DESIGN AND SYNTHESIS OF A BODIPY BASED PROBE FOR MERCURY IONS

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İZMİR

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ABSTRACT

DESIGN AND SYNTHESIS OF A BODIPY BASED PROBE FOR MERCURY IONS

The detection of heavy metal ions in living systems and aqueous environments has attracted significant attention in recent years, especially the detection of mercury, one of the most toxic heavy metals on Earth. To reduce mercury's lethal effects on the human body, animals, and marine life trace amounts of mercury species can be detected by using classical spectroscopic techniques for example atomic absorption and emission spectroscopy, high-performance liquid chromatography, and inductively coupled plasma mass spectrometry. However, because those techniques are time-consuming and expensive, fluorescence analysis, which offers high selectivity and sensitivity, has emerged as a suitable alternative for detecting mercury species.

In the work presented here, a new BODIPY -based fluorescent probe functionalised with a phenylhydrazine unit was designed and synthesised for the selective and sensitive detection of mercury species. The probe's detection limit was determined to be 29 nM, and the probe could detect mercury species in living cells without any changes in cell morphology.

ÖZET

CİVA İYONLARI İÇİN BODIPY BAZLI ALGILAYICININ TASARIMI VE SENTEZİ

Canlı sistemlerde ve sulu ortamlarda ağır metal iyonlarının tespiti, özellikle dünya üzerindeki en zehirli ağır metallerden biri olan cıvanın tespiti, son yıllarda büyük ilgi görmüştür. Cıvanın insan vücudu, hayvanlar ve deniz yaşamı üzerindeki öldürücü etkilerini azaltmak için eser miktarda cıva türü, atomik absorpsiyon ve emisyon spektroskopisi, yüksek performanslı sıvı kromatografisi ve endüktif olarak eşleştirilmiş plazma kütle spektrometrisi gibi klasik spektroskopik teknikler kullanılarak tespit edilebilir. Ancak, bu tekniklerin zaman alıcı ve pahalı olması nedeniyle, yüksek seçicilik ve hassasiyet sunan floresan analizi, cıva türlerinin tespiti için uygun bir alternatif olarak ortaya çıkmıştır.

Burada sunulan çalışmada, cıva türlerinin seçici ve hassas tespiti için bir fenilhidrazin ünitesi ile işlevselleştirilmiş yeni bir BODIPY bazlı floresan probu tasarlanmış ve sentezlenmiştir. Probun tespit limiti 29 nM olarak belirlenmiştir ve prob, hücre morfolojisinde herhangi bir değişiklik olmaksızın canlı hücrelerdeki cıva türlerini tespit edebilmiştir.

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LIST OF ABBREVIATIONS

BODIPY	4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacene
THF	Tetrahydrofuran
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
$POCl_3$	Phosphorous oxychloride
DCM	Dichloromethane
$BF_3.Et_2O$	Boron trifluoride diethyl etherate
ACN	Acetonitrile
PBS	Phospate-Buffered Saline
$CDCl_3$	Deutero Chloroform
NMR	Nuclear Magnetic Resonance
MnO_2	Manganese Oxide
NIS	N- Iodosuccinamide
MeOH	Methanol
EtOH	Ethanol
DAPI	4',6-diamidino-2-phenylindole

CHAPTER 1

INTRODUCTION

1.1. An Overview

Environmental pollution in air, soil, and water due to contamination with heavy metals is a vital problem that affects human life, marine life, and animals in general. Of all heavy metal, mercury is one of the most toxic because it accumulates in the body even at low concentrations, affects the central nervous system due to the thiophilic nature of proteins and enzymes, and can consequently cause cancer, skin disorders, and kidney failure, among other critical conditions. Therefore, designing selective, sensitive methods of detecting mercury in living cells is crucial.

To date, heavy metal ions have been detected by analytical methods such as atomic absorption spectroscopy, high-performance liquid chromatography, inductively coupled plasma mass spectrometry, and atomic fluorescence spectrometry. Despite the excellent performance of those techniques, their drawbacks include complicated sample preparation, expensive instrumentation, and lack of portability. For those reasons, fluorescence-based probes are highly preferred due to their high selectivity, high sensitivity, simplicity, low cost, and low detection limits.

Every fluorescent probe includes two units, a fluorophore unit and a receptor unit, the latter of which is an analyte-specific unit that determines the behaviour of a targeted analyte based on reaction or coordination chemistry. Highly selective, usually irreversible reactions that occur with the appearance of targeted molecules can be determined with a chemodosimeter. By contrast, chemosensors operate based on coordination, and reactions in molecules with the target analyte are reversible.

Numerous organic dye molecules are used in fluorescence-based probe strategies, including rhodamine derivatives, fluorescein, coumarin, and boron dipyrrin (BODIPY) derivatives. Owing to their unique properties, BODIPY fluorophores have attracted considerable attention in recent years.

In the work presented here, we took advantage of the alkynophilic nature of Hg^{2+} to design and synthesise a new BODIPY derivative functionalised with a phenylhydrazine moiety that is highly selective and sensitive to Hg^{2+} ions over relevant competing metal ions. We also investigated the derivative's potential for tracking Hg^{2+} ions in living cells.

1.2. BODIPY Fluorophore

BODIPY (4,4-difluoro-4-bora-3a, 4a-diaza-s-indacene) fluorophore was first noticed by Treibs and Kreuzer in 1968 (Treibs and Kreuzer, 1968). They discovered that the acylation of 2,4- dimetylpyrrole with acetic anhydride and boron trifluoride formed a highly fluorescent compound.

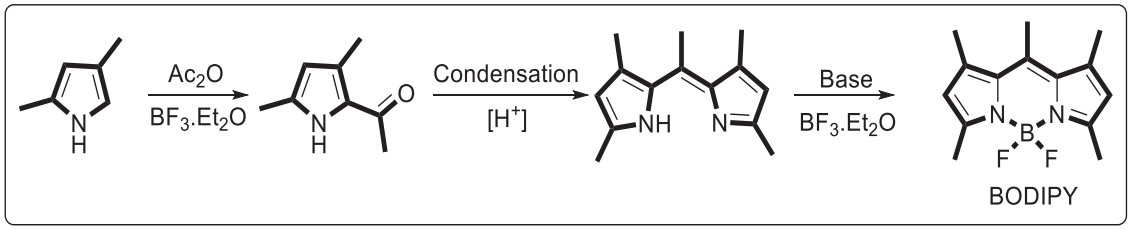

Figure 1.1. First synthesis of a BODIPY dye by Treibs and Kreuzer
(Source: Treibs and Kreuzer, 1968)

BODIPY dyes have remarkable properties, including excellent stability, high fluorescence quantum yields, large molar absorption coefficients, narrow absorption and emission bands in the visible wavelength range, and good solubility. Those features distinguish BODIPY among fluorescent dyes as candidates for use in biological systems. (Loudet et al., 2007; Ulrich et al., 2008; Leen et al., 2011)

The IUPAC's numbering system for a BODIPY (i.e. boron dipyrrin) compound is shown in Figure 1.2. Although BODIPY dyes are numbered differently from their precursors, the α-, β-, and meso-positions are the same for both. The carbon's position at the centre of the molecule is called the meso-position, the positions adjacent to nitrogen atoms are α-positions, and the other positions are β- positions.

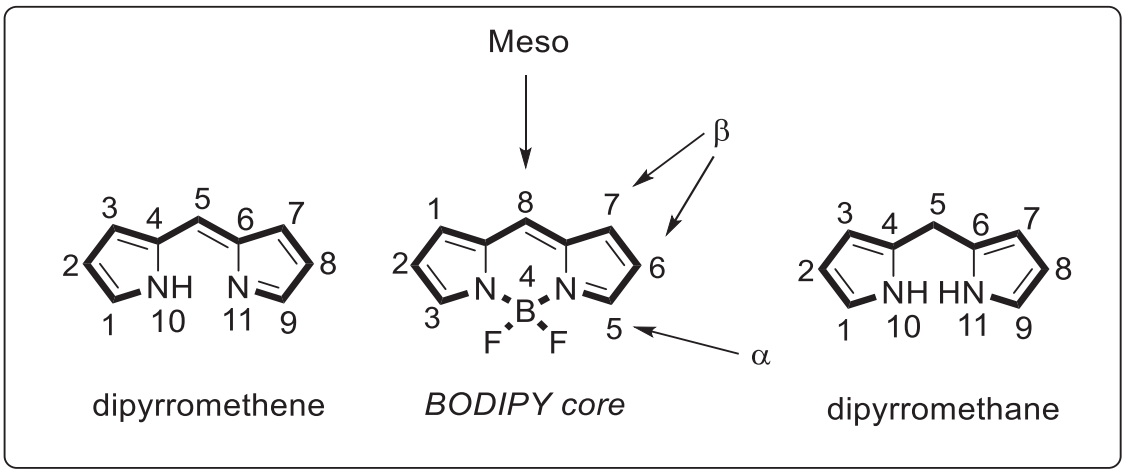

Figure 1.2. IUPAC numbering system of a boron dipyrrin compound
(Source: Wood and Thompson, 2007)

Two methods based on porphyrin chemistry can be used to synthesise the BODIPY structure (Wood et al., 2007). In the first method, an acid-catalysed condensation of pyrrole with an aldehyde yields a dipyrromethane. In that reaction, because the dipyrromethane compound is sensitive to light, air, and acid, it immediately oxidises to dipyrromethene with an oxidant such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or the milder 2,3,5,6-tetrachloro-1,4-benzoquinone *p*-chloranil. Once oxidation is complete, dipyrromethene reacts with $BF_3.Et_2O$ and a base to produce the BODIPY structure.

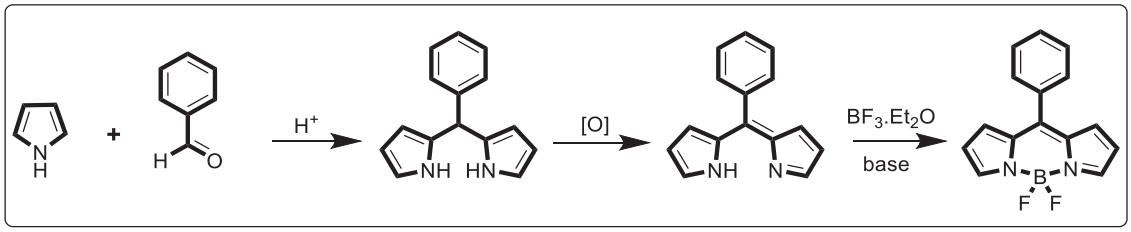

Figure 1.3. Acid catalyzed condensation of aromatic aldehydes with pyrroles
(Source: Boens et al., 2012)

The second method involves the condensation of pyrrole with an acylium derivative (e.g.an ortho ester, an acid anhydride, or an acid chloride) (Yakubovskyi et al., 2009; Li et al., 2006; Shah et al., 1990). With the reaction between the isolated acyl pyrrole and second pyrrole moiety occurs in an acidic environment, a dipyrrin compound is obtained,

and the BODIPY compound materialises with the addition of boron trifluoride etherate and a base.

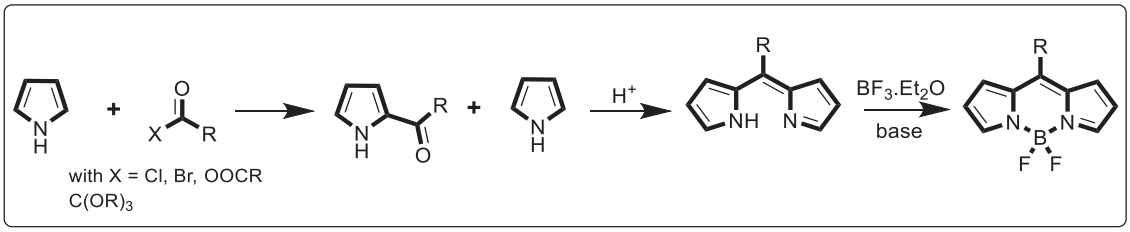

Figure 1.4. Synthesis of BODIPY dyes with the acylation of pyrrole followed by condensation and complexation
(Source: Boens et al., 2012)

However, in 2008, Wu and Burgess proposed that BODIPY complexes can be obtained without using a second pyrrole equivalent or other condensation reagents. (Wu et al., 2008). Instead, they discovered that phosphorus oxychloride can condense pyrrole-2-carbaldehyde with itself. (Figure 1.5.)

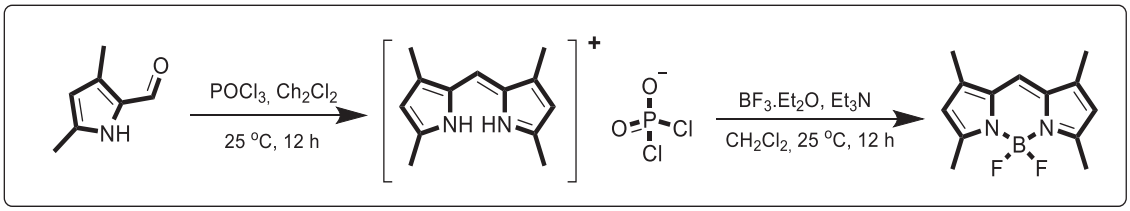

Figure 1.5. The new approach described by Wu and Burgess
(Source: Wu et al., 2008)

BODIPY dyes absorb and emit at lower wavelengths in the visible range (450- 500 nm) and generally have high quantum yields. Beyond that, the spectroscopic properties of BODIPY can be changed via chemical modification, including by attaching different groups to the proper positions or increasing the conjugation of the BODIPY core, which results in red shifts in its emission wavelength (700 nm) (Chen et al., 2000; Ni et al., 2014).

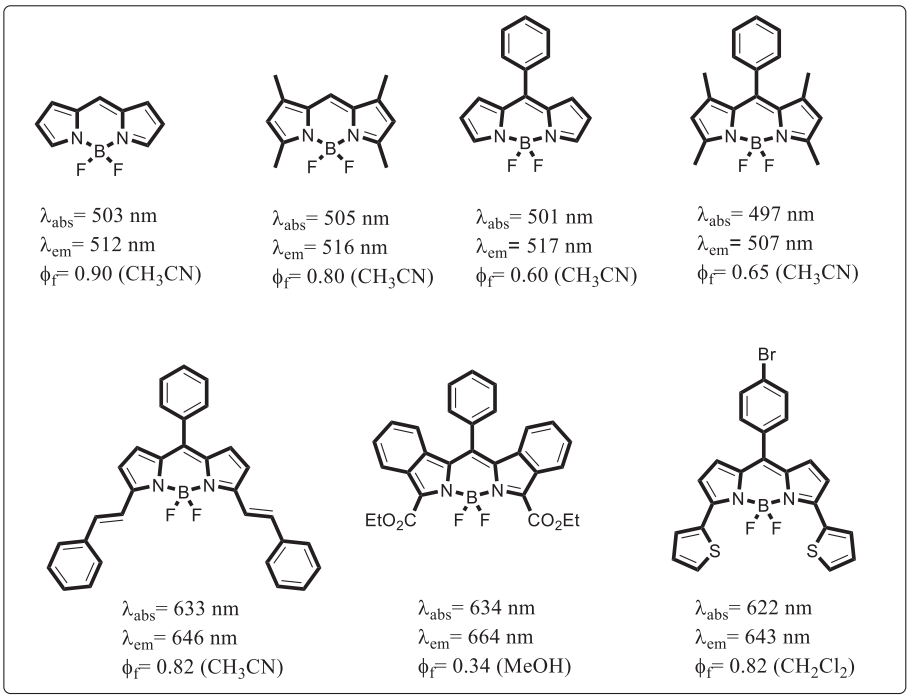

Figure 1.6. BODIPY structures with different absorption and emission wavelengths (Source: Ni and Wu, 2014)

In general, developing fluorescent probes involves two procedures. The first requires using a chemodosimeter such that a fluorophore and a receptor yield a new molecule through a reaction and cause changes in the fluorescence emission. In the second, based on coordination chemistry, the binding of the receptor and metal ion produces a fluorescence signal in which neither the fluorophore nor the receptor are fluorescent before the metal ion is introduced.

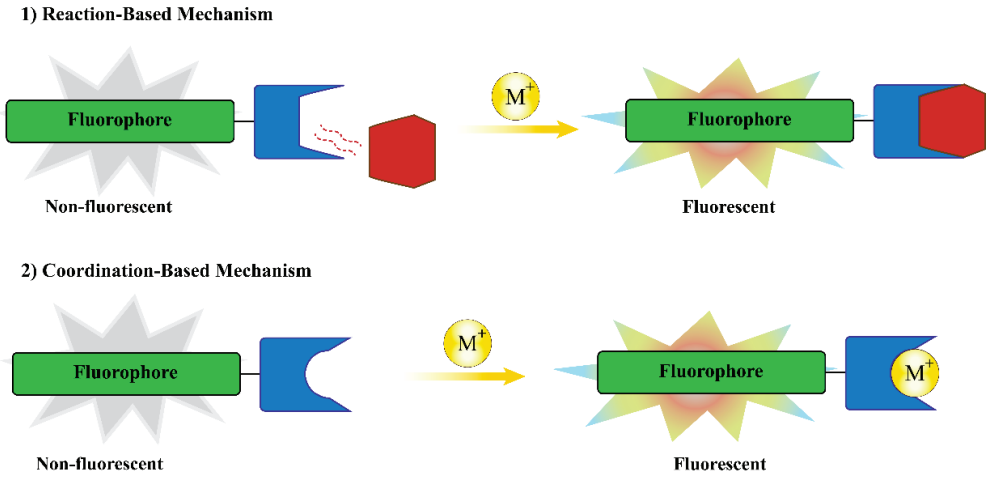

Figure 1.7. Reaction and coordination-based approaches (Source: Patil et al., 2012)

1.3. Literature Studies

Since the effects of heavy metals on human health and the environment are recognized, developing fluorescent probes to detect heavy metals has attracted attention over the past decades. Knowing that mercury is one of the most poisonous heavy metals, numerous probes were designed based on a chemodosimetric approach. Furthermore, the high reactivity of mercury ions towards unsaturated bonds has been known and several studies benefited from the strong thiophilic and alkynophilic nature of mercury ions.

In 1992, Czarnik and Chae reported the first mercury selective probe in which an anthracene unit was functionalized with a thioamide group (Chae and Czarnik, 1992). After the isomerization of C=S double bond, thiolate form was produced and as a result, fluorescence emission was quenched via PET mechanism. It is generally known that mercury ions have high affinity to sulphur-based compounds. After adding the mercury ions into the solution of the compound, an irreversible desulfation reaction took place, and the thioamide group was transformed to amide form and as a result, the PET mechanism was blocked. This publication was the first study in which the term chemodosimeter was used. After the discovery of Chae and Czarnik, different fluorescent probes have been developed to detect mercury ions.

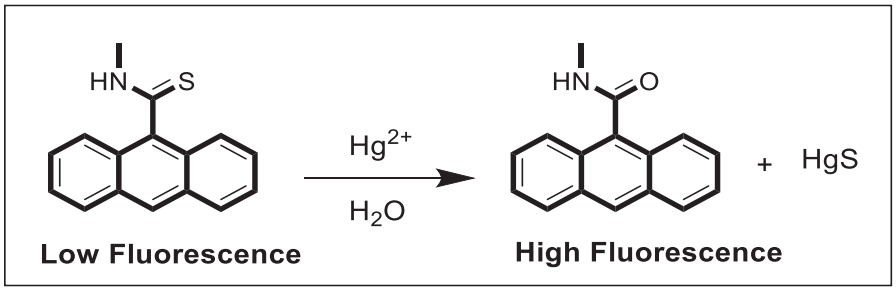

Figure 1.8. First developed Hg^{2+} chemodosimeter
(Source: Chae et al., 1992)

In 2007, Wu et al. reported a rhodamine based Hg^{2+} chemodosimeter (Wu et al., 2007). They took advantage of the well-known reaction in which thiourea derivatives with amine moiety can be converted into guanidine groups with the addition of the Hg^{2+} ion. The probe was nonfluorescent at first. With the promotion of mercury, a

desulfurization reaction took place, and this caused a ring-opening process of spirolactam of rhodamine which resulted in an increment of fluorescent response. Also, a color change from colorless to pink occurred as an outcome of a colorimetric response.

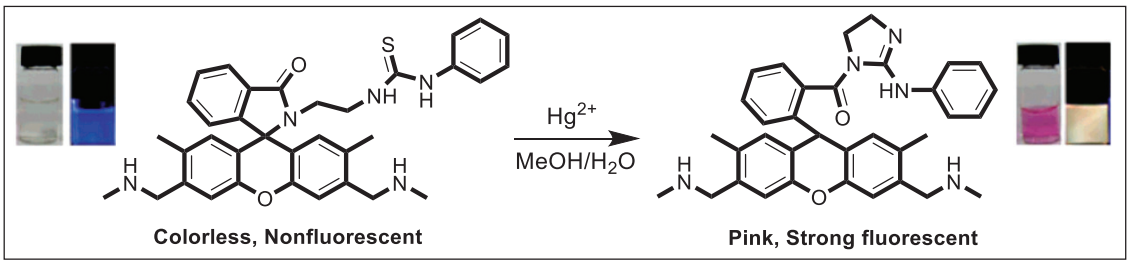

Figure 1.9. Proposed Hg^{2+}-Promoted Ring Opening of Spirolactam and Intramolecular Guanylation
(Source: Wu et al., 2007)

In 2008, Koide and co-workers published the first fluorescent probe bearing alkyne moiety to detect mercury ions (Song et al., 2008). They developed a fluorescent probe based on a fluorescein dye that was derivatized from an oxygen atom with an alkyne moiety. At first, the designed molecule was nonfluorescent. The cleavage of the fluorescence-masking alkyne moiety with the addition of Hg^{2+} resulted in an increment of fluorescent emission, providing a bright green fluorescent. The probe has enabled the detection of mercury ions in fish and dental samples.

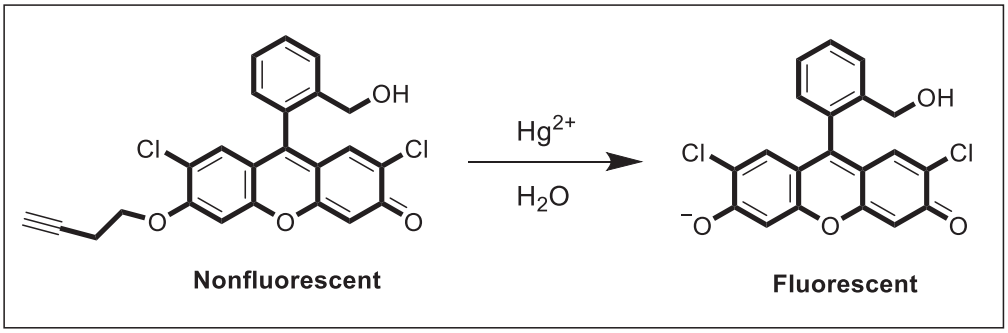

Figure 1.10. First example of Hg^{2+} sensor bearing alkyne moiety
(Source: Song et al., 2008)

At the beginning of 2010, Lin and co-workers were designed a new fluorescent probe bearing both thiol and alkyne moieties in the rhodamine structure for the mercury imaging in living cells (Lin et al., 2010). The interaction of mercury with thiol and alkyne

units in the presence of water enabled the transformation of the rhodamine dye from the non-fluorescent spirocyclic form to the fluorescent opened-ring form.

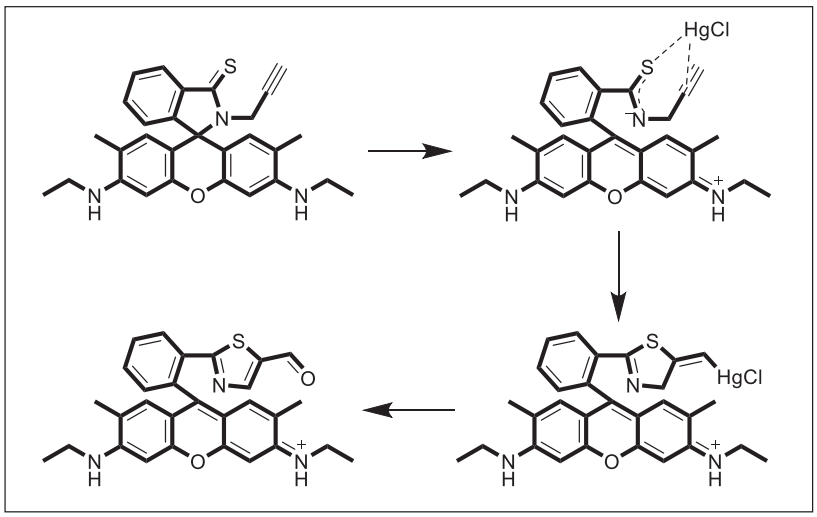

Figure 1.11. The proposed reaction mechanism of Rhodamine based probe with Hg^{2+} (Source: Lin et al., 2010)

Later in 2010, a nonsulfur probe based on a 1,8-naphthalimide bearing an alkyne unit was reported for the ratiometric fluorescent sensing for mercury ion in water at neutral pH by Dong et al (Dong et al., 2010). By changing the pH of the environment and the solvent system, they also observed the selectivity of probe towards gold ions. Addition of Hg^{2+} caused the change in the fluorescence emission band of probe from 543 to 486 nm.

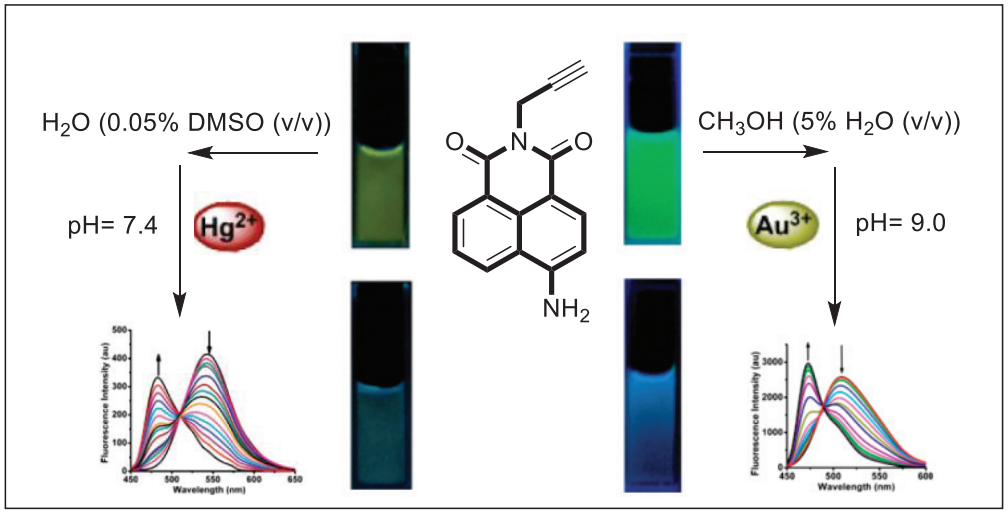

Figure 1.12. Ratiometric fluorescent sensing of Hg^{2+} and Au^{3+} (Source: Dong et al., 2010)

A ratiometric fluorescent probe functionalized with an alkyne moiety was published by Lee and Kim in 2011 (Lee et al., 2011). They designed an alkyne-tethered coumarin amide. The reaction known as the Kuscheroff reaction took place when mercury ions activated the alkyne unit in the presence of water and afforded an oxazole ring. This caused a significant red shift in the emission from 469 nm to 492 nm.

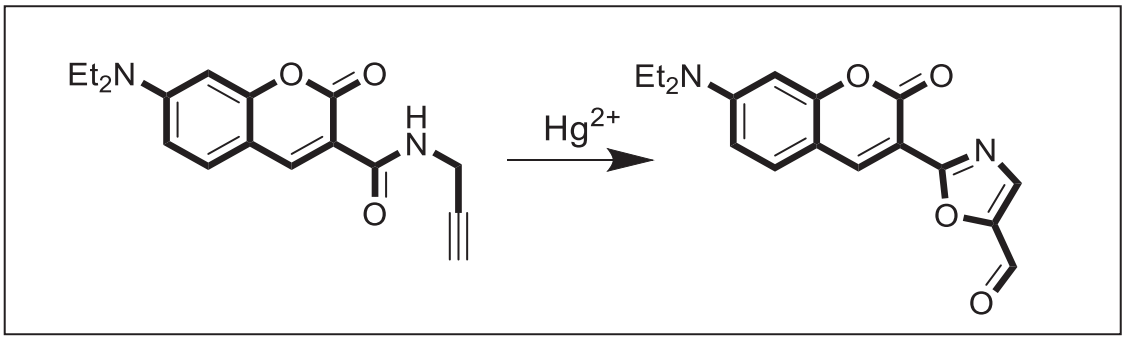

Figure 1.13. Hg (II)-mediated propargyl amide to oxazole transformation
(Source: Lee and Kim, 2011)

In 2013, Atta and co-workers reported a new fluorescent probe to detect mercury ions in semi aqueous media at ambient temperature. The addition of Hg^{2+} to the sensing solution triggered the intramolecular cyclization between phenol and acetylene, providing a form of a benzofuranylmercury chloride. The introduction of Hg^{2+} also resulted in a dramatic color change from blue to pale yellow with a very low detection limit of 0.13 μM. No color change was observed upon the addition of other metal ions, except gold ion which is also known for its alkynophilic nature. In the presence of gold ions, the color of the solution was slightly changed.

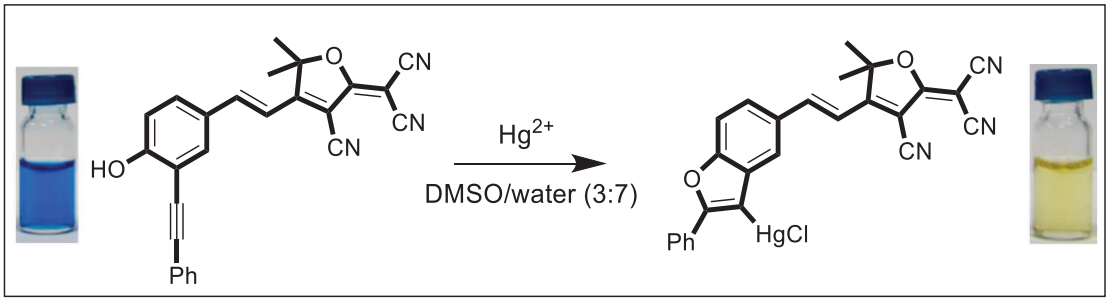

Figure 1.14. Proposed Mechanisms of Hg (II)-Mediated Intramolecular Cyclization
(Source: Atta et al., 2013)

Another fluorescent probe was reported by Kaur and Choi in 2014 (Kaur and Choi, 2014). Diketopyrrolopyrrole (DPP) dye, which normally used in organic electronic devices but very rare in fluorescent probe, was used as a fluorophore in this research. The probe functionalized with alkynyl and thiophene units was used to detect Hg^{2+} and Cu^{2+} ions at nanomolar concentrations. In the presence of Hg^{2+} in water, the conversion of alkyne to carbonyl took place and a significant color change from pink to blue was observed as a result of a colorimetric response.

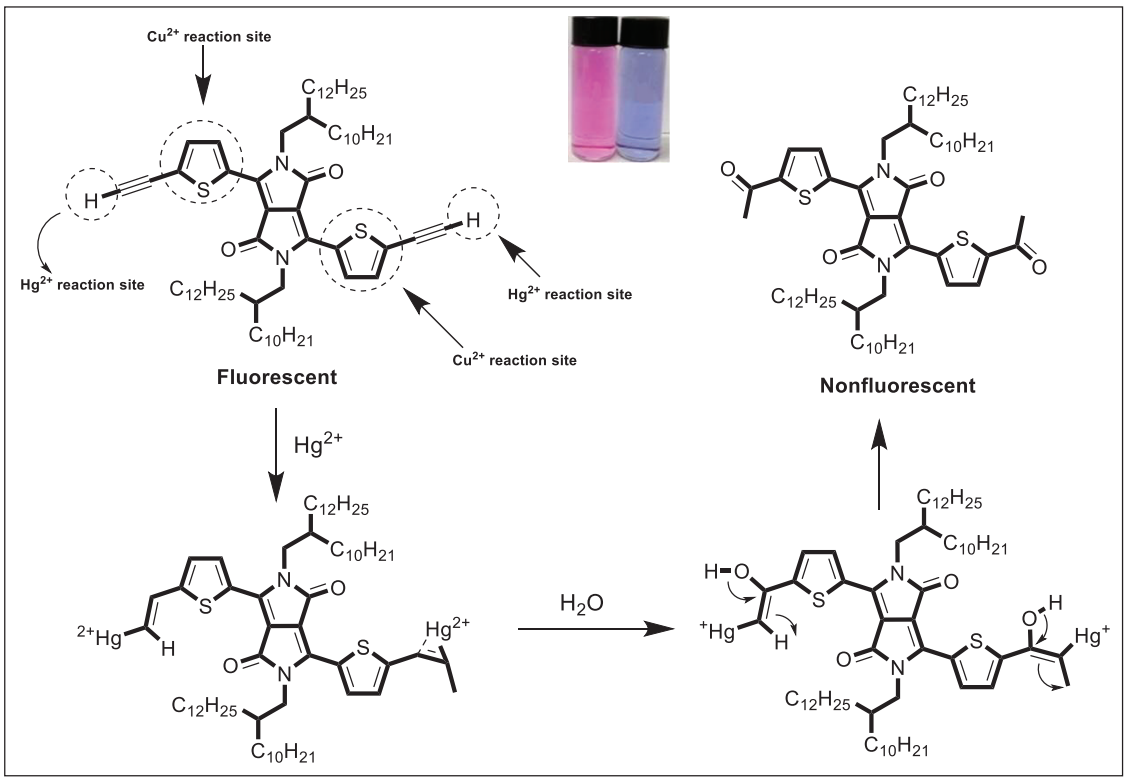

Figure 1.15. The proposed sensing mechanism of the probe towards Hg^{2+}
(Source: Kaur and Choi, 2014)

In 2015, our group has developed a BODIPY based fluorescent probe for detection of Au (III) and Hg (II) and this work was also benefited the alkynophilic nature of gold and mercury ions. The fluorescent probe showed a ratiometric response towards mercury and gold ions, providing a distinct color change from orange to green. With the extension of non-conjugated BODIPY derivative, a green fluorescence emission was observed.

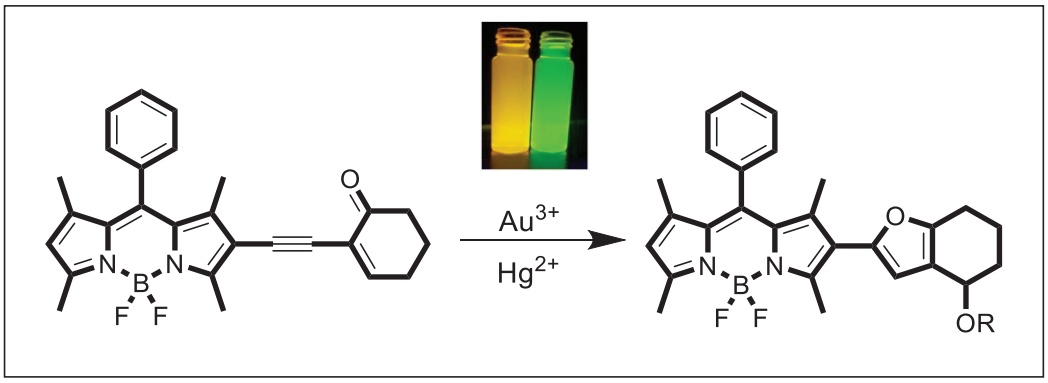

Figure 1.16. Proposed mechanism of BODIPY based probe towards to gold and mercury species
(Source: Üçüncü et al., 2015)

Duan and co-workers presented a novel coumarin based probe, 7-(propargylamino)-4-methyl-2H-chromene-2-one (CNP), bearing alkyne unit that converted into a stable ketone in the presence of mercury ions. (Duan et al., 2017) The probe was nonfluorescent at first due to the ICT process. The detection limit of the probe for the mercury ions was determined in nanomolar range. The sensing probe was designed to be used in environmental samples and on agar gels.

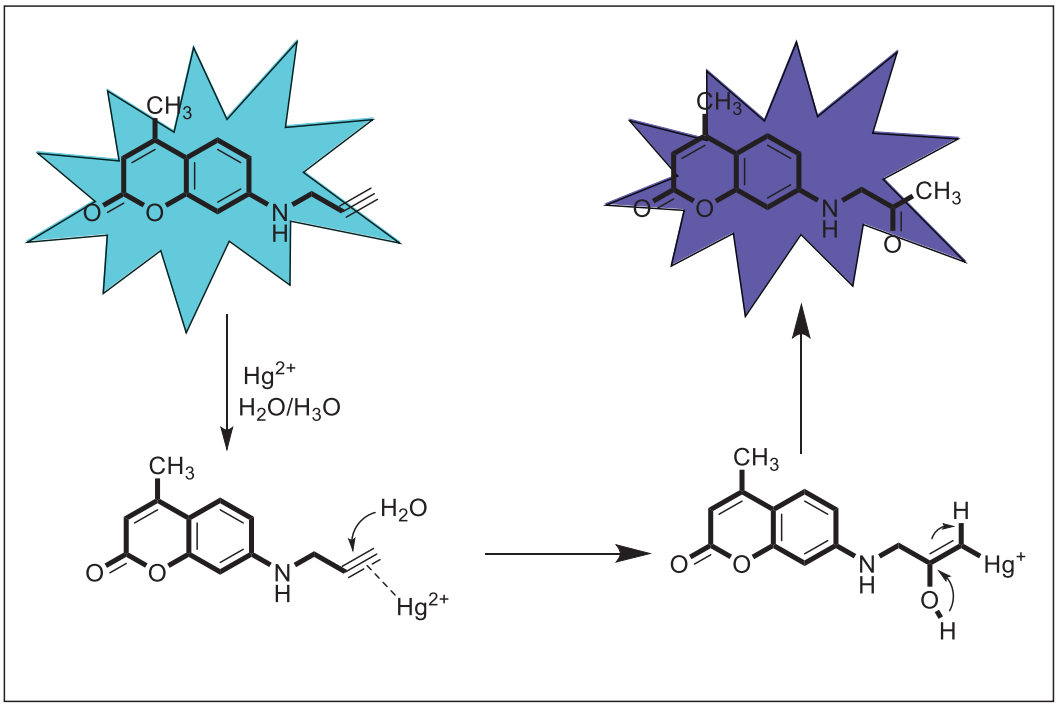

Figure 1.17. Detection mechanism of the probe
(Source: Duan et al., 2017)

CHAPTER 2

EXPERIMENTAL STUDY

2.1. General Methods

All used reagents were obtained from commercial suppliers (Sigma-Aldrich and Merck) and used without any purification. ^{1}H NMR and ^{13}C NMR spectrum were measured on a VNMRJ 400 Nuclear Magnetic Resonance Spectrometer (Varian Medical Systems). UV absorption spectra and Fluorescence emission spectra were obtained using HORIBA Duetta, the two-in-one Fluorescence and absorbance spectrometer. Samples were contained in quartz cuvettes with a path length of 10.0 mm (2.0 mL volume). Upon excitation at 480 nm, the emission spectra were integrated over the range 490 nm to 750 nm. The slit width was 5 nm for both excitation and emission. The pH was recorded by HI-8014 instrument (HANNA). All measurements were regulated at least in triplicate. The fluorescence images were obtained using Zeiss Axio Observer fluorescence microscope.

2.2. Determination of Quantum Yields

Fluorescence quantum yields of **BDP-BUS** and **BDP-PYRZL** were determined by using optically matching solutions of Rhodamine B (Φ_F=0.31 in water) as a standard. The quantum yield was calculated according to the equation;

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

2.3. Synthesis Section

The synthesis pathway for **BDP-BUS** and **BDP-PYRZL** were shown in Figure 2.1. **BODIPY**, **IODO-BODIPY**, **BDP-PRP, BDP-AL**, **BDP-BUS** and **BDP-PYRZL** were synthesized by using literature procedures.

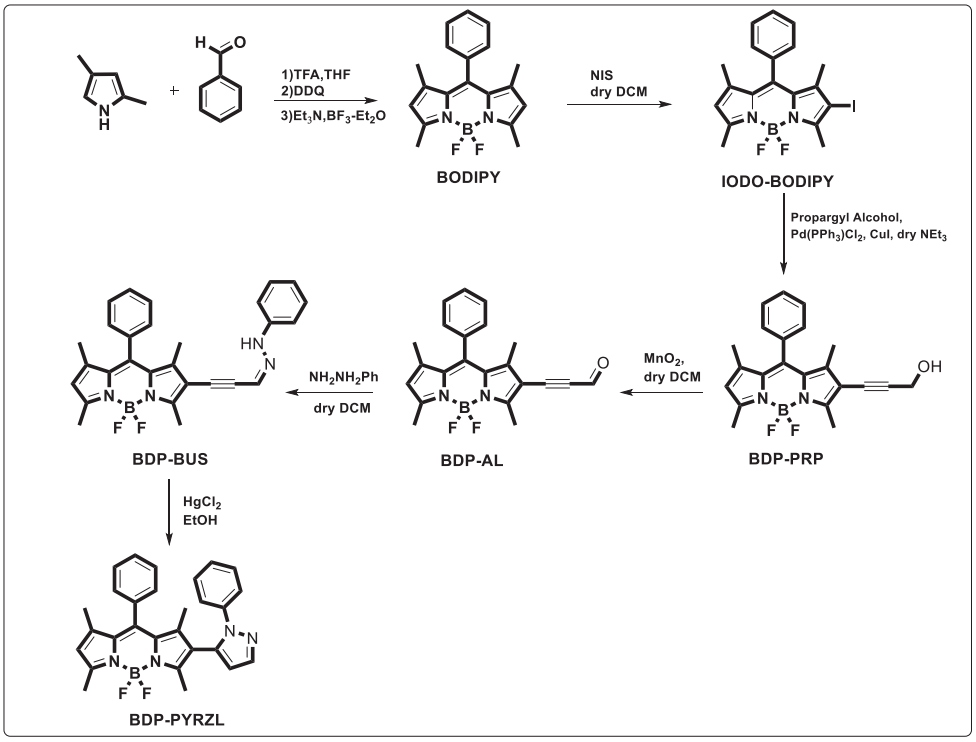

Figure 2.1. Synthesis pathway of **BDP-BUS** and **BDP-PYRZL**

2.3.1. Synthesis of BODIPY

BODIPY dye was synthesis according to literature procedure (Sauer et al., 2012). To a solution of 2,4-dimethylpyrrole (4 mmol, 411µL) in 25 ml dry THF, benzaldehyde (2 mmol, 200µL) and 60 µL trifluoroacetic acid were added under argon atmosphere. After 24 hours, DDQ (2 mmol, 255 mg) was dissolved in 15 ml of dry THF and added to reaction in ice. The resulting mixture was stirred for 4 hours. Then 12 ml of triethylamine was added dropwise in ice and the reaction was stirred further 45 minutes. Finally, 13 ml of $BF_3.Et_2O$ was added in ice and the reaction was stirred overnight. At the end of reaction solvent was removed under reduced pressure. Then, extracted with dichloromethane

(3x30 ml) and dried over $MgSO_4$. Solvent was evaporated in and the resultant compound purified by column chromatography (%40 yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49-7.47 (m, 3H), 7.28-7.26 (m, 2H), 5.98 (s, 2H), 2.55 (s, 6H), 1.37 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.4, 143.2, 141.7, 135.0, 129.1, 128.9, 127.9, 121.29, 15.0, 14.3.

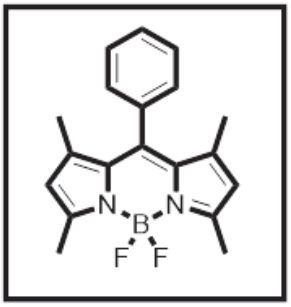

Figure 2.2. 5,5-difluoro-1,3,7,9-tetramethyl-10-phenyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine

2.3.2. Synthesis of IODO-BODIPY

IODO-BODIPY was synthesized by using literature procedure (Wu et al., 2011). N-iodosuccinimide (NIS) (140.0 mg, 0.62 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise into a solution of BODIPY (200 mg, 0.62 mmol) in CH_2Cl_2 (20 mL) within ca. 1 h at 0 ^{o}C. The reaction mixture was then concentrated under reduced pressure, and the crude product was purified by column chromatography (hexane/EtOAc, 20:1, v/v). The product was collected as a red solid. Yield: 191.7 mg, 68.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51- 7.48 (m, 3H), 7.27-7.25 (m, 2H), 6.04 (s, 1H), 2.63 (s, 3H), 2.57 (s, 3H), 1.38 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.9, 154.7, 145.3, 143.4, 141.7, 135.0, 132.0, 131.1, 129.8, 129.5, 129.4, 128.0, 122.5, 84.4, 16.8, 16.0, 14.9, 14.7.

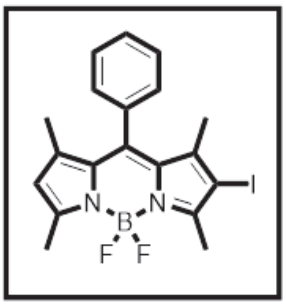

Figure 2.3. 5,5-difluoro-2-iodo-1,3,7,9-tetramethyl-10-phenyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine

2.3.3 Synthesis of BDP-PRP

BDP-PRP was synthesized by using literature procedure (Panteleev et al., 2015). Pd $(PPh_3)_2Cl_2$ (30 mg, 0.042 mmol, 0.1 equiv.) and CuI (16 mg, 0.084 mmol, 0.2 equiv.) were suspended in Et_3N (4 ml) under argon. Then, IODO-BODIPY (190 mg, 0.42 mmol) was added, followed by propargyl alcohol (182 μL, 175.6 mg, 0.46 mmol, 1.1 equiv), and the reaction was stirred at 40 °C for overnight. After the reaction was over, the extraction was done with the DCM and column chromatography (hexanes:EtOAc 10:1) yielded the title compound as an orange solid. Yield: 80 mg, 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.46 (m, 3H), 7.30 – 7.22 (m, 2H), 6.03 (s, 1H), 5.30 (s, 1H), 4.49 (d, *J* = 5.0 Hz, 2H), 2.62 (s, 3H), 2.57 (s, 3H), 1.42 (s, 3H), 1.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.86 (s), 156.32 (s), 144.91 (s), 143.16 (s), 142.18 (s), 134.54 (s), 132.47 (s), 129.20 (d, *J* = 6.7 Hz), 127.75 (s), 122.19 (s), 93.54 (s), 78.24 (s), 77.32 (s), 77.00 (s), 76.68 (s), 51.80 (s), 51.40 (s), 14.75 (s), 14.52 (s), 13.42 (s), 13.02 (s).

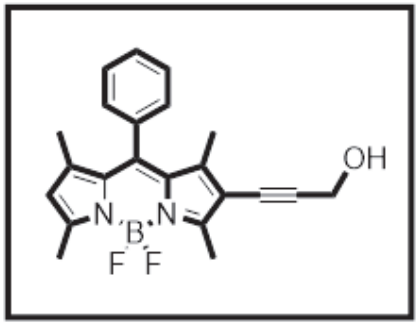

Figure 2.4. 3-(5,5-difluoro-1,3,7,9-tetramethyl-10-phenyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-yl)prop-2-yn-1-ol

2.3.4 Synthesis of BDP-AL

BODIPY-AL was synthesized by using literature procedure (Bobileva et al., 2017). To a solution of BDP-PRP (80 mg, 0.21 mmol) in dry DCM (5 ml) was added MnO_2 (184 mg, 2.11 mmol, 10 equiv.), and the solution was stirred at room temperature for 24 hr. The mixture was filtered through celite with DCM. Then, the filtrate was concentrated and column chromotography was done (hexane:EtOAc, 7:1) to obtain the product (68 mg, 86%) as an yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (d, *J* = 2.4 Hz, 1H), 7.53

(dd, *J* = 4.3, 2.1 Hz, 3H), 7.28 – 7.25 (m, 2H), 6.13 (s, 1H), 2.68 (s, 3H), 2.61 (s, 3H), 1.48 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 176.04 (s), 161.22 (s), 157.18 (s), 147.12 (s), 144.33 (s), 142.69 (s), 133.86 (d, *J* = 19.1 Hz), 129.48 (d, *J* = 8.6 Hz), 127.58 (s), 123.69 (s), 110.30 (s), 97.12 (s), 91.00 (s), 77.34 (s), 77.02 (s), 76.70 (s), 29.67 (s), 15.02 (s), 14.74 (s), 13.41 (s), 12.96 (s).

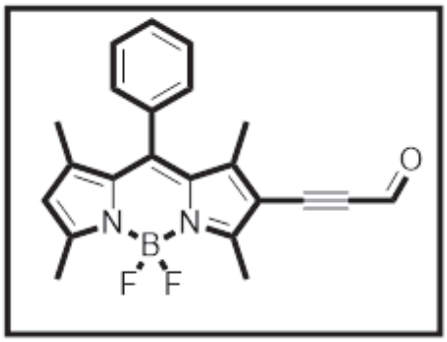

Figure 2.5. 3-(5,5-difluoro-1,3,7,9-tetramethyl-10-phenyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-yl)propiolaldehyde

2.3.5 Synthesis of BDP-BUS

BDP-BUS was synthesized by using literature procedure (Zora et al., 2011). BDP-AL (40 mg, 0.10 mmol) and phenylhydrazine (11.5 mg, 0.10 mmol, 1 equiv.) in a round-bottom flask was stirred at room temperature for 5 hours. After the reaction was over, the residue was purified by column chromatography using hexane/ethyl acetate (10:1) to afford the desired product. Yield: 20 mg, 50%. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.55 (s, 1H), 7.54 (dp, J = 6.8, 2.2 Hz, 3H), 7.30 – 7.26 (m, 3H), 7.25 – 7.20 (m, 1H), 7.03 (ddd, J = 8.6, 2.3, 1.1 Hz, 2H), 6.90 (tp, J = 7.4, 1.2 Hz, 1H), 6.63 (d, J = 2.0 Hz, 1H), 6.09 (s, 1H), 2.71 (s, 3H), 2.61 (s, 3H), 1.51 (s, 4H), 1.42 (d, J = 1.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 159.47 (s), 155.39 (s), 155.38 (s), 155.37 (s), 145.96 (s), 143.45 (s), 142.35 (s), 141.87 (s), 134.32 (s), 133.09 (s), 130.0 (s), 129.37 (s), 127.74 (s), 122.89 (s), 120.92 (s), 115.31 (s), 113.04 (s), 95.53 (s), 85.99 (s), 29.68 (s), 22.68 (s), 14.90 (s), 14.64 (s), 13.59 (s), 13.16 (s).

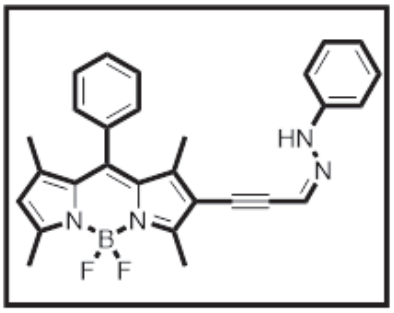

Figure 2.6. (Z)-5,5-difluoro-1,3,7,9-tetramethyl-10-phenyl-2-(3-(2 phenylhydrazono) prop-1-yn-1-yl)- 5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine

2.3.6 Synthesis of BDP-PYRZL

BDP-PYRZL was synthesized by using literature procedure. (Jeyaveeran et al., 2016). To a solution of BDP-BUS (20 mg, 0.042 mmol) in EtOH (2 ml) was added $HgCl_2$ (23.2 mg, 0.082 mmol, 2 equiv.) under argon and stirred at room temperature for 5 hr. After the reaction was completed, the reaction mixture was concentrated under reduced pressure and purified by column chromatography to afford the pure product of pyrazole. Yield: 10 mg, 52%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.63 (m, 1H), 7.54 – 7.43 (m, 2H), 7.36 – 7.29 (m, 5H), 7.25 (s, 1H), 7.24 – 7.19 (m, 3H), 6.06 (s, 1H), 2.57 (s, 3H), 2.18 (s, 3H), 1.40 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.64 (s), 159.08 (s), 151.58 (s), 145.80 (s), 144.49 (s), 142.47 (s), 139.83 (s), 134.38 (s), 129.51 (s), 129.27 (s), 129.02 (s), 127.76 (s), 127.58 (s), 127.52 (s), 123.48 (s), 42.69 (s), 30.92 (s), 29.68 (s), 29.30 (s), 22.68 (s), 14.85 (s), 14.62 (s), 14.14 (s), 13.15 (s), 12.81 (s).

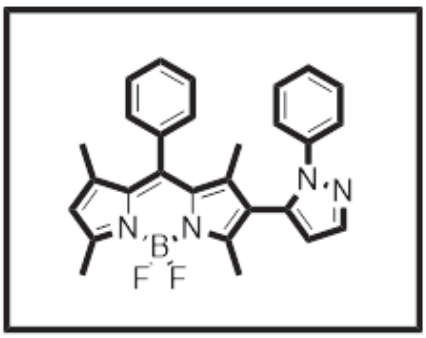

Figure 2.7. 5,5-difluoro-1,3,7,9-tetramethyl-10-phenyl-2-(1-phenyl-1H-pyrazol-5-yl)-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine

CHAPTER 3

RESULTS AND DISCUSSION

In this work, a new BODIPY-based fluorescent probe for the detection of mercury ions was developed. Based on previous studies, the activation of alkynes by Hg ions has long been known among chemists. However, a challenging point taken into consideration while designing the probe was the cross-affinity of other metal ions (e.g. Au^{+}, Au^{3+}, Pb^{2+}, Ag^{+}, Cu^{2+}) which has the same chemical nature as mercury ions. With this all in mind, a novel fluorescent probe was designed which was expected to be non-emissive at first due to the deactivation via isomerization of the C=N bond, and its spectroscopic behaviors were determined. BDP-BUS was synthesized by the synthetic route and characterized using ^{1}H-NMR, ^{13}C-NMR, and MS. For the applicability of mercury ions into the biological systems, the most suitable solvent system was determined. Among several combinations of solvent systems such as EtOH/PBS, HEPES/CH_3CN, EtOH/phosphate buffer, the maximum fluorescence intensity upon the addition of mercury ions was established in the CH_3CN/PBS (3:7, v/v, pH = 7.4) with 10 μM dye concentration. Then, the effect of pH was surveyed in different pH values from 4 to 9 and it can be seen from the results that there was no fluorescence response in the absence of mercury ions. In addition, the maximum fluorescence emission was found to be in the pH range of 6-7 which showed that the probe can be used for imaging of mercury ions in biological systems. (Figure 3.1)

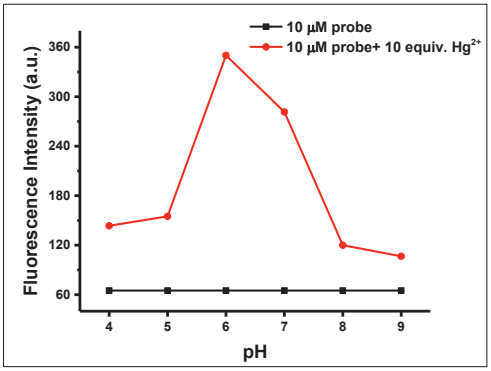

Figure 3.1. Effect of pH on the interaction of BDP-BUS (10.0 μM) with 10 equiv. Hg^{2+} in CH_3CN/PBS buffer solution (3:7, v/v, pH = 7.4). (λ_{ex}= 480 nm, λ_{em}= 530 nm at 25°C)

The photophysical behavior of BDP-BUS in response to the mercury ions was assessed with the UV-Vis and fluorescence spectroscopy. The free probe showed no fluorescent emission upon excitation at 480 nm due to non-radiative deactivation process. After the addition of Hg^{2+} (10 equiv.), fluorescent emission at 530 nm and a color change from colorless to green was observed which was the result of the intramolecular cyclization over the hydrazone unit which diminished the C=NNHR isomerization (Figure 3.2.)

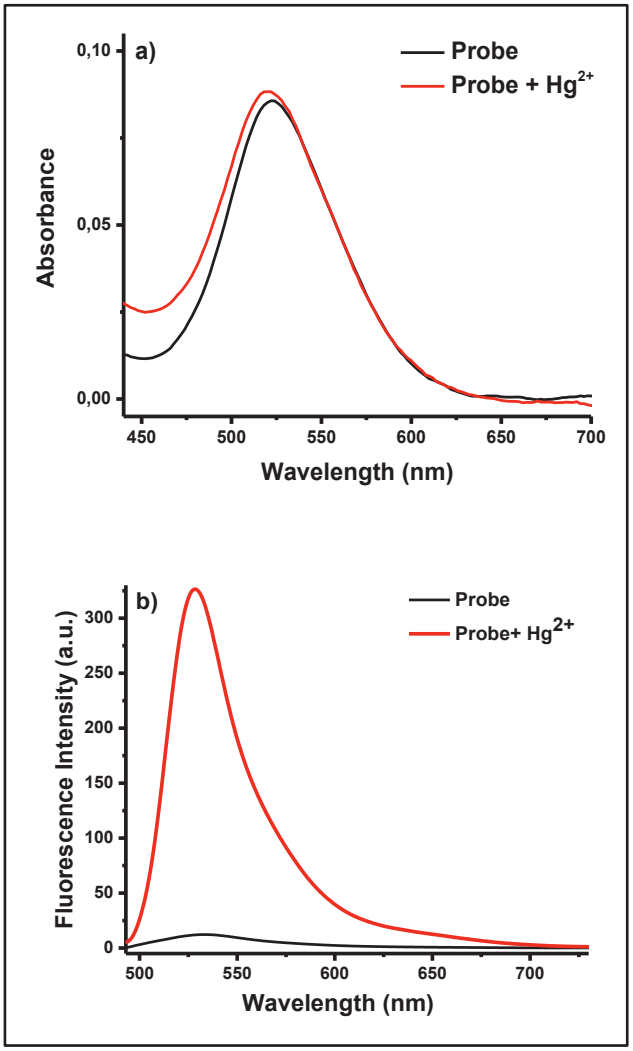

Figure 3.2. Absorption (a) and fluorescence (b) spectra of BDP-BUS (10 μM) in CH_3CN/PBS buffer solution (3:7, v/v, pH = 7.4) (λ_{ex}= 480 nm).

Next, the time dependency of BDP-BUS was investigated. The fluorescence intensity increased in the presence of Hg ions and became saturated within 10 minutes.

Based on this result, 10-minute standard was set for the further measurements. (Figure 3.3.)

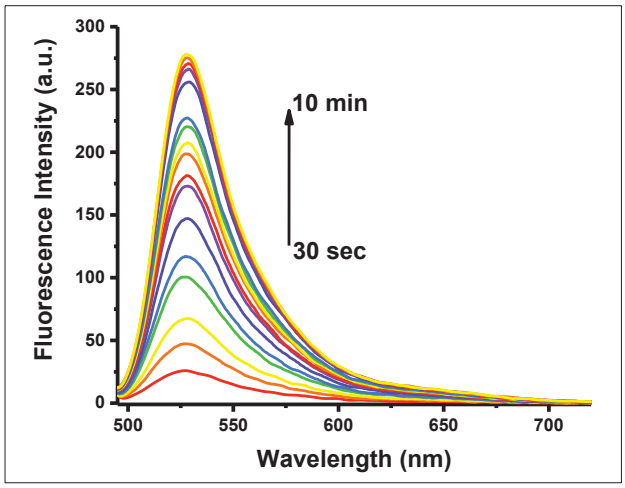

Figure 3.3. Time dependent fluorescence changes of BDP-BUS (10.0 μM) with 10 equiv. Hg^{2+} in CH_3CN/PBS buffer solution (3:7, v/v, pH = 7.4). (λ_{ex}= 480 nm, λ_{em}= 530 nm at 25 °C) within 10 min.

Then, the fluorescent titration experiment was determined with the systematic addition of mercury ions. (Figure 3.4.) It indicated that the fluorescence intensity of BDP-BUS was dramatically increased linearly upon the addition of Hg^{2+} (0-30 equiv.) and after addition of 20 equiv. of Hg^{2+}, it became saturated and remained stable.

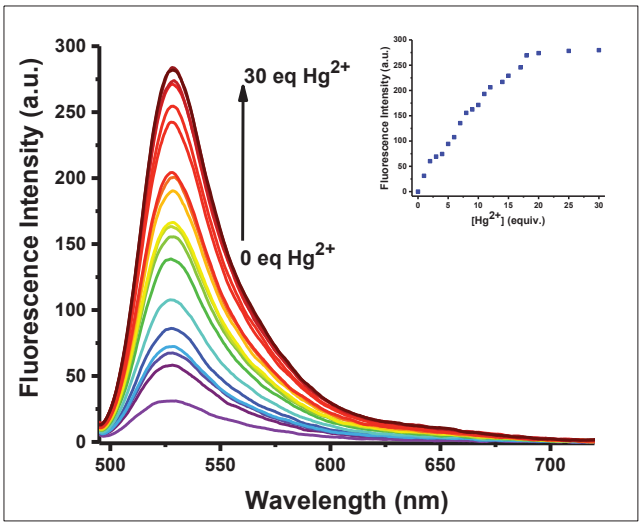

Figure 3.4. Fluorescence spectra of BDP-BUS (10 μM) in CH_3CN/PBS buffer solution (3:7, v/v, pH = 7.4) in the presence of different concentrations of Hg^{2+} (0–30 equiv). Inset: Calibration curve. (λ_{ex} = 480 nm at 25 °C).

Moreover, the selectivity of BDP-BUS was determined by screening its fluorescence response towards alkynophilic metal species, including Hg^{2+}, Au^{3+} Pd^{2+}, Cu^{2+}, Ag^{+} and other metal species Li^{+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Au^{+}, Cr^{2+}, Mg^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Al^{3+} in CH_3CN/PBS buffer solution (3:7, v/v, pH = 7.4). As can be seen in Figure 3.5., the probe showed good selectivity towards Hg^{2+}. The interference of other metal in the presence of Hg ions was also determined. Therefore, BDP-BUS showed a fluorescence response to mercury ions in the presence of 50 equiv. of all other metal ions. These results proved that the probe displays excellent sensitivity and selectivity towards Hg^{2+} over other competitive ions. (Figure 3.5)

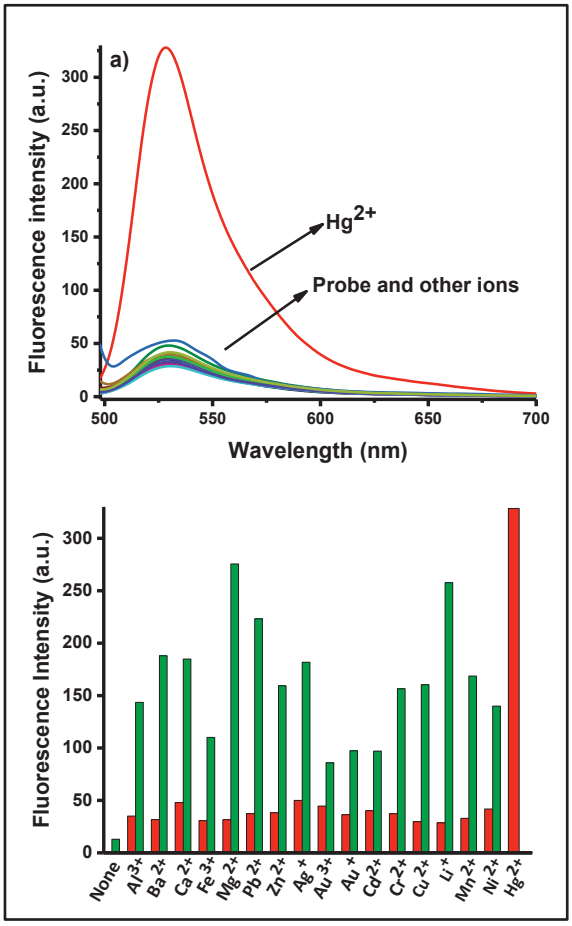

Figure 3.5. a) Fluorescence intensity of BDP-BUS (10 µM) in CH_3CN/PBS buffer (3:7, v/v, pH= 7.4) with representative cations (100 µM) b) fluorescence responses of BDP-BUS to various metal ions including Hg^{2+}, Au^{3+} Pd^{2+}, Cu^{2+}, Ag^{+}, Li^{+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Au^{+}, Cr^{2+}, Mg^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Al^{3+}. Red bars represent the addition of 50.0 equiv. of metal ions to a 10 µM solution of BDP-BUS (CH_3CN/PBS buffer (3:7, v/v, pH= 7.4). Green bars represent the addition of 10 equiv. of Hg^{2+} to the solutions containing BDP-BUS (10 µM) and the appropriate metal ions (50.0 equiv.). (λ_{ex} = 480 nm).

To determine the detection limit of BDP-BUS towards Hg^{2+}, the fluorescence titration experiment was performed. The emission intensity of the probe (10 μM) was measured 10 times to calculate the standard deviation of blank. A linear relationship between the fluorescence intensity and Hg^{2+} concentration was observed in the range 0 - 1 μM (R^2 = 0.9541). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 29 nM.

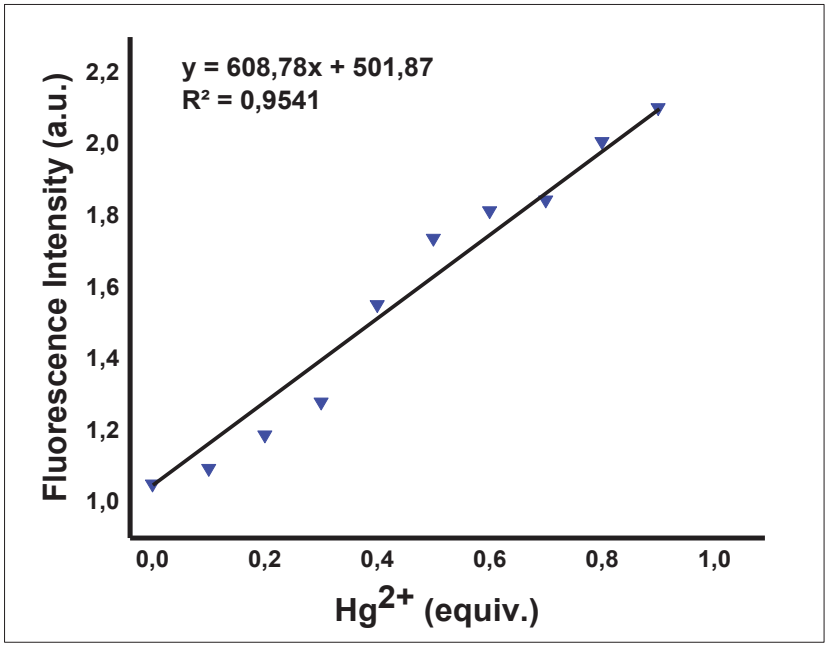

Figure 3.6. Fluorescence changes of BU-49 (10.0 μM) upon addition of Hg^{2+} (0.1 to 1 equiv.) in ACN/PBS buffer solution (3:7, v/v, pH = 7.4). (λ_{ex}= 480 nm, λ_{em}= 530 nm at 25 ºC)

As shown in Figure 3.7., the identification of Hg^{2+} was proceeded by a sequential process started with the activation of triplet bond by Hg^{2+}. Then with the addition of -NNHPh (phenylhydrazine), intermolecular cyclization promoted and produced a BODIPY derivative appended with a pyrazole ring. As a result of this process, a green emission was observed.

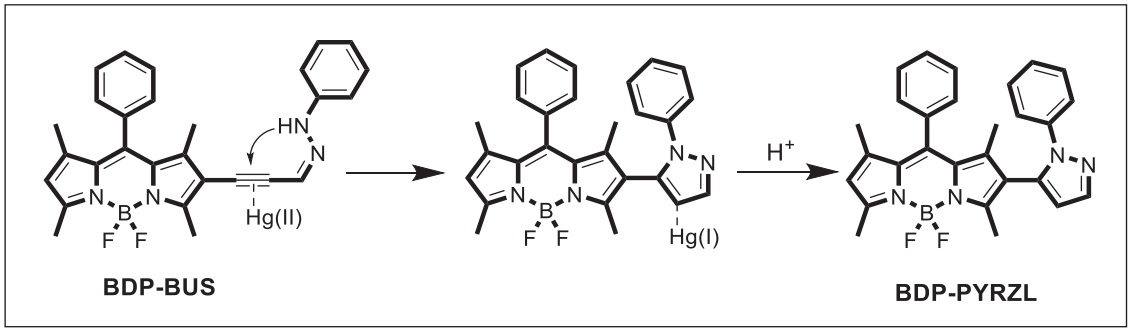

Figure 3.7. Cyclization reaction of BDP-BUS

Relying upon the promising properties of the designed probe, its sensing ability in living systems was examined. To this goal, human lung adenocarcinoma cells (A549) were incubated first with BDP-BUS (10 μM) for 30 min, then washed with PBS buffer three times. This process was followed by incubation with Hg^{2+} (100 μM) for 30 min and rinsed with PBS buffer three times. Then, the cells were stained with DAPI (1.0 mM) and the fluorescence images were obtained after 10 min. The images indicated that the cells showed a healthy spread, and no fluorescence signal was observed absence of Hg^{2+}. On the other hand, the cells incubated with Hg^{2+} exhibited a bright green fluorescence signal.

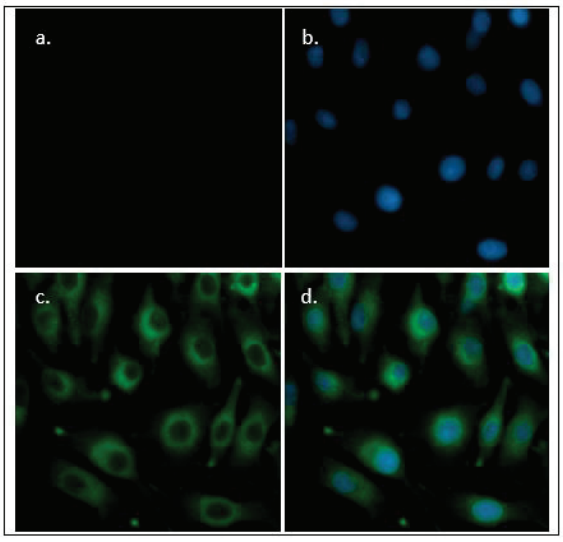

Figure 3.8. Fluorescence images of human lung adenocarcinoma (A549) cells. (a) Fluorescence image of A549 cells treated with only BDP-BUS (10 μM); (b) fluorescence image of cells treated with DAPI (control); (c) fluorescence image of cells treated with BDP-BUS (10 μM) and Hg^{2+} (100 μM) (λ_{ex}= 480 nm); (d) merged images of frames b- c.

CHAPTER 4

CONCLUSION

In this study, a new fluorescent probe derivatized with an alkyne moiety for the sensitive and selective detection of Hg ions was developed. Photophysical properties of the probe were examined and its imaging capacity for Hg^{2+} species in living cell was also determined.

BODIPY core was used as a fluorophore and the probe showed no fluorescence emission in absence of Hg ions (Φ_F= 0.09) due to the deactivation via isomerization of the C=N bond. In the presence of mercury species, intramolecular cyclization over the hydrazone unit was took place and a distinct green color was observed as a result of the formation of a desired product (BDP-PYRZL) ($\Phi_{F=}$0.7).

The probe was highly selective towards mercury species, with very low detection limit (29 nM) and there was no interference arising from other metal ions such as Ag^{+}, Au^{3+} and Pb^{2+} which also have an alkynophilic nature.

Furthermore, the sensing ability of BDP-BUS in human lung adenocarcinoma (A549) cell line was investigated. The probe exhibited a high selectivity towards mercury species in living cells, providing a bright green color in existence of Hg ions.

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APPENDIX A

^{1}H-NMR AND ^{13}C-NMR SPECTRA OF COMPOUNDS

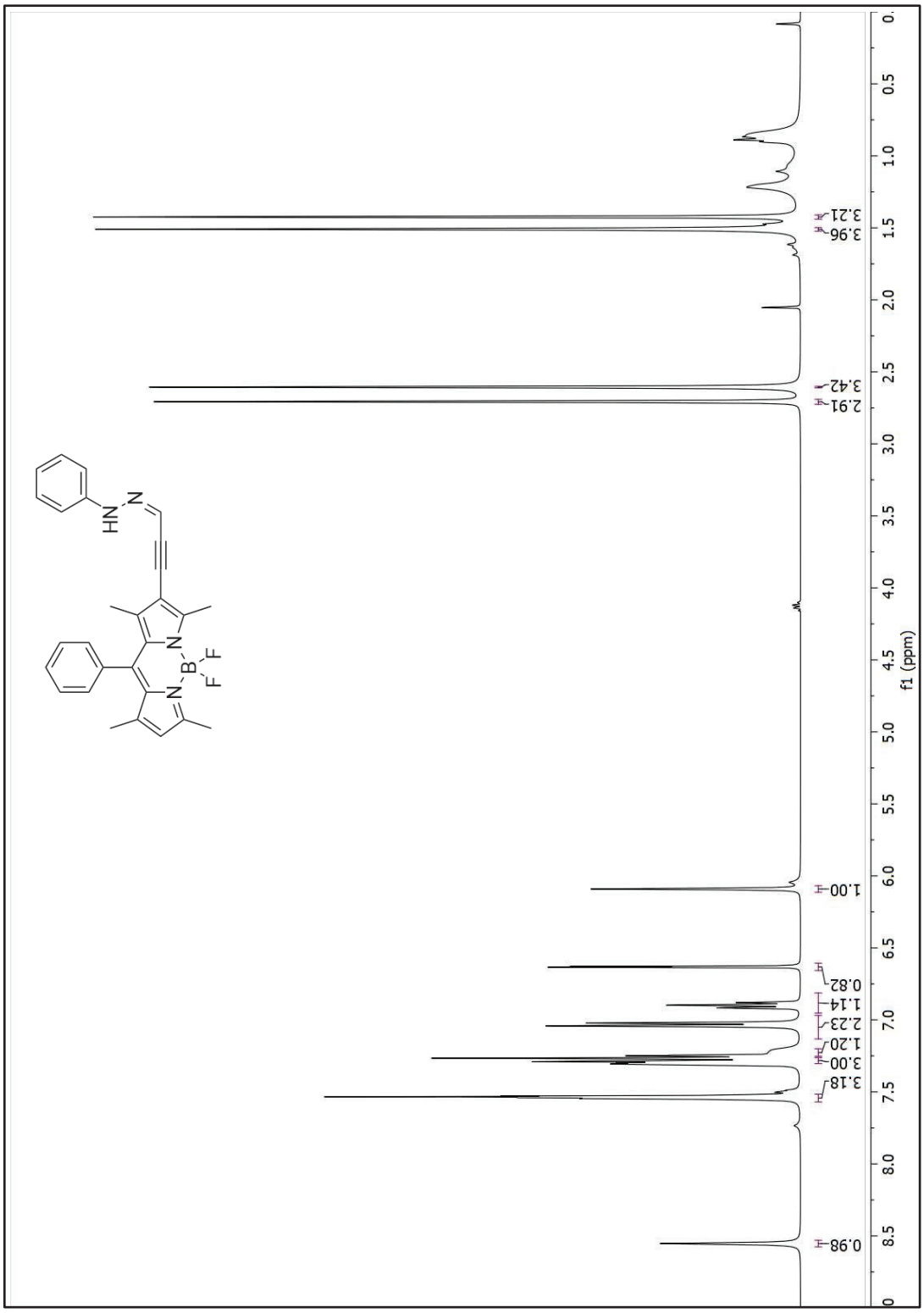

Figure A.1. ^{1}H NMR of **BDP-BUS**

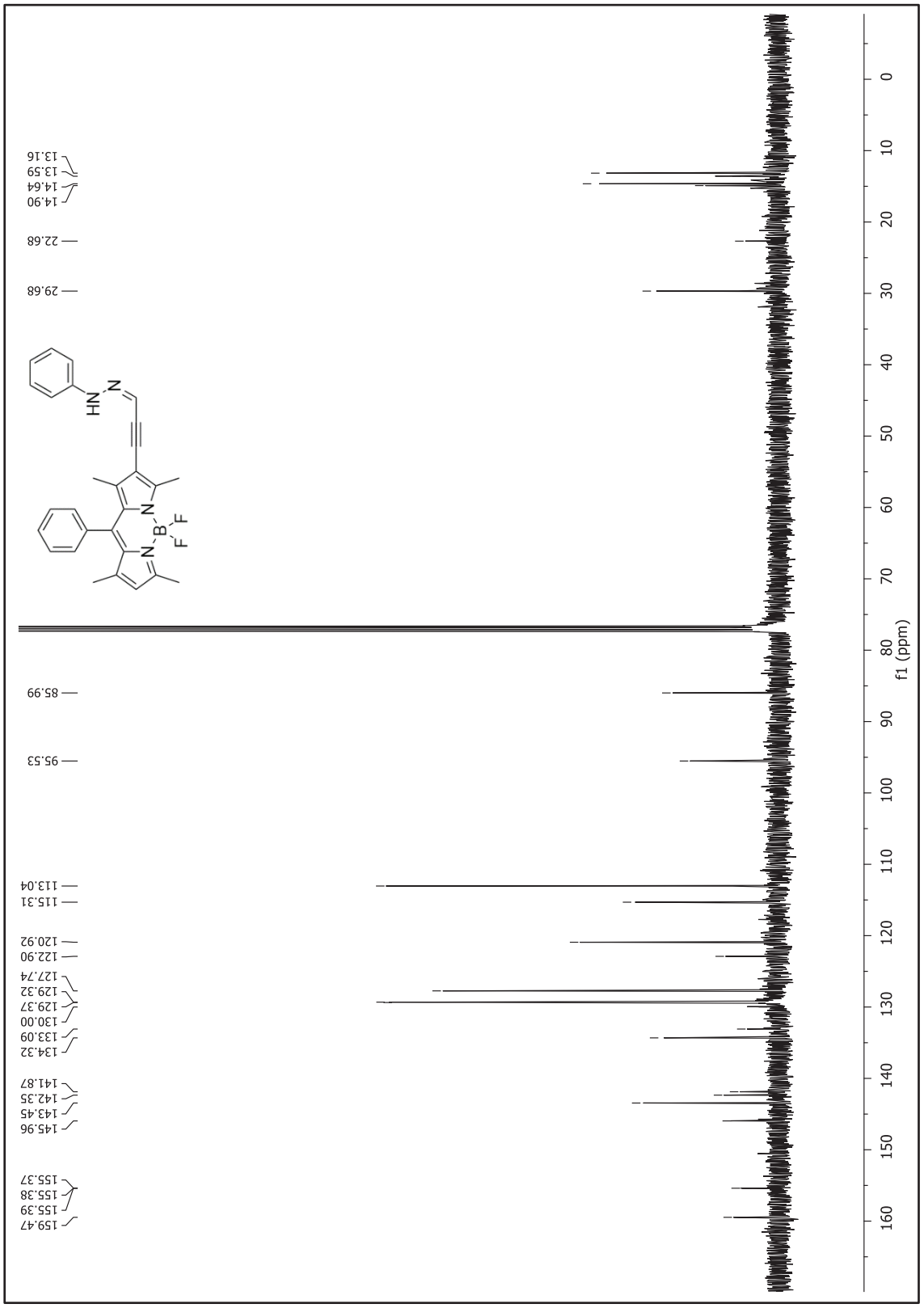

Figure A.2. ^{13}C NMR of **BDP-BUS**

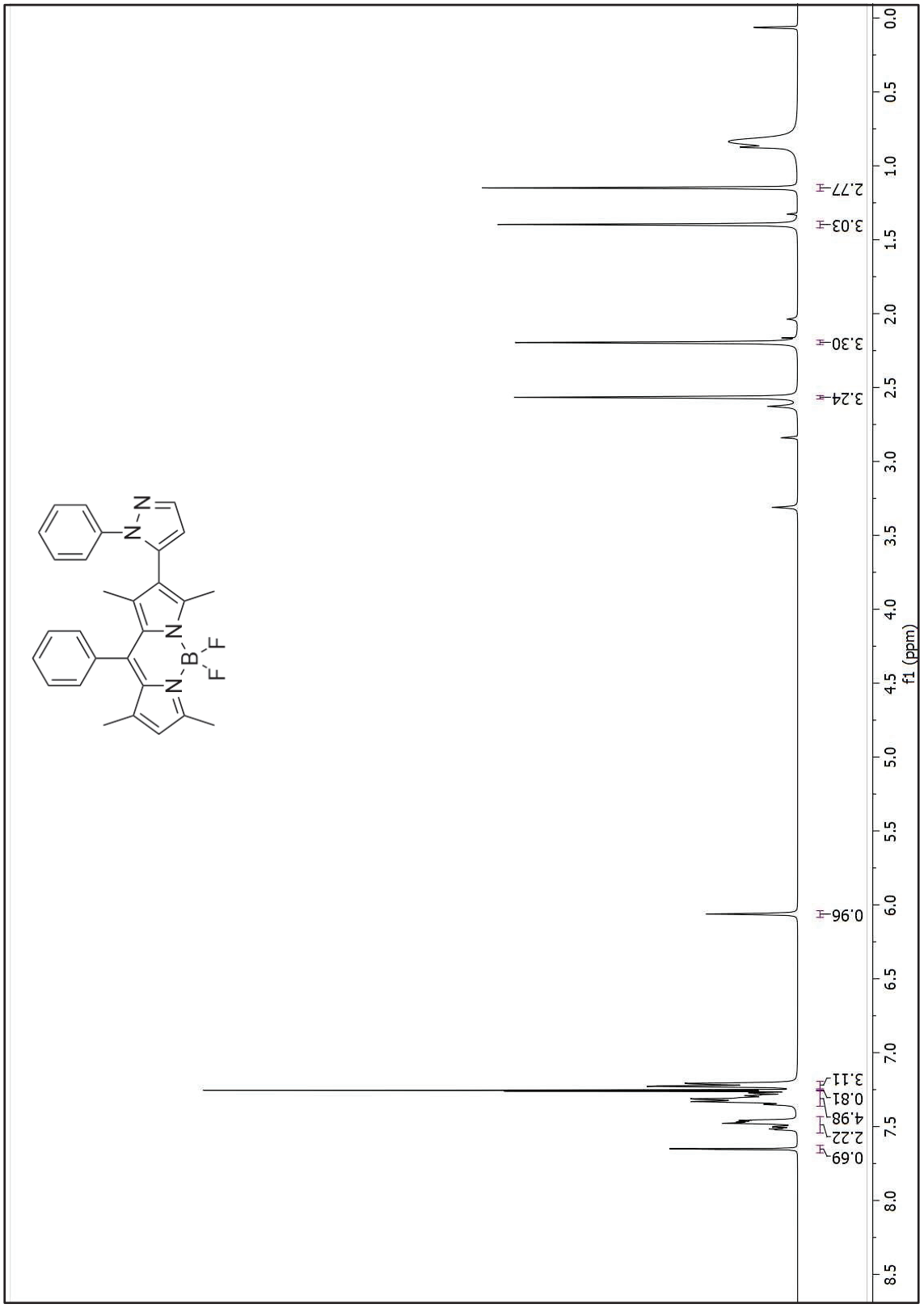

Figure A.3. ^{1}H NMR of **BDP-PYRZL**

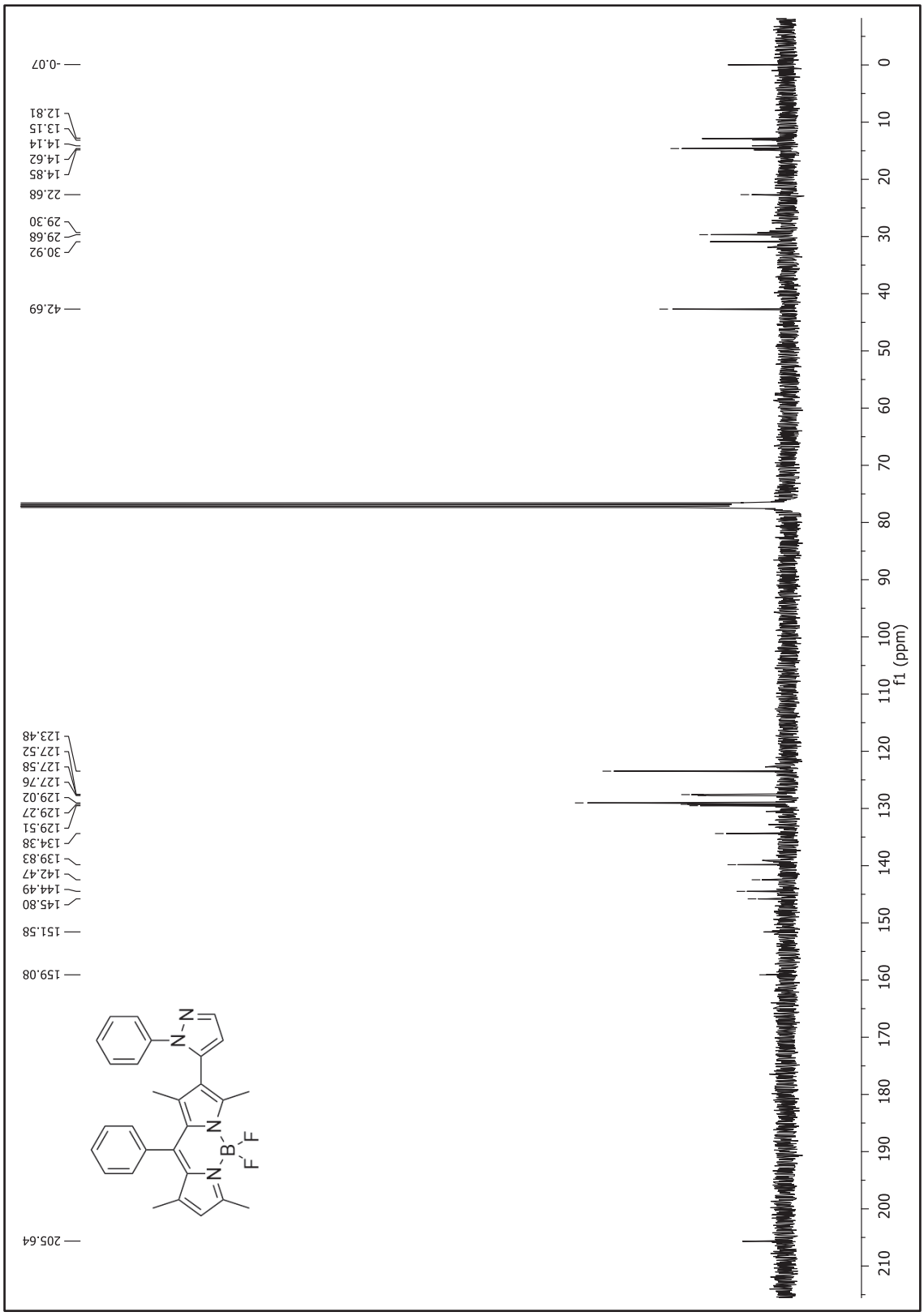

Figure A.4. ^{13}C NMR of **BDP-PYRZL**

APPENDIX B

MASS SPECTRA OF COMPOUNDS

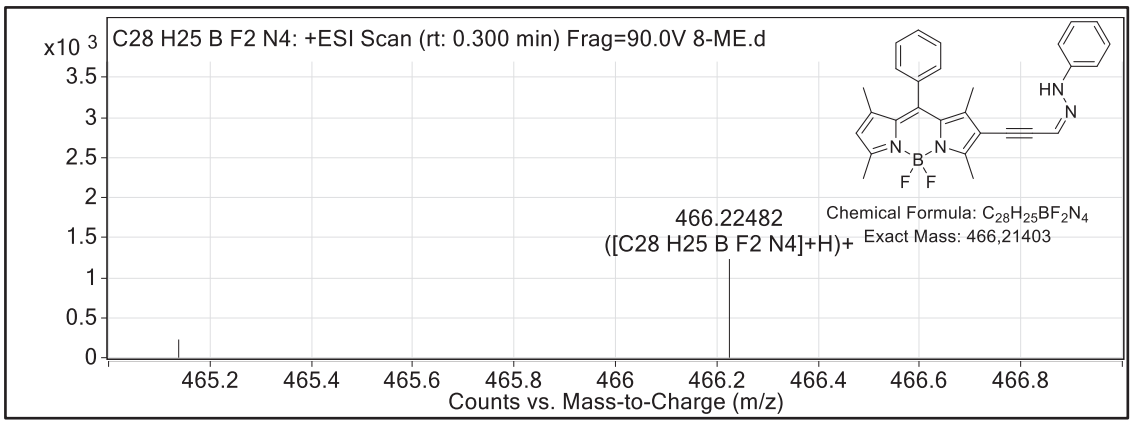

Figure B.1. Mass spectrum of **BDP- BUS** ((Z)- 5,5- difluoro- 1,3,7,9-tetramethyl-10-phenyl-2-(3-(2- phenylhydrazono) prop-1-yn-1-yl)-5*H*-5λ^4,6λ^4-dipyrrolo [1,2- c:2',1'-f][1,3,2]diazaborinine)

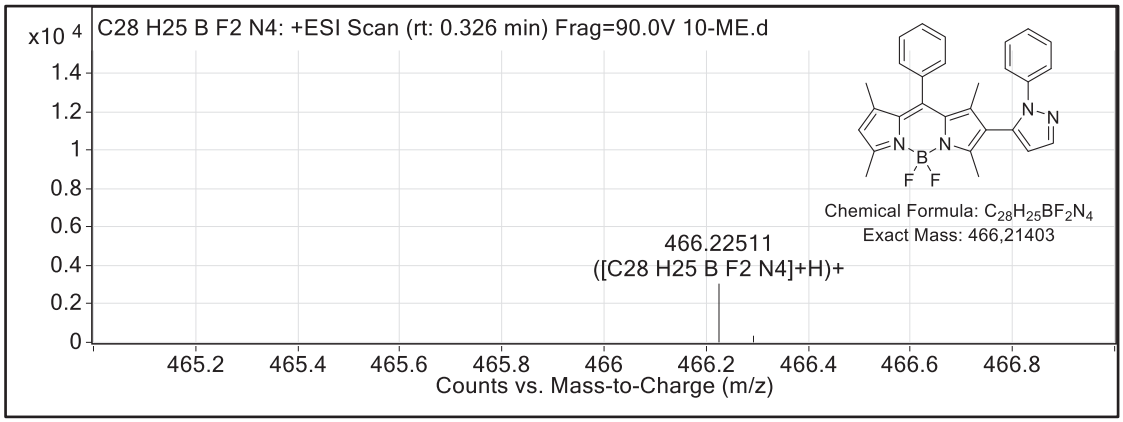

Figure B.2. Mass spectrum of **BDP-PYRZL** (5,5-difluoro-1,3,7,9-tetramethyl-10-phenyl-2-(1-phenyl-1*H*-pyrazol-5-yl)-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine)