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# Synthesis of the Ficellomycin Core using a Late-Stage [3+2]-azide Alkene Cycloaddition

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## Abstract

Antibiotics are crucial for fighting bacterial infections, and many antibiotics are produced by bacteria themselves in an effort to destroy their competition. One such compound, ficellomycin, which was first isolated from the *Streptomyces ficellus* bacterium in the 1970's, has been found to inhibit the growth of many Gram-positive bacteria, including some antibiotic-resistant organisms. Since its isolation, research on ficellomycin's synthesis and mechanism of action has been limited because of its highly unstable azabicyclo[3.1.0]hexane core. The objective of this research was to synthesize ficellomycin from L-serine and to investigate the use of a late-state [3+2]-azide alkene cycloaddition to construct the azabicycle[3.1.0]hexane core in a single step. Synthesis of the acyclic carbon backbone has been completed in 6 steps (51-91% yield) and [3+2]azide alkene cycloaddition studies are presented herein.

## 1. Introduction

### 1.1 Origin of Antibiotics

Infectious diseases and medicine have existed concurrently and spurred each other evolutionarily for thousands of years. For example, recent advances in science have allowed researchers to develop more efficient ways of combating infectious diseases. One example of the coevolution of medicine and infectious diseases is the relationship between antibiotics and bacteria. First termed in 1942 by Selman Waksman and his collaborators, antibiotics have become a widespread method of treatment for bacterial infections.[1] Most antibiotics are relatively small molecules, with molecular weights of less than 2000 atomic mass units, which are produced by bacteria in an effort to destroy their competition. Antibiotics target various mechanisms of reproduction in bacteria ranging from destruction of the cell wall to DNA replication inhibition; however, the overall result is the same: inhibiting the ability of bacteria to reproduce. Recent advances in pharmaceutical development have allowed scientists to utilize antibiotics for their own purposes; specifically the treatment of bacterial infections in humans. One bacterium that synthesizes and utilizes antibacterial compounds is *Streptomyces ficellus.* This bacterium produces three compounds identified as ficellomycin, feldamycin and, nojirimycin, all effectively inhibit the growth and reproduction of other bacterial species *in vitro*.[2]

## 1.2 Bacterial Structure

Bacteria are broadly classified as Gram-positive or Gram-negative based on their cell wall structure. Gram-positive bacteria have a peptidoglycan-containing thick cell wall with no outer membrane, while Gram-negative bacteria have a cell wall surrounded by a thin lipopolysaccharide layer, as shown in Figure 1. The terms Gram-positive and Gram-negative are derived from a method of bacteria differentiation created by Hans Christian Gram. Under the Gram staining test, bacteria that have been isolated are stained using a crystal violet dye (a triarylmethane dye), which is blue, followed by a red counterstain. Bacteria that are Gram-positive retain the crystal violet dye because the dye interacts with the peptidoglycan layer, while the lipopolysaccharide outer layer in Gram-negative bacteria does not interact with the dye and is instead colored by the red counterstain.[3]

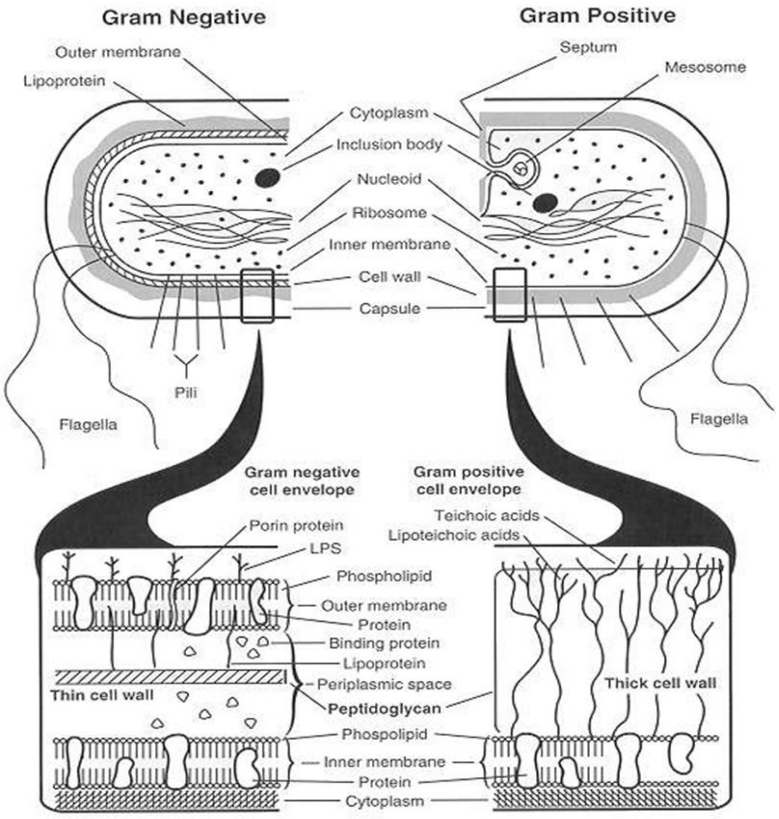



Figure 1. Structural difference between Gram-positive and Gram-negative bacteria (reprinted with permission).[4]

## 1.3 Mechanism of Various Antibiotics

Because of the structural differences between Gram-positive and Gram-negative bacteria, most antibiotics work to inhibit the function of only one or the other. For example, penicillin is a β-lactam antibiotic derived from the *Penicillium* fungi that targets gram-positive bacteria by inhibiting cell wall synthesis, as shown in Figure 2. In bacteria, the peptidoglycan layer is a crystal lattice structure that is formed from two linear chains of alternating amino sugars, which are connected by a β-(1,4)-glycosidic bond. Cross-linking between amino acids is aided by the enzyme transpeptidase, and results in a 3-dimensional cell-wall structure that is strong and rigid. The β-lactam ring of phenoxymethylpenicillin forms a covalent bond with the transpeptidase. This inhibits the amino acids cross-

linking reaction, which in turn inhibits formation of the cell wall. Penicillin therefore acts as an irreversible inhibitor and prevents further bacterial replication by inhibiting the stage of active multiplication.[5]

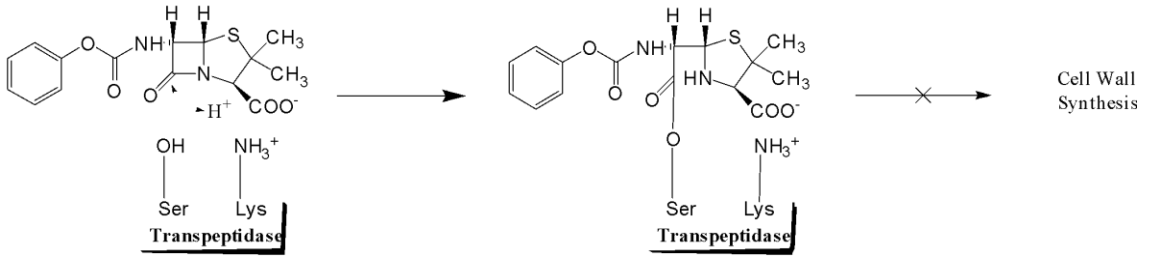



Figure 2. Mechanism by which penicillin inhibits cell-wall formation in Gram-positive bacteria.

Conversely, streptomycin is an aminoglycosides antibiotic isolated from the species *Streptomyces griseus* that eliminates Gram-negative bacteria by inhibiting the ability of the bacterium to synthesize proteins vital to its growth. Streptomycin enters the target bacterium and binds to the ribosomal subunit. In living organisms, the ribosomal subunit is responsible for synthesis of proteins. By binding to formyl-methionyl-tRNA (the starting residue in the synthesis of proteins in bacteria), streptomycin causes the bacterial ribosome to misread codons, which leads to the inhibition of protein synthesis, and ultimately bacterial death.[6]

## 1.4 Ficellomycin

First isolated from *Streptomyces ficellus* in the 1970's, ficellomycin, feldamycin, and norjirimycin are all potentially promising choices for antibiotic study. Ficellomycin specifically has been shown to inhibit the growth of Gram-positive bacteria including resistant strains of *Staphylococcus aureus* and *Escherichia coli*. In addition, it has been shown to work *in vitro* against bacteria that are resistant to penicillin, neomycin, streptomycin, macrolides, and lincosaminide antibiotics.[2,7] These antibiotic properties make ficellomycin a promising candidate for researchers who are hoping to synthesize new antibiotics to fight resistant strains of bacteria such as MRSA.

In 1988, the molecular structure of ficellomycin was determined by Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS) and derivatization studies to be valyl-2-[4-guanidyl-1-azabicyclo[3.1.0]hezan-2-yl]glycine, which is shown in Figure 3.[8] What is rare about its structure is that ficellomycin is one of the few natural products that contains an azabicycle core structure. It is believed that the aziridine ring, comprised of a three-membered heterocycle with one amine group and two methylene carbons, endows the molecule with its unique antibacterial properties, including activity against antibiotic-resistant organisms.[9]

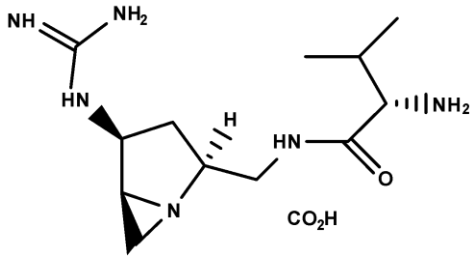



Figure 3. The structure of ficellomycin.

## 1.5 Structure Activity Relationship

Because ficellomycin has been shown to inhibit the growth of Gram-positive bacteria, it is hypothesized that this compound acts in a manner similar to that of penicillin; however the compound's mechanism has not been

determined. Another hypothesis is that the antibiotic restricts bacterial growth by inhibiting the semiconservative DNA replication of bacteria.[8] In 1977 Reusser showed that ficellomycin effectively inhibits the growth of *E. coli* in vitro. The antibiotic caused the accumulation of 34S DNA species in toulenized *E. coli* cells. The results of the experiment were compared with the effects of novobiocin, a compound that is known to inhibit DNA synthesis. It was found that while novobiocin appears to block an event associated with the initiation of the Okazaki fragments of DNA, ficellomycin inhibits an event before the initiation of the Okazaki fragments; however, the exact time of inhibition is not known.[9] The Okazaki fragments are short fragments of newly synthesized DNA that are formed on the lagging strand during DNA replication, and they must be linked together in order to from a new strand of DNA. By blocking the initiation of these fragments, novobiocin effectively inhibits the synthesis of a new strand of DNA. If ficellomycin inhibits an event before the initiation of the Okazaki fragments (as believed), then it too prevents DNA synthesis, and hence bacterial replication.

Another theory about the mode of action of ficellomycin is that it is similar to the mode of action of azinomycin A and B, the only other natural products known to possess an aziridine ring structure, shown in Figure 4a.[10] As depicted in Figure 4b, the azinomycins are known anticancer agents that intercalate DNA by interaction with the napthalene. In the azinomycins, the aziridine ring allows both molecules to alkylate DNA because of its specificity for the double strand of DNA. The naphthalene directs the noncovalent binding of the molecule to a specific site of DNA through intercalation, and then the azabicycle alkylates a DNA base. This process effectively reduces the DNA activity of cancerous cells. It was also shown that azinomycin A preferentially reacts by binding to a major groove of the DNA so that no DNA replication can occur.[11] Researchers theorize that ficellomycin interacts with DNA in a similar manner by attacking bacterial DNA via its strained azabicycle to form a covalent bond; however such hypotheses have yet to be proven.

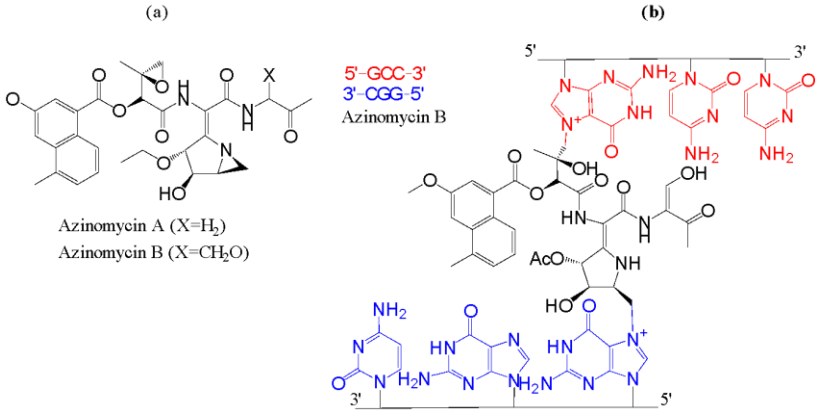



Figure 4. (a) Structure of the azinomycins. (b) DNA alkylation by Azinomycin B. Antiparallel DNA strands are colored in red and blue, and Azinomycin B is colored in black.

## 2. Motivation for Research

While it appears to be a promising new antibiotic for combating antibiotic-resistant organisms, there are several issues relating to ficellomycin's stability and biological activity. For example, in addition to endowing ficellomycin with its antibacterial properties, the azabicycle ring also lends a degree of instability to the molecule because the three-membered moiety is highly strained.[8] This strained configuration is possibly the reason researchers have been unable to synthesize the compound to date. In addition, while ficellomycin has been shown to inhibit bacterial growth *in vitro*, such antibacterial properties are limited and required large concentrations of the antibacterial agents when studied.[15] Previous studies show that the antibacterial properties of ficellomycin are observable only when there are large concentrations of the compound present. For example, the minimum inhibitory concentration (MIC) for ficellomycin *in vitro* was found to be >1000 ug/mL against most bacteria.[11] The MIC of a compound is minimum amount of the antibiotic required to visibly inhibit bacterial growth after a specific period of incubation. It

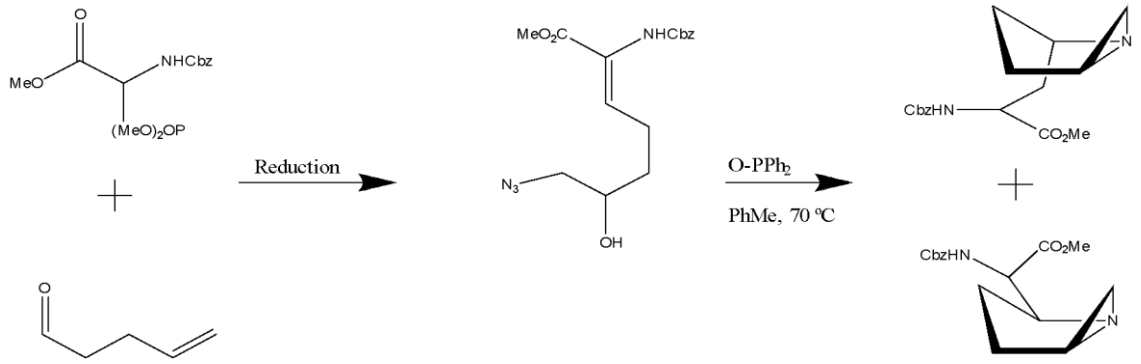
is possible that ficellomycin's high MIC is also due to the instability of the azabicycle indicating that ficellomycin may react with unwanted nucleophiles before reaching its biological target.

With its ability to inhibit antibiotic-resistant organisms, and its potentially unusual mode of action, ficellomycin is an antibiotic compound that has been overlooked for too long. While its instability and low antibacterial properties pose a potential problem for researchers, it is possible to enhance the antibacterial properties and stability of compounds by altering their structure.[16] However, before researchers can begin to enhance ficellomycin's antibacterial properties, a suitable synthesis scheme must be determined. Once a synthesis route and stable conformation for ficellomycin have been established, antibiotic bioassays can be employed to establish the most effective concentration and analog of the compound. In most antibiotic bioassays, agar plates (a plate with a nutrient broth for the bacteria to feed on) are used to culture the bacteria. The organism is usually allowed to grow on the agar plate at an appropriate pH for a certain number of days before being inoculated with the antibiotic that is being studied. The effectiveness of the antibiotic at inhibiting growth of the bacteria present on the agar is then recorded. The concentration/structure of the antibiotic can then be altered in order to determine the most effective amount/structure of antibiotic for combating bacterial growth. Such information would enable researchers to potentially manipulate the compound in a way that its antibacterial properties are maximized while minimizing its toxic side effects.[17] By applying this technique to ficellomycin, it would be possible to determine which analog of the compound most effectively inhibits bacterial growth.

## 3. Synthesis

### 3.1 Shipman Synthesis

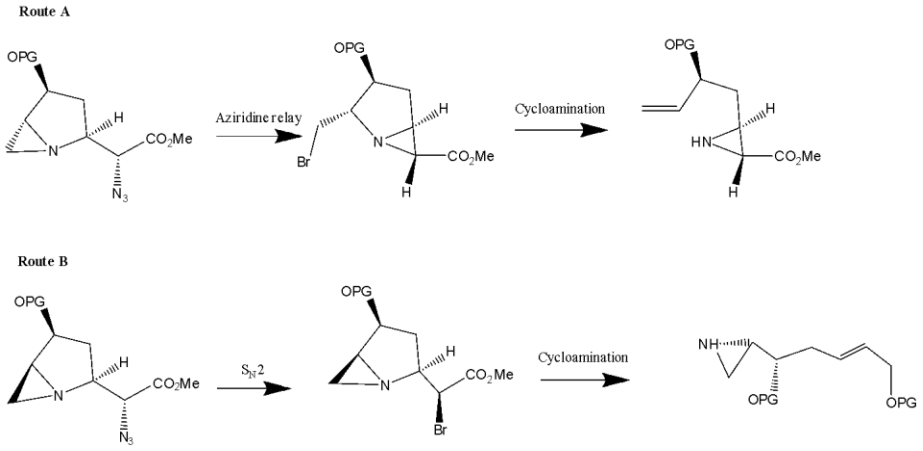
Attempts were made to synthesize ficellomycin in the early 1980's; however total synthesis of the compound had never been achieved. In 2007, researchers at Warwick University attempted to synthesize ficellomycin by two new methods. In the first attempt, the researchers started by synthesizing the 1-azabicycle[3.1.0]hexane core from an aziridine starting material (Scheme 1). Under these conditions, 4 stereoisomers were formed; however the total synthesis of ficellomycin was never achieved. In the second approach, the researchers took two open chain γ - aldehydes and added an azide using anti-1,4-addition. This compound was then reacted with a phosphonate using DBN to produces a pair of E/Z alkenes . Controlled cyclization of led to a piperidine structure. While the final product of this second reaction was not ficellomycin, the Warwick researchers hypothesized that ficellomycin could be made from these intermediates.[12]




Scheme 1. Attempted synthesis of ficellomycin's core by Warwick researchers. (a) Attempted synthesis starting from the 1-azabicycle[3.1.0]hexane core and reducing to an aziridine.

## 3.2 Chen Synthesis

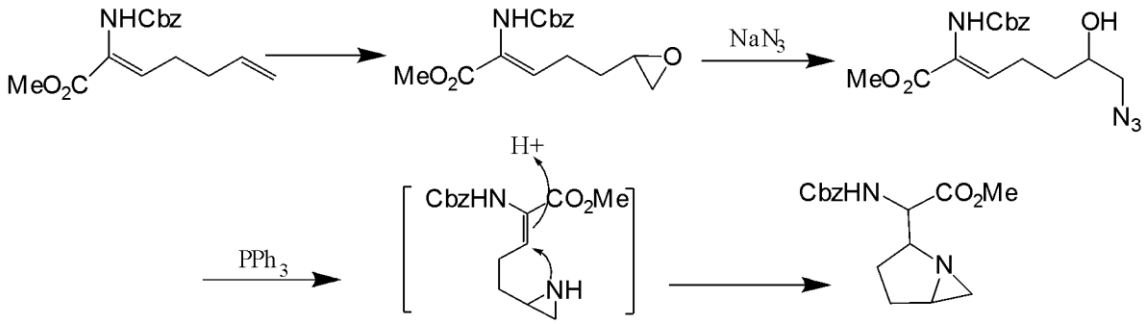
In a more recent study, researchers at the University of Toronto synthesized a racemic mixture of the bicyclic-aziridine structure present in ficellomycin starting from ethyl acetate. The bicyclic product, (2S,4S,5S)-2-((R)-1-azido-2-(4-methoxybenzyloxy)ethyl)-4-(4-methyloxybenzyloxy)-1-azabicycle[3.1.0]hexane, was afforded in 88% yield. The researchers theorized that either stereoisomer of the aziridine-containing structure could be used to synthesize the ficellomycin core by 2 routes, as illustrated in Scheme 2. In the first route, one stereoisomer of the bicyclic product could be subjected to cycloamination to form a bromoamine intermediate. The intermediate could then be subjected to aziridine relay by means of a nitrogen nucleophile, which would rearrange in order to form a more stable structure, the core of ficellomycin (Route A). In the second route, the other bicyclic stereoisomer that was synthesized was subjected to cycloamination to form the aziridine ring. This compound underwent a $S_N2$ Reaction, in which bromine replaced the azide group on the carbon chain, resulting in the core of ficellomycin. (Route B).[13]




Scheme 2. Synthesis scheme detailing the synthesis of ficellomcin's core. Route A: Synthesis via aziridine relay. Route B: Synthesis via $S_N2$ reaction.

## 3.3 Paumier Synthesis

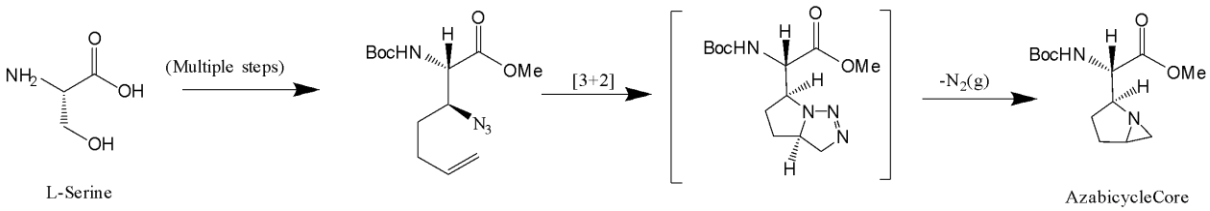
In 2004, Paumier and coworkers were able to successfully synthesize the core of ficellomycin starting from a benzyl-protected alkene. A simplified synthesis scheme is shown in Scheme 3. The researchers cyclized the benzyl-protected alkene into an epoxide, then added sodium azide to the epoxide. They then added triphenylphosphine ($PPh_3$) to perform a Wittig reaction, resulting in the core of ficellomycin. However, this synthesis scheme is not very effective, with percent yields as low as 30%. In addition, it is difficult to isolate the product from the $PPh_3$, and many of the other reaction steps include expensive and highly reactive agents.[14] Therefore, a more efficient, inexpensive synthesis scheme must be determined.




Scheme 3. Paumier synthesis of ficellomycin using an epoxide addition and $PPh_3$.

## 3.4 Proposed Synthesis of the Ficellomycin Core

Synthesis was centered around the reactivity of the azabicycle moiety. Therefore, synthesis planning was carried out based on the preparation of the azabicyclo3.2.1 core. Specifically, the synthesis of ficellomycin's core was attempted starting from L-serine and using a late stage [3+2] cycloaddition as shown in Scheme 4.

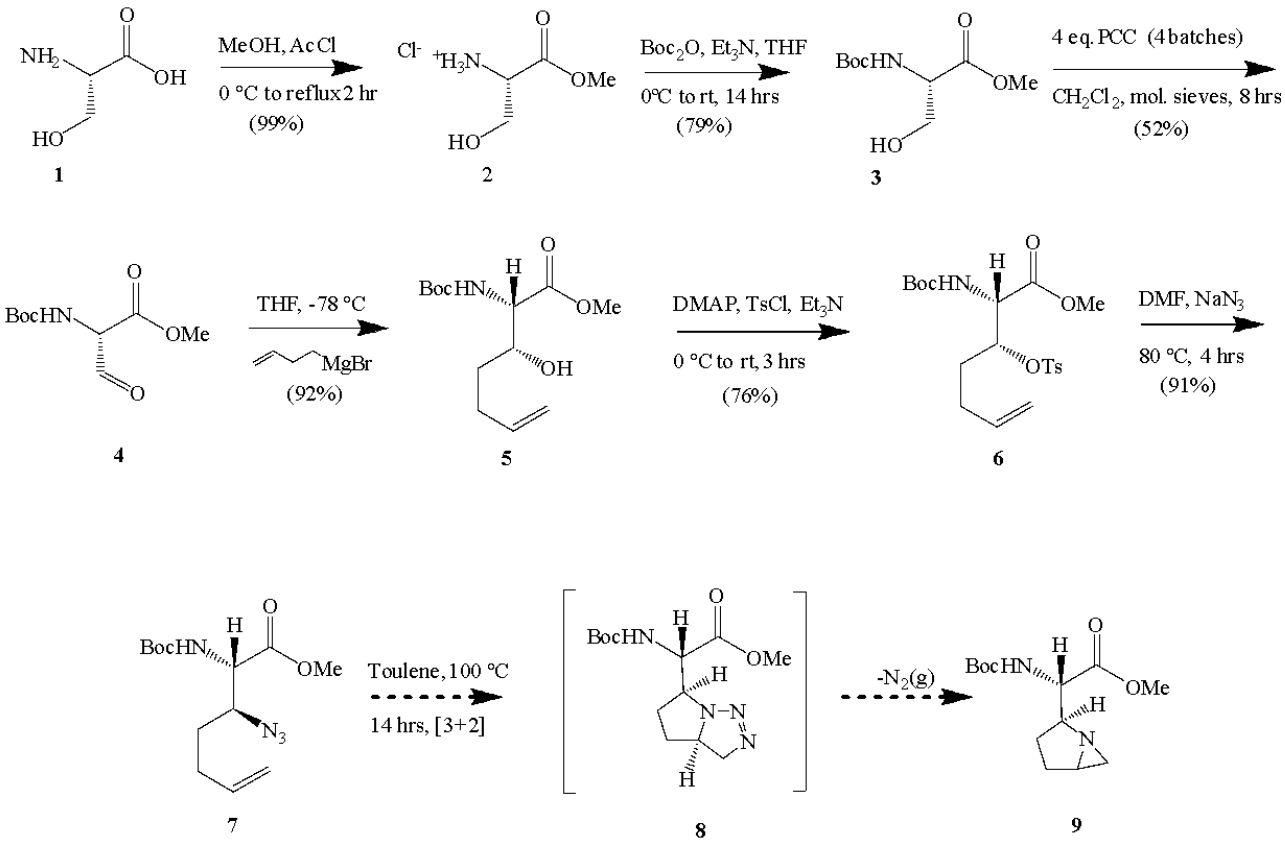



Scheme 4. Proposed scheme for the synthesis of ficellomycin's core starting with L-serine and utilizing a late [3+2]azide alkene cycloaddition.

# 4. Results and Discussion

## 4.1 Overview

Starting with L-serine **1**, the synthesis of the core of ficellomycin was attempted using a late stage [3+2]-azide alkene cycloaddition (Scheme 5). The synthesis scheme has been carried out to completion, which in theory will yield **9** as the final product. To date, the synthesis scheme has been successfully carried through the formation of **7**. The synthesis of **9** was attempted; however $^{1}H$ NMR data has not been collected to confirm the formation of the structure. H NMR and $^{13}C$ NMR were used to confirm the structures of each of the remaining compounds.




Scheme 5. Synthesis scheme for the synthesis of ficellomycin's core starting from L-serine and employing a late [3+2] azide alkene cycloaddition.

## 4.2 Synthesis of the Aldehyde **(4)**

In the first step, L-serine **1** was methylated using methanol (MeOH) and acetyl chloride (AcCl). This product was then treated with di-tert-butyl dicarbonate ($Boc_2O$), tetrahydrofuran (THF), and triethylamine ($Et_3N$) to protect the amine group as the tert-butyl carbamate. Initial syntheses of **3** resulted in acceptable percent yields of the methyl-ester hydrochloride (59%); however the efficiency of this reaction will have to be increased if this synthesis scheme is to be used for the synthesis of ficellomycin. Most likely, the largest amount of product is lost when normal phase flash column chromatography is run to purify the product and remove excess $Boc_2O$.

In the next step, an oxidation reaction was carried out, effectively oxidizing the alcohol on C-3 to an aldehyde, using pyridinium chlorochromate (PCC), dichloromethane (DCM) and 4 Å molecular sieves. Initial synthesis of Compound 4 using a Swern Oxidation resulted in essentially no yield of the desired product (Table 1); however some product was recovered after switching to PCC as the oxidizing agent. Most likely, no product was isolated using the Swern Oxidation because that specific reaction is typically ran at low temperatures ( -78 °C) and it is likely that the reaction cannot proceed at that temperature. In addition, significantly higher percent yields were obtained when a smaller diameter column was used to purify the product. Most likely, a significant amount of product was lost on the larger (500 mL) column during purification as opposed to the smaller (250 mL) column because an inadequate amount of mobile phase was used to extract the compound from the column. It was also found that 4 equivalents of PCC and a 7 hour run time are the ideal conditions for maximizing percent yield of the product. However, there are so many variables affecting this reaction that it is impossible to definitively determine which contributes most to the maximum production of product at this time. Additional experiments controlling all the variables but one would need to be performed in order to determine what the ideal conditions are for oxidation.

Table 1. Comparison of oxidation conditions.

| Trial # | Oxidizing Agent | Column Size (mL) | Starting Material (mg) | Equivalents Oxidizing Agent | Reaction Time (hours) | Percent Yield |
|---|---|---|---|---|---|---|
| 1 | $COCl_2$ | - | 200 | 0.35 | - | - |
| 2 | $COCl_2$ | - | 500 | 0.35 | - | - |
| 3 | PCC | 100 | 100 | 2 | 3.5 | 25 |
| 4 | PCC | 500 | 200 | 2 | 3.5 | 43 |
| 5 | PCC | 500 | 200 | 2 | overnight | 18 |
| 10 | PCC | 250 | 400 | 4 | 4 | 52 |
| 12 | PCC | 250 | 400 | 3 | 6 | 39 |
| 13 | PCC | 250 | 500 | 5 | 3.5 | 37 |
| 15 | PCC | 250 | 500 | 4 | 7 | 41 |

## 4.3 Synthesis of Alkene **(5)**

The aldehyde **4** was then subjected to a Grignard Reaction using 3-butene magnesium bromide in THF. Initial synthesis of **4** was successful following the Grignard procedure outlined by Chen, et al using 2.5 equivalents of 3-butene magnesium bromide.[21] However, it was found that using 1.5 equivalents of the Grignard reagent yielded maximum results (Table 2). This is most likely because at higher equivalents, the Grignard can interact with the aldehyde as desired and the ester as an undesired side reaction. Future plans for this step include running this reaction using only 1 equivalent of the Grignard reagent to see if this affects the efficiency of this step.

Table 2. Comparison of percent yields of Grignard reaction using various equivalents.

| Trial # | Starting Material (mg) | Grignard Equivalents | Percent Yield |
|---|---|---|---|
| 2 | 100 | 2.5 | 64% |
| 3 | 60 | 2.5 | 12% * |
| 4 | 50 | 3 | 11% * |
| 5 | 80 | 3 | 85% |
| 7 | 20 | 1.5 | 43% |

*denotes purified product

## 4.4 Synthesis of Azide **(7)**

The alkene **5** was reacted with toluenesulfonyl chloride (TsCl), dimethylaminopyridine (DMAP) and triethylamine ($Et_3N$) in order to convert the alcohol off of C-3 to a tosylated oxygen. Tosylation of **5**, while effective, yielded **6** in relatively low percent yields (64%) and this step is currently being modified to yield greater amounts of product.
$^1$H NMR analysis of this product was indicative of diastereomers, which were formed in the previous step. In theory, only one of these compounds will yield the desired product after undergoing the following reactions. Therefore, the diastereomers will need to be separated and the subsequent reactions ran separately in order to determine which compound is needed for the synthesis of ficellomycin's core. The presence of these diastereomers further complicates the synthesis of ficellomycin, adding the additional step of separation to the proposed synthesis scheme. In addition, once the required diastereomer has been identified, the preceding reactions will need to be modified in order to increase the production of that enantiomer.

The tosylation product **6** then underwent a $S_N2$ reaction using sodium azide ($NaN_3$) in dimethylformamide (DMF). This resulted in the substitution of the tosylated oxygen with the azide group on **7**.The azide addition synthesis resulting in **7** was extremely effective, with a percent yield of 91%. However, this compound appears to be relatively

unstable, requiring that **7** be stored in a nitrogen atmosphere with no light and that the [3+2] cycloaddition be carried out almost immediately after the addition.

### 4.5 [3+2]Azide/Alkene Cycloaddition **(9)**

In the final step of the synthesis sequence, **7** was refluxed in toluene at 100°C for 14 hours, causing it to undergo an intramolecular [3+2] cycloaddition in which the azide restructured to form an unstable triazole intermediate, **8**. This "green reaction" step caused **8** to rearrange to form the 3-membered ring containing bicycle, **9**, giving off $N_2$ as a byproduct. Synthesis of **9** using a [3+2]azide alkene cycloaddition in toluene appears to have been effective; however results from this step have yet to be verified by NMR or IR. In addition, because of the small amount of final product obtained, no percent yields have been determined.

## 5. Conclusion

Because of its instability and high MIC, there have been few efforts concentrated toward the synthesis of ficellomycin since the 1980's. However, research has shown that ficellomycin is effective against bacteria that have become resistant to other antibiotics and that its mechanism may be different from that of many other antibiotics.[4,5] Therefore, if the compound could be synthesized and its azabicycle stabilized, it is possible that ficellomycin could act against many strains of antibiotic-resistant bacteria. However, before such conclusions can be made regarding the effects of ficellomycin, it is imperative to determine a synthesis route for the compound, and an effective way to stabilize its azabicycle core.

Thus far, the proposed synthesis scheme for ficellomycin's core has proven effective; however, the results from the [3+2]azide alkene cycloaddition still need to be verified using NMR. Additionally, the percent yields for multiple steps of the synthesis need to be increased if this scheme is to be used to mass-produce the core of ficellomycin. In addition to completing the synthesis scheme and improving its efficiency, future goals include performing *in vitro* bioassays on the azabicycle core in order to examine the compound's stability and biological activity.

## 6. Acknowledgements

The author would like to express her gratitude to the members of the Wolfe research group for their support and comradery. In additional, she would like to recognize the University of North Carolina at Asheville Chemistry department faculty and staff for their guidance. The author would also like to offer special thanks to Dr. Amanda Wolfe for her support and guidance in lab. Funding for this project was provided by the University of North Carolina at Asheville Undergraduate Research Program.

## 7. Supplemental Information

### 7.1 Instrumentation

NMR spectra were obtained using $CDCl_3$ as the solvent on a Varian Gemini 2000 with an Oxford Instruments 400MHz superconducting magnet, Varian Unity Inova, with an Oxford Instruments 500MHz superconducting magnet.

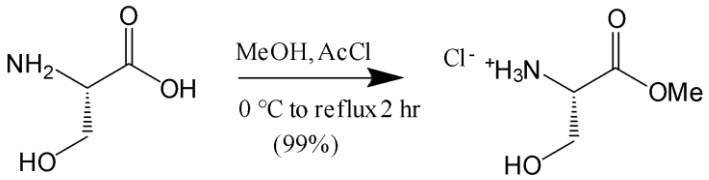
## 7.2 Materials

All reagents were purchased from commercial sources and were used as received. Prior to use in water sensitive reactions, the starting material was dissolved in an anhydrous solvent (DCM, DMF, DMSO, methanol) in a nitrogen-flushed flask.

## 7.3 Experimental Methods

The core of ficellomycin was synthesized in 7 steps starting with L-serine. $^{1}$H-NMR and $^{13}$C-NMR were used to confirm the results of each step. To date, the acyclic core has been synthesized in 6 steps, and cycloaddition studies are ongoing.

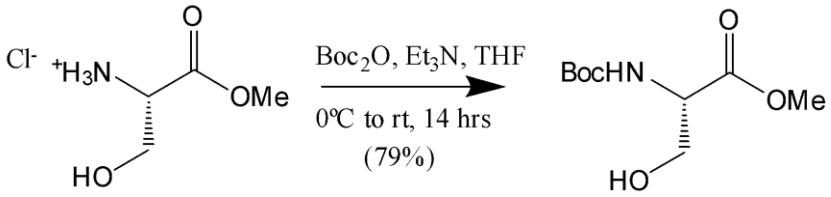
### *7.3.1 (R)-Methyl-2-amino-3-hydroxypropanoate hydrochloride **(2)***




**1** **2**

Acetyl chloride (5.8 mL, 0.677 mol) was added dropwise to anhydrous methanol (36.0 mL, 7.406 mol) at 0º C in a round bottom flask under nitrogen atmosphere. After complete addition, the solution was removed from the bath and 3.0 g (0.238 mol) of L-Serine **4** was added. The mixture was then placed in an oil bath and refluxed at 60-70º C for 2 hours. After 2 hours, the flask removed from heat. The solution was allowed to stir as it cooled to room temp (23 ºC) over 30 minutes. The solvent was then removed under reduced pressure and the white, crystalline product **2** was carried into the next step without further purification.[18]

### *7.3.2 (R)-Methyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate **(3)***

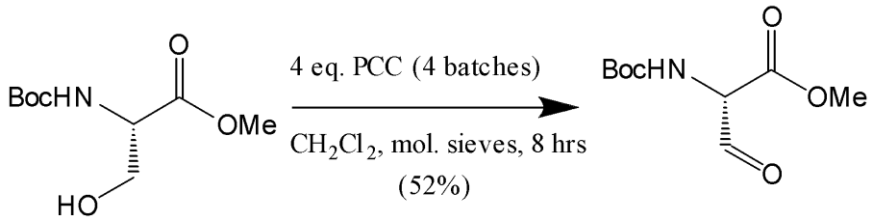



**2** **3**

The methyl chloride **2** (0.238 mol) was dissolved in 72.0 mL of tetrahydrofuran (7.399 mol) in a round bottom flask under nitrogen atmosphere. The solution was then cooled to 0º C and 8.6 mL (0.512 mol) of triethylamine was added. To the cooled solution, 6.0 g (0.236 mol) of di-tert-butyl dicarbonate was added and the reaction mixture was allowed to stir for 14 hours while slowly warming to room temperature. Upon completion, the reaction was diluted with diethyl ether (35 mL) and saturated aqueous sodium bicarbonate (35 mL). The organic layer was then extracted with an additional 2 times with 35 mL of saturated aqueous sodium bicarbonate. The organic layer was then dried over sodium sulfate and concentrated under reduced pressure. The crude mixture was purified by flash chromatography ($SiO_2$ 3cm x 10cm, 50% ethyl acetate/hexane) to yield **3** as a clear oil.[18]

$^{1}$H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, Boc), 3.45 (s, 3H, $CO_2CH_3$), 3.60-3.70 (m, 1H, CHNH), 4.15 (m, 2H, $CH_2OH$), 5.70 (br, s, 1H, OH) ppm; $^{13}$C NMR (150 MHz, $CDCl_3$): δ 28.2 ($C(CH_3)_3$), 54.5 ($OCH_3$), 77.5 (NCH), 148.7 ($CO_2$tBu), 169.4 ($CO_2$Me) ppm.

### *7.3.3 Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-oxopropanoate **(4)***

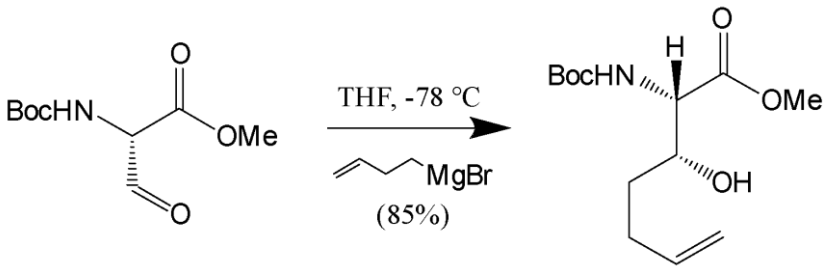



**3** **4**

The alcohol **3** (0.500 g, 0.001 mol) was dissolved in 25.0 mL (0.390 mol) of dichloromethane in a round bottom flask under nitrogen atmosphere. 4 Å molecular sieves and 0.750 g of pyridinium chlorochromate (1.5 mmol) were added and the mixture was allowed to stir at room temp for 2 hours. After 2 hours, added 0.250 g of pyridinium chlorochromate (0.5 mmol) and additional molecular sieves. Allowed to stir for another 2 hours, then added 0.25 g of pyridinium chlorochromate (0.5 mol) and allowed to stir for another 2 hours. After 2 hours 0.250 g of pyridinium chlorochromate (0.0005 mol) was added and the mixture was allowed to stir for another 2 hours. The reaction was then filtered through a plug ($SiO_2$ 3cm x 10cm) using 50% hexane/ethyl acetate until the liquid exiting the column was no longer yellow. The solution was then concentrated under reduced pressure to yield **4** as a light yellow oil.[19]

$^{1}$H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, Boc), 3.80 (d, 1H, CHNH), 3.95 (s, 3H, $OCH_3$), 8.18 (s, 1H, CHNH), 8.78 (d, 1H, CHO) ppm; $^{13}$C NMR (150 MHz, $CDCl_3$): δ 28.2 ($C(CH_3)_3$), 54.5 ($OCH_3$), 77.5 (NCH), 148.7 ($CO_2$tBu), 169.4 ($CO_2$Me), 198.0 (CHO) ppm.

### *7.3.4 Methyl (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-hydroxyhept-6-enoate **(5)***

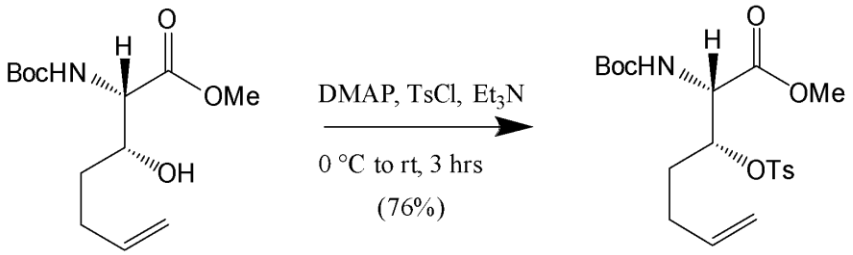



**4** **5**

The aldehyde **4** (0.165 g, 0.001 mol) was dissolved in 10 mL tetrahydrofuran (1.85 M) and placed in dry ice/acetone bath at – 78 ºC in a round bottom flask under a nitrogen atmosphere. To the cooled solution, 2.3 mL (1 M) of 3-butene magnesium bromide was added and the reaction mixture was allowed to stir for 2 hours. The solution was then removed from the bath and allowed to warm up to room temperature over 30 minutes. The reaction was then quenched with 15 mL of saturated aqueous ammonium chloride and the white precipitate that formed was removed by filtration. The reaction was then further diluted with ethyl acetate (20mL) and saturated aqueous ammonium chloride (35 mL). The organic layer was then extracted with an additional 2 times 35 mL of saturated aqueous ammonium chloride. The organic layer was then dried over sodium sulfate and concentrated under reduced pressure to yield **5** as a clear oil. [20]

$^1$H NMR (400Mhz, $CDCl_3$): δ 1.42 (s, 9H, Boc), 1.45-1.50 (m, 4H, $CH_2CH_2$), 3.66 (s, 3H, $OCH_3$), 4.35 (d, 1H, CHNH), 4.9-5.1 (m, 3H, $CHCH_2$), 5.25 (d, 1H, OH), 5.70-5.81 (m, 3H, $CHCH_2$) ppm. $^{13}$C NMR ($CDCl_3$): δ 14.1 ($C(CH_3)_3$), 21.4 ($CH_2CH_2$), 29.1 ($CH_2CH_2$), 61.1 ($OCH_3$), 82.9 (NCH), 116.2 (CHOH) 136.5 ($CHCH_2$), 137.9 ($CHCH_2$), 171.9 ($CO_2$tBu), 196.5 ($CO_2$Me) ppm.

### *7.3.5 Methyl (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-(tosyloxy)hept-6-enoate **(6)***

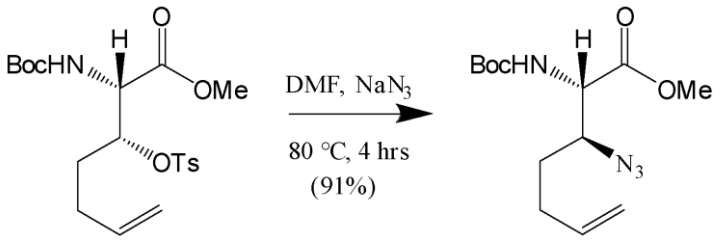



**5** **6**

The Grignard product **5** (0.090 g, 0.697 mol) was dissolved in 1.2 mL of dichloromethane (1 M) at 0º C in a round bottom flask under a nitrogen atmosphere. 0.027 g (0.042 mol) of 4-dimethylaminopyridine and then 0.080 g (0.084 mol) of 4-toluenesulfonyl chloride were sequentially added and the reaction was allowed to stir for 30 minutes. After 30 minutes, 0.05 mL (1 M) of trimethylamine was added dropwise (solution turned yellow) and the solution was stirred for 2 hours. After two hours, the solution was removed from the bath and allowed to warm to room temperature for 30 minutes. The reaction mixture was diluted with diethyl ether (0.07 mL) and allowed to stir for 30 minutes. After 30 minutes, the organic layer was extracted using diethyl ether (20 mL) and saturated aqueous sodium bicarbonate (20 mL). The organic layer was then dried over sodium sulfate and concentrated under reduced pressure to yield **6** as a clear liquid.[21]

$^1$H NMR (400Mhz, $CDCl_3$): δ 1.42 (s, 9H, Boc) 1.45-1.50 (m, 4H, $CH_2CH_2$), 2.43 (s, 3H, benz$CH_3$), 3.66 (s, 3H, $OCH_3$), 4.35 (d, 1H, CHNH), 4.41-4.51 (m, 3H, $CHCH_2$), 4.49-5.12 (M, 3H, $CHCH_2$), 5.71 (m, 1H, CHOTs), 6.96 (d, 1H, NH), 7.39 (m, 2H, benzene), 7.71 (m, 2H, benzene) ppm.

### *7.3.6 Methyl (2S,3S)-3-azido-2-((tert-butoxycarbonyl)amino)hept-6-enoate **(7)***

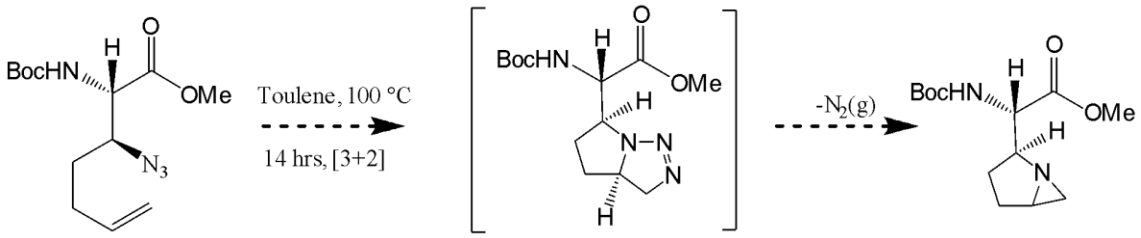



**6** **7**

The tosylation product **6** (0.0900 g, 1 mol) and 0.0175 g (1 mol) of sodium azide were dissolved in 3 mL (1 M) of dimethylformamide in a round bottom flask under a nitrogen atmosphere. The resultant mixture was then refluxed at 80 ºC for 4 hours. After 4 hours, the solution was allowed to cool to room temperature. The solution was then quenched with acetone (2 mL) and allowed to stir for 30 minutes. After 30 minutes, the organic layer was extracted using diethyl ether (20 mL) and deionized water (20 mL). The organic layer was then dried over sodium sulfate and concentrated under reduced pressure and the product **7** was stored as a white solid under high vacuum with no light.[22]

$^1$H NMR (400Mhz, $CDCl_3$): ): δ 1.11-1.19 (m, 4H, $CH_2CH_2$), 1.42 (s, 9H, Boc), 1.39-1.41 (m, 4H, $CH_2CH_2$), 3.28 (m, 1H, $CHN_3$), 3.79 (s, 3H, $OCH_3$), 3.90 (m, 1H, CHNH), 4.81-5.19 (m, 3H, $CHCH_2$), 5.3-5.49 (M, 3H, $CHCH_2$), 6.8 (m, 1H, $CHCH_2$), 7.61 (d, 1H, NH) ppm.

### *7.3.7 Methyl (2S)-2-((2S)-1-azabicyclo[3.1.0]hexan-2-yl)-2-((tert-butoxycarbonyl)amino)acetate **(9)***




7 8 9

The azide compound **7** (0.1889 mol) was dissolved in 20 mL (1 M) of toluene (0.1889 mol) in a round bottom flask under nitrogen atmosphere with no light. The solution was allowed to reflux at 100º C for 14 hours. After 14 hours, the product was allowed to cool to room temperature and then concentrated under reduced pressure.[23]

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