Fluorescence Quenching of PPV-SO and Bodipy-Naphthalene Systems

Brooklynd Dawn Saar
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Fluorescence Quenching of PPV-SO and Bodipy-Naphthalene Systems

Brooklynd Dawn Saar
Colorado Springs, Colorado

Bachelor of Science, The College of William and Mary, 2009

A Thesis presented to the Graduate Faculty of the College of William and Mary in Candidacy for the Degree of Master of Science

Department of Chemistry

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Brooklynd Dawn Saar

Approved by the Committee, November, 2011

Committee Chair
Associate Professor Elizabeth Harbron, Department of Chemistry
The College of William & Mary

Professor Deborah Bebout, Department of Chemistry
The College of William & Mary

Professor Lisa Landino, Department of Chemistry
The College of William & Mary
This work investigates the synthesis of fluorescent systems that turn off and on in response to external stimuli. The first project is largely materials-based and focuses on turning the fluorescence of a poly(phenylene vinylene)-spirooxazine system on/off in response to external light stimuli. Towards this end, the polymerization of poly(phenylene vinylene) (PPV) was optimized using t-butylbenzyl chloride to prevent the creation of insoluble, high molecular weight polymers. The synthesis of alkyne-functionalized PPV and azide-functionalized spirooxazine was explored so that Click chemistry could be used to postsynthetically attach the spirooxazine to the polymer backbone. While researchers were unable to conclusively identify alkyne-functionalized PPV using NMR, they are confident that the Williamson Ether addition of propargyl alcohol to the PPV polymer is a viable synthetic method. 9'- (4-bromobutyl)-spirooxazine was successfully synthesized. A simple SN2 reaction should add an azide to the 9'- (4-bromobutyl)-spirooxazine, and the SO-N3 will be ready to be “clicked” onto the alkyne-functionalized PPV. An initial Click trial reaction was completed, which was very low yielding and created both 1,4 and 1,5 substituted triazole ring products. The second half of this work was largely biology-based. In hopes of elucidating whether or not fish immune cells phagocytose a common environmental toxin, researchers attempted to synthesize a pH-sensing bodipy-naphthalene probe. The aminated naphthalene model compound was successfully synthesized; however, researchers were never able to create or isolate the aminated bodipy model compound because the final SN2 addition of triethyl diamine to brominated bodipy was not successful.
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INTRODUCTION

The main goal of the Harbron Lab is to manipulate fluorescence using external stimuli. Fluorescence is the emission of light that occurs when an electron relaxes to its ground electronic state \(S_0\) from an excited electronic state \(S_1\) of the same spin number. An excited state molecule can relax through a variety of competing radiative and non-radiative pathways including: fluorescence, phosphorescence, collisional quenching, internal conversion and intersystem crossing (Figure 1.1).¹ Fluorescence quantum yield is the efficiency of fluorescence for a particular molecule and is dependent on the rate of excited state energy decay through non-radiative pathways. As the rate of relaxation for non-radiative decay increases, the fluorescence quantum yield decreases. Therefore, fluorescence is typically observed in highly rigid molecules that have limited degrees of rotational freedom, which make them less likely to relax via non-radiative pathways.

![Jablonski Diagram of Fluorescence and Competing Relaxation Pathways](image)

Figure 1.1: Jablonski Diagram of Fluorescence and Competing Relaxation Pathways¹
Fluorescence spectroscopy is an inherently sensitive technique that is widely used. Since fluorescence does not occur in all molecules, it has very low background noise when compared to other forms of spectroscopy. This sensitivity allows fluorescence to be used in a variety of different ways including: system probing for substances present in only trace amounts, locating biological species of interest within a cell, and automated DNA sequencing.²

In this project, researchers investigated the synthesis of fluorescent systems that turn off and on in response to external stimuli. The first half of this work is materials-based and involves turning the fluorescence of a polymer-photochrome system on/off in response to light stimuli using fluorescence resonance energy transfer (FRET) as the quenching mechanism. The second study is more biological in nature and involves turning the fluorescence of a Bodipy fluorophore off/on in response to pH using photoinduced electron transfer (PET) as the quenching mechanism. Both fluorescent systems and their corresponding quenching mechanisms will be discussed in detail below.

**Polymer Fluorescence Study**

The main focus of the Harbron Lab is turning the fluorescence of conjugated polymers on/off through the use of fluorescence resonance energy transfer (FRET). FRET is a non-radiative energy transfer from a donor molecule to an acceptor molecule. The energy transfer efficiency is determined by the distance between the donor and
acceptor molecules and the amount of spectral overlap between the donor’s emission and the acceptor’s absorption spectra. In the Harbron Lab, a polymer’s excitation energy is transferred to a covalently attached acceptor molecule.

Photochromic molecules are the acceptor molecules of choice for the Harbron Lab because they allow researchers to control polymer fluorescence with external light stimuli. Photochromic molecules undergo photoinduced isomerizations when exposed to different wavelengths of light. These isomers not only have different structures but also have different absorption spectra. The photochromic molecule, Spiropyran, Figure 1.2, normally exists in its thermally stable and colorless SP isomer. UV light exposure cleaves the carbon-oxygen bond creating the colored merocyanine (MC) form of the molecule.

![Spiropyran SP and MC Isomers](image)

The MC isomer has increased conjugation, which causes the absorption of the molecule to shift from the UV region to the visible region. Over time the molecule relaxes back to its stable SP form in the absence of UV light. The emission spectrum of
poly(phenylene vinylene) (PPV) and the absorption spectra of the two forms of spiropyran are shown in Figure 1.3.

Spectral overlaps predict no energy transfer to SP from PPV and efficient energy transfer to MC from PPV. When spiropyran is exposed to UV light, it transitions into the MC isomer and accepts an energy transfer from the PPV backbone, quenching the polymer fluorescence. When the photochrome relaxes back to the SP isomer, the energy transfer will cease and the original fluorescence intensity will resume, thus allowing researchers to turn polymer fluorescence on/off using external light stimuli.

In order to manipulate the fluorescence of conjugated polymers, polymers must be synthesized with covalently-attached photochromic molecules. The usual synthetic procedure for making photochromic functionalized polymers in the Harbron Lab is often
referred to as a prepolymerization functionalization. In these procedures, both the monomer and the photochromes are synthesized together, resulting in a photochromic functionalized monomer which is later polymerized to form functionalized polymer.

Prepolymerization functionalization is highly inefficient for a number of different reasons, Figure 1.4. First of all, photochromic molecules are easily damaged by harsh conditions, so polymerization conditions often destroy the photochrome. A good example of this is found with the phototchrome spiropyran. Spiropyran is easily damaged by very basic conditions, but these same basic conditions are needed to synthesize the polymer backbone, which makes it virtually impossible to functionalize polymers with these photoresponsive molecules. Plus, prepolymerization functionalization makes it very difficult to synthesize and investigate a variety of polymer-photochrome systems. Since each synthetic scheme is different from start to finish, researchers have to start at the first step of the synthesis to attach a different photochromic molecule to the same polymer backbone.

Figure 1.4: Prepolymerization Functionalization
The alternative to prepolymerization functionalization is postpolymerization functionalization, Figure 1.5. In this procedure, the photochromic molecules are attached to the polymer after the polymerization, which enables researchers to efficiently create large libraries of functionalized polymers while simultaneously sparing the photomolecules from the harsh polymerization conditions. Postsynthetic functionalization of polymers has been successfully achieved using an active-ester containing thiophene monomer and by attaching halogens to polythiophenes.

While postpolymerization functionalization is a great synthetic alternative, it also has limitations. Since the functional groups are attached after the polymer itself is synthesized, the final polymer-photochrome product cannot be purified. Therefore, high yielding, specific reactions must be used to prevent the formation of side products and partially functionalized polymers. Lack of consistent and specific functionalization along the polymer backbone would result in polymer-photochrome systems that are difficult to characterize and are non-reproducible.

Figure 1.5: Postpolymerization Functionalization
Click chemistry is a synthetic method that should allow researchers to get around the caveats of postsynthetically functionalizing a polymer backbone. Specifically, click chemistry is a 1,3-dipolar cycloaddition of an azide to a terminal alkyne. These concerted pericyclic reactions are characterized by low activation barriers because bonds are simultaneously being created and broken. The mechanism involves an azide attacking a terminal alkyne as illustrated in Figure 1.6.

Figure 1.6: Azide attacking terminal alkyne to make 1,4- or 1,5-disubstituted triazol

Click reactions are high yielding and very specific, creating only 1,4- or 1,5-disubstituted triazol regioisomers. Therefore Click chemistry should allow researchers to create fully functionalized polymer-photochrome systems that are free of impurities. Click chemistry has been successfully used to postsynthetically functionalize poly(p-phenyleneethynylene) polymers (PPE). The post-modified PPE polymers were structurally and spectroscopically identical to those synthesized by pre-polymerization
The Harbron Lab expects to have similar success in using Click chemistry to functionalize poly(phenylene vinylene) (PPV).

PPV is a highly conjugated, fluorescent polymer that has many uses, including: LEDs, photovoltaic devices, biomedical imaging, field effects transistors, and electrochromic devices. Typically PPV is synthesized using the Gilch reaction, which is extremely basic and therefore damaging to attached photochromes, Figure 1.7.

![Gilch Polymerization](image)

Figure 1.7: Gilch Polymerization

The synthesis and functionalization of PPV polymer with spirooxazine will be investigated in this work. Spirooxazine (SO) is a photochromic molecule that is very similar to the previously discussed spiropyran. SO is preferred over spiropyran because it is more fatigue resistant. Like spiropyran, SO normally exists in its colorless and thermally stable Spiro form. When irradiated with UV light, the C-O bond is broken creating the highly conjugated MC form, Figure 1.8.
The absorption spectrum of the MC form is red-shifted due to increased conjugation. Figure 1.9 shows that the MC form of SO has great spectral overlap with PPV fluorescence. Therefore, when the SO-PPV is exposed to UV light, the SO will exist as an MC isomer that will act as an energy acceptor for the PPV, quenching the polymer fluorescence. When the photochrome relaxes back to the SP isomer, lack of spectral overlap between the Spiro isomer and PPV fluorescence should result in the restoration of polymer fluorescence.

Figure 1.9: MC-SO absorption and MEH-PPV emission spectra from Christina Davis
Figure 1.10 outlines the synthetic attempts made to create postsynthetically functionalized PPV-SO. First, researchers optimized the PPV polymerization procedure. Then researchers investigated three different synthetic methods to functionalize the PPV backbone with either an azide or an alkyne handle (steps 2-4). In step five, azide functionalized SO was synthesized. The final step in this project was the investigation of the click reaction using two model compounds.

pH-sensing Probe Study

In this biological study, researchers would like to turn the fluorescence of a fluorophore off/on in response to proton concentration. As previously mentioned, fluorescence is a sensitive technique that is often used as an environmental probing system. By creating a fluorophore whose fluorescence is turned off/on in response to
pH, researchers hope to elucidate whether or not fish immune cells phagocytose a common environmental toxin, polycyclic aromatic hydrocarbons.

It has long been understood that environmental toxins can alter the fish immune system. Evidence of this has been shown in field studies where fish from unpolluted sites have superior immune responses than fish from polluted sites. The reduced immune response of fish exposed to toxic chemicals has also been demonstrated in laboratory research. The immunosuppressant effects chemical toxins have on fish is concerning because the immune system is essential to warding off harmful bacteria, fungi, and viruses. Failure of the immune system to ward off these infectious agents can not only lead to decreased fish health but a drastic decrease in fish populations in polluted sites.

One type of environmental toxin that has been implicated in detrimentally affecting fish immune responses are polycyclic aromatic hydrocarbons (PAHs). PAH constitute a large class of organic compounds that are characterized by their fused aromatic ring systems. Figure 1.10 shows naphthalene, the smallest PAH and benzo[a]pyrene, one of the most well studied PAH due to its carcinogenicity. PAHs are created and released into the environment by both natural and man-made events. Volcanoes and forest fires are the predominant natural sources of PAHs. The man-made list of sources is much longer and includes: car exhaust, industrial power generators, production of coal tar, coke, asphalt and petroleum, oil, gas, tobacco, and charbroiled meat. When PAHs are released into the environment, they can partition
between air, water, and soil making them very tough to contain. Surface and ground water contamination can easily be obtained through a variety of factors including: airborne PAH, municipal wastewater discharge and oil spills. PAHs not only affect the plants and animals that initially absorb or consume them but they also affect many omnivorous and carnivorous animals when they find their way into the food chain.

Figure 1.11: Two well studied PAHs, Naphthalene and Benzo[a]pyrene

Extensive studies have been done to investigate the detrimental effects of PAHs on marine life. PAHs cause immunosuppressive effects in fish like reduced respiratory burst activity of macrophages, white blood cell reduction, and impaired T- and B-cell proliferation. Juvenile and embryonic fish are also adversely affected by PAH exposure during critical stages of immune organ development. PAH exposure often causes sublethal effects including anemia, germline mutations, and reduced growth. These defects can result in life-long immune deficiencies and early mortality.

The fish immune system is a highly complex. Healthy fish produce a large number of highly specific and diverse antibodies to effectively neutralize a variety of pathogens. These antibodies are produced during specific stages of B-cell maturation.
B-cell development in fish begins in the anterior kidney where lymphoid progenitor cells develop into progenitor B-cells. After extensive selection against self reactivity, progenitor B-cells eventually develop into mature B-cells and enter circulation. Mature B-cells are activated when their membrane bound immunoglobulins bind pathogens. These activated B-cells differentiate into plasma blasts that start secreting antibodies to fight the invader and memory B-cells that are stored in the fish for a later time. Eventually plasma blasts develop into plasma cells which secrete large amounts of antibodies to effectively neutralize the infectious agent and prevent disease.

PAHs are known to detrimentally affect fish B-cells in a number of ways. First, PAH toxicity in bone marrow causes the death of progenitor B-cells which leads to a reduced number of antibody generating cells and reduced humoral immunity. PAHs
also lead to the premature apoptosis of developing B-cells. The early demise of both progenitor and developing B-cells leads to a drastic reduction in both the quantity and diversity of mature B-cells produced by exposed fish resulting in a significantly compromised immune response.

The mechanisms responsible for the PAH destruction of B-cells are virtually unknown. Recently, researchers Li et al have discovered that fish B-cells are phagocytic and can therefore engulf pollutants. Li et al. showed that the majority of IgM+ fish B-cells can phagocytose latex beads up to 2 microns in diameter. This raises the question of whether or not PAH-induced B-cell destruction arises from the engulfment of PAHs.

Phagocytosis is the receptor mediated process where large particle are engulfed by the plasma membrane. This process is usually seen in macrophages and neutrophils and plays an important role in the host’s defense against invading pathogens. Phagocytosis occurs in four steps: attachment, engulfment, fusion with lysosomes and degradation. Attachment involves the recognition of the particle by the phagocyte and triggers a signal transduction cascade that leads to the extension of the plasma membrane. Once the plasma membrane engulfs the particle completely, a phagosome is created. This phagosome is directed into the cell interior for fusion with the acidic lysosomes creating a phagolysosome. Acidification of the phagolysosome is crucial to anti-infection response because microorganisms find low pH directly toxic.
and the lytic enzymes used to degrade the engulfed particle optimally function in acidic environments.\textsuperscript{23}

Due to the acidic environment of the phagolysosome, a pH responsive fluorescent probe attached to a PAH would be a great system to use to discover whether or not PAHs are phagocytosed by fish B-cells. Since fluorescence does not occur in all molecules and therefore has low background noise, it is an inherently sensitive technique that is very useful in system probing. Because pH is so important to
biological systems, extensive researcher has been done to discover increasingly better
ways to probe microenvironments of their proton concentration. Photoinduced
electron transfer (PET) fluorescence is an amenable technique to probe
microenvironments for pH. Thus researchers have endeavored to design and investigate
systems to detect varying proton concentrations with PET.

PET is a process where an electron is transferred from a receptor molecule to a
fluorophore, Figure 1.14. This transfer efficiently depletes the energy created by
excitation which quenches the fluorescence of the system. Since an electron is
transferred from one species to another, the process is very akin to a redox reaction.
Therefore the standard reduction potentials of the two species are needed to choose
components that will collaboratively participate in the exchange. The efficiency of this
transfer can be manipulated by the binding of the receptor to certain species of
interest. Thus this method can be used to probe environments for species that affect
the transfer efficiency, like proton concentration.
Figure 1.14: PET example with an amine as a receptor molecule

The target probe seen in Figure 1.15 which includes a naphthalene PAH linked to a bodipy fluorophore with a diamine linker was chosen as the pH sensitive probe for this investigation.

Figure 1.15: pH sensitive probe make up
In neutral or basic conditions, like the exterior or interior of the cell, the diamine will not be protonated and the bodipy fluorescence will be quenched via PET. However, in acidic conditions, like the phagolysosome, the diamine will be protonated and the bodipy molecule will fluoresce, thus creating an affective system for investigating whether or not PAHs are phagocytosed by B-cells.

![Diagram of probe at high and low pH](image)

Figure 1.16: Probe at high and low pH

This work investigates the synthesis of the naphthalene-diamine-Bodipy probe. The synthesis is broken up into two model compounds seen in Figure 1.17. The synthesis of these two molecules will be optimized and the fluorescence and pH-sensitive properties will be determined to ensure this is a viable method for probing a biological system. Then the two systems will be combined into one.
Figure 1.17: Model Compounds

12 Aaltonen, T; Jokinen, E.; Salo, H.; Markkula, S.; Lammi, R. Aquatic Toxicology 2000, 47, 277-289.


RESULTS AND DISCUSSION: Polymer Fluorescence Study

Step 1: PPV Gilch Polymerization Optimization

![Chemical structures](image)

Figure 2.1: Gilch Polymerization

The first step in the functionalization of PPV polymers is the synthesis of the polymer backbone. While there are multiple ways to make poly(phenylene vinylene), the Harbron Lab has learned, through the work of Katie Peth and Jordan Walk, that the Gilch polymerization is the most effective synthetic procedure. The mechanism of the Gilch polymerization, Figure 2.1, starts with the deprotonation of the acidic benzylic hydrogen by tert-butoxide. The resulting carbanion then undergoes a 1,6-elimination. The propagation step involves a carbanion attacking the benzylic carbon of another monomer. The double bond is formed by an elimination reaction, creating the PPV polymer backbone. The reaction is terminated when the base is completely consumed, which usually takes about 2 hours. After the reaction is complete, the polymer is precipitated in copious amounts of methanol and collected by Büchner filtration.
Figure 2.2: Gilch Mechanism

The traditional Harbron Lab procedure for the Gilch PPV polymerization has been previously devised by Jordan Walk and Katie Peth. In this procedure, a 500 ml 3-neck round bottom flask is attached to an addition funnel and the other two necks are each closed with a septum. All the glassware is flame dried at least twice under nitrogen or argon gas to remove any environmental water. Monomers A and B (Figure 2.1) are dissolved in dry THF and injected into the dry glassware via syringe. The monomer solution is heated to 50°C while a solution of t-BuOK in dry THF (0.07 volumetric equivalents of base to THF) is added to the addition funnel. The base / THF solution is added to the monomer solution dropwise over about 30 minutes or until the reaction
mixture turns a dark orange color and exhibits green fluorescence. Usually this color change requires the addition of about 4.2 equivalents of base. Once the solution changes color the reaction is stirred for 2 hours at 50°C. The polymer solution is then poured into vigorously stirring methanol. The polymer precipitates out of the methanol solution and is collected via Büchner filtration.

While the Glich polymerization has been previously determined to be the best synthetic procedure for PPV, this synthesis is not flawless. The biggest detriments to the reaction yield are the creation of insoluble globular polymer if the base is added to the monomers too quickly and the tendency of the polymer to stick to the Büchner filter paper in the final collection step. Hsieh suggested the addition of t-butyl-benzylchloride to prevent the formation of insoluble globular polymer.\(^1\) Harbron Lab decided to investigate the use of this additive to increase PPV polymer yields.

The mechanism of the Gilch polymerization with the t-butyl-benzylchloride additive is found below in Figure 2.3. This mechanism is very similar to the original Gilch polymerization. In the initiation step, a monomer molecule and t-butyl-benzylchloride are both deprotonated by t-BuOK. The monomer anion then undergoes a 1,6-elimination to form \((2)\). The anionic t-butyl-benzylchloride then acts as the nucleophile and attacks the electrophilic benzylic position creating \((4)\). Once \((4)\) is formed the polymer is extended via the traditional Gilch polymerization.
Figure 2.3: t-butyl-benzylchloride Mechanism\textsuperscript{1,2}

If the additive concentration is too high, two side reactions can decrease the polymer yield. Both of those competing reactions are shown above in Figure 2.3. In the first reaction, one anionic t-butyl-benzylchloride molecule simply reacts with another molecule of additive forming 4,4′-di-tert-butylstilbene.\textsuperscript{1} This decreases the yield because it consumes the t-BuOK, and a lack of base could cause the polymerization to terminate prematurely. The other side reaction involves (4) reacting with another additive molecule creating small oligomers instead of polymer. This decreases polymer yield because there is less monomer left to create polymers. While both of these side
products are easily removed when the polymer is precipitated in methanol, they should be avoided because they decrease the final polymer yield. The best way to eliminate these side reactions is to keep the concentration of t-butyl-benzylchloride low so there is kinetically less of a chance of two additives running into each other and forming side product.

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</tr>
</tbody>
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Table 2.1: Polymerizations
Since the t-butyl-benzylchloride additive had never been used in the Harbron Lab, the initial synthetic procedure was adopted from Hsieh’s work. The polymerizations attempted can be found in Table 2.1. Polymerizations 1 and 2 were a combination of old Harbron lab procedures and Hsieh’s procedures. Specifically the amount of additive used, the wet reaction conditions, and the absence of heat were from Hsieh’s work. Since the ratios of base to monomer and base to THF were not specified by Hsieh, these variables were adopted from studies done by Katie Peth and Jordan Walk. These two reaction conditions failed miserably. Instead of creating orange polymer, yellow oligomers were produced even with the addition of excess base.

Based on the Harbron Lab’s previous PPV polymerization experience, the failure of syntheses 1 and 2 was attributed to two factors. First, the t-BuOK appeared old and degraded, so the use of new base for the remaining polymerizations was essential. Second, the reaction was run in wet conditions. Wet conditions drastically reduce the efficacy of the base. For subsequent reactions, only dry THF was used and all glassware was flame dried under argon gas. While the lack of a heat source was also a deviation from the traditional Harbron PPV polymerization, this variable was not changed at this point.

The following dry reactions (3-7) appeared promising because they resulted in a red color change, the polymer was easily collected using Büchner filtration, and easily removed from the filter paper. However, the equivalents of base required to get the desired color change were drastically different from trial to trial. Also, the polymer
color was a little redder than usual and there were insoluble red flakes in the reaction flask which had never been observed before. Fluorescence testing revealed that these polymers had a fluorescence $\lambda_{\text{max}}$ of 580 nm which is much higher than the typical $\lambda_{\text{max}}$ of 545 nm for PPV in THF.

Researchers hypothesized that the shifted 580 nm peak was due to polymer crosslinking. In fact, PPV films and nanoparticles have a $\lambda_{\text{max}}$ of 580 so these crosslinked polymers are almost nanoparticle-like. The polymer solutions were filtered in hopes of removing the crosslinked polymers and their fluorescence was retested. Even after filtration, the $\lambda_{\text{max}}$ remained the same. Crosslinked polymer had never been previously observed in the Harbron Lab so the new additive method was abandoned.
Reactions 8-11 were done using the traditional Glich polymerization. These reactions were done under dry conditions, heat was introduced, and the amount of base and base to THF ratio varied. While this procedure had worked in the past, these 4 reactions were highly unsuccessful. Reaction 9 created only oligomers not polymer even with the addition of excess base. This failure was later attributed to wet THF when researchers discovered the seal on the THF bottle was broken. Reactions 8, 10 and 11 had a \( \lambda_{\text{max}} \) of 580 nm indicative of crosslinked polymers even when varying amounts of base were used.

The prevalence of crosslinked polymer was puzzling because the only way for a crosslinking reaction to occur in the PPV polymer is via a much less reactive mechanism, Figure 2.5. In this less favorable reaction, the alkyl carbon of monomer A is attacked by a carbanion forming a vinyl bond between the benzylic carbon of one monomer and the alkyl carbon of another monomer. This mechanism is less favorable because the alkyl carbon on monomer A is much less electrophilic than the resonance-stabilized benzylic carbon on intermediate 2. Therefore, the anion is more likely to attack the electrophilic benzylic carbon rather than attacking the alkyl carbon. However, since this is the only possible mechanism to create crosslinked polymers, a test reaction was conducted to investigate this pathway.
For the test reaction seen in Figure 2.6, monomer $A_i$ is used instead of monomer $A$. The only difference between monomer $A$ and $A_i$ is the lack of a terminal alkyl bromine. The lack of the terminal alkyl bromine makes crosslinking by the mechanism shown in Figure 2.5 impossible. This test reaction was carried out using the same...
amount of excess base as the previous polymerizations. The resulting red polymer was easily removed from the Büchner filter paper like the earlier polymerizations and had the same red precipitate. The fluorescence spectrum of the test polymerization is shown below in Figure 2.7. While there is a strong peak at 545 nm, there is an equally strong peak at 580 nm. Since crosslinking by the mechanism described above is impossible under these reaction conditions but a strong 580 nm peak is still observed, the large 580 nm peak must be due to another phenomenon besides crosslinking.

Figure 2.6: Crosslinking Test Reaction
Figure 2.7: Fluorescence of the Crosslinking Test Reaction in THF

While the exact reason for the 580 nm peak is still unknown, researchers concluded that the new base must have been much stronger than the old base and therefore is increasing the extent of polymerization and creating very long polymers (high molecular weight polymers). Hsieh reported that PPV solubility decreases as molecular weight increases. If researchers are creating high molecular weight polymers, polymer solubility could be reduced to the point where the polymer chains are coiling up on themselves to reduce polymer-solvent interactions. Coiled up polymers would have a fluorescence spectrum very similar to that of polymer films or nanoparticles—which are polymers that coil up to minimize polymer-solvent interactions. Thus an increase in polymer length explains the observed $\lambda_{\text{max}}$ shift and insoluble red flakes in the polymer reaction flask.
The additive method was further investigated in reactions 12-15 as a way to decrease the extent of polymerization. Since the t-butyl-benzylchloride only has one benzylic halogen it can only form a vinyl bond with one molecule of monomer. Therefore the additive terminates the PPV polymerization and effectively acts as a polymer cap, which could prevent the formation of extremely long polymer chains.

Reactions 12-15 used the additive in a room temperature environment under very dry reaction conditions. In reaction 12, the additive was reintroduced and 1.5 equivalents of base were used. This technique created polymers that initially had coloring closer to the orange color expected. However, reaction 12 eventually turned a darker red color and had a $\lambda_{\text{max}}$ of 580 nm, indicating the formation of long polymer chains despite the presence of the additive. To decrease the extent of polymerization, less base was used in reaction 13. Reaction 13 remained orange and had a $\lambda_{\text{max}}$ of 545 nm. However, reaction 9 had a very low yield, and the resulting polymer was almost impossible to collect on filter paper. Plus, the little polymer that was collected was stuck to the filter paper. Researchers decided to decrease the ratio of base to THF to 0.03 and keep the concentration of base low (<2 eq.) for reactions 14 and 15. Both of these reactions successfully made PPV polymer and the fluorescence of reaction 15 is seen below in Figure 2.8. This spectrum is very representative of what PPV polymer fluorescence should look like.
While this polymer had the desired fluorescence characteristics, it was extremely difficult to collect. In order to collect this polymer, after precipitation in methanol, some of the THF must be removed either through rotary evaporation or by leaving the solution uncovered in the vent hood over night. Once at least half of the THF is removed, the polymer can be collected on type 50 filter paper using Büchner filtration. If the final polymer sticks to the filter paper, THF should be used to rinse the polymer off the filter paper and can then be removed via rotary evaporation. The increased solubility of the 545 nm polymer versus the 580 nm polymer further supports the hypothesis that 545 nm polymer is shorter than 580 nm polymer because shorter polymer is more soluble than longer polymer.
In summary, the use of additive is imperative in PPV polymer synthesis because it acts as a cap and prevents the formation of extremely long polymer chains. While the additive concentration was kept constant in this work, the additive concentration could be varied to further refine the PPV synthesis. However, researchers must keep in mind that the additive concentration must be dilute enough to avoid yield reducing side reactions yet high enough to effectively cap the polymers. The concentration of base and base to THF ratio should also be kept low to decrease the extent of polymerization.

**Step 2: Attaching Terminal Alkynes to PPV Monomers**

Now that the polymer had been successfully synthesized, researchers turned their focus to attaching a terminal alkyne or azide to the polymer so the polymer could be used in a click chemistry reaction.

![Figure 2.9: Proposed Synthesis of Alkyne-Functionalized Monomers](image)

The above synthetic procedure was devised. This procedure involves the addition of a terminal alkyne through a Williamson Ether reaction to form alkyne-functionalized monomer. The Williamson Ether reaction was very straightforward and
resulted in a yellow oil. \(^1\)H NMR characterization showed the product was free of impurities and starting material. However, the peak integration was low for the methyl hydrogens in the protecting group. While this can partially be explained by the presence of a terminal alkyne hydrogen peak which indicates that some of the alkynes had become deprotected during the Williamson Ether experiment, the low integration could not fully be explained by the deprotection. However, the synthesis was continued despite the unexplained integration values.

The second step of the alkyne-functionalized monomer synthesis was largely based on the work of Jordan Walk. In this bromomethylation, formaldehyde is protonated to form a resonance stabilized cation. This cation is attacked by ring electrons in an electrophilic aromatic substitution reaction. A bromine ion removes the hydrogen reinstating ring resonance. The alcohol is protonated, becoming a good leaving group for the subsequent \(S_n2\) reaction with a nucleophillic bromine anion. The whole reaction is repeated on the opposite side forming a di-bromomethylated product.
While this reaction has successfully created other monomers, it created a black sludge that was impossible to characterize or purify. Therefore, another pathway was devised to create a handled polymer.
Step 3: Azide Functionalization of PPV Polymer Backbone

The above synthetic plan was suggested as a way to easily attach a handle to the PPV polymer backbone. In this reaction $\text{N}_3^-$ acts as a nucleophile in an $S_N 2$ reaction. After the addition of $\text{NaN}_3$ to the THF / polymer solution, a color change was observed. The solution color changed from red to orange to yellow over the course of the 48 hour reaction. The solution fluorescence also changed from green to blue. This fluorescence color change is indicative of the creation of oligomers. $^1\text{H}$ NMR confirmed the degradation of the polymer backbone.

While the exact mechanism is still unknown, researchers believe that the azide participated in a cyclization reaction with the alkene in the polymer backbone. This cyclization reaction would explain why the polymer backbone was degraded, resulting in the creation of oligomers. Unfortunately, the $^1\text{H}$ NMR spectrum was too busy to confirm this hypothesis.
Step 4: Alkyne Functionalization of PPV Polymer Backbone

Another synthetic scheme to attach a handle to the polymer backbone was devised and is shown in Figure 2.12. Again, this synthesis is a straightforward SN₂ reaction where the deprotonated propargyl alcohol acts as the nucleophile. Since this reaction involves using polymer, which is very time consuming to synthesize, a test reaction was devised and is shown below in Figure 2.13.
This test reaction uses a premonomer C, which is easily made in a one-step synthesis. t-BuOK is the base of choice because it is strong enough to deprotonate the propargyl alcohol but not strong enough to deprotonate the propargyl alkyne. This reaction was performed in THF under reflux conditions and revealed that 3 eqs of t-BuOK and propargyl alcohol were required to get 100% conversion.

The above test reaction was then applied to a 5% Br-functionalized PPV polymer. Unfortunately, the resulting polymer characterization was inconclusive. Since only 5% of the polymer could have been alkyne-functionalized and polymer NMR spectra have very broad peaks, there was no way to see the terminal alkyne peak in the polymer NMR. IR was used in an attempt to characterize the polymer. However, due to the limited solubility of the polymer, it was nearly impossible to get a good IR spectrum of the product. The best way to test whether or not this synthesis was successful was to move forward with the synthesis of azide-functionalized SO and the subsequent click reactions. If the click reactions between the SO-N₃ and alkyne-functionalized polymer worked, it would prove that the PPV polymer was functionalized with terminal alkyne.

Step 5: SO-N₃ Synthesis

In order to successfully make a SO-clicked polymer, SO must be synthesized with an azide or alkyne click handle. Since the synthesis of alkyne-functionalized PPV polymer was almost complete, researchers decided to functionalize SO with azide. The synthetic scheme is found below in Figure 2.14.
Figure 2.14: Azide-Functionalized Spirooxazine Synthetic Scheme 1

The first step of the synthesis involves a simple $S_{N2}$ reaction where the nitrogen is the nucleophile and the bromine is the leaving group. This reaction must be done in the presence of excess dibromobutane to avoid the creation of the side product shown below in Figure 2.15. The first reaction was completed in anhydrous acetonitrile, took 24 hours, and created dark purple crystals. All impurities were dissolved and removed when the crystals were sonicated in hexanes.
Figure 2.15: Di-indolenium salt side product

Step two of the SO synthesis is a quick room temperature reaction. This reaction is done in basic solution. The base quickly deprotonates the methyl group and the resulting electrons form a double bond. This reaction is high yielding and no further purification is necessary.

Step 3 of the reaction involves the resonance structure of the molecule that creates a zwitterion. The electrons from the carbanion attack the benzylic nitrogen on the naphthol ring forming a carbon-nitrogen bond. The oxygen of the naphthol ring then attacks the sp²-hybridized carbon forming a new C-C bond and the electrons return to the nitrogen.

This reaction yielded a black sludge, which was expected based on Jordan Walk’s previous experiments with another SO derivative. Multiple attempts were made to purify the black substance. Ethyl acetate was added to the product and the solution was sonicated for hours. The ethyl acetate turned a dark color and the large quartz like rocks were collected via Büchner filtration. After multiple sonication attempts in ethyl
acetate, the product still remained a dark color. Researchers then tried to recrystallize the dark crystals in ethanol. Even when the smallest amounts of ethanol were used, all the crystals quickly dissolved and were never recovered when the solution was cooled.

Since researchers could not successfully purify the SO derivative (12), another synthetic plan was devised to make azide-functionalized SO, Figure 2.16. This synthetic plan utilized a different SO derivative (14) that had been previously synthesized by Jordan Walk. The use of derivative (14) allowed researchers to take advantage of previous synthetic understanding of SO synthesis and purification to get around the challenging purification step.

Figure 2.16: Azide-Functionalized Spirooxazine Synthetic Scheme 2

The first step of synthetic plan 2 is the synthesis of SO derivative (14). This step has the same mechanism as step 3 in synthetic plan 1. In this reaction, nitrosonaphthalene (13) is suspended in absolute ethanol as a solution of tetramethyl
indolenine in ethanol is added dropwise. The reaction is then stirred for 2 hours under reflux conditions. While the synthetic procedure was really straightforward, the purification of this product was challenging. After the solvent was removed under vacuum, the remaining black viscous oil was dissolved in a minimal amount of ethyl acetate and filtered to collect purple crystals. These crystals were dissolved in hexanes and filtered to remove dark colored, insoluble biproducts. Pure SO crystals were then recrystallized out of hexanes and collected via Büchner filtration.

Once a pure SO derivative was made, the rest of the SO reactions were simple $S_n2$ reactions. For step 2, dibromobutane was added to a reaction mixture of (14) and $K_2CO_3$. The reaction was refluxed for 24 hours and the solvent was removed under vacuum. The resulting dark viscous oil was very similar to the oil collected in the previous step. The product was purified using the same filtration/recrystallization combination as described for the previous reaction.

Unfortunately, researchers did not have enough time to complete the final SO reaction. However, researchers Katie Peth and Elena Lepekhina were successfully able to make SO-N$_3$ using the simple $S_n2$ reaction outlined above.

**Step 6: Test Click Reactions**

While the polymer and SO were being synthesized researchers started a preliminary investigation of the click procedures in THF. THF must be used for the click reaction because it is one of the only solvents in which PPV is soluble. A model reaction
was devised (Figure 2.17) to allow researchers to perfect the click reaction in THF before applying it to the more challenging PPV polymer / SO system.

![Click Test Reaction](image)

Figure 2.17: Click Test Reaction

For the model reaction, a 1-azidohexane was created through a simple Sn2 reaction. This reaction was high yielding and required no further purification. Azidohexane was reacted with commercially available propargyl alcohol. The reaction was done in a dry environment with CuSO4 in excess THF to mimic the polymer reaction conditions. 1H NMR revealed that both the 1,4 and 1,5 substituted triazole ring products were formed in very low yields. To increase these yields, researchers planned on varying the reaction conditions.
RESULTS AND DISCUSSION: pH-sensing Probe Study

The first step in the investigation of the pH-sensing bodipy naphthalene system was to break up the system into two different model compounds seen in Figure 2.18. Researchers planned on testing the fluorescence and pH-sensitive properties of the model compounds before attempting the more difficult task of combining the two systems into one.

The naphthalene model compound synthetic plan was quite successful, Figure 2.19. In the first step methyl naphthalene is brominated in a 4-hour heated reaction. The reaction yielded yellow oil and no further purification was necessary. Step 2 of the synthesis is a simple $S_n2$ reaction with the amine acting as the nucleophile. The reaction was run in dry, heated conditions for 60 hours. Initial $^1H$ NMR characterization revealed the presence of excess amine. Column chromatography was used to purify the product.
Figure 2.19: Synthetic plan for the Naphthalene Test Product

The bodipy synthetic plan is outlined in Figure 2.20 below. The first step is a hydrolysis reaction. This reaction was done in the dark with the reaction vial covered in aluminum foil to ensure the molecules were not exposed to any light. The reaction was done in a 1:1 THF:water ratio. Initially the reaction was fairly high yielding at 75%; however, as the reaction was repeated it became very inconsistent. Researchers discovered that the reaction either worked perfectly and needed no purification or it failed miserably and was impossible to identify in the NMR. Researchers found that PM605 must be fully dissolved in a homogeneous water/THF mixture before the base was added in order for the synthesis to work. While not following this strict order of addition could prevent success, following this order did not ensure that the synthesis would work every time. The source of the inconsistency was never discovered so researchers can only say that this reaction is very sensitive.
Figure 2.20: Aminated Bodipy Synthetic Plan

Step 2 of involves the addition of PBr₃ to a chilled solution of DCM with hydrolyzed bodipy. This reaction worked very well, was high yielding, and required no further purification. Both step 1 and 2 were challenging to work with because the bodipy product sticks to glassware. For each reaction, great lengths were taken to minimize the glassware transfers in order to increase the yield.

The third step of the reaction was attempted two different ways. Initially only a weak base was used to facilitate this S₉₂ reaction. After multiple attempts, $^1$H NMR revealed that the amine was not adding to the PMBr. Then a strong base was used to deprotonate the amine to improve its nucleophilicity. Unfortunately after multiple
synthetic attempts and column purification the product could never be conclusively identified using NMR or isolated.
**CONCLUSIONS:**

While the synthesis of light-modulated fluorescent polymers using Click chemistry is not complete, its progress is very promising. Researchers discovered the PPV polymerization was creating high molecular weight polymers that were insoluble in THF. To synthesize lower molecular weight, soluble polymers, the equivalents of base should be kept low (<2), the ratio of base to THF should be kept around 0.03, and t-butylbenzyl chloride should be used to cap the polymers. The amount of t-butylbenzyl chloride added can be further investigated to fully optimize the polymerization conditions.

While researchers were unable to conclusively identify alkyne-functionalized PPV using NMR or IR spectroscopy, researchers are confident that the Williamson Ether addition of propargyl alcohol to the PPV polymer is a viable method for creating alkyne-functionalized PPV. Researchers suggest investigating two possible methods for proving that the Williamson Ether addition works. First, a test Williamson Ether addition should be conducted on highly functionalized PPV (>50%). The added functionalization should make it easier to see the terminal alkyne peak in the product NMR. If the terminal alkyne peak still cannot be identified, researchers should attempt a trial click reaction using SO-N₃. If the Click reaction works, it proves that the PPV polymer was functionalized with terminal alkyne.
The synthesis of SO-N$_3$ was attempted in two different ways. While the first synthetic plan was unsuccessful due to SO purification issues, the second synthetic plan was very promising. 9’-(4-bromobutyl)-spirooxazine was successfully synthesized and purified using the second synthetic plan and the Harbron Lab’s previous knowledge of this SO derivative through the work of Jordan Walk. All that remains, is a simple $S_N$2 reaction to add the azide, and the SO-N$_3$ will be ready to be “clicked” onto the alkyne-functionalized PPV.

An initial Click trial reaction was completed using azidohexane and propargyl alcohol. The click reaction synthesized both 1,4 and 1,5 substituted triazole ring products. While the presence and amount of regioisomers does not affect the final product and therefore is of no consequence, more research is needed to further optimize this reaction before it is applied to the polymer-SO system as this reaction is currently really low yielding.

The synthesis of a pH-sensing bodipy-naphthalene system was largely unsuccessful. While the aminated naphthalene model compound was successfully synthesized, researchers were never able to create or isolate the aminated bodipy model compound. The first step in the bodipy synthesis was very inconsistent. Even when researchers were able to successfully move past step 1, the final $S_N$2 addition of triethyl diamine to PMBr was not successful. Researchers tried using a strong base and the addition still didn’t create the desired product. Because of these detrimental issues, this project is not worth pursuing.
EXPERIMENTAL SECTION

$^1$H NMR and $^{13}$C NMR were obtained on a Varian 400 MHz instrument. All reagents were purchased from Sigma Aldrich or Across and were used as received unless specified otherwise. NMR data for all compounds can be found in the appendix.

Poly(phenylene vinylene) 20 % Functionalized Reaction # 15 (1)

A 3 neck round bottom flask was set up with an addition funnel attached to 1 neck and the other two necks were closed with septa. This apparatus was flame dried and cooled 3 times under Argon gas to remove environmental water. Monomer A (0.1015 g, 2 eq) and monomer B (0.4374 g, 8 eq) were weighed out using an analytical balance and added to an argon-charged Erlenmeyer flask. The monomers were dissolved in THF (anhydrous, 20ml) and added to the 3 neck round bottom flask via syringe. More THF (anhydrous, 180ml) was added to the 3 neck round bottom flask set up via syringe. The solution was stirred and 4-t-butyl-benzylchloride (0.18ml) was added to the stirring solution via syringe. THF (anhydrous, 60ml) was added to the addition funnel along with t-BuOK (1M in anhydrous THF, 2ml, 1eq). The base/THF solution was added to the monomer/THF solution dropwise (1 drop/sec). After all of the base/THF solution was added the monomer solution was a yellow color with blue fluorescence which is indicative of oligomers. More THF (anhydrous, 30ml) and more t-Bu-OK (1ml) were added to the addition funnel. The new base/THF solution was added dropwise until the monomer solution turned an orange color and had green fluorescence. After the
addition stopped the solution turned a much darker orange color and had green/yellow fluorescence. After stirring for 4 hours, the solution was poured into vigorously stirring MeOH (600ml). The polymer instantly precipitated turning the solution a bright red color. The MeOH/polymer solution stirred overnight and the polymer was collected via Büchner filtration on type 50 filter paper the next morning.

Alkyne-functionalized premonomer (5)

Freshly distilled acetone (70 ml) was added to a 250 ml round bottom flask along with K₂CO₃ (9g, 65 mmol), methoxyphenol (1.62 g, 13 mmol, 1 eq) and (3-bromoprop-1-yn-1-yl)trimethylsilane (3.2 ml, 3.73 g, 19.5 mmol, 1.5 eq). The flask was attached to a condenser and flushed with N₂. The solution was heated to 75°C and stirred for 48 hours. The solution turned a cloudy off white color after 12 hours. The solution cooled to room temperature and was diluted with DCM (150 ml). There was a lot of white precipitate present in the reaction flask. The solution was washed with HCL (1M, 2x75 ml). The precipitate dissolved in the washes, and the yellowish solution was rotovapped to remove the extra solvent. Yielded yellow oil with small white crystals, 1.97g, 64.7%.

Alkyne-functionalized Monomer (6)

(5) (1.9684 g, 8.4 mmol, 1 eq) was weighed out directly into a 100 ml round bottom flask. Paraformaldehyde (1.01 g), glacial acetic acid (15 ml), and HBr (4.5 ml) was added to the round bottom flask. The round bottom flask was flushed with N₂ and the solution
was heated to reflux. The solution turned black and the reaction was impossible to
work up or characterize.

**Azide functionalization of PPV polymer (7)**

PPV polymer (0.100g) was weighed out into a 50 ml round bottom flask that was
charged with N₂. THF (anhydrous, 30 ml) was added to the flask. The polymer did not
fully dissolve so THF (anhydrous, 120 ml) was added. The solution was heated to 50°C
and the polymer still did not fully dissolve. The solution was sonicated and the polymer
still would not fully dissolve. However, NaN₃ (0.027 g) was added anyway. More NaN₃
was added than the calculations suggested because the solution was so dilute. The
solution was heated overnight. After about 3 hours of reaction time, the solution
changed colors from orange to bright yellow. After 17 hours of reaction time an aliquot
was removed from the solution. The solvent was removed under vacuum and the
remaining polymer was redissolved in DCM and washed with H₂O. The organic layer
was dried on MgSO₄ and the DCM was removed under vacuum. The resulting polymer
has blue fluorescence instead of green indicating the creation of oligomers.

**Trial Reaction to form alkyne-functionalized linker (8)**

Linker (C) (250 mg, 1eq) was weighed directly into a 50 ml round bottom flask. The flask
was attached to a condenser and charged with N₂. THF (anhydrous, 10 ml) was added
to the flask via syringe along with propargyl alcohol (.13 ml, 3eq). Finally t-BuOK (1M in
anhydrous THF, 2.2 ml, 3eq) was added via syringe. The reaction was heated to reflux
and stirred for 24 hours. After 12 hours, a small aliquot was worked up by diluting the solution with DCM (10 ml), washing with HCl (1M, 4x10 ml), drying on MgSO₄, and removing the solvent under vacuum. The resulting crystals were pure in the NMR indicating a 100% conversion under these test conditions.

**Alkyne addition to 5% functionalized PPV-Br (9)**

PPV-Br polymer (0.2 g, 1eq, 5% functionalized) was added to a 500 ml 3 neck round bottom flask. The flask was charged with Argon. THF (anhydrous, 400 ml) was added to the flask and the solution was sonicated. The polymer did not fully dissolve. The flask was attached to a condenser, purged with N₂, and heated to reflux. An addition funnel containing THF (anhydrous, 20 ml), propargyl alcohol (0.1 ml, 3eq), and base (1.4 ml, 3eq) was attached to the 3 neck flask. The basic solution in the addition funnel was added slowly to the stirring polymer solution. The solution changed color from bright red to deep crimson red. After 72 hours, the reaction was cooled to room temperature. The polymer was precipitated in methanol and red polymer (0.2096g) was collected using Büchner filtration. The polymer was impossible to characterize using ¹H NMR.

**N-bromobutyl-functionalized indolenium salt (10)**

Acetonitrile (50 ml, anhydrous) was added via syringe to a N₂ charged 250 ml round bottom flask that was attached to a condenser. Dibromobutane (20 ml, 7 eq) was added to the flask via syringe. The solution was stirred and 2,3,3-trimethyl indolenine (3.9 ml, 1 eq) was added to the solution in 1 ml increments via syringe. The solution was
heated to reflux and stirred for 24 hours. The solvent was removed under vacuum leaving dark crystals behind. The crystals were purified by sonication in hexanes and recollected using Büchner filtration (yield: 6.6774 g, 94%).

**N-bromobutyl-functionalized indolenine (11)**

N-bromobutyl functionalized indolenium salt (10) (6.68 g, 0.0226 mol, 1 eq) was dissolved in water (DI, 30 ml) in a 250 ml round bottom flask. KOH (2.06 g, 1.6 eq, 0.0362 mol) was dissolved in water (DI, 85 ml) in a beaker. The KOH solution was added to the indolenium solution. Upon addition, the solution turned a brownish color and looked very cloudy. The reaction stirred for 20 minutes, and then the product was extracted out of the aqueous solution with diethyl ether (3x75 ml). The organic layer was dried over MgSO₄, and the solvent was removed under vacuum (yield: 5.32 g, 82%).

**N-bromobutyl functionalized Spirooxazine (12)**

Nitrosonaphthol (2.7252 g, 1 eq, 0.015 mol) was dissolved in freshly opened methanol (100 ml) in a 250 ml round bottom flask. The flask was attached to a condenser, flushed with N₂ and heated to reflux. N-bromobutyl functionalized indolenine (11) (5.32 g, 0.018 mol, 1.15 eq) was dissolved in methanol (20 ml) and transferred to an addition funnel. The addition funnel was attached to the reaction flask and the indolenine solution was added dropwise to the refluxing nitrosonaphthol solution. After 2 hours the reaction was removed from the heat and cooled to room temperature. The solution was black. An attempt was made to collect crystals via Büchner filtration but nothing
was collected. The solvent was removed under vacuum leaving a black viscous oil. Ethyl acetate was added to the black oil, and the mixture was sonicated. The oil still would not dissolve. The product was recollected via Büchner filtration and researchers attempted to recrystallize the oily/solid in EtOH. However, the product was very soluble in EtOH so the recrystallization failed. The product was isolated again by removing the EtOH under vacuum. The product was redissolved in MeOH, and then 2/3 of the solvent was removed under vacuum. The brown powder / black crystals were collected on a Büchner. The crystals were never successfully purified. Note: this reaction was also attempted in newly opened absolute ethanol but the resulting black oil product was the same.

**Hydroxyspirooxazine (14)**

Nitrosonaphthalene derivative (13) (2.43g, 1eq, synthesized by Jordan Walk) was dissolved in absolute ethanol (65ml) in a 250ml round bottom flask that was attached to a condenser and an addition funnel. The naphthalene/EtOH solution was heated to reflux, while N,2,3,3-tetramethyl indolenine (2.6ml, 1.15eq) was dissolved in EtOH (absolute, 65ml) in the attached addition funnel. The indolenine/EtOH solution was added dropwise to the refluxing naphthalene solution. The reaction was refluxed for two hours after the addition was completed. The solvent was removed under vacuum yielding black viscous oil. The dark oil was dissolved in a minimal amount of ethyl acetate (25ml). Purple crystals were collected using Büchner filtration. Hot hexanes was added to the crystals for a recrystallization procedure. However, researchers
noticed that not all the crystals dissolved. The hexanes/product mixture was gravity filtered to remove the dark crystalline impurities. Pure 14 was then recrystallized out of the filtrate and collected via Büchner filtration.

9’-(4-bromobutyl)-spirooxazine (15)

Hydroxyspirooxazine (14) (1.17g, 3.385 mmol, 1eq) was dissolved in a solution of freshly distilled acetone (93ml) and K$_2$CO$_3$ (0.53g) in a 3 neck round bottom flask attached to a condenser in an inert environment. Dibromobutane (0.404ml, 3.385 mmol, 1eq) was added dropwise via syringe. The solution was refluxed for 24 hours. The solvent was removed under vacuum, yielding viscous dark oil. The oil was dissolved in hot hexanes and filtered to remove the dark insoluble impurities. The product was then recrystallized out of the hexanes and collected via Büchner filtration.

1-azidohexane (16)

Sodium azide (1.73g, 26.6mmol) was dissolved in DMSO (53ml) in a 100ml round bottom flask. 1-Bromohexane (3.42 ml, 24.2 mmol) was added to the DMSO solution and the solution was stirred for 4 hours at room temperature. The solution was poured into water (DI, 100ml) and the product was extracted with diethyl ether (2x30ml). The organic layers were dried over Na$_2$SO$_4$ and the solvent was removed under vacuum to yield light yellow product (2.36g, 76.8%).

Trial Click Reaction to create (1-hexyl-1H-1,2,3-triazol-4-yl) methanol (17)
A 3-neck round bottom flask was flame dried under argon gas 3 times. 1-azidohexane (16) (1.8276g, 1eq) was weighed out into a nitrogen charged vial and dissolved in THF (anhydrous, 10ml). The THF/azidohexane solution was added to the 3-neck flask via syringe. Propargyl alcohol (0.40ml, 2.1eq) was also injected into the flask via syringe. More THF (anhydrous, 20ml), CuSO₄ (19mg), and sodium ascorbate (135 mg) were added to the reaction flask. The solution was heated to 50°C and stirred for 60 hours. After 60 hours, the solvent was removed under vacuum leaving a milky white solution. The solution was diluted with dichloromethane (30ml), washed with HCl (1M, 30ml), washed with NH₄OH (1M, 30ml), dried on MgSO₄ and the solvent was removed under vacuum. NMR confirmed the presence of 1,4- and 1,5-disubstituted triazol rings in very small quantities compared to the 1-azidohexane starting material peaks.

1-(bromomethyl)-naphthalene (18)

NBS (1.4g, 1.1eq), BPO (0.18 g, 0.1eq), and carbon tetrachloride (35 ml) were added to a 100 ml round bottom flask. 1-methylnapthalene (1ml, 1eq) was added to the solution. The flask was flushed with N₂, attached to a small condenser, and put in an oil bath at 90°C. The flask was removed from the heat after 4 hours and 20 minutes and cooled overnight. The solution was gravity filtered to remove the white solid, and the solvent was evaporated under vacuum. This yielded yellow oil (0.8g, 52%).

1-(N,N,N′-triethylene diamine methyl)-naphthalene (19)
K₂CO₃ (0.99g, 2 eq, 0.0036 mmol) was weighed out directly in a 50 ml 3 neck round bottom flask. The flask was attached to a condenser and flushed with N₂. The oily 1-(bromomethyl)-naphthalene (18) (0.8 g, 1 eq. 3.6 mmol) was transferred to the reaction flask using THF (anhydrous, 2x10ml). Lastly, N,N,N'-triethylene diamine (0.646ml, 1 eq, 0.0036 mmol) was added via syringe. NOTE: After all the additions were made, researchers discovered the THF septum was broken; therefore, the THF was not dry. The reaction was heated to 65°C in an oil bath and stirred for 60 hours. During the 60 hours the solution turned a dark brown color. The solution cooled to room temperature and was gravity filtered to remove the salt. The brown solution was rotovapped to remove the solvent, washed with HCL (2M) to remove the extra amine. Then researchers discovered the product was lost in the HCl washes.

PMOH (21)ii

In the dark, a 1:1 volume mixture of THF (200 ml) and DI water (200 ml) was stirred vigorously in a 500ml round bottom flask that was wrapped in aluminum foil. PM605 (20) (260 mg, 0.67 mmol, 1eq) and LiOH (0.34g, 20.2 eq) were added to the stirred solution. The solution was stirred in the dark for 2.5 hours. The reaction was then quenched with saturated NH₄Cl (50 ml). The bright pink aqueous mixture was extracted with CH₂Cl₂ (4x150 ml) until the aqueous layer was clear. [Note: the first organic layer is very large because the THF moves from the aqueous layer to the organic layer]. The organic layers were lowered out of the separatory funnel and directly into a flask containing MgSO₄ to minimize the loss of product due to glassware transfers. The
drying agent was rinsed extensively with CH₂Cl₂. Then the solvent was removed under vacuum yielding a reddish brown powder (yield 0.1740g, 75.4%).

**PMBr (22)**

PMOH (21) (0.047g, 1 eq) was added to CH₂Cl₂ (2.2 ml) in a 10 ml round bottom flask. The stirring dark purple solution was cooled in an ice water bath for 15 minutes. A solution of DCM (2.5 ml) and PBr₃ (13 microL, 1 eq) was added to the cool PMOH solution dropwise over 5 minutes using an addition funnel. The reaction was monitored using TLC (3:1 hexanes:ethylacetate eluent; rf<sub>product</sub>: 0.7; rf<sub>PMOH</sub>: 0.43). The reaction usually took about an hour. The reaction was quenched with cold brine (5 ml). The organic layer was dried on MgSO₄. All the glassware and the drying agent were rinsed excessively with DCM. The solvent was removed under vacuum to yield dark purple oil (0.17 g, 304%, which indicates the product was still wet). No further purification was necessary.

**PMamine (23)**

K₂CO₃ (2 eq, 0.118g) was weighed out into a 50 ml 3 neck round bottom flask. The flask was attached to a condenser and flushed with N₂. The oily PMBr (22) (0.17g, 1 eq) was dissolved in THF (anhydrous, 10 ml) and transferred to the N₂ charged flask via syringe. The PMBr flask was rinsed with excess THF (anhydrous, 10 ml). Lastly, the N,N,N′-triethylene diamine (0.08ml, 1eq) was added via syringe. The reaction was heated to 65 °C and stirred for 16 hours. The brown solution was cooled to room temperature and
gravity filtered to remove the salt. The solvent and excess amine were removed under vacuum, yielding dark purple viscous oil. Product peaks could not be identified using $^1$H NMR.

**PMamine trial 2 using a strong base (23)**

A 3 neck 25 ml round bottom flask was submerged in a dry ice/acetone bath and charged with N₂. THF (anhydrous, 3.5 ml) and N,N,N′-triethylene diamine (65 µL, 1eq) were added to the flask via syringe. After about 5 minutes, BuLi (0.23ml, 1eq) was added to the solution via syringe. The solution was stirred for 30 minutes. Then a stirred solution of PMBr (22) (0.1457g, 1eq) in THF (anhydrous, 1ml) was added to the chilled amine solution. THF (anhydrous, 1ml) was used to rinse extra PMBr out of the original flask. The reaction flask was charged with N₂, attached to a condenser and heated to reflux (68°C, oil bath temperature). The solution was refluxed for 16 hours and then cooled to room temperature. Water (DI, 10 ml) was added to the solution slowly. The aqueous mixture was washed with DCM (9x10ml) until all the color was removed from the aqueous layer. The organic layers were dried over MgSO₄, and the solvent was removed under vacuum. After the solvent was removed the product still appeared wet so it was placed in the vacuum oven to dry. Even though product peaks could not be conclusively identified using $^1$H NMR, researchers attempted to isolate the product using column chromatography (20:1 DCM:Methanol eluent). Unfortunately the product was never isolated.
APPENDIX: NMR Spectra

\[ ^1H \text{ NMR- Alkyne-functionalized premonomer (5)} \]

\[ ^1H \text{ NMR- Trial Reaction to form alkyne-functionalized linker (8)} \]
$^1$H NMR- $N$-bromobutyl-functionalized indolenium salt (10)

$^1$H NMR- $N$-bromobutyl-functionalized indolenine (11)
$^1$H NMR- Hydroxyspiroaxazine (14) Crude Product

$^1$H NMR- 1-azidohexane (16)
$^1$H NMR- Trial Click Reaction to create (1-hexyl-1H-1,2,3-triazol-4-yl) methanol (17)
Crude Product

$^1$H NMR- 1-(bromomethyl)-naphthalene (18)
$^1\text{H NMR- } 1\text{-}(N,N,N'-\text{triethylene diamine methyl})\text{-napthelene (19)}$

$^1\text{H NMR- PMOH (21)}$
$^1$H NMR- PMBr (22)