SYNTHESIS OF BROOKER'S MEROCYANINE DERIVATIVES AND THEIR APPLICATION AS A SENSOR OF HYDROGEN SULFIDE

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

ŞAFAK TEKİR

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

JANUARY 2022

Approval of the thesis:

SYNTHESIS OF BROOKER'S MEROCYANINE DERIVATIVES AND THEIR APPLICATION AS HYDROGEN SULFIDE

submitted by **ŞAFAK TEKİR** in partial fulfillment of the requirements for the degree of **Master of Science** i**n Chemistry, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Özdemir Doğan
Head of the Department, **Chemistry** _____

Assoc. Prof. Dr. Salih Özçubukçu
Supervisor, **Chemistry, METU** _____

Examining Committee Members:

Prof. Dr. Cihangir Tanyeli
Chemistry, METU _____

Assoc. Prof. Dr. Salih Özçubukçu
Chemistry, METU _____

Assoc. Prof. Dr. Görkem Günbaş
Chemistry, METU _____

Assist. Prof. Dr. Yunus Emre Türkmen
Chemistry, Bilkent University _____

Assist. Prof. Dr. Safacan Kölemen
Chemistry, Koç University _____

Date: 28.01.2022

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name: Şafak Tekir

Signature:

ABSTRACT

SYNTHESIS OF BROOKER'S MEROCYANINE DERIVATIVES AND THEIR APPLICATION AS A SENSOR OF HYDROGEN SULFIDE

Tekir, Şafak
Master of Science, Chemistry
Supervisor: Assoc. Prof. Dr. Salih Özçubukçu

January 2022, 59 pages

Brooker's merocyanine has some unique features related to its color properties. Its facile synthesis and derivatization broaden the applications in various areas. Since it can be modified with various functional groups, it can be applied as a sensor with necessary recognition modules. In this thesis, Brooker's merocyanine derivatives were designed and 10 different derivatives were synthesized for their application as sensor of hydrogen sulfide. Nitro and azide derivatives were tried to be reduced by hydrogen sulfide. Cell studies of nitro derivative were conducted using two different cell lines. Cyanate derivatives were also synthesized in order to get a turn-on fluorescent sensor for hydrogen sulfide.

Keywords: Brooker's Merocyanine, Merocyanine Dyes, Sensor Application, Hydrogen Sulfide Detection

ÖZ

BROOKER MEROSİYANİN TÜREVLERİNİN SENTEZİ VE HİDROJEN SÜLFÜR SENSÖRÜ OLARAK UYGULANMASI

Tekir, Şafak
Yüksek Lisans, Kimya
Tez Yöneticisi: Doç. Dr. Salih Özçubukçu

Ocak 2022, 59 sayfa

Brooker merosiyanin, renkler ile alakalı özellikleriyle bilinen bir moleküldür. Kolay sentezlenmesi ve türetilmesi, farklı alanlarda uygulanmasının yolunu açmaktadır. Brooker merosiyanin sensör çalışmalarında bilinen analit tanıyıcı modüller ile modifiye edilebildiği literatürde bilinmektedir. Bu tezde, Brooker merosiyanin türevleri tasarlanmış ve hidrojen sülfür sensörü olarak uygulamaları için 10 farklı türev sentezlenmiştir. Nitro ve azit türevleri hidrojen sülfür ile indirgenmeye çalışılmıştır. Nitro türevinin hücre çalışmaları, iki farklı hücre hattı kullanılarak gerçekleştirilmiştir. Hidrojen sülfür için floresan aktif sensör elde etmek için siyanat türevleri de sentezlendi.

Anahtar Kelimeler: Brooker Merosiyanin, Merosiyanin Boyaları, Sensör Uygulamaları, Hidrojen Sülfür Tanımlanması

To my beloved Pearl

ACKNOWLEDGMENTS

I would like to express my deepest gratitude towards my advisor Assoc. Prof. Dr. Salih Özçubukçu for his infinite patience and guidance through this journey.

I would like to thank Assoc. Prof. Dr. Görkem Günbaş and Dr. Zübeyir Elmazoğlu for conducting cell related studies.

I would like to thank Assoc. Prof. Dr. Özgül Persil Çetinkol and Dr. Mehrdad Farough for guiding me through fluorescence related studies and allowing me to use their instrument.

I would like to thank Dr. Aytül Saylam and Volkan Dolgun giving me infinite support.

I would like to express my gratitude to Özçubukçu Research Group member helping me survive through this lifetime of experiment.

I would like to show my deepest respect to my family for their infinite support during the hard times.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

BM	Brooker’s Merocyanine
RTD	Reactive Thiol Derivatives
TEA	Triethyl amine
HeLa	Henrietta Lacks
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 Dyes

A substance that can be natural or synthetic adhere to the surface of the substance is called dye.[1] Perkin first invented synthetic dyes in 1856, which was called Mauvine dye.[2,3] That invention enlarged the industry of creating new dyes for multiple purposes, which grows continually. In 2007, J. Seixas de Melo and his colleagues conducted Perkins's reaction and successfully isolated constituents of the Mauvine dyes (Figure 1.1), which motivates researchers to work upon dyes and their derivatives.[4]

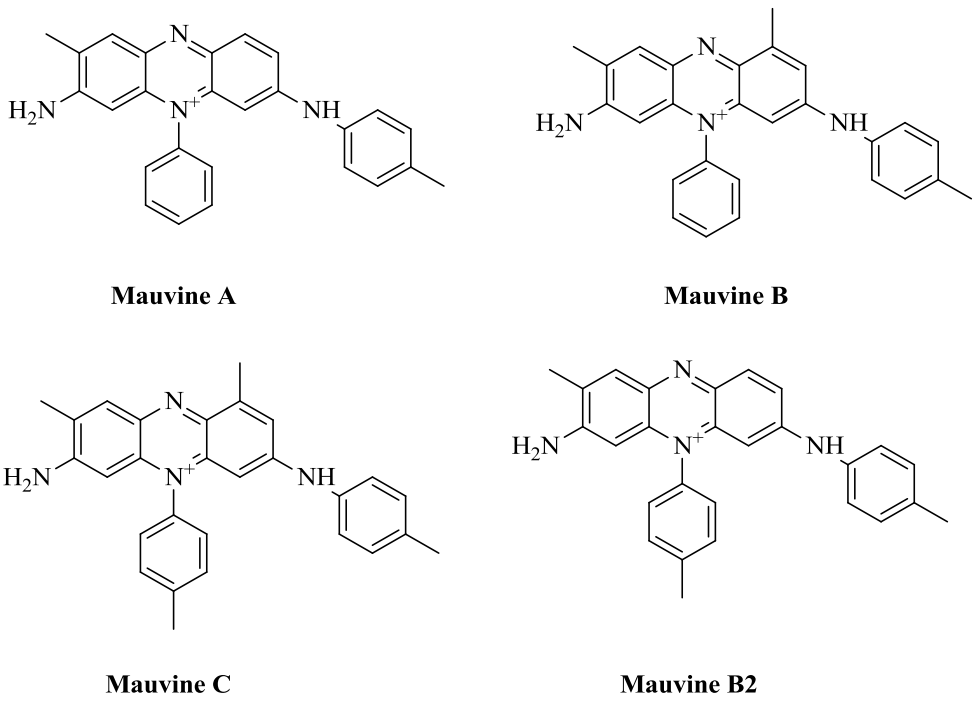


Figure 1.1. Mauvine dye constituents.

A typical dye contains two parts, and these are chromophore and auxochrome. The chromophore part contains a conjugated system that absorbs UV or visible light region. Chromophores can be colored by themselves, depending on the structure's ability to absorb light in a certain wavelength. Auxochrome, also known as a color enhancer, does not contain the color itself (Figure 1.2). However, attaching it to the chromophore will cause the dye to change its maximum absorbance value and molecule's molar absorptivity value.[5]

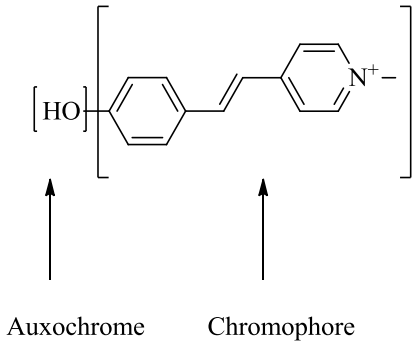


Figure 1.2. Chromophore and auxochrome displayed on Brooker's Merocyanine.

In a synthetic dye, a library of chromophores and auxochromes can form to target compounds with a specific purpose.

1.2 Reactive Dye

Reactive dyes are classified under the application methods of dyes. In the early stages of dye technology, dye molecules adhered to the surface, which causes them to become washed away after several uses. However, lately, technology has developed to decrease the wet-fastness of the dyes. Dyes attached to the surface via covalent bond achieved through the cellulose's hydroxyl functional group. This bond is obtained with a linker, which enables the dye to attach to the surface without altering the dyes' color-related properties (Figure 1.3).[5]

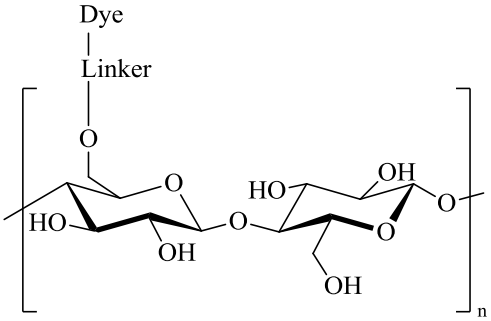


Figure 1.3. Generic reactive dye covalently bonded to cellulose.

1.3 Dye Classification by the Molecular Structure

Throughout the years, various forms of the dyes were synthesized. According to the literature, dyes can be classified in many ways, and these are some of the leading types with their examples;[5]

- Azo Dyes
- Anthraquinone Dyes
- Xanthene Dyes
- Merocyanine Dyes
- Phthalocyanine Dyes

1.3.1 Azo Dyes

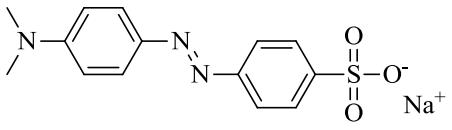


Figure 1.4. Methyl orange dye.

The name implies that azo dyes contain a nitrogen-nitrogen double bond in their molecular structure (Figure 1.4). Also, nitrogen atoms are attached to one or more aromatic groups to form their chromophore. This type of dye is derivatized upon

attachment of the electron donor-acceptor groups from the aromatic parts to form the required color.[6]

The ease of synthesis of this type of dye creates unlimited possibilities to apply in various areas.

On the other hand, recent studies show that azo dyes can be linked to various ways of pollution because of the low biodegradability and toxicity of its products.[7]

1.3.2 Anthraquinone Dyes

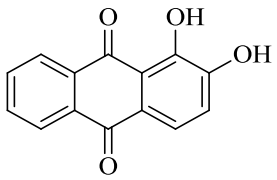


Figure 1.5. Alizarin dye.

Anthraquinone dyes contain two carbonyls fused with aromatic rings and can be modified through the aromatic parts, and they are one of the most important dye classes (Figure 1.5). Due to its properties related to the brightness and high molar absorptivity values allow users to apply in various fields.[8]

On the other hand, expenses related to synthesis and modification shape the future of the dye's applications.

1.3.3 Xanthene Dyes

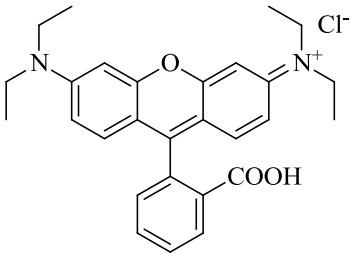


Figure 1.6. Rhodamine b dye.

The xanthene dyes contain dibenzopyran as a chromophore and are substituted from the meta position with auxochrome such as amine, hydroxy, and halides to form a xanthene derivative (Figure 1.6). The xanthene dyes, with proper modifications, can be achieved to reach many colors from pale yellow to dark blue. Also, they have fluorescent-related properties due to their rigid structure, increasing the application areas of these dyes.[9]

1.3.4 Phthalocyanine Dyes

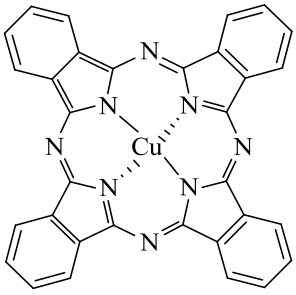


Figure 1.7. Copper phthalocyanine dye.

They were synthesized via tetramerization of phthalonitrile or phthalic anhydride at high temperatures (Figure 1.7). Besides their natural counters, they have exceptional stability. Also, they have a bright color with high molar absorptivity values. Since they are exceptionally stable, they are not much affected by modifications around the molecule, so their color can be blue or green. On the other hand, phthalocyanine

dyes contain free electrons over the nitrogen, acting as a ligand to form a complex with various metal ions. With that attachment, different colors can be achieved.[5]

1.3.5 Merocyanine Dyes

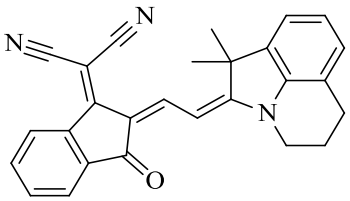


Figure 1.8. HB194 Dye.

Merocyanine dyes can be defined as a molecule containing donor-acceptor units connected via a conjugated bridge (Figure 1.8 and 1.9). Donor and acceptor units can be arranged for the requirements, and bridge length can be increased or decreased for the similar purpose. This sort of molecule exhibits exceptional brightness and molar absorptivity. Also, solubility in several solvents can be achieved with a dipolar structure. So, these properties broaden the applications of this kind of dyes.[10]

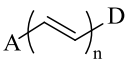


Figure 1.9. General merocyanine dye structure.

Brooker and coworkers published an article in 1951 about the merocyanine types of dyes. They investigated many forms of merocyanine types of dyes. They studied the effects of substituents and bridge lengths over the shift of the maximum absorbance value over-acidic/basic conditions. Some molecules in that study displayed extraordinary results, so in the following article Brooker and his coworkers extensively studied upon color features of the selected molecules.[11] One of these molecules is acknowledged as Brooker's Merocyanine, known for its solvatochromic properties.

1.3.5.1 Brooker's Merocyanine Dye

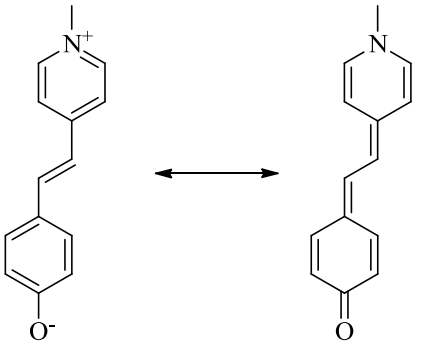


Figure 1.10. Brooker's Merocyanine resonance structure.

Brooker's color studies yield that some of the molecules have extraordinary properties.[12] One of these molecules is acknowledged as Brooker's Merocyanine (BM), known for its solvatochromic properties. Also, it has a high range of wavelength shifts with high molar absorptivity values.[13] Brooker's Merocyanine can be derivatized from several positions which actively participate in conjugation so that wavelength shifts would be controlled or arranged for a specific purpose (Figure 1.10). Synthesis of the molecule requires only several steps conducted with Knoevenagel condensation followed by the addition of iodomethane. Also, modification in starting materials might be done to derivatize BM to work on varied applications.

1.3.5.1.1 Solvatochromism

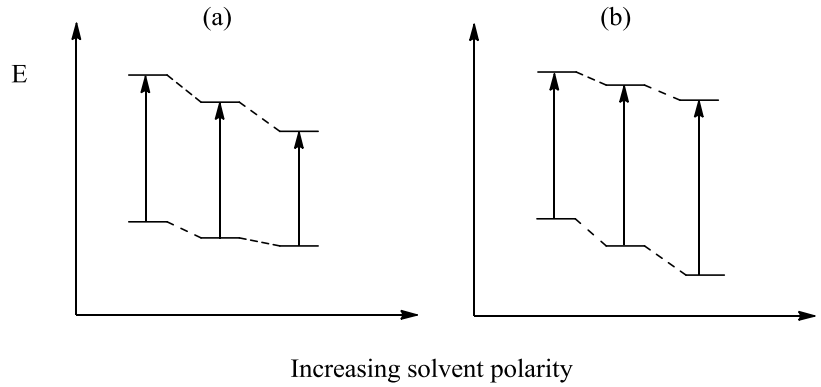


Figure 1.11. Solvents effect over solvatochromic dyes. (a) Positive solvatochromism. (b) Negative solvatochromism.

Solvatochromism can be called a molecule's energy difference between the ground state and excited state changes with the solvent's polarity changes. Sensitivity towards the solute-solvent interaction allows distinguished changes. Solvatochromic dyes, partitioned into two separate forms, one their zwitterionic form, and the other quinone form.[14,15] They are present in most solvent systems with various percentages. Solvatochromism is divided into two different subunits, and these are called positive and negative solvatochromism (Figure 1.11). In positive solvatochromism, molecules excited are the more polarized version of the dye and will be stabilized by the more polar solvents. Thus, the molecule's energy gap will be decreased, and the molecule will be red-shifted. On the other hand, in negative solvatochromism, the molecule's excited state is a less polar form of the dye. Thus, it will stabilize in its ground state when solvent polarity increases, so the dye will be blue-shifted.[16]

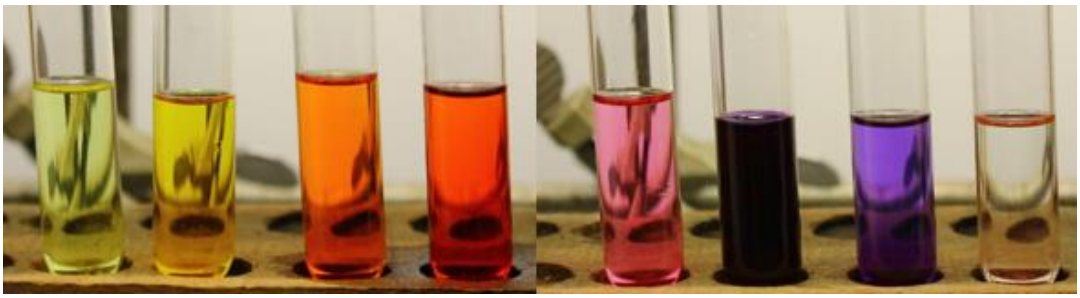


Figure 1.12. Solvatochromism of Brooker's Merocyanine. (From left to right) Acetic acid, water, methanol, ethanol, isopropanol, dimethyl sulfoxide, acetone, dichloromethane.

Solvatochromic dyes can display solvatochromic behavior in a mixture of solvents.[17] Polarity change in a moment will change the molecule's interaction with the environment, and color change will be observed (Figure 1.12). From that point of view, Jason Lye claimed the patent that solvatochromic dyes can be used to detect bacteria in water samples since most of the bacteria's outer parts are apolar.[18]

1.3.5.2 Intramolecular Charge Transfer (ICT)

In a bridged donor-acceptor molecules, donor part has higher electron density and electrons will flow through the bonds upon excitation, towards to acceptor part which has lower electron density. During this procedure, positions of the electrons will shift and alter the electronical structure of the molecule so energy difference would change which expressed as color change. Absorbed energy allow the molecule to reach to locally excited state and if the activation energy between the locally excited state and ICT state sufficiently low enough, system will shift towards ICT state and relaxation through radiation occurs.[19]

1.4 Applications of Brooker's Merocyanine Derivatives

1.4.1 Ligand Application of Brooker's Merocyanine Derivatives

The definition displays that merocyanine types of dyes contain donor-acceptor moieties, which can be tailored for the purpose such as ligand to make complex. In Cesar Zunica's article, BM derivative was used as a ligand to form a complex with the ruthenium (II) for the application of non-linear optics (Figure 1.13).[20]

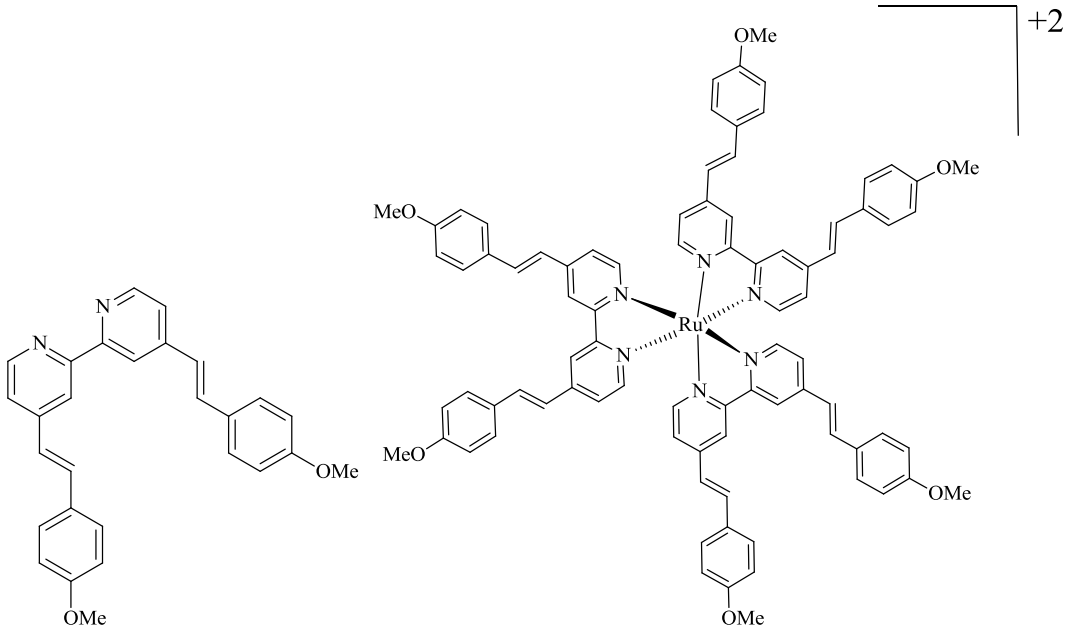


Figure 1.13. Ligand application of Brooker's Merocyanine derivative. (left) Ligand, (right) Ligand Metal Complex.

1.4.2 Sensor Application of Brooker's Merocyanine Derivatives

Sensor applications are common using such dyes. Since altering electronic structure with such labile system will be arranged by the scientists for targeted purposes. In chemosensor, a dye attached with recognition moiety, which is specific for the analyte, reacts with the analyte, and polarization of the molecule will be reverted, which will be observed as signal change. In literature, Jaqueline Nicolini and her co-

workers modified Brooker's Merocyanine with boronic acid, which reacts with fluoride anion and returns to the original form of Brooker's Merocyanine, and absorbance will shift towards the visible region (Figure 1.14).[21]

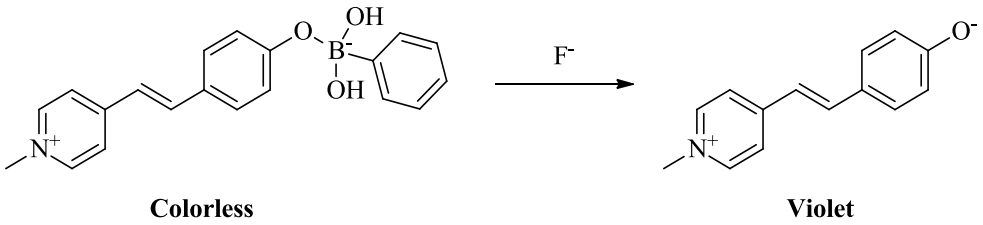


Figure 1.14. Sensor application of Brooker's Merocyanine derivative.

1.5 Importance of Hydrogen Sulfide

The human nose evolved so that we can detect hydrogen sulfide odor in low ppm levels since it is lethal to consume over 100 ppm.[22] However, recently scientists discovered that hydrogen sulfide is the third gasotransmitter that actively participates in regulating various pathways.[23,24] In vivo, it is estimated that hydrogen sulfide concentration reaches up to the low millimolar levels.[25] Endogenous hydrogen sulfide production is important due to fluctuations or over/under production affiliated with several diseases such as Alzheimer's disease,[26] Down's syndrome,[27] diabetes.[28] Thus, accurately measuring hydrogen sulfide reaches great importance.

1.5.1 Methods of Measuring Hydrogen Sulfide

Various methods were developed for the purpose with different strengths and weaknesses. Zhiqin Yuan and coworkers released an article in 2015 about colorimetric analysis of hydrogen sulfide by using carefully designed gold nanoparticles, which are reduced with hydrogen sulfide and products crosslink with each other. Results are observed as a red shift.[29] In 1993, Joel Radford-Knoery and coworker published an article about the determination of hydrogen sulfide in water samples by using gas chromatography.[30] Yuan Zhang and fellow researchers studied

hydrogen sulfide effects over the aging process. They used the electrochemical analysis method to measure the concentration of hydrogen sulfide in human plasma.[31] Also, in various articles, dyes modified with recognition modules react with hydrogen sulfide, and products are assessed using fluorescence as a method of study.[32–34]

1.5.1.1 Fluorescence

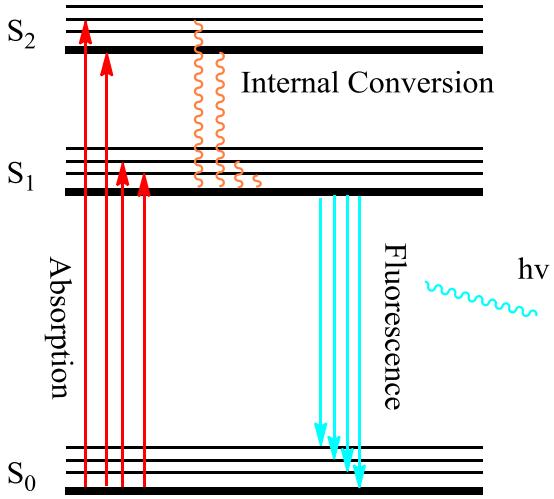


Figure 1.15. Jablonski diagram.

Jablonski introduced a diagram (Figure 1.15) that simplifies how fluorescence signals are produced. An electron absorbs energy from the ground level (S_0) to an excited level (S_n). At that level, the electron will relax through internal conversion, reaching the first excited level (S_1). Through there, the molecule relaxes through the radiative, which the observer will define as fluorescence. In this procedure, absorbance energy is lower than fluorescence energy molecules radiation will shift towards longer wavelengths.[35]

Fluorescence methodology is used for several reasons. It is a sensitive method. Also, it pierces through the skin, which allows users to observe the signal, and fluorescence methodology has its non-invasive nature is why fluorescence was chosen as a method of this study.[36]

1.5.1.1.1 Fluorescence Based Hydrogen Sulfide Sensors

Fluorescence methodology consists of various ways to approach problems. In this part, examples related to the methodology will be given. Fluorescence, active dye designed with the recognition part which reacts with the analyte and transformation of the type of donor-acceptor functional group will be observed as fluorescence signal change. It can be either turn-on or turn-off with a fluorescence signal.

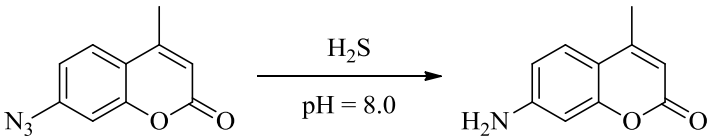


Figure 1.16. Hydrogen sulfide detection with azide functional group.

In the first example, there is an azide functional group, an acceptor group, reduced with hydrogen sulfide to an amine functional group; a donor group yielded as fluorescence turn-on in a certain emission wavelength (Figure 1.16).[34]

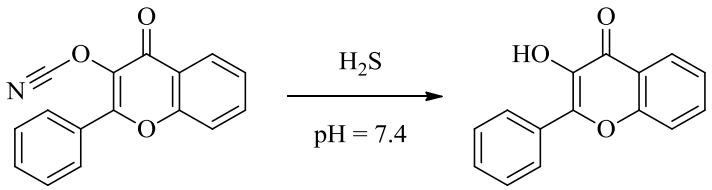


Figure 1.17. Hydrogen sulfide detection with electrophilic cyano functional group.

The electrophilic cyano functional group reacts with hydrogen sulfide. It leaves the dye to turn in the form of hydroxyl functional group derivative, which has a different electron density than starting molecule was observed as fluorescence turn-on in a certain wavelength (Figure 1.17).[33]

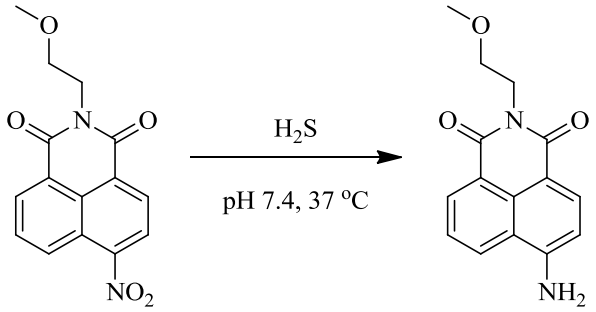


Figure 1.18. Hydrogen sulfide detection with nitro functional group.

In the literature, it was proven that aromatic nitro functional group can be reduced with hydrogen sulfide (Figure 1.18).[37] From that starting point, a dye designed with a nitro functional group is reduced with hydrogen sulfide, and again turn-on signal is observed.[32]

1.6 Aim of This Study

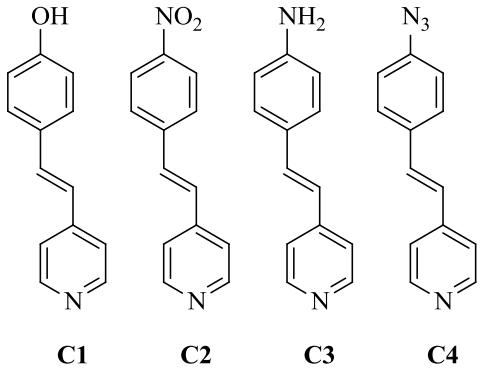


Figure 1.19. The structure of the molecules that will be synthesized in Part I.

This work consists of three parts. The first two parts are collaborative studies which cover only synthesis of the dye derivatives. In these two parts, the ability of Brooker's merocyanine derivatives to form donor-acceptor systems and responsiveness towards changes around the molecules allows the application of them as reactive dyes and ligands. **C1** was planned to be synthesized as a reactive dye to mimic the BM's responsiveness towards the polarity change around the molecule. **C1**, **C2**,

and **C4** were planned to be synthesized as ligands to form an iron complex (Figure 1.19).

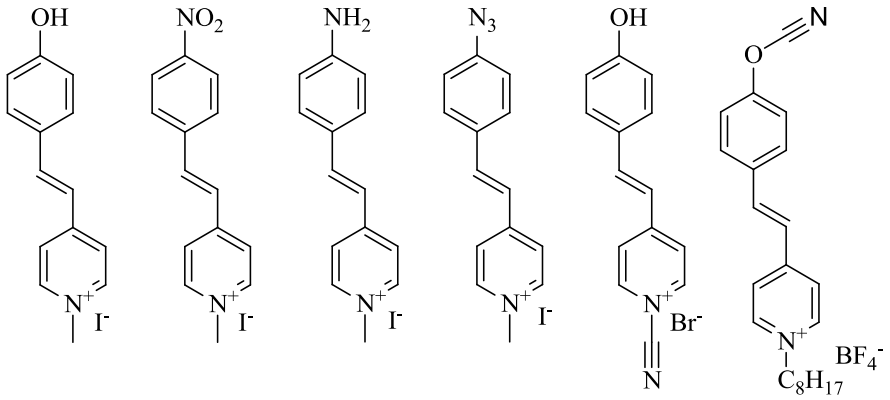


Figure 1.20. The structure of the molecules that will be synthesized in Part II.

The last and the main part of this work is about the application of Brooker's merocyanine derivatives as hydrogen sulfide sensor which is not related to their solvatochromic properties. **C5-C10** were planned to be synthesized as a hydrogen sulfide sensor with a recognition module known to react with hydrogen sulfide in literature (Figure 1.20). Although **C6** and **C8** were known in the literature, in this thesis, they were planned to be used in a different application. **C7** was considered as their control molecule. **C9** is new in literature, and **C1** was considered as control molecule of **C9**. Finally, **C10** was designed as a new molecule, and **C1-Alkylated** was planned as control molecule of **C10**.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Design of Brooker's Merocyanine Derivative as a Reactive Dye

The precursor of Brooker's Merocyanine, **C1** was synthesized by Knoevenagel condensation reaction using *p*-hydroxybenzaldehyde and 4-picoline.[38] **C1** was successfully obtained with 77% yield. A linker was attached using a substitution reaction. At the end, attachment of cellulose was successfully obtained (Figure 2.1).

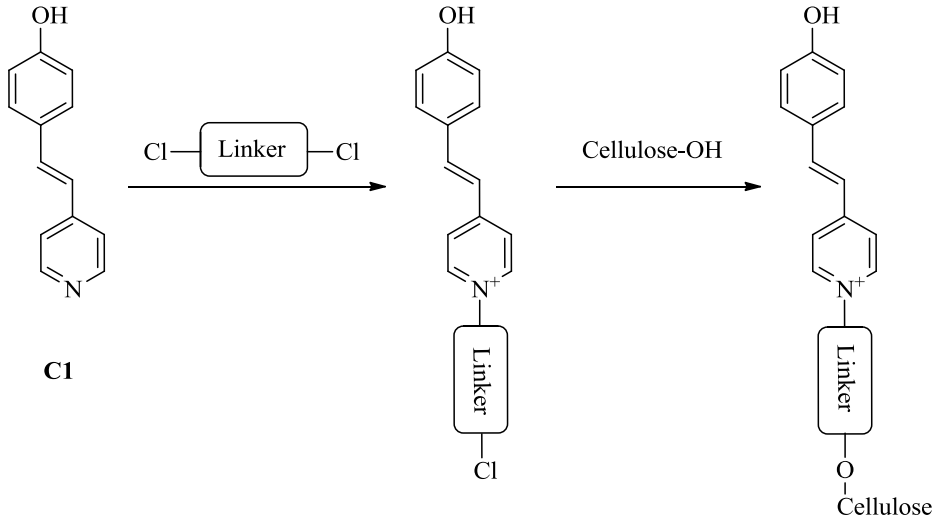
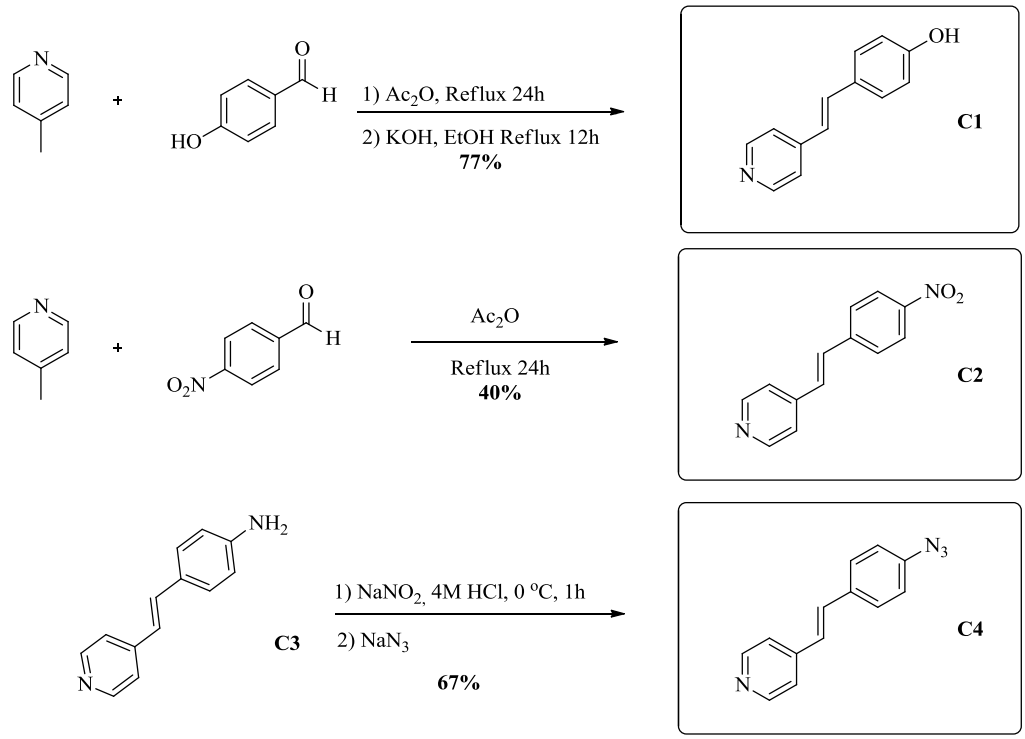


Figure 2.1. Synthesis of Brooker's Merocyanine derivative as reactive dye.

Solvatochromic dyes gave a response to the mixture of the solvent systems. Slight changes in the solutions polarity, can be observed as a color change. It was given to a textile firm to work on large scale dying process. The rest of the project will be followed by the textile firm. **C1-linker** as a reactive dye is considered responsive to the bacteria on the medium if the system's polarity shifts to either side.

2.2 Design of Brooker's Merocyanine Derivatives as a Ligand



Scheme 2.1. Synthesis of **C1**, **C3**, and **C4**.

BM derivatives having different functional groups were designed to be used as a ligand in a collaborative study with Karadaş research group. For that purpose, electron donating and withdrawing groups were substituted on the aromatic ring to form **C1**, **C2** and **C4**. **C1** was synthesized using Knoevenagel condensation. **C2** was also synthesized from 4-picoline and *p*-nitrobenzaldehyde by Knoevenagel condensation reaction.[37] **C4** was obtained from **C3** using Sandmeyer reaction with sodium azide (Scheme 2.1).[33]

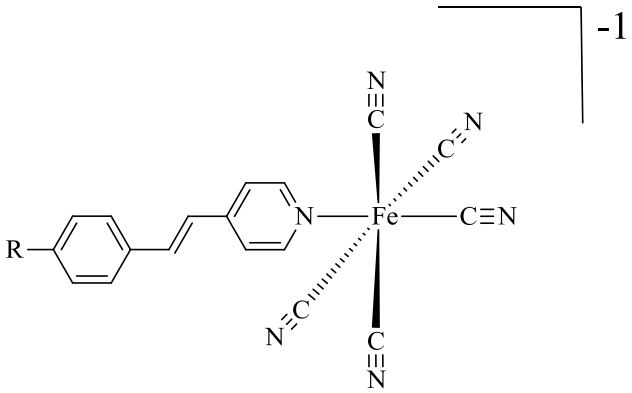


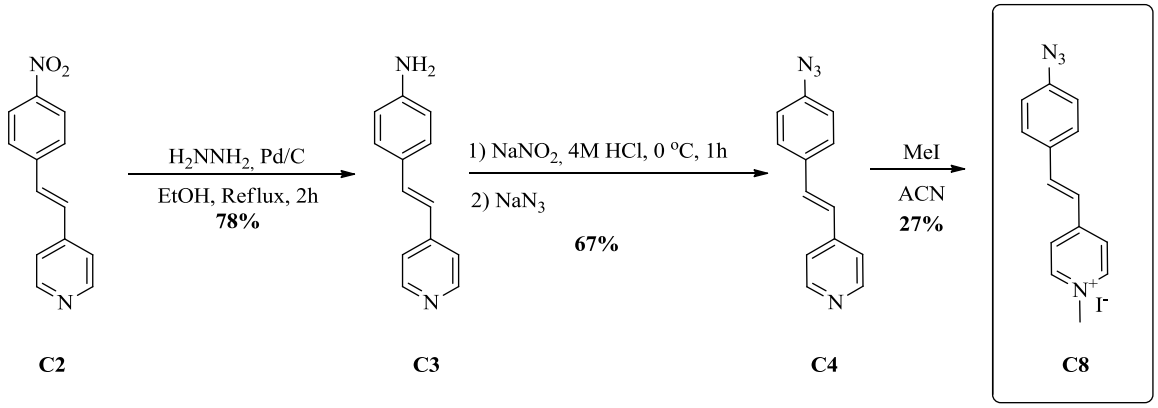
Figure 2.2. Iron complex of Brooker's Merocyanine derivatives.

C1, **C2** and **C4** will be used as ligands to form complexes with pentacyanoferrate by Karadaş Research Group (Figure 2.2).

2.3 Design Procedure of Brooker's Merocyanine Derivatives as a Sensor of Hydrogen Sulfide

2.3.1 Brooker's Merocyanine Derivatized with Azide Functional Group

2.3.1.1 Synthesis of C8



Scheme 2.2. Synthesis of **C8**.

C3 was synthesized from **C2** in 78% yield by reduction reaction with hydrazine hydrate and palladium charcoal.[37] Then, **C3** was used in Sandmeyer reaction to obtain **C4**. **C4** was successfully synthesized with a yield of 67%. After that, the methylation reaction was performed and **C8** was successfully obtained in 27% yield (Scheme 2.2).[38]

2.3.1.2 Sensor Studies of C8

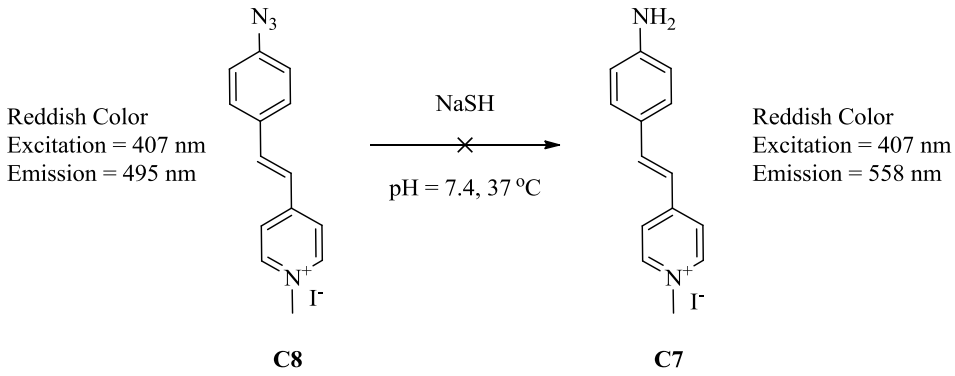


Figure 2.3. Reaction of **C8** with NaSH.

Stock solutions of **C8** and sodium hydrosulfide were prepared in dimethyl sulfoxide. The stock solution was diluted by pH 7.4 PBS buffer so that the final concentration of DMSO will not exceed 1%. 5 μM **C8** solution was treated with 100 equivalents of NaSH (Figure 2.3). In the fluorescence measurements, a red-shift was observed. Due to instability of the fluorescence signals within time, it has been hypothesized that this shift might be related to light induced decomposition of azide derivative. To test this, fluorescence measurements were performed without addition of NaSH, but in three different light conditions: total darkness, limited light exposure, and total exposure to light. As it is seen in Figure 2.4, the fluorescence signal of **C8** extinct over time when it was exposed to light. In literature, there are some dye derivatives that contain azide functional group which are reduced to the amine with intense exposure to light with certain wavelengths. For instance, a molecule structurally similar to **C8** was photo-dissociated after irradiation for 2900 seconds.[39] So, it was

thought that **C8** is very light sensitive and its fluorescence signals would have interfered with each other, and the isolation of sensor-related signals was impossible. Thus, the sensor application of **C8** was not continued.

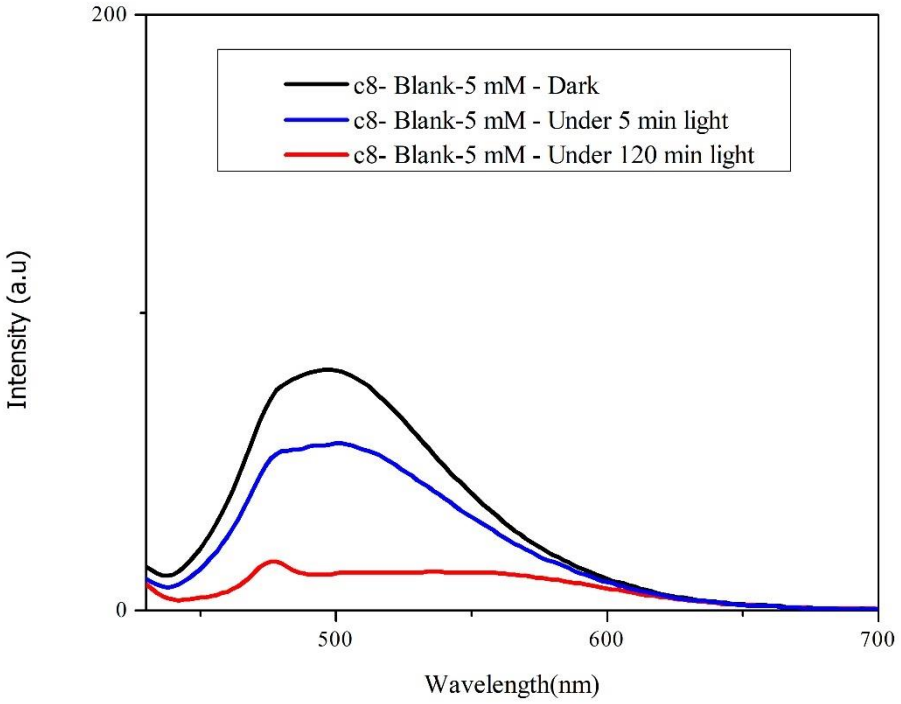
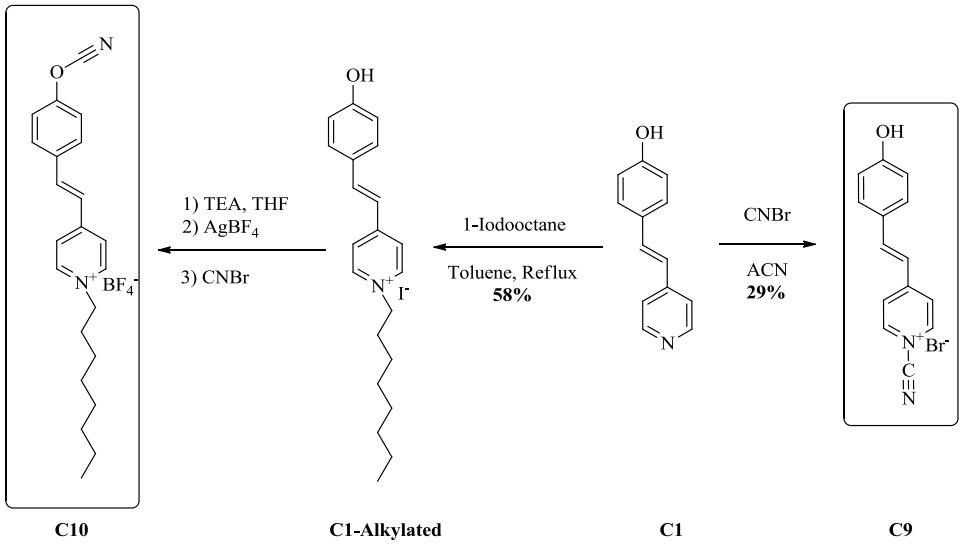


Figure 2.4. Fluorescence spectrum of **C8** light exposure in time.

2.3.2 Brooker's Merocyanine Derivatized with Electrophilic Cyano Functional Group

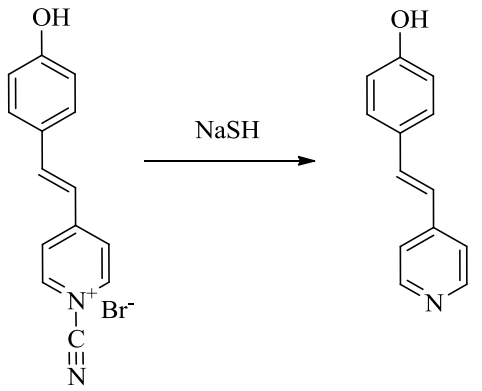
2.3.2.1 Synthesis of C9 and C10



Scheme 2.3. Synthesis of **C9** and **C10**.

C9 was obtained in 29% yield by a substitution reaction of cyanogen bromide in acetonitrile from **C1**.[40] For the synthesis of **C10**, **C1** was first alkylated by 1-iodooctane in toluene in 58% yield.[41] Then, it was treated by triethylamine and formed suspension in tetrahydrofuran. The solubility of **C1-Alkylated** was increased by salt exchange reaction using $AgBF_4$, and then cyanogen bromide was added into the reaction mixture (Scheme 2.3).[32] **C10** was obtained as a crude mixture including starting material.

2.3.2.2 Sensor Studies of C9 and C10



C9 **C1**

Orange Color in solid form Beige Color in solid form

Figure 2.5. Reaction of **C9** with Excess NaSH.

To test the reaction of **C9** with NaSH to form **C1** (Figure 2.5), NaSH was dissolved in D_2O and transferred to the solution of **C9** in DMSO-d_6. NMR spectrum was measured after 1 h. In ^1H NMR spectrum, there were expected chemical shifts for the hydrogens on the pyridine ring (Figure 2.6). In ^{13}C NMR spectrum, there was one extra peak at around 108 ppm which rises from cyanide carbon (Figure 4.11). Moreover, the resulting ^1H NMR spectrum was identical with that of pure **C1** (Figure 2.7).

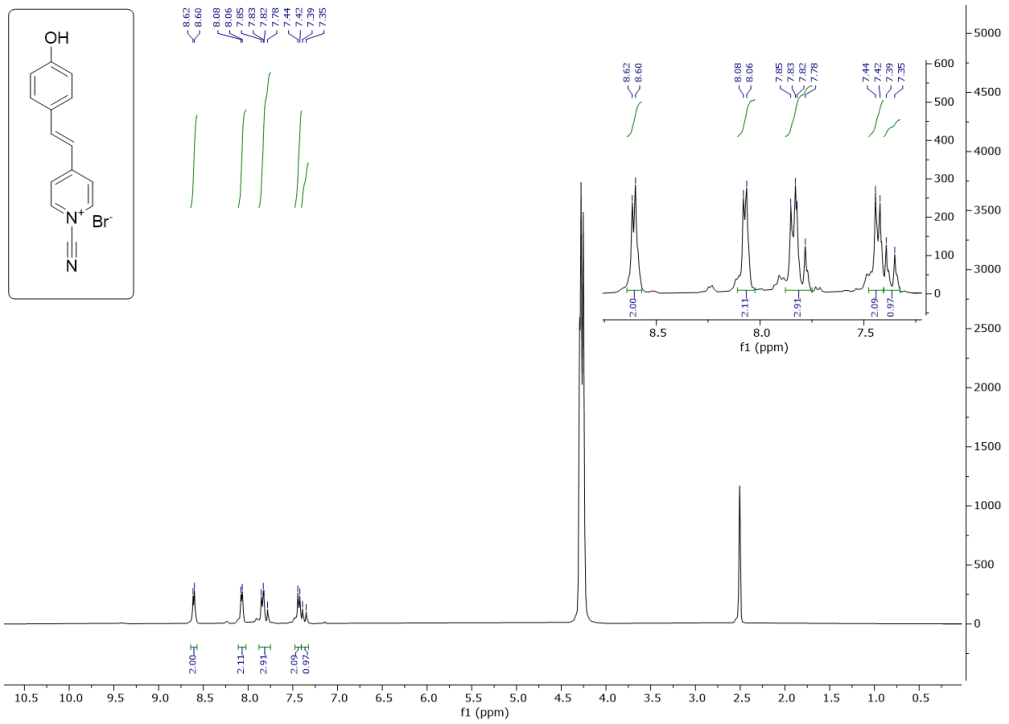


Figure 2.6. ^{1}H NMR spectrum of **C9** in DMSO-d_6:D_2O = 5:2.

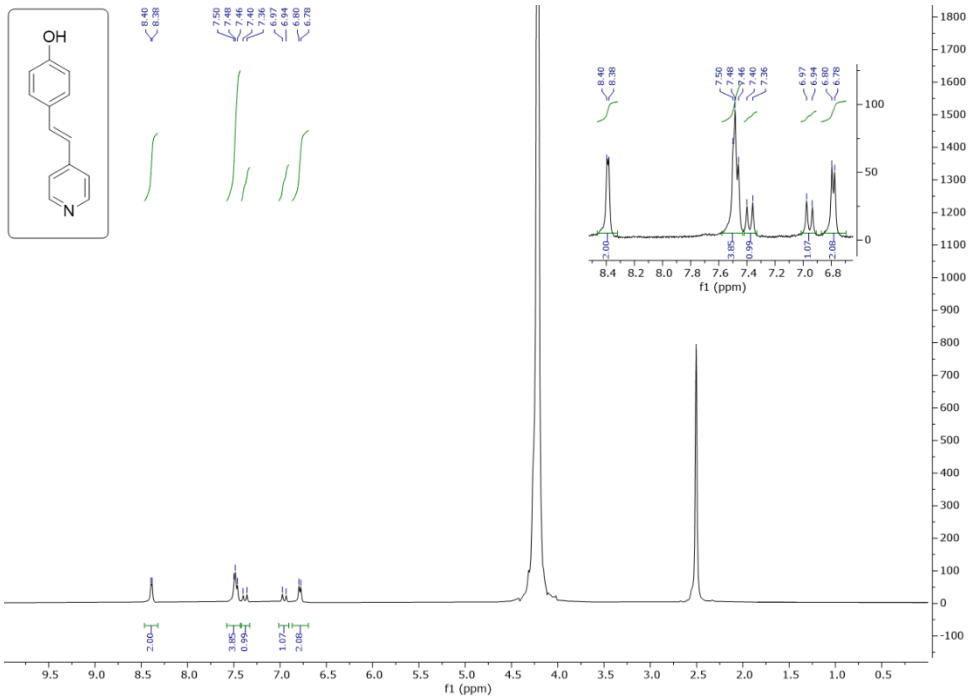


Figure 2.7. ^{1}H NMR spectrum of **C9** and excess H_2S after 1h in DMSO-d_6:D_2O = 5:2.

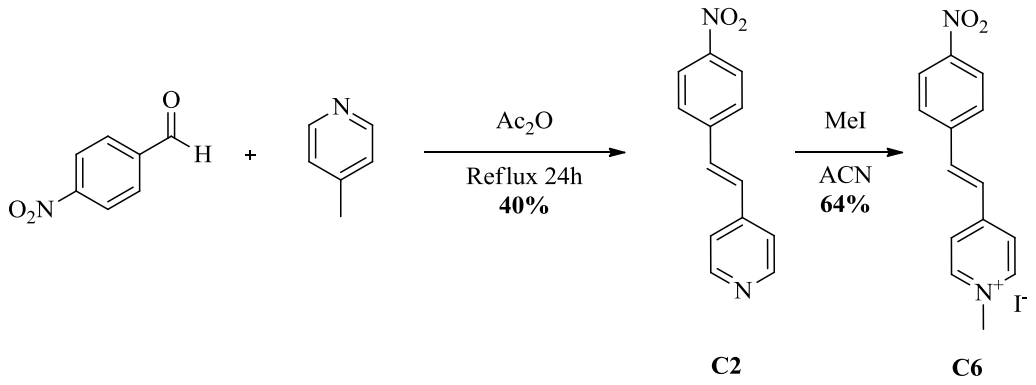
However, in literature, the reaction of cyanogen bromide with pyridine derivatives was used as a cyanation reagent that is prepared in situ.[40] This shows that cyanide group on the pyridine derivatives are quite reactive towards many different nucleophilic groups and therefore, it will not be selective towards hydrogen sulfide or any other type of thiols.

It is known in the literature that cyanates are mainly reactive towards hydrogen sulfide.[32] Therefore, **C10** was designed to overcome selectivity problem. However, in the last step of the synthesis, the reaction did not yield any product due to low solubility in THF. To overcome this problem, the alkyl group on pyridine was elongated, but it did not help to increase the solubility in THF. Salt exchange method was applied by adding $AgBF_4$ to increase the solubility in organic solvents. The corresponding salt became soluble in THF and the reaction yielded **C10** as a crude product. Stock solutions of **C10** and **C1-Alkylated** were prepared in dimethyl sulfoxide. Both were diluted by pH 7.4 PBS buffer so that the final concentration of DMSO will not exceed 1%. 5 μM **C10** solution was treated with 100 equivalents of NaSH. Turn-on fluorescence signal was observed.

In a second set of solutions, **C10** and **C1-alkylated** were diluted with dimethyl sulfoxide. 5 μM **C10** solution was treated with 1 equivalent of NaSH. Turn-on fluorescence signal was observed. However, 5 μM **C1-Alkylated** solution was treated with 1 equivalent of NaSH and there was also turn-on signal observed. Both **C10** and **C1-alkylated** were interacted with the NaSH and their fluorescence signal intensity increased. The interference caused from the turn-on signal of **C1-Alkylated** cannot be interpreted. So, **C10** transformation signal cannot be evaluated.

2.3.3 Brooker's Merocyanine Derivatized with Nitro Functional Group

2.3.3.1 Synthesis of C6



Scheme 2.4. Synthesis of **C6**.

C2 was obtained in 40% yield by Knoevenagel condensation of *p*-nitrobenzaldehyde and 4-picoline in acetic anhydride.[37] To synthesize **C6**, methylation reaction was performed with iodomethane in acetonitrile in 64% yield (Scheme 2.4).

2.3.3.2 Sensor Studies of C6

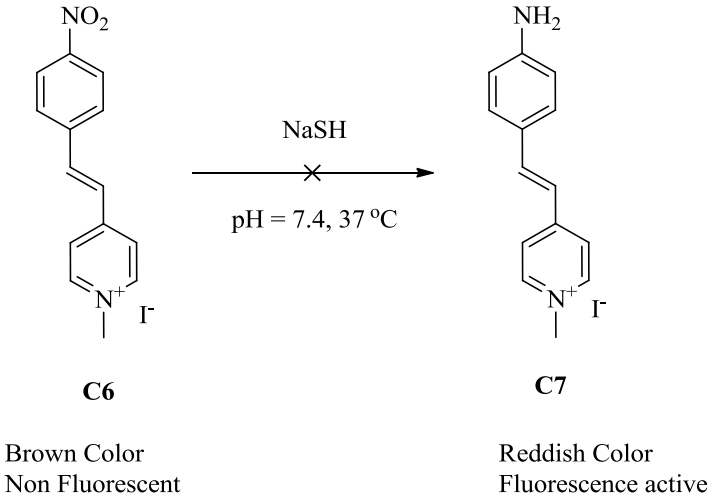


Figure 2.8. Reaction of **C6** with NaSH in pH 7.4 buffer.

Aromatic nitro groups can be reduced to corresponding amine derivatives after treatment with H_2S in buffer solutions. **C6** was tested for this reaction because after reduced by H_2S, it will be fluorescence active and can be used as a fluorescence turn-on sensor (Figure 2.8).

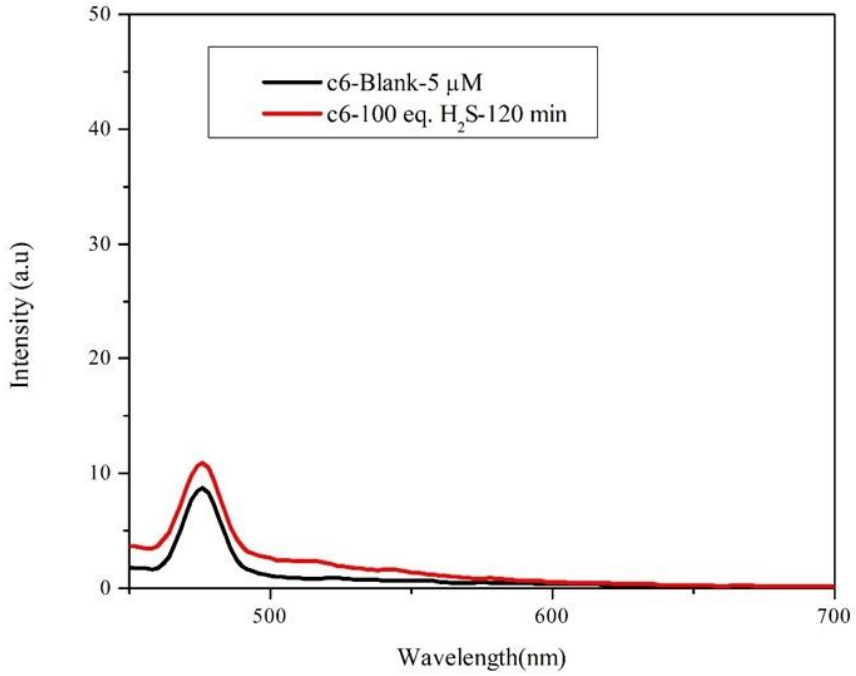


Figure 2.9. Fluorescence spectrum of **C6** with and without hydrogen sulfide (5 μM **C6**, 100 eq. H_2S, pH 7.4 PBS buffer, λ_{ex} = 407 nm).

Stock solutions of **C6** and sodium hydrosulfide were prepared in dimethyl sulfoxide. The stock solution was diluted by pH 7.4 PBS buffer so that the final concentration not to exceed 1% of dimethyl sulfoxide. 5 μM of the **C6** solution was treated with 100 equivalents of NaSH (Figure 2.8). A turn-on fluorescence signal was not observed (Figure 2.9). External salt addition and pH changes were done. However, there were still no turn-on signal observed.

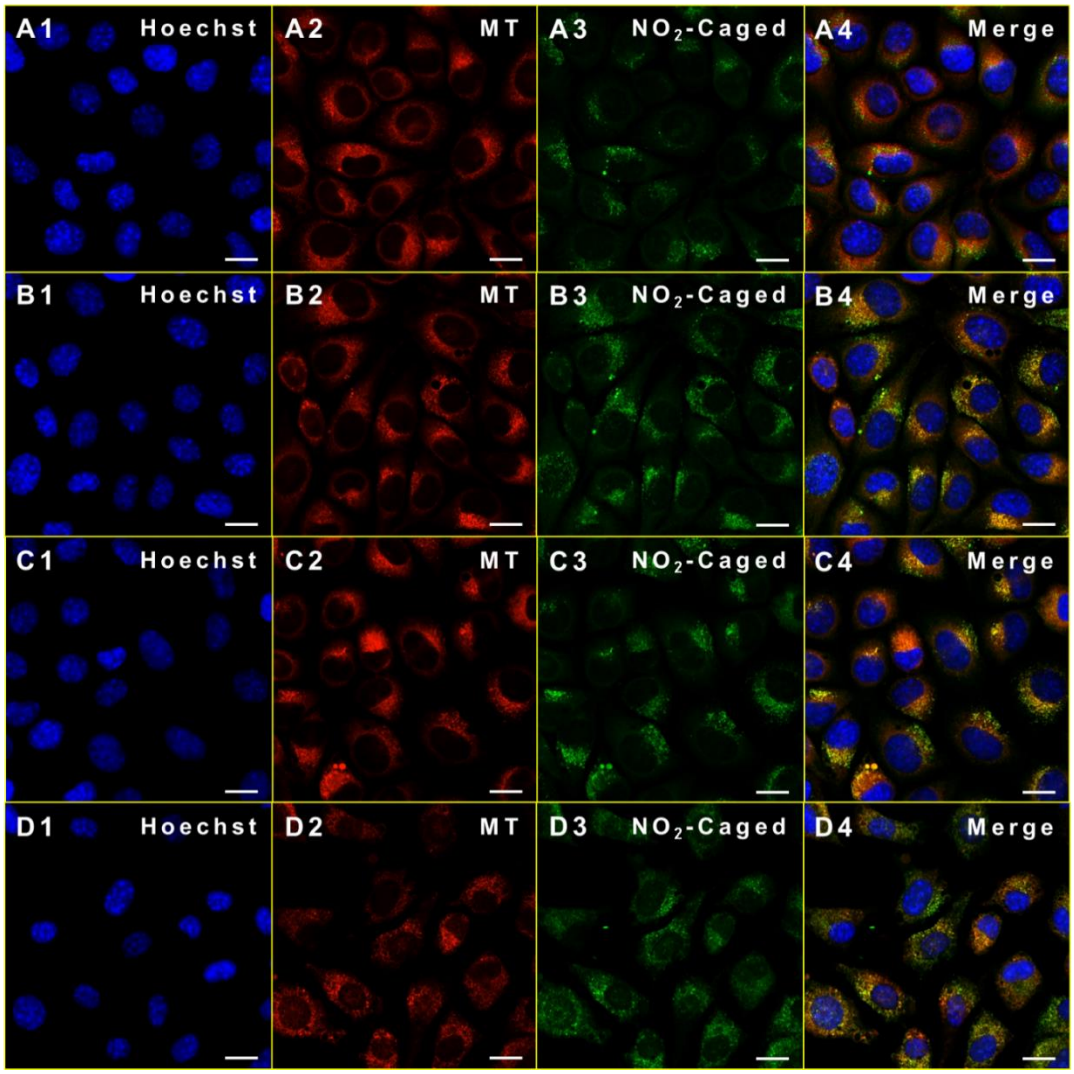


Figure 2.10. Confocal images of **L929 cells (healthy)** were treated with **C6** for 30 min alone **(A1-4)** or pretreated with 1 mM of NaSH **(B1-4)** or *N*-acetylcysteine (NAC) **(C1-4)** or *N*-ethylmaleimide (NEM) **(D1-4)** for 1 h, then followed by **C6** treatment in fresh media for 30 min. Blue: Hoechst 33342, Nucleus; Red: Mitotracker Red FM, Mitochondria; Green: **C6**. Scale bar: 10 μm.

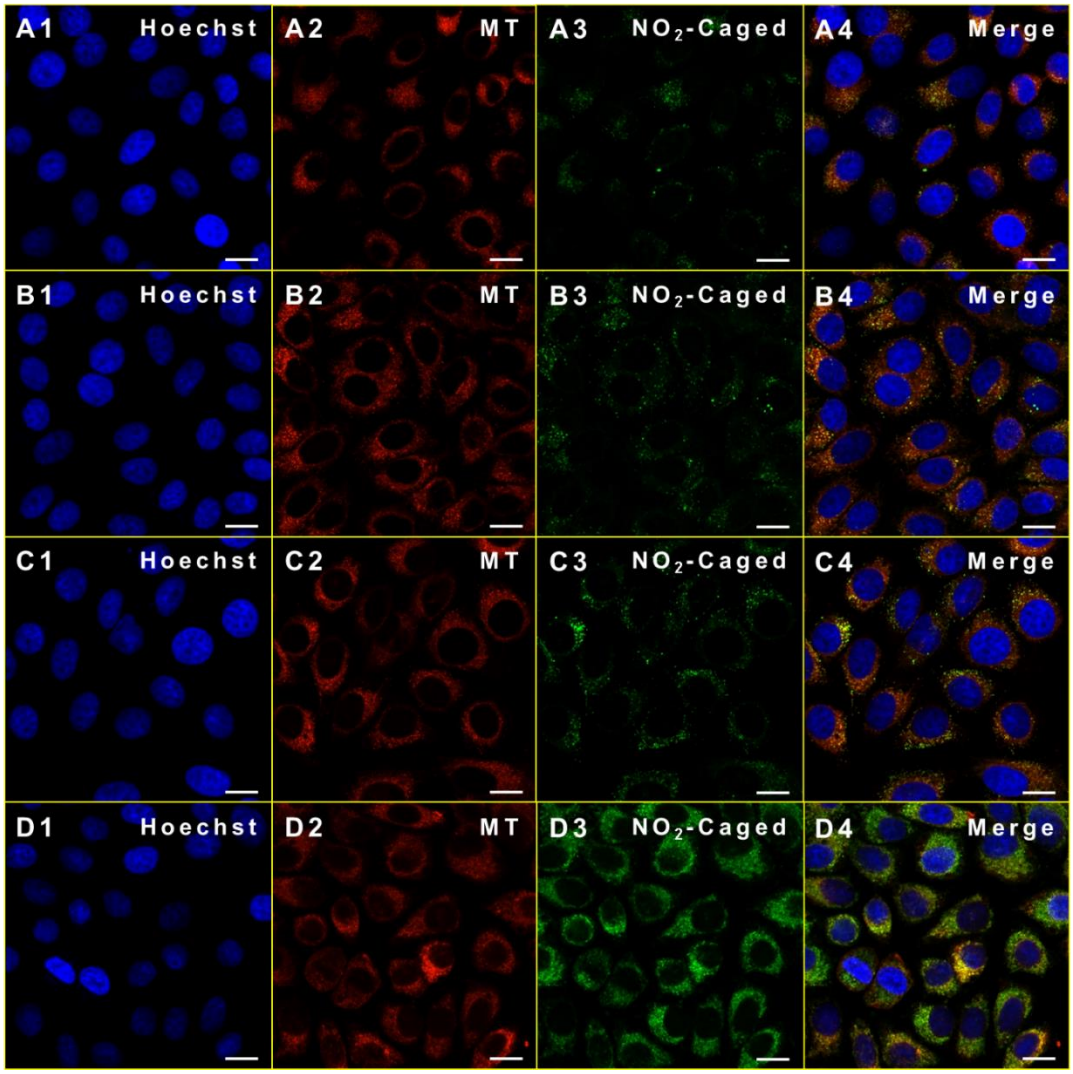


Figure 2.11. Confocal images of **Hela cells (Cervical cancer)** were treated with **C6** for 30 min alone **(A1-4)** or pretreated with 1 mM of NaHS **(B1-4)** or *N*-acetylcysteine (NAC) **(C1-4)** or *N*-ethylmaleimide (NEM) **(D1-4)** for 1 h, then followed by **C6** treatment in fresh media for 30 min. Blue: Hoechst 33342, Nucleus; Red: Mitotracker Red FM, Mitochondria; Green: **C6**. Scale bar: 10 μm.

Although, fluorescence studies showed that the nitro group on **C6** was not reduced to amine in the presence of NaSH in PBS buffer, additional experiment was required to confirm these results and cell studies were conducted. Two types of cells were used: HeLa cells and healthy L929 cells. Both cell types were treated with the **C6** solution for 30 min and incubated for another 30 min. Also, additional selectivity studies were conducted with the external addition of sodium hydrosulfide, *N*-

acetylcysteine, and *N*-ethylmaleimide. Cell nucleus tracked with Hoechst dye and mitochondria tracked with Mitotracker Red FM dye. Merge results were taken to observe the turn-on signal and its localization.

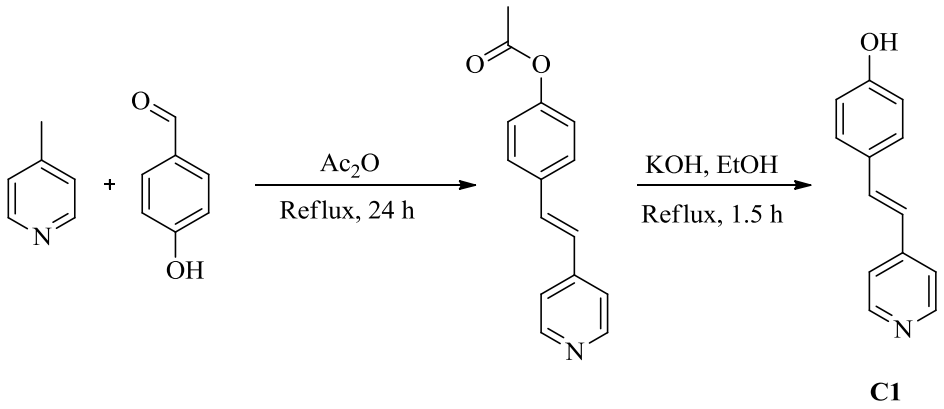
Both studies were conducted for 30 min., and in healthy cells, no distinguishable turn-on signal was observed (Figure 2.10). The **C6** was not responsive towards hydrogen sulfide and RTD (Figure 2.11). However, in the D3 column, there was a turn-on signal observed after treatment with *N*-ethylmaleimide (NEM). NEM was known as an inhibiting agent of RTD in cells. Therefore, if **C6** were not able to interact with the RTD in cells, it would have been interacted with another stimulant that convert C6 into fluorescence active form.

To find a possible reason of the turn-on signal on D3 column, additional literature survey was performed.[41,42] There is an enzyme called nitroreductase (NTR), and this enzyme reduces the aromatic nitro group in the cell environment. They are overexpressed in the cancer cells which explains the more intense turn-on signal on HeLa studies. However, in literature, NTR studies were conducted under a hypoxic environment. This study can be further extended by performing the cell studies under either hypoxic chamber or chemical inducing method with $CuCl_2$ as a future work.

CHAPTER 3

EXPERIMENTAL

3.1 Synthesis of (E)-4-(2-(pyridin-4-yl)vinyl)phenol

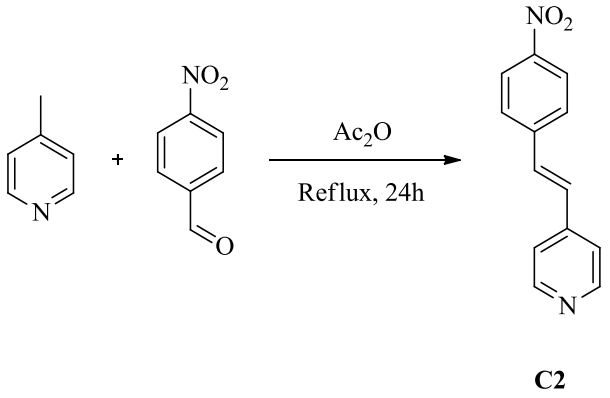


C1

Figure 3.1. Synthesis route of **C1**

In 25 mL two-necked round bottom flask, 4-methylpyridine (3.80 mL, 38.4 mmol) and 4-hydroxybenzaldehyde (4.67 g, 38.4 mmol) was dissolved in 9.50 mL acetic anhydride. The reaction was refluxed and stirred for 24 hours. The hot mixture was poured into ice-cold water and stirred for 1.5 hours. The precipitate was filtered and washed with water and recrystallized over ethanol. The reflux condition was arranged with alcoholic potassium hydroxide (0.75 M, 90 mL) for 1.5 hours. Precipitated over acetic acid and washed with water. The residue was lyophilized. Yellow solid was obtained (5.80 g, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, J = 4.9 Hz, 2H), 7.60 – 7.37 (m, 5H), 7.00 (d, J = 16.4 Hz, 1H), 6.80 (d, J = 8.5 Hz, 2H).

3.2 Synthesis of (E)-4-(4-nitrostyryl)pyridine

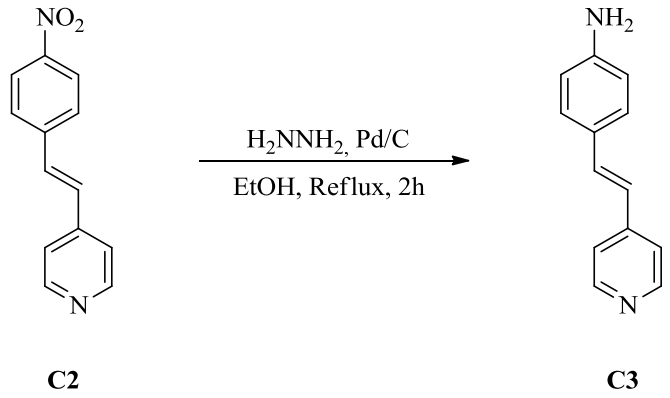


C2

Figure 3.2. Synthesis route of **C2**

In 25 mL round bottom flask, 4-methylpyridine (0.37 mL, 37 mmol) and 4-nitrobenzaldehyde (4.50 g, 29.8 mmol) were dissolved in 9 mL of acetic anhydride. The reaction was refluxed and stirred for 24 hours. After that, the hot mixture was poured into 500 mL of ice water and stirred for 90 minutes. The reaction was neutralized with NaOH and extracted with EtOAc. Concentrated under vacuum and recrystallized from EtOAc/Hex=1/1. A dark brown solid was obtained (2.70 g, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 6.3 Hz, 2H), 8.19 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 6.3 Hz, 2H), 7.29 (d, *J* = 16.4 Hz, 1H), 7.12 (d, *J* = 16.4 Hz, 1H).

3.3 Synthesis of (E)-4-(2-(pyridin-4-yl)vinyl)aniline

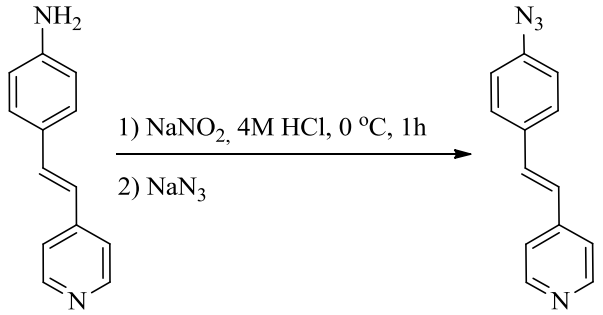


C2 **C3**

Figure 3.3. Synthesis route of **C3**

In 100 mL round bottom flask, **C2** (2.00 g, 8.40 mmol) and Pd/C (50 mg) catalyst were added to the 50 mL ethanol. 2 mL hydrazine dissolved in 5 mL ethanol was added dropwise for 0.5 hours. The system was arranged for reflux for 2 hours. Pd/C catalyst was filtered off, and the solvent was concentrated under a vacuum. Recrystallized over ethanol. Beige solid was obtained (1.35 g, 78% yield). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.45 (d, *J* = 5.0 Hz, 2H), 7.44 (d, *J* = 5.3 Hz, 2H), 7.39 – 7.29 (m, 3H), 6.86 (d, *J* = 16.4 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 2H), 5.53 (s, 2H).

3.4 Synthesis of (E)-4-(4-azidostyryl)pyridine

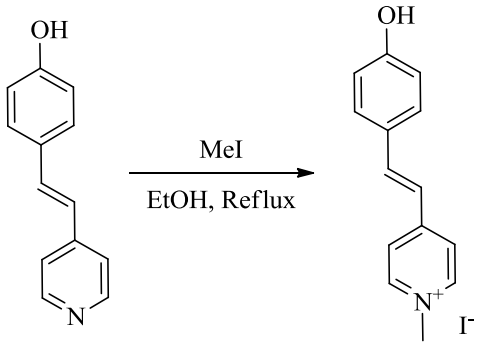


C3 **C4**

Figure 3.4. Synthesis route of **C4**.

In a 100 mL round bottom flask, **C3** (1.00 g, 5.10 mmol) dissolved in 50 mL of 4 M HCl at 2-3 °C. Sodium nitrite (0.88 g, 13 mmol) was dissolved in distilled water and added dropwise to the reaction solution. The solution was stirred for an hour at 2-3 °C temperature, and a solution of sodium azide (0.66 g, 10 mmol) in 5 mL of water was added dropwise to the mixture. The solution was kept at 2-3 °C for 30 minutes, and the mixture was allowed to reach room temperature. Reaction kept stirring overnight. The mixture was treated with saturated sodium bicarbonate until no more gas evolved. The precipitate was filtered and lyophilized. Yellow powder was obtained as 0.75 g in 67% yield. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.54 (d, *J* = 5.9 Hz, 2H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.66 – 7.50 (m, 3H), 7.31 – 7.11 (m, 3H).

3.5 Synthesis of (E)-4-(4-hydroxystyryl)-1-methylpyridin-1-ium iodide

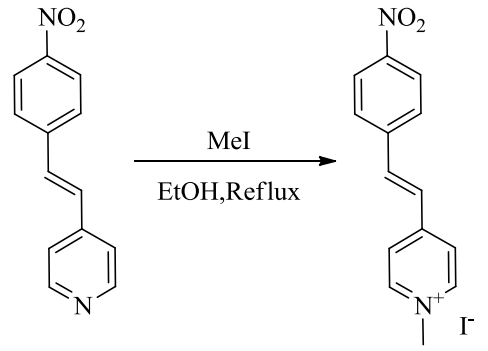


C1 **C5**

Figure 3.5. Synthesis route of **C5**.

In a 50 mL round bottom flask, **C1** (1.00 g, 5.07 mmol) dissolved with ethanol (25 mL). Iodomethane (380 µL, 6.08 mmol) was slowly added to the reaction, and the system refluxed for 24 hours while stirring. The system is followed by TLC. Reaction stopped when starting materials spot disappeared in the TLC. Then, the system was allowed to reach room temperature and concentrated under a vacuum. Recrystallized over ethanol. Red solid was obtained (1.52 g, 88% yield). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.64 (d, *J* = 7.0 Hz, 2H), 7.99 (d, *J* = 7.0 Hz, 2H), 7.87 (d, *J* = 16.1 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 15.9 Hz, 1H), 6.68 (d, *J* = 8.7 Hz, 2H), 4.14 (s, 3H).

3.6 Synthesis of (E)-1-methyl-4-(4-nitrostyryl)pyridin-1-ium iodide

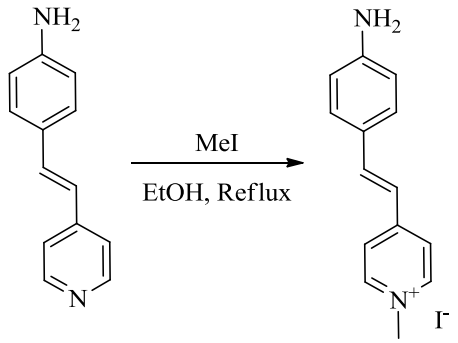


C2 **C6**

Figure 3.6. Synthesis route of **C6**.

In a 50 mL round bottom flask, **C2** (0.89 g, 3.93 mmol) was dissolved in ethanol (15 mL). Iodomethane (290 μL, 4.70 mmol) was slowly introduced to the system and refluxed for 24 hours. The procedure was followed by TLC. Reaction stopped when starting materials spot disappeared in the TLC. Then, the system was allowed to reach room temperature and concentrated under vacuum and recrystallized over ethanol. Beige solid was obtained (0.92 g, 64% yield). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.96 (d, *J* = 6.9 Hz, 2H), 8.35 (d, *J* = 8.9 Hz, 2H), 8.30 (d, *J* = 7.0 Hz, 2H), 8.14 (d, *J* = 16.4 Hz, 1H), 8.01 (d, *J* = 8.9 Hz, 2H), 7.77 (d, *J* = 16.6 Hz, 1H), 4.30 (s, 3H).

3.7 Synthesis of (E)-4-(4-aminostyryl)-1-methylpyridin-1-ium iodide

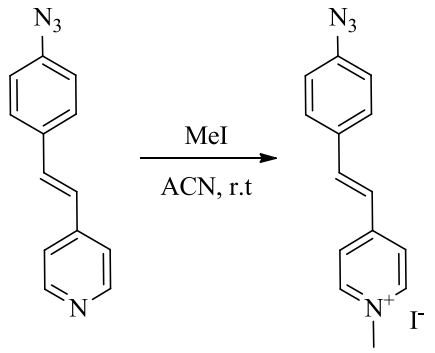


C3 **C7**

Figure 3.7. Synthesis route of **C7**.

In a 50 ml round bottom flask, **C3** (0.65 g, 3.31 mmol) was dissolved with ethanol (25 mL). Iodomethane (250 µL, 3.98 mmol) was slowly introduced to the system. The reaction was refluxed and stirred for 24 hours. The procedure was followed by TLC. Reaction stopped when starting materials spot disappeared in the TLC. Then, the system was allowed to reach room temperature and concentrated under vacuum and recrystallized over ethanol. Dark red solid was obtained (0.65 g, 58% yield). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.62 (d, *J* = 6.9 Hz, 2H), 7.98 (d, *J* = 7.0 Hz, 2H), 7.86 (d, *J* = 16.1 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.05 (d, *J* = 15.9 Hz, 1H), 6.68 (d, *J* = 8.7 Hz, 2H), 4.14 (s, 3H).

3.8 Synthesis of (E)-4-(4-azidostyryl)-1-methylpyridin-1-ium iodide

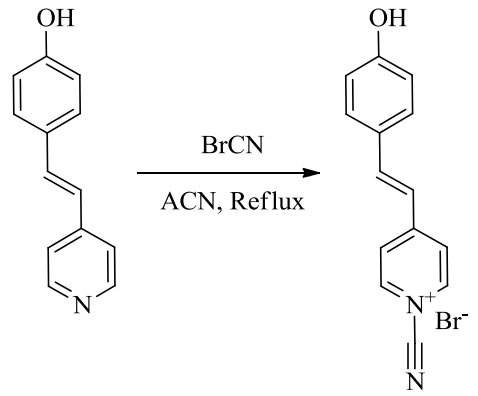


C4 **C8**

Figure 3.8. Synthesis route of **C8**.

In a 25 mL round bottom flask, **C4** (250 mg, 1.12 mmol) dissolved with acetonitrile (10 mL). The round-bottomed flask was covered with foil. Iodomethane (210 µL, 3.37 mmol) was slowly introduced to the system while stirring for 48 hours. Stirring stopped, and the reaction was allowed to precipitate. The reaction was filtered and rinsed with diethyl ether. Recrystallized over ethanol. Dark yellow solid was obtained (100 mg, 24% yield). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.88 (d, *J* = 7.2 Hz, 2H), 8.21 (d, *J* = 7.0 Hz, 2H), 8.03 (d, *J* = 16.3 Hz, 1H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 16.2 Hz, 1H), 7.26 (d, *J* = 8.5 Hz, 2H), 4.26 (s, 3H).

3.9 Synthesis of (E)-1-cyano-4-(4-hydroxystyryl)pyridin-1-ium bromide

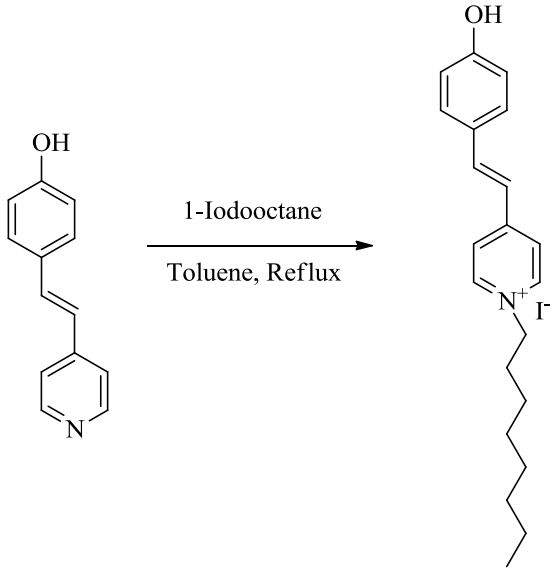


C1 **C9**

Figure 3.9. Synthesis route of **C9**.

In a 25 mL round bottom flask, **C1** (0.56 g, 2.8 mmol) was dissolved with acetonitrile (15 mL). Cyanogen bromide 3 M in DCM (2.8 mL) was slowly introduced to the system and refluxed for 24 hours. The procedure was followed by TLC. Reaction stopped when starting materials spot disappeared in the TLC. Then, the system was allowed to reach room temperature. The reaction was precipitated and followed by filtration rinsed with diethyl ether. Recrystallized over ethanol. Orange solid was obtained (220 mg, 26% yield). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.88 (d, *J* = 6.8 Hz, 2H), 8.21 (d, *J* = 5.4 Hz, 2H), 8.05 (d, *J* = 16.4 Hz, 1H), 7.94 (d, *J* = 9.0 Hz, 2H), 7.65 – 7.51 (m, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 108.7, 116.7, 123.7, 125.4, 130.7, 134.7, 138.3, 142.5, 153.5.

3.10 Synthesis of (E)-1-cyano-4-(4-hydroxystyryl)pyridin-1-ium bromide



C1 **C1-Alkylated**

Figure 3.10. Synthesis route of **C1-Alkylated**.

In 25 mL Schlenk tube, **C1** (150 mg, 0.76 mmol) and 1-iodooctane (220 mg, 0.913 mmol) dissolve with 10 mL of anhydrous toluene. The reaction mixture was arranged for reflux and kept stirring for 48 hours. The reaction was flushed with argon. The procedure was followed with regular TLC. The reaction was stopped when starting materials spot was disappeared from the TLC. Then, the reaction was allowed to reach room temperature, precipitated product was filtered and washed with hexane and ethyl acetate. By column chromatography using neutral alumina in the gradient elution of DCM/MeOH, red solid was obtained (90 mg, 28% yield). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.71 (d, *J* = 6.8 Hz, 2H), 8.01 (d, *J* = 6.6 Hz, 2H), 7.88 (d, *J* = 16.0 Hz, 1H), 7.53 (d, *J* = 8.7 Hz, 2H), 7.09 (d, *J* = 16.1 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 2H), 4.38 (t, *J* = 7.4 Hz, 2H), 1.85 (t, *J* = 6.0 Hz, 2H), 1.24 (m, 12H), 0.84 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-*d6*) δ 159.1, 153.9, 150.3, 143.7, 142.7, 131.4, 124.1, 122.7, 117.8, 59.5, 31.6, 30.9, 28.9, 28.8, 25.9, 22.5, 14.4.

3.11 (E)-4-(4-cyanatostyryl)-1-octylpyridin-1-ium tetrafluoroborate

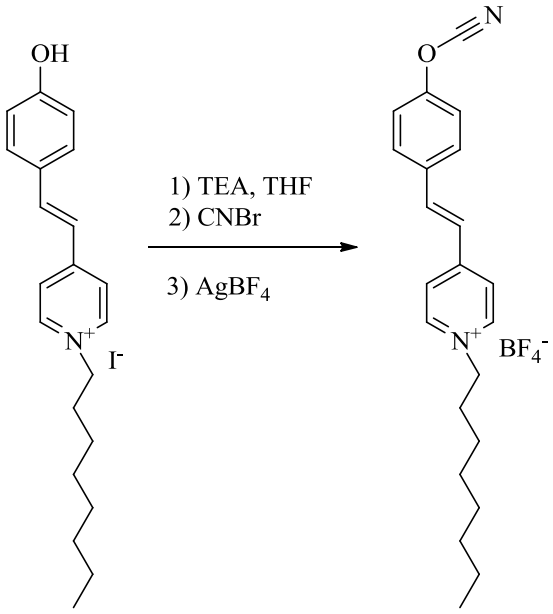


Figure 3.11. Synthesis Route of **C10**.

In a 25 mL Schlenk tube, **C1-Alkylated** (90 mg, 0.21 mmol) and TEA (25 mg, 0.25 mmol) were dissolved with 10 mL anhydrous tetrahydrofuran at 2-3 °C. The reaction was flushed with argon gas. The reaction mixture was allowed to reach equilibrium with TEA in the system for 30 minutes. Silver tetrafluoroborate (48 mg, 0.25 mmol) was introduced to the reaction, and increase in the solubility observed. Then, 3 M cyanogen bromide in dichloromethane (82 µL) injected dropwise and sudden precipitation was observed. The reaction was allowed to precipitate. By filtration method, liquid parts were separated from salts that was precipitated. Filter paper was washed with diethyl ether and dried under vacuum. Yellow/orange solid was obtained as crude product.

CHAPTER 4

CONCLUSION

In this thesis, BM derivatives were designed for the motivation of implementation. The molecules were designed to use as a reactive dye, ligand, and sensor. The synthesis of 10 different BM derivatives was completed, and three of them are not known in the literature (Figure 4.1).

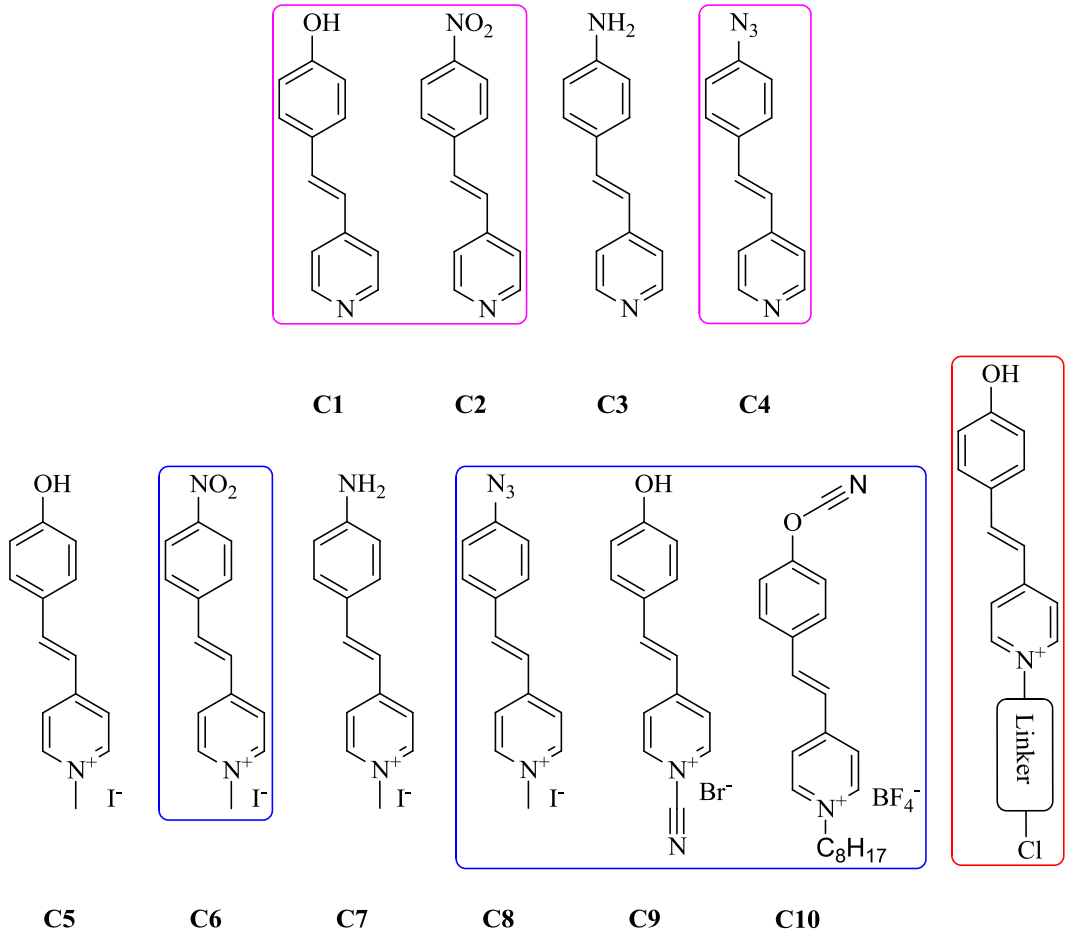


Figure 4.1. Synthesized Molecules (Purple: Ligands, Blue: Sensors, Red: Reactive Dye)

The reactive BM dye was synthesized for a textile company in order to use in fabrics that changes color when it detects bacteria, dirt, or sweat.

BM derivatives as ligands were synthesized for Karadaş Research Group to be used in photocatalytic water oxidation reactions.

For hydrogen sulfide sensor studies, four different BM derivatives were synthesized. **C8** was found to be highly sensitive to light exposure, yet application as a sensor would not be applicable. **C9** was successfully converted into **C1** with H_2S, however, its sensor studies were not continued due to selectivity problems. **C6** could not be converted into **C7** with H_2S but its cellular studies were conducted, and promising results were obtained.

Finally, **C10** was found to be fluorescence turn-on upon addition of hydrogen sulfide in dimethyl sulfoxide. However, **C1-alkylated** derivative which is formed from **C10** and H_2S reaction was also showing higher fluorescent signals in the presence of hydrogen sulfide. Therefore, further studies are required for the application of **C10** as a hydrogen sulfide sensor.

REFERENCES

1. Pereira, L., Alves, M., Malik, A., & Grohmann, E. (2012). Environmental Protection Strategies for Sustainable Development. *Strategies for Sustainability, Dordrecht Heidelberg London NewYork*.

2. Cañamares, M. V., Reagan, D. A., Lombardi, J. R., & Leona, M. (2014). TLC- SERS of Mauve, the First Synthetic Dye. *J. Raman Spectrosc. 45*(11-12), 1147-1152.

3. Travis, A. S. (1990). Perkin's Mauve: Ancestor of the Organic Chemical Industry. *Technol. Cult. 31*(1), 51-82.

4. de Melo, J. S., Takato, S., Sousa, M., Melo, M. J., & Parola, A. J. (2007). Revisiting Perkin's Dye(s): The Spectroscopy and Photophysics of Two New Mauveine Compounds (B2 and C). *Chem. Commun.* (25), 2624-2626.

5. Gürses, A., Açıkyıldız, M., Güneş, K., & Gürses, M. S. (2016). Dyes and Pigments: Their Structure and Properties. *Dyes and Pigments* (pp. 13-29). Springer, Cham.

6. Bafana, A., Devi, S. S. & Chakrabarti, T. (2011). Azo Dyes: Past, Present and the Future. *Environ. Rev.* **19**, 350–370.

7. Karthikeyan, K. T. & Jothivenkatachalam, K. (2014). Removal of Acid Yellow 17 Dye from Aqueous Solution using Turmeric Industrial Waste Activated Carbon. *J. Environ. Nanotechnol.* **3**, 69–80.

8. Bien, H.-S., Stawitz, J. and Wunderlich, K. (2000). Anthraquinone Dyes and Intermediates. In Ullmann's Encyclopedia of Industrial Chemistry, (Ed.).

9. Wright, P. and Staff, U.B. (2014). Xanthene Dyes. In Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, Inc (Ed.)

10. Kulinich, A. V & Ishchenko, A. A. (2009). Merocyanine Dyes: Synthesis,

Structure, Properties and Applications. *Russ. Chem. Rev*. **78**, 141–164.

11. Brooker, L. G. S., Keyes, G. H. & White, F. L. (1935). Studies in the Cyanine Dye Series. IV. *J. Am. Chem. Soc.* **57**, 2492–2496.

12. Brooker, L. G. S., Keyes, G. H. & Heseltine, D. W. (1951). Color and Constitution. XI.1 Anhydronium Bases of p-Hydroxystyryl Dyes as Solvent Polarity Indicators. *J. Am. Chem. Soc.* **73**, 5350–5356.

13. Morley, J. O., Morley, R. M., & Fitton, A. L. (1998). Spectroscopic Studies on Brooker's Merocyanine. *J. Am. Chem. Soc. 120*(44), 11479-11488.

14. Nandi, L. G., Nicoleti, C. R., Marini, V. G., Bellettini, I. C., Valandro, S. R., Cavalheiro, C. C. S., & Machado, V. G. (2017). Optical Devices for the Detection of Cyanide in Water Based on Ethyl(hydroxyethyl)cellulose Functionalized with Perichromic Dyes. *Carbohydr. Polym., 157*, 1548-1556.

15. Buncel, E. & Rajagopal, S. (1990). Solvatochromism and Solvent Polarity Scales. *Acc. Chem. Res.* **23**, 226–231.

16. Brian M. Dale; Mark A. Brown; Richard C. Semelka (2001). Principles and Principles and Applications. *Appl. Spectrosc.* **55**, 362A.

17. Lehmann, F., Mohr, G. J. & Grummt, U. W. (1997). Synthesis and Structure-property Relationships of Amphiphilic Acidochromic Hydroxystilbazolium Dyes. *Sens. Actuators B Chem.* **39**, 229–234.

18. Lye, J., MacDonald, J. G. & Wei, N. (2012). Solvatochromatic Bacterial Detection (U.S. Patent No. 8,338,128 B2). U.S. Patent and Trademark Office.

19. Misra, R., and Bhattacharyya, S.P. (2018) Intramolecular charge transfer: Theory and applications, *Wiley-VCH Verlag, Weinheim, Germany.*

20. Zúñiga, C., Crivelli, I. & Loeb, B. (2015). Synthesis, Characterization, Spectroscopic and Electrochemical Studies of Donor-acceptor Ruthenium(II) Polypyridine Ligand Derivatives with Potential NLO Applications.

Polyhedron **85**, 511–518.

21. Nicolini, J., Testoni, F. M., Schuhmacher, S. M. & Machado, V. G. (2007). Use of the Interaction of a Boronic Acid with a Merocyanine to Develop an Anionic Colorimetric Assay. *Tetrahedron Lett.* **48**, 3467–3470.

22. Ball, G. L., McLellan, C. J. & Bhat, V. S. (2012). Toxicological Review and Oral Risk Assessment of Terephthalic Acid (TPA) and its Esters: A Category Approach. *Crit. Rev. Toxicol.* **42**, 28–67.

23. Li, L., Rose, P. & Moore, P. K. (2011). Hydrogen Sulfide and Cell Signaling. *Annu. Rev. Pharmacol. Toxicol.* **51**, 169–187.

24. Li, L., Hsu, A., & Moore, P. K. (2009). Actions and Interactions of Nitric Oxide, Carbon Monoxide and Hydrogen Sulphide in the Cardiovascular System and in Inflammation—a Tale of Three Gases!. *Pharmacol. Ther. 123*(3), 386-400.

25. Ubuka, T. (2002). Assay Methods and Biological Roles of Labile Sulfur in Animal Tissues. *J. Chromatogr. B, 781*(1-2), 227-249.

26. Qu, K., Lee, S. W., Bian, J. S., Low, C. M. & Wong, P. T. H. (2008). Hydrogen Sulfide: Neurochemistry and Neurobiology. *Neurochem. Int.* **52**, 155–165.

27. Kamoun, P., Belardinelli, M.-C., Chabli, A., Lallouchi, K. & Chadefaux-Vekemans, B. (2003). Endogenous Hydrogen Sulfide Overproduction in Down Syndrome. *Am. J. Med. Genet.* **116A**, 310–311.

28. Szabo, C. (2012). Roles of Hydrogen Sulfide in the Pathogenesis of Diabetes Mellitus and its Complications. *Antioxid. Redox Signal.* **17**, 68–80.

29. Zhiqin Yuan, Fengniu Lu, Meihua Peng, Chia-Wei Wang, Yu-Ting Tseng, Yi Du, Na Cai, Chia-Wen Lien, Huan-Tsung Chang, Yan He, and Edward S. Yeung. (2015). Selective Colorimetric Detection of Hydrogen Sulfide Based on Primary Amine-Active Ester Cross-Linking of Gold Nanoparticles. *Anal.*

Chem. 87 (14), 7267-7273

30. Radford-Knoery, J. & Cutter, G. A. Determination of Carbonyl Sulfide and Hydrogen Sulfide Species in Natural Waters Using Specialized Collection Procedures and Gas Chromatography with Flame Photometric Detection. *Anal. Chem.* **65**, 976–982 (1993).

31. Zhang, Y., Tang, Z. H., Ren, Z., Qu, S. L., Liu, M. H., Liu, L. S., & Jiang, Z. S. (2013). Hydrogen sulfide, the Next Potent Preventive and Therapeutic Agent in Aging and Age-associated Diseases. *Cell. Mol. Biol. 33*(6), 1104-1113.

32. Montoya, L. A. & Pluth, M. D. (2012). Selective Turn-on Fluorescent Probes for Imaging Hydrogen Sulfide in Living Cells. *Chem. Commun.* **48**, 4767–4769.

33. Karakuş, E., Üçüncü, M. & Emrullahoğlu, M. (2016). Electrophilic Cyanate As a Recognition Motif for Reactive Sulfur Species: Selective Fluorescence Detection of H_2S. *Anal. Chem.* **88**, 1039–1043.

34. Thorson, M. K., Majtan, T., Kraus, J. P., & Barrios, A. M. (2013). Identification of Cystathionine β- Synthase Inhibitors Using a Hydrogen Sulfide Selective Probe. *Angew. Chem. Int. Ed.* **125**, 4739-4742.

35. Jabłoństski, A. (1933). Efficiency of Anti-stokes Fluorescence in Dyes. *Nature* **131**, 839–840.

36. Rao, J., Dragulescu-Andrasi, A. & Yao, H. (2007). Fluorescence Imaging in Vivo: Recent Advances. *Curr. Opin. Biotechnol.* **18**, 17–25.

37. Nickson, T. E. (1986). A Unique Application of the Sulfide Reduction Useful for the Preparation of Isomerically Pure Aromatic Nitro Compounds and Anilines. *J. Org. Chem.* **51**, 3903–3904.

38. Jahan, M., Bao, Q. & Loh, K. P. (2012). Electrocatalytically Active Graphene-porphyrin MOF Composite for Oxygen Reduction Reaction. *J. Am. Chem.*

Soc. **134**, 6707–6713.

39. Samuel J. Lord, Hsiao-lu D. Lee, Reichel Samuel, Ryan Weber, Na Liu, Nicholas R. Conley, Michael A. Thompson, Robert J. Twieg, and W. E. Moerner (2010). Azido Push-pull Fluorogens Photoactivate to Produce Bright Fluorescent Labels. *J. Phys. Chem. B* **114**, 14157–14167.

40. Budyka, M. F., Biktimirova, N. V., Gavrishova, T. N. & Kozlovskii, V. I. (2007). Azido Derivative of a Hemicyanine Dye with High Sensitivity to Visible Light. *Mendeleev Commun.* **17**, 159–160.

41. Li, H., Li, Y., Zhang, H., Xu, G., Zhang, Y., Liu, X., Zhou, H., Yang, X., Zhang, X. & Tian, Y. (2017). Water-soluble Small-molecule Probes for RNA Based on a Two-photon Fluorescence "off–on" Process: Systematic Analysis in Live Cell Imaging and Understanding of Structure–activity Relationships. *ChemComm.* **53**, 13245-13248.

42. Clark, S. A., Singh, V., Vega Mendoza, D., Margolin, W. & Kool, E. T. (2016). Light-Up 'channel Dyes' for Haloalkane-Based Protein Labeling in Vitro and in Bacterial Cells. *Bioconjug. Chem.* **27**, 2839–2843.

43. Whitten, J. P., Mccarthy, J. R., & Matthews, D. P. (1988). Cyanogen bromide-dimethylaminopyridine (CAP): a convenient source of positive cyanide for the synthesis of 2-cyanoimidazoles. *Synthesis* **6**, 470-472.

44. Wu, L. L., Wang, Q., Wang, Y., Zhang, N., Zhang, Q., & Hu, H. Y. (2020). Rapid Differentiation Between Bacterial Infections and Cancer Using a Near-Infrared Fluorogenic Probe. *Chem. Sci.* **11**, 3141-3145.

45. Chevalier, A., Zhang, Y., Khdour, O. M., Kaye, J. B. & Hecht, S. M. (2016). Mitochondrial Nitroreductase Activity Enables Selective Imaging and Therapeutic Targeting. *J. Am. Chem. Soc.* **138**, 12009–12012.

APPENDICES

A. NMR Spectra

Analysis conducted with Bruker Spectrospin Avance DPX-400 Spectrometer. Compounds were dissolved with $CDCl_3$ and DMSO-*d6*. ^{1}H NMR Spectrum of **C1** in DMSO-*d6*. ^{1}H NMR Spectrum of **C2** in $CDCl_3$.

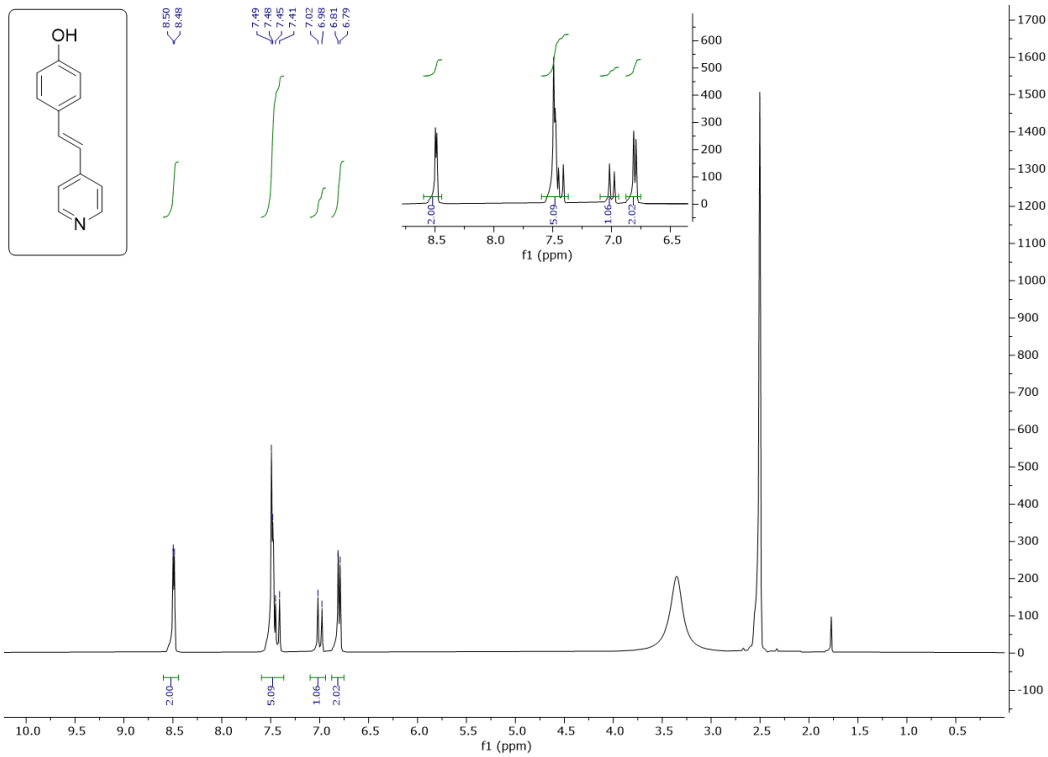


Figure 4.2. ^{1}H NMR Spectrum of **C1** in DMSO-*d6*.

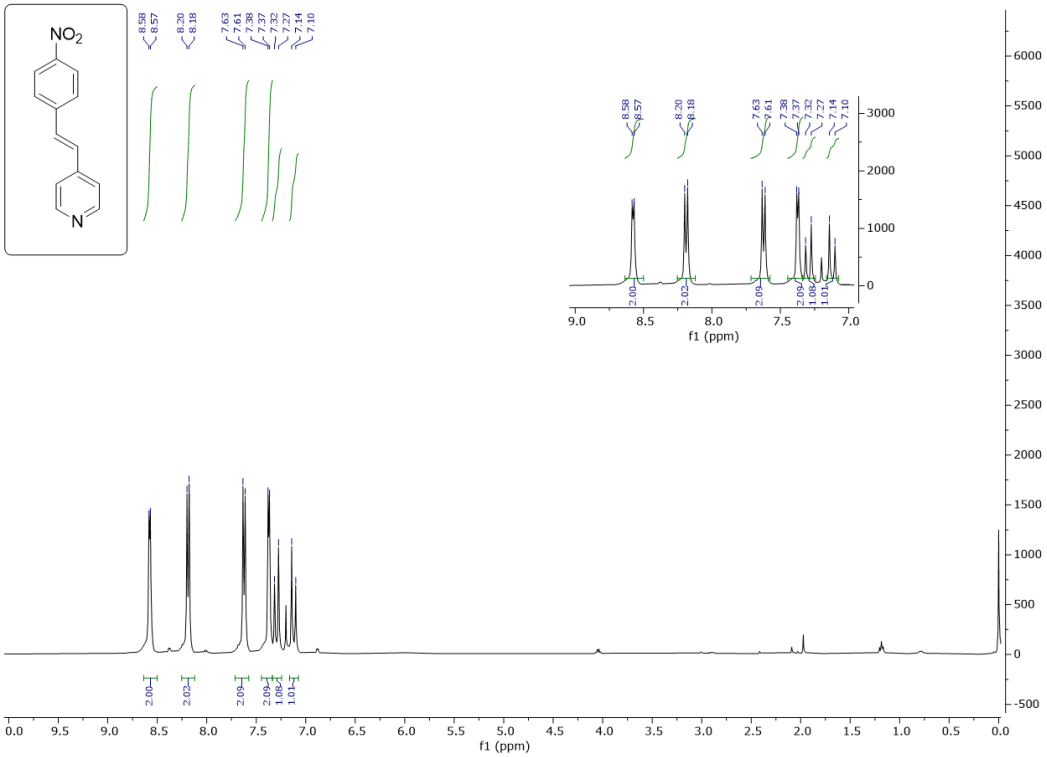


Figure 4.3. ^{1}H NMR Spectrum of **C2** in $CDCl_3$.

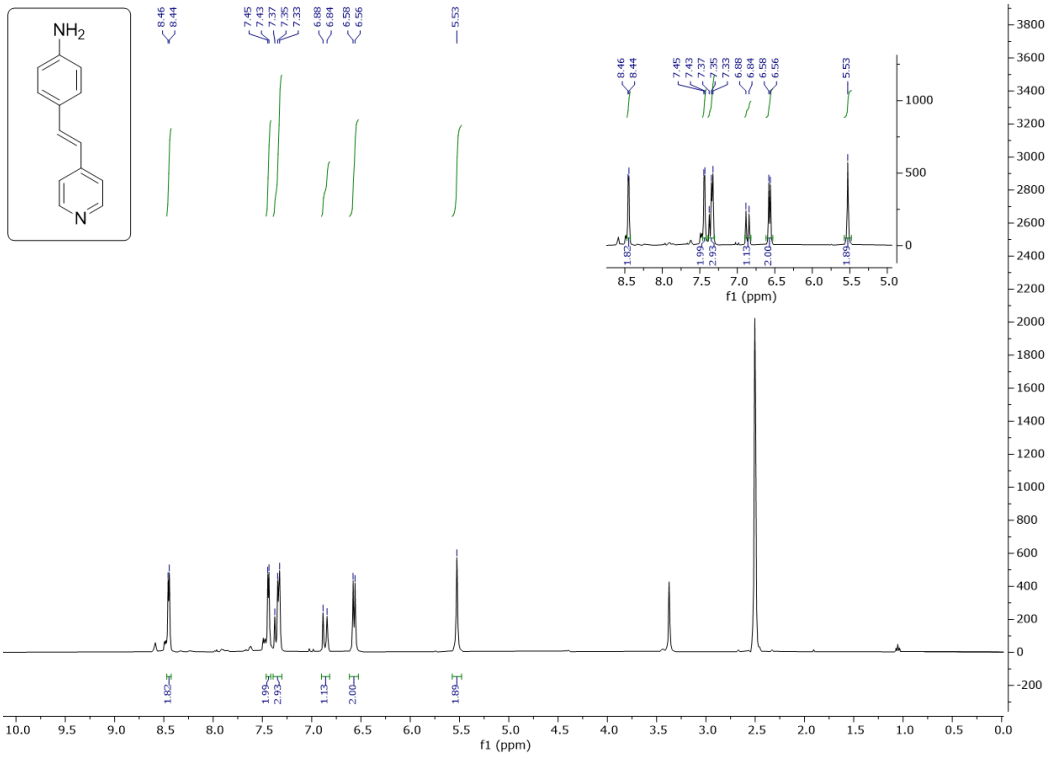


Figure 4.4. ^{1}H NMR Spectrum of **C3** in DMSO-*d6*.

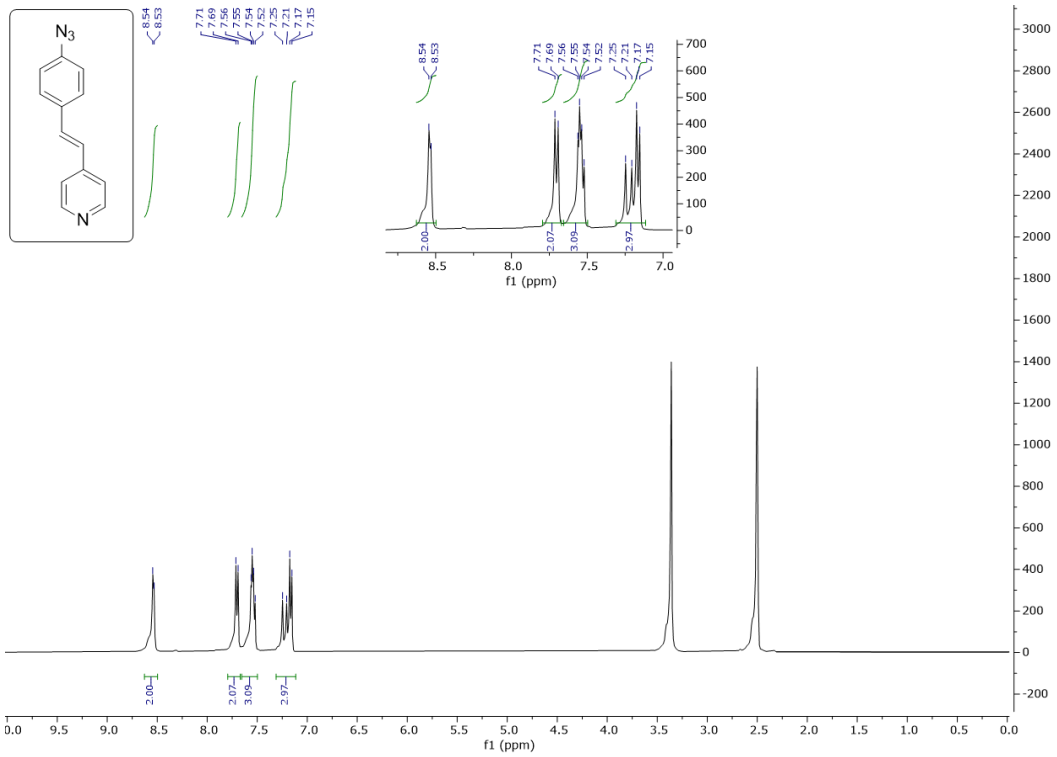


Figure 4.5. ^{1}H NMR Spectrum of **C4** in DMSO-*d6*.

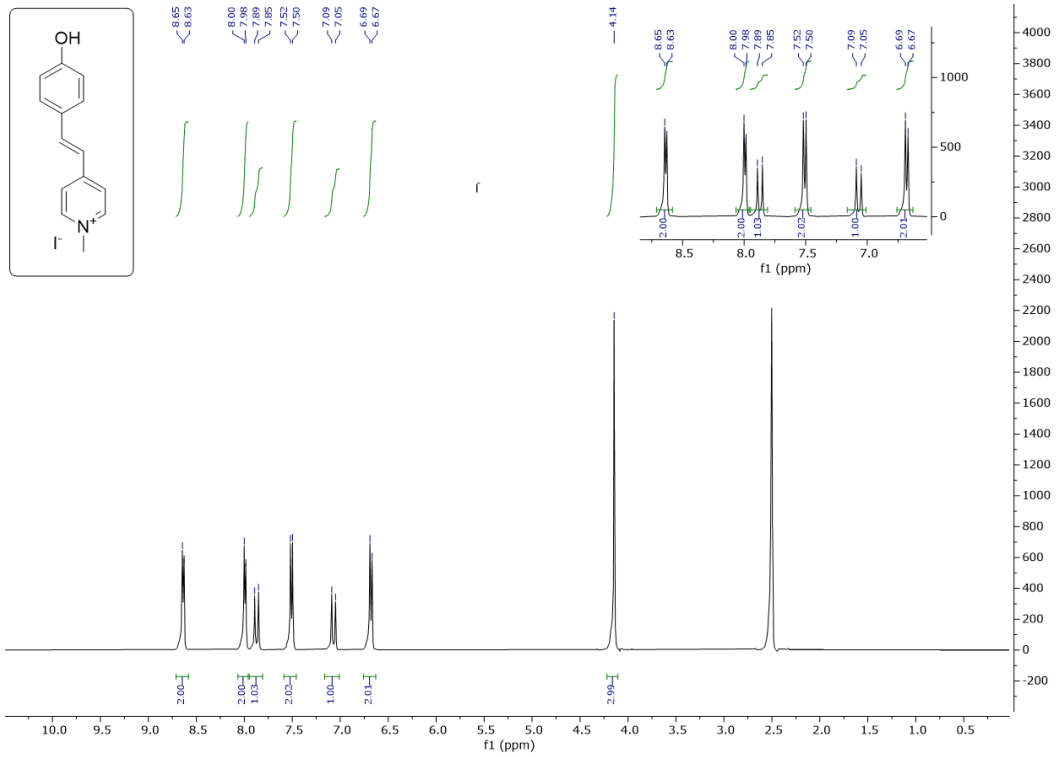


Figure 4.6. ^{1}H NMR Spectrum of **C5** in DMSO-*d6*.

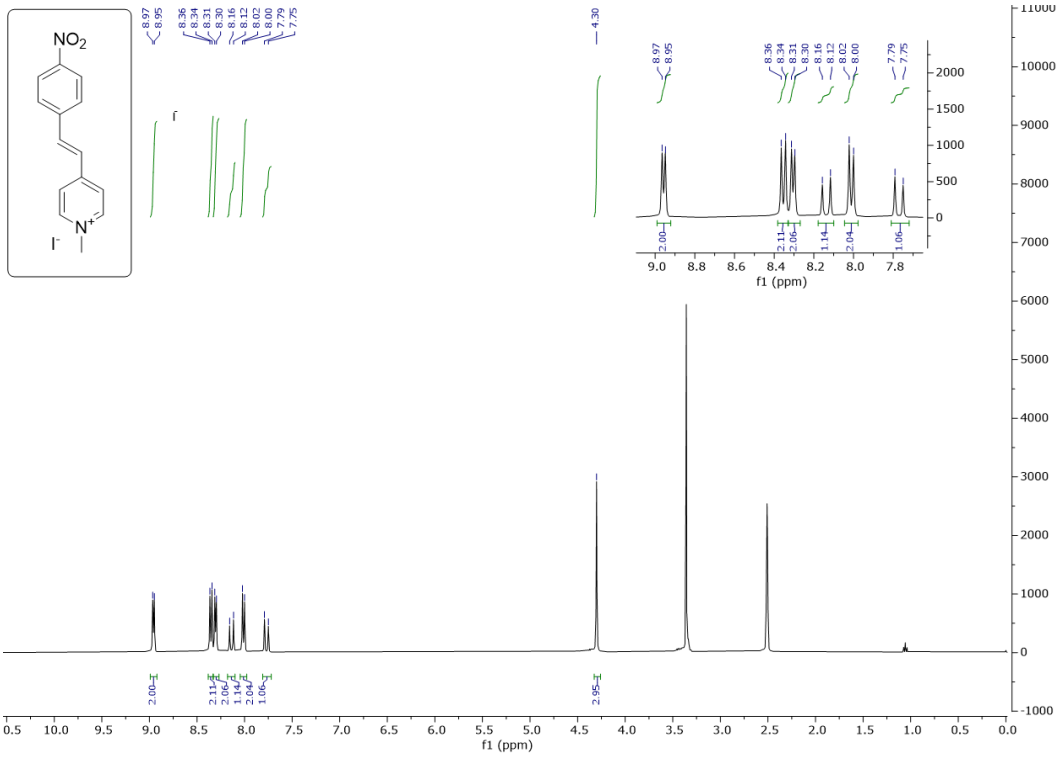


Figure 4.7. ^{1}H NMR Spectrum of **C6** in DMSO-*d6*.

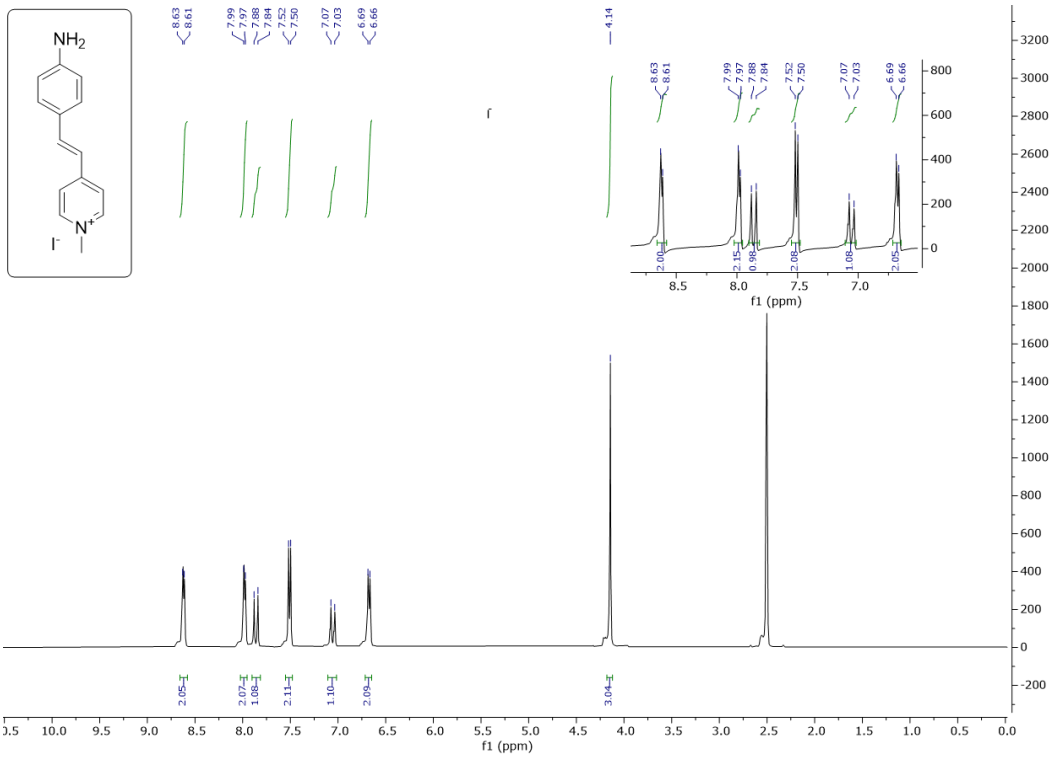


Figure 4.8. ^{1}H NMR Spectrum of **C7** in DMSO-*d6*.

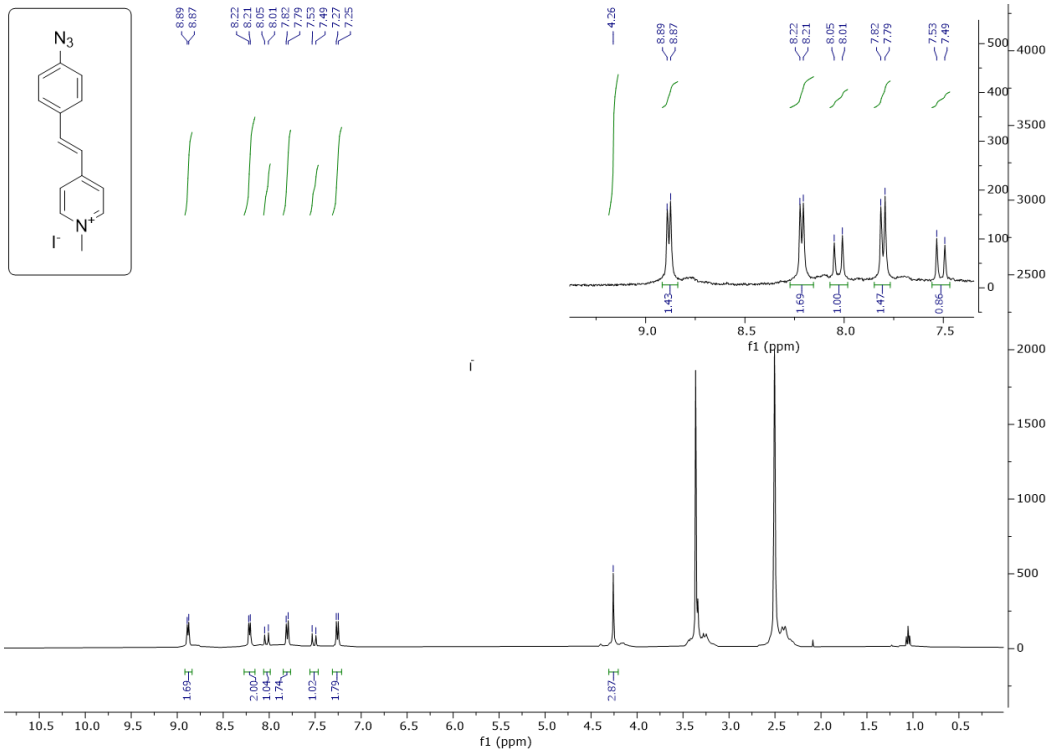


Figure 4.9. ^{1}H NMR Spectrum of **C8** in DMSO-*d6*.

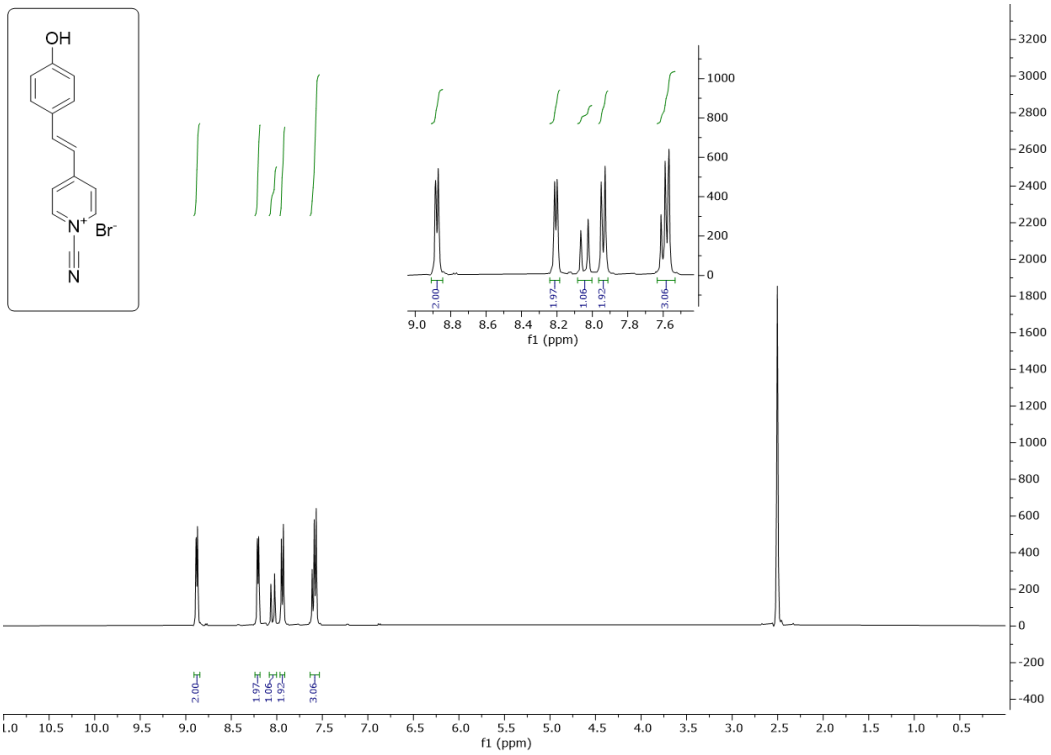


Figure 4.10. ^{1}H NMR Spectrum of **C9** in DMSO-*d6*.

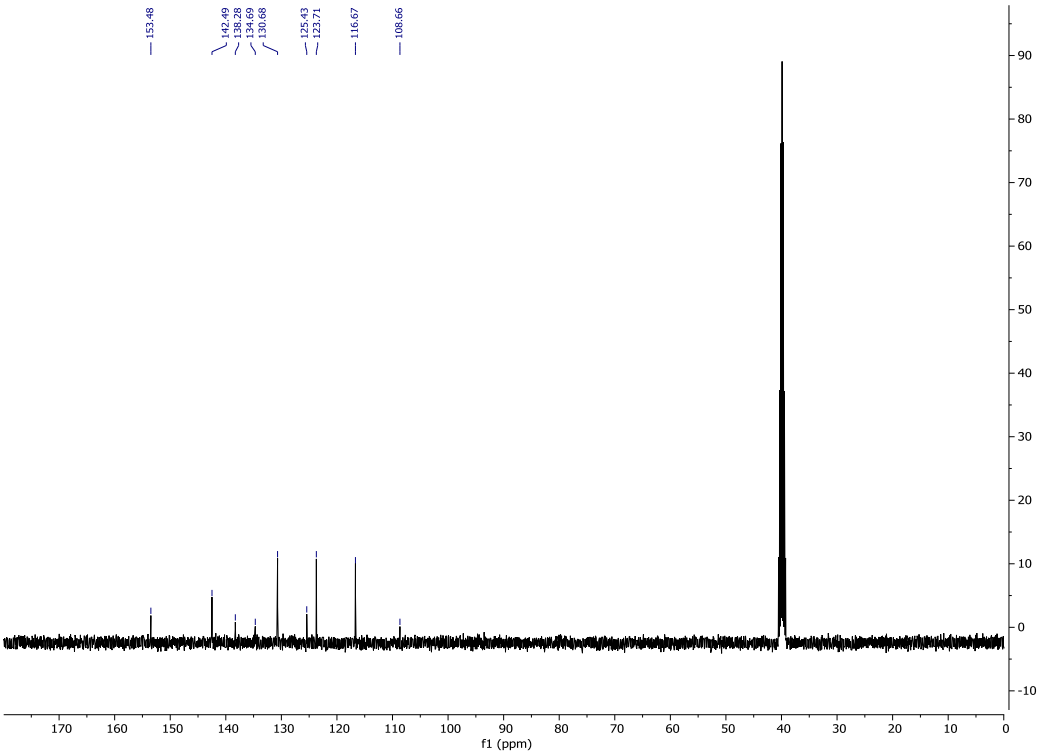


Figure 4.11. ^{13}C NMR Spectrum of **C9** in DMSO-*d6*.

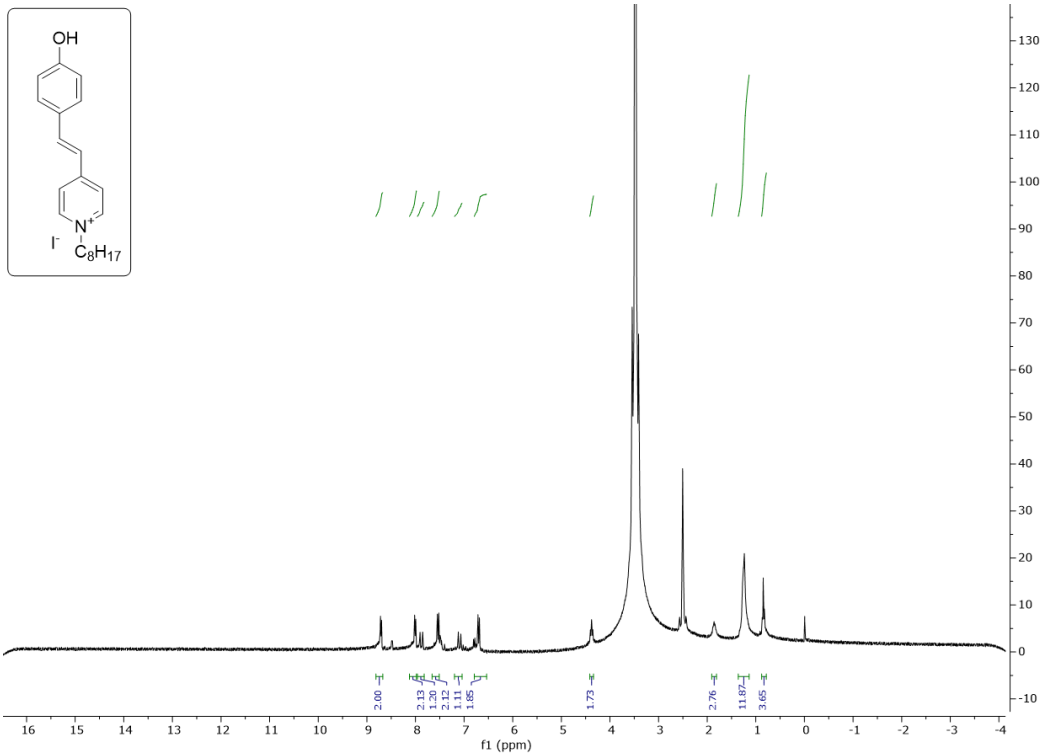


Figure 4.12. ^{1}H NMR Spectrum of **C1-Alkylated** in DMSO-*d6*.

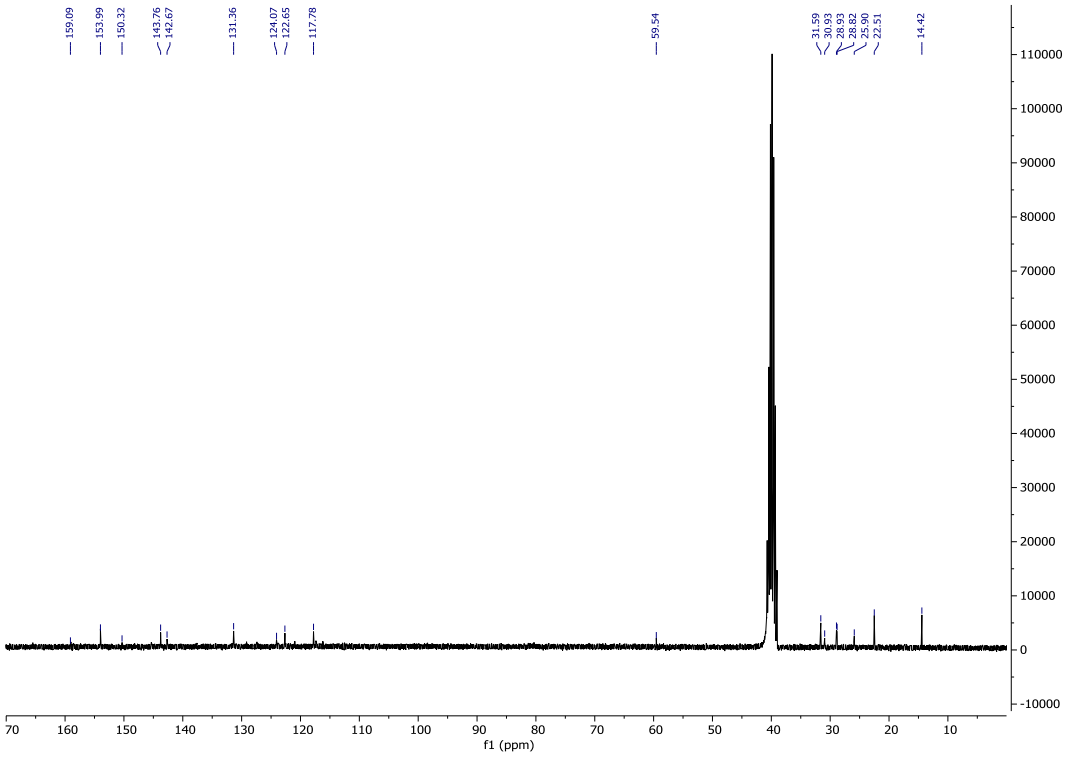


Figure 4.13. ^{13}C NMR Spectrum of **C1-Alkylated** in DMSO-*d6*.

B. IR Spectra

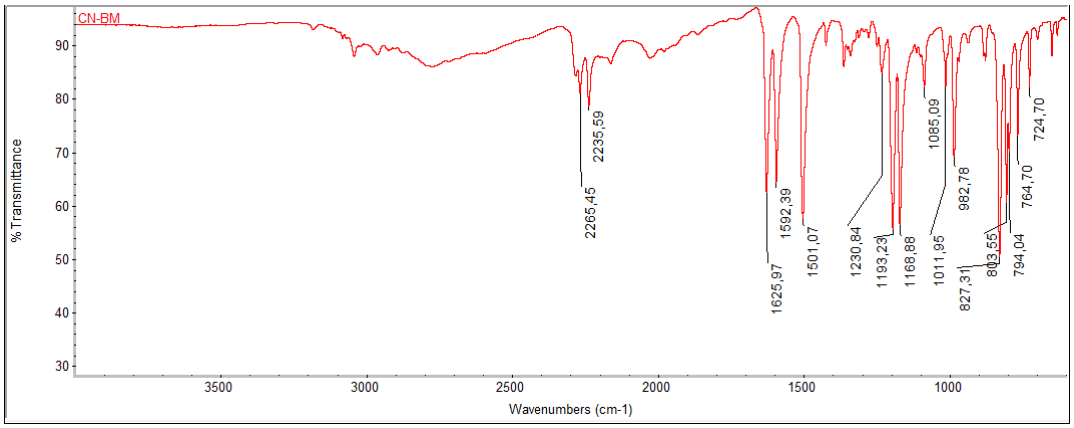


Figure 4.14. ATR-IR Spectrum of **C9**.

C. MS Spectra

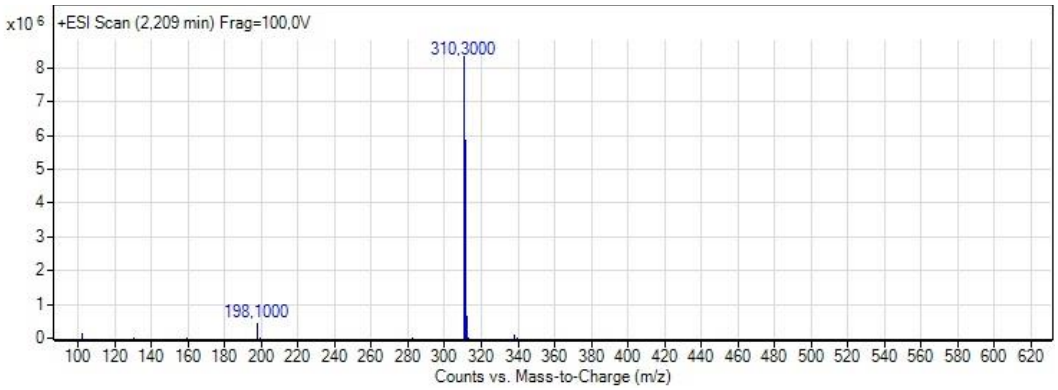


Figure 4.15. MS Spectrum of **C1-Alkylated**.

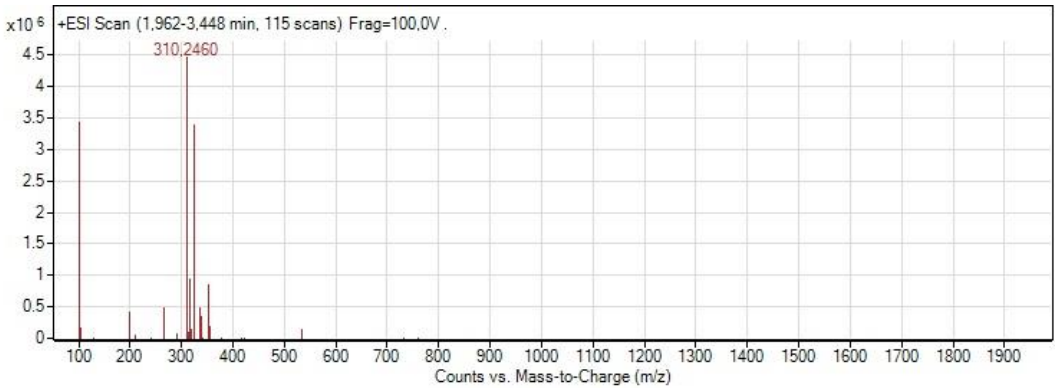


Figure 4.16. MS Spectrum of **C10**.

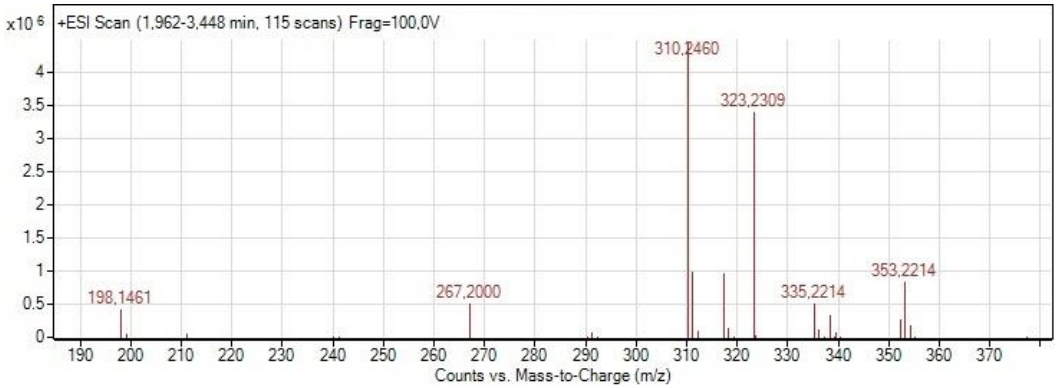


Figure 4.17. Detailed MS Spectrum of **C10**.