Design and Development of seco-B Ring Compounds as Effective Non-Classical Antifolates Against Gram-negative and Gram-positive Pathogens

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B.A., University of Iowa, 2010

A Thesis
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By

Kyle Wickersham

2016
Master of Science Thesis

Design and Development of seco-B Ring Compounds as Effective Non-Classical Antifolates Against Gram-negative and Gram-positive Pathogens

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University of Connecticut 2016
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When I first came to the University of Connecticut I wasn’t sure if I was going to find a Group where I could do research in an area I was interested in. Then I met Dr. Dennis Wright in the Pharmaceutical Science department. He allowed me to do the research that would help me in my future pursuit for a job in the pharmaceutical industry. While in his Lab I had the freedom and support to pursue my own ideas.

I would like to acknowledge Dr. Amy Anderson and her Lab Group for their help in connecting the dots between chemistry and biology. I would also like to thank Dr. Mark Peczuh for furthering at the time my limited knowledge in organic chemistry.

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Special thanks to Karla Arias and Jacob Loman, without them it would have been a very lonely two years in Connecticut.

Most Importantly I have to thank my parents. If it wasn’t for my parents and their sacrifices and support, and without it I may have never gone to college in the first place. From my Mom dropping off lunches to school that I forgot to waking up at 4:30 AM to take me to wrestling practice. My Dad missing many dinners at home to provide for us and give us the opportunities that many other kids never had, I thank you.

Lastly, I like to thank the sport of wrestling and the mentality it bestowed upon me because Tom Brand says it best, “The only thing you deserve is what you earn.”
# Table of Contents

Approval page..............................................................................................................iii  
Acknowledgements....................................................................................................iv  
Table of Contents.......................................................................................................v  
Chapter One: Introduction.............................................................................................1  
  1.1 The Current State of Antibacterials.................................................................1  
  1.2 Trimethoprim and the Folate Pathway.............................................................2  
  1.3 Bacterial Resistance in Antifolates.................................................................3  
  1.4 Propargyl-linked Antifolates.............................................................................4  
    1.4.1 First Generation Propargyl-linked Antifolates.........................................4  
    1.4.2 Second Generation Propargyl-linked Antifolates.................................5  
    1.4.3 Third Generation Propargyl-linked Antifolates.....................................6  
  1.5 Gram-negative Pathogens.................................................................................8  
    1.5.1 Outer Membrane of Gram-negative Pathogens.....................................8  
    1.5.2 Efflux Pumps of Gram-negative Pathogens..........................................9  
  1.6 Passage into Gram-negative Cytoplasm.......................................................10  
  1.7 Development of seco-B Ring Antifolates.....................................................10  
  1.8 Conclusion.......................................................................................................13  
References...................................................................................................................14  
Chapter Two: Design and Development of seco-B Ring Compounds......................17  
  2.1 Hetero straight chain seco-B Ring Antifolates............................................17  
  2.2 Sulfur-linked seco-B ring Compounds.........................................................18  
  2.3 Branched seco-B Ring Sulfide Compounds...............................................20  
  2.4 Sulfur-linked seco-B Rings in EcDHFR......................................................23  
  2.5 Hydrogen Bonding Network Observation in EcDHFR..............................25  
  2.6 Sulfate in EcDHFR.......................................................................................26  
  2.7 Conclusion.....................................................................................................27  
References...................................................................................................................28  
Chapter 3 Experimental Procedures..........................................................................30  
Appendix......................................................................................................................49
Chapter 1

Introduction

1.1 The Current State of Antibacterials

Community-associated and hospital-acquired infections of *Staphylococcus aureus* have increased in the past 20 years. *S. aureus* can cause skin and soft tissue infections such as folliculitis and impetigo or invasive infections like endocarditis and pneumonia. Which results in *S. aureus* being the most common cause of death and disease from an infection in the United States.¹ This rise can be explained by the rise in antibiotic-resistant strains, mainly methicillin-resistant *S. aureus* (MRSA) and more recently, vancomycin-resistant strains.²

To further complicate matter of antibiotic resistance, there has been a steep decline in antibiotic drug development for about two decades. Largely due to unexpected complications with newer innovative approaches to drug design. Large-scale genomics have sparked target-based efforts, where proteins important to bacterial growth are utilized in high-throughput screening efforts. These proteins are then used in high-throughput screens that produced small molecule “hits”, but the small molecules resulted in lack of permeability or solubility when applied to whole cell screens.³ Another cause for the decline in drug development is that major pharmaceutical companies and biotech companies have abandoned their antibiotic research, partly to pursue the larger profits of
chronic diseases and partly due to large amount of antibiotics already on the market such as the non-classical antifolate Trimethoprim (TMP).4

1.2 Trimethoprim and the Folate Pathway

Figure 1. TMP an inhibitor of DHFR

TMP-resistance was very rare before 1983, more recently 28% of MRSA have been reported resistant to TMP. What was a useful treatment for MRSA, has been largely negated.5 Trimethoprim (TMP), a diaminopyrimidine antimicrobial as seen in Figure 1, is

Figure 2. Folate Biosynthesis Pathway. Produced from Lehninger Principles of Biochemistry, 2008, 5th Edition
a broad-spectrum antibiotic which includes *S. aureus*. It exhibits an excellent safety profile, easily administered orally, relatively inexpensive, and works through competitive inhibition of dihydrofolate reductase (DHFR), a focal point in the folate biosynthesis pathway. TMP also works together with sulphonamides, to form the drug combination co-trimoxazole, which also targets folate biosynthesis. Figure 2 displays the essential steps in the folate pathway. DHFR catalyzes the reduction of dihydrofolate to tetrahydrofolate with the cofactor NADPH. Serine hydroxymethyltransferase then converts tetrahydrofolate to 5,10-methylene tetrahydrofolate, which is an important cofactor in biosynthesis of purine nucleotides, histidine and methionine amino acid, and the C1 unit transfer for the biosynthesis of deoxythimidine monophosphate (dTMP) production.7,8

### 1.3 Bacterial Resistance in Antifolates

Most TMP-resistant strains display one of two chromosomal mutations: H30N/F98Y or H149R/F98Y. The F98Y is assumed to be the biggest cause for the loss of affinity of TMP in the double mutant. The double mutant results in a 36-fold loss of activity of TMP against *S. aureus* DHFR, and it competes for hydrogen bonding with Leu5, which normally hydrogen bonds with N-4 amine nitrogen of TMP.9

This increase in resistance to antifolates warrants the development of new compounds that will overcome the loss of affinity brought on by the mutations in MRSA. Research in the Wright and Anderson groups focuses on Structure Activity Relationship
(SAR) design, synthesis, and assay of new non-classical antifolates against TMP resistant bacteria.

1.4 Propargyl-linked Antifolates

1.4.1 First Generation Propargyl-linked Antifolates

TMP is an antifolate that is a selective inhibitor of *E. coli* and *S. aureus* DHFR over human DHFR. Whereas, methotrexate (MTX) is a nonselective inhibitor, but is more potent than TMP and is used mostly in cancer chemotherapy. This difference in selectivity and potency is the focus of the Wright group, to optimize TMP to be as potent as MTX, but maintain its selectivity towards *E. coli* and *S. aureus*. Through structural analysis of DHFR with TMP it was observed that the interaction between 2,4-

![Figure 3. TMP and NADPH crystallized with S. aureus DHFR. Produced from Proteins, 2009, 76, 706-717.](image)
diaminopyrimidine and an acidic residue is conserved among many different species of DHFR. Shown in Figure 3, TMP forms four hydrogen bonding interactions with residues in the active site. The N1 and N2 on the diaminopyrimidine ring interacts with oxygen atoms of Asp27, and N4 amine hydrogen bonds with the carbonyl oxygen atoms of Leu5 and Phe92. There are also several van der Waals interactions with Leu5, Val6, Ala7, Val31, Phe92, and the nicotinamide ring of NADPH. The trimethoxybenzyl group is in a hydrophobic pocket interacting with Leu20, Leu28, Ile50, Leu54 residues. The oxygens of the methoxy groups form hydrogen bonds with water molecules.

A comparison of TMP with MTX displays that the trimethoxybenzyl group does not reach as far into the hydrophobic pocket as the pABA region of MTX. It is this observation that a plan was devised to extend the linker region to increase TMP activity. The linker length was increased to 3 carbon atoms and through trial and error of saturated, acetylene, and propargyl linkers it was determined that the 3 carbon propargyl linker afforded the best activity. The propargyl linker maintains the same number of degrees of freedom, and pushes the trimethoxybenzene group further into the hydrophobic pocket.

1.4.2 Second Generation Propargyl-linked Antifolates

Although the propargylic linker was relatively potent compared to TMP, its selectivity over human DHFR (hDHFR) was only 36-fold. To overcome this a C-ring was added on to the B-ring. It was observed that the opening to the active site in hDHFR is restricted by a four-residue loop of Pro61, Glu62, Lys63, and Asn64 also known as the
PEKN loop which is absent in ChDHFR. Through docking experiments as seen in Figure 4, it was observed that a functionality added meta or para to the B-ring would interact with a region of space filled by the PEKN loop in hDHFR. This functionality would destabilize binding to hDHFR and maintain activity in ChDHFR. Compounds that were synthesized with the added functionality to the B-ring did increase selectivity, but were found to have issues with permeability/solubility.

![Figure 4](image)

**Figure 4.** Comparison of ChDHFR (right) and hDHFR (left) highlighting PEKN loop region. *Produced from J. Med. Chem, 2008, 51, 6839-6852*

### 1.4.3 Third Generation Propargyl-linked Antifolates

The development of the propargyl-linked antifolates (PLA) occurred two fold, in the second generation increase potency and selectivity of enzyme inhibition and in the third generation improved the balance between solubility and permeability for these hydrophobic compounds. A pyridine ring was introduced to into these PLAs, and solubility was increased to an acceptable range of 40 ug/mL. Along with increased solubility it was
noticed through crystal structure studies of these compounds forced a different conformation of the nicotinamide ring of NADPH in order to maintain π-π stacking. Enantiomers were synthesized to understand the efficacy of a single enantiomer and because current regulations state drugs must be enantiomerically pure. The enantiomers that were developed demonstrated that a change in configuration of the stereocenter drove the selection of the alternate NADPH conformation and had no antagonistic effects.\textsuperscript{13} Along with being potent inhibitors, the third generation PLAs have minimal cytotoxicity when tested against MCF-10 and HepG2 mammalian cell lines.\textsuperscript{14}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Evolution of propargyl-linked antifolates from Trimethoprim}
\end{figure}
1.5 Gram-negative Pathogens

1.5.1 Outer Membrane of Gram-negative Pathogens

With the encouraging results from the optimization of TMP to the ABC ring, it is time to turn efforts towards activity against Gram-negative pathogens. They differ from Gram-positive pathogens in that they have a specialized outer membrane (OM) made up of a layer of lipopolysaccharides (LPS) and an inner membrane composed of phospholipids as seen in Figure 6. The main route of passage for compounds through the OM are through porins that allow non-specific diffusion of hydrophilic compounds with a
molecular weight less than 600 Da. The OmpF/OmpC-type porins have a preference for cations and don’t require phosphate starvation like PhoE-type porins.\textsuperscript{15}

1.5.2 Efflux pumps of Gram-negative Pathogens

Along with having an outer membrane, Gram-negative bacteria also possess a complex system of cytoplasmic membrane, periplasmic, and outer membrane efflux pumps. They can be categorized into 5 different families: ATP-binding cassette (ABC) transporters, small multidrug resistance (SMR), resistance-nodulation-division (RND), and major facilitator superfamily (MFS). \textit{E. coli} possess redundant efflux systems that have overlapping properties of the different classes, so a mutational loss of one efflux pump family does not make the pathogen susceptible to cytotoxic agents.\textsuperscript{16}
1.6 Passage into Gram-negative Cytoplasm

In order for a compound to enter the cytoplasm of a Gram-negative pathogen it needs to be hydrophilic and preferably cationic to pass into the periplasm, but also hydrophobic and uncharged to pass through the inner membrane into the cytoplasm. This of course creates a dilemma that is believed to be solved by designing a compound that is small, weakly charged or zwitterionic of low LogP that are uncharged at physiological pH. Figure 7 displays how most antibiotics on the market that are effective against Gram-negative bacteria have a cLogP between 0-2 and molecular weight <600 Da. Currently most of the compounds developed in the Wright lab have a cLogP between 3-5.

1.7 Development of seco-B Ring Antifolates

![Figure 7. MW vs. cLogP plot for antibacterials. Produced from Drug Discov. 2008, 3, 5](image)
In effort to increase activity of our compounds in Gram-negative pathogens a plan was devised to remove the B-ring reducing lipophilicity, increasing flexibility, and lowering the MW. Another possible outcome of increasing the flexibility is that compound might become less susceptible to activity loss due to acquired resistance and pass through the lipid bilayer more freely. As seen below in Scheme 1, UCP1016 has cLogP of 3.30 and when the B-ring is removed there is a significant decrease in cLogP. After testing 2, 3, and 4 saturated carbon chain lengths, it was found that the 3 saturated carbon chain gave the best activity in Gram-negative, and an improved MIC value compared to the B-ring compound as seen in Table 1.

**Scheme 1.** Development of seco-B ring compounds
<table>
<thead>
<tr>
<th>Compounds</th>
<th>EcIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>BW25113</th>
<th>cLogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMP</td>
<td>0.020</td>
<td>0.38</td>
<td>0.981</td>
</tr>
<tr>
<td>UCP1016</td>
<td>ND</td>
<td>&gt;20</td>
<td>3.30</td>
</tr>
<tr>
<td>UCP1028</td>
<td>ND</td>
<td>&gt;20</td>
<td>4.13</td>
</tr>
<tr>
<td>UCP1103</td>
<td>0.055</td>
<td>15</td>
<td>2.47</td>
</tr>
<tr>
<td>UCP1149</td>
<td>ND</td>
<td>10</td>
<td>3.30</td>
</tr>
</tbody>
</table>

Table 1. Comparison of enzyme activity, cell activity, and partition coefficients of seco-B ring compounds to three ring antifolates in *E. coli*
1.8 Conclusions

With the rise in resistance to antibiotics it is imperative that new antibiotics be developed to fight growing number of deaths caused by antibiotic resistant pathogens. DHFR is a key target in the folate pathway to develop new antibiotics, since it is essential to metabolic synthesis of nucleotides and amino acids. Resistance to TMP has sparked the design of Propargyl-linked antifolates, and further development is needed to design a potent pharmacophore that is active in both Gram-positive and Gram-negative bacteria.


9. Frey, K.; Lombardo, M.; Wright, D.; Anderson, A., Towards the understanding of resistance mechanisms in clinically isolated trimethoprim-resistant, methicillin-


17. Scocchera, E., Propargyl-linked antifolates: Rational design and synthesis of new antibacterials, UCONN: Medicinal Chemistry Seminar Presentation
Chapter 2
Design and Development of seco-B Ring Compounds

2.1 Hetero straight chain seco-B Ring Antifolates

\[ \text{Br} \rightarrow \text{alkyne} \]

\[ \text{X} = \text{NH}_2, \text{OH}, \text{or SH} \]

**Scheme 2.** Synthesis of heterochain seco-B ring compounds

To further lower the LogP a plan was devised to introduce heteroatoms into the straight chain of UCP1103. This resulted in UCP1150 (amine), UCP1152 (ether), and UCP1157 (sulfide). As you can see in Scheme 2 they were synthesized via nucleophilic attack into 4-bromobutyne with bromine as the leaving group resulting in the alkyne in high yield.\(^1\) Followed by a Sonagashira coupling of the alkyne to the diaminopyrimidine yielded the final compound.\(^2\) Although these compounds have a lower LogP, they displayed poor activity in both *S. aureus* and *E. coli* as seen in Table 2.
### Table 2. Hetero chain compounds compared against the all carbon chain

<table>
<thead>
<tr>
<th>Compound</th>
<th>cLogP</th>
<th>SaIC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Sa MIC (µg/mL)</th>
<th>EcIC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Ec MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP1103</td>
<td>2.47</td>
<td>0.019</td>
<td>0.625</td>
<td>0.55</td>
<td>15</td>
</tr>
<tr>
<td>UCP1150</td>
<td>1.91</td>
<td>2.322</td>
<td>&gt;10</td>
<td>18.92</td>
<td>&gt;20</td>
</tr>
<tr>
<td>UCP1152</td>
<td>1.86</td>
<td>0.405</td>
<td>&gt;10</td>
<td>ND</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

2.2 Sulfur-linked seco-B ring Compounds

After the poor activity of UCP1150 and UCP1152, a crystal structure of UCP1103 was obtained and it was observed that the failure in most of the heterochain compounds could be explained by the chain region being surrounded by nonpolar residues Phe31, Ile50, Leu54, and Ile94 (Figure 8). It was at this point that the use of a sulfur was envisioned because sulfur doesn’t hydrogen donate or accept like oxygen or nitrogen, and sulfur has similar electronegativity to carbon. In *S. aureus* UCP1157 displayed equal activity as the all carbon chain UCP1103 despite having a higher IC<sub>50</sub>. 
Using the exact same synthetic route shown in Scheme 2, but with 4-mercaptophenol instead of the pyridine, UCP1174 was synthesized, the sulfide version of UCP1149, and it also displayed equal activity as the all carbon chain in *S. aureus*. With sulfide compounds having the same activity as the all carbon chain in *S. aureus*, it was envisioned that using S$_{\text{N}2}$ substitution reactions with thiols would be possible to test a wide range of functional groups on the C ring relatively quickly compared to the all carbon seco-B ring compounds some of which are shown in Table 3.

**Figure 8.** Crystal structure of UCP1103 in *E. coli* DHFR
2.3 Branched seco-B Ring Sulfide Compounds

The risk with sulfur in our compounds is that it could have a shorter half-life compared to the all carbon chain due to the carbon-sulfur bond being weaker and the potential for the sulfur to be easily oxidized. To circumvent this anticipated problem and to increase contacts with the hydrophobic residues around the straight chain portion of SaDHFR, a methyl group off of the chain next to the sulfur was synthesized using a Thiol Michael addition\(^3\) followed by a Bestmann-Ohira homologation\(^4\) forming the alkyne.

<table>
<thead>
<tr>
<th>C ring Functional Group</th>
<th>S. aureus MIC</th>
<th>C ring Functional Group</th>
<th>S. aureus MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP1157</td>
<td>0.625</td>
<td>UCP1201</td>
<td>1.25</td>
</tr>
<tr>
<td>S</td>
<td>N</td>
<td>OEt</td>
<td></td>
</tr>
<tr>
<td>UCP1174</td>
<td>0.313</td>
<td>UCP1202</td>
<td>2.50</td>
</tr>
<tr>
<td>S</td>
<td>OH</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>UCP1199</td>
<td>1.25</td>
<td>UCP1203</td>
<td>&gt;10</td>
</tr>
<tr>
<td>S</td>
<td>NH(_2)</td>
<td>OH</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Various functional groups on the C ring of seco-B ring compounds.
Scheme 3. Synthesis of branched sulfide alkynes

The addition of a methyl group off of the chain resulted in a decrease in *S. aureus* MIC as seen in Table 4, 0.0781 µg/ mL. This prompted further development into increasing hydrophobic contacts in the chain region of SaDHFR resulting in *S. aureus* MIC of 0.0195 µg/ mL for UCP1192, the dimethyl version of the compound.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th><em>S. aureus</em> MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP1174</td>
<td>H</td>
<td>H</td>
<td>0.3125</td>
</tr>
<tr>
<td>UCP1176</td>
<td>H</td>
<td>-CH₃</td>
<td>0.0781</td>
</tr>
<tr>
<td>UCP1192</td>
<td>-CH₃</td>
<td>-CH₃</td>
<td>0.0195</td>
</tr>
<tr>
<td>UCP1193</td>
<td>H</td>
<td>-CH₂CH₃</td>
<td>0.0781</td>
</tr>
</tbody>
</table>

Table 4. Activity of branched sulfides in *S.Aureus*

A crystal structure of UCP1192 was obtained observed that the quartenary carbon with two methyl groups acted as the B ring by making the same contacts, Leu20, Thr46, and NADPH, as the B ring in UCP1106 as can be seen in Figure 9. Additionally, UCP1192
has a Log P of 3.78 compared to UCP1106 4.38. The key difference between UCP1106 and UCP1192 in terms of contacts with SaDHFR is that the zwitterion (UCP1106) makes a hydrogen bonding contact with Arg57 through a water molecule. Instead the C ring of

Figure 9. Comparison of UCP1106 (top) and UCP1192 (bottom)
UCP1192 favors Lys52 resulting in no hydrogen bonding contacts. There is no clear explanation for why UCP1192 has an alternate conformation than UCP1106, but the increase in degrees of freedom might be a possible explanation.

2.4 Sulfur-linked seco-B Rings in EcDHFR

Although the sulfur-linked seco-B rings have shown high activity in *S. aureus*, they have struggled in the *E. coli* cell lines. Even the most active compounds in *S. aureus* fail to get MIC values less than 20 µg/mL. As we mentioned earlier in order for compounds to pass through the OM of a Gram-negative pathogen it needs to be hydrophilic and cationic in nature. Sulfur having a large electron cloud possibly gives these compounds anionic character, making it difficult to pass through the OM. These sulfur-linked compounds are still a valuable resource when designing compounds because the two to three step synthesis of these compounds allow a person to test different functional groups relatively quickly. Comparing the results in Table 5 it can be inferred that UCP1192 could be a potent inhibitor of *E. coli* if transformed into the all carbon chain form. UCP1192 is relatively potent in NR698 cell line. This cell line has a compromised cell layer, and if a compound it potent in NR698 cell lines, but not in the fully functional BW25113 then it can be assumed that the poor activity is due to the compounds inability to pass through the outer membrane. For example, UCP1203 would be a poor inhibitor, but instead of using a 5 to 6 step synthesis to determine if a diol on the C ring would be an active inhibitor, it was synthesized in 2 steps using the sulfur linkage.
Table 5. Sulfur-linked seco-B ring compounds in *E. coli* DHFR

<table>
<thead>
<tr>
<th>Compound</th>
<th>BW25113</th>
<th>JW0451</th>
<th>NR698</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP1157</td>
<td>&gt;20</td>
<td>2.5</td>
<td>0.3125</td>
</tr>
<tr>
<td>UCP1174</td>
<td>&gt;20</td>
<td>5</td>
<td>0.0781</td>
</tr>
<tr>
<td>UCP1192</td>
<td>&gt;20</td>
<td>2.5</td>
<td>0.02</td>
</tr>
<tr>
<td>UCP1199</td>
<td>&gt;20</td>
<td>10</td>
<td>0.156</td>
</tr>
<tr>
<td>UCP1203</td>
<td>&gt;20</td>
<td>20</td>
<td>2.5</td>
</tr>
</tbody>
</table>
2.5 Hydrogen Bonding Network Observation in EcDHFR

While observing the crystal structure of UCP1103 it was noticed that there was a water network just off the straight chain involving Glu17, Thr46, Tyr100, and the nitrogen atom of Ile94, (Figure 10).

![Figure 10. Water network in EcDHFR](image)

To increase binding affinity, UCP1194 was designed and synthesized to have a hydroxy functional group on the carbon next to the alkyne which would interact with the water network. As shown in Scheme 4, first a Wittig reaction and then hydrogenation of 4-pyridinecarboxaldehyde to make the commercially available 4-pyridinepropanol. Dess-Martin oxidation to commercially available 4-pyridinepropanal followed by TMS-alkyne addition into the carbonyl gave the hydroxyl group of the chain. TMS deprotection
followed by the Sonagashira reaction gave UCP1194. Unfortunately, UCP1194 resulted in loss of activity in both *E. coli* and *S. aureus*.

Scheme 4. Synthesis of UCP1194

2.6 Sulfate in EcDHFR

Another observation made in the crystal structure of UCP1103 in EcDHFR is the presence of a sulfate ion that is hydrogen bonding with Lys32, Arg52, and Arg57 of the
enzyme and is located near the C ring of UCP1103. To improve enzyme affinity and activity UCP1202 was synthesized. Placing an acetic acid off the C ring should put the carboxylic acid functional group in the proper position to replace the sulfate. UCP1202 has shown no increase in activity in any of the *E. coli* cell lines. Having >20 µg/mL in BW25113, 20 µg/mL in JW0451 (efflux pump deficient), and 2.5 µg/mL in NR698 (compromised LPS layer). There are ongoing efforts to add a sulfate to UCP1174 or addition of sulfamide to UCP1199 to try and probe the sulfate network.

2.7 Conclusion

The speed and low cost of using thiols for substitution reactions makes for a useful tool in testing different functional groups relatively quickly compared to the all carbon seco-B ring compounds. Furthermore, the use of thiols in Michael additions can generate a wide range of substrates with varying carbon chain lengths alpha to the sulfur. The crystal structure of UCP1192 gives insight into future compound directions.
References


Chapter 3

Experimental Procedures

General Experimental. All reactions, unless specified, were conducted under an atmosphere of Argon in glassware that had been flame dried. Methylene chloride (CH$_2$Cl$_2$) was used from Baker Cycle-Tainers, and anhydrous toluene, dioxane, and trimethylamine purchased from Sigma-Aldrich. Dimethylformamide (DMF) was purchased from Acros. Josiphos was purchased from STREM. Where appropriate, control of temperature was achieved with an ice-bath or a heated oil bath. $^1$H NMR spectra were recorded at 400 MHz, and/or at 500 MHz and calibrated to the CDCl$_3$ peak at 7.28 ppm. $^{13}$C NMR spectra were recorded at 100MHz, and/or at 125 MHz and calibrated to the CDCl$_3$ peak at 77.23 ppm. Chemical shifts are reported in units of parts per million (ppm). High-resolution mass spectra were obtained on the JMS-AX505HA instruments and/or on an AccuTOF instrument at the University of Connecticut. IR spectra were obtained on the Bruker FT-IR instrument at the University of Connecticut. Flash chromatography was performed on Silica Gel, 40 microns, 32-63 flash silica and/or -NH$_2$ capped spherical silica gel. Thin layer chromatography was performed on silica gel (Silica Gel 60 F254) glass plates and the compounds were visualized by UV and/or potassium permanganate stain.

Synthesis of pyridine homopropargyl amine.

\[
\begin{align*}
\text{N} & \\
\text{H} & \\
\end{align*}
\]

To a solution of 4-amino pyridine (500 mg, 5.30 mmol) in Acetonitrile (25 mL), K$_2$CO$_3$ (2.95 g, 10.7 mmol) and was stirred for 10 minutes. 4-Bromo-butyne (1.00 mL, 10.7 mmol)
was added. The reaction vessel was sealed, and the mixture was kept stirring and heated for 16 hours at 85 °C. The reaction mixture was then filtered through celite and washed with ether. The solution was then concentrated on a rotary evaporator. Yielded yellow solid (779 mg, 98% yield); mp 145 °C; $^1$H NMR (500 MHz, Methanol-d4) $\delta$ 8.03 (d, $J = 7.3$ Hz, 2H), 6.86 (d, $J = 7.2$ Hz, 2H), 4.27 (t, $J = 6.2$ Hz, 2H), 2.77 (td, $J = 6.2$, 2.6 Hz, 2H), 2.27 (t, $J = 2.5$ Hz, 1H); $^{13}$C NMR (101 MHz, DMSO-d6) $\delta$ 159.18, 143.56, 109.60, 80.26, 74.94, 55.51, 20.54; IR (neat cm$^{-1}$) 3311, 3252, 3235, 3198, 3153, 1647, 1545, 1533, 1508; HRMS (DART, M$^+$+H) $m/z$ 147.0899 (calculated for C$_9$H$_{11}$N$_2$, 147.0922)

**Synthesis of inhibitor UCP1150.**

![Chemical Structure](https://example.com/struct.png)

To alkyne (111 mg, 0.758 mmol), iodo-ethyl pyrimidine (100 mg, 0.378 mmol), Cul (3.60 mg, 0.0190 mmol), PdCl$_2$(PPh$_3$)$_2$ (40.0 mg, 0.0560 mmol), and potassium acetate (372 mg, 3.78 mmol) was added followed by DMF (16 mL) and stirred at 65 °C for an hour. The reaction mixture was quenched with solid sodium bicarbonate and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography with amine silica gel (EtOAc followed by 1 % to 10% MeOH in CH$_2$Cl$_2$) to yield coupled pyrimidine as a brown solid (33.0 mg, 31% yield); mp 122 °C; $^1$H NMR (500 MHz, Methanol-d4) $\delta$ 8.16 (d, $J = 7.5$ Hz, 2H), 6.85 (d, $J = 7.5$ Hz, 2H), 4.37 (t, $J = 6.3$ Hz, 2H), 3.10 (t, $J = 6.3$ Hz, 2H), 2.46 (q, $J = 7.7$ Hz, 2H), 1.09 (t, $J = 7.6$ Hz, 3H); $^{13}$C NMR (126 MHz, Methanol-d4) $\delta$ 172.58, 164.90, 161.16, 159.57, 142.72, 109.26, 92.46, 88.44, 76.65, 56.07, 28.64, 21.37, 11.77; IR (neat cm$^{-1}$) 3311, 3252, 3235, 3198,
3153, 1653, 1620, 1565, 1540; HRMS (ESI, M^+H) m/z 283.1660 (calculated for C_{15}H_{19}N_{6} 283.1671)

**Synthesis of pyridine homopropargyl ether**

![Chemical structure](image)

To a solution of 4-hydroxy pyridine (500 mg, 5.26 mmol) in Acetonitrile (25 mL), K$_2$CO$_3$ (2.91 g, 21.0 mmol) and was stirred for 10 minutes. 4-Bromo-butyne (1.50 mL, 15.8 mmol) was added. The reaction vessel was sealed, and the mixture was kept stirring and heated for 16 hours at 100 °C. The reaction mixture was then diluted with water and extracted with EtOAc (100 mL) and then CH$_2$Cl$_2$ (100 mL). The CH$_2$Cl$_2$ portion was then dried with Na$_2$SO$_4$ and concentrated. Yielded a yellow semi-solid (200 mg, 26% yield); $^1$H NMR (500 MHz, Methanol-d$_4$) δ 7.70 (d, $J = 7.0$ Hz, 2H), 6.56 (d, $J = 7.1$ Hz, 2H), 4.08 (t, $J = 6.3$ Hz, 2H), 2.68 (td, $J = 6.3$, 2.6 Hz, 2H), 2.11 (t, $J = 2.6$ Hz, 1H); $^{13}$C NMR (126 MHz, Methanol-d$_4$) δ 141.80, 117.89, 78.64, 72.95, 55.81, 21.14; IR (neat cm$^{-1}$) 3285, 1635, 1529; HRMS (DART, M^+H) m/z 148.0744 (calculated for C$_9$H$_{10}$NO 148.0762);

**Synthesis of UCP1152**

![Chemical structure](image)

To alkyne (200 mg, 1.36 mmol), iodo-ethyl pyrimidine (180 mg, 0.680 mmol), CuI (6.48 mg, 0.0340 mmol), PdCl$_2$(PPh$_3$)$_2$ (71.5 mg, 0.100 mmol), and potassium acetate (670 mg, 6.79 mmol) was added followed by DMF (10 mL) and stirred at 65 °C for an hour. The reaction mixture was quenched with solid sodium bicarbonate and filtered through cotton,
and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (EtOAc followed by 5% to 20% MeOH in CH₂Cl₂) to yield coupled pyrimidine as a white solid (69.3 mg, 36% yield); mp 228 °C; ¹H NMR (500 MHz, Methanol-d4) δ 7.79 (d, J = 7.2 Hz, 2H), 6.48 (d, J = 7.3 Hz, 2H), 4.19 (t, J = 6.4 Hz, 2H), 3.04 (t, J = 6.4 Hz, 2H), 2.53 (q, J = 7.6 Hz, 2H), 1.14 (t, J = 7.6 Hz, 3H); ¹³C NMR (126 MHz, Methanol-d4) δ 175.39, 168.95, 160.36, 113.72, 88.63, 51.48, 25.03, 18.00, 8.24; IR (neat cm⁻¹) 3285, 3119, 1640, 1546; HRMS (DART, M⁺+H) m/z 284.1506 (calculated for C₁₅H₁₈N₅O 284.1511)

Synthesis of homopropargyl pyridine sulfide

To a solution of 4-mercapto pyridine (500 mg, 4.5 mmol) in Acetonitrile (15 mL), K₂CO₃ (1.3 g, 9.0 mmol) was added and allowed to stir for 10 minutes. 4-Bromo-butyne (0.84 mL, 9.0 mmol) was added. The reaction vessel was sealed and the mixture was stirred at 100 °C for an hour. The reaction mixture was then diluted with water (25 mL) and extracted with EtOAc (150 mL) and then dried with Na₂SO₄ and concentrated. The crude product was purified by flash chromatography (10% to 40 % EtOAc in Hexanes) to yield pure product as a brown solid (0.36 grams, 49% yield); mp 37 °C; ¹H NMR (500 MHz, Chloroform-d) δ 8.26 (d, J = 4.9 Hz, 2H), 6.97 (d, J = 4.9 Hz, 2H), 3.01 (t, J = 7.4 Hz, 2H), 2.43 (td, J = 7.3, 2.4 Hz, 2H), 2.03 (d, J = 2.6 Hz, 1H); ¹³C NMR (101 MHz, Chloroform-d) δ 149.49, 147.92, 120.84, 70.39, 29.59, 18.89; IR (neat cm⁻¹) 3290, 3119, 1640, 1546; HRMS (DART, M⁺+H) m/z 164.0545 (calculated for C₉H₁₀NS 164.0534)
Synthesis of UCP1157

\[
\begin{align*}
\text{Synthesis of UCP1157} & \\
\text{To alkyne (120 mg, 0.740 mmol), iodo-ethyl pyrimidine (100 mg, 0.370 mmol), JosiPhos} \\
\text{Copper (1.50 mg, 0.000920 mmol), Pd(PPh3)4 (43.4 mg, 0.0370 mmol), and potassium} \\
\text{acetate (180 mg, 1.84 mmol) was added followed by DMF (5 mL) and stirred at 85 ° C for} \\
\text{an hour. The reaction mixture was quenched with solid NaHCO3 and filtered through} \\
cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient 
flash chromatography (EtOAc followed by 1 % to 5% MeOH in CH2Cl2) to yield coupled 
pyrimidine as a yellow solid (43.8 mg, 36% yield); mp 170 °C; \text{1H NMR (500 MHz,} 
\text{Chloroform-d)} \delta 8.33 (d, J = 6.2 Hz, 2H), 7.16 (d, J = 6.2 Hz, 2H), 3.23 (t, J = 6.9 Hz, 2H), 
2.88 (t, J = 6.9 Hz, 2H), 2.62 (q, J = 7.6 Hz, 2H), 1.18 (t, J = 7.6 Hz, 3H); \text{13C NMR (126} 
MHz, Chloroform-d) \delta 173.10, 164.31, 160.45, 148.83, 121.06, 95.63, 89.88, 75.35, 
53.42, 30.27, 29.35, 19.85, 12.47; \text{IR (neat cm}^{-1}) 3313, 3113, 2971, 2925, 1635, 1560; 
\text{HRMS (DART, M}^{+}+\text{H)} m/z 300.1302 (calculated for C15H18N5S 300.1283) 
\end{align*}
\]

Synthesis of homopropargyl phenol sulfide

\[
\begin{align*}
\text{Synthesis of homopropargyl phenol sulfide} & \\
\text{To a solution of 4- mercapto phenol (500 mg, 3.96 mmol) in Acetonitrile (15 mL), K2CO3} \\
(603 mg, 4.36 mmol) was added and allowed to stir for 10 minutes. 4-Bromo-butynne 
(0.410 mL, 4.36 mmol) was added. The reaction vessel was sealed and the mixture was 
stirred at 100 °C for an hour. The reaction mixture was then diluted with water (25 mL) 
and extracted with EtOAc (150 mL) and then dried with Na2SO4 and concentrated. The}
\end{align*}
\]
The crude product was purified by flash chromatography (10% to 40% EtOAc in Hexanes) to yield pure product as a brown solid (691 mg, 98% yield); mp 48 °C; ¹H NMR (500 MHz, Chloroform-d) δ 7.36 (d, J = 8.6 Hz, 2H), 6.83 (d, J = 8.7 Hz, 2H), 6.17 (s, 1H), 2.98 (t, J = 7.4 Hz, 2H), 2.45 (td, J = 7.4, 2.6 Hz, 2H), 2.10 (t, J = 2.5 Hz, 1H); ¹³C NMR (126 MHz, Chloroform-d) δ 155.33, 134.37, 125.18, 116.35, 82.67, 69.87, 34.82, 19.37; IR (neat cm⁻¹) 3404, 3271, 1587; HRMS (DART, M⁺+H) m/z 179.0542 (calculated for C₁₀H₁₁OSe).

**Synthesis of UCP1174**

![Chemical Structure](image)

To alkyne (300 mg, 1.68 mmol), iodo-ethyl pyrimidine (220 mg, 0.84 mmol), Cul (7.98 mg, 0.042 mmol), PdCl₂(PPh₃)₂ (117.7 mg, 0.17 mmol), and potassium acetate (1,645 mg, 16.76 mmol) was added followed by DMF (10 mL) and stirred at 65 °C for an hour. The reaction mixture was quenched with solid NaHCO₃ and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (1% to 5% MeOH in CH₂Cl₂) to yield coupled pyrimidine as a white solid (42.1 mg, 16% yield); mp 138 °C; ¹H NMR (500 MHz, Chloroform-d) δ 7.32 (d, J = 8.4 Hz, 2H), 6.77 (d, J = 6.8 Hz, 2H), 2.96 (t, J = 6.7 Hz, 2H), 2.64 (m, J = 22.3, 7.1 Hz, 4H), 1.19 (t, J = 7.6 Hz, 3H); ¹³C NMR (126 MHz, Chloroform-d) δ 172.62, 164.49, 160.22, 157.06, 134.90, 123.19, 116.28, 97.24, 90.63, 74.64, 35.89, 29.35, 20.31, 12.58; IR (neat cm⁻¹) 3326, 1606, 1555, 1440; HRMS (DART, M⁺+H) m/z 315.1245 (calculated for C₁⁶H₁₉N₄OSe).
Synthesis of 3-((4-hydroxyphenyl)thio)butanal

![Chemical structure of 3-((4-hydroxyphenyl)thio)butanal](attachment:structure.png)

To a solution of 4-mercaptophenol (500 mg, 3.96 mmol) in dry CH₂Cl₂ (20 mL), TEA (0.610 mL, 4.35 mmol) was added and stirred. Crotonaldehyde (0.490 mL, 5.95 mmol) in dry CH₂Cl₂ (15 mL) was added dropwise over an hour and allowed to stir for 16 hours. Quenched with NH₄Cl and extracted with CH₂Cl₂ (150 mL) and concentrated. Purified by gradient flash chromatography (5% to 20% EtOAc in Hexanes) to yield pure product as colorless translucent liquid (420 mg, 54% yield); ¹H NMR (500 MHz, Chloroform-d) δ 9.71 (s, 1H), 7.34 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 3.52 (h, J = 6.8 Hz, 1H), 2.76 – 2.47 (m, 2H), 1.31 (d, J = 6.9 Hz, 3H); ¹³C NMR (126 MHz, Chloroform-d) δ 201.64, 156.30, 136.69, 123.27, 116.21, 50.07, 38.72, 21.14; IR (neat cm⁻¹) 3347, 2962, 2924, 1709, 1598, 1581, 1493; HRMS (DART, M⁺+H) m/z 197.0661 (calculated for C₁₀H₁₃O₂S 197.0636)

Synthesis of UCP1176

![Chemical structure of UCP1176](attachment:structure.png)

**Step 1** A solution of the aldehyde (300 mg, 1.53 mmol) in MeOH (2 mL) was cooled to 0 °C and a solution of Ohira-Bestmann reagent (0.534 mL, 2.29 mmol) in MeOH (14 mL) was added. To the reaction mixture K₂CO₃ (422 mg, 3.06 mmol) was added and stirred for 4 hours. The reaction was then quenched with NH₄Cl and extracted with EtOAc (100
mL) and concentrated. The crude product was purified by flash chromatography (5% EtOAc) to give the desired alkyne with inseparable impurity.

**Step 2** To alkyne (60.8 mg, 0.32 mmol), iodo-ethyl pyrimidine (41.8 mg, 0.16 mmol), Cul (1.52 mg, 0.0080 mmol), PdCl$_2$(PPh$_3$)$_2$ (22.2 mg, 0.03 mmol), and potassium acetate (155.3 mg, 1.58 mmol) was added followed by DMF (5 mL) and stirred at 65 ° C for an hour. The reaction mixture was quenched with solid NaHCO$_3$ and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (1 % to 5% MeOH in CH$_2$Cl$_2$) to yield coupled pyrimidine as a white solid (13.8 mg, 27% yield); mp 170 °C; $^1$H NMR (500 MHz, Chloroform-d) δ 7.30 (d, $J = 8.6$ Hz, 2H), 6.72 (d, $J = 8.7$ Hz, 2H), 3.15 (h, $J = 6.4$ Hz, 1H), 2.60 (q, $J = 7.6$ Hz, 2H), 2.55 (d, $J = 6.0$ Hz, 2H), 1.29 (d, $J = 6.8$ Hz, 3H), 1.16 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (101 MHz, Chloroform-d) δ 172.60, 164.56, 160.23, 157.39, 136.64, 122.04, 116.02, 96.31, 90.66, 44.02, 29.41, 27.34, 20.25, 12.56; IR (neat cm$^{-1}$) 3326, 1606, 1555, 1440; HRMS (DART, M$^+$+H) m/z 329.1440 (calculated for C$_{17}$H$_{21}$N$_4$OS 329.1436)

**Synthesis of 3-((4-hydroxyphenyl)thio)-3-methylbutanal**

![Chemical structure](attachment:structure.png)

To a solution of 4-mercaptophenol (500 mg, 3.96 mmol) in dry CH$_2$Cl$_2$ (20 mL), TEA (0.610 mL, 4.35 mmol) was added and stirred. 3-methyl-2-butenal (0.49 mL, 5.15 mmol) in dry CH$_2$Cl$_2$ (15 mL) was added dropwise over an hour and allowed to stir for 16 hours. Quenched with NH$_4$Cl and extracted with CH$_2$Cl$_2$ (150 mL) and concentrated. Purified by gradient flash chromatography (5% to 20% EtOAc in Hexanes) to yield pure product as colorless translucent liquid (510 mg, 60% yield); $^1$H NMR (500 MHz, Chloroform-d) δ 9.90
(s, 1H), 7.35 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 6.73 (s, 1H), 2.48 (d, J = 2.6 Hz, 2H), 1.38 (s, 6H); $^{13}$C NMR (126 MHz, Chloroform-d) δ 203.86, 157.30, 139.22, 121.39, 116.10, 53.98, 45.88, 28.93; IR (neat cm$^{-1}$) 3373, 2963, 2928, 2865, 1709, 1709, 1599, 1581; HRMS (DART, M$^{+}$+H) m/z 211.0803 (calculated for C$_{11}$H$_{15}$O$_2$S 211.0793)

**Synthesis of UCP1192**

![UCP1192](image)

**Step 1** A solution of the aldehyde (490 mg, 2.24 mmol) in MeOH (2 mL) was cooled to 0 °C and a solution of Ohira-Bestmann reagent (0.62 mL, 2.68 mmol) in MeOH (14 mL) was added. To the reaction mixture K$_2$CO$_3$ (620 mg, 2.70 mmol) was added and stirred for 4 hours. The reaction was then quenched with NH$_4$Cl and extracted with EtOAc (100 mL) and concentrated. The crude product was purified by flash chromatography (5% EtOAc) to give the desired alkyne with inseparable impurity.

**Step 2** To alkyne (142 mg, 0.690 mmol), iodo-ethyl pyrimidine (72.6 mg, 0.280 mmol), Cul (2.66 mg, 0.0140 mmol), PdCl$_2$(PPh$_3$)$_2$ (19.3 mg, 0.0300 mmol), and potassium acetate (275 mg, 2.80 mmol) was added followed by DMF (5 mL) and stirred at 65 °C for an hour. The reaction mixture was quenched with solid NaHCO$_3$ and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (1 % to 5% MeOH in CH$_2$Cl$_2$) to yield coupled pyrimidine as a white solid (25.9 mg, 28% yield); mp 168 0°C; $^1$H NMR (500 MHz, Methanol-d4) δ 7.40 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 2.68 (q, J = 7.6 Hz, 2H), 2.55 (s, 2H), 1.34 (s, 6H), 1.23 (t, J = 7.6 Hz, 3H); $^{13}$C NMR (126 MHz, Methanol-d4) δ 171.96, 164.96, 160.49, 158.41, 138.77, 120.50, 115.60, 96.28, 90.36, 75.37, 33.31, 29.14, 27.70, 13.54, 12.36;
IR (neat cm\(^{-1}\)) 3320, 2964, 1556, 1479; HRMS (DART, M\(^{+}\)H) m/z 343.1562 (calculated for C\(_{18}\)H\(_{23}\)N\(_4\)O\(_5\)S 343.1593)

**Synthesis of 3-((4-hydroxyphenyl)thio)pentanal**

![Chemical structure](image)

To a solution of 4-mercapto phenol (500 mg, 3.96 mmol) in dry CH\(_2\)Cl\(_2\) (20 mL), TEA (0.610 mL, 4.35 mmol) was added and stirred. Trans-2-pentenal (0.540 mL, 5.55 mmol) in dry CH\(_2\)Cl\(_2\) (15 mL) was added dropwise over an hour and allowed to stir for 16 hours. Quenched with NH\(_4\)Cl, extracted with CH\(_2\)Cl\(_2\) (150 mL), and concentrated. Purified by gradient flash chromatography (5% to 20% EtOAc in Hexanes) to yield pure product as colorless translucent liquid (422 mg, 51% yield); \(^1\)H NMR (500 MHz, Chloroform-d) \(\delta\) 9.74 (s, 1H), 7.34 (d, \(J = 8.6\) Hz, 2H), 6.82 (d, \(J = 8.6\) Hz, 2H), 3.32 (p, \(J = 6.7\) Hz, 1H), 2.63 (dd, \(J = 6.4, 1.9\) Hz, 2H), 1.63 (pd, \(J = 7.2, 2.7\) Hz, 2H), 1.07 (t, \(J = 7.3\) Hz, 3H); \(^{13}\)C NMR (126 MHz, Chloroform-d) \(\delta\) 201.81, 156.22, 136.73, 123.20, 116.19, 48.04, 45.83, 27.58, 11.46; IR (neat cm\(^{-1}\)) 3335, 2967, 2930, 2875, 1713, 1598, 1580; HRMS (DART, M\(^{+}\)H) m/z 211.0807 (calculated for C\(_{11}\)H\(_{15}\)O\(_2\)S 211.0793)

**Synthesis of UCP1193**

![Chemical structure](image)

**Step 1** A solution of the aldehyde (400 mg, 1.90 mmol) in MeOH (2 mL) was cooled to 0 °C and a solution of Ohira-Bestmann reagent (0.530 mL, 2.28 mmol) in MeOH (14 mL) was added. To the reaction mixture K\(_2\)CO\(_3\) (527 mg, 3.81 mmol) was added and stirred for 4 hours. The reaction was then quenched with NH\(_4\)Cl and extracted with EtOAc (100
mL) and concentrated. The crude product was purified by flash chromatography (5% EtOAc) to give the desired alkyne with inseparable impurity.

**Step 2** To alkyne (130 mg, 0.630 mmol), iodo-ethyl pyrimidine (111 mg, 0.420 mmol), Josiphos Cu (1.7 mg, 1.05x10^{-3} mmol), Pd(PPh₃)₄ (48.6 mg, 0.0400 mmol), and potassium acetate (206 mg, 2.10 mmol) was added followed by DMF (5 mL) and stirred at 85 °C for an hour. The reaction mixture was quenched with solid NaHCO₃ and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (1 % to 5% MeOH in CH₂Cl₂) to yield coupled pyrimidine as a white solid (38.1 mg, 27% yield); mp 81 °C; ¹H NMR (400 MHz, Methanol-d4) δ 7.36 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 8.6 Hz, 2H), 2.99 (p, J = 6.1 Hz, 1H), 2.72 – 2.58 (m, 4H), 1.67 (ddp, J = 28.6, 14.4, 7.0 Hz, 2H), 1.22 (t, J = 7.6 Hz, 3H), 1.07 (t, J = 7.3 Hz, 3H); ¹³C NMR (101 MHz, Methanol-d4) δ 172.07, 164.86, 160.52, 157.61, 136.42, 121.80, 115.85, 96.17, 90.32, 74.88, 51.08, 29.12, 26.41, 24.75, 12.29, 11.10; IR (neat cm⁻¹) 3326, 2963, 2923, 1559, 1480; HRMS (DART, M⁺+H) m/z 343.1613 (calculated for C₁₈H₂₃N₄OS 343.1593)

**Synthesis of 3-(pyridin-4-yl)propan-1-ol**

![Pyridine structure](image)

To a solution of (Formylmethyl)triphenylphosphonium chloride (2,340 mg, 6.86 mmol) in THF (15 mL) 1.0 M potassium tert-butoxide in THF (6.86 mL, 6.86 mmol) was added and allowed to stir for 15 minutes. 4-Pyridinecarboxaldehyde (0.620 mL, 6.54 mmol) was then added and allowed to stir for 1 hour. The reaction mixture was quenched with NH₄Cl, extracted with EtOAc (150 mL), and concentrated. The crude product was dissolved in EtOH (50 mL), and Pd/C (1 g) was added. The reaction flask was sealed and purged with
hydrogen gas and left stirring under hydrogen overnight. The reaction was filtered through celite and purified by flash chromatography (30% to 70% EtOAc in Hexanes) to yield a brown liquid (337 mg, 38% yield); Matched previous reported data.

**Synthesis of 3-(pyridin-4-yl)propan-1-ol**

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\end{align*}
\]

To a solution of 3- (pyridin-4-yl)propan-1-ol (300 mg, 2.22 mmol) in DCM (15 mL), DMP (1.41 g, 3.33 mmol) was added and allowed to stir for 2 hours. Then 0.05 mL of dH$_2$O was added dropwise. The reaction was then quenched with Na$_2$S$_2$O$_3$ and NaHCO$_3$, extracted with EtOAc (150 mL), and concentrated. It was then purified by flash chromatography (20% to 60% EtOAc in Hexanes) to yield a red-brown liquid (215 mg, 72.9% yield); Matched previous reported data

**Synthesis of 5-(pyridin-4-yl)pent-1-yn-3-ol**

\[
\begin{align*}
\text{OH} & \quad \text{N} \\
\end{align*}
\]

To a solution of Trimethylsilylacetylene (0.680 mL, 4.84 mmol) in THF (16 mL) at -78 °C, 2.5 M n-BuLi in Hexanes (1.94 mL, 4.84 mmol) was added dropwise. The reaction was allowed to stir for 30 minutes before 3-(pyridin-4-yl)propan-1-ol (215 mg, 1.61 mmol) in THF (2 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and after 2 hours was quenched with NH$_4$Cl, extracted with EtOAc, and concentrated. The product was then dissolved in dry MeOH and K$_2$CO$_3$ (667 mg, 4.83 mmol) was added and stirred at room temp. for 4 hours. The reaction was quenched with NaHCO$_3$, extracted with EtOAc, and concentrated. It was then purified by flash chromatography (50% to 80% EtoAC in Hexanes) to yield a brown solid (135 mg, 52%
yield in 2 steps); mp 58 °C; ¹H NMR (500 MHz, Chloroform-d) δ 8.42 (d, J = 6.1 Hz, 2H), 7.14 (d, J = 6.1 Hz, 2H), 6.09 (s, 1H), 4.38 (td, J = 6.5, 2.1 Hz, 1H), 2.81 (t, J = 8.0 Hz, 2H), 2.46 (d, J = 2.1 Hz, 1H), 2.03 (tq, J = 13.5, 6.6, 5.9 Hz, 2H); ¹³C NMR (126 MHz, Chloroform-d) δ 151.41, 148.99, 124.19, 85.23, 72.77, 60.40, 37.94, 30.70; IR (neat cm⁻¹) 3201, 3134, 2926, 2834, 1601; HRMS (DART, M⁺+H) m/z 162.0930 (calculated for C₁₀H₁₂NO 162.0919).

**Synthesis of UCP1194**

![Chemical Structure](image)

To alkyne (100 mg, 0.620 mmol), iodo-ethyl pyrimidine (126 mg, 0.480 mmol), JosiPhos Copper (2.00 mg, 0.00120 mmol), Pd(PPh₃)₄ (55.2 mg, 0.0480 mmol), and TEA (0.130 mL, 0.950 mmol) was added followed by DMF (5 mL) and stirred at 85 °C for an hour. The reaction mixture was quenched with solid NaHCO₃ and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (EtOAc followed by 1 % to 5% MeOH in CH₂Cl₂) to yield coupled pyrimidine as a white solid (33.9 mg, 24% yield); mp 135 °C; ¹H NMR (500 MHz, Methanol-d₄) δ 8.39 (d, J = 4.6 Hz, 2H), 7.18 (d, J = 4.5 Hz, 2H), 4.58 (t, J = 6.5 Hz, 1H), 2.82 (t, J = 7.9 Hz, 2H), 2.62 (q, J = 7.6 Hz, 2H), 2.06 (qq, J = 14.0, 7.9 Hz, 2H), 1.19 (t, J = 7.6 Hz, 3H); ¹³C NMR (126 MHz, Methanol-d₄) δ 171.29, 164.56, 160.10, 152.00, 148.69, 124.37, 98.82, 89.36, 76.91, 63.08, 61.33, 38.15, 30.81, 28.72, 12.17. IR (neat cm⁻¹) 3325, 3183, 2971, 2934, 2834, 1601; HRMS (DART, M⁺+H) m/z 298.1667 (calculated for C₁₆H₂₀N₅O 298.1668)
Synthesis of homopropargyl analine sulfide

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\text{CH}_3\text{CH}=\text{CHS-CH}_2\text{NH}_2
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To a solution of 4-mercapto aniline (900 mg, 7.19 mmol) in Acetonitrile (20 mL), K$_2$CO$_3$ (1,990 mg, 14.4 mmol) was added and allowed to stir for 10 minutes. 4-Bromo-butyne (1.35 mL, 14.4 mmol) was added. The reaction vessel was sealed and the mixture was stirred at 90 °C for an hour. The reaction mixture was then diluted with water (25 mL) and extracted with EtOAc (150 mL) and then dried with Na$_2$SO$_4$ and concentrated. The crude product was purified by flash chromatography (10% to 40 % EtOAc in Hexanes) to yield pure product as a brown solid (840 mg, 72% yield); mp 58 °C; $^1$H NMR (400 MHz, Chloroform-d) δ 7.29 (d, $J = 8.5$ Hz, 2H), 6.64 (d, $J = 8.5$ Hz, 2H), 3.75 (s, 2H), 2.91 (dd, $J = 7.9$, 7.1 Hz, 2H), 2.42 (td, $J = 7.4$, 2.6 Hz, 2H), 2.03 (t, $J = 2.6$ Hz, 1H); $^{13}$C NMR (126 MHz, Chloroform-d) δ 146.58, 134.81, 121.73, 115.62, 82.87, 69.54, 35.33, 19.43; IR (neat cm$^{-1}$) 3424, 3334, 3273, 1620, 1592, 1491; HRMS (DART, M$^+$H) m/z 178.0702 (calculated for C$_{10}$H$_{12}$NS 178.0690)

Synthesis of UCP1199

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\text{N}^\text{H}_2\text{N}^\text{H}_2
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To alkyne (111 mg, 0.630 mmol), iodo-ethyl pyrimidine (100 mg, 0.370 mmol), JosiPhos Copper (1.50 mg, 0.000920 mmol), Pd(PPh$_3$)$_4$ (42.5 mg, 0.0370 mmol), and KOAc (181 mg, 1.84 mmol) was added followed by DMF (5 mL) and stirred at 85 °C for an hour. The reaction mixture was quenched with solid NaHCO$_3$ and filtered through cotton, and
concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (EtOAc followed by 1 % to 5% MeOH in CH2Cl2) to yield coupled pyrimidine as a tan solid (35.9 mg, 31% yield); mp 139 °C; 1H NMR (500 MHz, Methanol-d4) δ 7.26 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 8.1 Hz, 2H), 2.94 (t, J = 6.8 Hz, 2H), 2.70 – 2.57 (m, 4H), 1.20 (t, J = 7.6 Hz, 3H); 13C NMR (126 MHz, Methanol-d4) δ 172.18, 164.64, 160.34, 147.17, 134.82, 120.93, 115.75, 97.25, 90.43, 74.31, 35.97, 29.17, 20.10, 12.39; IR (neat cm⁻¹) 3325, 3183, 2971, 2933, 2872, 1606, 1570, 1548; HRMS (DART, M⁺+H) m/z 314.1421 (calculated for C₁₆H₂₀N₅S 314.1439)

**Synthesis of ethyl 2-(4-(but-3-yn-1-ylthio)phenyl)acetate**

![Chemical structure](image)

To a solution of 4-mercapto-phenylacetic acid (320 mg, 1.90 mmol) in EtOH (10 mL), sulfuric acid (0.100 mL, 1.90 mmol) was added and allowed to stir for 2 hours. The reaction was then quenched with NaHCO₃ and extracted with EtOAc (3 x 25 mL). The extracts were combined, washed with brine (40 mL), dried (Na₂SO₄), and concentrated. The crude mixture was then dissolved in MeCN (10 mL) and K₂CO₃ was added and allowed to stir for 10 minutes. Then, 4-bromobutyne (0.360 mL, 3.80 mmol) was added and heated to 85 °C and stirred for 2 hours before being diluted with H₂O and extracted with EtOAc (3 x 25 mL). The extracts were combined, washed with brine (45 mL), dried (Na₂SO₄), and concentrated. The crude product was then purified by flash chromatography (0% to 20% EtOAc in hexanes) to yield a clear liquid (296 mg, 63% yield 2 steps); 1H NMR (500 MHz, Chloroform-d) δ 7.36 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.3 Hz,
2H), 4.18 (q, J = 7.2 Hz, 2H), 3.61 (s, 2H), 3.08 (t, J = 7.4 Hz, 2H), 2.50 (td, J = 7.5, 2.5 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H); \textsuperscript{13}C NMR (126 MHz, Chloroform-d) δ 171.30, 133.91, 132.72, 130.35, 129.99, 82.27, 69.79, 60.94, 40.89, 32.99, 19.40, 14.22; IR (neat cm\textsuperscript{-1}) 3292, 2981, 2934, 1730, 1494; HRMS (DART, M\textsuperscript{+}+H) m/z 249.0957 (calculated for C\textsubscript{14}H\textsubscript{17}O\textsubscript{2}S 249.0957)

**Synthesis of UCP1201**

![Synthesis of UCP1201](image)

To alkyne (196 mg, 0.790 mmol), iodo-ethyl pyrimidine (160 mg, 0.610 mmol), JosiPhos Copper (2.5 mg, 0.00150 mmol), Pd(PPh\textsubscript{3})\textsubscript{4} (70.3 mg, 0.0610 mmol), and KOAc (298 mg, 3.0 mmol) was added followed by DMF (7 mL) and stirred at 85 °C for an hour. The reaction mixture was quenched with solid NaHCO\textsubscript{3} and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (EtOAc followed by 1 % to 5% MeOH in CH\textsubscript{2}Cl\textsubscript{2}) to yield coupled pyrimidine as a clear semi-solid (50.2 mg, 22% yield); \textsuperscript{1}H NMR (500 MHz, Chloroform-d) δ 7.40 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 5.52 (s, 2H), 5.18 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 3.62 (s, 2H), 3.16 (t, J = 6.9 Hz, 2H), 2.79 (t, J = 6.9 Hz, 2H), 2.69 (q, J = 7.6 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H), 1.25 (t, J = 7.6 Hz, 3H); \textsuperscript{13}C NMR (126 MHz, Chloroform-d) δ 173.21, 171.39, 164.70, 160.86, 133.64, 132.95, 130.77, 130.06, 96.50, 90.28, 75.44, 61.02, 40.88, 34.00, 29.57, 20.51, 14.20, 12.63; IR (neat cm\textsuperscript{-1}) 3325, 3208, 2976, 2931, 2873, 1731, 1615, 1560, 1515, 1495; HRMS (DART, M\textsuperscript{+}+H) m/z 385.1683 (calculated for C\textsubscript{20}H\textsubscript{25}N\textsubscript{4}O\textsubscript{2}S 385.1698)
Synthesis of UCP1202

UCP1201 (40 mg, 0.104 mmol) was dissolved in THF (0.5 mL) then LiOH (17.4 mg, 0.730 mmol) in H₂O (0.4 mL) was added and allowed to react at room temperature for 4 hours. The reaction was quenched with 0.1M HCl in H₂O and extracted with DCM to yield white solid which was then washed with EtOAc and filtered through funnel with fritted disk to yield the carboxylic acid (39.2 mg, 98% yield); mp 178 °C; ¹H NMR (500 MHz, Methanol-d₄) δ 7.40 (d, J = 7.9 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 3.60 (s, 2H), 3.21 (t, J = 6.8 Hz, 2H), 2.82 (t, J = 6.8 Hz, 2H), 2.78 (q, J = 7.6 Hz, 2H), 1.31 (t, J = 7.6 Hz, 3H); ¹³C NMR (126 MHz, Methanol-d₄) δ 173.89, 165.22, 160.14, 154.40, 133.55, 133.52, 130.09, 129.83, 99.07, 92.23, 70.41, 40.01, 32.62, 25.09, 19.75, 10.98; IR (neat cm⁻¹) 3331, 3174, 2910, 1696, 1664, 1645, 1526, 1496; HRMS (ESI, M⁺+H) m/z 357.1358 (calculated for C₁₈H₂₀N₄O₂S 357.1395)

Synthesis of homopropargyl 3,4 dimethoxy sulfide

To a solution of 3,4- dimethoxythiophenol (500 mg, 2.94 mmol) in Acetonitrile (30 mL), K₂CO₃ (609 mg, 4.41 mmol) was added and allowed to stir for 10 minutes. 4-Bromo-butyne (0.41 mL, 4.41 mmol) was added. The reaction vessel was sealed and the mixture was stirred at 90 °C for an hour. The reaction mixture was then diluted with water (25 mL), extracted with EtOAc (150 mL), and then dried with Na₂SO₄ and concentrated. The crude product was purified by flash chromatography (10% to 40 % EtOAc in Hexanes) to
yield pure product as a colorless liquid (642 mg, 98% yield); $^1$H NMR (500 MHz, Chloroform-d) $\delta$ 6.95 (dd, $J = 8.2, 2.1$ Hz, 1H), 6.92 (d, $J = 2.1$ Hz, 1H), 6.74 (d, $J = 8.2$ Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 2.91 (t, $J = 7.4$ Hz, 2H), 2.37 (td, $J = 7.5, 2.6$ Hz, 2H), 2.00 (t, $J = 2.6$ Hz, 1H); $^{13}$C NMR (126 MHz, Chloroform-d) $\delta$ 149.12, 148.85, 125.51, 125.02, 115.65, 111.67, 82.41, 69.60, 55.88, 55.85, 34.63, 19.37; IR (neat cm$^{-1}$) 3287, 3000, 2935, 1582, 1501; HRMS (DART, M$^+$H) m/z 223.0791 (calculated for C$_{12}$H$_{15}$O$_2$S 223.0793)

**Synthesis of UCP1204**

![Chemical structure of UCP1204](image)

To alkyne (108 mg, 0.490 mmol), iodo-ethyl pyrimidine (100 mg, 0.380 mmol), JosiPhos Copper (1.50 mg, 0.000920 mmol), Pd(PPh$_3$)$_4$ (42.5 mg, 0.0380 mmol), and KOAc (180 mg, 1.84 mmol) was added followed by DMF (4 mL) and stirred at 85 °C for overnight. The reaction mixture was quenched with solid NaHCO$_3$ and filtered through cotton, and concentrated by azeotroping with toluene (3 x 50 mL) and purified by gradient flash chromatography (50-90% EtOAc in Hexanes) to yield coupled pyrimidine as an orange solid (40.7 mg, 30% yield); mp 129 °C; $^1$H NMR (500 MHz, Chloroform-d) $\delta$ 7.10 (dd, $J = 8.3, 2.1$ Hz, 1H), 7.05 (d, $J = 2.1$ Hz, 1H), 6.85 (d, $J = 8.3$ Hz, 1H), 5.56 (s, 2H), 5.12 (s, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.07 (t, $J = 6.9$ Hz, 2H), 2.74 (t, $J = 6.9$ Hz, 2H), 2.69 (q, $J = 7.6$ Hz, 2H), 1.25 (t, $J = 7.6$ Hz, 3H); $^{13}$C NMR (126 MHz, Chloroform-d) $\delta$ 173.15, 164.76, 160.84, 149.19, 149.12, 125.66, 125.09, 116.04, 111.67, 96.73, 90.38, 75.40, 56.02, 55.98, 35.75, 29.58, 20.45, 12.64; IR (neat cm$^{-1}$) 3440, 3313, 3095, 2935, 1582, 1501; HRMS (DART, M$^+$H) m/z 359.1517 (calculated for C$_{18}$H$_{23}$N$_4$O$_2$S 359.1542)
Synthesis of UCP1203

To a solution of UCP1204 (33.5 mg, 0.102 mmol) in DCM (1 mL), BBr₃ (0.0870 mL, 0.914 mmol) was added and allowed to stir for 2 hours. After the reaction was complete the reaction was quenched with concentrated NaHCO₃ in water and extracted with DCM to give pure white solid (7.47 mg, 22% yield); mp 168 °C; ¹H NMR (500 MHz, DMSO-d6) δ 9.10 (s, 1H), 9.10 (s, 1H), 6.86 (d, J = 2.0 Hz, 1H), 6.77 – 6.74 (m, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.33 (s, 2H), 6.14 (s, 2H), 3.01 (t, J = 7.0 Hz, 2H), 2.65 (t, J = 7.0 Hz, 2H), 2.55 (q, J = 7.6 Hz, 2H), 1.13 (t, J = 7.6 Hz, 3H); ¹³C NMR (126 MHz, DMSO-d6) δ 164.95, 161.53, 146.19, 145.70, 123.66, 123.29, 119.64, 116.69, 96.72, 88.43, 75.87, 34.91, 29.48, 29.18, 20.52, 12.94; IR (neat cm⁻¹) 3472, 3352, 3191, 2915, 1607, 1581, 1555, 1507; HRMS (ESI, M⁺+H) m/z 331.1208 (calculated from C₁₆H₁₉N₄O₂S 331.1229)
The image contains an NMR spectrum and a chemical structure. The chemical structure shows a molecule with amine groups (HN) and a pyridine ring. The NMR spectrum shows peaks at various ppm values, indicating the presence of different chemical environments.
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\text{HN} = \text{N} \quad \text{NH}_2 \\
\text{H}_2\text{N} \quad \text{OH}
\]