Synthesis and Biological Activity of N-Acyl Aziridines

A dissertation presented to

the faculty of

the College of Arts and Sciences of Ohio University

In partial fulfillment

of the requirements for the degree

Doctor of Philosophy

Greggory M. Wells

April 2016

© 2016 Greggory M. Wells. All Rights Reserved.
This dissertation titled

Synthesis and Biological Activity of N-Acyl Aziridines

by

GREGGORY M. WELLS

has been approved for

the Department of Chemistry and Biochemistry

and the College of Arts and Sciences by

Stephen C. Bergmeier

Professor of Chemistry and Biochemistry

Robert Frank

Dean, College of Arts and Sciences
ABSTRACT

WELLS, GREGGORY M., Ph.D., April 2016, Chemistry

Synthesis and Biological Activity of N-Acyl Aziridines

Director of Dissertation: Stephen C. Bergmeier

The development of new antimicrobial drugs is essential as the human population continues to build resistance to current treatments. Peptidomimetic compounds, those synthesized to mimic the behavior of naturally occurring biological proteins, have demonstrated promise in this area. Using bicyclic aziridine ring opening reactions, a library of N-acyl aziridinyl peptide isosteres has been synthesized and submitted to biological assays for cysteine protease inhibition, a common pathway to suppression of bacteria. Most of the compounds tested showed good activity against cathepsin B. This research required a novel synthetic approach to selectively generating oxazolidinones and aziridinyl ureas from fused bicyclic aziridines, which was accomplished with solvent selection, nucleophilic amine stoichiometry, and aziridine substitution. To extend the peptidic nature of these compounds, some success was achieved with acylation and amide coupling reactions. A practical approach to generating enantiomerically pure bicyclic aziridines was investigated, however the best enantioselective conditions provided only a 3 : 1 ratio. This stereoselective approach coupled with the ability to incorporate aziridines into peptide chains would ultimately lend a very powerful synthetic strategy for generating libraries of peptidomimetics with broad application potential.
I dedicate this dissertation to my mother, Shellie, whose unconditional love and support throughout my life has always given me the strength to carry on; and to my family and friends, who have waited with varying degrees of patience for its completion.
ACKNOWLEDGMENTS

I want to thank, first and foremost, my academic and research advisor, Professor Bergmeier. Over the years, he has supported me personally and professionally, and without his guidance, support, and encouragement, I would be a very different person. For the years of scientific discussions, professional suggestions, and personal advice, and for her unconditional friendship, I give a very special thank you to Dr. Susann H. Krake. I also want to thank Professor Klaus Himmeldirk, whose academic style was the catalyst for my interest in organic chemistry. For the countless hours together in the lab and all of the academic and scientific support, I give thanks to Dr. Crina Orac, Dr. Iwona Maciagiewicz, Dr. John Boughe, Alicia Frantz, Dennis Roberts, Ian Armstrong, Rumita Laha, and Zilong Zheng. Thanks to Dr. Kevin Alliston and Dr. William C. Groutas at Wichita State University for selecting and performing the initial cathepsin B, thrombin, and HLE assays. I want to thank Professor Jennifer Hines and Chunxi Zeng for their contributions and support of the cathepsin B assay. Thanks to Dr. Nigel Priestley for the antibacterial testing. Thank you to Professor Jeffrey L. Petersen at West Virginia University for the crystallography analysis. I want to thank Dr. Ralf Hoffmann for sponsoring my research at Biotechnologisch-Biomedizinisches Zentrum, Universität Leipzig. Thank you to the undergraduate students who contributed to this research, Zachary Farr, Patrick Gilson, and Jackie Sheridan. Finally, thank you to my committee members, Professor Mark C. McMills, Professor Hao Chen, and Professor Douglas Goetz.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>Dedication</td>
<td>4</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>4</td>
</tr>
<tr>
<td>List of Tables</td>
<td>10</td>
</tr>
<tr>
<td>List of Figures</td>
<td>12</td>
</tr>
<tr>
<td>List of Schemes</td>
<td>14</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
<td>18</td>
</tr>
<tr>
<td>Chapter 2: Optimization of Aziridinyl Urea Synthesis</td>
<td>26</td>
</tr>
<tr>
<td>2.1 Background and introduction</td>
<td>26</td>
</tr>
<tr>
<td>2.2 Synthesis of bicyclic aziridines</td>
<td>34</td>
</tr>
<tr>
<td>2.2.1 Selected allylic alcohols</td>
<td>35</td>
</tr>
<tr>
<td>2.2.2 Azidoformates</td>
<td>38</td>
</tr>
<tr>
<td>2.2.2.1 Synthesis of trityl bicyclic aziridine</td>
<td>38</td>
</tr>
<tr>
<td>2.2.2.2 Synthesis of propyl bicyclic aziridine</td>
<td>40</td>
</tr>
<tr>
<td>2.2.2.3 Synthesis of substituted bicyclic aziridines</td>
<td>42</td>
</tr>
<tr>
<td>2.2.3 N-Tosyloxy carbamates</td>
<td>44</td>
</tr>
<tr>
<td>2.2.3.1 Synthesis of N-tosyloxy carbamates</td>
<td>44</td>
</tr>
</tbody>
</table>
2.2.3.2 Bicyclic aziridination reactions of N-tosyloxycarbamates.........................46

2.2.4 Carbamates............................................................................................................49

2.3 Selectivity of bicyclic aziridine ring opening reactions ........................................52

2.3.1 Initial ring opening reactions ...............................................................................53

2.3.2 Effects of solvent polarity and amine stoichiometry..........................................58

2.3.3 Additive study.......................................................................................................66

2.3.4 Aziridine substitution ...........................................................................................68

2.4 Conclusions..................................................................................................................70

Chapter 3: Aziridinyl Ureas as Peptidomimetics .................................................................72

3.1 Introduction..................................................................................................................72

3.2 Aziridinyl urea compounds from amines ...................................................................80

3.3 Aziridinyl urea compounds from amino acid and peptide derivatives ..................81

3.3.1 Preparation of amino acid and peptide derivatives .............................................82

3.3.2 Trityl bicyclic aziridine reactions with amino acid derivatives .........................90

3.3.3 Substituted bicyclic aziridines with glycine benzylamide .................................91

3.3.4 Substituted bicyclic aziridines with amino acid and peptide derivatives ..........93

3.4 Modifications of aziridinyl urea compounds .............................................................98

3.4.1 Alcohol to amine conversion attempts .................................................................99

3.4.2 Acylations ...........................................................................................................104
3.4.2.1 Initial acylation attempts ................................................................. 104
3.4.2.2 DCC coupling reactions ................................................................. 106
3.4.2.3 CDI coupling reactions ................................................................. 107
3.4.2.4 Isocyanate substitution reactions .................................................. 110
3.4.2.5 Mitsunobu reaction ....................................................................... 111
3.4.3 Discussion of unsuccessful acylation attempts ............................... 111
3.5 Biological activity of aziridinyl urea compounds ............................... 114
3.5.1 Initial results for protease inhibition .................................................. 114
3.5.2 Cathepsin B activity .......................................................................... 117
3.5.3 Antibacterial results ......................................................................... 121
3.6 Conclusions ......................................................................................... 124
Chapter 4: Asymmetric Aziridination ..................................................... 125
4.1 Introduction ......................................................................................... 125
4.2 Selected chiral ligands ..................................................................... 132
4.3 Initial asymmetric aziridination reactions ......................................... 136
4.4 Quantification of enantioselectivity ................................................... 138
4.4.1 Chiral HPLC trials ......................................................................... 139
4.4.2 NMR trials ..................................................................................... 143
4.4.2.1 Crude analysis by $^1$H and COSY NMR ................................. 145
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Azidoformate and N-tosyloxycarbamate pathways to fused bicyclic aziridines</td>
<td>19</td>
</tr>
<tr>
<td>Table 2</td>
<td>Conditions and yields for bicyclic aziridination reactions</td>
<td>47</td>
</tr>
<tr>
<td>Table 3</td>
<td>Conditions for aziridination using iodosobenzene</td>
<td>51</td>
</tr>
<tr>
<td>Table 4</td>
<td>Yields and product ratios of ring opening reactions in methylene chloride</td>
<td>57</td>
</tr>
<tr>
<td>Table 5</td>
<td>Purified yields and product ratios in DMF and toluene</td>
<td>60</td>
</tr>
<tr>
<td>Table 6</td>
<td>Summarized product ratios in DMF and toluene</td>
<td>61</td>
</tr>
<tr>
<td>Table 7</td>
<td>Product ratios from allylamine stoichiometry study</td>
<td>64</td>
</tr>
<tr>
<td>Table 8</td>
<td>Product ratios from reactions using six molar equivalents of amines</td>
<td>65</td>
</tr>
<tr>
<td>Table 9</td>
<td>Product ratios from additive study</td>
<td>67</td>
</tr>
<tr>
<td>Table 10</td>
<td>Yields for ring opening reactions with propyl bicyclic aziridine</td>
<td>68</td>
</tr>
<tr>
<td>Table 11</td>
<td>Yields for aziridinyl urea compounds</td>
<td>81</td>
</tr>
<tr>
<td>Table 12</td>
<td>Yields of amino benzylamides via Cbz-protection pathway</td>
<td>83</td>
</tr>
<tr>
<td>Table 13</td>
<td>Yields of amino benzylamides via Boc-protection pathway</td>
<td>85</td>
</tr>
<tr>
<td>Table 14</td>
<td>Phenethyl bicyclic aziridine 1c opened with glycine benzylamide 12</td>
<td>92</td>
</tr>
<tr>
<td>Table 15</td>
<td>Results of aziridinyl urea alcohol conversion</td>
<td>100</td>
</tr>
<tr>
<td>Table 16</td>
<td>Initial acylation attempts for aziridinyl urea GWB-95</td>
<td>104</td>
</tr>
<tr>
<td>Table 17</td>
<td>Acylation attempts with DCC</td>
<td>106</td>
</tr>
<tr>
<td>Table 18</td>
<td>CDI stoichiometry study</td>
<td>108</td>
</tr>
<tr>
<td>Table 19</td>
<td>CDI stoichiometry study using benzyl alcohol</td>
<td>109</td>
</tr>
<tr>
<td>Table 20</td>
<td>Yields for acylation reactions with isocyanates</td>
<td>110</td>
</tr>
</tbody>
</table>
Table 21. Initial inhibition results for selected aziridinyl ureas ........................................ 116
Table 22. Cathepsin B inhibition results.................................................................................. 118
Table 23. Summary of antibacterial inhibition (MIC values) .............................................. 123
Table 24. Specific rotation of asymmetric aziridination products ...................................... 138
Table 25. Diastereomeric excess as determined by LCMS analysis .................................... 154
Table 26. Data collection details for 1e (C_{10}H_{15}NO_{2}) .............................................. 183
Table 27. Crystal data for 1e (C_{10}H_{15}NO_{2}) ................................................................ 185
Table 28. Data collection and structure refinement for 1e (C_{10}H_{15}NO_{2}) ..................... 185
Table 29. Atomic coordinates and equivalent isotropic atomic displacement parameters (Å^2) for 1e (C_{10}H_{15}NO_{2}). U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor ................................................................................................................................ 186
Table 30. Interatomic distance (Å) for 1e (C_{10}H_{15}NO_{2}) .............................................. 186
Table 31. Bond angles (°) for 1e (C_{10}H_{15}NO_{2}) ............................................................... 187
Table 32. Anisotropic atomic displacement parameters (Å^2) for 1e (C_{10}H_{15}NO_{2}). The anisotropic atomic displacement factor exponent takes the form: -2\pi^2[h^2 a^* a^* U_{11} + ... + 2 h k a^* b^* U_{12}] ...................................................................................................................... 187
Table 33. Hydrogen atom coordinates and isotropic atomic displacement (Å^2) for 1e (C_{10}H_{15}NO_{2}) .............................................................................................................. 188
LIST OF FIGURES

Figure 1. Fused bicyclic aziridines ................................................................. 18
Figure 2. Selected bisoxazoline and pyridine bisoxazoline ligands .................... 24
Figure 3. Commercially available allylic alcohols .......................................... 36
Figure 4. Crystal structure of cyclohexyl bicyclic aziridine 1e ......................... 48
Figure 5. Comparison of $^1$H NMR chemical shifts for selected protons ......... 54
Figure 6. $^1$H NMR comparison of oxazolidinone 8f to aziridinyl urea 9f .......... 56
Figure 7. Possible coordination of amine with carbonyl .................................. 66
Figure 8. Example of $\alpha$-peptoid structure .................................................... 72
Figure 9. Example of a $\beta$-peptoid structure .................................................. 73
Figure 10. Recently reported peptidomimetic containing $\alpha$- and $\beta$-peptoids .... 74
Figure 11. Structure of E-64 ............................................................................. 75
Figure 12. Structure of CA-074 ...................................................................... 76
Figure 13. Structure of Tokaramide A ............................................................... 76
Figure 14. Structure of Miraziridine A .............................................................. 77
Figure 15. Analogs of Miraziridine A ................................................................. 78
Figure 16. Amines selected to mimic amino acid side chains ............................ 79
Figure 17. Relationship between inhibition and fluorescence .......................... 117
Figure 18. Cathepsin B inhibition values ......................................................... 119
Figure 19. Inhibition values for GWB-98 and GWB-99 ..................................... 120
Figure 20. Chiral $bis$-geminal methyl bisoxazoline ligand ............................... 127
Figure 21. Selected bisoxazoline and pyridine bisoxazoline chiral ligands ..................... 132

Figure 22. Regis (R,R)-Whelk-O 1 chiral column stationary phase ................................. 139

Figure 23. Chiral HPLC separation results from Regis. .................................................... 142

Figure 24. Super-critical fluid chromatography from Regis. ............................................ 143

Figure 25. Homonuclear decoupling example for phenethyl aziridinyl urea 136 .......... 149

Figure 26. Chiral HPLC separation results from Regis. .................................................... 152
# LIST OF SCHEMES

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxazolidinone and aziridinyl urea compounds from trityl bicyclic aziridine</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Aziridinyl urea compounds from fused bicyclic aziridine 1b</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Aziridinyl ureas from fused bicyclic aziridines 1</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>Aziridinyl urea compounds from glycine benzylamide</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>Aziridinyl urea compounds from peptide benzylamides</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>Initial acylations of aziridinyl ureas</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>Isocyanate substitution reactions</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>Chiral aziridination of N-tosyloxy carbamates</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>Products from fused bicyclic aziridines</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>Early attempts to isolate fused bicyclic aziridines</td>
<td>27</td>
</tr>
<tr>
<td>11</td>
<td>Possible reaction mechanisms for chloride product</td>
<td>28</td>
</tr>
<tr>
<td>12</td>
<td>Azepine product</td>
<td>28</td>
</tr>
<tr>
<td>13</td>
<td>Synthesis of trityl bicyclic aziridine</td>
<td>29</td>
</tr>
<tr>
<td>14</td>
<td>Synthesis of propyl bicyclic aziridine</td>
<td>29</td>
</tr>
<tr>
<td>15</td>
<td>Bicyclic aziridines via N-tosyloxy carbamate pathway</td>
<td>30</td>
</tr>
<tr>
<td>16</td>
<td>Bicyclic aziridines via allylic carbamates</td>
<td>31</td>
</tr>
<tr>
<td>17</td>
<td>Ring opening reactions with several nucleophiles</td>
<td>32</td>
</tr>
<tr>
<td>18</td>
<td>Base-catalyzed ring opening reaction</td>
<td>32</td>
</tr>
<tr>
<td>19</td>
<td>Acid-catalyzed ring opening reactions</td>
<td>33</td>
</tr>
<tr>
<td>20</td>
<td>Considered phenyl bicyclic aziridine synthesis</td>
<td>34</td>
</tr>
</tbody>
</table>
Scheme 21. Synthetic precursors for bicyclic aziridines ..................................................... 35
Scheme 22. Synthesis of allylic alcohols .............................................................................. 36
Scheme 23. Reduction of ester 42 to alcohol 2h .................................................................. 37
Scheme 24. Synthesis of trityl bicyclic aziridine 1a ............................................................ 39
Scheme 25. Synthesis of propyl azidoformate 1b ............................................................... 40
Scheme 26. Alternative synthesis for propyl azidoformate 4b ........................................... 41
Scheme 27. Bicyclic aziridination of propyl azidoformate 4b ............................................ 41
Scheme 28. Synthesis of substituted bicyclic aziridines ...................................................... 42
Scheme 29. Synthesis of N-tosyloxy carbamates ................................................................. 44
Scheme 30. Synthesis of N-tosyloxy carbamate 7h ............................................................. 45
Scheme 31. Synthesis of N-tosyloxy carbamate 7k ............................................................. 46
Scheme 32. Aziridination to provide tricyclic aziridine 1h .................................................... 48
Scheme 33. Unsuccessful aziridination of 1k .................................................................... 49
Scheme 34. Preparation of phenethyl allylic carbamate 43 ................................................... 49
Scheme 35. Preparation of iodosobenzene 45 .................................................................. 50
Scheme 36. Oxazolidinones 8 and aziridinyl ureas 9 from bicyclic aziridine 1a .............. 52
Scheme 37. Aziridinyl ureas from bicyclic aziridine 1b ....................................................... 53
Scheme 38. Ring opening with benzylamine ....................................................................... 54
Scheme 39. Proposed transition states for ring opening reactions ...................................... 59
Scheme 40. Rearrangement of oxazolidinone 8 to imidazolidinone 46 .............................. 60
Scheme 41. Methyl bicyclic aziridine opening with allylamine .......................................... 69
Scheme 42. Selectivity of substituted aziridine in DMF ..................................................... 70
Scheme 43. Metabolism of Ximegalatran prodrug to give Megalatran........................................74

Scheme 44. Boc-protection of valine........................................................................................................86

Scheme 45. Synthesis of dipeptide benzylamides, 14 and 15 .............................................................87

Scheme 46. Boc-protection of isoleucine ...............................................................................................88

Scheme 47. Preparation of proline methyl ester ..................................................................................88

Scheme 48. Synthesis of Boc-protected isoleucinyl-proline methyl ester 68 ................................89

Scheme 49. Deamination of Boc-isoleucinyl-proline methyl ester 68 .............................................89

Scheme 50. Reactions of trityl bicyclic aziridine 1a with amino benzylamides .........................90

Scheme 51. Propyl bicyclic aziridine 1b opened with glycine benzylamide 12 .......................91

Scheme 52. Aziridinyl ureas from glycine benzylamide 12 .............................................................93

Scheme 53. Phenethyl bicyclic aziridine opened with phenylalanine benzylamide ..........93

Scheme 54. Phenethyl bicyclic aziridine 1c opened with proline benzylamide 61b .............94

Scheme 55. Phenethyl bicyclic aziridine opened with glycyl-glycine benzylamide ...........94

Scheme 56. Bicyclic aziridine 1c opened with valinyl-phenylalanine benzylamide 12 ..........95

Scheme 57. Bicyclic aziridine 1e opened with valinyl-glycine benzylamide 15 .....................95

Scheme 58. Reaction of bicyclic aziridine 1e with amino and dipeptide benzylamides ....96

Scheme 59. Bicyclic aziridine 1d opened with amino benzylamides ..............................................97

Scheme 60. Attempted reaction with isoleucyl-proline methyl ester 69 .................................98

Scheme 61. Typical conversions of an alcohol to an amine ..............................................................99

Scheme 62. Mitsunobu coupling of GWB-95 with carboxylic acid 17 .................................111

Scheme 63. Possible products from chloride addition to aziridine ............................................112

Scheme 64. Possible ring closing of activated aziridinyl urea .........................................................113
Scheme 65. Early use of chiral oxazoline-containing ligands .................. 126
Scheme 66. Early use of chiral bisoxazoline-substituted pyridine ligands .......... 126
Scheme 67. First use of chiral bisoxazoline ligands ..................................... 127
Scheme 68. mono-Substituted versus di-substituted PyBOX chiral ligands .......... 128
Scheme 69. Chiral bisoxazoline ligand 118 for asymmetric cyclopropanation ... 129
Scheme 70. Chiral bisoxazoline ligands for asymmetric cyclopropanation ........ 129
Scheme 71. Chiral PyBOX ligands for asymmetric epoxidation reactions .......... 130
Scheme 72. Intermolecular asymmetric aziridination of nitrostyrene ............... 131
Scheme 73. Intramolecular asymmetric aziridination .................................... 132
Scheme 74. Successful synthesis of chiral bisoxazoline ligand, 21h .................. 133
Scheme 75. Self-condensation side product 134 ........................................... 134
Scheme 76. Initial asymmetric aziridination reactions ................................... 136
Scheme 77. Racemic aziridination of phenethyl N-tosyloxycarbamate 7c .......... 137
Scheme 78. Chiral derivatization of bicyclic aziridines ................................... 144
Scheme 79. Imine formation from chiral amine 135 and acetone-d₆ .................... 147
CHAPTER 1: INTRODUCTION

This dissertation communicates two important developments using bicyclic aziridines and aziridinyl ureas. The first is a methodology study for the selective generation of oxazolidinones and aziridinyl ureas from bicyclic aziridines. The second is the demonstration of the biological value of a small library of aziridinyl ureas as potential peptidomimetics. An enantioselective study for the generation of chiral bicyclic aziridines is also described.

Bicyclic aziridines have been reported in recent literature via three different synthetic pathways, each of which has been explored to some extent in this work. A group of bicyclic aziridines, some previously reported and some novel examples, were generated following these literature precedents (Figure 1).

Figure 1. Fused bicyclic aziridines
The two major pathways to fused bicyclic aziridines are the thermal cyclization of azidoformates and copper-catalyzed aziridination of N-tosyloxy carbamates (Table 1).

Table 1. Azidoformate and N-tosyloxy carbamate pathways to fused bicyclic aziridines

Azidoformate Pathway:

\[
\begin{array}{c}
\text{R}^1 = \text{CH}_2\text{OTr} & \text{R}^2 = \text{H} & \text{R}^3 = \text{H} & \text{R}^4 = \text{H} \\
b & \text{H} & \text{CH}_2\text{CH}_3\text{CH}_3 & \text{H} \\
c & \text{H} & \text{H} & \text{CH}_2\text{Ph} \\
d & \text{H} & \text{H} & \text{cis-}(\text{CH}_2)_2(\text{CH})_2\text{CH}_2\text{CH}_3 \\
e & \text{H} & \text{H} & \text{C}_6\text{H}_{11} \\
f & \text{H} & \text{H} & \text{CH}_2\text{Ph} \\
g & \text{H} & \text{H} & \text{CH}(\text{CH}_3)_2 \\
h & \text{H} & \text{CH}_2\text{CH}_3 & \text{-CH}_2\text{CH}_3 \\
i & \text{H} & \text{H} & (\text{CH}_2)_2\text{CH}_3 \\
j & \text{H} & \text{H} & \text{CH}_3 \\
k & \text{H} & \text{H} & \text{CH}_3 \\
k \\
\end{array}
\]
Typically, bicyclic aziridines are reported for the purpose of generating oxazolidinones, with the earliest targets being chiral amino alcohols.\textsuperscript{1, 9} In addition to oxazolidinones, the generation of aziridinyl urea side products is also possible when opening bicyclic aziridines with amines. Aside from the research presented here, there have been very few literature examples where this product was observed, which prompted a comprehensive study for the selective generation of oxazolidinones and aziridinyl ureas.\textsuperscript{1} The study investigated the influence of differently substituted amines, solvent selection, and amine stoichiometry to optimize the conditions to produce either oxazolidinone 8 or aziridinyl urea 9 from starting bicyclic aziridine 1a (Scheme 1).

Scheme 1. Oxazolidinone and aziridinyl urea compounds from trityl bicyclic aziridine

Fused bicyclic aziridine 1b provided only aziridinyl urea compounds when opened with amines, due to the steric hindrance of the aziridine substitution (Scheme 2).

Scheme 2. Aziridinyl urea compounds from fused bicyclic aziridine 1b
Following the case of bicyclic aziridine 1b, several other bicyclic aziridines (1c-1i) were opened with amines to give aziridinyl urea compounds (Scheme 3).

![Scheme 3. Aziridinyl ureas from fused bicyclic aziridines 1](image)

An evaluation of these aziridinyl ureas as dipeptide isosteres prompted the generation of similar compounds using amino acid benzylamides and peptide benzylamides in place of amines. This provided a synthetic pathway for the incorporation of aziridines into short peptides effectively producing peptide-like compounds with an electrophilic site that could demonstrate biological activity, such as that exploited by serine protease inhibitors and cysteine protease inhibitors. The benzylamide substitution eliminated the usual acidity of the amino acid C-terminus and resulted in a more peptide-like structure. The most successful aziridinyl urea formations occurred with glycine benzylamide 12, which provided a racemic mixture of final compounds (Scheme 4).

![Scheme 4. Aziridinyl urea compounds from glycine benzylamide](image)
Additionally, a single reaction with glycine-glycine benzylamide 13 provided the expected racemic mixture 16 (Scheme 5). Chiral amino acid and peptide benzylamides provided mixtures of diastereomers; and although the aziridinyl urea formations were successful, separation of diastereomers in most cases was unsuccessful. In three cases, single diastereomers were isolated from peptide benzylamides (Scheme 5).

![Scheme 5. Aziridinyl urea compounds from peptide benzylamides](image)

In an effort to extend the peptidomimetic potential of aziridinyl ureas, several attempts were made to functionalize the left-hand primary alcohol. Most of these attempts were unsuccessful due to the reactivity of the aziridine ring leading to complex mixtures and no desired products. The following compounds were isolated in low to moderate yields.

A single Mitsunobu reaction provided acylated aziridinyl urea 18, which was also obtained via CDI-coupling with 3,4-dimethoxyphenyl acetic acid 17 (Scheme 6). This chemistry provided an additional acylated aziridinyl urea, 19.
The most successful acylation attempts resulted from electrophilic addition of aziridinyl urea alcohols toward isocyanates (Scheme 7).

Ten aziridinyl urea compounds, including some from amines and some from peptide benzylamides, were selected for biological testing against three different targets. Human leukocyte elastase (HLE) and thrombin are both serine proteases; and cathepsin B is a cysteine protease. The results of these assays indicated low inhibition of the serine proteases and comparatively higher inhibition of cathepsin B. This led to the biological evaluation of all available aziridinyl ureas, including one acylated compound, for inhibition of cathepsin B. All compounds were also tested for antibacterial activity against 10 bacteria lines.
To isolate single diastereomers of aziridinyl urea compounds generated from chiral amino acid and peptide benzylamides, an effort was made toward enantioselective cyclization of N-tosyloxycarbamates 7c-e to provide single enantiomers of bicyclic aziridines 1c-e (Scheme 8).

Scheme 8. Chiral aziridination of N-tosyloxycarbamates

This required the use of chiral ligands, for which bisoxazoline ligands have been employed in similar systems. A range of differently substituted bisoxazoline and pyridine bisoxazoline ligands were used (Figure 2).

Figure 2. Selected bisoxazoline and pyridine bisoxazoline ligands
These ligands were coordinated with three different copper catalysts, including Cu(CH$_3$CN)$_4$PF$_6$, 22a; [Cu(OTf)$_2$]$_2$•C$_6$H$_6$, 22b; and Cu(pyridine)$_4$(OTf)$_2$, 22c. A range of ligand/catalyst combinations provided limited enantioselectivity, which was reflected by a 3 : 1 ratio of diastereomers following chiral derivatization.
CHAPTER 2: OPTIMIZATION OF AZIRIDINYL UREA SYNTHESIS

Fused bicyclic aziridines have been a common research topic for graduate students under Professor Stephen C. Bergmeier. This work began at The Ohio State University where Dionne M. Stanchina utilized an intramolecular aziridination reaction with allylic azidoformates to provide fused bicyclic aziridines. The methodology was then optimized to provide these fused bicyclic aziridines as powerful intermediates for the synthesis of oxazolidinones, imidazolidinones, and now, aziridinyl ureas (Scheme 9).

![Scheme 9. Products from fused bicyclic aziridines](image)

2.1 Background and introduction

Bicyclic aziridines previously reported by Stanchina, Katz, and Lebel were used in our synthesis of aziridinyl ureas.\(^1\), \(^3\)-\(^8\) Although the synthesis of aziridines was well established via intermolecular reaction between azidoformates and olefins, Stanchina was among the earliest to attempt the intramolecular synthesis of bicyclic aziridines from
allylic azidoformates.\textsuperscript{9, 16} This thermal aziridination was performed in chlorinated solvents, which at first provided oxazolidinone products \textit{25} from nucleophilic chloride addition to the aziridine instead of the desired bicyclic aziridine \textit{24} (Scheme 10).\textsuperscript{9}

![Scheme 10. Early attempts to isolate fused bicyclic aziridines](image)

It was determined that the oxazolidinone \textit{24} was formed and immediately underwent addition by chloride anions, which were generated from \textit{1,1,2,2}-tetrachloroethane (TCE) at reflux temperatures. Two pathways were proposed to describe the initial cyclization. Either the azidoformate decomposed to a nitrene intermediate \textit{26}, which then underwent aziridination; or a dipolar cycloaddition occurred providing a triazoline intermediate \textit{27}, which could decompose to betaine \textit{28} and react with chloride anions (Scheme 11).
To determine the mechanism, the reaction was performed in toluene, which provided the azepine product 29 indicating that the reaction does proceed through a nitrene intermediate (Scheme 12).\textsuperscript{1,9}

Further work by Stanchina optimized the intramolecular thermolysis of allylic azidoformates to provide the desired bicyclic aziridines through solvent and temperature adjustments.\textsuperscript{1}
In 2002, Katz described the synthesis of trityl bicyclic aziridine (Scheme 13).³

This was prepared as a key intermediate in the pathway to substituted oxazolidinones. In contrast to most of the bicyclic aziridines reported to that point, trityl bicyclic aziridine 1a was obtained as a solid that was both stable and isolable via chromatography and recrystallization. That compound was used in the research reported here to determine the effects of solvent and amine stoichiometry upon the selectivity between oxazolidinone and aziridinyl urea products.

Although most bicyclic aziridines reported by Stanchina were synthesized with substitution alpha to the oxazolidinone oxygen, propyl bicyclic aziridine 1b was also prepared with substitution on the aziridine ring (Scheme 14).¹²
Compound 1b was used in the research reported here to demonstrate the effectiveness of aziridine ring substitution to direct nucleophilic attack to the carbonyl carbon of bicyclic aziridines providing exclusively the desired aziridinyl urea product.

In addition to the synthesis of bicyclic aziridines via the azidoformate pathway, the second of three pathways used in the research reported here is that from \( N \)-tosyloxy carbamates. In 2006, Lebel reported the synthesis of bicyclic aziridines from \( N \)-tosyloxy carbamates using \( \text{Rh}_2(\text{OAc})_4 \) as a catalyst (Scheme 15).

\[
\text{TsO} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{R} \quad \text{Rh catalysis} \quad \text{O} \quad \text{N} \quad \text{R}
\]

Scheme 15. Bicyclic aziridines via \( N \)-tosyloxy carbamate pathway

This research was reported more fully by Lebel in 2007 demonstrating that the same cyclization could be accomplished using copper catalysts with \( \text{Cu}(\text{pyridine})_4(\text{OTf})_2 \) providing the highest yield. Earlier in 2007, Fleming also reported the synthesis of bicyclic aziridines via copper catalysis with \( [\text{Cu}(\text{OTf})]_2\cdot\text{C}_6\text{H}_6 \) providing the most efficient conversion from a small set of attempted catalysts. Both \( [\text{Cu}(\text{OTf})]_2\cdot\text{C}_6\text{H}_6 \) and \( \text{Cu}(\text{pyridine})_4(\text{OTf})_2 \) were used in the synthesis of bicyclic aziridines reported here.

Fleming and Lebel applied the \( N \)-tosyloxy carbamate synthesis to the propyl bicyclic aziridine 1b previously reported by Stanchina, but with lower yields than that achieved via the azidoformate pathway. Several other aziridine substitutions were
reported by these two authors providing precedent for the novel substitutions presented here.

Finally, bicyclic aziridines have been reported from allylic carbamates 31 with Padwa describing the synthesis of tricyclic aziridines and Deng optimizing conditions for substituted bicyclic aziridines (Scheme 16).4,8

![Scheme 16. Bicyclic aziridines via allylic carbamates](image)

The latter determined that the use of hypervalent iodosobenzene in methylene chloride provides bicyclic aziridines in good yields. This method was used in the research reported here with poor results.

Stanchina performed bicyclic aziridine ring openings (upon unsubstituted aziridines) with many different nucleophiles to provide a range of final oxazolidinones (Scheme 17).1
In almost all cases, the nucleophilic attack occurred at the methylene of the aziridine ring. The most notable exception was the nucleophilic addition of benzylamine, which provided one of the few examples reported to provide an aziridinyl urea product.

Fleming also reported a few bicyclic aziridine ring opening reactions with nucleophilic addition at the benzyl-substituted aziridine ring. This included the addition of a secondary amine to the substituted aziridine, which required basic conditions and is likely due to the aziridine carbon also being a benzylic carbon (Scheme 18).
Deng reported ring opening reactions with nucleophilic attack upon the substituted aziridine carbon, but again these were benzylic carbons and occurred under acid catalysis (Scheme 19).\(^8\)

![Scheme 19. Acid-catalyzed ring opening reactions](image_url)

The ring opening reactions reported here will demonstrate that substituted aziridines undergo amine addition to provide the aziridinyl urea exclusively without a need for acidic or basic conditions.

The generation of oxazolidinone and aziridinyl urea compounds was accomplished by opening fused bicyclic aziridines (Figure 1). Initial reactions were performed using trityl bicyclic aziridine \(1a\). Selectivity studies employed reactions with propyl bicyclic aziridine \(1b\), which has been a common target throughout literature concerning bicyclic aziridines.\(^1,5-8,11,17\) To diversify the compound library, several new bicyclic aziridines were synthesized with varying substitution on the aziridine ring. These include phenethyl- (\(1c\)), \textit{cis}-hex-3-enyl- (\(1d\)), cyclohexyl- (\(1e\)), benzyl- (\(1f\)), and isopropyl bicyclic aziridine (\(1g\)), as well as cyclohexenyl tricyclic aziridine (\(1h\)). Pentyl- (\(1i\)) and methyl- (\(1j\)) bicyclic aziridines were also synthesized, but used only in a few
reactions. Dimethyl bicyclic aziridine 1k was not successfully isolated, though the synthesis attempt is presented here.

A simple phenyl substitution on the aziridine ring was considered, for which the starting material, \textit{trans}-cinnamyl alcohol 38, is commercially available (Scheme 20); however recent publications show that the benzyl aziridine carbon in 34 is particularly sensitive to nucleophilic substitution, which would not likely allow for stable storage.\textsuperscript{7, 8, 14, 17}

Scheme 20. Considered phenyl bicyclic aziridine synthesis

2.2 Synthesis of bicyclic aziridines

There are several pathways available for generating bicyclic aziridines (Scheme 21). Azidoformates were synthesized following procedures by Bergmeier.\textsuperscript{1-3} \textit{N}-tosyloxycarbamates were synthesized following procedures by Fleming and Lebel.\textsuperscript{5-7} Allylic carbamates were also synthesized following a procedure by Padwa and Deng.\textsuperscript{4, 8}
Scheme 21. Synthetic precursors for bicyclic aziridines

Some bicyclic aziridines were synthesized by more than one method and will be described in the appropriate section. All synthetic pathways started from allylic alcohols with several being commercially available and others prepared synthetically.

2.2.1 Selected allylic alcohols

Although some starting alcohols were commercially available (Figure 3), several were synthesized from other starting materials.
Wittig reagent 39 was prepared from commercially available ethyl bromoacetate then treated with commercially available aldehydes to give the corresponding ester, followed by DIBAL reduction to the alcohol (Scheme 22).\textsuperscript{18}

Scheme 22. Synthesis of allylic alcohols

Reaction between \textit{trans}-hydrocinnamaldehyde 40\textit{a} and ylide 39 was performed in methylene chloride, passed through a small pad of silica to remove triphenylphosphine oxide, then purified to provide ester 41\textit{a} in an 83\% yield. Reduction of ester 41\textit{a} was effected by treatment with DIBAL, which was subsequently quenched with water and
sodium fluoride. Silica gel purification provided phenethyl allylic alcohol 2c in a 92% yield. Cyclohexylcarboxaldehyde 40b was treated with ylide 39 in methylene chloride, filtered through silica gel to remove triphenylphosphine oxide then purified via silica gel to provide ester 41b in a 64% yield. This material was submitted to DIBAL reduction, quenched with water and sodium fluoride then purified on silica gel to provide an 84% yield of cyclohexyl alcohol 2e. Starting aldehydes 40c and 40d were treated with Wittig reagent 39 in methylene chloride then purified via flash chromatography. 1H NMR indicated that ester 41c was impure with starting aldehyde (12%) and the cis isomer (10%), which both coeluted with desired product. Purification of isopropyl ester 41d gave a very clean product by 1H NMR. Each ester was treated with DIBAL and purified via flash chromatography. Reduction of impure 41c provided the desired product as well as the reduction of each impurity, which again coeluted, providing an undetermined but approximately quantitative yield of impure alcohol 2f. Alcohol 2g was volatile reducing the yield from an initial 75% to 58%.

Commercially available methyl ester 42 was treated with DIBAL and purified on silica gel to provide a 96% yield of cyclohexenyl alcohol 2h (Scheme 23).

Scheme 23. Reduction of ester 42 to alcohol 2h
2.2.2 Azidoformates

Initial syntheses converted starting allylic alcohols to azidoformates following procedures reported by Bergmeier.\textsuperscript{1,3} In each of these reports, the final aziridination step was performed in a sealed tube at 110 °C to provide a racemic mixture of enantiomers. The products maintained relative trans stereochemistry, which was set by the olefin in the starting azidoformate. The syntheses described here were modified from the cited procedures to exclude 2,6-di-\textit{ tert}-butyl-4-methylphenol (BHT) for the sealed tube reactions.

2.2.2.1 Synthesis of trityl bicyclic aziridine

The synthesis of trityl-substituted bicyclic aziridine 1a was performed following a published procedure (Scheme 24).\textsuperscript{3} In the first step, commercially available 3,4-dihydroxy-1-butene 30 was treated with trityl chloride, DMAP, and triethylamine in methylene chloride to provide a 27% yield of the monoprotected diol 2a. This yield is not representative as a significant amount of slightly impure material was also obtained via flash chromatography.
Scheme 24. Synthesis of trityl bicyclic aziridine 1a

The mono-protected diol 2a was next treated with $p$-nitrophenylchloroformate and pyridine in methylene chloride to provide carbonate 3a in 73% yield after purification via flash chromatography. Allylic carbonate 3a was transformed into allylic azidoformate 4a upon treatment with sodium azide in acetone and water. This reaction progressed very slowly, requiring four days at room temperature for complete conversion, despite the use of five molar equivalents of sodium azide. Extraction with hexanes provided a clean product in 96% yield without further purification and $^1$H NMR data matched literature values. Material from this reaction was dissolved in methylene chloride, placed in a sealed tube, then cooled to -78 °C, evacuated, and warmed to room temperature. This process was repeated several times to remove atmospheric gases. The reaction was heated to 110 °C for eight hours, then cooled, concentrated, and purified via flash chromatography to provide a very low yield of bicyclic aziridine trans-rac-1a (9% yield). During the workup of a second iteration of this reaction, it was noted that a
considerable amount of solid did not dissolve in a solution of 25% ethyl acetate in hexanes. This solid was filtered to provide a clean $^1$H NMR that matched literature values for 1a in a 30% yield. Additionally, the crude filtrate was submitted to flash chromatography using silica gel to provide a second product in a 9% yield, which was determined by $^1$H and COSY NMR to be the cis stereoisomer cis-rac-1a.3

2.2.2.2 Synthesis of propyl bicyclic aziridine

Propyl bicyclic aziridine 1b was the first to be synthesized with substitution on the aziridine ring. Commercially available trans-2-hexen-1-ol 2b was selected and transformed into propyl bicyclic aziridine 1b (Scheme 25).1

![Scheme 25. Synthesis of propyl azidoformate 1b](image)

Alcohol 2b was first treated with CDI and pyridine to provide the acyloxyimidazolide, which was dissolved in DMF and treated with an excess of sodium azide. The reaction mixture was adjusted to a pH of 4 using concentrated hydrochloric acid and stirred overnight to provide an 82% yield of crude 4b. This product matched literature $^1$H NMR data and IR spectroscopy showed a distinct azide peak at 2139 cm$^{-1}$.1 Although this was an efficient synthesis, the pH adjustment using concentrated hydrochloric acid poses a safety consideration because an excess of HCl can generate
hydrazoic acid, which is explosive. For this reason, a second pathway to the azidoformate was also used (Scheme 26).

![Scheme 26. Alternative synthesis for propyl azidoformate 4b](image)

Alcohol 2b was treated with p-nitrophenylchloroformate and pyridine to provide carbonate 3b, which was then treated with sodium azide to give the propyl azidoformate 4b in 46-64% yields after purification on silica gel. Azidoformate 4b was dissolved in CH₂Cl₂ in a sealed tube, which was evacuated (as described for the bicyclic aziridination of trityl azidoformate 4a) and heated at 110 ºC for nine hours, then purified via flash chromatography to give a 64% yield of 1b (Scheme 27).

![Scheme 27. Bicyclic aziridination of propyl azidoformate 4b](image)

As noted earlier, the bicyclic aziridine is generated as a racemic mixture of products with relative trans-stereochemistry. Reaction times for the aziridination reaction varied from six to 13 hours and provided inconsistent degrees of conversion. Shorter
reaction times gave higher yields, but left some starting material unconverted. Longer reaction times returned no starting material, but provided lower yields. The speculation is that, although high temperatures are necessary for the formation of product, thermal decomposition occurs when high temperatures are maintained. Product loss occurred under high vacuum indicating compound volatility; and decomposition also occurred after a relatively short time in cold storage under inert atmosphere.

2.2.2.3 Synthesis of substituted bicyclic aziridines

Using the general \( p \)-nitrophenylchloroformate procedure established with propyl bicyclic aziridine \( 1b \), several other substituted bicyclic aziridines were synthesized (Scheme 28).

![Scheme 28. Synthesis of substituted bicyclic aziridines](image)

The synthesis and isolation of methyl bicyclic aziridine \( 1j \) from crotyl alcohol presented several issues. The alcohol was purchased as a mixture of \( cis \) and \( trans \)
isomers, which provided the final bicyclic aziridine as a mixture of two racemic products (four compounds total). The aziridination step did not go to completion, but provided a 60% conversion based on crude $^1$H NMR. The compound was not purified due to concerns about stability and volatility. These issues went unaddressed because the primary interest was the selectivity of the ring opening reaction (See Section 2.3.4.).

The synthesis of cis-hex-3-enyl bicyclic aziridine $1d$ was performed with contributions from Patrick Gilson. The first two steps provided clean $4d$ after purification on silica gel in a 57% yield. The sealed tube aziridination reaction was heated for 12 hours but did not go to completion. Silica gel purification provided a 33% yield of bicyclic aziridine $1d$ with 19% of starting azidoformate $4d$ recovered.

Patrick Gilson also contributed to the synthesis of pentyl bicyclic aziridine $1i$. The first two steps provided a 51% yield of mostly clean azidoformate $4i$, which underwent the sealed tube aziridination for 11 hours to provide a 17% yield of bicyclic aziridine $1i$ after flash chromatography.

Alcohol $2c$ was treated with $p$-nitrophenylchloroformate, followed by sodium azide, and then purified by flash chromatography to provide a 91% yield of $4c$ over two steps. The final aziridination step was performed in a sealed tube and purified by flash chromatography to provide a 48-50% yield of phenethyl bicyclic aziridine $1c$. In contrast to propyl bicyclic aziridine $1b$, this compound was less volatile with no loss during concentration under high vacuum. Treatment of alcohol $2e$ with $p$-nitrophenylchloroformate followed by sodium azide provided azidoformate $4e$ in a 26-
33% yield after purification. The final sealed tube aziridination provided bicyclic aziridine \(1e\) in a 37-50% yield after purification.

### 2.2.3 N-Tosyloxycarbamates

Another pathway to bicyclic aziridines utilized copper catalysis with \(N\)-tosyloxycarbamates. In contrast to the sealed tube, this reaction was performed under ambient conditions providing the ability to monitor reaction progress. This chemistry also provided the possibility for asymmetric aziridination using chiral ligands (Chapter 4). The syntheses in this section follow that reported by Fleming and Lebel.\(^6\)\(^7\)\(^13\)

#### 2.2.3.1 Synthesis of \(N\)-tosyloxylcarbamates

Alcohols were treated with CDI in methylene chloride to generate the acyloxyimidazolide intermediates that were very clean by \(^1\)H NMR following aqueous workup without the need for purification (Scheme 29). Clean crude material for each reaction was treated with hydroxylamine hydrochloride and pyridine, except for \(5c\), which was treated with hydroxylamine hydrochloride and imidazole directly.

![Scheme 29. Synthesis of \(N\)-tosyloxycarbamates](image-url)
The reaction with phenethyl acyloxyimidazolide 5c was performed several times with imidazole as the base and provided 64-72% yields. For acyloxyimidazolide 5e, replacement of imidazole with pyridine provided a higher yield, so those conditions were used for the remaining conversions. Yields continued to be high in most cases with the most notable exception being 6f, which was due to the persistent impurities that were carried through.

N-hydroxycarbamates 6c, 6d, 6f, and 6g were purified via flash chromatography to provide clean product with the exception of 6f, which still contained impurities. N-hydroxycarbamate 6e did not require purification. Reaction with tosyl chloride and triethylamine in diethyl ether provided N-tosyloxycarbamates 7c-7g in moderate yields after purification.

These conditions were used to convert cis-cyclohexenylmethanol 2h to the N-tosyloxycarbamate 7h (Scheme 30).

\[
\text{OH} \quad 1) \text{CDI, CH}_2\text{Cl}_2, \text{79\%} \quad \rightarrow \quad \begin{array}{c}
\text{HO-}\text{N-}\text{O} \\
\text{O}
\end{array} 
\text{6h} \quad \begin{array}{c}
\text{TsCl, Et}_3\text{N} \\
\text{Et}_2\text{O}
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{Tso-}\text{O-}\text{N-}\text{O} \\
\text{O}
\end{array} 
\text{7h}
\]

Scheme 30. Synthesis of N-tosyloxycarbamate 7h

Treatment of 2h with CDI gave a 79% yield of acyloxyimidazolide 5h without purification. The second step with hydroxylamine and pyridine gave an 81% yield of 6h
after silica gel purification. The tosylation reaction provided 7h in a 53% yield after flash chromatography.

A single disubstituted allylic alcohol 2k was carried through this synthesis as well, with moderate yields (Scheme 31).

Scheme 31. Synthesis of N-tosyloxycarbamate 7k

The initial CDI step provided a quantitative yield and a clean crude $^1$H NMR spectrum for acyloxyimidazolide 5k. The hydroxylamine step provided yields between 19-54% after aqueous workup and distillation to remove excess pyridine. Following tosylation and purification on silica gel, the desired N-tosyloxycarbamate 7k was isolated in a 43% yield, which is comparable to previous tosylation reactions.

2.2.3.2 Bicyclic aziridination reactions of N-tosyloxycarbamates

All aziridination reactions proceeded via a copper catalyst in the presence of potassium carbonate and 4Å molecular sieves and were carried out in either acetonitrile or methylene chloride (Table 2). N-Tosyloxycarbamate 7c was cyclized using Cu(pyridine)$_4$(OTf)$_2$ catalyst 22c, which was prepared by refluxing copper (II) triflate and excess pyridine in methanol, then recrystallizing from methanol and pyridine to give a
33% yield. All other aziridinations discussed in this section were performed using commercially-available Cu(CH$_3$CN)$_4$PF$_6$ 22a.

Table 2. Conditions and yields for bicyclic aziridination reactions

<table>
<thead>
<tr>
<th>Entry</th>
<th>R=, 7</th>
<th>Catalyst, 22</th>
<th>Solvent</th>
<th>Yield</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH$_2$CH$_2$Ph, 7c</td>
<td>Cu(pyridine)$_4$(OTf)$_2$ 22c</td>
<td>CH$_3$CN</td>
<td>42%</td>
<td>rac-1c</td>
</tr>
<tr>
<td>2</td>
<td>CH$_2$CH$_2$Ph, 7c</td>
<td>Cu(pyridine)$_4$(OTf)$_2$ 22c</td>
<td>CH$_2$Cl$_2$</td>
<td>38%</td>
<td>rac-1c</td>
</tr>
<tr>
<td>3</td>
<td>cC$<em>6$H$</em>{11}$, 7e</td>
<td>Cu(CH$_3$CN)$_4$PF$_6$ 22a</td>
<td>CH$_2$Cl$_2$</td>
<td>39%*</td>
<td>rac-1e</td>
</tr>
<tr>
<td>4</td>
<td>cis-(CH$_2$)$_2$(CH)$_2$CH$_2$CH$_3$, 7d</td>
<td>Cu(CH$_3$CN)$_4$PF$_6$ 22a</td>
<td>CH$_3$CN</td>
<td>30-47%</td>
<td>rac-1d</td>
</tr>
<tr>
<td>5</td>
<td>cis-(CH$_2$)$_2$(CH)$_2$CH$_2$CH$_3$, 7d</td>
<td>Cu(CH$_3$CN)$_4$PF$_6$ 22a</td>
<td>CH$_2$Cl$_2$</td>
<td>30%</td>
<td>rac-1d</td>
</tr>
<tr>
<td>6</td>
<td>CH$_2$Ph, 7f</td>
<td>Cu(CH$_3$CN)$_4$PF$_6$ 22a</td>
<td>CH$_3$CN</td>
<td>35%**</td>
<td>rac-1f</td>
</tr>
<tr>
<td>7</td>
<td>CH(CH$_3$)$_2$, 7g</td>
<td>Cu(CH$_3$CN)$_4$PF$_6$ 22a</td>
<td>CH$_2$Cl$_2$</td>
<td>45%</td>
<td>rac-1g</td>
</tr>
</tbody>
</table>

* - 34% starting material recovered  
** - 18% starting material recovered

Bicyclic aziridine 1c was isolated in a 42% yield (entry 1) in acetonitrile and 38% yield in methylene chloride (entry 2). Cyclohexyl bicyclic aziridine 1e was cyclized to provide a 39% yield, however 34% of the starting tosyloxycarbamate 7e was also recovered (entry 3). cis-Hex-3-enyl tosyloxycarbamate 7d gave a 30% yield of 1d in methylene chloride (entry 4) and a 30-47% yield of 1d in acetonitrile (entry 5). Benzyl bicyclic aziridine 1f was isolated in a 35% yield with 18% starting material 7f recovered (entry 6). Isopropyl bicyclic aziridine 1g was obtained in a 45% yield (entry 7). Each of these compounds was isolated as an oil with the exception of cyclohexyl bicyclic aziridine 1e, which was isolated as a white solid. This compound was crystallized from
hexanes and methylene chloride and submitted for crystallography to confirm the structure (Figure 4).

![Crystal structure of cyclohexyl bicyclic aziridine 1e](image)

**Figure 4. Crystal structure of cyclohexyl bicyclic aziridine 1e**

* cis-Hexene N-tosyloxycarbamate 7h was cyclized with Cu(CH$_3$CN)$_4$PF$_6$ 22a in acetonitrile to provide a 28% yield of tricyclic aziridine 1h (Scheme 32).*

![Scheme 32. Aziridination to provide tricyclic aziridine 1h](image)

The aziridination reaction with N-tosyloxycarbamate 7k was attempted twice using Cu(CH$_3$CN)$_4$PF$_6$ 22a and potassium carbonate in acetonitrile (Scheme 33).
Scheme 33. Unsuccessful aziridination of 1k

Despite consumption of starting material, the crude reaction mixture provided a complex $^1$H NMR spectrum and no product was isolated from flash chromatography. The low molecular weight and polarity of this compound likely led to product loss due to volatility. Synthesis of bicyclic aziridine 1k was discontinued.

2.2.4 Carbamates

Synthesis of bicyclic aziridines from allylic carbamates was reported by Deng and employed iodosobenzene catalysis.$^8$ To explore the possibility of using this chemistry for asymmetric aziridination, phenethyl allylic carbamate 43 was synthesized (Scheme 34).

Scheme 34. Preparation of phenethyl allylic carbamate 43

Phenethyl allylic alcohol 2c was first treated with trichloroacetyl isocyanate in methylene chloride. The concentrated crude material from this reaction was treated with
potassium carbonate in methanol then purified via flash chromatography to give allylic carbamate 43 in a quantitative yield.

Iodosobenzene 45 was prepared by treating iodobenzene diacetate 44 with sodium hydroxide, followed by trituration and filtration (Scheme 35). No purification or characterization of this product was performed mostly due to solubility. Melting point determination would typically offer an indication of purity; however this was not performed as the compound reportedly explodes at 210 °C rather than melting.

Scheme 35. Preparation of iodosobenzene 45

Several attempts were made to generate bicyclic aziridine 1c from carbamate 43 using the prepared iodosobenzene 45 as well as iodobenzene diacetate 44. These attempts were largely unsuccessful under the conditions summarized in Table 3.
Table 3. Conditions for aziridination using iodosobenzene

<table>
<thead>
<tr>
<th>Entry</th>
<th>PhI source</th>
<th>PhI Eq.</th>
<th>Additive*</th>
<th>Catalyst</th>
<th>Temperature**</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PhIO, 45</td>
<td>1.5</td>
<td>4Å MS</td>
<td>-</td>
<td>rt</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>PhIO, 45</td>
<td>2.2</td>
<td>4Å MS</td>
<td>-</td>
<td>rt</td>
<td>3%</td>
</tr>
<tr>
<td>3</td>
<td>PhIO, 45</td>
<td>2.2</td>
<td>4Å MS</td>
<td>-</td>
<td>rt</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>PhIO, 45</td>
<td>1.9</td>
<td>4Å MS</td>
<td>-</td>
<td>40 ºC</td>
<td>26%</td>
</tr>
<tr>
<td>5</td>
<td>PhIO, 45</td>
<td>1.9</td>
<td>4Å MS</td>
<td>Rh₂(OAc)₄</td>
<td>40-60 ºC, rt</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>PhI(OAc)₂, 44</td>
<td>1.9</td>
<td>4Å MS</td>
<td>Rh₂(OAc)₄</td>
<td>40-60 ºC, rt</td>
<td>-</td>
</tr>
</tbody>
</table>

* - MS = Molecular sieves
** - rt = room temperature

The first attempt (entry 1) showed no conversion after 18 hours; only starting material 43 and aromatic protons were observed by ¹H NMR. The concentrated reaction mixture was reconstituted and treated with another full equivalent of both molecular sieves and iodosobenzene 45. After another 19 hours, ¹H NMR analysis was complex showing no product and diminished presence of starting material. The reaction was repeated (entry 2) with an increase of iodosobenzene 45 providing only traces of product (3% yield). Although the literature indicated that iodosobenzene was only dried by vacuum suction during workup and filtration, the absence and removal of water is essential to the mechanism.²² For this reason, the aziridination reaction was repeated (entry 3) using iodosobenzene directly from the vacuum pump (room temperature, overnight) and molecular sieves directly from the vacuum oven (125 ºC, overnight). Despite these efforts, the reaction did not provide any clear product. Another procedure describes the reaction in a sealed tube at 40 ºC.¹⁰ This procedure provided a 26% yield of 1c with 23% starting material recovered (entry 4). Two reactions employed catalytic
Rh₂(OAc)₄ in a sealed tube; the first (entry 5) used iodosobenzene 45, prepared as in entry 3; the second used iodobenzene diacetate 44 as the hypervalent iodine source (entry 6). These reactions did not provide any product after 15 hours at 40 ºC or after another seven hours at 60 ºC. No product was observed after seven days at room temperature, despite an increase of Rh₂(OAc)₄ to one full equivalent. This work was discontinued due to the limited success with iodosobenzene and because concurrent reactions using N-tosyloxy carbamate starting materials consistently provided desired products.

2.3 Selectivity of bicyclic aziridine ring opening reactions

The original synthesis of aziridinyl urea compounds came from trityl bicyclic aziridine ring opening reactions with amines. This generated the expected oxazolidinone compounds, but also provided a secondary product, which was identified as aziridinyl ureas (Scheme 36).

![Scheme 36. Oxazolidinones 8 and aziridinyl ureas 9 from bicyclic aziridine 1a](image_url)

Reactions provided different product ratios even when amines with similar sterics or electronics were used. The product ratios were unpredictable and showed no trend or consistency across a small set of amines. Ultimately, product selectivity was accomplished via solvent selection and amine stoichiometry. Ring opening reactions with
increased stoichiometry of primary amines in toluene led to higher ratios of aziridinyl ureas; while lower stoichiometry in DMF led to the oxazolidinone product exclusively, in most cases. Ring opening reactions with secondary amines provided primarily oxazolidinones in either solvent and regardless of amine stoichiometry. Ring opening reactions with propyl bicyclic aziridine 1b were performed to determine product ratios when substitution was present on the aziridine ring (Scheme 37). This provided exclusively aziridinyl ureas 10a-i.

![Scheme 37. Aziridinyl ureas from bicyclic aziridine 1b](image)

### 2.3.1 Initial ring opening reactions

The first ring opening reactions of trityl bicyclic aziridine 1a with amines were performed in methylene chloride. Reactions were performed at room temperature, typically overnight, but occasionally for one to two days as convenient. An initial reaction with 110 mol% of benzylamine provided oxazolidinone 8a in an 81% yield (Scheme 38). Repeating this reaction provided aziridinyl urea 9a in a 19% yield.
It seemed likely that in each of these cases, only a single product was isolated and identified, despite the presence of both compounds. In support of this, the reaction was repeated again and crude $^1$H NMR showed a two to one mix of oxazolidinone 8a to aziridinyl urea 9a.

$^1$H and COSY NMR spectra give characteristic patterns for trityl-substituted oxazolidinones and aziridinyl ureas (Figure 5).

In oxazolidinones, protons adjacent to oxygen and nitrogen in the ring (H$_c$ and H$_d$) exhibit quartets around 4.4 ppm and 3.7 ppm, respectively. The protons adjacent to the trityloxy group (H$_a$ and H$_b$) exhibit AB-coupled pairs of doublets of doublets around 3.4 ppm and 3.2 ppm. Protons adjacent to the amine (H$_e$ and H$_f$) are more variable.
depending on the R-group, but for alkyl substitutions they typically also exhibit AB-coupled pairs of doublets of doublets between 2.7 - 2.3 ppm. The amide hydrogen is typically between 6.0 - 5.0 ppm and appears as a sharp singlet.

Aziridinyl ureas share much of the same connectivity with oxazolidinones, but the presence of the alcohol and aziridine give characteristic differences. In aziridinyl ureas, the methine proton adjacent to the alcohol (H\textsubscript{c}) exhibits a broad singlet or multiplet around 3.7 ppm while the methine proton adjacent to the aziridine nitrogen (H\textsubscript{d}) shows a doublet or multiplet around 2.7 ppm. The protons adjacent to the trityloxy group (H\textsubscript{a} and H\textsubscript{b}) show coupled multiplets around 3.5 ppm and 3.2 ppm. The aziridine methylene protons (H\textsubscript{e} and H\textsubscript{f}) typically exhibit signals around 2.5 ppm and 2.0 ppm. The urea proton is typically found between 6.0 - 5.5 ppm as a broad signal with multiplicity dependent on the nitrogen substitution, such as a broad triplet when the alpha carbon is a methylene or a broad doublet when the alpha carbon is a methine.

A comparison of the \textsuperscript{1}H NMR spectra for the oxazolidinone and aziridinyl urea products from trityl-substituted bicyclic aziridine opened by isobutylamine demonstrates this simple distinction (Figure 6).
Figure 6. $^1$H NMR comparison of oxazolidinone 8f to aziridinyl urea 9f
Several ring opening reactions with trityl bicyclic aziridine 1a were performed with a small set of amines, providing similar results to the initial benzylamine reaction (Table 4).

Table 4. Yields and product ratios of ring opening reactions in methylene chloride

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Purified Yields</th>
<th>Crude Product Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>benzylamine</td>
<td>0% (8a)</td>
<td>19% (9a)</td>
</tr>
<tr>
<td>2</td>
<td>morpholine</td>
<td>30% (8b)</td>
<td>0% (9b)</td>
</tr>
<tr>
<td>3</td>
<td>2-methoxyethylamine</td>
<td>26% (8c)</td>
<td>19% (9c)</td>
</tr>
<tr>
<td>4</td>
<td>aniline</td>
<td>49% (8d)</td>
<td>0% (9d)</td>
</tr>
<tr>
<td>5</td>
<td>N-methylbenzylamine</td>
<td>100% (8e)</td>
<td>0% (9e)</td>
</tr>
<tr>
<td>6</td>
<td>isobutylamine</td>
<td>43% (8f)</td>
<td>28% (9f)</td>
</tr>
<tr>
<td>7</td>
<td>allylamine</td>
<td>82% (8g)</td>
<td>0% (9g)</td>
</tr>
<tr>
<td>8</td>
<td>isopropylamine</td>
<td>83% (8h)</td>
<td>0% (9h)</td>
</tr>
<tr>
<td>9</td>
<td>propylamine</td>
<td>11% (8i)</td>
<td>26% (9i)</td>
</tr>
</tbody>
</table>

* - No crude $^1$H NMR available

These reactions were also carried out in methylene chloride and provided generally unpredictable results concerning the preference for oxazolidinone and aziridinyl urea products. Purified yields were determined after column chromatography and apparent product ratios were calculated from the integration of distinct peaks in the crude $^1$H NMR spectra.

Morpholine (entry 2) and N-methylbenzylamine (entry 5) were employed as examples of secondary amines. In each case, the oxazolidinone product (8b and 8e, respectively) was formed, exclusively. Aniline (entry 4), which was expected to have reduced nucleophilicity, and isopropylamine (entry 8) were examined to determine the
impact of steric bulk with each providing the oxazolidinone (8d and 8h, respectively), exclusively. Several primary amines with similar carbon chains were compared to determine the impact of electronic effects (Entries 3, 7, and 9). Despite the subtle differences between these three amines, the results were markedly different. 2-Methoxyethylamine showed a mix of products with a preference for oxazolidinone 8c over aziridinyl urea 9c in a ratio of 1.3 to 1.0 (2 : 1 by crude \(^1\)H NMR). Allylamine was purified to give a good yield of oxazolidinone 8g only; however a look at the crude \(^1\)H NMR shows a 3.0 to 2.0 mix of oxazolidinone 8g to aziridinyl urea 9g. Based on the product ratio from crude \(^1\)H NMR, it is possible that the reported yield had been artificially inflated by solvent. Propylamine led to a preference in favor of aziridinyl urea 9i with a purified ratio of 1.0 oxazolidinone 8i to 2.4 aziridinyl urea 9i, which can also be seen from the crude \(^1\)H NMR (1 : 2). Despite its similarity to isopropylamine, isobutylamine (entry 6) provided a mix of products with a 1.5 to 1.0 preference for oxazolidinone 8f over aziridinyl urea 9f.

2.3.2 Effects of solvent polarity and amine stoichiometry

Because there was no clear way to predict product preference based on amine substitution, we considered the transition states necessary to provide each product (Scheme 39). When amine addition occurs at the unsubstituted aziridine carbon, the aziridine is opened leaving a large charge separation, which would be better stabilized in a more polar solvent such as DMF. In contrast, amine addition at the carbonyl carbon would give a transition state with a much smaller charge separation. It is possible that a
less polar solvent, such as toluene, could provide a greater preference for the aziridinyl urea product.

Scheme 39. Proposed transition states for ring opening reactions

To investigate solvent implications, a computational study was performed by Travis Dudding, which supported the hypothesis that product selectivity could be controlled by reaction solvent. To test this hypothesis, reactions were performed in DMF and toluene using allylamine, which previously showed a preference for the oxazolidinone product; and propylamine, which previously favored the aziridinyl urea. Some discussion is necessary about the conditions and results of these reactions, which are summarized in Table 5.
### Table 5. Purified yields and product ratios in DMF and toluene

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Amine</th>
<th>Molar Eq.</th>
<th>Purified Yields</th>
<th>Product Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>allylamine</td>
<td>4.6</td>
<td>8% (34%)</td>
<td>1 (8g) : 0 (9g)</td>
</tr>
<tr>
<td></td>
<td>propylamine</td>
<td>4.3</td>
<td>5% (49%)</td>
<td>1 (8i) : 0 (9i)</td>
</tr>
<tr>
<td>Toluene</td>
<td>allylamine</td>
<td>4.9</td>
<td>14% (0%)</td>
<td>1 (8g) : 5 (9g)</td>
</tr>
<tr>
<td></td>
<td>propylamine</td>
<td>4.4</td>
<td>-</td>
<td>0 (8i) : 1 (9i)</td>
</tr>
</tbody>
</table>

Most important is that these reactions were not performed using 110 mol% of amine because the influence of amine stoichiometry was not a focus. As a consequence, the results of these reactions cannot be effectively compared to those in Table 4; however they still proved meaningful and will be discussed further. The second issue is that oxazolidinones 8 have the potential to rearrange to imidazolidinones 46 and reactions in DMF gave a much higher yield of the latter product (Scheme 40). As the focus was upon the product ratio of oxazolidinone and aziridinyl urea and because the imidazolidinone is evidence of oxazolidinone generation, the yields of these two compounds were combined when determining product ratios.

![Scheme 40. Rearrangement of oxazolidinone 8 to imidazolidinone 46](image-url)
In line with our hypothesis, reactions in DMF did provide the oxazolidinone product exclusively for both allylamine and propylamine, giving more consistent results than the reactions in methylene chloride. The reactions in toluene did increase the preference for the aziridinyl urea in each case; however some oxazolidinone was still generated when using allylamine. Several reactions were performed in DMF and toluene using a range of amine substitution and stoichiometry to provide a broader look at solvent influence (Table 6).

Table 6. Summarized product ratios in DMF and toluene

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Amine</th>
<th>Molar Eq.</th>
<th>Purified Yields</th>
<th>Product Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 (46)</td>
<td>9</td>
</tr>
<tr>
<td>DMF</td>
<td>Allylamine</td>
<td>1.3</td>
<td>57%</td>
<td>- 1.0 (8g) : 0.0 (9g)</td>
</tr>
<tr>
<td></td>
<td>Allylamine</td>
<td>4.6</td>
<td>8% (34%)</td>
<td>- 1.0 (8g) : 0.0 (9g)</td>
</tr>
<tr>
<td></td>
<td>benzylamine</td>
<td>1.8</td>
<td>45% (17%)</td>
<td>- 1.0 (8a) : 0.0 (9a)</td>
</tr>
<tr>
<td></td>
<td>propylamine</td>
<td>1.1</td>
<td>85%</td>
<td>- 1.0 (8i) : 0.0 (9i)</td>
</tr>
<tr>
<td></td>
<td>propylamine</td>
<td>4.3</td>
<td>5% (49%)</td>
<td>- 1.0 (8i) : 0.0 (9i)</td>
</tr>
<tr>
<td></td>
<td>2-methoxyethylamine</td>
<td>2.5</td>
<td>36% (16%)</td>
<td>- 1.0 (8c) : 0.0 (9c)</td>
</tr>
<tr>
<td></td>
<td>Allylamine</td>
<td>1.1</td>
<td>**</td>
<td>** 1.0 (8g) : 1.7 (9g)</td>
</tr>
<tr>
<td></td>
<td>Allylamine</td>
<td>4.9</td>
<td>14%</td>
<td>76% 1.0 (8g) : 5.4 (9g)</td>
</tr>
<tr>
<td></td>
<td>benzylamine</td>
<td>1.1</td>
<td>**</td>
<td>** 1.0 (8a) : 1.5 (9a)</td>
</tr>
<tr>
<td></td>
<td>benzylamine</td>
<td>1.8</td>
<td>7%</td>
<td>37% 1.0 (8a) : 5.3 (9a)</td>
</tr>
<tr>
<td></td>
<td>isopropylamine</td>
<td>1.1</td>
<td>**</td>
<td>** 1.0 (8h) : 0.0 (9b)</td>
</tr>
<tr>
<td></td>
<td>isopropylamine</td>
<td>2.2</td>
<td>37%</td>
<td>25% 1.5 (8h) : 1.0 (9b)</td>
</tr>
<tr>
<td></td>
<td>morpholine</td>
<td>1.1</td>
<td>**</td>
<td>** 1.0 (8b) : 0.0 (9b)</td>
</tr>
<tr>
<td></td>
<td>morpholine</td>
<td>2.0</td>
<td>52%</td>
<td>- 1.0 (8b) : 0.0 (9b)</td>
</tr>
<tr>
<td></td>
<td>propylamine</td>
<td>1.1</td>
<td>**</td>
<td>** 1.0 (8i) : 0.0 (9i)</td>
</tr>
<tr>
<td></td>
<td>propylamine</td>
<td>4.4</td>
<td>-</td>
<td>68% 0.0 (8i) : 1.0 (9i)</td>
</tr>
<tr>
<td></td>
<td>2-methoxyethylamine</td>
<td>1.1</td>
<td>**</td>
<td>** 1.0 (8c) : 2.7 (9c)</td>
</tr>
<tr>
<td></td>
<td>2-methoxyethylamine</td>
<td>2.5</td>
<td>-</td>
<td>53% 0.0 (8c) : 1.0 (9c)</td>
</tr>
</tbody>
</table>

* - Ratios calculated from isolated yields for purified products
** - Not purified. Product ratios based on crude 'H NMR
All reactions performed in DMF provided the oxazolidinone (and in some cases, the imidazolidinone) regardless of amine stoichiometry used. There was no evidence of aziridinyl urea production when using DMF.

For all reactions performed with 110 mol% of amine in toluene, the product ratios were determined by integration of distinct product peaks in the crude $^1$H NMR spectra. Reactions with morpholine and isopropylamine resulted in the same exclusive generation of oxazolidinones $8b$ and $8h$, respectively, in both methylene chloride and toluene. The reaction with propylamine also gave the same results in toluene as in methylene chloride with a product ratio of 1.0 oxazolidinone $8i$ to 2.0 aziridinyl urea $9i$. Benzylamine, which favored oxazolidinone $8a$ in methylene chloride, led to a preference for aziridinyl urea $9a$ in toluene with a ratio of 1.0 oxazolidinone $8a$ and 1.5 aziridinyl urea $9a$. Allylamine also went from a preference for oxazolidinone product $8g$ in methylene chloride to a product mix of 1.0 oxazolidinone $8g$ and 1.7 aziridinyl urea $9g$ in toluene. The most dramatic shift came from the reaction with 2-methoxyethylamine, which went from a ratio of 2.0 oxazolidinone $8c$ to 1.0 aziridinyl urea $9c$ in methylene chloride to a ratio of 1.0 oxazolidinone $8c$ to 2.7 aziridinyl urea $9c$ in toluene.

Reactions performed in toluene using greater than 110 mol% of amine, gave a clear increase in aziridinyl urea production for all reactions except morpholine. Reaction with 200 mol% of morpholine in toluene provided only oxazolidinone $8b$, showing no improvement over the reactions with 110 mol% in methylene chloride or toluene. Although reaction with propylamine showed a slight preference for aziridinyl urea $9i$ when using 110 mol% in methylene chloride or toluene, it provided aziridinyl urea $9i$
exclusively when 440 mol% were used. Reactions with 2-methoxyethylamine had already shown an increased preference for aziridinyl urea 9c when switching from methylene chloride to 110 mol% in toluene, but it provided exclusively aziridinyl urea 9c when using 250 mol%. Although isopropylamine gave only oxazolidinone 8h when using 110 mol% in methylene chloride or toluene, it showed a slight preference for aziridinyl urea 9h when using 220 mol%. The greatest improvement to aziridinyl urea product came with benzylamine and allylamine. Each of these reactions showed a slight preference for aziridinyl ureas (9a and 9g, respectively) previously when using 110 mol% in toluene, but provided a 5 to 1 preference for aziridinyl urea 9a and 9g, respectively, when using 180 mol% of benzylamine or 490 mol% of allylamine.

Several new reactions were performed using allylamine to determine the influence of amine stoichiometry upon product selectivity in toluene; the results of which are tabulated with previous allylamine results in Table 7.
Quantification of product ratio by integration of crude $^1$H NMR spectra shows a clear trend of increased aziridinyl urea preference with increased amine stoichiometry. Previously (Table 6), the reported ratio for the reaction with 490 mol% of allylamine (1 oxazolidinone to 5 aziridinyl urea) was taken from the isolated product ratio, but evaluation of the crude $^1$H NMR shows a ratio of 1 to 8. This result is a little out of line with the other results, but the general trend is obvious. Six molar equivalents mark the maximum preference for aziridinyl urea with 1020 mol% showing no improvement. In fact, performing the reaction in allylamine as the solvent (4890 mol %) provides the same product ratio. This seemed to provide reaction conditions necessary to selectively synthesize aziridinyl urea products. Several other amines were chosen in order to validate the applicability of these conditions (Table 8).
Table 8. Product ratios from reactions using six molar equivalents of amines

<table>
<thead>
<tr>
<th>Amine</th>
<th>Amine Eq.</th>
<th>$8$</th>
<th>$9$</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzylamine</td>
<td>6.0</td>
<td>0.0 (8a)</td>
<td>1.0 (9a)</td>
</tr>
<tr>
<td>morpholine</td>
<td>6.3</td>
<td>1.0 (8b)</td>
<td>0.0 (9b)</td>
</tr>
<tr>
<td>2-methoxyethylamine</td>
<td>6.3</td>
<td>1.0 (8c)</td>
<td>9.0 (9c)</td>
</tr>
<tr>
<td>isopropylamine</td>
<td>6.0</td>
<td>0.0 (8h)</td>
<td>1.0 (9h)</td>
</tr>
<tr>
<td>propylamine</td>
<td>6.5</td>
<td>1.0 (8i)</td>
<td>8.0 (9i)</td>
</tr>
</tbody>
</table>

Morpholine continued to provide only oxazolidinone $8b$ as has been the case under all conditions reported so far. The secondary nature of this amine is likely the cause for oxazolidinone preference. In all other reactions, the use of six molar equivalents of amine provided a strong preference for aziridinyl urea $9$, with benzylamine and isopropylamine providing only this product ($9a$ and $9h$, respectively). Benzylamine, which had already shown a strong preference with increased stoichiometry (180 mol%), provided only aziridinyl urea $9a$. Isopropylamine exhibited the greatest change from a ratio of 1.5 oxazolidinone $8h$ to 1.0 aziridinyl urea $9h$ using 220 mol% to a complete preference for aziridinyl urea $9h$. As shown earlier, 2-methoxyethylamine and propylamine provided only the aziridinyl urea products ($9c$ and $9i$) with fewer molar equivalents (250 and 440 mol%, respectively).
2.3.3 Additive study

It is possible that the increased amine stoichiometry plays a role in the reaction either by stabilizing the transition state or acting as a base or a hydrogen bond donor (Figure 7).

![Possible coordination of amine with carbonyl](image)

Figure 7. Possible coordination of amine with carbonyl

Several test reactions were performed to determine whether amine equivalents could be replaced with some reaction additive to obtain a preference for the aziridinyl urea product. The reactions were performed in toluene using 110 mol% of allylamine and many different additives, including non-nucleophilic amines and alcohols. All product ratios were determined by the integration of diagnostic peaks in the crude $^1$H NMR (Table 9).
Table 9. Product ratios from additive study

<table>
<thead>
<tr>
<th>Additive</th>
<th>Stoichiometry</th>
<th>8g : 9g</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>1.0 : 1.7</td>
</tr>
<tr>
<td>DIPEA</td>
<td>5.9</td>
<td>1.0 : 1.3</td>
</tr>
<tr>
<td>Pyridine</td>
<td>5.9</td>
<td>1.0 : 1.0</td>
</tr>
<tr>
<td>Pyridine (solvent)</td>
<td>41 (solvent)</td>
<td>1.0 : 0.0</td>
</tr>
<tr>
<td>p-trifluoromethylaniline</td>
<td>6.2</td>
<td>3.0 : 1.0</td>
</tr>
<tr>
<td>Methanol</td>
<td>6.3</td>
<td>1.0 : 1.6</td>
</tr>
<tr>
<td>Methanol</td>
<td>14.0</td>
<td>1.0 : 1.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>26.0</td>
<td>1.0 : 1.3</td>
</tr>
<tr>
<td>Methanol (solvent)</td>
<td>93 (solvent)</td>
<td>2.6 : 1.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>6.1</td>
<td>1.0 : 1.4</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>6.1</td>
<td>1.0 : 1.0</td>
</tr>
<tr>
<td>2-methoxyethanol</td>
<td>6.1</td>
<td>1.0 : 1.3</td>
</tr>
<tr>
<td>2,2,2-trifluoromethylethanol</td>
<td>6.3</td>
<td>1.6 : 1.0</td>
</tr>
</tbody>
</table>

The use of additives in these reactions left the product ratio largely unchanged, in most cases, relative to the original reaction. The use of p-trifluoromethylaniline and 2,2,2-trifluoromethylethanol; pyridine, as a solvent; and methanol, as a solvent, actually shifted the product preference in favor of oxazolidinone 8g. At any rate, if additional amine stoichiometry plays a role in the selectivity of the aziridinyl urea product, this effect was not achieved through any of the additives used.
2.3.4 Aziridine substitution

After demonstrating effective pathways to both the oxazolidinone and the aziridinyl urea product using solvent and amine stoichiometry, consideration was directed toward product selectivity via aziridine substitution. The oxazolidinone generation seen so far was not surprising, because the methylene group on the trityl bicyclic aziridine is very exposed. It was expected that adding steric bulk to this carbon should shift the product preference to the aziridinyl urea. To explore this possibility, propyl bicyclic aziridine 1b was treated with the same set of nucleophilic amines as trityl bicyclic aziridine 1a. In every case, the reactions provided aziridinyl urea 10 without any indication of the oxazolidinone product (Table 10).

Table 10. Yields for ring opening reactions with propyl bicyclic aziridine

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Purified Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>Benzyamine</td>
<td>26%</td>
</tr>
<tr>
<td>10b</td>
<td>Morpholine</td>
<td>20%</td>
</tr>
<tr>
<td>10c</td>
<td>2-methoxyethylamine</td>
<td>10%</td>
</tr>
<tr>
<td>10d</td>
<td>Aniline</td>
<td>27%</td>
</tr>
<tr>
<td>10e</td>
<td>N-methylbenzylamine</td>
<td>40%</td>
</tr>
<tr>
<td>10f</td>
<td>Isobutylamine</td>
<td>42%</td>
</tr>
<tr>
<td>10g</td>
<td>Allylamine</td>
<td>57%</td>
</tr>
<tr>
<td>10h</td>
<td>Isopropylamine</td>
<td>39%</td>
</tr>
<tr>
<td>10i</td>
<td>Propylamine</td>
<td>55%</td>
</tr>
</tbody>
</table>

Although purified yields were moderate, crude $^1$H NMR indicated relatively clean product in most cases. It was proposed that silica gel purification could be responsible for
the reduction in yield as its acidic nature could facilitate some aziridine ring opening. A few reactions were repeated to provide material for alternative purification attempts. In one case with benzylamine, a short pad of silica was effective at removing the slight excess of amine and provided the product in a 63% yield. Passing previously purified (via silica) material through neutral or acidic Alumina actually reduced the mass by approximately 25% for products from allylamine and benzylamine. It is possible that repeating these reactions and purifying them on a short pad of silica, instead of the long column used for flash chromatography, or the use of one molar equivalent of amine with no purification, would greatly improve the yields.

To determine the effectiveness of aziridine substitution for generating aziridinyl urea compounds, methyl bicyclic aziridine 1j was synthesized. A single ring-opening reaction was performed with this compound to see if the methyl substitution, representing the smallest alkyl group available, would maintain the selectivity exhibited by propyl bicyclic aziridine 1b. A sample of methyl bicyclic aziridine 1j was treated with allylamine in methylene chloride then analyzed by crude $^1$H NMR, which confirmed that aziridinyl urea 47 was the only product with no oxazolidinone present (Scheme 41).

![Scheme 41. Methyl bicyclic aziridine opening with allylamine](image-url)
This seemed to indicate that any substitution on the aziridine can lead to exclusive formation of the aziridinyl urea product.

Because all reactions with trityl bicyclic aziridine 1a in DMF provided exclusively the oxazolidinone and all reactions with propyl bicyclic aziridine 1b provided exclusively aziridinyl ureas, a single reaction with isopropyl bicyclic aziridine 1g was performed with 110 mol% of phenethylamine in DMF to demonstrate the dominant factor between solvent and substitution (Scheme 42).

![Scheme 42. Selectivity of substituted aziridine in DMF](image)

Crude $^1$H NMR indicated very clean conversion to aziridinyl urea GWB-126 and no evidence of oxazolidinone product.

2.4 Conclusions

It has been demonstrated that trityl bicyclic aziridine 1a can be opened with amines to give exclusively oxazolidinones 8 by performing the reactions in the polar solvent, DMF. Computational chemistry has been performed demonstrating that the stabilization of the transition state provides the oxazolidinone product. Trityl bicyclic aziridine 1a also gives exclusively aziridinyl urea products 9 upon reaction with primary
amines by using six or more molar equivalents of amines in toluene. Computational calculations support the hypothesis that less polar solvents lack the stabilizing effects necessary to form the oxazolidinone product allowing the aziridinyl urea to form. Secondary amines tend to give oxazolidinone product \(8\) even with increased stoichiometry in toluene, which is attributed to the steric hindrance of the amine. Substituted bicyclic aziridines can be synthesized and opened with amines to give exclusively aziridinyl urea products due to the steric bulk at the aziridine, which hinders nucleophilic attack at the aziridine carbon. This preference can occur even with methyl aziridine substitution, the smallest alkyl group; with as little as 110 mol\% of amine; and regardless of solvent polarity, as demonstrated by a representative sample in DMF.
CHAPTER 3: AZIRIDINYL UREAS AS PEPTIDOMIMETICS

3.1 Introduction

Peptidomimetics are analogs that are often modeled after naturally occurring peptides to exhibit enhanced therapeutic value. Biological proteins that act as enzymes to catalyze biochemical processes are peptides made up mostly of essential L-amino acid residues. In biological systems these peptides are readily hydrolyzed and either recycled or excreted after performing some function. This leads to short biological lifetimes and low bioavailability. Peptidomimetic compounds are designed to mimic the activity of naturally occurring peptides, but with modifications that protect them against proteolysis. These compounds may contain naturally-occurring L-amino acid residues, D-enantiomers of essential amino acids, heterocyclic modifications, acylated terminal amines, and/or esterified terminal carbon atoms. Peptidomimetics can be isosteres of peptide compounds with the carbon skeleton or side chain functional groups rearranged for increased stability without changing the three dimensional conformation.

A class of peptidomimetics called peptoids includes modifications to the peptide backbone.\textsuperscript{26} Compounds of the $\alpha$-peptoid class are synthesized as $N$-acylated glycine residues where the side chain is attached to the amine terminus (Figure 8).

![Figure 8. Example of $\alpha$-peptoid structure](image-url)
These isosteres show increased stability against proteolysis because the nitrogen has been alkylated making breakdown of the peptide bond more difficult. The tertiary nature of the amide can lead to a difference in the three dimensional conformation of the peptoid and also eliminates possible hydrogen bonding interactions with a target enzyme. β-peptoids are isosteres of amino acid residues where the amino group has been synthesized at the beta-position instead of the alpha-position (Figure 9).

β-peptoids are isosteres of amino acid residues where the amino group has been synthesized at the beta-position instead of the alpha-position (Figure 9).

These homologous amino acid analogs retain the amide proton and therefore the potential for hydrogen bonding, but place a methylene group between the nitrogen and the carbonyl group. A small set of peptidomimetic hybrids containing both of these types of peptoids was recently reported with biological activity against *E. coli* strains and several other targets. Figure 10 shows an example from this set, which contains 14 residues; an acylated terminal amine; α-peptoids with alkylated amines to mimic phenylalanine side chains; β-peptoids, which extend the carbon backbone with methylene groups; and a primary amide in place of the terminal carboxylic acid.
Figure 10. Recently reported peptidomimetic containing α- and β-peptoids

This set of compounds demonstrated MIC values of 1-8 µM against *E. coli.*, which is promising compared to a value greater than 128 µM for Vancomycin, a common antibiotic.\(^{26}\)

Ximegalatran 49 is a serine protease inhibitor that acts as a direct thrombin inhibitor and is used as an anticoagulant.\(^{27}\) It is an orally administered peptidomimetic prodrug, which is metabolized to the active ingredient, Megalatran 50 (Scheme 43).

Scheme 43. Metabolism of Ximegalatran prodrug to give Megalatran
Megalatran 50 contains an arginine mimic and an electrophilic \( N \)-acyl-azetidine, which leads to the inhibition of thrombin, the enzyme responsible for coagulation and blood clot formation.\(^{27-29}\)

Several selected peptidomimetic inhibitors of cathepsin B, including E-64 (51), CA-074 (52), Tokaramide A (53), and Miraziridine A (54) demonstrate a range of structural strategies for inhibition. Cathepsin B is a cysteine protease found in mammalian lysosomes and is responsible for the cleavage of peptide bonds.\(^{30}\) It can also cause cancer and tumor metastasis; inflammation and arthritis; pancreatitis; and is a common target for therapeutic drug discovery.\(^{31, 32}\)

A naturally occurring compound isolated from soil samples, designated E-64, was reported to inhibit the cysteine proteases, cathepsin B and L, papain, and calpains, though no specificity was demonstrated (Figure 11).\(^{33, 34}\)

![Figure 11. Structure of E-64](image)

This compound contains a guanidine group; leucine group; and an epoxide, which acts as an electrophile for covalent bonding and inhibition of cysteine proteases.
Several analogs of E-64 were later synthesized and tested as cysteine protease inhibitors. This led to the discovery of a compound, designated CA-074 that demonstrated specificity for cathepsin B (Figure 12).

![Figure 12. Structure of CA-074](image)

Compound CA-074 contained the peptide structure of L-leucyl-L-proline, but was synthesized with a propylamide in place of the terminal carboxylic acid. The electrophilic epoxide was determined to be essential to the inhibitory activity due to its electrophilicity.

Tokaramide A is a naturally occurring compound isolated from marine sponges by Fusetani, et al, and found to have biological activity with an IC\textsubscript{50} value of 29 ng/mL against cathepsin B (Figure 13).

![Figure 13. Structure of Tokaramide A](image)
The structure includes two L-valine and one L-arginine residues, but the C-terminus is an electrophilic aldehyde instead of a carboxylic acid. The mechanism of inhibition seems to follow that of the previously mentioned compounds in that a tetrahedral hemithioacetate forms via covalent bond with the cysteine residue of the protease.\textsuperscript{33}

Fusetani, \textit{et al}, reported that a second cysteine protease inhibitor was isolated from the same marine sponge as Tokaramide A, called Miraziridine A (Figure 14).\textsuperscript{36}

![Figure 14. Structure of Miraziridine A](image)

The structure was determined by several analytical methods and two total syntheses were accomplished to confirm the structure.\textsuperscript{36-38} Synthetic Miraziridine A was found to have very similar \( IC_{50} \) value (2.0 \( \mu \)M) compared to the natural source (2.1 \( \mu \)M). To determine the source of activity, three analogs of Miraziridine A were synthesized, the first excluded the vinylogous arginine (55); the second excluded the aziridine functionality with acetylation of the remaining terminal amine (56); and the third omitted both of these groups leaving a free amino-L-leucyl-statinyld-\( \alpha \)-aminobutyric acid tripeptide (57) (Figure 15).\textsuperscript{38}
Figure 15. Analogs of Miraziridine A

Based on these modifications and the corresponding IC\textsubscript{50} values, it is clear that the aziridine plays an important role in the inhibition of cathepsin B. The vinylogous arginine residue also seems to contribute to inhibition in a significant way.

The aziridinyl urea compounds that were generated from the selectivity studies in Section 2.3.4 were peptide isosteres and also contained an electrophilic site making them
reasonable candidates for protease inhibition. Two bicyclic aziridines contained substitution identical to the amino acid side chains of phenylalanine (bicyclic aziridine 1f) and valine (bicyclic aziridine 1g). To further enhance the biological potential of aziridinyl ureas, fused bicyclic aziridines were opened with amines that could act as amino acid mimics in a manner similar to α-peptoids (Figure 16).

![Figure 16. Amines selected to mimic amino acid side chains](image)

Additionally, several bicyclic aziridines were opened with amino acid benzylamides and dipeptide benzylamides generating amino acid containing aziridinyl
ureas. Several attempts were made to modify selected aziridinyl urea compounds by acylation, oxidation, coupling reagents, and alcohol to amine conversion with some success. Selected final compounds were tested for biological activity as antimicrobial therapeutics; and as inhibitors of human leukocyte elastase (HLE), thrombin, and cathepsin B.

3.2 Aziridinyl urea compounds from amines

Many of the bicyclic aziridines synthesized in Section 2.2 were submitted to ring opening reactions with amines to give a library of aziridinyl ureas (Table 11). A few amines used in the propyl aziridine selectivity study were used here and several more were selected to mimic amino acid residues.
### Table 11. Yields for aziridinyl urea compounds

<table>
<thead>
<tr>
<th>Amine, HNR&lt;sup&gt;3&lt;/sup&gt;R&lt;sup&gt;4&lt;/sup&gt;</th>
<th>1c</th>
<th>1d</th>
<th>1e</th>
<th>1f</th>
<th>1g</th>
<th>1h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isobutylamine</td>
<td>87%</td>
<td>88%</td>
<td>89%</td>
<td>82%</td>
<td>91%</td>
<td>86%</td>
</tr>
<tr>
<td>2-methoxyethylamine</td>
<td>86%</td>
<td>87%</td>
<td>87%</td>
<td>62%</td>
<td>91%</td>
<td>69%</td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>84%</td>
<td>83%</td>
<td>97%</td>
<td>86%</td>
<td>94%</td>
<td>78%</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>99%</td>
<td>88%</td>
<td>91%</td>
<td>56%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>Isoamylamine</td>
<td>89%</td>
<td>84%</td>
<td>68%</td>
<td>70%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>Tyramine</td>
<td>46%</td>
<td>98%</td>
<td>55%</td>
<td>56%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>Tryptamine</td>
<td>46%</td>
<td>91%</td>
<td>63%</td>
<td>62%</td>
<td>79%</td>
<td></td>
</tr>
<tr>
<td>3-(methylthio)propylamine</td>
<td>85%</td>
<td>88%</td>
<td>61%</td>
<td>75%</td>
<td>99%</td>
<td></td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>96%</td>
<td>54%</td>
<td>32%</td>
<td>63%</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>Allylamine</td>
<td>83%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyllamine</td>
<td>97%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reactions were carried out in methylene chloride using 150 mol% of each amine from the new set. The stoichiometry of the amine was increased slightly to expedite the reaction. This, and the choice of solvent, has no apparent effect on the reaction as the aziridinyl urea is the only product seen with substituted aziridines. Each reaction was purified via flash chromatography to provide good yields in almost every case.

### 3.3 Aziridinyl urea compounds from amino acid and peptide derivatives

Ring opening reactions of bicyclic aziridines with amino acid and peptide derivatives posed new challenges, which were not encountered in reactions with amines. All bicyclic aziridines reported to this point were generated as racemic mixtures. The
introduction of chiral non-racemic amino acid and peptide derivatives provided diastereomeric product mixes of aziridinyl ureas.

The resulting aziridinyl urea diastereomers exhibited little resolution on silica gel. In a few cases, aziridinyl urea products from dipeptide derivatives were successfully resolved to provide one or both diastereomers as isolated products. Glycine benzylamide provided the expected racemic mixture of aziridinyl urea products. All other amino acid and peptide derivatives provided diastereomers, which were not successfully isolated during purification.

3.3.1 Preparation of amino acid and peptide derivatives

This section includes the synthesis and preparation of all amino acid and dipeptide derivatives used in the synthesis of aziridinyl urea compounds. All starting amino acids used were commercially available and in all cases only the L-stereoisomer was employed. Glycinyl-glycine benzylamide 13 was synthesized and provided by Dr. Susann Krake.

The first synthetic pathway for the generation of amino benzylamides from free amino acids required the α-amine to be Cbz-protected, followed by conversion to the Cbz-protected amino benzylamide and hydrogenolysis to the desired product (Table 12).
Table 12. Yields of amino benzylationdides via Cbz-protection pathway

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amino Acid</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Glycine (Gly)</td>
<td>89%*</td>
<td>60%</td>
<td>65%*</td>
</tr>
<tr>
<td>b</td>
<td>Proline (Pro)</td>
<td>93%*</td>
<td>73%</td>
<td>94%*</td>
</tr>
<tr>
<td>c</td>
<td>Phenylalanine (Phe)</td>
<td>66%</td>
<td>69%</td>
<td>-</td>
</tr>
</tbody>
</table>

* - Clean crude yield

Cbz-chloride was dissolved in THF and added to a basic aqueous solution of glycine 58a at 0 °C, then stirred overnight to provide an 89% crude yield of Cbz-protected glycine 59a following aqueous workup. This material was treated with Cbz-chloride, N-methylmorpholine, and benzylamine in THF at -78 °C. The reaction was allowed to warm to room temperature over two hours then solid N-methylmorpholinium chloride was filtered off to provide crude Cbz-protected glycine benzylamide 60a. Purification of this compound via flash chromatography provided a 60% yield. Deprotection of the Cbz-group was effected by hydrogenolysis with hydrogen gas and 10% palladium on carbon in THF. The crude reaction mixture was filtered through Celite to remove palladium and solid matter then concentrated to give a 65% yield of clean 12 without purification as indicated by crude ¹H NMR.
Phenylalanine and proline benzylamides were also prepared following this procedure. Cbz-protection of proline 58b provided a 93% yield of 59b without purification, for which the crude $^1$H NMR looked reasonably clean. The next step was purified on silica gel to provide Cbz-protected proline benzylamide 60b in a 73% yield. Finally, the deprotection step afforded a 94% yield of proline benzylamide 61b following silica gel purification. Cbz-protected phenylalanine 59c was purified to provide a 66% yield of reasonably clean material. The next step provided a 69% yield of Cbz-protected phenylalanine benzylamide 60c after silica gel purification. The deprotection step was not performed as another synthetic pathway was being used concurrently and ultimately replaced this Cbz-protected pathway.

As an alternative to the Cbz-protected pathway, Boc-protection of the α-amine of each amino acid was followed by conversion to the Boc-protected amino benzylamide, which was easily deprotected to the desired amino benzylamide using TFA (Table 13).
Table 13. Yields of amino benzylamides via Boc-protection pathway

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amino Acid</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Glycine (Gly)</td>
<td>98%</td>
<td>90%*</td>
<td>90%</td>
</tr>
<tr>
<td>b</td>
<td>Proline (Pro)</td>
<td>90%*</td>
<td>73%*</td>
<td>53%</td>
</tr>
<tr>
<td>c</td>
<td>Phenylalanine (Phe)</td>
<td>100%*</td>
<td>100%*</td>
<td>67%</td>
</tr>
</tbody>
</table>

* - Clean crude yield

Boc-anhydride in THF was added to a basic aqueous solution of glycine 58a to provide Boc-protected glycine 62a after an aqueous work up. The product from this first step was purified on silica gel to provide a 98% yield, however $^1$H NMR spectrum for the crude material was very clean. This material was treated with two molar equivalents of CDI and 120 mol% of benzylamine in methylene chloride overnight, after which the reaction went through an aqueous workup. TLC and $^1$H NMR indicated that, in addition to 63a, some amount of benzylamine remained, however this reaction was not purified. The final deprotection step was effected with several equivalents of TFA, followed by aqueous workup and purification on silica gel to provide a 90% yield of glycine benzylamide 12.

Following the above steps, Boc-protection of proline 58b gave a 90% yield and a reasonably clean crude $^1$H NMR for 62b. This material was treated with CDI and 105
mol% of benzylamine to provide a 73% yield of Boc-protected proline benzylamide 63b and clean crude \textsuperscript{1}H NMR. The TFA-deprotection step was purified via silica gel to afford a 53% yield of clean proline benzylamide 61b.

The synthesis was also repeated for phenylalanine, which was Boc-protected to provide a clean crude \textsuperscript{1}H NMR spectrum and a quantitative yield of 62c. Step 2 also provided a quantitative yield and very clean crude \textsuperscript{1}H NMR for 63c. The final step was purified on silica gel to give a 67% yield of clean phenylalanine benzylamide 61c.

The preparation of peptides combined Boc-protected amino acids 62 and amino benzylamide compounds 61 to generate dipeptide benzylamides that could also be used as nucleophiles in aziridine ring opening reactions. Valinyl-glycine benzylamide and valinyl-phenylalanine benzylamide were synthesized, following the Boc-protection of valine 58d (Scheme 44).

![Scheme 44. Boc-protection of valine](image)

The same procedure as described for the Boc-protection of glycine was followed to provide a reasonably clean crude \textsuperscript{1}H NMR spectrum in a 97% yield of Boc-valine 62d.

Dipeptide benzylamides were generated by coupling Boc-protected valine 62d with glycine benzylamide 12 and phenylalanine benzylamide 61c using EDCI and HOBr, then deprotecting the Boc-group using TFA (Scheme 45).
The reaction between Boc-protected valine 62d and glycine benzylamide 12 was purified on silica gel to provide a 71% yield of clean 64. The coupling reaction between Boc-valine 62d and phenylalanine benzylamide 61c was purified on silica gel to provide 65 in a 72% yield. The deprotection step was performed with 10 molar equivalents of TFA to remove the Boc-group and generate the TFA-salt of the dipeptide, which was then treated with sodium hydroxide and extracted to obtain the salt-free dipeptide benzylamide. The deprotection of Boc-valinyl-glycine benzylamide 64 and Boc-valinyl-phenylalanine benzylamide 65 provided yields of 100% and 84%, of 15 and 14, respectively.

Isoleucyl-proline methyl ester was synthesized by coupling Boc-protected isoleucine and proline methyl ester, both of which were prepared from the corresponding amino acids. Boc-protected isoleucine 58e was prepared by treating isoleucine with Boc-anhydride and sodium hydroxide then performing an aqueous workup to give a reasonably clean crude $^1$H NMR of 62e (Scheme 46).
Scheme 46. Boc-protection of isoleucine

Proline methyl ester hydrochloride 66 was prepared by adding proline 58b to a mixture of thionyl chloride and methanol at 0 °C then concentrated under high vacuum to provide a reasonably clean crude $^1$H NMR without purification. Just prior to the coupling reaction, proline methyl ester HCl was neutralized with triethylamine in diethyl ether. The solid triethylammonium chloride was filtered off and the filtrate was concentrated to give a very clean $^1$H NMR and a crude yield of 65% for 67 (Scheme 47).

Scheme 47. Preparation of proline methyl ester

The two amino acid derivatives were coupled using DCC in ethyl acetate overnight, then purified by flash chromatography to provide an 88% yield of Boc-protected-isoleucinyl-proline methyl ester 68 (Scheme 48).
Scheme 48. Synthesis of Boc-protected isoleucinyl-proline methyl ester 68

Compound 68 was dissolved in methylene chloride and treated with TFA to deprotect the Boc-group, then treated with aqueous sodium hydroxide solution in an attempt to neutralize the TFA-salt and provide the free dipeptide methyl ester (Scheme 49).

Scheme 49. Deprotection of Boc-isoleucinyl-proline methyl ester 68

Organic extraction of the crude reaction returned almost no material, as the basic medium had likely removed the methyl ester providing 70 instead of desired product 69. The TFA-deprotection was repeated on a test scale, then neutralization was attempted in a biphasic solvent mix of CH₂Cl₂ and aqueous sodium bicarbonate (200 mol %), which did not result in complete neutralization, however the methyl ester was intact by ¹H NMR. Neutralization of the TFA salt was also attempted with aqueous potassium carbonate (500 mol%), which underwent complete neutralization without loss of the methyl ether,
however silica gel purification resulted in either the loss of the methyl ester or the desired product underwent self-condensation upon concentration. Ultimately, the dipeptide was used as the TFA-salt in an attempt to effect \textit{in situ} neutralization during a bicyclic aziridine opening reaction.

\textit{3.3.2 Trityl bicyclic aziridine reactions with amino acid derivatives}

Reactions performed between trityl bicyclic aziridine \textit{1a} and amino acid derivatives provided complicated mixtures including diastereomers of both oxazolidinones and aziridinyl ureas. Following the selectivity work of Section 2.3, initial reactions were performed in toluene using six molar equivalents of amino benzylamides in an attempt to generate exclusively aziridinyl urea compounds (Scheme 50).

\begin{center}
\textbf{Scheme 50. Reactions of trityl bicyclic aziridine \textit{1a} with amino benzylamides}
\end{center}

Opening trityl bicyclic aziridine \textit{1a} with 620 mol\% of glycine benzylamide \textit{12} provided a 19\% isolated yield of aziridinyl urea \textit{72a} after flash chromatography, however
another 40% yield of product was recovered as an undetermined ratio of oxazolidinone 71a to aziridinyl urea 72a. Reaction with 600 mol% of proline benzylamide 61b provided a mixture of aziridinyl urea diastereomers 72b and although purification provided the aziridinyl urea products free of oxazolidinone 71b in a 61% yield, the individual diastereomers were not separated. Reaction with 620 mol% of phenylalanine benzylamide 61c provided a 63% yield of undesired oxazolidinone 71c diastereomers only. This reaction was repeated with 110 mol% of phenylalanine benzylamide and again provided a 63% yield of oxazolidinone diastereomers 72c. Overall, the selectivity conditions that previously demonstrated success with amines did not provide aziridinyl ureas with amino acid derivatives when using trityl bicyclic aziridine.

3.3.3 Substituted bicyclic aziridines with glycine benzylamide

The reaction of propyl bicyclic aziridine 1b with glycine benzylamide 12 was monitored by $^1$H NMR in CDCl$_3$ and provided a 56% yield of clean racemic aziridinyl urea 73 after silica gel purification (Scheme 51).

Scheme 51. Propyl bicyclic aziridine 1b opened with glycine benzylamide 12
Several reactions were performed with phenethyl bicyclic aziridine 1c and glycine benzylamide 12 to provide good yields of the racemic product GWB-94 (Table 14).

Table 14. Phenethyl bicyclic aziridine 1c opened with glycine benzylamide 12

<table>
<thead>
<tr>
<th>Entry</th>
<th>61a equivalents</th>
<th>Temperature</th>
<th>Reaction Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6</td>
<td>Room temperature</td>
<td>42 hours</td>
<td>66%</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>50 ºC/Room temperature</td>
<td>48 hours</td>
<td>99%</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>Room temperature</td>
<td>68 hours</td>
<td>79%</td>
</tr>
</tbody>
</table>

Because glycine benzylamide 12 exhibited limited solubility in methylene chloride, consumption of starting material required either longer reaction times or increased stoichiometry of the nucleophile. The first reaction was performed at room temperature for nearly two days then purified by flash chromatography to provide a 66% yield (Table 14, entry 1). One reaction was heated to 50 ºC (to increase solubility) for the first 24 hours, after which reaction was continued at room temperature for another 24 hours (entry 2). That reaction provided material that was insoluble in methylene chloride, which was filtered off, washed with methylene chloride, and compared to the filtrate by $^1$H NMR. Both spectra showed clean product and the combined material a 99% yield. The third reaction ran over the weekend at room temperature and was purified by adding hexanes to precipitate the product from methylene chloride providing a 79% yield (entry 3).
Bicyclic aziridines 1d through 1h were also treated with 110 mol% of glycine benzylamide 12 in methylene chloride and purified to provide final aziridinyl urea compounds as racemic mixtures (Scheme 52).

Scheme 52. Aziridinyl ureas from glycine benzylamide 12

3.3.4 Substituted bicyclic aziridines with amino acid and peptide derivatives

Reaction between phenethyl bicyclic aziridine 1c and phenylalanine benzylamide 61c showed a mixture of diastereomers 74 by crude $^1$H NMR (Scheme 53).

Scheme 53. Phenethyl bicyclic aziridine opened with phenylalanine benzylamide

Chromatography provided an 11% yield of one diastereomer and a 4% yield of the other. These compounds exhibited resolution by TLC with $R_f$ values of 0.44 and 0.55
in 100% ethyl acetate, but were very difficult to separate by flash chromatography. The absolute stereochemistry of the isolated diastereomers was not determined.

Reaction between phenethyl bicyclic aziridine 1c and proline benzylamide 61b was attempted twice; and although in each case two diastereomeric products were clear by crude $^1$H NMR and several TLC solvent systems indicated a 10% difference between R$_f$ values, no isolation of diastereomers was successful (Scheme 54).

Scheme 54. Phenethyl bicyclic aziridine 1c opened with proline benzylamide 61b

Three dipeptide benzylamide nucleophiles were also used in reactions with phenethyl bicyclic aziridine. Reaction with glycinyl-glycine benzylamide 13 led to a white solid which was only soluble in DMSO (Scheme 55). The crude product was very clean by $^1$H NMR without further purification and provided an 82% yield of racemic 16.

Scheme 55. Phenethyl bicyclic aziridine opened with glycinyl-glycine benzylamide
Reaction with valinyl-phenylalanine benzylamide 14 provided a crude $^1$H NMR spectrum that indicated the expected diastereomeric mixture, however only a single diastereomer was isolated from flash chromatography in a 35% yield (Scheme 56). Once again, absolute stereochemistry was not determined.

Scheme 56. Bicyclic aziridine 1c opened with valinyl-phenylalanine benzylamide 12

The most successful result came from reaction between phenethyl bicyclic aziridine 1c and valinyl-glycine benzylamide 15 (Scheme 57).

Both expected diastereomers were evident by crude $^1$H NMR and TLC indicated an $R_f$ difference of 10%. Complete resolution did not occur during flash chromatography, but purification provided 37% of one diastereomer and 21% of the other. Fractions
containing both diastereomers were combined and analyzed by $^1$H NMR, which indicated a 3 : 2 ratio of diastereomers providing an estimated total of 86% yield for the reaction (54% and 32%, for respective diastereomers, overall). Peptide stereochemistry was known and aziridine stereochemistry was relative *trans*, but yields could not be assigned to either absolute stereochemistry.

Cyclohexyl bicyclic aziridine 1e was treated with 110 mol% of amino acid and peptide benzylamides in methylene chloride (Scheme 58).

Scheme 58. Reaction of bicyclic aziridine 1e with amino and dipeptide benzylamides

These compounds exhibited very low solubility in common $^1$H NMR solvents, with the exception of proline-containing product 76b, which was soluble in CDCl$_3$. All other compounds were submitted for crude $^1$H NMR analysis in DMSO-d$_6$. In all cases, the expected products were observed either as diastereomeric mixtures (from chiral nucleophiles) or as a racemic mixture (glycine benzylamide). With the exception of 76b, these compounds would not dissolve in common solvents at room temperature (CH$_2$Cl$_2$, MeOH, EtOH, acetone, CH$_3$CN, or THF). A small sample of each product was successfully dissolved in THF at 60 °C, in an attempt to recrystallize the compounds;
however no material was returned upon standing or cooling. Due to this solubility issue, no separation of diastereomers was performed. Based on crude masses and the NMR analysis, yields were approximately quantitative in all cases, even after discounting the excess of starting benzylamides.

Although reaction of cis-hex-3-enyl bicyclic aziridine 1d with proline benzylamide 61b and phenylalanine benzylamide 61c each appeared by crude $^1$H NMR to have gone to completion, no purification was attempted due to the consistent challenge of isolating diastereomers (Scheme 59).

![Scheme 59. Bicyclic aziridine 1d opened with amino benzylamides](image)

In addition to the amino benzylamide reactions, a single dipeptide reaction was attempted with cis-hex-3-enyl bicyclic aziridine 1d. This dipeptide was prepared as the TFA salt, so to determine whether neutralization could be performed in situ, cis-hex-3-enyl bicyclic aziridine 1d and the TFA salt of isoleucyl-proline methyl ester 69 were dissolved in methylene chloride and treated with an excess of triethylamine (Scheme 60).
Although all starting bicyclic aziridine appeared to be consumed based on crude $^1H$ NMR and the spectrum did not seem unreasonable for the expected mix of diastereomeric products, neither product was isolated after silica gel purification.

At this point, it was determined that the isolation of diastereomers, from a reasonably diverse range of bicyclic substrates and amino acid (and dipeptide) derivatives, presented too great of a challenge to persist. The issue prompted an investigation of asymmetric aziridination (Chapter 4).

3.4 Modifications of aziridinyl urea compounds

Using selected aziridinyl urea compounds, several modifications were considered in order to increase the functionality and enhance the peptidic nature of these compounds. Although the generation of aziridinyl ureas using chiral amino acids introduced resolution challenges, glycine benzylamide worked very well. This provided an aziridine-containing peptide analog that contained an amino acid, so if some modification could allow for amino acid addition at the primary alcohol then the peptide nature could be extended. The first consideration was converting the alcohol to amine such that common
peptide coupling reagents could be used for this purpose. When this work proved unsuccessful, the focus was simplified to the acylation of aziridinyl urea alcohols.

### 3.4.1 Alcohol to amine conversion attempts

Typical conditions for the conversion of an alcohol to an amine are summarized in Scheme 61, including the conversion of alcohols to sulfonates; the conversion of alcohols or sulfonates to azides or amines; and the conversion of azides to amines.

![Scheme 61. Typical conversions of an alcohol to an amine](image)

Several reactions were performed to convert the alcohol group of aziridinyl ureas into amines (Table 15). One method was attempted for the direct conversion in a one-pot reaction, but many other attempts were made to first convert the alcohol to an azide, tosylate, or triflate under various conditions. Some of these reactions were followed by ammonia gas addition in an effort to replace the leaving group with an amine. Azide
substitution was not successful, but would have been followed by reduction to give the amine. Two aziridinyl urea substrates were used for these substitution reactions.

Table 15. Results of aziridinyl urea alcohol conversion

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substitution</th>
<th>Target</th>
<th>Conditions</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Allyl, GWB-95</td>
<td>NH₂, 79</td>
<td>NaN₃, PPh₃, DMF:CCl₄</td>
<td>90 °C</td>
</tr>
<tr>
<td>2</td>
<td>Allyl, GWB-95</td>
<td>N₃, 80</td>
<td>PPh₃, DIAD, DPPA, THF</td>
<td>Room temp</td>
</tr>
<tr>
<td>3</td>
<td>Allyl, GWB-95</td>
<td>OTₛ, 81</td>
<td>TsCl, Et₃N, CH₂Cl₂</td>
<td>Room temp</td>
</tr>
<tr>
<td>4</td>
<td>Allyl, GWB-95</td>
<td>OTₛ, 81</td>
<td>TsCl, Et₃N, DMAP, CH₂Cl₂</td>
<td>Room temp</td>
</tr>
<tr>
<td>5</td>
<td>Allyl, GWB-95</td>
<td>OTₛ, 81</td>
<td>Ts₂O, pyridine, CH₂Cl₂</td>
<td>-78 °C</td>
</tr>
<tr>
<td>6</td>
<td>Allyl, GWB-95</td>
<td>OTₛ, 81</td>
<td>Ts₂O, pyridine, CH₂Cl₂</td>
<td>-78 °C</td>
</tr>
<tr>
<td>7</td>
<td>Phenethyl, GWB-102</td>
<td>NH₂, 82</td>
<td>Ts₂O, pyridine, THF, then NH₃</td>
<td>0 °C</td>
</tr>
<tr>
<td>8</td>
<td>Phenethyl, GWB-102</td>
<td>NH₂, 82</td>
<td>Ts₂O, pyridine, CH₂Cl₂, then NH₃</td>
<td>Room temp</td>
</tr>
<tr>
<td>9</td>
<td>Phenethyl, GWB-102</td>
<td>NH₂, 82</td>
<td>Tf₂O, pyridine, CH₂Cl₂, then NH₃</td>
<td>0 °C</td>
</tr>
<tr>
<td>10</td>
<td>Phenethyl, GWB-102</td>
<td>OTₛ, 83</td>
<td>Ts₂O, NaH, DMF</td>
<td>-78 °C</td>
</tr>
<tr>
<td>11</td>
<td>Phenethyl, GWB-102</td>
<td>OTₛ, 83</td>
<td>TsCl, pyridine, CH₂Cl₂</td>
<td>-78 °C, -23 °C</td>
</tr>
<tr>
<td>12</td>
<td>Phenethyl, GWB-102</td>
<td>OTₛ, 83</td>
<td>Ts₂O, pyridine, CH₂Cl₂</td>
<td>-78 °C, -23 °C</td>
</tr>
<tr>
<td>13</td>
<td>Phenethyl, GWB-102</td>
<td>OTf, 84</td>
<td>Tf₂O, pyridine, CH₂Cl₂</td>
<td>-78 °C, -23 °C</td>
</tr>
</tbody>
</table>

Reddy reported a procedure for the direct, one-pot conversion of alcohols to amines using sodium azide and two molar equivalents of triphenylphosphine in a solvent mix of carbon tetrachloride (CCl₄) and DMF at 90 °C. The proposed mechanism indicated that the alcohol would react with triphenylphosphine, which would then undergo azide substitution. The azide would react with the second equivalent of triphenylphosphine to give an iminophosphorane, which would decompose to the amine upon the addition of water.
A reaction was performed using these conditions in an effort to convert the primary alcohol of **GWB-95** to corresponding amine 79 (entry 1). After an aqueous workup, the $^1$H NMR for this reaction did not indicate any product or remaining starting material. Most notable was the absence of aziridine peaks, though the spectrum was missing most of the expected peaks. Because CCl$_4$ can generate chloride anions at elevated temperatures, it was suspected that the aziridine had undergone nucleophilic substitution. It is also possible that the aziridine ring of the product (or starting material) is susceptible to attack by the azide anion.

In an effort to minimize the presence of nucleophiles, entry 2 was attempted in THF using triphenylphosphine, diisopropylazodicarboxylate (DIAD), and diphenylphosphoryl azide (DPPA) to convert the alcohol of **GWB-95** to the corresponding azide 80.$^{41}$ No product or starting material was evident from the crude $^1$H NMR and purification provided no clean material.

Several tosylation attempts were made such that subsequent azide substitution could be performed. Two initial tosylation attempts with **GWB-95** were performed with tosyl chloride and triethylamine (entry 3), one of which also employed DMAP (entry 4). These reactions were submitted directly to flash chromatography to give fractions that showed a clear mixture of two unidentified side products by $^1$H NMR in each case, which was evident from separate pairs of tosyl and allyl signals. Entry 3 gave a mixture of side products in a 1 : 1 ratio and entry 4 provided a 2 : 1 mixture of unidentified compounds. These products could not be isolated for structure determination, but the expected aziridine methine protons were absent in each $^1$H NMR spectrum.
GWB-95 was treated with $p$-toluenesulfonic anhydride and pyridine in an attempt to generate tosylate 81 without the generation of chloride anions (entry 5). This reaction was submitted to an aqueous workup and purified via flash chromatography to provide a $^1$H NMR that looked like a single product, however in a very low yield. The reaction was repeated using the same conditions, but submitted directly the column without workup (entry 6). Although the crude $^1$H NMR spectra matched that from entry 5, no material was recovered from the column. It seemed that the desired tosylate product was being generated in some of these reactions, but because isolation was unsuccessful it was difficult to determine the impact of either aqueous workup or silica gel purification upon the yields.

Reactions were next attempted with GWB-102 to generate either tosylate 83 or triflate 84 in situ, then immediately treated with gaseous ammonia to convert the product into primary amine 82. First, GWB-102 was treated with tosyl anhydride and pyridine in THF at 0 ºC and allowed to warm to room temperature then ammonia was bubbled into the solution (entry 7). Although aziridinyl urea GWB-102 has two aromatic rings, a TLC of this reaction did not show any material under ultraviolet detection or after PMA stain. The reaction was filtered to recover insoluble material, which showed only tosyl peaks by $^1$H NMR. The rest of the material was submitted to column chromatography, which returned no mass, even after a complete flush with 10% methanol in ethyl acetate. The reaction was repeated at room temperature in methylene chloride to promote solubility of the tosyl anhydride (entry 8). Following ammonia addition, this reaction was submitted directly to crude $^1$H NMR, which was identical to that of entry 7.
**GWB-102** was treated with triflic anhydride with pyridine in methylene chloride at 0 ºC then allowed to warm to room temperature (entry 9). Ammonia was bubbled into the reaction, which was then sealed and stood overnight. Crude \(^1\)H NMR gave a reasonable spectrum, however purification with methanol in methylene chloride did not provide any clean material.

**GWB-102** was treated with tosyl anhydride and sodium hydride in DMF at -78 ºC then allowed to warm to room temperature (entry 10). Following aqueous workup, the crude \(^1\)H NMR spectrum showed only DMF, so the material was passed through a short pad of silica. The new \(^1\)H NMR showed no starting material and the expected aziridine peaks for the product were not present.

The final three reactions were performed with **GWB-102** initially at -78 ºC then kept at -23 ºC until workup in an effort to promote the kinetic acylations and slow nucleophilic addition at the aziridine. Tosylations were performed with tosyl chloride and pyridine in methylene chloride (entry 11) and with tosyl anhydride and pyridine in methylene chloride (entry 12). These reactions were diluted with methylene chloride and submitted directly to aqueous workup. The crude \(^1\)H NMR spectra were not clean, but could be rationalized to contain the desired product in low yields. Purification via flash chromatography did not provide any identifiable material. Finally, **GWB-102** was treated with triflic anhydride and pyridine in methylene chloride (entry 13) then submitted to aqueous workup and flash chromatography to provide no clean product.
3.4.2 Acylations

Following unsuccessful attempts to convert the alcohol of aziridinyl ureas to amines, several acylation pathways were pursued. This was attempted with acyl chlorides and anhydrides; and esterifications were performed using coupling reagents with carboxylic acids. These attempts had mixed results, but did provide the successful synthesis of some modified aziridinyl ureas. A few acylations were successful from reactions with isocyanates and a single Mitsunobu attempt was also successful, though yields for these reactions were modest.

3.4.2.1 Initial acylation attempts

Acyl chlorides are common reagents for acylation, so GWB-95 was first treated with acetyl chloride (Table 16, entry 1).

Table 16. Initial acylation attempts for aziridinyl urea GWB-95

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Target, X =</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AcCl</td>
<td>Ac</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>Ac₂O</td>
<td>Ac</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>(iPrCO)₂O, DMAP</td>
<td>COiPr</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>17, EDCI, DMAP</td>
<td>3,4-dimethoxyphenyl acetate</td>
<td>18</td>
</tr>
</tbody>
</table>

Following aqueous work up, the $^1$H NMR gave a fairly clean spectrum indicating a single compound and although some acylation product was evident by the methyl peak
around 2.0 ppm, this was not the desired product. The absence of aziridine methine signals indicated that the aziridine was opened by some nucleophile, most likely the chloride anion generated from acylation. COSY $^1$H NMR of the purified compound also showed a new amide doublet in addition to the expected amide triplet. The opening of the aziridine ring would generate a new amide bond further supporting this possibility.

To eliminate the potential for nucleophilic opening of the aziridine ring during acylation, GWB-95 was treated with acetic anhydride (entry 2). Both the crude $^1$H NMR and that following an aqueous workup showed the desired acylation product with aziridine methines intact and a new methyl group present in a 79% crude yield, however purification via flash chromatography returned no product. GWB-95 was also treated with isobutyric anhydride (entry 3) to provide a crude $^1$H NMR spectrum that was less clean than that of acetic anhydride, but nonetheless seemed reasonable for the desired product. Once again when flash chromatography was performed, no product was recovered.

Next, coupling reagents were considered with EDCI and DCC being among the most common. Esterification of GWB-95 with 3,4-dimethoxyphenyl acetic acid 17 was first attempted with EDCI (entry 4). Because the hydrochloride salt of EDCI was used, it was added last into the reaction mixture, which contained several equivalents of DMAP, in an effort to limit the liberated chloride anions from attack upon the aziridine ring. The aziridine methine protons were clearly intact in the crude $^1$H NMR spectrum, but were overshadowed by peaks with very large relative integration. Silica gel purification was performed but provided only impure product.
3.4.2.2 DCC coupling reactions

Dicyclohexylcarbodiimide (DCC) was used with different aziridinyl ureas and carboxylic acids to convert the alcohol to an ester (Table 17).

Table 17. Acylation attempts with DCC

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aziridinyl urea</th>
<th>$R^1$</th>
<th>$R^2$</th>
<th>Conditions</th>
<th>Target, $X \equiv$, product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GWB-95</td>
<td>PhEt</td>
<td>Allyl</td>
<td>AcOH, -23 °C to rt</td>
<td>Ac(17), 85</td>
</tr>
<tr>
<td>2</td>
<td>GWB-95</td>
<td>PhEt</td>
<td>Allyl</td>
<td>17, -78 °C to rt</td>
<td>3,4-dimethoxyphenyl acetate, 18</td>
</tr>
<tr>
<td>3</td>
<td>GWB-98</td>
<td>Pentyl</td>
<td>Bn</td>
<td>17, rt</td>
<td>3,4-dimethoxyphenyl acetate, 87</td>
</tr>
<tr>
<td>4</td>
<td>GWB-102</td>
<td>PhEt</td>
<td>PhEt</td>
<td>17, -78 °C to -23 °C</td>
<td>3,4-dimethoxyphenyl acetate, 88</td>
</tr>
</tbody>
</table>

GWB-95 was combined with acetic acid and DMAP, and then cooled to -78 °C before adding DCC (entry 1). The $^1$H NMR after aqueous workup seemed reasonable for the desired product, however silica gel chromatography did not improve the purity of the compound. It seemed that the dicyclohexyl urea (DCU) side product from the DCC coupling had a similar retention factor. This side product is known to be insoluble in methylene chloride and typically filtered, but this reaction was performed on a very small scale such that no solid was detected upon filtering. GWB-95 was next coupled with 3,4-dimethoxyphenyl acetic acid 17 by adding all reagents, cooling to -78 °C before adding DCC, then warming to room temperature (entry 2). The reaction was worked up and purified via flash chromatography to give a $^1$H NMR spectrum that looked reasonable, however the aziridine methines integrated to only 0.5 H in contrast to all other expected peaks giving the appropriate integration. 3,4-Dimethoxyphenyl acetic acid 17 was also
coupled with **GWB-98** (entry 3). This reaction did provide solid material that was insoluble in methylene chloride, which was filtered, submitted to $^1$H NMR, and identified as DCU. A comparison to impure product NMR spectra from Entries 1 and 2 confirmed that this side product is the major impurity in each case. The remaining filtrate from this reaction was purified via silica gel to provide a $^1$H NMR spectrum that seemed reasonable for the desired product, but also contained the DCU side product, with an overall impure yield of 69%. Finally, the coupling with 3,4-dimethoxyphenyl acetic acid 17 was performed with **GWB-102** and the reaction was carried out at -78 °C (entry 4). Once again, product fractions collected from flash chromatography contained DCU and the aziridine methine signals were not evident.

It is possible that the poor chromatographic resolution between dicyclohexyl urea and the acylated aziridinyl urea compounds is due to their structural similarity as both contain a urea functional group. NMR evidence seemed to show that this chemistry worked for different substrates and carboxylic acids, but a better purification method was necessary to remove the side product.

### 3.4.2.3 CDI coupling reactions

1,1-carbonyldiimidazole (CDI) was used next for coupling reactions, which seemed to provide better results than previous coupling attempts, though a stoichiometry study was required in order to optimize conditions (Table 18). CDI is appealing because the only side products are imidazole and carbon dioxide, the former of which can be removed by aqueous workup.
The most successful initial reaction coupled GWB-95 and 17 to provide a 46% yield of purified product from a one to two ratio of carboxylic acid to CDI (entry 1). This reaction was repeated with a one to one ratio of these reagents and only a catalytic amount of imidazole with the product yield expected to double (entry 2). This did not occur, in fact the crude $^1$H NMR looked very poor compared to the crude spectrum of the previously successful reaction and aziridine methines were not evident.

Several modifications were included in the next two reactions (Entries 3 and 4). The reactions were performed using two different aziridinyl ureas, GWB-96 and GWB-98, the stoichiometry of 17 was increased to ensure complete reaction of all CDI, and because reaction with CDI generates imidazole, no additional base was added. Again, $^1$H NMR showed no aziridine peaks and no clear product was present. At this point, it was noted that the only successful reaction so far involved excess base (entry 1). This prompted a series of reactions using GWB-96 with a notable increase in imidazole. Entry

Table 18. CDI stoichiometry study

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aziridinyl urea</th>
<th>$R_1^1$</th>
<th>$R_2^1$</th>
<th>ROOH 15 eq.</th>
<th>CDI eq.</th>
<th>Imid. eq.</th>
<th>Yield (%)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GWB-95 PhEt Allyl</td>
<td>1.2</td>
<td>2.3</td>
<td>2.0</td>
<td>46</td>
<td></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>GWB-95 PhEt Allyl</td>
<td>1.0</td>
<td>1.0</td>
<td>0.1</td>
<td>0</td>
<td>0%</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>GWB-96 PhEt Isobutyl</td>
<td>1.7</td>
<td>1.5</td>
<td>0.0</td>
<td>0%</td>
<td>0%</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>GWB-98 Pentyl Benzyl</td>
<td>1.8</td>
<td>1.5</td>
<td>0.0</td>
<td>0%</td>
<td>0%</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>GWB-96 PhEt Isobutyl</td>
<td>1.1</td>
<td>1.0</td>
<td>1.2</td>
<td>17%</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>GWB-96 PhEt Isobutyl</td>
<td>1.2</td>
<td>1.5</td>
<td>2.1</td>
<td>31%</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>GWB-96 PhEt Isobutyl</td>
<td>1.7</td>
<td>1.6</td>
<td>2.2</td>
<td>-</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>GWB-96 PhEt Isobutyl</td>
<td>1.6</td>
<td>1.6</td>
<td>2.4</td>
<td>-</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>GWB-96 PhEt Isobutyl</td>
<td>1.0</td>
<td>2.1</td>
<td>0.0</td>
<td>20%</td>
<td></td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>
2 was repeated once with an additional equivalent of imidazole (entry 5) and once with two equivalents (entry 6). Entry 7 (at room temperature) and entry 8 (50 °C) were compared to determine the effect of additional heating. A small amount of product was actually isolated from Entries 5 and 6, but the others still showed very little product by crude $^1$H NMR.

After tabulating these results, it was clear that the reaction should be monitored and a more systematic approach should be employed for optimization. Also, in order to conserve remaining aziridinyl urea stocks, studies were continued using benzyl alcohol. First, a series of reactions were used to determine the best ratio of carboxylic acid to CDI by $^1$H NMR in CDCl$_3$ (Table 19).

Table 19. CDI stoichiometry study using benzyl alcohol

<table>
<thead>
<tr>
<th>Entry</th>
<th>15 eq.</th>
<th>CDI eq.</th>
<th>Conversion</th>
<th>BnOH eq.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>1.05</td>
<td>50%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>4.00</td>
<td>100%</td>
<td>1.00</td>
<td>30%</td>
</tr>
<tr>
<td>3</td>
<td>4.00</td>
<td>2.00</td>
<td>30%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>2.00</td>
<td>100%</td>
<td>1.00</td>
<td>100%</td>
</tr>
</tbody>
</table>

From this it was discovered that the starting ratio of carboxylic acid 17 to CDI (1 : 2, entry 2) is very important to the formation of the required acyloxyimidazolide. This formation occurred quickly upon CDI addition evolving carbon dioxide gas. This was confirmed by $^1$H NMR, which indicated that no change occurred between five minutes and one hour after addition of carboxylic acid and CDI.
The product from entry 2, Step 1 was treated with one equivalent of benzyl alcohol and provided about a 30% conversion based on \(^1\)H NMR integration between product and remaining starting alcohol. The product from entry 4, Step 1 was treated with one full equivalent of benzyl alcohol to provide complete conversion by \(^1\)H NMR. These reactions were not purified to report yields, but this information was applied to the original coupling reaction with GWB-96 (Table 18, entry 9). Although the very first CDI reaction used a ratio of one carboxylic acid to two CDI equivalents to give a 46% yield, the optimization of the benzyl alcohol experiments did not translate well to a final aziridinyl urea case giving only 20% yield after purification. Even the crude \(^1\)H NMR did not look promising in this case.

3.4.2.4 Isocyanate substitution reactions

Acylation reactions with isocyanates were also explored and provided moderate yields using different aziridinyl urea compounds (Table 20).

Table 20. Yields for acylation reactions with isocyanates

<table>
<thead>
<tr>
<th>Aziridinyl urea</th>
<th>R(^1)=</th>
<th>R(^2)=</th>
<th>R(^3)=</th>
<th>R(^2)NCO</th>
<th>DMAP</th>
<th>Yield</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>GWB-95</td>
<td>PhEt</td>
<td>Allyl</td>
<td>4-MeO-benzyl</td>
<td>1.1</td>
<td>0.6</td>
<td>35%</td>
<td>90</td>
</tr>
<tr>
<td>GWB-96</td>
<td>PhEt</td>
<td>Isobutyl</td>
<td>4-N(_2)O-phenyl</td>
<td>2.0</td>
<td>1.1</td>
<td>66%</td>
<td>91</td>
</tr>
<tr>
<td>GWB-97</td>
<td>PhEt</td>
<td>Benzyl</td>
<td>4-N(_2)O-phenyl</td>
<td>2.3</td>
<td>0.3</td>
<td>68%</td>
<td>GWB-99</td>
</tr>
<tr>
<td>PGB-2</td>
<td>cis-Hex-3-ene</td>
<td>2-MeOEt</td>
<td>4-N(_2)O-phenyl</td>
<td>1.1</td>
<td>0.1</td>
<td>34%</td>
<td>92</td>
</tr>
</tbody>
</table>
From these reactions it seemed that a greater stoichiometry led to higher yields of acylation products. Based on entry 2 and 3, it also seemed that only catalytic amounts of DMAP were necessary with more equivalents having little impact on the yield.

### 3.4.2.5 Mitsunobu reaction

A single Mitsunobu reaction was performed to provide a 52% yield of the desired acylation of **GWB-95** (Scheme 62).[^42]

![Scheme 62. Mitsunobu coupling of GWB-95 with carboxylic acid 17](image)

3.4.3 Discussion of unsuccessful acylation attempts

The unsuccessful acylations of aziridinyl ureas were assumed to have undergone some nucleophilic addition at the aziridine ring, which could provide more than one side product leading to complex mixtures. This is illustrated in Scheme 63 using the acylation attempt with acetyl chloride.
A stability test was performed to determine if aziridinyl ureas, which are typically stable, would undergo nucleophilic attack in the presence of chloride anions. GWB-95 was taken up in CDCl$_3$, treated with 200 mol% of triethylammonium chloride, and monitored by $^1$H NMR over a 24 hour period. A decrease in integration of the aziridine methines was first noticed after four hours and after 24 hours the signals were completely diminished. This confirmed that nucleophilic addition at the aziridine is possible.

Despite this demonstration of chloride substitution, many other reactions described here were unsuccessful even in the absence of a nucleophile. One explanation for these cases could be that the alcohol is activated with the acyl group and a ring closing reaction occurs. This is illustrated in Scheme 64 using the reaction with acetic
anhydride, which could activate the alcohol as a leaving group, then undergo an intramolecular cyclization at the carbonyl oxygen.

Scheme 64. Possible ring closing of activated aziridinyl urea

This ring closing would result in a new bicyclic aziridine, which would restore the ring strain and activate this compound for subsequent nucleophilic attack or decomposition during flash chromatography.

These two proposed side reactions may explain why many of the acylation attempts with typical reagents led to an absence of expected product.
3.5 Biological activity of aziridinyl urea compounds

Initial biological testing was performed for ten selected aziridinyl ureas representing those generated from amines, amino acid benzylamides, and peptide benzylamides. Three assays were chosen to determine which biological targets may be most susceptible to inhibition by substituted aziridinyl ureas. Literature examples have shown that compounds with electrophilic centers, such as aziridines, can make covalent bonds with some serine- and cysteine- proteases and act as effective inhibitors. Therefore, the selected aziridinyl ureas were tested for inhibition against human leukocyte elastase (HLE) and thrombin, both of which are serine proteases; and cathepsin B, a cysteine protease. The selected aziridinyl ureas were shown to be most effective as cathepsin B inhibitors. Next, 60 available final aziridinyl urea compounds and one acylated aziridinyl urea compound were tested in an assay for cathepsin B inhibition. All 61 compounds were also tested for antibacterial activity against 12 common bacteria lines.

3.5.1 Initial results for protease inhibition

Selected aziridinyl ureas were tested for inhibition of HLE, thrombin, and cathepsin B by Kevin Alliston and Bill Groutas at Wichita State University. The assays were performed in a ratio of 250 : 1 inhibitor to enzyme, where any compound with 80-100% inhibition would be considered a good inhibitor. Several compounds showed low inhibition against one or more enzymes (Table 21). The best inhibition of cathepsin B was exhibited by GWB-104 (25%) and GWB-94 (14%), both of which contained glycine benzylamide urea substitution. The only difference between these compounds is the
aziridine substitution where the compound containing the cis-hexene substitution (GWB-104) showed about twice the inhibition of the compound containing the phenethyl substitution against cathepsin B (GWB-94). GWB-104 also exhibited 8% inhibition of thrombin, which is relatively low but the highest value observed against that target. Another comparison between cis-hexene and phenethyl aziridine substitutions can be made for PGB-3 and GWB-96, each of which contains isobutyl urea substitution. PGB-3 exhibited the third best inhibition of cathepsin B (11%), but no activity against other targets; in contrast, GWB-96 showed no inhibition of cathepsin B, low but comparable inhibition of thrombin (8%), and some inhibition of HLE (2%). Two compounds with dipeptide benzylamide urea substitution (GWB-107 and GWB-108) showed no inhibition of cathepsin B, but GWB-107 exhibited relatively good inhibition of HLE (8%) and thrombin (7%) and GWB-108 showed low inhibition of HLE (2%) and thrombin (3%).
Table 21. Initial inhibition results for selected aziridinyl ureas

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID</th>
<th>HLE Inhibition %</th>
<th>Thrombin Inhibition %</th>
<th>Cathepsin B Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="structure1.png" alt="Structure" /></td>
<td>GWB-94</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td><img src="structure2.png" alt="Structure" /></td>
<td>GWB-96</td>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td><img src="structure3.png" alt="Structure" /></td>
<td>GWB-98</td>
<td>0</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><img src="structure4.png" alt="Structure" /></td>
<td>GWB-101</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><img src="structure5.png" alt="Structure" /></td>
<td>GWB-103</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><img src="structure6.png" alt="Structure" /></td>
<td>GWB-104</td>
<td>0</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td><img src="structure7.png" alt="Structure" /></td>
<td>GWB-107</td>
<td>8</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td><img src="structure8.png" alt="Structure" /></td>
<td>GWB-108</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><img src="structure9.png" alt="Structure" /></td>
<td>PGB-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><img src="structure10.png" alt="Structure" /></td>
<td>PGB-3</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>
3.5.2 Cathepsin B activity

Based on the cathepsin B inhibition activity of the test set above, 60 aziridinyl urea compounds and one acylated aziridinyl urea compound were submitted to a cathepsin B assay kit purchased from Biovision Incorporated. This assay included a cathepsin B substrate modified with an AFC fluorophore. In the absence of an inhibitor, the AFC fluorophore would be cleaved resulting in high fluorescence values detected by a fluorometer (Figure 17, top). In the presence of an inhibitor, cleavage would be suppressed or completely prevented, resulting in a decrease or absence of fluorescence (Figure 17, bottom). The inhibition values for the 61 tested compounds are shown in Table 22, Figure 18, and Figure 19.

Figure 17. Relationship between inhibition and fluorescence
### Table 22. Cathepsin B inhibition results

<table>
<thead>
<tr>
<th>Amines</th>
<th>From</th>
<th>Inhibition%</th>
<th>From</th>
<th>Inhibition%</th>
<th>From</th>
<th>Inhibition%</th>
<th>From</th>
<th>Inhibition%</th>
<th>From</th>
<th>Inhibition%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isobutylamine</td>
<td>GWB-96</td>
<td>8%</td>
<td>PGB-3</td>
<td>18%</td>
<td>ZFB-3</td>
<td>23%</td>
<td>GWB-113</td>
<td>32%</td>
<td>GWB-123</td>
<td>36%</td>
</tr>
<tr>
<td>2-methoxyethylamine</td>
<td>GWB-100</td>
<td>14%</td>
<td>PGB-2</td>
<td>21%</td>
<td>ZFB-2</td>
<td>27%</td>
<td>GWB-114</td>
<td>24%</td>
<td>GWB-124</td>
<td>27%</td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>GWB-101</td>
<td>27%</td>
<td>PGB-1</td>
<td>23%</td>
<td>ZFB-4</td>
<td>29%</td>
<td>GWB-115</td>
<td>30%</td>
<td>GWB-125</td>
<td>37%</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>GWB-102</td>
<td>26%</td>
<td>GWB-103</td>
<td>28%</td>
<td>ZFB-5</td>
<td>23%</td>
<td>GWB-116</td>
<td>26%</td>
<td>GWB-126</td>
<td>0%</td>
</tr>
<tr>
<td>Isoamylamine</td>
<td>GWB-133</td>
<td>0%</td>
<td>GWB-105</td>
<td>23%</td>
<td>GWB-138</td>
<td>0%</td>
<td>GWB-118</td>
<td>27%</td>
<td>GWB-128</td>
<td>3%</td>
</tr>
<tr>
<td>Tyramine</td>
<td>GWB-134</td>
<td>11%</td>
<td>GWB-106</td>
<td>30%</td>
<td>GWB-139</td>
<td>0%</td>
<td>GWB-119</td>
<td>35%</td>
<td>GWB-129</td>
<td>0%</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>GWB-135</td>
<td>2%</td>
<td>GWB-110</td>
<td>42%</td>
<td>GWB-140</td>
<td>5%</td>
<td>GWB-120</td>
<td>45%</td>
<td>GWB-130</td>
<td>0%</td>
</tr>
<tr>
<td>3-(methylthio)propylamine</td>
<td>GWB-136</td>
<td>0%</td>
<td>GWB-111</td>
<td>27%</td>
<td>GWB-141</td>
<td>0%</td>
<td>GWB-121</td>
<td>37%</td>
<td>GWB-131</td>
<td>0%</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>GWB-137</td>
<td>0%</td>
<td>GWB-112</td>
<td>20%</td>
<td>GWB-142</td>
<td>0%</td>
<td>GWB-122</td>
<td>37%</td>
<td>GWB-132</td>
<td>0%</td>
</tr>
<tr>
<td>Allylamine</td>
<td>GWB-95</td>
<td>21%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzylamine</td>
<td>GWB-97</td>
<td>23%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine benzylamide</td>
<td>GWB-94</td>
<td>13%</td>
<td>GWB-104</td>
<td>36%</td>
<td>ZFB-1</td>
<td>24%</td>
<td>GWB-117</td>
<td>26%</td>
<td>GWB-127</td>
<td>0%</td>
</tr>
<tr>
<td>Val-Phe-NHBn (isomer 1)</td>
<td>GWB-107</td>
<td>33%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val-Gly-NHBn (isomer 1)</td>
<td>GWB-108</td>
<td>29%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val-Gly-NHBn(isomer 2)</td>
<td>GWB-109</td>
<td>22%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 18. Cathepsin B inhibition values
The top three rows of Table 22 show a general trend of increasing inhibition from the phenethyl substituted aziridines to the fused cyclohexyl substituted aziridines with isobutylamine, 2-methoxyethylamine, and pyrrolidine. The next six rows contain many outliers that have relatively low inhibition values, specifically for phenethyl, cyclohexyl, and isopropyl aziridine substitutions. This may be due to steric reasons especially in the case of cyclohexyl and isopropyl substituted aziridines with the larger amine groups, such as tyramine and tryptamine, though compounds containing phenethylamine still possess relatively good inhibition values. Electronics and polarity may also play a role in these decreased inhibition values, especially those containing 3-(methylthio)propylamine and ethanolamine, though no decreased inhibition is seen for compounds containing 2-methoxyethylamine. In general, compounds containing cis-hexene substituted aziridines and benzyl substituted aziridines exhibit very encouraging inhibition values. This observation applies to compounds containing the glycine benzylamide substitution also. The glycine benzylamide containing compounds have a range of inhibition values with cis-hexene substituted and benzyl substituted aziridines showing the highest inhibition, though the cyclohexyl substituted and fused cyclohexyl substituted aziridines have comparable values. For other compounds containing the glycine benzylamide
substitution, the phenethyl substituted and isopropyl substituted aziridines show significantly lower inhibition. The three dipeptide containing compounds exhibit average inhibition values compared to the rest of the set and are comparable to most of the glycine benzylamide containing compounds. The compounds with the highest inhibition are GWB-99 (47%, Figure 19), the only example of an acylated aziridinyl urea; and GWB-110 (42%) and GWB-120 (45%), both of which contain tryptamine substitution. It is also noteworthy the GWB-110 contains the cis-hexenyl aziridine substitution and GWB-120 contains the benzyl aziridine substitution. A comparison between GWB-99 (47%), the only acylated aziridinyl urea, and the parent aziridinyl urea (GWB-97, 23%) shows that the inhibition is doubled upon acylation.

3.5.3 Antibacterial results

All samples listed in Table 22 were also submitted to assays to investigate the potential for biological activity against twelve bacterial isolates including S. aureus (13709), S. pyogenes, C. glabrata, B. subtilis, C. albicans, B. anthracis, K. pneumonia, P. aeruginosa, E. faecalis, E. coli, B. cereus (11778), and B. cereus (10987). Samples were tested at concentrations of 500 µM, 250 µM, 125 µM, and 64 µM with most samples showing no inhibition even at the highest concentration (500 µM). These samples were not tested further. Minimum inhibitory concentrations (MIC, µg/mL⁻¹) were determined for samples showing any activity at the concentrations above (Table 23). This was true for five compounds, which had some degree of inhibition against six bacterial isolates. In general, the compounds that showed the best inhibition were those containing the
tryptamine substitution, with GWB-110 exhibiting the best inhibition across all six isolates. There was a single compound (GWB-106) that contained the tyramine substitution; however this compound only demonstrated activity against *B. anthracis*. It may also be noteworthy that both GWB-106 and GWB-110 possessed the *cis*-hexene-substituted aziridine.
Table 23. Summary of antibacterial inhibition (MIC values)

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>S. aureus (13709)</th>
<th>S. pyogenes</th>
<th>B. subtilis</th>
<th>B. anthracis</th>
<th>K. pneumonia</th>
<th>E. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
<td>µg/mL</td>
<td>µM</td>
<td>µg/mL</td>
<td>µM</td>
<td>µg/mL</td>
</tr>
<tr>
<td>GWB-106</td>
<td>&gt;500</td>
<td>-</td>
<td>&gt;500</td>
<td>-</td>
<td>&gt;500</td>
<td>125</td>
</tr>
<tr>
<td>GWB-110</td>
<td>125</td>
<td>43</td>
<td>125</td>
<td>43</td>
<td>125</td>
<td>64</td>
</tr>
<tr>
<td>GWB-120</td>
<td>500</td>
<td>175</td>
<td>500</td>
<td>175</td>
<td>500</td>
<td>175</td>
</tr>
<tr>
<td>GWB-130</td>
<td>500</td>
<td>151</td>
<td>&gt;500</td>
<td>-</td>
<td>&gt;500</td>
<td>-</td>
</tr>
<tr>
<td>GWB-135</td>
<td>500</td>
<td>182</td>
<td>500</td>
<td>182</td>
<td>500</td>
<td>182</td>
</tr>
</tbody>
</table>
3.6 Conclusions

In general, this set of aziridinyl urea compounds demonstrated a reasonable range of inhibition against cathepsin B. The best aziridine substitution seemed to be the cis-hexenyl and the benzyl; and the greatest inhibition was seen for tryptamine. Acylated aziridinyl ureas and those containing glycine benzylamide and peptide benzylamides also showed reasonable inhibition of cathepsin B. The potential biological activity from increasing the diversity of peptide substitution is certainly worth exploring.

For the small set of aziridinyl ureas tested against HLE and thrombin, some biological activity was observed. It may still be worthwhile to test all aziridinyl urea compounds against these targets to fully evaluate the trends of biological activity.

Also, some of the compounds tested against several bacterial lines suggested that aziridinyl ureas that contain the tryptamine substitution have an increased degree of inhibition.

All of these results suggest that generating more of these aziridinyl ureas could lead to an increased potential for biological activity towards cathepsin B, HLE, thrombin, and bacterial lines.
CHAPTER 4: ASYMMETRIC AZIRIDINATION

4.1 Introduction

Chiral bisoxazoline (BOX) and pyridine bisoxazoline (PyBOX) ligands have become very popular and effective for the enantioselective formation of chiral non-racemic cyclopropanes, epoxides, and aziridines. A wide range of oxazoline-containing ligands have been reported including $C_1$- and $C_2$- symmetries based on bridge substitution (connecting the oxazoline rings), which determines the bite angle; chiral element ($alpha$ to the nitrogen of each oxazoline ring), which is key for chiral discrimination; and methylene substitution ($alpha$ to the oxygen of each oxazoline ring), which adds steric bulk and can also exhibit chirality. Many different coordination metals have been explored including iron, magnesium, ruthenium, rhodium, and copper.

The earliest example of oxazoline-containing chiral ligands was reported for mono-phenylation with triphenylbismuth diacetate of cis-cyclohexane-1,2-diol 101 using mono-oxazoline substituted pyridines and copper diacetate, which improved enantioselectivity up to a 30% (Scheme 65).
The first use of chiral bisoxazoline-substituted pyridine (PyBOX) ligands was reported for asymmetric hydrosilation of ketones (Scheme 66).\(^{45}\)

Scheme 65. Early use of chiral oxazoline-containing ligands

Scheme 66. Early use of chiral bisoxazoline-substituted pyridine ligands
The first use of chiral bisoxazoline ligands for the enantioselective generation of Diels-Alder products was reported as an iron(III) complex (Scheme 67).\textsuperscript{46}

Scheme 67. First use of chiral bisoxazoline ligands

Enantioselectivity for these Diels-Alder products were further improved from 91 : 9 to 20 : 1 by adding additional geminal methyl groups to the bisoxazoline ligands to give 21h (Figure 20).\textsuperscript{47}

Figure 20. Chiral bis-geminal methyl bisoxazoline ligand

Following these examples, bisoxazoline and pyridine bisoxazoline chiral ligands have been employed in many different enantioselective reactions using a range of metal catalysts. Most relevant to the research presented here is the application to asymmetric cyclopropanation reactions, asymmetric epoxide reactions, and finally asymmetric aziridination reactions.
Asymmetric cyclopropanation of styrene was accomplished using PyBOX ligands with a single chiral substitution and ruthenium catalysts, which demonstrated that single chiral elements could be just as effective as the corresponding ligands possessing chiral elements on both bisoxazoline rings (Scheme 68).\(^{48}\)

![Scheme 68. mono-Substituted versus di-substituted PyBOX chiral ligands](image)

The asymmetric cyclopropanation of 2,5-dimethyl-2,4-hexadiene with several bisoxazoline ligands and copper catalysts demonstrated that the best trans/cis ratio was achieved when using chiral ligand 21e (Scheme 69).\(^{49}\)
Chiral bisoxazoline and pyridine bisoxazoline ligands have also been used with copper and ruthenium catalysts in an asymmetric cyclopropanation study with silyl enol ethers (Scheme 70). Although this study did not report significant success, the potential for these chiral ligands was demonstrated.

Asymmetric epoxidation of trans-stilbene using chiral PyBOX ligands and a ruthenium-center catalyst was first accomplished by Nishiyama in 1997 to provide decent
enantioselectivity (Scheme 71). This work was later expanded by Beller to include many PyBOX and other ligands with ruthenium for the epoxidation of olefins.52

![Scheme 71. Chiral PyBOX ligands for asymmetric epoxidation reactions](image)

The use of allylic N-tosyloxycarbamates to generate fused bicyclic aziridines via ruthenium or copper catalysis has been reported several times over the years and these procedures have been used in the research presented here to provide starting bicyclic aziridines.5,7, 11 At this time, there have been no reports of performing this chemistry asymmetrically, however Lebel has reported on the asymmetric intermolecular aziridination of a small set of N-tosyloxycarbamates with 4-nitrostyrene using chiral bisoxazoline ligands (Scheme 72).12
Optimization of this reaction by Lebel later determined that the best enantioselectivity for these *intermolecular* aziridinations was provided by using chiral bisoxazoline 118 with Cu(CH$_3$CN)$_4$ 22a, though [Cu(OTf)]$_2$•C$_6$H$_6$ 22b and Cu(pyridine)$_4$(OTf)$_2$ 22c catalysts also showed promise. As such, enantioselective, *intramolecular* aziridination attempts presented here from allylic N-tosyloxycarbamate starting materials are the first to be reported, however Lebel, has provided a strong foundation for this reaction series through her *intermolecular* research, including chiral ligand and copper catalyst optimization.

The intramolecular asymmetric aziridination attempts described here used eight selected chiral bisoxazoline and pyridine bisoxazoline ligands, all of which were commercially available except for ligand 21h; and three copper catalysts, Cu(CH$_3$CN)$_4$ 22a, [Cu(OTf)]$_2$•C$_6$H$_6$ 22b, and Cu(pyridine)$_4$(OTf)$_2$ 22c (Scheme 73).
4.2 Selected chiral ligands

Several bisoxazoline and pyridine bisoxazoline chiral ligands were obtained from commercial sources (Figure 21).

Figure 21. Selected bisoxazoline and pyridine bisoxazoline chiral ligands

Ligand 21h, which has been cited in recent literature as providing high enantiomeric excess in enantioselective reactions, was synthesized as follows (Scheme 74).12,13
(L)-(+)-α-Phenylglycine 128 was treated with thionyl chloride in methanol at 0 ºC overnight. The solid product was filtered and washed with THF to provide a 92% yield of crude 129. The 1H NMR spectrum and specific rotation matched that of commercially available material without purification.

The first attempt to prepare ligand 21h involved the conversion of phenylglycine methyl ester hydrochloride 129 directly to the free tertiary amino alcohol 132 by treatment with several equivalents of methyl magnesium chloride as reported by Davies. The crude 1H NMR indicated some desired product but also several extra peaks. The extra signals were consistent with the aminoester self-condensation product 134; most noteworthy was the doublet at 4.81 ppm from equivalent amide hydrogen signals split by equivalent α-hydrogen atoms (Scheme 75).
It is possible that incomplete methylation of the carbonyl had occurred before workup or that the Grignard reagent quality was poor. To check the integrity of the methyl magnesium chloride solution, a test reaction was setup with benzaldehyde, which showed the expected alcohol product by $^1$H NMR with only 3% estimated starting aldehyde remaining. After qualifying the Grignard reagent, a new synthesis was selected, which called for the protection of the amino group prior to methylation.

This conversion was performed from the free base by first treating the hydrochloride salt 129 with triethylamine in diethyl ether. The volume of organic filtrate was reduced but not concentrated to dryness, to avoid self condensation of the methyl ester, then treated with trifluoroacetic anydride and triethylamine at -78 °C. Aqueous workup provided white solid 130 in a 79-96% crude yield that provided a very clean $^1$H NMR. *N*-trifluoroacetyl-phenylglycine methyl ester 130 was treated with several equivalents of methyl magnesium chloride in THF following a procedure modified from that by Corey to generate tertiary alcohol 131 in a 64-100% yield. Deprotection of the trifluoroacetyl-group with two equivalents of potassium hydroxide in methanol at room temperature for one hour was unsuccessful. Crude $^1$H NMR indicated that the reaction
had only gone to 50% completion. Extending the reaction time would have likely provided complete conversion.

Deprotection of the amine was concurrently accomplished by refluxing 131 in a 5% solution of sodium hydroxide in methanol, followed by aqueous workup. The reaction provided quantitative yields of very clean crude material by $^1$H NMR. The specific rotation of purified 132 was determined to be $[\alpha]_D$ of $+18.4^\circ$, which did not perfectly match the literature value of $+25.0^\circ$.

Reaction with dimethylmalonyl dichloride was performed separately with each purified and crude 132 described above and purified to provide an 11% yield of 133 from purified amino alcohol and a 24% yield from the crude material. This diamidation reaction was also performed with the addition of DMAP, which provided a slight increase in yield at 30% after silica gel purification.

The final cyclization step was carried out at reflux in $p$-xylene with titanium tetraisopropoxide for 24 hours, and then purified by flash chromatography to provide a 67% yield of 21h as a white solid. Scale up of this reaction gave an 81% yield and clean $^1$H NMR spectrum after purification, but was treated with activated carbon to remove a light yellow color. This provided the expected white solid product, but reduced the yield to 60%. This compound was determined to have a specific rotation of $[\alpha]_D$ of $-146.1^\circ$, which is reasonably close to the literature value of $-149.5^\circ$, and the $^1$H NMR matched literature values.
4.3 Initial asymmetric aziridination reactions

Three initial asymmetric aziridination reactions were performed, following a report by Lebel, to ensure that successful cyclization would occur in the presence of a chiral ligand (Scheme 76).\(^{13}\)

![Scheme 76. Initial asymmetric aziridination reactions](image)

In the first reaction attempt, chiral ligand 21a and copper catalyst 22b were stirred in acetonitrile in the presence of molecular sieves for 30 minutes, then potassium carbonate was added and stirring continued for 15 minutes. Phenethyl toslyoxycarbamate 7c was then added and the reaction was stirred at room temperature overnight. The crude mixture was filtered through Celite then purified via flash chromatography to provide a 34% yield of 1c. This reaction was repeated in acetonitrile and in methylene chloride on the same scale, using the same stoichiometry of the same reagents, to compare solvent effect on the yield with each reaction providing a 47% yield after purification.

A racemic reaction was performed by treating phenethyl toslyoxycarbamate 7c with copper catalyst 22c and potassium carbonate in methylene chloride, then filtering and purifying on silica gel to provide a 38% yield of 1c (Scheme 77).\(^6\)
In order to establish specific rotations for phenethyl and \textit{cis}-hex-3-ethyl bicyclic aziridines, and to make some comparison between copper catalysts and solvents, reactions were performed using catalysts 22b and 22a, in methylene chloride and in acetonitrile (Table 24). Specific rotations were taken at several wavelengths to provide a range of values because at longer wavelengths the specific rotations appeared very similar. The ranges were wider at shorter wavelengths, which made it easier to compare reactions.
Table 24. Specific rotation of asymmetric aziridination products

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Reaction A</th>
<th>Reaction B</th>
<th>Reaction C</th>
<th>Reaction D</th>
</tr>
</thead>
<tbody>
<tr>
<td>365</td>
<td>7c 22b</td>
<td>7c 22a</td>
<td>7d 22b</td>
<td>7d 22a</td>
</tr>
<tr>
<td></td>
<td>CH₂Cl₂</td>
<td>CH₂CN</td>
<td>CH₂CN</td>
<td>CH₂CN</td>
</tr>
<tr>
<td>365</td>
<td>+28.3°</td>
<td>+45.8°</td>
<td>+82.5°</td>
<td>+83.4°</td>
</tr>
<tr>
<td>405</td>
<td>+16.1°</td>
<td>+26.7°</td>
<td>+59.6°</td>
<td>+57.7°</td>
</tr>
<tr>
<td>436</td>
<td>+14.9°</td>
<td>+24.6°</td>
<td>+48.8°</td>
<td>+47.0°</td>
</tr>
<tr>
<td>546</td>
<td>+7.4°</td>
<td>+11.8°</td>
<td>+26.5°</td>
<td>+26.0°</td>
</tr>
<tr>
<td>589</td>
<td>+3.1°</td>
<td>+8.8°</td>
<td>+22.1°</td>
<td>+20.5°</td>
</tr>
<tr>
<td>633</td>
<td>+4.4°</td>
<td>+3.9°</td>
<td>+18.4°</td>
<td>+16.5°</td>
</tr>
<tr>
<td>Yield</td>
<td>41%</td>
<td>48%</td>
<td>51%</td>
<td>48%</td>
</tr>
</tbody>
</table>

* - All measurements taken at 28 °C, c=0.57 in CHCl₃

For the asymmetric aziridination attempts to generate phenethyl bicyclic aziridine 1c, specific rotations indicated that copper catalyst 22a provided slightly higher enantioselectivity than copper catalyst 22b. With cis-hex-3-enyl bicyclic aziridine 1d, there seemed to be very little difference between these two copper catalysts. No comparison between aziridine substitutions can be made based on specific rotation alone.

4.4 Quantification of enantioselectivity

Chiral HPLC analysis was considered to be the most direct and effective way to quantify the enantiomeric excess from asymmetric aziridination reactions, however no method could be established to resolve the peaks for enantiomeric products. As an alternative to analysis of enantiomers, we also performed chiral derivatization to generate
diastereomers, which could possibly provide diastereomeric excess by NMR to reflect the enantioselectivity of our bicyclic aziridination reactions. Proton, carbon, COSY, and HOMODEC NMR analysis was explored but did not provide an effective way to quantify diastereomeric ratios. Eventually, LCMS analysis of diastereomers (from chiral-derivatized enantiomers) was attempted and, despite being an indirect and less desired reflection of enantioselectivity, was adopted as the primary analysis of asymmetric aziridination reactions.

### 4.4.1 Chiral HPLC trials

Method development was conducted using racemic phenethyl bicyclic aziridine 1c generated above with two available chiral columns, a Regis Whelk-O (R,R) column and an Astec Cyclobond I 2000 series column. The Whelk-O chiral column is noted specifically for resolution of enantiomers containing amides, ureas, carbamates, and aziridines, which seems well suited for the bicyclic aziridines and the subsequent aziridinyl urea products (Figure 22). This column is compatible with normal and reverse phase (aqueous) solvent systems.

![Figure 22. Regis (R,R)-Whelk-O 1 chiral column stationary phase](image)
The Cyclobond chiral column has a β-cyclodextrin solid phase and is noted for resolution of enantiomers of small molecules. Several solvent systems were employed including both normal- and reverse-phase systems. Except for the final attempts with isopropanol in hexanes, all method development was performed with a solvent flow rate of one milliliter per minute.

Initial HPLC method development for racemic phenethyl bicyclic aziridine 1c was performed using the Whelk-O column and a gradient running 10% to 90% acetonitrile in water, then back to 10% over 20 minutes, which showed a sharp peak in the chromatogram at 13.400 minutes. The gradient was modified to run from 20% to 80% acetonitrile in water, then back to 20% over 20 minutes, which gave a sharp peak at 17.025 minutes. A gradient from 50% to 90% acetonitrile in water, then back to 50% gave a slightly broader peak at 7.083 minutes.

Satisfied with the range of retention time, the gradient was replaced with an isocratic solvent system of lower organic content in an attempt to effect resolution of enantiomers. The sample was run isocratically at 40% acetonitrile in water to give a single, slightly broad peak in the chromatogram. Another isocratic method was attempted with 40% isopropanol in water for 30 minutes to give a fairly broad single peak with the Whelk column. This 40% isocratic method was repeated using the Cyclobond column, which seemed to give three overlapped peaks around 6.7 minutes. Reducing the organic content of the isocratic method to 25% isopropanol in water pushed these peaks together into one broad peak with a retention time of 12.450 minutes.
Method development continued with no successful resolution of the enantiomers of 1c despite attempts with both columns, using many different isocratic and gradient methods, different solvent systems, and run times of 20 to 60 minutes. Acetonitrile/water and isopropanol/water were the two solvent systems most explored with both columns, with lower organic content expected to give longer retention times and potentially better resolution. Normal phase solvent systems were briefly explored using ethyl acetate in hexanes and isopropanol in hexanes, which did not provide resolution.

Eventually, a purified, racemic sample of cis-hex-3-enyl bicyclic aziridine 1d was submitted to Regis Method Development. They reported that the best method used an isocratic solvent system with 20% isopropanol in hexanes at 1.5 milliliters per minute using the Whelk chiral column; however their chromatogram gave two overlapped peaks at 8.15 and 8.40 minutes (Figure 23).
Figure 23. Chiral HPLC separation results from Regis.

They also performed super-critical fluid chromatography using 15% isopropanol in liquid carbon dioxide, which was not only impractical based on instrument availability, it also provided no resolution with two overlapping peaks at 2.83 and 2.95 minutes (Figure 24).
No resolution of bicyclic aziridine 1c enantiomers was ever achieved by chiral HPLC, so the focus was switched to chiral derivatization of bicyclic aziridines to generate diastereomers that could potentially be quantified by NMR analysis.

4.4.2 NMR trials

Chiral derivatization was considered such that diastereomers could be quantified by NMR integration of equivalent peaks. NMR analysis of enantiomers only shows a single set of peaks because, although each stereoisomer is a mirror image of the other, the chemical environments of equivalent protons are identical. In contrast, diastereomeric protons have different chemical environments, which lead to separate signals by NMR analysis. Ideally, there would be some equivalent protons from each diastereomer that
would give signals that are resolved from one another and not overlapped with other signals, such that the integration would indicate the diastereomeric ratio and give some indication of enantiomeric excess for our asymmetric aziridination reactions. It would be optimal if this analysis could be performed on crude reaction samples, not only for the convenience of quickly assessing stereoselectivity for many samples, but also because purification via chromatography could artificially enhance the apparent presence of one diastereomer or the other.

Commercially-available chiral \((L)\text{-}(-)\alpha\text{-methylbenzylamine}\) 135 was selected as the chiral derivative and purified by distillation prior to use. A distilled sample provided a specific rotation of \([\alpha]_D\) of -38.2º, which matched the literature value of -39.0º reasonably well. This ensured that ring opening reactions would occur with an enantiomerically-pure source of amine.

Two bicyclic aziridines, 1c and 1d, were taken from asymmetric aziridination attempts (Table 24, Rxn A and C) and treated with chiral \((L)\text{-}(-)\alpha\text{-methylbenzylamine}\) 135 to generate aziridinyl urea diastereomers for NMR analysis (Scheme 78).

![Scheme 78. Chiral derivatization of bicyclic aziridines](image)
Crude $^1$H NMR analysis of the ring opening reactions with phenethyl and cis-hex-3-enyl bicyclic aziridine indicated incomplete consumption of the starting material. It is possible that one enantiomer of the bicyclic aziridine would react preferentially with the chiral amine; however it is essential that future reactions go to completion for an accurate assessment of diastereomeric ratio.

4.4.2.1 Crude analysis by $^1$H and COSY NMR

Because the goal of this initial analysis was to find resolved equivalent peaks, the crude material was submitted to $^1$H NMR in several deuterated solvents to determine which one might give the most useful spectrum. The initial analysis of phenethyl aziridinyl urea 136 diastereomers was performed in deuterated chloroform then repeated in deuterated acetone, benzene, methanol, and acetonitrile. The key diagnostic peaks were those from the aziridine, methine, and methyl protons, but each spectrum was reviewed for any useful signals. In each solvent, the aziridine protons exhibited sharp multiplets between 1.5 and 2.5 ppm; however diastereomeric peaks were overlapped in all cases. The methine proton geminal to the methyl group could be expected to give a separate quartet (or doublet of quartets, if also coupled with the amide hydrogen) for each diastereomer, likely in the 3-5 ppm region due to the proximity of the urea nitrogen atom. The methyl group of each diastereomer should exhibit a signal in the 0-2 ppm alkyl region, likely as a doublet as $^4J$-coupling to the amide proton was not expected. In CDCl$_3$, the crude $^1$H NMR spectrum seemed to show the diastereomeric methines as a multiplet at 4.91 ppm and the methyl groups as overlapped doublets at 1.39 ppm. In acetone-d$_6$, the
crude spectrum showed a multiplet at 4.90 ppm for the methine protons and what seemed like four or more overlapped doublets in the 1.5-1.3 ppm region where the methyl groups were expected. In benzene-d$_6$, a signal at 4.81 ppm was very broad where methine protons were expected and the methyl region showed several overlapped signals around 1.0 ppm. In MeOD-d$_4$, the methine region showed part of a multiplet at 4.86 ppm that was mostly hidden by the large solvent peak and the methyl region at 1.5-1.0 ppm was completely overlapped showing at least five peaks. In CD$_3$CN, a clear quartet at 4.72 ppm and several signals in the methyl region at 1.5-1.0 ppm gave no resolution for diastereomeric protons.

Initial analysis of cis-hex-3-enyl aziridinyl urea 137 diastereomers was performed in CDCl$_3$, MeOD-d$_4$, and acetone-d$_6$. Again, the diagnostic peaks for aziridine, methine, and methyl protons were all overlapped or obscured by other peaks. The aziridine protons exhibited multiplets in the 2.5-2.0 ppm region; the methine multiplet seemed to be around 4.0 ppm; and the methyl protons fell in the 1.5 ppm region, but in each solvent several peak sets were present.

Because reactions did not consume all starting bicyclic aziridine, it was possible that excess amine was present causing the crude analysis to be misleading. A $^1$H NMR of distilled amine was compared to the $^1$H NMR spectra of the crude reactions, each in acetone-d$_6$. This analysis indicated that no starting amine was present in the phenethyl aziridinyl urea sample; however it was not as clear for the cis-hex-3-enyl aziridinyl urea sample, as there were product peaks obscuring those regions where the amine signals were. It is noteworthy that the $^1$H NMR of the chiral amine seemed indicate that reaction
occurred with the deuterated acetone to give a mix of amine and resulting imine \textbf{138} (Scheme 79).

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\textbf{135}};
  \node (b) at (2,0) {\textbf{138}};
  \draw[-latex] (a) -- (b);
  \draw[latex-latex] (a.20) -- node[above] {$\text{Me}_2\text{N}$} node[below] {Ph} node[above] {$\text{H}$} node[below] {Ph} (a);
  \draw[latex-latex] (b.160) -- node[above] {$\text{Me}_2\text{N}$} node[below] {Ph} node[above] {$\text{H}$} node[below] {Ph} (b);
  \draw[latex-latex] (b.200) -- node[above] {$\text{Me}_2\text{N}$} node[below] {Ph} node[above] {$\text{H}$} node[below] {Ph} (b);
\end{tikzpicture}
\end{center}

Scheme 79. Imine formation from chiral amine \textbf{135} and acetone-d$_6$

Ultimately, the crude reactions were purified on silica gel in the hopes that cleaner material would give a better idea of which peaks may be useful. Purified material did give much cleaner spectra with $^1$H and COSY NMR analysis in CDCl$_3$, acetone-d$_6$, and benzene-d$_6$ for phenethyl aziridinyl urea \textbf{136} diastereomers, confirming the assignment of peaks in earlier crude analysis. For \textit{cis}-hex-3-enyl aziridinyl urea \textbf{137} diastereomers, analysis was continued in acetone-d$_6$ only. Unfortunately, the problem remained that all diagnostic peaks seemed to be completely overlapped for both expected diastereomers of each aziridinyl urea.

\textbf{4.4.2.2 Homonuclear decoupling experiments}

Homonuclear decoupling NMR allows for the selection of a particular wavelength to be subjected to irradiation such that the chosen proton signal is effectively removed from the spectrum, which eliminates the splitting pattern of any signals that were previously coupled to that proton. The plan was to systematically irradiate the methyl, methine, and aziridine peak region, as well as any signals determined to be coupled to
those protons, in order to reduce an overlapped multiplet for equivalent diastereomeric protons into a pair of singlets that could be integrated and compared. This analysis was performed on purified samples of both phenethyl aziridinyl urea 136 and cis-hex-3-enyl aziridinyl urea 137. This analysis was performed exclusively in acetone-d$_6$ for both samples.

By irradiating the methyl group region of the $^1$H NMR for 136 at 1.42 ppm, the multiplet signal for diastereomeric methines at 4.92 ppm seemed to be effectively reduced to two singlets of approximately equal intensity (Figure 25).
Figure 25. Homonuclear decoupling example for phenethyl aziridinyl urea 136

The two methine signals were slightly overlapped at the base, but they each integrated to 0.44 ppm. Although this seemed to provide the desired outcome, it was also
considered that the signal could be the result of the $^3J$ coupling with the amide proton at 6.91 ppm, which is unaffected by the irradiation of the methyl group and would split a single signal into a doublet as observed. Comparison of the $J$-coupling values of 8.1 Hz for the amide doublet and 6.6 Hz for the methine doublet seems to make this possibility unlikely. Decoupling experimentation continued by irradiating the methine signal at 4.92 ppm, which reduced the doublet of doublets for the methyl groups at 1.42 ppm to a very sharp singlet.

The analysis of cis-hex-3-enyl aziridinyl urea 137 was more thorough with 10 separate signals, including aziridine, methine, methyl, and adjacent protons, being systematically irradiated. First, the amide proton at 6.92 ppm was irradiated, which seemed to have no impact on either the methine signal at 4.91 or any other signal. Irradiation of the olefin multiplet at 5.38 ppm was expected to reduce splitting in the alkyl region, but in fact had no impact on any proton signals. As was the case with phenethyl aziridinyl urea 136, irradiation of the methine region at 4.92 ppm did effectively reduce the methyl group splitting at 1.45 ppm to a sharp singlet, but as before no separation of diastereomeric peaks occurred. Independent irradiation of methylene peaks (alpha to the alcohol) at 3.73 and 3.43 ppm reduced the amount of splitting on one another, but not significantly enough to improve integration and with no impact on the rest of the spectrum. Proton peaks at 2.87, 2.06, 1.36, and 0.94 ppm were all independently irradiated with no significant reduction of splitting and no improvement on resolving equivalent diastereomeric peaks. Irradiation of the methyl group region at 1.44
ppm did simplify the methine signals at 4.91 ppm, as was the case with \textbf{136}, but a small multiplet overlapped that region making effective integration impossible.

As a last effort on this topic, a sample of racemic \textit{cis}-hex-3-enyl bicyclic aziridine \textbf{1d} was opened with (\textit{L})-(\textit{S})-\alpha-methylbenzylamine \textbf{135} and purified on silica gel to provide a clean mix of diastereomers to be submitted to Ohio State University for NMR analysis. This analysis was requested because OSU had an 800 MHz NMR instrument, which could have provided a much higher resolution of $^1$H NMR peaks than the 300 MHz instrument used at Ohio University. Although peaks were stronger and sharper, there was no resolution between equivalent protons.

A number of other chiral nucleophiles were considered, for example valine methyl ester, as a different aziridinyl urea may have provided spectral signals that could more easily be differentiated and integrated. This option was not explored because concurrent work with LCMS, specifically method development performed by Regis using a mix of diastereomers, had provided a new avenue for the analysis of diastereomeric excess.

\textit{4.4.3 Successful LCMS analysis of diastereomeric ratios}

A sample of purified diastereomers from the chiral derivatization of racemic \textit{cis}-hex-3-enyl bicyclic aziridine \textbf{1d} was submitted to Regis Method Development for analysis. Although diastereomers can theoretically be resolved on a normal column, Regis performed LCMS analysis using the Whelk O column described in Section 4.4.1 with an isocratic solvent system of 20% isopropanol in hexanes at a flow rate of 1.5
milliliters per minute to provide three completely resolved peaks at 4.11, 4.86, and 7.35 minutes (Figure 26).

Some modifications were necessary to make the suggested Regis method give effective results on our instrument. The solvent system was made less polar to give longer retention times and allow a greater separation of peaks. After some trial and error
it was found that 10% isopropanol in hexanes at one milliliter per minute gave resolution of diastereomers with several minutes between peaks.

4.5 Results

With a successful analytical method finally established, focus was returned to the process of generating potentially enantio-enriched bicyclic aziridines from N-tosyloxycarbamates. *cis*-Hex-3-enyl bicyclic aziridine 1d and cyclohexyl bicyclic aziridine 1e were generated from copper catalysts 22a, 22b, and 22c and chiral ligands 21a-h. The crude reaction suspension was rinsed and centrifuged several times with solution being collected by pipette in order to exclude solid material. These collections were concentrated and purified through a short pad of silica to remove copper catalyst and decrease chiral ligand presence. This material was concentrated and opened with chiral (L)-(−)-α-methylbenzylamine 135 in methylene chloride to provide a mix of aziridinyl urea diastereomers. The crude products were submitted directly to LCMS analysis and integration was determined from diastereomeric product peaks in the UV chromatograms, which were confirmed by mass spectroscopy peaks at the corresponding retention times when possible. Diastereomeric excess was calculated from the integration of the area under each diastereomer peak, the data for which was provided by the LCMS software (Table 25).
Table 25. Diastereomeric excess as determined by LCMS analysis

<table>
<thead>
<tr>
<th>Aziridine Substitution</th>
<th>Copper Catalyst</th>
<th>21a</th>
<th>21b</th>
<th>21c</th>
<th>21d</th>
<th>21e</th>
<th>21f</th>
<th>21g</th>
<th>21h</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Hex-3-enyl</td>
<td>22a</td>
<td>52%</td>
<td>1%</td>
<td>20%</td>
<td>12%</td>
<td>10%</td>
<td>14%</td>
<td>5%</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>22b</td>
<td>47%</td>
<td>20%</td>
<td>12%</td>
<td>19%</td>
<td>2%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22c</td>
<td>50%</td>
<td>9%</td>
<td>22%</td>
<td>17%</td>
<td>12%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclohexyl</td>
<td>22a</td>
<td>49%</td>
<td>2%</td>
<td></td>
<td>22%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22b</td>
<td>4%</td>
<td></td>
<td>16%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22c</td>
<td></td>
<td></td>
<td></td>
<td>18%</td>
<td></td>
<td></td>
<td>5%</td>
<td></td>
</tr>
</tbody>
</table>

Although this analysis provided diastereomeric excess values, it was taken as a reflection of the enantioselectivity of the copper catalyst and chiral ligand combinations used in the asymmetric bicyclic aziridination reactions. Overall, the best enantioselectivity came from asymmetric aziridination using chiral ligand 21a; and the cis-hex-3-enyl results seem to indicate that this may occur regardless of which copper catalyst is used. Chiral ligand 21h provided decent enantioselectivity when paired with copper catalyst 22a for each aziridine substitution, but pairing it with the other catalysts significantly decreases its effectiveness. All other copper catalyst and chiral ligand combinations provided enantioselectivity that is considerably lower.

In addition to establishing a novel methodology for the intramolecular asymmetric aziridination of bicyclic aziridines, the driving force for this research was the generation
of highly enantio-enriched bicyclic aziridines. That would have allowed subsequent aziridine ring opening reactions with chiral amino acids and peptides to provide higher diastereomeric excesses, such that the lesser diastereomers could be more easily purified out leaving a single peptide-containing aziridinyl urea for characterization and biological testing. Most bisoxazoline and pyridine bisoxazoline chiral ligands are commercially-available as either \( R,R \) or \( S,S \) enantiomers, which would permit the independent synthesis of each enantiomer of a given bicyclic aziridine, which would then allow for the generation of each diastereomer of a given peptide-containing aziridinyl urea.

The best result for this study indicates an enantiomeric excess of 50%, which is effectively a 3 : 1 ratio of enantiomeric products. This does not significantly improve the isolation of a single aziridinyl urea diastereomer from chiral nucleophiles as racemic bicyclic aziridines previously generated aziridinyl ureas in a 1 : 1 ratio of diastereomers.

4.6 Conclusions

The results of this study did not provide conditions leading to significant enantioselectivity. Although the selection of copper catalysts and chiral bisoxazoline and pyridine bisoxazoline ligands was relatively limited, it represented those reported to be most effective for enantioselectivity of similar products.
CHAPTER 5: EXPERIMENTAL

5.1 General experimental

All reagents used were purchased from commercial sources and purified as necessary prior to use by standard literature procedures. Amines and acyl chlorides were purified by vacuum distillation. CH$_2$Cl$_2$ and THF were dried using a Solv-Tek solvent purification system. DMF was distilled from CaH$_2$ and degassed for 10 minutes prior to use. Thin layer chromatography (TLC) was performed using Whatman flexible plates Al Sil G/UV and Dynamic Adsorbents aluminum-backed, alumina neutral plates with visualization of developed plates using UV absorbance, an ethanolic solution of phosphomolybdic acid (5%), an ethanolic solution of vanillin, potassium permanganate, or Ninhydrin. Flash chromatography was performed using ICN silica gel 60A or Dynamic Adsorbents alumina neutral 32 – 60 µ and reagent grade solvents. Proton ($^1$H) and carbon ($^{13}$C) nuclear magnetic resonance (NMR) spectra including $^1$H COSY, $^1$H HOMODEC, and $^{13}$C APT were recorded on a Bruker Avance 300 MHz spectrometer, and chemical shift values are expressed in parts per million (δ) relative to tetramethylsilane (TMS, 0 ppm) as an internal reference or known solvent peak positions. Signal splitting is reported as singlet (s), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), triplet (t), doublet of triplets (dt), quartet (q), quintet (qu), sextet (sx), and multiplet (m); broad signals as denoted by prefixing the splitting code with (b), such as a broad singlet (bs). All coupling constants, $J$, are reported in Hz. Infrared analysis was performed using a Shimadzu Advantage FTIR-8400 and all samples were prepared as a thin layer on KBr plates. High performance liquid
chromatography (HPLC) was performed using a Shimadzu instrument equipped with SCL-10Avp (system controller), Sil-HTA (autosampler), SPD-10Avp (UV detector), ELSD-LT (low temperature evaporative light scattering detector) and two LC-10ATvp solvent pumps. Liquid chromatography / mass spectroscopy (LCMS) was performed using a Shimadzu instrument equipped with SCL-10Avp (system controller), Sil-HTc (autosampler), SPD-M10Avp (UV detector), and two LC-10ADvp solvent pumps with a DGU-14A (degasser), and an LCMS-2010A mass spectrometer. LC (HPLC or LCMS) method and detection mode are indicated in parentheses for each compound. LC Method 1 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 1.0 mL/min, CH₃CN in H₂O, solvent gradient: 0 - 5 min at 50%, 5 - 8 min from 50 - 70%, 8 - 20 min at 70%, 20 - 21 min from 70 - 50%, 21 - 30 min at 50%. LC Method 2 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 1.0 mL/min, CH₃CN in H₂O, solvent gradient: 0 - 2 min at 20%, 2 - 10 min from 20 - 80%, 10 - 20 min at 80%, 20 – 22 min from 80 - 20%, 22 - 30 at 20%. LC Method 3 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 1.0 mL/min, CH₃CN in H₂O, solvent gradient: 0 - 2 min at 50%, 2 - 5 min from 50 - 90%, 5 - 15 min at 90%, 15 - 18 min from 90 - 50%, 18 - 20 min at 50%. LC Method 4 used a Shimadzu C18 column (5 cm x 4.6 mm x 5µm), 1.0 mL/min, 50% CH₃CN in H₂O (isocratic), 20 min. LC Method 5 used a Supelco Hypersil Silica column (15 cm x 4.6 mm x 5µm), 1.0 mL/min, 15% isopropanol in hexanes (isocratic), 30 min. LC Method 6 used a Supelco Hypersil Silica column (15 cm x 4.6 mm x 5µm), 1.0 mL/min, isopropanol in hexanes, solvent gradient: 0 - 3 min at 5%, 3 - 4 min from 5 - 15%, 4 - 6 min at 15%, 6 - 7 min from 15 - 25%, 7 - 9 min at 25%, 9 - 10 min from 25 - 50%, 10 -
15 min at 50%, 15 - 16 min from 50 - 5%, 16 - 20 min at 5%. LC Method 7 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 0.5 mL/min, 70% CH₃CN in H₂O isocratic. LC Method 8 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 2.0 mL/min, 40% CH₃CN in H₂O (isocratic), 20 min. LC Method 9 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 1.0 mL/min, 50% CH₃CN in H₂O (isocratic), 20 min. LC Method 10 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 0.5 mL/min, 75% CH₃CN in H₂O (isocratic), 20 min. LC Method 11 used a Supelco Discovery C8 column (15 cm x 4.6 mm x 5 µm), 1.0 mL/min, 75% CH₃CN in H₂O (isocratic), 20 min. LC Method 12 used a Regis Whelk-O (R,R) chiral column (15 cm x 4.6 mm x 5 µm), 1.0 mL/min, 10% isopropanol in hexanes (isocratic), 30 min. Gas chromatography (GC) was performed using a Shimadzu GC-17A equipped with an AOC-20i auto injector and a Restek Ptx-5 column (15 m, 0.25 mm ID, 0.25 µm), injector temperature 325 ºC, detector temperature 330 ºC, flame ionization detector. GC Method used a temperature gradient: 0 – 1 min at 100 ºC, 1 - 5 min from 100 ºC - 260 ºC, 5 - 10 min at 260 ºC. All high resolution mass spectra (HRMS) were acquired using positive electrospray ionization (ESI) at the Old Dominion University College of Sciences Major Instrumentation Cluster. Specific rotation values were recorded from a Rudolph Research Analytical Autopol IV polarimeter using a 100 mm sample cell. Sample temperature is reported as a superscript and the concentration (in g/100 mL) and solvent are indicated in parentheses. Wavelength for measurements is 489 nm unless otherwise specified as a subscript. Melting point determinations were made using a Stanford Research Systems (SRS) Digimelt MPA 160 melting point apparatus.
5.2 Bicyclic aziridine synthesis

1-(Trityloxy)but-3-en-2-ol (2a). Commercially available racemic but-3-ene-1,2-diol 30 (9.9 g, 110 mmol) in anhydrous CH₂Cl₂ (300 mL) was treated with trityl chloride (33.3 g, 120 mmol) and DMAP (2.75 g, 22.5 mmol). Triethylamine (23 g, 230 mmol) was added dropwise over 1 h then the reaction was stirred at room temperature for 24 hours. The reaction mixture was washed with H₂O, HCl (aq, 1 M), NaHCO₃ (aq, sat), and brine then dried over MgSO₄, filtered, and concentrated. The mixture was purified via flash chromatography (25% EtOAc in hexanes) to provide 10.2 g (27% yield) of 2a as a colorless oil and 12.0 g of impure product. Rᶠ 0.62 (25% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.44 (m, 6H), 7.32 - 7.23 (m, 9H), 5.78 (m, 1H), 5.28 (m, 1H), 5.14 (m, 1H), 4.26 (m, 1H), 3.22 (dd, 1H, J₁ = 9.4, J₂ = 3.7), 3.11 (dd, 1H, J₁ = 9.4, J₂ = 7.5), 2.35 (d, 1H, J = 3.9). Analysis matched reported spectral data.

(Carbethoxymethylene)triphenylphosphorane (39). Ethyl bromoacetate (5.2 g, 31 mmol) in EtOAc (20 mL) was slowly added to triphenylphosphine (8.9 g, 34 mmol) in EtOAc (20 mL) and stirred at room temperature for 1 h. The precipitate was filtered off, washed with Et₂O, dissolved in CH₂Cl₂ (50 mL), then treated with NaOH (2.4 g, 60 mmol) in H₂O (50 mL) and stirred vigorously at room temperature for 4 h. The reaction was extracted with CH₂Cl₂, dried over MgSO₄, filtered and concentrated to provide 9.3 g
(93% yield) of clean crude 39. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.62 – 7.42 (m, 15H), 3.96 (bs, 2H), 2.88 (bs, 1H), 1.08 (bs, 3H). Analysis matched reported spectral data.$^{18}$

**General Procedure A:** Wittig reactions with aldehydes 40 to give esters 41

(Carbethoxymethylene)triphenylphosphorane 39 in CH$_2$Cl$_2$ (0.5 - 1.0 M) was added to a solution of aldehydes 40 (120 mol%) in CH$_2$Cl$_2$ (1.0 M), stirred at room temperature for 18 – 24 hours, then concentrated and purified as indicated to provide esters 41.

**(E)-Ethyl 5-phenylpent-2-enoate (41a).** Following **General Procedure A**, phosphorane 39 (9.3 g, 27 mmol) was added to hydrocinnamaldehyde 40a (4.48 g, 33.4 mmol) and purified through a short pad of silica (10% EtOAc in hexanes) to provide 4.94 g (90% yield) of 41a. R$_f$ 0.33 (10% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.28 – 7.15 (m, 5H), 7.04 – 6.95 (dt, 1H, $J_1 = 15.6$, $J_2 = 6.8$), 5.84 (d, 1H, $J = 15.6$), 4.17 (q, 2H, $J = 7.1$), 2.76 (m, 2H), 2.50 (q, 2H, $J = 7.4$), 1.27 (t, 3H, $J = 7.1$). Analysis matched reported spectral data.$^{56}$

**(E)-Ethyl 3-cyclohexylacrylate (41b).** Following **General Procedure A**, phosphorane 39 (10.0 g, 28.7 mmol) was added to cyclohexane carboxaldehyde 40b (3.98 g, 35.5 mmol)
then filtered through silica and purified via flash chromatography (0 – 2% EtOAc in Hexanes) to provide 3.90 g (75% yield, 16 : 1 ratio of \textit{trans}/\textit{cis}) of $41b$. $R_f$ 0.21 (2% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 6.91 (dd, 1H, $J_1 = 15.8$, $J_2 = 6.7$), 5.76 (d, 1H, $J = 15.8$), 4.18 (q, 2H, $J = 7.1$), 2.13 (m, 1H), 1.78 – 1.66 (m, 4H), 1.36 – 1.08 (m, 9H). Analysis matched reported spectral data.$^{56}$

(\textit{E})-Ethyl 4-phenylbut-2-enoate ($41c$). Following \textit{General Procedure A}, phosphorane 39 (15 g, 43 mmol) was added to phenylacetaldehyde $40c$ (6.20 g, 51.6 mmol) then suspended in hexanes, filtered to remove solid, and purified via flash chromatography (100% CH$_2$Cl$_2$) to provide 8.09 g (99% yield) of $41c$. $R_f$ 0.56 (100% CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.37 – 7.16 (m, 5H), 7.09 (m, 1H), 5.80 (d, 1H, $J = 15.6$), 4.17 (q, 2H, $J = 7.0$), 3.51 (d, 2H, $J = 6.7$), 1.26 (t, 3H, $J = 7.1$). Analysis matched reported spectral data.$^{57}$

(\textit{E})-Ethyl 4-methylpent-2-enoate ($41d$). Following \textit{General Procedure A}, phosphorane 39 (15 g, 43 mmol) was added to isobutyraldehyde $40d$ (3.71 g, 51.5 mmol) then suspended in hexanes, filtered to remove solid, and purified via flash chromatography (100% CH$_2$Cl$_2$) to provide 4.91 g (80% yield) of $41d$ as a light yellow oil. $R_f$ 0.55 (100%
CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 6.95 (dd, 1H, $J_1 = 15.8$, $J_2 = 6.6$), 5.77 (d, 1H, $J = 15.7$), 4.19 (q, 2H, $J = 7.1$), 2.46 (sex, 1H, $J = 6.7$), 1.29 (t, 3H, $J = 7.1$), 1.07 (d, 6H, $J = 6.8$). Analysis matched reported spectral data. 58

**General Procedure B**: DIBAL reduction of esters 41 to give alcohols 2

Esters 41 in CH$_2$Cl$_2$ (1 - 2 M) were treated dropwise with a solution of DIBAL (1 M in hexanes, 210 mol%) at -78 ºC, then allowed to warm to room temperature and stirred for 18 – 24 hours. The reactions were treated with H$_2$O and NaF (1000 - 2000 mol%), stirred at room temperature (30 - 90 minutes), filtered through Celite, then purified via silica gel chromatography to provide alcohols 2.

(E)-5-Phenylpent-2-en-1-ol (2c). Following General Procedure B, ester 41a (4.94 g, 24.2 mmol) was treated with DIBAL (51 mL, 51 mmol) to provide 2.99 g (76% yield) of 2c. R$_f$ 0.32 (25% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.31 – 7.17 (m, 5H), 5.70 (m, 2H), 4.09 (s, 2H), 2.71 (m, 2H), 2.36 (m, 2H). Analysis matched reported spectral data. 59

(E)-3-Cyclohexylprop-2-en-1-ol (2e). Following General Procedure B, ester 41b (3.90 g, 21.4 mmol) was treated with DIBAL (45 mL, 45 mmol) to provide 2.52 g (84% yield)
of 2e. Rf 0.19 (10% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 5.69 – 5.54 (m, 2H), 4.09 (m, 2H), 1.97 (m, 1H), 1.74 – 1.63 (m, 4H), 1.34 – 1.00 (m, 6H). Analysis matched reported spectral data.$^{60}$

![OH](Ph)

**(E)-4-Phenylbut-2-en-1-ol (2f).** Following General Procedure B, ester 41c (8.09 g, 42.5 mmol) was treated with DIBAL (90 mL, 90 mmol) to provide a quantitative yield of 2f. Rf 0.43 (40% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.32 – 7.18 (m, 5H), 5.86 (m, 1H), 5.71 (m, 1H), 4.12 (m, 2H), 3.39 (d, 2H, $J = 6.4$). Analysis matched reported spectral data.$^{61}$

![OH]( )

**(E)-4-Methylpent-2-en-1-ol (2g).** Following General Procedure B, ester 41d (4.91 g, 34.5 mmol) was treated with DIBAL (75 mL, 75 mmol) to provide 2.01 g (58% yield) of 2g. Rf 0.40 (30% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 5.71 – 5.54 (m, 2H), 4.09 (m, 2H), 2.31 (m, 1H), 1.49 (s, 1H), 1.00 (d, 6H, $J = 6.8$). Analysis matched reported spectral data.$^{62}$
Cyclohexenylmethanol (2h). Following General Procedure B, methyl-1-cyclohexene-1-carboxylate 42 (1.06 g, 7.54 mmol) was treated with DIBAL (17.3 mL, 17.3 mmol) to provide 811 mg (96% yield) of 2h as a clear, colorless oil. Rf 0.29 (20% EtOAc in hexanes); 1H NMR (CDCl3, 300 MHz) δ 5.68 (s, 1H), 3.98 (s, 2H), 2.23 – 2.02 (m, 4H), 1.80 – 1.55 (m, 4H). Analysis matched reported spectral data.63

General Procedure C: Conversion of alcohols 2 to p-nitrophenylcarbonates 3

Alcohols 2 in CH2Cl2 (~1 M) were treated with pyridine (300 mol%), cooled to 0 ºC before adding p-nitrophenylchloroformate (200 mol%), then stirred from 0 ºC to room temperature for 6 to 28 hours, as necessary. The reactions were quenched with NaHCO3 (aq, sat), washed with brine, dried over MgSO4, filtered, and concentrated to provide p-nitrophenylcarbonates 3, which were carried on to the next step without purification.

4-Nitrophenyl 1-(trityloxy)but-3-en-2-yl carbonate (3a). Alcohol 2a (2.65 g, 8.02 mmol) and pyridine (1.96 g, 24.7 mmol) were combined in CH2Cl2 (10 mL) and cooled to 0 ºC before adding p-nitrophenylchloroformate (3.24 g, 16.1 mmol) and stirring from 0 ºC to room temperature over 24 hours. The reaction was quenched with NaHCO3 (aq, sat), washed with brine, dried over MgSO4, filtered, concentrated and purified via flash
chromatography (10% EtOAc in hexanes) to provide 2.92 g (72% yield) of 3a. R$_f$ 0.34 (10% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.18 (d, 2H, $J = 9.1$), 7.47 (m, 6H), 7.32 - 7.21 (m, 11H), 5.84 (m, 1H), 5.45 (m, 1H), 5.38 (d, 1H, $J = 17.3$), 5.26 (d, 1H, $J = 10.6$), 3.35 (m, 2H).

(E)-Hex-2-enyl 4-nitrophenyl carbonate (3b). Following General Procedure C, (E)-hex-2-en-1-ol 2b (2.11 g, 21.1 mmol) was treated with $p$-nitrophenylchloroformate (8.51 g, 42.2 mmol) to provide 3b as a yellow solid in quantitative yield. R$_f$ 0.26 (5% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.26 (d, 2H, $J = 9.2$), 7.38 (d, 2H, $J = 9.2$), 5.91 (m, 1H), 5.65 (m, 1H), 4.71 (d, 2H, $J = 6.6$), 2.08 (dt, 2H, $J_1 = 14.2$, $J_2 = 7.1$), 1.44 (m, 2H), 0.92 (t, 3H, $J = 7.4$).

(E)-4-Nitrophenyl 5-phenylpent-2-enyl carbonate (3c). Following General Procedure C, alcohol 2c (2.99 g, 18.4 mmol) was treated with $p$-nitrophenylchloroformate (7.53 g, 37.4 mmol) to provide 3c as a fluffy yellow solid. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.24 (d, 2H, $J = 8.9$), 8.09 (d, 2H, $J = 9.0$), 7.44 - 7.25 (m, 5H), 5.93 (m, 1H), 5.68 (m, 1H), 4.70 (d, 2H, $J = 6.6$), 2.73 (m, 2H), 2.41 (m, 2H).
(E)-But-2-enyl 4-nitrophenyl carbonate (3j). Following General Procedure C, commerically available crotyl alcohol 2j (2.11 g, 29.3 mmol) was treated with p-nitrophenylchloroformate (6.54 g, 32.4 mmol) to provide 3j. R_f 0.59 (25% EtOAc in hexanes); ^1^H NMR (CDCl_3, 300 MHz) δ 8.28 (d, 2H, J = 9.2), 7.38 (d, 2H, J = 9.2), 5.94 (m, 1H), 5.69 (m, 1H), 4.70 (d, 2H, J = 6.8), 1.78 (d, 3H, J = 6.5).

General Procedure D: Conversion of carbonates 3 to azidoformates 4

p-Nitrophenylcarbonates 3 were dissolved in acetone (0.3 M, 4 parts) and treated with a solution NaN_3 (500 mol%) in H_2O (1 part) and stirred at room temperature for 2 - 6 days, as necessary. The reactions were extracted with EtOAc, the combined organic phases were washed with NaHCO_3 (aq, sat) and brine, dried over MgSO_4, filtered, concentrated and purified via flash chromatography to provide azidoformates 4.

1-(Trityloxy)but-3-en-2-yl carbonazidate (4a). p-Nitrophenylcarbonate 3a (1.02 g, 2.06 mmol) was dissolved in acetone (12 mL) and treated with NaN_3 (0.697 g, 10.8 mmol) in water (3 mL). The reaction was stirred at room temperature for 4 days, and then extracted with hexanes, washed with brine and H_2O, dried over MgSO_4, filtered, and concentrated to provide 795 mg (96% yield) of 4a. R_f 0.79 (25% EtOAc in hexanes); ^1^H NMR (CDCl_3,
300 MHz) δ 7.44 – 7.41 (m, 6H), 7.26 – 7.16 (m, 9H), 5.73 (m, 1H), 5.41 (m, 1H), 5.34 (d, 1H, J = 22.7), 5.21 (d, 1H, J = 18.5), 3.30 (dd, 1H, J = 10.2, J = 7.2), 3.20 (dd, 1H, J = 10.3, J = 3.8); 13C NMR (CDCl3, 75.47 MHz) δ 156.9, 132.1, 143.6, 128.7, 127.9, 127.2, 119.4, 86.8, 78.5, 64.9. Analysis matched reported spectral data.20

(E)-Hex-2-enyl carbonazidate (4b). Following General Procedure D, carbonate 3b (5.59 g, 21.1 mmol) was treated with NaN₃ (6.91 g, 106 mmol) to provide 1.65 g (46% yield) of 4b as a pale yellow oil. Rf 0.44 (5% EtOAc in hexanes); 1H NMR (CDCl3, 300 MHz) δ 5.84 (m, 1H), 5.58 (m, 1H), 4.62 (d, 2H, J = 6.6, J = 0.6), 2.04 (m, 2H), 1.42 (m, 2H), 0.90 (t, 3H, J = 7.4); IR 2139, 2189 cm⁻¹. Analysis matched reported spectral data.1

(E)-5-Phenylpent-2-enyl carbonazidate (4c). Following General Procedure D, carbonate 3c (assumed 18.4 mmol) was treated with NaN₃ (6.07 g, 93.4 mmol) to provide 3.89 g (91% yield over two steps) of 4c. Rf 0.46 (10% EtOAc in hexanes); 1H NMR (CDCl3, 300 MHz) δ 7.23 – 7.08 (m, 5H), 5.78 (m, 1H), 5.52 (m, 1H), 4.52 (d, 2H, J = 6.5), 2.62 (m, 2H), 2.30 (dd, 2H, J = 14.6, J = 7.2); IR 2137, 1751, 1736 cm⁻¹.
(2E,6Z)-Nona-2,6-dienyl carbonazidate (4d). Following General Procedure C, alcohol 2d (4.98 g, 35.5 mmol) was treated with p-nitrophenylchloroformate (10.8 g, 53.6 mmol), then following General Procedure D, carbonate 3d (assumed 35.5 mmol) was treated with NaN$_3$ (11.6 g, 179 mmol) to provide 5.28 g (71% yield over two steps) of 4d. R$_f$ 0.40 (25% CH$_2$Cl$_2$ in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 5.86 (m, 1H), 5.61 (m, 1H), 5.55 – 5.26 (m, 2H), 4.63 (d, 2H, $J = 6.5$), 2.12 (s, 4H), 2.03 (qu, 2H, $J = 7.3$), 0.95 (t, 3H, $J = 7.5$).

(E)-3-Cyclohexylallyl carbonazidate (4e). Following General Procedure C, alcohol 2e (2.47 g, 17.6 mmol) was treated with p-nitrophenylchloroformate (5.37 g, 26.6 mmol), then following General Procedure D, carbonate 3e (assumed 17.6 mmol) was treated with NaN$_3$ (5.87 g, 90.3 mmol) to provide 1.21 g (33% yield over two steps) of 4e. R$_f$ 0.61 (50% CH$_2$Cl$_2$ in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 5.78 (dd, 1H, $J_1 = 15.5, J_2 = 6.5$), 5.53 (m, 1H), 4.63 (d, 2H, $J = 6.6$), 1.99 (m, 1H), 1.74 – 1.71 (m, 4H), 1.46 – 1.05 (m, 6H); $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 157.4, 144.1, 119.9, 69.6, 40.4, 32.4, 26.2, 25.9; IR 2137, 1736 cm$^{-1}$. 
(E)-Oct-2-enyl carbonazidate (4i). Following General Procedure C, trans-2-octen-1-ol 2i (0.600 g, 4.68 mmol) was treated with p-nitrophenylchloroformate (1.59 g, 7.89 mmol), then following General Procedure D, carbonate 3i (assumed 4.68 mmol) was treated with a solution of NaN₃ (1.52 g, 23.4 mmol) to provide 700 mg (76% yield over two steps) of 4i. Rₚ 0.64 (10% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 5.85 (m, 1H), 5.57 (m, 1H), 4.64 (d, 2H, J = 6.6), 2.06 (dd, 2H, J₁ = 14.0, J₂ = 6.7), 1.41 – 1.27 (m, 6H), 0.89 (m, 3H); IR 2137, 1736 cm⁻¹.

(E)-But-2-enyl azidoformate (4j). Following General Procedure D, carbonate 3j (assumed 29.3 mmol) was treated with NaN₃ (9.5 g, 146.5 mmol) to provide 1.18 g (29% yield) of 4j. Rₚ 0.72 (25% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 5.75 (m, 1H), 5.51 (m, 1H), 4.51 (d, 2H, J = 6.6), 1.64 (d, 3H, J = 6.3); IR 2183, 2137 cm⁻¹.

General Procedure E: Conversion of alcohols 2 to acyloxyimidazolides 5

Alcohol 2 was dissolved in CH₂Cl₂ (0.3 - 0.5 M), treated with CDI (110 – 200 mol%), then stirred at room temperature for 18 hours. The reaction was washed with NH₄Cl (aq, sat), dried over MgSO₄, filtered, and concentrated to provide acyloxyimidazolides 5, which were used in the next step without purification.
(2E,6Z)-Nona-2,6-dienyl \textit{1H}-imidazole-1-carboxylate (5d). Following \textit{General Procedure E}, trans-2,cis-6-nonadien-1-ol 2d (8.73 g, 62.3 mmol) was treated with CDI (12.1 g, 74.6 mmol) to provide a quantitative yield of 5d as a clear yellow oil. R\textsubscript{f} 0.27 (10% EtOAc in hexanes); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) \(\delta\) 8.14 (s, 1H), 7.43 (s, 1H), 7.07 (m, 1H), 5.92 (m, 1H), 5.68 (m, 1H), 5.41 (m, 1H), 5.31 (m, 1H), 4.83 (d, 2H, \(J = 6.6\)), 2.16 (m, 4H), 2.04 (m, 2H), 0.96 (t, 3H, \(J = 7.5\)).

(E)-3-Cyclohexylallyl \textit{1H}-imidazole-1-carboxylate (5e). Following \textit{General Procedure E}, alcohol 2e (2.52 g, 18 mmol) was treated with CDI (3.25 g, 20 mmol) to provide 4.26 g (quantitative yield) of clean, crude 5e. R\textsubscript{f} 0.19 (20% EtOAc in hexanes); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) \(\delta\) 8.14 (s, 1H), 7.43 (s, 1H), 7.06 (s, 1H), 5.87 (dd, 1H, \(J_1 = 15.4\), \(J_2 = 6.5\)), 5.61 (m, 1H), 4.83 (d, 2H, \(J = 6.7\)), 2.03 (m, 1H), 1.76 – 1.62 (m, 4H), 1.34 – 1.04 (m, 6H).
(E)-4-Phenylbut-2-enyl 1H-imidazole-1-carboxylate (5f). Following General Procedure E, alcohol 2f (6.3 g, 43 mmol) was treated with CDI (13.8 g, 85.1 mmol) to provide 9.64 g (94% yield) of 5f. R$_f$ 0.11 (20% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.15 (s, 1H), 7.43 (s, 1H), 7.35 – 7.18 (m, 5H), 7.07 (m, 1H), 6.09 (m, 1H), 5.72 (m, 1H), 4.87 (d, 2H, $J$ = 6.3), 3.45 (d, 2H, $J$ = 6.7).

(E)-4-Methylpent-2-enyl 1H-imidazole-1-carboxylate (5g). Following General Procedure E, alcohol 2g (2.01 g, 20.1 mmol) was treated with CDI (6.56 g, 40.5 mmol) to provide 3.63 g (93% yield) of clean, crude 5g. R$_f$ 0.20 (20% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.15 (s, 1H), 7.43 (s, 1H), 7.07 (s, 1H), 5.90 (m, 1H), 5.61 (m, 1H), 4.84 (d, 2H, $J$ = 6.7), 2.36 (m, 1H), 1.03 (d, 6H, $J$ = 6.7).

Cyclohexenylmethyl 1H-imidazole-1-carboxylate (5h). Following General Procedure E, alcohol 2h (811 mg, 7.23 mmol) was treated with CDI (2.35 g, 14.5 mmol) to provide
1.18 (79% yield) of clean, crude 5h. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.14 (s, 1H), 7.43 (s, 1H), 7.07 (s, 1H), 5.88 (s, 1H), 4.77 (s, 2H), 2.08 – 2.06 (m, 4H), 1.75 – 1.57 (m, 4H).

3-Methylbut-2-enyl 1H-imidazole-1-carboxylate (5k). Following General Procedure E, 3-methyl-2-buten-1-ol 2k (5 mL, 50 mmol) was treated with CDI (16 g, 99 mmol) to provide a quantitative yield of clean, crude 5k as an orange oil. R$_f$ 0.33 (50% EtOAc in hexanes). $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.13 (s, 1H), 7.43 (s, 1H), 7.06 (s, 1H), 5.45 (t, 1H, $J$ = 7.5), 4.89 (d, 2H, $J$ = 7.5), 1.81 (s, 3H), 1.79 (s, 3H). Analysis matched reported spectral data.$^{64}$

General Procedure F: Conversion of imidazolides 5 to N-hydroxycarbamates 6

Acyloxyimidazolides 5 were dissolved in pyridine (0.3 – 1.0 M) and treated with hydroxylamine hydrochloride (300 mol%), typically stirred for 18 hours under argon at room temperature, then diluted with CH$_2$Cl$_2$, washed with 10% H$_2$SO$_4$, dried over MgSO$_4$, concentrated and either used as clean, crude material or purified via flash chromatography to provide N-hydroxycarbamates 6.
(E)-5-Phenylpent-2-enyl hydroxycarbamate (6c). Alcohol 2c (2.31 g, 14.2 mmol) in CH₂Cl₂ (50 ml, 0.3 M) was treated with CDI (4.57 g, 28.2 mmol) and stirred for 22 h, then treated with imidazole (6.47 g, 39.9 mmol) and hydroxylamine hydrochloride (5.0 g, 72 mmol) and stirred for 27 h. The reaction was concentrated, dissolved in EtOAc, washed with HCl (aq, 1 M) and brine, dried over MgSO₄, filtered, concentrated, and purified via flash chromatography (10% EtOAc in hexanes) to provide 2.27 g (72% yield) of 6c. Rᵣ 0.23 (10% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.31 – 7.15 (m, 6H), 5.84 (m, 1H), 5.60 (m, 1H), 4.59 (d, 2H, J = 6.2), 2.70 (m, 2H), 2.37 (dd, 2H, J₁ = 14.7, J₂ = 7.2). Analysis matched reported spectral data.¹¹

(2E,6Z)-Nona-2,6-dienyl hydroxycarbamate (6d). Following General Procedure F, acyloxyimidazolide 5d (62.3 mmol) was treated with hydroxylamine hydrochloride (13.0 g, 187 mmol) then purified via flash chromatography (10% EtOAc in hexanes) to provide 9.0 g (73% yield) of 6d. Rᵣ 0.19 (10% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.25 (s, 1H), 6.79 (bs, 1H), 5.81 (m, 1H), 5.44 – 5.26 (m, 2H), 4.60 (d, 2H, J = 6.4), 2.12 (m, 4H), 2.03 (m, 2H), 0.95 (t, 3H, J = 7.5).
(E)-3-Cyclohexylallyl hydroxycarbamate (6e). Following General Procedure F, acyloxyimidazolide 5e (4.21 g, 18.0 mmol) was treated with hydroxylamine hydrochloride (3.75 g, 54.0 mmol) then concentrated to provide 3.37 g (94% yield) of clean, crude 6e. $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.10 (bs, 1H), 5.84 (bs, 1H), 5.75 (dd, 1H, $J_1 = 15.5$, $J_2 = 6.4$), 5.54 (m, 1H), 4.61 (d, 2H, $J = 6.5$), 1.97 (m, 1H), 1.74 – 1.58 (m, 4H), 1.33 – 1.00 (m, 6H).

(1R)-4-Phenylbut-2-enyl hydroxycarbamate (6f). Following General Procedure F, acyloxyimidazolide 5f (9.64 g, 39.8 mmol) was treated with hydroxylamine hydrochloride (8.3 g, 120 mmol) then purified via flash chromatography (10% EtOAc in hexanes) to provide 1.47 g (49% yield, plus 15% side product by $^1$H NMR). R$_f$ 0.42 (20% EtOAc in CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.32 – 7.15 (m, 5H), 5.96 (m, 1H), 5.62 (m, 1H), 4.63 (d, 2H, $J = 6.4$), 3.39 (d, 2H, $J = 6.7$).
(E)-4-Methylpent-2-enyl hydroxycarbamate (6g). Following General Procedure F, acyloxyimidazolide 5g (3.63 g, 18.7 mmol) was treated with hydroxylamine hydrochloride (3.91 g, 56.3 mmol) then concentrated to provide 2.77 g (93% yield) of clean, crude 6g. $^1$H NMR (CDCl$_3$, 300 MHz) δ 6.94 (bs, 1H), 5.77 (dd, 1H, $J_1 = 15.4, J_2 = 6.3$), 5.51 (m, 1H), 4.60 (d, 2H, $J = 6.5$), 2.32 (m, 1H), 1.00 (d, 6H, $J = 6.7$).

Cyclohexenylmethyl hydroxycarbamate (6h). Following General Procedure F, acyloxyimidazolide 5h (1.18 g, 5.72 mmol) was treated with hydroxylamine hydrochloride (1.25 g, 18.0 mmol) then purified via flash chromatography (25% EtOAc in hexanes) to provide 792 g (81% yield) of 6h. $R_f$ 0.08 (20% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.74 (bs, 1H), 5.75 (s, 1H), 5.31 (s, 1H), 4.50 (s, 2H), 2.00 – 1.97 (m, 4H), 1.64 – 1.56 (m, 4H). Analysis matched reported spectral data.$^{11}$

3-Methylbut-2-enyl hydroxycarbamate (6k). Following General Procedure F, acyloxyimidazolide 5k (9 g, 50 mmol) was treated with hydroxylamine hydrochloride
(11.1 g, 160 mmol) then concentrated to provide 3.90 g (54% yield) of 6k. $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.22 (bs, 1H), 5.37 (m, 1H), 4.66 (d, 2H, $J = 7.1$), 1.76 (s, 3H), 1.72 (s, 3H). Analysis matched reported spectral data.$^7$

**General Procedure G**: Tosylation of N-hydroxycarbamates 6 to give 7

Hydroxycarbamate 6 was dissolved in Et$_2$O (0.1 - 0.5 M), treated with tosyl chloride (110 mol%), then cooled to 0 ºC and treated with triethylamine (110 mol%). Reaction was allowed to warm to room temperature and stirred for 18 hours, then diluted with Et$_2$O, washed with H$_2$O and brine, dried over MgSO$_4$, concentrated, and purified via flash chromatography to provide N-hydroxycarbamates 7.

(E)-5-Phenylpent-2-enyl tosyloxy carbamate (7c). Following General Procedure G, hydroxycarbamate 6c (2.27 g, 10.3 mmol) was treated with tosyl chloride (2.16 g, 11.3 mmol) to provide 3.08 g (80% yield) of 7c as a colorless oil. R$_f$ 0.33 (25% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.87 (d, 2H, $J = 8.2$), 7.76 (s, 1H), 7.35 – 7.15 (m, 7H), 5.75 (m, 1H), 5.40 (m, 1H), 4.43 (d, 2H, $J = 6.4$), 2.68 (m, 2H), 2.45 (s, 3H), 2.34 (m, 2H). Analysis matched reported spectral data.$^{11}$
(2E,6Z)-Nona-2,6-dienyl tosyloxycarbamate (7d). Following General Procedure G, hydroxycarbamate 6d (9.0 g, 45 mmol) was treated with tosyl chloride (9.5 g, 50 mmol) to provide 11.9 g (74% yield) of 7d as a pale yellow oil. Rf 0.17 (10% EtOAc in hexanes); ^1H NMR (CDCl₃, 300 MHz) δ 7.88 (d, 2H, J = 8.3), 7.71 (s, 1H), 7.36 (d, 2H, J = 8.0), 5.71 (m, 1H), 5.44 – 5.36 (m, 2H), 5.27 (m, 1H), 4.44 (d, 2H, J = 6.5), 2.46 (s, 3H), 2.11 – 2.00 (m, 6H), 0.96 (t, 3H, J = 7.5).

(E)-3-Cyclohexylallyl tosyloxycarbamate (7e). Following General Procedure G, hydroxycarbamate 6e (3.37 g, 16.9 mmol) was treated with tosyl chloride (3.54 g, 18.6 mmol) to provide 1.55 g (26% yield) of 7e. Rf 0.36 (100% CH₂Cl₂); ^1H NMR (CDCl₃, 300 MHz) δ 7.91 (d, 2H, J = 8.1), 7.78 (bs, 1H), 7.36 (d, 2H, J = 8.1), 5.66 (dd, 1H, J₁ = 15.5, J₂ = 6.5), 5.32 (m, 1H), 4.43 (d, 2H, J = 6.5), 2.46 (s, 3H), 1.94 (m, 1H), 1.75 – 1.60 (m, 4H), 1.34 – 0.97 (m, 6H).

(E)-4-Phenylbut-2-enyl tosyloxycarbamate (7f). Following General Procedure G, hydroxycarbamate 6f (1.47 g, 7.09 mmol) was treated with tosyl chloride (1.5 g, 7.9
mmol) to provide 1.51 g (59% yield) of 7f. Rf 0.33 (25% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.86 (d, 2H, $J = 8.3$), 7.35 – 7.12 (m, 7H), 5.86 (m, 1H), 5.44 (m, 1H), 4.46 (d, 2H, $J = 6.5$), 3.36 (d, 2H, $J = 7.2$), 2.43 (s, 3H).

(E)-4-Methylpent-2-enyl tosylloxycarbamate (7g). Following General Procedure G, hydroxycarbamate 6g (2.26 g, 14.2 mmol) was treated with tosyl chloride (2.87 g, 15.1 mmol) to provide 2.96 g (66% yield) of 7g. Rf 0.36 (25% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.88 (d, 2H, $J = 8.3$), 7.75 (s, 1H), 7.36 (d, 2H, $J = 8.2$), 5.69 (dd, 1H, $J_1 = 15.5$, $J_2 = 6.4$), 5.33 (m, 1H), 4.44 (d, 2H, $J = 6.6$), 2.46 (s, 3H), 2.19 (m, 1H), 0.98 (d, 6H, $J = 6.8$).

Cyclohexenylmethyl tosylloxycarbamate (7h). Following General Procedure G, hydroxycarbamate 6h (792 mg, 4.63 mmol) was treated with tosyl chloride (901 mg, 4.73 mmol) to provide 793 mg (53% yield) of 7h. Rf 0.21 (20% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.88 (d, 2H, $J = 8.3$), 7.71 (s, 1H), 7.36 (d, 2H, $J = 8.2$), 5.69 (s, 1H, $J_1 = 15.5$, $J_2 = 6.4$), 5.33 (m, 1H), 4.44 (d, 2H, $J = 6.6$), 2.46 (s, 3H), 2.19 (m, 1H), 1.82 (s, 2H), 1.82 (s, 2H), 1.64 – 1.53 (m, 4H). Analysis matched reported spectral data.$^{11}$
3-Methylbut-2-enyl tosyloxycarbamate (7k). Following General Procedure G, hydroxycarbamate 6k (1.40 g, 9.6 mmol) was treated with tosyl chloride (1.92 g, 10.1 mmol) to provide 1.25 g (43% yield) of 7k. Rf 0.40 (30% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) δ 7.88 (d, 2H, \(J = 8.3\)), 7.72 (bs, 1H), 7.36 (d, 2H, \(J = 8.3\)), 5.14 (t, 1H, \(J = 7.3\)), 4.50 (d, 2H, \(J = 7.3\)), 2.46 (s, 3H), 1.73 (s, 3H), 1.64 (s, 3H). Analysis matched reported spectral data.\(^7\)

General Procedure H: Bicyclic aziridines 1 from azidoformates 4

Azidoformates 4 were dissolved in CH\(_2\)Cl\(_2\) (0.02 – 0.33 M), placed in a sealed tube, which was cooled to -78 °C, evacuated, and returned to room temperature several times to degas the reaction mixture, then heated to 110°C for 10 - 16 h. Reactions were purified as indicated to provide bicyclic aziridines 1.

General Procedure I: Bicyclic aziridines 1 from N-tosyloxycarbamates 7

N-Tosyloxycarbamates 7 in either CH\(_2\)Cl\(_2\) or CH\(_3\)CN (0.05 – 0.1 M), as indicated, were treated with K\(_2\)CO\(_3\) (240 – 500 mol%) and either Cu(CH\(_3\)CN)\(_4\)PF\(_6\) \(22a\) or Cu(pyridine)\(_4\)(OTf)\(_2\) \(22c\) (7 – 13 mol%), as indicated, in the presence of 4Å molecular sieves, then stirred under argon at room temperature for 1 – 2 days. The reactions were filtered through silica gel, then purified via flash chromatography to provide bicyclic aziridines 1.
Copper (II) tetrakis(pyridine)bis(triflate) (22c). Copper (II) triflate (1.00 g, 2.77 mmol) in methanol (11 mL, 0.25 M) was treated with pyridine (5 mL, 61.8 mmol) then refluxed at 70 ºC for 2 hours. The reaction mixture was cooled to room temperature then to -23 ºC before collecting blue crystals at room temperature. Recrystallization from a 4 : 1 mix of pyridine and methanol provided 622 mg (33% yield) of 22c as a blue solid. Analysis matched reported spectral data.\textsuperscript{21}

\begin{center}
\includegraphics[width=0.2\textwidth]{copper_triflate.png}
\end{center}

4-(Trityloxymethyl)-3-oxa-1-azabicyclo[3.1.0]hexan-2-one (1a). Following General Procedure H, azidoformate 4a (3.3 g, 8.2 mmol) was heated for 16 h, then the crude product was suspended in 25% EtOAc in hexanes and filtered to provide 900 mg (30%) of 1a as a white solid. R\textsubscript{f} 0.35 (50% EtOAc in hexanes); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) δ 7.47 – 7.44 (m, 6H), 7.35 – 7.25 (m, 9H), 4.66 (t, 1H, \(J = 3.1\)), 3.56 (dd, 1H, \(J_1 = 10.5, J_2 = 3.7\)), 3.25 (dd, 1H, \(J_1 = 10.5, J_2 = 3.2\)), 3.03 (t, 1H, \(J = 4.2\)), 2.53 (d, 1H, \(J = 4.6\)), 2.16 (d, 1H, \(J = 4.0\)). Analysis matched reported spectral data.\textsuperscript{20}

\begin{center}
\includegraphics[width=0.2\textwidth]{trityloxymethyl.png}
\end{center}

6-Propyl-3-oxa-1-aza-bicyclo[3.1.0]hexan-2-one (1b). Following General Procedure H, azidoformate 4b (1.65 g, 9.74 mmol) was heated for 13 h, then the reaction mixture was concentrated and purified via flash chromatography (25% EtOAc in hexanes) to
provide 876 mg (64% yield) of 1b as a pale yellow oil. R_f 0.38 (50% EtOAc in hexanes); 

$^1$H NMR (CDCl$_3$, 250 MHz) δ 4.43 (m, 2H), 2.99 (m, 1H), 2.40 (dd, 1H, $J_1 = 9.3, J_2 = 5.6$), 1.55 (m, 4H), 0.98 (t, 3H, $J = 7.1$); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 167.4, 66.8, 48.4, 43.7, 33.0, 19.6, 13.6. Analysis matched reported spectral data.$^2$

6-Phenethyl-3-oxa-1-aza-bicyclo[3.1.0]hexan-2-one (1c). Following General Procedure H, azidoformate 4c (2.46 g, 10.6 mmol) was heated for 10 hours, then the reaction was concentrated and purified via flash chromatography (25% EtOAc in hexanes) to provide 900 mg (42% yield) of 1c. Following General Procedure I, N-tosyloxy carbamate 7c (640 mg, 1.7 mmol) and Cu(pyridine)$_4$(OTf)$_2$ 22c (150 mg, 0.22 mmol) in CH$_2$Cl$_2$ provided 132 mg (38% yield) of 1c. R_f 0.14 (25% EtOAc in hexanes); 

$^1$H NMR (CDCl$_3$, 300 MHz) δ 7.33 – 7.19 (m, 5H), 4.36 (d, 2H, $J = 3.2$), 2.93 (d, 1H, $J = 3.2$), 2.83 (m, 2H), 2.37 (dd, 1H, $J_1 = 9.8, J_2 = 6.1$), 1.91 (m, 2H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 167.2, 140.4, 128.6, 128.5, 126.3, 66.7, 47.7, 43.9, 32.9, 32.5; IR 2916, 1782, 1142 cm$^{-1}$. Analysis matched reported spectral data.$^{11}$

6-((Z)-Hex-3-enyl)-3-oxa-1-azabicyclo[3.1.0]hexan-2-one (1d). Following General Procedure H, azidoformate 4d (1.16 g, 5.54 mmol) was heated for 12 hours, then the
reaction was concentrated and purified via flash chromatography (25% EtOAc in hexanes) to provide 325 mg (33% yield) of 1d as a clear colorless oil. Following General Procedure I, N-tosyloxycarbamate 7d (1.02 mg, 2.89 mmol) and Cu(CH$_3$CN)$_4$PF$_6$ 22a (102 mg, 274 µmol) in CH$_2$Cl$_2$ provided 208 mg (40% yield) of 1d. R$_f$ 0.18 (25% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 5.46 (m, 1H), 5.32 (m, 1H), 4.41 (m, 2H), 3.01 (m, 1H), 2.40 (m, 1H), 2.24 (m, 2H), 2.06 (m, 2H), 1.67 (m, 2H), 0.97 (t, 3H, $J$ = 7.5); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 167.1, 133.5, 126.7, 66.6, 48.0, 43.8, 31.3, 24.0, 20.5, 14.3; IR 1783 cm$^{-1}$; LCMS (Method 4, ELSD) rt 3.99 min, 100%.

![6-Cyclohexyl-3-oxa-1-azabicyclo[3.1.0]hexan-2-one (1e)](image)

6-Cyclohexyl-3-oxa-1-azabicyclo[3.1.0]hexan-2-one (1e). Following General Procedure H, azidoformate 4e (301 mg, 1.43 mmol) was heated for 13 hours, then the reaction was concentrated and purified via flash chromatography (25% EtOAc in hexanes) to provide 131 mg (50% yield) of 1e. Following General Procedure I, N-tosyloxycarbamate 7e (1.04 g, 2.94 mmol) and Cu(CH$_3$CN)$_4$PF$_6$ 22a (110 mg, 0.29 mmol) in CH$_2$Cl$_2$ provided 210 mg (39% yield) of 1e. R$_f$ 0.31 (30% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 4.42 (s, 2H), 3.04 (s, 1H), 2.20 (m, 1H), 1.93 (m, 1H), 1.72 (m, 4H), 1.19 (m, 6H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 167.6, 66.8, 53.1, 42.4, 39.1, 29.9, 29.3, 26.0, 25.5, 25.4; IR 1767 cm$^{-1}$; HPLC (Method 4, ELSD) rt 3.99 min, 100%; mp 119.5 ºC.
Description of the X-ray analysis of 1e: A colorless crystal cleaved from a larger crystal of C₁₀H₁₅NO₂ was washed with the perfluoropolyether PFO-XR75 (Lancaster) and wedged in a glass capillary. The sample was optically aligned on a Bruker AXS D8 Venture fixed-chi X-ray diffractometer equipped with a Triumph monochromator, a Mo Kα radiation source (λ = 0.71073 Å), and a PHOTON 100 CMOS detector. Two sets of 12 frames each were collected using the omega scan method with a 10 s exposure time. Integration of these frames followed by reflection indexing and least-squares refinement produced a crystal orientation matrix for the monoclinic crystal lattice.

Data collection consisted of the measurement of a total of 1104 frames in three runs using omega scans with the detector held at 5.00 cm from the crystal. Frame scan parameters are summarized in Table 26 below:

<table>
<thead>
<tr>
<th>Run</th>
<th>2θ</th>
<th>Ω</th>
<th>φ</th>
<th>χ</th>
<th>Scan Width (°)</th>
<th>Frames</th>
<th>Exposure Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.01</td>
<td>-170.99</td>
<td>-144.00</td>
<td>54.74</td>
<td>0.50</td>
<td>368</td>
<td>20.00</td>
</tr>
<tr>
<td>2</td>
<td>11.01</td>
<td>-170.99</td>
<td>72.00</td>
<td>54.74</td>
<td>0.50</td>
<td>368</td>
<td>20.00</td>
</tr>
<tr>
<td>3</td>
<td>11.01</td>
<td>-170.99</td>
<td>0.00</td>
<td>54.74</td>
<td>0.50</td>
<td>368</td>
<td>20.00</td>
</tr>
</tbody>
</table>

The APEX2 software program (version 2014.1-1) was used for diffractometer control, preliminary frame scans, indexing, orientation matrix calculations, least-squares refinement of cell parameters, and the data collection. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 12946 reflections to a maximum θ
angle of 27.50° (0.77 Å resolution), of which 2222 were independent (average redundancy 5.826, completeness = 99.1%, R_{int} = 2.31%, R_{sig} = 1.61%) and 1835 (82.58%) were greater than 2σ(F²). The final cell constants of a = 37.5590(16) Å, b = 6.3920(3) Å, c = 8.0941(4) Å, β = 90.5632(14)°, volume = 1943.11(16) Å³, are based upon the refinement of the XYZ-centroids of 9949 reflections above 20 σ(I) with 6.466° < 2θ < 61.14°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.868. The calculated minimum and maximum transmission coefficients are 0.946 and 0.981.

The structure was solved by the direct methods and difference Fourier analysis using the programs provided by SHELXL-2013. Idealized positions for the hydrogen atoms were included as fixed contributions using a riding model with isotropic temperature factors set at 1.2 times that of the adjacent carbon atom. Full-matrix least-squares refinement, based upon the minimization of Σw_i |F_o^2 - F_c^2|^2, with weighting w_i^-1 = [σ²(F_o^2) + (0.0320 P)^2 + 1.6582 P], where P = (Max (F_o^2, 0) + 2 F_c^2)/3.² The final anisotropic full-matrix least-squares refinement on F² with 118 variables converged at R1 = 5.22%, for the observed data and wR2 = 11.42% for all data. The goodness-of-fit was 1.116.

A correction for secondary extinction was not applied. The largest peak in the final difference electron density synthesis was 0.198 e/Å³ and the largest hole was -0.146 e/Å³ with an RMS deviation of 0.033 e/Å³. The linear absorption coefficient, atomic scattering factors, and anomalous dispersion corrections were calculated from values found in the International Tables of X-ray Crystallography.
Table 27. Crystal data for 1e (C_{10}H_{15}NO_{2})

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification code</td>
<td>sb2cms</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C_{10}H_{15}NO_{2}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>181.23</td>
</tr>
<tr>
<td>Temperature</td>
<td>296(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.224 x 0.307 x 0.640 mm</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>C 2/c (No. 15)</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 37.5590(16) Å</td>
</tr>
<tr>
<td></td>
<td>b = 6.3920(3) Å</td>
</tr>
<tr>
<td></td>
<td>c = 8.0941(4) Å</td>
</tr>
<tr>
<td>Volume</td>
<td>1943.11(16) Å</td>
</tr>
<tr>
<td>Z</td>
<td>8</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.239 g/cm³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.086 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>784</td>
</tr>
</tbody>
</table>

Table 28. Data collection and structure refinement for 1e (C_{10}H_{15}NO_{2})

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theta range for data analysis</td>
<td>3.23 to 27.50°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-48 ≤ h ≤ 48, -7 ≤ k ≤ 8, -10 ≤ l ≤ 10</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>12946</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>2222 [R(int) = 0.0231]</td>
</tr>
<tr>
<td>Coverage of independent reflections</td>
<td>99.1%</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>multi-scan</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.981 and 0.946</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Refinement program</td>
<td>SHELXL-2013 (Sheldrick, 2013)</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>2222 / 0 / 118</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.116</td>
</tr>
<tr>
<td>Final R indices</td>
<td>R1 = 0.0522, wR2 = 0.1083</td>
</tr>
<tr>
<td></td>
<td>R1 = 0.0621, wR2 = 0.1142</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.198 and -0.146 e⁻¹/Å³</td>
</tr>
</tbody>
</table>
Table 29. Atomic coordinates and equivalent isotropic atomic displacement parameters (Å$^2$) for 1e (C$_{10}$H$_{15}$NO$_2$). U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor.

<table>
<thead>
<tr>
<th></th>
<th>x/a</th>
<th>y/b</th>
<th>z/c</th>
<th>U(eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>0.30699(3)</td>
<td>0.73728(14)</td>
<td>0.32885(16)</td>
<td>0.0807(3)</td>
</tr>
<tr>
<td>O2</td>
<td>0.28093(2)</td>
<td>0.04157(14)</td>
<td>0.38100(13)</td>
<td>0.0643(3)</td>
</tr>
<tr>
<td>N1</td>
<td>0.33356(2)</td>
<td>0.04306(14)</td>
<td>0.23898(11)</td>
<td>0.0424(2)</td>
</tr>
<tr>
<td>C1</td>
<td>0.30710(3)</td>
<td>0.92366(18)</td>
<td>0.31834(16)</td>
<td>0.0516(3)</td>
</tr>
<tr>
<td>C2</td>
<td>0.28606(3)</td>
<td>0.2581(2)</td>
<td>0.3370(2)</td>
<td>0.0677(4)</td>
</tr>
<tr>
<td>C3</td>
<td>0.32270(3)</td>
<td>0.26359(17)</td>
<td>0.26451(15)</td>
<td>0.0463(3)</td>
</tr>
<tr>
<td>C4</td>
<td>0.35201(3)</td>
<td>0.18112(15)</td>
<td>0.36380(12)</td>
<td>0.0339(2)</td>
</tr>
<tr>
<td>C5</td>
<td>0.39025(2)</td>
<td>0.22938(14)</td>
<td>0.32942(11)</td>
<td>0.0310(2)</td>
</tr>
<tr>
<td>C6</td>
<td>0.41420(3)</td>
<td>0.04287(15)</td>
<td>0.36808(12)</td>
<td>0.0364(2)</td>
</tr>
<tr>
<td>C7</td>
<td>0.45331(3)</td>
<td>0.09442(17)</td>
<td>0.34137(14)</td>
<td>0.0434(3)</td>
</tr>
<tr>
<td>C8</td>
<td>0.46462(3)</td>
<td>0.28552(18)</td>
<td>0.44080(14)</td>
<td>0.0457(3)</td>
</tr>
<tr>
<td>C9</td>
<td>0.44080(3)</td>
<td>0.47224(16)</td>
<td>0.40392(14)</td>
<td>0.0431(3)</td>
</tr>
<tr>
<td>C10</td>
<td>0.40173(3)</td>
<td>0.42064(15)</td>
<td>0.42941(13)</td>
<td>0.0390(2)</td>
</tr>
</tbody>
</table>

Table 30. Interatomic distance (Å) for 1e (C$_{10}$H$_{15}$NO$_2$)

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>O1-C1</td>
<td>1.1944(15)</td>
<td>O2-C1</td>
<td>1.3421(14)</td>
<td></td>
</tr>
<tr>
<td>O2-C2</td>
<td>1.4423(16)</td>
<td>N1-C1</td>
<td>1.4125(15)</td>
<td></td>
</tr>
<tr>
<td>N1-C3</td>
<td>1.4825(14)</td>
<td>N1-C4</td>
<td>1.5052(13)</td>
<td></td>
</tr>
<tr>
<td>C2-C3</td>
<td>1.5018(17)</td>
<td>C3-C4</td>
<td>1.4554(15)</td>
<td></td>
</tr>
<tr>
<td>C4-C5</td>
<td>1.4978(13)</td>
<td>C5-C6</td>
<td>1.5242(13)</td>
<td></td>
</tr>
<tr>
<td>C5-C10</td>
<td>1.5260(13)</td>
<td>C6-C7</td>
<td>1.5227(14)</td>
<td></td>
</tr>
<tr>
<td>C7-C8</td>
<td>1.5211(15)</td>
<td>C8-C9</td>
<td>1.5195(15)</td>
<td></td>
</tr>
<tr>
<td>C9-C10</td>
<td>1.5199(14)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 31. Bond angles (°) for 1e (C_{10}H_{15}NO_{2})

<table>
<thead>
<tr>
<th>Bond</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-O2-C2</td>
<td>110.21(10)</td>
</tr>
<tr>
<td>C1-N1-C4</td>
<td>109.44(8)</td>
</tr>
<tr>
<td>C3-N1-C4</td>
<td>58.30(7)</td>
</tr>
<tr>
<td>O1-C1-O2</td>
<td>122.00(12)</td>
</tr>
<tr>
<td>O1-C1-N1</td>
<td>125.06(11)</td>
</tr>
<tr>
<td>O2-C1-N1</td>
<td>112.93(10)</td>
</tr>
<tr>
<td>C4-C3-N1</td>
<td>61.63(7)</td>
</tr>
<tr>
<td>N1-C3-C2</td>
<td>106.66(9)</td>
</tr>
<tr>
<td>C3-C4-N1</td>
<td>115.59(8)</td>
</tr>
<tr>
<td>C4-C5-C6</td>
<td>111.47(8)</td>
</tr>
<tr>
<td>C6-C5-C10</td>
<td>110.77(8)</td>
</tr>
<tr>
<td>C7-C6-C5</td>
<td>111.70(8)</td>
</tr>
<tr>
<td>C8-C7-C6</td>
<td>111.31(9)</td>
</tr>
<tr>
<td>C9-C8-C7</td>
<td>111.46(9)</td>
</tr>
<tr>
<td>C8-C9-C10</td>
<td>111.73(9)</td>
</tr>
</tbody>
</table>

Table 32. Anisotropic atomic displacement parameters (Å²) for 1e (C_{10}H_{15}NO_{2}). The anisotropic atomic displacement factor exponent takes the form: -2π²[a²b²c²U_{11} + ... + 2hka^*b^*U_{12}]

<table>
<thead>
<tr>
<th>Atom</th>
<th>U_{11}</th>
<th>U_{22}</th>
<th>U_{33}</th>
<th>U_{23}</th>
<th>U_{13}</th>
<th>U_{12}</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>0.0687(6)</td>
<td>0.0405(4)</td>
<td>0.1330(9)</td>
<td>-0.0108(5)</td>
<td>0.0034(6)</td>
<td>-0.0150(4)</td>
</tr>
<tr>
<td>O2</td>
<td>0.0372(4)</td>
<td>0.0544(5)</td>
<td>0.1017(7)</td>
<td>-0.0068(5)</td>
<td>0.0104(4)</td>
<td>-0.0068(4)</td>
</tr>
<tr>
<td>N1</td>
<td>0.0401(4)</td>
<td>0.0408(4)</td>
<td>0.0462(4)</td>
<td>-0.0091(4)</td>
<td>-0.0043(4)</td>
<td>-0.0025(4)</td>
</tr>
<tr>
<td>C1</td>
<td>0.0407(5)</td>
<td>0.0427(5)</td>
<td>0.0713(7)</td>
<td>-0.0098(5)</td>
<td>-0.0056(5)</td>
<td>-0.0080(5)</td>
</tr>
<tr>
<td>C2</td>
<td>0.0377(6)</td>
<td>0.0524(7)</td>
<td>0.1130(11)</td>
<td>-0.0015(8)</td>
<td>0.0018(7)</td>
<td>0.0058(6)</td>
</tr>
<tr>
<td>C3</td>
<td>0.0410(5)</td>
<td>0.0387(5)</td>
<td>0.0594(6)</td>
<td>0.0037(5)</td>
<td>-0.0025(5)</td>
<td>0.0032(5)</td>
</tr>
<tr>
<td>C4</td>
<td>0.0371(5)</td>
<td>0.0316(4)</td>
<td>0.0331(4)</td>
<td>-0.0037(4)</td>
<td>0.0021(4)</td>
<td>-0.0029(4)</td>
</tr>
<tr>
<td>C5</td>
<td>0.0352(4)</td>
<td>0.0303(4)</td>
<td>0.0274(4)</td>
<td>0.0011(4)</td>
<td>0.0008(4)</td>
<td>-0.0024(4)</td>
</tr>
<tr>
<td>C6</td>
<td>0.0403(5)</td>
<td>0.0301(4)</td>
<td>0.0386(5)</td>
<td>0.0004(4)</td>
<td>0.0004(4)</td>
<td>-0.0010(4)</td>
</tr>
<tr>
<td>C7</td>
<td>0.0376(5)</td>
<td>0.0421(5)</td>
<td>0.0506(6)</td>
<td>-0.0034(5)</td>
<td>0.0009(4)</td>
<td>0.0037(4)</td>
</tr>
<tr>
<td>C8</td>
<td>0.0377(5)</td>
<td>0.0493(6)</td>
<td>0.0500(6)</td>
<td>-0.0036(5)</td>
<td>-0.0051(4)</td>
<td>-0.0035(5)</td>
</tr>
<tr>
<td>C9</td>
<td>0.0454(5)</td>
<td>0.0367(5)</td>
<td>0.0471(5)</td>
<td>-0.0021(5)</td>
<td>-0.0041(4)</td>
<td>-0.0100(4)</td>
</tr>
<tr>
<td>C10</td>
<td>0.0424(5)</td>
<td>0.0314(4)</td>
<td>0.0431(5)</td>
<td>-0.0034(4)</td>
<td>-0.0018(4)</td>
<td>-0.0008(4)</td>
</tr>
</tbody>
</table>
Table 33. Hydrogen atom coordinates and isotropic atomic displacement (Å²) for 1e (C₁₀H₁₅NO₂)

<table>
<thead>
<tr>
<th></th>
<th>x/a</th>
<th>y/b</th>
<th>z/c</th>
<th>U(eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2A</td>
<td>0.2847</td>
<td>0.3474</td>
<td>0.4336</td>
<td>0.081</td>
</tr>
<tr>
<td>H2B</td>
<td>0.2683</td>
<td>0.3030</td>
<td>0.2567</td>
<td>0.081</td>
</tr>
<tr>
<td>H3</td>
<td>0.3279</td>
<td>0.3665</td>
<td>0.1783</td>
<td>0.056</td>
</tr>
<tr>
<td>H4</td>
<td>0.3465</td>
<td>0.1521</td>
<td>0.4796</td>
<td>0.041</td>
</tr>
<tr>
<td>H5</td>
<td>0.3924</td>
<td>0.2628</td>
<td>0.2118</td>
<td>0.037</td>
</tr>
<tr>
<td>H6A</td>
<td>0.4108</td>
<td>0.0007</td>
<td>0.4820</td>
<td>0.044</td>
</tr>
<tr>
<td>H6B</td>
<td>0.4074</td>
<td>-0.0737</td>
<td>0.2978</td>
<td>0.044</td>
</tr>
<tr>
<td>H7A</td>
<td>0.4678</td>
<td>-0.0244</td>
<td>0.3740</td>
<td>0.052</td>
</tr>
<tr>
<td>H7B</td>
<td>0.4572</td>
<td>0.1205</td>
<td>0.2249</td>
<td>0.052</td>
</tr>
<tr>
<td>H8A</td>
<td>0.4636</td>
<td>0.2531</td>
<td>0.5577</td>
<td>0.055</td>
</tr>
<tr>
<td>H8B</td>
<td>0.4890</td>
<td>0.3208</td>
<td>0.4147</td>
<td>0.055</td>
</tr>
<tr>
<td>H9A</td>
<td>0.4443</td>
<td>0.5163</td>
<td>0.2905</td>
<td>0.052</td>
</tr>
<tr>
<td>H9B</td>
<td>0.4475</td>
<td>0.5876</td>
<td>0.4756</td>
<td>0.052</td>
</tr>
<tr>
<td>H10A</td>
<td>0.3873</td>
<td>0.5398</td>
<td>0.3967</td>
<td>0.047</td>
</tr>
<tr>
<td>H10B</td>
<td>0.3977</td>
<td>0.3944</td>
<td>0.5458</td>
<td>0.047</td>
</tr>
</tbody>
</table>

6-Benzyl-3-oxa-1-azabicyclo[3.1.0]hexan-2-one (1f). Following General Procedure I, N-tosyloxycarbamate 7f (1.51 g, 4.18 mmol) and Cu(CH₃CN)₄PF₆ 22a (153 mg, 0.41 mmol) in CH₃CN provided 275 mg (35% yield by mass, but purity was determined to be 74% by ¹H NMR integration relative to dimethyl fumarate, making the product yield 26%) of 1f. Rᵣ 0.34 (50% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.31 – 7.19 (m, 5H), 4.43 (m, 2H), 3.12 – 3.02 (m, 2H), 2.82 (dd, 1H, J₁ = 14.7, J₂ = 5.9), 2.64 (m, 1H); ¹³C NMR (CDCl₃, 300 MHz) δ 166.8, 136.2, 128.9, 128.8, 127.2, 66.6, 48.0, 43.2, 37.1; HPLC (Method 4, ELSD) rt 3.37 min, 89.6%.
6-Isopropyl-3-oxa-1-azabicyclo[3.1.0]hexan-2-one (1g). Following General Procedure I, N-tosyloxy carbamate 7g (2.96 g, 9.45 mmol) and Cu(CH₃CN)₄PF₆ 22a (370 mg, 0.99 mmol) in CH₂Cl₂ provided 600 mg (45% yield) of 1g. Rᵣ 0.16 (50% Et₂O in pentane); ¹H NMR (CDCl₃, 300 MHz) δ 4.46 – 4.38 (m, 2H), 3.03 (m, 1H), 2.19 (dd, 1H, J₁ = 7.1, J₂ = 3.7), 1.62 (m, 1H), 1.09 (d, 3H, J = 6.7), 1.01 (d, 3H, J = 6.8); ¹³C NMR (CDCl₃, 300 MHz) δ 167.4, 66.7, 54.1, 42.6, 30.0, 19.4, 18.8; IR 1771 cm⁻¹; HPLC (Method 9, ELSD) rt 3.37 min, 77.8%.

Tetrahydro-2-oxa-3a-azacyclopenta[1,3]cyclopropa[1,2]benzen-3-one (1h). Following General Procedure I, N-tosyloxy carbamate 7h (465 mg, 1.43 mmol) and Cu(CH₃CN)₄PF₆ 22a (36 mg, 0.097 mmol) in CH₃CN provided 94 mg (43% yield) of 1h. Rᵣ 0.29 (40% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 4.43 (d, 1H, J = 9.2), 4.07 (d, 1H, J = 9.2), 2.72 (d, 1H, J = 4.0), 2.17 – 2.00 (m, 2H), 1.91 – 1.78 (m, 2H), 1.59 – 1.26 (m, 4H). Analysis matched reported spectral data.⁴
6-Pentyl-3-oxa-1-azabicyclo[3.1.0]hexan-2-one (1i). Following General Procedure H, azidoformate 4i (700 mg, 3.55 mmol) was heated for 11 hours, then the reaction was concentrated and purified via flash chromatography (25% EtOAc in hexanes) to provide 100 mg (17% yield) of 1i. Rf 0.20 (25% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 4.41 (m, 2H), 2.98 (m, 1H), 2.39 (m, 1H), 1.63 – 1.46 (m, 4H), 1.34 – 1.32 (m, 4H), 0.90 (t, 3H, J = 6.7); ¹³C NMR (CDCl₃, 300 MHz) δ 167.2, 66.7, 48.6, 43.7, 31.3, 31.2, 26.0, 22.5, 14.0; IR 1782 cm⁻¹.

6-Methyl-3-oxa-1-aza-bicyclo[3.1.0]hexan-2-one (1j). Following General Procedure H, azidoformate 4j (750 mg, 5.3 mmol) was heated for 12 h, then the reaction was concentrated to provide a crude ¹H NMR spectrum, which showed approximately 60% product and 40% starting azide by integration. ¹H NMR (CDCl₃, 300 MHz) δ 4.41 (m, 2H), 2.95 (m, 1H), 2.47 (m, 1H), 1.37 (d, 3H, J = 5.6).

Carbamic acid 5-phenyl-pent-2-enyl ester (43). Allylic alcohol 2c (104 mg, 0.639 mmol) in CH₂Cl₂ (600 μL) was cooled to -78 ºC, treated with trichloroacetyl isocyanate
(92 μL, 0.772 mmol) in CH₂Cl₂ (400 μL) and stirred at room temperature for 4 hours, then concentrated. The reaction was dissolved in MeOH (1 mL), treated with potassium carbonate (11 mg, 82 μmol), and stirred at room temperature for another 4 hours, then concentrated and purified by flash chromatography (50% EtOAc in hexanes) to provide 138 mg (quantitative yield) of 43 as a white solid. Rf 0.55 (50% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.30 – 7.15 (m, 5H), 5.81 (m, 1H), 5.60 (m, 1H), 4.97 (bs, 2H), 4.49 (d, 2H, J = 6.3), 2.70 (m, 2H), 2.37 (m, 2H).

Iodosobenzene (45). [CAUTION: this compound may explode at high temperatures or during trituration] Iodobenzene diacetate 44 (1.0 g, 3.1 mmol) was treated dropwise with sodium hydroxide (aq, 3 M) and stirred with occasional trituration for 2 hours to give a dull yellow suspension. The solid was collected on a Buchner funnel, washed with H₂O, dried on vacuum pump, and then triturated to a fine powder in CH₂Cl₂. The solid was filtered and dried on the vacuum pump overnight to provide 555 mg (80% yield) of 45. No purification or analysis was performed.²²

5.3 Trityl-protected aziridinyl ureas and oxazolidinones

General Procedure J: Ring opening reactions of 1a to give 8 and 9
Trityl bicyclic aziridine 1a was suspended in anhydrous CH₂Cl₂ (0.7 M) and treated with freshly distilled amine (110 mol%). The reaction was stirred at room temperature until
complete as judged by TLC (typically 12 – 48 h). The reaction was concentrated and purified via flash chromatography to provide oxazolidinones 8 and aziridinyl ureas 9.

**General Procedure K**: Increased amine stoichiometry to give 9 from 1a

Trityl bicyclic aziridine 1a was suspended in anhydrous toluene (0.7 M) and treated with freshly distilled amine (610 mol%). The reaction was stirred at room temperature until complete as judged by TLC (typically 12 – 48 h). The reaction was concentrated and purified via flash chromatography to provide aziridinyl ureas 9.

---

4-((N-Benzylamino)methyl)-5-((trityloxy)methyl)oxazolidin-2-one (8a). Following General Procedure J, aziridine 1a (0.10 g, 0.270 mmol) was treated with benzylamine (30.4 mg, 0.284 mmol) to provide 105.0 mg (81%) of 8a as a white oil. Rf 0.20 (50% EtOAc in hexanes); 1H NMR (CDCl3, 300 MHz) δ 7.46 - 7.43 (m, 6H), 7.34 – 7.23 (m, 15H), 5.47 (s, 1H), 4.39 (dt, 1H, J1 = 9.5, J2 = 4.6), 3.76 (s, 2H), 3.70 (m, 1H), 3.32 (dd, 2H), 2.69 (dd, 2H); 13C NMR (CDCl3, 300 MHz) δ 159.5, 143.6, 139.9, 128.8, 128.6, 128.2, 128.1, 127.4, 127.3, 87.1, 79.2, 64.4, 54.8, 53.9, 52.7; IR 1751 cm⁻¹; HPLC (Method 3, 254 nm) rt 7.73 min, 100%; HRMS calculated for (C31H30N2O3)Na⁺, 501.2149, observed 501.2148.
2-(1-Hydroxy-2-trityloxy-ethyl)-aziridine-1-carboxylic acid benzylamide (9a).

Following General Procedure K, aziridine 1a (24.3 mg, 0.065 mmol) was treated with benzylamine (42.1 mg, 0.394 mmol) to provide 15.5 mg (50%) of 9a. Rf 0.23 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.36 – 7.22 (m, 18H), 6.96 (d, 2H, $J$ = 2.7), 6.24 (t, 1H, $J$ = 6.0), 4.28 (dd, 1H, $J_1$ = 14.9, $J_2$ = 7.3), 3.75 (bs, 1H), 3.58 (ddd, 2H, $J_1$ = 28.5, $J_2$ = 14.9, $J_3$ = 5.2), 2.98 (dd, 1H, $J_1$ = 6.0, $J_2$ = 2.9), 2.71 (d, 1H, $J$ = 9.0), 2.42 (s, 2H), 2.07 (s, 1H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 164.7, 143.1, 138.6, 128.6, 128.5, 128.5, 128.1, 127.6, 127.5, 127.3, 127.2, 87.4, 67.5, 44.6, 40.5; IR 3356, 1751, 1651 cm$^{-1}$; HPLC (Method 2, 254 nm) rt 14.20 min, 93.93%; HRMS calculated for (C$_{31}$H$_{30}$N$_2$O$_3$)Na$^+$, 501.2149, observed 501.2138.

4-(Morpholinomethyl)-5-((trityloxy)methyl)oxazolidin-2-one (8b). Following General Procedure J, aziridine 1a (59.1 mg, 0.159 mmol) was treated with morpholine (15.8 mg, 0.181 mmol) to provide 22 mg (30%) of 8b as a white oil. Rf 0.41 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.45 – 7.22 (m, 15H), 5.37 (s, 1H), 4.32 (dt, 1H, $J_1$ = 4.6, $J_2$ = 4.4), 3.81 (dt, 1H, $J_1$ = 7.2, $J_2$ = 5.8), 3.56 (d, 4H, $J$ = 2.4), 3.46 (dd, 1H, $J_1$ = 10.2, $J_2$ = 4.5), 3.19 (dd, 1H, $J_1$ = 10.3, $J_2$ = 4.1), 2.49 – 2.29 (m, 6H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 158.5, 143.4, 128.6, 128.0, 127.3, 86.9, 79.2, 66.8, 63.7, 63.0, 53.9, 51.1; IR
1751 cm$^{-1}$; HPLC (Method 1, 254 nm) rt 9.10 min, 88.8%; HRMS calculated for (C$_{28}$H$_{30}$N$_2$O$_4$)Na$^+$, 481.2098, observed 481.2095.

4-((2-Methoxyethylamino)methyl)-5-(((trityloxy)methyl)oxazolidin-2-one (8c) and 1-Hydroxy-2-((trityloxy)ethyl)-N-(2-methoxyethyl)aziridine-1-carboxamide (9c).

Following General Procedure J, aziridine 1a (52.9 mg, 0.142 mmol) was treated with 2-methoxyethylamine (12.0 mg, 0.160 mmol) to provide 17 mg (27%) of 8c as a white oil and 12 mg (19%) of 9c as a white oil. Analysis for 8c: R$_f$ 0.07 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.45 – 7.22 (m, 15H), 5.52 (s, 1H), 4.35 (dt, 1H, $J_1$ = 4.9, $J_2$ = 4.5), 3.71 (dt, 1H, $J_1$ = 4.7, $J_2$ = 3.0), 3.38 (m, 3H), 3.23 (s, 3H), 3.22 (dd, 1H, $J_1$ = 5.9, $J_2$ = 4.3), 2.69 (m, 4H); $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 158.7, 143.5, 128.6, 128.0, 127.3, 87.0, 78.9, 71.8, 64.1, 58.9, 54.6, 53.6, 49.3; IR 1751 cm$^{-1}$; HPLC (Method 4, 254 nm) rt 2.733 min, 80.5%; HRMS calculated for (C$_{27}$H$_{30}$N$_2$O$_4$)Na$^+$, 469.2098, observed 469.2092. Analysis for 9c: R$_f$ 0.20 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.46 – 7.22 (m, 15H), 5.89 (s, 1H), 3.62 (m, 1H), 3.44 (dd, 1H, $J_1$ = 9.7, $J_2$ = 4.7), 3.31 – 3.15 (overlapped multiplet, 4H), 3.28 (s, 3H), 3.04 (m, 1H), 2.76 (d, 1H, $J$ = 7.3), 2.48 (m, 1H), 2.35 (d, 1H, $J$ = 6.5), 2.03 (d, 1H, $J$ = 3.8); $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 164.7, 143.5, 128.6, 128.0, 127.3, 87.2, 71.0, 69.0, 66.6, 58.7, 40.3, 40.2, 27.1; IR 3333, 1682, 1651 cm$^{-1}$; HPLC (Method 1, 254 nm) rt 7.46 min, 82.2%; HRMS calculated for (C$_{27}$H$_{30}$N$_2$O$_4$)Na$^+$, 469.2098, observed 469.2090.
4-((Anilino)methyl)-5-((trityloxy)methyl)oxazolidin-2-one (8d). Following General Procedure J, aziridine 1a (58.0 mg, 0.156 mmol) was treated with aniline (16.3 mg, 0.175 mmol) to provide 36 mg (50%) of 8d as a white oil. Rf 0.39 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.42 – 7.22 (m, 15H), 7.13 (t, 2H, \(J = 7.9\)), 6.74 (t, 1H, \(J = 7.3\)), 6.54 (d, 2H, \(J = 7.8\)), 5.73 (s, 1H), 4.38 (dt, 1H, \(J_1 = 5.1\), \(J_2 = 4.7\)), 3.94 (dt, 1H, \(J_1 = 6.0\), \(J_2 = 6.0\)), 3.88 (bs, 1H), 3.36 (m, 2H), 3.22 (m, 2H); \(^13\)C NMR (CDCl\(_3\), 300 MHz), \(\delta\) 158.7, 147.1, 143.3, 129.4, 128.6, 128.1, 127.4, 118.4, 112.7, 118.4, 113.0, 87.3, 78.4, 63.8, 54.3, 47.8; IR 1751 cm\(^{-1}\); HPLC (Method 1, 254 nm) rt 13.46 min, 97.8%; HRMS calculated for \((C_{30}H_{28}N_2O_3)Na^+\), 487.1992, observed 487.1984.

4-((N-Benzylmethylamino)methyl)-5-((trityloxy)methyl)oxazolidin-2-one (8e). Following General Procedure J, aziridine 1a (62.1 mg, 0.167 mmol) was treated with \(N\)-benzylmethylamine (21.7 mg, 0.179 mmol) to provide 44 mg (54%) of 8e as a white oil. Rf 0.40 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.45 – 7.16 (m, 20H), 5.62 (s, 1H), 4.26 (dt, 1H, \(J_1 = 4.6\), \(J_2 = 4.3\)), 3.76 (dt, 1H, \(J_1 = 6.6\), \(J_2 = 5.9\)), 3.44 (s, 2H), 3.39 (dd, 1H, \(J_1 = 5.7\), \(J_2 = 4.7\)), 3.20 (dd, 1H, \(J_1 = 6.0\), \(J_2 = 4.2\)), 2.50 (dd, 1H, \(J_1 = 7.9\), \(J_2 = 4.4\)), 2.39 (dd, 1H, \(J_1 = 6.4\), \(J_2 = 6.0\)), 2.13 (s, 3H); \(^13\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 158.7, 143.4, 138.2, 128.8, 128.6, 128.4, 128.0, 127.4, 127.2, 86.8, 79.2, 63.9, 62.8,
4-((Isobutylamino)methyl)-5-((trityloxy)methyl)oxazolidin-2-one (8f) and 1-Hydroxy-2-(trityloxy)ethyl-N-isobutylaziridine-1-carboxamide (9f). Following General Procedure J, aziridine 1a (96.1 mg, 0.259 mmol) was treated with isobutylamine (21.9 mg, 0.299 mmol) to provide 50 mg (43%) of 8f as a white oil and 32 mg (28%) of 9f as a white oil. Analysis for 8f: Rf 0.09 (50% EtOAc in hexanes); 1H NMR (CDCl₃, 300 MHz) δ 7.45 – 7.21 (m, 15H), 6.19 (s, 1H), 4.39 (dt, 1H, J₁ = 4.8, J₂ = 4.5), 3.69 (m, 1H), 3.38 (dd, 1H, J₁ = 5.8, J₂ = 4.5), 3.23 (dd, 1H, J₁ = 5.8, J₂ = 4.5), 2.71 – 2.58 (m, 2H), 2.40 – 2.28 (m, 2H), 1.61 (m, 1H, J = 6.7), 1.47 (bs, 1H), 0.84 (d, 6H, J = 6.7); 13C NMR (CDCl₃, 300 MHz) δ 159.2, 143.5, 128.6, 128.0, 127.2, 86.9, 79.1, 64.2, 57.8, 54.6, 53.6, 28.3, 20.5; IR 1751 cm⁻¹; HPLC (Method 5, 254 nm) rt 4.95 min, 83.0%; HRMS calculated for (C₂₈H₃₂N₂O₃)Na⁺, 467.2305, observed 467.2296. Analysis for 9f: Rf 0.27 (50% EtOAc in hexanes); 1H NMR (CDCl₃, 300 MHz) δ 7.43 – 7.23 (m, 15H), 5.68 (s, 1H), 3.66 (m, 1H), 3.48 (dd, 1H, J₁ = 5.1, J₂ = 4.6), 3.17 (dd, 1H, J₁ = 5.6, J₂ = 4.1), 2.82 (m, 1H), 2.68 (m, 2H), 2.43 (m, 2H), 2.01 (d, 1H, J = 3.6), 1.4 (m, 1H, J = 6.8), 0.78 (dd, 6H, J₁ = 6.6, J₂ = 4.5); 13C NMR (CDCl₃, 300 MHz) δ 164.7, 143.4, 128.6, 128.1, 127.3, 87.3, 68.8, 66.7, 48.0, 40.5, 28.5, 26.9, 20.0, 19.9; IR 3348, 1682, 1651 cm⁻¹; HPLC
4-((Allylamino)methyl)-5-((trityloxy)methyl)oxazolidin-2-one (8g). Following General Procedure J, aziridine 1a (44.8 mg, 0.121 mmol) treated with allylamine (7.6 mg, 0.133 mmol) to provide 42.7 mg (82%) of 8g as a white oil. Rf 0.16 (75% EtOAc in hexanes); 1H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.45 – 7.22 (m, 15H), 5.86-5.73 (m, 1H), 5.40 (s, 1H), 5.15 – 5.07 (m, 2H), 4.37 (dt, 1H, $J_1 = 4.8, J_2 = 4.6$), 3.38 (dt, 1H, $J_1 = 6.8, J_2 = 5.3$), 3.40 (dd, 1H, $J_1 = 5.6, J_2 = 4.7$), 3.25 – 3.14 (m, 3H), 2.62 (dd, 1H, $J_1 = 7.4, J_2 = 4.8$); 13C NMR (CDCl$_3$, 300 MHz) $\delta$ 158.6, 143.4, 136.3, 128.6, 128.0, 127.2, 116.4, 87.0, 78.9, 64.0, 54.5, 52.7, 52.2; IR 1751 cm$^{-1}$; HPLC (Method 4, 254 nm) rt 4.53 min, 84.5%; HRMS calculated for (C$_{27}$H$_{28}$N$_2$O$_3$)Na$^+$, 451.1992, observed 451.1984.

2-(1-Hydroxy-2-trityloxy-ethyl)-aziridine-1-carboxylic acid allylamide (9g). Following General Procedure K, aziridine 1a (17.9 mg, 0.048 mmol) was treated with allylamine (16.7 mg, 0.293 mmol) to provide 12.0 mg (58%) of 9g. Rf 0.43 (75% EtOAc in hexanes); 1H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.47 – 7.26 (m, 15H), 5.88 (bt, 1H, $J = 5.6$), 5.56 (m, 1H), 4.96 (m, 2H), 3.75 (bd, 1H, $J = 5.9$), 3.56 (m, 2H), 3.27 (m, 1H), 3.10 (dd,
1H, J₁ = 9.7, J₂ = 3.4), 2.64 (d, 1H, J = 8.5), 2.40 (m, 2H); ¹³C NMR (CDCl₃, 300 MHz) δ 171.2, 164.5, 143.2, 134.3, 128.6, 128.1, 127.3, 116.0, 87.4, 67.8, 67.0, 42.8, 40.5; IR 3348, 1744, 1666; HPLC (Method 2, 254 nm) rt 13.29 min, 90.7%; HRMS calculated for (C₂₇H₂₈N₂O₃)Na⁺, 451.1992, observed 451.1982.

**Isopropylamino)methyl)-5-((trityloxy)methyl)oxazolidin-2-one (8h).** Following General Procedure J, aziridine 1a (39.4 mg, 0.106 mmol) treated with isopropylamine (6.9 mg, 0.117 mmol) to provide 38 mg (83%) of 8h as a white oil. Rₚ 0.12 (75% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.45 – 7.22 (m, 15H), 5.43 (s, 1H), 4.37 (dt, 1H, J₁ = 4.7, J₂ = 4.6), 3.66 (dt, 1H, J₁ = 7.3, J₂ = 5.1), 3.39 (dd, 1H, J₁ = 5.6, J₂ = 4.6), 3.23 (dd, 1H, J₁ = 5.9, J₂ = 4.3), 2.73 (m, 2H), 2.6 (dd, 1H, J₁ = 7.6, J₂ = 4.4), 1.00 (t, 6H, J = 5.7); ¹³C NMR (CDCl₃, 300 MHz) δ 158.8, 143.5, 128.7, 128.1, 127.3, 87.0, 79.1, 64.2, 55.1, 53.5, 51.2, 48.9, 23.2; IR 1751 cm⁻¹; HPLC (Method 4, 254 nm) rt 4.37 min, 89.6%; HRMS calculated for (C₂₇H₃₀N₂O₃)Na⁺, 453.2149, observed 453.2140.

![Chemical structure of 8h](image)

**2-(1-Hydroxy-2-trityloxy-ethyl)-aziridine-1-carboxylic acid isopropylamide (9h).** Following General Procedure K, aziridine 1a (21.1 mg, 0.057 mmol) was treated with isopropylamine (20.1 mg, 0.341 mmol) to provide 6 mg (25%) of 9h. Rₚ 0.21 (50%
EtOAc in hexanes; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.45 (m, 6H), 7.28 (m, 9H), 5.37 (d, 1H, $J$ = 8.1), 3.77 (m, 1H), 3.64 (d, 1H, $J$ = 3.3), 3.43 (dd, 1H, $J_1$ = 9.6, $J_2$ = 4.7), 2.62 (d, 1H, $J$ = 7.5), 2.46 (m, 1H), 2.38 (d, 1H, $J$ = 6.5), 2.00 (d, 1H, $J$ = 3.7), 0.94 (dd, 6H, $J_1$ = 16.0, $J_2$ = 6.5); $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 163.7, 143.4, 128.6, 128.0, 127.3, 87.2, 68.9, 66.5, 42.6, 40.5, 26.9, 22.4; IR 3317, 1743, 1651 cm$^{-1}$; HPLC (Method 2, 254 nm) rt 13.65 min, 92.84%; HRMS calculated for (C$_{27}$H$_{30}$N$_2$O$_3$)Na$^+$, 453.2149, observed 453.2140.

4-((Propylamino)methyl)-5-((trityloxy)methyl)oxazolidin-2-one (8i) and 1-Hydroxy-2-(trityloxy)ethyl-N-propylaziridine-1-carboxamide (9i). Following General Procedure J, aziridine 1a (118.5 mg, 0.319 mmol) was treated with propylamine (20 mg, 0.340 mmol) to provide 15 mg (11%) of 8i as a white oil and 35 mg (26%) of 9i as a white oil. Analysis for 8i: R$_f$ 0.08 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.45 – 7.22 (m, 15H), 5.62 (s, 1H), 4.37 (dt, 1H, $J_1$ = 4.8, $J_2$ = 4.6), 3.69 (dt, 1H, $J_1$ = 7.1, $J_2$ = 5.1), 3.39 (dd, 1H, $J_1$ = 5.6, $J_2$ = 4.6), 3.23 (dd, 1H, $J_1$ = 5.9, $J_2$ = 4.3), 2.67 (m, 2H), 2.50 (m, 2H), 1.41 (m, 2H), 0.87 (t, 3H, $J$ = 7.4); $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 158.6, 143.3, 128.5, 127.9, 127.1, 86.8, 78.8, 64.0, 54.4, 53.4, 51.6, 23.0, 11.5; IR 1751 cm$^{-1}$; HPLC (Method 4, 254 nm) rt 4.67 min, 79.8%; HRMS calculated for (C$_{27}$H$_{30}$N$_2$O$_3$)Na$^+$, 453.2149, observed 453.2143. Analysis for 9i: R$_f$ 0.43 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.46 – 7.23 (m, 15H), 5.74 (m, 1H), 3.68 (s, 1H), 3.49 (dd,
1H, \( J_1 = 5.1, J_2 = 4.6 \), 3.16 (dd, 1H, \( J_1 = 5.9, J_2 = 3.8 \)), 2.85 (m, 3H), 2.41 (m, 2H), 2.01 (d, 1H, \( J = 3.5 \)), 1.21 (m, 2H, \( J = 7.3 \)), 0.77 (t, 3H, \( J = 7.3 \)); \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \( \delta \) 164.7, 143.3, 128.6, 128.1, 127.3, 87.3, 68.5, 66.8, 42.2, 40.6, 26.6, 22.8, 11.2; IR 3333, 1682, 1651 cm\(^{-1}\); HPLC (Method 1, 254 nm) rt 10.70 min, 89.1%; HRMS calculated for \((C_{27}H_{30}N_2O_3)\), 453.2149, observed 453.2142.

racemic-trans-N-(2-(Benzylamino)-2-oxoethyl)-2-(1-hydroxy-2-(trityloxy)ethyl)aziridine-1-carboxamide (72a-isomer 1). Aziridine 1a (13.3 mg, 35.8 \( \mu \)mol) was treated with glycine benzylamide 12 (36.3 mg, 221 \( \mu \)mol) in toluene (100 \( \mu \)L) and placed on the shaker for 48 h. The reaction was concentrated and purified via flash chromatography (25% EtOAc in hexanes) to provide 3.7 mg (19% yield) of 72a-isomer 1. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 7.42 – 7.03 (m, 20H), 6.29 and 6.27 (overlapped bs+bs, 2H), 4.39 (m, 2H), 3.67 (bs, 1H), 3.50 (m, 3H), 3.16 (dd, 1H, \( J_1 = 9.6, J_2 = 3.7 \)), 2.82 (bd, 1H, \( J = 6.4 \)), 2.45 (m, 1H), 2.35 (d, 1H, \( J = 6.4 \)), 2.09 (d, 1H, \( J = 3.6 \)).

5.4 Aziridinyl ureas

**General Procedure L**

Reactions of bicyclic aziridines 1 to give aziridinyl ureas 11

Bicyclic aziridines 1 in anhydrous CH\(_2\)Cl\(_2\) (0.1 – 0.8 M, typically 0.7 M) were treated with freshly distilled amine (110 – 195 mol%, typically 110 mol%). The reactions were stirred at room temperature until complete as judged by TLC (24 – 72 h, typically 48 h).
The reactions were concentrated and, where necessary, purified as indicated to provide aziridinyl ureas.

2-Hydroxymethyl-3-propyl-aziridine-1-carboxylic acid benzylamide (10a). Following General Procedure L, aziridine 1b (30.0 mg, 0.213 mmol) was treated with benzylamine (25.5 mg, 0.238 mmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 14 mg (26% yield) of 10a as a colorless oil. R_f 0.33 (75% EtOAc in hexanes); ^1H NMR (CDCl_3, 300 MHz) δ 7.35 – 7.28 (m, 5H), 5.44 (s, 1H), 4.42 (m, 2H), 4.07 (d, 1H, J = 12.1), 3.43 (dd, 1H, J_1 = 7.9, J_2 = 4.2), 3.21 (bs, 1H), 2.55 (m, 1H), 2.31 (m, 1H), 1.41 (m, 4H), 0.94 (t, 3H, J = 7.1; ^13C NMR (CDCl_3, 300 MHz) δ 163.8, 138.4, 128.8, 127.6, 62.7, 44.8, 44.1, 41.7, 33.0, 20.5, 13.8; IR 3310, 1651 cm^{-1}; HPLC (Method 2, 254 nm) rt 5.48 min, 96.8%; HRMS calculated for (C_{14}H_{20}N_2O_2)Na^+, 271.1417, observed 271.1409.

2-(Hydroxymethyl)-3-propylaziridin-1-yl(morpholino)methanone (10b). Following General Procedure L, aziridine 1b (49.0 mg, 0.347 mmol) was treated with morpholine (33.7 mg, 0.387 mmol), then purified via flash chromatography (100% EtOAc) to provide
16 mg (20% yield) of 10b as a colorless oil. R_f 0.11 (100% EtOAc); ^1^H NMR (CDCl_3, 300 MHz) δ 3.95 (bddd, 1H, J_1 = 9.2, J_2 = 3.0), 3.70 – 3.49 (brm, 9H), 2.76 (bs, 1H), 2.63 (m, 1H), 2.42 (m, 1H), 1.78 (brm, 1H), 1.44 (brm, 2H), 1.17 (brm, 1H), 0.95 (t, 3H, J = 7.4); ^1^C NMR (CDCl_3, 300 MHz) δ 162.1, 66.2, 61.5, 43.6, 41.8, 31.6, 29.6, 19.6, 13.3; IR 3394, 1636 cm^-1; HPLC (Method 2, ELSD) rt 5.30 min, 93.7%; HRMS calculated for (C_{11}H_{20}N_{2}O_{3})Na^+, 251.1366, observed 251.1365.

![Image](https://example.com/image.png)

2-(Hydroxymethyl)-N-(2-methoxyethyl)-3-propylaziridine-1-carboxamide (10c). Following General Procedure L, aziridine 1b (49.0 mg, 0.347 mmol) was treated with 2-methoxyethylamine (29.2 mg, 0.389 mmol), then purified via flash chromatography (100% EtOAc) to provide 8 mg (11% yield) of 10c as a colorless oil. R_f 0.10 (100% EtOAc); ^1^H NMR (CDCl_3, 300 MHz) δ 5.52 (s, 1H), 4.06 (d, 1H, J = 11.8), 3.50 – 3.37 (overlapped multiplet, 6H), 3.37 (overlapped singlet, 3H), 2.53 (m, 1H), 2.32 (m, 1H), 1.49 (m, 4H), 0.97 (t, 3H, J = 7.1); ^1^C NMR (CDCl_3, 300 MHz) δ 163.7, 71.2, 62.4, 68.7, 44.5, 40.8, 40.2, 33.0, 20.3, 13.7; IR 3310, 1651 cm^-1; HPLC (Method 6, 254 nm) rt 3.41 min, 92.1%; HRMS calculated for (C_{10}H_{20}N_{2}O_{3})Na^+, 239.1366, observed 239.1366.
2-(Hydroxymethyl)-N-phenyl-3-propylaziridine-1-carboxamide (10d). Following General Procedure L, aziridine 1b (49.0 mg, 0.347 mmol) was treated with aniline (35.7 mg, 0.383 mmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 22 mg (27% yield) of 10d as a colorless oil. Rf 0.30 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.17 (t, 2H, $J$ = 7.9), 6.73 (t, 1H, $J$ = 7.3), 6.61 (d, 2H, $J$ = 7.7), 5.88 (s, 1H), 4.49 (t, 1H, $J$ = 8.9), 4.26 (dd, 1H, $J_1$ = 4.7, $J_2$ = 4.6), 3.93 (m, 1H), 3.44 (bs, 2H), 1.40 (m, 4H), 0.91 (t, 3H, $J$ = 6.9); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 159.9, 147.1, 129.4, 117.9, 112.9, 67.7, 55.9, 55.4, 32.9, 18.8, 13.9; IR 3348, 1751 cm$^{-1}$; HPLC (Method 2, 254 nm) rt 11.64 min, 93.7%; HRMS calculated for (C$_{13}$H$_{18}$N$_2$O$_2$)Na$^+$, 257.1261, observed 257.1260.

N-Benzyl-2-(hydroxymethyl)-N-methyl-3-propylaziridine-1-carboxamide (10e). Following General Procedure L, aziridine 1b (49.0 mg, 0.347 mmol) was treated with N-benzylmethylamine (47.1 mg, 0.389 mmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 36 mg (40% yield) of 10e as a colorless oil. Rf 0.23 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.33 – 7.27 (m, 5H), 4.91 (bd, AB quartet, 0.3H, $J$ = 16.0), 4.61 (bd, AB quartet, 0.7H, $J$ = 14.8), 4.49 (bd, AB quartet,
0.7H, J = 14.6), 4.39 (bs, AB quartet, 0.3H, J = 16.3), 4.01 – 3.97 (bm, 1H), 3.52 (m, 1H), 3.35 (bs, 0.3H), 3.14 (bs, 0.6H), 2.99 – 2.91 (bm, 3H), 2.63 (bm, 1H), 2.42 – 2.31 (m, 1H), 1.66 (m, 1H), 1.42 (m, 3H), 0.96 (t, 3H, J = 7.1); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 159.9, 147.1, 129.4, 117.9, 112.9, 67.7, 55.9, 55.4, 32.9, 18.8, 13.9; IR 3394, 1651, 1636 cm$^{-1}$; HPLC (Method 2, 254 nm) rt 11.41 min, 97.2%; HRMS calculated for (C$_{15}$H$_{22}$N$_2$O$_2$)Na$^+$, 285.1573, observed 285.1572.

2-(Hydroxymethyl)-N-isobutyl-3-propylaziridine-1-carboxamide (10f). Following General Procedure L, aziridine 1b (49.0 mg, 0.347 mmol) was treated with isobutylamine (27.7 mg, 0.379 mmol), then purified via flash chromatography (75% EtOAc in hexanes) to provide 31 mg (42% yield) of 10f as a colorless oil. R$_f$ 0.20 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 5.27 (bs, 1H), 4.05 (d, 1H, J = 11.3), 3.41 (m, 2H), 3.06 (t, 2H, J = 6.5), 2.52 (m, 1H), 2.28 (dd, 1H, J$_1$ = 5.9, J$_2$ = 3.3), 1.78 (m, 1H), 1.51 (m, 4H), 0.95 (m, 9H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.9, 62.7, 48.1, 44.0, 41.7, 33.1, 28.7, 20.6, 20.0, 13.8; IR 3294, 1682, 1666 cm$^{-1}$; HPLC (Method 2, ELSD) rt 9.90 min, 91.5%; HRMS calculated for (C$_{11}$H$_{22}$N$_2$O$_2$)Na$^+$, 237.1573, observed 237.1573.
N-Allyl-2-(hydroxymethyl)-3-propylaziridine-1-carboxamide (10g). Following General Procedure L, aziridine 1b (24.7 mg, 0.175 mmol) was treated with allylamine (11.4 mg, 0.200 mmol), then purified via flash chromatography (100% EtOAc) to provide 20 mg (58% yield) of 10g as a colorless oil. R\(_f\) 0.33 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta 5.85, 5.32, 5.16, 4.06, 3.86, 3.42, 2.53, 2.31, 1.48, 0.97\); \(^1\)C NMR (CDCl\(_3\), 300 MHz) \(\delta 163.7, 134.3, 116.3, 62.6, 44.1, 41.6, 33.0, 20.5, 13.8\); IR 3310, 1666, 1651 cm\(^{-1}\); HPLC (Method 2, ELSD) rt 5.78 min, 89.6%; HRMS calculated for (C\(_{10}\)H\(_{18}\)N\(_2\)O\(_2\))\(\text{Na}^+\), 221.1260, observed 221.1260.

2-(Hydroxymethyl)-N-isopropyl-3-propylaziridine-1-carboxamide (10h). Following General Procedure L, aziridine 1b (24.7 mg, 0.175 mmol) was treated with isopropylamine (11.8 mg, 0.200 mmol), then purified via flash chromatography (100% EtOAc) to provide 14 mg (40% yield) of 10h as a colorless oil. R\(_f\) 0.26 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta 5.00, 4.04, 3.93, 3.42, 2.51, 2.26, 1.17, 0.97\); \(^1\)C
NMR (CDCl₃, 300 MHz) δ 163.8, 62.7, 44.0, 42.4, 41.6, 33.0, 23.1, 20.6, 13.8, 11.3; IR 3294, 1651 cm⁻¹; HPLC (Method 2, ELSD) rt 6.94 min, 91.5%; HRMS calculated for \((\text{C}_{10}\text{H}_{20}\text{N}_{2}\text{O}_{2})\text{Na}^+\), 223.1417, observed 223.1416.

2-(Hydroxymethyl)-N,3-dipropylaziridine-1-carboxamide (10i). Following General Procedure L, aziridine 1b (24.7 mg, 0.175 mmol) was treated with propylamine (11.5 mg, 0.195 mmol), then purified via flash chromatography (100% EtOAc) to provide 19 mg (54% yield) of 10i as a colorless oil, \(R_f\) 0.27 (100% EtOAc); \(^1\)H NMR (CDCl₃, 300 MHz) δ 5.24 (bs, 1H), 4.06 (d, 1H, \(J = 11.6\)), 3.42 (m, 2H), 3.21 (m, 2H), 2.53 (m, 1H), 2.28 (dd, 1H, \(J_1 = 5.9\), \(J_2 = 3.2\)), 1.50 (m, 6H), 0.96 (m, 6H); \(^{13}\)C NMR (CDCl₃, 300 MHz) δ 162.9, 62.7, 43.9, 42.7, 41.6, 32.9, 23.5, 23.0, 22.8, 20.5, 13.8; IR 3310, 1666, 1636 cm⁻¹; GC (Method 1) rt 3.56 min, 86.5%; HRMS calculated for \((\text{C}_{10}\text{H}_{20}\text{N}_{2}\text{O}_{2})\text{Na}^+\), 223.1417, observed 223.1416.

2-Hydroxymethyl-3-propyl-aziridine-1-carboxylic acid (benzylcarbamoyl-methyl)-amide (73). Aziridine 1b (7.4 mg, 0.052 mmol) was treated with glycine benzylamide 12 (10.0 mg, 0.061 mmol) in CDCl₃ (0.700 mL) and monitored by \(^1\)H NMR until complete,
then concentrated and purified via flash chromatography (100% EtOAc) to provide 9.0 mg (56%) of 73. \( R_f \) 0.14 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 7.35 – 7.24 (m, 5H), 6.67 (bs, 1H), 6.00 (bm, 1H), 4.42 (m, 2H), 4.12 (m, 3H), 3.71 (dd, 1H, \( J_1 = 16.7 \), \( J_2 = 4.9 \)), 3.40 (dd, 1H, \( J_1 = 11.7 \), \( J_2 = 7.8 \)), 2.51 (m, 1H), 2.40 (m, 1H), 1.45 (m, 4H), 0.95 (t, 3H, \( J = 7.0 \)).

\[
\text{2-(Hydroxymethyl)-N-isobutyl-3-isopropylaziridine-1-carboxamide (GWB-123).}
\]

Following General Procedure L, bicyclic aziridine 1g (35 mg, 250 µmol) was treated with isobutylamine (20 mg, 270 µmol), then concentrated to provide 48 mg (91% yield) of clean, crude GWB-123 as a pale yellow oil. \( R_f \) 0.22 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 5.35 (bs, 1H), 4.09 (dd, 1H, \( J_1 = 12.5 \), \( J_2 = 2.6 \)), 3.36 (dd, 1H, \( J_1 = 12.5 \), \( J_2 = 3.8 \)), 3.05 (t, 2H, \( J = 6.4 \)), 2.56 (m, 1H), 2.05 (dd, 1H, \( J_1 = 7.4 \), \( J_2 = 3.2 \)), 1.78 (sept, 1H, \( J = 6.7 \)), 1.54 (m, 1H), 1.06 (d, 3H, \( J = 6.7 \)), 0.98 (d, 3H, \( J = 6.8 \)), 0.92 (d, 6H, \( J = 6.7 \)); \(^1^3\)C NMR (CDCl\(_3\), 300 MHz) \( \delta \) 164.2, 63.01, 48.0, 47.8, 43.4, 30.0, 28.7, 20.1, 20.0, 19.4; IR 3328, 1653 cm\(^{-1}\); HPLC (Method 8, ELSD) rt 2.07 min, 95.4%; HRMS calculated for (C\(_{11}\)H\(_{22}\)N\(_2\)O\(_2\))Na\(^+\), 237.1573, observed 237.1574.
2-(Hydroxymethyl)-3-isopropyl-N-(2-methoxyethyl)aziridine-1-carboxamide (GWB-124). Following General Procedure L, bicyclic aziridine 1g (35 mg, 250 µmol) was treated with 2-methoxyethylamine (21 mg, 270 µmol), then concentrated to provide 49 mg (91% yield) of clean, crude GWB-124 as a pale yellow oil. Rf 0.18 (100% EtOAc); 1H NMR (CDCl₃, 300 MHz) δ 5.70 (bs, 1H), 4.09 (dd, 1H, J₁ = 12.5, J₂ = 2.6), 3.55 – 3.29 (m, 8H), 2.56 (m, 1H), 2.12 (dd, 1H, J₁ = 7.4, J₂ = 3.3), 1.50 (sex, 1H, J = 6.8), 1.05 (d, 3H, J = 6.7), 0.97 (d, 3H, J = 6.8); 13C NMR (CDCl₃, 300 MHz) δ 164.0, 71.2, 62.6, 58.7, 46.9, 43.8, 40.2, 30.0, 20.0, 19.3; IR 3354, 1653 cm⁻¹; HPLC (Method 9, ELSD) rt 2.48 min, 98.6%; HRMS calculated for (C₁₀H₂₀N₂O₃)Na⁺, 239.1366, observed 239.1367.

2-(Hydroxymethyl)-3-isopropylaziridin-1-yl(pyrrolidin-1-yl)methanone (GWB-125). Following General Procedure L, bicyclic aziridine 1g (32 mg, 230 µmol) was treated with pyrrolidine (18 mg, 260 µmol), then concentrated to provide 45 mg (94% yield) of clean, crude GWB-125 as a yellow oil. Rf 0.18 (100% EtOAc); 1H NMR (CDCl₃, 300 MHz) δ 3.98 (dd, 1H, J₁ = 12.4, J₂ = 3.1), 3.53 - 3.41 (m, 5H), 2.61 (m, 1H), 2.30 (dd, 1H, J₁ = 5.3, J₂ = 3.4), 1.95 – 1.86 (m, 5H), 1.01 (d, 3H, J = 6.8), 0.87 (d, 3H, J = 6.8); 13C NMR (CDCl₃, 300 MHz) δ 162.6, 62.8, 46.8, 46.6, 46.5, 41.5, 28.0, 26.0, 24.6, 20.1,
209

17.7; IR 3422, 1636 cm\(^{-1}\); HPLC (Method 9, ELSD) rt 2.83 min, 98.1%; HRMS calculated for \((C_{11}H_{20}N_{2}O_{2})\)Na\(^+\), 235.1517, observed 235.1418.

\[
\text{2-(Hydroxymethyl)-3-isopropyl-N-phenethylaziridine-1-carboxamide (GWB-126).} 
\]

Following General Procedure L, bicyclic aziridine 1g (32 mg, 230 µmol) was treated with phenethylamine (30 mg, 250 µmol), then concentrated to provide 56 mg (95% yield) of clean, crude GWB-126 as a pale yellow oil. R\(_f\) 0.21 (75% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta 7.33 – 7.19 \text{ (m, 5H)}, 5.22 \text{ (bs, 1H)}, 4.02 \text{ (dd, 1H, } J_1 = 12.5, J_2 = 2.6), 3.49 \text{ (m, 2H)}, 3.27 \text{ (dd, 1H, } J_1 = 12.5, J_2 = 8.7), 2.82 \text{ (t, 2H, } J = 6.8), 2.50 \text{ (m, 1H)}, 1.91 \text{ (dd, 1H, } J_1 = 7.4, J_2 = 3.2), 1.43 \text{ (m, 1H)}, 0.92 \text{ (m, 6H)}; ^{13}\text{C NMR (CDCl}_3\text{, 300 MHz)} \delta 164.0, 138.7, 128.8, 128.7, 126.6, 62.9, 47.7, 43.4, 41.5, 35.8, 30.0, 20.0, 19.4; IR 3416, 1654 cm\(^{-1}\); HPLC (Method 8, ELSD) rt 3.24 min, 95.9%; HRMS calculated for \((C_{15}H_{22}N_{2}O_{2})\)Na\(^+\), 285.1573, observed 285.1574.

\[
\text{N-(2-(Benzylamino)-2-oxoethyl)-2-(hydroxymethyl)-3-isopropylaziridine-1-carboxamide (GWB-127).} 
\]

Following General Procedure L, bicyclic aziridine 1g (30 mg, 210 µmol) was treated with glycine benzylamide 61a (37 mg, 230 µmol), then purified via flash chromatography (5% MeOH in CH\(_2\)Cl\(_2\)) to provide 43 mg (66% yield)
of GWB-127 as a white solid. R_f 0.21 (5% MeOH in CH_2Cl_2); ^1^H NMR (MeOD-d_4, 300 MHz) δ 7.30 – 7.18 (m, 5H), 4.34 (s, 2H), 4.02 (m, 2H), 3.69 (m, 2H), 2.44 (s, 1H), 2.35 (m, 1H), 1.48 (m, 1H), 1.03 (dd, 3H, J_1 = 6.6, J_2 = 1.6), 0.95 (dd, 3H, J_1 = 6.7, J_2 = 1.7); ^1^C NMR (MeOD-d_4, 300 MHz) δ 172.6, 166.1, 139.8, 129.5, 128.4, 128.1, 58.9, 45.7, 44.8, 44.5, 43.8, 31.3, 20.3, 19.5; IR 3438, 1644 cm\(^{-1}\); HPLC (Method 9, ELSD) rt 4.00 min, 99.9%; HRMS calculated for (C\(_{16}\)H\(_{23}\)N\(_3\)O\(_3\))Na\(^+\), 328.1632, observed 328.1632; mp 131.9 ºC.

2-(Hydroxymethyl)-N-isopentyl-3-isopropylaziridine-1-carboxamide (GWB-128).

Following General Procedure L, bicyclic aziridine 1g (35 mg, 250 µmol) was treated with isoamylamine (24 mg, 280 µmol), then concentrated to provide 54 mg (95% yield) of clean, crude GWB-128 as a clear, colorless oil. R_f 0.19 (50% EtOAc in hexanes); ^1^H NMR (CDCl_3, 300 MHz) δ 5.27 (bs, 1H), 4.08 (dd, 1H, J_1 = 12.5, J_2 = 2.6), 3.36 (dd, 1H, J_1 = 12.5, J_2 = 8.7), 3.24 (m, 2H), 2.55 (m, 1H), 2.04 (dd, 1H, J_1 = 7.4, J_2 = 3.2), 1.67 – 1.46 (m, 2H), 1.40 (m, 2H), 1.05 (d, 3H, J = 6.7), 0.97 (d, 3H, J = 6.8), 0.92 (d, 6H, J = 6.6); ^1^C NMR (CDCl_3, 300 MHz) δ 164.1, 63.0, 47.7, 43.3, 39.1, 38.7, 30.0, 25.8, 22.5, 22.5, 20.1, 19.4; IR 3323, 1654 cm\(^{-1}\); HPLC (Method 8, ELSD) rt 2.91 min, 99.6%; HRMS calculated for (C\(_{12}\)H\(_{24}\)N\(_2\)O\(_2\))Na\(^+\), 251.1730, observed 251.1731.
2-(Hydroxymethyl)-N-(4-hydroxyphenethyl)-3-isopropylaziridine-1-carboxamide (GWB-129). Following General Procedure L, bicyclic aziridine 1g (38 mg, 270 µmol) was treated with tyramine (40 mg, 290 µmol), then purified via flash chromatography (100% EtOAc) to provide 29 mg (39% yield) of GWB-129 as a white oil. Rf 0.35 (100% EtOAc); ¹H NMR (CDCl₃, 300 MHz) δ 7.03 (d, 2H, J = 8.2), 6.79 (d, 2H, J = 8.4), 5.22 (bs, 1H), 4.04 (d, 1H, J = 11.9), 3.46 (q, 2H, J = 6.5), 3.28 (dd, 1H, J₁ = 12.3, J₂ = 8.7), 2.74 (t, 2H, J = 6.7), 2.52 (m, 1H), 1.94 (dd, 1H, J₁ = 7.4, J₂ = 3.1), 1.45 (sex, 1H, J = 6.8), 0.94 (d, 3H, J = 6.7), 0.91 (d, 3H, J = 6.8); ¹³C NMR (CDCl₃, 300 MHz) δ 164.3, 155.1, 129.8, 115.6, 62.8, 47.8, 43.3, 41.8, 34.8, 29.9, 20.0, 19.4; IR 3345, 1653 cm⁻¹; HPLC (Method 9, ELSD) rt 2.70 min, 100%; HRMS calculated for (C₁₅H₂₂N₂O₃)Na⁺, 301.1523, observed 301.1523.

N-(2-(1H-Indol-3-yl)ethyl)-2-(hydroxymethyl)-3-isopropylaziridine-1-carboxamide (GWB-130). Following General Procedure L, bicyclic aziridine 1g (38 mg, 270 µmol) was treated with tryptamine (47 mg, 290 µmol), then filtered and concentrated to provide 64 mg (79% yield) of GWB-130 as a yellow oil. Rf 0.14 (75% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 8.56 (bs, 1H), 7.58 (d, 1H, J = 7.7), 7.35 (d, 1H, J = 8.0), 7.24
2-(Hydroxymethyl)-3-isopropyl-N-(3-(methylthio)propyl)aziridine-1-carboxamide (GWB-131). Following General Procedure L, bicyclic aziridine 1g (46 mg, 330 µmol) was treated with 3-(methylthio)propylamine (37 mg, 360 µmol), then concentrated to provide 79 mg (99% yield) of clean, crude GWB-131 as a pale yellow oil. Rf 0.14 (75% EtOAc in hexanes); 1H NMR (CDCl3, 300 MHz) δ 5.69 (bs, 1H), 4.08 (dd, 1H, J1 = 12.5, J2 = 2.6), 3.40 – 3.27 (m, 3H), 2.57 – 2.53 (m, 3H), 2.11 – 2.06 (m, 4H), 1.83 (qu, 2H, J = 6.8), 1.52 (m, 1H), 1.06 (d, 3H, J = 6.7), 0.97 (d, 3H, J = 6.8); 13C NMR (CDCl3, 300 MHz) δ 164.1, 62.8, 47.4, 43.4, 39.9, 31.7, 30.0, 28.7, 20.1, 19.4, 15.5; IR 3422, 1646 cm⁻¹; HPLC (Method 8, ELSD) rt 2.03 min, 97.3%; HRMS calculated for (C_{11}H_{22}N_{2}O_{2}S)Na⁺, 269.1294, observed 269.1295.
N-(2-Hydroxyethyl)-2-(hydroxymethyl)-3-isopropylaziridine-1-carboxamide (GWB-132). Following General Procedure L, bicyclic aziridine 1g (43 mg, 310 µmol) was treated with ethanolamine (20 mg, 330 µmol), then purified via flash chromatography (5% MeOH in CH₂Cl₂) to provide 36 mg (58% yield) of GWB-132 as a white oil. R_f 0.18 (5% MeOH in CH₂Cl₂); ^1H NMR (CDCl₃, 300 MHz) δ 6.01 (bt, 1H, J = 5.4), 4.10 (d, 1H, J = 12.7), 3.76 – 3.60 (m, 2H), 3.56 – 3.39 (m, 2H), 3.23 (m, 1H), 2.55 (m, 1H), 2.20 (dd, 1H, J₁ = 7.3, J₂ = 3.2), 1.50 (sex, 1H, J = 6.8), 1.04 (d, 3H, J = 6.7), 0.96 (d, 3H, J = 6.8); ^13C NMR (CDCl₃, 300 MHz) δ 164.4, 61.8, 61.6, 46.0, 44.2, 43.0, 30.0, 20.0, 19.3; IR 3403, 1653 cm⁻¹; HPLC (Method 9, ELSD) rt 2.32 min, 100%; HRMS calculated for (C₉H₁₈N₂O₃)Na⁺, 225.1210, observed 225.1211.

N-Benzyl-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-97). Following General Procedure L, bicyclic aziridine 1c (23 mg, 110 µmol) was treated with benzylamine (13 mg, 120 µmol), then concentrated to provide 34 mg (97% yield) of clean, crude GWB-97 as a pale yellow oil. R_f 0.43 (75% EtOAc in hexanes); ^1H NMR (CDCl₃, 300 MHz) δ 7.30 – 7.14 (m, 10H), 5.31 (s, 1H), 4.26 (d, 2H, J = 5.9), 3.92 (d, 1H, J = 11.5), 3.55 (s, 1H), 3.29 (m, 1H), 2.77 (t, 2H, J = 7.3), 2.40 (m, 1H), 2.25 (m,
1H), 1.86 (m, 2H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.7, 140.5, 138.4, 128.7, 128.7, 128.5, 127.6, 127.5, 126.4, 62.5, 44.6, 44.1, 40.9, 33.3, 32.1; IR 3310, 1651 cm$^{-1}$; HPLC (Method 9, ELSD) rt 3.97 min, 90.1%; HRMS calculated for (C$_{19}$H$_{22}$N$_2$O$_2$)Na$^+$, 333.1573, observed 333.1571.

![Structure of GWB-100](image)

**2-(Hydroxymethyl)-N-(2-methoxyethyl)-3-phenethylaziridine-1-carboxamide (GWB-100).** Following General Procedure L, bicyclic aziridine 1c (202 mg, 995 µmol) was treated with 2-methoxyethylamine (111 mg, 1.47 mmol), then purified via flash chromatography (100% EtOAc) to provide 239 mg (86% yield) of GWB-100 as a pale yellow oil. R$_f$ 0.23 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.33 – 7.19 (m, 5H), 5.44 (bs, 1H), 3.94 (d, 1H, $J = 12.2$), 3.60 – 3.20 (m, 9H), 2.79 (m, 2H), 2.41 (m, 1H), 2.32 (m, 1H), 1.93 (m, 1H), 1.77 (m, 1H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.6, 140.9, 128.6, 128.5, 126.2, 71.2, 62.1, 58.7, 44.6, 40.2, 40.2, 33.3, 32.5; IR 3317, 1651 cm$^{-1}$; LCMS (Method 10, 254 nm) rt 4.65 min, 98.6%; HRMS calculated for (C$_{15}$H$_{22}$N$_2$O$_3$)Na$^+$, 301.1523, observed 301.1519.
2-(Hydroxymethyl)-N-isobutyl-3-phenethylaziridine-1-carboxamide (GWB-96).

Following *General Procedure L*, bicyclic aziridine 1c (102 mg, 503 μmol) was treated with isobutylamine (54 mg, 730 mmol), then purified via flash chromatography (75% EtOAc in hexanes) to provide 121 mg (87% yield) of **GWB-96** as a pale yellow oil. R<sub>f</sub> 0.35 (75% EtOAc in hexanes); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.32 – 7.21 (m, 5H), 5.30 (s, 1H), 3.97 (m, 1H), 3.46 (s, 1H), 3.30 (m, 1H), 2.93 (m, 2H), 2.82 (t, 2H, J = 7.2), 2.42 (m, 1H), 2.23 (m, 1H), 1.89 (m, 2H), 1.68 (m, 1H), 0.85 (d, 6H, J = 6.6); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) δ 163.8, 140.4, 128.7, 128.5, 126.4, 62.7, 48.1, 44.0, 41.0, 33.4, 32.1, 28.6, 20.0; IR 3317, 1651, cm<sup>-1</sup>; LCMS (Method 10, 254 nm) rt 4.81 min, 97.0%; HRMS calculated for (C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>)Na<sup>+</sup>, 299.1730, observed 299.1730.

N-Allyl-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-95).

Following *General Procedure L*, bicyclic aziridine 1c (19 mg, 93 μmol) was treated with allylamine (5.3 mg, 93 μmol), then concentrated to provide 20 mg (83% yield) of clean, crude **GWB-95** as a pale yellow oil. R<sub>f</sub> 0.30 (75% EtOAc in hexanes); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.34 – 7.21 (m, 5H), 5.75 (m, 1H), 5.13 (m, 2H), 4.81 (bs, 1H), 3.98 (d, 1H, J = 9.1), 3.71 (t, 2H, J = 5.5), 3.31 (m, 2H), 2.82 (t, 2H, J = 7.2), 2.43 (m, 1H), 2.26 (m,
2-(Hydroxymethyl)-3-phenethylaziridin-1-yl(pyrrolidin-1-yl)methanone (GWB-101). Following General Procedure L, bicyclic aziridine 1c (198 mg, 0.974 mmol) was treated with pyrrolidine (105 mg, 1.74 mmol), then purified via flash chromatography (75% EtOAc in hexanes) to provide 224 mg (84% yield) of GWB-101 as a yellow oil. R$_f$ 0.19 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.32 – 7.15 (m, 5H), 3.87 (dd, 1H, $J_1 = 12.3, J_2 = 2.7$), 3.40 (m, 5H), 2.72 (m, 2H), 2.43 (m, 1H), 2.33 (m, 1H), 2.21 (m, 1H), 1.88 (m, 4H), 1.52 (m, 1H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 162.3, 140.8, 128.5, 128.4, 126.3, 62.6, 46.8, 46.6, 44.4, 41.0, 32.8, 32.4, 26.0, 24.6; IR 3394, 1651 cm$^{-1}$; LCMS (Method 10, 254 nm) rt 4.78 min, 99.0%; HRMS calculated for (C$_{16}$H$_{22}$N$_2$O$_2$)Na$^+$, 297.1573, observed 297.1570.
N-(2-(Benzylamino)-2-oxoethyl)-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-94). Following General Procedure L, bicyclic aziridine 1c (11 mg, 54 µmol) was treated with glycine benzylamide 12 (12 mg, 73 µmol), then recrystallized from CH$_2$Cl$_2$ and hexanes to provide 19.5 mg (99% yield) of GWB-94 as an off white solid. R$_f$ 0.12 (75% EtOAc in hexanes); $^1$H NMR (CD$_3$OD, 300 MHz) δ 7.26 (m, 10H), 4.35 (s, 2H), 3.99 (d, 1H, $J = 17.1$), 3.84 (s, 1H), 3.68 (d, 1H, $J = 17.1$), 3.59 (dd, 1H, $J_1 = 12.9$, $J_2 = 3.7$), 2.75 (m, 2H), 2.55 (m, 1H), 2.25 (s, 1H), 1.99 (m, 1H), 1.62 (m, 1H); $^{13}$C NMR (CD$_3$OD, 300 MHz) δ 174.0, 167.6, 144.1, 141.3, 131.1, 131.0, 130.9, 130.0, 129.7, 128.5, 60.9, 48.3, 46.0, 45.4, 40.6, 36.0, 35.7.; IR 3394, 1651 cm$^{-1}$; LCMS (Method 10, 254 nm) rt 4.67 min, 97.8%; HRMS calculated for (C$_{21}$H$_{25}$N$_3$O$_3$)Na$^+$, 390.1788, observed 390.1787; mp 133.1 ºC.

2-(Hydroxymethyl)-N,3-diphenethylaziridine-1-carboxamide (GWB-102). Following General Procedure L, bicyclic aziridine 1c (201 mg, 990 µmol) was treated with phenethylamine (158 mg, 1.47 mmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 319 mg (99% yield) of GWB-102 as a bright yellow oil. R$_f$ 0.15 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.32 – 7.11 (m, 10H), 4.85
(bs, 1H), 3.92 (dd, 1H, $J_1 = 12.4$, $J_2 = 2.1$), 3.89 – 3.31 (m, 3H), 3.18 (dd, 1H, $J_1 = 12.3$, $J_2 = 8.2$), 3.27 (t, 4H, $J = 7.2$), 2.37 (m, 1H), 2.12 (m, 1H), 1.79 (dd, 2H, $J_1 = 13.5$, $J_2 = 6.9$); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.6, 140.5, 138.8, 128.9, 128.7, 128.6, 128.5, 126.6, 126.4, 62.6, 44.0, 41.7, 40.9, 35.8, 33.4, 32.1; IR 3340, 1651 cm$^{-1}$; LCMS (Method 10, 254 nm) rt 4.89 min, 98.3%, m/z 325; HRMS calcd for (C$_{20}$H$_{24}$N$_2$O$_2$)Na$^+$. 347.1730, observed 347.1726.

2-(Hydroxymethyl)-N-isopentyl-3-phenethylaziridine-1-carboxamide (GWB-133).

Following General Procedure L, bicyclic aziridine 1c (19 mg, 93 µmol) was treated with isoamylamine (8.3 mg, 95 µmol), then concentrated to provide 24 mg (89% yield) of clean, crude GWB-133 as a pale yellow oil. $R_f$ 0.19 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.34 – 7.20 (m, 5H), 4.84 (bs, 1H), 3.95 (d, 1H, $J = 12.2$), 3.53 (bs, 1H), 3.30 (dd, 1H, $J_1 = 12.2$, $J_2 = 8.1$), 3.11 (m, 2H), 2.81 (t, 2H, $J = 7.2$), 2.41 (m, 1H), 2.23 (dd, 1H, $J_1 = 9.0$, $J_2 = 5.9$), 1.95 – 1.80 (m, 2H), 1.55 (sex, 1H, $J = 6.6$), 1.30 (m, 2H), 0.89 (d, 6H, $J = 6.6$); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.6, 140.5, 128.7, 128.5, 126.4, 62.7, 44.0, 40.9, 39.0, 38.6, 33.4, 32.0, 25.7, 22.4, 22.4; IR 3311, 1654 cm$^{-1}$; HPLC (Method 8, 254 nm) rt 6.53 min, 100%; HRMS calculated for (C$_{17}$H$_{26}$N$_2$O$_2$)Na$^+$, 313.1886, observed 313.1887.
2-(Hydroxymethyl)-N-(4-hydroxyphenethyl)-3-phenethylaziridine-1-carboxamide (GWB-134). Following General Procedure L, bicyclic aziridine 1c (23 mg, 113 µmol) was treated with tyramine (16 mg, 117 µmol), then purified via flash chromatography (75% EtOAc in hexanes) to provide 18 mg (46% yield) of GWB-134 as a pale yellow oil. 

R_f 0.20 (75% EtOAc in hexanes); ^1H NMR (CDCl_3, 300 MHz) δ 7.29 – 7.13 (m, 5H), 6.97 (d, 2H, J = 8.2), 6.75 (d, 2H, J = 8.3), 4.89 (bt, 1H, J = 5.7), 3.89 (dd, 1H, J = 12.2, J = 2.3), 3.39 – 3.25 (m, 2H), 3.18 (dd, 1H, J = 12.3, J = 8.0), 2.76 – 2.60 (m, 4H), 2.37 (m, 1H), 2.13 (m, 1H), 1.80 (m, 2H); ^13C NMR (CDCl_3, 300 MHz) δ 163.8, 154.9, 140.5, 130.2, 129.9, 128.7, 128.5, 126.4, 115.6, 62.4, 44.0, 41.8, 41.0, 34.9, 33.3, 32.0; IR 3404, 1654 cm⁻¹; HPLC (Method 8, ELSD) rt 2.85 min, 98.0%; HRMS calculated for (C_{20}H_{24}N_2O_3)Na^+, 363.1679, observed 363.1680.

N-(2-(1H-Indol-3-yl)ethyl)-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-135). Following General Procedure L, bicyclic aziridine 1c (22 mg, 108 µmol) was treated with tryptamine (18 mg, 112 µmol), then purified via a short pad of silica (100% EtOAc) to provide 33 mg (85% yield) of GWB-135 as a yellow oil. R_f 0.25 (75%
EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.23 (s, 1H), 7.57 (d, 1H, $J = 7.7$), 7.34 (d, 1H, $J = 8.0$), 7.30 – 7.05 (m, 7H), 6.95 (s, 1H), 4.95 (bt, 1H, $J = 5.4$), 3.86 (d, 1H, $J = 11.9$), 3.45 (m, 2H), 3.17 (dd, 1H, $J_1 = 12.2$, $J_2 = 8.1$), 2.89 (t, 2H, $J = 6.6$), 2.64 (m, 2H), 2.34 (m, 1H), 2.06 (m, 1H), 1.74 (m, 2H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.7, 140.5, 136.4, 128.6, 128.4, 127.4, 126.3, 122.3, 122.2, 119.5, 118.7, 112.8, 111.4, 62.6, 44.0, 41.0, 40.9, 33.3, 32.2, 25.4; IR 3403, 1654 cm$^{-1}$; HPLC (Method 9, ELSD) rt 4.45 min, 100%; HRMS calculated for (C$_{22}$H$_{25}$N$_3$O$_2$)Na$^+$, 386.1839, observed 386.1839.

2-(Hydroxymethyl)-N-(3-(methylthio)propyl)-3-phenethylaziridine-1-carboxamide (GWB-136). Following General Procedure L, bicyclic aziridine 1c (21 mg, 103 µmol) was treated with 3-(methylthio)propylamine (11.5 mg, 110 µmol), then concentrated to provide 29 mg (91% yield) of clean, crude GWB-136 as a yellow oil. R$_f$ 0.25 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.34 – 7.17 (m, 5H), 5.13 (bs, 1H), 3.95 (dd, 1H, $J_1 = 12.4$, $J_2 = 2.5$), 3.30 (dd, 1H, $J_1 = 12.3$, $J_2 = 8.0$), 3.20 (dd, 2H, $J_1 = 12.9$, $J_2 = 6.5$), 2.81 (t, 2H, $J = 7.3$), 2.57 – 2.38 (m, 3H), 2.25 (m, 1H), 2.09 (s, 3H), 1.94 – 1.70 (m, 4H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.7, 140.6, 128.7, 128.5, 126.4, 62.5, 44.0, 40.8, 39.7, 33.4, 32.1, 31.6, 28.8, 15.5; IR 3318, 1653 cm$^{-1}$; HPLC (Method 8, ELSD) rt 3.47 min, 98.7%; HRMS calculated for (C$_{16}$H$_{24}$N$_2$O$_2$S)Na$^+$, 331.1451, observed 331.1451.
**N-(2-Hydroxyethyl)-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-137).** Following *General Procedure L*, bicyclic aziridine 1c (20 mg, 98 µmol) was treated with ethanolamine (6.1 mg, 99 µmol), then concentrated to provide 25 mg (96% yield) of clean, crude GWB-137 as a pale yellow oil. R<sub>f</sub> 0.09 (100% EtOAc); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.31 – 7.18 (m, 5H), 5.84 (bt, 1H, J = 5.5), 3.94 (d, 1H, J = 11.4), 3.62 (m, 2H), 3.44 – 3.29 (m, 2H), 3.19 (m, 1H), 2.77 (m, 2H), 2.37 (m, 2H), 1.95 (m, 1H), 1.71 (m, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) δ 164.2, 140.9, 128.5, 128.4, 126.2, 61.7, 61.6, 45.0, 43.1, 39.7, 33.3, 32.5; IR 3352, 1654 cm<sup>-1</sup>; HPLC (Method 9, ELSD) rt 2.50 min, 99.1%; HRMS calculated for (C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>)Na<sup>+</sup>, 287.1366, observed 287.1367.

**Racemic-trans-N-(2-(2-(Benzylamino)-2-oxoethylamino)-2-oxoethyl)-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (16).** Following *General Procedure L*, bicyclic aziridine 1c (15 mg, 75 µmol) was treated with glycinyl-glycine benzylamide 13 (19 mg, 86 µmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), then concentrated to provide 26 mg of crude product 16 (82% yield) as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz) δ 8.27 (m, 2H), 7.61 (t, 1H, J = 5.4), 7.33 – 7.15 (m, 10H), 5.03 (t, 1H, J = 5.6), 4.27 (d, 1H, J =
5.9), 3.79 – 3.50 (6H, m), 2.67 (m, 2H), 2.35 (m, 1H), 2.22 (m, 1H), 1.82 (m, 1H), 1.54 (m, 1H).

$N$-((S)-1-((S)-1-(Benzylationo)-1-oxo-3-phenylpropan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-107).

*Note that absolute stereochemistry for the aziridine is unknown.* Following General Procedure L, bicyclic aziridine 1c (20 mg, 98 µmol) was treated with L-valinyl-L-phenylalanine benzylamide 14 (42 mg, 120 µmol), then purified via flash chromatography (75% EtOAc in hexanes) to provide 16 mg (35% yield) of GWB-107 as a thick white oil. Rf 0.36 (75% EtOAc in CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.74 (d, 1H, $J = 8.9$), 7.30 – 7.17 (m, 13H), 6.99 (m, 2H), 6.89 (m, 1H), 5.53 (d, 1H, $J = 8.6$), 4.86 (m, 1H), 4.51 (bs, 1H), 4.26 (m, 3H), 3.97 (d, 1H, $J = 11.7$), 3.16 (m, 1H), 3.01 (m, 2H), 2.72 (m, 2H), 2.46 (m, 1H), 2.37 (m, 1H), 2.19 (m, 1H), 1.83 (m, 1H), 1.70 (m, 1H), 0.89 (d, 3H, $J = 6.8$), 0.66 (d, 3H, $J = 6.8$); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 171.9, 171.3, 163.2, 141.2, 137.3, 136.6, 129.3, 128.6, 128.5, 128.4, 127.6, 127.4, 126.9, 126.1, 61.1, 59.8, 54.5, 46.5, 43.5, 38.8, 37.7, 33.2, 30.0, 29.7, 19.3, 16.7; IR 3288, 1645, 1634 cm$^{-1}$; HPLC (Method 10, 254 nm) rt 5.39 min, 83.6%; HRMS calculated for (C$_{33}$H$_{40}$N$_4$O$_4$)Na$^+$, 579.2942, observed 579.2941; [α]$_D^{26.7}$ -15.9 (0.88, CH$_2$Cl$_2$).
N-((S)-1-(2-(Benzylamino)-2-oxoethylamino)-3-methyl-1-oxobutan-2-yl)-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-108) and N-((S)-1-(2-(Benzylamino)-2-oxoethylamino)-3-methyl-1-oxobutan-2-yl)-2-(hydroxymethyl)-3-phenethylaziridine-1-carboxamide (GWB-109). Note that absolute stereochemistry for the aziridine in each diastereomer is unknown. Following General Procedure L, bicyclic aziridine 1c (31 mg, 153 µmol) was treated with L-valinyl-glycine benzylamide 15 (50 mg, 190 µmol), then purified via flash chromatography (5% MeOH in CH₂Cl₂) to provide 26 mg (37% yield) of GWB-108 and 15 mg (21% yield) of GWB-109, each as a thick white oil. Analysis for GWB-108: Rₚ 0.16 (4% MeOH in CH₂Cl₂); ¹H NMR (CDCl₃, 300 MHz) δ 7.48 (bs, 1H), 7.29 – 7.17 (m, 11H), 5.74 (d, 1H, J = 7.3), 4.57 (bs, 1H), 4.38 (m, 2H), 4.07 (m, 1H), 3.91 (m, 3H), 3.19 (m, 1H), 2.71 (m, 2H), 2.41 (m, 1H), 2.25 (m, 1H), 2.16 (m, 1H), 1.72 (m, 2H), 0.99 (d, 3H, J = 6.7), 0.91 (d, 3H, J = 6.8); ¹³C NMR (CDCl₃, 300 MHz) δ 173.2, 169.1, 163.9, 141.2, 137.9, 128.6, 128.4, 127.7, 127.5, 126.1, 60.7, 46.6, 43.4, 43.2, 38.0, 33.2, 33.1, 30.0, 19.4, 17.8; IR 3298, 1645, 1634 cm⁻¹; HPLC (10, 254 nm) rt 4.78 min, 97.8%; HRMS calculated for (C₂₆H₃₄N₄O₄)Na⁺, 489.2472, observed 489.2472; [α]D²⁷.⁸ +10.6 (1.67, CH₂Cl₂). Analysis for GWB-109: Rₚ 0.07 (4% MeOH in CH₂Cl₂); ¹H NMR (CDCl₃, 300 MHz) δ 7.31 – 7.16 (m, 11H), 7.03 (bs, 1H), 5.84 (d, 1H, J = 7.1), 4.39 (d, 2H, J = 5.6), 3.99 (m, 1H), 3.90 (m, 3H), 3.36 (m, 1H), 2.76 (m, 2H), 2.43 (s, 1H), 2.34 (s, 1H), 2.11 (m, 1H), 1.95 (m, 1H), 1.70 (m, 1H), 0.93 (d, 3H, J = 7.3), 0.90 (d, 3H, J = 7.0); ¹³C NMR (CDCl₃, 300 MHz) δ 172.2, 168.9,
163.4, 140.6, 137.9, 128.6, 128.6, 128.3, 127.7, 127.4, 126.3, 60.6, 60.4, 43.4, 43.1, 40.1, 
33.3, 30.1, 21.1, 19.4, 18.2, 14.2; IR 3286, 1645 cm$^{-1}$; HPLC (Method 10, 254 nm) rt 
4.57 min, 94.6%; HRMS calculated for (C$_{26}$H$_{34}$N$_4$O$_4$)Na$^+$, 489.2472, observed 489.2472; 
$[\alpha]_D^{27.9} +7.6$ (0.93, CH$_2$Cl$_2$).

(2R,3S)-N-((S)-1-(Benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-(hydroxymethyl)-3-
phenethylaziridine-1-carboxamide (74-isomer 1) and (2R,3S)-N-((S)-1-
(Benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-(hydroxymethyl)-3-
phenethylaziridine-1-carboxamide (74-isomer 2). Note that absolute stereochemistry 
for the aziridine in each diastereomer is unknown. Following General Procedure L, 
bicyclic aziridine 1c (12 mg, 0.060 mmol) was treated with phenylalanine benzylamide 
61c (17 mg, 66 mmol), then purified via flash chromatography (50 – 100% EtOAc in 
hexanes) to provide 3 mg (11% yield) of 74-isomer 1 and 1 mg (4% yield) of 74-isomer 
2, each as a yellow oil. Analysis for 74-isomer 1: R$_f$ 0.44 (100% EtOAc); $^1$H NMR 
(CDC$_3$, 300 MHz) $\delta$ 7.31 – 7.09 (m, 15H), 6.11 (bs, 1H), 5.66 (d, 1H, $J = 7.7$), 4.46 (q, 
1H, $J = 7.5$), 4.34 (d, 2H, $J = 4.2$), 3.80 (m, 1H), 3.19 (m, 1H), 3.08 (d, 2H, $J = 7.4$), 2.74 
– 2.61 (m, 3H), 2.34 – 2.27 (m, 2H), 1.81 (m, 1H), 1.59 (m, 1H). Analysis for 74-isomer 
2: R$_f$ 0.55 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.28 – 7.12 (m, 15H), 6.18 (bs,
1H), 5.57 (d, 1H, J = 8.1), 4.51 (m, 1H), 4.37 (m, 2H), 3.89 (m, 1H), 3.74 (m, 1H), 3.03 (m, 3H), 2.71 (m, 2H), 2.38 (m, 1H), 2.18 (m, 1H), 1.83 – 1.71 (m, 2H).

2-((Z)-Hex-3-enyl)-3-(hydroxymethyl)-N-phenethylaziridine-1-carboxamide (GWB-103). Following General Procedure L, bicyclic aziridine 1d (202 mg, 1.11 mmol) was treated with phenethylamine (178 mg, 1.66 mmol), then purified through a short pad of silica (100% EtOAc) to provide 296 mg (88% yield) of GWB-103 as a yellow oil. R_f 0.20 (50% EtOAc in hexanes); ^1H NMR (CDCl_3, 300 MHz) ð 7.34 – 7.18 (m, 5H), 5.30 (m, 3H), 4.01 (bd, 1H, J = 11.4), 3.46 (m, 3H), 3.28 (dd, 1H, J_1 = 12.0, J_2 = 8.4), 2.81 (t, 2H, J = 6.8), 2.46 (m, 1H), 2.13 (m, 3H), 1.99 (m, 2H), 1.51 (m, 2H), 0.94 (t, 3H, J = 7.5); ^13C NMR (CDCl_3, 300 MHz) ð 163.9, 138.8, 133.2, 128.8, 128.6, 127.8, 126.6, 62.8, 44.1, 41.7, 41.3, 35.9, 31.0, 24.9, 20.6, 14.2; IR 3356, 1651 cm^{-1}; LCMS (Method 10, 254 nm) rt 4.89 min, 88.6%; HRMS calculated for (C_{18}H_{26}N_{2}O_{2})Na^+, 325.1886, observed 325.1883.
**N-(2-(Benzylamino)-2-oxoethyl)-2-((Z)-hex-3-enyl)-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-104).** Following *General Procedure L*, bicyclic aziridine 1d (195 mg, 1.07 mmol) was treated with glycine benzylamide 12 (275 mg, 1.66 mmol), then purified through a short pad of silica (100% EtOAc) to provide 280 mg (75% yield) of **GWB-104** as an off-white solid. Rf 0.16 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.30 – 7.24 (m, 5H), 6.65 (bs, 1H), 6.03 (bt, 1H, $J = 5.6$), 5.37 (m, 2H), 4.42 (m, 2H), 4.08 (m, 3H), 3.71 (dd, 1H, $J_1 = 16.6$, $J_2 = 4.9$), 3.38 (dd, 1H, $J_1 = 11.1$, $J_2 = 8.4$), 2.51 (m, 1H), 2.40 (m, 1H), 2.18 (dd, 2H, $J_1 = 14.1$, $J_2 = 7.1$), 2.04 (qu, 2H, $J = 7.2$), 1.5 (m, 2H), 0.95 (t, 3H, $J = 7.5$); $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 169.8, 163.9, 137.6, 132.9, 128.8, 127.8, 127.7, 61.4, 45.8, 43.8, 43.7, 39.4, 31.3, 24.6, 20.6, 14.3; IR 3286, 1651 cm$^{-1}$; LCMS (Method 10, 254 nm) rt 4.70 min, 99.6%; HRMS calculated for (C$_{19}$H$_{27}$N$_3$O$_3$)Na$^+$, 368.1945, observed 368.1940; mp 111.7 °C.

![Image](image_url)

**2-((Z)-Hex-3-enyl)-3-(hydroxymethyl)-N-isobutylaziridine-1-carboxamide (PGB-3).** Following *General Procedure L*, bicyclic aziridine 1d (154 mg, 0.85 mmol) was treated with isobutylamine (91 mg, 1.24 mmol), then purified through a short pad of silica (100% EtOAc) to provide 191 mg (88% yield) of **PGB-3** as a pale yellow oil. Rf 0.21 (50%
2-(Z)-Hex-3-enyl)-3-(hydroxymethyl)-N-(2-methoxyethyl)aziridine-1-carboxamide (PGB-2). Following General Procedure L, bicyclic aziridine 1d (154 mg, 0.85 mmol) was treated with 2-methoxyethylamine (125 mg, 1.66 mmol), then purified through a short pad of silica (100% EtOAc) to provide 190 mg (87% yield) of PGB-2 as a yellow oil. Rf 0.19 (100% EtOAc); 1H NMR (CDCl₃, 300 MHz) δ 5.81 (bs, 1H), 5.49 – 5.33 (m, 2H), 4.03 (dd, 1H, J₁ = 12.4, J₂ = 1.6), 3.74 (bs, 1H), 3.51 – 3.36 (m, 8H), 2.52 (m, 1H), 2.31 (m, 1H), 2.21 (q, 2H, J = 7.1), 2.06 (m, 2H), 1.66 (m, 1H), 1.48 (m, 1H), 0.97 (t, 3H, J = 7.5); 13C NMR (CDCl₃, 300 MHz) δ 163.7, 133.0, 127.6, 71.3, 62.4, 58.7, 44.5, 40.6, 40.3, 31.0, 24.7, 20.6, 14.2; IR 3317, 1651 cm⁻¹; LCMS (Method 10, 254 nm) rt 4.66 min, 97.1%; HRMS calculated for (C₁₃H₂₄N₂O₃)Na⁺, 279.1679, observed 279.1676.
(2-((Z)-Hex-3-enyl)-3-(hydroxymethyl)aziridin-1-yl)(pyrrolidin-1-yl)methanone (PGB-1). Following General Procedure L, bicyclic aziridine 1d (152 mg, 0.84 mmol) was treated with pyrrolidine (89 mg, 1.25 mmol), then purified through a short pad of silica (100% EtOAc) to provide 176 mg (83% yield) of PGB-1 as a yellow oil; Rf 0.19 (75% EtOAc in hexanes); 1H NMR (CDCl₃, 300 MHz) δ 5.43 (m, 1H), 5.29 (m, 1H), 4.03 (m, 1H), 3.88 (dd, 1H, J₁ = 8.8, J₂ = 4.2), 3.50 – 3.37 (m, 5H), 2.58 (m, 1H), 2.30 (m, 1H), 2.15 (q, 2H, J = 7.2), 2.08 – 1.84 (m, 7H), 1.27 (m, 1H), 0.96 (t, 3H, J = 7.5); 13C NMR (CDCl₃, 300 MHz) δ 162.4, 132.9, 127.3, 62.8, 46.9, 46.6, 44.4, 41.2, 30.7, 26.0, 24.7, 24.1, 20.5, 14.3; IR 3394, 1636 cm⁻¹; LCMS (Method 10, 254 nm) rt 4.67 min, 99.6%; HRMS calculated for (C₁₄H₂₄N₂O₂)Na⁺, 275.1730, observed 275.1727.

2-((Z)-Hex-3-enyl)-3-(hydroxymethyl)-N-isopentylaziridine-1-carboxamide (GWB-105). Following General Procedure L, bicyclic aziridine 1d (29 mg, 160 µmol) was treated with isoamylamine (15 mg, 170 µmol), then concentrated to provide 36 mg (84% yield) of clean, crude GWB-105 as a clear, yellow oil. Rf 0.15 (50% EtOAc in hexanes); 1H NMR (CDCl₃, 300 MHz) δ 5.49 – 5.34 (m, 3H), 4.05 (dd, 1H, J₁ = 12.2, J₂ = 1.9), 3.67 (bs, 1H), 3.37 (dd, 1H, J₁ = 12.2, J₂ = 8.2), 3.22 (m, 2H), 2.50 (m, 1H), 2.24 – 2.20
(m, 3H), 2.07 (m, 2H), 1.60 (m, 2H), 1.42 - 1.35 (m, 3H), 0.98 (t, 3H, J = 7.5), 0.92 (d, 6H, J = 6.6); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.8, 133.3, 127.9, 62.8, 44.0, 41.3, 39.0, 38.7, 31.0, 25.8, 24.9, 22.5, 22.4, 20.7, 14.2; IR 3416, 1653 cm$^{-1}$; HPLC (Method 8, ELSD) rt 5.87 min, 99.6%; HRMS calculated for (C$_{15}$H$_{28}$N$_2$O$_2$)Na$^+$, 291.2043, observed 291.2043.

2-((Z)-Hex-3-enyl)-3-(hydroxymethyl)-N-(4-hydroxyphenethyl)aziridine-1-carboxamide (GWB-106). Following General Procedure L, bicyclic aziridine 1d (25 mg, 140 µmol) was treated with tyramine (21 mg, 150 µmol), then concentrated to provide 43 mg (98% yield) of clean, crude GWB-106 as a clear, dark yellow oil. R$_f$ 0.18 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 6.99 (d, 2H, J = 8.3), 6.76 (d, 2H, J = 8.3), 5.58 (bt, 1H, J = 5.7), 5.41 - 5.24 (m, 2H), 3.97 (dd, 1H, $J_1 = 12.3$, $J_2 = 2.5$), 3.41 (dd, 2H, $J_1 = 12.6$, $J_2 = 6.4$), 3.29 (dd, 1H, $J_1 = 12.3$, $J_2 = 7.9$), 2.70 (t, 2H, J = 6.8), 2.46 (m, 1H), 2.17 - 2.13 (m, 3H), 2.00 (m, 2H), 1.56 (m, 1H), 1.44 (m, 1H), 0.94 (t, 3H, J = 7.5); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 164.0, 155.3, 155.2, 133.1, 129.8, 127.8, 115.7, 62.4, 44.1, 42.0, 41.3, 35.0, 30.8, 24.8, 20.6, 14.3; IR 3414, 1636 cm$^{-1}$; HPLC (Method 8, ELSD) rt 2.55 min, 96.2%; HRMS calculated for (C$_{18}$H$_{26}$N$_2$O$_3$)Na$^+$, 341.1836, observed 341.1836.
N-(2-(1H-Indol-3-yl)ethyl)-2-((Z)-hex-3-enyl)-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-110). Following General Procedure L, bicyclic aziridine 1d (25 mg, 140 µmol) was treated with tryptamine (24 mg, 150 µmol), then concentrated to provide 43 mg (91% yield) of clean, crude GWB-110 as a clear, dark yellow oil. Rf 0.31 (100% EtOAc); H NMR (CDCl3, 300 MHz) δ 8.32 (bs, 1H), 7.59 (d, 1H, J = 7.7), 7.36 (d, 1H, J = 8.0), 7.34 – 7.09 (m, 2H), 7.05 (s, 1H), 5.43 (bt, 1H, J = 6.3), 5.28 – 5.23 (m, 2H), 3.97 (dd, 1H, J1 = 12.4, J2 = 2.5), 3.54 (m, 2H), 3.26 (dd, 1H, J1 = 12.3, J2 = 8.2), 2.96 (t, 2H, J = 6.7), 2.44 (m, 1H), 2.09 – 2.04 (m, 3H), 1.94 (m, 2H), 1.46 (m, 2H), 0.90 (t, 3H, J = 7.5); C NMR (CDCl3, 300 MHz) δ 163.9, 136.4, 133.1, 127.7, 127.4, 122.2, 122.0, 119.4, 118.8, 112.8, 111.3, 62.7, 44.1, 41.2, 41.0, 30.9, 25.5, 24.7, 20.5, 14.3; IR 3415, 1636 cm⁻¹; HPLC (Method 8, ELSD) rt 5.34 min, 99.7%; HRMS calculated for (C20H27N3O2)Na⁺, 364.1995, observed 364.1996.

2-((Z)-Hex-3-enyl)-3-(hydroxymethyl)-N-(3-(methylthio)propyl)aziridine-1-carboxamide (GWB-111). Following General Procedure L, bicyclic aziridine 1d (45 mg, 250 µmol) was treated with 3-(methylthio)propylamine (20 mg, 270 µmol), then concentrated to provide 56 mg (88% yield) of clean, crude GWB-111 as a clear yellow
oil. R$_f$ 0.28 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) δ 5.67 (bs, 1H), 5.51 – 5.33 (m, 2H), 4.05 (d, 1H, $J$ = 11.9), 3.66 (bs, 1H), 3.41 – 3.28 (m, 3H), 2.56 – 2.49 (m, 3H), 2.28 – 2.19 (m, 3H), 2.10 – 2.02 (m, 5H), 1.81 (m, 2H), 1.67 – 1.52 (m, 2H), 0.98 (t, 3H, $J$ = 7.5); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.9, 133.2, 127.9, 62.6, 44.1, 41.2, 39.8, 31.6, 31.0, 28.9, 24.9, 20.6, 15.5, 14.3; IR 3442, 1645 cm$^{-1}$; HPLC (Method 8, ELSD) rt 3.08 min, 99.1%; HRMS calculated for (C$_{14}$H$_{26}$N$_2$O$_2$S)Na$^+$, 309.1607, observed 309.1608.

2-((Z)-Hex-3-enyl)-N-(2-hydroxyethyl)-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-112). Following General Procedure L, bicyclic aziridine 1d (54 mg, 300 µmol) was treated with ethanolamine (20 mg, 330 µmol), then purified via flash chromatography (5% MeOH in CH$_2$Cl$_2$) to provide 39 mg (54% yield) of GWB-112 as a clear colorless oil. R$_f$ 0.38 (10% MeOH in CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) δ 6.03 (bt, 1H, $J$ = 5.6), 5.49 – 5.33 (m, 2H), 4.07 (d, 1H, $J$ = 12.3), 3.69 (m, 2H), 3.41 (m, 2H), 3.28 (m, 1H), 2.50 (m, 1H), 2.37 (m, 1H), 2.21 (q, 2H, $J$ = 7.1), 2.05 (m, 2H), 1.68 (m, 1H), 1.45 (m, 1H), 0.96 (t, 3H, $J$ = 7.1); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 164.3, 133.0, 127.7, 61.9, 45.0, 43.1, 40.1, 31.0, 24.7, 20.6, 14.3, 14.2; IR 3447, 1636 cm$^{-1}$; HPLC (Method 8, ELSD) rt 1.50 min, 100%; HRMS calculated for (C$_{12}$H$_{22}$N$_2$O$_3$)Na$^+$, 265.1523, observed 265.1523.
2-Cyclohexyl-3-(hydroxymethyl)-N-isopentylcyclopropanecarboxamide (GWB-138).

Following *General Procedure L*, bicyclic aziridine **1e** (28 mg, 160 µmol) was treated with isoamylamine (14 mg, 160 µmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 28 mg (68% yield) of **GWB-138** as an off-white solid. R<sub>f</sub> 0.26 (50% EtOAc in hexanes); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 5.11 (bs, 1H), 4.08 (d, 1H, <i>J</i> = 12.4), 3.87 (bs, 1H), 3.35 (dd, 1H, <i>J</i><sub>1</sub> = 12.0, <i>J</i><sub>2</sub> = 8.9), 3.27 – 3.15 (m, 2H), 2.56 (d, 1H, <i>J</i> = 8.4), 2.02 (s, 1H), 1.85 – 1.55 (m, 6H), 1.40 (m, 2H), 1.26 – 1.02 (m, 6H), 0.93 (d, 6H, <i>J</i> = 3.3); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) δ 164.2, 63.2, 46.9, 43.1, 39.4, 39.1, 38.8, 31.0, 30.1, 26.1, 25.9, 25.7, 25.6, 22.5; IR 3318, 1651 cm<sup>-1</sup>; HPLC (Method 8, ELSD) rt 6.22 min, 100%; HRMS calculated for (C<sub>15</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>)Na<sup>+</sup>, 291.2043, observed 291.2043; mp 88.6 ºC.

![2-Cyclohexyl-3-(hydroxymethyl)-N-isopentylcyclopropanecarboxamide](image)

2-Cyclohexyl-3-(hydroxymethyl)-N-(4-hydroxyphenethyl)cyclopropanecarboxamide (GWB-139). Following *General Procedure L*, bicyclic aziridine **1e** (29 mg, 160 µmol) was treated with tyramine (23 mg, 170 µmol), then purified via flash chromatography (75% EtOAc in hexanes) to provide 28 mg (55% yield) of **GWB-139** as a white oil. R<sub>f</sub>
0.29 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.02 (d, 2H, $J = 8.3$), 6.79 (d, 2H, $J = 8.4$), 5.17 (t, 1H, $J = 5.6$), 4.03 (d, 1H, $J = 11.2$), 3.45 (dd, 2H, $J_1 = 12.9$, $J_2 = 6.4$), 3.26 (dd, 1H, $J_1 = 12.2$, $J_2 = 8.7$), 2.73 (t, 2H, $J = 6.7$), 2.53 (d, 1H, $J = 8.4$), 1.92 (dd, 1H, $J_1 = 7.0$, $J_2 = 3.0$), 1.71 – 1.62 (m, 5H), 1.28 – 0.83 (m, 7H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 164.4, 155.0, 129.9, 115.6, 62.9, 46.9, 43.1, 41.7, 39.2, 34.9, 30.8, 30.1, 26.1, 25.6, 25.5; IR 3327, 1655 cm$^{-1}$; HPLC (Method 8, ELSD) rt 2.85 min, 99.8%; HRMS calculated for (C$_{18}$H$_{26}$N$_2$O$_3$)Na$^+$, 341.1836, observed 341.1836.

$N$-(2-(1H-Indol-3-yl)ethyl)-2-cyclohexyl-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-140). Following General Procedure L, bicyclic aziridine 1e (27 mg, 150 µmol) was treated with tryptamine (25 mg, 160 µmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 32 mg (65% yield) of GWB-140 as a white glassy foam. R$_f$ 0.16 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.33 (bs, 1H), 7.60 (d, 1H, $J = 7.8$), 7.37 (d, 1H, $J = 8.0$), 7.25 – 7.10 (m, 2H), 7.04 (s, 1H), 5.15 (bs, 1H), 4.00 (d, 1H, $J = 11.6$), 3.57 (dd, 2H, $J_1 = 12.3$, $J_2 = 6.0$), 3.22 (dd, 1H, $J_1 = 12.0$, $J_2 = 9.1$), 2.98 (t, 2H, $J = 6.6$), 2.49 (d, 1H, $J = 8.6$), 1.80 (dd, 1H, $J_1 = 7.2$, $J_2 = 2.8$), 1.68 – 1.54 (m, 5H), 1.26 – 0.83 (m, 6H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 164.3, 136.4, 127.4, 122.2, 119.5, 118.7, 112.6, 111.3, 63.2, 46.8, 43.2, 40.9, 39.2, 30.5, 29.9, 26.0, 25.6, 25.5,
25.3; IR 3310, 1654 cm\(^{-1}\); HPLC (Method 8, ELSD) rt 6.27 min, 100%; HRMS calculated for \((C_{20}H_{27}N_{3}O_{2})Na^+\), 364.1995, observed 364.1995.

\[ \text{2-Cyclohexyl-3-(hydroxymethyl)-N-(3-(methylthio)propyl)aziridine-1-carboxamide (GWB-141). Following General Procedure L, bicyclic aziridine 1e (28 mg, 160 µmol) was treated with 3-(methylthio)propylamine (17 mg, 160 µmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 27 mg (61% yield) of GWB-141 as a white oil. R}_f 0.19 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta 5.41 (\text{bs, 1H}), 4.09 (d, 1H, } J = 12.5), 4.80 (bs, 1H), 3.38 – 3.30 (m, 3H), 2.55 (m, 3H), 2.11 (s, 3H), 2.04 (s, 1H), 1.87 – 1.70 (m, 7H), 1.26 – 0.83 (m, 6H); \(^1\)\(^3\)C NMR (CDCl\(_3\), 300 MHz) \(\delta 164.3, 63.2, 46.8, 43.2, 39.9, 39.4, 31.8, 31.0, 30.1, 28.8, 26.1, 25.7, 25.6, 15.5; IR 3327, 1655 cm\(^{-1}\); HPLC (Method 9, ELSD) rt 3.85 min, 98.6%; HRMS calculated for \((C_{14}H_{26}N_{2}O_{2}S)Na^+\), 309.1607, observed 309.1607.\]

\[ \text{2-Cyclohexyl-N-(2-hydroxyethyl)-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-142). Following General Procedure L, bicyclic aziridine 1e (28 mg, 160 µmol) was treated with ethanolamine (10 mg, 170 µmol), then purified via flash} \]
chromatography (10% MeOH in CH₂Cl₂) to provide 12 mg (32% yield) of **GWB-142** as a clear, colorless oil. R_f 0.07 (5% MeOH in CH₂Cl₂); \(^1\)H NMR (CDCl₃, 300 MHz) δ 5.66 (bs, 1H), 4.11 (d, 1H, J = 11.3), 3.86 – 3.64 (m, 3H), 3.39 (m, 1H), 2.90 (bs, 1H), 2.58 (dd, 1H, J₁ = 5.6, J₂ = 2.7), 2.16 (d, 1H, J = 2.6), 1.85 – 1.69 (m, 6H), 1.30 – 1.03 (m, 7H); \(^1^3\)C NMR (CDCl₃, 300 MHz) δ 164.7, 62.4, 62.1, 45.6, 43.8, 43.1, 39.4, 31.7, 30.0, 26.1, 25.7, 25.6; IR 3414, 1646 cm\(^{-1}\); HPLC (Method 9, ELSD) rt 2.80 min, 100%; HRMS calculated for (C₁₂H₂₂N₂O₃)Na\(^+\), 265.1523, observed 265.1523.

\[\text{N-} (2-(\text{Benzylamino})-2\text{-oxoethyl})-2\text{-cyclohexyl-3-(hydroxymethyl)aziridine-1-carboxamide (ZFB-1). Following General Procedure L, bicyclic aziridine 1e (20 mg, 110 µmol) was treated with glycine benzylamide 12 (20 mg, 120 µmol), then purified via flash chromatography (100% EtOAc) to provide 32 mg (84% yield) of ZFB-1 as a white solid. R_f 0.20 (100% EtOAc); \(^1\)H NMR (CD₃OD, 300 MHz) δ 7.27 – 7.20 (m, 5H), 4.34 (s, 2H), 4.05 – 3.96 (m, 2H), 3.74 – 3.61 (m, 2H), 2.44 (s, 1H), 2.35 (s, 1H), 1.82 (s, 1H), 1.72 – 1.67 (m, 4H), 1.27 – 1.13 (m, 6H); \(^1^3\)C NMR (CD₃OD, 300 MHz) δ 175.2, 168.6, 142.3, 132.0, 131.0, 130.7, 61.6, 48.4, 47.1, 46.3, 43.4, 34.2, 33.5, 33.3, 30.0, 29.5, 29.4; IR 1643 cm\(^{-1}\); HPLC (Method 9, ELSD) rt 3.37 min, 87.7%; HRMS calculated for (C₁₉H₂₇N₃O₃)Na\(^+\), 368.1945, observed 368.1946; mp 143.2 ºC.}
2-Cyclohexyl-3-(hydroxymethyl)-N-(2-methoxyethyl)aziridine-1-carboxamide (ZFB-2). Following General Procedure L, bicyclic aziridine 1e (20 mg, 110 µmol) was treated with 2-methoxyethylamine (8.6 mg, 110 µmol), then purified via flash chromatography (100% EtOAc) to provide 25 mg (87% yield) of ZFB-2 as a clear, colorless oil. R_f 0.23 (100% EtOAc); ^1H NMR (CDCl₃, 300 MHz) δ 5.54 (bs, 1H), 4.08 (dd, 1H, J₁ = 12.7, J₂ = 2.1), 3.54 – 3.32 (m, 8H), 2.58 (m, 1H), 2.11 (m, 1H), 1.85 (s, 1H), 1.74 – 1.68 (m, 4H), 1.26 – 1.10 (m, 6H); ^13C NMR (CDCl₃, 300 MHz) δ 164.1, 71.2, 62.9, 58.8, 46.2, 43.6, 40.3, 39.3, 30.8, 30.0, 29.7, 26.1, 25.7; IR 3340, 1643 cm⁻¹; HPLC (Method 9, ELSD) rt 2.87 min, 100%; HRMS calculated for (C₁₃H₂₄N₂O₃)Na⁺, 279.1679, observed 279.1681.

2-Cyclohexyl-3-(hydroxymethyl)-N-isobutylaziridine-1-carboxamide (ZFB-3). Following General Procedure L, bicyclic aziridine 1e (20 mg, 110 µmol) was treated with isobutylamine (8.8 mg, 120 µmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 25 mg (89% yield) of ZFB-3 as a clear, colorless oil. R_f 0.22 (50% EtOAc in hexanes); ^1H NMR (CDCl₃, 300 MHz) δ 5.21 (bs, 1H), 4.09 (dd, 1H, J₁ = 12.5, J₂ = 2.3), 3.35 (dd, 1H, J₁ = 12.5, J₂ = 8.8), 3.05 (t, 2H, J = 6.4), 2.58 (m,
1H), 2.02 (dd, 1H, $J_1 = 6.2$, $J_2 = 2.9$), 1.86 – 1.70 (m, 5H), 1.26 – 1.15 (m, 7H), 0.92 (d, 6H, $J = 6.7$); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 164.3, 63.2, 48.0, 47.0, 43.2, 39.4, 31.0, 30.1, 29.7, 26.1, 25.6, 25.6, 20.1, 20.0; IR 3310, 1643 cm$^{-1}$; HPLC (Method 9, ELSD) rt 4.31 min, 89.8%; HRMS calculated for (C$_{14}$H$_{26}$N$_2$O$_2$)Na$^+$, 277.1886, observed 277.1887.

**2-Cyclohexyl-3-(hydroxymethyl)aziridin-1-yl)(pyrrolidin-1-yl)methanone  (ZFB-4).**

Following *General Procedure L*, bicyclic aziridine 1e (20 mg, 110 µmol) was treated with pyrrolidine (8.7 mg, 120 µmol), then purified via flash chromatography (100% EtOAc) to provide 27 mg (97% yield) of **ZFB-4** as a pale yellow oil. $R_f$ 0.28 (90% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 3.98 (dd, 1H, $J_1 = 12.3$, $J_2 = 2.6$), 3.53 – 3.38 (m, 5H), 2.64 (m, 1H), 2.26 (dd, 1H, $J_1 = 5.2$, $J_2 = 3.4$), 1.97 – 1.49 (m, 10H), 1.27 – 0.83 (m, 5H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 162.7, 63.1, 46.8, 46.7, 46.0, 41.9, 37.7, 31.0, 28.5, 26.3, 26.0, 25.9, 25.8, 24.6; IR 3344, 1632 cm$^{-1}$; HPLC (Method 9, ELSD) rt 3.95 min, 89.6%; HRMS calculated for (C$_{14}$H$_{24}$N$_2$O$_2$S)Na$^+$, 275.1730, observed 275.1731.
2-Cyclohexyl-3-(hydroxymethyl)-N-phenethylaziridine-1-carboxamide (ZFB-5).

Following General Procedure L, bicyclic aziridine 1e (20 mg, 110 µmol) was treated with phenethylamine (14.4 mg, 119 µmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 30 mg (91% yield) of ZFB-5 as a yellow oil. Rf 0.35 (50% EtOAc in hexanes); 1H NMR (CDCl₃, 300 MHz) δ 7.32 – 7.19 (m, 5H), 5.05 (m, 1H), 4.04 (dd, 1H, J₁ = 12.5, J₂ = 2.0), 3.50 (dd, 2H, J₁ = 6.1, J₂ = 3.6), 3.25 (dd, 1H, J₁ = 12.3, J₂ = 8.9), 2.83 (t, 2H, J = 6.6), 2.52 (m, 1H), 1.87 (dd, 1H, J₁ = 7.0, J₂ = 2.9), 1.68 (m, 5H), 1.26 – 0.86 (m, 6H); 13C NMR (CDCl₃, 300 MHz) δ 164.2, 138.6, 128.9, 128.7, 126.4, 63.2, 46.9, 43.2, 41.6, 39.2, 35.8, 30.1, 29.7, 29.4, 26.7, 26.0; IR 3301, 1643 cm⁻¹; HPLC (Method 9, ELSD) rt 5.98 min, 90.4%; HRMS calculated for (C₁₈H₂₆N₂O₂)Na⁺, 325.1886, observed 325.1888.

2-Benzyl-3-(hydroxymethyl)-N-isobutylaziridine-1-carboxamide (GWB-113).

Following General Procedure L, bicyclic aziridine 1f (25 mg, 74% purity, 98 µmol) was treated with isobutylamine (10.2 mg, 140 µmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 21 mg (82% yield) of GWB-113 as a pale yellow oil. Rf 0.16 (50% EtOAc in hexanes); 1H NMR (CDCl₃, 300 MHz) δ 7.40 – 7.27 (m, 5H),
4.44 (bs, 1H), 4.13 (m, 1H), 3.94 (bs, 1H), 3.38 (dd, 1H, \(J_1 = 12.2, J_2 = 8.6\)), 3.03 (dd, 1H, \(J_1 = 12.8, J_2 = 2.9\)), 2.89 – 2.68 (m, 3H), 2.51 – 2.38 (m, 2H), 1.50 (sept, 1H, \(J = 6.7\)), 0.75 (m, 6H); \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 164.0, 139.0, 129.1, 128.7, 127.1, 62.7, 47.8, 44.9, 43.8, 38.0, 28.4, 19.8; IR 3313, 1653 cm\(^{-1}\); HPLC (Method 8, ELSD) rt 3.49 min, 100%; HRMS calculated for (C\(_{15}\)H\(_{22}\)N\(_2\)O\(_2\))Na\(^+\), 285.1573, observed 285.1574.

2-Benzyl-3-(hydroxymethyl)-N-(2-methoxyethyl)aziridine-1-carboxamide (GWB-114). Following General Procedure L, bicyclic aziridine 1f (25 mg, 74% purity, 98 µmol) was treated with 2-methoxyethylamine (10.3 mg, 137 µmol), then purified via flash chromatography (100% EtOAc) to provide 16 mg (62% yield) of GWB-114 as a pale yellow oil. R\(_f\) 0.13 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.38 – 7.26 (m, 5H), 5.03 (bs, 1H), 4.09 (d, 1H, \(J = 12.3\)), 3.75 (bs, 1H), 3.43 – 3.16 (m, 8H), 2.90 (dd, 1H, \(J_1 = 13.9, J_2 = 4.8\)), 2.66 (m, 2H), 2.50 (m, 1H); \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 163.8, 138.5, 128.9, 128.7, 126.9, 71.0, 62.4, 58.7, 44.9, 42.6, 40.2, 37.7; IR 3406, 1656 cm\(^{-1}\); HPLC (Method 8, ELSD) rt 1.84 min, 100%; HRMS calculated for (C\(_{14}\)H\(_{20}\)N\(_2\)O\(_3\))Na\(^+\), 287.1366, observed 287.1367.
2-Benzyl-3-(hydroxymethyl)aziridin-1-yl)(pyrrolidin-1-yl)methanone  (GWB-115).

Following General Procedure L, bicyclic aziridine 1f (25 mg, 74% purity, 98 µmol) was treated with pyrrolidine (10.4 mg, 150 µmol), then purified via flash chromatography (100% EtOAc) to provide 22 mg (86% yield) of GWB-115 as a pale yellow oil. Rf 0.18 (100% EtOAc); ^1H NMR (CDCl₃, 300 MHz) δ 7.33 – 7.18 (m, 5H), 3.98 (dd, 1H, J₁ = 12.3, J₂ = 2.8), 3.49 – 3.36 (m, 6H), 3.06 (dd, 1H, J₁ = 14.0, J₂ = 4.1), 2.71 – 2.57 (3H), 1.92 – 1.85 (m, 4H); ^13C NMR (CDCl₃, 300 MHz) δ 162.0, 137.5, 128.7, 128.6, 126.8, 62.3, 46.9, 46.6, 44.3, 41.8, 37.0, 26.0, 24.6; IR 3383, 1634 cm⁻¹; HPLC (Method 8, ELSD) rt 2.65 min 100%; HRMS calculated for (C₁₅H₂₀N₂O₂)Na⁺, 283.1417, observed 283.1417.

2-Benzyl-3-(hydroxymethyl)-N-phenethylaziridine-1-carboxamide  (GWB-116).

Following General Procedure L, bicyclic aziridine 1f (22 mg, 74% purity, 89 µmol) was treated with phenethylamine (15.4 mg, 127 µmol), then purified via flash chromatography (100% EtOAc) to provide 15 mg (56% yield) of GWB-116 as a pale yellow oil. Rf 0.14 (100% EtOAc); ^1H NMR (CDCl₃, 300 MHz) δ 7.32 – 7.16 (m, 7H), 7.07 (d, 2H, J = 7.0), 4.45 (bs, 1H), 4.06 (m, 1H), 3.69 (d, 1H, J = 6.5), 3.33 – 3.25 (m, 3H), 2.90 (dd, 1H, J₁ = 13.8, J₂ = 4.2), 2.68 – 2.57 (m, 3H), 2.46 (dd, 1H, J₁ = 13.7, J₂ =
2-Benzyl-N-(2-(benzylamino)-2-oxoethyl)-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-117). Following General Procedure L, bicyclic aziridine 1f (19 mg, 74% purity, 74 µmol) was treated with glycine benzylamide 12 (18 mg, 110 µmol), then purified via flash chromatography (100% EtOAc) to provide 13 mg (49% yield) of GWB-117 as a white solid. Rf 0.20 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.33 – 7.21 (m, 10H), 6.65 (bs, 1H), 5.75 (bs, 1H), 4.37 (m, 2H), 4.08 – 3.95 (m, 2H), 3.63 (dd, 1H, \(J_1 = 16.7, J_2 = 5.0\)), 3.40 (dd, 1H, \(J_1 = 12.8, J_2 = 7.2\)), 2.85 – 2.72 (m, 2H), 2.69 – 2.61 (m, 2H); \(^1\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 169.7, 163.8, 138.0, 137.7, 128.7, 127.8, 127.6, 126.8, 126.5, 61.0, 45.7, 43.8, 43.6, 40.3, 37.4; IR 3438, 1636 cm\(^{-1}\); HPLC (Method 8, ELSD) rt 2.93 min, 100%; HRMS calculated for (C\(_{20}\)H\(_{23}\)N\(_3\)O\(_3\))Na\(^+\), 376.1632, observed 376.1632; mp 121.6 °C.
2-Benzyl-3-(hydroxymethyl)-N-isopentylaziridine-1-carboxamide (GWB-118). Following General Procedure L, bicyclic aziridine 1f (21 mg, 74% purity, 81 µmol) was treated with isoamylamine (10.5 mg, 120 µmol), then purified via flash chromatography (50% EtOAc in hexanes) to provide 16 mg (70% yield) of GWB-118 as a pale yellow oil. Rf 0.18 (50% EtOAc in hexanes); 1H NMR (CDCl3, 300 MHz) δ 7.39 – 7.27 (m, 5H), 4.31 (bs, 1H), 4.12 (bd, 1H, J = 12.1), 4.87 (bs, 1H), 3.38 (dd, 1H, J₁ = 12.2, J₂ = 8.5), 3.10 – 2.94 (m, 3H), 2.69 (m, 1H), 2.52 – 2.37 (m, 2H), 1.36 (sept, 1H, J = 6.7), 1.12 (q, 2H, J = 7.0), 0.82 (d, 6H, J = 6.5); 13C NMR (CDCl3, 300 MHz) δ 163.9, 138.9, 129.0, 128.7, 127.0, 62.7, 44.9, 43.7, 38.6, 38.2, 37.9, 25.3, 22.2, 22.3; IR 3452, 1644 cm⁻¹; HPLC (Method 8, ELSD) rt 5.22 min, 100%; HRMS calculated for (C₁₂H₂₄N₂O₂)Na⁺, 299.1730, observed 299.1731.

2-Benzyl-3-(hydroxymethyl)-N-(4-hydroxyphenethyl)aziridine-1-carboxamide (GWB-119). Following General Procedure L, bicyclic aziridine 1f (21 mg, 74% purity, 81 µmol) was treated with tyramine (17 mg, 120 µmol), then purified via flash chromatography (100% EtOAc) to provide 15 mg (56% yield) of GWB-119 as a white glassy foam. Rf 0.39 (100% EtOAc); 1H NMR (CDCl₃, 300 MHz) δ 7.31 – 7.17 (m, 5H),
N-(2-(1H-Indol-3-yl)ethyl)-2-benzyl-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-120). Following General Procedure L, bicyclic aziridine 1f (19 mg, 74% purity, 74 µmol) was treated with tryptamine (17 mg, 110 µmol), then purified via flash chromatography (100% EtOAc) to provide 16 mg (62% yield) of GWB-120 as a pale yellow oil. R$_f$ 0.31 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) 8.10 (bs, 1H), 7.52 (d, 1H, $J = 7.8$), 7.38 (d, 1H, $J = 8.1$), 7.25 – 7.10 (m, 7H), 6.88 (s, 1H), 4.58 (bs, 1H), 4.04 (d, 1H, $J = 12.1$), 3.73 (bs, 1H), 3.39 (q, 2H, $J = 6.4$), 3.27 (dd, 1H, $J_1 = 12.3$, $J_2 = 8.5$), 2.87 – 2.77 (m, 3H), 2.63 (m, 1H), 2.46 (dd, 1H, $J_1 = 13.8$, $J_2 = 8.2$), 2.27 (m, 1H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.9, 138.5, 136.4, 128.8, 128.6, 127.4, 126.8, 122.2, 121.9, 119.5, 118.7, 112.7, 111.2, 62.6, 44.7, 43.2, 40.7, 37.7, 25.3; IR 3570, 1655 cm$^{-1}$; HPLC (Method 8, ELSD) rt 4.94 min, 100%; HRMS calculated for (C$_{21}$H$_{23}$N$_3$O$_2$)Na$^+$, 372.1683, observed 372.1682.
2-Benzyl-3-(hydroxymethyl)-N-(3-(methylthio)propyl)aziridine-1-carboxamide (GWB-121). Following General Procedure L, bicyclic aziridine 1f (22 mg, 74% purity, 89 µmol) was treated with 3-(methylthio)propylamine (13.4 mg, 128 µmol), then purified via flash chromatography (100% EtOAc) to provide 19 mg (75% yield) of GWB-121 as a pale yellow oil. Rf 0.30 (100% EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.40 – 7.27 (m, 5H), 4.55 (bs, 1H), 4.11 (bd, 1H, $J$ = 6.4), 3.76 (bs, 1H), 3.39 (dd, 1H, $J_1$ = 12.2, $J_2$ = 8.5), 3.20 – 3.05 (m, 2H), 2.98 (dd, 1H, $J_1$ = 13.5, $J_2$ = 3.8), 2.69 (m, 1H), 2.53 (dd, 1H, $J_1$ = 13.5, $J_2$ = 8.3), 2.43 (m, 1H), 2.34 (t, 2H, $J$ = 7.1), 2.05 (s, 3H), 1.59 (qu, 2H, $J$ = 6.9); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.9, 138.8, 129.0, 128.7, 127.1, 62.6, 44.9, 43.5, 39.4, 37.8, 31.3, 28.6, 15.5; IR 3470, 1653 cm$^{-1}$; HPLC (Method 8, ELSD) rt 2.84 min, 100%; HRMS calculated for (C$_{15}$H$_{22}$N$_2$O$_2$S)Na$^+$, 317.1294, observed 317.1294.

2-Benzyl-N-(2-hydroxyethyl)-3-(hydroxymethyl)aziridine-1-carboxamide (GWB-122). Following General Procedure L, bicyclic aziridine 1f (26 mg, 74% purity, 104 µmol) was treated with ethanolamine (9.1 mg, 150 µmol), then purified via flash chromatography (30% MeOH in EtOAc) to provide 16 mg (63% yield) of GWB-122 as a pale yellow oil. Rf 0.35 (35% 10% MeOH in EtOAc); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.36 – 7.25 (m, 5H), 5.37 (bs, 1H), 4.08 (d, 1H, $J$ = 11.6), 4.80 (bs, 1H), 3.62 – 3.55 (m, 2H),
3.34 – 3.29 (m, 2H), 3.20 – 3.12 (m, 1H), 2.87 – 2.71 (m, 2H), 2.67 – 2.56 (m, 2H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 164.3, 138.3, 128.8, 128.7, 126.9, 61.9, 61.8, 45.0, 43.0, 41.6, 37.4; IR 3438, 1636 cm$^{-1}$; HPLC (Method 8, ELSD) rt 1.44 min, 100%; HRMS calculated for (C$_{13}$H$_{18}$N$_2$O$_3$)Na$^+$, 273.1210, observed 273.1210.

1-(Hydroxymethyl)-N-isobutyl-7-azabicyclo[4.1.0]heptane-7-carboxamide (ZFB-6). Following General Procedure L, bicyclic aziridine 1h (20 mg, 130 µmol) was treated with isobutylamine (10.4 mg, 143 µmol), then purified via flash chromatography (60% EtOAc in hexanes) to provide 25 mg (86% yield) of ZFB-6 as a clear, colorless oil. R$_f$ 0.21 (60% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 5.16 (bs, 1H), 3.96 (bd, 1H, $J$ = 11.3), 3.83 (bs, 1H), 3.33 (d, 1H, $J$ = 11.9), 3.05 (t, 2H, $J$ = 6.5), 2.43 (m, 1H), 2.14 (m, 1H), 1.85 – 1.76 (m, 4H), 1.46 – 1.26 (m, 4H), 0.92 (d, 6H, $J$ = 6.5); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 165.1, 68.2, 48.0, 45.8, 40.3, 28.8, 26.8, 23.9, 20.6, 20.2, 20.0, 19.8; IR 3304, 1645 cm$^{-1}$; HPLC (Method 9, ELSD) rt 3.20 min, 97.9%; HRMS calculated for (C$_{12}$H$_{22}$N$_2$O$_2$)Na$^+$, 249.1573, observed 249.1576.

1-(Hydroxymethyl)-N-(2-methoxyethyl)-7-azabicyclo[4.1.0]heptane-7-carboxamide (ZFB-7). Following General Procedure L, bicyclic aziridine 1h (20 mg, 130 µmol) was
treated with 2-methoxyethylamine (10.8 mg, 143 µmol), then purified via flash chromatography (100% EtOAc) to provide 18 mg (59% yield) of ZFB-7 as a clear, colorless oil. Rf 0.17 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 5.40 (bs, 1H), 3.98 (m, 1H), 3.82 (bs, 1H), 3.54 – 3.32 (m, 8H), 2.51 (d, 1H, \(J = 3.8\)), 1.85 – 1.71 (m, 4H), 1.43 (m, 2H), 1.26 (m, 2H); \(^1^3\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 165.5, 71.3, 67.6, 58.7, 46.6, 40.2, 39.5, 26.7, 23.8, 20.3, 19.8; IR 3337, 1645 cm\(^{-1}\); HPLC (Method 11, ELSD) rt 2.28 min, 92.1%; HRMS calculated for (C\(_{11}\)H\(_{20}\)N\(_2\)O\(_3\))Na\(^+\), 251.1366, observed 251.1369.

(1-(Hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)(pyrrolidin-1-yl)methanone (ZFB-8). Following General Procedure L, bicyclic aziridine 1h (22 mg, 140 µmol) was treated with pyrrolidine (11 mg, 160 µmol), then purified via flash chromatography (100% EtOAc) to provide 25 mg (78% yield) of ZFB-8 as a clear, colorless oil. Rf 0.15 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 3.95 (d, 1H, \(J = 12.3\)), 3.50 – 3.34 (m, 5H), 2.43 (d, 1H, \(J = 4.3\)), 2.15 (m, 1H), 2.05 – 1.77 (m, 7H), 1.44 (m, 2H), 1.30 (m, 2H); \(^1^3\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 163.7, 68.2, 47.1, 46.7, 46.6, 40.5, 26.9, 26.1, 24.6, 23.7, 20.3, 19.9; IR 3366, 1614 cm\(^{-1}\); HPLC (Method 9, ELSD) rt 3.01 min, 100%; HRMS calculated for (C\(_{12}\)H\(_{20}\)N\(_2\)O\(_2\))Na\(^+\), 247.1417, observed 247.1419.
**N-(2-(Benzyamo)-2-oxoethyl)-1-(hydroxymethyl)-7azabicyclo[4.1.0]heptane-7-carboxamide (ZFB-10).** Following *General Procedure L*, bicyclic aziridine 1h (29 mg, 190 μmol) was treated with glycine benzylamide 12 (34 mg, 210 μmol), then purified via flash chromatography (10% MeOH in CH$_2$Cl$_2$) to provide 17 mg (29% yield) of **ZFB-10** as a white solid. R$_f$ 0.15 (10% MeOH in CH$_2$Cl$_2$); $^1$H NMR (CD$_3$OD, 300 MHz) δ 7.31 – 7.28 (m, 5H), 4.37 (s, 2H), 4.04 (d, 1H, $J = 17.3$), 3.89 (d, 1H, $J = 12.9$), 3.67 (m, 2H), 2.78 (d, 1H, $J = 4.0$), 1.93 – 1.84 (m, 3H), 1.61 – 1.43 (m, 3H), 1.31 – 1.28 (m, 2H); IR 3246, 1639 cm$^{-1}$.

**N-benzyl-2-(hydroxymethyl)-3-pentylaziridine-1-carboxamide (GWB-98).** Following *General Procedure L*, bicyclic aziridine 1i (100 mg, 591 μmol) was treated with benzylamine (100 μL, 916 μmol), then purified via flash chromatography (75% EtOAc in hexanes) to provide 107 mg (66% yield) of **GWB-98**. R$_f$ 0.24 (75% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.29 (m, 5H), 5.70 (s, 1H), 4.39 (d, 2H, $J = 5.8$), 4.01 (d, 1H, $J = 11.4$), 3.41 (m, 2H), 2.50 (m, 1H), 2.29 (d, 1H, $J = 3.0$), 1.61 (m, 1H), 1.35 (m, 7H), 0.87 (s, 3H); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 163.7, 138.5, 128.7, 127.6, 127.5, 62.5, 44.7, 44.2, 41.7, 31.5, 30.9, 26.8, 22.5, 14.0; IR 3310, 2932, 2855, 1651 cm$^{-1}$; HPLC
(Method 9, ELSD) rt 4.25 min, 89.6%; HRMS calculated for (C\textsubscript{16}H\textsubscript{24}N\textsubscript{2}O\textsubscript{2})Na\textsuperscript{+}, 299.1730, observed 299.1728.

\[
\text{2-Hydroxymethyl-3-methyl-aziridine-1-carboxylic acid allylamide (47). Following General Procedure L, bicyclic aziridine 1j (37.4 mg, 60\% purity, 0.198 mmol) was treated with allylamine (8.9 mg, 0.156 mmol), then purified via flash chromatography (10\% EtOAc in hexanes) to provide 11 mg (48\% yield) of 47.} \]

\[
\begin{align*}
\delta & \text{ 5.85 (m, 1H), 5.37 (bs, 1H), 5.19 (bm, 2H), 4.02 (dd, 1H, } J_1 = 12.2, J_2 = 2.7), \\
& \text{ 3.87 (t, 2H, } J = 5.7), 3.46 (dd, 1H, } J_1 = 12.2, J_2 = 7.2), 3.10 (bs, 1H), 2.51 (m, 1H), 2.42 (m, 1H), 1.30 (m, 3H).
\end{align*}
\]

5.5 Aziridinyl urea acylations

\[
\text{racemic-trans-((2,3)-1-(Allylcarbamoyl)-3-phenethylaziridin-2-yl)methyl 2-(3,4-dimethoxyphenyl)acetate (18). Aziridinyl urea GWB-95 (13.6 mg, 52.2 \mu mol) in THF (50 \mu L) was treated with triphenylphosphine (15.8 mg, 60.2 \mu mol), DIAD (12.6 \mu L, 65.1 \mu mol), and 3,4-dimethoxyphenylacetic acid 17 (12.2 mg, 62.2 \mu mol), then stirred at room temperature for 47 hours. The reaction was concentrated and purified via flash}
\]
chromatography (0 - 50% EtOAc in hexanes) to provide 12 mg (52% yield) of 18. Rf 0.28 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.32 - 7.27 (m, 2H), 7.23 - 7.15 (m, 3H), 6.82 - 6.78 (m, 3H), 5.80 (m, 1H), 5.19 - 5.09 (m, 2H), 5.04 (m, 1H), 3.92 - 3.72 (m, 10H), 3.57 (m, 2H), 2.72 (m, 2H), 2.47 (m, 1H), 2.39 (m, 1H), 2.06 - 1.95 (m, 2H).

\[ \text{racemic-trans-} ((2,3)-1-(\text{Isobutylcarbamoyl})-3-\text{phenethylaziridin-2-yl})\text{methyl 2-(3,4-dimethoxyphenyl)acetate (19).} \]

3,4-Dimethoxyphenylacetic acid 17 (13 mg, 66 \(\mu\)mol) and CDI (14 mg, 87 \(\mu\)mol) in CH\(_2\)Cl\(_2\) (50 \(\mu\)L) stirred for 30 minutes before adding aziridinyl urea GWB-96 (15.7 mg, 56.8 \(\mu\)mol) and imidazole (8.3 mg, 120 \(\mu\)mol) in CH\(_2\)Cl\(_2\) (150 \(\mu\)L). After 18 hours, the reaction was concentrated and purified via flash chromatography (40% EtOAc in hexanes) to provide 8 mg (31% yield) of 19. Rf 0.26 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.32 - 7.27 (m, 2H), 7.23 - 7.15 (m, 3H), 6.82 (m, 3H), 5.07 (bt, 1H, \(J = 6.0\)), 4.28 - 4.23 (dd, 1H, \(J_1 = 12.0, J_2 = 3.0\)), 3.94 - 3.85 (m, 7H), 3.58 (s, 2H), 3.02 (m, 1H), 2.90 (m, 1H), 2.81 - 2.65 (m, 2H), 2.47 (m, 1H), 2.38 (m, 1H), 2.01 (m, 1H), 1.76 - 1.51 (m, 2H), 0.88 (d, 6H, \(J = 6.0\)).
**racemic-trans-((2,3)-1-(Allylcarbamoyl)-3-phenethylaziridin-2-yl)methyl 4-methoxybenzylcarbamate (90).** Aziridinyl urea **GWB-95** (7.6 mg, 29 μmol) in CH$_2$Cl$_2$ (30 μL) was treated with 4-methoxybenzylisocyanate (4.7 μL, 33 μmol) and DMAP (2.0 mg, 16 μmol), then stirred at room temperature for 43 hours. The reaction was concentrated and purified via flash chromatography (50% EtOAc in hexanes) to provide 4 mg (35% yield) of 90. R$_f$ 0.22 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.32 - 7.27 (m, 2H), 7.22 - 7.18 (m, 5H), 6.87 (d, 2H, J = 9.0), 5.81 (m, 1H), 5.20 - 5.08 (m, 3H), 4.99 (bs, 1H), 4.33 - 4.24 (m, 4H), 3.93 (dd, 1H, J$_1$ = 12.0, J$_2$ = 6.0), 3.80 - 3.77 (m, 4H), 2.88 - 2.71 (m, 2H), 2.49 (m, 1H), 2.43 (m, 1H), 2.06 - 1.97 (m, 2H).

**racemic-trans-((2,3)-1-(Isobutylcarbamoyl)-3-phenethylaziridin-2-yl)methyl 4-nitrophenylcarbamate (91).** Aziridinyl urea **GWB-96** (13 mg, 47 μmol) in CH$_2$Cl$_2$ (100 μL) was treated with 4-nitrophenylisocyanate (15.3 mg, 93.2 μmol) and DMAP (6.1 mg, 50 μmol), then stirred at room temperature for 22 hours. The reaction was concentrated and purified via flash chromatography (50% EtOAc in hexanes) to provide 14 mg (66% yield) of 91. R$_f$ 0.25 (50% EtOAc in hexanes); $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.19 (d, 2H, J = 9.0), 7.72 (bs, 1H), 7.58 (d, 2H, J = 9.0), 7.32 - 7.16 (m, 5H), 5.31 (bs, 1H), 4.29
(dd, 1H, 1 = 12.0, 2 = 3.0), 3.98 (dd, 1H, 1 = 12.0, 2 = 6.0), 3.12 - 2.95 (m, 2H), 2.91 - 2.72 (m, 2H), 2.55 (m, 1H), 2.45 (m, 1H), 2.09 (m, 1H), 1.76 (m, 1H), 1.59 (m, 1H), 0.90 (d, 6H, 1 = 6.0).

\[
\text{racemic-trans-}(4\text{-Nitrophenyl})\text{-carbamic acid 1-benzylcarbamoyl-3-phenethyl-aziridin-2-ylmethyl ester (GWB-99). Aziridinyl urea GWB-97 (18.2 mg, 58.6 \mu\text{mol}) in CH}_2\text{Cl}_2 (100 \mu\text{L}) was treated with 4-nitrophenylisocyanate (21.7 mg, 132 \mu\text{mol}) and DMAP (1.9 mg, 15.6 \mu\text{mol}), then stirred at room temperature for 48 hours. The reaction was concentrated and purified via flash chromatography (10\% EtOAc in CH}_2\text{Cl}_2) to provide 19 mg (68\% yield) of GWB-99. R}_f 0.31 (10\% EtOAc in CH}_2\text{Cl}_2); ^1\text{H NMR (CDCl}_3, 300 MHz) \delta 8.17 (d, 1H, 1 = 9.1), 7.49 (d, 1H, 1 = 9.1), 7.33 - 7.14 (m, 1H), 5.48 (t, 1H, 1 = 5.7), 4.35 (m, 3H), 3.96 (dd, 1H, 1 = 12.0, 2 = 6.9), 2.79 (m, 2H), 2.54 (m, 1H), 2.47 (m, 1H), 2.03 (m, 1H), 1.63 (m, 1H).

\[
\text{racemic-trans-}((2,3)-3-((Z)-Hex-3-enyl)-1-(2-methoxyethylcarbamoyl)aziridin-2-yl)methyl 4-nitrophenylcarbamate (92). Aziridinyl urea PGB-2 (22 mg, 86 \mu\text{mol}) in}
CH₂Cl₂ (300 μL) was treated with 4-nitrophenylisocyanate (15 mg, 91 μmol) and DMAP (1.5 mg, 12 μmol) under argon at room temperature for 3 days. The reaction was concentrated and purified via flash chromatography (20 - 50% EtOAc in hexanes) to provide 11 mg (34% yield) of 92. R_f 0.30 (50% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 8.21 (d, 2H, J = 9.0), 7.99 (bs, 1H), 7.57 (d, 2H, J = 9.0), 5.67 (bs, 1H), 5.49 - 5.40 (m, 1H), 5.37 - 5.29 (m, 1H), 4.66 (dd, 1H, J₁ = 12.0, J₂ = 3.0), 3.96 (dd, 1H, J₁ = 12.0, J₂ = 9.0), 3.56 - 3.38 (m, 7H), 2.66 (m, 1H), 2.49 (m, 1H), 2.23 (m, 2H), 2.05 (m, 2H), 1.76 (m, 1H), 1.40 (m, 1H), 0.96 (m, 3H).

5.6 Amino acid and peptide benzylamide synthesis

**General Procedure M**: Cbz-protection of amino acids 58 to give 59

Commercially available L-amino acids 58 were dissolved in H₂O (1 M), treated with NaHCO₃ (200 mol%) and benzylchloroformate (150 mol%) at 0 ºC, then allowed to warm to room temperature and stirred for 18 hours. The reactions were diluted with H₂O and washed with EtOAc, the combined organic phase was back-extracted with NaHCO₃ (aq, sat), then combined aqueous phase were acidified to pH~1 with HCl (aq, 1 M). The aqueous phase was extracted with EtOAc, which was dried over MgSO₄, filtered, and concentrated. Crude material was purified where necessary to provide Cbz-protected amino acids 59.
2-(Benzyloxycarbonylamino)acetic acid (59a). Following *General Procedure M*, glycine 58a (1.0 g, 13 mmol) was treated with NaHCO₃ (2.2 g, 27 mmol) to provide 2.48 g (89% yield) of clean, crude 59a as a white solid. Rₚ 0.33 (50% EtOAc in hexanes); ¹H NMR (acetone-d₆, 300 MHz) δ 7.40 - 7.30 (m, 5H), 6.59 (bs, 1H), 5.10 (s, 2H), 3.90 (m, 2H). Analysis matched reported spectral data.⁶⁵

Cbz-protected L-proline (59b). Following *General Procedure M*, L-proline 58b (1.01 g, 8.74 mmol) was treated with NaHCO₃ (1.675 g, 19.94 mmol) to provide 2.02 (93% yield) of 59b. ¹H NMR (CDCl₃, 300 MHz) δ 10.80 (bs, 1H), 7.43 (m, 5H), 5.21 (m, 2H), 4.46 (m, 1H), 3.52 (m, 1H), 3.41 (m, 1H), 2.20 (m, 2H), 1.94 (m, 2H). Analysis matched reported spectral data.⁶⁶

(S)-2-(Benzyloxycarbonylamino)-3-phenylpropanoic acid (59c). Following *General Procedure M*, L-phenylalanine 58c (2.0 g, 120 mmol) was treated with NaHCO₃ (2.0 g, 24 mmol) and purified via flash chromatography (25% EtOAc in hexanes) to provide 2.4 g (66% yield) of 59c. Rₚ 0.18 (25% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ
10.10 (bs, 1H), 7.45-7.05 (m, 10H), 5.25 (bs, 1H), 5.08 (m, 2H), 4.70 (m, 1H), 3.15 (m, 2H). Analysis matched reported spectral data. 

**General Procedure N**: Cbz-protected amino benzylamides 60 from 59

Cbz-protected amino acids 59 were dissolved in anhydrous THF (0.1 M), cooled to -78 °C, and treated with N-methylmorpholine (110 mol%), benzylchloroformate (110 mol%), and benzylamine (110 mol%) with 5 minutes of stirring between reagents. The reaction was stirred from 0 °C to room temperature over 2 hours, then filtered, concentrated, and purified via flash chromatography to provide Cbz-protected amino benzylamides 60.

(Benzylcarbamoyl-methyl)-carbamic acid benzyl ester (60a). Following General Procedure N, Cbz-glycine 59a (34.9 mg, 0.167 mmol) providee 30 mg (60% yield) of 60a. Rf 0.17 (50% EtOAc in hexanes); 1H NMR (CDCl₃, 300 MHz) δ 7.32 – 7.23 (m, 10H), 6.59 (bs, 1H), 5.61 (bs, 1H), 5.05 (s, 2H), 4.40 (d, 2H, J = 5.7), 3.86 (d, 2H, J = 5.3). Analysis matched reported spectral data.

(S)-Benzyl 2-(benzylcarbamoyl)pyrrolidine-1-carboxylate (60b). Following General Procedure N, Cbz-proline 59b (2.0 g, 8.1 mmol) provided 2.0 g (73% yield) of 60b. Rf
0.30 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.35 - 7.10 (m, 10H), 6.80 (bs, 1H), 5.10 (s, 2H), 4.40 (m, 3H), 3.55 (m, 2H), 4.20 - 1.85 (m, 4H). Analysis matched reported spectral data.\(^{68}\)

(S)-Benzyl 1-(benzylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (60c). Following General Procedure N, Cbz-phenylalanine 59c (1.0 g, 3.4 mmol) provided 900 mg (69% yield) of 60c. R\(_f\) 0.65 (50% EtOAc in hexanes); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.45 - 7.05 (m, 15H), 6.35 (bs, 1H), 5.50 (bs, 1H), 4.95 (m, 2H), 4.45 (m, 1H), 4.35 - 4.20 (m, 2H), 3.05 (m, 2H). Analysis matched reported spectral data.\(^{69}\)

**General Procedure O:** Boc-protection of amino acids 58 to give 62

Commercially available L-amino acids 58 were added to a solution of NaOH (120 mol%) in H\(_2\)O (1 M), cooled to 0 °C, and treated with Boc\(_2\)O (110 mol%) in THF (1 M), then stirred for 24 – 48 hours. The reactions were diluted w/CH\(_2\)Cl\(_2\), acidified with HCl (aq, 1 M), extracted with CH\(_2\)Cl\(_2\), and the combined organics were dried over MgSO\(_4\), filtered, and concentrated to provide crude, clean Boc-protected amino acids 62.

**tert-Butoxycarbonylamino-acetic acid (62a).** Following General Procedure O, glycine 58a (2.02 g, 26.9 mmol) provided 4.33 g (92% yield) of 62a as a white crystalline solid.
$^1$H NMR (CDCl$_3$, 300 MHz) δ 10.71 (bs, 1H), 6.79 (bs, 0.40H), 5.14 (bs, 0.60H), 3.96 (m, 2H), 1.46 (m, 9H). Analysis matched reported spectral data.$^{70}$

**S-Pyrrolidine-1,2-dicarboxylic acid 1-tert-buty1 ester (62b).** Following General Procedure O, L-proline 58b (2.0 g, 17.4 mmol) provided 3.37 g (90% yield) of 62b as a white solid. $^1$H NMR (CDCl$_3$, 300 MHz) δ 9.35 (bs, 1H), 4.31 (bm, 1H), 3.44 (bm, 2H), 2.29 (bs, 1H), 2.01 (bm, 3H), 1.46 (bm, 9H). Analysis matched reported spectral data.$^{68}$

**S-2-tert-Butoxycarbonylamino-3-phenyl-propionic acid (62c).** Following General Procedure O, L-phenylalanine 58c (3.02 g, 18.3 mmol) provided 5.13 g (quantitative yield) of 62c as a clear oil. $^1$H NMR (CDCl$_3$, 300 MHz) δ 11.19 (bs, 1H), 7.26 – 7.17 (m, 5H), 6.66 (bs, 0.7 H), 5.03 (bs, 0.3H), 4.60 (m, 0.6 H), 4.35 (m, 0.4H), 3.25 (m, 1H), 3.09 (m, 0.7H), 2.92 (m, 0.3H), 1.45 (s, 5H), 1.22 (s, 4H).

**2-tert-Butoxycarbonylamino-3-methyl-butyric acid (62d).** Following General Procedure O, L-valine 58d (1.05 g, 9.0 mmol) provided 1.99 g (quantitative yield) of
clean, crude 62d. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 9.07 (bs, 1H), 6.03 (bs, 0.25H), 5.03 (bd, 0.75, \(J = 8.2\)), 4.26 (bs, 0.74H), 4.04 (bs, 0.26H), 2.20 (bs, 1H), 1.45 (s, 9H), 1.00 (d, 3H, \(J = 6.8\)), 0.94 (d, 3H, \(J = 6.3\)).

\[
\text{(2S,3R)-2-(tert-Butoxycarbonylamino)-3-methylpentanoic acid (62e).} \]

Following General Procedure O, \(L\)-isoleucine 58e (2.0 g, 16 mmol) provided 3.48 g (97% yield) of 62e. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 5.00 (d, 1H, \(J = 8.1\)), 4.29 (m, 1H), 1.93 (s, 1H), 1.45 (s, 9H), 0.99-0.91 (m, 8H).

**General Procedure P:** Boc-protected amino benzylamides 63 from 62

Boc-protected amino acids 62 in CH\(_2\)Cl\(_2\) (0.6 m) were treated with CDI (200 mol%), stirred for 1 hour, then treated with benzylamine (110 mol%) before stirring at room temperature for 48 hours. The reactions were washed with HCl (aq, 1 M) and NaHCO\(_3\) (aq, sat), dried over MgSO\(_4\), and concentrated to provide Boc-protected amino benzylamides 63.

\[
\text{(Benzylcarbamoyl-methyl)-carbamic acid tert-butyl ester (63a).} \]

Following General Procedure P, Boc-protected glycine 62a (2 g, 11.4 mmol) provided 2.73 g (90% yield) of
63a as a yellow oil. R<sub>f</sub> 0.28 (50% EtOAc in hexanes); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.35 – 7.25 (m, 5H), 6.51 (bs, 1H), 5.19 (bs, 1H), 4.45 (d, 2H, J = 5.8), 3.81 (d, 2H, J = 5.8), 1.42 (s, 9H).

S-2-Benzylcarbamoyl-pyrrolidine-1-carboxylic acid tert-butyl ester (63b). Following General Procedure P, Boc-protected proline 62b (3.74 g, 17.4 mmol) provided 3.86 g (73% yield) of 63b as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.31 – 7.25 (bm, 5H), 4.38 (bm, 3H), 3.42 (bs, 2H), 2.18 (bm, 4H), 1.40 (bs, 9H).

S-(1-Benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester (63c). Following General Procedure P, Boc-protected phenylalanine 62c (4.85 g, 18.3 mmol) provided 6.73 g (quantitative yield) of 63c as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.31 – 7.08 (m, 10H), 6.26 (bs, 1H), 5.11 (bs, 1H), 4.33 (m, 3H), 3.05 (d, 2H, J = 6.8), 1.37 (s, 9H).

General Procedure Q: Amino benzylamides 12 and 61 from 60
Cbz-protected amino benzylamides 60 in anhydrous toluene or THF (0.3 M) were treated with 10% Pd/C (10-100% mass ratio) and placed under H<sub>2</sub> (g) in an evacuated and oven-
dried flask and stirred at room temperature for 18 hours. The reactions were filtered through Celite with toluene or THF then concentrated to provide amino benzylamides 12 and 61.

**General Procedure R:** Amino benzylamides 12 and 61 from 63

Boc-protected amino benzylamides 63 in CH$_2$Cl$_2$ (0.5 M) were cooled to 0 °C and treated with trifluoroacetic acid (600 mol%), then allowed to warm to room temperature and stirred for 18 hours. The reactions were concentrated then taken up in CH$_2$Cl$_2$ (0.5 mL), cooled to 0 °C, treated with NaOH (1000 mol%) in H$_2$O (7 M) and stirred for 3 hours at room temperature. The reaction mixtures were extracted with CH$_2$Cl$_2$, dried over MgSO$_4$, concentrated, and purified by flash chromatography to provide amino benzylamides 12 and 61.

![Image](image.png)

**2-Amino-N-benzyl-acetamide (12).** Following General Procedure Q, Cbz-protected glycine benzylamide 60a (28.9 mg, 0.097 mmol) in toluene (0.5 mL) was treated with 10% Pd/C (28.7 mg, 100% m/m) to provide 10 mg (65% yield) of clean, crude 12. Following General Procedure R, Boc-protected glycine benzylamide 63a (2.65 g, 10 mmol) provided 950 mg (58% yield) of 12 as a yellow solid. R$_f$ 0.17 (8% MeOH in CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.60 (bs, 1H), 7.36 – 7.26 (m, 5H), 4.47 (d, 2H, J = 5.9), 3.39 (s, 2H), 1.50 (s, 2H).
L-proline benzylamide (61b). Following General Procedure Q, Cbz-protected proline benzylamide 60b (2.0 g, 5.91 mmol) in THF (20 mL) was treated with 10% Pd/C (204.7 mg, 10% m/m load) to provide 1.14 g (94% yield) of proline benzylamide 61b. Following General Procedure R, Boc-protected proline benzylamide 63b (3.86 g, 12.7 mmol) provided 1.37 g (53% yield) of 61b as a yellow oil. R_f 0.22 (5% MeOH in CH_2Cl_2); ^1H NMR (CDCl_3, 300 MHz) δ 7.97 (bs, 1H), 7.35 – 7.25 (m, 5H), 4.43 (d, 2H, J = 6.0), 3.79 (dd, 1H, J_1 = 9.0, J_2 = 5.3), 3.00 (m, 1H), 2.87 (m, 1H), 2.17 (m, 1H), 1.94 (m, 2H), 1.74 (m, 2H).

S-2-Amino-N-benzyl-3-phenyl-propionamide (61c). Following General Procedure R, Boc-protected phenylalanine benzylamide 63c (6.48 g, 18.3 mmol) provided 3.12 g (67% yield) of 61c as an off-white solid. R_f 0.10 (1% MeOH in CH_2Cl_2); ^1H NMR (CDCl_3, 300 MHz) δ 7.59 (bs, 1H), 7.34 – 7.20 (m, 10H), 4.44 (m, 2H), 3.65 (dd, 1H, J_1 = 9.1, J_2 = 4.0), 3.30 (dd, 1H, J_1 = 13.7, J_2 = 4.1), 2.75 (dd, 1H, J_1 = 13.7, J_2 = 9.2), 1.38 (bs, 2H).
{1-[(Benzylcarbamoyl-methyl)-carbamoyl]-2-methyl-propyl}-carbamic acid tert-butyl ester (64). Boc-\textsuperscript{L}-valine 62d (194 mg, 0.893 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (6 mL, 0.2 M) was treated with EDCI-HCl (300 mg, 1.6 mmol) and HOBt-H\textsubscript{2}O (300 mg, 1.96 mmol), cooled to 0 °C before adding DIPEA (1 mL, 5.74 mmol) and glycine benzylamide 12 (151 mg, 0.92 mmol) then stirred from 0 °C to room temperature over 48 hours. The reaction was washed with H\textsubscript{2}O, NaOH (aq, 1 M), HCl (aq, 1 M), then collected from a phase separator, concentrated, and purified by flash chromatography (50% EtOAc in CH\textsubscript{2}Cl\textsubscript{2}) to provide 230 mg (71% yield) of 64 as a white solid. R\textsubscript{f} 0.16 (50% EtOAc in CH\textsubscript{2}Cl\textsubscript{2}); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) δ 7.34 – 7.26 (m, 5H), 6.94 (bs, 1H), 6.69 (bs, 1H), 4.99 (d, 1H, J = 6.6), 4.44 (m, 2H), 3.99 (m, 2H), 3.84 (t, 1H, J = 6.4), 2.12 (m, 1H), 1.39 (s, 9H), 0.93 (dd, 6H, J\textsubscript{1} = 12.8, J\textsubscript{2} = 6.7).

2-Amino-N-(benzylcarbamoyl-methyl)-3-methyl-butyramide (15). Boc-val-gly-NHBn 64 (230 mg, 0.63 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (6.0 mL) was treated with TFA (470 μL, 6.33 mmol) and stirred at room temperature for 18 hours. The reaction was concentrated, dissolved in CH\textsubscript{2}Cl\textsubscript{2}, treated with NaOH (5 mL, aq, 3 M) and stirred at room temperature for 3 hours. The reaction was extracted with CH\textsubscript{2}Cl\textsubscript{2}, collected from a phase separator and concentrated to provide 167 mg (quantitative yield) of solid product 15 without purification. R\textsubscript{f} 0.21 (5% MeOH in CH\textsubscript{2}Cl\textsubscript{2}); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) δ 7.97 (bs,
1H), 7.29 (m, 5H), 6.67 (bs, 1H), 4.43 (m, 2H), 3.97 (m, 2H), 3.25 (d, 1H, J = 3.5), 2.26 (m, 1H), 1.41 (bs, 2H), 0.96 (d, 3H, J = 7.0), 0.77 (d, 3H, J = 6.9); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 175.5, 169.1, 137.9, 128.7, 127.7, 127.5, 60.0, 43.5, 43.4, 30.8, 19.6, 16.0; $[^\alpha]_D$ 28.1 -27.7 (0.22, CH$_2$Cl$_2$).

[1-(1-Benzylcarbamoyl-2-phenyl-ethylcarbamoyl)-2-methyl-propyl]-carbamic acid tert-butyl ester (65). Boc-L-valine 62d (163 mg, 0.75 mmol) in CH$_2$Cl$_2$ (6 mL) was treated with EDCI-HCl (242 mg, 1.26 mmol) and HOBT-H$_2$O (243 mg, 1.59 mmol), cooled to 0 ºC before adding DIPEA (1.0 mL, 5.74 mmol) and L-phenylalanine benzylamide 61c (195 mg, 0.77 mmol) then stirred at room temperature for 48 hours. The reaction was washed with H$_2$O, NaOH (aq, 1 M), HCl (aq, 1 M), then collected from a phase separator, concentrated, and purified via flash chromatography (30% EtOAc in CH$_2$Cl$_2$) to provide 246 mg (72% yield) of 65 as a white solid. R$_f$ 0.42 (50% EtOAc in hexanes), 0.76 (75% EtOAc in CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.26 – 7.01 (m, 12H), 5.22 (d, 1H, J = 6.2), 4.83 (m, 1H), 4.26 (m, 2H), 3.98 (bs, 1H), 3.08 (m, 2H), 2.00 (m, 1H), 1.35 (s, 9H), 0.84 (dd, 6H, J$_1$ = 15.1, J$_2$ = 6.7).
**2-Amino-\textit{N}-(1-benzylcarbamoyl-2-phenyl-ethyl)-3-methyl-butyramide (14).** Boc-val-phe-NHBn 65 (246 mg, 0.542 mmol) in CH$_2$Cl$_2$ (5.5 mL) was treated with TFA (400 μL, 5.39 μmol) and stirred at room temperature for 18 hours. The reaction was concentrated, dissolved in CH$_2$Cl$_2$, treated with NaOH (4 mL, aq, 3 M), and stirred at room temperature for 3 hours, then extracted with CH$_2$Cl$_2$, collected from a phase separator and concentrated to provide 161 mg (84% yield) of 14. R$_f$ 0.43 (5% MeOH in CH$_2$Cl$_2$); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.89 (bd, 1H, $J = 7.9$), 7.26 – 7.22 (m, 8H), 7.11 (d, 2H, $J = 6.6$), 6.42 (bs, 1H), 4.63 (m, 1H), 4.36 (m, 2H), 3.20 – 3.03 (m, 3H), 2.18 (m, 1H), 1.19 (bs, 2H), 0.87 (d, 3H, $J = 7.0$), 0.56 (d, 3H, $J = 6.9$); $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 174.9, 170.9, 137.8, 137.0, 129.2, 128.6, 128.6, 127.6, 127.4, 126.9, 59.9, 54.5, 43.4, 37.9, 30.6, 19.6, 15.5; [α]$_D$ $^{28.2}$ -45.5 (0.33, CH$_2$Cl$_2$).

**3-Methyl pyrrolidine-2-carboxylate (67).** MeOH (10 mL, 250 mmol) was cooled to 0 °C, treated with SOCl$_2$ (2 mL, 27 mmol), and stirred for 30 minutes at 0 °C before adding L-proline 58b (2.0 g, 17 mmol), then allowed to warm to room temperature and stirred for 18 hours to provide the L-proline methyl ester hydrochloride salt 66. The reaction was concentrated, suspended in Et$_2$O, cooled to 0 °C, treated with triethylamine (2.5 mL, 17.9 mmol), and stirred for 2 hours. The reaction was filtered and the solid was rinsed with
Et₂O, then the filtrate was concentrated to provide 1.45 g (65% yield over two steps) of 67. ¹H NMR (CDCl₃, 300 MHz) δ 3.80 - 3.73 (m, 4H), 3.09 (m, 1H), 2.92 (m, 1H), 2.14 (m, 2H), 1.91 - 1.72 (m, 3H).

(S)-Methyl 1-((2S,3R)-2-(tert-butoxycarbonylamino)-3-methylpentanoyl)pyrrolidine-2-carboxylate (68). Boc-protected isoleucine 62e (3.53 g, 15.5 mmol) in EtOAc (35 mL) was cooled to 0 °C, treated with DCC (3.14 g, 15.2 mmol) and stirred from 0 °C to room temperature over 4 hours before adding proline methyl ester 67 (1.45 g, 11.2 mmol), and then stirred for 18 hours at room temperature. The reaction was filtered, the solid was washed with EtOAc, then the filtrate was concentrated and purified via flash chromatography (20% EtOAc in hexanes) to provide 3.36 g (88% yield) of 68. Rₚ 0.55 (50% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 5.28 (d, 1H, J = 9.4), 4.54 (m, 1H), 4.30 (m, 1H), 3.76 (m, 1H), 3.72 (s, 3H), 3.67 (m, 1H), 2.22 (m, 1H), 2.03 - 1.95 (m, 3H), 1.76 (m, 1H), 1.59 (m, 1H), 1.42 (s, 9H), 1.14 (m, 1H), 1.01 (d, 3H, J = 6.8), 0.91 (t, 3H, J = 7.3).
(2S,3R)-1-((S)-2-(Methoxycarbonyl)pyrrolidin-1-yl)-3-methyl-1-oxopentan-2-aminium 2,2,2-trifluoroacetate (69). Boc-Ile-Pro-OMe 68 (200 mg, 600 µmol) in CH₂Cl₂ (3 mL) was cooled to 0 °C and treated with TFA (400 µL, 5.4 mmol), then allowed to warm to room temperature and stirred for 18 hours. The reaction was concentrated to provide 27 mg (19% yield) of 69. ¹H NMR (CDCl₃, 300 MHz) δ 6.57 (bs, 3H), 4.52 (m, 1H), 4.15 (m, 1H), 3.73 (s, 3H), 3.72-3.58 (m, 2H), 2.28 (m, 1H), 2.08 - 1.96 (m, 4H), 1.60 (m, 1H), 1.25 (m, 1H), 1.13 (m, 3H), 0.97 (t, 3H, J = 7.5).

5.7 Cathepsin B inhibition assay protocol

The cathepsin B inhibitor screening kit (fluorometric, catalog #K147-100) from BioVision, Inc included reaction buffer (15 mL), reagent (100 µL), human cathepsin B enzyme (5 µL), substrate (Ac-RR-AFC, 0.2 mL, 10 mM), and a known inhibitor (F-F-FMK, 20 µL, 1 mM). In the absence of an inhibitor, the cathepsin B enzyme cleaved the fluorescent AFC group from the substrate, which generated high values of fluorescence as measured by the fluorometer. When the provided inhibitor (or active test sample) was present in a sample well, the cathepsin B enzyme was inhibited, preventing the cleavage of the AFC unit, which resulted in a decrease or absence of fluorescence.

The reaction buffer was warmed to room temperature prior to use. The enzyme (5 µL) was diluted with buffer (105 µL) to provide a stock solution. For each well, 50 µL of enzyme solution was prepared from buffer (48 µL), reagent (1 µL), and enzyme stock
solution (1 µL). This enzyme solution (50 µL) was delivered to each well of a white 96-well plate. Controls were prepared per the protocol (0% DMSO) and also in 1% DMSO for comparison against test samples, which were all prepared with 1% DMSO. Inhibitor controls (0% DMSO) were prepared from the provided inhibitor (1 µL) diluted in buffer (9 µL) and added to the appropriate wells. Inhibitor controls (1% DMSO) were prepared from the provided inhibitor (1 µL) diluted with DMSO (1 µL) and buffer (8 µL) and added to the appropriate wells. Enzyme controls (0% DMSO) were prepared by adding buffer only (10 µL) to the appropriate wells. Enzyme controls (1% DMSO) were prepared by adding DMSO (1 µL) and buffer (9 µL) to the appropriate wells. Test samples (20 µL, 50 mM in DMSO) were diluted in DMSO (80 µL to give 100 µL of 10 mM solutions), then each test sample (1 µL) was diluted with buffer (9 µL) and delivered to the appropriate wells. This well plate was incubated at room temperature for 15 minutes, before adding the substrate solution. For each well, 40 µL of the substrate solution was prepared by diluting the substrate (2 µL per well) with buffer (38 µL per well), then delivered to each well. The plate was placed on a shaker for one minute, then placed in the fluorometer.

Measurements were made at 37 °C (Ex/Em = 400/505 nm) every 5 minutes for 60 minutes for a total of 13 measurements. The raw data was plotted as a line graph then two points (T_1 = 5 min and T_2 = 20 min) that were consistently linear in each graph were selected along with the corresponding RFU values (RFU_1 and RFU_2). The slope for enzyme controls (EC) and all test samples (S) were calculated by dividing the net ΔRFU (RFU_2 – RFU_1) by ΔT (T_2-T_1). The relative inhibition was determined by dividing the
difference (Slope of EC – Slope of S) by Slope of EC and multiplying by 100. This calculation provides an inhibition value of zero for enzyme controls and low inhibition values for poor inhibitors of cathepsin B. The calculation provided values of 100% for inhibitor controls and high inhibition values for good inhibitors of cathepsin B.

5.8 Chiral bisoxazoline ligand 21h synthesis

(S)-Methyl 2-amino-2-phenylacetate hydrochloride (129). MeOH (30 mL, 740 mmol) was cooled to 0 ºC, treated with thionyl chloride (5 mL, 70 mmol), and stirred for 2.5 hours at 0 ºC. (L)-(+)α-phenylglycine 128 (5.0 g, 33 mmol) was added at 0 ºC, then the reaction was allowed to warm to room temperature and stirred for 18 hours. The reaction mixture was concentrated and filtered and washed with THF to provide 6.05 g (91% yield) of 129 as a white solid. 1H NMR (DMSO-d6, 300 MHz) δ 9.23 (bs, 3H), 7.55 - 7.45 (m, 5H), 5.25 (s, 1H), 3.71 (s, 3H).

(S)-Methyl 2-phenyl-2-(2,2,2-trifluoroacetamido)acetate (130). (S)-Methyl 2-amino-2-phenylacetate hydrochloride 129 (6.0 g, 30 mmol) in Et2O (60 mL) was cooled to 0 ºC under argon then treated with triethylamine (4.6 mL, 33 mmol) and stirred from 0 ºC to room temperature over 4 hours. The solid was filtered, washed with Et2O, and concentrated to a volume of 60 mL. This mixture was cooled to -78 ºC under argon,
treated with triethylamine (4.6 mL, 33 mmol) and (CF₃CO)₂O (4.7 mL, 33 mmol), then allowed to warm to room temperature and stirred for 18 hours. The reaction mixture was washed with H₂O, HCl (aq, 1M), and NaHCO₃ (aq, sat), dried over MgSO₄, and concentrated to provide 7.46 g (96% yield) of 130 without purification. Rf 0.13 (10% EtOAc in hexanes), 0.40 (25% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.39 - 7.26 (m, 6H), 5.56 (d, 1H, J = 7.0), 3.78 (s, 3H).

(S)-2,2,2-Trifluoro-N-(2-hydroxy-2-methyl-1-phenylpropyl)acetamide (131).

MeMgCl (33 mL, 3M in THF, 99 mmol) was diluted with THF (70 mL), cooled to 0 ºC under argon, treated with (S)-methyl 2-phenyl-2-(2,2,2-trifluoroacetamido)acetate 130 (5.2 g, 20 mmol), then allowed to warm to room temperature and stirred for 18 hours. The completed reaction was cooled to 0 ºC, quenched with NH₄Cl (aq, sat), diluted with Et₂O, and extracted with Et₂O, then the organic phase was washed with brine, dried over MgSO₄, filtered, and concentrated to provide a quantitative yield of 131, which was used without further purification. Rf 0.30 (25% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.37 - 7.29 (m, 6H), 4.79 (d, 1H, J = 8.9), 1.57 (bs, 1H), 1.38 (s, 3H), 1.07 (s, 3H).
(S)-1-Amino-2-methyl-1-phenylpropan-2-ol (132). (S)-2,2,2-Trifluoro-N-(2-hydroxy-2-methyl-1-phenylpropyl)acetamide 131 (5.14 g, 19.7 mmol) was treated with a solution of 5% NaOH in MeOH (1.67 g, 42 mmol), and stirred at 70 °C for 5 hours. The reaction mixture was concentrated, dissolved in CH₂Cl₂, and washed with H₂O. The aqueous phase was back-extracted with CH₂Cl₂, the combined organic phases were washed with brine, the aqueous phase was back-extracted with CH₂Cl₂, and the combined organic phases were dried over MgSO₄, filtered, and concentrated to provide 3.19 g of 132 as a yellow solid, which was used without further purification. R_f 0.19 (10% MeOH in CH₂Cl₂); ¹H NMR (CDCl₃, 300 MHz) δ 7.33 - 7.28 (m, 5H), 3.80 (s, 1H), 2.04 (bs, 3H), 1.22 (s, 3H), 1.04 (s, 3H).

N₁,N₃-bis((S)-2-Hydroxy-2-methyl-1-phenylpropyl)-2,2-dimethylmalonamide (133). (S)-1-Amino-2-methyl-1-phenylpropan-2-ol 132 (3.19 g, 19.3 mmol) in anhydrous CH₂Cl₂ (40 mL) was cooled to -78 °C under argon, treated with triethylamine (3.2 mL, 23 mmol), DMAP (250 mg, 2.0 mmol), and dimethylmalonate dichloride (1.3 mL, 9.8 mmol), then allowed to warm to room temperature and stirred for 18 hours. The reaction was washed with HCl (aq, 1 M), NaHCO₃ (aq, sat), dried over MgSO₄, filtered, concentrated, and purified via flash chromatography (100% CH₂Cl₂, then 50% EtOAc in CH₂Cl₂) to provide 2.4 g (30% yield) of 133 as an off-white solid. R_f 0.16 (50% EtOAc
in CH₂Cl₂; ¹H NMR (CDCl₃, 300 MHz) δ 7.64 (bd, 2H, J = 8.7), 7.25 - 7.20 (m, 10H), 4.80 (d, 2H, J = 8.6), 1.85 (bs, 2H), 1.49 (s, 6H), 1.28 (s, 6H), 1.05 (s, 6H).

\[
(4S,4'S)-2,2'-(Propane-2,2-diyl)bis(5,5-dimethyl-4-phenyl-4,5-dihydrooxazole) (21h).
\]

\[\text{N1,N3-bis((S)-2-Hydroxy-2-methyl-1-phenylpropyl)-2,2-dimethylmalonamide 133 (290 mg, 670 µmol) in } p\text{-xylene (10 mL) was refluxed under argon with a Dean-Stark trap for 1 hour before adding Ti(OiPr)₄ (20 µL, 68 µmol) and refluxed for 18 hours. The reaction was concentrated and purified via flash chromatography (25% EtOAc in hexanes) to give a yellow solid, which was treated with activated carbon, filtered, and concentrated to provide 158 mg (60% yield) of 21h as an off-white solid. R_f 0.17 (25% EtOAc in hexanes); ¹H NMR (CDCl₃, 300 MHz) δ 7.32 - 7.20 (m, 10H), 4.85 (s, 2H), 1.68 (s, 6H), 1.57 (s, 6H), 0.86 (s, 6H); [α]_{25}^{25\text{°C}} -146.1 (1.00, CHCl₃). Analysis matched reported spectral data.}\]

5.9 Aziridinyl ureas from enantioenriched bicyclic aziridines 1d and 1e

**General Procedure S:** Enantioenriched bicyclic aziridines 1d and 1e

Chiral ligand 21a-h (12 mol%) and copper catalyst 22a-c (10 mol%) were dissolved in CH₃CN (0.28 – 0.31 M) and stirred at room temperature for 30 minutes. Molecular sieves (4Å, 200% by mass of N-tosyloxycarbamate) and K₂CO₃ (500 mol%) was added, the reaction was stirred at room temperature for 10 minutes, then N-Tosyloxycarbamate 7d
or 7e (100 mol%) was added in CH$_3$CN (0.28 – 0.31 M) and the reaction was stirred at room temperature under argon for 22 hours. The resulting suspension was centrifuged to separate solid material from the reaction solution, which was removed by pipette. The reaction was reconstituted with CH$_3$CN, mixed thoroughly, and centrifuged as above. Combined solutions were concentrated and passed through a short pad of silica to provide bicyclic aziridine 1d or 1e.

**General Procedure T**: Aziridinyl ureas from enantioenriched bicyclic aziridines

Enantioenriched bicyclic aziridine 1d or 1e was dissolved in CH$_2$Cl$_2$ (0.3 M), treated with (L)-(−)-α-methylbenzylamine 135 (110 mol%), and stirred at room temperature for 21 hours. An aliquot of the crude reaction mixture was diluted with CH$_2$Cl$_2$ and submitted directly to normal phase LCMS analysis. Enantioselectivity of bicyclic aziridines 1d and 1e was assessed from the LC integration (area %) of aziridinyl urea diastereomers.

![Diagram](image)

**2-((Z)-Hex-3-enyl)-3-(hydroxymethyl)-N-((S)-1-phenylethyl)aziridine-1-carboxamide (137-isomer 1 and 137-isomer 2)**. Following General Procedure S, chiral ligand 21a and copper catalyst 22a were treated with N-tosyloxycarbamate 7d (52 mg) then passed through a short pad of silica (50% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2.
LCMS (Method 12, 200 nm) rt 11.58 min (23.8%, M+1=303) and 14.57 min (76.2%, M+1=303).

Following General Procedure S, chiral ligand 21b and copper catalyst 22a were treated with N-tosyloxycarbamate 7d (50 mg) then passed through a short pad of silica (50% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.50 min (49.4%, M+1=303) and 14.57 min (50.6%, M+1=303).

Following General Procedure S, chiral ligand 21c and copper catalyst 22a were treated with N-tosyloxycarbamate 7d (50 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.51 min (40.1%, M+1=303) and 14.53 min (59.9%, M+1=303).

Following General Procedure S, chiral ligand 21d and copper catalyst 22a were treated with N-tosyloxycarbamate 7d (53 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.50 min (44.0%, M+1=303) and 14.53 min (56.0%, M+1=303).

Following General Procedure S, chiral ligand 21e and copper catalyst 22a were treated with N-tosyloxycarbamate 7d (50 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.25 min (45.2%, M+1=303) and 15.57 min (54.8%, M+1=303).
Following General Procedure S, chiral ligand 21f and copper catalyst 22a were treated with $N$-tosyloxy carbamate 7d (54 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.50 min (42.9%, M+1=303) and 14.54 min (57.1%, M+1=303).

Following General Procedure S, chiral ligand 21g and copper catalyst 22a were treated with $N$-tosyloxy carbamate 7d (52 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.29 min (47.4%, M+1=303) and 15.64 min (52.6%, M+1=303).

Following General Procedure S, chiral ligand 21h and copper catalyst 22a were treated with $N$-tosyloxy carbamate 7d (52 mg) then passed through a short pad of silica (50% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.76 min (67.2%, M+1=303) and 16.00 min (32.8%, M+1=303).

Following General Procedure S, chiral ligand 21a and copper catalyst 22b were treated with $N$-tosyloxy carbamate 7d (54 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.09 min (26.4%, M+1=303) and 15.15 min (73.6%, M+1=303).

Following General Procedure S, chiral ligand 21c and copper catalyst 22b were treated with $N$-tosyloxy carbamate 7d (56 mg) then passed through a short pad of silica (25%
EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.05 min (40.1%, M+1=303) and 15.17 min (59.9%, M+1=303).

Following General Procedure S, chiral ligand 21d and copper catalyst 22b were treated with N-tosyloxycarbamate 7d (55 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.99 min (44.2%, M+1=303) and 15.11 min (55.8%, M+1=303).

Following General Procedure S, chiral ligand 21f and copper catalyst 22b were treated with N-tosyloxycarbamate 7d (52 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.98 min (40.6%, M+1=303) and 15.08 min (59.4%, M+1=303).

Following General Procedure S, chiral ligand 21h and copper catalyst 22b were treated with N-tosyloxycarbamate 7d (51 mg) then passed through a short pad of silica (50% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.54 min (49.1%, M+1=303) and 15.75 min (50.9%, M+1=303).

Following General Procedure S, chiral ligand 21a and copper catalyst 22c were treated with N-tosyloxycarbamate 7d (54 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of
enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.01 min (25.0%, M+1=303) and 15.07 min (75.0%, M+1=303).

Following General Procedure S, chiral ligand 21c and copper catalyst 22c were treated with N-tosyloxy carbamate 7d (50 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.96 min (45.4%, M+1=303) and 15.09 min (54.5%, M+1=303).

Following General Procedure S, chiral ligand 21d and copper catalyst 22c were treated with N-tosyloxy carbamate 7d (54 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.97 min (38.9%, M+1=303) and 15.07 min (61.1%, M+1=303).

Following General Procedure S, chiral ligand 21f and copper catalyst 22c were treated with N-tosyloxy carbamate 7d (55 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 11.97 min (41.4%, M+1=303) and 15.08 min (58.6%, M+1=303).

Following General Procedure S, chiral ligand 21h and copper catalyst 22c were treated with N-tosyloxy carbamate 7d (51 mg) then passed through a short pad of silica (50% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 137-isomer 1 and 137-isomer 2. LCMS (Method 12, 200 nm) rt 12.57 min (44.2%, M+1=303) and 15.76 min (55.8%, M+1=303).
2-Cyclohexyl-3-(hydroxymethyl)-N-((S)-1-phenylethyl)aziridine-1-carboxamide (139-isomer 1 and 139-isomer 2). Following General Procedure S, chiral ligand 21a and copper catalyst 22a were treated with N-tosyloxy carbamate 7e (49 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 11.61 min (25.6%, M+1=303) and 17.46 min (74.4%, M+1=303).

Following General Procedure S, chiral ligand 21c and copper catalyst 22a were treated with N-tosyloxy carbamate 7e (49 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 11.44 min (49.2%, M+1=303) and 17.57 min (50.8%, M+1=303).

Following General Procedure S, chiral ligand 21f and copper catalyst 22a were treated with N-tosyloxy carbamate 7e (52 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 11.59 min (38.8%, M+1=303) and 17.66 min (61.2%, M+1=303).

Following General Procedure S, chiral ligand 21h and copper catalyst 22a were treated with N-tosyloxy carbamate 7e (48 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of
enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 12.61 min (63.5%, M+1=303) and 16.02 min (36.5%, M+1=303).

Following General Procedure S, chiral ligand 21b and copper catalyst 22b were treated with N-tosyloxy carbamate 7e (49 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 11.52 min (48.1%, M+1=303) and 17.62 min (51.9%, M+1=303).

Following General Procedure S, chiral ligand 21d and copper catalyst 22b were treated with N-tosyloxy carbamate 7e (50 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 11.54 min (42.1%, M+1=303) and 17.59 min (57.9%, M+1=303).

Following General Procedure S, chiral ligand 21e and copper catalyst 22c were treated with N-tosyloxy carbamate 7e (51 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 11.55 min (40.8%, M+1=303) and 17.61 min (59.2%, M+1=303).

Following General Procedure S, chiral ligand 21g and copper catalyst 22c were treated with N-tosyloxy carbamate 7e (52 mg) then passed through a short pad of silica (25% EtOAc in hexanes) and carried through General Procedure T to provide a mixture of enantiomers 139-isomer 1 and 139-isomer 2. LCMS (Method 12, 200 nm) rt 11.50 min (47.4%, M+1=303) and 17.62 min (52.6%, M+1=303).
REFERENCES


