substituent at the 2-quinoline position. This result, in comparison to the 2-phenyl substituted quinoline analogues **62**, **67**, **74** and **79**, suggests that, at the position where the quinoline substructure rests in the inhibitor binding domain of MKP-1, potentially both steric and electronic effects govern inhibitor binding. This result suggests that a second-generation library of quinoline-uracil based MKP-1 inhibitors may be extended to include other heteroaromatic substituents at the 2-quinoline position such as thiophene, pyrrole, benzofuran, thiazole and pyridine.

1.4 CONCLUSIONS

Collaborative research efforts have exposed a novel class of quinoline-uracil based MKP-1 inhibitors, which were found to be selective for MKP-1 over MKP-3. The active inhibitors were found to inhibit MKP-1 with low μM IC_{50} values comparable to those of the known inhibitors sanguinarine and NU-126.[6,7] A library of analogs provided valuable SAR data for the design of this class of phosphatase inhibitors. These results can guide the development of new quinoline-uracil based MKP-1 inhibitors.

2.0 ADDITION OF ORGANOMETALLIC REAGENTS TO NOVEL HETEROCYCLIC SULFONYL AND SULFINYL BENZALDIMINES

2.1 INTRODUCTION

This chapter explores the reactivity of heterocyclic sulfonyl- and sulfinylbenzaldimines towards a variety of organometallic nucleophiles. The synthesis of these uniquely protected aldimines and cleavage of the resulting heterocyclic protecting groups to generate the corresponding α-branched primary amine products are also discussed.

2.1.1 Overview of imines

The addition of organometallic carbon-based nucleophiles to the C=N bond of imines represents an important class of carbon-carbon bond forming reactions that has been heavily investigated throughout the past fifty years. More recently, developments in this field have focused on the synthesis of α-chiral amines through the asymmetric addition of organometallic reagents to imines. These asymmetric addition reactions have been shown to proceed effectively through the use of chiral auxiliaries and with both stoichiometric and catalytic amounts of chiral ligands. The success of these organometallic addition reactions is highly dependent on both the reactivity of the imine and on the nature of the organometallic reagent.

Imine reactivity is often mediated through the electron-withdrawing effects of the nitrogen atom substituent, a result which has led to the development of several classes of functionalized imines (Figure 5).[18,19] It has been reported that, in general, the relative reactivities of these imines decreases in the order of *N*-acyliminium ions > *N*-acylimines >> *N*-sulfonylimines > *N*-phosphinoylimines > *N*-alkyl and *N*-arylimines.[18,19] While the nature of the imine nitrogen atom substituent mediates the reactivity of the imine by controlling the electrophilicity of the C=N carbon terminus, it also serves as a protecting group for the resulting amine products.[18] Accordingly, it is important to be able to easily remove these protecting groups after the addition reaction.

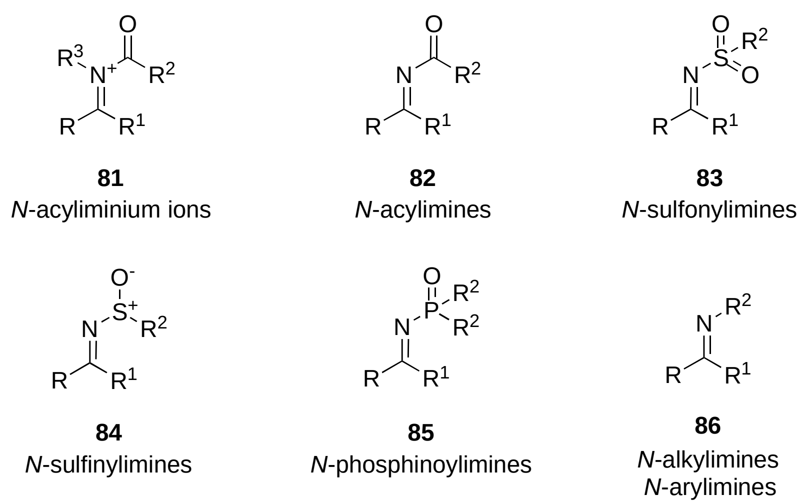
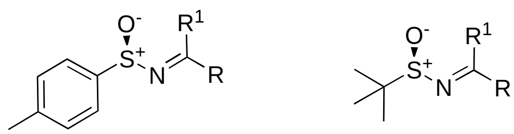


Figure 5. Representation of several known classes of imines.[18,19]

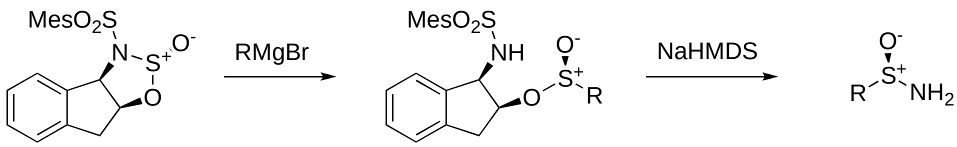
Deprotection of *N*-acylamines such as the *N*-Boc and *N*-formyl amines, as well as *N*-phosphinoylamides and *N*-sulfinamides, can be generally accomplished under acidic conditions with HCl in protic solvents such as methanol.[20-23] The deprotection of *N*-sulfonamides has traditionally been performed under much harsher reaction conditions, by reactions with reagents such as sodium in liquid ammonia, sodium naphthalene in 1,2-dimethoxyethane, and with SmI_2 and DMPU in THF at reflux.[24-26] A milder method for the deprotection of primary *N*-arylsulfonylamides requires the conversion of these products to their corresponding *t*-butyl *N*-arylsulfonylcarbamates by a DMAP-catalyzed reaction with Boc_2O.[27] The *N*-arylsulfonyl group can then be cleaved by reaction with Mg metal in methanol resulting in the corresponding *N*-Boc amines.[27]



87 **88**

Figure 6. Structures of Davis (**87**) and Ellman (**88**) *N*-sulfinylimines.

Among the several classes of imines shown in Figure 5, the *N*-sulfinylimines are unique in their ability to both activate the imine C=N bond towards nucleophilic addition and, when synthesized enantiomerically pure, serve as chiral auxiliaries to direct the diastereofacial selectivity for the nucleophilic addition reaction.[28] The most widely utilized *N*-sulfinylimines are the *N*-*p*-toluenesulfinyl imines **87**, pioneered by Davis, and the *N*-*tert*-butanesulfinyl imines explored by Ellman (**88,** Figure 6). Recently, a general procedure for the synthesis of enantiopure *N*-sulfinamides **91** from enantiomerically pure *N*-sulfonyl-1,2,3-oxathiazolidine-2-oxides **89** has emerged (Scheme 9).[29] The resulting *N*-sulfinamides can be condensed with a variety of aldehydes or ketones in the presence of $Ti(OEt)_4$ to form the enantiopure *N*-sulfinylimines.[28]



Scheme 9. Asymmetric synthesis of *N*-sulfinamides.[29]

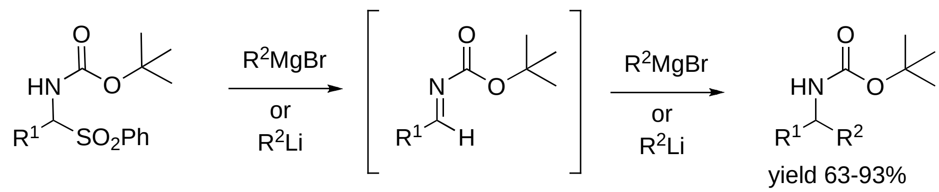
2.1.2 Addition of organometallic reagents to imines

The chemistry surrounding the addition of organometallic reagents to the imines listed above is rich in both scope and utility. Indeed, over the last few decades, many of these imines have been demonstrated to be excellent reaction partners for the addition of lithium, magnesium, zinc, copper, rhodium and aluminum based organometallic reagents. Also, more recently, asymmetric variants have been explored.

2.1.2.1 Organolithium and Grignard reagents

Organolithium and organomagnesium reagents have the dual properties of being good nucleophiles and strongly basic. The addition of these reagents to unactivated imines such as *N*-alkyl or *N*-arylimines is often difficult due to low imine reactivity and competitive α-deprotonation of imines that can form metalated enamines.[30] However, activated imines such as *N*-acylimines, *N*-sulfonylimines, *N*-phosphonylimines and *N*-sulfinylimines react quickly.

As a result of the high reactivity of *N*-acylimines, they are often unstable and cannot be isolated.[19] This has led to the development of many procedures for their *in situ* preparation.[18] A nice example has been demonstrated by Petrini, which utilizes both the nucleophilic and basic characteristics of organolithium and organomagnesium reagents for the *in situ* preparation and tandem nucleophilic addition to a variety of alkyl and aryl aldehyde based α-amidosubstituted sulfones **92** (Scheme 10).[30]



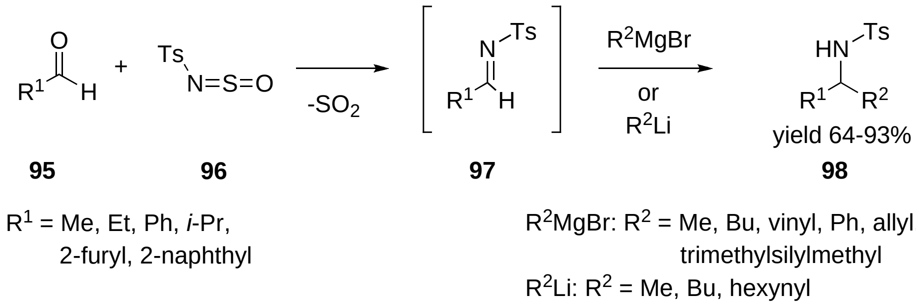
92 **93** **94**

R^1 = Ph, *i*-Bu, phenethyl

R^2MgBr: R^2 = vinyl
R^2Li: R^2 = phenethynyl, hexynyl, trimethylsilylethynyl

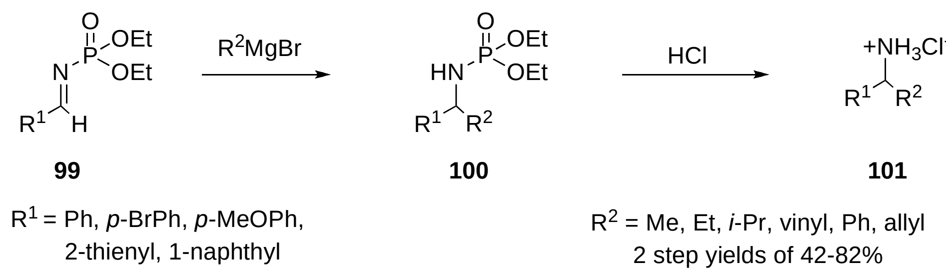
Scheme 10. Organometallic additions to *in situ* prepared *N*-acylimines.[30]

Along similar lines, methodology for the *in situ* preparation of alkyl and aryl aldehyde based *N*-sulfonylimines followed by the addition of organolithium or Grignard reagents has also been developed. Weinreb demonstrated a nice example through the use of the Kresze reaction (Scheme 11).[31] Aldehydes **95** were reacted with *N*-sulfinyl p-toluenesulfonamide **96** to generate the corresponding *N*-tosylimines **97** *in situ*, which were then reacted with a variety of alkyl, vinyl, allyl and alkynyl organolithium or Grignard reagents.[31]



Scheme 11. Organometallic additions to *in situ* prepared *N*-sulfonylimines.[31]

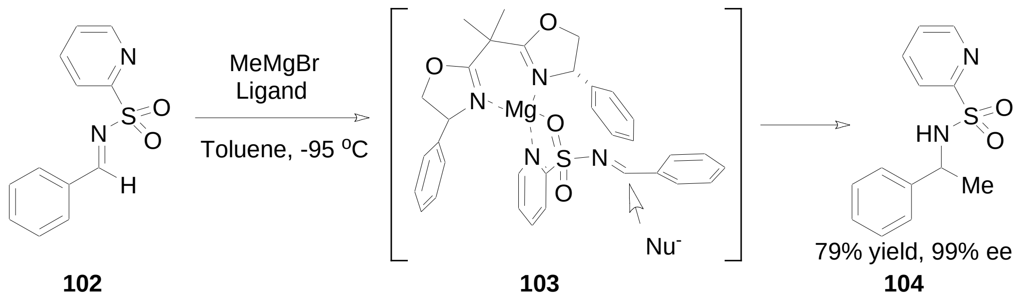
Zwierazk demonstrated that α-arylalkylamines could also be generated by the addition of alkyl, vinyl, allyl and aryl Grignard reagents to *N*-(diethoxyphosphoryl)aldimines **99** (Scheme 12).[23] The deprotected α-arylalkylamines **101** were generated in moderate to good yields over the two step addition/deprotection sequence.[23]



Scheme 12. Addition of organometallics to N-phosphinoylimines.[23]

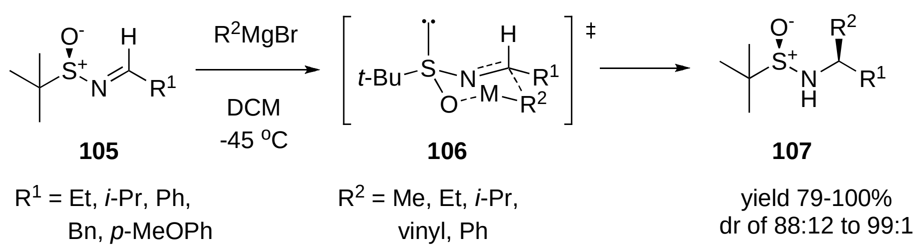
As a result of the high reactivity of organolithium and organomagnesium reagents, asymmetric addition reactions have generally been performed using stoichiometric amounts of chiral ligands or chiral auxiliaries attached directly to the imine or to the organometallic reagent.[18,19] An interesting example of the use of a stoichiometric amount of ligand has been demonstrated by Toru. Grignard reagents were found to asymmetrically add to *N*-(2-pyridylsulfonyl) aldimines **102** in the presence of a chiral phenyl-bis(oxazoline) ligand (Scheme

13).[32] The asymmetric induction for these reactions is rationalized by invoking a ligand-bound tetrahedral Mg(II) complex **103**, where the metal chelates with the oxygen atom and the nitrogen atom of the 2-pyridylsulfonyl protecting group.[32,33]



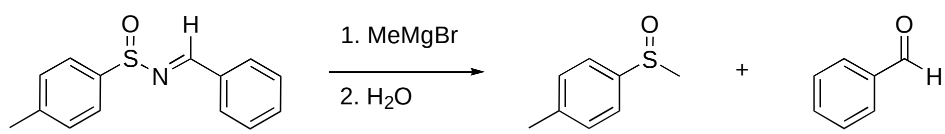
Scheme 13. Asymmetric addition of MeMgBr to *N*-(2-pyridylsulfonyl)aldimines.[32]

Ellman demonstrated that both aliphatic and aromatic *N*-*tert*-butanesulfinyl aldimines **105** reacted with Grignard reagents in non-coordinating solvents to produce the corresponding *N*-*tert*-butanesufinamides **107** in good yields and with excellent diastereoselectivities (Scheme 14).[34] Since the diastereoselectivities for these reactions decreased in the presence of coordinating solvents, a cyclic six membered transition state **106** was proposed.[34]



Scheme 14. Addition of Grignard reagents to *N*-tert-butylsulfinyl imines.[34]

Moreau demonstrated that aromatic *N*-*p*-toluenesulfinyl aldimines reacted with BnMgCl in toluene at -30 °C to give the corresponding *N*-*p*-toluenesulfinamides in moderate yields and diastereoselectivities.[35] However, reactions performed under the same conditions with MeMgBr and **108** resulted in the formation of the sulfur addition products **109** and **110** (Scheme 15).[35] In light of Moreau's findings, Chan demonstrated that the addition of a stoichiometric amount of CuI to the reaction mixture containing two equivalents of MeMgBr and **108** at -15 °C helped to suppress the formation of the sulfur adduct **109**. This reaction gave the desired *N*-*p*-toluenesulfinamide product in 49% yield and the sulfoxide **109** in only 21% yield.[36]

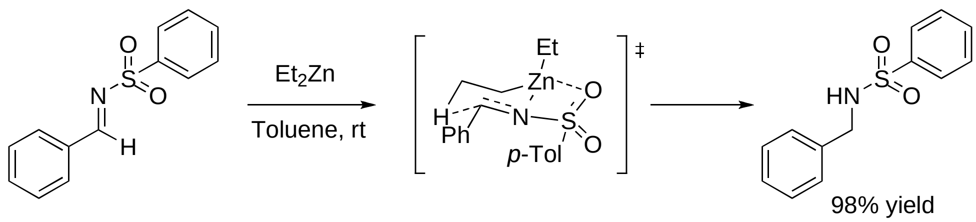


108 **109** **110**

Scheme 15. Addition of MeMgBr to the *N*-*p*-toluenesulfinyl aldimine **108**.[35]

2.1.2.2 Dialkylzinc reagents

The lower nucleophilicity of dialkylzinc reagents has hindered their direct addition to less activated imines such as *N*-alkyl and *N*-arylimines, as well as *N*-phosphinolyimines. This was demonstrated in a paper by Qian who studied the effects of diethylzinc coordination in reactions with *N*-tosylimines.[37] Qian reported that neither (*E*)-*N*-benzylidene-*P*,*P*-diphenylphosphinic amide nor (*E*)-*N*-benzylideneaniline reacted with Et_2Zn after 24 h in toluene at room temperature.[37] However, after 1 h in toluene, (*E*)-*N*-benzylidenebenzenesulfonamide **111** was shown to react with Et_2Zn to give the reduction product **113** (Scheme 16).[37] The product was proposed to arise from transfer of a β-hydrogen atom from one of the ethyl groups on zinc to the carbon terminus of the imine C=N bond *via* the cyclic six-membered transition state **112** shown in Scheme 16.[37] This transition state is supported by a solvent study which showed that when coordinating solvents such as THF are used, the ethyl adduct becomes the predominant product for the reaction.[37]

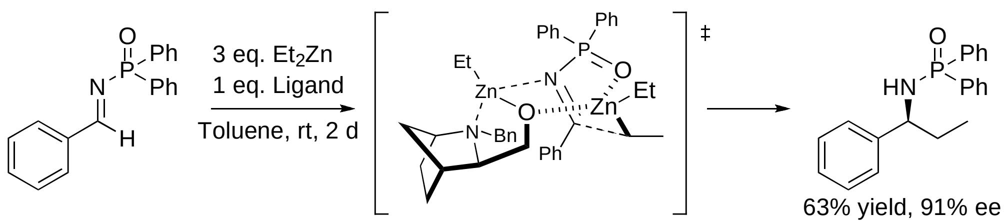


111 **112** **113**

Scheme 16. Reduction of *N*-tosylimines with Et_2Zn.[37]

Carretero demonstrated that *N*-(2-pyridinesulfonyl)aldimines **102** react with alkyl zinc bromides under copper catalyzed conditions to give the corresponding *N*-(2-pyridinesulfonyl)amides in good to excellent yields.[38] Primary and secondary alkyl zinc bromides containing alkene, ether, acetal, chloride, ester and nitrile groups were found to add efficiently to **102**.[38] The reactivity of *N*-(2-pyridinesulfonyl)aldimine was explained to arise from coordination of the imino and pyridyl nitrogen atoms to the copper catalyst, forming a five-membered chelate ring.[38] This metal coordination was used to explain the enhanced reactivity of *N*-(2-pyridinesulfonyl)aldimines over that of *N*-(heteroarylsulfonyl)imines which do not have the ability to form a bidentate chelate and were found not react under copper catalyzed conditions with organo zinc bromide reagents.[38]

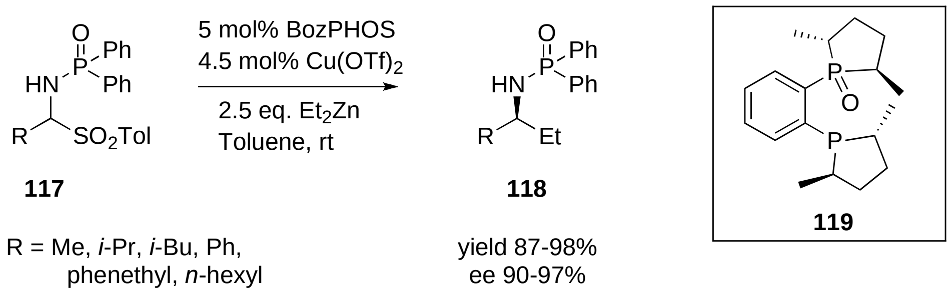
As a result of the low nucleophilicity of dialkylzinc reagents, many asymmetric addition reactions have been reported which utilize Lewis basic chiral ligands to activate the zinc reagent for addition to the imine. For example, Andersson discovered that the use of a stoichiometric amount of the ((1*S*,3*R*,4*R*)-2-methyl-2-azabicyclo[2.2.1]heptan-3-yl)methanol ligand effectively activated Et_2Zn for the asymmetric addition to (*E*)-*N*-benzylidene-*P*,*P*-diphenylphosphinic amide **114** (Scheme 17).[39] The proposed transition state **115** for this reaction is shown in Scheme 17.[39]



114 **115** **116**

Scheme 17. Ligand-activated asymmetric addition of Et_2Zn to *N*-phosphinoyl imine **114**.[39]

Charette has found that dialkylzinc reagents can be asymmetrically added to both alkyl and aryl *N*-phosphinoylaldimines, generated in situ, using a catalytic amount of $Cu(OTf)_2$ and the chiral BozPHOS ligand (**119**, Scheme 18).[40,41]



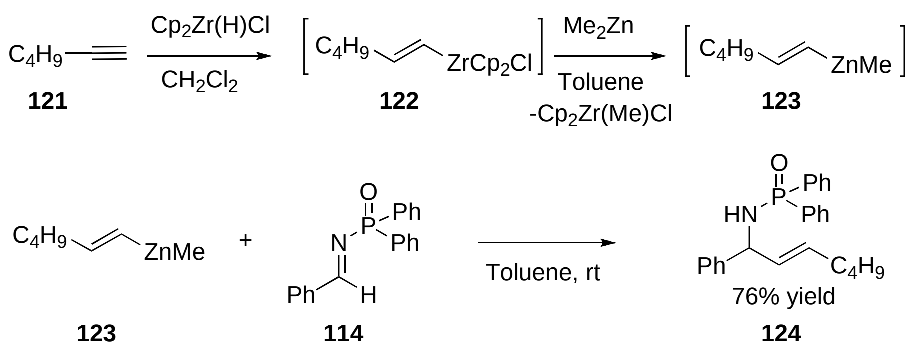
Scheme 18. Copper-catalyzed asymmetric addition of Et_2Zn to *N*-phosphinoyl aldimines.[41]

2.1.2.3 Hydrozirconation and transmetallation

Hydrozirconation of alkenes and alkynes by the use of Schwartz's reagent ($Cp_2Zr(H)Cl$, **120**) is a well established way to prepare alkyl and alkenylzirconocenes.[42] While these species are nucleophilic, the steric shielding of the cyclopentadienyl ligands on zirconium often prevents nucleophilic addition of the alkyl or alkenyl group to bulky elecrophiles.[42] As a result, transmetallation of these groups from zirconium to other metal centers such as zinc, rhodium and aluminum has led to the generation of a variety of organometallic nucleophiles capable of addition to electrophiles such as imines. Organometallic reagents generated from transmetallation of organozirconocenes have been effectively added to *N*-acyl, *N*-sulfonyl, *N*-phosphinoyl and *N*-sulfinylimines.

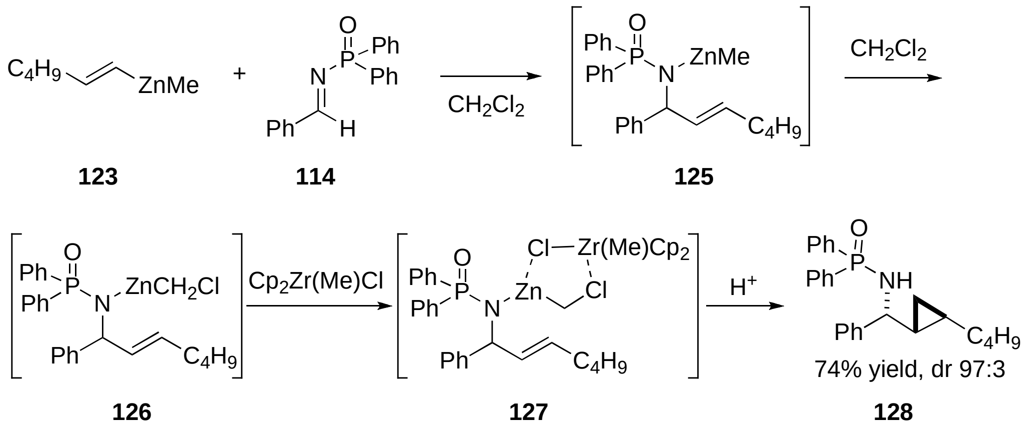
In 2002, Wipf reported that the organometallic reagents derived from hydrozirconation of alkynes followed by transmetallation with Me_2Zn effectively added to *N*-sulfonyl and *N*-phosphinoylaldimines.[43] Scheme 19 illustrates the hydrozirconation of 1-hexyne **121** with Schwartz's reagent **120** to generate the alkenylzirconocene species **122**. The alkenyl substituent is then transmetallated from **122** with Me_2Zn to form the vinylzinc reagent **123**, which can then be added to the *N*-phosphionylimine **114** to form the final allylic *N*-

phosphinoylamide **124**. This work showed that a range of vinylzinc reagents could be prepared *in situ* from symmetrical internal alkynes and terminal alkynes containing silyl ether, silyl ester, sulfonamide and carbamate functionality.[43] These zinc reagents were found to add in good overall yields (35-90%) to both aryl *N*-phosphinoylaldimines and alkyl and aryl *N*-sulfonylaldimines if the addition reactions were conducted in toluene.[43]



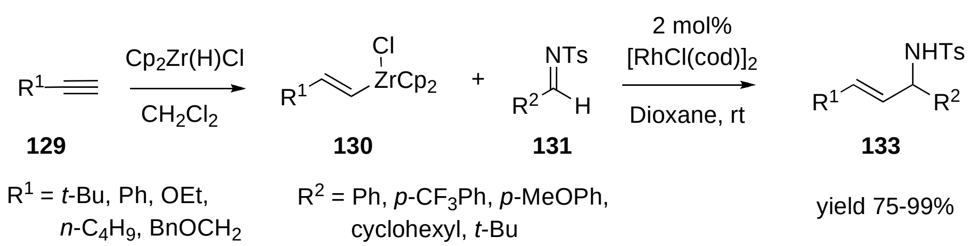
Scheme 19. *In situ* preparation of vinylzinc reagents and addition to aldimines.[43]

If the aldimine **114** was added to the vinylzinc reagent **123** in CH_2Cl_2, then the predominant product of the reaction was the *C*-cyclopropylalkylamide **128**.[43] This product was proposed to arise from the reaction pathway shown in scheme 20. The reaction was found to be general for a number of aldimines, preferentially producing the *anti* diastereoisomer of the *C*-cyclopropylalkylamide products in good overall yields (45-91%).[43]

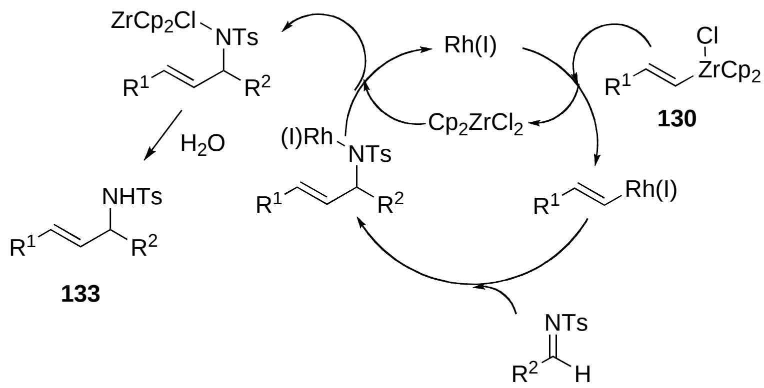


Scheme 20. Reaction pathway for the synthesis of *C*-cyclopropylalkylamides.[43]

It was later reported by Hanzawa that $[RhCl(cod)]_2$ **132** could catalyze the addition of alkenylzirconocene chlorides **130** to both aryl and alkyl *N*-sulfonylaldimines (**131**, Scheme 21).[44] The proposed catalytic cycle for these reactions is shown in Scheme 22.[44]

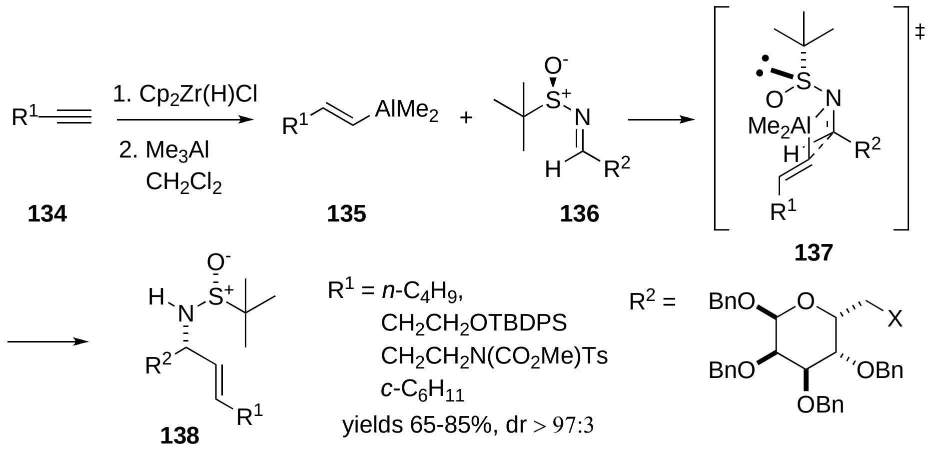


Scheme 21. Rhodium catalyzed addition of alkenylzirconocenes to aldimines.[44]



Scheme 22. Proposed catalytic cycle for the rhodium-catalyzed addition of alkenylzirconocenes to aldimines.[44]

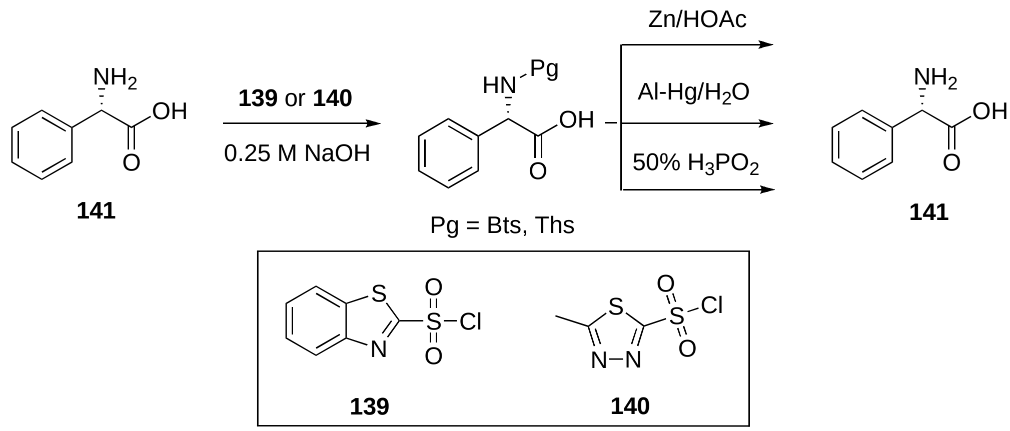
The catalytic enantioselective addition of alkenylzirconocenes through zirconocene-zinc transmetallation has been well-established for the synthesis of chiral allylic alcohols resulting from addition into aldehydes.[45] However, similar methodology for the synthesis of chiral allylic amines is still lacking. An interesting discovery was made in the Wipf group, demonstrating that alkenylzirconocenes, prepared via hydrozirconation of alkynes with $Cp_2Zr(H)Cl$, could be transmetallated to alane **135** and added to *N*-tert-butanesulfinylaldimines **136** (Scheme 23).[46] A four-membered chelate transition state **137** was proposed to explain the stereochemistry of the resulting products.[46]



Scheme 23. Addition of vinylalanes to *N*-tert-butanesulfinylaldimines.[46]

2.1.3 Introduction to Bts and Ths sulfonyl protecting groups

In 1996, Vedejs introduced benzothiazole-2-sulfonyl chloride (**139**, BtsCl) and 5-methyl-1,3,4-thiadiazole-2-sulfonyl chloride (**140**, ThsCl) as efficient nitrogen atom protecting reagents for use in peptide coupling reactions.[47] He reported that these protecting groups could be removed from amino acids in high yields without racemization under a variety of conditions, including treatment with Zn and acetic acid in ethanol, Al and $HgCl_2$ in water and THF, and H_3PO_2 in THF at reflux (Scheme 24).[47] The electron-withdrawing effects of the heteroaromatic group on the sulfone activate it for reduction with a variety of reagents, including SmI_2, Mg(0) and thiolate nucleophiles.[24,32,48] Many of these deprotection conditions are much more mild than those traditionally used for the cleavage of more electron rich sulfones.



Scheme 24. Protection and deprotection conditions for the Ths and Bts groups.[47]

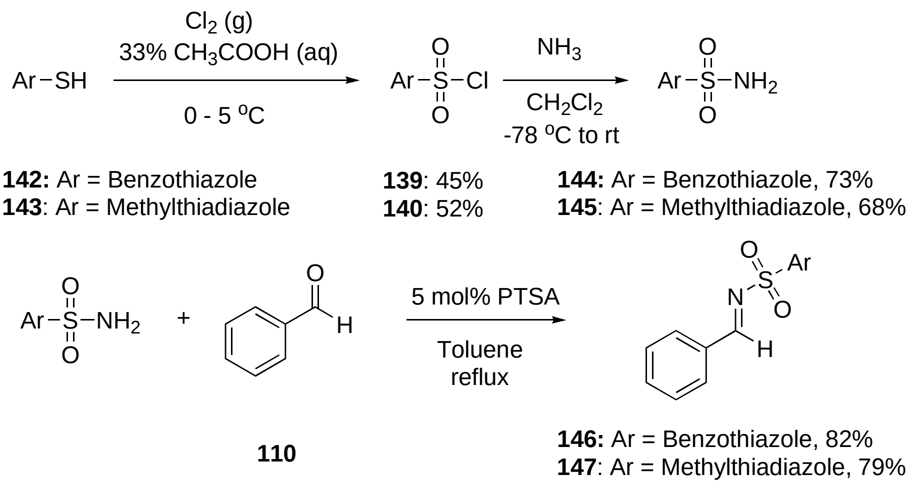
2.2 RESULTS AND DISCUSSION

We sought to explore the reactivity of *N*-Bts- and *N*-Ths-benzaldimines with a variety of organometallic carbon-based nucleophiles. This section will discuss the synthesis of these aldimines and their reactivity towards a number of organometallic reagents including lithium-, magnesium-, zinc-, aluminum-, rhodium- and copper-based nucleophiles.

2.2.1 Synthesis of *N*-Bts- and *N*-Ths-benzaldimines

N-Bts- and *N*-Ths-benzaldimines were synthesized according to the route shown in Scheme 25. Initially, benzo[d]thiazole-2-thiol **142** and 5-methyl-1,3,4-thiadiazole-2-thiol **143** were converted to their corresponding sulfonyl chlorides **139** and **140** by oxidative chlorination with Cl_2(g) in 33% aqueous acetic acid according to the procedure described by Vedejs.[47] While the yields for these products varied considerably, the average yields were 45% for Bts-Cl and 52% for Ths-Cl.

The sulfonyl chlorides **139** and **140** were further reacted with liquid NH_3 to form the sulfonamides **144** and **145** in 73% and 68% yield.



Scheme 25. Synthesis of *N*-Bts- and *N*-Ths-benzaldimines.

Initial attempts to synthesize *N*-Ths-benzaldimine **147** by $TiCl_4$ mediated condensation of benzaldehyde **110** with 5-methyl-1,3,4-thiadiazole-2-sulfonamide **145** resulted only in recovery of the starting materials. The failure of this reaction was attributed to rapid hydrolysis of the highly activated *N*-Ths-benzaldimine product. Further attempts to synthesize the α-amidosubstituted sulfone precursor by Petrini's methodology again resulted in unreacted starting material. It was found, however, that stock solutions of *N*-Ths-benzaldimine **147** could be prepared reproducibly by the reaction of **145** with one equivalent of benzaldehyde **110** in toluene under Dean-Stark conditions in the presence of 5.0 mol% of PTSA. These stock solutions were prepared in 0.16 M concentration by filtering reaction mixtures into a dry volumetric flask and diluting them with toluene or THF. The yields for these reactions were determined by ^{1}H-NMR integration using 1,2-dimethoxybenzaldehyde as the internal standard. It was found that the average yield for these reactions was 79% based on 12 individually prepared solutions with a range of 76-82%. All attempts to isolate the *N*-Ths-benzaldimine **147** from the reaction mixture led to rapid decomposition. The *N*-Bts-benzaldimine **146** was synthesized from benzo[d]thiazole-2-sulfonamide **144** and benzaldehyde **110** under the same reaction conditions as **147**; however, Bts-benzaldimine **146** was found to be isolable as a stable crystalline solid. The average yield for the synthesis of **146** was 82% over 6 reactions within a range of 80–87%.

2.2.2 Addition of organometallic reagents to the *N*-Bts- and *N*-Ths-benzaldimines

2.2.2.1 Lithium reagents

The addition of organolithium reagents to both *N*-Bts- and *N*-Ths-benzaldimines resulted in either poor yields or multiple decomposition products. As shown in Table 2, only the addition of MeLi to the *N*-Ths-benzaldimine **147** resulted in the formation of the desired product **148** in 23% yield. The addition of *t*-BuLi to the *N*-Ths-benzaldimine and MeLi to the *N*-Bts-benzaldimine resulted in multiple decomposition products by TLC analysis with no evidence of the desired product.

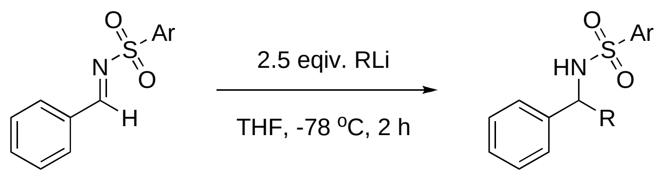


Table 2. Addition of Organolithium Reagents to **146** and **147**.

Entry	Ar	R	Product	Yield (%)
1	Ths (**147**)	Me	**148**	23
2	Ths (**147**)	*t*-Bu	**149**	0[a]
3	Bts (**146**)	Me	**150**	0[a]

[a] Multiple decomposition products with no evidence of desired product by TLC analysis.

2.2.2.2 Grignard reagents

In contrast to the results obtained for the addition of organolithium reagents, Grignard reagents were found to be suitable nucleophiles for addition to both *N*-Ths- and *N*-Bts-benzaldimines. For the addition of *i*-PrMgX to **147,** neither the coordinating ability of the solvent nor the nature of the halogen on the Grignard reagent had an appreciable effect on the yield of the product (Table 3, entries 1, 2 and 4). However, it was found that the reaction temperature was important and must be kept at -78 ^{o}C for the duration of the reaction (Table 3, entry 3). Under optimized reaction conditions, methyl, vinyl, 1-propynyl and phenyl magnesium bromide were reacted with **147** in THF at -78 ^{o}C to afford the corresponding sulfonamide products in moderate yields (46-61%) after recrystallization.

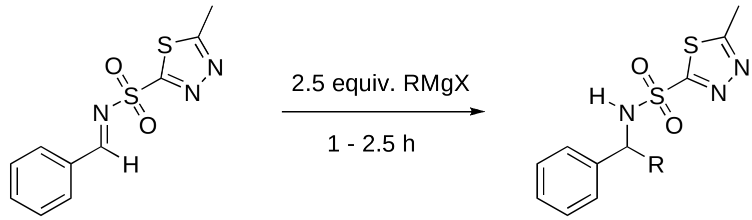


Table 3. Addition of Grignard Reagents to *N*-Ths-Benzaldimine **147**.

	Optimization Reactions				Yield (%)[a]	
	Solvent	X	R (product)	Temp. (°C)	2 Step[b]	1 Step
1	5:1 CH_2Cl_2: Toluene	Cl	*i*-Pr (**151**)	-78	45	57[c]
2	5:1 THF: Toluene	Cl	*i*-Pr (**151**)	-78	47	59[c]
3	5:1 THF: Toluene	Cl	*i*-Pr (**151**)	-78 → 25	15	19[c]
4	5:1 CH_2Cl_2: Toluene	Br	*i*-Pr (**151**)	-78	50	61[c]
	Grignard Reactions Using Optimized Conditions					
5	5:1 THF: Toluene	Br	Me (**148**)	-78	49	61[d]
6	5:1 THF: Toluene	Br	vinyl (**152**)	-78	37	46[d]
7	5:1 THF: Toluene	Br	1-propynyl (**153**)	-78	39	49[d]
8	5:1 THF: Toluene	Br	Ph (**154**)	-78	51	64[d]

[a]Yields based on recrystallized product; [b]Yield over two steps based on mmol of 5-methyl-1,3,4-thiadiazole-2-sulfonamide; [c]Yield based on average yield for aldimine stock solutions (79%); [d]Yield based on the yield of aldimine **147** determined by ^{1}H-NMR integration for the stock solution used.

Grignard reagents were found to add more effectively to the *N*-Bts-benzaldimine **146**. As shown in Table 4, MeMgBr, *i*-PrMgCl, vinylMgBr, 1-propynylMgBr and PhMgBr were reacted with **146** in THF at -78 °C to afford the corresponding sulfonamide products in good yields (71-87%).

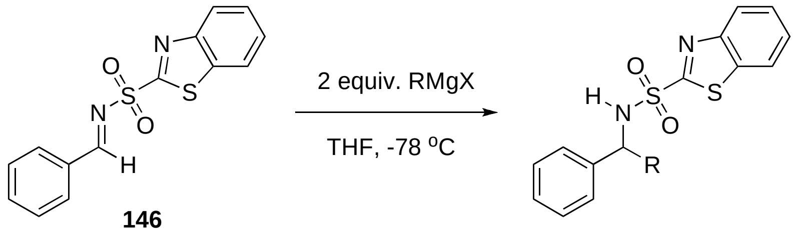


Table 4. Addition of Grignard Reagents to *N*-Bts-Benzaldimine **146**.

Entry	X	R (product)	Time (h)	Yield (%)[a]
1	Br	Me (**155**)	2.5	87%
2	Cl	*i*-Pr (**156**)	2.5	87%
3	Br	vinyl (**157**)	3	85%
4	Br	1-propynyl (**158**)	3	71%
5	Br	Ph (**159**)	3	77%

[a]Isolated yields.

2.2.2.3 Diethylzinc reactions

The addition of diethylzinc to both the *N*-Ths-benzaldimine and the *N*-Bts-benzaldimine succeeded with some surprising results. It was found that the addition of Et_2Zn to the *N*-Ths-benzaldimine **147** proceeded best in non-coordinating solvents, such as toluene, when 2.5 equivalents of Et_2Zn were added at room temperature (Table 5, entries 1, 2 and 3). Interestingly, it was also found that Et_2Zn added to the *N*-Ths-benzaldimine **147** at -78 ^{o}C in moderate yield over extended reaction times (Table 5, entry 4). A similar effect in the coordinating ability of the solvent was found for the addition of Et_2Zn to the *N*-Bts-benzaldimine **146** (Table 5, entries 5 and 6).

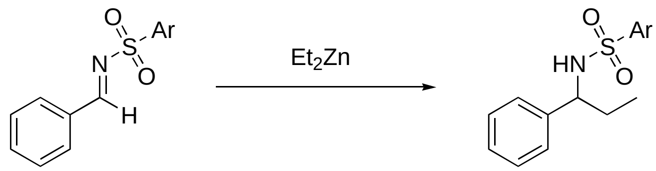


Table 5. Addition of Diethylzinc Reagents to **146** and **147**.

Entry	Ar (Product)	Solvent	Temp. (°C)	Et_2Zn (equiv.)	Time (h)	Yield (%)[a]
1	Ths (**160**)	THF	25	1.2	4	43
2	Ths (**160**)	Toluene	25	1.2	2.5	53
3	Ths (**160**)	Toluene	25	2	2.5	70
4	Ths (**160**)	Toluene	-78 °C	1.2	33	61
5	Bts (**161**)	THF	25	2	4	40
6	Bts (**161**)	Toluene	25	2	4	58

[a]Yields after recrystallization.

These results are different from what was reported by Qian for the reaction of (*E*)-*N*-benzylidenebenzenesulfonamide **111** with Et_2Zn, which primarily gave the reduced product **113** in non-coordinating solvents. This interesting difference is most likely due to the higher reactivity of the *N*-Bts- and *N*-Ths-benzaldimines resulting from the greater electron-withdrawing effect of the heterocyclic sulfonyl activating groups.

2.2.2.4 Hydrozirconation – transmetallation reactions

The alkenylzinc reagents derived from hydrozirconation of 1-hexyne and 3-hexyne with $Cp_2Zr(H)Cl$ (**120**) followed by *in situ* transmetallation to zinc, were added in moderate to good yields to both *N*-Bts- and *N*-Ths-benzaldimines (Table 6). Even though these reactions were conducted in the presence of CH_2Cl_2, the corresponding *C*-cyclopropylalkylamides were not detected. The methyl addition product was, however, isolated in 13% yield from the reaction of *N*-Bts-benzaldimine **146** with 1-hexyne derived alkenylzinc reagent (Table 6, entry 3).

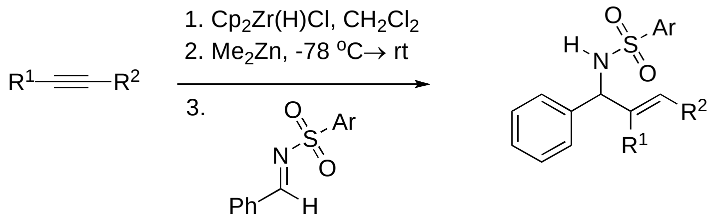


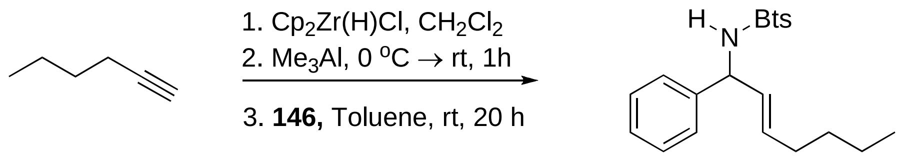
Table 6. Addition of Alkenylzinc Reagents to **146** and **147**.

Entry	Alkyne	Ar (product)	Solvent	Temp. (°C)	Time (h)	Yield (%)[a]
1	1-hexyne	Ths (**163**)	1:1 Toluene : CH_2Cl_2	25	2.5	35
2	1-hexyne	Ths (**163**)	1:1 Toluene : CH_2Cl_2	-40	10	57
3	1-hexyne	Bts (**164**)	CH_2Cl_2	25	4.5	71[b]
4	3-hexyne	Ths (**165**)	1:1 Toluene : CH_2Cl_2	25	2	47

[a]Isolated yields; [b]13% of the methyl addition product was also isolated in this reaction.

Multiple attempts were made to effect an asymmetric addition of either diethylzinc or the alkenylzinc reagent derived from hydrozirconation of 1-hexyne to *N*-Bts- and *N*-Ths-benzaldimines. However, after screening a wide range of Lewis basic chiral ligands and exploring the asymmetric copper-catalyzed systems popularized by Charette, the enantiomeric excesses for these reactions were never >12%. This result is presumably due to high levels of competitive background reactions for the addition of the zinc reagents to the *N*-Bts- and *N*-Ths-benzaldimines. In the absence of chiral ligands, addition to aldimines proceeds in moderate yields at low temperatures over extended reaction times (Table 5, entry 4 and Table 6, entry 2).

The addition of the vinylalane, resulting from the hydrozirconation of 1-hexyne followed by transmetallation to aluminum with Me_3Al, to the *N*-Bts-benzaldimine was also briefly explored. As shown in Scheme 26, this reaction was found to be sluggish in toluene at room temperature, affording the product **164** in only 16% yield after 20 h.



Scheme 26. Vinylalane addition to the *N*-Bts-benzaldimine **146**.

In light of the work reported by Hanzawa, we decided to investigate asymmetric rhodium(I) catalyzed addition reactions of alkenylzirconocenes to the *N*-Bts-benzaldimine **146** using the commercially available *R*,*R*-MeDUPHOS ligand **167** and chiral diene **168** (Table 7). As shown in Table 7, the rhodium-catalyzed addition of the 1-hexyne derived alkenylzirconocene succeeded with moderate enantiomeric excess in dioxane with both the chiral ligand **167** and the chiral diene **168** (Table 7, entry 3 versus entries 1 and 2). Unfortunately, the catalytic turnover for these reactions remained low, with yields of **164** never increasing above 19%.

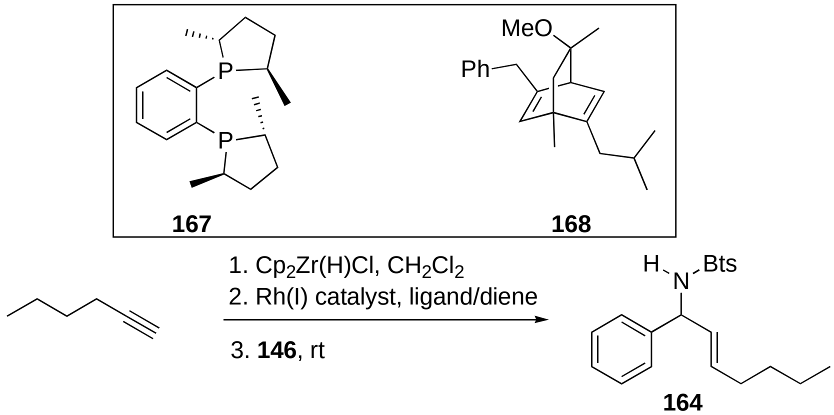
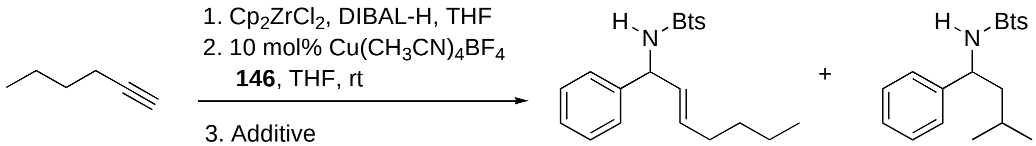


Table 7. Asymmetric Rh(I) Catalyzed Addition Reactions to **146.**

Entry	Catalyst (mol%)	Ligand (mol%)	Diene (mol%)	Solvent	Time (h)	Yield (%)[a]	ee (%)[b]
1	$[RhCl(ethylene)_2]_2$ (5)	X	**168** (10)	Dioxane	24	0	0
2	$[RhCl(cod)]_2$ (5)	**167** (10)	X	Toluene	16	19	1
3	$[RhCl(ethylene)_2]_2$ (5)	**167** (10)	**168** (10)	Dioxane	20	7	58

[a]Isolated yield; [b]Determined by chrial HPLC: chiralcel-OD column, 99:1 hexanes : *i*-PrOH, flow rate 1.0 mL/min, UV detection at 258 nm.

Along with rhodium(I)-catalyzed additions of alkenylzirconocenes to the *N*-Bts-benzaldimine, we also explored the use of the $Cu(CH_3CN)_4BF_4$ catalyst **169** for these addition reactions. An interesting result was found with a protocol for the *in situ* preparation of $Cp_2Zr(H)Cl$ by the reaction of Cp_2ZrCl_2 with DIBAL-H as recently reported by Negishi.[49] We found that the $Cu(CH_3CN)_4BF_4$ catalyst did not effectively catalyze the addition of the 1-hexenylzirconocene to *N*-Bts-benzaldimine **146** at room temperature in THF after 48 h (Table 8, entry 1). However, upon an attempt to activate the *N*-Bts-benzaldimine **146** with $BF_3{\bullet}OEt_2$, we discovered that the $Cu(CH_3CN)_4BF_4$ catalyst catalyzed the addition of the *iso*-butyl group from *i*-Bu_2AlCl to generate the product **170** in 63% yield (Table 8, entry 2). In the absence of the copper(I) catalyst, no reaction occurred (Table 8, entry 3).



164 **170**

Table 8. Copper(I)-Catalyzed Addition Reactions of Alkenylzirconocenes to **146**.

Entry	Catalyst (mol%)	Additive (mol%)	Time (h)	Yield (**164**) (%)[a]	Yield (**170**) (%)[a]
1	$Cu(CH_3CN)_4BF_4$ (5)	-	48	< 5	0
2	$Cu(CH_3CN)_4BF_4$ (10)	BF_3•OEt_2 (300)	18	0	63
3	-	BF_3•OEt_2 (300)	16	0	0

[a]Isolated yield.

2.2.3 Deprotection of *N*-Ths and *N*-Bts-sulfonamides

After exploring the addition of organometallic nucleophiles to the *N*-Ths and *N*-Bts-benzaldimines, several deprotection reactions were investigated for the removal of the heteroaromatic sulfonyl groups from the sulfonamide products. Utilizing the procedure reported by Vedejs, it was found after some optimization that the slow addition of a 50% H_3PO_2 solution to a mixture of either sulfonamides **148**, **154** or **153** in refluxing THF efficiently produced the corresponding amines. These amines were isolated crude and then converted to their more stable Boc-protected derivatives **171**, **172** and **173** in good yields over the two step reaction sequence (Table 9).

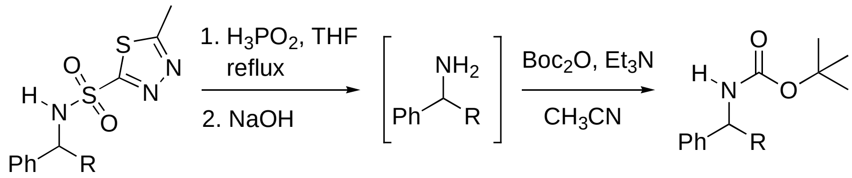


Table 9. H_3PO_2 Deprotection and Boc Protection of Crude Amines.

Deprotection				Protection		
Entry	R (substrate)	H_3PO_2 (equiv.)	Time (h)	$(Boc)_2O$ (eq.)	Time (h)	Yield (%, product)[a]
1	Me (**148**)	30	4.5	1.25	1	84 (**171**)
2	Ph (**154**)	30	4.5	1.25	1	83 (**172**)
3	1-propynyl (**153**)	30	4.5	1.25	1	88 (**173**)

[a]Isolated yields over two steps.

The use of SmI_2 was also investigated for the deprotection of the *N*-Bts- and *N*-Ths-sulfonamides **148** and **155** (Table 10). These reactions proceeded efficiently at room temperature after in the presence of excess SmI_2 to generate the corresponding 1-phenylethanamines. These amines were again isolated crude and then converted to their more stable Boc-protected derivatives **171** in good yields over the two-step reaction sequence.

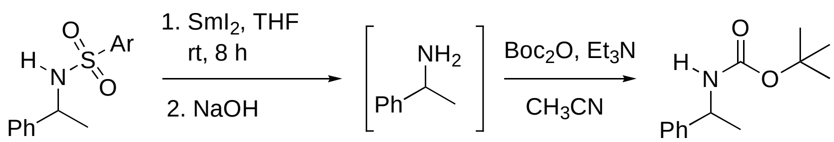


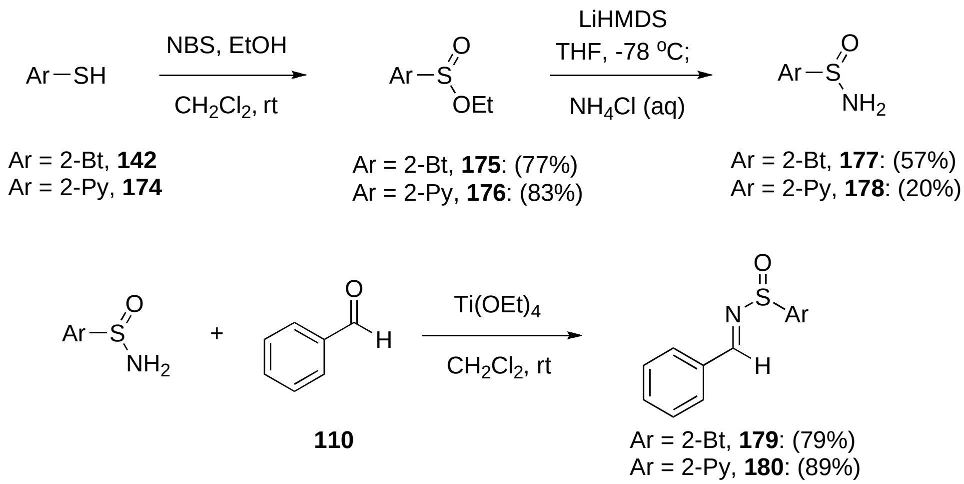
Table 10. SmI_2 Deprotection and Boc Protection of Crude Amines.

Deprotection				Boc Protection
Entry	Ar (substrate)	SmI_2 (equiv.)	Amine	Yield (%, Product)[a]
1	Ths (**148**)	7	Crude	84 (**171**)
2	Bts (**155**)	9	Crude	83 (**171**)

[a]Isolated yields over two steps.

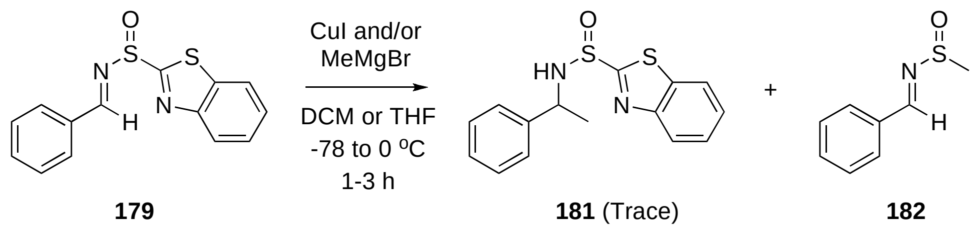
2.2.4 Addition of organometallic reagents to heterocyclic benzothiazole- and pyridyl-2-sulfinylbenzaldimines.

As a result of the low enantiomeric excess obtained for the asymmetric addition reactions of organometallic reagents to *N*-Bts- and *N*-Ths-benzaldimines, we decided to explore the novel benzo[d]thiazole-2-sulfinyl (Bt-2-sulfinyl) and pyridyl-2-sufinyl (Py-2-sulfinyl) protecting groups as chiral auxiliaries for the diastereomeric addition of organometallic reagents to the respective heterocyclic sulfinylbenzaldimines. Racemic benzothiazole- and pyridyl-2-sulfinylbenzaldimines were synthesized as shown in Scheme 27. Initially, benzo[d]thiazole-2-thiol **142** or pyridine-2-thiol **174** was reacted with NBS in EtOH/CH_2Cl_2 at room temperature to produce the corresponding sulfinates **175** and **176** in 77% and 83% yield, respectively.[50] These sulfinates were then reacted with LiHMDS at -78 ^{o}C in THF to generate the corresponding sulfinamides **177** and **178** in 57% and 20% yields after aqueous workup.[51] Sulfinamides **177** and **178** were then condensed with benzaldehyde **110** in the presence of $Ti(OEt)_4$ in CH_2Cl_2 at room temperature to form the desired heterocyclic-2-sulfinylbenzaldimines **179** and **180** in 79% and 89% yields.



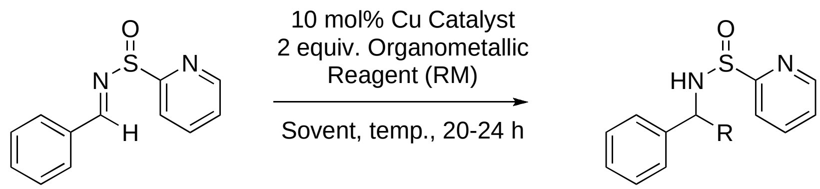
Scheme 27. Synthesis of heterocyclic-2-sulfinylbenzaldimines **179** and **180**.

The addition of organometallic reagents to benzothiazole-2-sulfinylbenzaldimine **179** was met with difficulty. The addition of MeMgBr to **179** gave results similar to those reported by Moreau for the reaction of *N*-*p*-toluenesulfinyl aldimines, with methylation taking place predominately at the sulfur atom of the sulfoxide, resulting in only trace amounts of the desired methylated product **181** (Scheme 28). The addition of 1 equivalent of CuI to the reaction mixture according to Chan's protocol unfortunately did not enhance the chemoselectivity for methylation, again resulting in only trace amounts of **181**. Imine **179** was also found to be unreactive toward dialkylzinc, vinylzinc and vinylaluminum reagents in DCM or toluene at room temperature over extended periods of time.



Scheme 28. Addition of MeMgBr to **179**.

Similarly, pyridyl-2-sulfinylbenzaldimine **180** was found in general to be unreactive toward vinylzirconocenes under copper- or rhodium-catalyzed conditions, vinylzinc reagents and vinylalanes. However, aldimine **180** did show moderate reactivity toward diethylzinc, cyclohexylzinc bromide and 1-propynylzinc halides under copper-catalyzed conditions as shown in Table 11.



180 **183**-**185**

Table 11. Reaction of **180** with Organometallic Reagents.

Entry	Reagent	Catalyst[b]	Solvent	Temp. (°C)	Yield (%, Product)	dr[c]
1	Et_2Zn	-	DCM	rt	17 (**183**)	1:1
2	Et_2Zn	$Cu(OTf)_2$	Toluene	rt	47 (**183**)	1:1
3	Et_2Zn	$Cu(OTf)_2$	DCM	0	47 (**183**)	2:1
4	Et_2Zn	$Cu(OTf)_2$	THF	0	Trace[d]	n.d.[e]
5	$C_6H_{11}ZnBr$	$Cu(OTf)_2$	2:1 DCM:THF	rt	64 (**184**)	3.5:1
6	$C_6H_{11}ZnBr$	$Cu(OTf)_2$	2:1 DCM:THF	0	63 (**184**)	4:1
7	$CH_3C{\equiv}CMgBr$	-	DCM	0	48 (**185**)	1:1
8	$CH_3C{\equiv}CZnCl$[a]	$Cu(OTf)_2$	2:1 DCM:THF	rt	13 (**185**)	1:1
9	$CH_3C{\equiv}CZnBr$[a]	$(CH_3CN)_4CuBF_4$	2:1 DCM:THF	rt	50 (**185**)	3.5:1
10	$CH_3C{\equiv}CZnI$[a]	$(CH_3CN)_4CuBF_4$	2:1 DCM:THF	rt	60 (**185**)	2.9:1

[a]Formed via transmetallation of 1-propynylmagnesium bromide with $ZnCl_2$, $ZnBr_2$, ZnI_2. [b]10 mol%. [c]Ratios determined by ^1H NMR integration. [d]As determined by TLC. [e]Not Determined.

As can be seen from Table 11, the reaction of aldimine **180** with various organozinc reagents under copper-catalyzed conditions could be optimized to afford moderate yields of the desired amines over reaction times of approximately 1 d. Variations of both solvent and temperature showed that the diastereomeric ratio for the addition of diethyl zinc to **180** was highest in polar, non-coordinating solvents at 0 ^{o}C. Interestingly, lowering the reaction temperature from room temperature to 0 ^{o}C had no observable influence on the diastereomeric ratio for the addition of cyclohexylzincbromide to **180**. Comparing the addition reactions of 1-propynylzinc halides to **180**, the highest diastereomeric ratios could be achieved from the $(MeCN)_4CuBF_4$ catalyzed addition of 1-propynylzinc bromide to **180** at room temperature, affording a dr of 3.5:1. Overall, pyridyl-2-sulfinylbenzaldimines have been shown to be moderately reactive towards the copper-catalyzed addition of organozinc reagents over the narrow substrate scope explored. The ability of this interesting class of chiral auxiliaries to chelate metal centers bodes well for their utility in the synthesis of α-branched amines.

2.3 CONCLUSION

N-Bts and *N*-Ths-benzaldimines were viable substrates for the addition of several organometallic reagents, including alkyl, vinyl, propynyl and aromatic organomagnesium reagents, dialkyl and alkenylzinc reagents, and alkylcuprates. The product sulfonamides were smoothly converted to the corresponding amines by reaction with either SmI_2 or H_3PO_2. Further exploration into the use of the pyridyl-2-sulfinyl protecting group as a heterocyclic auxiliary for the synthesis of chiral amines from the corresponding imines has led to promising results for the addition of organozinc reagents under copper catalysis. This interesting class of heterocyclic sulfonyl- and sulfinyl-benzaldimines has displayed good and in some cases unique reactivities toward a variety of organometallic reagents. They are therefore potential alternatives to conventional protecting groups in the synthesis of α-branched amines.

3.0 STRUCTURE ELUCIDATION AND SYNTHESIS OF A PUTATIVE POLO LIKE KINASE – POLO BOX DOMAIN (PLK1-PBD) INHIBITOR

3.1 OVERVIEW

This chapter will present the discovery of a novel Plk1-PBD inhibitor resulting from a HTS effort conducted by the PMLSC. The synthetic and analytical procedures utilized to identify the correct chemical structure of the biologically active substance are discussed. The composition of the initially unknown substance and the decomposition pathways of this substance are characterized. Finally, the synthesis of a library of 56 analogues targeted to the discovery of the biologically active species (scaffold) in the initially tested HTS sample is reported.

3.2 INTRODUCTION

Polo-like kinases (Plks) are named after the discovery of the polo gene of *Drosophila melanogaster*.[52,53] Mammalian Plks represent a unique family of four serine/threonine (S/T) kinases (Plk1-4) as they contain both an N-terminal kinase domain and a C-terminal polo-box domain (PBD); two structural features evolutionary conserved from yeast to mammals.[54,55] Investigations into the roles of Plks 1-4 as regulatory enzymes during cell division have shown Plks are active during mitosis, meiosis and cytokinesis.[55] Of the four mammalian Plks, the biological functions of Plk1 are the best understood. Plk1 is expressed mainly during the late G2 and M phases of the cell cycle and is a key enzyme involved in regulation and progression of mitotic events including mitotic entry, centrosome maturation and separation, bipolar spindle formation, chromosome segregation and cytokinesis.[56] Critical to the function of Plk1 is the PBD which serves as a (pSer/pThr) phosphopeptide binding domain that regulates the subcellular location of Plk1 during mitosis and stimulates the activity of the kinase domain through a conformational switching mechanism that releases the kinase domain from an inhibitory interaction with the PBD upon phosphopeptide binding.[57,58] The PBD of Plk1 serves to localize Plk1 to mitotic structures including chromosomes, kinetochores and the spindle midzone during mitosis.[59] In a study by Hanisch, overexpression of the PBD of Plk1 in HeLa S3 cells was shown to displace endogenous Plk1 from chromosomes and kinetochores resulting in spindle checkpoint-dependent mitotic arrest due to interference with proper chromosome congression.[60]

In adult tissues, Plk1 expression has been found to be highest in actively proliferating cell populations (spleen, ovary and testis) and very low in the liver, kidney, brain, thymus, intestine, lung, pancreas, heart, stomach and skin.[61,62] The important function of Plk1 in maintaining genomic stability during cell division suggests that the levels of Plk1 must be tightly regulated during mitotic progression. It has been found that either overexpression or down-regulation of Plk1 *in vivo* induced tumorigenesis in mice.[61,63,64] Plk1 overexpression has been reported in many cancers, including: lung, pancreases, endometrium, brain, breast, ovary, colon, skin, head and neck, esophagus, gastric tract and prostate; and overexpression of Plk1 often correlates with tumor aggressiveness and poor patient prognoses.[61,62,65] Plk1 is a validated target for cancer therapies as several studies using RNA interference-based strategies have shown that the depletion of Plk1 in different cancer cell lines led to cell death *in vitro* and tumor suppression in mice with human xenograft tumors.[66-69] The development of drugs targeting Plk1 is inspired by an approach designed to target key enzymes active during mitosis as a way to inhibit the hyperproliferative state of tumor cells and induce apoptosis.[70,71]

Several small molecule inhibitors of Plk1 have been identified which generally target Plk1 in one of two ways: a) ATP-competitive inhibition at the kinase domain or b) inhibition at the PBD. Inhibition of Plk1 at the kinase domain has led to the identification of several potent and selective ATP-competitive inhibitors including BI 2536, BI 6727 and GSK 461364A, which are currently in clinical trials as chemotherapeutics for non-small-cell lung cancer, leukemia and solid cancer (Figure 7, top).[62,72-76] Less well known are Plk1 small molecule inhibitors that target the PBD (Figure 7, bottom). Inhibitors of the PBD may have the advantage of being highly selective for Plks vs. other related protein kinases containing structurally similar catalytic domains.[77] Furthermore, specific inhibition of Plk1-PBD among the other mammalian Plks represents an important challenge for the development of small molecule PBD based inhibitors, since all Plks are active with nonoverlapping functions during the cell cycle and in tumor progression.[78] To date, three small molecule inhibitors of Plk1-PBD have been identified; purpurogallin IC_{50} ~ 0.3 μM, poloxin IC_{50} 4.8 ± 1.3 μM and thymoquinone IC_{50} 1.14 ± 0.04 μM.[79,80] Inhibition of Plk1-PBD by these small molecule inhibitors of the PBD showed the hallmarks of PBD overexpression found by Hanisch, resulting in Plk1 mislocation, chromosomal congression defects and mitotic arrest, suggesting that small molecule inhibitors of Plk1-PBD have the potential to become valuable anticancer drugs.[79,80]

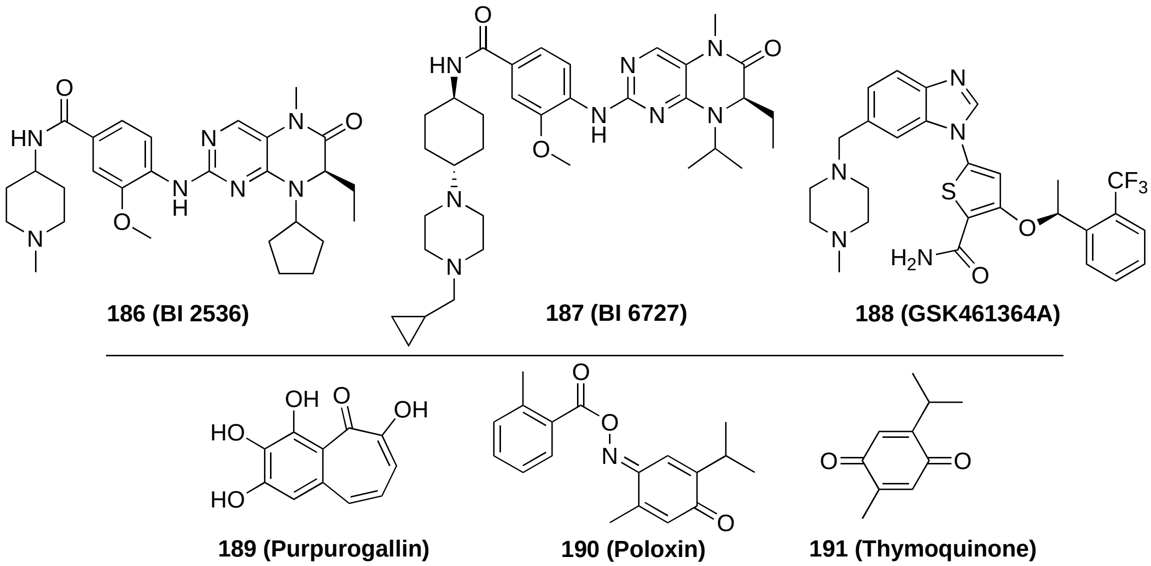
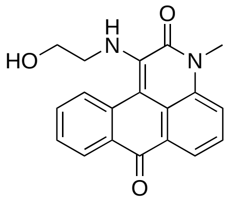


Figure 7. Selected inhibitors of Plk1: ATP-competitive inhibitors currently in clinical trials (top) and PBD selective inhibitors (bottom).

3.2.1 Project Background

The potential for kinase selectivity coupled with the important roles of Plk1-PBD during mitosis peaked our interest in the identification of PBD based Plk1 inhibitors. An HTS campaign conducted by the PMLSC of 97,090 samples provided by the NIH SMR identified 11 hits (0.011% of the originally screened compounds) as concentration-dependent inhibitors of phosphopeptide binding to Plk1-PBD with an IC_{50} < 50 μM (Figure 8).[81] A fluorescence polarization assay with recombinant Plk1-PBD was used to monitor for inhibition of Plk1. This assay was developed by the Yaffe group at MIT and was submitted to the MLSCN for this HTS campaign.[82] Of the 11 hits, **SID 861574** was found to be a 2.41 ± 0.71 μM inhibitor of Plk1-PBD and was chosen for further chemical hit-to-probe development as it's structure could be readily modified. **SID 861574** also did not have the undesirable electrophilic or hydrolytic structural features present in the remaining active compounds.



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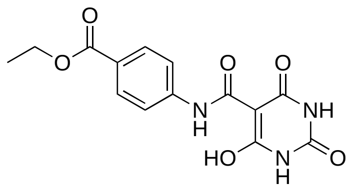

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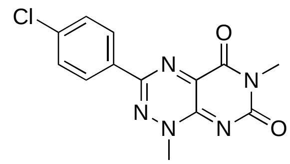

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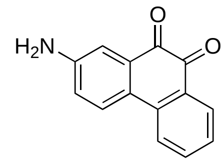

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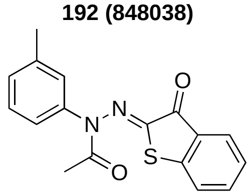

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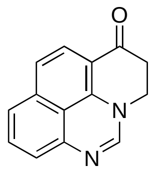

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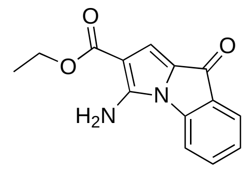

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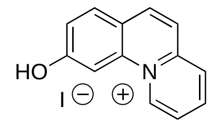

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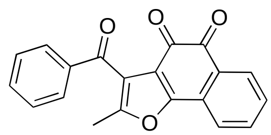

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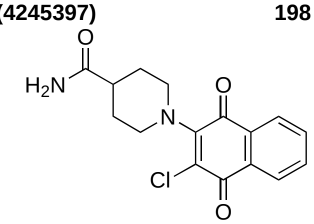

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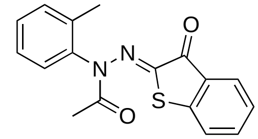
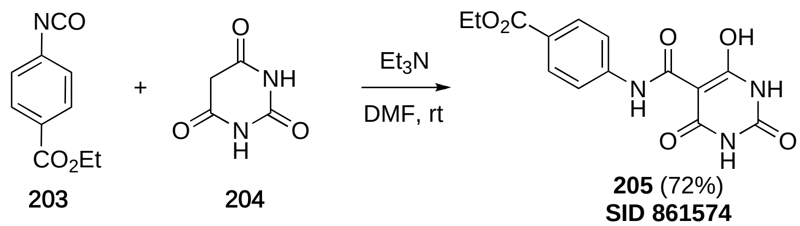


202 (17402739)

Figure 8. Validated inhibitors of phosphopeptide binding to Plk1-PBD with their corresponding PubChem SID numbers.[17]

Several rounds of medicinal chemistry optimizations led to the generation of a novel library of 38 analogues, including a synthetic sample of **SID 861574** (**205**, Scheme 29). However, all of these showed no affinity to Plk1-PBD in follow up assays.[83] The lack of affinity to Plk1-PBD of the resynthesized hit (**205**) as well as its 38 analogues from the first round of medicinal chemistry led us to examine the nature of the original sample of **SID 861574**. Low resolution EI mass spectral data demonstrated a strong molecular ion of *m/z* 319 (M^+, 57), consistent with the expected mass of **SID 861574**. The ^{1}H NMR spectra strongly supported the presence of an ethyl ester, a *para*-substituted aromatic ring system, and at least one heteroaromatic proton with a chemical shift similar to that found for a 6-amino or 6-hydroxyuracil ring system. An ATR-IR spectrum of **SID 861574** indicated the presence of a carboxylic acid functionality (peaks at 2863 (very broad) and 1644 cm^{-1}).

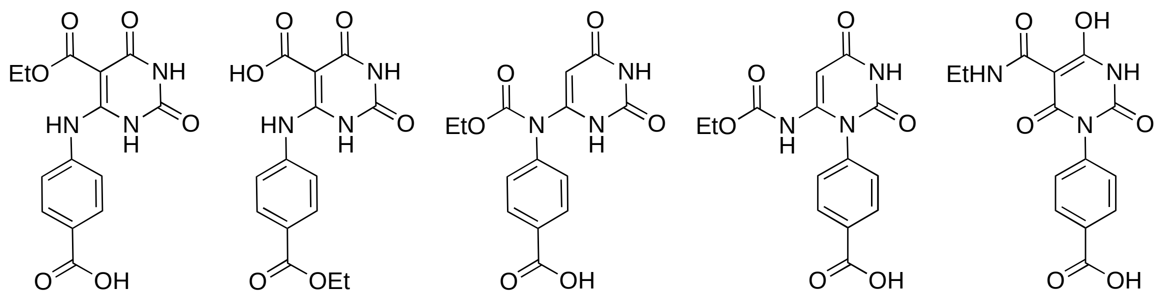


Scheme 29. Synthesis of **205**, the originally proposed compound/structure of **SID 861574**.

3.3 IDENTIFICATION OF THE STRUCTURE OF SID 861574

3.3.1 Initial synthetic attempts toward the identification of SID 861574

The results from the above spectral analyses of **SID 861574** guided us in the preparation of a set of analogues that would be consistent with both the mass and the structural features of the original **SID 861574** sample. We chose an approach in which the 6-amino or 6-hydroxyuracil ring system remained intact and the positions of the *para*-substituted aromatic ring and the ethyl ester, amide or carboxylic acid groups were varied along the uracil core. This strategy led to the synthesis of five isomers of the original hit (Figure 9). The synthetic routes utilized to generate these isomeric analogues as well as the carboxylic acid derivative of **205** are shown in Schemes 30-34 and are summarized below.

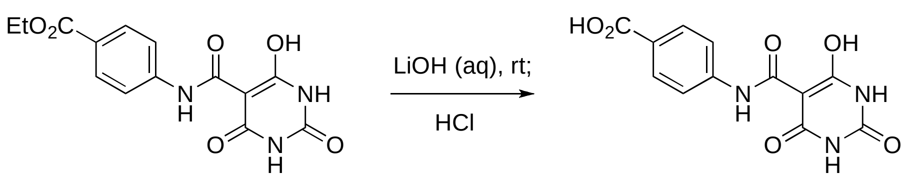


206 **207** **208** **209** **210**

Figure 9. Isomeric analogues of **SID 861574**.

3.3.1.1 Synthesis of the carboxylic acid analogue of 205

Compound **205** was converted to its carboxylic acid derivative by saponification with LiOH followed by acidification with HCl to produce **211** in 88% yield (Scheme 30). This derivative was synthesized as an analogue of the originally proposed structure of the HTS hit for further biological testing against Plk1-PBD.

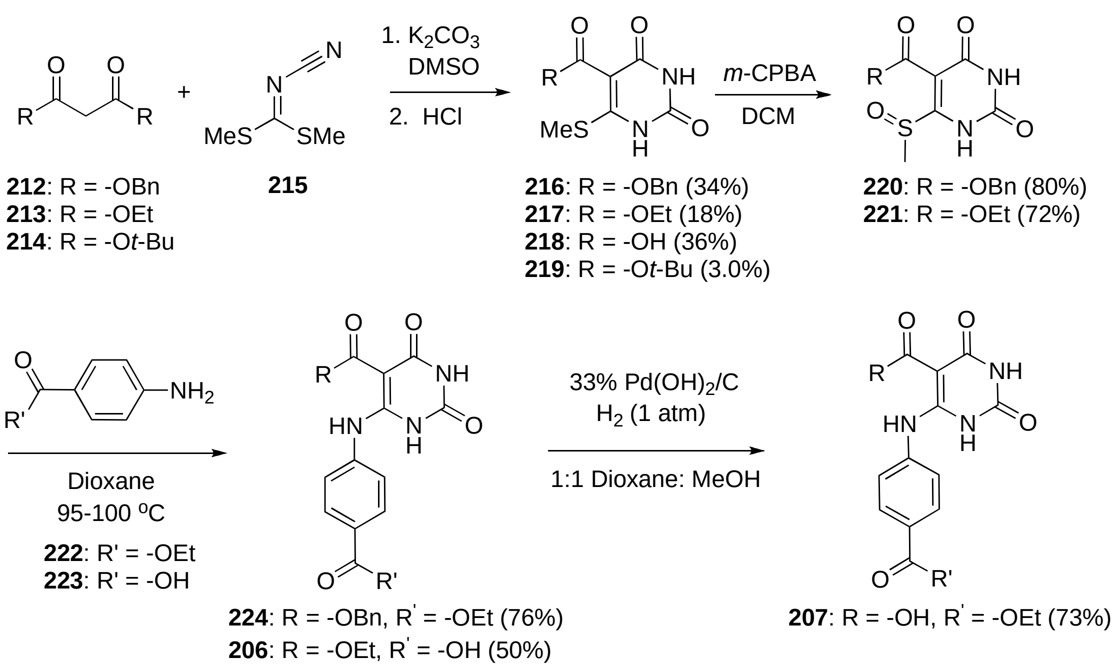


205 **211** (88%)

Scheme 30. Synthesis of carboxylic acid analogue **211**.

3.3.1.2 Synthesis of the isomeric 6-aminouracils 206 and 207

Analogues **206** and **207** were synthesized according to the route shown in Scheme 31. Initially, four 5-substituted-6-methylthiouracil intermediates **216**, **217**, **218** and **219** were synthesized by the cyclization of malonates **212**, **213** or **214**, with **215** according to the literature.[84] Direct aminolysis of these intermediates with 5 equivalents of either 4-aminobenzoic acid or ethyl 4-aminobenzoate under microwave conditions (120 – 150 ^{o}C) in DMF led to poor isolated yields of the respective 6-aminouracils. It was found, however, that the *m*-CPBA oxidation of intermediates **216** and **217** followed by aminolysis of the corresponding sulfoxides at 95-100 ^{o}C in dioxane produced the 6-aminouracil intermediate **224** and analogue **206** in 76% and 50% yield, respectively.[85] Compound **224** was converted to the final product, carboxylic acid **207**, by palladium-catalyzed hydrogenolysis of the benzyl ester in 73% yield.

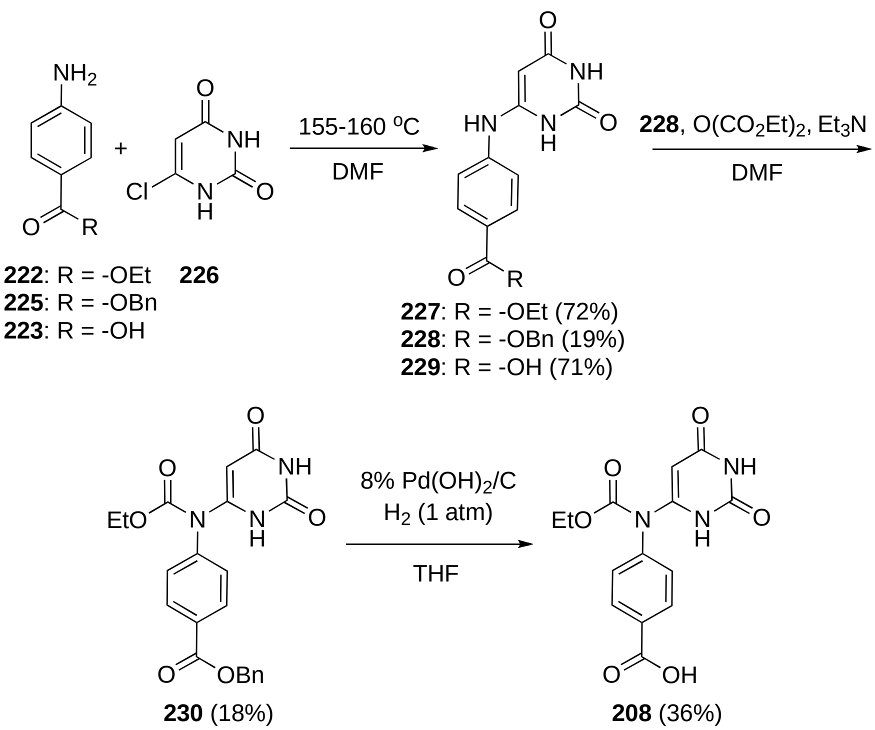


Scheme 31. Synthesis of **SID 861574** isomers **206** and **207**.

3.3.1.3 Synthesis of 6-aminouracil analogues 208 and 209

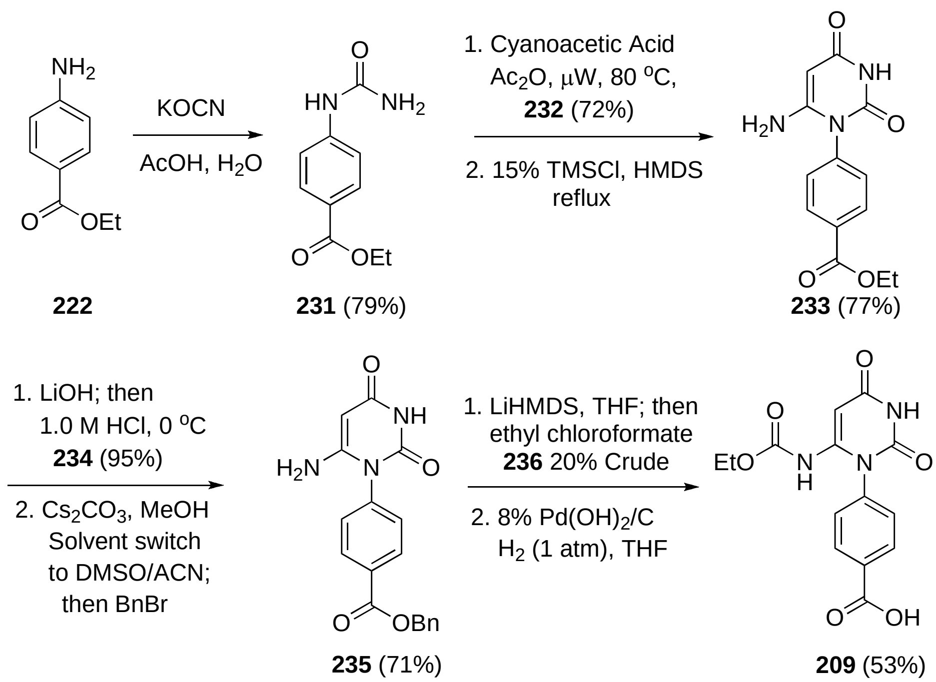
Compound **208** was synthesized according to the route shown in Scheme 32. In light of further SAR analyses, the three 6-aminouracils **227**, **228** and **229** were synthesized by aminolysis of 6-chlorouracil (**226**) with either 4-aminobenzoic acid, 4-ethylaminobenzoate or 4-benzylaminobenzoate in variable yields. Of these three 6-aminouracils, **228** was further converted to the corresponding carbamate **230** by reaction with diethyl pyrocarbonate in 18%

yield. Intermediate **230** was converted in 36% yield to the isomeric carboxylic acid analogue **208** by hydrogenolysis of the benzyl ester using Pearlman's catalyst.



Scheme 32. Synthesis of isomeric analogue **208**.

Analogue **209** was synthesized according to the route shown in Scheme 33. The requisite precursor, urea **231**, was obtained in 79% yield by a reaction of 4-aminobenzoate with potassium cyanate. The urea **231** was condensed with cyanoacetic acid under microwave conditions, producing **232**, which was cyclized by heating at reflux in HMDS in the presence of 15% TMSCl to furnish the 6-aminouracil **233** in 77% yield.[86,87] Saponification of the 6-aminouracil derivative with LiOH followed by acidification with HCl produced the corresponding acid **234** in 95% yield. This carboxylic acid intermediate was protected as the benzyl ester **235** by deprotonation with Cs_2CO_3 followed by reaction with benzyl bromide according to a literature procedure.[88] The benzyl protected 6-aminouracil was then deprotonated with excess LiHMDS and reacted with ethyl chloroformate, generating a mixture of products from which the 6-carboethoxyaminouracil derivative **236** was isolated crude in 20% yield. This intermediate was then converted to the final analogue **209** in moderate yield and in ~87% purity based on ^{1}H NMR.

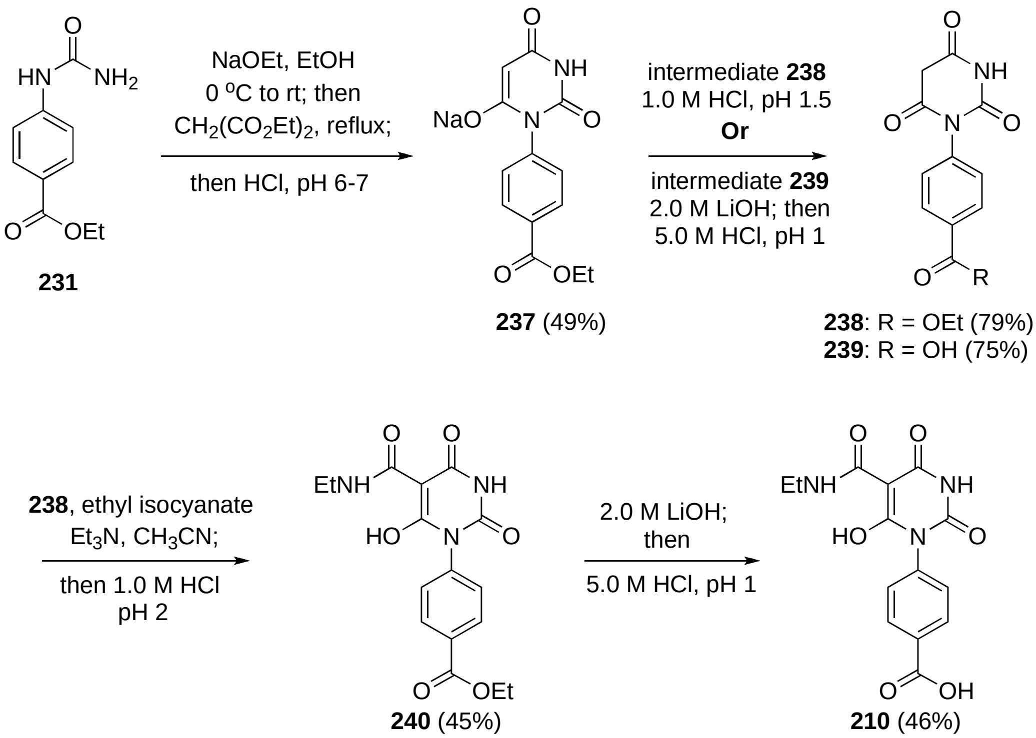


Scheme 33. Synthesis of isomeric analogue **209**.

3.3.1.4 Synthesis of the 6-hydroxyuracil analogue 210

Analogue **210** was synthesized according to the route shown in Scheme 34. Initially, the urea **231** was cyclized with diethyl malonate in the presence of NaOEt in ethanol at reflux.[89] The reaction mixture was acidified to a pH of 6-7 with HCl, furnishing the sodium salt of the corresponding 6-hydroxyuracil **237** in 49% yield. The isolated sodium salt was then either acidified to a pH of 1.5, generating the ethyl ester intermediate **238**, or further saponified with

LiOH and subsequently treated with HCl to produce the carboxylic acid **239**. The ethyl ester intermediate **238** was condensed with ethyl isocyanate, producing **240**, which was saponified with LiOH to reach the final analogue, **210**, upon acidification with HCl in 46% yield.[83]



Scheme 34. Synthesis of isomeric 6-hydroxyuracil analogue **210**.

3.3.1.5 Conclusions of the initial synthetic attempt to identify the structure of SID 861574

In this round of synthetic analoging, 25 new samples were synthesized including five analogues isomeric with the major component of the original HTS hit. Unfortunately, none of the five isomers prepared in this study were spectroscopically identical to **SID 861574** by ^{1}H or ^{13}C NMR. Consequently, the identity of this substance remained unknown.

3.3.2 Structure elucidation of SID 861574: Development of a controlled synthesis of N_1,N_3-differentially-substituted 5-methylene-6-hydroxy uracils

A breakthrough regarding the identity and structural connectivity of **SID 861574** came about via isolation of an X-ray quality crystal after slow evaporation of a 1:1 solution of MeOH:ACN at 5 ^{o}C, revealing the structure **241** shown in Figure 10. This surprising result showed a structure for **SID 861574** bearing the molecular formula $C_{14}H_{12}N_2O_7$ that contained an N_3-carbethoxy-substituted-6-*hydroxy* uracil core substituted in the N_1 position with a *p*-substituted benzoic acid moiety. In light of the differences between the X-ray structure and the originally proposed structure for **SID 861574**, evident by different atom connectivity's and molecular formulas/weights $C_{14}H_{13}N_3O_6$ (*m/z* 319 (M^+)) vs. $C_{14}H_{12}N_2O_7$ (*m/z* 320 (M^+)), a ^{1}H NMR (300 MHz, DMSO-d_6) was taken of a small set of the crystals sent for X-ray crystallography which showed no evidence of sample degradation/contamination. A second crystal from this set was also submitted for X-ray crystallographic analysis, giving an identical result to the first submission.

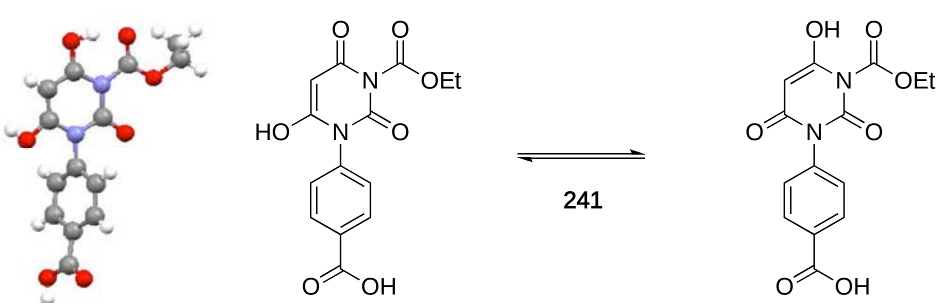
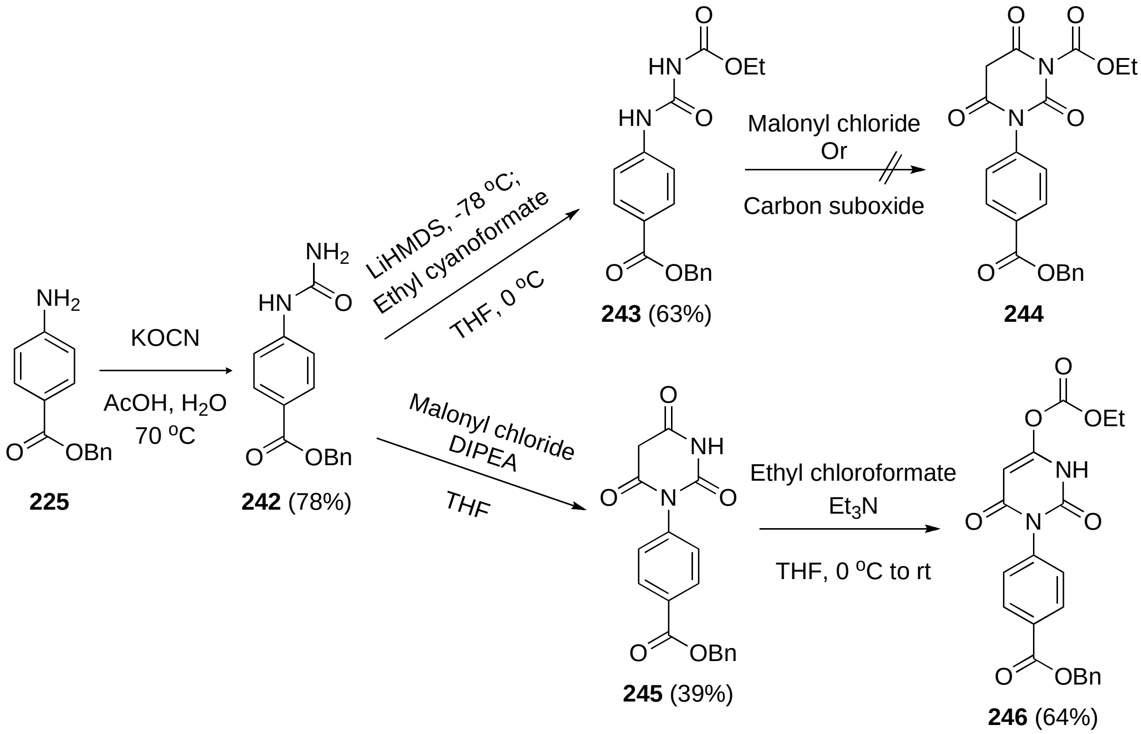


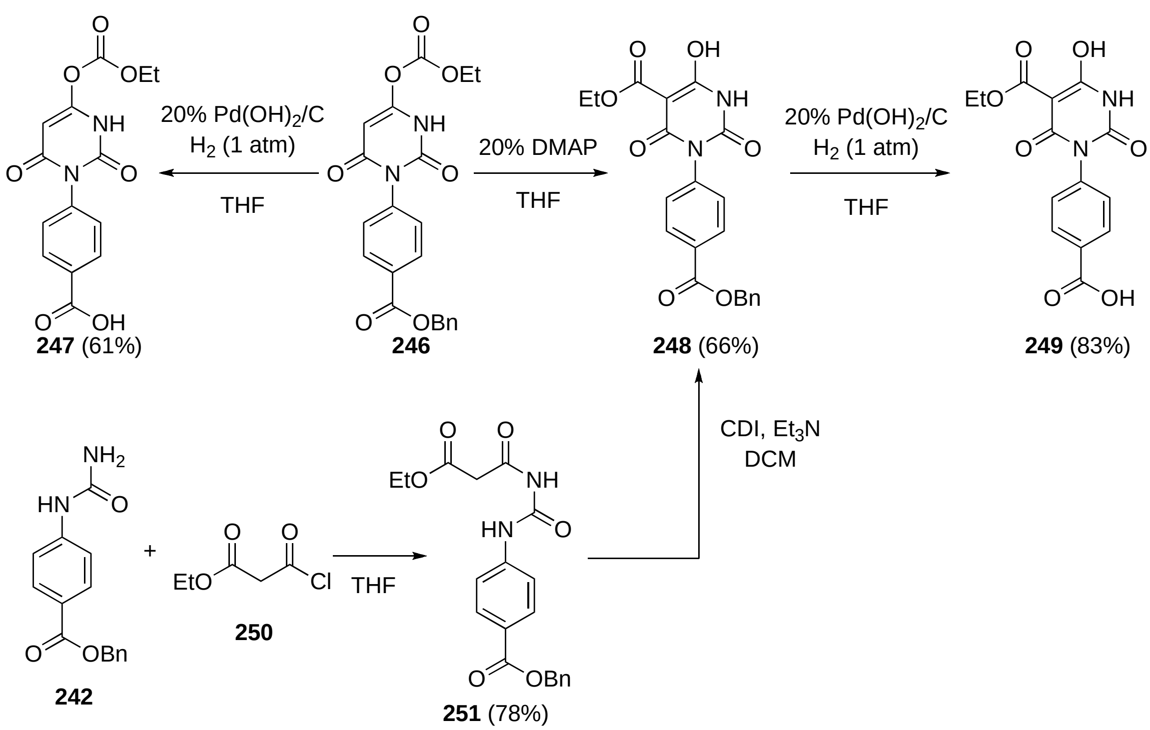
Figure 10. Originally obtained X-ray structure (**241**) for **SID 861574**.

Our initial synthetic approach to access the proposed structure of **SID 861574** was through a cyclization strategy. Urea **243** would be cyclized with a highly reactive electrophile such as malonyl chloride or carbon suboxide (C_3O_2) directly furnishing the appropriately N_1,N_3-subsituted-6-hydroxy uracil core. Subsequently, benzyl ester cleavage would unveil the requisite carboxylic acid (Scheme 35). Secondary urea **243** was synthesized by the initial conversion of amine **225** to the primary urea **242** by reaction with KOCN. Deprotonation of **242** with LiHMDS and subsequent reaction with Mander's reagent at -78 °C generated **243** in 63% yield.[90,91] Attempts to cyclize **243** by reaction with malonyl chloride in the presence of Et_3N, DIPEA or NaH in DCM or THF at 0 °C or room temperature resulted only in recovery of the starting urea. Similarly, the reaction of **243** with carbon suboxide in THF, Et_2O or DCM at -78 or 0 °C resulted in the isolation of **243** with no evidence of formation of **244**.[92] It was found, however, that the primary urea **242** could be cyclized with malonyl chloride in the presence of Hünig's base to furnish the N_1-subsituted-6-oxo-uracil **245** in 39% yield. Further elaboration of **245** to carbamate **244** was met with difficulty, as the reaction of **245** with ethyl chloroformate in the presence of triethylamine resulted in a selective *O*-carbethoxylation reaction forming carbonate **246** in 64% isolated yield (Scheme 35)**.** The carbonate **246** was found to be stable upon storage at -5 °C under nitrogen gas for months; however, **246** was found to decompose by ~50% after approximately 20 h in DMSO-d_6 at room temperature. Similar acylation reactions are known for piperidine-3,5-dione which was reported to undergo *N*,*O*-dicarbethoxylation upon reaction with excess ethyl chloroformate in the presence of Et_3N.[93]



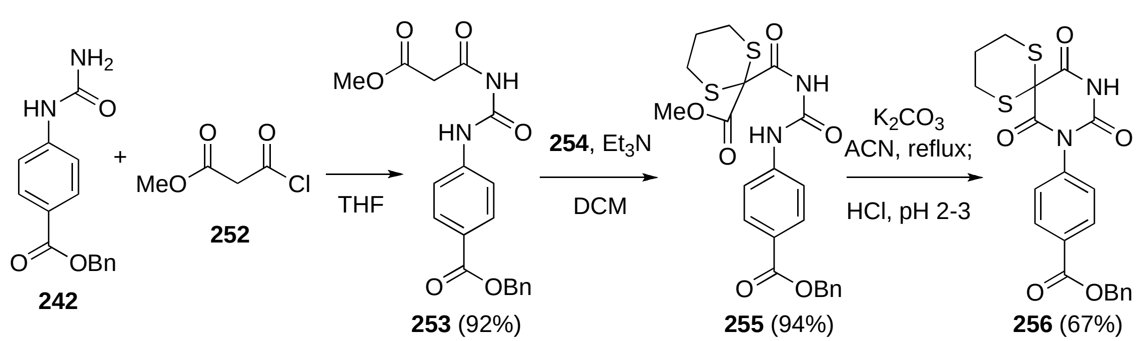
Scheme 35. Attempted synthesis of carbamate **244**. Synthesis of carbonate **246**.

Further, reaction of **246** in THF with 20 mol% of Pearlman's catalyst under a hydrogen gas atmosphere (1 atm) successfully cleaved the benzyl ester and gave carboxylic acid **247** in 61% yield. Efforts toward the conversion of carbonate **246** to carbamate **244** were investigated under thermodynamic reaction conditions by reacting **246** with 20 mol% DMAP in THF at room temperature (Scheme 36). After 4 h, this reaction resulted in the conversion of **246** to a 4:1 mixture of **248**:**245** by ^{1}H NMR analysis, demonstrating an interesting *O*- to *C*-carbethoxy transfer reaction catalyzed by DMAP and driven by the thermodynamic stability of the product ethyl ester **248**. It was found that while the conversion for this reaction was respectable, compound **248** was difficult to purify from the ~20% of **245** contaminating the final reaction mixture. Accordingly, an alternative cyclization reaction was developed in which **248** was prepared by the initial reaction of **242** with ethyl malonyl chloride generating intermediate **251** which was then cyclized over the N_1-C_5-uracil ring positions by reaction with CDI in the presence of triethylamine. This new cyclization strategy for uracil synthesis generated **248** in 66% isolated yield. The independently prepared products **248** from both routes were converted to their corresponding carboxylic acids **249** by hydrogenolysis of the benzyl esters. Product samples of **249** from both reaction pathways were combined and found to be identical by ^{1}H NMR (300 MHz, DMSO-d_6), offering further confirmation to the assigned structure of **248** isolated from the thermodynamic equilibration study. The spectral data for compounds **247** and **249** again proved these products to be distinct from the original HTS hit **SID 861574**.



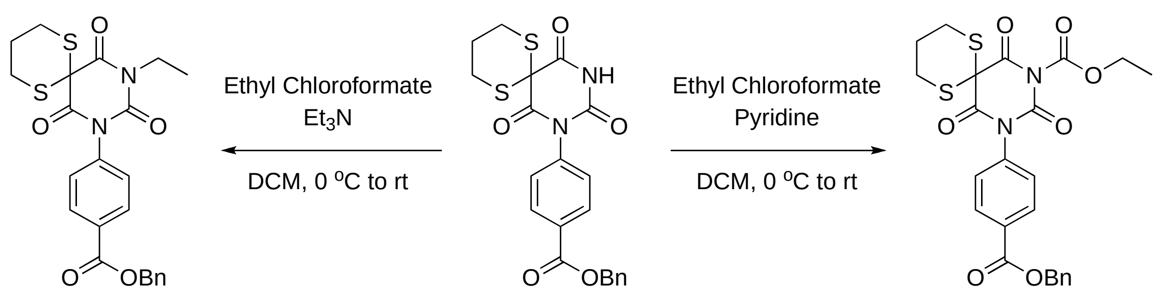
Scheme 36. Synthesis of *O*- and *C*-carbethoxylated derivatives **247** and **249**.

The results above indicate that while *O*- and *C*-carbethoxylation of **245** can be readily achieved, selective N_3-carbethoxylation is complicated by both the thermodynamic stability of the C_5-carbethoxy substitution and the lack of nucleophilicity of the N_3-nitrogen of the 6-oxo-uracil scaffold. As a result, we sought to develop a protecting group strategy for the uracil C_5-ring position. Initially, we attempted to implement this strategy by protecting the C_5-position of substrate **245** through a reaction with trimethylene dithiotosylate ($TsS(CH_2)_3STs$, **254**) to produce the corresponding dithiane **256** (see Scheme 37 for structure).[94,95] Unfortunately, the reaction of **245** with trimethylene dithiotosylate using KOAc as a base in absolute ethanol at reflux or using triethylamine as a base in either ACN or DCM at room temperature led to complex reaction mixtures, affording only slight evidence of product formation by ^1H NMR analysis.[96,97] The lack of efficiency for the formation of dithiane **256** from the cyclic precursor **245** led us to try a more stepwise approach to the formation of this compound (Scheme 37). In the first step, primary urea **242** was reacted with methyl malonyl chloride in THF at room temperature to afford **253** in 92% yield. After reaction optimization, it was found that **253** could be smoothly converted to **255** in 94% yield by reaction with trimethylene dithiotosylate in the presence of 2.5 equivalents of triethylamine in DCM at room temperature. Dithiane **255** was then cyclized by treatment with excess K_2CO_3 in ACN at reflux over 4 h to afford the desired C_5-protected-6-oxo-uracil intermediate **256** in 67% yield.



Scheme 37. Synthesis of the C_5-protected-6-oxouracil intermediate **256**.

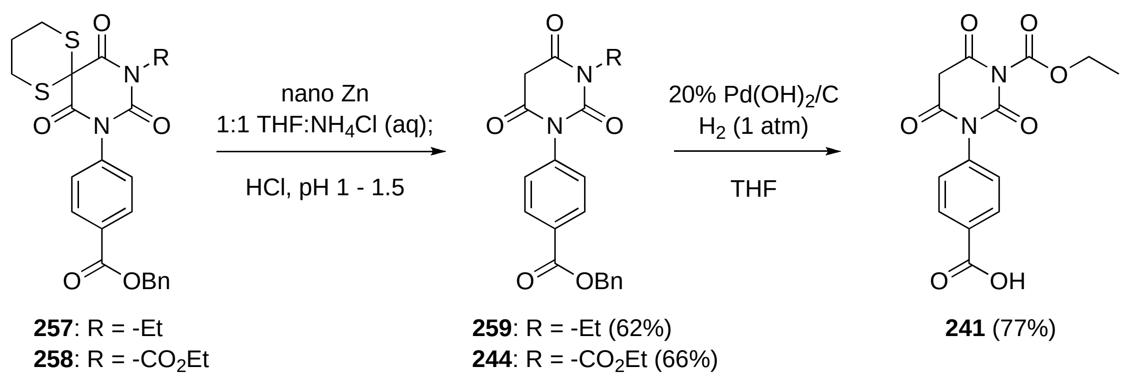
Initial attempts to introduce the ethyl ester at the N_3-position of **256** by reaction with a large excess of both triethylamine and ethyl chloroformate in DCM surprisingly afforded the ethyl adduct **257** in 71% yield (Scheme 38). This interesting reagent combination allows for a relatively mild method for N_3-ethylation of N_1-substituted-C_5-disubstituted-6-oxo-uracil scaffolds.[98,99] The scope and mechanism of this alkylation reaction has not been further investigated. It was found, however that **256** could be successfully N_3-carbethoxylated by reaction with ethyl chloroformate using pyridine as a base to form the carbamate **258** in 91% yield.



257 (71%) **256** **258** (91%)

Scheme 38. Synthesis of N_3-ethyl / N_3-carbethoxy-6-oxouracils **257** and **258**.

Utilizing **257** as a model substrate to optimize the reaction conditions for cleavage of the dithiane, it was found that common desulfurization conditions including reaction with activated Raney nickel under a hydrogen gas atmosphere (1 atm) or reaction with nickel or cobalt borohydride failed to produce the desired desulfurized product.[100] Reductive cleavage of the dithiane was ultimately accomplished via a modification of a procedure reported by Holton which demonstrated the efficiency of activated zinc to reductively desulfurize α-phenylthio ketones and esters under mild reaction conditions.[101-103] Thus, the reaction of **257** in a 1:1 solution of aqueous NH_4Cl:THF with 10 equivalents of nano-zinc at room temperature followed by an acidification of the reaction mixture to pH 1.0 – 1.5 with a 1.0 M HCl solution furnished **259** in 62% yield (Scheme 39). Gratifyingly, the application of this methodology to intermediate **258** smoothly cleaved the dithiane, forming carbamate **244** in 66% yield. Hydrogenolysis of the benzyl ester from **244** proceeded cleanly in 77% yield to the desired carboxylic acid **241**. This reaction represents the first example of the use of a dithiane protecting group strategy to synthesize C_5-unsubstituted-N_1,N_3-differentially-substituted-6-oxo-uracil scaffolds and should be beneficial for the development of barbiturates containing this unique and synthetically challenging substitution pattern.



Scheme 39. Synthesis of the originally proposed X-ray structure **241**.

To test the structural equivalency of **241** with the original HTS hit, a 1:1 mixture of **241** and **SID 861574** was prepared in DMSO-d_6 for ^{1}H NMR analysis. As can be clearly seen from the ^{1}H NMR spectrum shown in Figure 11, these two compounds were found not to be identical, allowing us to rule out the structure of **241** as that of **SID 861574**. In fact, in contrast to **SID 861574**, compound **241** was found to form exclusively the keto-tautomer in DMSO-d_6, as evidenced by the methylene peak at δ 3.97 ppm. While the structure of **SID 861574** remained elusive, this work allowed for the generation of a series of closely related **SID 861574** analogues and ultimately illuminate the structure of **SID 861574**.

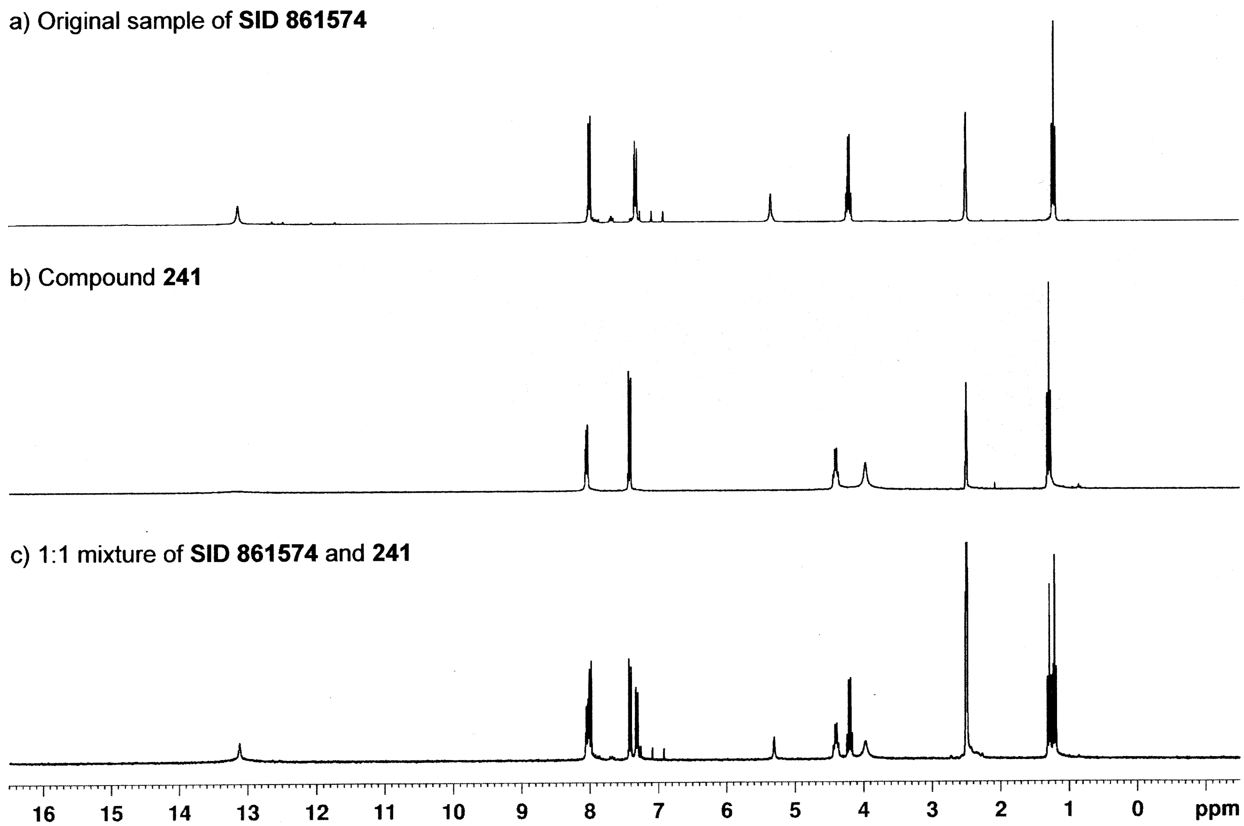
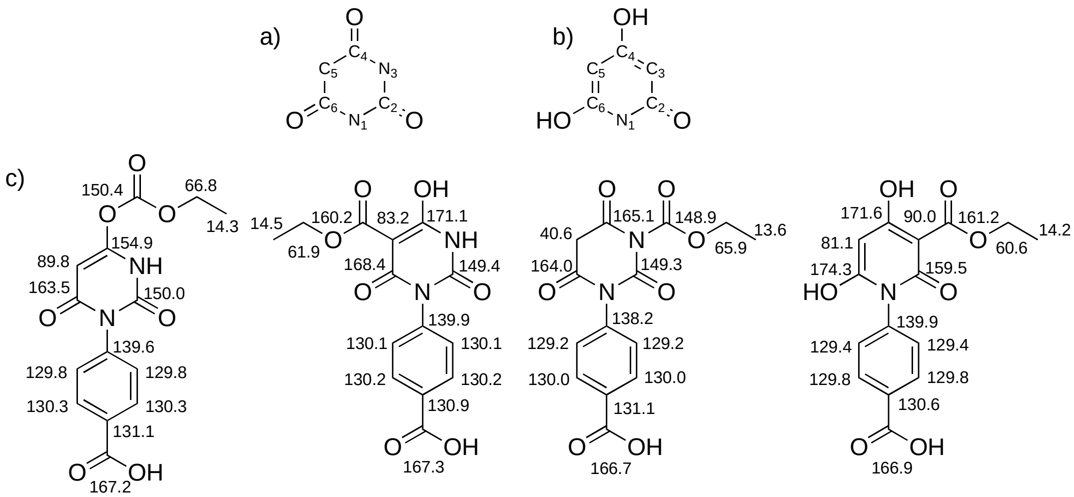


Figure 11. ^{1}H NMR (300 MHz, DMSO-d_6) stack plot of a) **SID 861574** b) compound **241** and c) a 1:1 mixture of **SID 861574** and **241**.

The X-ray crystal structure of **SID 861574** provided valuable information about the overall atom connectivity present in the molecular scaffold; however, the spectral inequivalency of **SID 861574** and **241** suggested an inconsistency between one or more assignments in the crystal structure and the atoms present in **SID 861574**. A close comparison of the ^{13}C NMR data of **SID 861574** and analogues **247**, **249** and **241** provided important insight regarding the nature of the core heterocycle and the point of attachment of the ethyl ester (Figure 12). First, the C_2-

carbonyl functionality in the 6-hydroxy uracil ring of substrates **247**, **249** and **241** consistently falls within the chemical shift range of δ 149.3 – 150.0. The closest carbonyl resonance found for **SID 861574** was well outside this range at δ 159.5, suggesting that the core heterocycle was not a 6-hydroxy uracil. Second, as can be seen from ethyl carbonate **247** and ethyl carbamate **241**, the chemical shift for the respective carbonyl resonances are found within a range of δ 148.9 – 150.4, signaling that the ethyl ester functionality of **SID 861574** was not attached to a *N*- or *O*-heteroatom but more likely to a *C*-atom as can be see by comparison with the ethyl ester analogue **249**. These two points together supported a structure in which the ethyl ester was attached to a carbon atom on the core heterocycle, which appeared to be a 4,6-dihydroxy-2-piperidone and not a 6-hydroxy uracil (Figure 12, compound **260**). Further evidence for this observation came about via a re-refinement of the original X-ray crystallography data in which the N_3-6-hydroxy uracil nitrogen atom was replaced with a carbon atom; hence, producing the 4,6-dihydroxy-2-piperidone core of **260** and decreasing the R-value for the original data set from 0.0809 to 0.0750. On the basis of this information, we sought to synthesize compound **260** and directly compare it to **SID 861574**.

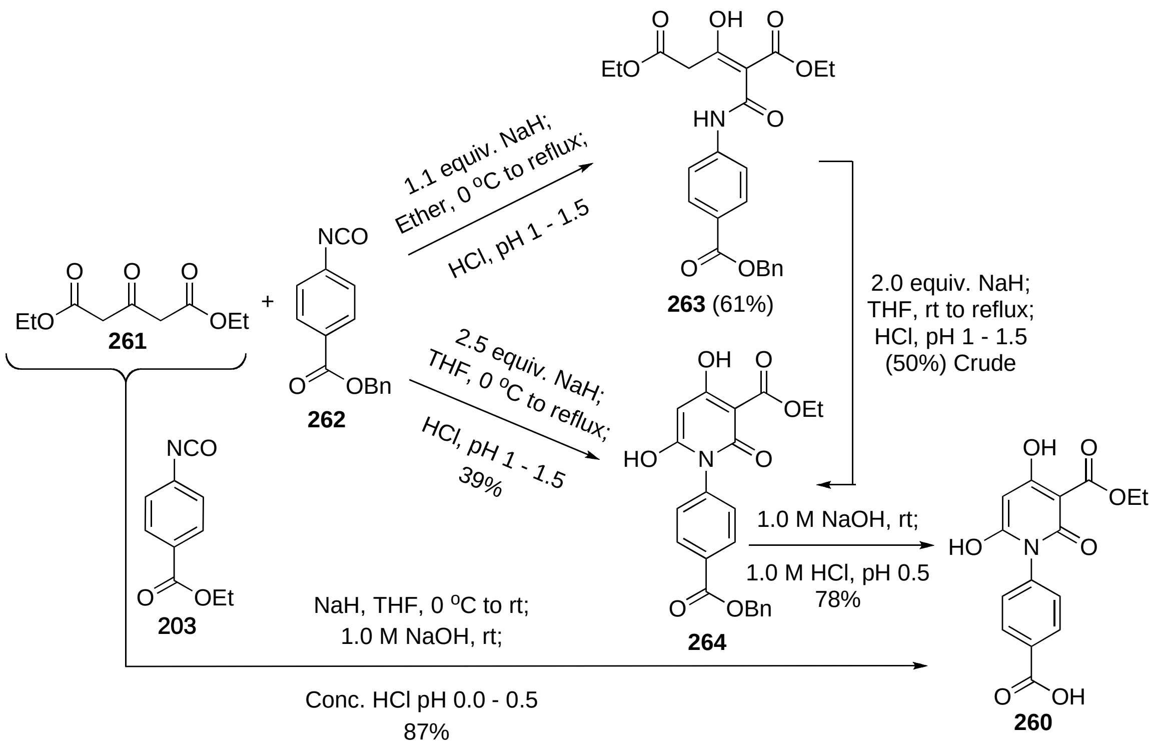


247 **249** **241** **SID 861574 (260)**

Figure 12. a) Numbering scheme for the 6-oxouracil scaffold. b) Numbering scheme for the 4,6-dihydroxy-2-piperidone scaffold. c) ^{13}C NMR chemical shift assignments in DMSO-d_6 for compounds **247**, **249**, **241** and **SID 861574** (**260**).

3.3.3 Positive identification of the structure of SID 861574 and biological testing results against Plk1-PBD

The synthesis of **260** was based on a procedure reported by Mee which demonstrated that reaction of sodium diethyl 3-oxoglutarate with phenyl isocyanate in ether at reflux produced as the major product ethyl 2,4,6-trioxo-1-phenylpiperidine-3-carboxylate.[104] Utilizing Mee's protocol, it was found that reaction of sodium diethyl 3-oxoglutarate with benzyl 4-isocyanatobenzoate **262** at 0 °C followed by heating in ether produced the uncyclized adduct **263** as the major product (Scheme 40).[105] Cyclization of **263** took place in the presence of NaH (2.0 equiv.) by heating in THF for 45 min to give **264** as the major product in 50% crude yield after acidification. These two reactions were then combined into one step by performing the reaction in THF at reflux in the presence of NaH (2.5 equiv.) to furnish **264** in 39% yield and 90-95% purity by ^{1}H NMR (Scheme 40). While the original synthetic plan was to selectively cleave the benzyl ester of intermediate **264** through a palladium-catalyzed hydrogenolysis, exquisite chemoselectivity was found for the cleavage of the benzyl ester via saponification with 1.0 M NaOH, leading to the desired compound **260** in 78% yield. A simplified one-pot reaction was developed in which a mixture of diethyl 3-oxoglutarate and readily available ethyl 4-isocyanatobenzoate **203** in THF were reacted in the presence of excess NaH at 0 °C to cleanly generate *in situ* the ethyl ester analog of **263**. Cyclization and saponification of this intermediate was accomplished by the addition of an equal volume of 1.0 M NaOH to THF with stirring at room temperature for 1 h. The resulting product was easily purified from this biphasic reaction mixture by removal of the THF layer and acidification of the aqueous layer with concentrated HCl (pH 0.0 - 0.5) at 0 °C, which cleanly precipitated **260** in 87% yield.



Scheme 40. Optimization of a one-pot synthesis of **260** (**SID 861574**).

Upon isolation, a 1:1 solution of **260** and **SID 861574** was prepared in DMSO-d_6 for ^1H NMR (300 MHz) analysis. This NMR analysis demonstrated that **260** and **SID 861574** were spectroscopically equivalent and hence *identical compounds*. Compound **260** and **SID 861574** were also found to be identical by ^{13}C NMR, IR, MS and X-ray crystallography. A sample of the newly synthesized compound **260** was tested against Plk1-PBD. Interestingly, unlike the initial assay results of the original HTS screen which showed **SID 861574** to inhibit Plk1-PBD with an IC_{50} of 2.41 ± 0.71 μM, freshly prepared **260** was found to be inactive against Plk1-PBD (IC_{50} > 50 μM).

3.4 INVESTIGATIONS INTO THE SAMPLE COMPOSITION OF SID 861574

As of 3/3/10, samples of two lots of **SID 861574** were obtained for analysis. The first lot, STOCK4S-15512 was the lot from which the originally tested sample of **SID 861574**, provided by the NIH SMR originated. The second lot PHAR065456 was obtained later and both batches are/were available from Ambinter. Samples from these lots were directly compared by ^{1}H NMR (300 MHz) analysis in DMSO-d_6 and it was found that both lots were identical in composition (Figure 13a, 13b and 13c). Further NMR analysis of these two lots (now referred to collectively as **SID 861574**) showed the major contaminant of the sample to be NH_4Cl (Figure 13d).

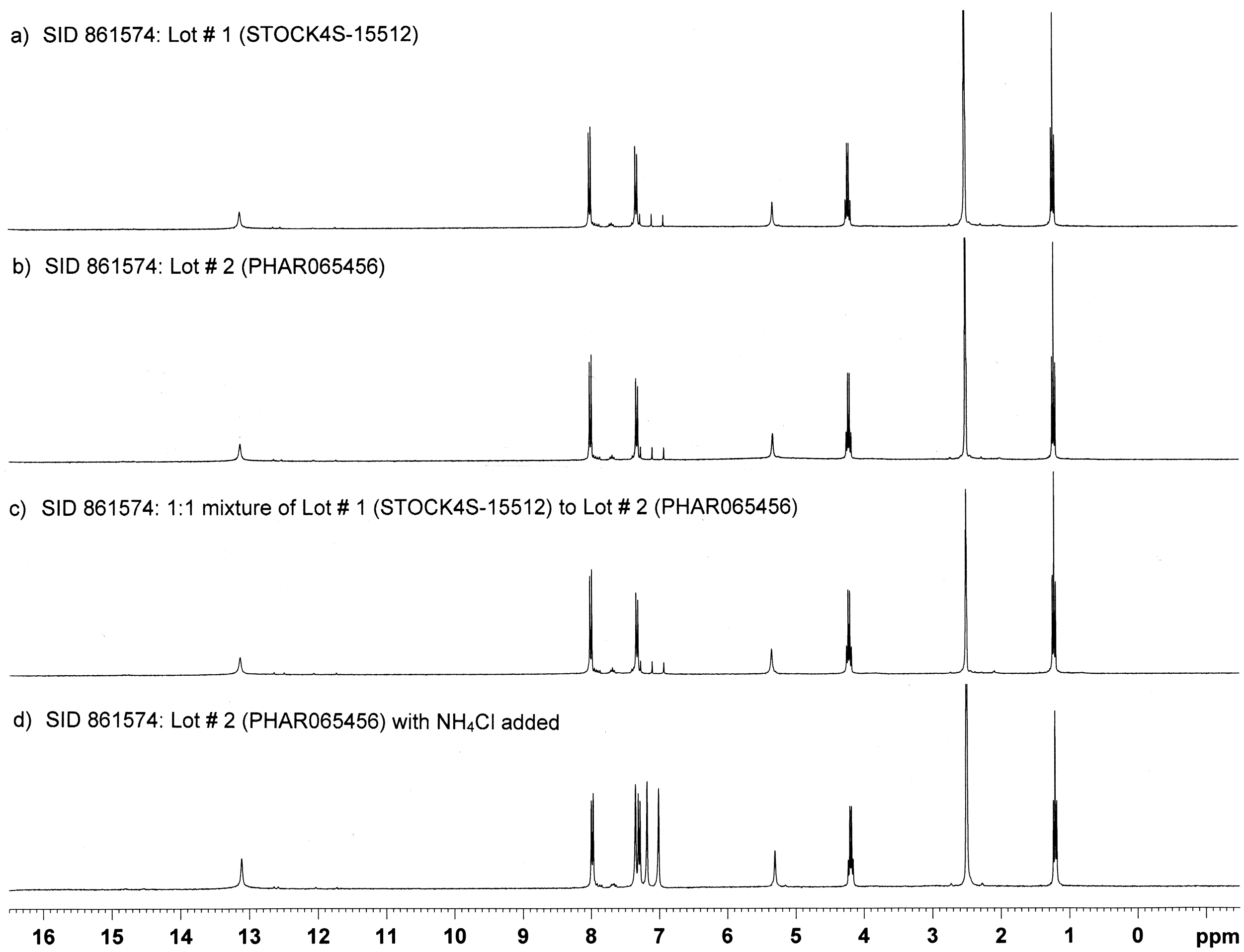
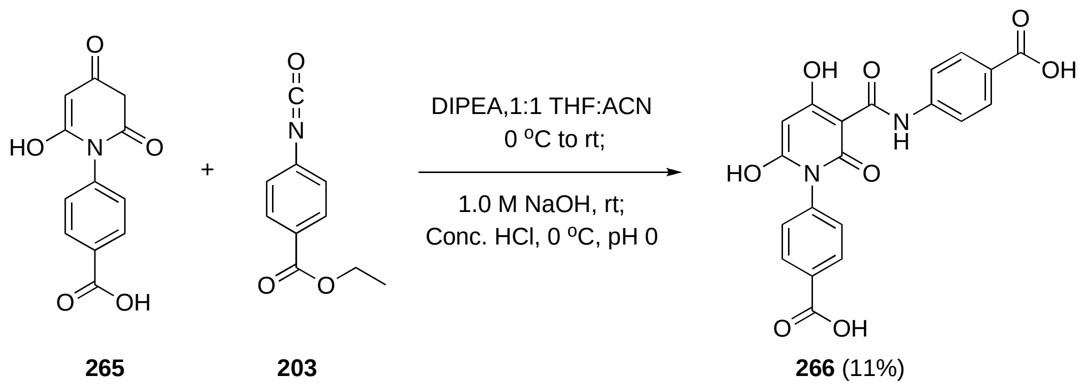


Figure 13. ^{1}H NMR (300 MHz, DMSO-d_6) stack plot of a) STOCK4S-15512, b) PHAR065456, c) 1:1 mixture of STOCK4S-15512 and PHAR065456, d) PHAR065456 with NH_4Cl added.

An LC/MS/UV analysis was performed on **SID 861574**, which showed **260** (retention time = 10.91 min, MS (ESI+) *m/z* 320 $[M + H]^+$) to be the major component of the sample along with two other impurities (< 5% by ^{1}H NMR) having retention times of 13.31 and 16.51 min (Figure 14). While the peak at 13.31 min ionized (ESI+) poorly and its origin remains unknown, the peak at 16.51 min was found to have a *m/z* = 411. This mass was consistent with a compound having the structure of **266** (*m/z* = 411 $[M + H]^+$), which was synthesized by the reaction of **265** (see Scheme 42 for synthesis) with ethyl 4-isocyanatobenzoate in the presence of

DIPEA (Scheme 41). A direct LC/MS/UV comparison of **266** with **SID 861574** showed a match between **266** and the impurity at 16.51 min in **SID 861574** by both retention time and mass (Figure 14c). As a result, **266** is very likely an impurity contained in the original sample of **SID 861574**, and, therefore, the biological activity of **266** against Plk1-PBD is also of interest.



Scheme 41. Synthesis of **266**, a likely impurity in **SID 861574**.

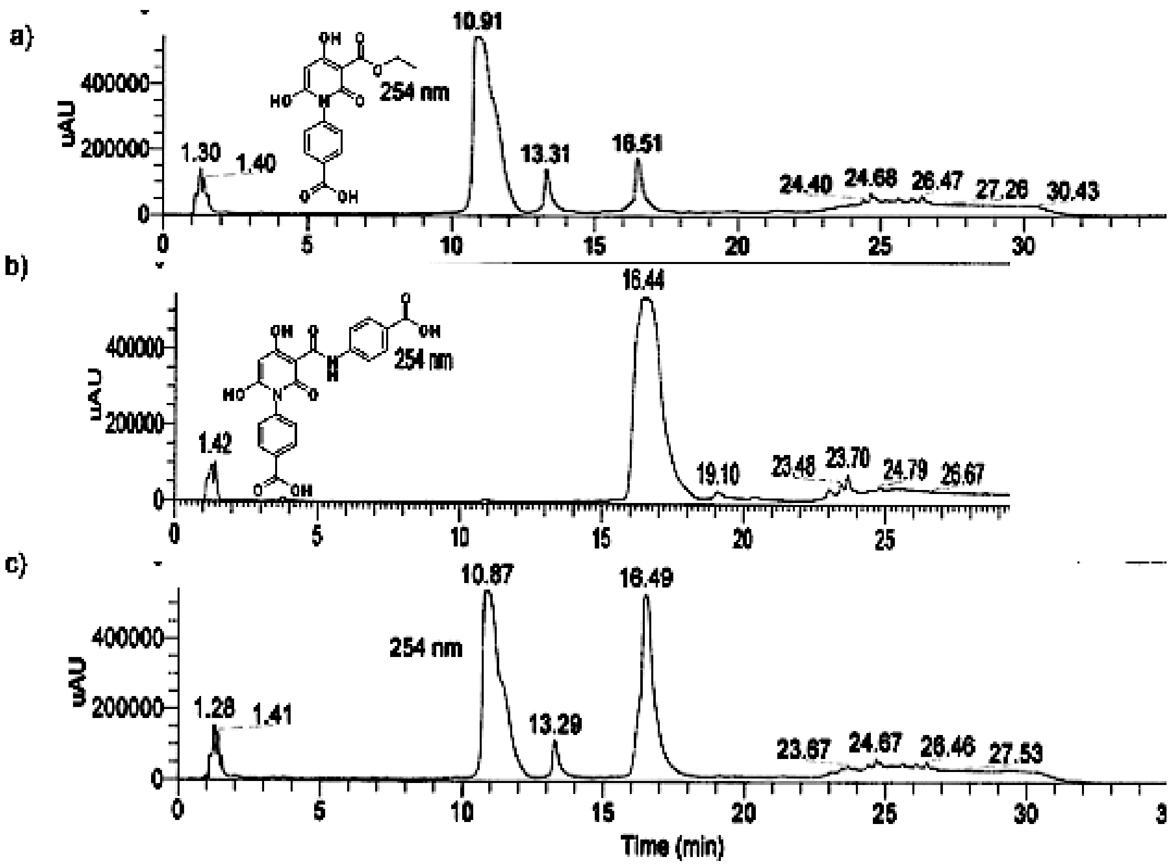


Figure 14. Direct LC/MS/UV comparison for a) **SID 861674**, b) compound **266** and c) a 3:1 mixture of **SID 861574** and compound **266**.[a]

3.5 INVESTIGATIONS INTO THE DECOMPOSITION OF 260 AND SID 861574

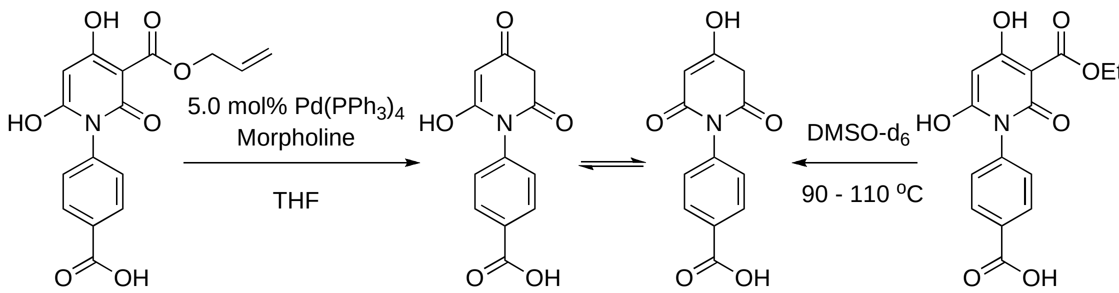
As a result of the differences in biological activity against Plk1-PBD of **SID 861574** and **260**, along with a thorough analysis of the composition of **SID 861574**, it was also important to analyze the decomposition patterns of **SID 861574** and **260** in wet DMSO. It is plausible that the

[a] LC/MS analysis performed by Peter Chambers.

originally tested sample of **SID 861574**, prepared in an aqueous DMSO solution, may have partially decomposed prior to testing against Plk1-PBD and that a decomposition product of this sample was actually responsible for the initial positive assay result.

3.5.1 High temperature decomposition of SID 861574

A high temperature ^{1}H NMR (300 MHz) decomposition experiment was performed on **SID 861574** by heating a sample of this compound to 90 °C for 3 h in DMSO-d_6. The experiment showed decomposition of the sample to predominately yield one product. We suspected that this decomposition product had the structure **265** as shown in Scheme 42. To confirm this structure, compound **265** was synthesized by an independent route, initially forming the allyl ester **267** via the one pot reaction of diallyl 3-oxopentanedioate (**268**) with ethyl 4-isocyanatobenzoate as described above.[106] Allyl ester **267** was then reacted with 5.0 mol% $Pd(PPh_3)_4$ in the presence of excess morpholine to cleave the allyl ester, following which decarboxylation was found to be spontaneous, forming **265** in 74% yield after acidification. Compound **265** was found to exist as a 4:1 mixture of tautomers in DMSO-d_6 by ^{1}H NMR. The most characteristic peaks for this tautomeric pair are the C_5-H resonances at δ 5.36 and 5.25 (Figure 15b and 15d).



Scheme 42. Synthesis of thermal decomposition fragment **265**.

To confirm that compound **265** was the genuine high temperature decomposition product of **260**, a 3:2 mixture of **260**:**265** was prepared in DMSO-d_6. This mixture was heated to 110 °C for 30 min. After cooling to room temperature, ^{1}H NMR showed a nearly complete decomposition of **260** to **265** and EtOH, confirming the structure of **265** as the thermal decomposition product of **260** (and therefore **SID 861574**) in DMSO (Figure 15). Compound **265** is thought to arise via the decomposition pathway shown in Scheme 43, in which an initial retro [4+2] reaction of **260** generates the corresponding α-oxo ketene intermediate **269**, which then either reacts with the eliminated EtOH to reform **260** or with H_2O (present in DMSO) to form the dicarboxylic acid intermediate **270**.[107,108] Intermediate **270**, may then either undergo a retro [4+2] reaction to reform **269** or irreversibly decarboxylate to form the final product **265**.

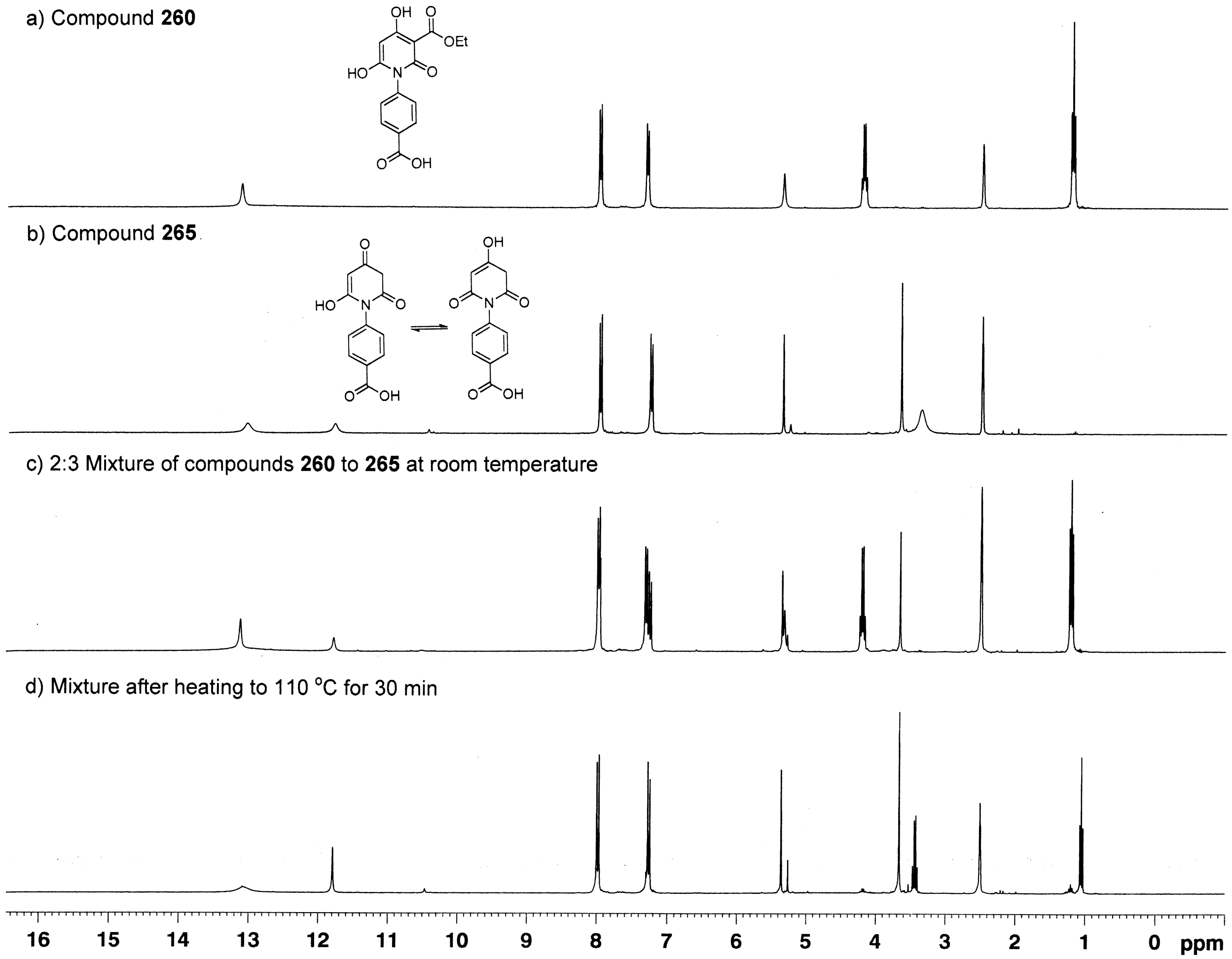
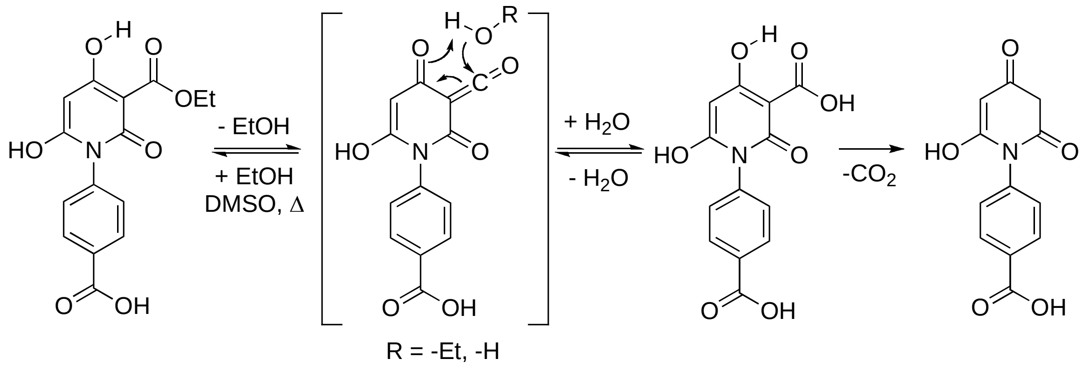


Figure 15. ^{1}H NMR (300 MHz, DMSO-d_6) stack plot of: a) compound **260**, b) compound **265**, c) a 2:3 mixture of **260** and **265** at room temperature, and d) a mixture after heating to 110 °C for 30 min, showing the thermal decomposition of **260** to **265**.



260 **269** **270** **265**

Scheme 43. Proposed high temperature decomposition pathway for **260** to **265** in wet DMSO.

3.5.2 Room temperature decomposition of 260 vs. SID 861574

The room temperature decomposition of a concentrated sample of compound **260** in wet DMSO-d_6 was monitored over 6 weeks (Figure 16a). It was found that over this time period **260** underwent a hydrolytic decomposition relatively cleanly to afford predominantly two products. The structure of the first decomposition product was found through synthesis and ^{1}H NMR comparison to be that of **271** (Figure 16b, Scheme 44a). Compound **272** was also synthesized via a modification of a procedure reported by Pericas et. al. for ^{1}H NMR comparison due to the expected similarity in the ^{1}H NMR resonances for compound **272** vs. **271**.[106] This experiment ruled out the possibility of the ^{1}H NMR resonances in Figure 16a to arise from **272** (Scheme 44b). The second room temperature decomposition product was found to be consistent with the high temperature decomposition product **265** (Figure 16c).

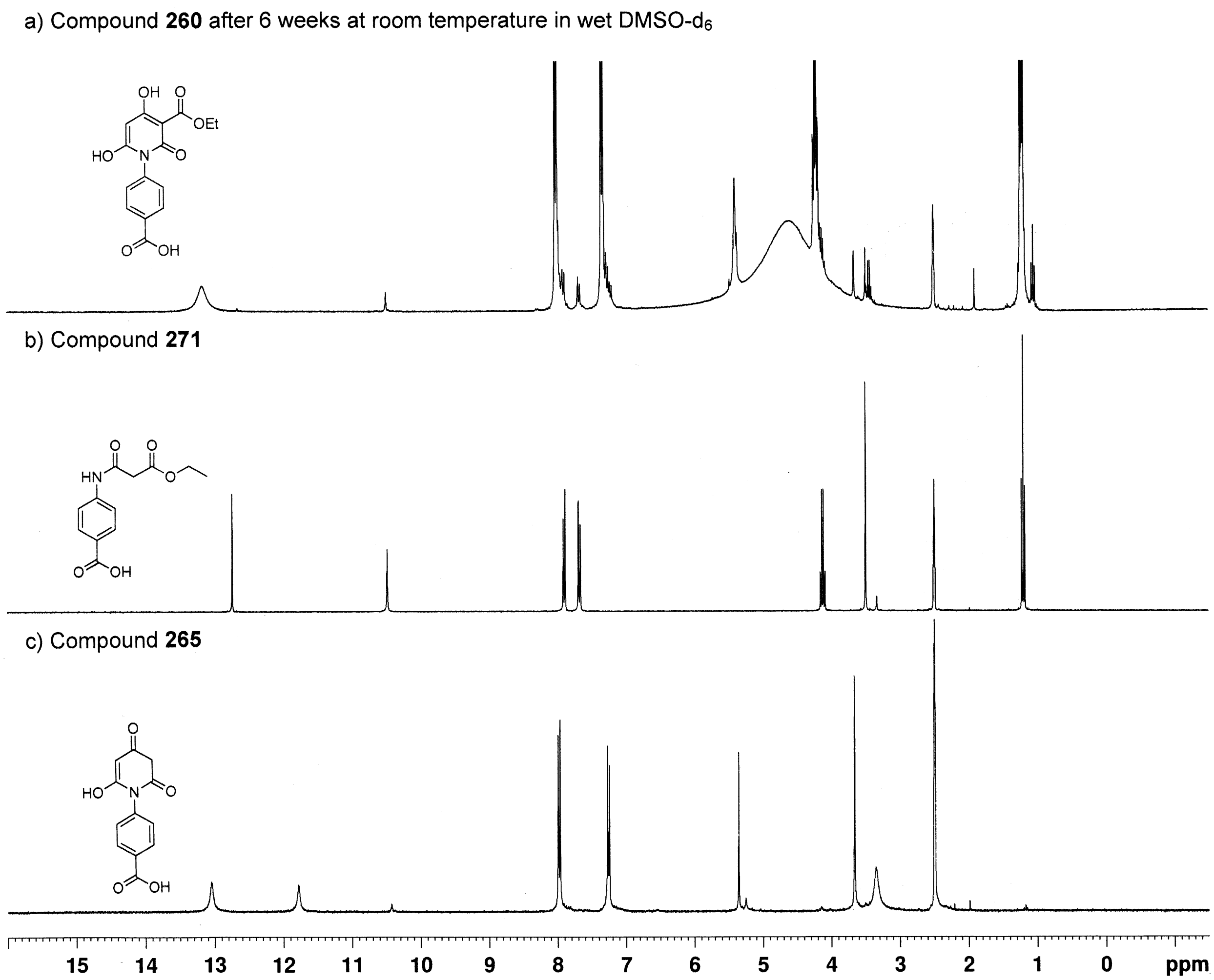
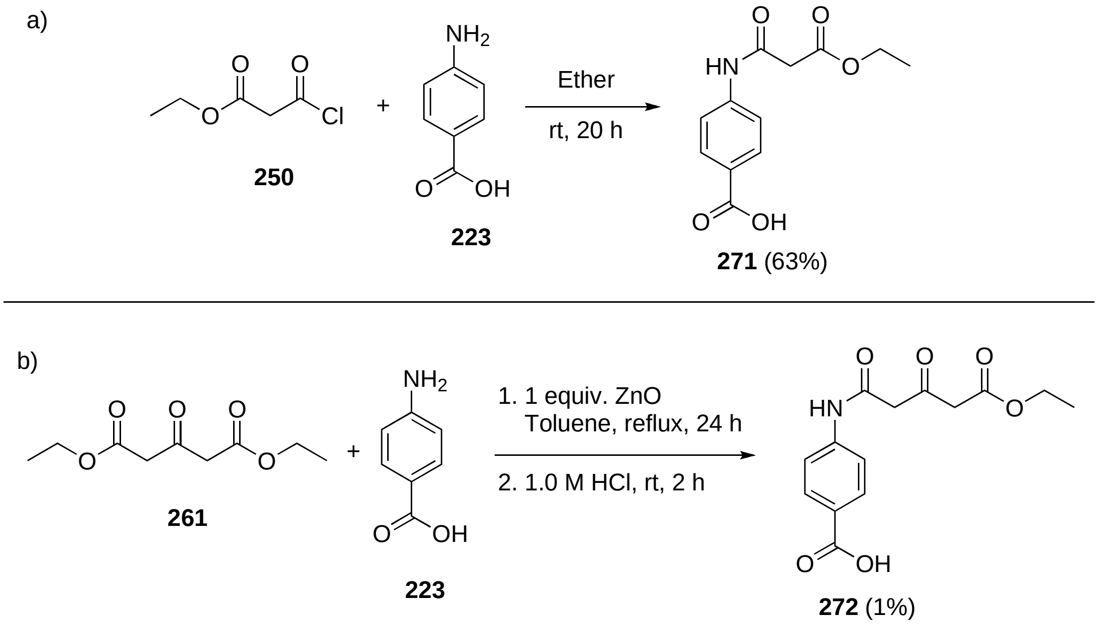


Figure 16. ^{1}H NMR (300 MHz, DMSO-d$_6$) stack plot for the room temperature decomposition of **260** over 6 weeks into compounds **271**, **265** and ethanol.



Scheme 44. Synthesis of possible decomposition products of **260**, **271** and **272**.

A control ^{1}H NMR (300 MHz) experiment of the room temperature decomposition of two samples of **260** was performed over 9 weeks (Figure 17). Both samples contained 2.5 mg of **260** in 1.0 mL of DMSO-d_6 containing 2.0 µL deionized water, however, one sample was doped with 1.3 equiv. NH_4Cl (Figure 17b) and the other was not (Figure 17a). As can be inferred by comparison with Figure 16a, both the rate and complexity of the decomposition of **260** appears to be enhanced in DMSO solutions heavily saturated with water. While **271** and **265** were detected in these spectra, the identities of the other decomposition products remain unknown. A comparison of the NMR spectra between the NH_4Cl doped sample and the non-doped sample shows only slight differences in both the rate and extent of decomposition of **260** over the period studied. After 9 weeks, both samples were stored at -20 ^{o}C to suppress further decomposition before biological testing against Plk1-PBD. The ^{1}H NMR decomposition profile of a sample of **SID 861574** stored under similar conditions was found to match the results of this control experiment (Figure 17c). These experiments reveal the instability of **SID 861574** in wet DMSO solutions and may provide a possible link between the decomposition of **SID 861574** and the biological activity against Plk1-PBD.

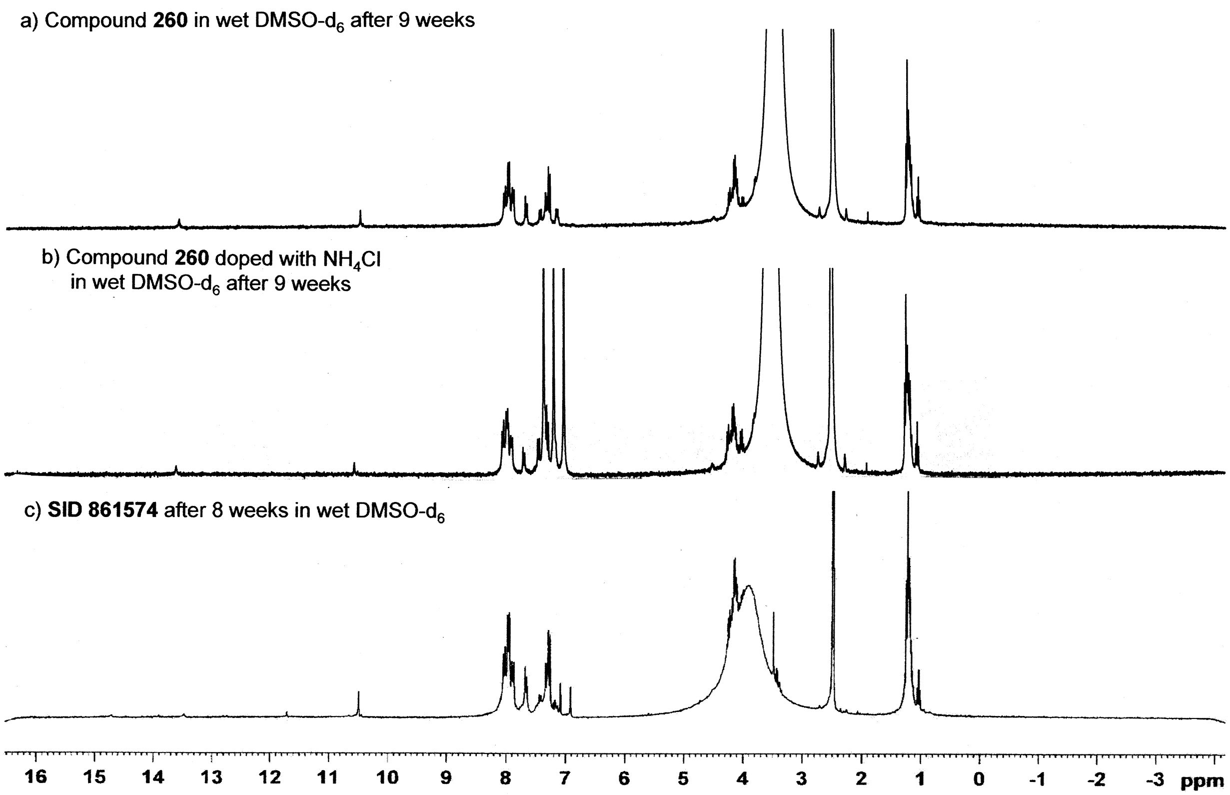
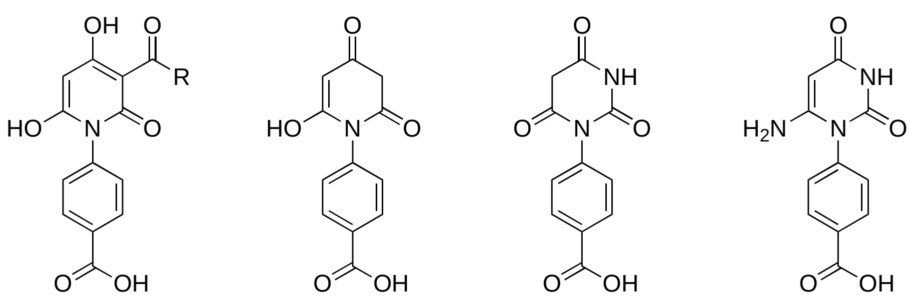


Figure 17. Comparison of the room temperature decomposition patterns in wet DMSO-d_6 of a) compound **260**, b) a mixture of **260** and NH_4Cl and c) **SID 861574**.

3.6 INITIAL BIOLOGICAL TESTING RESULTS AGAINST PLK1-PBD

Initially, three compounds were submitted for biological testing against Plk1-PBD (Figure 18, Table 12): a) compound **260**, batch DMA-NB205-92, a resynthesis of **SID 861574**, b) compound **267**, batch DMA-NB245-4, the allyl ester analogue of **260** and c) compound **265**, batch DMA-NB245-3, the freshly synthesized high temperature decomposition product of **260**. Of these three compounds, only **265** was found to inhibit Plk1-PBD with an IC_{50} of 2.079 µM. Following this result, an optimized synthesis of **265** was developed and the product of this reaction DMA-NB245-24 was submitted along with the previous samples and two additional closely structurally related compounds **234** and **239** for biological testing against Plk1-PBD. Curiously, of these samples again only compound **265** batch DMA-NB245-3 and *not* compound **265** batch DMA-NB245-24 was active against Plk1-PBD. This result strongly suggested that an impurity in the sample **265** batch DMA-NB245-3 (~90% pure) and not in DMA-NB245-24 ($\geq$ 95% pure) was responsible for the positive assay result. As can be seen from table 12, it was also found that **265** batch DMA-NB245-3 slightly decreased in potency from the first to the second testing. This decrease in potency may reflect the stability of the active agent in the sample over a storage period of 3 months.



260: R = -OEt
267: R = -OAllyl
265
239
234

Figure 18. Samples submitted for biological testing against Plk1-PBD.

Table 12. Biological Testing Results from Two Trials for Inhibitors of Plk1-PBD.

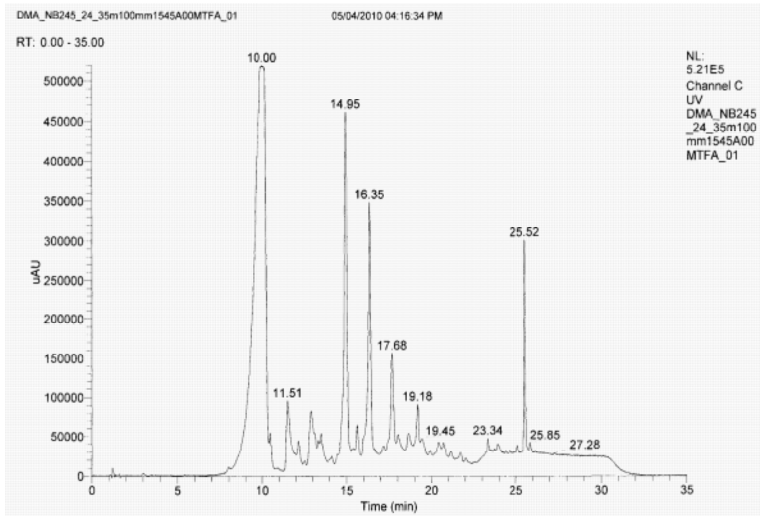
Entry	Compound	Batch (Notebook #)	Assay 1[a,c] IC_{50} (μM)	Assay 2[b,c] IC_{50} (μM)
1	**SID 861574**	-	-	2.95 ± 0.568
2	**260**	DMA-NB205-92	> 50	> 50
3	**267**	DMA-NB245-4	> 50	> 50
4	**265**	DMA-NB245-3	2.079	6.047
5	**265**	DMA-NB245-24	-	> 50
6	**239**	DMA-NB74-62	-	> 50
7	**234**	DMA-NB74-63	-	> 50

[a]Assay performed 12/2009. [b]Assay performed 3/2010. [c]Assays performed by Paul A. Johnston

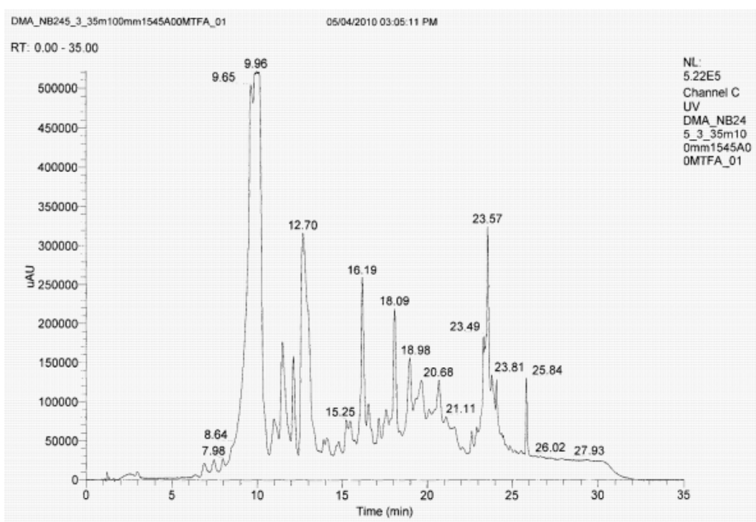
3.7 IDENTIFICATION AND SYNTHESIS OF PUTATIVE IMPURITIES FOUND IN THE BIOLOGICALLY ACTIVE BATCH OF COMPOUND 265, DMA-NB245-3

As a result of the large difference in biological activities between **265**, batches DMA-NB245-3 and DMA-NB245-24, a thorough analysis of these samples by LC/MS/UV and ^{1}H NMR was conducted. The LC/MS/UV analysis (Figure 19) reveals a predominant impurity at 12.70 min having a mass of *m/z* 335 $(M+H)^+$ in the active sample DMA-NB245-3. However, this impurity was absent in the inactive sample DMA-NB245. A ^{1}H NMR (300 MHz, DMSO-d_6) of **265** batch DMA-NB245-3 showed this sample to contain ~5-7% of an impurity having distinctive aromatic resonances at δ 7.89 (d) and 7.68 (m) (see figure 21a for ^{1}H NMR). Initially, we sought to identify this potentially active impurity through synthesis and isolation, by scaling up the synthetic route used to synthesize **265** batch DMA-NB245-3 200-fold (Scheme 45).

a)


DMA_NB245_24_35m100mm1545A00MTFA_01
05/04/2010 04:16:34 PM
RT: 0.00 - 35.00
NL:
5.21E5
Channel C
UV
DMA_NB245
_24_35m100
mm1545A00
MTFA_01
uAU
Time (min)
10.00
14.95
16.35
25.52
17.68
11.51
19.18
19.45
23.34
25.85
27.28

b)


DMA_NB245_3_35m100mm1545A00MTFA_01
05/04/2010 03:05:11 PM
RT: 0.00 - 35.00
NL:
5.22E5
Channel C
UV
DMA_NB24
5_3_35m10
0mm1545A0
0MTFA_01
uAU
Time (min)
9.96
9.65
12.70
23.57
16.19
18.09
23.49
18.98
20.68
23.81
25.84
15.25
21.11
8.64
7.98
26.02
27.93

c)

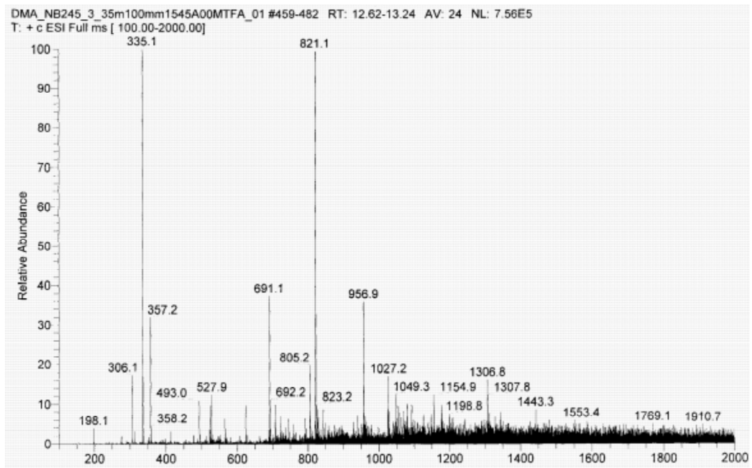
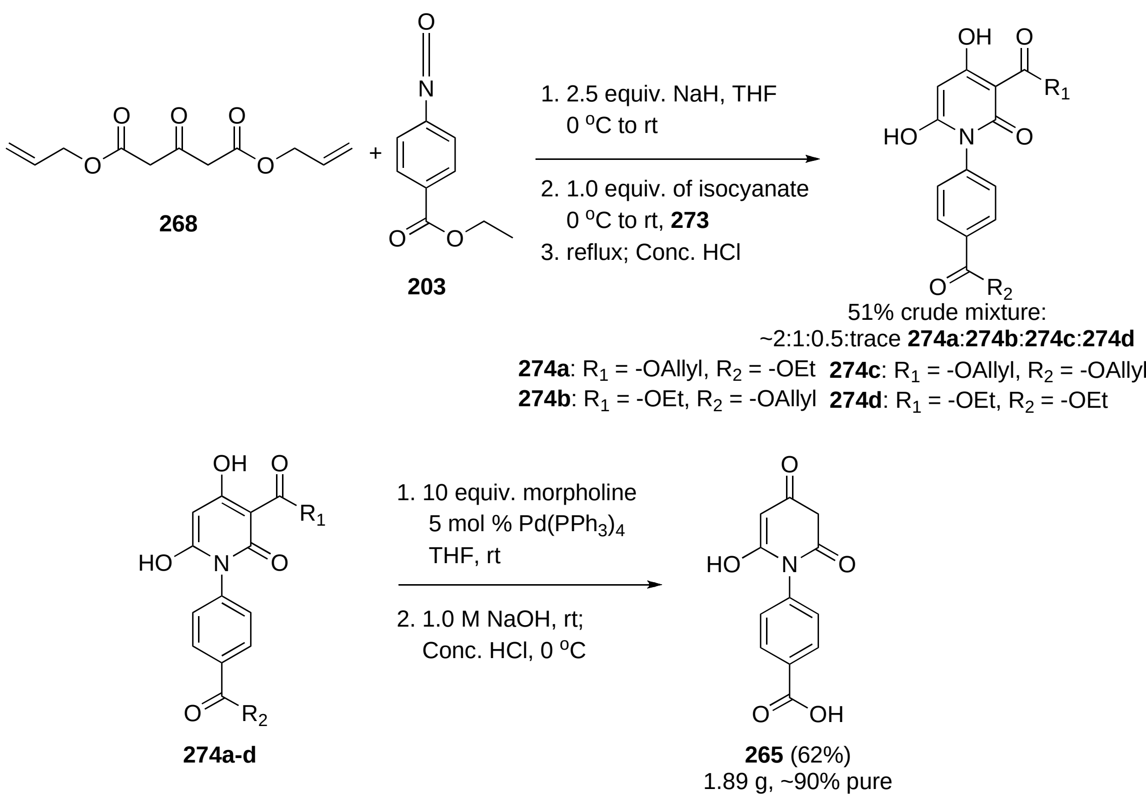


Figure 19. LC/MS/UV comparison of compound **265**: a) batch DMA-NB245-24 (inactive), b) batch DMA-NB245-3 (active) and c) MS (ESI+) trace for impurity peak at 12.70 min. Samples prepared as 1 mg substrate in 1 mL MeOH.[a]

In the first step of this scale up synthesis, the sodium salt of **268** was generated and reacted with isocyanate **203** to form adduct **273**, which was cyclized by heating in THF to give an approximately 2:1:0.5:trace mixture of **274a**: **274b**: **274c**: **274d** after acidification and recrystallization.[106] This mixture was then subjected to the deallylation conditions used for the synthesis of **265**, batch DMA-NB245-3. A mixture of **247a-d** was reacted with $Pd(PPh_3)_4$ in the presence of 10 equiv. morpholine in THF at room temperature for 1.5 h, followed by saponification of the ethyl ester by reaction with 1.0 M NaOH, providing 1.89 g of **265** in 62% yield (~90% purity by ^1H NMR) after acidification with HCl. This sample contained the identical impurity peaks by ^1H NMR as seen in batch DMA-NB245-3. After five recrystallizations from THF or EtOAc and hexanes, the majority of **265** was removed from the sample and the ratio of the impurity to **265** was enriched from 7:93 to 32:68 as judged by ^1H

[a] LC/MS analysis performed by Peter Chambers.

NMR. However, other unidentified impurities also remained in the sample. Following this series of recrystallizations, other methods were utilized to separate the remaining 32 mg of enriched sample. Unfortunately, isolation attempts by column chromatography, prep TLC, semiprep SFC, MPLC and HPLC failed to produce any pure samples. A sample from the remainder of this mixture was saved for further biological testing against Plk1-PBD (**265**, batch DMA-NB245-38). The identities of many of the impurities in this sample were finally determined after an elaborate series of experiments designed to optimize the synthesis of **265** with respect to the unknown but desired impurity(s). The interesting impurity profile of this sample will be discussed shortly.



Scheme 45. Scale up synthesis of **265** batch DMA-NB245-3.

Trials toward the optimization of the reaction conditions for the synthesis of **265** with respect to the desired impurity(s) identified by ^{1}H NMR in the biologically active sample of **265**, batch DMA-NB245-3, are depicted in Table 13. As can be seen from entries 1-4, the deallylation of **274** in the presence of high loadings of Pd(0) catalyst and morpholine (10 equiv.) led to higher percentages of the desired impurity(s) in the crude reaction mixtures as determined by ^{1}H NMR (DMSO-d_6). Entry 3 demonstrates that in the absence of an amine, the reaction product **265** acts as an allyl scavenging agent, forming a high percentage of the allylated reaction byproducts **277** and **278** in the crude reaction mixtures. The appearance of the impurity(s) of interest was also ruled out as arising from a side reaction of *N*-allylmorpholine (**276**), formed as a necessary byproduct in these reactions (Table 13, entries 4 and 7).

As a result of the correlation between higher percentages of the impurity in the crude reaction product mixtures with higher loadings of the Pd(0) catalyst, it was thought that possibly the Pd(II) formed in these reaction mixtures was responsible for catalyzing the reaction to the desired impurity(s). To test this hypothesis, the deallylation of **274** in the presence of $Pd(OAc)_2$ was explored with different catalyst loadings. The reaction succeeded at 60 °C, and catalyst loadings between 50-100 mol% were necessary for maximizing (32-35%) the percentage of the desired impurity(s) in these crude mixtures (Table 13, entries 5-9). Interestingly, entries 10-11 show that heating mixtures of **267** or **265** to 60 °C in the presence of $Pd(OAc)_2$ and morpholine also led to the formation of the desired impurity(s). Attempts to run this reaction in the presence of $ZnCl_2$ or in the absence of a Lewis acidic catalyst resulted in a large decrease in the formation of the desired impurity(s) by ^{1}H NMR (Table 13, entries 12 and 13). Finally, the optimized reaction conditions were found. The reaction of **265** in the presence of one equivalent of

$Pd(OAc)_2$ at 60 ^{o}C, in the absence of morpholine, generated a 1:1 mixture of **265**:**279** as determined by ^{1}H NMR integration (Table 13, entry 14 vs.15). These results suggest that the impurity(s) observed in sample **265** batch DMA-NB245-3 originate from a nucleophilic (H_2O, morpholine) ring opening reaction of product **265** that takes place in the presence of Lewis acidic palladium (II) salts.

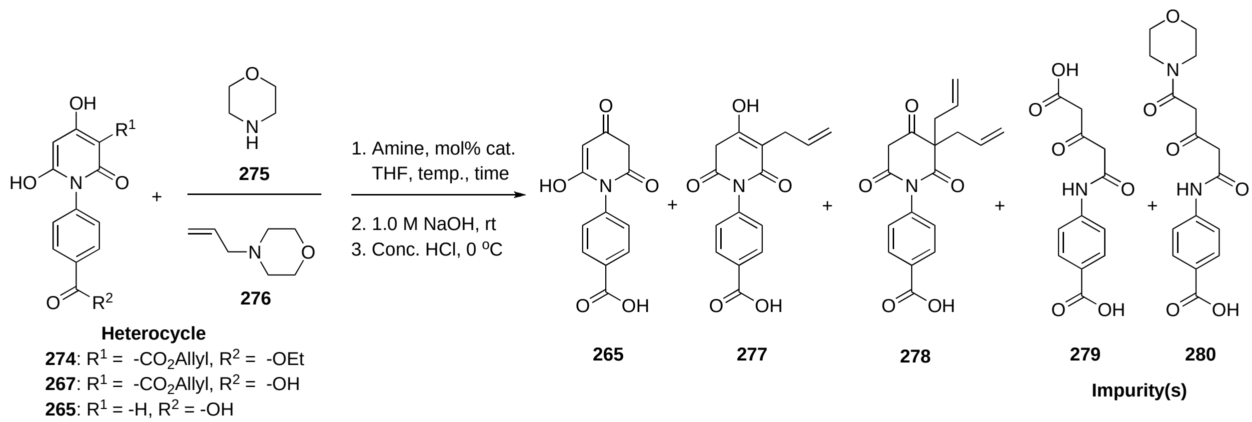
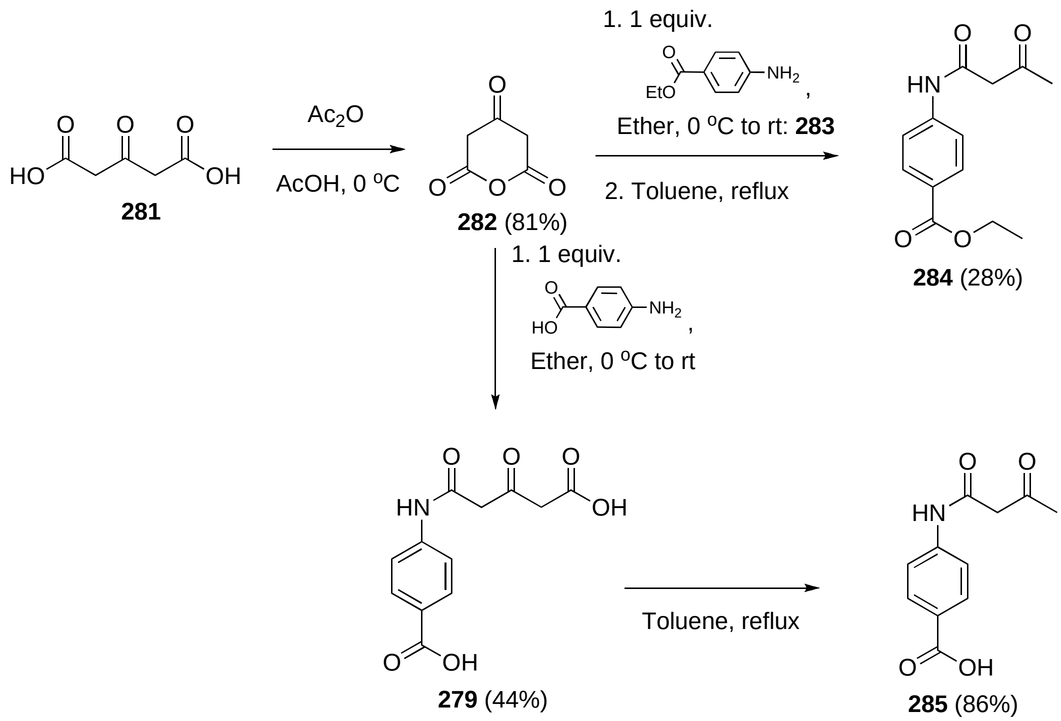


Table 13. Optimization of Reaction Conditions to Enhance the Ratio of the Major Impurity Found in the Biologically Active Sample of **265** (DMA-NB245-3).

Entry	Catalyst (mol %)	Heterocycle	Amine (equiv.)	Temp. (°C)	Time (h)	Crude yield[a]	265[c]	277[c]	278[c]	Impurity[c,d]	Other[c,e]
1	$Pd(PPh_3)_4$ (50)	**274**	**275** (10)	21	1.5	59%	55%	Trace[f]	Trace	22%	~ 23%
2	$Pd(PPh_3)_4$ (100)	**274**	**275** (10)	21	0.33	79%	42%	32%	Trace	17%	~9%
3	$Pd(PPh_3)_4$ (5)	**274**	-	21	2	68%	23%	35%	36%	Trace	~6%
4	$Pd(PPh_3)_4$ (5)	**274**	**275**(1) **276**(9)	21	1.5	75%	33%	16%	31%	11%	~9%
5	$Pd(OAc)_2$ (50)	**274**	**275** (10)	21	1.5	nr[b]	-	-	-	-	-
6	$Pd(OAc)_2$ (50)	**274**	**275** (10)	60	16	85%	59%	Trace	Trace	33%	8%
7	$Pd(OAc)_2$ (50)	**274**	**275**(10) **276**(10)	60	5	113%	53%	Trace	Trace	32%	15%
8	$Pd(OAc)_2$ (100)	**274**	**275** (10)	60	5	111%	50%	Trace	Trace	35%	15%
9	$Pd(OAc)_2$ (25)	**274**	**275** (10)	60	18	68%	54%	Trace	Trace	25%	21%
10	$Pd(OAc)_2$ (50)	**267**	**275** (10)	60	18	78%	68%	Trace	Trace	18%	14%
11	$Pd(OAc)_2$ (50)	**265**	**275** (10)	60	18	114%	62%	-	-	21%	17%
12	$ZnCl_2$ (50)	**265**	**275** (10)	60	18	85%	57%	-	-	Trace	43%
13	-	**265**	**275** (10)	60	18	114%	66%	-	-	Trace	34%
14	$Pd(OAc)_2$ (50)	**265**	-	60	24	96%	70%	-	-	30%	Trace
15	$Pd(OAc)_2$ (100)	**265**	-	60	16	nd[g]	50%	-	-	50%	Trace

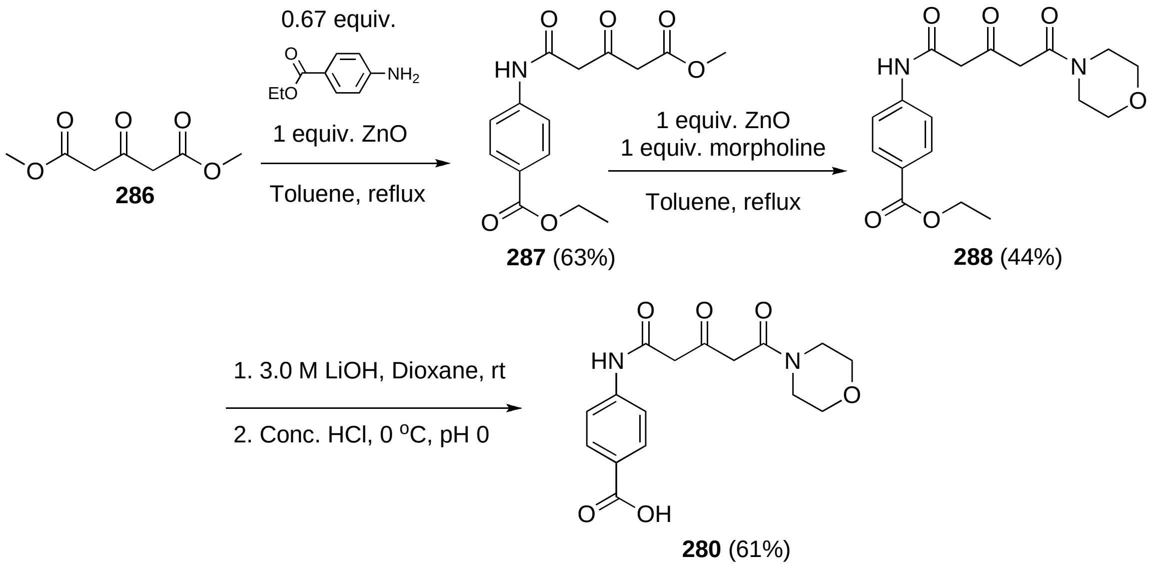
[a]Crude yield based on expected mass recovery of **265**. [b]No Reaction. [c]Precentages determined from ^1H NMR integration. [d]Compound(s) suspected of biological activity. [e]Precentage of other reaction byproducts. [f]Less than 5%. [g]Not Determined.

The structure of the putative impurity **279** formed in Table 13, entry 15 was confirmed after scaling up the reaction and carefully recrystallizing the products until the "impurity" was isolated in ~85-95% purity by ^1H NMR (300 MHz, DMSO-d_6). The product of this reaction was then directly compared to a genuine sample of **279** synthesized according to the route shown in Scheme 46.[104] Initial attempts to synthesize **279** by the reaction of ethyl 4-aminobenzoate with anhydride **282**, prepared according to a known literature procedure, were unsuccessful due to the spontaneous decarboxylation of **283** to form **284** which was isolated in 28% yield.[109,110] The reaction of anhydride **282** with 4-aminobenzoic acid **223**, however, resulted in the formation of the desired dicarboxylic acid **279** which was found to be more stable toward decarboxylation than the ethyl ester analogue **283**. A ^1H NMR (DMSO-d_6) comparison of **279** (isolated from Table 13, entry 15) and the freshly synthesized sample of **279** showed these samples to be identical. For further ^1H NMR comparisons and biological testing purposes, **279** was heated in toluene to form the decarboxylated analogue **285** in 86% yield.



Scheme 46. Synthesis of putative impurities **279** and **285** thought to be contained in **265** batch DMA-NB245-3.

This analysis provided an optimized procedure, resulting in the isolation of **279** as a possible impurity contained in the original sample of **265** batch DMA-NB245-3. Compound **279**, while a good match by ^{1}H NMR to the major impurity resonances in DMA-NB245-3, was found to differ by LC/MS from the major impurity contained in this sample. Consistent with both the ^{1}H NMR and LC/MS data for the major impurity in **265** batch DMA-NB245-3 is compound **280** (Scheme 47). Compound **280** may arise in **265** batch DMA-NB245-3 via a similar mechanism to that leading to compound **279**, with morpholine acting as the nucleophile opening the ring in compound **265**.



Scheme 47. Synthesis of the putative impurity **280** contained in **265** batch DMA-NB245-3.

Compound **280** was synthesized according to the route shown in Scheme 47. Initially, 1.5 equiv. of **286** were reacted with ethyl 4-aminobenzoate **222** in the presence of ZnO at reflux in toluene to afford the amide **287** in 63% yield. Reaction of **287** with morpholine under the same reaction conditions generated **288** in 44% yield. The ethyl ester of **288** was then saponified in the presence of 3.0 M LiOH, affording **280** in 61% yield following acidification and recrystallization from MeOH. A direct LC/MS/UV comparison of **280** and **265** batch DMA-NB245-3 was performed. As can be seen in Figure 20, compound **280** was identical in terms of both mass and retention time to the major impurity contained in **265** batch DMA-NB245-3. Compound **280** was also found to match the major impurity peaks found within **265** batch DMA-NB245-3 by ^{1}H NMR (Figure 21).

a)
b)

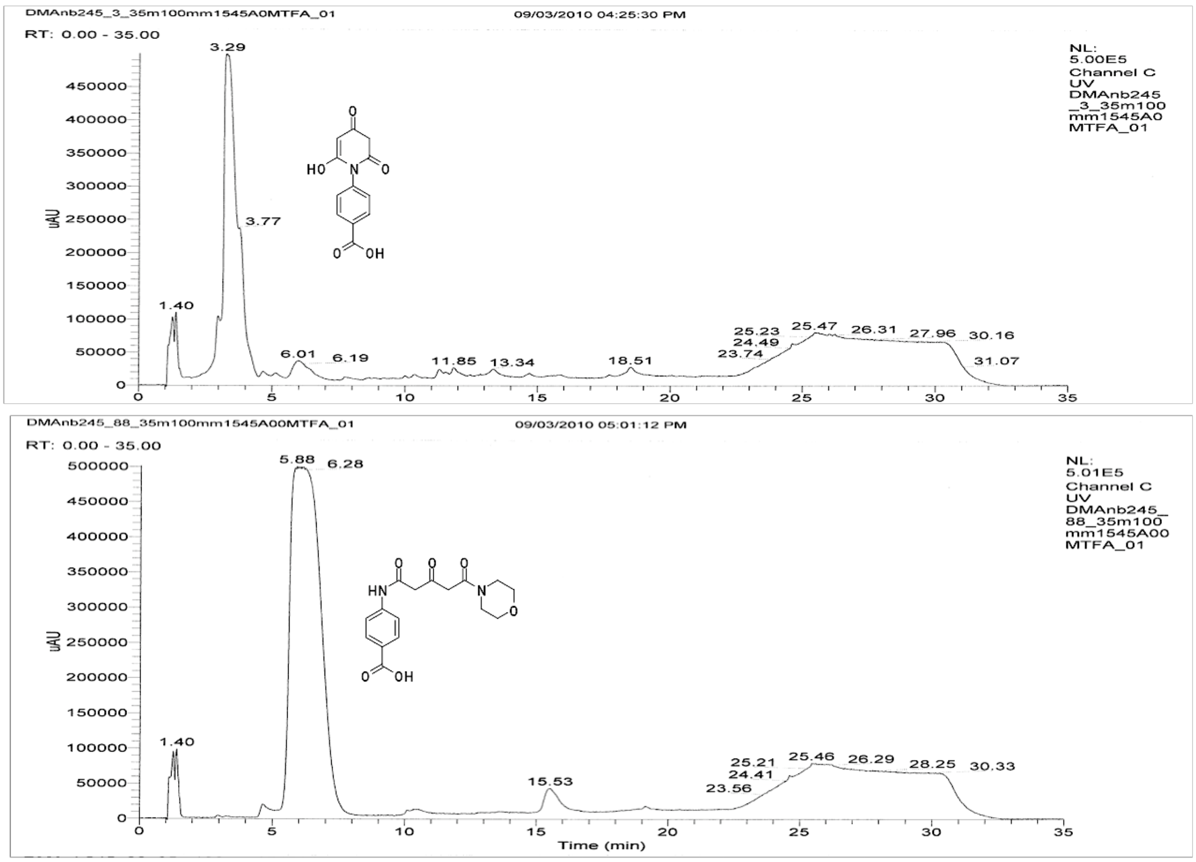


Figure 20. LC/MS/UV comparison of a) compound **265** batch DMA-NB245-3, and b) compound **280**. Samples prepared as 1 mg substrate in 1 mL DMSO.[a]

[a] LC/MS analysis performed by Peter Chambers.

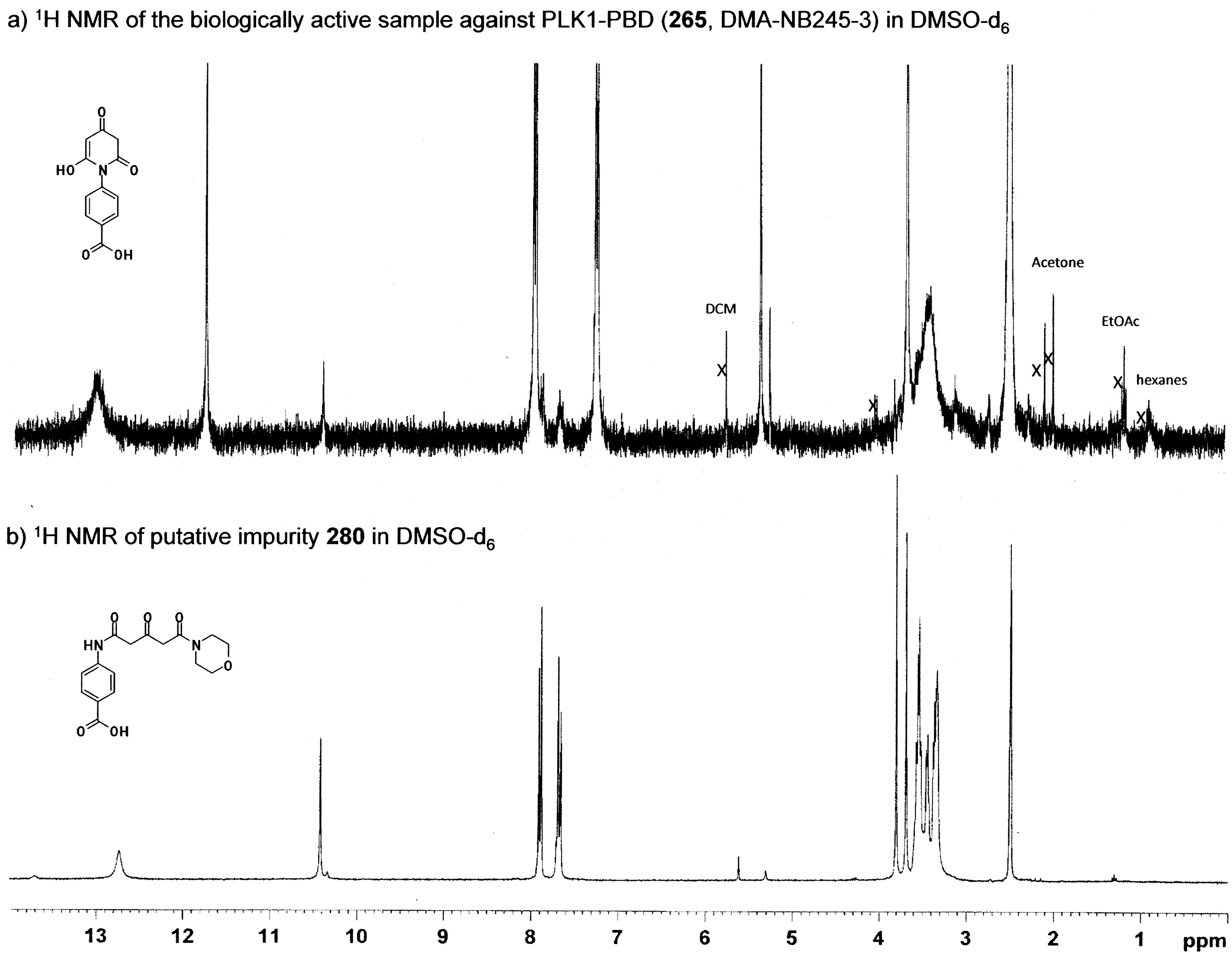
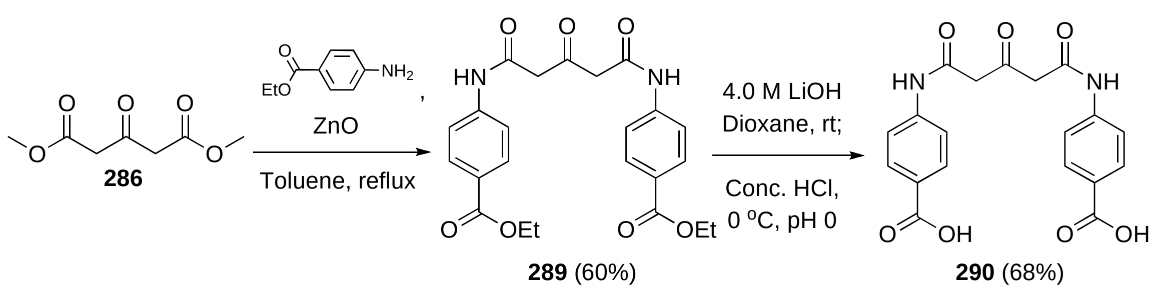


Figure 21. ^{1}H NMR (300 MHz, DMSO-d_6) stack plot of a) compound **265** batch DMA-NB245-3 and b) putative impurity **280**.

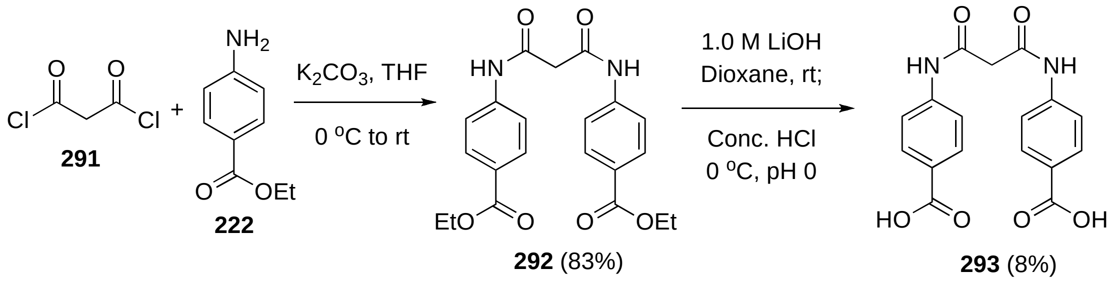
A ^{1}H NMR (DMSO-d_6) comparison of compounds **265**, **280**, **277**, **285, 279** and **260** with **265** batch DMA-NB245-38 (scaled up synthesis of DMA-NB245-3) showed that sample **265** batch DMA-NB245-38 was ~90% composed of a 51:22:9:8:6:4 ratio of **265**:**280**:**277**:**285**:**279**:**260** solvated with ~10% MeOH. This observation is also supported by LC/MS/UV analysis. The results of this analysis indicate that all of the compounds reported above in sample **265** batch DMA-NB245-38 are likely constituents of the biologically active sample **265** batch DMA-NB245-3 and are worthy candidates for biological testing against Plk1-PBD.

3.8 FURTHER SYNTHESIS OF ANALOGUES FOR BIOLOGICAL TESTING AGAINST PLK1-PBD

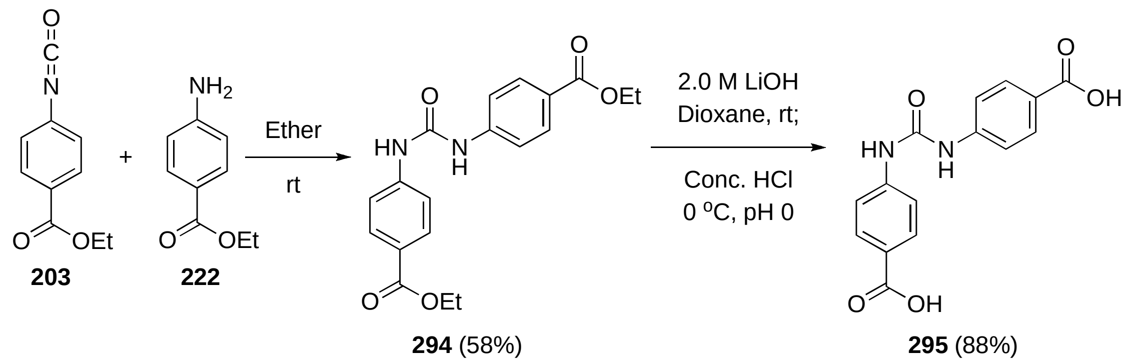
The different compound structures revealed through the synthesis and decomposition studies of **SID 861574** inspired the generation of an additional small set of analogues for biological testing against Plk1-PBD. Through these syntheses, several analogues were constructed in which either the ethyl 4-aminobenzoate or 4-aminobenzoic acid moieties were symmetrically linked over a diarylurea, diarylmalonamide or tricarbonyl framework as shown in Schemes 48 - 50. Pending further biological testing, these analogues may complement the findings of the above studies and generate a set of SAR data for this class of compounds at the PBD of Plk1.



Scheme 48. Synthesis of tricarbonyl analogues **289** and **290**.



Scheme 49. Synthesis of diarylmalonamide analogues **292** and **293**.



Scheme 50. Synthesis of diaylurea analogues **294** and **295**.

3.9 SUMMARY

Through the utilization of a fluorescence polarization assay with recombinant Plk1-PBD to monitor for inhibition of Plk1 via PBD binding by small molecules, a HTS campaign was conducted by the PMLSC and 97,090 compounds were screened as potential Plk1-PBD inhibitors. A total of 11 hits were identified as Plk1 inhibitors with IC_{50} < 50 μM, and one (**SID 861574**, IC_{50} = 2.41 ± 0.71 μM) was chosen for further hit-to-probe development. Medicinal chemistry studies resulted in the synthesis of a library of 38 novel analogues based on the assigned structure of **SID 861574**, all of which where found to be biologically inactive against

Plk1-PBD. A resynthesized sample of the initially proposed structure of **SID 861574** was found to be spectroscopically nonequivalent with the originally tested material and inactive against Plk1-PBD. Guided by NMR, IR, MS and X-ray crystallography data, a series of synthetic strategies led to uniquely functionalized 6-hydroxy- and 6-aminouracil scaffolds, all of which were spectroscopically nonequivalent to the originally tested sample of **SID 861574**. However, due to the characteristic structural features of these compounds as observed through rigorous spectroscopic analysis, the structure of the original HTS hit **SID 861574** could be identified. Following an independent synthesis of this compound (**260**), biological testing again revealed a lack of activity against Plk1-PBD.

Consequently, a thorough investigation of both the synthesis and decomposition profiles of the original **SID 861574** sample was conducted. These studies resulted in: a) the identification and synthesis of an impurity (**266**) contained in the original biologically active sample, b) the synthesis of compounds **265** and **271** resulting from the degradation of this sample, and c) a new set of potentially active analogues revealed through the synthesis of the strongly supported decomposition product **265** of **SID 861574**, which was found to be transiently active against Plk1-PBD. Testing of two different batches indicated that a separate agent formed during the synthesis of this compound was most likely responsible for the positive assay result. This work culminated in the synthesis of 56 compounds for biological testing against Plk1-PBD. It is hoped that a potent inhibitor of Plk1-PBD may be realized on the backdrop of several closely related analogues. SAR data for the binding of these compounds to Plk1-PBD could afford a scaffold for further hit-to-probe development.

4.0 EXPERIMENTAL PART

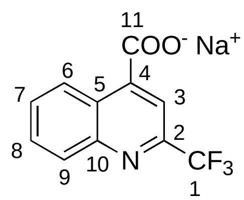
4.1 GENERAL EXPERIMENTAL

All moisture-sensitive reactions were performed under an atmosphere of nitrogen gas and all glassware was either dried in an oven at 140 ^{o}C or flame dried under high vacuum prior to use. THF and Et_2O were dried by distillation from Na/benzophenone ketyl, and CH_2Cl_2 and toluene were purified using an alumina column filtration system. Reactions were monitored by either ^{1}H NMR at 300 MHz in DMSO-d_6 or by TLC analysis (EM Science pre-coated silica gel 60 F254 plates, 250 m (layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a p-anisaldehyde solution (2.5 mL of p-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H_2SO_4 in 100 mL of 95% EtOH) and a $KMnO_4$ solution (1.5 g of $KMnO_4$ and 1.5 g of K_2CO_3 in 100 mL of a 0.1% NaOH solution). Flash chromatography on SiO_2 was used to purify the crude reaction mixtures. Melting points were determined using a Laboratory Devices Mel-Temp II. Infrared spectra were determined on a Smiths Detection IdentifyIR FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 300 instrument in $CDCl_3$ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard. ^{1}H NMR spectra were recorded at 300 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), number of protons, and coupling constant(s). ^{13}C NMR spectra were recorded at 76 MHz using a proton-decoupled pulse sequence with a d_1 of 3 sec, and are tabulated by observed peak. Mass spectra were obtained on a Micromass Autospec double focusing instrument.

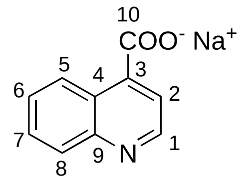
4.2 LIBRARY SYNTHESIS

4.2.1 Synthesis of 2,8-substituted-quinolinecarboxylic acid sodium salts

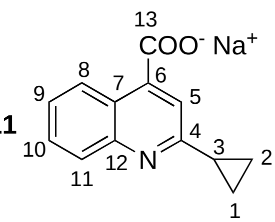
10



General procedure A. Sodium 2-(trifluoromethyl)quinoline-4-carboxylate (10). A suspension of 2-(trifluoromethyl)quinoline-4-carboxylic acid **21** (224 mg, 0.927 mmol) in an aqueous NaOH solution (0.371 mL, 0.926 mmol, 10%) was heated at 40-50 ^{o}C. After 1 h, the solution turned homogenous and the water was removed *in vacuo*. The resulting product **10** (242 mg, 99%) was isolated as a white solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 9.03 (d, 1 H, *J* = 8.5 Hz, H-6), 8.15 (s, 1 H, H-3), 8.05 (d, 1 H, *J* = 8.4 Hz, H-9), 7.73 (dt, 1 H, *J* = 8.4, 1.3 Hz, H-8), 7.44 (t, 1 H, *J* = 8.3 Hz, H-7).

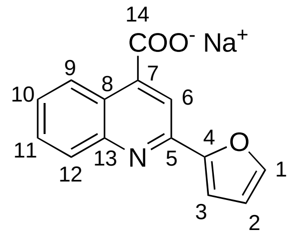
9 

Sodium quinoline-4-carboxylate (9). According to general procedure A, **20** (50.0 mg, 0.289 mmol) and aqueous NaOH (0.563 mL, 0.289 mmol, 0.513 M) were stirred at room temperature for 30 min. The resulting product **9** (56.4 mg, 100%) was isolated as a white solid: ^{1}H-NMR (methanol-d_4, 300 MHz) δ 8.85 (br, 1 H, H-1), 8.47 (br, 1 H, H-5), 8.04 (br, 1 H, H-8), 7.76 (br, 1 H, H-7), 7.61 (br, 2 H, H-6, H-2).

11 

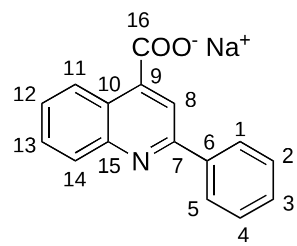
Sodium 2-cyclopropylquinoline-4-carboxylate (11). According to general procedure A, **22** (400 mg, 1.88 mmol) and aqueous NaOH (0.750 mL, 1.88 mmol, 10%) were heated at 40-50 ^{o}C for 1 h. The product **11** (440 mg, 100%) was isolated as a white solid: ^{1}H-NMR (methanol-d_4 300 MHz) δ 8.18 (dd, 1 H, *J* = 8.2, 0.8 Hz, H-8), 7.79 (d, 1 H, *J* = 8.4 Hz, H-11), 7.53 (dt, 1 H, *J* = 8.1, 1.4 Hz, H-10), 7.35 (dt, 1 H, *J* = 7.5, 1.2 Hz, H-9), 7.10 (s, 1 H, H-5), 2.21–2.12 (m, 1 H, H-3), 1.05–0.99 (m, 4 H, H-1, H-2).

12

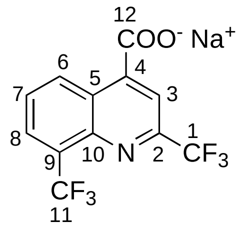


Sodium 2-(furan-2-yl)quinoline-4-carboxylate (12). According to general procedure A, **23** (400 mg, 1.67 mmol) and aqueous NaOH (0.699 mL, 1.67 mmol, 10%) were heated at 40–50 ^{o}C for 1 h. The product **12** (435 mg, 100%) was isolated as a light brown solid: ^{1}H-NMR (methanol-d_4, 300 MHz) δ 8.35 (d, 1 H, *J* = 8.3 Hz, H-9), 8.04 (d, 1 H, *J* = 8.3 Hz, H-12), 7.98 (s, 1 H, H-6), 7.76–7.69 (m, 2 H, H-11, H-1), 7.54 (t, 1 H, *J* = 7.5 Hz, H-10), 7.33 (d, 1 H, *J* = 3.4 Hz, H-3), 6.66 (dd, 1 H, *J* = 3.3, 1.7 Hz, H-2).

13



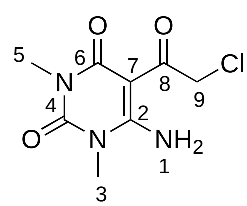
Sodium 2-phenylquinoline-4-carboxylate (13).[75] According to general procedure A, **24** (500 mg, 2.01 mmol) and aqueous NaOH (1.60 mL, 2.01 mmol, 5.0%) were combined over a 1 h period, affording **13** (540 mg, 99%) as a light yellow solid: ^{1}H-NMR (methanol-d_4, 300 MHz) δ 8.91 (d, 1 H, *J* = 8.1 Hz, H-11), 8.46 (s, 1 H, H-8), 8.15–8.13 (m, 2 H, H-1, H-5), 8.00 (d, 1 H, *J* = 8.3 Hz, H-14), 7.56 (t, 1 H, *J* = 7.0 Hz, H-13), 7.27–7.21 (m, 4 H, H-2, H-3, H-4, H-12).



14

Sodium 2,8-bis(trifluoromethyl)quinoline-4-carboxylate (14). According to general procedure A, **25** (279 mg, 0.901 mmol) and aqueous NaOH (0.360 mL, 0.91 mmol, 10%) were heated at 40-50 ^{o}C for 1 h. The product **14** (210 mg, 97%) was isolated as a white solid**:** ^{1}H-NMR (acetone-d_6, 300 MHz) δ 9.28 (d, 1 H, *J* = 8.6 Hz, H-8), 8.26 (s, 1 H, H-3), 8.05 (d, 1 H, *J* = 7.1 Hz, H-6), 7.40 (t, 1 H, *J* = 7.7 Hz, H-7).

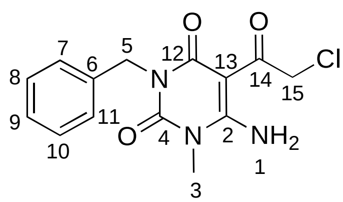
4.2.2 Synthesis of 1-*N*-alkyl-3-*N*-alkyl-5-chloroacetyl-6-aminouracils



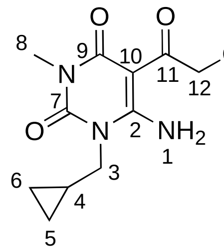
15

General procedure B. 6-Amino-5-(2-chloroacetyl)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (15, DMA-P77).[11] To a mixture of **36** (1.00 g, 6.45 mmol), chloroacetic acid (1.00 g, 10.6 mmol) and pyridine (0.512 mL, 6.46 mmol) was added 2-chloroacetyl chloride **41** (0.513 mL, 6.45 mmol) at room temperature. The reaction mixture was heated to 95 ^{o}C for 1 h under a nitrogen atmosphere. After 1 h, the mixture was cooled to 50 ^{o}C and deionized water (20.0 mL) was slowly added, with vigorous stirring, while cooling the mixture to 0 ^{o}C for 1 h. A white precipitate formed which was isolated by vacuum filtration, washed with additional H_2O (15.0 mL) and recrystallized from a 3:2 solution of EtOAc and hexanes. The resulting product **15** (789 mg, 53%) was isolated as a yellow crystalline solid: Mp 189–190 ^{o}C; IR (KBr) 3339, 3154, 1718, 1659, 1608, 1529, 1369, 1150, 781, 651 cm^{-1}; ^{1}H-NMR (DMSO-d_6 300 MHz) δ 10.79 (br, 1 H, H-1), 8.44 (br, 1 H, H-1), 4.91 (s, 2 H, H-9), 3.31 (s, 3 H, H-5), 3.14 (s, 3 H, H-3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 189.0 (C-8), 161.2 (C-6), 158.1 (C-2), 149.4 (C-4), 89.3 (C-7), 51.5 (C-9), 29.7 (C-3), 27.6 (C-5); MS (EI) *m/z* 231 (M^+, 9), 196 (100), 182 (77), 166 (23), 137 (27), 121 (68), 57 (92); HRMS (EI) *m/z* calculated for $C_8H_{10}N_3O_3Cl$ 231.0411, found 231.0411.

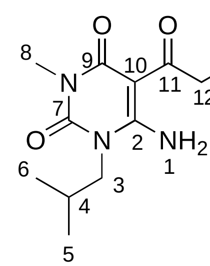
16



6-Amino-3-benzyl-5-(2-chloroacetyl)-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (16, DMA-P150). According to general procedure B, **37** (400 mg, 1.73 mmol) and 2-chloroacetyl chloride **41** (0.138 mL, 1.73 mmol) were combined to yield **16** (357 mg, 67%) as an off white crystalline solid: Mp 213.5–215 °C; IR (KBr) 3436, 3179, 3061, 1714, 1600, 1512, 1433, 1236, 1139, 778, 715, 649 cm^{-1}; ^{1}H-NMR (DMSO-d_6 300 MHz) δ 10.84 (br, 1 H, H-1), 8.52 (br, 1 H, H-1), 7.28–7.21 (m, 5 H, H-7, H-8, H-9, H-10, H-11), 4.97 (s, 2 H, H-5), 4.92 (s, 2 H, H-15), 3.32 (s, 3 H, H-3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 189.2 (C-14), 161.0 (C-12), 158.2 (C-2), 149.4 (C-4), 137.3 (C-6), 128.2 (C 2, C-8, C-10), 127.4 (2 C, C-7, C-11), 126.9 (C-9), 89.3 (C-13), 51.3 (C-15), 43.6 (C-5), 29.8 (C-3); MS (EI) *m/z* 307 (M^+, 13), 271 (100), 258 (43), 231 (58), 180 (17), 132 (26), 106 (26), 65 (42); HRMS (EI) *m/z* calculated for $C_{14}H_{14}N_3O_3Cl$ 307.0724, found 307.0718.

17 

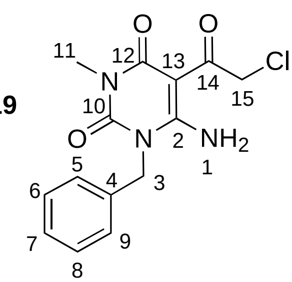
6-Amino-5-(2-chloroacetyl)-1-(cyclopropylmethyl)-3-methylpyrimidine-2,4 (1*H*,3*H*)-dione (17, DMA-P164). According to general procedure B, **38** (220 mg, 1.12 mmol) and 2-chloroacetyl chloride **41** (0.0895 mL, 1.12 mmol) were combined to yield **17** (145 mg, 47%) as a yellow crystalline solid: Mp 185–186 ^{o}C; IR (KBr) 3317, 3066, 3010, 1723, 1655, 1603, 1523, 1387, 1144, 1019, 827 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$ 300 MHz) δ 10.96 (br, 1 H, H-1), 8.54 (br, 1 H, H-1), 4.93 (s, 2 H, H-12), 3.88 (d, 2 H, *J* = 6.8 Hz, H-3), 3.15 (s, 3 H, H-8), 1.15 (m, 1 H, H-4), 0.47–0.39 (m, 4 H, H-5, H-6); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 189.4 (C-11), 161.1 (C-9), 157.4 (C-2), 149.6 (C-7), 89.2 (C-10), 51.3 (C-12), 45.8 (C-3), 27.6 (C-8), 9.2 (C-4), 3.5 (2 C, C-5, C-6); MS (EI) *m/z* 271 (M$^+$, 9), 236 (74), 206 (71), 168 (82), 139 (42), 80 (51); HRMS (EI) *m/z* calculated for $C_{11}H_{14}N_3O_3Cl$ 271.0724, found 271.0731.

18 

6-Amino-5-(2-chloroacetyl)-1-isobutyl-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (18, DMA-P92). According to general procedure B, **39** (250 mg, 1.27 mmol) and 2-chloroacetyl chloride **41** (0.101 mL, 1.27 mmol) were combined to yield **18** (122 mg, 35%) as a white crystalline solid: Mp 184–185 ^{o}C; IR (KBr) 3488, 3219, 3155, 2970, 1712, 1655, 1611, 1522, 1375, 1231, 773 cm^{-}

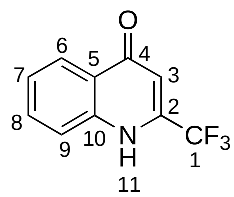
[1]; ^{1}H-NMR (DMSO-d_6 300 MHz) δ 11.00 (br, 1 H, H-1), 8.44 (br, 1 H, H-1), 4.92 (s, 2 H, C-12), 3.78 (d, 2 H, *J* = 7.8 Hz, H-3), 3.14 (s, 3 H, H-8), 2.04–1.97 (m, 1 H, H-4), 0.85 (d, 6 H, *J* = 6.6 Hz, H-5, H-6); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 189.3 (C-11), 161.2 (C-9), 157.6 (C-2), 149.6 (C-7), 89.2 (C-10), 51.5 (C-12), 48.0 (C-3), 27.6 (C-8), 26.0 (C-4), 19.3 (2 C, C-5, C-6); MS (EI) *m/z* 273 (M^+, 16), 238 (95), 224 (39), 182 (53), 168 (100); HRMS (EI) *m/z* calculated for $C_{11}H_{16}N_3O_3Cl$ 273.0880, found 273.0881.

19

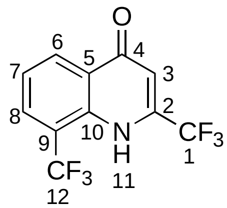


6-Amino-1-benzyl-5-(2-chloroacetyl)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (19, DMA-P143). According to general procedure B, **40** (365 mg, 1.58 mmol) and 2-chloroacetyl chloride **41** (0.126 mL, 1.58 mmol) were combined to yield **19** (350 mg, 72%) as a white crystalline solid: Mp 247–248.5 ^{o}C; IR (KBr) 3471, 3215, 3157, 1709, 1655, 1616, 1513, 1379, 1236, 772 cm^{-1}; ^{1}H-NMR (DMSO-d_6 300 MHz) δ 10.92 (br, 1 H, H-1), 8.44 (br, 1 H, H-1), 7.37–7.21 (m, 5 H, H-5, H-6, H-7, H-8, H-9), 5.21 (s, 2 H, H-3), 4.95 (s, 2 H, H-15), 3.18 (s, 3 H, H-11); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 189.4 (C-14), 161.2 (C-12), 157.6 (C-2), 149.6 (C-10), 135.0 (C-4), 128.5 (2 C, C-5, C-9), 127.3 (C-7), 126.2 (2 C, C-6, C-8), 89.4 (C-13), 51.4 (C-15), 44.8 (C-3), 27.7 (C-11); MS (EI) *m/z* 307 (M^+, 19), 272 (100), 258 (40), 139 (7); HRMS (EI) *m/z* calculated for $C_{14}H_{14}N_3O_3Cl$ 307.0724, found 307.0719.

4.2.3 Synthesis of the 2,8-substituted-4-quinoline carboxylic acids

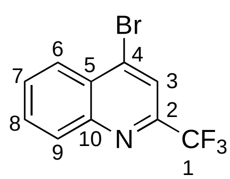
29 

General procedure C. 2-(Trifluoromethyl)quinoline-4(1*H*)-one (29).[9] To a vigorously stirred solution of aniline **26** (0.456 mL, 5.00 mmol) in polyphosphoric acid (6.30 g) was added 4,4,4-trifluoro-3-oxobutanoate **28** (0.731 mL, 5.00 mmol) over a 2 min period at 100 ^{o}C. The reaction mixture was heated to 150 ^{o}C for 2 h under a nitrogen atmosphere. After 2 h of heating, the reaction mixture was cooled to room temperature and neutralized with an aqueous NaOH solution (16.0 mL, 5.0 %). A precipitate formed which was isolated by vacuum filtration, washed with excess deionized water and re-dissolved in an aqueous NaOH solution (8.00 mL, 10 %) with gentle heating. The basic solution was filtered and then acidified to a pH of 4.5 with concentrated HCl. The white precipitate which formed was isolated by vacuum filtration, washed with deionized water and recrystallized from a 2:1 solution of ethanol and water. The resulting product **29** (515 mg, 46%) was isolated as a clear crystalline solid: ^{1}H-NMR (acetone-d_6 300 MHz) δ 11.19 (br, 1 H, H-11), 8.30 (dd, 1 H, *J* = 8.3, 1.0 Hz, H-6), 7.96 (d, 1 H, *J* = 8.4 Hz, H-9), 7.85 (td, 1 H, *J* = 6.9, 1.5 Hz, H-8), 7.62 (t, 1 H, *J* = 7.1 Hz, H-7), 7.04 (s, 1 H, H-3).



30

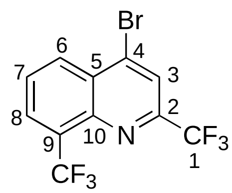
2,8-Bis(trifluoromethyl)quinolin-4(1*H*)-one (30, DMA-P104).[17] According to general procedure C, 2-(trifluoromethyl)aniline **27** (3.72 mL, 30.0 mmol) and 4,4,4-trifluoro-3-oxobutanoate **28** (4.38 mL, 30.0 mmol) were reacted over a 2 h period at 150 ^{o}C. An alternative workup was preformed in which the product was extracted from the aqueous acidified mixture (pH = 5) with ether (4 x 100 mL) and chromatographed on SiO_2 (1:1 EtOAc: hexanes). The product was recrystallized from hexanes to afford **30** (2.66 g, 32%) as a white crystalline solid: Mp 133-134 ^{o}C; ^{1}H-NMR (acetone-d_6 300 MHz) δ 11.75 (s, 1 H, H-11), 8.60 (d, 1 H, *J* = 8.4 Hz, H-8), 8.29 (d, 1 H, *J* = 7.5 Hz, H-6), 7.84 (t, 1 H, *J* = 7.8 Hz, H-7), 7.37 (s, 1 H, H-3); MS (EI) m/z *281* (M^{+}, 100), 261 (66), 233 (63), 214 (24), 183 (16), 75 (7).



31

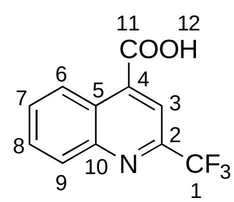
General Procedure D. 4-Bromo-2-(trifluoromethyl)quinoline (31).[17] To a melt of $POBr_3$ (1.35 g, 4.69 mmol) at 75 ^{o}C, under a nitrogen atmosphere, was added **29** (1.00 g, 4.69 mmol) over a 15 min period. After the addition, the reaction mixture was heated to 150 ^{o}C for 2 h and then cooled to room temperature. At room temperature, the reaction was quenched by the addition of deionized water (20.0 mL). A white precipitate formed which was extracted from the aqueous mixture with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with a saturated brine solution (50.0 mL), dried (Na_2SO_4), filtered through a plug of SiO_2 and concentrated by rotary evaporation. The resulting crude solid was recrystallized from ethanol and water to yield **31** (652 mg, 50%) as a slightly impure white solid: ^{1}H-NMR (chloroform-d, 300 MHz) δ 8.22 (m, 2 H, H-6, H-9), 8.01 (s, 1 H, H-3), 7.89-7.73 (m, 2 H, H-7, H-8).

32



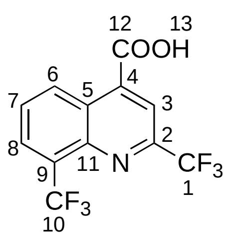
4-Bromo-2,8-bis(trifluoromethyl)quinoline (32, DMA-P108).[17] According to general procedure D, **30** (2.01 g, 7.13 mmol) and $POBr_3$ (2.05 g, 7.13 mmol) were combined to yield **32** (2.15 g, 88%) as a white crystalline solid: Mp 60-62 ^{o}C; ^{1}H-NMR (chloroform-d, 300 MHz) δ 8.48 (d, 1 H, *J* = 8.4 Hz, H-6), 8.23 (d, 1 H, *J* = 7.3 Hz, H-8), 8.12 (s, 1 H, H-3), 7.83 (t, 1 H, *J* = 7.9 Hz, H-7); MS (EI) *m/z* 343 (M^+, 97), 345 (100), 267 (29), 169 (18), 84 (94).

21



General procedure E. 2-(Trifluoromethyl)quinoline-4-carboxylic acid (21, DMA-P114). [17] To a solution of *n*-butyl lithium in hexanes (0.825 mL, 1.11 mmol, 1.34 M) in THF (2.00 mL) was added, over a 15 min period at -78 ^{o}C, a solution of 4-bromo-2-(trifluoromethyl)quinoline **31** (305 mg, 1.11 mmol) in THF (0.50 mL). The reaction mixture was stirred under a nitrogen atmosphere for 2 h and then slowly poured into an Erlenmeyer flask containing an excess of powdered dry ice. This mixture was stirred for 15 min while warming to room temperature and was then diluted with deionized water (28.0 mL). The resulting aqueous solution was washed with ether (3 x 10.0 mL), acidified to a pH of 3-4 with an HCl solution (1.18 M) and extracted with EtOAc (3 x 30.0 mL). The EtOAc extracts were combined and concentrated by rotary evaporation. The resulting crude solid was recrystallized from EtOAc and hexanes to afford **21** (142 mg, 53%) as a white crystalline solid: Mp 195.5–197 ^{o}C; IR (KBr) 3113, 2946, 1728, 1358, 1256, 1199, 1103, 901, 767, 663 cm^{-1}; ^{1}H-NMR (acetone-d$_6$ 300 MHz) δ 8.96 (d, 1 H, J = 8.6 Hz, H-6), 8.36 (s, 1 H, H-3), 8.28 (d, 1 H, J = 8.4 Hz, H-9), 8.02 (t, 1 H, J = 8.3 Hz, H-8), 7.95–7.89 (m, 1 H, H-7); ^{13}C-NMR (acetone-d$_6$, 75 MHz) δ 166.6 (C-11), 149.0 (C-8), 147.9 (q, J_{CF} = 34.6 Hz, C-2), 138.8 (C-6), 132.0, 131.1 (2 C), 126.8, 126.7, 122.4 (q, J_{CF} = 272.8 Hz, C-1), 118.8 (C-3); MS (EI) *m/z* 241 (M^+, 100), 223 (12), 196 (20), 185 (9), 176 (7), 128 (5); HRMS (EI) *m/z* calculated for $C_{11}H_6NO_2F_3$ 241.0351, found 241.0349.

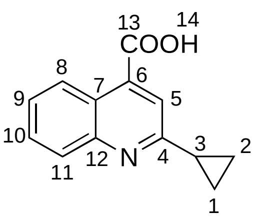
25



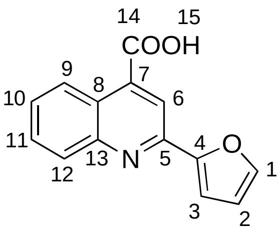
2,8-Bis(trifluoromethyl)quinoline-4-carboxylic acid (25, DMA-P115).[18] According to general procedure E, **32** (300 mg, 0.872 mmol), *n*-butyl lithium (0.65 mL, 0.87 mmol, 1.34 M) and CO_2 (excess dry ice) were converted to **25** (144 mg, 53%). The product was obtained as a white crystalline solid: Mp 226–228 ^{o}C; IR (KBr) 3027, 1710, 1519, 1430, 1361, 1311, 1148, 1036, 781, 687 cm^{-1}; ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 12.32 (s, 1 H), 9.12 (d, 1 H, J = 8.7 Hz), 8.36 (s, 1 H), 8.28 (d, 1 H, J = 7.1 Hz), 7.91 (t, 1 H, J = 7.9 Hz); ^{13}C-NMR (acetone-d$_6$, 75 MHz) δ 166.1, 148.5 (q, J_{CF} = 35.5 Hz), 145.0, 139.3, 131.4, 130.6 (q, J_{CF} = 5.6 Hz), 129.9, 129.0 (q, J_{CF} = 30.0 Hz), 127.5, 124.6 (q, J_{CF} = 271.4 Hz), 122.0 (q, J_{CF} = 273.1 Hz), 119.1; MS (EI) *m/z* 309

(M^+, 100), 290 (21), 264 (28), 240 (17), 214 (17), 176 (30); HRMS (EI) *m/z* calculated for $C_{12}H_5NO_2F_6$ 309.0224, found 309.0216.

22



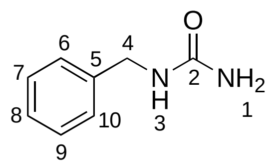
2-Cyclopropylquinoline-4-carboxylic acid (22, DMA-P121).[19] A solution of indoline-2,3-dione **33** (1.00 g, 6.80 mmol) in aqueous KOH (4.00 mL, 35.0 mmol, 8.75 M) was stirred for 10 min at room temperature. The solution changed color from black to transparent yellow. To this mixture was added 1-cyclopropylethanone **34** (1.01 mL, 10.2 mmol) and ethanol (2.5 mL). The reaction mixture was heated at reflux for 4.5 h, cooled to 0 ^oC and acidified to pH of 5-6 with glacial acetic acid. A precipitate developed which was isolated by vacuum filtration and was recrystallized from a 10:1 solution of EtOAc and hexanes to afford **22** (1.13 g, 78%) as a white crystalline solid: Mp 214–214.8 ^oC; IR (KBr) 3448, 3095, 3007, 1667, 1616, 1405, 1338, 882, 772 cm^{-1}; ^1H-NMR (acetone-d_6, 300 MHz) δ 8.74 (dd, 1 H, *J* = 8.6, 0.8 Hz, H-11), 7.97–7.94 (m, 2 H, H-5, H-8), 7.74 (dt, 1 H, *J* = 7.6, 1.4 Hz, H-10), 7.57 (dt, 1 H, *J* = 7.7, 1.4 Hz, H-9), 2.43–2.34 (m, 1 H, H-3), 1.23–1.07 (m, 4 H, H-1, H-2); ^{13}C-NMR (acetone-d_6, 75 MHz) δ 169.7 (C-13), 164.9 (C-4), 147.6 (C-12), 142.3 (C-6), 131.9 (C-10), 128.2 (C-11), 127.4 (C-9), 127.3 (C-5), 124.9 (C-8), 120.1 (C-7), 18.1 (C-15), 11.6 (C 2, C-1, C-2); MS (EI) *m/z* 213 (M^+, 60), 212 (100), 187 (14), 167 (63), 128 (12); HRMS (EI) *m/z* calculated for $C_{13}H_{11}NO_2$ 213.0790, found 213.0784.



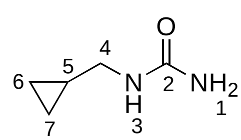
23

2-(Furan-2-yl)quinoline-4-carboxylic acid (23, DMA-P117).[20] A solution of indoline-2,3-dione **33** (1.00 g, 6.80 mmol) in aqueous KOH (4.00 mL, 35.0 mmol, 8.75 M) was stirred for 10 min at room temperature. The solution turned from black to transparent yellow. To this mixture was added 1-(furan-2-yl)ethanone **35** (1.02 mL, 10.2 mmol). The reaction mixture was heated at reflux for 4.5 h, cooled to room temperature and acidified to pH of 3-4 with concentrated hydrochloric acid (36 %). A precipitate developed which was isolated by vacuum filtration, dissolved in a solution of 10:1 EtOAc:MeOH and filtered through a plug of silica gel. The solvents were then removed by rotary evaporation and the resulting orange solid was recrystallized three times from a 3:1 solution of ethanol and water to afford **23** (548 mg, 34 %) as a brown solid: Mp 234–237 ^{o}C (dec.); IR (KBr) 3435, 3141, 1714, 1600, 1372, 1232, 1018, 761 cm^{-1}; ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 8.82 (dd, 1 H, J = 8.5, 0.8 Hz, C-12), 8.44 (s, 1 H, C-6), 8.11 (dd, 1 H, *J* = 8.5, 0.6 Hz, C-9), 7.85–7.64 (m, 2 H, H-1, H-11), 7.66 (dt, 1 H, *J* = 7.8, 1.3 Hz, H-10), 7.40 (dd, 1 H, *J* = 3.4, 0.7 Hz, H-3), 6.72 (dd, 1H, *J* = 3.4, 1.6 Hz, H-2); ^{13}C-NMR (acetone-d$_6$, 75 MHz) δ 167.4 (C-14), 154.2 (C-4), 150.0 (C-5), 149.5 (C-13), 145.7 (C-1), 137.0 (C-7), 131.0 (C-11), 130.5 (C-12), 128.4 (C-10), 126.6 (C-9), 124.8 (C-6), 119.3 (C-8), 113.4 (C-3), 111.3 (C-2); MS (EI) *m/z* 239 (M$^+$, 100), 211 (16), 166 (25), 140 (20), 110 (26); HRMS (EI) *m/z* calculated for $C_{14}H_9NO_3$ 239.0582, found 239.0590.

4.2.4 Synthesis of the 1-*N*-alkyl-3-*N*-alkyl-6-aminouracils

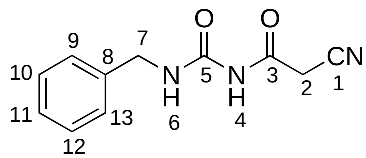
44 

1-Benzylurea (44, DMA-P105).[21] Neat phenylmethanamine **42** (5.00 g, 46.7 mmol) was added to an aqueous HCl solution (39.4 mL, 46.5 mmol, 1.18 M) at room temperature. This solution was stirred for 5 min, diluted with deionized water (100 mL) and then mixed with an aqueous solution of potassium cyanate (40.0 mL, 70.0 mmol, 1.75 M). The reaction mixture was stirred for 30 h and the resulting precipitate was removed by vacuum filtration. The isolated solid was then recrystallized from a 1:3 solution of ethanol and water affording **44** (4.04 g, 58%) as large colorless crystals: Mp 151-152 ^{o}C; ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 7.49–7.17 (m, 5 H, H-6, H-7, H-8, H-9, H-10), 6.07 (br, 1 H, H-3), 5.22 (br, 2 H, H-1), 4.27 (d, 2 H, *J* = 12.0 Hz, H-4).

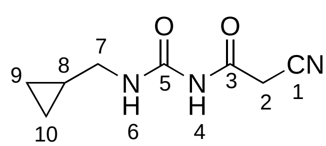
45 

1-(Cyclopropylmethyl)urea (45, DMA-P126).[22] To a solution of cyclopropylmethanamine hydrochloride **43** (3.00 g, 27.9 mmol) in deionized water (60.0 mL) was added an aqueous solution of potassium cyanate (20.0 mL, 41.8 mmol, 2.10 M) at room temperature. The reaction mixture was stirred for 44 h and then extracted with EtOAc (11 x 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting solid was recrystallized from a 9:1 solution of EtOAc and hexanes affording **45** (2.31 g, 76%) as colorless crystals: Mp 121-122 ^{o}C; ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 5.84 (br, 1 H, H-3), 5.25

(br, 2 H, H-1), 2.97 (t, 2 H, J = 6.0 Hz, H-4), 0.87–0.96 (m, 1 H, H-5), 0.12–0.42 (m, 4 H, H-6, H-7).

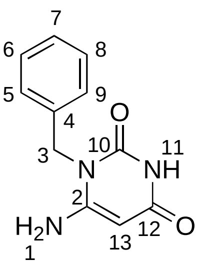
47 

***N*-(Benzylcarbamoyl)-2-cyanoacetamide (47).**[23] A solution of 1-benzylurea **44** (3.84 g, 25.6 mmol) and 2-cyanoacetic acid **46** (2.40 g, 28.2 mmol) in acetic anhydride (30.0 mL) was heated to 77 °C for 1.25 h. After 1.25 h, the reaction mixture was cooled to room temperature and a pale yellow precipitate formed. The reaction mixture was then diluted with ether (50.0 mL) and cooled to 0 °C for 30 min. The resulting precipitate was isolated by vacuum filtration, washed with ether (30.0 mL) and dried in vacuo. The crude product **47** (4.18 g, 75%) was used for the next step without further purification: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 9.83 (br, 1 H, H-4), 8.50 (br, 1 H, H-6), 7.26-7.37 (m, 5 H, H-9, H-10, H-11, H-12, H-13), 4.48 (d, 2 H, J = 12.0 Hz, H-7), 3.93 (s, 2 H, H-2).

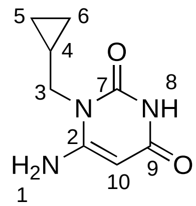
48 

2-Cyano-*N*-(cyclopropylmethylcarbamoyl)acetamide (48, DMA-P130).[22] A solution of 1-(cyclopropylmethyl)urea **45** (2.28 g, 20.0 mmol) and 2-cyanoacetic acid **46** (1.87 g, 22.0 mmol) in acetic anhydride (23.0 mL) was heated to 77 °C for 1 h. The reaction mixture was then cooled to room temperature and the solvent was removed by rotary evaporation. The resulting crude residue was recrystallized twice from a 5:1 solution of EtOAc and hexanes affording **48** (2.02 g, 56%) as a light orange crystalline solid: Mp 162-163 ^{o}C; ^{1}H-NMR (acetone-d_6, 300 MHz) δ 9.62 (br, 1 H, H-4), 8.12 (br, 1 H, H-6), 3.93 (s, 2H, H-2), 3.11–3.16 (m, 2 H, H-7), 0.97-1.10 (m, 1 H, H-8), 0.21-0.50 (m, 4 H, H-9, H-10); MS (EI) *m/z* 181 (M^+, 8), 166 (9), 153 (58), 97 (67), 85 (100), 70 (99), 54 (79).

49

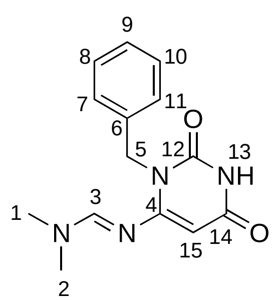


6-Amino-1-benzylpyrimidine-2,4(1*H*,3*H*)-dione (49, DMA-P111).[13] A suspension of **47** (4.15 g, 19.1 mmol) in deionized water (25.0 mL) and ethanol (2.50 mL) was heated to 85–90 ^{o}C. To this mixture was added a NaOH solution (4.89 mL, 10%) and the reaction mixture was heated for 1 h. A white precipitate developed. After 1 h of heating, the reaction mixture was cooled to room temperature and acidified to pH 7 with an HCl solution (1.18 M). The neutralized reaction mixture was then diluted with deionized water (30.0 mL) and cooled to 0 ^{o}C. The resulting white precipitate was isolated by vacuum filtration, washed with deionized water (30.0 mL) and recrystallized from a 1:10 solution of methanol and EtOAc. The resulting product **49** (2.08 g, 50%) was obtained as a white crystalline solid: Mp 283-284 ^{o}C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.50 (br, 1 H, H-11), 7.17–7.36 (m, 5 H, H-5, H-6, H-7, H-8, H-9), 6.78 (br, 2 H, H-1), 5.02 (s, 2 H, H-3), 4.60 (s, 1 H, H-13); MS (EI) *m/z* 217 (M^+, 25), 91 (100), 78 (55), 63 (75).



50

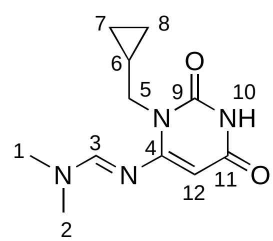
6-Amino-1-(cyclopropylmethyl)pyrimidine-2,4(1*H*,3*H*)-dione (50, DMA-P132).[24] A suspension of **48** (1.99 g, 11.0 mmol) in deionized water (20.0 mL) and ethanol (1.50 mL) was heated to 85–90 °C. To this mixture was added a NaOH solution (2.80 mL, 10%). The reaction mixture was heated for 1 h, cooled to room temperature and acidified to pH 7 with an HCl solution (1.18 M). The neutralized reaction mixture was then cooled to 0 °C and a light orange crystalline precipitate formed which was isolated by vacuum filtration and dried *in vacuo*. The resulting product **50** (0.71 g, 36%) was obtained as a light orange crystalline solid: Mp 265-266 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.29 (br, 1 H, H-8), 6.77 (br, 2 H, H-1), 4.53 (s, 1 H, H-10), 3.66 (d, 2 H, *J* = 6.6 Hz, H-3), 1.07–1.15 (m, 1 H, H-4), 0.33–0.45 (m, 4 H, H-5, H-6); MS (EI) *m/z* 181 (M^+, 43), 152 (14), 127 (87), 68 (31), 55 (100).



52

General procedure F. (*E*)-*N'*-(3-Benzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin -4-yl)-*N*,*N*-dimethylformimidamide (52, DMA-P113).[14] To a suspension of **49** (1.50 g, 6.91 mmol) in DMF (34.0 mL) was added DMF-DMA (3.72 mL, 27.7 mmol). The reaction mixture was heated to 40 °C for 24 h under a nitrogen atmosphere. After 24 h, the solvent was removed by rotary evaporation and the crude residue was dissolved in a minimum amount of hot DMSO. The product was crystallized by slowly adding a 2:1 mixture of hexanes to EtOAc, followed by cooling at 0 °C for 5 h. The resulting precipitate was isolated by vacuum filtration and dried under high vacuum affording **52** (1.42 g, 76%) as a white crystalline solid: Mp 220–221 °C; IR (KBr) 3489, 3126, 2980, 2846, 1651, 1609, 1552, 1347, 1226, 1127 cm^{-1}; ^{1}H- NMR (DMSO-d_6 300 MHz) δ 10.70 (br, 1 H, H-13), 8.01 (s, 1 H, H-3), 7.27–7.16 (m, 5 H, H-7, H-8, H-9, H-10, H-11), 5.04 (s, 2 H, H-5), 4.99 (s, 1 H, H-15), 3.02 (s, 3 H, H-2), 2.88 (s, 3 H, H-1); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 163.2 (C-14), 160.3 (C-3), 156.0 (C-4), 151.8 (C-12), 138.5 (C-6), 128.2 (2 C, C-8, C-10), 127.1 (2 C, C-7, C-11), 126.8 (C-9), 82.2 (C-15), 44.2 (C-5), 40.3 (C-2), 34.4 (C-1); MS (EI) *m/z* 272 (M^+, 24), 256 (6), 192 (15), 108 (100), 91 (54), 69 (64); HRMS (EI) *m/z* calculated for $C_{14}H_{16}N_4O_2$ 272.1273, found 272.1274.

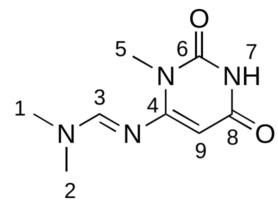
53



(*E*)-*N'*-(3-(Cyclopropylmethyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-*N*,*N*-dimethylformimidamide (53, DMA-P139). According to general procedure F, **50** (673 mg, 3.71 mmol) and DMF-DMA (19.7 mL, 14.9 mmol) were heated in DMF (18.0 mL) at 40 °C for 14 h. The resulting product **53** (819 mg, 93%) was isolated as an orange crystalline solid: Mp 248.7–250 °C; IR (KBr) 3416, 3325, 3182, 3035, 2812, 1634, 1584, 1491, 1384, 1285, 1115, 1021, 835, 803 cm^{-1}; ^{1}H-NMR (methanol-d_4, 300 MHz) δ 7.89 (s, 1 H, H-3), 4.97 (s, 1 H, H-12), 3.81 (d, 2 H, J = 7.1 Hz, H-5), 3.09 (s, 3 H-2), 2.99 (s, 3 H, H-1), 1.24–1.12 (m, 1 H, H-6), 0.40–0.26 (m, 4 H, H-7, H-8); ^{13}C-NMR (methanol-d_4, 75 MHz) δ 167.2 (C-11), 164.0 (C-3), 157.3

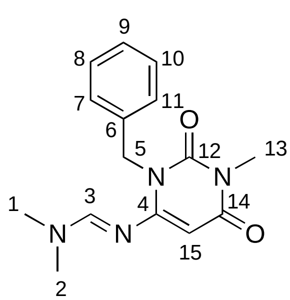
(C-4), 154.0 (C-9), 83.5 (C-12), 47.6 (C-5), 41.2 (C-1), 35.4 (C-2), 11.5 (C-6), 4.2 (2 C, C-7, C-8); MS (EI) *m/z* 236 (M^+, 100), 207 (17), 181 (24), 152 (28), 138 (16), 123 (23), 99 (46), 84 (42), 73 (56), 55 (81); HRMS (EI) *m/z* calculated for $C_{11}H_{16}N_4O_2$ 236.1273, found 236.1274.

54



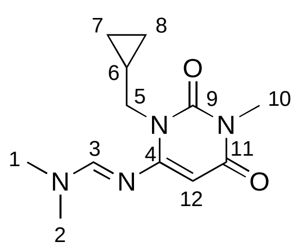
(*E*)-*N*,*N*-Dimethyl-*N′*-(3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl) formimidamide (54, DMA-P128).[14] According to general procedure F, **51** (1.50 g, 10.6 mmol) and DMF-DMA (5.70 mL, 42.4 mmol) were heated in DMF (45.0 mL) at 40 °C for 8 h. The resulting product **54** (1.37 g, 65%) was isolated as a white crystalline solid: Mp 254-256 °C; IR (KBr) 3155, 3024, 1689, 1655, 1618, 1557, 1359, 1227, 1109, 996 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.61 (s, 1 H, H-7), 8.02 (s, 1 H, H-3), 4.96 (s, 1 H, H-9), 3.33 (s, 3 H, H-5), 3.13 (s, 3 H, H-2), 2.98 (s, 3 H, H-1); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 163.1 (C-8), 160.8 (C-3), 155.8 (C-4), 151.8 (C-6), 82.2 (C-9), 40.3 (C-5), 34.4 (C-2), 28.6 (C-3); MS (EI) *m/z* 196 (M^+, 100), 152 (5), 99 (21), 82 (43), 55 (26).

55



General Procedure G. (*E*)-*N′*-(3-Benzyl-1-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-*N*,*N*-dimethylformimidamide (55, DMA-P129). A suspension of **52** (797 mg, 2.93 mmol) in acetonitrile (13.0 mL) and DMF (2.00 mL) was reacted with DBU (0.882 mL, 5.85 mmol) at 50 °C for 1 h under a nitrogen atmosphere. After 1 h, the reaction mixture became homogenous and was cooled to room temperature. After addition of iodomethane (0.911 mL, 14.6 mmol), the reaction mixture was stirred at room temperature for 2 d and then glacial acetic acid (8 drops) was added. The solvents were removed by rotary evaporation and the crude yellow oil was added to a saturated $NaHCO_3$ solution (20.0 mL). The aqueous phase was extracted with EtOAc (4 x 50.0 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting residue was chromatographed on SiO_2 (20:1 EtOAc: methanol). The crude product was recrystallized from a 1:1 solution of EtOAc and hexanes and **55** (429.1 mg, 51%) was obtained as a white crystalline solid: Mp 163-164 °C; ^{1}H-NMR (methanol-d$_4$, 300 MHz) δ 7.85 (s, 1 H, H-3), 7.19–7.11 (m, 5 H, H-7, H-8, H-9, H-10, H-11), 5.18 (s, 2 H, H-5), 5.11 (s, 1 H, H-15), 3.19 (s, 3 H, H-13), 3.04 (s, 3 H, H-2), 2.94 (s, 3 H, H-1); MS (EI) *m/z* 286 (M^+, 98), 242 (37), 186 (36), 91 (100).

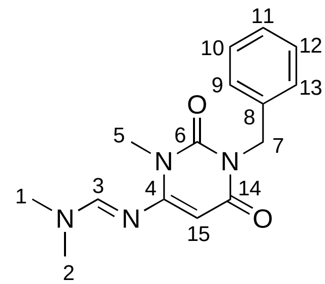
56



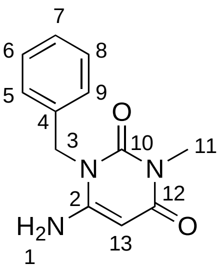
(*E*)-*N'*-(3-(Cyclopropylmethyl)-1-methyl-2,6-dioxo-1,2,3,6 tetrahydropyrimidin-4-yl)-*N*,*N*-dimethylformimidamide (56, DMA-P142). According to general procedure G, **53** (785 mg, 3.32 mmol), DBU (2.50 mL, 16.6 mmol) and iodomethane (2.06 mL, 33.2 mmol) were reacted for 3 d. The resulting product **56** (382 mg, 46%) was isolated as a colorless crystalline solid: Mp 176-177 °C; ^{1}H-NMR (methanol-d$_4$, 300 MHz) δ 7.88 (s, 1 H, H-3), 5.07 (s, 1 H, H-12), 3.86 (d, 2 H, *J* = 7.1 Hz, H-5), 3.18 (s, 3 H, H-10), 3.08 (s, 3 H, H-2), 3.02 (s, 3 H, H-1), 1.22–1.14

(m, 1 H, H-6), 0.40–0.27 (m, 4 H, H-7, H-8); MS (EI) *m/z* 250 (M^+, 100), 221 (14), 195 (19), 152 (23), 122 (31), 99 (36), 69 (49), 55 (59); HRMS (EI) *m/z* calculated for $C_{12}H_{18}N_4O_2$ 250.1430, found 250.1433.

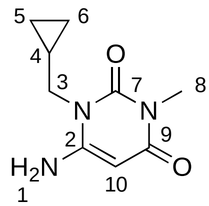
57



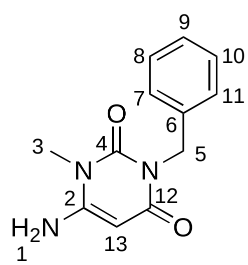
(*E*)-*N′*-(1-Benzyl-3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-*N*,*N*-dimethylformimidamide (57, DMA-P131).[25] According to general procedure G, **54** (1.10 g, 5.61 mmol), DBU (1.69 mL, 11.2 mmol) and (bromomethyl)benzene (1.33 mL, 11.2 mmol) were heated at 80 ^{o}C for 5 h. The resulting product **57** (192 mg, 15%) was isolated as a white solid: Mp 201–202 ^{o}C; IR (KBr) 3079, 2966, 1683, 1650, 1619, 1573, 1410, 1363, 1112, 760, 694 cm^{-1}; ^{1}H-NMR (DMSO-d_6 300 MHz) δ 8.08 (s, 1 H, H-3), 7.25 (s, 5 H, H-9, H-10, H-11, H-12, H-13), 5.18 (s, 1 H, H-15), 4.94 (s, 2 H, H-7), 3.29 (s, 3 H, H-5), 3.11 (s, 3 H, H-2), 3.00 (s, 3 H, H-1); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 161.9 (C-14), 159.4 (C-3), 156.0 (C-4), 151.9 (C-6), 138.0 (C-8), 128.1 (2 C, C-10, C-12), 127.4 (2 C, C-9, C-13), 126.8 (C-11), 81.7 (C-15), 43.1 (C-7), 34.4 (C-2), 29.6 (C-5) (one signal buried in DMSO-d_6, C-1)**;** MS (EI) *m/z* 286 (M^+, 100), 269 (6), 181 (9), 111 (13), 99 (22), 82 (32), 73 (24); HRMS (EI) *m/z* calculated for $C_{15}H_{18}N_4O_2$ 286.1430, found 286.1440.

40 

General procedure H. 6-Amino-1-benzyl-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (40, DMA-P136).[26] An aqueous ammonia solution was prepared by slowly bubbling ammonia gas through deionized water (250 mL) at 0 °C for 30 min. A portion of the ammonia solution (20.0 mL) was added to **55** (658 mg, 2.30 mmol) dissolved in methanol (20.0 mL). This mixture was stirred at room temperature in a stoppered flask. After 3 d, the solvents were removed by rotary evaporation and the resulting residue was chromatographed on SiO_2 (EtOAc, with a few drops of Et_3N). The product was recrystallized from a 1:1 solution of EtOAc and hexanes to afford **40** (417 mg, 77%) as a white crystalline solid: Mp 171–172 °C; IR (KBr) 3357, 3213, 1693, 1663, 1626, 1498, 1454, 1431, 1379, 1283, 1130, 783, 696 cm^{-1}; ^{1}H-NMR (methanol-d_4, 300 MHz) δ 7.25–7.11 (m, 5 H, H-5, H-6, H-7, H-8, H-9), 5.04 (br, 2 H, H-1), 4.87 (s, 1 H, H-13), 3.15 (s, 3 H, H-11); ^{13}C-NMR (methanol-d_4, 75 MHz) δ 165.6 (C-12), 157.1 (C-10), 153.7 (C-2), 137.1 (C-4), 129.9 (C 2, C-6, C-8), 128.8 (C-7), 127.5 (2 C, C-5, C-9), 77.1 (C-13), 46.8 (C-3), 28.3 (C-11); MS (EI) *m/z* 231 (M^+, 100), 173 (7), 145 (9), 111 (7), 106 (11); HRMS (EI) *m/z* calculated for $C_{12}H_{13}N_3O_2$ 231.1008, found 231.1005.

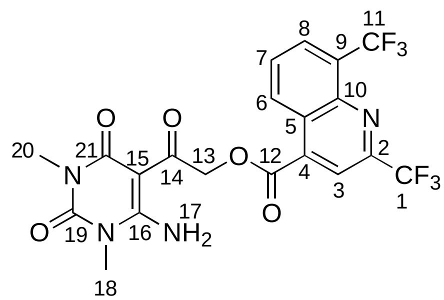
38 

6-Amino-1-(cyclopropylmethyl)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (38).[27] According to general procedure H, **56** (350 mg, 1.40 mmol) was reacted with an aqueous ammonia solution (20 mL) for 4 d. The crude product **38** (220 mg, 80%) was isolated as white solid and was used for the next step without further purification: ^{1}H-NMR (DMSO-d_6 300 MHz) δ 6.79 (br, 2 H, H-1), 4.68 (s, 1 H, H-10), 3.72 (d, 2 H, *J* = 7.1 Hz, H-3), 3.06 (s, 3 H, H-8), 1.16–1.11 (m, 1 H, H-4), 0.43–0.32 (m, 4 H, H-5, H-6).

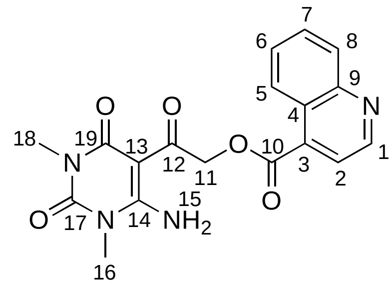
37 

6-Amino-3-benzyl-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (37, DMA-P137).[28] According to general procedure H, **57** (756 mg, 2.64 mmol) was reacted with an aqueous ammonia solution (20 mL) for 3 d. The concentrated crude reaction mixture was chromatographed on SiO_2 (9:1 CH_2Cl_2: methanol). The product **37** (446 mg, 73%) was isolated as a white solid: Mp 202-203 ^{o}C; ^{1}H-NMR (DMSO-d_6 300 MHz) δ 7.29–7.21 (m, 5 H, H-7, H-8, H-9, H-10, H-11), 6.87 (br, 2 H, H-1), 4.90 (s, 2 H, H-5), 4.73 (s, 1 H, H-13), 3.23 (s, 3 H, H-3); MS (EI) *m/z* 231 (M^+, 42), 220 (52), 205 (100), 145 (19), 99 (15).

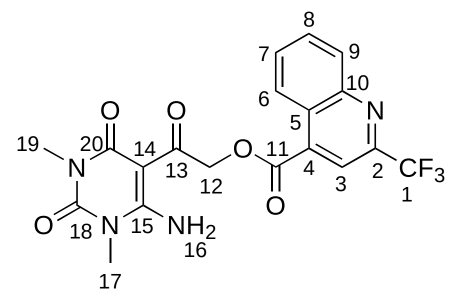
4.2.5 Synthesis of the final library analogues

63 

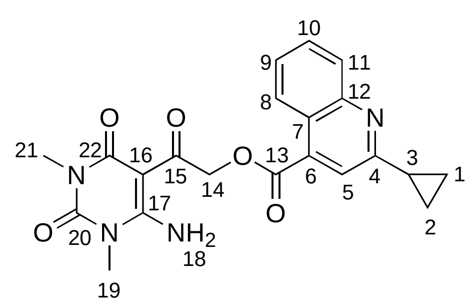
General procedure I. 2-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydro- pyrimidin-5-yl)-2-oxoethyl 2,8-bis(trifluoromethyl) quinoline-4-carboxylate (63, DMA-P146). A solution of **15** (40.5 mg, 0.175 mmol) and **14** (60.8 mg, 0.184 mmol) in DMF (5.00 mL) was heated to reflux under a nitrogen atmosphere for 1 h, then cooled to room temperature. The product was precipitated by the slow addition of deionized water (20 mL) over 1 h while the mixture was cooled to 0 ^{o}C. The resulting precipitate was isolated by vacuum filtration, washed with deionized water (40 mL), and dried in a Genevac for 17 h at 40 ^{o}C followed by 19 h at 50 ^{o}C to remove residual DMF. The product **63** (49.6 mg, 56%) was isolated as a white solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.04 (br, 1 H, H-17), 9.23 (d, 1 H, *J* = 8.6 Hz, H-8), 8.52 (s, 1 H, H-3), 8.45 (d, 1 H, *J* = 7.1 Hz, H-6), 8.07 (t, 1 H, *J* = 8.0 Hz, H-7), 7.70 (br, 1 H, H-17), 5.69 (s, 2 H, H-13), 3.54 (s, 3 H, H-20), 3.28 (s, 3 H, H-18).

58 

2-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl quinoline-4-carboxylate (58, DMA-P78). According to general procedure I, **15** (47.0 mg, 0.203 mmol) and **9** (44.0 mg, 0.225 mmol) were converted to **58** (55.0 mg, 74 %) as a tan solid: Mp 249–250 ^{o}C; IR (KBr) 3416, 3137, 1712, 1639, 1605, 1531, 1448, 1247, 1152, 775 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 10.73 (s, 1 H, H-15), 9.10 (d, 1 H, *J* = 4.4 Hz, H-1), 8.72 (d, 1 H, *J* = 8.7 Hz, H-8), 8.46 (br, 1 H, H-15), 8.15 (d, 1 H, *J* = 8.5 Hz, H-5), 8.02 (d, 1 H, *J* = 4.3 Hz, H-2), 7.87 (t, 1 H, *J* = 7.2 Hz, H-7), 7.75 (t, 1 H, *J* = 7.2 Hz, H-6), 5.55 (s, 2 H, H-11), 3.33 (s, 3 H, H-18), 3.19 (s, 3 H, H-16); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 189.8 (C-12), 165.6 (C-10), 161.4 (C-19), 158.0 (C-14), 150.3 (C-17), 149.5 (C-1), 148.3 (C-9), 135.1 (C-3), 129.9 (C-7), 129.6 (C-8), 128.1 (C-6), 125.4 (C-4), 124.0 (C-5), 122.0 (C-2), 88.9 (C-13), 69.9 (C-11), 29.6 (C-16), 27.4 (C-18); MS (EI) *m/z* 368 (M^{+}, 8), 212 (60), 182 (100), 157 (89), 129 (56), 101 (23), 57 (56); HRMS (EI) *m/z* calculated for $C_{18}H_{16}N_4O_5$ 368.1120, found 368.1126.

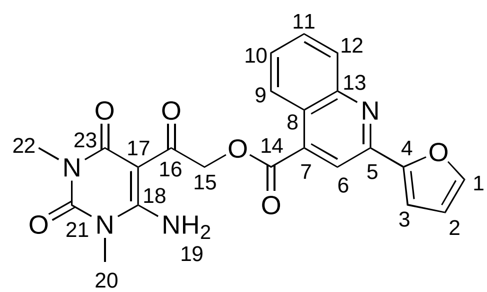
59 

2-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(trifluoromethyl)quinoline-4-carboxylate (59, DMA-P145). According to general procedure I, **15** (53.3 mg, 0.230 mmol) and **10** (66.6 mg, 0.253 mmol) were converted to **59** (45.6 mg, 45%) as a tan solid: ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 11.06 (br, 1 H, H-16), 8.96 (d, 1 H, *J* = 7.8 Hz, H-9), 8.39 (s, 1 H, H-3), 8.30 (d, 1 H, *J* = 8.2 Hz, H-6), 8.04 (t, 1 H, *J* = 7.3 Hz, H-8), 7.93 (t, 1 H, *J* = 7.1 Hz, H-7), 7.68 (br, 1 H, H-16), 5.67 (s, 2 H, H-12), 3.54 (s, 3 H, H-19), 3.28 (s, 3 H, H-17).

60 

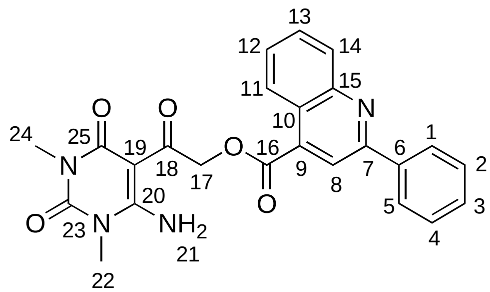
2-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-cyclopropylquinoline-4-carboxylate (60, DMA-P140). According to general procedure I, **15** (57.9 mg, 0.250 mmol) and **11** (64.7 mg, 0.275 mmol) were converted to **60** (59.8 mg, 59%) as a white crystalline solid: Mp 241–242 ^{o}C; IR (KBr) 3399, 3294, 3094, 3009, 1716, 1647, 1621, 1513, 1446, 1243, 1024, 696, 774 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 10.78 (br, 1 H, H-18), 8.67 (d, 1 H, *J* = 8.2 Hz, H-11), 8.48 (br, 1 H, H-18), 7.99–7.97 (m, 2 H, H-5, H-8), 7.79 (t, 1 H, *J* = 7.0 Hz, H-9), 7.63 (t, 1 H, *J* = 7.1 Hz, H-10), 5.57 (s, 2 H, H-14), 3.36 (s, 3 H, H-21), 3.22 (s, 3 H, H-19), 2.55–2.42 (m, 1 H, H-3), 1.17–1.12 (m, 4 H, H-1, H-2); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 189.8 (C-15), 165.8 (C-13), 162.8 (C-22), 161.4 (C-17), 158.0 (C-4), 149.5 (C-20), 148.2 (C-12), 135.1 (C-6), 129.8 (C-10), 128.9 (C-11), 126.6 (C-9), 125.3 (C-5), 122.6 (C-8), 121.4 (C-7), 88.9 (C-16), 69.8 (C-14), 29.6 (C-19), 27.5 (C-21), 17.3 (C-3), 10.8 (2 C, C-1, C-2); MS (EI) *m/z* 408 (M^+, 10), 212 (100), 195 (66), 167 (79), 139 (20), 101 (15); HRMS (EI) *m/z* calculated for $C_{21}H_{20}N_4O_5$ 408.1434, found 408.1445.

61

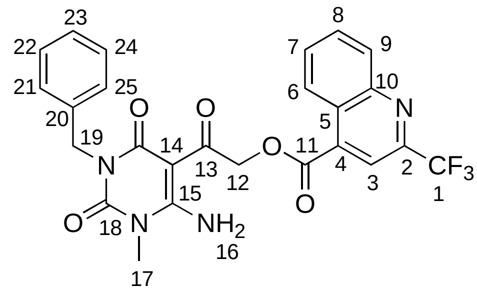


2-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(furan-2-yl)quinoline-4-carboxylate (61, DMA-P138). According to general procedure I, **15** (46.3 mg, 0.200 mmol) and **12** (57.5 mg, 0.220 mmol) were converted to **61** (61.5 mg, 70%) as a brown solid: Mp 272–273 °C; IR (KBr) 3366, 3100, 1710, 1619, 1530, 1459, 1383, 1238, 1169, 973, 780 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.73 (br, 1 H, H-19), 8.67 (d, 1 H, *J* = 8.3 Hz, H-12), 8.46 (br, 1 H, H-19), 8.38 (s, 1 H, H-6), 8.11 (d, 1 H, *J* = 8.3 Hz, H-9), 7.99 (br, 1 H, H-1), 7.86 (t, 1 H, *J* = 7.6 Hz, H-11), 7.70 (t, 1 H, *J* = 7.7 Hz, H-10), 7.45 (d, 1 H, *J* = 3.4 Hz, H-3), 6.76 (dd, 1 H, *J* = 3.4, 1.8 Hz, H-2), 5.58 (s, 2 H, H-15), 3.34 (s, 3 H, H-22), 3.20 (s, 3 H, H-20); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 189.8 (C-16), 165.4 (C-14), 161.4 (C-23), 158.0 (C-18), 152.3 (C-4), 149.5 (C-5), 148.3 (C-21), 148.0 (C-13), 145.5 (C-1), 136.3 (C-7), 130.6 (C-12), 129.3 (C-11), 127.7 (C-10), 125.5 (C-9), 123.0 (C-6), 118.0 (C-8), 112.8 (C-3), 111.2 (C-2), 88.9 (C-17), 70.0 (C-15), 29.6 (C-20), 27.5 (C-22); MS (EI) *m/z* 434 (M^+, 54), 365 (9), 239 (100), 212 (34), 195 (98), 182 (53), 166 (34), 139 (14), 101 (10); HRMS (EI) *m/z* calculated for $C_{22}H_{18}N_4O_6$ 434.1226, found 434.1221.

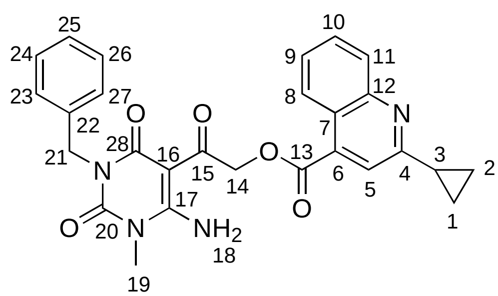
62



2-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-phenylquinoline-4-carboxylate (62, DMA-P100). According to general procedure I, **15** (52.0 mg, 0.225 mmol) and **13** (67.1 mg, 0.246 mmol) were converted to **62** (66.0 mg, 66%) as a tan solid: Mp 270–272 ^{o}C (dec); IR (KBr) 3381, 3097, 2951, 1713, 1619, 1529, 1460, 1234, 1165, 970, 777, 695 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.75 (br, 1 H, H-21), 8.71 (d, 1 H, *J* = 8.7 Hz, H-14), 8.54 (s, 1 H, H-8), 8.47 (br, 1 H, H-21), 8.29 (d, 2 H, *J* = 6.8 Hz, H-1, H-5), 8.20 (d, 1 H, *J* = 8.0 Hz, H-11), 7.89 (t, 1 H, *J* = 7.8 Hz, H-13), 7.74 (t, 1 H, *J* = 7.8 Hz, H-12), 7.57 (m, 3 H, H-2, H-3, H-4), 5.59 (s, 2 H, H-17), 3.34 (s, 3 H, H-24), 3.20 (s, 3 H, H-22); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 189.8 (C-18), 165.7 (C-16), 161.5 (C-25), 158.0 (C-20), 155.7 (C-7), 149.5 (C-23), 148.3 (C-15), 137.7 (C-9), 136.6 (C-6), 130.4 (C-13), 130.0 (C-14), 129.8 (C-12), 129.0 (2 C, C-2, C-4), 127.9 (C-3), 127.1 (2 C, C-1, C-5), 125.4 (C-11), 123.2 (C-8), 119.3 (C-10), 88.9 (C-19), 70.0 (C-17), 29.6 (C-22), 27.5 (C-24); MS (ESI) *m/z* 445 ($[M + H]^+$); HRMS (ESI) *m/z* calculated for $C_{24}H_{21}N_4O_5$ (M+H) 445.1512, found 445.1502.

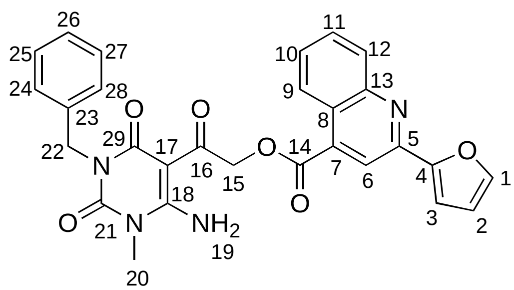
64 

2-(6-Amino-3-benzyl-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(trifluoromethyl)quinoline-4-carboxylate (64, DMA-P158). According to general procedure I, **16** (56.9 mg, 0.185 mmol) and **10** (51.1 mg, 0.194 mmol) were converted to **64** (58.9 mg, 62%) as a tan solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.09 (br, 1 H, H-16), 8.95 (d, 1 H, *J* = 8.0 Hz, H-9), 8.39 (s, 1 H, H-3), 8.30 (d, 1 H, *J* = 8.2 Hz, H-6), 8.03 (t, 1 H, *J* = 6.9 Hz, H-8), 7.92 (t, 1 H, *J* = 7.1 Hz, H-7), 7.74 (br, 1 H, H-16), 7.44 (d, 2 H, *J* = 7.1 Hz, H-21, H-25), 7.34–7.25 (m, 3 H, H-22, H-23, H-24), 5.68 (s, 2 H, H-12), 5.13 (s, 2 H, H-19), 3.55 (s, 3 H, H-17).

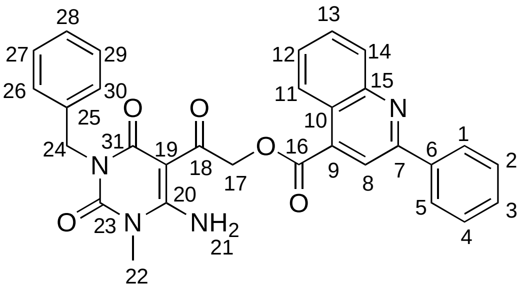
65 

2-(6-Amino-3-benzyl-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-cyclopropylquinoline-4-carboxylate (65, DMA-P161). According to general procedure I, **16** (60.3 mg, 0.196 mmol) and **11** (48.4 mg, 0.206 mmol) were converted to **65** (47.5 mg, 50%) as an orange solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.12 (br, 1 H, H-18), 8.74 (d, 1 H, *J* = 7.9, H-11), 7.98–7.95 (m, 2 H, H-5, H-8), 7.77–7.72 (m, 2 H, H-10, H-18), 7.57 (t, 1 H, *J* = 7.7 Hz, H-9), 7.45–7.25 (m, 5 H, H-23, H-24, H-25, H-26, H-27), 5.62 (s, 2 H, H-14), 5.13 (s, 2 H, H-21), 3.54 (s, 3 H, H-19), 2.44–2.36 (m, 1 H, H-3), 1.25–1.10 (m, 4 H, H-1, H-2).

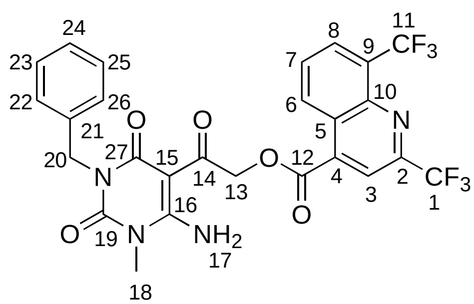
66



2-(6-Amino-3-benzyl-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(furan-2-yl)quinoline-4-carboxylate (66, DMA-P160). According to general procedure I, **16** (57.3 mg, 0.186 mmol) and **12** (51.0 mg, 0.195 mmol) were converted to **66** (57.7 mg, 59%) as a brown solid: Mp 143–146 °C (dec.); IR (KBr) 3378, 3116, 2961, 1717, 1623, 1531, 1435, 1232, 1190, 1012, 775 cm^{-1}; ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.11 (br, 1 H, H-19), 8.80 (d, 1 H, *J* = 8.1 Hz, H-12), 8.48 (s, 1 H, H-6), 8.11 (d, 1 H, *J* = 8.0 Hz, H-9), 7.84–7.81 (m, 2 H, H-1, H-11), 7.72 (br, 1 H, H-19), 7.66 (t, 1 H, *J* = 7.7 Hz, H-10), 7.44 (d, 2 H, H-24, H-28), 7.40 (d, 1 H, *J* = 3.3 Hz, H-3), 7.34-7.25 (m, 3 H, H-25, H-26, H-27), 6.72 (dd, 1 H, *J* = 3.4, 1.6 Hz, H-2), 5.67 (s, 2 H, H-15), 5.13 (s, 2 H, H-22), 3.54 (s, 3 H, H-20); ^{13}C-NMR (acetone-d_6, 75 MHz) δ 191.7 (C-16), 166.7 (C-14), 162.6 (C-29), 160.0 (C-18), 154.4 (C-4), 150.8 (C-5), 150.0 (C-21), 149.6 (C-13), 145.8 (C-1), 138.8 (C-23), 137.8 (C-7), 131.2 (C-11), 130.5 (C-12), 129.1 (C 4, C-24, C-25, C-27, C-28), 128.4 (C-10), 128.1 (C-9), 126.8 (C-26), 124.7 (C-6), 119.1 (C-8), 113.5 (C-3), 111.4 (C-2), 90.3 (C-17), 71.1 (C-15), 44.8 (C-22), (C-20, buried in solvent peak). MS (EI) *m/z* 510 (M^+, 100), 288 (24), 239 (15), 195 (17), 91 (26); HRMS (EI) *m/z* calculated for $C_{28}H_{22}N_4O_6$ 510.1539, found 510.1554.

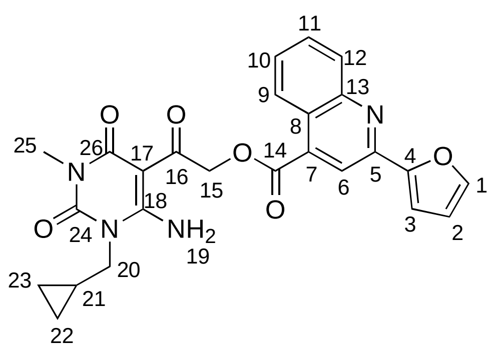
67 

2-(6-Amino-3-benzyl-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-phenylquinoline-4-carboxylate (67, DMA-P162). According to general procedure I, **16** (59.1 mg, 0.192 mmol) and **13** (54.7 mg, 0.202 mmol) were converted to **67** (46.5 mg, 47%) as a tan solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.13 (br, 1 H, H-21), 8.84 (d, 1 H, *J* = 7.8 Hz, H-14), 8.63 (s, 1 H, H-8), 8.37–8.35 (m, 2 H, H-1, H-5), 8.23 (d, 1 H, *J* = 8.4 Hz, H-11), 7.87 (t, 1 H, *J* = 7.6 Hz, H-13), 7.73–7.67 (m, 2 H, H-12, H-21), 7.62–7.54 (m, 3 H, H-2, H-3, H-4), 7.46–7.43 (m, 2 H, H-26, H-30), 7.34-7.26 (m, 3 H, H-27, H-28, H-29), 5.68 (s, 2 H, H-17), 5.14 (s, 2 H, H-24), 3.55 (s, 3 H, H-22).

68 

2-(6-Amino-3-benzyl-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2,8-bis(trifluoromethyl)quinoline-4-carboxylate (68, DMA-P159). According to general procedure I, **16** (47.7 mg, 0.155 mmol) and **14** (53.8 mg, 0.165 mmol) were converted to **68** (47.9 mg, 53%) as a yellow solid: Mp 116–118 ^{o}C (dec.); IR (KBr) 3391, 3115, 1720, 1624, 1534, 1437, 1313, 1244, 1199, 1145, 1108, 949, 776, 690 cm^{-1}; ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.08 (br, 1 H, H-17), 9.23 (d, 1 H, J = 8.8 Hz, H-8), 8.52 (s, 1 H, H-3), 8.45 (d, 1 H, J = 7.4 Hz, H-6), 8.07 (t, 1 H, J = 8.0 Hz, H-7), 7.78 (br, 1 H, H-17), 7.43 (d, 2 H, J = 7.0 Hz, H-22, H-26), 7.34–7.25 (m, 3 H, H-23, H-24, H-25), 5.70 (s, 2 H, H-13), 5.13 (s, 2 H, H-20), 3.55 (s, 3 H, H-18); ^{13}C-NMR (acetone-d$_6$, 150 MHz) δ 190.2 (C-14), 164.5 (C-12), 161.6 (C-27), 159.0 (C-16), 149.7 (C-10), 147.6 (q, J_{CF} = 34.5 Hz, C-2), 144.1 (C-19), 139.5 (C-21), 137.7 (C-4), 130.8 (C-6), 130.1 (q, J_{CF} = 6.0 Hz, C-8), 129.2 (C-7), 128.1 (C 4, C-22, C-23, C-25, C-26), 128.0 (q, J_{CF} = 30.0 Hz, C-9), 127.0 (C-24), 126.3 (C-5), 123.7 (q, J_{CF} = 270.0 Hz, C-1), 121.1 (q, J_{CF} = 271.5 Hz, C-11), 118.5 (C-3), 89.2 (C-15), 70.6 (C-13), 43.8 (C-20), 29.4 (C-18); MS (EI) *m/z* 580 (M$^+$, 100), 309 (26), 214 (6), 132 (6), 91 (17); HRMS (EI) *m/z* calculated for $C_{26}H_{18}F_6N_4O_5$ 580.1181, found 580.1191.

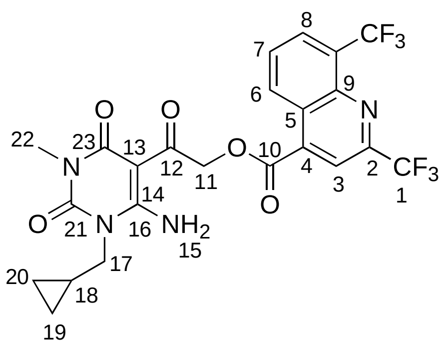
69



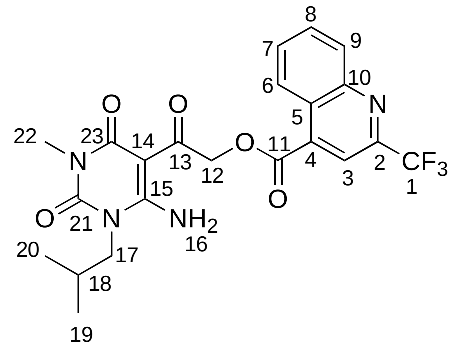
2-(6-Amino-1-(cyclopropylmethyl)-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(furan-2-yl)quinoline-4-carboxylate (69, DMA-P165). According to general procedure I, **17** (54.3 mg, 0.200 mmol) and **12** (54.9 mg, 0.210 mmol) were converted to **69** (53.3 mg, 56%) as a brown solid: Mp 138–142 ^{o}C (dec); IR (KBr) 3399, 6116, 2960, 1716, 1651, 1617, 1525, 1458, 1277, 1148, 962, 776 cm^{-1}; ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 11.26 (br, 1 H, H-19), 8.81 (d, 1 H, J = 8.3 Hz, H-12), 8.49 (s, 1 H, H-6), 8.11 (d, 1 H, J = 8.3 Hz, H-9), 7.85–7.81 (m, 2 H, H-1, H-11), 7.71 (br, 1 H, H-19), 7.66 (t, 1 H, J = 7.2 Hz, H-10), 7.40 (d, 1 H, J =

3.3 Hz, H-3), 6.73 (dd, 1 H, *J* = 3.3, 1.6 Hz, H-2), 5.66 (s, 2 H, H-15), 4.04 (d, 2 H, *J* = 7.0 Hz, H-20), 3.29 (s, 3 H, H-25), 1.29 (m, 1 H, H-21), 0.57–0.49 (m, 4 H, H-22, H-23); ^{13}C-NMR (acetone-d$_6$, 75 MHz) δ 191.8 (C-16), 166.6 (C-14), 162.6 (C-26), 159.1 (C-18), 154.3 (C-4), 151.0 (C-5), 149.9 (C-24), 149.5 (C-13), 145.7 (C-1), 137.7 (C-7), 131.1 (C-11), 130.5 (C-12), 128.3 (C-10), 126.7 (C-9), 124.6 (C-6), 119.0 (C-8), 113.4 (C-3), 111.3 (C-2), 90.2 (C-17), 71.0 (C-15), 46.8 (C-20), 28.0 (C-25), 10.0 (C-21), 4.2 (C-22, C-23); MS (EI) *m/z* 474 (M^+, 57), 239 (100), 206 (66), 139 (41), 91 (41); HRMS (EI) *m/z* calculated for $C_{25}H_{22}N_4O_6$ 474.1539, found 474.1519.

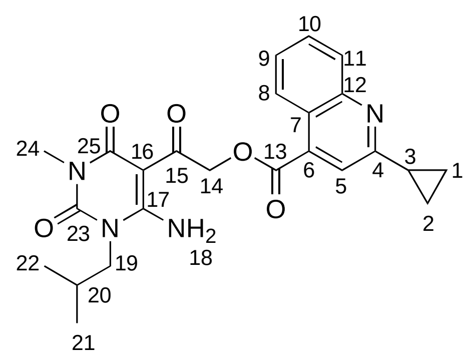
70



2-(6-Amino-1-(cyclopropylmethyl)-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2,8-bis(trifluoromethyl)quinoline-4-carboxylate (70, DMA-P166). According to general procedure I, **17** (40.7 mg, 0.150 mmol) and **14** (52.2 mg, 1.575 mmol) were converted to **70** (44.7 mg, 55%) as a tan solid: ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 11.22 (br, 1 H, H-15), 9.24 (d, 1 H, *J* = 9.1 Hz, H-8), 8.53 (s, 1 H, H-3), 8.45 (d, 1 H, *J* = 6.6 Hz, H-6), 8.08 (t, 1 H, *J* = 8.1 Hz, H-7), 7.77 (br, 1 H, H-15), 5.70 (s, 2 H, H-11), 4.05 (d, 2 H, *J* = 7.1 Hz, H-17), 3.29 (s, 3 H, H-22), 1.29 (m, 1 H, H-18), 0.56–0.48 (m, 4 H, H-19, H-20).

71 

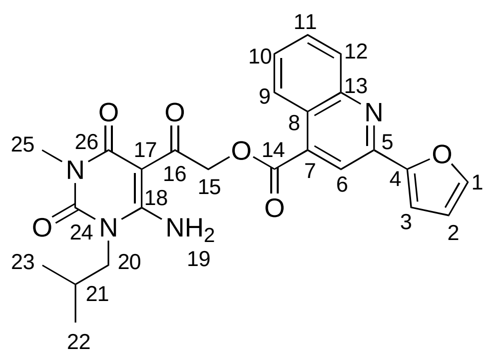
2-(6-Amino-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(trifluoromethyl)quinoline-4-carboxylate (71, DMA-P151). According to general procedure I, **18** (57.2 mg, 0.209 mmol) and **10** (57.9 mg, 0.220 mmol) were converted to **71** (33.4 mg, 58%) as an orange solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.25 (br, 1 H, H-16), 8.95 (d, 1 H, *J* = 8.5 Hz, H-9), 8.39 (s, 1 H, H-3), 8.30 (d, 1 H, *J* = 8.5 Hz, H-6), 8.03 (dt, 1 H, *J* = 7.7, 1.4 Hz, H-8), 7.92 (dt, 1 H, *J* = 7.8, 1.3 Hz, H-7), 7.65 (br, 1 H, H-16), 5.68 (s, 2 H, H-12), 3.98 (d, 2 H, *J* = 7.9, H-17), 3.29 (s, 3 H, H-22), 2.29–2.20 (m, 1 H, H-18), 0.97 (d, 6 H, *J* = 6.7 Hz, H-19, H-20).

72 

2-(6-Amino-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-cyclopropylquinoline-4-carboxylate (72, DMA-P149). According to general procedure I, **18** (75.0 mg, 0.274 mmol) and **11** (67.7 mg, 0.288 mmol) were converted to **72** (37.8 mg, 31%) as an orange solid: Mp 124.5–126 ^{o}C (dec); IR (KBr) 3413, 3138, 2961, 1718, 1655, 1613, 1520, 1459, 1243, 1145, 968, 772 cm^{-1}; ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.29 (br, 1 H, H-18), 8.75

(d, 1 H, *J* = 8.7 Hz, H-11), 7.98–7.94 (m, 2 H, H-5, H-8), 7.75 (dt, 1 H, *J* = 7.6, 1.4 Hz, H-10), 7.60 (br, 1 H, H-18), 7.57 (dt, 1 H, *J* = 7.7, 1.4 Hz, H-9), 5.62 (s, 2 H, H-14), 3.98 (d, 2 H, *J* = 7.9 Hz, H-19), 3.29 (s, 3 H, H-24), 2.44–2.36 (m, 1 H, H-3), 2.30–2.20 (m, 1 H, H-20), 1.25–1.08 (m, 4 H, H-1, H-2), 0.97 (d, 6 H, *J* = 6.7 Hz, H-21, H-22); ^{13}C-NMR (acetone-d_6, 75 MHz) δ 190.2 (C-15), 165.8 (C-13), 162.8 (C-25), 161.4 (C-17), 157.6 (C-4), 149.8 (C-23), 148.2 (C-12), 135.1 (C-6), 129.9 (C-10), 128.8 (C-11), 126.6 (C-9), 125.3 (C-5), 122.6 (C-8), 121.4 (C-7), 88.8 (C-16), 69.9 (C-14), 47.9 (C-19), 27.6 (C-24), 26.0 (C-20), 19.3 (2 C, C-21, C-22), 17.3 (C-3), 10.8 (2 C, C-1, C-2); MS (EI) *m/z* 450 (M^+, 8), 254 (35), 212 (100), 197 (21), 182 (76), 168 (78), 139 (28); HRMS (EI) *m/z* calculated for $C_{24}H_{26}N_4O_5$ 450.1903, found 450.1900.

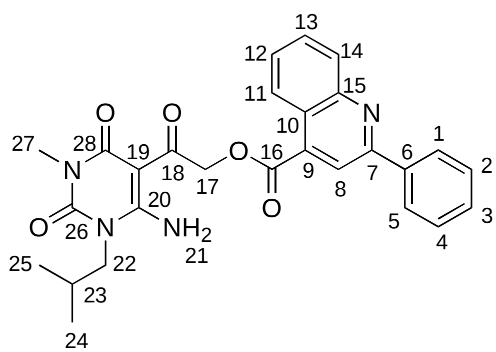
73



2-(6-Amino-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(furan-2-yl)quinoline-4-carboxylate (73, DMA-P153). According to general procedure I, **18** (54.7 mg, 0.200 mmol) and **12** (57.5 mg, 0.210 mmol) were converted to **73** (57.7 mg, 61%) as a brown solid: Mp 148–149 ^{o}C (dec); IR (KBr) 3410, 3137, 2962, 1718, 1654, 1614, 1522, 1459, 1231, 1190, 993, 775 cm^{-1}; ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.27 (br, 1 H, H-19), 8.81 (d, 1 H, *J* = 8.5 Hz, H-12), 8.49 (s, 1 H, H-6), 8.11 (d, 1 H, *J* = 8.2 Hz, H-9), 7.85–7.81 (m, 2 H, H-1, H-11), 7.67 (t, 2 H, *J* = 7.1 Hz, H-10, H-19), 7.40 (d, 1 H, *J* = 3.4 Hz, H-3), 6.73 (dd, 1 H, *J* = 3.3, 1.7 Hz, H-2), 5.66 (s, 2 H, H-15), 3.98 (d, 2 H, *J* = 7.9 Hz, H-20), 3.29 (s, 3 H, H-25), 2.27–

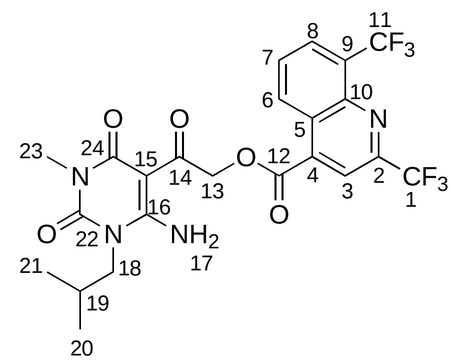
2.23 (m, 1 H, H-21), 0.97 (d, 6 H, *J* = 6.6 Hz, H-22, H-23); ^{13}C-NMR (acetone-d_6, 75 MHz) δ 190.1 (C-16), 165.4 (C-14), 161.4 (C-26), 157.6 (C-18), 152.3 (C-4), 149.8 (C-24), 148.3 (C-5), 148.0 (C-13), 145.5 (C-1), 136.3 (C-7), 130.6 (C-12), 129.3 (C-11), 127.8 (C-10), 125.5 (C-9), 123.0 (C-6), 118.1 (C-8), 112.8 (C-3), 111.2 (C-2), 88.8 (C-17), 70.1 (C-15), 47.9 (C-20), 27.5 (C-25), 26.0 (C-21), 19.3 (2 C, C-22, C-23); MS (EI) *m/z* 476 (M^+, 68), 254 (77), 239 (96), 224 (49), 195 (100), 182 (81), 168 (78), 139 (36), 101 (19); HRMS (EI) *m/z* calculated for $C_{25}H_{24}N_4O_6$ 476.1696, found 476.1709.

74

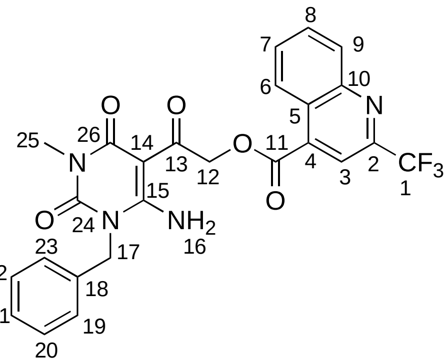


2-(6-Amino-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-phenylquinoline-4-carboxylate (74, DMA-P101). According to general procedure I, **18** (54.7 mg, 0.200 mmol) and **13** (59.6 mg, 0.220 mmol) were converted to **74** (58.5 mg, 60%) as a white crystalline solid: Mp 233–234 °C; IR (KBr) 3481, 3055, 2958, 1722, 1654, 1633, 1512, 1392, 1233, 1145, 995, 770 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.95 (br, 1 H, H-21), 8.72 (d, 1 H, *J* = 8.2 Hz, H-14), 8.55 (s, 1 H, H-8), 8.46 (br, 1 H, H-21), 8.30 (d, 2 H, *J* = 7.0 Hz, H-1, H-5), 8.20 (d, 1 H, *J* = 8.6 Hz, H-11), 7.89 (t, 1 H, *J* = 7.2 Hz, H-13), 7.74 (t, 1 H, *J* = 7.3 Hz, H-12), 7.58 (m, 3 H, H-2, H-3, H-4), 5.61 (s, 2 H, H-17), 3.81 (d, 2 H, *J* = 7.5 Hz, H-22), 3.20 (s, 3 H, H-27), 2.05 (m, 1 H, H-23), 0.88 (d, 6 H, *J* = 6.5 Hz, H-24, H-25); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 190.1 (C-18), 165.7 (C-16), 161.4 (C-28), 157.6 (C-20), 155.7 (C-7), 149.8 (C-26), 148.4 (C-15), 137.7 (C-9), 136.6 (C-6), 130.4 (C-13), 130.1 (C-14), 129.8 (C-12), 129.0 (2 C, C-2, C-4), 128.0 (C-3), 127.2 (2 C, C-1, C-5), 125.4 (C-11), 123.2 (C-8), 119.3 (C-10), 88.8 (C-19), 70.1 (C-17), 47.9 (C-22), 27.6 (C-27), 26.0 (C-23), 19.3 (2 C, C-24, C-25); MS (ESI) *m/z* 487 ($[M + H]^+$); HRMS (ESI) *m/z* calculated for $C_{27}H_{27}N_4O_5$ (M+H) 487.1981, found 487.1943.

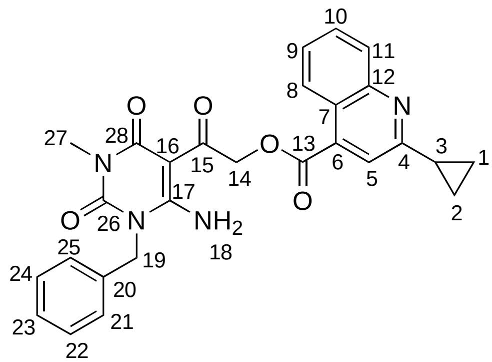
75



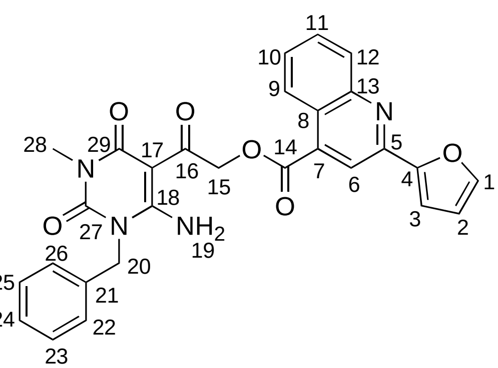
2-(6-Amino-1-isobutyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2,8-bis(trifluoromethyl)quinoline-4-carboxylate (75, DMA-P152). According to general procedure I, **18** (45.1 mg, 0.165 mmol) and **14** (57.3 mg, 0.173 mmol) were converted to **75** (49.3 mg, 55%) as a tan solid: ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 11.25 (br, 1 H, H-17), 9.24 (d, 1 H, *J* = 9.3 Hz, H-8), 8.52 (s, 1 H, H-3), 8.45 (d, 1 H, *J* = 7.3 Hz, H-6), 8.07 (t, 1 H, *J* = 8.0 Hz, H-7), 7.66 (br, 1 H, H-17), 5.70 (s, 2 H, H-13), 3.99 (d, 2 H, *J* = 7.9 Hz, H-18), 3.29 (s, 3 H, H-23), 2.30–2.20 (m, 1 H, H-19), 0.97 (d, 6 H, *J* = 6.7 Hz, H-20, H-21).

76 

2-(6-Amino-1-benzyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(trifluoromethyl)quinoline-4-carboxylate (76, DMA-P154). According to general procedure I, **19** (54.1 mg, 0.176 mmol) and **10** (48.6 mg, 0.185 mmol) were converted to **76** (46.2 mg, 51%) as an orange solid: Mp 113–116 °C (dec.); IR (KBr) 3435, 3067, 2962, 1718, 1654, 1618, 1523, 1454, 1198, 1142, 1002, 779 cm^{-1}; ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.15 (br, 1 H, H-16), 8.94 (d, 1 H, J = 8.8 Hz, H-9), 8.38 (s, 1 H, H-3), 8.29 (d, 1 H, J = 8.0 Hz, H-6), 8.03 (t, 1 H, J = 8.5 Hz, H-8), 7.91 (t, 1 H, J = 7.1 Hz, H-7), 7.48 (br, 1 H, H-16), 7.40–7.32 (m, 5 H, H-19, H-20, H-21, H-22, H-23), 5.70 (s, 2 H, H-12), 5.41 (s, 2 H, H-17), 3.34 (s, 3 H, H-25); ^{13}C-NMR (acetone-d_6, 150 MHz) δ 191.5 (C-13), 165.8 (C-11), 162.6 (C-26), 159.2 (C-15), 151.0 (C-24), 149.0 (C-10), 147.9 (q, J_{CF} = 34.5 Hz, C-2), 139.5 (C-18), 135.6 (C-4), 132.3 (C-8), 131.2 (2 C, C-7, C-9), 129.6 (2 C, C-22, C-20), 128.5 (C-5), 127.2 (2 C, C-19, C-23), 126.9 (C-21), 126.6 (C-6), 122.4 (q, J_{CF} = 273.0 Hz, C-1), 118.5 (C-3), 90.2 (C-14), 71.3 (C-12), 45.9 (C-17), 28.1 (C-25); MS (EI) m/z 512 (M^+, 6), 493 (2), 288 (87), 258 (80), 241 (17), 225 (44), 196 (58), 146 (16), 101 (46), 91 (100); HRMS (EI) m/z calculated for $C_{25}H_{19}F_3N_4O_5$ 512.1308, found 512.1331.

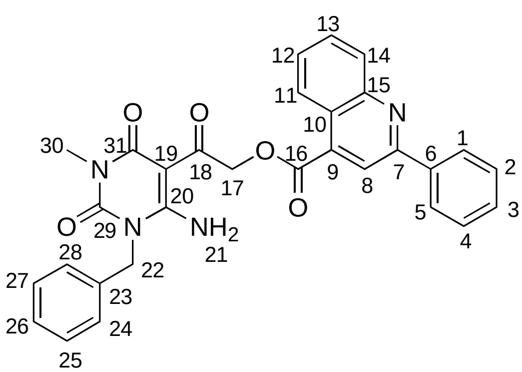
77 

2-(6-Amino-1-benzyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-cyclopropylquinoline-4-carboxylate (77, DMA-P157). According to general procedure I, **19** (57.2 mg, 0.186 mmol) and **11** (45.9 mg, 0.195 mmol) were converted to **77** (43.2 mg, 48%) as an orange solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.18 (br, 1 H, H-18), 8.73 (d, 1 H, *J* = 8.4 Hz, H-11), 7.97–7.94 (m, 2 H, H-5, H-8), 7.74 (dt, 1 H, *J* = 7.6, 1.4 Hz, H-10), 7.56 (dt, 1 H, *J* = 7.7, 1.3 Hz, H-9), 7.40–7.32 (m, 6 H, H-18, H-21, H-22, H-23, H-24, H-25), 5.64 (s, 2 H, H-14), 5.41 (s, 2 H, H-19), 3.34 (s, 3 H, H-27), 2.43–2.35 (m, 1 H, H-3), 1.24–1.07 (m, 4 H, H-1, H-2).

78 

2-(6-Amino-1-benzyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-(furan-2-yl)quinoline-4-carboxylate (78, DMA-P156). According to general procedure I, **19** (54.2 mg, 0.176 mmol) and **12** (48.3 mg, 0.185 mmol) were converted to **78** (48.9 mg, 54%) as a brown solid: Mp 146–150 ^{o}C (dec.); IR (KBr) 3435, 3116, 1717, 1653, 1618, 1522, 1454, 1233, 1149, 962, 776 cm^{-1}; ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 11.18 (br, 1 H, H-19), 8.78 (d, 1 H, *J* = 8.5 Hz, H-12), 8.47 (s, 1 H, H-6), 8.10 (d, 1 H, *J* = 8.5 Hz, H-9), 7.83–7.79 (m, 2 H, H-1, H-11), 7.65 (t, 1 H, *J* = 7.6 Hz, H-10), 7.39–7.32 (m, 7 H, H-3, H-19, H-22, H-23, H-24, H-25, H-26), 6.72 (dd, 1 H, *J* = 3.4, 1.7 Hz, H-2), 5.68 (s, 2 H, H-15), 5.40 (s, 2 H, H-20), 3.34 (s, 3 H, H-28); ^{13}C-NMR (acetone-d$_6$, 75 MHz) δ 191.8 (C-16), 166.6 (C-14), 162.5 (C-29), 159.2 (C-18), 154.3 (C-4), 151.0 (C-27), 149.9 (C-5), 149.5 (C-13), 145.7 (C-1), 137.7 (C-7), 135.7 (C-21), 131.1 (C-11), 130.5 (C-12), 129.6 (2 C, C-23, C-25), 128.5 (C-10), 128.3 (C-9), 127.2 (C 2, C-22, C-26), 126.7 (C-24), 124.6 (C-6), 119.0 (C-8), 113.4 (C-3), 111.3 (C-2), 90.1 (C-17), 71.0 (C-15), 45.9 (C-20), 28.1 (C-28); MS (EI) *m/z* 510 (M$^+$, 100), 288 (25), 271 (17), 239 (20), 195 (23), 166 (15), 91 (93); HRMS (EI) *m/z* calculated for $C_{28}H_{22}N_4O_6$ 510.1539, found 510.1524.

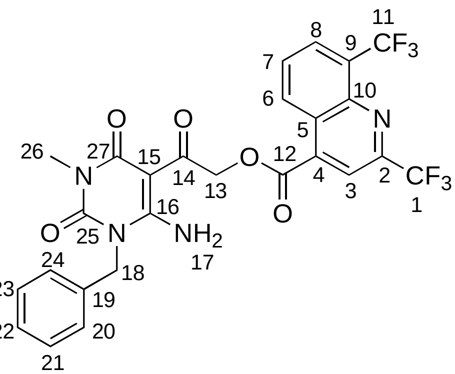
79



2-(6-Amino-1-benzyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2-phenylquinoline-4-carboxylate (79, DMA-P148). According to general procedure I, **19** (56.2 mg, 0.182 mmol) and **13** (54.5 mg, 0.201 mmol) were converted to **79** (36.5 mg, 39%) as an orange solid: ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 11.19 (br, 1 H, H-21), 8.83 (d, 1 H, *J* = 8.5 Hz, H-14), 8.62 (s, 1 H, H-8), 8.36–8.34 (m, 2 H, H-1, H-5), 8.21 (d, 1 H, *J* = 8.4 Hz, H-11), 7.86 (t, 1 H, *J* = 6.9 Hz, H-13), 7.69 (t, 1 H, *J* = 7.4 Hz, H-12), 7.62–7.51 (m, 3 H, H-2, H-3, H-4), 7.36–

7.32 (m, 6 H, H-21, H-24, H-25, H-26, H-27, H-28), 5.69 (s, 2 H, H-17), 5.41 (s, 2 H, H-22), 3.34 (s, 3 H, H-30).

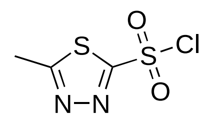
80



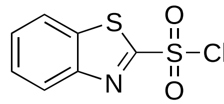
2-(6-Amino-1-benzyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-oxoethyl 2,8-bis(trifluoromethyl)quinoline-4-carboxylate (80, DMA-P155). According to general procedure I, **19** (47.7 mg, 0.155 mmol) and **14** (53.9 mg, 0.163 mmol) were converted to **80** (52.5 mg, 58%) as a tan solid: ^{1}H-NMR (acetone-d_6, 300 MHz) δ 11.14 (br, 1 H, H-17), 9.22 (d, 1 H, *J* = 8.8 Hz, H-8), 8.51 (s, 1 H, H-3), 8.44 (d, 1 H, *J* = 7.3 Hz, H-6), 8.06 (t, 1 H, *J* = 7.9 Hz, H-7), 7.49 (br, 1 H, H-17), 7.36–7.33 (m, 5 H, H-20, H-21, H-22, H-23, H-24), 5.71 (s, 2 H, H-13), 5.41 (s, 2 H, H-18), 3.34 (s, 3 H, H-26).

4.3 ADDITION OF ORGANOMETALLIC REAGENTS TO BENZOTHIAZOLE AND METHYLTHIADIAZOLE ALDIMINES

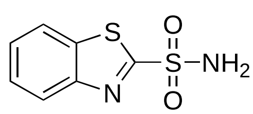
4.3.1 Synthesis of *N*-Bts- and *N*-Ths-benzaldimines

140 

General procedure J. 5-Methyl-1,3,4-thiadiazole-2-sulfonyl chloride (140).[56] To a solution of acetic acid (45.0 mL, 33%) cooled to -5-5 ^{o}C (internal thermometer) was introduced a vigorous stream of Cl_2 gas. After 5 min, the solution became yellow and a yellow solid precipitated. At this time, 5-methyl-1,3,4-thiadiazole-2-thiol **143** (5.00 g, 37.8 mmol) was slowly added to the reaction mixture over a 30 min period, carefully keeping the temperature of the reaction mixture <5 ^{o}C and the solution thoroughly saturated with Cl_2 gas at all times. The reaction mixture was stirred for an additional 15 min after all of **143** was added. The resulting white precipitate which formed during the course of the reaction was quickly isolated by vacuum filtration through an ice cooled Buchner funnel and was washed with ice cold deionized water (30.0 mL). The solid was then dissolved in cold ether (125 mL) and the ether solution was washed with a cold saturated $NaHCO_3$ solution (1 X 75.0 mL) followed by a cold saturated brine solution (1 X 50.0 mL). The ether solution was dried (Na_2SO_4), concentrated by rotary evaporation (10 ^{o}C), and the resulting white solid was recrystallized from ether (50.0 mL) at -78 ^{o}C, isolated by vacuum filtration and dried under high vacuum for 2 h. The product **140** (3.94 g, 53%) was isolated as a white crystalline solid: Mp 48-49 ^{o}C (dec); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.97 (s, 3 H).

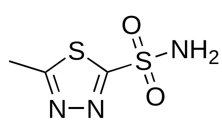
139 

Benzo[d]thiazole-2-sulfonyl chloride (139).[56] According to general procedure J, benzo[d]thiazole-2-thiol **142** (4.00 g, 23.9 mmol) was slowly added over a 1.5 h period to a mixture of acetic acid (45 mL, 33%) and Cl_2 gas (large excess). The reaction mixture was stirred for an additional 30 min after all of **142** was added. A modified work up procedure was performed in which the crude reaction product was isolated by vacuum filtration, washed with ice cooled deionized water (40.0 mL), dissolved in cold CH_2Cl_2 (100 mL) and washed with a cold saturated $NaHCO_3$ solution (1 X 50.0 mL), followed by a cold saturated brine solution (1 X 50.0 mL). The CH_2Cl_2 solution was then filtered through a 1 in plug of celite, concentrated by rotary evaporation (13 ^oC) and the crude orange solid was recrystallized from ether (20.0 mL) at -78 ^oC. The product **139** (3.24 g, 56%) was isolated as a white crystalline solid: Mp 104.5-108 ^oC; ^1H-NMR ($CDCl_3$, 300 MHz) δ 8.33 (d, 1 H, *J* = 7.5 Hz), 8.08 (d, 1 H, *J* = 6.9 Hz), 7.74-7.73 (m, 2 H).

144 

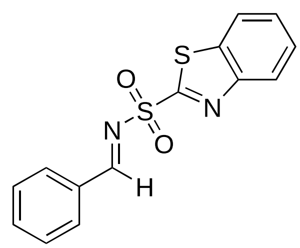
Benzo[d]thiazole-2-sulfonamide (144).[76] To a solution of **139** (2.46 g, 10.5 mmol) in CH_2Cl_2 (25.0 mL) cooled to -78 ^oC under a nitrogen atmosphere, was added condensed NH_3 (5 drops, acetone/dry ice condenser) over a 15 min period. The reaction mixture was then slowly warmed to room temperature. After 17 h, the heterogeneous reaction mixture was cooled to 0 ^oC and diluted with hexanes (25.0 mL). The resulting white precipitate was isolated by vacuum filtration, washed with a 1:1 solution of CH_2Cl_2 and hexanes, and added to a boiling solution of

EtOAc (100 mL) with stirring for 20 min. After 20 min, the EtOAc solution was cooled to room temperature, filtered through a 1.5 in plug of SiO_2 and concentrated by rotary evaporation. The resulting white solid was recrystallized from a 5:1 solution of hexanes and EtOAc. The recrystallized product was isolated by vacuum filtration, washed with hexanes (40 mL) and dried under high vacuum for 10 h. The product **144** (1.64 g, 73%) was isolated as a white crystalline solid: Mp 179-180 ^{o}C, IR (KBr) 3312, 3151, 3039, 1472, 1356, 1164, 929, 765 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.33 (s, 2 H), 8.27 (d, 1 H, J = 7.8 Hz), 8.17 (d, 1 H, J = 8.1 Hz), 7.70-7.60 (m, 2 H, J = 7.8 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 169.4, 151.7, 135.6, 127.5, 127.4, 124.2, 123.2; MS (EI) *m/z* 214 (M^+, 85), 150 (100), 135 (94), 108 (80), 90 (74), 80 (58), 68 (88); HRMS (EI) *m/z* calculated for $C_7H_6N_2O_2S_2$ 213.9871, found 213.9874.

145 

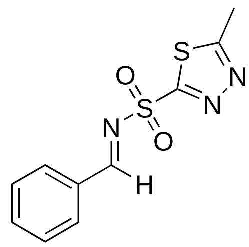
5-Methyl-1,3,4-thiadiazole-2-sulfonamide (145).[77] To a solution of **140** (3.57 g, 18.0 mmol) in CH_2Cl_2 (40.0 mL) cooled to -78 ^{o}C under a nitrogen atmosphere, was added condensed NH_3 (7-8 drops, acetone/dry ice condenser) over a 5 min period. The reaction mixture was then slowly warmed to room temperature. After 16 h, the heterogeneous reaction mixture was cooled to 0 ^{o}C and the white precipitate was isolated by vacuum filtration. The solid was stirred in boiling EtOAc (100 mL) for 20 min to dissolve the sulfonamide. The resulting heterogeneous mixture was cooled to room temperature and the remaining ammonium salts were removed by vacuum filtration. The EtOAc solution was concentrated by rotary evaporation and the resulting white solid was recrystallized from a 1:1 solution of EtOAc and hexanes. The precipitate was isolated by vacuum filtration, washed with hexanes (40.0 mL) and dried under high vacuum for 2 h. The product **145** (2.20 g, 68%) was isolated as a white crystalline solid: Mp 164.5-166 °C; IR (KBr) 3319, 3148, 3035, 1418, 1358, 1209, 1175, 930 cm^{-1}; ^{1}H-NMR (acetone-d$_6$, 300 MHz) δ 7.59 (s, 2 H), 2.85 (s, 3 H); ^{13}C-NMR (acetone-d$_6$, 75 MHz) δ 171.3, 170.5, 15.7; MS (EI) *m/z* 179 (M$^+$, 17), 115 (100), 99 (71), 80 (25), 59 (15); HRMS (EI) *m/z* calculated for $C_3H_5N_3O_2S_2$ 178.9823, found 178.9831.

146



(*E*)-*N*-Benzylidenebenzo[d]thiazole-2-sulfonamide (146). To a suspension of **144** (500 mg, 2.33 mmol) and *p*-TsOH (22.3 mg, 0.117 mmol) in toluene (20.0 mL) was added freshly distilled benzaldehyde **110** (0.237 mL, 2.33 mmol). The reaction mixture was heated at reflux under a nitrogen atmosphere for 20 h. Water formed during the course of the reaction was azeotropically removed via a Dean-Stark trap. After 20 h, the reaction mixture was cooled to room temperature and filtered through a flame-dried piece of glass wool contained inside a disposable 9 in pipette. The toluene was removed in vacuo and the resulting crude yellow oil was recrystallized from a 7:1 mixture of hexanes to EtOAc. The precipitate was isolated by vacuum filtration, washed with hexanes (30.0 mL) and dried under high vacuum for 15 h. The product **146** (614 mg, 87%) was isolated as an off-white crystalline solid: Mp 128-129 °C (dec); IR (KBr) 3067, 1596, 1566, 1313, 1338, 1161, 870, 769 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 9.24 (s, 1 H), 8.20 (d, 1 H, *J* = 7.7 Hz), 8.06-8.01 (m, 3 H), 7.76-7.60 (m, 5 H); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 175.5, 164.2, 153.2, 137.6, 136.7, 132.6, 132.5 (2), 129.9 (2), 128.6, 128.2, 125.9, 122.9; MS (EI) *m/z* 302 (M^+, 1.7), 237 (5), 214 (12), 150 (18), 135 (100), 105 (33); HRMS (EI) *m/z* calculated for $C_{14}H_{10}N_2O_2S_2$ 302.0184, found 302.0180.

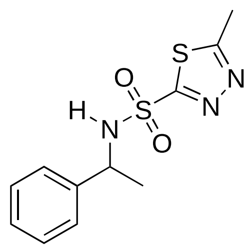
147



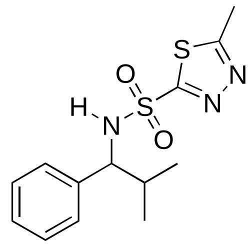
Representative procedure for the synthesis of (*E*)-*N*-benzylidene-5-methyl-1,3,4-thiadiazole-2-sulfonamide stock solutions (147). To a suspension of **145** (179 mg, 1.00 mmol) and PTSA (9.50 mg, 0.0500 mmol) in toluene (8.0 mL) was added freshly distilled benzaldehyde **110** (0.102 mL, 1.00 mmol). The reaction mixture was heated at reflux under a nitrogen atmosphere. Water formed during the course of the reaction was azeotropically removed *via* a Dean-Stark trap. After 21 h, the reaction mixture was cooled to room temperature and filtered through a flame dried piece of glass wool, contained inside a disposable 9 in pipette, into a dry 5.0 mL volumetric flask. The resulting solution was immediately sealed with a septum under nitrogen gas and then diluted to the 5.0 mL mark with dry toluene. An aliquot (0.500 mL, 0.100 mmol) of the aldimine stock solution was removed and added to a dry NMR tube containing a 98% pure sample of 2,3-dimethoxybenzladehyde (17.5 mg, 0.103 mmol) dissolved in dry CD_2Cl_2 (0.50 mL). The 2,3-dimethoxybenzaldehyde served as an internal standard for ^{1}H-NMR (300 MHz, pulse delay = 10.00 s) integration. Integration of the Ths-benzaldimine proton (δ 9.54, area = 1.00) versus the internal standard aldehyde proton (δ 10.84, area = 1.31) showed the reaction yield to be 79%.

4.3.2 Organometallic additions to *N*-Bts- and *N*-Ths-benzaldimines

148



5-Methyl-*N*-(1-phenylethyl)-1,3,4-thiadiazole-2-sulfonamide (148) by reaction of 147 with MeLi. To a solution of MeLi (0.36 mL, 0.40 mmol, 1.1 M) in THF (1.50 mL) was added a solution of **147** (1.00 mL, 0.160 mmol, 0.160 M) at -78 ^{o}C under a nitrogen atmosphere. The reddish-brown reaction mixture was stirred at -78 ^{o}C for 2 h, was quenched by the addition of a saturated NH_4Cl solution (5.0 mL) and was warmed to room temperature. After stirring for 10 min, the quenched reaction mixture was partitioned between a saturated brine solution (10.0 mL) and ether (10.0 mL). The ether layer was removed and the aqueous layer was extracted with ether (2 X 10.0 mL). The ether layers were combined, dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO_2 (1:1 EtOAc: hexanes). The product was recrystallized from EtOAc and hexanes, isolated by vacuum filtration, washed with hexanes (15.0 mL) and dried under high vacuum for 1 h to give **148** (10.4 mg, 23%) as a white crystalline solid: Mp 130-131 ^{o}C; IR (KBr) 3065, 2981, 2866, 1468, 1447, 1350, 1208, 1169, 1095, 1016, 768 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 7.27-7.21 (m, 5 H), 5.85 (s, 1 H), 4.74 (q, 1 H, *J* = 6.9 Hz), 2.75 (s, 3 H), 1.55 (d, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 170.9, 169.7, 142.1, 128.9 (2), 127.9, 126.9 (2), 55.3, 23.4, 15.8; HRMS (TOF MS ES+) *m/z* calculated for $C_{11}H_{13}N_3O_2S_2Na$ (M+Na) 306.0347, found 306.0328.

151 

General procedure K. Grignard reactions. 5-Methyl-*N*-(2-methyl-1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (151, Table 3 entry 2). To a solution of 147 (0.385 mL, 0.237 mmol, 0.615 M) in THF (2.00 mL) cooled to -78 ^{o}C under a nitrogen atmosphere, was added a solution of *i*-PrMgCl in ether (0.30 mL, 0.60 mmol, 2.0 M). The resulting red-orange solution was stirred at -78 ^{o}C for 2 h and then quenched with a saturated NH_4Cl solution (5.0 mL). After stirring for 10 min, the quenched reaction mixture was partitioned between a saturated brine solution (10.0 mL) and ether (10.0 mL). The ether layer was removed and the aqueous layer was extracted with ether (2 X 10.0 mL). The ether layers were combined, dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO_2 (1:1 EtOAc: hexanes). The isolated product was recrystallized from a 1:10 mixture of EtOAc and hexanes, washed with hexanes (15.0 mL) and dried under high vacuum for 1 h to give 151 (43.5 mg, 59%) as a white crystalline solid: Mp 164-165 ^{o}C; IR (KBr) 3118, 2975, 2870, 1452, 1352, 1097, 1051, 922, 705 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 7.20-7.18 (m, 3 H), 7.07-7.04 (m, 2 H), 5.90 (d, 1 H, *J* = 8.7 Hz), 4.24 (t, 1 H, *J* = 8.3 Hz), 2.67 (s, 3 H), 2.01 (m, 1 H, *J* = 6.8 Hz), 1.03 (d, 3 H, *J* = 6.6 Hz), 0.76 (d, 3 H, *J* = 6.6 Hz); ^{13}C-NMR δ 170.1, 169.6, 140.0, 128.6 (2), 127.9 (2), 127.6, 65.9, 34.4, 19.6, 19.4, 15.8; HRMS (TOF MS EI+) *m/z* calculated for $C_{13}H_{18}N_3O_2S_2$ (M+H) 312.0840, found 312.0835.

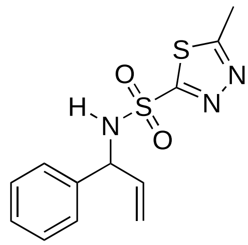
5-Methyl-*N*-(2-methyl-1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (151, Talbe 3 entry 1). According to general procedure K, a solution of **147** (0.385 mL, 0.237 mmol, 0.615 M) and *i*-PrMgCl in THF (0.30 mL, 0.60 mmol, 2.0 M) were reacted in CH_2Cl_2 (2.00 mL) at -78 ^{o}C for 2.5 h. The same workup procedure was followed except CH_2Cl_2 was used in place of ether. The product **151** (41.9 mg, 57%) was isolated as a white crystalline solid.

5-Methyl-*N*-(2-methyl-1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (151, Table 3 entry 3). According to general procedure K, a solution of **147** (0.358 mL, 0.237 mmol, 0.666 M) and *i*-PrMgCl in THF (0.30 mL, 0.60 mmol, 2.0 M) were reacted in THF (2.00 mL) initially at -78 ^{o}C. The reaction mixture was warmed to room temperature for 1.5 h following the addition of *i*-PrMgCl. The product **151** (13.9 mg, 19%) was isolated as a white crystalline solid.

5-Methyl-*N*-(2-methyl-1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (151, Table 3 entry 4). According to general procedure K, a solution of **147** (0.358 mL, 0.316 mmol, 0.883 M) and *i*-PrMgBr in THF (0.40 mL, 0.80 mmol, 2.0 M) were reacted in CH_2Cl_2 (2.00 mL) at -78 ^{o}C for 2.5 h. The same workup procedure was followed except CH_2Cl_2 was used in place of ether. The product **151** was isolated as a white crystalline solid (60.2 mg, 61%).

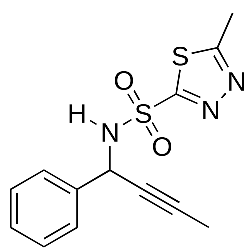
5-Methyl-*N*-(1-phenylethyl)-1,3,4-thiadiazole-2-sulfonamide (148, Table 3 entry 5). According to general procedure K, a solution of **147** (1.00 mL, 0.160 mmol, 0.160 M) and MeMgBr in ether (0.13 mL, 0.40 mmol, 3.0 M) were reacted in THF (2.00 mL) at -78 ^{o}C for 2 h. The resulting product **148** (27.7 mg, 61%) was isolated as a white crystalline solid.

152



5-Methyl-*N*-(1-phenylallyl)-1,3,4-thiadiazole-2-sulfonamide (152, Table 3 entry 6). According to general procedure K, a solution of **147** (1.00 mL, 0.160 mmol, 0.160 M) and vinylMgBr in THF (0.40 mL, 0.40 mmol, 1.0 M) were reacted in THF (1.50 mL) at -78 ^{o}C for 2 h. The resulting product **152** (21.8 mg, 46%) was isolated as a white crystalline solid: Mp 122-123 ^{o}C; IR (KBr) 3141, 3024, 2870, 1443, 1360, 1172, 1054, 939, 702 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 7.32-7.21 (m, 5 H), 6.05-5.94 (m, 1 H), 5.86 (d, 1 H *J* = 7.2 Hz), 5.23-5.17 (m, 3 H), 2.77 (s, 3 H); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 170.2, 169.0, 139.0, 136.7, 129.1 (2), 128.5, 127.7 (2), 117.8, 61.1, 16.0; HRMS (TOF MS ES+) *m/z* calculated for $C_{12}H_{14}N_3O_2S_2$ (M+H) 296.0527, found 296.0506.

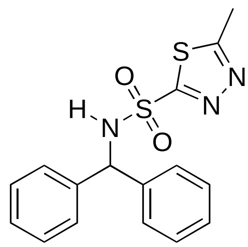
153



5-Methyl-*N*-(1-phenylbut-2-ynyl)-1,3,4-thiadiazole-2-sulfonamide (153, Table 3 entry 7). According to general procedure K, a solution of **147** (1.00 mL, 0.160 mmol, 0.160 M) and 1-propynylMgBr in THF (0.80 mL, 0.40 mmol, 0.50 M) were reacted in THF (1.50 mL) at -78 ^{o}C for 2 h. The resulting product **153** (23.9 mg, 49%) was isolated as a white crystalline solid: Mp 169-170 ^{o}C; IR (KBr) 3055, 2858, 1455, 1351, 1211, 1173, 1019, 734 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 7.50-7.46 (m, 2 H), 7.39-7.33 (m, 3 H), 5.67 (d, 1 H, *J* = 8.4 Hz), 5.43 (dd, 1 H, *J* =

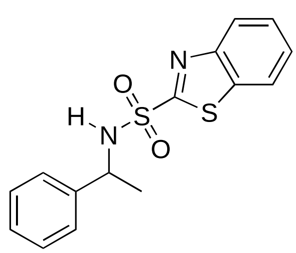
6.6, 2.1 Hz), 2.84 (s, 3 H), 1.72 (d, 3 H, *J* = 2.4 Hz); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 170.5, 168.8, 137.8, 129.3 (2), 129.2, 128.0 (2), 84.7, 75.5, 51.0, 16.2, 3.7; HRMS (TOF MS ES+) *m/z* calculated for $C_{13}H_{13}N_3O_2S_2Na$ (M+Na) 330.0347, found 330.0345.

154



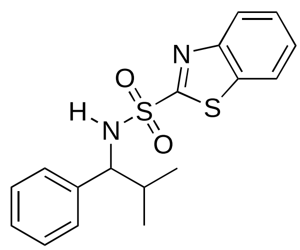
***N*-Benzhydryl-5-methyl-1,3,4-thiadiazole-2-sulfonamide (154, Table 3 entry 8).** According to general procedure K, a solution of **147** (1.00 mL, 0.160 mmol, 0.160 M) and PhMgBr in ether (0.13 mL, 0.40 mmol, 3.0 M) were reacted in THF (1.50 mL) at -78 ^{o}C for 2 h. The resulting product **154** (35.2 mg, 64%) was isolated as a white crystalline solid: Mp 185-186 ^{o}C; IR (KBr) 3174, 3066, 2869, 1447, 1359, 1174, 1045, 702 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 7.33-7.21 (m, 10 H), 6.08 (d, 1 H, *J* = 6.9 Hz), 5.85 (d, 1 H, *J* = 7.5 Hz), 2.73 (s, 3 H); ^{13}C-NMR (CD_2Cl_2, 300 MHz) δ 170.3, 168.8, 140.1 (2), 129.1 (4), 128.3 (2), 127.8 (4), 62.5, 15.9; HRMS (TOF MS ES+) *m/z* calculated for $C_{16}H_{16}N_3O_2S_2$ (M+H) 346.0684, found 346.0671.

155



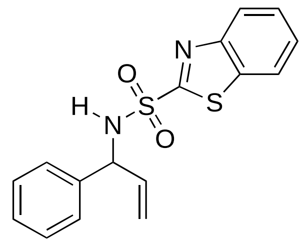
***N*-(1-Phenylethyl)benzo[d]thiazole-2-sulfonamide (155, Table 4 entry 1).** According to general procedure K, **146** (90.7 mg, 0.300 mmol) and MeMgBr in ether (0.20 mL, 0.60 mmol, 3.0 M) were reacted in THF (1.80 mL) at -78 ^{o}C for 2.5 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO_2 (3:1 hexanes: EtOAc). The product **155** (83.2 mg, 87%) was isolated as a white crystalline solid: Mp 144-145 ^oC; IR (KBr) 3116, 2973, 2874, 1477, 1350, 1163, 1062, 982, 764 cm^{-1}; ^1H-NMR (CD_2Cl_2, 300 MHz) δ 8.07 (dd, 1 H, *J* = 8.0, 1.5 Hz), 7.92 (dd, 1 H, *J* = 8.1, 1.2 Hz), 7.57 (dt, 2 H, *J* = 7.4, 1.2 Hz), 7.24 (d, 2 H, *J* = 6.9 Hz), 7.15-7.04 (m, 3 H), 6.35 (d, 1 H, *J* = 7.2 Hz), 4.80 (quintet, 1 H, *J* = 7.1 Hz), 1.53 (d, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 167.3, 152.8, 142.2, 137.0, 128.9 (2), 128.1, 128.0, 127.8, 126.9 (2), 125.3, 122.6, 55.3, 23.7; HRMS (TOF MS ES+) *m/z* calculated for $C_{15}H_{14}N_2O_2S_2Na$ (M+Na) 341.0394, found 341.0389.

156

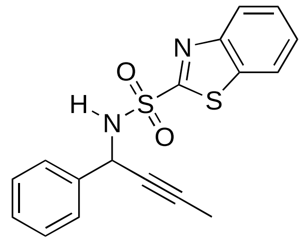


***N*-(2-Methyl-1-phenylpropyl)benzo[d]thiazole-2-sulfonamide (156, Table 4 entry 2).** According to general procedure K, **146** (90.7 mg, 0.300 mmol) and *i*-PrMgCl in ether (0.30 mL, 0.60 mmol, 2.0 M) were reacted in THF (1.70 mL) at -78 ^oC for 2.5 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO_2 (3:1 hexanes: EtOAc) to give **156** (90.1 mg, 87%) as a white crystalline solid: Mp 157-158 ^oC; IR (KBr) 3265, 2959, 2872, 1455, 1336, 1041, 915, 771 cm^{-1}; ^1H-NMR (CD_2Cl_2, 300 MHz) δ 7.99 (dd, 1 H, *J* = 8.1, 1.2 Hz), 7.87 (dd, 1 H, *J* = 7.8, 1.2 Hz), 7.53 (dt, 2 H, *J* = 7.2, 1.2 Hz), 7.02-6.93 (m, 5 H), 5.58 (d, 1 H, *J* = 8.7 Hz), 4.26 (t, 1 H, *J* = 8.1 Hz), 2.01 (m, 1 H, *J* = 6.9 Hz), 1.00 (d, 3 H, *J* = 6.6 Hz), 0.75 (d, 3 H, *J* = 6.6 Hz); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 166.7, 152.8, 140.0, 137.0, 128.4 (2), 127.9, 127.7, 127.6 (2), 125.3, 122.5, 65.8, 34.8, 19.7, 19.3; HRMS (TOF MS ES+) *m/z* calculated for $C_{17}H_{19}N_2O_2S_2$ (M+H) 347.0888, found 347.0862.

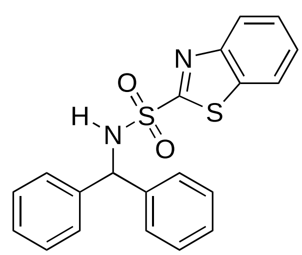
157



***N*-(1-Phenylallyl)benzo[d]thiazole-2-sulfonamide (157, Table 4 entry 3).** According to general procedure K, **146** (90.7 mg, 0.300 mmol) and vinylMgBr in THF (0.60 mL, 0.60 mmol, 1.0 M) were reacted in THF (1.20 mL) at -78 ^{o}C for 3 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO_2 (3:1 hexanes: EtOAc) to give **157** (84.5 mg, 85%) as a white crystalline solid: Mp 141-142 ^{o}C; IR (KBr) 3117, 2872, 1450, 1346, 1161, 1039, 930, 765 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.07 (d, 1 H, *J* = 8.1 Hz), 7.90 (d, 1 H, *J* = 7.2 Hz), 7.54 (dt, 2 H, *J* = 7.2, 1.5 Hz), 7.16-7.12 (m, 5 H), 5.93 (m, 1 H), 5.52 (s, 1 H), 5.27-5.12 (m, 3 H); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 167.1, 152.9, 139.5, 137.2, 137.0, 129.1 (2), 128.5, 128.1, 127.9 (3), 125.4, 122.7, 117.8, 61.4; HRMS (TOF MS ES+) *m/z* calculated for $C_{16}H_{15}N_2O_2S_2$ (M+H) 331.0575, found 331.0549.

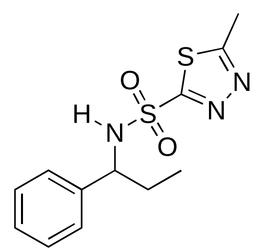
158 

***N*-(1-Phenylbut-2-ynyl)benzo[d]thiazole-2-sulfonamide (158, Table 4 entry 4).** According to general procedure K, **146** (90.7 mg, 0.300 mmol) and 1-propynylMgBr in THF (1.2 mL, 0.60 mmol, 0.50 M) were reacted in THF (1.30 mL) at -78 ^{o}C for 3 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO_2 (3:1 hexanes: EtOAc) to give **158** (72.8 mg, 71%) as a white crystalline solid: Mp 155.5-156.5 ^{o}C; IR (KBr) 3073, 2866, 1454, 1351, 1168, 1038, 764 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 8.14 (d, 1 H, *J* = 7.2 Hz), 8.00 (d, 1 H, *J* = 7.5 Hz), 7.61-7.55 (m, 2 H), 7.48 (d, 2 H, *J* = 6.6 Hz), 7.28-7.25 (m, 3 H), 6.18 (s, 1 H), 5.46 (s, 1 H), 1.29 (s, 3 H); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 166.6, 153.2, 138.0, 137.3, 129.2 (2), 129.1, 128.3, 128.0 (3), 125.5, 122.8, 84.5, 75.3, 51.0, 3.3; HRMS (TOF MS ES+) *m/z* calculated for $C_{17}H_{14}N_2O_2S_2Na$ (M+Na) 365.0394, found 365.0358.

159 

***N*-Benzhydrylbenzo[d]thiazole-2-sulfonamide (159, Table 4 entry 5).** According to general procedure K, **146** (90.7 mg, 0.300 mmol) and PhMgBr in ether (0.20 mL, 0.60 mmol, 3.0 M) were reacted in THF (1.80 mL) at -78 ^{o}C for 3 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO_2 (3:1 hexanes: EtOAc). The product was recrystallized from a 5:1 mixture of hexanes and EtOAc to afford **159** (88.4 mg, 77%) as a white crystalline solid: Mp 187-188 °C; KBr (IR) 3156, 3068, 1448, 1350, 1167, 1045, 945 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 8.06 (dd, 1 H, *J* = 8.4, 1.2 Hz), 7.93 (dd, 1 H, *J* = 8.1, 1.2 Hz), 7.58 (dt, 2 H, *J* = 7.5, 1.2 Hz), 7.19-7.15 (m, 10 H), 5.87 (s, 2 H); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 166.3, 152.4, 139.9 (2), 136.7, 128.7 (4), 128.0 (2), 127.7 (5), 127.4, 125.2, 122.1, 62.3; HRMS (TOF MS ES+) *m/z* calculated for $C_{20}H_{16}N_2O_2S_2Na$ (M+Na) 403.0551, found 403.0520.

160

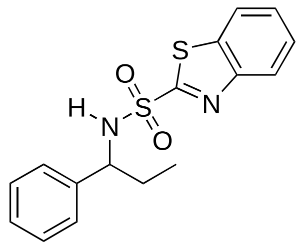


General procedure L. Diethylzinc Reactions. 5-Methyl-N-(1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (160, Table 5 entry 2). To a solution of **147** (2.00 mL, 0.312 mmol, 0.156 M) in toluene (2.50 mL) was slowly added a solution of Et_2Zn in toluene (0.48 mL, 0.48 mmol, 1.0 M). The resulting dark red-orange reaction mixture was stirred at room temperature for 2.5 h and quenched with a saturated NH_4Cl solution (5.0 mL). After stirring for 10 min, the quenched mixture was partitioned between a saturated NH_4Cl solution (10.0 mL) and ether (20.0 mL). The aqueous layer was extracted with ether (2 X 20.0 mL). The combined ether layers were washed with a saturated brine solution (5.0 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO_2 (1:1 EtOAc: hexanes). The product was recrystallized from a 1:10 mixture of EtOAc and hexanes, isolated by vacuum filtration, washed with hexanes (15.0 mL) and dried under high vacuum for 1 h to give **160** (48.8 mg, 53%) as a white crystalline solid: Mp 144-145 °C; IR (KBr) 3105, 2941, 2864, 1458, 1350, 1167, 1100, 1011, 913, 760 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 7.23-7.13 (m, 5 H), 6.23 (d, 1 H, J = 7.2 Hz), 4.45 (q, 1 H, J = 7.4 Hz), 2.70 (s, 3 H), 2.01-1.72 (m, 2 H, J = 7.3 Hz), 0.85 (t, 3 H, J = 7.2 Hz); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 170.3, 169.8, 140.9, 129.1 (2), 128.1, 127.7 (2), 61.6, 30.8, 16.0, 10.9; HRMS (TOF MS ES+) *m/z* calculated for $C_{12}H_{15}N_3O_2S_2Na$ (M+Na) 320.0503, found 320.0493.

5-Methyl-*N*-(1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (160, Table 5 entry 1). According to general procedure L, a solution of **147** in THF (2.00 mL, 0.312 mmol, 0.156 M) and a solution of Et_2Zn in toluene (0.48 mL, 0.48 mmol, 1.0 M) were reacted in THF (2.00 mL) at room temperature for 4 h. The product **160** (39.9 mg, 43%) was isolated as a white crystalline solid.

5-Methyl-*N*-(1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (160, Table 5 entry 3). According to general procedure L, a solution of **147** in toluene (2.00 mL, 0.316 mmol, 0.158 M) and a solution of Et_2Zn in toluene (0.80 mL, 0.80 mmol, 1.0 M) were reacted in toluene (2.80 mL) at room temperature for 2.5 h. The product **160** (65.7 mg, 70%) was isolated as a white crystalline solid.

5-Methyl-*N*-(1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (160, Table 5 entry 4). According to general procedure L, a solution of **147** in toluene (1.00 mL, 0.158 mmol, 0.158 M) and a solution of Et_2Zn in toluene (0.20 mL, 0.20 mmol, 1.0 M) were reacted in toluene (2.20 mL) at -78 ^oC for 33 h. The product **160** (28.6 mg, 61%) was isolated as a white crystalline solid.

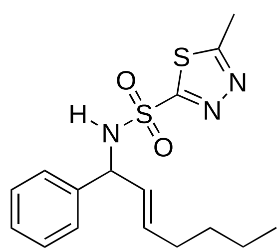
161 

***N*-(1-Phenylpropyl)benzo[d]thiazole-2-sulfonamide (161, Table 5 entry 5).** According to general procedure L, **146** (90.7 mg, 0.300 mmol) and a solution of Et_2Zn in toluene (0.60 mL, 0.60 mmol, 1.0 M) were reacted in THF (1.40 mL) at room temperature for 4 h. The same workup procedure was followed except the crude reaction mixture was chromatographed on SiO_2 (3:1, hexanes: EtOAc). The product was recrystallized from a 5:1 mixture of hexanes and CH_2Cl_2 to afford **161** (39.6 mg, 40%) as a white crystalline solid: Mp 126.5-127.5 ^{o}C; IR (KBr) 3171, 2967, 2873, 1456, 1357, 1163, 1037, 766 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, 1 H, *J* = 7.4 Hz), 7.82 (d, 1 H, *J* = 7.2 Hz), 7.50 (dt, 2 H, *J* = 7.6, 1.8 Hz), 7.09-6.95 (m, 5 H), 5.97 (s, 1 H), 4.48 (d, 1 H, *J* = 4.2 Hz), 1.97-1.73 (m, 2 H, *J* = 7.5 Hz), 0.81 (t, 3 H, *J* = 7.5 Hz); ^{13}C-NMR δ 166.6, 152.1, 139.8, 136.4, 128.2 (2), 127.4, 127.3, 127.1, 126.7 (2), 124.8, 121.9, 60.8, 30.3, 10.5; HRMS (TOF MS ES+) *m/z* calculated for $C_{16}H_{16}N_2O_2S_2Na$ (M+Na) 355.0551, found 355.0557.

***N*-(1-Phenylpropyl)benzo[d]thiazole-2-sulfonamide (161, Table 5 entry 6).** According to general procedure L, **146** (90.7 mg, 0.300 mmol) and a solution of Et_2Zn in toluene (0.60 mL, 0.60 mmol, 1.0 M) were reacted in toluene (1.40 mL) at room temperature for 4 h. The same workup procedure was followed except that the crude reaction mixture was chromatographed on SiO_2 (3:1, hexanes: EtOAc). The product was recrystallized from a 5:1 mixture of hexanes and CH_2Cl_2 to afford **161** (57.8 mg, 58%) as a white crystalline solid.

Preparation of zirconocene hydrochloride from $LiAlH_4$ (120).[58] To a solution of Cp_2ZrCl_2 (5.00g, 17.1 mmol) in THF (45.0 mL) under a nitrogen atmosphere was added a solution of $LiAlH_4$ in ether (4.4 mL, 4.4 mmol, 1.0 M) slowly *via* a syringe pump over a 45 min period. The reaction mixture was stirred for an additional 30 min. The resulting heterogeneous solution was filtered through a Schlenk filter under vacuum and the resulting solid was sequentially washed with THF (4 X 10 mL), CH_2Cl_2 (1 X 10 mL), THF (1 X 10 mL) and ether (1 X 5.0 mL). The product was then dried under high vacuum in the dark for 15 h to yield **120** (3.16 g, 72%) as a white solid.

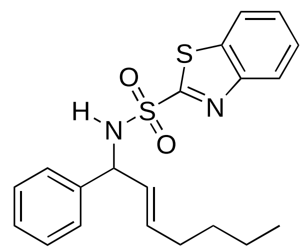
163



General procedure M. Addition of alkenylzinc reagents. (*E*)-5-Methyl-*N*-(1-phenylhept-2-enyl)-1,3,4-thiadiazole-2-sulfonamide (163, Table 6 entry 1). To a suspension of $Cp_2Zr(H)Cl$ (163.8 mg, 0.636 mmol) in CH_2Cl_2 (2.00 mL) under a nitrogen atmosphere was added 1-hexyne (0.0820 mL, 0.720 mmol) at room temperature. After 15 min, the resulting clear pale yellow solution was cooled to -78 ^{o}C and a Me_2Zn solution in toluene (0.30 mL, 0.60 mmol, 2.0 M) was added. This mixture was warmed to room temperature for 1 h and then slowly added to a solution of **147** in toluene (2.00 mL, 0.316 mmol, 0.158 M). The resulting dark red-orange reaction mixture was stirred at room temperature for 2.5 h and then quenched by the addition of a saturated NH_4Cl solution (5.0 mL). After stirring for 1 h, the quenched mixture was partitioned between a saturated NH_4Cl solution (10.0 mL) and ether (20.0 mL). The aqueous layer was extracted with ether (2 X 20.0 mL). The ether layers were combined, washed with a saturated brine solution (5.0 mL), filtered through a 1 in pad of SiO_2 which was washed with additional EtOAc (20.0 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO_2 (1:2 EtOAc: hexanes). The product was recrystallized from a 5:1 mixture of hexanes and CH_2Cl_2, isolated by vacuum filtration, washed with hexanes (15.0 mL) and dried under high vacuum for 10 h to give **163** (38.4 mg, 35%) as a white crystalline solid: Mp 88.5-90 ^oC; IR (KBr) 3106, 2956, 2927, 2871, 1453, 1354, 1172, 1085, 979 cm^{-1}; ^1H-NMR (CD_2Cl_2, 300 MHz) δ 7.33-7.21 (m, 5 H), 6.30 (d, 1 H, *J* = 7.8 Hz), 5.58 (m, 2 H), 5.15 (dt, 1 H, *J* = 6.3, 2.7 Hz), 2.75 (s, 3 H), 1.95 (m, 2 H), 1.26 (septet, 4 H, *J* = 3.6 Hz), 0.86 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 170.3, 169.6, 140.2, 135.5, 129.2 (2), 128.5, 128.3, 127.8 (2), 61.2, 32.3, 31.5, 22.7, 16.1, 14.2; HRMS (TOF MS ES+) *m/z* calculated for $C_{16}H_{21}N_3O_2S_2Na$ (M+Na) 374.0937, found 374.0964.

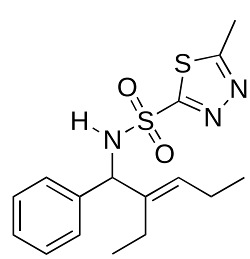
(*E*)-5-Methyl-*N*-(1-phenylhept-2-enyl)-1,3,4-thiadiazole-2-sulfonamide (163, Table 6 entry 2). According to general procedure M, $Cp_2Zr(H)Cl$ (163.8 mg, 0.636 mmol), 1-hexyne (0.0820 mL, 0.720 mmol), Me_2Zn (0.30 mL, 0.60 mmol, 2.0 M) and a solution of **147** in toluene (2.00 mL, 0.320 mmol, 0.160 M) were combined in CH_2Cl_2 (2.00 mL) for 10 h. The same workup procedure was performed except that the crude reaction mixture was chromatographed on SiO_2 (3:1 hexanes: EtOAc) to give **163** (64.4 mg, 57%) as a white crystalline solid.

164



(*E*)-*N*-(1-Phenylhept-2-enyl)benzo[*d*]thiazole-2-sulfonamide (164, Table 6 entry 3). According to general procedure M, $Cp_2Zr(H)Cl$ (123.0 mg, 0.477 mmol), 1-hexyne (0.0620 mL, 0.540 mmol), Me_2Zn (0.225 mL, 0.450 mmol, 2.0 M), and **146** (90.7 mg, 0.300 mmol) were combined in CH_2Cl_2 (1.50 mL) for 4.5 h. The same workup procedure was performed except that the crude reaction mixture was chromatographed on SiO_2 (4:1 hexanes: EtOAc). The methyl addition product **146** (12.0 mg, 13%) was isolated as a white solid. The desired product **164** (82.3 mg, 71%) was isolated as a white crystalline solid: Mp 98.5-99.5 ^{o}C; IR (KBr) 3131, 2923, 2872, 1452, 1351, 1168, 1036, 986, 756 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.11 (d, 1 H, *J* = 7.8 Hz), 7.92 (d, 1 H, *J* = 7.5 Hz), 7.57 (quintet, 2 H, *J* = 8.4 Hz), 7.27-7.14 (m, 5 H), 5.60-5.50 (m, 3 H), 5.20 (t, 1 H, *J* = 6.8 Hz), 1.78 (q, 2 H, *J* = 6.3 Hz), 1.12-1.02 (m, 4 H), 0.75 (t, 3 H, *J* = 6.6 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 152.9, 140.1, 137.1, 135.3, 129.1 (2), 128.3 (2), 128.0, 127.8, 127.7 (2), 125.5, 122.5, 61.1, 32.2, 31.3, 22.7, 14.4; HRMS (TOF MS ES+) *m/z* calculated for $C_{20}H_{22}N_2O_2S_2Na$ (M+Na) 409.1020, found 409.1022.

165



(*E*)-*N*-(2-Ethyl-1-phenylpent-2-enyl)-5-methyl-1,3,4-thiadiazole-2-sulfonamide (165, Table 6 entry 4). According to general procedure M, $Cp_2Zr(H)Cl$ (81.9 mg, 0.138 mmol), 1-hexyne

(0.0410 mL, 0.360 mmol), Me_2Zn (0.15 mL, 0.15 mmol, 1.0 M) and a solution of **147** in toluene (1.00 mL, 0.150 mmol, 0.150 M) were combined in CH_2Cl_2 (1.00 mL) for 2 h. The same workup procedure was performed except that the crude reaction mixture was chromatographed on SiO_2 (1:1 hexanes: EtOAc) to give **165** (25.1 mg, 47%) as a white solid: Mp 98.5-99.5 ^oC; IR (KBr) 3122, 2965, 2872, 1455, 1357, 1170, 1043, 940 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 7.29-7.81 (m, 5 H), 5.80 (d, 1 H, *J* = 8.1 Hz), 5.31 (t, 1 H, *J* = 7.1 Hz), 5.19 (d, 1 H, *J* = 8.1 Hz), 2.77 (s, 3 H), 2.13-1.86 (m, 4 H), 0.90 (dt, 6 H, *J* = 7.5, 3.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 169.5, 168.9, 138.9, 138.5, 130.8, 128.7 (2), 127.9, 127.8 (2), 62.9, 22.1, 21.1, 15.9, 14.3, 13.7; HRMS (TOF MS ES+) *m/z* calculated for $C_{16}H_{21}N_3O_2S_2Na$ (M+Na) 374.0973, found 374.0951.

Synthesis of (*E*)-*N*-(1-phenylhept-2-enyl)benzo[d]thiazole-2-sulfonamide (164) by the addition of (*E*)-hex-1-enyldimethylaluminum. To a suspension of $Cp_2Zr(H)Cl$ (87.7 mg, 0.340 mmol) in CH_2Cl_2 (1.00 mL) under a nitrogen atmosphere was added 1-hexyne (0.0402 mL, 0.350 mmol) at room temperature. After 15 min, the volatiles were removed from the zirconocene solution under high vacuum and the resulting yellow oil was dissolved in toluene (1.00 mL). This mixture was cooled to 0 ^oC and a solution of Me_3Al in toluene (0.34 mL, 0.34 mmol, 1.0 M) was added. The reaction mixture was warmed to room temperature for 1 h and then canulated under nitrogen gas into a solution of **146** (45.5 mg, 0.150 mmol) in toluene (0.50 mL). The resulting yellow mixture was stirred at room temperature for 20 h and then quenched by the addition of a saturated NH_4Cl solution (5.0 mL). After 30 min, the quenched mixture was partitioned between a saturated brine solution (10.0 mL) and ether (20.0 mL). The aqueous layer was extracted with ether (2 X 20.0 mL). The combined ether layers were filtered through a 1 in pad of SiO_2 which was washed with additional EtOAc (20.0 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO_2 (1:3 EtOAc: hexanes) to give **164** (9.4 mg, 16%) as a clear oil.

General procedure N. Rhodium(I) catalyzed additions of alkenyl- zirconocnes. (*E*)-*N*-(1-Phenylhept-2-enyl)benzo[d]thiazole-2-sulfonamide (164, Table 7 entry 3). To a suspension of $Cp_2Zr(H)Cl$ (87.7 mg, 0.340 mmol) in CH_2Cl_2 (1.00 mL) under a nitrogen atmosphere was added 1-hexyne (0.0402 mL, 0.350 mmol) at room temperature. After 15 min, the volatiles were removed from the zirconocene solution under high vacuum and the resulting yellow oil was dissolved in degassed dioxane (0.50 mL). The zirconocene solution was then canulated under nitrogen gas into a premixed solution of (1*S*,4*S*,8*S*)-5-benzyl-8-methoxy-1,8-dimethyl-2-(2'-methylpropyl)-bicyclo[2.2.2]octa-2,5-diene **168** (4.66 mg, 0.0150 mmol) and $[RhCl(ethylene)_2]_2$ (2.9 mg, 0.0075 mmol) in degassed dioxane (0.50 mL) at room temperature. To this mixture was then added, *via* cannulation under nitrogen gas, a solution of *R*,*R*-MeDUPHOS **167** (4.6 mg, 0.015 mmol) and **146** (45.4 mg, 0.150 mmol) in degassed dioxane (0.50 mL). The reaction mixture was stirred under nitrogen gas for 20 h and then quenched with a saturated NH_4Cl solution (5.0 mL) with stirring for 50 min. The quenched mixture was partitioned between a saturated brine solution (10.0 mL) and ether (20.0 mL). The aqueous layer was extracted with ether (2 X 20.0 mL). The combined ether layers were filtered through a 2 in pad of SiO_2 which was washed with additional EtOAc (25.0 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO_2 (1:3 EtOAc: hexanes) to give **164** (4.30 mg, 7.0%) as a slightly impure yellow oil. A sample of this product was dissolved in a 99:1 mixture of hexanes and *i*-PrOH for chiral HPLC (chiralcel-OD column; 99:1

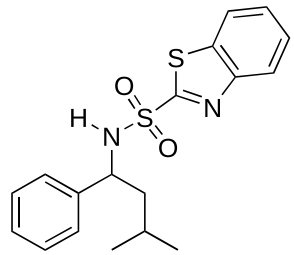
hexanes : *i*-PrOH; flow rate 1.0 mL/min; UV detection at 258 nm; product enantiomer retention times of 39.3 and 59.7 min and areas of 61.4 and 16.2) which showed 58% ee.

(*E*)-*N*-(1-Phenylhept-2-enyl)benzo[d]thiazole-2-sulfonamide (164, Table 7 entry 2). According to general procedure N, a solution of $Cp_2Zr(H)Cl$ (58.0 mg, 0.225 mmol), 1-hexyne (0.0287 mL, 0.250 mmol), $[RhCl(cod)]_2$ (2.9 mg, 0.0075 mmol), **168** (4.66 mg, 0.0150 mmol) and **146** (45.4 mg, 0.150 mmol) were combined in degassed dioxane for 24 h. No product was detected by TLC.

(*E*)-*N*-(1-Phenylhept-2-enyl)benzo[d]thiazole-2-sulfonamide (164, Table 7 entry 2). According to general procedure N, a solution of $Cp_2Zr(H)Cl$ (87.7 mg, 0.340 mmol) and 1-hexyne (0.0340 mL, 0.300 mmol) were combined, after a solvent switch to toluene (0.50 mL), with a solution of $[RhCl(cod)]_2$ (3.7 mg, 0.0078 mmol), *R*,*R*-MeDUPHOS (4.6 mg, 0.015 mmol) and **146** (45.4 mg, 0.150 mmol) in toluene (1.00 mL) for 16 h. The product **164** (11.0 mg, 19%) was obtained as a clear oil in 1% ee (determined by chiral HPLC analysis).

Synthesis of Tetrakis[acetonitrile]copper(I) tetrafluoroborate (169).[60] To a suspension of Cu_2O (0.750 g, 5.24 mmol) in acetonitrile (14.0 mL) was slowly added a solution of HBF_4 (2.77 mL, 15.1 mmol, 48 %) over a 5 min period under a nitrogen atmosphere. After 10 min, the reaction mixture was quickly filtered and transferred to a dry 50 mL round bottom flask. This solution was then cooled to -30 ^oC for 20 min under nitrogen gas and a crystalline solid formed. The resulting solid was isolated by filtration, washed with ether (20 mL) and dried under high vacuum for 3 h. The resulting $Cu(CH_3CN)_4BF_4$ (1.24 g, 75%) was isolated as a clear crystalline solid: Mp 160-162 ^oC.

General procedure O. Copper(I) catalyzed additions of alkenylzirconocenes. (*E*)-*N*-(1-Phenylhept-2-enyl)benzo[d]thiazole-2-sulfonamide (164, Table 8 entry 1). To a solution of Cp_2ZrCl_2 (49.7 mg, 0.170 mmol) in THF (0.50 mL) cooled to 0 ^oC under a nitrogen atmosphere was added a solution of DIBAL-H in hexanes (0.17 mL, 0.17 mmol, 1.0 M) over a 15 min period. After 30 min at 0 ^oC, a white $Cp_2Zr(H)Cl$ suspension formed and 1-hexyne (0.0172 mL, 0.150 mmol) was added. This mixture was warmed to room temperature and became homogenous after 1 h. The mixture was then added to a premixed solution of **146** (45.4 mg, 0.150 mmol) and $Cu(CH_3CN)_4BF_4$ (2.40 mg, 0.00750 mmol) in THF (0.50 mL) at 0 ^oC under a nitrogen atmosphere. The reaction mixture was warmed to room temperature and stirred for 2 d. The reaction was quenched by the addition of a saturated NH_4Cl solution (5.0 mL) with stirring for 50 min, and partitioned between a saturated brine solution (10.0 mL) and ether (20.0 mL). The aqueous layer was extracted with ether (2 X 20.0 mL). The combined ether layers were filtered through a 1 in pad of SiO_2 which was washed with additional EtOAc (25.0 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO_2 (1:3 EtOAc: hexanes) to give **164** (1.5 mg, 3%) as an impure clear oil.

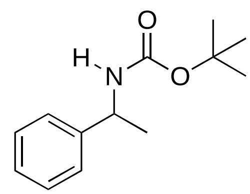
170 

***N*-(3-Methyl-1-phenylbutyl)benzo[d]thiazole-2-sulfonamide (170, Table 8 entry 2).** According to general procedure O, Cp_2ZrCl_2 (99.4 mg, 0.340 mmol), DIBAL-H (0.34 mL, 0.34 mmol, 1.0 M), 1-hexyne (0.0344 mL, 0.300 mmol), $Cu(CH_3CN)_4BF_4$ (4.80 mg, 0.0150 mmol) and **146** (45.4 mg, 0.150 mmol) were combined, and then BF_3•OEt_2 (0.0565 mL, 0.450 mmol) was added. The resulting purple reaction mixture was stirred at room temperature for 18 h to give **170** (34.0 mg, 63%) as a white crystalline solid: Mp 122-123 ^{o}C; IR (KBr) 3182, 2957, 1455, 1426, 1356, 1170, 1052, 930, 763 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.01 (dd, 1 H, *J* = 8.1, 1.2 Hz), 7.84 (dd, 1 H, *J* = 7.2, 1.5 Hz), 7.52 (dt, 2 H, *J* = 7.5, 1.2 Hz), 7.08 (dd, 2 H, *J* = 8.1, 1.2 Hz), 7.01-6.93 (m, 3 H), 5.74 (d, 1 H, *J* = 8.1 Hz), 4.65 (q, 1 H, *J* = 7.5 Hz), 1.82-1.73 (m, 1 H), 1.66-1.50 (m, 2 H), 0.88 (t, 6 H, *J* = 7.8 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.5, 152.1, 140.3, 136.5, 128.2 (2), 127.4, 127.3, 127.1, 126.6 (2), 124.9, 121.9, 57.7, 46.5, 24.6, 22.3, 22.2; HRMS (TOF MS ES+) *m/z* calculated for $C_{18}H_{20}N_2O_2S_2Na$ (M+Na) 383.0864, found 383.0868.

(*E*)-*N*-(1-Phenylhept-2-enyl)benzo[d]thiazole-2-sulfonamide (164, Table 8 entry 3). According to general procedure O, Cp_2ZrCl_2 (99.4 mg, 0.340 mmol), DIBAL-H (0.34 mL, 0.34 mmol, 1.0 M), 1-hexyne (0.0344 mL, 0.300 mmol), and **146** (45.4 mg, 0.150 mmol) were combined and then $BF_3.OEt_2$ (0.0565 mL, 0.450 mmol) was added. The reaction mixture was stirred at room temperature for 16 h after which time no product **164** or **170** was noted by TLC.

4.3.3 Deprotection of *N*-Ths and *N*-Bts-sulfonamides

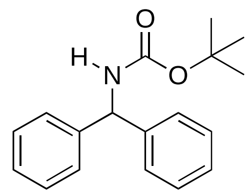
171



General procedure P. Deprotection of sulfonamides with H_3PO_2 and re-protection with Boc_2O. *Tert*-butyl 1-phenylethylcarbamate (171, Table 9 entry 1).[78] To a solution of sulfonamide **148** (200 mg, 0.706 mmol) in refluxing THF (9.0 mL) was slowly added a H_3PO_2 solution (2.3 mL, 21 mmol, 50%) via syringe pump over a 3.5 h period under a nitrogen atmosphere. The reaction mixture was heated at reflux for an additional 1 h after all of the H_3PO_2 solution was added. After this time, the reaction mixture was cooled to room temperature, diluted with deionized water (5.0 mL) and washed with hexanes (5.0 mL). The hexane layer was then washed with an HCl solution (2.0 mL, 0.50 M) and the aqueous layers were combined, cooled to 0 ^{o}C, basified to a pH > 13 with a NaOH solution (10 mL, 5.0 M) and extracted with ether (3 X 30 mL). The combined ether layers were washed with a saturated brine solution (5.0 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting clear oil was then dissolved in CH_3CN (3.0 mL) and reacted with Boc_2O (192 mg, 0.883 mmol) in the presence of Et_3N (0.0980 mL, 0.760 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h and the solvent was then removed by rotary evaporation. The crude residue was chromatographed on SiO_2 (20:1, hexanes: EtOAc to remove the excess Boc_2O followed by 5:1 hexanes: EtOAc) to give **171** (132 mg, 84%) as a clear crystalline solid: Mp 87-88 ^{o}C; IR (KBr) 3383, 2983, 1687, 1519, 1367, 1248, 1171, 1059, 756 cm^{-1}; ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ 7.32-7.23 (m, 5 H) 4.95 (s, 1 H), 4.74 (s, 1 H), 1.43 (s, 3 H), 1.41 (s, 9 H);

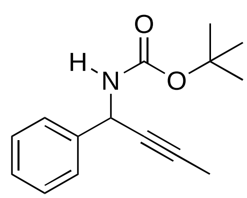
^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 155.5, 145.2, 129.0 (2), 127.5, 126.4 (2), 79.6, 50.8, 28.7 (3), 23.3; HRMS (TOF MS EI+) *m/z* calculated for $C_{13}H_{19}NO_2Na$ (M+Na) 244.1313, found 244.1309.

172



***Tert*-butyl benzhydrylcarbamate (172, Table 9 entry 2).**[79] According to general procedure P, sulfonamide **154** (50.0 mg, 0.145 mmol) and H_3PO_2 (0.48 mL, 4.3 mmol, 50%) were combined in THF (2.0 mL) for 4.5 h. The isolated crude amine was then combined with Boc_2O (39.5 mg, 0.181 mmol) and Et_3N (0.020 mL, 0.145 mmol) in CH_3CN (1.5 mL) for 1 h to give **172** (34.2 mg, 83%) as a clear crystalline solid: Mp 123-124 ^{o}C; IR (KBr) 3373, 2978, 1690, 1520, 1363, 1173, 1022, 744 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.36-7.25 (m, 10 H), 5.93 (s, 1 H), 2.20 (s, 1 H), 1.46 (s, 9 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 155.0, 142.1 (2), 128.6 (4), 127.3 (2), 127.2 (4), 79.9, 58.5, 28.4 (3); HRMS (TOF MS ES+) *m/z* calculated for $C_{18}H_{21}NO_2Na$ (M+Na) 306.1470, found 306.1468.

173



***Tert*-butyl 1-phenylbut-2-ynylcarbamate (173, Table 9 entry 3).** According to general procedure P, sulfonamide **153** (70.0 mg, 0.228 mmol) and H_3PO_2 (0.75 mL, 6.8 mmol) were combined in THF (5.0 mL) for 4.5 h. The isolated crude amine was then combined with Boc_2O

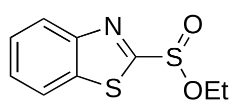
(59.7 mg, 0.274 mmol) and Et_3N (0.0318 mL, 0.228 mmol) in CH_3CN (3.0 mL) for 1 h to give **173** (49.5 mg, 88%) as a white crystalline solid: Mp 90-91 ^oC; IR (KBr) 3318, 2978, 1713, 1682, 1523, 1365, 1246, 1157, 1021, 880 cm^{-1}; ^1H-NMR (CD_2Cl_2, 300 MHz) δ 7.47 (d, 2 H, *J* = 6.0 Hz), 7.38-7.27 (m, 3 H), 5.55 (s, 1 H), 5.18 (s, 1 H), 1.88 (s, 3 H), 1.44 (s, 9 H); ^{13}C-NMR (CD_2Cl_2, 75 MHz) δ 155.1, 140.8, 128.9 (2), 128.1, 127.1 (2), 81.1, 80.1, 77.9, 46.8, 28.4 (3), 3.6; HRMS (TOF MS ES+) *m/z* calculated for $C_{15}H_{19}NO_2Na$ (M+Na) 268.1313, found 268.1302.

General procedure Q. Deprotection of sulfonamides with SmI_2 and re-protection with Boc_2O. *Tert*-butyl 1-phenylethylcarbamate (171, Table 10 entry 1). To a solution of sulfonamide **148** (100 mg, 0.353 mmol) in degassed THF (1.0 mL) was added a solution of SmI_2 in THF (25 mL, 2.5 mmol, 0.10 M) slowly over a 1 h period at room temperature under a nitrogen atmosphere. The reaction mixture turned from a dark blue color to yellow over 8 h. The mixture was quenched by pouring it into KOH (2.0 g) and ice (20 g). This solution was warmed to room temperature and extracted with ether (3 X 50 mL). The combined ether layers were concentrated by rotary evaporation (13 ^oC). The resulting yellow residue was acidified with an HCl solution (10 mL, 1.0 M). The aqueous solution was washed with hexanes (1 X 10 mL), cooled to 0 ^oC and basified with a NaOH solution (5.0 mL, 5.0 M) to pH > 13. The basic mixture was extracted with ether (3 X 25 mL). The combined ether layers were washed with a saturated brine solution (1 X 5.0 mL), dried (Na_2SO_4) and concentrated by rotary evaporation (13 ^oC). The crude amine was then dissolved in CH_3CN (1.5 mL) and reacted with Boc_2O (96.3 mg, 0.353 mmol) in the presence of Et_3N (0.0490 mL, 0.353 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1.5 h and the solvent was then removed by rotary evaporation. The crude residue was chromatographed on SiO_2 (10:1 hexanes: EtOAc) to give **171** (65.4 mg, 84%) as a clear crystalline solid.

***Tert*-butyl 1-phenylethylcarbamate (171, Table 10 entry 2).** According to general procedure Q, sulfonamide **155** (100 mg, 0.314 mmol) and SmI_2 (28 mL, 2.8 mmol, 0.10 M) were reacted in THF for 8 h. The isolated crude amine was then combined with Boc_2O (85.7 mg, 0.393 mmol) and Et_3N (0.0438 mL, 0.314 mmol) in CH_3CN (1.5 mL) for 1.5 h to give **171** (57.4 mg, 83%) as a white crystalline solid.

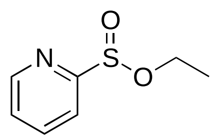
4.3.4 Addition of organometallic reagents to (*E*)-*N*-benzylidenebenzo[d]thiazole-2-sulfinamide and (*E*)-*N*-benzylidenepyridine-2-sulfinamide.

175

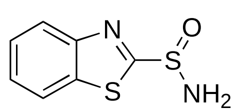


Ethyl benzo[d]thiazole-2-sulfinate (175). To a solution of benzo[d]thiazole-2-thiol **142** (1.50 g, 8.97 mmol) in EtOH (20 mL) and CH_2Cl_2 (20 mL) cooled to 0 ^oC was added NBS (3.19 g, 17.9 mmol) in one portion. The reaction mixture turned a dark red color, was warmed to room temperature for 4 h, and quenched by the addition of a saturated solution of $NaHCO_3$ (40 mL). This mixture was diluted with CH_2Cl_2 (50 mL) and the organic layer was washed with a saturated brine solution (40 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. The resulting oily suspension was filtered and chromatographed on SiO_2 (5:1 hexanes: EtOAc). The isolated product was dried by rotary evaporation, followed by high vacuum for 5 h affording **175** (1.57 g, 77%) as a tan oil: IR (salt plate) 2983, 2934, 1733, 1471, 1314, 1139, 1002, 883, 762 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 8.18 (d, 1 H, J = 8.4 Hz), 8.01 (d, 1 H, J = 7.8 Hz), 7.63-

7.51 (m, 2 H), 4.43-4.36 (m, 1 H), 4.00-3.94 (m, 1 H), 1.39 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 176.2, 154.1, 136.6, 127.7 (2), 125.4, 122.9, 63.4, 16.1; MS (EI) *m/z* 227 (M^+, 30), 183 (98), 162 (89), 151 (58), 135 (100), 108 (81), 90 (58), 69 (43); HRMS (EI) *m/z* calculated for $C_9H_9NO_2S_2$ 227.0075, found 227.0079.

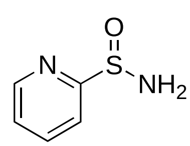
176 

Ethyl pyridine-2-sulfinate (176). To a solution of **174** (1.67 g, 14.7 mmol) in a 1:1 mixture of EtOH and DCM (60 mL) was added NBS (5.23 g, 29.4 mmol) in one portion at 0 °C. The reaction mixture immediately turned orange in color, was warmed to room temperature over 1 h and was then diluted with a saturated $NaHCO_3$ solution (50 mL) followed by DCM (50 mL). This mixture was stirred for 30 min, the aqueous layer was removed and the DCM layer was washed with a saturated brine solution (2 X 50 mL). The DCM layer was then diluted with hexanes (50 mL), filtered through a plug of SiO_2 (2 in), washed through with DCM (100 mL) and dried in vacuo to afford **176** as an orange oil (2.19 g, 83%). This compound was used crude for the next step: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.63 (d, 1 H, *J* = 4.2 Hz), 8.03-7.93 (m, 2 H), 7.49-7.46 (m, 1 H), 4.27-4.17 (m, 1 H), 3.89-3.78 (m, 1 H), 1.32 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.8, 150.0, 137.8, 126.2, 119.8, 62.6, 15.6.

177 

Benzo[d]thiazole-2-sulfinamide (177). To a solution of **175** (250 mg, 1.10 mmol) in THF (5.0 mL) cooled to -78 ^{o}C under a nitrogen atmosphere was added a solution of LiHMDS in THF (1.43 mL, 1.43 mmol, 1.00 M) dropwise over a 5 min period. The reaction mixture turned a deep red color, stirred at -78 ^{o}C for 2 h, and quenched by pouring it at -78 ^{o}C into an Erlenmeyer flask containing a saturated NH_4Cl solution (20 mL). This mixture was stirred for 30 min, while warming to room temperature, and then extracted with EtOAc (3 X 10 mL). The combined EtOAc layers were washed with a saturated brine solution (10 mL), dried (Na_2SO_4), concentrated to 20 mL by rotary evaporation and filtered through a 1 in plug of SiO_2 which was washed with additional EtOAc (50 mL). The filtrate was concentrated by rotary evaporation and the resulting solids were recrystallized from a 2:1 solution of EtOAc and hexanes (30 mL). The solid was washed with a 2:1 solution of EtOAc and hexanes (20 mL) and dried under high vacuum for 15 h to give **177** (125 mg, 57%) as a light yellow crystalline solid: Mp 141-142 ^{o}C (dec); IR (KBr) 3310, 3174, 3083, 1474, 1426, 1040, 892, 761 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 8.24 (d, 1 H, *J* = 7.5 Hz), 8.12 (d, 1 H, *J* = 7.8 Hz), 7.59 (m, 2 H), 7.18 (s, 2 H); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 179.5, 153.4, 136.2, 126.9, 126.3, 123.7, 122.8; MS (EI) *m/z* 198 (M$^+$, 4.5), 182 (40), 167 (14), 150 (81), 135 (100), 108 (86), 90 (67), 69 (86); HRMS (EI) *m/z* calculated for $C_7H_6N_2OS_2$ 197.9922, found 197.9919.

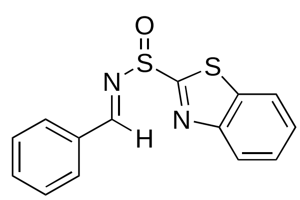
178



Pyridine-2-sulfinamide (178). To a solution of **176** (6.47 g, 34.4 mmol) in dry THF (100 mL) was added a solution of LiHMDS (37.8 mL, 37.8 mmol, 1.0 M) in THF, over 5 min at -65 ^{o}C. The reaction mixture turned a dark brown/red color during the addition, was stirred at -65 ^{o}C for 1 h and was quenched by addition to a saturated NH_4Cl solution (100 mL). The resulting mixture was stirred at room temperature for 1 h and then diluted with EtOAc (50 mL). The EtOAc layer was removed and the aqueous layer was extracted with EtOAc (2 X 100 mL). The

EtOAc extracts were combined, dried (Na_2SO_4) and filtered through a plug of SiO_2 (1 in) with additional EtOAc (200 mL). The EtOAc was removed in vacuo and the resulting solids were recrystallized from a 1:2 mixture of hexanes to EtOAc (a seed crystal was added to initiate crystallization). The resulting light brown precipitate was isolated by vacuum filtration, washed with hexanes (20 mL) and dried in vacuo to afford **178** (985 mg, 20%): Mp 108.0-109.5 ^{o}C (dec.); IR (KBr) 3226, 3075, 2699, 1580, 1451, 1426, 1086, 1056, 877 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (d, 1 H, *J* = 4.2 Hz), 7.97-7.89 (m, 2 H), 7.46-7.42 (m, 1 H), 7.47 (br-s, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.4, 150.0, 138.0, 125.6, 120.6; MS (EI) *m/z* 142 (M^{+}, 2.6), 126 (4.7), 110 (4.5), 96 (36), 79 (100); HRMS (EI) *m/z* calculated for $C_5H_6N_2OS$ 142.0201, found 142.0199.

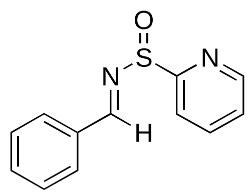
179



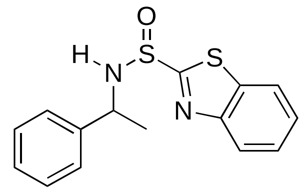
(*E*)-*N*-Benzylidenebenzo[d]thiazole-2-sulfinamide (179). To a suspension of **177** (200 mg, 1.00 mmol) in CH_2Cl_2 (15.0 mL) under a nitrogen atmosphere was added benzaldehyde **110** (0.102 mL, 1.00 mmol) and $Ti(OEt)_4$ (0.418 mL, 2.02 mmol) at room temperature. After 1.5 h, the solution turned a dark orange color and the reaction was quenched by the addition of SiO_2 (1.00 g) with stirring for 15 min. The resulting suspension was filtered through a 1 in plug of SiO_2 which was washed with additional CH_2Cl_2 (75 mL). The filtrate was concentrated by rotary evaporation and the resulting pale yellow oil was placed under high vacuum for 12 h when it crystallized affording **179** (228 mg, 79%) as a pale yellow crystalline solid: Mp 101-102.5 ^{o}C; IR (KBr) 3055, 1604, 1569, 1312, 1097, 994, 760 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.93 (s, 1

H), 8.22 (d, 1 H, *J* = 8.1 Hz), 7.96 (t, 3 H, *J* = 6.6 Hz), 7.61-7.48 (m, 5 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 174.0, 163.7, 153.3, 136.3, 133.6, 133.4, 130.2 (2), 129.2 (2), 127.0, 126.9, 124.8, 122.1; MS (EI) *m/z* 286 (M^+, 4.4), 183 (33), 167 (67), 135 (28), 103 (100), 76 (36); HRMS (EI) *m/z* calculated for $C_{14}H_{10}N_2OS_2$ 286.0235, found 286.0242.

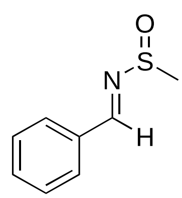
180



(*E*)-*N*-benzylidenepyridine-2-sulfinamide (180). To a suspension of **178** (400 mg, 2.81 mmol) and **110** (0.286 mL, 2.81 mmol) in dry DCM (30 mL) was added $Ti(OEt)_4$ (1.17 mL, 5.63 mmol). The reaction mixture turned a deep orange color and cleared after 2 h. After 2 h, SiO_2 (1.00 g) was added with stirring for 15 min. The resulting slurry was vacuum filtered through a plug of SiO_2 (1.5 in) and washed with additional DCM (150 mL). The clear pale yellow filtrate was dried in vacuo to afford **180** as an off white solid (577 mg, 89%): Mp 90.0 - 91.0 ^{o}C (dec.); IR (KBr) 3057, 1605, 1573, 1448, 1423, 1214, 1098, 1070, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.85 (s, 1 H), 8.75 (d, 1 H, *J* = 4.2 Hz), 7.94–7.88 (m, 4 H), 7.54–7.44 (m, 4 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.1, 161.8, 150.1, 138.1, 133.8, 132.8, 129.8 (2 C), 128.9 (2 C), 125.5, 119.2; MS (EI) *m/z* 230 (M^+, 3), 181 (27), 152 (31), 127 (99), 103 (95), 79 (100); HRMS (EI) *m/z* calculated for $C_{12}H_{10}N_2OS$ 230.0514, found 230.0503.

181 

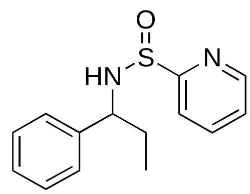
General procedure R. *N*-(1-Phenylethyl)benzo[d]thiazole-2-sulfinamide (181). To a solution of aldimine **179** (30.0 mg, 0.105 mmol) in THF (1.0 mL) at -78 °C was added dropwise a solution of MeMgBr in ether (0.070 mL, 0.21 mmol, 3.0 M) under a nitrogen atmosphere. The reaction mixture was stirred for 1 h, quenched by adding a saturated NH_4Cl solution (3.0 mL) at -78 °C, slowly warmed to room temperature over a 30 min period and partitioned between a saturated brine solution (10 mL) and ether (10 mL). The aqueous layer was extracted with ether (2 X 10 mL), the combined ether extracts were dried (Na_2SO_4), condensed by rotary evaporation and dissolved in a minimum amount of EtOAc for chromatography on SiO_2 (2:1 hexanes: EtOAc) to give two products (A and B). These products were dried in vacuo to afford **181** (Product A, 2.1 mg, 7%) isolated impure as an off white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.56 (d, 2 H, *J* = 8.1 Hz), 7.45-7.11 (m, 6 H), 7.09 (t, 1 H, *J* = 7.5 Hz), 5.76 (s, 1 H), 4.87 (q, 1 H, *J* = 6.0 Hz), 1.68 (d, 3 H, *J* = 6.6 Hz). A ^{1}H-NMR ($CDCl_3$) of fraction B showed **182** (4.2 mg, 24%) as clear oil.

182 

(*E*)-*N*-Benzylidenemethanesulfinamide (182). According to general procedure R, aldimine **179** (30.0 mg, 0.105 mmol) was combined with a solution of MeMgBr in ether (0.039 mL, 0.12 mmol, 3.0 M) in CH_2Cl_2 (1.0 mL) at -78 °C. The reaction mixture was warmed to 0 °C for 3 h.

The product **182** (9.8 mg, 56%) was isolated as a clear oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.66 (s, 1 H), 7.89 (d, 2 H, J = 7.2 Hz), 7.57-7.51 (m, 3 H), 2.74 (s, 3 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 161.8, 133.9, 132.7, 129.5 (2), 129.0 (2), 42.9; MS (EI) *m/z* 167 (M^+, 34), 156 (100), 104 (36), 77 (73). HRMS (EI) *m/z* calculated for C_8H_9NOS 167.0405, found 167.0400.

183



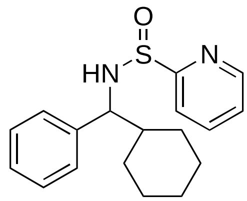
General Procedure S. *N*-(1-phenylpropyl)pyridine-2-sulfinamide (183, Table 11 entry 3). To a solution of **180** (23.0 mg, 0.100 mmol) and $Cu(OTf)_2$ (3.6 mg, 0.010 mmol) in DCM (1.0 mL) was added a solution of Et_2Zn (0.200 mL, 0.200 mmol, 1.0 M) in DCM at 0 ^oC. The reaction mixture turned from yellow to purple upon the addition, was stirred under nitrogen gas for 20 h and was quenched by the addition of a saturated $NaHCO_3$ solution (5.0 mL) with stirring for 30 min. The resulting mixture was partitioned between DCM (10 mL) and a saturated brine solution (10 mL). The DCM layer was kept and the aqueous layer was extracted with DCM (2 X 10 mL). The combined DCM layers were dried (Na_2SO_4) and concentrated in vacuo. The resulting yellow residue was dissolved in DCM (~ 0.5 mL) and chromatographed on SiO_2 (3:1 EtOAc:Hexanes). The product was isolated (R_f = 0.32) and dried in vacuo to afford **183** as a clear oil (12.3 mg, 47% yield, 2:1 d.r.): IR (KBr) 3216, 2966, 2931, 2875, 1577, 1453, 1423, 1089, 1041, 773 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz, 2:1 mixture of diastereoisomers) δ 8.75 (s, 0.67 H), 8.50 (s, 0.34 H), 8.20-7.90 (m, 1.25 H), 7.86-7.83 (m, 0.33 H), 7.76-7.71 (m, 0.38 H), 7.41-7.27 (m, 3.67 H), 7.25-7.23 (m, 0.34 H), 7.18-7.08 (m, 1.22 H), 4.85 (br-s, 0.66 H), 4.72 (br-s, 0.34 H), 4.44-4.42 (m, 1 H), 2.07-1.96 (m, 0.45 H), 1.89-1.08 (m, 1.66 H), 0.89-0.84 (m, 3

H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.5, 163.8, 150.7, 150.3, 142.4, 141.9, 138.4, 137.9, 129.2 (2 C), 128.8 (2 C), 128.3 (4 C), 127.8 (2 C), 126.2, 125.6, 121.7, 121.4, 60.2, 57.7, 31.6, 31.4, 11.1, 11.0; HRMS (TOF MS ES+) *m/z* calculated for $C_{14}H_{16}N_2OSNa$ 283.0881, found 283.0901.

***N*-(1-phenylpropyl)pyridine-2-sulfinamide (183, Table 11 entry 1)**. According to general procedure S, **180** (23.0 mg, 0.100 mmol) and Et_2Zn in DCM (0.200 mL, 0.200 mmol, 1.0 M) were reacted in DCM (1.0 mL) at room temperature for 24 h to afford **183** crude (6.0 mg, 17%, dr 1:1).

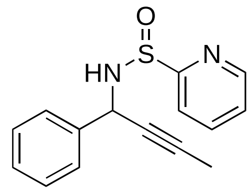
***N*-(1-phenylpropyl)pyridine-2-sulfinamide (183, Table 11 entry 2)**. According to general procedure S, **180** (23.0 mg, 0.100 mmol), $Cu(OTf)_2$ (3.6 mg, 0.010 mmol) and Et_2Zn in toluene (0.200 mL, 0.200 mmol, 1.0 M) were reacted in toluene (1.0 mL) at room temperature for 20 h to afford **183** (12.2 mg, 47%, dr 1:1).

***N*-(1-phenylpropyl)pyridine-2-sulfinamide (183, Table 11 entry 4)**. According to general procedure S, **180** (23.0 mg, 0.100 mmol), $Cu(OTf)_2$ (3.6 mg, 0.010 mmol) and Et_2Zn in THF (0.200 mL, 0.200 mmol, 1.0 M) were reacted in THF (1.0 mL) at 0 ^{o}C for 15 h to afford a trace amount of **183** by TLC (SiO_2, 3:1 EtOAc:hexanes).

184 

***N*-(cyclohexyl(phenyl)methyl)pyridine-2-sulfinamide (184, Table 11 entry 5)**. According to general procedure S, **180** (23.0 mg, 0.100 mmol), $Cu(OTf)_2$ (3.6 mg, 0.010 mmol) and cyclohexylzinc bromide in THF (0.400 mL, 0.200 mmol, 0.50 M) were reacted in DCM (1.0 mL) at room temperature for 24 h. The product was isolated by chromatography on SiO_2 (2:1 EtOAc:hexanes; R_f = 0.30) to afford **184** as a clear oil (20.1 mg, 64%, dr 3.5:1): IR (KBr) 3228, 2926, 2852, 1577, 1451, 1423, 1089, 1039 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, 3.5:1 mixture of diastereoisomers) δ 8.72 (br-s, 0.23 H), 8.63 (br-s, 0.77 H), 7.85-7.76 (m, 1.24 H), 7.68-7.63 (m, 0.79 H), 7.42-7.34 (m, 1.24 H), 7.15-7.06 (m, 2.80 H), 6.94 (br-s, 1.56 H), 5.00 (br-s, 0.23 H), 4.84 (br-s, 0.79 H), 4.23 (br-s, 1 H), 1.98-1.82 (m, 1.22 H), 1.79-1.75 (m, 1.44 H), 1.41 (d, 1.44 H, J = 10.8 Hz), 1.26-1.00 (m, 5 H), 0.96-0.85 (m, 2 H); HRMS (TOF MS ES+) *m/z* calculated for $C_{18}H_{22}N_2OSNa$ 337.1351, found 337.1321.

***N*-(cyclohexyl(phenyl)methyl)pyridine-2-sulfinamide (184, Table 11 entry 6)**. According to general procedure S, **180** (23.0 mg, 0.100 mmol), $Cu(OTf)_2$ (3.6 mg, 0.010 mmol) and cyclohexylzinc bromide in THF (0.400 mL, 0.200 mmol, 0.50 M) were reacted in DCM (1.0 mL) at 0 ^oC for 24 h. The product was isolated by chromatography on SiO_2 (2:1 EtOAc:hexanes; R_f = 0.30) to afford **184** (19.9 mg, 63%, dr 4:1).

185 

General Procedure T. *N*-(1-phenylbut-2-ynyl)pyridine-2-sulfinamide (185, Table 11 entry 9). To a solution of $ZnBr_2$ (45.0 mg, 0.200 mmol) in THF (0.20 mL) cooled to 0 °C was added a solution of 1-propynylmagnesium bromide in THF (0.400 mL, 0.200 mmol, 0.50 M). The resulting white suspension was slowly warmed to room temperature for 30 min and was then added via syringe to a yellow/orange homogenous mixture of **180** (23.0 mg 0.100 mmol) and $(CH_3CN)_4CuBF_4$ (3.2 mg, 0.010 mmol) in DCM (1.0 mL). The resulting green/yellow slightly heterogeneous reaction mixture was stirred at room temperature for 22 h and then a saturated $NaHCO_3$ solution (5.0 mL) was added with stirring for 30 min. The resulting mixture was diluted with DCM (10 mL), washed with a saturated brine solution (10 mL) and the aqueous layer was extracted with DCM (2 X 10 mL). The combined organic layers were dried (Na_2SO_4), condensed in vacuo and dissolved in a minimum amount of DCM for chromatography on SiO_2 (2:1 EtOAc: hexanes; R_f = 0.33). The product was isolated and dried in vacuo to afford **185** as a clear oil (13.4 mg, 50%, dr 3.5:1): IR (KBr) 3441, 3203, 2918, 1577, 1453, 1424, 1092, 1064, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, 3.5:1 mixture of diastereoisomers) δ 8.75 (d, 0.22 H, *J* = 4.5 Hz), 8.62 (d, 0.78 Hz, *J* = 4.2 Hz), 8.04 (d, 0.22 H, *J* = 7.8 Hz), 7.94 (d, 0.78 H, *J* = 7.5 Hz), 7.93-7.91 (m, 0.22 H), 7.83 (dt, 0.78 H, *J* = 7.4, 1.2 Hz), 7.58 (d, 0.44 H, *J* = 7.2 Hz), 7.44-7.20 (m, 6 H), 5.39-5.33 (m, 0.22 H), 5.28-5.26 (m, 0.78 H), 4.93 (d, 0.78 H, *J* = 4.5 Hz), 4.79 (d, 0.22 H, *J* = 4.8 Hz), 1.91 (d, 2.29 H, *J* = 2.1 Hz), 1.72 (d, 0.66 H, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.6, 150.6, 150.5, 139.9, 138.3, 138.2, 129.3 (2 C), 129.1 (2 C), 128.9, 128.7, 128.5 (2 C), 128.1 (2 C), 126.0, 121.8, 121.6, 84.0, 78.0, 48.6, 47.8, 4.5, 4.3; HRMS (TOF MS ES+) *m/z* calculated for $C_{15}H_{14}N_2OSNa$ 293.0725, found 293.0732.

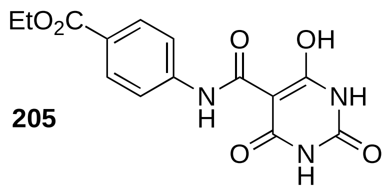
***N*-(1-phenylbut-2-ynyl)pyridine-2-sulfinamide (185, Table 11 entry 7)**. According to general procedure T, **180** (23.0 mg, 0.100 mmol) and 1-propynylmagnesium bromide in THF (0.300 mL, 0.150 mmol, 0.50 M) were reacted in DCM (1.0 mL) at 0 ^{o}C for 3 h to afford **185** (12.9 mg, 48%, dr 1:1).

***N*-(1-phenylbut-2-ynyl)pyridine-2-sulfinamide (185, Table 11 entry 8)**. According to general procedure T, 1-propynylmagnesium bromide in THF (0.400 mL, 0.200 mmol, 0.5 M) and $ZnCl_2$ in THF (0.200 mL, 0.200 mmol, 1.0 M) were mixed at -78 ^{o}C, warmed to room temperature and then reacted with **180** (23.0 mg, 0.100 mmol) and $Cu(OTf)_2$ (3.6 mg, 0.010 mmol) in DCM (1.0 mL) at room temperature for 21 h to afford **185** (3.6 mg, 13%, dr 1:1).

***N*-(1-phenylbut-2-ynyl)pyridine-2-sulfinamide (185, Table 11 entry 10)**. According to general procedure T, ZnI_2 (63.8 mg, 0.200 mmol), 1-propynylmagnesium bromide in THF (0.400 mL, 0.200 mmol, 0.5 M), **180** (23.0 mg, 0.100 mmol) and $(CH_3CN)_4CuBF_4$ (3.2 mg, 0.010 mmol) in DCM (1.0 mL) were reacted at room temperature for 17 h to afford **185** (16.3 mg, 60%, dr 2.9:1).

4.4 SYNTHESIS OF SID 861574 AND RELATED PLK1-PBD INHIBITORS

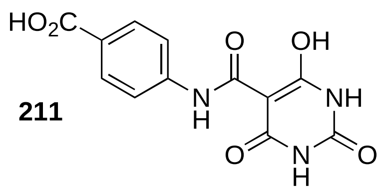
4.4.1 First generation analogues of SID 861574



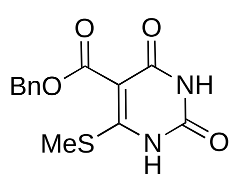
205

Ethyl 4-(6-hydroxy-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamido) benzoate (205, DMA-NB74-1). To a solution of barbituric acid (124 mg, 0.970 mmol) in DMF (1.5 mL) was added dropwise Et_3N (0.273 mL, 1.94 mmol) at room temperature. A white precipitate formed during the addition. The resulting suspension was stirred at room temperature for 30 min and then ethyl-4-isocyanatobenzoate (191 mg, 0.970 mmol) was added. The suspension turned pale yellow following the addition and was stirred at room temperature for 15 h. After 15 h, the reaction mixture was acidified with an HCl solution (~ 3.0 mL, 1.2 M, pH = 0). The resulting white solids were isolated by vacuum filtration, washed with deionized water (30 mL) and heated in boiling EtOH (20 mL) for 15 min followed by cooling to room temperature. The white precipitate was isolated by vacuum filtration, washed with EtOH (30 mL) and dried in vacuo to afford **205** as a white solid (223 mg, 72%): Mp 340-340.5 ^{o}C; IR (KBr) 3214, 2986, 2808, 1744, 1705, 1637, 1603, 1495, 1417, 1280, 1111, 853 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 17.33 (s, 1 H), 11.86 (s, 2 H), 11.71 (s, 1 H), 7.95 (d, 2 H, J = 8.4 Hz), 7.66 (d, 2 H, J = 8.4 Hz), 4.29 (q, 2 H, J = 6.9 Hz), 1.32 (t, 3 H, J = 7.0 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 169.5, 165.5 (3 C), 148.9, 141.0, 130.9 (2 C), 126.3, 120.7 (2 C), 81.5, 61.1, 14.6; MS (EI) *m/z* 319 (M$^+$, 34), 165

(54), 137 (26), 120 (100), 69 (37); HRMS (EI) *m/z* calculated for $C_{14}H_{13}N_3O_6$ 319.080435, found 319.081014.

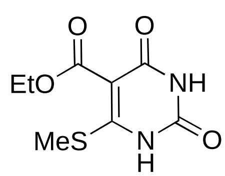
211 

General Procedure U. 4-(6-Hydroxy-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamido)benzoic acid (211, DMA-NB74-83). A mixture of **205** (40.0 mg, 0.125 mmol) in a LiOH solution (2.0 mL, 1.0 M) was stirred at room temperature for 2.5 h. The reaction mixture was acidified with an aqueous HCl solution (1.0 M, pH=1-2) at 0 ^oC and the resultant carboxylic acid product precipitated. The solid was isolated by vacuum filtration, washed with deionized water (15 mL) and dried in vacuo to afford **211** as a white solid (32.0 mg, 88%): Mp >380 ^oC; IR (ATR) 3245, 3038, 2855, 1718, 1671, 1592, 1528, 1414, 1254, 1174, 798 cm^{-1}; ^1H-NMR (DMSO-d_6, 300 MHz) δ 17.17 (s, 1 H), 12.85 (s, 1 H), 11.79 (s, 3 H), 7.95 (d, 2 H, J = 8.4 Hz), 7.66 (d, 2 H, J = 8.7 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 169.3, 167.2 (3 C), 149.0, 140.9, 131.1 (2 C), 127.3, 120.6 (2 C), 81.7; MS (EI) *m/z* 291 (M^+, 18), 154 (34), 137 (100), 120 (79), 68 (35); HRMS (EI) *m/z* calculated for $C_{12}H_9N_3O_6$ 291.049135, found 291.048553.

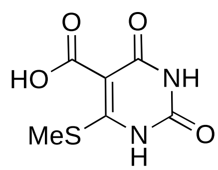
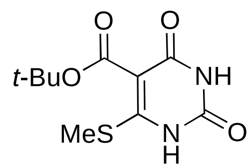
216 

Benzyl 6-(methylthio)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (216, DMA-NB74-6). To a suspension of K_2CO_3 (2.55 g, 18.5 mmol) in DMSO (20 mL) was added dibenzyl malonate (2.91 mL, 13.0 mmol) followed by 3,3-dimethylthio-2-azaprop-2-enenitrile (1.50 g, 9.23 mmol). The reaction mixture was stirred at room temperature for 5 h and was then slowly added to an ice cold HCl solution (50 mL, 10%). A white precipitate formed. After stirring for 15 min, the precipitate was isolated by filtration, washed with deionized water (30 mL) and recrystallized from a 4:1 mixture of EtOH:water (50 mL). The resulting crude product was recrystallized a second time from a 9:1 mixture of EtOAc:EtOH (65 mL) to afford **216** as a clear crystalline solid (921 mg, 34%): Mp 215-216 ^{o}C; IR (ATR) 3453, 3030, 1718, 1650, 1411, 1273, 1130 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 11.37 (s, 1 H), 10.94 (s, 1 H), 7.45-7.31 (m, 5 H), 5.21 (s, 2 H), 2.55 (s, 3 H); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 163.9, 159.4, 158.9, 149.7, 135.9, 128.3 (2 C), 127.9, 127.8 (2 C), 104.3, 66.1, 14.3; HRMS (TOF MS ES+) m/z calculated for $C_{13}H_{12}N_2O_4SNa$ (M+Na) 315.0415 found 315.0414.

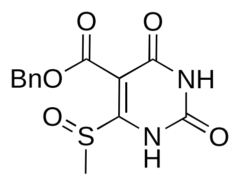
217



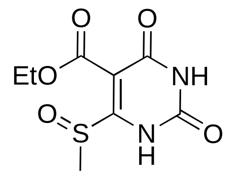
Ethyl 6-(methylthio)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (217, DMA-NB74-7). To a suspension of K_2CO_3 (3.40 g, 24.6 mmol) in DMSO (27 mL) was added diethyl malonate (2.70 mL, 17.2 mmol) followed by 3,3-dimethylthio-2-azaprop-2-enenitrile (2.00 g, 12.3 mmol). The reaction mixture was stirred at room temperature for 5 h and was then added to deionized water (125 mL) and acidified (pH = 0.5) by the slow addition of an HCl solution (~21 mL, 10%). A precipitate formed after cooling to 0 ^{o}C for 15 min and was removed by filtration. The filtrate was neutralized with KOH (pH = 7) and a white solid slowly formed over 15 h. The solid was isolated by filtration and was dissolved in an aqueous HCl solution (15 mL, 10%) for 15 min. A white solid precipitated which was isolated by vacuum filtration, washed with water (30 mL) and recrystallized from EtOAc (20 mL) to afford **217** as a clear crystalline solid (522 mg, 18%): Mp 237-238.5 °C; IR (ATR) 3453, 3157, 3019, 2824, 1713, 1687, 1647, 1480, 1405, 1285, 1103, 1021 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 11.35 (s, 1 H), 10.94 (s, 1 H), 4.17 (q, 2 H, *J* = 6.9 Hz), 2.55 (s, 3 H), 1.22 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 164.3, 159.9, 157.7, 150.2, 105.8, 61.2, 14.8, 14.5; MS (EI) *m/z* 230 (M$^+$, 7), 184 (33), 125 (22), 111 (29), 97 (53), 83 (54), 69 (88), 57 (100); HRMS (EI) *m/z* calculated for $C_8H_{10}N_2O_4S$ 230.036129, found 230.036411.

218  **219** 

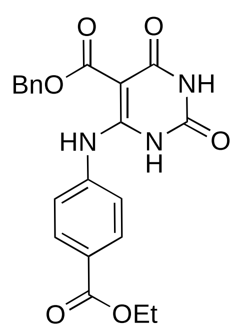
6-(Methylthio)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (218, DMA-NB74-14) and *tert*-butyl 6-(methylthio)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (219). To a suspension of K_2CO_3 (1.70 g, 12.3 mmol) in DMSO (15 mL) was added ditert-butyl propane-1,3-dioate (2.70 mL, 7.69 mmol) followed by 3,3-dimethylthio-2-azaprop-2-enenitrile (1.00 g, 6.15 mmol). The reaction mixture was stirred at room temperature for 18 h and was then added in small portions to an aqueous HCl solution (30 mL, 10%). A precipitate formed which was isolated by vacuum filtration, washed with water (40 mL) and stirred in boiling EtOAc (100 mL). An insoluble white solid was filtered from the EtOAc solution. The filtrate was concentrated and recrystallized three times from a minimum amount of boiling EtOAc to afford **219** as a fluffy white solid (41.6 mg, 3.0%): Mp 327-328 °C (dec.); IR (ATR) 3381, 3151, 3038, 1718, 1696, 1642, 1528, 1299, 1118 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 11.29 (s, 1 H), 10.90 (s, 1 H), 2.56 (s, 3 H), 1.45 (s, 9 H); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 162.7, 159.6, 155.0, 149.9, 107.4, 81.4, 27.7 (3 C), 14.3; HRMS (ESI) *m/z* calculated for $C_{10}H_{14}N_2O_4SNa$ 281.0572, found 281.0574. The solid isolated from the filtration was purified by solid/liquid extraction using EtOAc (4 X 60 mL) to afford **218** as a white crystalline solid (450 mg, 36%): Mp 329-330 °C (dec.); IR (ATR) 3276, 3120, 1713, 1612, 1511, 1374, 1174 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 13.85 (s, 1 H), 12.18 (s, 1 H), 10.90 (s, 1 H), 2.58 (s, 3 H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 169.8, 166.5, 164.9, 148.7, 96.7, 14.7; MS (EI) *m/z* 202 (M^+, 0.7), 158 (76), 115 (11), 68 (100); HRMS (EI) *m/z* calculated for $C_6H_6N_2O_4S$ 202.004829, found 202.004120.

220 

General procedure V. Benzyl 6-(methylsulfinyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (220, DMA-NB74-16). To a suspension of **216** (292 mg, 1.00 mmol) in CH_2Cl_2 (20 mL) was added *m*-CPBA (591 mg, 2.50 mmol, 73%) at room temperature. After 15 min, the suspension cleared and after 30 min a white solid precipitated from the reaction mixture. After 1.5 h, the white precipitate was isolated by vacuum filtration, washed with CH_2Cl_2 (10 mL) and dried in vacuo to afford **220** as a fluffy white solid (247 mg, 80%): Mp 204-205 °C; IR (ATR) 3524, 3038, 1739, 1715, 1681, 1666, 1394, 1301, 1124 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 11.67 (s, 1 H), 10.34 (s, 1 H), 7.46-7.33 (m, 5 H), 5.27 (s, 2 H), 2.93 (s, 3 H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 168.0, 163.5, 159.8, 148.9, 136.2, 128.9 (2 C), 128.5, 128.3 (2 C), 101.4, 66.8, 41.4; HRMS (ESI) *m/z* calculated for $C_{13}H_{12}N_2O_5SNa$ (M+Na) 331.0365, found 331.0388.

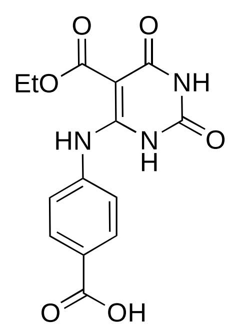
221 

Ethyl 6-(methylsulfinyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (221, DMA-NB74-21). According to general procedure V, **217** (150 mg, 0.651 mmol) and *m*-CPBA (385 mg, 1.63 mmol, 73%) were mixed in CH_2Cl_2 (13 mL) at room temperature for 1.5 h. The product was precipitated from the reaction mixture at 0 ^oC with hexanes (10 mL) and the resulting solid was isolated by vacuum filtration, washed with a 1:1 mixture of CH_2Cl_2:hexanes (10 mL) and dried in vacuo to afford **221** as a clear crystalline solid (115 mg, 72%): Mp 239-240 ^oC; IR (ATR) 3174, 3055, 2831, 1735, 1705, 1674, 1592, 1400, 1314, 1120, 1006 cm^{-1}; ^1H-NMR (DMSO-d_6, 300 MHz) δ 11.64 (s, 1 H), 10.31 (s, 1 H), 4.21 (q, 2 H, *J* = 6.9 Hz), 2.96 (s, 3 H), 1.25 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 167.2, 163.4, 159.8, 148.9, 101.7, 61.6, 41.4, 14.5; MS (EI) *m/z* 246 (M^+, 30), 200 (25), 155 (52), 140 (87), 112 (100), 94 (31); HRMS (EI) *m/z* calculated for $C_8H_{10}N_2O_5S$ 246.031043, found 246.031121.

224 

General Procedure W. Benzyl 6-(4-(ethoxycarbonyl)phenylamino)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (224, DMA-NB74-19). A suspension of **220** (200 mg, 0.649 mmol) and ethyl 4-aminobenzoate (1.03 g, 6.17 mmol) in dioxane (2.0 mL) was heated to 95-100 ^oC for 6 h. A white solid precipitated from the reaction mixture during this time. The reaction mixture was cooled to room temperature, diluted with acetone (1.0 mL) and further cooled to 0 ^{o}C for 30 min. The cold reaction mixture was vacuum filtered and the isolated white solid was washed with acetone (5.0 mL) and dried in vacuo to afford **224** as a white crystalline solid (202 mg, 76%): Mp 379-380 ^{o}C (dec.); IR (ATR) 3381, 3230, 3038, 1718, 1696, 1642, 1528, 1299, 1118 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 11.08 (s, 1 H), 11.01 (s, 1 H), 10.80 (s, 1 H), 7.97 (d, 2 H, *J* = 8.4 Hz), 7.48-7.44 (m, 4 H), 7.39-7.30 (m, 3 H), 5.24 (s, 2 H), 4.32 (q, 2 H, *J* = 6.9 Hz), 1.32 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 168.3, 165.7, 161.0, 156.8, 149.6, 141.2, 137.1, 131.0 (2 C), 128.7 (2 C), 128.0, 127.7 (2 C), 127.6, 124.8 (2 C), 83.1, 65.5, 61.2, 14.7; MS (EI) *m/z* 409 (M$^+$, 7), 365 (24), 301 (13), 275 (23), 256 (33), 91 (100); HRMS (EI) *m/z* calculated for $C_{21}H_{19}N_3O_6$ 409.127386, found 409.127861.

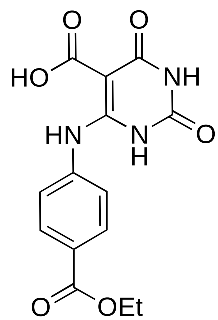
206



4-(5-(Ethoxycarbonyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino)benzoic acid (206, DMA-NB74-25). According to general procedure W, **221** (50.0 mg, 0.203 mmol) and 4-aminobenzoic acid (264 mg, 1.90 mmol) were heated in dioxane (1.0 mL) at 95-100 ^{o}C for 5 h. The resulting product **206** was isolated as a light white solid (32.1 mg, 50%): Mp >380 ^{o}C; IR (ATR) 3217, 3172, 1743, 1705, 1666, 1634, 1575, 1420, 1301, 1204, 788 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 12.94 (s, 1 H), 11.02 (s, 2 H), 10.73 (s, 1 H), 7.95 (d, 2 H, *J* = 8.4 Hz), 7.41 (d, 2 H, *J* = 8.4 Hz), 4.15 (q, 2 H, *J* = 7.2 Hz), 1.22 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-

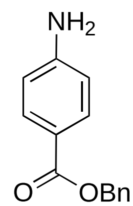
d_6, 75 MHz) δ 168.5, 167.3, 161.0, 156.6, 149.6, 141.0, 131.1 (2 C), 128.4, 124.5 (2 C), 83.3, 60.3, 14.8; MS (EI) *m/z* 319 (M^+, 11), 273 (100), 163 (19), 91 (25), 57 (36); HRMS (EI) *m/z* calculated for $C_{14}H_{13}N_3O_6$ 319.080435, found 319.080744.

207

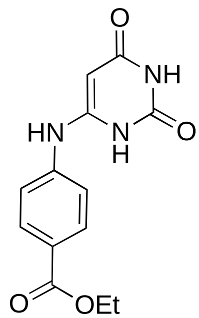


General procedure X. 6-(4-(Ethoxycarbonyl)phenylamino)-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid (207, DMA-NB74-27). To a solution of **224** (25.0 mg, 0.0611 mmol) in a 1:1 mixture of dioxane:MeOH (15 mL) was added 20 wt% $Pd(OH)_2$/C (10.7 mg, 0.020 mmol/Pd). The dark back suspension was placed under a H_2 atmosphere (1 atm). The reaction mixture was stirred for 3 h and filtered through a plug of celite (1.5 in, disposable pipette) with EtOAc (50 mL). The filtrate was concentrated by rotary evaporation (40 ^{o}C) and the resulting white solid was purified by warming in a 3:1 solution of EtOAc:hexane (10 mL) for 15 min. The solid was isolated by vacuum filtration, washed with hexanes (10 mL) and dried in vacuo to afford **207** as a white crystalline solid (14.2 mg, 73%): Mp 324.5-326 ^{o}C (dec.); IR (ATR) 3161, 2991, 2823, 1722, 1681, 1625, 1575, 1390, 1286, 1128 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 14.52 (s, 1 H), 11.68 (s, 1 H), 11.59 (s, 1 H), 11.52 (s, 1 H), 8.00 (d, 2 H, *J* = 8.4 Hz), 7.52 (d, 2 H, *J* = 8.4 Hz), 4.33 (q, 2 H, *J* = 7.2 Hz), 1.33 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 169.5, 168.5, 165.7, 157.0, 148.8, 139.9, 131.0 (2 C), 128.6, 125.8 (2 C), 80.1, 61.3, 14.6; MS (EI) *m/z* 319 (M^+, 1), 275 (100), 230 (63), 145 (16), 120 (24); HRMS (EI) *m/z* calculated for $C_{14}H_{13}N_3O_6$ 319.080435, found 319.08111.

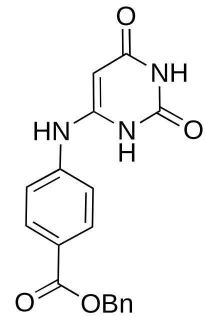
225



Benzyl 4-aminobenzoate (225, DMA-NB74-77).[90] To a solution of 4-aminobenzoic acid (3.00 g, 21.9 mmol) in MeOH (50 mL) was added Cs_2CO_3 (3.92 g, 12.0 mmol). The reaction mixture was stirred at room temperature for 30 min, concentrated by rotary evaporation (40 ^oC) and dried in vacuo. The resulting cesium salt was suspended in a 3:1 solution of CH_3CN (30 mL): DMF (10 mL) and a solution of benzyl bromide (2.73 mL, 23.0 mmol) in CH_3CN (10 mL) was added dropwise over 2 h. The reaction mixture was stirred at room temperature for 17 h, concentrated by rotary evaporation (40 ^oC) and the resulting crude yellow oil was dissolved in EtOAc (150 mL). The EtOAc solution was washed with a saturated $NaHCO_3$ solution (1 X 50 mL), followed by a saturated brine solution (1 X 50 mL). The EtOAc layer was dried with Na_2SO_4, concentrated by rotary evaporation (40 ^oC) and the resulting yellow oil was recrystallized from a 10:1 solution of hexanes:EtOAc (150 mL) to afford **225** as a fluffy white solid (2.23 g, 45%): Mp 87-88 ^oC; IR (ATR) 3453, 3354, 3222, 1681, 1631, 1597, 1277, 1169, 1114, 1075 cm^{-1}; ^1H-NMR (DMSO-d_6, 300 MHz) δ 7.67 (d, 2 H, J = 8.4 Hz), 7.43-7.32 (m, 5 H), 6.57 (d, 2 H, J = 8.7 Hz), 6.31 (s, 2 H), 5.48 (s, 2 H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 166.1, 154.1, 137.3, 131.7 (2 C), 128.9 (2 C), 128.3, 128.2 (2 C), 116.0, 113.1 (2 C), 65.5; MS (EI) *m/z* 227 (M^+, 54), 182 (17), 120 (100), 91 (94); HRMS (EI) *m/z* calculated for $C_{14}H_{13}NO_2$ 227.094629, found 227.094483.

227 

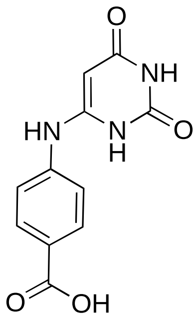
General procedure Y. Ethyl 4-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino)benzoate (227, DMA-NB74-57). A mixture of 6-chloro-1,3-dihydropyrimidine-2,4-dione (220 mg, 1.50 mmol) and ethyl 4-aminobenzoate (991 mg, 6.00 mmol) was heated to 155-160 ^{o}C for 3 h. The solids melted during heating forming a clear solution. After 3 h, the reaction mixture was cooled to room temperature, diluted with acetone (4.0 mL) and stirred for 30 min. The remaining solid was isolated by vacuum filtration and washed with acetone (10 mL). The resulting solid was boiled in EtOAc (30 mL) for 30 min and the mixture was cooled to room temperature. The purified solid was isolated by vacuum filtration, washed with acetone (10.0 mL) and dried in vacuo to afford **227** as a white solid (299 mg, 72%): Mp 363-365 ^{o}C (dec.); IR (ATR) 3086, 3014, 1716, 1683, 1647, 1485, 1407, 1260, 1127 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 10.64 (s, 1 H), 10.36 (s, 1 H), 8.79 (s, 1 H), 7.92 (d, 2 H, *J* = 8.7 HZ), 7.26 (d, 2 H, *J* = 8.7 Hz), 4.98 (s, 1 H), 4.27 (q, 2 H, *J* = 6.9 Hz), 1.29 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 165.2, 164.4, 150.8, 150.6, 143.2, 130.9 (2 C), 124.2, 120.0 (2 C), 78.9, 60.5, 14.2; MS (EI) *m/z* 275 (M$^+$, 100), 247 (6), 120 (32), 69 (41), 57 (53); HRMS (EI) *m/z* calculated for $C_{13}H_{13}N_3O_4$ 275.090606, found 275.090923.



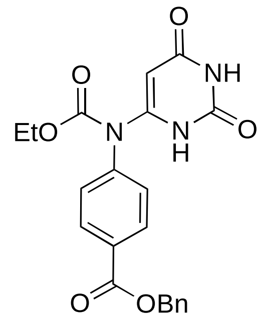
228

Benzyl 4-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino)benzoate (228, DMA-NB74-55). According to general procedure Y, **225** (1.39 g, 6.12 mmol) and 6-chloro-1,3-dihydropyrimidine-2,4-dione (225 mg, 1.54 mmol) were dissolved in DMF (1.0 mL) and heated to 155-160 ^{o}C for 1 h. The reaction mixture was worked up according to the general procedure. The isolated product was further purified by dissolving in DMSO (10 mL) and adding distilled water (20 mL) slowly to re-precipitate the product. The resulting solid was isolated by vacuum filtration, boiled in EtOAc (10 mL) for 5 min to remove residual DMSO, re-isolated by vacuum filtration, washed with hexanes (15 mL) and dried in vacuo to afford **228** as a pale yellow solid (98.7 mg, 19%): Mp 301-303 ^{o}C (dec.); IR (ATR) 3194, 3086, 2932, 1752, 1711, 1592, 1560, 1267, 1101, 751 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 10.64 (s, 1 H), 10.37 (s, 1 H), 8.82 (s, 1 H), 7.95 (d, 2 H, J = 8.4 Hz), 7.46-7.37 (m, 5 H), 7.27 (d, 2 H, J = 8.4 Hz), 5.32 (s, 2 H), 4.98 (s, 1 H); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 165.5, 164.9, 151.3, 151.1, 143.9, 136.7, 131.3 (2 C), 129.0 (2 C), 128.6, 128.4 (2 C), 124.3, 120.5 (2 C), 79.5, 66.4; MS (EI) *m/z* 337 (M^+, 25), 187 (12), 146 (18), 91 (100), 65 (17); HRMS (EI) *m/z* calculated for $C_{18}H_{15}N_3O_4$ 337.106256, found 337.105811.

229

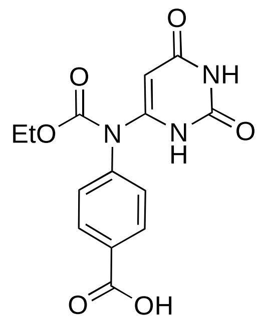


4-(2,6-Dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino)benzoic acid (229, DMA-NB74-5). According to general procedure Y, 6-chloro-1,3-dihydropyrimidine-2,4-dione (293 mg, 2.00 mmol) and 4-aminobenzoic acid (1.02 g, 7.50 mmol) were dissolved in DMF (1.0 mL) and heated to 155-160 ^{o}C for 2 h to afford **229** as a light yellow solid (352 mg, 71%, ~90% pure by ^{1}H-NMR). A 50.0 mg sample was further purified by recrystallization from a 3:1 solution of deionized water:DMSO initially heated to 90 ^{o}C then cooled to rt. The recrystallized solid was isolated by vacuum filtration and heated in distilled water for 30 min at 90 ^{o}C two times to remove any residual DMSO. After cooling to room temperature the solid was isolated by vacuum filtration, washed with additional water (5.0 mL) and dried under high vacuum for 24 h. Following the recrystallization, **229** was isolated as a white powder (33.2 mg, 66% recovery from recrystallization): Mp >380 ^{o}C; IR (KBr) 3354, 3012, 1719, 1600, 1398, 1276 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 12.79 (s, 1 H), 10.63 (s, 1 H), 10.35 (s, 1 H), 8.75 (s, 1 H), 7.91 (d, 2 H, *J* = 8.7 Hz), 7.26 (d, 2 H, *J* = 8.7), 4.97 (s, 1 H); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 167.3, 164.9, 151.3 (2 C), 143.2, 131.3 (2 C), 125.9, 120.6 (2 C), 79.0; MS (EI) *m/z* 247 (M$^+$, 53), 155 (64), 120 (40), 84 (77), 68 (100); HRMS (EI) *m/z* calculated for $C_{11}H_9N_3O_4$ 247.059306, found 247.057526.

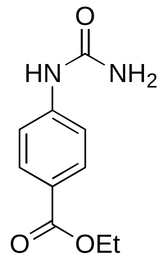


230

Benzyl 4-((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)(ethoxycarbonyl)amino)benzoate (230, DMA-NB108-3). To a solution of **228** (70.0 mg, 0.208 mmol) and Et_3N (32.1 µL, 0.228 mmol) in DMF (1.0 mL) was added diethyl pyrocarbonate (33.7 µL, 0.228 mmol) at 0 ^oC. The reaction mixture turned a light green color and was warmed to room temperature for 48 h. After 48 h, the reaction mixture was filtered through a 1 in plug of SiO_2 (pretreated with a 99.9:0.1 solution of EtOAc:Et_3N) and the product was flushed through with a 99.9:0.1 solution of EtOAc:Et_3N (85 mL). The filtrate was concentrated by rotary evaporation (30 ^oC) and the resulting orange residue was diluted with EtOAc (1.0 mL) followed by hexanes (30 mL) with cooling to 0 ^oC for 30 min. The resulting solid was isolated by vacuum filtration, washed with hexanes (10 mL) and dried *in vacuo*. This crystallization procedure was performed twice to afford **230** (15.2 mg, 16% crude yield, ~85% pure by ^{1}H-NMR). The mother liquors from the previous two recrystallizations were combined, concentrated by rotary evaporation (30 ^oC) and the resulting pale yellow oil was diluted with EtOAc (1.0 mL) followed by hexanes (30 mL) at 0 ^oC. The resulting solid was isolated by vacuum filtration, washed with hexanes (5.0 mL) and dried in vacuo to afford pure **230** as a fluffy off-white solid (2.0 mg, 2.3%): Mp 171-172 ^oC; IR (ATR) 3196, 2987, 2789, 1715, 1647, 1591, 1418, 1273, 1223, 1107, 1016 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 11.55 (s, 1 H), 11.24 (s, 1 H), 8.02 (d, 2 H, *J* = 8.7 Hz), 7.54 (d, 2 H, *J* = 8.7 Hz), 7.48-7.38 (m, 5 H), 5.36 (s, 3 H), 4.19 (q, 2 H, *J* = 6.9 Hz), 1.20 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 165.3, 164.6, 152.4, 151.3, 150.7, 144.2, 136.5, 130.7 (2 C), 129.0 (2 C), 128.6, 128.4 (2 C), 128.3, 126.5 (2 C), 98.4, 66.8, 63.5, 14.5; MS (EI) *m/z* 409 (M^+, 20), 302 (7), 230 (21), 91 (100); HRMS (EI) *m/z* calculated for $C_{21}H_{19}N_3O_6$ 409.127386, found 409.127299.

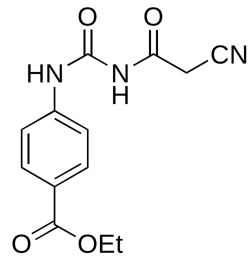
208 

4-((2,6-Dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)(ethoxycarbonyl)amino)benzoic acid (208, DMA-NB108-6). According to general procedure X, **230** (8.80 mg, 0.0183 mmol, 85% pure) and 20 wt% $Pd(OH)_2/C$ (0.8 mg, 0.0015 mmol/Pd) in distilled THF (0.50 mL) were stirred under a H_2 gas (1.0 atm) atmosphere for 6 h at room temperature. After filtration through celite, the resulting white solid was triturated with a 5:1 mixture of hexanes:EtOAc (10 mL) while cooling to 0 ^oC. The resulting white solid was isolated by vacuum filtration, washed with hexanes (3.0 mL), and dried in vacuo to afford **208** as a white powdery solid (2.1 mg, 36%): IR (ATR) 3492, 3187, 2987, 2818, 1702, 1599, 1413, 1284, 1027 cm^{-1}; ^1H-NMR (DMSO-d_6, 300 MHz) δ 13.10 (s, 1 H), 11.57 (s, 1 H), 11.24 (s, 1 H), 7.96 (d, 2 H, *J* = 8.7 Hz), 7.50 (d, 2 H, *J* = 8.7 Hz), 5.34 (d, 1 H, *J* = 1.5 Hz), 4.19 (q, 2 H, *J* = 7.2 Hz), 1.20 (t, 3 H, *J* = 7.2 Hz); MS (EI) *m/z* 319 (M^+, 100), 275(13), 247 (52), 137 (33), 68 (87); HRMS (EI) *m/z* calculated for $C_{14}H_{13}N_3O_6$ 319.080435, found 319.081124.



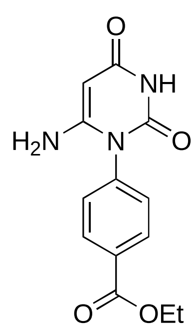
231

Ethyl 4-ureidobenzoate (231, DMA-NB74-59).[111] A solution of ethyl 4-aminobenzoate (3.00 g, 17.8 mmol) in glacial acetic acid (30 mL) was stirred at room temperature for 30 min at which time the solution turned homogenous. To this solution was added a solution of KOCN (2.17 g, 26.7 mmol) in deionized water (20 mL). The reaction mixture was heated to 70 ^{o}C for 14 h, cooled to room temperature and concentrated by rotary evaporation (80 ^{o}C). The resulting clear oil was crystallized by stirring in deionized water (60 mL) at 0 ^{o}C for 30 min. The resulting white solid was isolated by vacuum filtration, washed with water (40 mL) and recrystallized from a 2:1 solution of EtOAc:hexanes (65 mL) to afford **231** as a white crystalline solid (2.93 g, 79%): Mp 180-181 ^{o}C; IR (ATR) 3405, 3358, 3181, 1705, 1675, 1586, 1528, 1286, 1174, 1019 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 8.94 (s, 1 H), 7.81 (d, 2 H, *J* = 8.7 Hz), 7.51 (d, 2 H, *J* = 8.4 Hz), 6.04 (s, 2 H), 4.24 (q, 2 H, *J* = 7.2 Hz), 1.28 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 166.0, 156.0, 145.6, 130.7 (2 C), 122.4, 117.2 (2 C), 60.6, 14.7; HRMS (TOF MS ES+) *m/z* calculated for $C_{10}H_{12}N_2O_3Na$ (M+Na) 231.0746, found 231.0762.



232

Ethyl 4-(3-(2-cyanoacetyl)ureido)benzoate (232, DMA-NB74-40). A suspension of **231** (312 mg, 1.50 mmol) and cyanoacetic acid (129 mg, 1.50 mmol) in Ac_2O (0.50 mL) was heated in a microwave reactor for 30 min at 80 ^oC. The heterogeneous reaction mixture was cooled to room temperature, diluted with a 3:1 mixture of EtOAc:hexanes (3.0 mL) and stirred vigorously for 20 min. The resulting solids were isolated by vacuum filtration, washed with a 1:1 mixture of EtOAc:hexanes (10 mL) and dried in vacuo to afford **232** as a white crystalline solid (299 mg, 72%): Mp 232-233 ^oC; IR (ATR) 3248, 2987, 1713, 1694, 1685, 1593, 1507, 1273, 1174, 701 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.95 (s, 1 H), 10.23 (s, 1 H), 7.92 (d, 2 H, J = 8.7 Hz), 7.67 (d, 2 H, J = 8.7 Hz), 4.28 (q, 2 H, J = 6.9 Hz), 4.06 (s, 2 H), 1.30 (t, 3 H, J = 6.9 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 166.3, 165.7, 150.4, 142.3, 130.8 (2 C), 125.3, 119.6 (2 C), 115.3, 61.0, 27.6, 14.7; MS (EI) m/z 275 (M^+, 100), 57 (6); HRMS (EI) m/z calculated for $C_{13}H_{13}N_3O_4$ 275.090606, found 275.090412.

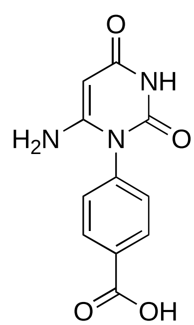


233

Ethyl 4-(6-amino-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzoate (233, DMA-NB74-43). A suspension of **232** (663 mg, 2.41 mmol) and TMSCl (0.047 mL, 0.361 mmol) in HMDS (7.2

mL) was heated to reflux for 17 h. After 17 h, the reaction mixture was cooled to room temperature and the solvent was removed by rotary evaporation (70 °C). The crude orange residue was cooled to 0 °C and hydrolyzed with a saturated $NaHCO_3$ solution (20 mL) with stirring for 30 min while warming to room temperature. The resulting precipitate was isolated by vacuum filtration, washed with distilled water (30 mL), dried in vacuo and recrystallized from a 1:1 solution of EtOAc:hexanes (50 mL) to afford **233** as a pale orange solid (512 mg, 77%): Mp 268-269 °C (dec.); IR (ATR) 3414, 3319, 3085, 2963, 1713, 1698, 1627, 1575, 1366, 1284, 1101 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.54 (s, 1 H), 8.07 (d, 2 H, *J* = 8.4 Hz), 7.49 (d, 2 H, *J* = 8.4 Hz), 6.22 (s, 2 H), 4.66 (s, 1 H), 4.36 (q, 2 H, *J* = 7.2 Hz), 1.33 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 165.7, 163.2, 155.7, 151.2, 139.0, 131.0 (2 C), 130.9, 130.6 (2 C), 75.6, 61.5, 14.6; MS (EI) *m/z* 275 (M^+, 74), 232 (32), 165 (39), 146 (44), 120 (100), 65 (33); HRMS (EI) *m/z* calculated for $C_{13}H_{13}N_3O_4$ 275.090606, found 275.090591.

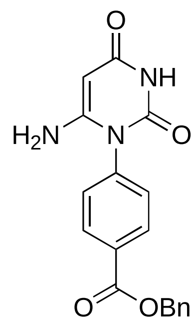
234



4-(6-Amino-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzoic acid (234, DMA-NB74-63). According to general procedure U, **233** (400 mg, 1.45 mmol) and an aqueous solution of LiOH (10.0 mL, 2.0 M) were combined at room temperature for 1.5 h to afford **234** as a light orange solid: (341 mg, 95%): Mp >380 °C; IR (ATR) 3435, 3333, 3200, 1694, 1620, 1605, 1473, 1263, 1027 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 13.16 (s, 1 H), 10.53 (s, 1 H), 8.03 (d, 2 H, *J* = 8.4

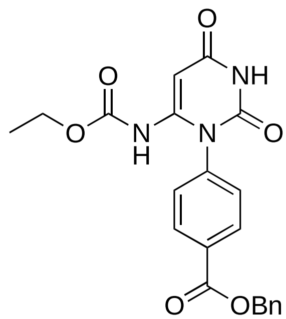
Hz), 7.44 (d, 2 H, *J* = 8.1 Hz), 6.21 (s, 2 H), 4.65 (s, 1 H); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 166.8, 162.7, 155.3, 150.8, 138.1, 131.6, 130.7 (2 C), 129.9 (2 C), 75.1; MS (EI) *m/z* 247 (M$^+$, 57), 204 (28), 163 (31), 137 (33), 120 (33), 83 (44), 68 (71), 53 (100); HRMS (EI) *m/z* calculated for $C_{11}H_9N_3O_4$ 247.059306, found 247.059011.

235

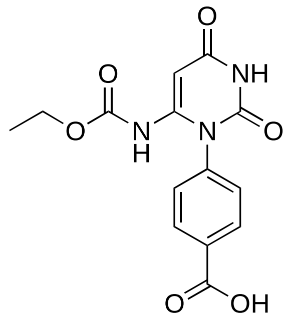


Benzyl 4-(6-amino-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzoate (235, DMA-NB74-69). To a suspension of **234** (150 mg, 0.607 mmol) in MeOH (6.0 mL) was added Cs_2CO_3 (108.6 mg, 0.333 mmol). The suspension was stirred at room temperature for 30 min, concentrated by rotary evaporation (40 ^{o}C) and dried in vacuo. The resulting off white cesium salt was dissolved in DMSO (3.0 mL) and benzyl bromide (90.6 μL, 0.758 mmol) was added. The reaction mixture was stirred at room temperature for 2 h and then distilled water (15 mL) was added with cooling to 0 ^{o}C for 30 min. The precipitate was isolated by vacuum filtration, washed with distilled water (15 mL) and dried in vacuo. The resulting solid was boiled in a 1:1 mixture of EtOAc:hexanes (30 mL) for 15 min, isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford **235** as a light orange solid (145 mg, 71%): Mp 256-257 ^{o}C (dec.); IR (ATR) 3424, 3322, 3239, 3086, 2762, 1698, 1627, 1603, 1575, 1474, 1267, 1093 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 10.54 (s, 1 H), 8.09 (d, 2 H, *J* = 8.4 Hz), 7.50-7.35 (m, 7 H), 6.21 (s, 2 H), 5.39 (s, 2 H), 4.65 (d, 1 H, *J* = 1.8 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 165.5, 163.2, 155.7, 151.2, 139.2, 136.5, 131.1 (2 C), 130.7 (3 C), 129.0 (2 C), 128.7, 128.5 (2 C), 75.6, 66.7; MS (EI) *m/z* 337 (M^+, 24),187 (13), 146 (19), 91 (100), 65 (18); HRMS (EI) *m/z* calculated for $C_{18}H_{15}N_3O_4$ 337.106256, found 337.107044.

236

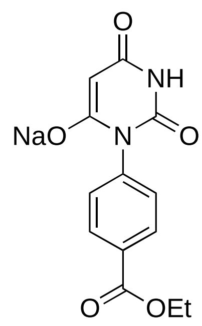


Benzyl 4-(6-(ethoxycarbonylamino)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzoate (236). To a suspension of **235** (55.0 mg, 0.163 mmol) in dry THF (0.50 mL) was added a solution of LiHMDS (42.2 mg, 0.245 mmol) in THF (0.75 mL) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 30 min upon which time the suspension cleared and ethyl chloroformate (17.1 µL, 0.179 mmol) was added. The reaction mixture was slowly warmed to room temperature and stirred for 5 h. The product was isolated by column chromatography on SiO_2 (EtOAc; R_f = 0.63) and recrystallized from a 1:5 mixture of EtOAc:hexanes (15 mL) to afford **236** as an off white solid (13.1 mg, 20%, ~80% pure by 1H-NMR). This product was used crude for the next step: ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 11.43 (s, 1 H), 9.06 (s, 1 H), 8.04 (d, 2 H, *J* = 8.1 Hz), 7.48-7.34 (m, 7 H), 5.88 (s, 1 H), 5.38 (s, 2 H), 3.81 (q, 2 H, *J* = 6.9 Hz), 1.05 (t, 3 H, *J* = 6.9 Hz).

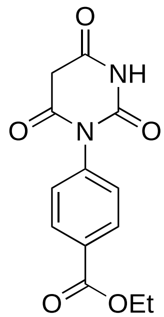


209

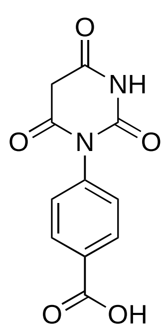
4-(6-(Ethoxycarbonylamino)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzoic acid (209). According to general procedure X, **236** (9.0 mg, 0.0176 mmol, 80% pure) and 20 wt% $Pd(OH)_2/C$ (0.7 mg, 0.0013 mmol/Pd) in distilled THF (0.50 mL) were stirred under a H_2 atmosphere (1.0 atm) for 10 h at room temperature. After filtration through celite, the resulting white solid was triturated with a 5:1 mixture of hexanes:EtOAc (10 mL) while cooling to 0 ^{o}C. The resulting white solid was isolated by vacuum filtration, washed with hexanes (3.0 mL), and dried in vacuo to afford **209** as a white solid (3.4 mg, 53%, ~87% pure by ^{1}H-NMR): Mp 289-290 ^{o}C (dec.); IR (ATR) 3399, 3214, 2987, 2791, 1755, 1705, 1702, 1639, 1610, 1522, 1377, 1204, 1036 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 13.19 (s, 1 H), 11.42 (s, 1 H), 9.04 (s, 1 H), 7.99 (d, 2 H, *J* = 8.4 Hz), 7.41 (d, 2 H, *J* = 8.1 Hz), 5.88 (d, 1 H, *J* = 1.8 Hz), 3.97 (q, 2 H, *J* = 6.9 Hz), 1.06 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d$_6$, 175 MHz) δ 166.8, 163.1, 152.5, 150.5, 147.4, 138.3, 131.2, 130.2 (2 C), 129.9 (2 C), 92.7, 61.5, 14.1; MS (EI) *m/z* 319 (M$^+$, 29), 273 (88), 230 (93), 188 (82), 68 (73), 53 (100); HRMS (EI) *m/z* calculated for $C_{14}H_{13}N_3O_6$ 319.080435, found 319.079582.

237 

Sodium 3-(4-(ethoxycarbonyl)phenyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate (237). A sodium ethoxide solution was freshly prepared by dissolving Na (280 mg, 12.2 mmol) in distilled EtOH (10 mL) under a nitrogen atmosphere. This solution was cooled to 0 ^{o}C and then **231** (1.00 g, 4.80 mmol) was added. The heterogeneous solution was stirred at room temperature for 30 min and upon clearing, diethyl malonate (0.729 mL, 4.80 mmol) was added. The homogenous reaction mixture was refluxed for 13 h, a white precipitate formed and the reaction mixture was cooled to room temperature. The reaction mixture was diluted with EtOH (5.0 mL) and acidified to a pH = 6.0-7.0 with a concentrated HCl solution. The solvent was removed by rotary evaporation (60 ^{o}C) and the product was dissolved in boiling EtOH (100 mL). The remaining inorganic solids were removed by vacuum filtration and the filtrate was concentrated by rotary evaporation (40 ^{o}C). The resulting clear oil was crystallized by boiling in acetone (75 mL) for 15 min and the resulting solid was isolated by vacuum filtration, washed with acetone (50 mL) and dried in vacuo to afford **237** as a white solid (743 mg, 49%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 9.30 (s, 1 H), 7.91 (d, 2 H, *J* = 8.4 Hz), 7.22 (d, 2 H, *J* = 8.4 Hz), 4.32 (q, 2 H, *J* = 7.2 Hz), 3.96 (d, 1 H, *J* = 2.1 Hz), 1.31 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 166.0, 164.5, 164.3, 152.9, 143.1, 130.7 (2 C), 129.2 (2 C), 128.3, 75.4, 61.1, 14.7.

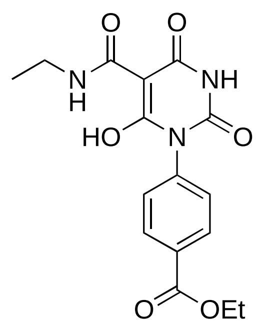
238 

Ethyl 4-(2,4,6-trioxotetrahydropyrimidin-1(2*H*)-yl)benzoate (238, DMA-NB74-61).[112] To a solution of **237** (250 mg, 0.838 mmol) in distilled water (3.0 mL) was slowly added an aqueous HCl solution (1.0 M, pH = 1.5) dropwise over a 15 min period at 0 ^oC. The reaction mixture was stirred at 0 ^oC for 30 min. The resulting precipitate was isolated by vacuum filtration, washed with deionized water (10 mL) and dried in vacuo to afford **238** as a white solid (184 mg, 79%): Mp 224-225 ^oC; IR (ATR) 3252, 3086, 2987, 1702, 1687, 1411, 1275, 1100 cm^{-1}; ^1H-NMR (DMSO-d_6, 300 MHz) δ 11.58 (s, 1 H), 8.05 (d, 2 H, *J* = 8.4 Hz), 7.40 (d, 2 H, *J* = 8.1 Hz), 4.34 (q, 2 H, *J* = 7.2 Hz), 3.74 (s, 2 H), 1.33 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 167.6 (2 C), 166.2, 152.3, 140.3, 130.8, 130.7 (2 C), 130.5 (2 C), 61.9, 15.1 (1 C in DMSO peak); MS (EI) *m/z* 276 (M^+, 23), 248 (24), 231 (55), 163 (37), 146 (100), 84 (53); HRMS (EI) *m/z* calculated for $C_{13}H_{12}N_2O_5$ 276.074622, found 276.074522.

239 

4-(2,4,6-Trioxotetrahydropyrimidin-1(2*H*)-yl)benzoic acid (239, DMA-NB74-62). According to general procedure U, **237** (225 mg, 0.641 mmol) and an aqueous solution of LiOH (2.0 mL, 2.0 M) were combined at room temperature for 2 h to afford **239** as a white crystalline solid (120 mg, 75%): Mp 303-304 ^{o}C (dec.); IR (ATR) 3390, 3219, 3086, 1716, 1690, 1685, 1606, 1351, 1204, 1114 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 13.11 (s, 1 H), 11.55 (s, 1 H), 8.01 (d, 2 H, *J* = 8.1 Hz), 7.35 (d, 2 H, *J* = 8.1 Hz), 3.73 (s, 2 H); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 167.2 (2 C), 167.1, 151.8, 139.5, 131.2, 130.3 (2 C), 129.8 (2 C), (1 C in DMSO peak); MS (EI) *m/z* 248 (M$^+$, 45), 220 (15), 163 (100), 146 (70), 90 (23); HRMS (EI) *m/z* calculated for $C_{11}H_8N_2O_5$ 248.043322, found 248.042989.

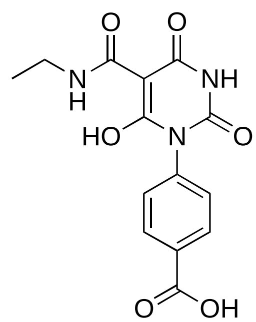
240



Ethyl 4-(5-(ethylcarbamoyl)-6-hydroxy-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzoate (240, DMA-NB74-96). To a solution of **238** (60.0 mg, 0.217 mmol) in acetonitrile (1.5 mL) was added Et_3N (46.3 μL, 0.326 mmol) followed by ethanisocyanate (18.8 μL, 0.239 mmol). The reaction mixture was stirred at room temperature for 5 h, cooled to 0 ^{o}C and acidified with an aqueous HCl solution (1.0 M, pH 2) over a 10 min. The product precipitated and the mixture was stirred at 0 ^{o}C for an additional 30 min. The precipitated product was isolated by vacuum filtration, washed with deionized water (5.0 mL), dried in vacuo and recrystallized from a 3:1 mixture of hexanes:EtOAc (10 mL) to afford **240** as a fluffy white solid (33.8 mg, 45%): Mp 241-242 ^{o}C; IR (ATR) 3209, 3086, 3002, 2829, 1718, 1709, 1685, 1592, 1446, 1282, 1096 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 17.43 (s, 1 H), 11.83 (s, 1 H), 9.69 (s, 1 H), 8.03 (d, 2 H, *J* =

8.4 Hz), 7.46 (d, 2 H, *J* = 8.4 Hz), 4.34 (q, 2 H, *J* = 7.2 Hz), 3.41-3.32 (m, 2 H), 1.33 (t, 3 H, *J* = 6.9 Hz), 1.13 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 170.3, 165.7 (3 C), 149.4, 139.6, 130.3 (2 C), 130.2, 130.0 (2 C), 79.7, 61.4, 35.0, 14.9, 14.6; MS (EI) *m/z* 347 (M$^+$, 100), 274 (12), 257 (39), 231 (12); HRMS (EI) *m/z* calculated for $C_{16}H_{17}N_3O_6$ 347.111736, found 347.110260.

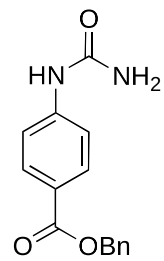
210



4-(5-(Ethylcarbamoyl)-6-hydroxy-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzoic acid (210, DMA-NB108-1). According to general procedure U, **240** (23.8 mg, 0.0685 mmol) and an aqueous LiOH solution (0.50 mL, 2.0 M) were combined at room temperature for 2 h to afford **210** as a pale yellow crystalline solid (10.1 mg, 46%): Mp 296-297 ^{o}C (dec.); IR (ATR) 3576, 3496, 3151, 2993, 2851, 1675, 1575, 1457, 1413, 1282, 1169, 1094 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 17.42 (s, 1 H), 13.13 (s, 1 H), 11.82 (s, 1 H), 9.69 (s, 1 H), 8.01 (d, 2 H, *J* = 8.1 Hz), 7.43 (d, 2 H, *J* = 8.4 Hz), 3.39 (m, 2 H), 1.13 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 170.3, 167.2 (3 C), 149.4, 139.2, 131.1, 130.1 (4 C), 79.7, 35.0, 14.9; MS (EI) *m/z* 319 (M$^+$, 74), 275 (21), 248 (15), 163 (100), 146 (83), 137 (46), 69 (44), 57 (45); HRMS (EI) *m/z* calculated for $C_{14}H_{13}N_3O_6$ 319.080435, found 319.079829.

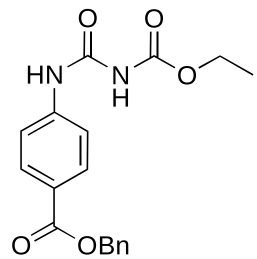
4.4.2 Second generation analogues of SID 861574: Synthesis of *N*$_1$,*N*$_3$-differentially-substituted-5-methylene-6-hydroxy uracils.

242

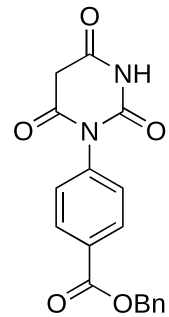


Phenylmethyl 4-(aminocarbonylamino)benzoate (242). A solution of **225** (1.25 g, 5.50 mmol) in AcOH (12.5 mL) was stirred at room temperature for 5 min until all of the amine dissolved. A solution of KOCN (669 mg, 8.25 mmol) in distilled water (6.25 mL) was then added at room temperature and the resulting clear homogenous solution was warmed to 70 °C for 15 h. The resulting suspension was cooled to room temperature, diluted with water (10 mL) and further cooled to 0 °C for 30 min. The precipitate was isolated by vacuum filtration, washed with water (30 mL), dried in vacuo and recrystallized by dissolving in boiling EtOAc (50 mL) and slowly adding hexanes (50 mL) followed by cooling to 0 °C for 30 min. The product was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford **242** as a white crystalline solid (1.15 g, 78%): Mp 196.5-197.5 ^{o}C; IR (ATR) 3440, 3308, 3202, 1705, 1655, 1580, 1530, 1265, 1109 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 8.98 (s, 1 H), 7.86 (d, 2 H, *J* = 8.7 Hz), 7.53 (d, 2 H, *J* = 8.7 Hz), 7.46-7.34 (m, 5 H), 6.05 (s, 2 H), 5.30 (s, 2 H); ^{13}C NMR (DMSO-d$_6$, 75 MHz) δ 165.8, 156.0, 145.9, 136.9, 130.9 (2 C), 129.0 (2 C), 128.5, 128.4 (2 C), 122.0, 117.3 (2 C), 66.1; MS (EI) *m/z* 270 (M$^+$, 9), 163 (23), 120 (49), 91 (100), 65 (73); HRMS (EI) *m/z* calculated for $C_{15}H_{14}N_2O_3$ 270.1004, found 270.1008.

243

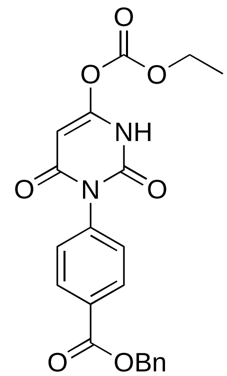


Benzyl 4-(3-(ethoxycarbonyl)ureido)benzoate (243, DMA-NB139-67). To a solution of 242 (550 mg, 2.03 mmol) in THF (20 mL) was added, at -78 ^oC, a solution of LiHMDS (418 mg, 2.42 mmol, 0.48 M) in THF, dropwise over 5 min. The mixture was stirred at -78 ^oC for 30 min and then ethyl cyanoformate (0.219 mL, 2.24 mmol) was added. The reaction mixture was slowly warmed to 0 ^oC over 5 h and was quenched by the addition of a saturated NH_4Cl solution (10 mL) with stirring for 20 min. The mixture was diluted with EtOAc (50 mL) and washed with a saturated $NaHCO_3$ solution (1 X 50 mL). The EtOAc layer was dried (Na_2SO_4), concentrated in vacuo and the yellow residue was chromatographed on SiO_2 (2:1 hexanes:EtOAc). The isolated product was recrystallized from a 10:1 solution of hexane:EtOAc (40 mL) to afford **243** as a light yellow crystalline solid (438 mg, 63%): Mp 156-157 ^oC; IR (ATR) 3239, 3133, 2969, 1726, 1694, 1593, 1549, 1505, 1273, 1241, 1090 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 10.47 (s, 1 H), 10.12 (s, 1 H), 7.95 (d, 2 H, J = 8.7 Hz), 7.66 (d, 2 H, J = 9.0 Hz), 7.47-7.34 (m, 5 H), 5.32 (s, 2 H), 4.18 (q, 2 H, J = 7.2 Hz), 1.24 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.0, 154.4, 150.2, 142.4, 136.2, 130.4 (2 C), 128.4 (2 C), 128.0, 127.9 (2 C), 124.1, 118.8 (2 C), 65.9, 61.7, 14.1; MS (EI) *m/z* 342 (M^+, 26), 235 (42), 146 (42), 91 (100); HRMS (EI) *m/z* calculated for $C_{18}H_{18}N_2O_5$ 342.1216, found 342.1202.



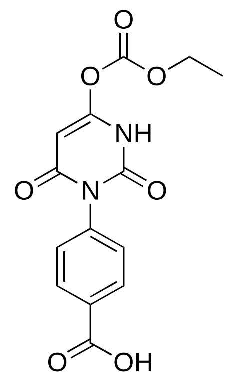
245

Phenylmethyl 4-(2,4,6-trioxo-1,3,5-trihydropyrimidinyl)benzoate (245, DMA-NB166-38). To a solution of malonyl dichloride (804 µL, 8.55 mmol) in distilled THF (55.0 mL) was added **242** (1.10 g, 4.07 mmol) at room temperature. The homogeneous light yellow reaction mixture was stirred for 5 min and then DIPEA (744 µL, 4.27 mmol) was added. After 2 h, the reaction mixture was diluted with a saturated brine solution (150 mL) and extracted with DCM (2 X 150 mL). The extracts were combined, filtered through a 1 in plug of SiO_2, washed through the plug with excess EtOAc (250 mL), dried (Na_2SO_4) and concentrated in vacuo. The resulting orange solids were dissolved in boiling EtOAc (25 mL) and hexanes (150 mL) were slowly added to precipitate the product. After cooling to 0 °C, the solid was isolated by vacuum filtration, washed with hexanes (50 mL) and dried in vacuo to afford **245** as a yellow/orange crystalline solid (532 mg, 39%): Mp 220.5-222.0 °C; IR (ATR) 3194, 3101, 2881, 1694, 1423, 1360, 1273, 1114 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.60 (s, 1 H), 8.10 (d, 2 H, *J* = 7.8 Hz), 7.49-7.35 (m, 7 H), 5.39 (s, 2 H), 3.75 (s, 2 H); ^{13}C NMR (DMSO-d_6, 175 MHz) δ 167.1 (2 C), 165.5, 151.8, 140.0, 136.5, 130.3 (2 C), 130.1 (2 C), 130.0, 129.0 (2 C), 128.6, 128.4 (2 C), 66.8, 40.8; MS (EI) *m/z* 338 (M^+, 8), 231 (27), 146 (23), 91 (100), 65 (44); HRMS (EI) *m/z* calculated for $C_{18}H_{14}N_2O_5$ 338.0903, found 338.0902.

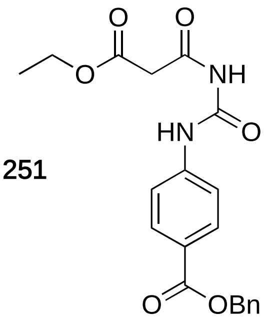


246

Phenylmethyl 4-(4-ethoxycarbonyloxy-2,6-dioxo-1,3-dihydropyrimidinyl)benzoate (246). To a homogeneous light orange solution of **245** (150 mg, 0.443 mmol) in THF (5.0 mL) was added ethyl chloroformate (48.1 µL, 0.488 mmol) followed by Et_3N (68.5 µL, 0.488 mmol) at 0 °C. The reaction mixture turned heterogeneous and dark orange upon the addition of Et_3N, was warmed to room temperature over 1 h, stirred at room temperature for 2 h, diluted with distilled water (5.0 mL) and acidified to a pH of 3-4 with an HCl solution (1.0 M, ~ 4 drops). The acidified mixture was then diluted with DCM (50 mL) and washed with a saturated brine solution (2 X 50 mL). The DCM layer was dried (Na_2SO_4), vacuum filtered through a 1 in plug of SiO_2, washed through the plug with 3:1 EtOAc:hexanes (250 mL) and concentrated by rotary evaporation (30 °C). The residue was triturated with a 3:1 solution of DCM:hexanes (75 mL), cooled to 0 °C for 15 h and the remaining solids were isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford **246** as a light yellow solid (117 mg, 64%): Mp 209.5-211.0 °C (dec); IR (ATR) 3183, 3096, 2969, 2831, 1787, 1713, 1644, 1260, 1196, 1103, 1016 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.43 (s, 1 H), 8.08 (d, 2 H, *J* = 8.4 Hz), 7.50 – 7.36 (m, 7 H), 5.82 (s, 1 H), 5.39 (s, 2 H), 4.33 (q, 2 H, *J* = 7.2 Hz), 1.31 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.1, 163.0, 154.4, 149.9, 149.5, 139.7, 136.1, 129.9 (2 C), 129.7 (2 C), 129.5, 128.6 (2 C), 128.2, 127.9 (2 C), 89.5, 66.4 (2 C), 13.9; MS (EI) *m/z* 410 (M^+, 100), 338 (67), 303 (24), 146 (9), 91 (26); HRMS (EI) *m/z* calculated for $C_{21}H_{18}N_2O_7$ 410.1114, found 410.1113.

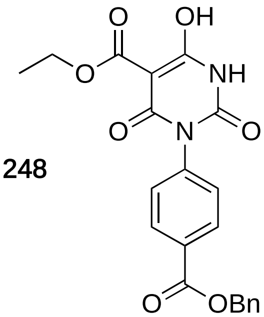
247 

4-(4-Ethoxycarbonyloxy-2,6-dioxo-1,3-dihydropyrimidinyl)benzoic acid (247). To a solution of **246** (50.0 mg, 0.122 mmol) in distilled THF (3.0 mL) was added 20 wt% $Pd(OH)_2$/C (17.1 mg, 0.0240 mmol) and the reaction mixture was placed under a hydrogen (1 atm) atmosphere at room temperature for 2 h. At this time, the reaction mixture was directly filtered through a 1 in plug of SiO_2, washed through with additional EtOAc (35 mL) and dried by rotary evaporation (25 °C). The resulting light yellow solid was triturated with a 2:1 solution of hexanes:EtOAc (25 mL), isolated by vacuum filtration, washed with hexanes (10 mL), triturated a second time with acetone (1.0 mL, 0 °C, 5 min), filtered and dried in vacuo to afford **247** as a white powdery solid (23.7 mg, 61%): Mp 210-211 °C (dec); IR (ATR) 3075, 2969, 2825, 2674, 1787, 1694, 1649, 1423, 1260, 1185, 1016 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 13.12 (s, 1 H), 12.42 (s, 1 H), 8.01 (d, 2 H, *J* = 8.1 Hz), 7.42 (d, 2 H, *J* = 8.1 Hz), 5.81 (s, 1 H), 4.33 (q , 2 H, *J* = 7.2 Hz), 1.32 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.2, 163.5, 154.9, 150.4, 150.0, 139.6, 131.1, 130.3 (2 C), 129.8 (2 C), 89.8, 66.8, 14.3; MS (EI) *m/z* 320 (M^+, 11), 248 (43), 163 (100), 146 (71); HRMS (EI) *m/z* calculated for $C_{14}H_{12}N_2O_7$ 320.0645, found 320.0650.



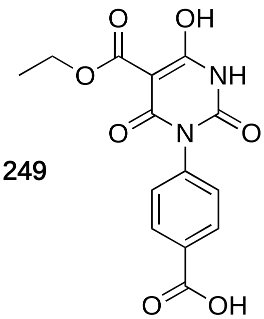
251

Ethyl 2-[*N*-(*N*-{4-[benzyloxycarbonyl]phenyl}carbamoyl)carbamoyl]acetate (251, DMA-NB205-51). To a solution of ethyl malonyl chloride (186 µL, 1.20 mmol) in THF (7.0 mL) was added **242** (270 mg, 1.00 mmol) in one portion at room temperature. The resulting dark orange suspension cleared after 5 h. After 20 h, the reaction mixture was directly filtered through a 1 in plug of SiO_2 and flushed through with EtOAc (75 mL). The filtrate was concentrated in vacuo and the resulting orange solids were dissolved in boiling EtOAc (20 mL) to which hexanes (80 mL) was slowly added to precipitate the product. After slowly cooling to room temperature and then to 0 °C for 30 min, the precipitate was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford methyl **251** as light yellow crystalline needles (300 mg, 78%): Mp 136.5-137.5 °C; IR (ATR) 3233, 3120, 2982, 1750, 1694, 1599, 1554, 1506, 1273, 1247, 1154, 1096, 1021 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.62 (s, 1 H), 10.00 (s, 1 H), 8.06 (d, 2 H, J = 8.7 Hz), 7.60 (d, 2 H, J = 8.7 Hz), 7.46-7.35 (m, 5 H), 5.36 (s, 2 H), 4.25 (q, 2 H, J = 7.2 Hz), 3.52 (s, 2 H), 1.31 (t, 3 H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.8, 167.4, 166.1, 150.8, 141.5, 136.2, 131.2 (2 C), 128.8 (2 C), 128.4 (3 C), 126.1, 119.6 (2 C), 66.9, 62.5, 42.5, 14.2; MS (EI) *m/z* 384 (M^+, 57), 277 (27), 146 (100), 91 (80); HRMS (EI) *m/z* calculated for $C_{20}H_{20}N_2O_6$ 384.1321, found 384.1323.



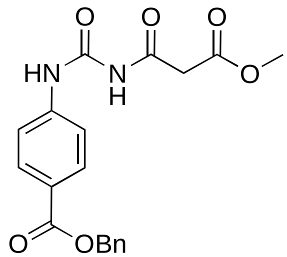
248

Phenylmethyl 4-[5-(ethoxycarbonyl)-4-hydroxy-2,6-dioxo-1,3-dihydropyrimidinyl]benzoate (248, DMA-NB205-60). To a pale yellow solution of **251** (150 mg, 0.390 mmol) in DCM (8.0 mL) was added Et_3N (114 µL, 0.820 mmol) followed by CDI (94.8 mg, 0.585 mmol) at room temperature. The reaction mixture was stirred for 36 h, diluted with deionized water (5.0 mL), acidified to a pH of 1.5 with an HCl solution (1.0 M) and then diluted with DCM (30 mL). The aqueous layer was removed and the DCM layer was washed with an HCl solution (30 ml deionized water, 2 drops of 1.0 M HCl), dried (Na_2SO_4) and concentrated by rotary evaporation (20 °C). The resulting tan solids were dissolved in EtOAc (20 mL) and hexanes (60 mL) were added to precipitate the product. After cooling to 0 °C for 30 min, the resulting solid was isolated by vacuum filtration, washed with hexanes (20 mL) and dried in vacuo to afford **248** as a light tan solid (106 mg, 66%): Mp 214-216 °C (dec); IR (ATR) 3164, 2982, 1737, 1681, 1605, 1517, 1415, 1277, 1150, 1105, 1005 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.50 (s, 1 H), 8.07 (d, 2 H, J = 8.4 Hz), 7.50-7.36 (m, 7 H), 5.39 (s, 2 H), 4.27 (q, 2 H, J = 7.2 Hz), 1.24 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 171.7, 168.5, 165.6, 160.1, 149.3, 140.4, 136.6, 130.4 (2 C), 130.2 (2 C), 129.8, 129.0 (2 C), 128.6, 128.4 (2 C), 83.1, 66.8, 61.9, 14.5; MS (EI) *m/z* 410 (M^+, 2), 364 (57), 257 (88), 231 (57), 146 (78), 91 (100); HRMS (EI) *m/z* calculated for $C_{21}H_{18}N_2O_7$ 410.1114, found 410.1100.

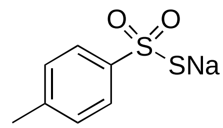


249

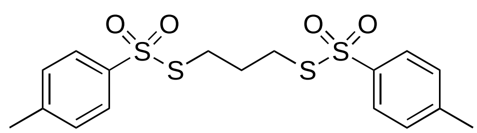
4-[5-(Ethoxycarbonyl)-4-hydroxy-2,6-dioxo-1,3-dihydropyrimidinyl]benzoic acid (249, DMA-NB205-71). To a solution of **248** (50.0 mg, 0.122 mmol) in THF (3.0 mL) was added $Pd(OH)_2/C$ (17.0 mg, 0.0244 mmol) at room temperature. The resulting dark black suspension was stirred under hydrogen (1 atm) for 3 h and was then filtered through a plug of SiO_2 (1 in, 5 3/4 in disposable glass pipette) with EtOAc (40 mL). The filtrate was concentrated by rotary evaporation (25 °C) and the resulting solids were dried in vacuo to afford **249** as a light tan powder (32.3 mg, 83%): Mp 241-242 °C (dec); IR (ATR) 3233, 3101, 2974, 2894, 1750, 1718, 1694, 1592, 1474, 1424, 1379, 1286, 1228, 1165 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.09 (s, 1 H), 12.41 (s, 1 H), 8.00 (d, 2 H, *J* = 8.4 Hz), 7.37 (d, 2 H, *J* = 8.4 Hz), 4.27 (q , 2 H, *J* = 6.9 Hz), 1.24 (t, 3 H, *J* = 6.9 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 171.7, 168.4, 167.3, 160.2, 149.4, 139.9, 130.9, 130.2 (2 C), 130.1 (2 C), 83.2, 61.9, 14.5; MS (EI) *m/z* 320 (M^+, 4.5), 303 (42), 274 (20), 163 (57), 146 (63), 91 (76), 69 (80), 57 (100); HRMS (EI) *m/z* calculated for $C_{14}H_{12}N_2O_7$ 320.0645, found 320.0633.

253 

Benzyl 4-(3-(3-methoxy-3-oxopropanoyl)ureido)benzoate (253). To a solution of methyl malonyl chloride (162 µL, 1.51 mmol) in THF (8.0 mL) was added **242** (370 mg, 1.37 mmol) in one portion at room temperature. The resulting light brown suspension cleared after 5 h at room temperature and a precipitate reformed after 20 h. The reaction mixture was then diluted with EtOAc (10 mL), filtered through a 1 in plug of SiO_2, flushed through with EtOAc (100 mL) and concentrated in vacuo. The resulting solids were dissolved in boiling EtOAc (20 mL) and hexanes (80 mL) was slowly added to precipitate the product. After slowly cooling to room temperature and then to 0 °C for 30 min, the precipitate was isolated by vacuum filtration, washed with hexanes (50 mL) and dried in vacuo to afford **253** as a tan solid (467 mg, 92%): Mp 163.5-164.5 °C; IR (ATR) 3233, 3144, 3000, 1756, 1731, 1726, 1700, 1599, 1411, 1265, 1236, 1154 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.60 (s, 1 H), 9.91 (s, 1 H), 8.06 (d, 2 H, *J* = 8.4 Hz), 7.60 (d, 2 H, *J* = 8.4 Hz), 7.47-7.35 (m, 5 H), 5.36 (s, 2 H), 3.81 (s, 3 H), 3.54 (s, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.6, 167.4, 165.9, 150.8, 141.2, 136.0, 131.0 (2 C), 128.6 (2 C), 128.3, 128.2 (2 C), 125.9, 119.5 (2 C), 66.7, 53.0, 42.3; MS (EI) *m/z* 370 (M^+, 15), 263 (13), 146 (77), 91 (100); HRMS (EI) *m/z* calculated for $C_{19}H_{18}N_2O_6$ 370.1165, found 370.1174.

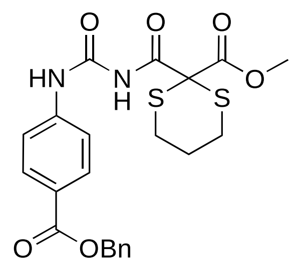
296 

Sodium 4-methylbenzenesulfonothioate (296).[94] To a suspension of *para*-toluenesulfinic acid sodium salt (5.00 g, 28.1 mmol) in anhydrous pyridine (30.0 mL) was added sulfur (900 mg, 28.1 mmol) at room temperature. The initially pale yellow suspension began to clear and a heavy white precipitate formed after 16 h. After 16 h, the reaction mixture was diluted with ether (30 mL) and was stirred at room temperature for 5 min before cooling to 0 ^{o}C for 30 min. The white precipitate was then isolated by vacuum filtration, washed with ether (4 X 25 mL) and dried in vacuo to afford **296** (5.43 g, 92%): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.65 (d, 2 H, J = 7.8 Hz), 7.15 (d, 2 H, J = 7.5 Hz), 2.29 (s, 3 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.9, 138.9, 128.6 (2 C), 124.5 (2 C), 21.3.

254 

S,S'-propane-1,3-diyl bis(4-methylbenzenesulfonothioate) (254).[94] To a suspension of KI (19.1 mg, 0.115 mmol) and **296** (5.00 g, 23.8 mmol) in 95% ethanol (20 mL) was added freshly distilled 1,3-dibromopropane (1.17 mL, 11.5 mmol) at room temperature. The heterogeneous reaction mixture was heated to reflux for 5 h, cooled to room temperature and diluted with deionized water (30 mL) followed by DCM (75 mL). The DCM layer was removed, washed with a saturated brine solution (2 X 50 mL), dried (Na_2SO_4) for 30 min and concentrated by rotary evaporation (40 ^{o}C). The resulting clear oil was dissolved in DCM (2.0 mL) for chromatography on SiO_2 (gradient elution 1:1 hexanes:DCM -> DCM). The isolated product fractions were combined, concentrated by rotary evaporation (40 ^{o}C), transferred to a preweighed 10 mL RBF with DCM (2.0 mL), re-concentrated by rotary evaporation (40 °C) and dried in vacuo afford **254** as a white crystalline solid (3.66 g, 73%, ~95% pure): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.79 (d, 4 H, *J* = 8.1 Hz), 7.36 (d, 4 H, *J* = 8.1 Hz), 3.01 (t, 4 H, *J* = 6.9 Hz), 2.47 (s, 6 H), 2.06 (m, 2 H, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 145.2 (2 C), 141.5 (2 C), 130.0 (4 C), 127.1 (4 C), 34.1 (2 C), 28.1, 21.7 (2 C).

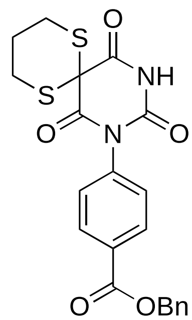
255



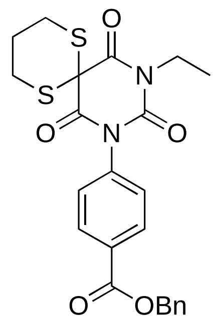
Methyl 2-(4-(benzyloxycarbonyl)phenylcarbamoylcarbamoyl)-1,3-dithiane-2-carboxylate (255). To a suspension of **253** (450 mg, 1.22 mmol) in DCM (12.0 mL) was added Et_3N (423 µL, 3.04 mmol) followed by **254** (557 mg, 1.34 mmol) at room temperature. The reaction mixture cleared and turned light orange within 5 min. After 3 h, the reaction mixture was diluted with DCM (50 mL), washed with a saturated brine solution (2 X 50 mL), dried (Na_2SO_4) and concentrated in vacuo. The resulting yellow oil was dissolved in CH_2Cl_2 (1.5 mL) and chromatographed on SiO_2 (gradient elution 3:1 -> 2:1 hexanes:EtOAc). The product fractions were combined and concentrated in vacuo to afford **255** as a white solid (540 mg, 94%): Mp 134-135 °C; IR (ATR) 3265, 3226, 3114, 2956, 1694, 1592, 1543, 1260, 1204, 1172, 1109 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.46 (br-s, 1 H), 9.28 (br-s, 1 H), 8.08 (d, 2 H, *J* = 8.7 Hz), 7.63 (d, 2 H, *J* = 8.7 Hz), 7.47-7.34 (m, 5 H), 5.36 (s, 2 H), 3.85 (s, 3 H), 3.11-2.94 (m, 4 H), 2.10-2.06 (m, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.6, 166.4, 165.8, 149.6, 141.2, 136.1, 131.0 (2 C), 128.6 (2 C), 128.3, 128.2 (2 C), 126.0, 119.4 (2 C), 66.7, 61.2, 54.6, 28.1 (2 C), 23.4; MS

(EI) *m/z* 474 (M^+, 4), 253 (24), 227 (86), 177 (99), 91 (100); HRMS (EI) *m/z* calculated for $C_{22}H_{22}N_2O_6S_2$ 474.0919, found 474.0908.

256

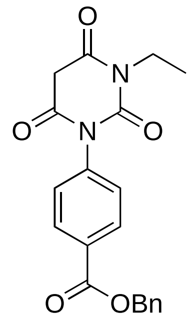


Benzyl 4-(7,9,11-trioxo-1,5-dithia-8,10-diazaspiro[5.5]undecan-8-yl)benzoate (256, DMA-NB166-96). To a suspension of **255** (488 mg, 1.03 mmol) in ACN (50 mL) was added K_2CO_3 (157 mg, 1.13 mmol) at room temperature. The reaction mixture was heated to reflux for 4 h, cooled to room temperature, diluted with distilled water (30 mL) and acidified with an HCl solution (1.0 M, pH = 2-3). The resulting cloudy solution was diluted with a saturated brine solution (150 mL) and was extracted with EtOAc (2 X 150 mL). The extracts were combined, dried (Na_2SO_4) and concentrated by rotary evaporation (40 °C). The resulting yellow solids were chromatographed on SiO_2 (gradient elution 3:1 -> 2:1 hexanes:EtOAc) and the product fractions were combined and concentrated in vacuo to afford **256** as white solid (306 mg, 67%): Mp 112.0-113.5 °C; IR (ATR) 3220, 3114, 2932, 1694, 1392, 1323, 1265 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.61 (br-s, 1 H), 8.22 (d, 2 H, *J* = 8.1 Hz), 7.46-7.27 (m, 7 H), 5.40 (s, 2 H), 3.36-3.27 (m, 2 H), 3.18-3.10 (m, 2 H), 2.19-2.16 (m, 2 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.1, 165.6, 164.7, 148.3, 137.7, 135.9, 131.3, 131.1 (2 C), 128.8 (4 C), 128.5, 128.4 (2 C), 67.3, 52.0, 27.6 (2 C), 23.1; MS (EI) *m/z* 442 (M^+, 7), 335 (13), 263 (21), 146 (93), 118 (81), 91 (100); HRMS (EI) *m/z* calculated for $C_{21}H_{18}N_2O_5S_2$ 442.065716, found 442.063885.

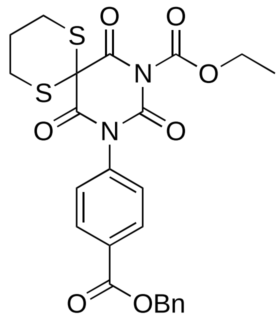


257

Benzyl 4-(10-ethyl-7,9,11-trioxo-1,5-dithia-8,10-diazaspiro[5.5]undecan-8-yl)benzoate (257, DMA-NB205-38). To a clear solution of **256** (303 mg, 0.680 mmol) in DCM (20 mL) was added Et_3N (474 µL, 3.40 mmol) followed by ethyl chloroformate (325 µL, 3.40 mmol) dropwise over 25 min at 0 °C. The clear reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature over 30 min and then filtered directly through a 1 in plug of SiO_2 and washed through with a 3:1 mixture of hexanes:EtOAc (100 mL). The filtrate was concentrated in vacuo and the resulting white solids were purified by recrystallization from a boiling 2:1 mixture of hexanes:EtOAc (30 mL) followed by cooling to 0 °C for 15 h. The resulting solid was isolated by vacuum filtration, washed with hexanes (15 mL) and dried in vacuo to afford **257** as a clear crystalline solid (225 mg, 71%): Mp 161-162 °C; IR (ATR) 2974, 1713, 1681, 1398, 1385, 1314, 1273, 1109, 846 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.22 (d, 2 H, *J* = 8.1 Hz), 7.47-7.31 (m, 7 H), 5.40 (s, 2 H), 4.00 (q, 2 H, *J* = 6.9), 3.40-3.31 (m, 2 H), 3.15-3.07 (m, 2 H), 2.20-2.13 (m, 2 H), 1.26 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 165.6 (2 C), 164.9, 149.3, 138.6, 135.9, 131.1 (3 C), 128.8 (2 C), 128.7 (2 C), 128.5, 128.3 (2 C), 67.2, 52.6, 38.9, 27.9 (2 C), 23.3, 13.1; MS (EI) *m/z* 470 (M^+, 2), 400 (93), 259 (49), 65 (100); HRMS (EI) *m/z* calculated for $C_{23}H_{22}N_2O_5S_2$ 470.0970, found 470.0973.

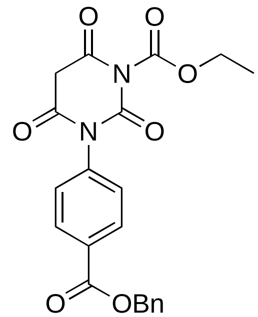
259 

Phenylmethyl 4-(3-ethyl-2,4,6-trioxo-1,3,5-trihydropyrimidinyl)benzoate (259, DMA-NB205-58). To a solution of **257** (130 mg, 0.267 mmol) in THF (2.0 mL) was added nano-zinc (181 mg, 2.76 mmol) followed by a saturated NH_4Cl solution (2.0 mL). The heterogeneous biphasic black reaction mixture was stirred at room temperature for 45 min, carefully acidified to pH 1.5 with an HCl solution (1.0 M) and then filtered through a plug of celite (1 in) with EtOAc (75 mL). The filtrate was washed with a saturated brine solution (2 X 50 mL), dried (Na_2SO_4) and concentrated by rotary evaporation (35 °C). The resulting pale yellow oil was re-filtered through a 1 in plug of celite (9 in disposable pipette) with EtOAc (25 mL) and the filtrate was concentrated by rotary evaporation (20 °C). The resulting clear oil was dissolved in DCM (3.0 mL) and hexanes (15 mL) were added. The cloudy white solution was cooled to -78 °C for 30 min and the resulting solid was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford **259** as a white powdery solid (62.8 mg, 62%): Mp 152.5-154.0 °C; IR (ATR) 2974, 2894, 1700, 1681, 1398, 1273, 1122, 1228, 1172 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.22 (d, 2 H, *J* = 8.4 Hz), 7.43-7.38 (m, 5 H), 7.29 (d, 2 H, *J* = 8.4 Hz), 5.39 (s, 2 H), 4.00 (q, 2 H, *J* = 6.9 Hz), 3.86 (s, 2 H), 1.26 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 165.6, 164.4, 164.2, 150.9, 138.3, 135.9, 131.1 (3 C), 128.9 (2 C), 128.8 (2 C), 128.6, 128.4 (2 C), 67.2, 40.2, 37.9, 13.4; MS (EI) *m/z* 366 (M^+, 58), 259 (93), 146 (88), 91 (100); HRMS (EI) *m/z* calculated for $C_{20}H_{18}N_2O_5$ 366.1216, found 366.1199.



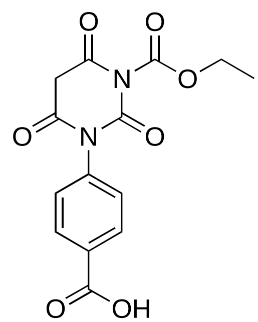
258

Phenylmethyl 4-[4-(ethoxycarbonyl)-1,3,5-trioxo-7,11-dithia-2,4-diazaspiro[5.5]undec-2-yl]benzoate (258, DMA-NB205-59). To a solution of **256** (300 mg, 0.678 mmol) in dry DCM (7.0 mL) cooled to 0 °C was added anhydrous pyridine (274 µL, 3.39 mmol) followed by ethyl chloroformate (334 µL, 3.39 mmol). The clear reaction mixture was stirred at 0 °C for 30 min and was slowly warmed to room temperature over 30 min. The reaction mixture was then diluted with DCM (50 mL), washed with deionized water (4 X 50 mL), dried (Na_2SO_4) and concentrated by rotary evaporation (10-15 °C). The resulting pale yellow oil was dissolved in DCM (5.0 mL) and hexanes (30 mL) were added at room temperature. The cloudy solution was then cooled to -78 °C under nitrogen for 30 min. A precipitate formed which was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford **258** as a white powdery solid (317 mg, 91%): Mp 147.5-148.5 °C; IR (ATR) 2987, 1787, 1705, 1694, 1398, 1334, 1273, 1215, 1109, 1008 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.23 (d, 2 H, *J* = 8.7 Hz), 7.47-7.33 (m, 7 H), 5.40 (s, 2 H), 4.51 (q, 2 H, *J* = 6.9 Hz), 3.27-3.19 (m, 4 H), 2.21-2.15 (m, 2 H), 1.43 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 165.5, 164.8, 162.6, 148.2, 146.6, 137.5, 135.9, 131.5, 131.2 (2 C), 128.9 (2 C), 128.7 (2 C), 128.6, 128.4 (2 C), 67.3, 67.1, 52.6, 27.8 (2 C), 23.1, 13.9; MS (EI) *m/z* 514 (M^+, 100), 346 (29), 178 (33), 91 (48); HRMS (EI) *m/z* calculated for $C_{24}H_{22}N_2O_7S_2$ 514.0868, found 514.0852.

244 

Ethyl 2,4,6-trioxo-3-{4-[benzyloxycarbonyl]phenyl}-1,3,5-trihydropyrimidinecarboxylate (244, DMA-NB245-23). To a solution of **258** (90.0 mg, 0.175 mmol) in THF (3.0 mL) was added nano-zinc (229 mg, 3.50 mmol) followed by a saturated NH_4Cl solution (3.0 mL) at room temperature. The heterogeneous biphasic black reaction mixture was vigorously stirred at room temperature for 2 h and was then slowly acidified to pH 1-1.5 with an HCl solution (1.0 M, the mixture clears and the Zn clumps together near this pH). The resulting mixture was vacuum filtered through a plug of SiO_2 (1 in) with EtOAc (100 mL). The filtrate was transferred to a separatory funnel, the water layer was removed, and the EtOAc layer was dried (Na_2SO_4) and concentrated by rotary evaporation (25 °C). The resulting clear oil was re-filtered through a plug of SiO_2 with a solution of EtOAc (0.10% TFA, 30 mL) and the filtrate was concentrated by rotary evaporation (20 °C). The clear oil was dissolved in DCM (3.0 mL) and hexanes (40 mL) were slowly added. The turbid solution was cooled to -5 °C for 15 h over which time a fluffy white solid crystallized. This solid was isolated by vacuum filtration, washed with hexanes (10 mL) and dried in vacuo. An attempt to dissolve the white solid in $CDCl_3$ for ^1H NMR resulted in a turbid solution in which a small amount of a sticky pale yellow oil did not dissolve. This solution was gravity filtered through a plug of sand (0.5 in, contained in a 5 3/4 in disposable pipette packed tightly with a plug of glass wool) with additional DCM (2.5 mL). The filtrate was concentrated and dried in vacuo (20 °C) to afford **244** as a white powdery solid (47.5 mg, 66%): Mp 140.5-142.0 °C; IR (ATR) 2981, 2913, 1787, 1763, 1694, 1411, 1347, 1247, 1215, 1191, 1016, 934, 857 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (d, 2 H, *J* = 8.4 Hz), 7.46-7.35 (m, 5 H), 7.29 (d, 2 H, *J* = 8.4 Hz), 5.38 (s, 2 H), 4.48 (q, 2 H, *J* = 7.2 Hz), 3.87 (s, 2 H), 1.40 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 165.5, 163.9, 162.2, 148.7, 148.3, 137.2, 135.8, 131.3, 131.1 (2 C), 128.8 (4 C), 128.5, 128.3 (2 C), 67.2, 67.0, 39.8, 13.8; MS (EI) *m/z* 410 (M^+, 24), 303 (100); HRMS (EI) *m/z* calculated for $C_{21}H_{18}N_2O_7$ 410.1114, found 410.1112.

241

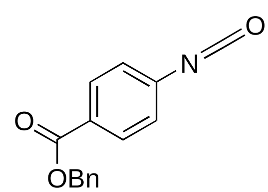


4-[3-(ethoxycarbonyl)-2,4,6-trioxo-1,3,5-trihydropyrimidinyl]benzoic acid (241, DMA-NB205-75). To a solution of **244** (53.0 mg, 0.129 mmol) in THF (3.0 mL) was added $Pd(OH)_2$/C (18.0 mg, 0.0256 mmol, 20%) and the resulting dark back suspension was placed under hydrogen (1 atm) for 2 h at room temperature. At this time, due to low conversion, a second batch of $Pd(OH)_2$/C (9.0 mg, 0.0128 mmol, 10%) was added and the reaction went to completion after an additional 2 h. The reaction mixture was directly filtered through a 1 in plug of SiO_2 (5 3/4 in disposable pipette, pre-treated once with a 100:0.1 solution of EtOAc:TFA and then flushed with EtOAc (10 mL)) with EtOAc (45 mL). The filtrate was condensed by rotary evaporation (25 °C) and the resulting solids were dissolved in EtOAc (4.0 mL). Hexanes were added (50 mL) and the solution was cooled to 0 °C for 30 min. The resulting precipitate was isolated by vacuum filtration, washed with a 5:1 solution of hexanes:DCM (10 mL), hexanes (20 mL) and dried in vacuo to afford **241** as a white powdery solid (31.8 mg, 77%): Mp 219-220 °C; IR (ATR) 2995, 2900, 2686, 1795, 1756, 1694, 1675, 1385, 1347, 1247, 1215, 1016 cm^{-1}; ^{1}H

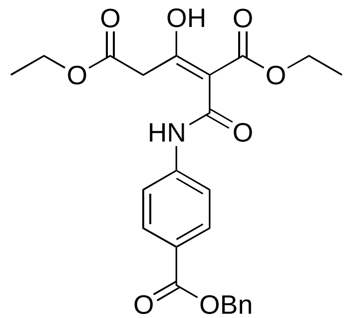
NMR (DMSO-d_6, 300 MHz) δ 13.20 (s, 1 H), 8.04 (d, 2 H, *J* = 8.1 Hz), 7.41 (d, 2 H, *J* = 8.1 Hz), 4.41 (q, 2 H, *J* = 6.9 Hz), 3.97 (s, 2 H), 1.29 (t, 3 H, *J* = 6.9 Hz); ^{13}C-NMR (DMSO-d_6, 175 MHz) δ 166.7, 165.1, 164.0, 149.3, 148.9, 138.2, 131.1, 130.0 (2 C), 129.2 (2 C), 65.9, 40.6, 13.6; MS (EI) *m/z* 320 (M^+, 97), 303 (9), 248 (18), 163 (100), 146 (31); HRMS (EI) *m/z* calculated for $C_{14}H_{12}N_2O_7$ 320.0645, found 320.0638.

4.4.3 Synthesis and identification of the structure of SID 861574

262



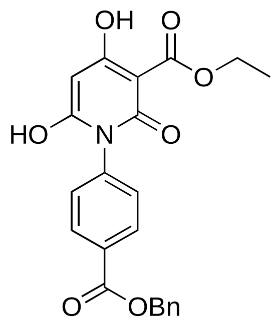
Phenylmethyl 4-isocyanatobenzoate (262). To a mixture of **225** (600 mg, 2.64 mmol) in DCM (25 mL) was added a saturated $NaHCO_3$ solution (25 mL) and the resulting biphasic mixture was vigorously stirred while cooling to 0 °C over 15 min (ice bath). Stirring was stopped, the layers were allowed to separate and phosgene (2.78 mL, 5.28 mmol, 20% in toluene) was added in a single portion via syringe to the lower organic layer. Stirring was resumed immediately and continued at 0 °C for 30 min. The reaction mixture was then transferred to a separatory funnel, diluted with distilled water (30 mL) and the aqueous layer was extracted with DCM (2 X 60 mL). The DCM extracts were combined, dried (Na_2SO_4) and concentrated in vacuo to afford **262** as a pale yellow-orange oil (658 mg, 98%). This product was used crude for the next step: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05 (d, 2 H, *J* = 8.7 Hz), 7.47-7.36 (m, 5 H), 7.15 (d, 2 H, *J* = 8.7 Hz), 5.37 (s, 2 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 165.6, 138.1, 135.8, 131.3 (2 C), 128.7 (2 C), 128.4, 128.3 (2 C), 127.5, 125.6, 124.8 (2 C), 67.0.



263

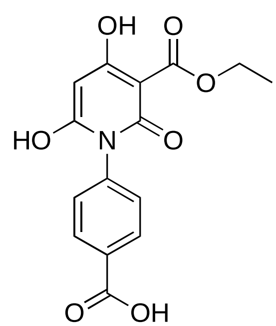
Diethyl (2*Z*)-3-hydroxy-2-(*N*-{4-[benzyloxycarbonyl]phenyl}carbamoyl)pent-2-ene-1,5-dioate (263, DMA-NB205-79). To a solution of diethyl 3-oxopentane-1,5-dioate (72.7 µL, 0.400 mmol) in dry ether (5.0 mL) was added NaH (11.1 mg, 0.440 mmol) in one portion at room temperature. A hydrogen gas release was realized immediately and the initially white suspension cleared after 5 min. The reaction mixture was cooled to 0 °C and a solution of 262 (101 mg, 0.400 mmol) in dry ether (1.0 mL) was added dropwise over 5 min. The resulting white suspension was warmed to room temperature over 5 min, heated to reflux for 2.5 h, cooled to room temperature, diluted with distilled water (10 mL) and the resulting suspension was acidified with an HCl solution (1.0 M, pH = 1-1.5). The aqueous layer was extracted with ether (2 X 20 mL). The ether layers were combined, dried (Na_2SO_4) and concentrated by rotary evaporation (25 °C). The resulting white solids were dissolved in DCM (3.0 mL) and hexanes (30 mL) were slowly added at room temperature. The resulting cloudy white solution was cooled at 0 °C for 1 h and a crystalline solid precipitated, which was isolated by vacuum filtration, washed with hexanes (40 mL) and dried in vacuo to afford **263** as clear crystalline needles (112 mg, 61%): Mp 96-97 °C; IR (ATR) 3125, 3070, 2982, 2937, 1718, 1681, 1586, 1530, 1379, 1273, 1172, 1090, 1008 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 18.20 (s, 1 H), 11.56 (s, 1 H), 8.08 (d, 2 H, *J* = 9.0 Hz), 7.64 (d, 2 H, *J* = 8.7 Hz), 7.48-7.35 (m, 5 H), 5.37 (s, 2 H), 4.30 (q, 2 H, *J* = 7.2 Hz), 4.22 (q, 2 H, *J* = 7.2 Hz), 3.83 (s, 2 H), 1.35 (t, 3 H, *J* = 7.2 Hz), 1.30 (t, 3 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 186.6, 170.9, 168.2, 167.8, 165.9, 141.1, 136.1, 130.9 (2 C), 128.6 (2 C), 128.3, 128.2 (2 C), 126.2, 120.5 (2 C), 96.3, 66.7, 61.5, 61.4, 45.5, 14.2, 14.1; HRMS (ESI) *m/z* calculated for $C_{24}H_{25}NO_8Na$ 478.1478, found 478.1448.

264



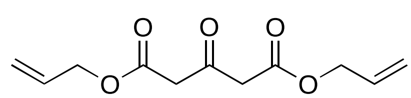
Ethyl 1-(4-(benzyloxycarbonyl)phenyl)-4,6-dihydroxy-2-oxo-1,2-dihydropyridine-3-carboxylate (264). To a solution of diethyl 3-oxopentane-1,5-dioate (179 µL, 0.987 mmol) in distilled THF (20 mL) cooled to 0 °C was added NaH (56.4 mg, 2.35 mmol) in one portion. The ice bath was immediately removed and the white suspension was slowly warmed to room temperature over 15 min. At this time, hydrogen gas release ceased and the light suspension was re-cooled to 0 °C over 10 min. To the reaction mixture was added, dropwise over 5 min, a solution of **262** (238 mg, 0.940 mmol) in THF (2.0 mL). The ice bath was immediately removed and the heterogeneous reaction mixture was warmed to room temperature over 20 min and to reflux for 2 h. After 2 h, the reaction mixture cleared, became light yellow in color and was cooled to room temperature. To the mixture was then added an HCl solution (1.0 M, 0.50 mL, to quench any un-reacted NaH) followed by deionized water (20 mL). The biphasic mixture was neutralized (1.0 M HCl, pH 7-8) and then extracted with ether (1 X 30 mL). The aqueous layer was acidified (1.0 M HCl, pH = 1-1.5) and the resulting cloudy solution was extracted with DCM (2 X 50 mL). The DCM extracts were combined, dried (Na_2SO_4) and concentrated in vacuo. The resulting light tan solids were recrystallized (2 X) by dissolving in 2-3 mL of DCM and then slowly adding hexanes (20 mL) with cooling to 0 °C for 30 min. After each recrystallization, the resulting tan solids were isolated by vacuum filtration, washed with a 2:1 mixture of hexanes:DCM (20 mL) and dried in vacuo. The product of the second recrystallization showed **264** (149 mg, 39%, ~92% pure by ^{1}H NMR): Mp 180.5-182.0 °C; IR (ATR) 3082, 2982, 2693, 2441, 1718, 1636, 1586, 1485, 1424, 1334, 1265, 1236, 1103, 1003 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 13.14 (s, 1 H), 11.01 (br-s, 1 H), 8.06 (d, 2 H, J = 8.1 Hz), 7.50-7.35 (m, 7 H), 5.39 (s, 2 H), 5.32 (s, 1 H), 4.18 (q, 2 H, J = 6.9 Hz), 1.21 (t, 3 H, J = 6.9 Hz); ^{13}C-NMR (DMSO-d$_6$, 75 MHz) δ 174.4, 172.0, 165.6, 161.9, 160.2, 141.3, 136.6, 130.2 (2 C), 130.1 (2 C), 129.5, 129.0 (2 C), 128.6, 128.4 (2 C), 89.6, 81.8, 66.8, 60.8, 14.6; HRMS (ESI) m/z calculated for $C_{22}H_{19}NO_7Na$ 432.1059, found 432.1064.

260



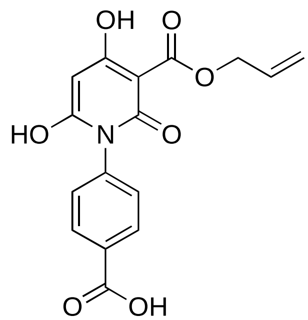
4-[3-(Ethoxycarbonyl)-4,6-dihydroxy-2-oxohydropyridyl]benzoic acid (260, DMA-NB245-14). To a clear solution of diethyl 3-oxopentane-1,5-dioate (229 µL, 1.26 mmol) and ethyl 4-isocyanatobenzoate (200 mg, 1.05 mmol) in distilled THF (20 mL) cooled to 0 °C was added NaH (66.0 mg, 2.62 mmol) in one portion. Hydrogen gas release was noted upon the addition of NaH. The reaction mixture was stirred at 0 °C for 30 min and then warmed to room temperature for 30 min. To the heavy white suspension was then added a sodium hydroxide solution (1.0 M, 20 mL) and the biphasic mixture was vigorously stirred for 1 h at room temperature. The THF layer was removed and the aqueous layer was washed with ether (2 X 40 mL) and acidified with concentrated HCl to pH 0.0-0.5 at 0 °C. The resulting white suspension was stirred at 0 °C for an additional 30 min following the acidification and the precipitate was isolated by vacuum filtration, washed with deionized water (50 mL) and dried in vacuo to afford **260** as a white powdery solid (291 mg, 87%): Mp 225-227 °C (dec); IR (ATR) 3082, 2969, 2863, 2699, 1687, 1644, 1586, 1517, 1498, 1429, 1416, 1273, 1228, 1109 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 13.14 (s, 1 H), 13.00 (br-s, 1 H), 10.84 (br-s, 1 H), 8.00 (d, 2 H, *J* = 8.4 Hz), 7.32 (d, 2 H, *J* = 8.4 Hz), 5.35 (s, 1 H), 4.21 (q, 2 H, *J* = 7.2 Hz), 1.22 (t, 2 H, *J* = 7.2 Hz); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 174.3, 171.6, 166.9, 161.2, 159.5, 139.9, 130.6, 129.8 (2 C), 129.4 (2 C), 90.0, 81.1, 60.6, 14.2; HRMS (ESI) *m/z* calculated for $C_{15}H_{13}NO_7Na$ 342.0590, found 342.0583.

4.4.4 Synthesis of SID 861574 decomposition products and other related analogues

268 

Diprop-2-enyl 3-oxopentane-1,5-dioate (268). A suspension of dimethyl 1,3-acetone-dicarboxylate (1.00 mL, 6.80 mmol), allyl alcohol (4.60 mL, 67.1 mmol) and ZnO (280 mg, 3.40 mmol, 50 mol %) in dry toluene (5.0 mL) was heated to reflux for 27 h. The heterogeneous, pale yellow reaction mixture was cooled to room temperature and then filtered through a 1 in plug of SiO_2 with EtOAc (75 mL). The cloudy white filtrate was concentrated by rotary evaporation (60 °C) and the oily residue was chromatographed on SiO_2 (10:1 hexanes:EtOAc). The product fractions were combined and concentrated in vacuo to afford **268** as a clear oil (666 mg, 43%, 90:10 mixture of keto- to enol- tautomers): IR (ATR) 2995, 2900, 2686, 1795, 1694, 1675, 1385, 1347, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) keto-tautomer δ 5.97-5.86 (m, 2 H), 5.38-5.25 (m, 4 H), 4.65 (dt, 4 H, *J* = 5.7, 1.4 Hz), 3.66 (s, 4 H); enol-tautomer δ 12.01 (s, 1 H), 5.98-5.86 (m, 2 H), 5.38-5.25 (m, 4 H), 5.19 (s, 1 H), 5.47-4.67 (m, 4 H), 3.27 (s, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) keto-tautomer δ 195.3, 166.6 (2 C), 131.5 (2 C), 119.3 (2 C), 66.4 (2 C), 49.0 (2 C); enol-tautomer δ 172.1, 170.2, 167.8, 132.0, 131.7, 119.0, 118.7, 92.1, 66.2, 65.2, 41.1; HRMS (ESI) *m/z* calculated for $C_{11}H_{14}O_5Na$ 249.0739, found 249.0749.

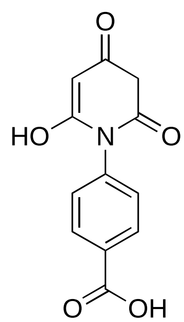
267



4-[4,6-Dihydroxy-2-oxo-3-(prop-2-enyloxycarbonyl)hydropyridyl]benzoic acid (267, DMA-NB245-18). To a clear solution of **268** (213 mg, 0.942 mmol) and **203** (150 mg, 0.785 mmol) in distilled THF (15.0 mL) cooled to 0 °C was added NaH (49.6 mg, 1.96 mmol) in one portion. Hydrogen gas release was noted upon the addition of NaH and the reaction mixture was stirred at 0 °C for 30 min and then warmed to room temperature for 30 min. To this white suspension was then added a sodium hydroxide solution (1.0 M, 15 mL) and the resulting clear biphasic mixture was vigorously stirred for 1 h at room temperature. The THF layer was then removed and the aqueous layer was washed with ether (2 X 30 mL) and acidified with concentrated HCl to pH 0.0-0.5 at 0 °C. The resulting white suspension was stirred at 0 °C for an additional 1 h following the acidification. The precipitate was isolated by vacuum filtration, washed with deionized water (50 mL) and dried in vacuo to afford **267** as a white powdery solid (208 mg, 80%): 211-212 °C (dec); IR (ATR) 3088, 2855, 1694, 1636, 1586, 1493, 1424, 1273, 1191, 1103 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.01 (br-s, 2 H), 10.72 (br-s, 1 H), 8.00 (d, 2 H, J = 8.4

Hz), 7.33 (d, 2 H, J = 8.4 Hz), 5.99-5.87 (m, 1 H), 5.47-5.36 (m, 2 H), 5.19 (dd, 1 H, J = 10.5, 1.2 Hz), 4.67 (d, 2 H, J = 4.8 Hz); ^{13}C NMR (DMSO-d$_6$, 75 MHz) δ 174.2, 171.1, 166.9, 161.3, 159.6, 140.0, 132.3, 130.5, 129.8 (2 C), 129.4 (2 C), 117.5, 89.9, 81.0, 64.7; MS (EI) *m/z* 331 (M^+, 82), 273 (24), 163 (47), 137 (93), 69 (100); HRMS (EI) *m/z* calculated for $C_{16}H_{13}NO_7$ 331.0692, found 331.0695.

265



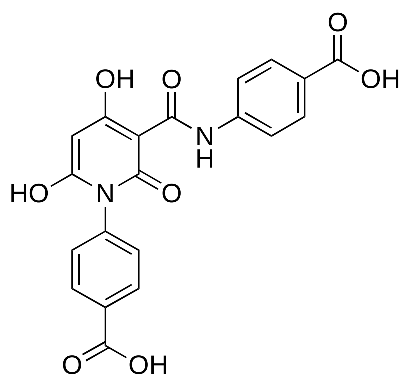
4-(4-Hydroxy-2,6-dioxo-1,5-dihydropyridyl)benzoic acid (265, DMA-NB245-24). To a white suspension of **267** (100 mg, 0.302 mmol) and $Pd(PPh_3)_4$ (17.4 mg, 0.0151 mmol, 0.05 mol%) in dry THF (5.0 mL) was added morpholine (266 μL, 3.02 mmol) at room temperature. The resulting heterogeneous light yellow reaction mixture was stirred at room temperature for 1 h. The reaction mixture was then diluted with a NaOH solution (1.0 M, 5.0 mL), followed by ether (10 mL) and the organic layer was removed. The pale yellow/orange aqueous layer was washed with ether (2 X 10 mL), cooled to 0 °C and acidified to pH 0.0-0.5. Upon acidification, a light yellow precipitate formed and the aqueous mixture was cooled to and gently stirred at 0 °C for 30 min. The resulting brown solid was removed by vacuum filtration and the filtrate was immediately extracted with EtOAc (3 X 40 mL). The extracts were combined, dried (Na_2SO_4), vacuum filtered through a 1 in plug of SiO_2, flushed through with EtOAc (100 mL) and the clear filtrate was concentrated in vacuo. The resulting yellow solids were dissolved in EtOH (15.0 mL), filtered through a small plug of glass wool and then diluted by slow addition of hexanes

(100 mL) at room temperature. The homogeneous pale yellow solution was brought to a boil and concentrated to 75 mL when a light yellow precipitate began to form. At this time, the mixture was slowly cooled to room temperature and then to -5 °C for 10 h. The crystallized solid was isolated by vacuum filtration, washed with hexanes (10 mL), dissolved in HPLC grade water (8.0 mL) with heating and the light orange solution was concentrated in vacuo to afford **265** as an orange solid (55.1 mg, 74%): 240-245 °C (slow dec.); IR (ATR) 3459, 3075, 2894, 1694, 1631, 1605, 1392, 1366, 1210, 1165, 1122 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) major tautomer (83%) δ 13.04 (br-s, 1 H), 11.78 (br-s, 1 H), 7.98 (d, 2 H, *J* = 8.4 Hz), 7.26 (d, 2 H, *J* = 8.4 Hz), 5.36 (s, 1 H), 3.66 (s, 2 H); distinct resonances for minor tautomer (17%) δ 5.25 (s, 1 H), 10.43 (s, 1 H); ^{13}C NMR (DMSO-d_6, 75 MHz) major tautomer δ 168.8, 168.5, 166.9, 166.5, 139.9, 130.3, 129.7 (2 C), 129.5 (2 C), 93.9, 37.4; MS (EI) *m/z* 247 (M^+, 63), 219 (21), 163 (72), 146 (100), 137 (42), 90 (51), 69 (66); HRMS (EI) *m/z* calculated for $C_{12}H_9NO_5$ 247.0481, found 247.0483; LC/MS/UV data: Phenomenex C_{18} (4.6 X 100 nm) column, 1.0 mL/min, 254 nm, gradient elution with 15 to 45% acetonitrile in water containing 0.10% TFA, 1 mg sample/mL DMSO or MeOH; Retention time = 9.96 min(MeOH), 3.29 (DMSO); MS(ESI+) *m/z* 248 $[M + H]^+$. This sample was found to have an IC_{50} > 50 μM against PLK1-PBD.

4-(4-Hydroxy-2,6-dioxo-1,5-dihydropyridyl)benzoic acid (265, DMA-NB245-3). To a light yellow solution of **267** (20.0 mg, 0.0557 mmol) and $Pd(PPh_3)_4$ (3.2 mg, 0.00279 mmol, 5 mol%) in dry THF (1.5 mL) was added morpholine (48.2 μL, 0.557 mmol, 10 equiv.) at room temperature. The resulting heterogeneous light orange reaction mixture was stirred at room temperature for 1 h, diluted with an NaOH solution (1.0 M, 2.0 mL) and stirred at room temperature for an additional 1.5 h. The resulting light orange mixture was extracted with ether

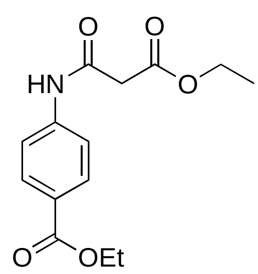
(3 X 3.0 mL) and the aqueous layer was cooled to 0 ^{o}C and acidified with an HCl solution (1.0 M, pH 0). No precipitate formed upon acidification. The aqueous solution was extracted with EtOAc (4 X 10 mL) and the EtOAc extracts were combined, dried (Na_2SO_4) and concentrated by rotary evaporation (25 ^{o}C). The resulting light orange residue was dissolved in EtOAc (6.0 mL) and hexanes (30 mL) was slowly added at room temperature. The resulting orange precipitate was isolated by vacuum filtration, washed with DCM (10 mL) and dried in vacuo to afford **265** (9.3 mg, 68%). This sample was found to have an IC_{50} = 2.079 μM against PLK1-PBD in an initial testing and an IC_{50} = 6.047 μM in a follow-up testing.

266



4-(1-(4-Carboxyphenyl)-4,6-dihydroxy-2-oxo-1,2-dihydropyridine-3-carboxamido)benzoic acid (266, DMA-NB245-67). To a solution of **265** (200 mg, 0.809 mmol) and **203** (155 mg, 0.809 mmol) in a 1:1 mixture of THF:ACN (10.0 mL) cooled to 0 ^{o}C was added DIPEA (0.270 mL,1.62 mmol, 2 equiv.) in one portion. An orange precipitate formed immediately upon addition of DIPEA and re-dissolved within 3 min. After 5 min, the ice bath was removed. The reaction mixture was warmed to room temperature over 45 min and was then diluted with a 1.0 M NaOH solution (10 mL) to saponify the ethyl ester. After 1.5 h, the deep red aqueous layer was diluted with de-ionized water (15 mL) and extracted with ether (3 X 20 mL). The aqueous layer was cooled to 0 ^{o}C, acidified with concentrated HCl (pH = 0) and the resulting cloudy purple solution was extracted with EtOAc (4 X 30 mL). The EtOAc extracts were combined, dried (Na_2SO_4) and concentrated by rotary evaporation (30 ^{o}C). The resulting solids were recrystallized from a boiling 1:7 mixture of THF:hexanes (150 mL) affording the desired product (188 mg, ~ 80% pure). This product was further purified by a second recrystallization from a boiling 1:2 mixture of EtOAc:hexanes (75 mL), which was slowly cooled to -5 ^{o}C. The resulting light tan solid was isolated by vacuum filtration, triturated with MeOH (5 mL) at 45 ^{o}C for 30 min (to remove EtOAc and hexanes) and concentrated in vacuo to afford **266** solvated with approximately 5% MeOH (35.3 mg, 11%): Mp 275.0-276.5 ^{o}C (dec.); IR (ATR) 3221, 3107, 2887, 2656, 2516, 1719, 1700, 1581, 1536, 1410, 1366, 1254, 1174, 1090 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 14.88 (br-s, 1 H), 12.42 (s, 1 H), 8.03 (d, 2 H, *J* = 8.4 Hz), 7.89 (d, 2 H, *J* = 8.7 Hz), 7.67 (d, 2 H, *J* = 8.7 Hz), 7.41 (d, 2 H, *J* = 8.1 Hz), 5.39 (s, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 175.2, 169.5, 166.8 (2 C), 163.1, 160.8, 141.7, 139.7, 130.7, 130.6 (2 C), 129.9 (2 C), 129.4 (2 C), 125.6, 119.5 (2 C), 89.8, 82.8; LC/MS/UV data: Phenomenex C_{18} (4.6 X 100 nm) column, 1.0 mL/min, 254 nm, gradient elution with 15 to 45% acetonitrile in water containing 0.10% TFA, 1 mg sample/mL DMSO; Retention time = 16.44 min; MS(ESI+) *m/z* 411 $[M + H]^+$.

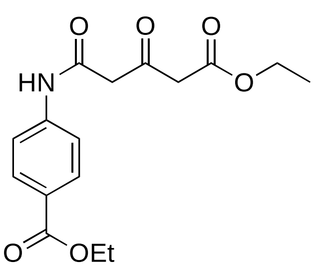
271



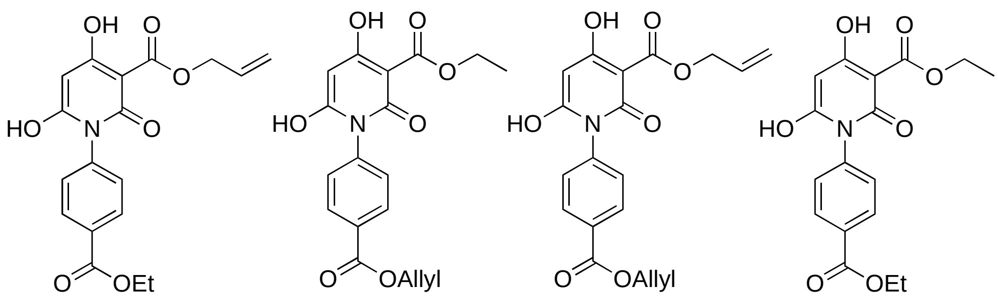
4-[2-(Ethoxycarbonyl)acetylamino]benzoic acid (271, DMA-NB245-90).[113] To a solution of ethyl malonylchloride (282 μL, 1.97 mmol, 90%) in dry ether (5.0 mL) was added 4-

aminobenzoic acid (250 mg, 1.82 mmol) at room temperature. The resulting light orange suspension was stirred at room temperature for 4 h before an additional (250 mg, 1.82 mmol) of 4-aminobenzoic acid was added. After 20 h, the reaction mixture was diluted with EtOAc (50 mL), extracted with 1.0 M HCl (3 x 50 mL) and brine (2 X 50 mL). The EtOAc layer was dried (Na_2SO_4), concentrated by rotary evaporation (40 ^oC) and the resulting orange solids were recrystallized by dissolving in boiling EtOAc (40 mL) to which hexanes (5 mL) were added upon dissolution of the product. After slowly cooling to room temperature, the resulting light orange solid was isolated by vacuum filtration, washed with hexanes (20 mL) and dried in vacuo to afford **271** (309 mg, 63%): ^1H NMR (DMSO-d_6, 300 MHz) δ 12.74 (s, 1 H), 10.49 (s, 1 H), 7.90 (d, 2 H, J = 8.7 Hz), 7.68 (d, 2 H, J = 8.7 Hz), 4.12 (q, 2 H, J = 7.2 Hz), 3.50 (s, 2 H), 1.20 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.6, 167.0, 164.7, 142.9, 130.6 (2 C), 125.5, 118.5 (2 C), 60.8, 43.8, 14.1; HRMS (TOF MS ES+) *m/z* calculated for $C_{12}H_{13}NO_5Na$ (M+Na) 274.0691, found 274.0692.

272



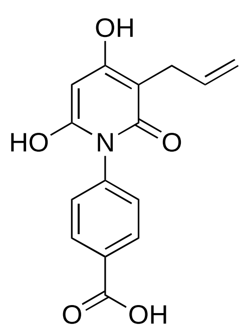
4-[4-(Ethoxycarbonyl)-3-oxobutanoylamino]benzoic acid (272, DMA-NB245-91). A suspension of ZnO (297 mg, 3.65 mmol), **261** (994 μL, 5.47 mmol) and 4-aminobenzoic acid (500 mg, 3.65 mmol) in dry toluene was prepared at room temperature and heated to reflux for 24 h. At this time, the reaction mixture was cooled to room temperature, diluted with EtOAc (50 mL) and filtered through a plug of SiO_2 (1 in) with EtOAc (250 mL). The solid remaining on top of the SiO_2 plug was found by ^{1}H NMR (DMSO-d6) to contain the majority of the major product (possibly as the zinc salt). These solids were then carefully removed and triturated with a boiling solution of 50:1 THF:AcOH (60 mL) for 30 min. The hot suspension was vacuum filtered and the filtrate was concentrated in vacuo to afford crude 4-[(1-{[*N*-(4-carboxyphenyl)carbamoyl]methyl}-2-(ethoxycarbonyl)vinyl)amino]benzoic acid (olefin position is uncertain) as a white solid (160 mg, ~65-70% pure): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.53 (br-s, 2 H), 10.41 (s, 1 H), 8.94 (s, 1 H), 7.94 (d, 2 H, J = 8.7 Hz), 7.89 (d, 2 H, J = 8.7 Hz), 7.71 (d, 2 H, J = 8.7 Hz), 7.29 (d, 2 H, J = 8.7 Hz), 5.43 (s, 1 H), 3.99 (s, 2 H), 3.97 (q, 2 H, J = 7.2 Hz), 1.13 (t, 3 H, J = 7.2 Hz). The remainder of this solid was suspended in a 1.0 M HCl solution (20 mL) and stirred at room temperature for 2 h, at which time the remaining white solid was isolated by vacuum filtration, washed with deionized water (20 mL), dried in vacuo and suspended in EtOAc (2.5 mL) for chromatography on SiO_2 (EtOAc). The product (R_f = 0.66; SiO_2; EtOAc) fractions were combined and concentrated by rotary evaporation (40 ^{o}C). The resulting white solid was dissolved in MeOH (10 mL) and re-concentrated in vacuo to afford **272** containing ~2% of 4-aminobenzoic acid (15.2 mg, 1%, ~95% pure): Mp 242-243 ^{o}C (dec); IR (ATR) 3334, 3208, 2975, 2667, 2548, 1735, 1683, 1597, 1534, 1409, 1308, 1258, 1169, 1025 cm^{-1}; keto-tautomer (83%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.71 (s, 1 H), 10.42 (s, 1 H), 7.90 (d, 2 H, J = 8.7 Hz), 7.67 (d, 2 H, J = 8.7 Hz), 4.10 (q, 2 H, J = 7.2 Hz), 3.74 (s, 2 H), 3.71 (s, 2 H), 1.18 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 198.1, 166.9, 166.8, 165.1, 142.8, 130.5 (2 C), 125.5, 118.4 (2 C), 60.7, 51.6, 49.2, 14.0; distinguished resonances for major-enol-tautomer (13%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.64 (s, 1 H), 10.43 (s, 1 H), 5.37 (s, 1 H), 3.36 (s, 2 H); distinguished resonances for minor-enol-tautomer (4%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.93 (br-s, 1 H), 5.25 (s, 1 H), 3.39 (s, 2 H); HRMS (TOF MS ES+) *m/z* calculated for $C_{14}H_{15}NO_6Na$ (M+Na) 316.0797, found 316.0810.



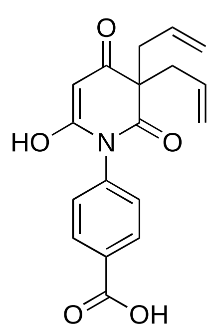
274a **274b** **274c** **274d**

Allyl 1-(4-(ethoxycarbonyl)phenyl)-4,6-dihydroxy-2-oxo-1,2-dihydropyridine-3-carboxylate (274a). To a solution of **268** (6.54 g, 28.9 mmol, 1.11 equiv.) in distilled THF (500 mL) cooled to 0 °C was added NaH in three 551 mg batches over 5 min (1.65 g, 65.4 mmol, 2.5 equiv.). The ice bath was immediately removed and the resulting white suspension was slowly warmed to room temperature over 30 min. At this time, gas release ceased, the light suspension was re-cooled to 0 °C and a solution of **203** (5.00 g, 26.2 mmol) in THF (10.0 mL) was added dropwise over 5 min. The ice bath was immediately removed. The heterogeneous reaction mixture was warmed to room temperature over 30 min and heated to reflux for 2.45 h over which time reaction mixture became orange in color. After cooling to 0 °C, a concentrated HCl solution (reaction mixture pH ~7-8, reaction mixture turned deep red upon neutralization) was added to the reaction mixture, followed by deionized water (500 mL) and ether (200 mL). The organic layer was removed and the deep red aqueous layer was extracted with ether (2 X 300 mL). The aqueous layer was then further acidified with an HCl solution (1.0 M, pH = 0.5-1.0) and the resulting heterogeneous solution was extracted with EtOAc (3 X 300 mL). The EtOAc layers were combined, dried (Na_2SO_4) and concentrated by rotary evaporation (30 °C). The resulting orange/tan solids were triturated with DCM (100 mL) at room temperature for 15 min. The remaining solids were isolated by vacuum filtration, washed with DCM (50 mL) and hexanes (50 mL), and dried in vacuo to afford a mixture of ~3.5:1:2:trace **274a**: **274b**: **274c**: **274d** determined by ^{1}H NMR integration (3.23 g, 35%). The remaining dark red mother liquor was diluted with hexanes (250 mL) and cooled to -5 °C for 1 h. The light yellow precipitate was isolated by vacuum filtration, washed with a 1:1 DCM:hexanes solution (30 mL) and hexanes (50 mL), and dried in vacuo to afford a second batch of this crude mixture (1.41 g, 15%). The product mixture was carried on crude to the next step. A LC/MS/ELS analysis was performed (ESI industries epic C_{18} column; 1.0 mL/min; 30 min run; gradient elution = 30 to 60% ACN in water, ELS/MS detection). **247a**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.03 (br-s, 1 H), 11.89 (br-s, 1 H), 8.02 (d, 2 H, *J* = 8.7 Hz), 7.36 (d, 2 H, *J* = 8.7 Hz), 5.99-5.86 (m, 1 H), 5.47-5.41 (m, 1 H), 5.35 (s, 1 H), 5.20-5.16 (m, 1 H), 4.70 (dt, 2 H, *J* = 4.8, 1.5 Hz), 4.35 (q, 2 H, *J* = 7.2 Hz), 1.34 (t, 3 H, *J* = 7.2 Hz); LC/MS (ESI+) *m/z* 360.2 $[M+H]^+$; LC/ELS retention time 12.99 min. **247b**: LC/MS (ESI+) *m/z* 360 $[M+H]^+$; LC/ELS retention time 12.99 min. **247c**: LC/MS (ESI+) *m/z* 372 $[M+H]^+$; LC/ELS retention time 14.37 min. **247d**: LC/MS (ESI+) *m/z* 348 $[M+H]^+$; LC/ELS retention time 11.53 min.

277



278

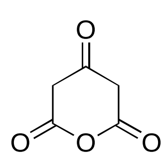


General Procedure for the Optimization Experiments in Table 13. To a solution of heterocycle (25 – 50 mg) and catalyst (5-100 mol%) in distilled THF was added the amine (1-10 equiv.) at room temperature. The reaction mixture was stirred at room temperature or heated to the specified temperature for the specified time period. At this time, a NaOH solution (1.0 M, 2.0 mL) was added to the room temperature reaction mixture. After 2 h, the biphasic mixture was diluted with deionized water (15 mL) and extracted with ether (3 X 20 mL). The aqueous layer was then cooled to 0 ^{o}C and acidified slowly with a concentrated HCl solution just to pH 0. The resulting heterogeneous solution was stirred gently at 0 ^{o}C for 30 min, vacuum filtered (filter paper) and the isolated solids were washed with deionized water (10.0 mL). The aqueous filtrate was extracted with EtOAc (3 X 20 mL). The extracts were combined, dried (Na_2SO_4) and concentrated in vacuo. The crude reaction mixtures were then analyzed by ^{1}H NMR (DMSO-d_6 or MeOD) and the respective product ratios were reported. Products **277** and **278** were observed in the crude reaction mixtures and their NMR characterization data is reported:

4-(3-Allyl-4-hydroxy-2,6-dioxo-5,6-dihydropyridin-1(2*H*)-yl)benzoic acid (277). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.89 (br-s, 1 H), 11.15 (s, 1 H), 7.98 (d, 2 H, *J* = 8.4 Hz), 7.25 (d, 2 H, *J* = 8.1 Hz), 5.82-5.73 (m, 1 H), 5.05-4.89 (m, 2 H), 3.74 (s, 2 H), 3.02 (d, 2 H, *J* = 6.6 Hz).

4-(3,3-diallyl-2,4,6-trioxopiperidin-1-yl)benzoic acid (278). ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.11 (d, 2 H, *J* = 6.3 Hz), 7.14 (d, 2 H, *J* = 6.3 Hz), 5.82-5.72 (m, 2 H), 5.20-5.15 (m, 4 H), 2.82-2.67 (m, 4 H); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 174.4, 172.2, 167.6, 166.2, 139.7, 131.6 (2 C), 130.6, 130.1 (2 C), 128.7 (2 C), 118.7 (2 C), 54.5, 41.9, 1C in MeOD solvent peak.

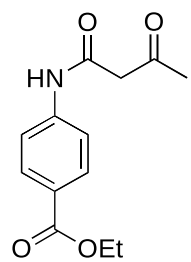
282



2*H*-Pyran-2,4,6(3*H*,5*H*)-trione (282).[109,110] To a 1.4:1 mixture of AcOH:Ac_2O (25.8 mL) cooled to 0 ^{o}C was added 1,3-acetonedicarboxylic acid (10.0 g, 66.4 mmol) portion wise over a

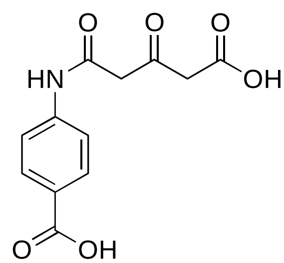
20 min period. The resulting light brown heterogeneous mixture was stirred at 0-5 °C for 3.5 h. At this time, the white solid was isolated by vacuum filtration, washed with AcOH (30 mL) followed by hexanes (100 mL), and was dried in vacuo to afford **282** (6.85 g, 81%): ^{1}H NMR (Acetone-d$_6$, 300 MHz) δ 10.98 (br-s, 1 H), 5.35 (s, 1 H), 3.70 (d, 2 H, *J* = 0.9 Hz); MS (EI) *m/z* 128 (M^+, 100), 120 (63), 116 (43).

284



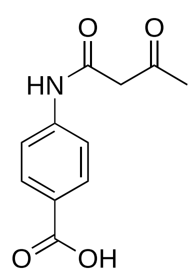
Ethyl 4-(3-oxobutanamido)benzoate (284, DMA-NB245-72).[114] To a suspension of **282** (1.00 g, 7.81 mmol) in anhydrous ether (50 mL) cooled to 0 °C was added a solution of ethyl 4-aminobenzoate (1.32 g, 7.81 mmol) in anhydrous ether (25 mL) dropwise over 30 min. The resulting white suspension was stirred at 0 ^{o}C for 3 h and at room temperature for 16 h affording a pale yellow nearly homogeneous reaction mixture, which was diluted with hexanes (50 mL) and vacuum filtered. The filtrate was cooled to 0 ^{o}C and hexanes (125 mL) was slowly added with stirring. The resulting turbid solution was cooled to -5 ^{o}C for 40 h over which time a white solid crystallized, which was isolated by vacuum filtration, washed with hexanes (50 mL) and dried in vacuo to afford a nearly 1:1 mixture of **283** and **284** (1.14 g crude, ~50 %). Compound **283**: ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 12.69 (br-s, 1 H), 10.45 (s, 1 H), 7.92 (d, 2 H, *J* = 8.7 Hz), 7.70 (d, 2 H, *J* = 8.4 Hz), 4.28 (q, 2 H, *J* = 7.2 Hz), 3.17 (s, 2 H), 3.62 (s, 2 H), 1.31 (t, 3 H, *J* = 7.2 Hz). After storage of the recrystallized solid at room temperature, it was found by ^{1}H NMR that the mixture further decomposed to ~88% of **284** over 2 d and to ~95% after 4 d. This mixture was recrystallized from boiling wet ether (100 mL); however, the purity did not improve. Complete decarboxylation was accomplished by refluxing the mixture in toluene (50 mL) for 20 min. Concentration of the mixture in vacuo afforded **284** (539 mg, 28%): keto tautomer (88%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.40 (s, 1 H), 7.91 (d, 2 H, *J* = 8.7 Hz), 7.70 (d, 2 H, *J* = 8.7 Hz), 4.28 (q, 2 H, *J* = 7.2 Hz), 3.60 (s, 2 H), 2.21 (s, 3 H), 1.31 (t, 3 H, *J* = 7.2 Hz); distinguished resonances for enol tautomer (12%): δ 13.61 (s, 1 H), 10.24 (s, 1 H), 5.23 (s, 1 H), 1.94 (s, 3 H); MS (EI) *m/z* 249 (M^+, 13), 165 (16), 146 (20), 137 (18), 120 (100).

279



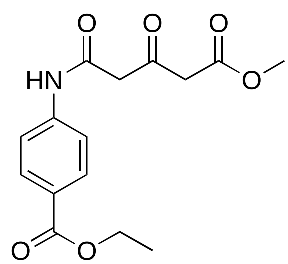
4-(4-Carboxy-3-oxobutanamido)benzoic acid (279, DMA-NB245-70). To a suspension of **282** (250 mg, 1.95 mmol) in anhydrous ether (10 mL) cooled to 0 °C was added a solution of 4-aminobenzoic acid (270 mg, 1.95 mmol) dissolved in anhydrous ether (25 mL) dropwise over 30. The resulting white suspension was slowly warmed to room temperature for 15 h, vacuum filtered and the resulting white solid was washed with wet ether (50 mL). The crude solids were dissolved in THF (20 mL) at room temperature and hexanes (20 mL) was slowly added. Initially, a pale yellow solid precipitated which was removed by vacuum filtration. The filtrate was diluted with hexanes (100 mL) and the resulting turbid solution was cooled to -5 °C for 3 h. The precipitate was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford **279** (176 mg, 34%, ~90% pure). Product further crystallized from the mother liquor over 16 h at room temperature. The precipitate was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo to afford an approximately 94:6 mixture of **279**:**285** (54.1 mg, 10%, ~94% pure) as a white solid: IR (ATR) 3240, 2914, 2675, 2556, 1732, 1668, 1627, 1592, 1428, 1286, 1258, 1174, 940 cm^{-1}; keto tautomer (80%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.71 (s, 1 H), 10.41 (s, 1 H), 7.89 (d, 2 H, *J* = 8.0 Hz), 7.67 (d, 2 H, *J* = 9.0 Hz), 3.71 (s, 2 H), 3.62 (s, 2 H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 198.4, 168.4, 166.9, 165.1, 142.8, 130.5 (2 C), 125.4, 118.4 (2 C), 51.6, 49.4; distinguished resonances for major enol tautomer (14%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.6 (s, 1 H), 5.38 (s, 1 H), 3.26 (s, 2 H); distinguished resonances for minor enol tautomer (6%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 5.18 (s, 1 H), 3.37 (s, 2 H); LC/MS/UV data: Phenomenex C_{18} (4.6 X 100 nm) column, 1.0 mL/min, 254 nm, gradient elution with 15 to 45% acetonitrile in water containing 0.10% TFA, 1 mg sample/mL DMSO; Retention time = 4.94 min; MS(ESI+) *m/z* 266 $[M + H]^+$.

285



4-(3-Oxobutanoylamino)benzoic acid (285, DMA-NB245-77).[114] A mixture of **279** (127 mg, 0.479 mmol) in toluene (20 mL) was heated to 110 °C. The decarboxylation experiment was monitored over three heating cycles of 30 min (35%), 3 h (~98%), and 2.5 h (complete by ^{1}H NMR) for a total heating time of 6 h. The product was isolated and worked up as follows after each heating cycle. Upon cooling to room temperature, the reaction mixture was diluted with hexanes (30 mL), further cooled to -5 °C for 1 h and vacuum filtered. The isolated yellow crystalline solid was washed with hexanes (20 mL) and dried in vacuo to afford **285** (91.6 mg, 86%): keto tautomer (91%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.72 (s, 1 H), 10.37 (s, 1 H), 7.90 (d, 2 H, *J* = 8.7 Hz), 7.68 (d, 2 H, *J* = 8.7 Hz), 3.60 (s, 2 H), 2.21 (s, 3 H); distinguished resonances for enol tautomer (9%): δ 13.63 (s, 1 H), 10.21 (s, 1 H), 5.23 (s, 1 H), 1.94 (s, 3 H); MS (EI) *m/z* 221 (M^+, 32), 137 (100), 127 (14), 120 (98); LC/UV data: Phenomenex C_{18} (4.6 X 100 nm) column, 1.0 mL/min, 254 nm, gradient elution with 15 to 45% acetonitrile in water containing 0.10% TFA, 1 mg sample/mL DMSO; Retention time = 5.06 min.

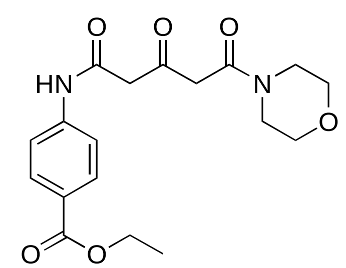
287



Ethyl 4-(5-methoxy-3,5-dioxopentanamido)benzoate (287, DMA-NB245-75). To a suspension of **286** (1.35 mL, 8.90 mmol) and ZnO (483 mg, 5.93 mmol) in dry toluene (30 mL) was added ethyl 4-aminobenzoate (1.00 g, 5.93 mmol, 1 equiv.) at room temperature. The white suspension was heated to reflux for 2 h, cooled to room temperature, filtered through a plug of SiO_2 (1 in) with EtOAc (150 mL), concentrated by rotary evaporation (40 ^{o}C) and the resulting yellow oil was chromatographed on SiO_2 (2:1 hexanes:EtOAc). The product (R_f = 0.20) fractions were combined, concentrated by rotary evaporation (30 ^{o}C) and the resulting oil was dissolved twice in MeOH (8.0 mL) followed by re-concentration in vacuo, to aid in the removal of residual EtOAc, to afford **287** as a white crystalline solid (1.15 g, 63%): Mp 68.5-72.0 ^{o}C; IR (ATR) 3321, 3195, 3115, 2988, 2956, 1745, 1681, 1594, 1536, 1316, 1279, 1247, 1146, 1003 cm^{-1}; keto tautomer (86%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.45 (s, 1 H), 7.92 (d, 2 H, *J* = 8.7 Hz), 7.70 (d, 2 H, *J* = 8.7 Hz), 4.28 (q, 2 H, *J* = 7.2 Hz), 3.76 (s, 2 H), 3.72 (s, 2 H), 3.63 (s, 3

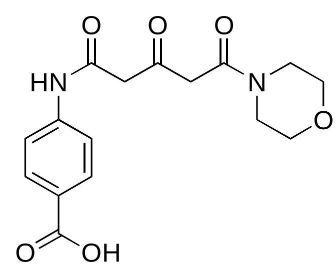
H), 1.30 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 198.0, 167.3, 165.3, 165.1, 143.1, 130.3 (2 C), 124.6, 118.5 (2 C), 60.9, 52.3, 52.0, 49.4, 14.6; distinguished resonances for major enol tautomer (12%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.61 (s, 1 H), 10.43 (s, 1 H), 5.39 (s, 1 H), 3.66 (s, 3 H), 3.39 (s, 2 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.5, 169.3, 168.7, 142.8, 119.0, 93.9, 52.4, 41.0; distinguished resonances for minor enol tautomer (2%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.81 (s, 1 H), 5.27 (s, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.9, 171.4, 166.0, 118.2, 91.5, 51.7, 43.5; HRMS (TOF MS ES+) *m/z* calculated for $C_{15}H_{17}NO_6Na$ (M+Na) 330.0954, found 330.0953.

288



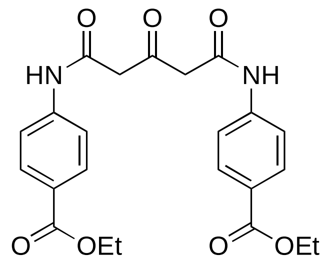
***N*-ethyl-4-(5-morpholino-3,5-dioxopentanamido)benzamide (288, DMA-NB245-82)**. To a suspension of **287** (350 mg, 1.14 mmol) and ZnO (92.7 mg, 1.14 mmol) in toluene (20 mL) was added morpholine (100 μL, 1.14 mmol) at room temperature. The resulting mixture was heated to reflux for 36 h, cooled to room temperature and vacuum filtered through a plug of SiO_2 (2 in) with EtOAc (800 mL). The cloudy filtrate was concentrated by rotary evaporation (40 ^{o}C) and the resulting light yellow solids were dissolved in EtOAc (4.0 mL) for chromatography on SiO_2 (EtOAc). The product fractions (R_f = 0.26) were combined, concentrated by rotary evaporation (40 ^{o}C) and the resulting light orange oil was dissolved in MeOH (5.0 mL, to remove residual EtOAc), re-concentrated by rotary evaporation (40 ^{o}C) and further dried in vacuo to afford **288** as a light orange solid (180 mg, 44%). The tautomeric ratios reported below as percentages reflect the ratio of the tautomer in solution immediately after sample preparation vs. after equilibration (20 h) as measured by ^{1}H NMR integration: Mp 133.5-134.5 °C; IR (ATR) 3271, 3195, 3115, 2964, 2869, 1717, 1692, 1592, 1543, 1266, 1234, 1107 cm^{-1}; keto tautomer (88% vs. 80%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.45 (s, 1 H), 7.92 (d, 2 H, *J* = 8.7 Hz), 7.70 (d, 2 H, *J* = 8.7 Hz), 4.28 (q, 2 H, *J* = 7.2 Hz), 3.81 (s, 2 H), 3.69 (s, 2 H), 3.57-3.52 (m, 4 H), 3.46-3.44 (m, 2 H), 3.38-3.32 (m, 2 H), 1.31 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 199.5, 165.4, 165.3, 165.2, 143.1, 130.3 (2 C), 124.5, 118.5 (2 C), 66.1, 66.0, 60.5, 51.5, 48.5, 46.1, 41.5, 14.2; distinguished resonances for major enol tautomer (0% vs. 12%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 14.97 (s, 1 H), 5.62 (s, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.2, 170.1, 166.5, 143.2, 118.9, 88.7, 43.8; distinguished resonances for minor enol tautomer (12 vs. 8%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.68 (s, 1 H), 10.37 (s, 1 H), 5.31 (s, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.6, 165.8, 142.8, 93.1, 41.5; HRMS (TOF MS ES+) *m/z* calculated for $C_{18}H_{22}N_2O_6Na$ (M+Na) 385.1376, found 385.1368.

280



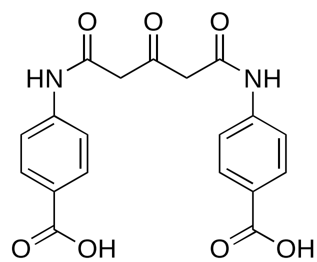
4-(5-Morpholino-3,5-dioxopentanamido)benzoic acid (280, DMA-NB245-88). To a homogeneous yellow solution of **288** (120 mg, 0.331 mmol) in dioxane (10 mL) was added in one portion a 3.0 M LiOH solution (10 mL). The resulting slightly heterogeneous pale yellow reaction mixture was stirred at room temperature for 6 h, cooled to 0 °C and acidified to pH 0 with a concentrated HCl solution. The resulting pale yellow homogeneous aqueous solution was extracted with EtOAc (4 X 30 mL). The extracts were combined, dried (Na_2SO_4) and concentrated by rotary evaporation (40 ^oC). The resulting yellow residue was triturated with MeOH (10 mL) at 40 ^oC for 30 min and the heterogeneous solution was cooled to -5 ^oC for 1 h. The precipitate was isolated by vacuum filtration, washed with MeOH (5 mL, cooled to 0 ^oC) and dried in vacuo to afford **280** as a white solid (67.1 mg, 61%). The tautomeric ratios reported below as percentages reflect the ratio of the tautomer in solution immediately after sample preparation vs. after equilibration (6 h) as measured by ^1H NMR integration: Mp 177.5-178.5 ^oC; IR (ATR) 3290, 3202, 2975, 2924, 2863, 2662, 2548, 1683, 1596, 1545, 1407, 1314, 1295, 1226, 1169, 1113 cm^{-1}; keto-tautomer (90% vs. 82%): ^1H NMR (DMSO-d_6, 300 MHz) δ 12.75 (s, 1 H), 10.43 (s, 1 H), 7.90 (d, 2 H, J = 8.7 Hz), 7.67 (d, 2 H, J = 8.7 Hz), 3.81 (s, 2 H), 3.69 (s, 2 H), 3.75-3.52 (m, 4 H), 3.46-3.43 (m, 2 H), 3.38-3.34 (m, 2 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 199.6, 167.0, 165.5, 165.3, 142.9, 130.5 (2 C), 125.5, 118.5 (2 C), 66.1 (2 C), 51.6, 48.5, 46.2, 41.6; distinguished resonances for major-enol-tautomer (1% vs. 11%): ^1H NMR (DMSO-d_6, 300 MHz) δ 14.98 (s, 1 H), 5.62 (s, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.4, 170.2, 166.5, 143.0, 118.9, 88.8, 43.9; distinguished resonances for minor-enol-tautomer (8% vs. 7%): ^1H NMR (DMSO-d_6, 300 MHz) δ 13.71 (s, 1 H), 10.35 (s, 1 H), 5.31 (s, 1 H); HRMS (TOF MS ES+) *m/z* calculated for $C_{16}H_{18}N_2O_6Na$ (M+Na) 357.1063, found 357.1054; LC/MS/UV data: Phenomenex C_{18} (4.6 X 100 nm) column, 1.0 mL/min, 254 nm, gradient elution with 15 to 45% acetonitrile in water containing 0.10% TFA, 1 mg sample/mL DMSO; Retention time = 6.00 min; MS(ESI+) *m/z* 375 $[M + Na]^+$.

289



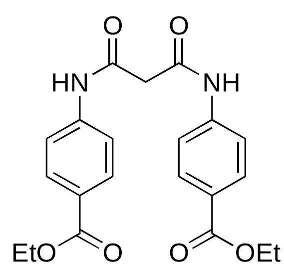
Ethyl 4-(4-{*N*-[4-(ethoxycarbonyl)phenyl]carbamoyl}-3-oxobutanoylamino)benzoate (289, DMA-NB245-78). To a suspension of dimethyl 1,3-acetonedicarboxylate (0.40 mL, 2.64 mmol) and ZnO (0.215 mg, 2.64 mmol) in dry toluene (12 mL) was added ethyl 4-aminobenzoate (979 mg, 5.81 mmol, 2.2 equiv.) at room temperature. The reaction mixture was heated to reflux for 9 h, cooled to room temperature and vacuum filtered 3 times through a plug of SiO_2 (1 in) with EtOAc (~300 mL/filtration). The cloudy filtrate was condensed by rotary evaporation (40 ^oC) after each filtration and this process was repeated until the filtrate was clear. After the third filtration, the resulting white solids were dissolved in boiling EtOAc (25 mL) and diluted with hexanes (50 mL). The turbid solution was slowly cooled to room temperature and the resulting white crystalline solid was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo. The mother liquor was recrystallized 3 additional time utilizing the same procedure affording **289** (701 mg, 60%, total yield from 4 recrystallizations): Mp 194.0-195.0 ^oC; IR (ATR) 3353, 3295, 3195, 3126, 2975, 1719, 1656, 1599, 1536, 1411, 1273, 1172, 1103 cm^{-1}; keto tautomer (72%): ^1H NMR (DMSO-d_6, 300 MHz) δ 10.47 (s, 2 H), 7.92 (d, 4 H, *J* = 8.7 Hz), 7.71 (d, 4 H, *J* = 9.0 Hz), 4.28 (q, 4 H, *J* = 7.2 Hz), 3.76 (s, 4 H), 1.31 (t, 6 H, *J* = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 198.9, 165.3 (2 C), 165.2 (2 C), 143.1 (2 C), 130.3 (4 C), 124.5 (2 C), 118.5 (4 C), 60.5 (2 C), 51.8 (2 C), 14.2 (2 C); distinguished resonances for enol tautomer (28%): ^1H NMR (DMSO-d_6, 300 MHz) δ 13.70 (s, 1 H), 10.56 (s, 1 H), 10.41 (s, 1 H), 7.75 (d, 2 H, *J* = 9.0 Hz), 5.42 (s, 1 H), 3.40 (s, 2 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.8, 170.5, 166.3, 143.2, 142.8, 124.5, 118.9, 118.5, 93.4, 43.4; HRMS (TOF MS ES+) *m/z* calculated for $C_{23}H_{24}N_2O_7Na$ (M+Na) 463.1481, found 463.1440.

290



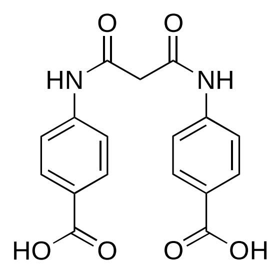
4-{4-[*N*-(4-carboxyphenyl)carbamoyl]-3-oxobutanoylamino}benzoic acid (290, DMA-NB245-81). To a solution of **289** (200 mg, 0.454 mmol) in dioxane (20 mL) was added a 4.0 M LiOH solution (20 mL) in one portion at room temperature. The resulting heterogeneous yellow reaction mixture was stirred at room temperature for 2.5 h, cooled to 0 ^{o}C and acidified to pH 0 with concentrated HCl (10 mL). The resulting white oily suspension was stirred at 0 ^{o}C for 18 h; however, the suspension did not sufficiently coagulate to afford an isolable solid. The mixture was warmed to room temperature and diluted with deionized water (100 mL) followed by EtOAc (150 mL) for extraction. A white solid formed between the two layers, was isolated by vacuum filtration, washed with EtOAc (20 mL) and dried in vacuo to afford the product (81.7 mg, batch 1). The aqueous layer was extracted with EtOAc (3 X 150 mL). The EtOAc layers were combined, dried (Na_2SO_4) and concentrated by rotary evaporation (40 ^{o}C). The resulting light brown residue was triturated with boiling EtOAc (40 mL) for 30 min. This mixture was cooled to room temperature and the resulting solid was isolated by vacuum filtration, washed with hexanes (30 mL) and dried in vacuo (52.7 mg, batch 2). ^{1}H NMRs (DMSO-d_6) of both batches of solid showed a similar composition for the isolated products with the major contaminants being residual solvents; dioxane and EtOAc. The products from both batches were combined and triturated with boiling MeOH (50 mL) for 30 min. The white suspension was slowly cooled to room temperature, the resulting light pink solid was isolated by vacuum filtration, washed with MeOH (10 mL) and dried in vacuo to afford **290** solvated with ~4% dioxane (118 mg, 68%): Mp 264.0-265.0 ^{o}C; IR (ATR) 3303, 3070, 2831, 2667, 2561, 1663, 1594, 1530, 1405, 1316, 1292, 1172 cm^{-1}; keto tautomer (77%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.72 (s, 2 H), 10.45 (s, 2 H), 7.90 (d, 4 H, J = 8.7 Hz), 7.68 (d, 4 H, J = 8.7 Hz), 3.67 (s, 4 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 199.0, 166.9 (2 C), 165.1 (2 C), 142.8 (2 C), 130.5 (4 C), 125.4 (2 C), 118.4 (4 C), 51.8 (2 C); distinguished resonances for enol tautomer (23%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.75 (br-s, 1 H), 10.53 (s, 1 H), 5.40 (s, 1 H), 3.39 (s, 2 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.8, 170.5, 166.2, 118.8, 93.3, 43.5.

292



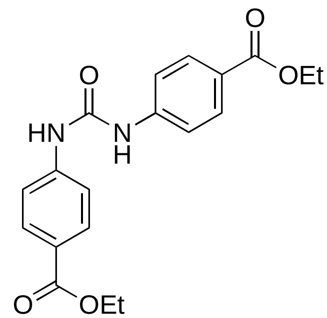
***N*₁,*N*₃-Bis(4-(carbethoxy)phenyl)malonamide (292, DMA-NB245-80).**[113] To a solution of ethyl 4-aminobenzoate (882 mg, 5.24 mmol) in THF (15 mL) cooled to 0 ^{o}C was added malonyl chloride (0.250 mL, 2.49 mmol) in one portion and the reaction mixture turned heterogeneous and light yellow in color. After stirring at 0 ^{o}C for 5 min, K_2CO_3 (861 mg, 6.23 mmol) was added, the ice bath was removed, the reaction mixture was warmed to room temperature for 2 h and was then diluted with EtOAc (100 mL) and washed with 1.0 M HCl (2 X 100 mL) followed by a saturated brine solution (2 X 100 mL). The EtOAc layer was then dried (Na_2SO_4) and concentrated by rotary evaporation (40 ^{o}C). The resulting light yellow solids were dissolved in boiling EtOAc (100 mL) and then hexanes (100 mL) was added to precipitate the product. The resulting suspension was slowly cooled to room temperature and then to -5 ^{o}C for 3 h. The fluffy white precipitate was isolated by vacuum filtration, washed with hexanes (50 mL) and dried in vacuo to afford **292** (819.9 mg, 83%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.55 (s, 2 H), 7.93 (d, 4 H, *J* = 8.7 Hz), 7.74 (d, 4 H, *J* = 8.7 Hz), 4.28 (q, 4 H, *J* = 7.2 Hz), 3.57 (s, 2 H), 1.31 (t, 6 H, *J* = 7.2 Hz); MS (EI) *m/z* 398 (M^+, 80), 207 (57), 165 (82), 120 (90), 95 (100).

293



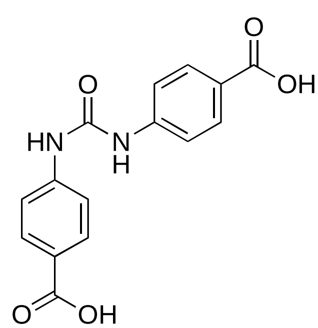
***N*$_1$,*N*$_3$-Bis(4-benzoic acid)malonamide (293, DMA-NB245-85).**[113] To a suspension of **292** (250 mg, 0.627 mmol) in dioxane (20 mL) was added a 1.0 M LiOH solution (20 mL) at room temperature. The resulting homogenous yellow reaction mixture was stirred at room temperature for 2.5 h, cooled to 0 ^{o}C and acidified with concentrated HCl (10 mL, reaction pH = 0). After 1 h at 0 ^{o}C, a white solid precipitated from the mixture which was isolated by vacuum filtration, washed with deionized water (30 mL) and recrystallized from boiling MeOH (50 mL). After slowly cooling the suspension to room temperature, the recrystallized solid was isolated by vacuum filtration, washed with MeOH (10 mL) and dried in vacuo to afford **293** (45.8 mg, 21%, ~93% pure). This sample was recrystallized a second time from boiling MeOH (20 mL) to afford **293** as a white crystalline solid (17.1 mg, 8%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.73 (s, 2 H), 10.50 (s, 2 H), 7.91 (d, 4 H, *J* = 8.7 Hz), 7.72 (d, 4 H, *J* = 8.7 Hz), 3.56 (s, 2 H); MS (EI) *m/z* 342 (M^+, 42), 149 (100).

294



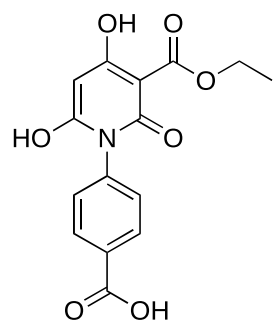
Diethyl 4,4'-carbonylbis(azanediyl)dibenzoate (294, DMA-NB245-86).[115] To a solution of ethyl 4-isocyanatobenzoate (300 mg, 1.57 mmol) in anhydrous ether (20 mL) was added ethyl 4-aminobenzoate (259 mg, 1.57 mmol) in one portion. The resulting homogeneous reaction mixture became cloudy after ~ 1 h and a clear crystalline solid precipitated slowly over 40 h. At this time, the reaction mixture was further diluted with ether (15 mL), stirred at room temperature for 15 min and vacuum filtered. The resulting white solid was isolated by vacuum filtration, washed with ether (15 mL) and dried in vacuo to afford **294** (327 mg, 58%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.19 (s, 2 H), 7.90 (d, 4 H, *J* = 8.7 Hz), 7.60 (d, 4 H, *J* = 8.7 Hz), 4.28 (q, 4 H, *J* = 7.2 Hz), 1.30 (t, 6 H, *J* = 7.2 Hz); MS (EI) *m/z* 356 (M^+, 21), 311 (9), 165 (66), 120 (100).

295



4,4'-Carbonylbis(azanediyl)dibenzoic acid (295, DMA-NB245-87).[115] To a suspension of **294** (220 mg, 617 mmol) in dioxane (10 mL) was added a 2.0 M LiOH solution (10 mL) at room temperature. The heterogeneous reaction mixture was stirred at room temperature for 8 h, cooled to 0 ^{o}C and acidified with concentrated HCl to pH 0. The resulting turbid solution was cooled to -5 ^{o}C for 18 h. The white precipitate was isolated by vacuum filtration, triturated with boiling MeOH (200 mL) for 1 h and dried in vacuo to afford **295** ~7% solvated with dioxane (164 mg, 88%): ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 12.63 (s, 2 H), 9.24 (s, 2 H), 7.88 (d, 4 H, *J* = 8.7 Hz), 7.58 (d, 4 H, *J* = 8.7 Hz).

SID 861574



SID 861574. LC/MS/UV data: Phenomenex C_{18} (4.6 X 100 nm) column, 1.0 mL/min, 254 nm, gradient elution with 15 to 45% acetonitrile in water containing 0.10% TFA, 1 mg sample/mL DMSO; Retention time = 16.92 min; MS(ESI+) *m/z* 320 $[M + H]^+$. X-Ray crystal structure: A 2.5 mg sample of **SID 861574** was dissolved in a 1:1 mixture of MeOH:ACN (1.0 mL). The solvent was slowly evaporated at 5 ^{o}C over several months and the resulting orange crystals were used for X-Ray crystallographic analysis.

APPENDIX A

A.1 X-RAY CRYSTALLOGRAPHIC DATA FOR SID 861574

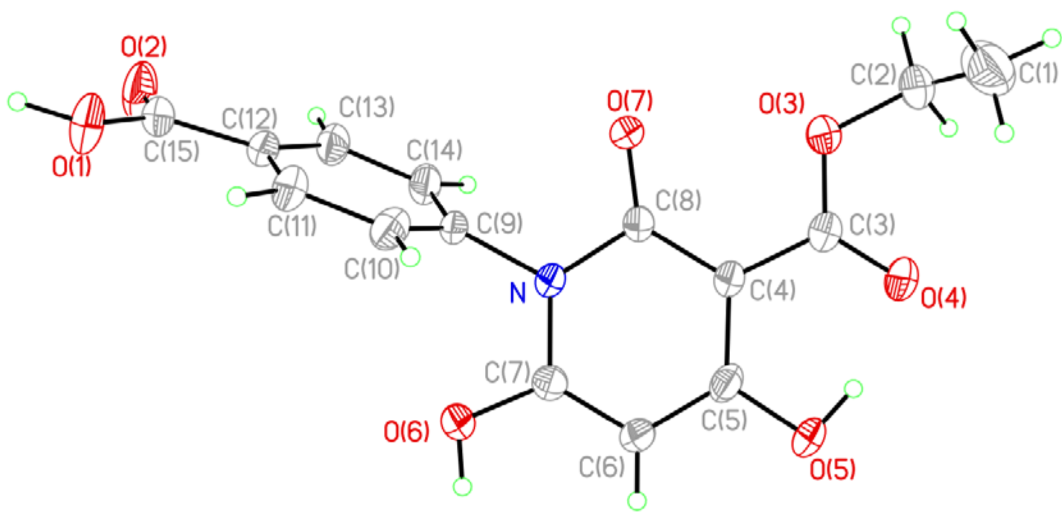


Figure 22. X-Ray crystal structure of SID 861574

Table 1. Crystal data and structure refinement for daw1016.

Identification code	daw1016	
Empirical formula	C15 H13 N O7	
Formula weight	319.26	
Temperature	203(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	a = 21.011(7) Å	a= 90°.
	b = 13.139(5) Å	b= 94.689(7)°.
	c = 10.438(4) Å	g = 90°.
Volume	2871.9(17) $Å^3$	
Z	8	
Density (calculated)	1.477 Mg/m^3	
Absorption coefficient	0.119 mm^{-1}	
F(000)	1328	
Crystal size	0.29 x 0.26 x 0.14 mm^3	
Theta range for data collection	1.83 to 27.50°.	
Index ranges	-27<=h<=27, -17<=k<=17, -13<=l<=13	
Reflections collected	13676	
Independent reflections	3298 [R(int) = 0.0618]	
Completeness to theta = 27.50°	100.0 %	
Absorption correction	None	
Max. and min. transmission	0.9835 and 0.9663	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3298 / 0 / 241	
Goodness-of-fit on F^2	1.059	
Final R indices [I>2sigma(I)]	R1 = 0.0527, wR2 = 0.1376	
R indices (all data)	R1 = 0.0750, wR2 = 0.1513	
Largest diff. peak and hole	0.490 and -0.280 e.$Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for daw1016. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	863(1)	-1216(2)	5421(3)	60(1)
N(1)	2641(1)	1769(1)	2492(1)	25(1)
O(1)	5056(1)	1058(1)	-711(2)	45(1)
O(2)	4266(1)	855(1)	-2255(2)	50(1)
C(2)	696(1)	-834(2)	4100(2)	49(1)
O(3)	1211(1)	-174(1)	3704(2)	46(1)
C(3)	1208(1)	786(2)	4081(2)	33(1)
O(4)	797(1)	1114(1)	4746(2)	46(1)
C(4)	1715(1)	1427(1)	3640(2)	27(1)
O(5)	1306(1)	2841(1)	4761(1)	36(1)
C(5)	1730(1)	2458(2)	4007(2)	27(1)
O(6)	3083(1)	3323(1)	2370(1)	34(1)
C(6)	2185(1)	3128(1)	3615(2)	28(1)
O(7)	2244(1)	182(1)	2421(1)	33(1)
C(7)	2633(1)	2773(1)	2851(2)	26(1)
C(8)	2187(1)	1054(1)	2850(2)	25(1)
C(9)	3108(1)	1442(1)	1620(2)	26(1)
C(10)	3748(1)	1436(2)	2036(2)	31(1)
C(11)	4195(1)	1251(2)	1158(2)	32(1)
C(12)	3993(1)	1054(1)	-116(2)	28(1)
C(13)	3344(1)	989(2)	-500(2)	32(1)
C(14)	2898(1)	1200(2)	367(2)	30(1)
C(15)	4452(1)	964(1)	-1127(2)	32(1)

Table 3. Bond lengths [Å] and angles [°] for daw1016.

C(1)-C(2)	1.483(4)
C(1)-H(1A)	0.9700
C(1)-H(1B)	0.9700
C(1)-H(1C)	0.9700
N(1)-C(7)	1.372(2)
N(1)-C(8)	1.410(2)
N(1)-C(9)	1.458(2)
O(1)-C(15)	1.313(3)
O(1)-H(1O)	1.05(4)
O(2)-C(15)	1.218(3)
C(2)-O(3)	1.471(3)
C(2)-H(2A)	0.9800
C(2)-H(2B)	0.9800
O(3)-C(3)	1.321(2)
C(3)-O(4)	1.230(2)
C(3)-C(4)	1.461(3)
C(4)-C(5)	1.407(3)
C(4)-C(8)	1.428(2)
O(5)-C(5)	1.334(2)
O(5)-H(5O)	0.90(3)
C(5)-C(6)	1.386(3)
O(6)-C(7)	1.321(2)
O(6)-H(6O)	0.88(3)
C(6)-C(7)	1.366(3)
C(6)-H(6)	0.88(2)
O(7)-C(8)	1.240(2)
C(9)-C(10)	1.378(3)
C(9)-C(14)	1.382(3)
C(10)-C(11)	1.386(3)
C(10)-H(10)	0.90(2)
C(11)-C(12)	1.386(3)
C(11)-H(11)	0.99(2)
C(12)-C(13)	1.392(3)
C(12)-C(15)	1.491(3)

C(13)-C(14)	1.383(3)
C(13)-H(13)	0.94(2)
C(14)-H(14)	0.97(2)

C(2)-C(1)-H(1A)	109.5
C(2)-C(1)-H(1B)	109.5
H(1A)-C(1)-H(1B)	109.5
C(2)-C(1)-H(1C)	109.5
H(1A)-C(1)-H(1C)	109.5
H(1B)-C(1)-H(1C)	109.5
C(7)-N(1)-C(8)	122.98(15)
C(7)-N(1)-C(9)	118.58(15)
C(8)-N(1)-C(9)	118.26(15)
C(15)-O(1)-H(1O)	105(2)
O(3)-C(2)-C(1)	109.7(2)
O(3)-C(2)-H(2A)	109.7
C(1)-C(2)-H(2A)	109.7
O(3)-C(2)-H(2B)	109.7
C(1)-C(2)-H(2B)	109.7
H(2A)-C(2)-H(2B)	108.2
C(3)-O(3)-C(2)	117.23(16)
O(4)-C(3)-O(3)	121.52(18)
O(4)-C(3)-C(4)	122.69(18)
O(3)-C(3)-C(4)	115.79(17)
C(5)-C(4)-C(8)	119.09(17)
C(5)-C(4)-C(3)	118.01(17)
C(8)-C(4)-C(3)	122.89(17)
C(5)-O(5)-H(5O)	100.0(17)
O(5)-C(5)-C(6)	116.40(17)
O(5)-C(5)-C(4)	121.46(17)
C(6)-C(5)-C(4)	122.14(17)
C(7)-O(6)-H(6O)	108.6(19)
C(7)-C(6)-C(5)	118.73(18)
C(7)-C(6)-H(6)	120.6(13)
C(5)-C(6)-H(6)	120.7(13)
O(6)-C(7)-C(6)	125.76(17)

O(6)-C(7)-N(1)	113.41(16)
C(6)-C(7)-N(1)	120.83(17)
O(7)-C(8)-N(1)	115.68(16)
O(7)-C(8)-C(4)	128.11(17)
N(1)-C(8)-C(4)	116.20(16)
C(10)-C(9)-C(14)	121.65(17)
C(10)-C(9)-N(1)	119.61(17)
C(14)-C(9)-N(1)	118.63(16)
C(9)-C(10)-C(11)	119.25(19)
C(9)-C(10)-H(10)	121.7(14)
C(11)-C(10)-H(10)	119.0(14)
C(10)-C(11)-C(12)	119.74(19)
C(10)-C(11)-H(11)	117.7(13)
C(12)-C(11)-H(11)	122.3(12)
C(11)-C(12)-C(13)	120.19(17)
C(11)-C(12)-C(15)	121.93(18)
C(13)-C(12)-C(15)	117.78(18)
C(14)-C(13)-C(12)	119.96(19)
C(14)-C(13)-H(13)	119.9(15)
C(12)-C(13)-H(13)	120.1(15)
C(9)-C(14)-C(13)	118.95(18)
C(9)-C(14)-H(14)	120.9(13)
C(13)-C(14)-H(14)	120.1(13)
O(2)-C(15)-O(1)	123.69(18)
O(2)-C(15)-C(12)	121.29(19)
O(1)-C(15)-C(12)	114.95(18)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x 10^3$) for daw1016. The anisotropic displacement factor exponent takes the form: $-2p^2[h^2 a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	49(2)	48(2)	86(2)	12(1)	17(1)	-5(1)
N(1)	26(1)	21(1)	30(1)	0(1)	13(1)	2(1)
O(1)	29(1)	68(1)	41(1)	0(1)	17(1)	2(1)
O(2)	39(1)	77(1)	36(1)	-13(1)	20(1)	-10(1)
C(2)	46(1)	37(1)	69(2)	-2(1)	35(1)	-9(1)
O(3)	47(1)	30(1)	66(1)	-8(1)	36(1)	-10(1)
C(3)	33(1)	32(1)	38(1)	-2(1)	17(1)	0(1)
O(4)	43(1)	40(1)	62(1)	-8(1)	36(1)	-5(1)
C(4)	28(1)	25(1)	30(1)	0(1)	13(1)	1(1)
O(5)	38(1)	32(1)	41(1)	-6(1)	24(1)	3(1)
C(5)	29(1)	29(1)	26(1)	0(1)	11(1)	7(1)
O(6)	35(1)	22(1)	46(1)	1(1)	20(1)	-2(1)
C(6)	34(1)	21(1)	29(1)	-1(1)	12(1)	2(1)
O(7)	38(1)	20(1)	44(1)	-2(1)	22(1)	0(1)
C(7)	29(1)	21(1)	29(1)	3(1)	8(1)	2(1)
C(8)	25(1)	24(1)	28(1)	2(1)	10(1)	1(1)
C(9)	29(1)	20(1)	30(1)	1(1)	17(1)	0(1)
C(10)	32(1)	34(1)	29(1)	-3(1)	8(1)	4(1)
C(11)	25(1)	38(1)	34(1)	-1(1)	9(1)	3(1)
C(12)	30(1)	24(1)	32(1)	-1(1)	15(1)	1(1)
C(13)	32(1)	36(1)	29(1)	-4(1)	12(1)	-3(1)
C(14)	25(1)	34(1)	32(1)	1(1)	10(1)	1(1)
C(15)	32(1)	30(1)	36(1)	-2(1)	15(1)	-3(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for daw1016.

	x	y	z	U(eq)
H(1A)	1248	-1623	5434	90
H(1B)	516	-1631	5690	90
H(1C)	934	-645	6004	90
H(1O)	5306(18)	1010(30)	-1540(30)	110(13)
H(2A)	635	-1409	3504	59
H(2B)	296	-449	4074	59
H(5O)	1063(13)	2290(20)	4860(30)	63(8)
H(6O)	3012(14)	3970(20)	2530(30)	65(9)
H(6)	2183(9)	3770(16)	3847(18)	22(5)
H(10)	3883(10)	1559(16)	2860(20)	36(6)
H(11)	4651(11)	1329(16)	1460(20)	40(6)
H(13)	3209(11)	804(17)	-1350(20)	47(7)
H(14)	2446(11)	1203(16)	90(20)	38(6)

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