Synthesis of 5- and 6-Aminopyridin-3-ol Quinone Methide Precursors

Thesis

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By

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Abstract

Organophosphorus (OP) nerve agents are compounds that contain phosphoryl or thiophosphoryl functional groups. These molecules have been continuously developed and prepared since the late 1930's, with newer generations (V- and A-series) having much higher toxicity relative to the original G-series of OP nerve agents. OP pesticides are also the cause of many poisonings, attributed to over 100,000 suicide deaths per year. OP molecules act as covalent inhibitors of the enzyme acetylcholinesterase (AChE), but their therapeutic window for treatment is small due the rapid inhibition and also due to a subsequent and spontaneous *O*-dealkylation event. This *O*-dealkylation causes the "aging" of AChE, and current therapeutics are not able to regenerate the native enzyme. Quinone methide precursors (QMPs) are hypothesized to reverse the aged state of AChE through either an S_N2 reaction or the formation of a quinone methide (QM) inside the active site of AChE. The focus of this thesis is the development and synthesis of 5-aminopyridin-3-ol and 6-aminopyridin-3-ol QMPs as potential therapeutic treatments for OP poisoning.

Acknowledgments

I would like to thank Dr. Craig McElroy for giving me the opportunity to join and work in his lab during my graduate studies. He has always been helpful and levelheaded in difficult situations, like starting graduate school during the COVID pandemic. I would also like to thank Dr. Christopher Hadad for allowing me to spend time working in his lab with his graduate students. These periods were extremely helpful for the development of my synthetic chemistry laboratory skills. I would like to specifically thank William Clay, Benjamin Clark, and Dr. Jeremy Lear for their help during my time in the Hadad Lab. Financial support from the Defense Threat Reduction Agency (CB10791 MCDC1905-006), via a sub-contract from MRIGlobal, is gratefully acknowledged.

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Last, but certainly not last, I would like to thank my wife, Elizabeth. Without your support, both academic and emotional, I would not be half the man I am today.

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Chapter 1: Introduction

1.1 Background, History and Prior Use of OP Compounds

Organophosphorus (OP) molecules are a class of organic molecules that contain either a phosphorus-oxygen (phosphoryl) double bond or a phosphorus-sulfur (thionophosphoryl) double bond (**Figure 1**) [1]. These molecules are extremely toxic due to their propensity to bind the enzyme acetylcholinesterase (AChE) as covalent inhibitors.

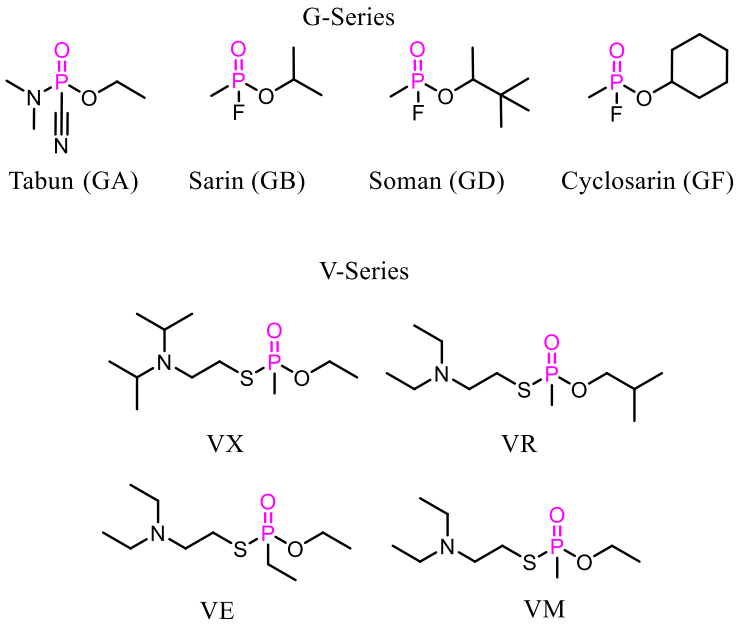


Figure 1: Organophosphorus Nerve Agents

The original class of OP nerve agents, the G series, was synthesized by the German chemist Gerhard Schrader in the later 1930's [1]. He was working to develop novel insecticides but when one of his target molecules, later named tabun, showed extreme human toxicity, the German government took over the once humanitarian project. The "G" in G series comes from their country of origin, Germany. While these G-series OP

nerve agents were not used in World War II, which came shortly after their synthesis, the Allied powers did uncover their existence. In response, the United Kingdom and United States worked to develop their own OP nerve agents called the V-series during the 1950s. As tensions grew during the Cold War, Russia began to develop its own OP nerve agents, which later became known as the A-series [1].

There are multiple examples of OP nerve agent use in recent years, but one of the most historically significant was their use in the Iran-Iraq war from 1980 to 1988 (Error! Reference source not found.) [2]. Iraq was the first country to use OP nerve agents in a warfare setting in 1984, with the use of tabun against Iranian troops in the Manjoon Islands [3]. Other uses of OP nerve agents were much more sinister, being used on civilian masses and for secret attempts at executions. In 1995, sarin gas was released by the Aum Shinrikyo cult in the subway system of Tokyo (Error! Reference source not found.**3**) [4]. This attack caused the death of twelve people and injured thousands more, however the Aum Shinrikyo completed four more sarin attacks on the Tokyo subway before their leader was arrested [5]. One of the most recent publicized uses of OP nerve agents was the use of novel Novichok agents (A-series OP nerve agents) on Alexei Navalny. An outspoken critic of the Russian president Vladimir Putin, Navalny was unknowingly poisoned prior to his travel to the Tomsk airport. Due to the quick actions of those around him, he was hospitalized in Russia and then eventually was able to be transported to Germany where he received the necessary treatment [6].

Figure 2: Iran-Iraq War Casualties from OP nerve agents

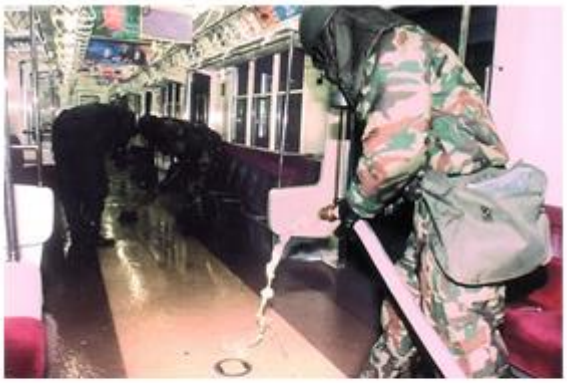


Figure 3: Tokyo Subway Cleanup post OP exposure

Not all OP compounds are agents of chemical warfare, most of them are pesticides. These pesticides are used mainly in developing nations, unfortunately, often without the required personal protective equipment. Examples of OP pesticides include demeton, diisopropylfluorophosphate (DFP), paraoxon, and many others (Error! Reference source not found.). Organophosphorus pesticides were found to have been used in between 110,000 and 168,000 suicide deaths per year from 2006 to 2015 [7].

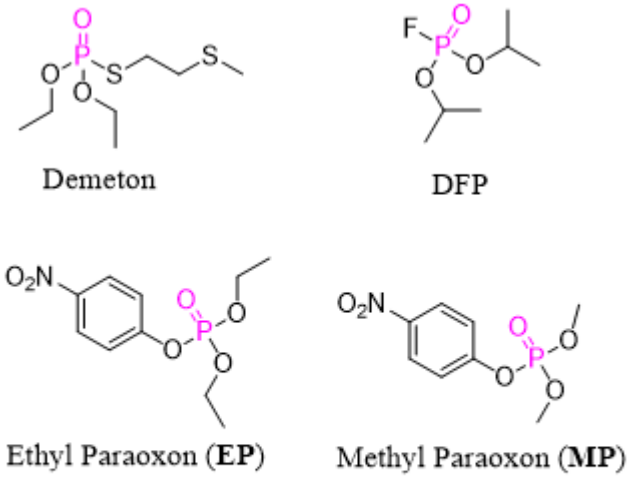


Figure 4: OP Pesticides

1.2 Acetylcholinesterase and OP Poisoning

The enzyme acetylcholinesterase (AChE) is a common serine hydrolase present in the synapses and neuromuscular junction of nerve cells, as well as on erythrocytes, in many different organisms. The enzyme acts to degrade acetylcholine, a neurotransmitter, into acetate and choline within neuromuscular junctions and synapses. The enzyme is found in both the central and peripheral nervous system and is incredibly efficient, with the ability to cleave 25,000 molecules of acetylcholine per second. The active site of AChE is deep inside the enzyme, so its substrate must pass through an active site gorge to reach the catalytic triad of His447, Ser203, and Glu334 [8] (Error! Reference source not found.**5**)

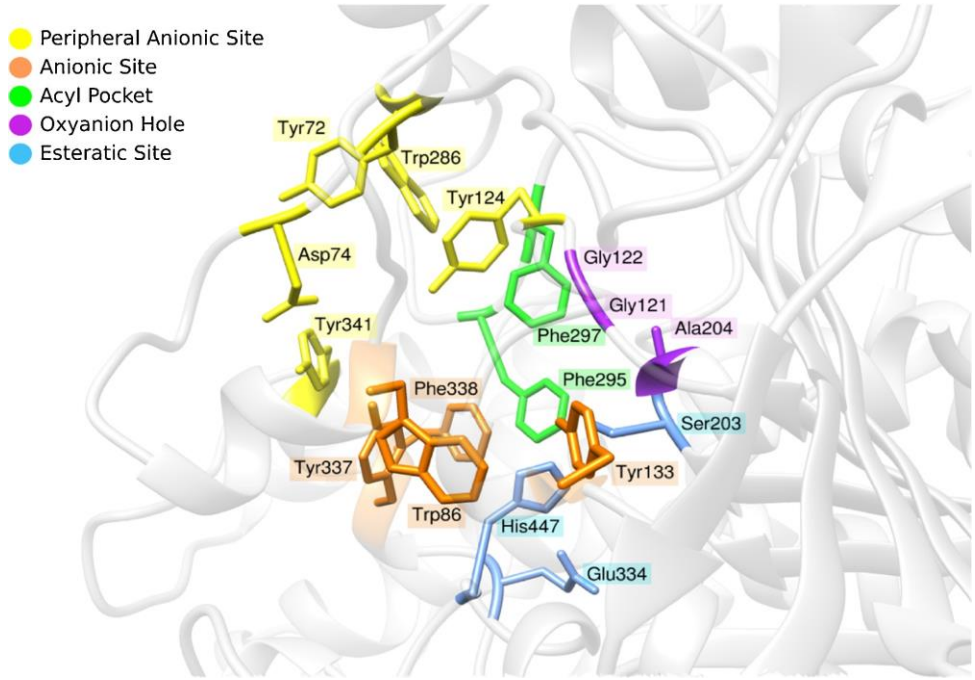


Figure 5: Acetylcholinesterase Crystal Structure (PDB: 4EY4)

[9].

The catalytic cycle of AChE is completed by a catalytic triad containing a histidine, serine, and glutamate residues. The cycle begins with a proton transfer from the serine

residue to the histidine residue, creating a histidinium ion. The resulting negatively charged oxygen on the serine will perform a nucleophilic attack on the carbonyl group of acetylcholine, forming a serine bound tetrahedral intermediate. This intermediate quickly degrades due to the reformation of the carbonyl group, elimination of choline, and the deprotonation of the histidinium ion. The histidinium ion is then reformed through a proton transfer from water, resulting in a hydroxide ion that will perform another nucleophilic attack on the serine bound acetate. This will form another tetrahedral intermediate that degrades to release acetate as well as to regenerate the native serine and

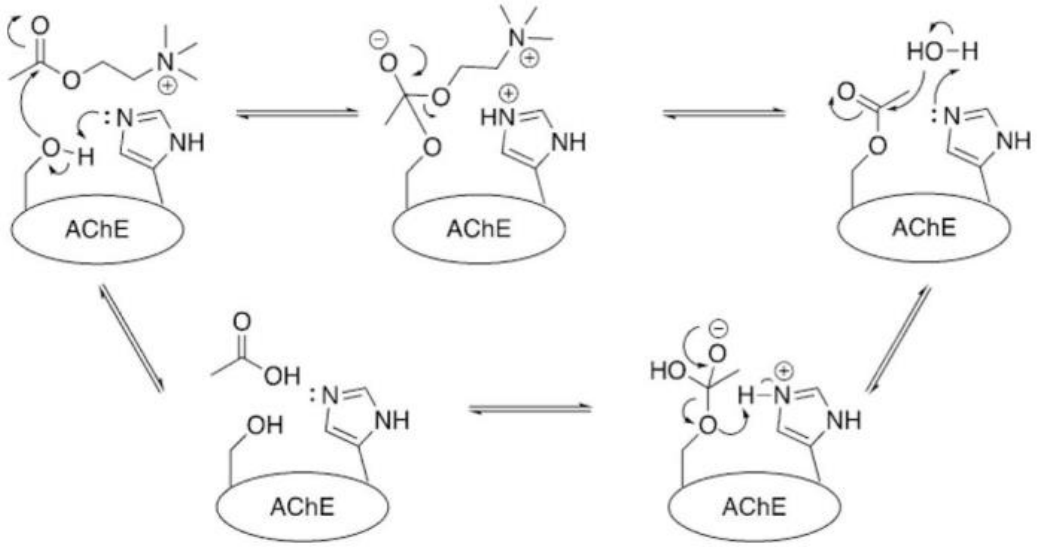


Figure 6: Catalytic Cycle of Acetylcholinesterase

histidine residues [10] (**Figure**) [1].

If organophosphorus compounds are present in the synapse or neuromuscular junction, they will act as competitive inhibitors of AChE. Instead of the catalytic serine attacking the carbonyl of ACh, it will instead attack the phosphorus center of the OP.

This will form a pentacoordinate (trigonal bipyramidal) intermediate followed by the loss of a leaving group, which creates the inhibited form of the enzyme.

If OP inhibited AChE is left untreated, a further spontaneous O-dealkylation event can occur. This creates an oxyanion that will form a salt bridge with the nearby histidinium ion. This event is called aging, and it creates the aged form of AChE that is no longer susceptible to current therapeutics [11] (**Figure 7**) [1].

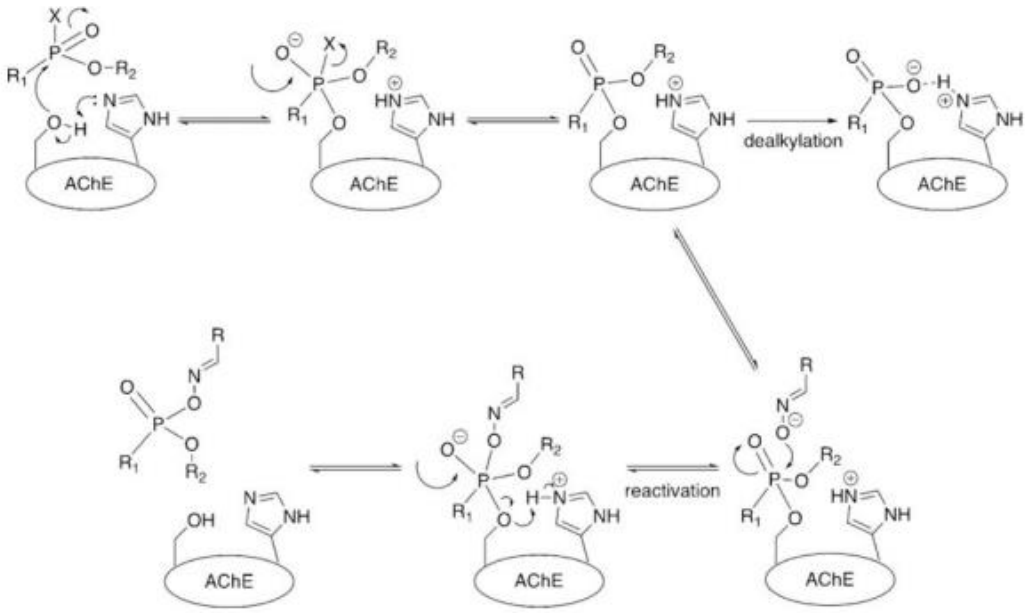


Figure 7: OP Aged and Inhibited AChE

Poisoning by organophosphorus compounds is characterized by a cholinergic crisis, meaning that there is an overaccumulation of acetylcholine in cholinergic synapses or neuromuscular junctions. This cholinergic crisis affects both muscarinic and nicotinic receptors in the peripheral nervous system (PNS), as well as the central nervous system (CNS). Muscarinic symptoms include excessive secretions, including salvation, lacrimation, urination, diarrhea, gastrointestinal distress, and emesis. Nicotinic symptoms include muscle dysfunction, and CNS symptoms include impairment of respiratory drive,

loss of consciousness, and seizures [12]. This is illustrated in Error! Reference source not found., which was adapted from Mousavi et al [13].

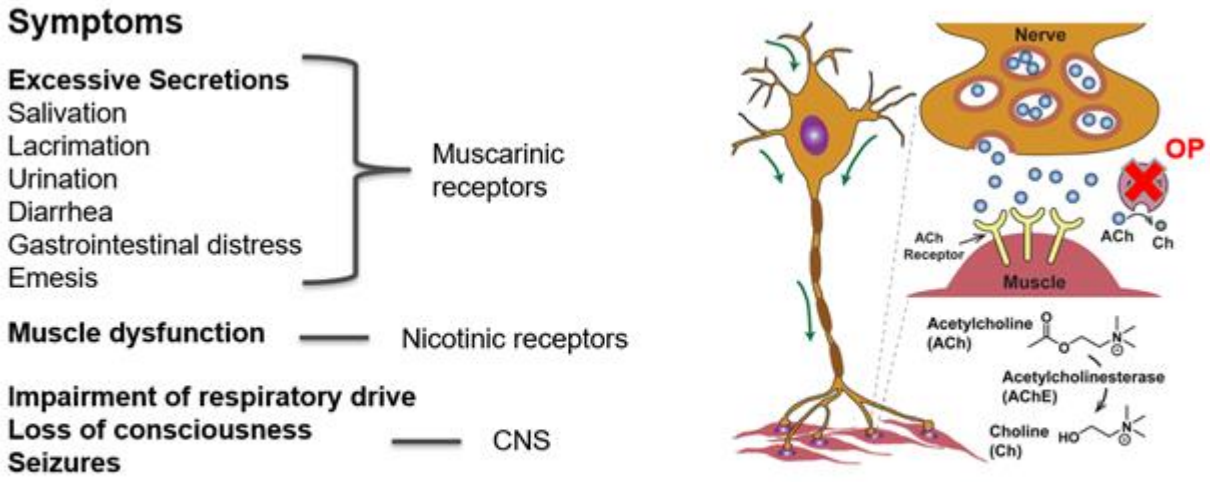


Figure 8: OP Poisoning Cholinergic Crisis

1.3 Current and Future Therapeutics

There is only one currently FDA approved prophylactic treatment for OP poisoning, pyridostigmine bromide (PB). This molecule acts as a reversible cholinesterase inhibitor, meaning that it also covalently binds AChE, but is not permanently bound, with a half-life of under two hours. Continuous dosing of pyridostigmine bromide shows around 40% inhibition of serum cholinesterase [14]. While PB does block the binding of OP agents to AChE, it also keeps the enzyme from executing its normal catalytic hydrolysis of ACh. This means that muscarinic and nicotinic symptoms can still arise.

Some therapeutics are provided to help manage the symptoms of OP poisoning. These drugs do not address the OP-inhibited AChE but do aid in mitigating the symptoms of cholinergic crisis. Atropine, a natural product from *Atropa belladonna*, is a muscarinic

receptor antagonist that helps alleviate the excessive secretions and diazepam, a benzodiazepine, is given for seizure control [16, 17] (Error! Reference source not found.**9**).

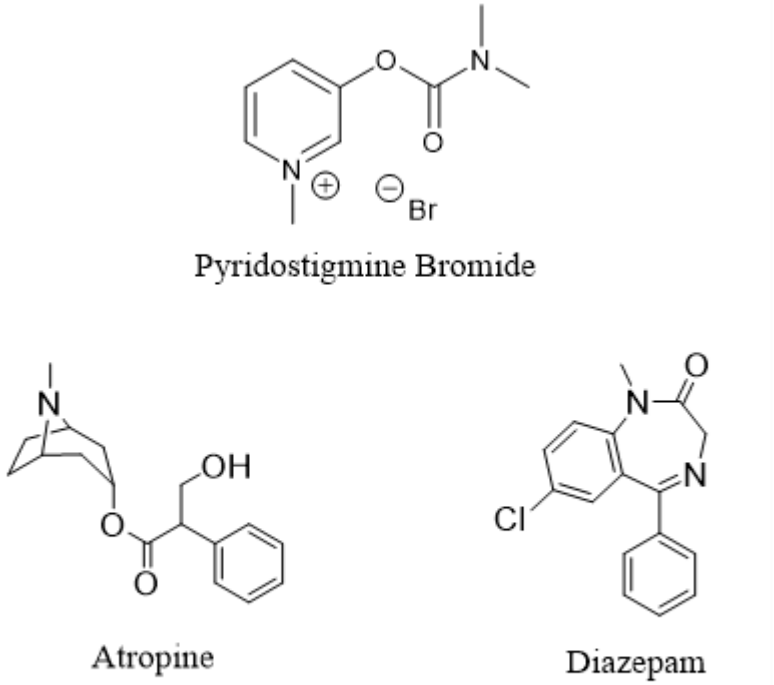


Figure 9: Structures of Current Prophylaxis and Symptom Therapeutics

Reactivators are a class of therapeutics that reverse the OP inhibited form of AChE. These molecules are typically characterized by a high density of heteroatoms and at least one quaternary amine, typically an aromatic amine such as pyridine. Examples of these drugs include pralidoxime (2-PAM), obidoxime, and HI-6 (Error! Reference source not found.**10**). While they do have the ability to reverse the inhibited state of AChE, these drugs come with quite a few downsides. They exhibit poor CNS availability and are only effective in the time between exposure and the spontaneous dealkylation event [1].

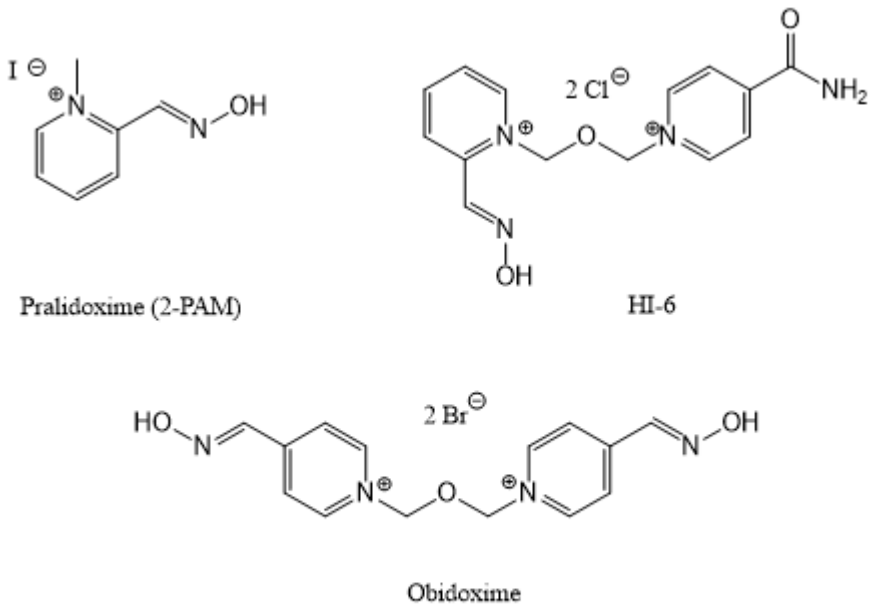


Figure 10: Current AChE Reactivators

Quinone methide precursors, or QMPs, are a class of resurrecting molecules. This means that they are able to reverse the aged state of AChE. QMPs are hypothesized to resurrect AChE through two proposed mechanisms. The first is an S_N2 reaction between the anionic phosphoryl oxygen and the benzylic carbon of the QMP leading to realkylation of the OP in the active site of AChE. The other, is that the QMP will form a quinone methide (QM) in the active site of AChE, allowing it to realkylate the phosphorylated serine residue. Once realkylated, reactivation of the resultant OP inhibited AChE by the oxygen of the same or a second molecule of the QMP leads to the removal of the OP, yielding native AChE (**Figure 11**) [11]. The rate of formation, and the selectivity of the quinone methide are heavily dependent on the electronics of the QMP [18].

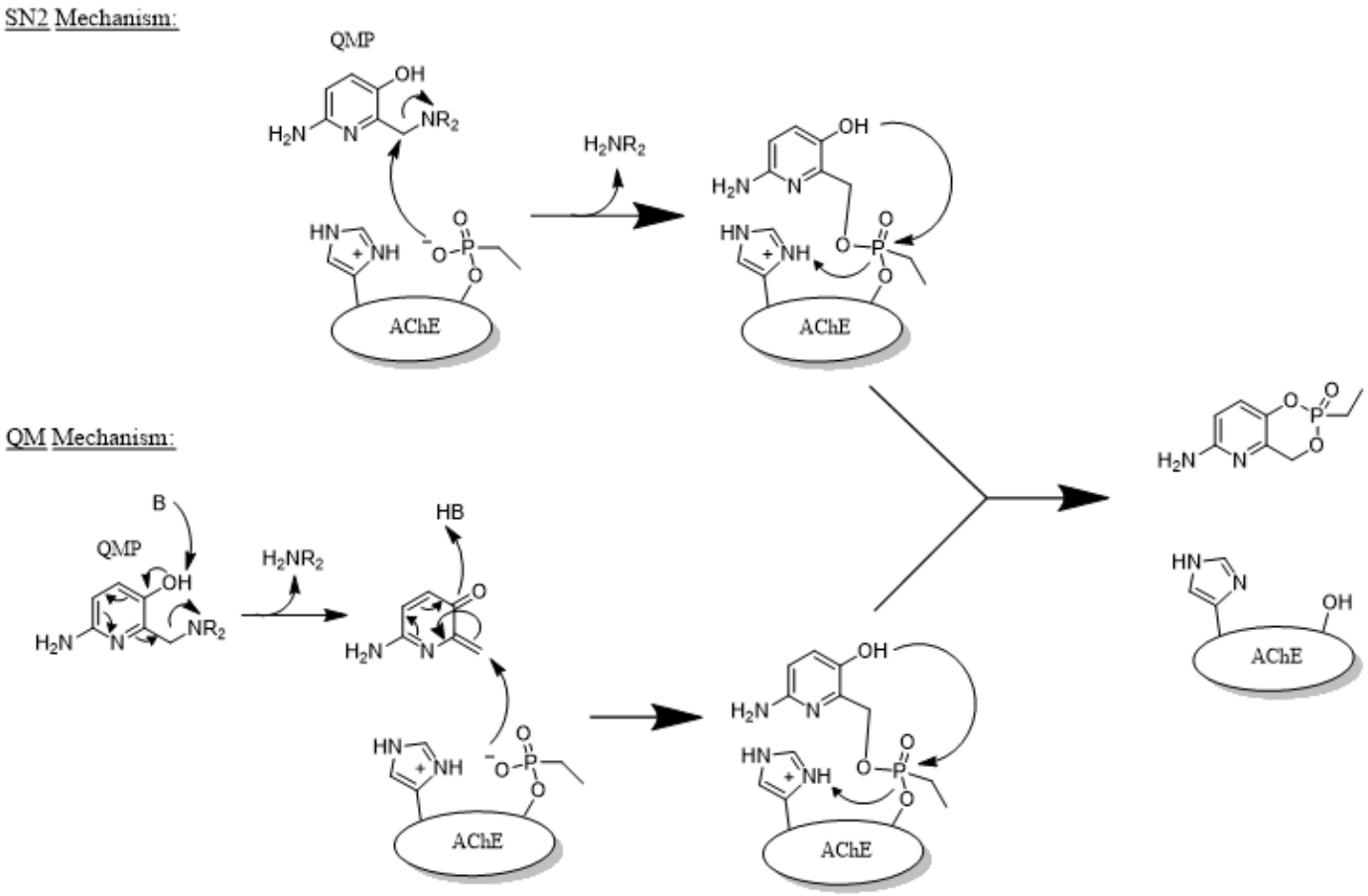
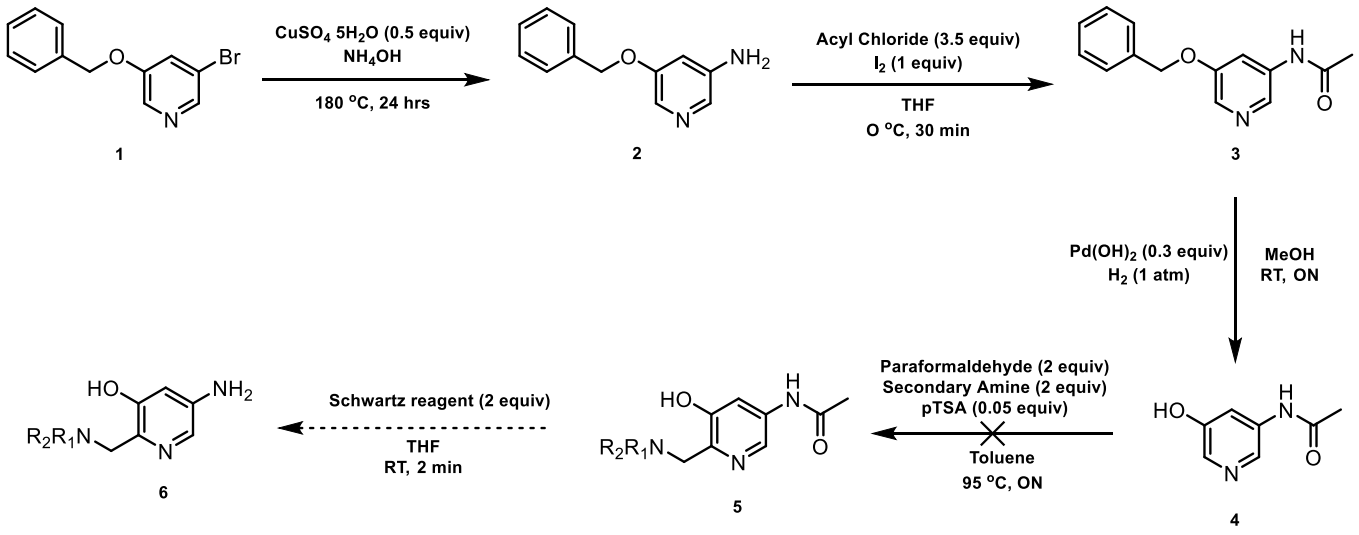


Figure 11: Two potential mechanisms for QMPs

Chapter 2: 5-Aminopyridin-3-ol QMPs

2.1 Synthetic Scheme

The 5-aminopyridin-3-ol QMP scheme (**Scheme 1**) begins with 5-bromo-3-benzyloxypyridine (**1**) which is subjected to an Ullmann amination [29] to provide 3-amino-5-benzyloxypyridine (**2**). **2** is then acylated to protect the free amine **3** prior to hydrogenolysis to remove the benzyl protecting group on the hydroxyl **4**. **4** is then subjected to Mannich conditions to install a variety of secondary amines. This has been the sticking point in the scheme as will be discussed later in this thesis. Half of the Mannich products **5** would be retained to maintain the amide and tested for biological activity, the other half would be subjected to Schwartz reagent to yield the free amine **6**.



Scheme 1: 5-aminopyridine QMP Scheme

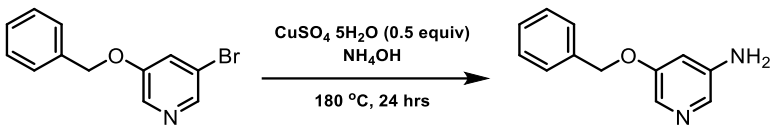
2.2 Results and Discussion

2.2.1 Ullmann Amination

The Ullmann amination was the most frequently performed reaction during my research. The initial procedure used was adapted from Holladay et al.[19] who reports a procedure using CuBr as the copper source, and liquid ammonia as the amine source. I initially started with ammonium hydroxide as my amine source, but this provided no product, most likely due to the age and condition of the reagent. After purchasing fresh ammonium hydroxide, experiments utilizing both methanol and DMF as solvents were attempted, but neither provided product. I then tried replacing the ammonium hydroxide with a methanolic ammonia solution. This did provide product, however; the reaction workup that I was using in the previous reactions was no longer compatible. I worked with Dr. Gopichand Gutti to develop an acid/base workup for this reaction, which provided yields in the 30-40% range. Subsequent reactions provided yields upwards of 50%. However, later repetitions of this procedure began to yield dirtier and dirtier product. Even after replacing all the components of the reaction, it continued to yield product that was very difficult to purify, so I began to search for a new procedure. I found a different patent from Dull et al.[20] that utilized $CuSO_4$ pentahydrate as their copper source and ammonium hydroxide as their amine source. While I could not utilize the quick acid/base workup of the previous reactions, this procedure was worth the extra effort as it provides consistent 50-60% yields of my aminated product.

I have also attempted other Ullmann aminations utilizing other amines, including methylamine, ethylamine and dimethylpyrrole. These reactions were not successful using

either the Holladay et al. or Dull et al. procedures and were not investigated further. I also utilized other copper sources as well as copper-binding ligands in an attempt to boost yields and efficiency. Other than CuBr and $CuSO_4$, I also tried CuBr-DMS, Cu^o, and CuI. These provided lower yields than the standard procedure and were not pursued further. The same is true for the ligands I tried, which included 1,10-phenanthroline, *N*,*N*-dimethylethylenediamine, and 4-hydroxy-L-proline.

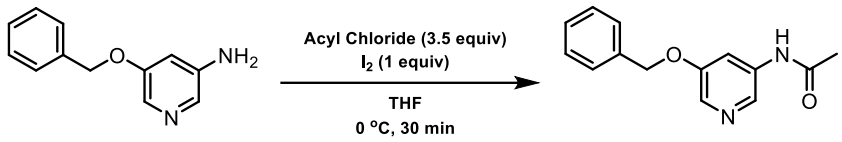


Scheme 2: Ullmann Amination

2.2.2 *N*-Acylation Protection

The second reaction in the scheme is an *N*-acylation reaction to protect the free amine by converting it to an amide. The original conditions were from Ishii et al. [21] and utilized triethylamine as the base and acyl chloride as the acylating species. While this reaction is extremely common, it was not as easily completed as I had expected. This reaction did not work very well, producing inconsistent yields around 30%. After altering the time, temperature, and base used, I went in search of different reaction conditions. I found a procedure from Phukan et al. [22] that utilized I_2 as the base under very mild conditions. This produced consistent product yields in the 70-80% range. One of the largest sources of error for this reaction is the volatility of the acyl chloride, causing it to hydrolyze quickly even when stored with desiccant. As a consequence, reaction

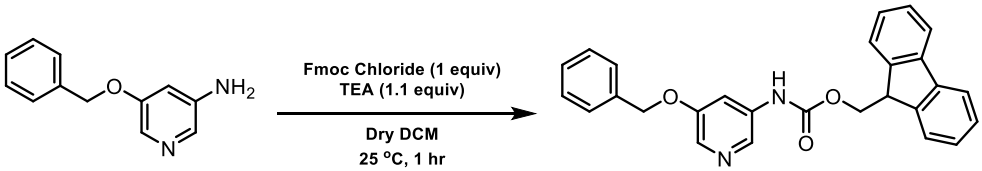
efficiency and yields were best when using a fresh supply of acyl chloride and decreased with the age of the reagent.



Scheme 3: *N*-acylation Reaction

2.2.3 *N*-Fmoc Protection

An alternate amine protecting group, fluorenylmethyloxycarbonyl (Fmoc), was also used. The Fmoc protection was adapted from Debnath et al. [23] and utilized Fmoc-Cl and triethylamine in dry dichloromethane (DCM). The rational for the change from an acyl protecting group to an Fmoc protecting group is that Fmoc is significantly larger and more nonpolar. This increased the organic solubility of the scaffold, which was originally thought to be an issue during subsequent reactions. Yields in the 55-85% range were obtained.

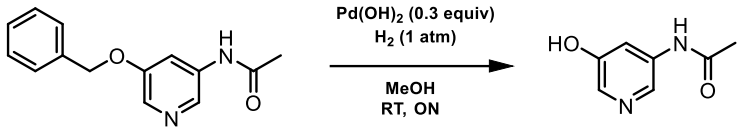


Scheme 4: *N*-Fmoc Protection

2.2.4 Hydrogenolysis Deprotection

The third reaction in the scheme is a hydrogenolysis reaction to remove the benzyl protecting group on the alcohol. This was completed using $Pd(OH)_2$, also known as Pearlman's catalyst. The reactions were solvated with MeOH and treated with 0.3

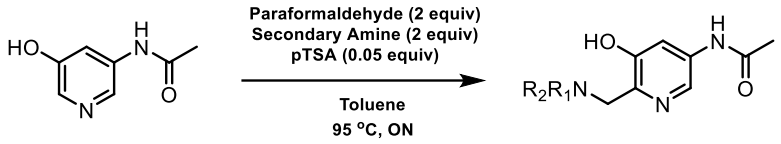
equivalents of catalyst. Reactions were run for anywhere from four to 18 hours, depending on when in the day they were started. Regardless of time, yields in the 80% or higher range were common.



Scheme 5: Hydrogenolysis Deprotection

2.2.5 Mannich Reaction

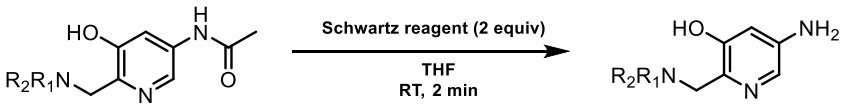
The fourth reaction in the scheme is the Mannich reaction. This is a very useful reaction to install a secondary amine with an intervening methylene on an aromatic framework. This reaction proceeds via an iminium intermediate and is directed by either a free hydroxyl or free amine on the aromatic framework. Both directing groups were tested, as well as a multitude of various solvents, including toluene, tetrahydrofuran (THF), 1,4-dioxane, ethanol, and *N*,*N*-dimethylformamide (DMF). The main procedure used was provided by the Hadad group and utilized a pre-stir with the secondary amine and paraformaldehyde to form the iminium ion, followed by the addition of the aromatic molecule. The initial solvent used was toluene, but THF was also used often. A secondary procedure from Wu et al. [24] utilized dibromomethane and a secondary amine to form the iminium intermediate, then addition of the aromatic molecule containing the directing group.



Scheme 6: Mannich Reaction

2.2.6 *N*-deacylation Reaction

The final reaction in the 5-aminopyridin-3-ol synthetic scheme is the removal of the acyl protecting group on the amine. The procedure for this reaction was adapted from Sultane et al. [25] who used zirconocene chloride hydride, also known as Schwartz reagent. This reaction was chosen due to its mild and efficient conditions. This reaction was not attempted but has good literature precedence.

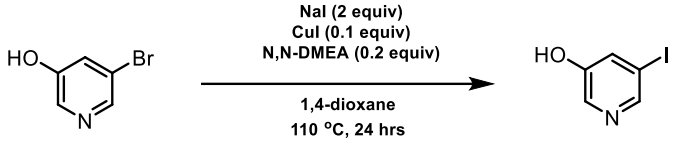


Scheme 7: *N*-deacylation Reaction

2.2.7 Finkelstein Reaction

A few Finkelstein reactions were unsuccessfully attempted with the intention of improving the yields of the Ullmann reaction. The Finkelstein reaction is a halogen exchange reaction that would swap the bromine for an iodine, which was expected to improve the yields of the subsequent Ullmann reactions. The procedure was adapted from Boyington et al.[26] and used CuI as the copper source, NaI as the iodine source, *N*,*N*-dimethylethylenediamine as the copper ligand, and 1,4-dioxane as the solvent. I was able

to get very low and dirty yields from this reaction, but it became unnecessary as the alternative Ullmann conditions gave improved yields.



Scheme 8: Finkelstein Reaction

2.3 Experimental Procedures

General Procedures

All flash chromatography was completed using a Combiflash® NextGen 300+ Flash Chromatography system. Unless otherwise stated, all flash chromatography was completed with Buchi FlashPure EcoFlex Silica 25g (40-63 μm) at a 40 mL/min flow rate. Reaction progress was observed via thin-layer chromatography (TLC) using Silica Gel 60 F_{254} plates. All NMR spectra was obtained using Bruker AVIII 400 MHz.

Ullmann Amination

CuBr Procedure

3-(Benzyloxy)-5-bromopyridine (1 equiv) and copper (I) bromide (0.1 equiv) were added to a dry pressure tube and solvated generously with methanolic ammonia. The tube was sealed and heated to 130 ˚C for 24 hours. After 24 hours, the reaction was allowed to cool to room temperature and was diluted with water. The solution was acidified to pH 2 using 1N HCl and extracted with ethyl acetate. The aqueous portion was basified to pH 8 with 1N NaOH and extracted using ethyl acetate. The basic organic extract was dried over Na_2SO_4 and concentrated to provide pure product, 5-

(benzyloxy)pyridine-3-amine (30-55% yield). ^{1}H NMR (400 MHz, CDCl3) δ 7.85 (d, J = 2.4 Hz, 1H), 7.77 (d, J = 2.3 Hz, 1H), 7.48 – 7.37 (m, 4H), 7.40 – 7.32 (m, 1H), 6.60 (t, J = 2.4 Hz, 1H), 5.09 (s, 2H), 3.70 (s, 2H).

$CuSO_4$ Procedure

3-(Benzyloxy)-5-bromopyridine (1 equiv) and copper (II) sulfate (0.5 equiv) were added to a dry pressure tube and solvated generously with 10% ammonium hydroxide solution. The tube was sealed and heated to 180 ˚C for 24 hours. After 24 hours, the reaction was cooled to room temperature, and then to 0 ˚C in an ice bath. The reaction mixture was then diluted with equal parts water and saturated potassium carbonate. The solution was extracted four times with chloroform, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated to afford crude product. The product, 5-(benzyloxy)pyridine-3-amine (45-60% yield), was purified via silica gel chromatography ($CHCl_3$/MeOH 3:1 v/v) [20]. ^{1}H NMR (400 MHz, MeOD) δ 7.59 (dd, J = 8.2, 2.3 Hz, 2H), 7.48 – 7.33 (m, 4H), 7.37 – 7.29 (m, 1H), 6.76 (t, J = 2.3 Hz, 1H), 5.10 (s, 2H).

N-acylation

Standard Procedure

5-(Benzyloxy)pyridine-3-amine (1 equiv) and triethylamine (5 equiv) were added to a clean round bottom flask and solvated in a minimal amount of tetrahydrofuran. Acyl chloride (4 equiv) was added dropwise with strong stirring at room temperature. The reaction was allowed to stir at room temperature for one hour. The reaction mixture was concentrated, re-solvated with water, and extracted with ethyl acetate. The organic extract

was washed with sodium bicarbonate and brine, dried over Na_2SO_4, and concentrated to afford crude product. The crude *N*-(5-benzyloxy)pyridine-3-yl)acetamide (35-75% yield) was purified via silica gel flash chromatography (EtOAc/Hex 4:1 v/v) [21]. ^{1}H NMR (400 MHz, DMSO) δ 8.34 – 8.27 (m, 1H), 8.06 (d, J = 2.6 Hz, 1H), 7.80 (t, J = 2.3 Hz, 1H), 7.50 – 7.31 (m, 5H), 5.16 (s, 2H), 3.32 (s, 1H), 2.07 (s, 3H).

Iodine Procedure

5-(Benzyloxy)pyridine-3-amine (1 equiv) and iodine (1 equiv) were added to a clean round bottom flask before being solvated in a minimal amount of THF. Acyl chloride (4 equiv) was added dropwise to the flask at room temperature. After addition, the reaction was allowed to stir for 10 minutes. The reaction was then placed in an ice bath, followed by addition of a saturated sodium dithionite solution to consume any unreacted iodine, and extraction with diethyl ether. The organic extract was washed with sodium bicarbonate and brine, dried over Na_2SO_4, and concentrated to provide pure product, *N*-(5-benzyloxy)pyridine-3-yl)acetamide (60-90% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.34 – 8.27 (m, 1H), 8.06 (d, J = 2.6 Hz, 1H), 7.80 (t, J = 2.3 Hz, 1H), 7.50 – 7.31 (m, 5H), 5.16 (s, 2H), 3.32 (s, 31H), 2.07 (s, 3H).

N-Fmoc

5-(Benzyloxy)pyridine-3-amine, Fluorenylmethyloxycarbonyl chloride (1 equiv) and triethylamine (1.1 equiv) were added to a dry round bottom flask and solvated with dry dichloromethane. The reaction was mixed at room temperature for one hour. The reaction was then filtered and concentrated to afford crude product. The crude (9H-fluoren-9-yl)methyl (5-(benzyloxy)pyridin-3-yl)carbamate (55-85% yield) was purified

via silica gel flash chromatography (DCM/MeOH 5-10% v/v). ^{1}H NMR (400 MHz, CDCl3) δ 8.11 (d, J = 2.5 Hz, 1H), 8.07 – 8.02 (m, 1H), 7.81 (d, J = 7.6 Hz, 3H), 7.64 (d, J = 7.5 Hz, 2H), 7.49 – 7.39 (m, 7H), 7.36 (td, J = 7.4, 1.3 Hz, 4H), 6.79 (s, 1H), 5.12 (s, 2H), 4.61 (d, J = 6.4 Hz, 2H), 4.30 (t, J = 6.5 Hz, 1H).

Hydrogenolysis

N-(5-benzyloxy)pyridine-3-yl)acetamide and $Pd(OH)_2$ were added to a dry round bottom flask and solvated with methanol. The flask was then purged and backfilled twice with nitrogen, and then twice with hydrogen. The reaction was allowed to stir at room temperature for 12 hours. The crude mixture was filtered through Celite and concentrated to afford crude product. The crude *N*-(5-hydroxypyridine-3-yl)acetamide (quantitative yield) was purified via silica gel chromatography (DCM/MeOH 10-20% v/v). ^{1}H NMR (400 MHz, DMSO) δ 10.05 (s, 1H), 9.90 (s, 1H), 8.12 (d, J = 2.1 Hz, 1H), 7.79 (d, J = 2.5 Hz, 1H), 7.62 (t, J = 2.3 Hz, 1H).

Mannich

Standard Procedure

Paraformaldehyde (2 equiv), a secondary amine (2 equiv) and *p*-toluenesulfonic acid (0.05 equiv) were added to a dry pressure tube, solvated in toluene, and stirred at room temperature for two hours. *N*-(5-hydroxypyridine-3-yl)acetamide (1 equiv) was added to the tube, and the reaction was heated to 95 $^{\circ}$C overnight. The next morning the reaction was allowed to cool to room temperature, then it was partitioned between ethyl acetate and water. The organic extract was dried over sodium sulfate and concentrated.

Purification was attempted via silica gel flash chromatography (EtOAc/Hex 4:1 v/v), but isolation of Mannich products from starting material and side products was unsuccessful.

DBM Procedure

Dibromomethane (15 equiv) and a secondary amine (4 equiv) were added to a dry round bottom flask. The flask was purged and backfilled with N_2 twice and stirred at room temperature for three hours. *N*-(5-hydroxypyridine-3-yl)acetamide (1 equiv) was solvated in tetrahydrofuran and added to the flask. The reaction was allowed to mix at room temperature for 12 hours. The mixture was then concentrated, redissolved in a minimal amount of dichloromethane, and treated with *n*-hexane at 0 $^{\circ}$C. The precipitate was filtered and washed with hexanes. The filtrate was then basified with 1N sodium hydroxide and extracted with dichloromethane. The organic extract was dried over Na_2SO_4 and concentrated. Purification was attempted via silica gel flash chromatography (DCM/MeOH 9:1 v/v), but isolation of Mannich products from starting material and side products was unsuccessful.

N-deacylation

The acylated product was solvated in dry THF, then zirconocene hydrochloride (Schwartz reagent, 2 equiv) was added. The reaction was stirred at room temperature for two minutes. Water was then added, followed by two equal portions of ethyl acetate. The organic extracts were dried over Na_2SO_4 and concentrated to afford crude product. The amine was purified via silica gel flash chromatography. *This reaction was not completed*

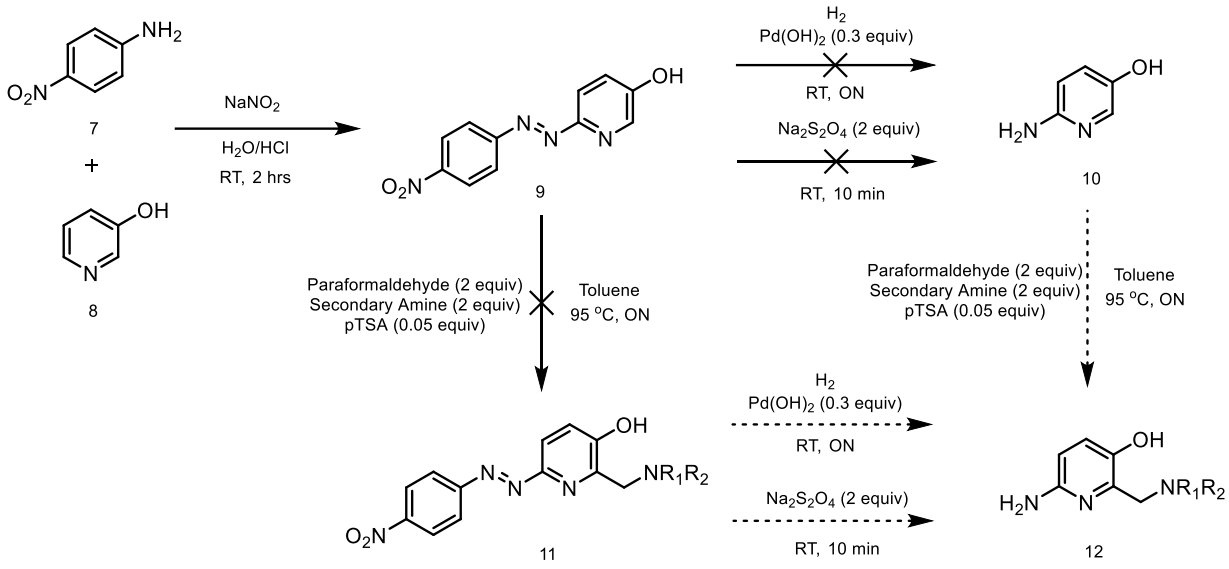
Finkelstein

To a dry round bottom flask, 5-bromopyridin-3-ol (1equiv), copper iodide (0.1 equiv), and sodium iodide (2 equiv) were added, then a condenser was added, and the system was sealed under N_2. Next the contents were solvated with 1,4-dioxane and *N,N*-dimethylethylenediamine (0.2 equiv) were added to the flask. The reaction was heated to 110 $^{\circ}$C and allowed to react for 24 hours with vigorous stirring. After 24 hours, the system was cooled to room temperature and the reaction mixture was poured into a solution of 30% aqueous ammonium hydroxide. The solution was diluted with water, using twice the volume of the 30% NH_4OH, and was then acidified with 3M HCl to pH 6. The aqueous solution was extracted three times with an equal volume of dichloromethane, and the organic extracts were dried over Na_2SO_4 and concentrated to afford the product [26]. ^{1}H NMR (400 MHz, CDCl3) δ 8.85 – 8.80 (m, 1H), 8.13 (t, J = 1.7 Hz, 1H), 7.05 (dd, J = 2, 1.7 Hz, 1H), 5.92 (s, 1H).

3.1 Synthetic Scheme

The first 6-aminopyridin-3-ol scheme (

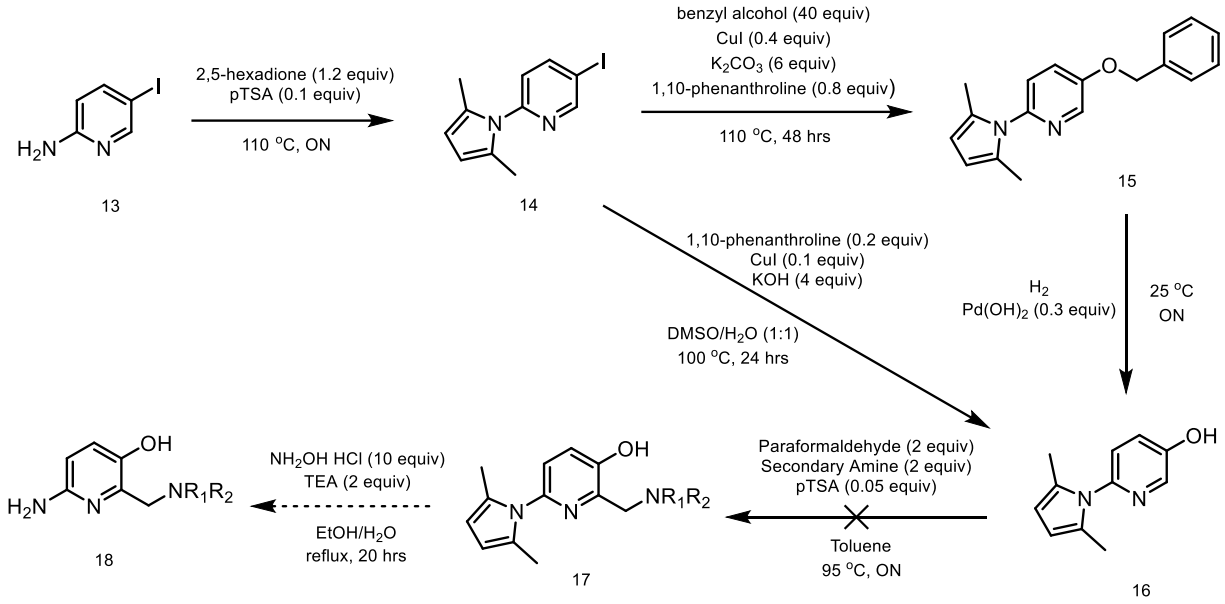
Scheme 9) utilizes 4-nitroaniline (**7**) and 3-hydroxypyridine (**8**) in a diazonium formation reaction to afford (E)-6-((4-nitrophenyl)diazenyl)pyridin-3-ol (**9**). The synthesis then diverges, using **9** in either a diazonium cleavage reaction to yield 6-aminopyridin-3-ol (**10**) or in a Mannich reaction to yield **11**. The cleavage product **10** was then subjected to Mannich conditions to provide the final Mannich product **12**, or the Mannich product, **11**, was cleaved to provide the same final product, **12**.



Scheme 9: 6-aminopyridine Diazonium QMP Scheme

The second 6-aminopyridine scheme (**Scheme 10**) starts with 5-iodopyridin-2-amine (**13**), protecting the amine via a Paal-Knorr pyrrole synthesis to make 2-(2,5-dimethyl-1H-pyrrol-1-yl)-5-iodopyridine (**14**). **14** was then subjected to Ullmann ether conditions to install a benzyl protected alcohol, making 5-(benzyloxy)-2-(2,5-dimethyl-1H-pyrrol-1-yl)pyridine (**15**). The alcohol of **15** was subsequently deprotected in a

standard hydrogenolysis reaction to yield 6-(2,5-dimethyl-1H-pyrrol-1-yl)pyridin-3-ol (**16**). This seemed inefficient, so an Ullmann hydroxylation reaction was employed to install the free alcohol and eliminate a synthetic step. **16** was then subjected to standard Mannich conditions to yield **17**, which was then deprotected to yield the free amine as the final product, **18**.



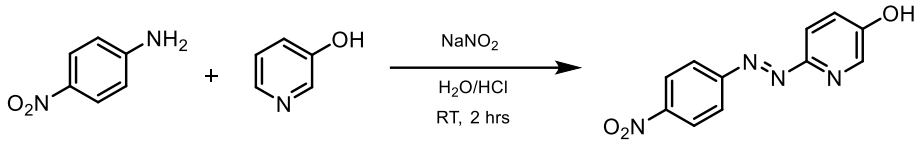
Scheme 10: 6-aminopyridine QMP Scheme

3.2 Results and Discussion

3.2.1 Diazonium Formation

The first reaction in the 6-aminopyridine scheme was the formation of a diazonium compound, adapted from Chaudhary et al. [27] This reaction utilized diazotized 4-nitroanliline, made in situ from the addition of sodium nitrite under acidic conditions. This solution is then added dropwise to a solution of 3-hydroxypyridine and subsequently basified. While yields were decent, around 70%, the products of the subsequent cleavage reaction were extremely difficult to separate. I next tried to make the separation easier by

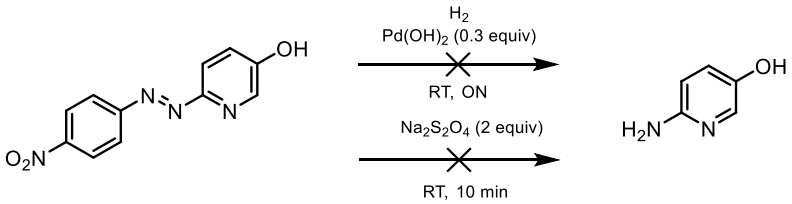
changing the 4-nitroanline for *p*-aminobenzoic acid (PABA). Although the carboxylic acid on PABA is not as forgiving as the nitro on 4-nitroaniline when it comes to changes in pH. I was eventually able to isolate this diazonium compound after changing the workup. The reaction conditions were not changed.



Scheme 11: Diazonium Formation

3.2.2 Diazonium Cleavage

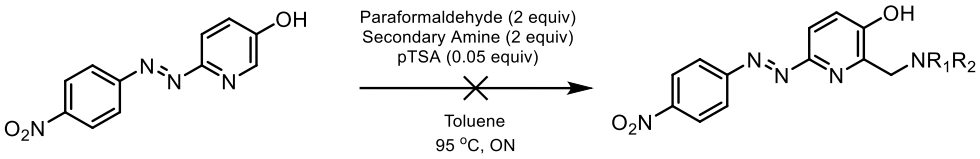
The next reaction in the first 6-aminopyridin-3-ol scheme was the cleavage of the diazonium compound. I attempted two different cleavage conditions, one from the same Chaudhary et al. paper [27], and another as suggested to me by Dr. McElroy. The conditions from the paper utilized Pd/C and a H_2 atmosphere. I was very familiar with these conditions as they are the same that I have used in the past for hydrogenolysis reactions. The second set of conditions were to use sodium dithionite, which is an industrial reducing agent. Unfortunately, neither of these conditions worked consistently, and when they did work the products were extremely difficult to separate. I was hoping that the change from 4-nitroaniline to PABA would make the products easier to separate, but this was not the case. Separation of products could be expedited utilizing a cationic column, where the carboxylic acid of PABA would bind and the desired cleavage product would elute.



Scheme 12: Diazonium Cleavage

3.2.3 Diazonium Mannich

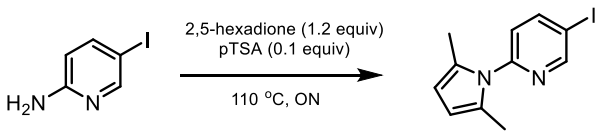
The next reaction, whether pre- or post-cleavage, was the Mannich reaction. Standard conditions from the Hadad lab and the dibromomethane conditions from Wu et al. [24] were used, as discussed in the previous chapter. Mannich reactions on the diazonium compounds provided little to no product, had many side products, and were very difficult to separate. These difficulties lead to the development of the second 6-aminopyridin-3-ol synthetic scheme.



Scheme 13: Diazonium Mannich

3.2.4 Pyrrole Protection

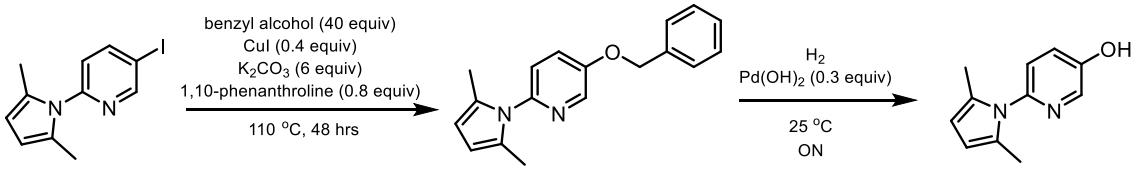
The first reaction of the second 6-aminopyridine scheme is the protection of the amine utilizing a Paal-Knorr pyrrole synthesis. This reaction, as well as the following Ullmann ether synthesis and hydrogenolysis were adapted from Nara et al. [28]. This protection method worked consistently and effectively, providing consistent near quantitative yields. It also required no purification, so after extraction the product could be efficiently taken into the next synthetic step.



Scheme 14: Pyrrole Protection

3.2.5 Ullmann Hydroxylation/Hydrogenolysis

As mentioned in the previous section, the first three reactions of the second 6-aminopyridine scheme were adapted from Nara et al. [28]. The second step, an Ullmann hydroxylation, installed a benzyl protected alcohol. This was completed using copper iodide as the copper source, 1,10-phenanthroline as the copper ligand, potassium carbonate as the base, and benzyl alcohol. This reaction took quite a while, with a 48-hour reaction time. This paired with the subsequent overnight hydrogenolysis took nearly a full week to react and purify. Hydrogenolysis conditions were the same as previously mentioned in chapter two. These long reaction times were what caused me to search for an alternative expedited procedure.

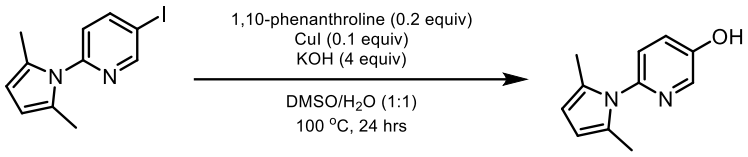


Scheme 15: Ullmann Hydroxylation/Hydrogenolysis

3.2.6 Direct Ullmann Hydroxylation

After searching for new Ullmann hydroxylation conditions, Dr. McElroy and I came across a fantastic review paper from Sambiagio et al. [29] that cataloged the timeline of development of Ullmann type reactions. Through this review, I found a paper from

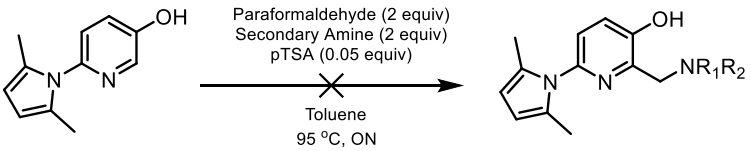
Zhao et al. [30] that provided conditions utilizing a variety of copper ligands and solvents to give direct Ullmann hydroxylation of *p*-iodotoluene. I was pleasantly surprised that conditions from the paper worked well for my 6-aminopyridine scaffold, providing consistent 40-50% yields. This also saved me about two days of work when compared to the previous hydroxylation/hydrogenolysis steps.



Scheme 16: Direct Ullmann Hydroxylation

3.2.7 Mannich Reaction

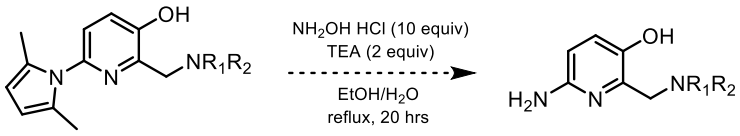
After the direct hydroxylation, the next step is to perform Mannich reactions with our standard set of secondary amines. This followed the same general procedure as mentioned in previous sections, but again various combinations of solvent, temperature, and reaction time were tested. I was unable to isolate the products of these reactions. While the crude mixture ran well on TLC those conditions did not translate to other forms of chromatography; manual columns, flash columns, and prep TLC were all unsuccessful.



Scheme 17: Mannich Reaction

3.2.8 Pyrrole Deprotection

The final step of the second 6-aminopyridine scheme was the deprotection of the pyrrole protected amine, which was again adapted from Nara et al. [28]. This was completed with relative ease utilizing hydroxylamine hydrocholoride and triethylamine in a refluxing ethanol/water solvent system. This reaction was tested on a sample of 2-(2,5-dimethyl-1H-pyrrol-1-yl)-5-iodopyridine (**2**) to give a 36% yield. It was also tested on the crude mixture of a piperidine Mannich product, but no deprotected product was obtained.



Scheme 18: Pyrrole Deprotection

3.3 Experimental Procedures

General Procedures

All flash chromatography was completed using a Combiflash® NextGen 300+ Flash Chromatography system. Unless otherwise stated, all flash chromatography was completed with Buchi FlashPure EcoFlex Silica 25g (40-63 μm) at a 40 mL/min flow rate. Reaction progress was observed via thin-layer chromatography (TLC) using Silica Gel 60 F254 plates. All NMR spectra was obtained using Bruker AVIII 400 MHz.

Diazonium Formation

To a clean round bottom flask, 4-nitroaniline (1 equiv) (or *p*-aminobenzoic acid) was slowly added to a cooled 6M HCl solution. To this cold, acidic solution, sodium nitrite (1 equiv) in water was added and the solution was stirred for 5 minutes. In a

separate clean round bottom flask, an aqueous solution of 3-hydroxypyridine (1 equiv) was prepared. The 4-nitroaniline/sodium nitrite solution was added dropwise to the 3-hydroxypyridine solution on ice. A pH of 8 was maintained with sodium hydroxide during the addition. After the addition was complete, the reaction was brought to room temperature and stirred for 90 minutes. The reaction was then acidified to pH 3 with 6M HCl and stirred at room temperature for an additional 30 minutes. The reaction was filtered through a fritted funnel and the solid was washed sequentially with water, hexanes, ether, and dichloromethane to provide crude product [27]. ^{1}H NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 8.49 – 8.41 (m, 2H), 8.31 (d, J = 2.8 Hz, 1H), 8.28 – 8.18 (m, 1H), 8.16 – 8.04 (m, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.42 (dd, J = 8.8, 2.9 Hz, 1H).

Diazonium Cleavage

Hydrogenolysis Procedure

(E)-6-((4-Nitrophenyl)diazenyl)pyridin-3-ol (**9**) (1 equiv) and $Pd(OH)_2$ (0.3 equiv) were added to a dry round bottom flask and solvated with methanol. The flask was then purged and backfilled twice with nitrogen, and then twice with hydrogen. The reaction was allowed to stir at room temperature for 24 hours. The crude mixture was filtered through Celite and concentrated. Purification was attempted via silica gel flash chromatography (DCM/MeOH 9:1 v/v), but isolation of product was unsuccessful.

Sodium dithionite Procedure

(E)-6-((4-Nitrophenyl)diazenyl)pyridin-3-ol (**9**) (1 equiv) was solubilized in methanol and added to a clean and dry round bottom flask. With strong stirring, sodium dithionite (2 equiv) was added, and the reaction was monitored via TLC. When sufficient

product was formed, the reaction was concentrated. Purification was attempted via silica gel flash chromatography (DCM/MeOH 9:1 v/v), but isolation of product was unsuccessful.

Mannich Reaction

The procedure for the Mannich reactions can be found in the chapter two experimental procedures section. All equivalents are maintained, the only change is the starting material. Mannich products were not able to be isolated.

Pyrrole Protection

5-Iodopyridin-2-amine (**13**) (1 equiv), 2,5-hexadione (1.2 equiv) and *p*-toluenesulfonic acid (0.1 equiv) were added to a clean and dry round bottom flask. The reactants were solvated with toluene, a Dean-Stark apparatus was added, and the whole system was wrapped in aluminum foil. The reaction was heated to 110 $^{\circ}$C and allowed to react overnight. The reaction was then cooled to room temperature and washed sequentially with sodium bicarbonate, water, and brine. The organic layer was concentrated to provide pure 2-(2,5-dimethyl-1H-pyrrol-1-yl)-5-iodopyridine (**14**) [27]. ^{1}H NMR (400 MHz, CDCl3) δ 8.83 (d, J = 2.3 Hz, 1H), 8.13 (dd, J = 8.3, 2.3 Hz, 1H), 7.05 (d, J = 8.3 Hz, 1H), 5.93 (s, 2H), 2.16 (s, 6H).

Ullmann Hydroxylation/Hydrogenolysis

To a clean and dry round bottom flask, copper iodide (0.4 equiv), 1,10-phenanthroline (0.8 equiv), potassium carbonate (6 equiv), and 2-(2,5-dimethyl-1H-

pyrrol-1-yl)-5-iodopyridine (**14**) (1 equiv) were added. The flask was purged and backfilled with nitrogen twice before adding benzyl alcohol (40 equiv) and a minimal amount of toluene. The reaction was heated to 110 °C for 24 hours. Then a TLC was performed to assess the reactions progress. If not complete, copper iodide (0.1 equiv) and 1,10-phenanthroline (0.2 equiv) were added and the reaction was heated at 110 °C for another 24 hours. After the full 48 hours, the reaction was cooled to room temperature, filtered through a silica plug and concentrated. The 6-(2,5-dimethyl-1H-pyrrol-1-yl)pyridin-3-ol (**16**) product was purified via silica gel flash chromatography (EtOAc/Hex 1:9 v/v) [27]. ^{1}H NMR (400 MHz, CDCl3) δ 8.81 (dd, J = 2.3, 0.7 Hz, 1H), 8.11 (dd, J = 8.3, 2.3 Hz, 1H), 7.45 – 7.35 (m, 4H), 7.35 – 7.21 (m, 1H), 7.03 (dd, J = 8.3, 0.7 Hz, 1H), 5.91 (s, 2H), 5.18 (s, 2H), 2.14 (s, 6H).

Direct Ullmann Hydroxylation

To a clean and dry round bottom flask, copper iodide (0.1 equiv), 1,10-phenanthroline (0.2 equiv), potassium hydroxide (4 equiv) and 2-(2,5-dimethyl-1H-pyrrol-1-yl)-5-iodopyridine (**14**) (1 equiv) were added. The flask was purged and backfilled with nitrogen twice before heating to 100 °C for 24 hours. The reaction was then cooled to room temperature and diluted with water and ethyl acetate. The mixture was filtered through a silica plug, and the aqueous layer was removed. The organic layer was concentrated and purified via silica gel flash chromatography (EtOAc/Hex 40% v/v) to provide pure 6-(2,5-dimethyl-1H-pyrrol-1-yl)pyridin-3-ol (**16**) [28]. ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H), 8.10 (dd, J = 3.0, 0.6 Hz, 1H), 7.33 (dd, J = 8.5, 3.0 Hz, 1H), 7.19 (dd, J = 8.5, 0.7 Hz, 1H), 5.74 (s, 2H), 1.98 (s, 6H).

Pyrrole Deprotection

To a clean and dry round bottom flask, hydroxylamine hydrochloride (10 equiv), triethylamine (2 equiv) and Mannich product (1 equiv) were added. The reactants were solvated with ethanol/water (2:1 v/v), a condenser was attached, and the reaction was refluxed for 20 hours. The reaction was then cooled to room temperature and then to 0 $^{\circ}$C in an ice bath. The cooled reaction mixture was quenched with cold HCl, washed with ether, and adjusted to pH 10 with 6M NaOH. Dichloromethane was added to extract the product, the extracts were dried over sodium sulfate and purified via silica gel flash chromatography (EtOAc/Hex 70% v/v).

Chapter 4: Conclusions and Future Work

While I was not successful in synthesizing and isolating the final QMP products I was tasked with, I learned a great deal from my time in the McElroy Lab. For better or for worse, I was forced to be independent and self-sufficient in the lab. I started graduate school during the peak of the COVID pandemic, so shadowing and typical learning experiences were made extremely difficult. Even so, I was able to be mentored by some fantastic scientists. To say I am more confident in my lab skills would be an understatement. I have not only honed skills that I had learned in the past, but also developed new skills that I had no idea existed prior to graduate school.

The future work of this project would be to use my work on the synthesis and isolation of 5- and 6-aminopyridin-3-ol intermediates to create a library. This would include the optimization of Mannich reactions, utilization of different protecting groups on the primary amine, and the biochemical testing of the QMPs.

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