THE SYNTHESIS AND IN VITRO TESTING OF STRUCTRALLY NOVEL ISOXAZOLIDINE ANTIBACTERIAL AGENTS VIA AN ACYLNITROSO DIELS-ALDER REACTION

A Dissertation

Submitted to the Graduate School of the University of Notre Dame in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy by

George P. Nora, B.S., M.S.

________________________________________
Marvin J. Miller, Director

Graduate Program in Chemistry and Biochemistry
Notre Dame, Indiana
April 2007
THE SYNTHESIS AND IN VITRO TESTING OF STRUCTURALLY NOVEL ISOXAZOLIDINE ANTIBACTERIAL AGENTS VIA AN ACYLNITROSO DIELS-ALDER REACTION

Abstract

by

George P. Nora

The research in this dissertation focuses on the synthesis and in vitro testing of structurally novel antibacterial agents derived from acylnitroso Diels Alder adducts. The striking structural similarity of β-lactam antibacterial agents to isoxazolidines derived from acylnitroso Diels Alder adducts led to the hypothesis that appropriately elaborated isoxazolidines might act as a β-lactam surrogate. In order to test this hypothesis, several isoxazolidines were synthesized and tested against E. coli X-580, which is a stain of E. coli that is hypersensitive to β-lactam antibacterial agents, and were found to have activity.

In order to increase the potency and broad spectrum antibacterial activity, additional isoxazolidines were synthesized which incorporate side chains which are analogous to physiologically relevant side chains that many effective β-lactam antibiotics have. These side chains included phenylglycine and amino thiazol methoxy oxime (ATMO). The current progress towards a diketopiperazine derived from an isoxazolidine
is presented which could also exhibit antibacterial activity. The efforts towards an isoxazolidine containing an alcohol in the side chain are shown and the attachment of it to deferoxamine (DFO) which is a siderophore is proposed. In addition, the potential synthesis of a macrocyclic oligopeptide is proposed which incorporates an isoxazolidine and DFO. This could increase the antibacterial activity of the oligopeptide to include Gram (+) bacteria.
To Stephanie
FIGURES

Figure 1.1 Diagram of Gram positive and Gram negative bacteria................................. 3
Figure 1.2 General Structure of Penicillins................................................................... 4
Figure 1.3 Commonly Used Antibiotics ....................................................................... 5
Figure 1.4 Mechanism of Cross Linking ................................................................. 6
Figure 1.5 β-lactamase inhibitors............................................................................... 7
Figure 1.6 Baldwin γ-lactam analogs to β-lactam compounds.................................... 16
Figure 1.7 Lilly β-Lactam Mimics............................................................................. 17
Figure 1.8 Wolfe β-Lactam Mimics.......................................................................... 17
Figure 1.9 Comparison of Penicillin to acyl-D-Ala-D-Ala and an Isoxazolidine .......... 19
Figure 2.1 Previous Methods to Functionalize Acynitroso Diels-Alder Adducts ........ 22
Figure 2.2 Molecules Synthesized from Amino cyclopentenols ................................. 23
Figure 2.3 Additional Products from the Acynitroso Diels Alder Adducts.................. 24
Figure 2.4 Products from the oxidative cleavage of the double bond ....................... 25
Figure 2.5 Antibacterial kinetic growth assay of isoxazolidine 63a............................. 30
Figure 2.6 Antibacterial kinetic growth assay of isoxazolidine 63b ............................ 31
Figure 2.7 Picture of Kirby Bauer Diffusion Assay .................................................... 32
Figure 2.8 Picture of MIC Value Determination......................................................... 38
Figure 3.1 Graph of Isoxazolidine 87 Testing Against *E. coli* X580 ............................... 54

Figure 4.1 Examples of Siderophores ............................................................................. 66

Figure 4.2 Diagram of a Siderophore Drug Conjugate .................................................... 66

Figure 4.3 Boc Protection ............................................................................................... 71
SCHEMES

Scheme 1.1.......................................................................................................................... 7
Scheme 1.2 Chemistry of Weinreb Amides................................................................. 17
Scheme 1.3 Examples of Saponification of Weinreb Amides...................................... 18
Scheme 2.1 Retro Synthetic Pathway to Isoxazolidines............................................ 26
Scheme 2.2 Synthesis of Diels Alder Adducts 60a-c ................................................. 27
Scheme 2.3 Synthesis of Hydroxamic Acids 59a-c ..................................................... 28
Scheme 2.4 Synthesis of Isoxazolidines 63a-c ............................................................ 29
Scheme 3.1 Retrosynthetic Analysis to Synthesize Isoxazolidine 65 ....................... 42
Scheme 3.2 Synthesis of ATMO D-Alanine HOBT Ester 73 ...................................... 43
Scheme 3.3 Synthesis of Isoxazolidine 77 ................................................................. 44
Scheme 3.4 Attempted coupling ................................................................................. 45
Scheme 3.5 Aqueous Coupling Attempt ..................................................................... 47
Scheme 3.6 Acid Fluoride Attempt ................................................................................ 48
Scheme 3.7 Attempts to Synthesize isoxazolidine 62b ............................................. 49
Scheme 3.8 Coupling Attempts With Isoxazolidine 82 ............................................ 50
Scheme 3.9 Synthesis of Isoxazolidine 87 ................................................................. 53
Scheme 3.10 Synthesis of Isoxazolidine 88 ................................................................. 55
Scheme 3.11 Attempted Coupling of \(O\)-TBS Hydroxyl Amine ........................................... 56

Scheme 3.12 \(O\)-\(\text{tert}\)-Butyl Hydroxyl Amine Coupling.................................................. 57

Scheme 3.13 Synthesis of Hydroxamic Acid 105 ................................................................. 58

Scheme 3.14 Synthesis of Isoxazolidine 108 with a Phenylglycine Side Chain ............ 59

Scheme 3.15 Synthesis of ATMO Glycine HOBt Ester 111 .................................................. 60

Scheme 3.16 Synthesis of ATMO Appended Isoxazolidine 115 ........................................ 61

Scheme 4.1 Synthesis of \(\gamma\)-Sultam .................................................................................... 63

Scheme 4.2 Current Progress Towards Isoxazolidine 126 ................................................ 64

Scheme 4.3 Potential Synthesis of Siderophore Analog ....................................................... 68

Scheme 4.4 Current Progress Towards the Synthesis of Diketopiperzine 138 .......... 69

Scheme 4.5 Progress Towards the Synthesis of Isoxazolidine 145 .................................... 70

Scheme 4.6 Methyl esters \(via\) Ozonolysis ........................................................................ 72

Scheme 4.7 Attempts to Synthesize Additional Esters....................................................... 72

Scheme 4.8 Cleavage of Diels-Alder Adduct ................................................................... 73
TABLES

Table 1.1 Various Penicillin Structures ................................................................. 8
Table 1.2 Selected Cephalosporin Structures ....................................................... 10
Table 1.3 Selected Carbapenem Structures .......................................................... 11
Table 1.4 Selected Nocardicin Structures ............................................................. 13
Table 1.5 Monocyclic β-lactams and their antibacterial Activity against Various Bacteria reported as Zones of Inhibition (mm) ................................................. 14
Table 2.1 KIRBY BAUER DIFFUSION ASSAY RESULTS ON A BROAD SELECTION OF BACTERIA ................................................................. 34
Table 2.2 KIRBY BAUER DIFFUSION ASSAY RESULTS ON RESISTANT BACTERIA ......................................................................................... 36
Table 2.3 MIC values (μg/mL) ............................................................................ 39
Table 2.4 MIC VALUES AGAINST CLINICLLY RELEVANT BACTERIA ........ 40
Table 3.1 Coupling Conditions Used to Attempt the Synthesis of Isoxazolidine 78 ..... 46
Table 3.2 List of Acids Used in Coupling Reaction ............................................. 51
ACKNOWLEDGMENTS

First and foremost I would like to thank my doctoral research advisor Dr. Marvin J. Miller. Without his tireless efforts to obtain the funding and the unique insight into β-lactam antibacterial agents the research that is presented in this dissertation would not be possible. In addition, I would like to thank him for all the extra time and personal help that he has given me though out my stay here. It has been a real privilege and a honor to be a member of his group.

I would like to thank my committee members Dr. Brad Smith, Dr. Rich Taylor, and Dr. Olaf Wiest for their time and being helpful to me during time here. I would like to express extra gratitude to Dr. Brad Smith for writing numerous letters of recommendation and for giving a seminar at Illinois State University which led to my decision to come to Notre Dame.

I would like to thank several past and present members of the Miller group. Matt Surman is an invaluable person to have around in the lab not just for his chemical knowledge but as a good friend. Dr. Tim Durham, Dr. Li Dong, and Dr. Aaron Murray were also very valuable to me. I would like to thank Dr. Fangzheng Li and say that I am looking forward to working with him in the near future. Dr. Timothy Long was instrumental in helping with the biological testing and invaluable to have around. There are many current group members Kelly Fennell, Brian Bodnar, Leslie Patterson, Baiyuan Yang, and Weiqiang Huang, Gaiying Zhao, Tim Wencewicz who are all wonderful friends and very valuable colleagues. I will miss them all a great deal.
I would like to thank several other past and present members of the department. Especially Dr. Kevin Miller for his continuous support and friendship. I would also like to give special thanks to Doug Schauer, John Markiewicz, Jake Plummer, Jeremey Weitgenant, Casey Cosner, Ed O’Neal, and Dr. Roger Hanshaw who I had numerous valuable conversations with.

Special thanks to Theresa Bollinger who makes a tough job look easy. Dr. Jaroslav Zajicek, Don Schifferl provided valuable assistance with the NMR facilities and I want to thank Dr. Bill Boggess and Nonka Sevova for their efforts in the obtaining mass spectroscopic data. Several collaborators were instrumental in helping in one way or another in obtaining data such as Dr. Ute Möllmann at the Lebnitz Institute for Infection Biology, Hans Knöll Institute for Natural Products Research, Jena, Germany and Doug Zeckner at Eli Lilly and Company for testing of our compounds. I am also grateful for the NIH (GM068012) funding of this research.
ABBREVIATIONS

(±)...............................racemic
ºC..............................degrees Celsius
1H................................proton
13C..............................carbon-thirteen

α..................................alpha
Ac...............................acetyl
AcOH............................acetic acid
Ac2O............................acetic anhydride
anal..............................analysis
aq...............................aqueous
Ar...............................argon or aromatic
Atm.............................atmosphere

β..................................beta
Bn...............................benzyl
Boc.............................tert-butoxycarbonyl
bs...............................broad singlet

calcld.........................calculated
Cbz..............................benzyloxy carbonyl
CDCl3..........................deuterochloroform
CDI..............................carbonyldiimidazole
CHCl3..........................chloroform
CH2Cl2..........................dichloromethane

δ..............................delta (chemical shift)

d..............................doublet
DCC.............................N,N'-dicyclohexylcarbodiimide
dd..............................doublet of doublets
DMAP..........................dimethylaminopyridine
DMF.............................dimethylformamide
DMSO..........................dimethylsulfoxide

EDC...........................1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ee..............................enantiomeric excess
eq..............................equivalents (equal molar amount)
Et..............................ethyl
Et3N............................triethyl amine
Et2O............................diethyl ether
EtOAc...............ethyl acetate
EtOH...............ethanol
EWG...............electron withdrawing group
FAB...............fast atom bombardment
FT-IR...............fourier transform infrared spectroscopy

γ...............gamma
g...............gram

h...............hour
H₂...............hydrogen
HRMS...............high resolution mass spectroscopy
Hz...............hertz

i-Pr...............isopropyl
i-PrOH.............2-propanol
IR...............infrared

J...............coupling constant

L...............liter or ligand

m...............multiplet
M...............molarity
Me...............methyl
MeCN.............acetonitrile
MeOH...............methanol
mg...............milligram
MHz.............megahertz
Min...............minute
mL...............milliliter
mmol...............millimole
mol...............mole
mp...............melting point
MS...............mass spectroscopy
m/z...............mass to charge

NMR...............nuclear magnetic resonance
Nuc...............nucleophile

[O]...............oxidation
OH...............hydroxyl
OMe...............methoxy

PDC...............pyridinium dichlorochromate
Pd/C………………..palladium on carbon
Pd(0)………………..palladium zero
Ph………………..phenyl
PhCH₃………………..toluene
PhH………………..benzene
(Ph)₃P……………triarylphosphine
pKa………………..pk for association
ppm………………..parts per million
Pyr………………..pyridine

q………………..quartet

rt………………..room temperature

s………………..singlet
SAR………………..structure-activity relationship
sec………………..second

t………………..triplet
TBDPS………………..tert-butyldiphenylsilyl
t-butyl………………..tert-butyl
t-BuOH………………..tert-butyl alcohol
TFA………………..trifluoracetic acid
THF………………..tetrahydrofuran
TLC………………..thin layer chromatography
TMS………………..tetramethylsilyl
Ts………………..tosyl
TsOH………………..p-toluenesulfonic acid

UV………………..ultraviolet

Z………………..benzyloxy carbonyl
CHAPTER 1:

INTRODUCTION: THE MECHANISM OF ACTION OF B-LACTAM ANTIBACTERIAL AGENTS, WAYS TO ALTER THE EFFECTIVENESS AND SUCCESSFULL ATTEMPTS TO MIMIC THE B-LACTAM MECHANISM OF ACTION IN NON B-LACTAM SYSTEMS

1.1 Introduction

Bacteria are a diverse group of unicellular organisms which flourish virtually everywhere. They have adapted to survive in some of the harshest environments from inside antarctic snow\(^1\) and hydrothermal vents\(^2\) to inside the human stomach.\(^3\) Some of these microorganisms play a pivotal role in making yogurt, butter, and cheese but others can cause food spoilage and food poisoning. More importantly, bacteria can also cause life threatening illnesses and were a major cause of death until antibacterial agents were developed. The widespread use of these “wonder drugs” has created numerous resistant strains of pathogenic bacteria that once again are becoming a serious threat.\(^4\) This chapter will first briefly focus on bacteria and the history of effective antibiotics with an emphasis on β-lactam antibiotics. The remainder of the chapter will describe the successful attempts to mimic β-lactam antibiotics and the rational of why an isoxazolidine might mimic β-lactam antibiotics.
1.2 Bacteria and Gram Staining

The overall structure of various types of bacteria is rather simple and all types of bacteria have numerous features in common (Figure 1.1).5-6 Most bacteria have flagella which allow them to move. Each species of bacteria differs in the quantity and the location of where the flagella are located on the cell surface. Some bacteria, usually only Gram negative bacteria, have pili which are used to transfer DNA to other cells.7 The cytoplasm of all bacteria contain ribosomes8 where RNA is located and proteins are synthesized and the nucleoid where DNA is kept.9

Bacteria can be classified into two distinct groups which can be identified by staining the cells with crystal violet followed by rinsing consecutively with water, iodine, water, an ethanol acetone (3:1) mixture, water, Safranin O stain, and water. The process called Gram staining, named after the inventor Hans Christian Gram, leaves some cells a violet color and others red.10-13 The reason for this stems from differences in the cell walls (Figure 1.1).14-15 Gram positive bacteria those that turn violet do so because they have a thick cell wall that absorbs the crystal violet stain and Gram negative bacteria which turn red from the Safranin O stain because the ethanol acetone (3:1) mixture helps the stain penetrate the outer membrane.
Figure 1.1 Diagram of Gram positive and Gram negative bacteria
1.3 Antibiotics

Competition among microbes for vital resources has created a competition among them which has generated an array of structurally diverse antibiotics. In our own fight against pathogenic bacteria, we have taken advantage of this and utilize these weapons. This strategy has provided some of the most clinically relevant antibacterial agents to date. The first such compound to be discovered was penicillin 1, which is a β-lactam antibiotic. Since its discovery, it has become the primary line of defense against bacterial infections (Figure 1.2). However, several other compounds have been isolated and found to be useful antibacterial agents. 

![Figure 1.2 General Structure of Penicillins](image)

There are several additional antibacterial agents that are used to augment β-lactam antibiotics. For instance, tetracycline 2 and erythromycin A 3 are useful antibacterial agents (Figure 1.3). One of the last lines of defense is vancomycin 4 a glycopeptide that targets bacterial cell wall synthesis. Some antibacterial agents are not derived from a natural source like ciprofloxacin 5 a fluorinated quinolone. Despite these powerful antibiotics, bacteria are developing resistance to all of these antibiotics.
How do bacteria become resistant to antibacterial agents? Quite simply this is accomplished by their ability to reproduce very rapidly. After a bacterial infection is wiped out, a few survivors remain that have some resistance to the antibiotic that was used. These bacteria not only pass on their resistance to their “children” but can also pass this resistance on to other bacteria through the use of plasmids.
1.4 The Mechanism of Action of β-Lactam Antibiotics

The cell wall of bacteria is made up of polypeptide and polysaccharide chains with alternating sugar units of N-acetyl glucosamine (NAG) and N-acetylmuramic acid (NAM) (Scheme 1.1). The NAM unit has a peptide that is attached to it which ends in an unnatural D-alanine D-alanine terminus to avoid lipases. The ends are crosslinked together by a transpeptidase enzyme called a penicillin binding protein (PBP). This occurs by a hydroxyl on a serine residue attacking and releasing the terminal D-alanine thus forming an active ester that an amino group can attack from another peptidoglycan unit to form a cross link.\(^{39}\)

β-Lactam antibiotics work by preventing the cross-linking of the peptidoglycan layer of the cell wall. This is done by the penicillin binding protein attacking the β-lactam carbonyl \(6\) which causes the ring to open up thus blocking the active site of the enzyme from the natural substrate \(8\) (Scheme 1.2).\(^{40}\)

![Figure 1.4 Mechanism of Cross Linking](image-url)
The primary method that bacteria are using to avoid being destroyed by β-lactam antibiotics is through the production and use of β-lactamase enzymes. These enzymes inactivate the β-lactam ring by hydrolyzing it before it can inactivate the penicillin binding protein.\(^{29}\) One method to combat β-lactamase enzymes is to administer an inhibitor to the β-lactamase enzyme in conjunction with a β-lactam antibiotic. This strategy is rather successful and is already being used in the clinic. Two of the top β-lactamase inhibitors are clavulanic acid \(9\) and sulbactam \(10\) (Figure 1.4).

**Figure 1.5 β-lactamase inhibitors**

### 1.5 β-Lactam Antibiotics

In order to combat resistance, several modifications to the penicillin structure have been made.\(^{41}\) The first and most obvious is alteration of the groups attached to the amino group. An extensive number of these analogs have been synthesized (Table 1.1).
Initially penicillin G 11a and penicillin V 11b were clinically useful antibiotics but resistance developed to such an extent that another generation of semi-synthetic penicillins were utilized in the clinic such as methicillin 11c, ampicillin 11d, and amoxicillin 11e.

**TABLE 1.1**

**VARIOUS PENICILLIN STRUCTURES**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a</td>
<td></td>
<td>Penicillin G</td>
</tr>
<tr>
<td>11b</td>
<td></td>
<td>Penicillin V</td>
</tr>
<tr>
<td>11c</td>
<td></td>
<td>Methicillin</td>
</tr>
<tr>
<td>11d</td>
<td></td>
<td>Ampicillin</td>
</tr>
</tbody>
</table>
Cephalosporins were discovered shortly after the penicillins but could not be made by fermentation like penicillin. Chemical modification of them was impeded until a process was developed to make the cephalosporin core from penicillin. Unlike penicillin, cephalosporins have two positions that are easily derivatized, the amino side chain and the double bond substituent. Similar to the penicillins, bacteria became resistant to the initial cephalosporin antibiotics like cephalosporin C, 12a, and cephalothin, 12b. Additional analogs were synthesized to overcome this resistance such as cefazolin, 12c, cefuroxime, 12d, and cefotaxime, 12e. The amino thiazol methoxy oxime (ATMO) side chain on cefotaxime 12e is an excellent side chain to overcome β-lactamases.
### TABLE 1.2

**SELECTED CEPHALOSPORIN STRUCTURES**

![Cephalosporin Structures](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>12a</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;N</td>
<td>O</td>
<td>Cephalosporin C</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12b</td>
<td></td>
<td>O</td>
<td>Cephalothin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;O</td>
<td></td>
</tr>
<tr>
<td>12c</td>
<td>N=N</td>
<td>N=N</td>
<td>Cefazolin</td>
</tr>
<tr>
<td></td>
<td>N=Z</td>
<td>Z=N</td>
<td></td>
</tr>
<tr>
<td>12d</td>
<td></td>
<td>O</td>
<td>Cefuroxime</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>12e</td>
<td></td>
<td>O</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;O</td>
<td></td>
</tr>
</tbody>
</table>
Carbapenems are a unique union of the penicillin and cephalosporin structures by incorporating the unsaturation of the cephalosporin with the five membered ring of the penicillin. Another unique feature of the carbapenems is that they replace the amino side chain of penicillin and cephalosporins have with a hydroxyl or sulfonic acid group. Like the cephalosporins the carbapenems have an additional side chain connected to the double bond. Several of the carbapenems are very active compounds such as thienamycin 13a, olivanic acid 13b, and imipenem 13c.

### TABLE 1.3

SELECTED CARBAPENEM STRUCTURES

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>* Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>OH</td>
<td>H</td>
<td>S₈-NH₂</td>
<td>S         Thienamycin</td>
</tr>
<tr>
<td>13b</td>
<td>H</td>
<td>OSO₃H</td>
<td>R</td>
<td>Olivanic</td>
</tr>
</tbody>
</table>

![Carbapenem Structure Diagram](image-url)
Additional analogs of penicillin have been made with increasingly higher molecular weights with some modest success.\textsuperscript{45-49} With the increased size and complexity, the question arises whether a monocyclic β-lactam which would be more efficient to make would be an effective antibacterial agent. Originally this was believed not to be true and was substantiated by a class of natural products called the nocardicins which have a monocyclic β-lactam ring. Several examples of these compounds have been isolated from natural sources or synthesized such as nocardicin D \textit{14a}, nocardicin E \textit{14b}, and nocardicin G \textit{14c}.\textsuperscript{50-52} Despite having side chains that are similar to ones on very active penicillin compounds like amoxicillin \textit{11e}, the nocardicins do not demonstrate any significant clinical antibacterial activity but do have some \textit{in vitro} activity.
TABLE 1.4

SELECTED NOCARDICIN STRUCTURES

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>14a</td>
<td>HO₂C⁺</td>
<td>Nocardicin</td>
</tr>
<tr>
<td>14b</td>
<td>HO⁻</td>
<td>Nocardicin</td>
</tr>
<tr>
<td>14c</td>
<td>HO⁻</td>
<td>Nocardicin</td>
</tr>
</tbody>
</table>
Modification of the nocardicin core structure to include an electron withdrawing group attached to the \( \beta \)-lactam nitrogen increases the activity of the compounds to a clinically useful level.\(^{53-56}\) The first of these compounds 15a and 15b contains an oxygen as a electron withdrawing group and a phenylacetyl side chain (Figure 1.5). These first compounds, termed oxamazins, were remarkably active and they contained an oxygen as the electron withdrawing group. When the phenylacetyl side chain was replaced with an ATMO side chain 16a and 16b the antibacterial activity was greatly increased (Table 1.5). In addition to the electron withdrawing group being an oxygen atom, a sulfonic acid residue was found 17a and 17b to be affective as well.

### TABLE 1.5

**MONOCYCLIC B-LACTAMS AND THEIR ANTIBACTERIAL ACTIVITY AGAINST VARIOUS BACTERIA REPORTED AS ZONES OF INHIBITION (MM)**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>15a</th>
<th>15b</th>
<th>16a</th>
<th>16b</th>
<th>17a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>R= C(CH(_3))(_2)CO(_2)H</td>
<td>R= CH(_3)</td>
<td>R= H</td>
<td>R= CH(_3)</td>
<td>R= CH(_3)</td>
</tr>
</tbody>
</table>

14
1.6 Non-β-Lactams That Mimic β-Lactams

Several attempts have been made to synthesize a molecule that would mimic the mechanism of action of β-lactam antibiotics without a β-lactam ring present in the molecule. The major advantage in creating such a compound would be to cause a bad fit into a β-lactamase enzyme but still be effective as an antibacterial agent. On the other hand, it would also be beneficial if such a compound would preferentially bind to β-lactamases and inactivate them.

Jack Baldwin and coworkers were some of the first to attempt related syntheses and studies.\textsuperscript{57-60} They were able to synthesize three γ-lactam analogs. Each compound contained other essential functionality for antibacterial activity that are incorporated into β-lactam compounds, such as a carboxylic acid adjacent to the nitrogen and an amino
group which can be appended with various side chains. They choose the penicillin V side chain. Their initial compound 18 which contains a 5-5 fused system was not active nor was the 5-4 19 compound active. In an effort to tie the nitrogen lone pair of electrons up, a double bond was placed in the attached ring 20. This produced an active compound but with poor activity.

![Image of molecules](image-url)

Figure 1.6 Baldwin γ-lactam analogs to β-lactam compounds

Another approach pursued at Eli Lilly and company differed from the Baldwin approach by adding a heteroatom attached to the surrogate β-lactam nitrogen. They chose to use a nitrogen atom to attach to the β-lactam nitrogen. The most active examples that were reported 21a-e and 22a-c utilized a ATMO side chain and various electron withdrawing groups attached to the double bond. Despite extensive effort to synthesize these compounds, they were only moderately active.

![Image of molecules](image-url)

Figure 1.7 Lilly β-Lactam Mimics
1.7 Wolfe examples

The idea of heteroatom activation in making a β-lactam mimic was used on different structure by Wolfe and coworkers. These compounds used an oxygen attached to the nitrogen in order to promote activation. The most active compound that was reported contained a phenyl acetyl amino side chain. The other compounds were not as active probability due to the lack of recognition in the active site of the PBP due to the absence of a side chain.

![Chemical structures](image)

Figure 1.8 Wolfe β-Lactam Mimics

1.8 Weinreb Amides as non-β-lactam “β-lactams”

The non β-lactam mimics synthesized by Wolf and coworkers look like N,O-dimethyl hydroxamic acids which are commonly referred to as Weinreb amides. These compounds were developed by Weinreb and coworkers to form ketones when treated with Grignard or alkyl lithium reagents.

![Chemical reaction](image)

Scheme 1.2 Chemistry of Weinreb Amides
The chemical hydrolysis of penicillin and other β-lactam antibiotics has been shown to be an indicator of biological activity.\textsuperscript{77-79} Though rates of the saponification of Weinreb amides have not been reported, several examples of base catalyzed hydrolysis of the Weinreb functionality have been reported. For instance, Weinreb amide 28 can be readily removed with LiOH accompanied with ester hydrolysis.\textsuperscript{80} The use of KOH is also affective in the hydrolysis of Weinreb amide 30.\textsuperscript{81} The use of LiOH though preferred for some substrates such as 32, was not ideal for sterically hindered molecules like 34. In cases such as 34, a preformed mixture of water and t-BuOK works best.\textsuperscript{82-84} Other examples have also been published.\textsuperscript{85-88}

\begin{align*}
\text{Scheme 1.3 Examples of Saponification of Weinreb Amides}
\end{align*}
1.9 Isoxazolidines

In designing a β-lactam mimic, it is desirable to use heteroatom activation to activate an amide carbonyl. This technique has shown some promising results in Wolf’s work and was affective in the oxamazins example. Another desirable characteristic is for the molecule to contain a carboxylic acid adjacent to the β-lactam nitrogen and an amino group next to the carbonyl which could easily be appended. In addition, the potential β-lactam mimic should be structurally similar to penicillin which mimics the terminal acyl-D-Ala-D-Ala of the growing peptidoglycan layer of a cell wall. An isoxazolidine fits all of these traits (Figure 1.9).

![Figure 1.9 Comparison of Penicillin to acyl-D-Ala-D-Ala and an Isoxazolidine](image)

1.10 Summary

Antibacterial agents have greatly improved the quality and in many cases extended the life of many people. Penicillin and other β-lactam antibiotics have proven themselves to be incredibly useful in this regard. This chapter has highlighted how β-lactam antibiotics work and some of the more useful members of this class of antibacterial agents.
Resistant strains of bacteria are beginning to become a serious threat and if they are not checked they will become a serious heath issue. The primary method of dealing with this is to modify existing compounds to adapt to the new resistant bacteria. Eventually modification of current antibiotics will no longer be productive.

An alternate approach to combat resistant bacteria is to make molecules that mimic the action of current antibiotics. In doing so, the potential new antibacterial agents may avoid the resistance mechanisms. Successful attempts at mimicking β-lactam antibiotics have been summarized and the rational why isoxazolidines could mimic β-lactam antibiotics has been given.
CHAPTER 2:
INITIAL SYNTHESIS AND ANTIBACTERIAL TESTING OF ISOXAZOLIDINES

2.1 Introduction

The acylnitroso Diels-Alder reaction is a powerful tool which converts a simple inexpensive diene into a platform that can be readily functionalized into numerous biologically relevant molecules.\textsuperscript{89-92} There are three synthetically useful ways that acylnitroso Diels Alder adducts \textsuperscript{36} can be transformed into numerous biologically valuable products (Figure 1). The first involves reduction of the NO bond (path a) which gives amino cyclopentenols \textsuperscript{37} and the second is oxidative cleavage of the double bond to form dicarboxylic acids \textsuperscript{39} (path c). The third method constructs cyclopentenes \textsuperscript{38} when the acylnitroso Diels Alder adduct is reacted with Pd(0) followed by the addition of a nucleophile or an electrophile if In(0) and Pd(0) are present at the same time (path b).
Amino cyclopentenols can be converted into numerous biologically active molecules (Figure 2.2). For instance, several carbocyclic nucleosides such as polyoxin C \textsuperscript{40,93} aristeromycin \textsuperscript{41,94} and noraristeromycin \textsuperscript{42,95} have recently been synthesized. Natural products such as Streptazolin \textsuperscript{44,96} have also been synthesized \textit{via} an amino cyclopentenol. In addition, analogues to a phosphodiesterase inhibitor \textsuperscript{43,97} and analogues of LY354740 \textsuperscript{45a} and \textsuperscript{45b,98} (a conformationally constrained analogues of L-glutamic acid) have been synthesized.
Figure 2.2 Molecules Synthesized from Amino cyclopenentols

Treatment of the acylnitroso Diels Alder adduct 36 with Pd(0) followed by the addition of a nucleophile is a powerful method to synthesize a diverse array of disubstituted cyclopentenes. This methodology is augmented to work with electrophiles when In(0) is added in conjunction with Pd(0) (Figure 2.3). Several biologically active molecules have been synthesized which include a benzodiazepine 46, 5-lipoxygenase inhibitor 47, and a conformationally restricted substrate analogue of siderophore biosynthetases 48.
The synthesis of a number of interesting molecules can be obtained from the oxidative cleavage of the double bond (Figure 2.4). Novel amino acids can be obtained such as an interesting proline analog 50,\textsuperscript{102} and, in conjunction with the cleavage of the NO bond, diaminopimelic acid 51.\textsuperscript{103} With the appropriate elaboration, the proline analog 50 can be transformed into a diketopiperazine 52.\textsuperscript{104}
Isoxazolidine 50 not only can be viewed as a proline analog but potentially as a β-lactam mimic. As was described in the introduction chapter the molecule contains all the necessary functionality that is known to be needed for antibacterial activity and the Weinreb amide-like carbonyl has potential to be reactive like a β-lactam carbonyl. In order to test whether isoxazolidines can mimic the β-lactam antibiotic mechanism of action a series of isoxazolidines were made and then tested against bacteria to determine any growth inhibition.

The retrosynthetic pathway that was chosen to give our initial compounds (Scheme 1) has a number of aspects that made it very attractive. The first is the relatively short number of steps to obtain a testable compound. In addition this route allows for elaboration at several different positions. These include the side chain $R_1$, the amino acid $R_2$, and groups attached to the ring.
2.2 Preparation of Isoxazolidines 9a-c

Acylation of glycine 56a, D-alanine 56b, and L-alanine 56c with phenylacetyl chloride gave the corresponding N-acylated amino acids 57a-c in good yield. The hydroxamic acids 59a-c were formed in two steps by first coupling OBHA (O-benzyl hydroxyl amine) to the N-acylated amino acids 58a-c using an aqueous EDC coupling procedure followed by removal of the benzyl group by hydrogenation.\textsuperscript{105-106} Oxidation of hydroxamic acids 59a-c using NaIO\textsubscript{4} produced the corresponding transient acyl nitroso species which was trapped with cyclopentadiene to give the corresponding Diels-Alder adducts 60a-c as undetermined mixtures of diastereomers.
Scheme 2.2 Synthesis of Diels Alder Adducts 60a-c

Hydroxamic acids 59a-c were also synthesized via methyl esters 61a-c, which were formed by treatment of N-acylated amino acids 57a-c with thionyl chloride and methanol. Addition of alkaline hydroxylamine to methyl esters 61a-c generated the crude hydroxamic acids 59a-c. There were two drawbacks to this approach making the previous one more attractive. The first was that the hydroxamic acids were difficult to purify due to their high affinity for iron and other metals, which the pathway in Scheme 1 avoided. The second drawback to using the methyl ester pathway was that it limited the functionality of the side chain due to the molecule being exposed to an acidic environment followed by a strongly basic one.
With the acyl nitroso Diels-Alder adducts 60a-c in hand, they were oxidatively cleaved using \textit{in situ} generated RuO$_4$ to form the crude isoxazolidine-3,5-diacids 63a-c in low yield. Due to the difficulty in purifying the crude diacids 63a-c, the dimethylesters 62a-c were made using diazomethane and then saponified using LiOH after rigorous purification to give pure isoxazolidines 63a-c.
Scheme 2.4 Synthesis of Isoxazolidines 63a-c

2.3 Preliminary Antibacterial Testing

Initial antibacterial testing of isoxazolidines 63a-c was conducted using a kinetic growth assay.\textsuperscript{107} This involved treating \textit{Escherichia coli} X580, a strain of bacteria that is hypersensitive to β-lactam antibiotics, with various concentrations of isoxazolidine and monitoring the growth of the bacteria by measuring the absorbance (650nm) using a plate reader taking readings every half hour. A graph of the absorbance over a period of 24 hours was plotted containing four concentrations of isoxazolidine 63a ranging from 0.84 µg/mL to 5.04 µg/mL and a control containing only bacteria (Figure 2.5). The graph clearly shows the growth of \textit{Escherichia coli} X580 was inhibited at all the concentrations.
tested. The graph also shows that the growth of *Escherichia coli* X580 was completely inhibited at concentrations of 1.68 µg/mL or greater.

![Graph showing antibacterial kinetic growth assay of isoxazolidine 63a](image)

**Figure 2.5 Antibacterial kinetic growth assay of isoxazolidine 63a**

Isoxazolidine 63b was tested in the same kinetic growth assay as isoxazolidine 63a and a similar graph was constructed containing four concentrations of isoxazolidine 63b ranging from 0.88 µg/mL to 5.25 µg/mL in addition to a control containing only bacteria (Figure 2.2). The graph indicates the inhibition of growth of *Escherichia coli* X580 by isoxazolidine 63b at concentrations of 1.75 µg/mL or higher and completely prevents the growth at 5.25 µg/mL.
Figure 2.6 Antibacterial kinetic growth assay of isoxazolidine 63b

2.4 Broad Screen Testing

Armed with these exciting results, isoxazolidines 63a-c were tested against a broad range of bacteria. A different assay was utilized in order to determine if isoxazolidines 63a-c were broadly active against bacteria. The Kirby Bauer diffusion assay was chosen for this task due to the speed and ease of data collection. This assay involves making a lawn of bacteria on the surface of a Mueller Hinton II agar filled petri dish followed by making holes in the agar and filling them with a 1mg/mL solution of the isoxazolidine. After incubation at 37°C for 24h, the diameter of the zones of inhibition were measured and compared to the zone produced by a positive control such as penicillin 11a or ampicillin 11d and the negative control of DMSO which does not
produce a zone of inhibition. An example of the final result of this assay is shown in Figure 2.3.

![Figure 2.7 Picture of Kirby Bauer Diffusion Assay](image)

The Kirby Bauer diffusion assay was conducted with isoxazolidines 63a-c against a variety of bacteria and the data was compiled in Table 2.1. There are several pieces of information that are noteworthy. The first and most obvious is that isoxazolidines 63a-c are only active against *Escherichia coli* X580 and *Micrococcus luteus* X186, which were both obtained from Eli Lilly and company. They are bacteria that are hypersensitive to β-lactam antibiotics and are intended to be used as a screen to determine if there is any inherent activity with newly discovered compounds. The second fact to point out is that the table clearly shows that ampicillin 11d is more effective than penicillin G 11a and ampicillin 11d inhibits more stains of bacteria than does penicillin G 11a. The last point
is that both ampicillin 11d and penicillin G 11a are not as effective against the other strains of *Escherichia coli* as they were against *Escherichia coli* X580 thus reinforcing the notion that *Escherichia coli* X580 is a hypersensitive stain of bacteria to β-lactam antibiotics.
### TABLE 2.1

**KIRBY BAUER DIFFUSION ASSAY RESULTS ON A BROAD SELECTION OF BACTERIA**

<table>
<thead>
<tr>
<th>Species</th>
<th>S</th>
<th>train</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>1a</th>
<th>1d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram (-)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>A</td>
<td>TCC®</td>
<td>0</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25922</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>A</td>
<td>TCC®</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>33475</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>A</td>
<td>TCC®</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>33476</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Samonella typhimurium</em></td>
<td>TCC®</td>
<td>0</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13311</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organism</td>
<td>Type</td>
<td>Catalog Number</td>
<td>Plate</td>
<td>Colonies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------</td>
<td>----------------</td>
<td>-------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Klebsiella</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>pneumoniae</em></td>
<td></td>
<td>TCC® 8308</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudomonas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>aeruginosa</em></td>
<td></td>
<td>TCC® 10145</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Agrobacterium</strong></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>radiobacter</em></td>
<td></td>
<td>TCC® 1700</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gram (+)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>faecalis</em></td>
<td></td>
<td>TCC® 49532</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td></td>
<td>TCC® 10876</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>aureus</em></td>
<td></td>
<td>TCC®</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Staphylococcus epidermidis

Micrococcus luteus

Zones reported in mm

Additional antibacterial testing was conducted at the Hans Knöll Institute for Natural Products Research in Jena, Germany by Dr. Ute Möllmann and coworkers. This testing was done using the Kirby Bauer diffusion assay similar to the previous method except that the agar was impregnated with bacteria throughout the agar and not just on the surface. The bacteria that were screened against are clinically relevant strains of bacteria most of which have developed resistance to some important antibiotics. The data shows that isoxazolidines 63a-c have no activity against these strains.

### TABLE 2.2

KIRBY BAUER DIFFUSION ASSAY RESULTS ON RESISTANT BACTERIA

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Relevant property</th>
<th>63a</th>
<th>64</th>
<th>12e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>TCC®</td>
<td></td>
<td>A</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Micrococcus</td>
<td></td>
<td></td>
<td>X</td>
<td>186</td>
<td>4</td>
</tr>
<tr>
<td>luteus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Gram (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------------------------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>SG</td>
<td>Wild</td>
<td>0</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td><em>aureus</em></td>
<td>511</td>
<td>type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>134/93</td>
<td>MRSA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>1528</td>
<td>VRE</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td><em>faecalis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium</em></td>
<td>IMET</td>
<td>Wild</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>vaccae</em></td>
<td>10670</td>
<td>type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram (−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>DC0</td>
<td>Wild</td>
<td>0</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td><em>coli</em></td>
<td>type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>IV-3-2</td>
<td>TEM1</td>
<td>0</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td><em>coli</em></td>
<td>type</td>
<td>β-lactamase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>P99</td>
<td>ampC β-lactamase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>cloacae</em></td>
<td></td>
<td>lactamase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>IV-3-1</td>
<td>PSE1</td>
<td>0</td>
<td>0</td>
<td>14p</td>
</tr>
<tr>
<td><em>aeruginosa</em></td>
<td>13</td>
<td>β-lactamase</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Another way to gauge the activity of isoxazolidines 63a-c was through the determination of the values for the minimum inhibitory concentration (MIC). This was conducted by adding incremental amounts of each isoxazolidine to a 96 well plate containing *Escherichia coli* X580 and incubating it over night at 37°C. The MIC value was then determined by visually observing which wells did not become turbid due to growth of the bacteria (Figure 2.8). The lowest concentration that no growth could be observed was considered the MIC value. The values calculated for isoxazolidines 63a-c were all the same at 50 μg/mL and penicillin G was 6.25 μg/mL (Table 2.3).

Figure 2.8 Picture of MIC Value Determination
TABLE 2.3

MIC VALUES (MG/ML)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>6.25 µg/mL</td>
</tr>
<tr>
<td>63a</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>63b</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>63c</td>
<td>50 µg/mL</td>
</tr>
</tbody>
</table>

Additional testing of isoxazolidines 63a-c was also conducted at Eli Lilly and company by Dr. Douglas Zeckner. This testing focused on the determination of the MIC values on a few test bacteria in relation to penicillin G 11a, ampicillin 11d, and vancomycin 4. Despite the data in this table showing that isoxazolidines 63a-c are not active against the stains that were tested, the trend that ampicillin 11d is more effective than penicillin G 11a is again highlighted.
<table>
<thead>
<tr>
<th>Species Strain</th>
<th>Strain 3a</th>
<th>Strain 3b</th>
<th>Strain 3c</th>
<th>Strain 1a</th>
<th>Strain 1d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram (+) Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> AT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> 02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> SP 409</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram (-) Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 2.5 Summary and Conclusions

The data in this chapter has illustrated the synthesis and extensive antibacterial testing of isoxazolidines \( 63a-c \). The fact that the testing shows that isoxazolidines \( 63a-c \) have an inhibitory effect against \textit{Escherichia coli} X580 has generated some credibility to the hypothesis that isoxazolidines can mimic the β-lactam mechanism of action. Additionally, these results parallel how oxamazins were developed. The oxamazins had activity against \textit{Escherichia coli} X580 with the phenyl acetyl side chain \( 15a \), but it was markedly improved with the ATMO side chain \( 16a \). Related studies were also done on monobactams by Bristol-Meyers-Squibb scientists which led to the commercially used antibiotic Aztreonam \( 17a \). However, the lack of broad activity of \( 63a-c \) dictates the synthesis of additional isoxazolidines which are modified to increase the activity.

The first logical adaptation to be made is to alter the side chain from the phenylacetyl side to one that exhibits a stronger and broader antibacterial effect. The side chain on ampicillin (phenylglycine) would be an attractive target based on the testing data in this chapter showing that superior antibacterial activity compared to penicillin G (phenylacetyl) and its ability to survive all of the chemical transformations necessary to synthesize an isoxazolidine which utilizes it as a side chain.
CHAPTER 3:
SYNTHESIS OF ISOXAZOLIDINES APPENDED WITH AN PHENYL GLYCINE
AND ATMO AMINO SIDE CHAINS

3.1 Introduction

As was previously shown in chapters 1 and 2, the ideal side chain to attach to an isoxazolidine would be the ATMO group. The most expedient method to synthesize such a compound would be through a convergent approach where isoxazolidine 67 is coupled to an amino acid which has the ATMO group already attached. Isoxazolidine 67 can be readily accessed from an acylnitroso Diels Alder adduct 68.

Scheme 3.1 Retrosynthetic Analysis to Synthesize Isoxazolidine 65
3.2 Synthesis of ATMO D-Alanine Active Ester

The first step to synthesize isoxazolidine 65 is to make the active ester 73 followed by the synthesis of isoxazolidine 77. The synthesis of active ester 73 was accomplished starting with commercially available (syn)-2-amino-α-(methoxyimimo)-4-thiazoleacetic acid 70, followed by the formation of the HOBt active ester 71 using DCC as the coupling agent (Scheme 3.2). Addition of active ester 71 to D-alanine tert-butyl ester generated ATMO alanine 72. Removal of the tert-butyl group using TFA and triethylsilane delivered free acid 66, which was treated with DCC and HOBT to afford HOBT active ester, 73.

Scheme 3.2 Synthesis of ATMO D-Alanine HOBT Ester 73
3.3 Synthesis of Isoxazolidine 77

With the ATMO D-alanine HOBT ester 73 in hand, the synthesis of isoxazolidine 77 was attempted (Scheme 3.3). To this end, Boc cycloadduct 75 was synthesized by oxidation of Boc-hydroxylamine 74 with NaIO₄ to give the corresponding transient acyl nitroso species which was trapped with freshly cracked cyclopentadiene. Cycloadduct 75 was then treated with in situ prepared RuO₄ which oxidatively cleaved the double bond and the resulting diacid compound was esterified using diazomethane to give diester 76. The removal of the Boc group using HCl gas afforded isoxazolidine, 77.

![Scheme 3.3 Synthesis of Isoxazolidine 77](image)

3.4 Initial Coupling Attempts

With isoxazolidine 77 and active ester 73 in hand, coupling of the two was attempted (Scheme 3.4). The coupling was tried in three different solvents: CH₂Cl₂, CH₃CN, and DMF without success. Addition of TEA to the reaction was also fruitless. Even though ¹H NMR and mass spectral data indicated the existence of isoxazolidine 77 and active ester 73, the lack of coupling suggests that one or both of them was
decomposing in the reaction. Despite numerous attempts to couple them together a different strategy was needed to synthesize isoxazolidine 78.

Scheme 3.4 Attempted coupling

In an effort to determine if the active ester 73 was the source of the problem, a number of different coupling attempts were performed using different active esters. The first alternative coupling method employed making the HOBt active ester in situ and adding isoxazolidine 77 to it without success (Scheme 3.5). The DCC mediated formation of the HOObt active ester was also unproductive. An attempt was made to generate the HOAt active ester in situ using EDC and coupling isoxazolidine 77 also fell short. An unsuccessful attempt to couple isoxazolidine 77 to ATMO D-alanine 66 was also made using HATU.
TABLE 3.1

COUPLING CONDITIONS USED TO ATTEMPT THE SYNTHESIS OF ISOXAZOLIDINE 78

<table>
<thead>
<tr>
<th>Attempt</th>
<th>Coupling</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EDC, HOBt</td>
<td>CH$_3$CN</td>
</tr>
<tr>
<td>2</td>
<td>DCC, HOBt</td>
<td>CH$_3$CN</td>
</tr>
<tr>
<td>3</td>
<td>EDC, HOAt</td>
<td>CH$_3$CN</td>
</tr>
<tr>
<td>4</td>
<td>HATU</td>
<td>CH$_2$Cl$_2$</td>
</tr>
<tr>
<td>5</td>
<td>EDC, HOBt</td>
<td>CH$_2$Cl$_2$</td>
</tr>
<tr>
<td>6</td>
<td>DCC, HOBt</td>
<td>CH$_2$Cl$_2$</td>
</tr>
<tr>
<td>7</td>
<td>EDC, HOAt</td>
<td>CH$_2$Cl$_2$</td>
</tr>
<tr>
<td>8</td>
<td>EDC, HOBt</td>
<td>DMF</td>
</tr>
</tbody>
</table>
An additional approach utilized an aqueous EDC mediated coupling similar to the method used to couple OBHA to acid 57b (Scheme 3.6). In this attempt, freshly prepared isoxazolidine 77 was added to aqueous mixture of 57b. A minimum amount of THF was added to bring everything into solution followed by adjustment of the pH to 4.5. EDC was added in portions over a period of an hour but upon work none of the desired isoxazolidine 62b was found. The only identifiable compound that was isolated was 57b.

Scheme 3.5 Aqueous Coupling Attempt

Carpino and coworkers have reported the use of acid fluorides in coupling reactions which have been shown to be as reactive as an acid chloride without encountering racemization problems associated with the use of acid chlorides (Scheme 3.7).\textsuperscript{114-118} This seemed like a logical alternative approach to form isoxazolidine 78. Acid fluoride 79 was synthesized by treating ATMO D-alanine 66 with cyanuric fluoride.\textsuperscript{119} FTIR showed a carbonyl stretch of 1840 cm\textsuperscript{-1} which is characteristic for an acid fluoride.\textsuperscript{118} Despite all this effort addition of isoxazolidine 77 to acid fluoride 79 did not produce the desired product.
After numerous attempts to synthesize isoxazolidine 78 with an ATMO side chain, the stability of isoxazolidine 77 was considered questionable. This idea was reinforced when N-phenylacetyl-D-alanine 57b was used as the substrate in similar coupling reactions (Scheme 3.8). For instance when 57b was treated with EDC and HOBt followed by the addition of isoxazolidine 77, none of desired product was formed. In addition when acid fluoride 80, which was formed from acid 57b, was reacted with isoxazolidine 77 no reaction took place.
3.5 Attempts to Couple Isoxazolidine 82

One possible explanation for the lack of reactivity of isoxazolidine 77 is that the methyl esters may be labile or act as an electron withdrawing groups that would decrease the nucleophilicity of the nitrogen isoxazolidine 77. An isoxazolidine with an alternate protecting group was anticipated to avoid these possibilities. To this end, isoxazolidine 81 was synthesized from Boc adduct 75 using in situ generated RuO$_4$ to oxidatively cleave the double bond followed by allyl protection using allyl bromide and cesium carbonate. Removal of the Boc group was accomplished using HCl gas and the resulting HCl salt 82 was neutralized with NaHCO$_3$. The free amine was then added to a series of acids which were premixed with EDC and HOBr (Table 3.9). Though none of the
reactions were successful, it calls into question if isoxazolidines are nucleophilic enough and or stable enough to work in a successful coupling reaction.

Scheme 3.8 Coupling Attempts With Isoxazolidine 82
TABLE 3.2

LIST OF ACIDS USED IN COUPLING REACTION

<table>
<thead>
<tr>
<th>Acid</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-hydroxybutyric acid</td>
<td>CH$_3$CH(OH)CH$_2$CO$_2$H</td>
</tr>
<tr>
<td>D-mandelic acid</td>
<td>PhCH(OH)CO$_2$H</td>
</tr>
<tr>
<td>L-mandelic acid</td>
<td>PhCH(OH)CO$_2$H</td>
</tr>
<tr>
<td>L-lactic acid</td>
<td>CH$_3$CH(OH)CO$_2$H</td>
</tr>
<tr>
<td>DL-lactic acid</td>
<td>CH$_3$CH(OH)CO$_2$H</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>CH$_3$CO$_2$H</td>
</tr>
<tr>
<td>Glycolic acid</td>
<td>HOCH$_2$CO$_2$H</td>
</tr>
<tr>
<td>Malonic acid</td>
<td>(HO)$_2$CCH$_2$CO$_2$H</td>
</tr>
<tr>
<td>Chloro acetic acid</td>
<td>ClCH$_2$CO$_2$H</td>
</tr>
<tr>
<td>Cyanacetic acid</td>
<td>NCCH$_2$CO$_2$H</td>
</tr>
<tr>
<td>2-chloro propionic acid</td>
<td>CH$_3$CH(Cl)CO$_2$H</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>CH$_3$CH$_2$CH$_2$CO$_2$H</td>
</tr>
<tr>
<td>Glutaric acid</td>
<td>(HO)$_2$CCH$_2$CH$_2$CH$_2$CO$_2$H</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>CH$_3$(CH$<em>2$)$</em>{12}$CO$_2$H</td>
</tr>
<tr>
<td>Mandelic acid</td>
<td>PhCH(OH)CO$_2$H</td>
</tr>
<tr>
<td>Mercapto acetic</td>
<td>HSCH$_2$CO$_2$H</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Methoxy acetic</td>
<td>CH$_3$OCH$_2$CO$_2$H</td>
</tr>
<tr>
<td>Phenyl acetic</td>
<td>PhCH$_2$CO$_2$H</td>
</tr>
</tbody>
</table>

3.6 Linear Approach to ATMO Appended Isoxazolidine

Since the convergent approach was not producing the desired isoxazolidine with a ATMO side chain, a more linear approach was pursued. The first attempt to do this involved the synthesis of a Boc protected glycine appended to an isoxazolidine 88 (Scheme 3.10). This synthesis began with Boc protection of glycine under standard conditions followed by coupling with OBHA using aqueous EDC coupling conditions. Removal of the benzyl group by hydrogenation produced the hydroxamic acid 85. Diels-Alder adduct 86 was synthesized from hydroxamic acid 85 using NaIO$_4$ and freshly cracked cyclopentadiene. Oxidative cleavage of the double bond was then accomplished to give isoxazolidine 87. Removal of the Boc group proved to be problematic despite multiple attempts to remove it using either HCl gas or TFA.
3.7 Antibacterial Testing of Isoxazolidine 87

Though isoxazolidine 87 could not be deprotected, it represented an interesting analog to isoxazolidines 63a-c that could be tested for antibacterial activity. The kinetic growth assay was used to test isoxazolidine 87 against *Escherichia coli* X580. This was done using the same method and similar concentrations as isoxazolidines 63a-b were. The graph of the absorbance over a period of 24 hours was plotted containing four concentrations of isoxazolidine 87 ranging from 0.8 µg/mL to 4.8 µg/mL and a control containing only bacteria (Figure 3.1). The graph clearly shows that isoxazolidine 87 is not active at all of the concentrations that were tested. Despite isoxazolidine 87 not being active, it does show that the side chain can substantially affect the antibacterial activity.
Obviously the protecting group needed to be changed, so a Cbz group was chosen to replace the Boc group. The synthesis began by making glycine methyl ester 90 by treating Cbz glycine 89 with thionyl chloride and methanol (Scheme 3.11). The addition of alkaline hydroxylamine to methyl ester 90 produced the desired hydroxamic acid 91 which was subjected to NaIO₄ to oxidize it to the corresponding transient acyl nitroso species which was trapped with freshly cracked cyclopentadiene to give Diels Alder adduct 92. Oxidative cleavage using in situ generated RuO₄ of adduct 92 followed by esterification with phenyldiazomethane resulted in isoxazolidine 93. Global deprotection of isoxazolidine 93 using 10% palladium on carbon under one atmosphere of hydrogen produced isoxazolidine 88. Isoxazolidine 88 was treated with N, O-bis(trimethylsilyl)acetamide (BSA), which is known to silylate amino acids with TMS
groups, thus making the molecule soluble and then ATMO-OBt ester 71 was added. Unfortunately this initial attempt to couple the ATMO side chain failed. Antibacterial testing of 88 using the agar diffusion assay demonstrated that it was unable to inhibit the growth of Escherichia coli X580.

![Scheme 3.10 Synthesis of Isoxazolidine 88](image)

3.8 Synthesis of Isoxazolidine Appended with a D-Phenyl Glycine Side Chain

The obvious difficulty shown so far in synthesizing an isoxazolidine with an ATMO group dictated that an alternate side chain should be tried. In order to guarantee the successful synthesis of an isoxazolidine that incorporates a side chain with greater activity than isoxazolidines 63a-c, a completely linear approach was taken analogous to the method used for preparation of isoxazolidines 63a-c. The side chain would need to withstand the harsh oxidation conditions and have the potential to be more active than the
phenyl acetyl side chain. The phenylglycine side chain was chosen for these reasons and due to its commercial availability. However, great care had to be taken to keep base away from the phenyl glycine due to the ease of its racemization.\textsuperscript{120}

The first method to make isoxazolidine 108 began by coupling Cbz D-phenyl glycine to D-alanine tert-butyl ester using EDC and HOBt (Scheme 3.12). The resulting peptide 95 was treated with TFA and triethylsilane in order to remove the tert-butyl ester. Two attempts to couple O-TBS protected hydroxyl amine were attempted without success.\textsuperscript{121-122} The first involved an aqueous coupling using EDC at pH= 4.5 and the other used an anhydrous coupling with EDC and HOBt.

![Scheme 3.11 Attempted Coupling of O-TBS Hydroxyl Amine](image)

The problem with the O-TBS hydroxylamine was likely the instability of it, so O-\textit{tert}-butyl hydroxylamine was chosen to replace it. Aqueous EDC-mediated coupling with peptide 96 was accomplished with \textit{O-tert}-butyl hydroxylamine (Scheme 3.13). Deprotection of the \textit{tert}-butyl group could not be achieved and hence this pathway was abandoned.
In order to circumvent the problem of making hydroxamic acid 99, an alternative protecting group was needed to replace the CBZ group. A Boc group was employed to replace the CBZ group. To this end, Boc D-phenyl glycine was coupled to D-alanine benzyl ester using EDC (Scheme 3.14). Removal of the benzyl group using catalytic hydrogenation provided peptide 102. Hydroxamic acid 104 was synthesized by first coupling O-benzyl hydroxylamine to peptide 102 followed by removal of the benzyl group by catalytic hydrogenation. With hydroxamic acid 104 in hand, the Diels-Alder adduct 105 was synthesized.
Scheme 3.13 Synthesis of Hydroxamic Acid 105

The synthesis of benzyl protected isoxazolidine 106 was accomplished in two steps from Diels-Alder adduct 105. This was done in succession by oxidatively cleaving the double bond followed by esterification with phenyl diazomethane (Scheme 3.15). The removal of the Boc group was accomplished by treatment of isoxazolidine 106 with HCl gas. Catalytic hydrogenation then afforded the desired isoxazolidine 108.
3.9 Synthesis of ATMO Appended Isoxazolidine

While the synthesis of isoxazolidine 108 was in progress, a new strategy was devised to create isoxazolidine 115 by using a minimum protecting group strategy. This would involve coupling an isoxazolidine with free acids and an activated ester. The first step would be to make the active ester. Hence, the synthesis of ATMO glycine HOBT ester 111 began by reacting ATMO-OBt ester 71 with glycine tert-butyl ester to make ATMO-glycine tert-butyl ester 109 (Scheme 3.16). Removal of the tert-butyl ester was achieved using TFA and triethylsilane to make acid 110 which transformed into HOBT ester 111 after stirring with DCC and HOBT.
With the active ester 111 in hand, isoxazolidine 114 was prepared. This involved coupling the unprotected free amine 114 which was obtained from removing the Boc group from 112 using HCl gas and subsequent treatment with TEA. Coupling was achieved when isoxazolidine 114 was mixed with active ester 111. Antibacterial testing will be performed on isoxazolidine 115 in the near future.
3.10 Summary and Conclusions

The synthesis of additional analogs has been completed. The ATMO appended isoxazolidine 115 proved to be a challenging molecule to synthesize. After numerous attempts to make it a minimum protecting group strategy proved to be successful. Additionally, a phenyl glycine appended isoxazolidine 108 was fashioned starting from D-phenyl glycine.
CHAPTER 4:
ADDITIONAL SYNTHESSES OF POTENTIAL β-LACTAM MIMICS AND FUTURE DIRECTIONS

4.1 Introduction

The syntheses of several isoxazolidines which exhibit modest antibacterial activity have been completed. Improving that activity would ideally involve increasing the electrophilicity of the carbonyl that is proposed to be acting as the β-lactam carbonyl and at the same time improving how well the isoxazolidine mimics the D-Ala D-Ala structure. This chapter presents a number of different ways that the increase in electrophilicity of the simulated β-lactam carbonyl might be achieved.

In addition, the progress towards the synthesis of an isoxazolidine that could mimic the D-Ala D-Ala structure more closely will be shown. A proposed method to help determine if isoxazolidines are passing into the cell as well as a synthesis of an oligopeptide which contains an isoxazolidine are presented.

4.2 Synthesis of γ-Sultam

It seemed logical that attaching a stronger electron withdrawing group than oxygen would increase the electrophilicity of the carbonyl and thus potentially increase the antibacterial activity. With this in mind, the synthesis of an interesting γ-sultam 119 has been achieved. This endeavor began by treating DL-cystine dimethyl ester 116 with
chlorine gas, concentrating the resulting mixture, and subsequently treating the residue with TEA to produce γ-sultam 117 (Scheme 4.1). Addition of γ-sultam 117 to a preformed N-phenylacetyl-D-alanine NHS active ester in the presence of N-methylmorpholine formed γ-sultam-containing peptide 118. Careful saponification of the methyl ester produced the final target molecule 119 which was tested against *Escherichia coli* X580 and was found not to have an inhibitory effect.

Scheme 4.1 Synthesis of γ-Sultam
4.3 Progress Towards the Synthesis of An Isoxazolidine Analog of Thienamycin

The synthesis of a thienamycin analog 126 is well under way. The synthesis of hydroxamic acid 122 was completed in two steps starting with 3-hydroxybutyric acid. The first step formed the O-benzyl protected hydroxamate 121 using an aqueous EDC mediated coupling procedure and the second removed the benzyl group using catalytic hydrogenation. Hydroxamic acid 122 was then transformed into Diels-Alder adduct 123 upon treatment with NaIO₄ and freshly cracked cyclopentadiene. The alcohol in adduct 123 was protected as a benzyl ether 124 using phenyldiazomethane and a catalytic amount of HBF₄. All that remains to be done to complete the synthesis of thienamycin analog 126 is to form the dibenzyl ester 125 and then globally deprotect the molecule.
4.4 Siderophores

The assimilation of iron by micro organisms is an immense challenge due to elemental iron being virtually insoluble under physiological conditions (Ksp of Fe(OH)$_3$ < $10^{-18}$ at pH=7). To overcome this problem, micro organisms construct and utilize low molecular weight iron chelators called siderophores. Molecules such as the mycobactins 127 and Ferrichrome 128 sequester iron from outside the cell and bring it in for the use of the organism (Figure 4.1). Some siderophores have been adapted to serve human kind. For instance, thalessemia (Cooley’s anemia) is a genetic blood disorder and the only treatment for those who have it is to receive multiple red blood cell transfusions. In order to avoid death from the iron overload caused by so many transfusions, they must take Deferoxamine 129 (DFO).
Siderophores can also be used as drug delivery agents in addition to their use in chelation therapy. This is done by the siderophore acting as a “Trojan Horse” where the bacteria will bring the siderophore into the cell with a drug that is attached. Such a complex is termed a drug conjugate which typically consists of a siderophore and a linker which connects a drug to the siderophore (Figure 4.2).

Figure 4.2 Diagram of a Siderophore Drug Conjugate
4.5 The Proposed Use of Siderophores to Increase The Antibacterial Activity of Isoxazolidine 126

The ability of isoxazolidines to cross through biological membranes is one question that could potentially be answered by isoxazolidine 126. To answer this question isoxazolidine 126 will need to be attached to a siderophore such as DFO 129. The DFO isoxazolidine conjugate 134 would then be tested for antibacterial activity and if the complex 134 had a greater antibacterial effect than isoxazolidine 126 by itself then there would be evidence to suggest that the isoxazolidine 126 was not crossing the membrane in an efficient manner.

Thus, the synthesis of the siderophore isoxazolidine conjugate 134 was envisioned to be synthesized by the following route that begins with TBS protection of the alcohol on Diels Alder adduct 123 (Scheme 4.3). Oxidative cleavage of the olefin to the diacid followed by esterification using phenyldiazomethane should produce the dibenzyl ester 131. Removal of the TBS protecting group will be accomplished by treatment with TBAF. With the free hydroxyl in hand it could be coupled to DFO 129 via a linker. Once coupling conditions have been worked out deprotection using hydrogenation would give the final DFO isoxazolidine conjugate 134.
4.6 Progress Towards the Synthesis of a Diketopiperzine

Diketopiperazines are privileged structures that could mimic β-lactam antibacterial agents. A diketopiperzine which incorporates an isoxazolidine ring has more potential for antibacterial activity because of its bicyclic nature, a feature that is common to most active β-lactam antibiotics. The synthesis of such a molecule is in progress and was initiated by oxidative cleavage of the double bond on Diels Alder adduct 92 using in situ generated RuO₄, followed by esterification of the crude diacid produced isoxazolidine 135. The remainder of the synthesis requires removal of the Cbz group to induce diketopiperzine formation followed by acylation and saponification.
4.7 Progress Towards the Synthesis of a Isoxazolidine With Only One Carboxylic Acid

The synthesis of an isoaxazolidine 145 containing only one carboxylic acid is in progress and takes advantage of a literature procedure for the synthesis of an isoaxazolidine (Scheme 4.5). The synthesis starts by reacting N-hydroxyphtalimide 139 with 3-butene-1-ol under Mitsunobu conditions to append the hydroxyl with an alkene tether which was epoxidized using MCPBA to obtain epoxide 140. Treatment of epoxide 140 with tert-butyl amine opened the phthalimide which in turn attacked the epoxide to give isoaxazolidine 141. Refluxing isoaxazolidine 141 in HCl followed by protection of the amine with a Boc group under standard conditions produced
isoxazolidine 142. The remaining steps that need to be done to complete the synthesis involve oxidation of the alcohol using PDC to a carboxylic acid followed by benzyl protection with phenyl diazomethane. Removal of the Boc and subsequent coupling of 57b should give isoxazolidine 144. The final desired product 145 would then be obtained by removal of the benzyl ester by hydrogenation.

Scheme 4.5 Progress Towards the Synthesis of Isoxazolidine 145

The ability to attach a Boc group to isoxazolidine 141 has shown this pathway to be quite interesting. This could represent a solution to the direct coupling problem that was experienced with the diacid isoxazolidine. The reason for the direct attachment of the Boc group to make isoxazolidine 142 could potentially be due to a hydrogen bond effect from the hydroxyl group. This could occur in a number of ways such as a
hydrogen bond between one of the carbonyls like in structure A or form a six membered ring in structure B (Figure 4.3).

![Figure 4.3 Boc Protection](image)

4.8 The Use of Ozone for Oxidative Cleavage of Diels-Alder Adducts

One major obstacle in synthesizing additional analogs and thus attempting to optimize the antibacterial activity of these compounds is the oxidative cleavage of the acylnitroso Diels-Alder adducts which has been achieved in poor yield using in situ generated RuO$_4$. In addition to the low yield of the desired products, rigorous purification required the preparation of the dimethyl ester compounds and then saponification of the esters. Alternate oxidation reagents such as KMnO$_4$ did not improve the yield. An appealing alternative to the use of heavy metal oxidants is ozone.

It has been reported that treatment of alkenes at -78°C with ozone in the presence of NaOH and methanol will generate a dimethyl ester. This procedure has the advantage of turning the two step procedure of RuO$_4$ oxidation followed by esterification into one step and avoids the use and disposal of ruthenium. In order to test if these ozone conditions would work, the acylnitroso Diels-Alder adduct 146 was treated with ozone at -78°C in the presence of NaOH and methanol. The desired isoxazolidine 147 was obtained in good yield. The reaction was repeated under the exact same conditions.
except freshly prepared NaOCH$_3$ was used to replace the NaOH. These conditions did not improve the yield or purity of the crude product. However when KOH was used as the base, the crude $^1$H NMR spectrum was much cleaner than when NaOH or NaOCH$_3$ was used as the base with a similar yield. This observation, coupled with the KOH being much more soluble compared to NaOH, lead to the use of KOH as the base for the remainder of this work.

**Scheme 4.6 Methyl esters via Ozonolysis**

In order to determine if other synthetically useful esters could be made using this methodology, three alternate reactions were performed using the same reaction conditions on Diels-Alder adduct 146 except each reaction used either benzyl alcohol, isopropanol, or tert-butanol instead of methanol (Scheme 3). The dibenzyl ester 148a and diisopropyl ester 148b both formed albeit in modest yield but di-tert-butyl ester 148c could not be isolated.

**Scheme 4.7 Attempts to Synthesize Additional Esters**
The previous reactions worked well enough to try the same reaction conditions on advanced intermediate \( 62a \). Diels-Alder adduct \( 62a \) in hand dimethyl ester \( 149 \) was formed in good yield upon treatment with ozone, KOH, methanol, and \( \text{CH}_2\text{Cl}_2 \) at \(-78^\circ\text{C}\). The dibenzyl ester \( 149 \) was also synthesized using the same conditions except benzyl alcohol was used in place of methanol.

![Scheme 4.8 Cleavage of Diels-Alder Adduct](image)

In conclusion, oxidative cleavage of double bonds to diesters can be achieved using ozone with KOH and either methanol, benzyl alcohol, and isopropanol. This reaction gives very pure material. Despite the modest yields of the benzyl alcohol and isopropyl alcohol this method offers an attractive alternative to heavy metal oxidants such as \( \text{RuO}_4 \).

### 4.9 Summary

Progress towards the synthesis of Isoxazolidines derived from an acylnitroso Diels-Alder reaction which have the potential to have increased antibacterial activity compared to isoxazolidines \( 63a-c, 108 \) and \( 115 \) has been shown. This included the current efforts towards making a thienamycin analog \( 126 \) and to create a
diketopiperazine (138) which could have biological activity other than antibacterial. The current synthetic effort towards an isoxazolidine 145 containing only one carboxylic acid was also presented. The complete synthesis of a γ-sultam 119 was also discussed.

In an effort to determine if isoxazolidines have trouble crossing the cell membrane, the synthesis of a DFO isoxazolidine siderophore conjugate 134 was proposed. This synthesis would utilize isoxazolidine 126 which is intended to mimic thienamycin. DFO (129) was chosen for its commercial availability and low toxicity. This chapter as also shown that ozone can be used as a viable alternative to ruthenium tetroxide to oxidatively cleave the acylnitroso Diels-Alder adducts.
CHAPTER 5:
EXPERIMENTAL SECTION

5.1 General Methods

All NMR spectra were recorded on a Varian instrument at 300MHz (\textsuperscript{1}H), 75MHz (\textsuperscript{13}C), 500MHz (\textsuperscript{1}H), 125MHz (\textsuperscript{13}C). Chemical shifts are indicated in \( \delta \) values (ppm) from internal reference peaks of TMS, CDCl\(_3\), CD\(_3\)OD or DMSO-d6. Coupling constants are given in hertz (Hz). Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FTIR spectrometer using KBr pellets for solids and oils were taken as neat thin films. Optical rotations were measured using an Autopol III\textsuperscript{\textregistered} automatic polarimeter. Melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected. TLC was performed with aluminum backed Merck silica gel 60-F254 using 254-nm UV light, p-anisaldehyde, PMA, aq KMnO\(_4\), FeCl\(_3\), or ninhydrin for visualization. Flash chromatography was performed on Sorbent Technologies silica gel 60 (32-63 \( \mu \)m) mesh. All reagents were purchased from Aldrich, Arcos, Advanced ChemTech, and Fisher and used without purification unless otherwise indicated. THF was distilled from a mixture of sodium metal and benzophenone ketyl and CH\(_3\)CN, CH\(_2\)Cl\(_2\) and TEA were distilled from CaH\(_2\). DI water was further purified through a mixed bed type II filter made by US Filter. All other solvents were used without purification.
5.2 Chapter 2 Experimental Section

Preparation of N-phenylacetylamino acetic acid (57a). To a biphasic mixture (CH$_2$Cl$_2$, 250mL) of glycine (5g, 66.6 mmol) and NaOH (33mL, 6M, 199 mmol) was added freshly distilled phenylacetyl chloride (9.3 mL, 69.9 mmol) and the reaction was stirred for 25.5 h. To the mixture was added 6M HCl until it was acidic by pH paper and a white solid precipitated which was collected by suction filtration. The resulting white solid was recrystallized with EtOAc/hexanes to give the title compound in 91% yield. (11.67g, 60.4 mmol) Mp: 142-143$^\circ$ C; $^1$H NMR (500MHz, CD$_3$OD): 3.57 (s, 2H), 3.90 (s, 2H), 7.21-7.32 (m, 5H); $^{13}$C NMR (125MHz, CDCl$_3$): 40.8, 42.0, 126.4, 128.8, 129.2, 136.3, 170.7, 171.4 FTIR(KBr): 3374, 3034, 2935, 1728, 1610, 1228, 764; HRMS(FAB): calcd: 194.0817, found: 194.0820.

(R)-N-Phenylacetyl-2-aminopropionic acid (57b): 93% yield (15.16 g, 73.1 mmol) Mp: 102-103$^\circ$ C; [$\alpha$]$^D$= -3.05 (c=1 dm, THF); $^1$H NMR (500 MHz,CDCl$_3$): 1.37 (d, 3H, J=6.93 Hz), 3.60 (s, 2H), 4.55 (q, 1H, J= 7.42 Hz), 6.28 (bd, 1H, J=6.93 Hz), 7.24-7.36 (m, 5H), 11.31 (bs, 1H); $^{13}$C NMR (1250 MHz, CDCl$_3$): 18.1, 43.4, 48.5, 127.7, 129.2, 129.6, 134.2, 172.1, 176.1 FTIR(KBr): 3334, 3073, 2981, 1706, 1627, 1042, 724. HRMS (FAB): calcd: 208.0974, found: 208.0984.

(S)-N-Phenylacetyl-2-aminopropionic acid (57c): 92 % yield (10.76 g, 51.9 mmol) Mp:102-103$^\circ$ C;[$\alpha$]$_{D}$= 11.1 (c= 1dm, THF) $^1$H NMR (500 MHz, CDCl$_3$): 1.38 (d, 3H, J=7.42 Hz), 3.62 (s, 2H), 4.56 (q, 1H, J=6.93), 6.12 (bd, 1H, J= 6.44Hz), 7.25-7.37 (m,
Representative Procedure for the Synthesis of Hydroxamates. The Preparation of N-phenylacetyl-O-benzyl-2-aminoaceto hydroxamic acid (58a). A solution of 57a (1.0 g, 5.18 mmol) and OBHA (0.87 g, 5.4 mmol) in THF/H2O (15/25 mL) was prepared and the pH was adjusted to 4.5 using 1M NaOH and 1M HCl. EDC•HCl (1.99 g, 10.35 mmol) was added as a solid in small portions over a period of one hour. As time progressed, a white solid formed and 1M HCl was added in order to keep the pH at 4.5. The resulting white suspension was extracted with EtOAc (3x50) and the combined organics were washed sat NaHCO3 (3x25), brine (25), dried (MgSO4), filtered and concentrated to give the desired crude product (2.1 g) which was purified by flash chromatography (silica, CH2Cl2:EtOAc, 3:1) to give the product in 92% yield (1.42 g, 4.76 mmol). Mp = 145-146°C; 1H NMR (500 MHz, (CD3)2SO): δ 3.48 (s, 2H), 3.61 (d, 2H, J= 5.78), 4.78 (s, 2H), 7.20-7.23 (m, 1H), 7.26-7.31 (m, 4H), 7.34-7.40 (m, 5H), 8.35 (s, 1H), 11.19 (s, 1H); 13C NMR (125 MHz, (CD3)2SO): δ 39.9, 42.0, 76.9, 126.3, 128.2, 128.3, 128.8, 129.1, 135.9, 136.2, 166.2, 170.5; FTIR (KBr): 3297.4, 3221.1, 3084, 1677.1, 1645.5, 1028.2, 959.9, 730.8, 695.5 cm⁻¹; HRMS (FAB): calcd: 299.1396, found: 299.1399.

58b 91% yield (1.37 g, 4.40 mmol). Mp= 142-143°C; [α]20D = 17.43 (c= 0.009, DMSO); 1H NMR (500 MHz, (CD3)2SO): δ 1.17 (d, 3H, J= 7.17), 3.46 (s, 2H), 4.14 (p, 1H,
J=7.18), 4.77 (dd, 2H, J= 3.19, J= 10.97), 7.19-7.39 (m, 10H), 8.35 (d, 1H, J=7.37), 11.24 (s, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 17.9, 43.3, 46.5, 78.3, 127.5, 128.6, 128.8, 129.1, 129.3, 129.4, 134.5, 135.3, 169.8, 171.6; FTIR (KBr): 3272.5, 3161.3, 2992.7, 1673.3, 1643.6, 1047.9, 971.9, 741.5, 694.4 cm$^{-1}$; HRMS (FAB): calcd: 313.1552, found: 313.1566; MS (FAB): 313, 220, 190.

$^{58c}$ 91% yield (2.74 g, 8.78 mmol). Mp= 140-141°C; [$\alpha$]$^2_D$= -21.96 (c=0.011, CH$_2$Cl$_2$);

$^1$H NMR (500 MHz, (CD$_3$)$_2$SO): δ 1.17 (d, 3H, J=6.98), 3.45 (s, 2H), 4.12 (p, 1H, J=7.18), 4.76 (dd, 2H, J=2.99, J=10.97), 7.19-7.39 (m, 10H), 8.34 (d, 1H, J=7.58), 11.22 (s, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 18.2, 43.2, 46.5, 78.2, 127.4, 128.6, 128.7, 128.9, 129.3, 129.4, 134.6, 135.2, 169.8, 171.6; FTIR (KBr): 3272.1, 3161.1, 2992.7, 1689.5, 1643.4, 1048.2, 971.7, 741.4, 694.3 cm$^{-1}$; HRMS (FAB): calcd: 313.1552, found: 313.1529; MS (FAB): 190, 307, 313.

Representative Procedure for the Synthesis of Hydroxamic acids. The Preparation of $N$-phenylacetyl-2-aminoaceto hydroxamic acid ($59a$). Method A To a methanolic (50mL) solution of O-benzyl hydroxamate $58a$ (4.0 g, 13.4 mmol) under argon was added 10% palladium on carbon (0.4g) followed by the exchange of argon for a hydrogen atmosphere which was maintained for 5h. The hydrogen was then removed by purging with argon and the mixture was filtered and concentrated under reduced pressure to give the desired hydroxamic acid in 98% yield (2.73g, 13.1 mmol)
Method B: An alkaline methanolic solution of hydroxylamine was formed by adding a 0°C methanolic (50 mL) solution of KOH (3.24 g, 57.9 mmol) to a 0°C methanolic (50 mL) solution of hydroxylamine hydrochloride (3.02 g, 43.4 mmol). Immediately upon addition, a white solid was formed and was removed by suction filtration and the resulting solution was added to a 0°C methanolic (30 mL) solution of glycine N-phenylacetyl methyl ester (3.00 g, 14.5 mmol). The reaction was allowed to come to room temperature after 3h. The colorless solution was then acidified to a pH=4 using concentrated HCl and then concentrated to a white solid which was triturated with EtOAc until the remaining solid was no longer ferric chloride positive. The combined extracts were dried (MgSO₄), filtered, and then concentrated to give the title compound as a white solid in 77% yield. Mp: 126-128°C; \( ^1 \text{H NMR (500MHz, (CD}_3\text{)}_2\text{SO): } \delta 3.48 (s, 2H), 3.51 (s, 2H), 3.62 (d, 2H, J= 5.93), 3.95 (d, 2H, J=5.0), 7.20-7.31 (m, 5H), 8.11 (bs, 1H), 8.34 (bt, 1H, J=5.44), 8.86 (bs, 1H), 9.17 (bs, 1H), 10.10 (bs, 1H), 10.58 (bs, 1H); \( ^{13} \text{C NMR (125 MHz, (CD}_3\text{)}_2\text{SO): } \delta 40.0, 42.0, 126.3, 128.2, 129.1, 136.3, 165.8, 170.5; \) FTIR (KBr): 3130, 2877, 1642, 1602, 1072, 695 cm\(^{-1}\); HRMS (FAB): calcd: 209.0926, found: 209.0932.

\((R)-N\text{-Phenylacetyl-2-aminopropyl hydroxamic acid (59b): Method A: 95\% yield (0.202 g, 0.912 mmol). Method B: 72\% yield Mp: 132-133 \degree \text{C; } \left[\alpha\right]^{20}_D= 17.27 (c= 0.014, DMSO); \( ^1 \text{H NMR (500 MHz, CD}_3\text{OD): } \delta 1.32 (d, 3H, J=6.93), 3.39 (s, 1H), 3.53 (s, 2H), 4.27 (q, 1H, J=6.93), 7.20-7.30 (m, 5H) 8.31(bd, 1H, J=6.92); \( ^{13} \text{C NMR (125 MHz, (CD}_3\text{)}_2\text{SO): } \delta 18.6, 41.9, 45.9, 126.3, 128.1, 128.9, 136.4, 169.0, 169.7; \) FTIR (KBr): 3283, 3063, 2930, 1678, 1638, 1031, 699 cm\(^{-1}\); HRMS (FAB): calcd: 223.1083, found: 223.1067.}
(S)-N-Phenylacetyl-2-aminoproyl hydroxamic acid (59c): Method A: 97% yield (0.798g, 3.59 mmol) Method B: 71% yield Mp: 132-133 °C; $\left[\alpha\right]^\circ_{D} = -53$ (c= 0.008, CH₃OH); ¹H NMR (500MHz, CD₃OD): 1.32 (d, 3H, $J = 6.98$), 3.53 (s, 2H), 4.30 (q, 1H, $J=7.18$), 7.20-7.29 (m, 5H); ¹³C NMR (125MHz, CD₃OD): δ 18.4, 43.5, 48.3, 128.0, 129.6, 130.3, 136.8, 172.1, 173.7; FTIR (KBr): 3309, 1636, 1537, 1030, 699 cm⁻¹; HRMS (FAB) calcd: 313.1552, found: 313.1555.

Representative Procedure for the Synthesis of Acylnitroso Diels Alder Adducts.

Preparation of N-[N-phenylacetyl-glycyl]-2, 3-oxazabicyclo[2.2.1] hept-5-ene (60a).

A methanolic solution (100 mL) of hydroxamic acid 59a (1.90 g, 9.12 mmol) was cooled to 0°C and then freshly distilled cyclopentadiene (4.5 mL, 54.7 mmol) was added followed by the addition of aqueous (25 mL) NaIO₄ (2.09 g, 9.58 mmol) [formed by heating over a steam bath then cooling to room temp] dropwise over a period of 30 min. The reaction was stirred for an additional 2h at 0°C and then brought to room temperature and stirred for an additional 1h. The methanol was then removed by rotary evaporation making sure not to let the bath temperature rise above 40°C. The remaining aqueous layer was then extracted (EtOAc, 3x75 mL), washed (brine), dried (MgSO₄), filtered, and then concentrated to give the crude product as a brown oil which was chromatographed
(silica, 1:4, EtOAc/hexanes) to give a yellow oil that was induced to crystallize by cooling in hexanes to give the desired product in 65% yield (3.13 g, 11.5 mmol). Mp: 104-105°C; $^1$H NMR (500MHz, CDCl$_3$): $\delta$ 1.85 (d, 1H, $J=8.9$), 1.98 (d, 1H, $J=8.41$), 3.60 (s, 2H), 3.78 (bd, 1H, $J=18.31$), 4.05 (dd, 2H, $J=19.3, J=4.45$), 5.30 (s, 1H), 6.19 (s, 1H), 6.35 (s, 1H), 6.51 (s, 1H), 7.28-7.37 (m, 5H); $^{13}$C NMR (125MHz, CDCl$_3$): $\delta$ 43.1, 43.7, 48.6, 62.9, 84.8, 127.5, 129.1, 129.6, 133.0, 134.8, 136.2, 171.0, 172.7; FTIR (KBr): 3364, 3064, 2961, 1661, 1641, 1175, 721 cm$^{-1}$; HRMS (FAB): calcd 273.1239, found: 273.1250.

(60b): 68% yield (4.0 g, 13.99 mmol). Mp: 106-108°C; $\left[\alpha\right]_{D}^{20}$= 36.5 (c= 0.017, THF); $^1$H NMR (500MHz, CDCl$_3$): $\delta$ 1.06 (d, 3H, $J=5.39$), 1.33 (d, 3H, $J=6.98$), 1.80 (d, 1H, $J=8.77$), 1.94 (d, 1H, $J=8.58$), 3.53 (s, 2H), 4.75 (bs, 1H), 5.29 (bd, 2H, $J=10.5$), 6.34 (bs, 1H), 6.48 (bs, 1H), 6.7 (bs, 1H), 7.22-7.32 (m, 5H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 15.9, 18.8, 43.8, 47.3, 48.6, 62.0, 84.9, 127.4, 129.1, 129.5, 133.4, 134.9, 136.3, 170.3, 173.0; FTIR (KBr): 3264, 3066, 1637, 1548, 1172, 1102, 848, 731 cm$^{-1}$; HRMS (FAB+): calcd: 287.1396, found: 287.1375.

(60c): 71% yield (2.76 g, 9.66 mmol). Mp: 105-107°C; $\left[\alpha\right]_{D}^{20}$= -27.2(c= 0.013, CH$_3$OH); $^1$H NMR (500MHz, CDCl$_3$): $\delta$ 1.00 (d, 3H, $J=5.39$), 1.27 (d, 3H, $J=6.78$), 1.74 (d, 1H, $J=8.78$), 1.87 (d, 1H, $J=8.57$), 3.47 (s, 2H), 4.7 (bs, 1H), 5.23 (bd, 2H, $J=11.37$), 6.28 (bs, 1H), 6.42 (bs, 1H), 6.81 (bs, 1H), 7.154-7.25 (m, 5H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 15.2, 18.2, 43.0, 46.9, 48.1, 61.6, 84.5, 126.7, 128.4, 129.0, 133.0, 134.9, 135.7, 170.1, 172.8; FTIR (KBr): 3263, 3065, 1635, 1547, 1456, 1328, 848, 725 cm$^{-1}$; HRMS (FAB) calcd: 287.1396, found: 287.1378.
Representative Procedure for the Preparation of methyl esters. Preparation of N-phenylacetylaminomethyl acetate (61a). To a methanolic solution (100 mL) of N-phenylacetyl glycine (5.0g, 24.1mmol) was added SO\textsubscript{2}Cl\textsubscript{2} (3.5 mL, 48.2 mmol) dropwise. The colorless solution was stirred for 3h and then concentrated by rotary evaporation. The resulting white solid was dissolved in EtOAc and washed with NaOH then brine, dried (MgSO\textsubscript{4}), filtered, and concentrated. The crude product was recrystallized using EtOAc/hexanes to give the title compound in 92% yield. (4.61g, 22.2 mmol) Mp: 87-88°C; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): 3.62 (s, 2H), 3.71 (s, 3H), 3.99 (d, J=4.95), 6.07 (bs, 1H), 7.28-7.38 (m, 5H); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): 41.4, 43.5, 52.2, 127.4, 129.0, 129.5, 134.8, 170.3, 171.3 FTIR (KBr): 3249, 3077, 2938, 1754, 1644, 1212, 713; HRMS (FAB): calcd: 208.0974, found: 208.0981.

(R)-N-Phenylacetyl-2-aminomethyl propionate (61b): 91% yield. (4.85g, 21.9 mmol) Mp: 64-65°C; [\alpha]\textsubscript{D}\textsuperscript{20} = 22.5 (c= 1dm, THF); \textsuperscript{1}H NMR (500MHz, CDCl\textsubscript{3}): 1.34 (d, J=6.92), 3.58 (s, 2H), 3.70 (s, 3H), 4.58 (q, J=7.42), 6.15 (bs, 1H), 7.29-7.37 (m, 5H); \textsuperscript{13}C NMR (125MHz, CDCl\textsubscript{3}): 18.4, 43.6, 48.2, 52.5, 127.5, 129.1, 129.5, 134.7, 170.6, 173.5; FTIR(KBr): 3343, 3034, 2956, 1755, 1651, 1169, 732; HRMS (FAB): calcd: 222.1130, found: 222.1115.

(S)-N-Phenylacetyl-2-aminomethyl propionate (61c): 90% yield. (4.79g, 21.6 mmol) Mp: 64-65°C; [\alpha]\textsubscript{D}\textsuperscript{20} = -22.5 (c= 1, THF); \textsuperscript{1}H NMR (500MHz, CDCl\textsubscript{3}): 1.34 (d, J=6.93), 3.60 (s, 2H), 3.71 (s, 3H), 4.58 (q, J=7.42), 6.04 (bs, 1H), 7.29-7.38 (m, 5H);
$^{13}$C NMR (125 MHz, CDCl$_3$): 18.5, 43.7, 48.2, 52.6, 127.6, 129.1, 129.5, 134.7, 170.7, 173.5; FTIR (KBr): 3343, 3033, 2956, 1744, 1649, 1169, 732.

Representative Procedure for the preparation of isoxazolidines dimethylesters.

Preparation of 2-($N$-phenylacetylamino)acetylisoxazolidine-3,5-dimethyl ester. (62a)

To a vigorously stirring solution of cycloadduct 60a (1.5g, 5.5 mmol) in 1:1 CCl$_4$/CH$_3$CN (70mL) was added DI water (70mL) and NaIO$_4$ (5.89g, 27.5 mmol). The resulting biphasic mixture was cooled to 0°C followed by the addition of RuCl$_3$•H$_2$O$_n$ (0.286 g, 1.37 mmol). Upon the addition of ruthenium, the mixture turned brown and was kept at 0°C for 30 min and allowed to stir at room temperature for an additional 2 h during which time the mixture gradually turned black. The mixture was then filtered through celite and diluted with EtOAc (50mL) and 1M HCl (10mL). The aqueous layer was extracted (EtOAc 3x30) then the combined extracts were dried (MgSO$_4$), filtered, and concentrated to give the crude diacid compound. The crude product was treated with freshly prepared diazomethane (from Diazald®) at 0°C in THF/ether (1:1, 40mL) until the yellow of diazomethane persisted. The remaining diazomethane was quenched with the addition of AcOH and the resulting mixture was washed with NaHCO$_3$ (3x30), dried (MgSO$_4$), filtered, and concentrated to give the crude product which was purified by column
chromatography (1:1, EtOAc:hexanes, Silica) to give the desired product in 42% yield (0.841g, 2.31 mmol). \( ^1H \) NMR (500 MHz, CDCl\(_3\)): \( \delta \) 2.75-2.83 (m, 2H), 3.75 (s, 3H), 3.80 (s, 3H), 3.83 (d, 1H, J=14.35), 3.94 (d, 1H, J=14.55), 4.33 (t, 1H, J=6.58), 4.91 (t, 1H, J= 6.78), 7.24-7.32 (m, 5H); \( ^13C \) NMR (125MHz, CDCl\(_3\)): \( \delta \) 35.7, 40.2, 53.0, 53.2, 56.7, 77.6, 127.2, 128.8, 129.6, 134.0, 168.7, 169.8, 171.5; FTIR (neat): 2955, 1744, 1664, 1438, 1216, 1052 cm\(^{-1}\); Mass Spec (FAB): 130, 190, 308.

\( 62b \): 44% yield (0.61g, 1.68 mmol). \([\alpha]_{20}^{20} = \) 22.3 (c= 0.007, CH\(_3\)OH); \( ^1H \) NMR (CDCl\(_3\)): \( \delta \) 1.42 (d, 3H, J= 6.98), 2.75-2.80 (m, 1H), 2.89-2.95 (m, 1H), 3.57 (s, 2H), 3.75 (s, 3H), 3.80 (s, 3H), 4.83 (t, 1H, J=7.17), 4.95-5.00 (m, 1H), 6.09 (bs, 1H), 7.28-7.37 (m, 5H); \( ^13C \) NMR (CDCl\(_3\)): \( \delta \) 18.2, 35.1, 43.6, 46.4, 53.0, 53.2, 56.8, 78.0, 127.6, 129.2, 129.6, 134.6, 168.4, 169.5, 171.0, 173.6; FTIR (neat): 3327, 3019, 2956, 1752, 1655, 1509, 1217, 1052, 755 cm\(^{-1}\); HRMS (FAB): calcd: 379.1505, found: 379.1517.

\( 62c \): 35% yield (0.69g, 1.83 mmol). \([\alpha]_{20}^{20} = -33.6 \) (c= 0.017, CHCl\(_3\)); \( ^1H \) NMR (500MHz, CDCl\(_3\)): \( \delta \) 1.41 (d, 3H, J=6.97), 2.71-2.76 (m, 1H), 2.87-2.93 (m, 1H), 3.54 (s, 2H), 3.71 (s, 3H), 3.77 (s, 3H), 4.83 (t, 1H, J=7.18), 4.93-4.98 (m, 2H), 6.59 (d, 1H, J=6.98), 7.23-7.33 (m, 5H); \( ^13C \) NMR (125MHz, CDCl\(_3\)): \( \delta \) 17.7, 34.8, 43.1, 46.1, 52.7, 52.9, 56.6, 77.7, 127.1, 128.8, 129.2, 134.7, 168.2, 169.3, 170.9, 173.5; FTIR (neat): 3412, 3019, 2956, 1752, 1655, 1509, 1217, 909, 755 cm\(^{-1}\); HRMS (FAB) calcd: 379.1505, found: 379.1481.
Representative Procedure for the preparation of isoxazolidines diacids. (63a): To a solution of isoxazolidine dimethylester 62a (0.13g, 0.36 mmol) in THF (3 mL) was added an aqueous solution of LiOH (7.5mL, 0.1 M) and the reaction mixture was allowed to stir at room temperature for 20 min. The resulting mixture was acidified to pH=3 using 1M HCl and then extracted (EtOAc 3x5), dried (MgSO₄), filtered, and concentrated to give the desired product in 85% yield (0.105g, 0.31 mmol). Mp: 170-171°C; ¹H NMR (500MHz, CD₃OD): δ 2.68-2.73 (m, 1H), 3.04-3.10 (m, 1H), 3.62 (s, 2H), 4.12 (s, 1H), 4.15 (s, 1H), 4.34 (bs, 1H), 4.37 (bs, 1H), 4.84 (t, 1H, J=6.78), 4.88-4.91 (m, 1H), 7.24-7.36 (m, 5H); ¹³C NMR (125MHz, CD₃OD): δ 27.0, 32.2, 34.0, 49.0, 69.4, 118.4, 120.1, 120.8, 127.1, 161.8, 162.6, 165.2; FTIR (KBr): 3406, 2992, 1727, 1628, 1335, 1028, 704 cm⁻¹; HRMS (FAB): calcd: 337.1036, found: 337.1028.

(63b): 81% yield (0.095g, 0.27 mmol). [α]²⁰/D= 46.03(c= 0.011, CH₃OH); ¹H NMR (500MHz, CD₃OD): δ 1.42 (d, 3H, J=7.38), 1.49 (d, 3H, J=7.18), 2.64-2.69 (m, 1H), 3.02-3.08 (m, 1H), 3.57 (s, 2H), 4.84-4.93 (m, 4H), 7.23-7.32 (m, 5H); ¹³C NMR(125MHz, CD₃OD): δ 17.3, 36.4, 43.3, 47.8, 58.4, 79.2, 128.0, 129.6, 130.3, 136.9,
171.2, 172.3, 174.1; FTIR (KBr): 3348, 2943, 1735, 1621, 1530, 1230, 1046, 702 cm$^{-1}$; HRMS (FAB): calcd: 351.1192, found: 351.1192.

(63c): 83% yield (0.075g, 0.21 mmol). [$\alpha$]$^D_{20}$=48.5 (c= 0.012, CH$_3$OH); $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 1.39 (d, 3H, J=7.17), 1.47 (d, 3H, J=7.17), 2.61-2.67 (m, 1H), 2.98-3.04 (m, 1H), 3.55 (s, 2H), 4.82-4.91 (m, 4H), 7.20-7.30 (m, 5H); $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta$ 17.3, 36.3, 43.3, 47.8, 58.4, 79.2, 128.0, 129.6, 130.3, 136.9, 171.3, 172.3, 174.1; FTIR (KBr): 3379, 2943, 1735, 1621, 1530, 1231, 1046, 703 cm$^{-1}$; HRMS (FAB) calcd: 351.1192, found: 351.1188.

Procedure for Concentration Dependent Antibacterial Testing

Luria broth was prepared by filling a 1L flask with 900 mL of distilled deionized water and to it was added 10 g of Bacto tryptone, 5.0 g of bacto yeast extract, and 0.5 g of sodium chloride. The mixture was stirred until it became homogenous and to it was added 2 mL of 1M NaOH. The pH of the resulting solution was adjusted to 7.0 using 1M HCl. Two 500 mL Erlenmeyer flasks were each filled with 90 mL of the Luria broth and a 250 mL Erlenmeyer flask was filled with 4 g of glucose and 20 mL of distilled deionized water. All of the flasks were sterilized in an autoclave. To each of the Luria broth flasks was added 1 mL of the sterilized glucose solution and 200 µL of E. coli X-580 suspended in sterile lactose-glycerol was added to one of the Luria broth flasks. The inoculated flask was then incubated in a shaker for 24h at 37°C. After the start up culture was grown, 70 µL was transferred to the previously prepared flask containing sterile Luria broth and glucose and shaken for 5min. Once this was obtained, a 10 µL solution of the compound to be tested was placed in a well in a sterile 96-well plate followed by
the addition of 190 µL of culture broth. The 96-well plate was then incubated at 37°C in
a Molecular Devices Thermomax® microplate reader with readings being taken at 650
nM every 30 min. for a 24h period.

General Procedure for Antimicrobial Agar Diffusion Assay

Mueller Hinton agar plates were prepared using standard procedures with commercially
available Mueller Hinton agar (Fisher). A lawn of the bacteria was then placed on the
surface of the agar using a sterile cotton swab dipped in an aqueous suspension of fresh
bacteria culture. A pipet tip was then used to bore three 6mm (diameter) holes in the
agar. One hole was filled with penicillin G, another with DMSO, and the final hole with
the compound being tested. The plate was then incubated for 18-24h at 37°C. The
diameter of the zones of inhibition were then recorded in millimeters using a ruler.

5.3 Chapter 3 Experimental Section

(R,Z)-2-(2-(Aminothiazol-4-yl)-2-(methoxyimino)acetamido)propanoic acid (66):
To a CH₂Cl₂ (2mL) solution 72 (1.50 g, 4.57 mmol) at 0°C was added HSi(Et)₃ (1.82 mL,
11.4 mmol) followed by the addition of TFA (4 mL, 52 mmol). After 15 min. the ice
bath was removed. The colorless solution was stirred for 2.5 h and then freeze dried with
benzene. The white waxy solid was taken up in EtOAc (30mL) and diluted with 1M
NaOH (30mL). The layers were separated and the aqueous was washed with EtOAc
(3x20). The aqueous was then made acidic with 1M HCl (pH paper) and extracted with
EtOAc (3x20), dried (MgSO$_4$), filtered, and concentrated to give none of the desired compound. The original organic was dried, filtered, and concentrated, and then put under vacuum desiccation over night to give the desired product in 45 % yield (0.556 g, 2 mmol). $^1$H NMR (DMSO-d$_6$, 500MHz): 1.32 (d, 3H, J=7.42Hz), 3.87 (s, 3H), 4.28 (apparent quentent, 1H, J= 7.42 Hz), 7.02 (s, 1H), 7.89 (bs, 1H), 9.00 (d, H, J= Hz); $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO): 16.7, 33.3, 47.6, 62.4, 110.8, 147.4, 161.1, 169.2, 173.6; HRMS (FAB): calcd: 273.0658, found: 273.0652.

(Z)-1H-Benzo[d][1,2,3]triazol-1-yl2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetate (71): To a colorless solution of DCC (5.12 g, 24.9 mmol) in THF (80 mL) was added HOBt (3.35 g, 24.8 mmol). After 15 min. ATMO (5.0 g, 24.9mmol) was added in one portion to the colorless solution. A white solid began to precipitated immediately followed by the mixture turning a slight yellow color. The suspension was allowed to stir at room temperature for 4.45 h under a nitrogen atmosphere. The mixture was then cooled to 0°C and the solid was filtered out. The filtrate was diluted with cold hexanes to precipitate a solid which was filtered, washed (hexanes), and dried to give the desired product in 76 % yield (6.02 g, 18.97 mmol) as a light yellow solid. Product decomposed at 128°C when a melting point was attempted. Product is uv active and stains with PMA. Rf (EtOAc): 0.67; $^1$H NMR (DMSO-d6): 4.12(s, 3H), 7.28 (s, 1H), 7.5-7.6 (m, 2H), 7.72-7.80 (m,1H), 8.17 (d, 1H, J=9.42); FTIR (KBr): 3394, 3263, 3115, 2934, 1812, 1624, 1541, 1101, 1087, 936, 746.
(R, Z)-tert-Butyl 2-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)propanoate (72): To a solution of D-alanine-tert-butyl ester hydrochloride (1.5 g, 8.25 mmol) in freshly distilled DMF (50 mL) was added TEA (1.15 mL, 8.25 mmol) followed by the addition of ATMO-ester 71 (2.63 g, 8.82 mmol). The resulting yellow solution was stirred for 15.5 h and then diluted with EtOAc (30 mL). The solution was washed with brine (6x15), dried (MgSO₄), filtered, and concentrated to give 3.86 g of a crude yellow oil. The crude product was put on under high vacuum rotovap for 1h and then was diluted with EtOAc and placed in a freezer overnight. A white crystalline solid was then obtained which could be recrystallized to give the desired pure product in 86% yield (2.34 g, 7.12 mmol) as a white solid. $^1$H NMR (500 MHz, CDCl₃): δ 1.44 (m, 12H), 3.93 (s, 3H), 4.64 (q, 1H, J= 7.42), 5.85 (s, 2H), 6.75 (s, 1H), 7.80 (bd, 1H, J= 7.91); $^{13}$C NMR (125 MHz, CDCl₃): δ 18.1, 28.0, 48.7, 62.7, 82.4, 111.6, 142.7, 148.1, 162.2, 168.4, 172.8; FTIR (KBr): 3415, 3326, 3006, 2939, 1726, 1661, 1534, 1151, 1051, 756 cm⁻¹; HRMS (FAB) calcd: 329.1284, found: 329.1279.

(±)-3,5-Diallyl 2-tert-butyl isoxazolidine-2,3,5-tricarboxylate (81): To a biphasic mixture of boc cycloadduct (2.5 g, 12.7 mmol) in CCl₄ (25 mL), CH₃CN (25 mL), and H₂O (40 mL) was added NaIO₄ (11.33 g, 51.9 mmol). The mixture was cooled
to 0°C followed by the addition of RuCl₃·H₂O (0.058 g, 0.278 mmol). The mixture initially turned a brown color and stayed that way through out the course of the reaction. A small amount of white precipitate formed though out the course of the reaction. The reaction was brought to room temperature after 30 min. and after 2 h TLC determined that the reaction was completed Et₂O (20 mL) was added which turned the mixture a dark black color. The solid was filtered out then the liquid was passed through celite and then the layers were then separated. The aqueous was extracted with ether (3x10) then the combined organics were dried (Na₂SO₄), filtered, and concentrated to give 3.32 g of a crude colorless oil. The crude oil was then diluted with ether and then filtered through celite, dried, filtered and concentrated.

The crude diacid was diluted with DMF (50 mL) and allyl bromide (2.74 mL, 31.7 mmol) and Cs₂CO₃ (8.26 g, 25.3 mmol) were added. The solution was allowed to stir for 15 h and then diluted with H₂O and EtOAc. The layers were separated and the aqueous was extracted with EtOAc (3x25) and then the combined organic layers were washed with water (3x50), brine (3x25), dried (MgSO₄) and then concentrated to give the desired crude compound which was purified by flash chromatography (1:4, EtOAc:hexanes) in 46.7% yield (2.03 g, 5.94 mmol). ¹H NMR (CDCl₃, 500 MHz): 1.51 (s, 9 H), 2.80-2.89 (m, 2H), 4.58-4.67 (m, 4 H), 4.79-4.83 (m, 2H), 5.26-5.29 (m, 2H), 5.34-5.39 (m, 2H), 5.85-5.97 (m, 2H); ¹³C(CDCl₃, 125 MHz): 28.29, 35.91, 60.18, 66.51, 83.53, 119.20, 131.52, 156.10, 168.28, 169.50. FTIR (neat) : 2981, 1744, 1649. HRMS (FAB): calcd: 342.1553, found: 342.1552.
***tert-Butyl 2-(benzyloxyamino)-2-oxoethylcarbamate (84):*** To a solution of Boc glycine (1.0 g, 5.25 mmol) and OBHA (0.92 g, 5.78 mmol) in THF/H2O (10/50 mL) and the pH was adjusted to 4.5 using 1M NaOH and 1M HCl. To the resulting mixture was added EDC (2.02 g, 10.5 mmol) was added in small portions over a period of one hour. As time progressed a white solid formed. The resulting white suspension was extracted with EtOAc (3x50) and the combined organics were washed sat NaHCO3(3x25), brine(25), dried(MgSO4), filtered and concentrated to give the desired crude product which was purified by flash chromatography (SiO, EtOAc hexanes,1:1,) in 95% yield (1.39g, 4.99 mmol) Mp = 141-142 °C; \(^1\)H NMR (500MHz, (CD\(_3\))\(_2\)SO): 1.40 (s, 9H), 3.45 (bs, 2H), 4.79 (s, 2H), 6.98 (bs, 1H), 7.36-7.42(m, 5H), 11.1(bs, 1H); \(^13\)C NMR (125MHz, (CD\(_3\))\(_2\)SO): 28.2, 41.1, 77.0, 78.1, 128.2, 128.3, 128.8, 136.0, 155.8, 166.8; FTIR(CHCl\(_3\)): 3233, 2979, 1682, 1169, 1028, 753; HRMS(FAB+): calcd: 281.1501, found: 281.1506; Mass Spec (FAB+): 281, 277, 225, 221, 181, 176, 120.

![Chemical Structure](image)

***tert-Butyl 2-(hydroxyamino)-2-oxoethylcarbamate (85):*** An alkaline methanolic solution of hydroxylamine was formed by adding a 0\(^\circ\)C methanolic (125 mL) solution of KOH (37.3 g, 665 mmol) [formed by heating over a steam bath] to a 0\(^\circ\)C methanolic (255 mL) solution of hydroxylamine hydrochloride (39.6 g, 570 mmol) [formed by heating over a steam bath]. Immediately upon addition, a white solid was formed and was removed by suction filtration and the resulting colorless solution was added to a 0\(^\circ\)C methanolic (50 mL) solution of glycine N-Boc methyl ester (18.04g, 95 mmol). The reaction was allowed to come to room temperature after 12h. The colorless solution was
then concentrated by rotary evaporation to a white solid which was triturated with EtOAc (5x30) to give the title compound as a white solid in 99% yield (17.9g, 94 mmol). Mp = 119-120 °C; $^1$H NMR (500MHz, (CD$_3$)$_2$SO): 1.37 (s, 9H), 3.42 (d, H, J=5.98), 3.51(d, H, J=5.98), (m, 2H); $^{13}$C NMR (125MHz, (CD$_3$)$_2$SO): 28.1, 40.9, 77.8, 155.6, 166.3; FTIR(KBr): 3322, 2979, 2933, 1675, 1552, 1289, 1174, 1057, 972, 864; HRMS(FAB+): calcd: 191.1033, found: 191.1033.

**tert-Butyl 2-(2-oxa-3-azabicyclo[2.2.1]hept-5-en-3-yl)-2-oxoethylcarbamate (86):** A methanolic solution (80 mL) of boc glycine hydroxamic acid (1.0 g, 5.26 mmol) was cooled to 0°C and then freshly cracked cyclopentadiene (2.16 mL, 26.2 mmol) was added followed by the addition of aqueous(20 mL) NaIO$_4$ (1.18 g, 5.52 mmol) [formed by heating over a steam bath] dropwise over a period of 30 min. The reaction was stirred for an additional 2h at 0°C and then brought to room temperature and stirred for an additional 30 min. The methanol was then removed by rotary evaporation making sure not to let the bath temperature get to 40°C. The remaining aqueous layer was then extracted (EtOAc, 3x75 mL), washed (brine), dried (MgSO$_4$), filtered, and then concentrated to give the crude product (3.21g) as a brown oil which was chromatographed (SiO$_2$, 20% EtOAc/hexanes) to give a yellow oil that was induced to crystallize by cooling in hexanes to give 68% yield (0.90g, 3.57 mmol). Mp = 98-100 °C; $^1$H NMR (500MHz, (CDCl$_3$): 1.44 (s, 9H), 1.86 (d, 1H, J=8.58), 1.99 (bs, 1H), 3.67 (bs, 1H), 3.95-4.00 (m, 1H), 5.22-5.32(m, 2H), 6.37 (bs, 1H), 6.55 (bs, 1H); $^{13}$C NMR (125MHz, (CDCl$_3$): 28.4,
43.9, 48.4, 62.8, 79.6, 84.6, 132.8, 136.2, 155.7, 173.7; FTIR(KBr): 3423, 2982, 1707, 1673, 1500; HRMS(FAB+): calcd: 255.1345, found: 255.1386.

(+/−)-2-[N-Cbzglycinyl]aza-1-oxabicyclo[2.2.1]hept4-ene (92): An alkaline methanolic solution of hydroxylamine was formed by adding a 0°C methanolic (50 mL) solution of KOH (5.02 g, 89.6 mmol) [formed by heating over a steam bath] to a 0°C methanolic (50 mL) solution of hydroxylamine hydrochloride (4.67 g, 67.2 mmol) [formed by heating over a steam bath]. Immediately upon addition, a white solid was formed and was removed by suction filtration and the resulting colorless solution was added to a 0°C methanolic (50 mL) solution of glycine N-Z methyl ester (5g, 22.4 mmol). The reaction was allowed to come to room temperature and was stirred for an additional 12h. The colorless solution was then concentrated by rotary evaporation to a white solid which was extracted with boiling EtOAc until the extract was no longer ferric chloride positive to give the crude hydroxamic in 95% yield (4.8g, 21.4 mmol) and was used without further purification.

A methanolic solution (300 mL) of CBZ-glycinylhydroxamic acid (4.8 g, 21.4 mmol) was cooled to 0°C and then freshly cracked cyclopentadiene (8.8 mL, 107 mmol) was added followed by the addition of a room temperature aqueous(75 mL) NaIO₄ (6.86 g, 32.1 mmol) [formed by heating over a steam bath] dropwise over a period of 30 min. The reaction was stirred for an additional 2h at 0°C and then brought to room temperature and stirred for an additional 30 min. The methanol was then removed by rotary evaporation making sure not to let the bath temperature get to 40°C. The remaining
aqueous layer was then extracted (EtOAc, 3x75 mL) and the combined organics were washed (brine), dried (MgSO₄), filtered, and then concentrated to give the crude product as a brown oil which was chromatographed (SiO₂, 50% EtOAc/hexanes) to give a yellow oil in 72% yield (4.42 g, 15.33 mmol). ¹H NMR (500MHz, (CDCl₃): 1.85 (bd, 1H, J=8.58), 1.98 (bs, 1H), 3.74-3.77 (bm, 1H), (dd, 1H, J=4.79, J= 13.76), 5.10 (s, 2H), 5.30 (bs, 2H), 5.44(bs, 1H), 6.35 (bs, 1H), 6.53 (bs, 1H), 7.28-7.35 (m, 5H); ¹³C NMR (125MHz, CDCl₃): 44.4, 48.6, 63.0, 67.0, 84.8, 128.2, 128.3, 128.7, 133.0, 136.6, 156.3, 173.1; FTIR (CHCl₃): 3339, 3032, 2959, 1720, 1522, 1239, 1051, 845, 737; HRMS(FAB+): calcd: 289.1188, found: 289.1174.

(R)-tert-Butyl-2-((R)-2-(benzyloxy carbonylamino)-2-phenylacetamido)propanoate (95): To a solution of D-Z-phenylglycine (1.0 g, 3.5 mmol) in CH₃CN (15 mL) was added HOBr (0.52g, 3.85 mmol) and D-ala-t-butylester (0.70 g, 3.85 mmol) at 0°C. EDC (0.738 g, 3.85 mmol) was then added and the resulting yellow solution was allowed to stir for 15 h. The resulting light yellow solution was diluted with EtOAc (20mL) and washed with sat. NaHCO₃ (3x20), brine(2x20), dried (MgSO₄), filtered and concentrated to give 2.29 g of a crude white solid which was recrystallized using hexanes and EtOAc to give the desired product in 71 % yield (1.02g, 2.48 mmol). ¹H NMR (500 MHz, (CD₃OD): 1.29 (d, 3H, J= 7.18) 1.43 (s, 9H), 4.20-4.25 (m, 1H), 5.10 (q, 2H, J= 12.37), 5.30 (s, 1H), 7.26-7.36 (m, 8H), 7.43-7.44 (m, 2H); ¹³C NMR (125MHz, CD₃OD): 17.4,
(R)-2-((R)-2-(Benzyloxycarbonylamino)-2-phenylacetamido)propanoic acid (96): To a 0°C CH$_2$Cl$_2$ (20 mL) solution of 95 (0.40 g, 0.96 mmol) was added HSi(Et)$_3$ (0.46 mL, 2.9 mmol) followed by the addition of TFA (2 mL, mmol). The yellow solution was stirred for 5h and then freeze dried with benzene. A white solid formed which was confirmed to be the desired crude product was purified by flash chromatography (1:1, CH$_2$Cl$_2$:EtOAc) to give the desired product in 92% yield (0.31 g, 0.88 mmol). $^1$H NMR (500 MHz, CD$_3$OD): 1.32 (d, 3H, $J = 7.18$), 4.38 (m, 1H), 5.10 (abq, 2H, $J = 12.76$, $J = 6.78$) 5.31 (s, 1H), 7.27-7.36 (m, 8H), 7.43-7.44 (m, 2H); $^{13}$C NMR (125 MHz, CD$_3$OD): 17.6, 60.3, 67.9, 128.7, 129.0, 129.1, 129.4, 129.6, 129.8, 138.2, 139.4, 158.0, 172.6, 175.8; HRMS (FAB+): calcd: 357.1450, found: 357.1449.

(R)-Benzyl-2-((R)-2-(tert-butoxycarbonylamino)-2-phenylacetamido)propanoate (101): To a solution of D-Boc-phenyl glycine (5.0 g, 19.9 mmol) in CH$_3$CN/CH$_2$Cl$_2$ (50mL/50mL) was added D-alanine benzylester PTSA salt (7.38 g, 21.9 mmol), HOBt (2.96 g, 21.9 mmol), and TEA (3.05 mL, 21.9 mmol). After the resulting yellow solution was brought to 0°C EDC HCl (4.19g, 21.9 mmol) was added and stirred for 1h at 0°C and
then allowed to come to room temperature and stirred for an additional 12 h. The resulting solution was diluted with EtOAc (100 mL) and washed with saturated NaHCO$_3$ (3x 50 mL), dried (MgSO$_4$), filtered, and concentrated (rotary evaporation). The resulting crude yellow solid was purified by silica gel chromatography (1:3, EtOAc: CH$_2$Cl$_2$) to give the desired compound in 77% yield (6.31 g, 15.31 mmol). Mp= 120-121°C; $\alpha^{D}_{20}$ = -32.22° (c=0.032); $^1$H NMR (500 MHz, (CD$_3$)$_2$SO): 1.30 (d, 3H, J= 7.18), 1.38 (s, 9H), 4.36 (ap, 2H, J= 7.18), 5.04 (s, 2H), 5.24 (d, 1H, J= 8.97), 7.25-7.40 (m, 10H), 8.65 (d, 1H, J= 6.98); $^{13}$C NMR (500 MHz, (CD$_3$)$_2$SO): 16.9, 28.2, 47.8, 57.2, 65.9, 78.4, 127.2, 127.4, 127.7, 128.0, 128.1, 128.4, 135.9, 138.6, 146.2, 154.9, 169.8, 172.1; FTIR(KBr): 3309, 3065, 2981, 1740, 1657, 1530, 1251, 1170, 733, 696; HRMS (FAB+): calcd: 415.2076, found: 415.2085.

(R)-2-((R)-2-(tert-Butoxycarbonylamino)-2-phenylacetamido)propanoic acid (102): A solution of 101 (6.0 g, 14.5 mmol) in a solution of CH$_2$Cl$_2$/CH$_3$OH (40 mL/60 mL) was purged several times with Ar followed by the addition of Pd on carbon (0.1 g). The mixture was purged with Ar again and a balloon of hydrogen was added. The reaction was allowed to progress for 24 h and was filtered to give a 98% yield (4.58 g, 14.21 mmol) of the desired product. $\alpha^{D}_{20}$ = -53.5° (c=0.0131, CH$_3$OH); $^1$H NMR (500 MHz, (CD$_3$)$_2$SO): 1.28 (d, 3H, J=7.37), 1.39 (s, 9H), 4.24 (ap, 1H, J= 7.18), 5.25 (d, 1H, J=8.97), 7.24-7.33 (m, 3H), 7.40-7.42 (bd, 2H, J= 7.58), 8.47 (bd, 1H, J=7.18); $^{13}$C NMR (500 MHz, (CD$_3$)$_2$SO): 17.4, 28.2, 47.7, 57.4, 78.5, 127.3, 127.5, 128.2, 138.9, 154.9,
169.7, 173.8; FTIR(KBr): 3320, 3034, 2981, 1721, 1661, 1523, 1164, 756, 699; HRMS (FAB+): calcd: 323.1607, found: 323.1610.

![Structure of 103](image)

**tert-butyl-(R)-2-((R)-1-(Benzyloxyamino)-1-oxopropan-2-ylamino)-2-oxo-1-phenylethylcarbamate (103)**: A solution of 102 (4.25 g, 13.2 mmol) and OBHA (2.53 g, 15.8 mmol) in THF/H$_2$O (25/70 mL) was adjusted to pH= 4.5 using 1M HCl followed by the addition of solid EDC HCl (10.12 g, 52.7 mmol) in small portions over a period of one hour. As time progressed a white solid formed and the pH increased and was brought back to 4.5 using 1M HCl. The resulting white suspension was extracted with EtOAc (3x50) and the combined organics were washed with saturated NaHCO$_3$ (3x25), brine (25), dried (MgSO$_4$), filtered and concentrated to give the desired crude product which was purified by flash chromatography (silica gel, CH$_2$Cl$_2$: EtOAc, 3:2,) in 91 % yield (5.14 g, 12.0 mmol). Mp: 197-200°C; $\alpha$$_{D}^{20}$ = -51.21° ($c= 0.0074$, 9:1, EtOAc:DMSO); $^1$H NMR (500MHz, (CD$_3$)$_2$SO): 1.18 (d, 3H, J=6.98), 1.39 (s, 9H), 4.16 (ap, 1H, J=7.18), 4.72 (q, 2H, J=10.97), 5.24 (d, 1H, J=8.77), 7.24-7.41 (m, 10H), 8.40(bd, 1H, J=7.57), 11.24(bs, 1H); $^{13}$C NMR (500M Hz, (CD$_3$)$_2$SO): 18.3, 28.2, 46.0, 57.5, 76.7, 78.5, 127.2, 127.4, 128.2, 128.3, 128.9, 135.8, 138.8, 154.9, 168.7, 169.5; FTIR(KBr): 3256, 2981, 1706, 1644, 1523, 1169, 749; HRMS (FAB+): calcd: 428.2185, found: 428.2176.
**tert-Butyl-(R)-2-((R)-1-(hydroxyamino)-1-oxopropan-2-ylamino)-2-oxo-1-phenylethylcarbamate (104):** A solution of 103 (3.25 g, 7.60 mmol) in a solution of CH$_2$Cl$_2$ /CH$_3$OH (40 mL/ 60 mL) was purged several times with Ar followed by the addition of 10% Pd on carbon (0.325g). The mixture was purged with Ar again and a balloon of hydrogen was added. The reaction was allowed to progress for 24 h and was filtered to give a 98% yield (2.51 g, 7.45 mmol) of the desired product. Mp: 160-163 °C; $\alpha^D_{20} = -63.2$ ° (c= 0.00958, DMSO); $^1$H NMR (500MHz, (CD$_3$)$_2$SO): 1.20 (t, 3H, J=6.98), 1.38(s, 9H), 4.22 (sep, 1H, J=6.98), 5.20 (dd, 1H, J=8.77, J=8.17), 7.01 (bs, 1H), 7.24-7.41 (m, 5H), 8.23 (bs, 1H, J=7.37), 8.34(bd, 1H, J=7.78), 8.87 (s, 1H), 10.61 (s, 1H); $^{13}$C NMR (500MHz, (CD$_3$)$_2$SO): ; FTIR (KBr): 3274, 2981, 1644, 1524, 1168, 699; HRMS (FAB+): calcd: 338.1716, found: 338.1694.

**tert-Butyl-(1R)-2-((2R)-1-(2-oxa-3-azabicyclo[2.2.1]hept-5-en-3-yl)-1-oxopropan-2-ylamino)-2-oxo-1-phenylethylcarbamate (105):** A methanolic solution (200 mL) of hydroxamic acid 104 (2.93 g, 8.6 mmol) was cooled to 0°C and then freshly cracked cyclopentadiene (2.86 g, 43.4 mmol) was added followed by the addition of a room temperature aqueous (50 mL) NaIO$_4$ (2.04 g, 9.5 mmol) [formed by heating over a steam bath] dropwise over a period of 30 min. The reaction was stirred for an additional 2h at
0°C and then brought to room temperature and stirred for an additional 3h. The methanol was then removed by rotary evaporation making sure not to let the bath temperature get to 40°C. The remaining aqueous layer was extracted (EtOAc, 3x75 mL) and the combined organics were washed (brine), dried (MgSO₄), filtered, and concentrated to give the crude product as a brown oil which was chromatographed (SiO₂, 50% EtOAc/hexanes) to give a yellow oil in 65% yield (2.24 g, 5.59 mmol). \( \alpha^D_{20} = -56.3^\circ \) (c=0.00917, CHCl₃); \(^1\)H NMR (500MHz, CDCl₃): 1.13 (bs, 3H), 1.42 (bs, 9H), 1.83-1.84 (bm, 1H), 1.94-1.96 (bm, 1H), 4.52 (bs, 1H), 4.69 (bs, 1H), 5.22 (bs, 1H), 5.29-5.34 (m, 1H), 5.84 (bs, 1H), 6.09-6.16 (m, 1H), 6.29 (bs, 1H), 6.37 (bs, 1H), 6.46-6.53 (m, 1H), 6.84 (bs, 1H), 7.28-7.37 (m, 5H); \(^1^3\)C NMR (500M Hz, CDCl₃): 15.8, 18.6, 28.4, 47.7, 48.5, 58.6, 62.0, 62.4, 80.0, 84.9, 127.3, 128.3, 129.0, 133.0, 133.2, 136.3, 136.6, 138.6, 155.2, 169.4, 172.3, 174.8; FTIR(KBr): 3324,3065, 2978, 1715, 1663, 1497, 1168, 1049, 847, 801; HRMS (FAB+): calcd: 402.2029, found: 402.2026.

![Chemical Structure](image)

(±)-Dibenzyl-2-((R)-2-((R)-2-(tert-butoxycarbonylamino)-2-phenylacetamido)propanoyl)isoxazolidine-3,5-dicarboxylate (106): To a biphasic mixture of Diels-Alder adduct 105 (0.8g, 1.99 mmol) in CCl₄ (15 mL), CH₃CN (15 mL), and H₂O (30 mL) was added NaIO₄ (2.13 g, 9.96 mmol). The mixture was cooled to 0°C followed by the addition of RuCl₃ H₂O (0.080 g, 0.39 mmol). The mixture initially turned a brown color and turned a black color and stayed that way though out the course of the reaction and a small amount of white precipitate formed. The reaction was brought to room
temperature after 30 min and, after 6 h, the reaction was diluted with EtOAc (50 mL). The solid was filtered through celite and the resulting mixture was acidified using citric acid and the layers were separated. The aqueous was extracted with EtOAc (3x10) then the combined organics were dried (MgSO₄), filtered, and concentrated to give a crude product as a yellow oil.

The crude diacid was diluted with THF (30 mL) and then treated with excess PhCHN₂ at 0°C for 1 h. The excess PhCHN₂ was destroyed with the addition of AcOH (5 mL) and the resulting light yellow solution was diluted with EtOAc and washed with saturated Na₂HCO₃ (3x 25 mL). The resulting combined organic layers were dried (MgSO₄), filtered, and concentrated (rotary evaporation) to give a yellow oil which was chromatographed on silica gel (1:1, EtOAc: hexanes) to give desired compound in 42% yield (g, mmol). α₂⁰D = -0.203° (c=0.0103 , EtOAc), ¹H NMR (500MHz, (CD₃)₂SO): 0.84-1.77 (m, 12H), 2.70-2.9 (m, 2H), 3.4-3.55 (m, 2H), 3.99-4.2 (m, 2H), 4.45-5.20 (4m, H), 5.62-5.86 (m, 1H), 6.40-6.76 (m, 1H), 7.25-7.35 (m, 15H); ¹³C NMR (500M Hz, CDCl₃ : 18.3, 25.5, 26.2, 28.4, 29.8, 35.0, 35.5, 46.9, 47.6, 56.6, 66.1, 67.8, 69.7, 73.1, 127.4, 127.8, 128.4, 128.6, 128.7, 128.8, 128.9, 129.1, 129.2, 155.2, 167.8, 168.2, 168.7, 169.8, 171.6; HRMS (FAB+): calcd: 646.2765, found: 646.2805.

(Z)-tert-Butyl 2-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)acetate (109):

To a solution of glycine-tert-butyl ester hydrochloride (0.7 g, 4.18 mmol) in freshly distilled CH₂Cl₂ (15 mL) was added TEA (0.58 mL, 4.18 mmol) followed by the addition of ATMO-ester 71 (1.4 g, 4.39 mmol). The resulting yellow solution was stirred
for 15.5 h and then diluted with EtOAc (30 mL). The solution was washed with brine (3x15), dried (MgSO₄), filtered, and concentrated to give a crude yellow solid. A white crystalline solid was then obtained after flash chromatography (1:1, CH₂Cl₂, EtOAc) to give the desired pure product in 68% yield (0.89 g, 2.84 mmol) as a white solid. H NMR (500 MHz, CDCl₃): δ 1.48 (s, 9H), 4.04 (s, 3H), 4.10 (d, 2H, J=5.39) 6.98 (bs, 1H), 7.05 (s, 1H); 13C NMR (125 MHz, CDCl₃): δ 28.3, 42.1, 63.6, 82.9, 112.7, 142.2, 147.3, 162.0, 168.1, 168.8; HRMS (FAB) calcd: 315.1127, found: 315.1135.

5.4 Chapter 4 Experimental Section

N-(Benzyloxy)-3-hydroxybutanamide (121): A solution of 3-hydroxybutyric acid sodium salt (1.65g, 13.1 mmol) and OBHA (2.29g, 14.4 mmol) in THF/H₂O (15/50 mL) was adjusted to pH= 4.5 using 1M HCl followed by the addition of solid EDC HCl (7.53g, 39.3 mmol) in small portions over a period of one hour. As time progressed a white solid formed and the pH increased and was brought back to 4.5 using 1M HCl. The resulting white suspension was extracted with EtOAc (3x50) and the combined organics were washed with saturated NaHCO₃ (3x25), brine (25), dried (MgSO₄), filtered and concentrated to give the desired crude product which was purified by flash chromatography (silica gel, CH₂Cl₂: EtOAc, 3:2,) in 74 % yield ( 2.03g, 9.7 mmol). Mp: 91-93 °C; α D 20 = -36.7° (c=0.006 , DMSO); H NMR (500 MHz, (CDCl₃): 1.19 (d, 3H, J= 6.18), 2.13-2.25 (bm, H),  2.35 (bs, H), 2.50-2.53 (bm, H), 3.44 (bs, 1H), 4.13(bs, 1H), 4.81(bs, H), 4.89 (bs, H), 7.36-7.39 (bm, 5H), 8.68 (bs, H), 9.28 (bs, H); 13C NMR (125
MHz, CDCl$_3$): 23.1, 41.7, 64.8, 78.4, 128.7, 128.9, 129.3, 135.3, 170.1; FTIR (KBr): 3165, 3007, 2921, 1665, 1131, 1068, 850; HRMS (FAB+): calcd: 210.1130, found: 210.1119.

**tert-Butyl 3-(hydroxymethyl)isoxazolidine-2-carboxylate (142):** A solution of 141 (0.9g, 3.43mmol) in 4M HCl (10 mL) was heated to reflux for 4h. The resulting solution was brought to room temperature and the solvent removed by freeze drying. The resulting solid was diluted with water and the solid filtered off. The filtrate was evaporated and the solid was stirred in ether which precipitated the desired product which was used without further purification.

To a THF (15mL) solution of the crude product (0.354g, 3.43 mmol) was added Na$_2$CO$_3$ (1.09g, 10.3mmol), water (15mL), and (Boc)$_2$O (1.57g, 7.2mmol). The resulting mixture was stirred for 10h and then brought to pH=2 using citric acid. The solution was diluted with EtOAc (20mL) and the aqueous extracted (3x10). The combined organics were dried (MgSO$_4$), filtered, and concentrated to give the crude product which was chromatographed (silica, 80/20, hexanes: EtOAc) to give the desired product in 50% yield (0.49g, 1.71mmol). $^1$H NMR (500 MHz, CDCl$_3$): 1.51 (d, 9H, J=4.2), 2.05-2.11 (m, 1H), 2.38-2.45 (m, 1H), 3.76 (q, 1H, J= 7.91), 4.01-4.17 (m, 3H), 4.42-4.47 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$): 28.0, 28.4, 30.2, 57.8, 67.9, 69.0, 82.1, 82.2, 153.8, 157.1; HRMS (FAB+): calcd: 204.1236, found: 204.1230.
CHAPTER 6:
SELECTED NMR SPECTRA

The following section contains $^1$H and $^{13}$C NMR spectra for selected compounds. Each spectrum is labeled with the appropriate chemical structure, the solvent used to obtain the spectra, and the corresponding number that was used to identify the compound throughout the dissertation.
Figure 6.1 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 57a
Figure 6.2 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 57a
Figure 6.3 1H NMR (500 MHz, CDCl$_3$) 57b
Figure 6.4 $^{13}$C NMR (125 MHz, CDCl$_3$) 57b
Figure 6.5 1H NMR (500 MHz, CDCl$_3$) 57c
Figure 6.6 $^{13}$C NMR (125 MHz, CDCl$_3$) 57c
Figure 6.7 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 58a
Figure 6.8 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 58a
Figure 6.9 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 58b
Figure 6.10 $^{13}\text{C}$ NMR (125 MHz, (CD$_3$)$_2$SO) 58b
Figure 6.11 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 58c
Figure 6.12 $^{13}$C NMR (125 MHz, CDCl$_3$) $^{58}c$
Figure 6.13 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 59a
Figure 6.14 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 59a
Figure 6.15 $^1$H NMR (500 MHz, CD$_3$OD) 59b
Figure 6.16 $^{13}$C NMR (125 MHz, CD$_3$OD) $^{59b}$
Figure 6.17 $^1$H NMR (500 MHz, CD$_3$OD) 59c
Figure 6.18 $^{13}$C NMR (125 MHz, CD$_3$OD) 59c
Figure 6.19 $^1$H NMR (500 MHz, CDCl$_3$) 60a
Figure 6.20 $^{13}$C NMR (125 MHz, CDCl$_3$) 60a
Figure 6.21 $^1$H NMR (500 MHz, CDCl$_3$) 60b
Figure 6.22 $^{13}$C NMR (125 MHz, CDCl$_3$) 60b
Figure 6.23 $^1$H NMR (500 MHz, CDCl$_3$) 60c
Figure 6.24 $^{13}$C NMR (125 MHz, CDCl$_3$) 60c
Figure 6.25 $^1$H NMR (500 MHz, CDCl$_3$) 61a
Figure 6.26 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 61a
Figure 6.27 $^1$H NMR (500 MHz, CDCl$_3$) 61b
Figure 6.28 $^{13}$C NMR (125 MHz, CDCl$_3$) \textit{61b}
Figure 6.29 $^1$H NMR (500 MHz, CDCl$_3$) 61c
Figure 6.30 $^{13}$C NMR (125 MHz, CDCl$_3$) 61c
Figure 6.31 $^1$H NMR (500 MHz, CDCl$_3$) 62a
Figure 6.32 $^{13}\text{C}$ NMR (125 MHz, CDCl$_3$) 62a
Figure 6.33 $^1$H NMR (500 MHz, CDCl$_3$) 62b
Figure 6.34 $^{13}$C NMR (125 MHz, CDCl$_3$) 62b
Figure 6.35 $^1$H NMR (500 MHz, CDCl$_3$) 62c
Figure 6.36 $^{13}$C NMR (125 MHz, CDCl$_3$) 62c
Figure 6.37 $^1$H NMR (500 MHz, CDOD) 63a
Figure 6.38 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 63a
Figure 6.39 $^1$H NMR (500 MHz, CD3OD) 63b
Figure 6.40 $^{13}$C NMR (125 MHz, CD$_3$OD) 63b
Figure 6.41 $^1$H NMR (500 MHz, CD$_3$OD) 63c
Figure 6.42 $^{13}$C NMR (125 MHz, CD$_3$OD) 63c
Figure 6.44 1H NMR (500 MHz, CDCl$_3$) 66
Figure 6.45 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 66
Figure 6.46 $^1$H NMR (125 MHz, CDCl$_3$) 72
Figure 6.47 $^{13}$C NMR (125 MHz, CDCl$_3$) 72
Figure 6.48 $^1$H NMR (500 MHz, CDCl$_3$) 81
Figure 6.49 $^{13}$C NMR (125 MHz, CDCl$_3$) 81
Figure 6.50 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 84
Figure 6.51 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 84
Figure 6.52 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 85
Figure 6.53 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 85
Figure 6.54 $^1$H NMR (500 MHz, CDCl$_3$) 86
Figure 6.55 $^{13}$C NMR (125 MHz, CDCl$_3$) 86
Figure 6.56 $^1$H NMR (500 MHz, CDCl$_3$) 92
Figure 6.57 $^{13}$C NMR (125 MHz, CDCl$_3$) 92
Figure 6.58 $^1$H NMR (500 MHz, CD$_3$OD) 95
Figure 6.59 $^{13}$C NMR (125 MHz, CD$_3$OD) 95
Figure 6.60 $^1$H NMR (500 MHz, CD$_3$OD) 96
Figure 6.61 $^{13}$C NMR (125 MHz, CD$_3$OD) 96
Figure 6.62 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 101
Figure 6.63 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) **101**
Figure 6.64 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 102
Figure 6.65 $^{13}\text{C}$ NMR (125 MHz, (CD$_3$)$_2$SO) 102
Figure 6.66 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) **103**
Figure 6.67 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) **103**
Figure 6.68 $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 104
Figure 6.69 $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 104
Figure 6.70 $^1$H NMR (500 MHz, CDCl$_3$) **105**
Figure 6.71 $^{13}$C NMR (125 MHz, CDCl$_3$) 105
Figure 6.72 $^1$H NMR (500 MHz, CDCl$_3$) **106**
Figure 6.73 $^{13}$C NMR (125 MHz, CDCl$_3$) 106
Figure 6.74 $^1$H NMR (500 MHz, CDCl$_3$) 109
Figure 6.75 $^{13}$C NMR (125 MHz, CDCl$_3$) 109
Figure 6.76 $^1$H NMR (500 MHz, CDCl$_3$) 102
Figure 6.77 $^{13}$C NMR (125 MHz, CDCl$_3$) 121
Figure 6.78 $^1$H NMR (500 MHz, CDCl$_3$) 142
Figure 6.79 $^{13}$C NMR (125 MHz, CDCl$_3$) 142
REFERENCES


Acids (Oxamazins) and the Corresponding Sulfur Analouges (Thiamazins)” *J. Med. Chem.* 1987, 30, 528-536.


102. Shireman, B. T.; Miller, M. J.; Jonas, M.; Wiest, O. “Conformational Study and Steroselective, Regiospecific Syntheses of Novel Aminoxy trans-Proline


