CHAPTER I
INTRODUCTION

1.1 Introduction of 1,4-dihydropyridines

The 1,4-dihydropyridine (DHP) is a heterocyclic molecule semi-saturated with two hydrogens replacing one double bond at the position of 1 and 4 of the pyridine ring, as shown in **Figure 1.1**.

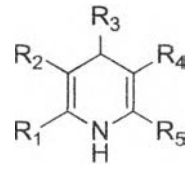


Figure 1.1 Basic structure of 1,4-dihydropyridine (DHP).

DHP was successfully carried out by Hantzsch reaction in 1882 [1]. Since then their derivatives have been used for a variety of applications [2-8]; e.g. modeling NADH in biochemistry [2] such as nifedipine, lacidipine, and diludine, (**Figure 1.2**) photosensitive polymers and pharmaceutical medicines [4-8]. Some of these bioactive DHPs are applied in the class of pharmacological agents known such as an antioxidants and calcium channel blockers (CCB) [4, 5]. For instance, the inhibition of calcium ion cell penetration by several DHP-CCBs was reported to weaken the contractility of the cardiac muscle [6]. These compounds were also proved to be effective vasodilators and useful in the treatment of hypertension, ischemic heart disease and other cardiovascular disorders [7, 8].

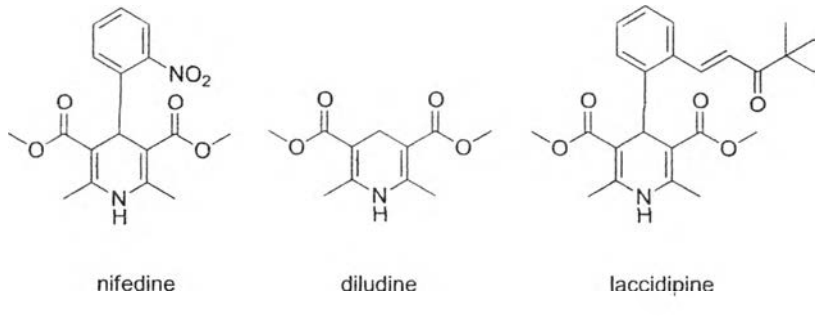
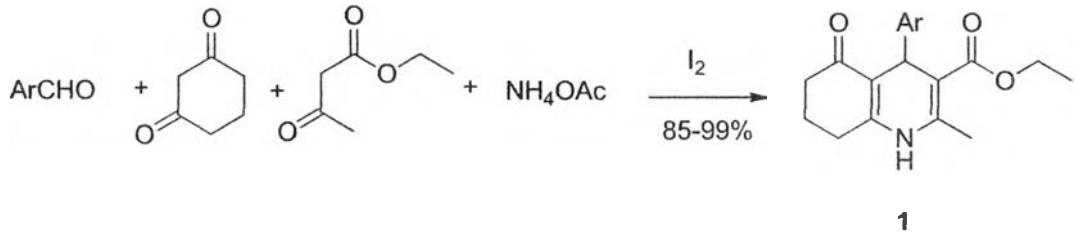


Figure 1.2 Bioactive DHP derivatives.

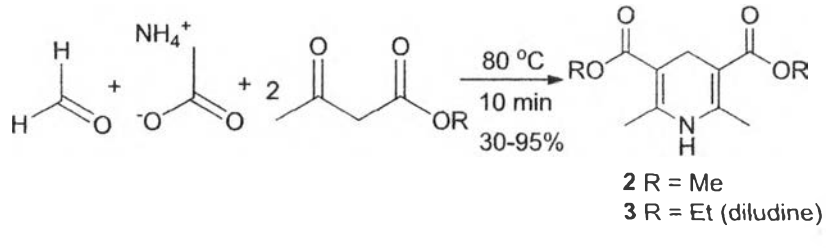
In 2005, Ko, S. and co-workers [9] reported the simple, inexpensive, and efficient one pot synthesis reaction of DHP derivatives **1** (**Figure 1.3**) using aryl aldehyde, 1,3-cyclohexanedione, ethyl acetoacetate and ammonium acetate in the presence of catalytic amount of iodine at room temperature. Asymmetric DHPs were gained with excellent product yield without any other possible symmetric DHP by-products.



1

Figure 1.3 The synthetic scheme of compound **1**.

In 2010, Cheung L. and co-workers [10] synthesized DHP **2** and **3** (diludine) (**Figure 1.4**) from methyl acetoacetate or ethyl acetoacetate, ammonium acetate, and formaldehyde at 80 °C for 10 min. This coupling reaction leading to symmetric DHP **2** and **3** in range of 30-95% yield was successfully performed in one pot. This work has overcome the difficult and uncontrollable use of formaldehyde.



2 R = Me
3 R = Et (diludine)

Figure 1.4 The synthetic scheme of compound **2** and **3**.

In 2011, Sueki and co-workers [11] used $Yb(OTf)_3$ as a suitable catalyst in one-pot reaction for 2,6-unsubstituted DHP derivatives **4** (**Figure 1.5**). Various Lewis acids were attempted for the condensation reaction of aniline, aldehyde and acetal protected β-ketoester. The favorable condition for synthesis was the catalytic use of 2.5 mol% $Yb(OTf)_3$ in 1,4-dioxane at 90 °C for 16 hours.

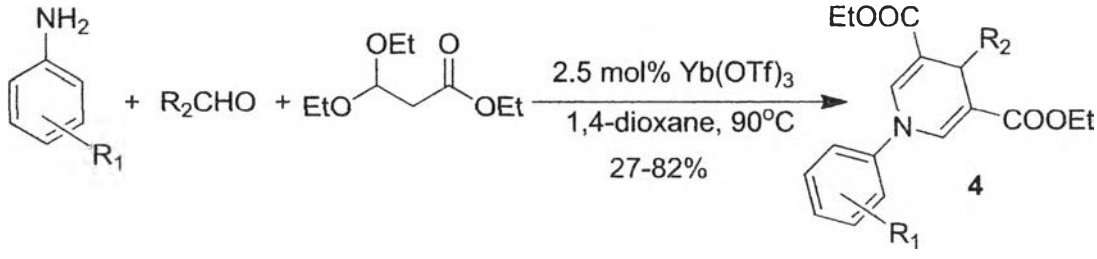


Figure 1.5 The synthetic scheme of compound **4.**

In 2010, Sirijindalert and co-workers [12] demonstrated that DHPs can be easily obtained from the cyclotrimerization of β-amino acrylates **5** under simple conditions by treatment with $TiCl_4$ in CH_2Cl_2 at room temperature (**Figure 1.6**). The cyclotrimerization from β-amino acrylates to *N*-substituted DHP **6** was achieved through Michael addition reaction in 2 steps followed by cyclization in high yields (70–83%). Our group also reported the mercury(II) detecting DHP fluorescence chemosensor by using this synthetic method [13].

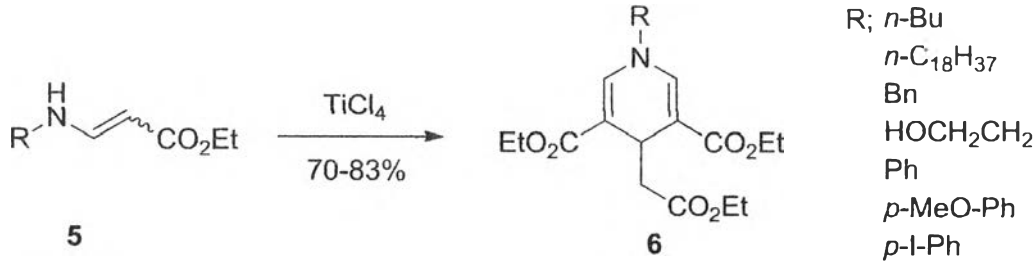


Figure 1.6 The synthetic scheme of DHP derivatives **6**.

Most of DHPs consisting of the π-conjugated commonly give the strong absorption with the maximum around 350 nm and maximum emission around 450 nm [13-15]. Due to their optical properties, the photophysical characteristics of DHP have been continuously reported.

1.2 Introduction of fluorometry

Fluorometry is also a class of techniques that assay the state of a biological chemical system by studying its interaction with fluorescence probe molecules. This interaction is monitored by measuring the changes of the optical properties fluorescence probe. The fluorescence characterizes the relationship between absorbed and emitted photons at specified wavelength. It is a precise quantitative and qualitative analytical technique that is not only highly sensitive and highly specific but with even greater advantages of rapid testing, inexpensive and easy to use. The development of highly selective and trustworthy methods for the quantification of environmentally toxic heavy metal of wastewater by using fluorescence spectroscopy was reported [16].

Fluorescence is a photon emission process that occurs when a molecule absorbs photons from the UV visible light, known as excitation, and then rapidly emits light photons when return to their ground state. The phenomenon is usually illustrated by the Jablonski diagram [17], which offers a convenient representation of the excited state structure and the relevant transitions, to illustrate possible various molecular processes. From Franck-Condon principle, most molecules absorb light more rapidly (10^{15} s) than molecular vibration (10^{8} s). A simplified Jablonski diagram shown in **Figure 1.7**, demonstrates that, this causes electrons to become excited to second electronic state (S_2), then the electrons lose the energy by internal conversion (vibration or rotation) evacuate to first excited state (S_1). After that, the fluorescence signal is observed when the electrons relax to singlet ground electronic state (S_0) via photon emission (radiative decay). The time required to complete the whole process takes only around nano-second.

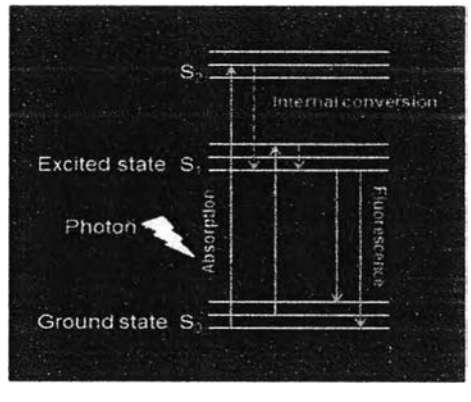


Figure 1.7 Jablonski diagram illustrating the fluorescence processes.

1.3 Fluorescence chemosensor

A fluorescence signal change normally resulted from the interaction between fluorophore (target compound) and analyte, which can be a metal ion, an anion or an organic molecule. Fluorescence chemosensor will then exhibit a new fluorescence behavior detectable by the fluorometry.

Fluorescence chemosensors are usually composed of three components: fluorophore, receptor, and spacer as linker between fluorophore and receptor in (**Figure 1.8**). When a fluorescence sensor specifically interacts with analyte, the read-out data is usually measured from the signal change such as fluorescence intensity, radiatve decay lifetime, and the maximum emission wavelength. The mechanism which controls the quenching response of a fluorophore to substrate binding includes photo-induced electron transfer (PET), intramolecular charge transfer (ICT), fluorescence (FÖrster) resonance energy transfer (FRET), and excimer/exciplex formation or extinction. An important feature of the fluorescence chemosensors is that signal transduction of the analytes leading to the readout can happen in a very short time (less than nanoseconds) and without any other assistances. This makes a real-time and real-space detection of the analyte possible as well as the imaging associated with analyte distribution [18].

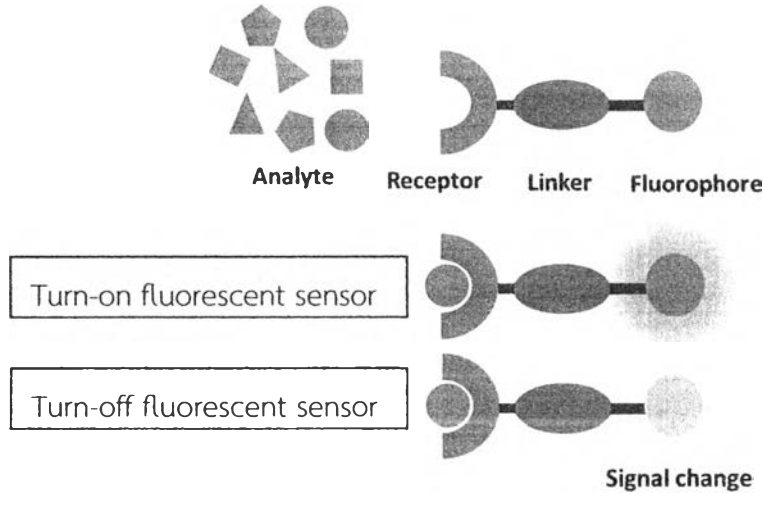


Figure 1.8 Schematic illustration of a fluorescence sensor device.

1.4 The operation of fluorescence chemosensor

1.4.1 Photo induced electron transfer (PET)

PET process is known as the fluorescence quenching mechanism that occurred though the electron transfers when the electrons in the ground state of fluorophore excited by light energy to higher energy level. The electron of fluorophore normally moves from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). However, some when there is a molecule as suitable receptor unit in the system and its HOMO is between LUMO and HOMO of fluorophore. The electron in the HOMO of receptor can move to the HOMO of fluorophore. As a result, the electron from the LUMO will move to HOMO of receptor instead causing the fluorescence diminution. (**Figure 1.9**)

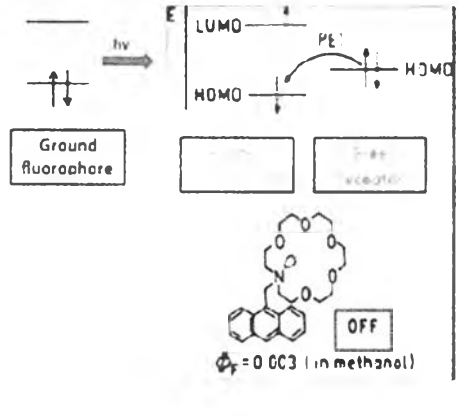


Figure 1.9 Photoinduced electron transfer (PET). [7]

1.4.2 Intramolecular charge transfer (ICT)

In the case of molecule consisting of both donor site and receptor site within itself, electron transfer can be occurred intramolecularly inhibiting the fluorescence, this phenomenon is "Intramolecular charge transfer" (ICT). The structural molecular change from locally exited state (LE) to lower energy (ICT state) can occasionally occur before the electron returns to its ground state. As a result, the fluorescence intensity of molecules is reduced in either visible light or non-visible light territory in **Figure 1.10**. Especially, if molecule can be dissolved in polar solvent, the extinction coefficient become lower [19], because the ICT excited state is more stable than that of the locally exited state.

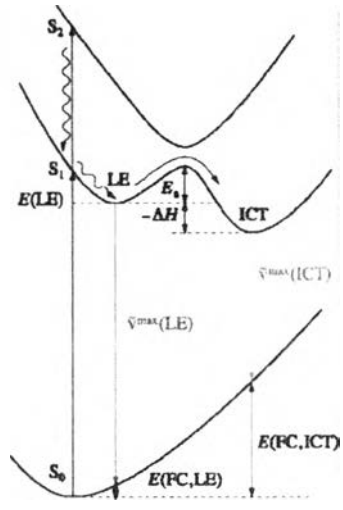


Figure 1.10 Intramolecular charge transfer (ICT) process. [20]

1.4.3 Fluorescence resonance energy transfer (FRET)

Fluorescence (or Förster) Resonance Energy Transfer (FRET) has been widely used in all applications of fluorescence, including medical diagnostic, optical imaging and DNA analysis. FRET occurs between a donor molecule in the excited state and acceptor molecule in the ground state. If the emission spectrum of donor molecule overlaps with the absorption spectrum of the acceptor, the energy transfer will occur as the result of long-range dipole-dipole interaction between the donor and acceptor. The emission signal of the acceptor (solid yellow arrow, **Figure 1.11**) occurs through the excitation of the donor molecule (solid purple arrow), while the emission of the donor molecule is reduced (dashed blue arrow). The donor fluorescence (solid blue arrow) is diminished during the transition to a lower quantum state. The efficiency of the transferred energy depends on the molecular distance between 1-10 nm, the extent of spectral overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, the quantum yield of the donor, the relative orientation of the donor and acceptor transition dipoles, and the distance between the donor and acceptor molecules [21].

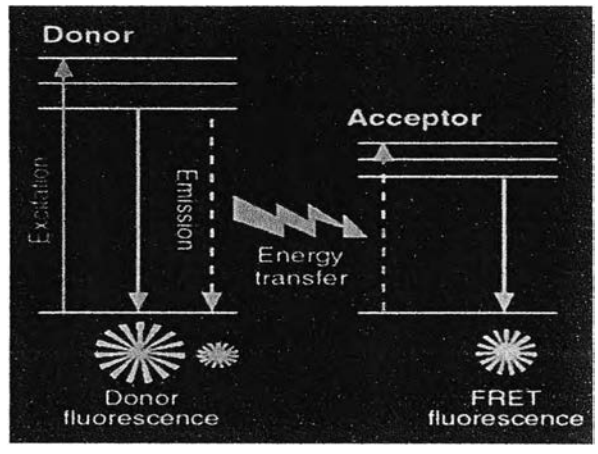


Figure 1.11 Jablonski diagram showing the energy transfer between the fluorescence donor and acceptor concerned with FRET [21].

1.5 Allosteric

Allosteric molecule is the molecule with the shape that specifically reacts with substrate after the structural modulation under treatment condition. Most of the concerned knowledge was used in biochemistry and chemistry field. Allosteric site within the molecule which the small ion or small molecule (effector or modulator) can be bound to. The binding leads to structural change of allosteric molecule resulting in the specific reaction.

In the field of chemistry, allosteric is used to describe the molecule with the ability for selective binding of detection to target analyte in different states. The allosteric system can be generally classified into 2 types; heterotropic and homotropic.

The simplest mode of allosteric action takes place in the form of heterotropic allosterism, in which the binding of first chemical species (guest 1) consequently cause a negative or positive effect with binding of second chemical species (guest 2). From the recent report, the heterotropic was studied in calix [4] arene that can detect γ-lactam once Na^+ added as an effector **Figure 1.12**.

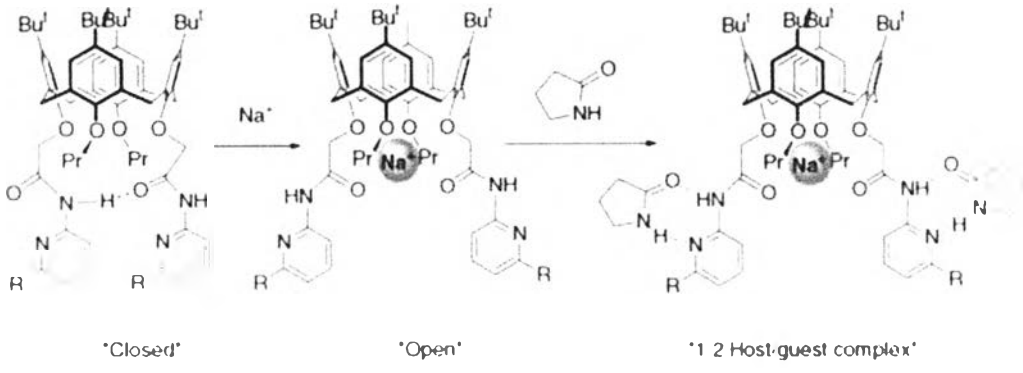


Figure 1.12 Heterotropic allosteric of binding between Na^+ and calix [4] arene. [22]

Homotropic allosterism or artificial system has the concept of allosteric binding to the guest molecule for producing new binding modes for the same guest molecule. It is literally reported to have difficultly for the efficient regulation of equilibria and catalyses. In details, the initial binding of the host with a guest species must have a different effect from that of the subsequent interactions between host and the same guest molecules. (**Figure 1.13**)

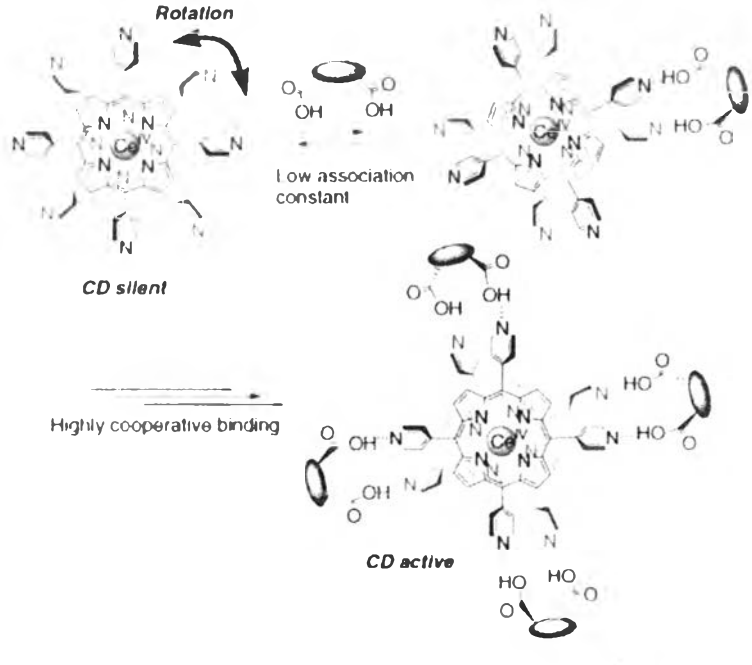


Figure 1.13 Heterotropic allosteric of binding between Na^+ and porphyrin. [22]

1.6 Applications of fluorescence chemosensors

Small molecule fluorescence chemosensors have become an important sensors in a wide variety of applications, including small ions [23] or biomolecules i.e. proteins and nucleic acids [24]. Responsive small-molecule chemosensors that immediately provide optical feedback can overcome limitations because their use does not require sophisticated instrumentation or sample preparation. The most important consideration influences the design of this small molecule ligands for sensing metal ion. The probe should be highly selective for the metal over all other components in the environmental or biological sample. Some examples reported herein show the focus on the design and synthesis of such small-molecule-based metal ion sensors including their successful detections.

1.6.1 Fluorescence chemosensors containing azacrown ether unit

In 2008, Zhang and co-workers [25] designed a rhodamine derivative containing an azacrown ether that gives low fluorescence at 575 nm in acetonitrile. Addition of metal ion leads to spirocycle opening via coordination or chemical reaction. This sensor can detect either Fe^{3+} (give green color) or Hg^{2+} (give orange color) (**Figure 1.14**). The fact that, containing azacrown ether unit of compound **7** inducing the metal ion coordination has evidently seen from the broadening and downfield shifting of the spectra of compound **7**.

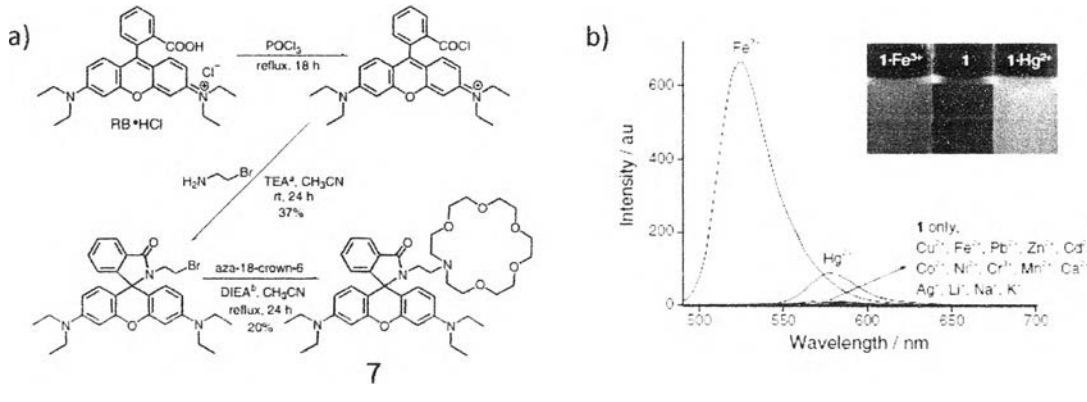


Figure 1.14 a) Synthesis of rodamine-azacrown ether **7** b) Fluorescence enhancement of **7** with Fe^{3+} and Hg^{2+}.

In 2010, Móczár and co-workers [26] synthesized acridinone derivative containing various sizes of azacrown ether (**Figure 1.15**) from chloromethyl-substituted acridinone substrate. Substituted mono azacrown ether as receptor unit contributed for the coordination with the metal ion that also affects to inhibit PET process. Thus, fluorescence chemosensor with capability of enhancing intensity and strong fluorescence could selectively detect Cu^{2+} in MeOH.

a) b)

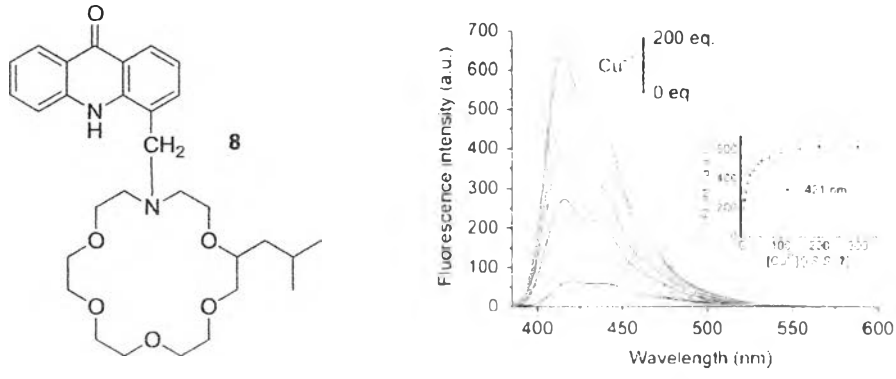


Figure 1.15 a) Chromofluorophore compound **8**. b) Fluorescence emission series of spectra of compound **8** on incremental addition of Cu^{2+}.

In 2011, Kondo and colleagues [27] successfully synthesized 9,9′-dimethyl-2,2′-bianthracene as new chromofluorophore by reductive coupling of 2-chloro-9-methylanthracene. The conformational change trigged by single bond rotation between two anthracene units results in the intramolecular sandwich complexation between two azacrown ether and Ba^{2+} (**Figure 1.16**). This inhibits the PET process and also causes enhancement observed by naked-eyes under blank light.

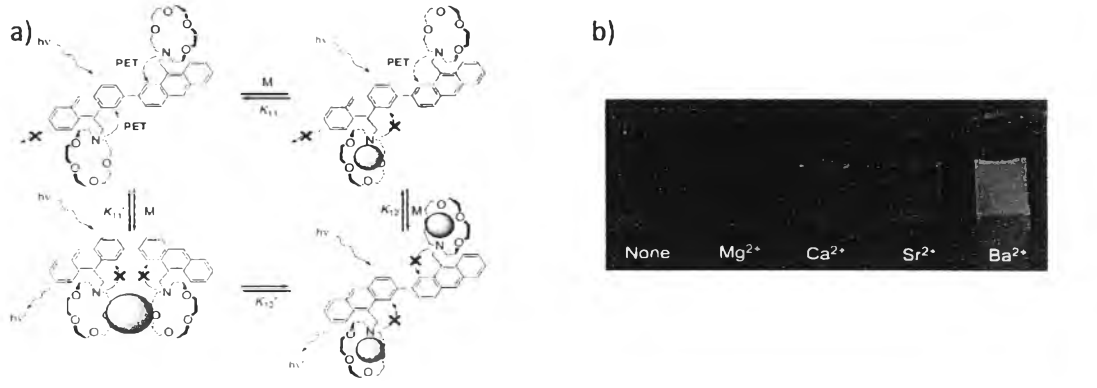


Figure 1.16 a) Intramolecular sandwich complex of azacrown ether. b) Fluorescence enhancement of bis(aza-15-crown-5)ether-2,2'-biantracene.

1.6.2 Au^{3+} sensors

In 2009, Jou and co-workers [28] reported a rhodamine-alkene derivative **9** as the first fluorescence and colorimetric chemodosimeter for Au^{3+}. Probe **9** displayed a selective turn-on fluorescence mode and colorimetric change from colorless to pink (**Figure 1.17**) with Au^{3+} in EtOH and HEPES buffer (0.01 M, pH 7.4) (1:1, v/v). The fluorescent oxazolecarbaldehyde **10** was obtained by spirocyclic ring opening followed by the cyclization of propargylamide. Finally, probe **9** was successfully applied to the Hela cell.

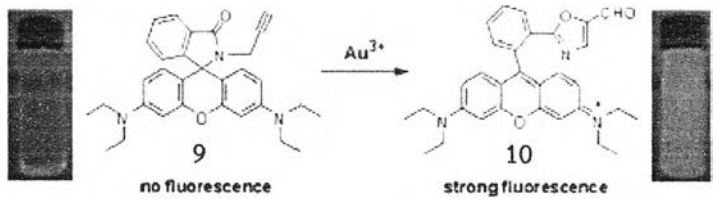


Figure 1.17 Highly selective fluorescence probe **9** for Au^{3+} based on spirocyclic ring opening followed the cyclization of propargylamide.

In 2010, Dong and co-workers [29] reported propagyl 1,8-naphthalimide **11** for the ratiometric fluorescence sensing for Hg^{2+} and Au^{3+}. Propagyl compound **11** was proved to have a selective fluorescence enhancement and colorimetric change (from green to blue) with Au^{3+} in MeOH-H_2O (95:5 v/v, pH = 9.0) (**Figure 1.18**). After increasing the concentration (33 mM) of Hg^{2+} and Au^{3+}, probe **11** was turned into methyl lactone **12** through Kucherov reaction mechanism.

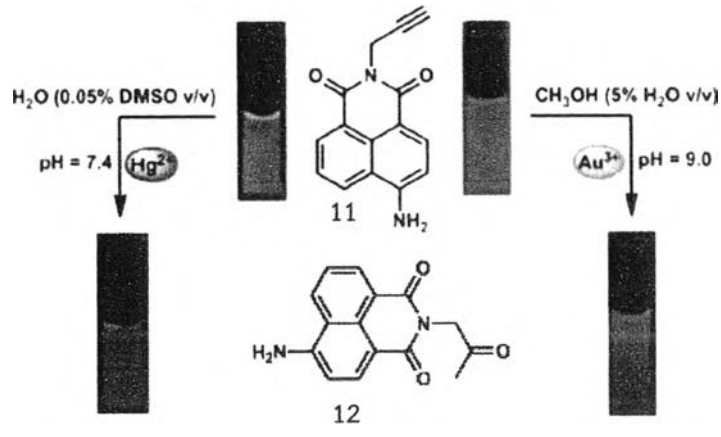


Figure 1.18 Color and structural change of compound **11** for Hg^{2+} and Au^{3+}.

In 2013, Choi and colleages [30] demonstrated that 4-propargylamino-1,8-naphthalimide based fluorescence probe **13** selectively detect Au^{3+}. Propargylamine moiety attached on the probe **13** reacts with Au^{3+} associated with a color change from green to blue under fluorescence light with distinctly different electronic properties. They also applied to treat probe **13** and Au^{3+} in Hela cells and adipocytes. The results exhibit that cells incubated by both probe **13** and Au^{3+} show blue fluorescence at 405 nm and also are observed in attenuated green fluorescence at 488 nm. Moreover, both types of cell can be separately incubated with **13** and Au^{3+} resulting in the fluorescence intensity change.

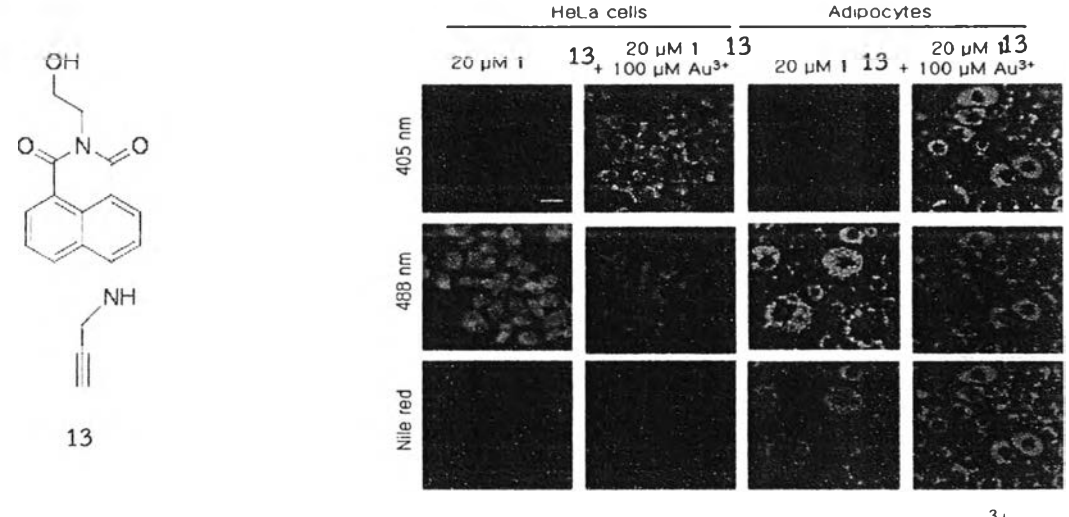


Figure 1.19 Fluorescence images of HeLa cells and adipocytes treated with Au^{3+} and probe **13**.

1.6.3 DHP sensors

In 2013, Homraruen and teams [13] reported a new fluorescence 1,4-dihydropyridine (DHP) tricaboxylic acid **14** (**Figure 1.20**), which can be used as a selective chemodosimeter for Hg^{2+} in aqueous solution. The decrease of fluorescence signal was proportional to Hg^{2+} concentration with high quenching efficiency (K_{sv}=78,300) providing a detection limit of 0.2 μM. The quenching process involves an oxidation of the DHP into a pyridinium ring **15** specifically induced by Hg^{2+} that brought about its remarkable selectivity over other metal ions.

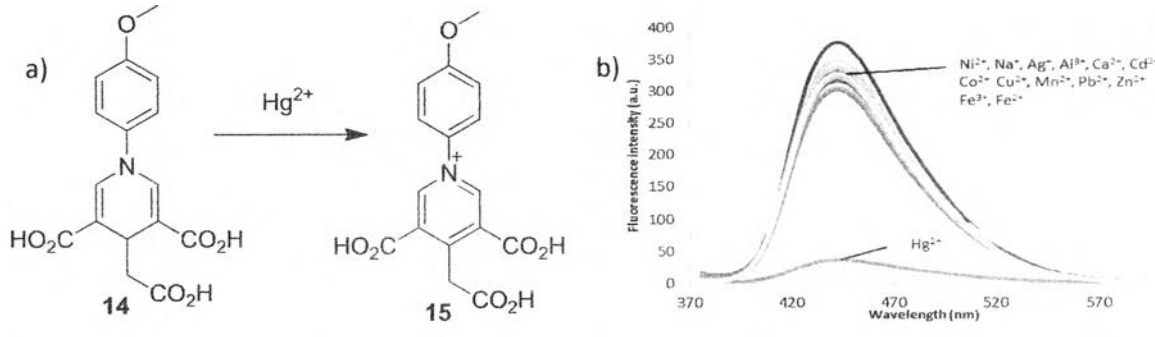


Figure 1.20 a) Structure of DHP fluorescence sensor **14** b) its selectivity toward Hg^{2+} ion.

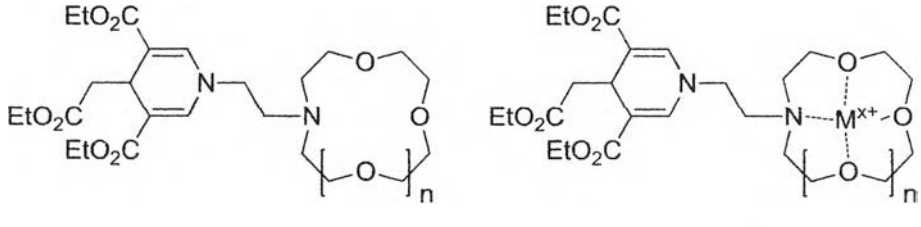
1.7 Statement of problem

According to most literature reviews related to the fluorescence chemosensor, enormous numbers of fluorescent organic molecule were reported but not many of them can be used in aqueous media without mixed organic solvents. On top of that, only few of them that possess allosteric property were presented. The hetero allosteric chemosensors containing crown ether are particular one of the most favorite systems in order to detect the effector such as alkaline or alkaline earth in the system. These modulated complexes between allosteric chemosensors and effector have more rigidity within the molecule with different molecular energy level and can be used to selectively or specifically detect other chemical species such as heavy metal ion or nitro aromatic compound. From reported reviews, many sensing molecules in heteroallosteric system are often large and complicated which make their syntheses difficult.

Considering all mentioned reasons, this project is focused on fluorescence chemosensors that consist of DHP derivative as fluorophore linked with various sizes of azacrown ether. The DHP can be easily obtained from the cyclotrimerization of β-amino acrylates by treatment of $TiCl_4$ at room temperature (mild condition) in 70-83 %yield. Based on its fluorescence property, DHP derivative can be applied to be fluorescence chemosensor. The hypothesis of this work is that hetero allosteric system with appropriate size of metal ion e.g. Li^+, Na^+, and K^+ cooperated into the corresponding azacrown ether may be utilized to selectively detect another chemical species as different system to its sole system without alkaline or alkaline earth metal ion. Moreover, azacrown ether is not only used as allosteric site but also favorable to improve the water solubility of the target DHP in aqueous media due to its high polarity, since the structure of triester DHP is difficult to dissolve in aqueous media.

1.8 Objectives of this research

In this study, fluorescence sensors include DHP moiety as fluorophore and azacrown ether as water soluble and tuning hetero allosteric moiety. The synthetic preparations of series of DHP linked with various sizes of azacrown ether as fluorescence chemosensors will be achieved (**Figure 1.21**). The photophysical properties of Et-DHP-AC(1-3) and also allosteric test of Et-DHP-AC(1-3)•M^{x+} will be investigated in aqueous solution (milliQ water) and in mixed solvent between acetonitrile or THF and milliQ water. The applications of series of Et-DHP-AC(1-3)•M^{x+} focus on allosteric effect with the hope that might alter the selectivity towards the specific chemical species. Moreover, the applications of Et-DHP-AC(1-3) for Au^{3+} sensors will be also investigated in aqueous media.



Et-DHP-AC(1); n=1
Et-DHP-AC(2); n=2
Et-DHP-AC(3); n=3

Et-DHP-AC(1)•M^{x+}; n=1, Li^+
Et-DHP-AC(2)•M^{x+}; n=2, Na^+
Et-DHP-AC(3)•M^{x+}; n=3, K^+

Figure 1.21 Target molecules 1 and 2.