การสังเคราะห์สีย้อมอินทรีย์ที่มีฐานเป็นโบดิพีเพื่อใช้เป็นฟลูออเรสเซนต์เซนเซอร์ในการตรวจจับไอออนของโลหะ

SYNTHESIS OF BODIPY-BASED ORGANIC DYE AS A FLUORESCENT SENSOR FOR DETECTION OF METAL IONS

โดย

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ปริญญาวิทยาศาสตรบัณฑิต

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จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2557

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โดย นายณัฐกร อุปถัมภ์

ได้รับอนุมัติให้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรบัณฑิต ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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ชื่อโครงการ การสังเคราะห์สีย้อมอินทรีย์ที่มีฐานเป็นโบดิพีเพื่อใช้เป็นฟลูออเรสเซนต์เซนเซอร์ในการตรวจจับไอออนของโลหะ
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บทคัดย่อ

ในงานวิจัยนี้ได้ออกแบบโมเลกุลของสีย้อมอินทรีย์ที่มีฐานเป็นโบดิพี โดยมีชิฟเบสเป็นหมู่แทนที่ในตำแหน่ง *เมโซ-* เพื่อใช้ในการศึกษาการเกิดสารประกอบเชิงซ้อนกับไอออนของโลหะหนัก การสังเคราะห์เริ่มต้นจากไพโรลและ 4-คาร์บอกซีเบนซาลดีไฮด์ ทำปฏิกิริยาเกิดเป็นสารประกอบไดไพโรมีเทน สารผลิตภัณฑ์ที่ได้ไม่สามารถทำปฏิกิริยาขั้นต่อไปได้ เนื่องจากเกิดการสลายตัวระหว่างทำปฏิกิริยา จึงได้เปลี่ยนวิธีการสังเคราะห์โดยให้ไพโรลและ 4-คาร์บอกซีเบนซาลดีไฮด์ทำปฏิกิริยาเกิดเป็นสารประกอบโบดิพีก่อน แล้วจึงนำไปทำปฏิกิริยาต่อในขั้นถัดไป แต่สารผลิตภัณฑ์ที่สังเคราะห์ได้ยังคงมีสิ่งปนเปื้อนที่ไม่สามารถกำจัดออกได้หมด ทำให้ไม่สามารถสังเคราะห์สารประกอบในขั้นถัดไป ซึ่งเป็นสารตั้งต้นในการสังเคราะห์สารประกอบเป้าหมายได้

คำสำคัญ: โบดิพี, ฟลูออเรสเซนต์เซนเซอร์, ไอออนของโลหะ

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Title Synthesis of BODIPY-based Organic Dye as a Fluorescent Sensor for Detection of Metal Ions
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ABSTRACT

In this study, a novel BODIPY-based organic dye was designed to be used in a heavy-metal ion complexation studies. It was derivatized by Schiff base at *meso*-position of the BODIPY. In the first proposed synthesis route, pyrrole and 4-carboxybenzaldehyde condensed to give dipyrromethane. The product cannot be used in the next step because it was decomposed. The modified version of synthesis route was proposed. BODIPY dye was synthesized from pyrrole and 4-carboxybenzaldehyde. The product was contaminated by impurities which cannot be completely eliminated. Therefore, the target molecule was not obtained and the consequential experiments were not conducted.

Keyword: BODIPY, fluorescent sensor, metal ions

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LIST OF ABBREVEATIONS

d	doublet (NMR)
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
Et	ethyl
h	hour
Hz	hertz
m	multiplet (NMR)
MHz	megahertz
min	minute
mL	milliliter
mmol	millimole
mol	mole
NMR	Nuclear Magnetic Resonance
ppm	part per million
s	singlet (NMR)
t	triplet (NMR)
TFA	trifluoroacetic acid
TLC	thin layer chromatography
UV	ultraviolet
δ	chemical shift
%	percent

ศูนย์วิทยทรัพยากร
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CHAPTER I

INTRODUCTION

1.1 Statement of the Problems

Heavy-metal ions (e.g. Cd^{2+}, Hg^{2+}, and Pb^{2+}) are toxic to the organisms including human. The air, soil and water can be contaminated by heavy-metals ions. Human receives heavy-metal into the body by food consumption because of the accumulation in the food chain. Heavy-metals cannot be eliminated by metabolism.[1] Accumulation of heavy-metals can cause many diseases in human. Over exposure of cadmium (Cd) can cause renal impairment and bones softening. The disease caused by cadmium poisoning is called itai-itai disease.[2] Mercury (Hg) is one of the most toxic pollutants. Accumulation of mercury can cause many symptoms such as liver and kidney impairment, heart failure, and anemia.[3] Lead (Pb) is not a transition metal, but it is categorized as heavy-metal. The symptoms caused by accumulation of lead are insomnia, delirium, convulsion, anemia, and kidney failure.[4] Some of heavy-metals are essential nutrition in plant and animal including human such as copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn). Intaking of these elements are not dangerous, if they are in optimal concentration range.

Many techniques such as titration, atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), anodic stripping voltammetry, and X-ray fluorescence spectrometry are used to identify and detect heavy-metal ions. Some techniques require multi-step of sample preparation and expensive instruments. Some heavy-metal ions may change their oxidation state after sampling and error results may be obtained.[5] As a result, low-cost, highly selective and sensitive technique is more needed to discriminate and detect metals ion.[6]

Using fluorescent sensors which can interact with metal ions is another technique to detect and measure metal ions. Color change of analytes can be easily observed by naked-eye. The change of absorption and emission bands of the sensor is used to identify metal ions in an analyte. Moreover, it is also used to quantify the concentration of metal,

sensitivity, selectivity, stoichiometry, binding constant, etc. Fluorescent sensors of metal ions consist of two parts. One is a fluorophore which is able to absorb and emit light. There are many kinds of organic dyes being used as fluorophore such as azo dyes, BODIPY dyes, coumarin dyes, fluorescein dyes, and rhodamine dyes. The other part of the sensor is a metal chelating molecule. When this part is bound with a metal ion, the electronic structure or the molecular structure of the sensor is altered. The wavelength of light absorption and emission of the sensor is changed due to the change of electronic structure.[7] This change is used in quantification process and identification of metal ions.

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene dyes or BODIPY dyes are widely used as a chemosensor to detect and discriminate metal ions because they are strongly UV-visible absorbing small molecules that emit sharp fluorescence peaks with high quantum yield and small Stokes' shift. They have high solubility in many organic solvents. Moreover, they are stable and insensitive to polarity and pH change.[8]

In this study, a novel BODIPY-based organic dye was designed in purpose of fluorescent sensor for metal ions detection. This molecule consists of BODIPY as a fluorophore and Schiff base which is substituted at *meso*-position as a metal chelating part.

1.2 Theory

1.2.1 Fluorescence

When the elementary particles in their ground state are excited by absorbing the light or electromagnetic radiation which has suitable energy, electrons move to excited state from the ground state with the process called absorption.

$$M + h\nu \rightarrow M^*$$

M represents an elementary particle in a ground state

M* represents an elementary particle in an excited state

Relaxation of the excited particles to ground state leads to light emission and the luminescence is observed. Fluorescence is a type of relaxation of excited particles to ground state which is spin-allowed. There are many types of relaxation which do not have a light emission in de-excited process such as internal conversion, intersystem crossing,

intramolecular charge transfer, and conformational change. Moreover, electron transfer, energy transfer, and excimer formation are the types of relaxation of excited molecule that can interact to other molecules (Figure 1.1).

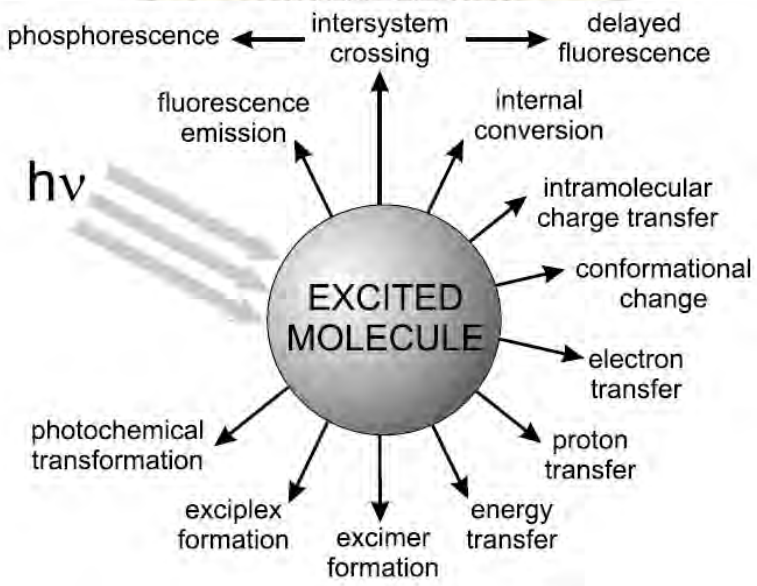

Figure 1.1 Possible de-excitation pathways of excited molecules. (reference 9)

The Jablonski diagram[9] (Figure 1.2) is a diagram that shows the electronic states of the molecule and transition pathways after absorption of photon. S_0 is a ground state. S_1, S_2, and S_n are represented as singlet energy levels, while T_1 and T_2 are triplet energy levels. Energy levels which are between singlet energy levels and also triplet energy levels are vibrational levels. When a molecule is irradiated with photon or electromagnetic radiation, an electron is excited from S_0 to upper singlet energy levels (S_n). Internal conversion occurs when the excited electron relaxes to lowest vibrational level of S_1 non-radiatively. At this point, there are two pathways to be occurred. First way is fluorescence. In this process, the excited electron at S_1 relaxes to ground state and leads to light emission. This process is a radiative pathway. The other way is intersystem crossing. Spin of the electron changes in this process because electron moves from singlet excited state, S_1 to triplet excited state, T_1. This process is non-radiative. After this process, the excited electron at T_1 relaxes to ground state and leads the light emission called phosphorescence.

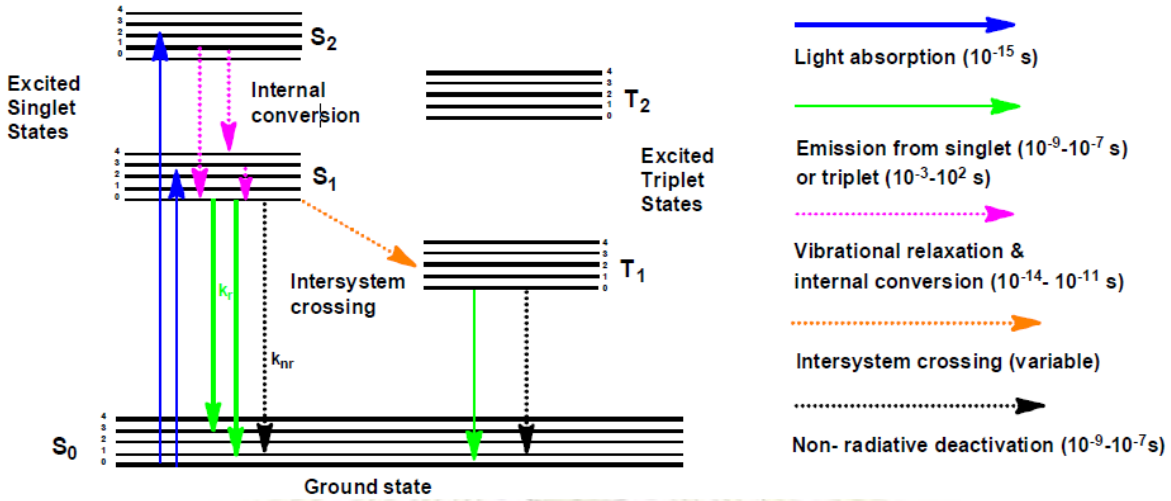

Figure 1.2 The Jablonski diagram. (Adapted from reference 9)

From the Jablonski diagram, the emitted light has less energy compared to the absorbed light. The emission occurs in the longer wavelength because of the rapid relaxation of the excited electron to lowest vibrational state of S_1 non-radiatively. This difference between excitation and emission wavelength is called Stokes' shift (Figure 1.3).[9]

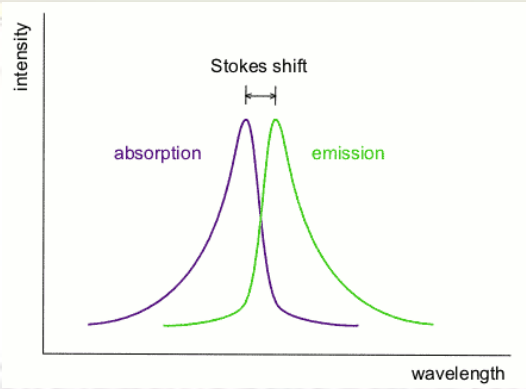

Figure 1.3 Stokes' Shift.

1.2.2 Fluorescent Dyes

Non-fluorescent dyes absorb part of the light and the remaining part is reflected. An observer sees the remaining part of the light that is reflected and that is its color. For example, a green dye reflects green light to the observer because that dye absorbs the red part of visible wavelength, which is complementary light of green. Each fluorescent dye has difference fluorescent properties because each dye can absorb and emit light at particular wavelength and uniquely gives fluorescent signals.[10]

There are many kinds of fluorescent dye: coumarins, pyrenes, naphthalenes, fluoresceins, rhodamines, and BODIPY. Most dyes absorb light in UV-visible region, which enable them to use in broad application. Organic dyes have some advantages and drawbacks. Their advantages are high quantum yield, good solubility and conjugation, but self-quenching and easy photobleaching are considered as their drawbacks.

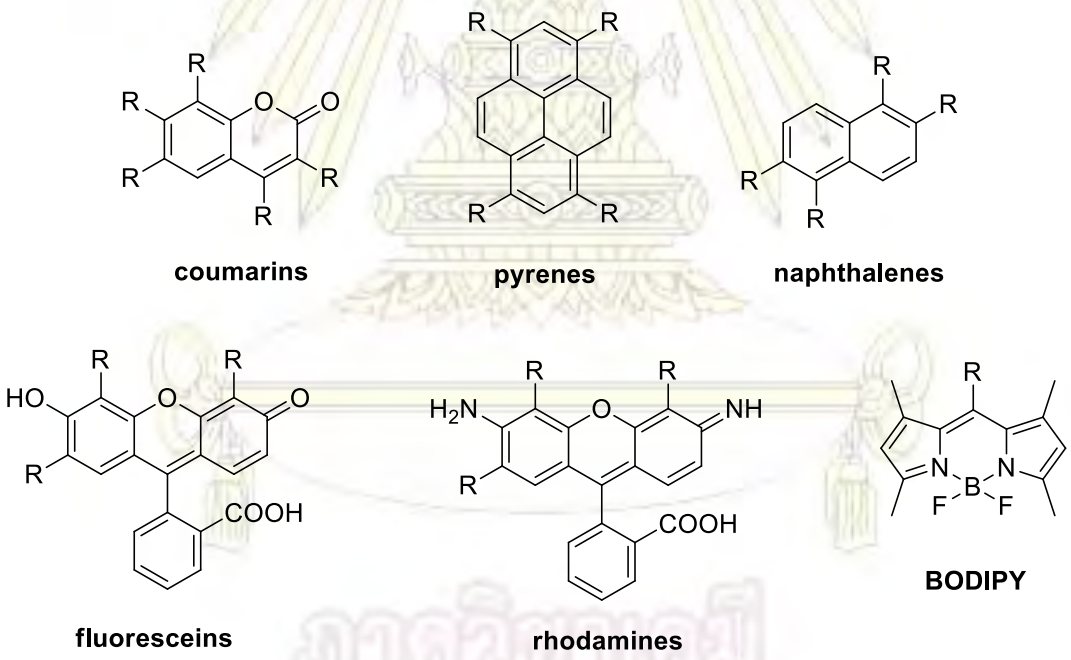

Figure 1.4 Structure of organic dyes.

1.2.3 BODIPY

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacenes or BODIPY dyes were first discovered by Treibs and Kreuzer in 1968.[11] BODIPY is a fluorescent dyes which are widely used in many research areas such as ion sensing, protein labeling, DNA labeling, sensitizers for solar cells, drug delivery reagents and light harvesting systems.

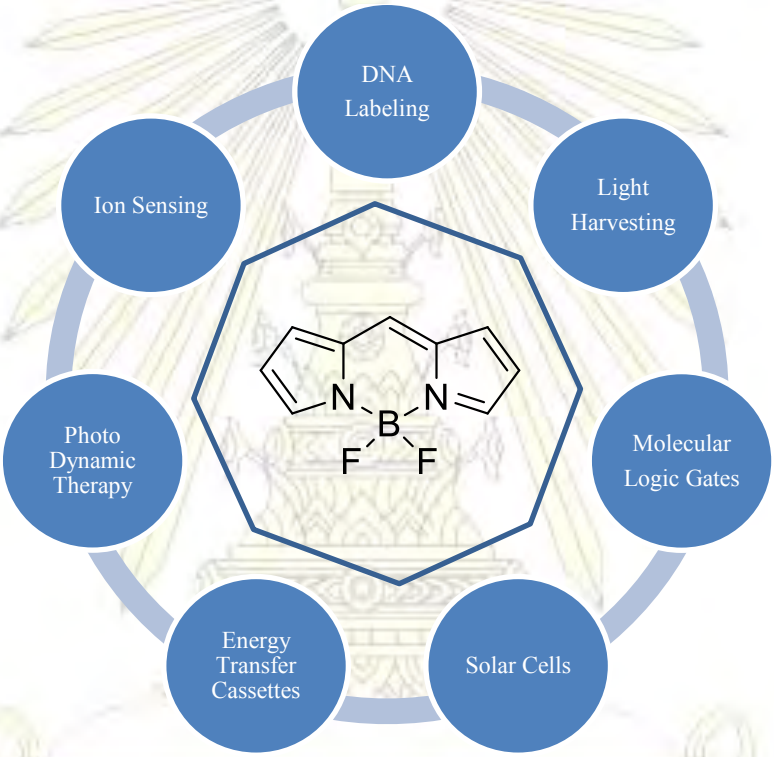

Figure 1.5 Application areas of BODIPY. (Adapted from reference 8)

BODIPY is composed by dipyrromethene complexed with disubstituted boron atom with fluorine atom (BF_2). BODIPY dyes have great properties and can be applied in many research areas. They are strongly UV-visible absorbing small molecules that emit sharp fluorescence peaks with high quantum yield. They have high molar absorptivity and high solubility in many organic solvents. Also, they are insensitive to polarity and pH of their environment.[8] BODIPY is easy functionalized and modified. All of the positions (1 to 8) of BODIPY can be functionalized or modified.

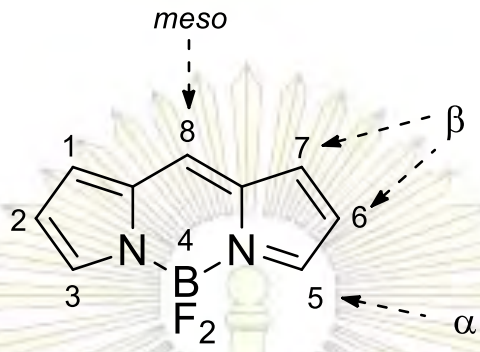

Figure 1.6 The BODIPY core.

1.3 Related Research

meso-Substituted BODIPY dyes tend to be easy to prepare via condensation of pyrroles and aldehydes. As shown in Figure 1.7, the aldehyde was condensed with pyrrole to give excellent yields of dipyrromethane. *p*-Chloranyl was used as oxidizing agents to turn the dipyrromethane into dipyrromethene. The dipyrromethene was then complexed with boron trifluoride and give **BDP-1** as a product. However, the byproducts from the reaction had to be removed after complexation was done. [12]

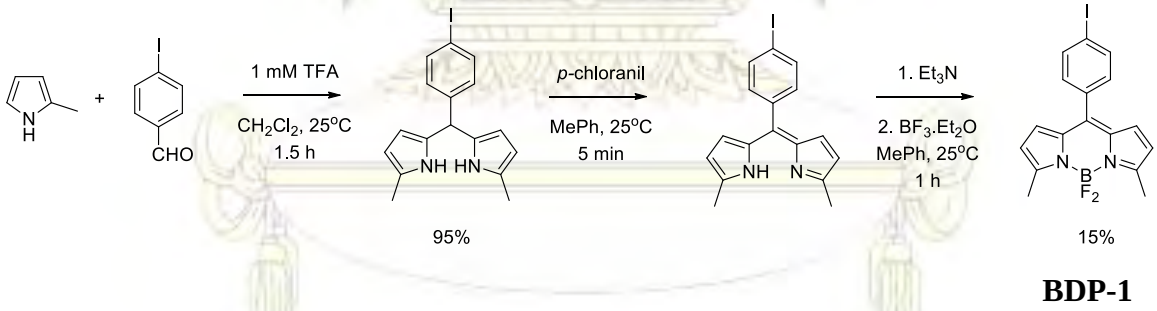

Figure 1.7 Synthesis of BODIPY dye using *p*-chloranil as oxidizing agent.

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) can be used as oxidizing agent instead of *p*-chloranyl (Figure 1.8). **BDP-2** was prepared from aldehyde using neat condition. The aldehyde was dissolved in excess pyrrole at room temperature. The dipyrromethane was isolated and then oxidized by DDQ. **BDP-2** was obtained after the complexation with boron trifluoride. [12]

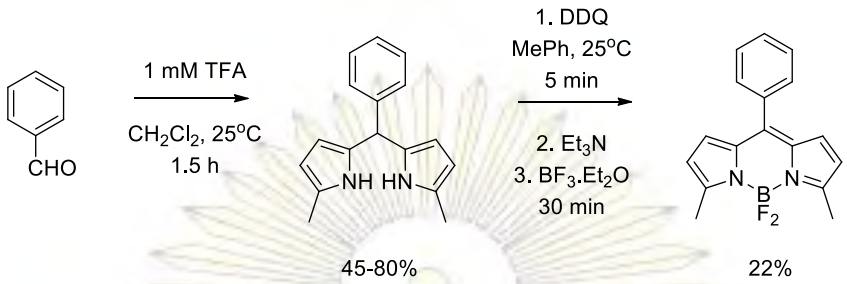

Figure 1.8 Synthesis of BODIPY dye using DDQ as oxidizing agent.

BODIPY dyes are easily functionalized, have high photostability and high fluorescence quantum yields, so there are many organic dyes based on BODIPY as fluorescent sensors in literature.

In 2012, Yin *et al.*[13] reported that **BDP-3** can sensitively and selectively detect Cu^{2+} in CH_3CN and act as fluorescence 'turn on' sensor for Cu^{2+}. Moreover, the obvious changes in the color and fluorescence after binding with Cu^{2+} make **BDP-3** as a good colorimetric sensor. The mechanism and stoichiometry of binding with Cu^{2+} were not reported. **BDP-4** was synthesized by Zhao *et al.* in 2014.[14] **BDP-4** exhibits a strong visible absorption band and Cu^{2+} ion cause the greatest fluorescence quenching of **BDP-4**. However, addition of biothiol such as glutathione (GSH) can promote the removal of Cu^{2+} from **BDP-2**-Cu^{2+}complex and gives fluorescence recovery. In addition, **BDP-4** was used as fluorescence bioimaging by detection of Cu^{2+} in living cells.

In 2014, Zhang *et al.*[15] reported that **BDP-5** detected Cu^{2+} in aqueous media as a "turn-on" fluorescent sensor and colorimetric sensor. The fluorescence titration resulted that **BDP-5** and Cu^{2+} formed a complex with ratio 1:1. **BDP-5** showed the effective and selective Cu^{2+} detection at low detection limit. Moreover, **BDP-5** can be used as fluorescence "turn-on" chemosensor for Cu^{2+}in HepG2 cells.

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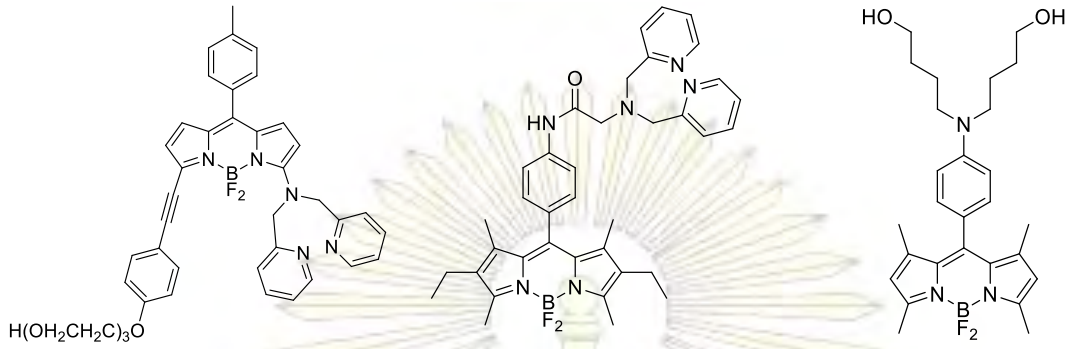
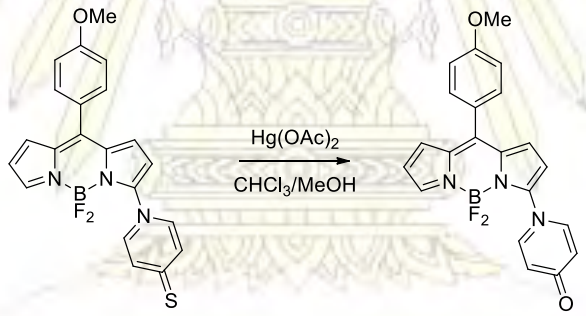


BDP-3 **BDP-4** **BDP-5**

Figure 1.9 Example of BODIPY dyes as fluorescent sensors for Cu^{2+} detection.[13-15]

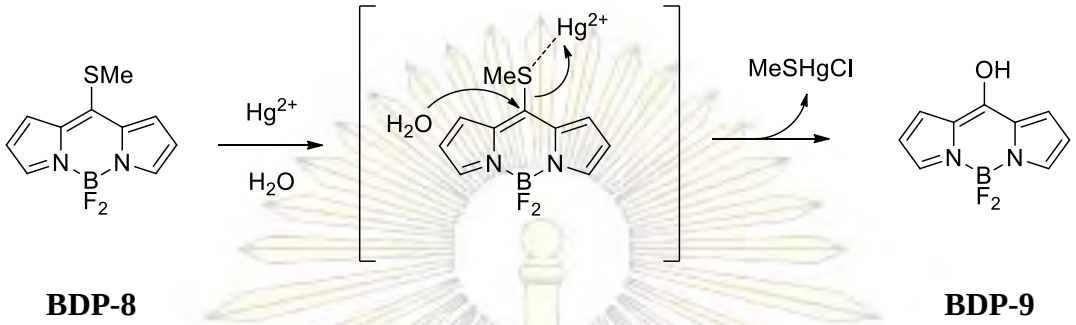
3-(Pyridine-4-thione)BODIPY, **BDP-6** was synthesized as a fluorescent sensor for Hg^{2+} detection by Khan *et al.* in 2012.[16] It can be used as a colorimetric sensor for Hg^{2+}. **BDP-6** was highly selective with Hg^{2+} due to the desulfurization reaction. Desulfurization of **BDP-6** gives **BDP-7** which is strongly fluorescent compound as a product.



BDP-6 **BDP-7**

Figure 1.10 Desulfurization of 3-(Pyridine-4-thione)BODIPY after detection of Hg^{2+}.

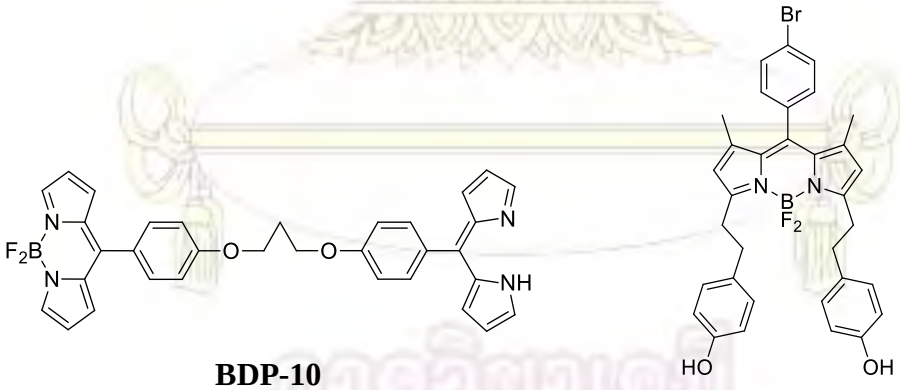
In 2012, Kim *et al.*[17] also reported that 8-thiomethyl-BODIPY, **BDP-8** discriminated Hg^{2+} ion through desulfurization reaction and gave hydroxy-BODIPY, **BDP-9** as a product. **BDP-9** gave a blue emission of fluorescence, while **BDP-8** gave a bright green fluorescence.



BDP-8 **BDP-9**

Figure 1.11 Desulfurization mechanism of 8-thiomethyl-BODIPY after detection of Hg^{2+}.

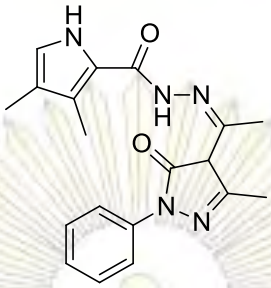
Many of BODIPY derivatives were developed. Not only for Cu^{2+} and Hg^{2+}, but BODIPY dye with different substituted groups also discriminate other metal ions. **BDP-10** was synthesized by Kursunlu *et al.* in 2012.[18] It selectively and sensitively detected Fe^{2+} and Zn^{2+} with the mole ratio (Metal ion:**BDP-10**) 1:3 and 1:2, respectively. **BDP-11** developed by Wang *et al.* in 2015[19] can be a fluorescent sensor and colorimetric sensor for Fe^{3+}. In addition, **BDP-11** can be used to detect Fe^{2+} in living cell.



BDP-10 **BDP-11**

Figure 1.12 Example of BODIPY dyes for sensing metal ion.[18-19]

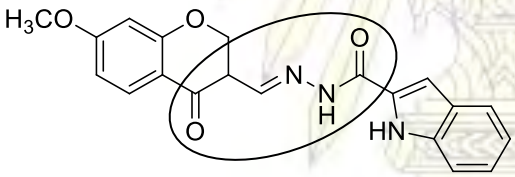
Schiff bases have been also reported as a fluorescent sensor. In 2014, Cheng *et al.*[20] reported that Schiff base **SB-1** can be used as fluorescent sensor. **SB-1** was selective and sensitive with Al^{3+}.



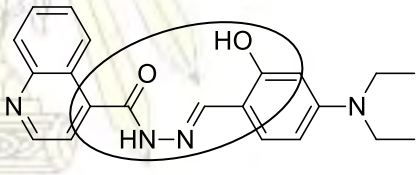
SB-1

Figure 1.13 Schiff base as fluorescent sensor.

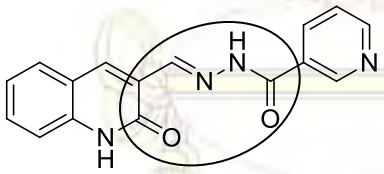
Many organic dyes derivatized by Schiff base have been reported.[21-24] All of them are selective and sensitive with Al^{3+} and have the same structure of Schiff base part which is metal binding part.



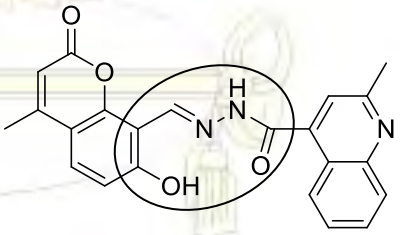
SB-2



SB-3



SB-4



SB-5

Figure 1.14 Organic dyes derivatized by Schiff base as fluorescent sensor for Al^{3+}.[21-24]

As circled in Figure 1.14, all of organic dyes have a similar structure of Schiff base part. That structure bound sensitively and selectively with Al^{3+} and gave fluorescent signal. In this study, design of novel BODIPY-based organic dye is based on reported Schiff base organic dyes. The Schiff base part, which gives a fluorescent signal when

bound with metal ions, is designed as a metal binding part. The BODIPY dye is derivatized by Schiff base in order to increase intensity of fluorescent signal when it chelates metal ions. However, to the best of my knowledge, derivatization of BODIPY by Schiff base has not been reported.

1.4 Objectives and Scope of Study

- Synthesize BODIPY-based organic dye, compound **1**, which is substituted by Schiff base at *meso*-position as fluorescent sensor for metal ions detection. As shown in Figure 1.15, Schiff base is a metal chelating part and BODIPY is fluorophore.

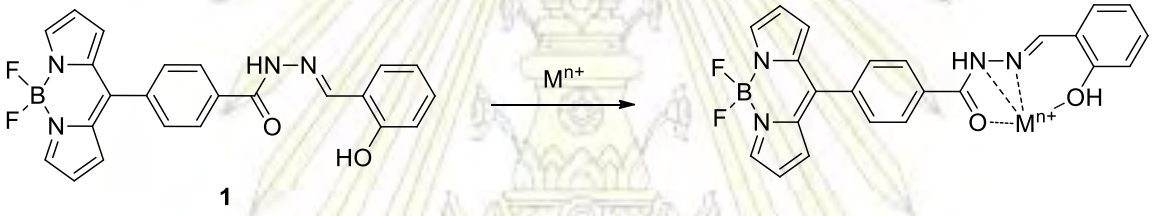


Figure 1.15 Possible complexation mechanism of compound **1**.

Compound **1** can be synthesized by using pyrrole and 4-cayboxybenzaldehyde as starting material as shown in Figure 1.16.

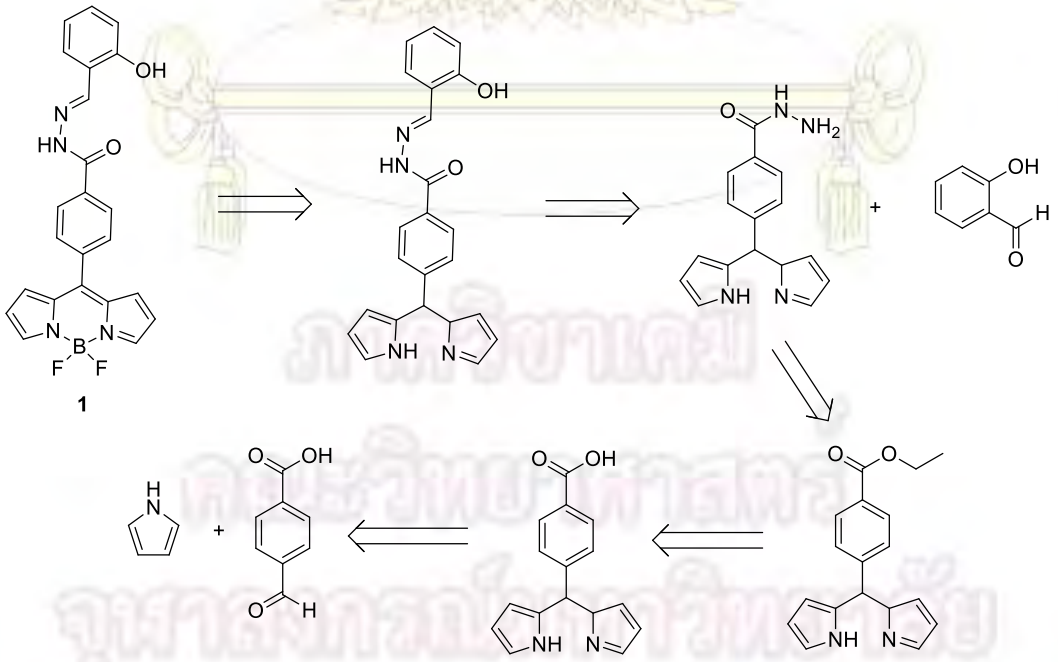


Figure 1.16 Retrosynthetic scheme of compound **1**.

- Study selectivity of compound **1** when binding to metal ions. Thirteen of metal ions are chosen to study: Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Ag^{+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}.

- Study photophysical properties of compound **1** and its complex with metal ions: color change, UV-Vis absorption pattern change, and fluorescence absorption pattern change in terms of qualitative and quantitative analysis.

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CHAPTER II

EXPERIMENTAL

2.1 Materials and Chemicals

All reactions were performed in oven-dried glassware. The progress of the reactions and the isolation of products by column chromatography were monitored by thin layer chromatography (TLC) performed on Merck D.C. silica gel 60 F_{254} 0.2 mm. Column chromatography was performed on Merck 70-230 mesh ASTM silica gel (particle size 0.063-0.200 mm).

Solvents for reaction set up including dichloromethane, ethyl acetate, hexanes, and ethanol were AR grade. Solvents for chromatographic purification including hexanes, ethyl acetate, and dichloromethane were commercial grade and were distilled before use.

All chemicals used in the reaction were reagent grade and were used as received without further purification. They were purchased from the following vendors:

- Aldrich Chemical Co., Inc. (Milwaukee, Wisconsin, USA) : pyrrole, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), salicylaldehyde
- Carlo Erba Reagenti (Milan, Italy) : triethylamine, dichloromethane, ethyl acetate, and hexane
- Fluka Chemical Corp. (Buchs, Switzerland) : 4-carboxybenzoic acid, boron trifluoride diethyl etherate
- Merck Co., Ltd. (Darmstadt, Germany) : anhydrous sodium sulfate, absolute ethanol, concentrated sulfuric acid, hydrazine hydrate
- Thermo Fisher Scientific Inc. (Massachusetts, USA) : trifluoroacetic acid (TFA)
- Suksapan Panit (Bangkok, Thailand) : sodium hydrogen carbonate.

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2.2 Instruments and Equipments

Evaporation of solvents was carried out on a Büchi Rotavapor R-200 equipped with a Büchi Heating Bath B-490.

All reported proton (^{1}H) nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury plus 400 operating at 400 MHz. The spectra were taken in deuterated chloroform ($CDCl_3$) or deuterium oxide (D_2O). The chemical shifts (δ) are reported in parts per million (ppm) and are relative to that of tetramethylsilane (TMS) or relative to the residue protonated signal of deuterated solvents as a reference. Multiplicities were abbreviated as followed: s = singlet, d = doublet, t = triplet, m = multiplet.

2.3 Synthesis of 5-(4-ethoxycarbonylphenyl)dipyrromethane (3)

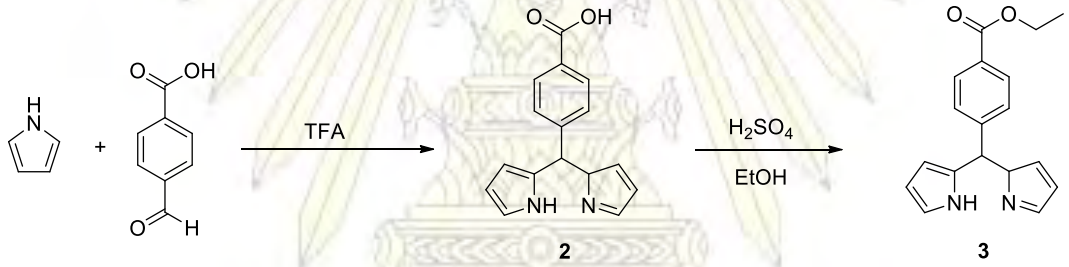

Figure 2.1 Synthesis of compound **3**.

The synthetic method was adapted from reported literature.[25] Pyrrole (4.5 mmol) and 4-carboxybenzaldehyde (2.0 mmol) were dissolved in dichloromethane (50 mL) at room temperature. The mixture was purged with nitrogen gas for 30 minutes. A few drops of trifluoroacetic acid (TFA) were added and the mixture was stirred until 4-carboxybenzaldehyde was completely consumed. Purple solid of compound **2** was filtered with suction and washed with dichloromethane. The solid was directly used for next step without further purification. The solid was dissolved in ethanol (9 mL), then concentrated sulfuric acid (1 mL) was added dropwise. After refluxing for 2 hours, purple solution was turn into brown solution. The mixture was treated with 10% sodium hydrogen carbonate and partitioned by dichloromethane. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was removed which resulted in a black solid. The desired product was not obtained by this method.

2.4 Synthesis of 4,4-difluoro-8-(4-carboxyphenyl)-4-bora-3a,4a-diaza-s-indacene (6)

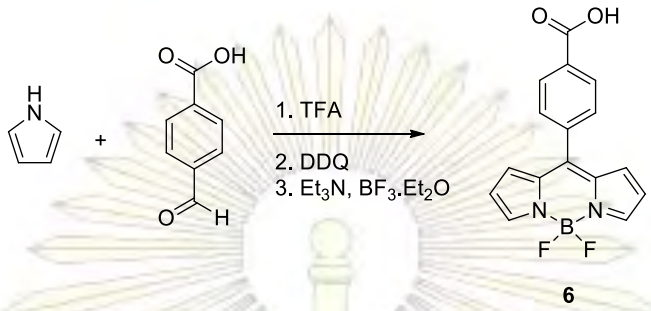

Figure 2.2 Synthesis of compound **6**.

The synthetic method was adapted from reported literature.[25] Pyrrole (4.5 mmol) and 4-carboxybenzaldehyde (2.0 mmol) were dissolved in dichloromethane (150 mL) at room temperature. The mixture was purged with nitrogen gas for 30 minutes. A few drops of trifluoroacetic acid (TFA) were added and the mixture was stirred until 4-carboxybenzaldehyde was completely consumed. At this point, 2.0 mmol of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was added. After stirring for 10 minutes, trimethylamine (4 mL) and boron trifluoride diethyl etherate (4 mL) were rapidly added. The mixture was further stirred for 2 hours, washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated. The residue was chromatographed on a silica column using ethylacetate as an eluent. The product was obtained as a red solid: yield: 11%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.57 (d, 2H), 6.90 (d, 2H), 7.70 (d, 2H, phenyl), 7.99 (s, 2H), 8.26 (d, 2H, phenyl).

2.5 Synthesis of 4,4-difluoro-8-(4-hydrazinecarbonylphenyl)-4-bora-3a,4a-diaza-s-indacene (8)

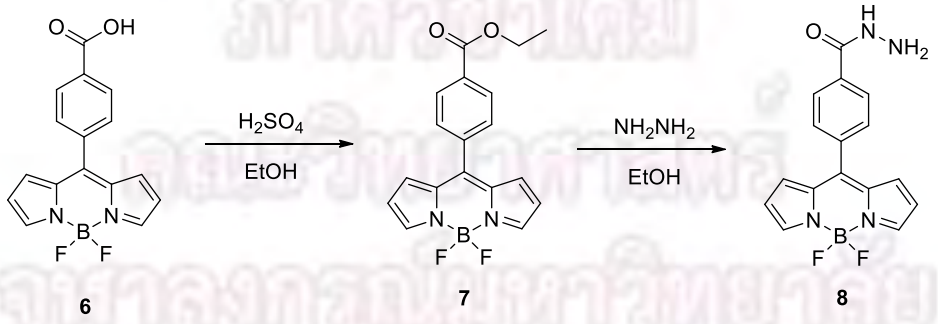

Figure 2.3 Synthesis of compound **8**.

A 50 mL round-bottomed flask was charged with a solution of 0.001 g of compound **6** in 4.5 mL of ethanol. Concentrated sulfuric acid (0.5 mL) was added dropwise to a solution. The mixture was stirred under reflux for 12 hours. The solvent was evaporated and the residue was partitioned between dichloromethane (50 mL) and 10% sodium hydrogen carbonate solution (50 mL). The organic layer was washed with water and dried over anhydrous sodium sulfate. After the solvent was removed, orange solid of compound **7** was obtained. The ester was directly used for next step without further purification. The ester was dissolved in ethanol (4 mL). Hydrazine hydrate (1 mL) was added dropwise to the solution. The reaction mixture was refluxed for 12 hours to give yellow solution. The mixture was evaporated and the residue was kept in refrigerator for 24 hours. The crystal of compound **8** was not obtained.

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CHAPTER III

RESULTS AND DISCUSSION

Fluorescent dyes based on BODIPY have been rapidly developed because of their great photophysical property. BODIPY dyes can be used to detect and discriminate metal ions especially transition metals and heavy-metals.

Recently, Schiff bases have been reported that they can be used as fluorescent sensor. Many organic dyes were functionalized with Schiff base in order to increase their photophysical property when they bind to metal ions. The organic dyes which have been reported have similar part of Schiff base structure.

In the current study, BODIPY-based organic dye, which is substituted by Schiff base at *meso*-position, was designed. Design of Schiff base structure is based on previous reported organic dyes.[18-22] The structure of target molecule is presented in Figure 3.1.

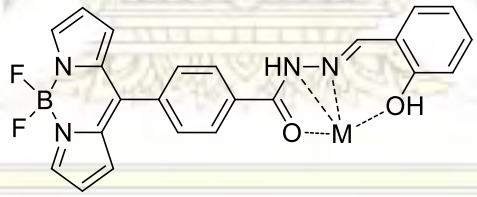

Figure 3.1 Complexation model of the target molecule.

As shown in Figure 3.1, Schiff base is proposed to form a complex with metal ions in the manner depicted. The complexation behavior by the Schiff base moiety can be detected by UV-Vis spectroscopy. Once the BODIPY moiety is incorporated, the complex shows clear optical properties which can also be detected by fluorescence spectroscopy

Proposed synthesis route of the target molecule, compound **1**, is shown in Figure 3.2 where pyrrole and 4-carboxybenzaldehyde were used as starting materials. Compound **2** was synthesized under nitrogen atmosphere and trifluoroacetic acid was used as catalyst.

Compound **2** was obtained as a purple solid in excellent yields (70-95%). However, attempts to purify compound **2** by column chromatography and recrystallization failed because compound **2** rapidly decomposed when it was dissolved in any solvent and turning from a purple to a black solution. Consequently, compound **2** was used in the next step without further purification.

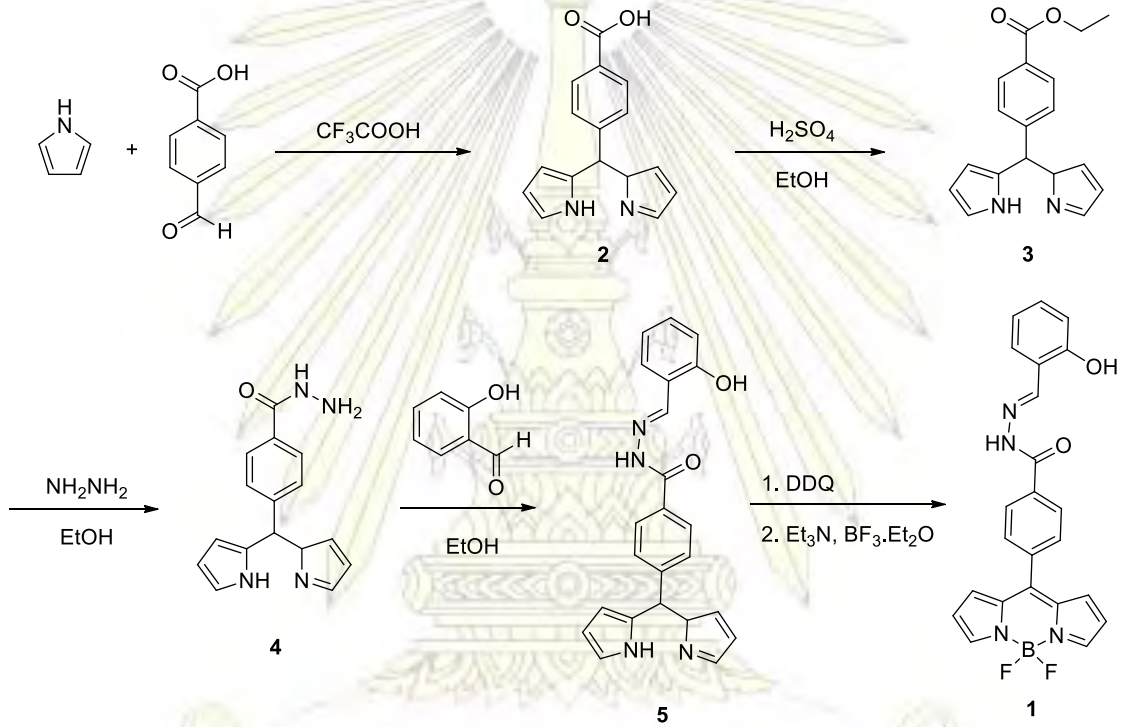

Figure 3.2 Proposed synthesis route of compound **1**.

Compound **2** was synthesized from pyrrole and 4-carboxybenzaldehyde via electrophilic substitution. Two equivalents of pyrrole were condensed with one equivalent of 4-carboxybenzaldehyde. Mechanism of synthesis of compound **2** is shown in Figure 3.3.

According to the mechanism scheme, after a protonation of the carbonyl oxygen with a proton, the nucleophilic carbon of the pyrrole attacks the electrophilic carbonyl carbon of the aldehyde resulting in carbocation intermediate. After deprotonation occurs, oxygen of hydroxyl group is protonated. The nucleophilic carbon of the other molecule of pyrrole attacks the same position of electrophilic carbon to give a product as compound **2**.

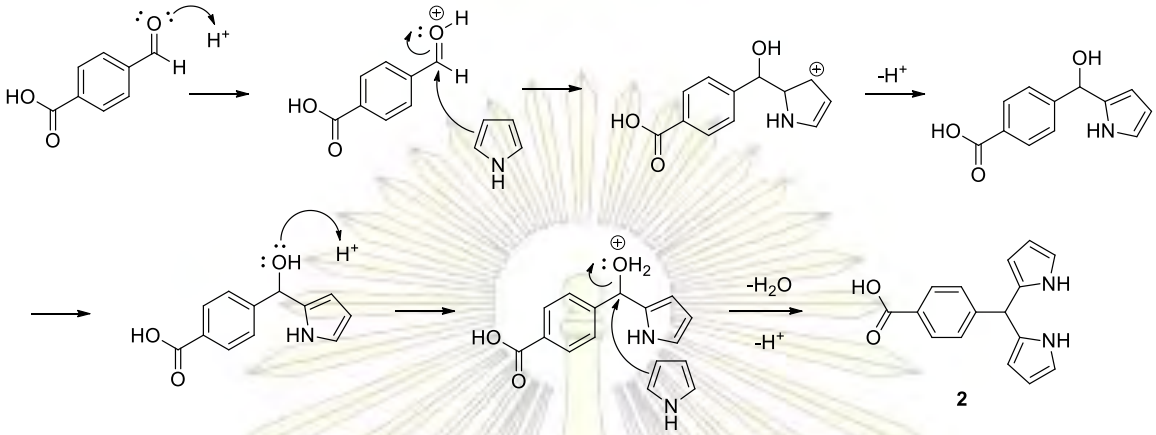

Figure 3.3 Mechanism of synthesis of compound **2**.

In the next step, compound **2** was further converted to compound **3** via esterification reaction. Compound **2** was refluxed with 10% sulfuric acid in ethanol. After 2 hours of refluxing, the purple solution of compound **2** turned into a brown solution. At this point, the solution was treated with 10% sodium hydrogen carbonate solution to quench all the acid. The mixture was partitioned by dichloromethane and the organic layer was separated. The organic layer was washed with water, and then the solvent was removed. The product was obtained as a black solid. The absence of signals of substituted phenyl protons and protons of pyrrole ring in its ^{1}H NMR spectrum confirmed that the solid was not compound **3**. Since the decomposition of compound **2** during the reaction run, the desired product was not obtained. The synthesis route of compound **1** was, therefore, modified as shown in Figure 3.4. In order to prevent the decomposition of compound **2**, formation BODIPY should be done before functionalization step because BODIPY is not easily decomposed.

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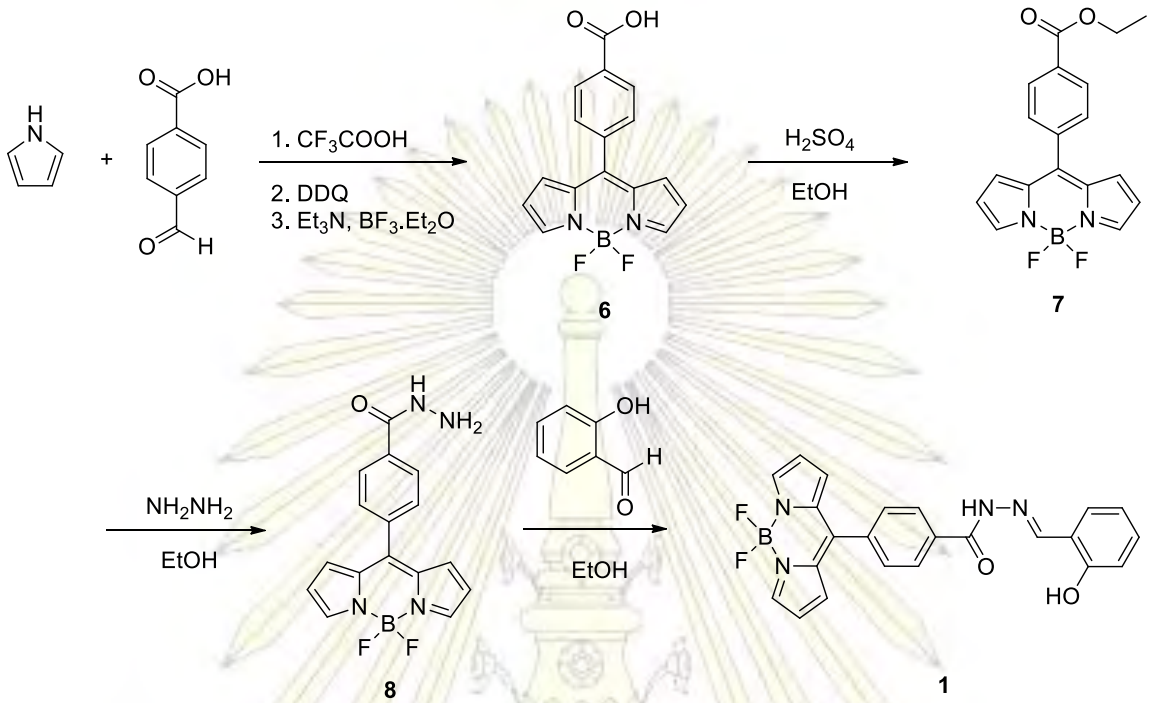

Figure 3.4 Proposed modified synthesis route of compound **1**.

Compound **6** was prepared according to a literature method by one-pot condensation of pyrrole with 4-carboxybenzaldehyde using trifluoroacetic acid as catalyst and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as oxidizing agent under nitrogen atmosphere.[25] After the aldehyde was totally consumed, DDQ was added and the mixture immediately turned into black. The mixture was further stirred for 10 minutes. Triethylamine was added in order to deprotonate the amine protons. The intermediate was then complexed with boron trifluoride diethyl etherate. The crude was chromatographed on a silica column using ethyl acetate as an eluent. Red solid of compound **6** was obtained with poor yield (11%). The structure of compound **6** was characterized by the analysis of its ^{1}H NMR spectrum. However, ^{13}C NMR was not performed because compound **6** was obtained in a small amount.

According to ^{1}H NMR spectrum of compound **6** (Figure 3.5), a group of signals at 0-4 ppm was signals from impurities and remaining solvent. The strong and singlet signal at 7.26 ppm was assigned as a proton of $CHCl_3$ which contaminated in $CDCl_3$.[26] The doublet signals at 6.57 and 6.90 ppm were assigned as four protons of BODIPY core, protons b and c, respectively. The singlet at 7.99 ppm was assigned as two protons of

BODIPY core, proton a. The signals at 7.70 and 8.26 ppm appeared as doublet. The chemical shift of these signals fell into the aromatic region and was therefore assigned as substituted phenyl protons, protons d and e, respectively.

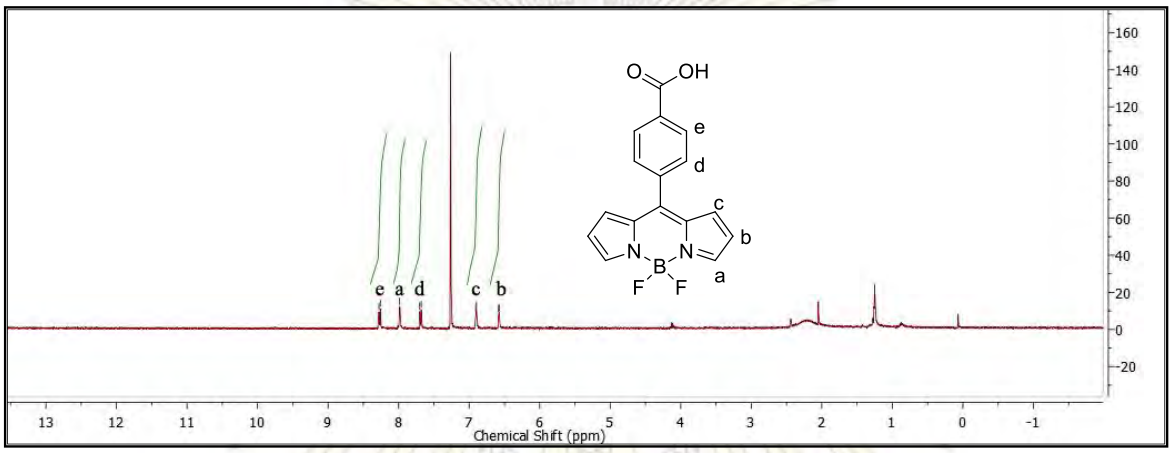

Figure 3.5 ^{1}H NMR spectrum of compound **6**.

Compound **6** was converted to compound **7** via esterification reaction. The mixture of compound **6** and sulfuric acid in ethanol was refluxed for 12 hours. After refluxing, the mixture was partitioned between 10% sodium hydrogen carbonate solution and dichloromethane. The organic layer was separated and evaporated to give orange solid as a product. The product was obtained in a small amount and was used in the next step without purification and characterization.

The product was further converted to compound **8** via nucleophilic substitution reaction. Hydrazine was used as nucleophile in this reaction. After addition of hydrazine, an orange solution was refluxed for 12 hours. After refluxing, the mixture was changed from orange to yellow. The mixture was subjected to a rotatory evaporator and the solution was concentrated. The residue then was kept in refrigerator for 24 hours. The crystal of compound **8** was not obtained. The yellow crude could not be dissolved in dichloromethane, hexanes, and ethyl acetate. The crude was evaporated until only yellow solid was remained in a flask. ^{1}H NMR spectrum of the solid in D_2O confirmed that yellow solid was not compound **8** due to the absence of signals of substituted phenyl protons and BODIPY core's protons. However, several attempts to synthesize compound

8 failed because compound **6** were not pure. Compound **6** was re-purified, but impurities which cannot be identified were not eliminated while the ester product of compound **6** was not purified and characterized before use.

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CHAPTER IV

CONCLUSION

A novel BODIPY-based organic dye was designed. The dye was derivatized by placing a Schiff base at *meso*-position of the BODIPY core. The first synthetic route was proposed as pyrrole condensed with 4-carboxybenzoic acid to give 5-(4-carboxyphenyl) dipyrromethane. The product cannot be used in the next synthesis step because it was rapidly decomposed. The alternative version of synthesis route was proposed. Pyrrole and 4-carboxybenzoic acid were condensed and formed into a complex with boron trifluoride diethyl etherate to give a 4,4-difluoro-8-(4-carboxyphenyl)-4-bora-3a,4a-diaza-s-indacene in poor yield (11%) with small amount impurities. The product was then purified by column chromatography but the impurities remained. Attempts to convert the product to 4,4-difluoro-8-(4-hydrazinecarbonylphenyl)-4-bora-3a,4a-diaza-s-indacene failed. The target molecule was not obtained. Therefore, the consequential experiments were not conducted. To achieve the goal, synthesis conditions have to be optimized. Synthesis route can be modified to be a convergent synthesis.

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VITAE

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