12-1-2014

Part I: Synthesis and Biological Evaluations of Potent Class L Selective Histone Deacetylase Inhibitors
Part II: Aqueous Complexes for Efficient Sizebased Separation of Americium from Curium
Part III: Designing Strong Chiral Bronsted Acids and Their Application for Oxaxinanones Derivatization and the Aza-henry Reaction

Joseph Steve Ulicki
University of Wisconsin-Milwaukee

Follow this and additional works at: http://dc.uwm.edu/etd

Part of the Biology Commons, and the Chemistry Commons

Recommended Citation
PART I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEACETYLASE INHIBITORS

Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

By

Joseph Steve Ulicki

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry

at The University of Wisconsin-Milwaukee

December 2014
ABSTRACT

PART I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEAcetylASE INHIBITORS

Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICAN FROM CURIUM

Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

BY

Joseph Steve Ulicki
The University of Wisconsin-Milwaukee, 2014
Under the Supervision of Professor M. Mahmun Hossain

Part I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEACETYLASE INHIBITORS

Histone deacetylase inhibitors (HDACi) have found a wide variety of medicinal uses and are most noted for their specific apoptotic action towards cancer cells¹. Several hydroxamates and tetrapeptides HDACi have since been moved on to phase 1 and 2 clinical trials, with FK228 & SAHA having already been approved for treatment of advanced cutaneous T-cell lymphoma (CTCL). FK228

¹ Histone deacetylase inhibitors (HDACi) are a class of drugs that are used to treat a variety of diseases, including cancer. They work by inhibiting the deacetylation of histones, which are proteins that help to compact DNA into chromosomes. By inhibiting this process, HDACi can help to activate genes that were previously repressed, leading to cell death in cancer cells. FK228 and SAHA are examples of HDACi that have been approved for treatment of advanced cutaneous T-cell lymphoma (CTCL).
& SAHA are pan-HDACi which describes the unselective inhibition for any of the 11 Zn$^{2+}$-dependent HDAC isoforms. Research in the past five years, has shifted towards developing selective HDACi instead of developing pan-HDACi since they have higher cytotoxicity in vitro and vivo. The research presented involves exploration of a new class of HDACi that resemble the natural product Thailandepsin A, (TDP-A) but still retains adequate HDAC inhibitory activity and antitumor activity while increasing the maximum tolerated dose levels (MTD).

**Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM**

Separation of the adjacent actinide (An) elements americium and curium (An = Am, or Cm) is important to concepts for advanced nuclear fuel cycles proposed to reduce the transuranic content of nuclear waste placed in geological repositories, but the similar chemistries of Am and Cm make this separation among the most difficult in the periodic table. The work presented in this section examines the design and synthesis of new aqueous ligands with complexation of the adjacent actinide ions americium(III) and curium(III). The ligand $N,N'$-bis[6-carboxy-2-
pyridylm-ethyl]-1,7-diaza-18crown6 (H₂bp18c6) and its derivatives in an aqueous solution were studied to quantify and characterize their americium/curium selectivity.

**Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-HENRY REACTION**

Nature uses chiral Brønsted acids catalytically for numerous organic reactions. Organic chemists have until only very recently realized the importance of this unique class of catalyst. As a result, there are very few known chiral Brønsted acids used in traditional asymmetric organic reactions. In recent years, due to the tremendous need for new asymmetric organic reactions in the pharmaceutical and food industries, interest in strong chiral Brønsted acids has been steadily growing. Currently, there are only a few of these acids known that have been used successfully for organic reactions. The research presented here involves exploration of a new class of chiral Brønsted acids for organic reactions. In addition, this research focuses on the importance of strong acidity as well as asymmetry for aza-Henry reaction. Binaphthyl sulfur-containing acid systems, which are analagous to sulfuric acid (H₂SO₄), were found to be especially attractive. This dissertation reports on the use of an innovative application of these asymmetric chiral sulfonate/sulfate systems in conjunction with a strong Brønsted acid. These new catalytic systems were very successful for obtaining asymmetry in the aza-Henry reaction.
DEDICATION

To

My Mother and Father, who always supported my work as a student throughout my life.

   My father whose wisdom has always gave me great insight.

   My mother, whose loving touch and caring soul has always been there when dealing
   with me.

“A grad student in procrastination tends to stay in procrastination unless an external force is applied to it”

Also known as Newton’s first law of graduation or the “Law of Inertia” originally discovered experimentally by Galileo when he threatened to cut his grad student’s funding!
(From http://www.phdcomics.com, originally published 2001)
Acknowledgments

I am tremendously delighted to have this opportunity to express my gratitude to my advisor Professor M. Mahmum Hossain for his steady support, guidance and friendship throughout the course of this work. Dr. Hossain is the main reason I initially found interest in research and in organic chemistry. He has taught me many great lessons in life pertaining to becoming a great group leader, and more importantly, how to conduct myself as a chemist in and around this community.

I respectfully express my deepest sense of gratitude to the enthusiastic support of Monzur Morshed, Matthew Dudley, Nazim Uddin, Robert Todd, Matthew Huisman, Maria Shevyrev, Eduardo A. Gracia, Md. Sharif Al Asad, Shamsul Arefin Ahmed, Brian Spindler, Chris Anipelli, Dan Murphy, Brad Endres, James Ulicki, and any other members of Hossain group both past and present. All my committee members: Dr. Alexander Arnold, Dr. Jian Chen, Dr. A. Andy Pacheco, and Dr. Xiaohua Peng. Dr. Eric Cheng, Dr. Mark Jensen, and Dr. Mark Steeber were all of great help and assistants by giving me the opportunity to work in collaboration with their labs. Dr. Wang and Dr. Lui for showing me the principles in LCMS and enzyme assays. The achievement of my graduate studies would not have been possible without the support from the University of Wisconsin-Milwaukee’s Department of Chemistry/Biochemistry and the Graduate school. A special thanks to Neil Korfhage for his artistic ability with glass to specially make any request and to fix anything that broke and Dr. F. Holger Forsterling for all his guidance and knowledge in the NMR lab. Also a special
thanks to Pat Hayes for all the help she has given me over the years with graduation, writing my thesis and especially her enthusiasm with the brewers. A particular thanks to Elise Nicks and Wendy Grober for keeping me on the right track to graduate for the many years I was at UWM. All of the support staff of the department has also been very helpful in my endeavors.

I am very grateful to my family for their encouragement, direction and support in every step of this degree. I am also thankful to my mother and father, whose continuous encouragement and support helped me every moment of the journey. My brothers, Chris and James deserve a very special appreciation for their help and support. All my relatives and family have played a very important role towards this accomplishment with their constant help and support.

I dedicate this thesis to my parents. They have been my inspiration and have made my work meaningful. They are truly my best friends and again I would not have been able to do this without their constant help and support.
# TABLE OF CONTENTS

## PART I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEACETYLASE INHIBITORS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1.1 Gene Expression by Histone Deacetylase (HDAC) proteins</td>
<td>1</td>
</tr>
<tr>
<td>1.1.2 Classification of HDAC protein</td>
<td>4</td>
</tr>
<tr>
<td>1.1.3 Histone Deacetylases Involvement with Different Types of Cancers</td>
<td>8</td>
</tr>
<tr>
<td>1.1.4 HDAC Inhibitors</td>
<td>12</td>
</tr>
<tr>
<td>1.1.5 Mechanisms of HDACs</td>
<td>15</td>
</tr>
<tr>
<td>1.1.6 Use of HDAC Inhibitor in Combination with Other Chemotherapies</td>
<td>18</td>
</tr>
<tr>
<td>1.2 Rationale design and biological evaluations of Fragments</td>
<td>20</td>
</tr>
<tr>
<td>1.2.1 Rationale design Fragments</td>
<td>20</td>
</tr>
<tr>
<td>1.2.2 In vitro and in vivo testing parameters</td>
<td>24</td>
</tr>
<tr>
<td>1.3 Synthesis of natural product derivatives</td>
<td>26</td>
</tr>
<tr>
<td>1.3.2 Synthesis of fragments related to TDP-A</td>
<td>28</td>
</tr>
<tr>
<td>1.3.3 Trouble-shooting synthesis of fragments</td>
<td>35</td>
</tr>
<tr>
<td>1.4 Biological evaluations</td>
<td>39</td>
</tr>
<tr>
<td>1.4.1 Isozyme Selectivity</td>
<td>39</td>
</tr>
<tr>
<td>1.4.2 Cellular Assays</td>
<td>43</td>
</tr>
<tr>
<td>1.4.3 Maximum Tolerated Dose Studies and Solubility</td>
<td>45</td>
</tr>
<tr>
<td>1.4.4 Molecular Modeling</td>
<td>46</td>
</tr>
<tr>
<td>1.5 Conclusion and Future Directions</td>
<td>53</td>
</tr>
<tr>
<td>1.6 Experimental for Biological Evaluations</td>
<td>54</td>
</tr>
<tr>
<td>1.7 Experimental for chemical synthesis</td>
<td>57</td>
</tr>
<tr>
<td>1.8 References</td>
<td>102</td>
</tr>
</tbody>
</table>

## Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Overview</td>
<td>116</td>
</tr>
<tr>
<td>2.2 Introduction</td>
<td>118</td>
</tr>
<tr>
<td>2.3 Rationally Design of bp18c6 Derivatives</td>
<td>123</td>
</tr>
<tr>
<td>2.4 Results and Discussion</td>
<td>127</td>
</tr>
<tr>
<td>2.5 Conclusion</td>
<td>148</td>
</tr>
<tr>
<td>2.6 Experimental</td>
<td>149</td>
</tr>
<tr>
<td>2.7 References</td>
<td>159</td>
</tr>
</tbody>
</table>
### Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAZINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>Introduction</td>
<td>168</td>
</tr>
<tr>
<td>3.1</td>
<td>Aza-Henry Reaction</td>
<td>172</td>
</tr>
<tr>
<td>3.2</td>
<td>Brønsted Acids for Organic Reactions</td>
<td>182</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Designing Chiral Brønsted Acids for Organic Reactions</td>
<td>184</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Metal-assisted Chiral Brønsted Acids</td>
<td>188</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Chiral Diol Based Brønsted Acid</td>
<td>192</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Chiral Phosphoric Acids</td>
<td>200</td>
</tr>
<tr>
<td>3.2.4.1</td>
<td>Mannich-type Reaction</td>
<td>204</td>
</tr>
<tr>
<td>3.2.4.2</td>
<td>Nucleophilic Addition to Aldimines</td>
<td>211</td>
</tr>
<tr>
<td>3.2.4.3</td>
<td>Aza Diels Alder Reactions</td>
<td>218</td>
</tr>
<tr>
<td>3.2.4.4</td>
<td>Friedel-Crafts Reaction</td>
<td>226</td>
</tr>
<tr>
<td>3.2.4.5</td>
<td>Transfer Hydrogenations</td>
<td>227</td>
</tr>
<tr>
<td>3.2.5</td>
<td>Novel Phosphoric Acids</td>
<td>233</td>
</tr>
<tr>
<td>3.3</td>
<td>Results and Discussion</td>
<td>242</td>
</tr>
<tr>
<td>3.4</td>
<td>Conclusion</td>
<td>261</td>
</tr>
<tr>
<td>3.5</td>
<td>Experimental</td>
<td>262</td>
</tr>
<tr>
<td>3.6</td>
<td>General Procedure</td>
<td>262</td>
</tr>
<tr>
<td>3.7</td>
<td>References</td>
<td>291</td>
</tr>
<tr>
<td>3.8</td>
<td>Results and Discussion for Oxazinanones</td>
<td>262</td>
</tr>
<tr>
<td>3.9</td>
<td>Experimental</td>
<td>314</td>
</tr>
<tr>
<td>3.7</td>
<td>References</td>
<td>326</td>
</tr>
</tbody>
</table>

### INDEX

#### APPENDIX A

NMR Spectroscopic data for Part I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEACETYLASE INHIBITORS

#### APPENDIX B

NMR Spectroscopic data for Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

#### APPENDIX C

NMR Spectroscopic data for Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAZINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

Vitae

---

xi
LIST OF FIGURES

PART I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEACETYLASE INHIBITORS

Figure 1.1. Cartoon of chromosome to DNA (left diagram), cartoon and molecular diagram of octamer of core histones (H2A, H2B, H3 and H4).........................1
Figure 1.2. The equilibrium activities of histone acetyltransferase (HAT) and deacetylase (HDAC).......................................................................................3
Figure 1.3 Evolutionary relationship between the HDACs........................................5
Figure 1.4 Class I (HDAC1, 2, 3 and 8), class IIa (HDAC4, 5, 7 and 9), class IIb (HDAC6 and 10) and class IV (HDAC11) HDACs with the various structural/functional domain ........................................7
Figure 1.5 HDAC2 Crystal structure (three domains are in different colors) and the active site with TDP-A bound (PDB: 3MAX)..................................11
Figure 1.6 Four Main classes of HDACi..................................................................13
Figure 1.7 SAHA bound to active site of HDAC1....................................................16
Figure 1.8 How HDACi mechism of Apoptosis, Differentiation, Cell-cycle arrest, and Immune modulation work.........................................................17
Figure 1.9 Structure of Triciferol ...........................................................................19
Figure 1.10 How TDP-A is produced from the bacteria Burkholderia thailandensis E264........................................................................................................20
Figure 1.11 Rational design and synthesis of 24 designer compounds...............23
Figure 1.12 The lowest energy conformation of crystal structure, HDAC 2 (PDB: 3MAX) using the software packages of MOE...............................47
Figure 1.13 The lowest energy conformation of TDP-A in HDAC2 catalytic core............................................................................................................48
Figure 1.14 The lowest energy conformation of TDF1 and TDF3 in HDAC2 catalytic core....................................................................................................50
Figure 1.15 The lowest energy conformation of TDF1 in the HDAC2 catalytic core...........................................................................................................52

PART II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

Figure 2.1. Energy comparison of Uranium, coal, oil, and natural gas................116
Figure 2.2. Nuclear power cycle ...........................................................................118
Figure 2.3 Radiotoxicity inventory for 1 ton of spent fuel from a pressurized water reactor (PWR) with a 4% 235U enrichment and burn-up of 40 GWd/tHM (thHM = Ton heavy metal)......................................................119
Figure 2.4 Contributions to the decay heat generated by spent PWR fuel irradiated to 50 GWd/tHM.................................................................120
Figure 2.5 Structure of H2bp18c6(1).....................................................................122
Figure 2.6 Ligands 1-3, increases in the size of the picolinic acid arms.............124
Figure 2.7 Ligands 4-9 have electron donating groups or a more rigid crown rings.............................................................................................................125
Figure 2.8 Ligands 10-12, with electron donating methoxy groups.................125
Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

Figure 3.1 Imine provides a vast array of nitrogen containing compounds............................................................170

Figure 3.2 Cyclopentadienyl dicarbonyl iron Lewis acid is also known as Hossain’s catalyst.........................................................182

Figure 3.3 Increasing number of publications in recent years in the area of organocatalyst. (SciFinder Scholar) ..............................187

Figure 3.4 Continuous publications about chiral Brønsted acid reflects the importance of this area. (SciFinder Scholar) ..................187

Figure 3.5 Increasing importance of chiral phosphoric acid is revealed by the number of increasing publications in this area. (SciFinder Scholar) .................................................................201

Figure 3.6 Representative derivatives of chiral BINOL-phosphoric acids........................................................................202

Figure 3.7 Structures of transition states (TSs) in (a) dicoordination pathway and (b) monocoordination pathway. Bond lengths are in Å, and natural charges are underlined and bold.................206

Figure 3.8 3D structures of transition states (TSs) in (a) re-face attack and (b) si-face attack. Bond lengths are in Å......................206

Figure 3.9 Chiral H$_8$-BINOL phosphoric acids.................................................................207

Figure 3.10 Developed chiral Brønsted acids which have great potential..................................................................................256

Figure 3.11 $^1$H NMR (δ=1.77 and 4.71ppm) of 2-methylpropene and phenylmethaniuminium ion (δ=8.55 and 11.9ppm) in reaction mixture.......................................................................................307

Figure 3.12 $^1$H NMR (δ=1.77 and 4.71ppm) and $^2$H NMR (δ=1.77 and 4.71ppm) of 2-methylpropene and of 4-phenyl-6-aryl-1,3-oxazinan-2-one-5,5-d$_2$ (δ=8.55 and 11.9ppm) in a mixture of 18% to 82% respectively......................................................309
LIST OF TABLES

PART I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS
I SELECTIVE HISTONE DEACETYLASE INHIBITORS

Table 1.1. Four classes of HDAC proteins........................................4
Table 1.2 Implication of zinc dependant HDACs in different types of
  cancers......................................................................................10
Table 1.3 Properties of HDACi including stuctural class, IC50,
 Activated genes, Repressed genes and in vitro effect..............14
Table 1.4 Enzyme Inhibition on HDAC proteins (IC50 values are in µM).....21
Table 1.5 National Cancer Institute-60 (NCI-60) screening..............22
Table 1.6 Acid trials to remove trityl protecting group................35
Table 1.7 Hg²⁺ and Ag⁺ trials to remove trityl protecting group..........36
Table 1.8 Iodine reactions to cleave trityl protecting group on
  β-hydroxyacid (4)......................................................................37
Table 1.9 HDAC inhibitory activity (IC₅₀ in µM).............................42
Table 1.10 Antiproliferative Activity (IC₅₀ in µM).........................44
Table 1.11 Antiproliferative Activity (IC₅₀ in µM)..........................44
Table 1.12 Solubility and MTD (mg/kg)........................................45

Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED
  SEPARATION OF AMERICIUM FROM CURIUM

Table 2.1. Optimization of coupling reactions for the synthesis of
  methyl vinyl-picolinate (22)....................................................133
Table 2.2. Optimization of hydroboration workup for the synthesis
  of methyl 6-(2-hydroxyethyl) picolinate (20).........................135
Table 2.3 Optimization of halogenation of 6-(2-hydroxyethyl)
  picolinate (20) for synthesis of (methyl 6-(2-chloroethyl)
  picolinate (21a) or methyl 6-(2-bromoethyl)picolinate (22b).....136
Table 2.4 Optimization of coupling reactions for the synthesis
  of methyl 6-allylpicolinate (22).............................................141
Table 2.5 Optimization of hydroboration workup for the synthesis
  of methyl 6-(2-hydroxyethyl)picolinate (20).........................144

Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR
  APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-
  HENRY REACTION

Table 3.1 Published pKa values for different Brønsted acids............185
Table 3.2 Use of chiral dioxathiepin and diylsulfate derivatives bound
  with Brønsted acid for asymmetric aza-Henry reactions...........257
Table 3.3 Effect of changing Brønsted acid bound to chiral amines
  for asymmetric aza-Henry reaction.....................................259
Table 3.4 Effect of different ratio of chiral component 63a and HBF₄....260
| Table 3.5  | Optimization of the solvent system                          | 304 |
| Table 3.6  | Optimized HBF₄.OEt₂-catalyzed reactions of Boc-imine     | 305 |
| Table 3.7  | HBF₄.OEt₂-catalyzed reactions of Boc-imine and styrene derivatives | 312 |
List of Schemes

PART I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEACETYLASE INHIBITORS

Scheme 1.1 Synthesis of Natural product derivatives TDC1, TDC2, and TDC3 ..............................................................27
Scheme 1.2 Retrosynthesis of TDF3 ...............................................................28
Scheme 1.3 Synthesis of TDF2m, TDF2, TDF3 and TDF3m ......................32
Scheme 1.4 Synthesis of TDF1 and TDF3t .................................................33
Scheme 1.5 Synthesis of TDF4, TDF4m, TDF5, TDF5m, TDF6 and TDF6m ..........................................................34

Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

Scheme 2.1 Synthesis of H2bp18c6 (1) .........................................................127
Scheme 2.2 Proposed synthesis of 6,6'-(1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane-7,16-diyl)bis(ethane-2,1-diyl)dipicolinic acid (2) .........................................................128
Scheme 2.3 Synthesis of 2-(6-(methoxycarbonyl)pyridin-2-yl)acetic acid (18) and methyl 6-(2-oxoethyl)picolinate (24) from methyl 6-(cyanomethyl)picolinate (23) .....................................................................................130
Scheme 2.4 Proposed synthesis of Ligand (2) using a coupling reaction for carbon extension .................................................131
Scheme 2.5 Final steps of synthesis of 6,6'-(1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane-7,16-diyl)bis(ethane-2,1-diyl)dipicolinic acid (2) .....................................................................................137
Scheme 2.6 Proposed synthesis of Ligand (2) 6,6'-(1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane-7,16-diyl)bis(propane-2,1-diyl)dipicolinic acid (3) .....................................................................................138
Scheme 2.7 Proposed synthesis of Ligand (3) using a coupling reaction for carbon extension .................................................140

Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

Scheme 3.1 Asymmetric Diels-Alder reactions catalyzed by Brønsted acid assisted Lewis acids (BLA) .........................190
<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 3.2</td>
<td>Proposed reaction mechanism of the Mannich-type reaction with chiral Phosphoric acid catalysts</td>
<td>205</td>
</tr>
<tr>
<td>Scheme 3.3</td>
<td>Proposed mechanism of 1,3-oxazinan-2-one</td>
<td>308</td>
</tr>
<tr>
<td>Scheme 3.4</td>
<td>HBF₄·OEt₂ reaction of Boc-imine and 2-methyl propene-1,1-d₂</td>
<td>309</td>
</tr>
<tr>
<td>Scheme 3.5</td>
<td>HBF₄·OEt₂ reaction of Boc-imine and styrene.</td>
<td>310</td>
</tr>
<tr>
<td>Scheme 3.6</td>
<td>Proposed rationale for the stereoselective formation of 4,6-Diphenyloxazinanone</td>
<td>311</td>
</tr>
</tbody>
</table>
LIST OF EQUATIONS

Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICICUM FROM CURIUM

Equation 2.1 2-(6-(methoxycarbonyl)pyridin-2-yl)acetic acid (18) ……129
Equation 2.2 Methyl 6-(cyanomethyl)-picolinate (23)…………………130
Equation 2.3 Coupling reactions for the synthesis of methyl 6-vinylpicolinate (22)………………………………………..132
Equation 2.4 Final conditions for synthesis of methyl 6-vinylpicolinate (22)………………………………………………134
Equation 2.5 Synthesis of methyl 6-(2-hydroxyethyl)picolinate (20)….134
Equation 2.6 Halogenation of 6-(2-hydroxyethyl)picolinate (20) for synthesis of (methyl 6-(2-chloroethyl)picolinate (21a) or methyl 6-(2-bromoethyl)picolinate (22b)…………………136
Equation 2.7 Optimization of reactions for the synthesis of methyl 6-allylpicolinate (28)……………………………………141
Equation 2.8 Final conditions for synthesis of methyl 6-allylpicolinate (28) using Suzuki coupling………………143
Equation 2.9 Hydroboration reaction with borane used for the synthesis of methyl 6-(2-hydroxyethyl)picolinate (20)….143
Equation 2.10 Halogenation of 6-(2-hydroxyethyl)picolinate (25) produced 5-(methoxycarbonyl)-2,3-dihydro-1H-indolizin-4-ium (29a or 29b) with corresponding anion………145
Equation 2.11 Synthesis of dimethyl 6,6',((1,4,10,13-tetraoxa-7,16-diazaacyclo-octadecane-7,16-diyl)bis(propane-3,1-diyl))dipicolinate……………………………………146
Equation 2.12 Synthesis of 7,16-bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-1,4,10,13-tetraoxa-7,16-diazaacyclooctadecane (31) and synthesis of dimethyl 6,6'-(1,4,10,13-tetraoxa-7,16-diazaacyclo-octadecane-7,16-diyl)bis(propane-3,1-diyl)) dipicolinate (3)………………………………………………147

Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

Equation 3.1 First catalytic aza-Henry reaction reported by Shibasaki…………………………………………………………173
Equation 3.2 Shibasaki’s (R)-ALB-KO'Bu catalyst for asymmetric aza-Henry reaction ................................................................. 174
Equation 3.3 Synthesis of CP-99994 antagonist using asymmetric aza-Henry reaction ............................................................... 175
Equation 3.4 Cu-(R,S)-bisoxazoline complex catalyzed aza-Henry reaction ........................................................................ 176
Equation 3.5 Cu-(R,R)-bisoxazoline complex catalyzed asymmetric aza-Henry reaction ..................................................... 177
Equation 3.6 Mechanism for the formation of chiral nitronate complex ....................................................................................... 178
Equation 3.7 Asymmetric aza-Henry reaction using thiourea derivative as a catalyst .............................................................. 179
Equation 3.8 Asymmetric aza-Henry reaction catalyzed by chiral bis-amidinium salt ................................................................. 180
Equation 3.9 Bulkier chiral bis-amidinium salt catalyzed asymmetric aza-Henry reaction ..................................................... 180
Equation 3.10 Scope of aza-Henry reaction catalyzed by chiral phosphoric acid ................................................................. 181
Equation 3.11 3-Hydroxyacrylate formation from an aldehyde and ethyl diazoacetate (EDA) .............................................................. 183
Equation 3.12 Brensted acid catalyzed cyclopropanation reaction .......................................................................................... 183
Equation 3.13 Brensted acid catalyzed hemiacetal formation reaction ................................................................................... 183
Equation 3.14 Brensted acid catalyzed aziridination reaction .............................................................................................. 184
Equation 3.15 Brensted acid catalyzed aza-Henry reaction .............................................................................................. 184
Equation 3.16 Asymmetric Diels-Alder reaction using chiral complex of boron and (R,R)-(+)/tartaric acid diamide ............. 188
Equation 3.17 Asymmetric Diels-Alder reaction using chiral complex of boron and BINOL .......................................................... 189
Equation 3.18 Asymmetric Michael reaction catalyzed by lanthanum-binaphthol (La-BINOL) complex (14) ............................ 191
Equation 3.19 Asymmetric BINOL and SnCl4 complex (15) as a catalyst ............................................................................... 191
Equation 3.20 Planer-chiral pyrrolidinopyridine derivative as an asymmetric catalyst through H-transfer mechanism .... 192
Equation 3.21 BINOL catalyzed asymmetric Morita-Baylis-Hillman reaction ........................................................................... 193
Equation 3.22 Asymmetric Morita-Baylis-Hillman reaction with modified BINOL at 3-position ............................................... 194
Equation 3.23 Tetrol catalyzed addition reaction of methylene amino pyrrolidine to imines ......................................................... 195
Equation 3.24 Asymmetric diol catalyzed enamine Mannich reaction ....................................................................................... 195
Equation 3.25 Asymmetric nitroso Diels-Alder reaction catalyzed by BINOL derivative .......................................................... 196
Equation 3.26 Asymmetric nitroso Diels-Alder reaction followed by Michael addition reaction ........................................... 197
Equation 3.27 Asymmetric Mannich-type reaction of ketene silyl acetal with aldmines .......................................................... 197
Equation 3.28 Asymmetric nitroso aldol reaction .................................................................................................................. 198
Equation 3.29  Asymmetric boron-diol complex catalyzed aziridination reaction.................................199
Equation 3.30  TADDOL catalyzed asymmetric Diels-Alder reaction........ 201
Equation 3.31  Chiral phosphoric acid catalyzed asymmetric Mannich-type reaction.........................................................204
Equation 3.32  Chiral phosphoric acid catalyzed direct Mannich reaction.................................................................207
Equation 3.33  Asymmetric phosphoric acid catalyzed three-component direct Mannich reaction ..................................208
Equation 3.34  Chiral phosphoric acid catalyzed Mukaiyama-Mannich reaction..........................................................209
Equation 3.35  Chiral phosphoric acid catalyzed asymmetric Mannich reaction of acyclic silyl dienolates...............210
Equation 3.36  Asymmetric hydrophosphonylation of aldime with dialkyl phosphate..............................................................211
Equation 3.37  Asymmetric aza Friedel-Crafts alkylation of furan to aldimes...............................................................212
Equation 3.38  Asymmetric nucleophilic addition of indoles...............213
Equation 3.39  Asymmetric phosphoric acid catalyzed Pictet-Spengler reaction starting from geminally disubstituted tryptamines..............................................................213
Equation 3.40  Asymmetric synthesis of tetrahydro-β-carbolines via an (R)-BINOL-phosphoric acid-catalyzed asymmetric Pictet-Spengler reaction of N-benzyltryptamine ..........214
Equation 3.41  Asymmetric direct alkylation of aldimes with α-diazo ester to furnish β-amino-α-diazoester..............................215
Equation 3.42  Asymmetric Strecker reaction catalyzed by chiral phosphoric acid..........................................................215
Equation 3.43  Asymmetric phosphoric acid-catalyzed aza ene-type addition to aldimes .................................................216
Equation 3.44  Electrophilic addition using chiral phosphoric acid as a catalyst...............................................................216
Equation 3.45  Asymmetric iminoazaenamine reaction.........................................................................................217
Equation 3.46  Asymmetric addition of alcohols to N-acyl imines catalyzed by chiral phosphoric acids.............................217
Equation 3.47  Enantioselective Brønsted acid catalyzed iminoazaenamine reaction of various N-Boc-protected aldimes and methylene-aminopyrrolidine.................................218
Equation 3.48  Asymmetric aza Diels-Alder reaction with aldime in the presence of phosphoric acid to give cycloadducts.................................................................219
Equation 3.49  Aza Diels-Alder reaction of Brassard’s diene with aldimes................................................................................220
Equation 3.50  Asymmetric reverse electron-demand aza Diels-Alder reaction of electron-rich alkene with 2-aza diene ..........221
Equation 3.51  Rueping’s asymmetric aza Diels-Alder reaction of aldime with cyclohexenone .............................................222
Equation 3.52  Gong’s asymmetric aza Diels-Alder reaction of aldime with cyclohexenone .............................................222
Equation 3.53  Asymmetric Biginelli reaction catalyzed by chiral phosphoric acid ........................................ 223
Equation 3.54  Asymmetric synthesis of SNAP-7941 using chiral phosphoric as a catalyst ................................... 224
Equation 3.55  Enantioselective Brønsted acid catalyzed Nazarov reaction ......................................................... 225
Equation 3.56  Friedel-Crafts reaction between pyrrole derivatives and N-acyl imines catalyzed by chiral phosphoric acids .......................................................... 226
Equation 3.57  Chiral phosphoric acid catalyzed Friedel–Crafts alkylation of indoles with nitroalkenes ............. 227
Equation 3.58  Chiral phosphoric acid catalyzed asymmetric hydrogenation reaction ........................................... 228
Equation 3.59  List’s asymmetric transfer hydrogenation by Brønsted acids .......................................................... 229
Equation 3.60  MacMillan’s three component transfer hydrogenation reaction ......................................................... 230
Equation 3.61  Asymmetric reduction of quinolines using chiral phosphoric acid ................................................... 231
Equation 3.62  Asymmetric reduction of benzoazines, benzothiazines, and benzoazinones using chiral phosphoric acid .................................................................................. 231
Equation 3.63  List’s catalytic reductive amination of R-branched aldehydes .......................................................... 231
Equation 3.64  Asymmetric synthesis of trans-alkenyl α-amino esters using chiral phosphoric acid ......................... 232
Equation 3.65  Guo’s transfer hydrogenation reaction using double axially chiral phosphoric acid .................... 233
Equation 3.66  Asymmetric Mannich-type reaction using TADDOL-based phosphoric acid diesters catalyst ........ 234
Equation 3.67  Asymmetric addition of sulfonamide to aldimines ................................................................. 235
Equation 3.68  Terada’s new stronger chiral Brønsted acid catalyzed direct Mannich reaction .............................. 236
Equation 3.69  Synthesis of the super chiral Brønsted acid ................................................................................. 237
Equation 3.70  Asymmetric Dield-Alder Reaction using the super chiral Brønsted acid ........................................ 237
Equation 3.71  Yamamoto’s Brønsted acid catalyzed asymmetric protonation reactions of silyl enol ethers using a chiral Brønsted acid catalyst ...................................................... 238
Equation 3.72  Gong’s asymmetric three-component 1,3-dipolar cycloaddition reaction using chiral phosphoric acids ................................................................. 240
Equation 3.73  Uraguchi’s asymmetric direct Henry reaction ................................................................................. 241
Equation 3.74  Synthesis of (R)-1,1'-dinaphthyl-2,2'-dioxathiepin (60a) and formation of catalyst 61a and 62a ......................................................................................... 242
Equation 3.75  Synthesis of (R)-1,1'-dinaphthyl-2,2'-dihydrogen sulfate (63a) and formation of catalyst 64a and 65a .................................................................................. 243
Equation 3.76  Multistep synthesis of (R)-3,3'-Bis(4'-methylphenyl)-1,1'-dinaphthyl-2,2'-dioxathiepin (60b) and subsequent catalyst 61b ....................................................................... 244
Equation 3.77  Multistep synthesis of (R)-3,3'-Bis(4''-methylphenyl)-2,2'-dihydroxy-1,1''-dinaphthyl-2,2''-diylsulfate (63b) and subsequent catalyst 64b ..................................................245

Equation 3.78  Multistep synthesis of (R)-3,3'-Bis(naphthyl)-2,2'-dihydroxy-1,1''-dinaphthyl-2,2''-diylsulfate (63c) and subsequent catalyst 64c ..................................................246

Equation 3.79  Multistep synthesis of (R)-3,3'-Bis(mesitylene)-2,2'-dihydroxy-1,1''-dinaphthyl-2,2''-diylsulfate (63d) and subsequent catalyst 64d ..................................................248

Equation 3.80  Multistep synthesis of (R)-3,3'-Bis(anthracenyl)-2,2'-dihydroxy-1,1''-dinaphthyl-2,2''-diylsulfate (63e) and subsequent catalyst 64e ..................................................249

Equation 3.81  Multistep synthesis of (R)-3,3'-Bis(phenanthryl)-2,2'-dihydroxy-1,1''-dinaphthyl-2,2''-diylsulfate (63f) and subsequent catalyst 64f ..................................................251

Equation 3.82  Multistep synthesis of (R)-3,3'-Bis(triphenylsilyl)-2,2'-dihydroxy-1,1''-dinaphthyl-2,2''-diylsulfate (63g) and subsequent catalyst 64g ..................................................253

Equation 3.83  Multistep synthesis of (R)-3,3'-Bis(tribenzylsilyl)-2,2'-dihydroxy-1,1''-dinaphthyl-2,2''-diylsulfate (63h) and subsequent catalyst 64h ..................................................224

Equation 3.84  Reaction of 1,3-oxazinan-2-one ..................................................302

Equation 3.85  Reaction of 1,3-oxazinan-2-one ..................................................302
PART I: SYNTHESIS AND BIOLOGICAL EVALUATIONS OF POTENT CLASS I SELECTIVE HISTONE DEACETYLASE INHIBITORS

Part II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

Part III: DESIGNING STRONG CHIRAL BRONSTED ACIDS AND THEIR APPLICATION FOR OXAXINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

By
Joseph Steve Ulicki
The University of Wisconsin-Milwaukee
1.1 Introduction

1.1.1 Gene Expression by Histone Deacetylase (HDAC) proteins

The nucleosome is a basic unit of DNA in eukaryotes which is wrapped in sequence around eight histone protein cores (two molecules of each core histone) which can be compared to string wrapped around a rod. Nucleosomes are used to pack the enormous eukaryotic genomes into the nucleus while still ensuring appropriate

**Figure 1.1:** Cartoon of chromosome to DNA (left diagram), cartoon and molecular diagram of octamer of core histones (H2A, H2B, H3 and H4)(right diagrams).
access to it (mammalian cells have approximately 2 m of linear DNA which is packed into a nucleus that is 10 µm in diameter)\(^1\). Nucleosomes are folded through a sequence of successively advanced ordered structures to ultimately form chromosomes (figure 1.1). The nucleosome core particle consists of about 146 base pairs (bp) of DNA wrapped in 1.67 left-handed helical turns around the octameric core\(^2\). This octamer consist of two copies of each core histones, which are H2A, H2B, H3, and H4\(^3\) (figure 1.1). Linker histones (connects adjacent octameric core histones) such as H1 and its isoforms are involved in chromatin condensing and sit at the base of the nucleosome near the DNA entry and exit site which bind to the linker region of the DNA\(^4\). Histone H1 is similar to a clamp by holding the DNA to the octamer when attached and releasing the DNA from the octamer when not clamped\(^5\)-\(^8\). Non-condensed nucleosomes without the linker histone resemble “beads on a string of DNA” under an electron microscope. Neighboring octamers are joined by a fragment of free DNA deemed the “linker DNA” which varies from 12-85 bp in length depending on the cell’s class and tissue type\(^9\)-\(^12\). The nucleosomes carry epigenetically inherited material in the covalent alterations of the core histones.

The N-terminal tails of histones protruding from the nucleosome are subject to various posttranslational modifications. These include phosphorylation\(^13\), acetylation\(^14\), methylation\(^15\), sumoylation\(^16\), ADP-ribosylation\(^17\) and ubiquitination\(^18\). The manner that these posttranslational modifications take place can modulate the chromatin structure and therefore regulate gene expression. By an overwhelming
amount, acetylation/deacetylation\textsuperscript{19-25} of core histones has been the most studied and therefore, best understood of the posttranslational modifications.

**Figure 1.2:** The equilibrium activities of histone acetyltransferase (HAT) and deacetylase (HDAC)

In cells, acetylation/deacetylation regulates many protein functions such as protein-protein interactions\textsuperscript{26}, DNA recognition\textsuperscript{27} and protein stability\textsuperscript{28}, which acts as a signaling mechanism that is similar to phosphorylation\textsuperscript{29-31}. Histone acetylation and deacetylation in eukaryotic cells is delicately maintained by histone acetyltransferases (HATs) and histone deacetylases (HDACs). These enzymes are responsible for modifications to chromatin structures that can regulate gene transcription\textsuperscript{32}. In general, HAT acetylation activity leads to an increase in gene transcription\textsuperscript{33-35} by neutralizing the positive charge on lysine residuals of histones, which loosens their interactions with the negatively charged DNA backbone, leading to a more active chromatin framework. Acetyl CoA\textsuperscript{36} transfers the acetyl group to the lysine terminal of the histone. In contrast, HDACs catalyze the removal of the acetyl groups on lysine residuals located on the amino-terminal tails of core histones, which leads to gene repression by chromatin condensation\textsuperscript{37}.
As a result, inhibition of HATs leads to a gene that is always deactivated while inhibition of HDACs leads to general hyperacetylation of histones, which is followed by the transcriptional activation of certain genes through relaxation of the DNA conformations. HAT/HDAC inhibition and posttranslational modifications are essential for the regulation of many cellular processes such as transcription\(^{38}\), cell division\(^{39}\), cell survival\(^{40}\) and differentiation\(^{40}\).

**1.1.2: Classification of HDAC proteins**

**Table 1.1**: Four classes of HDAC proteins\(^{42-45}\)

<table>
<thead>
<tr>
<th>Zn(^{2+}) cationic dependent proteins</th>
<th>NAD(^{+}) dependent proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class I</strong></td>
<td><strong>Class II</strong></td>
</tr>
<tr>
<td>Class Ia</td>
<td>Class Iib</td>
</tr>
<tr>
<td>HDAC 1</td>
<td>HDAC 6</td>
</tr>
<tr>
<td>HDAC 2</td>
<td>HDAC 10</td>
</tr>
<tr>
<td>HDAC 3</td>
<td>HDAC 4</td>
</tr>
<tr>
<td>HDAC 8</td>
<td>HDAC 5</td>
</tr>
<tr>
<td>HDAC 8</td>
<td>HDAC 7</td>
</tr>
<tr>
<td>HDAC 9</td>
<td>HDAC 9</td>
</tr>
<tr>
<td><strong>Class IV</strong></td>
<td></td>
</tr>
<tr>
<td>HDAC 11</td>
<td>SIRT 1</td>
</tr>
<tr>
<td>SIRT 2</td>
<td>SIRT 3</td>
</tr>
<tr>
<td>SIRT 4</td>
<td>SIRT 5</td>
</tr>
<tr>
<td>SIRT 6</td>
<td>SIRT 7</td>
</tr>
</tbody>
</table>

Histone deacetylases or HDACs are a family of epigenetic enzymes that catalyze the hydrolysis of acetylated \(\varepsilon\)-amino groups (not the alpha carbon amino group) of lysine in histone tails. There are roughly 30 lysine residuals on each histone, (H3, H4, H2A and H2B) which are all subjects to deacetylation by HDACs\(^{45}\). Removal of the acetyl groups restores the positive charge of lysine
residues, enabling them to interact back with negative charged phosphate groups of DNA, following chromatin condensing and transcriptional repression\textsuperscript{46}. The first human HDAC was discovered and cloned by the Schreiber laboratory\textsuperscript{47} (Stuart L. Schreiber, Ph.D. Director of Chemical Biology at and a Founding Member of the Broad Institute of Harvard and MIT). To date, there are 18 known human HDACs proteins\textsuperscript{48-51} that are grouped into four classes based on their size\textsuperscript{51}, number of catalytic sites\textsuperscript{51}, subcellular localization\textsuperscript{51}, and their sequence homology to yeast counterparts\textsuperscript{51}. Class I (HDACs 1, 2, 3 and 8), class II (HDACs 4, 5, 6, 7, 9 and 10), and class IV (HDAC 11) are all Zn\textsuperscript{2+}-dependent enzymes\textsuperscript{48-52} (table 1.1) (figure 1.3). Class III proteins, called sirtuins, (SIRTs 1–7) are defined by their dependency on the coenzyme, electron transporter, nicotinamide adenine

**Figure 1.3 Evolutionary relationship between the HDACs**

The actual distances may be greater than shown. Class I HDACs (red) are related to yeast (Saccharomyces cerevisiae, RPD3) and class II HDACs (blue) are related to the yeast HDA1 enzyme. HDAC 11 (Black) does not show enough identity with Class I or Class II HDACs to be placed in either class\textsuperscript{53}. 

![Diagram of HDACs evolutionary relationship](image)
dinucleotide (NAD\(^+\)). Class I HDACs are predominantly located in the nucleus while class II HDACs can shuttle between the nucleus and cytoplasm\(^54\). Classes I, II and IV HDACs are all Zn\(^{2+}\)-dependent proteins and are the main proteins that are studied in this dissertation. Class I HDACs are widely expressed in most cells whereas class II and IV show various degrees of tissue specificity. It has been revealed that Zn\(^{2+}\)-dependent HDACs, especially class I and class II enzymes play a role in tumorigenesis and cell development\(^54\).

Class I HDACs (HDAC1, 2, 3, and 8) are smaller in size of the zinc dependent enzymes and are the most closely related to the yeast (Saccharomyces cerevisiae) transcriptional regulator RPD3 (figure 1.4). They have any range between 377 to 488 base pairs of amino acids\(^51-54\). These proteins are all located in the nucleus. The main role of HDAC 1, 2, and 8 are all transcription corepressor while HDAC3 is not well understood yet. Knockout studies have shown that class I HDACs are involved in cell proliferation and survival\(^3\). Also, research has shown that class I enzymes are the most important class when implied in cancer progression/tumorigenesis\(^54\).

Class II is the largest of the three groups of zinc dependent enzymes, and is divided into two subgroups: class IIa and IIb (figure 1.4). Class IIa is represented by HDAC4, HDAC 5, HDAC 7 and HDAC 9, while class IIb is HDAC6 and HDAC10. These proteins range in size from 669 to 1215 bp and can shuttle between the nucleus and cytoplasm\(^55\). They are most closely related to the yeast (Saccharomyces cerevisiae) transcriptional regulator HAD1\(^55\).
Class IV is the smallest class consisting of its solo protein, HDAC11 (figure 1.4). This isoform is only located in the nucleus and main role is unknown. It is the smallest protein of all the HDACs containing only 347 base pairs. The reason it is in its own class is due to the fact it is not related to any other HDAC protein and its catalytic domain is very different from all other HDAC proteins.

Figure 1.4: The figure shows the class I (HDAC1, 2, 3 and 8), class IIa (HDAC4, 5, 7 and 9), class IIb (HDAC6 and 10) and class IV (HDAC11) HDACs with the various structural/functional domains listed. Schematic depictions of HDAC isoforms and their sub-cellular localization. Bars depict the length of each isoform. Colored (green/blue) regions depict the metal ion-dependent catalytic domain. Light grey depicts the inactive domain sequence (IDS). Each protein starts with the N-terminus (left) and ends with the C-terminus (right).
1.1.3: Histone Deacetylases Involvement with Different Types of Cancers

Class I HDACs are expressed at much higher rates than normal cells in numerous types of cancers such as prostate, ovarian (HDAC 1, HDAC 2, & HDAC 3), colorectal, lung cancers (HDAC 1 and HDAC 3), gastric pancreatic (HDAC 2) and hepatocellular carcinomas, but are not found in normal, resting endothelial cells and normal organs\textsuperscript{56} (table 1.2). Class I HDACs are produced at elevated
levels in ovarian cancers compared to normal ovarian tissues, as measured using small interfering RNA procedures\textsuperscript{56}.

HDAC 3 was produced at higher levels in 92\% of non-small cell lung carcinomas by immunoblot analysis\textsuperscript{57}. HDAC 2 was elevated in 44/71 gastric tumors\textsuperscript{58}. Class IIa HDAC 6 protein was observed in breast and ovarian cancer tissues\textsuperscript{58}. HDAC 5 is down regulated in colon cancers and acute myeloid leukemia. HDAC inhibition in cancer cells induces expression of genes including tumor suppressor genes that lead to cell growth arrest, differentiation, apoptosis, antiangiogenic effects and regression of cancer\textsuperscript{56-61}. Learning what HDAC proteins/classes are associated with what types of cancer formation has been given much attention over the last decade to pharmaceutical and carcinoma studies.

Since discovery of higher levels of each individual HDAC protein in the formation of specific types of cancer tumors, the role of each isoform in tumorigenesis is not yet well understood. Therefore, clarifying the detailed mechanism linking the HDAC activity of each isoform to cancer formation would facilitate studies that lead to faster treatment and more precise classification of each type of tumor. Key development of selective HDAC inhibitors is fully required to completely understand the role of each individual HDAC protein in all stages of cancer development.
Table 1.2: Implication of zinc dependant HDACs in different types of cancers

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor Implication</th>
<th>Tumor expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I (type RPD3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDAC1</td>
<td>Possible prognostic indicator for lung and breast cancers. Over expressed in prostate cancers (hormone-refractory), gastric, and colorectal.</td>
<td>++</td>
</tr>
<tr>
<td>HDAC2</td>
<td>Over expressed in colorectal and gastric cancers. Loss of antigen presenting cells in colorectal cancers gave HDAC2 over expression.</td>
<td>++</td>
</tr>
<tr>
<td>HDAC3</td>
<td>Over expressed in lung cancers and several solid tumors</td>
<td>++</td>
</tr>
<tr>
<td>HDAC8</td>
<td>Knock down inhibits cell growth in several human tumor cells.</td>
<td>++</td>
</tr>
<tr>
<td>Class II (type HAD1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDAC4</td>
<td>Unknown</td>
<td>++</td>
</tr>
<tr>
<td>HDAC5</td>
<td>Down regulated in colon cancers and acute myeloid leukemia</td>
<td>- -</td>
</tr>
<tr>
<td>HDAC6</td>
<td>Ambiguous prognostic in breast cancer</td>
<td></td>
</tr>
<tr>
<td>HDAC7</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>HDAC9</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>HDAC10</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Class IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDAC11</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

*: ++ indicated an over expression in tumor. - - indicates a reduced expression

To date, X-ray co-crystallographic information is available for HDACs 2, 4, 7 and 8 in complex with several different small molecule inhibitors. This data is of great use for the development of molecular modeling methods and also run computational studies of new targets that are very selective for few HDAC’s active site.
Figure 1.5: HDAC2 Crystal structure (three domains are in different colors) and the active site with TDP-A bound (PDB: 3MAX)

Zinc dependent class I histone deacetylase (HDAC) enzymes, HDAC1, HDAC2, HDAC3 and HDAC8, are known to play important roles in tumorigenesis and are often over-expressed in many different types of cancers. Inhibiting the
functionalities of class I HDACs by using small molecules, known as HDAC inhibitors (HDACi) resulted in terminal differentiation, growth arrest and apoptosis of cancerous cells.\(^6_4\).

1.1.4 HDAC Inhibitors

Over the last decade, there has been extensive research and development of many HDACi which has lead to very promising results in treating cancer cells and other various diseases. Several HDACi drugs are in clinical trials for treatment of cancers and diseases. The first HDACi, **suberoyl anilide hydroxamic acid** (SAHA, Vorinostat) was approved by the Food and Drug Administration (FDA) for treatment of T-Cell lymphoma (CTCL) in the early 2000s. Then, in 2009 the FDA approved **romidepsin/FK-228** also for CTCL.\(^6_5\) **SAHA** is considered to be a pan-inhibitor, which means it has no selective to any class or specific HDAC protein and inhibits the majority of the 11 zinc dependent HDAC isoforms. However, **FK-228** is considered a class I selective inhibitor (inhibiting only HDAC1 and HDAC2). This is the selectivity in HDACi that research is trying to design.

There are many different types of HDACi but the four most promising classes of HDACi discovered thus far are 1) hydroxamic acids, 2) short-chain fatty acids (SCFA), cyclic peptides and benzamide/ketone derivatives\(^6_6\)\(^-\)\(^6_7\) (**figure 1.6** and **table 1.3**).
Figure 1.6. Four Main classes of HDACi

Cyclic peptides

FK228 (istodax®)  Thailandepsin A, TDP-A (R=SCH₃)  Thailandepsin B, TDP-B (R=CH₂CH₃)  Largazole

Hydroxamic acids

SAHA (Zolinza®)  Trichostatin A, TSA

Benzamides

MS-27-275  CI-994

Short-chain fatty acids

Butyrate  Valproate  Phenylbutyrate
**Table 1.3**: Properties of HDACi including structural class, IC$_{50}$, Activated genes, Repressed genes and *in vitro* effect$^{68}$

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Structural</th>
<th>Range</th>
<th>Activated genes</th>
<th>Repressed genes</th>
<th><em>In vitro</em> effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td>Short chain fatty acids</td>
<td>mM</td>
<td>CDKN1A, GATA2, PKCD, MHC1, MHC2, BAK, IL8, RARβ, TG1, cyclin E, CPA3, CD86, ICAM1</td>
<td>Cyclin D1, cyclin A, BCL2, IL2, BCLXL</td>
<td>Apoptosis Differentiation Cell-cycle arrest</td>
</tr>
<tr>
<td>Trichostatin</td>
<td>hydroxamic acid</td>
<td>nM</td>
<td>CDKN1A, GATA2, HSP86, CDKN1B, PKCD, HDAC1, IGFBP3, DHFR, TGFβ1, ER, CD86, cyclin E, FNG, IFNB, TP53, VHL, MHC1, MHC2, CPA3, P107, BAX, BAK, TG1, CDNK2A, MLH1, TIMP3</td>
<td>Cyclin A, CDKN1C, BCLXL, PU.1, HIF1A, VEGF, IL2, IL10</td>
<td>Apoptosis Differentiation Cell-cycle arrest</td>
</tr>
<tr>
<td>Suberoylanilid hydroxamic acid (SAHA)</td>
<td>hydroxamic acid</td>
<td>μM</td>
<td>CDKN1A</td>
<td>CMYC, CMYB, BMYB</td>
<td>Apoptosis Differentiation Cell-cycle arrest</td>
</tr>
<tr>
<td>FR901228 (Depsipetide)</td>
<td>Tetrapeptide</td>
<td>μM</td>
<td>CDKN1A, MAGE3, NY–ESO1, CD86</td>
<td>CD95L, CMYC cyclin D,</td>
<td>Apoptosis Cell-cycle arrest</td>
</tr>
<tr>
<td>MS-27-275</td>
<td>Benzamidine</td>
<td>μM</td>
<td>CDKN1A, gelsolin, TGFBR2</td>
<td></td>
<td>Cell-cycle arrest</td>
</tr>
</tbody>
</table>

_BAK_, BCL2 antagonist/killer protein; _BAX_, B-cell-associated _X_ protein; _BCL2_, B-cell lymphoma protein 2; _BCLXL_, BCL2-related protein, long form; _CD95L_, CD95 ligand; _CDKN1A_, cyclin-dependent-kinase inhibitor 1A; _CDKN1B_, cyclin-dependent-kinase inhibitor 1B; _CDKN1C_, cyclin-dependent-kinase inhibitor 1C; _PCPA3_, carboxypeptidase A3; _CT_, cyclic tetrapeptide; _DHFR_, dihydrofolate reductase; _ER_, oestrogen receptor; _GATA2_, GATA-binding protein 2; _HA_, hydroxamic acid; _HDAC_, histone deacetylase; _HIF1A_, hypoxia-inducible factor 1, α-subunit inhibitor; _HSP86_, heat-shock protein (90 kDa); _ICAM1_, intercellular adhesion molecule 1; _IFNB_, interferon-β; _IFNG_, interferon-γ; _IGFBP3_, insulin-like-growth-factor binding protein 3; _IL_, interleukin; _MAGE3_, melanoma antigen, family A, 3; _MHC_, major histocompatibility complex; _MLH1_, mutL homologue 1; _BMYB_, _myb_ myeloblastosis viral oncogene homolog (avian)-like 2; _CMYB_, _myb_ myeloblastosis viral oncogene homolog (avian); _CPA3_, _carboxypeptidase A3_; _CT_, cyclic tetrapeptide; _HSP86_, heat-shock protein (90 kDa); _ICAM1_, intercellular adhesion molecule 1; _IFNB_, interferon-β; _IFNG_, interferon-γ; _IGFBP3_, insulin-like-growth-factor binding protein 3; _IL_, interleukin; _MAGE3_, melanoma antigen, family A, 3; _MHC_, major histocompatibility complex; _MLH1_, mutL homologue 1; _BMYB_, _myb_ myeloblastosis viral oncogene homolog (avian)-like 2; _CMYB_, _myb_ myeloblastosis viral oncogene homolog (avian); _AI2_, plasminogen activator inhibitor type 2; _PKCD_, protein kinase C-δ; _PU.1_, _PU.1_ transcription factor; _RARβ_, retinoic-acid receptor-β; _SCFA_, short-chain fatty acid; _TG1_, transglutaminase type 1; _TGFBR2_, transforming growth factor-β1; _TGFB1_, transforming growth factor-β1; _TIMP3_, tissue inhibitor of metalloproteinase 3; _VEGF_, vascular endothelial growth factor; _VHL_, von Hippel–Lindau syndrome

All of the four groups of HDACi have similar trends structurally. The structure of HDACi are characterized by these main features: a coordinating group/zinc binding group (ZBG) (such as a thiol or hydroxamic acid) to chelate to Zn$^{2+}$ in the active site, a hydrophobic region (capping group), and a 5 to 7 carbon
linker that connects the cap group to ZBG\textsuperscript{68}. HDAC inhibitors generally adopt a “monkey wrench” scaffold in which a C5 (or C6 in some cases) linker connects a cap region and a zinc-binding group (ZBG)\textsuperscript{68}. The cap is relatively flexible and mediates surface-to-surface interactions between drug and protein target; the ZBG is critical for HDAC inhibitory activity by chelating a zinc ion in the catalytic center of HDACs.

1.1.5 Mechanisms of HDACs

In 1999, the first experiments were carried out to study and to understand how HDACi bind to their enzymes\textsuperscript{69}. The enzyme used in this binding studying was histone deacetylase-like protein (HDLP), a homolog from the hyperthermophilic bacterium \textit{Aquifex aeolicus}. This HDLP shares a similar sequence with Class I protein, HDAC1 by 35.2\%\textsuperscript{69}. The main goal of Dr. Finnin’s work was to determine the structure of the binding pocket and also what main parts of the inhibitors were binding to the enzyme/pocket. The structure of the complexes of TSA and SAHA with HDLP were clearly measured to 2.0 angstroms (Å) resolution. Analysis of the X-ray crystal revealed that the region interacting with TSA or SAHA of HDLP contains three main features (figure 1.7), 1) a surface recognition section, 2) a tube-like, 11 Å deep channel and 3) a 14 Å long, tapered pocket which attaches to the channel\textsuperscript{70}. 
HDACi treatment is estimated to alter the expression level of approximately 2%–10% of genes, with as many genes downregulated as upregulated\textsuperscript{70}. The most studied of the upregulated genes is CDKN1A, which encodes the cell cycle inhibitor kinase p21\textsuperscript{70}. Although the antiproliferative effect of HDACi can be explained by having this gene re-expressed, cell-cycle arrest is not crucial for HDACi-mediated tumour suppression because HDACis causes cell-cycle arrest in normal and cancer cells alike, and unlike many chemotherapeutic drugs, HDACis can kill proliferating cancer cells\textsuperscript{71}. As for the method of this tumour cell killing, there are a plethora of routes through which it can occur. The main process through which HDACis induce cancer cell cytotoxicity is apoptosis\textsuperscript{71} (programmed
cell death). Apoptosis can occur through the extrinsic (death-receptor) or the intrinsic (characterized by cytochrome C release from mitochondria$^{71}$).

**Figure 1.8:** How HDACi mechisam of Apoptosis, Differentiation, Cell-cycle arrest, and Immune modulation$^{72}$

Pathway$^{72}$. Interestingly, the pro-apoptotic transcription factor p53$^{72}$ does not appear to be required for HDACi-induced apoptosis. Rather, the pro-apoptotic Bax and BH3-only proteins Bid or Bim are necessary for this effect$^{73}$. Lastly, overexpression of the antiapoptotic proteins Bcl2 or BclXL leaves apoptosis
induction by HDACi\textsuperscript{73}. Apoptosis induced by HDACi also depends on high levels in reactive oxygen species (ROS) fueled by HDACi treatment. This occurrence likely mimics the inability of tumour cells to upregulate thioredoxin (an antioxidant protein) during HDACi-induced ROS accumulation\textsuperscript{73}. HDACis can also stimulate genomic volatility by impairing DNA damage repair\textsuperscript{34} and by causing mitotic disaster (mis-segregation of chromosomes because of defects in the mitotic spindle checkpoint)\textsuperscript{74}. Also, HDACis can still induce cancer cell death by an autophagy-dependent processes\textsuperscript{74} which were demonstrated in an experiment with conditions blocking apoptosis. This is exciting for future research in the development of new HDACi, because it shows that HDACis may still be valuable against cancers with apoptosis defects\textsuperscript{74}. Additionally, HDACis can sensitize tumour cells for destruction by the immune system\textsuperscript{75} with stimulation of the host immune system. In summary, HDACis can stimulate diverse cellular and physiologic processes that operate together to prevent the initiation and progression of cancer.

1.1.6 Use of HDAC Inhibitor in Combination with Other Chemotherapies

The most beneficial and favorable use of HDACis is extremely likely to be in combination with other anticancer therapies. Many studies have shown that HDACis can join with a large set of other chemotherapeutic drugs (etoposide, cisplatin, bortezomib, and gemcitabine).\textsuperscript{76-77}
Recent research has shown synergism between HDACis and other compounds which can be combined to form a single hybrid compound. The first compound of this kind is triciferol (figure 1.9), in which the side chain of 1,25-vitamin D₃ is replaced with the dienyl hydroxamic acid of TSA⁷⁸ which ultimately possesses characteristics of both 1,25-D³ (vitamin D receptor agonism) and TSA (histone hyperacetylation) and exhibits property similar to co-treatment with the two original compounds in terms of cancer cell cytotoxicity in vitro⁷⁸. It will be a tremendous step forward to see in the future if this compound is just as effective in animal studies and human clinical trials, as it was in cancer cell cytotoxicity in vitro. HDACis will be combined with other new drugs to form new antitumorigenic, antibacterial and antiviral hybrid compounds.

**Figure 1.9:** Triciferol exhibits effects similar to co-treatment with both compounds (cholecalciferol and TSA) in terms of cancer cell cytotoxicity in vitro.⁷⁹
1.2 Rationale design and biological evaulauions of Frangents

Thailandepsin A (TDP-A) is a natural product that was isolated from the bacteria *Burkholderia thailandensis* E264 by post-doctoral student Dr. Cheng Wang in Dr. Yi-Qiang Cheng’s laboratory in the Biological Sciences Department at the University of Wisconsin-Milwaukee. Dr. Cheng’s extensive studies of FK228 biosynthesis in Chromobacterium violaceum no. 968 have led to the discovery of thailandepsin A (TDP-A) and thailandepsin B (TDP-B) (figure 1.10), two new natural analogues of FK228. This work represents a remarkable example of genomics-guided smart discovery of new chemical entities. TDPs exhibited potent inhibitory activities against class I HDACs, which is outlined in table 1.4.

Figure 1.10: How TDP-A is produced from the bacteria *Burkholderia thailandensis* E264
**TDP-A** had IC\textsubscript{50} values of 14nM and 3.5nM in HDAC1 and HDAC2 respectively. National Cancer Institute-60 (NCI-60) screening showed that **TDP-A** and **TDP-B** possess broad-spectrum antiproliferative activities (GI\textsubscript{50} values in single to sub nM range) and potent cytotoxic activities (LC\textsubscript{50} values in sub µM range) towards human cancer cell lines derived from colon, melanoma, ovarian, breast, CNS, non-small cell lung and renal cancers. However, one problem that currently exists is **TDPs** have low solubility in water and showed undesirable levels of cytotoxicity (LC\textsubscript{50}) in NCI testing (**table 1.5**).

**Table 1.4:** Enzyme Inhibition on HDAC proteins (IC\textsubscript{50} values are in µM)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>HDAC1</th>
<th>HDAC2</th>
<th>HDAC 3</th>
<th>HDAC4</th>
<th>HDAC6</th>
<th>HDAC7</th>
<th>HDAC8</th>
<th>HDAC9</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6.7</td>
<td>1.5</td>
<td>0.018</td>
<td>&gt;50</td>
<td>12</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>2\textsuperscript{a}</td>
<td>0.0053</td>
<td>0.0039</td>
<td>0.0053</td>
<td>0.47</td>
<td>0.33</td>
<td>3.2</td>
<td>0.026</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>7.5</td>
<td>39</td>
<td>0.087</td>
<td>&gt;50</td>
<td>9.9</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>6\textsuperscript{a}</td>
<td>0.014</td>
<td>0.0035</td>
<td>0.0048</td>
<td>42</td>
<td>0.38</td>
<td>11</td>
<td>1.2</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>7.7</td>
<td>4.5</td>
<td>0.023</td>
<td>22</td>
<td>11</td>
<td>27</td>
<td>30</td>
<td>&gt;50</td>
</tr>
<tr>
<td>7\textsuperscript{a}</td>
<td>0.0065</td>
<td>0.0067</td>
<td>0.0094</td>
<td>18</td>
<td>0.61</td>
<td>24</td>
<td>1.0</td>
<td>30</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Compounds were reduced/activated prior to being assayed.
Table 1.5: National Cancer Institute-60 (NCI-60) screening

<table>
<thead>
<tr>
<th>Agent</th>
<th>Pairwise correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Agent coefficients with Z (FK228)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Agent</th>
<th>Non-Small Cell Lung Cancer</th>
<th>Small Cell Lung Cancer</th>
<th>Colorectal Cancer</th>
<th>Ovarian Cancer</th>
<th>Breast Cancer</th>
<th>Prostate Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCRF-CEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL-60 (TB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k-562</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOLT-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPMI-8226</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS49/ATCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CECX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOP-62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOP-92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H295</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H322M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H660</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COLO205</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCC-2998</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT-116</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV-40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-295</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-339</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNB-16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNB-75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U251</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOXIMV1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALME-3M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-MEL-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-MEL-28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-MEL-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UACC-255</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UACC-42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGROV1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVCAR-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVCAR-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVCAR-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVCAR-8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI/ADR-RES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-OV-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>786-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A498</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-370</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA-MB-231/ATCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT-549</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-47D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean percent response values (µM)
In order to obtain new compound derivatives or fragments that have reduced cytotoxicity but still retain adequate HDAC inhibitory activity as well as antitumor activity, structure activity relationship (SAR) studies must be carried out on TDP-A. The goal of these SAR studies is to find a compound that is more selective to one or two Class I HDAC proteins. To reduce cytotoxicity, it is crucial to design a compound that is selective to just one or two HDAC proteins because in theory, the inhibitor will be more specific to the substrate it binds to and not bind to a general protein class. Rational design and synthesis of 18 designer compounds (figure 1.11) are based on the scaffolds of

**Figure 1.11:** Rational design and synthesis of 24 designer compounds
TDP-A, TDP-B, SAHA and FK228.

Therefore, our designer molecules will keep a critical ZBG (the thiol group from FK228/TDPs or the hydroxamic acid group from SAHA) and the C5 linker (figure 1.11). TDF1, TDF2, TDF3 and TDF1t, TDF2m, TDF3m (Set 1) are TDP-A fragments with gradual increasing sizes; this set of compounds is designed to test the influence of molecule size on bioactivity and has the highest priority. TDF4, TDF5, TDF6 and TDF4m, TDF5m, TDF6m (Set 2) all have the same size as TDF2 but with different hydrophobic side chains. This set of compounds is designed to test the influence of different side chains on bioactivities. TDF7, TDF8, TDF9 and TDF7m, TDF8m, TDF9m (Set 3) contain a hydroxamic acid ZBG inspired by SAHA in place of a thiol ZBG as seen in all activated FK228/TDP-class HDAC inhibitors. This set of compounds is designed to test the effect of an alternative ZBG in combination with different molecule sizes. Once all compounds are synthesized, they will be purified by HPLC and characterized by LC-MS, HRMS and NMR (¹H and ¹³C).

1.2.2 In vitro and in vivo testing parameters:

After characterization is complete, the in vitro testing will begin. The HDAC inhibitory activity of compounds (including all synthetic compounds, and SAHA, FK228, TDP-A and TDP-B as references) will be determined with a fluorogenic assay which had been successfully applied to TDPs⁷⁹. Each compound will be assayed against three isoforms of human HDACs (HDAC1, HDAC4 and HDAC6,
which represent class I, class IIa and class IIb HDACs, respectively), and the IC50 value (in μM) will be determined accordingly.

After in vitro testing is complete, in vivo testing will begin. The antiproliferative activity of compounds will be evaluated with an MTT assay used routinely by the NCI-DTP program (detailed protocol available at http://dtp.nci.nih.gov/branches/ btb/ivclsp.html). The cancer lines that will be tested are cervical (HeLa), colon (HCT-116 and COLO-205), renal (RFX-393), breast (MDA-MB-231) and prostate (DU-145). These cells lines have shown to be sensitive to TDPs in our previous study. The GI50 value (in μM) will be determined accordingly.

Next, the acute toxicity of compounds will be assessed in healthy mice following the standard NCI-DTP protocol (available at http://dtp.nci.nih.gov/branches/btb/acute_tox.html)79. Because HDAC inhibitors are known or expected to be very potent or toxic, a single mouse is given a single IV dose of 100 mg/kg, a second mouse receives a dose of 50 mg/kg and a third mouse receives a single dose of 25 mg/kg. The mice are observed for a period of two weeks. They are sacrificed if they lose more than 20% of their body weight or if there are other signs of significant toxicity. If all three mice must be sacrificed, the next three dose levels (12.5, 6.25 and 3.125 mg/kg) are tested in a similar manner. This process is repeated until a tolerated dose is found, and the MTD value is determined according.
1.3 Synthesis of natural product derivatives

Natural HDAC inhibitors such as SAHA, FK228 TDP-A and TDP-B have synthetic routes previously published, but none of the previous work focused on fragments or modifications from the natural product TDP-A. Having a good amount of TDP-A in-hand, we decided to synthesis three derivatives from the natural product. The initial three compounds that were synthesized were TDC1, TDC2, and TDC3. This first set of compounds was designed from natural product TDP-A by modifying the hydroxyl handle. Dr. Weiping Tang’s group (Xiaoyun Li) at the University of Wisconsin-Madison added the corresponding group on these three derivatives. The synthetic procedure for TDP-A analogues is shown in scheme 1.1. TDP-A was reacted under basic conditions (pyridine, DMAP) with the corresponding acid chloride to produce TDC1 (82% yield, R=benzoyl), TDC2 (85% yield, R=ethyl formate) and TDC3 (92% yield, R=acetyl).
**Scheme 1.1.** Reagents and conditions: (a) pyridine (1 mL), DMAP (one crystal), RCOCl (1.5 equiv), 0°C to 25°C, 40 h, **TDC1-82%, TDC2-85%, TDC3-92%,**
1.3.2 Synthesis of fragments related to TDP-A

The rest of the compounds designed in this project were small molecule fragments related to the natural product TDP-A which all test specific traits of the natural product (toxicity, solubility and amino acid specificity). We envisioned that the preparation of the key intermediate, achiral β-hydroxy acid 4 (scheme 1.2), would follow a similar procedure developed by Simon and Ganesan from the 3 carbon starting material acrolein. Next, we would assemble the two amino acids
by a peptide coupling reaction to yield dipeptide (MetCys). Then finally, 4 and MetCys would be coupled together with another peptide coupling reaction.

The actual synthetic route for the β-hydroxy acid and fragments TDF1 and TDF1t are outlined in scheme 1.3. The S_N2 reaction with Triphenylmethyl mercaptan and acrolien 1 followed by the Wittig reaction provided aldehyde 2. This one pot protocol to form α,β-unsaturated aldehyde 2 gave 65% overall yield in two steps. The desired aldol product 3 was achieved in 85% yield from the condensation of aldehyde 2 and t-butyl acetate as the enolate source. Hydrolysis of the t-butyl ester 3 with lithium hydroxide provided the β-hydroxy acid 4 in 95% yield. The β-hydroxy acid 4 and the β-hydroxy t-butyl ester 3 were treated with iodine in the presence of sodium acetate to give the disulfide dimers TDF1 and TDF1t in 68% and 52% yield, respectively. NMR, HRMS and HPLC data confirmed the products.

Turning our attention to the synthesis of TDF2 and TDF3 (scheme 1.4), β-hydroxy acid 4 was reacted with D-methionine methyl ester hydrochloride in the presence of the coupling reagent PyBOP (benzotriazol-1-yloxytriyrrolidinophosphonium hexafluoro-phosphate) and Hunig’s base (DIPEA). This yielded the coupled product 6 in 81% yield. Saponification with lithium hydroxide of the methyl ester 6 provided carboxylic acid 7 in 90% yield. The coupling reaction with the amino acid D-cystine methyl ester hydrochloride produced dipeptide product 8 in 81% yield. The hydrolysis of the methyl ester 9 with lithium hydroxide gave the free carboxylic acid 10. Deprotection of trityl with
iodine and sodium acetate of the carboxylic acid 9 produced the disulfide product TDF3 in 36% yield.

**Scheme 1.3.** Reagents and conditions: (a) (1) HSCPh$_3$ (1.0 equiv), Et$_3$N (1.4 equiv), CH$_2$Cl$_2$, 25 °C, 1 h, 99%, (2) (triphenylphosphoranylidene) acetaldehyde (1.0 equiv), benzene, 80 °C, 12 h, 60%, (b) t-butyl acetate (5.0 equiv), n-BuLi (5.5 equiv), DIPEA (5.5 equiv), -78 °C, 2 h, 82% (c) LiOH (20 equiv), THF/H$_2$O(4:1), 50 °C, 12 h, 95% (d) I$_2$ (0.5 equiv), NaOAc (1.0 equiv) MeOH/CH$_2$Cl$_2$ (10:1) 0 °C, 12 h, TDF1t 52%, TDF1 68%.
Other disulfide products \textbf{TDF2, TDF2m, TDF3m} were synthesized from \textbf{6, 7, 8, and 9} under the same iodine conditions giving \textbf{35\%, 26\%, and 30\%}, yields respectively. From a practical point of view, the aldol sequence is concise, providing the disulfide product of \textbf{TDF2} in six steps and \textbf{TDF3} in eight steps from acrolein \textbf{1}. The overall yield for disulfide products of \textbf{TDF2} and \textbf{TDF3} was \textbf{13\%} and \textbf{7\%}, respectively, from acrolien. The synthesis can routinely be performed on a multigram scale. All compounds were confirmed by NMR, HRMS and HPLC and are in the appendix.

\textbf{Scheme 1.4.} Reagents and conditions: (a) D-methionine methyl ester hydrochloride (1.0 equiv), PyBop (1.0 equiv), DIPEA (3.0 equiv), $\text{CH}_2\text{Cl}_2$, 25 °C, 6 h, 81\% (b) LiOH (20 equiv), THF/H$_2$O(4:1), 25 °C, 12h, 90\% (c) D-cystine (Trt)-OH (1.5 equiv), PyBop (1.0 equiv), DIPEA (3.0 equiv), $\text{CH}_2\text{Cl}_2$, 25 °C, 6 h, 81\% (d) LiOH (20 equiv), THF/H$_2$O(4:1), 25 °C, 12h, 90\% (e) I$_2$ (0.5 equiv), NaOAc (1.0 equiv) MeOH/$\text{CH}_2\text{Cl}_2$ (10:1) 0 °C, 12 h. % Yield: \textbf{TDF2m: 25.7\%, TDF2: 34.8\%, TDF3m: 29.4\%, TDF3: 35.6\%}. 
Derivatives **TDF4, TDF5 and TDF6** which have different aliphatic side chain amino acids were synthesized by a similar sequence using the β-hydroxy acid and PyBop coupling agent to attach the amino acid followed by deprotection of the methyl esters with lithium hydroxide to produce **14, 15, and 16 (scheme 1.5)**. Then carboxylic acids **14, 15, 16** were treated in iodine and sodium acetate conditions to yield disulfide products **TDF4 (25.4%), TDF5 (32.4%) and TDF6 (42.4%).** The corresponding methyl esters **11, 12, 13** were reacted using similar iodine/sodium acetate conditions to yield the disulfide dimers **TDF4m (52%), TDF5m (34%) and TDF6m (15%),** respectively.
1.3.3 Trouble-shooting synthesis of fragments

In amino acid chemistry, protecting groups are vital in peptide coupling. Triphenylmethylene, or trityl (Trt), is an important protecting group commonly used for sulfur containing amino acids such as in the amino acid cysteine, and is often employed in reactions that involve disulfide bond formation. Common methods to cleave trityl groups (R-S-Trt) involve acidification\textsuperscript{82-84} to form the thiol (R-S-H). The difficulty of this process is that the reaction with acid would cleave allylic alcohols to yield a dehydrated product. If we wanted to use this method, employing a protecting group on the allylic alcohol would be the most plausible opinion.

Iodine (I\textsubscript{2}) has been an attractive tool used previously in the literature for disulfide bond formation with the protecting group triphenylmethylene. Using iodine deprotects the sulfur (R-STrt) while simultaneously forming the coupled product (R-S-S-R).

**Table 1.6: Acid trials to remove trityl protecting group**

<table>
<thead>
<tr>
<th>entry</th>
<th>reagent(s) (equiv)</th>
<th>solvent</th>
<th>temp(°C)</th>
<th>time</th>
<th>yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TFA (&gt;10)</td>
<td>DCM</td>
<td>25</td>
<td>1 h</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>TFA (5)</td>
<td>DCM</td>
<td>25</td>
<td>2 h</td>
<td>N.R.</td>
</tr>
<tr>
<td>3</td>
<td>TFA (1)</td>
<td>DCM</td>
<td>25</td>
<td>24 h</td>
<td>trace</td>
</tr>
<tr>
<td>4</td>
<td>TFA(1),(i-Pr)\textsubscript{3}SiH(2)</td>
<td>DCM</td>
<td>25</td>
<td>24 h</td>
<td>N.R.</td>
</tr>
</tbody>
</table>

\[
\text{HO} \quad \text{OH} \quad \text{\textsuperscript{S}} \quad \text{Trt} \quad \xrightarrow{TFA \text{ EtOH}} \quad \text{HO} \quad \text{\textsuperscript{S}} \quad \text{SH}
\]
Table 1.6: Hg$^{2+}$ and Ag$^+$ cations trials to remove trityl protecting group

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent(s) (equiv)</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hg(OAc)$_2$ (1 eq.), DTE (1 eq.), NaOAc (1 eq.)</td>
<td>EtOH</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>Hg(OAc)$_2$ (1 eq.), DTE (1 eq.), MeOH/THF (3:1)</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hg(OAc)$_2$ (1 eq.), DTE (1 eq.), MeOH/THF (3:1)</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hg(OAc)$_2$ (1 eq.), DTE (1 eq.), MeOH/THF (3:1)</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ag(NO$_3$) (1 eq.), DTE (1 eq.), MeOH/THF (3:1)</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ag(NO$_3$) (1 eq.), DTE (1 eq.), MeOH/THF (3:1)</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ag(NO$_3$) (1 eq.), DTE (1 eq.), MeOH/THF (3:1)</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ag(NO$_3$) (1 eq.), DTE (1 eq.), MeOH/THF (3:1)</td>
<td>25</td>
<td>10 h</td>
<td>N.R.</td>
<td></td>
</tr>
</tbody>
</table>
When running β-hydroxy acid 4 in trifluoroacetic acid (TFA) we observed the deprotection of the trityl group, and also the α,β-unsaturated alcholol group dehydrated to form the conjugated product 40. The results are shown in table 1.6. Furthermore, cleavage of the protecting group with reactive metals such as silver and mercury react readily to give the metal-sulfur intermediate, but are difficult to further remove the metal and obtain the thiol product (table 1.7). Next we turned to iodine. After studying a large variety of reaction conditions with iodine, we found a straightforward approach to deprotect the sulfur (R-STrt) and simultaneously form the disulfide bond (R-S-S-R) without affecting the allylic alcohols to any extent. Those results are summarized in table 1.8.
Table 1.8: Iodine reactions to cleave tryt protecting group

<table>
<thead>
<tr>
<th>entry</th>
<th>reagent(s)</th>
<th>solvent</th>
<th>temp (ºC)</th>
<th>time</th>
<th>yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I$_2$ (10)</td>
<td>MeOH</td>
<td>25</td>
<td>1 h</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>I$_2$ (5)</td>
<td>MeOH</td>
<td>25</td>
<td>1 h</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>I$_2$ (1)</td>
<td>MeOH</td>
<td>25</td>
<td>1 h</td>
<td>trace</td>
</tr>
<tr>
<td>4</td>
<td>I$_2$ (5), NaOAc (5)</td>
<td>MeOH/DCM (1:1)</td>
<td>25</td>
<td>1 h</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>I$_2$ (2), NaOAc (1)</td>
<td>MeOH/DCM (1:1)</td>
<td>25</td>
<td>2 h</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>I$_2$ (10)</td>
<td>MeOH/DCM (1:10)</td>
<td>25</td>
<td>1 h</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>I$_2$ (5)</td>
<td>MeOH/DCM (1:10)</td>
<td>25</td>
<td>1 h</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>I$_2$ (1)</td>
<td>MeOH/DCM (1:10)</td>
<td>25</td>
<td>1 h</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>I$_2$ (10), NaOAc (10)</td>
<td>MeOH/DCM (1:10)</td>
<td>25</td>
<td>1 h</td>
<td>13</td>
</tr>
<tr>
<td>10</td>
<td>I$_2$ (5), NaOAc (5)</td>
<td>MeOH/DCM (1:10)</td>
<td>25</td>
<td>1 h</td>
<td>34</td>
</tr>
<tr>
<td>11</td>
<td>I$_2$ (2), NaOAc (2)</td>
<td>MeOH/DCM (1:10)</td>
<td>25</td>
<td>2 h</td>
<td>59</td>
</tr>
<tr>
<td>12</td>
<td>I$_2$ (2), NaOAc (1)</td>
<td>MeOH/DCM (1:10)</td>
<td>25</td>
<td>2 h</td>
<td>68</td>
</tr>
<tr>
<td>13</td>
<td>I$_2$ (2), NaOAc (1)</td>
<td>MeOH/DCM (1:10)</td>
<td>-78</td>
<td>2 h</td>
<td>N/A</td>
</tr>
</tbody>
</table>
1.4 Biological evaluations

1.4.1 Isozyme Selectivity.

The pure compounds of all fragments and natural product derivatives were evaluated for inhibitory effects on three different groups of HDAC proteins (class I, IIa, and IIb). The natural products FK228, SAHA, TDP-A, and TDP-B were used as controls. All activity was determined by using a fluorescence-based in vitro assay (HDAC-GloTM I/II Assay and Screening System (Promega)). Class I HDAC proteins evaluated were HDAC1, HDAC2, HDAC3 and HDAC8. Class IIa HDAC protein was HDAC4 and the Class IIb HDAC protein was HDAC6. The results of the enzyme assays are shown in table 1.9.

The first group designed and analyzed was the TDP-A derivatives TDC1, TDC2, and TDC3 which had different aliphatic groups on the alcohol. TDC1 had the largest group (benzoyl), TDC3 had the smallest chain (acetyl group) and TDC2 had a medium chain attached (ethyl formate). All showed similar HDAC inhibition compared to the natural products to all class of HDAC proteins (HDAC I, IIa, and IIb). TDC3 had the best inhibition of the three derivatives. Having a large group (benzoyl group) on the hydroxyl handle in TDC1 nearly abolished selectivity toward Class IIb HDAC (HDAC4) (table 1.9). TDC3 showed internal selectivity to class I HDACs (HDAC1: 2.7nm, HDAC2: 44nm, HDAC3:245nm, and HDAC8: 10,000nm). By adding a group to the alcohol in TDP-A, the general results in similar inhibition to HDAC1, HDAC2 and HDAC8, a 100 fold decrease in activity to HDAC3, and roughly a 10 fold decrease to HDAC4 and HDAC6. This decrease in activity is presumably due to the cap group interacting with the protein surface
outside the catalytic pocket. On TDP-A, the alcohol group has a hydrogen bond donor while the R groups added are a hydrogen bond accepter. Overall, the natural product derivatives (TDC1, TDC2, TDC3) are less selective than the pan-HDAC inhibitor TDP-A, while increasing selectivity to Class I HDACs. Next, with the linker and ZBG groups (R-S-H) very specific in our natural products (TDP-A, TDP-B and FK228), we investigated the cap group. The first fragment designed was TDF1, which had the same linker and ZBG as the natural products (an allylic alcohol connected by 5 carbons to a thiol). TDF1 had very potent inhibitory activity to HDAC1 (6nm) and HDAC2 (44nm) while exhibiting very poor activity against Class IIa (HDAC4) and IIb (HDAC 6) (table 1.9). Intrigued by the selectivity to class I HDACs, we sought to further increase the selectivity for HDAC1 and HDAC2 inhibition. The first change we made was to add a t-butyl group onto the carboxylic acid cap end (TDF1t) to decrease the hydrogen bonding to surface interactions. However, this change actually resulted in increased inhibition of HDAC6 (class IIa) by roughly five-fold.

Encouraged by the initial results, we added two amino acids to our next fragments (methionine first, cysteine second) in sequence for the next four fragments (TDF2, TDF2m TDF3 and TDF3m) (table 1.9). In general, HDAC inhibition for this set of fragments was similar to that observed for TDF1. Interestingly, while TDF2 and TDF2m showed some inhibitory activity toward Class II HDACs, HDAC6 (high micromolar), TDF3 and TDF3m showed no inhibitory activity. TDF3 and TDF3m was the first closed ring fragments used in
this study. Also, replacing the carboxylic acid and adding a methyl ester to the fragments instead kept inhibition the same.

The last set of compounds that were designed was based on the first fragment **TDF1** which had the best selectivity for class I HDACs. **TDF4**, **TDF5** and **TDF6** tested the different aliphatic side chains to see the importance between the natural product side chains (**TDP-A**: R=SCH$_3$, **TDP-B**: R=CH$_2$CH$_3$) with the amino acids norleucine and valine. This set of fragments had very similar activity, except all of the methyl esters fragments (**TDF4m**, **TDF5m** and **TDF6m**), which were about 10-fold less active than the carboxylic acid derivative toward all class I HDACs.

In summary, **TDC3** had the best inhibition for the natural product derivatives and for the fragments **TDF1t** and **TDF5** showed similar inhibition. All fragments showed high selective inhibition to Class I over Class II. Furthermore, all fragments showed 100 to 1000-fold better selectivity than FDA approved **SAHA** for HDAC1 to HDAC8 ratios, which exemplify and highlight the internal selectivity to class I HDACs (last two columns in **table I.9**).
Table 1.9. HDAC inhibitory activity (IC$_{50}$ in µM)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ of HDAC1</th>
<th>IC$_{50}$ of HDAC2</th>
<th>IC$_{50}$ of HDAC3</th>
<th>IC$_{50}$ of HDAC4</th>
<th>IC$_{50}$ of HDAC6</th>
<th>IC$_{50}$ of HDAC8</th>
<th>IC$_{50}$ of HDAC4</th>
<th>IC$_{50}$ of HDAC6</th>
<th>Ratio HDAC1/HDAC8</th>
<th>Ratio HDAC1/HDAC6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAHA</td>
<td>0.0207</td>
<td>0.0694</td>
<td>0.1697</td>
<td>7.04</td>
<td>116.73</td>
<td>0.1794</td>
<td>0.01399</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FK228</td>
<td>0.0038</td>
<td>0.0183</td>
<td>0.0187</td>
<td>1.48</td>
<td>2.37</td>
<td>0.5075</td>
<td>0.00035</td>
<td>0.0075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF-P</td>
<td>0.0022</td>
<td>0.0256</td>
<td>0.0482</td>
<td>10.90</td>
<td>27.93</td>
<td>0.6503</td>
<td>0.000026</td>
<td>0.0034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF-A</td>
<td>0.0024</td>
<td>0.0081</td>
<td>0.0077</td>
<td>8.44</td>
<td>20.48</td>
<td>0.2734</td>
<td>0.000065</td>
<td>0.0088</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDC1</td>
<td>0.0109</td>
<td>0.0510</td>
<td>0.6398</td>
<td>3.67</td>
<td>~500</td>
<td>5.49</td>
<td>0.00252</td>
<td>0.0020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDC2</td>
<td>0.0096</td>
<td>0.0370</td>
<td>0.7010</td>
<td>4.33</td>
<td>134.57</td>
<td>1.49</td>
<td>0.00096</td>
<td>0.0064</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDC3</td>
<td>0.0027</td>
<td>0.0442</td>
<td>0.2452</td>
<td>10.03</td>
<td>40.48</td>
<td>1.34</td>
<td>0.00006</td>
<td>0.0020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF1</td>
<td>0.0068</td>
<td>0.0443</td>
<td>0.5791</td>
<td>41.91</td>
<td>&gt;1000</td>
<td>165.94</td>
<td>0.000020</td>
<td>0.000004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF1t</td>
<td>0.0024</td>
<td>0.0245</td>
<td>0.5401</td>
<td>34.21</td>
<td>~500</td>
<td>41.31</td>
<td>0.00016</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF2</td>
<td>0.0095</td>
<td>0.0902</td>
<td>0.8098</td>
<td>27.03</td>
<td>&gt;1000</td>
<td>166.37</td>
<td>0.00043</td>
<td>0.00006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF2m</td>
<td>0.0109</td>
<td>0.0726</td>
<td>0.3528</td>
<td>22.31</td>
<td>&gt;1000</td>
<td>46.32</td>
<td>0.00017</td>
<td>0.00024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF3</td>
<td>0.0229</td>
<td>0.0875</td>
<td>0.8186</td>
<td>62.85</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>0.00036</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF3m</td>
<td>0.0125</td>
<td>0.0942</td>
<td>0.6226</td>
<td>63.03</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>0.00028</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF4</td>
<td>0.0039</td>
<td>0.0365</td>
<td>0.7135</td>
<td>43.89</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>0.00003</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF4m</td>
<td>0.0303</td>
<td>2.12</td>
<td>0.0971</td>
<td>116.90</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>0.00061</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF5</td>
<td>0.0026</td>
<td>0.0459</td>
<td>0.8393</td>
<td>49.38</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>0.00002</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF5m</td>
<td>0.0265</td>
<td>1.66</td>
<td>0.1241</td>
<td>133.30</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>0.00159</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF6</td>
<td>0.0035</td>
<td>0.1106</td>
<td>0.0416</td>
<td>16.63</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>0.00003</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF6m</td>
<td>0.0302</td>
<td>1.93</td>
<td>0.1002</td>
<td>100.41</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.4.2 Cellular Assays.

The anti-proliferative activities of the compounds described above were tested against breast, colon, cervical prostate, and renal tumor cell lines in an MTT using the natural products FK228, SAHA, TDP-A and TDP-B as positive controls. The MTT assay is a colorimetric assay for assessing cell viability\textsuperscript{[86]}\textsuperscript{86}. NAD(P)H-dependent cellular oxidoreductase enzymes may reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (normally between 500 to 600 nm) by a spectrophotometer. The degree of light absorption depends on the solvent and how many living cells reduced MTT (yellow) to formazan (purple)\textsuperscript{[87]}\textsuperscript{87}.

Surprisingly, modifications of TDP-A (TDC1, TDC2, and TDC3) increased the GI\textsubscript{50} by 100 to 1000-fold (table 1.10 and table 1.11) despite having similar HDAC inhibition profiles (table 1.9). The synthetic compounds TDF1t, TDF3, TDF3m, and TDF5 had GI\textsubscript{50} values 8-fold lower (GI\textsubscript{50} values of 0.5 µM, 0.6µM, 0.4µM and 0.5µM, respectively) against all tumor cell lines that were tested compared to SAHA, a synthetic FDA approved analogue known to have GI\textsubscript{50} values in the range of 2.5-3 µM. By contrast, TDF2, TDF2m, TDF4 and TDF6 had moderate levels of anti-proliferative activity with GI\textsubscript{50} values within 2µM, 6µM, 5µM and 4µM, respectively, against all three tumor cell lines. Since different tumor cells are known to have different sensitivity to HDAC inhibitors, the top four fragments (TDC1, TDF1t, TDF3m, and TDF5) were tested against four other tumor cell lines derived from ovarian, prostate, breast and colon cancers. In general, the results of these studies were consistent with those obtained using the previous tumor cell lines. Specifically, TDP-A showed the strongest anti-proliferative activity and all fragments showing great inhibitory activity when compared to SAHA (table 1.11).
### Table 1.10. Antiproliferative Activity (GI<sub>50</sub> in µM)

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Colon HCT-116</th>
<th>Cervical HeLa</th>
<th>Renal RFX39 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAHA</td>
<td>2.7</td>
<td>2.8</td>
<td>2.5</td>
</tr>
<tr>
<td>FK228</td>
<td>0.0012</td>
<td>0.0013</td>
<td>0.0012</td>
</tr>
<tr>
<td>TDP-B</td>
<td>0.002</td>
<td>0.0021</td>
<td>0.0022</td>
</tr>
<tr>
<td>TDP-A</td>
<td>0.00093</td>
<td>0.00036</td>
<td>0.00047</td>
</tr>
<tr>
<td>TDC1</td>
<td>0.0247</td>
<td>0.2248</td>
<td>0.0351</td>
</tr>
<tr>
<td>TDC2</td>
<td>0.056</td>
<td>0.1412</td>
<td>0.0510</td>
</tr>
<tr>
<td>TDC3</td>
<td>0.1205</td>
<td>0.332</td>
<td>0.1067</td>
</tr>
<tr>
<td>TDF1</td>
<td>2.1</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>TDF1t</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>TDF2</td>
<td>1.4</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>TDF2 m</td>
<td>2.3</td>
<td>2.6</td>
<td>5.9</td>
</tr>
<tr>
<td>TDF3</td>
<td>0.3</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>TDF3 m</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>TDF4</td>
<td>3.7</td>
<td>4.8</td>
<td>4.1</td>
</tr>
<tr>
<td>TDF4 m</td>
<td>31</td>
<td>20.1</td>
<td>32.1</td>
</tr>
<tr>
<td>TDF5</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>TDF5 m</td>
<td>32.6</td>
<td>30.2</td>
<td>40.1</td>
</tr>
<tr>
<td>TDF6</td>
<td>1.3</td>
<td>3.6</td>
<td>3.1</td>
</tr>
<tr>
<td>TDF6 m</td>
<td>28.4</td>
<td>25.6</td>
<td>31.7</td>
</tr>
</tbody>
</table>

Individual values were derived from the average of triplicate experiments with standard error within 20% margin.

### Table 1.11. Antiproliferative Activity (GI<sub>50</sub> in µM)

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Ovarian nSKOV-3</th>
<th>Prostate DU-145</th>
<th>Breast MDA-MB-231</th>
<th>Colon COLO-205</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAHA</td>
<td>1.5</td>
<td>2</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>TDP-A</td>
<td>0.002</td>
<td>0.001</td>
<td>0.003</td>
<td>0.0035</td>
</tr>
<tr>
<td>TDC1</td>
<td>0.03</td>
<td>0.08</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>TDF1t</td>
<td>0.3</td>
<td>0.2</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>TDF3m</td>
<td>0.5</td>
<td>0.3</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>TDF5</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Individual values were derived from the average of triplicate experiments with standard error within 20% margin.
### Table 1.12. Solubility and MTD (mg/kg)

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Solubility</th>
<th>MTD (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAHA</td>
<td>Not soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>FK228</td>
<td>Partially soluble</td>
<td>3.125</td>
</tr>
<tr>
<td>TDP-B</td>
<td>Not soluble</td>
<td>6.25</td>
</tr>
<tr>
<td>TDP-A</td>
<td>Not soluble</td>
<td>6.25</td>
</tr>
<tr>
<td>TDC1</td>
<td>Not soluble</td>
<td>50</td>
</tr>
<tr>
<td>TDC2</td>
<td>Not soluble</td>
<td>12.5</td>
</tr>
<tr>
<td>TDC3</td>
<td>Not soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF1</td>
<td>Soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF1t</td>
<td>Mostly soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF2</td>
<td>Soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF2m</td>
<td>Partially soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF3</td>
<td>Soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF3m</td>
<td>Partially soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF4</td>
<td>Mostly soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF4m</td>
<td>Soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF5</td>
<td>Mostly soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF5m</td>
<td>Soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF6</td>
<td>Soluble</td>
<td>&gt;200</td>
</tr>
<tr>
<td>TDF6m</td>
<td>Soluble</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

10mg/ml cpd in 20% DMSO/saline and in healthy BALB/c mice, IP. Individual values were derived from the average of triplicate experiments with standard error within 20% margin.

### 1.4.3 MTD Studies

**Maximum Tolerated Dose (MTD) Studies and Solubility.** Synthetic compounds demonstrated significantly higher MTD levels than the natural products studied in this work. To determine the MTD in vivo, the compounds listed above were injected intraperitoneally into BALB/c mice and morbidity and mortality were monitored over two weeks. The parental compounds TDP-A, TDP-B and FK228 were used as positive controls and showed very high toxicity with animals being able to tolerate doses of 6.25 mg/kg for both TDP-A and TDP-B and 3.25 mg/kg for FK228 (Table 1.12). By contrast, the synthetic compounds (fragments and natural product derivatives) were found to be much less toxic to animals with a MTD of more than 200 mg/kg for all except natural product derivatives TDC1 and TDC2. Solubility increased for all fragments that contained an acidic carboxylic acid group (Table 1.12). Generally, the smaller molecules were much more soluble as well.
1.4.4 Molecular Modeling

Among the 11 classified HDAC isoforms, four have their X-ray structures in the Protein Data Bank (PDB): HDAC2 (3MAX), HDAC3 (4A69), HDAC7 (3C0Z), and HDAC8 (1T69). Many of these X-ray crystals have their active site bound with small molecules. For this study we docked TDP-A and fragments TDF1 and TDF3 in HDAC2 (3MAX). HDAC2 (figure 1.12) is also the most potent isoform available of the four X-ray structures and is the protein structure we are trying to selectively inhibit.

The results showed that the natural product TDP-A have similar binding modes and orientation as FK228 and SAHA in the active site (figure 1.13). The thiol of TDP-A chelates with the zinc ion and residuals His 146 and Tyr 308 which centers the molecule in the middle of the catalytic pocket. Also, amino acid residual Asp 104 is important in hydrogen bonds on the surface of the protein with all molecules (figure 1.14). From the data we also concluded that small fragments like TDF1 could chelate to the zinc ion in reverse manner (figure 1.15) while the carboxylic acid is hydrogen bonding to the Tyr 29 residual and water. This may be a great reason why the smaller fragments have increased HDAC1 and HDAC2 inhibition.
Figure 1.12: The lowest energy conformation of crystal structure, HDAC 2(PDB: 3MAX), using the software packages of MOE. It is a trimer which has 3 individual catalytic sites and has a MW of 55.4 kDa and has approximately 488 amino acid residuals.
Figure 1.13: The lowest energy conformation of TDP-A in HDAC2 catalytic core. Natural product TDP-A was docked into a model based on the crystal structure of HDAC2 (PDB: 3MAX), using the software packages of MOE. Surface view of TDP-A was docked in HDAC2 catalytic pocket (1st diagram). Schematic representation (2nd diagram) of TDP-A binding in HDAC 2. The thiol is coordinating to the zinc (Zn$^{2+}$) cation deep in the pocket. The residuals His 146 and Tyr 308 play a key role in centering the molecule in the active site. Also, residual Asp 104 hydrogen bonds to the amide hydrogen in TDP-A. The areas that are highlighted in blue on the protein interaction diagram (2nd diagram) are areas of TDP-A that are outside of the active site. These highlighted areas would have surface interactions with the protein.
Figure 1.14: The lowest energy conformation of TDF1 and TDF3 in HDAC2 catalytic core. Fragments TDF1 and TDF3 were docked into a model based on the crystal structure of HDAC 2 (PDB: 3MAX), using the software packages of MOE. Surface view of TDF1 and TDF3 docked in HDAC2 catalytic pocket (1st diagram). Schematic representation (2nd diagram) of TDF3 binding in HDAC 2. The thiol in both inhibitors (yellow) is coordinating to the zinc (Zn$^{2+}$) cation in the middle of the pocket (1st diagram). The residuals His 145, His 146 and Gly 154 play a key role in centering the two fragments in the active site. Also, residual Asp 104 hydrogen bonds to the amide hydrogen and the carboxylic acid hydrogen in TDF3. The areas that are highlighted in blue on the protein interaction diagram (2nd diagram) are areas of TDF3 that are outside of the active site. These highlighted areas would have surface interactions with the protein.
**Figure 1.15:** The lowest energy conformation of TDF1 in the HDAC2 catalytic core. Fragment TDF1 was docked into a model based on the crystal structure of HDAC 2 (PDB: 3MAX), using the software packages of MOE. Surface view of TDF1 docked in HDAC2 catalytic pocket (1st diagram). Schematic representation (2nd diagram) of TDF1 binding in HDAC 2. The thiol in TDF1 (yellow) is coordinating to the zinc (Zn$^{2+}$) cation in the middle of the pocket. The residuals His 145, His 146 and Gly 154 play a key role in centering the fragments in the active site. Also, residual Asp 104 hydrogen bonds to the carboxylic acid hydrogen in TDF1. This figure is especially important due to the fact that the smallest fragment (TDF1) that was synthesized was the only fragment that can potential bind in the active site in the reverse direction so it does not “stick” out of the pocket.
I.5 Conclusion and Future Directions

HDAC inhibitors have broad implications for the treatment of cancer and other human diseases. The design of this project was to develop HDAC inhibitors that resemble TDP-A as anticancer drugs. The need for additional HDAC inhibitors is well justified by the recent approval of SAHA and FK228 for clinical use. Our successful discovery of thailandepsins and their demonstrated potent HDAC inhibitory activities warrant systematic SAR studies of thailandepsin-based fragment compounds. Future research can take lead compounds from these studies for further evaluations by NCI DTP, and can be used to utilize the obtained data for application of extramural funding.

Since some HDAC inhibitors also have positive implications in central nerve system (CNS) disorders and autoimmune disease, it could be interesting to assess the applications of our acquired compounds for those disorders and diseases as well in the future.
1.6 Experimental for Biological Evaluations

HDAC inhibition assays

HDAC-GloTM I/II Assay and Screening System (Promega) was used to determine the HDAC inhibitory activity. Recombinant human HDAC1, HDAC2, HDAC3, HDAC4, HDAC6 and HDAC8 were purchased from BPS Bioscience Inc. The inhibitory activities of the test compounds against recombinant HDAC were performed according to reagent suppliers’ protocols.

All test compounds were firstly dissolved in DMSO to make 10 mM stock solution. A certain amount of stock solution was diluted to 1 mM by DMSO and then reduced +→+(TCEP) in a molar ration of 1:1.5 for 20 min at ambient temperature prior to being assayed. After reduction, a series of fivefold dilutions of each reduced compound were prepared with HDAC-Glo buffer and 25 µl of each dilution was added into a well on a 96-well plate. HDAC enzymes were diluted to the desired concentrations with HDAC-Glo buffer and 25 µl of each diluted enzyme was dispensed into a well to mix with 25 µl of testing compound at room temperature up for 30-60 seconds. After the reaction mixture had been incubated at room temperature for 1 hour, 50 µl of HDAC-Glo reagent was added to each reaction well and mixed up for 30-60 seconds. Finally, the plate was incubated at room temperature for an additional 30 minutes before the luminescence was measured on a Synergy HT plate reader (Bio-Tek). The luminescence intensity data were analyzed using GraphPad Prism 5 (GraphPad Software). In the absence of any test compound, the luminescence intensity (I100) in each data set
was defined as 100% activity. In the absence of both of HDAC enzymes and test compound, the luminescence intensity (I0) in each data set was defined as 0% activity. The relative activity (%) in the presence of each compound was calculated according to the following equation: %activity = (I-I0)/(I100-I0), where I = the luminescence intensity if a compound is present in the reaction. Experiments were performed in triplicate and the calculated mean values were used for plotting.

**Anti-proliferative assay**

The cytotoxicity of the compounds against the HeLa (cervical cancer, ATCC), RXF393 (renal cancer, ATCC) and HCT-116 (colon cancer, ATCC), SKOU-3 (ovarian cancer, ATCC), DU-145 (prostate cancer, ATCC), MDA-MB-231 (breast cancer, ATCC), and COLO-205 (colon cancer, ATCC) cell lines was determined using the MTT assay as previously described (Ref). Briefly, the tumor cells (1x10^4 cells/well) were seeded in 96-well microtiter plates and incubated for 24 h at 37°C. Following incubation, the media was replaced with fresh media containing the above compounds at concentrations ranging from 1 µM to 1 pM diluted in DMSO and incubated for an additional 48 h. Drugs were run in duplicate or triplicate and control cells received fresh media with DMSO concentration equivalent to the treatment groups. Thereafter, the wells were washed twice with warm PBS and incubated for another 4 h with RPMI 1640 media containing 250 µg/mL of MTT. After aspirating the culture medium, 200 µL of DMSO was added to dissolve the precipitate and the resulting solution was measured for absorbance at 570 nm with a reference wavelength of 690 nm using a microplate reader.
(Infinite M200 Pro TECAN). Results were used to determine the growth inhibition-50% (GI₅₀) of each drug.

**Solubility and Maximum tolerated dose (MTD)**

BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used between 7 and 10 weeks of age. Mice were housed in a conventional animal vivarium and were given free access to food and water. All studies and procedures were approved by the Animal Care and Use Committee of the University of Wisconsin, Milwaukee.

Each animal was initially weighed using a digital scale and then intraperitoneally (IP) injected with the compounds (listed in Table 1). A single mouse was given a single IP dose of 200 mg/kg, body weight, a second mouse received a dose of 100 mg/kg and a third mouse received a single dose of 50 mg/kg. The mice were observed and weighed every day for a period of 8 days. They were euthanized if they lost more than 20% of their body weight or if there were other signs of significant toxicity. If none of the mice survived with the first three doses, the next 3 dose levels (25 mg/kg, 12.5 mg/kg and 6.25 mg/kg) were tested in a similar manner. This process was repeated until a tolerated dose was found, and the MTD value was determined.
1.7 Experimental for chemical synthesis

General Considerations.

All reactions were performed under argon atmosphere via schlenk line in oven-dried glassware with magnetic stirring unless otherwise stated. Air and moisture-sensitive liquids and solutions were transferred via oven-dried, stainless steel syringe and were introduced into the reaction vessel through rubber septa. All reactants and reagents were purchased from Ark Pharm, Inc. unless otherwise stated. CH2Cl2 and DMF (dimethylformamide) were distilled from calcium hydride. THF (tetrahydrofuran) was distilled from sodium-benzophenone. Anhydrous acetonitrile was purchased from Sigma Aldrich. Previously reported compounds were identified by 1H NMR (nuclear magnetic resonance) spectrum. All new compounds were characterized by additional 13C NMR and mass spectroscopy. 1H and 13C NMR spectra were performed on a Bruker NMR at 300 and 75 MHz, respectively. 1H NMR data are reported as follows: chemical shift (δ) in parts per million (ppm) from tetramethylsilane as an internal standard (CDCl3 δ7.26 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets). 13C data were reported as follows: chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane with the solvent as an internal indicator (CDCl3 δ77.16 ppm).
(E)-5-(tritylthio)pent-2-enal. A round bottom flask was charged with 3-(tritylthio)propanal (3.77 g, 11.3 mmol) and 2-(triphenylphosphoranylidene) (3.8 g, 12.5 mmol, 1.1 equiv.). The flask was put under argon and the contents of the flask were dissolved in benzene (90 mL). The solution was then refluxed overnight. The reaction mixture was allowed to cool to room temperature and was then concentrated in vacuo. The crude product was separated twice via column chromatography. The first column was run with a 20% ethyl acetate/hexane solution until the product spot eluted. A second column was run and the product eluted with 10% ether/hexane to give 2.83 g (70%) of pure product. $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) δ (ppm) 9.45-9.44 (d, 1H), 7.44-7.24 (m, 15H), 6.64 (t, 1H), 6.02-5.97 (dd, 1H), 2.36-2.32 (m, 4H); <ju112211> Exp 1 $^{13}$C NMR (CDCl$_3$, 150 MHz) δ (ppm) 193.6, 155.6, 144.5, 133.6, 129.5, 127.9, 126.7, 31.6, 29.9 Fuse, Shinichiro, et al. Total Synthesis of Spiruchostatin B Aided by an Automated Synthesizer. Org. Biomol. Chem. 2011, 9, 3825-3833.

20% EtOAc/Hexane
(E)-1-tert-butoxy-4-hydroxy-8-(tritylthio)oct-5-en-2-one. To a 50 mL round bottom flask was charged with THF (100 mL) and cooled to -78°C. Next, diisopropylethylamine (DIPEA) (4.86 mL, 27.89 mmol, 5.5 equiv.) and n-butyllithium (12.27 mL, 30.68 mmol, 5.5 equiv.) were added dropwise at -78°C and let stir for 1 hr. Tert-butyl acetate (3.7 mL, 27.6 mmol, 5 eqv.) was added at -78°C and was allowed to stir for 1 hr. Lastly, (E)-5-(tritylthio)pent-2-enal (2.0 g, 5.579 mmol, 1 equiv.) was added and let stir for 45 min at -78°C. The reaction was quenched with a saturated solution of NH₄Cl (50 mL) at -78°C and then concentrated in vacuo to remove the organic solvent. Then dichloromethane was added to aqueous mixture and the two phases were separated. Then the aqueous layer was extracted two more times with dichloromethane and the organic layers were combined. The organic layer was washed with NaHCO₃, brine, then dried over anhydrous Na₂SO₄, and concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/ hexane, 1:9) gave 2.06 g (78%) of product as a white solid. ¹H NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 7.43 (d, 6H), 7.30 (m, 9H), 5.62-5.55 (m, 1H), 5.47-5.40 (dd, 1H), 4.42 (s, 1H), 3.09 (bs, OH), 2.43 (m, 2H), 2.23 (m, 2H), 2.10 (m, 2H), 1.47 (s, 9H) <ju010411> Exp 2; ¹³C NMR (75 MHz, CDCl₃, 25 °C) δ (ppm) 172.8, 144.9, 132.1, 129.9, 129.6, 127.9, 126.6, 81.3, 69, 66.6, 42.4, 31.5, 31.4, 28.2 <bs061812> Exp 5.
20% EtOAc/Hexane

(E)-3-hydroxy-7-(tritylthio)hept-4-enoic acid. (E)-1-tert-butoxy-4-hydroxy-8-(tritylthio)oct-5-en-2-one (400 mg, 0.843 mmol, 1.0 equiv.) was dissolved in a 4:1 ratio of THF/water (10 mL). Next, lithium hydroxide (620 mg, 25.89 mmol, 25 equiv.) was added. The solution was then heated to 50°C and stirred for 12 hr. The reaction was then diluted with water (10 mL) and then acidified to a pH of 4-5 with KHSO₄. The aqueous layer was extracted with ethyl acetate (10 mL) four times. The organic layers were combined and washed with water, brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 9:10) gave 353 mg (95%) of product as a white solid. ¹H NMR (300 MHz, CDCl₃, 25 °C) δ 7.47-7.23 (m, 15H), 5.64-5.57 (m, 1H), 5.49-5.42 (dd, 1H), 4.49 (q, 1H), 2.55 (d, 2H) 2.24 (m, 2H), 2.09 (m, 2H) Exp 5; ¹³C NMR (125 MHz, CDCl₃, 25 °C) δ (ppm) 171.9, 144.9, 131.7, 130.7, 129.6, 128.0, 126.7, 68.6, 66.7, 41.3, 31.5, 31.4 Exp 6.
(4E,4'E)-7,7'-disulfanediylbis(3-hydroxyhept-4-enoic acid). Iodine (52.2 mg, 0.206 mmol, 0.5 equiv.) and sodium acetate (33.7 mg, 0.411 mmol, 1.0 equiv.) were dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (10 mL). (E)-3-hydroxy-7-(tritylthio)hept-4-enoic acid (172 mg, 0.411 mmol, 1 equiv.) was dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na$_2$S$_2$O$_3$) solution until the reaction mixture turned clear. Then, brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was
collected at 8-12min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H₂O was used to elute the column under the flow rate of 8 ml/min. The wavelength was set at 200 nm to detect the compound and 46 mg of the pure compound was collected at 12-14.5 min. (31.9% yield.) ¹H NMR (500 MHz, DMSO, 25 °C) δ (ppm) 5.71 (m, 1H), 5.54 (dd, 1H), 4.43 (m, 1H), 2.69 (m, 2H), 2.48 (m, 2H), 2.38 (m, 2H) <ju062012> Exp 1; ¹³C NMR (125 MHz, CD₃OD, 25 °C) δ (ppm) 173.6, 133.3, 128.7, 68.6, 42.0, 37.7, 31.5 <ju062112> Exp 2.

Di-tert-butyl 7,7′-disulfanediyl(4E,4′E)-bis(3-hydroxyhept-4-enoylate). Iodine (58.9 mg, 0.232 mmol, 1.0 equiv.) and sodium acetate (19.0 mg, 0.464 mmol, 2.0 equiv.) were dissolved in a 10:1 solution of CH₂Cl₂/MeOH (5 mL). (E)-1-tert-butoxy-4-hydroxy-8-(tritylthio)oct-5-en-2-one (110 mg, 0.232 mmol, 1.0 equiv.) was dissolved in a 10:1 solution of CH₂Cl₂/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na₂S₂O₃) solution until the reaction mixture turned clear. Then, brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na₂SO₄, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash
Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 15-20 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 70 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wavelength was set at 200 nm to detect the compound and 140 mg of the pure compound was collected at 22-25 min. (65.5 % yield.) $^1$H NMR (300 MHz, CD$_3$OD, 25 °C) $\delta$ (ppm) 5.75 (m, 1H), 5.66 (dd, 1H), 4.47 (m, 1H), 2.78 (m, 2H), 2.44 (m, 4H), 1.50 (s, 9H) $<$bs062712> Exp 2; $^{13}$C NMR (75 MHz, CD$_3$OD, 25 °C) $\delta$ (ppm) 170.9, 133.4, 128.8, 80.5, 68.8, 43.4, 37.7, 31.5, 27.1 $<$bs062712> Exp 4.

20% EtOAc/Hexane

\[
\text{(2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanoic acid. Methyl (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanoate (333 mg,}
\]
0.591 mmol, 1.0 equiv.) was dissolved in a 4:1 ratio of THF/water (10 mL). Next was added lithium hydroxide (353 mg, 14.8 mmol, 25 equiv.). The solution was then heated to 50ºC and stirred for 12 hr. The reaction was then diluted with water (10 mL) and then acidified to a pH of 4-5 with KHSO₄. The aqueous layer was extracted with ethyl acetate (10 mL) four times. The organic layers were combined and washed with water, brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 1:1) gave 290 mg (89%) of product as a white solid. ¹H NMR (500 MHz, CDCl₃, 25 ºC) δ (ppm) 7.43-7.21 (m, 15H), 6.95-6.85 (m, NH), 5.60 (m, 1H), 5.46 (m, 1H), 4.68 (m, 1H), 4.46 (m, 1H), 4.44 (bs, OH), 2.56 (t, 2H), 2.43 (m, 2H), 2.23 (t, 3H), 2.10 (s, 3H) 2.08 (m, 2H), 2.02 (m, 1H) <ju072412> Exp 8; ¹³C NMR (125 MHz, CDCl₃, 25 ºC) δ (ppm) 174.4, 172.71, 144.9, 132.0, 131.9, 130.4, 130.3, 129.6, 126.7, 69.2, 66.7, 51.8, 43.4, 42.8, 31.4, 31.0, 30.1, 15.4 <ju072412> Exp 12.

65% EtOAc/Hexane

Methyl (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-{methylsulfanyl}butanoate. (E)-3-hydroxy-7-(tritylthio)hept-4-enoic acid (410
mg, 0.980 mmol, 1.2 equiv.) and D-methionine methyl ester hydrochloride salt (163 mg, 0.816, 1.0 equiv.) were dissolved in dichloromethane (anhydrous) (35 mL) under Ar. The reaction was cooled to 0ºC and then benzotriazol-1-yl-oxypyrrolidinophosphonium hexafluorophosphate (PyBOP) (510 mg, 0.980 mmol, 1.2 equiv.) was added. The solution stirred for 10 min and then DIPEA (0.208 mL, 1.19 mmol, 4 equiv.) was added. The reaction was allowed to warm to 25ºC and stirred for 12 hr. It was then quenched with a saturated NH₄Cl, extracted with dichloromethane (3 x 15 mL), washed with brine, dried over Na₂SO₄, and then concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 2:3) gave 360 mg (81%) of product as a white solid. ¹H NMR (300 MHz, CDCl₃, 25 ºC) δ 7.43-7.41 (m, 6H), 7.27 (m, 9H), 6.67 (s, NH), 5.56 (m, 1H), 5.45 (dd, 1H), 4.73 (m, 1H), 4.44 (m, 1H), 3.76 (s, 3H), 3.51 (s, OH), 2.52 (t, 2H), 2.41 (m, 2H), 2.21 (m, 3H), 2.15-1.95 (m, 6H) <ju012712> Exp 2; ¹³C NMR (125 MHz, CDCl₃, 25 ºC) δ (ppm) 172.7, 171.4, 144.9, 132.4, 129.9, 129.6, 127.9, 126.7, 69.1, 66.6, 52.7, 51.4, 42.8, 36.7, 31.4, 30.0, 24.8, 15.5 <ju072412> Exp 4.

50% EtOAc/Hexane
Methyl (2R)-2-[(4E)-3-hydroxy-7-[(3E)-5-hydroxy-6-[(2R)-1-methoxy-4-(methyl-sulfanyl)-1-oxobutan-2-yl]carbamoyl]hex-3-en-1-yl]disulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanoate. Iodine (59.2 mg, 0.233 mmol, 1 equiv.) and sodium acetate (38.2 mg, 0.466 mmol, 2 equiv.) were dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (5 mL). Methyl (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanoate (526 mg, 0.233 mmol, 1 equiv.) was dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na$_2$S$_2$O$_3$) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wavelength was set at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column.
under the flow rate of 8 ml/min. The wavelength was set at 200 nm to detect the compound and 40 mg of the pure compound was collected at 19-21 min. (25.7 % yield.) \(^1\)H NMR (300 MHz, CDCl\(_3\), 25 °C) \(\delta\) (ppm) 7.60 (s, \(\text{NH}\)), 5.77-5.70 (m, 1H), 5.67-5.61 (dd, 1H), 4.62 (m, 1H), 4.49-4.40 (m, 1H), 4.39 (s, \(\text{OH}\)), 3.71 (s, 3H), 2.86-2.77 (m, 2H), 2.55 (m, 2H), 2.45-2.41 (m, 4H), 2.15-2.05 (m, 1H) 2.09 (s, 3H), 1.96 (m, 1H) <djm070312> Exp 4; \(^{13}\)C NMR (125 MHz, (CD\(_3\))\(_2\)CO, 25 °C) \(\delta\) (ppm) 173.5, 172.4, 135.5, 135.4, 129.1, 129.0, 70.0, 52.8, 52.4, 44.4, 39.3, 33.0, 32.7, 31.0, 15.6 <ju071112> Exp 5.

(2R)-2-[(4E)-7-{{[(1R)-1-carboxy-3-(methylsulfanyl)propyl]carbamoyl}-5-hydroxyhex-3-en-1-yl}disulfanyl]-3-hydroxyhept-4-enamido]-4-(methylsulfanyl)butanoic acid. Iodine (66.8 mg, 0.258 mmol, 1 equiv.) and sodium acetate (42.4 mg, 0.517 mmol, 2 equiv.) were dissolved in a 10:1 solution of CH\(_2\)Cl\(_2\)/MeOH (5 mL). (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanoic acid (142 mg, 0.258 mmol, 1 equiv.) was dissolved in a 10:1 solution of CH\(_2\)Cl\(_2\)/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by
adding a saturated sodium thiosulfate (Na$_2$S$_2$O$_3$) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wavelength was set at 200 nm to detect the compound and 55 mg of the pure compound was collected at 16-20 min. (34.8 % yield.) $^1$H NMR (300 MHz, CD$_3$OD, 25 °C) δ (ppm) 5.72 (m, 1H), 5.62 (dd, 1H), 4.57 (m, 1H), 4.45 (m, 1H), 2.76 (m, 2H), 2.57 (m, 2H), 2.45 (m, 4H), 2.20-2.14 (m, 1H), 2.11 (s, 3H), 1.96 (m, 1H) <djmo70312> Exp 9; $^{13}$C NMR (75 MHz, CD$_3$OD, 25 °C) δ (ppm) 173.8, 172.2, 133.4, 129.1, 69.1, 51.3, 43.4, 37.6, 31.6, 30.9, 29.7, 13.8 <djmo70312> Exp 1.

![Chemical Structure](image-url)

**Methyl-(2S)-2-[(2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)-butanamido]-4-[(triphenylmethyl)sulfanyl]propanoate.**

(2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]-hept-4-enamido]-4-(methylsulfanyl)butanoic acid. (416
mg, 0.757 mmol, 1.2 equiv.) and S-trityl-D-cysteine (275 mg, 0.631 mmol 1.0 equiv.) were dissolved in dichloromethane (anhydrous) (35 mL) under Ar. The reaction was cooled to 0°C and then benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (433 mg, 0.832 mmol, 1.1 equiv.) was added. The solution stirred for 10 min and then DIPEA (0.394 mL, 2.27 mmol, 3.0 equiv.) was added. The reaction was allowed to warm to 25°C and stirred for 14 hr. It was then quenched with a saturated NH₄Cl, extracted with dichloromethane (3 x 15 mL), washed with brine, dried over Na₂SO₄, and then concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 2:3) gave 381 mg (55%) of product as a white solid. ¹H NMR (500 MHz, CDCl₃, 25 °C) δ (ppm) 7.48-7.40 (m, 12H), 7.35-7.21 (m, 18H), 7.20-6.85 (m, 2H, NH), 5.55 (m, 1H), 5.45 (m, 1H), 4.70 (m, 1H), 4.54 (m, 1H), 4.46 (bs, 1H), 3.70 (d, 3H), 3.62-3.28 (bs, OH), 2.80-2.63 (m, 2H), 2.60 (t, 2H), 2.37 (m, 2H), 2.25 (m, 2H), 2.19-2.03 (m, 6H), 1.99 (m, 1H) <ju073012> Exp 1; ¹³C NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 171.7, 170.9, 170.7, 144.9, 144.2, 132.5, 130.1, 129.6, 129.5, 128.1, 127.9, 127.0, 126.6, 69.5, 66.9, 66.6, 52.7, 52.1, 51.4, 43.8, 42.7, 33.5, 31.5, 31.2, 30.0, 15.3 < BS072612> Exp 2.

Methyl (2S)-2-[(2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanamido]-3-[(triphenylmethyl)sulfanyl]-propanoate (400 mg, 0.440 mmol, 1.0 equiv.) was dissolved in a 4:1 ratio of THF/water (50 mL). Next was added lithium hydroxide (263 mg, 11.0 mmol, 25 equiv.). The solution was then stirred for 12 hr. The reaction was then diluted with water (30 mL) and then acidified to a pH of 4-5 with KHSO₄. The aqueous layer was extracted with ethyl acetate (15 mL) four times. The organic layers were combined and washed with water, brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 3:1) gave 327 mg (83%) of product as a white solid. ¹H NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 7.54-7.14 (m, 30H), 7.09 (m, NH), 6.59 (m, NH), 6.48 (bs, OH), 5.54 (m, 1H), 5.42 (dd, 1H), 4.66 (m, 1H), 4.44-4.28 (m, 2H), 2.85-2.65 (m, 2H), 2.52 (t, 2H), 2.38 (m, 2H), 2.24 (m, 2H), 2.15-1.90 (m, 7H) <BS073112> Exp 4; ¹³C NMR (75 MHz, CDCl₃, 25 °C) δ (ppm) 172.4, 172.2, 171.4, 144.9, 144.3, 132.3, 130.0, 129.6, 129.5, 128.2, 127.9, 127.0, 126.7, 69.3, 67.1, 66.7, 52.4, 51.7, 43.4, 33.2, 31.5, 31.4, 31.1, 30.0, 15.3 <BS073112> Exp 5.
Iodine (44.5 mg, 0.172 mmol, 1.0 equiv.) and sodium acetate (28.2 mg, 0.344 mmol, 2.0 equiv.) were dissolved in a 10:1 solution of CH₂Cl₂/MeOH (25 mL). (2S)-2-[(2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanamido]-3-[(triphenylmethyl)sulfanyl]propanoic acid (154 mg, 0.172 mmol, 1.0 equiv.) was dissolved in a 10:1 solution of CH₂Cl₂/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na₂S₂O₃) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The
aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in *vacuo*. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wavelength was set at 210 nm. The peak was collected at 8-10min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H$_2$O was used to elute the column under the flow rate of 8 ml/min. The wavelength was set at 200 nm to detect the compound and 25 mg of the pure compound was collected at 10-13.5 min. (35.6 % yield.) $^1$H NMR (500 MHz, CD$_3$OD, 25 °C) $\delta$ (ppm) 5.74 (m, 1H), 5.62-5.42 (m, 1H), 4.79 (bs, 2 OH), 4.64-4.20 (m, 3H), 2.93-2.78 (m, 2H), 2.78-2.65 (m, 2H), 2.64-2.44 (m, 4H), 2.42-2.31 (m, 2H), 2.14-2.08 (d, 3H), 2.05-1.87 (m, 2H) <ju081512> Exp 5; $^{13}$C NMR (125 MHz, CD$_3$OD, 25 °C) $\delta$ (ppm) 172.3, 170.7, 169.3, 133.2, 129.8, 70.0, 52.4, 51.9, 43.8, 39.3, 33.0, 31.4, 29.4, 28.7, 13.6 <ju081512> Exp 8.
Methyl (4S,7R,12E)-11-hydroxy-7-[2-(methylsulfanyl)ethyl]-6,9-dioxo-1,2-dithia-5,8-diazacyclopentadec-12-ene-4-carboxylate. Iodine (93 mg, 0.364 mmol, 1.1 equiv.) and sodium acetate (52 mg, 0.661 mmol, 2 equiv.) were dissolved in a 10:1 solution of CH₂Cl₂/MeOH (25 mL). Methyl (2S)-2-[(2R)-2-[4E]-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-4-(methylsulfanyl)butanamido]-3-[(triphenylmethyl)sulfanyl]-propanoate (300 mg, 0.330 mmol, 1 equiv.) was dissolved in a 10:1 solution of CH₂Cl₂/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na₂S₂O₃) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na₂SO₄, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20 min 20-100% ACN/H₂O 20 ml/min and the detection wavelength was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H₂O was used to elute the column.
under the flow rate of 8 ml/min. The wavelength was set up at 200 nm to detect the compound and 41 mg of the pure compound was collected at 17-19 min. (29.4 % yield.) $^1$H NMR (300 MHz, (CD$_3$)$_2$SO, 25 °C) $\delta$ (ppm) 8.85-8.30 (m, NH), 8.15-7.95 (m, NH), 5.65-5.48 (m, 1H), 5.48-5.26 (m, 1H), 5.06 -4.73 (m, OH), 4.72-4.51 (m, 1H), 4.50-4.32 (m, 1H), 4.31-4.10 (m, 1H), 3.70-3.60 (m, 3H), 3.26-3.10 (m, 2H), 2.96-2.69 (m, 2H), 2.67-2.12 (m, 6H), 2.04 (s, 3H), 1.92-1.65 (m, 2H)

Exp 1; $^{13}$C NMR (75 MHz, (CD$_3$)$_2$SO, 25 °C) $\delta$ (ppm) 172.2, 171.3, 170.4, 133.9, 128.7, 70.0, 52.8, 51.6, 51.2, 44.8, 40.0, 33.5, 32.7, 32.2, 29.7, 16.5

Exp 6.

methyl (2R)-2-[(4E)-3-hydroxy-7-[(triphénylmethyl)sulfanyl]hept-4-enamido]-3-methylbutanoate. (E)-3-hydroxy-7-(tritylthio)hept-4-enoic acid (150 mg, 0.358 mmol, 1.2 equiv.) and D-valine methyl ester hydrochloride salt (50 mg, 0.298 mmol, 1.0 equiv.) were dissolved in dichloromethane (anhydrous) (25 mL) under Ar. The reaction was cooled to 0°C and then benzotriazol-1-yl-oxytrypyrrolidinophosphonium hexafluorophosphate (PyBOP) (186 mg, 0.298 mmol, 1.2 equiv.) was added. The solution stirred for 10 min and then DIPEA (0.208 mL, 1.19 mmol, 4 equiv.) was added. The reaction was allowed to warm to 25°C and stirred for 14 hr. It was then quenched with a saturated NH$_4$Cl, extracted with dichloromethane (3 x 15 mL), washed with brine, dried over Na$_2$SO$_4$, and then
concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 3:7) gave 127 mg (82%) of product as a white solid. ¹H NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 7.50-7.25 (m, 15H), 6.41 (m, NH), 5.57 (m, 1H), 5.46 (dd, 1H), 4.57 (m, 1H), 4.44 (m, 1H), 3.76 (s, 3H), 3.36 (s, OH), 2.43 (m, 2H), 2.20-2.11 (m, 5H), 0.94 (m, 6H) <ju011712> Exp 3; ¹³C NMR (75 MHz, CDCl₃, 25 °C) δ (ppm) 172.6, 171.8, 144.9, 132.2, 130.2, 129.6, 127.9, 126.6, 69.3, 66.6, 56.9, 52.3, 43.0, 31.5, 31.4, 31.0, 19.0, 17.8 <ju011812> Exp 2.

50%EtOAc/Hexane

(2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylbutanoic acid. Methyl (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylbutanoate (122 mg, 0.229 mmol, 1.0 equiv.) was dissolved in a 4:1 ratio of THF/water (15 mL). Next was added lithium hydroxide (137 mg, 5.74 mmol, 25 equiv.). The solution was then
stirred for 12 hr. The reaction was then diluted with water (10 mL) and then acidified to a pH of 4-5 with KHSO₄. The aqueous layer was extracted with ethyl acetate (15 mL) four times. The organic layers were combined and washed with water, brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 1:2) gave 99.5 mg (83%) of product as a white solid. ¹H NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 7.41 (m, 6H), 7.29 (m, 9H), 6.95-6.82 (m, NH), 5.54 (m, 1H), 5.45 (dd, 1H), 4.62-4.38 (m, 2H), 2.44 (m, 2H), 2.22 (m, 3H), 2.07 (m, 2H), 0.95 (m, 6H) <djm061912> Exp 1; ¹³C NMR (75 MHz, CDCl₃, 25 °C) δ (ppm) 173.9, 172.4, 144.9, 132.18, 130.1, 129.6, 127.9, 126.6, 69.3, 66.6, 56.9, 43.0, 31.5, 31.4, 30.6, 19.0, 17.8 <BS013112> Exp 2.

50% EtOAc/Hexane
(2R)-2-[(4E)-7-{{[(3E)-6-{{[(1R)-1-carboxy-2-methylpropyl]carbamoyl}-5-hydroxyhex-3-en-1-yl}disulfanyl]-3-hydroxyhept-4-enamido}-3-methylbutanoic acid. Iodine (128 mg, 0.504 mmol, 1.1 equiv.) and sodium acetate (75.1 mg, 0.916 mmol, 2.0 equiv.) were dissolved in a 10:1 solution of CH₂Cl₂/MeOH (7 mL). (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylbutanoic acid (237 mg, 0.458 mmol, 1.0 equiv.) was dissolved in a 10:1 solution of CH₂Cl₂/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na₂S₂O₃) solution until the reaction mixture turned clear. Then, brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na₂SO₄, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20 min 20-100% ACN/H₂O 20ml/min and the detection wavelength was set at 210 nm. The peak was collected at 8-12 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H₂O was used to elute the column under the flow rate of 8 ml/min. The wavelength was set at 200 nm to detect the compound and 40 mg of the pure compound was
collected at 13-18.5 min. (15.5 % yield.) $^1$H NMR (300 MHz, CD$_3$OD, 25 °C) $\delta$ (ppm) 5.75 (m, 1H), 5.62 (m, 1H), 4.49 (m, 1H), 4.37 (m, 1H), 2.75 (t, 2H), 2.58-2.37 (m, 4H), 2.25 (m, 1H), 1.00 (m, 6H) $<$BS062612> Exp 2; $^{13}$C NMR (75 MHz, CDCl$_3$/CD$_3$OD, 25 °C) $\delta$ (ppm) 178.0, 176.2, 136.8, 133.2, 72.9, 61.3, 42.9, 35.6, 34.7, 34.6, 22.9, 21.5 $<$ju062012> Exp 1.

![Structure Image]

methyl 2-[(4E)-3-hydroxy-7-[(3E)-5-hydroxy-6-[(1-methoxy-3-methyl-1-oxobutan-2-yl)carbamoyl]hex-3-en-1 yl]disulfanyl]hept-4-enamido]-3-methylbutanoate. Iodine (118 mg, 0.465 mmol, 1.1 equiv.) and sodium acetate (69.4 mg, 0.846 mmol, 2 equiv.) were dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (15 mL). Methyl (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylbutanoate (225 mg, 0.423 mmol, 1 equiv.) was dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na$_2$S$_2$O$_3$) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash
Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20 min 20-100% ACN/H₂O 20ml/min and the detection wavelength was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H₂O was used to elute the column under the flow rate of 8 ml/min. The wavelength was set up at 200 nm to detect the compound and 90 mg of the pure compound was collected at 12-17 min. (37.0 % yield.) ¹H NMR (300 MHz, CD₂Cl₂, 25 °C) δ (ppm) 6.82 (m, NH), 5.75 (m, 1H), 5.62 (dd, 1H), 4.49 (m, 2H), 3.95 (bs, OH), 3.75 (s, 3H), 2.79 (t, 2H), 2.54-2.39 (m, 4H), 2.25 (m, 1H), 0.95 (m, 6H) ¹³C NMR (75 MHz, CD₂Cl₂, 25 °C) δ (ppm) 172.3, 171.8, 133.2, 129.1, 69.1, 57.1, 52.1, 42.7, 38.3, 31.7, 31.1, 18.9, 17.6

Exp 1; Exp 2.


(E)-3-hydroxy-7-(tritylthio)hept-4-enolic acid (1.0 g, 2.39 mmol, 1.0 eqv.) and D-norleucine methyl ester hydrochloride salt (346 g, 0.264 mmol, 1.1 equiv.) were dissolved in dichloromethane (anhydrous) (75 mL) under Ar. The reaction was cooled to 0°C and then benzotriazol-1-yl-oxytripyrrolidinophosphonium
hexafluorophosphate (PyBOP) (1.5 g, 2.88 mmol, 1.2 equiv.) was added. The solution stirred for 10 min and then DIPEA (1.51 mL, 8.32 mmol, 3.5 equiv.) was added. The reaction was allowed to warm to 25°C and stirred for 14 hr. It was then quenched with a saturated NH₄Cl, extracted with dichloromethane (3 x 50mL), washed with brine, dried over Na₂SO₄, and then concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 3:7) gave 1.12 g (86%) of product as a white solid. "H NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 7.50-7.20 (m, 15H), 6.61 (t, 1H), 5.65 (m, 1H), 5.46 (dd, 1H) 4.62 (m, 1H), 4.45 (m, 1H), 3.74 (s, 3H), 2.40 (m, 2H), 2.23 (m, 2H), 2.10 (m, 2H), 1.83 (m, 1H), 1.68 (m, 1H), 1.32 (m, 4H), 0.91 (t, 3H)  

13C NMR (75 MHz, CDCl₃, 25 °C) δ (ppm) 172.5, 171.7, 144.9, 132.2, 129.9, 129.1, 127.5, 126.6, 69.2, 66.6, 52.5, 52.3, 43.2, 31.5, 31.4, 30.0, 27.9, 22.2, 14.2  

50%EtOAc/Hexane
(2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]hexanoic acid. Methyl (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]hexanoate (320 mg, 0.586 mmol, 1.0 equiv.) was dissolved in a 4:1 ratio of THF/water (15 mL). Next was added lithium hydroxide (351 mg, 14.7 mmol, 25 equiv.). The solution was then stirred for 12 hr. The reaction was then diluted with water (10 mL) and then acidified to a pH of 4-5 with KHSO₄. The aqueous layer was extracted with ethyl acetate (15 mL) four times. The organic layers were combined and washed with water, brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 4:6) gave 296 mg (95%) of product as a white solid. 

¹H NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 7.50-7.15 (m, 15H), 7.03-6.91 (m, 1H), 5.55 (m, 1H), 5.44 (dd, 1H), 4.56 (q, 1H), 4.45 (m, 1H), 2.43 (m, 2H), 2.24 (m, 2H), 2.10 (m, 2H), 1.87 (m, 1H), 1.71 (m, 1H), 1.35-1.26 (m, 4H), 0.91 (t, 3H) <BS013112> Exp 3; ¹³C NMR (75 MHz, CDCl₃, 25 °C) δ (ppm) 175.4, 172.5, 145.1, 132.0, 129.7, 129.6, 127.9, 126.7, 69.2, 66.6, 52.5, 43.4, 31.5, 31.4, 30.4, 27.5, 22.3, 13.9 <BS013112> Exp 4.

50% EtOAc/Hexane
(2R)-2-[(4E)-7-{{[(3E)-6-{{[(1R)-1-carboxypentyl]carbamoyl}-5-hydroxyhex-3-en-1-yl}disulfanyl}]3-hydroxyhept-4-enamido}hexanoic acid. Iodine (134 mg, 0.562 mmol, 1.0 equiv.) and sodium acetate (173 mg, 1.12 mmol, 2.0 equiv.) were dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (10 mL). (2R)-2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]hexanoic acid (280 mg, 0.526 mmol, 1.0 equiv.) was dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na$_2$S$_2$O$_3$) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20 ml/min and the detection wavelength was set at 210 nm. The peak was collected at 8-12 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wavelength was set at 200 nm to detect the compound and 75 mg of the pure compound was collected at 17-24.5 min. (24.1 % yield.) $^1$H NMR (500 MHz, DMSO, 25 °C) $\delta$ (ppm) 5.62 (m, 1H), 5.49 (m, 1H), 4.35 (s, 2H), 2.60 (t, 2H), 2.37
(m, 4H), 1.75 (m, 1H), 1.57 (m, 1H), 1.23 (m, 4H), 0.75 (m, 3H) \( \delta \) (ppm) 174.2, 172.1, 133.2, 128.9, 69.0, 52.2, 43.3, 37.6, 31.6, 29.4, 27.6, 22.0, 13.0 <BS062612> Exp 4.

\(^{13}\)C NMR (75 MHz, CD\(_3\)OD, 25 °C) \( \delta \) (ppm) 174.2, 172.1, 133.2, 128.9, 69.0, 52.2, 43.3, 37.6, 31.6, 29.4, 27.6, 22.0, 13.0 <BS062612> Exp 4.

methyl \( (2R)-2-[(4E)-3\text{-hydroxy-7-} \{(3E)-5\text{-hydroxy-6-}\{[(2R)-1\text{-methoxy-1-oxohexan-2-yl}]\text{carbamoyl}\}hex-3\text{-en-1-yl}]\text{disulfanyl}\}\text{hept-4-enamido}\}\text{hexanoate}. \) Iodine (169 mg, 0.669 mmol, 1.1 equiv.) and sodium acetate (99.7 mg, 1.22 mmol, 2.0 equiv.) were dissolved in a 10:1 solution of CH\(_2\)Cl\(_2\)/MeOH (15 mL). Methyl \( (2R)-2-[(4E)-3\text{-hydroxy-7-} \{(\text{triphenylmethyl})\text{sulfanyl}\}\text{hept-4\text{-enamido}\}\text{hexanoate} \) (332 mg, 0.608 mmol, 1.0 equiv.) was dissolved in a 10:1 solution of CH\(_2\)Cl\(_2\)/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na\(_2\)S\(_2\)O\(_3\)) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na\(_2\)SO\(_4\), and concentrated in vacuo. The crude reaction system was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H\(_2\)O 20 ml/min and the detection wavelength was set up at
210 nm. The peak was collected at 12-15 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H₂O was used to elute the column under the flow rate of 8 ml/min.

The wavelength was set up at 200 nm to detect the compound and 135 mg of the pure compound was collected at 27-32 min. (36.7 % yield.)

¹H NMR (300 MHz, CD₂Cl₂, 25 °C) δ (ppm) 7.11 (d, 1H), 5.71 (m, 1H), 5.58 (dd, 1H), 4.53 (m, 1H), 4.45 (m, 1H), 4.18 (bs, OH), 3.73 (s, 3H), 2.76 (t, 2H), 2.44 (m, 4H), 1.88 (m, 1H), 1.68 (m, 1H), 1.32 (m, 4H), 0.91 (t, 3H) <BS101512> Exp. 3; ¹³C NMR (75 MHz, CD₂Cl₂, 25 °C) δ (ppm) 173.1, 171.9, 133.3, 128.9, 69.1, 52.2, 52.1, 42.9, 38.2, 31.8, 31.7, 27.5, 22.3, 13.7 <BS101512> Exp. 4.

![Methyl 2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylpentanoate.](image)

(E)-3-hydroxy-7-(tritylthio)hept-4-enoic acid (600 mg, 1.43 mmol, 1.0 eqv.) and DL-isoleucine methyl ester (188 mg, 1.43 mmol, 1.0 equiv.) were dissolved in dichloromethane (anhydrous) (40 mL) under Ar. The reaction was cooled to 0ºC and then benzotriazol-1-yl-oxytrypyrrolidinophosphonium hexafluorophosphate (PyBOP) (821 mg, 1.58 mmol, 1.1 equiv.) was added. The solution stirred for 10 min and then DIPEA (0.781 mL, 4.30 mmol, 3.0 equiv.) was added. The reaction was allowed to warm to 25ºC and stirred for 14 hr. It was then quenched with a saturated NH₄Cl, extracted with dichloromethane (3 x 20 mL), washed with brine,
dried over Na$_2$SO$_4$, and then concentrated in *vacuo*. Chromatography on SiO$_2$ (ethyl acetate/hexane, 3:7) gave 457 mg (58%) of product as a white solid. $^1$H NMR (300 MHz, CDCl$_3$, 25 °C) $\delta$ (ppm) 7.44-7.15 (m, 15H), 6.48 (m, 1H), 5.54 (m, 1H), 5.45 (dd, 1H), 4.75-4.58 (m, 1H), 4.43 (m, 1H), 3.9 (m, OH), 3.74 (s, 3H), 2.40 (m, 2H), 2.22 (q, 2H), 2.09 (m, 2H), 1.90 (m, 1H), 1.39 (m, 1H), 1.16 (m, 1H), 0.95 (m, 6H) <ju021712> Exp 1; $^{13}$C NMR (75 MHz, CDCl$_3$, 25 °C) $\delta$ (ppm) 172.8, 171.8, 144.9, 132.2, 130.1, 129.5, 127.9, 126.6, 69.2, 66.6, 56.3, 52.2, 43.0, 37.7, 31.5, 31.4, 26.3, 16.3, 11.6 <ju021712> Exp 2.

50% EtOAc/Hexane

2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylpentanoic acid.

Methyl 2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylpentanoate (456 mg, 0.836 mmol, 1.0 equiv.) was dissolved in a 4:1 ratio of THF/water (15 mL). Next was added lithium hydroxide (500 mg, 20.9 mmol, 25
equiv.). The solution was then stirred for 12 hr. The reaction was then diluted with water (15 mL) and then acidified to a pH of 4-5 with KHSO₄. The aqueous layer was extracted with ethyl acetate (20 mL) four times. The organic layers were combined and washed with water, brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. Chromatography on SiO₂ (ethyl acetate/hexane, 4:6) gave 422 mg (95%) of product as a white solid. ¹H NMR (300 MHz, CDCl₃, 25 °C) δ (ppm) 7.43 (m, 6H), 7.30-7.25 (m, 9H), 7.08-6.79 (m, 1H), 5.55 (m, 1H), 5.46 (m, 1H), 4.67-4.55 (m, 1H), 4.48 (m, 1H), 2.44 (m, 2H), 2.22 (m, 2H), 2.00 (m, 2H), 1.97-1.85 (m, 1H), 1.40 (m, 1H), 1.20 (m, 1H), 0.91 (m, 6H) <djm061912> Exp 2; ¹³C NMR (75 MHz, CDCl₃, 25 °C) δ (ppm) 175.8, 172.3, 145.1, 132.0, 130.3, 129.6, 127.9, 126.6, 69.3, 66.5, 56.5, 43.2, 37.3, 31.4, 29.7, 26.3, 16.8, 11.6 <ju022012> Exp 2.

50% EtOAc/Hexane
2-[(4E)-7-{[(3E)-6-[(1-carboxy-2-methylbutyl)carbamoyl]-5-hydroxyhex-3-en-1-yl]disulfanyl]-3-hydroxyhept-4-enamido]-3-methylpentanoic acid. Iodine (200 mg, 0.788 mmol, 1.0 equiv.) and sodium acetate (129 mg, 1.57 mmol, 2.0 equiv.) were dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (15 mL). 2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylpentanoic acid (420 mg, 0.788 mmol, 1.0 equiv.) was dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na$_2$S$_2$O$_3$) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20 min 20-100% ACN/H$_2$O 20ml/min and the detection wavelength was set up at 210 nm. The peak was collected at 8-12 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8 ml/min. The wavelength was set up at 200 nm to detect the compound and 63 mg of the pure compound was collected at 15.5-21 min. (13.9 % yield.) $^1$H NMR (300 MHz, CD$_3$OD, 25 °C) δ (ppm) 5.75 (m, 1H), 5.61 (m, 1H), 4.57-4.40 (m, 2H), 3.37
(s, OH), 2.75 (t, 2H), 2.47 (m, 4H), 2.01-1.88 (m, 1H), 1.60-1.40 (m, 1H), 1.27 (m, 1H), 0.96 (m, 6H) <ju062112> Exp 1; $^{13}$C NMR (75 MHz, CD$_3$OD, 25 °C) δ (ppm) 173.5, 172.0, 133.3, 129.0, 69.0, 56.7, 43.1, 37.6, 37.0, 31.6, 26.4, 15.3, 10.7 <ju062112> Exp 2.

Methyl 2-[(4E)-3-hydroxy-7-[(3E)-5-hydroxy-6-[(1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl]hex-3-en-1-yl]disulfanyl]hept-4-enamido]-3-methylpentanoate. Iodine (134 mg, 0.529 mmol, 1.0 equiv.) and sodium acetate (86.8 mg, 1.06 mmol, 2.0 equiv.) were dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (10 mL). Methyl 2-[(4E)-3-hydroxy-7-[(triphenylmethyl)sulfanyl]hept-4-enamido]-3-methylpentanoate (289 mg, 0.529 mmol, 1.0 equiv.) was dissolved in a 10:1 solution of CH$_2$Cl$_2$/MeOH (5 mL) and was added dropwise at 0°C over 20 minutes to the first solution containing iodine and sodium acetate. This solution was then allowed to stir for 2 hr. The reaction was quenched by adding a saturated sodium thiosulfate (Na$_2$S$_2$O$_3$) solution until the reaction mixture turned clear. Then brine (5 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL) and then with ethyl acetate (3 x 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude reaction mixture was first purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20 min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set at 210 nm. The peak
was collected at 12-15 min. Then this peak was injected in the HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 µm) system and 50 % ACN/H₂O was used to elute the column under the flow rate of 8 ml/min. The wavelength was set at 200 nm to detect the compound and 52 mg of the pure compound was collected at 25-31 min. (16.3 % yield.)  

\(^1\)H NMR (300 MHz, CD₂Cl₂, 25 °C) δ (ppm) 6.60 (s, NH), 5.74 (m, 1H), 5.65 (dd, 1H), 4.70-4.54 (m, 1H), 4.53-4.43 (s, 1H), 3.75 (s, 3H), 3.70 (bs, OH), 2.78 (t, 2H), 2.56-2.35 (m, 4H), 1.90 (m, 1H), 1.45 (m, 1H), 1.23 (m, 1H), 0.99-0.89 (m, 6H) <ju101412> Exp 1;  

\(^13\)C NMR (75 MHz, CD₂Cl₂, 25 °C) δ (ppm) 172.4, 171.6, 133.1, 129.2, 69.2, 56.4, 52.2, 42.6, 38.3, 37.7, 31.7, 26.2, 15.3, 11.3 <ju101412> Exp 2.

(2R)-1-methoxy-4-(methylsulfanyl)-1-oxobutan-2-aminium chloride.

Chlorotrimethylsilane (8.51 mL, 67.02 mmol, 2 equiv.) was added dropwise to a round bottom flask containing D-methionine hydrochloride (5.00g, 33.51mmol, 1.0 equiv.) under Ar. Then anhydrous methanol (50 mL) was added and the solution was stirred for 22 hr. The contents of the flask were then concentrated in vacuo.  

\(^1\)H NMR (300 MHz, D₂O, 25 °C) δ (ppm) 4.24 (t, 1H), 3.79 (s, 3H), 2.62 (t, 2H), 2.33-2.09 (m, 2H), 2.05 (s, 3H) <ju072312> Exp 2;  

\(^13\)C NMR (125 MHz, D₂O, 25 °C) δ (ppm) 170.51, 53.67, 51.72, 28.72, 28.44, 13.87 <ju072412> Exp 7.
1-methoxy-3-methyl-1-oxopentan-2-aminium chloride. Chlorotrimethylsilane (1.93 mL, 15.25 mmol, 2 equiv.) was added dropwise to a round bottom flask containing DL-Isoleucine hydrochloride (1.0 g, 7.62 mmol, 1.0 equiv.) under Ar. Then anhydrous methanol (20 mL) was added and the solution was stirred for 22 hr. The contents of the flask were then concentrated in vacuo. $^1$H NMR (300 MHz, D$_2$O, 25 °C) δ (ppm) 4.05 (m, 1H), 3.76 (s, 3H), 2.15-1.94 (m, 1H), 1.49-1.32 (m, 1H), 1.32-1.16 (m, 1H), 0.95-0.81 (m, 6H) <djm091312> Exp 1; $^{13}$C NMR (75 MHz, D$_2$O, 25 °C) δ (ppm) 170.20, 57.22, 53.40, 35.50, 24.87, 14.01, 10.68 <djm091212> Exp 4.

(2R)-1-methoxy-1-oxohexan-2-aminium chloride. Chlorotrimethylsilane (1.93 mL, 15.247 mmol, 2 equiv.) was added dropwise to a round bottom flask containing D-norleucine hydrochloride (1.00 g, 7.62 mmol, 1.0 equiv.) under Ar. Then anhydrous methanol (20 mL) was added and the solution was stirred for 22 hr. The contents of the flask were then concentrated in vacuo. $^1$H NMR (300 MHz, D$_2$O, 25 °C) δ (ppm) 4.06 (t, 1H), 3.76 (s, 3H), 1.95-1.75 (m, 2H), 1.35-1.27 (m, 4H), 0.79 (t, 3H) <ju091412> Exp 1; $^{13}$C NMR (75 MHz, D$_2$O, 25 °C) δ (ppm) 171.0, 53.4, 52.9, 29.3, 26.1, 21.4, 12.8 <ju091412> Exp 2.
methyl (2S)-2-[[9H-fluoren-9-ylmethoxy]carbonyl]amino]-3-[(triphenylmethyl)sulfanyl]propanoate. Chlorotrimethylsilane (2.17 mL, 15.07 mmol, 2 equiv.) was added dropwise to a round bottom flask containing (2S)-2-[[9H-fluoren-9-ylmethoxy]carbonyl]amino]-3-[(triphenylmethyl)sulfanyl]propanoic acid (5.00 g, 8.54 mmol, 1.0 equiv.) under Ar. Then anhydrous methanol (40 mL) was added and the solution was stirred for 22 hr. The contents of the flask were then concentrated in vacuo.

methyl (2R)-2-amino-2-[(triphenylmethyl)sulfanyl]acetate. (9H-fluoren-9-yl)methyl (R)-(methoxycarbonyl)(tritylthio)methylcarbamate (450 mg, 0.750 mmol, 1 equiv.) was dissolved in anhydrous acetonitrile (10 mL). Then, excess diethylamine (2 mL) was added and the solution was stirred for 2 hr. The contents of the flask were concentrated in vacuo. The solid was re-dissolved in chloroform and the solvent was removed in vacuo. $^1$H NMR (300 MHz, CDCl$_3$, 25 °C) δ (ppm) 7.50-7.20 (m, 15H), 3.66 (s, 3H), 3.24 (t, 1H), 2.63 (m, 1H), 2.51 (m, 1H), 2.00-1.42 (bs, NH$_2$) (Exp 1; $^{13}$C NMR (125 MHz, CDCl$_3$, 25 °C) δ (ppm) 171.0, 144.8, 129.1, 128.0, 126.8, 66.5, 53.8, 52.1, 37.0 (Exp 14).
**General remarks**

All reactions in non-aqueous media were conducted under a positive pressure of dry argon in glassware that had been oven dried prior to use unless noted otherwise. Anhydrous solutions of reaction mixtures were transferred via an oven dried syringe or cannula. All solvents were dried prior to use unless noted otherwise. \(^1\)H and \(^{13}\)C Nuclear magnetic resonance spectra (NMR) were obtained on a Varian Unity-Inova 400 MHz or 500 MHz recorded in ppm (\(\delta\)) downfield of TMS (\(\delta = 0\)). Signal splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), or multiplet (m), with coupling constants (J) in hertz. High resolution mass spectra (HRMS) were performed by UW Biotech Center on an Electron Spray Injection (ESI) mass spectrometer.

**General strategies for the preparation of substrates:**

\[ \text{RCOCl, DMAP, Py} \]

\[ \begin{align*}
\text{TDDPA} & \quad \rightarrow \quad \text{OCOR} \\
\end{align*} \]

\[ R = \text{Bz, TDPA-18} \]
\[ \text{CO2Et, TDPA-19} \]
\[ \text{Ac, TDPA-20} \]
**Representative experimental procedures:**

For substrate 18:

To a stirred solution of TDPA (27 mg, 0.5 mmol) and DMAP (one crystal) in anhydrous pyridine (1 mL) was added benzoyl chloride (12 mg, 0.75 mmol) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 40 h. The reaction mixture was poured into H₂O (20 mL) and extracted with Et₂O (3 × 10 mL). The combined organic layers were washed with brine (1 × 10 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (Hexane/Ethyl acetate = 50 : 50) to yield TDPA-18 (27 mg, 82%).

TDPA-19 (25mg, 85%) and TDPA-20 (30mg, 92%) were obtained according to the same procedure described for TDPA-18.

![TDPA-18](image)

**¹H NMR** (400 MHz, CDCl₃, TMS): δ 0.88-0.98 (m, 7H), 1.19-1.28 (m, 1H), 1.39-1.48 (m, 1H), 1.88-1.91 (m, 1H), 2.09-2.16 (m, 1H), 2.21 (s, 3H), 2.30-2.36 (m, 1H), 2.46 (s, 1H), 2.63-2.85 (m, 6H), 2.96 (dd, J = 11.6, 3.2 Hz, 1H), 3.20-3.33 (m,
3H), 3.40-3.48 (m, 2H), 4.41-4.44 (m, 1H), 5.00 (s, 1H), 5.59 (s, 1H), 5.71 (d, \( J = 12.8 \) Hz, 1H), 6.10-6.13 (m, 1H), 6.32 (s, 1H), 6.83 (d, \( J = 7.6 \) Hz, 1H), 7.43-7.48 (m, 3H), 7.56-7.59 (m, 1H), 7.67 (d, \( J = 2.8 \) Hz, 1H), 8.10 (d, \( J = 6.8 \) Hz, 1H). \(^{13}\)C NMR (100 MHz, CDCl$_3$, TMS): \( \delta \) 11.8, 15.5, 15.7, 20.5, 22.5, 27.1, 28.0, 28.6, 31.5, 36.9, 37.3, 41.4, 57.7, 58.1, 59.9, 69.9, 70.2, 109.9, 128.7, 130.0, 130.3, 133.3, 133.3, 165.9, 169.2, 169.4, 170.4, 171.3. HRMS (ESI) m/z calcd for C$_{30}$H$_{41}$N$_3$O$_7$S$_3$ (M+Na) 674.1999, found 674.2016.

\[
\text{TDPA-19}
\]

\(^1\)H NMR (400 MHz, CDCl$_3$, TMS): \( \delta \) 0.90-0.93 (m, 6H), 1.18-1.27 (m, 2H), 1.33 (t, \( J = 5.6 \) Hz, 3H), 1.39-1.47 (m, 1H), 1.84-1.87 (m, 1H), 2.05-2.12 (m, 1H), 2.20 (s, 3H), 2.29-2.35 (m, 1H), 2.47 (s, 1H), 2.58-2.66 (m, 2H), 2.66-2.73 (m, 3H), 2.78-2.81 (m, 1H), 2.95 (dd, \( J = 11.6, 3.2 \) Hz, 1H), 3.05-3.10 (s, 1H), 3.19 (dd, \( J = 10.8, 5.6 \) Hz, 2H), 3.27-3.31 (m, 1H), 4.20-4.26 (m, 2H), 4.37-4.41 (m, 1H), 4.98 (s, 1H), 5.63 (s, 1H), 5.67-5.72 (m, 2H), 6.33 (s, 1H), 6.76 (d, \( J = 8.0 \) Hz, 1H), 7.35 (d, \( J = 5.6 \) Hz, 1H), 7.58 (d, \( J = 3.5 \) Hz, 1H). \(^{13}\)C NMR (100 MHz, CDCl$_3$, TMS): \( \delta \) 11.8,
14.5, 15.2, 15.8, 27.2, 28.0, 31.6, 36.7, 37.1, 41.4, 57.8, 58.0, 64.5, 70.1, 73.2,
129.6, 133.2, 154.4, 169.0, 169.4, 170.3, 171.2. HRMS (ESI) m/z calcd for
C_{26}H_{41}N_{3}O_{8}S_{3} (M+Na) 642.1948, found 642.1959.

1H NMR (400 MHz, CDCl\textsubscript{3}, TMS): \( \delta \) 0.91 (t, \( J = 6.0 \) Hz, 6H), 1.15-1.31 (m, 1H),
1.37-1.45 (m, 1H), 1.81-1.86 (m, 1H), 2.07-2.12 (m, 1H), 2.13 (s, 3H), 2.20 (s, 3H),
2.28-2.34 (m, 1H), 2.44-2.46 (m, 1H), 2.60-2.72 (m, 5H), 2.78-2.85 (m, 1H), 2.87
(dd, \( J = 8.4, 4.8 \) Hz, 1H), 3.05-3.12 (s, 1H), 3.20-3.27 (m, 3H), 4.38-4.41 (m, 1H),
4.96 (s, 1H), 5.61 (s, 1H), 5.69 (d, \( J = 12.4 \) Hz, 1H), 5.79-5.83 (m, 1H), 6.30 (s,
1H), 6.78 (d, \( J = 7.6 \) Hz, 1H), 7.35 (d, \( J = 5.6 \) Hz, 1H), 7.62 (d, \( J = 3.5 \) Hz, 1H). \textsuperscript{13}C
NMR (100 MHz, CDCl\textsubscript{3}, TMS): \( \delta \) 11.7, 15.4, 15.7, 20.3, 21.5, 22.5, 27.1, 28.0,
28.6, 31.5, 36.7, 36.9, 41.4, 57.7, 58.0, 59.6, 69.7, 70.1, 129.5, 133.2, 169.3,
170.4, 170.5, 171.2. HRMS (ESI) m/z calcd for C_{25}H_{39}N_{3}O_{7}S_{3} (M+Na) 612.1842,
found 612.1858.
HPLC Data for separation

<table>
<thead>
<tr>
<th></th>
<th>FC condition</th>
<th>Rt-FC</th>
<th>HPLC condition</th>
<th>Rt-HPLC</th>
<th>Pure amount</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TDF1</strong></td>
<td>0-20min 20-100%ACN 20ml/min</td>
<td>8-12min</td>
<td>40% ACN</td>
<td>12-14.5min</td>
<td>95mg</td>
</tr>
<tr>
<td><strong>TDF1t</strong></td>
<td>15-20min</td>
<td>70% ACN</td>
<td>22-25 min</td>
<td>185mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF2</strong></td>
<td>8-12min</td>
<td>35% ACN</td>
<td>16-20min</td>
<td>25mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF2m</strong></td>
<td>12-15min</td>
<td>40% ACN</td>
<td>19-21min</td>
<td>185mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF3</strong></td>
<td>8-10min</td>
<td>35% ACN 8ml/min</td>
<td>10-13.5min</td>
<td>125mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF3m</strong></td>
<td>12-15min</td>
<td>40% ACN 8ml/min</td>
<td>17-19 min</td>
<td>158mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF4</strong></td>
<td>8-12min</td>
<td>40% ACN</td>
<td>15.5-21min</td>
<td>31mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF4m</strong></td>
<td>12-15min</td>
<td>50% ACN</td>
<td>25-31 min</td>
<td>87mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF5</strong></td>
<td>8-12min</td>
<td>40% ACN</td>
<td>17-24.5min</td>
<td>146mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF5m</strong></td>
<td>12-15min</td>
<td>50% ACN</td>
<td>27-32 min</td>
<td>130mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF6</strong></td>
<td>8-12min</td>
<td>35% ACN</td>
<td>13-18.5min</td>
<td>40mg</td>
<td></td>
</tr>
<tr>
<td><strong>TDF6m</strong></td>
<td>12-15min</td>
<td>50% ACN</td>
<td>12-17 min</td>
<td>90mg</td>
<td></td>
</tr>
</tbody>
</table>

**TDF1**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H₂O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 %
ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 12-14.5min

**TDF1t:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 15-20 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 70 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 22-25min.

**TDF2:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 16-20min.

**TDF2m:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was
collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 19-21min.

**TDF3:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-10min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 10-13.5min.

**TDF3m:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 17-19 min.
TDF4: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 15.5-21min.

TDF4m: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 25-31min.

TDF5: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The
wave length was set up at 200 nm to detect the compound and the pure compound was collected at 17-24.5min.

**TDF5m:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H₂O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H₂O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 27-32min.

**TDF6:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H₂O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H₂O was used to eluted the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 13-18.5min.

**TDF6m:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H₂O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar)
with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 12-17min.
1.8 References


   Yoshida, M.; Wang, X.-F.; Yao, T.-P. HDAC6 is a microtubule-associated 

   HDAC-6 interacts with and deacetylates tubulin and microtubules in vivo. 

31. Zou, H.; Wu, Y.; Navre, M.; Sang, B.-C. Characterization of the two catalytic 
   *341*, 45–50.

32. Zhang, Y.; Gilquin, B.; Khochbin, S.; Matthias, P. Two catalytic domains are 

33. (a) Verdin, E.; Dequiedt, F.; Kasler, H. Class II histone deacetylases: Versatile 

34. Haberland, M.; Montgomery, R. L.; Olson, E. N. The many roles of histone 
   deacetylases in development and physiology: Implications for disease and 


50. Karagiannis, T. C.; El-Osta, A. Will broad-spectrum histone deacetylase inhibitors be superseded by more specific compounds? Leukemia 2007, 21, 61–65.


Chapter II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

2.1 Overview

Nuclear Energy has much better curb appeal to older energy methods such as burning coal or oil. A single 20 gram fuel pellet of Uranium can produce the same amount of energy as 400 kilograms of coal, 410 litres of oil, or 350 cubic metres of natural gas\(^1\)(figure 2.1). When the whole life cycle of power generation is taken into account, nuclear power is one of the cleanest forms of energy, only behind hydroelectric and wind power\(^5\). The main problem with nuclear power is the radioactive waste that is generated. The high level wastes generated by reprocessing spent nuclear fuels are vitrified or converted in to glass today. These wastes, which will ultimately be disposed of in deep underground repositories\(^2\), contain long-lived radionuclides, including the minor actinides neptunium (Np), americium (Am) and curium (Cm) with half-lives ranging from hundreds to millions of years\(^3\). A new management protocol for these wastes could be developed if the long-lived radionuclides are separated from the short/medium lived radionuclides and then transmuted into short lived nuclides by nuclear means\(^4\).

Figure 2.1: Energy comparison of Uranium, coal, oil, and natural gas\(^5\)
However, it is well-known that the chemical properties of these elements are similar: they form trivalent cations (M$^{3+}$) in solution as their most stable oxidation states, and they have similar ionic radii$^{6-11}$. These similarities give rise to the difficulty in the separation of these elements. The research presented in this chapter aims to investigate and develop of new molecules for the difficult separation of the minor actinides americium (Am) from curium (Cm).
2.2 Introduction

A drawback of the generation of electricity by nuclear energy is its unavoidable by-product of radioactive waste. Electricity production by nuclear power/nuclear reactions is responsible for more than 95% of the radioactivity of the total amount of nuclear waste\textsuperscript{12-14}. Radioactive waste arises from all stages of the nuclear fuel cycle (\textbf{figure 2.2}), but more than 99% of the radioactivity involved in electricity generation by nuclear power plants is concentrated within the spent fuel discharged from reactors\textsuperscript{15-18}. Public approval of nuclear power as a long term source of sustainable energy highly depends on the impact of the radioactive waste on the environment and the atmosphere.

\textbf{Figure 2.2: Nuclear power cycle }\textsuperscript{19}
The removal of these radioactive wastes is a serious environmental crisis for which there is, as of yet, no universally accepted solution. It is one of the most pressing scientific and political problems that face mankind globally.

Radioactive waste from nuclear reactors usually contains radionuclides with a wide range of half-lives\(^{20-22}\). The majority of these nuclides have small half-lives ranging from less than a second up to a couple of years and disappear in a moderately short period of time representing very small risks during waste handling and storage. However, some radionuclides still exist after thousands or even millions of years, so their separation from all global ecosystems must be guaranteed for an extremely long time. An example of a storage system like this would be a deep geological formation of rocks or a deep ocean valley. The majority of the long-lived radionuclides determining the long-term safety of the waste within a repository belongs to the actinide group and more specifically, neptunium (Np), americium (Am) and curium (Cm)\(^{23}\) (figure 2.3 and figure 2.4).

**Figure 2.3**: Radiotoxicity inventory for 1 ton of spent fuel from a pressurized water reactor (PWR) with a 4% 235U enrichment and burn-up of 40 GWd/tHM (tHM = Ton heavy metal)\(^{24}\)
Figure 2.4: Contributions to the decay heat generated by spent PWR fuel irradiated to 50 GWd/tHM\textsuperscript{25}

With the current reprocessing technology today, the time perspective for the confinement of radioactive waste in a repository decreases from one-million years to 100,000 years of storage\textsuperscript{26-29}. This is still too large of a time table for the storage of radioactive waste geological formation. Also, if 99.9% of radioactive uranium and plutonium could be separated during reprocessing, the radiotoxicity of the remaining waste would only decrease one order of magnitude\textsuperscript{30}. This statistic makes it very that the small byproducts of nuclear reactions contain the majority of the radioactive waste. The contaminated lifetime high level waste is determined by the presence of minor actinides (\textsuperscript{237}Np, \textsuperscript{241}Am, \textsuperscript{243}Am, and \textsuperscript{245}Cm)\textsuperscript{31-33} and long-lived fission products (\textsuperscript{99}Tc, \textsuperscript{129}I, \textsuperscript{79}Se, \textsuperscript{93}Zr, \textsuperscript{135}Cs)\textsuperscript{34-35}.

Separating the minor actinide americium (Am) from curium (Cm) and the lanthanide fission products in used nuclear fuel is a critical part of minor actinide
recycle/transmutation in advanced nuclear fuel cycles. The first step in recycling the waste the lanthanides elements must be separated first because they are much more abundant than the minor actinides and also are powerful neutron poisons which would inhibit fission of $\text{Am}^{37-40}$. Removal of Cm is important to simplify fuel fabrication and to halt the buildup of neutron-emitting transcurium actinides when the used fuel is recycled repeatedly. To date, several processes have been developed to co-separate Am and Cm from the lanthanides by-products in nuclear waste. Am and Cm separation to date is still a very difficult processes and still remains very challenging and problematic. The objective of this project is to rationally design new molecules that can efficiently separate Am from both Cm and the lanthanides.

Recent work in the Office of Nuclear Energy-funded Sigma Team for Minor Actinide Separations at Argonne national laboratory shows that steric strain in coordination complexes can amplify normally small differences in the complexation free energies of similar metal ions, such as $\text{Am}^{3+}$ and $\text{Cm}^{3+}$. This produces free energy differences (or separation factors) large enough for convenient solvent extraction separations. That work also demonstrated that the sterically constrained ligand, $\text{N,N'}$-bis[(6-carboxy-2-pyridyl)methyl]-4,13-diaza-18-crown-6 ($\text{H}_2\text{bp18c6}$, figure 2.5) shows a distinct thermodynamic preference for $\text{Am}^{3+}$ over $\text{Cm}^{3+}$ and could produce a high purity Am stream from a mixture of equal amounts of Am and Cm in as few as six contacts. Computational studies also suggested derivatives of bp18c6 could be even more selective for Am over Cm.
While the Am/Cm selectivity of bp18c6 is impressive, a more selective ligand would make practical applications more economical by reducing the number of separation stages and eliminating contamination of the Am product with light lanthanides (i.e. La and Ce). The work presented in this chapter is the rationally design, synthesize and screen for new ligands that could improve the separation of americium from curium and the lanthanides.

Our general hypothesis is that tuning the steric constraints and electron donating strength of the bp18c6 molecule will produce molecules with better selectivity for Am. Our two specific aims are for this project are (1) Rationally design new aqueous ligands and chemically synthesize these designer ligands, and (2) Evaluate the potential of these synthetic ligands for americium selectivity. The outcome of this work will be a unique aqueous ligand for a straightforward and efficient separation of americium from curium and the lanthanides. This will enable a simple and efficient separation process that will be important for improving the efficiency of advanced fuel cycles. As an aqueous ligand, we expect the derivatives that will be developed would be deployable as a simple americium-
selective variation on one or more established or developing process concepts such as TALSPEAK, ALSEP, or SANEX\textsuperscript{43}.

2.3 Rationally Design of bp18c6 Derivatives

The hypothesis underlying this research program is that tuning the steric constraints and electron donating strength of the already sterically constrained bp18c6 molecule will produce molecules with even better selectivity for Am. This hypothesis is based on work conducted by the Sigma Team for Minor Actinide Separations at Argonne National Laboratory, with whom we are collaborating with Dr. Mark Jensen. We propose that changing the steric constraints of the ligands will alter their cation size-based selectivity (i.e. the Am/Cm selectivity), while changing the chemical softness of the pyridine nitrogen donors by adding electron donating groups to the pyridine groups of bp18c6 will alter the Am/lanthanide selectivity. The ligand H\textsubscript{2}bp18c6 has already been shown to have different affinities for americium and curium and lanthanides, but improving this selectivity is needed to reduce the number of stripping stages (making the separation more efficient and economical) and to eliminate light lanthanide
Figure 2.6: Ligands 1-3, increases in the size of the picolinic acid arms

contamination of the Am. Thus we will focus on understanding the interplay between the most relevant steric and electronic factors underlying the Gibbs free energies that drive the actual separation. Computational studies of the bp18c6 ligand system suggest that ligand strain indeed increases as the actinide or lanthanide cations become smaller, and that the crown ether nitrogen atoms are key donor groups affected by the strain. The computations also suggest that increasing the length of the arms attached to the crown ether could tune the relative strain in the complexes and improve the size selectivity of the ligands. These findings are supported by literature reports on the structures of lanthanide-crown ether complexes. Therefore, our designer molecules will keep the critical 6-carboxy-2-pyridyl (picolinic acid) group and the diaza-18-crown-6 ring size. Ligands 2 and 3 (Figure 2.6) with longer picolinic acid arms are designed to test the influence of the strain induced in the metal complexes by the complexing picolinate arms.
Figure 2.7: Ligands 4-9 have electron donating groups or a more rigid crown rings system. This set of ligands is designed to test how further constraining the ring system affects the Am/Cm selectivity. To make constrained ligands such as 6 and 7 more water soluble, which is critical for aqueous separation, we will introduce hydrophilic groups (-OCH₃) at the benzene ring (ligands 8-9).

Figure 2.8: Ligands 10-12 with electron donating methoxy groups
Ligands 10-12 (figure 2.8) all have the same ring size as bp18c6 with electron donating methoxy group(s) on the pyridine ring to enhance the softness of the nitrogen atom. The methoxy group(s) will also enhance the aqueous solubility of the ligands. Computational modeling suggested that softer pyridine nitrogen will improve Am/lanthanide selectivity. The synthesized ligands will be screened for solubility and size selectivity using the lanthanides Nd$^{3+}$ and Sm$^{3+}$ (which are close in cationic size with Am$^{3+}$ and Cm$^{3+}$, respectively$^{44}$). Ligands passing the size selectivity screen will be further evaluated for Am/Cm and Am/lanthanide selectivity and when suitable, full thermodynamic characterization by our Sigma Team collaborator will take place. The results from these studies will be used to inform additional improvements to the base ligand, particularly with respect to substituents on the pyridine rings (ligands 10-12). From these combined studies, we will design a unique ligand for Am separation that will enable new separations important to advanced fuel cycles.
2.4 Results and Discussion

Ligand H2bp18c6 (Scheme 1)(N,N'-bis[(6-carboxy-2-pyridyl)methyl]-4,13-diaza-18-crown-6)(1) was synthesized by the produce of Rodriguez-Blas and coworkers with a few small modifications\(^{45-46}\). This ligand was obtained in four steps from dimethylpyridine-2,6- dicarboxylate (13) with an overall yield of 28% (scheme 2.1). This first step is the reduction of one methyl ester of the starting material dimethylpyridine-2,6- dicarboxylate (13) to yield 6-Hydroxymethylpyridine-2-carboxylic Acid Methyl Ester (14) in 51%\(^{47}\). Next, Bromination of (14) with PBr\(_3\) at 0\(^\circ\) C in CHCl\(_3\) formed 6-Bromomethylpyridine-2-carboxylic Acid Methyl Ester (15) in good yield of 99%\(^{48}\). In the Alkylation reaction 15 was treated with 4,13-diaza-18-crown-6 (16) in refluxing acetonitrile in the presence of Na\(_2\)CO\(_3\) to give compound N,N'-Bis[(6-methoxycarbonyl-2-pyridyl)methyl]-4,13-diaza-18-crown-6 (17) in 67% yield. Full deprotection of the methyl esters of 17 was cleanly achieved with 6 M HCl at reflux to yield the desired ligand, H2bp18c6 (1)(scheme 2.1)(N,N'-bis[(6-carboxy-2-pyridyl)methyl]-4,13-diaza-18-crown-6). After ligand 1 was successfully characterized and confirmed by NMR and HRMS, we began synthesis on ligand 2.

Scheme 2.1: Synthesis of H\(_2\)bp18c6 (1)
We envisioned again starting with the same starting material as for ligand (1), dimethylpyridine-2,6- dicarboxylate (13). Reduction of starting material dimethylpyridine-2,6- dicarboxylate (13) and then brominated with PBr₃ yielded 6-Bromomethylpyridine-2-carboxylic Acid Methyl Ester (15) in two step overall yield of 50%. The carbon extension step followed and gave a lot more problems than we originally hoped. The first proposed synthesis is outline in scheme 2.2.

**Scheme 2.2**: Proposed synthesis of 6,6'-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl))dipicolinic acid (2).
addition of a strong organic base (n-Butyl lithium) followed by quenching the reaction with CO$_2$, and then acid which would produce 2-(6-(methoxycarbonyl)pyridin-2-yl)acetic acid (18). We tried numerous attempts to use n-butyllithium with different ratios of equivalents of base and S.M. and also at different temperatures. None were successful. Next, our lab used Mg metal in hopes that the Grignard reagent would form. After repeated reactions (equation 2.1), none were found to have any 2-(6-(methoxycarbonyl)pyridin-2-yl)acetic acid (18) present.

Equation 2.1: 2-(6-(methoxycarbonyl)pyridin-2-yl)acetic acid

After the unsuccessful attempts to add the carbon with the CO$_2$, we turned to sodium cyanide for the carbon extension. The reaction of 6-Bromomethylpyridine-2-carboxylic acid methyl ester (15) with 3 eqv. NaCN refluxed in ethanol and yielded (equation 2.2) methyl 6-(cyanomethyl)-picolinate (23) in quantitative yield. The nitrile was then subjected to hydrolysis with HCl to
form the carboxylic acid (18). The basic function of the pyridine ring made this reaction extremely hard to work up and isolate the product. We also tried Diisobutylaluminium hydride (DIBAL or DIBALH) to reduce the nitrile with an aqueous workup. Neither attempt was successful (scheme 2.3).

**Equation 2.2: Methyl 6-(cyanomethyl)-picolinate**

![Equation 2.2 Diagram]

**Scheme 2.3: Synthesis of 2-(6-(methoxycarbonyl)pyridin-2-yl)acetic acid (18) and methyl 6-(2-oxoethyl)picolinate (24) from methyl 6-(cyanomethyl)-picolinate (23).**

![Scheme 2.3 Diagram]
With no successful method for the carbon extension, we decided to switch gears and try coupling reactions for addition of the carbon to the pyridyl ring (Suzuki\textsuperscript{50}, Negishi\textsuperscript{51}, Heck\textsuperscript{52}, Sonogashira\textsuperscript{53}, and Stille\textsuperscript{54}). A new proposed synthesis which includes the coupling reaction is outlined in scheme 2.4. Methyl 6-bromopyridine-2-carboxylate is commercial available from Aldrich for $50 US for 1.0 gram.

**Scheme 2.4:** Proposed synthesis of Ligand (2) using a coupling reaction for carbon extension
The Suzuki reaction (equation 2.3) was carried out with Pd(PPh$_3$)$_4$ (tetrakis (triphenyl-phosphine) palladium), a boronic acid and a base. The best Suzuki coupling reaction (table 2.1, entry 8) was run with 10 mol% Pd(PPh$_3$)$_4$, 1.5 eqv. of vinylboronic acid pinacol ester, 2 eqv. of Na$_2$CO$_3$, and run in toluene to yield 44%. Professor Molander’s (Penn University) BF$_3$K salts (boronic acid) produced very small amounts of coupled products (table 2.1, entry 11-24).

**Equation 2.3:** Coupling reactions for the synthesis of methyl 6-vinylpicolinate (22).
Table 2.1: Optimization of coupling reactions for the synthesis of methyl 6-vinyl-picolinate (22)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Pd</th>
<th>Boronic Acid or BF₃K salt (1.5 eqv.)</th>
<th>Base (2 eqv.)</th>
<th>Solvent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>Na₂CO₃</td>
<td>Dioxane</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>Na₂CO₃</td>
<td>DME</td>
<td>8%</td>
</tr>
<tr>
<td>3</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>NaOH</td>
<td>Dioxane</td>
<td>N.R.</td>
</tr>
<tr>
<td>4</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>NaOH</td>
<td>DME</td>
<td>13%</td>
</tr>
<tr>
<td>5</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>CsCO₃</td>
<td>Toluene</td>
<td>trace</td>
</tr>
<tr>
<td>6</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>CsCO₃</td>
<td>Dioxane</td>
<td>N.R.</td>
</tr>
<tr>
<td>7</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>CsCO₃</td>
<td>DME</td>
<td>N.R.</td>
</tr>
<tr>
<td>8</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>Na₂CO₃</td>
<td>Toluene</td>
<td>44%</td>
</tr>
<tr>
<td>9</td>
<td>Tetrakis (5 mol %)</td>
<td>Pinacol Ester</td>
<td>Na₂CO₃</td>
<td>Toluene</td>
<td>23%</td>
</tr>
<tr>
<td>10</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>Na₂CO₃</td>
<td>Tolu./MeOH (10:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>11</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>t-BuNH₂</td>
<td>i-PrOH/H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>12</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>t-BuNH₂</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>13</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>(i-Pr)₂NEt</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>14</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>CsCO₃</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>14%</td>
</tr>
<tr>
<td>15</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>Na₂CO₃</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>16</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>CsCO₃</td>
<td>THF</td>
<td>8%</td>
</tr>
<tr>
<td>17</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>Na₂CO₃</td>
<td>THF</td>
<td>20%</td>
</tr>
<tr>
<td>18</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>NaOH</td>
<td>THF</td>
<td>N.R.</td>
</tr>
<tr>
<td>19</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>Et₃N</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>20</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>Et₃N</td>
<td>DME/DMF</td>
<td>N.R.</td>
</tr>
<tr>
<td>21</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>Na₂CO₃</td>
<td>DME/DMF</td>
<td>N.R.</td>
</tr>
<tr>
<td>22</td>
<td>Tetrakis (10 mol %)</td>
<td>BF₃K salt</td>
<td>CsCO₃</td>
<td>THF/ H₂O</td>
<td>Trace</td>
</tr>
<tr>
<td>23</td>
<td>Tetrakis (10 mol %)</td>
<td>BF₃K salt</td>
<td>Na₂CO₃</td>
<td>THF/ H₂O</td>
<td>Trace</td>
</tr>
<tr>
<td>24</td>
<td>Pd(dppf)Cl₂CH₂Cl₂</td>
<td>BF₃K salt</td>
<td>CsCO₃</td>
<td>THF/ H₂O</td>
<td>N.R.</td>
</tr>
<tr>
<td>25</td>
<td>Tetrakis (10 mol %)</td>
<td>MIDA with XPhos</td>
<td>K₃PO₄</td>
<td>i-PrOH/H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>26</td>
<td>Tetrakis (10 mol %)</td>
<td>MIDA with XPhos</td>
<td>Na₂CO₃</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>27</td>
<td>Tetrakis (10 mol %)</td>
<td>MIDA with SPhos</td>
<td>K₃PO₄</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>28</td>
<td>Tetrakis (10 mol %)</td>
<td>MIDA with SPhos</td>
<td>N/A</td>
<td>i-PrOH/ H₂O (2:1)</td>
<td>N.R.</td>
</tr>
<tr>
<td>29</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester (2.0 eqv)</td>
<td>Na₂CO₃</td>
<td>Toluene</td>
<td>35%</td>
</tr>
<tr>
<td>30</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester (2.5 eqv)</td>
<td>Na₂CO₃</td>
<td>Toluene</td>
<td>41%</td>
</tr>
<tr>
<td>31</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester (3.0 eqv)</td>
<td>Na₂CO₃</td>
<td>Toluene</td>
<td>45%</td>
</tr>
<tr>
<td>32</td>
<td>Tetrakis (10 mol %)</td>
<td>Tribuyl(vinyl) tin</td>
<td>Na₂CO₃</td>
<td>Dioxane</td>
<td>55%</td>
</tr>
<tr>
<td>33</td>
<td>Tetrakis (10 mol %)</td>
<td>Tribuyl(vinyl) tin</td>
<td>Na₂CO₃</td>
<td>DME</td>
<td>45%</td>
</tr>
<tr>
<td>34</td>
<td>Tetrakis (10 mol %)</td>
<td>Tribuyl(vinyl) tin</td>
<td>N/A</td>
<td>Dioxane</td>
<td>85%</td>
</tr>
<tr>
<td>35</td>
<td>Tetrakis (5 mol %)</td>
<td>Tribuyl(vinyl) tin</td>
<td>N/A</td>
<td>Dioxane</td>
<td>70%</td>
</tr>
</tbody>
</table>
Stille coupling was the next reaction carried out. The conditions were similar to Suzuki coupling just the boronic acid was changed to tribuyl(vinyl) tin (table 2.1, entry 32-35). Stille coupling by far worked the best on (equation 2.4) methyl 6-bromopyridine-2-carboxylate (21) with the conditions of 10mol% Pd(PPh₃)₄, 1.5 eqv. tribuyl(vinyl) tin, and dioxane for a solvent to yield 85% methyl 6-vinylpicolinate (22)

**Equation 2.4:** Final conditions for synthesis of methyl 6-vinylpicolinate (22)

With much motivation that the reaction yield was high, we continued to the hydroboration (equation 2.5) of the alkene (22) with borane (BH₃). The results are highlighted in table 2.2 for which entry 8 has the best yield of methyl 6-(2-hydroxyethyl)picolinate (20) at 41% isolated.

**Equation 2.5:** Synthesis of methyl 6-(2-hydroxyethyl)picolinate (20).
Table 2.2: Optimization of hydroboration workup for the synthesis of methyl 6-(2-hydroxyethyl)picolinate (20).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>Oxidation method</th>
<th>Solvent</th>
<th>Temp</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BH₃-S(CH₃)₂ (1eqv.)</td>
<td>NaOOH (4eqv.)</td>
<td>THF</td>
<td>27°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>BH₃-S(CH₃)₂ (2.2 eqv.)</td>
<td>NaOOH (4eqv.) in Methanol</td>
<td>THF</td>
<td>0°C</td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>BH₃-S(CH₃)₂ (0.3 eqv.)</td>
<td>NaOOH 4eqv.) in Methanol</td>
<td>THF</td>
<td>0°C</td>
<td>8%</td>
</tr>
<tr>
<td>4</td>
<td>BH₃-S(CH₃)₂ (0.3 eqv.)</td>
<td>NaOOH (excess) in MeOH</td>
<td>THF</td>
<td>0°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>5</td>
<td>BH₃-S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃ (4 eqv.)</td>
<td>THF</td>
<td>0°C</td>
<td>17%</td>
</tr>
<tr>
<td>6</td>
<td>BH₃-S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃ (10 eqv.) in 100eqv H₂O</td>
<td>THF</td>
<td>0°C</td>
<td>20%</td>
</tr>
<tr>
<td>7</td>
<td>BH₃-S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃ (10eqv.) 100eqv H₂O</td>
<td>THF</td>
<td>27°C</td>
<td>15%</td>
</tr>
<tr>
<td>8</td>
<td>BH₃-S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃ (10 eqv.) 100eqv H₂O</td>
<td>THF</td>
<td>0°C</td>
<td>41%</td>
</tr>
</tbody>
</table>

Next, the alkyl alcohol (20) was screened with halogenations reactions. For bromination, PBr₃ and CH₃Br were tested but unfortunately no product (methyl 6-(2-chloroethyl)-picolinate (21)) formation was observed. Next, chlorination was examined with POCl₃ and SOCl₂. Thioyl chloride (SOCl₂) in dichloromethane at room temperature achieved the alkyl halide (21a) in quantitative yield. These results are outlined in equation 2.6 and table 2.3.
**Equation 2.6:** Halogenation of 6-(2-hydroxyethyl)picolinate (20) for synthesis of (methyl 6-(2-chloroethyl)picolinate (21a) or methyl 6-(2-bromoethyl)picolinate (22b).

![Equation 2.6: Halogenation reaction](image)

**Table 2.3:** Optimization of halogenation of 6-(2-hydroxyethyl)picolinate (20) for synthesis of (methyl 6-(2-chloroethyl)picolinate (21a) or methyl 6-(2-bromoethyl)picolinate (22b).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBr₃ (1 eqv.)</td>
<td>CH₂Cl₂</td>
<td>27°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>PBr₃ (1 eqv.)</td>
<td>CH₂Cl₂</td>
<td>0°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>3</td>
<td>PBr₃ (1 eqv.)</td>
<td>CH₂Cl₂</td>
<td>27°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>4</td>
<td>CBr₄, PPh₃ (1 eqv.)</td>
<td>CH₂Cl₂</td>
<td>27°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>5</td>
<td>POCl₃ (1 eqv.)</td>
<td>CH₂Cl₂</td>
<td>27°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>6</td>
<td>SOCl₂</td>
<td>CH₂Cl₂</td>
<td>0°C</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>SOCl₂ (1 eqv.)</td>
<td>CH₂Cl₂</td>
<td>27°C</td>
<td>Quant</td>
</tr>
</tbody>
</table>
Now with the alkyl halide in hand, we focused on the alkylation with 1,10-diaza-18-crown-6 in basic conditions (scheme 2.5). The reaction was refluxed for 30 hours and yielded dimethyl 6,6′-((1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl))dipicolinate (22) in 67% isolated yield. Next, (22) reacted with 6 M hydrochloric acid for 12 hrs to deprotect the methyl esters to carboxylic acids to produce the final ligand 6,6′-((1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl))dipicolinic acid (2) in 43% yield.

**Scheme 2.5:** Final steps of synthesis of 6,6′-((1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethane-2,1-diyl))dipicolinic acid (2).
Characterization of ligand (2) was carried out with $^1H$, $^{13}C$ NMR, and HRMS. The overall synthesis' yield is 10% in a short, concise 5 step procedure. The most problematic step in synthesizing ligand (2) was the addition of a carbon in the two position of the pyridyl ring.

With ligand (2) completed, we turned our attention to ligand (3) (6,6'-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(propane-3,1-diyl))dipicolinic acid) which had a 3 carbon extension from the crown function to the pyridyl ring. The proposed synthesis is outlined in scheme 2.6.

Scheme 2.6: Proposed synthesis of Ligand (2) 6,6'-(1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane-7,16-diyl)bis(propane-2,1-diyl))dipicolinic acid (3).
From the previous synthesis of ligand 2, there were many grams of intermediate 15 which worked out in our advantage due to using it in this synthesis. The first reaction is conversion of the alkyl halide 15 to methyl 6-(3-hydroxypropyl)picolinate 25 with formation of the Grignard reagent with Magnesium metal (scheme 2.6). This method would also have countless problems with any product formation which was the same case in the synthesis of ligand 2. With no successful method for the carbon extension again, we decided to try coupling reactions (Suzuki, Negishi, Heck, Sonogashira, and Stille). A proposed synthesis for ligand (3) based on the first reaction being a C-C formation reaction (scheme 2.7). Methyl 6-bromopyridine-2-carboxylate is commercial and readily available from Aldrich which makes it a good starting material.
Scheme 2.7: Proposed synthesis of Ligand (3) using a coupling reaction for carbon extension

Next, we turned to coupling reactions for the carbon extension in ligand 3 since we had previous success for ligand 2. These reactions are in equation 2.7 and table 2.4.
Equation 2.7: Optimization of reactions for the synthesis of methyl 6-allylpicolinate (28)

![Chemical structure and reaction scheme]

**Sources of Pd**
- Pd(Ph3)4: Tetrakis (triphenylphosphine) palladium
- Pd(dppf)Cl2(CH2)2: 1,1'-Bis(diphenyl-phosphino)ferrocene dichloropalladium

**Boronic Acid Sources**
- Vinylboronic Acid Pinacol Ester
- Potassium vinyltrifluoroborate
- MIDA = Vinylboronic acid MIDA ester

<table>
<thead>
<tr>
<th><strong>Entry</strong></th>
<th><strong>Pd</strong></th>
<th><strong>Boronic Acid or BF3K salt (1.5 eqv.)</strong></th>
<th><strong>Base</strong> (2 eqv.)</th>
<th><strong>Solvent</strong></th>
<th><strong>Yield</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>Na2CO3</td>
<td>Dioxane</td>
<td>32%</td>
</tr>
<tr>
<td>2</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>Na2CO3</td>
<td>DME</td>
<td>24%</td>
</tr>
<tr>
<td>3</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>NaOH</td>
<td>Dioxane</td>
<td>21%</td>
</tr>
<tr>
<td>4</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>NaOH</td>
<td>DME</td>
<td>13%</td>
</tr>
<tr>
<td>5</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>CsCO3</td>
<td>Toluene</td>
<td>9%</td>
</tr>
<tr>
<td>6</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>CsCO3</td>
<td>Dioxane</td>
<td>N.R.</td>
</tr>
<tr>
<td>7</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>CsCO3</td>
<td>DME</td>
<td>N.R.</td>
</tr>
<tr>
<td>8</td>
<td>Tetrakis (10 mol %)</td>
<td>Pinacol Ester</td>
<td>Na2CO3</td>
<td>Toluene</td>
<td>44%</td>
</tr>
</tbody>
</table>

Table 2.4: Optimization of coupling reactions for the synthesis of methyl 6-allylpicolinate (22)
Suzuki coupling was carried out first with allylboronic acid pinacol ester in Na$_2$CO$_3$ and dioxane with palladium having an oxidation state of zero (table 4, entry 1, yield =32%). BF$_3$K salts (boronic acid) did not work the best and produced at the highest yield of only 22% of coupled product (table 4, entry 11-22).

Suzuki coupling by far worked the best on methyl 6-bromopyridine-2-carboxylate (21) with allylboronic acid pinacol ester (3.0-3.5 eqv.) in Na$_2$CO$_3$ (2 eqv.) and dioxane with 15 mol% Pd(PPh$_3$)$_4$ (table 4, entry 31, yield: 68%) to yield methyl 6-allylpicolinate (28) (equation 2.8).
**Equation 2.8:** Final conditions for synthesis of methyl 6-allylpicolinate (28) using Suzuki coupling.

![Chemical structure](image)

With much excitement that the reaction yield was high and working well, we continued to the hydroboration (**equation 2.9**) of the alkene (28) with borane (BH$_3$). The results are highlighted in **table 5** for which entry 8 has the best isolated yield of methyl 6-(2-hydroxyethyl)picolinate (20) at 41%.

**Equation 2.9:** Hydroboration reaction with borane used for the synthesis of methyl 6-(2-hydroxyethyl)picolinate (20).
Table 2.5: Optimization of hydroboration workup for the synthesis of methyl 6-(2-hydroxyethyl)picolinate (20).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>Oxidation method</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BH₃ S(CH₃)₂ (1eqv.)</td>
<td>NaOOH (4eqv.)</td>
<td>THF</td>
<td>27°C</td>
<td>10%</td>
</tr>
<tr>
<td>2</td>
<td>BH₃ S(CH₃)₂ (2.2 eqv.)</td>
<td>NaOOH (4eqv.) in Methanol</td>
<td>THF</td>
<td>0°C</td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>BH₃ S(CH₃)₂ (0.3 eqv.)</td>
<td>NaOOH 4eqv.) in Methanol</td>
<td>THF</td>
<td>0°C</td>
<td>8%</td>
</tr>
<tr>
<td>4</td>
<td>BH₃ S(CH₃)₂ (0.3 eqv.)</td>
<td>NaOOH (excess) in Methanol</td>
<td>THF</td>
<td>0°C</td>
<td>N.R.</td>
</tr>
<tr>
<td>5</td>
<td>BH₃ S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃(4 eqv.)</td>
<td>THF</td>
<td>0°C</td>
<td>17%</td>
</tr>
<tr>
<td>6</td>
<td>BH₃ S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃(10 eqv.) in 100equiv H₂O</td>
<td>THF</td>
<td>0°C</td>
<td>20%</td>
</tr>
<tr>
<td>7</td>
<td>BH₃ S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃(10equiv.) 100equiv H₂O</td>
<td>THF</td>
<td>27°C</td>
<td>31%</td>
</tr>
<tr>
<td>8</td>
<td>BH₃ S(CH₃)₂ (2.2 eqv.)</td>
<td>NaBO₃(10 eqv.) 100equiv H₂O</td>
<td>THF</td>
<td>0°C</td>
<td>57%</td>
</tr>
</tbody>
</table>

Next, the alkyl alcohol (28) was screened with halogenations reactions. For bromination, PBr₃ and CH₂Br were tested. No product (methyl 6-(2-chloropropyl)-picolinate (25)) formation was observed. Next, chlorination was examined with POCl₃ and SOCl₂. No product formation was formed with these chlorinated agents as well. So this was very strange due to the halogenations reaction worked well for ligand (2). When purification was being conducted for any of these reactions, the amount of mass to the column was always about 80% less than the mass out of the column. So we used very polar solvents to wash anything that was left in the column out and we found
Equation 2.10: Halogenation of 6-(2-hydroxyethyl)picolinate (25) produced 5-(methoxycarbonyl)-2,3-dihydro-1H-indolizin-4-ium (29a or 29b) with corresponding anion.

\[
\begin{align*}
\text{PBr}_3(3 \text{ eqv}) \text{ CH}_2\text{Cl}_2 & \text{ rt} \rightarrow 5\text{-}(\text{methoxycarbonyl})\text{-}2,3\text{-dihydro}\text{-}1\text{H}\text{-}\text{indolizin}\text{-}4\text{-ium (29a or 29b)} \\
\text{PBr}_3(1 \text{ eqv}) \text{ CH}_2\text{Cl}_2 & \text{ rt} \rightarrow 48\% \\
\text{CBr}_4(3 \text{ eqv}) \text{ PPh}_3\text{CH}_2\text{Cl}_2 & \text{ rt} \rightarrow 39\% \\
\text{SOCl}_2(1.2 \text{ eqv})\text{CH}_2\text{Cl}_2 & \text{ rt} \rightarrow 52\%
\end{align*}
\]

a large enough quantity in mass to analyze by \textsuperscript{1}H NMR and HRMS. After spectrum were generated and analyzed, it was clear that the product was a cyclized version of the alkyl hailde (equation 2.10).

5-(methoxycarbonyl)-2,3-dihydro-1H-indolizin-4-ium (29a or 29b) could be reacted under basic conditions (NaH) with the crown ether molecule (equation 2.11) to theoretically synthesize (27) dimethyl 6,6'-(1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane-7,16-diyl)bis(propane-3,1-diyl)dipicolinate. The reaction did not work.
Equation 2.11: Synthesis of dimethyl 6,6’-((1,4,10,13-tetraoxa-7,16-diazacyclo-
 octadecane-7,16-diyl)bis(propane-3,1-diyl))dipicolinate.

\[ \text{Equation 2.11:} \]

Next, Aldrich had compound (30) 2-(3-bromopropyl)-4,4,5,5-tetramethyl-
1,3,2-dioxaborolane commercial available for $38 US for 1.0 gram. The crown 
ether was reacted with n-butyllithium and then with (30) 2-(3-bromopropyl)-
4,4,5,5-tetramethyl-1,3,2-dioxaborolane to produce 7,16-bis(3-(4,4,5,5-tetramethyl-
1,3,2-dioxaborolan-2-yl)propyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane
in a 68\% yield (equation 2.12). Following this basic reaction, the coupling reaction 
was tested with many different bases and solvents in which product was not 
formed in any case.
Equation 2.12: Synthesis of 7,16-bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)-1,4,10,13-tetraoxa-7,16-diazacycloclooctadecane (31) and synthesis of dimethyl 6,6’-((1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(propane-3,1-diyl))dipicolinate (3).
2.5 Conclusion

In conclusion, this chapter investigates the synthesis of ligands for the separation of Am/Cm from nuclear waste. Ligands 1 and ligand 2 were sent to the Office of Nuclear Energy-funded Sigma Team for Minor Actinide Separations at Argonne national laboratory under the supervision of Dr. Mark Jensen. A paper was recently published in Inorganic chemistry with ligand 1 for the separation of americium (Am) from curium (Cm) (DOI: 10.1021/ic500244p). Future work will make new ligands in hopes to have more efficiently separation of americium from curium. Further derivatization of ligands and exploring further applications of this new class of is still under investigation.
2.6 Experimental

I. General Considerations.

All reactions were performed under argon atmosphere via schlenk line in oven-dried glassware with magnetic stirring unless otherwise stated. Air and moisture-sensitive liquids and solutions were transferred via oven-dried, stainless steel syringe and were introduced into the reaction vessel through rubber septa. All reactants and reagents were purchased from Ark Pharm, Inc. unless otherwise stated. CH$_2$Cl$_2$ and DMF (dimethylformamide) were distilled from calcium hydride. THF (tetrahydrofuran) was distilled from sodium-benzophenone. Anhydrous acetonitrile was purchased from Sigma Aldrich. Previously reported compounds were identified by $^1$H NMR (nuclear magnetic resonance) spectrum. All new compounds were characterized by additional $^{13}$C NMR and mass spectroscopy. $^1$H and $^{13}$C NMR spectra were performed on a Bruker NMR at 300 and 75 MHz, respectively. $^1$H NMR data are reported as follows: chemical shift (δ) in parts per million (ppm) from tetramethylsilane as an internal standard (CDCl$_3$ δ7.26 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets). $^{13}$C data were reported as follows: chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane with the solvent as an internal indicator (CDCl$_3$ δ77.16 ppm).
II. Experimental Procedures

a. 6-Hydroxymethylpyridine-2-carboxylic Acid Methyl Ester (1)

NaBH₄ (4.310 g, 114.02 mmol) was added in small portions over a period of 0.5 h to a stirred solution of dimethylpyridine-2,6-dicarboxylate (7.445 g, 38.17 mmol) in MeOH (300 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h and then a saturated NaHCO₃ aqueous solution (200 mL) was added. The MeOH was removed in a rotary evaporator and the resulting aqueous solution was extracted with CHCl₃ (5 × 100 mL). The combined organic extracts were dried over MgSO₄ and evaporated to give 4.370 g of product (69%) as a white solid. Anal. Calcd for C₈H₉NO₃: C, 57.5; H, 5.4; N, 8.4%. Found: C, 56.9; H, 5.3; N, 8.3%. δH (solvent CDCl₃, 295 K, 300 MHz): 8.02 (d, 1H, py, J = 7.7 Hz); 7.84 (t, 1H, py); 7.54 (d, 1H, py, J = 7.8 Hz); 4.86 (s, 2H, -CH₂-); 3.99 (s, 3H, -OCH₃). δC (solvent CDCl₃, 295 K, 75.5 MHz): 52.6 (primary C); 64.6 (secondary C); 137.7, 124.0, 123.8 (tertiary C); 165.5, 160.3, 146.9 (quaternary C). IR: 1740 ν(C=O), 1591 ν(C=N)py cm⁻¹. FAB-MS (m/z(%BPI)): 168 (100) [M + H]⁺.
b. 6-Bromomethylpyridine-2-carboxylic Acid Methyl Ester (2)

PBr$_3$ (5 mL) was added over 6-Hydroxymethylpyridine-2-carboxylic Acid Methyl Ester (1.20 g, 7.18 mmol) at 0 °C under an inert atmosphere (Ar). The mixture was stirred at 0 °C for 1 h, and the excess of PBr$_3$ was removed under reduced pressure. The residue was dissolved in toluene (50 mL), and the organic solution, washed with a 1 M NaHCO$_3$ aqueous solution. The organic extract was evaporated, and the resulting oily residue was precipitated by addition of diethyl ether to give 1.09 g of product (92%) as a pale yellow solid. Anal. Calcd for C$_8$H$_8$BrNO$_2$: FAB-MS (m/z(%BPI)): 22.973(100) [M + 1]$^+$.δ$_H$(solvent CDCl$_3$, 295 K, 300 MHz): 8.09 (d, 1H, py, $^3J$ = 7.6 Hz); 7.90 (t, 1H, py); 7.75 (d, 1H, py, $^3J$ = 7.6 Hz); 4.59 (s, 2H, -CH$_2$-); 3.94 (s, 3H, -OCH$_3$). δ$_C$ (solvent CDCl$_3$, 295 K, 75.5 MHz): 33.1 (primary C); 53.1 (secondary C); 138.2, 127.1, 124.4 (tertiary C); 165.3, 157.4, 147.6 (quaternary C). IR: 1738 v(C=O), 1581 v(C=N)$_{py}$ cm$^{-1}$. 
c. \(N,N^\prime\)-Bis[(6-methoxycarbonyl-2-pyridyl)methyl]-1,7-diaza-12-crown-4(3).

6-Bromomethyl-pyridine-2-carboxylic Acid Methyl Ester (2.000 g, 10.78 mmol) and \(\text{Na}_2\text{CO}_3\) (3.400 g, 32.08 mmol) were added to a solution of 1,7-diaza-12-crown-4 (0.946 g, 5.43 mmol) in acetonitrile (75 mL). The mixture was heated to reflux with stirring for a period of 24 h, and then the excess of \(\text{Na}_2\text{CO}_3\) was filtered off. The filtrate was concentrated to dryness, and the yellow residue partitioned between equal volumes (200 mL) of \(\text{H}_2\text{O}\) and \(\text{CH}_2\text{Cl}_2\). The organic phase was separated, dried over \(\text{MgSO}_4\), filtered, and evaporated to dryness. Addition of hexane gave 1.922 g of 3 (75%) as a pale yellow solid. Anal. Calcd for \(\text{C}_{24}\text{H}_{32}\text{N}_4\text{O}_6\): C, 61.0; H, 6.8; N, 11.8%. Found: C, 60.4; H, 6.7; N, 11.5%. \(\delta_\text{H}\) (solvent \(\text{CDCl}_3\), 295 K, 300 MHz): 8.09 (d, 2H, py, \(3J = 8.6\) Hz); 8.01 (d, 2H, py, \(3J = 7.6\) Hz); 7.82 (t, 2H, py); 4.00 (s, 6H, -OCH\(_3\)); 3.97 (s, 4H, -CH\(_2\)-); 3.63 (t, \(3J = 4.6\) Hz 8H, -CH\(_2\)-); 2.83 (m, 8H, -CH\(_2\)-). \(\delta_\text{C}\) (solvent \(\text{CDCl}_3\), 295 K, 75.5 MHz): 52.9 (primary C); 55.3, 62.2, 69.5 (secondary C); 123.5, 126.3, 137.3 (tertiary C); 147.1, 161.4, 165.9 (quaternary C). IR: 1709 \(\nu(\text{C}==\text{O})\), 1589 \(\nu(\text{C}==\text{N})\) \(\text{py}\) cm\(^{-1}\). FAB-MS (\(m/z\)(%BPI)): 473(100) [M + H]\(^+\).
d. *N*,*N*-Bis[[6-carboxy-2-pyridyl)methyl]-1,7-diaza-12-crown-4 (H₂bp12c4·4HCl).

A solution of compound *N*,*N*-Bis[[6-methoxycarbonyl-2-pyridyl)methyl]-1,7-diaza-12-crown-4 (3) (1.000 g, 2.12 mmol) in 6 M HCl (10 mL) was heated to reflux for 12 h. After cooling to room temperature the white solid formed was collected by filtration to give 1.044 g (81%) of the desired product. Anal. Calcd for C₂₂H₂₈N₄O₆·4HCl·H₂O: C, 43.4; H, 5.6; N, 9.2%. Found: C, 43.4; H, 6.4; N, 9.1%. δH (solvent D₂O, 295 K, 300 MHz, pD = 8.0): 7.98 (t, 2H, py); 7.89 (d, 2H, py, ³J = 7.7 Hz); 7.65 (d, 2H, py, ³J = 7.6 Hz); 4.07 (s, 4H, -CH₂-); 3.55 (t, ³J = 4.6 Hz, 8H, -CH₂-); 3.02 (t, ³J = 4.6 Hz, 8H, -CH₂-). δC (solvent D₂O, 295 K, 75.5 MHz, pD = 8.0): 55.4, 62.8, 68.3 (secondary C); 140.3, 128.7, 124.9 (tertiary C); 174.6, 156.1, 155.1 (quaternary C). IR: 1697 ν(C=O), 1592 ν(C=N)py cm⁻¹. FAB-MS (m/z(%BPI)): 445(100) [H₃bp12c4]⁺.
e. **Methyl 6-(2-vinyl)picolinate.** To a flask containing methyl-6-bromopyridine-2-carboxylate (12.0g) (55.6mmol) dissolved in freshly distilled DMF (125ml) was dry loaded tetrakis(triphenylphosphine) palladium(0) (3.21g) (2.78mmol) under positive argon. To the reaction mixture was added tributyl(vinyl) tin (16.23ml) (55.6mmol) via syringe and then the reaction mixture was stirred at 90 °C for 24 h. The reaction mixture was cooled to r.t. and extracted by adding EtOAc (250mL) and washing with DI water (3x125mL) to remove DMF. The aqueous portions were added together and extracted with EtOAc (3x250mL). The organic layers were combined and dried over anhydrous Na₂SO₄ and the solvent was removed by rotovap. Pure methyl 6-(2-vinyl)picolinate was obtained via silica column with 20% ethyl acetate/hexane as the eluent giving the desired product (7.33g) (81% yield) as a pale yellow oil. **¹H NMR (300MHz, CDCl₃)** δ 7.86 (d, 1H, J = 9.0), 7.67 (t, 1H, J = 9.0), 7.47 (d, 1H, J = 9.0), 6.81 (dd, 1H, J = 12, 18), 6.10 (d, 1H, J = 18), 5.45 (d, 1H J = 12), 4.00 (s, 3H)
f. **Methyl 6-(2-hydroxyethyl)picolinate.** To a flask containing methyl 6-(2-vinyl)picolinate (1.00g, 6.13mmol) dissolved in freshly distilled THF (20ml) was added BH₃·S(Me)₂ (6.44mL, 2M solution in THF, 12.9mmol) at 0°C via syringe. This was allowed to warm to r.t. while stirring for 2 h. The reaction flask was then cooled to 0°C and removed from positive argon. NaBO₃·4H₂O (4.72g, 30.6mmol) and DI water (1ml) were added and the reaction mixture was warmed to r.t. and allowed to mix overnight. THF was removed under reduced pressure by rotovap. EtOAc (100ml) was added and the solution was washed with DI water (100ml) and the wash was extracted with ethyl acetate (3x100ml). The organic layers were combined and dried over anhydrous Na₂SO₄ and the solvent was removed by rotovap. The crude mixture was purified by silica column with 45, 65, 100% EtOAc/hexane as the eluent giving the desired product (223mg) (20% yield) as a cloudy yellowing oil. **¹H NMR** (300MHz, CDCl₃) δ 7.97 (d, 1H, J = 6.0), 7.76 (t, 1H, J = 7.5), 7.38 (d, 1H, J = 9.0), 4.04 (t, 2H, J = 4.5), 3.96 (s, 3H), 3.09 (t, 2H, J = 4.5)
g. **Methyl 6-(2-chloroethyl)picolinate.** To a dry round bottom flask containing methyl 6-(2-hydroxyethyl)picolinate (500mg, 2.76mmol) dissolved in distilled dichloromethane (25ml) was added SOCl₂ (242µL, 3.31mmol) at 0°C via syringe. The reaction mixture was warmed to r.t. and stirred for 3 h. The solution was washed with DI water (50mL) and extracted with dichloromethane (3x50mL), giving pure methyl 6-(2-chloroethyl)picolinate (545mg) (99% yield). ¹H NMR (300MHz, CDCl₃) δ 8.04 (d, 1H, J = 9.0), 7.80 (t, 1H, J = 7.5), 7.43 (d, 1H, J = 6.0), 4.01 (s, 3H), 3.95 (t, 2H, J = 6.0), 3.35 (t, 2H, J = 7.5)
h. 2-Pyridinecarboxylic acid, 6,6’-[(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethylene)]bis-, 2,2'-dimethyl ester. To a dry two neck flask equipped with condenser and stir bar was dry loaded 1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane (178mg, 0.564mmol) and Na₂CO₃ (398mg, 3.76) while under positive N₂. To this was added methyl 6-(2-chloroethyl)picolinate (250mg, 1.25mmol) dissolved in anhydrous acetonitrile (2mL) via syringe. The reaction mixture was then heated to 90 °C and stirred overnight. The reaction mixture was cooled to r.t. and passed through a celite plug and the acetonitrile was removed by rotovap. The crude mixture was purified via neutral alumina column (0, 2, 10% MeOH/EtOAc) giving pure 2-Pyridinecarboxylic acid, 6,6’-[(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethylene)]bis-, 2,2'-dimethyl ester (79mg) (20% yield).¹H NMR (300MHz, CDCl₃) δ 7.92 (d, 1H, J = 6.0), 7.70 (t, 1H, J = 7.5), 7.39 (d, 1H, J = 9.0), 3.96 (s, 3H), 3.53 (t, 16H), 3.02 (t, 2H, J = 6.6), 2.91 (t, 2H, J = 6.6), 2.80 (t, 8H, J = 6.0) ¹³C NMR (75 MHz, CDCl₃): δ 165.97, 161.31, 147.44, 136.97, 126.92, 122.74, 70.66, 69.91, 55.51, 53.90,
To a round bottom flask containing 2-Pyridinecarboxylic acid, 6,6’-[(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethylene)]bis(2,2'-dimethyl ester (150mg, 0.255mmol) was added 6M HCl (2mL) and refluxed overnight. The reaction mixture was cooled to r.t. and crystalized by the slow addition of MeOH, THF then acetone with intermediate cooling to -78 °C. Giving pure 2-Pyridinecarboxylic acid, 6,6’-[(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl)bis(ethylene)]bis (28mg) (20% yield) as a white powdery solid.  

\[ ^1H\text{ NMR}\ (300MHz, \text{CDCl}_3) \delta 8.12-8.09\ \text{(comp, 4H)},\ 7.71\ \text{(comp, 2H)},\ 4.00\ \text{(comp, 8H)},\ 3.81-3.68\ \text{(comp, 16H)},\ 3.45\ \text{(comp, 4H)},\ 3.33\ \text{(t, 4H)}\]

**MS (DUIS):** Calculated (m/z) for C_{28}H_{41}N_{4}O_{8} (M+H)^+ : 561.29, Found 561.7.
2.7 References


34. Smith, R. M.; Martell, A. E.; Motekaitis, R. J., *Critically Selected Stabilities Constants of Metal Complexes Database Version 8.0*. NIST Standard Reference Data: Gaithersburg, Maryland, USA, 2004; Vol. 46.


PART III: DESIGNING STRONG CHIRAL BRØNSTED ACIDS AND THEIR APPLICATION FOR OXAZINANONES DERIVATIZATION AND THE AZA-HENRY REACTION

Overview:

An essential challenge of organic chemistry is the future development and synthesis of complex organic molecules. The addition of useful functional groups in a short, concise and cheap fashion is vital in synthesizing these types of molecules. There is an overwhelming need for these types of reactions that install numerous functionalities, in few synthetic steps and with high selectivity and yield. Carbon-carbon (C-C) bond formation is the framework of synthesizing any advanced molecule/drug candidate. C-C bond formation allows for the transformation of cheap starting material into expensive/important organic compounds. In the last decade, much effort has been devoted to organocatalytic C-C bond forming reactions. The work in this chapter focuses on the first Brønsted acid catalyzed carbon-carbon bond formation by an unprecedented tandem Mannich reaction. In the presence of a simple, achiral Brønsted acid, tetrafluoroboric acid or triflic acid, catalyze N-tert-butoxycarbonl (N-Boc)-imines to yield cyclic 1, 3-oxazinan-2-ones in very good yields. Following the achiral reactions, we then turn to developing a chiral Brønsted acid reaction that is used in place of tetrafluoroboric acid or triflic acid.
3.1 Introduction

The Mannich reaction is a classic method for the synthesis of β-amino carbonyl compounds which makes it very attractive in complex molecule formation development which is why it is such an important carbon-carbon bond formation reaction in organic synthesis. The imagination of chemist’s has long been motivated by this reaction’s flexibility and capability to generate both functional and structural diversity compounds. For example, the Mannich reaction has been used for many decades in natural product synthesis and in medicinal chemistry derivatization.

Chiral nitrogen-containing compounds form an enormous group in biologically active natural products. These nitrogen-containing units play a key role in biologically active molecules to provide binding sites. Imines are an important class of starting materials and play a key role in synthesizing various nitrogen-containing biologically active target molecules.

Nucleophilic addition to imines also provides an easy route to a vast array of nitrogen-containing biologically active compounds (Figure 3.1). Without
Figure 3.1: Imine provides a vast array of nitrogen containing compounds

Additional enantiocontrol elements, the obtained product would be racemic since the nucleophile will have equal opportunity to attack from either the *re* or *si* face of the prochiral substrate. Traditionally, the control elements are attached to the imines as one or more substituents, or as the attacking nucleophiles. This requires pre-existing stereochemistry or a chiral auxiliary. In the former case, the stereochemical outcome is hard to control; and, in the later case, the chiral auxiliary has to be removed after the initial reaction has been performed. In any case, the enantiocontrol element must be used in a stoichiometric (1eqv.) amount for efficient induction. Catalysis can be applied to avoid wasteful
stoichiometric amounts. The development of efficient chiral catalysts in recent years has revolutionized the way in which chiral compounds are made.

Imines are less electrophilic compared to carbonyl compounds. One or more α-hydrogen containing imines are more susceptible to deprotonation and side reactions (such as tautomerization to enamines). The electrophilicity of an imine can be enhanced by using an activating group that can coordinate to the nitrogen atom, typically a Lewis acid. Unfortunately, the nitrogen containing imine and its product after nucleophilic addition reaction can also strongly bind to the activator. Therefore, the catalytic process is difficult to achieve. Predicting and controlling stereochemistry of the reaction of imines are also difficult due to the presence of both syn- and anti-isomers, which may be present in equilibrium. The catalytic asymmetric addition to C=N is in an early stage even though it has great potential in organic synthesis. These difficulties and potentials have attracted a variety of research groups in recent years. As a result, a significant amount of advancement has been witnessed in this area. Many of the successful methods involve transition metal catalysis and organocatalysis. The most effective catalysts almost always operate in a multifunctional mode. They usually bind with both the imine substrate and the nucleophile, although not necessarily at the same center; and are arranged such a way that they can interact only in a very specific fashion, thereby not only reducing the activation energy, but also providing the desired enatioselectivity – as in much the same way enzymes catalyze chemical reactions.
3.1 Aza-Henry Reaction

Continuous growth in high-throughput screening for enantiomerically pure biologically active agents has increased the demand for the development of enantioselective synthetic methods that can provide exceptional stereoselectivity. Organic compounds containing vicinal nitrogenated functionalities are receiving ever-increasing attention by synthetic organic chemists because of this growing need. The nucleophilic additions of nitroalkanes to imines using aza-Henry or nitro-Mannich reactions are a powerful transformation that can generate the carbon-carbon bond with simultaneous generation of two vicinal stereogenic centers bearing both nitro and amino functional groups.\textsuperscript{3,4,5,6}

The first catalytic aza-Henry reaction was reported by Shibasaki in 1999 following their successful introduction of a catalyst for the imine class of compounds involving the analogous nitro-aldol reaction (Equation 1).\textsuperscript{7} The reaction of nitromethane and \textit{N}-phosphinoyl benzaldimine was catalyzed by a hetero-bimetallic complex (1) prepared in situ from Yb(O\textsuperscript{3}Pr\textsubscript{3}), KO\textsuperscript{t}Bu, and binaphthol in a 1:1:3 molar ratio. Slow addition of excess nitromethane to the imines, and the use of non-polar solvents (toluene/THF 7/1) at low temperature (-40\textdegree C) and high catalyst loading (20 mol\%) were essential to ensure good yield and %ee. This catalyst prepared from (R)-binaphthol produced the nitroamine product with an (R)-stereoconfiguration at the newly formed stereogenic center.
**Equation 3.1:** First catalytic aza-Henry reaction reported by Shibasaki.

\[
\text{Ph} \underset{\text{Ph}_2}{\text{N}} \underset{\text{P}}{\text{O}} + \text{CH}_3\text{NO}_2 \xrightarrow{\text{Catalyst (20 mo\%)}} \text{toluene/THF (7/1)} \xrightarrow{-40^\circ\text{C}, 60\text{h}} \text{Ph} \underset{\text{Ph}_2}{\text{N}} \underset{\text{P}}{\text{O}} \text{NO}_2
\]

79% yield, 91% ee

Shibasaki *et. al.* later reported that an \((R)-\text{ALB-KO}^\text{Bu}\) combination was a more effective catalyst for nitro compounds other than nitromethane (Equation 3.2).° The ee range was, however, not quite as impressive (60-83%). It was found that the diastereoselectivity of the reaction could therefore be controlled by choosing an appropriate solvent. In CH\(_2\)Cl\(_2\), formation of the anti-isomer was favored over the syn-isomer by ratios ranging from 75:25 – 88:12. The catalyst prepared from \((R)\)-binaphthol provided the nitroamine product with a \((1R, 2S)\)-configuration. The \((R)\)-ALB catalyst (2) alone did not promote the reaction at low temperature, while at room temperature, only racemic product was formed in good yield. An example of a two-step transformation to optically active 1,2-
diamine is presented in Equation 3.3. The methodology has been applied to the synthesis of the substance-P antagonist, CP-99994.

**Equation 3.2:** Shibasaki's (R)-ALB-KO\(^t\)Bu catalyst for asymmetric aza-Henry reaction.

\[
\text{Ar} = \text{Ph, 4-MeOC}_{6}H_{4}, 4-\text{CH}_{3}C_{6}H_{4}, 4-\text{ClC}_{6}H_{4}; \text{R = Me, Et, (CH}_{2}\rangle_{2}\text{OBn}
\]

yield = 68 - 98\%, \textit{anti:syn} = 3:1 - 7:1; \textit{ee (anti)} = 60 - 83%
**Equation 3.3:** Synthesis of CP-99994 antagonist using asymmetric aza-Henry reaction.

![Synthesis reaction diagram]

Jørgensen later used Cu-bisoxazoline complexes as catalysts for addition of silyl notronates to an imine of glyoxalic ester (Equation 3.4). In this case even N-PMP imine could be a good substrate due to the more electrophilic nature of glyoxylic imines compared to benzaldimines, and the additional coordinating ability of the CO$_2$Et group to the catalyst. The catalysts prepared from CuPF$_6$ or Cu(SbF$_6$)$_2$ and (4R,5S)-DiPhBOX ligand (3a) were found to be the best, providing almost or over 90% $ee$ of silylnitronates derived from 1-nitropropane, 1-nitropentane and 2-phenyl nitroethane. For silylnitronate of nitroethane, (S,S)-PhBOX ligand (3b) gave better results. In accordance to previous works, formation of the *anti*-products is favored up to 97:3 diastereoselectivity. A similar
Cu-(R,R)-PhBOX (3c) catalyst in the combination with a catalytic amount of triethylamine allowed direct nitro-Mannich reaction of unmodified nitroalkanes and glyoxal imines (Equation 3.5). The reaction could be performed in CH$_2$Cl$_2$ under ambient condition without the requirement of dry or inert atmosphere. The same set of nitroalkanes gave very good ee (74-99%) and diastereoselectivity (anti:syn > 90:10 for nitroalkanes with $\alpha$-substituents larger than methyl group) (Equation 5). Nitroethane gave only 70:30 selectivity and phenylnitromethane gave almost 50:50 diastereomeric mixture, probably due to Et$_3$N assisted equilibrium. Nitroethanol and ethyl nitroacetate gave the products with lower enantioselectivity than unfunctionalized nitroalkanes. Yet ee of 70% was obtained with nitroethanol. A model was proposed to explain the selectivity. Reduction of nitro group with Raney Ni followed by oxidative deprotection of the PMP group by ceric ammonium nitrate (CAN) provided a convenient route to $\alpha,\beta$-
diamino acid. A similar bis(oxazoline)-Cu(II) complex conveniently grafted onto a mesoporous silica provided comparable enantioselectivities and even higher diastereoselectivities than the unsupported catalyst under similar conditions.\textsuperscript{11}

**Equation 3.5:** Cu-(R,R)-bisoxazoline complex catalyzed asymmetric aza-Henry reaction.

A protocol for the enantioselective nitro-Mannich coupling between alkyl, aryl, and heterocyclic p-methoxybenzylimines and trimethylsilylnitropropanate catalyzed by a chiral t-Bu-BOX Cu(II) catalyst is reported by Anderson et. al.\textsuperscript{12} It uses the lowest reported loading of commercially available metal catalyst and chiral ligand, and gives the highest yields and selectivities for a broad substrate range including nonaromatic aldimines. The resultant nitroamines are obtained in 70-94% enantiomeric excess in good yield and can be readily reduced to synthetically useful 1,2-diamines.

Recently metal-free organocatalysts were reported to catalyze asymmetric aza-Henry reaction. A structurally simple thiourea derivative from 1,2-diaminocyclohexane was prepared by Takemoto and was found to be the
effective organocatalyst for the reaction between nitromethane and imines.\textsuperscript{13} The catalyst was designed with a hypothesis that the nitro compound would bind to the urea by hydrogen bonding, and its proton would be abstracted by the vicinal tertiary amine to create a chiral nitronate complex, which could add to imine with high selectivity (Equation 6). Indeed a range of $N$-phosphinoylaldimines lacking $\alpha$-hydrogens was good substrates providing $ee$ in the range of 63-76\% (Equation 3.7). Nitroethane could also be the source of nitronate although the diastereoselectivity and enantioselectivity were not quite satisfactory (73:27 and 67\%). The reaction worked best in nonpolar solvents and the presence of an amino group in the vicinity of the urea function was essential for the catalytic activity. It should also be noted that $N$-tosylimine gave almost racemic product and $N$-phenylimine was unreactive, therefore, phosphinoyl group must play some important role in determining the enantioselectivity.

\textbf{Equation 3.6:} Mechanism for the formation of chiral nitronate complex.
**Equation 3.7:** Asymmetric aza-Henry reaction using thiourea derivative as a catalyst.

![Chemical Structure](image)

Equation: \[ \text{Equation 3.7: Asymmetric aza-Henry reaction using thiourea derivative as a catalyst.} \]

A chiral bis-amidinium salt (5) found to catalyze the enantioselective aza-Henry reaction between $N$-Boc-benzaldimines and nitroalkanes (Equation 3.8).\(^{14}\) Addition of nitromethane and nitroethane provided the β-nitro-$N$-Boc-amines in moderate yield and a fairly good to excellent enantiocontrol. In addition, a good diastereoccontrol was observed for addition of nitroethane, with the *anti*-isomer being favored (*anti*: *syn* = 88 : 12 – 95 : 5).

Further improvement was attained by modification of bulkiness of chiral bis-amidinium salt (6).\(^{15}\) The new bulkier catalyst (6) helps to achieve better selectivity than the previous catalyst (5) (Equation 3.9).
**Equation 3.8:** Asymmetric aza-Henry reaction catalyzed by chiral bis-amidinium salt.

\[
\text{Ar} = \text{Ph, 2-, 3-, and 4-NO}_2\text{C}_6\text{H}_4, \text{4-CF}_3\text{C}_6\text{H}_4, \text{4-CIC}_6\text{H}_4, \text{4-CF}_3\text{OC}_6\text{H}_4.;} \\
\text{R = H, Me; Yield = 50 - 69%; ant\textit{i}:syn = 88:12 - 95:5; ee = 60 - 95%}
\]

**Equation 3.9:** Bulkier chiral bis-amidinium salt catalyzed asymmetric aza-Henry reaction.
Rueping and co-workers have reported the development of chiral Brønsted-acid-catalysed diastereo- and enantioselective nitro-Mannich reactions between \( \alpha \)-imino esters and diverse nitroalkanes, providing valuable \( \beta \)-nitro-\( \alpha \)-amino esters (Equation 3.10).\(^{16}\) The authors postulated a plausible reaction mechanism in which they assume that the chiral Brønsted acid 10 plays a bifunctional role. On the one hand, the \( \alpha \)-imino ester would be activated by protonation, resulting in the formation of the chiral ion pair. It can be assumed that the adjustment of the nitroalkane/nitronate equilibrium should also be accelerated by 10.

**Equation 3.10:** Scope of aza-Henry reaction catalyzed by chiral phosphoric acid.

<table>
<thead>
<tr>
<th>( R )</th>
<th>Yield (%)</th>
<th>( d.r. ) (anti/syn)</th>
<th>e.r.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Et</td>
<td>64</td>
<td>9:1</td>
<td>96:4</td>
</tr>
<tr>
<td>Me</td>
<td>61</td>
<td>10:1</td>
<td>96:4</td>
</tr>
<tr>
<td>( nC_3H_11 )</td>
<td>65</td>
<td>9:1</td>
<td>96:4</td>
</tr>
<tr>
<td>PhCH(_2)</td>
<td>93</td>
<td>13:1</td>
<td>94:6</td>
</tr>
<tr>
<td>( p-Br-C_6H_4CH_2 )</td>
<td>84</td>
<td>11:1</td>
<td>93:7</td>
</tr>
<tr>
<td>( p-MeO-C_6H_4CH_2 )</td>
<td>73</td>
<td>13:1</td>
<td>94:6</td>
</tr>
<tr>
<td>2-thienyl-( CH_2 )</td>
<td>69</td>
<td>13:1</td>
<td>92:8</td>
</tr>
<tr>
<td>PhCH(_2)CH(_2)CH(_2)</td>
<td>57</td>
<td>7:1</td>
<td>96:4</td>
</tr>
<tr>
<td>( p-Me-C_6H_4 )</td>
<td>64</td>
<td>2:1</td>
<td>92:8</td>
</tr>
</tbody>
</table>

![Chemical structure of 10](image-url)
3.2 Brønsted Acids for Organic Reactions

The cyclopentadienyl dicarbonyl iron Lewis acid, \([(\eta^5-C_5H_5)Fe^+\text{(CO)}_2(\text{THF})]\text{BF}_4^-, \text{11} \) (Figure 3.2), has been found by the Hossain group to catalyze a variety of organic reactions including cyclopropanation,\(^{17}\) aziridination,\(^{18}\) and Diels-Alder reactions.\(^{19}\) Recently Hossain group reported the synthesis of 3-hydroxy-2-arylacrylic acid ethyl esters (3-hydroxyacrylates) along with 3-oxo-3-arylpropanoic acid ethyl esters (\(\beta\)-keto esters) from aromatic aldehydes and ethyl diazoacetate (EDA) catalyzed by \text{11} \) (Equation 3.11).\(^{20}\) The potential wide scope of this reaction encouraged further investigation of the reaction and catalyst. This endeavor led to the exploration of the catalytic activity of HBF\(_4\).Et\(_2\)O.\(^{21}\) which proved to be a unique Brønsted acid to perform this reaction.

**Figure 3.2:** Cyclopentadienyl dicarbonyl iron Lewis acid is also known as Hossain’s catalyst.

\[
\text{[\text{Fe}\overbrace{\text{C}}^{\text{C}}\overbrace{\text{O}}^{\text{O}}\text{CO}}_{\text{CO}}\text{BF}_4^+]_{\text{11}}
\]
Equation 3.11: 3-Hydroxyacrylate formation from an aldehyde and ethyl diazoacetate (EDA).

\[
\begin{align*}
\text{CHO} & + \text{N}_2\text{CHCOOEt} \xrightarrow{11 \text{ or HBF}_4} \text{COOEt} \\
\text{arylaldehydes} & (\text{EDA}) & \text{3-hydroxypropenoic acid esters}
\end{align*}
\]

There are numerous examples of use of HBF\(_4\) and its complexes as a strong Brønsted acid catalyst.\(^{22}\) HBF\(_4\).Et\(_2\)O in particular works as a catalyst for cyclopropanation (Equation 3.12)\(^{23}\), hemiacetal formation (Equation 3.13)\(^{24}\) and

Equation 3.12: Brønsted acid catalyzed cyclopropanation reaction.

\[
\begin{align*}
\text{CHO} & + \text{N}_2\text{CHCOOEt} \xrightarrow{10 \% \text{ HBF}_4\text{.Et}_2\text{O}} \text{COOEt} \\
\text{Ethyl-2-formylcyclopropanecarboxylate}
\end{align*}
\]

Equation 3.13: Brønsted acid catalyzed hemiacetal formation reaction.
aziridination (Equation 3.14). These findings opened the possibility of developing a new metal-free chiral Brønsted acid catalyst. The present research presents the development of a completely new metal-free chiral Brønsted acid catalyst and its application to the asymmetric aza-Henry reaction (Equation 3.15).5,25

**Equation 3.14:** Brønsted acid catalyzed aziridination reaction.

**Equation 3.15:** Brønsted acid catalyzed aza-Henry reaction.

3.2.1 **Designing Chiral Brønsted Acids for Organic Reactions**

The concept of Brønsted acid has been known for long time. The quest to understand the nature and use of Brønsted acids is continuously growing, but manipulations and tailoring of different Brønsted acids to the needs of different asymmetric reactions are new. Lewis acids are convenient to tune by changing the electron donating and withdrawing groups bound to the metal center. Steric features of a Lewis acid can also be tuned by designing the steric environment of
the ligands precisely. On the other hand, it is very hard to control the steric feature and hydrogen donating ability of a small Brønsted acid.

By definition, the strength of a Brønsted acid depends on the capability of its proton donation. This implies that the stronger the acid, the easier it is to remove the hydrogen from the loosely bound anionic part. Since hydrogen is a very small cation, controlling acid strength has always been a challenge to the chemists. At the same time adding a steric directional feature to generate a chiral Brønsted acid has increased the challenge while this idea has been very attractive. To get an idea of relative acidity of different acids, a table of pKₐ values for different Brønsted acids is presented in Table 3.1.

**Table 3.1:** Published pKₐ values for different Brønsted acids²⁶,²⁷,²⁸

<table>
<thead>
<tr>
<th>Substrate</th>
<th>pKₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In water</td>
</tr>
<tr>
<td>H₂O</td>
<td>15.7</td>
</tr>
<tr>
<td>H₃O</td>
<td>-1.7</td>
</tr>
<tr>
<td>HBF₄</td>
<td>-5</td>
</tr>
<tr>
<td>H₂SO₃</td>
<td>1.9, 7.21</td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>-3.0, 1.99</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>2.12, 7.21, 12.32</td>
</tr>
<tr>
<td>PhOH</td>
<td>18.0</td>
</tr>
<tr>
<td>PhSH</td>
<td>10.3</td>
</tr>
<tr>
<td>PhSeH</td>
<td>7.1</td>
</tr>
<tr>
<td>![PhSO₃H]</td>
<td>-1.8</td>
</tr>
<tr>
<td>![PhSO₂OH]</td>
<td>2.1</td>
</tr>
</tbody>
</table>
In recent years there has been a developing need of organo-catalysts in the competitive pharmaceutical industry and food industry because of residual metal contamination from organometallic catalysts. This phenomenon is reflected in the increase in the number of research publications in this area (Figure 3.3). This new trend in the area of chiral Brønsted acids is helping to meet the increasing need of organocatalyst (Figure 3.4). Recently, the Hossain group found the Brønsted acid (HBF₄) to possess catalytic activity that is comparable to the Iron Lewis acid 11 in the same reactions. With these findings our aim is to develop an efficient chiral Brønsted acid or chiral Brønsted acid-containing complex as a catalyst for different reactions, namely cyclopropanation (Equation 3.12), hemiacetal formation (Equation 3.13), azirdination (Equation 3.14), and aza-henry reaction (Equation 3.15). It is envisioned that the newly developed
catalyst will also be applicable in other acid catalyzed reactions for organic synthesis.

**Figure 3.3:** Increasing number of publications in recent years in the area of organocatalyst. (SciFinder Scholar).

![Graph showing increasing number of publications](image)

**Figure 3.4:** Continuous publications about chiral Brønsted acid reflects the importance of this area. (SciFinder Scholar).

![Graph showing continuous publications about chiral Brønsted acids](image)
1.2.2  **Metal-assisted Chiral Brønsted Acids**

Metal-assisted designer chiral Brønsted acids played an important role in the continuing advancement of the chiral Brønsted acids.\textsuperscript{30,31} Yamamoto et al reported the asymmetric Diels-Alder reaction of naphthoquinone derivatives and various dienes in the presence of a chiral boron reagent derived from B(OMe)\textsubscript{3} and an (R,R)-(+)‐tartaric acid diamide (Equation 3.16).\textsuperscript{32} The rate enhancement and

**Equation 3.16:** Asymmetric Diels-Alder reaction using chiral complex of boron and (R,R)-(+)‐tartaric acid diamide.

![Chemical reaction diagram](image)
high enantioselectivity observed in this reaction was attributed to Brønsted acid assisted Lewis acid catalysis.\textsuperscript{33} Brønsted acid assisted chiral Lewis acid (BLA) promotes high selectivity through the dual effects of intramolecular hydrogen-bonding interactions and the attractive $\pi-\pi$ donor-acceptor interactions in the transition state (Equation 3.17).\textsuperscript{34} High enantioselectivity (> 99% $ee$) and exo-selectivity (> 99%) are obtained for the cycloaddition in presence of BLA. Yamamoto also developed other catalysts that give high selectivity for Diels-Alder reactions (Scheme 3.1).

**Equation 3.17:** Asymmetric Diels-Alder reaction using chiral complex of boron and BINOL.

\[
\text{BrCH} + \text{CHO} \xrightarrow{\text{BLA 2 (5 mol %)}} \text{CHO} \\
\text{CH}_2\text{Cl}_2, -78^\circ\text{C} \\
\text{99 % yield (exo/endo > 99:1)} \\
\text{99 % ee}
\]

Shibasaki developed a lanthanum-binaphthol (La-BINOL) complex for the asymmetric Michael reaction (> 99% $ee$) with broad generality (Equation 18).\textsuperscript{35}
This powdered complex was found to be very stable and reusable. The activity of the chiral Lewis acid is increased by the added Brønsted acid. There are numerous other examples where a chiral Lewis acid accompanied by Brønsted acid give better selectivity and reactivity.\textsuperscript{36,37}

**Scheme 3.1:** Asymmetric Diels-Alder reactions catalyzed by Brønsted acid assisted Lewis acids (BLA).

Lewis acid assisted Brønsted acid (LBA), 15 can be generated \textit{in situ} from optically pure BINOL and SnCl$_4$.\textsuperscript{38} The protonation of silyl enol ether was performed by LBA in 97\% \textit{ee} (Equation 3.19). Later LBA was used for the regio and stereoselective reactions.\textsuperscript{39}
Equation 3.18: Asymmetric Michael reaction catalyzed by lanthanum-binaphthol (La-BINOL) complex (14).

\[
\begin{align*}
\text{Equation 3.19: Asymmetric BINOL and SnCl}_4 \text{ complex (15) as a catalyst.}
\end{align*}
\]

The planar-chiral pyrrolidinopyridine derivative (16) proved to possess high Brønsted acid catalytic activity (Equation 3.20). Through a series of kinetic
studies it was established that the planer-chiral catalyst functions as a Brønsted acid catalyst in the rate-limiting and enantioselectivity-determining step.

**Equation 3.20:** Planer-chiral pyrrolidinopyridine derivative as an asymmetric catalyst through H-transfer mechanism.

3.2.3 **Chiral Diol Based Brønsted Acid**

In recent years development of different derivatives of weakly acidic chiral BINOL-derived provided great tools for organocatalysis. Schaus and co-workers reported the BINOL-derived enantioselective catalyst for Morita-Baylis-Hillman process (Equation 3.21). The phenolic hydroxyl groups have weak acidity (Table 3.1). Thus, this acid catalytic activity may be considered as a borderline
case between the hydrogen bond catalysis and chiral Brønsted acid catalysis. The reaction yielded 39-88% product with high enantioselectivity (67-96% ee).

**Equation 3.21**: BINOL catalyzed asymmetric Morita-Baylis-Hillman reaction.

![Equation 3.21](image)

Sasai and co-workers developed a bifunctional BINOL-derived organocatalyst (19) for the aza Morita-Baylis-Hillman reaction (Equation 3.22). The 3-pyridyl moiety functiones as a Lewis-basic site and the binaphthol moiety as a Brønsted-acidic site. Introduction of the N-isopropyl-N-3-pyridylaminomethyl moiety at the 3-position is essential to attain high enantioselectivity. Development of catalyst 20 with phosphine ligand improved yield and enantioselectivity of the reaction.
**Equation 3.22:** Asymmetric Morita-Baylis-Hillman reaction with modified BINOL at 3-position.

![Chemical structures](image)

Dixon and co-workers reported that tetroltr (21) catalyzes the addition reaction of methyleneaminopyrrolidine to imines, thereby giving rise to R-aminohydrazone in 17-75% ee (Equation 3.23).\(^{44}\)

Use of enamine as nucleophile resulted in the enamine Mannich reaction in which diol 22 shows high catalytic activity (Equation 3.24).\(^{45}\)
Equation 3.23: Tetrol catalyzed addition reaction of methyleneaminopyrrolidine to imines.

![Equation 3.23](image)

Equation 3.24: Asymmetric diol catalyzed enamine Mannich reaction.

![Equation 3.24](image)

Yamamoto and co-workers reported the nitroso Diels-Alder reaction of diene with nitrosobenzene catalyzed by a binaphthol (BINOL) derivative
produces bicyclo ketones with excellent enantioselectivities (Equation 3.25).\textsuperscript{46} Interestingly, use of morpholino-4,4-dimethylcyclohexene resulted in R-amination with excellent enantioselectivity (Equation 3.26). This suggests the involvement of a sequential process, namely, the N-nitroso aldol reaction, followed by Michael addition.

**Equation 3.25:** Asymmetric nitroso Diels-Alder reaction catalyzed by BINOL derivative.

Yamamoto and co-workers designed a Brønsted-acid-assisted chiral Brønsted-acid catalyst bearing a bis(triflyl)methyl group 24 (Equation 3.27).\textsuperscript{47} The enantioselective Mannich-type reaction of a ketene silyl acetal with aldimines catalyzed by 24 in the presence of a stoichiometric amount of an achiral proton source gave (S)-$\beta$-amino esters in high yields with good enantiomeric excesses.
**Equation 3.26:** Asymmetric nitroso Diels-Alder reaction followed by Michael addition reaction.

\[
\text{N} = \text{O} + \text{Ph} \text{N} = \text{O} \xrightarrow{\text{Catalyst 13 (30 mol\%)} \text{ Pentane-CH}_2\text{Cl}_2 \text{ -78\(^\circ\)C, 24 h}} \text{O} \text{OH} \]

\[54\% (94\% \text{ ee})\]

**Equation 3.27:** Asymmetric Mannich-type reaction of ketene silyl acetal with aldmines.

\[
\text{Ph-} \text{N} \text{Ar} + \text{OMe} \xrightarrow{\text{Catalyst 24 (10 mol\%)} \text{ 2,6-xylene (100 mol\%)} \text{ n-PrCl, -78\(^\circ\)C}} \text{Ph-} \text{N} \text{Ar} \text{OMe}
\]

Yamamoto *et. al.* reported that an enamine reacted with nitrosobenzene by means of 25 gives an \(N\)-nitroso aldol product (Equation 3.28). In contrast, use of glycolic acid derivative 26 gave an \(O\)-nitroso aldol product with high enantioselectivity.\(^{48}\)
**Equation 3.28:** Asymmetric nitroso aldol reaction.

\[
\text{Ar} \quad \text{OH} \quad \text{Ar} \\
\text{OH} \\
\text{OH} \\
\text{Ar} \quad \text{Ar} \\
\text{Ar} = 1\text{-naphthyl}
\]

\[
\begin{align*}
\text{N} & \quad \text{P} \\
\text{H} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{O}
\end{align*}
\]

\[
\text{Ar} \quad \text{Ar} \\
\text{Ar} \quad \text{Ar} \\
\text{Ar} \quad \text{Ar}
\]

VAPOL (27)-BH₃ found to catalyze the asymmetric aziridination reaction of benzhydryl imines with ethyl diazo acetate (EDA). This catalyst was prepared by treating S-VAPOL with 3 equivalent of BH₃-THF complex, heating at 55°C for 1h to remove the volatiles and then heating the residue at 55 °C for 30 minutes under high vacuum. The aziridination reaction yielded very high cis diastereoselectivity and up to 99% ee (Equation 3.29). Success of this reaction
depends on the purity of the BH₃-THF complex used to generate the catalyst. This observation led to the development of a new more reliable catalyst system.

**Equation 3.29:** Asymmetric boron-diol complex catalyzed aziridination reaction.
Among different borate systems, $\text{B(OPh)}_3$ was found to form a better catalytic system. Further optimization was done by making the catalyst from $\text{B(OPh)}_3$ and VAPOL (27) or VANOL (28). Other ligand systems such as BINOL (29) or BANOL (30) were not found to be as efficient as VAPOL (27) or VANOL (28). The antibiotic (–)-choramphenicol has been synthesized in only four steps from $p$-nitro-benzaldehyde in optically pure form from an asymmetric catalytic aziridination reaction with a chiral catalyst prepared from $\text{B(OPh)}_3$ and the (R)-VAPOL (27) ligand. Catalysts generated from the VAPOL (27) and VANOL (28) ligands give much higher asymmetric induction than do catalysts prepared from 6,6‘-diphenyl-VAPOL (31), BINOL (29), and BANOL (30) ligands.

Rawal et. al. used TADDOL (32) as a catalyst for Diels-Alder reactions of aminosiloxydienes and substituted acroleins to afford the products in good yields and high enantioselectivities (up to 92% ee) (Equation 3.30). The credit of these remarkable reactions were assigned to hydrogen bonding. The gross structure of the TADDOL (32) has been found to have a profound influence on both the rate and the enantioselectivity of the cycloadditions.

### 3.2.4 Chiral Phosphoric Acids

Chiral phosphoric acid (33) is already known as a ligand system in different organometallic reactions. Akiyama et. al. (Equation 3.31) and Terada et. al. (Equation 3.32) independently developed the most popular use of chiral phosphoric acid and their different derivatives as chiral Brønsted acid catalysts.
Chiral phosphoric acids claimed enormous attention after these phenomenal discoveries (Figure 3.5 and Figure 3.6).

**Equation 3.30:** TADDOL catalyzed asymmetric Diels-Alder reaction.

![TADDOL catalyzed asymmetric Diels-Alder reaction](image)

**Figure 3.5:** Increasing importance of chiral phosphoric acid is revealed by the number of increasing publications in this area. (SciFinder Scholar).
Figure 3.6: Representative derivatives of chiral BINOL-phosphoric acids.
3.2.4.1 Mannich-type Reaction

Akiyama et al used chiral phosphoric acid as an efficient catalyst for Mannich-type reactions (Equation 3.31). Theoretical studies found that the two-point hydrogen bonding interaction makes the dicoordination pathway overwhelmingly favored over the monocoordination pathway in the Mannich-type reaction (Scheme 3.2, Figure 3.7 and Figure 3.8). The reaction proceeds through the protonation followed by the nucleophilic attack via zwitterionic and nine-membered cyclic transition state (TS). The intriguing issue of the re-facial selectivity was also well rationalized. The two-point hydrogen-bonding moiety and aromatic stacking interaction between the 4-nitrophenyl group and N-aryl group are significantly important to fix the geometry of aldimine on the zwitterionic TS. The si-facial attacking TS is structurally less favored than the re-facial alternative due to repulsive interaction of the 3,3'-aryl substituents.

Equation 3.31: Chiral phosphoric acid catalyzed asymmetric Mannich-type reaction.

![Chemical reaction diagram](image)
Scheme 3.2: Proposed reaction mechanism of the Mannich-Type reaction with chiral Phosphoric acid catalysts.

Terada *et. al.* used chiral phosphoric acids for the direct Mannich reaction (Equation 3.32).\(^5\) Electron withdrawing groups were found to play an important role in the reactivity and selectivity of the reactions. The beneficial effects of the 3,3'-bisaryl substituents of the catalysts on the enantioselectivity are greatly appreciated. This process provide an attractive way to construct β-aminoketones under extremely mild conditions. The stereochemical course of this reaction was established through the synthesis of Boc-(S)-phenylglycine methylester. The
transformation thus demonstrated is applicable as a useful method for the synthesis of various phenylglycine derivatives.

**Figure 3.7**: Structures of transition states (TSs) in (a) dicoordination pathway and (b) monocoordination pathway. Bond lengths are in Å, and natural charges are underlined and bold.

**Figure 3.8**: 3D structures of transition states (TSs) in (a) re-face attack and (b) si-face attack. Bond lengths are in Å
**Equation 3.32**: Chiral phosphoric acid catalyzed direct Mannich reaction.

\[
\text{Ar}_N^\text{Boc} + \text{Acetylketone} \xrightarrow{33 \ (2\text{ mol\%})} \text{CH}_2\text{Cl}_2, \text{RT} \quad \text{Boc}^\text{Ar}_N^\text{Ac}
\]

The three-component direct Mannich reaction (Equation 3.33) was recently reported by Guo et al, wherein H₈-BINOL derivatives 34 were employed (Figure 3.9). A catalytic amount of the chiral phosphoric acid was sufficient to promote an

**Figure 3.9**: Chiral H₈-BINOL phosphoric acids.

34a

34b

34c

34d
anti-selective direct asymmetric Mannich reaction of cycloketones with high diastereo- (anti/syn 98:2) and enantioselectivity (up to 98% ee). Mannich reactions between aldimines and aromatic ketones were performed with fairly good enantioselectivity (ee). Cyclohexanone derivatives were found to be good substrates for this direct Mannich reaction.

**Equation 3.33:** Asymmetric phosphoric acid catalyzed three-component direct Mannich reaction.

\[
\begin{align*}
\text{ArCHO} + \text{PhNH}_2 + \text{XO}_\text{Ph} & \xrightarrow{33d (0.5 \text{ mol}) \text{ or } 34b (2 \text{ mol})} \text{toluene, 0°C} \xrightarrow{} \text{PhHN}_R \text{O}_X
\end{align*}
\]

Giere *et. al.* have shown that vinylketene silyl N,O-acetals (35) readily participate in Brønsted acid-catalyzed vinylogous Mukaiyama-Mannich reactions with aromatic and heteroaromatic aldimines and furnish δ-amino-α,β-unsaturated amides (36) in good yields and enantioselectivities (Equation 3.34). Direct three-component vinylogous Mannich reactions deliver the products with almost identical yield and enantioselectivity, thus avoiding the synthesis of the imines in a separate step. The utility of the vinylogous Mannich products are demonstrated through their conversion into various functional building blocks including a short
synthesis of the enantiomerically highly enriched 2-phenylpiperidine. 33q resulted higher ee compared to 33h.

**Equation 3.34:** Chiral phosphoric acid catalyzed Mukaiyama-Mannich reaction.

\[
\text{Ar} + 33\text{h or } 33\text{q (1 mol\%)} \rightarrow 36
\]

Vinylogous Mannich reaction of acyclic silyl dienolates reported by Schneider et. al. furnished valuable δ-amino α,β-unsaturated carboxylic esters in high yields, complete regioselectivity and good to very good enantioselectivities (Equation 3.35). Although an excess of nucleophile has been typically employed for the purpose of increasing the reaction rate, almost identical results have been obtained with a nearly stoichiometric ratio of reactants and longer reaction times. This process is further facilitated by the discovery that it may be executed successfully as a three component Mannich reaction, which avoids the need to synthesize the imine in a separate step. Wide application of the reaction was explored with various substituents.
Equation 3.35: Chiral phosphoric acid catalyzed Mannich reaction of acyclic silyl dienolates.

\[
\begin{align*}
\text{N}^{\text{PMP}} & \quad + \quad \text{O}T\text{BS} & \quad \xrightarrow{\text{Catalyst (3 mol\%)} } & \quad \text{O}E\text{T}O
\end{align*}
\]

Hydrophosphonylation of aldimine with dialkyl phosphate proceeded using a catalytic amount (10 mol\%) of chiral phosphoric acid (33) to give R-aminophosphonates in a highly enantioselective manner (Equation 3.36).\textsuperscript{63}
Aldimines, in particular, derived from cinnamaldehyde derivatives exhibit high enantioselectivity. Bulky electron withdrawing substituent on the binephthyl system of the catalyst (33g) helped to attain higher yield and ee.

Equation 3.36: Asymmetric hydrophosphonylation of aldimine with dialkyl phosphate.

3.2.4.2 Nucleophilic Addition to Aldimines

Terada and co-workers reported the aza Friedel-Crafts alkylation of furan to aldimines by means of 2 mol % of 33q (Equation 3.37). The present reaction provided an atom-economical route to furan-2-ylamine derivatives (37) in a highly
enantioselective fashion. The synthetic utility of these products is demonstrated by oxidative cleavage of the furan ring (aza-Achmatowicz reaction) to form a 1,4-dicarbonyl compound (38) that could, in turn, be derivatized to a γ-butenolide (39).

**Equation 3.37**: Asymmetric aza Friedel-Crafts alkylation of furan to aldimines.

You and co-workers demonstrated that indoles are also suitable nucleophiles for addition to aldimines catalyzed by ent-33r (Equation 3.38). High yields and excellent ee’s were achieved for a wide range of aromatic aldimines.
**Equation 3.38:** Asymmetric nucleophilic addition of indoles.

\[
\begin{align*}
\text{N} & \quad X \\
\text{H} & \\
\text{X} & \\
\text{H} \\
\end{align*}
\]

The Pictet-Spengler reaction is important for preparation of tetrahydro-\(\beta\)-carbolines and tetrahydroquinolines.\(^6\) List and co-workers used phosphoric acid to catalyze the Pictet-Spengler reaction starting from geminally disubstituted tryptamines (Equation 3.39).\(^7\) The presence of the bis-(ethoxycarbonyl) group facilitated the cyclization reactions by virtue of the Thorpe-Ingold effect. Aliphatic as well as aromatic aldehydes were found to be good substrates. The requirement of a geminal diester functionality is a major constraint of this procedure. This limitation could be used to advantage in organic synthesis with a good retro-synthetic plan.

**Equation 3.39:** Asymmetric phosphoric acid catalyzed Pictet-Spengler reaction starting from geminally disubstituted tryptamines.
Recently optically active tetrahydro-β-carbolines were synthesized via an 
(R)-BINOL-phosphoric acid-catalyzed asymmetric Pictet-Spengler reaction of N-benzyltryptamine with a series of aromatic and aliphatic aldehydes (Equation 3.40). The tetrahydro-β-carbolines were obtained in yields ranging from 77% to 97% and with ee values up to 87%. The 3,3′-triphenylsilyl-substituted BINOL-phosphoric acid (33r) proved to be the catalyst of choice for the reaction with aromatic aldehydes. For the aliphatic aldehydes, 3,5-bistrifluoromethylphenyl-substituted BINOL-phosphoric acid (33g) was identified as the best catalyst. In addition, this scaleable method shortens the synthesis toward the pharmaceutically very relevant PDE5 inhibitors of the pyrroloquinolone class by three steps.

**Equation 3.40:** Asymmetric synthesis of tetrahydro-β-carbolines via an (R)-BINOL-phosphoric acid-catalyzed asymmetric Pictet-Spengler reaction of N-benzyltryptamine.

Phosphoric acid 33m, bearing the 9-anthryl group, catalyzed the direct alkylation of aldimines with α-diazo ester to furnish β-amino-α-diazoester with excellent enantioselectivity (Equation 3.41). Diazoacetate is commonly
employed in aziridine ring formation reactions (aza-Darzens reaction) under Lewis acidic\textsuperscript{70} and Brønsted-acidic\textsuperscript{71} conditions.

**Equation 3.41:** Asymmetric direct alkylation of aldimines with $\alpha$-diazo ester to furnish $\beta$-amino-$\alpha$-diazoester.

\[
\begin{align*}
t-	ext{BuO}_2\text{C}-\text{H} & \quad + \quad \text{O} & \quad N & \quad \xrightarrow{33\text{m} \ (2 \text{ mol\%})} & \quad t-	ext{BuO}_2\text{C}-\text{Ar} \\
& & & \text{toluene, rt} & \\
\end{align*}
\]

Rueping and co-workers reported the Strecker reaction catalyzed by 33I (Equation 3.42).\textsuperscript{72} They proposed a transition state model based on the X-ray crystal structure of 33I and optimized the structure with a complex derived from 33I and an imine.

**Equation 3.42:** Asymmetric Strecker reaction catalyzed by chiral phosphoric acid.

\[
\begin{align*}
\text{Ph} & \quad \text{N} & \quad \text{Ph} & \quad + \quad \text{HCN} & \quad \xrightarrow{33\text{I} \ (10 \text{ mol\%})} & \quad \text{HN} \\
& \quad \text{Ar} & \quad \text{Ar} & \quad \text{toluene, -40}^\circ\text{C} & \quad \text{Ar} & \\
\end{align*}
\]
Enecarbamate proved to be an efficient nucleophile in the phosphoric acid-catalyzed aza ene-type addition to aldmines.\textsuperscript{73} It is noted that a concentration as low as 0.1 mol % of chiral phosphoric acid catalyst worked well to afford the adducts with excellent enantioselectivities (Equation 3.43).\textsuperscript{74,75}

**Equation 3.43:** Asymmetric phosphoric acid-catalyzed aza ene-type addition to aldmines.

\[
\begin{align*}
\text{N} & \quad \text{O} \\
\text{N} & \quad \text{O} \\
\text{C} & \quad \text{Ph} \\
\text{C} & \quad \text{H} \quad \text{R} \quad \text{H} \\
\text{MeO} & \quad \text{NH} \\
\text{Allyl} & \quad \text{Ph} \\
1) & \quad 33 \ (0.1 \text{ mol}%) \\
\text{toluene, -40°C} & \\
2) & \quad \text{H}3\text{O}+ \\
\text{R} & \quad \text{Ph} \\
\text{R} & \quad \text{Ph} \\
\end{align*}
\]

Terada and co-workers subsequently employed the enecarbamate as a precursor of iminium salt, which was trapped by the indole (Equation 3.44).\textsuperscript{76} The phosphoric acid activates the electron-rich alkene in place of imine to give 1-indolyl-1-alkylamine derivatives, which have pharmaceutical and biological importance, with excellent enantioselectivity.

**Equation 3.44:** Electrophilic addition using chiral phosphoric acid as a catalyst.
Rueping and co-workers recently reported the iminoazaenamine reaction catalyzed by partially hydrogenated phosphoric acid 34b (Equation 3.45).\textsuperscript{77}

**Equation 3.45:** Asymmetric iminoazaenamine reaction.

\[
\begin{align*}
\text{Ar} & \text{N}_\text{Boc} \quad + \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{N}  \\
& \quad \text{C} \quad \text{H} \quad \text{C} \quad \text{Cl} \quad 0^\circ \text{C} \quad \text{CHCl}_3  \\
\end{align*}
\]

Antilla and co-workers have successfully developed the first catalytic asymmetric addition of alcohols to \(N\)-acyl imines catalyzed by chiral phosphoric acids (Equation 3.46).\textsuperscript{78} The chiral \(N,O\)-aminal could be prepared in a straightforward procedure in high yields with excellent enantiomeric excess under mild reaction conditions.

**Equation 3.46:** Asymmetric addition of alcohols to \(N\)-acyl imines catalyzed by chiral phosphoric acids.

\[
\begin{align*}
\text{R}^1 & \text{NCOPh} \quad + \quad \text{R}^2 \text{OH}  \\
& \quad \text{Catalyst 33 (5 mol\%)} \quad \text{EtOAc, RT, 24 h}  \\
\end{align*}
\]

A new highly enantioselective Brønsted acid catalyzed imino-azaenamine reaction of various \(N\)-Boc-protected aldimines and methylene-aminopyrrolidine
was developed by Rueping et al (Equation 3.47). The corresponding valuable aminohydrazones have been isolated in good yields and with high enantioselectivities. The mild reaction conditions of this metal-free process together with the operational simplicity and practicality not only render this a useful procedure for the synthesis of optically active aminohydrazones but also further expand the repertoire of enantioselective BINOL-phosphate catalyzed transformations.

**Equation 3.47:** Enantioselective Brønsted acid catalyzed imino-azaenamine reaction of various N-Boc-protected aldimes and methylene-aminopyrrolidine.

\[
\begin{align*}
\text{R}N&\text{R} \quad \text{N} \quad \text{H} \quad + \quad \text{N} \quad \text{PG} \quad \text{Ar} \quad \text{H} \\
\text{CHCl}_3, 0^\circ\text{C} \quad \text{Catalyst 33 (5-10 mol\%)} \\
\text{PG} \quad \text{HN} \quad \text{Ar} \\
\text{R}N&\text{R}
\end{align*}
\]

### 3.2.4.3 Aza Diels Alder Reactions

Akiyama et al found that Danishefsky's diene undergoes the aza Diels-Alder reaction with aldime in the presence of phosphoric acid to give cycloadducts (Equation 3.48). Interestingly, addition of acetic acid significantly improved both the chemical yield and enantioselectivity. Bulkiness of chiral phosphoric acid was found to be effective in increasing the yield and ee of the reaction.
Equation 3.48: Asymmetric aza Diels-Alder reaction with aldimine in the presence of phosphoric acid to give cycloadducts.

Brassard’s diene\textsuperscript{82} is more reactive than Danishefsky’s diene. Although the diastereoselective aza Diels-Alder reaction of Brassard’s diene with aldimines has already been reported,\textsuperscript{83} the catalyzed enantioselective version of the reaction has not yet been realized. A phosphoric acid bearing a 9-anthryl group at the 3,3’-position (33m) is effective for the aza-Diels-Alder reaction. Interestingly, use of a pyridinium salt, 40, improved the yield. Supposedly, the use of less acidic pyridinium salt 40 suppressed decomposition of Brassard’s diene in the reaction medium. Aldimines derived from aliphatic aldehydes as well as aromatic aldehydes worked well to give the corresponding cycloadducts after acid treatment with excellent enantioselectivity (Equation 3.49).\textsuperscript{84} The promising success of this reaction directed toward scale-up the reaction to 1.01g scale.

The reverse electron-demand aza Diels-Alder reaction of electron-rich alkene with 2-aza diene was catalyzed by chiral phosphoric acid to give tetrahydroquinoline derivatives in favor of the \textit{cis}-isomer with excellent enantioselectivities (Equation 3.50).\textsuperscript{85} Cyclic enol ethers as well as acyclic enol ether were found to be excellent substrates.
Equation 3.49: Aza Diels-Alder reaction of Brassard’s diene with aldimines.

![Equation 3.49](image)

The aza Diels-Alder reaction of aldimine with cyclohexenone was independently developed by Rueping$^{86}$ and Gong.$^{87}$ While Rueping employed 33l and 33m in combination with acetic acid (Equation 3.51), Gong used H8-BINOL-derived phosphoric acid 34c bearing $p$-ClC$_6$H$_4$ groups at the 3,3'-positions (Equation 3.52). Gong proposed that cyclohexenone is equilibrated to the enol form by acetic acid, thus promoting the aza Diels-Alder reaction. To optimize the reaction further, binol phosphate catalysts, various imine protecting groups, solvent, temperature, catalyst loading, and enone concentrations were varied (Equation 3.51).$^{86}$ The best reactivities and selectivities were observed with 10 mol% of the
binol phosphate 33m and 20 mol% acetic acid in toluene at room temperature. Various aldimines were applied in the double Brønsted acid

**Equation 3.50:** Asymmetric reverse electron-demand aza Diels-Alder reaction of electron-rich alkene with 2-aza diene.

- **89% yield, 94% ee**
  - (47h, -10°C)

- **82% yield, 96% ee**
  - (19h, -10°C)

- **86% yield, 89% ee**
  - (39h, -10°C)

- **76% yield, 91% ee**
  - (42h, 0°C)

- **86% yield, 90% ee**
  - (21h, -10°C)

- **95% yield, 97% ee**
  - (145h, 4°C)

catalyzed enantioselective synthesis of isoquinuclidines under these optimized conditions. In general, aromatic as well as heteroaromatic substituted isoquinuclidines with electron withdrawing and electron-donating substituents
could be isolated in good yields, with high enantiomeric ratios and high \textit{exo/endo} ratios. The relative and absolute structural configurations of the products were obtained by X-ray crystal structure analysis.

**Equation 3.51:** Rueping's asymmetric aza Diels-Alder reaction of aldimine with cyclohexenone.

![Equation 3.51](image)

**Equation 3.52:** Gong's asymmetric aza Diels-Alder reaction of aldimine with cyclohexenone.

![Equation 3.52](image)

Although the Biginelli reaction is a useful multicomponent reactions, in providing ready access to multifunctionalized 3,4-dihydropyrimidin-2-(1\textit{H})-ones, the catalyzed enantioselective version of the Biginelli reaction has been little explored. The highly enantioselective Biginelli reaction catalyzed by chiral phosphoric acid was reported by Gong and co-workers (Equation 3.53).
range of 3,4-dihydropyrimidin-2-(1H)-ones were obtained with excellent enantioselectivities. The optimal chiral phosphoric acid, derived from H8-binol,

**Equation 3.53**: Asymmetric Biginelli reaction catalyzed by chiral phosphoric acid.

![Equation 3.53](image-url)
afforded the reaction in high yields with excellent enantioselectivities of up to 97% ee. A wide variety of substrates, including aldehydes and β-keto esters, could be tolerated. This reaction has the advantage of avoiding contamination by transition metals in the manufacture of the medicinally relevant chiral 3,4-dihydropyrimidin-2-(1H)-ones.

The asymmetric Biginelli reaction catalyzed by BINOL-derived phosphoric acids was investigated to produce the desired SNAP-7941 (41) dihydropyrimidones (DHPM) core (Equation 54). The use of methoxy

**Equation 3.54:** Asymmetric synthesis of SNAP-7941 using chiral phosphoric as a catalyst.
methylacetoacetate 42a in the multicomponent reaction gave DHPM 44a in low yield and enantioselectivity. This observation is, again, attributed to an unfavorable hydrogen-bonding environment, because the use of methylacetoacetate 42b greatly improved yield and selectivity of the reaction. Optimal reaction conditions were achieved with a limiting amount of urea and an excess of both aldehyde and methylacetoacetate. DHPM 44b was synthesized in 96% yield with 94.5:5.5 er, utilizing 3,3′-diphenyl-substituted BINOL-derived phosphoric acid catalyst 33b.

The enantioselective Brønsted acid catalyzed Nazarov reaction was developed by Rueping and co-workers (Equation 3.55). This efficient method is not only the first example of an organocatalytic electrocyclic reaction but it also provides the corresponding cyclopentenones in good yields and with excellent enantioselectivities (86–98% ee). Compared to the metal-catalyzed reaction, special features of this new Brønsted acid catalyzed electrocyclization are the lower catalyst loadings (2 mol%), higher enantioselectivities, access to all possible stereoisomers, milder conditions and faster reaction times.

**Equation 3.55: Enantioselective Brønsted acid catalyzed Nazarov reaction.**

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>% yield</th>
<th>45/46</th>
<th>45 ee</th>
<th>46 ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>331</td>
<td>95</td>
<td>3.5:1</td>
<td>90, 93</td>
<td></td>
</tr>
<tr>
<td>33m</td>
<td>86</td>
<td>2:1</td>
<td>89, 95</td>
<td></td>
</tr>
</tbody>
</table>
3.2.4.4 Friedel-Crafts Reaction

Antilla and co-workers have developed an efficient method for the highly enantioselective Friedel-Crafts reaction between pyrrole derivatives and \( N \)-acyl imines catalyzed by chiral phosphoric acids (Equation 3.56).\(^2\) When the observed

**Equation 3.56**: Friedel-Crafts reaction between pyrrole derivatives and \( N \)-acyl imines catalyzed by chiral phosphoric acids.

![Reaction Scheme]

steric and electronic effects of the substrates were controlled excellent enantiomeric excess and yields could be achieved using catalyst 33r. The
reaction conditions were optimized by studying different solvent systems, temperatures, studying the substituent effect on the imine, and the substituent effect on the pyrroles. The developed methodology was applied to the synthesis of a pyrrolo- [1,2-α]pyrazine without racemization.

Akiyama et al have developed a chiral phosphoric acid catalyzed Friedel–Crafts alkylation of indoles with nitroalkenes to generate Friedel–Crafts adducts with excellent enantioselectivities (Equation 3.57). They found that 3Å molecular sieves lead to an efficient Friedel–Crafts alkylation in the presence of a chiral phosphoric acid (33r).

**Equation 3.57:** Chiral phosphoric acid catalyzed Friedel–Crafts alkylation of indoles with nitroalkenes.

\[
\begin{align*}
\text{R}^1\text{Indole} + \text{R}^2\text{NO}_2 & \xrightarrow{\text{Catalyst R-33r (10 mol%)}} \text{3Å MS (10-40 mg)} \xrightarrow{-35^\circ\text{C}} \\
\text{R}^1\text{Pyrrrole} & \quad \text{R}^2\text{NO}_2
\end{align*}
\]

3.2.4.5 Transfer Hydrogenations

Although chemical hydrogenation normally requires a metal catalyst or use of stoichiometric amounts of metal hydrides, living organisms typically employ organic cofactors such as nicotinamide adenine dinucleoside (NADH) in combination with metalloenzymes. The organo-catalyzed transfer hydrogenation and reductive Michael reaction, both of which employ Hantzsch ester (46) as a model of NADH, were reported quite recently. A number
of groups have independently reported the enantioselective reduction of imines catalyzed by different phosphoric acids as a Brønsted acid. Rueping and co-workers employed 20 mol % of chiral phosphoric acid, bearing 3,5-(CF₃)₂C₆H₃ groups at the 3,3'-positions (33g), as the catalyst (Equation 3.58) for asymmetric hydrogenation.⁹⁷

**Equation 3.58**: Chiral phosphoric acid catalyzed asymmetric hydrogenation reaction.

![Equation 3.58](image)

List et al have reported highly efficient and enantioselective transfer hydrogenation of ketimines catalyzed by Brønsted acids (Equation 3.59).⁹⁸ They synthesized and screened a variety of chiral phosphoric acid catalysts for the reduction of imine in the presence of Hantzsch ester. Highest selectivity was attained using triisopropylbenzene substituted chiral phosphoric acid (33i). A reasonable mechanism was proposed for the reaction using chiral Brønsted acids. Remarkable features of this process includes (a) its high yields and enantioselectivities, (b) its wide scope, including both aliphatic and aromatic amines, (c) its simplicity and practicality (in situ generation and reduction of
imines), and (d) the remarkably low catalyst loading, which so far is unprecedented in asymmetric Brønsted acid catalysis.

**Equation 3.59:** List's asymmetric transfer hydrogenation by Brønsted acids.

![Equation 3.59](image)

MacMillan and co-workers found that bulky chiral phosphoric acid, bearing Ph₃Si groups at the 3,3'-positions (33r), is the catalyst of choice for the reductive amination reaction (Equation 3.60). The three component transfer hydrogenation reaction starting from the aldehyde, amine, and Hantzsch ester proceeded smoothly in the presence of MS 5Å to give secondary amines with excellent enantioselectivities. Dialkyl ketones as well as alkyl aryl ketones proved to be good substrates, and even ethyl methyl ketone was reductively aminated with 88% ee. The single-crystal X-ray structure of a catalyst bound aryl imine was obtained which exhibited a remarkable correlation with MM3 calculations.

The mechanism of the Hantzsch ester hydrogenation of imines catalyzed by chiral BINOL-phosphoric acid has been investigated using density functional theory (DFT) methods. Despite the importance of this reaction, there are a number of possible detailed mechanisms, and the preferred pathway has not been firmly established. Calculations show that the catalyst not only activates the imine group for the reaction by acting as a Brønsted acid, but also establishes an
interaction with the Hantzsch ester that can explain the enantioselectivity of the reaction.

**Equation 3.60:** MacMillan’s three component transfer hydrogenation reaction.

Rueping and co-workers extended the chiral phosphoric acid-catalyzed transfer hydrogenation to the reduction of quinolines (Equation 3.61),
benzoxazines, benzothiazines, and benzoxazinones (Equation 3.62). Reduction of benzoxazines, in particular, is highly selective, reducing the catalyst loading of 33k to as low as 0.01 mol % without considerable loss of reactivity or selectivity. They applied this methodology to the enantioselective synthesis of biologically active tetrahydroquinoline alkaloids such as (+)-galipinine. This reaction was further developed for the cascade reaction.
Equation 3.61: Asymmetric reduction of quinolines using chiral phosphoric acid.

\[
\text{N}H\text{CO}_2\text{Et} + \text{Et}_2\text{C}_2\text{HCO}_2\text{Et} \xrightarrow{\text{Catalyst 33k (1-5 mol\%)}} \text{N}H\text{R} + \text{Et}_2\text{C}_2\text{HCO}_2\text{Et}
\]

Benzene, 60°C

Equation 3.62: Asymmetric reduction of benzoxazines, benzothiazines, and benzoxazinones using chiral phosphoric acid.

\[
\text{N}H\text{X}R + \text{Et}_2\text{C}_2\text{HCO}_2\text{Et} \xrightarrow{\text{Catalyst 33k (0.1-1 mol\%)}} \text{N}H\text{R} + \text{Et}_2\text{C}_2\text{HCO}_2\text{Et}
\]

CHCl₃, RT

\(X = O, S\)

(48 as 1.4 eqv.)

(51-95% (93-99% ee))

List and co-workers achieved the catalytic reductive amination of R-branched aldehydes (Equation 3.63). On treatment of \(\alpha\)-branched aldehydes and \(p\)-anisidine with 48 as a hydrogen source in the presence of 5 mol % of 33i, \(\alpha\)-substituted amines were obtained with high chemical yields with good to

Equation 3.63: List’s catalytic reductive amination of R-branched aldehydes.

\[
\text{R}_2\text{CHO} + \text{Et}_2\text{C}_2\text{HCO}_2\text{Et} + \text{H}_2\text{NR}^3 \xrightarrow{\text{Catalyst 33i (5 mol\%)}} \text{R}_2\text{NHNR}^3
\]

5Å MS, Benzene, 60°C
excellent enantioselectivities by dynamic kinetic resolution. Different derivatives of 48 were also used as a hydrogen source in the reaction. Organocatalytic cascade reactions using chiral phosphoric acid derivative was subsequently achieved.

Asymmetric synthesis of trans-alkenyl α-amino esters was realized by chiral phosphoric acid catalyzed transfer hydrogenation of β,γ-alkynyl α-imino esters (Equation 3.64). Utilizing Hantzsch esters as the hydrogen donor, both the alkyne and imine moieties of β,γ-alkynyl α-imino esters were reduced to afford trans-alkenyl α-amino esters with up to 96% ee.

**Equation 3.64:** Asymmetric synthesis of trans-alkenyl α-amino esters using chiral phosphoric acid.

\[
\begin{align*}
\text{HN} &\overset{\text{PMP}}{\text{CO}_2\text{R'}} + \overset{\text{EtO}_2\text{C}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{CO}_2\text{Et}}{\overset{\text{N}}{\overset{\text{PMP}}{\text{H}}}}}}} & \overset{\text{Catalyst 33m (1 mol%)}}{\text{Et}_2\text{O, rt}} \rightarrow \overset{\text{HN}}{\overset{\text{CO}_2\text{R'}}{\text{R}}} \\
& \text{up to 96% ee}
\end{align*}
\]

Guo et al reported a transfer hydrogenation reaction using double axially chiral phosphoric acid (Equation 3.65). The use of the bis-BINOL scaffold to synthesize the chiral phosphoric acid is unique. In comparison to other chiral phosphoric acids, this bis-BINOL phosphoric acid shows higher efficiency.
**Equation 3.65:** Guo’s transfer hydrogenation reaction using double axially chiral phosphoric acid.

\[
\begin{align*}
\text{Catalyst} (1 \text{ mol\%}) & \quad \text{Et}_2\text{O, 35°C, 20h} \\
\text{N} & \quad \text{H} \\
\text{F} & \quad \text{O} \\
threebond & \quad \text{Et}_2\text{O}, 35°C, 20h \\
\text{N} & \quad \text{H} \\
\text{R}^2 & \quad \text{R}^1 \\
\end{align*}
\]

**Novel Phosphoric Acids**

TADDOL-based phosphoric acid diesters 50 were synthesized and subjected to the Mannich-type reaction by Akiyama et. al. (Equation 3.66). Although 50a did not show significant catalytic activity, its derivative with \( p-\)
CF$_3$C$_6$H$_4$ group 50b exhibited high catalytic activity. The Mannich-type reaction proceeded smoothly to give β-amino esters with high enantioselectivity.$^{108}$

**Equation 3.66:** Asymmetric Mannich-type reaction using TADDOL-based phosphoric acid diesters catalyst.

Antilla and co-workers synthesized a novel phosphoric acid derivatives, starting from BINOL derivatives and (S)-VAPOL,$^{109}$ and demonstrated its catalytic activity in the addition of sulfonamide to aldimines to produce protected aminals, which have been incorporated into peptide chains as retro-inverso peptide mimics (Equation 3.67).$^{110}$
Equation 3.67: Asymmetric addition of sulfonamide to aldimines.

\[
\begin{align*}
\text{Ph} & \quad \text{N}^{\text{Boc}} + \quad \text{H}_2\text{NTs} \\
\text{Catalyst (5-10 mol\%)} \\
\text{Et}_2\text{O, RT} \\
\text{Boc} & \quad \text{NH} \\
\text{Ph} & \quad \text{NHTs}
\end{align*}
\]

\[
\begin{align*}
\text{33o} & \quad \text{96\% yield, 60\% ee} \\
\text{33p} & \quad \text{99\% yield, 71\% ee}
\end{align*}
\]

Terada et al. reported a new stronger chiral Brønsted acid \(52\) by modifying the chiral phosphoric acid.\(^{111}\) This catalyst was applied for direct Mannich reaction resulting in high yield and promising \%ee (Equation 3.68).
Though the reaction is high yielding, the selectivity (%ee) is low. This could be reasoned as a compromise between the acidity and selectivity or functionality rotation around the nitrogen atom thereby reducing the commitment to selectivity.

**Equation 3.68:** Terada’s new stronger chiral Brønsted acid catalyzed direct Mannich reaction.

Yamamoto et al reported the formation of the super chiral Brønsted acid (53) (Equation 3.69) and using it for asymmetric Diels-Alder Reaction (Equation 70).\textsuperscript{112} The previously known chiral phosphoric acids did not give any product or ee for asymmetric Diels-Alder Reaction, while the new super chiral Brønsted acid (53) gave high yield and ee for asymmetric Diels-Alder Reaction. Non-polar aromatic solvents were found to give higher yield and higher ee. Substrate scopes were studied by changing different functionality in the substrate system.
**Equation 3.69:** Synthesis of the super chiral Brønsted acid.

\[
\begin{align*}
&\text{POCl}_3 \text{ (1.2 equiv)} \quad \text{DMAP (2 equiv)} \\
&\text{Et}_3\text{N (7 equiv)} \quad \text{CH}_2\text{Cl}_2 \\
\end{align*}
\]

0°C – r.t., 2 h

\[
\begin{align*}
&\text{TfNH}_2 \text{ (2 equiv)} \quad \text{EtCN} \\
\end{align*}
\]

\[\text{r.t. } \rightarrow \text{ reflux}\]

\[
\begin{align*}
&\begin{array}{c}
\begin{array}{c}
\text{Ar}
\end{array}
\end{array} \\
&\begin{array}{c}
\text{O}
\end{array} \\
&\begin{array}{c}
\text{H}
\end{array} \\
&\begin{array}{c}
\text{Ar}
\end{array}
\end{align*}
\]

\[
\begin{align*}
&\begin{array}{c}
\text{Ar}
\end{array} \\
&\begin{array}{c}
\text{O}
\end{array} \\
&\begin{array}{c}
\text{H}
\end{array} \\
&\begin{array}{c}
\text{Tf}
\end{array}
\end{align*}
\]

\[
\begin{align*}
&\begin{array}{c}
\text{Ar}
\end{array} \\
&\begin{array}{c}
\text{O}
\end{array} \\
&\begin{array}{c}
\text{H}
\end{array} \\
&\begin{array}{c}
\text{Tf}
\end{array}
\end{align*}
\]

53a \(\text{Ar} = \text{Ph}\)

53b \(\text{Ar} = 1,3,5-(\text{i-Pr})_3\text{C}_6\text{H}_2\)

**Equation 3.70:** Asymmetric Dield-Alder Reaction using the super chiral Brønsted acid.

\[
\begin{align*}
&\begin{array}{c}
\text{Me}
\end{array} \\
&\begin{array}{c}
\text{COEt}
\end{array} \\
\end{align*}
\]

\[
\begin{align*}
&\begin{array}{c}
\text{R}_3\text{Si}
\end{array} \\
&\begin{array}{c}
\text{R}_1
\end{array} \\
\end{align*}
\]

up to 99% yield

and 92% ee

Yamamoto *et. al.* have reported Brønsted acid catalyzed asymmetric protonation reactions of silyl enol ethers using a chiral Brønsted acid catalyst in the presence of achiral Brønsted acid media (Equation 3.71).\(^{113}\) The versatility of the reaction was revealed using a wide variety of substituents. Additionally, the reactivity of this Brønsted acid is especially appealing for chiral phosphoric acid
Equation 3.71: Yamamoto’s Brønsted acid catalyzed asymmetric protonation reactions of silyl enol ethers using a chiral Brønsted acid catalyst.

\[
\text{OTMS Ph} \xrightarrow{\text{Catalyst (10 mol%)}} \begin{array}{c}
\text{2,4,6-(CH}_3)_2\text{C}_6\text{H}_2\text{CO}_2\text{H (1eqv.)} \\
\text{toluene, rt} \\
\end{array} \text{Ph}
\]
catalysis in that the catalyst loading for this reaction can be decreased down to 0.05 mol % without any significant loss of enantioselectivity.

Gong et al. reported an asymmetric three-component 1,3-dipolar cycloaddition reaction using different chiral phosphoric acids (33) and modified chiral phosphoric acid (57) between aldehydes, amino esters, and dipolarophiles (Equation 3.72). This reaction results in high yields and excellent enantioselectivities under mild conditions. The procedure is easy to perform and allows a rapid, diversity-oriented, and enantioselective synthesis of pyrrolidine derivatives. The concept that the stereoselectivity may be controlled by use of a chiral Brønsted acid bonded dipole may lead to new findings in asymmetric catalytic 1,3-dipolar addition reactions with dipolarophiles other than electron-deficient olefins.

Uraguchi et al designed a chiral tetraaminophosphonium salt (58) possessing the P-spirocyclic structure with its potential as an organic molecular catalyst has demonstrated in the application to the asymmetric direct Henry reaction (Equation 3.73). High yield and ee were attained with this new class of organocatalyst.
Equation 3.72: Gong’s asymmetric three-component 1,3-dipolar cycloaddition reaction using chiral phosphoric acids.

Catalyst (10 mol%) CH₂Cl₂, rt, 24 h

33b 78% yield, 8% ee
33g 88% yield, 25% ee
33c 87% yield, 46% ee

33i 78% yield, 23% ee
57 96% yield, 98% ee
Equation 3.73: Uruguchi's asymmetric direct Henry reaction.

\[
\text{PhCHO} + \text{NO}_2^+\rightarrow \text{PhOH} + \text{R}_1^\text{NO}_2
\]

Catalyst 58 (5 mol\%) (M,S)-3/KO\text{tBu} THF -78°C, 8 h
3.3 Results and Discussion

(R)-1,1’-dinaphthyl-2,2’-dioxathiepin (60a) and (R)-1,1’-dinaphthyl-2,2’-diylsulfate (63a) were synthesized following the procedure of Zhang et al (Equation 74) and Koy et al (Equation 3.75).\textsuperscript{116,117} Catalyst 61a was formed after addition of 1.0 equivalent of HBF\textsubscript{4}.Et\textsubscript{2}O to 60a. Catalyst 62a was formed through the same procedure by addition of 0.5 equivalent of HBF\textsubscript{4}.Et\textsubscript{2}O. In a similar way, catalyst 64a and 65a was also formed from compound 63a by adding the required amount of HBF\textsubscript{4}.Et\textsubscript{2}O (Equation 3.75).

\textbf{Equation 3.74:} Synthesis of (R)-1,1’-dinaphthyl-2,2’-dioxathiepin (60a) and formation of catalyst 61a and 62a.
Equation 3.75: Synthesis of \((R)-1,1\textquotesingle\)-dinaphthyl-2,2'-diylsulfate (63a) and formation of catalyst 64a and 65a.

OMe or MOM following the standard procedure\(^{118}\). The protected BINOL was then lithiated by n-BuLi in the presence of an organic base such as tetramethyl diaminooethane (TMEDA)\(^{119}\). This was followed by subsequent addition of B(OMe)\(_3\) to form the appropriate boronic acid\(^{120}\). These lithiated BINOLs were derivatives of binaphthol were synthesized following the procedure of Wipf et al (Equation 3.76-82)\(^{120}\). Initially, the phenolic OH group of BINOL was protected with
Equation 3.76: Multistep synthesis of \((R)-3,3'\text{-Bis}(4''\text{-methylphenyl})-1,1'\text{-dinaphthyl-2,2'}\text{-dioxathiepin (60b)}\) and subsequent catalyst 61b.
Equation 3.77: Multistep synthesis of \((R)-3,3'\)-Bis(4"-methylphenyl)-2,2'-dihydroxy-1,1'-dinaphthyl-2,2'-diylsulfate (63b) and subsequent catalyst 64b.
Equation 3.78: Multistep synthesis of (R)-3,3’-Bis(naphthyl)-2,2′-dihydroxy-1,1′-dinaphthyl-2,2′-diylsulfate (63c) and subsequent catalyst 64c.
Equation 3.79: Multistep synthesis of \((R)-3,3\text{'-Bis(mesitylene)-2,2\text{'-dihydroxy-}1,1\text{'-dinaphthyl-2,2\text{'-diylsulfate (63d} and subsequent catalyst 64d.}
Equation 3.80: Multistep synthesis of (R)-3,3'-Bis(anthracenyl)-2,2'-dihydroxy-1,1'-dinaphthyl-2,2'-diylsulfate (63e) and subsequent catalyst 64e.
Equation 3.81: Multistep synthesis of (R)-3,3'-Bis(phenanthryl)-2,2'-dihydroxy-1,1'-dinaphthyl-2,2'-diylsulfate (63f) and subsequent catalyst 64f.

59

66

Quantitative yield

67

70% yield

67

Pd(PPh₃)₄

Bromanthracene

Water/Dioxane

68e
Equation 3.82: Multistep synthesis of \((R)-3,3\text{-}Bis\text{(triphenylsilyl)}\text{-}2,2\text{-}dihydroxy-1,1\text{-}dinaphthyl\text{-}2,2\text{-}diylsulfate (63g}\) and subsequent catalyst 64g.
Equation 3.83: Multistep synthesis of $(R)$-3,3'-'Bis(tribenzylsilyl)-2,2'-dihydroxy-1,1'-dinaphthyl-2,2'-diylsulfate (63h) and subsequent catalyst 64h.
also reacted with silyl compounds to form silyl derivatives.\textsuperscript{119} The BINOL-boronic acids were then subjected to Suzuki-coupling reaction to form the desired derivatized-BINOL.\textsuperscript{120} The final BINOL derivative was formed after deprotection of OMe or MOM. The overall yields of these derivatized BINOLs varies depending on the different substituents involved. Thus BINOL derivatives were reacted under basic conditions with SOCl\textsubscript{2} or SO\textsubscript{2}Cl\textsubscript{2} to form dioxathiepin (60) and diylsulfate (63) derivatives.

Initially, the aza-Henry reaction (Equation 3.15) was performed with HBF\textsubscript{4}Et\textsubscript{2}O to attain the racemic product of the reaction. After purification using gradient column chromatography (pentane:ethyl acetate), the pure sample was injected in the HPLC to determine the retention time for each of the two enantiomers. Then the reaction was performed with 0.1 equivalent amount of catalyst 61a (Figure 3.10). This led us to 3% ee of the product (Table 3.2). Using 62a as a catalyst did not produce any ee for the reaction. On the other hand, using 64a (0.1 equivalent) as a catalyst generated 8% ee. This comparative study helped us to conclude that catalyst 64, in general, has a better asymmetric outcome in the reaction. Essentially, diylsulfates (63) are superior to their dioxathiepin (60) counterpart.

Study of catalysts 71 and 72 showed very interesting results (Table 3.2, and Table 3.3) (Figure 3.10). Catalyst 71 produced 26% ee, while catalyst 72 shows very
Figure 3.10: Developed chiral Brønsted acids which have great potential.

61a $R=H$
61b $R=\text{phenyl}$

62a $R=H$

64a $R=H$
64b $R=\text{phenyl}$
64c $R=\text{benzene}$
64d $R=\text{indane}$
64e $R=\text{indenyl}$
64f $R=\text{SiPh}_3$
64g $R=\text{SiBn}_3$

65a $R=H$
65b $R=\text{phenyl}$

71

72
Table 3.2: Use of chiral dioxathiepin and diylsulfate derivatives bound with Brønsted acid for asymmetric aza-Henry reactions.

Table 3.2: Use of chiral dioxathiepin and diylsulfate derivatives bound with Brønsted acid for asymmetric aza-Henry reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Catalyst loading (eqv.)</th>
<th>Time (hr)</th>
<th>% conversion by NMR</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61a</td>
<td>0.1</td>
<td>192</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>61b</td>
<td>0.1</td>
<td>549</td>
<td>56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>64a</td>
<td>0.1</td>
<td>72</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>64b</td>
<td>0.1</td>
<td>102</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>64c</td>
<td>0.1</td>
<td>45</td>
<td>39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>65b</td>
<td>0.05</td>
<td>439</td>
<td>64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>71</td>
<td>0.1</td>
<td>76</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>8</td>
<td>72</td>
<td>0.1</td>
<td>72</td>
<td>25</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolated yield

low selectivity (6% ee). These results suggest that the ratio of chiral component to the Brønsted acid component could play an important role in asymmetry of the reaction. This phenomenon was also supported via changing the Brønsted acid to CF$_3$SO$_3$H.

Applying this concept to our new chiral Brønsted acid system directed us to a very exciting avenue of results (Table 3.2). The attention switched towards catalyst 64 because of the better selectivity compared to catalyst 61. The next point of interest was to check the effect on ratio of the chiral component 63 to the Brønsted acid (HBF$_4$:Et$_2$O) (Table 3.4). This set of experiments revealed that 64a : HBF$_4$ ratio 2:1 attains the highest amount of asymmetry. This finding does not
hold in the case of bulkiness at the 3,3'-position (Entry 6, Table 3.2). As a result, the focus was to find the best catalyst using a 1:1 ratio of the chiral component 63 and the Brønsted acid. Next attempt was to increase the bulkiness at the 3,3'-position of 63. Interestingly, 64c expressed a very promising result by attaining higher yield and ee in a shorter reaction time (Entry 5, Table 3.2). As expected the interest continued to increase the bulkiness at the 3,3'-position of chiral component 63 with the goal of high yield and selectivity. So far all the attempts to synthesize 63d, 63e, and 63g failed because of strong chlorinating capability of SO₂Cl₂. 63f was synthesized by changing the base to dimethylaminopyridine (DMAP) instead of pyridine. Further experiments are in the process to find the catalytic effect of 63f as a catalyst.
**Table 3.3**: Effect of changing Brønsted acid bound to chiral amines for asymmetric aza-Henry reaction.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Catalyst loading (equiv.)</th>
<th>Time (hr)</th>
<th>% conversion by NMR</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol</td>
<td>0.1</td>
<td>72</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>HBF₄</td>
<td>0.1</td>
<td>76</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>CF₃SO₂H</td>
<td>0.1</td>
<td>93</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>CF₃SO₂H</td>
<td>0.05</td>
<td>77</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>
**Table 3.4:** Effect of different ratio of chiral component 63a and HBF$_4$.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>HBF$_4$</th>
<th>Time (hr)</th>
<th>% conversion by NMR</th>
<th>% ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 eqv.</td>
<td>72</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>0.1 eqv.</td>
<td>102</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>0.15 eqv.</td>
<td>92</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>0.2 eqv.</td>
<td>92</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>0.3 eqv.</td>
<td>92</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>0.1 eqv.</td>
<td>92</td>
<td>2</td>
<td>14</td>
</tr>
</tbody>
</table>

1 eqv. 20 eqv.
3.3.1 Conclusion

In conclusion, this reports the use of a complete new chiral Brønsted acid for asymmetric organic reactions. At present, further derivatization of these catalysts and exploring further applications of this new class of organocatalysts is under investigation.
3.4 Experimental

3.4.1 General Procedure

All organometallic operations were performed under a dry nitrogen atmosphere with standard Schlenk techniques. All of the glass flasks were flame dried under vacuum and filled with nitrogen prior to use. Column chromatography was performed with silica gel (40-140 mesh). HPLC grade CH$_2$Cl$_2$ was distilled under N$_2$ from P$_2$O$_5$. HPLC grade pentane was distilled from sodium under an inert atmosphere immediately prior to use. Reagent grade Et$_2$O and tetrahydrofuran were freshly distilled under a N$_2$ atmosphere from sodium benzophenone ketyl.

Benzaldehyde, p-tolualdehyde, and p-anisaldehyde were purified by extraction with NaHCO$_3$ solution, washed with water, dried over Na$_2$SO$_4$, and distilled under vacuum. p-Nitrobenzaldehyde and p-chlorobenzaldehyde were purified by recrystallization from ethanol and dried under vacuum for several days. Ethyl diazoacetate (EDA) was obtained from Aldrich Chemical Co.

All 1H (300 MHz), 13C NMR (75.5 MHz) were performed with a Bruker 300 MHz NMR system and samples were dissolved in deuterated solvents. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane, and CDCl$_3$ was used as the solvent. FAB-MS was performed on a Micromass Autospec (Manchester, UK). Chiral HPLC was performed at room temperature utilizing a Regis chiral (S,S) Whelk-O-1 column. All HPLC were run using 1.5 mL/min flow rate with 98.5% hexane and 1.5% isopropyl alcohol mixture.
3.4.2 Synthesis of binephthyl derivative systems

3.4.2.1 \((R)-2,2'-\text{Dimethoxy}-1,1'-\text{dinaphthyl}\)

A suspension of \((R)-1,1'-\text{bi-2-naphthol}\) (28.10 g, 98.14 mmol) was heated in acetone to give a homogeneous solution (Equation 3.84). Potassium carbonate (47.47 g, 343.49 mmol) and methyl iodide (24.4 mL, 392.57 mmol) were added to the solution, and the mixture was heated at reflux for 24 h. Additional methyl iodide (24.4 mL, 392.57 mmol) was added, and heating was continued for 12 h. The solvent was evaporated to leave a volume of 200 mL, which was cooled to 25 °C and treated with 200 mL of water. The mixture was stirred for 8 h, and the resulting solid was washed with water and dried to afford 30.14 g (98%) of \((R)-2,2'-\text{Dimethoxy}-1,1'-\text{dinaphthyl}\) as a white powder: 1H NMR δ: 7.98 (d, 2H), 7.86 (d, 2H), 7.46 (d, 2H), 7.31 (t, 2H), 7.21 (t, 2H), 7.10 (d, 2H), 3.77 (s, 6H).

Equation 3.84: Protection of BINOL with methoxy group.
3.4.2.2  \((R)-2,2^\prime\)-Dimethoxymethyl-1,1\(^\prime\)-dinaphthyl

\((R)\)-binephthol ((\(R\))-BINOL, 2 g, 6.98 mmol) and NaH (60% dispersion in mineral oil, 0.42 g, 17.46 mmol) was taken in a round bottom flask under \(N_2\)-environment at 0\(^\circ\)C (Equation 3.85). This was dissolved in 40 mL THF followed by addition of MOMBr (1.14 mL, 13.97 mmol) at 0\(^\circ\)C. The resulting mixture was gradually allowed to warm to room temperature and left stirring for 1 h. Water was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with \(Et_2O\). The combined organic layers were dried with \(Na_2SO_4\), and after evaporation \((R)-1,1\(^\prime\)-bi-2-methoxymethyloxynaphthalene was obtained in a quantitative yield.\(^{118}\)

**Equation 3.85:** Protection of BINOL with methoxy methyl group.

\[
\begin{align*}
\text{OMOM} & \quad \text{OMOM} \\
\text{MOMBr} & \quad \text{NaH} \\
\text{THF, rt} & \quad \text{OMOM}
\end{align*}
\]

3.4.2.3  \((R)-3,3^\prime\)-Bis(dihydroxyborane)-2,2\(^\prime\)-dimethoxy-1,1\(^\prime\)-dinaphthyl

To a solution of TMEDA (3.20 mL, 21.38 mmol) in ether (50 mL) was added at room temperature 2.5 M \(n\)-BuLi in hexane (8.55 mL, 21.38 mmol) (Equation 3.86). The solution was stirred for 30 min, solid \((R)-2,2^\prime\)-dimethoxy-1,1\(^\prime\)-dinaphthyl (2.24 g, 7.12 mmol) was added in one portion, and the reaction mixture was stirred for 3 h. The resulting light brown suspension was cooled to -78 \(^\circ\)C, and ethyl borate (7.47 mL, 43.89 mmol) was added over a period of 10
min. The solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was cooled to 0 °C, 1 M HCl solution (100 mL) was added, and the resulting solution was stirred for 2 h at room temperature. The organic layer was washed with 1 M HCl solution and brine, dried (Na₂SO₄), and concentrated in vacuum. The resulting pale yellow solid was recrystallized from toluene to give 2.04 g (71%) of (R)-3,3'-Bis(dihydroxyborane)-2,2'-dimethoxy-1,1'-dinaphthyl as colorless crystals.¹²⁰ ¹H NMR δ: 8.62 (s, 2H), 7.99 (d, 2H), 7.44 (t, 2H), 7.32 (td, 2H), 7.16 (d, 2H), 6.10 (s, 4H, OH), 3.31 (s, 6H).

**Equation 3.86:** Synthesis of methoxy protected BINOL boronic acid.

![Equation 3.86]  

3.4.2.4  (R)-3,3'-Bis(4'-methylphenyl)-2,2'-dihydroxy-1,1'-dinaphthyl

4-Bromotoluene (1.49 g, 8.69 mmol), Ba(OH)₂ (1.49 g, 8.69 mmol), and Pd(PPh₃)₄ (134 mg, 116 mmol) were added to a solution of (R)-3,3'-Bis(dihydroxyborane)-2,2'-dimethoxy-1,1'-dinaphthyl (980 mg, 2.89 mmol) in degassed dioxane/water (20 mL, 3:1) (Equation 3.87).¹²⁰ The reaction mixture was heated at reflux for 24 h and cooled to room temperature. Dioxane was removed, and the resulting residue was redissolved in methylene chloride, the washed with 1 N HCl solution and brine, dried (using Na₂SO₄), and concentrated
in vacuum to give crude coupling products. Then it was dissolved in 50 mL CH$_2$Cl$_2$. 1.0 M solution of BBr$_3$ in CH$_2$Cl$_2$ (17.39 mL, 17.39 mmol) was added to the solution at 0 °C. The reaction mixture was warmed to room temperature, stirred for 24 h, and quenched with water (1 mL) in an ice bath. The mixture was poured into a stirred mixture of CH$_2$Cl$_2$ and water. The organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated under vacuum. Chromatography on SiO$_2$ (hexanes/EtOAc, 8:1) gave 660 mg (50%) of (R)-3,3'-Bis(4"-methylphenyl)-2,2'-dihydroxy-1,1'-dinaphthyl as a solid.$^{121}$ 1H NMR δ: 8.12 (s, 2H), 8.00 (d, 2H), 7.76 (d, 4H), 7.45 (td, 2H), 7.37 (t, 8H), 5.61 (s, 2H); 13C NMR δ: 150.3, 137.5, 137.1, 134.6, 133.0, 131.1, 130.7, 130.3, 129.5, 129.2, 128.8, 128.4, 127.7, 127.1, 126.8, 124.3, 124.2, 112.6, 21.3.

**Equation 3.87:** Synthesis of (R)-3,3'-bis(4"-methylphenyl)-2,2'-dihydroxy-1,1'-dinaphthyl by Suzuki coupling followed by deprotection reaction.

![Equation 3.87](image)

3.4.2.5 **(R)-3,3'-Bis(1"-naphthyl)-2,2'-dihydroxy-1,1'-dinaphthyl**

1-Bromonaphthalene (4.28 g, 20.68 mmol), Ba(OH)$_2$• (3.54 g, 20.68 mmol), and Pd(PPh$_3$)$_4$ (318 mg, 0.27 mmol) were added to a solution of (R)-3,3'-bis(dihydroxyborane)-2,2'-dimethoxy-1,1'-dinaphthyl (2.62 g, 6.89 mmol) in
degassed dioxane/water (100 mL, 3:1) (Equation 3.88). The reaction mixture was heated at reflux for 24 h and cooled to room temperature. Dioxane was removed, and the resulting residue was redissolved in methylene chloride, washed with 1 N HCl acid solution and brine, dried (using Na$_2$SO$_4$), and concentrated in vacuum to give crude coupling products. Then it was dissolved in 35 mL CH$_2$Cl$_2$. 2.60 mL BBr$_3$ (27.58 mmol) was added to the solution at 0 °C. The reaction mixture was warmed to room temperature, stirred for 24 h, and quenched with water (1 mL) in an ice bath. The mixture was poured into a stirred mixture of CH$_2$Cl$_2$ and water. The organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated under vacuum. Chromatography on SiO$_2$ (hexanes/EtOAc, 8:1) gave 1.41 g (38%) of (R)-3,3'-bis(1''-naphthyl)-2,2'-dihydroxy-1,1'-dinaphthyl as a solid.\textsuperscript{122} 1H NMR $\delta$: 8.03 (d, 2H), 7.96 (q, 6H), 7.84 (d, 2H), 7.30-7.8 (m, 20H), 5.1-5.3 (b, OH). 13C NMR $\delta$: 131.9, 128.6, 128.3, 127.9, 127.2, 126.3, 125.9, 125.4, 124.6, 124.4, 124.1.

**Equation 3.88:** Synthesis of (R)-3,3'-bis(1''-naphthyl)-2,2'-dihydroxy-1,1'-dinaphthyl by Suzuki coupling followed by deprotection reaction.
3.4.2.6  (R)-3,3'-Bis-(2-mesityl)-2,2'-dihydroxy-1,1'-dinaphthyl

2-Bromomesitylene (5.66 g, 19.97 mmol), Ba(OH)$_2$ (3.42 g, 19.97 mmol), and Pd(PPh$_3$)$_4$ (307 mg, 0.27 mmol) were added to a solution of (R)-3,3'-bis(dihydroxyborane)-2,2'-dimethoxy-1,1'-dinaphthyl (2.53 g, 6.66 mmol) in degassed dioxane/water (100 mL, 3:1) (Equation 3.89). The reaction mixture was heated at reflux for 24 h and cooled to room temperature. Dioxane was removed, and the resulting residue was redissolved in methylene chloride, washed with 1 N HCl acid solution and brine, dried (using Na$_2$SO$_4$), and concentrated in vacuum to give crude coupling products. Then it was dissolved in

Equation 3.89: Synthesis of (R)-3,3'-bis-(2-mesityl)-2,2'-dihydroxy-1,1'-dinaphthyl by Suzuki coupling followed by deprotection.

35 mL CH$_2$Cl$_2$. 6.17 mL BBr$_3$ (64.74 mmol) was added to the solution at 0 °C. The reaction mixture was warmed to room temperature, stirred for 24 h, and quenched with water (1 mL) in an ice bath. The mixture was poured into a stirred mixture of CH$_2$Cl$_2$ and water. The organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated under vacuum. Chromatography on SiO$_2$ (hexanes/EtOAc, 8:1) gave 0.63 g (14.27%) of (R)-3,3'-bis(2'-mesityl)-2,2'-
dihydroxy-1,1'-dinaphthyl as a solid.\(^{113}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta = 7.84\) (d, 2H), 7.72 (s, 2H), 7.36-7.23 (m, 6 H), 6.98 (s, 4H), 5.00 (s, 2H), 2.31 (s, 6H), 2.13 (s, 6H), 2.06 (s, 6H)

### 3.4.2.7 \((R)-3,3'\)-Bis(9''-phenanthryl)-2,2'-dihydroxy-1,1'-dinaphthyl

9-Bromonaphanthrene (2.13 g, 8.28 mmol), Ba(OH)\(_2\) (1.42 g, 8.29 mmol), and Pd(PPh\(_3\))\(_4\) (128 mg, 0.11 mmol) were added to a solution of \((R)-3,3'\)-Bis(dihydroxyborane)-2,2'-dimethoxy-1,1'-dinaphthyl (1.05 g, 2.76 mmol) in degassed dioxane/water (40 mL, 3:1) (Equation 3.90).\(^{120}\) The reaction mixture was refluxed for 24 h and cooled to room temperature. Dioxane was removed, and the resulting residue was redissolved in CH\(_2\)Cl\(_2\), washed with 1 N HCl acid solution and brine, dried (using Na\(_2\)SO\(_4\)), and concentrated in vacuum to give crude coupling products. Then it was dissolved in 20 mL CH\(_2\)Cl\(_2\). 1.04 mL

**Equation 3.90**: Synthesis of \((R)-3,3'\)-bis(9''-phenanthryl)-2,2'-dihydroxy-1,1'-dinaphthyl by Suzuki coupling followed by deprotection reaction.
BBR₃ (11.05 mmol) was added to the solution at 0 °C. The reaction mixture was warmed to room temperature, stirred for 24 h, and quenched with water (1 mL) in an ice bath. The mixture was poured into a stirred mixture of CH₂Cl₂ and water. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated under vacuum. Chromatography on SiO₂ (hexanes/EtOAc, 8:1) gave 0.66 g (44%) of (R)-3,3'-Bis(9"-phenanthryl)-2,2'-dihydroxy-1,1'-dinaphthyl as a solid.¹²³

1H NMR δ: 8.70 – 8.90 (m, 2H), 8.10 (dq, 1H), 7.80-8.00 (m, 4H), 7.60-7.8 (m, 5H), 7.40-7.60 (m, 4H), 5.2-5.3 (b, 2H).

3.4.2.8 (R)-3,3'-Bis(9"-anthracenyl)-2,2'-dihydroxy-1,1'-dinaphthyl

9-Bromoanthracene (1.00 g, 3.88 mmol), Ba(OH)₂ ⋅ (666 mg, 3.88 mmol), and Pd(PPh₃)₄ (60 mg, 0.05 mmol) were added to a solution of (R)-3,3'-Bis(dihydroxyborane)-2,2'-dimethoxy-1,1'-dinaphthyl (492 mg, 1.29 mmol) in degassed dioxane/water (40 mL, 3:1) (Equation 3.91).¹²⁰ The reaction mixture was refluxed for 24 h and cooled to room temperature. Dioxane was removed, and the resulting residue was redissolved in CH₂Cl₂, washed with 1 N HCl solution and brine, dried (using Na₂SO₄), and concentrated in vacuum to give crude coupling products. Then it was dissolved in 50 mL CH₂Cl₂. 1.82 mL BBR₃ (19.29 mmol) was added to the solution at 0 °C. The reaction mixture was warmed to room temperature, stirred for 24 h, and quenched with water (1 mL) in an ice bath. The mixture was poured into a stirred mixture of CH₂Cl₂ and water. The organic layer was washed with brine, dried over granular Na₂SO₄, and concentrated under vacuum. Chromatography on SiO₂ (hexanes/EtOAc, 8:1)
gave 0.60 g (73%) of (R)-3,3'-Bis(9''-anthracenyl)-2,2'-dihydroxy-1,1'-dinaphthyl as a solid.\textsuperscript{124} 1H NMR (CDCl\textsubscript{3}, 300 MHz) \( \delta \): 8.24 (s, 2H), 8.03 (m, 2H), 7.86 (m, 2H), 7.80 (d), 7.63-7.75 (m, 8H), 7.10-7.30 (m, 12H), 6.94 (m, 2H), 5.10 (s, 2H, OH).

**Equation 3.91:** Synthesis of (R)-3,3'-bis(9''-anthracenyl)-2,2'-dihydroxy-1,1'-dinaphthyl.

\[ \text{O} \text{Me} \text{B(OH)}_2 \text{OMe} \text{B(OH)}_2 \rightarrow \begin{align*} 1. \text{9-Bromoanthracene} \\
\text{Pd(PPh}_3)_4 \\
\text{Ba(OH)}_2 \\
dioxane/water \\
2. \text{BBr}_3 \end{align*} \]

**4.2.9 (R)-3,3'-Bis(triphenylsilyl)-2,2'-dihydroxy-1,1'-dinaphthyl**

MOM protected BINOL (2.61 g, 6.98 mmol) was dissolved in Et\textsubscript{2}O (20 mL) followed by dropwise addition of \( \text{^6} \text{BuLi} \) (6.98 mL, 2.5 M, 17.46 mmol) over 5 mins at room temperature (Equation 3.92).\textsuperscript{99} The resulting suspension was stirred at room temperature for 1 hour 30 mins and a color change was observed over the initial 30 mins from a yellow solution to a brown suspension. The mixture was cooled to 0 °C and THF (40 mL) was added. After a further 15 mins at 0°C a solution of Ph\textsubscript{3}SiCl (5.15 g, 17.46 mmol) in THF (20 mL) was added. The reaction mixture was warmed to room temperature and stirred for 30 hours. After addition
of the THF and Ph₃SiCl the color darkened from light brown to very dark brown over the first one hour. The color faded through green to orange and finally pale yellow over the 30 hour period. The reaction was quenched by addition of saturated NH₄Cl, then extracted with CH₂Cl₂ as an organic solvent. The organics were washed with brine and dried over granular Na₂SO₄, filtered and concentrated to yield the crude product as viscous yellow oil. The product was purified by gradient silica column chromatography using pentane and ethylacetate solvent system. Then the product was dissolved in dioxane (20 mL) and added 5 mL concentrated HCl acid to the stirring solution. After 24 hours the reaction mixture was neutralized by adding saturated NaHCO₃ solution and the product was extracted using CH₂Cl₂ as an organic solvent. Then it was dried over granular Na₂SO₄ and solvent was removed by rotary evaporation. The product was separated by a column chromatography using gradient pentane/EtOAc mixture.¹⁹⁹ ¹H NMR (300 MHz, C₆D₆) δ = 4.68 (s, 2H, OH), 6.90-7.38 (m, 26H, ArH), 7.80-7.84 (m, 12H, ArH), 8.14 (s, 2H, ArH);

Equation 3.92: Synthesis of (R)-3,3′-bis(triphenylsilyl)-2,2′-dihydroxy-1,1′-dinaphthyl.
3.4.2.10 \((R)-1,1'-\text{dinaphthyl}-2,2'-\text{dioxathiepin}\)

Under N\(_2\)-environment \((R)\)-BINOL (0.38 g, 1.33 mmol) was dissolved in 10 mL CH\(_2\)Cl\(_2\) at 0\(^\circ\)C followed by addition of pyridine (2.146 mL, 26.54 mmol). SOCl\(_2\) (0.24 mL, 3.31 mmol) was slowly added to the stirring mixture (Equation 3.93). After addition of SOCl\(_2\) the solution was refluxed for 3 hours. Then it was cooled to room temperature. Saturated citric acid solution was added to quench the reaction and then extracted the product three time with 15mL portion of CH\(_2\)Cl\(_2\). Then washed the collected organic layer three times with saturated NaHCO\(_3\) solution and dried over MgSO\(_4\). After removing the solvent by rotary evaporation 75\% (0.33 g) product was isolated.\(^\text{116}\) \(^1\text{H}\) NMR (300 MHz, CDCl\(_3\)) \(\delta = 8.04\text{-}8.07\) (d, 2H), 7.98\text{-}7.99 (d, 2H), 7.48\text{-}7.64 (m, 6H), 7.34\text{-}7.44 (m, 2H).

**Equation 3.93**: Synthesis of \((R)-1,1'-\text{dinaphthyl}-2,2'-\text{dioxathiepin}\).

4.2.11 \((R)-3,3'-\text{Bis}(4''\text{-methylphenyl})-1,1'-\text{dinaphthyl}-2,2'-\text{dioxathiepin}\)

Under N\(_2\)-environment \((R)-3,3'-\text{Bis}(4''\text{-methylphenyl})-2,2'-\text{dihydroxy}-1,1'-\text{dinaphthyl}\) (0.70 g, 1.50 mmol) was dissolved in 10 mL CH\(_2\)Cl\(_2\) at 0\(^\circ\)C followed by addition of pyridine (2.40 mL, 30.04 mmol) (Equation 3.94). SOCl\(_2\) (0.27 mL, 3.75 mmol) was slowly added to the stirring mixture. After addition of SOCl\(_2\) the solution was refluxed for 3 hours. Then it was cooled to
room temperature. Saturated citric acid solution was added to quench the reaction and the extracted the product three times with 15mL portion of CH$_2$Cl$_2$. Then washed the organic layer three times with NaHCO$_3$ solution and dried over MgSO$_4$. After removing the solvent by rotary evaporation 70% product was isolated. $^1$H NMR (300 MHz, CDCl$_3$) $\delta = 7.64$–$8.6$ (m, 8H), 7.50–7.6 (m, 10H), 7.34–7.43 (m, 10H), 2.50 (s, 6H). $^{13}$C NMR (DMSO, 75.4 MHz) $\delta$: 152.7, 137.9, 135.9, 130.7, 130.6, 129.8, 129.7, 129.4, 129.3, 128.9, 128.5, 128.4, 127.0, 126.9, 126.7, 126.6, 126.5, 126.3, 124.2, 117.7, 21.2. MS calculated for C$_{34}$H$_{24}$O$_3$S 512, found 512.

**Equation 3.94:** Synthesis of (R)-3,3'-bis(4''-methylphenyl)-1,1'-dinaphthyl-2,2'-dioxathiepin.

![Chemical structure](image)

3.4.2.12 **(R)-1,1'-dinaphthyl-2,2'-diylsulfate**

Under N$_2$-atmosphere (R)-1,1'-bi-2-naphthol (6.84 g, 23.88 mmol) was dissolved in 30.78 mL (380 mmol) pyridine. At 0°C SO$_2$Cl$_2$ (2.51 mL, 31.05 mmol) was added slowly (Equation 3.95). Then the solution was heated to 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl solution. The total mixture was heated to boiling for 10 minutes. The product was filtered as
nice orange colored solid in 40% yield.\textsuperscript{117} $^1$H NMR (300 MHz, CDCl$_3$) $\delta = 8.13$-$8.17$ (d, 2H), 8.03-$8.06$ (d, 2H), 7.66-$7.71$ (d, 2H), 7.55-$7.65$ (m, 4H), 7.40-$7.47$ (m, 2H).

**Equation 3.95:** Synthesis of (R)-1,1'-dinaphthyl-2,2'-diylsulfate.

\[
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{O} \\
\text{Cl}_2 \\
\text{Pyridine} \\
80^\circ\text{C} \\
\text{SO}_2\text{Cl}_2 \\
\end{array}
\rightarrow
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{S} \\
\text{O} \\
\text{Cl}_2 \\
\text{Pyridine} \\
80^\circ\text{C} \\
\text{SO}_2\text{Cl}_2 \\
\end{array}
\]

### 3.4.2.13 (R)-3,3'-Bis(4''-methylphenyl)-1,1'-dinaphthyl-2,2'-diylsulfate

Under N$_2$-atmosphere (R)-3,3'-Bis(4''-methylphenyl)-2,2'-dihydroxy-1,1'-dinaphthyl (0.49 g, 1.05 mmol) was dissolved in 6 mL pyridine. At 0°C SO$_2$Cl$_2$ (0.11 mL, 1.44 mmol) was added slowly (Equation 3.96).\textsuperscript{117} Then the solution was heated at 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl solution. The total mixture was heated to boiling for 10 minutes. The

**Equation 3.96:** Synthesis of (R)-3,3'-bis(4''-methylphenyl)-1,1'-dinaphthyl-2,2'-diylsulfate.

\[
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{O} \\
\text{Cl}_2 \\
\text{Pyridine} \\
80^\circ\text{C} \\
\text{SO}_2\text{Cl}_2 \\
\end{array}
\rightarrow
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{S} \\
\text{O} \\
\text{Cl}_2 \\
\text{Pyridine} \\
80^\circ\text{C} \\
\text{SO}_2\text{Cl}_2 \\
\end{array}
\]
product (0.15 g, 0.28 mmol, 27% yield) was filtered as nice orange colored solid. 

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 8.15 (s, 2H), 8.05 (m, 4H), 7.55-7.65 (m, 8H), 7.40-7.7 (m, 2H), 7.30-7.37 (d, 6H), 2.45 (s, 6H). 

$^{13}$C NMR (CDCl$_3$, 75.4 MHz) $\delta$: 132.8, 129.5, 129.2, 128.7, 128.6, 127.6, 127.0, 126.8, 119.9, 21.2. MS calculated for C$_{34}$H$_{24}$O$_4$S 528, found 528.

3.4.2.14 (R)-3,3'-Bis(1''-naphthyl)-1,1'-dinaphthyl-2,2'-diylsulfate

Under N$_2$-atmosphere (R)-3,3'-Bis(1''-naphthyl)-2,2'-dihydroxy-1,1'-qdinaphthyl (2.54 g, 4.71 mmol) was dissolved in 20 mL pyridine (Equation 3.97). At 0°C SO$_2$Cl$_2$ (0.57 mL, 7.07 mmol) was added slowly. Then the solution was heated at 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl acid solution. The total mixture was heated to boiling for 10 minutes. The product

Equation 3.97: Synthesis of (R)-3,3'-bis(1''-naphthyl)-1,1'-dinaphthyl-2,2'-diylsulfate.
(0.0050 g) was filtered as nice white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta = 8.16$ (m, 2H), 8.06 (m, 2H), 7.94 (m, 4H), 7.30-7.9 (m, 12H). $^{13}$C NMR (CDCl$_3$ 75.4 MHz) $\delta = 133.8, 133.1, 128.8, 128.6, 128.2, 127.2, 127.1, 126.3, 125.7, 125.3, 124.6. MS calculated for C$_{40}$H$_{24}$O$_4$S 600, found 600.

### 3.4.2.15 (R)-3,3'-Bis(mesityl)-1,1'-dinaphthyl-2,2'-diylsulfate

Under N$_2$-atmosphere (R)-3,3'-Bis(mesityl)-2,2'-dihydroxy-1,1'-dinaphthyl (0.2 g, 0.31 mmol) was dissolved in 20 mL pyridine (Equation 3.98).\textsuperscript{117} At 0°C SO$_2$Cl$_2$ (0.03 mL, 0.37 mmol) was added slowly. Then the solution was heated at 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl solution. The total mixture was heated to boiling for 10 minutes. Different fractions were isolated by column chromatography. None of the fraction was found to be the product.

**Equation 3.98:** Failed synthesis of (R)-3,3'-Bis(mesityl)-1,1'-dinaphthyl-2,2'-diylsulfate.
3.4.2.16 (R)-3,3'-Bis(9'-anthracenyl)-1,1'-dinaphthyl-2,2'-diylsulfate

Under N$_2$-atmosphere (R)-3,3'-Bis(9'-anthracenyl)-2,2'-dihydroxy-1,1'-dinaphthyl (0.2 g, 0.31 mmol) was dissolved in 20 mL pyridine (Equation 3.99). At 0°C SO$_2$Cl$_2$ (0.03 mL, 0.37 mmol) was added slowly. Then the solution was heated at 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl solution. The total mixture was heated to boiling for 10 minutes. Different fractions were isolated by column chromatography. None of the fraction was found to be the product.

Equation 3.99: Failed synthesis of (R)-3,3'-Bis(9'-anthracenyl)-1,1'-dinaphthyl-2,2'-diylsulfate.

3.4.2.17 (R)-3,3'-Bis(phenanthryl)-1,1'-dinaphthyl-2,2'-diylsulfate

Under N$_2$-atmosphere (R)-3,3'-Bis(phenanthryl)-2,2'-dihydroxy-1,1'-dinaphthyl (0.93 g, 1.46 mmol) was dissolved in 30 mL pyridine (Equation 3.100). At 0°C SO$_2$Cl$_2$ (0.17 mL, 2.18 mmol) was added slowly. Then the
solution was heated at 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl acid solution. The total mixture was heated to boiling for 10 minutes. Different fractions were isolated by column chromatography. None of the fraction was found to be the product.

**Equation 3.100:** Failed synthesis of \((R)-3,3'-\text{Bis(phenanthryl)}-1,1'-\text{dinaphthyl-2,2'}-\text{diylsulfate.}\)

4.2.18 \((R)-3,3'-\text{Bis(triphenylsilyl)}-2,2'-\text{dihydroxy-1,1'-dinaphthyl-2,2'}-\text{diylsulfate}\)

Under N\(_2\)-atmosphere \((R)-3,3'-\text{Bis(triphenylsilyl)}-2,2'-\text{dihydroxy-1,1'-dinaphthyl}\) (0.1 g, 0.13 mmol) was dissolved in 10 mL pyridine (Equation 3.101).\(^{117}\) At 0°C SO\(_2\)Cl\(_2\) (0.01 mL, 0.19 mmol) was added slowly. Then the solution was heated at 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl solution. The total mixture was heated to boiling for 10
minutes. Different fractions were isolated by column chromatography. None of the fraction was found to be the product.

**Equation 3.101**: Failed synthesis of \((R)-3,3'-\text{bis(triphenylsilyl)}-2,2'-\text{dihydroxy-1,1'}-\text{dinaphthyl-2,2'}-\text{diylsulfate.}

\[
\begin{align*}
\text{Under N}_2\text{-atmosphere } (R)-3,3'-\text{Bis(triphenylsilyl)}-2,2'-\text{dihydroxy-1,1'}-\text{dinaphthyl } (0.1 \text{ g, 0.13 mmol}) \text{ and dimethylaminopyridine } (0.31 \text{ g, 2.55 mmol}) \text{ were dissolved in 2 mL CH}_2\text{Cl}_2 \text{ (Equation 3.102). At 0°C SO}_2\text{Cl}_2 \text{ (0.01 mL, 0.19 mmol)} \text{ was added slowly. Then the solution was allowed to reach room temperature and stirred for 24hrs. The reaction was quenched by neutralizing with saturated solution of citric acid. The total mixture was heated to boiling for 10 minutes. 0.02 g product was isolated by column chromatography. } ^1\text{H NMR (300 MHz, CDCl}_3 \text{)} \delta = 8.21 \text{ (s, 2H), 7.87 (d, 2H), 7.61-7.70 (m, 23H), 7.35-7.50 (m, 41H).} ^{13}\text{C NMR (CDCl}_3 \text{ 75.4 MHz)} \delta = 151.0, 142.3, 142.0, 136.6, 136.2, 135.9, 134.9, 133.4, 133.2, 131.5, 130.0, 129.6, 129.4, 128.8, 127.8, 127.7, 126.9, 126.5, 123.8, 123.3. \text{ MS calculated for C}_{56}\text{H}_{40}\text{O}_4\text{Si}_2 \text{ 865, found 865.}
\end{align*}
\]
Equation 3.102: Synthesis of (R)-3,3’-bis(triphenylsilyl)-2,2’-dihydroxy-1,1’-dinaphthyl-2,2’-diylsulfate.

3.4.2.19 (R)-3,3’-Bis(tribenzylsilyl)-2,2’-dihydroxy-1,1’-dinaphthyl-2,2’-diylsulfate

Under N$_2$-atmosphere (R)-3,3’-Bis(tribenzylsilyl)-2,2’-dihydroxy-1,1’-dinaphthyl (4.39 g, 4.97 mmol) was dissolved in 100 mL pyridine (Equation 3.103). At 0°C SO$_2$Cl$_2$ (0.60 mL, 7.42 mmol) was added slowly. Then the solution was heated at 90°C for 30 minutes. The reaction was quenched by neutralizing with 5 M HCl solution. The total mixture was heated to boiling for 10 minutes. Different fractions were isolated by column chromatography. None of the fraction was found to be the product.

Equation 3.103: Failed synthesis of (R)-3,3’-bis(tribenzylsilyl)-2,2’-dihydroxy-1,1’-dinaphthyl-2,2’-diylsulfate.
3.4.3 General procedure for the synthesis of \( N \)-benzylidene arylimines

Amine and aldehyde were dissolved in equimolar amount in ethanol solvent and refluxed for an hour. After cooling it to room temperature water was added slowly to crystallize the product in almost quantitative yield.\(^\text{125}\)

3.4.3.1 Synthesis of \( N \)-benzylidenephenylimines

Benzaldehyde (1g, 9.42 mmol) and aniline (0.88g, 9.45 mmol) were dissolved in 20 mL ethanol (Equation 3.104). The solution was cooled to room temperature after refluxing for an hour. The water was slowly added to crystallize the product. The product was isolated by vacuum filtration in 85% yield.\(^\text{125}\) \(^\text{1H}\) NMR (300 MHz, CDCl\(_3\)) \(\delta = 8.64\) (s, 1H), 7.39-7.48 (m, 4H), 7.29-7.34 (m, 3H), 7.06-7.09 (dd, 1H), 6.95-6.7.00 (dt, 1H).

Equation 3.104: Synthesis of \( N \)-benzylidenephenylimines.

3.4.3.2 Synthesis of \( N \)-benzylidene-4-methoxyphenyylimines
\( p \)-Methoxybenzaldehyde (4.93 g, 36.21 mmol) and aniline (3.37 g, 36.21 mmol) were dissolved in 20 mL ethanol (Equation 3.105).\(^{125}\) The solution was cooled to room temperature after refluxing for an hour. The water was slowly added to crystallize the product. The product was isolated by vacuum filtration in 90\% yield.\(^{126}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta = 8.41 \) (s, 1H), 7.87 (d, 2H), 7.50 (d, 2H), 7.30 (m, 3H), 7.00 (d, 2H), 3.89 (s, 3H).

**Equation 3.105:** Synthesis of \( N \)-benzylidene-4-methoxyphenylimines.

\[
\begin{align*}
\text{OCH}_3 & \quad + \quad \text{OCH}_3 \\
\text{OH} & \quad \text{Reflux}\quad \text{Ethanol} \\
\end{align*}
\]

\[
\begin{align*}
\text{N} & \quad \text{OCH}_3 \\
\end{align*}
\]

3.4.3.3 **Synthesis of \( N \)-benzylidene-2,4,5-trimethoxyphenylimines**

2,4,5-Trimethoxybenzaldehyde (1.00 g, 5.09 mmol) and aniline (0.47 g, 5.1 mmol) were dissolved in 10 mL ethanol (Equation 3.106).\(^{125}\) The solution was cooled to room temperature after refluxing for an hour. The water was slowly added to crystallize the product. The product was isolated by vacuum filtration in 92\% yield.\(^{127}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta = 8.86 \) (s, 1H), 7.85 (s, 1H), 7.36 (m, 2H), 7.20 (m, 3H), 6.53 (s, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 3.90 (s, 3H).

**Equation 3.106:** Synthesis of \( N \)-benzylidene-2,4,5-trimethoxyphenylimines.
3.4.3.4 Synthesis of \( N \)-benzylidene-2-nitrophenylimines

2-Nitrobenzaldehyde (1.00 g, 6.62 mmol) and aniline (0.62 g, 6.62 mmol) were dissolved in 10 mL ethanol (Equation 3.107).\textsuperscript{125} The solution was cooled to room temperature after refluxing for an hour. The water was slowly added to crystallize the product. The product was isolated by vacuum filtration in 88% yield.\textsuperscript{128} \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta = 9.23 \) (s, 1H), 8.36 (d, 1H), 8.10 (d, 1H), 7.77 (m, 1H), 7.72 (m, 1H), 7.65 (m, 2H), 7.32 (m, 3H).


3.4.3.5 Synthesis of \( N \)-benzylidene-4-chlorophenylimines

4-Chlorobenzaldehyde (1.00 g, 7.11 mmol) and aniline (0.66 g, 7.11 mmol) were dissolved in 10 mL ethanol (Equation 3.108).\textsuperscript{125} The solution was cooled to room temperature after refluxing for an hour. The water was slowly
added to crystallize the product. The product was isolated by vacuum filtration in 89% yield.\textsuperscript{126} $^1$H NMR (300 MHz, CDCl$_3$) $\delta = 8.45$ (s, 1H), 7.87 (d, 2H), 7.50 (m, 4H), 7.30 (m, 3H).

**Equation 3.108**: Synthesis of $N$-benzylidene-4-chlorophenylimines.

\[
\begin{array}{c}
\text{Cl} \quad \text{O} \\
\text{Cl} \quad \text{N}_2
\end{array}
\xrightarrow{\text{Ethanol, Reflux}}
\begin{array}{c}
\text{Cl} \quad \text{N}_2
\end{array}
\]

### 3.4.3.6 Synthesis of $N$-benzylidene-2,4-dichlorophenylimines

2,4-Dichlorobenzaldehyde (1.00 g, 5.71 mmol) and aniline (0.53 g, 5.71 mmol) were dissolved in 10 mL ethanol (Equation 3.109).\textsuperscript{126} The solution was cooled to room temperature after refluxing for an hour. The water was slowly added to crystallize the product. The product was isolated by vacuum filtration in 80% yield.\textsuperscript{127} $^1$H NMR (300 MHz, CDCl$_3$) $\delta = 9.10$ (s, 1H), 8.45 (d, 1H), 7.30-7.60 (m, 8H).

**Equation 3.109**: Synthesis of $N$-benzylidene-2,4-dichlorophenylimines.
3.4.3.7 Synthesis of \(N\)-benzylidene-4-phenylphenylimines

4-Phenylbenzaldehyde (1.00 g, 5.48 mmol) and aniline (0.51 g, 5.48 mmol) were dissolved in 10 mL ethanol (Equation 3.110). The solution was cooled to room temperature after refluxing for an hour. The water was slowly added to crystallize the product. The product was isolated by vacuum filtration in 90% yield. \(^{130}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta = 8.50\) (s, 1H), 8.02 (d, 2H), 7.69 (m, 4H), 7.50 (m, 5H), 7.28 (m, 3H).

Equation 3.110: Synthesis of \(N\)-benzylidene-4-phenylphenylimines.

3.4.4 General procedure for the aza-Henry reaction
Catalytic amount of the sulfate was dissolved in CH$_2$Cl$_2$ in a round-bottom flask followed by addition of equimolar amount of HBF$_4$.Et$_2$O. The solvent was removed by rotary evaporator. Imine (1 equivalent) was added to the flask under N$_2$-atmosphere. Then excess CH$_3$NO$_2$ (100 equivalent amount) was added to the reaction mixture as a solvent as well as a reagent. The reaction mixture was allowed to stir at room temperature for time till the completion of the reaction. Then the reaction mixture was passed through a silica plug to quench the reaction. The product was isolated after column chromatography.

3.4.4.1 Synthesis of 2-(phenyl)-2-(phenylamino)-nitroethane

Under nitrogen, $N$-benzylidenephenylimines (0.5g, 2.76 mmol) was dissolve in nitromethane (15.03 mL, 276 mmol) followed by addition of HBF$_4$.Et$_2$O (37µL, 0.276 mmol) (Equation 3.111). Then the reaction mixture was stirred until the reaction was deemed complete. Then the product was isolated by doing column chromatography with a gradient pentane and ethyl acetate mixture.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta = 7.36$-$7.47$ (m, 5H), $7.14$-$7.20$ (t, 2H), $6.75$-$6.80$ (t, 1H), $6.62$-$6.65$ (d, 2H), $5.15$-$5.25$ (m, 1H), $4.73$-$4.75$ (d, 2H), $4.42$ (s, 1H, NH).

**Equation 3.111**: Synthesis of 2-(phenyl)-2-(phenylamino)-nitroethane.
3.4.4.2 Synthesis of 2-(2,4,5-trimethoxyphenyl)-2-(phenylamino)-nitroethane

Under nitrogen, N-benzylidene-2,4,5-trimethoxyphenylimines (0.5g, 1.51 mmol) was dissolve in nitromethane (8.20 mL, 151 mmol) followed by addition of HBF₄·Et₂O (20µL, 0.15 mmol) (Equation 112). Then the reaction mixture was stirred until the reaction was deemed complete. Then the product was isolated by doing column chromatography with a gradient pentane and ethyl acetate mixture. ¹H NMR (300 MHz, CDCl₃) δ: 7.07-7.13 (t, 2H), 6.84 (s, 1H), 6.62-6.72 (m, 3H), 6.54 (s, 1H), 5.34-5.38 (t, 1H), 4.67-4.76 (m, 2H), 4.09 (s, 3H), 3.92 (s, 3H), 3.71 (s, 3H).

Equation 3.112: Synthesis of 2-(2,4,5-trimethoxyphenyl)-2-(phenylamino)-nitroethane.

3.4.4.3 Synthesis of 2-(4-phenyl-phenyl)-2-(phenylamino)-nitroethane

Under nitrogen, N-benzylidene-4-phenylphenylimines (0.5g, 1.57 mmol) was dissolve in nitromethane (8.56 mL, 157 mmol) followed by addition of HBF₄·Et₂O (21µL, 0.16 mmol) (Equation 3.113). Then the reaction mixture was stirred until the reaction was deemed complete. Then the product was isolated by
doing column chromatography with a gradient pentane and ethyl acetate mixture.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 8.06-8.09 (d, 1H), 7.70-7.80 (m, 2H), 7.55-7.64 (t, 1H), 7.46-7.52 (t, 1H), 7.07-7.13 (t, 2H), 6.70-6.75 (t, 1H), 6.49-6.52 (d, 2H), 5.84 (m, 1H), 4.98-5.03 (dd, 1H), 4.79 (dd, 1H).

**Equation 3.113:** Synthesis of 2-(4-phenyl-phenyl)-2-(phenylamino)-nitroethane.

3.4.4.4 Synthesis of 2-(2-nitrophenyl)-2-(phenylamino)-nitroethane

Under nitrogen, $N$-benzylidene-2-nitrophenylimines (0.5g, 1.74 mmol) was dissolve in nitromethane (9.49 mL, 174 mmol) followed by addition of HBF$_4$.Et$_2$O (24µL, 0.17 mmol) (Equation 3.114). Then the reaction mixture was stirred until the reaction was deemed complete. Then the product was isolated by doing column chromatography with a gradient pentane and ethyl acetate mixture. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 8.10-8.11 (d, 1H), 7.70-7.74 (d, 1H), 7.58-7.64 (t, 1H), 7.44-7.54 (t, 1H), 7.08-7.14 (2H), 6.70-6.78 (t, 1H), 6.49-6.53 (d, 2H), 5.85 (m, 1H), 4.98-5.07 (dd, 1H), 4.9 (s, 1H).
**Equation 3.114:** Synthesis of 2-(2-nitrophenyl)-2-(phenylamino)-nitroethane.

![Chemical structure](image)

3.4.4.5 **Synthesis of 2-(4-methoxyphenyl)-2-(phenylamino)-nitroethane**

Under nitrogen, *N*-benzylidene-4-methoxyphenylimines (0.5g, 1.82 mmol) was dissolved in nitromethane (9.93mL, 182 mmol) followed by addition of HBF$_4$.Et$_2$O (25µL, 0.18 mmol) (Equation 3.115). Then the reaction mixture was stirred until the reaction was deemed complete. Then the product was isolated by doing column chromatography with a gradient pentane and ethyl acetate mixture.

$^1$H NMR (300 MHz, CDCl$_3$) δ: 7.31-7.34 (d, 2H), 7.14-7.20 (t, 2H), 6.90-6.93 (d, 2H), 6.72-6.80 (t, 1H), 6.63-6.66 (d, 2H), 5.10-5.20 (t, 1H), 4.69-4.72 (dd, 2H), 3.81 (s, 3H).

**Equation 3.115:** Synthesis of 2-(4-methoxyphenyl)-2-(phenylamino)-nitroethane.

![Chemical structure](image)


26 http://www.chem.wisc.edu/areas/reich/pkatable/index.htm

27 http://www2.lsdiv.harvard.edu/labs/evans/pdf/evans_pKa_table.pdf


    **2006**, 8, 3175.


3.8 Results and Discussion for Oxazinanones

**Equation 3.84:** Reaction of 1,3-oxazinan-2-one

Nitro-Mannich (or aza-Henry) reactions involve catalytic formation of β-nitro amines from Boc-imines and nitroalkanes. Recently, there has been a surge of research in this area of organic chemistry due to the importance of β-nitro amines in organic syntheses. For example, the organocatalytic nitro-Mannich reaction has newly become an essential reaction in this area of research, while Brønsted acids have been found to be especially effective catalysts. Johnston et al. have investigated several aspects of the chiral Brønsted acid-catalyzed nitro-Mannich reactions of Boc-imines and nitromethane. At about the same time, using various nitroalkanes to aromatic N-Boc imines, Jacobsen et. al. reported thiourea-catalyzed nitro-Mannich reactions. These reactions ultimately form β-nitro amines in excellent yields and in high enantioselectivity.

Our group is actively working with strong Brønsted acid-catalyzed organic reactions in the synthesis of important building blocks of biologically active compounds. In order to expand the scope of strong Brønsted acid-catalyzed reactions in organic chemistry; we investigated nitro-Mannich reactions using Boc-imine with nitromethane in the presence of fluoroboric acid (HBF$_4$•OEt$_2$). Surprisingly, nitromethane did not participate in the reaction and no formation of β-nitro amine product was observed. Instead, we observed the formation of 1,3-oxazinan-2-one in high yield (equation 3.85).
Herein, we report on an unprecedented catalytic tandem elimination-cycloaddition reaction in which the formation of oxazinanones occurs when employing various Boc-imines in the presence of a strong Brønsted acid.

**Equation 3.85:** Reaction of 1,3-oxazinan-2-one instead of Aza henry product

![Chemical Structure](image)

The major difference in our reaction conditions compared to the aforementioned nitro-Mannich reactions is the use of fluoroboric acid. In order to determine whether fluoroboric acid is exclusively unique, we screened several Brønsted acids (Table 1). Several attempts, including a control reaction and reactions employing weaker acids such as acetic acid or triflyl amide, did not yield any oxazinanone product. However, by employing stronger acids such as trifluoroacetic acid, oxazinanone product was efficiently produced in yields of 40% (**Table 3.5**, entries 4-5). Indeed, Brønsted acids with lower $pK_a$s provided even greater yields of oxazinanone product. Ultimately, the stronger triflic and fluoroboric acids were found to be the most effective catalysts for the reaction as shown in Table 1. A 10 mol% catalyst loading was also found to be most effective; however, an increase in the catalyst loading does not improve the yield.
In order to determine the scope of this unprecedented reaction, a number of various Boc-imines were subjected to the newly optimized reaction conditions. Both aromatic and aliphatic Boc-imine provided the corresponding oxazinanones in good yields. However, aromatic imines (Table 3.5, entries 1-8) were found to be more reactive in these cyclization reactions, resulting in high yields of oxazinanones (65-75%). Electron donating or electron withdrawing groups did not appreciably affect the yields of the products. An aliphatic N-Boc-imine (Table 3.5, entry 9) could also be used in these reactions with a moderate yield of 50%.

Table 3.5. Optimization of the solvent system.

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dichloromethane</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>THF</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>acetonitrile</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>DMF</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>toluene</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>75% toluene in dichloromethane</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>methanol</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>nitromethane</td>
<td>70</td>
</tr>
</tbody>
</table>

To understand the mechanism of the reaction, we investigated the reaction by NMR spectroscopy. $^1$H NMR revealed the presence of 2-methylpropene (Figure 1) in the reaction mixture which is likely formed from the decomposition of the protonated N-Boc imine 3 (Scheme 2). The resulting 2-methylpropene reacts with Boc-imine in the presence of acid, followed by cyclization to form the oxazinanone product 2. Evidence of this process is also supported directly by observation using NMR. Indeed, the formation of the phenylmethaniminium ion (5,
Figure 3.11) in the reaction mixture is believed to result from the rapid decarboxylation of 4. Several other intermittent species are observed by low temperature NMR, the exact nature of which is still under investigation.

Table 3.6. Optimized HBF₄·OEt₂-catalyzed reactions of Boc-imine

<table>
<thead>
<tr>
<th>entry</th>
<th>imine</th>
<th>product</th>
<th>Yield[a] (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Imine 1" /></td>
<td><img src="image2.png" alt="Product 1" /></td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td><img src="image3.png" alt="Imine 2" /></td>
<td><img src="image4.png" alt="Product 2" /></td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td><img src="image5.png" alt="Imine 3" /></td>
<td><img src="image6.png" alt="Product 3" /></td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td><img src="image7.png" alt="Imine 4" /></td>
<td><img src="image8.png" alt="Product 4" /></td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>![Chemical Structure 5]</td>
<td>![Chemical Structure 6]</td>
<td>72</td>
</tr>
<tr>
<td>---</td>
<td>------------------------</td>
<td>------------------------</td>
<td>----</td>
</tr>
<tr>
<td>5</td>
<td>![Chemical Structure 7]</td>
<td>![Chemical Structure 8]</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>![Chemical Structure 9]</td>
<td>![Chemical Structure 10]</td>
<td>74</td>
</tr>
<tr>
<td>7</td>
<td>![Chemical Structure 11]</td>
<td>![Chemical Structure 12]</td>
<td>50</td>
</tr>
</tbody>
</table>

[a] Isolated yields, the average of at least two runs. In a typical reaction, the N-Boc-imines (1.5 mmol) were dissolved in 5 ml CH$_2$Cl$_2$ under a N$_2$ atmosphere and stirred 2-3h at room temperature with 10 mol% of the Brønsted acid such as fluoroboric acid or triflic acid. Acid was removed from the reaction mixture by passing the solution through a plug of neutral alumina. The solvent was removed under reduced pressure and the pure oxazinanone was recrystallized from 5-10% CH$_2$Cl$_2$ in pentane.
Figure 3.11. $^1$H NMR ($\delta=1.77$ and 4.71ppm) of 2-methylpropene and phenylmethaniminium ion ($\delta=8.55$ and 11.9ppm) in reaction mixture.
Scheme 3.3. Proposed mechanism

In order to substantiate the formation of 2-methylpropene as an intermediate, the reaction was carried out by adding excess 2-methylpropene. The yield of oxazinanone product 2 was improved up to 95%, corroborating our proposed mechanism. In addition, to verify the incorporation of added 2-methylpropene in the product, a reaction was conducted with fresh Boc-imine and 2-methylpropene-1,1-d$_2$ in the presence of fluoroboric acid. The results were just as expected, 82% of the product was incorporated with deuterium (Scheme 3.3) confirming our assumptions of 2-methylpropene in the reaction.
Scheme 3.4. HBF₄·OEt₂ reaction of Boc-imine and 2-methylpropene-1,1-d₂.

Figure 3.12. ¹H NMR (δ=1.77 and 4.71ppm) and ²H NMR (δ=1.77 and 4.71ppm) of 2-methylpropene and of 4-phenyl-6-aryl-1,3-oxazinan-2-one-5,5-d₂ (δ=8.55 and 11.9ppm) in a mixture of 18% to 82% respectively.
To further understand the mechanism of the reaction, a competitive experiment was carried out in the presence of 1.2 equiv of styrene. In this situation, both alkenes, 2-methylpropene from 1 and styrene, competed with fresh Boc-imine to form the corresponding oxazinanone product. The reaction provided 4,6-diphenyl-(1,3)-oxazinan-2-one 6 along with 4-Phenyl-6,6-dimethyl-(1,3)-oxazinan-2-one, 2 (Scheme 3.4), which strongly supports the proposed mechanism.

Recently, a similar mechanism was proposed for the formation of selective oxazinanone ring systems using triflic acid-catalyzed reactions of vinylidene cyclopropanes with Boc-imines.8

Scheme 3.5 HBF$_4$·OEt$_2$ reaction of Boc-imine and styrene.

This intermolecular reaction facilitated the generation of oxazinanones simply by Boc-imines reacting with different styrene derivatives. More exactly, several styrene derivatives were reacted with Boc-imines to form the corresponding oxazinanones in good yield (Table 4). Interestingly, α-methylstyrene (Table 4, entry 4) produced an oxazinanone possessed of a fairly congested tertiary carbon center with the highest yield of all. As shown in the earlier proposed mechanism (scheme 3.3), the high yield of this product is accounted for by the increased relative stability of the tertiary benzylic carbocation. This latter result, concerning tolerance of steric congestion and the lack of discrimination concerning styrenes containing either EWG or EDG groups (table 3.7, entries 2 and 3), further exemplifies the robustness and versatility of this reaction. In 1990, Overman reported a closely related reaction which involved intramolecular
Boc-iminium ion-alkyne cycloaddition to form tricyclic systems containing the oxazinanone ring system.\(^9\)

In all these intermolecular reactions, we observed formation of only the syn-diastereomer. The configuration of the product was determined by comparing the spin-spin coupling constants with known reported syn-compounds.\(^{10}\) The six-membered transition state A, where both bulky aryl groups are equatorial positions (syn) will be favorable over transition state B with one aryl group at an axial position (Scheme 3.6). As a result, syn-4,6-diaryloxazinanone 6 is expected to be the only or a major product.

**Scheme 3.6.** Proposed rationale for the stereoselective formation of 4,6-Diphenyloxazinanone.

To our knowledge, the reaction depicted in Scheme 1 is the first recorded tandem elimination-cycloaddition reaction for the synthesis of an oxazinanone ring system. To date, all of the reported syntheses for making the oxazinanones 2 require three or more steps.\(^{11}\) The chiral oxazinanones are employed as auxiliaries for asymmetric enolate alkylation and aldol
reactions,$^{11b}$ and also in the synthesis of oxazolidinone derivatives.$^{12}$ Oxazinanone derivatives are of interest because they exhibit antibacterial activity against Gram-positive bacteria and are useful as PR modulators.$^{13}$ Chiral Brønsted acid-catalyzed syntheses of optically active analogs of oxazinanones are currently under investigation.

Table 3.7  HBF$_4$.OEt$_2$-catalyzed reactions of Boc-imine and styrene derivatives.

<table>
<thead>
<tr>
<th>entry</th>
<th>styrene</th>
<th>product</th>
<th>yield$^a$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="styrene" /></td>
<td><img src="image2" alt="product" /></td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td><img src="image3" alt="styrene" /></td>
<td><img src="image4" alt="product" /></td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="styrene" /></td>
<td><img src="image6" alt="product" /></td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td><img src="image7" alt="styrene" /></td>
<td><img src="image8" alt="product" /></td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td><img src="image9" alt="styrene" /></td>
<td><img src="image10" alt="product" /></td>
<td>68</td>
</tr>
</tbody>
</table>
Isolated yields, the average of at least two runs. In a typical reaction, the N-Boc-imines (1.3 mmol) and styrene derivative (1.6 mmol) were dissolved in 5 ml CH₂Cl₂ under a N₂ atmosphere and stirred 2-3h at room temperature with 10 mol% fluorobric acid. Acid was removed from the reaction mixture by passing the solution through a plug of neutral alumina. The solvent was removed under reduced pressure and the pure oxazinanone was recrystallized from 10% CH₂Cl₂ in pentane.

In summary, a new carbon-carbon bond formation reaction has been developed using Brønsted acid catalysis. Cyclic oxazinanones are produced from Boc-imines in one step, resulting in medium to high yields. The atom-economic process presented here in Scheme 1 is unprecedented in the area of organic chemistry and should extend the scope of C-C bond forming reactions.
3.6 Experimental

General considerations: All oxazinanones syntheses were carried out under standard Schlenk techniques. All of the glass flasks were dried in the oven. Proton and carbon spectra were obtained on 300 MHz NMR spectrometer unless stated otherwise. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane and CDCl$_3$ was used as the solvent. Previously reported compounds were identified by $^1$H NMR. All new compounds were additionally characterized by $^{13}$C NMR and elemental analysis.

HPLC reagent grade CH$_2$Cl$_2$ was distilled under nitrogen from P$_2$O$_5$. Technical grade pentane was stirred with sulfuric acid overnight and then washed with saturated NaHCO$_3$ and stored over Na$_2$SO$_4$. The pentane was then distilled under nitrogen from sodium. Reagent garde diethyl ether and THF were freshly distilled under a nitrogen atmosphere from sodium benzophenone ketyl. HPLC grade methanol was distilled under nitrogen from magnesium iodide. Boc-imines were freshly prepared based on the adaptation of reported procedures$^1$. All other reagents were used as supplied.
**General Procedure for the Synthesis of 4-Aryl-6,6-dimethyl-(1,3)-oxazinan-2-one**: For each experiment described in *Scheme 1*, 1.0-5.0 mmol of the Boc-protected imine was dissolved in 5-25 ml of freshly distilled dichloromethane under nitrogen. A 0.1 equiv sample of the appropriate catalyst was added and then the reaction mixture was stirred. The reaction mixture was allowed to stir until the completion, monitored by NMR spectroscopy. The reaction mixture was then filtered through an alumina plug and the solvent removed by rotary evaporation. Product was crystallized from 5-10% dichloromethane in pentane and identified by comparing spectra to known $^1$H NMR. $^1$H, $^{13}$C NMR, High resolution Mass spectra and elemental analysis were applied to characterize the new compounds.

![Chemical structure](image)

**General Procedure for the Synthesis of 4-phenyl-6-aryl-1,3-oxazinan-2-one**: For each experiment described in *Scheme 2*, 1.0-5.0 mmol of the Boc-protected imine was dissolved in 5-25 ml of freshly distilled dichloromethane under nitrogen. A 0.1 equiv sample of the appropriate catalyst was added followed by 1.2 equiv of respective alkenes and then the reaction mixture was stirred. The reaction mixture was allowed to stir until the completion, monitored by NMR spectroscopy. The reaction mixture was then filtered through an alumina plug and the solvent removed by rotary evaporation. Desired product was then crystallized from 20% dichloromethane in pentane and identified by comparing spectra to known $^1$H NMR. $^1$H, $^{13}$C NMR and High resolution Mass spectra were applied to characterize the new compounds.
**General Procedure for the Synthesis of 4-phenyl-6-aryl-1,3-oxazinan-2-one-5,5-d$_2$:** 1.0-5.0 mmol of the Boc-protected imine was dissolved in 5-25 ml of freshly distilled dichloromethane under nitrogen. A 0.1 equiv sample of the appropriate catalyst was added followed by excess of methylpropene-1,1-d$_2$ and then the reaction mixture was stirred. The reaction mixture was allowed to stir until the completion, monitored by TLC. The reaction mixture was then filtered through an alumina plug and the solvent removed by rotary evaporation. Desired product was then crystallized from 20% dichloromethane in pentane and identified by comparing spectra to known $^1$H NMR. $^1$H, $^{13}$C NMR and High resolution Mass spectra were applied to characterize the new compounds.
Characterization data for products shown in Table 3

4-Phenyl-6,6-dimethyl-(1,3)-oxazinan-2-one (Table 3, entry 1).

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.5 mmol), HBF₄.OEt₂ (200 µL, 0.15 mmol) and CH₂Cl₂ (5 mL) were used to obtained the product (225 mg, 75% yield) as a yellow solid, m. p. 155-156 °C. Crystallization solvent was 5% CH₂Cl₂ in pentane.

¹H NMR (300 MHz, CDCl₃): δ 1.45 (s, 3H), 1.53 (s, 3H), 1.85 (t, 1H, J= 13.7,11.9 Hz), 2.03 (dd, 1H, J= 13.8, 4.8 Hz), 4.62 (dd, 1H, J= 11.8,4.7 Hz), 6.25 (s, br, 1H, NH), 7.30-7.45 (m, 5H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 25.6, 29.4, 42.0, 53.0, 78.5, 126.1, 128.5, 129.1, 140.9, and 154.0.

HRMS (ESI) (M + H)+, Calcd. for C₁₂H₁₅NO₂ 206.1181; Found 206.1175.
4-methoxyphenyl-6,6-dimethyl-(1,3)-oxazin-2-one (Table 3, entry 2). The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), HBF$_4$.OEt$_2$ (175 µL, 0.13 mmol) and CH$_2$Cl$_2$ (5 ML) were used to obtained the product (210 mg, 70% yield) as a yellow solid, m. p. 146-147 °C. Crystallization solvent was 5% CH$_2$Cl$_2$ in pentane.

$^1$H NMR (300 MHz, CDCl$_3$): δ 1.52 (s, 3H), 1.67 (s, 3H), 1.85 (t, 1H, $J$=11.8 Hz), 2.02 (dd, 1H, $J$= 13.8, 4.7 Hz), 3.83 (s,3H), 4.58 (dd, 1H, $J$= 11.8,4.7 Hz), 5.31 (s, br, 1H, NH),6.94 (d,2H, Ph),7.25-7.29 (d, 2H, Ph).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 25.2, 29.4, 42.0, 52.4, 55.2, 78.4, 114.3, 127.3, 132.7, 153.6, and 159.6.

HRMS (ESI) (M + H)$^+$, Calcd. for C$_{13}$H$_{17}$NO$_3$ 236.1287; Found 236.1281.
4-Chlorophenyl-6,6-dimethyl-(1,3)-oxazinan-2-one (Table 3, entry 3).

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), HBF₄.OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (5 mL) were used to obtained the product (225 mg, 75% yield) as a white solid, m. p. 170-171 °C. Crystallization solvent was 5-10% CH₂Cl₂ in pentane.

¹H NMR (300 MHz,CDCl₃): δ 1.45 (s, 3H), 1.52 (s, 3H), 1.80 (t, 1H, J=13.8, 11.8 Hz), 2.00 (dd, 1H, J= 13.8, 4.7 Hz), 4.61 (dd, 1H, J= 11.8,4.7 Hz), 5.90 (s, br, 1H, NH), 7.27-7.30 (d, 2H, Ph) 7.36-7.39 (d, 2H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 25.2, 29.3, 41.9, 52.4, 78.4, 127.4, 129.2, 139.3, and 153.9.

HRMS (ESI) (M + H)⁺, Calcd. for C₁₃H₁₇NO₃ 236.1287; Found 236.1294.

4-Fluorophenyl-6,6-dimethyl-(1,3)-oxazinan-2-one (Table 3, entry 4).

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), HBF₄.OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (5 mL) were used to obtained the product (225 mg, 75% yield) as a yellow solid, m. p. 125-126 °C. Crystallization solvent was 5% CH₂Cl₂ in pentane.

¹H NMR(300 MHz,CDCl₃): δ 1.45 (s, 3H), 1.52 (s, 3H), 1.80 (t, 1H, J=13.8,12.0 Hz), 2.00 (dd, 1H, J= 13.8, 4.7 Hz), 4.60 (dd, 1H, J= 12.0,4.7 Hz), 5.85 (s, br, 1H, NH), 7.05-7.11 (t, 2H, Ph) 7.28-7.35 (m, 2H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 25.2, 29.3, 42.0, 52.3, 78.4, 115.8, 116.0, 127.7, 127.8, 128.2, 136.5, 153.7.

HRMS (ESI) (M + H)⁺, Calcd. for C₁₂H₁₄FNO₂ 224.1087; Found 224.1080.
4-Methylphenyl-6,6-dimethyl-(1,3)-oxazinan-2-one (Table 3, entry 5).

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.4 mmol), HBF₄.OEt₂ (190 µL, 0.14 mmol) and CH₂Cl₂ (5 mL) were used to obtained the product (215 mg, 72% yield) as a white solid, m. p. 132-133 °C. Crystallization solvent was 5% CH₂Cl₂ in pentane.

¹H NMR(300 MHz,CDCl₃): δ 1.42 (s, 3H), 1.50 (s, 3H), 1.80 (t, 1H, J=13.7, 11.8 Hz), 2.00 (dd, 1H, J = 13.8, 4.7 Hz), 2.35 (s,3H), 4.60 (dd, 1H, J = 11.8,4.7 Hz), 5.92 (s, br, 1H, NH), 7.2-7.3 (m, 4H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 21, 25.2, 29.3, 42.0, 52.6, 78.3, 126.4, 129.5, 137.8, 138.5, 153.9.

HRMS (ESI) (M + H)+, Calcd. for C₁₃H₁₇NO₂ 220.1338; Found 220.1331.

4-Ethylphenyl-6,6-dimethyl-(1,3)-oxazinan-2-one (Table 3, entry 6).

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), HBF₄.OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (5 mL) were used to obtained the product (215 mg, 72% yield) as a yellow solid, m. p. 145-147 °C. Crystallization solvent was 5% CH₂Cl₂ in pentane.

¹H NMR(300 MHz,CDCl₃): δ 1.25 (t, 3H, J = 7.6 Hz), 1.46 (s, 3H), 1.51 (s, 3H), 1.82 (t, 1H, J=13.7, 11.8 Hz), 2.00 (dd, 1H, J = 13.8, 4.7 Hz), 2.65 (q, 2H, J=7.6 Hz), 4.60 (dd, 1H, J = 11.7,4.7 Hz), 5.72 (s, br, 1H, NH), 7.2-7.3 (m, 4H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 15.4, 25.2, 28.4, 29.4, 42.0, 52.6, 78.4, 126.0, 128.4, 138.0, 144.5, 153.9.

HRMS (ESI) (M + H)+, Calcd. for C₁₄H₁₉NO₂ 234.1494; Found 234.1490.
4-isopropylphenyl-6,6-dimethyl-(1,3)-oxazinan-2-one (Table 3, entry 7).

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.2 mmol), HBF₄·OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (5 mL) were used to obtained the product (210 mg, 70% yield) as a yellow solid, m. p. 185-187 °C. Crystallization solvent was 5% CH₂Cl₂ in pentane.

¹H NMR (300 MHz, CDCl₃): δ 1.25 (d, 6H, J = 6.9 Hz), 1.45 (s, 3H), 1.53 ( s, 3H), 1.85 (t, 1H, J=12 Hz), 2.00 (ddd, 1H, J= 13.8, 4.7 Hz, 1.5 Hz), 2.92 (m, 1H, J= 6.9 Hz), 4.60 (dd, 1H, J= 12,4.8 Hz), 5.42 (s, br, 1H, NH), 7.2-7.3 (m, 4H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 23.8, 25.2, 29.3, 33.7, 42.0, 52.7, 78.4, 81.6, 126.0, 127.0, 138.1, 149.2, 153.7.

HRMS (ESI) (M + H)⁺, Calcd. for C₁₅H₂₁NO₂ 248.1651; Found 248.1659.

4-(3-methoxyphenyl)-6,6-dimethyl-1,3-oxazinan-2-one (Table 3, entry 8).

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), HBF₄·OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (5 mL) were used to obtained the product (223 mg, 74% yield) as a yellow-white solid, m. p. 157-158 °C. Crystallization solvent was 5% CH₂Cl₂ in pentane.

¹H NMR (300 MHz, CDCl₃): δ 1.44 (s, 3H), 1.51 ( s, 3H), 1.83 (t, 1H, J=12 Hz), 2.03 (ddd, 1H, J= 13.8, 4.7 Hz, 1.5 Hz), 3.82 (s, 3H), 4.60 (dd, 1H, J= 12,4.8 Hz), 5.67 (s, br, 1H, NH), 6.85-6.92 (m, 3H, Ph) 7.26-7.32 (m, 1H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 25.2, 29.3, 41.8, 52.9, 55.2, 78.4, 111.5, 113.8, 118.2, 130.0, 142.5, 153.8, 160.0.
HRMS (ESI) (M + H)$^+$, Calcd. for $\text{C}_{13}\text{H}_{17}\text{NO}_3$ 236.1287; Found 236.1294

4-cyclohexyl-6,6-dimethyl-1,3-oxazinan-2-one (Table 3, entry 9): The general procedure was followed (2-3 hrs). Boc-imine (Table 3, entry 9) (280 mg, 1.3 mmol), HBF$_4$.OEt$_2$ (175 µL, 0.13 mmol) and CH$_2$Cl$_2$ (5 mL) were used to obtained the product (142 mg, 50% yield) as a white solid, m. p. 136-137 °C. Crystallization solvent was 5% CH$_2$Cl$_2$ in pentane.

$^1$H NMR (300 MHz, CDCl$_3$): δ 1.00 (m, 2H), 1.13 (m, 3H), 1.39 (s, 3H), 1.42 (s, 3H), 1.63 (m, 3H), 1.65 (m, 5H), 3.30 (m, 1H), 5.17 (s, 1H, NH).

$^{13}$C NMR (300 MHz, CDCl$_3$): δ 24.9, 25.8, 26.2, 27.7, 28.4, 29.6, 35.1, 42.1, 52.6, 77.8, and 154.6.

HRMS (ESI) (M + H)$^+$, Calcd. for $\text{C}_{12}\text{H}_{21}\text{NO}_2$ 212.1651; Found 212.165
4,6-diphenyl-1,3-oxazinan-2-one (Table 4, entry 1):

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), styrene (166 mg, 1.6 mmol), HBF$_4$·OEt$_2$ (175 µL, 0.13 mmol) and CH$_2$Cl$_2$ (10 mL) were used to obtained the product (237 mg, 72% yield) as a white solid, m. p. 198-199 °C. Crystallization solvent was 10% CH$_2$Cl$_2$ in pentane.

$^1$H NMR (300 MHz, CDCl$_3$): δ 2.06 (ddd, 1H, \( J= 14.4, 11.9, 11.6 \) Hz), 2.42 (dddd, 1H, \( J= 14.1, 4.2, 2.1, 1.8 \) Hz), 4.80 (dd, 1H, \( J= 11.5, 4.5 \) Hz), 5.40 (dd, 1H, \( J= 11.7, 2.0 \) Hz), 5.52 (s, br, 1H, NH), 7.32-7.45 (m, 10H, Ph).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 39.1, 55.8, 78.4, 125.7, 126.0, 128.5, 128.6, 129.1, 138.4, 140.4, 153.8.

HRMS (ESI) (M + H)$^+$, Calcd. for C$_{16}$H$_{15}$NO$_2$ 254.1181; Found 254.1177.
6-(4-chlorophenyl)-4-phenyl-1,3-oxazinan-2-one (Table 4, entry 2):

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), 1-chloro-4-vinylbenzene (221 mg, 1.6 mmol), HBF₄·OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (10 mL) were used to obtain the product (254 mg, 70% yield) as a white solid, m. p. 194-195 °C. Crystallization solvent was 10% CH₂Cl₂ in pentane.

\(^1\)H NMR (300 MHz, CDCl₃): δ 2.1 (ddd, 1H, J= 13.9, 11.9, 11.6 Hz), 2.4 (dddd, 1H, J= 14.1, 4.5, 2.4, 2.1 Hz), 4.80 (dd, 1H, J= 11.5, 4.5 Hz), 5.31 (s, br, 1H, NH), 5.44 (dd, 1H, J= 11.7, 2.0 Hz), 7.35-7.45 (m, 9H, Ph).

\(^13\)C NMR (75 MHz, CDCl₃): δ 39.1, 55.7, 77.7, 125.9, 127.1, 128.8, 128.6, 129.1, 136.9, 140.1, 153.5.

HRMS (ESI) (M + H)^+, Calcd. for C₁₆H₁₄ClNO₂ 280.0791; Found 280.0782.

4-phenyl-6-p-tolyl-1,3-oxazinan-2-one (Table 4, entry 3):

The general procedure was followed (2-3 hrs). Boc-imine 1 (300 mg, 1.3 mmol), 1-methyl-4-vinylbenzene (190 mg, 1.6 mmol), HBF₄·OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (10 mL) were used to obtain the product (245 mg, 70% yield) as a white solid, m. p. 182-183 °C. Crystallization solvent was 10% CH₂Cl₂ in pentane.

\(^1\)H NMR (300 MHz, CDCl₃): δ 2.1 (ddd, 1H, J= 14.0, 11.8, 11.6 Hz), 2.36 (s, 3H), 2.41 (ddddd, 1H, J= 4.5, 2.4, 2.1 Hz, some peaks overlap with -CH₃ absorption), 4.78 (dd, 1H, J= 11.5, 4.5 Hz), 5.40 (s, br, 1H, NH), 5.45 (m, 1H), 7.2-7.4 (m, 9H, Ph).

\(^13\)C NMR (75 MHz, CDCl₃): δ 21.0, 39.1, 55.8, 78.4, 125.3, 125.7, 126.0, 128.6, 129.1, 129.2, 135.4, 138.3, 140.5, 153.9.
HRMS (ESI) (M + H)$^+$, Calcd. for $\text{C}_{17}\text{H}_{17}\text{NO}_2$ 268.1338; Found 268.1343.

6-methyl-4,6-diphenyl-1,3-oxazinan-2-one (Table 4, entry 4):

The general procedure was followed (2-3 hrs). Boc-imine 1 (300 mg, 1.3 mmol), 1-(prop-1-en-2-yl)benzene (190 mg, 1.6 mmol), HBF$_4$.OEt$_2$ (175 µL, 0.13 mmol) and CH$_2$Cl$_2$ (10 mL) were used to obtain the product (243 mg, 75% yield) as a white solid, m. p. 198-199 °C. Crystallization solvent was 10% CH$_2$Cl$_2$ in pentane.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.87 (s, 3H), 2.1 (t, 1H, $J$= 13.8, 11.7 Hz), 2.41 (dd, 1H, $J$= 13.9, 3.8 Hz), 4.78 (dd, 1H, $J$= 11.4, 4.7 Hz), 5.37 (s, br, 1H, NH), 7.3-7.5 (m, 10H, Ph).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 26.1, 42.6, 53.1, 81.2, 123.9, 126.1, 127.5, 128.3, 128.4, 129.0, 140.4, 153.4.

HRMS (ESI) (M + H)$^+$, Calcd. for $\text{C}_{17}\text{H}_{17}\text{NO}_2$ 268.1338; Found 268.1335.
6-(naphthalen-1-yl)-4-phenyl-1, 3-oxazinan-2-one (Table 4, entry 5):

The general procedure was followed (2-3 hrs). Boc-imine (300 mg, 1.3 mmol), 2-vinylnaphthalene (246 mg, 1.6 mmol), HBF₄.OEt₂ (175 µL, 0.13 mmol) and CH₂Cl₂ (10 mL) were used to obtained the product (269 mg, 68% yield) as a yellow solid, m. p. 217-218 °C. Crystallization solvent was 10% CH₂Cl₂ in pentane.

¹H NMR (300 MHz, CDCl₃): δ 2.15 (ddd, 1H, J= 14.0, 11.9, 11.6 Hz), 2.50 (dddd, 1H, J= 13.8, 4.5, 2.1, 1.8 Hz ), 4.84 (dd, 1H, J= 11.5,4.5 Hz), 5.39 (s, br, 1H, NH), 5.63 (dd, 1H, J= 11.7, 1.7 Hz), 7.3-7.5 (m, 10H, Ph).

¹³C NMR (75 MHz, CDCl₃): δ 39.1, 55.8, 78.5, 123.3, 124.8, 126.0, 126.3, 127.6, 128.0, 128.5, 128.5, 129.1, 133.2, 135.7, 140.3, 153.9.

HRMS (ESI) (M + H)⁺, Calcd. for C₂₀H₁₇NO₂ 304.1338; Found 304.1346.
3.7 References


3. For reviews on organocatalysis


Appendix
Appendix A

Part I: Synthesis and Biological Evaluations of Potent Class I Selective Histone Deacetylase inhibitors

\[ \text{STr} \]

\[ \text{O} - \text{C=C} - \text{STr} \]

1\text{H}: ju112211 Exp 1. 500 user: ju
BS110111 Exp 1. 300 user: nmr

\[ \text{t-BuO} \text{CH} - \text{CH} - \text{CH} - \text{CH}_2 - \text{S} \text{Trt} \]

$^1$H: ju010411 Exp 2. 300 user: nmr

$^{13}$C: BS061812 Exp 5. 300 user: BS
$^1$H: BS072312 Exp 5. 300 user: BS

$^{13}$C: ju072412 Exp 6. 500 user: ju
$^{1}H$: ju062012 Exp 1. 500 user: ju
extra$^{1}H$: ju062112 Exp. 1 500 user: ju
$^{13}C$: ju062112 Exp 2. 500 user: ju
\[ \left( \text{t-BuO}^- \right) \text{C} = \text{O} \xrightarrow{\text{C} = \text{C}} \text{S} \right) _2 \]

\[ ^{1}H: \text{BS062712 Exp 2. 300 user: BS} \]

\[ ^{13}C: \text{BS062712 Exp 4. 300 user: BS} \]
$^{1}H$: ju072412 Exp 8. 500 user: ju

$^{13}C$: ju072412 Exp 12. 500 user: ju
\[ ^1H: ju012712 \text{ Exp. 2. } 300 \text{ user: nmr} \]

\[ ^{13}C: ju072412 \text{ Exp. 4. } 500 \text{ user: ju} \]
$^{1}H$: djm070312 Exp 4. 300 user: djm

$^{1}H$: djm060112 Exp 3. 300 user: djm

$^{13}C$: ju071112 Exp 5. 500 user: ju

$^{13}C$: djm060112 Exp 4. 300 user: djm
\[^1\text{H}: \text{dj}m070312 \text{ Exp} 9. \ 300 \text{ user: djm}\]

\[^{13}\text{C}: \text{dj}m070312 \text{ Exp} 10. \ 300 \text{ user: djm}\]
HSQC: $^1H/^13C$

COSY: $^1H/^1H$
\[ ^1H: BS073112 \text{ Exp 4. } 300 \text{ user: BS} \]

\[ ^{13}C: BS073112 \text{ Exp 5. } 300 \text{ user: BS} \]

HSQC: BS073112 Exp 11. 300 user: BS

COSY: BS073112 Exp 6. 300 user: BS

\[ ^{13}C \text{ dept-135 BS080112 Exp 1. } 300 \text{ user: BS} \]

\[ ^{13}C \text{ dept-135 BS080112 Exp 2. } 300 \text{ user: BS} \]
HSQC: $^1\text{H}/^{13}\text{C}$

COSY: $^1\text{H}/^1\text{H}$
$^{13}$C: Dep-135

$^{13}$C: Dep-90
$^1$H: ju081512 Exp 5. 500 user: ju

$^{13}$C: ju081512 Exp 8. 500 user: ju

COSY: ju081512 Exp. 6 500 user: ju

$^{13}$C Dept-135: ju081512 Exp 9. 500 user: ju

$^{13}$C Dept-90: ju081512 Exp 10. 500 user: ju

HSQCGP: ju081512 Exp 7. 500 user: ju
\(^1\)H: ju091312 Exp 1. 300 user: ju
COSY: ju091312 Exp 2. 300 user: ju
\(^{13}\)C: BS122912 Exp 6. 300 user: BS
\(^{13}\)C Dept-135: ju091312 Exp 7. 300 user: ju
\(^{13}\)C Dept-90: ju011713 Exp 1. 300 user: ju
1.) HSQCGP: ju091312 Exp 3. 300 user: ju
2.) Extra: HSQCGP: BS010313-2 Exp 3. 300 user: BS
\[ \text{O} \quad \text{O} \quad \text{OH} \quad \text{STrt} \]

\[ ^1\text{H}: \text{ju011712 Exp 3. 300 user: nmr} \]

\[ ^1\text{C}: \text{ju011812 Exp 2. 300 user: nmr} \]
^H: djm061912 Exp 1.  300 user: djm

^13C: BS013112 Exp 2.  300 user: BS
$^{1}H: \text{BS062612 Exp 2. 300 user: BS}$

$^{13}C: \text{ju062012 Exp 1. 300 user: ju}$
$^{1}H$: BS101512 Exp 1. 300 user: BS

$^{13}C$: BS101512 Exp 2. 300 user: BS
$^1\text{H}: \text{BS0127122 Exp 1. 300 user: BS}$

$^{13}\text{C}: \text{ju012712 Exp 3. 300 user: nmr}$
$\text{H: BS013112 Exp 3. 300 user: BS}$

$\text{\textsuperscript{13}C: BS013112 Exp 4. 300 user: BS}$
$^{1}H$: ju062012 Exp 4. 500 user: ju

$^{13}C$: BS062612 Exp 4. 300 user: BS
$^{1}H$: BS101512 Exp. 3 300 user: BS

$^{13}C$: BS101512 Exp. 4 300 user: BS
$^{1}H$: ju021712 Exp 1. 300 user: nmr

$^{13}C$: ju021712 Exp 2. 300 user: nmr

\[
\begin{align*}
\text{Structure Image} \\
\text{NMR Spectra Images}
\end{align*}
\]
\[ ^1\text{H}:\text{djmo61912 Exp 2. 300 user: djm} \]
\[ ^13\text{C}:\text{ju022012 Exp 2. 300 user: nmr} \]
$^{1}H: \text{ju062112 Exp 1. 300 user: ju}$

$^{13}C: \text{ju062112 Exp 2. 300 user: ju}$
$\left(\text{O}^2\text{C}\text{H}2\text{N}\text{O}2\text{S}\right)_2$

$^{1}H$: ju101412 Exp 1. 300 user: ju

$^{13}C$: ju101412 Exp 2. 300 user: ju

$^{13}C$ Dept-135: ju101412 Exp 3. 300 user: ju
\[ \text{\textsuperscript{1}H: ju072312 Exp 2.  300 user: ju} \]
\[ \text{\textsuperscript{13}C: ju072412 Exp 7.  500 user: ju} \]

\[ \text{\textsuperscript{1}H: djm091312 Exp 1.  300 user: djm} \]
\[ \text{\textsuperscript{13}C: djm091312 Exp 4.  300 user: djm} \]
$\text{O}$

$\text{O}$

$\text{NH}_3\text{Cl}^-$

$^1\text{H}: \text{ju091412 Exp 1} \quad 300 \text{ user: ju}$

$^{13}\text{C}: \text{ju091412 Exp 2.} \quad 300 \text{ user: ju}$
$^1$H: djm072412 Exp 1. 300 user: djm

$^{13}$C: ju072412 Exp. 14 500 user: ju
$^{1}H$: ju072312 Exp 1. 300 user: ju
TDPA-18
TDPA-18
TDPA-20
TDPA-20

[Diagram of a chemical structure with peaks in a spectrum chart]

ppm (δ)
MS (MALDI) Exact Mass Calc for C_{15}H_{24}N_{2}O_{5}S_{3} (M-1)^-: 407.08, Found: 407.05

MS (MALDI) Exact Mass Calc for C_{15}H_{24}N_{2}O_{5}S_{3} (M+Na)^+: 431.78, Found: 431.07
MS (MALDI) Exact Mass Calc for C_{26}H_{44}N_{2}O_{8}S_{2} (M-1)^-: 575.25, Found: 575.26

MS (MALDI) Exact Mass Calc for C_{26}H_{44}N_{2}O_{8}S_{2} (M+Na)^+: 599.23, Found: 599.23
MS (MALDI) Exact Mass Calc for $C_{28}H_{48}N_2O_8S_2 (M^·): 604.29$, Found: 604.02

4700 Reflector Spec #1 MC [BP = 620.0, 43305]
MS (MALDI) Exact Mass Calc for $\text{C}_{28}\text{H}_{48}\text{N}_{2}\text{O}_8\text{S}_2$ (M)$^- \cdot 604.29$, Found: 604.05
MS (MALDI) Exact Mass Calc for $\text{C}_{26}\text{H}_{44}\text{N}_{2}\text{O}_{8}\text{S}_{2} (\text{M+Na})^+ : 599.24$, Found: 599.23

MS (MALDI) Exact Mass Calc for $\text{C}_{26}\text{H}_{44}\text{N}_{2}\text{O}_{8}\text{S}_{2} (\text{M-1})^- : 575.25$, Found: 575.26
MS (MALDI) Exact Mass Calc for $\text{C}_{24}\text{H}_{40}\text{N}_{2}\text{O}_{8}\text{S}_{2}$ (M-1)$^{-}$: 547.22, Found: 547.23

MS (MALDI) Exact Mass Calc for $\text{C}_{24}\text{H}_{40}\text{N}_{2}\text{O}_{8}\text{S}_{2}$ (M+Na)$^{+}$: 571.22, Found: 571.21
MS (MALDI) Exact Mass Calc for C_{26}H_{44}N_{2}O_{8}S_{2} (M) 604.06, Found: 604.05
MS (MALDI) Exact Mass Calc for C_{24}H_{40}N_{2}O_{8}S_{4} (M+22.99)^{+}: 635.16, Found: 635.16

MS (MALDI) Exact Mass Calc for C_{24}H_{40}N_{2}O_{8}S_{4} (M-1)^{-}: 611.17, Found: 611.15
MS (MALDI) Exact Mass Calc for \( \text{C}_{26}\text{H}_{44}\text{N}_2\text{O}_{8}\text{S}_4 \) (M+22.99): 663.19, Found: 663.19

MS (MALDI) Exact Mass Calc for \( \text{C}_{26}\text{H}_{44}\text{N}_2\text{O}_{8}\text{S}_4 \) (M-1): 639.20, Found: 642.20
MS (MALDI) Exact Mass Calc for $\text{C}_{14}\text{H}_{22}\text{O}_{6}\text{S}_{2}$ (M-1): 349.08, Found: 349.07

MS (MALDI) Exact Mass Calc for $\text{C}_{14}\text{H}_{22}\text{O}_{6}\text{S}_{2}$ (M+Li)$^+$: 361.08, Found: 361.07
MS (MALDI) Exact Mass Calc for C\textsubscript{22}H\textsubscript{38}O\textsubscript{6}S\textsubscript{2} (M+Na): 485.19, Found: 485.18

\[ 4700 \text{ Reflector Spec #1 MC[BP = 379.1, 95526]} \]

MS (MALDI) Exact Mass Calc for C\textsubscript{22}H\textsubscript{38}O\textsubscript{6}S\textsubscript{2} (M-1): 461.21, Found: 460.13

\[ 4700 \text{ Reflector Spec #1 MC[BP = 333.1, 97569]} \]
MS (MALDI) Exact Mass Calc for C_{53}H_{54}N_{2}O_{5}S_{3} (M+Na)^+: 917.31, Found: 917.30

MS (MALDI) Exact Mass Calc for C_{53}H_{54}N_{2}O_{5}S_{3} (M-1)^−: 893.34, Found: 893.40
MS (MALDI) Exact Mass Calc for \( \text{C}_{54}\text{H}_{56}\text{N}_{2}\text{O}_{5}\text{S}_{3} \) (M+Na): 931.32, Found: 931.31

\[
\text{4700 Reflector Spec} \#1 \text{ MC⇒BC[BP = 931.3, 660]}
\]

MS (MALDI) Exact Mass Calc for \( \text{C}_{54}\text{H}_{56}\text{N}_{2}\text{O}_{5}\text{S}_{3} \) (M-1): 907.18, Found: 924.18

\( \text{M+H}_{2}\text{O = 924.18} \)
TDPA-18
TDPA-20
HPLC Data for separation

<table>
<thead>
<tr>
<th></th>
<th>FC condition</th>
<th>Rt-FC</th>
<th>HPLC condition</th>
<th>Rt-HPLC</th>
<th>Pure amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF1</td>
<td>0-20min 20-100%ACN</td>
<td>8-12min</td>
<td>40% ACN</td>
<td>12-14.5min</td>
<td>95mg</td>
</tr>
<tr>
<td></td>
<td>20ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF1t</td>
<td>15-20min</td>
<td></td>
<td>70% ACN</td>
<td>22-25 min</td>
<td>185mg</td>
</tr>
<tr>
<td>TDF2</td>
<td>8-12min</td>
<td></td>
<td>35% ACN</td>
<td>16-20min</td>
<td>25mg</td>
</tr>
<tr>
<td>TDF2m</td>
<td>12-15min</td>
<td></td>
<td>40% ACN</td>
<td>19-21min</td>
<td>185mg</td>
</tr>
<tr>
<td>TDF3</td>
<td>8-10min</td>
<td></td>
<td>35% ACN 8ml/min</td>
<td>10-13.5min</td>
<td>125mg</td>
</tr>
<tr>
<td>TDF3m</td>
<td>12-15min</td>
<td></td>
<td>40% ACN</td>
<td>17-19 min</td>
<td>158mg</td>
</tr>
<tr>
<td>TDF4</td>
<td>8-12min</td>
<td></td>
<td>40% ACN</td>
<td>15.5-21min</td>
<td>31mg</td>
</tr>
<tr>
<td>TDF4m</td>
<td>12-15min</td>
<td></td>
<td>50% ACN</td>
<td>25-31 min</td>
<td>87mg</td>
</tr>
<tr>
<td>TDF5</td>
<td>8-12min</td>
<td></td>
<td>40% ACN</td>
<td>17-24.5min</td>
<td>146mg</td>
</tr>
<tr>
<td>TDF5m</td>
<td>12-15min</td>
<td></td>
<td>50% ACN</td>
<td>27-32 min</td>
<td>130mg</td>
</tr>
<tr>
<td>TDF6</td>
<td>8-12min</td>
<td></td>
<td>35% ACN</td>
<td>13-18.5min</td>
<td>40mg</td>
</tr>
<tr>
<td>TDF6m</td>
<td>12-15min</td>
<td></td>
<td>50% ACN</td>
<td>12-17 min</td>
<td>90mg</td>
</tr>
</tbody>
</table>

TDF1: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to eluted the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 12-14.5min.
**TDF1t:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 15-20 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 70 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 22-25min.

**TDF2:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 16-20min.

**TDF2m:** The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 19-21min.
**TDF3**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H₂O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-10min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H₂O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 10-13.5min.

**TDF3m**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H₂O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H₂O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 17-19 min.

**TDF4**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H₂O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H₂O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 15.5-21min.
**TDF4m**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 25-31min.

**TDF5**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 40 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 17-24.5min.

**TDF5m**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 27-32min.
**TDF6**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 8-12min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 35 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 13-18.5min.

**TDF6m**: The crude reaction system was firstly purified by Hi-Flash Column (ODS-C18, 3.0x16.5 cm, 50 um, Yamazen A1-580), 0-20min 20-100% ACN/H$_2$O 20ml/min and the detection wave length was set up at 210 nm. The peak was collected at 12-15 min. Then this peak was injected in HPLC (Varian ProStar) with the column (Prep-C18, 21.2x250 mm, 10 um) system and 50 % ACN/H$_2$O was used to elute the column under the flow rate of 8ml/min. The wave length was set up at 200 nm to detect the compound and the pure compound was collected at 12-17min.
Updated list of derivatives from UWM Chemistry

List of Chemical purity with $^1$H $^{13}$C NMR data and pure amount.

<table>
<thead>
<tr>
<th>Derivatives</th>
<th>Pure amount</th>
<th>LC/MS</th>
<th>NMR $^1$H</th>
<th>NMR $^{13}$C</th>
<th>HRMS</th>
<th>Exact</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF1</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>95mg</td>
</tr>
<tr>
<td>TDF1t</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>185mg</td>
</tr>
<tr>
<td>TDF2</td>
<td>25mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>25mg</td>
</tr>
<tr>
<td>TDF2m</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>185mg</td>
</tr>
<tr>
<td>TDF3</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>125mg</td>
</tr>
<tr>
<td>TDF3m</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>158mg</td>
</tr>
<tr>
<td>TDF4</td>
<td>31mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>31mg</td>
</tr>
<tr>
<td>TDF4m</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>87mg</td>
</tr>
<tr>
<td>TDF5</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>146mg</td>
</tr>
<tr>
<td>TDF5m</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>130mg</td>
</tr>
<tr>
<td>TDF6</td>
<td>40mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>40mg</td>
</tr>
<tr>
<td>TDF6m</td>
<td>&gt;50mg</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>90mg</td>
</tr>
</tbody>
</table>
1. **TDF1**: After purification, about 95mg pure sample was obtained and the molecular weight was confirmed by LC/MS.

![Chemical Structure and Spectra](image-url)
2. **TDF1t**: After purification, about 185mg pure compound was obtained and the molecular weight was confirmed by LC/MS.

Chemical Formula: $C_{22}H_{39}O_5S_2$

Exact Mass: 462.21
Molecular Weight: 462.66

![Chemical structure and mass spectra](image-url)
3. **TDF2**: the pure compound was purified, obtained 25mg and confirmed by LC/MS,

Chemical Formula: $C_{24}H_{40}N_2O_8S_4$

Exact Mass: 612.17

Molecular Weight: 612.84
4. **TDF2m**: After purification, three fragments were collected 15mg, 185mg, and 12mg. Their molecular weight matched the expected one by LC/MS.

Chemical Formula: C_{29}H_{44}N_{8}O_{6}S_{4}
Exact Mass: 640.20
Molecular Weight: 640.90
5. **TDF3**: The sample is very polar and had to increase solvent system to dissolve. 125mg was obtained

![Chemical Structure of TDF3]

Chemical Formula: C_{18}H_{18}N_{2}O_{5}S_{3}
Exact Mass: 408.08
Molecular Weight: 408.56

6. **TDF3m**: The sample was run and the target compound was determined by LC/MS. Total amount: 158mg.

![Chemical Structure of TDF3m]

Chemical Formula: C_{18}H_{18}N_{2}O_{5}S_{3}
Exact Mass: 422.10
Molecular Weight: 422.58
7. **TDF4**: the structure was confirmed by LC/MS. Total amount: 31mg.

![Chemical Structure](image)

Chemical Formula: $C_{26}H_{44}N_2O_8S_2$

Exact Mass: 576.25

Molecular Weight: 576.77
9. **TDF5**: After purification, total 146mg pure compound was obtained. The molecular weight was confirmed by LC/MS.

![Chemical Structure](image)

**Chemical Formula:** C$_{28}$H$_{44}$N$_{2}$O$_{9}$S$_{2}$

**Exact Mass:** 576.25

**Molecular Weight:** 576.77
10. **TDF6**: After purification, total 40mg pure compound was obtained. The molecular weight was confirmed by LC/MS.

![Chemical structure of TDF6](image1)

**Chemical Formula:** $C_{24}H_{40}N_2O_6S_2$

**Exact Mass:** 548.22

**Molecular Weight:** 548.71

![UV Chromatogram, 200 nm](image2)
Working Group:  Dr. Cheng

Joe Ulicki

Dr. Cheng Wang

Sreya Biswas

Approval Date:  06/2012

Expiration Date:  to 05/2015

Modification:

University of Wisconsin - Milwaukee

Department of University Safety & Assurances

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE PROTOCOL REVIEW FORM

Coversheet

Principal Investigator/Project Director: Yi-Qiang Cheng

Department: Biological Sciences

Telephone Number: 229-4739

Email address: ycheng@uwm.edu

Protocol Title: Assessment of Acute Toxicity and Efficacy of New Chemical Compounds in Mice

Pain or Distress Classification (B,C,D,E): E

See Appendix B for USDA Classifications.

This protocol is a (an):

NOTE: to check a box, highlight the box and then right click and click “Properties” then under “Default Value” click the option as “Checked” then click “OK”:

☑ Original  □ Continuation  □ Modification (FAQ4)  □ Resubmission
If resubmission or continuation application, please give previous protocol number:

Do you have current (or pending) funding for this project: Yes

Funding Source (grant, Department, start-up, etc.): NIH/NCI

Grant #: R01CA512212

Title of Grant (if different from protocol title): Discover and Engineer New Histone Deacetylase Inhibitors as Anticancer Agents

If the funding agency is NIH or NSF or any agency requiring an “official” ACUC approval letter, list program area, contact person, title, and address:

This protocol is for:

- ☒ Research
- ☐ Teaching*
- ☐ Breeding
- ☐ Holding

(*note: the observation of ongoing approved protocols is exempt)

Duration of project (3 year maximum)

Start date/End date (month/year): 06/2012 to 05/2015

Building, facility or site where the animals will be housed: Lapham Hall - UWM ARC

(If in ARC facilities complete the application for Use of ARC Facilities Form.)

Identify the person or unit responsible for daily animal care: UWM ARC

List all personnel working with animals below:

1. Last name, first name: Cheng, Yi-Qiang
   Phone/email: 229-4739/ycheng@uwm.edu
   UWM student or employee? Faculty member
   UWM certification for animal use? (Certification pending)

2. Last name, first name: Ulicki, Joseph
   Phone/email: jsulicki@uwm.edu
   UWM student or employee? Graduate student
   UWM certification for animal use? Yes
3 Last name first name: Matson, Vyara

Phone/email: kamenova@uwm.edu

UWM student or employee? Graduate student

UWM certification for animal use? Yes

4 Last name first name: Biswas, Sreya

Phone/email: biswas@uwm.edu

UWM student or employee? Graduate student

UWM certification for animal use? (Certification pending)

☑ Personnel unknown at this time. Animal Care Program will be notified prior to animal use and personnel will be certified.

UW-Milwaukee has on file with the Public Health Service a written Assurance, which commits UW-Milwaukee to following the standards and regulations established by the Animal Welfare Act and the Health Research Extension Act.

UW-Milwaukee has established the Institutional Animal Care Committee (IACUC) to review and approve proposals for the use of animals in research and teaching. The IACUC will determine if these proposals are consistent with these regulations and requirements. The IACUC is responsible for the welfare of “any live vertebrate animal used or intended for use in research, experimentation, testing, training, or related purpose” if these animals are maintained at UW-Milwaukee or used under funds administered by UW-Milwaukee.

I certify that I have read the above statement and will adhere to all regulations concerning the use of animals in research and teaching, and that I will notify the IACUC
in writing of any changes in this Protocol Form before proceeding with any animal experimentation.

I further certify that personnel will not perform any animal procedures until they have been certified by the animal care program personnel.

I will ensure that all personnel are enrolled in the **Occupational Health and Safety Program** prior to their contact with animals.

I will provide emergency SOPs and after hours contact if needed.

---

_June 21, 2012_

**Signature**

**Date**

---

**PROTOCOL FORM**

1*st* **Species of Animals** (Scientific and common name): *Mus musculus* (mice), regular (BALB/c) and athymic (*nu/nu*)

**Total Number Per Year:** 110 regular mice and 440 athymic nude mice (10 regular mice and 44 athymic nude mice per compound; estimated testing of 7 new compounds and 3 reference compounds per year; plus 10 regular mice for training).

**Source of animals** (e.g., commercial, UWM breeding colony, outside institution, or other): Athymic (*nu/nu*) mice will be purchased from the Jackson Laboratory (Bar Harbor, ME), regular mice will be either purchased from the same source or obtained from the surplus of the Steeber Laboratory’s mouse colony.
(NOTE: for transgenic/knockout, mutagenesis or selective breeding programs, complete Appendix A).

2) Will any animals listed in question 1 be used for training purposes for laboratory personnel? ☑Yes ☐ No

If yes, how many?: 10

3) Is the species endangered or protected? If no, check the appropriate box and proceed to question 4. If yes, provide appropriate documentation (e.g., license number, copy of scientific collection permit, etc.).

☑No
☐Yes

Specify:

4) Is any special care necessary for the animals? (i.e., beyond basic husbandry practices?)

☑No
☐Yes

Specify:

Provide a scientific justification if the care requirements are outside of the recommendations in the “Guide for the Care and Use of Laboratory Animals”:

5) Are there any special housing requirements for the animals?

☐No
☑Yes

Specify: Mice will be kept in barrier housing.

Provide a scientific justification if the housing requirements are outside of the recommendations in the “Guide for the Care and Use of Laboratory Animals”:

6) Briefly explain in language understandable to a layperson the aim of the study and why the study is important to human or animal health, the advancement of knowledge or the good of society. Note: If this is an educational project, clearly state the educational goals.
The overall goal of the federally funded project is to identify new anticancer lead compounds generated through de novo (i.e., new) discovery and biological or chemical engineering. The term “lead compound” is a standard usage in the field of drug discovery and development; it does not mean a compound made from the element lead (Pb). A qualified lead compound has to be either more effective against human tumors and/or less toxic to normal cells/tissues/organs than three reference compounds of the class (FK228, thailandepsin A, thailandepsin B), thus can lead to additional studies. Acute toxicity will be assessed first in normal mice, while effectiveness will be assessed subsequently in immune system deficient (i.e., nude) mice that have been transplanted with human tumors.

This study will provide critical experimental data required for the continuous assessment of newly discovered or engineered chemical compounds on large animal models and perhaps on cancer patients.

7) Are the animals from a UWM breeding colony? If no, check the appropriate box and proceed to question 8. If yes, answer questions 7a, 7b, 7c, and 7d. NOTE: for transgenic/knockout, mutagenesis or selective breeding programs also complete appendix A. See FAQ 1

☒ No
☐ Breeding protocol on file; Protocol number:

7a) Number of adult breeders used per year:

7b) Anticipated total number of offspring for each species that will be produced (total per year):

7c) Anticipated number of offspring that will be used for experiments:

7d) What will be done with the surplus? (Note: the number of animals listed in 7b should total 7c plus 7d).
8) Check off all procedures listed below that apply to this study. If you did not check off any of the procedures, check N/A and proceed to question 9. If you checked off one or more of the procedures answer questions 8a, 8b, 8c and 8d.

☑️ N/A

☐ Experimental procedures that require food restriction (do not check box for pre-anesthetic fasting, or restricted feeding to control obesity)

☐ Experimental procedures that require fluid restriction. (More than 5 hours for rodents).

8a) Provide the length of time of the food and/or fluid restriction on both daily basis and throughout the experiment:

8b) Provide a scientific justification for the food and/or fluid restriction:

8c) Describe how animal health is monitored (e.g. body weight, urine/fecal output, food/fluid consumed, etc.) :

8d) Describe steps taken to ensure adequate nutrition/hydration during the restricted period:

9) If the animals require a special diet (commercial or non-commercial prepared diet that has ingredient(s) altered compared to maintenance diets) answer the following questions. If not, check N/A and proceed to question 10.

☑️ N/A

For commercially prepared diets:

9a) Provide the name/source of the diet:

9b) Describe how the diet is altered:

9c) Provide a scientific justification for the use of the diet here or in question 11:

For non-commercially prepared diets:
9d) What is the formulation of the diet:

9e) Location of diet preparation:

9f) Who will prepare the diet and what are their qualifications to do so:

9g) Provide a scientific justification for the use of the diet:

10) Will environmental enrichment devices be used (e.g. group housing, toys, foraging devices or treats)?

☑ Yes List types of enrichment: Group housing, Enviro-Dri bedding.

☐ No Provide a scientific justification:

11) Specify all animal procedures outside of normal husbandry. For holding protocols check N/A and proceed to question 12. For breeding protocols describe the breeding scheme, weaning ages, etc. For all other protocols, this description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study.

☐ N/A Holding Protocol

Specifically address the following in essay format:

Sequence, frequency and duration of procedures;

Animal numbers used in each procedure;

Substances given to animals (drugs, infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules);

Blood withdrawals (volume, frequency, withdrawal sites, and methodology);

How surgical procedures are involved (provide details of survival and non-survival surgical procedures in question 27 and perfusions as part of question 25b);

How nutritional intervention is involved (provide specific details in questions 8 or 9) see FAQ2;

Describe social or environmental manipulation;

Describe biological samples taken;
Describe methods of physical restraint and length of time animal(s) are restrained.

Describe acclimation procedures.

Prior to the actual experiments described in the following paragraphs, 10 surplus regular mice from the Steeber Lab’s colony will be used for initial training purpose and Dr. Douglas Steeber will act as a collaborator on the project and provide training.

Standard protocols posted by the US National Cancer Institute (NCI) Developmental Therapeutic Program (DTP) (http://dtp.cancer.gov/screening.html) will be closely followed to assess the acute toxicity (as expressed by maximal tolerated dose, MTD) and efficacy of a given lead anticancer compound.

(1) Compounds selected for in vivo testing should have been pre-screened by in vitro enzyme assays (performed separately) to show adequate histone deacetylase inhibitory activities. Compounds (including 3 reference compounds) will be dissolved in the standard DST vehicle (10% DMSO in saline/0.05% Tween 80) for both acute toxicity testing and efficacy testing.

(2) Cultivation of colon cancer cell lines. One human colon tumor cell line (COLO205) will be tested in this study. This cell line will be grown in RPMI medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 0.1% β-mercaptoethanol, at 37°C in a humidified chamber under 5% CO₂ in air.

(3) Establishment of human colon cancer xenografts. Athymic nude mice (nu/nu) (4-6 wks of age) will be purchased from the Jackson Laboratory (Bar Harbor, ME). All animals will be maintained on a 12 hr light/12 hr dark schedule with free access to laboratory chow and water. Tumors will be induced by injecting tumor cells (5 x 10⁴ cells) subcutaneously into the rear flank of nude mice. Following injection the mice will be randomized into control or treatment groups and identified using numbered ear tags. Primary tumor growth will be monitored daily by palpation. Once a tumor is detected its growth will be monitored every
other day using caliper measurements with size calculated as: tumor volume = length \times width^2/2.

(4) **Evaluation of the acute toxicity of anticancer lead compounds.** Generally, the determination of maximum tolerated dose (MTD) is performed in a way that conserves compound and minimizes the number of animals sacrificed. Thus, a single mouse is given a single injection (IP, IV or SC, to be determined) of 200 mg/kg (or lower if the compound is anticipated to be extremely potent); a second mouse receives a dose of 100 mg/kg and a third mouse receives a single dose of 50 mg/kg. The mice are observed for a period of 2 weeks. They are sacrificed if they lose more than 20% of their body weight or if there are other signs of significant toxicity. If all 3 mice must be sacrificed, the next 3 dose levels (25, 12.5 and 6.25 mg/kg) are tested in a similar manner. This process is repeated until a tolerated dose is found. The last testing will be repeated once. This dose is then designated the MTD and is used to calculate the amount of material administered to mice during efficacy testing. The mice are allowed ad libitum feed and water. Dose volumes are generally 0.1 ml/10 grams body weight. It is estimated that 10 regular mice [(3 + 3 x 2) for actual testing, plus 1 additional mouse as backup] will be needed for each compound.

(5) **Evaluation of the efficacy of anticancer lead compounds.** For efficacy studies, mice bearing human xenograft tumors will be used once the tumor volume reaches 150-200 mm$^3$ as described above. When the tumor volume reaches the appropriate size, the mice will receive an intravenous injection of the appropriate volume of DST buffer (vehicle control; n = 10), or escalating doses of compound solution up to one-fifth of the previously determined MTD (e.g., if the MTD = 50 mg/kg, then doses of 0.1, 1.0 and 10 mg/kg will be used) (n = 10). Tumor volumes will be measured as above for 3-6 weeks following injection in a blinded manner. Mice will be euthanized at the end of the study or when tumors become ulcerated or when tumor growth surpasses the maximum volume of 2000 mm$^3$. Tumors will be excised at appropriate time points for additional ex vivo evaluations. Thus, for each compound, 1
tumor line x (3 + 1) doses x 10 mice/group = 40 total mice. In our experience ~10% of the mice will be lost due to technical problems (e.g., failure to grow tumor, unsuccessful tail vein injection) or overly aggressive tumor growth. Therefore, we are increasing the number of mice to 40 + 4 = 44 for each compound to be tested.

(6) **Statistical analysis.** Statistical differences between treatment groups will be assessed by ANOVA and Student’s *t*-test for unpaired data between two groups. The level of significance is set at *P* < 0.05. If *P* < 0.05, Tukey-Kramer multiple comparisons post-tests will be performed. Power calculations for the efficacy studies are based on the assumption that a meaningful decrease in tumor volume is 20%, which will be detected using a one-tailed (two-group) *t*-test with alpha set at 0.05. The intra-group standard deviation is set at 15% of the mean value. With a group size of 10 animals per group, the power is 0.89. With a smaller inter-group decrease in tumor volume of 10%, the study would still have adequate power (0.70) with 10 animals per group.

12) **Are you mixing your own non-pharmaceutical grade compounds for administration to the animals?** If not, check No and proceed to question 13.

Note: OLAW and USDA consider that the use of non-pharmaceutical grade compounds should be based on:

- scientific necessity;
- no availability of an acceptable veterinary or human pharmaceutical-grade compound; and
- specific review and approval by the IACUC.

☐ No
☒ Yes

If yes, please check the following boxes:
I assure that steps will be taken during the preparation of the compound to ensure sterility (if injectable), that the appropriate pH will be established and that an appropriate vehicle will be used.

I assure that the compound will be labeled with an appropriated shelf life and that monitoring procedures will be established to adhere to this.

Comments: We will prepare the highest possible quality of compounds (≥98% purity) in the standard vehicle for this proposed animal study.

13) Standard Operating Procedures (SOP) for Animal Husbandry: Have you provided an SOP for animal care and a disaster plan to the Department of University Safety and Assurances for review and approval? Note: These plans need to be approved prior to receiving animals. Check N/A if animals are housed in ARC facilities where these plans are in place and proceed to question 14.

☐ Yes
☒ N/A

14a) Transportation Procedures: Will animals be transported to/from UWM facilities or between UWM facilities and other institutions or facilities? NOTE: Does not apply to animals received by commercial vendors. If no, check No and go to question 15. If Yes, check appropriate boxes below and complete the questions.

☒ No
☐ Yes

Where will the animals be transported to/from:

Describe the care of the animals during transport:

15) Quarantine Procedures: Will you be using vertebrate animals that were acquired outside of UWM breeding colonies or commercial vendors? If no, check No and proceed to
question 16. If yes, explain how your quarantine procedures are designed to reduce the possibility of disease transmission to vertebrate animals within the new facility.

☑️ N/A

☐ Yes

**Quarantine Procedures** (See Quarantine Procedures for Mammals):

16) **Will you be capturing animals in the field?** If no, check No and proceed to question 17. If yes, answer question 16a.

☐ No

☑️ Yes

16a) **Describe the specific capture techniques you will use for each species:**

17) **Explain your rationale for animal use.** Check N/A for Holding or Breeding protocol. The rationale should include reasons why non-animal models cannot be used. Recommended key words for literature search for alternatives include, “alternative, reduction, refinement, replacement”. See Alternatives website. NOTE: If this is an educational project, provide assurance that the use of alternative methods (i.e. computer simulation models, videotapes) has been considered to meet these goals:

**Rationale:** This research design of animal studies is to identify new anticancer lead compounds that are either more efficacious against human tumors than three reference compounds of the same class (FK228, thailandepsin A and thailandepsin B) and/or less toxic to normal cells/tissues/organs. It will provide critical experimental data required for the continuous assessments of newly discovered or engineered chemical compounds on large animal models and perhaps on cancer patients. There are no non-animal alternatives because cell cultures or other in vitro methods cannot provide a holistic assessment of the toxicity or efficacy of anticancer effects.

18) **Justify the appropriateness of the species selected.** (The species should be the lowest possible on the phylogenetic scale.) Check N/A for Holding or Breeding protocol.
Justification: The regular and athymic \((nu/nu)\) nude mice are the standard laboratory animal models for in vivo anticancer studies. Standard protocols posted by the US National Cancer Institute (NCI) Developmental Therapeutic Program (DTP) (http://dtp.cancer.gov/screening.html) will be closely followed to assess the acute toxicity (as expressed by maximal tolerated dose, MTD) and efficacy of a given lead anticancer compound.

19) Justify the numbers of animals to be used. (check all that apply)

- Pilot study or preliminary project, group variances unknown at present (Suggestion: minimal number of animals should be requested)

For the acute toxicity testing, the determination of maximum tolerated dose (MTD) is performed in a way that conserves compound and minimizes the number of animals sacrificed. For each compound, each round of testing will need only 3 mice, each to receive a single injection (IP, IV or SC) of 200 mg/kg, 100 mg/kg or 50 mg/kg body weight of compound. The mice are observed for a period of 2 weeks. They are sacrificed if they lose more than 20% of their body weight or if there are other signs of significant toxicity. If all 3 mice must be sacrificed, the next 3 dose levels (25, 12.5 and 6.25 mg/kg) are tested in a similar manner. This process is repeated until a tolerated dose is found. The last testing will be repeated once. It is estimated that 10 regular mice \([(3 + 3 \times 2) \text{ for actual testing, plus 1 additional mouse as backup}]\) will be needed for each compound.

For efficacy studies, mice bearing human xenograft tumors will be used once the tumor volume reaches 150-200 mm\(^3\) as described above. When the tumor volume reaches the appropriate size, the mice will receive an intravenous injection of the appropriate volume of DST buffer (vehicle control; \(n = 10\)), or escalating doses of compound solution up to one-fifth of the previously determined MTD (e.g., if the MTD = 50 mg/kg, then doses of 0.1, 1.0 and 10 mg/kg will be used) \((n = 10)\). Tumor volumes will be measured as above for 3-6 weeks following
injection in a blinded manner. Mice will be euthanized at the end of the study or when tumors become ulcerated or when the tumor weight exceeds 10% of the animal’s normal body weight. Tumors will be excised at appropriate time points for additional ex vivo evaluations. Thus, for each compound, 1 tumor line x (3 + 1) doses x 10 mice/group = 40 total mice. In our experience ~10% of the mice will be lost due to technical problems (e.g., failure to grow tumor, unsuccessful tail vein injection) or overly aggressive tumor growth. Therefore, we are increasing the number of mice to 40 + 4 = 44 for each compound to be tested.

☑ Based on the statistical analysis, the number of animals requested is the minimum necessary to achieve significant results

☑ Based on the PI’s experience using these methods in similar studies, and review of related use in peer reviewed published studies, the number of animals requested is the minimum necessary to meet the scientific objectives of the proposed research and to provide scientifically meaningful data

Based on the standard protocols posted by the US National Cancer Institute (NCI) Developmental Therapeutic Program (DTP) (http://dtp.cancer.gov/screening.html).

20) Provide written assurance that the activity does not unnecessarily duplicate previous experiments. Check N/A for Holding or Breeding protocols.

☐ N/A

☑ Literature search conducted.

Name of search utilized: Over the last two years of project development, we have routinely and extensively searched the literature and patents to ensure that our studies will not duplicate any previous studies and to ensure that our project will have the highest probability to succeed. Our methods of literature and patent search include MEDLINE/PubMed, US National Library of Medicine, SciFinder Scholar, USPTO Patent search.

Key words used: depsipeptide, FK228, histone deacetylase inhibitor, thailandepsin

Date the search was completed: October 2009, and again in May 2012
Years searched: all up to 2009 and 2012, respectively

☐ Consultation with colleagues (qualifications, dates and content of consult):

21) CHECK OFF ALL PROCEDURES LISTED BELOW THAT APPLY TO THIS STUDY:

(These procedures may cause more than slight or momentary pain and/or distress.) Check N/A and proceed to question 22 if none of these procedures apply. If you checked off one or more procedures, answer questions 21a, 21b, 21c and 21d.

NOTE: Non-invasive procedures such as needle aspiration, parenteral injections of non-irritating substances, blood collection from a common peripheral vein per standard veterinary practice and manual restraint that is no longer than would be required for a simple exam are not considered to cause more than momentary pain or distress.)

☐ N/A

☐ a. Surgical procedures (including terminal surgeries which are considered painful procedures alleviated by anesthesia- USDA Policy 11). NOTE: do not check for tissue perfusions-answer question 25b

☐ b. Administration of Freunds complete adjuvant.

☐ c. Ocular and skin irritancy testing.

☐ d. Complete Food or water deprivation beyond that necessary for normal pre-surgical preparation.

☐ e. Noxious electrical shock that is not immediately escapable.

☐ f. Paralysis or immobility in a conscious animal.

☐ g. Electro-fishing, gill netting, seining, fyke netting, hook-and-line fishing, mist netting of birds, live trapping.

☐ h. Administration of chemical or biological agents at doses known to cause more than momentary pain or distress or alter the normal physiology of the animal over time.

☐ i. Any activity intended to induce a stress response.
j. Prolonged forced confinement in a small enclosure.

k. Other: Although very common procedures, tumor implantation (xenograft) and injection of a lead compound may cause more than momentary pain and distress.

22) Note: If you did not check off any of the items listed in question 21 you should check non-applicable in this question and proceed to question 23. Federal regulations require consideration of the use of alternatives to the use of animals in procedures that may cause more than momentary or slight pain or distress to animals whether relieved or not (examples listed above). Alternatives include the concepts of replacement of vertebrate animals with in vitro modes, computer models or less sentient animals; refinement of experimental procedures to minimize pain or distress (e.g. early endpoints, use of analgesics, anesthetics or sedatives; techniques that reduce stress to the animal) or reduction in the number of animals by using appropriate statistical methods in the design and analysis of the study, reduction in variability by using animals of defined genetic or microbiological status, and maximizing the data gained from an individual animals.

N/A

22a) On what basis are the procedures that may cause more than momentary pain or distress justified: There are no non-animal alternatives because cell cultures or other in vitro methods cannot provide a holistic assessment of the toxicity or efficacy of anticancer effects of lead compounds.

22b) Indicate how alternatives to procedures (checked off in 21) which may cause more than momentary pain or distress were considered in the design of the study, or why alternatives to procedures that may cause more than momentary pain or distress were not found to be suitable for accomplishing the goals of the study: Animal model studies provide the best pre-clinical information on the efficacy of lead compounds in the treatment of human tumors. There are no non-animal alternatives because cell cultures or
other in vitro methods cannot provide a holistic assessment of the toxicity or efficacy of anticancer effects of lead compounds.

22c) **Methods used to search for alternatives to procedures that may cause more than momentary pain or distress (indicate all that apply):**

- Literature search conducted. (See Alternatives website for resources to assist in the search) Note: Recommended key words include: “alternative, reduction, refinement, replacement”

  **Name of search utilized:** MEDLINE/PubMed, National Library of Medicine, SciFinder Scholar, USPTO patent search.

  **Key words used:** Cancer therapy, pre-clinical test, animal model, non-animal model, in combination with alternative, reduction, refinement, or replacement

  **Date the search was completed:** May 2012

  **Years searched:** all up to 2012

- Consultation with colleagues (qualifications, dates and content of consult):

- Other information services utilized. Elaborate, providing specific information.

22d) Describe any discomfort or pain or alterations in normal physiology that may result from this procedure in the short term and long term. What will you do to minimize the chances of or relieve this discomfort and assure that no animal experiences undue pain or distress during the course of your research? **Behavioral and physiological endpoints** Include drugs, dosages, nursing care, mechanical devices, humane euthanasia, etc. Provide a scientific justification if appropriate anesthetics, analgesics or tranquilizer drugs cannot be utilized:

As a standard procedure, human tumors xenografted onto athymic (nu/nu) mice does not seem to invoke chronic pain and discomfort, according to the vast pool of literature. However, if the tumors grow to the point in which pain may become a factor (e.g. tumor leads to bleeding or
rupture, or tumor impedes mobility, or tumor growth surpasses the maximum volume of 2000 mm$^3$), the animals will be humanely euthanized.

23) Describe how frequently you will monitor your animals to ensure they are not experiencing pain or discomfort from your procedures or from unanticipated illness or injury, and state what you will do with any animals that may become ill or injured:

Immediately after administration of lead compound solution, animals will be observed for at least two hours, and if acute undesirable reactions to injected compounds are observed (e.g. shock and/or seizure), or the animals become apparently ill or injured during the course of testing, they will be humanely euthanized with CO$_2$. Otherwise the animals will be visually checked daily, including all tumor-bearing mice, and weighed once every three days for 3-6 weeks, in concurrence with the measuring of tumor sizes. All animals will be euthanized at appropriate times (see Item 24). Visible tumors from euthanized mice will be harvested, photographed and tested for redox content. The remaining corpse of euthanized mice will be kept in the radiation/biohazard freezer for disposal.

24) Will the experiments induce chronic disease, tumors or radiation sickness? If no, check No and proceed to question 25. If yes, describe the criteria (behavioral and physiological endpoints) for termination of the affected animals.

☐ No

☒ Yes

Criteria for termination: Tumors will be induced subcutaneously in animals as a consequence of xenografting and for the purpose of experiment before the administration of lead compound solution. It is expected that tumor growth will be curbed, reduced or completely cured after the administration of anticancer compounds. However, if acute undesirable reactions to the lead compounds (e.g. shock and/or seizure) are observed, or when the weight loss reaches 20% compared to vehicle controls, or tumor impedes mobility, or the animals show inability to eat or drink, or infection develops, of signs of severe organ system dysfunction are observed, or the
animals are found in a moribund state, or the tumor growth surpasses the maximum volume of 2000 mm³, the animals will be euthanized immediately. All surviving mice will be euthanized upon completion of the studies.

25) Describe the ultimate disposition of animals at the end of the study. If some or all are euthanized, answer yes to 25a and describe the methods of euthanasia (see CO₂ SOP) used and include all drugs, doses and routes of injection. If euthanasia methods differ from the 2007 AVMA Panel on Euthanasia provide a scientific justification.

If some or all the animals will be perfused, check the box in 25b, list the person responsible for anesthesia, the dose of anesthetic used and the procedure and agents used to perfuse.

If some or all the animal will not be euthanized check the appropriate box in 25c.

25a) ☑ Yes (euthanized)

Method (include drugs, doses and routes of injection): The animals will be euthanized with CO₂, following the SOP.

If a physical method is used, name the person performing the procedure:

Experience/training:

26) Does the project involve the use of hazardous agents to the humans working with the living animals (not tissues)? If no, check No and proceed to question 27. If yes, check the appropriate box(s) below and list the agent(s). Note: the appropriate forms listed below will need to be submitted to appropriate personnel in the Department of University Safety and Assurances for review along with the IACUC protocol. Approval is needed prior to receiving IACUC final approval. Delays in approval will result if the forms are not submitted.

☐ No

☑ Yes   Appropriate forms have been submitted

☐ Yes   Date if form previously approved:

☑ Chemicals hazardous to humans (see FAQ 5 for definition) DMSO
If checked, please list agent(s): Anticancer compound FK228, thailandepsina, and derivatives.

If checked, please complete the **Investigations Involving the Use of Hazardous Chemicals form** or check the appropriate box(s) below:

- [ ] MS-222. I have read the SOP and am using MS-222 as prescribed by the SOP. I have trained my staff accordingly.

- [ ] I have modified the SOP to accurately reflect the handling and control measures for use of MS-222 and will submit the form. (Click “Enable Macros” when opening the SOP and “Save As” when completed.)

- [ ] Isoflurane. I am familiar with the safety procedures for the use of Isoflurane link and have trained my staff accordingly.

- [ ] Formaldehyde. I have read the SOP and am using Formaldehyde as prescribed by the SOP. I have trained my staff accordingly.

- [ ] I have modified the SOP to accurately reflect the handling and control measures for use of Formaldehyde and will submit the form. (Click “Enable Macros” when opening the SOP and “Save As” when completed.)

- [x] DMSO. I have read the SOP and am using DMSO as prescribed by the SOP. I have trained my staff accordingly.

- [ ] I have modified the SOP to accurately reflect the handling and control measures for use of DMSO and will submit the form. (Click “Enable Macros” when opening the SOP and “Save As” when completed.)

- [ ] Radionuclides
  
  - If checked, please list agent(s):

  - If checked, please complete the **Animal Use of Radionuclides form**

- [ ] rDNA
  
  - If checked, please list agent(s):
If checked, please complete the cover sheet and one of the registration forms below:

**Registration Cover Sheet for all Recombinant DNA Research**

**Recombinant DNA Registration Form for Exempt Research**

**Recombinant DNA Registration Form for Non-exempt Research**

☑ Biological agents or Biological Toxin  If checked, please list agent(s):

If checked, please complete one of the registration forms below:

**Biological Agent Registration Form**

**Biological Toxin Registration Form**

**QUESTIONS FOR PROJECTS INVOLVING SURGICAL PROCEDURES**

27) **Does the project involve surgical procedures?** If no, check No. If yes, check the appropriate box(s) and proceed to 27a. See **Guidelines for Rodent/Avian Surgery**

☑ No

☑ Major--Penetrates and/or exposes body cavity or produces substantial impairment of physical or physiological functions (i.e. laparotomy, thoracotomy and craniotomy.)

☑ Minor --Does not expose body cavity and causes little or no physical impairment (i.e. subcutaneous implants.)

☑ Survival

☑ Non-survival--An animal is euthanized before recovery from anesthesia. (for perfusion procedures answer question 23b)
Pictures from the mice lab
Appendix B

Chapter II: AQUEOUS COMPLEXES FOR EFFICIENT SIZE-BASED SEPARATION OF AMERICIUM FROM CURIUM

Table of Contents

TABLE OF CONTENTS .................................................................................................................. 431

I. ANALYTICAL DATA .................................................................................................................. 432
   I. $^1$H AND $^{13}$C OF DIMETHYL PYRIDINE-2,6-DICARBOXYLATE ........................................ 432
   II. $^1$H AND $^{13}$C OF METHYL 6-(HYDROXYMETHYL)PICOLINATE .................................. 433
   III. $^1$H AND $^{13}$C OF METHYL 6-(BROMOMETHYL)PICOLINATE ..................................... 434
   IV. $^1$H, $^{13}$C AND COSY ($^1$H, $^1$H) OF 1,4,10,13-TETRAOXA-7,16-DIAZACYCLO-OCTADECANE ............................................................ 435
   V. $^1$H AND $^{13}$C OF N,N'-BIS[(6-METHOXYCARBONYL-2-PYRIDYL)METHYL]-1,7-DIAZA-12-CROWN-4 ................................................................. 437
   VI. $^1$H AND $^{13}$C OF N,N'-BIS[(6-CARBOXY-2-PYRIDYL)METHYL]-1,7-DIAZA-12-CROWN-4 (H2BP12c4·4HCl) ......................................................... 437
   VII. $^1$H OF METHYL 6-(2-VINYL)PICOLINATE .................................................................... 440
   VIII. $^1$H OF METHYL 6-(2-HYDROXYETHYL)PICOLINATE .................................................. 441
   IX. $^1$H OF METHYL 6-(2-CHLOROETHYL)PICOLINATE ..................................................... 442
   X. $^1$H, $^{13}$C AND MS OF METHYL 2-PYRIDINECARBOXYLIC ACID, 6,6'-[(1,4,10,13-TETRAOXA-7,16-DIAZACYCLOOCTADECANE-7,16-DIYL]BIS-(ETHYLENE)]BIS- 2,2'-DIMETHYL ESTER ........................................................................................................ 443
   XI. $^1$H AND MS OF METHYL 2-PYRIDINECARBOXYLIC ACID, 6,6'-(1,4,10,13-TETRAOXA-7,16-DIAZACYCLOOCTADECANE-7,16-DIYL]BIS(ETHYLENE)]BIS ............................................................... 446
I. Analytical Data

i. $^1$H and $^{13}$C of dimethyl pyridine-2,6-dicarboxylate

$^1$H: ju020413 Exp 1 User: ju 500

$^{13}$C: ju020413 Exp 2 User: ju 500
ii. \(^1\text{H}\) and \(^{13}\text{C}\) of methyl 6-(hydroxymethyl)picolinate

\[
\begin{array}{c}
\text{O} \\
\text{C} \\
\text{O} \\
\text{I} \\
\text{OH}
\end{array}
\]
iii. $^1\text{H}$ and $^{13}\text{C}$ of methyl 6-(bromomethyl)picolinate

$^1\text{H}$: djm020813 Exp 1 User: djm 300

$^{13}\text{C}$: ju020413 Exp 7 User: ju 300
iv. $^1$H, $^{13}$C and COSY ($^1$H, $^1$H) of 1,4,10,13-tetraoxa-7,16-
diazacyclo-octadecane

$^1$H: ju020613 Exp 1 User: ju 300

$^{13}$C: ju020613 Exp 2 User: ju 300
v. $^1$H and $^{13}$C of N,N'-Bis[(6-methoxycarbonyl-2-pyridyl)methyl]-1,7-diaza-12-crown-4

\[
\begin{align*}
\text{H: djm021213 Exp 1 User: ju 300} \\
\text{C: djm021213 Exp 2 User: ju 300} \\
\text{COSY: djm021213 Exp 4 User: djm 300}
\end{align*}
\]

vi. $^1$H and $^{13}$C of N,N'-Bis[(6-carboxy-2-pyridyl)methyl]-1,7-diaza-12-crown-4 (H2bp12c4·4HCl)
Carbon Spectrum
vii. $^1\text{H}$ of Methyl 6-(2-vinyl)picolinate
viii. $^1$H of Methyl 6-(2-hydroxyethyl)picolinate

Proton Spectrum
ix. $^1$H of Methyl 6-(2-chloroethyl)picolinate
x. $^1$H, $^{13}$C and MS of Methyl 2-Pyridinecarboxylic acid, 6,6'-\[\{(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyldiy)bis-(ethylene)\}bis-, 2,2'-dimethyl ester
Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample: CB-05

Operator: Mark Wang

Data filename: C:\LabSolutions\Data\Hossain, Mahmuni\CB-05-1.lcd
Spectrum Mode: Single
Retention Time: 0.133 min.
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode (Scan, SIM, Profile): Scan
Polarity (+,-): +

Intensity

![Mass Spectrum Graph]

m/z
xi. $^1$H and MS of Methyl 2-Pyridinecarboxylic acid, 6,6'-[[1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl]bis(ethylene)]bis
Appendix C

PART III: DESIGNING STRONG CHIRAL BRØNSTED ACIDS AND THEIR APPLICATION FOR OXAZINANONES DERIVATIZATION AND THE AZA-HENRY REACTION
Carbon Spectrum

Current Data Parameters

HNM 4000000
HE 4

FID - Acquisition Parameters

Type 2
EXTREM 4 mm Multi
SOLVENT agp
TD 6414
HE 10-13
SH 0
SW 61115 Hz
FIDRES 0.27407 Hz
AD 1.0080 Hz/sec
DF 312
DW 21.400 ppm
DF 4.00 ppm
DI 0.1000 Hz/sec
DS 0.00000000 Hz/sec

********** CHARMS C1 **********

HNC 12
HF 5.80 ppm
FL1 -6.00 Hz
FPOD 79.474069 MHz

********** CHARMS C2 **********

C303200 emai1
HNC 50
HF 150.00 ppm
FL1 -10.00 Hz
FPOD 50.00 ppm
FPOD 150.00 ppm

********** CHARMS C3 **********

C303200 emai1
HNC 50
HF 150.00 ppm
FL1 -10.00 Hz
FPOD 50.00 ppm
FPOD 150.00 ppm

FID - Processing parameters

SH 20720
DF 75.46750 MHz
DOW 1.00 Hz
DI 1.60 Hz
DMS 1.25 Hz
DMS 1.25 Hz

1D NMR plot parameters

FLP 127.124 ppm
FP 127.124 ppm

FID 127.124 ppm

UMCN 12.0113 ppm/cm
DCON 62.4110 Hz/cm
Proton Spectrum
Carbon Spectrum

Current Data Parameters

Channel 1: 2000 Hz/s
Time: 20 min
DATETIM: 04/04/02
CABL: 1 mm HN
PC: 180
TC: 25°C
FREQUENCY: 400.13 MHz
SNR: 10315.94 dB
PK: 1.60943 Hz
DO: 27.000 usec
GS: 5.000 usec
TE: 150.0 ms
G1: 0.1000000 sec
G2: 0.0360000 sec

CHANNEL 1:

DEC 15
P1 5.00 usw
P3 10.00 usw
PG 100.00 MHz

CHANNEL 2:

DEC 15
P4 5.00 usw
P5 10.00 usw
PG 100.00 MHz

Processing parameters:

SI: 12768
SW: 95.467787 MHz
GV: 0
LG: 1.00 Hz
G2: 1.40

LC RMS plot parameters:

CM: 206.00 ppm
TC: 180.00 Hz
PS: 0.00 ppm
SI: 0.00 Hz
EM: 2.3705 ppm/cm
DT: 0.0500 Hz/cm
Proton Spectrum
Carbon Spectrum

**Current data parameters**

**NAME**
methanol

**EPR**
1

**PROC**
1

**F1 - Acquisition parameters**

**Data**
03100526

**LURC**
23.22

**SPECTRUM**
1821.86 MHz

**POLARIZATION**
5 mm Multi

**POLPOL**
ngc

**T8**
16.14

**SOURCE**
CD2CN

**DS**
0

**SV**
1821.86 MHz

**FDR**
0.274611 Hz

**LG**
1.209265 mm

**SG**
162.3

**D1**
4.06 ppm

**D1**
0.1666666 mm

**D1**
0.2030000 mm

--------- CHANNEL F1 ---------

**WID**
100

**D**
4.00 ppm

**S**
-1.00 mm

**ST**
76.476648 MHz

--------- CHANNEL F2 ---------

**WID**
100

**PC**
100.00 ppm

**S**
1.00 mm

**ST**
76.476648 MHz

**W**
EN

**S2R**
5

**LB**
1.00 Hz

**DB**
0

**PC**
1.40

**1.5**

**ppm**

--------- EPR plot parameters ---------

**F1**
200.000 ppm

**F2**
80.000 ppm

**D**
0.02 Hz

**SM00**
9.0948 ppm/cm

**SM**
162.0700 mm/cm
Proton Spectrum
Carbon Spectrum
Carbon Spectrum

Current Data Parameters
NAME: msf10216
RESULT 5
PROMO:
1
FL - ACQUISITION PARAMETERS
DATE: 20100312
TIME: 13:13
DE$/,M 1 (ppm Atrum
PULSPER 1000
TO 2500
SOLVENT: DMSO-
HE: 0
DS: 0
SNR 100.25.94 Hz
FINPES 0.214637 Hz
AD 1.0098416 sec
PR 1.01% sec
DW 27.6540 sec
N 2.00 ppm
DK 0.00 ppm
DL 0.002109 ppm
DL 0.0031090 ppm

------- CHANNEL f1 -------

M0 11
FL 6.00 sec
F6 0.00 dB
XPD1 104.61659 MHz

------- CHANNEL f2 -------

CPD1 0.2585 MHz
CPD2 100.00 MHz
FL 0.00 dB
F6 0.00 dB
XPD1 104.61659 MHz

RF - PROCESSING PARAMETERS
ST 157.98
SF 75.46775 MHz
HE: 0
SR 0
LG 1.00 kHz
CD 0
FC 1.40

2D WAX plot parameters
FLP 222.000 ppm
FPL 123.81 Hz
FS 9.000 ppm
FSS 2.32 Hz
DPLW 3.2599 ppm/Hz
DPLH 2.833 Hz/Hz

---
Proton Spectrum

Current Data Parameters

- **Date:** 2005-12-11
- **Frequency:** 670.1 MHz
- **Type:** 5 mm 600 MHz
- **Solvent:** DMSO-d6
- **Temperature:** 300 K
- **Spin Rate:** 10000 Hz
- **Acquisition Parameters:**
  - RF Field: 7.3 T
  - Gain: 250000 mV
  - **Channels:**
    - **Chemical Shift:** 6.0 ppm
    - **First Black Box:** 3.0 ppm
    - **Second Black Box:** 4.0 ppm
  - **Processing Parameters:**
    - **Deconvolution:** 0.1 Hz
    - **Filter:** 1.0 Hz
- **2D NMR Plot Parameters:**
  - **Chemical Shift:** 6.0 ppm
  - **Spin Rate:** 10000 Hz
  - **Line Width:** 0.02 Hz
Proton Spectrum
Carbon Spectrum

---

**Carbon Data Parameters**
- **NAME**: nmr12345
- **PREP**: 2
- **REMARK**: 1

**FI - Acquisition Parameters**
- **DE**: 25.00°
- **TIME**: 1.00°
- **SPECTRUM**: dpm300
- **REPO**: 5 Hz Multilin

**DE**
- **DEL**: 45.00°
- **AV**: 45.00°
- **DS**: 30.00°

**PM**
- **HF**: 1010.00 Hz
- **FL**: 1.00 Hz

**CHE**: 1299.00 Hz
- **CM**: 27.000000 Hz
- **CE**: 4.00 Hz
- **CF**: 50.0 Hz
- **CN**: 0.0000000 Hz

**------------------ CHANNEL Fe ------------------**
- **FL**: 5.00 Hz
- **FIL**: 1.00 Hz
- **FIR**: 75.0 Hz

**------------------ CHANNEL Co ------------------**
- **FL**: 6.00 Hz
- **FIL**: 1.00 Hz
- **FIR**: 75.0 Hz

**-------------------- FI - PROCESSING Parameters -------------------**
- **ST**: 3500 Hz
- **SS**: 1500.0000 Hz

**-------------------- ED - NMR plot parameters -------------------**
- **F1**: 200.0000 pm
- **F2**: 500.0000 pm
- **F3**: 0.0000 pm

---

**CHEM**: 2.0000 ppm/cm
**S**: 490.0000 Hz/cm
Proton Spectrum
Proton Spectrum
Proton Spectrum

Chemical structure:

\[
\text{HNCO} \\
\text{Ph} - \text{CH} - \text{Ph}
\]

Data Parameters:

Current Data Parameters

- ppm: 4.00ppm
- linewidth: 0.00ppm
- peak gain: 1.00
- gate width: 0.50ms
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz
- peak frequency: 100.1MHz
- peak phase: 0.00
- peak gain: 1.00
- peak linewidth: 0.00ppm
- peak phase: 0.00
- peak gain: 1.00
- sweep time: 5.00s
- sweep frequency: 100.1MHz

ppm scale:

- 9
- 8
- 7
- 6
- 5
- 4
- 3
- 2
- 1

Intensities:

- 4.00ppm
- 5.00ppm
- 6.00ppm
- 7.00ppm
- 8.00ppm
- 9.00ppm
Carbon Spectrum

Current Data Parameters

F2 - Acquisition Parameters

Data: 20108603
Date: 11.30
INSTRUM: dp3100
PRESIDO: 5 mm multinou
MULITIP: 61036
EXTRNT: C6D3
RC: 10.94

1H
37115.94 Hz
T31HM: 0.074427 Hz
T32: 1.856150 Hz
T33: 4.5082 Hz
T34: 4.5082 Hz
STK: 410.30 Hz
STT: 0.1000000 sec
STT: 0.0300000 sec

--- CHANNEL 1 ---

F1: 1.30 Hz
F2: 4.00 ppm
F3: 0.475769 Hz

--- CHANNEL 2 ---

PROC: water
F1: 10.00 ppm
F2: 16.00 Hz
F3: 100.111000 Hz

F2 - Processing parameters

S1: 12768
S2: 75.477167 Hz
SNR: 0
SNR: 1.00 Hz
SNR: 1.40

1D HMN plot parameters

2000 cm
2F: 220.000 ppm
F2: 10.00 ppm
F3: 1.00 Hz
F3: 0.000 ppm
F3: 0.0100 ppm
F3: 0.0100 ppm
F3: 686.000 ppm
$^1$H NMR for

\[ \text{A} = \text{NH}_2 \]

\[ \text{A} = \text{CH} \]

\[ \text{A o-CH} \]

\[ \text{A p-CH} \]

\[ \text{A m-CH} \]
$^{13}$C NMR for

$$\text{NH}_2$$

[Diagram of NMR spectrum with peaks labeled]
2D NMR for

\[
\text{NH}_2
\]
6,6-dimethyl-4-phenyl-1,3-oxazinan-2-one-5,5-d$_2$

Proton Spectrum

2H Spectrum
CURRICULUM VITAE

Joseph Steve Ulicki
Place of Birth: Menomonee Falls, WI

Education

**High School**
Menomonee Falls High School, Menomonee Falls, WI

**College**
University of Wisconsin Milwaukee, Milwaukee, WI
B.S. Major: Chemistry and Biochemistry (2009)
Ph.D. Major: Organic/Drug development Minor: Biophysical Chemistry

Research Experience

**Graduate Student Studies** September 2009-2014
Advisor: Prof. M. Mahmun Hossain, Ph.D
Dissertation Title

**Undergraduate Student Studies** September 2007-2009
Advisor: Prof. M. Mahmun Hossain, Ph.D
Research Title: Chiral Bronsted Acids

Affiliations/Honors
Member of the American Chemical Society (2008-Present)