## SYNTHESIS OF BIOTINYLATED AND C4/N1

# SUBSTITUTED $\beta$ -LACTAMS

By

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Chair:

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'IQI' Date

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## DEDICATION

To my grandmother, Galina Gutman, for selflessness.

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### ABSTRACT

The spread of antibiotic resistance inexorably diminishes effective treatments available against bacterial infections.  $\beta$ -lactam antibiotics inhibit bacterial cell wall synthesis, pathways unique to bacteria, and thus are generally safe and effective. Monocyclic  $\beta$ -lactam antibiotics with aryl-thio moieties at C4 were shown to be effective against *Moraxella catharralis* (M. cat) and *Mycobacterium tuberculosis* (Mtb), bacteria traditionally resistant to  $\beta$ -lactams. A set of monocylic  $\beta$ -lactams with trifluoromethylsubstituted thiophenol moieties were synthesized and then carbamoylated. Their activity against M.cat. and Mtb. is reported. The mechanism and cellular target of these  $\beta$ lactams is currently unknown. To that end, we have developed a novel synthesis to tag a derivative of these monocyclic  $\beta$ -lactams with Biotin (Vitamin H). The design, synthesis and evaluation of antimicrobial activity are described.

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## CHAPTER 1

#### **INTRODUCTION**

β-lactam antibiotics, such as penicillin, are safe and potent bactericidal molecules.<sup>1</sup> They are suicide inhibitors of D,D- transpeptidases, enzymes responsible for catalyzing cross-linking in the peptidoglycan layer of the cell wall.<sup>2</sup> The effectiveness of these antibiotics is in sharp decline though due to the emergence of multiple drug-resistant microorganisms.<sup>3</sup> The most common form of β-lactam antibiotic resistance is production of β-lactamases.<sup>1</sup> These enzymes inactivate the antibiotic by hydrolizing the β-lactam ring. For example, in 2008 the NDM-1 gene was identified in Klebsiella pneumoniae; it encodes for a metallo- β-lactamase that inactivates all known β-lactam antibiotics except aztreonam.<sup>4</sup> Another form of resistance, for example expressed by methicillin-resistant *Staphylococcus aureus* (MRSA), is the production of modified transpeptidases with low or no affinity to currently known β-lactam antibiotics.<sup>5</sup> It is thus necessary to synthesize novel β-lactam antibiotics capable of circumventing bacterial defenses while retaining the low toxicity and high efficacy common to this type of antibiotics.<sup>6</sup>

It has been shown (unpublished results from our laboratory) that monocyclic  $\beta$ lactams substituted with thiophenol at C4 (Figure 1) and derivatives thereof have activity against *Mycobacterium tuberculosis* (Mtb) and *Moraxella catharralis* (M. cat.).

1



Figure 1. 4-(phenylthio)azetidin-2-one.

Aggressive  $\beta$ -lactamase activity normally precludes  $\beta$ -lactam antibiotic use against Mtb and M.cat. Furthermore, Mtb can enter a resilient dormant state in which L,D- transpeptidases are substituted for D,D- transpeptidases. This not only enhances the impermeability of the cell wall to antibiotics by changing the crosslink motif from  $4 \rightarrow 3$ to  $3 \rightarrow 3$ , but it also changes the nucleophile in the active site of the transpeptidases from traditional serine to cysteine residue.<sup>2, 7</sup>

It has been demonstrated that the bacteria *Enterococcus faecium* (Ldt<sub>fm</sub>) also uses L,D-transpeptidases, and imipenem, a carbanem which belongs to the  $\beta$ -lactam class, inhibits its growth. There is evidence that the imipenem  $\beta$ -lactam ring acylates the cysteine residue in L,D-transpeptidase, thus preventing cross-linking catalysis.<sup>7</sup> The basis for our synthetic scheme is that monocyclic  $\beta$ -lactams containing potential leaving groups at C4 that have been developed as mechanism-based inhibitors of bacterial and mammalian serine and cysteine enzymes.<sup>8,9,10</sup> Although the requirement of leaving-group (LG) departure to achieve irreversible inhibition is still controversial, we hypothesize that the mechanism of action of the thiophenol is interaction with intracellular sulfur-containing moieties.<sup>11</sup> It follows then that increasing the vulnerability of the  $\beta$ -lactam

ring to nucleophilic attack at the lactam carbonyl at C2 by increasing the electrophilicity of the latter with electron-withdrawing groups (EWG) might enhance its activity. Several compounds were synthesized to test this hypothesis, and the results are presented.

The cellular target of our monocyclic  $\beta$ -lactams is experimentally unknown though, and its identification would be a boon to future drug design. To that end, we proposed to tag 4-((4-mercaptophenyl)thio)azetidin-2-one (Figure 2) with biotin (Vitamin H).



Figure 2. 4-((4-mercaptophenyl)thio)azetidin-2-one.

Biotin has a strong interaction (Ka = 1015  $M^{-1}$ ) with avidin or streptavidin, and thus can be easily removed from the cellular matrix by an avidin column or beads. Biotin is specifically advantageous because it is non-toxic and relatively small (~500 g/mol). It is available with variable length "spacer arms", so that its reactive section can be spatially isolated from the lactam ring. Its small size and spatial separation reduces the likelihood of interference in the mechanism. Moreover, it can be attached to the free sulfhydryl or the nitrogen (N1) in the  $\beta$ -lactam ring. If, as theorized, the mechanism of activity of compound **2** involves the departure of the dithiol at C4 from the lactam, then both the thiophenol and the lactam should be followed. Compound **2** was chosen because biotinylation at the free sulfhydryl would produce a thioether linkage. The other target functional groups options were carboxylic acid or amine, and biotinylation at those sites produces peptide bonds, which can be targeted by bacterial peptidases. The design and novel synthesis of biotinylated  $\beta$ -lactams is presented.

### CHAPTER 2

#### **RESULTS AND DISCUSSION**

## Design, Synthesis, and Activity of Trifluoromethyl (CF3)-Substituted Monocyclic β-lactams

Two types of modifications to the lactam **1** were proposed in the interest of increasing its efficacy against Mtb and M.cat.

First, trifluoromethyl-substituted thiophenyl moieties were attached at C4 (Scheme 1). The trifluoromethyl group was chosen because is a strong EWG and cannot be easily altered in the living systems. Even though, a nitro (-NO<sub>2</sub>) functional group is a stronger EWG, it is reduced by Mtb enzymes to an amino group (-NH<sub>2</sub>), which can be harmful to the human host.  $\beta$ -Lactams with CF<sub>3</sub> substituents at *ortho*-, lactam **4**, *meta*-, lactam **5**, and *para*-, lactam **6**, positions at the thiophenol ring at C4 were prepared in order to determine the effect of the position of the CF<sub>3</sub> group on the antimicrobial activity. Substitution at the *meta*-positions, compound **7**, was synthesized in order to test the effect on the antimicrobial activity of increasing the number of CF<sub>3</sub> groups at the thiophenol. Theoretically, the closer the functional groups are to C2, the greater the effect of the EWG. Increasing the number of functional groups, theoretically, should have even greater electron-withdrawing effect. As previously mentioned, it is expected

that both aforementioned approaches will increase the electrophilicity of C2, and thus the effectiveness of the antibiotic.

Scheme 1 shows the synthesis of these  $\beta$ -lactam derivatives following the procedures of Clauss et al.<sup>12</sup> and Wasserman et al.<sup>13</sup> To the commercially available  $\beta$ -lactam **3** in acetone/water solution the corresponding thiophenol was added in the presence of NaHCO<sub>3</sub>. Several hours, in most cases no more than four hours, were needed for the completion of the reaction. Recrystallization of the crude lactams from ethylacetate/hexanes yielded slightly yellow crystals. Attempts to purify some of the compounds, such as lactam **4**, by a flash chromatography using silicagel, were unsuccessful due to the compound's degradation during separation.



**Figure 3**. Synthesis of C4 Trifluoromethyl-thiophenol β-lactams.

The second type of modification was made with the same intent in mind. The nitrogen in the  $\beta$ -lactam ring, N1, was carbamoylated (Scheme 2).<sup>8</sup> The carbamoyl group

is electron-withdrawing, but it also has been shown to help bypass the bacterial cell wall. Carbamoylated  $\beta$ -lactams **8-11** were synthesized by adding benzyl isocyanate and then triethylamine to each respective product of Scheme 1, **4-7**, in methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>). After purification, the compounds presented waxy solids.



**Figure 4**. Synthesis of Carbamoylated β-lactams.

Kirby-Bauer disc diffusion assays were used to initially screen compounds **4-7** for antibacterial activity by our collaborators, followed by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these compounds using the microdilution protocol. Organisms (*Eschericia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus auerus* ATCC 25923, *Enterococcus faecalis* ATCC 29212) used to screen synthesized compounds represent highly stable quality control strains routinely used for antimicrobial testing.

Compounds	Moraxella 1-3	Mtb H37Rv	Mtb H37Rv
•	& 5-9	without	with clavulanic
	MIC/MBC	clavulanic acid	acid µg/mL
	µg/mL	µg/mL	
7	12.5/100	>100	>100
11	6.25/6.25	>100	>100
5	50/200	6.25	1.55
9	3.125/6.25	NT	NT
6	50/200	12.5	6.25
10	<1.625/12.5	12.5	6.25
4	NT	>100	NT
8	NT	>100	NT
1	NE	100	25
Penicillin	3.1/NT	NT	3.1
Carbamoylated 1	50/50	6.25	6.25

**Table 1.** Minimum Inhibitory Concentrations (MIC) of Novel Compounds.

The most pronounced difference in activity was against M.cat.  $\beta$ -lactam **1**, lacking the thiophenol group and any CF<sub>3</sub> functional groups, respectively, had no effect on the microorganisms tested. Once the CF<sub>3</sub> groups were introduced to either the *para*-, lactam **6**, or *meta*-, lactam **9**, positions of the thiophenol at C4, significant inhibitory and bactericidal properties were observed. The *ortho*-CF<sub>3</sub> substituted lactam **4**, was not tested against M.cat. Both lactams **5** and **6** have the same activity, thus, the data indicates that the position of the  $CF_3$  functional group does not have an effect on the antimicrobial activity of these compounds. Lactam **7** has four-fold the inhibitory and two-fold the bactericidal activity of either lactam **5** or lactam **6**. It appears that the addition of another  $CF_3$  group, increases the activity significantly.

These trends were not observed against Mtb. Instead, the position of the functional group has an effect on the compounds'activity. When the CF<sub>3</sub> group is at the *ortho*-position, lactam **4**, the antimicrobial activity is modest, >100 µg/mL, while when the CF<sub>3</sub> group is at the *meta*-, lactam **5**, and *para*-, lactam **6**, positions the antimycobacterial activity increases, 6.25 µg/mL and 12.5 µg/mL respectively. While a single *meta*- CF<sub>3</sub> substition, lactam **5**, was the most active, further substitution at the second *meta*- position, lactam **7**, reduced the activity back to >100 µg/mL. Both lactams **5** and **6** were more active than lactam **1**.

Most of the CF<sub>3</sub>-substituted lactams were tested against Mtb with and without clavulanic acid, a  $\beta$ -lactamase inhibitor. By inhibiting the  $\beta$ -lactamases, we could determine whether these enzymes have effect on, more specifically hydrolyze, our compounds. The presence of clavulanic acid increased the activity of lactam **6** by two-fold and the activity of lactam **5** by four-fold. This is consistent with the results for lactam **1**, which also increased in activity by four-fold in the presence of clavulanic acid. It appears that the  $\beta$ -lactamases weakly bind to these lactams, but do not destroy their activity altogether, like they do against penicillin. Compounds **4-7** are categorically more effective than penicillin without clavulanic acid, but only compound **5** is more effective when clavulanic acid is co-administered. Therefore this set of compounds resists

hydrolisys by  $\beta$ -lactamase, which is a very attractive feature for a  $\beta$ -lactam based antimicrobials.

Carbamoylation at the lactam nitrogen, N1, was significant for activity against M.cat. Whereas lactam **1** had no effect gainst this bacterium, carbamoylation of lactam **1** had 50  $\mu$ g/mL MIC and MBC. Similarly, the activity of lactams **5**, **6**, **and 7** was increased by carbamoylation at N1. When the lactams are carbamoylated at N1, there is no clear trend for the effect of the position and number of the CF<sub>3</sub> groups at the thiophenol ring at C4. For inhibiting the growth of M.cat. , *para*-CF<sub>3</sub>, lactam **10**, was better than *meta*-CF<sub>3</sub>, lactam **9**, but the activities were reversed for the MBC. Adding another CF<sub>3</sub> functional group to the second *meta*-position, lactam **11**, actually reduced the MIC compared to lactam **9**, and did not change the MBC. Most importantly, carbamoylation in tandem with CF<sub>3</sub> substitution produced significant MBC properties not present with each isolated modification. Only lactam **10** was more inhibitory than penicillin, and it was twice as effective.

Of the compounds tested, only lactam **10** showed significant activity against Mtb, and its activity was not changed from its precursor, lactam **6**. Carbamoylation did not increase the activity of lactams **4** or **7** either. On the other hand, it increased the activity of lactam **1** significantly. The presence of clavulanic acid had no effect on carbamoylated lactam **1**. This is surprising because the activity of lactam **1**, without the carbamoyl group at N1, increased four-fold in the presence of clavulanic acid. Clavulanic acid also increased the activity of compounds **6** and **10**. It might be that carbamoylation is "protecting" these lactams against  $\beta$ -lactamases, e.g. the carbamoylated lactam **1**, but this "protection" has no effect in the presence of CF<sub>3</sub> substituted thiophenols. CF<sub>3</sub>-thiophenol substitution actually reduced the effectiveness of lactam **10** compared to carbamoylated lactam **1** in the absence of clavulanic acid. CF<sub>3</sub> substitution is effective alone- lactam**6** is more effective than lactam **1**, but carbamoylation at N1 and CF<sub>3</sub> substitution at the thiophenol at C4 together, is a drawback. This data suggests that, in Mtb, both modifications may be fulfilling the same role, but CF<sub>3</sub> substitution renders the lactam ring more vulnerable to  $\beta$ -lactamase activity. If carbamoylation at N1 and CF<sub>3</sub> substitution at the thiophenol at C4 compete, then CF<sub>3</sub> substitution at the *meta*-position, lactam **5**, with clavulanic acid produces better results than carbamoylation without CF<sub>3</sub> substitution. As previously mentioned, this combination is the only one more active than penicillin with clavulanic acid. Compound **9** needs to be tested against Mtb to support this theory.

## Design and Synthesis of Biotinylated Monocyclic β-lactams

Compound 2 is not commercially available, so it needed to be synthesized from  $\beta$ -lactam 3 and 1, 4-dithiolbenzene 12. The procedure used for the syntheses of 4-7 was not viable in this case. Both reagent 12 and product 2 have free sulfhydryl functional groups, and these easily form stable disulfide bonds between one another in the basic aqueous conditions used in the earlier syntheses (Scheme 1). A synthesis using water/acetone and NaHCO<sub>3</sub> was initially attempted and the yield of the desired lactam 2 was low due to dimerization, lactam 13 (Scheme 3).



Figure 5. Synthesis of Tag Reagent and Subsequent Dimerization.

As a result, we sought to develop an organic, anhydrous, and aprotic solvent/base system.  $\beta$ -lactam **2** was first successfully synthesized using anhydrous dimethylformamide (DMF), triethylamine, and a catalytic amount of dimethylaminopyridine (DMAP). Control reactions combining both reactants in pure DMF or triethylamine yielded no product. The reaction was also successfully completed sans DMAP in deuterated chloroform, to directly take NMR scans. In chloroform, the reaction proceeded more slowly, and for that reason the mixture was pushed to completion by irradiation.

It is important to note that the characteristic shift of the C4 hydrogen peak is different in the NMR figure of compound **2**. Normally, the reagent peak is about 5.9 ppm, and this peak moves upfield to about 5.0 ppm when the acetyl group is replaced with a less electron-withdrawing thiophenol substituent. Synthesis of lactam **2** yields two signals at 4.9 ppm and 4.8 ppm with a stoichiometric relationship of 1:1 lactam **3** to thiol 12. At first, only the signal at 4.8 ppm was present, but, as the reaction reached completion, the ratio of these signals approached a 1:1 ratio. Over time and with environmental exposure, the 5.8 ppm signal degraded into a multiplet. When the reagent ratio was changed to 5:1 lactam 3 to thiol 12, the first peak to appear was at 5.9 ppm. Furthermore, this peak remained prominent as the reaction progressed. These shifts and ratios suggest that lactam 14 is formed during the synthesis.



Figure 6. 4,4'-(1,4-phenylenebis(sulfanediyl))bis(azetidin-2-one).

The C4 signal in lactam **14** should be slightly more downfield because the presence of a second lactam group is electron-withdrawing. When lactam **3** was in excess, the 5.9 ppm was first produced and remained the major product. The degradation of only the 5.8 ppm indicates the eventual formation of dimers and chains of lactam **2**. The corresponding peaks in the aromatic region further support lactam **14** as a by-product. The hydrogen atoms on the aromatic ring of thiol **12** produce a singlet at about 7.1 ppm because of the molecule's symmetry, and this singlet splits into two doublets, indicating the splitting expected of the asymmetrical lactam **2**. Lactam **14** is also

symmetrical, and this singlet was found at about 7.4 ppm. In some spectra, it was possible to see two juxtaposed singlets, each one referring to a diastereomer of **14**. Unfortunately, the lactam **14** produced an insoluble film after vacuum rotary evaporation or attempts at purification via column chromatography. Methanol washes extracted some product from the film, but subsequent evaporation continuously produced more precipitate. The film was only soluble in DMSO, and NMR analysis suggested that  $\beta$ lactam **14** was degrading and that dimer **13** was being formed.

Dimerization only occurred with prolonged environmental exposure, so this new approach was successful in preventing dimerization in the short-term. Storage and purification were still not possible without significant dimerization though, and lactam 14 remained as a significant byproduct. Lactam 14 sequesters two equivalents of lactam 3 for every equivalent of thiol 12, so there will inevitably be leftover thiol 12. Proceeding to the addition of sulfhydryl-reactive biotin would be inefficient. In response, we considered protecting the labile sulfhydryl group to further minimize dimerization and formation of the byproduct. Preliminary research on sulfhydryl-reactive biotin also favored this strategy. While sulfhydryl-reactive biotin mainly attaches to the intended – SH moiety, it has some reactivity towards amines or amides in basic conditions. A protected sulfhydryl would then force biotin to favor attachment to the  $\beta$ -lactam ring at N1. With this strategy, we could feasibly control the location of the biotin tag. As previously mentioned, the mechanism of action of our lactams might involve disassociation of the thiol moiety at C4, necessitating tracking of both the thiophenol and the lactam.

To that end, the synthetic approach presented in Scheme 4 was developed. Hexamethyldisilazane **14** was added to thiol **12** in chloroform-d to protect the sulfhydryl with a trimethylsilyl (TMS) group. HMDS was used because, though it is a weak silylating agent, the only byproduct is ammonia. As the ammonia evaporated, the reaction was easily monitored with wet pH paper.



Reagents and Conditions: (a) Et<sub>3</sub>N in Chlorform-d

Figure 7. Sulfhydryl Protection by HMDS.

HMDS slowly protected one of the thiol functional groups with and without triethylamine in both chloform-d and DMF. Irradiating the mixture in chloform-d in the presence of triethylamine pushed the reaction to completion. Protection was easily discerned due to splitting of the aromatic hydrogens in an asymmetric molecule. While there is some evidence of a shifted singlet at about 6.8 ppm, which could indicate doubly silylated thiol **12**, this peak was minor even in excess HMDS **15**. The TMS group is electron-withdrawing and thus weakens the nucleophilicity of the other thiol functional group.

Preliminary evidence shows that subsequent addition of lactam **3** was successful though slow, even after irradiation. This is probably because the acetyl group is not as good of a leaving group in the organic chloroform. The NMR spectrum shows a shift to 5.0 ppm of the C4 hydrogen. The silylated lactam **16** appeared slightly more downfield than lactam **2** as expected because, as previously mentioned, the TMS group is electron-withdrawing, and thus shifts the peak downfield to its unprotected counterpart. This reaction needs to be repeated to obtain an MS spectrum to validate the results.

While this reaction was attempted in many other ways unsuccessfully, it is interesting to note that without triethylamine, lactam **2** catalyzes both the silylation of thiol **12** and itself. After about 2 hours, the aromatic peak of thiol **12** turns into its characteristic doublets and the proton peaks on the lactam **2** shift slightly upfield. For example, the 6.0 ppm peak shifts to about 5.5 ppm. At first, it was thought this might be evidence that the two are attached, but subsequent cleavage by methanol-d or trifluoro acetic acid both returned a NMR spectrum identical to both starting reagents. It is thus important to monitor the reaction accordingly, because once lactam **2** undergoes protection, most likely through a silyl enol ether at the acetyl group, it is difficult to react it further with thiol **12**. So far this same behavior is seen when using NaHMDS. Unlike HMDS, NaHMDS is a very strong base. Thus we hope to eliminate the need for triethylamine and simplify future purification.

While the methodology for silulation of lactam **2** was being developed, we explored biotinylation without silulation.

All types of biotin were purchased commercially from Thermo Fisher Scientific. Biotin-HPDP, **17**, was the one of the first biotin used in this synthetic strategy.



Figure 8. Structure of Biotin HPDP.

This biotin forms a disulfide bond with the free thiol on  $\beta$ -lactam **2**, and pyridine-2-thione is the resulting leaving group. Pyridine-2-thione is UV-active at 343 nm, and therefore the reaction can be monitored at that wavelength. Moreover, pyridine-2-thione can react with excess of the starting reagent -  $\beta$ -lactam **3** to form another derivative of lactam **1**. Biotin HPDP has a hydrophobic spacer arm that is 29.2 angstroms long.

The reaction was first monitored by UV absorbance by UV/VIS spectrophotometer. 1,4 benzenedithiol, **13**, in DMF was used to blank the spectrum. After addition of Biotin HPDP and triethylamine, absorbance at 343 nm was immediately detected, but there was no following change in absorbance after 15 minutes. This showed that biotinylation was instantaneous, and that it was not necessary to monitor the progress of the reaction. After addition of excess  $\beta$ -lactam **12**, analysis of the crude mixture by <sup>1</sup>H NMR and MS indicated that biotinylated lactam **2** was produced in addition to lactam bound to the pyridine-2-thione leaving group, lactam **18**.



Figure 9. 4-(pyridin-2-ylthio)azetidin-2-one.

Purification was attempted by diluting DMSO with brine and extracting with ethyl acetate or methylene chloride. A white precipitate appeared in the water layer. Analysis of the precipitate indicated that it was unreacted starting material. Unfortunately, an insoluble film was produced after vacuum rotary evaporation of the organic layer. Methanol washes extracted some product from the film, but subsequent evaporation continuously produced more precipitate indicating the same degradation observed with lactam **14**.

The two types of biotin that yielded the most promising results were Biotin-BMCC, **19**, and Maleimide-PEG2-Biotin (Biotin-PEG2), **20**.



Figure 10. Biotin-BMCC and Maleimide-PEG2-Biotin.

Both types of biotin use a maleimide group to attach to a thiol functional group via a Michael Addition mechanism. Biotin-BMCC is soluble in DMF or DMSO and Biotin-PEG2, because of the relatively hydrophilic polyethylene glycol spacer arm, is soluble in methylene chloride or chloroform.

Biotin-BMCC attachment was monitored in an NMR tube using deuterated DMSO using the same strategy as in Figure 6, except that Biotin-BMCC was used as a reagent instead of HMDS. NMR and MS analysis of the crude showed that biotinylated lactam **2** was produced. The C4 proton peak was present at 5.0 ppm and the doublets of an asymmetrical aromatic region were present. In the NMR spectrum of the Biotin-BMCC standard, the protons attached to double-bonded carbons are represented by the peak at around 7.0 ppm. This peak completely disappears in the crude and instead a peak appears at about 4.2 ppm, showing that these protons are much more shielded, as expected. A positive ion MS scan of the crude shows a molecular peak at 745.4 m/z as well as a complex with sodium at 767.4 m/z. The MS scan shows that the reaction did not go to completion because there is a peak at 676.3 m/z, the molecular weight of biotinylated thiol **12** without attached lactam.

Purification was attempted by diluting DMSO with brine and extracting with ethyl acetate or methylene chloride. Both extracts did not yield the product, but suggested that starting material was extracted instead. Evaporation of the water layer and subsequent NMR analysis in DMSO again showed that the biotin peaks were retained, but that the yield was very low. MS analysis needs to be performed, and the extraction needs to be repeated for consistency.

Biotin-PEG2 attachment was more easily purified because the reaction was performed in chloform-d, and the solvent readily evaporates at room temperature. The synthetic strategy was slightly altered to first attach thiol **12** to lactam **3**, and then to follow with addition of Biotin-PEG2. The crude NMR showed the lactam C4 peak at 5.0 ppm as well as the aromatic thiol doublets at about 7.3 ppm. In the Biotin-PEG2 standard, the signal for the hydrogen atoms bound to the double bond in the maleimide group appeared at about 6.7 ppm. This signal disappeared in the crude and was replaced by two signals at around 4.0 ppm. MS analysis clearly showed two peaks in the high molecular weight range, 737.4 m/z and 597.3 m/z. The former refers to the desired biotinylated product and the latter refers to biotinylated lactam **2** not bound to thiol **12**. These two products explain the multiple signals at 4.0 ppm in the NMR spectra of the

crude product. In a repeat attempt of the same methods, both the 737.4 m/z and 597.3 m/z peaks appeared only as complexes with sodium (23 amu). Fragmentation analysis of the 759.3 m/z peak yielded 716.3 m/z and 690.5 m/z fragments. These fragments show loss of 43 and 69 amu respectively. This fragmentation pattern is characteristic of the lactam ring. The former mass characterizes the split of the bottom half the ring, and the latter mass characterizes loss of the entire ring itself.

Purification was attempted by thin-layer chromatography (TLC). The optimal mobile phase was determined to be 8% methanol, 16% hexanes, and 76% methylene chloride. This separation yielded 5 products, and MS analysis of the third dot yielded only 759.5 m/z peak in the high mass range. Preliminary analysis of other mobile phases with less effective separation suggests that the identity of dot 4 is the 597.3 m/z peak.

Using this data we hope to further develop a robust methodology for controlling the stereoselectivity and stereochemistry of biotinylation of compound **2**.

## CHAPTER 3

#### CONCLUSION

It is imperative to synthesize new antibiotics more quickly than new resistances appear among bacteria. We designed and synthesized eight derivatives of a known monocyclic  $\beta$ -lactam antibiotic. Several derivatives showed increased inhibitory and bactericidal activity against Mtb. and M.cat. We also successfully tagged a monocylic  $\beta$ lactam with biotin, and we aim to determine the cellular target in Mtb. and M.cat. using this tagged molecule. The patterns elicited from the derivatives and identification of the cellular target will all better future antibiotic design in this class of  $\beta$ -lactam antibiotics.

### CHAPTER 4

#### EXPERIMENTAL

#### Equipment and Materials

All reactions were monitored by thin-layer chromatography (TLC) using EM Reagents plates with fluorescence indicator (SiO<sub>2</sub>-60, F<sub>254</sub>) and layer thickness: 250 μm; purchased from EMD Chemicals, Inc. (Gibbstown, NJ). Unless otherwise noted, the compounds were detected under UV light and iodine vapors. Melt-Temp II melting point apparatus was used to determine the melting points of the synthesized compounds. NMR spectra (25°C) were obtained at 400 MHz for <sup>1</sup>H NMR and 125 MHz for <sup>13</sup>C NMR with a Bruker 400 spectrometer (Billerica, MA) in CDCl<sub>3</sub> or DMSO-D<sub>6</sub>. IR spectra were obtained as a thin film on NaCl plates and in solid form (KBr standard) on a Shimadzu FT-IR-8300 (Columbia, MD). Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. HRMS was carried out by University of Iowa, (Iowa City, IA). All other solvents, chemicals and enzymes were purchased from Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Pittsburgh, PA) and Acros Organics (Geel, Belgium). Unless stated otherwise, solutions in organic solvents were dried with anhydrous magnesium sulfate, and concentrated under vacuum conditions using rotatory evaporation. Mass spectra were measured on LC/MS 2010 Shimadzu mass spectrometer (Columbia, MD), equipped with electrospray ionization source. ESI/MS spectra were recorded in the positive-ion setting. Acidified LC/MS grade methanol was purchased from Fisher and used as received. Electrospray mass spectrometric analysis was performed using high purity nitrogen as nebulizing gas at pressure of 80/80kgf/cm<sup>2</sup>, with a gas-flow rate of 4.5 L/min and temperature of the probe at 25°C. Each sample was introduced into the system at 10  $\mu$ L/min.

#### **Reactions and Synthesis**

General procedure for the preparation of trifluoromethyl-substituted thiophenol βlactams. A solution of 50 mL acetone, 30 mL acetone, and 4.0 molar equivalent (0.03 mol, 2.6g) of NaHCO<sub>3</sub> was prepared. 1.00 molar equivalent (7.74 mmol, 1.00g) of of 4acetoxy-2-azetidinone was added followed by 1.03 molar equivalent of the respective trifluoromethyl benzenethiol. The solution was stirred overnight at room temperature. The mixture was then filtered to remove NaHCO<sub>3</sub>. The aqueous layer was extracted with ethyl acetate (3x50mL), and NaCl was added to aid in the formation of two layers. The resultant yellow-white powder was purified by crystallization in a solution of ethyl acetate/hexanes.

### 4-((2-(trifluoromethyl)phenyl)thio)azetidin-2-one.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δH 2.95 (1H, ddd, *J* = 14.28, 2.30, 1.02); 3.44 (1H, ddd, *J* = 8.68, 3.10, 1.80); 5.03 (1H, dd, *J* = 2.62, 1.99); 6.91 (1H, singlet); 7.47 (1H, triplet, *J* =

7.16); 7.56 (1H, triplet, J = 7.11); 7.62 (1H, doublet, J = 7.55); 7.75 (1H, doublet, J = 7.55); 7.75 (1H, doublet, J = 7.16); 7.62 (1H, doublet, J = 7.55); 7.75 (1H, doublet, J = 7.55); 7.55]

7.63). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 31.11, 45.99, 55.05, 127.47, 128.80, 131.42,

132.63, 135.88, 166.26. IR (neat)  $v_{max}$  (C=O) 1766 cm<sup>-1</sup>. MP: 81-82 °C. Yield: 90%.

### 4-((3-(trifluoromethyl)phenyl)thio)azetidin-2-one.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$ H 2.92 (1H, ddd, J = 14.50, 1.12, 0.80); 3.45 (1H, ddd, J =

8.52, 3.05, 1.88); 5.07 (1H, dd, *J* = 2.62, 2.27); 6.80 (1H, singlet); 7.51 (1H, triplet, J =

7.75), 7.62 (1H, doublet, *J* = 9.85), 7.67 (1H, doublet, *J* = 9.15), 7.72 (1H, singlet).

<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 45.83, 54.38, 125.43 (1C, quartet, J = 0.03), 129.75 (1C,

quartet, J= 0.03), 130.03, 131.95 (1C, quartet, J = 0.33), 133.41, 136.32, 166.39. IR

(neat)  $v_{max}$  (C=O) 1766 cm<sup>-1</sup>. MP: 73-75 °C. Yield: 82.5%.

### 4-((4-(trifluoromethyl)phenyl)thio)azetidin-2-one.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δH 2.95 (1H, ddd, *J*=11.72, 1.40, 0.85); 3.47 (1H, ddd,

J=8.45, 3.06, 1.88); 5.11 (1H, dd, J=2.65, 2.30); 7.01 (1H, singlet); 7.51 (2H, doublet,

J=8.08); 7.61 (2H, doublet, J=8.17). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 45.91, 53.78,

126.34, 130.13 (1C, quartet, J = 0.33), 131.75, 137.57, 166.43. IR (neat)  $v_{max}$  (C=O) 1764 cm<sup>-1</sup>. MP: 95-96.5 °C. Yield: 80.5%.

### 4-((3,5-bis(trifluoromethyl)phenyl)thio)azetidin-2-one.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δH 3.00 (1H, ddd, J = 11.90, 1.26, 0.98); 3.55 (1H, ddd, J = 8.28, 2.89, 2.11); 5.17 (1H, dd, J = 2.65, 2.32); 6.46 (1H, singlet); 7.86 (1H, singlet); 7.88 (2H, singlet). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 46.04, 54.22, 121.59, 122.11, 124.30, 132.11, 132.77 (1C, quartet, J = 0.33), 135.93, 166.04. IR (neat)  $v_{max}$  (C=O) 1771 cm<sup>-1</sup>. MP: 77-79 °C. Yield: 79%.
### General procedure for the preparation of carbamoylated β-lactams.

1.00 molar equivalent of each respective compound **4-7** was dissolved in ~3 mL of dichloromethane. 1.1 molar equivalent (2.48 mmol, 0.304 mL) of benzyl isocyanate was added to solution followed by 1.1 molar equivalent (2.48 mmol, 0.345 mL) of triethylamine. The solution was stirred overnight at room temperature, and the solvent was allowed to evaporate the next day. The resultant viscous oil was purified by crystallizing the impurities using a solution of ethyl acetate/hexanes. Over time, some samples originally characterized as a viscous oil hardened into a wax.

#### N-benzyl-2-oxo-4-((2-(trifluoromethyl)phenyl)thio)azetidine-1-carboxamide.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δH 2.87 (1H, dd, J = 13.74, 2.83); 3.42 (1H, dd, J = 10.80, 5.75); 4.33 - 4.47 (m); 5.28 (1H, dd, J = 2.92, 2.78); 6.76 (1H, singlet); 7.19 - 7.31 (m); 7.39 -7.48 (m); 7.67 (1H, doublet, J = 7.65); 7.84 (1H, doublet, J = 7.49). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 43.87, 44.62, 58.18, 127.22 (1C, quartet, J = 0.05), 127.81, 127.86, 128.95, 129.23, 130.49, 132.54, 134.46 (1C, quartet, J = 0.3), 137.69, 137.92, 149.77, 165.49. IR (neat)  $v_{max}$  (C=O) 1778 cm<sup>-1</sup>, 1706 cm<sup>-1</sup>. MP: Viscous Oil (N/A). Yield: 66%.

## N-benzyl-2-oxo-4-((3-(trifluoromethyl)phenyl)thio)azetidine-1-carboxamide.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$ H 2.82 (1H, dd, *J* = 13.66, 2.77); 3.41 (1H, dd, *J* = 10.72, 5.92); 4.41 (1H, doublet, *J* = 5.95); 5.26 (1H, dd, *J* = 2.77, 1.55); 6.72 (1H, singlet); 7.18-7.29(m); 7.39 (1H, triplet, *J* = 7.81); 7.54 (1H, doublet, *J* = 7.80); 7.74 (1H, doublet, *J* = 7.85); 7.77 (1H, singlet). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  43.91, 44.57, 57.21, 125.90,

127.85, 127.88, 128.95, 129.88, 131.14, 131.74 (1C, quartet, *J* = 0.32), 132.13, 137.74, 137.79, 149.71, 165.20.

IR (neat)  $v_{max}$  (C=O) 1777 cm<sup>-1</sup>, 1706 cm<sup>-1</sup>. MP: Viscous Oil (N/A). Yield: 67.5% **N-benzyl-2-oxo-4-((4-(trifluoromethyl)phenyl)thio)azetidine-1-carboxamide.** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$ H 2.99 (<sup>1</sup>H, dd, *J*=13.63, 2.81); 3.57 (1H, dd, *J* = 10.71, 5.74); 4.52 (1H, doublet, *J* = 5.99), 5.41 (1H, dd, *J* = 2.93, 2.81); 6.87 (1H, singlet); 7.29-7.41 (m); 7.67 (1H, dd, *J* = 43.02, 8.11). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  43.91, 44.86, 57.01, 126.20, 127.87, 127.93, 128.98, 130.62 (1C, quartet, *J* = 0.33), 133.38, 136.34, 137.81, 149.75, 165.26. IR (neat)  $v_{max}$  (C=O) 1778 cm<sup>-1</sup>, 1708 cm<sup>-1</sup>. MP: 72-73 °C. Yield: 64%.

**N-benzyl-2-**((**3**,**5**-bis(trifluoromethyl)phenyl)thio)-4-oxoazetidine-1-carboxamide. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$ H 2.91 (1H, dd, *J* = 13.62, 2.89); 3.53 (1H, dd, *J* = 10.64, 5.82); 4.36-4.46 (m); 5.33 (1H, dd, *J* = 2.94, 2.89); 6.72 (1H, singlet); 7.19 - 7.30 (m); 7.77 (1H, singlet); 8.05 (1H, singlet). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  43.99, 44.83, 57.49, 121.68, 122.45, 124.40, 127.88, 127.94, 128.97, 132.52 (2C, quartet, *J* = 0.33), 133.26, 135.40, 137.61, 149.62, 164.80. IR (neat)  $v_{max}$  (C=O) 1780 cm<sup>-1</sup>, 1707 cm<sup>-1</sup>. MP: 61-62 °C. Yield: 65%.

## APPENDICES

# APPENDIX A: NMR SPECTRA



Figure 11. <sup>1</sup>H NMR Spectrum of Compound 4



Figure 12. <sup>1</sup>H NMR Spectrum of Compound 5



Figure 13. <sup>1</sup>H NMR Spectrum of Compound 6



Figure 14. <sup>1</sup>H NMR Spectrum of Compound 7.



Figure 15. <sup>1</sup>H NMR Spectrum of Compound 8



Figure 16. <sup>1</sup>H NMR Spectrum of Compound 9



Figure 17. <sup>1</sup>H NMR Spectrum of Compound 10



Figure 18. <sup>1</sup>H NMR Spectrum of Compound 11



Figure 19. <sup>13</sup>C NMR Spectrum of Compound 4



Figure 20. <sup>13</sup>C NMR Spectrum of Compound 5



Figure 21. <sup>13</sup>C NMR Spectrum of Compound 6



Figure 22. <sup>13</sup>C NMR Spectrum of Compound 7



Figure 23. <sup>13</sup>C NMR Spectrum of Compound 8



Figure 24. <sup>13</sup>C NMR Spectrum of Compound 9



Figure 25. <sup>13</sup>C NMR Spectrum of Compound 10



Figure 26. <sup>13</sup>C NMR Spectrum of Compound 11



Figure 27. <sup>1</sup>H NMR Spectrum of Compound 3



Figure 28. <sup>1</sup>H NMR Spectrum of Compound 12 in CDCl<sub>3</sub>



Figure 29. <sup>1</sup>H NMR Spectrum of Compound 12 in DMSO-d<sub>6</sub>



Figure 30. <sup>1</sup>H NMR Spectrum of Compound 15



Figure 31. <sup>1</sup>H NMR Spectrum of Compound 17



Figure 32. <sup>1</sup>H NMR Spectrum of Compound 19



Figure 33. <sup>1</sup>H NMR Spectrum of Compound 20



Figure 34. <sup>1</sup>H NMR Spectrum of Compound Hydrolyzed Compound 20



**Figure 35.** <sup>1</sup>H NMR Spectrum of Compound **2** and **14** Synthesized with 1:1 Ratio of Compound **3** and Compound **12** 



**Figure 36.** <sup>1</sup>H NMR Spectrum of Compounds **14** Synthesized with 5:1 Ratio of Compound **3** and Compound **12** 



Figure 37. <sup>1</sup>H NMR Spectrum of Compound 13



Figure 38. <sup>1</sup>H NMR Spectrum of Crude Biotinylated (HPDP) Compound 2



**Figure 39.** <sup>1</sup>H NMR Spectrum of the Precipitate Formed in the Aqueous Layer during Purification of Biotinylated (HPDP) Compound **2** 



**Figure 40.** <sup>1</sup>H NMR Spectrum of MeCl<sub>2</sub> Layer during Purification of Biotinylated (HPDP) Compound **2** 



Figure 41. <sup>1</sup>H NMR Spectrum of Biotinylated (BMCC) Compound 2



Figure 42. <sup>1</sup>H NMR Spectrum of  $H_2O$  Layer during Purification of Biotinylated (BMCC) Compound 2



Figure 43. <sup>1</sup>H NMR Spectrum of Biotinylated (PEG2) Compound 2


Figure 44. <sup>1</sup>H NMR Spectrum of Compound 13a



Figure 45. <sup>1</sup>H NMR Spectrum of Compound 15

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Figure 46. <sup>1</sup>H NMR Spectrum of Silylated Compound 3 and 12



**Figure 47.** <sup>1</sup>H NMR Spectrum of Deprotected Compound **3** and **12** (see Figure 46) Utilizing Trifluoroacetic Acid

APPENDIX B: MS SPECTRA



Figure 48. MS Spectrum of Compound 14



Figure 49. MS Spectrum of Crude Biotinylated (HPDP) Compound 2



Figure 50. MS Spectrum of Crude Biotinylated (BMCC) Compound 2



Figure 51. MS Spectrum of Crude Biotinylated (PEG2) Compound 2



Figure 52. MS Spectrum of Biotinylated (PEG2) Compound 2



Figure 53. MS2 Fragmentation Spectrum of Biotinylated (PEG2) Compound 2



Figure 54. MS Spectrum of Slightly Impure Biotinylated (PEG2) Compound 3

APPENDIX C: IR SPECTRA



Figure 55. IR Spectrum of Compound 4



Figure 56. IR Spectrum of Compound 5

## SHIMADZU



Figure 57. IR Spectrum of Compound 6.



Figure 58. IR Spectrum of Compound 7.

🕀 SHIMADZU



Figure 59. IR Spectrum of Compound 8.



Figure 60. IR Spectrum of Compound 9.



Figure 61. IR Spectrum of Compound 10.

🕀 SHIMADZU



Figure 62. IR Spectrum of Compound 11.

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