ฟลูออเรสต์เซนต์คีโมเซ็นเซอร์ชนิดใหม่ฐานเฟอร์โรซีน

นางสาวพรชนก จินะแปง

NEW FERROCENE-BASED FLUORESCENT CHEMOSENSOR

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พรชนก จินะแปง : ฟลูออเรสต์เซนต์คีโมเซ็นเซอร์ชนิดใหม่ฐานเฟอร์โรซีน. (NEW FERROCENE-BASED FLUORESCENT CHEMOSENSOR) อ.ที่ปรึกษาวิทยานิพนธ์ หลัก: รศ. ดร.ไพฑูรย์ รัชตะสาคร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร.มงคล สุขวัฒนา สินิทธ์, 61 หน้า.

สารเรืองแสงที่มีคุณสมบัติละลายน้ำได้เป็นสารที่มีความน่าสนใจสำหรับการวิเคราะห์ สารเคมีหรือไอออนของโลหะที่ต้องการความว่องไวและความแม่นยำสูง ดังนั้นในวิทยานิพนธ์ฉบับ นี้จึงได้ ออกแบบและสังเคราะห์สารอนุพันธ์ใหม่จำนวนสองชนิดขึ้นจากเฟอร์โรซีนที่มีสมบัติเป็น หมู่ให้อิเล็กตรอนที่ดีและ1,8-แนพทาลิไมด์ซึ่งมีความพิเศษในสมบัติทางกายภาพเชิงแสง นอกจากนี้การเชื่อมต่อหมู่ไตรเอธิลีนไกลคอลในโครงสร้างยังช่วยเพิ่มความสามารถในการละลาย น้ำ ซึ่งช่วยในการกระจายตัวของสารอนุพันธ์ในตัวกลางที่มีน้ำเป็นองค์ประกอบได้ดีมากขึ้น จาก ผลการศึกษาพบว่าสารอนุพันธ์ใหม่ทั้งสองชนิด มีความความยาวคลื่นการดูดกลืนแสงที่ยาวที่สุด อยู่ในช่วง 360-370 นาโนเมตร เมื่อสารอนุพันธ์นี้อยู่ในสภาวะสารละลายผสมระหว่างน้ำและอะซิ โตรไนไตรล์ จะให้ประสิทธิภาพการเรืองแสงฟลูออเรสเซนต์ที่ต่ำ เนื่องจากการมีหมู่เฟอร์โรซินิลที่ ทำหน้าที่เป็นตัวระงับสัญญาณการเรืองแสงฟลูออเรสเซนต์ของส่วน 1,8-แนพทาลิไมด์ ผ่าน กระบวนการโฟโต้อินดิวซ์อิเล็กตรอนทรานส์เฟอร์ (PET) อย่างไรก็ตามหนึ่งในสองของสารอนุพันธ์ ที่สังเคราะห์ขึ้น สามารถแสดงสมบัติการขยายสัญญาณการเรืองแสงในสภาวะที่มีไอออน Au (III) หรือ Au (I) ในตัวกลางผสมของฟอตเฟตบัฟเฟอร์และอะซิโตรไนไตรล์ โดยปราศจากการรบกวน จากไอออนโลหะชนิดอื่น แม้ว่าจะมีความเข้มข้นสูงกว่าถึง 10 เท่าก็ตาม ในงานวิจัยนี้ได้ ทำการศึกษาผลของอัตราส่วนของน้ำในตัวกลางผสมอะซิโตรไนไตรล์และเวลาการตอบสนองที่ สัญญาณคงที่ ตลอดจนผลของ pH และสารลดแรงตึงผิว เพื่อให้ได้มาซึ่งสภาวะการตรวจวัดที่ เหมาะสมที่สุด โดยภายใต้สภาวะดังกล่าวพบว่าสารเรืองแสงจะให้ค่าต่ำสุดที่สามารถตรวจวัดได้ (LOD) คือ 80 ppb

ภาควิชา	เคมี	ลายมือชื่อนิสิต	....
สาขาวิชา	เคมี	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก	....
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PONDCHANOK CHINAPANG: NEW FERROCENE-BASED FLUORESCENT CHEMOSENSOR. ADVISOR: ASSOC. PROF. PAITOON RASHATASAKHON, Ph.D., CO-ADVISOR: ASSOC. PROF. MONGKOL SUKWATTANASINITT, Ph.D., 61 pp.

Two new compounds containing a ferrocene unit as a well-behaved electron-donor and a naphthalimide moiety as a strongly fluorescent transducer are designed and successfully synthesized. The incorporation of a hydrophilic triethyleneglycol unit can enhance the water-solubility and facilitate the dispersion of compounds in aqueous media. The incorporation of the ferrocene unit in both compounds results in molecules with low quantum efficiencies due to the photoinduced electron transfer (PET) from ferrocenyl to naphthalimide moiety. However, one of these compounds exhibits a selective fluorescent turn-on by the addition of Au (III) ion or Au (I) with low to neglible interference by 10-fold concentration of analytes to other metal ions in PBS buffer/acetonitrile. The effect of water content towards the response time and the effect of pH on sensitivity also the effect of surfactants of this sensor are investigated. Under optimum conditions, the detection limit for Au (III) ion was 80 ppb.

Department:	Chemistry	Student's Signature	....
Field of Study:	Chemistry	Advisor's Signature	....
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LIST OF ABBREVIATIONS

Ar	aromatic
calcd	calculated
^{13}C NMR	carbon-13 nuclear magnetic resonance
CDCl$_3$	deuterated chloroform
CH$_2$Cl$_2$	Methylene chloride
CuI	Copper iodide
DMSO-d_6	deuterated dimethyl sulfoxide
DMSO	dimethylsulfoxide
d	doublet (NMR)
dd	doublet of doublet (NMR)
ESIMS	electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
equiv	equivalent (s)
FT-IR	fourier transform infrared spectroscopy
g	gram (s)
^{1}H NMR	proton nuclear magnetic resonance
Hz	Hertz
HRMS	high resolution mass spectrum
h	hour (s)
IR	infrared
J	coupling constant
mg	milligram (s)
mL	milliliter (s)
mmol	millimole (s)
m.p.	Melting point
nm	Nanometer (s)
m/z	mass per charge

m	multiplet (NMR)
M.W.	molecular weight
M	molar
MHz	megaHertz
rt	room temperature
s	singlet (NMR)
THF	tetrahydrofuran
TLC	thin layer chromatography
UV	ultraviolet
δ	chemical shift
°C	degree Celsius
µL	microliter (s)
μM	micromolar (s)
ε	Molar absorptivity
λ	Wavelength
Φ	quantum yield
%yield	percentage yield

CHAPTER I

INTRODUCTION

1.1 Introduction

Metallic elements are the major group of elements on earth [1], which can be classified into three principal categories, i.e. main group, transition and heavy metals. Almost all of the metal ions play important role in either biological system or environment. For example, Co (II), Ni (II), Cu (II), Zn (II), Fe (II) and Fe (III) are very important in biochemistry and are essential metal ions for human health. In contrary, some of these elements are toxic or act as harmful pollutants. Ions such as Cd (II), Hg (II) and Pb (II) are recognized as toxic metal ions that are very dangerous for human beings. For example, the abnormally high concentrations of mercury in the form of MeHgSMe in fish caused Minamata disaster in Japan in 1952. Thus, efficient methods concerning the qualitative and quantitative analysis of heavy metals need to be developed.
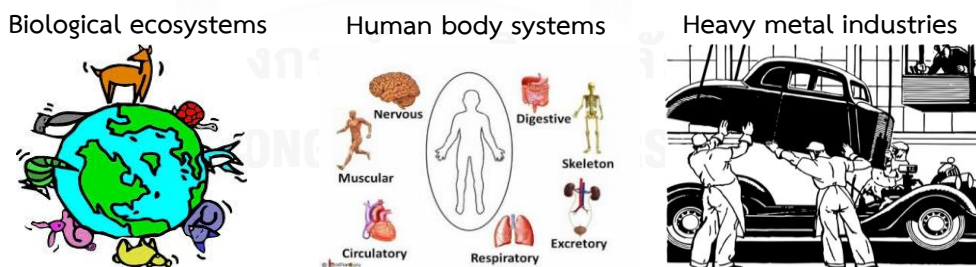
Figure 1.1 The important role of metallic elements.

1.2 Gold-detect fluorescent chemosensor

1.2.1 Gold: One of the most noble-metal

During the last decade, there has been much interests in gold species with respect to the unique chemical and physical properties of gold ions. Gold-related chemistry has triggered variable research. Gold species as gold-catalyst have been extensively in organic transformation owing to the *"alkynophilicity"* characteristic of gold ions, which have been known to activate carbon-carbon triple bond towards nucleophilic addition. The alkynophilic Lewis acidity of gold ions expands wide range potential applications in the area of gold-catalysed for organic transformations [2-8]. The gold catalysts are often in a form of cationic gold species **a** (**Scheme 1.1**) can coordinate to the alkyne to produce the gold-alkyne complex **b** containing a electrophilic site. Further reaction with intramolecular nucleophile then produces the structurally rearranged product.
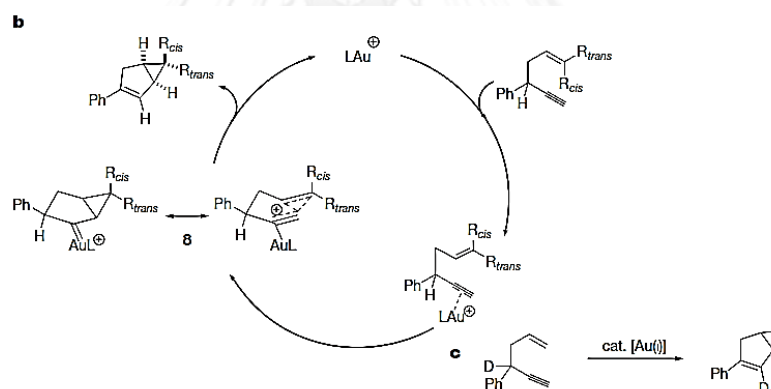
Scheme 1.1 Example of the catalytic mechanism of gold complex [9].

In addition, gold nanoparticles (AuNPs), which possess distinct physical and chemical attributes make them excellent scaffolds for the fabrication of novel chemical and biological sensors [10, 11]. Several gold compounds have anti-inflammatory properties and used as pharmaceuticals. The well-known gold-based drugs such as Sanocrysin is valuable treatment of tuberculosis. Sologanol and Auronofin are also important in Rheumatic arthritis treatment and reducing viral reservoir of HIV [12].
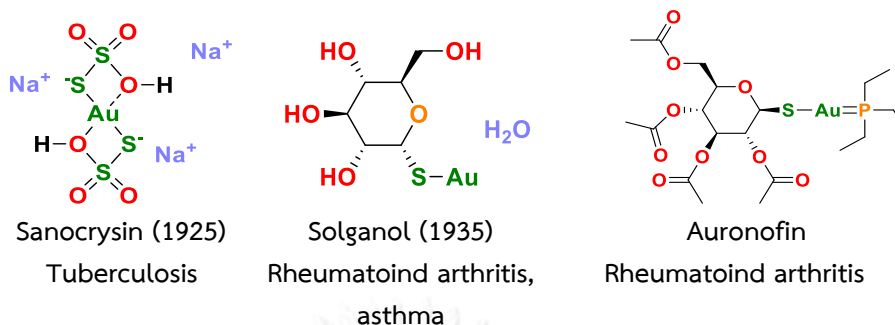
Figure 1.2 Some well-known gold-based drugs.

However, there are a few reports on toxicity of gold ions. Their soluble salts such as gold chloride are known to cause damage to the liver and kidneys because gold ions are able to bind strongly with DNA or biological tissue. In particular, some gold ion such as Au (III) are highly toxic to biological systems and cause undesirable DNA cleavage [13-18]. Therefore, it is highly urgent to develop gold ion probes to access the quantity in environmental and biological samples.
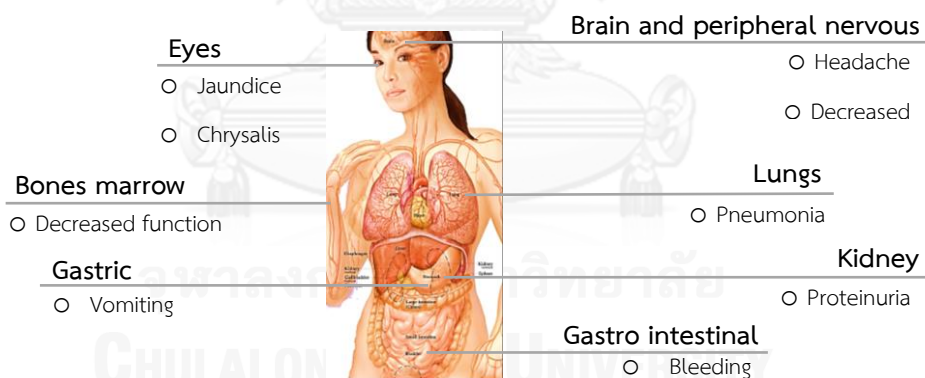
Figure 1.3 Main symptoms of gold toxicity on human body.

(http://www.prism.princeton.edu/PRISM_cleanroom/MSDS/Gold.pdf)

1.2.2 Recent progress on fluorescent chemosensors for gold ions

The trace analysis of metal ions in biological, clinical, or industrial samples are often carried out by many techniques. For example; spectroscopic methods (atomic absorption/emission with various atomizers), mass spectrometry (ICP-MS) and electrochemical technique (anodic stripping voltammetry). By the way, most of these

analysis methods have several drawbacks, for example, expensive installation and complicated maintenance of instruments, requirement of skilled specialists or operators, demands of sample pre-treatment and time-consuming.

On the other hand, the fluorescence method is more convenient in terms of simple operation, cost effectiveness, rapid and real-time operation, and high sensitivity. These could provide a simple detection method for determination of trace elements outside the laboratory. Therefore, this research focuses on development of new fluorescent chemosensor for detection of metal ions.

1.3 Fluorescent chemosensors

As defined by the Oxford English Dictionary, sensors are devices that detect or measure a physical property and record, indicate or otherwise respond to a certain analyte. A chemical sensor is a device that qualitatively or quantitatively detects the presence of specific chemical substances using a specific chemical interaction or reaction [19]. Chemosensors based on fluorescence signal changes are commonly referred to as fluorescent chemosensors [20] which are gaining increased attention due to their high sensitivity and specificity.

Generally, a fluorescent sensor device contains two main components: a receptor (binding site) and a signal transducer (signaling unit) linked by a spacer. (**Figure 1.4**). In most cases, the spacer is not responsible for signal transduction. The receptor is a fragment designed to response to a specific stimulus or locate a specific target substance (the analyte). A powerful sensor must exhibit a selective receptor-to-analyte complex formation depending on the size, shape, binding energy, or interaction of the receptor and analyte.
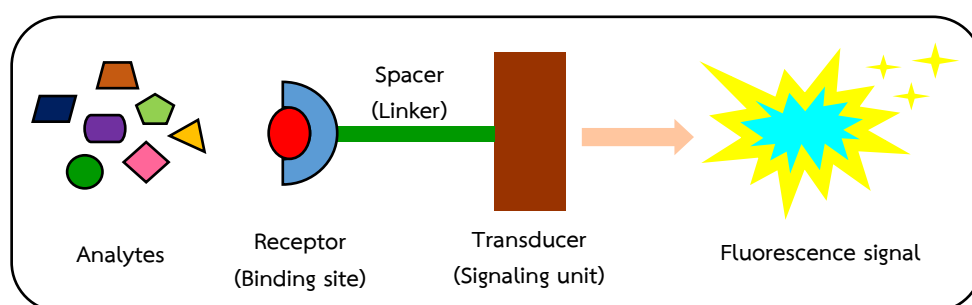
Figure 1.4 Schematic illustration of a chemical sensors.

Signal transduction is the process through which an interaction of receptor with analyte yields a measurable form of energy change and is converted to a signal change that can be read and quantified. The read-out domain is the part responsible for reporting either the binding or reactivity event [21]. It is usually measured as a change in fluorescence signal intensity, intensity decay lifetime or shift of emission wavelength.

Based on their changes in fluorescent intensities, fluorescent sensors can be categorized into two groups. The "turn-on" sensors are those which exhibit stronger intensity under the presence of analyte. Sensors which show opposite changes in fluorescent signal would be called "turn-off" sensors (**Figure 1.5**).
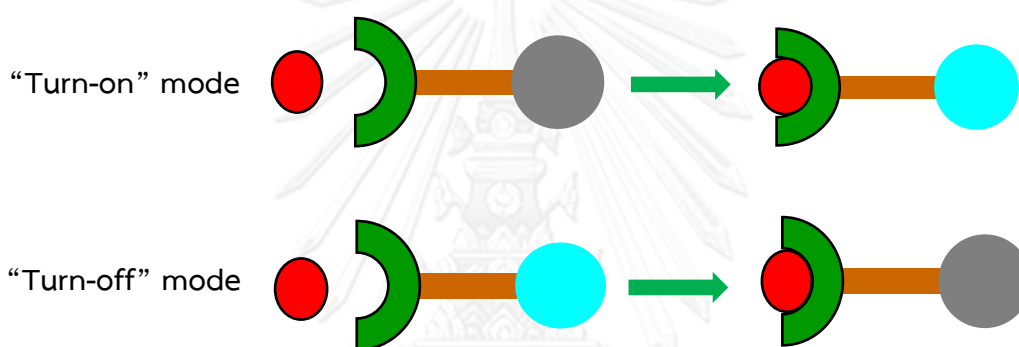
Figure 1.5 Schematic illustration of two modes fluorescent chemosensor.

1.4 The principle of fluorescence spectroscopy

Fluorescence is the emission of photons following relaxation from an excited electronic state to the ground state [22]. Basically, fluorescence occurs in certain conjugated molecules (generally polyaromatic hydrocarbons or heterocycles) called fluorophores or fluorescent dyes. To describe the process occur between absorption and emission of light, Jablonski's diagram is used to explain the fluorescence process that occur in fluorescence chemosensors.
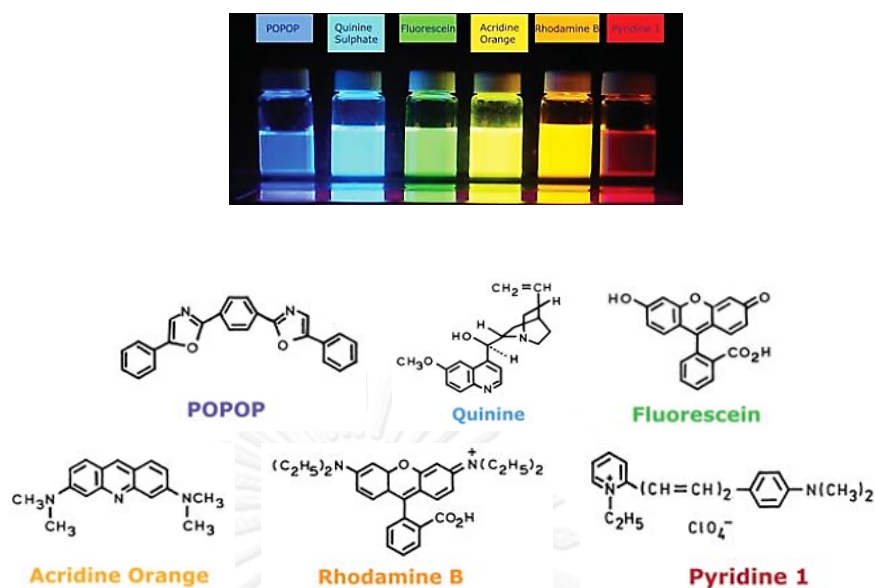
Figure 1.6 Typical of some fluorescence substances [22].

1.4.1 The Jablonski's diagram

The processes that occur between the absorption and emission of light which is responsible for the fluorescence is illustrated by the Jablonski's diagram (**Figure 1.7**). In this diagram, the singlet ground, first, and second electronic states are depicted by S_0, S_1, and S_2, respectively. At each of these electronic energy levels the fluorophores can exist in a number of vibrational energy levels. The transitions between states are depicted as vertical lines to illustrate the instantaneous nature of light absorption. Transitions occur in about 10^{-15} s, a time too short for significant displacement of nuclei [22].
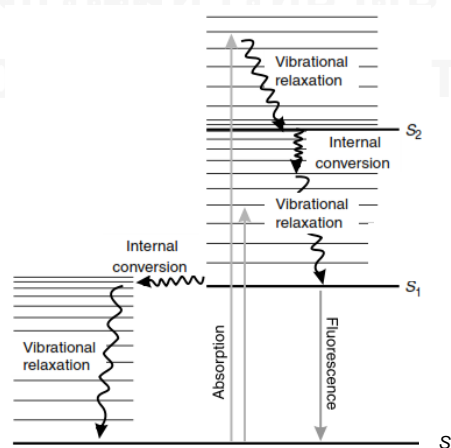
Figure 1.7 Jablonski diagram.

Following light absorption, several processes can occur. A fluorophore is usually excited to some higher vibrational level of either S_1 or S_2. With a few rare exceptions, molecules in higher excited state phases rapidly relax to the lowest vibrational level of S_1. This process is called internal conversion and generally occurs within 10^{-12} s or less. Since fluorescence lifetimes are typically near 10^{-8} s, internal conversion is generally complete prior to emission. Hence, fluorescence emission generally results from a thermally equilibrated excited state, that is, the lowest energy vibrational state of S_1.

1.4.2 Fluorescence spectra

Fluorescence data are generally presented as emission spectra which are plots of the fluorescence intensity versus either wavelength (nm) or wavenumber (cm^{-1}). In general, the highest emission intensity can be obtained when the compound is irradiated by light of maximum absorption wavelength. The excitation using light at other wavelengths will lead to an emission band at the same position, albeit in lower intensity (**Figure 1.8**). It is also important to note that emission spectra vary widely and are dependent upon the chemical structure of the fluorophore and the solvent in which it is dissolved [22].
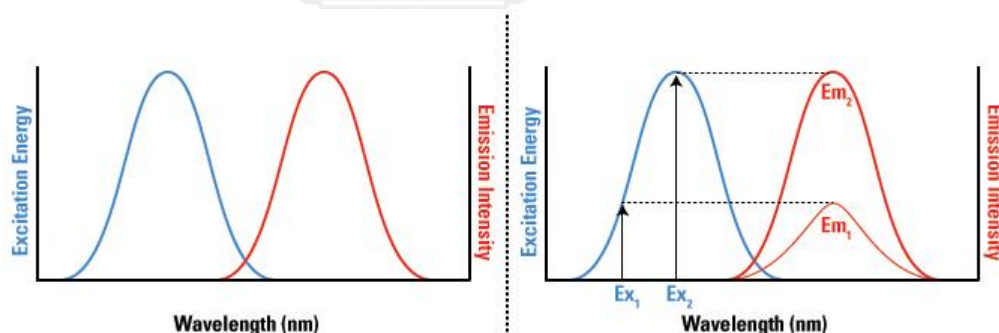
Figure 1.8 General diagram of the excitation and emission spectra for a fluorophore (left) and the intensity of the emitted light (Em₁ and Em₂) using different excitation wavelength.

1.4.3 Fluorescence transduction mechanism

An important feature of the fluorescent chemosensors is that signal transduction of analyte binding event into the readout signal can happen in a very short time and without any other assistance. Currently, fluorescent sensors can be designed on basis of various binding mechanism between analyte and receptor unit involves photophysical such as photo-induced electron transfer (PET), photo-induced charge transfer (PCT), internal charge transfer (ICT), fluorescence resonance energy transfer (FRET) and eximer/exciplex formation or extinction. These mechanisms make real-time and real-space detection of the analyte distribution [22]. Thus, it is not surprising that in the last decade the development of fluorescent sensors for metal ions on various principles has become a very active area of research [20]. Great effort has recently been devoted to the design and construction of molecular sensory system for a broad range of environmental and biological analyses.

1.4.3.1 Photo-induced Electron Transfer (PET)

When the fluorophore is excited, the electron in the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO). This enables the electron transfer from the HOMO of the donor (belonging of the free cation receptor) to that of the fluorophore (**Figure 1.9**, left) resulting in low or undetecTable emission. When this system (sensors) is in the presence of the analyte, the energy of bound receptor will be lower, thus the PET can be disrupted and the system can emit upon excitation (**Figure 1.9,** right).
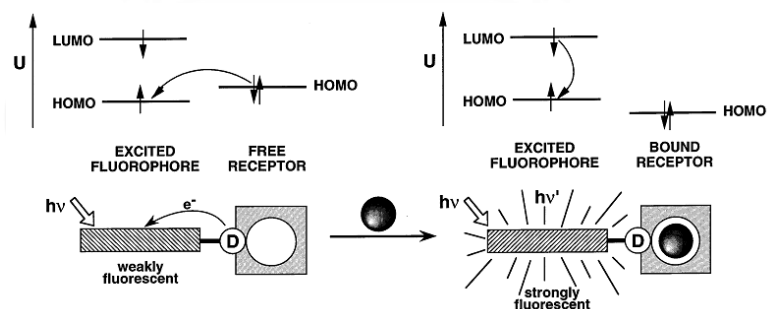
Figure 1.9 Principle of the PET quenching mechanism [22].

1.4.3.2 Internal Charge Transfer (ICT)

If the fluorophore possesses both electron-donating and electron-withdrawing groups, its structure at excited states may be those with a charge separation. When these structures are relatively stable, the molecule may be well-populated in the more stable electronic state compared to the locally excited state, as known as the ICT state. This process gives rise to the emission at a longer wavelength or lower intensity due to significant geometry changes (**Figure 1.10**).
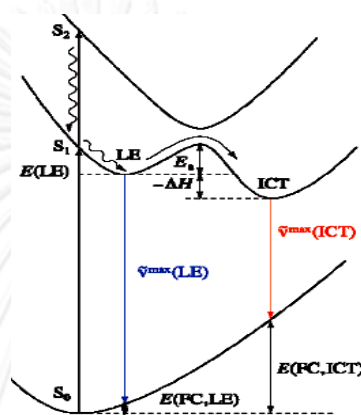
Figure 1.10 Principle of the ICT quenching mechanism [22].

1.4.4 Fluorescence properties

1.4.4.1 Quantum yield

For any fluorescence compounds, the ability to give the intense signal could be estimated from the magnitude of a quantum yield the fraction of the number of quanta absorbed by a molecule that are emitted as fluorescence is termed the fluorescence quantum yield. In the other word, the quantum yield is value that relates to their emission efficiency.

The most widely used method of determining quantum yield is by the relative method. In this procedure, the quantum yields are measured between the absorbance at the maximum absorption wavelength and the sum of all fluorescent intensity at the same absorbance. Then the slope or gradient from plot of sum fluorescence intensity and absorbance of compound was calculated following the equation [23], where Φ is the quantum yield, *Grad* is the gradient from the plot of integrated fluorescence intensity *vs* absorbance and η is the refractive index of the solvent. Subscript STD and x refer to standard and tested compound, respectively.

$$\Phi_{X=}\ \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

1.4.4.2 The Stokes shift

In general, the energy of the emission is typically less than that of absorption. Fluorescence typically occurs at lower energies or longer wavelengths. When a photon of energy is emitted, returning the fluorophore to its ground state S_0. Due to energy dissipation during the excited-state lifetime, the energy of this photon is lower, and therefore of longer wavelength, than the excitation photon. The difference in energy or wavelength represented by Stokes shift ($h\mathbf{V}_{EX}$ – $h\mathbf{V}_{EM}$) (**Figure 1.11**).

The Stokes Shift is a key aspect in the detection of the emitted fluorescence in biological applications and also a distinct characteristic of each fluorophore. For example, the detection of emitted fluorescence can be difficult to distinguish from the excitation light when using fluorophores with very small Stokes shifts (left), because the excitation and emission wavelengths greatly overlap. Conversely, fluorophores with large Stokes shifts (right) are easy to distinguish because of the large separation between the excitation and emission wavelengths. The Stokes shift is fundamental to the sensitivity of fluorescence techniques because it allows emission photons to be detected against a low background, isolated from excitation photons. In contrast, absorption spectrophotometry requires measurement of transmitted light relative to high incident light levels at the same wavelength [22].
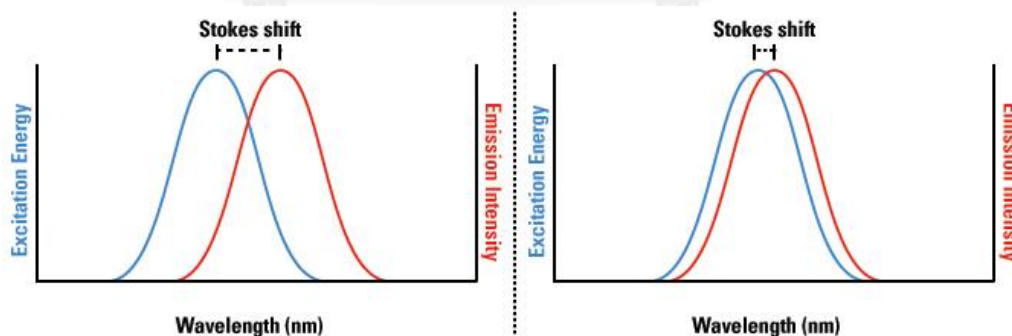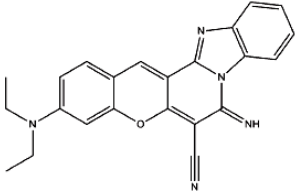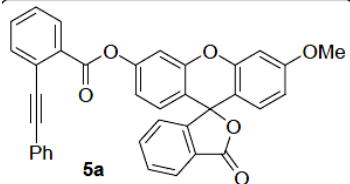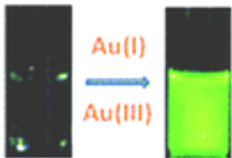
Figure 1.11 Fluorophores with large (left) and small Stokes shifts (right) [22].

1.5 Fluorescent sensors for gold detections

Table 1.1 summarized precedent works related to fluorescent gold sensors available in the literature over the past five years.

Compound [24]:	Conditions: MeCN-HEPES buffer
	(10 mmol L^{-1}, pH 7.4, 1:1 v/v)
	Detection mode: Turn-off
	Reaction time: 30 sec.
	LOD: 1.23 μmol L^{-1}
Photophysical properties:	
λ_{abs} = 552 nm	**Fluorescence change:**
λ_{exc} = 390 nm	
Φ = 0.22	**Visible color change:**
Compound [25]:	**Conditions:** MeCN-PBS buffer
	(10 mmol L^{-1}, pH 7.4, 1:1 v/v)
	Ion detections: Au(I) and Au(III)
	Detection mode: Turn-on
	Reaction time: Abs, after 40 min.
	Ems, after 30 min. (sat. after 3h.)
Photophysical properties:	**LOD:** 5-80 μmol L^{-1}
λ_{abs} = 313 to 452 nm	**Fluorescence change:**
λ_{ems} = 515 nm	
	Visible color change:

Compound [26]:	Conditions: MeCN-Acetate buffer
Photophysical properties: λ_{abs} = 366, 520 nm λ_{ems} = 511 nm	(10 mmolL^{-1}, pH 4.7, 1:1 v/v) **Detection mode:** turn on **Reaction time:** 1 min. **LOD:** 0.11 μmol L^{-1} **Fluorescence and spectral change:**
Compound [27]:	Conditions: EtOH-PBS buffer
Photophysical property: λ_{ems} = 511 nm	(10 mmol L^{-1}, pH 7.4, 1:1 v/v) **Ion detections:** Au(I) and Au(III) **Detection mode:** Turn-on **Reaction time:** measured after 30 min then saturated after 60 min. **LOD:** 320 nmol L^{-1}, 63 ppb **Metal ion interferences:** Hg^{2+} and Pd^{2+} **Fluorescence change:**
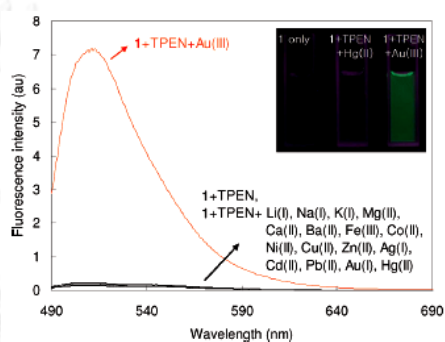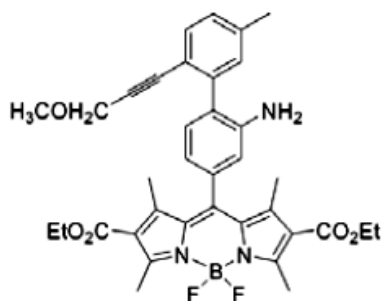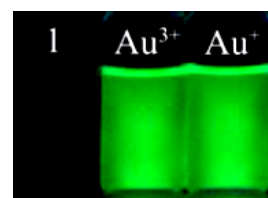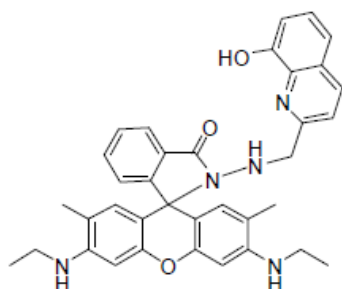
Compound [28]:	Conditions: H_2O-EtOH (7:3 v/v) pH: 5.5 – 8.0
Photophysical properties: λ_{abs} = 543 nm λ_{ems} = 556 nm	**Ion detection:** Au(III) **Detection mode:** Reversible turn-on **LOD:** 48 nmol L^{-1} **Fluorescence and spectral change:** **Visible color changes:**
Compound [29]:	Conditions: MeOH-H_2O (95:5 v/v, pH 9.0)
Photophysical properties: λ_{ems} = 543 nm in H_2O (0.05% DMSO v/v) = 509 nm in CH_3OH (5% H_2O v/v)	**Ion detections:** Hg(II) or Au(III) **Detection mode:** Fluorescence color change (Blue shift) **LOD:** 100 µmol L^{-1} **Fluorescent spectral change:**
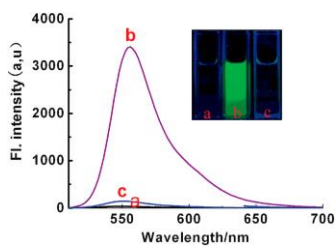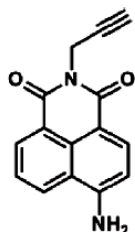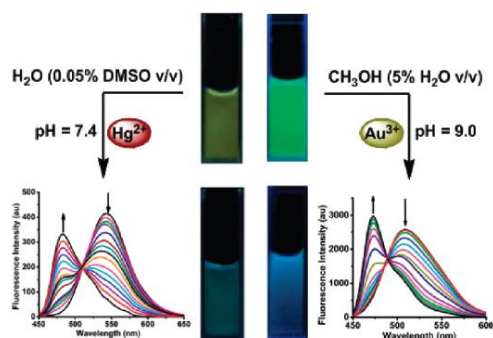
1.6 1,8-Naphthalimide as fluorophores

During the past few years, there has been upsurge in the number of reports on the photophysics and applications on 1,8-naphthalimide-based compound [30-35]. 1,8-Naphthalimide is obtained from reaction of 1,8-naphthalic anhydride with various amines which can be decorated on their applications. Due to their advantageous optical properties, such as strong absorption and emission in visible region, high fluorescence quantum yields, large Stokes shift, high photo stability and insensitive to pH. These remarkable photophysical properties are because of the ICT process that is caused by "push-pull" substituent pairs (electron donor-acceptor pairs) between electron donor at 4-position and electron acceptor at *N*-imide [36].
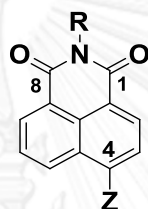
Figure 1.12 Structure of 1,8-naphthalimide.

In this research, 1,8-naphthalimide has chosen as fluorophore unit with excellent stability and high fluorescence quantum yield. Some of them have been used as fluorescent brightening agents for polymer. In 2001, Vladimir Bojinov and Ivo Grabchev [37] reported a new method for synthesis of four (**a-d**) 4-allyloxy-1,8-naphthalimide fluorescent brighteners. All product are colorless and displayed intense blue fluorescence in toluene with quantum yields of 0.43, 0.47, 0.48 and 0.51, respectively. Then, resulted polystyrene copolymer with 4-allyloxy-1,8-naphthalimide derivatives could emit intensive blue fluorescence emission.
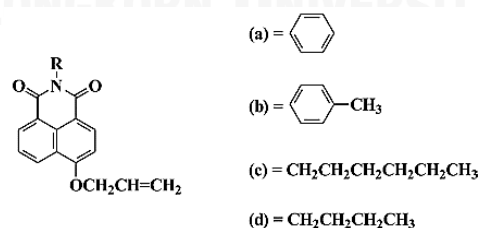
Figure 1.13 The general structure of synthesized 4-allyloxy-1,8-naphthalimide fluorescent brighteners for polystyrene copolymer (a-d) [37].

And also, as the blue emissive polymers have been established by Vladimir Bojinov in 2002 [38]. Monomeric 4-alkoxy-1,8-naphthalimide derivatives have been investigated as blue emitting fluorophore. In this study, the copolymerization of acrylonitrile with 4-alkoxy-1,8-naphthalimdes resulted high photostability and intensive blue excellent fluorescence quantum yield emission in range 0.49-0.75.
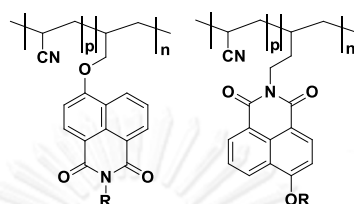
Where R = CH_3 (Ia), C_2H_5 (Ib), C_3H_8 (Ic)

R = C_4H_9 (IIa), C_6H_{13} (IIb), C_6H_5 (IIc), p-C_6H_5-CH_3 (IId)

Figure 1.14 Blue fluorescent polyacrylonitrile copolymers with 1,8-naphthalimides as fluorophore and their photophysical characteristics in DMF [38].

In 2011, Ivo Grabchev et al. prepared a new green fluorescent poly(popyleneamine) dendrimers second generation, having eight 4-*N*-methylpyperazine-1,8-naphthalimide signaling units in its dendrimers periphery. To investigated in THF, this new dendrimers shown good fluorescent emission with quantum yield determined to be 0.69 [39].
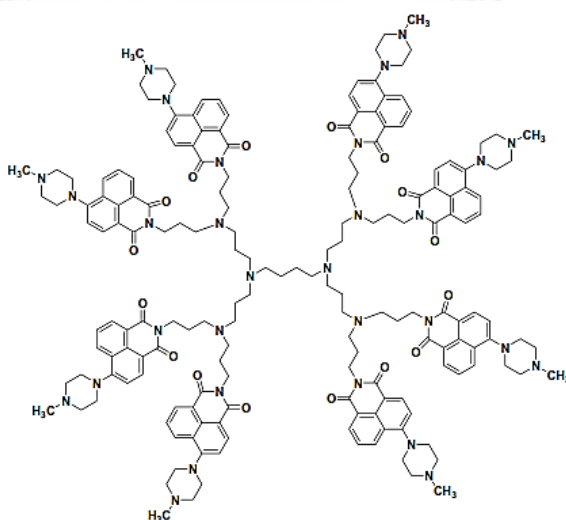
Figure 1.15 Structure of 4-N-methyl piperazine-1,8-naphthalimide PPA dendrimer [39].

Consequently, the 1,8-naphthalimide structure has been extensively used within the ion recognition and sensing of cations or anions, as a fluorescent chemosensor with strongly absorbing and emitting characteristic. Interestingly, some of them could be also used as fluorescent markers in biological systems. The examples presented herein has variable receptor moiety on naphthalimide fluorophore, commonly, 4-position or 1,8-imide site. Thus, up until now, there have been enormous researches on an ion recognition and sensing system involving the naphthalimide fluorophore.

In 2011, Lingxin Chen et al. have been reported a novel "off-on" fluorescent chemosensor of naphthalimide modified rhodamine B, based on the equilibrium between spirolactam (non-fluorescence) and the ring-opened amide (fluorescence). The chemosensor shown Cu(II)-selectivity over various metal ions and anions in neutral ethanol-HEPES solution [40].
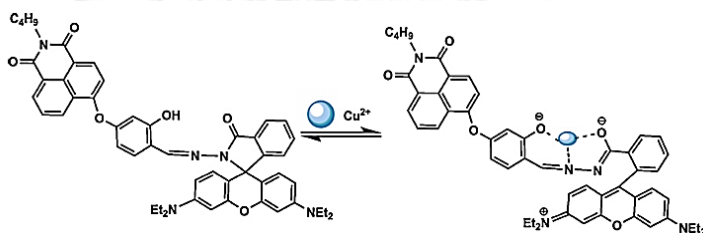
Figure 1.16 Binding mode of naphthalimide modified rhodamine B with Cu (II) in aqueous media (left: off-state; right: on-state) [40].

And also, in this report has been demonstrated the practical applicability of the visualizing fluorescence image of Cu (II) ions in HeLa cell samples. Their fluorescence images were recorded at 37°c before and after the addition of Cu (II) ion 30 min.
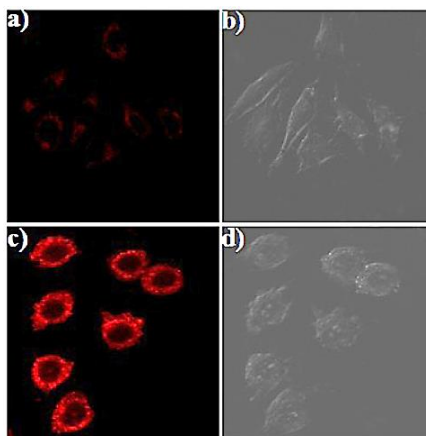
Figure 1.17 Confocal fluorescence images in HeLa cells [40]. (a) Cells incubated with 20 μM chemosensor in PBS buffer for 30 min; (b) brightfield image of (a); (c) cells incubated with 1 μM of Cu(II), and then incubated with 20 μM of chemosensor for 30 min; (d) brightfield image of (c).

In the same year, Hui Xu et al. have been successfully designed and synthesized a new simple water-soluble "turn-on" fluorescent chemosensor containing two acetic carboxylic moieties. The chemosensor exhibits high selectivity and selectivity with fluorescent enhancement on Hg (II) in water *via* inclusion complex over other heavy and transition metal (HTM) ions which are considered as severe environmental pollutants [41].
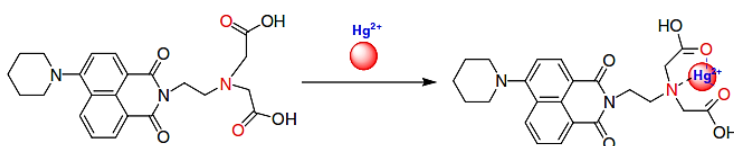
Figure 1.18 Proposed binding mechanism of 1,8-naphthalimide chemosensor with Hg (II) ion [41].

Haishi Cao et al. [42] have reported a new *N*-imidazole functionalized naphthalimide for sensing of F⁻, a design that displays strong fluorescence due to coplanar geometry formed by intramolecular H-bond between imidazole and naphthalimide moieties. The addition of F⁻ resulted decreasing in the fluorescence emission at 442 nm with 94% quenching, due to the disruption of the H-bond, which is referred to "fluoride-triggered disruption". Consequently causes fluorescence signal quenching. This quenching strategy provides a powerful "on-off" signal change for fluorescence sensing on the basis of 1,8-naphthalimide.
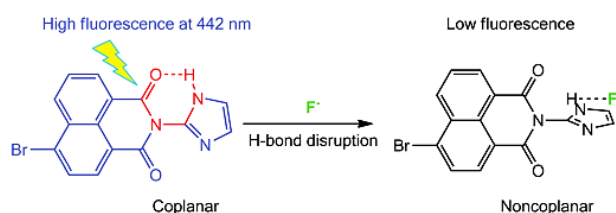
Figure 1.19 Proposed mechanism of fluorescence quenching for the chemosensor upon addition of fluoride involved a disrupting intramolecular H-bond [42].

Zijian Guo et al. recently developed a new fluorescent sensor [43], Naph-BPEA, shows a specific turn-on response to Zn (II) ion in the green emission region. The titration profile based on emission at 540 nm and that based on absorption profile at 400 nm suggest a 1:1 of Zn (II) binding stoichiometry to a sensor probe. The quantum yield of Zn (II)-Naph-BPEA complex was determined to be 0.19. In addition, the detection limit of this sensor was calculated to be 57 nm. Thus, this sensor might be a powerful candidate as fluorescent imaging agent for intracellular Zn (II) ion. Consequently, this sensor is able to Zn (II) ion imaging in HepG2 cells were demonstrated.
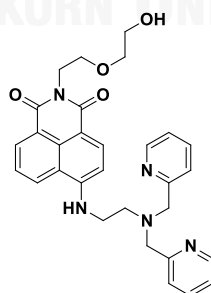
Figure 1.20 Chemical structure of a Naph-BPEA fluorescent probe for sensing of Zn (II) ion [43].
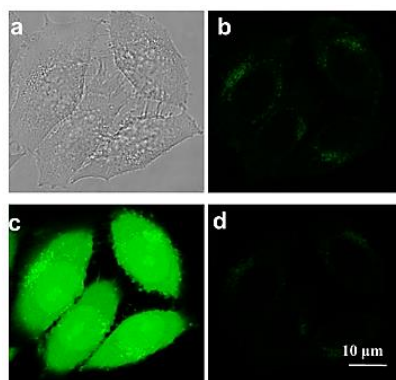
Figure 1.21 Confocal fluorescence images of HepG2 by 5 μM of Naph-BPEA sensor. (a) bright field image of the stain cells; (b) fluorescence images of stain cells; (c) fluorescence image of (b) incubate with 5 μM of ZnSO$_4$/pyrithione 20 min.; (d) fluorescence image of (c) treated by TPEN solution [43].

1.7 Ferrocenyl compounds as luminescent quencher and their applications

Ferrocene and ferrocenyl derivatives fulfill many of broad criteria for development of molecular materials for specific technological applications. As far as we known, their chemistry are well-known and often appreciated for their outstanding stability. For example, ferrocene has high photo-stability under visible irradiation, good electron donating moieties, excellent redox reversible. Ferrocene has been widely used as luminescence excited state quenchers and may undergo chemical modification to a number of various ferrocene-based compounds. Their chemistry are well known and considerable interest in various areas, like asymmetric catalysis [44, 45], artificial photosynthesis [46-50] and electrochemistry [51-53].

Furthermore, ferrocenyl derivatives are well known as efficient fluorescence quenchers which led to numerous applications in the areas of analytical chemistry, molecular organized systems, and biology [52, 54, 55]. On the other hand, literature reports indicate that ferrocene has proved to be a simple building block for the preparation of derivatives which can be explored for the quenching state of analytes.

Owing to its remarkable photophysical properties, In 2002, Jiaan Gan et al. [56] has been reported a series of ferrocene derivatives containing 4-amino-1,8-naphthalimide linked on 4-position of naphthalimide ring through –CH=N- spacer. After incorporated of ferrocene into the naphthalimide, the significantly decrease of fluorescence quantum yield was found. Thus, their electrochemical and photophysical

resulted interaction between ferrocene and naphthalimide moieties was appended *via* PET quenching mechanism.

Later in 2010, Tei Tagg et al. [57] prepared and characterized dyads containing ferrocene donor and naphthalimide acceptor unit, separated by alkyne bond spacer. It has been found that the attachment of ferrocenyl substituent in to 4-position of 1,8-naphthalimide which it caused dramatically decrease of fluorescence emission efficiency.
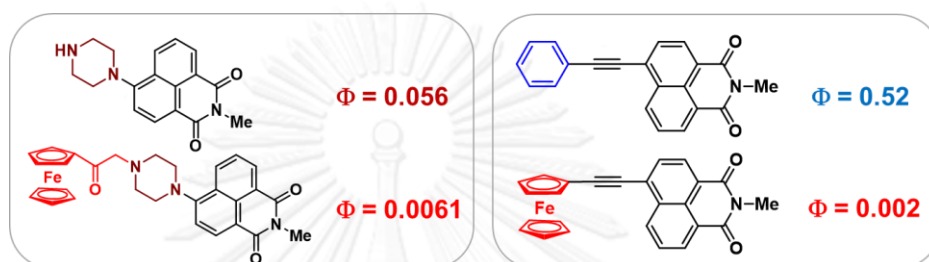
Figure 1.22 The change of fluorescence quantum yield after the incorporating of ferrocenyl unit into 1,8-naphthalimide plateforms. (left: linked by saturated piperine spacer [56], right: linked by alkyne spacer [57])

In the same year, Huimin Ma et al. [58] has been prepared and characterized a highly specific ferrocene-based fluorescent probe, (9-anthryl)ethynylferrocene, 9-AEF for HOCl sensing. The designed strategy is based-on the strong quenching effect of good electron-donor ferrocene on anthracene fluorescence *via* an ICT quenching process. Then, in the presence of HOCl, the fluorescence was enhancement, the quantum yield from less than 0.001 to be 0.12. The limit of detection was determined to be 0.3 μM.
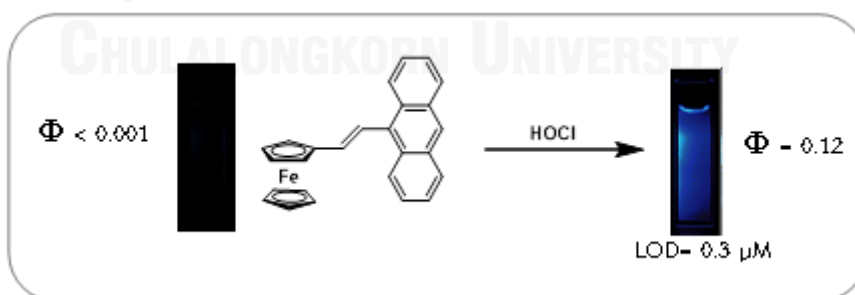
Figure 1.23 Fluorescence response of 9-AEF to HOCl [58].

1.8 Objectives and scope of this research

From the information described above, it is our intention to design and synthesize new 1,8-naphthalimide derivatives containing a ferrocene moiety. These new materials should exhibit relatively low emission efficiency due to the PET process between the naphthalimide and ferrocene. Analytes which can prevent this quenching mechanism could therefore cause an enhancement or restoration of the fluorescence signal. After that, the photophysical properties of these compounds are investigated. The application of these new target compounds as metal ion sensors in aqueous media and their optimal measurement conditions will also explored. The structures of target compounds are shown in **Figure 1.24**.
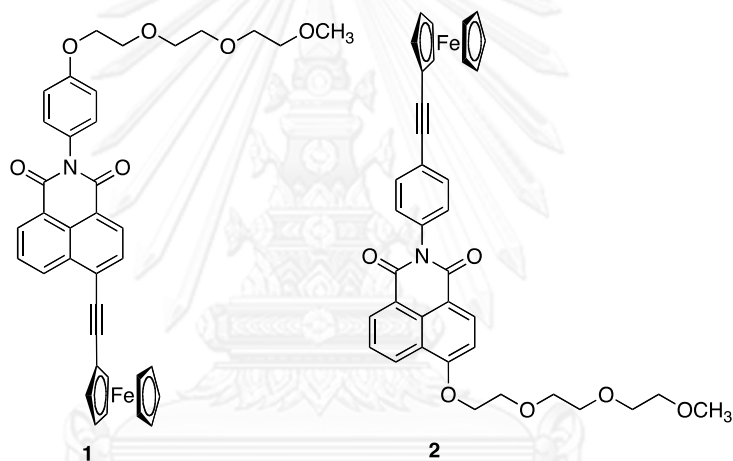
Figure 1.24 Structures of target compound **1** and **2**.

CHAPTER II

EXPERIMENTAL

2.1. Materials and instruments

4-Bromo-1,8-Naphalic anhydride, 4-methoxy aniline, 4-iodo aniline, triethylene glycol monomethyl ether, sodium hydride, 4Å molecular sieve, tetrakis(triphenylphosphine)palladium and tris(dibenzylideneacetone)dipalladium, were purchased from Sigma-Aldrich (USA) and used without purification. Potassium carbonate, 1H-imidazole, copper iodide, triphenyl phosphine were purchased from Fluka (Switzerland) and used without purification. Ethynyl ferrocene was purchased from Chemieliva (China) and used without purification. All chemicals were reagent grades. Spectroscopic grade acetronitrile for fluorescence measurement was purchased from Fluka (Switzerland). All organic solvents for reaction work up and chromatography were commercial grades, which were distilled prior to use. $HAuCl_4$ and AuCl were purchased from Sigma-Aldrich (USA). Other inorganic salts were in perchlorate, acetate, chloride or nitrate form. Thin layer chromatography (TLC) was performed on aluminum sheets pre-coated with silica gel (Merck Kiesegel 60 F254) (Merck KgaA, Darmstadt, Germany). Column chromatography was performed on silica gel (Merck Kieselgel 60G) (Merck KGaA, Darmstadt, Germany). All ^1H- and ^{13}C-NMR spectra were obtained on a Varian Mercury NMR spectrometer, which operated at 400 MHz for ^1H and 100 MHz for ^{13}C nuclei (Varian Company, CA, USA). Absorption spectra were measured by a Varian Cary 50 UV-Vis spectrophotometer. Fluorescence spectra were obtained from a Varian Cary Eclipse spectrofluorometer.

2.2. Synthesis of 1 and 2

2.2.1 Synthesis of 4─bromo─*N*─(4'-hydroxyphenyl)─1,8─naphthalimide (3).

3
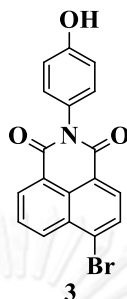
This compound was synthesized by a modified method described in the literature [35]. A mixture of commercially available 4─bromo─1,8─naphthalic anhydride (1.0 g, 3.61 mmol), 10 mL of glacial acetic acid, and 4─hydroxyaniline (0.79 g, 7.22 mmol) was refluxed overnight. After the reaction was cooled to room temperature, it was poured into ice water and the dark purple precipitate was filtered and washed with water. The crude product was purified by silica gel column chromatography using ethyl acetate/hexane (1:4) as eluent. The product (**3**) was obtained as an off-white powder in 63% yield. ^{1}H NMR (400 MHz, CDCl$_3$, δ ppm): 8.64 (1H, d, J = 8.0 Hz), 8.57 (1H, d, J = 8.0 Hz), 8.39 (1H, d, J = 8.0 Hz), 8.01 (1H, d, J = 8.0 Hz), 7.80 (1H, t, J = 8.0 Hz), 7.15 (2H, d, J = 8.8 Hz), 7.00 (2H, d, J = 8.8 Hz).

2.2.2 Synthesis of 4─bromo─*N*─(4'(2-(2-(2-methoxyethoxy)ethoxy)ethoxy) phenyl)─1,8─naphthalimide (4).

4
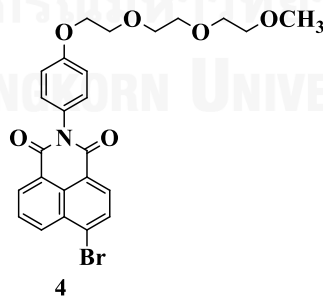
A mixture of **3** (0.25 g, 0.68 mmol), K$_2$CO$_3$ (0.19 g, 1.36 mmol) and TBAF (0.04 g, 0.14 mmol) in CH$_3$CN (7 mL) was stirred at room temperature for 1 h. Then, 1-iodo-2-(2-(2-methoxyethoxy)ethoxy)ethane (0.35 g, 1.36 mmol) [59] was added in one portion and the reaction was heated under reflux conditions and monitored by TLC. After the

reaction completed, the mixture was concentrated under reduced pressure. The residue was re-dissolved in CH_2Cl_2 and the solution was sequentially washed with water, 5% HCl, and brine. After drying of the organic solution over anhydrous $MgSO_4$ and evaporation of the solvent, the residue was purified by silica gel chromatography using gradient eluents from EtOAc/hexane (4:1) to pure EtOAc to afford **4** as an off-white solid in 46% yield. ^{1}H NMR (400 MHz, CDCl$_3$, δ ppm): 8.68 (1H, d, J = 7.3 Hz), 8.60 (4H, d, J = 8.5 Hz), 8.43 (1H, d, J = 7.8 Hz), 8.05 (1H, d, J = 7.9 Hz), 7.86 (1H, t), 7.14 (2H, d, J = 8.6 Hz), 7.06 (2H, d, J = 8.6 Hz), 4.19 (2H, t), 3.89 (2H, t), 3.79 – 3.73 (2H, m), 3.73 – 3.64 (4H, m), 3.60 – 3.52 (2H, m), 3.39 (3H, s). ^{13}C NMR (100 MHz, CDCl$_3$, δ ppm): 164.1, 164.0, 159.0, 130.9, 133.6, 132.5, 131.7, 131.3, 130.7, 129.4, 129.4, 128.3, 127.8, 123.4, 122.6, 115.5, 72.1, 71.1, 70.8, 70.7, 69.8, 67.8, 59.1. MS (MALDI-TOF) Calcd for $C_{25}H_{24}BrNO_6$: 514.37; Found: 513.08

2.2.3 Synthesis of fluorophore 1
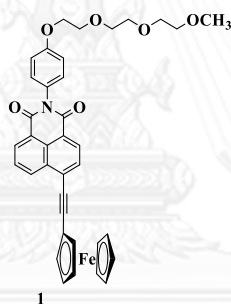
A mixture of **4** (0.2 g, 1.01 mmol), ethynylferrocene (0.28 g, 1.31 mmol), Pd(PPh$_3$)$_4$ (0.18 g, 0.15 mmol), PPh$_3$ (26.6 mg, 0.10 mmol), CuI (19.0 mg, 0.10 mmol) and triethylamine (3 mL) in toluene (3 mL) was stirred at 40°C under nitrogen atmosphere for 1.5 h. The reaction was then subjected to a typical aqueous-organic extractive work-up followed by flash chromatography using EtOAc/hexane (1:4) as the eluent to afford **1** as a dark red solid in 90% yield. Melting point 166.2 – 167.0 °C (decomposed). ^{1}H NMR (400 MHz, CDCl$_3$, δ ppm): 8.73 (1H, d, J = 8.9 Hz), 8.66 (1H, d, J = 6.8 Hz), 8.56 (1H, d, J = 7.2 Hz), 7.91 (1H, d, J = 6.6 Hz), 7.86 (1H, s), 7.21 (2H, d, J = 8.5 Hz), 7.06 (2H, d, J = 8.2 Hz), 4.66 (2H, s), 4.38 (2H, s), 4.31 (5H, s), 4.19 (2H, s), 3.89 (2H, s), 3.75 (2H, s), 3.69 (4H, M), 3.56 (2H, s), 3.38 (3H, s). ^{13}C NMR (100 MHz, CDCl$_3$, δ ppm): 164.4, 164.0, 160.9, 135.3, 134.4, 132.7, 132.5, 130.4, 129.8, 129.3, 126.5, 124.9, 124.3, 123.0, 115.7, 106.7, 89.7, 85.8, 77.8, 77.6, 77.4, 77.2, 72.4, 72.0, 71.6, 71.2, 71.1, 70.5, 69.6, 69.3, 69.1, 67.7, 59.0. MS (MALDI-TOF) Calcd for $C_{37}H_{33}FeNO_6$: 643.16 Found: 643.31

2.2.4 Synthesis of 4—bromo—*N*—(4—iodo—phenyl)—1,8—naphthalimide (5)
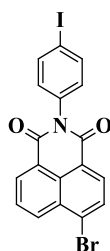
5

This compound was synthesized by a modified method described in the literature [60]. A mixture of commercially available 4—bromo—1,8—naphthalic anhydride (1.00 g, 3.61 mmol), 4—iodoaniline (1.58 g, 7.22 mmol), and imidazole (5.16 g, 75.8 mmol) was refluxed in $CHCl_3$ (30 mL). After 1.5 h, the reaction was cooled to room temperature and the solvent was removed under reduced pressure. The residue was taken up in absolute ethanol and the resulting suspension was sonicated for 15 min. A light yellow solid was filtered and dried under vacuum to afford the product (**5**) in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.67 (1H, d, *J* = 7.3 Hz), 8.62 (1H, d, *J* = 8.5 Hz), 8.42 (1H, d, *J* = 7.8 Hz), 8.06 (1H, d, *J* = 7.9 Hz), 7.86 (1H, m), 7.04 (2H, d, *J* = 8.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.5, 163.5, 138.6, 134.8, 133.8, 132.6, 131.7, 131.2, 130.9, 130.8, 130.6, 129.3, 128.2, 123.0, 122.1, 94.6.

2.2.5 Synthesis of 4—(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)—*N*-(4'-iodophenyl)—1,8-naphthalimide (6)
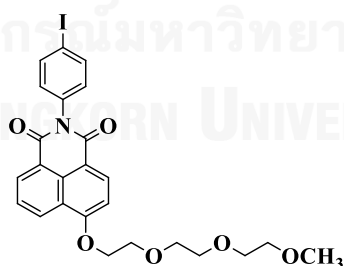
6

To a slurry mixture of NaH (60%, 0.16 g, 3.95 mmol) and anhydrous DMF (3 mL) under nitrogen atmosphere chilled in an ice bath, triethylene glycol monomethyl ether (2.84 g, 2.37 mmol) was slowly added. After the mixture was stirred for 1 h, a solution of **5** (0.38 g, 0.79 mmol) in 1 mL of anhydrous DMF was added, and the mixture was stirred while the temperature was gradually returned to room temperature for 24 h. The reaction was quenched by addition of water and the resulting solution was

extracted three times with EtOAc. The combined organic layers were sequentially washed with 5% HCl, 10% Na_2CO_3, and water. The crude product after evaporation of solvent was purified by silica gel chromatography using gradient eluents from EtOAc/hexane (1:1) to pure EtOAc to afford **6** as a yellow solid in 42% yield. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 8.67 (1H, d, *J* = 7.3 Hz), 8.62 (1H, d, *J* = 8.5 Hz), 8.42 (1H, d, *J* = 7.8 Hz), 8.14 (1H, d, *J* = 7.9 Hz), 7.86 (1H, m), 7.04 (2H, d, *J* = 8.6 Hz). 4.20 (2H, s), 3.90 (2H, s), 3.76 (2H, s), 3.71 (4H, s), 3.57 (2H, s), 3.40 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.1, 164.0, 159.0, 133.6, 132.5, 131.7, 131.3, 130.9, 130.7, 129.5, 129.4, 128.3, 127.8, 123.4, 122.6, 115.5, 72.1, 71.0, 70.8, 70.7, 69.8, 67.8, 59.1. MS (MALDI-TOF) Calcd for $C_{25}H_{24}NIO_6$: 561.15 Found: 561.67

2.2.6 Synthesis of fluorophore 2
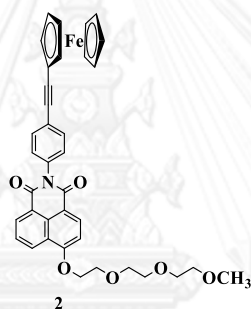
The mixture of **6** (0.57 g, 1.11 mmol), ethynylferrocene (0.30 g, 1.44 mmol), $Pd_2(dba)_3$ (0.15 g, 0.17 mmol), PPh_3 (29.26 mg, 0.11 mmol), CuI (20.95 mg, 0.11 mmol) and triethylamine (3 mL) in toluene (3 mL) was stirred at 40 °C under nitrogen atmosphere for 1.5 h. After the reaction mixture was subjected to a typical aqueous-organic extractive work-up followed by flash chromatography using EtOAc/hexane (1:4) as the eluent, fluorophore **2** was obtained as a yellow solid in 96% yield. Melting point 155.4 – 155.8 °C (decomposed). ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 8.67 (1H, d, *J* = 7.3 Hz), 8.62 (1H, d, *J* = 8.5 Hz), 8.42 (1H, d, *J* = 7.8 Hz), 8.06 (1H, d, *J* = 7.9 Hz), 7.86 (1H, m), 7.23 (2H, d, *J* = 8.5 Hz), 7.08 (2H, d, *J* = 8.2 Hz), 4.67 (2H, s), 4.40 (2H, s), 4.32 (5H, s), 4.20 (2H, s), 3.90 (2H, s), 3.76 (2H, m), 3.71 (4H, m), 3.57 (2H, m), 3.38 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.4, 164.0, 160.9, 135.3, 134.4, 132.7, 132.5, 130.4, 129.8, 129.3, 126.5, 124.9, 124.3, 123.0, 115.7, 106.7, 89.7, 85.8, 77.8, 77.6, 77.4, 77.2, 72.4, 72.0, 71.6, 71.2, 71.1, 70.5, 69.9, 69.3, 69.1, 59.5. MS (MALDI-TOF) Calcd for $C_{37}H_{33}FeNO_6$: 643.16 Found: 642.99

## 2.3 Measurement of UV-Vis and fluorescence spectra

Stock solution of compound **1** and **2** (100 μM) were prepared in acetonitrile. Stock solutions (10mM) were prepared in deionized water by dissolving of perchlorate, acetate, chloride or nitrate form of Ag (I), Au (I), , Au (III), Bi (III), Ca (II), Cd (II), Co (II), Cr (II), Cu (II), Fe (III), Ga (III), Hg (II), Mn (II), Ni (II), Pb (II), Sr (II) and Zn (II) ions except for HAuCl$_4$ for Au (III) ions.

In typical experiment, test solutions were prepared by placing 50 μL of the probe stock solution into 2.0 mL Eppendorf® tube, adding an aliquot of each metal stock, then dilute the solution to 1.0 mL with 10 mM PBS (pH 8.0) and acetonitrile. Normally, excitation was at 369 nm. Both excitation and emission slit widths were 5 nm. Fluorescence spectra were measured after addition of Au (III) 15 min. For low concentration of Au (III), fluorescence spectra were measured after addition of Au (III) for 30 min.

2.4 Interference behaviors from other metal species on Au (III) signaling

Under the same measurement conditions, competitive signaling behavior of probe **2** toward Au (III) ions in the presence of coexistence metal ions as background were studied. Final concentration of **2**, Au (III), and the other various competing metal ions were 5 μM, 20 μM and 0.2 mM, respectively, in CH$_3$CN-PBS buffer (pH 8.0, 10 mmoL^{-1}, 4/6, v/v).

2.5 Water content effect on reaction time and Au (III) signaling

The water effect of probe **2** on Au (III) signaling investigated by measuring of fluorescence emission spectra at 467 nm and reaction time at highest fluorescence intensity in series of water/acetonitrile was 4/6 to 8/2 (v/v). The sample solution with different water content in acetonitrile were freshly prepared prior each measurement. Final concentrations of probe and Au (III) and each buffer solution were 5 μM and 0.5 mM, respectively.

2.6 pH effect on Au (III) signaling

The pH effect on Au (III) signaling of probe **2** was investigated by measuring of fluorescence emission spectra in the series of buffer between pH 4.0-9.0. The pH of the solution was fixed by using acetate buffer (pH 4.0-6.0), phosphate (7.0-8.0) and tris-HCl (9.0). Final concentrations of probe, Au (III) and each buffer solution were 5 μM, 0.5 mM and 10 mM, respectively, in CH_3CN-PBS buffer (pH 8.0, 10 $mmoL^{-1}$, 4/6, v/v).

2.7 Surfactant effect on Au (III) signaling

The surfactant effect on Au (III) signaling of probe **2** was investigated by measuring of fluorescence emission spectra with three types of surfactant; cationic (TTAB and CTAB), anionic (Tween-20, Brij and Triton X-100) and non-ionic (SDBS) surfactant. Final concentrations of probe and Au (III) were 5 μM and 0.5 mM, respectively. Concentration each surfactant solution was used at its CMC point. All experiments were carried in CH_3CN-PBS buffer (pH 8.0, 10 $mmoL^{-1}$, 4/6, v/v). Under same measurement conditions.

2.8 Limit of detection

The limit of detection of fluorophore was estimate by plotting of fluorescence change of probe **2** in the presence of Au (III) (0.0-3.0 μM, $HAuCl_4$ as Au (III) source) as a function of fluorescence intensity (taken as peak height at 467 nm) following the reported procedure [61]. Also, the minimum concentration of Au (III) that gives the fluorescence intensity was determined with a signal-to-background ratio of three.

2.9 Elucidation of sensing mechanism by ^{13}C-NMR spectroscopy

To have evidence for the complexation between Au (III) and **2**, the ^{13}C-NMR spectra **2** and the reaction product of **2** with $HAuCl_4$ were obtained. To a solution of **2** (70 mM, in CD_3CN) was added $HAuCl_4$ solution, and the mixture was mixed properly at room temperature. The final concentration of **2** and $HAuCl_4$ is 60 mM and 0.15 mM, respectively. The ^{13}C-NMR spectra of compound **2** and the reaction product of **2**–Au (III) were measured in CD_3CN.

CHAPTER III

RESULTS AND DISCUSSION

3.1. Synthesis and characterization of fluorophores

The synthesis of our fluorophores began with an imidation of commercially available 4-bromo-1,8-naphthalic anhydride (**Scheme 3.1**). Refluxing of such compound with *p*-aminophenol in glacial acetic acid provided naphthalimide **3** in 63% yield, whereas the reaction with *p*-iodoaniline and imidazole in CHCl$_3$ yielded naphthalimide **5** in 80% yield. The installation of a hydrophilic triethylene glycol group into **3** was carried out by *O*-alkylation of the phenolic –OH group using 1-iodo-2-(2-(2-methoxyethoxy)ethoxy)ethane under basic condition. Meanwhile, a nucleophilic aromatic substitution of the bromine in **5** by triethylene glycol monomethyl ether in the presence of NaH afforded **6** in 42% yield. Finally, the Sonogashira coupling with ethynyl ferrocene transformed **4** and **6** into target compound **1** as reddish solid in 26% total yield (3 steps) and **2** as yellowish solid in 32% total yield (3 steps). All of the compounds were then fully characterized by ^{1}H-NMR spectroscopy (**Figure 3.1** and **3.2**). The physical appearance, ^{1}H-NMR (**Figure 3.3** and **3.4**) and its ^{13}C-NMR, IR and mass spectrometry of target compound **1** and **2** are shown in appendix.
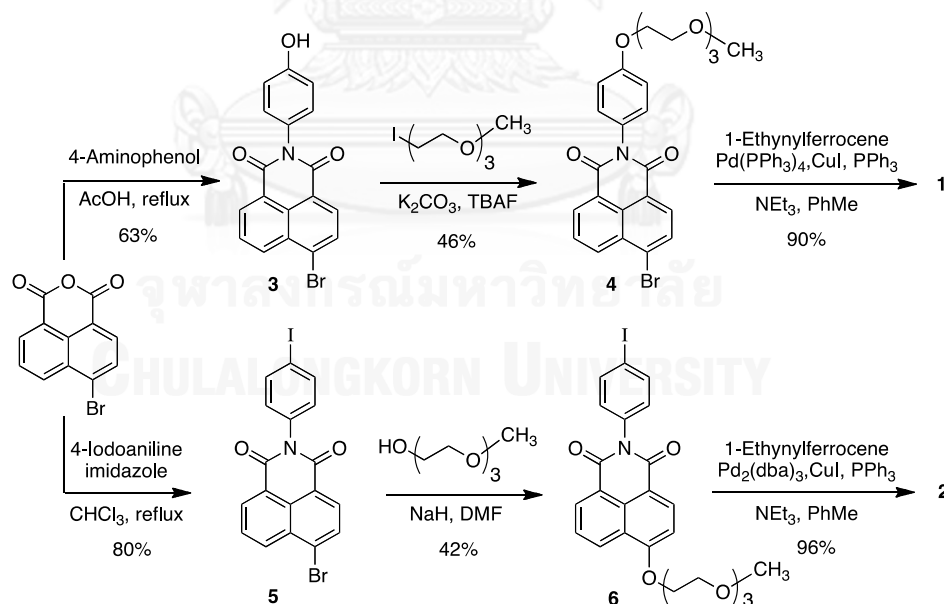
Scheme 3.1 Synthesis of **1** and **2**.
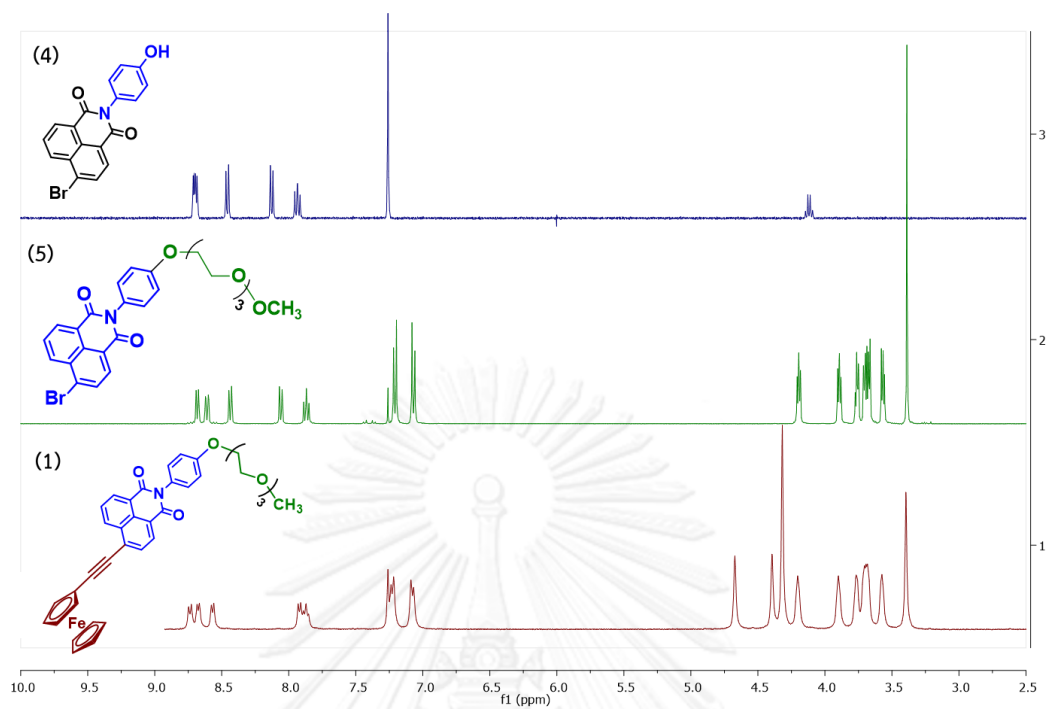
Figure 3.1 The comparison of ¹H-NMR of compound **3**, **4** and **1**.
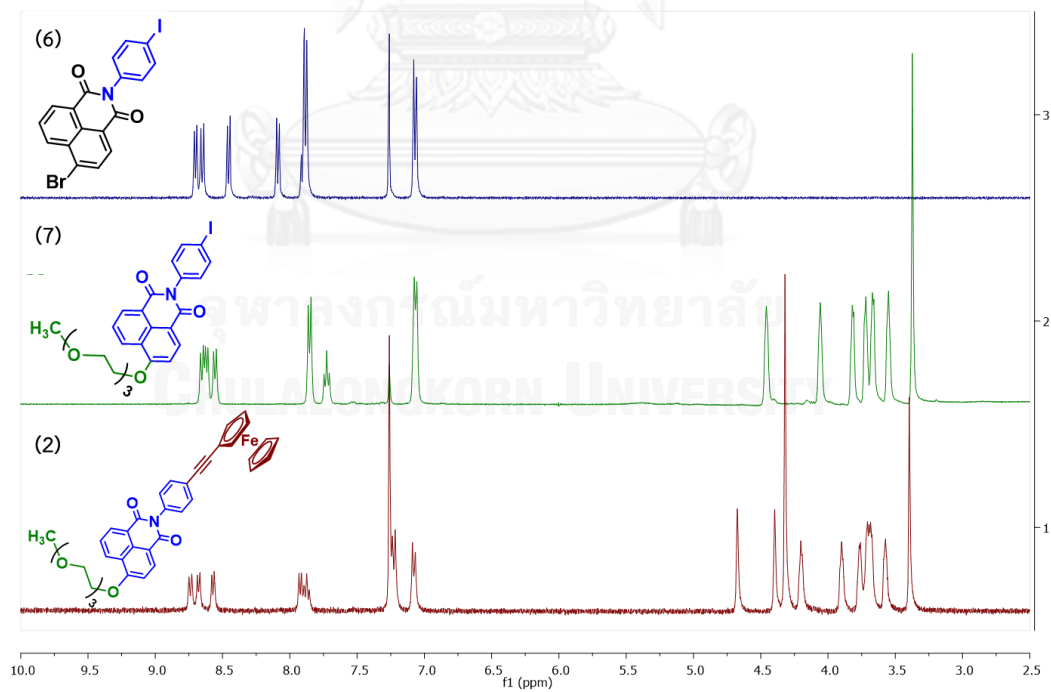
Figure 3.2 The comparison of ¹H-NMR of compound **5**, **6** and **2**.
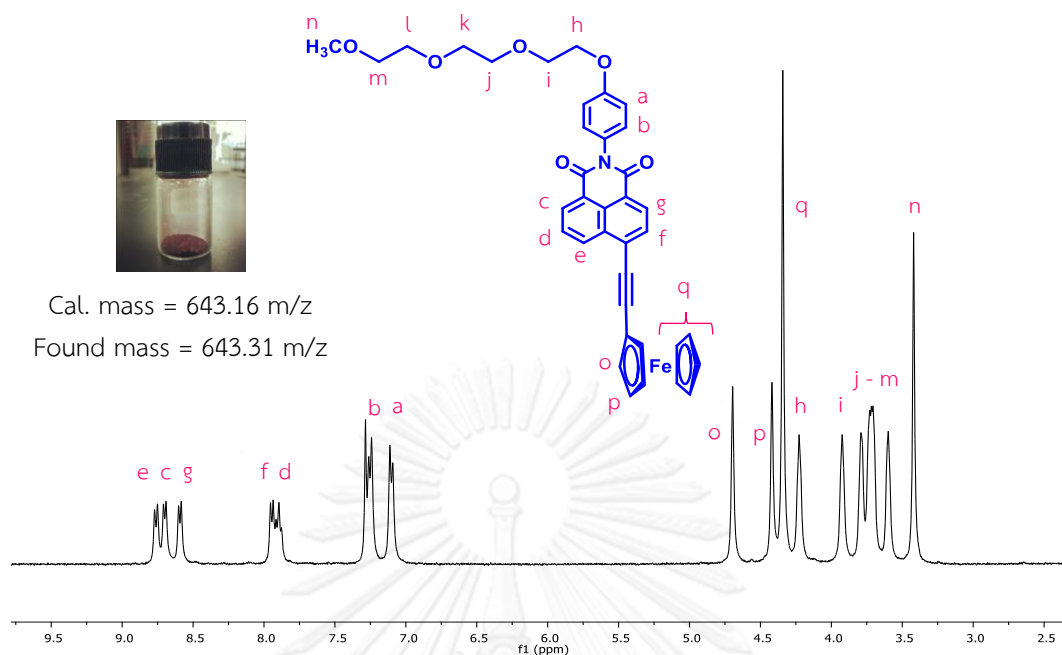
Figure 3.3 The physical appearance, mass spectroscopic analysis and ^{1}H-NMR of compound **1**.
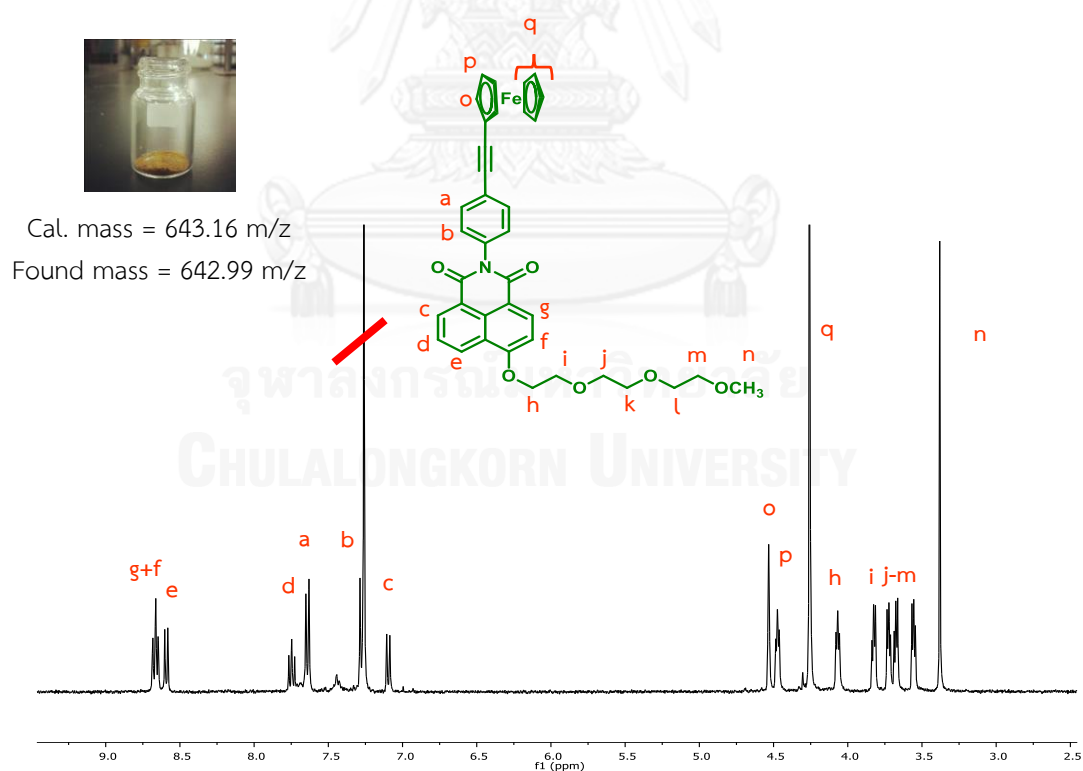
Figure 3.4 The physical appearance, mass spectroscopic analysis and 1H-NMR of compound **2**.

3.2. Photophysical properties of fluorophores

The photophysical properties of **1** and **2** were investigated using their solutions in 1:1 mixture between CH_3CN and H_2O. The results are tabulated in **Table 3.1** and the normalized absorption and emission spectra are shown in **Figure 3.3**.

Table 3.1 Photophysical properties of **1** and **2** in CH_3CN-H_2O (v:v = 1:1).

Compound	Absorption		Emission		Stokes shift (cm^{-1})
	λ_{max} (nm)	ε (M^{-1}cm^{-1})	λ_{max} (nm)	Φ^a_F	
1	380	9000	405	<0.01	1624
2	369	17,000	453	0.01	5025

^{a}quinine sulfate in 0.1 M H_2SO_4 (Φ = 0.54) was the reference.
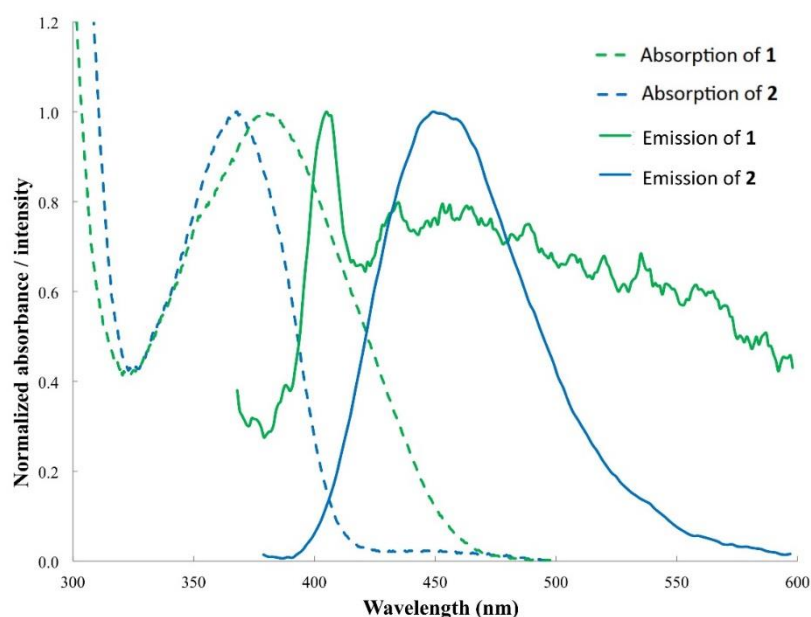
Figure 3.5 Normalized absorption (solid line) and emission spectra (dashed line) of 1,8-naphthalimide **1** and **2** in CH_3CN-H_2O (1/1; v/v).

For the absorption, **1** and **2** showed a maximum absorption wavelength at 380 nm and 369 nm, respectively. The longer maximum absorption wavelength of **1** corresponds to a longer conjugated system caused by the extension of pi-system of the 1,8-naphthalimide fluorophore by the ethynylferrocene appended at the

4-position. The substitution at this position by a heteroatom such as O in **2** leads to the compounds with absorption around 360-370 nm [62].
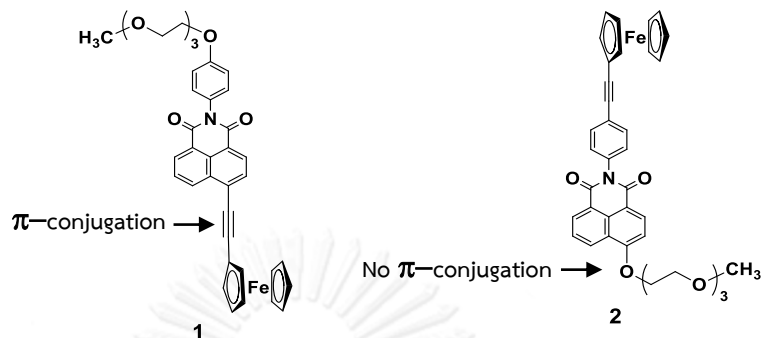
Figure 3.6 Representative of π-conjugation at 4-position of **1** and **2**.

From the molar extinction coefficient data, **2** appears to be a more absorptive compound than **1**. This might correspond to the absorption of light in **2** that generates a charge transfer interaction between the substituent at 4-position and the imide carbonyl group as depicted in **Figure 3.5** [63].
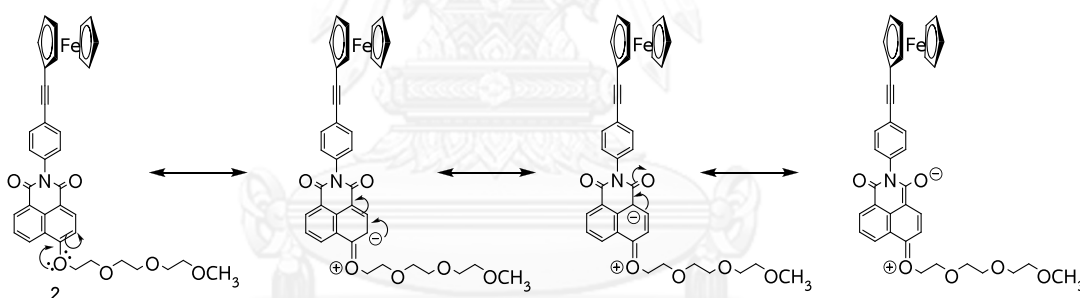
Figure 3.7 Proposed charge separation in **2**.

For the fluorescence data, **1** shows maximum emission wavelength at 405 nm. Compound **2**, on the other hand, exhibits maximum emission wavelength at 453 nm in the blue region which is generally characteristic electron transition in the naphthalimide derivatives with halogen or good electron-donating alkoxy group at C-4 position (**Figure 3.6**) [38].
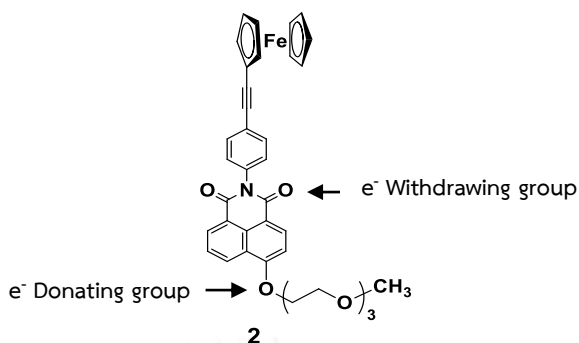
Figure 3.8 Electron donating and withdrawing in **2**.

The Stokes shift values of **1** and **2** were calculated to 1624 and 5025 cm^{-1}, respectively. The larger Stokes shift in **2** corresponds to the presence of an electron-donating (alkoxy group) and withdrawing groups (carbonyl group) in the structure causing an ICT process (**Figure 3.7**). Polar solvents such as a mixture of CH$_3$CN and H$_2$O can stabilize the excited molecule with a charge separation and also lower the energy of the lone pair electrons by a strong H-bond formation. In addition, a larger geometry difference between its excited state and ground state may result from various modes of vibration and rotation of the triethylene glycol unit attached to the fluorophore pendant. This large Stokes shift is a prominent point for fluorescent sensor applications since it could prevent self-absorption between the emission and the excitation.
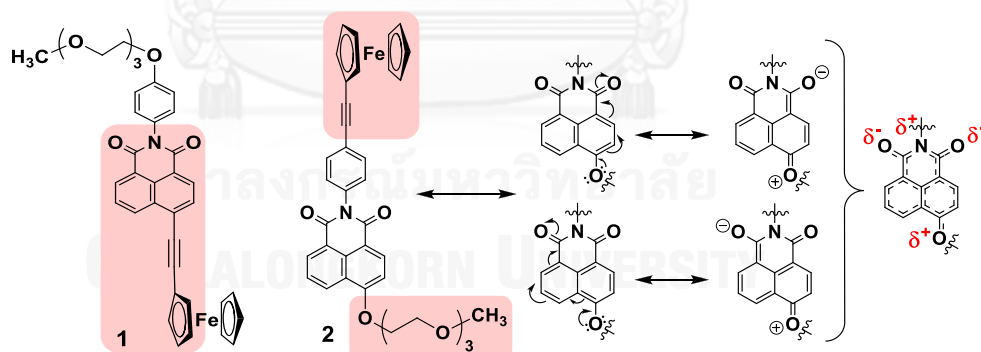
Figure 3.9 Rational description of low fluorescence emission of **1** and **2**.

The efficiency of molecule to emit of the absorbed light energy is characterized by the fluorescence quantum yield. As expected, both 1,8-naphthalimide derivatives exhibit unusually low quantum efficiency, in the comparison with the parent 1,8-naphthalimide, due to the effect of the fluorescence quenching process (PET) from

ethynyl ferrocene to the excited state of naphthalimide part. However, the fluorescence quantum yield of **1** cannot be determined since its signal is extremely low. For compound **1**, there is a direct conjugation between naphthalimide and one of the cyclopentadienyl ligands (**Figure 3.7, left**). Thus, there might be a possibility for metal to ligand charge transfer (MLCT) or ligand to metal charge transfer (LMCT) that leads to a non-emissive behavior.

For compound **2**, the fluorescence emission can be observed under the same conditions with a low quantum efficiency of around 1%. This weakly fluorescent property may cause by the combination of ICT effect between the electron donor at the 4-position to naphthalimide and the carbonyl group, as well as the through-space PET effect by the ferrocenyl group (**Figure 3.7, right**).

3.3. Selectivity screening of compound 1 and 2 toward various metal cations in aqueous media.

With both **1** and **2** in our hands, the selectivity of these compounds toward various metal ions were investigated by monitoring their fluorescent signal changes in the presence of Ag (I), Al (III), Au (I), Au (III), Bi (III), Ca (II), Cd (II), Co (II), Cr (II), Cu (II), Fe (III), Ga (III), Hg (II), Mn (II), Ni (II), Pd (II), Pb (II), Sr (II) and Zn (II) in aqueous media.

In the case of **1**, no significant change in fluorescent signal was found in the presence of metal ions examined (**Figure 3.8**). However, the signal of **2** was enhanced to approximately 8 and 2 folds of its original intensity in the presence of Au (III) and Au (I), respectively (**Figure 3.9** and **3.10**).
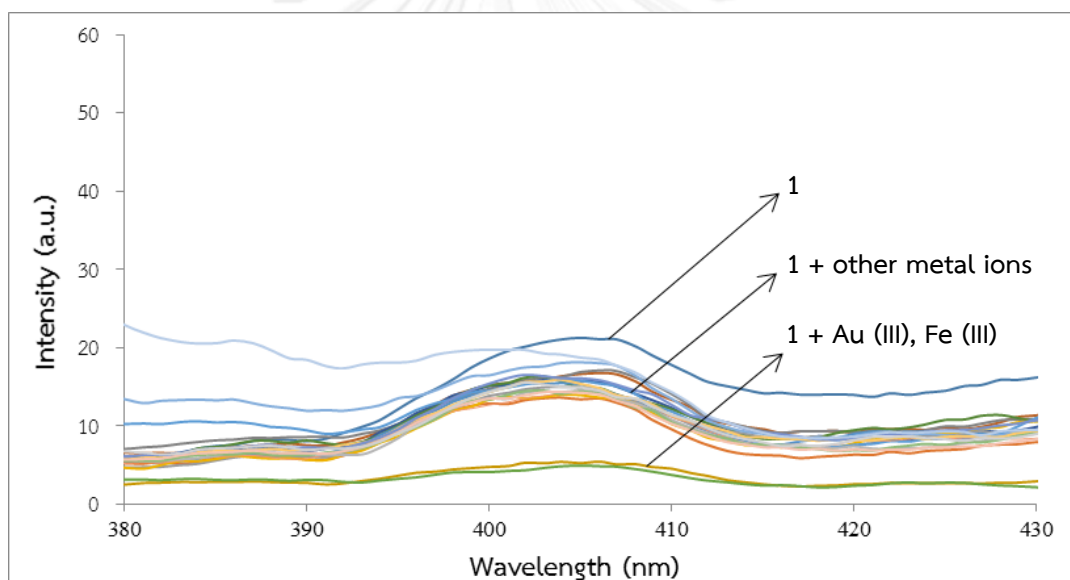
Figure 3.10 Fluorescence spectra of compound **1** (5 μM, λ_{ex} 369 nm) in a mixture of CH$_3$CN-PBS buffer solution (pH 8.0, 10 mM, 1/1, v/v) upon addition of various metal ions (0.5 mM).
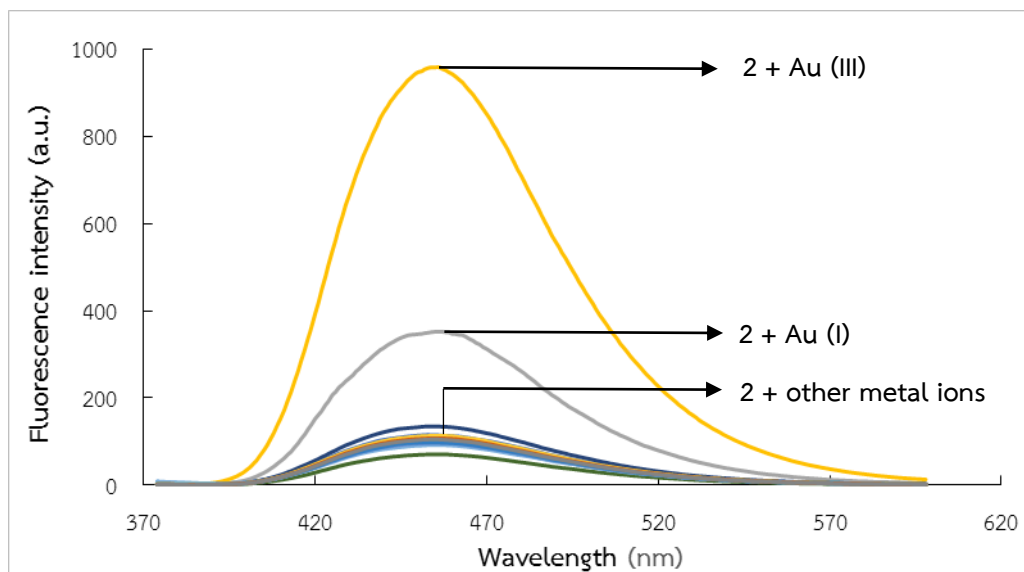
Figure 3.9 Fluorescence spectra of compound **2** (5 µM, λ_{ex} 369 nm) in a mixture of CH$_3$CN-PBS buffer solution (pH 8.0, 10 mM, 1/1, v/v) upon addition of various metal ions (0.5 mM).
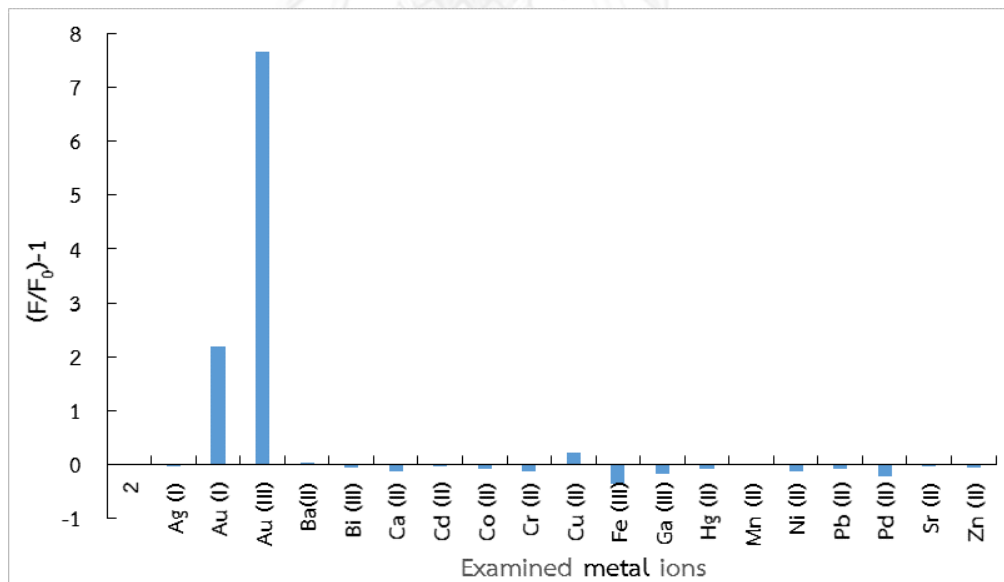
Figure 3.10 Relative fluorescence responses at λ_{em} 456 nm, where F is the fluorescence intensity in the presence of metal ion analyte and F$_0$ is the original fluorescent intensity of **2**. [Inset: fluorescence spectra of **2** (5 µM, λ_{ex} 369 nm) in a mixture of CH$_3$CN-PBS buffer solution (pH 8.0, 10 mM, 1/1, v/v) upon addition of various metal ions (0.5 mM)].

3.4. Elucidation of the proposed sensing mechanism of compound 2 toward gold ions.

This selectivity towards gold ions is attributed to the special alkynophilicity of the gold ions toward the triple bond. For compound **1**, the complexation of Au species to the triple bond may not completely disturb the conjugation between fluorophore and ferrocene group because the complexation usually exploits one of the two pi-bonds, leaving another bond to maintain the conjugation. Therefore, the PET process could still take place after the complexation.

For compound **2** which does not possess a direct conjugation between naphthalimide and ferrocene groups, the through-space PET process could be somewhat disturbed after alkyne-Au complexation. On the basis of these fluorescence quenching process, the fluorescence signal can be enhanced when the coordination of the gold ion at the triple bond occurs. The proposed sensing mechanism is shown in **Scheme 3.2**.
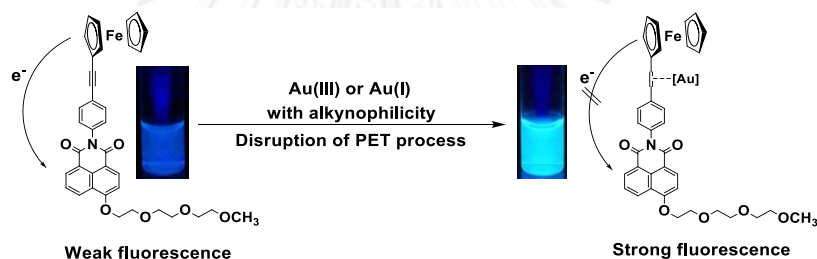
Scheme 3.2 Proposed sensing mechanism of **2** towards Au (III) or Au (I) ion.

To elucidate these proposed sensing mechanism, we decided to use ^{13}C-NMR spectroscopy technique for monitoring the change of alkynyl-carbon of compound **2** in the absence and the presence of Au (III). The results were shown in **Figure 3.11** and **3.12**. It is clear that the addition of Au (III) caused the disappearance of alkynyl-carbon signals at 90 and 85 ppm, even after 10,000 scans. This data supports our proposed sensing mechanism to some extents.
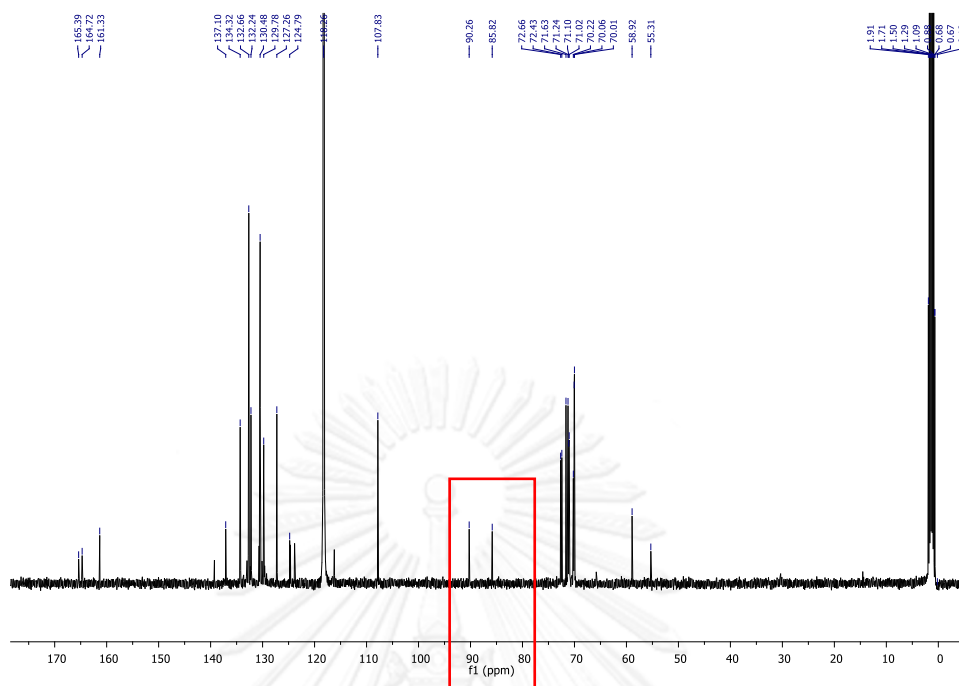
Figure 3.11. ^{13}C-NMR (400 MHz) of compound **2** (70 mM) in CD$_3$CN (3,000 scans).
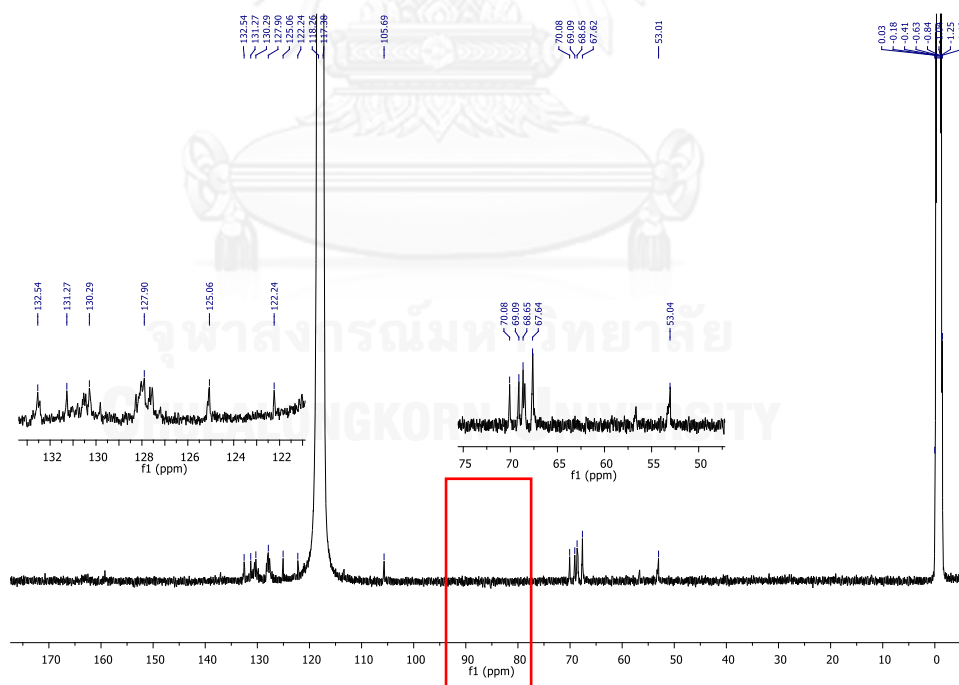
Figure 3.12. ^{13}C-NMR (400 MHz) of compound **2** (60 mM) with Au (III) 0.15 mM in CD$_3$CN (10,000 scans).

3.5. Optimization of sensing conditions of compound 2 towards Au (III)

The conditions in which fluorescent sensors operate usually affect their performance. Besides high selectivity, a short response time is strongly considered for a fluorescent sensor to monitor target metal in environmental samples in real time. In our study, the effect of water content in acetonitrile on selectivity and response time was first investigated.

It should be noted that, the fluorescence enhancement of **2** in a mixture of CH_3CN-H_2O (1/1, v/v) depends on the amount of the gold ion. Typically, when 10 eq. of Au (III) was used, the fluorescence intensity gradually increased and saturated within 2 h. For a faster measurement, higher concentration of Au (III) must be used. For example, the fluorescence intensity was enhanced within 15 min when 100 eq. of Au (III) was used. **Table 3.2** summarizes the data on the enhanced fluorescent intensity and the time required to reach a constant intensity (response time) under the various ratios of water and CH_3CN.

Table 3.2. Effect of water content on fluorescence intensity of compound **2** towards Au (III) ion

Entry	Volume ratio of water in CH_3CN	Without Au(III)	With Au(III)		F/F_0
		F_0 (a.u.)	F (a.u.)	T_F (min)	
1	0.1 : 0.9	86.2	417.9	>60	4.8
2	0.2 : 0.8	85.1	600.7	50	7.1
3	0.3 : 0.7	84.2	671.3	40	8.0
4	0.4 : 0.6	85.7	715.6	40	8.4
5	0.5 : 0.5	85.5	734.6	30	8.6
6	**0.6 : 0.4**	**82.2**	**751.0**	**15**	**9.1**
7	0.7 : 0.3	124.5	696.4	10	5.6
8	0.8 : 0.2	111.3	621.0	10	5.6

Measurement conditions: [**2**] = 5 µM and [Au(III)] = 0.5 mM in CH_3CN-PBS (pH 8.0, 10 mM) (λ_{ex} 369 nm, λ_{em} 456 nm)

When the data from **Table 3.2** was transformed into **Figure 3.13**, it is obvious that the fluorescence intensity of **2** in the presence of Au (III) were gradually increased when the fraction of aqueous in CH_3CN increase from 0.1 to 0.5, and reached a maximum point when the aqueous fraction was 0.6. In addition, the aqueous fraction of 0.6 could also offer the highest fluorescence enhancement (9.1 fold, blue triangle) in a practicable experimental time of 15 min (orange circle). When this aqueous fraction is raised to 0.7 and 0.8, the enhanced fluorescence intensities were obviously lower. The increase in water content was not only increase the polarity of solutions, it also promotes the hydrogen bonding between solvents and carbonyl groups of naphthalimide (which acts as H-bonding acceptor), which in turn stabilizes the geometry of the excited state and yields a weaker fluorescence signal. Therefore, this mixture of solvents at 0.6 aqueous fraction was selected for further optimization study.
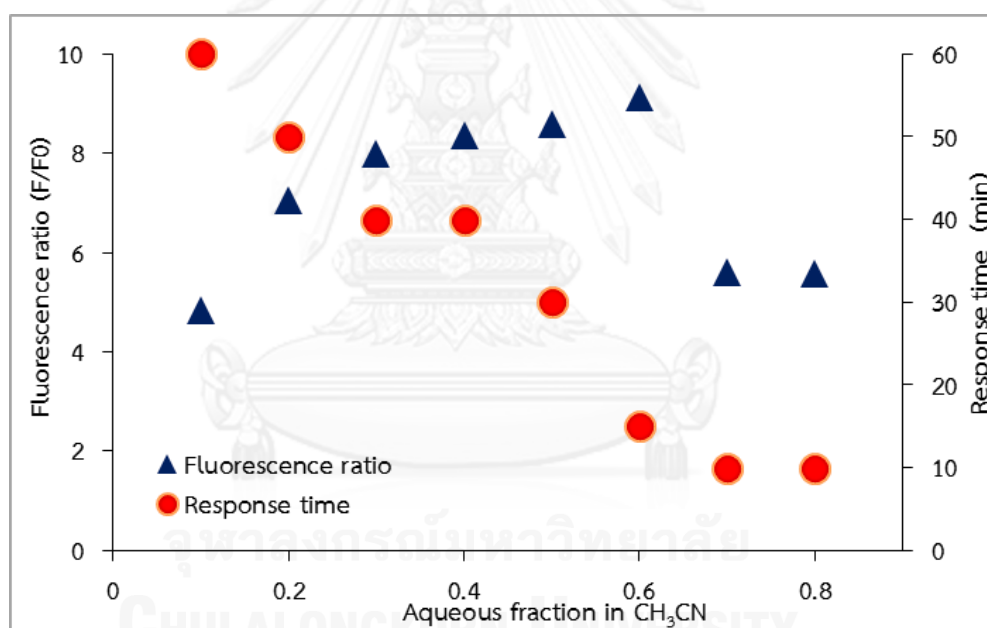
Figure 3.13. Fluorescence intensity change and saturate time of compound **2** (5 μM) with Au (III) (500 μM) in the mixture of PBS buffer—CH_3CN in various fraction (λ_{ex} = 367 nm).

Next, the effect of pH of the media on the enhanced fluorescent signal was examined (**Figure 3.14**). We carried out a series of experiments using solutions of **2** in 6/4 (v/v) mixture of aqueous buffer solution at various pH and CH_3CN (acetate buffer for pH 4 to 5, phosphate buffer for pH 6.0 to 8.0, and tris-HCl buffer for pH 9.0). Upon addition of Au (III), the fluorescent signals were enhanced to a saturated level within 15 minutes for every conditions, but the levels of enhancement depended on the pH of the buffer. Results indicated that the best sensitivity of the Au (III) detection by **2** could be achieved at pH 7 to 8.
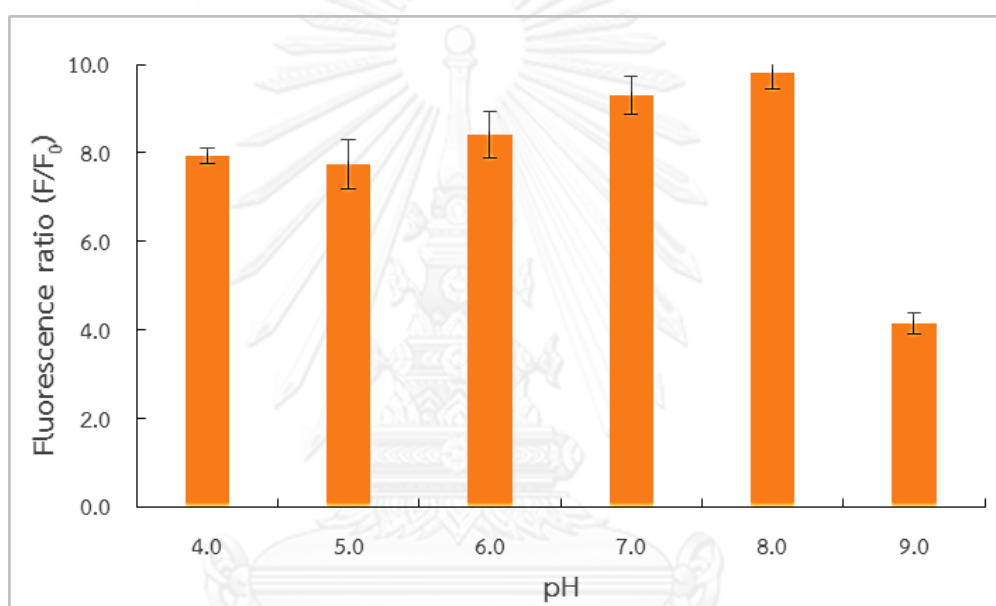
Figure 3.14. Fluorescent enhancement of **2** (5 µM) by Au (III) (50 mM) at various point of pH.

There are several examples on the use of surfactants to improve the sensitivity of aqueous-based chemosensors [64-67]. In this study, the emission enhancement behavior of **2** by Au (III) was investigated using a variety of surfactants at the concentration above their critical micelle concentration (CMC), for example anionic (SDBS), cationic (TTAB and CTAB) and non-ionic (Brij and Triton X-100). **Figure 3.15.** showed that the enhanced signals worsen when the cationic surfactants were used. However, both non-ionic and anionic-type surfactants could not improve the sensitivity since there was no significant difference fluorescence ratio compare to sample which is without any surfactant.
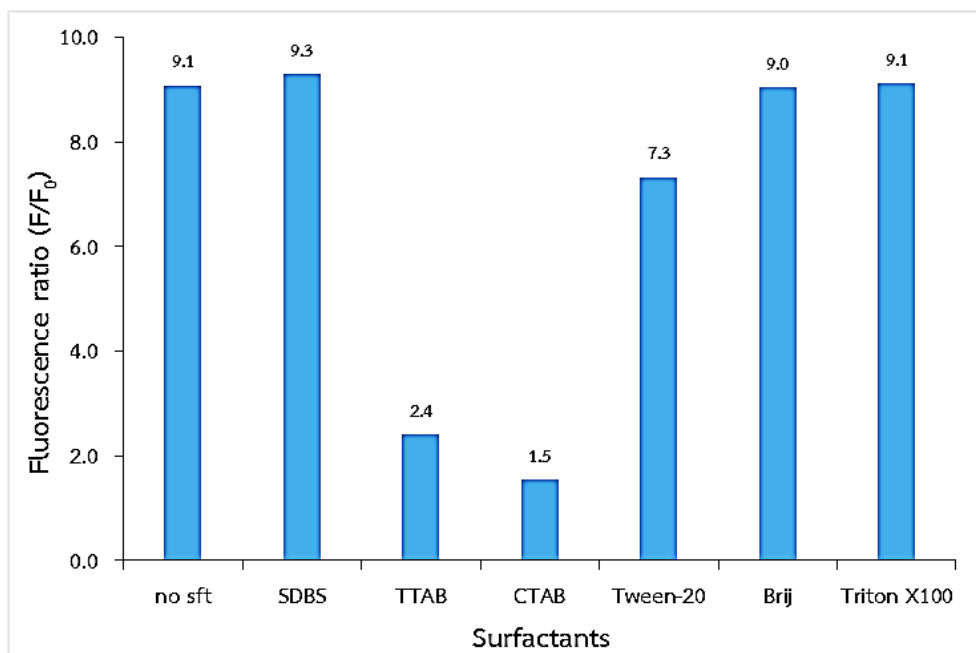
Figure 3.15 Relative fluorescence responses of **2** (5 µM) with Au (III) (0.5 mM) in the presence of various surfactants (near CMC point of each surfactant) in CH_3CN and 10 mM PBS buffer pH 8.0 (4/6, v/v). All fluorescent intensities were acquired 15 minutes after the addition of Au (III) and surfactants, respectively. (λ_{ex} = 367 nm).

3.6 Selectivity and sensitivity determination of compound 2 towards Au (III)

The interference of other metal ions in Au (III) detection was examined using solutions of **2** (5 µM), Au (III) (20 µM), and each of the interfering ion at 10-fold concentration of Au (III) (200 µM) (**Figure 3.16.**). With the exception of Fe (III), other interfering ions did not cause significant differences in fluorescent signal in the presence of Au (III). The interference by Fe (III) may result from the filtering effect as the ferric ion possesses a significant absorption in the absorption range of **2** [68]. In the case of Hg (II) ions can activate the alkynes, as well as Pd (II), especially terminal-alkynes [25, 69], so we designed the probe with non-terminal alkyne. Unfortunately, the effect from Hg (II) ion still remained.
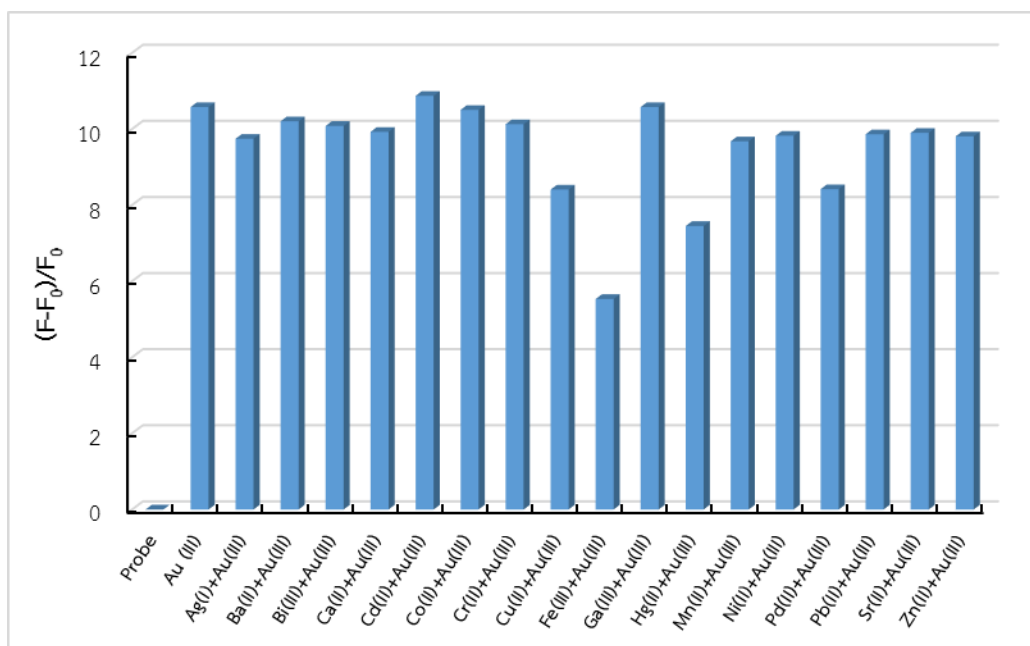
Figure 3.16. Relative fluorescence responses of **2** (5 µM) with Au (III) (20 µM) in the presence of various metal ions (200 µM) in CH_3CN and 10 mM PBS buffer pH 8.0 (4/6, v/v). All fluorescent intensities were acquired 15 minutes after the addition of Au (III) and interfering ions. (λ_{ex} = 367 nm).

In order to determine the detection limit, we treated compound **2** (1µM) with 0.00 to 3.00 µM of Au (III) in mixture of PBS buffer solution (10 mM, pH 8.0) in CH_3CN (6/4, %v/v) then its fluorescence spectra were taken after 1h at same measurement conditions. It should be noted that for a faster measurement, more than 1 µM can be used. For example, the fluorescence saturation was attained within 15 min when 5 µM of compound **2** were used. Results were showed in **Table 3.3**.

Then, we constructed a plot between fluorescent signal and concentration of Au (III) as shown in **Figure 3.17**. From the linearity obtained in the concentration range between 0.5 and 3.0 µM, the limit of detection of three-times-noise [61] was determined to be 4.0×10^{-4} µM or 80 ppb. Then, the calculations are shown.

Table 3.3. Fluorescence intensity of **2** (1 μM) when treated with 0.00 to 3.00 μM of Au (III) in mixture of PBS buffer solution (10 mM, pH 8.0) in CH₃CN (6/4, v/v).

[Analyte] (μM)	Average fluorescence intensity (a.u.)	SD
0.00	19.72	1.24
0.50	42.76	5.45
1.00	79.73	6.53
1.50	98.65	5.41
2.00	121.08	3.22
2.50	142.11	6.65
3.00	159.34	4.32

$$From\ S/N = 3;$$
$$y = 3SD + F_0$$
$$y = (3 \times 1.24) + 19.72$$
$$= 23.44$$

$$From\ the\ standard\ curve;$$
$$y = 47.28x + 23.421$$
$$23.44 = 47.28x + 23.421$$
$$\textbf{\textit{LOD}} = x = 4.0 \times 10^{-4} \mu M$$
$$= \frac{(0.0004\ \mu mol) \times 196.97 g/mol}{1\ L}$$
$$= 0.079\ ppm$$

In graph: y = 47.28x + 23.421, R² = 0.99163
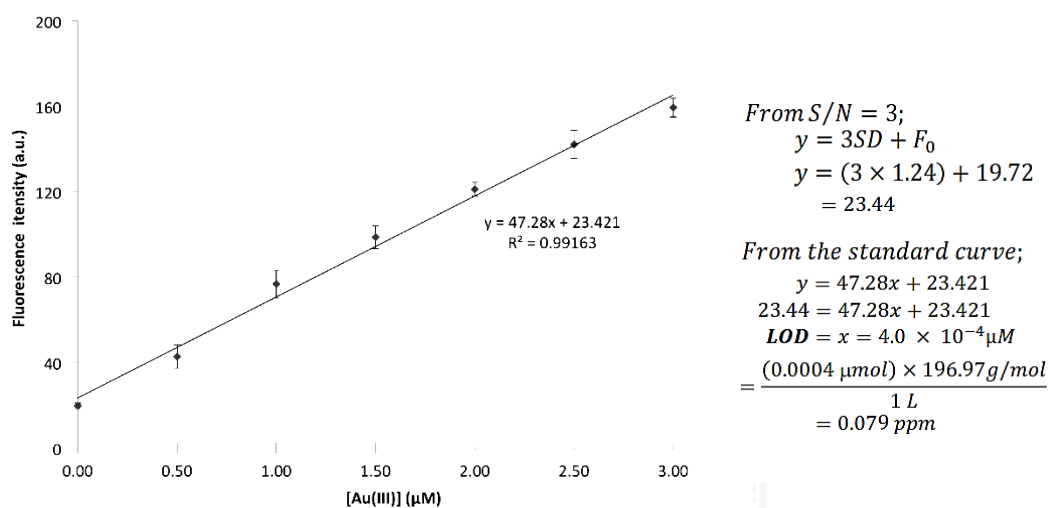
Figure 3.17. Fluorescence response of **2** (1μM) in CH₃CN-PBS buffer pH 8.0, 10 mM, (4/6, v/v) when treated with 0 – 3 μM of Au (III) (λ_{ex} = 367 nm; λ_{em} = 467 nm, measured after 15 minutes) and calculation of the limit of detection (LOD).

CHAPTER IV

CONCLUSIONS

In conclusions, two new *N*—aryl—1,8—naphthalimides containing an ethynylferrocene and triethylene glycol monomethyl ether chain were successfully synthesized from the commercially available 4—bromo—1,8—naphthalic anhydride with appropriated aniline derivatives. The installation of triethylene glycol chain was for improving their solubilities in aqueous solution. The incorporation of an ethynylferrocene relied on the Sonogashira coupling reaction. Both of these compounds were fully characterized by various spectroscopic techniques.

The investigation of their photophysical properties show the maximum absorption wavelengths around 360-370 nm. The larger stoke shift in **2** corresponds to the presence of an electron-donating and withdrawing groups in the structure causing an ICT process. While compound **1** is virtually non-emissive due to the MLCT or LMCT, compound **2** exhibits the maximum emission wavelength in the blue region which is generally characteristic electron transition in the naphthalimide derivatives with halogen or good electron-donating alkoxy group at C-4 position. However, as expected, its quantum efficiency is relatively low due to the PET effect from ethynyl ferrocene to naphthalimide part.

The selectivity investigation of these fluorophores indicated that compound **2** shows a selective fluorescent enhancement by Au (III) or Au (I) ions. This selectivity towards gold ions is attributed to the special alkynophilicity of the gold ions toward the triple bond as proven by ^{13}C-NMR experiments. The conditions in which fluorescent sensors operate were optimized. In this study, the aqueous fraction of 0.6 in acetonitrile with pH 7-8 could also offer the highest fluorescence enhancement (9.1 fold) in a practicable experimental time of 15 min. The selectivity towards Au (III) ion of **2** under these optimal conditions make it prospective candidates for chemical sensors or optical materials.

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Coordination and Solvent Polarity Effect in Photoinduced Charge Separation Process. Inorganic Chemistry 49(6) (2010): 2872-2880.

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APPENDIX
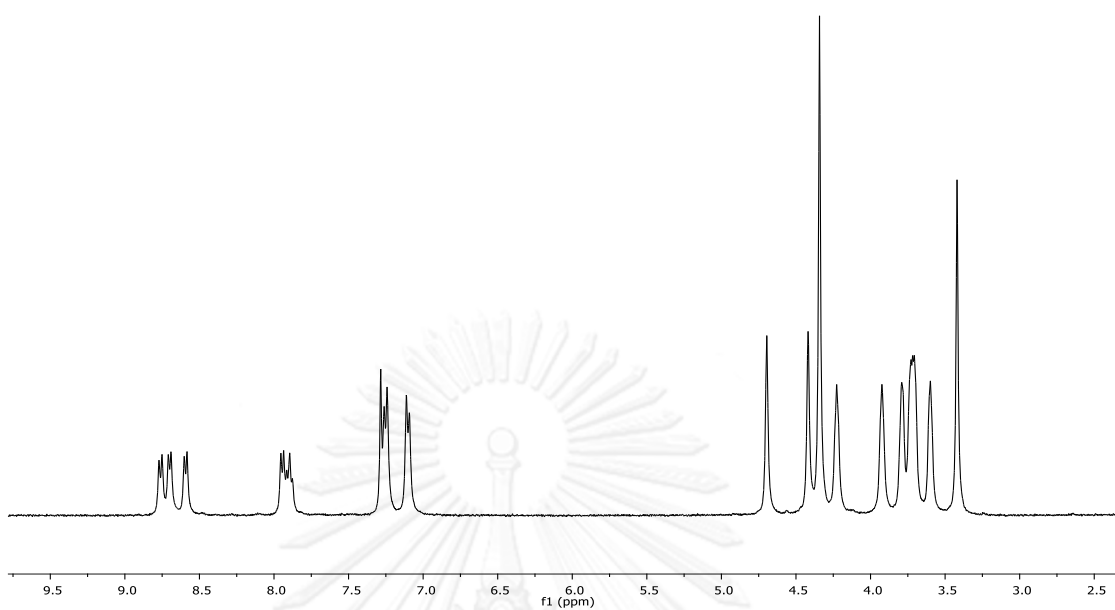
Figure A.1 ^{1}H-NMR (400 MHz) of compound **1** in CDCl$_3$.
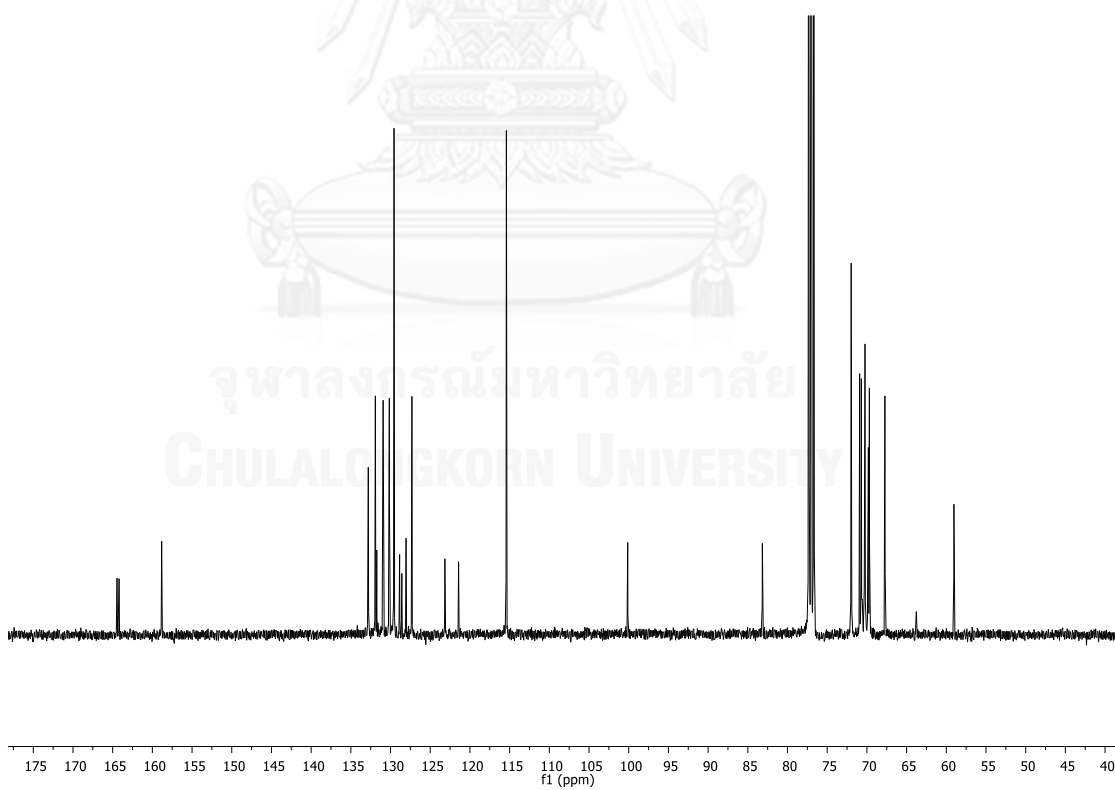
Fig.A.2 ^{13}C-NMR (400 MHz) of compound **1** in CDCl$_3$
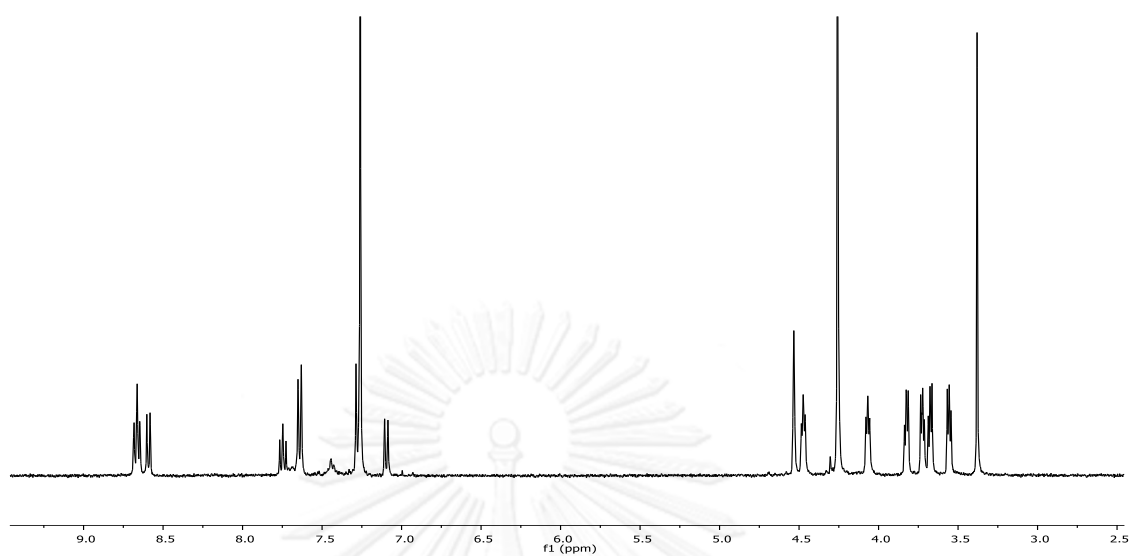
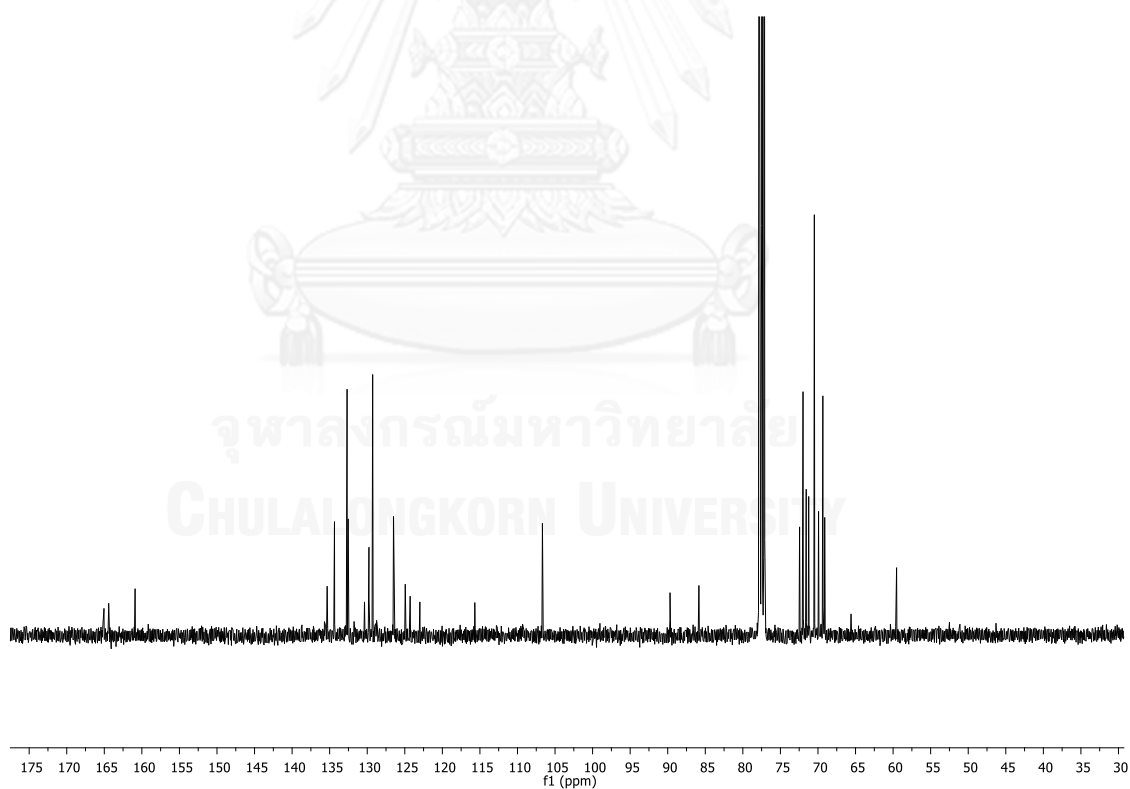**Figure A.3** ^{1}H-NMR (400 MHz) of compound **2** in CDCl$_3$.

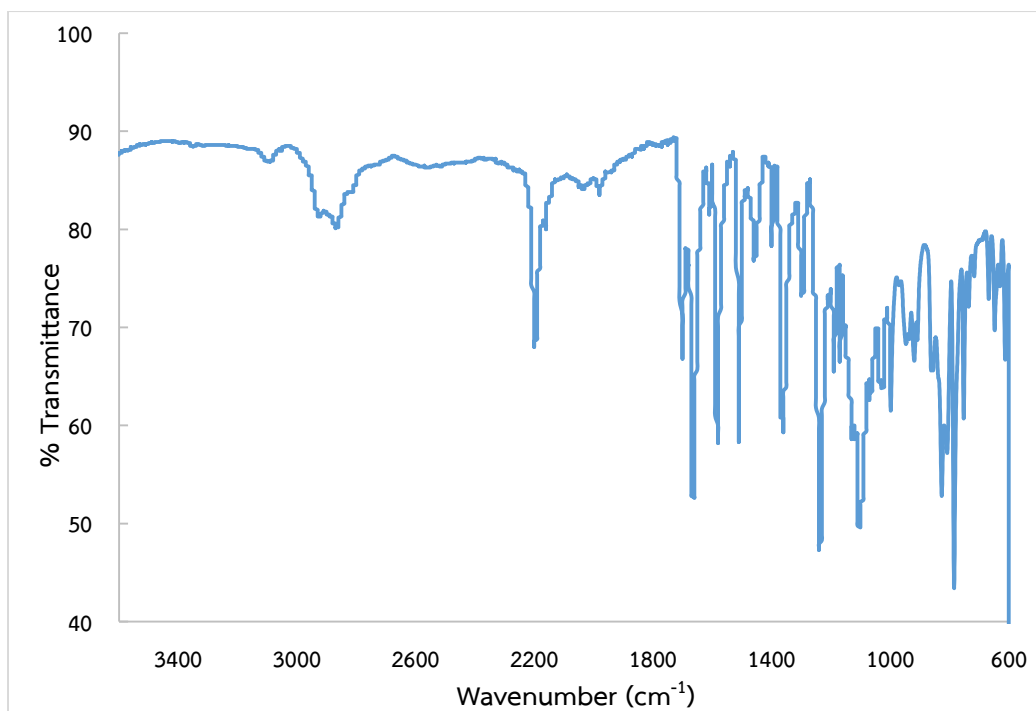**Figure A.4** ^{13}C-NMR (400 MHz) of compound **2** in CDCl$_3$.

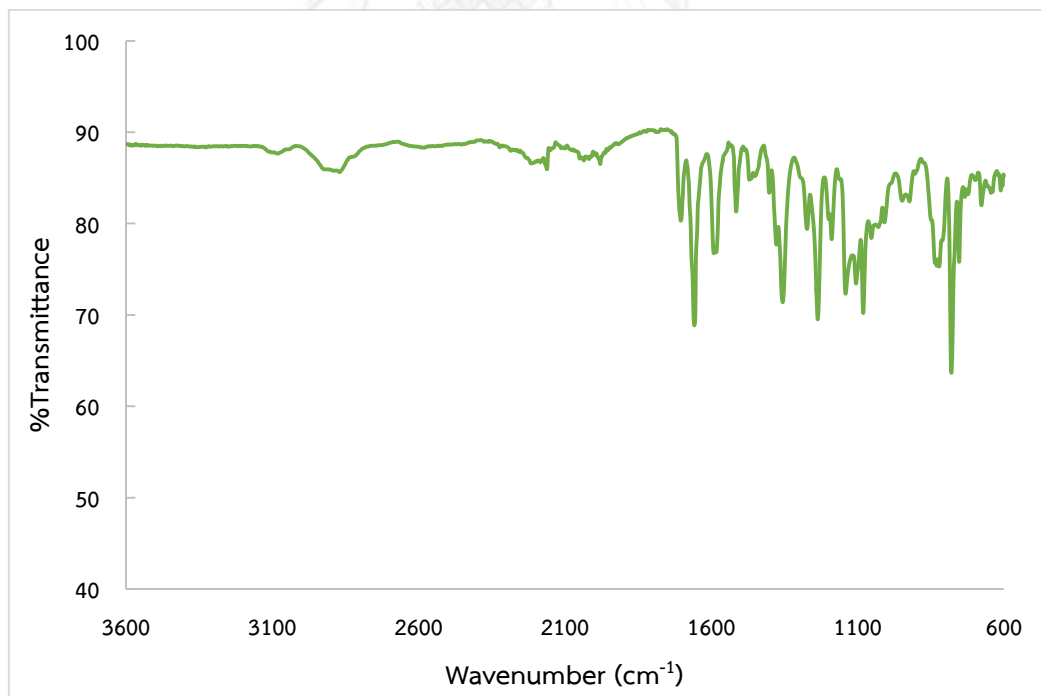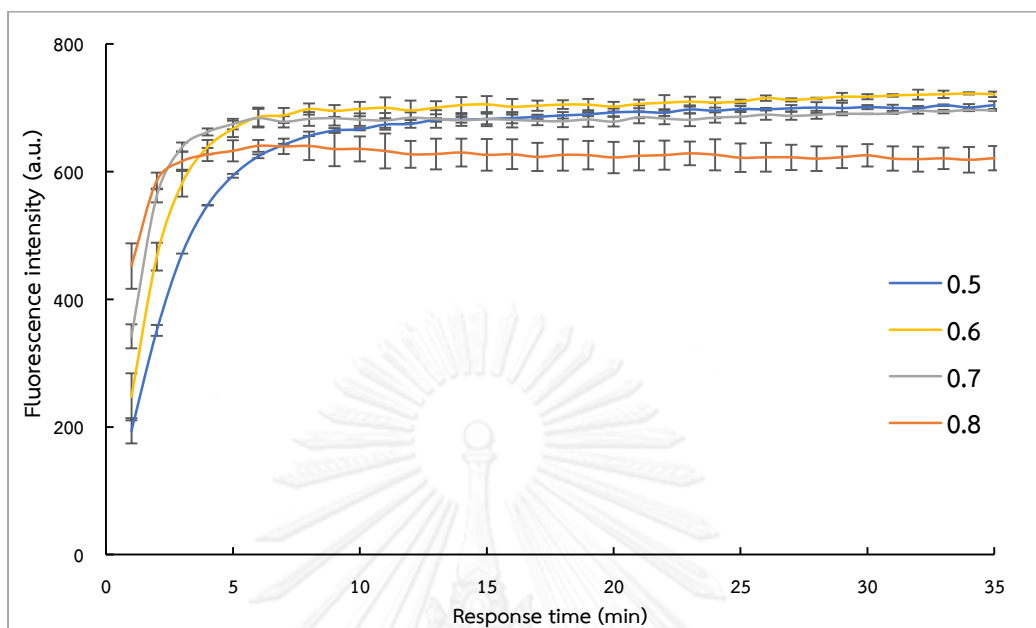**Figure A.5** Infrared spectrum of compound **1** (solid).

Figure A.6 Infrared spectrum of compound **2** (solid).

[2] = 5 μM; [Au(III)] = 0.5 mM in CH_3CN-PBS (pH 8.0, 10 mM) (λ_{ex} 369 nm, λ_{em} 456 nm)

Figure A.7 Pre-screening of effect of water content on fluorescence intensity of 2 towards Au(III) in various volume ration of aqueous 0.5 – 0.8.

The structures of compound **1** and **2** optimized using density functional theory (DFT) method at the B3LYP/LANL2DZ level of theory are shown in Figure S1. The B3LYP/LANL2DZ-computed HOMO energies of the compound **1** and **2** are -5.535 and -6.470 eV, respectively. All the computations were performed using GAUSSIAN 09 package program. (Frisch, M. J. et al; Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT, 2014.)
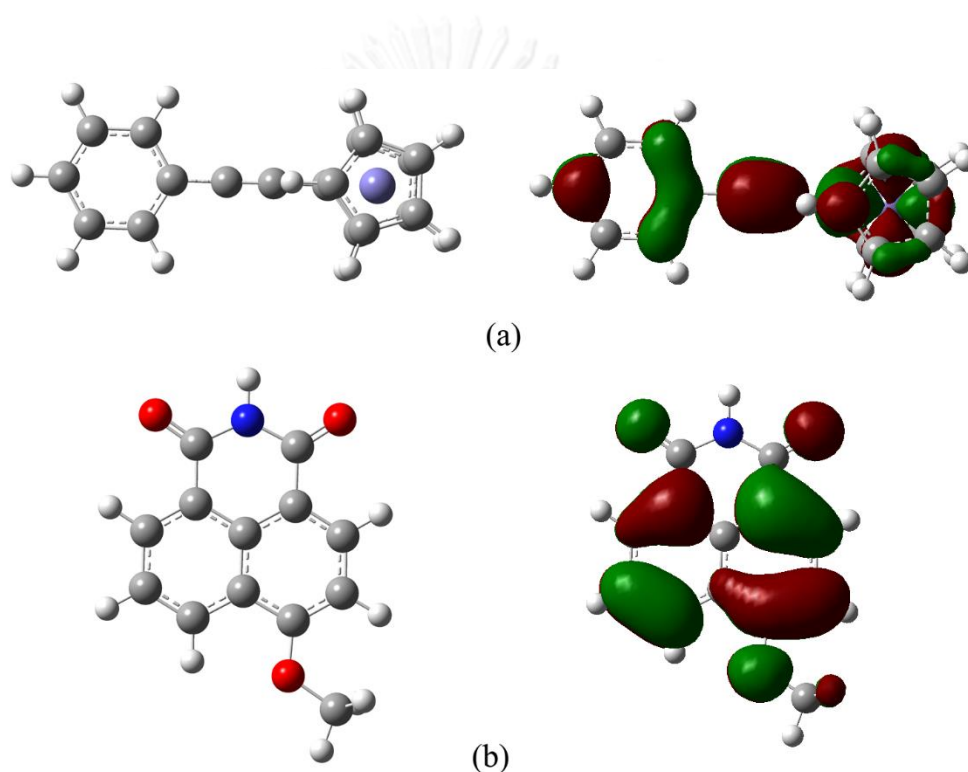

(a)

(b)

Figure A.8 The B3LYP/LANL2DZ-optimzed structures of compound (a) **1** and (b) **2** and their HOMOs illustrated on the right side were plotted at an isovalue of 0.02 e.

VITA

Pondchanok Chinapang received her B.Sc. degree from Chiang Mai University in March 2011 and continues her graduate studied at Department of Chemistry, Faculty of Science, Chulalongkorn University from June 20011, under supervision of Assoc. Prof. Paitoon Rashtasakhon. During her master course, she joined Masaoka's group at Institute for Molecular Science (IMS), Okazaki, Japan as exchange student for 5 months. Her recent research is focus on the design and synthesis of novel fluorescent materials for selective recognition of metal ions.