SYNTHESIS OF ASYMMETRIC THIAZOLO[5,4-D] THIAZOLE DERIVATIVES FOR MOLECULAR SENSING APPLICATIONS

by

Jared Christian Meadows

A thesis submitted to the faculty of
The University of North Carolina at Charlotte
in partial fulfillment of the requirements
for the degree of Master of Science in
Chemistry

Charlotte

2021

Approved by:

_____
Dr. Michael G. Walter

_____
Dr. Jerry M. Troutman

_____
Dr. Swarnapali De Silva Indrasekara

_____
Dr. Yong Zhang

ABSTRACT

JARED MEADOWS. Synthesis of Asymmetric Thiazolo[5,4-*d*] Thiazole Derivatives for Molecular Sensing Applications. (Under the direction of DR. MICHAEL G. WALTER)

This thesis outlines the synthesis and characterization of new asymmetrically substituted thiazolo[5,4-d]thiazole (TTz) compounds. This class of compounds contains a central bicyclic heteroaromatic system that is electron-deficient, and when substituted asymmetrically with both electron-donating and withdrawing groups, shows strong solvatofluorochromism. These asymmetric TTz compounds typically have very strong excited-state dipole moments and exhibit a "push-pull" electronic effect when photoexcited. These compounds also demonstrate sensitivity to their chemical environments and are interesting target compounds for various molecular sensing applications for this reason. A variety of synthetic routes have been pursued to access addition push-pull compounds containing amino, nitro, and acetamido functional groups. In particular, introducing primary amine functionality to asymmetrically substituted TTz compounds was a major synthetic focus of the research described in this thesis. These new TTz compounds show new and interesting photophysical properties. One of these synthetic routes has also provided a significantly more efficient way of yielding an asymmetric TTz compounds with these functionalities. The synthetic efforts as well as the optoelectronic properties of these new TTz materials will be presented. In particular, this class of compounds was being investigated for use as a fluorescent probe to study the

antibacterial target enzyme undecaprenyl pyrophosphate synthase (UppS). Similar fluorophores have been used in the past, but the push-pull electronic nature of these compounds typically results in solvatofluorochromism, and the shift in fluorescence is often quite sensitive to the chemical environment of the TTz. For these reasons, this class of compounds is being investigated not only for the UppS application, but for general use as molecular sensors as well. The synthetic efforts toward developing, and photophysical properties of, TTz derivatives with amine, nitro, and acetamido functionalities will be described in this thesis.

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Dr. Michael Walter and the Walter Research Group as they integrated me into the research group and helped me to learn and understand the background and methods of the research that we conduct here at the University of North Carolina at Charlotte. Similarly, I would like to specifically thank Dr. Jay Troutman and the Troutman Research Group for helping me understand the background and methods of the biochemical side of this collaborative project. I would also like to thank the rest of my thesis committee, Dr. Swarnapali De Silva Indrasekara and Dr. Yong Zhang for being part of my thesis committee and for giving me constructive feedback throughout my time here at UNCC. This feedback was helpful in my development as a scientist in academia and gave me many opportunities to improve both my thesis and my research skills in general. I feel that my experience at UNCC has more than prepared me for my Ph.D. program that I began in Fall 2021. Lastly, I would like to specifically thank Ana Ledezma Montoya and Andrew Brotherton, two undergraduate researchers in the Walter group, who helped me collect a lot of photophysical data while I was away starting my first semester as a PhD student at the University of Georgia.

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LIST OF ABBREVIATIONS

TTz	thiazolo[5,4-d]thiazole
UppS	undecaprenyl pyrophosphate synthase
FPP	farnesyl diphosphate
GPP	geranyl diphosphate
IPP	isopentenyl diphosphate
BPP	bactoprenyl diphosphate
DFT	density functional theory
HOMO	highest occupied molecular orbital
LUMO	lowest unoccupied molecular orbital
OFET	organic field effect transistor
VSD	voltage-sensitive dye

CHAPTER 1: INTRODUCTION

1.1. Background on Thiazolo[5,4-*d*] Thiazole Derivatives

Thiazolo[5,4-*d*] thiazole (TTz) derivatives are a class of heterocyclic aromatic compounds that are formed via a double condensation reaction by which dithiooxamide reacts with two aryl aldehydes (often *para*-substituted) simultaneously to form the bicyclic TTz core, typically yielding little to no partial condensation products.[1-4] There are two main types of TTz derivatives that were synthesized, which we refer to as symmetric and asymmetric TTz's. A symmetric TTz is one that is functionalized by the same group on the 2 and 5 positions of the bicyclic core, while the asymmetric TTz is one that is functionalized with two different groups. These can both be synthesized via the reaction shown in **Figure 1**. In the case of asymmetric TTz synthesis, multiple TTz derivatives will be formed. The mechanism by which the TTz core is formed involves an oxidation step so the reaction does not require inert conditions. Aryl aldehydes that are substituted with electron donating groups result in characteristically lower yields due to the deactivation of the aldehyde as an electrophile through resonance.[1-4]

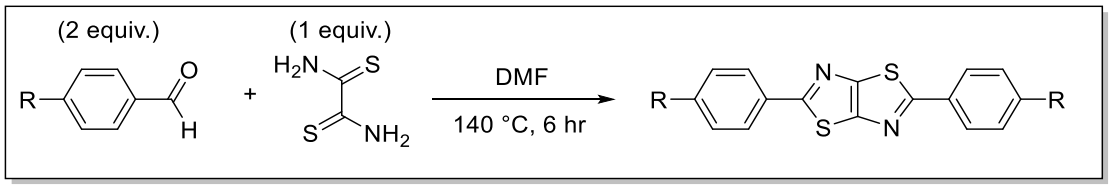

Figure 1: General reaction scheme for the synthesis of symmetrically substituted TTz compounds. This reaction undergoes a double condensation and oxidation to form the TTz core. Polar, high boiling solvents are often used for this reaction.

A symmetrically substituted TTz can be formed using the reaction shown in **Figure 1**. Synthesizing symmetric TTz compounds using this method is advantageous, as it is a simple, one reaction pot synthesis that results in the precipitation of the product. Because of the rigid and planar structure of these compounds, they typically aggregate and are not very soluble in various polar synthetic solvents, such as DMF. This usually results in the precipitation of large amounts of product when using this reaction. This is particularly advantageous, as the reaction solution can be filtered off and the precipitate can be washed and collected as is in most cases. This reaction can also be done on a large scale with little to no impurities observed in the filtered product.

These TTz compounds have many interesting properties and are photostable and thermally stable. They also show a high degree of chemical and oxidative stability. Additionally, they exhibit a high radiative rate of fluorescence due to their highly planar and rigid structure.[3-4] When an asymmetric TTz is formed with an electron donating substituent and an electron withdrawing substituent opposite each other, the electronics of these compounds changes remarkably. The electron-deficient nature of the TTz core allows these compounds to exhibit a “push-pull” effect whereby the electron density shifts very significantly throughout the molecule when excited by photons, a process called intramolecular charge transfer (ICT). This results in a characteristically strong excited-state dipole moment. In some cases, the excited-state dipole moment of asymmetric TTz derivatives can exceed 15 Debye.[3] This strong excited-state dipole moment is the main contributor to one of the asymmetric TTz compounds most interesting properties: its solvatofluorochromic effect.

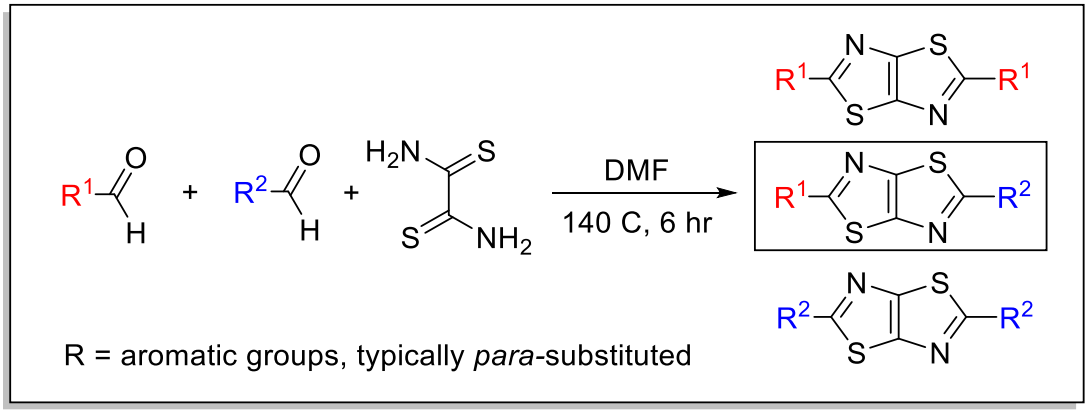

Figure 2: General reaction scheme for the synthesis of asymmetrically substituted TTz compounds. This reaction forms three major products as a result.

Asymmetrically substituted TTz's can be synthesized using a mixed pot version of the symmetric synthesis, as illustrated in **Figure 2**. One major drawback of this reaction is the formation of multiple TTz products, typically resulting in a lower yield of the desired product. Since post-synthetic modifications can be difficult due to the chemical stability and solubility of symmetrically substituted TTz's, it can sometimes be advantageous to take this approach to produce asymmetrically substituted moieties. Similar to the symmetric TTz synthesis, TTz's that are symmetrically functionalized with electron withdrawing groups will tend to be the major products of this reaction, as the aldehyde is more electrophilic, allowing the condensation with dithiooxamide to occur more readily. This causes the symmetric TTz with more electron-withdrawing substituents to typically be the major product in most asymmetric TTz syntheses. Another promotor of the formation of TTz compounds with electron withdrawing groups is that they generally have lower solubility in DMF or related synthetic solvents than TTz compounds that are functionalized with electron donating groups. This causes the TTz compound to precipitate out of solution, pushing the reaction to further completion.[3]

1.2. Solvatofluorochromism of Asymmetric TTz Derivatives

Solvatofluorochromism is defined as a change in the emission wavelength of fluorescence when a compound is dissolved in solvents with significantly different polarities. This effect is seen when the excited state dipole moment of a compound is strong enough that the shift in polarity in the molecule causes polar solvents to rearrange to stabilize the excited state. Because the speed of fluorescence is relatively much faster than the solvent relaxation, when the solvent relaxes after stabilizing the excited state, it lowers the energy of the excited state (**Figure 3**). The more polar the solvent, the more apparent this energy change, which causes the energy gap between the ground state and excited state to decrease, causing a red-shift in the emission wavelength of fluorescence.[5]

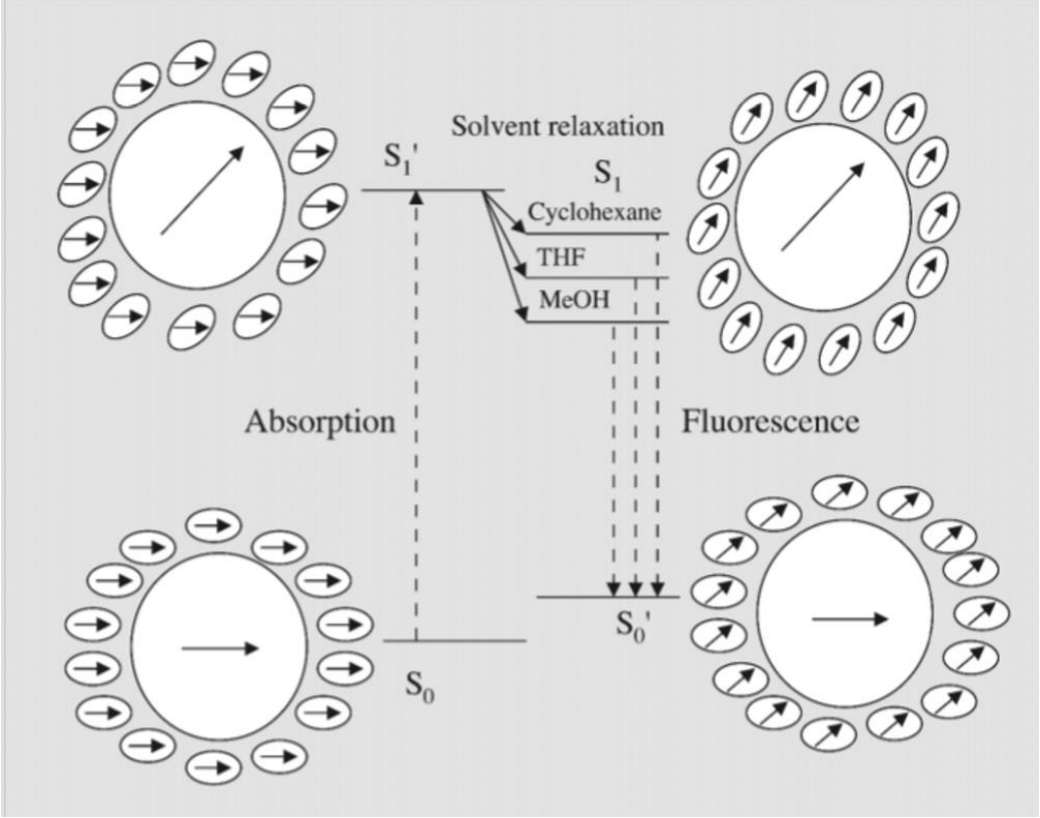

Figure 3: Simplified Jablonski scheme of the solvent relaxation effect that causes solvatofluorochromism in compounds with strong excited-state dipole moments.[14]

Whenever a TTz is functionalized with an electron donating group and an electron withdrawing group *para* to one another, this class of compounds consistently shows strong solvatofluorochromism. The rigid and planar nature of this heterocyclic compound, along with its relatively electron-deficient nature, facilitates intramolecular charge transfer from one part of the molecular to another.[3] This significant shift in electron density upon excitation is referred to as a "push-pull" system. This push-pull system refers to the arrangement of electron density in the molecule while in the ground state, compared to the excited state. In asymmetrically substituted TTz systems, the electron density shifts significantly to the electron accepting side of the molecule upon

photoexcitation. This shift in electron density is what causes the strong excited-state dipole moment, which subsequently causes the solvatofluorochromic effect.[6-8]

1.3. Using Asymmetric TTz Derivatives as a Fluorescent Biomarker

The purpose of the research described in this thesis was to develop a new TTz derivative to be used as a fluorophore specifically to study the antibacterial target enzyme UppS. UppS is a *cis*-prenyltransferase that is ubiquitous among most species of bacteria that is critical to the formation of surface polysaccharides that act as a chemical defense for bacteria. This enzyme catalyzes the carbon chain elongation of FPP by adding eight IPP molecules to the diphosphate end of the substrate. FPP consists of three *trans* IPP units, and each IPP unit added by UppS is a *cis* IPP unit, resulting in a 3E, 8Z compound called bactoprenyl diphosphate, or BPP. This reaction scheme for this process can be seen in **Figure 4**. This compound is also called undecaprenyl pyrophosphate, as it is comprised of eleven repeating units of IPP.[9-10] This compound has been associated not only with surface polysaccharide synthesis, but also for the synthesis of peptidoglycans that form the bacterial cell wall. Using a shorter isoprenoid, GPP, a fluorescent molecule can be tagged to mimic the size of the natural substrate, FPP, that can react with UPPS *in vitro* to form a fluorescent analog of BPP.[11-13] This fluorescence can be monitored with HPLC and a fluorescence detector to see different sized isoprenoids being synthesized by UPPS. Surfactants are necessary to aid in the dissociation of the substrate from UPPS *in vitro*, and the size of the surfactant used influences the size of the isoprenoid that is formed as a result.[14]

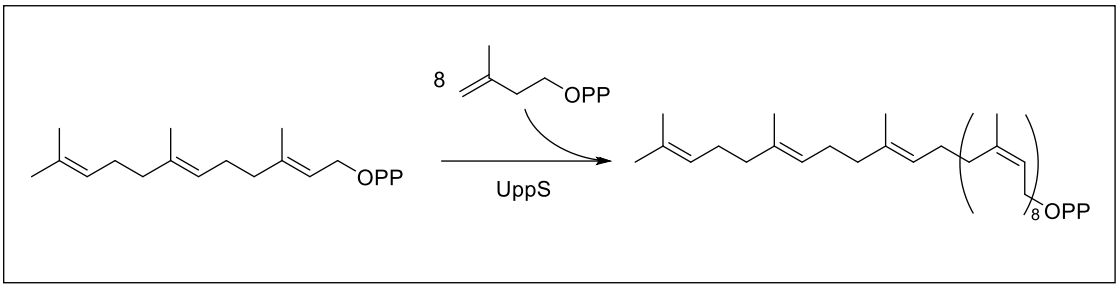

Figure 4: A simplified scheme of the natural production of BPP *in vivo.* This reaction is eight subsequent reactions that add one IPP to the phosphate end of the molecule with each reaction. The reaction continues until eight IPP units have been added, forming BPP. In this scheme, "PP" is meant to represent a diphosphate group.

Antibiotic resistance is a growing concern in the world, with over twenty bacterial and fungal species listed on the Center of Disease Control and Prevention's list of antibiotic-resistant threats as of 2019, and five of those organisms being listed as "urgent threats" (**Figure 5**).[15] Because of the increasing resistance to current antibiotic medicines, new medications must be developed, and research into new antibacterial target enzymes, such as UppS, is critical. Many current antibiotic medications, such as amoxicillin, are antibiotics that contain a beta-lactam ring within their structure that inhibit cell wall biosynthesis by competitively binding to transpeptidases within the bacteria, which inhibits the synthesis of peptidoglycan within the cell wall.[16] With the recent pandemic, it is apparent that diseases caused by microorganisms can be detrimental to society, and new antibiotic medicines will be an essential development to mitigate bacterial and fungal outbreaks in the future. Before new medications can be developed, the scientific community must first learn more about the mechanisms that these microorganisms use to protect themselves from the human immune system.[17]

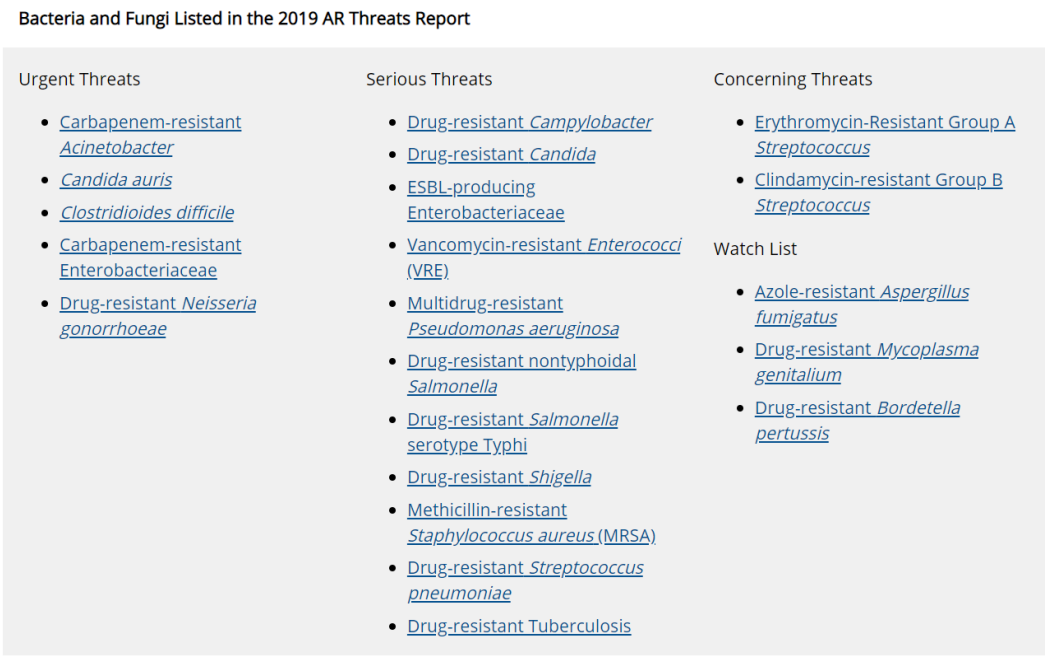

Figure 5: List of organisms that are showing a concerning level of antibiotic resistance, reported by the Center of Disease Control and Prevention in 2019.

Several other compounds have been utilized as fluorophores to study this enzyme, but none display the degree of solvatofluorochromism that asymmetric TTz compounds have shown. This solvatofluorochromic effect that is displayed by this class of compounds makes them very interesting for many molecular sensing applications, as the shift in fluorescence is very sensitive to the chemical environment of the TTz molecule. While UppS is ubiquitous among most bacterial species, there are species-specific differences in some of the amino acids that are present within the binding domain of UppS. This means that for any given species of bacteria, certain fluorophores may not be as effective, as the intermolecular interactions between the fluorophore and the UppS binding domain are unique for that species of bacteria.[18] For example, a fluorophore with hydroxyl or amino functionalities might bind more tightly with a binding domain that contains amino acid residues that can hydrogen bond, such as tyrosine. Having a probe that could also

function as a molecular sensor for the chemical environment of the binding site could be beneficial for understanding the species-specific chemical environments and the effect they have on the effectiveness of different fluorophores.

The way these fluorophores have been used to probe UppS is by mimicking the natural substrate, farnesyl diphosphate (FPP), by attaching a fluorescent molecule to a shorter isoprenoid, such as a geranyl acetate derivative.[19] Once the fluorophore is bound to the isoprenoid, the acetate is deprotected, brominated, and then phosphorylated to form the FPP analog.[18-20] The fluorescent FPP analog can then be elongated by UppS *in vitro* to form a fluorescent BPP analog (**Figure 6**). Fluorescence can be monitored during this process to detect the degree of chain elongation, since IPP is added one unit at a time.

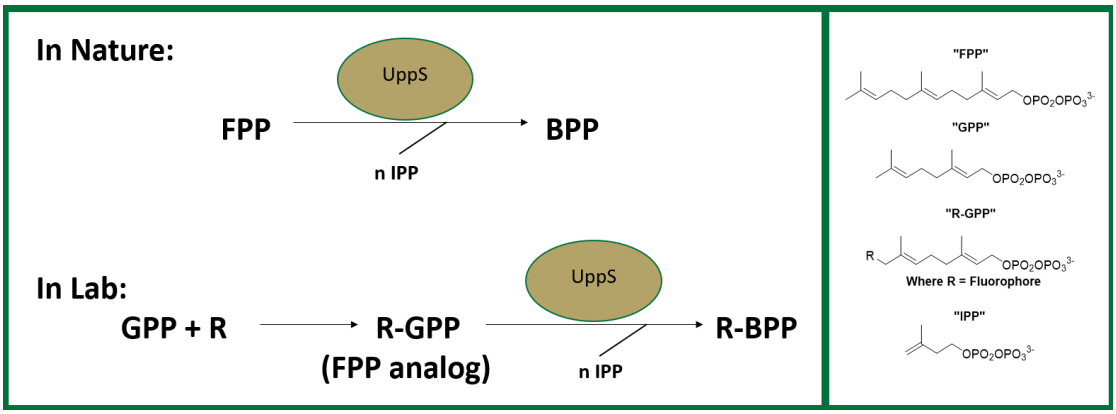

Figure 6: A simplified diagram of the natural production of BPP *in vivo* versus the production of fluorescent BPP analogs *in vitro*. In this figure, "R" represents any fluorophore that can be tagged to GPP to form a fluorescent FPP analog.

For this purpose, a specific compound has been the synthetic target of this research: 2-(4-aminophenyl)-5-(4-nitrophenyl)thiazolo[5,4-d]thiazole, ($PhNO_2$)TTz($PhNH_2$). It has a similar functionality to a fluorophore that has been used for this application, 4-nitroaniline, and was also expected to have solvatofluorochromic properties, since it is a

TTz that is functionalized with a strong electron withdrawing group in the *para* position relative to a strong electron donating group. The DFT calculations using Spartan also seemed to suggest that there was a very strong push-pull electronic interaction upon excitation of this molecule, which is what is thought to cause the solvatofluorochromic effect in this class of compounds.[21] Because of the functional groups present and the presence of a push-pull system (**Figure 7**), ($PhNO_2$)TTz($PhNH_2$) was chosen as the synthetic target to be used for the UppS project.

Several synthetic pathways to arrive at this target compound will be discussed in detail. Synthesizing ($PhNO_2$)TTz($PhNH_2$) proved to be more challenging than anticipated, and so much of the work in this thesis was developing an efficient means of synthesizing and purifying this compound.

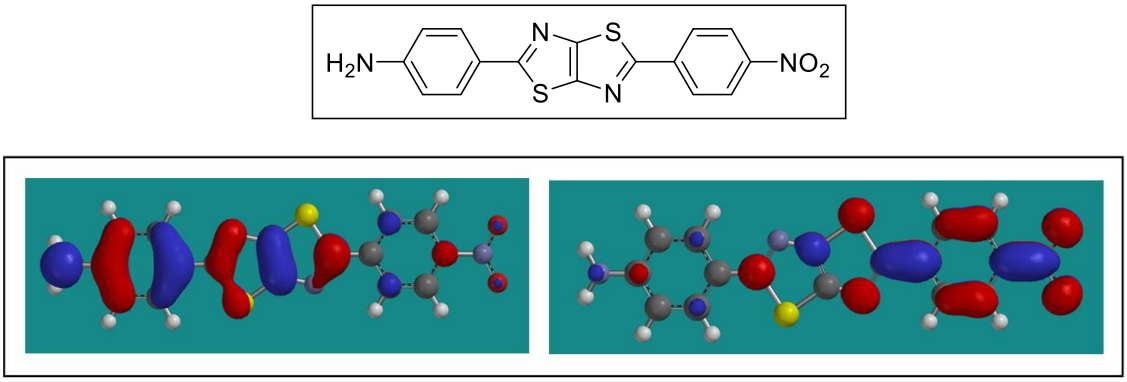

Figure 7: Spartan DFT calculations of the HOMO (left) and LUMO (right) of ($PhNO_2$)TTz($PhNH_2$). The electron density of the molecule significantly shifts to the electron accepting nitro group when excited into the LUMO. This illustrates the push-pull effect that causes solvatofluorochromism in this class of compounds.

Several fluorophores with similar functionality to ($PhNO_2$)TTz($PhNH_2$) have been utilized in past studies for studying this enzyme (**Figure 8**), but a TTz-derived fluorophore would not only function as a fluorophore, but as a sensor for the molecular

environment of the binding site. Since the solvatofluorochromism is so sensitive to its chemical environment, it is hypothesized that the fluorescence of these compounds would shift according to the chemical environment of the binding site of UppS as well. This information could be useful for understanding how the intermolecular interactions within the binding site could affect the efficacy of a certain fluorescent-labeled FPP analog, and therefore the function of that fluorophore within UppS. Since it is known that the binding site can vary from species to species within bacteria, which affects the efficacy of the fluorophore, having a sensor to probe these enzymes could provide useful insight on the chemical environment of specific binding domains.

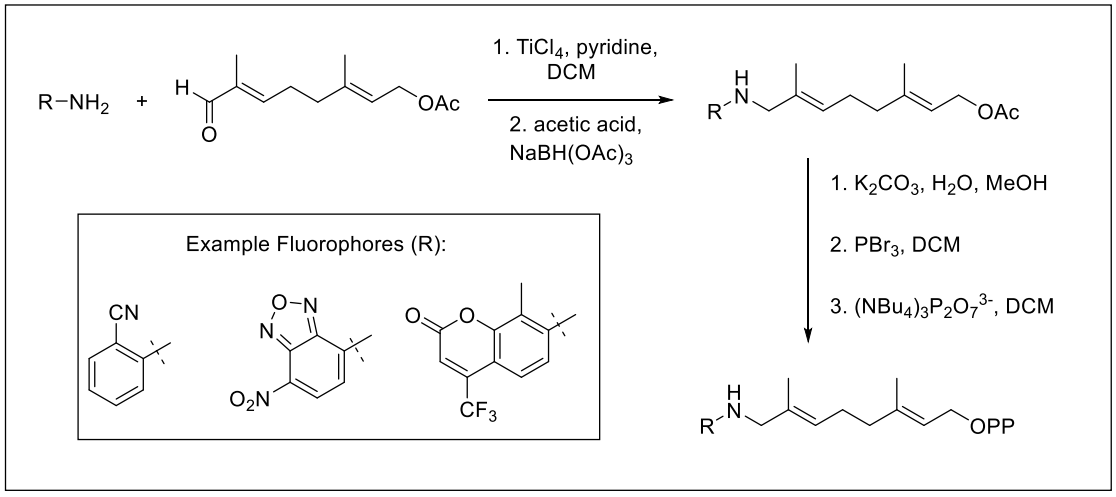

Figure 8: Reaction scheme of the preparation of fluorophores that have been reported in the literature as being useful for probing UppS in past studies.[18]

1.4. Other Potential Applications of Asymmetric TTz Derivatives

While the focus of this project was to design a TTz fluorophore for studying UppS, the electronic properties of this class of compounds makes them an attractive choice for several other applications like electrochromic applications, light-harvesting for solar

cells, and other molecular sensing applications.[3, 22-35] The push-pull electronic system that is present in asymmetrically substituted TTz's make them particularly sensitive to their chemical environments, and they have been used as sensitizers for solar cells, they have been integrated into polymers as high-hole mobility FET materials, and have also been used in biological applications as non-toxic biological stains.[23-32]

The electronic properties of the TTz core make it a very attractive material as a semiconductor for use in solar cell polymers as well. The electron-deficient nature of thiazolo[5,4-d]thiazole derivatives makes them more able to facilitate intermolecular charge transfers, making them useful for these kinds of applications.[22-23] Not only can they be used within the polymers, but they are also used as light-harvesting chromophores for photovoltaic applications as well.[24-26] As for biological applications, this class of compounds has been used for live cell imaging, and could also be useful for monitoring voltages in neurons due to their interesting electronic properties. Some TTz's are known to be voltage-sensitive and electrochromic, and fluorescence can be affected by applying a current to these compounds. [3, 27-29, 33]

Because of the semiconducting properties of TTz-based compounds, they are very attractive compounds for use in organic field-effect transistors (OFETs) as well, particularly for the use of being density of charge carriers. They can function as either electron-transport materials or hole-transport materials. This is because heteroaromatic compounds tend to form π-π stacking interactions with a large surface area, which is very favorable for this type of application as OFETs. Also, the electron-deficient nature of the TTz makes it an attractive choice for increasing the ionization potential of these devices as well, which would make them more stable.[30-32]

CHAPTER 2: MATERIALS AND METHODS

2.1. Materials and Instrumentation

Outlined here are all the materials and reagents that were utilized throughout this thesis study. The following reagents were used in syntheses and were obtained through Sigma-Aldrich without further purification: dithiooxamide, 4-formylbenzoic acid, 4-nitrobenzaldehyde, 4-acetamidobenzaldehyde, hydroxylamine hydrochloride, polyphosphoric acid, sulfuric acid, hydrochloric acid, sodium hydroxide, potassium hydroxide, tin (II) chloride, D-glucose monohydrate, and all organic solvents that were used in syntheses or for spectroscopic measurements. Reactions were completed using VWR stir bars and heating stir plates, while reaction vessels and setups consisted mainly of round bottom flasks and reflux condensers along with heating oil baths.

^{1}H NMR measurements were obtained using either a JEOL 300 MHz NMR or a JEOL 500 MHz NMR, using chloroform-*d*, *d*6-dimethyl sulfoxide, and acetonitrile-*d*3 as solvents. Mass spectra were obtained using a MALDI-TOF mass spectrometer. UV-Vis measurements were taken in solution using a Cary 300 UV-Vis spectrophotometer. Fluorescence spectra were taken using a Shimadzu RF-5301PC spectrofluorophotometer. Time-resolved fluorescence lifetime measurements were collected using a Jobin Yvon-Spex Fluorolog while using a 398 nm diode laser. Density functional theory calculations were performed with Spartan computational software, using B3LYP as the density functional setting and 6-31G* as the basis set.

2.2. Synthesis of Symmetrically Substituted TTz Compounds

For the synthesis of the symmetrically substituted TTz compounds, one equivalence of dithiooxamide was reacted with two equivalences of the corresponding aryl aldehyde (**Figure 9**). This scale is usually done using 0.250 g of dithiooxamide and 25 mL of DMF. For the compounds *bis*-2,5-(4-nitrophenyl)thiazolo[5,4-d] thiazole ($(PhNO_2)_2TTz$) and *bis*-2,5-(4-carboxyphenyl)thiazolo[5,4-d] thiazole ($(PhCOOH)_2TTz$), dithiooxamide was reacted with two equivalences of 4-nitrobenzaldehyde (0.629 g) and 4-formylbenzoic acid (0.625 g), respectively. These reactions were heated at 140 °C for 6 h in a round bottom flask using DMF as a solvent.[1,2] Better results were observed as the concentration of this reaction mixture increases, as the resulting symmetrically substituted TTz compounds generally have poor solubility in DMF and precipitate out. Once the reaction was completed, the solutions were allowed to cool and placed in a refrigerator to facilitate precipitation of the product. This mixture was then vacuum-filtered and the precipitate was washed with DI water and isopropyl alcohol to remove starting material. The resulting solid was then collected and vacuum dried overnight to remove residual organic solvents. This reaction produces: ($(PhNO_2)_2TTz$), a reddish-brown solid in approximately 40-50% yield or ($(PhCOOH)_2TTz$), a mustard yellow solid, in approximately 60-70% yield. $(PhCOOH)_2TTz$ was structurally characterized via ^1H NMR (300 MHz, d6-DMSO, δ): 8.06 (d, 2H), 8.13 (d, 2H), and 8.28 (s, 1H) and by MALDI-TOF (m/z: 384.37). $(PhNO_2)_2TTz$ was structurally characterized via ^1H NMR (300 MHz, d6-DMSO, δ): 8.32 (d, 2H), 8.39 (d, 2H) and by MALDI-TOF (m/z: 387.47).

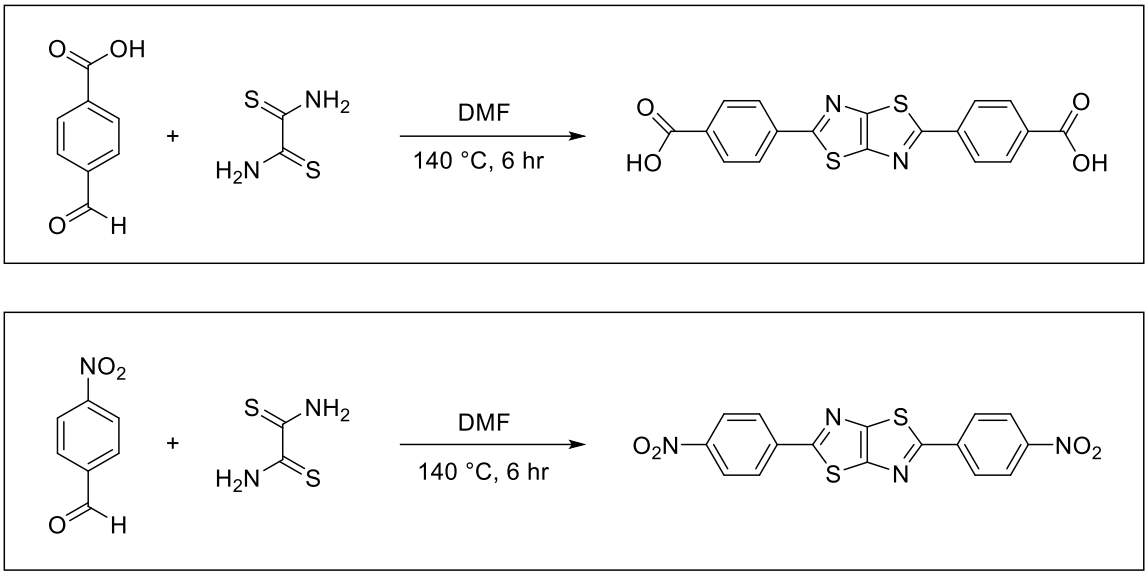

Figure 9: Reaction schemes of the preparation of $(PhCOOH)_2TTz$ (top) and $(PhNO_2)_2TTz$ (bottom). In both reactions, one equivalence of dithiooxamide were reacted with two equivalences of the corresponding aromatic aldehyde.

2.3. Synthesis of 2-(4-nitrophenyl)-5-(4-acetamidophenyl)thiazolo[5,4-d] thiazole

For the synthesis of the 2-(4-nitrophenyl)-5-(4-acetamidophenyl)thiazolo[5,4-d] thiazole, $(PhNO_2)TTz(PhNHAc)$, one equivalence of dithiooxamide (0.250 g) was reacted with one equivalence of 4-nitrobenzaldehyde (0.314 g) and one equivalence of 4-acetamidobenzaldehyde (0.339 g) (**Figure 10**). This reaction was heated at 140 °C for 6 h in a round bottom flask using DMF (25 mL) as a solvent.[1,2] Better results were observed as the concentration of this reaction mixture increases, as the resulting symmetrically substituted TTz compounds generally have poor solubility in DMF and precipitate out. Once the reaction was completed, the solutions were allowed to cool and placed in a refrigerator to facilitate precipitation of the product. This mixture was then vacuum-filtered, and the precipitate was washed with DI water to remove starting material. The resulting solid was then collected, and vacuum dried overnight to remove residual organic solvents.

This mixture was then separated via column chromatography by dry loading 50 mg of the mixture onto 1.00 g of silica gel using acetone. This silica was then dry loaded onto a 1-inch diameter, ~5-inch-tall silica gel column and separated using 1:1 chloroform:ethyl acetate as an eluent. $(PhNO_2)_2TTz$ will elute first from the column, and is a band with a characteristic green fluorescence. The next compound to elute is $(PhNO_2)TTz(PhNHAc)$, which has a characteristically yellow-orange fluorescence. This separation of the loaded 50 mg of the crude mixture yields $(PhNO_2)TTz(PhNHAc)$, a bright orange solid, in approximately 40-50% yield. $(PhNO_2)TTz(PhNHAc)$ was structurally characterized via ^1H NMR (300 MHz, d6-DMSO, δ): 2.06 (s, 3H), 7.74 (d, 2H), 7.97 (d, 2H), 8.25 (d, 2H), 8.34 (d, 2H), 10.25 (s, 1H) and by MALDI-TOF (m/z: 397.60).

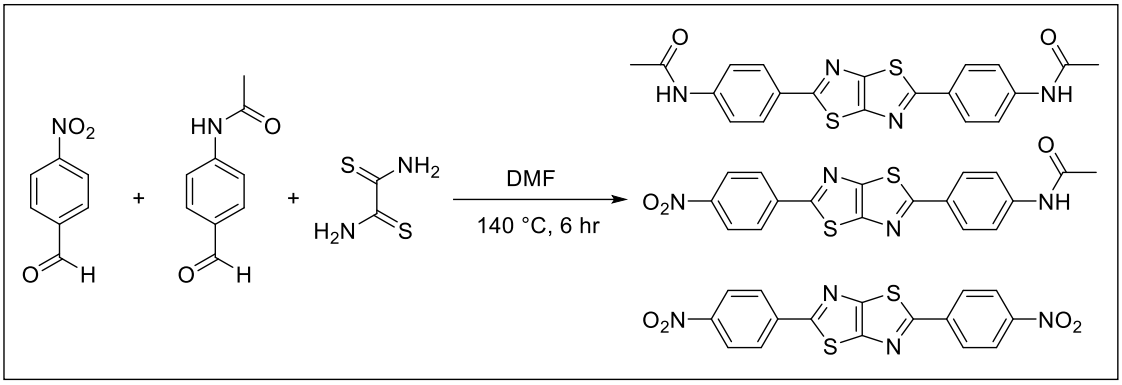

Figure 10: Reaction scheme of the synthesis of $(PhNO_2)TTz(PhNHAc)$. A mixed pot synthesis reaction that produces three separate TTz compounds as a result. Crude product was purified using the chromatography technique described in 2.3.

2.4. Deprotection of 2-(4-nitrophenyl)-5-(4-acetamidophenyl)thiazolo[5,4-d] thiazole

To 50 mL of a 5:1 mixture of concentrated hydrochloric acid and n-butanol, 50 mg of $(PhNO_2)TTz(PhNHAc)$ was added and heated at 100 °C for 48 h (**Figure 11**). This solution was neutralized using sodium hydroxide and heated for 2 h. After neutralization, the mixture was cooled and vacuum filtered. The precipitate was then washed with water

and hexanes to remove any residual solvents. The precipitate was then collected and dried in a vacuum oven overnight. This reaction yields 2-(4-nitrophenyl)-5-(4-aminophenyl)[5,4-d]thiazolo thiazole ($PhNO_2$)TTz($PhNH_2$), a dark red solid, in approximately 85% yield. ($PhNO_2$)TTz($PhNH_2$) was structurally characterized via ^1H NMR (300 MHz, d6-DMSO, δ): 5.99 (s, 2H), 6.63 (d, 2H), 7.69 (d, 2H), 8.21 (d, 2H), 8.33 (d, 2H) and by MALDI-TOF (m/z: 354.54).

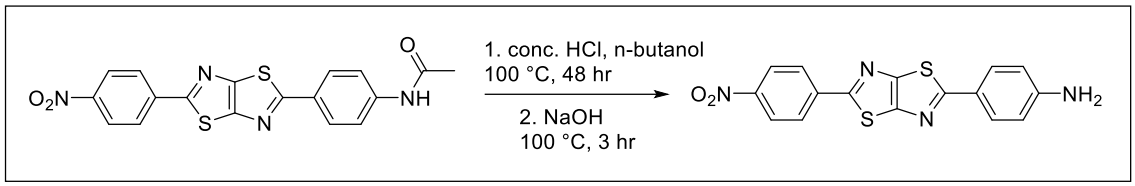

Figure 11: Reaction scheme of the acid-catalyzed deprotection of ($PhNO_2$)TTz(PhNHAc) to form the target compound ($PhNO_2$)TTz($PhNH_2$).

2.5. Single Amination of *bis*-2,5-(4-carboxyphenyl)thiazolo[5,4-d] thiazole

0.050 g (1 eq) of $(PhCOOH)_2$TTz and 0.009 g (1 eq.) of hydroxylamine hydrochloride was added to 20 mL of polyphosphoric acid and slowly heated to 160 °C over 3 h (**Figure 12**). After the 3 h, 10 mL of 10% (v/v) of sulfuric acid was added to the mixture and refluxed for 24 h. Once this reaction was complete, the mixture was neutralized and extracted with ethyl acetate. This reaction only yielded pure material with obvious solvatofluorochromism once, but in poor yield (< 5%). These results were not reproducible. Other purification methods need to be developed for this separation.

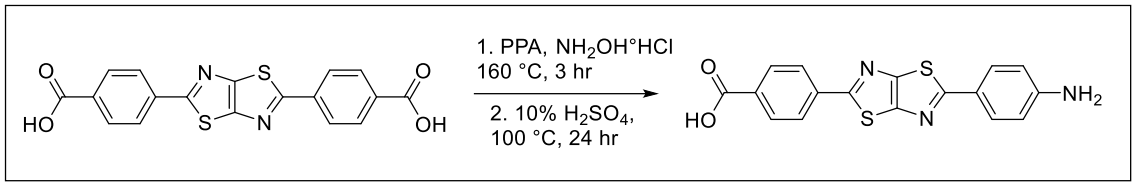

Figure 12: Scheme of the proposed reaction for forming ($PhNH_2$)TTz(PhCOOH) via a Lossen rearrangement mechanism.

2.6. Reduction of *bis*-2,5-(4-nitrophenyl)thiazolo[5,4-d]thiazole

0.050 g (1 eq.) of ($PhNO_2$)$_2$TTz) and 0.246 g (10 eq.) of tin (II) chloride were added to 20 mL of a 5:1 mixture of hydrochloric acid and n-butanol (**Figure 13**). This reaction was heated to 100 °C for 24 h. This solution was then made slightly basic by adding sodium hydroxide and heated at 100 °C for 2 h. Once this neutralization step was completed, this mixture was vacuum filtered and washed with DI water and hexanes to remove any excess solvent. The solid was then collected and dried in a vacuum oven overnight. The reaction produced ($PhNH_2$)$_2$TTz, a bright yellow solid in approximately 80% yield. ($PhNH_2$)$_2$TTz was structurally characterized via ^{1}H NMR (300 MHz, ACN-d3, δ): 4.62 (s, 2H), 6.69 (d, 2H), 7.70 (d, 2H) and by MALDI-TOF (m/z: 324.56).

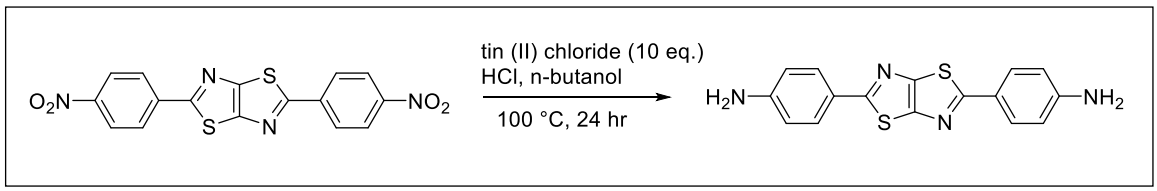

Figure 13: Reaction scheme of the reduction of ($PhNO_2$)$_2$TTz) in the presence of excess tin (II) chloride in concentrated hydrochloric acid and n-butanol to form ($PhNH_2$)$_2$TTz.

2.7. Single Reduction of *bis*-2,5-(4-nitrophenyl)thiazolo[5,4-d] thiazole

0.050 g (1 eq.) of ($PhNO_2$)$_2$TTz was reacted with 0.129 g (5 eq.) of D-glucose monohydrate in a mixture of 15 mL of 2M potassium hydroxide and 5 mL of n-butanol

(**Figure 14**). This reaction was refluxed at 110 °C for 48 h. Once the reaction was complete, the vessel was allowed to cool to room temperature before neutralizing with hydrochloric acid and extracting with ethyl acetate. This reaction was tried several times using several different organic solvents to increase solubility, but no product was isolated using this method.

Another method was attempted when trying to singly reduce this compound. This method involved using tin (II) chloride like in the previous reaction, but by using 1 equivalence instead of 10. Those reaction conditions are as follows: 0.053 g of $(PhNO_2)_2TTz$ (1 eq) was reacted with 0.025 g of tin (II) chloride in 20 mL of a 5:1 mixture of concentrated hydrochloric acid and n-butanol, respectively (**Figure 14**). This reaction was heated at 100 °C for 48 h and then neutralized and heated at 100 °C for another 3 h. This reaction produced $(PhNH_2)_2TTz$ in ~35% yield, but no asymmetric product formation was observed. There was also unreacted $(PhNO_2)_2TTz$ left over from the reaction.

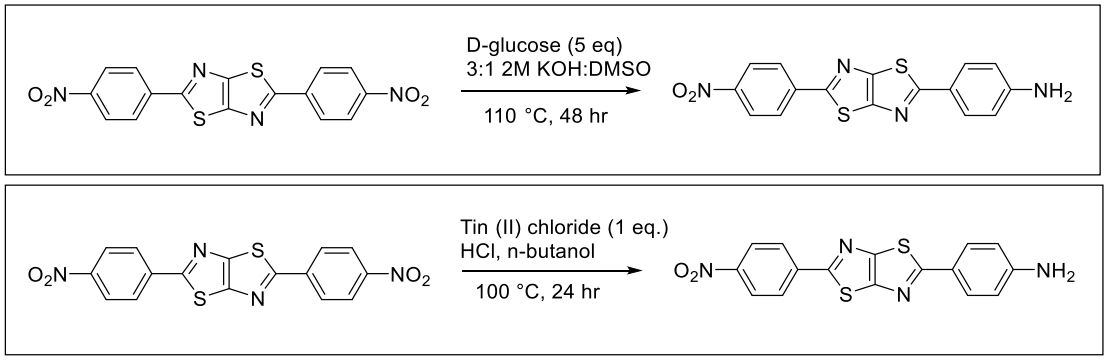

Figure 14: Reaction schemes of the attempted reactions to selectively reduce one nitro group in $(PhNO_2)_2TTz$ to produce $(PhNO_2)TTz(PhNH_2)$. The glucose reduction method (top) showed no signs of reactivity, while the known tin (II) chloride method (bottom) showed little to no formation of the desired product.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Choosing an Asymmetric TTz Synthetic Pathway

When deciding on how to synthesize the desired asymmetric push-pull TTz compounds, there were several factors to consider. From the very first reaction, it had to be decided if the synthetic pathway would begin with a symmetric or an asymmetric TTz synthesis, each of which having their own unique tradeoffs. Symmetric TTz's are relatively simple to make and generally have higher yields than asymmetric TTz's, since they produce only one TTz derivative, rather than three. However, post-synthetic modifications to these TTz compounds can be difficult due to their generally poor solubility. The general reaction schemes for a symmetric TTz synthesis and an asymmetric synthesis are illustrated in **Figure 15**.

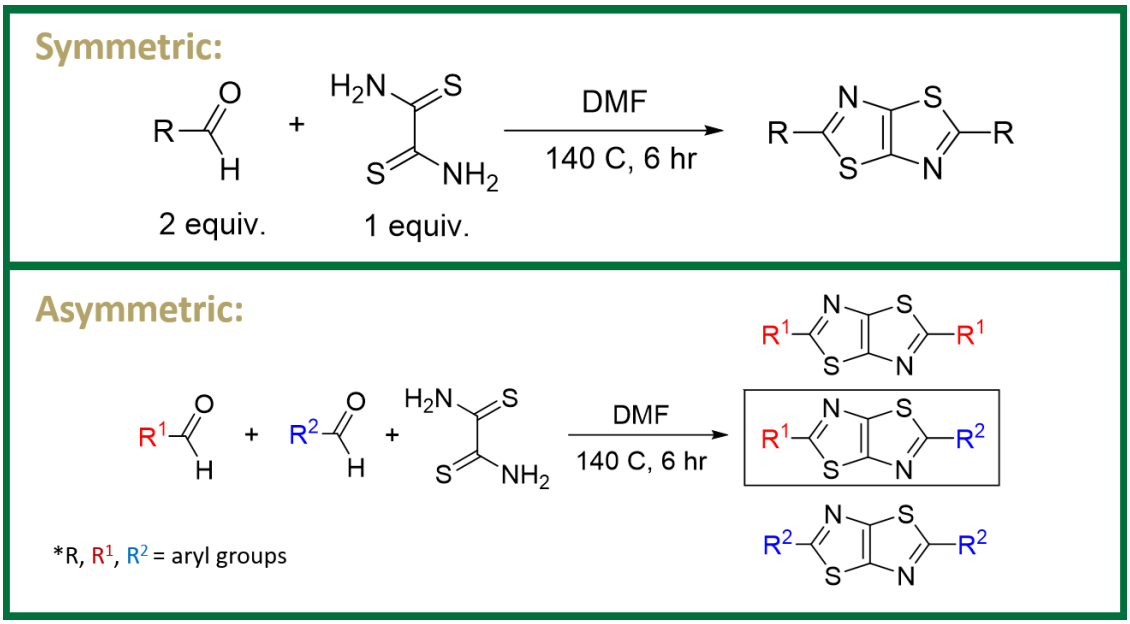

Figure 15: Side-by-side comparison of the symmetric TTz synthesis versus the asymmetric TTz synthesis. Symmetric syntheses typically have better yields, but are generally more difficult to modify once synthesized.

At the beginning of this study, the post-synthetic method for introducing aminophenyl functionalities to a TTz were not known. As such, the rational starting point was to use a symmetric TTz synthesis so that there would be more starting material to further develop new asymmetric pathways. The rationale was that if there was more starting material, then yields of the final product would theoretically be higher. However, modifying these starting materials was proved to be more challenging than anticipated, and yields were unexpectedly low, as described in the following sections.

3.2. Lossen Rearrangement Pathway

The first synthetic pathway that was investigated to develop a push-pull system containing an aminophenyl substituent was by using a Lossen rearrangement reaction to attempt to convert on of the carboxyl groups in $(PhCOOH)_2TTz$ into an amino group

(**Figure 16**). This partial Lossen rearrangement has been successful in the past with porphyrin compounds, so this was a natural starting point using $(PhCOOH)_2TTz$ as the starting material.[36] Synthesizing asymmetrically substituted TTz compounds with push-pull systems using the one-pot TTz synthesis has proven to have poor yields and so alternative synthetic pathways needed to be investigated to produce push-pull systems in greater yields.

The synthesis of $(PhCOOH)_2TTz$ is done by reacting one equivalence of dithiooxamide with two equivalences of 4-formylbenzoic acid in DMF. This reaction forms the bicyclic TTz core via a double condensation reaction. $(PhCOOH)_2TTz$ is quite insoluble in DMF and most other synthetic solvents, and readily precipitates out of solution upon formation. This reaction also has relatively high yields (~65%) due to the electron withdrawing carboxyl group on 4-formylbenzoic acid activating the aldehyde as an electrophile. This is also why electron donating groups in the para position of the aldehyde are thought to inhibit the formation of the TTz core, as they tend to deactivate the aldehydes as electrophiles. It is for this reason that $(PhCOOH)_2TTz$ was chosen as the starting point for this pathway, since it was a symmetrically substituted TTz that was substituted with electron withdrawing groups. This compound was able to be made in gram amounts, at relatively high yields (~65%), so a large amount of starting material could be made in one reaction.

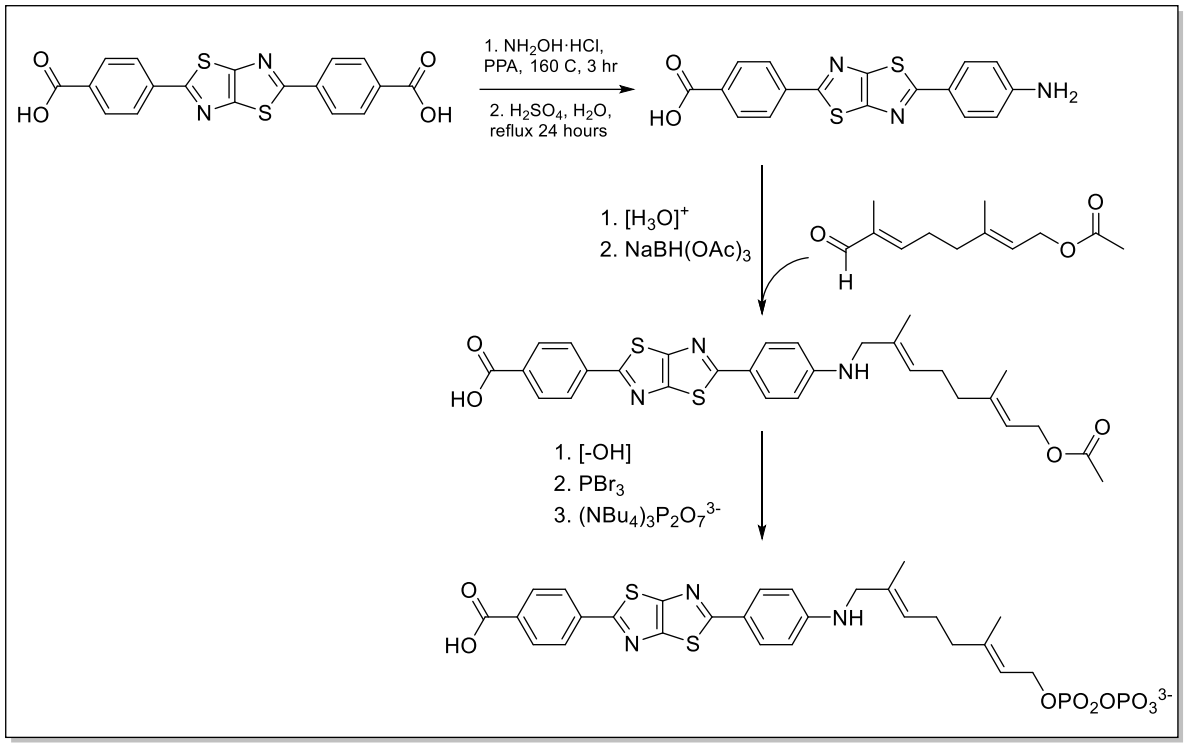

Figure 16: Scheme of the synthetic pathway by which $(PhCOOH)_2TTz$ was to be aminated via a Lossen rearrangement mechanism. From there, it would be reacted with the selected geranyl acetate to be tagged to it, and further modified to be phosphorylated before being used to probe UppS.

However, modifying $(PhCOOH)_2TTz$ proved to be more difficult than anticipated. It has characteristically poor solubility in most organic solvents and seemed to be unresponsive to the Lossen rearrangement reaction. Spectroscopic evidence of a strongly solvatofluorochromic compound was created during this reaction, suggesting that an asymmetrically substituted carboxy/aminophenyl TTz was produced. This solvatofluorochromism was observed and noted (**Figure 17**), but these results were ultimately not reproducible, and alternative synthetic pathways were pursued. The solvatofluorochromism that was present was very strong and not at all characteristic of neither $(PhCOOH)_2TTz$ nor $(PhNH_2)_2TTz$, which both fluoresce blue independently of the solvent they are dissolved in. This strong solvatofluorochromism was indication that

this reaction did work and did in fact form the desired product that had the predicted electronic properties. There is evidence that this compound was synthesized, however, difficulties isolating and characterizing the pure form of this compound led us to investigate a new asymmetric TTz synthetic pathway. While this method of synthesis is still potentially feasible, it is likely that a more elaborate purification technique is required.

Figure 17: Strong solvatofluorochromism observed in TTz compound ($PhNH_2$)TTz(PhCOOH) when excited by a 405 nm black light. Sample was dissolved in cyclohexane (left), chloroform (middle), and isopropanol (right). More polar solvents tend to red-shift the emission wavelength in this class of compounds.

3.3. Reduction of $(PhNO_2)_2TTz$ Pathway

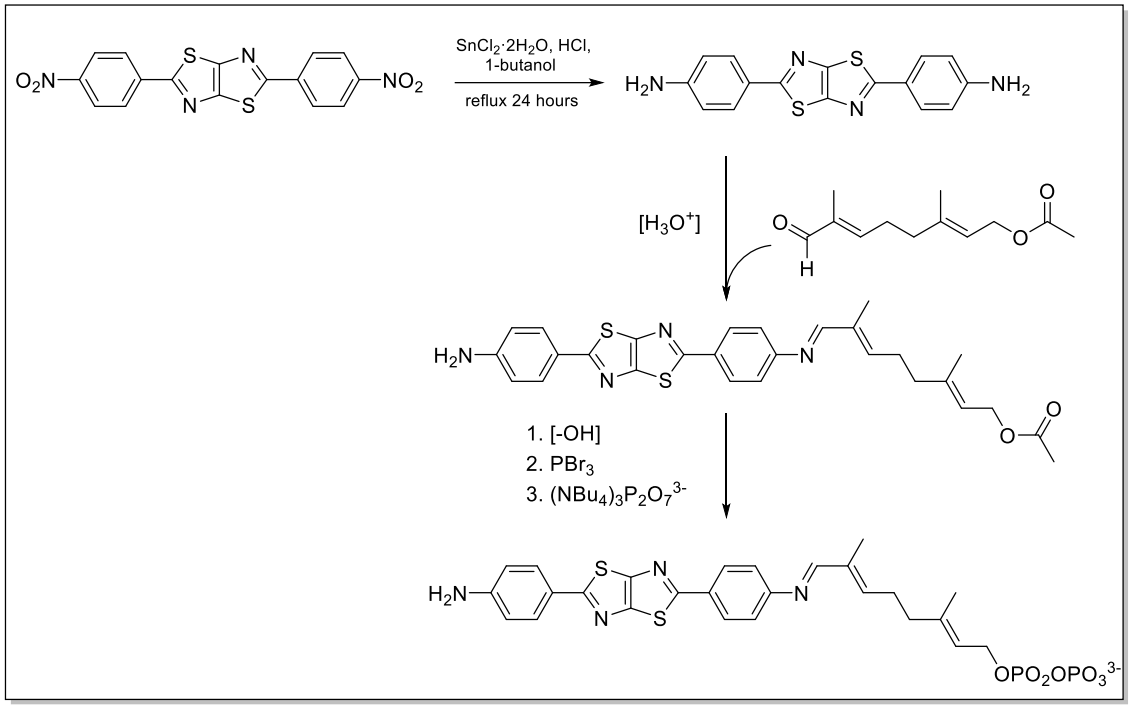

Figure 18: Scheme of the synthetic pathway by which $(PhNO_2)_2TTz$ was reduced by a reaction with tin (II) chloride and then was to be used to fluorescently label the geranyl acetate derivative shown.

Like the previous synthetic pathway, this pathway begins with the synthesis of a TTz compound that is symmetrically substituted with electron withdrawing groups, but in this case, those withdrawing groups were nitro groups rather than carboxyl groups. $(PhNO_2)_2TTz$ was synthesized using the same reaction conditions, but by using 4-nitrobenzaldehyde instead of 4-formylbenzoic acid. Similarly, this reaction formed $(PhNO_2)_2TTz$ in relatively high yields (~50%) due to the strongly electron-withdrawing nitro groups. $(PhNO_2)_2TTz$ also showed poor solubility in most organic solvents but was more soluble in chlorinated solvents than the $(PhCOOH)_2TTz$ derivative. Using $(PhNO_2)_2TTz$ as a starting point, the compound was reduced using tin (II) chloride in 10:1 molar excess in hydrochloric acid and n-butanol to form $(PhNH_2)_2TTz$, a

symmetrically substituted TTz compound with electron-donating groups rather than electron withdrawing groups in ~80% yield. This method was being investigated to introduce amine groups as a strong electron donating group to this class of compounds. (**Figure 18**)

Once $(PhNH_2)_2TTz$ was synthesized and characterized, it was tested using geranyl acetate to fluorescently label this isoprenoid (**Figure 19**). $(PhNH_2)_2TTz$ and 1-formyl geranyl acetate were reacted in 1:1 molar equivalences in DCM in the presence of catalytic acetic acid to form an imine bridge between the two compounds. This reaction was done on a very small scale to get qualitative data about the nucleophilicity of amine-functionalized TTz systems.

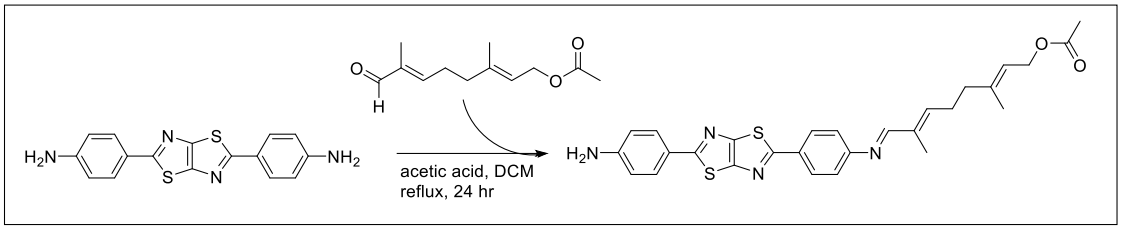

Figure 19: Scheme of the reaction between $(PhNH_2)_2TTz$ and 1-formyl geranyl acetate, the selected geranyl acetate derivative for this functionality.

This reaction yielded a new fluorescent compound that showed properties that were indicative of this imine product being formed: solvatofluorochromism (a sign that there is an asymmetric push-pull system), and a significantly higher Rf when compared to $(PhNH_2)_2TTz$ on TLC using 2:1 ethyl acetate:chloroform as the eluent, suggesting that alkylation was successful. DFT calculations were performed in Spartan to determine if the imine would be a strong enough electron-withdrawing group to cause the push-pull

effect, and the results were consistent with the solvatofluorochromism that was observed from the new product (**Figure 20**).

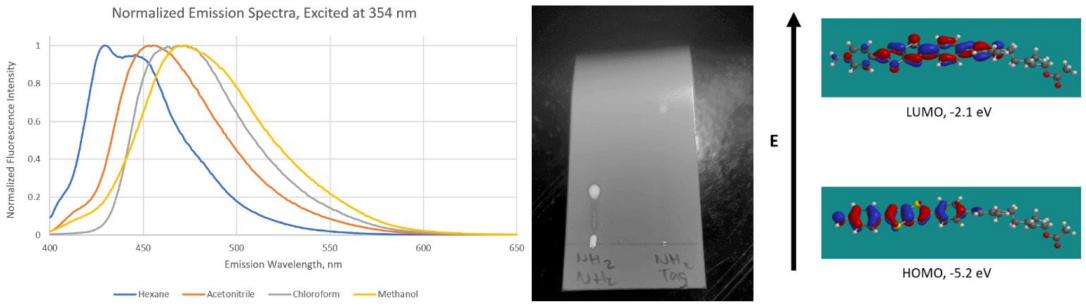

Figure 20: A normalized emission spectra of the fluorescence of the imine TTz compound (left) showing strong positive solvatofluorochromism and TLC (middle) of $(PhNH_2)_2TTz$, labeled "NH_2 NH_2" compared to the imine, labeled "NH_2 Tag". This was evidence that suggested that the alkylation of $(PhNH_2)_2TTz$ was successful. The DFT calculation showing the push-pull effect is shown on the right, and is consistent with the solvatofluorochromism that was observed.

Once the amine groups were determined to be nucleophilic, and therefore potentially useful fluorophores for the UppS application, the next step was to attempt to singly reduce $(PhNO_2)_2TTz$ to $(PhNO_2)TTz(PhNH_2)$. This pathway was pursued because of the concern that both amine groups on the symmetric $(PhNH_2)_2TTz$ could be acting as nucleophiles, which would render this compound useless for the UppS application, as it would doubly alkylate the TTz core. For this reason, a way to singly reduce $(PhNO_2)_2TTz$ to $(PhNO_2)TTz(PhNH_2)$ was the main target of the study. This was first attempted by using the same conditions that were used to fully reduce $(PhNO_2)_2TTz$ to $(PhNH_2)_2TTz$, except by using 1:1 molar equivalences of tin (II) chloride and $(PhNO_2)_2TTz$, rather than using tin (II) chloride in excess. This reaction worked, but produced $(PhNH_2)_2TTz$ as the major product, with unreacted $(PhNO_2)_2TTz$ left over. There was also no observable spectroscopic solvatofluorochromism, strongly indicating that these conditions were not favorable for producing the desired compound, $(PhNO_2)TTz(PhNH_2)$.

Additional partial reduction methods were also investigated, including a reaction developed by Manoranian et al.[37] The reaction uses D-glucose under basic conditions and a polar organic solvent to selectively reduce one nitro group in dinitro aromatic compounds. Glucose serves as a hydrogen source as it is broken down into other compounds under hot basic conditions. This mild reduction had relatively good yields with similar compounds, so this method was used to try to produce the asymmetric TTz, ($PhNO_2$)TTz($PhNH_2$). This reaction was attempted using DMSO and other polar organic solvents, however, no aminophenyl-containing TTz compounds were isolated. $(PhNO_2)_2$TTz was unreactive using these reaction conditions.

Post-synthetic modifications to produce asymmetrically substituted amino-nitrophenyl TTz's using symmetric nitrophenyl TTz's as starting materials were very challenging. Therefore, the next strategy used was to attempt to synthesize an asymmetric TTz using a mixed-pot reaction and then modifying to form the target ($PhNO_2$)TTz($PhNH_2$) compound (**Figure 21**).

3.4. Deprotection of ($PhNO_2$)TTz(PhNHAc) Pathway

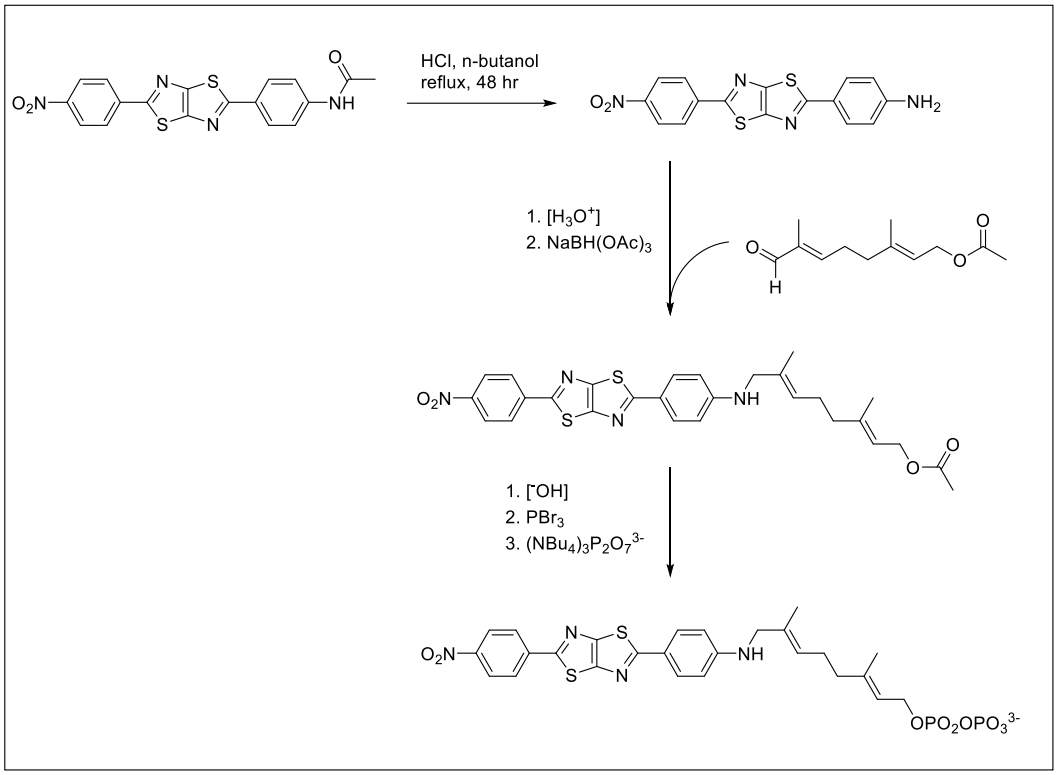

Figure 21: Scheme of the successful synthetic pathway by which ($PhNO_2$)TTz(PhNHAc) was synthesized using the mixed pot TTz reaction and purified via flash column chromatography. From there, the compound was deprotected using an acid-catalyzed deprotection reaction to form ($PhNO_2$)TTz($PhNH_2$).

All the reactions described in the materials and methods section were conducted with the intent of creating new push-pull asymmetric TTz compounds. Amine groups are strongly electron donating but have been difficult to add to these TTz compounds using other methods. The mechanism by which the TTz core is formed involves an oxidation step that is inhibited by strong electron donating groups, so using aryl aldehydes with amine functionalities generally result in very low yields.[12] However, using an acetamide-functionalized aryl aldehyde to form an asymmetric TTz compound with two different electron withdrawing groups in the first synthesis step showed significantly higher yields (~40% observed). Using this method, amine functionalized TTz compounds are an easier

target to achieve than by attempting to create an asymmetrically substituted TTz by using the respective electron withdrawing and donating aryl aldehydes reacting with dithiooxamide. Once the ($PhNO_2$)TTz(PhNHAc) compound was synthesized, it was purified via column chromatography and deprotected using concentrated hydrochloric acid and n-butanol, forming ($PhNO_2$)TTz($PhNH_2$) (**Figure 21**). It is worth noting that during TLC analysis and flash column purification, a negligible amount of the symmetrical phenylacetamide TTz was purified from the mixed aryl aldehyde synthesis.

Having stronger electron donating and withdrawing groups typically results in a stronger push-pull system, so having a more efficient way to attach a strong donating group like an amine is critical for synthesizing new push-pull TTz systems with amine functionalities. This push-pull effect is what causes the solvatofluorochromic effect that is typical of this class of compounds and is also what makes them interesting target compounds for use as molecular sensors. Being able to selectively add a strong donating group to these TTz compounds should allow a new generation of TTz compounds with amine functionality that could have potential use as molecular sensors.

3.5. Photophysical Properties of ($PhNO_2$)TTz($PhNH_2$)

Using a comparative method, quantum yield measurements were collected by plotting absorbance versus integrated fluorescence intensity at five different concentrations with absorbances below 0.1. This same technique was then used on a standard of known quantum yield, and the quantum yield of the sample compound in question was calculated using the following expression: $\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$, where ST and X represent standard and test samples, respectively, Φ is the quantum yield, “Grad” is the

gradient of integrated fluorescence intensity versus absorbance, and η represents the refractive index of the solvent used to dissolve the sample. This was performed in nine solvents for both the ($PhNO_2$)TTz($PhNH_2$) and the final product, ($PhNO_2$)TTz($PhNH_2$) to see how the quantum yield changes depending on the solvent polarity. Optoelectronic properties of the compounds are presented in the following data table (**Table 1**). Much of the photophysical data collected in **Table 1** was collected by two undergraduate researchers in our group, Andrew Brotherton and Ana Ledezma Montoya.

Compound	Solvent	Abs (nm) λ_{max}	Abs (eV) E_{ex}	Ext. Coeff. ε	Em (nm) λ_{max}	Em (ev) E_{em}	Stokes Shift (nm) SS (nm)	Stokes Shift (eV) SS (eV)	Quantum Yield Φ_F	Fluorescence Lifetime (ns) τ_F	Radiative Rate (1/s) k_r	Nonradiative Rate (1/s) k_{nr}
(PhNHAc) TTz ($PhNO_2$)	THF	397	3.12	33900	533	2.33	136	0.80	0.18	1.89	9.47×10^7	4.34×10^8
	Toluene	395	3.14	21000	496	2.50	101	0.64	0.34	0.87	1.38×10^8	1.01×10^7
	Benzene	398	3.12	26000	493	2.52	95	0.60	0.11	1.05	1.04×10^8	8.49×10^8
	Chlorobenzene	402	3.08	7000	524	2.37	122	0.72	0.36	2.32	1.55×10^8	2.76×10^8
	o-Dichlorobenzene	404	3.07	-	544	2.28	140	0.79	0.35	2.42	1.45×10^8	2.69×10^8
	Cyanobenzene	404	3.07	14300	463	2.68	59	0.39	0.05	1.27	3.86×10^7	7.49×10^8
	Chloroform	400	3.10	44500	557	2.23	157	0.87	0.09	1.72	4.94×10^7	5.32×10^8
	Ethanol	395	3.14	-	496	2.50	101	0.64	-	-	-	-
	Acetonitrile	394	3.15	-	508	2.44	114	0.71	-	-	-	-
($PhNH_2$) TTz ($PhNO_2$)	THF	407	3.05	14700	508	2.44	101	0.61	0.01	1.09	1.01×10^7	9.07×10^8
	Toluene	418	2.97	17000	536	2.31	118	0.65	0.34	2.51	1.36×10^8	2.63×10^8
	Benzene	408	3.04	9000	544	2.28	136	0.76	0.03	2.15	1.26×10^7	4.53×10^8
	Chlorobenzene	418	2.97	31000	568	2.18	150	0.78	0.06	1.66	3.60×10^9	5.64×10^8
	o-Dichlorobenzene	422	2.94	-	544	2.28	122	0.66	0.05	1.88	2.70×10^9	5.13×10^8
	Cyanobenzene	412	3.01	9900	509	2.44	97	0.57	0.02	1.07	2.06×10^7	9.14×10^8
	Chloroform	409	3.03	12600	529	2.34	120	0.69	0.02	2.07	7.25×10^6	4.76×10^8
	Ethanol	422	2.94	-	480	2.58	58	0.36	-	-	-	-
	Acetonitrile	416	2.98	-	510	2.43	94	0.55	-	-	-	-

Table 1: Photophysical properties of target compound ($PhNO_2$)TTz($PhNH_2$) and precursor ($PhNO_2$)TTz(PhNHAc) in different solvents.

"Quantum yield" or "quantum efficiency" can be described as the efficiency of the process of fluorescence in a compound. It is defined as the probability that upon photoexcitation, the compound will decay back to the ground state through a radiative process rather than a nonradiative one. In other words, fluorescence quantum yield can be thought of as the likelihood that a molecule will emit a photon upon relaxation after being excited by a photon. Solvent polarity and concentration are important factors when considering the fluorescence quantum efficiency of a fluorophore. Solvent polarity

becomes important, as more polar solvents tend to better stabilize the photoexcited state of the molecule (causing the red-shift in fluorescence), and concentration is relevant due to the aggregation, and sub-sequent self-quenching of fluorescence, that can occur at high concentrations. Solvent polarity becomes especially important for molecules with large excited-state dipole moments.

The quantum yield of the synthesized nitro/amino TTz compounds in several different solvents, was generally lower than previous asymmetric TTz compounds. Previous TTz compounds that have been synthesized by the Walter group have had quantum yields as high as 0.93.[12] However, these same compounds were also reported to have a quantum yield of 0.17 in acetonitrile and 0.35 in dichloromethane,[12] so it is important to note that the solvent chosen for these measurements has a very significant impact on the quantum efficiency. The highest quantum yields obtained were $\Phi_F = 0.36$ for the amide/nitrophenyl TTz derivative in chlorobenzene, and $\Phi_F = 0.34$ for the amino/nitrophenyl TTz derivative in toluene.

While the quantum efficiencies were not as high as some of the other TTz derivatives that have been made,[12] the Stokes shifts could make them potentially useful for biological fluorophores. Common biological fluorophores, such as fluorescein and rhodamine derivatives, have a very small Stokes shift (lower than 50 nm), and self-quenching of the fluorescence poses a problem for the efficiency of these fluorophores in this application. While these fluorophores have high quantum efficiency (nearly 100%), the self-quenching caused by the overlapping of the absorption and emission wavelengths is a disadvantage of using these compounds for these types of applications. An ideal fluorophore for this application would have a very high quantum efficiency and a high

Stokes shift to avoid self-quenching of fluorescence. While the TTz derivatives that were synthesized (**Table 1**) do not have a high quantum efficiency, they could still be potentially useful as biological fluorophores due to their high Stokes shifts. The Stokes shifts for these materials was quite large, 0.8 eV, which is the largest observed emission shift for these asymmetric TTz derivatives.

Fluorescence lifetimes are also an important parameter when considering the efficiency of fluorophores for applications like biological imaging. The fluorescent lifetimes shown in Table 1 are comparable to other organic dyes, which can range from 0.1 to 20 ns. Some organic compounds can reach fluorescence lifetimes of up to 90 ns, but this is not typically the case. An ideal fluorophore for a biological imaging application would have a high quantum efficiency and a long fluorescence lifetime.[38-39] A long lifetime in this context is considered to be >1 nanosecond for small organic molecules. In this context, rhodamine and fluorescein derivatives would be good probes, as they have "long" fluorescence lifetimes and very high quantum efficiencies.[38-39] Although the quantum efficiency of the compounds presented in **Table 1** is relatively low compared to these benchmark compounds, the presented TTz compounds do have a relatively high fluorescence lifetime. It is also worth noting that there are examples of fluorophores that have low quantum efficiencies, but have been useful for biological imaging, such as Evan W. Miller's voltage-sensitive dyes (VSD). While the VSD's developed by Miller's group have low quantum yields (around 0.24), they have been shown to be effective dyes for these types of applications due to their high contrast.[40] Since the presented TTz compounds in **Table 1** notably do not show any spectroscopic fluorescence in water, it is possible that these compounds could be useful for this

application since there would be little to no background fluorescence observed, resulting in a high fluorescence contrast.

3.6. Unique Fluorescent Properties of ($PhNO_2$)TTz($PhNH_2$)

($PhNO_2$)TTz($PhNH_2$) was the most important target synthetic compound of this study, as was a natural "model compound" for a strong push-pull system. It contains a nitro group, which is strongly electron withdrawing, in the *para* position relative to an amine group, which is strongly electron donating. Before this compound was synthesized, DFT calculations were done in Spartan to estimate how strong the push-pull effect would be. The HOMO/LUMO electron density maps are shown in **Figure 7**.

Based on these calculations, we assumed that this compound would exhibit strong solvatofluorochromism. While our assumption was technically true, it exhibited a different type of solvatofluorochromism in which more polar solvents tended to blue-shift rather than red-shift the emission wavelength, which is a separate phenomenon known as negative solvatofluorochromism.

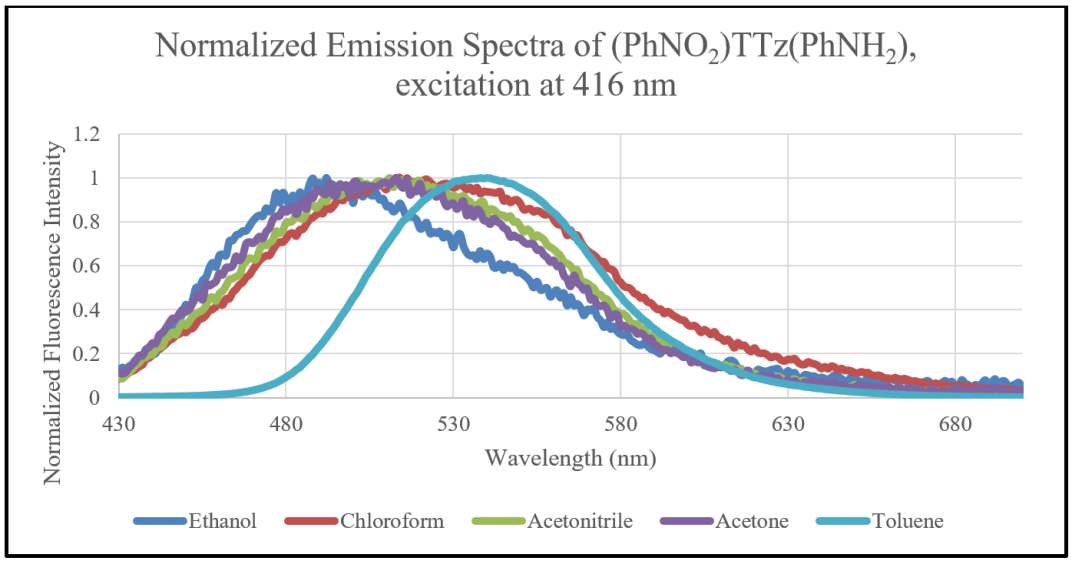

Figure 22: Normalized emission spectra of $(PhNO_2)TTz(PhNH_2)$ when dissolved in various solvents. In general, the emission wavelength is blue-shifted by more polar solvents. The λ_{max} of emission for each solvent is as follows: ethanol 492 nm, acetonitrile 511 nm, acetone 513 nm, chloroform 514 nm, and toluene 540 nm.

This phenomenon is seen in compounds that can perform intramolecular charge transfers (ICTs) that can hydrogen bond with the solvent, or excited-state intramolecular proton transfers (ESIPTs) in the form of tautomerization by which the molecule relaxes by an intramolecular proton transfer.[41-42] Therefore, there are two possible explanations for the negative solvatofluorochromism that is associated with $(PhNO_2)TTz(PhNH_2)$. The first explanation, which seems to be more likely, is that the amine group is hydrogen bonding with the solvents that were chosen, which interrupts the solvent relaxation effect that causes the solvatofluorochromism. Creating more amine-functionalized push-pull dyes will give more insight to this hypothesis.

The second explanation would be that the amine group is tautomerizing to become an imine and is relaxing to the ground state using that proton transfer mechanism. This proposed mechanism, however unlikely, is illustrated in **Figure 23**. This is the less likely explanation, as the compound would have to break aromaticity in order to tautomerize,

presumably creating a transitional state that would be higher in energy, and therefore not feasible for a relaxation mechanism. Compounds that exhibit negative solvatofluorochromism through this mechanism typically use enol functionalities and do not break aromaticity to accomplish this.[41]

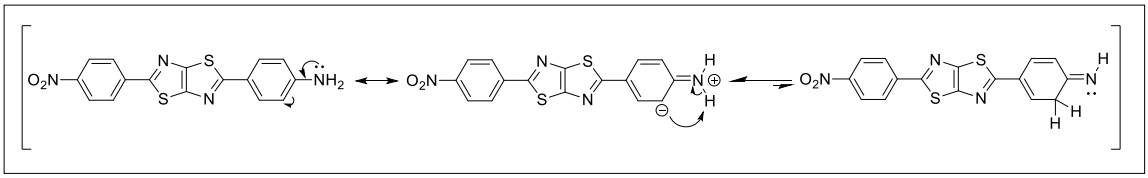

Figure 23: Proposed ESIPT mechanism of ($PhNO_2$)TTz($PhNH_2$). The final state would be very high in energy due to breaking aromaticity of the molecule, and is therefore highly unlikely to be the mechanism by which ($PhNO_2$)TTz($PhNH_2$) shows negative solvatofluorochromism.

3.7. ($PhNO_2$)TTz($PhNH_2$) as a Fluorescent Probe for UppS

The synthesis and purification of ($PhNO_2$)TTz($PhNH_2$) is deceptively difficult. Because of the difficulty in obtaining this compound, it has not been tested as a fluorophore for the aforementioned UppS application. However, it did display solvatofluorochromism and also contains a primary amine functional group and can theoretically be used for this application. Many fluorophores that are currently used for this application are attached to the geranyl acetate derivative via an imine linkage and then reduced to an amine.[12, 43] Using the same procedure, it is reasonable to assume that ($PhNO_2$)TTz($PhNH_2$) could be used as a fluorophore by using this coupling mechanism. Specifically, the nitrobenzoxadizol (NBD) fluorophore has been used and employs a nitro group that is similarly electron withdrawing.[16] p-Nitroaniline has also been employed, which is a more direct comparison to ($PhNO_2$)TTz($PhNH_2$), as the nitro group is also

para-substituted, and should be electronically similar.[37] One major difference is that the thiazolo-thiazole core of ($PhNO_2$)TTz($PhNH_2$) acts as an intramolecular charge transfer complex, which facilitates the movement of electrons throughout the molecule, which is what causes its highly solvatofluorochromic properties. For this reason, it is believed that ($PhNO_2$)TTz($PhNH_2$) will be sensitive to the chemical environment of the binding site of UppS, and may be able to detect the subtle chemical differences in the binding sites of Upps from different organisms.[18]

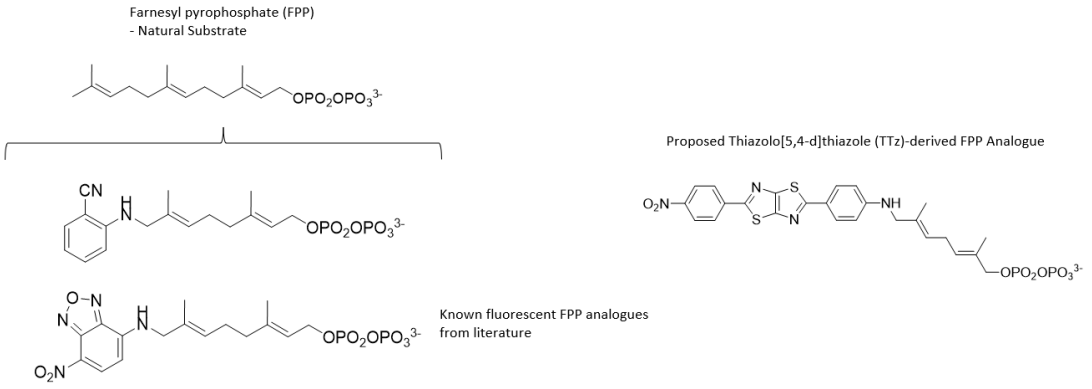

Figure 24: Proposed TTz-tagged FPP fluorescent analog for use as a probe for the antibiotic target enzyme UppS. Known fluorescent FPP analogs from literature are shown to illustrate the amine linkage to this compound.[16]

One consideration that is notable is the relatively large size of the TTz fluorophore in comparison to some of the smaller molecules that are typically used for this application. The size of the molecule certainly brings a concern that the molecule will be too large for the binding site of UppS. While it would certainly not be ideal for the molecule to be too large for the binding site, it would give some insight into the tolerance of the size of molecules that are allowed into this pocket. It is also worth noting that the TTz-derived fluorophores are also highly planar and rigid: a significant property of these compounds

that allows the intramolecular charge transfer to occur. This causes the compound to have a rod shape, and could also give some insight into the shape of the binding site if it is allowed to bind and facilitate the chain elongation of the tagged isoprenoid.

The photophysical properties of ($PhNO_2$)TTz($PhNH_2$) seem to suggest a higher sensitivity for intermolecular interactions with other aromatic compounds. This is shown from its higher solubility in, and higher quantum efficiencies while dissolved in, aromatic solvents. While the compound has not yet been tested within UppS, it is predicted that it will have more solvatofluorochromic sensitivity when interacting with aromatic amino acids within the binding site. Because of its rigid and planar molecular structure, along with its high degree of unsaturation, π-π stacking intermolecular interactions will most likely be the most notable and have the most potential to shift the wavelength of emission while using this compound as a fluorophore. Because of this, it is therefore predicted that this compound, if compatible with UppS, will be more effective in species of UppS that contain more aromatic amino acid residues within the binding site. As chain elongation is facilitated by this enzyme, it is likely that these π-π stacking interactions will be greatly reduced, resulting in a significant shift in the emission wavelength as well, potentially giving another indication, other than mass, of the degree of chain elongation by UppS. It has been shown that alkylation of this class of compounds greatly reduces the aggregation of these molecules in solution and has a significant impact on the solubility in alkylated solvents.[3]

If there is a significant enough shift in fluorescence from isoprenoid chain elongation by UppS, it could be possible to determine the degree of chain elongation by fluorescence spectroscopy, rather than by mass spectrometry. This would be a particularly useful

means of analyzing the length of elongation, as product would not be destroyed in the process, as it would during the process of mass spectrometry.

CHAPTER 4: CONCLUSION AND FUTURE WORK

In this study, the major goal was to synthesize a FPP analog using a TTz as a fluorophore. However, post-synthetic modifications of this class of compounds proved not to be a trivial task. The target compound, ($PhNO_2$)TTz($PhNH_2$) was chosen specifically to be used as a fluorophore to probe UppS, and in synthesizing ($PhNO_2$)TTz($PhNH_2$), much was learned about this class of compounds and how to incorporate new functionalities to them that were not as accessible in the past. Also, ($PhNO_2$)TTz($PhNH_2$) should still be pursued as the fluorophore to probe UppS unless a better candidate is synthesized. This compound should be tagged to a geranyl acetate derivative and phosphorylated as soon as possible to see if the UppS binding site will allow such a rigid, rod-shaped molecule into its binding site. If binding is successful, then optimization of the fluorophore by using a weaker electron withdrawing group and keeping the amine as the donor could potentially lead to more favorable properties for this application, such as a high quantum yield.

Previously, introducing a strong electron donor to this class of compounds meant doing it during the formation of the TTz core in the first synthetic reaction, which typically resulted in extremely inefficient yields. During this study, a significantly more efficient method of introducing amine functionalities to TTz compounds will make this class of compounds more accessible for future research. Access to the strong amine electron donating group means also having access to stronger push-pull systems, which

are important for the utilization of these compounds as molecular sensors, charge carriers, light harvesting chromophores, or other material applications.

With the synthetic pathway that was developed during this research, these amine functionalized TTz's should be more accessible to study. This synthetic route could be a more feasible route than the Lossen rearrangement pathway to synthesize ($PhNH_2$)TTz(PhCOOH), potentially making that compound more accessible as well. (**Figure 25**).

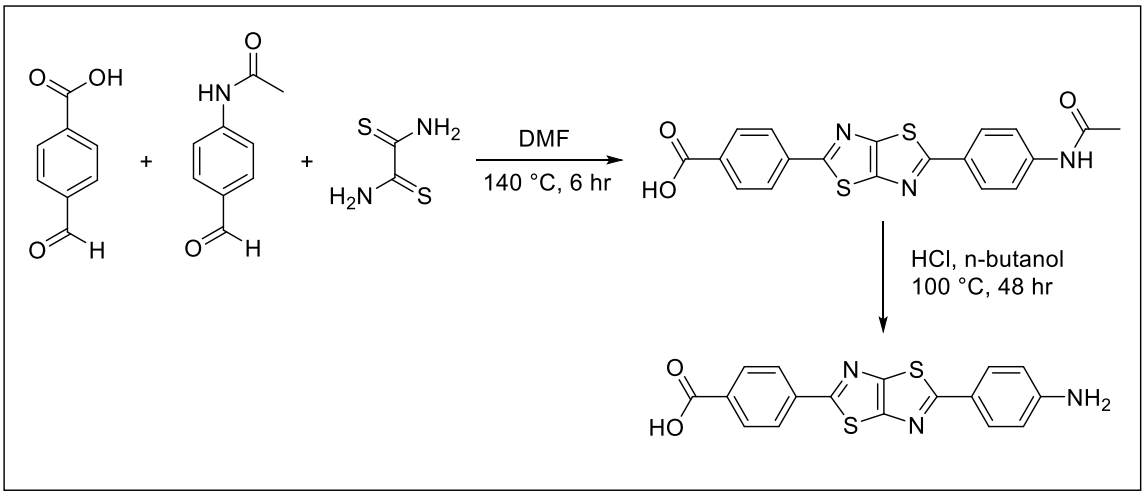

Figure 25: Suggested synthetic pathway for producing ($PhNH_2$)TTz(PhCOOH). This pathway could potentially be more efficient than the Lossen rearrangement pathway that was explored in this study.

The negative solvatofluorochromism that ($PhNO_2$)TTz($PhNH_2$) displayed was truly an interesting development as well, as this phenomenon has not been seen with TTz compounds that have been synthesized by the Walter group in the past. Of the TTz compounds that the group has synthesized that show solvatofluorochromism, only this compound has shown negative solvatofluorochromism, and this needs to be explored. It is hypothesized that this compound is showing this trend among solvents that can

hydrogen bond with the molecule but will revert to a trend of positive solvatofluorochromism among solvents that it can not hydrogen bond with. More photophysical studies should be done to confirm this.

While the focus of this study was to synthesize ($PhNO_2$)TTz($PhNH_2$) to be used as a fluorophore for the UppS application, the study did not make it to this phase of the research. Synthesizing this compound proved to be significantly more difficult than anticipated, and an efficient method to synthesize asymmetric TTz compounds with acetamide and amine functionalities has been developed as a result. This can potentially lead to a new generation of TTz compounds that incorporate these functionalities, that could be used for a wide range of molecular sensing applications, including to probe UppS. As previously mentioned, if the solvatofluorochromic effect of this class of compounds is significant enough, it could allow for less destructive means of characterizing the isoprenoids that are produced during the enzymatic chain elongation that UppS catalyzes.

The photophysical properties of the synthesized TTz derivatives are not discouraging, however, and seem to suggest that these compounds could be useful fluorescent tags for biological applications, or as stains for biological imaging applications. While these compounds do not boast a high quantum efficiency, their very high Stokes shift could be advantageous for these applications, as it would not exhibit the self-quenching that is present in common fluorophores used in these applications, such as fluorescein and rhodamine derivatives. These compounds should be further investigated and optimized for use as fluorescent probes for biological applications such as the UppS application that is described in this thesis. These compounds should also be investigated for their use in

biological imaging applications. Alkylation of the compound may be necessary to promote staining of cell membranes, but it should be noted that this process could affect the photophysical properties of these compounds.

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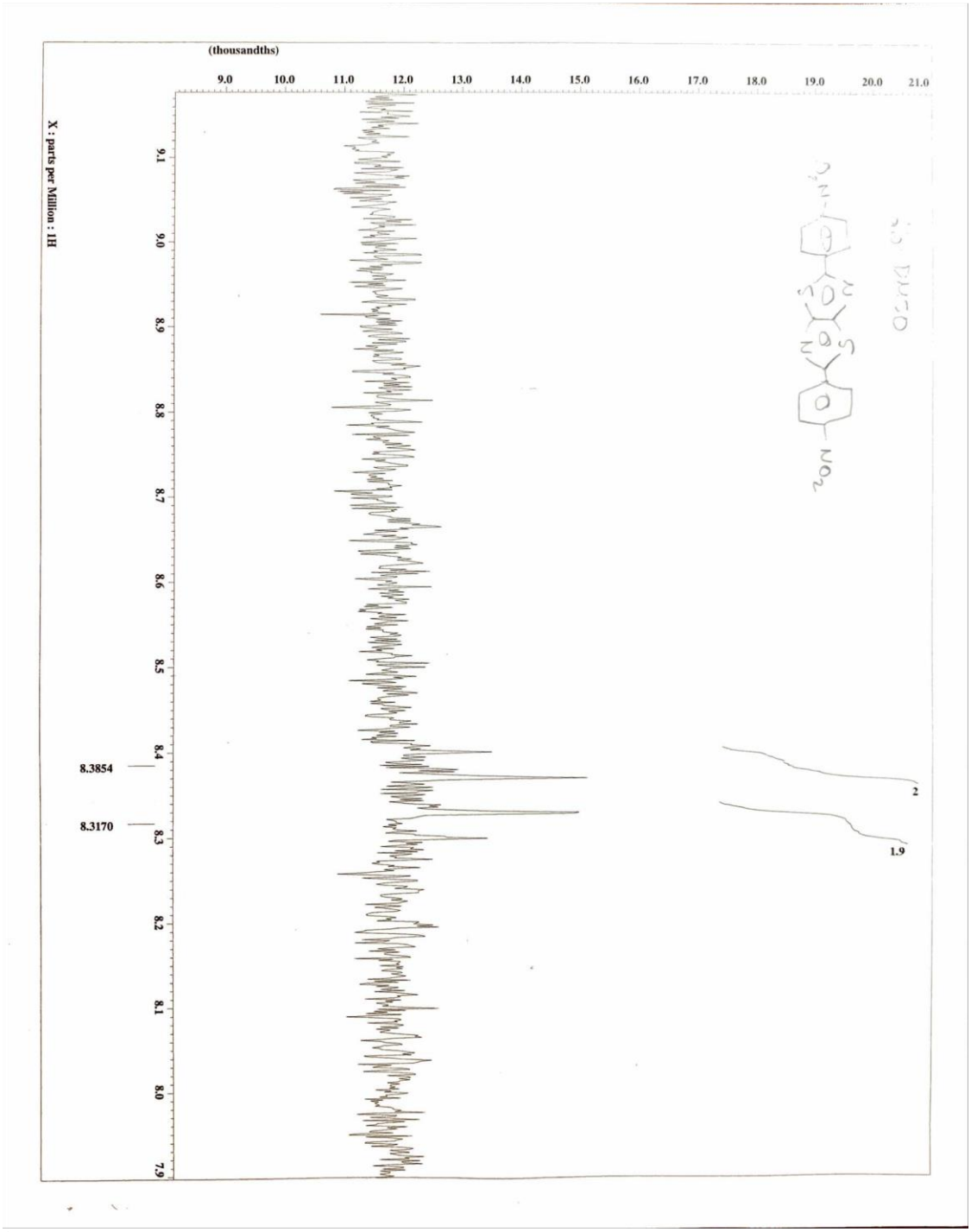
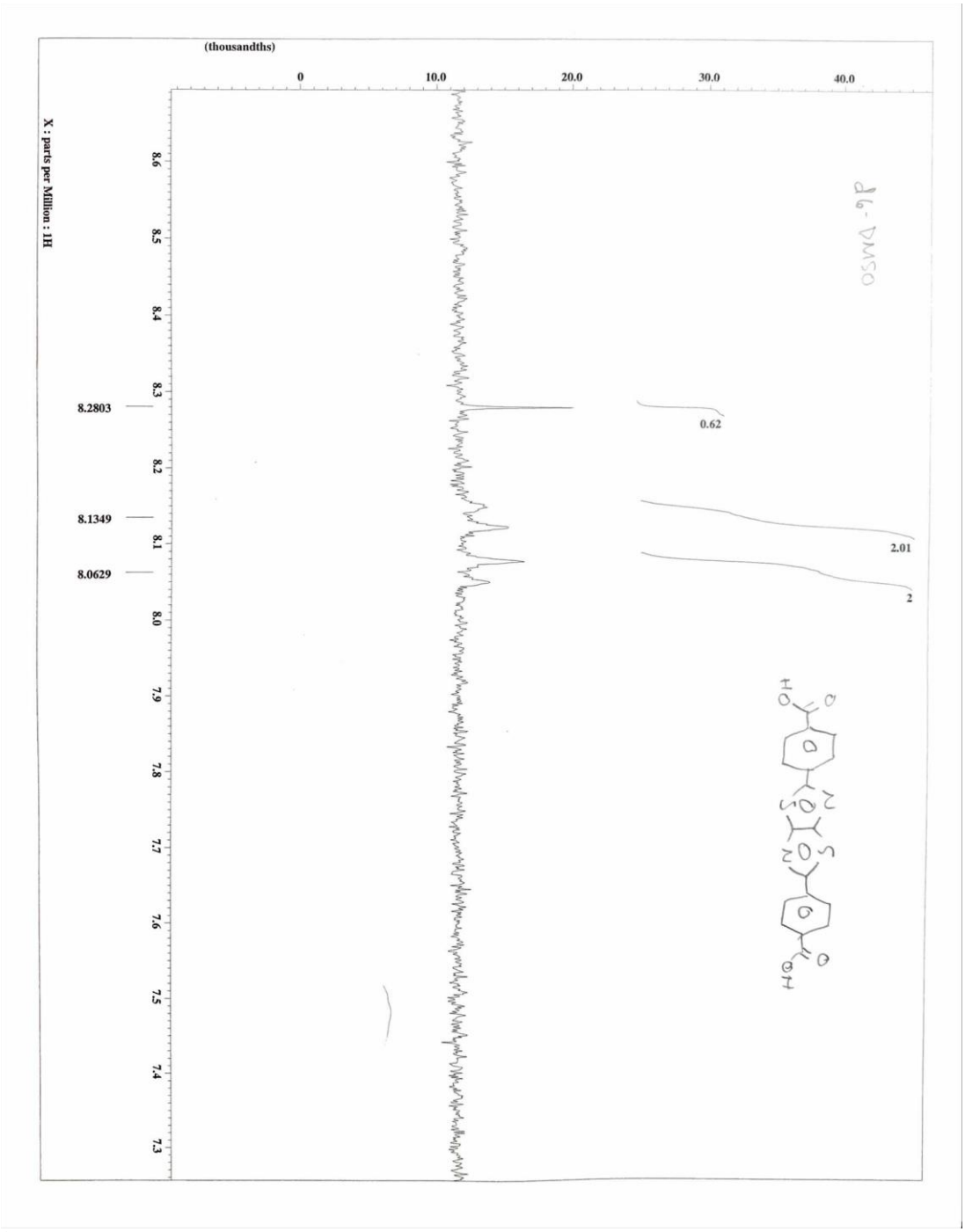
APPENDIX: SUPPLEMENTAL DATA

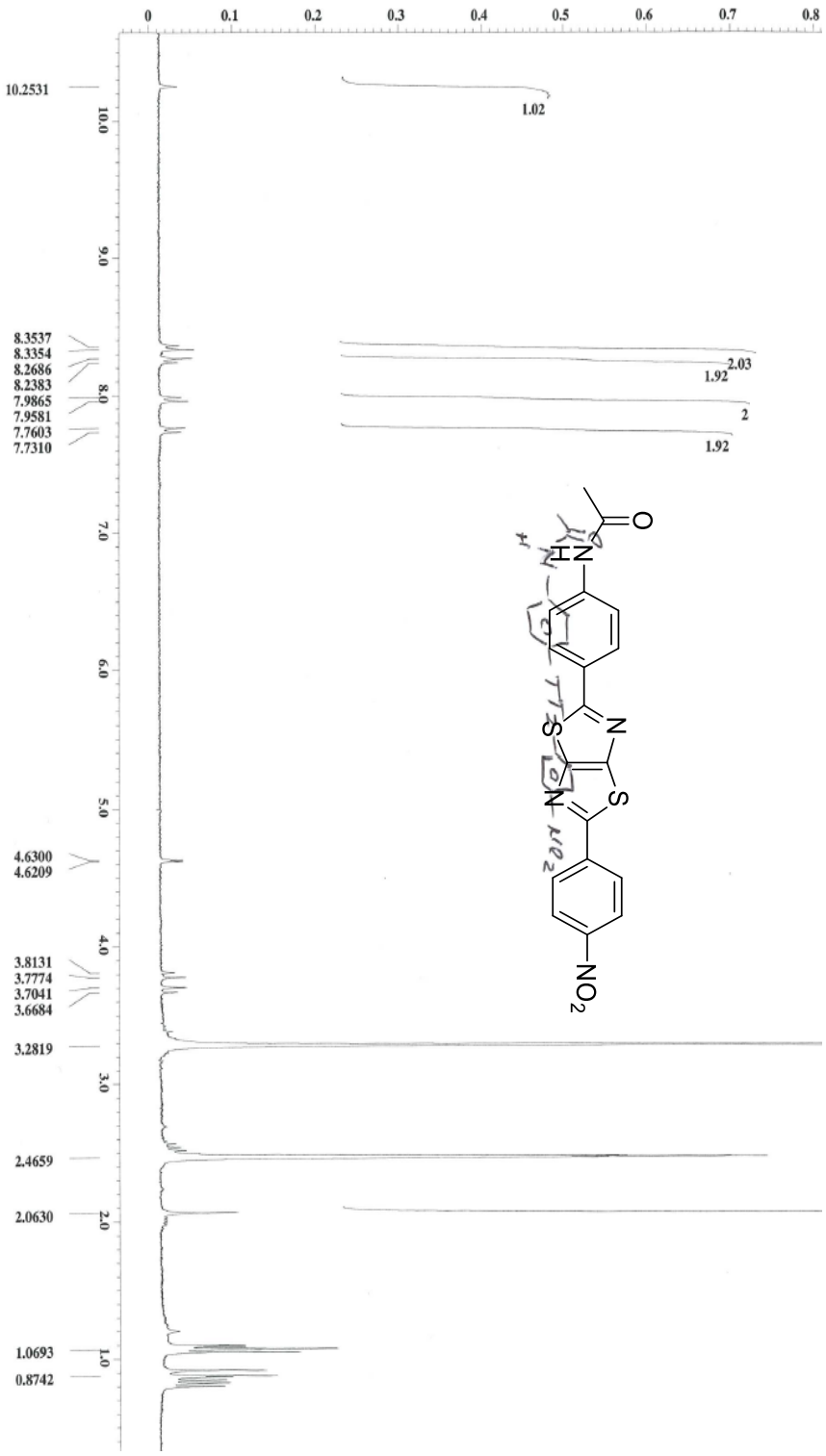


^{1}H NMR Spectrum of $(PhNO_2)_2TTz$ in d6-DMSO



^{1}H NMR Spectrum of $(PhCOOH)_2TTz$ in d6-DMSO

^{1}H NMR Spectrum of $(PhNH_2)_2TTz$ in d6-DMSO



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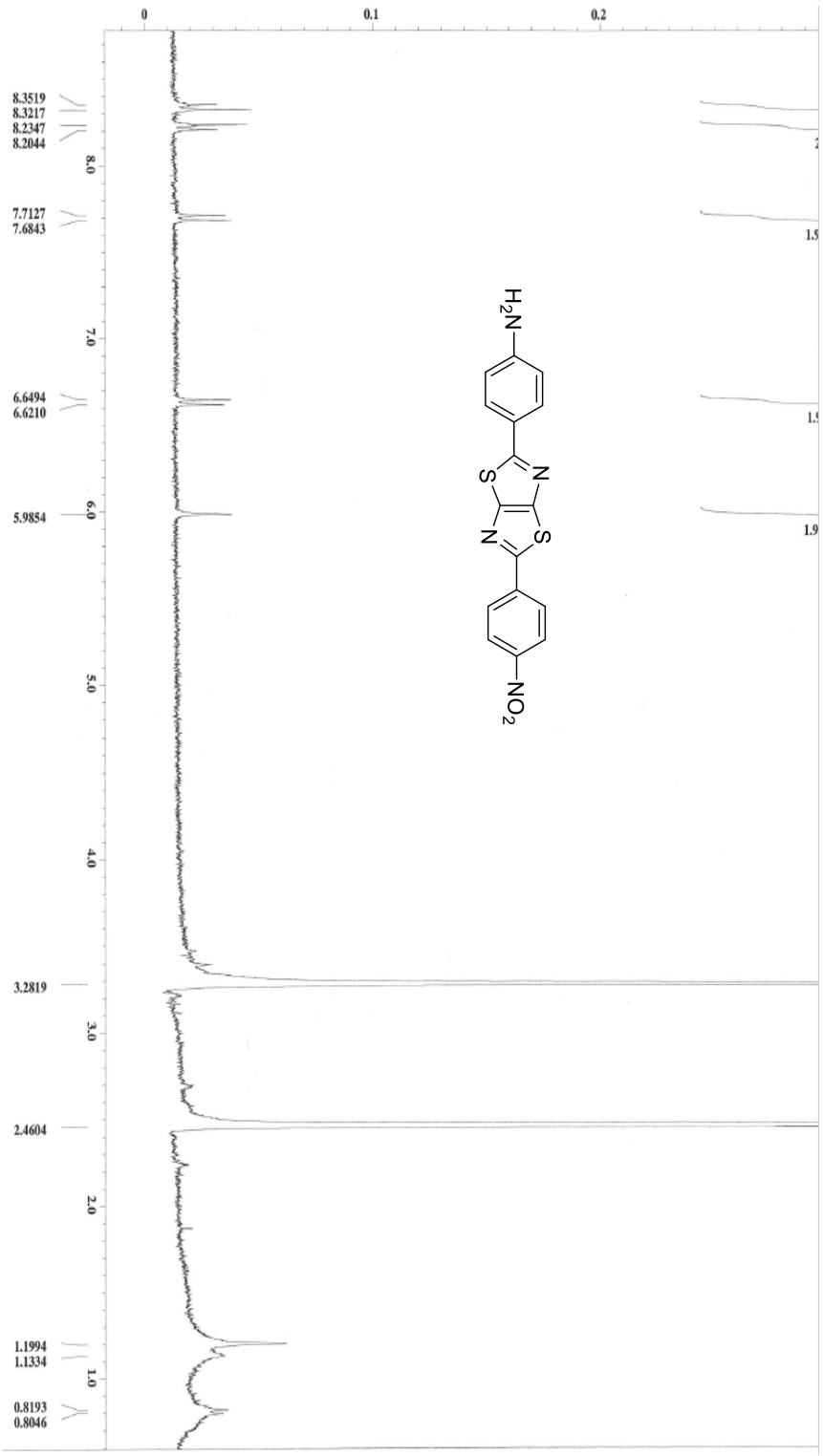

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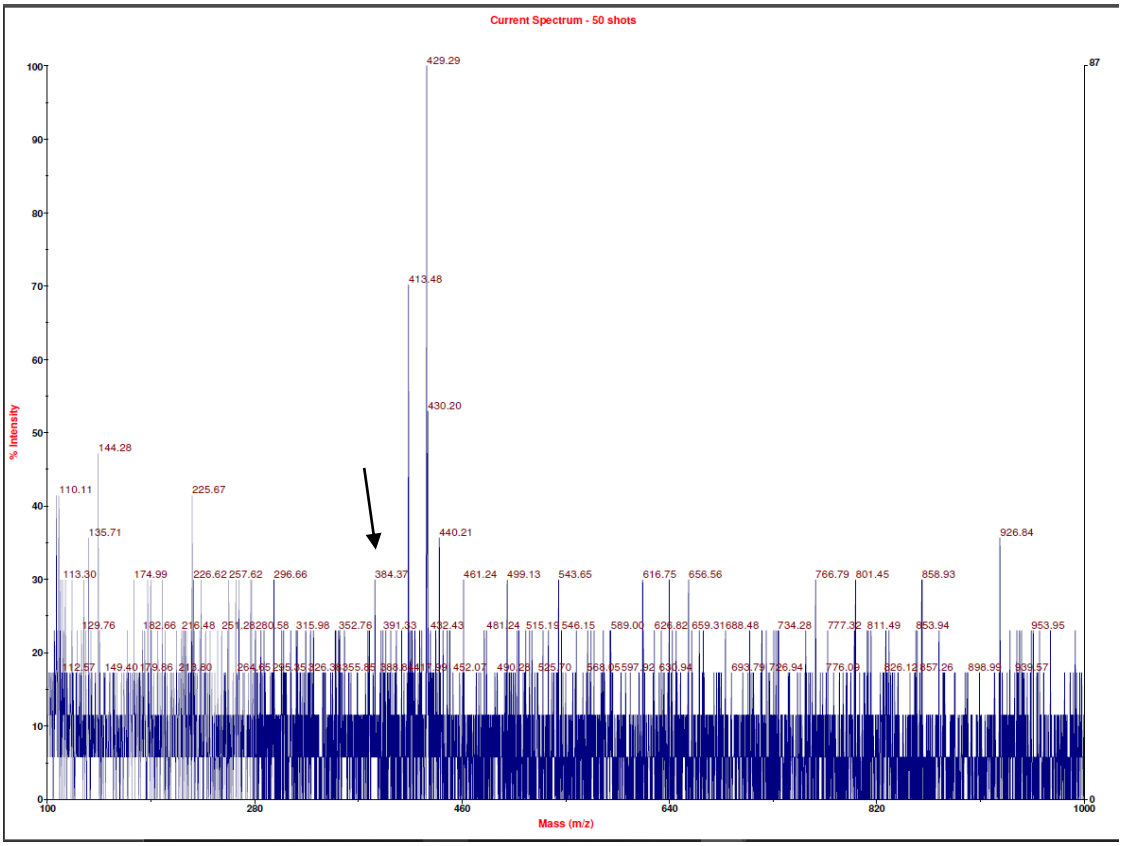
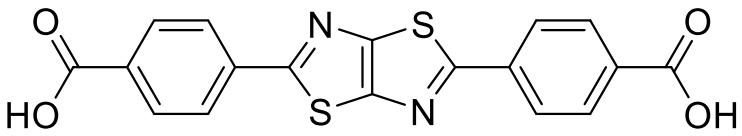
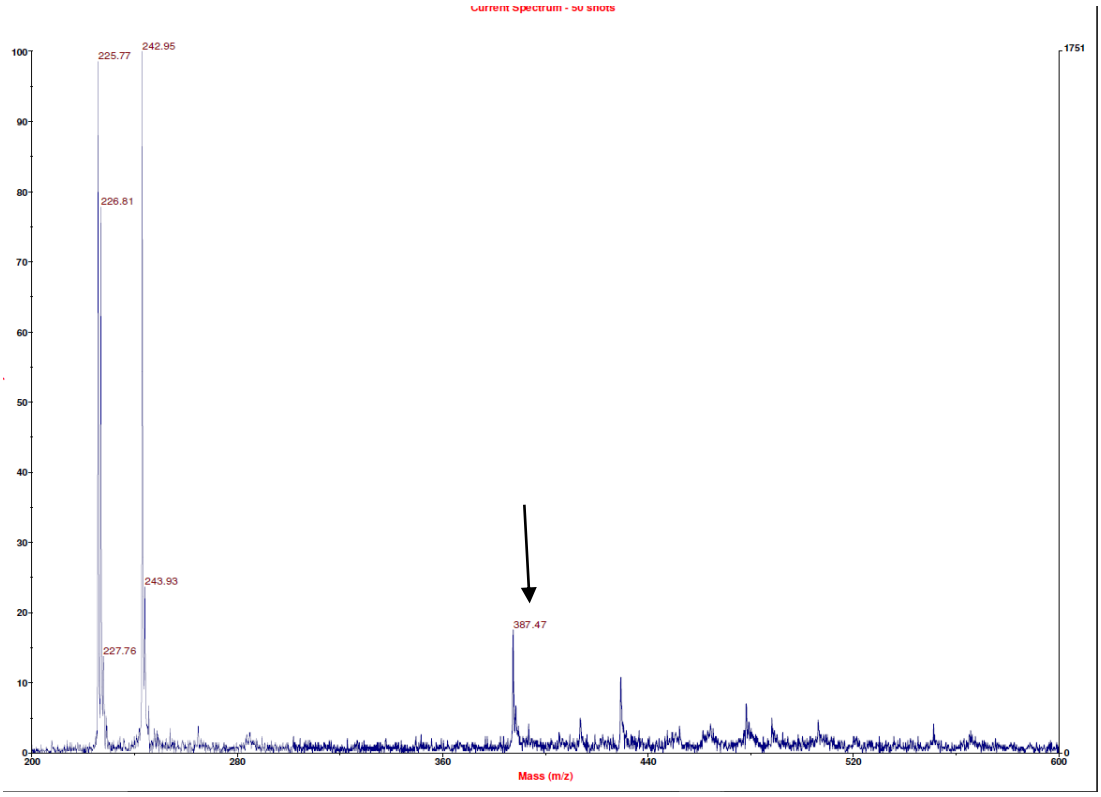
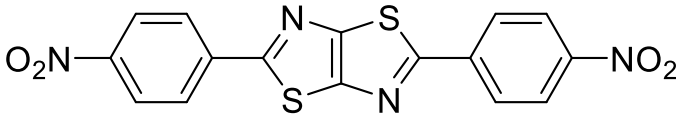




m/z: 382.01 (100.0%), 383.01 (22.1%), 384.00 (9.1%), 384.01 (3.1%), 385.01 (1.8%)

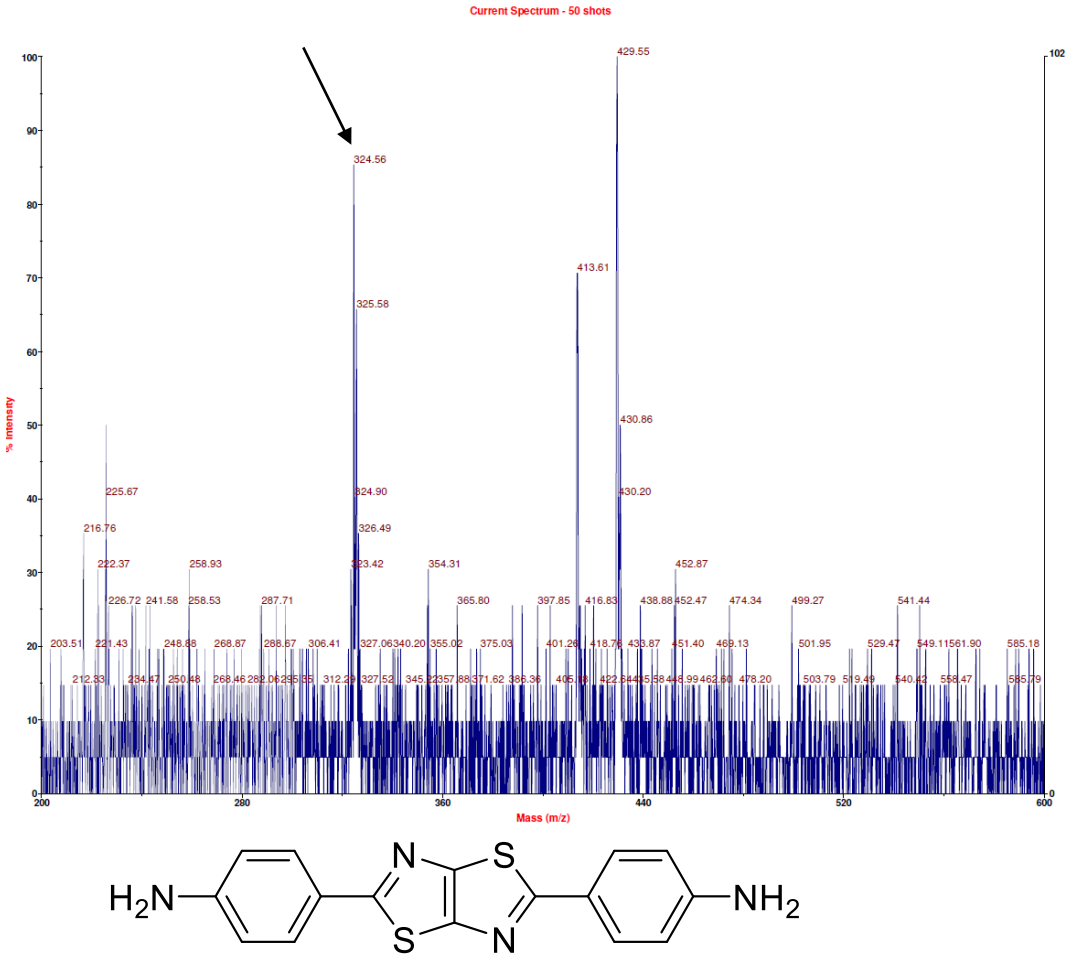
MALDI-TOF Spectrum of $(PhCOOH)_2TTz$ and m/z peaks as calculated by ChemDraw. Poor solubility made this compound difficult to load onto the MALDI plate for analysis.





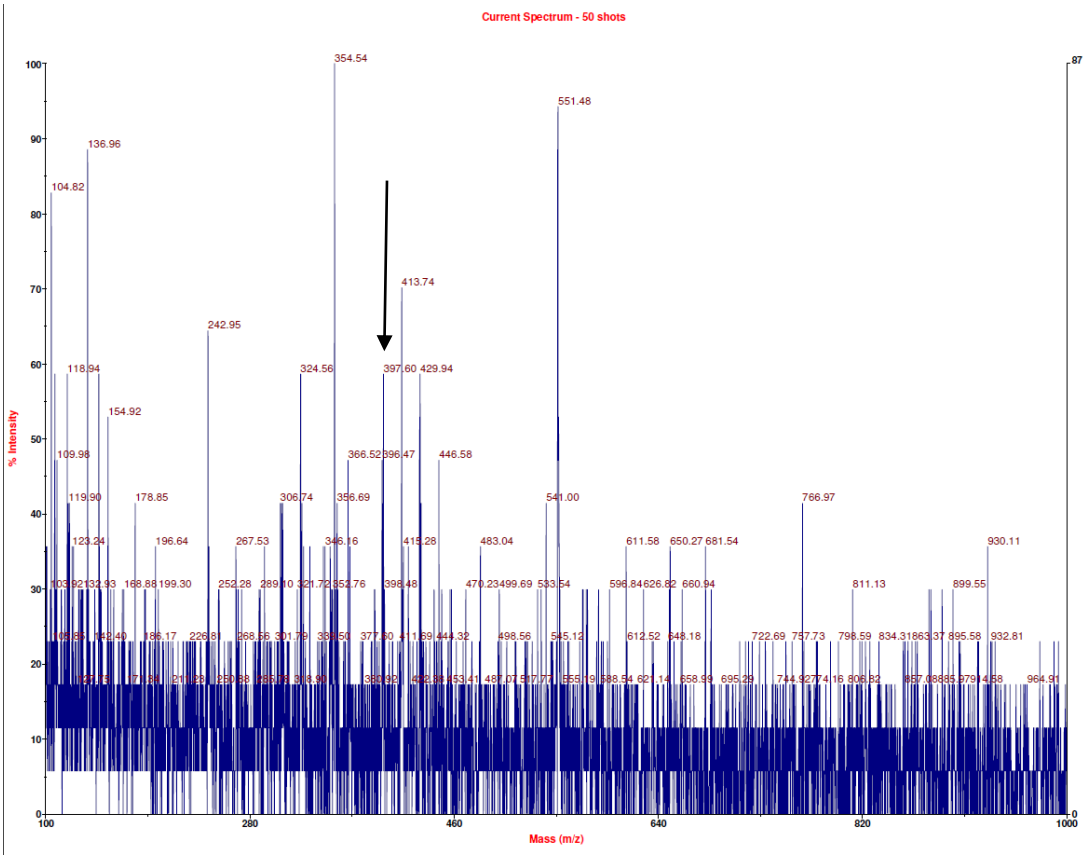
m/z: 384.00 (100.0%), 385.00 (20.6%), 385.99 (9.0%), 387.00 (1.7%), 386.01 (1.4%), 386.00 (1.4%)

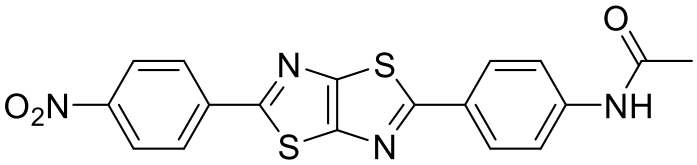
MALDI-TOF Spectrum of $(PhNO_2)_2TTz$ and m/z peaks as calculated by ChemDraw.



m/z: 324.05 (100.0%), 325.05 (20.4%), 326.05 (9.6%), 327.05 (1.7%), 326.06 (1.4%)

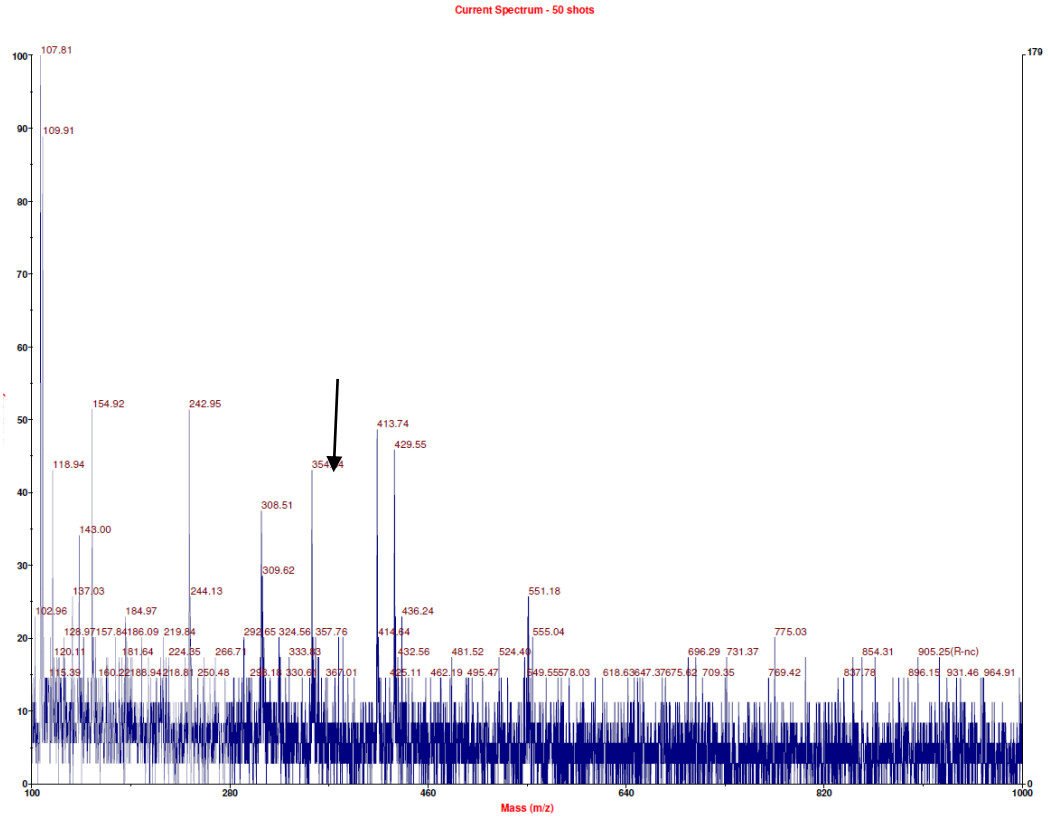
MALDI-TOF Spectrum of $(PhNH_2)_2TTz$ and m/z peaks as calculated by ChemDraw.

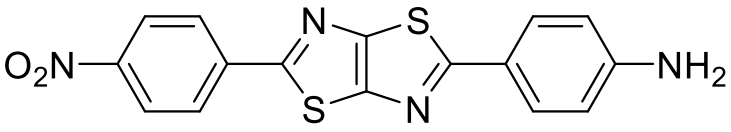




m/z: 396.04 (100.0%), 397.04 (19.7%), 398.03 (9.1%), 397.03 (3.1%), 398.04 (3.1%), 399.03 (2.0%)

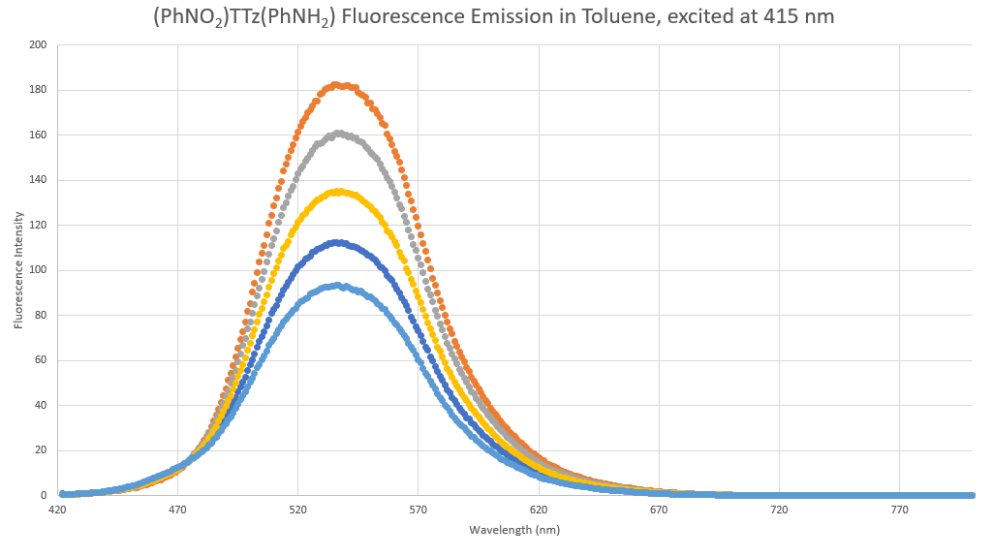
MALDI-TOF Spectrum of ($PhNO_2$)TTz(PhNHAc) and m/z peaks as calculated by ChemDraw.

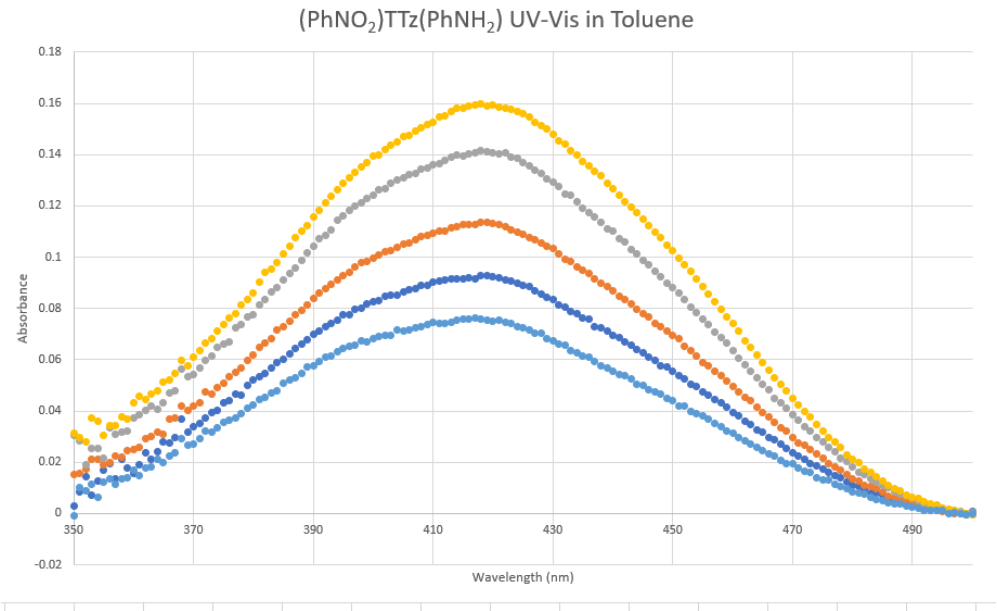




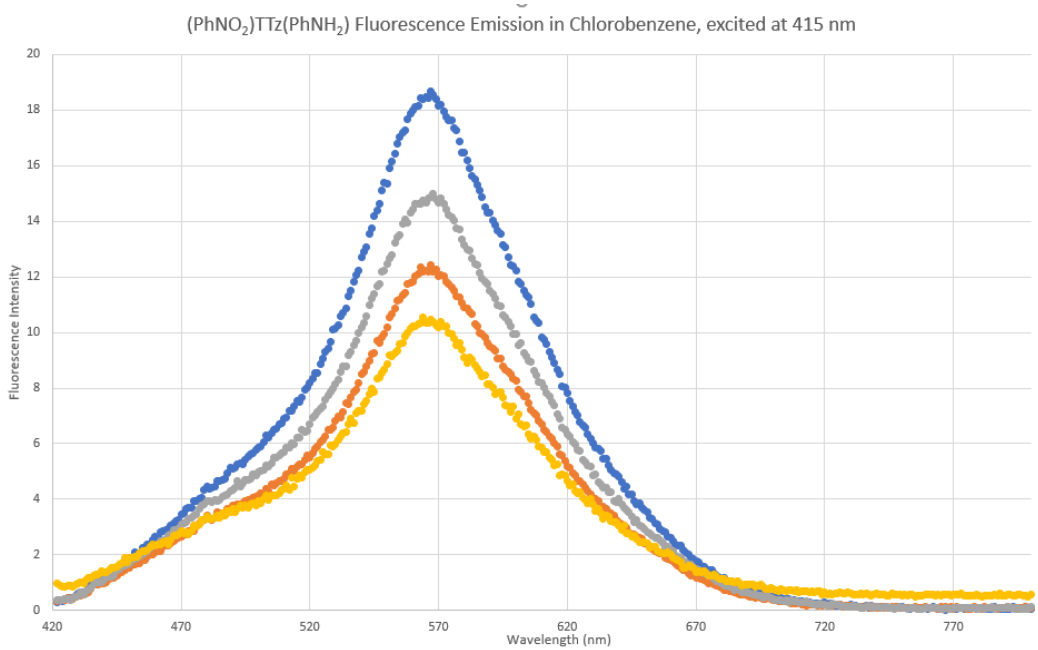
m/z: 354.02 (100.0%), 355.03 (17.5%), 356.02 (9.3%), 355.02 (3.1%), 356.03 (2.1%), 357.02 (1.8%)

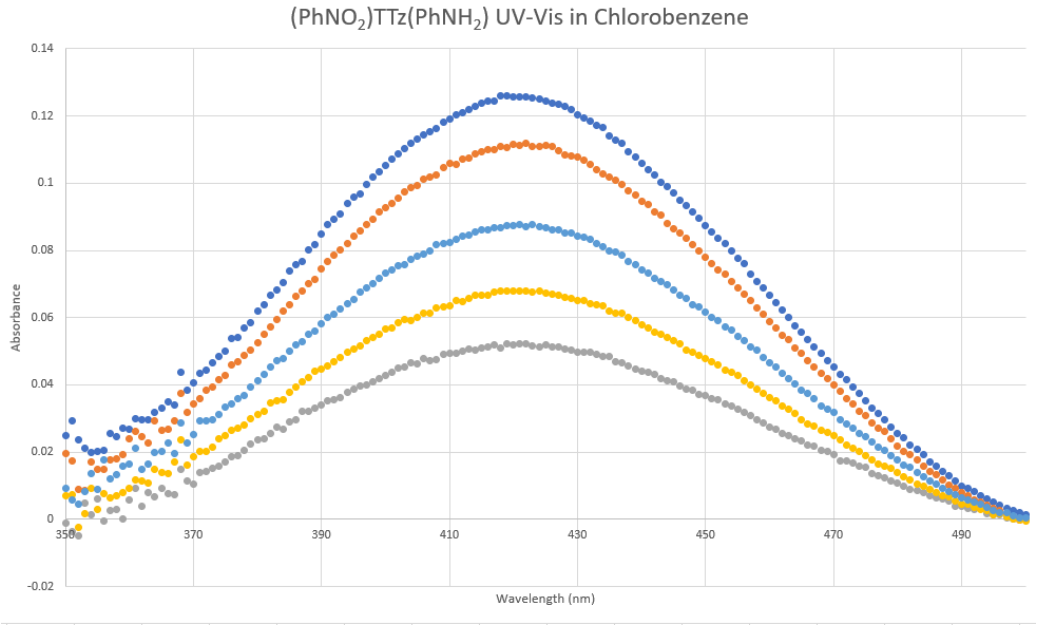
MALDI-TOF Spectrum of ($PhNO_2$)TTz($PhNH_2$) and m/z peaks as calculated by ChemDraw.



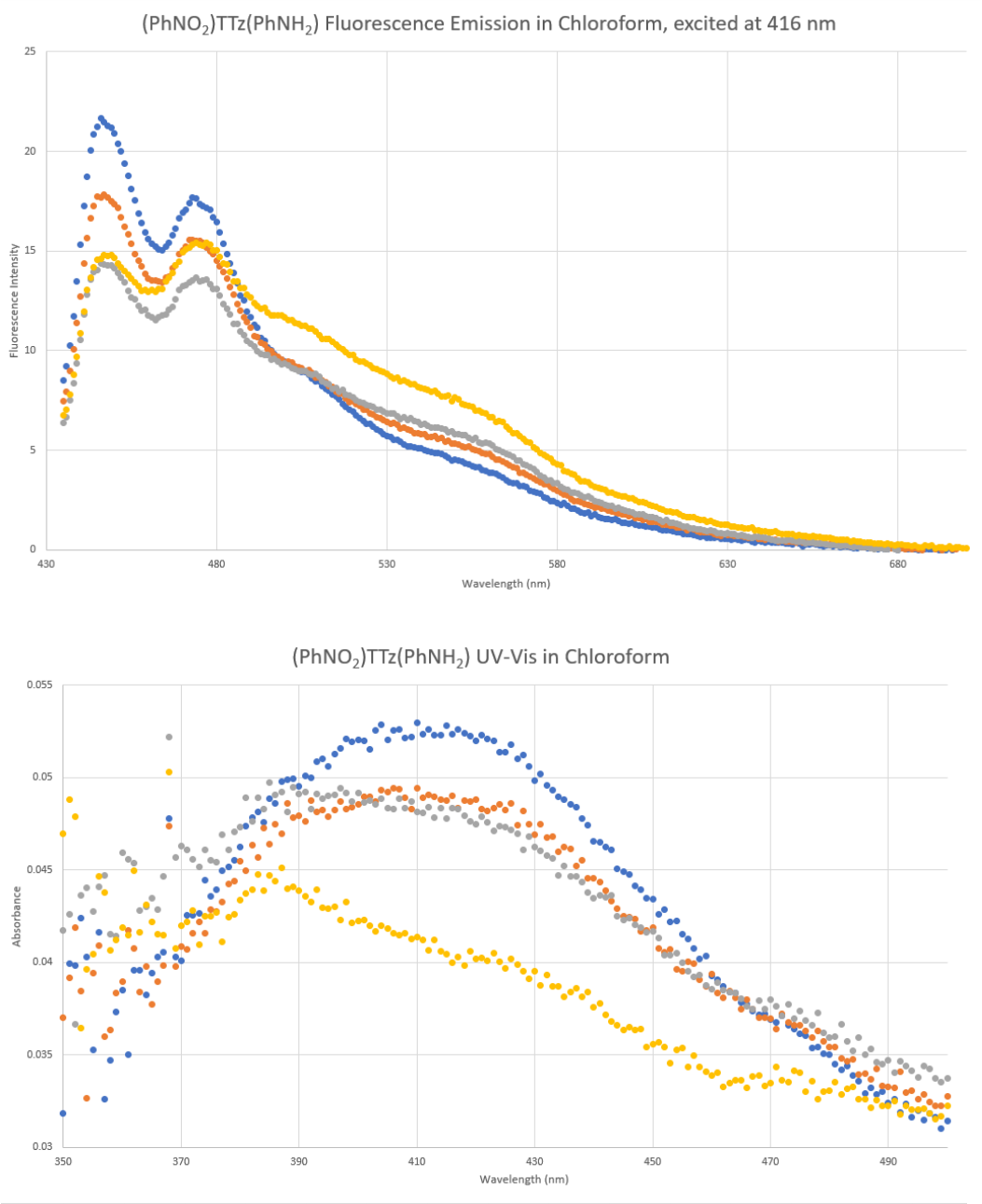


UV-Vis and emission spectra of ($PhNO_2$)TTz($PhNH_2$) in toluene at 5 different concentrations. These spectra were used to calculate a quantum yield for this compound in this solvent.





UV-Vis and emission spectra of ($PhNO_2$)TTz($PhNH_2$) in chlorobenzene at 5 different concentrations. These spectra were used to calculate a quantum yield for this compound in this solvent.



UV-Vis and emission spectra of ($PhNO_2$)TTz($PhNH_2$) in chloroform at 4 different concentrations. These spectra were used to calculate a quantum yield for this compound in this solvent. Solubility was notably poorer in chloroform than in aromatic solvents.

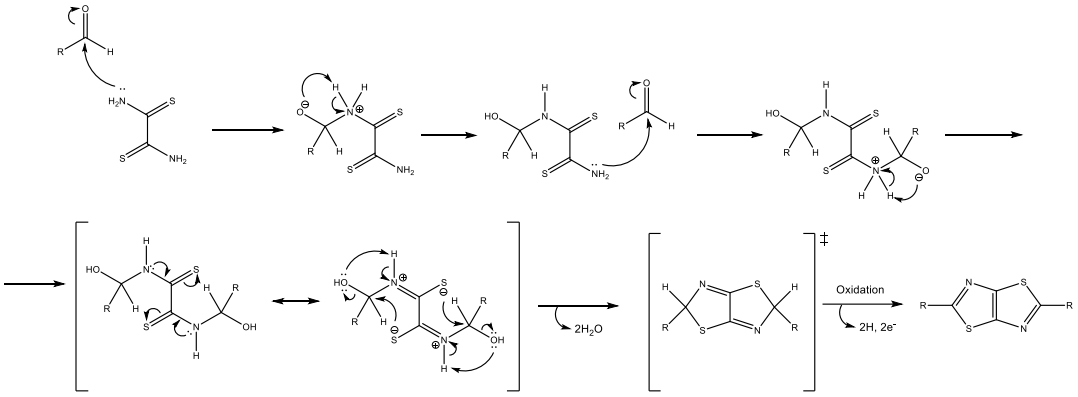
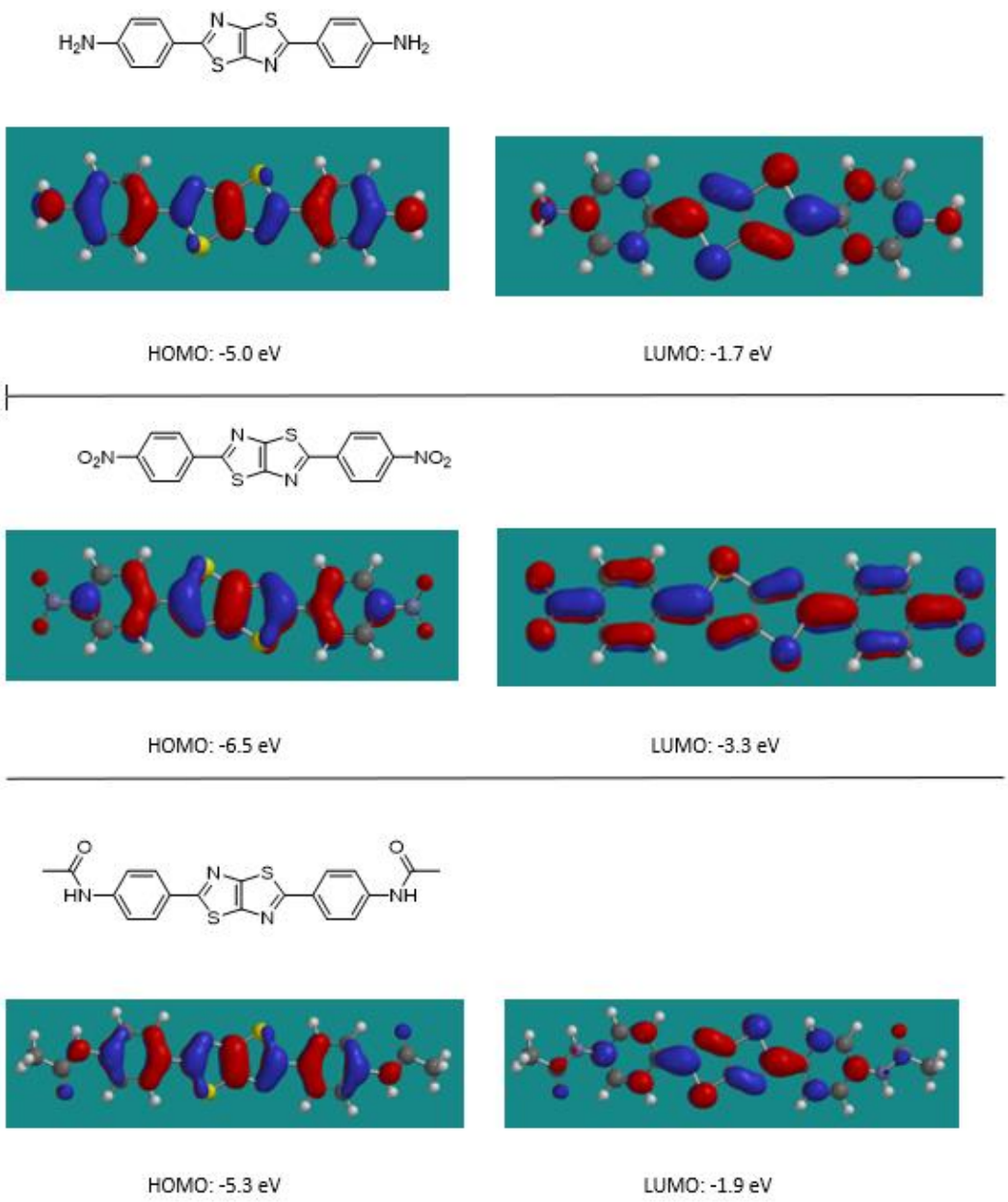
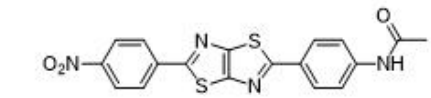
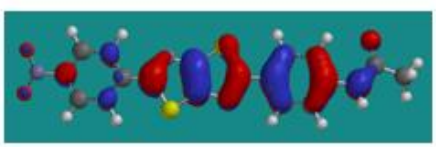


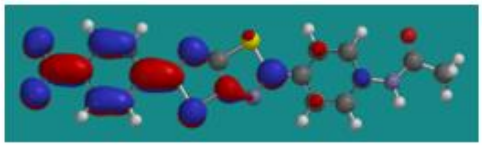
Figure 26. Proposed mechanism of the formation of the TTz core. It is a concerted double condensation reaction with a critical oxidation step that removes two electrons from the core, causing it to become electron deficient.



DFT calculations in Spartan of synthesized compounds, Part 1 of 2

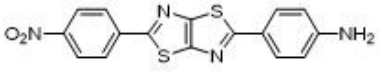


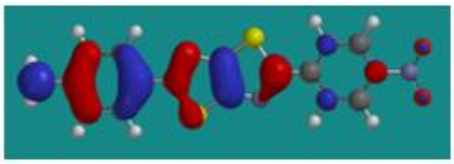


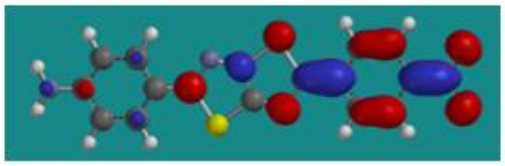


HOMO: -5.8 eV

LUMO: -2.8 eV



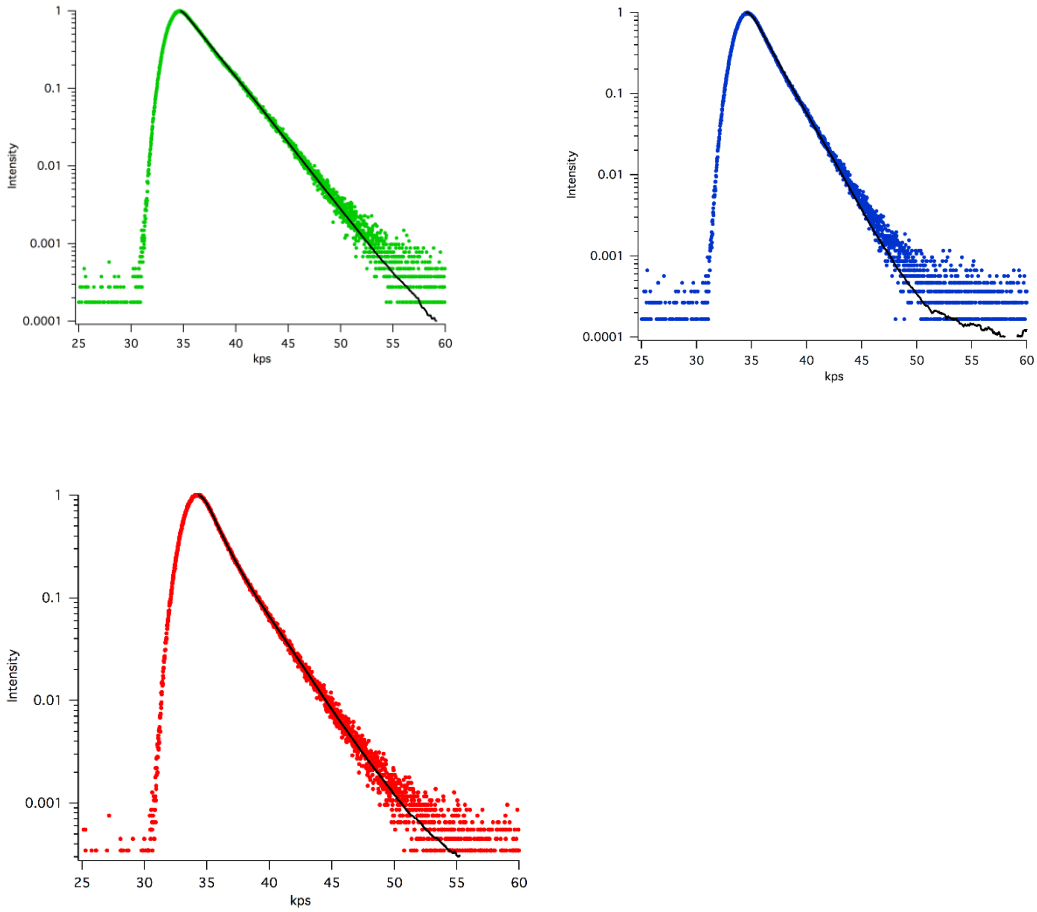




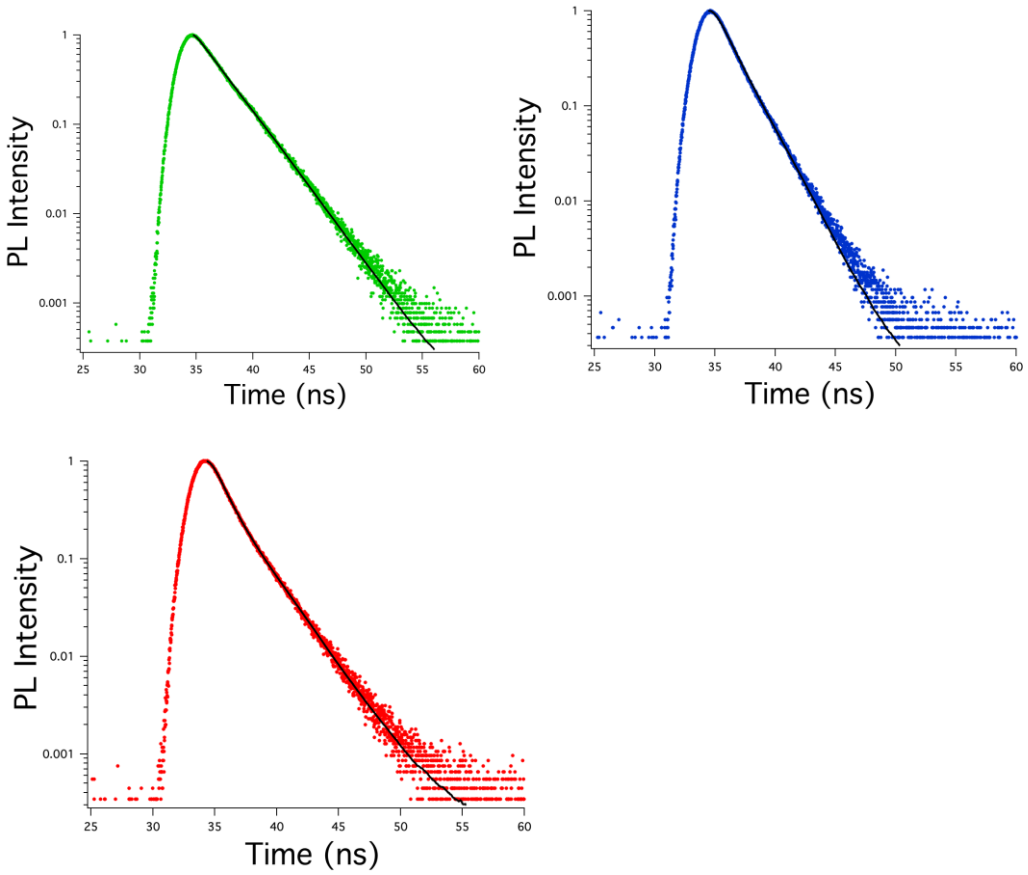
HOMO: -5.5 eV

LUMO: -2.7 eV

DFT calculations in Spartan of synthesized compounds, Part 2 of 2



TCSPC PL Decay measurements of ($PhNO_2$)TTz(NH_2) in toluene (green, top-left), chlorobenzene (blue, top-right), and o-chlorobenzene (red, bottom-left)



TCSPC PL Decay measurements of ($PhNO_2$)TTz(NHAc) in toluene (green, top-left), chlorobenzene (blue, top-right), and o-chlorobenzene (red, bottom-left)