Synthesis of Biologically Active Heterocycles and Development of New Organometallic Methodologies

by

David M. Arnold

B.S., Kings College, 2005

Submitted to the Graduate Faculty of
Arts and Sciences in partial fulfillment
of the requirements for the degree of

Master of Science

University of Pittsburgh

2010
This thesis was presented

by

David M. Arnold

It was defended on

12/9/10

and approved by

Dr. Dennis P. Curran, Distinguished Service Professor of Chemistry and Bayer Professor,
Department of Chemistry

Dr. Kazunori Koide, Associate Professor, Department of Chemistry

Thesis Director: Dr. Peter Wipf, Distinguished University Professor, Department of Chemistry
Copyright © by David M. Arnold

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 Grignard reagents, organozinc and organocuprates. The heterocyclic sulfonyl protecting groups were easily cleaved from the \( \alpha \)-branched amines, affording a useful protecting group strategy for the synthesis of this important class of compounds.
TABLE OF CONTENTS

TABLE OF CONTENTS ........................................................................................................... VI
LIST OF TABLES ................................................................................................................ XI
LIST OF FIGURES ............................................................................................................... XII
LIST OF SCHEMES ........................................................................................................... XIV
ACKNOWLEDGEMENTS ................................................................................................. XVII
ABBREVIATIONS ............................................................................................................. XVIII

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

1.1 INTRODUCTION ........................................................................................................ 1
  1.1.1 Brief biological background ........................................................................... 1
  1.1.2 Project background ........................................................................................ 2
  1.1.3 Library design ................................................................................................ 4

1.2 LIBRARY SYNTHESIS ............................................................................................. 5
  1.2.1 Synthesis of 2,8-substituted quinolinecarboxylic acid sodium salts .......... 5
  1.2.2 Synthesis of 1-N-alkyl-3-N-alkyl-5-chloroacetyl-6-aminouracils .......... 8
  1.2.3 Synthesis of the final library compounds ................................................... 11

1.3 BIOLOGICAL RESULTS FOR MKP-1 INHIBITORS ........................................ 15

1.4 CONCLUSIONS ....................................................................................................... 19
2.0 ADDITION OF ORGANOMETALLIC REAGENTS TO NOVEL HETEROCYCLIC SULFONYL AND SULFINYL BENZALDIMINES

2.1 INTRODUCTION

2.1.1 Overview of imines

2.1.2 Addition of organometallic reagents to imines

2.1.2.1 Organolithium and Grignard reagents

2.1.2.2 Dialkylzinc reagents

2.1.2.3 Hydrozirconation and transmetallation

2.1.3 Introduction to Bts and Ths sulfonyl protecting groups

2.2 RESULTS AND DISCUSSION

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

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

2.2.2.1 Lithium reagents

2.2.2.2 Grignard reagents

2.2.2.3 Diethylzinc reactions

2.2.2.4 Hydrozirconation – transmetallation reactions

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

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

2.3 CONCLUSION

3.0 STRUCTURE ELUCIDATION AND SYNTHESIS OF A PUTATIVE POLO LIKE KINASE – POLO BOX DOMAIN (PLK1-PBD) INHIBITOR
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>OVERVIEW</td>
<td>52</td>
</tr>
<tr>
<td>3.2</td>
<td>INTRODUCTION</td>
<td>52</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Project Background</td>
<td>55</td>
</tr>
<tr>
<td>3.3</td>
<td>IDENTIFICATION OF THE STRUCTURE OF SID 861574</td>
<td>57</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Initial synthetic attempts toward the identification of SID 861574</td>
<td>57</td>
</tr>
<tr>
<td>3.3.1.1</td>
<td>Synthesis of the carboxylic acid analogue of 205</td>
<td>58</td>
</tr>
<tr>
<td>3.3.1.2</td>
<td>Synthesis of the isomeric 6-aminouracils 206 and 207</td>
<td>58</td>
</tr>
<tr>
<td>3.3.1.3</td>
<td>Synthesis of 6-aminouracil analogues 208 and 209</td>
<td>59</td>
</tr>
<tr>
<td>3.3.1.4</td>
<td>Synthesis of the 6-hydroxyuracil analogue 210</td>
<td>61</td>
</tr>
<tr>
<td>3.3.1.5</td>
<td>Conclusions of the initial synthetic attempt to identify the structure of SID 861574</td>
<td>62</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Structure elucidation of SID 861574: Development of a controlled synthesis of $N_1,N_3$-differentially-substituted 5-methylene-6-hydroxy uracils</td>
<td>63</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Positive identification of the structure of SID 861574 and biological testing results against Plk1-PBD</td>
<td>72</td>
</tr>
<tr>
<td>3.4</td>
<td>INVESTIGATIONS INTO THE SAMPLE COMPOSITION OF SID 861574</td>
<td>75</td>
</tr>
<tr>
<td>3.5</td>
<td>INVESTIGATIONS INTO THE DECOMPOSITION OF 260 AND SID 861574</td>
<td>78</td>
</tr>
<tr>
<td>3.5.1</td>
<td>High temperature decomposition of SID 861574</td>
<td>79</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Room temperature decomposition of 260 vs. SID 861574</td>
<td>82</td>
</tr>
<tr>
<td>3.6</td>
<td>INITIAL BIOLOGICAL TESTING RESULTS AGAINST PLK1-PBD</td>
<td>85</td>
</tr>
</tbody>
</table>
3.7 IDENTIFICATION AND SYNTHESIS OF PUTATIVE IMPURITIES FOUND IN THE BIOLOGICALLY ACTIVE BATCH OF COMPOUND 265, DMA-NB245-3
                                                                                     87
3.8 FURTHER SYNTHESIS OF ANALOGUES FOR BIOLOGICAL TESTING AGAINST PLK1-PBD
                                                                                     99
3.9 SUMMARY ...................................................................................................... 100

4.0 EXPERIMENTAL PART ....................................................................................... 102
4.1 GENERAL EXPERIMENTAL .............................................................................. 102
4.2 LIBRARY SYNTHESIS ...................................................................................... 103
        4.2.1 Synthesis of 2,8-substituted-quinolinecarboxylic acid sodium salts.... 103
        4.2.2 Synthesis of 1-N-alkyl-3-N-alkyl-5-chloroacetyl-6-aminouracils .... 106
        4.2.3 Synthesis of the 2,8-substituted-4-quinoline carboxylic acids......... 110
        4.2.4 Synthesis of the 1-N-alkyl-3-N-alkyl-6-aminouracils ..................... 116
        4.2.5 Synthesis of the final library analogues .......................................... 126
4.3 ADDITION OF ORGANOMETALLIC REAGENTS TO BENZOTHIAZOLE AND METHYLTHIADIAZOLE ALDIMINES.............. 144
        4.3.1 Synthesis of N-Bts- and N-Ths-benzaldimines ................................ 144
        4.3.2 Organometallic additions to N-Bts- and N-Ths-benzaldimines ......... 149
        4.3.3 Deprotection of N-Ths and N-Bts-sulfonamides ............................. 168
        4.3.4 Addition of organometallic reagents to (E)-N-benzylidenebenzo[d]thiazole-2-sulfinamide and (E)-N-benzylideneapyridine-2-sulfinamide... 171
4.4 SYNTHESIS OF SID 861574 AND RELATED PLK1-PBD INHIBITORS .......................................................................................................................... 182

4.4.1 First generation analogues of SID 861574 .................................................................................................................. 182

4.4.2 Second generation analogues of SID 861574: Synthesis of $N_1,N_3$-differentially-substituted-5-methylene-6-hydroxy uracils .......................................................... 206

4.4.3 Synthesis and identification of the structure of SID 861574 .............. 223

4.4.4 Synthesis of SID 861574 decomposition products and other related analogues .................................................................................................................. 227

APPENDIX A .......................................................................................................................................................................................... 251

BIBLIOGRAPHY .................................................................................................................................................................................. 259
LIST OF TABLES

Table 1. Biological Results$^a$ Against MKP-1 and MKP-3.$^1$ ......................................................... 16
Table 2. Addition of Organolithium Reagents to 146 and 147.......................................................... 38
Table 3. Addition of Grignard Reagents to $\text{Name}$-Ths-Benzaldimine 147................................. 39
Table 4. Addition of Grignard Reagents to $\text{Name}$-Bts-Benzaldimine 146..................................... 40
Table 5. Addition of Diethylzinc Reagents to 146 and 147............................................................ 41
Table 6. Addition of Alkenylzinc Reagents to 146 and 147........................................................... 42
Table 7. Asymmetric Rh(I) Catalyzed Addition Reactions to 146................................................... 44
Table 8. Copper(I)-Catalyzed Addition Reactions of Alkenylzirconocenes to 146......................... 45
Table 9. H$_3$PO$_2$ Deprotection and Boc Protection of Crude Amines........................................... 46
Table 10. SmI$_2$ Deprotection and Boc Protection of Crude Amines.............................................. 46
Table 11. Reaction of 180 with Organometallic Reagents. ............................................................... 50
Table 12. Biological Testing Results from Two Trials for Inhibitors of Plk1-PBD......................... 87
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). ................................................. 93
LIST OF FIGURES

Figure 1. Structure of sanguinarine and related analogues tested for MKP-1 inhibition.\textsuperscript{7} .......... 3

Figure 2. Structure of NU-126 (7).\textsuperscript{6} ................................................................. 3

Figure 3. Structure of MKP-1 inhibitor 8. ................................................................. 4

Figure 4. Library analogues found to inhibit MKP-1 with a similar potency as to the original hit. ................................................................. 17

Figure 5. Representation of several known classes of imines.\textsuperscript{18,19} ......................................... 21

Figure 6. Structures of Davis (87) and Ellman (88) $N$-sulfinylimines ...................................... 22

Figure 7. Selected inhibitors of Plk1: ATP-competitive inhibitors currently in clinical trials (top) and PBD selective inhibitors (bottom) ......................................................................................... 55

Figure 8. Validated inhibitors of phosphopeptide binding to Plk1-PBD with their corresponding PubChem SID numbers.\textsuperscript{17} ............................................................................................................................................. 56

Figure 9. Isomeric analogues of SID 861574. .............................................................................. 58

Figure 10. Originally obtained X-ray structure (241) for SID 861574. ........................................ 63

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..................................................................................................................... 70
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).

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$_4$Cl added.

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.

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.

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.

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$_4$Cl and c) SID 861574.

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

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.

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.

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.

Figure 22. X-Ray crystal structure of SID 861574.
LIST OF SCHEMES

Scheme 1. Main retrosynthetic disconnection for the library analogues. ........................................ 5
Scheme 2. Synthesis of the quinolinecarboxylic acid sodium salts 9, 10, 11, 12, 13, and 14 .......... 6
Scheme 3. Synthesis of the mono- and bis-(trifluoromethyl)-4-quinoline carboxylic acids 21 and 25 ................................................................................................................................................... 7
Scheme 4. Synthesis of quinolinecarboxylic acids 22 and 23. ....................................................... 8
Scheme 5. Synthesis of 1-N-alkyl-3-N-alkyl-5-chloroacetyl-6-aminouracils 15, 16, 17, 18 and 19. ......................................................................................................................................................... 9
Scheme 6. Synthesis of 1-N-alkyl-6-aminouracils 49 and 50. ........................................................ 9
Scheme 7. Synthesis of 1-N-alkyl-3-N-alkyl-6-aminouracils 37, 38 and 40. ............................... 10
Scheme 8. Synthesis of the final MKP-1 inhibitor library................................................................. 14
Scheme 9. Asymmetric synthesis of N-sulfinamides.29 ................................................................. 23
Scheme 10. Organometallic additions to in situ prepared N-acylimines.30 ................................. 24
Scheme 11. Organometallic additions to in situ prepared N-sulfonylimines.31 ............................ 25
Scheme 12. Addition of organometallics to N-phosphinoylimines.23 ............................................. 25
Scheme 13. Asymmetric addition of MeMgBr to N-(2-pyridylsulfonyl)aldimines.32 ................. 26
Scheme 14. Addition of Grignard reagents to N-tert-butylnfinsulfonyl imines.34 .......................... 26
Scheme 15. Addition of MeMgBr to the N-n-toluenesulfonyl aldimine 108.35 ........................... 27
Scheme 16. Reduction of N-tosylimines with Et2Zn.37 ................................................................. 28
Scheme 17. Ligand-activated asymmetric addition of Et₂Zn to N-phosphinoyl imine 114. 39...... 29
Scheme 18. Copper-catalyzed asymmetric addition of Et₂Zn to N-phosphinoyl aldimines. 41 .... 30
Scheme 19. In situ preparation of vinylzinc reagents and addition to aldimines. 43 ............... 31
Scheme 20. Reaction pathway for the synthesis of C-cyclopropylalkylamides. 43 .................. 32
Scheme 21. Rhodium catalyzed addition of alkenylzirconocenes to aldimines. 44 ................. 32
Scheme 22. Proposed catalytic cycle for the rhodium-catalyzed addition of alkenylzirconocenes to aldimines. 44 .................................................................................................................. 33
Scheme 23. Addition of vinylalanes to N-tert-butanesulfinylaldimines. 46 ............................ 34
Scheme 24. Protection and deprotection conditions for the Ths and Bts groups. 47 ............... 35
Scheme 25. Synthesis of N-Bts- and N-Ths-benzaldimines. .................................................. 36
Scheme 26. Vinylalane addition to the N-Bts-benzaldimine 146. ......................................... 43
Scheme 27. Synthesis of heterocyclic-2-sulfinylbenzaldimines 179 and 180. .................... 48
Scheme 28. Addition of MeMgBr to 179. ............................................................................... 49
Scheme 29. Synthesis of 205, the originally proposed compound/structure of SID 861574. ..... 57
Scheme 30. Synthesis of carboxylic acid analogue 211. ....................................................... 58
Scheme 31. Synthesis of SID 861574 isomers 206 and 207.................................................. 59
Scheme 32. Synthesis of isomeric analogue 208. ................................................................. 60
Scheme 33. Synthesis of isomeric analogue 209. ................................................................. 61
Scheme 34. Synthesis of isomeric 6-hydroxyuracil analogue 210. ...................................... 62
Scheme 35. Attempted synthesis of carbamate 244. Synthesis of carbonate 246. ................. 65
Scheme 36. Synthesis of O- and C-carbethoxylated derivatives 247 and 249. ..................... 66
Scheme 37. Synthesis of the C₃-protected-6-oxouracil intermediate 256. ......................... 67
Scheme 38. Synthesis of N₃-ethyl / N₃-carbethoxy-6-oxouracils 257 and 258..................... 68
Scheme 39. Synthesis of the originally proposed X-ray structure 241. ........................................ 69
Scheme 40. Optimization of a one-pot synthesis of 260 (SID 861574). ................................. 74
Scheme 41. Synthesis of 266, a likely impurity in SID 861574. .................................................. 77
Scheme 42. Synthesis of thermal decomposition fragment 265. .................................................. 80
Scheme 43. Proposed high temperature decomposition pathway for 260 to 265 in wet DMSO. 82
Scheme 44. Synthesis of possible decomposition products of 260, 271 and 272 ................. 84
Scheme 45. Scale up synthesis of 265 batch DMA-NB245-3 ............................................................... 90
Scheme 46. Synthesis of putative impurities 279 and 285 thought to be contained in 265 batch DMA-NB245-3................................................................. 95
Scheme 47. Synthesis of the putative impurity 280 contained in 265 batch DMA-NB245-3...... 96
Scheme 48. Synthesis of tricarbonyl analogues 289 and 290 ....................................................... 99
Scheme 49. Synthesis of diarylmalonamide analogues 292 and 293 ........................................ 100
Scheme 50. Synthesis of diarylurea analogues 294 and 295 ....................................................... 100
ACKNOWLEDGEMENTS

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
Ac₂O: Acetic anhydride
AcOH: Acetic acid
ATP: Adenosine triphosphate
ATR: Attenuated total reflectance
BnBr: Benzyl bromide
Boc₂O: Di-tert-butyl dicarbonate
BozPHOS: (2R,5R)-1-\{2-[(2R,5R)-2,5-Dimethylphospholan-1-yl]phenyl\}-2,5-dimethylphospholane 1-oxide
Bt: Benzothiazole
Bts: Benzothiazole-2-sulfonyle
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(1H)-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[(2R,5R)-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 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. 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. 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. Recent 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.
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). 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. 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
In 2006, a second paper demonstrated the potency of novel benzofuran based inhibitors of MKP-1, such as NU-126 (7, Figure 2). The potency of 7 was similar to that of 1, inhibiting MKP-1 with an *in vitro* IC\(_{50}\) value of 28.2 \(\mu\)M. 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 \(\mu\)M), Cdc25B (IC\(_{50}\) > 400 \(\mu\)M), VHR (IC\(_{50}\) = 38.1 \(\mu\)M) and PTP1B (IC\(_{50}\) > 100 \(\mu\)M).

![Figure 1. Structure of sanguinarine and related analogues tested for MKP-1 inhibition.](image)

![Figure 2. Structure of NU-126 (7).](image)
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. 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 $19.2 \pm 5.6 \mu$M (8, Figure 3). 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. This fact, along with the low $\mu$M 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.

![Figure 3. Structure of MKP-1 inhibitor 8.](image)

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, methylecyclopropyl, 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](image)

**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. The resulting 2,8-substituted quinolinecarboxylic acid sodium salts were isolated in yields ranging from 97 – 100%.

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.

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. Quinolines 29 and 30 were then reacted with POBr₃ at 150 °C, forming the corresponding 4-bromo-(trifluoromethyl)quinolines 31 and 32 in 50% and 88% yield, respectively. 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 CO₂ (dry ice) and protonated with HCl to generate the final mono- and bis-(trifluoromethyl)-4-quinoline carboxylic acids 21 and 25 in 53% yield.

The remaining two quinoline carboxylic acids, 2-cyclopropylquinoline-4-carboxylic acid 22 and 2-(furan-2-yl)quinoline-4-carboxylic acid 23, were synthesized by using the
Pfitzinger reaction.\textsuperscript{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.\textsuperscript{11}

![Reaction scheme](image)

**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).\textsuperscript{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.
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-methyl-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.

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. The urea products 44 and 45 were then condensed with 2-cyanoacetic acid 46 at 77 °C in acetic anhydride to produce the corresponding cyanoacetylureas 47 and 48 in 75% and 56% yields. The products 47 and 48 cyclized by treatment with 10% NaOH in ethanol and water at 95 °C, affording the 1-N-alkyl-6-aminouracils 49 and 50 in 50% and 36% yield upon acidification.

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. 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. 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.

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 SN2 reaction in DMF at reflux. 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 1H 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.\textsuperscript{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.
Scheme 8. Synthesis of the final MKP-1 inhibitor library.
Among the five 1-\textit{N}-alkyl-3-\textit{N}-alkyl-5-chloroacetyl-6-aminouracils, only the 1,3-\textit{N},\textit{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-\textit{N}-alkyl-3-\textit{N}-alkyl-5-chloroacetyl-6-aminouracils and the final library analogues. Only four of the 1-\textit{N}-alkyl-3-\textit{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.

Curiously, the resynthesized 73 suffered from an almost three-fold loss of potency with an IC\textsubscript{50} value of 50 \textmu M, versus 19.2 \textmu M 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\textsubscript{50} value comparable to

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{PubChem CID\textsuperscript{a}} & \textbf{R\textsuperscript{1}} & \textbf{R\textsuperscript{2}} & \textbf{R\textsuperscript{3}} & \textbf{R\textsuperscript{4}} & \textbf{MKP-1 IC\textsubscript{50} (\textmu M)} & \textbf{MKP-3 IC\textsubscript{50} (\textmu M)} \\
\hline
58 & 9547710 & H & H & CH\textsubscript{3} & CH\textsubscript{3} & >50 & >50 \\
59 & 9547724 & H & CF\textsubscript{3} & CH\textsubscript{3} & CH\textsubscript{3} & >50 & >50 \\
60 & 9547721 & H & cyclopropyl & CH\textsubscript{3} & CH\textsubscript{3} & >50 & >50 \\
61 & 2364091 & H & furan & CH\textsubscript{3} & CH\textsubscript{3} & 20.6 & >50 \\
62 & 2358568 & H & Ph & CH\textsubscript{3} & CH\textsubscript{3} & >50 & >50 \\
63 & 9547725 & CF\textsubscript{3} & CF\textsubscript{3} & CH\textsubscript{3} & CH\textsubscript{3} & >50 & >50 \\
64 & 9547733 & H & CF\textsubscript{3} & CH\textsubscript{3} & Bn & >50 & >50 \\
65 & 9547736 & H & cyclopropyl & CH\textsubscript{3} & Bn & 28.9 & >50 \\
66 & 9547735 & H & furan & CH\textsubscript{3} & Bn & 16.9 & >50 \\
67 & 9547737 & H & Ph & CH\textsubscript{3} & Bn & >50 & >50 \\
68 & 9547734 & CF\textsubscript{3} & CF\textsubscript{3} & CH\textsubscript{3} & Bn & >50 & >50 \\
69 & 9547740 & H & furan & methylcyclopropyl & CH\textsubscript{3} & 24.6 & >50 \\
70 & 9547741 & CF\textsubscript{3} & CF\textsubscript{3} & methylcyclopropyl & CH\textsubscript{3} & >50 & >50 \\
71 & 9547738 & H & CF\textsubscript{3} & isobutyl & CH\textsubscript{3} & >50 & >50 \\
72 & 9547722 & H & cyclopropyl & isobutyl & CH\textsubscript{3} & >50 & >50 \\
73 & 2094474 & H & furan & isobutyl & CH\textsubscript{3} & 50 & >50 \\
74 & 2098087 & H & Ph & isobutyl & CH\textsubscript{3} & >50 & >50 \\
75 & 9547728 & CF\textsubscript{3} & CF\textsubscript{3} & isobutyl & CH\textsubscript{3} & >50 & >50 \\
76 & 9547729 & H & CF\textsubscript{3} & Bn & CH\textsubscript{3} & >50 & >50 \\
77 & 9547732 & H & cyclopropyl & Bn & CH\textsubscript{3} & >50 & >50 \\
78 & 9547731 & H & furan & Bn & CH\textsubscript{3} & 13.4 & >50 \\
79 & 9547736 & H & Ph & Bn & CH\textsubscript{3} & 50 & >50 \\
80 & 9547730 & CF\textsubscript{3} & CF\textsubscript{3} & Bn & CH\textsubscript{3} & >50 & >50 \\
\hline
\end{tabular}

\textsuperscript{a}Pubchem compound identification number
\end{table}

\textsuperscript{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.

![Chemical structures](image)

**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 IC₅₀ 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).\textsuperscript{18,19} It has been reported that, in general, the relative reactivities of these imines decreases in the order of \textit{N}-acyliminium ions > \textit{N}-acylimines >> \textit{N}-sulfonylimines > \textit{N}-phosphinoylimines > \textit{N}-alkyl and \textit{N}-arylimines.\textsuperscript{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.\textsuperscript{18} Accordingly, it is important to be able to easily remove these protecting groups after the addition reaction.

\begin{figure}
\centering
\begin{tikzpicture}
\node (nac) at (0,0) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{+}{\text{R}}^1 \\
  \text{R}^3 \\
\end{array}$}
node (nacim) at (1.5,0) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{\text{R}}{\text{R}}^1 \\
\end{array}$}
node (nsul) at (3,0) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{=}{\text{R}}^1 \\
\end{array}$}
node (nacim) at (4.5,0) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{=}{\text{R}}^1 \\
\end{array}$}
node (nacim) at (0,-1.5) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{\text{R}}{\text{R}}^1 \\
\end{array}$}
node (nacim) at (1.5,-1.5) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{\text{R}}{\text{R}}^1 \\
\end{array}$}
node (nacim) at (3,-1.5) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{\text{R}}{\text{R}}^1 \\
\end{array}$}

\node (nacim) at (4.5,-1.5) {$\begin{array}{c}
  \text{R}^2 \\
  \text{N} \overset{\text{R}}{\text{R}}^1 \\
\end{array}$}

\end{tikzpicture}
\caption{Figure 5. Representation of several known classes of imines.\textsuperscript{18,19}}
\end{figure}

Deprotection of \textit{N}-acylamines such as the \textit{N}-Boc and \textit{N}-formyl amines, as well as \textit{N}-phosphinoylamides and \textit{N}-sulfinamides, can be generally accomplished under acidic conditions with HCl in protic solvents such as methanol.\text superscript{20-23} The deprotection of \textit{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 SmI₂ and DMPU in THF at reflux.²⁴⁻²⁶ A milder method for the deprotection of primary \( \text{N-arylsulfonylamides} \) requires the conversion of these products to their corresponding \( \text{t-butyl N-arylsulfonylcarbamates} \) by a DMAP-catalyzed reaction with Boc₂O.²⁷ The \( \text{N-arylsulfonyl group} \) can then be cleaved by reaction with Mg metal in methanol resulting in the corresponding \( \text{N-Boc amines} \).²⁷

\[
\begin{align*}
\text{O}^+ & \quad \text{R}^1 \\
\text{S}^- & \quad \text{N} & \quad \text{R}
\end{align*}
\]

\( 87 \)

\[
\begin{align*}
\text{O}^+ & \quad \text{R}^1 \\
\text{S}^- & \quad \text{N} & \quad \text{R}
\end{align*}
\]

\( 88 \)

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

Among the several classes of imines shown in Figure 5, the \( \text{N-sulfinylimines} \) are unique in their ability to both activate the imine \( \text{C=N} \) 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 \( \text{N-sulfinylimines} \) are the \( \text{N-p-toluenesulfinyl imines} \) 87, pioneered by Davis, and the \( \text{N-tert-butanesulfinyl imines} \) explored by Ellman (88, Figure 6). Recently, a general procedure for the synthesis of enantiopure \( \text{N-sulfinamides} \) 91 from enantiomerically pure \( \text{N-sulfonyl-1,2,3-oxathiazolidine-2-oxides} \) 89 has emerged (Scheme 9).²⁹ The resulting \( \text{N-sulfinamides} \) can be condensed with a variety of aldehydes or ketones in the presence of Ti(OEt)₄ to form the enantiopure \( \text{N-sulfinylimines} \).²⁸
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 \(\alpha\)-deprotonation of imines that can form metalated enamines.\(^\text{30}\) 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.\(^\text{19}\) This has led to the development of many procedures for their \textit{in situ} preparation.\(^\text{18}\) 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 \( \alpha \)-amidosubstituted sulfones \(^92\) (Scheme 10).\(^{30}\)

![Scheme 10. Organometallic additions to *in situ* prepared \( \text{N-acylimines.} \)^30](image)

Along similar lines, methodology for the *in situ* preparation of alkyl and aryl aldehyde based \( \text{N-sulfonylimines} \) 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 \( \text{N-sulfinyl p-toluenesulfonamide} \) \(^96\) to generate the corresponding \( \text{N-tosylimines} \) \(^97\) *in situ*, which were then reacted with a variety of alkyl, vinyl, allyl and alkynyl organolithium or Grignard reagents.\(^{31}\)
Zwierzak 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

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
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.\textsuperscript{32,33}

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

![Scheme 15. Addition of MeMgBr to the $N$-$p$-toluenesulfinyl aldimine 108.](image)

### 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 $N$-phosphinoylimines. This was demonstrated in a paper by Qian who studied the effects of diethylzinc coordination in reactions with $N$-tosylimines. Qian reported that neither ($E$)-$N$-benzylidene-$P, P$-diphenylphosphinic amide nor ($E$)-$N$-benzylideneaniline reacted with Et$_2$Zn after 24 h in toluene at room temperature. However, after 1 h in toluene, ($E$)-$N$-benzylidenebenzenesulfonamide 111 was shown to react with Et$_2$Zn to give the reduction product 113 (Scheme 16). 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 \textit{via} the cyclic six-membered transition state 112 shown in Scheme 16.\textsuperscript{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.\textsuperscript{37}

\begin{center}
\textbf{Scheme 16.} Reduction of N-tosylimines with Et$_2$Zn.\textsuperscript{37}
\end{center}

Carretero demonstrated that \textit{N-}(2-pyridinesulfonyl)aldimines \textbf{102} react with alkyl zinc bromides under copper catalyzed conditions to give the corresponding \textit{N-}(2-pyridinesulfonyl)amides in good to excellent yields.\textsuperscript{38} Primary and secondary alkyl zinc bromides containing alkene, ether, acetal, chloride, ester and nitrile groups were found to add efficiently to \textbf{102}.\textsuperscript{38} The reactivity of \textit{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.\textsuperscript{38} This metal coordination was used to explain the enhanced reactivity of \textit{N-}(2-pyridinesulfonyl)aldimines over that of \textit{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.\textsuperscript{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 \((1S,3R,4R)-2\text{-methyl-2-azabicyclo[2.2.1]heptan-3-yl})\text{methanol ligand effectively activated} \text{Et}_2\text{Zn for the asymmetric addition to} (E)-N\text{-benzylidene-}P,P\text{-diphenylphosphinic amide 114 (Scheme 17).}^{39} \text{The proposed transition state 115 for this reaction is shown in Scheme 17.}^{39}

![Scheme 17. Ligand-activated asymmetric addition of \text{Et}_2\text{Zn to} N\text{-phosphinoyl imine 114.}^{39}](image)

Charette has found that dialkylzinc reagents can be asymmetrically added to both alkyl and aryl \(N\text{-phosphinoylaldimines, generated in situ, using a catalytic amount of} \text{Cu(OTf)}_2 \text{and the chiral BozPHOS ligand (119, Scheme 18).}^{40,41}
2.1.2.3 Hydrozirconation and transmetallation

Hydrozirconation of alkenes and alkynes by the use of Schwartz’s reagent (Cp₂Zr(H)Cl, 120) is a well established way to prepare alkyl and alkenylzirconocenes. 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 electrophiles. 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 Me₂Zn effectively added to N-sulfonyl and N-phosphinoylaldimines. 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 Me₂Zn 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. 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.

![Scheme 19. In situ preparation of vinylzinc reagents and addition to aldimines.](image)

If the aldimine 114 was added to the vinylzinc reagent 123 in CH₂Cl₂, then the predominant product of the reaction was the C-cyclopropylalkylamide 128. 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%).
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 Cp₂Zr(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
2.1.3 Introduction to Bts and Ths sulfonyle 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. 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 HgCl₂ in water and THF, and H₃PO₂ in THF at reflux (Scheme 24). The electron-withdrawing effects of the heteroaromatic group on the sulfone activate it for reduction with a variety of reagents, including SmI₂, Mg(0) and thiolate nucleophiles. Many of these deprotection conditions are much more mild than those traditionally used for the cleavage of more electron rich sulfones.
**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 \(\text{Cl}_2(\text{g})\) 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\textsubscript{3} to form the sulfonamides 144 and 145 in 73\% and 68\% yield.

\[
\begin{align*}
\text{Ar-SH} & \xrightarrow{\text{Cl}_2(\text{g})} \text{Ar-S-Cl} \xrightarrow{\text{NH}_3} \text{Ar-S-NH}_2 \\
0 - 5 \text{oC} & \quad \text{CH}_2\text{Cl}_2 & -78 \text{oC to rt} \\
142: \text{Ar} = \text{Benzothiazole} & \quad 139: 45\% \quad 144: \text{Ar} = \text{Benzothiazole, 73\%} \\
143: \text{Ar} = \text{Methylthiadiazole} & \quad 140: 52\% \quad 145: \text{Ar} = \text{Methylthiadiazole, 68\%} \\
\end{align*}
\]

\[
\begin{align*}
\text{Ar-S-NH}_2 & + \text{PhCHO} \xrightarrow{\text{5 mol}\% \text{PTSA}} \text{Ar-N=S} \\
\text{Toluene reflux} & \quad 110 \\
146: \text{Ar} = \text{Benzothiazole, 82\%} & \quad 147: \text{Ar} = \text{Methylthiadiazole, 79\%}
\end{align*}
\]

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

Initial attempts to synthesize N-Ths-benzaldimine 147 by TiCl\textsubscript{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 \(\alpha\)-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 \textsuperscript{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.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar</th>
<th>R</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ths (147)</td>
<td>Me</td>
<td>148</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>Ths (147)</td>
<td>t-Bu</td>
<td>149</td>
<td>0\textsuperscript{a}</td>
</tr>
<tr>
<td>3</td>
<td>Bts (146)</td>
<td>Me</td>
<td>150</td>
<td>0\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{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 °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 °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.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>X</th>
<th>R (product)</th>
<th>Temp. (°C)</th>
<th>2 Step</th>
<th>1 Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1 CH₂Cl₂: Toluene</td>
<td>Cl</td>
<td>i-Pr (151)</td>
<td>-78</td>
<td>45</td>
<td>57</td>
</tr>
<tr>
<td>5:1 THF: Toluene</td>
<td>Cl</td>
<td>i-Pr (151)</td>
<td>-78</td>
<td>47</td>
<td>59</td>
</tr>
<tr>
<td>5:1 THF: Toluene</td>
<td>Cl</td>
<td>i-Pr (151)</td>
<td>-78 → 25</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>5:1 CH₂Cl₂: Toluene</td>
<td>Br</td>
<td>i-Pr (151)</td>
<td>-78</td>
<td>50</td>
<td>61</td>
</tr>
</tbody>
</table>

Grignard Reactions Using Optimized Conditions

<table>
<thead>
<tr>
<th>Solvent</th>
<th>X</th>
<th>R (product)</th>
<th>Temp. (°C)</th>
<th>2 Step</th>
<th>1 Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1 THF: Toluene</td>
<td>Br</td>
<td>Me (148)</td>
<td>-78</td>
<td>49</td>
<td>61</td>
</tr>
<tr>
<td>5:1 THF: Toluene</td>
<td>Br</td>
<td>vinyl (152)</td>
<td>-78</td>
<td>37</td>
<td>46</td>
</tr>
<tr>
<td>5:1 THF: Toluene</td>
<td>Br</td>
<td>1-propynyl (153)</td>
<td>-78</td>
<td>39</td>
<td>49</td>
</tr>
<tr>
<td>5:1 THF: Toluene</td>
<td>Br</td>
<td>Ph (154)</td>
<td>-78</td>
<td>51</td>
<td>64</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>R (product)</th>
<th>Time (h)</th>
<th>Yield (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Br</td>
<td>Me (155)</td>
<td>2.5</td>
<td>87%</td>
</tr>
<tr>
<td>2</td>
<td>Cl</td>
<td>i-Pr (156)</td>
<td>2.5</td>
<td>87%</td>
</tr>
<tr>
<td>3</td>
<td>Br</td>
<td>vinyl (157)</td>
<td>3</td>
<td>85%</td>
</tr>
<tr>
<td>4</td>
<td>Br</td>
<td>1-propynyl (158)</td>
<td>3</td>
<td>71%</td>
</tr>
<tr>
<td>5</td>
<td>Br</td>
<td>Ph (159)</td>
<td>3</td>
<td>77%</td>
</tr>
</tbody>
</table>

\(^a\)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\(_2\)Zn to the N-Ths-benzaldimine 147 proceeded best in non-coordinating solvents, such as toluene, when 2.5 equivalents of Et\(_2\)Zn were added at room temperature (Table 5, entries 1, 2 and 3). Interestingly, it was also found that Et\(_2\)Zn added to the N-Ths-benzaldimine 147 at -78 °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\(_2\)Zn to the N-Bts-benzaldimine 146 (Table 5, entries 5 and 6).
Table 5. Addition of Diethylzinc Reagents to 146 and 147.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar (Product)</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Et₂Zn (equiv.)</th>
<th>Time (h)</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ths (160)</td>
<td>THF</td>
<td>25</td>
<td>1.2</td>
<td>4</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>Ths (160)</td>
<td>Toluene</td>
<td>25</td>
<td>1.2</td>
<td>2.5</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>Ths (160)</td>
<td>Toluene</td>
<td>25</td>
<td>2</td>
<td>2.5</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>Ths (160)</td>
<td>Toluene</td>
<td>-78 °C</td>
<td>1.2</td>
<td>33</td>
<td>61</td>
</tr>
<tr>
<td>5</td>
<td>Bts (161)</td>
<td>THF</td>
<td>25</td>
<td>2</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>Bts (161)</td>
<td>Toluene</td>
<td>25</td>
<td>2</td>
<td>4</td>
<td>58</td>
</tr>
</tbody>
</table>

<sup>a</sup>Yields after recrystallization.

These results are different from what was reported by Qian for the reaction of (E)-N-benzylidenebenzenesulfonamide 111 with Et₂Zn, 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 Cp₂Zr(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 CH₂Cl₂, 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).
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 \( \text{Me}_3\text{Al} \), to the \( N \)-Bts-benzalimine was also briefly explored. As shown in Scheme 26, this reaction was found to be sluggish in toluene at room temperature, affording the product \( \text{164} \) in only 16% yield after 20 h.
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%.
Along with rhodium(I)-catalyzed additions of alkenylzirconocenes to the \( N \)-Bts-benzaldimine, we also explored the use of the \( \text{Cu(CH}_3\text{CN})_4\text{BF}_4 \) catalyst 169 for these addition reactions. An interesting result was found with a protocol for the \textit{in situ} preparation of \( \text{Cp}_2\text{Zr(H)Cl} \) by the reaction of \( \text{Cp}_2\text{ZrCl}_2 \) with DIBAL-H as recently reported by Negishi.\(^{49}\) We found that the \( \text{Cu(CH}_3\text{CN})_4\text{BF}_4 \) 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 \( \text{BF}_3\text{\cdot OEt}_2 \), we discovered that the \( \text{Cu(CH}_3\text{CN})_4\text{BF}_4 \) catalyst catalyzed the addition of the \textit{iso}-butyl group from \( i\)-\( \text{Bu}_2\text{AlCl} \) 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).

\[
\begin{array}{cccccccc}
\text{Entry} & \text{Catalyst (mol\%)} & \text{Ligand (mol\%)} & \text{Diene (mol\%)} & \text{Solvent} & \text{Time (h)} & \text{Yield (\%)} & \text{ee (\%)} \\
1 & [\text{RhCl(ethylene)}_2]_2 & X & 168 & \text{Dioxane} & 24 & 0 & 0 \\
2 & [\text{RhCl(cod)}]_2 & 167 (10) & X & \text{Toluene} & 16 & 19 & 1 \\
3 & [\text{RhCl(ethylene)}_2]_2 & 167 (10) & 168 & \text{Dioxane} & 20 & 7 & 58 \\
\end{array}
\]

\( ^{a}\text{Isolated yield;} ^{b}\text{Determined by chiral HPLC: chiralcel-OD column, 99:1 hexanes : \text{-PrOH, flow rate 1.0 mL/min, UV detection at 258 nm.} \}

---

\( 167 \) 

\( 168 \) 

\( 164 \) 

\( 169 \) 

\( 170 \)
1. Cp₂ZrCl₂, DIBAL-H, THF
2. 10 mol% Cu(CH₃CN)₄BF₄
3. Additive

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst (mol%)</th>
<th>Additive (mol%)</th>
<th>Time (h)</th>
<th>Yield (164) (%)ᵃ</th>
<th>Yield (170) (%)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cu(CH₃CN)₄BF₄ (5)</td>
<td>-</td>
<td>48</td>
<td>&lt; 5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Cu(CH₃CN)₄BF₄ (10)</td>
<td>BF₃•OEt₂ (300)</td>
<td>18</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>BF₃•OEt₂ (300)</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

ᵃIsolated 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% H₃PO₂ 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$_3$PO$_2$ Deprotection and Boc Protection of Crude Amines.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R (substrate)</th>
<th>H$_3$PO$_2$ (equiv.)</th>
<th>Time (h)</th>
<th>(Boc)$_2$O (eq.)</th>
<th>Time (h)</th>
<th>Yield (%), product$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Me (148)</td>
<td>30</td>
<td>4.5</td>
<td>1.25</td>
<td>1</td>
<td>84 (171)</td>
</tr>
<tr>
<td>2</td>
<td>Ph (154)</td>
<td>30</td>
<td>4.5</td>
<td>1.25</td>
<td>1</td>
<td>83 (172)</td>
</tr>
<tr>
<td>3</td>
<td>1-propynyl (153)</td>
<td>30</td>
<td>4.5</td>
<td>1.25</td>
<td>1</td>
<td>88 (173)</td>
</tr>
</tbody>
</table>

$^a$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.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar (substrate)</th>
<th>SmI$_2$ (equiv.)</th>
<th>Amine</th>
<th>Yield (%), product$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ths (148)</td>
<td>7</td>
<td>Crude</td>
<td>84 (171)</td>
</tr>
<tr>
<td>2</td>
<td>Bts (155)</td>
<td>9</td>
<td>Crude</td>
<td>83 (171)</td>
</tr>
</tbody>
</table>

$^a$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-sulfanyl (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\(_2\)Cl\(_2\) 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 °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\(_2\)Cl\(_2\) at room temperature to form the desired heterocyclic-2-sulfinylbenzaldimines 179 and 180 in 79% and 89% yields.
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.
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.
Table 11. Reaction of 180 with Organometallic Reagents.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>Catalyst&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Yield (%&lt;sup&gt;c&lt;/sup&gt;, Product)</th>
<th>dr&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;Zn</td>
<td>-</td>
<td>DCM</td>
<td>rt</td>
<td>17 (183)</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;Zn</td>
<td>Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Toluene</td>
<td>rt</td>
<td>47 (183)</td>
<td>1:1</td>
</tr>
<tr>
<td>3</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;Zn</td>
<td>Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>DCM</td>
<td>0</td>
<td>47 (183)</td>
<td>2:1</td>
</tr>
<tr>
<td>4</td>
<td>Et&lt;sub&gt;2&lt;/sub&gt;Zn</td>
<td>Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>THF</td>
<td>0</td>
<td>Trace&lt;sup&gt;d&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;ZnBr</td>
<td>Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2:1 DCM:THF</td>
<td>rt</td>
<td>64 (184)</td>
<td>3.5:1</td>
</tr>
<tr>
<td>6</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;ZnBr</td>
<td>Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2:1 DCM:THF</td>
<td>0</td>
<td>63 (184)</td>
<td>4:1</td>
</tr>
<tr>
<td>7</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;C≡CMgBr</td>
<td>-</td>
<td>DCM</td>
<td>0</td>
<td>48 (185)</td>
<td>1:1</td>
</tr>
<tr>
<td>8</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;C≡CZnCl&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2:1 DCM:THF</td>
<td>rt</td>
<td>13 (185)</td>
<td>1:1</td>
</tr>
<tr>
<td>9</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;C≡CZnBr&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;CN)&lt;sub&gt;4&lt;/sub&gt;CuBF&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2:1 DCM:THF</td>
<td>rt</td>
<td>50 (185)</td>
<td>3.5:1</td>
</tr>
<tr>
<td>10</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;C≡CZnI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;CN)&lt;sub&gt;4&lt;/sub&gt;CuBF&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2:1 DCM:THF</td>
<td>rt</td>
<td>60 (185)</td>
<td>2.9:1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Formed via transmetallation of 1-propynylmagnesium bromide with ZnCl<sub>2</sub>, ZnBr<sub>2</sub>, ZnI<sub>2</sub>.<sup>b</sup>10 mol%.<sup>c</sup>Ratios determined by <sup>1</sup>H NMR integration.<sup>d</sup>As determined by TLC.<sup>e</sup>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 °C. Interestingly, lowering the reaction temperature from room temperature to 0 °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)₄CuBF₄ catalyzed addition of 1-propynylzinc bromide to 180 at room temperature, affording a dr of 3.5:1. Overall, pyridyl-2-sulfinylbenzalimines 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-benzalimines 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 SmI₂ or H₃PO₂. 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-benzalimines 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*. 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. Investigations into the roles of Plks 1-4 as regulatory enzymes during cell division have shown Plks are active during mitosis, meiosis and cytokinesis. 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.\textsuperscript{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.\textsuperscript{57,58} The PBD of Plk1 serves to localize Plk1 to mitotic structures including chromosomes, kinetochores and the spindle midzone during mitosis.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 \textit{in vivo} induced tumorigenesis in mice.\textsuperscript{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.\textsuperscript{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 \textit{in vitro} and tumor suppression in mice with human xenograft tumors.\textsuperscript{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.\textsuperscript{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).\textsuperscript{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.\textsuperscript{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.\textsuperscript{78} To date, three small molecule inhibitors of Plk1-PBD have been identified; purpurogallin $IC_{50} \sim 0.3$ $\mu$M, poloxin $IC_{50} 4.8 \pm 1.3$ $\mu$M and thymoquinone $IC_{50} 1.14 \pm 0.04$ $\mu$M.\textsuperscript{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.\textsuperscript{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). 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. 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 its structure could be readily modified. SID 861574 also did not have the undesirable electrophilic or hydrolytic structural features present in the remaining active compounds.
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. 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 ¹H 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).
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.
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.

Scheme 30. Synthesis of carboxylic acid analogue 211.

3.3.1.2 Synthesis of the isomeric 6-aminouracils 206 and 207

Analogue 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. Direct aminolysis of these intermediates with 5 equivalents of either 4-aminobenzoic acid or ethyl 4-aminobenzoate under microwave conditions (120 – 150 °C) in DMF led to poor isolated yields of
the respective 6-aminouracils. It was found, however, that the \textit{m}-CPBA oxidation of intermediates 216 and 217 followed by aminolysis of the corresponding sulfoxides at 95-100 °C in dioxane produced the 6-aminouracil intermediate 224 and analogue 206 in 76% and 50% yield, respectively.\textsuperscript{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.](image)

### 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.
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. 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 Cs₂CO₃ followed by reaction with benzyl bromide according to a literature
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.

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. 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 \( \text{N}_1,\text{N}_3 \)-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 °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\(_2\)O\(_7\) that contained an \( \text{N}_3 \)-carbethoxy-substituted-6-hydroxy uracil core substituted in the \( \text{N}_1 \) position with a \( \text{p} \)-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\(_3\)O\(_6\) (\( m/z \) 319 (M\(^+\)) vs. C\(_{14}\)H\(_{12}\)N\(_2\)O\(_7\) (\( m/z \) 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.](image)
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 (C₃O₂) directly furnishing the appropriately N₁,N₃-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. Attempts to cyclize 243 by reaction with malonyl chloride in the presence of Et₃N, 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, Et₂O or DCM at -78 or 0 °C resulted in the isolation of 243 with no evidence of formation of 244. 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 N₁-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-d₆ 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 Et₃N.
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.](attachment:image.png)

The results above indicate that while \(O\)- and \(C\)-carbethoxylation of 245 can be readily achieved, selective \(N_2\)-carbethoxylation is complicated by both the thermodynamic stability of
the C₅-carbethoxy substitution and the lack of nucleophilicity of the N₃-nitrogen of the 6-oxo-
uracil scaffold. As a result, we sought to develop a protecting group strategy for the uracil C₅-
ring position. Initially, we attempted to implement this strategy by protecting the C₅-position of
substrate 245 through a reaction with trimethylene dithiotosylate (TsS(CH₂)₃STs, 254) to
produce the corresponding dithiane 256 (see Scheme 37 for structure). 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. 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 K₂CO₃ in ACN at reflux over 4 h to afford the desired C₅-
protected-6-oxo-uracil intermediate 256 in 67% yield.

Scheme 37. Synthesis of the C₅-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.

![Scheme 38. Synthesis of \( N_3 \)-ethyl / \( N_3 \)-carbethoxy-6-oxouracils 257 and 258.](image)

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 \( \alpha \)-phenylthio ketones and esters under mild reaction conditions.\(^{101-103}\) Thus, the reaction of 257 in a 1:1 solution of aqueous \( \text{NH}_4\text{Cl}:\text{THF} \) 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 C5-unsubstituted-N1,N3-differentially-substituted-6-oxo-uracil scaffolds and should be
beneficial for the development of barbiturates containing this unique and synthetically
challenging substitution pattern.


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-d6 for 1H NMR analysis. As can be clearly seen from
the 1H 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-d6, 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 $\delta$ 149.3 – 150.0. The closest carbonyl resonance found for SID 861574 was well outside this range at $\delta$ 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 $\delta$ 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.
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. 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). 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 $^1$H 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$_4$Cl (Figure 13d).
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.](image_url)
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

\[ \text{\textsuperscript{a} LC/MS analysis performed by Peter Chambers.} \]
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. 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).
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-d₆. This mixture was heated to 110 °C for 30 min. After cooling to room temperature, ¹H 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 H₂O (present in DMSO) to form the dicarboxylic acid intermediate 270. 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.
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-d₆ 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 ⁱH 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 ⁱH NMR comparison due to the expected similarity in the ¹H NMR resonances for compound 272 vs. 271.¹⁰⁶ This experiment ruled out the possibility of the ¹H 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.
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$_4$Cl (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$_4$Cl 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 °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$_4$Cl and c) SID 861574.](image)

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 (≥ 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.

![Chemical structures](image)

**Figure 18.** Samples submitted for biological testing against Plk1-PBD.
Table 12. Biological Testing Results from Two Trials for Inhibitors of Plk1-PBD.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Batch (Notebook #)</th>
<th>Assay 1&lt;sup&gt;ac&lt;/sup&gt; IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Assay 2&lt;sup&gt;bc&lt;/sup&gt; IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SID 861574</td>
<td>-</td>
<td>-</td>
<td>2.95 ± 0.568</td>
</tr>
<tr>
<td>2</td>
<td>260</td>
<td>DMA-NB205-92</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>3</td>
<td>267</td>
<td>DMA-NB245-4</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>4</td>
<td>265</td>
<td>DMA-NB245-3</td>
<td>2.079</td>
<td>6.047</td>
</tr>
<tr>
<td>5</td>
<td>265</td>
<td>DMA-NB245-24</td>
<td>-</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>6</td>
<td>239</td>
<td>DMA-NB74-62</td>
<td>-</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>7</td>
<td>234</td>
<td>DMA-NB74-63</td>
<td>-</td>
<td>&gt; 50</td>
</tr>
</tbody>
</table>

<sup>a</sup>Assay performed 12/2009. <sup>b</sup>Assay performed 3/2010. <sup>c</sup>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 <sup>1</sup>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)<sup>+</sup> in the active sample DMA-NB245-3. However, this impurity was absent in the inactive sample DMA-NB245. A <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) 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 <sup>1</sup>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).
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(PPh3)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

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

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.

\[
\begin{align*}
\text{268} & \quad + \quad \text{203} \\
1. & \quad 2.5 \text{ equiv. NaH, THF, } 0 \degree \text{C to rt} \\
2. & \quad 1.0 \text{ equiv. of isocyanate, } 0 \degree \text{C to rt, 273} \\
3. & \quad \text{reflux; Conc. HCl} \\
\end{align*}
\]

\[
\begin{align*}
\text{51% crude mixture:} \\
\text{~2:1:0.5:trace} & \quad 274a:274b:274c:274d \\
274a: & \quad R_1 = -\text{OAllyl, } R_2 = -\text{OEt} \\
274b: & \quad R_1 = -\text{OEt, } R_2 = -\text{OAllyl} \\
274c: & \quad R_1 = -\text{OAllyl, } R_2 = -\text{OAllyl} \\
274d: & \quad R_1 = -\text{OEt, } R_2 = -\text{OEt}
\end{align*}
\]

\[
\begin{align*}
\text{265} & \quad (62\%)
\end{align*}
\]

\[
\begin{align*}
\text{1.} & \quad 10 \text{ equiv. morpholine, 5 mol \% Pd(PPh}_3)_4 \text{, THF, rt} \\
\text{2.} & \quad 1.0 \text{ M NaOH, rt; Conc. HCl, } 0 \degree \text{C} \\
\end{align*}
\]

\[
\begin{align*}
\text{265} & \quad (62\%)
\end{align*}
\]

\[
\begin{align*}
274a-d
\end{align*}
\]

\[
\begin{align*}
\text{Scheme 45. Scale up synthesis of 265 batch DMA-NB245-3.}
\end{align*}
\]
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 °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$_2$O, morpholine) ring opening reaction of product 265 that takes place in the presence of Lewis acidic palladium (II) salts.
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 $^1$H 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 $^1$H 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 $^1$H 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).
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.\textsuperscript{a}

\textsuperscript{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
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.
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 IC₅₀ < 50 µM, and one (SID 861574, IC₅₀ = 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 were 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 °C or flame dried under high vacuum prior to use. THF and Et₂O were dried by distillation from Na/benzophenone ketyl, and CH₂Cl₂ and toluene were purified using an alumina column filtration system. Reactions were monitored by either ¹H NMR at 300 MHz in DMSO-d₆ or by TLC analysis (EM Science pre-coated silica gel 60 F254 plates, 250 μ (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. H₂SO₄ in 100 mL of 95% EtOH) and a KMnO₄ solution (1.5 g of KMnO₄ and 1.5 g of K₂CO₃ in 100 mL of a 0.1% NaOH solution). Flash chromatography on SiO₂ 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. ¹H and ¹³C NMR spectra were obtained on a Bruker Avance 300 instrument in CDCl₃ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard. ¹H 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). ¹³C 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

![Diagram of compound 10 with chemical formula]

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 °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) $\delta$ 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).
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) $\delta$ 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).

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 °C for 1 h. The product 11 (440 mg, 100%) was isolated as a white solid: $^1$H-NMR (methanol-d$_4$, 300 MHz) $\delta$ 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).
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 °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) $\delta$ 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).

Sodium 2-phenylquinoline-4-carboxylate (13). 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) $\delta$ 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).
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 °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

General procedure B. 6-Amino-5-(2-chloroacetyl)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (15, DMA-P77). 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 °C for 1 h under a nitrogen atmosphere. After 1 h, the mixture was cooled to 50 °C and deionized water (20.0 mL) was slowly added, with vigorous stirring, while cooling the mixture to 0 °C for 1 h. A white precipitate formed which was isolated by vacuum filtration, washed with additional H$_2$O (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 °C; IR (KBr) 3339, 3154,
1718, 1659, 1529, 1150, 781, 651 cm\(^{-1}\); \(^1\)H-NMR (DMSO-d\(_6\) 300 MHz) \(\delta\) 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); \(^1^3\)C-NMR (DMSO-d\(_6\), 75 MHz) \(\delta\) 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\(_8\)H\(_{10}\)N\(_3\)O\(_3\)Cl 231.0411, found 231.0411.

\[ \text{6-Amino-3-benzyl-5-(2-chloroacetyl)-1-methylpyrimidine-2,4(1H,3H)-dione (16, DMA-P150).} \]

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 ⁰C; IR (KBr) 3436, 3179, 3061, 1714, 1600, 1512, 1433, 1236, 1139, 778, 715, 649 cm\(^{-1}\); \(^1\)H-NMR (DMSO-d\(_6\) 300 MHz) \(\delta\) 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); \(^1^3\)C-NMR (DMSO-d\(_6\), 75 MHz) \(\delta\) 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\(_3\)O\(_3\)Cl 307.0724, found 307.0718.
6-Amino-5-(2-chloroacetyl)-1-(cyclopropylmethyl)-3-methylpyrimidine-2,4 (1\textit{H},3\textit{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 °C; IR (KBr) 3317, 3066, 3010, 1723, 1655, 1603, 1523, 1387, 1144, 1019, 827 cm\(^{-1}\); \(^1\)H-NMR (DMSO-d\(_6\) 300 MHz) \(\delta\) 10.96 (br, 1 H, H-1), 8.54 (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) \(\delta\) 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 (El) \(m/z\) 271 (M\(^+\), 9), 236 (74), 206 (71), 168 (82), 139 (42), 80 (51); HRMS (El) \(m/z\) calculated for C\(_{11}\)H\(_{14}\)N\(_3\)O\(_3\)Cl 271.0724, found 271.0731.

6-Amino-5-(2-chloroacetyl)-1-isobutyl-3-methylpyrimidine-2,4(1\textit{H},3\textit{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 °C; IR (KBr) 3488, 3219, 3155, 2970, 1712, 1655, 1611, 1522, 1375, 1231, 773 cm\(^{-1}\)


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$_3$O$_3$Cl 273.0880, found 273.0881.

6-Amino-1-benzyl-5-(2-chloroacetyl)-3-methylpyrimidine-2,4(1H,3H)-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 °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$_3$O$_3$Cl 307.0724, found 307.0719.
4.2.3 Synthesis of the 2,8-substituted-4-quinoline carboxylic acids

General procedure C. 2-(Trifluoromethyl)quinoline-4(1H)-one (29).

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 °C. The reaction mixture was heated to 150 °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) $\delta$ 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).
2,8-Bis(trifluoromethyl)quinolin-4(1H)-one (30, DMA-P104).\textsuperscript{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 °C. An alternative workup was performed 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 °C; \textsuperscript{1}H-NMR (acetone-d\textsubscript{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\textsuperscript{+}, 100), 261 (66), 233 (63), 214 (24), 183 (16), 75 (7).

General Procedure D. 4-Bromo-2-(trifluoromethyl)quinoline (31).\textsuperscript{17} To a melt of POBr\textsubscript{3} (1.35 g, 4.69 mmol) at 75 °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 °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\textsubscript{2}Cl\textsubscript{2} (3 x 100 mL). The combined organic extracts were washed with a saturated brine solution (50.0 mL), dried (Na\textsubscript{2}SO\textsubscript{4}), filtered through a plug of SiO\textsubscript{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) $\delta$ 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).

![structure of 32](image)

**4-Bromo-2,8-bis(trifluoromethyl)quinoline (32, DMA-P108).** 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 °C; $^1$H-NMR (chloroform-d, 300 MHz) $\delta$ 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).

![structure of 21](image)

**General procedure E. 2-(Trifluoromethyl)quinoline-4-carboxylic acid (21, DMA-P114).** 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 °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 °C; IR (KBr) 3113, 2946, 1728, 1358, 1256, 1199, 1103, 901, 767, 663 cm⁻¹; ¹H-NMR (acetone-d₆ 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); ¹³C-NMR (acetone-d₆, 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 C₁₁H₆NO₂F₃ 241.0351, found 241.0349.

2,8-Bis(trifluoromethyl)quinoline-4-carboxylic acid (25, DMA-P115). According to general procedure E, 32 (300 mg, 0.872 mmol), n-butyl lithium (0.65 mL, 0.87 mmol, 1.34 M) and CO₂ (excess dry ice) were converted to 25 (144 mg, 53%). The product was obtained as a white crystalline solid: Mp 226–228 °C; IR (KBr) 3027, 1710, 1519, 1430, 1361, 1311, 1148, 1036, 781, 687 cm⁻¹; ¹H-NMR (acetone-d₆, 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); ¹³C-NMR (acetone-d₆, 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
2-Cyclopropylquinoline-4-carboxylic acid (22, DMA-P121).\textsuperscript{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 \degree C 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 \degree C; IR (KBr) 3448, 3095, 3007, 1667, 1616, 1405, 1338, 882, 772 cm\(^{-1}\); \(^1\)H-NMR (acetone-d\(_6\), 300 MHz) \(\delta\) 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) \(\delta\) 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.
2-(Furan-2-yl)quinoline-4-carboxylic acid (23, DMA-P117). 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 °C (dec.); IR (KBr) 3435, 3141, 1714, 1600, 1372, 1232, 1018, 761 cm\(^{-1}\); \(^1\)H-NMR (acetone-d\(_6\), 300 MHz) \(\delta\) 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, 1 H, J = 3.4, 1.6 Hz, H-2); \(^13\)C-NMR (acetone-d\(_6\), 75 MHz) \(\delta\) 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\(_9\)NO\(_3\) 239.0582, found 239.0590.
4.2.4 Synthesis of the 1-N-alkyl-3-N-alkyl-6-aminouracils

1-Benzylurea (44, DMA-P105). 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 °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).

1-(Cyclopropylmethyl)urea (45, DMA-P126). 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$_2$SO$_4$) 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 °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).

**N-(Benzylcarbamoyl)-2-cyanoacetamide (47).**

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: 1H-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).

**2-Cyano-N-(cyclopropylmethylcarbamoyl)acetamide (48, DMA-P130).**

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 °C; \(^1\)H-NMR (acetone-\(d_6\), 300 MHz) \(\delta\) 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).

6-Amino-1-benzylpyrimidine-2,4(1\(H,3H\))-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 °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 °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 °C; \(^1\)H-NMR (DMSO-\(d_6\), 300 MHz) \(\delta\) 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).
6-Amino-1-(cyclopropylmethyl)pyrimidine-2,4(1H,3H)-dione (50, DMA-P132).\textsuperscript{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 \textit{in vacuo}. The resulting product 50 (0.71 g, 36%) was obtained as a light orange crystalline solid: Mp 265-266 °C; \textsuperscript{1}H-NMR (DMSO-d\textsubscript{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, \textit{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) \textit{m/z} 181 (M\textsuperscript{+}, 43), 152 (14), 127 (87), 68 (31), 55 (100).

General procedure F. (\textit{E})-\textit{N'-(3-Benzyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin -4-yl)-N,N-dimethylformimidamide} (52, DMA-P113).\textsuperscript{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 \( \text{52} \) (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) \( \delta \) 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) \( \delta \) 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\(_4\)O\(_2\) 272.1273, found 272.1274.

\[
\text{(E)-N’-(3-(Cyclopropylmethyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-dimethylformimidamide (53, DMA-P139).} \quad \text{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) \( \delta \) 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) \( \delta \) 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_{4}O_{2} 236.1273, found 236.1274.

\[
\begin{array}{c}
\text{54} \\
\end{array}
\]

(E)-N,N-Dimethyl-N’-(3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)
formimidamide (54, DMA-P128). 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) \(\delta\) 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); \(^1^3\)C-NMR (DMSO-d\(_6\), 75 MHz) \(\delta\) 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).

\[
\begin{array}{c}
\text{55} \\
\end{array}
\]

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 NaHCO₃ solution (20.0 mL). The aqueous phase was extracted with EtOAc (4 x 50.0 mL) and the organic layers were combined, dried (Na₂SO₄) and concentrated by rotary evaporation. The resulting residue was chromatographed on SiO₂ (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; ¹H-NMR (methanol-d₄, 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).

![Image of molecule 56](image)

(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; ¹H-NMR (methanol-d₄, 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_{4}O_{2} 250.1430, found 250.1433.

\[
\begin{array}{c}
\text{(E)-N'-(1-Benzyl-3-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-N,N-
\text{dimethylformimidamide (57, DMA-P131).}}^{25}
\end{array}
\]

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 °C for 5 h. The resulting product 57 (192 mg, 15%) was isolated as a white solid: Mp 201–202 °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_{4}O_{2} 286.1430, found 286.1440.
General procedure H. 6-Amino-1-benzyl-3-methylpyrimidine-2,4(1H,3H)-dione (40, DMA-P136).\textsuperscript{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\textsubscript{2} (EtOAc, with a few drops of Et\textsubscript{3}N). 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\textsuperscript{-1}; \textsuperscript{1}H-NMR (methanol-d\textsubscript{4}, 300 MHz) \delta 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); \textsuperscript{13}C-NMR (methanol-d\textsubscript{4}, 75 MHz) \delta 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) \textit{m/z} 231 (M\textsuperscript{+}, 100), 173 (7), 145 (9), 111 (7), 106 (11); HRMS (EI) \textit{m/z} calculated for C\textsubscript{12}H\textsubscript{13}N\textsubscript{3}O\textsubscript{2} 231.1008, found 231.1005.
6-Amino-1-(cyclopropylmethyl)-3-methylpyrimidine-2,4(1\textit{H},3\textit{H})-dione (38).\textsuperscript{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: $^1\text{H}-\text{NMR (DMSO-d}_6$ 300 MHz) $\delta$ 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).

6-Amino-3-benzyl-1-methylpyrimidine-2,4(1\textit{H},3\textit{H})-dione (37, DMA-P137).\textsuperscript{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\textsubscript{2} (9:1 CH\textsubscript{2}Cl\textsubscript{2}: methanol). The product 37 (446 mg, 73%) was isolated as a white solid: Mp 202-203 °C; $^1\text{H}-\text{NMR (DMSO-d}_6$ 300 MHz) $\delta$ 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\textsuperscript{+}, 42), 220 (52), 205 (100), 145 (19), 99 (15).
4.2.5 Synthesis of the final library analogues

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 °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 °C followed by 19 h at 50 °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) $\delta$ 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).
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 °C; IR (KBr) 3416, 3137, 1712, 1639, 1605, 1531, 1448, 1247, 1152, 775 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₈H₁₆N₄O₅ 368.1120, found 368.1126.
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).

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 °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,
$J = 7.0 \text{ Hz, H-9}$), 7.63 (t, 1 H, $J = 7.1 \text{ Hz, H-10}$), 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) $\delta$ 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$_4$O$_5$ 408.1434, found 408.1445.

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) $\delta$ 10.73 (br, 1 H, H-19), 8.67 (d, 1 H, $J = 8.3 \text{ Hz, H-12}$), 8.46 (br, 1 H, H-19), 8.38 (s, 1 H, H-6), 8.11 (d, 1 H, $J = 8.3 \text{ Hz, H-9}$), 7.99 (br, 1 H, H-1), 7.86 (t, 1 H, $J = 7.6 \text{ Hz, H-11}$), 7.70 (t, 1 H, $J = 7.7 \text{ Hz, H-10}$), 7.45 (d, 1 H, $J = 3.4 \text{ Hz, H-3}$), 6.76 (dd, 1 H, $J = 3.4, 1.8 \text{ Hz, H-2}$), 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) $\delta$ 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), 129.
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\(_4\)O\(_6\) 434.1226, found 434.1221.

\[
\begin{align*}
2-(6-\text{Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl})-2\text{-oxoethyl} \\
2\text{-phenylquinoline-4-carboxylate (62, DMA-P100). According to general procedure I, 15 (52.0} \text{ 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 °C (dec); IR (KBr) 3381, 3097, 2951, 1713, 1619, 1529, 1460, 1234, 1165, 970, 777, 695 cm\(^{-1}\). \text{\textsuperscript{1}H-NMR (DMSO-d\(_6\), 300 MHz) } \delta \text{ 10.75 (br, 1 H, H-21), 8.71 (d, 1 H, } J = 8.7 \text{ 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 \text{ Hz, H-1, H-5), 8.20 (d, 1 H, } J = 8.0 \text{ Hz, H-11), 7.89 (t, 1 H, } J = 7.8 \text{ Hz, H-13), 7.74 (t, 1 H, } J = 7.8 \text{ 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); } \text{\textsuperscript{13}C-NMR (DMSO-d\(_6\), 75 MHz) } \delta \text{ 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 \text{ 445 ([M + H] \(^+\)); HRMS (ESI) } m/z \text{ calculated for C\(_{24}\)H\(_{21}\)N\(_4\)O\(_5\) (M+H) 445.1512, found 445.1502.}
\end{align*}
\]
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) \(\delta\) 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).

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) \(\delta\) 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,
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; ¹H-NMR (acetone-d₆, 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); ¹³C-NMR (acetone-d₆, 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 C₂₈H₂₂N₄O₆ 510.1539, found 510.1554.
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) $\delta$ 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).

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 °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)
$\delta$ 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) $\delta$ 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$_6$N$_4$O$_5$ 580.1181, found 580.1191.

![Structure of compound 69](image_url)

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 °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) $\delta$ 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$_4$O$_6$ 474.1539, found 474.1519.

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).
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) $\delta$ 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).

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 °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) $\delta$ 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) \( \delta \) 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\(_{4}\)O\(_{5}\) 450.1903, found 450.1900.

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 °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) \( \delta \) 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) $\delta$
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$_4$O$_6$ 476.1696, found 476.1709.

$\text{N}$
$\text{N}$
$\text{O}$
$\text{O}$
$\text{NH}_2$
$\text{O}$
$\text{N}$
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) $\delta$ 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) $\delta$ 190.1 (C-18), 165.7 (C-16), 161.4 (C-28), 157.6 (C-20), 155.7 (C-7), 149.8 (C-26),
138
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$_4$O$_5$ (M+H) 487.1981, found 487.1943.

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) $\delta$ 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).
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; ¹H-NMR (acetone-d₆, 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); ¹³C-NMR (acetone-d₆, 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, J₇CF = 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, J₇CF = 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 C₂₅H₁₉F₃N₄O₅ 512.1308, found 512.1331.
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) $\delta$ 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).

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 ºC (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$_4$O$_6$ 510.1539, found 510.1524.

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).

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: ¹H NMR (acetone-d₆, 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

General procedure J. 5-Methyl-1,3,4-thiadiazole-2-sulfonyl chloride (140). To a solution of acetic acid (45.0 mL, 33%) cooled to -5-5 °C (internal thermometer) was introduced a vigorous stream of Cl₂ gas. After 5 min, the solution became yellow and a yellow solid precipitated. At this time, 5-methyl-1,3,4-thiadiazole-2-thiol (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 °C and the solution thoroughly saturated with Cl₂ 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 NaHCO₃ solution (1 X 75.0 mL) followed by a cold saturated brine solution (1 X 50.0 mL). The ether solution was dried (Na₂SO₄), concentrated by rotary evaporation (10 °C), and the resulting white solid was recrystallized from ether (50.0 mL) at -78 °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 °C (dec); ¹H-NMR (CDCl₃, 300 MHz) δ 2.97 (s, 3 H).
Benzo[d]thiazole-2-sulfonyl chloride (139).\textsuperscript{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\textsubscript{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\textsubscript{2}Cl\textsubscript{2} (100 mL) and washed with a cold saturated NaHCO\textsubscript{3} solution (1 X 50.0 mL), followed by a cold saturated brine solution (1 X 50.0 mL). The CH\textsubscript{2}Cl\textsubscript{2} solution was then filtered through a 1 in plug of celite, concentrated by rotary evaporation (13 °C) and the crude orange solid was recrystallized from ether (20.0 mL) at -78 °C. The product 139 (3.24 g, 56\%) was isolated as a white crystalline solid: Mp 104.5-108 °C; $^1$H-NMR (CDCl\textsubscript{3}, 300 MHz) $\delta$ 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).

Benzo[d]thiazole-2-sulfonamide (144).\textsuperscript{76} To a solution of 139 (2.46 g, 10.5 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (25.0 mL) cooled to -78 °C under a nitrogen atmosphere, was added condensed NH\textsubscript{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 °C and diluted with hexanes (25.0 mL). The resulting white precipitate was isolated by vacuum filtration, washed with a 1:1 solution of CH\textsubscript{2}Cl\textsubscript{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 SiO₂ 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 °C, IR (KBr) 3312, 3151, 3039, 1472, 1356, 1164, 929, 765 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₇H₆N₂O₂S₂ 213.9871, found 213.9874.

5-Methyl-1,3,4-thiadiazole-2-sulfonamide (145). To a solution of 140 (3.57 g, 18.0 mmol) in CH₂Cl₂ (40.0 mL) cooled to -78 °C under a nitrogen atmosphere, was added condensed NH₃ (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 °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) \(\delta\) 7.59 (s, 2 H), 2.85 (s, 3 H); \(^{13}\)C-NMR (acetone-\(d_6\), 75 MHz) \(\delta\) 171.3, 170.5, 15.7; MS (El) \(m/z\) 179 (M\(^+\), 17), 115 (100), 99 (71), 80 (25), 59 (15); HRMS (El) \(m/z\) calculated for C\(_3\)H\(_5\)N\(_3\)O\(_2\)S\(_2\) 178.9823, found 178.9831.

\(\text{(E)-N-Benzylidenebenzo[d]thiazole-2-sulfonamide (146).}\) 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\(_2\)Cl\(_2\), 300 MHz) \(\delta\) 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\(_2\)Cl\(_2\), 75 MHz) \(\delta\) 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\(_2\)O\(_2\)S\(_2\) 302.0184, found 302.0180.

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-dimethoxybenzaldehyde (17.5 mg, 0.103 mmol) dissolved in dry CD\(_2\)Cl\(_2\) (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 (\(\delta\) 9.54, area = 1.00) versus the internal standard aldehyde proton (\(\delta\) 10.84, area = 1.31) showed the reaction yield to be 79%.
4.3.2 Organometallic additions to $N$-Bts- and $N$-Ths-benzaldimines

![Chemical Structure](image)

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 °C under a nitrogen atmosphere. The reddish-brown reaction mixture was stirred at -78 °C for 2 h, was quenched by the addition of a saturated NH$_4$Cl 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$_2$SO$_4$) 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 °C; IR (KBr) 3065, 2981, 2866, 1468, 1447, 1350, 1208, 1169, 1095, 1016, 768 cm$^{-1}$; $^1$H-NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 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$_2$Cl$_2$, 75 MHz) $\delta$ 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$_3$O$_2$S$_2$Na (M+Na) 306.0347, found 306.0328.
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 °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 °C for 2 h and then quenched with a saturated NH₄Cl 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 (Na₂SO₄) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO₂ (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 °C; IR (KBr) 3118, 2975, 2870, 1452, 1352, 1097, 1051, 922, 705 cm⁻¹; ¹H-NMR (CD₂Cl₂, 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); ¹³C-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 El+) m/z calculated for C₁₃H₁₈N₃O₂S₂ (M+H) 312.0840, found 312.0835.

5-Methyl-N-(2-methyl-1-phenylpropyl)-1,3,4-thiadiazole-2-sulfonamide (151, Table 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 CH₂Cl₂ (2.00 mL) at -78 °C for 2.5 h. The same workup procedure was followed except CH₂Cl₂ 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 °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 CH₂Cl₂ (2.00 mL) at -78 °C for 2.5 h. The same workup procedure was followed except CH₂Cl₂ 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 °C for 2 h. The resulting product 148 (27.7 mg, 61%) was isolated as a white crystalline solid.
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 °C for 2 h. The resulting product 152 (21.8 mg, 46%) was isolated as a white crystalline solid: Mp 122-123 °C; IR (KBr) 3141, 3024, 2870, 1443, 1360, 1172, 1054, 939, 702 cm⁻¹; ¹H-NMR (CD₂Cl₂, 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); ¹³C-NMR (CD₂Cl₂, 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 C₁₂H₁₄N₃O₂S₂ (M+H) 296.0527, found 296.0506.

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 °C for 2 h. The resulting product 153 (23.9 mg, 49%) was isolated as a white crystalline solid: Mp 169-170 °C; IR (KBr) 3055, 2858, 1455, 1351, 1211, 1173, 1019, 734 cm⁻¹; ¹H-NMR (CD₂Cl₂, 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\(_2\)Cl\(_2\), 75 MHz) \( \delta \) 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\(_3\)O\(_2\)S\(_2\)Na (M+Na) 330.0347, found 330.0345.

**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 °C for 2 h. The resulting product 154 (35.2 mg, 64%) was isolated as a white crystalline solid: Mp 185-186 °C; IR (KBr) 3174, 3066, 2869, 1447, 1359, 1174, 1045, 702 cm\(^{-1}\); \(^1\)H-NMR (CD\(_2\)Cl\(_2\), 300 MHz) \( \delta \) 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\(_2\)Cl\(_2\), 300 MHz) \( \delta \) 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\(_3\)O\(_2\)S\(_2\) (M+H) 346.0684, found 346.0671.

**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 °C for 2.5 h. The same workup procedure was
performed except that the product was isolated by chromatography on SiO₂ (3:1 hexanes: EtOAc). The product 155 (83.2 mg, 87%) was isolated as a white crystalline solid: Mp 144-145 °C; IR (KBr) 3116, 2973, 2874, 1477, 1350, 1163, 1062, 982, 764 cm⁻¹; ^1H-NMR (CD₂Cl₂, 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); ^13C-NMR (CD₂Cl₂, 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 C₁₅H₁₄N₂O₂S₂Na (M+Na) 341.0394, found 341.0389.

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 °C for 2.5 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO₂ (3:1 hexanes: EtOAc) to give 156 (90.1 mg, 87%) as a white crystalline solid: Mp 157-158 °C; IR (KBr) 3265, 2959, 2872, 1455, 1336, 1041, 915, 771 cm⁻¹; ^1H-NMR (CD₂Cl₂, 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); ^13C-NMR (CD₂Cl₂, 75 MHz) δ 166.7, 152.8,
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 °C for 3 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO<sub>2</sub> (3:1 hexanes:EtOAc) to give 157 (84.5 mg, 85%) as a white crystalline solid: Mp 141-142 °C; IR (KBr) 3117, 2872, 1450, 1161, 1039, 930, 765 cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 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); <sup>13</sup>C-NMR (CD<sub>2</sub>Cl<sub>2</sub>, 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<sub>16</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub> (M+H) 331.0575, found 331.0549.  

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<sub>17</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub> (M+H) 347.0888, found 347.0862.
\textit{N-}(1\text{-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 °C for 3 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO\textsubscript{2} (3:1 hexanes: EtOAc) to give 158 (72.8 mg, 71\%) as a white crystalline solid: Mp 155.5-156.5 °C; IR (KBr) 3073, 2866, 1454, 1351, 1168, 1038, 764 cm\textsuperscript{-1}; \textsuperscript{1}H-NMR (CD\textsubscript{2}Cl\textsubscript{2}, 300 MHz) \delta 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); \textsuperscript{13}C-NMR (CD\textsubscript{2}Cl\textsubscript{2}, 75 MHz) \delta 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+) \textit{m/z} calculated for C\textsubscript{17}H\textsubscript{14}N\textsubscript{2}O\textsubscript{2}S\textsubscript{2}Na (M+Na) 365.0394, found 365.0358.

\textit{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 °C for 3 h. The same workup procedure was performed except that the product was isolated by chromatography on SiO\textsubscript{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; ¹H-NMR (CD₂Cl₂, 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); ¹³C-NMR (CDCl₃, 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 C₂₀H₁₆N₂O₂S₂Na (M+Na) 403.0551, found 403.0520.

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 Et₂Zn 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 NH₄Cl solution (5.0 mL). After stirring for 10 min, the quenched mixture was partitioned between a saturated NH₄Cl 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 (Na₂SO₄) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO₂ (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; ¹H-NMR (CD₂Cl₂, 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); ¹³C-NMR (CD₂Cl₂, 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 C₁₂H₁₅N₃O₂S₂Na (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 Et₂Zn 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 Et₂Zn 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 Et₂Zn in toluene (0.20 mL, 0.20 mmol, 1.0 M) were reacted in toluene (2.20 mL) at -78 °C for 33 h. The product 160 (28.6 mg, 61%) was isolated as a white crystalline solid.
**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 Et₂Zn 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 SiO₂ (3:1, hexanes: EtOAc). The product was recrystallized from a 5:1 mixture of hexanes and CH₂Cl₂ to afford 161 (39.6 mg, 40%) as a white crystalline solid: Mp 126.5-127.5 °C; IR (KBr) 3171, 2967, 2873, 1456, 1357, 1163, 1037, 766 cm⁻¹; ¹H-NMR (CDCl₃, 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); ¹³C-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 C₁₆H₁₆N₂O₂S₂Na (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 Et₂Zn 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 SiO₂ (3:1, hexanes: EtOAc). The product was recrystallized from a 5:1 mixture of hexanes and CH₂Cl₂ to afford 161 (57.8 mg, 58%) as a white crystalline solid.
Preparation of zirconocene hydrochloride from LiAlH₄ (120). ⁵⁸  To a solution of Cp₂ZrCl₂ (5.00g, 17.1 mmol) in THF (45.0 mL) under a nitrogen atmosphere was added a solution of LiAlH₄ 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), CH₂Cl₂ (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.

General procedure M. Addition of alkenylzinc reagents. (E)-5-Methyl-N-(1-phenylept-2-enyl)-1,3,4-thiadiazole-2-sulfonamide (163, Table 6 entry 1).  To a suspension of Cp₂Zr(H)Cl (163.8 mg, 0.636 mmol) in CH₂Cl₂ (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 °C and a Me₂Zn 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 NH₄Cl solution (5.0 mL). After stirring for 1 h, the quenched mixture was partitioned between a saturated NH₄Cl 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$_2$SO$_4$) 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$_2$Cl$_2$, 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 °C; IR (KBr) 3106, 2956, 2927, 2871, 1453, 1354, 1172, 1085, 979 cm$^{-1}$; $^1$H-NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 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$_2$Cl$_2$, 75 MHz) $\delta$ 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$_3$O$_2$S$_2$Na (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$_2$Zr(H)Cl (163.8 mg, 0.636 mmol), 1-hexyne (0.0820 mL, 0.720 mmol), Me$_2$Zn (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$_2$Cl$_2$ (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.
(E)-N-(1-Phenylhept-2-enyl)benzo[d]thiazole-2-sulfonamide (164, Table 6 entry 3). According to general procedure M, Cp₂Zr(H)Cl (123.0 mg, 0.477 mmol), 1-hexyne (0.0620 mL, 0.540 mmol), Me₂Zn (0.225 mL, 0.450 mmol, 2.0 M), and 146 (90.7 mg, 0.300 mmol) were combined in CH₂Cl₂ (1.50 mL) for 4.5 h. The same workup procedure was performed except that the crude reaction mixture was chromatographed on SiO₂ (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 °C; IR (KBr) 3131, 2923, 2872, 1452, 1351, 1168, 1036, 986, 756 cm⁻¹; ¹H-NMR (CDCl₃, 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); ¹³C-NMR (CDCl₃, 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 C₂₀H₂₂N₂O₂S₂Na (M+Na) 409.1020, found 409.1022.

(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, Cp₂Zr(H)Cl (81.9 mg, 0.138 mmol), 1-hexyne
(0.0410 mL, 0.360 mmol), Me₂Zn (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 CH₂Cl₂ (1.00 mL) for 2 h. The same workup procedure was performed except that the crude reaction mixture was chromatographed on SiO₂ (1:1 hexanes: EtOAc) to give 165 (25.1 mg, 47%) as a white solid: Mp 98.5-99.5 °C; IR (KBr) 3122, 2965, 2872, 1455, 1357, 1170, 1043, 940 cm⁻¹; ¹H-NMR (CDCl₃, 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); ¹³C-NMR (CDCl₃, 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 C₁₆H₂₁N₃O₂S₂Na (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 Cp₂Zr(H)Cl (87.7 mg, 0.340 mmol) in CH₂Cl₂ (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 °C and a solution of Me₃Al 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 NH₄Cl 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 SiO₂ which was washed with additional EtOAc (20.0 mL), dried (Na₂SO₄) and
concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO₂ (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 Cp₂Zr(H)Cl (87.7 mg, 0.340 mmol) in CH₂Cl₂ (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 (1S,4S,8S)-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 NH₄Cl 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 SiO₂ which was washed with additional EtOAc (25.0 mL), dried (Na₂SO₄) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO₂ (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.

\((E)-N-(1-\text{Phenylhept-2-}}\text{-enyl)}\text{benzo[}\text{d}\text{]}\text{thiazole-2-}}\text{sulfonamide (164, Table 7 entry 2).}\) According to general procedure N, a solution of Cp2Zr(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.

\((E)-N-(1-\text{Phenylhept-2-}}\text{-enyl)}\text{benzo[}\text{d}\text{]}\text{thiazole-2-}}\text{sulfonamide (164, Table 7 entry 2).}\) According to general procedure N, a solution of Cp2Zr(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).** To a suspension of Cu2O (0.750 g, 5.24 mmol) in acetonitrile (14.0 mL) was slowly added a solution of HBF4 (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 °C 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(CH₃CN)₄BF₄ (1.24 g, 75%) was isolated as a clear crystalline solid: Mp 160-162 °C.

**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 Cp₂ZrCl₂ (49.7 mg, 0.170 mmol) in THF (0.50 mL) cooled to 0 °C 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 °C, a white Cp₂Zr(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(CH₃CN)₄BF₄ (2.40 mg, 0.00750 mmol) in THF (0.50 mL) at 0 °C 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 NH₄Cl 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 SiO₂ which was washed with additional EtOAc (25.0 mL), dried (Na₂SO₄) and concentrated by rotary evaporation. The resulting crude residue was chromatographed on SiO₂ (1:3 EtOAc: hexanes) to give 164 (1.5 mg, 3%) as an impure clear oil.
N-(3-Methyl-1-phenylbutyl)benzo[d]thiazole-2-sulfonamide (170, Table 8 entry 2).

According to general procedure O, Cp$_2$ZrCl$_2$ (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$_3$CN)$_4$BF$_4$ (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 °C; IR (KBr) 3182, 2957, 1455, 1426, 1356, 1274, 1273, 1271, 1266 (2), 1249, 1219, 57.7, 46.5, 24.6, 22.3, 22.2; HRMS (TOF MS ES+) m/z calculated for C$_{18}$H$_{20}$N$_2$O$_2$S$_2$Na (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$_2$ZrCl$_2$ (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

**General procedure P. Deprotection of sulfonamides with H$_3$PO$_2$ and re-protection with Boc$_2$O. Tert-butyl 1-phenylethylcarbamate (171, Table 9 entry 1).**

To a solution of sulfonamide 148 (200 mg, 0.706 mmol) in refluxing THF (9.0 mL) was slowly added a H$_3$PO$_2$ 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$_3$PO$_2$ 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 °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$_2$SO$_4$) and concentrated by rotary evaporation. The resulting clear oil was then dissolved in CH$_3$CN (3.0 mL) and reacted with Boc$_2$O (192 mg, 0.883 mmol) in the presence of Et$_3$N (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$_2$O followed by 5:1 hexanes: EtOAc) to give 171 (132 mg, 84%) as a clear crystalline solid: Mp 87-88 °C; IR (KBr) 3383, 2983, 1687, 1519, 1367, 1248, 1171, 1059, 756 cm$^{-1}$; $^1$H-NMR (CD$_2$Cl$_2$, 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$_2$Cl$_2$, 75 MHz) $\delta$ 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$_2$Na (M+Na) 244.1313, found 244.1309.

Tert-butyl benzhydrylcarbamate (172, Table 9 entry 2). According to general procedure P, sulfonamide 154 (50.0 mg, 0.145 mmol) and H$_3$PO$_2$ (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$_2$O (39.5 mg, 0.181 mmol) and Et$_3$N (0.020 mL, 0.145 mmol) in CH$_3$CN (1.5 mL) for 1 h to give 172 (34.2 mg, 83%) as a clear crystalline solid: Mp 123-124 °C; IR (KBr) 3373, 2978, 1690, 1520, 1363, 1173, 1022, 744 cm$^{-1}$; $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 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) $\delta$ 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$_2$Na (M+Na) 306.1470, found 306.1468.

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$_3$PO$_2$ (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$_2$O
(59.7 mg, 0.274 mmol) and Et$_3$N (0.0318 mL, 0.228 mmol) in CH$_3$CN (3.0 mL) for 1 h to give 
173 (49.5 mg, 88%) as a white crystalline solid: Mp 90-91 °C; IR (KBr) 3318, 2978, 1713, 1682,
1523, 1365, 1157, 1021, 880 cm$^{-1}$; $^1$H-NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 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$_2$Cl$_2$, 75 MHz) $\delta$ 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$_2$Na (M+Na) 268.1313, found 268.1302.

General procedure Q. Deprotection of sulfonamides with SmI$_2$ and re-protection with 
Boc$_2$O. 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 °C). 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 °C 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$_2$SO$_4$) and concentrated by rotary evaporation (13 
°C). The crude amine was then dissolved in CH$_3$CN (1.5 mL) and reacted with Boc$_2$O (96.3 mg, 
0.353 mmol) in the presence of Et$_3$N (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$_2$O (85.7 mg, 0.393 mmol) and Et$_3$N (0.0438 mL, 0.314 mmol) in CH$_3$CN (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.

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$_2$Cl$_2$ (20 mL) cooled to 0 °C 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$_2$Cl$_2$ (50 mL) and the organic layer was washed with a saturated brine solution (40 mL), dried (Na$_2$SO$_4$) 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$^{-1}$; $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 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) \( \delta \) 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\(_9\)H\(_9\)NO\(_2\)S\(_2\) 227.0075, found 227.0079.

**Ethyl pyridine-2-sulfinate (176).** To a solution of 176 (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 × 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) \( \delta \) 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) \( \delta \) 163.8, 150.0, 137.8, 126.2, 119.8, 62.6, 15.6.

**Benzo[d]thiazole-2-sulfinamide (177).** To a solution of 175 (250 mg, 1.10 mmol) in THF (5.0 mL) cooled to -78 °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 °C for 2 h, and quenched by pouring it at -78 °C into an Erlenmeyer flask containing a saturated NH₄Cl 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 (Na₂SO₄), concentrated to 20 mL by rotary evaporation and filtered through a 1 in plug of SiO₂ 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 °C (dec); IR (KBr) 3310, 3174, 3083, 1474, 1426, 1040, 892, 761 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₇H₆N₂O₂S₂ 197.9922, found 197.9919.

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 °C. The reaction mixture turned a dark brown/red color during the addition, was stirred at -65 °C for 1 h and was quenched by addition to a saturated NH₄Cl 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$_2$SO$_4$) 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 °C (dec.); IR (KBr) 3226, 3075, 2699, 1580, 1451, 1426, 1086, 1056, 877 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 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) $\delta$ 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$_2$Cl$_2$ (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$_2$Cl$_2$ (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 °C; IR (KBr) 3055, 1604, 1569, 1312, 1097, 994, 760 cm$^{-1}$; $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 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) $\delta$ 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$_2$OS$_2$ 286.0235, found 286.0242.

\[ \text{(E)-N-benzylidenepyridine-2-sulfonamide (180)} \]

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 °C (dec.); IR (KBr) 3057, 1605, 1573, 1448, 1423, 1214, 1098, 1070, 756 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 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) $\delta$ 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$_2$OS 230.0514, found 230.0503.
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 \(\text{NH}_4\text{Cl}\) 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 (\(\text{Na}_2\text{SO}_4\)), 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: \(^1\text{H-NMR}\) (CDCl\(_3\), 300 MHz) \(\delta\) 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 \(^1\text{H-NMR}\) (CDCl\(_3\)) of fraction B showed 182 (4.2 mg, 24%) as clear oil.

\((E)-N\)-Benzyldenemethanesulfinamide (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\(_2\)Cl\(_2\) (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) $\delta$ 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) $\delta$ 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$_8$H$_9$NOS 167.0405, found 167.0400.

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$_2$Zn (0.200 mL, 0.200 mmol, 1.0 M) in DCM at 0 °C. 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$_2$SO$_4$) 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) $\delta$ 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
\(^{13}\)C NMR (CDCl\(_3\), 75 MHz) \(\delta\) 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\(_2\)OSNa 283.0881, found 283.0901.

\(\text{N-(1-phenylpropyl)pyridine-2-sulfinamide (183, Table 11 entry 1).}\) According to general procedure S, 180 (23.0 mg, 0.100 mmol) and Et\(_2\)Zn 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).

\(\text{N-(1-phenylpropyl)pyridine-2-sulfinamide (183, Table 11 entry 2).}\) According to general procedure S, 180 (23.0 mg, 0.100 mmol), Cu(OTf)\(_2\) (3.6 mg, 0.010 mmol) and Et\(_2\)Zn 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).

\(\text{N-(1-phenylpropyl)pyridine-2-sulfinamide (183, Table 11 entry 4).}\) According to general procedure S, 180 (23.0 mg, 0.100 mmol), Cu(OTf)\(_2\) (3.6 mg, 0.010 mmol) and Et\(_2\)Zn in THF (0.200 mL, 0.200 mmol, 1.0 M) were reacted in THF (1.0 mL) at 0 °C for 15 h to afford a trace amount of 183 by TLC (SiO\(_2\), 3:1 EtOAc:hexanes).
**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$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz, 3.5:1 mixture of diastereoisomers) $\delta$ 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$_2$OSNa 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 $^\circ$C 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).
General Procedure T. N-(1-phenylbut-2-ynyl)pyridine-2-sulfinamide (185, Table 11 entry 9). To a solution of ZnBr₂ (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 (CH₃CN)₄CuBF₄ (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 NaHCO₃ 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 (Na₂SO₄), condensed in vacuo and dissolved in a minimum amount of DCM for chromatography on SiO₂ (2:1 EtOAc: hexanes; Rf = 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 (CDCl₃, 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); ¹³C NMR (CDCl₃, 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$_{2}$OSNa 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 °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 °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$_{3}$CN)$_{4}$CuBF$_{4}$ (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

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 Et3N (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 °C; IR (KBr) 3214, 2986, 2808, 1744, 1705, 1637, 1603, 1495, 1417, 1280, 1111, 853 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 (El) m/z 319 (M⁺, 34), 165
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 °C 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 °C; IR (ATR) 3245, 3038, 2855, 1718, 1671, 1592, 1528, 1414, 1254, 1174, 798 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₂H₉N₃O₆ 291.049135, found 291.048553.

Benzyl 6-(methylthio)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (216, DMA-NB74-6). To a suspension of K₂CO₃ (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,
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 °C; IR (ATR) 3453, 3030, 1718, 1650, 1411, 1273, 1130 cm⁻¹;¹H-NMR (DMSO-d₆, 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);¹³C-NMR (DMSO-d₆, 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 C₁₃H₁₂N₂O₄SNa (M+Na) 315.0415 found 315.0414.

Ethyl 6-(methylthio)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (217, DMA-NB74-7). To a suspension of K₂CO₃ (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 °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; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₈H₁₀N₂O₄S 230.036129, found 230.036411.

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 K₂CO₃ (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; ¹H-NMR (DMSO-d₆, 300 MHz) δ 11.29 (s, 1 H), 10.90 (s, 1 H), 2.56 (s, 3 H), 1.45 (s, 9 H); ¹³C-NMR (DMSO-d₆, 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 C₁₀H₁₄N₂O₄SNa 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; ¹H-NMR (DMSO-d₆, 300 MHz) δ 13.85 (s, 1 H), 12.18 (s, 1 H), 10.90 (s, 1 H), 2.58 (s, 3 H); ¹³C-NMR (DMSO-d₆, 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 C₆H₆N₂O₄S 202.004829, found 202.004120.

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 CH₂Cl₂ (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 CH₂Cl₂ (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; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₃H₁₂N₂O₅SNa (M+Na) 331.0365, found 331.0388.
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 CH₂Cl₂ (13 mL) at room temperature for 1.5 h. The product was precipitated from the reaction mixture at 0 °C with hexanes (10 mL) and the resulting solid was isolated by vacuum filtration, washed with a 1:1 mixture of CH₂Cl₂:hexanes (10 mL) and dried in vacuo to afford 221 as a clear crystalline solid (115 mg, 72%): Mp 239-240 °C; IR (ATR) 3174, 3055, 2831, 1735, 1705, 1674, 1592, 1400, 1314, 1120, 1006 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₈H₁₀N₂O₅S 246.031043, found 246.031121.

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 °C 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 °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 °C (dec.); IR (ATR) 3381, 3230, 3038, 1718, 1696, 1642, 1528, 1299, 1118 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₂₁H₁₉N₃O₆ 409.127386, found 409.127861.

![Chemical Structure](image)

**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 °C for 5 h. The resulting product **206** was isolated as a light white solid (32.1 mg, 50%): Mp >380 °C; IR (ATR) 3217, 3172, 1743, 1705, 1666, 1634, 1575, 1420, 1301, 1204, 788 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-
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 °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 °C (dec.); IR (ATR) 3161, 2991, 2823, 1722, 1681, 1625, 1575, 1390, 1286, 1128 cm$^{-1}$; $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 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) $\delta$ 169.5, 168.5, 165.7, 157.0, 148.8, 139.9, 131.0 (2 C), 128.6, 125.8 (2 C), 128.4, 124.5 (2 C), 112.8, 110.8, 106.8, 104.8, 102.8, 100.8, 98.8, 96.8, 94.8, 92.8, 90.8, 88.8, 86.8, 84.8, 82.8, 80.8, 78.8, 76.8, 74.8, 72.8, 70.8, 68.8, 66.8, 64.8, 62.8, 60.8, 58.8, 56.8, 54.8, 52.8, 50.8, 48.8, 46.8, 44.8, 42.8, 40.8, 38.8, 36.8, 34.8, 32.8, 30.8, 28.8, 26.8, 24.8, 22.8, 20.8, 18.8, 16.8, 14.8, 12.8, 10.8, 8.8, 6.8, 4.8, 2.8, 0.8; 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$_3$O$_6$ 319.080435, found 319.080744.
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 C₁₄H₁₃N₃O₆ 319.080435, found 319.08111.

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 Cs₂CO₃ (3.92 g, 12.0 mmol). The reaction mixture was stirred at room temperature for 30 min, concentrated by rotary evaporation (40 °C) and dried in vacuo. The resulting cesium salt was suspended in a 3:1 solution of CH₃CN (30 mL): DMF (10 mL) and a solution of benzyl bromide (2.73 mL, 23.0 mmol) in CH₃CN (10 mL) was added dropwise over 2 h. The reaction mixture was stirred at room temperature for 17 h, concentrated by rotary evaporation (40 °C) and the resulting crude yellow oil was dissolved in EtOAc (150 mL). The EtOAc solution was washed with a saturated NaHCO₃ solution (1 X 50 mL), followed by a saturated brine solution (1 X 50 mL). The EtOAc layer was dried with Na₂SO₄, concentrated by rotary evaporation (40 °C) 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 °C; IR (ATR) 3453, 3354, 3222, 1681, 1631, 1597, 1277, 1169, 1114, 1075 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₄H₁₃NO₂ 227.094629, found 227.094483.
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 °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 °C (dec.); IR (ATR) 3086, 3014, 1716, 1683, 1485, 1407, 1260, 1127 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₃H₁₃N₃O₄ 275.090606, found 275.090923.
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 °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 °C (dec.); IR (ATR) 3194, 3086, 2932, 1752, 1711, 1592, 1560, 1267, 1101, 751 cm⁻¹; ¹H-NMR (DMSO-d₆, 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), 7.27 (d, 2 H, J = 8.4 Hz), 5.32 (s, 2 H), 4.98 (s, 1 H); ¹³C-NMR (DMSO-d₆, 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 C₁₈H₁₅N₃O₄ 337.106256, found 337.105811.
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 °C for 2 h to afford 229 as a light yellow solid (352 mg, 71%, ~90% pure by ¹H-NMR). A 50.0 mg sample was further purified by recrystallization from a 3:1 solution of deionized water:DMSO initially heated to 90 °C then cooled to rt. The recrystallized solid was isolated by vacuum filtration and heated in distilled water for 30 min at 90 °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 °C; IR (KBr) 3354, 3012, 1719, 1600, 1398, 1276 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 (El) m/z 247 (M⁺, 53), 155 (64), 120 (40), 84 (77), 68 (100); HRMS (El) m/z calculated for C₁₁H₉N₃O₄ 247.059306, found 247.057526.
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$_3$N (32.1 µL, 0.228 mmol) in DMF (1.0 mL) was added diethyl pyrocarbonate (33.7 µL, 0.228 mmol) at 0 °C. 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$_3$N) and the product was flushed through with a 99.9:0.1 solution of EtOAc:Et$_3$N (85 mL). The filtrate was concentrated by rotary evaporation (30 °C) and the resulting orange residue was diluted with EtOAc (1.0 mL) followed by hexanes (30 mL) with cooling to 0 °C 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 °C) and the resulting pale yellow oil was diluted with EtOAc (1.0 mL) followed by hexanes (30 mL) at 0 °C. 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 °C; 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 C₂₁H₁₉N₃O₆ 409.127386, found 409.127299.

![Chemical structure of 208](image)

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 H₂ 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 °C. 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-d₆, 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 C₁₄H₁₃N₃O₆ 319.080435, found 319.081124.
Ethyl 4-ureidobenzoate (231, DMA-NB74-59). 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 °C for 14 h, cooled to room temperature and concentrated by rotary evaporation (80 °C). The resulting clear oil was crystallized by stirring in deionized water (60 mL) at 0 °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 °C; IR (ATR) 3405, 3358, 3181, 1705, 1675, 1586, 1528, 1286, 1174, 1019 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₀H₁₂N₂O₃Na (M+Na) 231.0746, found 231.0762.
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 Ac₂O (0.50 mL) was heated in a microwave reactor for 30 min at 80 °C. 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 °C; IR (ATR) 3248, 2987, 1713, 1694, 1685, 1593, 1507, 1273, 1174, 701 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₃H₁₃N₃O₄ 275.090606, found 275.090412.

Ethyl 4-(6-amino-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-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 NaHCO₃ 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; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₃H₁₁N₃O₄ 275.090606, found 275.090591.

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; ¹H-NMR (DMSO-d₆, 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) \(\delta\) 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\(_9\)N\(_3\)O\(_4\) 247.059306, found 247.059011.

Benzyl 4-(6-amino-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)benzoate (235, DMA-NB74-69). To a suspension of 234 (150 mg, 0.607 mmol) in MeOH (6.0 mL) was added Cs\(_2\)CO\(_3\) (108.6 mg, 0.333 mmol). The suspension was stirred at room temperature for 30 min, concentrated by rotary evaporation (40 °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 °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 °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) \(\delta\) 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) \(\delta\) 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) \textit{m/z} 337 (M+, 24), 187 (13), 146 (19), 91 (100), 65 (18); HRMS (EI) \textit{m/z} calculated for C_{18}H_{15}N_{3}O_{4} 337.106256, found 337.107044.

\[
\text{Benzyl 4-(6-(ethoxycarbonylamino)-2,4-dixo-3,4-dihydropyrimidin-1(2H)-yl)benzoate (236).} \quad \text{To a suspension of 235 (55.0 mg, 0.163 mmol) in dry THF (0.50 mL) was added a solution of LiHMDS (42.2 mg, 0.245 mmol) in THF (0.75 mL) at 0 \degree C. The reaction mixture was stirred at 0 \degree C for 30 min upon which time the suspension cleared and ethyl chloroformate (17.1 \mu L, 0.179 mmol) was added. The reaction mixture was slowly warmed to room temperature and stirred for 5 h. The product was isolated by column chromatography on SiO}_{2} (EtOAc; R_f = 0.63) and recrystallized from a 1:5 mixture of EtOAc:hexanes (15 mL) to afford 236 as an off white solid (13.1 mg, 20%, ~80% pure by 1H-NMR). This product was used crude for the next step: }^{1} \text{H-NMR (DMSO-d}_6, 300 MHz) \delta 11.43 (s, 1 H), 9.06 (s, 1 H), 8.04 (d, 2 H, } J = 8.1 \text{ Hz), 7.48-7.34 (m, 7 H), 5.88 (s, 1 H), 5.38 (s, 2 H), 3.81 (q, 2 H, } J = 6.9 \text{ Hz), 1.05 (t, 3 H, } J = 6.9 \text{ Hz).} \]
4-(6-(Ethoxycarbonylamino)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-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 °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 °C (dec.); IR (ATR) 3399, 3214, 2987, 2791, 1755, 1705, 1702, 1639, 1610, 1522, 1477, 1204, 1036 cm$^{-1}$; $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 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) $\delta$ 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$_3$O$_6$ 319.080435, found 319.079582.
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 °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 °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 °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) $\delta$ 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) $\delta$ 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.
Ethyl 4-(2,4,6-trioxotetrahydropyrimidin-1(2H)-yl)benzoate (238, DMA-NB74-61). 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 °C. The reaction mixture was stirred at 0 °C 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 °C; IR (ATR) 3252, 3086, 2987, 1702, 1687, 1411, 1275, 1100 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₃H₁₂N₂O₅ 276.074622, found 276.074522.

4-(2,4,6-Trioxotetrahydropyrimidin-1(2H)-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 °C (dec.); IR (ATR) 3390, 3219, 3086, 1716, 1690, 1685, 1606, 1351, 1204, 1114 cm⁻¹; ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₁H₈N₂O₅ 248.043322, found 248.042989.

Ethyl 4-(5-(ethylcarbamoyl)-6-hydroxy-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)benzoate (240, DMA-NB74-96). To a solution of 238 (60.0 mg, 0.217 mmol) in acetonitrile (1.5 mL) was added Et₃N (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 °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 °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 °C; IR (ATR) 3209, 3086, 3002, 2829, 1718, 1709, 1685, 1592, 1446, 1282, 1096 cm⁻¹; ¹H-NMR (DMSO-d₆, 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) $\delta$ 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$_3$O$_6$ 347.111736, found 347.110260.

![Chemical Structure](image)

**4-(5-(Ethylcarbamoyl)-6-hydroxy-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-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 °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) $\delta$ 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) $\delta$ 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$_3$O$_6$ 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.

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 °C; IR (ATR) 3440, 3308, 3202, 1705, 1655, 1580, 1530, 1265, 1109 cm⁻¹; $^1$H NMR (DMSO-d₆, 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-d₆, 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 C₁₅H₁₄N₂O₃ 270.1004, found 270.1008.
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 °C, a solution of LiHMDS (418 mg, 2.42 mmol, 0.48 M) in THF, dropwise over 5 min. The mixture was stirred at -78 °C for 30 min and then ethyl cyanoformate (0.219 mL, 2.24 mmol) was added. The reaction mixture was slowly warmed to 0 °C over 5 h and was quenched by the addition of a saturated NH₄Cl solution (10 mL) with stirring for 20 min. The mixture was diluted with EtOAc (50 mL) and washed with a saturated NaHCO₃ solution (1 X 50 mL). The EtOAc layer was dried (Na₂SO₄), concentrated in vacuo and the yellow residue was chromatographed on SiO₂ (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 °C; IR (ATR) 3239, 3133, 2969, 1726, 1694, 1593, 1549, 1505, 1273, 1241, 1090 cm⁻¹; ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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 C₁₈H₁₈N₂O₅ 342.1216, found 342.1202.
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 SiO₂, washed through the plug with excess EtOAc (250 mL), dried (Na₂SO₄) 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; ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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 C₁₈H₁₄N₂O₅ 338.0903, found 338.0902.
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 Et₃N (68.5 µL, 0.488 mmol) at 0 °C. The reaction mixture turned heterogeneous and dark orange upon the addition of Et₃N, 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 (Na₂SO₄), vacuum filtered through a 1 in plug of SiO₂, 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; ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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_{2}O_{7} 410.1114, found 410.1113.

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 trituated with a 2:1 solution of hexanes:EtOAc (25 mL), isolated by vacuum filtration, washed with hexanes (10 mL), trituated 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$^{-1}$; $^1$H NMR (DMSO-d$_6$, 300 MHz) $\delta$ 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) $\delta$ 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$_2$O$_7$ 320.0645, found 320.0650.
Ethyl 2-\(N\)-(4-\(N\)-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) \(\delta\) 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) \(\delta\) 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\(_2\)O\(_6\) 384.1321, found 384.1323.
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$_3$N (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$_2$SO$_4$) 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$^{-1}$; $^1$H NMR (DMSO-d$_6$, 300 MHz) $\delta$ 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) $\delta$ 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$_2$O$_7$ 410.1114, found 410.1100.
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$_2$O$_7$ 320.0645, found 320.0633.
**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 SiO2, 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 (CDCl₃, 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); ¹³C NMR (CDCl₃, 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 C₁₉H₁₈N₂O₆ 370.1165, found 370.1174.
Sodium 4-methylbenzenesulphonothioate (296). 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 °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%): ¹H-NMR (CDCl₃, 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); ¹³C-NMR (CDCl₃, 75 MHz) δ 152.9, 138.9, 128.6 (2 C), 124.5 (2 C), 21.3.

S,S'-propane-1,3-diyl bis(4-methylbenzenesulphonothioate) (254). 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 (Na₂SO₄) for 30 min and concentrated by rotary evaporation (40 °C). The resulting clear oil was dissolved in DCM (2.0 mL) for chromatography on SiO₂ (gradient elution 1:1 hexanes:DCM -> DCM). The isolated product fractions were combined, concentrated by rotary evaporation (40 °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) \(\delta\) 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) \(\delta\) 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).

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\(_3\)N (423 \(\mu\)L, 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\(_2\)SO\(_4\)) and concentrated in vacuo. The resulting yellow oil was dissolved in CH\(_2\)Cl\(_2\) (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) \(\delta\) 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) \(\delta\) 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\(_2\)O\(_6\)S\(_2\) 474.0919, found 474.0908.

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\(_2\)CO\(_3\) (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\(_2\)SO\(_4\)) 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) \( \delta \) 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); \(^1^3^C\)-NMR (CDCl\(_3\), 75 MHz) \( \delta \) 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\(_2\)O\(_3\)S\(_2\) 442.065716, found 442.063885.
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 Et₃N (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 SiO₂ 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 (CDCl₃, 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); ¹³C-NMR (CDCl₃, 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 C₂₃H₂₂N₂O₅S₂ 470.0970, found 470.0973.
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$_4$Cl 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$_2$SO$_4$) 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$^{-1}$; $^1$H 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$_2$O$_5$ 366.1216, found 366.1199.
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 (Na₂SO₄) 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 (CDCl₃, 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); ¹³C-NMR (CDCl₃, 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 C₂₄H₂₂N₂O₇S₂ 514.0868, found 514.0852.
Ethyl 2,4,6-trioxo-3-\{4-\text{[benzyloxy carbonyl]}phenyl\}-1,3,5-trihydropyrimidine carboxylate (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 NH₄Cl 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 SiO₂ (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 (Na₂SO₄) and concentrated by rotary evaporation (25 °C). The resulting clear oil was re-filtered through a plug of SiO₂ 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 CDCl₃ 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) \(\delta\) 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); \(^1^3\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 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\(_2\)O\(_7\) 410.1114, found 410.1112.

\[
\text{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) $\delta$ 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) $\delta$ 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$_2$O$_7$ 320.0645, found 320.0638.

4.4.3 Synthesis and identification of the structure of SID 861574

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$_2$SO$_4$) 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) $\delta$ 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) $\delta$ 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.
Diethyl (2Z)-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 (Na₂SO₄) 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; ¹H NMR (DMSO-d₆, 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$_8$Na 478.1478, found 478.1448.

Ethyl 1-(4-(benzylxycarbonyl)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$_2$SO$_4$) 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 1H NMR): Mp 180.5-182.0 °C; IR (ATR) 3082, 2982, 2693, 2441, 1718, 1636, 1586, 1485, 1334, 1265, 1236, 1103, 1003 cm⁻¹; 1H NMR (DMSO-d₆, 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); 13C-NMR (DMSO-d₆, 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 C₂₂H₁₉NO₇Na 432.1059, found 432.1064.

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-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 C₁₅H₁₃NO₇Na 342.0590, found 342.0583.

4.4.4 Synthesis of SID 861574 decomposition products and other related analogues

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 SiO₂ with EtOAc (75 mL). The cloudy white filtrate was concentrated by rotary evaporation (60 °C) and the oily residue was chromatographed on SiO₂ (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 (CDCl₃, 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$_5$Na 249.0739, found 249.0749.

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](https://example.com/265.png)

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$_2$SO$_4$), 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; ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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 C₁₂H₉NO₅ 247.0481, found 247.0483; LC/MS/UV data: Phenomenex C₁₈ (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 IC₅₀ > 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(PPh₃)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 °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 (Na₂SO₄) and concentrated by rotary evaporation (25 °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 IC₅₀ = 2.079 µM against PLK1-PBD in an initial testing and an IC₅₀ = 6.047 µM in a follow-up testing.

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 °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 °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$_2$SO$_4$) and concentrated by rotary evaporation (30 °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 °C. The resulting light tan solid was isolated by vacuum filtration, triturated with MeOH (5 mL) at 45 °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 °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) $\delta$ 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) $\delta$ 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]$^+$.

![Structure](271.png)

4-[2-(Ethoxycarbonyl)acetylamino]benzoic acid (271, DMA-NB245-90).\textsuperscript{113} To a solution of ethyl malonylchioride (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$_2$SO$_4$), concentrated by rotary evaporation (40 °C) 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%): $^1$H NMR (DMSO-d$_6$, 300 MHz) $\delta$ 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) $\delta$ 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$_5$Na (M+Na) 274.0691, found 274.0692.

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 SiO₂ plug was found by ¹H 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): ¹H NMR (DMSO-d₆, 300 MHz) δ 12.53 (br-s, 2 H), 10.41 (s, 1 H), 8.94 (d, 2 H, J = 8.7 Hz), 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 SiO₂ (EtOAc). The product (R_f = 0.66; SiO₂; EtOAc) fractions were combined and concentrated by rotary evaporation (40 °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 °C (dec); IR (ATR) 3334, 3208, 2975, 2667, 2548, 1735, 1683, 1597, 1534, 1409, 1308, 1258, 1169, 1025 cm⁻¹; keto-tautomer (83%): ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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%): ¹H NMR (DMSO-d₆, 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%): ¹H NMR (DMSO-d₆,
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_{6}Na (M+Na) 316.0797, found 316.0810.

** 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 (Na₂SO₄) 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 \textbf{274a}: \textbf{274b}: \textbf{274c}: \textbf{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). \textbf{247a}: $^1$H NMR (DMSO-d$_{6}$, 300 MHz) $\delta$ 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. \textbf{247b}: LC/MS (ESI+) $m/z$ 360 [M+H]$^+$; LC/ELS retention time 12.99 min. \textbf{247c}: LC/MS (ESI+) $m/z$ 372 [M+H]$^+$; LC/ELS retention time 14.37 min. \textbf{247d}: LC/MS (ESI+) $m/z$ 348 [M+H]$^+$; LC/ELS retention time 11.53 min.

**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 °C and acidified slowly with a concentrated HCl solution just to pH 0.
The resulting heterogeneous solution was stirred gently at 0 °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 (Na₂SO₄)
and concentrated in vacuo. The crude reaction mixtures were then analyzed by ¹H NMR
(DMSO-d₆ 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(2H)-yl)benzoic acid (277). ¹H NMR
(DMSO-d₆, 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). ¹H NMR (MeOH-d₄, 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); ¹³C NMR (MeOH-d₄, 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.

2H-Pyran-2,4,6(3H,5H)-trione (282).¹⁰⁹,¹¹⁰ To a 1.4:1 mixture of AcOH:Ac₂O (25.8 mL)
cooled to 0 °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](image)

**Ethyl 4-(3-oxobutanamido)benzoate (284, DMA-NB245-72).** 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 °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 °C and hexanes (125 mL) was slowly added with stirring. The resulting turbid solution was cooled to -5 °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) $\delta$ 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%): $\delta$ 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).

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%): ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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%): ¹H NMR (DMSO-d₆, 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%): ¹H NMR (DMSO-d₆, 300 MHz) δ 5.18 (s, 1 H), 3.37 (s, 2 H); LC/MS/UV data: Phenomenex C₁₈ (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]⁺.

4-(3-Oxobutanoylamino)benzoic acid (285, DMA-NB245-77). 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 ¹H 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) $\delta$ 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%): $\delta$ 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.

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 °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 °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 °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) $\delta$ 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) $\delta$ 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) $\delta$ 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) $\delta$ 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) $\delta$ 11.81 (s, 1 H), 5.27 (s, 1 H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 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$_6$Na (M+Na) 330.0954, found 330.0953.

$\text{N-ethyl-4-(5-morpholino-3,5-dioxopentanamido)benzamide (288, DMA-NB245-82).}$ 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 °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 °C) and the resulting light orange oil was dissolved in MeOH (5.0 mL, to remove residual EtOAc), re-concentrated by rotary evaporation (40 °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$_2$O$_6$Na (M+Na) 385.1376, found 385.1368.

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 (Na₂SO₄) and concentrated by rotary evaporation (40 °C). The resulting yellow residue was triturated with MeOH (10 mL) at 40 °C for 30 min and the heterogeneous solution was cooled to -5 °C for 1 h. The precipitate was isolated by vacuum filtration, washed with MeOH (5 mL, cooled to 0 °C) 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 °C; 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-d₆, 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); ¹³C NMR (DMSO-d₆, 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-d₆, 300 MHz) δ 14.98 (s, 1 H), 5.62 (s, 1 H); ¹³C NMR (DMSO-d₆, 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-d₆, 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 C₁₆H₁₈N₂O₆Na (M+Na) 357.1063, found 357.1054; LC/MS/UV data: Phenomenex C₁₈ (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]⁺.
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 SiO₂ (1 in) with EtOAc (~300 mL/filtration). The cloudy filtrate was condensed by rotary evaporation (40 °C) 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 °C; IR (ATR) 3353, 3295, 3195, 3126, 2975, 1719, 1656, 1599, 1536, 1411, 1273, 1172, 1103 cm⁻¹; keto tautomer (72%): ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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-d₆, 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); ¹³C NMR (DMSO-d₆, 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_{2}O_{7}Na (M+Na) 463.1481, found 463.1440.

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 °C and acidified to pH 0 with concentrated HCl (10 mL). The resulting white oily suspension was stirred at 0 °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_{2}SO_{4}) and concentrated by rotary evaporation (40 °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). ^1H 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 °C; IR (ATR) 3303, 3070, 2831, 2667, 2561, 1663, 1594, 1530, 1405, 1316, 1292, 1172 cm⁻¹; keto tautomer (77%): ¹H NMR (DMSO-d₆, 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); ¹³C NMR (DMSO-d₆, 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%): ¹H NMR (DMSO-d₆, 300 MHz) δ 13.75 (br-s, 1 H), 10.53 (s, 1 H), 5.40 (s, 1 H), 3.39 (s, 2 H); ¹³C NMR (DMSO-d₆, 100 MHz) δ 170.8, 170.5, 166.2, 118.8, 93.3, 43.5.

\[ \text{HN} \quad \text{O} \quad \text{O} \quad \text{NH} \quad \text{EtO} \quad \text{O} \quad \text{O} \quad \text{Et} \]

\(N₁,N₃\)-Bis(4-(carbethoxy)phenyl)malonamide (292, DMA-NB245-80). To a solution of ethyl 4-aminobenzoate (882 mg, 5.24 mmol) in THF (15 mL) cooled to 0 °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 °C for 5 min, K₂CO₃ (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 (Na₂SO₄) and concentrated by rotary evaporation (40 °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 °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).

\[
\text{HN} \quad \text{O} \\
\text{O} \\
\text{NH} \\
\text{HO} \quad \text{O} \\
\text{O} \\
\text{OH}
\]

$N_1,N_3$-Bis(4-benzoic acid)malonamide (293, DMA-NB245-85). 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 °C and acidified with concentrated HCl (10 mL, reaction pH = 0). After 1 h at 0 °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).
Diethyl 4,4'-carbonylbis(azanediyl)dibenzoate (294, DMA-NB245-86). 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) $\delta$ 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).

4,4'-Carbonylbis(azanediyl)dibenzoic acid (295, DMA-NB245-87). 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 °C and acidified with concentrated HCl to pH 0. The resulting turbid solution was cooled to
-5 °C for 18 h. The white precipitate was isolated by vacuum filtration, trituated with boiling
MeOH (200 mL) for 1 h and dried in vacuo to afford 295 ~7% solvated with dioxane (164 mg,
88%): 1H NMR (DMSO-d6, 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. LC/MS/UV data: Phenomenex C18 (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 °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.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification code</td>
<td>daw1016</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C15 H13 N O7</td>
</tr>
<tr>
<td>Formula weight</td>
<td>319.26</td>
</tr>
<tr>
<td>Temperature</td>
<td>203(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>C2/c</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 21.011(7) Å, b = 13.139(5) Å, c = 10.438(4) Å</td>
</tr>
<tr>
<td></td>
<td>a = 90°, b = 94.689(7)°, g = 90°</td>
</tr>
<tr>
<td>Volume</td>
<td>2871.9(17) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>8</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.477 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.119 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>1328</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.29 x 0.26 x 0.14 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>1.83 to 27.50°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-27&lt;=h&lt;=27, -17&lt;=k&lt;=17, -13&lt;=l&lt;=13</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>13676</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>3298 [R(int) = 0.0618]</td>
</tr>
<tr>
<td>Completeness to theta = 27.50°</td>
<td>100.0 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>None</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.9835 and 0.9663</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>3298 / 0 / 241</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.059</td>
</tr>
<tr>
<td>Final R indices [I&gt;2sigma(I)]</td>
<td>R1 = 0.0527, wR2 = 0.1376</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0750, wR2 = 0.1513</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.490 and -0.280 e.Å⁻³</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U(eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(1)</td>
<td>863(1)</td>
<td>-1216(2)</td>
<td>5421(3)</td>
<td>60(1)</td>
</tr>
<tr>
<td>N(1)</td>
<td>2641(1)</td>
<td>1769(1)</td>
<td>2492(1)</td>
<td>25(1)</td>
</tr>
<tr>
<td>O(1)</td>
<td>5056(1)</td>
<td>1058(1)</td>
<td>-711(2)</td>
<td>45(1)</td>
</tr>
<tr>
<td>O(2)</td>
<td>4266(1)</td>
<td>855(1)</td>
<td>-2255(2)</td>
<td>50(1)</td>
</tr>
<tr>
<td>C(2)</td>
<td>696(1)</td>
<td>-834(2)</td>
<td>4100(2)</td>
<td>49(1)</td>
</tr>
<tr>
<td>O(3)</td>
<td>1211(1)</td>
<td>-174(1)</td>
<td>3704(2)</td>
<td>46(1)</td>
</tr>
<tr>
<td>C(3)</td>
<td>1208(1)</td>
<td>786(2)</td>
<td>4081(2)</td>
<td>33(1)</td>
</tr>
<tr>
<td>O(4)</td>
<td>797(1)</td>
<td>1114(1)</td>
<td>4746(2)</td>
<td>46(1)</td>
</tr>
<tr>
<td>C(4)</td>
<td>1715(1)</td>
<td>1427(1)</td>
<td>3640(2)</td>
<td>27(1)</td>
</tr>
<tr>
<td>O(5)</td>
<td>1306(1)</td>
<td>2841(1)</td>
<td>4761(1)</td>
<td>36(1)</td>
</tr>
<tr>
<td>C(5)</td>
<td>1730(1)</td>
<td>2458(2)</td>
<td>4007(2)</td>
<td>27(1)</td>
</tr>
<tr>
<td>O(6)</td>
<td>3083(1)</td>
<td>3323(1)</td>
<td>2370(1)</td>
<td>34(1)</td>
</tr>
<tr>
<td>C(6)</td>
<td>2185(1)</td>
<td>3128(1)</td>
<td>3615(2)</td>
<td>28(1)</td>
</tr>
<tr>
<td>O(7)</td>
<td>2244(1)</td>
<td>182(1)</td>
<td>2421(1)</td>
<td>33(1)</td>
</tr>
<tr>
<td>C(7)</td>
<td>2633(1)</td>
<td>2773(1)</td>
<td>2851(2)</td>
<td>26(1)</td>
</tr>
<tr>
<td>C(8)</td>
<td>2187(1)</td>
<td>1054(1)</td>
<td>2850(2)</td>
<td>25(1)</td>
</tr>
<tr>
<td>C(9)</td>
<td>3108(1)</td>
<td>1442(1)</td>
<td>1620(2)</td>
<td>26(1)</td>
</tr>
<tr>
<td>C(10)</td>
<td>3748(1)</td>
<td>1436(2)</td>
<td>2036(2)</td>
<td>31(1)</td>
</tr>
<tr>
<td>C(11)</td>
<td>4195(1)</td>
<td>1251(2)</td>
<td>1158(2)</td>
<td>32(1)</td>
</tr>
<tr>
<td>C(12)</td>
<td>3993(1)</td>
<td>1054(1)</td>
<td>-116(2)</td>
<td>28(1)</td>
</tr>
<tr>
<td>C(13)</td>
<td>3344(1)</td>
<td>989(2)</td>
<td>-500(2)</td>
<td>32(1)</td>
</tr>
<tr>
<td>C(14)</td>
<td>2898(1)</td>
<td>1200(2)</td>
<td>367(2)</td>
<td>30(1)</td>
</tr>
<tr>
<td>C(15)</td>
<td>4452(1)</td>
<td>964(1)</td>
<td>-1127(2)</td>
<td>32(1)</td>
</tr>
</tbody>
</table>
Table 3. Bond lengths [Å] and angles [°] for daw1016.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(1)-C(2)</td>
<td>1.483(4)</td>
</tr>
<tr>
<td>C(1)-H(1A)</td>
<td>0.9700</td>
</tr>
<tr>
<td>C(1)-H(1B)</td>
<td>0.9700</td>
</tr>
<tr>
<td>C(1)-H(1C)</td>
<td>0.9700</td>
</tr>
<tr>
<td>N(1)-C(7)</td>
<td>1.372(2)</td>
</tr>
<tr>
<td>N(1)-C(8)</td>
<td>1.410(2)</td>
</tr>
<tr>
<td>N(1)-C(9)</td>
<td>1.458(2)</td>
</tr>
<tr>
<td>O(1)-C(15)</td>
<td>1.313(3)</td>
</tr>
<tr>
<td>O(1)-H(1O)</td>
<td>1.05(4)</td>
</tr>
<tr>
<td>O(2)-C(15)</td>
<td>1.218(3)</td>
</tr>
<tr>
<td>C(2)-O(3)</td>
<td>1.471(3)</td>
</tr>
<tr>
<td>C(2)-H(2A)</td>
<td>0.9800</td>
</tr>
<tr>
<td>C(2)-H(2B)</td>
<td>0.9800</td>
</tr>
<tr>
<td>O(3)-C(3)</td>
<td>1.321(2)</td>
</tr>
<tr>
<td>C(3)-O(4)</td>
<td>1.230(2)</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
<td>1.461(3)</td>
</tr>
<tr>
<td>C(4)-C(5)</td>
<td>1.407(3)</td>
</tr>
<tr>
<td>C(4)-C(8)</td>
<td>1.428(2)</td>
</tr>
<tr>
<td>O(5)-C(5)</td>
<td>1.334(2)</td>
</tr>
<tr>
<td>O(5)-H(5O)</td>
<td>0.90(3)</td>
</tr>
<tr>
<td>C(5)-C(6)</td>
<td>1.386(3)</td>
</tr>
<tr>
<td>O(6)-C(7)</td>
<td>1.321(2)</td>
</tr>
<tr>
<td>O(6)-H(6O)</td>
<td>0.88(3)</td>
</tr>
<tr>
<td>C(6)-C(7)</td>
<td>1.366(3)</td>
</tr>
<tr>
<td>C(6)-H(6)</td>
<td>0.88(2)</td>
</tr>
<tr>
<td>O(7)-C(8)</td>
<td>1.240(2)</td>
</tr>
<tr>
<td>C(9)-C(10)</td>
<td>1.378(3)</td>
</tr>
<tr>
<td>C(9)-C(14)</td>
<td>1.382(3)</td>
</tr>
<tr>
<td>C(10)-C(11)</td>
<td>1.386(3)</td>
</tr>
<tr>
<td>C(10)-H(10)</td>
<td>0.90(2)</td>
</tr>
<tr>
<td>C(11)-C(12)</td>
<td>1.386(3)</td>
</tr>
<tr>
<td>C(11)-H(11)</td>
<td>0.99(2)</td>
</tr>
<tr>
<td>C(12)-C(13)</td>
<td>1.392(3)</td>
</tr>
<tr>
<td>C(12)-C(15)</td>
<td>1.491(3)</td>
</tr>
<tr>
<td>Bond</td>
<td>Distance or Angle</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>C(13)-C(14)</td>
<td>1.383(3)</td>
</tr>
<tr>
<td>C(13)-H(13)</td>
<td>0.94(2)</td>
</tr>
<tr>
<td>C(14)-H(14)</td>
<td>0.97(2)</td>
</tr>
<tr>
<td>C(2)-C(1)-H(1A)</td>
<td>109.5</td>
</tr>
<tr>
<td>C(2)-C(1)-H(1B)</td>
<td>109.5</td>
</tr>
<tr>
<td>H(1A)-C(1)-H(1B)</td>
<td>109.5</td>
</tr>
<tr>
<td>C(2)-C(1)-H(1C)</td>
<td>109.5</td>
</tr>
<tr>
<td>H(1A)-C(1)-H(1C)</td>
<td>109.5</td>
</tr>
<tr>
<td>H(1B)-C(1)-H(1C)</td>
<td>109.5</td>
</tr>
<tr>
<td>C(7)-N(1)-C(8)</td>
<td>122.98(15)</td>
</tr>
<tr>
<td>C(7)-N(1)-C(9)</td>
<td>118.58(15)</td>
</tr>
<tr>
<td>C(8)-N(1)-C(9)</td>
<td>118.26(15)</td>
</tr>
<tr>
<td>C(15)-O(1)-H(1O)</td>
<td>105(2)</td>
</tr>
<tr>
<td>O(3)-C(2)-C(1)</td>
<td>109.7(2)</td>
</tr>
<tr>
<td>O(3)-C(2)-H(2A)</td>
<td>109.7</td>
</tr>
<tr>
<td>C(1)-C(2)-H(2A)</td>
<td>109.7</td>
</tr>
<tr>
<td>O(3)-C(2)-H(2B)</td>
<td>109.7</td>
</tr>
<tr>
<td>C(1)-C(2)-H(2B)</td>
<td>109.7</td>
</tr>
<tr>
<td>H(2A)-C(2)-H(2B)</td>
<td>108.2</td>
</tr>
<tr>
<td>C(3)-O(3)-C(2)</td>
<td>117.23(16)</td>
</tr>
<tr>
<td>O(4)-C(3)-O(3)</td>
<td>121.52(18)</td>
</tr>
<tr>
<td>O(4)-C(3)-C(4)</td>
<td>122.69(18)</td>
</tr>
<tr>
<td>O(3)-C(3)-C(4)</td>
<td>115.79(17)</td>
</tr>
<tr>
<td>C(5)-C(4)-C(8)</td>
<td>119.09(17)</td>
</tr>
<tr>
<td>C(5)-C(4)-C(3)</td>
<td>118.01(17)</td>
</tr>
<tr>
<td>C(8)-C(4)-C(3)</td>
<td>122.89(17)</td>
</tr>
<tr>
<td>C(5)-O(5)-H(5O)</td>
<td>100.0(17)</td>
</tr>
<tr>
<td>O(5)-C(5)-C(6)</td>
<td>116.40(17)</td>
</tr>
<tr>
<td>O(5)-C(5)-C(4)</td>
<td>121.46(17)</td>
</tr>
<tr>
<td>C(6)-C(5)-C(4)</td>
<td>122.14(17)</td>
</tr>
<tr>
<td>C(7)-O(6)-H(6O)</td>
<td>108.6(19)</td>
</tr>
<tr>
<td>C(7)-C(6)-C(5)</td>
<td>118.73(18)</td>
</tr>
<tr>
<td>C(7)-C(6)-H(6)</td>
<td>120.6(13)</td>
</tr>
<tr>
<td>C(5)-C(6)-H(6)</td>
<td>120.7(13)</td>
</tr>
<tr>
<td>O(6)-C(7)-C(6)</td>
<td>125.76(17)</td>
</tr>
</tbody>
</table>
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 ($\AA^2 \times 10^3$) for daw1016. The anisotropic displacement factor exponent takes the form: $-2p^2[ h^2 a^*2U_{11} + \ldots + 2hk a^*b^*U_{12} ]$

<table>
<thead>
<tr>
<th></th>
<th>U$^{11}$</th>
<th>U$^{22}$</th>
<th>U$^{33}$</th>
<th>U$^{23}$</th>
<th>U$^{13}$</th>
<th>U$^{12}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(1)</td>
<td>49(2)</td>
<td>48(2)</td>
<td>86(2)</td>
<td>12(1)</td>
<td>17(1)</td>
<td>-5(1)</td>
</tr>
<tr>
<td>N(1)</td>
<td>26(1)</td>
<td>21(1)</td>
<td>30(1)</td>
<td>0(1)</td>
<td>13(1)</td>
<td>2(1)</td>
</tr>
<tr>
<td>O(1)</td>
<td>29(1)</td>
<td>68(1)</td>
<td>41(1)</td>
<td>0(1)</td>
<td>17(1)</td>
<td>2(1)</td>
</tr>
<tr>
<td>O(2)</td>
<td>39(1)</td>
<td>77(1)</td>
<td>36(1)</td>
<td>-13(1)</td>
<td>20(1)</td>
<td>-10(1)</td>
</tr>
<tr>
<td>C(2)</td>
<td>46(1)</td>
<td>37(1)</td>
<td>69(2)</td>
<td>-2(1)</td>
<td>35(1)</td>
<td>-9(1)</td>
</tr>
<tr>
<td>O(3)</td>
<td>47(1)</td>
<td>30(1)</td>
<td>66(1)</td>
<td>-8(1)</td>
<td>36(1)</td>
<td>-10(1)</td>
</tr>
<tr>
<td>C(3)</td>
<td>33(1)</td>
<td>32(1)</td>
<td>38(1)</td>
<td>-2(1)</td>
<td>17(1)</td>
<td>0(1)</td>
</tr>
<tr>
<td>O(4)</td>
<td>43(1)</td>
<td>40(1)</td>
<td>62(1)</td>
<td>-8(1)</td>
<td>36(1)</td>
<td>-5(1)</td>
</tr>
<tr>
<td>C(4)</td>
<td>28(1)</td>
<td>25(1)</td>
<td>30(1)</td>
<td>0(1)</td>
<td>13(1)</td>
<td>1(1)</td>
</tr>
<tr>
<td>O(5)</td>
<td>38(1)</td>
<td>32(1)</td>
<td>41(1)</td>
<td>-6(1)</td>
<td>24(1)</td>
<td>3(1)</td>
</tr>
<tr>
<td>C(5)</td>
<td>29(1)</td>
<td>29(1)</td>
<td>26(1)</td>
<td>0(1)</td>
<td>11(1)</td>
<td>7(1)</td>
</tr>
<tr>
<td>O(6)</td>
<td>35(1)</td>
<td>22(1)</td>
<td>46(1)</td>
<td>1(1)</td>
<td>20(1)</td>
<td>-2(1)</td>
</tr>
<tr>
<td>C(6)</td>
<td>34(1)</td>
<td>21(1)</td>
<td>29(1)</td>
<td>-1(1)</td>
<td>12(1)</td>
<td>2(1)</td>
</tr>
<tr>
<td>O(7)</td>
<td>38(1)</td>
<td>20(1)</td>
<td>44(1)</td>
<td>-2(1)</td>
<td>22(1)</td>
<td>0(1)</td>
</tr>
<tr>
<td>C(7)</td>
<td>29(1)</td>
<td>21(1)</td>
<td>29(1)</td>
<td>3(1)</td>
<td>8(1)</td>
<td>2(1)</td>
</tr>
<tr>
<td>C(8)</td>
<td>25(1)</td>
<td>24(1)</td>
<td>28(1)</td>
<td>2(1)</td>
<td>10(1)</td>
<td>1(1)</td>
</tr>
<tr>
<td>C(9)</td>
<td>29(1)</td>
<td>20(1)</td>
<td>30(1)</td>
<td>1(1)</td>
<td>17(1)</td>
<td>0(1)</td>
</tr>
<tr>
<td>C(10)</td>
<td>32(1)</td>
<td>34(1)</td>
<td>29(1)</td>
<td>-3(1)</td>
<td>8(1)</td>
<td>4(1)</td>
</tr>
<tr>
<td>C(11)</td>
<td>25(1)</td>
<td>38(1)</td>
<td>34(1)</td>
<td>-1(1)</td>
<td>9(1)</td>
<td>3(1)</td>
</tr>
<tr>
<td>C(12)</td>
<td>30(1)</td>
<td>24(1)</td>
<td>32(1)</td>
<td>-1(1)</td>
<td>15(1)</td>
<td>1(1)</td>
</tr>
<tr>
<td>C(13)</td>
<td>32(1)</td>
<td>36(1)</td>
<td>29(1)</td>
<td>-4(1)</td>
<td>12(1)</td>
<td>-3(1)</td>
</tr>
<tr>
<td>C(14)</td>
<td>25(1)</td>
<td>34(1)</td>
<td>32(1)</td>
<td>1(1)</td>
<td>10(1)</td>
<td>1(1)</td>
</tr>
<tr>
<td>C(15)</td>
<td>32(1)</td>
<td>30(1)</td>
<td>36(1)</td>
<td>-2(1)</td>
<td>15(1)</td>
<td>-3(1)</td>
</tr>
</tbody>
</table>
Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for daw1016.

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


(10) Pfitzinger, W. J. Prakt. Chem. 1886, 33, 100.


(80) Watanabe, N.; Sekine, T.; Takagi, M.; Iwasaki, J. I.; Imamoto, N.; Kawasaki, H.; Osada, 


  28, 1039.


(86) Fulle, F.; Muller, C. E. Heterocycles 2000, 53, 347.


(88) Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; 


(96) Uneme, H.; Mitsudera, H.; Yamada, J.; Kamikado, T.; Kono, Y.; Manabe, Y.; Numata, 

  59, 5700.


(99) Lyakhov, S. A.; Lyakhova, E. A.; Panchenko, N. N.; Litvinova, L. A.; Andronati, S. A. 

(110) Song, J.; Lei, Y. X.; Rappoport, Z. J. Org. Chem. 2007, 72, 9152.
(112) Ashkinazi, R. I. 1999 U. S. Patent # 7074925