Design, Synthesis, and Evaluation of C5 Substituted Carbapenem Antibiotics To Counter Resistant Pathogens

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DESIGN, SYNTHESIS, AND EVALUATION OF C5 SUBSTITUTED CARBAPENEM ANTIBIOTICS TO COUNTER RESISTANT PATHOGENS

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DESIGN, SYNTHESIS, AND EVALUATION OF C5 SUBSTITUTED CARBAPENEM ANTIBIOTICS TO COUNTER RESISTANT PATHOGENS

A Thesis Presented to the Graduate Faculty of Dedman College Southern Methodist University in Partial Fulfillment of the Requirements for the degree of Master of Chemistry with a Major in Chemistry by Noora Mohammed S.A Al-Kharji B.S., Chemistry, Southern Methodist University December 15, 2018
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The 21st century has seen a dramatic acceleration in the evolution of bacterial resistance. Many classes of commercial antibiotics, including most current commercial β-lactams, were structurally optimized to counter 20th century pathogens. The carbapenems represent the most potent and broadest spectrum of the β-lactams, representing crucial last line agents to treat life-threatening infections. While the 21st century has seen the appearance of carbapenem-resistant strains, the basic substitution pattern of commercial carbapenem antibiotics has remained constant, other than minor modifications at the C2 position. This research will investigate whether substitution at an atypical carbapenem position, the C5 position, can improve the antibiotic’s potency against resistant pathogens, particularly including *Mycobacterium tuberculosis*, *Mycobacterium abscessus* and *Acinetobacter baumannii*. This project produced improved synthetic methodology to generate this atypically substituted class of carbapenem, helped define the effect of substituents at this position on the carbapenemase susceptibility and binding to transpeptidase targets, and demonstrated that selected C5-substituted carbapenems can be superior to current commercial carbapenem antibiotics against clinically important pathogen
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</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii</td>
<td><em>Acinetobacter baumannii</em></td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>AcO</td>
<td>acetate</td>
</tr>
<tr>
<td>AgOTf</td>
<td>silver trifluoromethanesulfonate</td>
</tr>
<tr>
<td>Aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BlaC</td>
<td>Ambler β-lactamase</td>
</tr>
<tr>
<td>n-BuLi</td>
<td>n-butyllithium</td>
</tr>
<tr>
<td>cat</td>
<td>catalyst</td>
</tr>
<tr>
<td>CDC</td>
<td>Disease Control and Prevention</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>CH$_3$CN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>CH$_3$ZnI</td>
<td>methyl zinc iodide</td>
</tr>
<tr>
<td>DHP-1</td>
<td>dehydropeptidase</td>
</tr>
<tr>
<td>DIPEA</td>
<td>diisopropyl ethylamine</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>Et$_2$O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>Et$_3$N</td>
<td>triethylamine</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>GlCNAc</td>
<td>N-acetylglucosamine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>GN</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>GP</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HCOOH</td>
<td>formic acid</td>
</tr>
<tr>
<td>HF</td>
<td>hydrofluoric acid</td>
</tr>
<tr>
<td>IM</td>
<td>inner membrane</td>
</tr>
<tr>
<td>KN₃</td>
<td>potassium azid</td>
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<tr>
<td>Ldt</td>
<td>L,D-transpeptidases</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>$M_{ab}$</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>MATE</td>
<td>multidrug and toxic compound extrusion</td>
</tr>
<tr>
<td>MCPBA</td>
<td><em>meta</em>-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MDR</td>
<td>multi drug-resistant</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MeLi</td>
<td>methyllithium</td>
</tr>
<tr>
<td>Me₂S</td>
<td>dimethyl sulfide</td>
</tr>
<tr>
<td>Me₂S-CuI</td>
<td>dimethyl sulfide copper iodide complex</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>magnesium sulfate</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>MFS</td>
<td>major facilitator superfamily</td>
</tr>
<tr>
<td>Min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>MRSA</td>
<td>methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>$M_{tb}$</td>
<td><em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td>MurNac</td>
<td>N-acetylMuramic acid</td>
</tr>
<tr>
<td>NAG</td>
<td>linked N-acetylgalactosamine</td>
</tr>
<tr>
<td>NaH</td>
<td>sodium hydride</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>sodium bicarbonate</td>
</tr>
<tr>
<td>NaH$_2$PO$_4$</td>
<td>monosodium phosphate</td>
</tr>
<tr>
<td>NaN$_3$</td>
<td>sodium azide</td>
</tr>
<tr>
<td>NaOAc</td>
<td>sodium acetate</td>
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<tr>
<td>NaSO$_4$</td>
<td>sodium sulfate</td>
</tr>
<tr>
<td>NGM</td>
<td>N-glycolylmuramic acid</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>ammonium chloride</td>
</tr>
<tr>
<td>NTM</td>
<td>nontuberculous mycobacterium</td>
</tr>
<tr>
<td>OM</td>
<td>outer membrane</td>
</tr>
<tr>
<td>OMPs</td>
<td>outer membrane protein</td>
</tr>
<tr>
<td>PAA</td>
<td>peracetic acid</td>
</tr>
<tr>
<td>PBP</td>
<td>penicillin-binding-protein</td>
</tr>
<tr>
<td>Pd/c</td>
<td>palladium on charcoal</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PNB</td>
<td>$p$-nitrobenzyl</td>
</tr>
<tr>
<td>PNB-OH</td>
<td>$p$-nitrobenzyl alcohol</td>
</tr>
<tr>
<td>PNZ</td>
<td>$p$-nitrobenzylloxy carbonyl</td>
</tr>
<tr>
<td>PPh$_3$</td>
<td>triphenylphosphine</td>
</tr>
<tr>
<td>Rh$_2$(OAc)$_4$</td>
<td>rhodium acetate</td>
</tr>
</tbody>
</table>
RND | resistance-nodulation-division
---|---
rt | room temperature
Ru(acac)$_3$ | ruthenium(III) acetylacetonate
SARs | structure-activity relationships
SCSRs | structure carbapenemase susceptibility relationships
SMR | small multidrug resistance
SPPRs | structure porin permeability relationships
STARs | structure target affinity relationships
TB | tuberculosis
TBS | *tert*-butyldimethylsilyl
TBS-OTf | *tert*-butyldimethylsilyl
THF | tetrahydrofuran
TiCl$_4$ | titanium (IV) tetrachloride
XDR | extensively drug-resistant
ZnBr$_2$ | zinc (II) bromide
ZnCl$_2$ | zinc (II) chloride
ZnI$_2$ | zinc (II) iodide
18-crown 6 | 1,4,7,10,13,16-hexaoxacyclooctadecane
Acknowledgement

I would first like to thank my advisor, Dr. John Buynak, for his patience, motivation, knowledge and his continuous support of my MA degree. I could not have imagined having a better advisor for my MA study. Dr. Buynak has always been encouraging and always available when I needed help. His guidance helped me in all the time of research and writing of this thesis. I would also like to thank Dr. Lattman, Dr. Isaac, Dr. Lippert and Dr. Zoltowski who gave access to their laboratories equipment and reagent. I would like to thank my committee members, Dr. Lattman, Dr. Tao, and Dr. Son. Dr. Son motivated me to pursue my B.S in and MA in chemistry, and I am very grateful for his encouragement and support throughout both my degrees.

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Overview

Not surprisingly, under constant antibiotic pressure, bacteria are developing unprecedented levels of antibiotic resistance. Carbapenem antibiotics represent the most potent of the β-lactam class. The carbapenems were structurally optimized in the 1970s and 1980s to treat pathogens of that era. Bacterial mechanisms of antibiotic resistance have evolved substantially in the past 40 to 50 years, and it is time to reevaluate crucial structure-activity relationships (SARs) to determine whether selective modifications can improve efficacy. Carbapenems are delicate molecules, generated by total synthesis. Current commercial carbapenem antibiotics are simple C2 derivatives of a common scaffold. Atypical modifications (i.e. non-C2) are particularly synthetically challenging. The objective of this research is to prepare atypically substituted carbapenem antibiotics and, in collaboration with numerous investigators, both industrial and academic, determine the potential for improvement in activity in treatment of selected 21st century pathogens, particularly including *Mycobacterium tuberculosis*, *Mycobacterium abscessus*, and Gram-negative pathogens including *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. In addition to inherent antimicrobial efficacy, our collaborators are capable of evaluating outer membrane porin penetration, penicillin-binding (target) affinity, and β-lactamase (carbapenemase) susceptibility, prospectively enabling us to develop structure porin permeability relationships (SPPRs), structure target affinity relationships (STARs), and structure carbapenemase susceptibility relationships (SCSRs) of the carbapenem scaffold.
Gram-negative bacteria, Gram-positive bacteria, and *Mycobacteria* have developed resistance to antibiotics through β-lactamases, efflux pumps, and penicillin-binding-protein (PBP) modifications. This antibiotic resistance is particularly troublesome in the case of Gram-negative bacteria due to the presence of an outer membrane and sequestered periplasmic space that provides them with additional defenses against incoming antibiotics. Gram-negative bacteria *Acinetobacter* spp and *Pseudomonas aeruginosa* cause hospital-acquired infections with mortality rates as high as 43% and 18-61%, respectively, in the United States. 22 Several strains of *Enterobacteriaceae*, a large family of Gram-negative bacteria that include some common pathogens, have been found to have multi-drug antibiotic resistance and pan-resistance, i.e. resistance to all known antibiotics. Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium*, have also been found to develop resistance to many antibiotics. *Mycobacterium tuberculosis* (*Mtbc*), the pathogen responsible for tuberculosis (TB), is another problematic pathogen due to its special L,D transpeptidase.15 TB affects one fourth of the world’s population and in 2016, there are 1.3 million TB-related deaths recorded in 2017. TB is one of the top 10 causes of death and a leading killer of individuals simultaneously infected with HIV. 6

Today’s most commonly prescribed antibiotics are the β-lactams, which target bacterial cell wall biosynthesis. Therefore, one way to address the problem of resistance is to structurally modify β-lactams to overcome the resistance mechanisms of bacteria. Carbapenems are a class of β-lactams that have the broadest spectrum of activity against Gram-negative, Gram-positive, and *Mycobacterium* species, thus are referred to as “last-line agents” or antibiotics of last resort.
Characterized by C1β-methyl group and a substituted sulfur at C2, past studies have extensively explored carbapenems with different side chains at C2. Based on examination of existing crystallographic studies of carbapenem antibiotics with their targets, the PBP transpeptidases, and with numerous β-lactamases, we decided to try an alternative approach and explore the effect of carbapenem antibiotics with a substituent other than hydrogen at the C5 position. Even small modifications in structure can improve properties, and it is noted that very early studies had shown that the addition of the methyl group to the C1β position made a significant difference in the carbapenem antibacterial-spectrum, and enhanced stability towards β-lactamase and human renal dehydropeptidase, DHP-1.33

In this study, atypical substitutions of carbapenems were explored to design better antibiotics for the 21st century evolved pathogens. This was done by looking at previously made carbapenems, understanding which functional groups add stability to the compound, and understanding which carbon positions make a difference to synthesize drugs with improved permeability, stability against β-lactamases, and stability to efflux pump mediated extrusion. Our research discovered an atypical modification of carbapenem that decreased minimum inhibitory concentration (MIC) of Mtb and Mab (combined with clavulanic acid) compared to meropenem. Our research is still exploring and synthesizing new carbapenems (discussed below).

The Bacterial Cell Envelope

Bacteria maintain and shape their cell envelopes to shelter them against stresses they encounter, allowing them to grow as needed and to reproduce. Depending on the cell envelope composition, most bacteria are classified as either Gram-negative or Gram-positive bacteria.
However, some cell envelopes are more complex and have the characteristics of both Gram-negative and Gram-positive cell walls, such as that of *Mycobacteria*. 1, 37

**Gram-Negative Bacteria**

The cell envelope of Gram-negative (GN) bacteria (Figure 1) is composed of the outer membrane (OM), the inner membrane (IM), the periplasmic space between these two membranes, and the peptidoglycan cell wall, located in the periplasm. The relatively thin GN cell wall is made out of thin mesh of peptidoglycan. The OM excludes toxins and provides stabilization to the organism. There are two types of OM proteins, which include lipoproteins (non-transmembrane) and β-barrel proteins (transmembrane). The β-barrel proteins (such as porins, OmpF, and OmpC) allow the diffusion of small molecules, limiting the hydrophilic molecules < 700 Da. The IM is a protective barrier (especially for hydrophobic molecules) composed of lipopolysaccharide (LPS), which is a glucosamine disaccharide with polysaccharide chain, approximately seven saturated acyl chains, and a polysaccharide core. The periplasm is established in an aqueous cellular compartment between OM and IM. Proteins are synthesized in the cytoplasm, and then are transferred to the IM, OM or periplasm. The shape of Gram-negative bacteria varies from rod shaped, comma shaped, perpendicular, etc. 37, 45
**Figure 1.** Diagram of Gram-negative and Gram-positive bacteria cell envelope. It shows 1) the outer membrane, including the porins. 2) periplasmic space, including the lipoprotein that peptidoglycan. 3) and the inner cell membrane for Gram-negative. Also, shows the lipoprotein that peptidoglycan and the inner cell membrane, with no outer membrane for Gram-positive.

**Gram-Positive Bacteria**

Unlike Gram-negative bacteria, Gram-positive bacteria (Figure 1) do not have an OM. The thick GP cell wall consists of layers of peptidoglycan with anionic polymers through the layers. The anionic polymer is called teichoic acid, which differs in composition based on the bacterial species. Some are made out of glycerol phosphate, glucosyl phosphate, and ribitol phosphate polymers, and the makeup 60% of the Gram-positive cell wall. The peptidoglycan in Gram-positive bacteria also differs from Gram-negative with the nature of the peptide crosslinks, where the peptides are connected through pentaglycine branch. The branch stem peptides are very important to Gram-positive bacteria as they are the attachment site for proteins. Also, these stem peptides can be modified leading to resistance to the β-lactam antibiotics. 36, 37
Mycobacteria

The cell envelopes of Mycobacteria (Figure 2), such as \( M_{tb} \), are very complex and have the characteristics of both Gram-positive and Gram-negative bacteria. They have an OM (with porins), IM, and peptidoglycan cell wall. The peptidoglycan layer surrounds the IM, and it has arabinogalactan that is covalently attached to mycolic acids. The mycolic acids are fatty acids that contain highly long alkyl groups that can reach as much as 90 carbons, and they are very important to the OM. These fatty acids maintain a rigid cell shape, and protect the cell from hydrophobic antibiotics. As mentioned above, \( M_{tb} \) and \( M_{ab} \) have L,D-transpeptidase with (3→3) linkage, which plays a role in drug-resistance.\(^{37,35,9}\)

Figure 2. Diagram of mycobacterium in general showing mycolic acids, porin, peptidoglycan and cell membrane.\(^{46}\)

Penicillin-binding Proteins (PBPs)

Penicillin-binding proteins, or PBPs, are enzymes involved in cell wall biosynthesis. They are a subgroup enzymes of transpeptidases called serine D,D transpeptidases. They catalyze the
formation of cross-linked peptidoglycan by the linkages between the fourth and third residues of two adjacent peptide side chains (4 → 3 linkage) as a final step for the bacteria to form peptidoglycan. Peptidoglycan is constituted of β-linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNac) polysaccharide chains cross-linked through peptide chains. It serves as a three-dimensional “net” that protects the cell by lining the exterior of the cell membrane (Figure 3). It is very important to the cell as it has multiple functions including cell wall synthesis, cell division, determination of the shape of the bacteria’s cell, attachment site for virulence factors, helping the bacteria in morphological transformation when it is under stress, and in regulating osmotic pressure. PBPs are critical to cell wall biosynthesis and represent the targets of β-lactam antibiotics. Inhibition of PBPs leads to abnormalities in the cell such as deformation of structure and loss of selective permeability, eventually causing cell lysis. 25, 4, 3

Figure 3. Formation of 4,3- and 3,3-cross-linked peptidoglycan by D,D- and L,D-transpeptidases.
The PBPs in Gram-negative and Gram-positive bacteria have different compositions. In Gram-positive bacteria, the peptidoglycan is produced in multiple layers and it expands. On the other hand, in Gram-negative bacteria, 50% of its peptidoglycan is recycled and is composed of a thin layer. Different bacteria may have different peptidic sequence, but is usually terminated at the D-Ala, D-Ala moiety. 25, 4, 3

**The discovery of β-lactams as antibiotics**

The use of antibiotics dates back to the Ancient Egypt, starting with eating garlic—known today for its antibacterial properties, and with putting molds on injuries. This concept of an organism inhibiting the growth of another organism was not too apparent, but it was discovered when *Penicillium* mold effects were observed. In 1921, Alexander Fleming left culture plates with *Staphylococcus* in his laboratory and left for vacation. When he came back, he discovered *Penicillium notatum* mold in the culture plates, and he observed that the *Staphylococcus* colonies were surrounded by mold were undergoing lysis. Based on his studies, he concluded that mold had antibacterial properties. In 1928, Fleming, Dr. Stuart Craddock and Mr. Frederick Ridley purified the lytic agent from the *Penicillium notatum* and called it penicillin (Figure 4), the structure of which is characterized by a four-membered β-lactam ring, fused to a five-membered thiazolidine ring. This success lead to the use of penicillin as antibiotic in trials in the 1940s penicillin finally became available commercially from large scale production laboratories such as fermentation laboratories in the U.S. and isolation laboratories from *Penicillium chrysogenum* in Peoria. After the success of penicillin, other classes of β-lactams with different chemical structures were developed. 10, 14
**Carbapenem Antibiotics**

Carbapenems are a sub-class of β-lactam with a five-membered ring fused to the β-lactam ring (Figure 5). The carbapenem structure is similar to penicillin, but with a carbon instead of sulfur at the first position, a double bond between C2 and C3, and a C1 β-hydroxyethyl group, replacing the C1β-acylamido groups of the penicillin and cephalosporin classes. These structural differences render carbapenems poor substrates for many β-lactamases, enzymes that causes β-lactam hydrolysis. This class of β-lactams plays a critical role in the antibiotic world, where it possesses the broadest spectrum of activity. Due to its high effectiveness, it is used for high-risk bacterial infections as a “last resort” antibiotic. However, carbapenems have a low oral bioavailability, so they are administered intravenously.  

**Figure 4.** General structures of penicillin and cephalosporin.

**Figure 5.** Structure of carbapenem.
The Mechanism of Action of Carbapenems

Like all β-lactam antibiotics, the carbapenem targets are the PBP transpeptidases responsible for cross linking peptidoglycan strands during bacterial cell wall biosynthesis. In the case of GN pathogens, the carbapenems must traverse the outer membrane through the water-filled porin channels to enter the periplasm, where this cross-linking process occurs. In the case of GP bacteria, however, the cell wall is external, so no such barrier exists. The carbapenem antibiotics form a covalent ester linkage with the active site serine residue of the PBP transpeptidase, resulting in the inhibition of formation of the peptidoglycan cross-linkage. This leads to the inhibition of the PBP’s ability to catalyze the formation of peptidoglycan (cell wall composition), causing an increase in osmotic pressure. As a result, the cell wall weakens, and the cell will burst.

The term “penicillin-binding-protein” is specifically used for the enzymes that recognize the β-lactams. β-lactams inhibit transpeptidation/carboxypeptidation by structurally being similar to D-Ala-D-Ala–peptide moiety (Figure 6). The PBP is acylated by the antibiotic (Scheme 1), with concomitant opening of the β-lactam ring. The stable acylated PBP can no longer catalyze the hydrolysis of the acyl-enzyme intermediate, which causes inactivation. This weakens the peptidoglycan, which eventually causes cell death. 8,32.

Scheme 1. Acylation of Ldt by carbapenem.
Figure 6. β-lactam mechanism of action.⁴⁷
**Thienamycin**

The first carbapenem isolated was thienamycin (Figure 7) which demonstrated a potent broad-spectrum activity and resistance toward the class A and C β-lactamases common in the 20\textsuperscript{th} century. Thienamycin binds to penicillin-binding proteins (PBPs) and is effective towards both Gram-negative bacteria (such as *Pseudomonas aeruginosa*) and Gram-positive bacteria (such as *Staphylococcus aureus* and *streptococci*). However, thienamycin was considered too unstable for commercial development, as it was sensitive to mild bases above pH 8 and nucleophiles. Therefore, there was a need for a better carbapenem.\textsuperscript{32}

![Structure of thienamycin](image)

**Figure 7.** Structure of thienamycin.

**Imipenem**

Imipenem and panipenem (Figure 8) are carbapenems that are potent against both GP and GN bacteria. Imipenem, a C2 derivative closely structurally related to thienamycin, became the first carbapenem used to treat infections. This carbapenem was better than thienamycin due to its stability in mild base. Like thienamycin, it also had stability against β-lactamase and had great affinity for PBPs. However, imipenem was sensitive to dehydropeptidase I (DHP-I) and required
a DHP inhibitor to protect it from being hydrolyzed by DHP-I. So, the search continued to generate a better carbapenem that was not sensitive towards DHP-I.\textsuperscript{32}

\textbf{Figure 8.} Structure of imipenem and panipenem.

\textbf{Meropenem}

Other carbapenem derivatives that were not as sensitive as imipenem towards DHP-I were made and found to be more effective against Gram-negative bacteria. It was discovered that a methyl group at the C1 position of the β-lactam protects the molecule from being hydrolyzed by DHP-I. This type of carbapenem has a broader antibacterial spectrum, better stability, and less susceptibility to many β-lactamases mostly due to the methyl group on the C1 position. Crystallographic analyses indicated that the hydroxyethyl R\textsubscript{2} function group helps the carbapenem resist hydrolysis by β-lactamases. Moreover, the \textit{trans} configuration at C5 and C6 further improves stability against β-lactamases, with the hydroxyl group displacing the hydrolytic water. (\textbf{Figure 10}) The carbapenems with a methyl at C1 included meropenem, ertapenem, biapenem, and doripenem (\textbf{Figure 11}). These carbapenems showed broader antimicrobial spectrum due to the pyrrolidine moiety. Doripenem showed better potency against \textit{P. aeruginosa} and \textit{A. baumannii}, and it turned to be least hydrolyzed by certain β-lactamases. On the other hand, meropenem
showed potency against the multi drug-resistant (MDR) *Mycobacterium tuberculosis* when it is combined with clavulanic acid, a β-lactamase inhibitor (**Figure 9**).\(^{19, 24, 32, 34}\)

**Figure 9.** Clavulanic acid mechanism of action.
Figure 10. Structures of meropenem, ertapenem, biapenem, and doripenem.
**Figure 11.** A. shows a methyl at C1, which reduces susceptibility to DHP-1. B. Shows the pyrrolidine ring, which increases stability of the β-lactam. C. shows penicillin to show difference between it and carbapenem, where carbapenem has a methyl at C1 position while penicillin has a sulfur. D. Shows cephalosporin with sulfur group. E. points out the hydroxyethyl group at R configuration and trans configuration at C5 and C6, which increases the β-lactam’s potency. F. Shows the hydroxyl group at S configuration.
**Bacterial Resistance to Carbapenem**

Bacterial pathogens have several mechanisms to protect themselves against carbapenems. For example, *Pseudomonas aeruginosa* (Gram-negative) can both release β-lactamase into the periplasm (location of PBP) and use efflux pumps to export antibiotics. Other bacteria, such as streptococci and *Staphylococcus aureus* (Gram-positive), produce mutated PBPs. 19, 32

**β-lactamase**

β-lactamase is an enzyme that hydrolyzes the β-lactam ring (Scheme 2) resulting in the opening of the ring and rendering the antibiotic unable to bind to PBP. There are four molecular classes of β-lactamas, A, B, C, and D. Each class of β-lactamase has one or more types of β-lactamase. Class A consists of β-lactamases with varying substrate specificity. For example, it contains penicillinases, which attack penicillin. It contains, extended-spectrum β-lactamases, which attack penicillin, cephalosporins and monobactams. It also consists of carbapenemases, which attack most β-lactams. Class B contains metallo carbapenemases, which attack most β-lactams. Class C contains expended-spectrum cephalosporinases, which attacks some penams, some cephalosporins, and monobactams. Class D contains narrow-spectrum penicillinases, extended-spectrum β-lactamases, and carbapenemases, which attack many penams, carbapenems, oxacillin, some monobactams and fourth generation cephalosporins.

Classes A, C, and D are serine β-lactamases that inactivate the β-lactam by hydrolyzing it through a nucleophilic attack by serine. On the other hand, Class B β-lactamases use Zn^{2+} and a Zn-complexed hydroxide to hydrolyze the β-lactam. Carbapenemases, which are members of class
A, B, and D β-lactamases, are considered to be a major player behind carbapenem resistance due to their versatile hydrolytic ability.\textsuperscript{10, 14, 18, 32}

\begin{center}
\includegraphics[width=\textwidth]{scheme2.png}
\end{center}

\textbf{Scheme 2.} Hydrolysis of carbapenem by serine-based β-lactamase

\textbf{Class A, C and D}

\textbf{Class A}

Classes A, C and D are active-site-serine β-lactamases, which are grouped according to sequence homology. Until recently, the carbapenem class of antibiotics remained stable to most of these enzymes. Particularly in Class A and Class D, however, the past 20 years has seen the evolution of some of these serine β-lactamases into carbapenemases, thereby effectively bestowing carbapenem-resistance to the producing species. One recent class A carbapenemase has a disulfide bond (such as KPC2 carbapenemase) that alters the distance between certain amino acids (for example, Ser70 and Thr237), which causes the active site length to decrease. As a result, the steric hindrance that is made by the hydroxyethyl group on the C6 position (which is a major function group in inhibiting class A β-lactamses) of the imipenem decreases. Thus, the class A KPC
carbapenemases hydrolyze imipenem and other carbapenems. Another example includes non-
carbapenemase GES-1, which can turn into a GES-2 carbapenemase only by the substitution of
Gly170Asn amino acid. In addition, if Gly170 was mutatated to Ser, the GES-2 mimics a stronger
carbapenemase called GES-5, which has a higher rate of acylation for imipenem by 5,000-fold
than GES-2. 10, 14, 18, 32

Class D

Class D β-lactamases have a few OXA carbapenemases types. *Acinetobacter baumannii*
strains commonly harbor a class D β-lactamase, which produces resistance against carbapenems.
The major carbapenemase type in *Acinetobacter baumannii* is the OXA-23 β-lactamase. This type
of carbapenemase is a major problem because it has resistance to meropenem, knowing that
meropenem is a last-resort antibiotic against *Acinetobacter baumannii*. Class D β-lactamases have
high structural diversity, which allows them to have high resistance to a broad-spectrum of
antibiotics. Class D resembles class A β-lactamase where the serine undergoes acylation by the β-
lactam with a lysine residue that promotes the process. In class D, however, the acylation and
deacylation occur through the N-carboxylated lysine residue. It was suggested that class D β-
lactamases have a hydrophobic tunnel barrier that only allows certain substrates to reach the active
site. 10, 14, 18, 32
Class B

When it comes to class B β-lactamase, it is divided to three sub-classes; 1, 2, and 3. B2 (such as CphA) functions when it binds to one Zn$^{+2}$, while B1 and B3 (metallo-β-lactamases) are optimally active when they bind to two Zn$^{2+}$. The metallo-β-lactamase subclasses function by having a Zn$^{2+}$ ion lower the pK$_a$ of a water molecule. The water molecule then generates hydroxide, which acts as a nucleophile to attack the carbapenem. The second Zn$^{2+}$ ion stabilizes the formed intermediate. (Figure 12). 10, 14, 18, 32

Figure 12. Subclass B1 and B3 metallo-β-lactamase active sites. 32

When it comes to class B2, hydrolysis occurs when the carbapenem bond is cleaved by a water molecule nucleophile that is coordinated with His118 and Asp120. Another water molecule
(coordinated with Asp120, His263, Cys221 and Zn$^{+2}$) bonds with the carboxylate of the β-lactam and donates a proton to the nitrogen in the carbapenem (Scheme 3). 10, 14, 18, 32, 33

**Scheme 3.** Stabilization of the anionic intermediate. 32

**Porins**

Outer membrane protein (OMPs), known as porins, allow molecules to pass through the outer membrane to the periplasm. There are four types of porins in bacteria—general, specific, gated, and efflux porins. Since different types of bacteria have different OMPs, not all carbapenems are affected the same way by porins. Specific and gated porins permit entry of carbapenems into the cell. These types of porins are present in *K. pneumoniae* and *E. coli*. On the other hand, efflux pumps force certain carbapenems out of the cell. There are five major families of efflux pumps which include the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, the resistance-nodulation-division (RND) family, the small multidrug resistance
(SMR) family, the major facilitator superfamily (MFS) family, and the multidrug and toxic compound extrusion (MATE) family. Multidrug efflux pumps are present in all bacteria, and many have more than one type of efflux pump (such as *Mycobacterium tuberculosis*). Moreover, some types of bacteria have low efflux expressions while some have higher efflux expression, and expression can be either be induced (transiently) or due to mutations (constitutively). A single efflux pump can extrude multiple antibiotics; thus, using efflux pump inhibitors can eventually cause an increase in a bacteria’s susceptibility to several antibiotics. Another way to overcome the efflux pump mechanism is by modifying the β-lactam’s functional groups in order to reduce its affinity to the efflux pump, depending on the targeted bacteria and their efflux pump affinities. 4,13, 31, 38

**PBP Mutations**

As mentioned, β-lactams bind to the PBP by mimicking D-Ala, D-Ala moiety (Figure 13). However, certain bacterial pathogens can achieve resistance to β-lactam antibiotics by mutating their PBPs. The mutated PBP are not recognized by the β-lactams, and thus, are highly drug resistant.

![Figure 13. Structural similarity between D-Ala, D-Ala and penicillin](image)

Figure 13. Structural similarity between D-Ala, D-Ala and penicillin
When it comes to *Mycobacterium abscessus* (\(M_{ab}\)) and *Mycobacterium tuberculosis* (\(M_{tb}\)), the majority of peptidoglycan linkages are between the third residue (3 \(\rightarrow\) 3) of two adjacent peptide stems. This is formed by L,D-transpeptidases (Ldt), which is a class of cysteine transpeptidase, and is not recognized by most classes of \(\beta\)-lactam antibiotics. The glycan strand is composed of a polymer of alternating B(1-4) linked N-acetyglucosamine (NAG) and N-glycolylmuramic acid (NGM) with L-Ala-D-iGlu- meso-2,6-diaminopimelate[DAP]-D-Ala-D-Ala stem peptide attached though a lactic acid moiety. The bacteria’s structure gets its strength and long-term survival from the mesh formed by the cross linkage of the peptide stem (missing a D-Ala) with a nearby stem peptide from a different glycan strand. This process is catalyzed by PBP and Ldt to form both linkages of (4 \(\rightarrow\) 3) and (3 \(\rightarrow\) 3) (Figure 14), with (3 \(\rightarrow\) 3) cross links being the majority and as high as 80% of links in \(M_{tb}\). \(^{20, 24, 25, 28, 30, 40}\)
Figure 14. Difference between $4 \rightarrow 3$ linkage, which is shown at the top, and $3 \rightarrow 3$ linkage, which is shown at the bottom.
**Scheme 4.** Hydrolysis of β-lactam by BlaC β-lactamase

**M<sub>tb</sub> Resistance**

*M<sub>tb</sub>*, which causes tuberculosis (TB), is a multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogen. *M<sub>tb</sub>* has been spreading at a high rate. According to Center for Disease Control and Prevention (CDC), TB is one of the top 10 causes of death. In 2017, TB caused an estimated 1.3 million deaths among HIV-negative people and 300,000 deaths among HIV-positive people. 10 million people developed TB in 2017. Approximately 1.7 billion people are estimated to have latent TB, which means 23% of the world’s population is at risk of developing active TB.

TB treatment is composed of a combination of chemotherapy for two months followed by four months of isoniazid and rifamycin. This extended duration of treatment makes it hard for patients to comply. Although this noncompliance is a factor of the spreading of the disease, the high resistance of *M<sub>tb</sub>* to β-lactam antibiotics is a major problem. This resistance is caused by the chromosomally encoded gene of class A Ambler β-lactamase (BlaC), which is a broad-spectrum enzyme that hydrolyzes all class of β-lactams and which can be inactivated by the β-lactamase inhibitor, clavulanic acid. In a study by Hugonnet *et al*, they showed that a combination of meropenem and clavulanate were effective against 13 strains of *M<sub>tb</sub>* due to poor hydrolyzation of the β-lactam by BlaC. As mentioned above, *M<sub>tb</sub>* has 80% 3→3 L,D-transpeptidase linkage,
specifically type 2 (LdtM\textsubscript{2}). The 3→3 cross-link is between the carboxy terminus of a DAP on the peptide stem with NH\textsubscript{2} of a DAP on another stem (Figure 14). Carbapenems were reported to target this type of enzyme (Scheme 4). \textsuperscript{24} More specifically, the combination of meropenem-clavulanic acid have been proven to stop the formation of transpeptidase and inactivate β-lactamase. \textsuperscript{11, 13, 16}

\textit{Mab} Resistance

\textit{Mycobacterium abscessus (M\textsubscript{ab})} is a rapidly growing nontuberculous mycobacterium (NTM), which is responsible for as high as 80\% of NTM pulmonary infections. It also causes skin and soft-tissue infections, central nervous system infections, bacteremia, and ocular infections. M\textsubscript{ab} is highly resistant to antibacterial agents and causes dangerous diseases such as respiratory disorders and soft tissue disorders. M\textsubscript{ab} is a major problem because not only are they resistant against various antibiotics, but they are also resistant to disinfectants. Also, M\textsubscript{ab} causes a lot of pulmonary diseases, which are very hard to cure and require a combination of antibiotics a macrolide and intravenous agents for 2 weeks to 12 months, depending on the type of infection or disease. For example, pulmonary diseases are treated with a macrolide in combination with other antibiotics and central nervous system infections are treated with clarithromycin combined with other antibiotic and both have a 12-month recommended treatment duration. Skin and soft tissue infections are treated with a macrolide in combination with imipenem and other antimicrobial therapy for a minimum of 4 months. \textsuperscript{34} In 1990a, the combination clarithromycin, amikacin, and imipenem (or cefoxitin) were reported as the best multidrug therapy. \textsuperscript{7, 8, 34, 40}

Imipenem and cefoxitin have shown MICs of 16 and 32 mg/L respectively, against M\textsubscript{ab}. Mab is highly resistant to other β-lactams. M\textsubscript{ab} has hydrophilic porins that potentially allow β-lactams into the cell. However, the major challenges when facing M\textsubscript{ab} is its low permeability and
β-lactamase, which make it resistant to antibiotics. It has a gene encoding an Amber class A β-lactamase (Bla\textsubscript{Mab}), which is inactivated via avibactam via reversible formation of a covalent adduct.

**Acinetobacter baumannii Resistance**

*Acinetobacter baumannii* is an MDR pleomorphic aerobic Gram-negative coccobacillus, and a major pathogen, of increasing clinical concern, causing nosocomial infections. It is one of the most successful types of pathogens that are spread in hospitals. For example, in 2018, 180 strains of *Acinetobacter baumannii* out of 1259 GN bacilli isolates were identified in 13 hospitals. The mortality rate of inpatients with *Acinetobacter baumannii* is 26-55%.

*Acinetobacter baumannii* is responsible for many soft tissue infections, such as skin infections and urinary tract infections. It is one of the most successful types of pathogens in escaping antimicrobial antibiotics. It degrades the antibiotics through enzymes (β-lactamase), target modification, efflux pump and permeability defects.\textsuperscript{22} All four types of β-lactamas (A, B, C and D) were identified in *Acinetobacter baumannii*. A number of class A β-lactamas (including carbapenemases) have been identified in *Acinetobacter baumannii*, which are generally inhibited by clavulanate. These enzymes hydrolyze penicillin and cephalosporins. A variety of class B β-lactamas have also been identified, which hydrolyze carbapenems. A number of class C β-lactamas have also been identified, including cephalosporinas. Finally, class D β-lactamas, including more than 400 OXA-type (oxacillinases- type) have been identified, and many have carbapenemase activity, such as OXA-23. Carbapenems are used to treat *Acinetobacter baumannii* are imipenem, meropenem and doripenem. Carbapenems are usually combined with a few other antibiotics and β-lactamase inhibitor.
CHAPTER II
SYNTHETIC STRATEGY

Synthetic Strategy and Discussion

There are many challenges that need to be taken into consideration when synthesizing carbapenem antibiotics. The structure of new antibiotics should have affinity to PBPs but not for β-lactamases. It must be able to penetrate the GN outer membrane efficiently and not be pumped out by efflux pumps. Meropenem is one of the most successful types of carbapenems. Carbapenems are a successful class of β-lactam due to the many special features in the structure. Carbapenems are different from penicillin in having a carbon in place of sulfur at the C1 position, a double bond between C2 and C3, special C2 side chains, and a C6 hydroxyethyl side chain. Each functional group has a role in improving the potency of the drug. The C6 hydroxyethyl group is important to activity as it aids in resistance to hydrolysis by β-lactamases, and its stereochemistry is very important to the carbapenem’s activity. As mentioned above, the hydroxyethyl group causes steric hindrance that is important in inhibiting class A β-lactamase. The stereochemistry of the functional groups of the β-lactam are important as well. For example, positions C5 and C6 show an increase in stability against β-lactamase in trans configuration, and a hydroxyl group at C8 shows better potency in the R configuration. 17, 22, 39

The methyl group at the C1 position of the carbapenem, meropenem, improves the stability of the antibiotic against β-lactamases and protection against DHP-I hydrolysis, which improves the in vivo half-life of the antibiotic. The addition of a methyl at C1 showed broader antimicrobial activity. The basicity of the nitrogen group on the side chains helps the antibiotic penetrate more efficiently through the outer membrane. 17, 22, 39
Modification at C5

The C5 position has not been investigated. There is only one paper about the C5 position written in 1989 by Onoue, Hiroshi, and Yukitoshi Narukawa. Therefore, we decided to evaluate C5 substituted carbapenems for better potency against certain pathogens. Therefore, it was decided to make a carbapenem with a methyl at the C5 position and a carbapenem with an ethyl at the C5 position in order to add hydrophobicity to the antibiotic and fill the small hydrophobic pockets near Met 303 and Val322 of the binding site in *M. tuberculosis* as shown in Figure 16.

![Figure 15. Stereochemistry of C5 and C6 positions in carbapenem.](image)
**Figure 16.** Shows a part of Mtb binding with a meropenem analogue acyl-enzyme binding to Cys354 and His352. It shows the hydrophobic pockets, Met303 and Val322, that would potentially bind to a meropenem analogue with methyl at the C5 position.

The molecule needs to be small in order to pass through the porins of the bacteria. It is also preferable to have the nitrogen group in the C2 side chain, as it gains a positive charge, and goes through the hydrophilic porins of the bacteria more efficiently. The hydrophilic porins are intended to uptake positively charged amino acids.

Meropenem, a commercial antibiotic, is one of the most successful antibiotics because of its side chain (at the C2 position) ability to bind to several PBPs and penetrate through the OM. The first antibiotics synthesized in this research were made with a meropenem side chain at the C2 position (10, 21), knowing that meropenem showed activity against *Mtb* when combined with clavulanic acid.48 The second antibiotics were made with an ethyl sulfur side chain, which is a hydrophobic side chain that can potentially bind to *Mtb*’s hydrophobic pockets (13, 24)

a) THF, 1) (CuI)$_4$, 3[(CH$_3$)$_2$S], -60 °C, 2) CH$_3$MgI if R = CH$_3$, CH$_2$CH$_3$MgI if R = CH$_2$CH$_3$, 10 °C.

b) EtOAc, NaOAc, AcOH, RuCl$_3$, O$_2$, MeCHO, 12-15 °C. c) CH$_2$Cl$_2$, Compound 5, ZnCl$_2$, 42 °C.

d) CH$_3$CN, HF. e) EtOAc, Rh$_2$(OAc)$_4$, 60 °C. f) CH$_3$CN, (PhO)$_2$P(O)Cl, DIPEA, 4-nitrobenzyl
The carbapenem was synthesized by starting with commercial azetidinone with an acetoxy group at the C4 position (47). Several synthetic methods of the generation of the first step (3) were evaluated. The first method included methyl zinc iodide (Scheme 6), which was made by reacting methyl iodide with Zn in CH2Cl2. However, the reaction did not succeed. So, it was repeated, but in the presence of ZnCl2 in order to coordinate with the acetate oxygen and make it a better leaving group. However, the reaction failed again. Then, it was decided to repeat the reaction, but in the presence of copper iodide dimethyl sulfide complex. Once again, the reaction did not yield any product.

Consequently, we decided to use a different method. So, a Gilman reagent was prepared by reacting methyl iodide with lithium metal in Et2O to yield methylthiolithium (Scheme 7), and a copper iodide dimethyl sulfide complex was prepared with it at -60 °C. Then, the mixture was treated with the commercial azetidinone (47). However, yield was very low (2% yield). After repeating the experiment six times with varying the temperature, it was decided to use another method.

Scheme 6. Attempted synthesis of compound 3 using CH3ZnI
Since we desired to avoid reactive organolithium reagent and to increase the yield, a Grignard reagent was employed to synthesize methyl magnesium iodide by using methyl iodide and magnesium turnings in the presence of a few iodine crystals. In the case of synthesizing an ethyl functional group at the C5 position (15), iodoethane was used in place of iodomethane. Then copper iodide dimethyl sulfide complex was prepared in anhyd. THF at rt, the reaction subsequently cooled to -60 °C, treated with methyl magnesium iodide in Et₂O, warmed to -20 for 10 min, then cooled back to -60 °C, treated with azetidinone (47), and warmed to room temperature. The magnesium dimethyl cuprate served as a nucleophile and replace the acetoxy group with a methyl group (3) in a reasonable yield (83%).

Acetoxylation of 3 and 15 was then performed by adding activated ruthenium trichloride to catalyze the oxidation with O₂ and freshly twice distilled acetaldehyde at 12-15 °C to yield the intermediate, compound 4, 16. Prior to the reaction, it was critical for the ruthenium trichloride to be heated using a flame while it was on the high vacuum until the catalyst turned into fine powder (approximately 5-10 min of the rb flask containing the RuCl₃ in the Bunsen burner flame at 0.1 mm pressure). The heating of the catalyst activates it and removes the water to generate anhydrous RuCl₃. The activation does not succeed if a heating-gun was used, so it was important to use a Bunsen burner. The RuCl₃ reacts with the acetaldehyde and oxygen to yield oxoruthenium

**Scheme 7.** Synthesis of compound 3 using methyllithium.
species (49) as shown in Scheme 8. There are two pathways to this reaction. The first pathway includes acetaldehyde reaction with the molecular oxygen to yield peracetic acid. The peracetic acid then reacts with Ru$^{\text{III}}$ to yield the oxoruthenium species 49. The second possible pathway includes the formation of metalacyclic intermediate (48). After protonylysis, the reaction give acetic acid and oxoruthenium 49 as shown in Scheme 8.$^{51}$

Acetaldehyde had to be freshly distilled twice before each reaction in order for the reaction to succeed. The distillation remove water from the acetaldehyde and any of the formed polymers in the container. It was hypothesized that water was extremely damaging to the reaction. If water enters the flask, it might act as a nucleophile and open the β-lactam ring (52) as shown in Scheme 9. Therefore, a few reagents were used to try to minimize the water in the reaction (shown in Table 1) such as MgSO$_4$ and ground molecular sieves (4Å), but the reagent did not improve the results. It was also very important to add all the acetaldehyde at once in the beginning of the reaction. If acetaldehyde is added in aliquots, side product increases. The possible side product is shown in Scheme 9.

Scheme 8. Mechanism of synthesis of compound 4.$^{51}$
Increasing the oxygen pressure also improved yield. The reaction was run with an oxygen balloon only at first, and the septum of the reaction was opened to obtain NMR samples. It was observed that the reaction needed an increase in pressure, and that it was extremely sensitive to moisture. Therefore, 12 psi O₂ pressure was applied, the vessel was closed at all times, and samples were taken out for monitor using a syringe. If oxygen pressure was lost, the reaction ended. The syntheses of compound 4 (with methyl at C5) and compound 15 (with ethyl at C5) were repeated 104 times, varying the temperature, reagents, and the equivalents of the reagents, and it was shown that the optimized results used 11 mol % of activated RuCl₃, 0.27 equivalence of NaOAc, 9.6 equivalences of acetic acid, 13.4 equivalences of acetaldehyde, and 12 psi dry O₂ pressure at 16-18 °C and increased amount of EtOAc. (Table 1).

The product was then reacted with the TBS enol ether of p-nitrobenzyl 2-diazoacetoacetate (5) in the presence of ZnCl₂ to coordinate with the acetate (53), making the acetate a better leaving group. in order to provide the structure for the five-membered ring next to the β-lactam ring (Scheme 10). This reaction was also performed multiple times (32 times) with multiple variations (Table 2). Therefore, silica gel was used as a reagent to tautomerize the product to the desired isomer by adding it to the reaction. However, that technique did not work. Therefore, it was
decided to use ZnCl$_2$ in Et$_2$O, which improved results. The variations of reagents and conditions are shown in Table 2.

Scheme 10. synthesis of compound 6 and 7.

The product (6) was then treated with 1 mL of HF in order to remove the TBS protecting group (7) as shown in Scheme 10. The β-lactam was then treated with catalytic rhodium acetate at 68 °C to cyclize the compound 7. The diazo functional produced N$_2$ gas and generated a carbene (54). The generated rhodium carbenoid (56) inserts into the N-H group, forming a bicyclic structure 8 (Scheme 11).
Scheme 11. Synthesis of compound 8

Product 8 was then treated with diphenyl phosphoryl chloride and diisopropylethylamine at -35 °C. The diisopropylethylamine removed the acidic proton at C3, generating an enolate anion (58), which is phosphorylated at the oxygen (11) as shown in Scheme 12. The phosphate then acts as a good leaving group for the next reaction (60). The thiol side chain was then added with diisopropylethylamine to the intermediate (9, 12) (Scheme 12). After purification, the compound is hydrogenated at 55 psi in EtOAc and phosphate buffer to remove the p-nitrobenzyl protecting group to yield antibiotics 10, 13, 21, 24.

Scheme 12. Synthesis of enol phosphate intermediate with substitution at the C5 position.
<table>
<thead>
<tr>
<th>Entry</th>
<th>RuCl₃ (eq)</th>
<th>NaOAc (eq)</th>
<th>AcOH (eq)</th>
<th>MeCHO (eq)</th>
<th>EtOAc (eq)</th>
<th>O₂ (psi)</th>
<th>Temp (°C)</th>
<th>Other Reagents</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0665</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>MgSO₄</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>Molecular sieves</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>35</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0.33</td>
<td>0.54</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>MgSO₄</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>55</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>Molecular sieves</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>AgOTf</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.33</td>
<td>0.27</td>
<td>0</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>Ac₂O</td>
<td>0</td>
</tr>
</tbody>
</table>

Ru(acac)₃: Ruthenium(III) acetylacetonate
AgOTf: Silver trifluoromethanesulfonate
Ac₂O: Acetic anhydride
MeCHO: Acetaldehyde
Table 1.2. Compound 4. RuCl$_3$ heated using a Bunsen burner under high vacuum.

<table>
<thead>
<tr>
<th>Entry</th>
<th>RuCl$_3$ (eq)</th>
<th>NaOAc (eq)</th>
<th>AcOH (eq)</th>
<th>MeCHO (eq)</th>
<th>EtOAc (psi)</th>
<th>O$_2$ (psi)</th>
<th>Temp (°C)</th>
<th>Other Reagent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>35</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>11</td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>40</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>0.074 eq Ru(acac)$_3$</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>Ru(acac)$_3$</td>
<td>6%</td>
</tr>
<tr>
<td>14</td>
<td>0.0723</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>AgOAc</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>0</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>PAA</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>0.43</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>30 mL</td>
<td>3</td>
<td>rt</td>
<td>PAA</td>
<td>25%</td>
</tr>
<tr>
<td>17</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>3</td>
<td>rt</td>
<td>PAA</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>3</td>
<td>rt</td>
<td>0.24 eq of 5% Ru/C + 1.5 eq of MCPBA</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0.66</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>3</td>
<td>rt</td>
<td>AgOTf</td>
<td>0</td>
</tr>
</tbody>
</table>

Ru(acac)$_3$: Ruthenium(III) acetylacetonate
AgOAc: silver acetate
PAA: Peracetic acid
AgOTf: Silver trifluoromethanesulfonate
MeCHO: Acetaldehyde
Table 1.3. Compound 4. RuCl$_3$ heated using a Bunsen burner under high vacuum. Freshly twice distilled acetaldehyde, and increased O$_2$ pressure.

<table>
<thead>
<tr>
<th>Entry</th>
<th>RuCl$_3$ (eq)</th>
<th>NaOAc (eq)</th>
<th>AcOH (eq)</th>
<th>MeCHO (eq)</th>
<th>EtOAc</th>
<th>O$_2$ (psi)</th>
<th>Temp (°C)</th>
<th>Other Reagent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>12</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>12</td>
<td>15</td>
<td>9 eq HCOOH</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>17.9 eq</td>
<td>15 mL</td>
<td>12</td>
<td>0</td>
<td></td>
<td>33%</td>
</tr>
<tr>
<td>23</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>12</td>
<td>0</td>
<td>0.0113 eq di tert butyl methyl phenol</td>
<td>0%</td>
</tr>
<tr>
<td>24</td>
<td>0.33</td>
<td>0.90</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>12</td>
<td>0</td>
<td></td>
<td>33%</td>
</tr>
<tr>
<td>25</td>
<td>0.33</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>15 mL</td>
<td>12</td>
<td>18</td>
<td></td>
<td>66%</td>
</tr>
<tr>
<td>26</td>
<td>0.11</td>
<td>0.27</td>
<td>9.6</td>
<td>13.4</td>
<td>56 mL</td>
<td>12</td>
<td>16</td>
<td></td>
<td>95%</td>
</tr>
</tbody>
</table>

HCOOH: formic acid
MeCHO: Acetaldehyde
Table 2.1 Variation of reagents for compound 6 using ZnCl₂ salt.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound 5</th>
<th>ZnCl₂ salt</th>
<th>CH₂Cl₂</th>
<th>Temp (°C)</th>
<th>Other reagent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 eq</td>
<td>0.5 eq</td>
<td>2 mL</td>
<td>rt</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>1 eq</td>
<td>0.5 eq</td>
<td>4 mL</td>
<td>36</td>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>2.5 eq</td>
<td>1 eq</td>
<td>1 mL</td>
<td>36</td>
<td>0.5 eq ZnBr₂</td>
<td>5%</td>
</tr>
<tr>
<td>4</td>
<td>1.5 eq</td>
<td>0</td>
<td>1 mL</td>
<td>36</td>
<td>0.5 eq ZnI₂</td>
<td>0%</td>
</tr>
<tr>
<td>5</td>
<td>1.5 eq</td>
<td>0</td>
<td>1 mL</td>
<td>36</td>
<td>0.5 eq ZnBr₂</td>
<td>5%</td>
</tr>
<tr>
<td>6</td>
<td>1.5 eq</td>
<td>0</td>
<td>4 mL</td>
<td>40</td>
<td></td>
<td>5%</td>
</tr>
</tbody>
</table>

Table 2.2 Variation of reagents for compound 6 using 1 M ZnCl₂ in Et₂O.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound 5</th>
<th>ZnCl₂ in Et₂O</th>
<th>CH₂Cl₂</th>
<th>Temp (°C)</th>
<th>Other reagent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.5 eq</td>
<td>0.5 eq</td>
<td>2 mL</td>
<td>35</td>
<td></td>
<td>30%</td>
</tr>
<tr>
<td>8</td>
<td>2 eq</td>
<td>0.5 eq</td>
<td>2 mL</td>
<td>35</td>
<td></td>
<td>30%</td>
</tr>
<tr>
<td>9</td>
<td>3 eq</td>
<td>0.5 eq</td>
<td>2 mL</td>
<td>35</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>10</td>
<td>4 eq</td>
<td>1 eq</td>
<td>2 mL</td>
<td>35</td>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>11</td>
<td>3.5 eq</td>
<td>0.5 eq</td>
<td>0</td>
<td>70</td>
<td>2 mL toluene</td>
<td>30%</td>
</tr>
<tr>
<td>12</td>
<td>2 eq</td>
<td>0.70</td>
<td>2 mL</td>
<td>35</td>
<td></td>
<td>50%</td>
</tr>
</tbody>
</table>
Results of C5 Substituted Carbapenems

Antibiotics 10, 13, 21 and 24 were all designed against \( M_{tb} \) and \( M_{ab} \), which have shown that they need a hydrophobic functionality at the C5 position according to previous results. The antibiotic was microbiologically evaluated against \( M_{tb} \) at UCF, in the laboratory of Dr. Kyle Rohde. Antibiotic 10 showed that it was more than twice as active as the best commercial antibiotic, meropenem, against \( M_{tb} \) and more than four times as potent against \( M_{ab} \). The MIC (\( \mu M \)) of antibiotic 10 against \( M_{tb} \) is 0.4 and 1.2 against \( M_{ab} \), whereas meropenem show MIC of 1.8 against \( M_{tb} \) and 15.0 against \( M_{ab} \) as shown in Table 3.0. Antibiotic 10 and meropenem were evaluated with and without an inhibitor, and in both cases antibiotic 10 showed lower MIC as shown in Figure 17. All other antibiotics, 13, 21, and 24, did not generally show better activity than meropenem. Antibiotic 13 showed better potency against \( M_{tb} \) than the commercial meropenem as shown in Table 3.0.

Table 3.0. MICs of antibiotics 10, 13, 21, and 24 against \( M_{tb} \) with and without clavulanic acid and \( M_{ab} \) with and without avibactam.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>( M_{tb} ) MIC (( \mu M ))</th>
<th>( M_{ab} ) MIC (( \mu M ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No CLA</td>
<td>CLA</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>13</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>21</td>
<td>3.4</td>
<td>3.0</td>
</tr>
<tr>
<td>24</td>
<td>24.3</td>
<td>16.8</td>
</tr>
</tbody>
</table>

CLA = clavulanic acid
AVA = Avibactam
Figure 17. A. shows structure of commercial meropenem, 10, 13, 21, and 24. B. Progress curve for inhibition of Mtb and Mab, with and without β-lactamase inhibitors, clavulanic acid or avibactam, respectively.

Antibiotics 10, 13, 21, and 24 were also sent to Notre Dame labs to be evaluated against Acinetobacter baumannii (A. baumannii) with OXA-23 β-lactamase. Antibiotic 10 and antibiotic 21 displayed superior activity against Acinetobacter baumannii. Both antibiotics have a meropenem side chain at C2 and a modified C5 position. Antibiotic 10 has a methyl group at the
C5 position while antibiotic 21 has an ethyl group at the C5 position. Since both compounds showed activity against OXA-23 producing A. baumannii, the compounds were also tested as inhibitors of the OXA-23 β-lactamase. The compounds were shown to increase meropenem potency against Acinetobacter baumannii producing OXA-23 by 2024 fold indicating that it is an inhibitor of the OXA-23 carbapenemase. OXA-23 is a class D β-lactamase that produces resistance to carbapenems, as described in the introduction. Antibiotics with a C2 thioethyl side chain did not show potency against Acinetobacter baumannii as shown in Tables 3.1, 3.2, and 3.3. All antibiotics showed activity against E. coli JM83, especially 10. Antibiotic 10 showed an MIC value of 0.5, however, commercial meropenem has an MIC value of 0.030 against E. coli JM83.

Table 3.1. MICs of compounds 10, 13, 21 and 24 against E. coli JM83 background with and without the OXA-23 β-lactamase.

<table>
<thead>
<tr>
<th>Compound #</th>
<th>JM83 (no β-lactamase)</th>
<th>OXA-23 (pNT165) MIC (µg/ml)</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>0.030</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0.5, 0.5, 0.5, 0.5</td>
<td>0.5, 0.5</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>2, 2, 2</td>
<td>4, 4</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>4(8), 8(4), 8</td>
<td>4(8), 8</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>32</td>
<td>&gt;64</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.2. MICs of meropenem against A. baumannii CIP 70.10 producing OXA-23 in the presence of compounds 10, 13, 21 and 24 treated as inhibitors.

<table>
<thead>
<tr>
<th>Compound (4 µg/ml)</th>
<th>OXA-23 (pNT165) MIC (µg/ml) of meropenem</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>64, 64, 64</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>≤1, ≤0.031</td>
<td>≥2048</td>
</tr>
<tr>
<td>13</td>
<td>2, 4</td>
<td>16-32</td>
</tr>
<tr>
<td>21</td>
<td>≤1, ≤0.031</td>
<td>≥2048</td>
</tr>
<tr>
<td>24</td>
<td>32, 64</td>
<td>0-2</td>
</tr>
</tbody>
</table>
Table 3.3. MICs of compounds 10, 13, 21 and 24 against *A. baumannii* CIP 70.10 background with and without the OXA 23 β-lactamase.

<table>
<thead>
<tr>
<th>Compound #</th>
<th>CIP 70.10 (no β-lactamase) MIC (µg/ml)</th>
<th>OXA-23 (pNT165)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>0.5, 0.5</td>
<td>64, 32/64</td>
</tr>
<tr>
<td>10</td>
<td>0.5, 0.5, 0.5</td>
<td>4, 4</td>
</tr>
<tr>
<td>13</td>
<td>8, 8, 8</td>
<td>8, 8</td>
</tr>
<tr>
<td>21</td>
<td>2, 2, 2</td>
<td>4, 4</td>
</tr>
<tr>
<td>24</td>
<td>&gt;32</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

Table 3.4. MICs of meropenem against *A. baumannii* CIP 70.10 producing the OXA-23 β-lactamase in the presence of compounds 10 and 21 treated as inhibitors.

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Concentration (µg/ml)</th>
<th>OXA-23 (pNT165) MIC (µg/ml) of meropenem</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.5</td>
<td>32(64)</td>
<td>0-2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>32, 16/32</td>
<td>0-4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>≤0.031, ≤0.002</td>
<td>≥16000</td>
</tr>
<tr>
<td>21</td>
<td>0.5</td>
<td>32</td>
<td>0-2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>32, 32</td>
<td>0-2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>≤0.031, ≤0.002</td>
<td>≥16000</td>
</tr>
<tr>
<td>Meropenem</td>
<td>-</td>
<td>64, 32</td>
<td>-</td>
</tr>
</tbody>
</table>
Modification at C5 and C1 Simultaneously

It was very clear that methyl or ethyl at the C5 position improves the observed MIC values against M_{tb}, M_{ab} and Acinetobacter baumannii. On the other hand, 1 β-methylcarbapenem also exhibit potent antibacterial activity. Meropenem exhibit a broad spectrum of antibacterial activity against Gram-negative, Gram-positive bacteria, and mycobacteria. As shown in Table 2, antibiotics 10 and 21 were superior against Acinetobacter baumannii when combined with meropenem. Therefore, our lab desired to generate a carbapenem with methyl at both C1 and C5 position. The desired scheme for this compound is shown on Scheme 13. Many different methods were used, but this work is still in progress.
Scheme 13. Anticipated scheme for adding a methyl group on C5 and C1 positions on β-lactam using chiral oxazolidine.

The synthesis of this carbapenem started by treating compound 4 with chiral oxazolidine (61), which can be effectively used as a chiral auxiliary for a stereoselective reaction. Compound 4 was treated with compound 61 at -35 °C using titanium tetrachloride in the presence of an organic base. The titanium tetrachloride forms an enolate on the chiral oxazolidine to couple with the β-lactam.

In order to avoid TiCl4, oxazolidine was treated with tert-butylidimethylsilyl trifluoromethanesulfonate (TBS-OTf) to yield compound 33. Compound 33 was then dissolved in
CH$_2$Cl$_2$, cooled to -78 °C, treated with compound 4 and then treated with ZnCl$_2$. However, the desired product shown on **Scheme 14** was not obtained. Three undesired products were obtained according to the NMR. The first two products were ring-open products of the β-lactam, and the third product was a coupling of two β-lactams. The N-H can act as a nucleophile by attacking the β-lactam intermediate to yield the product of two β-lactams attached to each other as shown in **Scheme 15**. This reaction was also attempted with oxazolidine with a bromine (70 shown in **Figure 18**) using TiCl$_4$, however, undesired product 4 shown in **Scheme 15** was obtained according to the $^1$H NMR.

**Figure 18.** Used chiral oxazolidine compounds in try to add methyl at the C1 position.

**Scheme 14.** Alternate scheme to add methyl on C1 position.
Since the oxazolidine methods were unsuccessful, we decided to approach the product differently by using methyl 3-oxovalerate (73). First, the reaction was attempted with commercial azetidione (47). The first method included treating the methyl 3-oxovalerate with NaH in order to get a dianion methyl 3-oxovalerate (74) and couple it with compound 4 using ZnCl$_2$ in the presence of $n$-butyllithium. However, the NMR shows that the methyl 3-oxovalerate was added to the $\beta$-lactam through the anion in between the carbonyls, which yielded the undesired product 76 shown in Scheme 16. Therefore, another approach was taken by converting methyl 3-oxovalerate to methyl 2-diazo-3-oxovalerate, treating it with TMS-OTf (44), then coupling it with compound 4 using ZnCl$_2$ as shown in desired mechanism on Scheme 17.
Scheme 16. Observed undesired product after adding dianion methyl 3-oxovalerate with commercial azetidinone.

Therefore, it was decided to take methyl propionylacetate, add diazo group and convert it to the enolate form using TMS-OTf (44) in the presence of a base. Then, it was coupled with the commercial acetate (47) using ZnCl₂ as a trial reaction. After successful trial using methyl
propionylacetate, compound 4 was tested using the same conditions as shown in Scheme 17. The synthesis started by adding PNB protecting group to methyl propionylacetate (73) by mixing them together at 170 °C. The PNB alcohol attacks the carbonyl that belongs to the ester, and the methanol is distilled out of the system (78). After column purification, the product 42 was taken into CH₂Cl₂ and triethylamine was added to remove the proton between the carbonyls to form an enolate (79). The double bond of the enolate is reacted with methanesulfonylazide, transferring a diazo group (80) as shown in Scheme 18. A TMS group is then attached to the carbonyl to prepare the compound 44 to be coupled with compound 4. Compound 4 is then taken with the TMS enol ether (44) and ZnCl₂. The ZnCl₂ forms a bond with the acetate, which allows it to leave form the β-lactam intermediate as shown in previous reaction of Scheme 11. The TMS enol ether is then coupled with the β-lactam to yield 45 R and 45 S as shown in Scheme 18.
Scheme 18. Adding a methyl at the C1 position to β-lactam with methyl at the C5 position

The diastereomers mixture 45 R and 45 S was treated with HF to remove the TBS group. The next steps involve treating the β-lactam with catalytic rhodium acetate at 68 °C to cyclize the compound 66. The product would then be treated with diphenyl phosphoryl chloride and diisopropylethylamine at -35 (67). The thiol side chain would then be added with diisopropylethylamine to form the intermediate 68 shown in Scheme 18, which would then hydrogenated at 55 psi in EtOAc and pH = 6 phosphate buffer to remove the p-nitrobenzyl
protecting group and produce the anticipated antibiotic 69 shown in Scheme 18. This scheme continues to be explored.
CHAPTER IV
C2 SUBSTITUTION

Modification of the C2 Position

The position at C2 is very important to carbapenem antibiotics. Most β-carbapenems have a sulfur linked to a basic amino group at the C2 position to produce porin-mediated transport across the OM. Although primary amines decrease the stability of a β-lactam due to the ability of the of primary amine to act as a nucleophile and attack the β-lactam carbonyl group, like the case of thienamycin, the basicity of this amine functionality increases the antibiotic activity. Therefore, our lab attempted to synthesize an antibiotic with amino group directly bonded at the C2 position as shown in Scheme 19.

![Scheme 19](image)

Scheme 19. Scheme of compound 32 synthesis.
a) \( \text{CH}_2\text{Cl}_2 \), Compound 5, \( \text{ZnCl}_2 \), 48 °C. b) \( \text{CH}_3\text{CN} \), HF. c) \( \text{EtOAc} \), \( \text{Rh}_2(\text{OAc})_4 \), 60 °C. d) \( \text{CH}_3\text{CN} \), \( (\text{PhO})_2\text{P(O)Cl} \), DIPEA, 0 °C. e) \( \text{EtOAc} \), 18-crown 6, \( \text{KN}_3 \). f) THF, \( \text{Et}_3\text{N} \), H\( _2\text{S} \), 0 °C. g) \( \text{EtOAc} \), pH = 6 Na\( _2\text{HPO}_4 \) (0.3 M) buffer, Pd/C, H\( _2 \).

It was decided to make the antibiotic with a primary amine at C2 and evaluate it against 21\textsuperscript{st} century pathogens. If it showed potency, the same compound would be synthesized with a methyl group at C5 to add stability to the structure and make it a potential antibiotic against MTB. The synthesis of the amine compound 30 was successful (Scheme 19), but the deprotection of the \( p \)-nitrobenzyl by hydrogenolysis to yield antibiotic 31 was never successful. Different methods to deprotect compound 30 were used but were unsuccessful. After observing the instability of the amine compound, it was decided to acylate it in order to add stability through the resonance between the nitrogen and the carbonyl. However, the acylation of the amine was also unsuccessful with different methods described in Scheme 20.

The carbapenem was synthesized by starting with commercial azetidinone with the acetoxy group at C4 (47). The product was then coupled with TBS enol ether \( p \)-nitrobenzyl ester (5) in order to provide the structure for the five-membered ring next to the \( \beta \)-lactam ring (25). The product was then treated with 1 mL of HF in order to remove the TBS protecting group (26). The \( \beta \)-lactam was then treated with rhodium acetate at 68 °C to cyclize the compound (27). The diazo functional group generated \( \text{N}_2 \) gas and generated a carbene. The generated rhodium carbenoid formed the ring closure of the five-membered ring (28). The product was then treated with diisopropylethylamine at -35 °C. The base and diphenyl phosphoryl chloride converted the ketone to the enol phosphate (28), which is a good leaving group for the next reaction. The product was then reacted with potassium azide in the presence of 18-crown-6 in order to for the azide to displace the phosphate (29) as shown in Scheme 19. The 18-crown-6 complexes the potassium into the
solution allowing the azide to couple with the compound. The azide β-lactam was then dissolved in anhyd THF, treated with triethylamine, and hydrogen sulfide was bubbled through the flask at 0 °C for a few minutes to reduce the azide to the amine (30). The product was then hydrogenated at 55 psi in EtOAc and phosphate buffer solution (pH 6) to remove the p-nitrobenzyl protecting group (31), however, this step failed despite multiple attempts. Knowing that amines are very sensitive, ethanol was substituted in the last step with 1 equiv of NaHCO₃ in DI water and THF. However, this attempt was also unsuccessful.

Since carbapenem-derived amines are very sensitive, I attempted to acylate compound 30 to add stability through the resonance between the carbonyl and nitrogen. It was thought that the acylated nitrogen would add some chemical stability and allow the compound to go through hydrogenolysis without decomposition. This reaction was carried out by taking the azide β-lactam (compound 30) in dichloromethane. The flask was charged with triphenylphosphine to convert the azide to amine through the Staudinger reaction, which is a mild reduction. Triphenylphosphine reacts with the azide to generate a phosphazide as shown in Scheme 20. Iminophosphorane is formed (80) as a part of the intermediate as shown in Scheme 20, and N₂ is generated (81). A proton source is provided by the work up of the reaction for the nitrogen to form an amine, and a stable phospine oxide is formed as a side product. However, this reaction failed. It was hypothesized that acetyl chloride might have been too strong of an acylating reagent, therefore, it was replaced with acetic anhydride. However, the reaction still did not show any product formation. Then, the amine β-lactam (compound 32) was treated with acetic anhydride in attempt to acylate the amine, however, the reaction failed once more.
Scheme 20. Method A) Reacting compound 29 with triphenylphosphine then acetyl chloride in method 1 and acetic acid in method 2 in attempt to yield acylated amine β-lactam. Method B) reacting compound 29 with hydrogen sulfide to yield compound 30, and then reacting compound 30 in attempt to yield acylated amine.

Realizing that acetic acid was not a suitable reagent for this reaction, the azide compound was dissolved in dichloromethane and treated with thioacetic acid in the presence of lutidine (Scheme 21). The lutidine deprotonates thioacetic acid (83) to allow it to react with the azide to form a thiotriazoline as an intermediate (84) as shown in Scheme 21, and then the nitrogen is proposed to acylate (33). However, that reaction failed as well.
**Scheme 21.** Attempt of acylating compound 30 using thioacetic acid.

The acylation of the azide carbapenem and compound 30 were attempted using several methods Table 4, however, all methods failed. Acetyl chloride along with PPh₃, acetyl chloride with lutidine and H₂S, and acetyl chloride with triethylamine and H₂S have all failed. Neither did exchanging acetyl chloride with acetic anhydride in all three different methods not using thioacetic acid help the reaction succeed. It was concluded that the amine compound was too unstable to be converted to an antibiotic.
Table 4. Methods in attempt to acylate compound 30.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound 29</th>
<th>Compound 30</th>
<th>Acylating reagent</th>
<th>Base</th>
<th>Other reagent</th>
<th>Solvent</th>
<th>yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>1 eq</td>
<td>2 eq Acetyl Chloride</td>
<td>4 eq Lutidine</td>
<td>H₂S</td>
<td>THF</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1 eq</td>
<td>-</td>
<td>1.9 eq Acetyl Chloride</td>
<td>-</td>
<td>1.4 eq PPh₃</td>
<td>CH₂Cl₂</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1 eq</td>
<td>-</td>
<td>0.6 eq Acetyl Chloride</td>
<td>-</td>
<td>0.8 eq PPh₃</td>
<td>CDCl₃</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1 eq</td>
<td>-</td>
<td>0.6 eq Acetic anhydride</td>
<td>-</td>
<td>0.8 eq PPh₃</td>
<td>CDCl₃</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1 eq</td>
<td>0.6 eq Acetic anhydride</td>
<td>Triethylamine</td>
<td>H₂S</td>
<td>CH₂Cl₂</td>
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<tr>
<td>6</td>
<td>1 eq</td>
<td>-</td>
<td>1.3 eq Thioacetic acid</td>
<td>1.3 eq Lutidine</td>
<td>-</td>
<td>MeOH</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1 eq</td>
<td>-</td>
<td>1.3 eq Thioacetic acid</td>
<td>1.3 eq Lutidine</td>
<td>-</td>
<td>CH₂Cl₂</td>
<td>0</td>
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<tr>
<td>8</td>
<td>1 eq</td>
<td>-</td>
<td>0.8 eq Acetyl Chloride</td>
<td>-</td>
<td>0.65 eq PPh₃</td>
<td>CDCl₃</td>
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</tr>
</tbody>
</table>
Summary

During this research, I discovered that an amine group directly bonded to the C2 position reduces the stability of the carbapenem, making it hard to produce antibiotics with amine at C2. Also, during this research I was able to optimize reaction conditions for compounds 3, 4, 15 and 16, which now permits large productions. I generated compounds that are active against Mtb, Mab and A. bumannii, and stable in the presence of OXA-23 β-lactamase. I was able to synthesize an antibiotic that is better than the commercial antibiotic, meropenem, against Mtb and Mab. Also, the synthesized antibiotics worked as inhibitors against A. bumannii in the presence of OXA-23 β-lactamase. Antibiotics were combined with meropenem and increased its potency by more than 2000 fold.
Experimental

Methylmagnesium iodide (1). A dry 500 mL 3-necked round-bottom flask (w/stir bar) with a reflux condenser was charged with magnesium turnings (30.5 g, 1.26 mol, 1.5 eq) in 200 mL of anhyd Et₂O and a catalytic amount of iodine crystals were added. A solution of methyl iodide (118.6 g, 52 mL, 0.835 mmol) in 50 mL of anhyd Et₂O was then added to the solution dropwise at a rate as to maintain gentle reflux. The reaction was allowed to stir for 3-24 h.

Copper iodide dimethyl sulfide complex (2). A dry 5 L 3-neck round-bottom-flask (w/overhead stir) was charged with copper iodide (79 g, 0.42 mol) in 2 L of anhyd THF. Dimethyl sulfide (25.8 g, 30.72 mL, 0.42 mol) was added to the solution at rt, and the solution was then cooled to −60 °C.

4-Methyl-3-[1-(t-butyldimethylsilyloxy)ethyl]-2-azetidinone (3). The copper iodide dimethyl sulfide (2) mixture was cooled to -60 °C and methylmagnesium iodide solution (1) was added to the flask. The mixture was warmed to −10 to 0 °C and was allowed to stir for 30 min. The mixture was then cooled to −60 °C and azetidinone (4-Oxo-3-[1-(1,1,2,2-tetramethylpropoxy)ethyl]-2-azetidinyl acetate) (60 g, 0.208 mol) was added to the flask. The reaction was allowed to warm to rt over the course of 45 min. The reaction was quenched with slow addition of satd aq NH₄Cl. The mixture was poured into a 3 L round-bottom flask and the THF was removed. The product was dissolved in EtOAc and washed with 2x dilute aq NH₄OH solution. The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The product was purified by silica gel flash chromatography via gradient elution (2.5:97.5 EtOAc/CH₂Cl₂ to 40/60 EtOAc/CH₂Cl₂) to afford 3 (50 g, 83% yield) as a white solid.

1H NMR (400 MHz, CDCl₃): δ 6.38 (s, 1H), 5.29 (s, 1H), 4.19 (m, 1H), 3.83 (m, 1H), 2.69 (m, 1H), 1.3 (dd, J= 54 Hz, 3H), 0.86 (s, 9H), 0.067 (S, 6H).
\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): δ 168.99, 65.74, 65.50, 47.88, 25.74, 25.59, 22.33, 20.59, 17.77, -4.34, -4.62, -4.75.

IR: 3415.79, 3229.86, 2959.39, 2929.27, 2893.85, 2857.09, 2708.17, 2249.20, 1754.52, 1471.48, 1462.92, 1446.72, 1378.34, 1347.43, 1333.39, 1299.97, 1254.06, 1187.16, 1143.14, 1096.71, 1037.92, 1005.93, 986.68, 956.49, 909.25, 835.17, 809.60, 766.45, 734.96, 661.74, 646.36.

2-Methyl-4-oxo-3-[1-(t-butyldimethylsilyloxy)ethyl]ethyl]-2-azetidinyl acetate (4). A dry 100 mL round-bottom flask (w/ stir bar) was charged with compound 3 (5 g, 20 mmol) in 70 mL of dry EtOAc. The solution was subsequently treated with sodium acetate (1.5 g, 18.3 mmol, 0.9 eq), acetic acid (11.55 g, 11 mL, 192 mmol, 9.6 eq) and ruthenium trichloride (dried using a Bunsen burner under vacuum) (0.3 g, 1.44 mmol, 7 mol%). The flask was cooled to 12-15 °C and oxygen pressure (12 psi) was applied to the reaction. Acetaldehyde (11.8 g, 15 mL, 268 mmol, 13.4 eq) was freshly distilled twice and added via syringe. The reaction was monitored by NMR. Once completed, the reaction was poured into cold hexane (1L). The mixture was extracted with cold hexane and washed with cold satd aq NaCl until the pH reached 7. The organic layer was dried over Na\(_2\)SO\(_4\) and evaporated in vacuo to afford 4 (4.55 g, 91% yield) as a purple oil form. \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.05 (S, 1H), 4.31 (m, 1H), 3.05 (d, J= 9.2 Hz, 1H), 2.04 (s, 3H), 1.82 (s, 3H), 1.33 (d, J= 6Hz, 3H), 0.86 (s, 9H), 0.067 (s, 6H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): δ 170.22, 166.39, 88.61, 70.25, 68.75, 67.32, 64.69, 25.49, 21.90, 19.69, 17.64, 0.82, -3.98, -4.41.

IR: 3327.71, 2957.92, 2931.39, 2887.40, 2858.22, 2253.32, 1781.31, 1472.47, 1463.20, 1416.50, 1362.49, 1254.34, 1222.80, 1171.03, 1092.37, 1015.43, 963.78, 914.20, 835.01, 812.13, 778.15, 733.78, 647.79.
(p-Nitrophenyl)methyl 2-diazo-3-(t-butyldimethylsilyloxy)-3-butenoate (5). A dry 1 L round-bottom flask (w/ stir bar) was charged with p-nitrobenzyl-2-diazoacetoacetate (52.6 g, 0.2 mol) in 400 mL dry CH₂Cl₂, and the flask was cooled to 0 °C. Triethylamine (40 mL, 0.29 mol, 1.4 eq) was added to the flask and TBS-OTf (63.44 g, 55 ml, 0.24 mol, 1.2 eq) was added. The reaction was warmed to room temperature and allowed to stir until completed. Once completed, the solution was washed three times with ice water. The organic layer was dried over Na₂SO₄, evaporated in vacuo and further dried under high vacuum overnight.

¹H NMR (400 MHz, CDCl₃): δ 7.92 (dd, J= 287 Hz, 8.4 Hz 4H), 5.31 (s, 2H), 4.98 (s, 1H), 4.98 (s, 1H), 0.86 (s, 9H), 0.07 (s, 6H).

¹³C NMR (100 MHz, CDCl₃): δ 163.64, 160.90, 147.91, 147.69, 143.04, 142.18, 140.29, 128.62, 128.28, 123.96, 123.82, 90.56, 65.34, 64.73, 28.25, 25.60, 25.52, 18.04, 17.93, -3.64, -4.84.

IR: 2929.35, 2858.14, 2138.91, 2109.84, 1698.47, 1667.19, 1603.85, 1522.60, 1532.99, 1385.52, 1344.24, 1297.1254, 1153.99, 1109.18, 1082.80, 837.67, 739.32.

(p-Nitrophenyl)methyl2-diazo-4-{2-methyl-4-oxo-3-[1-(t-butyldimethylsilyloxy)ethyl]-2-azetidinyl}acetoacetate (6). A dry 100 mL round-bottom flask (w/ stir bar) with a reflux condenser was charged with compound 4 (3 g, 10.4 mmol) and compound 5 (5.9 g, 15.6 mmol, 1.5 eq) in 25 mL dry CH₂Cl₂. 1M ZnCl₂ in ether (7.3 mL, 7.28 mmol, 0.7 eq) was added to the reaction and the flask was heated to 42 °C for 20 min at reflux. Once completed, the reaction was cooled to rt and then diluted with EtOAc. The solution was washed with sated aq NaHCO₃ once and extracted with EtOAc twice. The organic layer was dried over Na₂SO₄ and evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (2.5:97.5 EtOAc/ CH₂Cl₂ to 40:60 EtOAc/ CH₂Cl₂) to afford 6 (0.92 g, 31% yield) as solid.
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.92 (dd, $J= 287$ Hz, 8.4 Hz 4H), 6.38 (s, 1H), 5.35 (d, $J= 14$Hz, 2H), 4.27 (t, $J= 2.8$ Hz, 1H), 3.59 (dd, $J= 292$ Hz, 16.8 Hz, 2H), 2.85 (s, 1H), 1.538 (s, 3H), 1.40 (d, $J=12$, 3H), 1.33 (t, $J= 13.6$ Hz, 2 H), 0.085 (s, 9H), 0.866 (S, 6H).

$^{13}$C NMR (100 MHz, CDCl$_3$): 189.84, 167.09, 160.56, 147.85, 141.95, 128.66, 128.56, 123.88, 123.81, 66.80, 65.43, 65.21, 55.71, 49.90, 25.33, 25.29, 21.15, 19.78, 17.76, -3.30, -4.82.

($p$-Nitrophenyl)methyl2-diazo-4-[3-(1-hydroxyethyl)-2-methyl-4-oxo-2-azetidinyl]acetoacetate (7). A 50 mL round-bottom flask (w/ stir bar) was charged with compound 6 (2g, 7.8 mmol) in 20 mL of dry acetonitrile, and 1 mL of HF was added. The reaction was stirred at rt. Once completed (1-3 h), finely ground NaHCO$_3$ was added to the reaction to attain pH= 7. The reaction was filtered to remove the NaF and evaporated in vacuo to afford 7 (1.4 g, 71% yield) as white solid.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.91 (dd, $J= 280$ Hz, 8.3 Hz, 4H), 5.34 (q, $J=18.4$ Hz, 13.2 Hz, 2H), 4.84 (s, 1H), 4.39 (m, 1H), 3.22 (d, $J= 10$ Hz, 1H), 2.7 (dd, $J= 44.4$, 18 Hz, 2H), 1.63 (s, 3H), 1.42 (d, $J= 6.4$ Hz, 3H), 0.89 (s, 1H), 0.085 (s, 2H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 190.53, 167.18, 160.14, 147.43, 141.86, 128.37, 123.50, 65.32, 63.51, 54.89, 49.67, 49.07, 48.64, 48.00 21.24, 20.29.

IR: 3362.70, 2969.02, 2143.11, 1722.43, 1648.53, 1522.75, 1347.90, 1306.60, 1216.14, 1127.81, 1025.25, 853.56, 739.51.

($p$-Nitrophenyl)methyl6-(1-hydroxyethyl)-5-methyl-3,7-dioxoazabicyclo[3.2.0]heptane-2-carboxylate (8). A 100 mL round-bottom flask (w/ stir bar) with a reflux condenser was charged with compound 7 (1.3g, 3.33 mmol) and Rh$_2$(OAc)$_4$ (35 mg, 0.08 mm, 0.024 eq) in 50 mL dry EtOAc. The reaction was heated to 60 °C for 30 min. Once completed, reaction was cooled to room temperature and was evaporated in vacuo.
1H NMR (400 MHz, CDCl3): δ 7.96 (dd, J= 232 Hz, 8.4 Hz, 4H), 5.31 (q, J= 19.6 2H), 4.15 (q, J= 8 Hz, 1H), 3.68 (q, J= 10 Hz, 1H), 3.18 (d, J= 4Hz, 1H), 2.65 (dd, J= 40 Hz, 20 Hz, 1H), 2.08 (s, 3H), 1.55 (dd, J= 30 Hz, 15 Hz, 3H), 1.45 (d, J= 15 Hz, 1H), 1.28 (m, 1H).

(p-Nitrophenyl)methyl6-(S)-6-[(R)-1-hydroxyethyl]-3-{(3S,5S)-5-(dimethylamino)carbonyl-1-[(p-nitrophenyl)methyl]-3-pyrrolidinylthio]-5-methyl-7-oxazabicyclo[3.2.0]hept-2-ene-2-carboxylate (9). A 50 mL round-bottom flask (w/ stir bar) was charged with compound 8 (1.3g, 3.59 mmol) in 10 mL of dry CH3CN and was cooled to -35 °C. Diphenyl phosphoryl chloride (0.93 g, 0.72 mL, 3.59 mmol, 1 eq) was then added to the flask followed by a slow addition of N,N-diisopropylethylamine (0.45 g, 3.5 mmol, 1 eq), and the reaction was allowed to stir for 30 minutes. Once completed, 4-nitrobenzyl (2S,4S)-2-(dimethylcarbamoyl)-4-mercapto-1-pyrrolidinecarboxylate (1.26 g, 0.9 mL, 3.59 mmol, 1 eq) and an additional 1 eq of DIPEA was added. Once completed, the reaction was extracted with CH2Cl2 and washed with satd aq NH4Cl. The resultant solution was then evaporated in vacuo to afford 9 (1.1 g, 85% yield) as solid.

1H NMR (400 MHz, CDCl3): δ 8.25 (d, J= 8.8 Hz, 2H), 7.67 (d, J= 8.4 Hz, 2H), 7.50 (dd, J= 28.8 Hz, 8.4 Hz, 2H), 5.54 (d, J= 13.6 Hz, 2H), 5.24 (m, 1H), 4.74, (m, 1H), 4.31 (m, 1H), 3.57 (m, 1H), 3.25 (dd, J= 14.4 Hz, 2H), 3.09 (dd, J= 78 Hz, 4 Hz, 6H), 2.8 (m, 1H), 1.99 (m, 1H), 1.62 (d, J= 6 Hz, 3H), 1.45 (d, J= 6 Hz, 3H).

IR: 3429.25, 3114.77, 3080.51, 2970.22, 1773.76, 1708.32, 1648.96, 1606.81, 1520.54, 1429.55, 1403.63, 1375.21, 1345.86, 1287.63, 1208.91, 1173.33, 1149.68, 1111.84, 1046.69, 1014.02, 853.63, 853.63, 803.04, 778.75, 765.87, 736.85, 684.52.

(6S)-6-[(R)-1-Hydroxyethyl]-3-[(3S,5S)-5-(dimethylamino)carbonyl-3-pyrrolidinylthio]-5-methyl-7-oxazabicyclo[3.2.0]hept-2-ene-2-carboxylate (10). A 100 mL round-bottom flask (w/ stir bar) was charged with compound 9 (0.55 g, 0.79 mmol) in 80 mL of dry EtOAc and 40 mL of
pH6 phosphate buffer solution (0.3 M). After compound 9 was dissolved, 10% Pd on carbon (0.55 g, 5.2 mmol, 6.6 eq) was added, and the vessel was degassed and then subjected to hydrogen pressure 55 psi in a Parr hydrogenation device for 90 min. Once completed, the solution was filtered through celite, the aqueous layer was separated and washed with Et₂O. The organic solvents were then removed from the aqueous layer and the product isolated by column chromatography on Diaion CHP20P resin. Tubes containing the product were identified by inspection of the UV of each fraction, combined, and the water was partially removed in vacuo. The remaining aqueous solution was lyophilized to produce the purified antibiotic 10 (0.1385 g, 25% yield) as white solid.

¹H NMR (400 MHz, CDCl₃): δ 4.85 (s, 1H), 4.60 (t, J= 8.4 Hz, 1H), 4.33 (m, 1H), 3.98 (t, J= 6.4 Hz, 1H), 3.64 (q, J= 6.8 Hz, 1H), 3.36 (m, 2H), 3.09 (d, J= 47 Hz, 6H), 2.90 (d, J= 22 Hz, 2H), 1.87 (m, 1H), 1.55 (s, 3H), 1.34 (d, 66 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃): δ 178.0, 170.0, 168.5, 137.5, 129.7, 66.5, 66.4, 63.00, 60.5, 58.8, 52.0, 47.6, 41.7, 37.5, 36.5, 36.4, 22.0, 21.5.

(p-Nitrophenyl)methyl (6S)-6-[(R)-1-hydroxyethyl]-3-(diphenylphosphate) -5-methyl-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (11). A 50 mL round-bottom flask (w/ stir bar) was charged with compound 8 (1.2 g, 3.31 mmol) in 10 mL of dry CH₃CN and was cooled to -35 °C. Diphenyl phosphoryl chloride (0.86 g, 0.66 mL, 3.31 mmol, 1 eq) was added to the flask followed by a slow addition of N,N-diisopropylethylamine (0.42g, 0.56 mL, 3.31 mmol, 1 eq). Once completed, the reaction was washed with aqueous NH₄Cl and extracted with CH₂Cl₂, and then evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (0.5/99.5 MeOH/CH₂Cl₂ to 6/96 MeOH/ CH₂Cl₂) to afford 11 (0.92 g, 31% yield) as solid.
\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.88 (dd, J= 236 Hz, 4 Hz, 4H), 7.30 (dd, J= 63 Hz, 6.8 Hz, 4 Hz, 10H), 5.36 (dd, J= 82 Hz, 14 Hz, 2 H), 4.25 (m, 1H), 3.15 (d, J= 173 Hz, 21 Hz, 2H), 3.16 (d, J= 10 Hz, 1H), 1.74 (d, J= 10 Hz, 1H), 1.58 (s, 3H), 1.43 (d, J= 18.4 Hz, 3H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 176.42, 159.18, 152.42, 150.03, 147.62, 142.83, 129.93, 128.42, 126.03, 123.78, 120.17, 117.47, 68.58, 65.28, 64.68, 59.73, 45.18, 22.68, 21.63, 1.12.

IR: 3455.73, 1778.25, 1724.91, 1637.25, 1522.40, 1488.46, 1345.79, 1297.66, 1194.34, 1012.38, 971.78, 851.65, 773.25.

\((p\text{-Nitrophenyl})\text{methyl}(6S)-6-[(R)-1\text{-hydroxyethyl}]-3-(ethylthio)-5\text{-methyl-7-}

\text{oxazabicyclo[3.2.0]hept-2-ene-2-carboxylate (12). A 10 mL tube with 4 mL of anhyd THF was}

cooled to -78°C and was charged with ethanethiol (0.20g, 0.30 mL, 3.175 mmol, 2.5 eq) followed

by nBuLi (0.49 ml, 1.27 mmol, 1 eq). The mixture was allowed to stir for 15 minutes. A separate

25 mL round-bottom flask (w/ stir bar) was charged with compound 11 (0.75 g, 1.27 mmol) in 10

mL of dry CH\(_3\)CN. The 10 mL tube-mixture was added to the flask slowly. Once completed, the

reaction was diluted with EtOAc and washed once with satd aq NH\(_4\)Cl and once with satd aq

NaHCO\(_3\). The resultant solution was evaporated in vacuo to afford 12 (0.25 g, 33% yield) as solid.

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.05 (dd, J= 224 Hz, 8.4 Hz, 4H), 5.41 (dd, J= 128 Hz, 14 Hz, 2H),

4.30 (m, 1H), 3.11 (dd, J= 138 Hz, 17.6 Hz, 1H), 3.23 (d, J= 10Hz, 1H), 2.95 (d, J= 10 Hz), 3.9

(m, 2H), 1.69 (s, 3H), 1.45 (d, 6 Hz, 3H), 1.37 (t, 7.2 Hz, 3H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 174.92, 160.99, 149.97, 147.48, 143.28, 128.00, 123.69, 121.68, 76.66, 67.79, 65.08, 64.98, 60.93, 48.57, 26.56, 22.64, 21.24, 14.81.

IR: 3418.95, 1764.33, 1696.54, 1606.15, 1520.10, 1378.46, 1331.14, 1290.03, 1212.07, 1150.74, 851.88, 736.74.
(65)-6-[(R)-1-Hydroxyethyl]-3-(ethythio)-5-methyl-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (13). A 100 mL round-bottom flask (w/stir bar) was charged with compound 12 (0.25 g, 0.59 mmol) in 25 mL of EtOAc and 10 mL of pH 6 phosphate buffer solution (0.3 M). After solution was dissolved, 10% Pd on carbon (0.3 g, 2.8 mmol, 4.7 eq) was added, and the vessel was degassed and then subjected to hydrogen pressure 55 psi in a Parr hydrogenation device for 90 min. Once completed, the solution was filtered through celite, the aqueous layer was separated and washed with Et₂O. The organic solvents were then removed from the aqueous layer in vacuo, and the product isolated by column chromatography on Diaion CHP20P resin. Tubes containing the product were identified by inspection of the UV of each fraction, combined, and the water was partially removed in vacuo. The remaining aqueous solution was lyophilized to produce the purified antibiotic 13 (15.7 mg, 6.4% yield) as white solid.

**1H NMR (400 MHz, CDCl₃):** δ 4.94 (s, 1H), 4.33 (m, 1H), 3.14 (dd, J = 117 Hz, 6.8 Hz, 2H), 2.82 (m, 1H), 1.51 (s, 3H), 1.34 (d, J = 6 Hz, 3H), 1.28 (t, J = 7.6 Hz, 3H).

**13C NMR (100 MHz, CDCl₃):** δ 191.14, 181.55, 154.21, 140.66, 78.60, 76.99, 73.72, 59.56, 38.47, 33.98, 32.43, 27.41, 9.83.

**Ethylmagnesium iodide (14).** A dry 500 mL- 3-neck round bottom flask. (w/ stir bar) with a reflux condenser was charged with magnesium turnings (30.5 g, 1.26 mol, 1.5 eq) and a catalytic amount of iodine crystals in 200 mL of anhyd Et₂O. A solution of ethyl iodide (130 g, 67 mL, 0.835 mol) in Et₂O was added to the solution dropwise at a rate to maintain gentle reflux.

(3S)-3-[(R)-1-(1-butyldimethylsilyloxy)ethyl]-4-ethyl-2-azetidinone (15). The copper iodide dimethyl sulfide (2) mixture was cooled to -60 °C and ethylmagnesium iodide (14) was added to the flask. The mixture was warmed to –10 °C and was allowed to stir for 5 minutes. The flask was cooled to -60 °C and acetate Azetidinone (60g, 0.209 mmol) was added to the flask. Once the
reaction completed, it was warmed to room temperature. The reaction was quenched with slow addition of satd aq NH$_4$Cl. The mixture was poured into a 3 L round-bottom flask and evaporated in vacuo. The product was dissolved in EtOAc and washed with dilute NH$_4$OH. The EtOAc layer was dried over Na$_2$SO$_4$ and evaporated. The product was then purified by silica gel flash chromatography via gradient elution (2.5:97.5 EtOAc/CH$_2$Cl$_2$ to 40/60 EtOAc/CH$_2$Cl$_2$) to afford 15 (48.7 g, 81% yield) as a white solid.

$^1$H NMR (400 MHz, CDCl$_3$): δ 5.32 (s, 1H), 2.5 (m, 1H), 2.14 (s, 1H), 1.87 (t, j= 3.2 Hz, 3H), 1.26 (q, j= 2.4 Hz, 2H), 0.92 (s, 9H), 0.094 (s, 6H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 169.20, 65.66, 63.92, 52.69, 27.98, 25.88, 22.75, 22.74, 17.94, 10.70, -4.26, -4.91.

IR: 3311.64, 2931.54, 2886.10, 2857.70, 2739.21, 2709.67, 1782.31, 1464.07, 1369.46, 1251.31, 1160.75, 1102.39, 1016.07, 990.17, 945.01, 885.89, 834.36, 812.53, 776.38, 752.23, 660.51.

(3R)-3-[(R)-1-(t-butyldimethylsilyloxy)ethyl]-2-ethyl-4-oxo-2-azetidinyl acetate (16). A dry 500 mL round-bottom flask (w/ stir bar) was charged with compound 15 (10 g, 38.8 mmol) in 280 mL of dry EtOAc. The solution was subsequently treated with sodium acetate (1.8 g, 22.0 mmol, 0.6 eq), acetic acid (23.1 g, 22 mL, 384 mmol, 9.9 eq) and ruthenium trichloride (dried using a Bunsen burner under vacuum) (3 g, 14 mmol, 37 mol%) was added to the flask. The flask was then cooled to 12-15 °C and oxygen pressure (12psi) was applied to the reaction. Acetaldehyde (23.6 g, 30 mL, 536 mmol, 13.8 eq) was freshly distilled twice and added via syringe. The flask was kept closed at all times. Once completed, the reaction was poured into cold hexane. The mixture was extracted with cold hexane and washed with iced satd aq NaCl until pH reached 7. The organic layer was dried over Na$_2$SO$_4$ and evaporated in vacuo to afford 16 (4.55 g, 91% yield) as purple oil form.
H NMR (400 MHz, CDCl₃): δ 5.89 (s, 1H), 4.29 (m, 1H), 2.64 (q, J = 0.8 Hz, 2H), 1.27 (d, J= 3Hz, 3H), 1.05 (t, J = 3.2 Hz, 3H), 0.873 (s, 9H), 0.088 (s, 6H).

C NMR (100 MHz, CDCl₃): 170.38, 166.22, 90.01, 66.10, 64.47, 28.77, 25.51, 22.21, 21.52, 17.72, 9.02, 0.84, -4.13, -4.76.

IR: 3092.33, 2963.11, 2927.92, 2895.10, 2853.16, 2282.96, 1754.39, 1710.67, 1463.12, 1444.34, 1381.09, 1369.22, 1348.24, 1332.02, 1300.65, 1252.41, 1185.02, 1139.74, 1099.13, 1066.49, 1047.11, 961.20, 835.24, 1139.74, 1109.13, 1066.49.

(p-Nitrophenyl)methyl2-diazo-4-{2-ethyl-4-oxo-3-[1-(t-butyldimethylsilyloxy)ethyl]-2-azetidinyl}acetoacetate (17). A dry 250 mL round-bottom flask (w/stir bar) with a reflux condenser was charged with compound 16 (10 g, 38.85 mmol) and compound 5 (21.2 g, 56.5 mmol, 1.5 eq) in 50 mL dry CH₂Cl₂. 1M ZnCl₂ in Et₂O (27 mL, 27.7 mmol, 0.7 eq) was added to the reaction and the flask was heated to 48 °C. Once completed, the reaction was cooled to room temperature and then diluted with EtOAc. The solution was washed with satd aq NaHCO₃ once and extracted with EtOAc twice. The organic layer was dried over Na₂SO₄ and then evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (2.5:92.5 EtOAc/ CH₂Cl₂ to 40/60 EtOAc/ CH₂Cl₂) to afford 17 (3.9 g, 39% yield) as solid.

H NMR (400 MHz, CDCl₃): δ 7.93 (d, J = 282 Hz, 20.4 Hz, 4 H), 6.21 (s, 1H), 5.36 (d, J = 36.8 Hz, 2 H), 4.29 (m, 1 H), 3.74 (dd, J = 343 Hz, 14.8 Hz, 2 H), 3.14 (d, J= 8.8 Hz, 1 H), 2.02 (q, J= 7.6 Hz, 1H), 1.90 (q, J = 7.6 Hz, 1 H), 1.34 (d, J = 36.8 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H), 0.932 (s, 9H), 0.138 (s, 6H).

C NMR (100 MHz, CDCl₃): δ 199.20, 189.52, 168.88, 167.61, 160.90, 147.94, 141.85, 128.63, 123.95, 65.73, 61.61, 59.41, 52.50, 46.92, 27.89, 25.69, 22.64, 22.68, 17.82, 10.60, 8.05, 0.92, -3.33, -4.36.
IR: 3258.47, 2958.53, 2930.65, 2884.27, 2856.98, 2140.77, 1751.98, 1651.27, 1608.18, 1525.54, 1471.60, 1462.71, 1375.46, 1347.80, 1258.48, 1214.02, 1188.42, 1103.18, 1039.56, 987.42, 957.23, 896.93, 834.10, 811.33, 776.83, 739.51.

\((p\text{-Nitrophenyl})\text{methyl2-diazo-4-[3-(1-hydroxyethyl)-2-ethyl-4-oxo-2-azetidinyl]acetoacetate}\) (18). A 100 mL round-bottom flask (w/ stir bar) was charged with compound 17 (6g, 7.8 mmol) in 20 mL of acetonitrile, and 1 mL of HF was then added. The reaction stirred at rt and monitored by TLC. Once completed, finely ground NaHCO₃ was added to the reaction to retain pH = 7. The reaction was filtered to remove the NaF and evaporated in vacuo to afford 17 (2.34 g, 39% yield) as white solid.

\(^1\)H NMR (400 MHz, CDCl₃): δ 7.90 (dd, J= 289 Hz, 8.5 Hz, 4H), 5.35 (s, 2H), 4.23 (m, 1H), 3.35 (dd, J= 260 Hz, 18.4 Hz, 2 H), 2.05 (m, 1H), 1.88 (m, 1H), 1.35, (d, J= 4 Hz, 3H), 0.92 (t, J= 7 Hz, 3H).

\(^{13}\)C NMR (100 MHz, CDCl₃): δ 190.98, 167.27, 160.44, 147.99, 141.88, 128.82, 124.02, 66.76, 65.74, 63.57, 58.61, 46.13, 26.04, 21.85, 8.57.

IR: 3457.32, 2967.10, 2348.64, 2145.69, 1722.53, 1640.40, 1607.84, 1523.19, 1347.83, 1316.92, 1261.20, 1214.38, 1129.57, 1040.55, 1015.59, 853.38, 799.72, 738.72.

\((p\text{-Nitrophenyl})\text{methyl5-ethyl-6-(1-hydroxyethyl)-3,7-dioxoazabicyclo[3.2.0]heptane-2-carboxylate}\) (19). A dry 100 mL round-bottom flask (w/ stir bar) with a reflux condenser was charged with compound 18 (2.34 g, 5.79 mmol) and Rh₂(OAc)₄ (50 mg, 0.11 mmol, 0.019 eq) in 40 mL dry EtOAc. The reaction was heated to 80 °C for 30 min. Once completed, reaction was cooled to room temperature and was evaporated in vacuo.
1H NMR (400 MHz, CDCl$_3$): $\delta$ 7.94 (dd, J= 272 Hz, 88 Hz, 4 H), 5.40 (dd, J= 38 Hz, 13.2 Hz, 2 H), 4.83 (s, 1H), 4.45 (m, 1H), 3.25 (d, J= 9.6 Hz, 1 H), 2.5 (dd, J= 158 Hz, 17.6 Hz, 2H), 1.95 (m, 2H), 1.49 (d, J= 3.5 Hz, 3H), 1.16 (dt, J=108 Hz, 7.2 Hz, 3H).

13C NMR (100 MHz, CDCl$_3$): $\delta$ 189.62, 167.71, 161.00, 141.95, 128.73, 124.05, 65.55, 61.71, 52.60, 46.02, 27.46, 25.75, 22.68, 17.92, 10.70, 8.15, 1.02, -3.23, -4.26.

(p-Nitrophenyl)methyl (6S)-6-[(R)-1-hydroxyethyl]-3-[(3S,5S)-5-(dimethylamino)carbonyl-1-[(p-nitrophenyl)methyl]-3-pyrrolidinylthio]-5-ethyl-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (20). A dry 50 mL round-bottom flask (w/ stir bar) was charged with compound 19 (2.34 g, 6.22 mmol) in 30 mL of dry CH$_3$CN and was cooled to -35° C. Diphenyl phosphoryl chloride (1.4 g, 1.1 mL, 6.22 mmol, 1 eq) was added to the flask followed by a slow addition of N,N-diisopropylethylamine (0.74 g, 1 mL, 6.22 mmol, 1 eq). Once completed, the side chain, 4-nitrobenzyl (2S,4S)-2-(dimethylcarbamoyl)-4-mercapto-1-pyrrolidinecarboxylate (1.26 g, 0.9 mL, 3.59 mmol, 1 eq) and an additional 1 eq of DIPEA was added. Once completed, the reaction was extracted with CH$_2$Cl$_2$ and washed with satd aq NH$_4$Cl. The resultant solution was then evaporated in vacuo to afford 20 (1.8 g, 77% yield) as solid.

1H NMR (400 MHz, CDCl$_3$): $\delta$ 8.20 (m, 4H), 7.64 (d, J= 8.8 Hz, 2H), 7.47 (dd, J= 35 Hz, 8.8 Hz, 2 H), 5.52 (d, J= 14 Hz, 2H), 5.22 (m, 1H), 5.18 (dd, J= 77.2 Hz, 14 Hz, 2H), 4.15 (m, 1H), 3.65 (m, 1H), 3.55 (m, 1H), 3.26 (m, 2H), 3.09 (d, J= 78 Hz, 6H), 2.75 (m, 1H), 2.04 (m, 1H), 1.91 (m, 2H), 1.40 (d, J= 6 Hz, 3H), 0.99 (t, J= 7.2 Hz, 3 H).

13C NMR (100 MHz, CDCl$_3$): $\delta$ 175.86, 170.52, 160.54, 153.49, 153.02, 147.43, 146.01, 143.75, 143.06, 127.95, 124.34, 124.21, 123.66, 123.57, 69.04, 65.73, 65.13, 64.28, 64.17, 56.18, 55.85, 53.80, 52.90, 45.98, 41.44, 40.70, 37.14, 36.89, 36.06, 27.60, 22.67, 7.68, 0.91.
IR: 3412.08, 2917.18, 2282.69, 1770.98, 1606.39, 1520.45, 1403.11, 1345.69, 1281.09, 1206.45, 1147.09, 1110.40, 1036.35, 735.94.

(6S)-6-[(R)-1-Hydroxyethyl]-3-[(3S,5S)-5-(dimethylamino)carbonyl-3-pyrrolidinylthio]-5-ethyl-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (21). A Parr hydrogenation vessel was charged with compound 20 (0.6 g, 0.86 mmol) in 30 mL of EtOAc and 30 mL of pH6 NaH$_2$PO$_4$ buffer solution (0.3 M). After compound 20 was dissolved, 10 % Pd on carbon (0.6 g, 5.6 mmol, 6.5 eq) was added, and the vessel was degassed and then subjected to hydrogen pressure 55 psi in a Parr hydrogenation device for 90 min. Once completed, the solution was filtered, the aqueous layer was separated and washed with Et$_2$O. The organic solvents were then removed from the aqueous layer and the product isolated by column chromatography on Diaion CHP20P resin. Tubes containing the product were identified by inspection of the UV of each fraction, combined, and the water was partially removed in vacuo. The remaining aqueous solution was lyophilized to produce the purified antibiotic 21 (0.15 g, 25% yield) as white solid.

$^1$H NMR (400 MHz, CDCl$_3$): δ 4.58 (s, 1H), 3.43 (m, 2H), 3.29 (d, J=17.6 Hz, 2H), 3.02 (d, J= 4.4 Hz, 6H), 1.98 (m, 1H), 1.85 (m, 1H), 1.33 (d, J= 6.4 Hz, 3H), 0.97 (t, J= 7.2 Hz, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 182.09, 171.06, 170.79, 138.38, 134.87, 67.02, 66.44, 61.01, 54.74, 47.27, 43.88, 39.31, 38.53, 38.05, 29.90, 214.12, 18.83, 9.83.

(P-Nitrophenyl)methyl (6S)-6-[(R)-1-hydroxyethyl]-3-(diphenylphosphate)-5-ethyl-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (22). A dry 50 mL round-bottom flask (w/ stir bar) was charged with compound 19 (2.34g, 6.22 mmol) in 30 mL of dry CH$_3$CN and was cooled to -35° C. Diphenyl phosphoryl chloride (1.4 g, 1.1 mL, 6.22 mmol, 1 eq) was added to the flask followed by a slow addition of N,N-diisopropylethylamine (0.74 g, 1 mL, 6.22 mmol, 1 eq) and the reaction stirred for 30 min. Once completed, the reaction was washed with satd aq NH$_4$Cl and extracted with CH$_2$Cl$_2$. The organic layer was dried over Na$_2$SO$_4$ and then evaporated
in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (0.5/99.5 MeOH/CH₂Cl₂ to 6/96 MeOH/CH₂Cl₂) to afford 22 (1.8 g, 77% yield) as solid form.

¹H NMR (400 MHz, CDCl₃): δ 7.83 (dd, J= 248 Hz, 5.2 Hz, 4 H), 7.28 (m, 5H), 5.32 (dd, J= 81.2 Hz, 4 Hz, 2H), 4.21 (m, 1H), 3.17 (d, J= 4 Hz, 1H), 3.17 (dd, J= 49.6 Hz, 4 Hz, 2H), 2.18 (s, 1H), 2.02 (m, 1 H), 1.84 (m, 1H), 1.30 (d, J= 6 Hz, 3H), 0.923 (t, J= 4 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃): δ 177.26, 158.85, 152.25, 152.19, 149.77, 147.49, 142.64, 129.98, 129.79, 129.52, 128.00, 126.12, 123.64, 119.93, 118.62, 118.51, 69.54, 65.16, 64.22, 62.95, 41.50, 27.74, 22.59, 7.36.

IR: 3330.57, 3076.23, 2973.44, 2252.61, 1762.98, 1639.31, 1590.35, 1522.95, 1489.09, 1457.42, 1385.69, 1347.37, 1296.95, 1186.99, 1163.02, 1106.84, 1072.32, 1046.82, 1025.78, 1012.10, 969.62, 912.30, 850.68, 776.44, 735.26, 689.20, 647.55.

(p-Nitrophenyl)methyl(6S)-6-[(R)-1-hydroxyethyl]-5-ethyl-3-(ethylthio)-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (23). A 10 mL tube (w/ stir bar) with 1.5 mL of DMF was cooled to 0 °C and was charged with compound 22 (180 mg, 0.25 mmol) and ethanethiol (25.8 mg, 30 µl, 0.375 mmol, 1.5 eq) followed by diisopropylamine (35.9 mg, 50 µl, 0.32 mmol, 1.3 eq). The mixture was allowed to stir for 1.5 h. Once completed, the reaction was diluted with EtOAc and washed once with satd aq NH₄Cl and once with satd aq NaHCO₃. The organic layer was evaporated in vacuo and purified by silica gel flash chromatography via gradient elution (0.5/99.5 MeOH/CH₂Cl₂ to 6/96 MeOH/CH₂Cl₂) to afford 23 (100 mg, 56% yield) as solid form.

¹H NMR (400 MHz, CDCl₃): δ 7.93 (dd, J= 224 Hz, 8.8 Hz, 4 H), 5.42 (dd, J= 122 Hz, 14 Hz, 2 H), 4.33 (m, 1H), 3.17 (m, 2H), 2.88 (m, 2H), 1.98 (m, 2H), 1.64 (m, 1 H), 1.44 (d, J= 64 Hz, 3H), 1.36 (t, J= 7.6 Hz, 3H), 1.03 (t, J= 7.6 Hz, 3H).
(6S)-6-[(R)-1-Hydroxyethyl]-5-ethyl-3-(ethylthio)-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate 75 (24). A Parr hydrogenation vessel was charged with compound 23 (185 mg, 0.43 mmol) in 20 mL of EtOAc and 20 mL of pH6 NaH2PO4 (0.3 M) buffer solution. After compound was dissolved, 10% Pd on carbon (0.26 g, 2.4 mmol, 5.6 eq) was added, and the vessel was degassed and then subjected to hydrogen pressure 55 psi in a Parr hydrogenation device for 90 min. Once completed, the solution was filtered through celite, the aqueous layer was separated and washed with Et2O. The organic solvents were then removed from the aqueous layer and the product isolated by column chromatography on Diaion CHP20P resin. Tubes containing the product were identified by inspection of the UV of each fraction, combined, and the water was partially removed in vacuo. The remaining aqueous solution was lyophilized to produce the purified antibiotic 24 (19.4 mg, 10 % yield) as white solid.

1H NMR (400 MHz, CDCl3): δ 4.63 (s, 1H), 4.19 (m, 1H), 3.21 (d, 9.6Hz, 1H), 3.08 (dd, J= 54, 18 Hz, 2H), 2.73 (m, 2H), 1.82 (m, 2 H), 1.194 (m, 6H), 0.816 (t, 5.2 Hz, 3 H).

13C NMR (100 MHz, CDCl3): δ 192.32, 181.42, 154.19, 142.01, 150.89, 83.18, 56.42, 39.96, 38.45, 35.30, 34.17, 32.21, 19.85, 10.83.

(p-Nitrophenyl)methyl2-diazo-4-3-[1-(t-butyldimethylsilyloxy)ethyl]-4-oxo-2-azetidinyl]acetoacetate (25). A dry 50 mL round-bottom flask (w/ stir bar) with reflux condenser was charged with 4-oxo-3-[1-(1,1,2-trimethyl-2-propenyloxy)ethyl]-2-azetidinyl acetate (6 g, 20.8
mmol) and compound 5 (11.8 g, 31.2 mmol, 1.5 eq) in 40 mL of dry CH₂Cl₂ followed by 1 M ZnCl₂ in Et₂O (14.6 ml, 14.6 mmol, 0.7 eq) and the flask was heated to 48 °C. Once completed, the reaction was cooled to room temperature and diluted with EtOAc. The solution was washed with satd aq NaHCO₃. The organic layer was dried over Na₂SO₄ and evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (2.5:97.5 EtOAc/CH₂Cl₂ to 40/60 EtOAc/CH₂Cl₂) to afford 25 (3 g, 100% yield) as yellowish solid.

1H NMR (400 MHz, CDCl₃): δ 7.89 (dd, J= 275 Hz, 8.8 Hz, 4H), 6.26 (s, 1H), 5.36 (s, 1H), 6.22 (dd, J= 70.8 Hz, 15 Hz, 2H), 3.66 (d, J= 21.6, 1H), 3.01 (m, 1H), 2.85 (s, 1H), 1.21 (d, J= 11 Hz, 3H), 0.867 (s, 9 H), 0.099 (s, 6H).

(p-Nitrophenyl)methyl2-diazo-4-[3-(1-hydroxyethyl)-4-oxo-2-azetidinyl]acetoacetate (26). A 50 mL round-bottom flask (w/ stir bar) was charged with compound 25 (3.19 g, 8.17 mmol) in 30 mL of CH₃CN followed by 1 mL of HF and the reaction was stirred for rt. Once completed, finely ground NaHCO₃ was added to the reaction to retain pH = 7. The reaction was then filtered to remove the NaF and evaporated in vacuo to afford 26 (1.6 g, 50% yield) as white solid.

1H NMR (400 MHz, CDCl₃): δ 7.88 (dd, J= 287 Hz, 2Hz, 4H), 5.32 (d, J= 23 Hz, 2H), 4.10 (t, J= 6.4 Hz, 1H), 3.415 (s, 1H), 3.12 (dd, J= 87 Hz, 5.6 Hz, 2H), 2.86 (d, J= 2Hz, 1H), 1.27 (d, J= 6Hz, 1H), 0.883 (d, J= 2.8 Hz, 3H).

13C NMR (100 MHz, CDCl₃): δ 190.39, 160.47, 141.87, 128.65, 123.86, 65.50, 64.94, 63.50, 49.45, 49.24, 49.03, 48.81, 48.60, 47.15, 44.95, 20.88.

(p-Nitrophenyl)methyl 6-(1-hydroxyethyl)-3,7-dioxoazabicyclo[3.2.0]heptane-2-carboxylate (27) A dry 250 mL round-bottom flask (w/ stir bar) with a reflux condenser was charged with compound 26 (1.64g, 4.2mmol) and Rh₂(OAc)₄ (45 mg, 0.1 mmol, 0.024 eq) in 100 mL EtOAc.
The reaction was heated to 60 °C and stirred for 30 min. Once completed, reaction was cooled to
room temperature and was evaporated in vacuo.

$^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.91 (dd, J= 283 Hz, 8.8 Hz, 4H), 5.33 (q, J= 13.2 Hz, 2H), 4.57
(m, 1H), 4.16 (m, 1H), 3.24 (d, J= 5.2 Hz, 1H), 2.75 (dd, J= 185 Hz, 12 Hz, 2H), 1.43 (d, J= 6.4 Hz, 3H).

(p-Nitrophenyl)methyl 3-diphyenyl-phosphate-(1-hydroxyethyl)-7-
oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (28). A dry 50 mL round-bottom flask (w/ stir
bar) was charged with compound 27 (1.64g, 4.7 mmol) in 12 mL of dry CH$_3$CN and was cooled
to -35 °C. Diphenyl phosphoryl chloride (1.38 g, 1.1 mL, 5.17 mmol, 1.1 eq) was added to the
flask followed by a slow addition of N,N-diisopropylethylamine (0.61 g, 0.82 mL, 4.7 mmol, 1 eq), and the reaction was stirred for 30 min. Once completed, the reaction was washed with satd
aq NH$_4$Cl and extracted with CH$_2$Cl$_2$. The organic layer was dried over Na$_2$SO$_4$ and evaporated
in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution
(0.5/99.5 MeOH/CH$_2$Cl$_2$to 6/96 MeOH/CH$_2$Cl$_2$) to afford 28 (1.0 g, 61% yield) as solid form.

$^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.16 (dd, J= 235 Hz, 4.8 Hz, 4H), 7.29 (m, 10H), 5.41 (s, 1H), 5.35
(dd, J= 71 Hz, 14 Hz, 2H), 4.24 (q, J= 6.4 Hz, 1H), 4.14 (q, J= 7.2 Hz), 3.25 (m, 1H), 2.06 (s, 1H),
1.30 (dd, J= 35 Hz, 6.4 Hz, 3H), 1.28 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 177.51, 158.73, 152.78, 152.71, 149.74, 149.67, 149.60, 147.39,
142.50, 129.91, 129.63, 127.99, 126.07, 123.54, 119.85, 119.84, 119.81, 118.31, 118.20, 67.22,
65.24, 65.13, 50.97, 36.53, 21.53, 0.89.

IR: 3338.49, 3075.45, 2981.08, 2252.36, 1944.03, 1814.40, 1744.37, 1667.35, 1491.51, 1522.67,
1489.28, 1456.48, 1386.76, 1348.11, 1290.48, 1186.77, 1072.50, 1025.52, 1011.00, 965.93,
910.95, 852.03, 776.59, 734.56, 689.66, 647.91.
(p-Nitrophenyl)methyl 6-(1-hydroxyethyl)-7-oxo-3-
(phenylsulfonyloxy)azabicyclo[3.2.0]hept-2-ene-2-carboxylate (29). A dry 500 mL round-bottom flask (w/ stir bar) was charged with compound 28 (12 g, 33.12 mmol) in 330 mL of dry CH₃CN and was cooled to 0°C. p-Toluenesulfonic anhydride (11.36 g, 36.1 mmol, 1.09 eq) and N,N-diisopropylethylamine (4.72 g, 6.36 ml, 36.4 mmol, 1.1 eq) were added to the flask, and the flask was stirred for an hour at 0°C. 1H NMR (400 MHz, CDCl₃): δ 8.03 (dd, J= 152 Hz, 2 Hz, 4H), 7.54 (dd, J= 80.8 Hz, 2 Hz, 4H), 5.27 (dd, J= 69.6 Hz, 14 Hz, 2H), 4.28 (m, 1H), 3.23 (dd, 10 Hz, 10.4 Hz, 2H), 2.46 (s, 1H), 1.82 (d, J= 16.8 Hz, 1H), 1.61 (s, 3H), 1.37 (d, J= 6.4 Hz, 3H). 13C NMR (100 MHz, CDCl₃): δ 176.77, 158.29, 151.02, 147.53, 146.44, 142.35, 131.88, 129.97, 128.28, 127.89, 123.6, 121.31, 67.46, 65.42, 65.26, 60.35, 60.36, 60.37, 51.13, 36.48, 21.72, 14.12. IR: 3337.89, 2983.01, 2257.45, 1926.10, 1816.27, 1747.59, 1666.59, 1607.38, 1522.59, 1495.62, 1448.50, 1347.96, 1318.05, 1177.42, 1096.84, 1033.61, 957.21, 912.36, 849.71, 815.64, 735.07, 682.60, 667.72, 648.58.

(p-Nitrophenyl)methyl 3-azido-6-(1-hydroxyethyl)-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (30). A 500 mL round-bottom flask (w/ stir bar) with compound (29) was charged with 18-crown-6 (8.7 g, 33.12 mmol, 1 eq) and KN₃ (8 g, 99.36 mmol, 3 eq) was allowed to stir for 1.5 h. The flask was taken out of the bath and was allowed to stir in room temperature for an hour. The reaction was diluted with EtOAc and washed once with satd aq NaHCO₃. Once with satd aq NH₄Cl. The organic layer was dried over Na₂SO₄ and then evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (50/50 EtOAc/CH₂Cl₂ to 100/0 EtOAc/CH₂Cl₂) to afford 30 (2.6 g, 22% yield) as yellow solid.
$^1$H NMR (400 MHz, CDCl$_3$): δ 7.94 (dd, J= 230 Hz, 6.8 Hz, 4H), 5.38 (d, J= 84 Hz, 14 Hz, 2H), 5.31 (s, 1H), 4.26 (m, 1H), 3.26 (m, 1H), 3.16 (d, J= 12.8 Hz, 2H), 2.32 (s, 1H), 1.84 (s, 1H), 1.33 (d, J= 12.8 Hz, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 176.37, 159.58, 147.62, 144.28, 142.89, 128.16, 123.91, 123.78, 66.77, 65.55, 65.23, 51.18, 37.16, 21.85, 1.03.

IR: 3476.86, 2970.94, 2348.37, 2120.05, 1754.69, 1708.23, 1606.69, 1522.84, 1347.87, 1215.97, 1108.53, 852.14, 802.64, 736.65, 698.91.

### 3-Amino-6-(1-hydroxyethyl)-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (31). A Parr hydrogenation vessel was charged with compound 30 (0.615 g, 1.59 mmol) in 60 mL of EtOAc and 30 mL of pH6 NaH$_2$PO$_4$ (0.3 M) buffer solution. Then, 10% Pd on carbon (0.6 g, 5.6 mmol, 3.5 eq) was added, and the vessel was degassed and then subjected to hydrogen pressure 55 psi in a Parr hydrogenation device for 90 min. Once completed, the solution was filtered, the aqueous layer was separated and washed with Et$_2$O. The organic solvents were then removed from the aqueous layer and the product isolated by column chromatography on Diaion CHP20P resin. Tubes containing the product were identified by inspection of the UV of each fraction, combined, and the water was partially removed in vacuo. The remaining aqueous solution was lyophilized to produce the purified antibiotic 31 (35 mg, 5.7 % yield) as yellow solid, however, no product was observed.

### (p-Nitrophenyl)methyl(6S)-6-[(S)-1-hydroxyethyl]-3-amino-7-oxoazabicyclo[3.2.0]hept-2-ene-2-carboxylate (32). A 50 mL round-bottom flask (w/ stir bar) was charged with compound 30 (0.6 g, 1.55 mmol) in anhyd THF, followed by triethylamine (0.22 mL, 1.55 mmol, 1 eq) and was cooled to 0 °C. Hydrogen sulfide gas was then bubbled into the flask for five minutes. Once completed, mixture was diluted with EtOAc, washed once with satd aq NaHCO$_3$ and once with
brine. The organic layer was dried over Na$_2$SO$_4$ and then evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (100% EtOAc) to afford **32** (0.1 g, 17% yield) as yellow solid.

$^1$H NMR (400 MHz, CDCl$_3$): δ 7.96 (dd, J= 229 Hz, 8.4 Hz, 4H), 5.38 (dd, J= 137Hz, 7.6 Hz, 2H), 3.09 (d, J= 1.2 Hz, 2H), 2.95 (dd, J= 28.4 Hz, 8.8 Hz, 2H), 2.07 (s, 1H), 1.36 (d, J= 6Hz, 1H), 1.27 (m, 3H).

$^1$H NMR (400 MHz, CDCl$_3$): δ 7.39 (m, 5H), 5.0 (m, 1H), 4.0 (t, J= 4Hz, 1H), 1.56 (s, 2H), 4.76 (m, 1H), 1.6 (s, 6H), 1.511 (d, J= 4 Hz, 3H), 0.027 (s, 9H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 155.93, 137.58, 129.10, 128.93, 126.94, 104.34, 100.1, 70.09, 69.64, 59.14, 25.56, 17.98, 11.59, 10.83, -3.65, -4.49, -4.72, -5.74.
IR: 2956.44, 2930.44, 2859.23, 1770.53, 1688.32, 1393.85, 1362.30, 1347.09, 1319.74, 1254.78, 1204.54, 1147.49, 1080.43, 1055.88, 946.90, 865.94, 840.70, 784.02, 699.88.

(S)-3-[(Z)-1-(Trimethylsilyl)-1-propenyl]-4-phenyl-1,3-oxazolidin-2-one (35). A 100 mL round-bottom flask (w/ stir bar) was charged with (S)-4-phenyl-3-propionyl-1,3-oxazolidin-2-one (3 g, 13.7 mmol) in 30 mL of dry CH₂Cl₂. Trimethylsilyl trifluoromethanesulfonate (4.57 g, 3.8 mL, 1.5 eq) was added followed by triethylamine (2.7 g, 3.85 mL, eq) and the reaction was stirred for 1 h at rt. The reaction was diluted with CH₂Cl₂ and washed with satd aq NaHCO₃. The organic layer was dried over Na₂SO₄ and evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (100% CH₂Cl₂) to afford 35 (7.0 g, 60% yield) as liquid form.

¹H NMR (400 MHz, CDCl₃): δ 7.39 (m, 5 H), 5.05 (m, 1H), 4.75 (m, 2H), 4.05 (t, J= 4 Hz, 1H), 3.15 (q, J= 4Hz, 1H), 1.5 (d, J= 8 Hz, 3H), 1.4 (t, J= 8 Hz, 2 H), 0.2 (s, 6 H).

¹³C NMR (100 MHz, CDCl₃): δ 155.13, 137.48, 137.26, 128.13, 127.95, 125.80, 101.71, 98.59, 69.36, 68.99, 58.46, 11.59, 10.83, -0.81, -0.1.0.

IR: 3513.67, 3035.71, 2961.24, 2918.74, 2360.22, 2341.91, 1766.75, 1711.67, 1687.98, 1605.69, 1494.74, 1478.67, 1458.21, 1394.38, 1348.02, 1321.39, 1254.00, 1152.39, 1057.68, 1030.40, 946.96, 873.02, 848.63, 804.03, 760.85, 701.09, 638.62.

3-(2-{3-[(R)-1-(Dimethyl-tert-butyldisiloxyl)ethyl]-2-methyl-4-oxo-2-azetidinyl}acetyl)-1,3-oxazolidin-2-one (36). A 10 mL tube (w/ stir bar) was charged with chiral oxazolidinone (3-propionyl-1,3-oxazolidin-2-one) (0.56 g, 1.99 mmol, eq) in dry CH₂Cl₂ and was cooled to 0 °C. Titanium tetrachloride (0.175 mL, 1.59 mmol, 1.6 eq) was added and the reaction was stirred for 10 min. N, N-diisopropylethylamine (0.350 mL, 1.99 mmol, 2 eq) was added to the reaction. The mixture was allowed to stir for 30 min, and it was allowed to warm to room temperature.
Compound 4 (0.3 g, 0.995 mmol) was added to the reaction. Once completed, reaction was diluted with CH₂Cl₂ and washed with water. No product was observed.

(Azidosulfonyl)methane (37). A 1-L round-bottom flask (w/ stir bar) was charged with 400 mL of acetone, and the flask was cooled to 0 °C. Methanesulfonyl chloride (14.4 mL, 186 mmol) was added to the cold acetone followed by slow addition of sodium azide (19 g, 0.3 mmol, 1.5 eq) in 120 mL of water. Once competed, reaction was diluted with Et₂O and washed with satd aq NaHCO₃. The organic layer was dried over Na₂SO₄ and evaporated in vacuo to afford 37 (16 g, 65% yield) in solid form.

¹H NMR (400 MHz, CDCl₃): δ 3.23 (s, 3H).

Methyl 2-diazo-3-oxovalerate (38). A 1-L round-bottom flask (w/ stir bar) was charged with commercial methyl propionylacetate (10 g, 9.6 mL, 76.8 mmol) in 400 mL of dry CH₂Cl₂ and triethylamine was added to the flask at rt. The reaction was cooled to 0 °C and (azidosulfonyl)methane was added drop wise. The reaction was allowed to stir for one hour at rt. Once completed, the solvent was concentrated, and the reaction was diluted with 250 mL Et₂O and washed three times with 10% NaOH. The organic layer was dried over Na₂SO₄ and evaporated in vacuo to afford 38 in liquid form.

¹H NMR (400 MHz, CDCl₃): δ 3.82 (s, 3H), 2.89 (q, J= 21.6 Hz, 7.2 Hz, 2H), 1.16 (t, J= 3 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃): δ 192.11, 160.96, 74.38, 51.24, 32.85, 7.23. IR: 1659.36, 1437.45, 1364.39, 1308.12, 1219.44, 1138.15, 1081.30, 1024.53

Methyl (Z)-2-diazo-3-(trimethylsilyloxy)-3-pentenoate (39). A 50 mL round-bottom flask (w/ stir bar) was charged with compound 38 (2 g, 11.3 mmol) in 25 mL of dry CH₂Cl₂ and triethylamine (2.12 mL, 15.82 mmol1.4 eq) was added to the flask at 0 °C. TMS-OTf (2.5 mL,
13.6 mmol, 1.2 eq) was added to the reaction mixture dropwise. The reaction was allowed to stir for 30 min at 0 °C. Once completed, the reaction mixture was diluted with hexane, washed with satd aq NaHCO₃ once and washed with brine once. The organic layer was dried over Na₂SO₄ and then evaporated in vacuo to afford 39 (2.78 g, 99% yield) in liquid form.

¹H NMR (400 MHz, CDCl₃): δ 5.35 (q, J= 7.2 Hz, 1H), 3.797 (s, 3H), 1.69 (d, J= 6.8 Hz, 3H), 0.233 (s, 9H).

¹³C NMR (100 MHz, CDCl₃): δ 193.24, 161.71, 75.35, 52.00, 33.59, 8.09. IR: 2956.74, 2348.51, 2147.00, 1736.56, 1438.91, 1261.18, 1200.17, 1080.48, 843.12, 801.64, 736.89.

Methyl (Z)-2-diazo-3-(dimethyl-tert-butyilsilyloxy)-3-pentenoate (40). A 50 mL round-bottom flask (w/ stir bar) was charged with compound 38 (2 g, 11.3 mmol) in 25 mL of dry CH₂Cl₂ and triethylamine (2.12 mL, 15.82 mmol, 1.4 eq) was added to the flask at 0 °C. TBS-OTf (3.12 mL, 13.6 mmol, 1.2 eq) was added to the reaction mixture dropwise. The reaction was allowed to stir for 30 min at 0 °C. Once completed, the reaction mixture was diluted with hexane, washed with satd aq NaHCO₃ once and washed with brine once. The organic layer was dried over Na₂SO₄ and then evaporated in vacuo to afford 40 (3.2 g, 99% yield) in liquid form.

¹H NMR (400 MHz, CDCl₃): δ 5.28 (q, J= 4.8 Hz, 1H), 3.81 (s, 3H), 1.69 (d, J= 7.2 Hz, 3H), 0.937 (s, 9H), 0.174 (s, 6H).

¹³C NMR (100 MHz, CDCl₃): δ 179.70, 172.03, 51.70, 36.98, 35.30, 33.37, 17.45, 13.83, 10.34, 16.55, 8.26, 7.94.

IR: 2952.71, 2859.58, 1923.33, 1834.72, 1726.78, 1626.07, 1472.80, 1436.08, 1411.48, 1361.95, 1255.82, 1145.84, 1042.97, 940.40, 841.00, 723.29, 693.85.

Methyl 2-diazo-3-oxo-4-[4-oxo-3-[1-(trimethylsilyloxy)ethyl]-2-azetidinyl]valerate (41). A 10 mL tube (w/ stir bar) was charged with compound commercial acetate (3-[(R)-1-(Dimethyl-
tert-butyldimethoxy)silyl]-4-oxo-2-azetidinyl acetate) (0.1 g, 0.35 mmol) in 2 mL of dry CH₂Cl₂ and compound 39 (0.24 g, 1.05 mmol, 3 eq) was added followed by 1 M ZnCl₂ in Et₂O (0.35 mL, 0.35 mmol, 1 eq) and the reaction was heated to 45 °C. Once completed, reaction mixture was diluted with EtOAc, washed once with satd aq NaHCO₃. The organic layer was dried over Na₂SO₄ and then evaporated in vacuo to yield 41 in oil form.

¹H NMR (400 MHz, CDCl₃): δ 7.28 (s, 2H), 5.38, (s, 1H), 4.37 (m, 1H), 4.20 (m, 1H), 3.81 (s, 3H), 1.67 (s, 6H), 1.26 (m, 6H), 0.92 (s, 9H),

(p-Nitrophenyl)methyl 3-oxovalerate (42). A 250 mL round-bottom flask (w/ stir bar) with distillation device was charged with commercial methyl propionylacetate (30 g, 28.9 mL, 0.23 mol) and (p-nitrophenyl)methanol (33.54 g, 0.219 mol) and heated to 170 °C for 24 hours. Once completed, the crude material was purified by silica gel flash chromatography via gradient elution (50/50 CH₂CL₂/Hexane to 100/0 CH₂Cl₂/Hexane then 2.5/97.5 EtOAc/CH₂Cl₂) to afford 42 (27 g, 47% yield) as oil form.

¹H NMR (400 MHz, CDCl₃): δ 7.81 (dd, J= 258 Hz, 5.6 Hz, 4H), 5.21 (s, 2H), 3.54 (s, 2H), 2.52 (q, J= 6.8 Hz, 2H), 1.02 (t, J= 7 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃): δ 203.35, 166.90, 147.40, 143.52, 128.18, 123.52, 65.1348.44, 36.26, 30.62, 7.36.

(p-Nitrophenyl)methyl 2-diazo-3-oxovalerate (43). A 1-L round-bottom flask (w/ stir bar) was charged with compound 42 (27 g, 0.1075 mol) in 400 mL of dry CH₂Cl₂ and triethylamine was added to the flask at rt. The reaction was cooled to 0 °C and (azidosulfonyl)methane was added slowly. The reaction was allowed to stir for one hour at rt. Once completed, the solvent was concentrated, and the reaction was diluted with 250 mL Et₂O and washed three times with 10% NaOH. The organic layer was dried over Na₂SO₄ and evaporated in vacuo to afford 43 in oil form.
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.38 (d, J= 7.2 Hz, 2H), 7.95, (d, J= 6.8 Hz, 2H), 5.33 (s, 2H), 2.80 (q, J= 8 Hz, 2H), 1.06 (t, J= 8 Hz, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 192.58, 160.69, 147.55, 142.26, 128.36, 123.62, 75.14, 65.02, 33.52, 7.87.

(4-Nitro-2,4,6-cycloheptatrien-1-yl)methyl (E)-2-diazo-3-(trimethylsilyloxy)-3-pentenoate (44). A 250 ml round-bottom flask (w/ stir bar) was charged with compound 43 (8 g, 36.7 mmol) in 80 mL of CH$_2$Cl$_2$ and triethylamine (6.8 mL, 51.33 mmol, 1.4 eq) was added to the flask at 0°C. TMS-OTf (7.96 mL, 44.00 mmol, 1.2 eq) was then added slowly to the flask at 0°C. Once completed, the reaction was washed with satd aq NaHCO$_3$ and once with sat brine. The organic layer was dried over Na$_2$SO$_4$ and then evaporated in vacuo. The crude material was purified by a short silica gel flash chromatography (DCM) to afford 44 (4 g, 50% yield) as yellowish/orange solid.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.12 (d, J= 8 Hz, 2H), 7.47 (d, J= 8Hz, 2H), 5.285 (m, 3H), 1.62 (d, J= 8 Hz, 3H), 0.23 (s, 9H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 163.84, 147.33, 143.01, 132.17, 127.92, 123.43, 106.43, 64.58, 64.50, 11.53, -0.28, -0.42, 1006.31, 843.33, 802.82, 733.83, 668.61.

IR: 3296.58, 2981.78, 2957.04, 2880.38, 2848.98, 2605.60, 2525.25, 2425.07, 2348.69, 2254.13, 2144.43, 1724.38, 1658.35, 1437.77, 1364.63, 1308.02, 1219.17, 1137.95, 1081.23, 1024.48, 975.48, 919.49, 798.68, 743.53, 699.09, 647.84.

(4-Nitro-2,4,6-cycloheptatrien-1-yl)methyl 4-{(2R,3S)-3-[(S)-1-(t-butyldimethylsilyloxy)ethyl]-2-methyl-4-oxo-2-azetidinyl}-2-diazo-3-oxovalerate (45) A dry 100 mL round-bottom flask (w/ stir bar) with a reflux condenser was charged with compound 4 (5 g, 16.6 mmol) and compound 44 (8.4 g, 28.9 mmol, 1.7 eq) in 15 mL of dry CH$_2$Cl$_2$. 1M
ZnCl₂ in Et₂O (7.3 mL, 7.28 mmol, 0.7 eq) was added to the reaction, and the flask was heated to 42 °C. Once completed, the reaction was cooled to rt and diluted with EtOAc. The solution was washed with sated aq NaHCO₃ once and extracted with EtOAc twice. The organic layer was dried over Na₂SO₄ and evaporated in vacuo. The crude material was purified by silica gel flash chromatography via gradient elution (2.5:97.5 EtOAc/CH₂Cl₂ to 40/60 EtOAc/CH₂Cl₂) to afford 44 (0.65 g, 7.5% yield) as solid.

1H NMR (400 MHz, CDCl₃): δ 8.152 (d, J= 8 Hz, 2H), 7.493 (d, J= 8 Hz, 2H), 6.980 (s, 1H), 5.295 (m, 2H), 4.065 (m, 1H), 3.714 (m, 1H), 2.69 (d, J= 9.6 Hz, 1 H), 2.56 (d, J= 5.2 Hz, 1H), 1.95 (s, 1H), 1.43 (s, 3H), 1.17 (d, J= 9.2 Hz, 3H), 0.798 (s, 17H).

13C NMR (100 MHz, CDCl₃): δ 193.37, 170.79, 168.82, 167.60, 160.17, 147.56, 142.16, 128.73, 123.62, 67.20, 65.54, 65.28, 65.20, 60.05, 59.37, 50.73, 46.80, 25.62, 25.44, 22.17, 21.73, 20.71, 17.79, 17.12, 13.90, 12.98, 0.74.

(4-Nitro-2,4,6-cycloheptatrien-1-yl)methyl 4-{[(2R,3S)-3-[(S)-1-hydroxyethyl]-2-methyl-4-oxo-2-azetidinyl]-2-diazo-3-oxovalerate (46) A 100 mL round-bottom flask (w/ stir bar) was charged with compound 45 (0.65 g, 0.125 mmol) in 5 mL of acetonitrile, and 1 mL of HF was then added. The reaction stirred at rt for 30 min and monitored by TLC. Once completed, finely ground NaHCO₃ was added to the reaction to retain pH = 7. The reaction was diluted with EtOAc and washed with satd aq NaHCO₃. The EtOAc was dried over Na₂SO₄ and evaporated to afford 45 (0.27 g, 53% yield) as white solid.

1H NMR (400 MHz, CDCl₃): δ 8.152 (d, J= 8 Hz, 2H), 7.493 (d, J= 8 Hz, 2H), 6.65 (s, 1H), 5.32 (m, 2H), 4.08 (m, 1H), 3.84 (m, 1H), 3.07 (d, J= 9.6 Hz, 1 H), 2.00 (s, 1H), 1.52 (s, 3H), 1.34 (d, J= 7.2 Hz, 3H), 1.22, (d, J= 8 Hz, 4H).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 195.46, 167.44, 159.90, 147.75, 141.81, 128.63, 123.81, 65.50, 63.71, 62.49, 60.23, 58.36, 49.39, 21.96, 20.30, 13.98, 12.29.

IR: 3467.33, 3059.41, 2971.72, 1720.35, 1649.03, 1608.18, 1526.31, 1348.26, 1294.62, 1220.89, 1120.06, 1092.07, 991.65, 934.14, 906.57, 853.12, 805.95, 737.22, 699.50.
Carbapenem Spectra
Reference


45. “Protein Synthesis Inhibitors.” all_antibiotics [TUSOM | Pharmwiki], tmedweb.tulane.edu/pharmwiki/doku.php/antibiotic_pharmacology.


