การสังเคราะห์และสมบัติการรับรู้เชิงแสงของคาลิกซ์[4]เอรีน ที่มีฟลูออโรฟอร์เป็นองค์ประกอบ

นาง นงนิตย์ มรกต

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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SYNTHESIS AND OPTICAL RECOGNITION PROPERTIES OF CALIX[4]ARENE CONTAINING FLUOROPHORES

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry

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ได้สังเคราะห์ฟลูออโรไอโอโนฟอร์ **17** และ **18** ชนิดใหม่จากการทำปฏิกิริยาของพารา-เทอร์เชอรี-บิวทิวคาลิกซ์[4]เอรีนไทรเอสเทอร์โมโนเอซิดคลอไรด์ ด้วย 2-แอมิโน-4-(1,3-เบนโซไทอาโซล-2-อิล)ฟีนอล และ 4-แอมิโนควินาลดีน ตามลำดับ ควอนตัมยิลด์ของสารประกอบ **17** และ **18** เท่ากับ 0.67 และ 0.01 ตามลำดับ จากนั้นศึกษาสมบัติการเกิดสารประกอบเชิงซ้อนของสารประกอบ **17** และ **18** กับไอออนของโลหะแอลคาไล เช่น ไอออนลิเทียม, ไอออนโซเดียม และไอออนโพแทสเซียม โดยการไทเทรตด้วยเทคนิคฟลูออริเมตริกพบว่า สารประกอบ **18** ให้ความเข้มของการเรืองแสงต่ำมาก ในขณะที่สารประกอบ **17** ให้ความเข้มของการเรืองแสงสูง ดังนั้นเฉพาะสารประกอบ **17** เท่านั้นที่นำมาตรวจสอบการรับรู้เชิงแสงพบว่าสารประกอบ **17** มีความจำเพาะต่อไอออนโซเดียม ในขณะที่ไอออนลิเทียมและไอออนโพแทสเซียมไม่แสดงการเปลี่ยนแปลงอย่างชัดเจนภายใต้ภาวะการทดลองเดียวกัน นอกจากนี้พบว่าความเข้มของการเรืองแสงต่อไอออนโซเดียมลดลงแบบเส้นตรงเมื่อเพิ่มความเข้มข้นของไอออนโซเดียม และสามารถหาค่าคงที่ของการเกิดสารประกอบเชิงซ้อนกับสารประกอบ **17** ได้ในเทอมลอการิทึม เท่ากับ 2.91

ภาควิชา……..เคมี……..	ลายมือชื่อนิสิต…….
สาขาวิชา……เคมี……..	ลายมือชื่ออาจารย์ที่ปรึกษา…….……
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New fluoroionophores **17** and **18** derived from *p-tert*-butylcalix[4]arene triester monoacid chloride with 2-amino-4-(1,3-benzothiazol-2yl)phenol and 4-aminoquinaldine respectively, were synthesized. The quantum yield of compounds **17** and **18** were 0.67 and 0.01 respectively. Complexation studies of compounds **17** and **18** with alkali metal ions such as Li^+, Na^+ and K^+ were carried out by fluorimetric titration. The fluorescence intensity of compound **18** was very low. Only compound **17** was subjected to the recognition investigation. Compound **17** was selective to Na^+, while Li^+ and K^+ showed no significant change under the same experimental condition. In methanol, the fluorescence intensity of compound **17** was quenched by Na^+ and decreased linearly with increasing Na^+ concentration with the stability constant of log K = 2.91.

Department……Chemistry…… student's signature……..
Field of study…Chemistry……. Advisor's signature…….
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LIST OF ABBREVIATIONS AND SYMBOLS

^{o}C	Degree Celsius
A	Absorbance
Equiv.	Equivalent
g	Gram
mmol	Millimol
mL	Milliliter
mp	Melting point
M	Molar
M^{-1}	Per molar
F	Formal
Hz	Hertz
δ	Chemical shift
J	Coupling constant
ppm	Part per million
^{1}H-NMR	Proton nuclear magnetic resonance
s, d, t, m	Splitting patterns of ^{1}H-NMR (singlet, doublet, triplet, multiplet)
THF	Tetrahydrofuran
DMF	N, N-dimethylformamide
Ar	Aryl group

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CHAPTER I

INTRODUCTION

1.1 Alkali metals in living bodies

The internal environment of humans is maintained, in health, in a state of homeostasis in which the chemical and physical properties of body fluid exist in a state of relative constancy. The homeostatic balance is maintained by tightly controlled regulatory mechanism that prevent large fluctuations of body water and specific chemical constituents of cells, the electrolytes. A change in either the water or electrolytes composition reflects or results in a change in the other constituents. The major cations of the body are Na^+, K^+, Ca^{2+} and Mg^{2+}.

Sodium is the predominant cation of the extracellular fluid (ECF). Its concentration ranges from 136 to 145 mM. Its major function is maintaining the normal water distribution and osmotic pressure of the plasma. Other functions include its role in the maintenance of acid-base balance and the excitation of nerve and muscle. [1] Potassium is the major intracellular metal ion of the body. Intracellular K^+ concentration is 150 mM compared to a plasma K^+ concentration of 3.5 to 5.0 mM. Potassium has two major physiological functions. It has an important role in cell metabolism and neuromuscular excitation. [1] Lithium occurs naturally in blood at very low levels. Lithium ion in blood must be monitored closely when administrating lithium salts for treatment of manic depressive psychosis (bipolar personality). It is therefore important to maintain the blood concentration in the 0.5-1.5 mM range. Lithium is ineffective for treatment if it is too low. [2]

1.2 Chemical sensors (Chemosensors)

The accurate measurement of physiological metal ions, such as sodium and potassium, is essential in clinical diagnosis. The reference method for measuring Na^+ concentrations is flame atomic emission spectroscopy, ion-exchange sodium separation [3] and inductively coupled plasma spectrometry. [4] The reference

method for measuring K^+ concentrations is flame atomic emission spectroscopy, ion-exchange with measurement by photometry, neutron activation analysis and ion-selective electrode. [5] However, most clinical analysis involves use of flame emission spectrophotometry (FES), ion-selective electrodes (ISEs). Besides, there is increasing demand for portable systems utilizing small disposable sensors capable of whole-blood measurements. [6-9] Consequently, the development of practical and inexpensive optical sensors and systems for the clinical determination of these analytes in biological fluids remains important area of research. Therefore, the need for real-time measurement can take advantage of the development of optical fibers. This technology has extended the possibility of performing remote real-time measurements with optical sensors by monitoring changes in a absorption or luminescence spectra of chromogenic or fluorescence compounds immobilized on a polymer matrix on the tip of a fiber. It is clear indeed that light is a very versatile tool and micro sized optical fibers can allow analysis at almost any location.

A chemosensor can be defined as a molecule able to bind selectively and reversibly the analyte of interest with a concomitant change in the property of the system, such as a redox potential and absorption or luminescence spectra. To obtain the detection of the target analyte, two different procures are needed: molecular recognition and signal transduction. This means that chemosensors have to be built by components able to perform these functions. It usually consists of two molecular units: a receptor or ionophore (able to selectively bind the analyte) and an active unit or a signaling unit such as a chromophore or a fluorophore (that changes one or more of its properties upon analyte complexation) (Figure 1.1). [10-11]

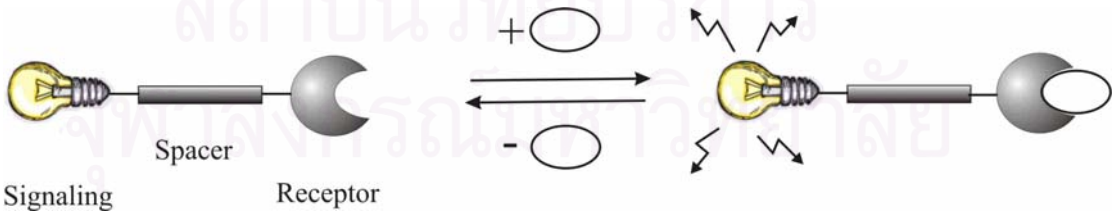


Figure 1.1 A general concept of chemosensors [10]

The design of chemical sensors that are specific for the detection of important alkali metal ions such as Li^+ , Na^+ and K^+ in biological fluid, continues to be a field of

active research. The selective detection of these ions in the presence of others in mixed solutions remains a challenging task.

1.3 Chromoionophores and fluoroionophores

A number of direct sensing schemes have been described using colorimetric and fluorescent chemosensor molecules whose optical properties change upon direct binding of the cation (*chromoionophores* and *fluoroionophores*). For example, chromoionophores have been prepared by covalently linking colored dyes to simple crown ether and calix[4]arenes [12-15] and fluoroionophores have been prepared by covalently linking fluorophores with crown ether and calix[4]arenes. [16-18]

1.3.1 Chromoionophores

Color reactions are popular criteria for the identification and quantitative determination of substances. For alkali metal ions, only a few color reagents were known before 1977 and these did not allow the photometric ion determination of practical samples. Until discovery and development of the crown ether chemistry, optical detection methods are practicable, especially complex formation with dye ligands.

In the alkali metal salt complexes of ionophores, the positive cation charge influence the donor heteroatom (O, N, S) and their electronic surrounding by ion-dipole forces. If one of the heteroatoms is a constituent of chromoionophores, the electronic disturbance propagates the whole ($n+\pi$) system. Due to different influences on the ground and photoexcited states by cations, changes occur in the absorption spectra. Basically, the change of color of chromoionophores occurs through two mechanisms. [13,19]

1.3.1 (a) Cation-induced hypsochromic band shifts (blue shift)

When a chromoionophore contains an electron donor group (often an amino group) conjugated to aromatic ring (electron acceptor), it can complex a cation as well as being a chromophore. The amine nitrogen atoms of the chromoionophore are positively polarized, the excited states are more strongly destabilization by cations than the ground state, therefore, resulting in hypsochromic shifts. (Figure 1.2)

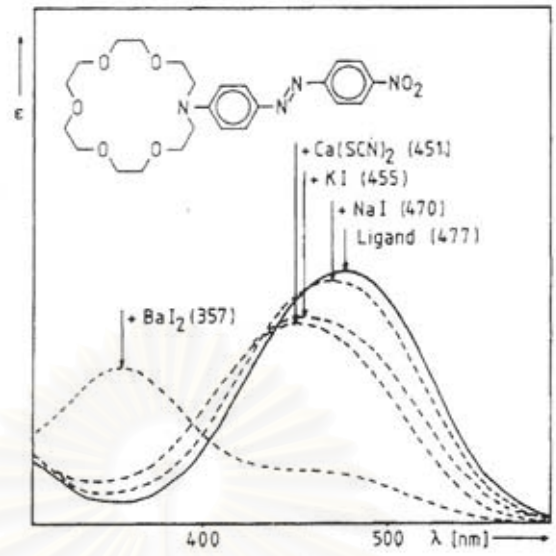


Figure 1.2 A chromoionophore which induces blue-shift upon binding with guests [13]

The influence of the ligand ring size on the selectivity is obvious by the rule that always the strongest maximum wavelength is effected by the cation which fits best into the ligand cavity. The observable changes of the absorption spectra can be described satisfactorily by the electronic states. The chromophores are influenced by these ion-dipole forces, depending on the size and direction of the dipole moment. The more the dipole moment alters during the excitation, the more the absorption band shifts.

1.3.1 (b) Cation-induced bathochromic shifts (red shift)

When a chromoionophore contains an electron acceptor, it can give color change upon complexation. Because the donor atom will surely be polarized positively in the ground state, the excited states are more strongly stabilization by binding of a cation at the acceptor site, causing bathochromic band shifts (Figure 1.3).

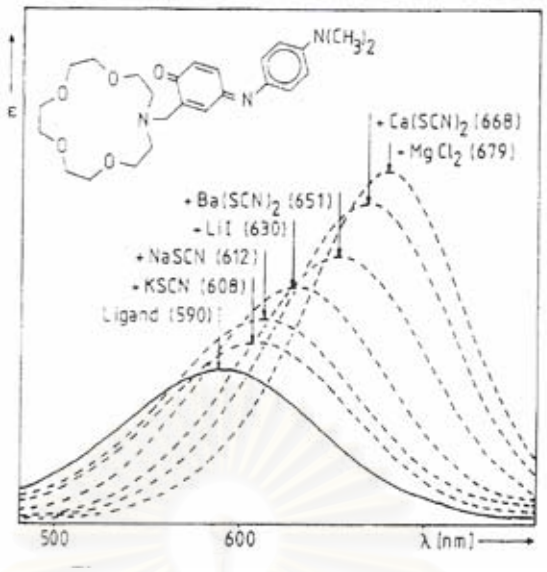


Figure 1.3 A chromoionophore which induces red-shift upon binding with guests [13]

1.3.2 Fluoroionophores

Fluoroionophores or fluorosensors are generally multi-component systems comprising of a signaling moiety (fluorophore) and a guest-binding site (receptor). The two units are often separated by a space group. The components are chosen such that the communication between the receptor and the fluorophore results in "switching off" of fluorescence of the system or fluorescence quenching in the absence of a guest (cation). However, in the presence of a guest, the communication between the receptor and fluorophore gets turned-off leading to the recovery of the fluorescence of the system. Thus, the presence of the guest is indicated by a switching-on the fluorescence or fluorescence enhancement. [20-22] A number of fluorophores, a signaling subunit which shows a change in fluorescence properties such as emission frequency or intensity during a cation recognition events, has been reviewed by Valeur and LeRay, [20] Jiang and Guo [21] and Bren. [22] Basically, the change occurs through two mechanisms. [20-22]

1. *Fluorescence PET (Photoinduced Electron Transfer).* The recognition subunit is an electron donor and the fluorophore acts as an acceptor. When the fluorophore is excited, the electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO). This enables PET from the HOMO of the donor (belonging of the free cation receptor) to that of

fluorophore, causing fluorescence quenching of the latter. Upon binding a cation, the HOMO of the donor becomes lower in energy than that of the fluorophore; consequently, PET is not possible anymore and fluorescence quenching is suppressed. (Figure 1.4)

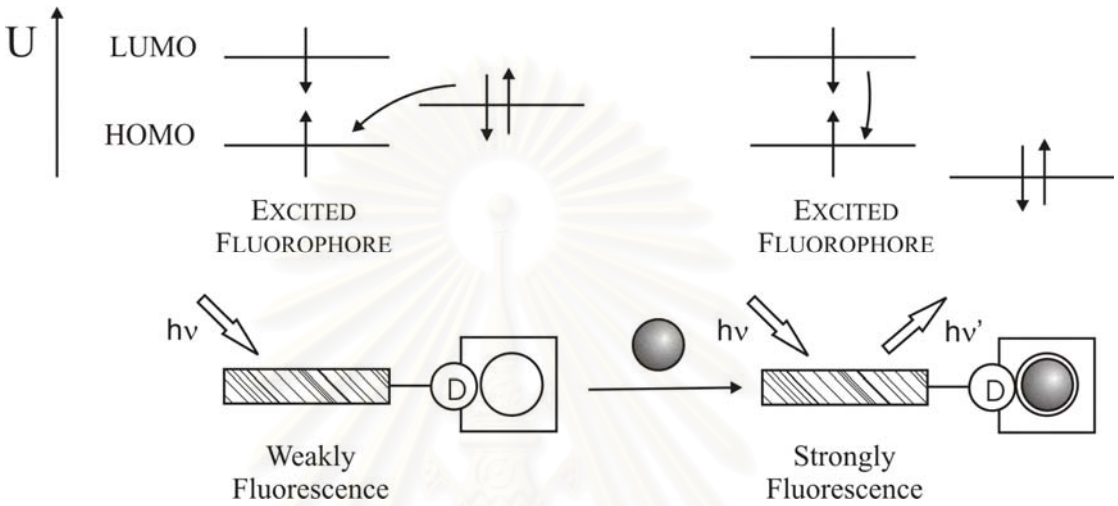


Figure 1.4 Principle of the PET sensor [20]

A calix[4]arene ester containing pyrene and nitrobenzene as fluorophores [23] shown in Figure 1.5 possessed a PET mechanism.

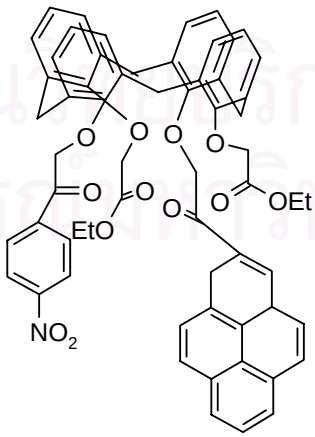


Figure 1.5 A calixarene-based PET fluoroionophore

Another fluoroionophore design involved *excimer-monomer PET system.* Some fluorophores such as anthracene and pyrene can form an excimer (excited dimer) when an excited molecule come close to another one during the lifetime of excited state, dual fluorescence is then observed with monomer band at shorter wavelength and excimer broad band at longer wavelength. When the proximity of the fluorophore is disturbed, for example by cation binding, the excimer formation is altered and the monomer/ excimer fluorescence intensity ratio is altered.

2. *Fluorescence PCT (Photoinduced charge transfer).* When a fluorophore contains an electron donor group (often an amino group) conjugated to an electron-withdrawing group, it undergoes intramolecular charge transfer from the donor to the acceptor upon excitation by light. The consequent change in dipole moment results in a stoke shift that depends on the microenvironment of the fluorophore. Upon cation binding, the electron-donating character of the group is diminished causing reduction in conjugation. Therefore, the blue shift together with a decrease in molar extinction coefficient is expected. Conversely, a cation interacting with the acceptor group enhances the electron-withdrawing character of the group; the absorption spectrum is thus red-shift and the molar extinction coefficient is increased (Figure 1.6).

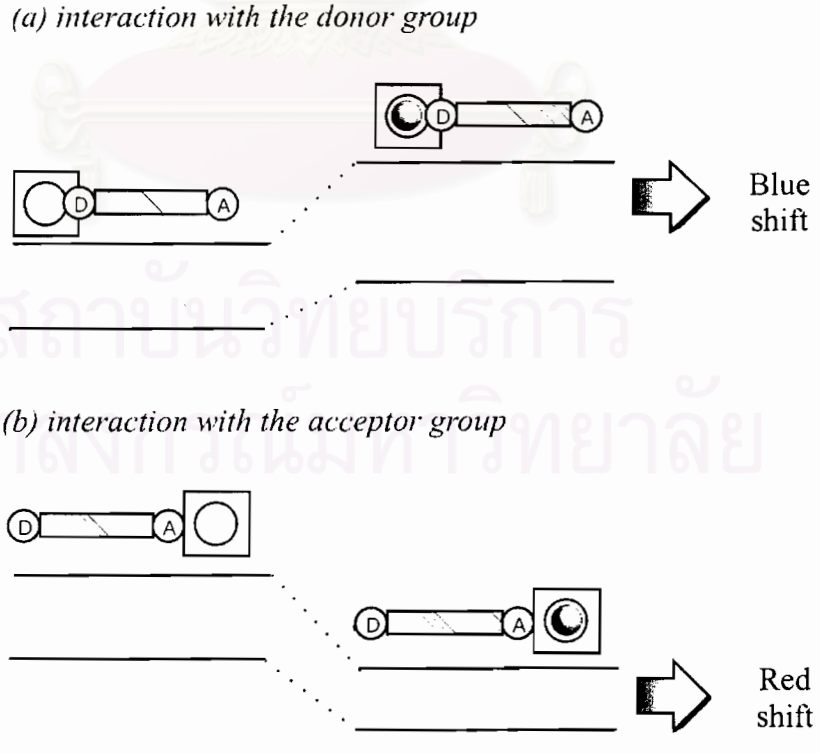


Figure 1.6 Principle of cation recognition by fluorescence PCT sensors. [20]

A calix[4]arene containing ester as a receptor and benzothiazolyl group as a fluorophore [24] shown in Figure 1.7 possesses a PCT mechanism.

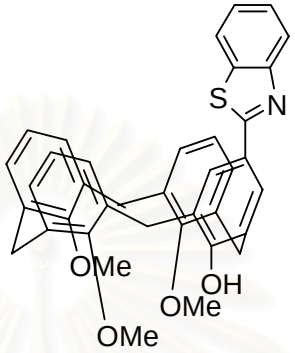


Figure 1.7. A calixarene-based PCT fluoroionophore

1.4 Design of chromoionophores and fluoroionophores

In order to design a host that will selectively bind an alkali metal ion, we use the chelate and macrocyclic effects as well as the concept of complementarity (matching of host and guest and electronic requirements) and crucially, host preorganization.

Having defined parameters such as the required host size, charge, characters of the donor atoms etc, the intellectual process of ligands tailoring can begin. Host-guest interactions occur through binding sites. The type and number of binding sites must be selected in a fashion that is most complementary to the characteristics of the binding sites of the guest. These binding sites must be arranged on a organic framework of suitable size to accommodate the guest. Binding site should be spaced somewhat apart from one another to minimize repulsions between them, but arranged so that can all interact simultaneously with the guest. [25]

The nature of the organic framework of the host itself, whether lipophilic or hydrophilic, plays a fundamental role in a host behavior. This determines the solubility characteristics of host and its complexes.

The part of the signaling unit must be selected with suitable. Normally the chromophore has conjugated bonds between a donor and receptor and gives the color.

1.5 Alkali metal ion sensors based on *p-tert-* butylcalix[4]arene and calix[4]arene

The term “calixarene” was introduced by Gutsche in 1976 to describe a homologous series of macrocyclic phenol-formaldehyde condensates whose constitution and structure had been the subject of much speculation during the past 30 years. Calixarenes are phenolic metacyclophanes annulated by single methylene groups. Structures are ranging from the relatively rigid tetrameric calix[4]arene to the much larger (32-membered ring), and more flexible, octameric calix[8]arene (Figure 1.8). [26-28]

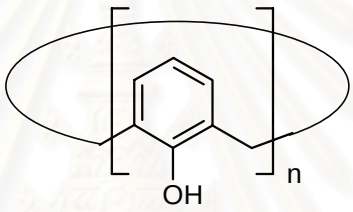


Figure 1.8. Structure of calix[n]arenes (n = 4 -14)

The most accessible are the tetramer, the hexamer and the octamer, although all members of the series from n equals 4 to 14 are known. All calixarenes are not static at room temperature since rotations about the Ar-CH_2-Ar bonds are possible. For instance the tetramer can adopt four different conformations: cone, partial cone, 1,2-alternate and 1,3-alternate conformation which differ by the respective orientation of the aromatic rings (Figure 1.9). [26-28]

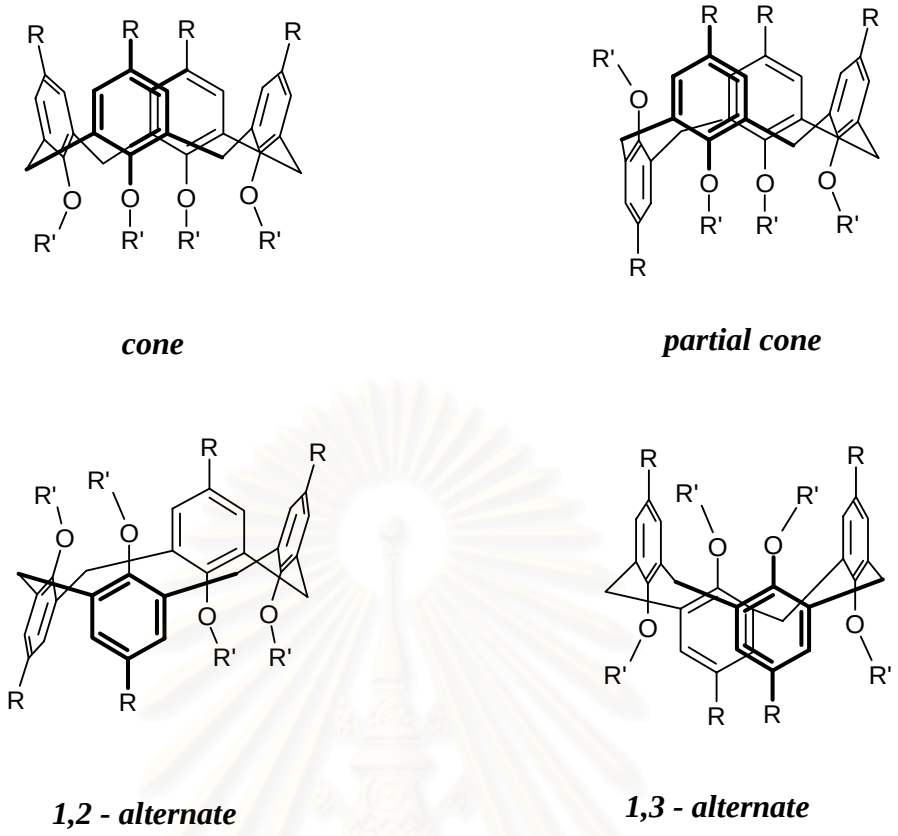


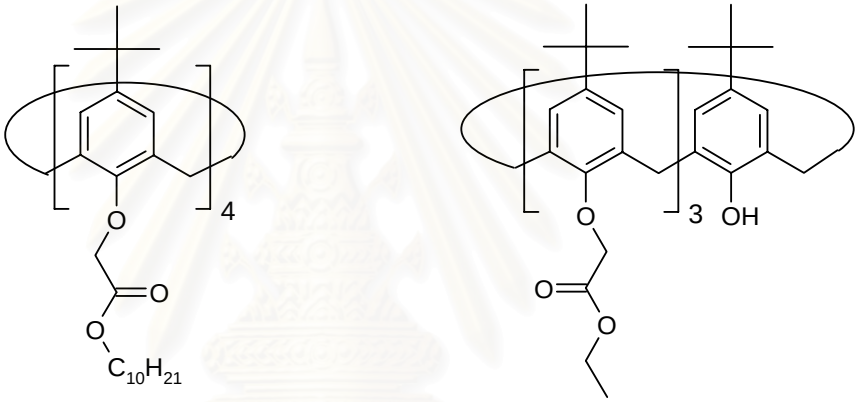
Figure 1.9. Four possible conformations of calix[4]arene

Calixarene have been shown to complex alkali metal ions, but the low solubility in most organic solvents has severely limited the study of their complexing abilities. Calixarene shows definitely many advantages such as: (i) easy and cheap synthesis; (ii) availability in several sizes; (iii) amenability to chemical modifications such as substitutions at the para-positions (upper rim), derivation at the bridging CH_2 groups and at the phenolic hydrogens (lower rim). Their different types of modifications, which can be operated separately or combined, lead to a great variety of derivatives with modified solubility, conformational mobility and complexing abilities. [27]

Conformations and the nature of functional groups attached to calixarene were found to be important parameters that affect the selectivity of calixarene derivatives. For alkali metal ions, calixarene ester and ketone derivatives, known for their affinity for this series of the metal ions and their lack of affinity for the alkaline earth metal ions have already proved to be good candidates to meet this demand. In contrast, amide derivatives have been used for affinity for alkaline earth metal ions. When

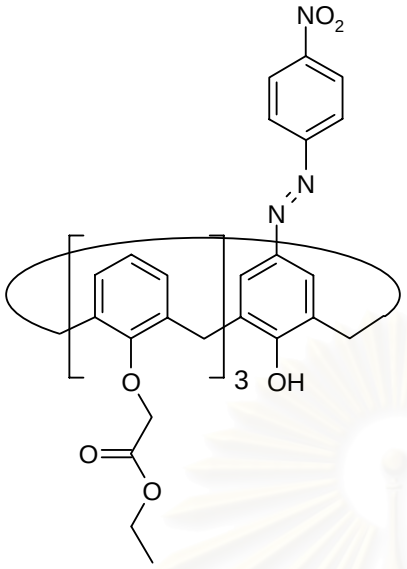
playing with the degree of condensation the selectivity is shifted from one metal ion to another: for instance, Na^+ and Cs^+ are based on tetramers and hexamers, respectively. In addition, calixarene derivatives bearing chromophores and fluorophores capable of optically signaling complexation are being developed and attracting intense interest in metal ion detection.

Without signaling subunit, the tetraester was found to preferentially extract Na^+. [29] The decyl acetate derivatives of *p-tert*-butylcalix[4]arene (**L1**) showed high selectivity when used as neutral carrier in polyvinyl chloride membrane electrode. [30] The same was true for the triester derivative of *p-tert*-butylcalix[4]arene (**L2**). [31]

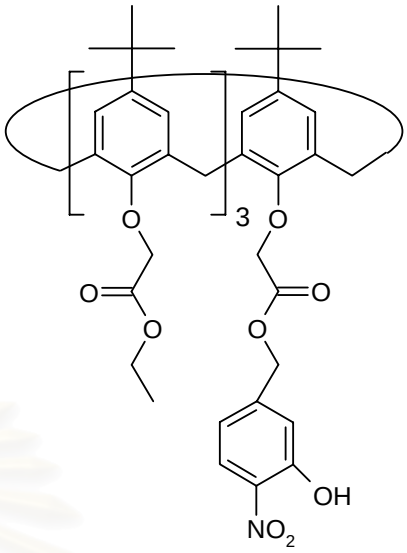


L1 **L2**

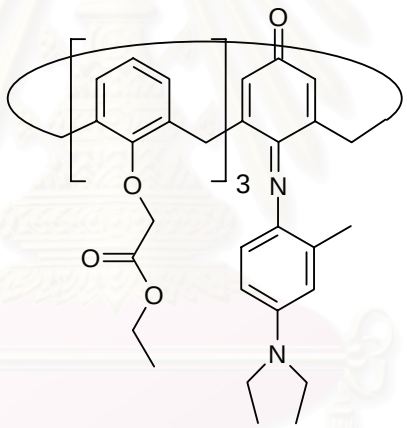
Although calixarene shows metal ion binding ability similar to crown ether, there has been a few reports of the combination of calixarene with photochromic molecules. In the case of calixarene, incorporation of several photochromic molecules is possible. Therefore, interactions between the calixarene and the photochromic moieties are expected to produce additional function when calixarene adopt the cone conformation to form a rigid cavity. [32] Shimizu *et al*. [33] synthesized azophenol calix[4]arene triester (**L3**) and McCarrick *et al*. [34-35] synthesized nitrophenol *p-tert*-butylcalix[4]arene triester (**L4**) which showed high Li^+ selectivity and induced a color change. Kubo *et al.* [36] synthesized indoaniline dyes containing calix[4]arene triester (**L5**) which showed a high selectivity for Na^+ and sodium ion binding induced a bathochromic shift in absorption spectrum. The indoaniline chromophore system might be one of important candidates because the optical property can be perturbed significantly by chemical stimuli. [14,36-38]



L3



L4

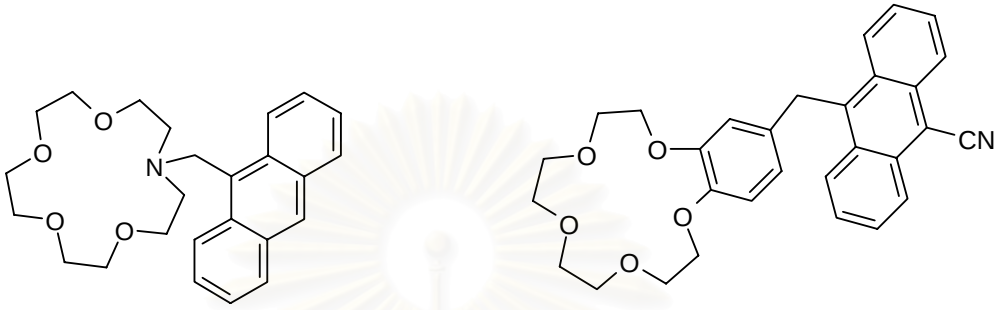


L5

Among chemosensory systems, the fluorescence method is very important and interesting due to its simpler instrumentation, high sensitivity and direct visual perception even in very dilute solution. Fluorescence chemosensors, which combine two fundamental functional units: a fluorophore and an ionophore, play an important role in supramolecular chemistry. The part of ionophores can selectively bind the substrate, and the part of fluorophores is attached to the vicinity of the binding site for signal detection and transduction.

While numerous fluoroionophores based on crown ethers have been synthesized and studied, ones based on calixarene are limited in number. This is

probably due to the versatility in terms of synthetic strategies in incorporating fluorophores into crown ethers and resulted structures where electron donor groups can be situated in close proximity to both ion docking site and fluorophore. (Figure 1.10) Nontheless, some calixarene-derived fluoroionophores have been synthesized with certain selectivity.



L6 **L7**

Figure 1.10 Example of crown ether-based fluoroionophores: *p*-cyanoanthryl-benzocrown-6 (**L6**) [39] and 9-anthryl-azacrown-5 (**L7**) [40]

It was mentioned earlier that calix[4]arene could be functionalized to suit the specificity required. In general, calixarene with oxygen donor atoms is suitable for binding alkali ions selectively. In the case of ester or ketone lower-rim function, specificity stems from the size of a pocket formed from phenolic-oxygen and carbonyl-oxygen together with a number of coordination sites. To retain the binding ability, this structure must be retained. This results in the limitation of the way fluorophores be cooperated into calixarene structures. Fluorophores should be linked while retained the functional groups responsible for binding an ion. The fluorophore also altered selectivity in terms of signal sensing. Searching fluorophores is thus another research area of interest. [41,42]

Calixarene with fluorophores at the upper-rim is illustrated in Figure 1.7. In a medium such that phenolic group is not deprotonated, complexation with Li^+ leads to the ejection of phenolic protons forming the enolate-cation pair (Figure 1.11) which results in a red shift of the emission spectra. No change in emission spectra was observed with Na^+ and K^+. The same fluorophore when applied to calix[4]arene-

crown-5 ether resulted in a compound **L8** exhibited selective fluoroionophoric behavior toward Ca^{2+} ion in dioxane-water. [42]

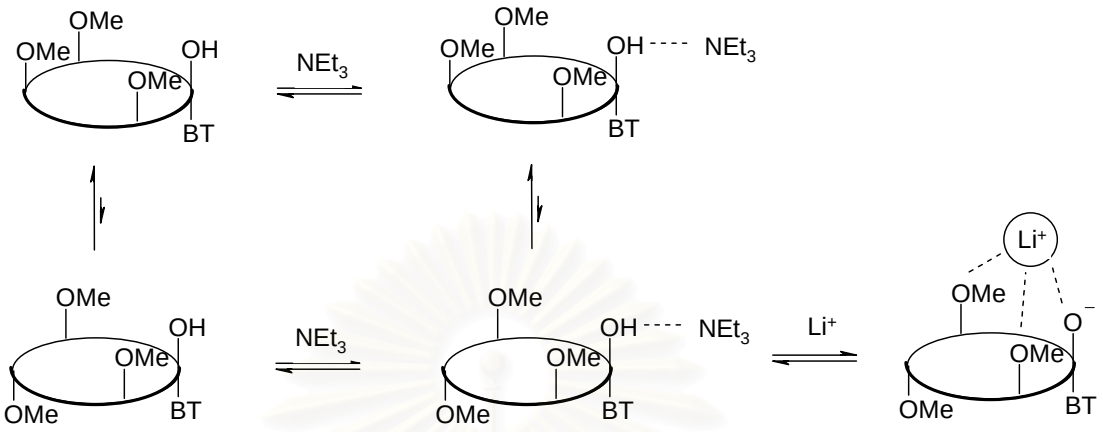
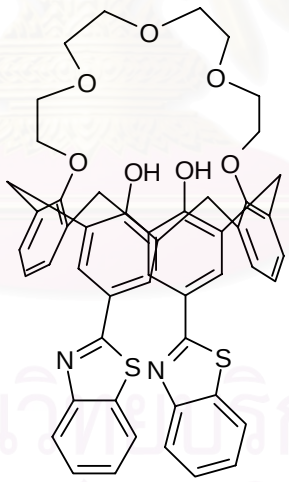
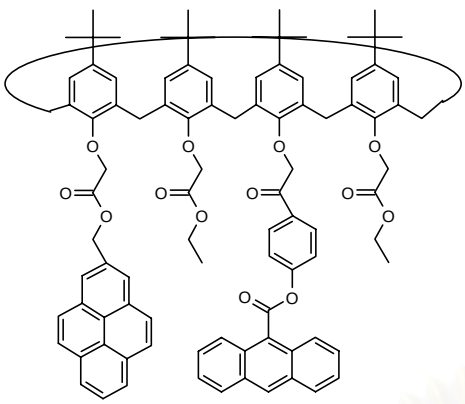


Figure 1.11 The mechanism of binding between 5-benzothiazol-1-yl-28-hydroxy-25,26,27-trimethoxycalix[4]arene with lithium ion. BT denotes benzothiazol [24].

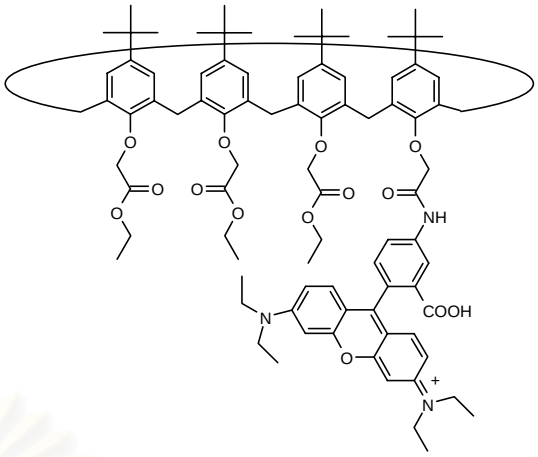


L8

Calixarene with fluorophores at the lower-rim is shown in Figure 1.5. Compound **L9** containing anthracene and pyrene fluorophores showed selectivity towards sodium in MeOH-THF. [43] An aminorhodamine B, connected through the amide bond at the lower rim, resulted in a fluoroionophore **L10** which shows selectivity for Na^+ over K^+ in aqueous media. [44]



L9



L10

From above examples, *p-tert*-butylcalix[4]arene has been derivatized at the lower rim where the four carbonyls together with four phenolic oxygens binding sodium ions. In fact, it has been shown that the ester substituents on the aromatic affected the selectivities of the calixarene receptor. (Figure 1.12 and Table 1.1) [29]

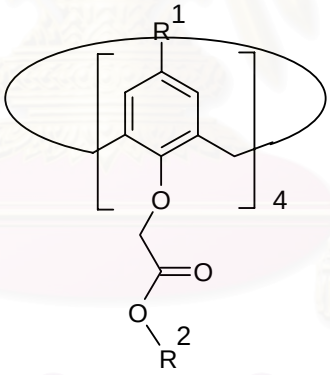


Figure 1.12 Calix[4]arene ester with different substituents. [29]

Table 1.1 Percent extraction of alkali metal ions into CH_2Cl_2 with compounds **1-5**. [29]

	1	**2**	**3**	**4**	**5**
Li^+	15.0	1.8	6.7	1.1	27.6
Na^+	94.6	60.4	85.7	34.2	94.0
K^+	49.1	12.9	22.3	4.8	75.8
Rb^+	23.6	4.1	9.8	1.9	53.4
Cs^+	48.9	10.8	25.5	4.6	81.9

1 R^1 = *t*-Bu, R^2= Et ; **2** R^1 = *t*-Bu, R^2= Et ; **3**. R^1 =*t*-Bu, R^2= Et; **4**. R^1 = *t*-Bu, R^2= Et; **5** R^1 =*t*-Bu, R^2= Et

It can be seen from Table 1.1 that calix[4]arene tetraester is a good receptor for sodium ion regardless of substituents but the selectivity changes upon changing *para*-substituents and the alkyl groups of the ester. It is suspected that the alkyl group of the fluorophore may affect the selectivity of the calix[4]arene receptor in the same way.

1.6 Some characteristics of fluoronoionophores.

1.6.1 Selectivity

A selectivity of a receptor is a preference of that receptor towards a guest. This is customarily estimated from a magnitude of a thermodynamic *stability constant* of a receptor-guest or, in more general terms, a ligand-metal complex. For fluoroionophore, the method of obtaining a binding constant is through fluorimetric titration-a measurement of fluorescence intensity at different metal-ligand ratios. [45-47]

The complexation of a metal ion M by a ligand L in solution can be represented by the equilibrium

$$M + L \leftrightarrow ML$$

which is controlled by the stability constant

$$K_s = \frac{[ML]}{[L][M]}$$

where the bracket denotes the concentration of each species in mole per liter.

In fluorimetric titration, the fluorescence intensity of the solutions containing free ligand L (I_F^0) and ligand complexed with metal ion ML (I_F) at the chosen emission wavelength are measured and are related to the initial concentration of the ligand (C_0) by

$$I_F^0 = kaC_0 \quad (1)$$

I_F depend directly on [L] and [ML]

$$I_F = ka[L] + kb[ML] \quad (2)$$

Where k, a and b is a constant

Since $[ML] = C_0 - [L]$

Therefore $I_F - I_F^0 = k(b-a)(C_0 - [L])$ (3)

After rearrangement

$$\frac{I_F^0}{I_F - I_F^0} = \left(\frac{a}{b-a}\right)\left(\frac{1}{K_s[M]} + 1\right) \quad (4)$$

Equation 4 shows that a plot of $I_F^0 / (I_F - I_F^0)$ against 1/ [M] would be a straight line in which stability constant K_s can be calculated.

1.6.2 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. Quantum yield are measured either on a relative basis with reference to a standard or by using an absolute method.

The most widely used method of determining quantum yields is by the relative method and the quantum yield of the unknown, Q_x, is calculated according to the following equation [48] :

$$Q_x = Q_{STD}\left(\frac{A_{STD}}{A_x}\right)\left(\frac{E_x}{E_{STD}}\right)\left(\frac{\eta_x^2}{\eta_{STD}^2}\right) \quad (5)$$

Or $$Q_x = Q_{STD}\left(\frac{Grad_x}{Grad_{STD}}\right)\left(\frac{\eta_x^2}{\eta_{STD}^2}\right) \quad (6)$$

Where Q is the quantum yield, A is the absorbance of the solution, E is the corrected emission intensity, 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 unknown sample, respectively.

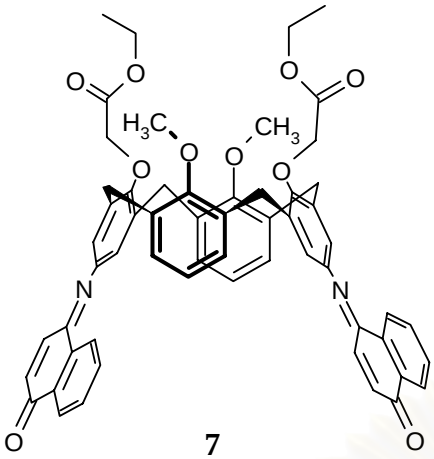
1.7 Objective and the scope of this research

The objective of this research is to synthesize fluoroionophores, employing calix[4]arene as a building block,**7**, **11**, **17** and **18**, as sensors for alkali metal ions. This dissertation described an attempt to synthesize two type of fluoroionophores:

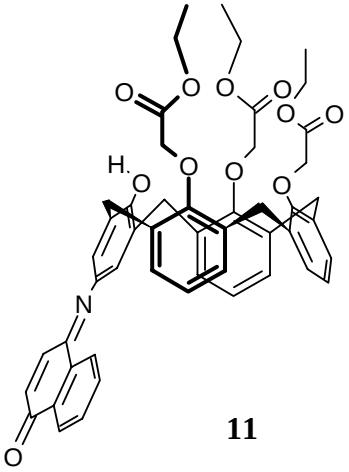
(i) Fluoroionophores containing quinone-imine connecting through the wide rim.

(ii) Fluoroionophores possessing amide functions connecting through the narrow rim.

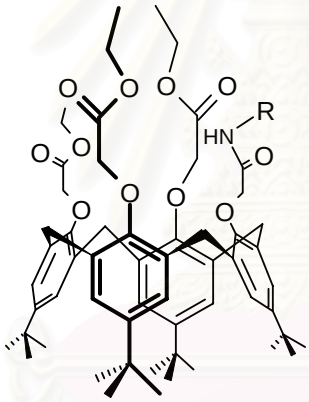
The binding ability, sensitivity and selectivity towards alkali metal ions will be evaluated by means of fluorescence spectrometry. The target molecules were shown below.

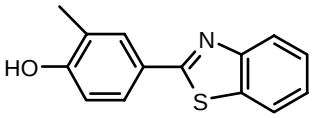


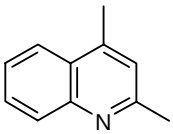
7



11



17, R = 

18, R = 

CHAPTER II

EXPERIMENTAL SECTION

2.1 General procedures

2.1.1 Analytical instrument

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 200, 400 and a Bruker DRX 400 MHz nuclear resonance spectrometers. In all cases, samples were dissolved in deuterated chloroform. The chemical shifts were recorded in part per million (ppm) using a residue proton solvents as internal reference. Elemental analysis was carried out on CHNS/O analyzer (Perkin Elmers PE 2400 series II). EI mass spectra were recorded on a Micromass Platform II. Absorption spectra were measured by a Varian Cary 50 UV-Vis spectrophotometer. Fluorescence spectra were performed on a Varian Cary Eclipse spectrofluorometer by a personal computer data processing unit. The light source is a pulsed xenon lamp and a detector is a photomultiplier tube.

2.1.2 Materials

Unless otherwise specified, the solvent and all materials were reagent grades purchased from Fluka, BDH, Aldrich, Carlo erba, Merck or Lab scan and were used without further purification. Commercial grade solvents such as acetone, dichloromethane, hexane, methanol, toluene and ethyl acetate were purified by distillation before used. Acetonitrile, dimethylformamide and dichloromethane for set up the reaction were dried over calcium hydride and freshly distilled under nitrogen atmosphere prior to use. Tetrahydrofuran was freshly distilled from sodium/benzophenone.

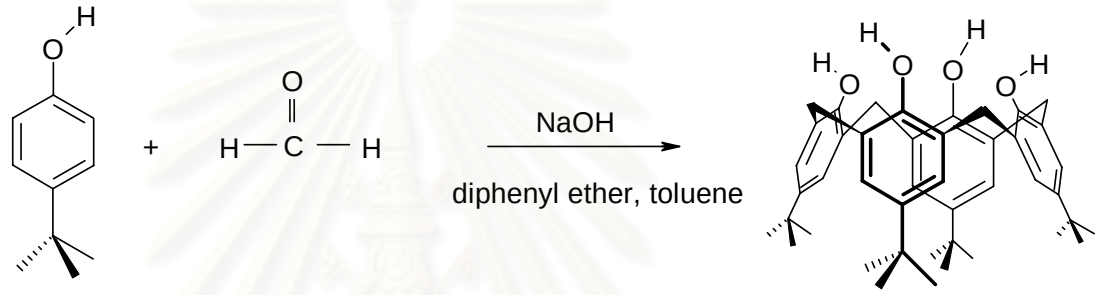
Column chromatography was carried out on silica gel (Kieselgel 60, 0.063-0.200 mm, Merck). Thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60, F_{254}, 1mm, Merck). Compounds on TLC plates were detected by

the UV-light. Methanol and ethanol for fluorescence measurement (AR grade, Merck) were dried with molecular sieves.

All synthesized compounds were characterized by ^{1}H-NMR spectroscopy, mass spectrometry and elemental analysis.

2.2 Synthesis

2.2.1 Preparation of *p-tert*-butylcalix[4]arene (1)



1

a. Preparation of precursor

In a 250 ml two-necked round bottom flask equipped with a magnetic bar, *p-tert*-butylphenol (51.808 g, 0.38 mol), 37% formaldehyde solution (34 mL, 0.42 mol) and sodium hydroxide (1 g, 0.025 mol) were stirred. The contents of the open flask were heated in silicone bath for *ca*. 2 h at 110-120 ^{o}C. Until there was considerable fronting and the reaction mixture may fill most of the flask before shrinking back to the original volume. The reaction mixture was allowed to cool to room temperature and diphenyl ether (900 mL) was added to the flask and transferred to 1-L one-necked round bottom flask.

b. Preparation of *p-tert*-butylcalix[4]arene

The 1-L one-necked flask equipped with a reflux condenser. The contents of the flask were heated with a heating mantle. The reaction mixture was refluxed under nitrogen for 1.5-2 h. until no vapor water. The reaction mixture was cooled to room temperature for 30 min and ethyl acetate 300 mL was added to precipitate the product. The resulting mixture was stirred for 30 mins and allow to stand for at least 30 mins.

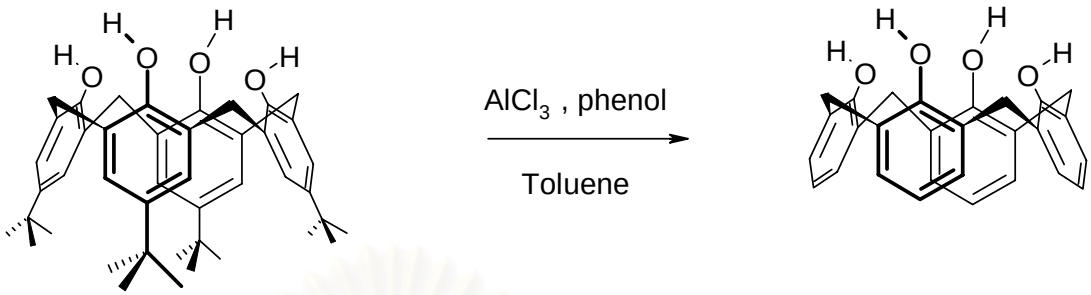
The solution over precipitate was discarded and filtrate yields a material which was washed three times with 100 mL portions of a mixed solvents (acetic acid : ethyl acetate = 1:1) to yield *p-tert*-butylcalix[4]arene (30.125 g, 50%).

Characterization data for 1

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 10.38 (s, 4H, ArO*H*), 7.09 (s, 8H, *m*-Ar*H*), 4.31 (d, *J* = 14 Hz, $4H_A$, ArCH_2Ar), 3.55 (d, *J* = 13.2 Hz, $4H_B$, ArCH_2Ar), 1.25(s, 36H, t-C_4H_9)

2.2.2 Preparation of 25,26,27,28-tetrahydroxycalix[4]arene (2)



1 **2**

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, *p-tert*-butylcalix[4]arene **1** (9.873 g, 15.2 mmol), aluminium chloride (9.847 g, 73.8 mmol), phenol (6.950 g, 73.8 mmol) and toluene (50 mL) were stirred under nitrogen atmosphere at room temperature for 1 h. The reaction was poured into 100 mL of 3 M hydrochloric and stirred for 10 mins. The mixture was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The methanol was subsequently added to precipitate a white powder **2** (5.168 g, 80 %).

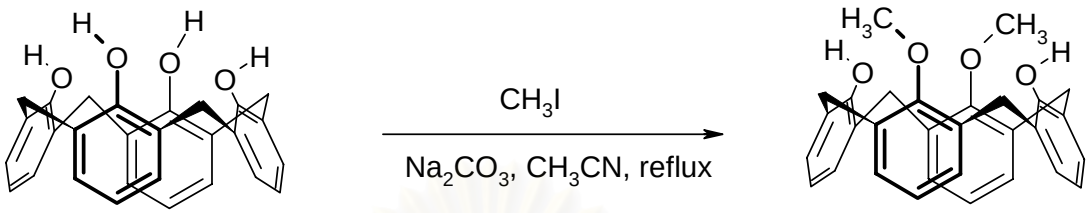
Charaterization data for 2

^{1}H NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 10.28 (s, 4H, ArO*H*), 7.10 (d, *J* = 8 Hz, 8H, *m*-Ar*H*), 6.79 (t, *J* = 8 Hz, 4H, *p*-Ar*H*), 4.30 (AB, 4H_A, ArCH_2Ar), 3.53 (AB, 4H_B, ArCH_2Ar)

Preparation of chromoionophore

2.2.3 Preparation of 25,27-dimethoxy-26,28-dihydroxycalix[4]arene (3)



2 **3**

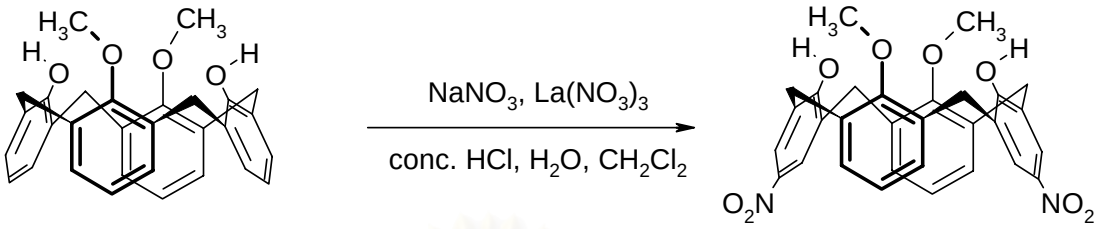
In a 250 mL two-necked round bottom flask equipped with a magnetic bar, 25,26,27,28-tetrahydroxycalix[4]arene **2** (3.395 g, 8.0 mmol), anhydrous sodium carbonate (8.479 g, 80.0 mmol) and acetonitrile (120 mL) were stirred under nitrogen atmosphere at room temperature for 1 h. Methyl iodide (2.50 mL, 20 mmol) was then added and the mixture was heated at reflux for 20 h. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and treated with 2 M hydrochloric acid and extracted with dichloromethane (2 x 25 mL). The organic layer was extracted with water (25 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The methanol was subsequently added to precipitate a white powder **3** (2.957 g, 82 %).

Charaterization data for 3

^{1}H NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 7.78 (s, 2H, ArO*H*), 7.12 (d, *J* = 7 Hz, 4H, *m*-*H*ArOCH$_3$), 6.92 (d, *J* = 8 Hz, 4H, *m*-*H*ArOH), 6.78 (t, *J* = 8 Hz, 2H, *p*-*H*ArOCH$_3$), 6.74 (t, *J* = 8 Hz, 2H, *p*-*H*ArOH),4.36 (AB, 4H$_A$, ArC*H*$_2$Ar), 4.02 (s, 6H, OC*H*$_3$), 3.46 (AB, 4H$_B$, ArC*H*$_2$Ar)

2.2.4 Preparation of 5,17-dinitro-25,27-dimethoxy-26,28-dihydroxycalix[4]arene (4)



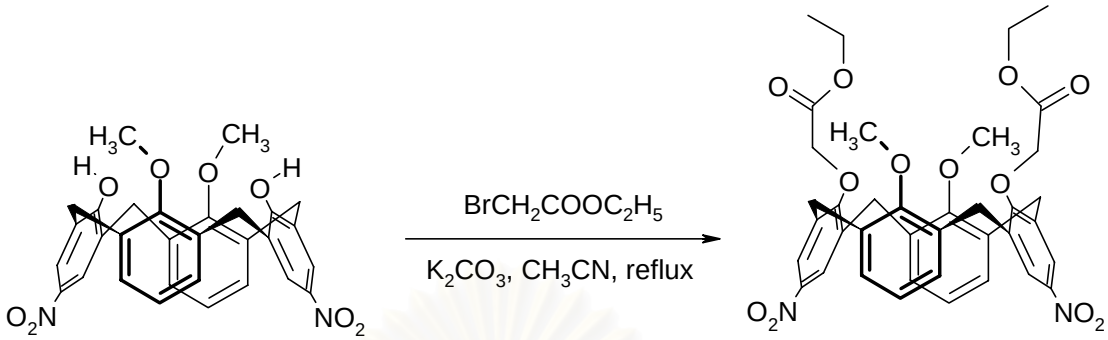
3 **4**

In a 500 mL one-necked round bottom flask equipped with a magnetic bar, 25,27-dimethoxy-26,28-dihydroxycalix[4]arene **3** (2.018 g, 4.4 mmol) and dichloromethane (70 mL) were stirred at room temperature for 1 h.. The mixture of sodium nitrate (1.142 g, 13.2 mmol), a catalytic amount of lanthanide nitrate and water (30.4 mL) was then added and followed by concentrated hydrochloric acid. The mixture was stirred overnight at room temperature. The colour of the mixture turned yellow. The aqueous layer was then separated and extracted with dichloromethane (2 x 25 mL). The organic layer was extracted with saturated aqueous ammonium chloride (2 x 25 mL) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the product was crystallized by adding hexane to give a yellow solid **4** (2.169 g, 89 %).

Charaterization data for 4

^{1}H NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)
δ 8.98 (s, 2H, ArO*H*), 8.10 (s, 4H, *H*ArNO_2), 7.00 (d, *J* = 8 Hz, 4H, *m*-*H*ArOCH_3), 6.89 (t, *J* = 8 Hz, 2H, *p*-*H*ArOCH_3), 4.35 (d, *J* = 14 Hz, $4H_A$, ArCH_2Ar), 4.08 (s, 6H, OCH_3), 3.59 (d, *J* = 14 Hz, $4H_B$, ArCH_2Ar)

2.2.5 Preparation of 5,17-dinitro-25,27-dimethoxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene (5)



4 **5**

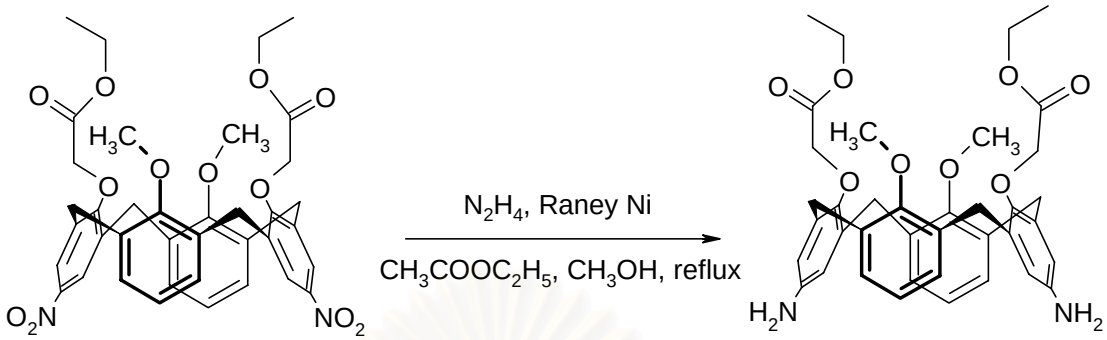
In a 250 mL two-necked round bottom flask equipped with a magnetic bar, 5,17-dinitro-25,27-dimethoxy-26,28-dihydroxycalix[4]arene **4** (0.519 g, 1.2 mmol), anhydrous potassium carbonate (0.800 g, 5.8 mmol) and acetonitrile (50 mL) were stirred under nitrogen atmosphere at room temperature for 1 h. Ethylbromoacetate (0.60 mL, 6.3 mmol) was then added and the mixture was heated at reflux for 40 h. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and treated with 2 M hydrochloric acid until no bubble and extracted with dichloromethane (2 x 25 mL). The organic layer was extracted with saturated sodium chloride (2 x 25 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The methanol was subsequently added to precipitate a pale yellow solid **5** (0.342 g, 50 %).

Charaterization data for 5

^{1}H NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 7.88 - 7.10 (m, 10H, *H*Ar), 4.51 (s, 4H, -OC*H*$_2$CO), 4.35-4.29 (q, *J* = 7 Hz, 4H, OC*H*$_2$CH$_3$), 4.16 (d, *J* = 14, 2H$_A$, ArC*H*$_2$Ar), 4.02 (s, 6H, OC*H*$_3$), 3.81 (d, *J* = 14 Hz, 2H$_A$, ArC*H*$_2$Ar), 3.34 - 3.24 (d, *J* = 3.2, 4H$_B$, ArC*H*$_2$Ar), 1.38 – 1.34 (td, *J* = 7 Hz, 6H, OCH$_2$C*H*$_3$)

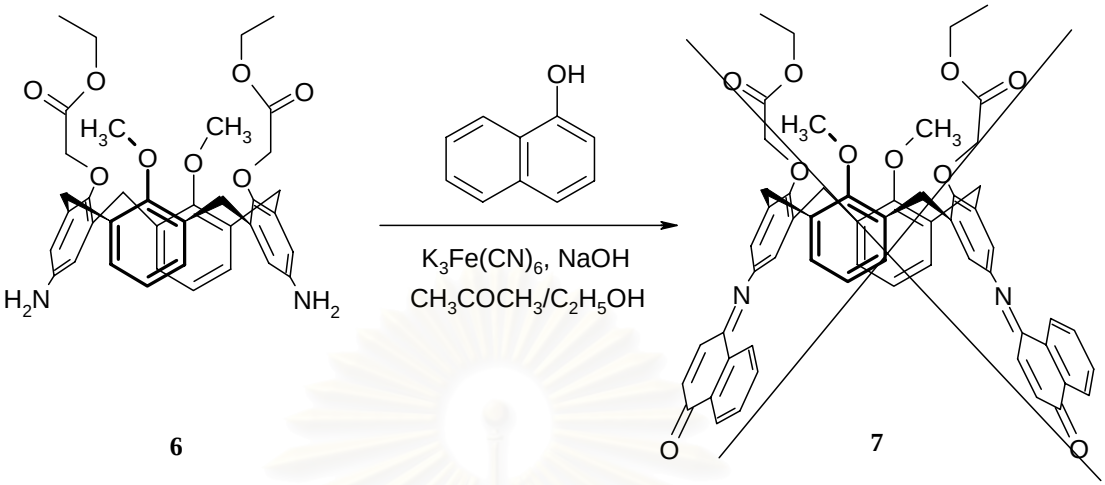
2.2.6 Preparation of 5,17-diamino-25,27-dimethoxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene (6)



5 **6**

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, 5,17-dinitro-25,27-dimethoxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene **5** (0.735 g, 1.1 mmol), Raney Ni (1.076 g, 18.8 mmol) and ethylacetate (40 mL) and methanol (30 mL) were stirred under nitrogen atmosphere at room temperature. Hydrazine (1.2 mL, 140 mmol) was then added and the mixture was heated at reflux for 2 h. The mixture was cooled to room temperature and the reaction mixture was filtered under nitrogen atmosphere. The filtrate was concentrated under reduced pressure. The residue was dissolved in dichloromethane (10 mL) and extracted with water (5 x 15 mL). The organic layer was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the compound **6** was used immediately for further reaction.

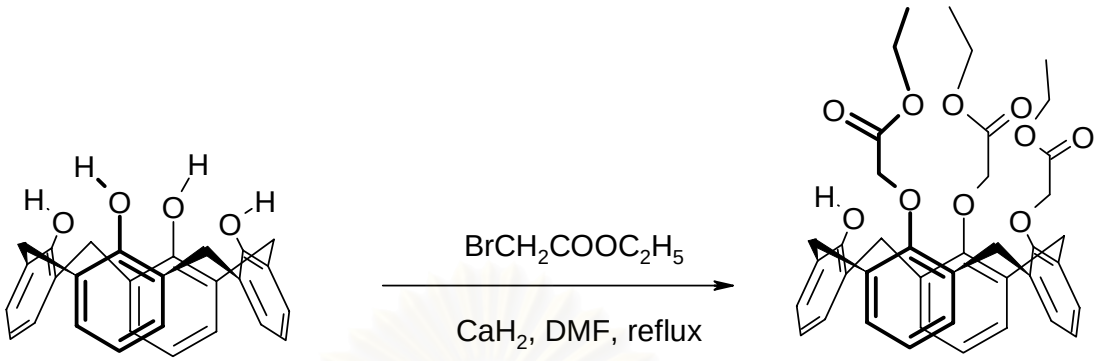
2.2.7 Preparation of 5,17-bis(benzylquinoimine)-25,27-dimethoxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene (7)



6 **7**

In a 250 mL one-necked round bottom flask equipped with a magnetic bar, 5,17-diamino-25,27-dimethoxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene **6** (0.780 g, 1.2 mmol), naphthol (5.443 g, 37.8 mmol) and NaOH (1.184 g, 29.6 mmol, in 2 mL H_2O) in acetone (15 mL) and ethanol (10 mL) were stirred at room temperature for 1 h. The aqueous solution of $K_3Fe(CN)_6$ (21.5343 g, 65.4 mmol) was then added dropwise and the mixture was stirred for 40 min at room temperature. The reaction mixture was poured into water (100 mL). The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane. Compound **6** and naphthol were recovered from the reaction.

2.2.8 Preparation of 25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene (8)



2 **8**

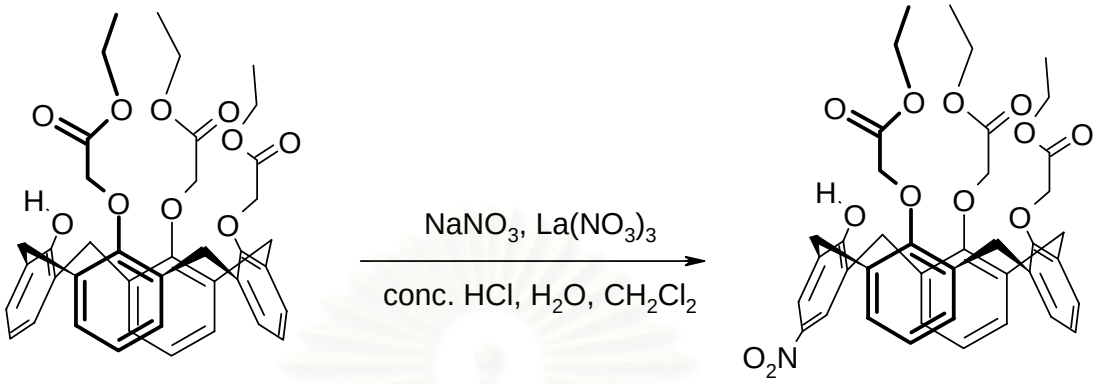
In a 250 mL two-necked round bottom flask equipped with a magnetic bar, 25,26,27,28-tetrahydroxycalix[4]arene **2** (1.080 g, 2.5 mmol), anhydrous calcium hydride (0.316 g, 7.5 mmol) and DMF (25 mL) were stirred under nitrogen atmosphere at room temperature for 1 h.. ethylbromoacetate (0.80 mL, 7.5 mmol) was then added and the mixture was heated at reflux for 24 h. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and treated with 2 M hydrochloric acid until no bubble and stirred overnight. The organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 25 mL). The organic layer was extracted with water (5 x 25 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The ethanol was subsequently added to precipitate a pale yellow powder **8** (1.309 g, 75 %).

Charaterization data for 8

^{1}H NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 7.04 (d, *J* = 7 Hz, 2H, m-*H*ArOH), 6.99 (d, *J* = 8 Hz, 2H, *m*-*H*Ar), 6.87 (t, *J* = 8 Hz, 1H, *p*-*H*ArOH), 6.66 (t, *J* = 8 Hz, 1H, *p*-*H*Ar), 6.48 (m, 6H, *H*Ar), 6.10(s, 1H, ArO*H*), 5.03 (s, 2H, OCH_2CO), 4.90 (d, *J* = 14, 2H_A, ArCH_2Ar), 4.61 (s, 4H, OCH_2CO), 4.57 (d, *J* = 16, 2H_A, ArCH_2Ar), 4.21 (q, 4H, OCH_2CH$_3$), 4.09 (q, 2H, OCH_2CH$_3$), 3.27 (m, 4H_B, ArCH_2Ar), 1.28 (t, *J* = 7, 6H, OCH$_2$CH_3), 1.20 (t, *J* = 7, 3H, OCH$_2$CH_3)

2.2.9 Preparation of 5-nitro-25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene (9)



8 **9**

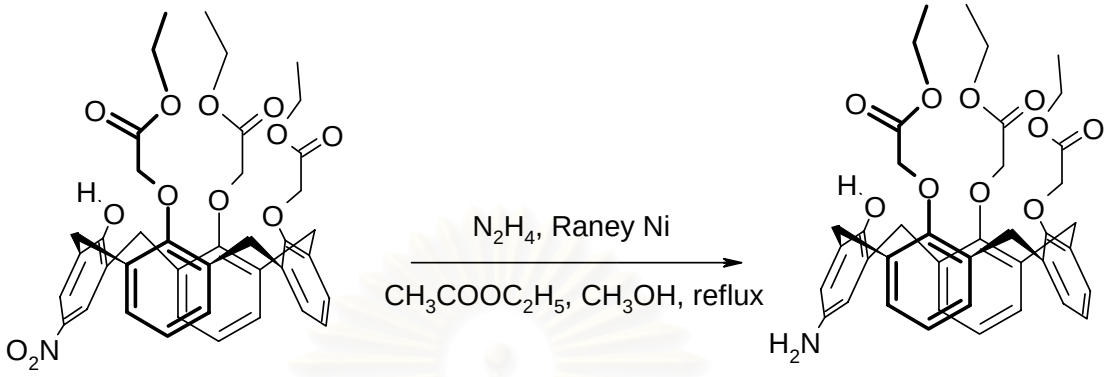
In a 250 mL one-necked round bottom flask equipped with a magnetic bar, 25,26,27-tris(ethoxycarbonylmethoxy)28-hydroxycalix[4]arene **8** (0.359 g, 0.5 mmol) and dichloromethane (14 mL) were stirred at room temperature for 1 h.. The mixture solution of sodium nitrate (0.270 g, 3.2 mmol), a catalytic amount of lanthanum nitrate and water (8.8 mL) was then added and followed by concentrated hydrochloric acid. The mixture was stirred overnight at room temperature. The colour of the mixture turned yellow. The aqueous layers were then separated and extracted with dichloromethane (2 x 25 mL). The organic layer was extracted with saturated aqueous ammonium chloride (2 x 25 mL) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the product was precipitated by adding hexane to give a yellow solid **9** (0.426 g, 80 %).

Charaterization data for 9

^{1}H NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 8.02 (s, 2H, *m*-*H*Ar-NO_2), 7.62 (s, 1H, ArO*H*), 7.08 (t, *J* = 7, 1H, *p*-*H*Ar), 6.93 (d, *J* = 7, 2H, *m*-*H*Ar), 6.90 (d, *J* = 7, 4H, *m*-*H*Ar), 6.75 (t, *J* = 8, 2H, *p*-*H*Ar), 5.06 (s, 2H, -OCH_2CO), 4.63 (s, 4H, -OCH_2CO), 4.59 (m, 4H_A, ArCH_2Ar), 4.32 (q, *J* = 7 Hz, 4H, OC$H_2$$CH_3$), 4.22 (q, *J* = 8, 2H, OC$H_2$$CH_3$), 3.56 (m, 4$H_B$, ArC$H_2$Ar), 1.42 (t, *J* = 7, 6H, OCH_2CH_3), 1.36 (t, *J* = 7 Hz, 3H, OCH_2CH_3)

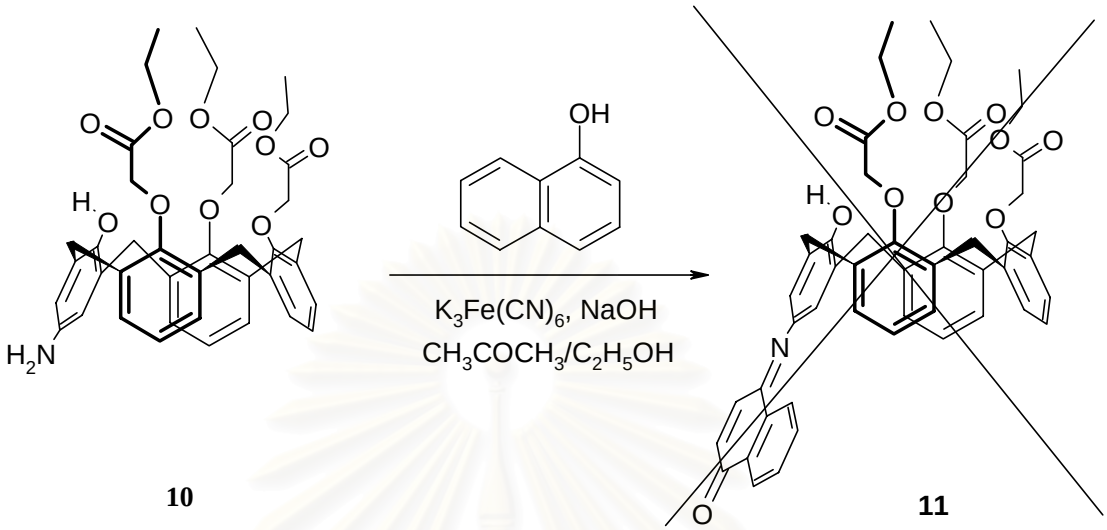
2.2.10 Preparation of 5-amino-25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene (10)



9 **10**

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, 5-nitro-25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene **9** (0.390 g, 0.5 mmol), Raney Ni (0.538 g, 18.8 mmol) and ethylacetate (20 mL) and methanol (15 mL) were stirred under nitrogen atmosphere at room temperature. Hydrazine (2.1 mL, 140 mmol) was then added and the mixture was heated at reflux for 2 h. The mixture was cooled to room temperature and the reaction mixture was filtered under nitrogen atmosphere. The filtrate was concentrated under reduced pressure. The residue was dissolved in dichloromethane (10 mL) and extracted with water (5 x 15 mL). The organic layer was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the compound **10** was used immediately for further reaction.

2.2.11 Preparation of 5-benzylquinoimine-25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene (11)

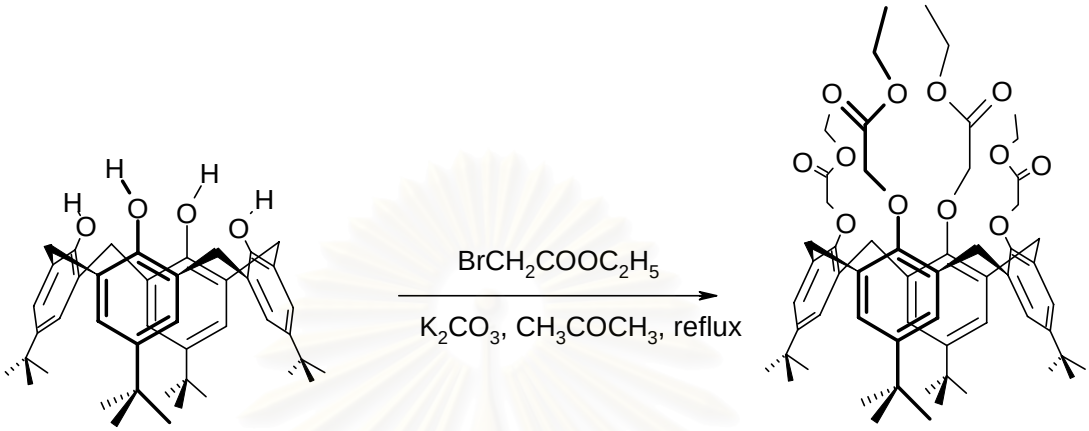


10 **11**

In a 250 mL one-necked round bottom flask equipped with a magnetic bar, 5-amino-25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene **10** (0.837 g, 1.2 mmol), naphthol (5.443 g, 37.8 mmol) and NaOH (1.184 g, 29.6 mmol, in 2 mL H_2O) in acetone (15 mL) and ethanol (10 mL) were stirred at room temperature for 1 h. The aqueous solution of $K_3Fe(CN)_6$ (21.5343 g, 65.4 mmol) was then added dropwise and the mixture was stirred for 40 min at room temperature. The reaction mixture was poured into water (100 mL). The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane. Compound **10** and naphthol were recovered.

Preparation of Fluoroionophore

2.2.12 Preparation of 5,11,17,23-tetra-*p-tert*-butyl-25,26,27,28-tetrakis(ethyoxycarbonylmethoxy)calix[4]arene (12)



1 **12**

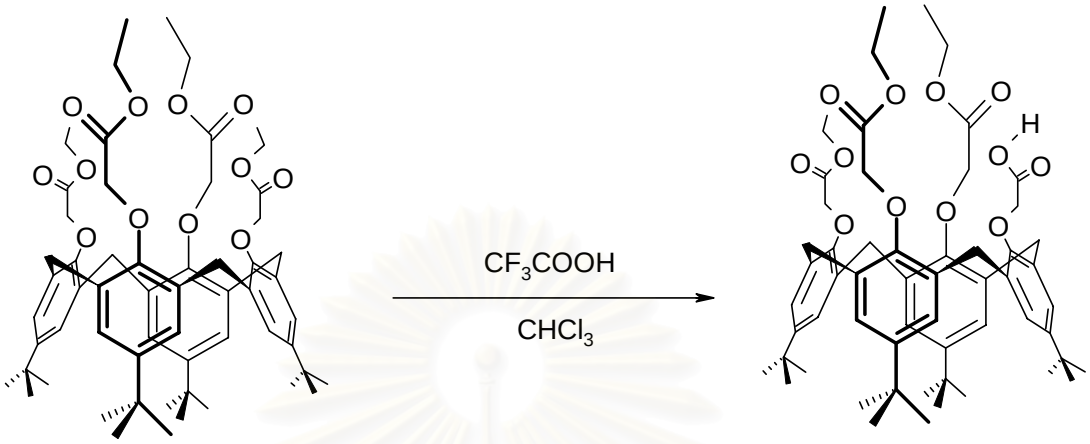
5,11,17,23-tetra-*p-tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene **1** (1.968 g, 3.03 mmol) and potassium carbonate (8.293 g, 60 mmol) was suspended in dry acetone in a 100 mL two-necked round bottom flask (40 mL) and the mixture was stirred at room temperature for 2 h. Ethylbromoacetate (3.34 mL, 30 mmol) was then added and the mixture was heated at reflux for 5 d. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (20 mL) and treated with 2 M hydrochloric acid until no bubble and extracted with dichloromethane (2 x 25 mL). The organic layer was extracted with water (25 mL), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to obtain a yellow oil. Upon adding ethanol, a white solid of **12** was obtained (1.968 g, 68%).

Characterization data for 8

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 6.81 (s, 8H, *m*-Ar*H*), 4.90 (d, *J* = 13 Hz, 4H, ArCH_2Ar), 4.84 (s, 8H, -OCH_2CO), 4.27 (q, *J* = 8 Hz, OCH_2CH$_3$), 3.24 (d, *J* = 14 Hz, 4H, ArCH_2Ar), 1.34 (t, *J* = 7 Hz, 12H, OCH$_2$CH_3), 1.11 (s, 36H, *t*-C$_4H_9$)

2.2.13 Preparation of 5,11,17,23-tetra-*p*-*tert*-butyl-28-carboxymethoxy-25,26,27-tris(ethoxycarbonylmethoxy)-calix[4]arene (13)



12 **13**

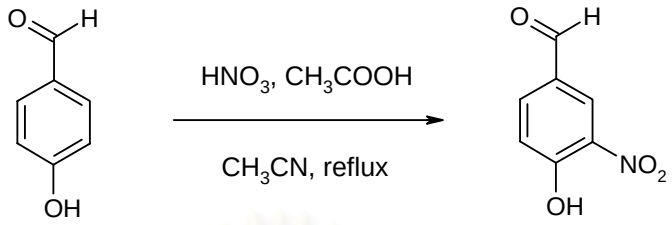
In a 50 mL two-necked round bottom flask, compound **12** (0.509 g, 0.51 mmol) was dissolved in chloroform (10 mL). Trifluoroacetic acid (0.10 mL) was added. The mixture was stirred at room temperature for 24 h., rinsed with dichloromethane and extracted with water (2 x 25 mL). The organic layer was dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure. A white solid of **13** was obtained (0.455 g, 88 %).

Characterization data for 9

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 7.20, 7.19 (d, *J* = 7 Hz, 4H, *m*-Ar*H*), 6.68, 6.58 (d, *J* = 8 Hz, 4H, *m*-Ar*H*), 5.00 (d, *J* = 12 Hz, 1H$_A$, ArC*H*$_2$Ar), 4.90 (d, *J* = 13 Hz, 2H$_A$, ArC*H*$_2$Ar), 4.66 (s, 2H, OC*H*$_2$CO), 4.63 (s, 6H, OC*H*$_2$CO), 4.34 (d, *J* = 16 Hz, 1H$_A$, *ArCH*$_2$*Ar*), 4.30 (m, 6H, OC*H*$_2$CH$_3$), 3.32-3.23 (dd, *J* = 13 Hz, 4H$_B$, *CH*$_2$*ArCH*$_2$), 1.37, 1.36 (s, 27H, *t*-C$_4$*H*$_9$), 1.34-1.32 (t, *J* = 8 Hz, 6H, OCH$_2$C*H*$_3$), 1.13-1.11 (t, *J* = 7 Hz, 3H, OCH$_2$C*H*$_3$), 0.87 (s, 9H, *t*-C$_4$*H*$_9$)

2.2.14 Preparation of 2-nitro-hydroxybenzaldehyde (19)



19

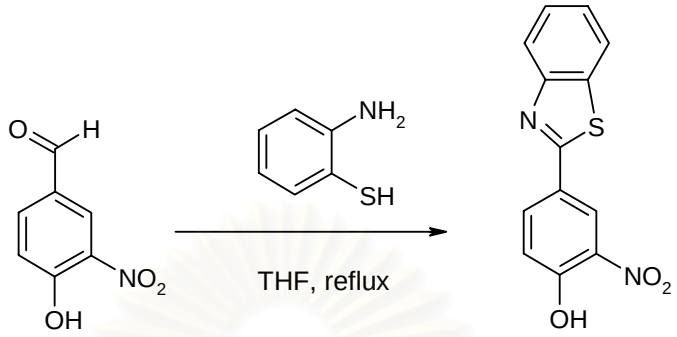
A mixture of *p*-hydroxybenzaldehyde (2.446 g, 20.03 mmol), acetonitrile (40 mL) and glacial acetic acid (20 mL) was stirred in a 100 mL two-necked round bottom flask at room temperature. A solution of concentrated nitric acid (65%, 1.46 mL, 20.05 mmol) in a acetonitrile (20 mL) was then added dropwise over 20 min and refluxed gently under nitrogen for 3 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (50 mL), water (25 mL) and 2 M HCl (10 mL) and extracted with ethylacetate (2 x 50 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to dryness to obtain a yellow oil. Upon adding ethanol, a brown solid of **19** was obtained (3.153 g, 93%).

Characterization data for 19

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 9.98 (s, 1H, ArC*H*O), 8.68 (s, 1H, ArO*H*), 8.19-8.17 (q, *J* = 2 Hz, 1H, *p*-*H*ArNO_2), 7.39 (s, 1H, *o*-*H*ArNO_2), 7.37 (d, *J* = 8 Hz, 1H, *m*-*H*ArNO_2)

2.2.15 Preparation of 5-(1,3-benzothiazol-2-yl-2-hydroxyl) nitrobenzene(20)



19 **20**

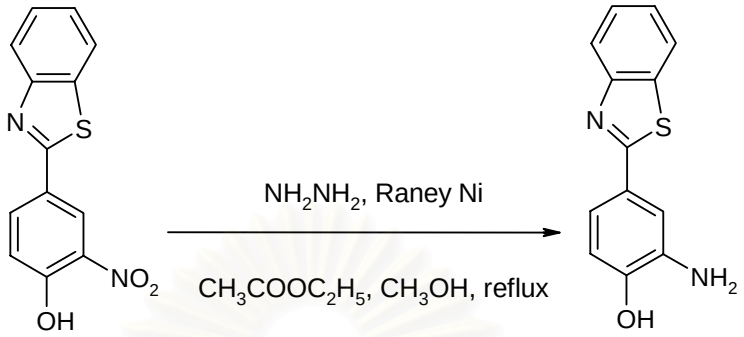
To solution (THF, 20 mL) of 2-nitro-hydroxybenzaldehyde **19** (0.101 g, 0.60 mmol) was added 2-aminothiophenol (0.09 mL, 0.70 mmol). The mixture solution was heated at reflux for 16 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in 25% hexane in dichloromethane and placed on a silica gel chromatography column. Compound **20** was eluted from the column by hexane : chloromethane (1:3) as eluent. Compound **20** was obtained as a yellow solid (0.079 g, 48 %).

Characterization data for 20

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 10.76 (s, 1H, ArO*H*), 8.75 (s, 1H, *H*ArOH), 8.31 (d, *J* = 8 Hz, 1H, ArO*H*), 8.03 (d, *J* = 8 Hz, 1H, ArO*H*), 7.87 (d, *J* = 8 Hz, 1H, Ar*H*), 7.48 (t, *J* = 7 Hz, 1H, Ar*H*), 7.38(t, *J* = 7 Hz, 1H, Ar*H*), 7.26 (d, *J* = 9 Hz, 1H, Ar*H*)

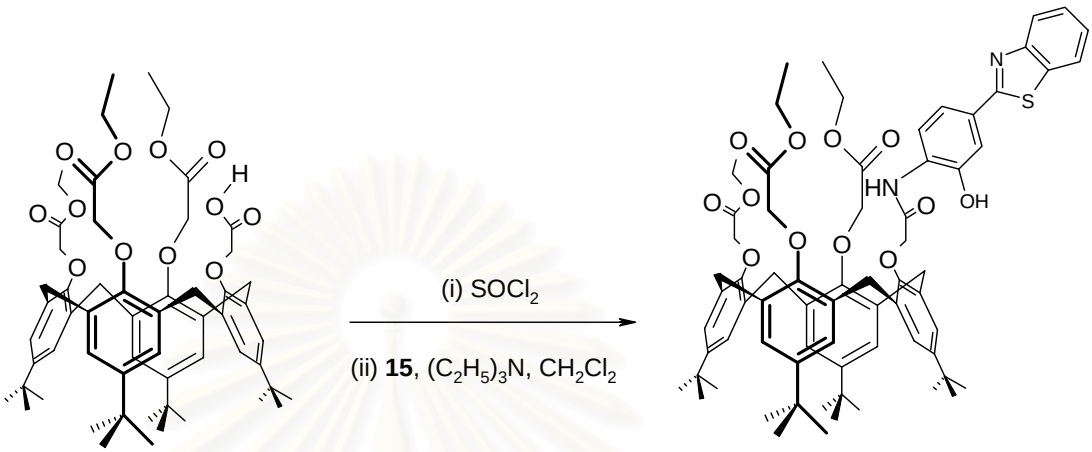
2.2.16 Preparation of 5-(1,3-benzothiazol-2-yl-2-hydroxyl) aminobenzene (15)



20 **15**

Compound **20** (0.174 g, 0.64 mmol) and Raney Ni (0.0338 g, 24.1 mmol) were suspended in the mixture of ethylacetate (19 mL) and methanol (14 mL). Hydrazine (1.3 mL, 179 mmol) was then added into the mixture. The mixture was refluxed for 2 h. and allowed to cool to room temperature. The solvent was subsequently removed under reduced pressure. The residue was dissolved in dichloromethane and extracted with water (5 x 15 mL). The organic layer was separated and dried over anhydrous sodium sulphate. The solvent was removed under pressure to give a pale violet solid **15**. The compound **15** was used immediately for further reaction.

2.2.17 Preparation of 5,11,17,23-tetra -*tert*-butyl-25,26,27-tris(ethoxycarbontlmethoxy)-28-methoxy-N-(1,3-benzothiazol-2yl-2-hydroxyl)amide-calix[4]arene (17)



13 **17**

Under the nitrogen atmosphere, compound **13** (0.323 g, 0.33 mmol) was dissolved in thionyl chloride (2.5 mL) and refluxed for 30 min. Excess thionylchloride was evacuated, the residue was dissolved in dried dichloromethane and the solution again vacuated to remove all thionyl chloride. The white crystalline residue (**14**) was dissolved in dried dichloromethane (10 mL). At 0 ^{o}C, a mixture of compound **15**, triethylamine (0.4 mL) in dried dichloromethane (20 mL) were stirred under nitrogen atmosphere. The solution of the acid chloride compound was transferred into the mixture via cannula. The mixture was stirred at 0 ^{o}C under nitrogen atmosphere for 16 h. It was then washed with water (5 x 15 mL). The organic layer was dried over magnesium sulphate. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on a silica gel with a mixture of hexane and ethylacetate (1:2). The product **17** was isolated as a white solid (0.195 g, 47%).

Characterization data for 17

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 10.11 (s, 1H, ArO*H*), 9.44 (s, 1H, N*H*), 8.48 (s, 1H, *m*-*H*ArOH), 8.08 (d, *J* = 8 Hz, 1H, *m*-*H*ArOH), 7.93 (d, *J* = 8 Hz, 1H, *o*-*H*ArOH), 7.53 (t, *J* = 8 Hz, 1H, Ar*H*), 7.42 (t, *J* = 8 Hz, 1H, Ar*H*), 7.18 (d, *J* = 9 Hz, 1H, Ar*H*), 6.94 (d, *J* = 6 Hz, 1H, Ar*H*), 6.92, 6.81 (s, 8H, Ar*H*-calix), 5.10 (d, *J* = 16 Hz, $1H_A$, ArCH_2Ar), 4.95 (d, *J* = 13 Hz, $1H_A$, ArCH_2Ar), 4.83 (s, 2H, OCH_2), 4.75 (d, *J* = 14 Hz, $2H_A$, ArCH_2Ar), 4.73(s, 6H, OCH_2), 4.67 (d, *J* = 16 Hz, $1H_B$, ArCH_2Ar), 4.27 (q, *J* = 7 Hz, 2H, CH_2CH_3), 4.03-3.91 (m, 4H, CH_2CH_3), 3.30 (d, *J* = 14 Hz, $3H_B$, ArCH_2Ar), 1.3 (t, *J* = 8 Hz, 3H, CH_3), 1.18(s, 9H, *t*-C_4H_9), 1.08 (s, 27H, t-C_4H_9), 1.07 (t, H, *J* = 8 Hz, CH_3).

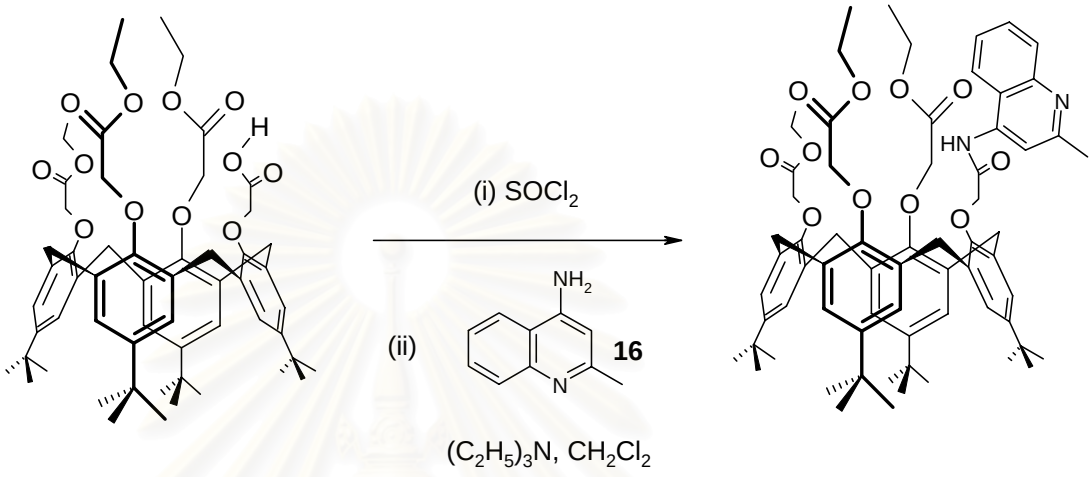
ESI-TOF mas spectrum : $C_{71}H_{84}N_2O_{12}S$, m/z = 1212 ($M+Na^+$)

Elemental Analysis

Anal calc. for $C_{71}H_{84}N_2O_{12}S$: C, 71.69; H, 7.12; N, 2.36

Found : C, 71.66; H, 7.17; N, 2.35

2.2.18 Preparation of 5,11,17,23-tetra -*tert*-butyl-25,26,27-tris(ethoxycarbonylmethoxy)-28-methoxy-N-(4-aminoquinaldine)amidecalix[4]arene (18)



13 **18**

Compound **18** was synthesized by a similar procedure to compound **17**, but fluorophore **15** was replaced by 4-aminoquinalidine **16** (0.089 g, 25 %)

Characterization data for 18

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

δ 9.90 (s, 1H, N*H*), 8.19 (d, *J* = 8 Hz, 1H, Ar*H*), 8.12 (d, *J* = 8 Hz, 1H, Ar*H*), 7.74 (t, *J* = 7 Hz, 1H, Ar*H*), 7.70 (t, *J* = 9 Hz, 1H, Ar*H*), 7.18 (s, 1H, *H*ArN), 6.87, 6.81 (s, 8H, Ar*H*-calix), 4.97 (s, 2H, OCH_2), 4.81 (s, 6H, OCH_2), 4.76 (m, 4H, ArCH_2Ar), 3.81 (m, 2H, CH_2CH$_3$), 3.66 (m, 4H, CH_2CH$_3$), 3.46 (m, 4H, ArCH_2Ar), 2.80 (s, 3H, CH_3ArN), 1.28 (s, 9H, *t*-C$_4H_9$), 1.20 (s, 27H, *t*-C$_4H_9$), 1.00 (t, *J* = 7 Hz, 6H, CH_3), 0.90 (t, *J* = 7 Hz, 3H, CH_3)

ESI-TOF mas spectrum : $C_{68}H_{84}N_2O_{11}$, m/z = 1128 ($M+Na^+$)

Elemental Analysis

Anal calc. for $C_{68}H_{84}N_2O_{11}.CH_2Cl_2$: C, 69.62; H, 7.28; N, 2.35

Found: C, 70.89; H, 7.61; N, 2.53

2.3 Cation complexation studies of ligand by fluorescence titrations

A solution of $5.1x10^{-6}$ M of a ligand in a 0.01 M tetrabutyl ammonium perchlorate were prepared by adding 5 mL of a stock solution of ligand ($5.1x10^{-5}$ M) in a 50 mL volumetric flask. A stock solution of $2.04x10^{-3}$ M of an alkali metal ion (Li^+, Na^+ and K^+ as perchlorate salt) in dried methanol were prepared in a 25 mL volumetric flask.

Fluorescence spectra of all ligands and cation complexes were recorded at ambient temperature. The solution of a guest was added directly to 2.00 mL of $5.1x10^{-5}$ M ligand in a cuvette by microburette and stirred for 40 seconds. Fluorescence spectra were measured after each addition. Table 2.1 shows guest : host ratios used in titration experiments.

Table 2.1 Amounts of solutions of the alkali metal ion used to prepare various alkali metal ion: ligand ratios

Ratio of cation : ligand	Volume cation added(mL)
0.0 : 1.0	0
8.0 : 1.0	0.04
20.0 : 1.0	0.06
40.0 : 1.0	0.10
60.0 : 1.0	0.10
80.0 : 1.0	0.10
100.0 : 1.0	0.10
200.0 : 1.0	0.50
300.0 : 1.0	0.50

2.4 Determination of quantum yield of fluoroionophores 17 and 18

The standard should be chosen to ensure it absorption at the excitation wavelength of choice for the test sample, and, if possible, emit in a similar region to the test sample. In addition, the chosen concentration range of standard solution and sample solution was prepared according to absorbance in the 10 mm fluorescence cuvette should never exceed 0.1 at the excitation wavelength.

The standard used was anthracene which was prepared in a concentration range of 2.37 – 14.23 micromolar. The concentration range of **17** and **18** were 2.27 - 6.82 and 5.28 - 42.26 micromolar, respectively. All of them were dissolved in ethanol.

Procedure for determination of quantum yield: (i) Record the UV-vis absorbance of each solution, (ii) Record the fluorescence spectrum of the same solution in the same cuvette. Calculate and note down the integrated fluorescence intensity. (iii) Plot a graph of the integrated fluorescence intensity *vs* absorbance. The result should be a straight line with gradient (Grad.) and intercept = 0.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Design concept

It is known that calix[4]arene can be modified to be a receptor for alkali metal ions. For example calix[4]arene tetraester is a good receptor for sodium ion, while calix[4]arene triester is a good receptor for lithium ion. Chromophores and fluorophores can be attached to the narrow or wide rim of calix[4]arene. Chromophores can be signaling units by giving different color in the presence and absence of metal ions whereas fluorophores can give emission signals that are sensitive to guest binding.

The alkali metal ion receptors containing chromophores and fluorophores by attaching on the wide rim of calix[4]arenes were designed. Receptor units are put on the narrow rim of calix[4]arenes. Another fabrication for calix[4]arenes containing fluorophores is to put both receptor units and signalling units on the narrow rim.

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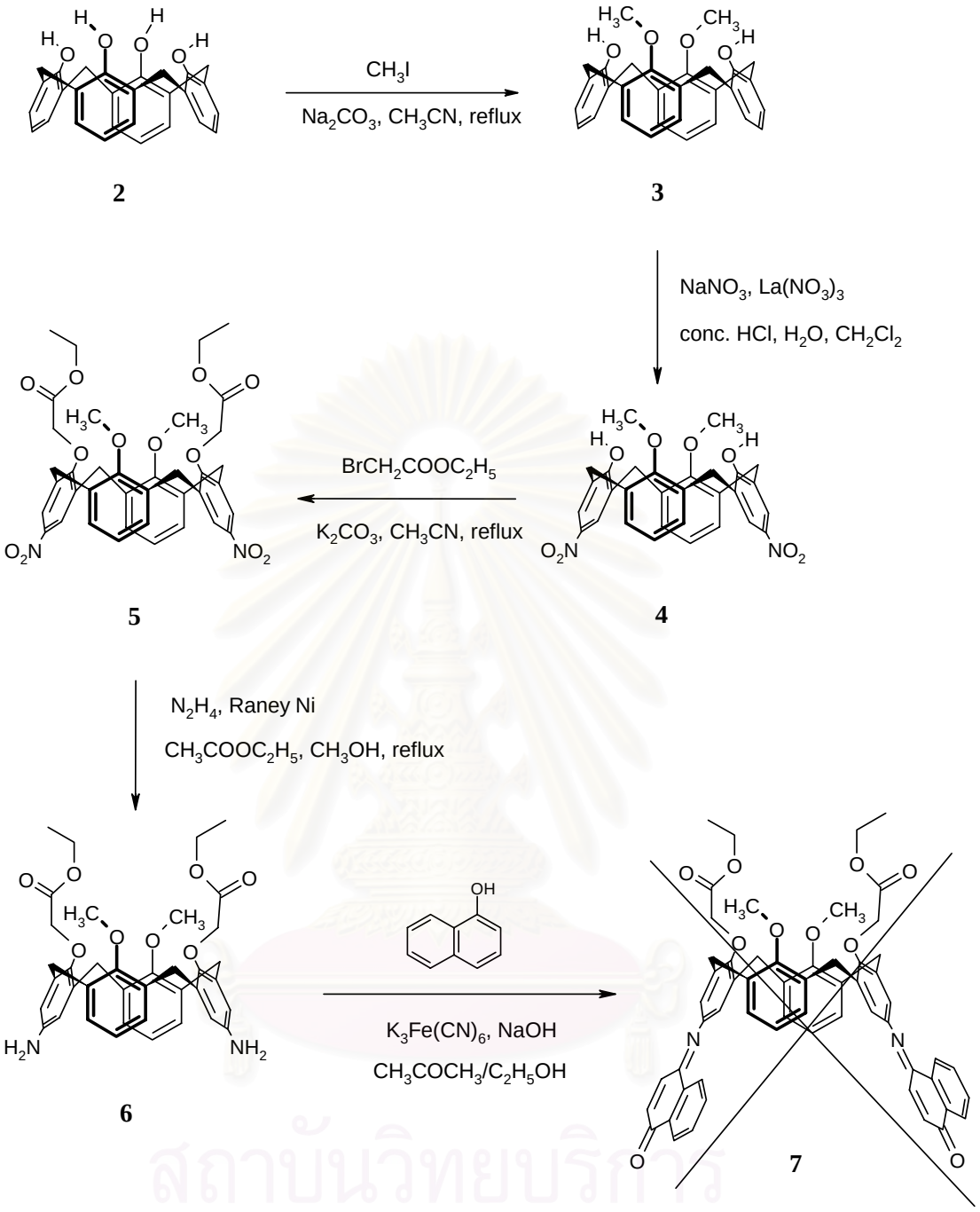
3.2 Attempts to synthesize calix[4]arenes containing fluorophores

3.2.1 Synthesis and characterization of compound 7

Synthesis of compounds **1** and **2** were carried out following the literature procedure. [49-50] Synthesis and characterization of compound **7** was outlined in Scheme 3.1.



Scheme 3.1 Synthesis pathway of compound **7**

In the first step, calix[4]arene **2** was reacted with iodomethane in the presence of sodium carbonate or potassium carbonate in acetonitrile. [51-52] Nitration with sodium nitrate, in an aqueous hydrochloric acid solution and a catalytic amount of $La(NO_3)_3$, in dichloromethane produced compound **4**. [51-52]

According to the method described in the literature, [4,51,53] the reaction of compound **4** with ethylbromoacetate was carried out in the presence of sodium hydride in THF or DMF. Unfortunately no matter how hard it was tried, this reaction did not give the desired product.

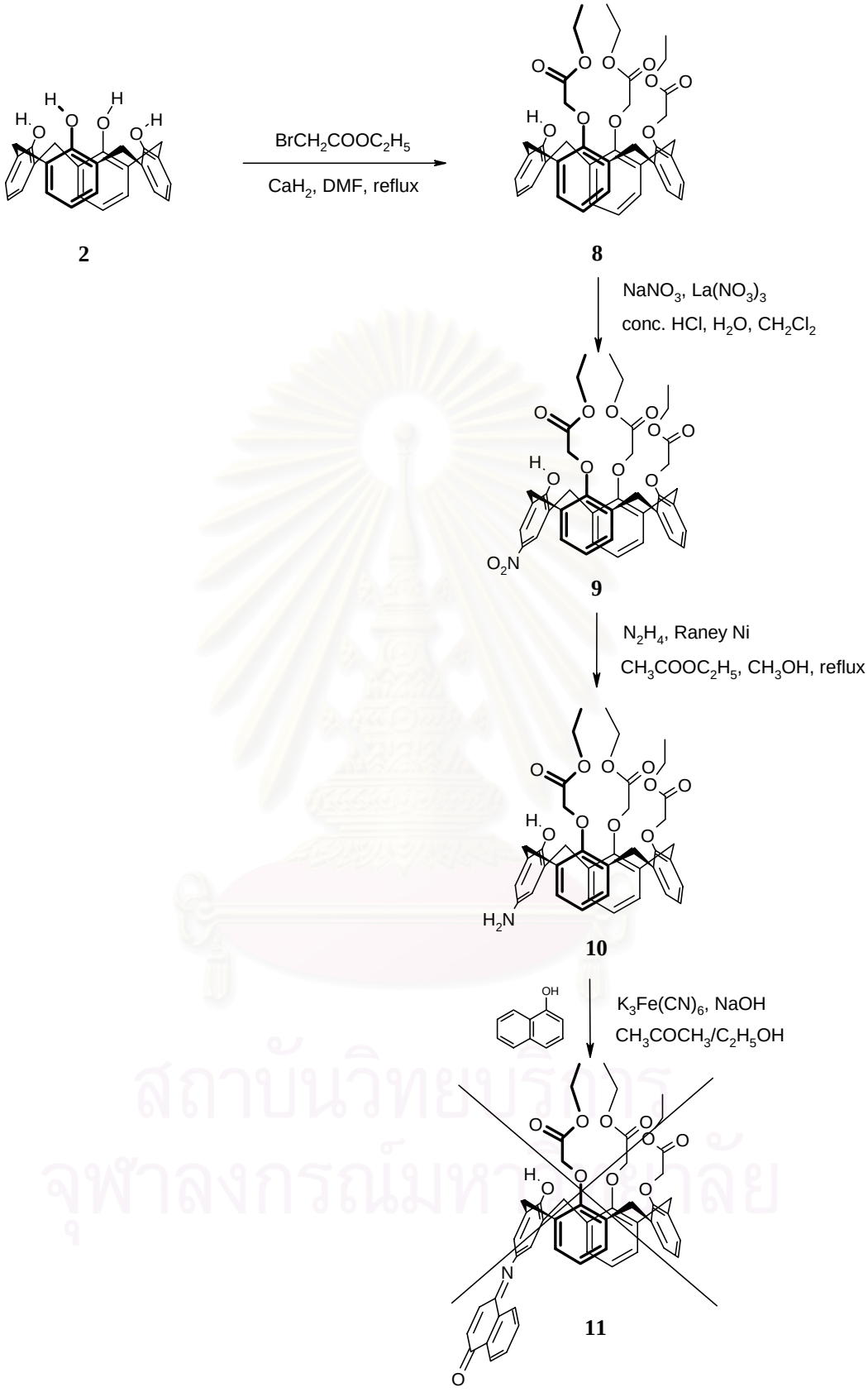
Therefore, compound **5** was synthesized by the reaction between compound **4** and ethylbromoacetate in the presence of potassium carbonate as base and acetonitrile as solvent for 2 days. The yield of compound **5** was 50% and the structure was confirmed by ^{1}H-NMR (Figure A5). Reduction of **5** by Raney nickel and hydrazine gave a white solid **6**.

The coupling reaction of compound **6** with α-naphthol was carried out using air as oxidant [38] and NaOH as base. The desired product cannot be obtained. Although this reaction was carried out using $K_3Fe(CN)_6$ as oxidant, [14,36-37] the product **7** was not obtained probably because *para*-substitution of amine in compound **6** was not a good donor group.

3.2.2 Attempt to synthesize of compound 11

The synthesis of compound **11** was outlined in Scheme 3.2.

According to the method described in the literature, the trisubstituted calix[4]arene can be synthesized by the reaction of calix[4]arene with alkylating agents in the presence of BaO or $Ba(OH)_2$ in DMF [54] or by reaction of calix[4]arene by $BrCH_2Ph$, followed with $BrCH_2COOC_2H_5$ in the presence of K_2CO_3 in CH_3CN and reductive dealkylation with Pd/C, HCO_2NH_4 in ethanol. [55-56] However, alkylation with ethylbromoacetate and BaO or $Ba(OH)_2$ as base causes the decomposition of the ester. [33] This reaction thus was used CaH_2 as base in DMF [33] instead. It was found that CaH_2 must be freshly opened and DMF must be freshly distilled before use.



Scheme 3.2 Synthesis pathway of compound **11**

Therefore, compound **8** was synthesized by the reaction between calix[4]arene and CaH_2 as base in freshly distilled DMF as solvent for 1 day. The reaction temperature was kept at 70°C. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 added with 2 M HCl and then stirred overnight. The structure was confirmed by ^{1}H-NMR (Figure A6).

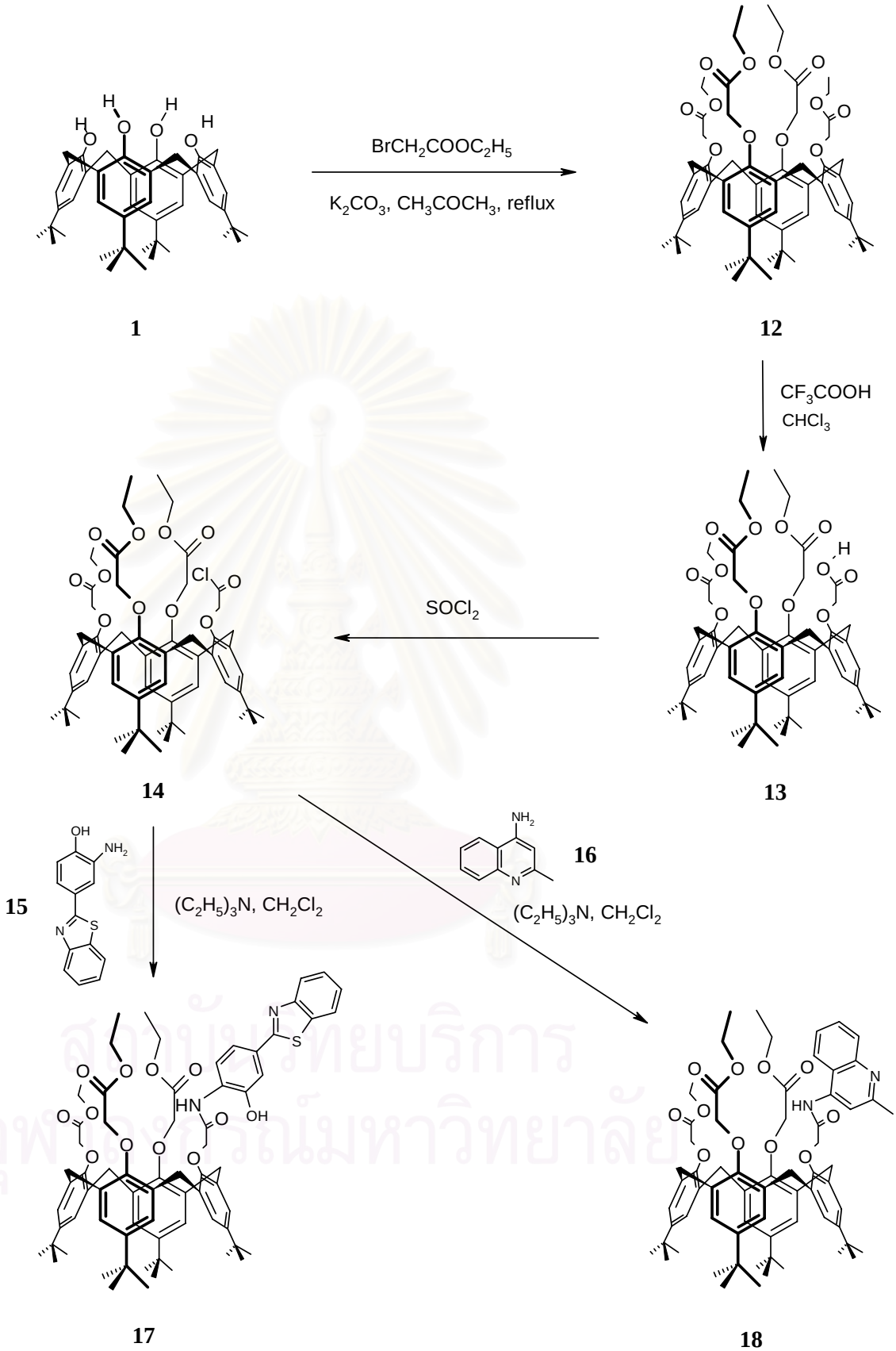
The coupling reaction of compound **10** with α-naphthol was carried out using $K_3Fe(CN)_6$ or air as oxidant in NaOH as base. The starting material was recovered from the reaction and no desired product was found.

3.3 Synthesis and characterization of calix[4]arene containing fluorophores

3.3.1 Synthesis and characterization of compounds 17 and 18

The synthetic pathway of compounds **17** and **18** was illustrated in Scheme 3.3.

Compound **12** was obtained by reaction of compound **1** with 10 equivalents of ethyl bromoacetate in the presence of potassium carbonate as base and dried acetone as solvent for 5 days. [29] Only one spot was observed in the TLC plate using 8% MeOH in CH_2Cl_2 as eluent and *p-tert*-butylcalix[4]arene diester was not observed. The yield of compound **12** was 66%. ^{1}H-NMR spectrum of compound **12** showed a triplet of CH_3 and a quartet of CH_2 at 1.34 and 4.27, respectively (Figure A9). Compound **12** existed in a cone confirmation as evidenced by two doublets of $ArCH_2Ar$ (AB system) appearing at 4.90 and 3.24 ppm with the coupling constant (J = 13 Hz) as well as a singlet peak of *tert*-butyl protons at 1.11 ppm.



1 **12** **13** **14** **15** **16** **17** **18**

Scheme 3.3 Synthesis pathway of **17** and **18**

The hydrolysis of one ester unit with a specific reagent, trifluoroacetic acid, in chloroform resulted in a white solid **13**. [57] ^{1}H-NMR (Figure A10) spectrum of **13** showed a splitting of $ArCH_2Ar$ (AB system), *m*-ArH and CH_2 of C_2H_5 signals indicated an asymmetric structure of **13**.

For fluorophore **15** , nitration of *p*-hydroxybenzaldehyde using acetic acid and nitric acid in acetonitrile resulted in a brown solid **19** (93%). Condensation of **19** with *o*-aminothiophenol in tetrahydrofuran gave compound **20** as a yellow-green solid (45%). Reduction of **20** by Raney nickel and hydrazine yielded **15** as a white solid. To avoid the decomposition, compound **15** was used immediately for further reaction.

Compound **14** was prepared by chlorination reaction with thionyl chloride and further reacted immediately with 2 equilvalents of fluorophore **15** or **16** in dichloromethane *via* cannular under nitrogen atmosphere. The final products **17** and **18** were obtained in 47% and 25% yields, respectively.

The characterization of **17** was carried out by ^{1}H-NMR spectroscopy (Figure A11), elemental analysis (Table 3.1) and mass spectrometry (Figure 3.1). The results agree with the proposed structure.

Table 3.1 Elemental analysis data for **17**

	%C	**%H**	**%N**
Calculated	71.69	7.12	2.36
Found	71.66	7.17	2.35

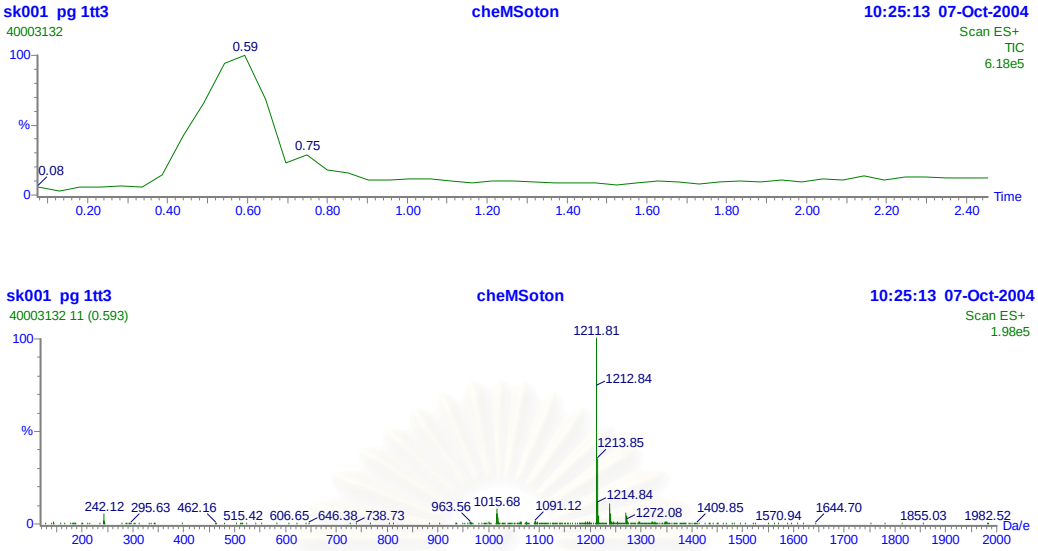


Figure 3.1 The mass spectrum of **17**

For compound **18**, the structure was confirmed by ^{1}H-NMR spectroscopy (Figure A12), elemental analysis (Table 3.2) and mass spectrometry (Figure 3.2). The results agree with the proposed structure.

Table 3.2 Elemental analysis data for **18.CH_2Cl_2**

	%C	**%H**	**%N**
Calculated	69.66	7.29	2.36
Found	70.89	7.61	2.53

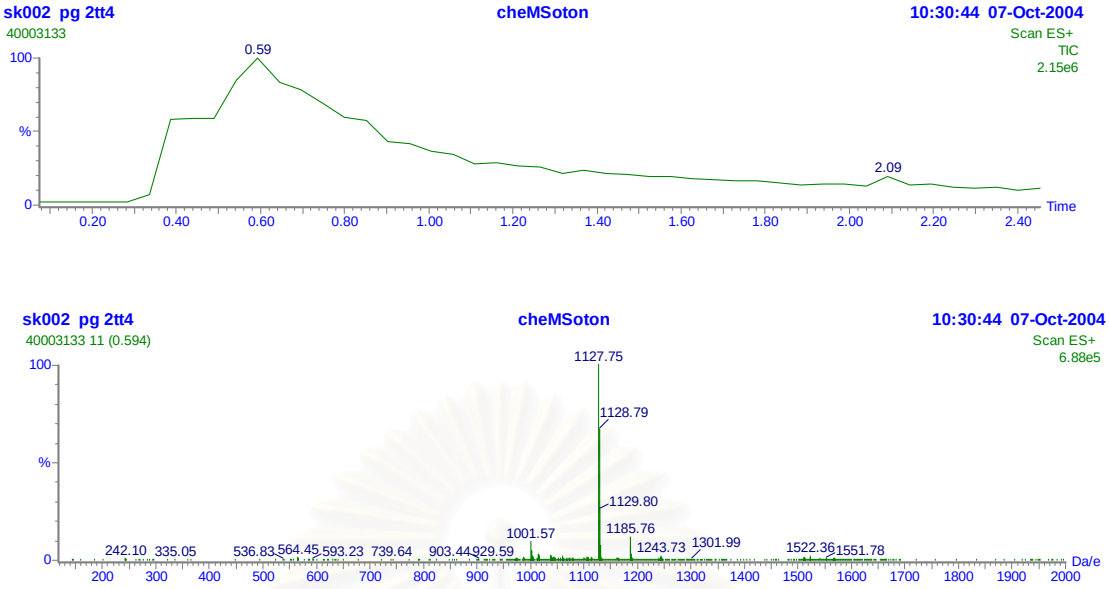


Figure 3.2 The mass spectrum of **18**

The typical AB pattern for the methylene bridge protons (J = 13-16 Hz) [52] of ^{1}H-NMR spectra of **17** and **18** indicate that calix[4]arene unit is in the cone conformation . Signals of the amide protons of both **17** and **18** appear at 9.44 and 9.90 ppm, respectively suggesting the existence of the intramolecular hydrogen bonding.

3.4 Cation complexation studies

Compounds **17** and **18** contain the tetraester group which binds alkali metal ions. Thus complexation studies of compounds **17** and **18** with Li^{+}, Na^{+} and K^{+} were carried out in polar solvents such as methanol and ethanol.

Compounds **17** and **18** consisted of 5-(1,3-benzothiazol-2-yl)-2-hydroxyl-aminobenzene **15** and 4-aminoquinaldine **16** as two different fluorophores. The cation recognition *via* ion-dipole interactions can also be easily monitored by cation complexation induced change in fluorescence intensity by fluorimetric titrations.

3.4.1 Complexation studies of compounds 17 and 18 to determine the selectivity

From fluorimetric titrations of compound **17** with alkali metal ions, the fluorescence intensity of **17** upon addition of alkali metal ions are shown in Table 3.3, and Figure 3.3.

Table 3.3 Fluorescence intensity of **17** upon addition of alkali metal ions

equivalent	Fluorescence intensity (a.u.)			
	Ligand (**17**)	Li-complex	Na-complex	K-complex
8	240.66	248.21	238.82	235.64
20	240.66	249.33	231.48	233.63
40	240.66	250.13	225.55	232.55
60	240.66	250.94	219.14	231.68
80	240.66	252.29	212.65	231.54
100	240.66	252.39	205.73	236.32
200	240.66	251.75	200.70	235.29

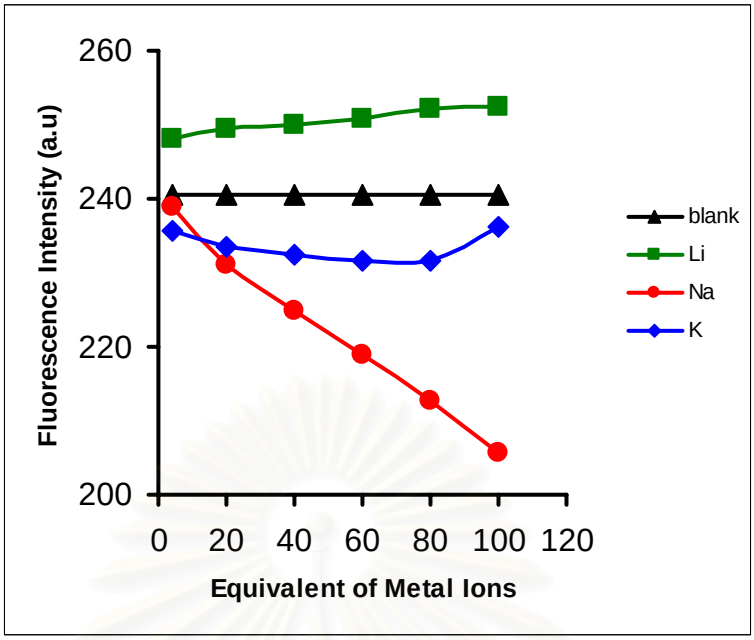


Figure 3.3 A change of fluorescence intensity of fluoroionophore **17** upon addition of the alkali metal ions up to 100 equiv. [**17**] = 5.1×10^{-6} M in methanol

From fluorimetric titrations of compound **18** with alkali metal ions, the fluorescence intensity of **18** upon addition of alkali metal ions are shown in Table 3.4, and Figure 3.4

.

Table 3.4 Fluorescence intensity of **18** upon addition of alkali metal ions

Equivalent	Fluorescence intensity(a.u.)			
	Ligand (**18**)	Li-complex	Na-complex	K-complex
8	48.92	50.27	48.74	49.23
20	48.92	52.53	48.67	48.95
40	48.92	52.45	48.41	48.98
60	48.92	51.57	48.18	48.60
80	48.92	52.67	47.89	48.99
100	48.92	52.54	47.52	48.87
200	48.92	51.99	46.51	47.62

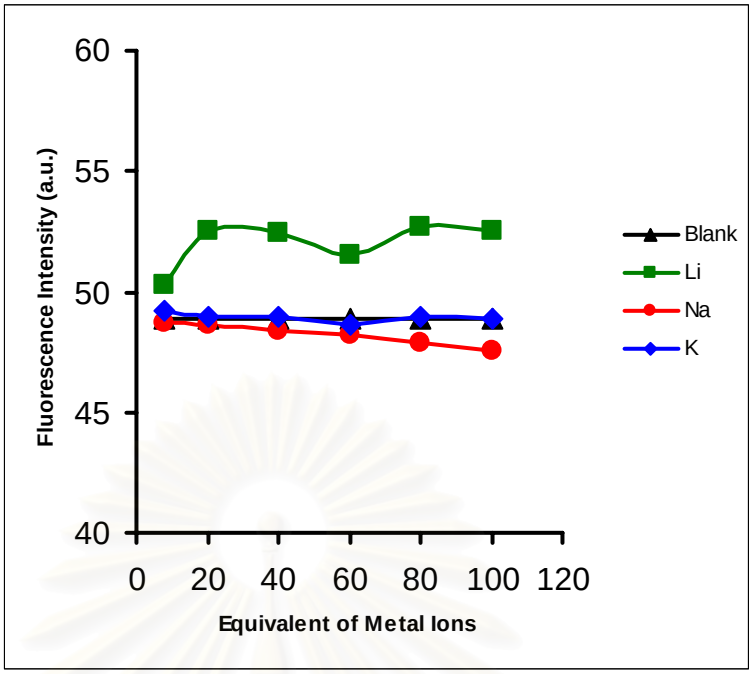


Figure 3.4 A change of fluorescence intensity of fluoroionophore **18** upon addition of the alkali metal ions up to 100 equiv. [**18**] = 5.1×10^{-6} M in methanol

Unfortunately, Table 3.4 and Figure 3.4 indicate that fluorescence intensity of fluoroionophore **18** was very low. Therefore, addition of the alkali metal ions to the solution of **18** gave a negligible change in its emission spectra or intensity. Therefore, the fluoroionophore **17** was subjected for further investigation and it binds with Na^+ selectively while other metal ions such as Li^+ and K^+ gave no significant response.

3.4.2 Complexation studies of compound 17

Figure 3.5 shows the absorption and emission spectra of **17** in methanol. It was found that the excitation wavelength and the emission wavelength were 370 nm and 442 nm, respectively.

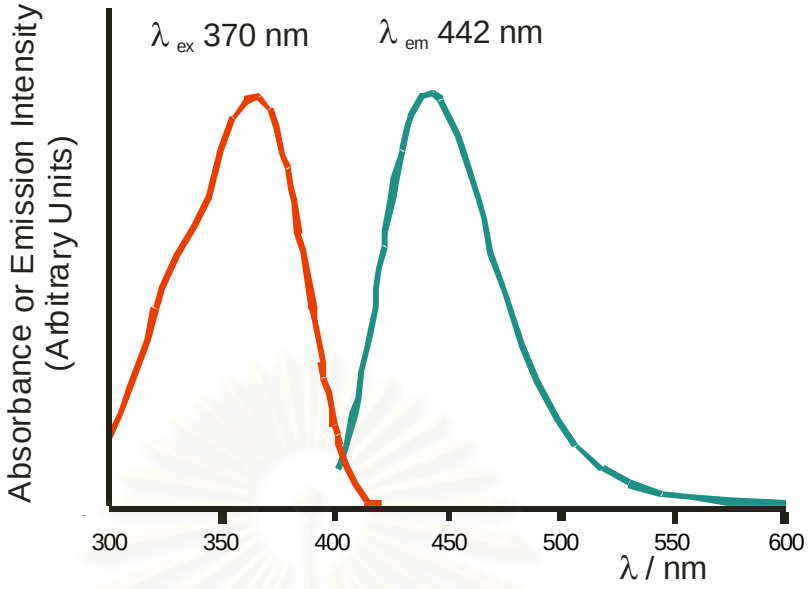


Figure 3.5 The absorption and the emission spectra of fluoroionophore **17** in methanol

The emission spectra (λ_{ex} = 370 nm) of **17** (5.1 x 10^{-6} M) in the presence of various equivalent of Na^+ are shown in Figure 3.6 from which it can be seen that the fluorescence intensity (λ_{em} = 442 nm) of **17** decreases continually upon addition of Na^+ with no significant change in the position of the emission maxima. Therefore, compound **17** is sensitive to Na^+.

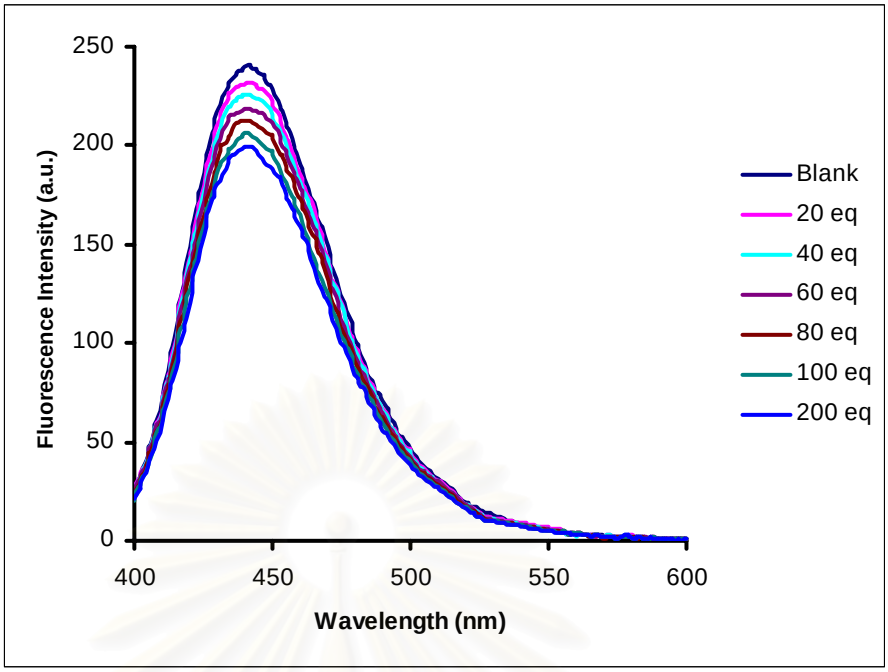


Figure 3.6 Emission spectra of **17** ($5.1x10^{-6}$M) was quenched by $NaClO_4$ (λ_{ex} =370 nm) in methanol

When a graph was plotted between fluorescence intensity and concentration of sodium ion, the sensitivity of **17** towards Na^+ was found to be 83 a.u./nM.

The ^{1}H-NMR spectrum of fluoroionophore **17** also showed that the signals due to NH, OH, aromatic proton on the benzylbenzothiazol group and H_A ($ArCH_2Ar$) shifted upfield, while the signals corresponding to H_B, $ArCH_2Ar$, Ar-calix and $OCH_2(C=O)$ shifted downfield (Table 3.5) upon complexation with Na^+. This probably indicates that the complexation of Na^+ into the cavity of **17** induces a break of intramolecular hydrogen bonding between RNH-(C=O) and the adjacent oxygen atom [58-59] (Figure 3.7) because the lone pair of nitrogen of amide NH donated to benzylbenzothiazol group with results in the upfield shift and Na^+ also withdrawn the electron cloud from aromatic units of the calixarene with results in the downfield shift.

Table 3.5 ^{1}H-NMR chemical shift (ppm) for compound **17** (in $CDCl_3$) in the absence and presence of Na^+

^{1}H-NMR in species	Absence of Na^+	Presence of Na^+
ArOH	10.11	9.86
NH	9.44	9.29
m-ArOH	8.48	8.25
H_A, $ArCH_2Ar$	5.10, 4.95	4.77, 4.71
H_B, $ArCH_2Ar$	3.30	3.49, 3.41
Ar-calix	6.92, 6.81	7.14
$OCH_2(C=O)$	4.83, 4.73	4.90

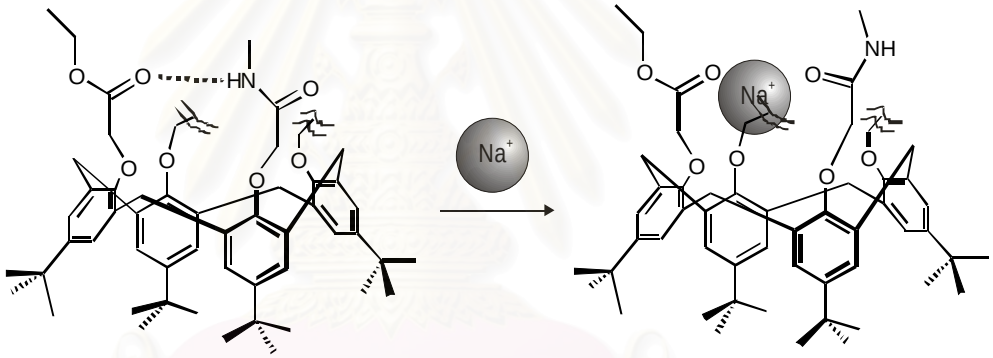


Figure 3.7 The probability of complexation of Na^+ into the cavity of **17** (two ester bonds are omitted for clarity)

The possible quenching mechanism is that the free fluoroionophore **17** has intramolecular hydrogen bonding. Then the HOMO of receptor (amide nitrogen) becomes lower energy than the HOMO of fluorophore. The excited electron can go back to group state and emit light. Upon binding with Na^+, the hydrogen bond is broken. Therefore, the HOMO of receptor becomes higher energy than the HOMO of fluorophore. The excited electron cannot go back to ground state and the fluorescence is quenched. [60]

3.4.3 Complexation studies of compound 17 to determine the stability constant

Figure 3.8 shows changes in emission spectra of fluoroionophore **17** (recorded in methanol and 0.01M tetrabutylammonium perchlorate at a concentration of 5.1 x 10^{-6} M) upon addition of sodium perchlorate (2.4 x 10^{-3} M in methanol). The fluorimetric titration was performed 3 times and the maxima fluorescence intensity was recorded in Table 3.6. When I^{o}_{F} / (I_F - I^{o}_{F}) was plotted against the reciprocal of the Na^+ concentration $[M]^{-1}$ (Figures 3.8-3.10), the stability constant is obtained from the ratio of intercept/slope (Table 3.7) with log K of 2.91 ± 0.03 and when I_F is plotted against the Na^+ concentration (nM).

Table 3.6 The fluorescence intensity of compound **17** with adding Na^+ (I^o_F = 240.67)

Titration	Equiv. Na^+	$[Na^+]$ (mM)	I_F (a.u.)	$I_F - I^o_F$ (a.u.)	$I^o_F / I_F - I^o_F$	1/[Na+] (M^{-1})
1	20	0.09714	231.48	-9.19	-26.20	1029.1
	40	0.18546	225.55	-15.12	-15.92	5392.2
	60	0.26609	219.14	-21.52	-11.18	3758.2
	80	0.34000	212.65	-28.02	-8.59	2941.2
	100	0.40800	205.73	-34.94	-6.89	2451.0
	200	0.68000	200.70	-39.96	-6.02	1470.6
2	20	0.09714	232.34	-9.16	-26.65	1029.1
	40	0.18546	226.63	-14.77	-16.34	5392.2
	60	0.26609	218.45	-22.94	-10.52	3758.2
	80	0.34000	214.60	-26.80	-9.01	2941.2
	100	0.40800	207.71	-33.69	-7.17	2451.0
	200	0.68000	197.81	-43.59	-5.54	1470.6
3	20	0.09714	233.52	-7.87	-30.66	1029.1
	40	0.18546	227.53	-13.86	-17.41	5392.2
	60	0.26609	223.24	-18.16	-13.29	3758.2
	80	0.34000	218.64	-22.76	-10.61	2941.2
	100	0.40800	212.35	-29.05	-8.31	2451.0
	200	0.68000	204.74	-36.66	-6.58	1470.6

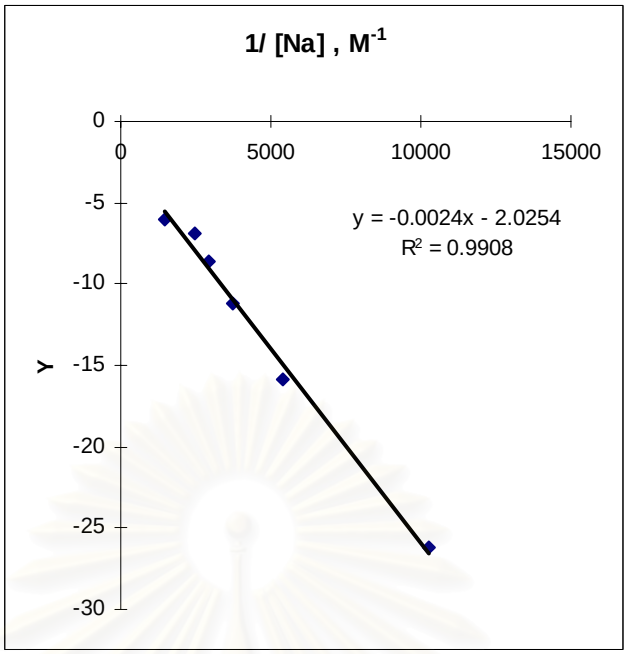


Figure 3.8 The linear plot between Y{ = I^{o}_{F} /(I_F - I^{o}_{F})}and 1/[Na^{+}] for the first fluorimetric titration

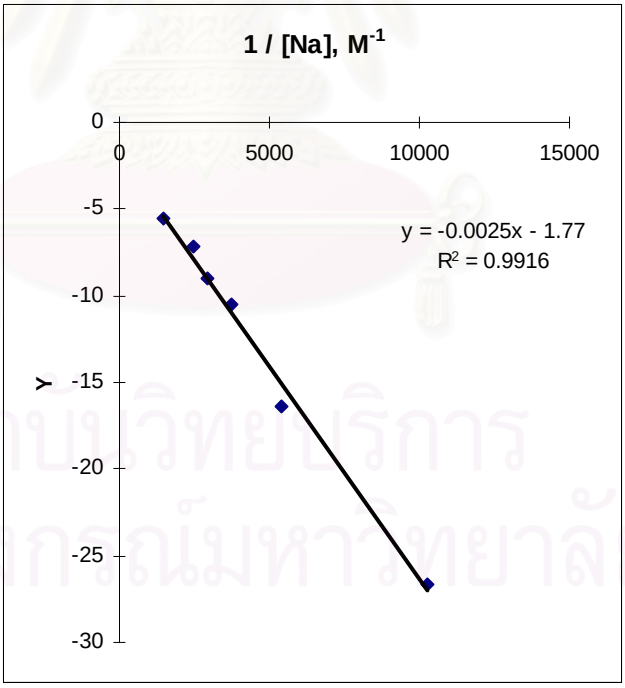


Figure 3.9 The linear plot between Y{ = I^{o}_{F} /(I_F - I^{o}_{F})} and 1/[Na^{+}] for the second fluorimetric titration

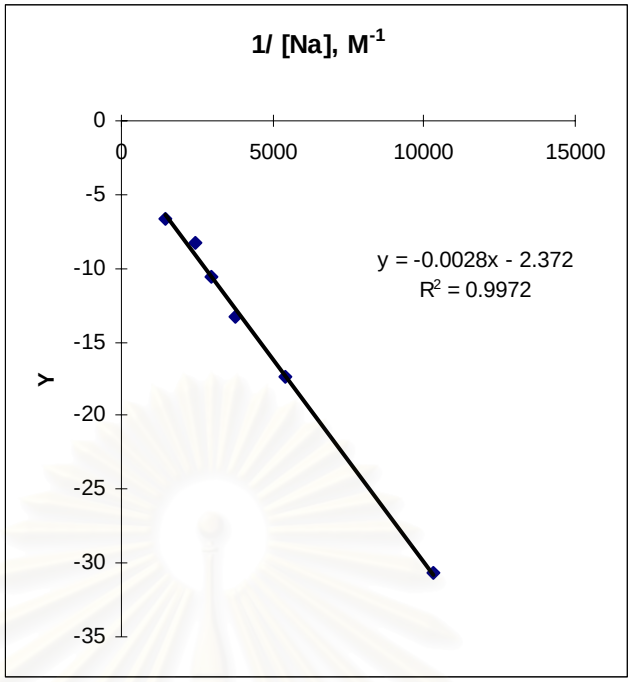


Figure 3.10 The linear plot between Y{ = I^o_F /(I_F - I^o_F)} and 1/[Na^+] for the third fluorimetric titration

Table 3.7 The stability constant of **17** towards Na^+

Titration	Intercept	slope	R^2	Log K
1	-2.025	-0.002381	0.9908	2.93
2	-1.770	-0.002456	0.9916	2.86
3	-2.372	-0.002761	0.9972	2.93
Average stability constant				2.91 ±0.03

3.5 Determination of quantum yields

Condition to determine quantum yields showed in Table 3.8 and the fluorescence spectrum was measured in between 400 to 600 nm.

Table 3.8 Condition for determination of the quantum yields

Compound	$\lambda_{ex(nm)}$	$\lambda_{em(nm)}$
Anthracence (STD)	375	422
17	370	442
18	380	450

The maximum absorbance and the fluorescence spectrum of the standard anthracene solution, the solutions of **17** and **18** were recorded (Table 3.9). When the integrated fluorescence intensity was plotted against absorbance of each compound (Figure 3.11-3.13), the gradient (slope) was determined. Finally, the quantum yields can be calculated (Table 3.10).

Table 3.9 Experimental data for determining the quantum yields

Compound	[Compound] μM	A	Integrated Fluorescence intensity
Anthracene	0	0	0
	2.371	0.017	2344
	4.742	0.034	4687
	7.113	0.051	7031
	9.484	0.068	9374
	14.226	0.102	14061
17	0	0	0
	2.273	0.030	10172
	3.409	0.045	15383
	4.546	0.060	20493
	5.682	0.075	25479
	6.818	0.090	30766
18	0	0	0
	10.564	0.020	97
	15.846	0.030	146
	21.128	0.040	194
	26.410	0.050	243
	42.256	0.080	388

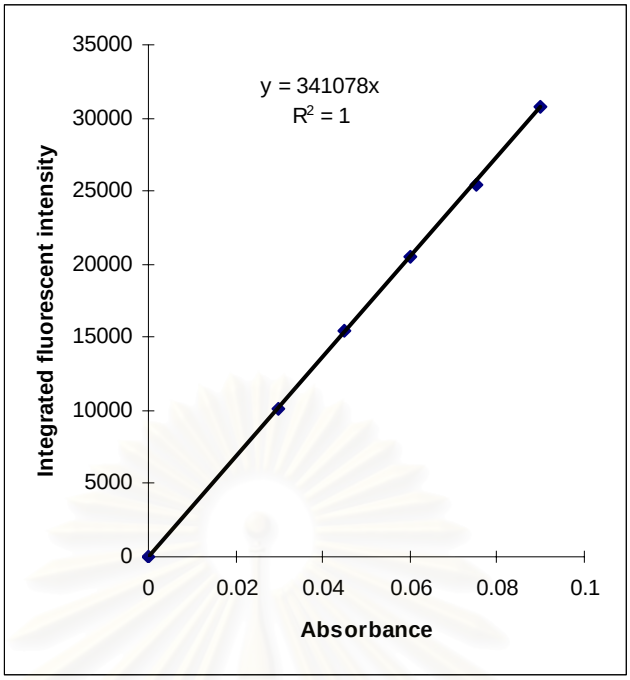


Figure 3.11 The linear plot between integrated fluorescence intensity (arbitrary units) and absorbance for **17**.

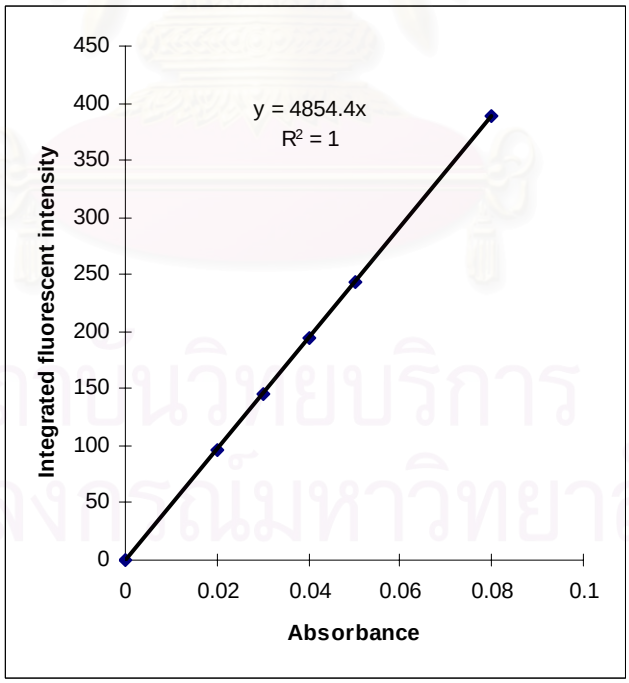


Figure 3.12 The linear plot between integrated fluorescence intensity (arbitrary units) and absorbance for **18**.

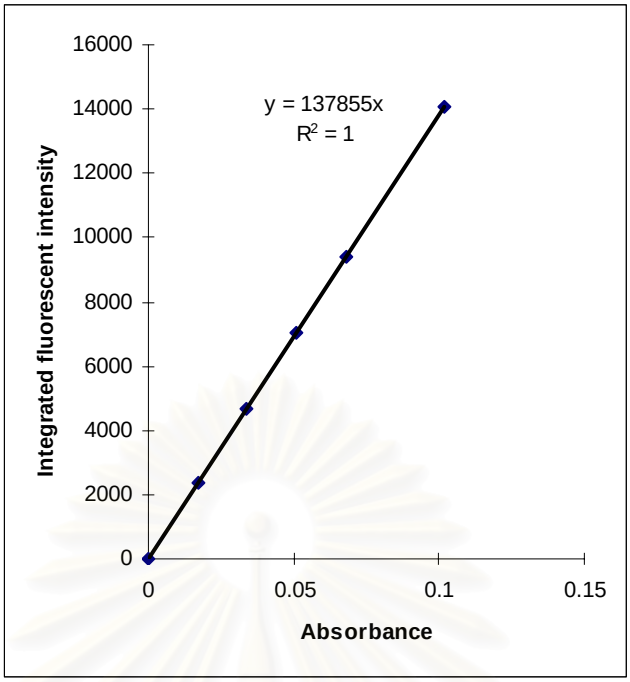


Figure 3.13 The linear plot between integrated fluorescence intensity (arbitrary units) and absorbance for **anthracence**.

Table 3.10 The quantum yields for fluoroionophores **17** and **18** in ethanol

Compound	Grad	R2	Quantum yields
Anthracene	137855	1	0.27 [61]
17	341078	1	0.67
18	4854.4	1	0.01

From Tables 3.9 and 3.10, the value of quantum yields is corresponding to the absorbance and integrated fluorescence intensity. The more absorbance and integrated fluorescence intensity resulted in the more quantum yields.

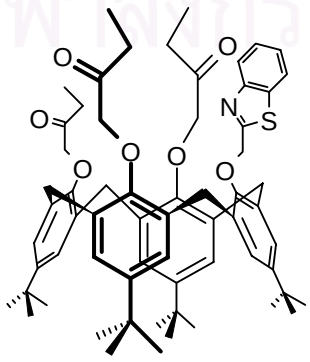
CHAPTER IV

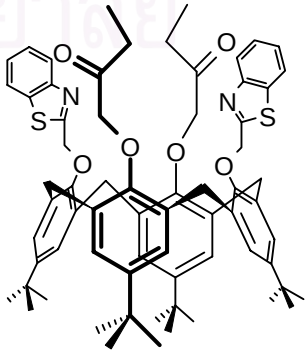
Conclusion

The fluoroionophores **17** and **18** consisting of calix[4]arene triester and amide were synthesized by reacting *p-tert*-butylcalix[4]arene triester acid chloride **14** with **15** and 4-aminoquinaldine (fluorophore **16**), respectively. The quantum yield of fluoroionophores **17** and **18** (in ethanol) are 0.67 and 0.10, respectively as referred to anthracene (0.27 in ethanol). The carbonyl oxygen on **17** formed a cavity which was selective to sodium ion while the amide nitrogen played a major in signaling. The rupture of intramolecular hydrogen bonding by sodium ion complexation resulted in quenching of fluorescence intensity of **17**. The stability constant of fluoroionophore **17** measured by fluorimetric titration in methanol showed the selectivity for sodium ion over other alkali metal ions with log K of 2.91. Other alkali ions (Li^+ and K^+) were not fit into cavity of **17** resulting in negligible signal change. In contrast, fluoroionophore **18** was not selective to the alkali metal ion under study.

Suggestion for future works:

Future works should be aimed at design and synthesize other calix[4]arenes containing fluorophores in which the sensory units are attached to the wide rim of calix[4]arenes. Furthermore, the new fluoroionphore should have a PET mechanism upon binding metal ions.





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APPENDICES

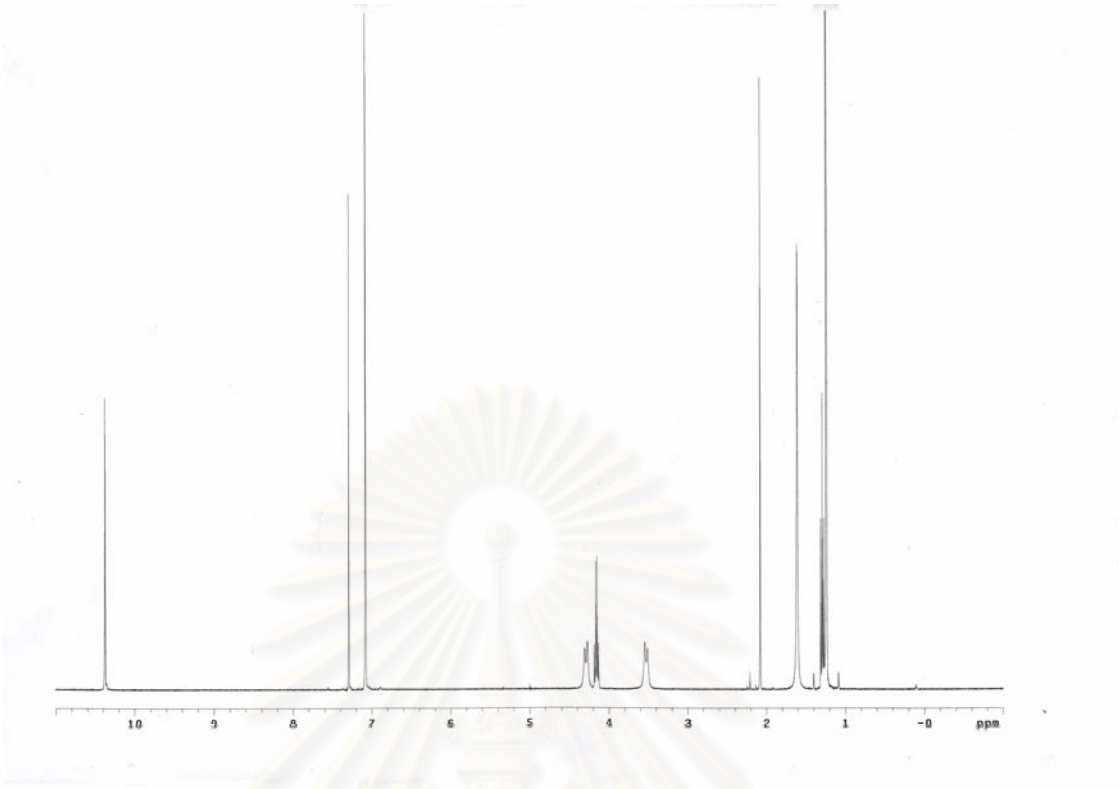

Figure A. 1: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of p-*tert*-butylcalix[4]arene (**1**)

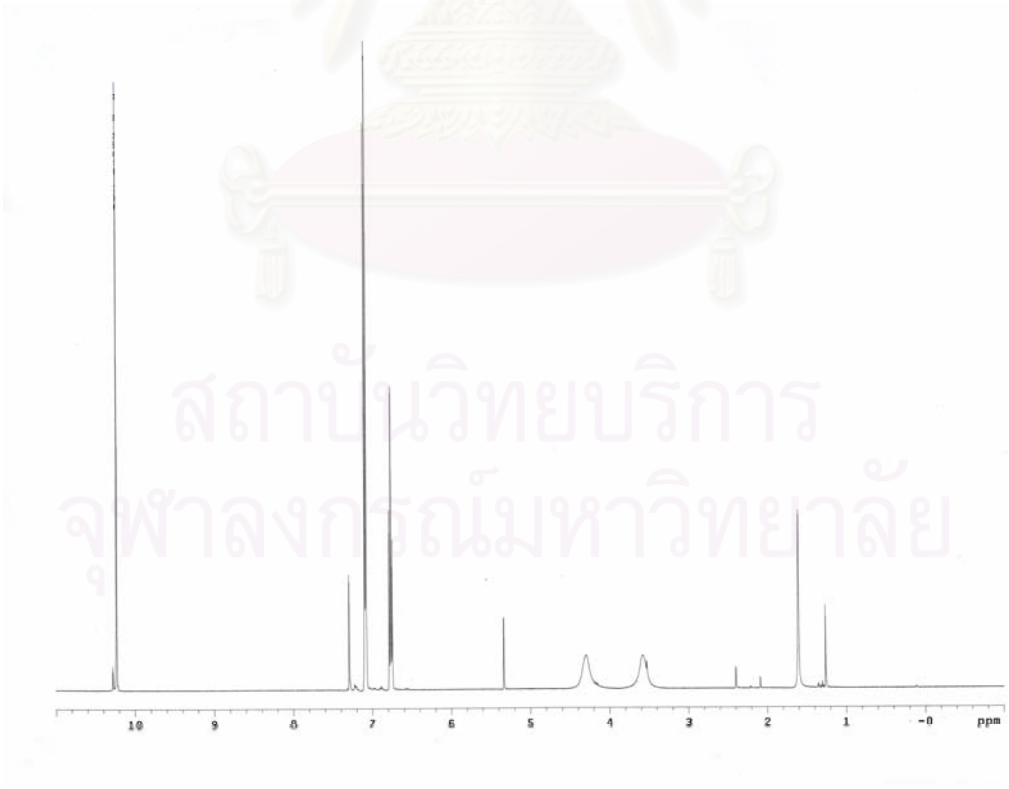

Figure A. 2: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of calix[4]arene (**2**)

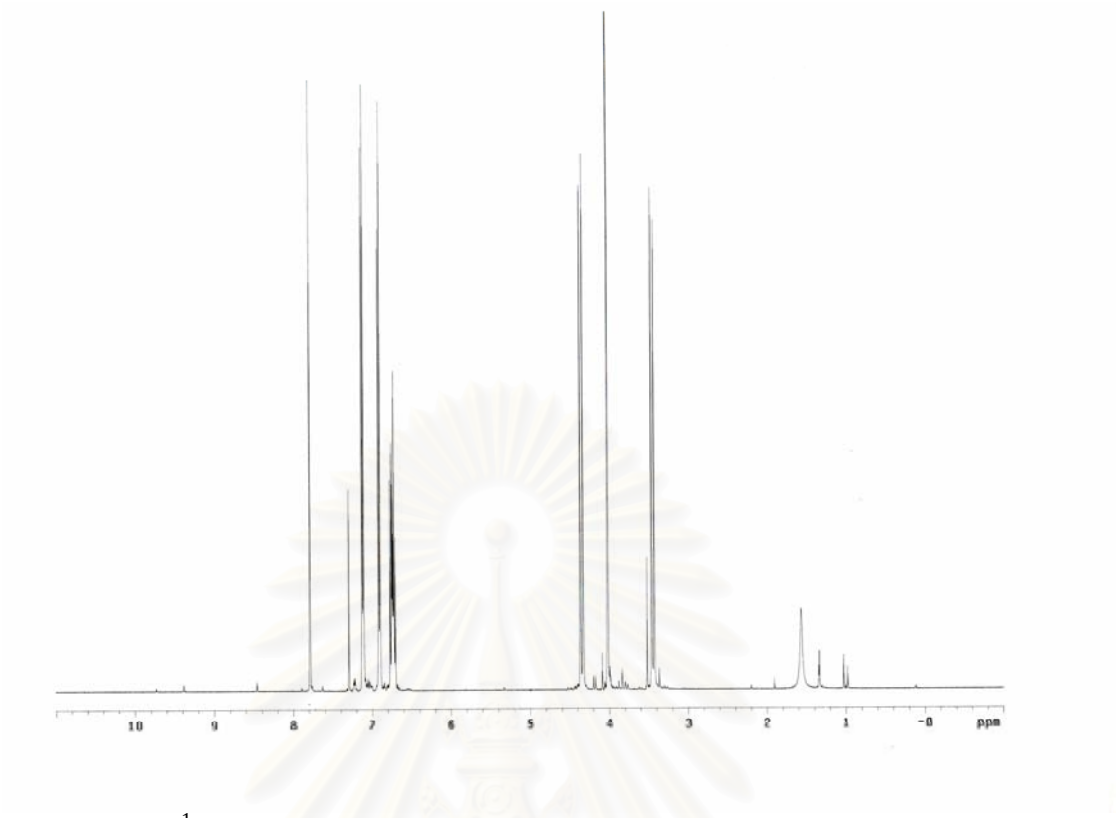

Figure A. 3: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 25,27-dimethoxy-26,28-dihydroxycalix[4]arene (**3**)

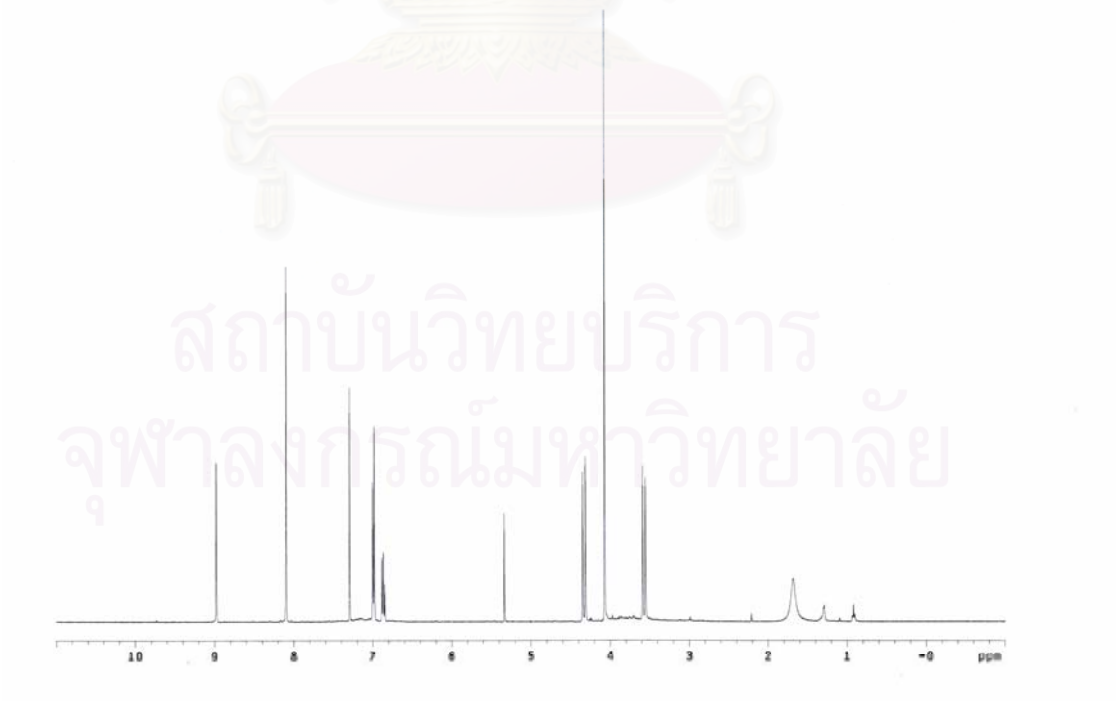

Figure A. 4: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5,17-dinitro-25,27-dimethoxy-26,28-dihydroxycalix[4]arene (**4**)

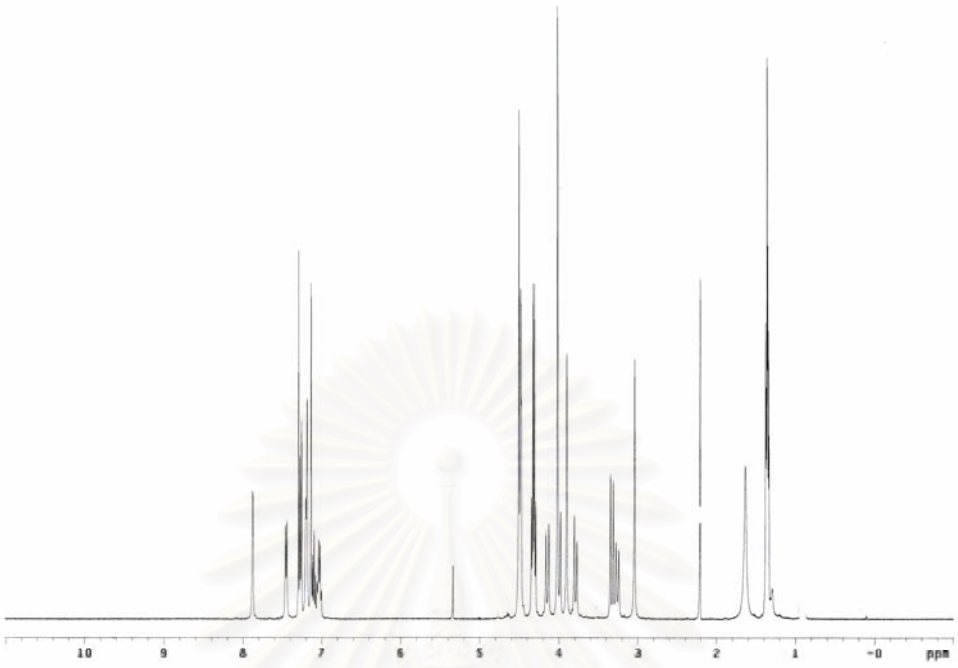

Figure A. 5: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5,17-dinitro-25,27-dimethoxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene (**5**)

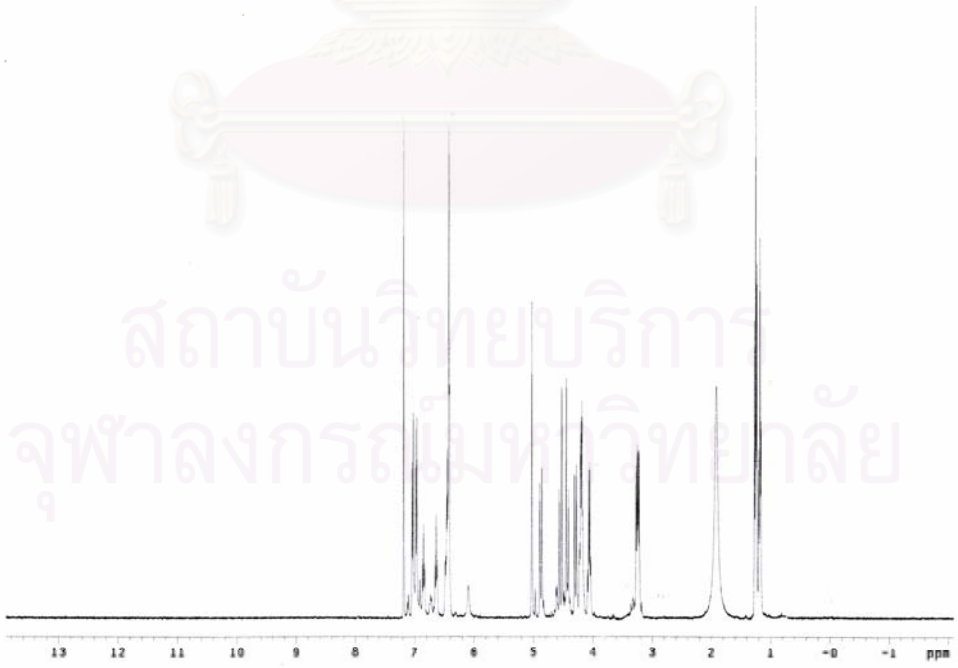

Figure A. 6: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene (**8**)

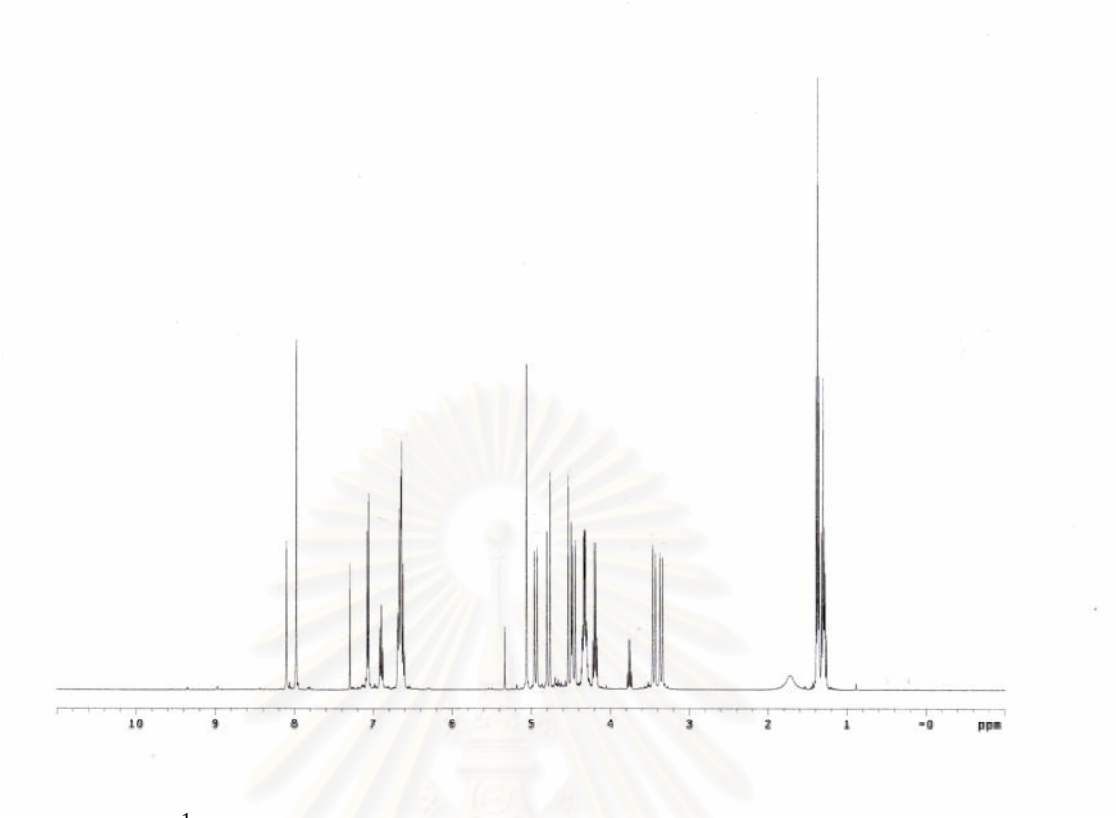

Figure A. 7: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5-nitro-25,26,27-tris(ethoxycarbonylmethoxy)-28-hydroxycalix[4]arene (**9**)

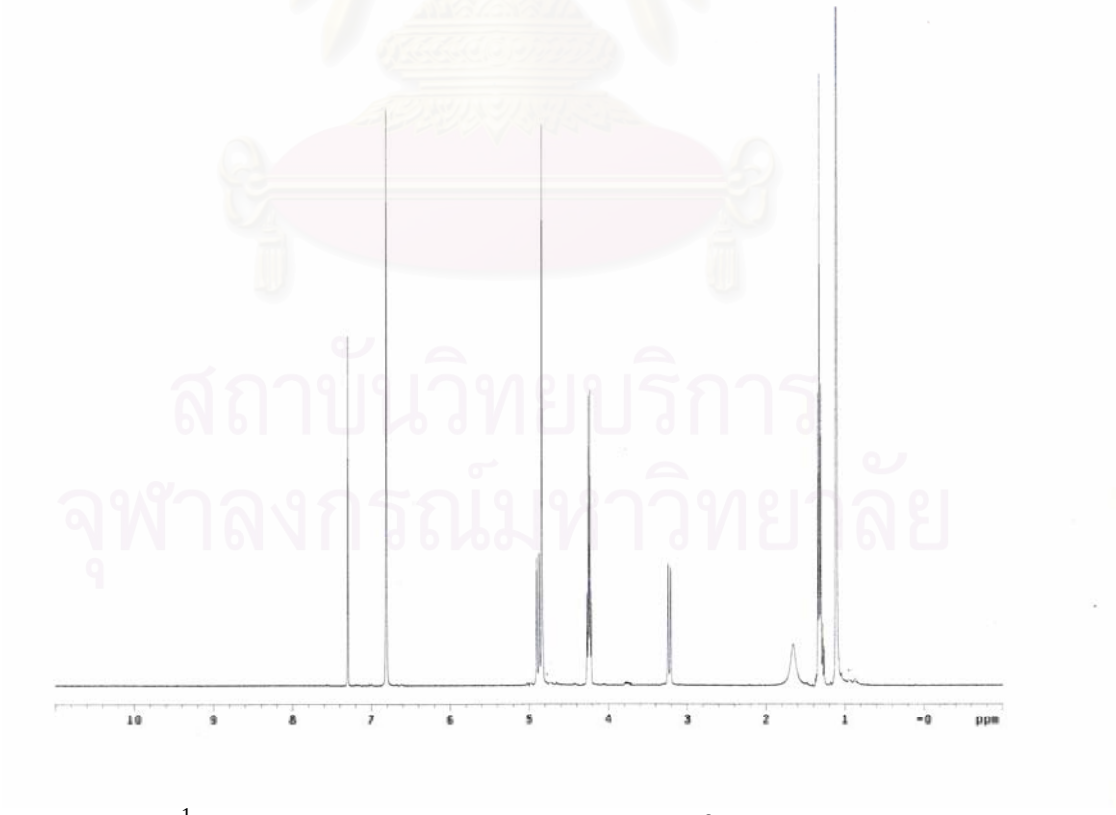

Figure A. 8: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(ethoxycarbonylmethoxy)calix[4]arene (**12**)

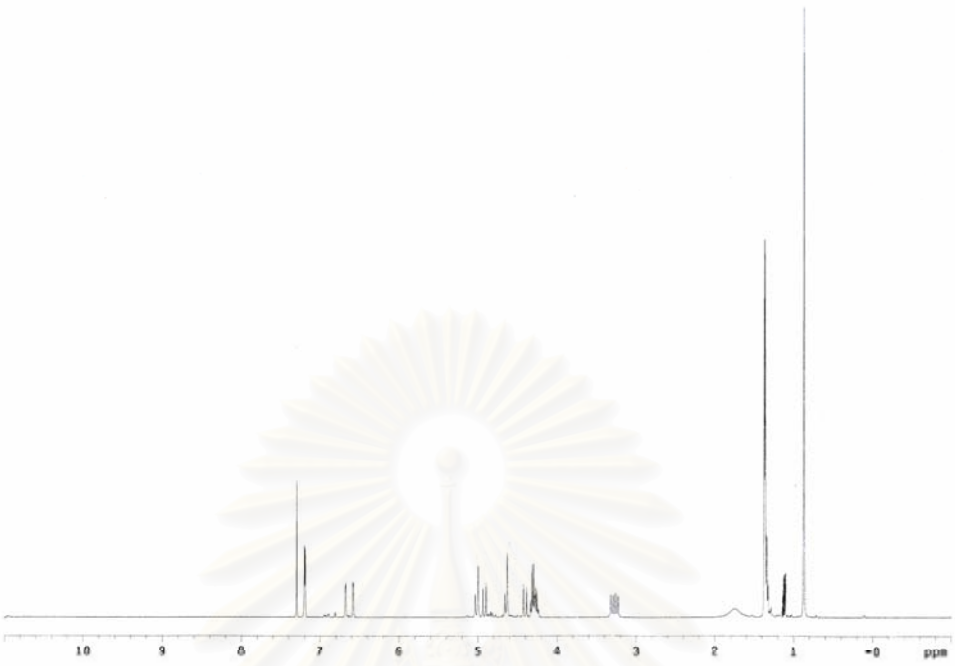

Figure A. 9: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5,11,17,23-tetra-*tert*-butyl-28-carboxymethoxy-25,26,27-tris(ethoxycarbonylmethoxy)calix[4]arene (**13**)

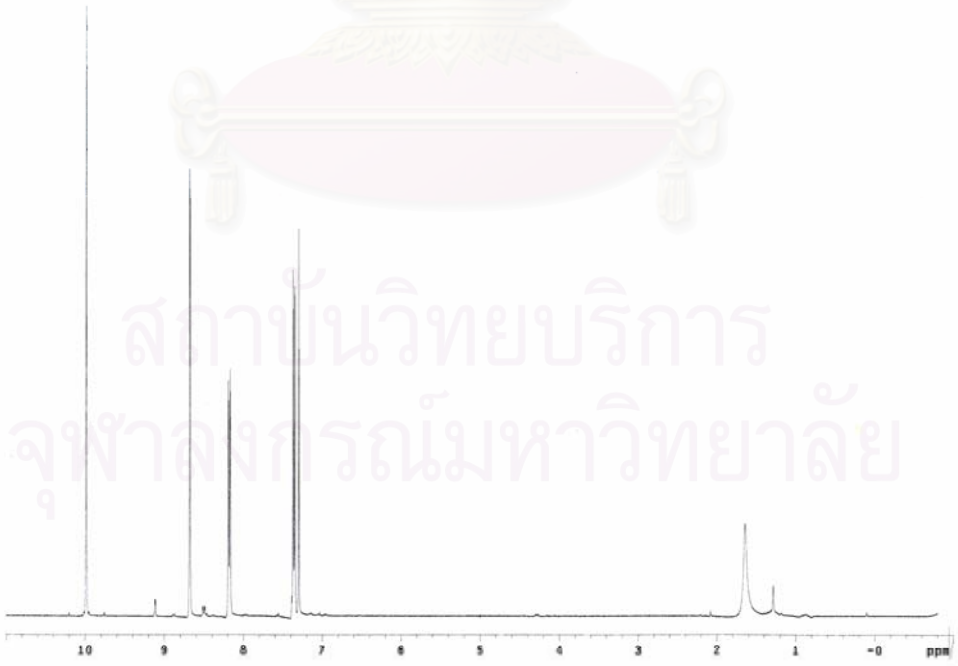

Figure A. 10: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-nitro-hydroxybenzadehyde (**19**)

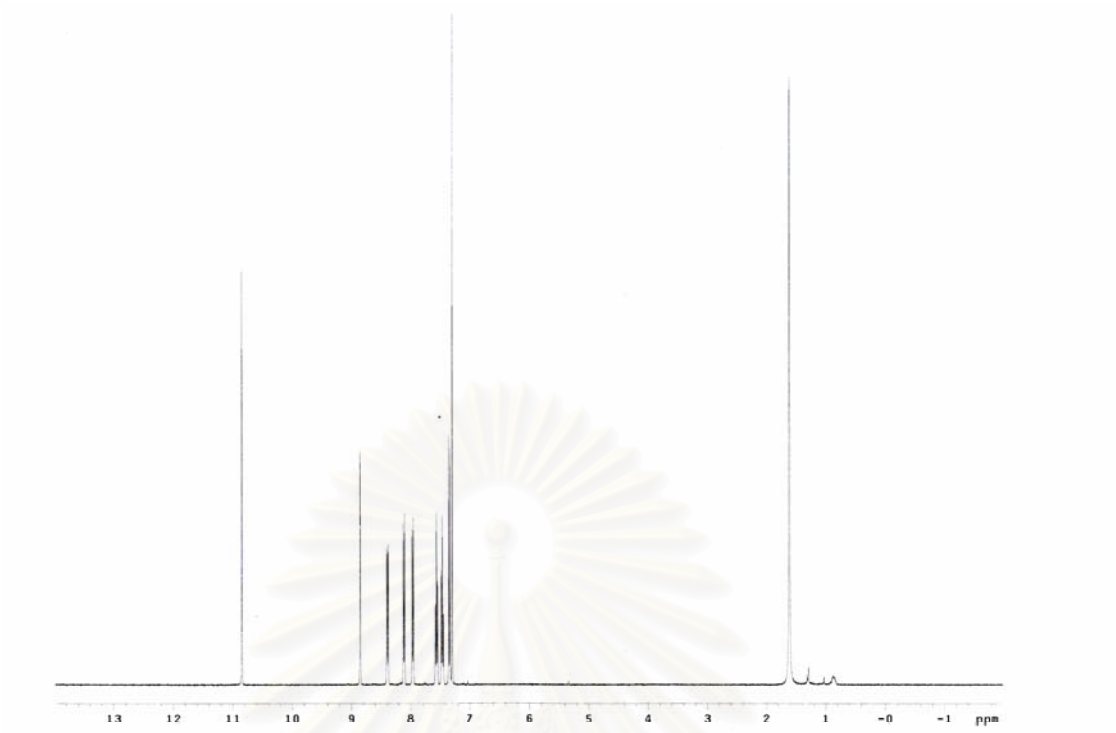

Figure A. 11: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5-(1,3-benzothiazol-2-yl)-2-hydroxyl-nitrobenzene (**20**)

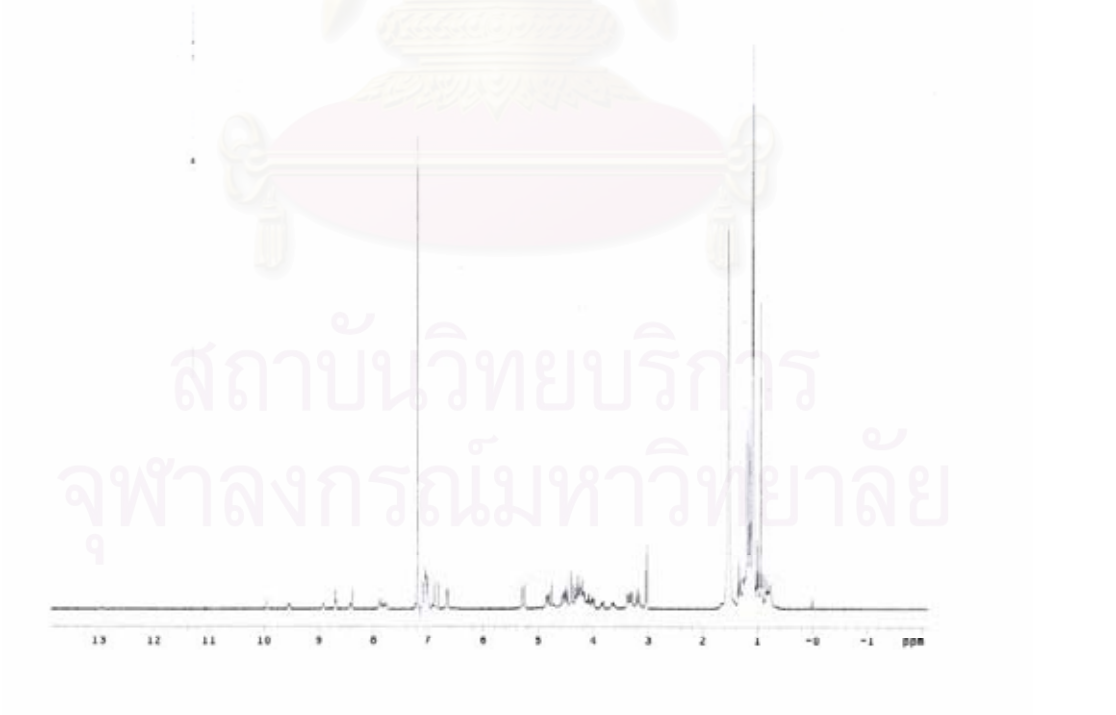

Figure A. 12: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5,11,17,23-tetra-*tert*-butyl-25,26,27-tris(ethoxycarbonylmethoxy)-28-methoxy-*N*-(4-aminoquinaldine) amidecalix[4]arene (**18**)

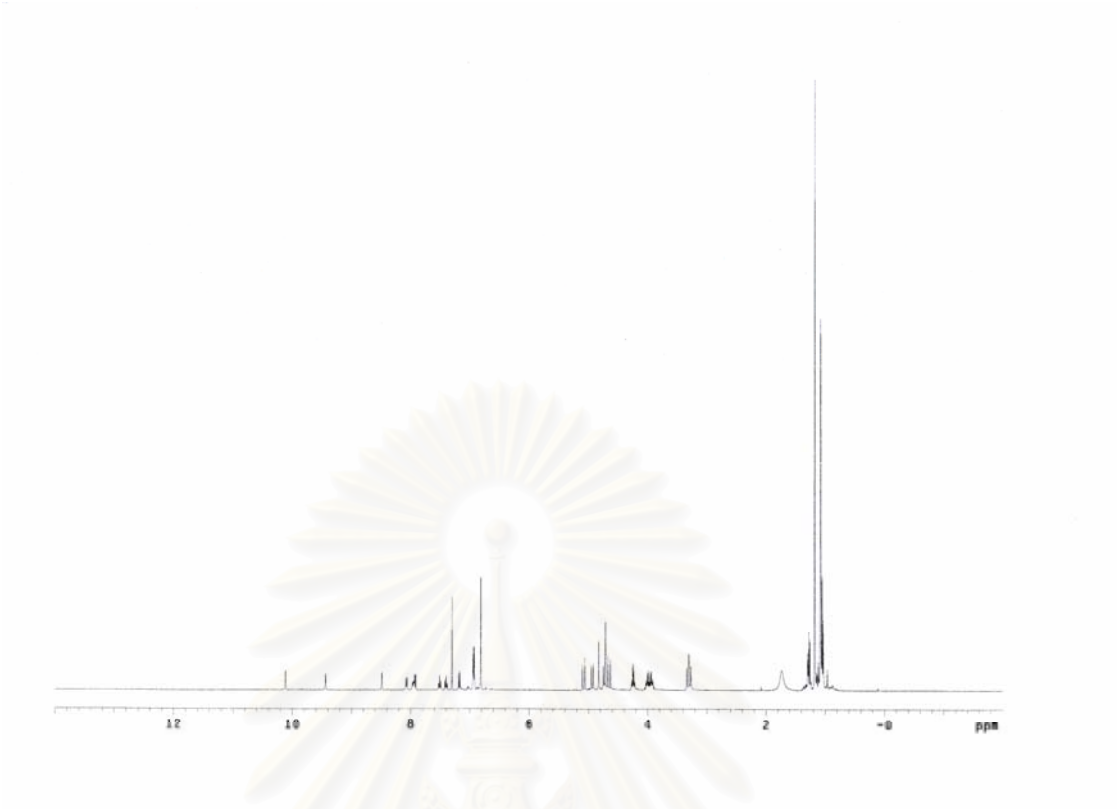

Figure A. 13: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5,11,17,23-tetra-*tert*-butyl-25,26,27-tris(ethoxycarbonylmethoxy)-28-methoxy-*N*-(1,3-benzothiazol-2-yl-2-hydroxy)amidecalix[4]arene (**17**)

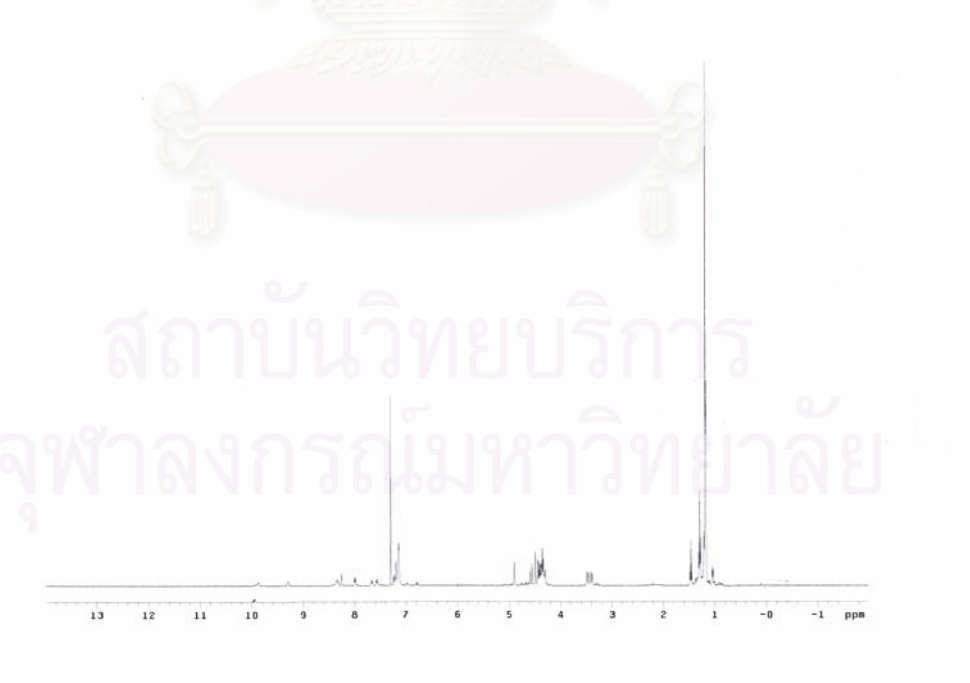

Figure A. 14: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 5,11,17,23-tetra-*tert*-butyl-25,26,27-tris(ethoxycarbonylmethoxy)-28-methoxy-*N*-(1,3-benzothiazol-2-yl-2-hydroxy)amidecalix[4]arene-Na complex (**17 + Na**)

VITA

Mrs. Nongnit Morakot was born on August 16, 1955 in Phuket, Thailand. She received her Bachelor degree of Science in Chemistry in 1977 and Master degree of Science in Chemistry from Chiangmai University in 1983. Since 1999, she has been a graduate student at the Department of Chemistry, Chulalongkorn University and become a member of the Supramolecular Chemistry Research Laboratory under the supervision of Associate Professor Dr. Thawatchai Tuntulani and Dr. Boosayarat Tomapatanaget. She finished her Doctor of Philosophy degree in the academic year 2004.