Turning 10-Methylphenylthiazine into a Hydrogen Atom Transfer Agent
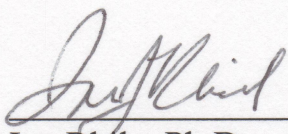
Joseph I. Polinski

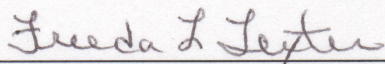Candidate for the degree

Bachelor of Science

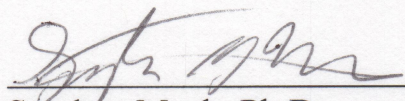Submitted in partial fulfillment of the requirements for

College Honors

Departmental Distinction in Biochemistry

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Radical Chemistry and PCET:

Turning 10-Methylphenylthiazine into a Hydrogen Atom Transfer Agent

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Introduction

Electron transfer and hydrogen atom transfer are processes in chemistry that are at the fundamental core of countless chemical applications. In electron transfer, as the name implies, only an electron is transferred, and in proton transfer, only a proton is transferred. Likewise, in hydrogen atom transfer (HAT), both an electron and a proton are transferred, abbreviated as H•. HAT is a subclass of proton-coupled electron transfer (PCET), a much broader mechanism where the proton and electron are transferred in one mechanistic step, although not necessarily to the same site.[1] These processes find applicative importance in environmental chemistry, biochemistry, and alternative energy research. As an example, photosynthesis involves a proton gradient to convert solar energy to chemical energy. This proton gradient is generated as the movement of electrons respond to the absorbance of light.

In the area of biomolecules, flavins and DNA can undergo PCET and/or HAT processes causing a variety of effects. HAT can cause damages to DNA, which produces radicals as an important component of oxidative stress. Alternately, a PCET can occur during which the electron is transferred to a remote site and the proton is transferred to the hydrogen-bonded DNA base or the solvent.[2] In another simple example related to biological systems, PCET can be seen in the oxidation of an alcohol with flavin, as the flavin accepts a hydrogen atom from an alcohol.[3]

In each case above, the chief molecule of the reaction is a nitrogen heterocycle with a relatively acidic proton. In the absence of such an acidic proton, these heterocycles are only able to undergo electron transfer. The chemistry of the system mandates the presence of a proton, but leaves a variety of questions that still need to be

answered. The minimal acidity of the proton, along with the reduction potential required for the heterocycle and the reactivity of different nitrogen heterocycles is in question. The goal of the synthesis is to provide a starting point to answer the questions presented. The project will also help to clarify the types of structures that will undergo reactivity.

In the summer of 2005 and in the winter of 2006, I explored radical chemistry and hydrogen atom transfer through the organic synthesis project "Synthesis of a Stable, Hydrogen-Bonded Phenoxyl Radical" under the mentorship of Dr. Ian Rhile.[4] This project was important as it introduced me to the multistep synthesis, column chromatography, and spectroscopic techniques fundamental to radical chemistry. Most importantly, this project introduced me to air-sensitive reactions, a reaction type that is mandated by each step of this synthesis.

The project sought to synthesize a molecule that was capable of forming a stable radical, stabilized by system conjugation and two internal hydrogen bonds. The attempted three-step synthesis is seen below. During the first synthesis, protonation of hexamethylenetetramine by trifluoroacetic acid allows the ortho-directed electrophilic aromatic substitution that yields aldehydes in the 2- and 6-position after hydrolysis.[5] In the second step, these undergo condensation to create imidazoles in the 2- and 6-positions.[6] Two procedures were tested for this final step, the first involving the hydrogen atom acceptor lead(IV) oxide and the second used potassium ferricyanide. This ACRE project was an important first step introducing me to radical chemistry that is applicable to this project.
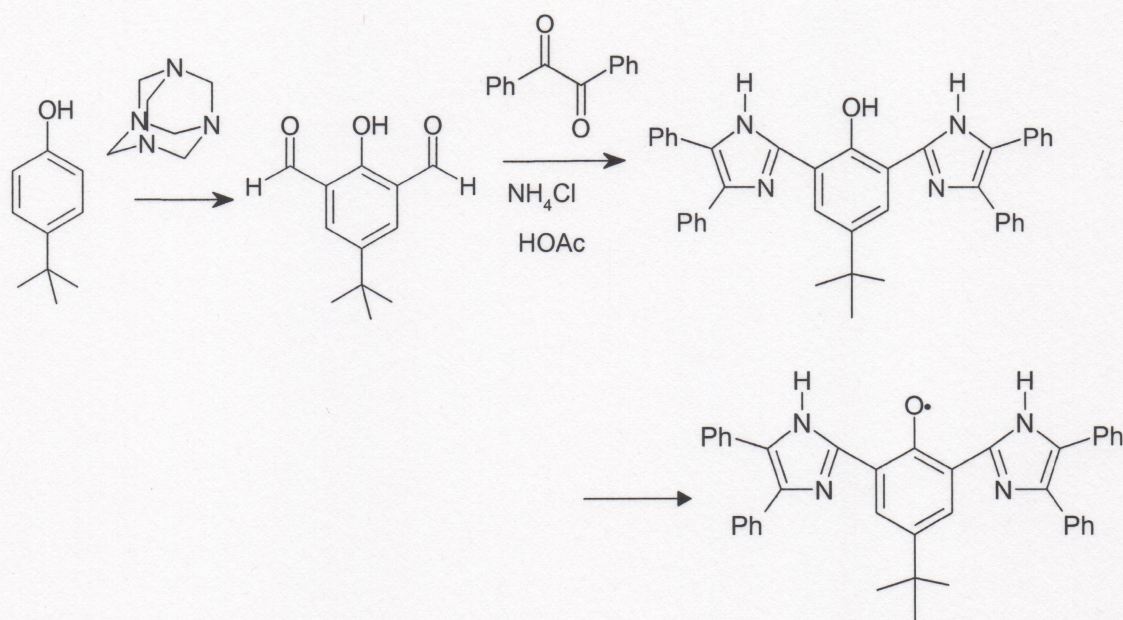
Figure 1. ACRE phenoxyl radical synthesis.

The current project of "Turning 10-Methylphenylthiazine into a Hydrogen Atom Transfer Agent" involves similar chemistry as the previous two ACREs. In this instance, a persistent radical cation with a basic site is the model molecule for the study of the PCET system. Phenothazinum ions, where the radical cation readily gains an electron to form stable and reduced organic molecules are known and documented.[7] The target synthesis proposes to append to the radical cation, which is only capable of electron transfer, a pyridyl ring, which is then capable of gaining a proton. Hence, the radical cation-base will be capable of gaining an electron and a proton, the required components for PCET. This proposed mechanism is both a HAT and the form of PCET where the proton and electron originate from orthogonal bonding systems, with the proton in the σ system and the electron in the π system of the molecule. The proposed molecule that is derived from the stable radical cation will be stable with and without both a proton and

electron as seen below in Figure 2, which differs from the aforementioned biological

system and adds to the ease of the characterization process after synthesis. While these

are not the actual biological systems where these processes occur, the similarities

between the target synthesis and the actual biological systems allows an understanding to

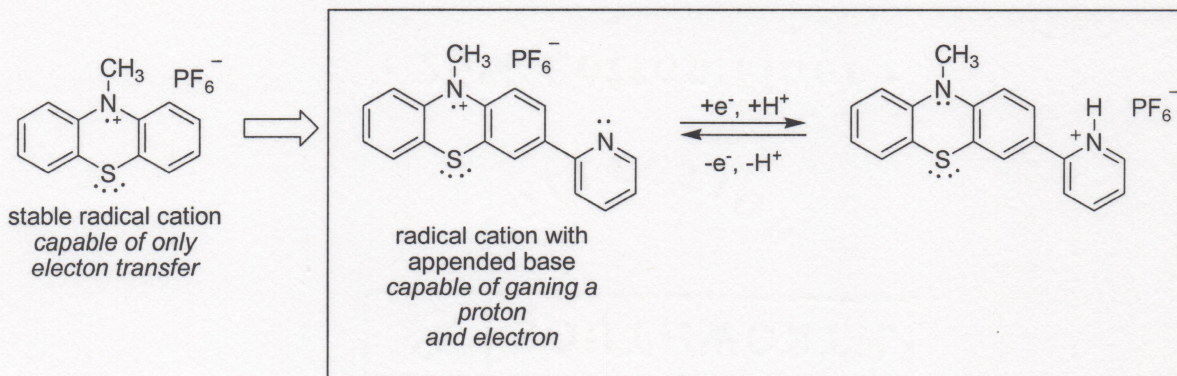be gained as to the relationships between structure and mechanisms.

Figure 2. The proposed molecules are derived from stable radical cations. The proposed molecules will be stable with and without both a proton and electron, and the electron and proton movement in each case are to separate sites.

The intended synthesis of the desired compound is a three-step process that

parallels literature syntheses, seen in Figure 3. As reported by Lai *et al.*, reacting

bromine with 10-methylphenothiazene (**1**) will provide the 3-bromo-10-methyl-

phenothiazene (**2**).[8] As reported by Krämer *et al.*, palladium coupling with a boron

compound will produce compound **3**.[9] Performing another palladium coupling with 2-

bromopyridine will produce the neutral precursor to the radical cation (**4**).
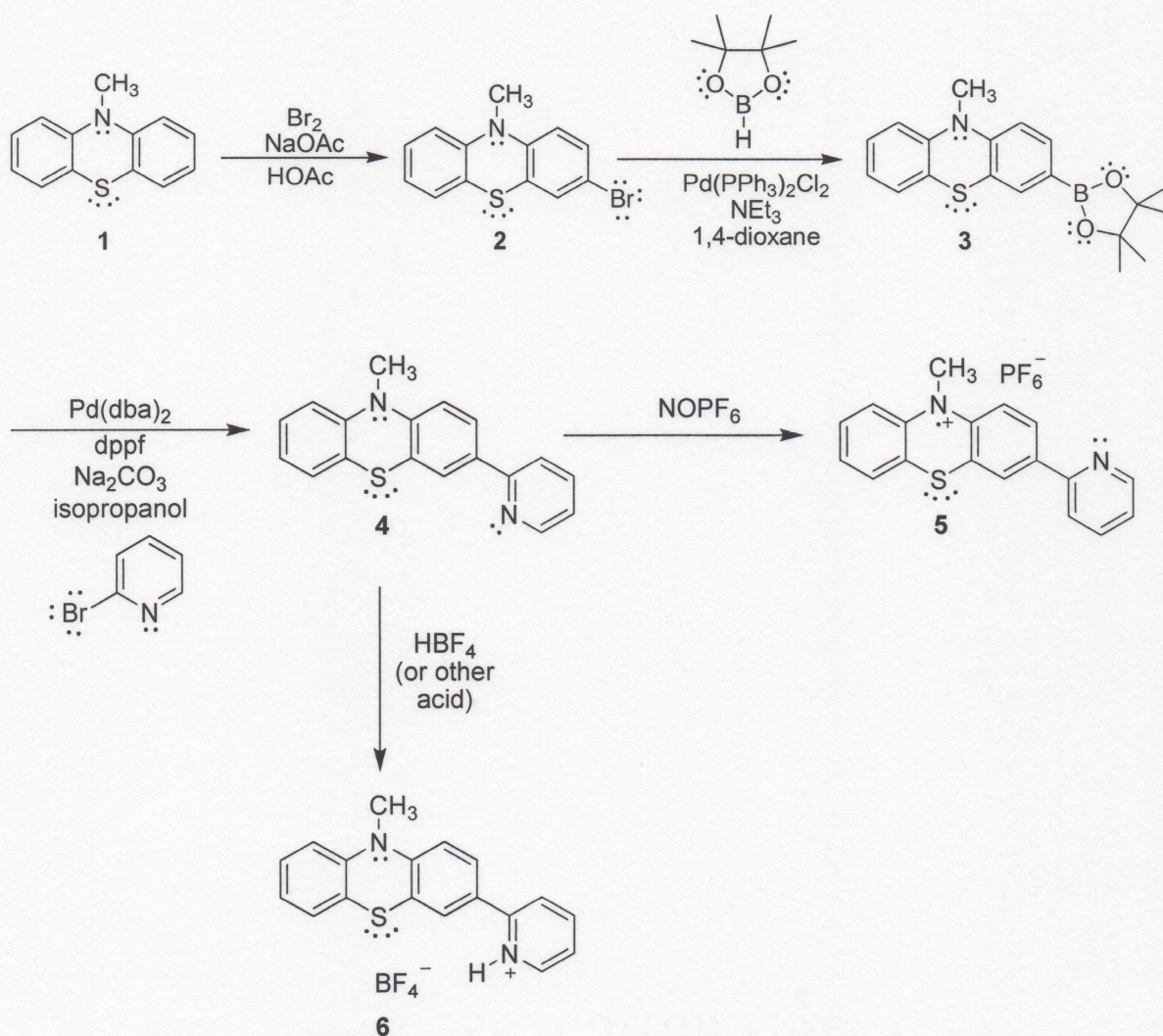
Figure 3. Syntheses of target molecules.

The radical cation and the product of hydrogen atom transfer, which are the two relevant forms of the target molecule, will be generated. Oxidants such as NOPF$_6$ or AgBF$_4$ will oxidize the parent substrate to generate the radical cations. Similarly, the protonated forms will be generated with the corresponding acids (HBF$_4$ and HPF$_6$). Crina Sasaran completed the initial steps of this synthesis on a small scale during a

summer 2008 ACRE project,[10] and her initial work allowed small scale testing to be done that preceded the large-scale synthesis of this project.

The products of each step of synthesis were analyzed and compared closely to products generated by Crina Sasaran's small-scale summer steps. Unfortunately, nuclear magnetic resonance (NMR) analysis and characterization could not be performed due to instrumentation malfunctions early in the year. Due to the lack of NMR analysis, the success of the target synthesis has been based chiefly on GS/MS data. The first two steps of the synthesis have been completed on a large scale, and UV-vis analysis has been completed on product (4) with attempts to characterize the formation of the protonated species to parallel previous data collected by Crina Sasaran on the radical cation.

Experimental and Results

Synthesis of 3-bromo-10-methylphenothiazine (1).[8]

1
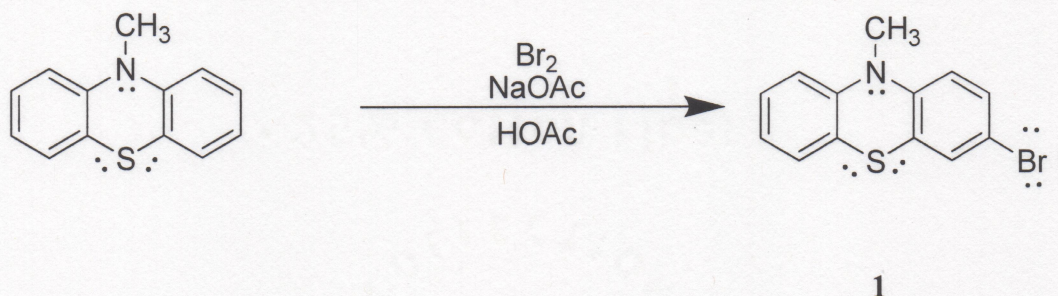
KOH (3.85 g, 68.6mmol) was dissolved in glacial acetic acid (167 ml), under nitrogen and stirring. 10-Methylphenothiazine (5.00 g, 22.1 mmol) was added to the mixture, followed by chloroform (33 ml). The solution was cooled to 0-5 °C, and liquid Br_2 (1.20 ml, 23.3 mmol) dissolved in glacial acetic acid (20 ml) was added dropwise. The suspension was stirred at room temperature for 4 h and acetic acid was removed by rotary evaporation, affording a purple powder. The solid was dissolved in aqueous $NaHCO_3$ and CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and filtered through a short silica gel column. The crude product was purified by flash chromatography on silica gel (ethyl acetate:hexanes, 5:95). After recrystallization with toluene-ethanol (1:10), 4.8 g (16.19 mmol, 73%) of white solid (1) was recovered. GC/MS (*m/z*): 291.3 (M), 294.2 (M+2).

Synthesis of 10-Methyl-3-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-10*H*-phenothiazine (2).[9]

1   2
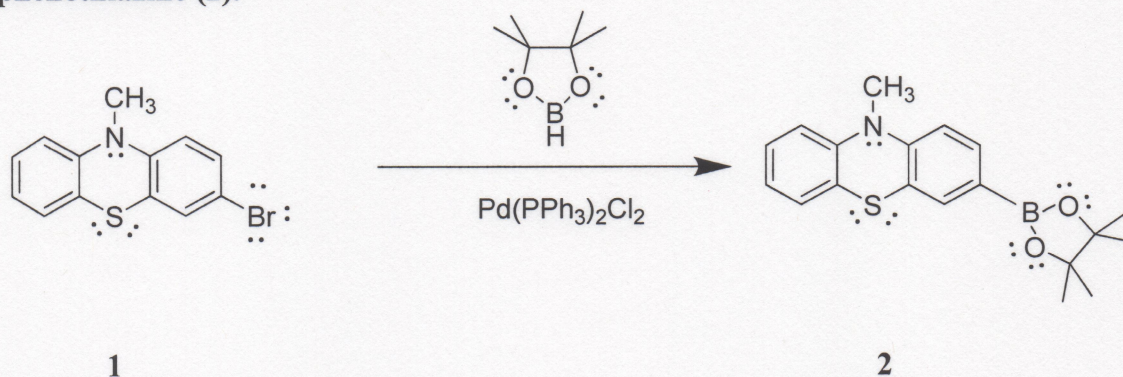
1,4-Dioxane was dried over sodium and benzophenone under nitrogen and reflux, and triethylamine (NEt₃) was dried under nitrogen and reflux prior to being used in the reaction. To 3-bromo-10-methylphenothiazine **(1)** (2.0 g, 6.84 mmol) was added NEt₃ (3.82 ml), 1,4-dioxane (40 ml), PdCl₂(PPh₃)₂ (0.029 g, 0.04 mmol) and pinacolborane (13.69 ml, 1 M solution in tetrahydrofuran). The reaction mixture was stirred and heated under nitrogen and reflux for 48 h. The suspension was cooled down to room temperature, diluted with ethyl acetate (50 ml) and brine. The aqueous layer was extracted with ethyl acetate and the organic layer was dried over $MgSO_4$ and concentrated. Purification and analysis of synthesis product is pending.

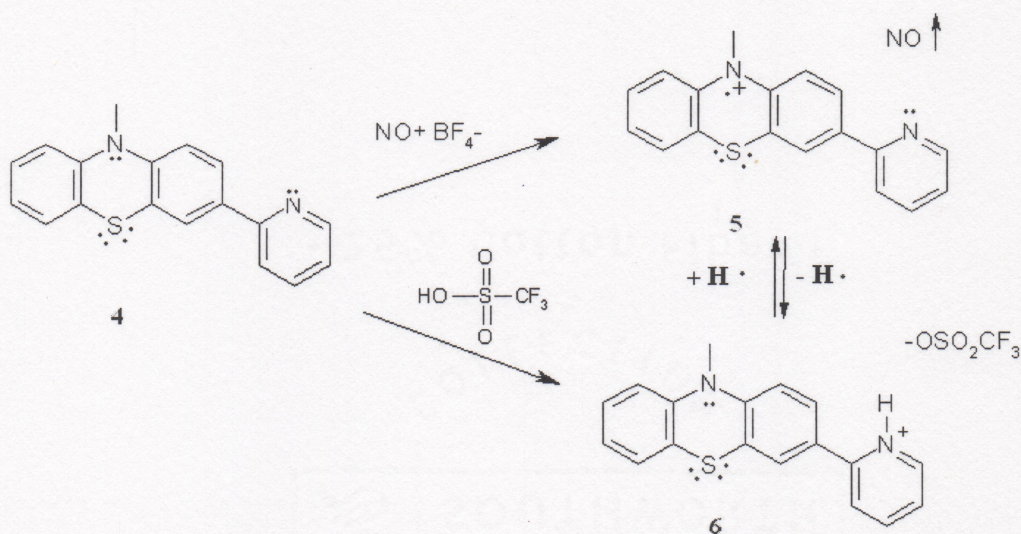**Protonating 3-(2-pyridyl)-10-methylphenothiazine (4) from Sarasan Summer ACRE**

Figure 4. Two separate pathways for protonation or radical cation transformation of **4**.

In her ACRE project over the summer, Crina Sarasan added NOBF$_4$ to the product **4** and observed a drastic change in the UV-vis spectrum, indicating the formation of the radical cation product (**5**).[10] Attempting to produce the protonated product **6** to illustrate that product **4** can undergo both protonation and radical cation formation, two experiments were performed. Both experiments attempted to protonate Crina Sarasan's product **3** to yield product **6**. The first attempt used HBF$_4$, but the resulting UV-vis spectrum was insufficient in determining if the reaction was successful. The yellow color of the acid, whether it was contaminant or inherent color, produced a UV-vis signal that overpowered the spectrum, providing inconclusive results.

The second experiment used trifluoromethanesulfonic (triflic) acid as the proton donor, and the decrease in the acid background spectrum was enough to observe a considerable change in the UV-vis spectrum of **4** upon addition of the acid. 50 µL of

triflic acid was added to 25 mL of acetonitrile (ACN) yielding a solution of 1.08×10^{-6}

moles/mL. 0.0015 g of 3-(2-pyridyl)-10-methylphenothiazine (**4**) was added to 25 mL of

ACN, and then 0.5 mL of this solution was added to 10 mL of ACN yielding a $1.08 \times$

10^{-6} moles/mL solution. The samples were mixed 1:1 and with large acid excess and

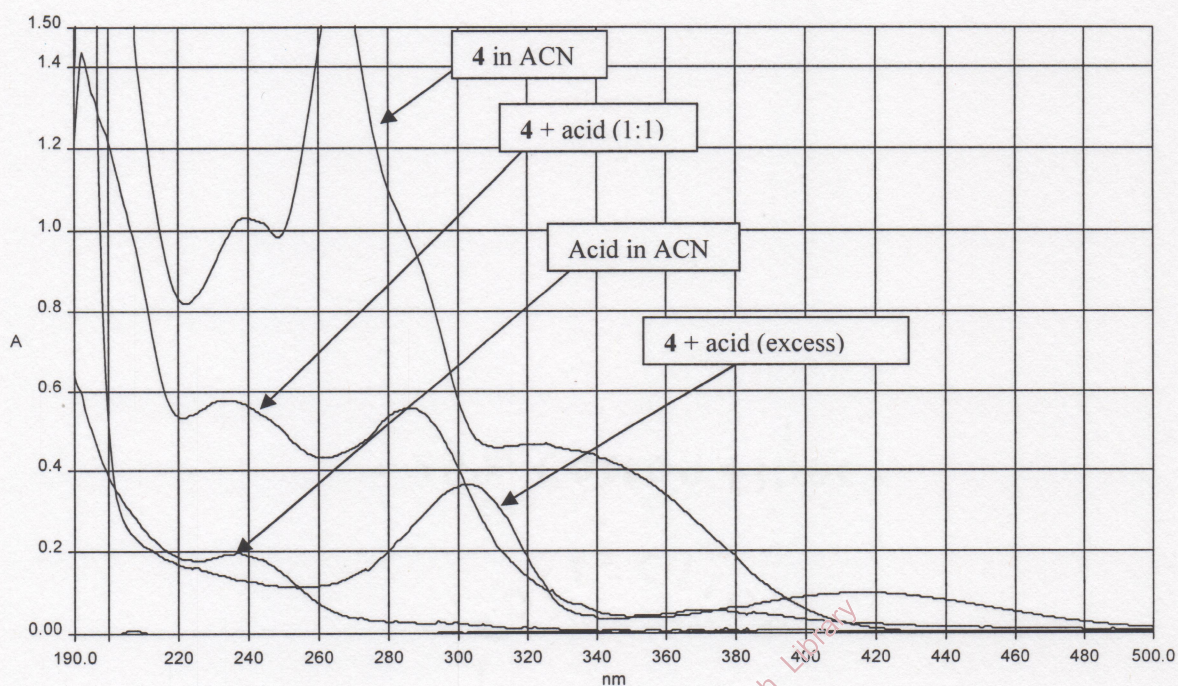monitored on the UV-vis which yielded the spectrum seen as Figure 5 below.

Figure 5. UV-vis combined spectrum for protonation of **4.**

Discussion

The first step of synthesis to create 3-bromo-10-methylphenothiazine (**1**) was an

electrophilic aromatic substitution. The nitrogen, and its associated lone pairs in the

fused ring system, act as a strong ring activating group, donating electron density into either ring allowing the system overall to be ring activating, ortho,para-directing. The ring acts as a nucleophile, and the major product of the reaction has the bromine substituted in position 3 (para to the nitrogen) most likely due to the steric hindrance of the methyl group that would accompany attempted bromination of the 1-position (the ortho). The curved arrow formalism for this mechanistic step is seen below in Figure 6.
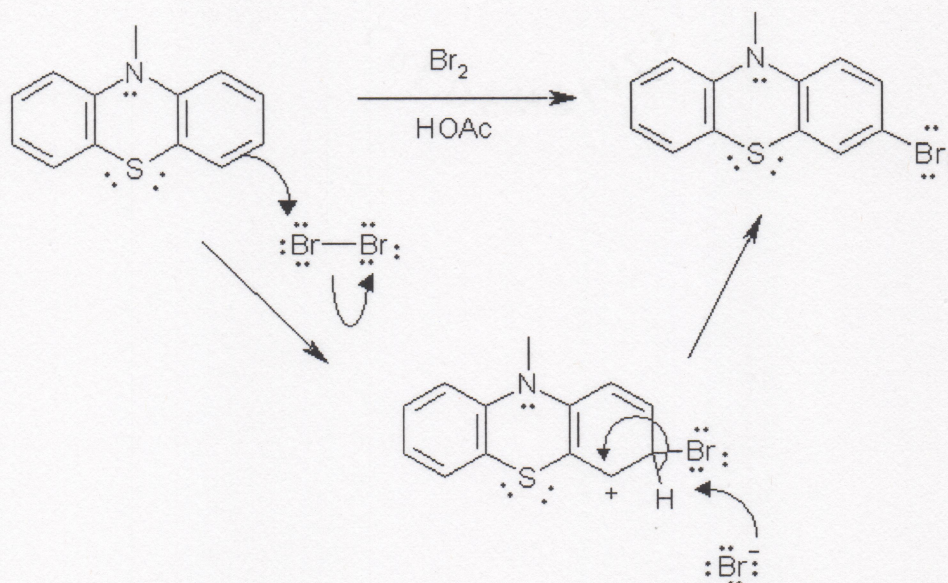

Figure 6. Electrophilic aromatic substitution of 10-methylphenothiazine.

The purification of the bromo compound steps was difficult. Initial recrystallization attempts with the aforementioned solvent system were difficult to perform correctly and ineffective at purification due to an unknown oil-like impurity. After multiple failed recrystallizations, a flash column was performed which eliminated the oil contaminant from the sample. However, after the column, the pooled samples still contained a mixture of three compounds, as seen in TLC analysis. Even after the recrystallization following the column, trace amounts of two impurities remained, again

determined by TLC analysis. The product integrated to 85.6% in the GC/MS analysis,

which was sufficient to continue the next reaction. The GC/MS indicated that the

starting material and a dibromo compound were the impurities.

The second step of the reaction was an organometallic reaction involving a

palladium catalyst as seen mechanistically below. In this instance, L symbolizes any

possible Pd ligand (:NEt$_3$, 1,4-dioxane, PPh$_3$ etc.). Ar-Br symbolizes 3-bromo-10-

methylphenothiazine.
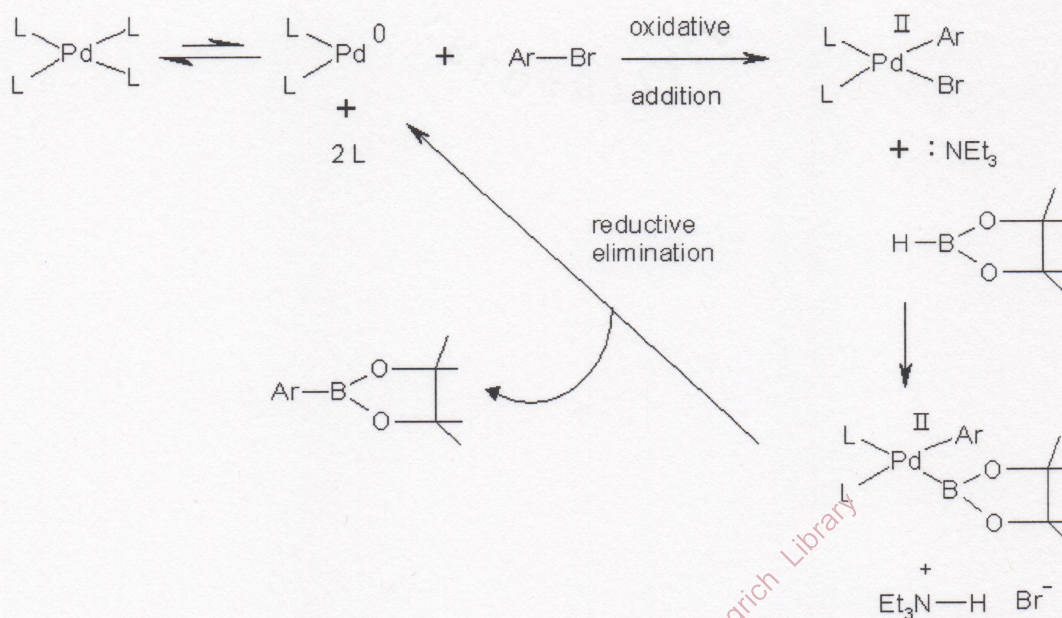
Figure 7. Synthesis of 10-Methyl-3-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-10*H*-phenothiazine (**2**).

Undergoing constant ligand exchange, Pd^0 undergoes oxidative addition as it is

oxidized to Pd^{II}. The base then deprotonates the pinacolborane and the deprotonated

pinacolborane and 10-methylphenothiazine become Pd ligands. A final reductive

elimination yields the 10-methyl-3-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-10*H*-

phenothiazine (**2**) and regenerates the catalyst. This reaction was my first experience with organometallic reactions, which introduced me to chemistry that is vastly different from the nucleophile/electrophile chemistries of all my previous experiences. The purification and analysis of the product of this step is planned before graduation.

The final completed part of the project was the protonation and UV-vis analysis of 3-(2-pyridyl)-10-methylphenothiazine (**4**). This facet of the project attempted to build on Crina Sarasan's successes with creating a radical cation by making the associated protonated form of **4**, thus fulfilling the paradigm that is the basis of these reactions. This reaction was performed on a small, analytical scale, mandated not only by limitations of **4,** but also by the intense UV-vis activity of the species under study.

Conclusion

Although there was a considerable amount of difficulty in the purification steps of the first step of synthesis, the first two steps have been performed on a large scale, promising further testing for the synthesis, which promises to generate the desired product eventually. UV-vis experiments with 3-(2-pyridyl)-10-methylphenothiazine (**4**) were also performed which illustrated, to a certain degree, the success of not only Crina Sarasan's ACRE synthesis, but also the ability of **4** to become a radical cation and be protonated. This synthesis will be continued until graduation, and a student in the future will perform further work on this project.

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