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# STUDIES ON DISCRIMINATION OF METAL IONS AND AMMONIUM IONS BY CROWN ETHER ANALOGUES WITH POLYCYCLIC AROMATIC RINGS

**2005**

**YOSHIO NAKAHARA**

*Department of Molecular Chemistry*
*Graduate School of Engineering*
*Osaka University*

# STUDIES ON DISCRIMINATION OF METAL IONS AND AMMONIUM IONS BY CROWN ETHER ANALOGUES WITH POLYCYCLIC AROMATIC RINGS

（多環式芳香環を有するクラウンエーテル類縁体による
金属イオンおよびアンモニウムイオンの識別に関する研究）

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# Preface

The work in this thesis was carried out under the guidance of Professor Mitsuru Akashi at the Department of Molecular Chemistry, Graduate School of Engineering, Osaka University.

The object of this thesis is to prepare new crown ether analogues and to clarify their recognition abilities towards metal ions and ammonium ions. The author hopes that the results in this work will contribute to further development of crown ether chemistry and molecular recognition chemistry.

Yoshio Nakahara

Department of Molecular Chemistry
Graduate School of Engineering
Osaka University
Yamada-oka, Suita, Osaka, Japan

January, 2005

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# General Introduction

Research on supramolecular host-guest interactions and molecular recognition is a subject of considerable interest due to its applications to many fields such as chemistry, biology, medicine, and environmental studies.[1] Over the past two decades, the design of supramolecular systems has developed, leading to host molecules that are able to express recognition events in the form of measurable signals such as redox potentials, absorption, or emission characteristics.[2] Such host molecules, molecular recognition sensors, usually consist of a macrocyclic receptor (binding part) and an organic chromophore (sensing part).[3] Among the various molecular recognition sensors, much attention has been paid to the design of cationic-specific sensors for the selective detection of biologically important ions such as metal ions, especially, alkali metal and alkaline earth metal ions, and organic ions, especially, ammonium ions and amino acids.[4] In this context, designing molecular recognition sensors for practical applications is a challenging task.

Since crown ethers have both a hydrophobic exterior and an electron-rich cavity which can coordinate guest molecules, they are very effective for the separation and resolution of guest molecules such as alkali metal ions, alkaline earth metal ions, and ammonium ions that play various important roles in biochemistry and environmental science.[5] There is a real need to establish a simple and reliable analytical method to detect these important ions, and the well-known high sensitivity of various absorption and fluorescence spectroscopies has led to the design and development of crown ethers functionalized by UV and fluorescence active groups for analytical use. In 1977, Takagi et al. developed the first examples of these crown ethers,[6] which are also called chromogenic crown ethers. These molecules contain both a crown ether for the binding site with metal ions and an ionic chromophore moiety that transduces the

chemical information produced by the metal binding event into an optical signal. Since then, many kinds of chromogenic and fluorometric crown ethers have been developed.[7] However, the relationship between their structures and their detection abilities has remained unclear.

The construction of molecular recognition sensors that function in water is urgently required for clinical diagnostics and environmental analysis. However, most of the host compounds lose their specificity in aqueous media because their complexation abilities are drastically decreased by strong hydration.[8] The principal challenges in the design of molecular recognition sensors in aqueous solution are: (a) strong binding properties; (b) good selectivity; (c) large signal response; and (d) high sensitivity. To increase the binding strength and guest selectivity, several groups have developed chromophores and fluorophores based on cryptand scaffolds.[9] Although these indicators did improve complexation abilities towards guest molecules, and there still remain several unsolved problems before practical use is possible.

Chiral crown ethers represent an important set of discriminating agents in NMR spectroscopy.[10] Lanthanide shift reagents are the most famous chiral NMR shift reagents,[11] but their use has been limited because of the paramagnetic line broadening caused by them.[12] Therefore, it is of interest to provide an alternative concept based on molecular recognition associated with chiral NMR shift reagents for organic compound structural analysis.

Based on these studies, the objectives of the present studies is to prepare new types of crown ether analogues with polycyclic aromatic rings, which can discriminate metal ions and ammonium ions with high selectivity and sensitivity.

This thesis consists of three chapters.

In **Chapter 1**, fluorometric sensing of alkali metal and alkaline earth metal cations by new types of double-armed lariat ethers with pyrene moieties at each end of two sidearms was described.

In **Chapter 2**, fluorescent characteristics of new types of photosensitive monoazacryptand derivatives with a pyrene ring in aqueous micellar solutions were described.

In **Chapter 3**, synthesis of new types of $C_2$-symmetric chiral double-armed crown ethers and their enatiomeric recognition abilities as chiral NMR discriminating agents towards ammonium salts were described.

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# Chapter 1

# Fluorometric Sensing of Alkali Metal and Alkaline Earth Metal Cations by New Types of Double-Armed Lariat Ethers with Pyrene Moieties at Each End of Two Sidearms

## 1-1. Introduction

Recently a variety of artificial optical sensors that selectively respond to a specific molecule have been developed.[1] High selectivity toward specific guest molecules should be achieved by using appropriate host molecules based on host-guest interactions. As for realizing high sensitivity, fluorometric sensing has been used. The pyrenyl group has often been used as an effective fluorescence probe because of its high detection sensitivity.[2] Host molecules containing plural pyrenyl groups show an intramolecular excimer emission due to π-π stacking of the pyrene rings in the free state. Their structures change upon the addition of guest molecules as a direct result of complexation, which results in a decrease of the excimer emission intensity with an increase of monomer emission intensity. Since the degree of structural change highly depends on the kind of guest molecules, these compounds show selectivity towards specific molecules. Thus, a certain event that brings about structural changes in response to specific guest molecules is important in the molecular design of new fluorophores. Indeed, a variety of detection systems for guest molecules and ions using fluorescence changes in intramolecular excimer emission or fluorescence quenching of various pyrene functionalized ligands have been developed.[3] Lariat ethers are known to be effective host molecules for alkali metal and alkaline earth metal cations on the basis of the cooperative coordination of the crown ring and the

electron-donating sidearm to the cations.[4-7] In addition, other types of double-armed lariat ethers having two sidearms on the different carbon of the crown ring[8,9] show complexation behaviors different from those of lariat ethers containing two sidearms on the same carbon of the crown ring.[10]

In this chapter, the author reports the synthesis of two new types of double-armed lariat ethers (Types A and B), Type A has two sidearms containing two pyrene rings on the same carbon atom of (3x+1)-crown-x, (x = 4, 5, 6) and Type B has two sidearms on the different carbon atoms of 3y-crown-y, (y = 5, 6). Complexation properties toward alkali metal and alkaline metal cations were evaluated using fluorescence spectroscopy.

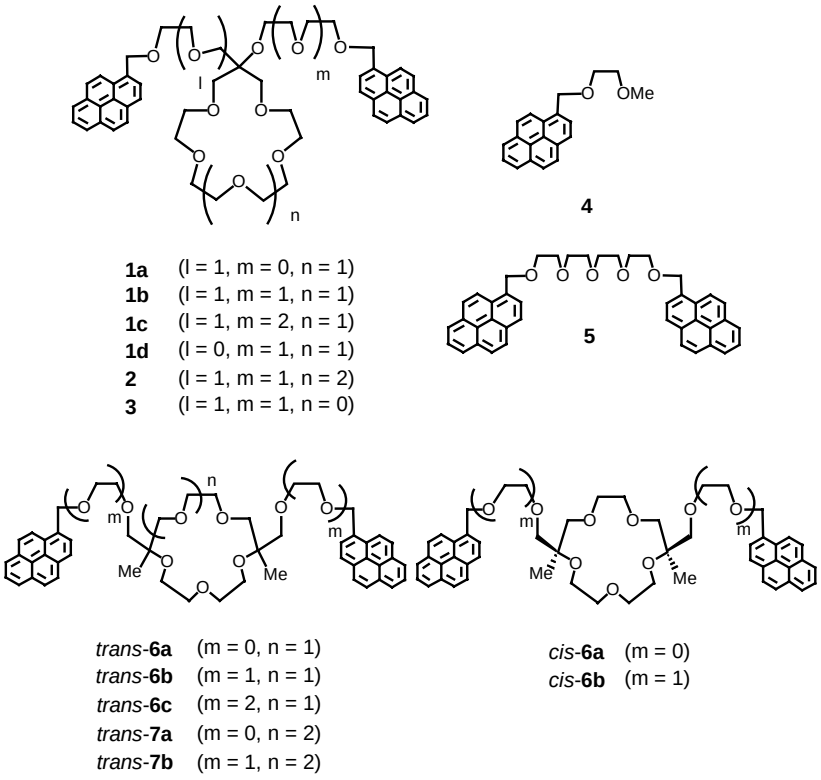


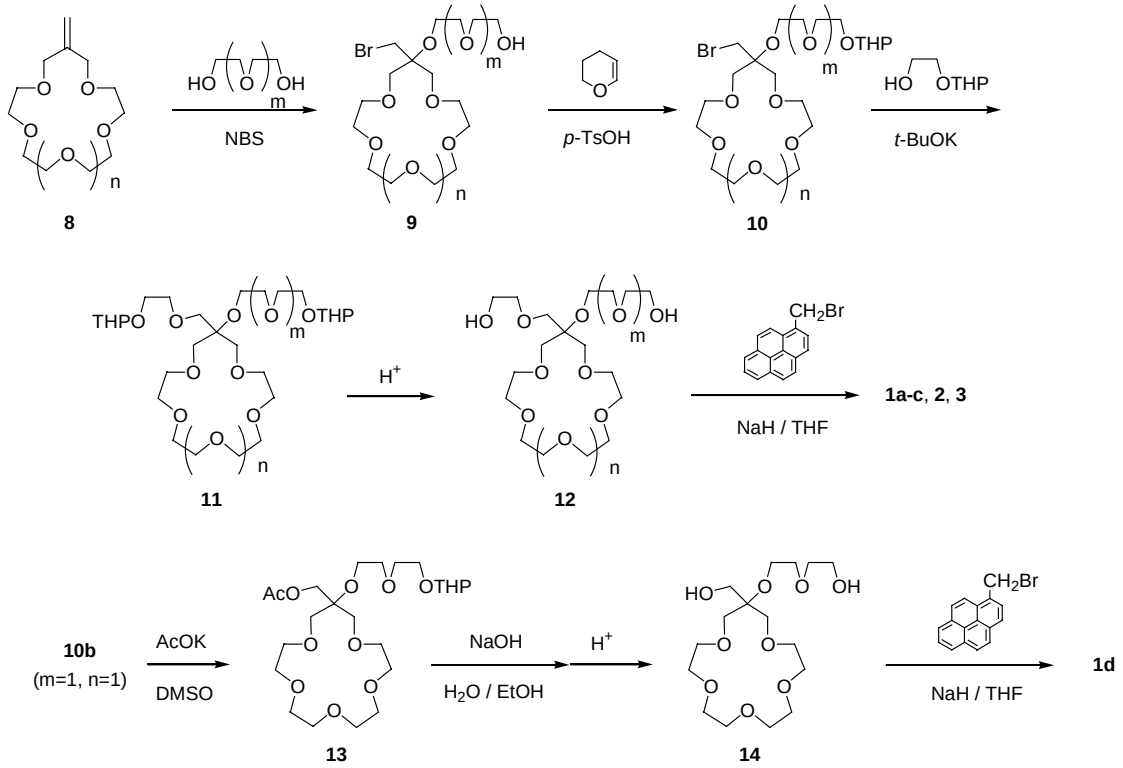

**FIGURE 1.** Structures of fluorophores.

## 1-2. Results and Discussion

### 1-2-1. Design and Synthesis of Fluorophores

Two types of double-armed lariat ethers containing two pyrene rings were designed to afford systematic variations of crown ring size, sidearm type, and sidearm location on the crown ring. Pyrene moieties were introduced into the end of the sidearm of crowns as optical responding units. The general synthetic procedures[10] for compounds **1**-**3** (Type A) are summarized in Scheme 1.

**SCHEME 1.**

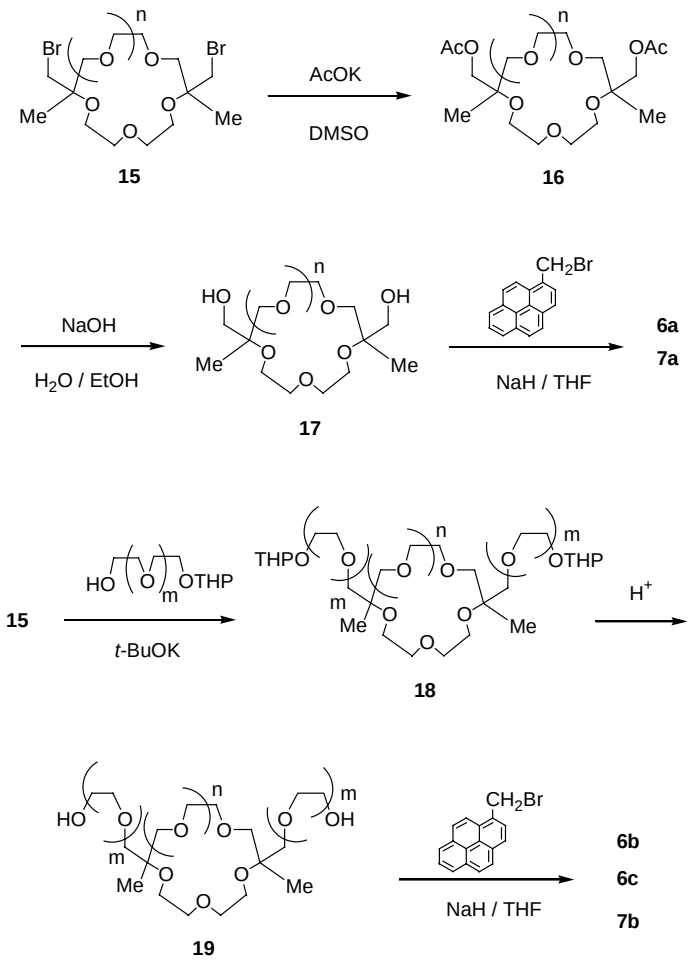



Starting materials 3x-methylene-(3x+1)-crown-x **8a** (x=5) and **8b** (x=6) were prepared according to the literature.[11] Compound **8c** (x=4) was prepared in a similar method to **8a** and **8b** with minor modifications. Compounds **9a-e** were obtained by bromoalkoxylation of 3x-methylene-(3x+1)-crown-x (x=4, 5, 6) (8a-c) using N-bromosuccinimide (NBS) and oligoethylene glycol. Hydroxyl groups of compounds **9** were protected by treatment with 3,4-dihydro-2H-pyrane according to conventional methods to give corresponding tetrahydropyranyl ethers **10**, which were

reacted with ethylene glycol monotetrahydropyran-2-yl ether under basic conditions, followed by deprotection under acidic conditions to give compounds **12**. Compounds **12** were reacted with 1-bromomethylpyrene in THF in the presence of NaH at refluxed temperature for 36 h to produce **1**-**3**.

Another type of fluorophores **6** and **7** (Type B) were synthesized according to the procedure summarized in Scheme 2.

**SCHEME 2**




The presence of methyl groups at the pivot positions of compounds **6** and **7** was previously verified to play an important role in increasing their complexation ability toward alkali metal and alkaline earth metal cations by us[7] and others.[6] *Trans*- and

*cis*-[2,(3y-6)-bis(bromomethyl)-2,(3y-6)-dimethyl-3y-crown-y, (y = 5, 6)] **15** were treated with potassium acetate in DMSO at 100˚C for 48 h to give the corresponding diacetyl derivatives **16**. Crown diols **17** were obtained by hydrolysis of **16** in EtOH-$H_2O$ in the presence of sodium hydroxide. Crown diols **19** having two oxyethylene chains were acquired by the reaction of **15** with ethylene glycol monotetrahydropyran-2-yl ether under basic conditions, followed by deprotection under acidic conditions.

### 1-2-2. Fluorescence Spectra of Fluorophores in the Free State

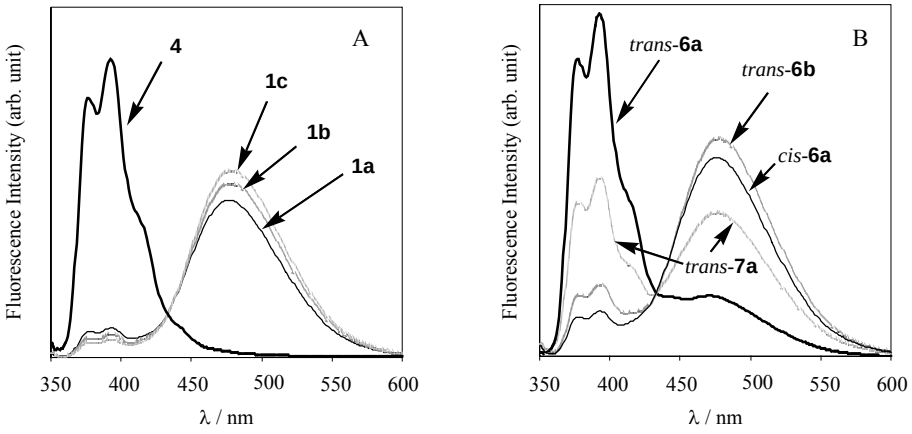



**FIGURE 2.** Fluorescence spectra of **1a-c** ($1 \times 10^{-6}$ M), **4** ($2 \times 10^{-6}$ M), *trans*-**6a** ($1 \times 10^{-6}$ M), *cis*-**6a** ($1 \times 10^{-6}$ M), *trans*-**6b** ($1 \times 10^{-6}$ M), and *trans*-**7a** ($1 \times 10^{-6}$ M) in $CH_3CN/CHCl_3$ (99:1 v/v). Excitation wavelength: 340 nm.

Figure 2A shows the fluorescence spectra of fluorophores **1a-c** and **4**. Compound **4** containing one pyrene ring showed only monomer emission at 395 nm at a concentration of $2 \times 10^{-6}$ M, whereas compounds **1a-c** containing two pyrene rings showed strong excimer emission at 480 nm at a concentration of $1 \times 10^{-6}$ M. Judging from the concentration of fluorophore, excimer emission of **1a-c** is attributed to intramolecular π-π stacking of two pyrene rings. In comparison with Type A crowns

(**1-3**), Type B crowns (**6-7**) are featured by the presence of *cis* and *trans* isomers. Interestingly, the fluorescent behavior of *trans* isomers was found to be highly dependent on the combination of crown ring size and sidearm length (Figure 1B). Excimer emission of *trans* isomer **6a** is rather weak in comparison to those of *trans* isomer **6b** that has more flexible sidearms, *trans* isomer **7a** that has a larger ring size, and the corresponding *cis* isomer **6a**. The rigidity of the 15-crown-5 ring may prevent the approach of two pyrene rings of *trans*-**6a**, located in the opposite side of the crown ring plane.

### 1-2-3. Fluorescence Spectra of Fluorophores in the Presence of Metal Cations

To evaluate the complexation behavior of fluorophores, fluorescence spectra were measured as functions of the concentrations of alkali metal and alkaline earth metal cations in a mixed $CH_3CN/CHCl_3$ (99/1) solvent at room temperature. As a typical example, spectral changes of fluorophores **1a** and *trans*-**6b** upon complexation with $Ca^{2+}$ are shown in Figure 3.

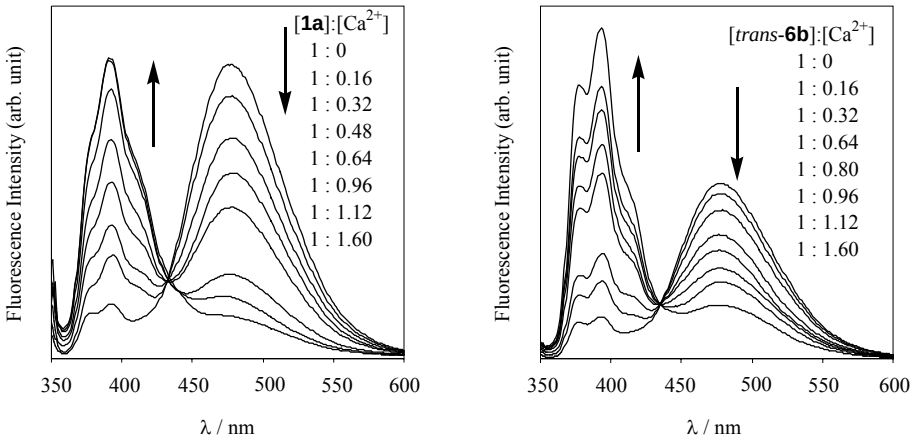



**FIGURE 3.** Fluorescence spectral changes of **1a** ($1 \times 10^{-6}$ M) and *trans*-**6b** ($1 \times 10^{-6}$ M) with different concentrations of $Ca(ClO_4)_2$ in $CH_3CN/CHCl_3$ (99:1 v/v). Excitation wavelength: 340 nm.

The fluorescence intensity of the excimer emissions of **1a** and *trans*-**6b** decreases with increasing $Ca^{2+}$ concentrations, and the fluorescence intensity of monomer emissions increases. This drastic spectral change is ascribed to conformational changes of each fluorophore caused by the cooperative coordination of the crown ring and the electron-donating sidearm to $Ca^{2+}$, resulting in inhibition of intramolecular π-π stacking of the pyrene rings. An isoemissive point at 433 nm indicates only one type of fluorophore-$Ca^{2+}$ complex is involved. On the other hand, an acyclic fluorophore **5** as a reference hardly responds to any kinds of alkali metal and alkaline earth metal cations, indicating that higher complexation ability towards metal ions is needed for the fluorophore and that the presence of a crown ring is effective.

The existence of the **1a**·$Ca^{2+}$ complex in solution was evidenced by $^1$H NMR spectroscopy. The $^1$H NMR spectrum of **1a** showed two signals at 4.82 and 4.84 ppm based on the methylene protons adjacent to two pyrene rings in $CD_3CN/CDCl_3$ (2:1 v/v). Upon addition of an equimolar amount of $Ca(ClO_4)_2$, the methylene protons were shifted downfield to 4.87 and 5.04 ppm. This downfield shift observed clearly indicates the effective coordination of the electron-donating sidearm to $Ca^{2+}$.[7b] The difference in the extent of the peak shifts between two kinds of the methylene protons by the addition of $Ca^{2+}$ suggests that only one of the two sidearms participates in complexation with the cation. The sidearm constituting the glycerol structure at the pivot carbon should be responsible for the complexation, according to our previous work.[10b]

The stability constants (*K*) of the complex were evaluated from plots of excimer emission intensities vs. [metal]/[ligand] by means of a nonlinear least-square curve fitting method.[12] The curve showed that all the ligands formed 1:1 complexes. Stability constants (*K*) for fluorophores toward alkali metal and alkaline earth metal cations are summarized in Tables 1 and 2.

**TABLE 1. Ratio of Excimer Fluorescence Intensity to Total Fluorescence Intensity $I_E$ / ($I_E$ + $I_M$) of Fluorophore 1-3 in the Presence of Alkaline Earth Metal Cations and Stability Constants of Corresponding Complexes**

| Probe [$I_E$ / ($I_E$ + $I_M$)][a] | $M^{2+}$ (x $10^{-6}$ M) | $I_E$ / ($I_E$ + $I_M$)[b] | log $K$ ($M^{-1}$) |
|---|---|---|---|
| **1a** [0.85] | $Mg^{2+}$ (3) | 0.83 | N.D.[c] |
| | $Ca^{2+}$ (3) | 0.17 | 7.6 |
| | $Sr^{2+}$ (3) | 0.45 | 6.8 |
| | $Ba^{2+}$ (3) | 0.74 | 6.9 |
| **1b** [0.89] | $Mg^{2+}$ (3) | 0.89 | N.D.[c] |
| | $Ca^{2+}$ (3) | 0.71 | 6.7 |
| | $Sr^{2+}$ (3) | 0.40 | 8.2 |
| | $Ba^{2+}$ (3) | 0.66 | 7.4 |
| **1c** [0.92] | $Mg^{2+}$ (3) | 0.92 | N.D.[c] |
| | $Ca^{2+}$ (3) | 0.85 | N.D.[c] |
| | $Sr^{2+}$ (3) | 0.65 | 8.6 |
| | $Ba^{2+}$ (3) | 0.71 | 8.7 |
| **1d** [0.94] | $Mg^{2+}$ (3) | 0.94 | N.D.[c] |
| | $Ca^{2+}$ (3) | 0.54 | 6.3 |
| | $Sr^{2+}$ (3) | 0.33 | 8.7 |
| | $Ba^{2+}$ (3) | 0.51 | 8.2 |
| **2** [0.95] | $Mg^{2+}$ (3) | 0.94 | N.D.[c] |
| | $Ca^{2+}$ (3) | 0.82 | 5.5 |
| | $Sr^{2+}$ (3) | 0.88 | 6.9 |
| | $Ba^{2+}$ (3) | 0.88 | 9.0 |
| **3** [0.93] | $Mg^{2+}$ (10) | 0.88 | N.D.[c] |
| | $Ca^{2+}$ (10) | 0.15 | 6.5 |
| | $Sr^{2+}$ (10) | 0.62 | 6.7 |
| | $Ba^{2+}$ (10) | 0.56 | 7.0 |

[a] These values were calculated by the fluorescence intensity, $I_E$ (480 nm) and $I_M$ (395 nm), in the free state. [b] These values were calculated by the fluorescence intensity, $I_E$ (480 nm) and $I_M$ (395 nm), in the presence of metal ions. [c] Not determined.

**TABLE 2. Ratio of Excimer Fluorescence Intensity to Total Fluorescence Intensity $I_E$ / ($I_E$ + $I_M$) of Fluorophore 6-7 in the Presence of Alkali Metal Cations and Alkaline Earth Metal Cations and Stability Constants of Corresponding Complexes**

| Probe [$I_E$ / ($I_E$ + $I_M$)][a] | $M^{n+}$ (x $10^{-6}$ M) | $I_E$ / ($I_E$ + $I_M$)[b] | log $K$ ($M^{-1}$) |
|---|---|---|---|
| *trans*-**6b** [0.78] | $Mg^{2+}$ (3) | 0.24 | 6.3 |
| | $Ca^{2+}$ (3) | 0.07 | 6.2 |
| | $Sr^{2+}$ (3) | 0.34 | 6.8 |
| | $Ba^{2+}$ (3) | 0.42 | 6.3 |
| *trans*-**6c** [0.91] | $Mg^{2+}$ (3) | 0.84 | N.D.[c] |
| | $Ca^{2+}$ (3) | 0.66 | 5.2 |
| | $Sr^{2+}$ (3) | 0.35 | 5.5 |
| | $Ba^{2+}$ (3) | 0.86 | N.D.[c] |
| *trans*-**7a** [0.45] | $Li^{+}$ (10) | 0.42 | N.D.[c] |
| | $Na^{+}$ (10) | 0.43 | N.D.[c] |
| | $K^{+}$ (10) | 0.22 | 6.1 |
| | $Mg^{2+}$ (3) | 0.38 | 6.6 |
| | $Ca^{2+}$ (3) | 0.11 | 7.0 |
| | $Sr^{2+}$ (3) | 0.14 | 8.5 |
| | $Ba^{2+}$ (3) | 0.19 | 8.2 |
| *trans*-**7b** [0.61] | $Mg^{2+}$ (3) | 0.58 | N.D.[c] |
| | $Ca^{2+}$ (3) | 0.15 | 8.1 |
| | $Sr^{2+}$ (3) | 0.24 | 8.0 |
| | $Ba^{2+}$ (3) | 0.46 | 8.7 |
| *cis*-**6a** [0.80] | $Mg^{2+}$ (10) | 0.18 | 7.4 |
| | $Ca^{2+}$ (10) | 0.40 | 7.0 |
| | $Sr^{2+}$ (10) | 0.43 | 6.7 |
| | $Ba^{2+}$ (10) | 0.49 | 7.5 |
| *cis*-**6b** [0.87] | $Mg^{2+}$ (10) | 0.80 | N.D.[c] |
| | $Ca^{2+}$ (10) | 0.68 | N.D.[c] |
| | $Sr^{2+}$ (10) | 0.67 | 6.1 |
| | $Ba^{2+}$ (10) | 0.81 | 7.1 |

[a] These values were calculated by the fluorescence intensity, $I_E$ (480 nm) and $I_M$ (395 nm), in the free state. [b] These values were calculated by the fluorescence intensity, $I_E$ (480 nm) and $I_M$ (395 nm), in the presence of metal ions. [c] Not determined.

The ratio of excimer fluorescence intensity to the total fluorescence intensity $I_E$ / ($I_E$ + $I_M$) of **1a-d**, **2**, and **3** was plotted against the ratio of [metal]/[ligand] (Figure 4), where $I_E$ and $I_M$ are fluorescence intensities at 480 nm (excimer emission) and at 395 nm (monomer emission), respectively, and all graphs were normalized to compare $I_E$ / ($I_E$ + $I_M$) changes upon metal cation additions.

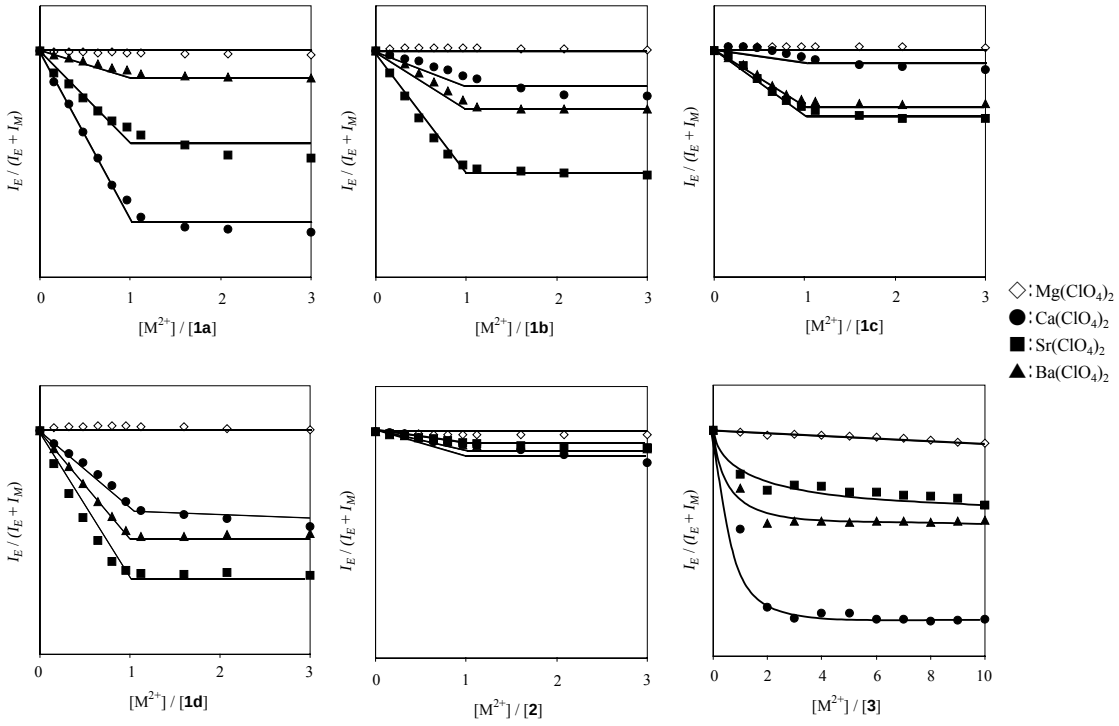



**FIGURE 4.** Changes of $I_E$ / ($I_E$ + $I_M$) of **1a-d** ($1 \times 10^{-6}$ M), **2** ($1 \times 10^{-6}$ M), and **3** ($1 \times 10^{-6}$ M) upon the addition of perchlorate salts of alkaline earth metal cations in $CH_3CN/CHCl_3$ (99:1 v/v). Excitation wavelength: 340 nm.

As shown in Figure 4, the presence of alkaline earth metal cations except for $Mg^{2+}$ noticeably decreased excimer emission fluorescence intensities of **1** containing a 16-crown-5 ring with an increase in corresponding monomer emission intensities. On the other hand, alkali metal cations ($Li^+$, $Na^+$, $K^+$) caused no changes in the fluorescence spectra. Compounds **1a**, **1b**, and **1c** showed selectivity toward $Ca^{2+}$, $Sr^{2+}$, and $Sr^{2+}$ or $Ba^{2+}$, respectively, among seven alkali metal and alkaline earth metal ions examined.

In this work, "selectivity" corresponds to the largest change in $I_E$ / ($I_E$ + $I_M$), and not the highest stability constant (*K*). Cation selectivity was clearly affected by the number of oxyethylene units in the sidearm. This finding is reasonably explained by considering the enlargement of the cavity by elongation of the oxyethylene chain. It should be noted that cation selectivity of compound **1d** is almost the same as that of **1b**. This result clearly indicates that only the sidearm constituting the glycerol structure at the pivot position is responsible for metal cation complexation as expected from our previous work.[10] Changes in fluorescence spectra of **2** containing a 19-crown-6 ring upon addition of alkaline earth metal cations were much smaller compared to those of the 16-crown-5 derivative **1b** bearing equivalent sidearms. However, the log *K* values of **2** were almost comparable to those of **1b**. In the case of **1b** this result suggests that the cooperative participation of the electron-donating sidearm during complexation of the crown ring with the metal ion effectively inhibits the π-π stacking of the pyrene rings. On the other hand, in the case of **2** only crown ring oxygen atoms coordinate the metal ion; in other words, the electron-donating sidearm of **2** hardly participates in metal cation coordination. Compound **3** containing a 13-crown-4 ring also responded to alkaline earth metal cations, but needed the addition of larger amount of metal salts to bring about complete conformational changes, because of a low metal cation complexation ability. Accordingly, a proper combination of crown ring size and electron-donating sidearms is important in the molecular design of new fluorophores.

Figure 5 shows fluorescent properties of fluorophores **6** and **7** (Type B) upon complexation with alkaline earth metal cations.

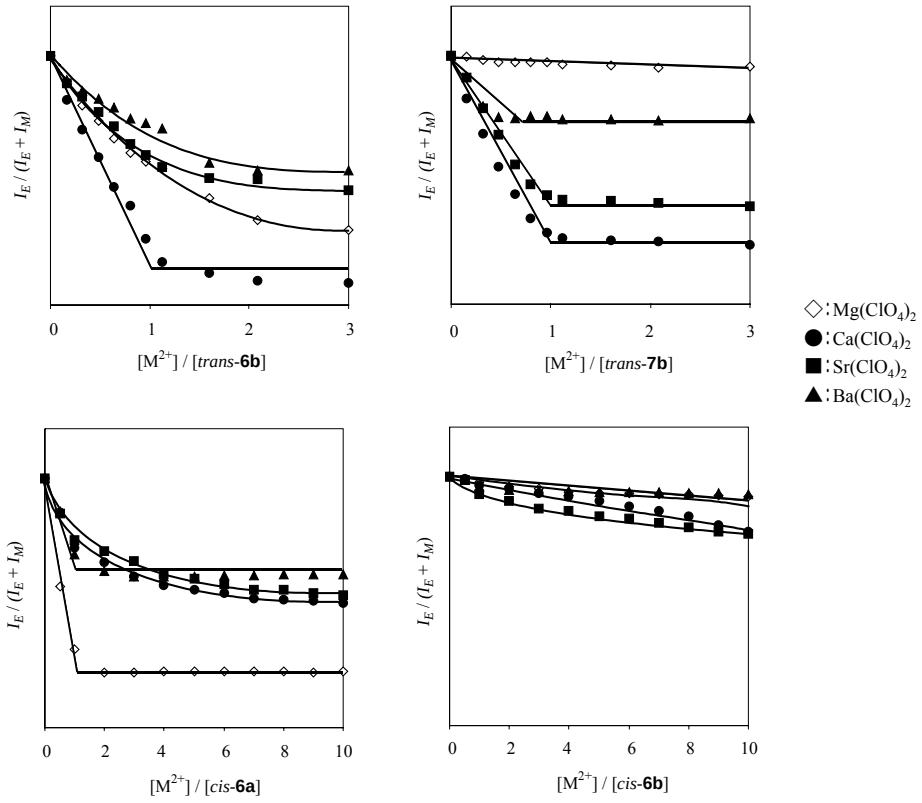



**FIGURE 5.** Changes of $I_E$ / ($I_E$ + $I_M$) of *trans*-**6b** ($1 \times 10^{-6}$ M), *trans*-**7b** ($1 \times 10^{-6}$ M), *cis*-**6a** ($1 \times 10^{-6}$ M), and *cis*-**6b** ($1 \times 10^{-6}$ M) upon the addition of perchlorate salts of alkaline earth metal cations in $CH_3CN/CHCl_3$ (99:1 v/v).
Excitation wavelength: 340 nm.

A few characteristic trends in cation selectivity were observed in these fluorophore types, when compared to Type A fluorophores (**1**). For example, both *trans*-**6b** containing a 15-crown-5 ring with one oxyethylene unit per each sidearm and the corresponding 18-crown-6 derivative, *trans*-**7b**, showed the same cation selectivity ($Ca^{2+}$) regardless of crown ring size. However, the fitness of the cavity size of the host molecule and the metal cation size seems to be still important in this series of fluorophores, judging from the fact that *trans*-**6b** and *trans*-**7b** also showed high affinity toward $Mg^{2+}$ and $Sr^{2+}$, respectively. In addition, *trans*-**6c** containing a 15-crown-5 ring with two oxyethylene units per each sidearm showed $Sr^{2+}$ selectivity. As for stability constants (*K*), the 18-crown-6 derivative *trans*-**7b** possesses much higher values than

the 15-crown-5 derivatives *trans*-**6b** and *trans*-**6c** (Table 2). Another important finding in this fluorophore series (Type B) is that the *cis* isomer **6a** containing a 15-crown-5 ring showed $Mg^{2+}$ selectivity, by considering that even 13-crown-4 derivative **3** did not show $Mg^{2+}$ selectivity in a series of Type A. Although $Mg^{2+}$ is one of several important divalent cations relevant to living organisms, there are few studies on $Mg^{2+}$ sensors.[13] Therefore *cis*-**6a** is expected to be a promising $Mg^{2+}$-specific indicator. Interestingly, when $Ba^{2+}$ was added to the *cis*-**6a** solution, monomer emission did not increase in response to the excimer emission decrease. To our regret, at the present stage we have no explanation for this phenomenon. In contrast to *cis*-**6**a, the *cis* isomer **6b** containing a 15-crown-5 ring with one oxyethylene unit per one sidearm showed little fluorescence spectral change. This indicates that the spatial distance of two pyrenes of *cis*-**6b** is not largely changed upon metal cation complexation because of the presence of flexible oxyethylene units.

Fluorescence characteristics in the presence of alkali metal cations were also examined in Type B series fluorophores. Although fluorescence spectra of most fluorophores were not changed by adding alkali metal cations, only *trans*-**7a** containing an 18-crown-6 ring responded to $K^+$. On the other hand, $Na^+$ affected little change in the fluorescence spectra (Figure 6). This demonstrates that *trans*-**7a** is potentially useful as a selective fluorescent detection device for $K^+$ over $Na^+$.

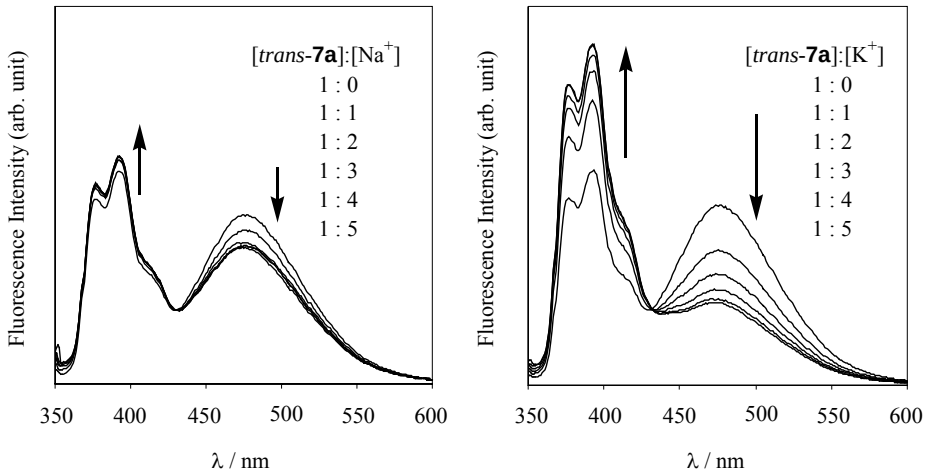



**FIGURE 6.** Fluorescence spectral changes in *trans*-**7a** ($1 \times 10^{-6}$ M) with different concentrations of NaSCN and KSCN in $CH_3CN/CHCl_3$ (99:1 v/v).
Excitation wavelength: 340 nm.

## 1-3. Conclusion

In this chapter, the author described the synthesis and complexation properties of two types of fluorophores based on double-armed lariat ethers. The difference in crown ring size and oxyethylene sidearm length affected metal ion selectivity and as a result, selectivity for a variety of metal cations was achieved. It is noteworthy that among fluorophores examined in this study only *trans*-**7a** containing an 18-crown-6 ring responded to $K^+$ with very high selectivity versus $Li^+$ and $Na^+$. This is significant because there are few examples of selective fluorescence detection for alkali metal cations by monomer-excimer emission systems.[3a,3b,3e,14]

## 1-4. Experimental Section

### General Methods.

$^1$H NMR spectra were taken at 400 MHz on a JEOL JNM-GSX-400 spectrometer

using tetramethylsilane as the internal standard. IR spectra were obtained on a Horiba FT-710 spectrometer. Mass spectra were measured on a JEOL JMS-DX-303 mass spectrometer. Elemental analyses were measured with a Yanagimoto CHN-Corder. Fluorescence measurements were carried out on a Shimadzu fluorescence spectrometry (RF-1500).

All chemicals were of commercially available reagent grade and the starting crown ethers of Type A, 15-methylene-16-crown-5 (**8a**), and 18-methylene-19-crown-6 (**8b**), were prepared according to the method described in the literature.[11] The starting crown ethers of Type B, *trans*-bis(bromomethyl)-2,9-dimethyl-15-crown-5 (*trans*-**15a**), *trans*-bis(bromomethyl)-2,12-dimethyl-18-crown-6 (*trans*-**15b**), *cis*-bis(bromomethyl)-2,9-dimethyl-15-crown-5 (*cis*-**15a**), and *cis*-bis(bromomethyl)-2,12-dimethyl 18-crown-6 (*cis*-**15b**), were prepared according to the method described in the literature.[8c]

**12-Methylene-13-crown-4 (8c).**

A solution of 3-chloro-2-chloromethyl-1-propene (10.0 g, 80.0 mmol) and triethylene glycol (12.0 g, 80.0 mmol) in *t*-BuOH (60 mL) was dropwise added to a suspension of *t*-BuOH (80 mL) containing *t*-BuOLi (19.2 g, 0.240 mol) for 3 h at 60˚C. The mixture was stirred at refluxing temperature for another 24 h. The insoluble matter was removed by filtration and *t*-BuOH was evaporated in vacuo. Water (200 mL) was added to the residue and the solution was extracted with dichloromethane (200 mL × 3). The organic layer was dried over $MgSO_4$ and evaporated in vavuo to give a brown viscous liquid. The crude product was distilled under reduced pressure (0.06 Torr, 120˚C) to give **8c** (5.22 g, 25.8 mmol) as a colorless oil. Yield 32%. $^1$H NMR ($CDCl_3$) δ 3.61-3.74 (m, 12H), 4.17 (s, 4H), 5.13-5.15 (m, 2H). IR (neat, $cm^{-1}$) 3080, 2850, 1650, 1450, 1350, 1300, 1120, 920. MS (FAB) (m/z, relative intensity) 203 ($M^+$+1, 68), 154 (100).

**General Procedure for Bromoalkoxylation of 8a-c Using *N*-Bromosuccinimide (NBS) and Oligoethylene Glycol to Give Compounds 9a-e.**

To a stirred suspension of NBS (20 mmol) in oligoethylene glycol (0.200 mol) was added **8** (20 mmol) under cooling in an ice bath for 1 h. The resulting mixture was further stirred at 50˚C for 1 h. After cooling to room temperature, 10% sodium carbonate aqueous solution (200 mL) was added to the mixture, and the product was extracted with dichloromethane (200 mL × 3). The solvent was evaporated to give a slightly yellowish liquid. The crude product was used for the next step without further purification.

**General Procedure for the Synthesis of the Protected Compounds 10a-e.**

After dissolving crude compound **9** (20 mmol) and *p*-toluenesulfonic acid (1.2 mmol) in 1,2-dichloroethane (3 mL), 3,4-dihydro-2H-pyrane (30 mmol) was added dropwise to the mixture under cooling in an ice bath for 10 min. The resulting mixture was further stirred at room temperature for 3 h. Sodium carbonate aqueous solution (10%, 200 mL) was added to the mixture, and the product was extracted with dichloromethane (200 mL × 3). After evaporation, the residue was purified by silica gel chromatography (dichloromethane:acetone = 95:5).

**15-Bromomethyl-15-[2-(2-tetrahydropyranyloxy)ethoxy]-1,4,7,10,13-pentaoxacyclohexadecane (10a).**

By following the general procedure, **10a** was obtained from **9a** as a slightly yellowish liquid. Yield 76% (based on **8a**). $^1$H NMR ($CDCl_3$) δ 1.40-1.83 (m, 6H), 3.42-3.90 (m, 28H), 4.65-4.67 (m, 1H). IR (neat, $cm^{-1}$) 2950, 1460, 1350, 1300, 1200, 1120, 970. MS (CI) (m/z, relative intensity) 471 ($M^+$+1, 5), 389 (100).

**15-Bromomethyl-15-[2-[2-(2-tetrahydropyranyloxy)ethoxy]ethoxy]-1,4,7,10,13-pentaoxacyclohexadecane (10b).**

By following the general procedure, **10b** was obtained from **9b** as a slightly yellowish liquid. Yield 73% (based on **8a**). $^1$H NMR ($CDCl_3$) δ 1.40-1.87 (m, 6H), 3.42-3.91 (m, 32H), 4.53-4.64 (m, 1H). IR (neat, $cm^{-1}$) 2900, 1450, 1350, 1300, 1200, 1120, 950. MS (FAB) (m/z, relative intensity) 515 ($M^+$+1, 10), 154 (100).

**15-Bromomethyl-15-[2-[2-[2-(2-tetrahydropyranyloxy)ethoxy]ethoxy]ethoxy]-1,4,7,10,13-pentaoxacyclohexadecane (10c).**

By following the general procedure, **10c** was obtained from **9c** as a slightly yellowish liquid. Yield 96% (based on **8a**). $^1$H NMR ($CDCl_3$) δ 1.46-1.87 (m, 6H, m), 3.44-3.90 (m, 36H), 4.59-4.63 (m, 1H). IR (neat, $cm^{-1}$) 2900, 1400, 1350, 1300, 1250,1120, 970. MS (FAB) (m/z, relative intensity) 581 ($M^+$+Na, 35), 136 (100).

**18-Bromomethyl-18-[2-[2-(2-tetrahydropyranyloxy)ethoxy]ethoxy]-1,4,7,10,13,16-hexaoxacyclononadecane (10d).**

By following the general procedure, **10d** was obtained from **9d** as a slightly yellowish liquid. Yield 64% (based on **8b**). $^1$H NMR ($CDCl_3$) δ 1.46-1.87 (m, 6H), 3.44-3.90 (m, 36H), 4.59-4.63 (m, 1H). IR (neat, $cm^{-1}$) 2950, 1400, 1360, 1300, 1240, 1100, 970. MS (FAB) (m/z, relative intensity) 559 ($M^+$+1, 3), 169 (100).

**12-Bromomethyl-12-[2-[2-(2-tetrahydropyranyloxy)ethoxy]ethoxy]-1,4,7,10-tetraoxacyclotridecane (10e).**

By following the general procedure, **10e** was obtained from **9e** as a slightly yellowish liquid. Yield 81% (based on **8c**). $^1$H NMR ($CDCl_3$) δ 1.43-1.85 (m, 6H), 3.44-3.89 (m, 28H), 4.60-4.64 (m, 1H). IR (neat, $cm^{-1}$) 2860, 1690, 1450, 1350, 1280, 1200, 1120, 990. MS (FAB) (m/z, relative intensity) 509 ($M^+$+K, 94) 169 (100).

**General Procedure for the Synthesis of 11a-e.**

After potassium *t*-butoxide (40 mmol) was dissolved in ethylene glycol monotetrahydropyranyl ether (80 mmol), **10** (10 mmol) was added to the mixture, followed by stirring at 120˚C for 36 h. After cooling to room temperature, the mixture was filtered and evaporated. Water (200 mL) was added to the mixture, and the product was extracted with dichloromethane (200 mL × 3). The solvent was evaporated to give a yellowish liquid. The crude product was used for the next step without further purification.

**General Procedure for the Deprotection of 11a-e to Give 12a-e.**

After crude compound **11** was dissolved in methanol (150 mL), conc. $H_2SO_4$ (5 drops) was added to the solution, followed by stirring at room temperature for 24 h. After neutralization with sodium hydroxide, methanol was evaporated in vacuo. The residue was purified by alumina chromatography (chloroform:methanol = 95:5).

**15-(2-Hydroxyethoxy)-15-[(2-hydroxyethoxy)methyl]-1,4,7,10,13-pentaoxacyclohexadecane (12a).**

By following the general procedure, **12a** was obtained from **11a** as a slightly yellowish viscous liquid. Yield 53% (based on **10a**). $^1$H NMR ($CDCl_3$ + $D_2O$) δ 3.56-3.81 (m, 30H). IR (neat, $cm^{-1}$) 3570-3230, 2870, 1960, 1730, 1660, 1450, 1360, 1290, 1120, 950. MS (FAB) (m/z, relative intensity) 369 ($M^+$+1, 37), 154 (100).

**15-[2-(2-Hydroxyethoxy)ethoxy]-15-[(2-hydroxyethoxy)methyl]-1,4,7,10,13-pentaoxacyclohexadecane (12b).**

By following the general procedure, **12b** was obtained from **11b** as a slightly yellowish viscous liquid. Yield 33% (based on **10b**). $^1$H NMR ($CDCl_3$ + $D_2O$) δ

3.53-3.81 (m, 34H). IR (neat, $cm^{-1}$) 3540-3250, 2730, 1950, 1730, 1650, 1460, 1360, 1300, 1070, 940. MS (FAB) (m/z, relative intensity) 413 ($M^+$+1, 41), 45 (100).

**15-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]-15-[(2-hydroxyethoxy)methyl]-1,4,7,10, 13-pentaoxacyclohexadecane (12c).**

By following the general procedure, **12c** was obtained from **11c** as a slightly yellowish viscous liquid. Yield 28% (based on **10c**). $^1H$ NMR ($CDCl_3$ + $D_2O$) δ 3.54-3.83 (m, 38H). IR (neat, $cm^{-1}$) 3590-3190, 2900, 1960, 1700, 1650, 1460, 1360, 1300, 1100, 940. MS (FAB) (m/z, relative intensity) 457 ($M^+$+1, 21), 45 (100).

**18-[2-(2-Hydroxyethoxy)ethoxy]-18-[(2-hydroxyethoxy)methyl]-1,4,7,10,13,16-hexaoxacyclononadecane (12d).**

By following the general procedure, **12d** was obtained from **11d** as a slightly yellowish viscous liquid. Yield 28% (based on **10d**). $^1H$ NMR ($CDCl_3$ + $D_2O$) δ 3.53-3.79 (m, 38H). IR (neat, $cm^{-1}$) 3560-3270, 2860, 1960, 1650, 1460, 1350, 1300, 1100, 950. MS (FAB) (m/z, relative intensity) 457 ($M^+$+1, 40), 154 (100).

**12-[2-(2-Hydroxyethoxy)ethoxy]-12-[(2-hydroxyethoxy)methyl]-1,4,7-tetraoxacyclotridecane (12e).**

By following the general procedure, **12e** was obtained from **11e** as a slightly yellowish viscous liquid. Yield 28% (based on **10e**). $^1H$ NMR ($CDCl_3$ + $D_2O$) δ 3.47-3.80 (m, 30H). IR (neat, $cm^{-1}$) 3570-3210, 2840, 1960, 1630, 1450, 1360, 1300, 1020, 890. MS (FAB) (m/z, relative intensity) 369 ($M^+$+1, 61), 154 (100).

**General Procedure for the Synthesis of 1-3.**

To a suspension of NaH (7.86-11.4 mmol) and **12** (1.31-1.90 mmol) in THF (15 mL) was added dropwise a solution of 1-bromomethylpyrene (5.24-7.60 mmol) in THF

(30 mL) and the resulting mixture was stirred for 36 h at refluxing temperature. After cooling to room temperature, a small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. Water (200 mL) was added to the residue, and then extracted with dichloromethane (200 mL × 3). The combined organic layer was dried over $MgSO_4$ and the dichloromethane was evaporated off. The residue was purified by alumina chromatography (benzene:ethyl acetate = 95:5-70:30).

**15-[2-(1-Pyrenylmethoxy)ethoxy]-15-[[2-(1-pyrenylmethoxy)ethoxy]methyl]-1,4,7, 10,13-pentaoxacyclohexadecane (1a).**

By following the general procedure, **1a** was obtained from **12a** as a slightly yellowish viscous liquid. Yield 29%. $^1$H NMR ($CDCl_3$) δ 3.45-3.92 (m, 30H), 5.15 (s, 2H), 5.18 (s, 2H), 7.89-8.34 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2860, 1730, 1590, 1450, 1350, 1300, 1120, 850, 710. MS (FAB) (m/z, relative intensity) 796 ($M^+$, 1), 215 (100). Anal. Calcd for $C_{50}H_{52}O_9$: C, 75.36; H, 6.58. Found: C, 75.63; H, 6.30.

**15-[2-[2-(1-Pyrenylmethoxy)ethoxy]ethoxy]-15-[[2-(1-pyrenylmethoxy)ethoxy] methyl]-1,4,7,10,13-pentaoxacyclohexadecane (1b).**

By following the general procedure, **1b** was obtained from **12b** as a slightly yellowish viscous liquid. Yield 30%. $^1$H NMR ($CDCl_3$) δ 3.38-3.82 (m, 34H), 5.19 (s, 4H), 7.88-8.35 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2870, 1730, 1600, 1460, 1350, 1300, 1120, 850, 710. MS (FAB) (m/z, relative intensity) 840 ($M^+$, 2), 215 (100). Anal. Calcd for $C_{52}H_{56}O_{10}$: C, 74.26; H, 6.71. Found: C, 73.92; H, 6.52.

**15-[2-[2-[2-(1-Pyrenylmethoxy)ethoxy]ethoxy]ethoxy]-15-[[2-(1-pyrenylmethoxy) ethoxy]methyl]-1,4,7,10,13-pentaoxacyclohexadecane (1c).**

By following the general procedure, **1c** was obtained from **12c** as a slightly

yellowish viscous liquid. Yield 21%. $^1$H NMR ($CDCl_3$) δ 3.42-3.78 (m, 38H), 5.21 (s, 4H), 7.93-8.36 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2860, 1730, 1590, 1460, 1350, 1290, 1090, 850, 710. MS (FAB) (m/z, relative intensity) 884 ($M^+$, 1), 215 (100). Anal. Calcd for $C_{54}H_{60}O_{11}$: C, 73.28; H, 6.83. Found: C, 73.46; H, 6.72.

**18-[2-[2-(1-Pyrenylmethoxy)ethoxy]ethoxy]-18-[[2-(1-pyrenylmethoxy)ethoxy] methyl]-1,4,7,10,13,16-hexaoxacyclononadecane (2).**

By following the general procedure, **2** was obtained from **12d** as a slightly yellowish viscous liquid. Yield 29%. $^1$H NMR ($CDCl_3$) δ 3.45-3.79 (m, 38H), 5.18 (s, 4H), 7.92-8.37 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2860, 1730, 1600, 1460, 1350, 1300, 1100, 850, 710. MS (FAB) (m/z, relative intensity) 884 ($M^+$, 2), 215 (100). Anal. Calcd for $C_{54}H_{60}O_{11}$: C, 73.28; H, 6.83. Found: C, 73.63; H, 6.46.

**12-[2-[2-(1-Pyrenylmethoxy)ethoxy]ethoxy]-12-[[2-(1-pyrenylmethoxy)ethoxy] methyl]-1,4,7,10-tetraoxacyclotridecane (3).**

By following the general procedure, **3** was obtained from **12e** as a slightly yellowish viscous liquid. Yield 31%. $^1$H NMR ($CDCl_3$) δ 3.42-3.78 (m, 30H), 5.16 (s, 2H), 5.17 (s, 2H), 7.90-8.34 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2860, 1740, 1590, 1460, 1350, 1300, 1080, 850, 710. MS (FAB) (m/z, relative intensity) 796 ($M^+$, 1), 215 (100). Anal. Calcd for $C_{50}H_{52}O_9$: C, 75.36; H, 6.58. Found: C, 75.56; H, 6.31.

**15-Acetoxymethyl-15-[2-[2-(2-tetrahydropyranyloxy)ethoxy]ethoxy]-1,4,7,10,13-pentaoxacyclohexadecane (13).**

After potassium acetate (11.5 g, 0.117 mol) was dissolved in DMSO (40 mL), **10b** (10.1 g, $1.96 \times 10^{-2}$ mol) was added to the mixture followed by stirring at 100˚C for 48 h. After cooling to room temperature, the mixture was filtered and evaporated to give a yellowish liquid. The crude product was used for the next step without further

purification.

**15-Hydroxymethyl-15-[2-[2-(2-tetrahydropyranyloxy)ethoxy]ethoxy]-1,4,7,10,13-pentaoxacyclohexadecane (14).**

After sodium hydroxide (0.700 g, 17.6 mmol) was dissolved in a mixed solvent of water/ethanol (2:1 v/v, 30 mL), **13** (6.90 g, 15.2 mmol) was added to the mixture followed by stirring at room temperature for 24 h. The mixture was filtered and evaporated. The crude compound was dissolved in methanol (150 mL), conc. $H_2SO_4$ (5 drops) was added to the solution, followed by stirring at room temperature for 24 h. After neutralization with sodium hydroxide, methanol was evaporated in vacuo. The residue was purified by alumina chromatography (chloroform:methanol = 95:5) to give **14** (2.62 g, 7.11 mmol) as a slightly yellowish liquid. Yield 37% (based on **10b**). $^1$H NMR ($CDCl_3$ + $D_2O$) δ 3.56-3.79 (m, 30H). IR (neat, $cm^{-1}$) 3520-3250, 2860, 1730, 1630, 1460, 1360, 1300, 1120, 940. MS (FAB) (m/z, relative intensity) 369 ($M^+$+1, 31), 85 (100).

**15-[2-[2-(1-Pyrenylmethoxy)ethoxy]ethoxy]-15-[1-pyrenylmethoxy]methyl]-1,4,7,10,13-pentaoxacyclohexadecane (1d).**

The synthetic procedure was almost the same as that used for **1**. The crude product was purified by alumina chromatography (benzene:ethyl acetate = 85:15) to give 0.581 g of a slightly yellowish viscous liquid. Yield 40%. $^1$H NMR ($CDCl_3$) δ 3.34-3.83 (m, 30H), 5.17 (s, 4H), 7.90-8.36 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2850, 1730, 1590, 1460, 1350, 1300, 1130, 850, 710. MS (FAB) (m/z, relative intensity) 796 ($M^+$, 3), 215 (100). Anal. Calcd for $C_{50}H_{52}O_9$: C, 75.36; H, 6.58. Found: C, 75.57; H, 6.38.

**1-Methoxy-2-(1-pyrenylmethoxy)ethane (4).**

The synthetic procedure was almost the same as that used for compounds **1**. The crude product was purified by chromatography over alumina (benzene:ethyl acetate = 98:2) to give **4** as a slightly yellowish viscous liquid. Yield 65%. $^1$H NMR ($CDCl_3$) δ 3.39 (s, 3H), 3.58 (t, 2H, *J* = 4.8Hz), 3.72 (t, 2H, *J* = 4.8Hz), 5.29 (s, 2H), 7.97-8.43 (m, 9H). IR (neat, $cm^{-1}$) 3040, 2860, 1790, 1590, 1460, 1350, 1240, 1090, 820, 710. MS (FAB) (m/z) 290 ($M^+$+1, 84), 215 (100). Anal. Calcd for $C_{20}H_{18}O_2$: C, 82.73; H, 6.25. Found: C, 82.47; H, 6.11.

**Tetraethylene glycol bis(1-pyrenylmethyl) ether (5).**

The synthetic procedure was almost the same as that used for compounds **1**. The crude product was purified by chromatography over alumina (benzene:ethyl acetate = 95:5) to give **5** as a slightly yellowish viscous liquid. Yield 22%. $^1$H NMR ($CDCl_3$) δ 3.57-3.71 (m, 16H), 5.21 (s, 4H), 7.94-8.37 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2860, 1720, 1600, 1460, 1350, 1240, 1090, 850, 710. MS (FAB) (m/z, relative intensity) 622 ($M^+$, 9), 215 (100). Anal. Calcd for $C_{42}H_{38}O_5$: C, 81.00; H, 6.15. Found: C, 80.73; H, 6.13.

**General Procedure for the Synthesis of 16a-b.**

After potassium acetate (96 mmol) was dissolved in DMSO (20 mL), **15** (8 mmol) was added to the mixture followed by stirring at 100˚C for 48 h. After cooling to room temperature, the mixture was filtered and evaporated to give a yellowish liquid. The crude compound was purified by silica gel chromatography (ethyl acetate) to give a slightly yellowish liquid.

***trans*-2,9-bis(Acetoxymethyl)-2,9-dimethyl-1,4,7,10,13-pentaoxacyclopentadecane (*trans*-16a).**

By following the general procedure, *trans*-**16a** was obtained from *trans*-**15a** as a

slightly yellowish viscous liquid. Yield 75%. $^1$H NMR ($CDCl_3$) δ 1.15 (s, 6H), 2.05 (s, 6H), 3.41-3.77 (m, 16H), 4.07 (d, 2H, *J* = 11.6 Hz), 4.12 (d, 2H, *J* = 11.6 Hz). IR (neat, $cm^{-1}$) 2840, 1720, 1640, 1450, 1370, 1310, 1040, 960. MS (FAB) (m/z, relative intensity) 393 ($M^+$+1, 86), 43 (100).

**_cis_-2,9-bis(Acetoxymethyl)-2,9-dimethyl-1,4,7,10,13-pentaoxacyclopentadecane (_cis_-16a).**

By following the general procedure, *cis*-**16a** was obtained from *cis*-**15a** as a slightly yellowish viscous liquid. Yield 64%. $^1$H NMR ($CDCl_3$) δ 1.17 (s, 6H), 2.06 (s, 6H), 3.35-3.82 (m, 16H), 4.04 (d, 2H, *J* = 12.3 Hz), 4.22 (d, 2H, *J* = 12.3 Hz). IR (neat, $cm^{-1}$) 2850, 1730, 1640, 1450, 1370, 1300, 1040, 960. MS (FAB) (m/z, relative intensity) 393 ($M^+$+1, 22), 87 (100).

**_trans_-2,12-bis(Acetoxymethyl)-2,12-dimethyl-1,4,7,10,13,16-hexaoxacyclooctadecane (_trans_-16b).**

By following the general procedure, *trans*-**16b** was obtained from *trans*-**15b** as a slightly yellowish viscous liquid. Yield 75%. $^1$H NMR ($CDCl_3$) δ 1.17 (s, 6H), 2.06 (s, 6H), 3.44-3.71 (m, 20H), 4.06 (d, 2H, *J* = 11.4 Hz), 4.12 (d, 2H, *J* = 11.4 Hz). IR (neat, $cm^{-1}$) 2850, 1720, 1640, 1450, 1370, 1300, 1100, 960. MS (FAB) (m/z, relative intensity) 437 ($M^+$+1, 57), 87 (100).

**General Procedure for the Synthesis of 17a-b.**

After sodium hydroxide (5 mmol) was dissolved in a mixed solvent of water/ethanol (2:1 v/v, 30 mL), **16** (2.5 mmol) was added to the mixture followed by stirring at room temperature for 24 h. The mixture was filtered and evaporated. The residue was purified by alumina chromatography (chloroform:methanol = 95:5).

***trans*-2,9-Dimethyl-2,9-bis(hydroxymethyl)-1,4,7,10,13-pentaoxacyclopentadecane (*trans*-17a).**

By following the general procedure, *trans*-**17a** was obtained from *trans*-**16a** as a slightly yellowish viscous liquid. Yield 89%. $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.10 (s, 6H), 3.51-3.76 (m, 20H). IR (neat, $cm^{-1}$) 3550-3250, 2880, 1650, 1460, 1370, 1290, 1090, 960. MS (FAB) (m/z, relative intensity) 309 ($M^+$+1, 81), 154 (100).

***cis*-2,9-Dimethyl-2,9-bis(hydroxymethyl)-1,4,7,10,13-pentaoxacyclopentadecane (*cis*-17a).**

By following the general procedure, *cis*-**17a** was obtained from *cis*-**16a** as a slightly yellowish viscous liquid. Yield 58%. $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.09 (s, 6H), 3.49-3.74 (m, 20H). IR (neat, $cm^{-1}$) 3510-3250, 2880, 1660, 1460, 1380, 1290, 1090, 960. MS (FAB) (m/z, relative intensity) 309 ($M^+$+1, 87), 154 (100).

***trans*-2,12-Dimethyl-2,12-bis(hydroxymethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (*trans*-17b).**

By following the general procedure, *trans*-**17b** was obtained from *trans*-**16b** as a slightly yellowish viscous liquid. Yield 75%. $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.09 (s, 6H), 3.46-3.75 (m, 24H). IR (neat, $cm^{-1}$) 3550-3250, 2920, 1650, 1470, 1360, 1300, 1090, 960. MS (FAB) (m/z, relative intensity) 353 ($M^+$+1, 75), 45 (100).

**General Procedure for the Synthesis of 6-7.**

The synthetic procedure was almost the same as that used for **1**. The crude compound was purified was purified by alumina chromatography (benzene:ethyl acetate = 90:10-80:20).

***trans*-2,9-Dimethyl-2,9-bis[(1-pyrenylmethoxy)methyl]-1,4,7,10,13-**

**pentaoxacyclopentadecane (*trans*-6a).**

By following the general procedure, *trans*-**6a** was obtained from *trans*-**17a** as a slightly yellowish viscous liquid. Yield 20%. $^1$H NMR ($CDCl_3$) δ 1.08 (s, 6H), 3.17-3.65 (m, 20H), 5.14 (d, 2H, *J* =12.1 Hz), 5.25 (d, 2H, *J* =12.1 Hz), 7.93-8.39 (m, 18H). IR (neat, $cm^{-1}$) 3050, 2860, 1710, 1590, 1460, 1360, 1290, 1090, 890, 750. MS (FAB) (m/z, relative intensity) 736 ($M^+$, 1), 215 (100) Anal. Calcd for $C_{48}H_{48}O_7$: C, 78.24; H, 6.57. Found: C, 78.00; H, 6.43.

***cis*-2,9-Dimethyl-2,9-bis[(1-pyrenylmethoxy)methyl]-1,4,7,10,13-pentaoxacyclopentadecane (*cis*-6a).**

By following the general procedure, *cis*-**6a** was obtained from *cis*-**17a** as a slightly yellowish viscous liquid. Yield 23%. $^1$H NMR ($CDCl_3$) δ 1.14 (s, 6H), 3.29-3.68 (m, 20H), 5.15 (d, 2H, *J* =12.1 Hz), 5.22 (d, 2H, *J* =12.1 Hz), 7.84-8.40 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2870, 1720, 1600, 1450, 1360, 1290, 1090, 850, 730. MS (FAB) (m/z, relative intensity) 736 ($M^+$, 15) 215 (100) Anal. Calcd for $C_{48}H_{48}O_7$: C, 78.24; H, 6.57. Found: C, 78.08; H, 6.44.

***trans*-2,12-Dimethyl-2,12-bis[(1-pyrenylmethoxy)methyl]-1,4,7,10,13,16-hexaoxacyclooctadecane (*trans*-7a).**

By following the general procedure, *trans*-**7a** was obtained from *trans*-**17b** as a slightly yellowish viscous liquid. Yield 50%. $^1$H NMR ($CDCl_3$) δ 1.11 (s, 6H), 3.38-3.63 (m, 24H), 5.18 (d, 2H, *J* =11.7 Hz), 5.23 (d, 2H, *J* =11.7 Hz), 7.93-8.39 (m, 18H). IR (neat, $cm^{-1}$) 3050, 2850, 1710, 1630, 1450, 1360, 1240, 1100, 840, 710. MS (FAB) (m/z, relative intensity) 780 ($M^+$, 3), 215 (100). Anal. Calcd for $C_{50}H_{52}O_8$: C, 76.90; H, 6.71. Found: C, 77.17; H, 6.44.

**General Procedure for the Synthesis of 18a-c.**

After potassium *t*-butoxide (32 mmol) was dissolved in oligoethylene glycol monotetrahydropyranyl ether (80 mmol), **15** (4 mmol) was added to the mixture followed by stirring at 120˚C for 36 h. After cooling to room temperature, the mixture was filtered and evaporated. Water (200 mL) was added to the mixture, and the product was extracted with dichloromethane (200 mL × 3). The solvent was evaporated to give a yellowish liquid. The crude product was used for the next step without further purification.

**General Procedure for the Deprotection of 18a-c to Give 19a-c.**

After crude compound **18** was dissolved in methanol (150 mL), conc. $H_2SO_4$ (5 drops) was added to the solution, followed by stirring at room temperature for 24 h. After neutralization with sodium hydroxide, methanol was evaporated in vacuo. The residue was purified by alumina chromatography (chloroform:methanol = 95:5).

***trans*-2,9-Dimethyl-2,9-bis[(2-hydroxyethoxy)methyl]-1,4,7,10,13-pentaoxacyclopentadecane (*trans*-19a).**

By following the general procedure, *trans*-**19a** was obtained from *trans*-**15a** as a slightly yellowish viscous liquid. Yield 44% (based on *trans*-**15a**). $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.15 (s, 6H), 3.44-3.76 (m, 28H). IR (neat, $cm^{-1}$) 3500-3270, 2880, 1650, 1460, 1360, 1290, 1090, 960. MS (FAB) (m/z, relative intensity) 397 ($M^+$+1, 43), 115 (100).

***cis*-2,9-Dimethyl-2,9-bis[(2-hydroxyethoxy)methyl]-1,4,7,10,13-pentaoxacyclopentadecane (*cis*-19a).**

By following the general procedure, *cis*-**19a** was obtained from *cis*-**15a** as a slightly yellowish viscous liquid. Yield 58% (based on *cis*-**15a**). $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.15 (s, 6H), 3.43-3.74 (m, 28H). IR (neat, $cm^{-1}$) 3510-3250, 2870, 1650, 1450,

1360, 1290, 1120, 960. MS (FAB) (m/z, relative intensity) 397 ($M^+$+1, 19), 115 (100).

**_trans_-2,9-Dimethyl-2,9-bis[[2-(2-hydroxyethoxy)ethoxy]methyl]-1,4,7,10,13-pentaoxacyclopentadecane (_trans_-19b).**

By following the general procedure, *trans*-**19b** was obtained from *trans*-**15a** as a slightly yellowish viscous liquid. Yield 27% (based on *trans*-**15a**). $^1$H NMR ($CDCl_3$ + $D_2O$): δ 1.16 (s, 6H), 3.35-3.78 (m, 36H). IR (neat, $cm^{-1}$) 3510-3270, 2860, 1650, 1470, 1350, 1290, 1050, 890. MS (FAB) (m/z, relative intensity) 485 ($M^+$+1, 21), 45 (100).

**_trans_-2,12-Dimethyl-2,12-bis[(2-hydroxyethoxy)methyl]-1,4,7,10,13,16-hexaoxacyclooctadecane (_trans_-19c).**

By following the general procedure, *trans*-**19c** was obtained from *trans*-**15b** as a slightly yellowish viscous liquid. Yield 35% (based on *trans*-**15b**). $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.14 (s, 6H), 3.43-3.75 (m, 32H). IR (neat, $cm^{-1}$) 3530-3230, 2870, 1650, 1460, 1370, 1300, 1090, 960. MS (FAB) (m/z, relative intensity) 441 ($M^+$+1, 22), 115 (100).

**_trans_-2,9-Dimethyl-2,9-bis[[2-(1-pyrenylmethoxy)ethoxy]methyl]-1,4,7,10,13-pentaoxacyclopentadecane (_trans_-6b).**

By following the general procedure, *trans*-**6b** was obtained from *trans*-**19a** as a slightly yellowish viscous liquid. Yield 32%. $^1$H NMR ($CDCl_3$) δ 1.12 (s, 6H), 3.35-3.77 (m, 28H). 5.25 (s, 4H), 7.95-8.39 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2940, 1730, 1600, 1460, 1350, 1290, 1100, 850, 710. MS (FAB) (m/z, relative intensity) 824 (M+, 9), 215 (100). Anal. Calcd for $C_{52}H_{56}O_9$: C, 75.70; H, 6.84. Found: C, 75.69; H, 6.70.

***cis*-2,9-Dimethyl-2,9-bis[[2-(1-pyrenylmethoxy)ethoxy]methyl]-1,4,7,10,13-pentaoxacyclopentadecane (*cis*-6b).**

By following the general procedure, *cis*-**6b** was obtained from *cis*-**19a** as a slightly yellowish viscous liquid. Yield 19%. $^1$H NMR ($CDCl_3$) δ 1.15 (s, 6H), 3.37-3.74 (m, 28H). 5.23 (s, 4H), 7.94-8.37 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2870, 1730, 1610, 1460, 1350, 1290, 1090, 850, 710. MS (FAB) (m/z, relative intensity) 824 (M+, 23), 215 (100). Anal. Calcd for $C_{52}H_{56}O_9$: C, 75.70; H, 6.84. Found: C, 75.65; H, 6.80.

***trans*-2,9-Dimethyl-2,9-bis[[2-[2-(1-pyrenylmethoxy)ethoxy]ethoxy]methyl]-1,4,7,10,13-pentaoxacyclopentadecane (*trans*-6c).**

By following the general procedure, *trans*-**6c** was obtained from *trans*-**19b** as a slightly yellowish viscous liquid. Yield 48%. $^1$H NMR ($CDCl_3$) δ 1.08 (s, 6H), 3.30-3.76 (m, 36H). 5.26 (s, 4H), 7.95-8.40 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2870, 1730, 1600, 1460, 1350, 1290, 1090, 850, 710. MS (FAB) (m/z, relative intensity) 912 (M+, 1), 215 (100). Anal. Calcd for $C_{56}H_{64}O_{11}$: C, 73.66; H, 7.06. Found: C, 73.84; H, 6.83.

***trans*-2,12-Dimethyl-2,12-bis[[2-(1-pyrenylmethoxy)ethoxy]methyl]-1,4,7,10,13,16-hexaoxacyclooctadecane (*trans*-7b).**

By following the general procedure, *trans*-**7b** was obtained from *trans*-**19c** as a slightly yellowish viscous liquid. Yield 17%. $^1$H NMR ($CDCl_3$) δ 1.14 (s, 6H), 3.32-3.75 (m, 32H). 5.25 (s, 4H), 7.95-8.40 (m, 18H). IR (neat, $cm^{-1}$) 3040, 2860, 1730, 1590, 1450, 1350, 1290, 1090, 850, 710. MS (FAB) (m/z, relative intensity) 868 (M+, 1), 215 (100). Anal. Calcd for $C_{54}H_{60}O_{10}\cdot H_2O$: C, 73.12; H, 7.04. Found: C, 73.22; H, 6.72.

**Measurement of Fluorescence Spectra.**

Fluorescence spectra were measured at room temperature. The concentration of fluorescent reagents was $1 \times 10^{-6}$ M in a mixed solvent of $CH_3CN/CHCl_3$ (99/1). Alkali metal cations and alkaline earth metal cations were added into the solution of fluorescent reagent as perchlorate salts ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$) and thiocyanate salts ($Li^+$, $Na^+$, $K^+$). To prevent nonlinearity of the fluorescence intensities, the excitation wavelength was set to 340 nm, which was an isosbestic point in the absorption spectra. Before each experiment, nitrogen was bubbled through the samples for 15 min.

**Measurement of Stability Constants.**

All of the stability constants herein reported were determined from the curve by means of a nonlinear least-square curve fitting method. The curve showed that all the ligands formed 1:1 complexes. Typically, the concentration of the host compound was fixed to be $1 \times 10^{-6}$ mol/L and the molar ratios of the guest to host were changed in the range from 0 to 10 by changing the concentrations of the guest salt. Eight data were collected for each host-guest system and the stability constant (*K*) was calculated using an iterative nonlinear least squares curve-fitting program.[12]

**1-5. References**

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# Chapter 2

# Fluorescent Characteristics of Novel Photosensitive Monoazacryptand Derivatives with a Pyrene Ring in Aqueous Micellar Solutions

## 2-1. Introduction

The design of chemosensors, molecules that can selectively recognize the presence of a specific analyte, is an important goal of supramolecular chemistry.[1] Since the fluorescent detection of metal ions using fluorophores is one of the most powerful methods to discriminate between metal cations including alkali metal and alkaline earth metal cations, much effort has been devoted to the design and development of new types of fluorophores exhibiting high selectivity in addition to high sensitivity. Crown ethers and their analogous compounds are specific hosts for alkali metal and alkaline earth metal cations, and can be functionalized by the introduction of fluorescent substituents.[2] These hosts are effective as selective fluorophores in organic media, but most of them lose their specificity in aqueous media because their complexation abilities are drastically decreased by strong hydration.[3] For example, lariat ether-type fluorophores with a pyrene moiety at each end of their two sidearms can selectively detect alkaline earth metal cations in acetonitrile[4] using the effective coordination of one of their electron-donating sidearms, but become ineffective in aqueous solutions. This result indicates that much stronger complexation ability is required for fluorophores when used in aqueous media. Thus, the number of fluorophores effective in aqueous media has been rather limited,[5] and it remains a challenge to design a new fluorescence detection system for a specific metal cation in water. Cryptands are well known to be very effective host molecules for alkali metal

and alkaline earth metal cations than crown ethers because of their highly preorganized three-dimensional structures.[6] Our strategy for this design is based on the use of a cryptand scaffold, together with the use of the less polar regions of aqueous micelles[7] as complexation fields for cations. In this chapter, the author reports the design and synthesis of novel monoazacryptand types of fluorophores **1**-**2** and monoazacrown ether types of fluorophores **3**-**4**. Furthermore, their fluorescence properties for alkali metal and alkaline earth metal cations in water in the presence of nonionic surfactants such as polyoxyethylene(10) isooctylphenyl ether (Triton X-100), polyoxyethylene(9) nonylphenyl ether (Tergitol NP-9), polyoxyethylene(20) sorbitan monolaurate (Tween-20), polyoxyethylene(20) sorbitan monopalmitate (Tween-40), polyoxyethylene(20) sorbitan monostearate (Tween-60), polyoxyethylene(20) sorbitan monooleate (Tween-80), polyoxyethylene(23) lauryl ether (Brij-35), polyoxyethylene(10) cetyl ether (Brij-56), polyoxyethylene(20) stearyl ether (Brij-78), *n*-dodecyl-β-maltopyranoside, and poly(ethylene oxide)-ploy(propylene oxide)-poly(ethylene oxide) block copolymer (Pluronic F-127), and ionic surfactants such as sodium dodecyl sulfate (SDS), sodium *n*-dodecylbenzenesulfonate (SDBS), sodium cholate (NaC), sodium deoxycholate (NaDC), tetramethylammonium dodecyl sulfate (TMADS), tetramethylammonium dodecanoate (TMADC), disodium 5,12-bis(decyloxymethyl)-4,7,10,13-tetraoxahexadecane-1,16-disulfonates (Gemini Surfactant), and cetyltrimethylammonium bromide (CTAB) are described.

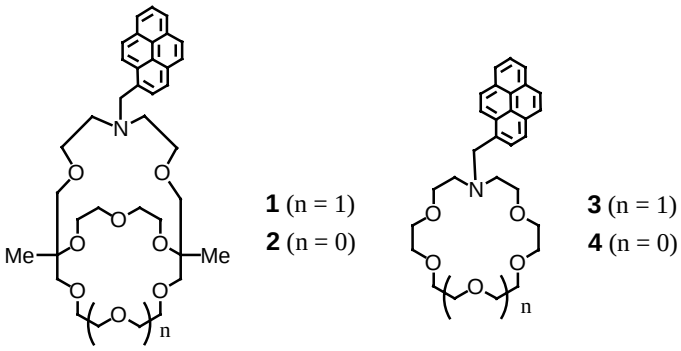



**FIGURE 1.** Structures of fluorophores.

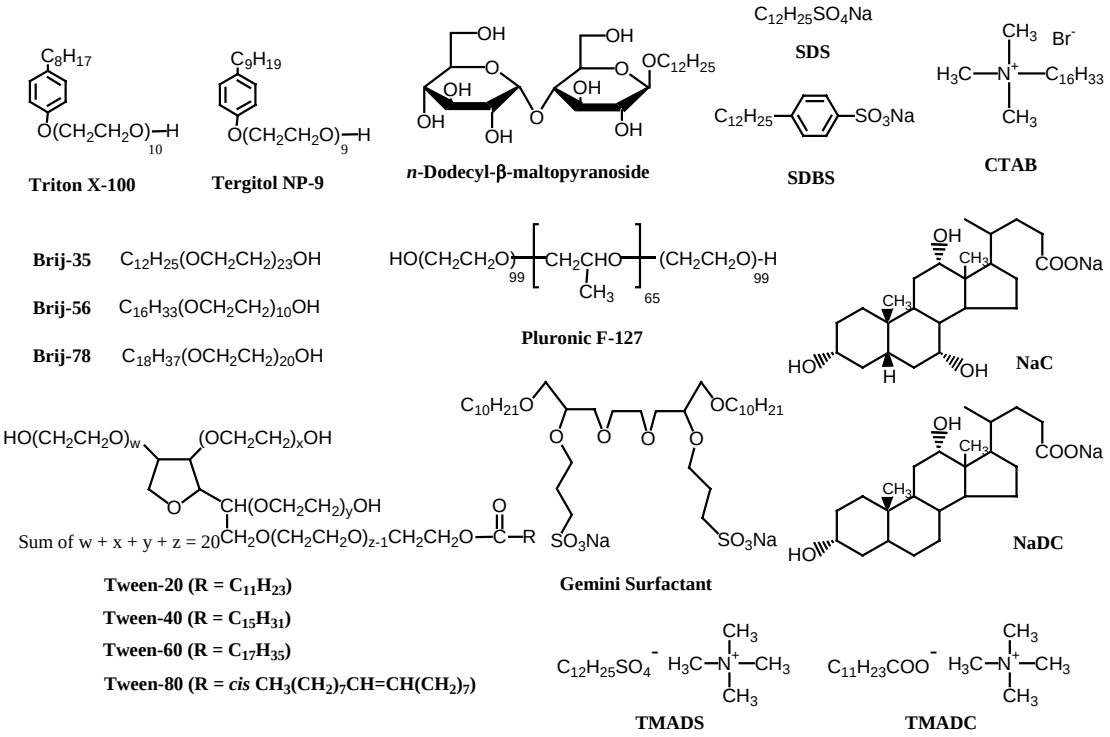


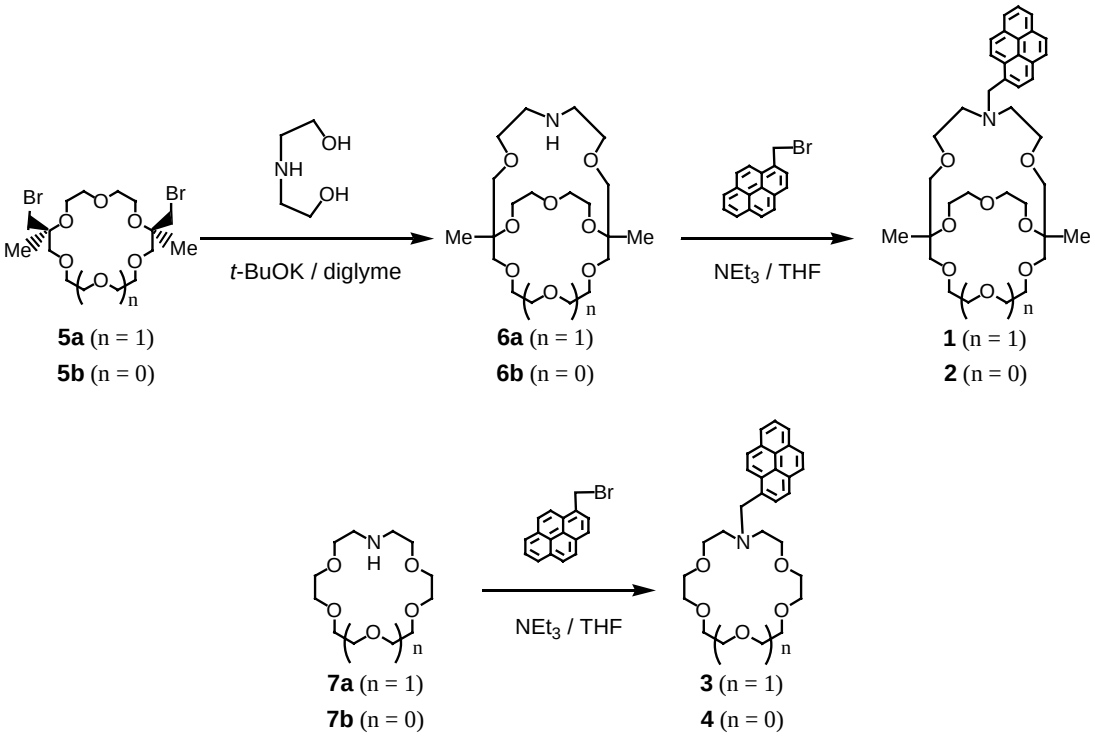

**FIGURE 2.** Structures of surfactants.

## 2-2. Results and Discussion

### 2-2-1. Design and Synthesis of Fluorophores

Pyrene-functionalized monoazacryptands were synthesized according to the procedure summarized in Scheme 1. The starting materials **5** were prepared according to a previous report.[8] The compounds **6** were prepared by the reaction of **5** with diethanolamine, under basic conditions.[9] Fluorophores **1**-**2** were prepared by the *N*-alkylation of the monoazacryptands with 1-pyrenylmethyl bromide in THF in the presence of triethylamine.[10] Pyrene-functionalized monoazacrown ethers **3**-**4** were prepared from the monoazacrown ethers **7**[11] by the same method as **1**-**2**.

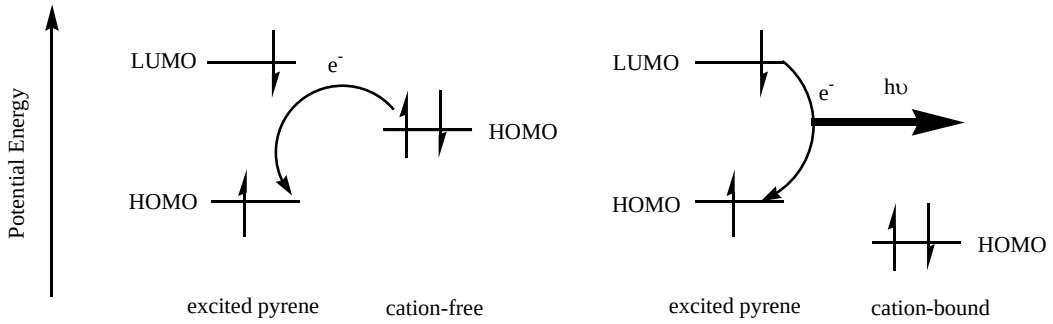
**SCHEME 1**




## 2-2-2. Fluorometric Sensing of Alkali Metal and Alkaline Earth Metal Cations in Aqueous Micellar Solutions

Photoinduced electron transfer (PET)[12] types of fluorophores have proven to be highly successful as direct fluorescent cation sensing molecules.[13] The fluorescence of a monoazacryptand is based on its pyrene ring, and is quenched due to PET from the amino nitrogen atom in the free state. Upon complexation with a metal cation, the nitrogen lone pair no longer participates in PET, causing a recovery of the fluorescence (Scheme 2). The degree of fluorescence recovery upon the addition of metal cations is dependent on the complexation ability of the host compounds. Therefore, this results in a fluorescence intensity-based sensor governed by ion binding.

**SCHEME 2**



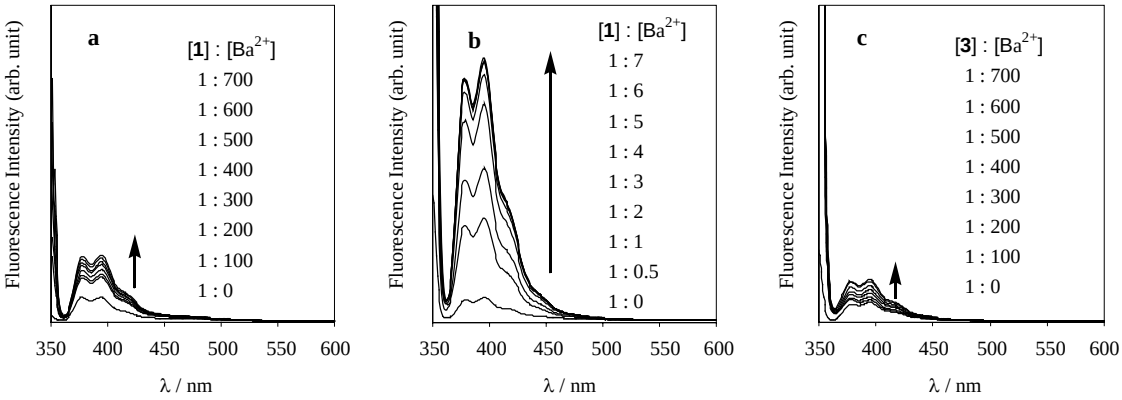




**FIGURE 3.** Fluorescence spectral changes of **1** ($1.0 \times 10^{-6}$ M) and **3** ($1.0 \times 10^{-6}$ M) with different concentrations of $Ba(SCN)_2$ in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the absence (a) and presence (b), (c) of Triton X-100 ($5.0 \times 10^{-3}$ M). Excitation wavelength: 342 nm.

At first, we measured the fluorescence spectra of **1** in Tris solutions as a function of the concentration of $Ba(SCN)_2$ in order to evaluate its complexation behavior. In this case, the addition of a large excess of $Ba^{2+}$ to **1** only slightly changed the fluorescence intensity (Figure 3a). This result clearly indicates that the complexation ability of **1** toward $Ba^{2+}$ is insufficient for its fluorescent detection in aqueous solution. In order to improve the complexation ability of **1**, we added Triton X-100 surfactant micelles into the aqueous solution. Our hypothesis was that the solubilization of **1** into these micelles would make enable the complexation of **1** with $Ba^{2+}$ in the less polar regions.

The fluorescence spectral changes of **1** in the presence of Triton X-100 surfactant micelles are shown in Figure 3b. The addition of $Ba^{2+}$ remarkably affected the fluorescence intensity of **1**, in agreement with our expectations. It should be noted that the addition of only a small excess of $Ba^{2+}$ to the ligand dramatically increased the fluorescence intensity of **1**. When $Ba^{2+}$ is added, the amino nitrogen atom becomes involved in the complexation with $Ba^{2+}$ and loses its ability to donate an electron to the excited state of the pyrene ring. Thus, the addition of $Ba^{2+}$ caused a recovery of the fluorescence. When the corresponding monoaza-18-crown-6 ether derivative (**3**)[10] was used as a fluorophore instead of **1**, the addition of a large amount of $Ba^{2+}$ barely changed the fluorescence behavior in the presence of the Triton X-100 micelles (Figure 3c). This result clearly demonstrates that the strong complexation ability of the cryptand scaffold is necessary for the detection of metal ions, even in aqueous micellar solutions.

**TABLE 1. Stability Constants (log *K*) of 1-3 for $Ba^{2+}$ in $CH_3OH/CH_3CN$ (99:1 v/v), Aqueous Micellar Solutions of Triton X-100, and $H_2O/CH_3CN$ (99:1 v/v)**

| | log *K* ($M^{-1}$) | | |
|---|---|---|---|
| | $CH_3OH$ | aquous micellar solutions of Triton X-100 | $H_2O$ |
| **1** | 9.7 | 6.6 | 3.4 |
| **2** | 6.1 | 4.7 | N.D.[a] |
| **3** | 4.9 | N.D.[a] | N.D.[a] |

[a] Not determined.

The stability constants (*K*) of the complexes were evaluated from a curve of the fluorescent intensity plotted against the ratio of $[Ba^{2+}]$/[Host] by means of a nonlinear least-squares curve-fitting method.[14] The *K* values of **1-3** towards $Ba^{2+}$ in $CH_3OH/CH_3CN$ (99:1 v/v), aqueous micellar solutions of Triton X-100, and

$H_2O/CH_3CN$ (99:1 v/v) are summarized in Table 1. These results show that the polar environment around the fluorophores in the Triton X-100 micelles is intermediate between methanol and water.

To clarify the effects of the Triton X-100 surfactant micelles, the fluorescence intensity of **1** in the presence of $Ba^{2+}$ ($5.0 \times 10^{-6}$ M, 5 equiv.) was plotted against the Triton X-100 concentration. Figure 4 shows that the recovery of the fluorescence intensity began when the Triton X-100 concentration reached the critical micellar concentration (CMC) (0.24 mM).[15] This result supports our assumption that **1** is incorporated into the micelle, and that the hydrophobic environment of the micelle promotes the complexation ability of **1** with $Ba^{2+}$.

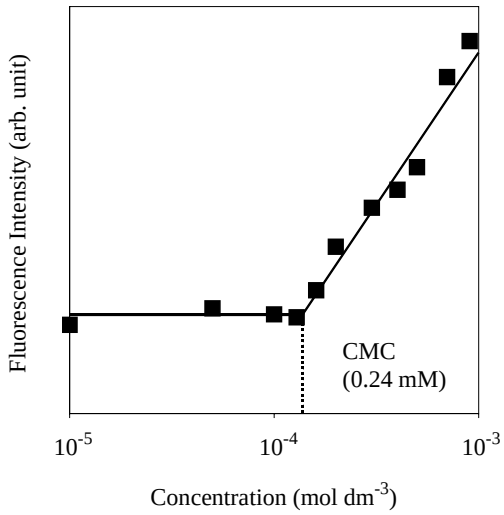



**FIGURE 4.** Changes of the fluorescence intensity of **1** ($1.0 \times 10^{-6}$ M) with different concentrations of Triton X-100 in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) containing $Ba(SCN)_2$ ($5.0 \times 10^{-6}$ M, 5 equiv.) and. Excitation wavelength: 342 nm.

The selectivity towards other cations was also examined (Figure 5). The thiocyanate salts of alkali metal cations ($Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$) and the perchlorate salts of alkaline earth metal cations ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$) were used to evaluate the binding ability. Surprisingly, **1** displayed a large chelation-enhanced fluorescence

(CHEF)[16] effect ($I_{complex}$-$I_{free}$), where $I_{free}$ and $I_{complex}$ are the fluorescence intensities (monitored at 394 nm) in the absence and presence of metal cations ($1.0 \times 10^{-5}$ M, 10 equiv.), respectively, with only $Ba^{2+}$ among these metal cations. This result demonstrates that **1** (derived from 18-crown-6) is a highly sensitive and selective sensor for $Ba^{2+}$ in water. Indeed, the response of fluorophore **1** towards $Ba^{2+}$ was barely affected, even in the presence of a large excess of other alkali metal and alkaline earth metal cations. On the other hand, in the case of **2** (derived from 15-crown-5), the addition of all alkali metal and alkaline earth metal cations barely caused any CHEF effects. This demonstrated that the stronger complexation ability of the cryptand based on the 18-crown-6 is necessary for the high sensitive detection of $Ba^{2+}$.

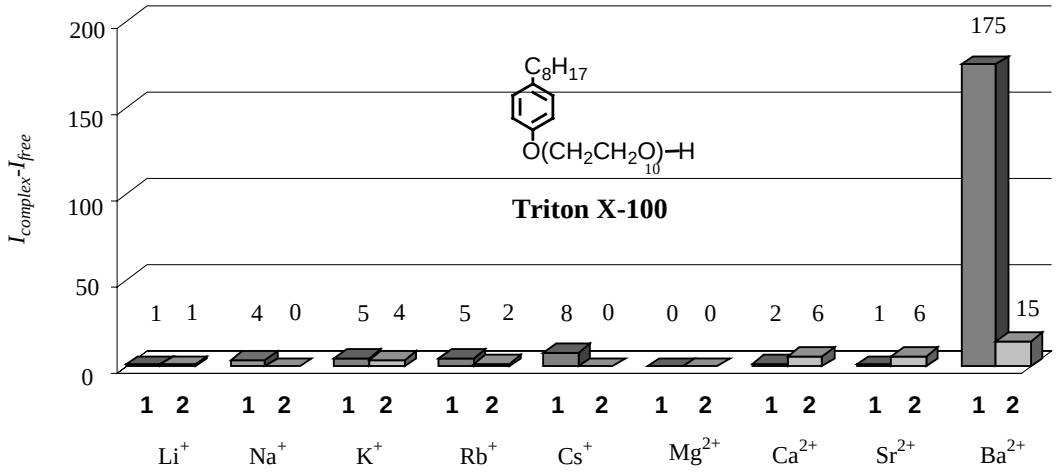



**FIGURE 5.** CHEF effects of **1** ($1.0 \times 10^{-6}$ M) and **2** ($1.0 \times 10^{-6}$ M) with thiocyanate salts ($1.0 \times 10^{-5}$ M, 10 equiv.) of alkali metal and alkaline earth metal cations in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the presence of Triton X-100 ($5.0 \times 10^{-3}$ M). Excitation wavelength: 342 nm.

Next, the selective fluorescent detection of important metal cations ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$) relevant to living organisms using fluorophores was carried out. Selective fluorescent discrimination among these metal cations in water is required in fields such as clinical diagnosis and environmental analysis. Therefore, the fluorescent properties

of photosensitive monoazacryptands for these metal cations were examined in aqueous micellar solutions of nonionic surfactants such as Triton X-100, Tween-20, Tween-60, Brij-78, *n*-dodecyl-β-maltopyranoside, and Pluronic F-127, and ionic surfactants such as SDS, SDBS, NaC, NaDC, TMADS, TMADC, and CTAB. Here, the chloride salts of alkali metal and alkaline earth metal cations ($1.0 \times 10^{-3}$ M, 1000 equiv.) were used in consideration of practical analysis.

Figure 6 shows the CHEF effects of **1**-**2** upon the addition of alkali metal and alkaline earth metal cations in the presence of various nonionic surfactants. Among these nonionic surfactants, in the presence of Tween-60, the monoazacryptand-type fluorophores showed relatively large CHEF effects as compared to those in the presence of other nonionic surfactants. In particular, **2** showed excellent $K^+$ selectivity in aqueous micellar solutions of Tween-60. To investigate the effects of the pore size of the monoazacryptands, the metal cation selectivity towards all alkali metal cations ($Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$) was also examined. Figure 7 showed that the largest CHEF effects of **1** and **2** were observed upon the addition of $Rb^+$ and $K^+$, respectively, which suggests that the amino nitrogen atom is effectively coordinated to the metal cation in these cases. Therefore, it is expected that the pore sizes of **1** and **2** are similar to the ionic sizes of $Rb^+$ and $K^+$, respectively. Previously, we examined the metal cation selectivity of this type of monoazacryptand using solvent extraction experiments,[9a] but no clear metal cation selectivity was observed at that time. In this study, the metal cation selectivity of monoazacryptand-type fluorophores was determined from the fluorescence intensity changes upon the addition of metal cations in aqueous micellar solutions of Tween-60.

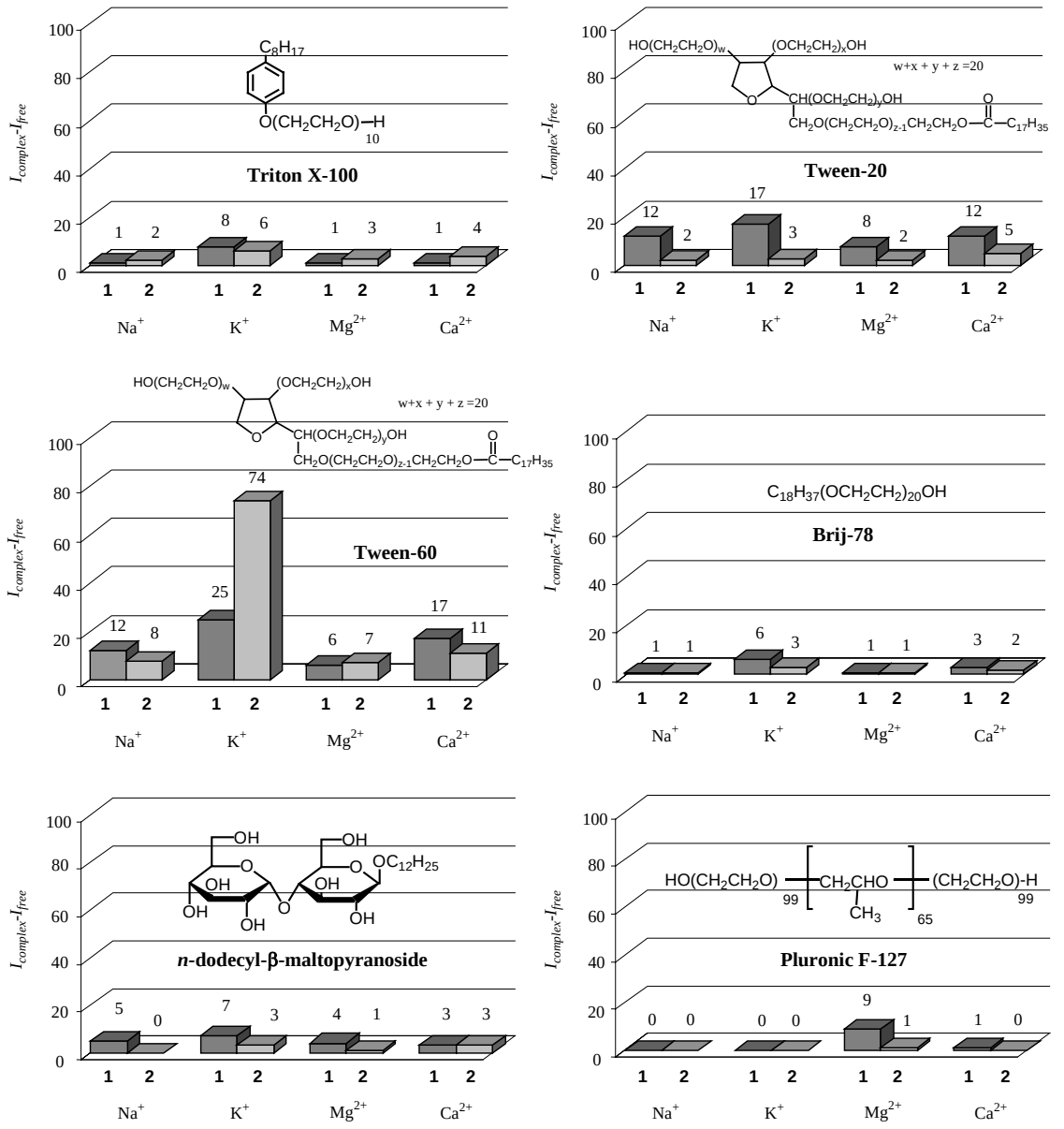



**FIGURE 6.** CHEF effects of **1** ($1.0 \times 10^{-6}$ M) and **2** ($1.0 \times 10^{-6}$ M) with chloride salts ($1.0 \times 10^{-3}$ M, 1000 equiv.) of alkali metal and alkaline earth metal cations in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the presence of Triton X-100 ($1.0 \times 10^{-3}$ M), Tween-20 ($1.0 \times 10^{-3}$ M), Tween-60 ($1.0 \times 10^{-3}$ M), Brij-78 ($1.0 \times 10^{-3}$ M), *n*-dodecyl-β-maltopyranoside ($1.0 \times 10^{-3}$ M), and Pluronic F-127 (1mg/mL). Excitation wavelength: 342 nm.

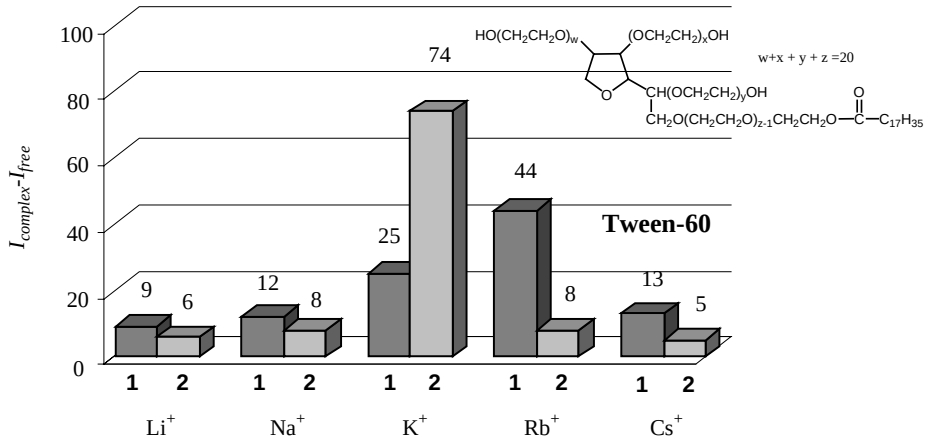



**FIGURE 7.** CHEF effects of **1** ($1.0 \times 10^{-6}$ M) and **2** ($1.0 \times 10^{-6}$ M) with chloride salts ($1.0 \times 10^{-3}$ M, 1000 equiv.) of alkali metal cations in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the presence of Tween-60 ($1.0 \times 10^{-3}$ M).
Excitation wavelength: 342 nm.

In the presence of Tween-20, *n*-dodecyl-β-maltopyranoside and Pluronic F-127, the CHEF effects of the fluorophores were very small. This result suggests that these surfactants with shorter alkyl chains do not offer sufficient hydrophobic complexation fields for cations to fluorophores. Interestingly, even in the presence of Brij-78, which has the same carbon number of lipophilic groups and the same number of oxyethylene units as Tween-60, the fluorophores showed minimal CHEF effects. To clarify the difference between Tween-60 and Brij-78, the association abilities of the surfactants toward $K^+$ were examined by UV spectroscopy. The absorption wavelength in the UV spectrum of the picrate anion is a measure of the type of ion pair formed.[17] The surfactant was mixed with potassium picrate in THF, and the maximal wavelength of the picrate anion was plotted (Figure 8). A larger bathochromic shift of the picrate anion was observed in the presence of Brij-78, which indicates that a loose ion pair of potassium picrate was formed as compared to that in the presence of Tween-60. In other words, the complexation ability of Brij-78 towards $K^+$ was stronger than that of Tween-60 in THF. Compounds with a long and linear polyoxyethylene unit are known

to form cationic complexes with alkali metal and alkaline earth metal cations similar to crown ethers.[18] Thus, it can be hypothesized that Tween-60, which has a branched polyoxyethylene unit, gives the host compounds more effective complexation fields for cations than Brij-78, which has a linear polyoxyethylene unit that can interrupt the complexation between fluorophores and metal cations. When the corresponding monoazacrown ethers **3** and **4** were used instead of **1** and **2**, the addition of all metal cations barely caused any CHEF effects in aqueous micellar solutions of Triton X-100 and Tween-60 (Figure 9). This result demonstrates that the cryptand scaffold is necessary for the detection of metal cations, even in aqueous micellar solutions of Tween-60, which creates effective complexation fields for cations..

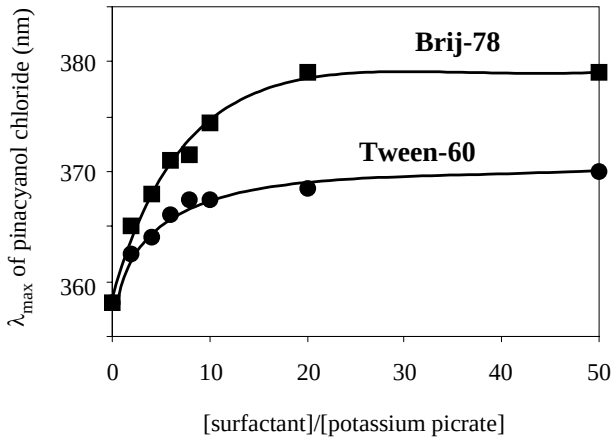



**FIGURE 8.** Plots of the wavelength of maximal absorption ($\lambda_{max}$) of potassium picrate ($5.0 \times 10^{-5}$ M) upon the addition of surfactants in THF.

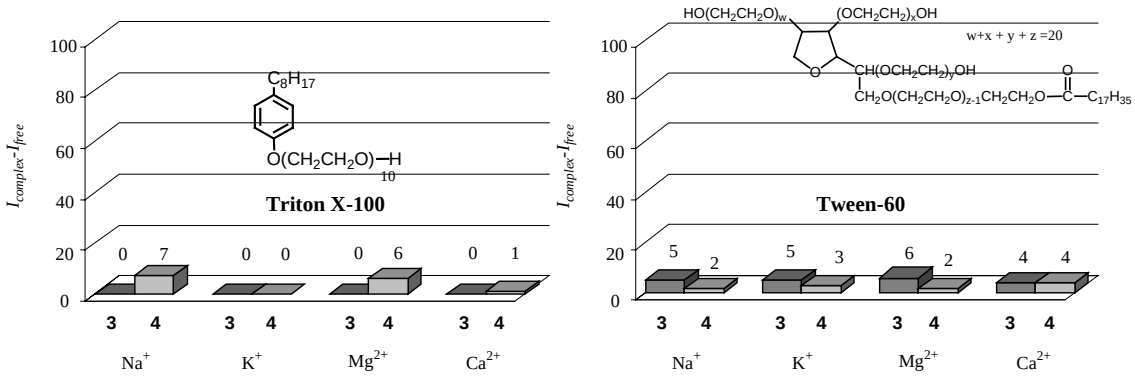



**FIGURE 9.** CHEF effects of **3** ($1.0 \times 10^{-6}$ M) and **4** ($1.0 \times 10^{-6}$ M) with chloride salts ($1.0 \times 10^{-3}$ M, 1000 equiv.) of alkali metal and alkaline earth metal cations in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the presence of Triton X-100 ($1.0 \times 10^{-3}$ M) and Tween-60 ($1.0 \times 10^{-3}$ M). Excitation wavelength: 342 nm.

In the presence of anionic surfactants, it is probable that anionic charges are present at the micellar interface, and the micelle region becomes favorable as a complexation field for cations. That may be the reason why target metal cations are concentrated at the micelle interface by the electrostatic attractive force of the anionic charges on the surfactants. However, it is also possible that the counter cations of the anionic surfactants may interfere with the host-guest (target metal cations) interaction. In fact, in the presence of SDS, a strong fluorescence of **1** was observed, even in the absence of additional metal cations, whereas the, a fluorescence of **2** remained fully quenched. This result demonstrated that **1** (derived from a 18-crown-6) can complex with the counter cations ($Na^+$) of SDS, causing a recovery of the fluorescence, but **2** (derived from a 15-crown-5) could barely complex with $Na^+$. Therefore, we chose **2** as a fluorescent indicator for metal cations in aqueous micellar solutions of anionic surfactants. The selective fluorescent detection was performed in water in the presence of anionic surfactants such as SDS,[19] SDBS,[20] NaC,[21] NaDC,[22] TMADS,[23] and TMADC.[24]

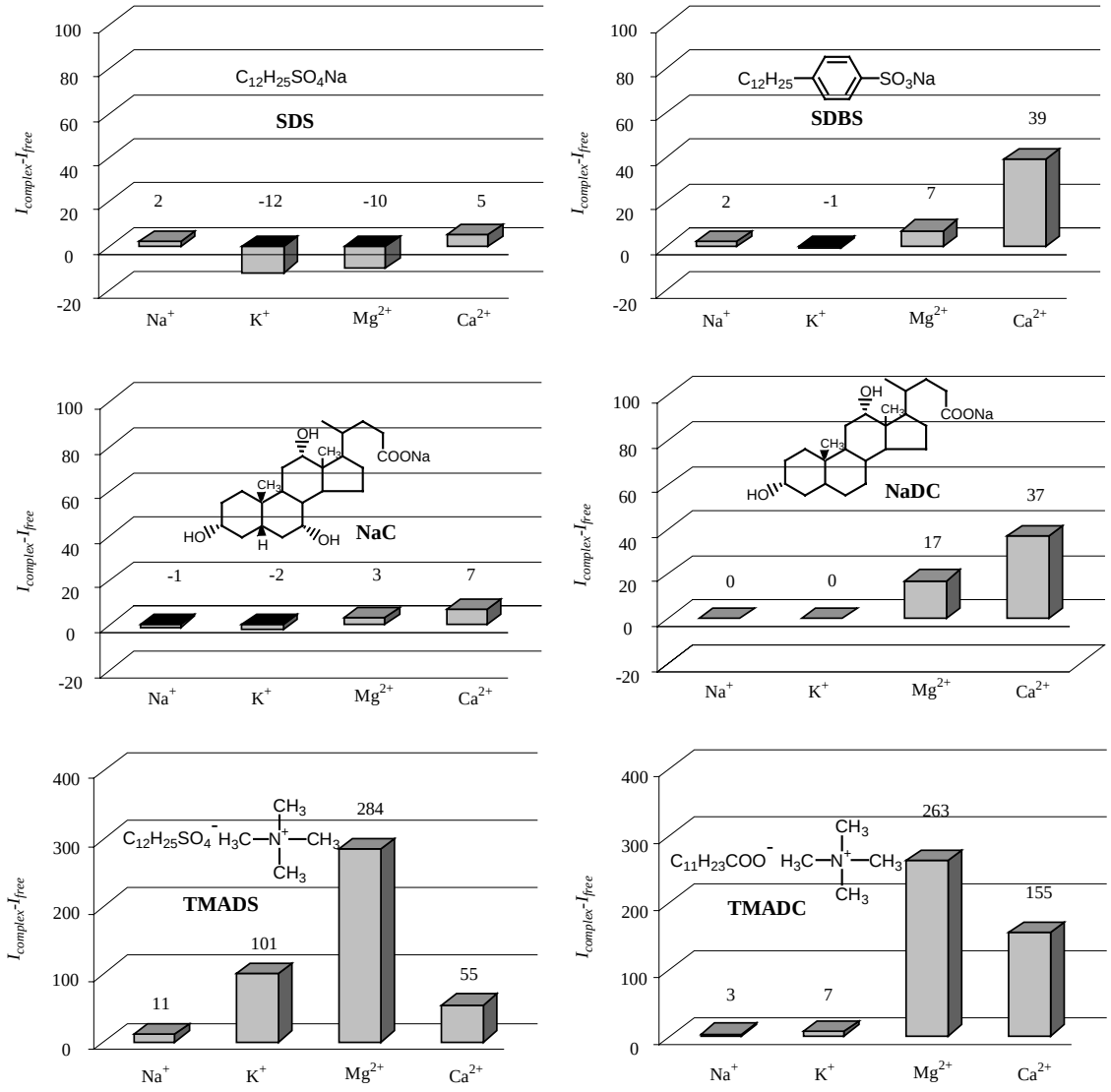



**FIGURE 10.** CHEF effects of 2 ($1.0 \times 10^{-6}$ M) with chloride salts ($1.0 \times 10^{-3}$ M, 1000 equiv.) of alkali metal and alkaline earth metal cations in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the presence of SDS ($8.3 \times 10^{-3}$ M), SDBS ($1.3 \times 10^{-3}$ M), NaC ($1.1 \times 10^{-2}$ M), NaDC ($5.0 \times 10^{-3}$ M), TMADS ($5.4 \times 10^{-3}$ M), and TMADC ($2.5 \times 10^{-2}$ M). Excitation wavelength: 342 nm.

Figure 10 shows the CHEF effects of **2** upon the addition of alkali metal and alkaline earth metal cations in aqueous micellar solutions of anionic surfactants. **2** showed relatively high CHEF effects with $Ca^{2+}$ in the presence of SDBS and NaDC, but

showed little CHEF effects with all other metal cations in the presence of SDS and NaC. This result suggests that the larger hydrophobic portions of SDBS and NaDC, when compared with SDS and NaC, may give the fluorophores more effective complexation fields for cations. To lower the effects of the counter cations, TMADS and TMADC, which have tetramethylammonuim ions as the counter cations, were used. Since cryptands have little complexation ability with quaternary ammonium cations, it is expected that an inhibition of the formation of the fluorophore-metal complex by the counter cations will be weakened. In the presence of both surfactants, **2** showed very large CHEF effects with $Mg^{2+}$. Overall, there was a tendency that **2** detected alkaline earth metal cations more effectively than alkali metal cations in aqueous micellar solutions of anionic surfactants. This result may be due to an enhancement of the electrostatic interaction between the anionic surfactants and alkaline earth metal cations which have a larger charge density.

In the presence of cationic surfactants, it is probable that cationic charges are present at the micellar interface, and the micelle region becomes unfavorable as a complexation field for cations. This may be the reason why it is difficult for target metal cations to approach the micellar interface because of the electrostatic repulsion between the metal cations and the cationic charges on the surfactants. In fact, both fluorophores failed to detect all kinds of metal cations in aqueous micellar solutions of CTAB (Figure 11).

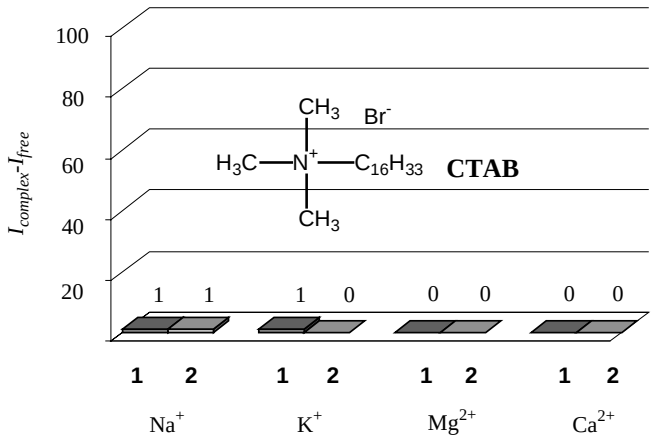



**FIGURE 11.** CHEF effects of **1** ($1.0 \times 10^{-6}$ M) and **2** ($1.0 \times 10^{-6}$ M) with chloride salts ($1.0 \times 10^{-3}$ M, 1000 equiv.) of alkali metal and alkaline earth metal cations in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the presence of CTAB ($1.0 \times 10^{-3}$ M). Excitation wavelength: 342 nm.

### 2-2-3. A New Fluorescence Method for the Determination of the Critical Micelle Concentration by Photosensitive Monoazacryptands

The critical micelle concentration (CMC) is one of the most important physical parameters of surfactants.[25] A variety of methods to determine the CMC have been reported thus far.[26] In the most common methods, the CMC can be determined from breaks in the surface tension, electrical conductivity, and dynamic light scattering curves. Fluorescent methods have also been used extensively for the CMC determination due to their relative quickness and ease.[27] In particular, methods using pyrene as fluorescent probes have attracted much attention because of their high sensitivity.[28] It is well known that the optical properties of fluorescent probes are strongly affected by the polarity of their environments.[25] Below the CMC, fluorescent probes will exist mainly in aqueous medium, whereas above the CMC they are incorporated into a micelle;[29] thus the environmental-sensitive fluorescent probes exhibit a break point when the surfactant concentration reaches the CMC. However, it has been reported that pyrene probes are not completely partitioned into the

hydrophobic region when the volume of the micelle is insufficient to solubilize pyrene.[30] Therefore, it was difficult to find a clear break point in the CMC measurements of surfactants with very low CMC values.[31] Several studies have been directed towards the development of ionic pyrene probes to improve their solubility.[29,32]

In the former section of this chapter, the author reported that a monoazacryptand-type fluorophore with a pyrene ring functioned as a probe to detect $Ba^{2+}$ in aqueous micellar solutions, with high sensitivity and selectivity. In aqueous solution, this fluorophore showed little fluorescence upon the addition of $Ba^{2+}$ because of the very weak complexation with $Ba^{2+}$. On the other hand, in the presence of the Triton X-100 micelles, the fluorescence was dramatically recovered by an inhibition of the PET process based on the complexation ability with $Ba^{2+}$. Furthermore, it was shown that the fluorescence linearly increased with an increase in the Triton X-100 concentration above the CMC. This fact motivated us to utilize the complexation between a monoazacryptand-type fluorophore and $Ba^{2+}$ as a novel probe for the CMC determination. The PET systems have been used as a powerful means of translating molecular recognition events into large fluorescence signals.[13] If the PET system is applied to the CMC determination, one can observe a remarkable change in the fluorescence spectra of the PET probe when the surfactant concentration reaches the CMC. Despite this, there has been only one report on the CMC determination using the PET system, which describes only the CMC of SDS and nothing about the scope and limitations of this system.[33]

In this section, the author determined the CMC of nonionic surfactants such as Triton X-100, *n*-dodecyl-β-maltopyranoside, Tween-20, Tween-60, and Brij-56, and anionic surfactants such as SDS, TMADS, SDBS, and disodium 5,12-bis(2-oxadodecyl)-4,7,10,13-tetraoxa-1,16-hexadecanedisulfonate (Gemini Surfactant) using the monoazacryptand-$Ba^{2+}$ complex, and compared these values with those obtained by conventional fluorescent methods utilizing

1,6-diphenyl-1,3,5-hexatriene (DPH), 8-anilinonaphthalenesulfonic acid magnesium salt (ANS), or pyrene as probes (Figure 12).

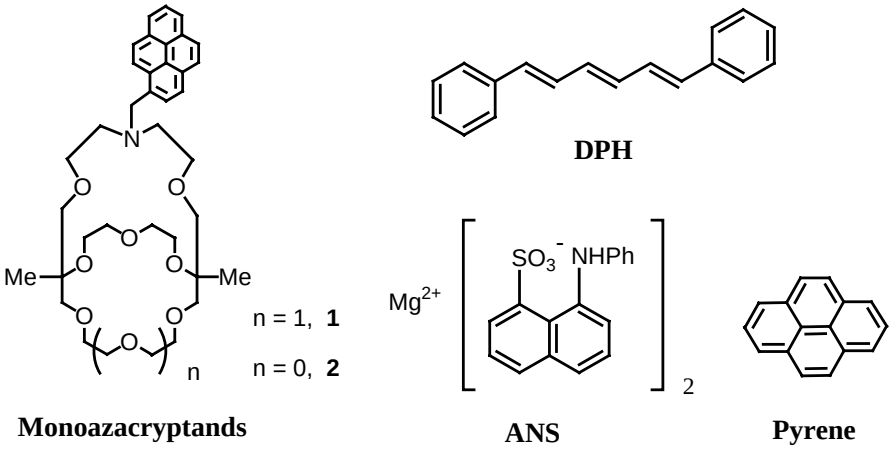
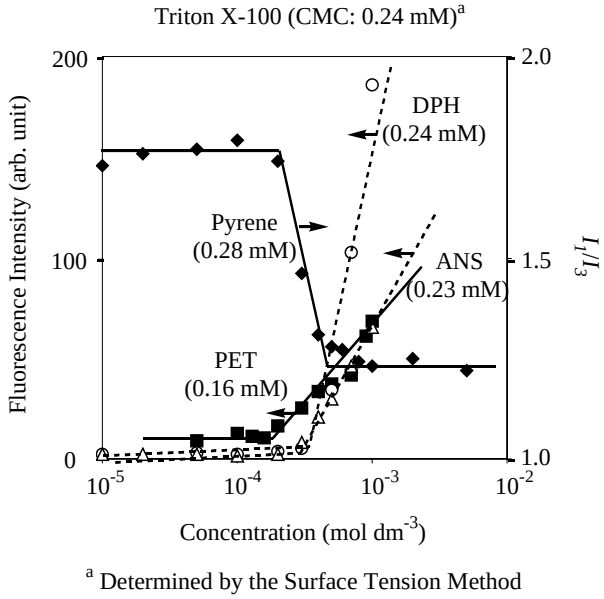



**FIGURE 12.** Fluorescent probes for the CMC determination.

Figure 5 (see the page 45) shows that the recovery of the fluorescence intensity begins when the Triton X-100 concentration almost reached the CMC. In short, the fluorescence recovery upon complexation with $Ba^{2+}$ is expected to be a good mechanism for determining the CMC, which we termed the PET method. The CMC determination for various surfactants was carried out by the PET method to determine its scope and limitations. The CMC values obtained were compared with those determined by other fluorescent methods using DPH, ANS, and pyrene as probes, as well as the literature values. Figure 13 shows plots of the fluorescence intensity (ratio) of these four probes against the Triton X-100 concentration. In the cases of DPH and ANS, the CMCs were determined by a linear-squares fitting of the fluorescence intensity against the surfactant concentration (DPH[34] and ANS[35] method). The pyrene spectrum shows several vibronic peaks, and the ratio of the intensities of the first (at 373 nm) and third (at 383 nm) vibronic peaks, $I_1/I_3$, is a sensitive indicator of the polarity of the pyrene microenvironment.[28a,36] The ratio $I_1/I_3$ is plotted against the surfactant concentration, and the CMC was determined from the center of the sigmoid

(Pyrene method).[30] The CMC values obtained by these four methods gave almost the same CMC, and were in good agreement with the reported CMC values[15] (determined by the surface tension method). This result clearly demonstrates that PET method is useful for the CMC determination.




[a] Determined by the Surface Tension Method

**FIGURE 13.** Changes in the fluorescence intensity or $I_1/I_3$ of $1.0 \times 10^{-6}$ M **1** (■), $5.0 \times 10^{-6}$ M DPH (○), $1.0 \times 10^{-5}$ M ANS (△), $1.0 \times 10^{-6}$ M pyrene (◆) with different concentrations of Triton X-100 in water.

Next, the CMC values of other nonionic surfactants (*n*-dodecyl-β-maltopyranoside, Tween-20, Tween-60, and Brij-56) were determined by the PET method. Figure 14 shows the results of the CMC measurements of nonionic surfactants by four different types of fluorescent probes. In the case of *n*-dodecyl-β-maltopyranoside, these methods gave almost the same CMC values. Furthermore, these CMC values were in very good agreement with the CMC values determined by the surface tension method.[37] On the other hand, in the cases of Tween-20 and Tween-60, the CMC values obtained by the PET method were consistent with the values obtained by the DPH method, but were different from the values

obtained by the ANS method. In the pyrene method, there was no abrupt change in the fluorescence intensities, and hence that the CMC could not be determined. The PET and DPH methods gave CMC values, closer to the values reported in the literature[15a] (determined by the surface tension method).

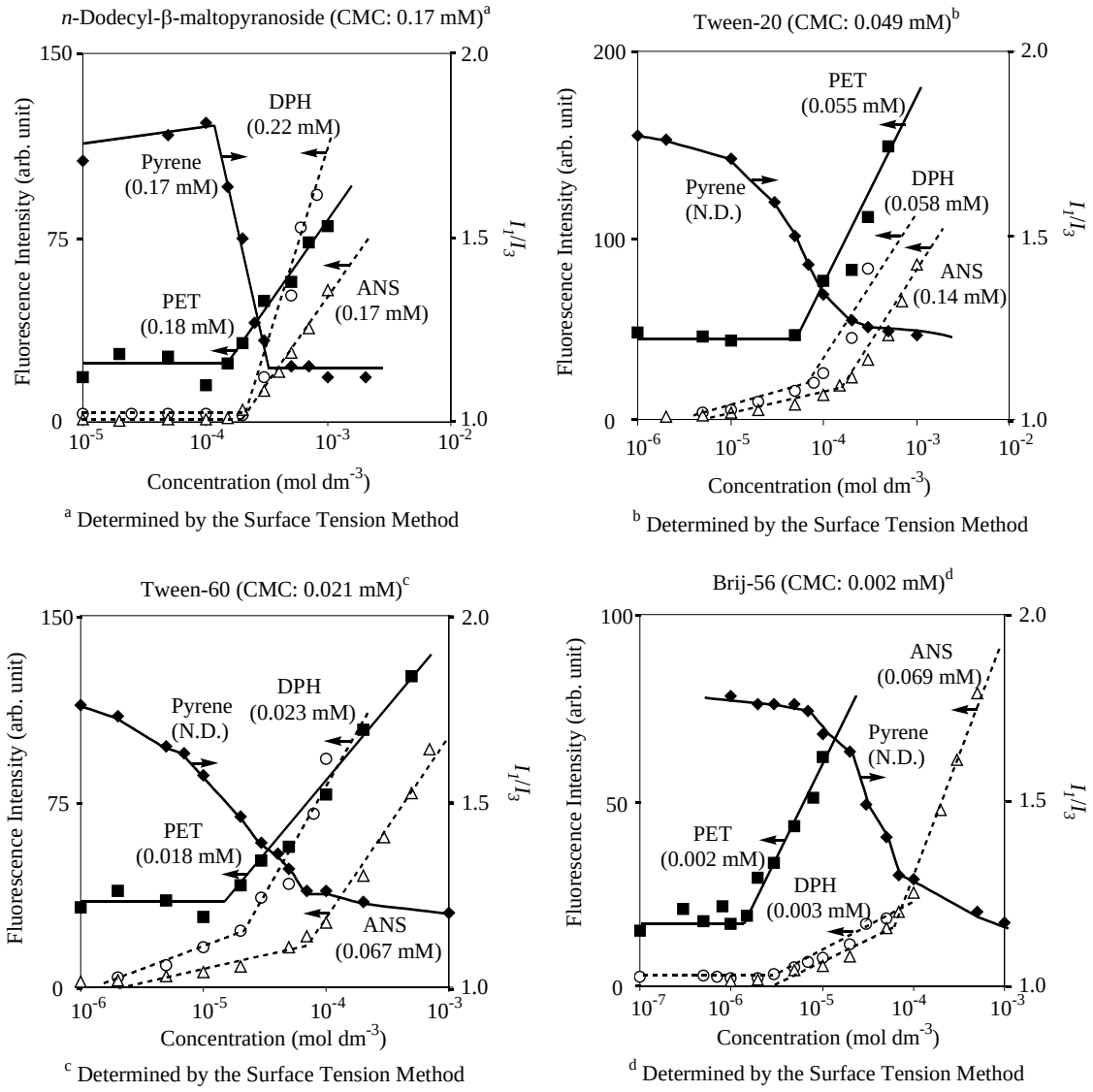



**FIGURE 14.** Changes in the fluorescence intensity or $I_1/I_3$ of $1.0 \times 10^{-6}$ M **1** (■), $5.0 \times 10^{-6}$ M DPH (○), $1.0 \times 10^{-5}$ M ANS (△), $1.0 \times 10^{-6}$ M pyrene (◆) with different concentrations of *n*-dodecyl-β-maltopyranoside, Tween-20, Tween-60, and Brij-56 in water.

In the case of Brij-56, the CMC value obtained by the PET method was different from those obtained by the DPH and ANS methods, and the former was in the best agreement with the reported value[15a] (determined by the surface tension method). In the pyrene method, no abrupt change in the fluorescence intensities was observed similar to the cases of Tween surfactants. It is noteworthy that in the PET method, a clear discernible curvature point was observed, even for Brij-56, which has a very low CMC. This profile may be due to the high sensitivity of the pyrene ring in the PET probe. In the DPH and ANS methods, the number of probe molecules exceeds the number of surfactant molecules at the low concentration ranges ($< 10^{-5}$ M). When the CMC measurements of Brij-56 using the DPH and ANS methods were performed at the same probe concentrations ($1.0 \times 10^{-6}$ M) as the PET method, neither method revealed any clear break points. In the pyrene method, for those surfactants (Tween-20, Tween-60, and Brij-56) with a very low CMC, no abrupt change was observed. This behavior can be explained by the fact that for surfactants with a very low CMC, pyrene is solubilized gradually in the core of the micelle, thereby reporting an average environment between the micelle and the bulk phase.[38] In the PET method, it is believed that the fluorescence intensity is affected by not only the polar environment change around the probe upon formation of the micelle, but also by the inhibition of PET process based on its complexation with $Ba^{2+}$, which enabled the CMC determination at a very low concentration.

Table 2 shows a comparison between the CMC values determined and the reported literature values for nonionic surfactants. The excellent agreement between the CMC values determined by the PET method and the literature values suggests that the presence of the monoazacryptand-type probe does not affect the micellization process appreciably.

**TABLE 2. Comparison between the Determined CMCs and Literature Values of Nonionic Surfactants**

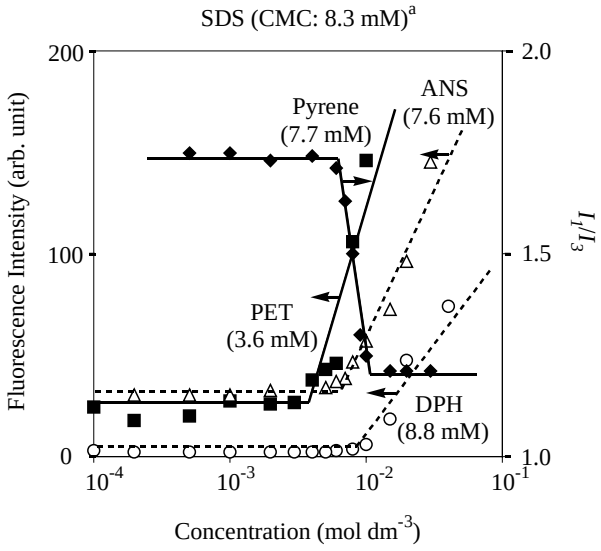
| | | CMC (mM) | | | | | |
|---|---|---|---|---|---|---|---|
| Surfactant | M.w. | PET | DPH | ANS | Pyrene | Lit. | Source |
| Triton X-100 | 625 | 0.16 | 0.24 | 0.23 | 0.28 | 0.24 | 15 |
| *n*-Dodecyl-β–maltopyranoside | 511 | 0.18 | 0.22 | 0.17 | 0.17 | 0.17 | 37 |
| Tween-20 | 1228 | 0.055 | 0.058 | 0.14 | N.D.[a] | 0.049 | 15a |
| Tween-60 | 1312 | 0.018 | 0.023 | 0.067 | N.D.[a] | 0.021 | 15a |
| Brij-56 | 682 | 0.002 | 0.003 | 0.069 | N.D.[a] | 0.002 | 15a |

[a] Not determined.

Next, the CMC values of anionic surfactants were examined by the PET method. In the former section of this chapter (see the page 51), the author reported that in the presence of SDS, a strong fluorescence of **1** was observed, even in the absence of metal cations. On the other hand, the fluorescence of **2** remained fully quenched. Therefore, we chose **2** as a fluorescent probe for the CMC determination of the anionic surfactants.

Figure 15 shows the results of the CMC measurements of SDS. In the PET method using **2**, $Ba^{2+}$ of 100 equivalents to **2** was added into the host solution containing Tris ($1.0 \times 10^{-2}$ M, pH = 10.2). In this case, the DPH, ANS, and Pyrene methods showed almost the same CMC values as the reported value[19] (determined by the capillary electrophoresis method). However, the PET method gave a much lower CMC value. In the PET method, the excimer emission of probe **2** was also observed in the presence of SDS below the CMC (Figure 16). This phenomenon can be ascribed to the formation of undefined aggregates between SDS and the fluorophores. Previtali et al. discussed the excimer formation of ionic pyrene derivatives at variable concentrations of ionic surfactants with opposite charges.[32a] They reported that at the low surfactant concentration, premicellar aggregates containing more than two pyrene

groups were responsible for the excimer emission.




[a] Determined by the Capillary Electrophoresis Method

**FIGURE 15.** Changes in the fluorescence intensity or $I_1/I_3$ of $1.0 \times 10^{-6}$ M **1** (■), $5.0 \times 10^{-6}$ M DPH (○), $1.0 \times 10^{-5}$ M ANS (△), $1.0 \times 10^{-6}$ M pyrene (◆) with different concentrations of SDS in water.

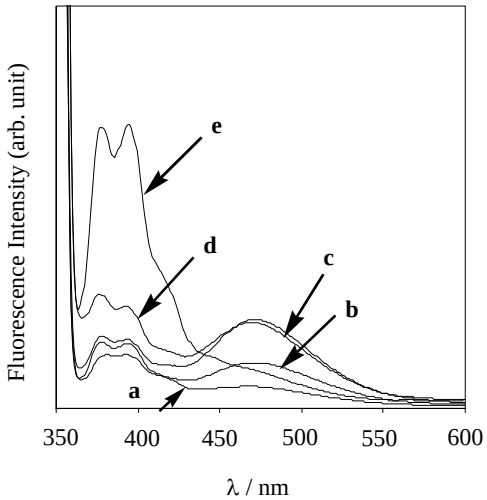



**FIGURE 16.** Fluorescence spectra of **2** ($1.0 \times 10^{-6}$ M) in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) containing $Ba^{2+}$ ($1.0 \times 10^{-4}$ M, 100 equiv.) in the presence of SDS of (a) 0.1, (b) 0.5, (c) 2, (d) 4, and (e) 8 mM.

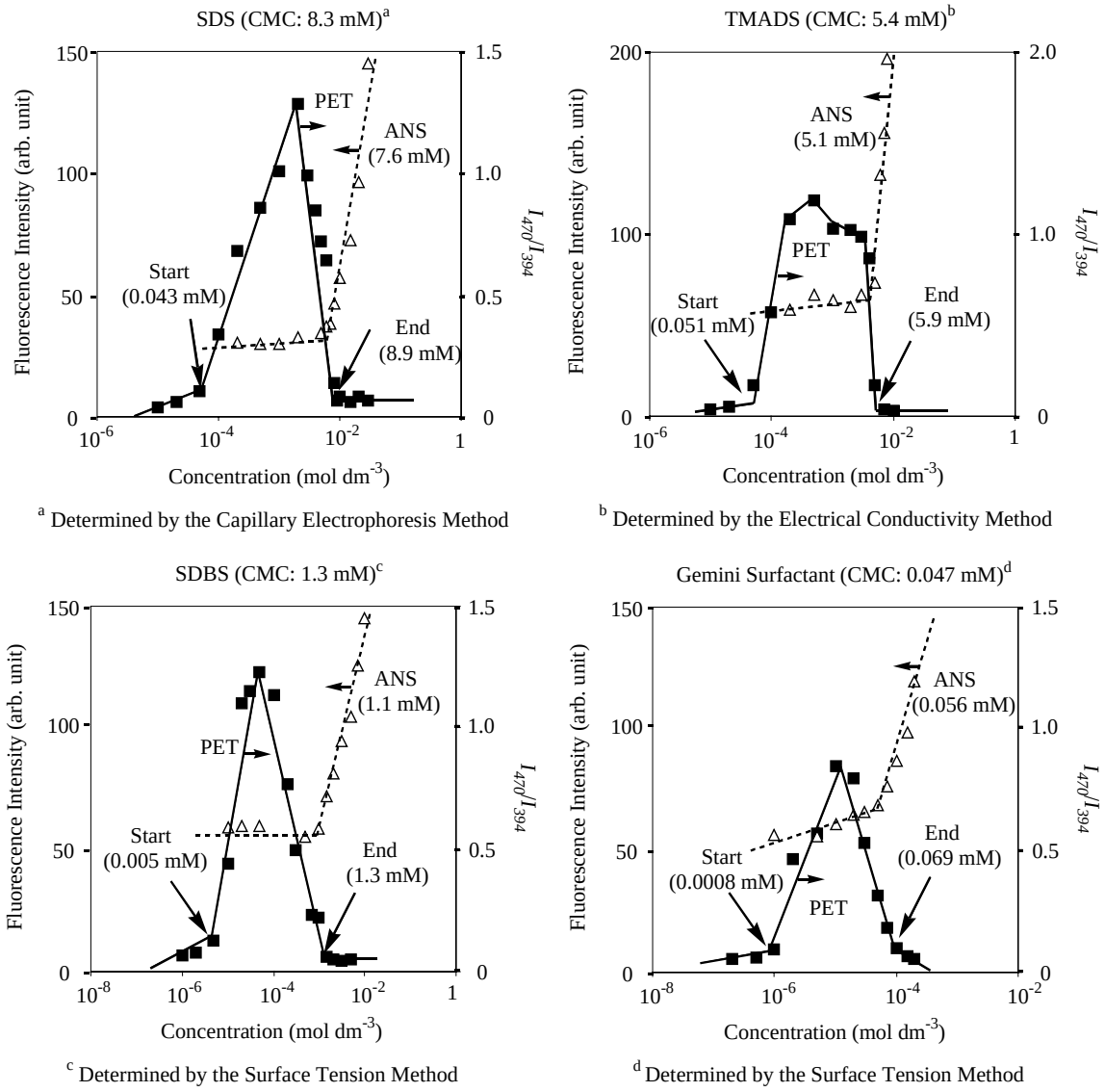



**FIGURE 17.** Changes in the fluorescence intensity or $I_{excimer}/I_{monomer}$ ratios of $1.0 \times 10^{-6}$ M **2** (■) and $1.0 \times 10^{-5}$ M ANS (△) with different concentrations of SDS, TMADS, SDBS, and Gemini Surfactant in water.

In the cases of TMADS, SDBS, and Gemini surfactant, a similar phenomenon was observed and the ratios of the excimer to the monomer fluorescence intensity, $I_{excimer}/I_{monomer}$, were plotted against the surfactant concentration (Figure 17), where $I_{excimer}$ and $I_{monomer}$ are the fluorescence intensities at 470 nm and 394 nm, respectively. First, the $I_{excimer}/I_{monomer}$ ratio increased with an increase in the surfactant concentration,

and reached a maximum at a certain concentration below the CMC. At this concentration range, it is believed that the premicellar aggregates were comprised of several surfactant molecules plus several fluorescent probes, and these aggregates were in equilibrium with the surfactant monomer and the fluorescent probe monomer, thus increasing with an increase in the surfactant concentration. After reaching a maximum, the $I_{excimer}/I_{monomer}$ ratio decreased with an increase in the surfactant concentration and reached a minimum in the proximity of the reported CMC. These results showed that in this region, the number of probes incorporated into one premicellar aggregate decreased with an increase in the surfactant concentration, thus resulting in a decrease of the $I_{excimer}$. In all anionic surfactants examined in this study, these end points of the $I_{excimer}/I_{monomer}$ were in agreement with their reported CMCs, (TMADS[23] determined by the electrical conductivity method; SDBS[20] determined by the surface tension method; and Gemini Surfactant[39] determined by the surface tension method), and therefore the end points of $I_{excimer}/I_{monomer}$ can be regarded as the CMC of the anionic surfactants. In general, gemini surfactants with two alkyl chains and two hydrophilic groups show much lower CMC values in comparison with those of conventional surfactants bearing one alkyl chain and one hydrophilic group.[40] In this study, even in the case of Gemini Surfactant with terminal sulfate groups, premicellar aggregates were observed. Recently, the aggregation behavior of gemini surfactants below the CMC was investigated intensely,[41] and Mathias et al. demonstrated the premicellar concentration range of four series of cationic gemini surfactants using time-resolved fluorescence spectroscopy.[41c] The PET method showed that premicellar aggregates of Gemini Surfactant with terminal sulfate groups began to appear from a concentration of $10^{-6}$ M.

Table 3 shows a comparison between the CMC values of the anionic surfactants determined in this study and the reported values in the literature. In the previous studies, charged fluorescent probes did not work when the probes and surfactants had opposite charges.[35,42] However, the PET method using the monoazacryptand-$Ba^{2+}$

complex gave reliable CMC values, even in the case of anionic surfactants, whose charges are opposite to the probes.

**TABLE 3. Comparison between the Determined CMCs and Literature Values of Anionic Surfactants**

| | | CMC (mM) | | | | |
|---|---|---|---|---|---|---|
| | | PET | | ANS | Lit. | Source |
| Surfactant | M.w. | Start | End | | | |
| SDS | 288 | 0.043 | 8.8 | 7.6 | 8.3 | 19 |
| TMADS | 340 | 0.051 | 5.9 | 5.1 | 5.4 | 23 |
| SDBS | 348 | 0.005 | 1.3 | 1.1 | 1.3 | 20 |
| Gemini Surfactant | 779 | 0.0008 | 0.069 | 0.056 | 0.047 | 39 |

In the presence of cationic surfactants, the author reported that the fluorescence of **1** remained attenuated in aqueous micellar solutions of CTAB, even upon the addition of $Ba^{2+}$. Therefore, the PET method is not applicable for cationic surfactants.

### 2-2-4. Estimation of Hydrophobicity in Different Micelles Utilizing the Fluorophore-$Ba^{2+}$ Complexation Strength

Studies on estimating the hydrophobicity of different micelles, which are sometimes regarded as model systems such as larger bioaggregates membranes, have been the subject of active research.[27e,43] On the other hand, a monoazacryptand-type fluorophore **1**-$Ba^{2+}$ complex is believed to exist around the micelle-water interface because of its ionic group, and in this case the fluorescent intensity may be governed mainly by the **1**-$Ba^{2+}$ binding strength. It is believed that the fluorescence intensity of **1** is a good indicator of the hydrophobicity around the probe. For preliminary experiments, the hydrophobicity around the probe [**1** ($1.0 \times 10^{-6}$ M) with $Ba^{2+}$ ($5.0 \times$

$10^{-6}$ M, 5 equiv.)] in various solvents was compared to the reported $I_1/I_3$ values[36] of pyrene, which is a sensitive indicator of the polarity of the microenvironment around the probe (Table 4). Interestingly, the lower the $I_1/I_3$ values were, the larger the fluorescence intensities were. This result demonstrates that the fluorescence intensity of the **1**-$Ba^{2+}$ complex is sensitive to the polarity of the medium. In this section, the **1**-$Ba^{2+}$ complex can be utilized to estimate the hydrophobicity of diverse nonionic micelles such as Tween-20, Tween-40, Tween-60, Tween-80, Triton X-100, Tergitol NP-9, Brij-35, Brij-56, and *n*-dodecyl-β-maltopyranoside.

**TABLE 4. Comparison between the $I_1/I_3$ Values of Pyrene and the Fluorescence Intensities of Monoazacryptand (1)-$Ba^{2+}$ Complex in Various Solvents**

| | Pyrene[a] ($I_1/I_3$) | **1**-$Ba^{2+}$ complex[b] (fluorescence intensity) |
|---|---|---|
| Methanol | 1.35 | 411 |
| Ethylene glycol | 1.64 | 106 |
| *N*,*N*-Dimethylformamide | 1.81 | 28 |
| Water | 1.87 | 20 |
| Dimethyl sulfoxide | 1.95 | 5 |

[a] Reference 36.

[b] This study.

Figure 18 shows the fluorescence intensities of the **1**-$Ba^{2+}$ complex [**1** ($1.0 \times 10^{-6}$ M) with $Ba^{2+}$ ($5.0 \times 10^{-6}$ M, 5 equiv.)] in the presence of various non-ionic surfactants ($1 \times 10^{-3}$ M). There was a tendency that the longer the alkyl chain was, the larger the fluorescence intensity was among the same series (Tween, Triton, and Brij series) of surfactants. This result shows that the longer alkyl chain offers a more hydrophobic environment to the fluorophores. When comparing Tween-60 with Tween-80, the fluorescence intensity observed in the micelles of Tween-60 was larger than that of the

Tween-80 micelles, despite the fact that they have the same alkyl chain length. This result suggests that the presence of a *cis*-orientation in the alkyl chain weakens the packing of the alkyl chains, and as a result the polar environment around **1** in the micelles of Tween-80 became less hydrophobic. In the presence of Tween-20 and *n*-dodecyl-β-maltopyranoside, **1** showed relatively high fluorescence intensities as compared to that in the presence of Brij-35 with the same alkyl chain length. The author already reported that nonionic surfactants with a long and linear polyoxyethylene units formed cationic complexes with metal cations (see the page 50), and the author hypothesized that a less effective complexation fields for $Ba^{2+}$ was created for **1** in aqueous micellar solutions of Brij-35, which has a significant complexation ability with $Ba^{2+}$. In consideration of the results in organic solvents, it was demonstrated that hydrophobicity around the probe in the most nonionic surfactant micelles was almost intermediate between methanol and ethylene glycol.

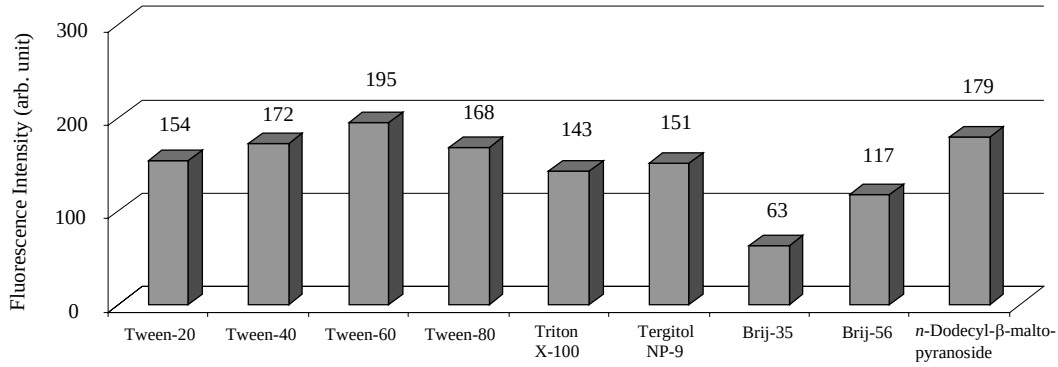



**FIGURE 18.** The fluorescence intensities of **1** ($1.0 \times 10^{-6}$ M) with $Ba^{2+}$ ($5.0 \times 10^{-6}$ M, 5 equiv.) in Tris solutions ($1.0 \times 10^{-2}$ M Tris, pH = 10.2) in the presence of nonionic surfactants ($1.0 \times 10^{-3}$ M).

## 2-3. Conclusion

In this chapter, a new type of fluorescent detection device for metal cations in

aqueous media was designed by combining pyrene-functionalized monoazacryptands and surfactant micelles. The monoazacryptand **1** derived from the 18-crown-6 exhibited very high selectivity and sensitivity towards $Ba^{2+}$ among alkali metal and alkaline earth metal cations in aqueous micellar solutions of Triton X-100. Therefore, **1** is expected to be a promising practical $Ba^{2+}$-specific indicator in aqueous micellar solutions. With respect to the selective fluorescent detection of important metal cations ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$) relevant to living organisms, **2** derived from 15-crown-5 was found to detect $K^+$ with high selectivity in aqueous micellar solutions of Tween-60. In the presence of anionic surfactants, this type of fluorophore detected alkaline earth metal cations ($Mg^{2+}$, $Ca^{2+}$) more effectively than alkali metal cations ($Na^+$, $K^+$). As a result, selectivity for a variety of metal cations was achieved.

In addition, the author proposed a novel fluorescent method (PET method) to determine the CMC of nonionic and ionic surfactants utilizing the complexation behavior between a monoazacryptand-type fluorophore and $Ba^{2+}$. Based on a comparison of the CMC values obtained by the PET method versus the CMC values obtained by conventional fluorescent methods utilizing DPH, ANS and pyrene probes as well as the literature values, one can conclude that the PET method was useful for the determining the CMC of various nonionic and anionic surfactants. Furthermore, for determining the CMC of nonionic surfactants with very low CMC values, the PET method was more effective than any other fluorescent method. This notable achievement may be due to the high sensitivity of the pyrene ring, the higher solubility of the probe (due to the presence of the cryptand functionality) over pyrene, and the drastic change in the fluorescence intensity based on the PET mechanism. With respect anionic surfactants, the PET method also revealed the formation concentration range of premicellar aggregates comprised of surfactant molecules plus fluorescent probes in aqueous micellar solutions of anionic surfactants. Since premicellar aggregates have potentially novel applications such as microelectronics and separation

materials, the PET method will be useful to investigate the premicellar effect of anionic surfactants. In the next study, this CMC determination method will be used to assess the critical association concentration (CAC) of other types of assemblies such as polymers, biological membranes, and vesicles, which are difficult to find a clear break point by the conventional fluorescent methods. When the PET method can be applied to the above-mentioned systems, we believe that new and useful insight will be gained.

## 2-4. Experimental Section

### General Methods.

$^{1}$H NMR spectra were taken at 400 MHz on a JEOL JNM-GSX-400 spectrometer using tetramethylsilane as the internal standard. IR spectra were obtained on a Horiba FT-710 spectrometer. Mass spectra were measured on a JEOL JMS-DX-303 mass spectrometer. Elemental analyses were measured with a Yanagimoto CHN-Corder. The fluorescence measurements were carried out on a Shimadzu fluorescence spectrophotometer (RF-1500). The UV-visible spectra were measured with a Hitachi U-3010 spectrophotometer.

All chemicals were of commercially available reagent grade, and the surfactants, polyoxyethylene(10) isooctylphenyl ether (Triton X-100), polyoxyethylene(9) nonylphenyl ether (Tergitol NP-9), polyoxyethylene(20) sorbitan monolaurate (Tween-20), polyoxyethylene(20) sorbitan monopalmitate (Tween-40), polyoxyethylene(20) sorbitan monostearate (Tween-60), polyoxyethylene(20) sorbitan monooleate (Tween-80), polyoxyethylene(23) lauryl ether (Brij-35), polyoxyethylene(10) cetyl ether (Brij-56), polyoxyethylene(20) stearyl ether (Brij-78), *n*-dodecyl-β-maltopyranoside, poly(ethylene oxide)-ploy(propylene oxide)-poly(ethylene oxide) block copolymer (Pluronic F-127), and sodium dodecyl sulfate (SDS), sodium *n*-dodecylbenzenesulfonate (SDBS), sodium cholate (NaC),

sodium deoxycholate (NaDC), and cetyltrimethylammonium bromide (CTAB) were used without further purification. Tetramethylammonium dodecyl sulfate (TMADS)[20], tetramethylammonium dodecanoate (TMADC)[24], and disodium 5,12-bis(2-oxadodecyl)-4,7,10,13-tetraoxa-1,16-hexadecanedisulfonate (Gemini Surfactant)[39] were prepared according to previous reports. The starting crown ethers, *cis*-bis(bromomethyl)-dimethyl-crown ethers, were prepared according to the literature.[8] The starting monoazacrown ethers were prepared according to the literature.[11]

**General Procedure for the Preparation of Monoazacryptands 6.**

A solution of diethanolamine (14.7-21.1 mmol) and *cis*-bis(bromomethyl)-dimethyl-crown ethers **5** (4.91-7.05 mmol) in diglyme (80 mL) was added dropwise to a suspension of diglyme (80 mL) containing NaH (29.4-42.3 mmol) over a 5 h period at 120 ºC. The mixture was stirred at that temperature for another 24 h. The insoluble matter was removed by filtration, and the diglyme was evaporated in vacuo. The residue was purified by chromatography over alumina (chloroform).

**1,11-Dimethyl-3,6,9,13,19,21,24,27-octaoxa-16-azabicyclo[9.9.7]heptacosane (6a).**

By following the general procedure, **6a** was obtained from **5a** as a slightly yellowish viscous liquid. Yield 28%. $^1$H NMR ($CDCl_3$, + $D_2O$) δ 1.08 (s, 6H), 2.79 (t, 4H, $J$ = 5.1 Hz), 3.32-3.85 (m, 28H). IR (neat, $cm^{-1}$) 3560-3300, 2880, 1960, 1660, 1450, 1360, 1300, 1090, 750. MS (FAB) (m/z, relative intensity) 422 ($M^+$+1, 100). Anal. Calcd for $C_{20}H_{39}NO_8 \cdot H_2O$: C, 57.27; H, 9.35; N, 3.71. Found: C, 57.10; H, 9.38; N, 3.44.

**1,11-Dimethyl-3,9,12,15,18,20,23-heptaoxa-6-azabicyclo[9.7.6]tetracosane (6b).**

By following the general procedure, **6b** was obtained from **5b** as a slightly

yellowish viscous liquid. Yield 68%. $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.09 (s, 6H), 2.72-2.89 (m, 4H), 3.29-3.91 (m, 24H). IR (neat, $cm^{-1}$) 3530-3240, 2880, 1660, 1450, 1360, 1290, 1100, 760. MS (FAB) (m/z, relative intensity) 378 ($M^+$+1, 100). Anal. Calcd for $C_{18}H_{35}NO_7$: C, 57.27; H, 9.35; N, 3.71. Found: C, 57.10; H, 9.38; N, 3.44.

**General Procedure for the Synthesis of 1-4.**

A THF-toluene solution (30 mL, 1:1 v/v) of **6** (1.59-1.97 mmol) or **7** (1.82-3.04 mmol), triethylamine (1-2 mL), and 1-bromomethylpyrene (4.77-9.11 mmol) was refluxed for 12 h. After cooling to room temperature, the mixture was filtered and the solvent was evaporated. The crude product was purified by chromatography over alumina (benzene:ethyl acetate = 98:2-90:10).

**1,11-Dimethyl-16-(1-pyrenylmethyl)-3,6,9,13,19,21,24,27-octaoxa-16-azabicyclo[9.9.7]heptacosane (1).**

By following the general procedure, **1** was obtained from **6a** as a slightly yellowish viscous liquid. Yield 57%. $^1$H NMR ($CDCl_3$) δ 1.08 (s, 6H), 2.87-2.98 (m, 4H), 3.48-3.85 (m, 28H), 4.33 (s, 2H), 7.95-8.56 (m, 9H). IR (neat, $cm^{-1}$) 3040, 2820, 1930, 1740, 1590, 1450, 1370, 1300, 1040, 850, 710. MS (FAB) (m/z, relative intensity) 636 ($M^+$+1, 66), 215 (100). Anal. Calcd for $C_{37}H_{49}NO_8$: C, 69.90; H, 7.77; N, 2.20. Found: C, 69.96; H, 7.79; N, 1.91.

**1,11-Dimethyl-6-(1-pyrenylmethyl)-3,9,12,15,18,20,23-heptaoxa-6-azabicyclo[9.7.6] tetracosane (2).**

By following the general procedure, **2** was obtained from **6b** as a slightly yellowish viscous liquid. Yield 40%. $^1$H NMR ($CDCl_3$) δ 1.06 (s, 6H), 2.90-2.96 (m, 4H), 3.33-3.93 (m, 24H), 4.29 (s, 2H), 7.95-8.56 (m, 9H). IR (neat, $cm^{-1}$) 3040, 2860, 1930, 1740, 1590, 1450, 1370, 1290, 1100, 850, 710. MS (FAB) (m/z, relative

intensity) 592 ($M^+$+1, 48), 215 (100). Anal. Calcd for $C_{35}H_{45}NO_7$: C, 71.04; H, 7.67; N, 2.37. Found: C, 70.81; H, 7.69; N, 2.22.

***N*-(1-Pyrenylmethyl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (3).**

By following the general procedure, **3** was obtained from **7a** as a slightly yellowish viscous liquid. Yield 42%. $^1$H NMR ($CDCl_3$) δ 2.91 (t, 4H, *J* = 5.9 Hz), 3.57-3.71 (m, 20H), 4.35 (s, 2H), 7.94-8.60 (m, 9H). IR (neat, $cm^{-1}$) 3040, 2790, 1930, 1740, 1590, 1450, 1350, 1300, 1040, 850, 710. MS (FAB) (m/z, relative intensity) 478 ($M^+$+1, 48), 215 (100). Anal. Calcd for $C_{29}H_{35}NO_5$: C, 72.93; H, 7.39; N, 2.93. Found: C, 72.66; H, 7.20; N, 2.79.

***N*-(1-Pyrenylmethyl)-1,4,7,10-tetraoxa-13-azacyclopentadecane (4).**

By following the general procedure, **4** was obtained from **7b** as a slightly yellowish viscous liquid. Yield 67%. $^1$H NMR ($CDCl_3$) δ 2.92 (t, 4H, *J* = 5.9 Hz), 3.37 (s, 4H), 3.53-3.70 (m, 12H), 4.35 (s, 2H), 7.95-8.58 (m, 9H). IR (neat, $cm^{-1}$) 3040, 2840, 1930, 1740, 1590, 1450, 1350, 1290, 1100, 850, 710. MS (FAB) (m/z, relative intensity) 434 ($M^+$+1, 69) 215 (100). Anal. Calcd for $C_{27}H_{31}NO_4$: C, 74.00; H, 7.21; N, 3.23. Found: C, 73.90; H, 7.00; N, 3.15.

**Measurement of Fluorescence Spectra.**

The fluorescence spectra were measured at room temperature. The concentration of fluorescent reagents was fixed at a concentration of $1 \times 10^{-6}$ M in the surfactant solutions. Alkali metal cations and alkaline earth metal cations were added into the solution of fluorescent reagent as thiocyanate or chloride salts. To prevent nonlinearity of the fluorescence intensities, the excitation wavelength was set to 342 nm.

**Measurement of Stability Constants.**

All of the stability constants reported herein were determined from a curve of the fluorescence intensity by means of a nonlinear least-square curve fitting method. Typically, the concentration of the host compound was fixed at a concentration of $1 \times 10^{-6}$ M, and the molar ratios of the guest to host were changed by adding the guest salt. Eight data were collected for each host-guest system and the stability constant (*K*) was calculated using an iterative nonlinear least squares curve-fitting program.[14]

**Measurement of UV.**

UV spectra were measured at room temperature. The concentration of potassium picrate was fixed at a concentration of $5 \times 10^{-5}$ M in THF. The molar ratios of the surfactant to potassium picrate were changed in the range from 0 to 50 by adding the surfactant.

**Fluorescent Determination of the CMC.**

Stock solutions of the fluorescent probe at a concentration of $1.0 \times 10^{-4}$ M (PET method), $5.0 \times 10^{-4}$ M (DPH method), and $1.0 \times 10^{-3}$ M (ANS method) in acetonitrile were prepared. For the CMC determination, a series of surfactant solutions were prepared and a small portion of the fluorescent probe solution was added. The concentrations of the fluorescent probe were fixed at a concentration of $1.0 \times 10^{-6}$ M (PET method), $5.0 \times 10^{-6}$ M (DPH method), and $1.0 \times 10^{-5}$ M (ANS method). The samples were incubated for 30 min in the dark at room temperature before measuring the fluorescence. In the PET method, $1.0 \times 10^{-2}$ M Tris was added (pH = 10.2). The CMC from the PET method was determined by a linear-squares fitting of the fluorescence intensity at 394 nm upon excitation at 342 nm against the surfactant concentration, the fluorescence intensity at 430 nm upon excitation at 360 nm for the DPH method, and the fluorescence intensity at 490 nm upon excitation at 370 nm for

the ANS method. Two straight lines can be drawn through these points, and their intersection is taken as the CMC.

**Pyrene Fluorescence Measurement of the CMC.**

The pyrene spectrum shows several vibronic peaks, and the intensity ratio of the first (at 373 nm) and third (at 383 nm) vibronic peaks, $I_1/I_3$, is a sensitive indicator of the polarity of the pyrene microenvironment.[36] The excitation wavelength was 335 nm. Pyrene was recrystallized from ethanol twice, and a stock solution of pyrene at a concentration of $1.0 \times 10^{-3}$ M in acetonitrile was prepared. For the CMC determination, a series of surfactant solutions were prepared, and the fluorescent probe was added. The concentration of pyrene was fixed at a concentration of $1.0 \times 10^{-6}$ M. The samples were incubated for 30 min in the dark at room temperature before measuring the fluorescence. The ratio $I_1/I_3$ was plotted against the surfactant concentration, and the CMC was determined from the center of the sigmoid.[30]

**2-5. References**

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# Chapter 3

# Synthesis of New Types of $C_2$-Symmetric Chiral Double-Armed Crown Ethers and Their Enantiomeric Recognition Abilities as Chiral NMR Discriminating Agents towards Primary Ammonium Salts

## 3-1. Introduction

In the field of supramolecular chemistry, a variety of chiral host compounds have recently been developed for discriminating the molecular chirality of organic compounds, such as ammonium salts and carboxylic acids, with good enantioselectivity.[1] Optically pure chiral reagents can be used for the determination of absolute configuration and enantiomeric excess of chiral compounds in NMR spectroscopy,[2] and in particular chiral crown ethers are known to be effective chiral solvating agents (CSAs) toward protonated primary amines and amino acids methyl ester salts.[3] The use of these CSAs for NMR spectroscopy is one of the most effective and convenient methods for the assessment of the enantiomeric composition of chiral compounds. On the other hand, with respect to the molecular design of *C*-pivot lariat ethers,[4] it is known that the presence of a methyl group on the *C*-pivot carbon atom containing an electron-donating sidearm remarkably raises the complexation ability,[4b,4d,4e] and we recently prepared a novel $C_2$-symmetric chiral crown ether (2*R*,12*R*)-2,12-dimethyl-2,12-bis(hydroxymethyl)-18-crwon-6 (*R*,*R*)-**1**, which has two *C*-pivot carbon atoms as the chiral centers.[5] Since the methyl group on the *C*-pivot carbon atom is believed to restrict the movement of another substituent attached to the same carbon, two sidearms of *C*-pivot-type of chiral crown ethers should strongly affect

the complexation property towards a guest molecule. From this standpoint, our molecular design is based on the introduction of the functional groups into *C*-pivot positions of the $C_2$-symmetric chiral crown ether skeleton. So far, there has been no report on *C*-pivot-type of chiral crown ethers using as CSAs. In many CSAs, the host compounds have aromatic rings, and upon complexation with guest compounds, different chemical shift changes between the enantiomers are induced on the guest signals due to the strong ring current effect.[6] Accordingly, the introduction of aromatic rings into two hydroxyl groups of (*R*,*R*)-**1** is regarded as a useful strategy for the design of new chiral hosts. In this chapter, the author describes the design and synthesis of the new types of $C_2$-symmetric chiral double-armed crown ethers with two hydroxyl groups **1**-**2**, and chiral crown ethers with two aromatic sidearms **3**-**5**. The author also reports the enantiomeric recognition abilities of chiral crown ethers **3**-**5** as chiral NMR discriminating agents towards primary ammonium salts **6**-**10**.

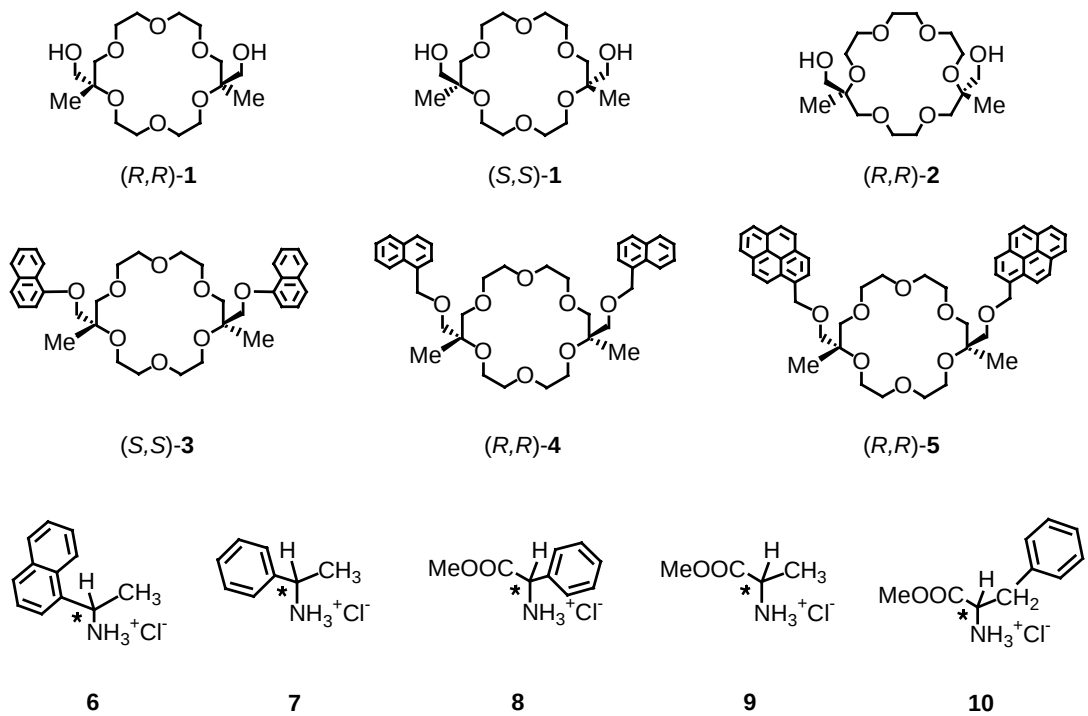



**FIGURE 1.** Structures of chiral crown ethers and ammonium salts.

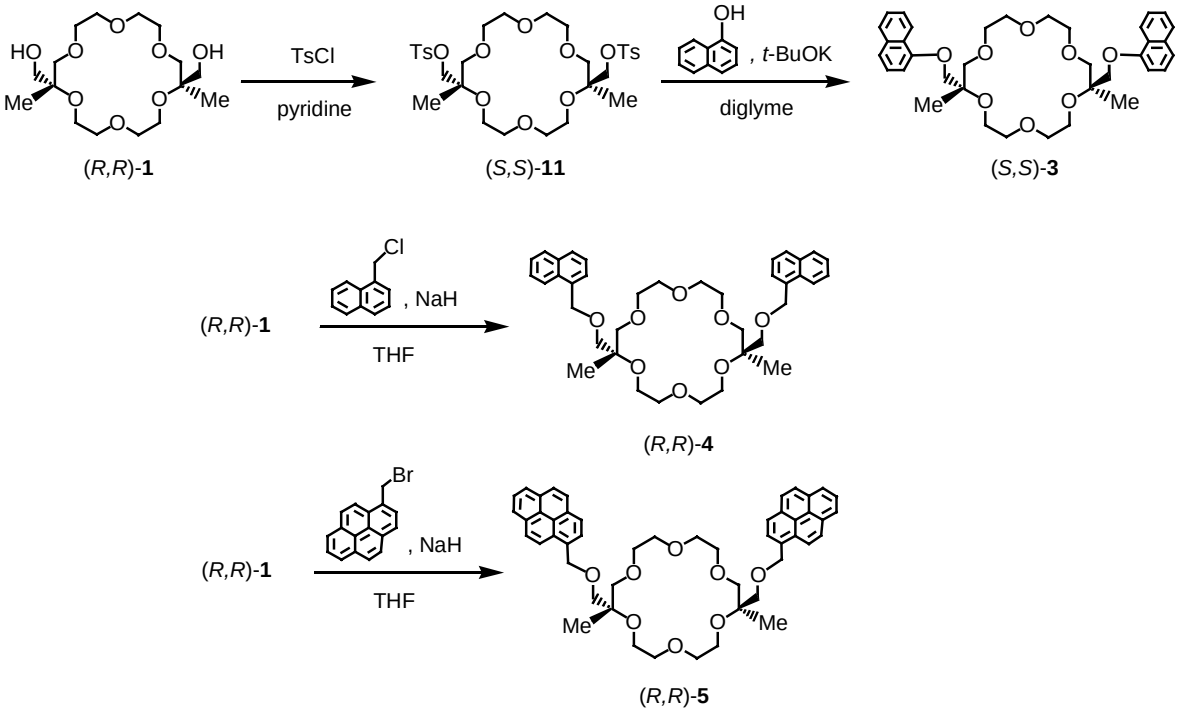
## 3-2. Results and Discussion

### 3-2-1. Design and Synthesis of $C_2$-symmetric Chiral Crown Ethers

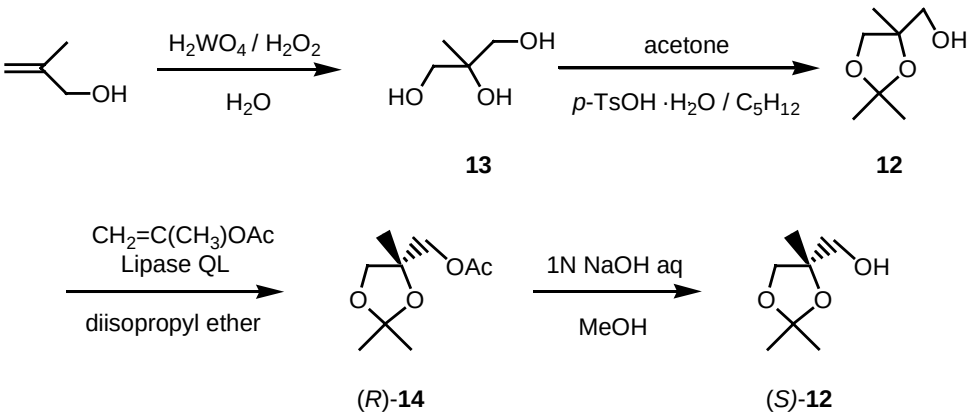
Chiral crown ethers with **3**-**5** were prepared according to the procedures shown in Scheme 1. (2*R*,12*R*)-2,12-bis(hydroxymethyl)-2,12-dimethyl-18-crown-6 (*R*,*R*)-**1** was synthesized according to our previously reported procedure.[5] Optical resolution was achieved by acetylation of a racemic mixture of **1** with isopropenyl acetate in isopropyl ether at 35 ˚C using lipase QL. Then (*R*,*R*)-**1** was obtained by hydrolysis of the corresponding acetylated chiral crown ether. Two hydroxy groups of (*R*,*R*)-**1** were tosylated to give (*S*,*S*)-**11**, and the reaction of (*S*,*S*)-**11** with 1-naphthol to afforded (*S*,*S*)-**3**.[7] (*R*,*R*)-**4** and (*R*,*R*)-**5** were obtained by the reactions of (*R*,*R*)-**1** with 1-chloromethylnaphthalene and 1-bromomethylpyrene, respectively.[4h]

Here, we also report other synthetic routes of chiral crown ether diols, (2*S*,12*S*)-2,12-dimethyl-2,12-bis(hydroxymethyl)-18-crown-6 (*S*,*S*)-**1** and (2*R*,9*R*)-2,9-dimethyl-2,9-bis(hydroxymethyl)-18-crown-6 (*R*,*R*)-**2**, which were obtained from the chiral subunit, (4*S*)-2,2,4-trimethyl-1,3-dioxolane-4-methanol (*S*)-**12**. This synthetic method is improved, compared to our previous synthetic method about (*R*,*R*)-**1**, by the fact that the optical resolution process is achieved in advance before the cyclization step. Therefore, this synthetic method offers the possibilities to construct various types of chiral crown ethers with the combination of architecting blocks.

**SCHEME 1**



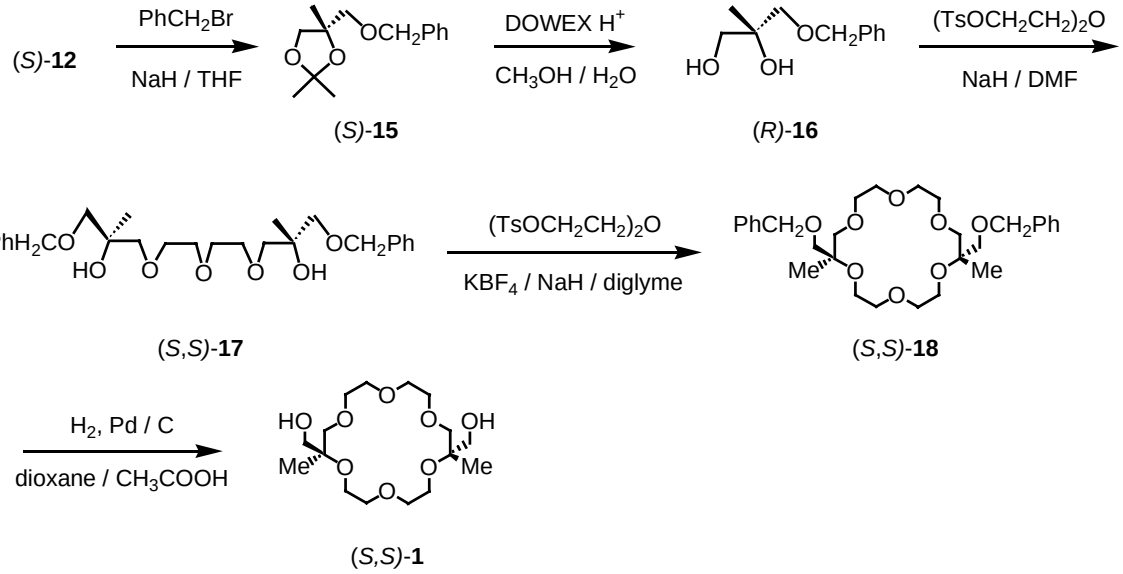

**SCHEME 2**




(*S*)-**12** was synthesized as shown in Scheme 2. The synthetic procedure was referred to the literature.[8] 2-Methylglycerol was prepared from 3-chloro-2-methylpropene using $H_2WO_4/H_2O_2$ as a reagent.[9] In the subsequent ketalization step, the addition of TsOH reduced the formation of unwanted 6-membered

ring isomers using acetone. In the optical resolution process, the acetylation of **12** with isopropenyl acetate was carried out in isopropyl ether at 35 ˚C, using commercially available lipase QL as the catalyst. The reaction was pursued by gas chromatography (GC) and was stopped when the conversion to acetate (*R*)-**14** reached 40%. (*R*)-**14** was isolated by chromatography over silica in 35% yield. Then (*S*)-**12** was obtained by the hydrolysis of (*R*)-**14**. The enantiomeric excess of (*S*)-**12** was determined by optical GC (>97% ee).

**SCHEME 3**

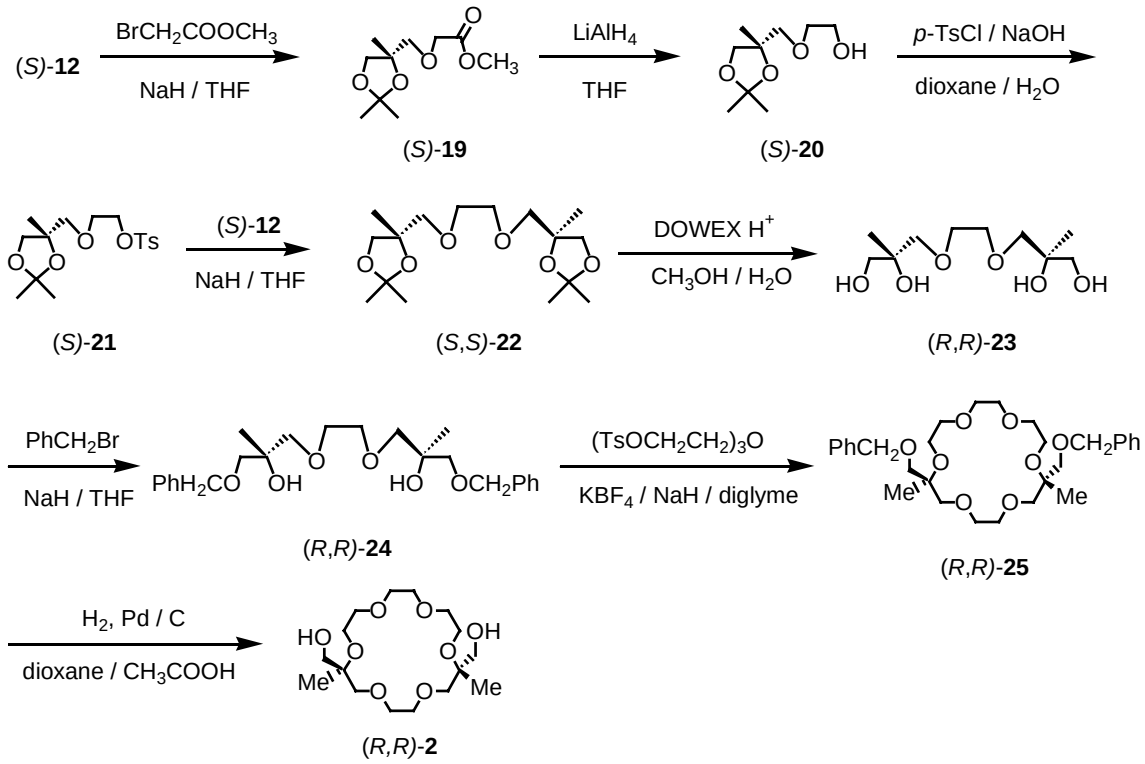



(2*S*,12*S*)-2,12-bis(hydroxymethyl)-2,12-dimethyl-18-crown-6 (*S*,*S*)-**1** was synthesized as shown in Scheme 3. (*S*)-**12** was protected with benzyl bromide to give (*S*)-**15**.[10] Then the acetal group was cleaved by the treatment of (*S*)-**15** with DOWEX ($H^+$) to give (*R*)-**16**. (*S*,*S*)-**17** was synthesized by the reaction of (*R*)-**16** and diethylene glycol ditosylate in the presence of NaH (2 equivalents to OH group) in 81% yield. In this reaction, only the primary hydroxyl groups reacted with the tosyl groups. The $K^+$-templated reaction of the conjugated base of diol (*S*,*S*)-**17** with diethylene glycol

ditosylate resulted in the formation of crown ether (*S*,*S*)-**18** in 39% yield.[11] Subsequent hydrogenation of (*S*,*S*)-**18** led to the chiral crown ether diol (*S*,*S*)-**1**.

The enantiomeric excess of (*S*,*S*)-**1** was determined by $^1$H NMR spectroscopy, using (*R*)-(+)-α-(1-naphthyl)ethylammonium hydrochloride) ((*R*)-NapEt-HCl) as a shift reagent. Upon complexation with ((*R*)-NapEt-HCl), the signal of the methyl protons on the *C*-pivot position of racemic-**1** shifted to upfield region and split into two equal area signals due to the ring current effects of the naphthyl ring of ((*R*)-NapEt-HCl. Therefore, the enantiomeric excess of **1** was calculated by comparing the peak areas of the two split signals. In this study, (*S*,*S*)-**1** showed a considerably high enantiomeric excess (>97% ee). Interestingly, optical absolute configuration of (*S*,*S*)-**1** is converse of that of (*R*,*R*)-**1**.

**SCHEME 4**




(2*R*,9*R*)-2,9-bis(hydroxymethyl)-2,9-dimethyl-18-crown-6 (*R*,*R*)-**2** was

synthesized as shown in Scheme 4. The elongation of the oxyethylene unit of (*S*)-**12** was achieved by a conventional method[7] using the reaction with methyl bromoacetate in THF, followed by reduction with $LiAlH_4$ to give (*S*)-**20**. The tosylation was carried out under the basic condition.[4d] (*S*,*S*)-**22** was prepared by the reaction between tosylate (*S*)-**21** and the chiral subunit (*S*)-**12** in THF in the presence of NaH in 78% yield. Then the acetal group was cleaved by the treatment of (*S*,*S*)-**22** with DOWEX ($H^+$) to give (*R*,*R*)-**23**. Without further purification, (*R*,*R*)-**23** was protected with benzyl bromide in the presence of NaH (2 equivalents to OH group) to give (*R*,*R*)-**24**. In this reaction, only the primary hydroxyl groups react with the benzyl bromide. The $K^+$-templated reaction of the conjugated base of diol (*R*,*R*)-**24** with triethylene glycol ditosylate resulted in the formation of the crown ether (*R*,*R*)-**25** in 35% yield.[11] Subsequent hydrogenation of (*R*,*R*)-**25** produced the chiral crown ether diol (*R*,*R*)-**2**.

### 3-2-2. Enantiomeric Recognition Abilities as Chiral NMR Discriminating Agents towards Primary Ammonium Salts

The enantiomeric recognition abilities of chiral crown ethers **3-5** with two aromatic sidearms as chiral NMR discriminating agents towards protonated primary amines and amino acid methyl ester salts were examined. Figure 2 shows the methyl proton signals of racemic naphthylethylammonium chloride **6** ($1.25 \times 10^{-3}$ M) in the absence and presence of chiral crown ethers **3-5** ($2.50 \times 10^{-3}$ M) in a mixed solvent of $CDCl_3/CD_3OD$ (9:1 v/v). A comparison of the results obtained in the presence of (*S*,*S*)-**3** with naphthyl sidearms against that in the presence of (*R*,*R*)-**4** with naphthylmethyl sidearms gave an interesting suggestion for the design of effective chiral discriminating agents. (*R*,*R*)-**4** caused a significant splitting as well as large upfield shift of the methyl proton signal of **6**, in sharp contrast to the case of (*S*,*S*)-**3**, where only a slight downfield shift of the methyl proton signal of **6** and no splitting of the signal were obtained, demonstrating that the naphthalene rings of (*R*,*R*)-**4** are located

near the methyl group of **6**, on the other hand, the naphthalene rings of (*S*,*S*)-**3** are not. These results may be due to the higher mobility of the aromatic sidearms of (*R*,*R*)-**4** based on the presence of the methylene spacer between the oxygen atom of the sidearm and the naphthalene ring. To generate the more effective splitting, we introduced larger aromatic rings, pyrenylmethyl groups possessing a stronger ring current effect than naphthylmethyl groups, into the host compound. As expected, (*R*,*R*)-**5** gave the larger chemical shift change and splitting of the methyl signal of **6** than (*R*,*R*)-**4**.

It is known that the formation of diastereomeric host-guest complexes between a chiral host and racemic guests can cause the enantiomeric splitting of the guest signals, which is due to a difference in stability and/or predominant geometry between the diastereomeric complexes.[12] The stability constants between (*R*,*R*)-**4** and the chiral ammonium salts of (*R*)-**6** and (*S*)-**6**, which were examined by the $^1$H NMR titration method in a mixed solvent of $CDCl_3/CD_3OD$ (3:7 v/v),[1g] showed that a difference in the stability constant between (*R*,*R*)-**4**/(*R*)-**6** complex and (*R*,*R*)-**4**/(*S*)-**6** complex was relatively small (log $K$ = 3.61 for the (*R*,*R*)-**4**/(*R*)-**6** complex, and log $K$ = 3.67 for the (*R*,*R*)-**4**/(*S*)-**6** complex, respectively). These results suggest that a different predominant complex structure between those diastereomeric complexes is mainly responsible for the enantiomeric splitting of the guest signals. Figure 3 shows the plausible structures of (*R*,*R*)-**4**/(*R*)-**6** complex and (*R*,*R*)-**4**/(*S*)-**6** complex, which were guided from the chemical shift changes upon complexation between (*R*,*R*)-**4** with (*R*)-**6** and (*S*)-**6**, assuming the following issues: (i) a distance between the naphthalene ring of (*R*,*R*)-**4** and the methyl group of (*S*)-**6** in the (*R*,*R*)-**4**/(*S*)-**6** complex is smaller than that between the naphthalene ring of (*R*,*R*)-**4** and the methyl group of (*R*)-**6** in the (*R*,*R*)-**4**/(*R*)-**6** complex. (ii) a distance between the naphthalene ring of (*R*)-**6** and the methyl group of (*R*,*R*)-**4** in the (*R*,*R*)-**4**/(*R*)-**6** complex is smaller than that between the naphthalene ring of (*S*)-**6** and the methyl group of (*R*,*R*)-**4** in the (*R*,*R*)-**4**/(*S*)-**6** complex. On the basis of these assumptions, the conformations of the guest compounds in the

complex were determined. These structures support our hypothesis that aromatic rings of (*R*,*R*)-**4** strongly affect the enantiomeric recognition of racemic ammonium salts.

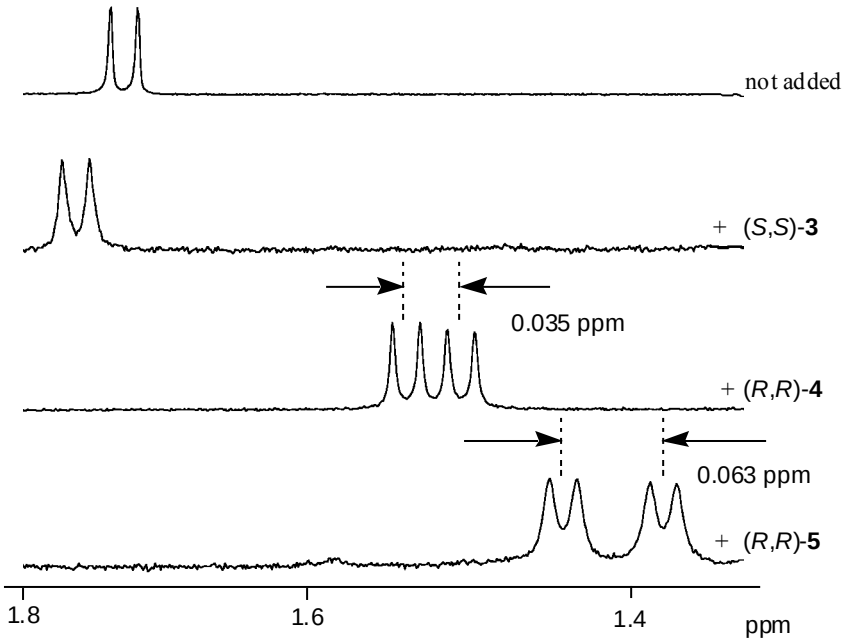



**FIGURE 2.** Resonance for the methyl group of **6** ($1.25 \times 10^{-3}$ M) in the $^1$H NMR spectra before and after the addition of **3**-**5** ($2.50 \times 10^{-3}$ M) in $CDCl_3/CD_3OD$ (9:1 v/v) at 25 ˚C.

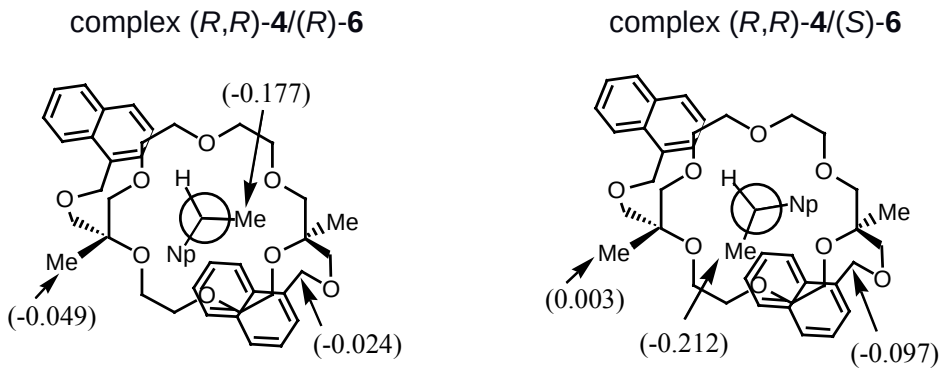



**FIGURE 3.** Plausible structures of (*R*,*R*)-**4**/(*R*)-**6** complex and (*R*,*R*)-**4**/(*S*)-**6** complex. The values in parenthesis are the chemical shift changes based on complexation between (*R*,*R*)-**4** with (*R*)-**6** and (*S*)-**6**.

Table 1 lists the chemical shift changes ($\Delta\delta^R$ and $\Delta\delta^S$) and the enantiomeric splitting ($\Delta\Delta\delta^{R-S}$) observed in the $^1$H NMR spectra of racemic ammonium salts **6**-**10** in the presence of chiral crown ethers. In those cases using another four ammonium salts **6**-**9** as guest compounds, the effective enantiomeric splitting of the guest signals was observed only in the presence of chiral crown ethers with the methylene spacer between the oxygen atom of the sidearm and the aromatic ring [(*R*,*R*)-**4** and (*R*,*R*)-**5**]. This result clearly demonstrates that the presence of the methylene spacer is necessary for chiral recognition using this type of crown ether as a chiral NMR shift reagent. In addition, it is noteworthy that (*R*,*R*)-**5** showed remarkable $\Delta\Delta\delta^{R-S}$ towards any of the guest compounds, including the phenylalanine methyl ester ammonium chloride **10** whose proton signals could not be split, even in the presence of a conventional chiral NMR shift reagent, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid.[3b] Thus, (*R*,*R*)-**5** is highly effective as a chiral $^1$H NMR shift reagent.

**TABLE 1. Differences in the Chemical Shifts [$\Delta\delta^{RS}$ (ppm)] between Enantiomers in the $^1$H NMR Spectra of 6-10 ($1.25 \times 10^{-3}$ M) in the Presence of Chiral Crown Ethers ($2.50 \times 10^{-3}$ M) in $CDCl_3/CD_3OD$ (9/1 v/v) at 25˚C**

| host | | guest (resonance) **6** (**Me**) | **7** (**Me**) | **8** (COO**Me**) | **9** (COO**Me**) | **10** (COO**Me**) |
|---|---|---|---|---|---|---|
| (*R*,*R*)-**1** | $\Delta\delta^{R}$ [a] | -0.009 | -0.011 | -0.010 | -0.001 | -0.057 |
| | $\Delta\delta^{S}$ [a] | -0.009 | -0.011 | -0.010 | -0.001 | -0.057 |
| | $\Delta\Delta\delta^{R-S}$ [b] | 0 | 0 | 0 | 0 | 0 |
| (*S*,*S*)-**3** | $\Delta\delta^{R}$ [a] | 0.029 | 0.031 | -0.009 | 0.004 | -0.045 |
| | $\Delta\delta^{S}$ [a] | 0.029 | 0.031 | -0.002 | 0.004 | -0.045 |
| | $\Delta\Delta\delta^{R-S}$ [b] | 0 | 0 | 0.007 | 0 | 0 |
| (*R*,*R*)-**4** | $\Delta\delta^{R}$ [a] | -0.177 | -0.224 | -0.136 | -0.074 | -0.184 |
| | $\Delta\delta^{S}$ [a] | -0.212 | -0.237 | -0.109 | -0.064 | -0.184 |
| | $\Delta\Delta\delta^{R-S}$ [b] | 0.035 | 0.013 | 0.027 | 0.010 | 0 |
| (*R*,*R*)-**5** | $\Delta\delta^{R}$ [a] | -0.276 | -0.348 | -0.213 | -0.187 | -0.222 |
| | $\Delta\delta^{S}$ [a] | -0.339 | -0.377 | -0.194 | -0.153 | -0.238 |
| | $\Delta\Delta\delta^{R-S}$ [b] | 0.063 | 0.029 | 0.019 | 0.034 | 0.016 |

[a] (Chemical shift values of the methyl protons speified in bold in the presence of chiral crown ethers)-(chemical shift values in the absence of chiral crown ethers).
[b] $\Delta\Delta\delta^{R-S}$ (ppm) = $\left|\Delta\delta^{R} - \Delta\delta^{S}\right|$.

### 3-3. Conclusion

In this chapter, the author reported the synthesis of new types of $C_2$-symmetric chiral double-armed crown ethers, and introduced these host compounds as chiral NMR shift reagents. Among these chiral crown ethers, (*R*,*R*)-**5** with two pyrenylmethyl groups was found to be an excellent chiral $^1$H NMR shift reagent for all the ammonium salts examined in this study. The pyrene rings linked to the oxygen atom of the

sidearm through the methylene spacer strongly affected the chiral recognition of ammonium salts. Lanthanide shift reagents are the most famous chiral NMR shift reagents,[13] but their use has been limited because of the paramagnetic line broadening caused by them.[14] When chiral crown ethers were used as CSAs for NMR spectroscopy, line-broadening of the guest signals was rarely observed in contrast to the cases of lanthanide shift reagents. Accordingly, (*R*,*R*)-**5** is potentially useful as a widely applicable chiral NMR shift reagent.

And much more, the study of host-guest chiral recognition is of great value in a variety of fields, such as asymmetric catalysis reactions,[15] separating of enantiomers[16] enantiometric sensing,[17] and enzyme mimics.[18] In the future, there will be increasing interest focused on the design and synthesis of chiral compounds.

## 3-4. Experimental Section

### General Methods.

Optical rotations were measured using a JASCO DIP-370 polarimeter at ambient temperature and $[\alpha]_D$-values were given in units of $10^{-1}$ deg $cm^2$ $g^{-1}$. $^1H$ NMR spectra were taken at 400 MHz on a JEOL JNM-GSX-400 spectrometer using tetramethylsilane as the internal standard. IR spectra were obtained on a Horiba FT-710 spectrometer. Mass spectra were measured on a JEOL JMS-DX-303 mass spectrometer. Elemental analyses were measured with a Yanagimoto CHN-Corder. GC analyses were performed with a SHIMADZU Gas Chromatograph GC-14B. The starting crown ether, (2*R*,12*R*)-2,12-bis(hydroxymethyl)-2,12-dimethyl-18-crwon-6, was prepared according to the literature.[5]

### (2*S*,12*S*)-2,12-Dimethyl-2,12-bis(tosyloxymethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane [(*S*,*S*)-11].

To a solution of (*R*,*R*)-**1** (0.150 g, $4.26 \times 10^{-4}$ mol) in pyridine (2 mL) was added *p*-toluenesulfonyl chloride in pyridine (4 mL) at room temperature. The resulting mixture was stirred at ambient temperature for 40 h. After concentration under reduced pressure, water (50 mL) was added to the residue, and then extracted with dichloromethane (50 mL × 3). The combined organic layer was dried over $MgSO_4$ and the dichloromethane was evaporated off. The crude product was purified by chromatography over alumina (benzene:ethyl acetate = 95:5) to give (*S*,*S*)-**11** (0.201 g, $3.04 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 71%. $^1$H NMR ($CDCl_3$) δ 1.10 (s, 6H), 2.43 (s, 6H), 3.32-3.60 (m, 20H), 3.86 (d, 2H, *J* = 9.5 Hz), 4.02 (d, 2H, *J* = 9.5 Hz), 7.31 (d, 4H, *J* = 8.1 Hz), 7.76 (d, 4H, *J* = 8.1 Hz). IR (neat, $cm^{-1}$) 3060, 2860, 1730, 1600, 1460, 1350, 1300, 1100, 990, 810. MS (FAB) (m/z, relative intensity) 661 ($M^+$+1, 2), 91 (100). Anal. Calcd for $C_{30}H_{44}O_{12}S_2$: C, 54.53; H, 6.71; S, 9.71. Found: C, 54.25; H, 6.46; S, 9.34.

**(2*S*,12*S*)-2,12-Dimethyl-2,12-bis[(1-naphthyloxy)methyl]-1,4,7,10,13,16-hexaoxacyclooctadecane [(*S*,*S*)-3].**

To a suspension of *t*-BuOK (1.08 g, $9.65 \times 10^{-3}$ mol) and 1-Naphthol (2.79 g, $1.93 \times 10^{-2}$ mol) in diglyme (10 mL) was added a solution of (*S*,*S*)-**11** (0.638 g, $9.65 \times 10^{-4}$ mol) in diglyme (4 mL) at 100˚C. The resulting mixture was stirred for 42 h at 150˚C. After cooling to room temperature, the mixture was filtered and concentrated in vacuo. Water (100 mL) was added to the residue, and then extracted with chloroform (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and the chloroform was evaporated off. The crude product was purified by chromatography over silica (hexane:ethyl acetate = 80:20) to give (*S*,*S*)-**3** (0.328 g, $5.42 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 56%. $[\alpha]_D^{27}$= -8.17 (c 0.30, $CHCl_3$). $^1$H NMR ($CDCl_3$) δ 1.41 (s, 6H), 3.60-3.87 (m, 20H), 4.03 (d, 2H, *J* = 9.2 Hz), 4.24 (d, 2H, *J* = 9.2 Hz), 6.84 (d, 2H, *J* = 7.7 Hz), 7.33-7.50 (m, 8H), 7.78 (d, 2H, *J* = 7.3 Hz), 8.22 (d,

2H, $J$ = 7.7 Hz). IR (neat, $cm^{-1}$) 3050, 2880, 1720, 1580, 1460, 1360, 1270, 1110, 770, 730. MS (FAB) (m/z, relative intensity) 605 ($M^+$+1, 9), 197 (100). Anal. Calcd for $C_{36}H_{44}O_8$: C, 71.50; H, 7.33. Found: C, 71.32; H, 7.11.

**(2*R*,12*R*)-2,12-Dimethyl-2,12-bis[(1-naphthylmethoxy)methyl]-1,4,7,10,13,16-hexaoxacyclooctadecane [(*R*,*R*)-4].**

To a suspension of NaH (0.280 g, $7.02 \times 10^{-3}$ mol) and (*R*,*R*)-**1** (0.414 g, $1.17 \times 10^{-3}$ mol) in THF (35 mL) was added dropwise a solution of 1-chloromethylnaphthalene (1.66 g, $9.40 \times 10^{-3}$ mol) in THF (30 mL) and the resulting mixture was stirred for 50 h at refluxing temperature. After cooling to room temperature, a small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. Water (100 mL) was added to the residue, and then extracted with dichloromethane (50 mL × 3). The combined organic layer was dried over $MgSO_4$ and the dichloromethane was evaporated off. The residue was purified by chromatography over silica (hexane:ethyl acetate = 80:20) to give (*R*,*R*)-**4** (0.260 g, $4.11 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 35%. $[\alpha]_D^{26}$= -6.97 (c 0.30, $CHCl_3$). $^1$H NMR ($CDCl_3$) δ 1.12 (s, 6H), 3.38-3.66 (m, 24H), 4.92 (d, 2H, $J$ = 12.1 Hz), 4.97 (d, 2H, $J$ = 12.1 Hz), 7.37-7.51 (m, 8H), 7.75-7.84 (m, 4H), 8.10 (d, 2H, $J$ = 8.4 Hz). IR (neat, $cm^{-1}$) 3040, 2820, 1720, 1600, 1450, 1370, 1100, 800, 740. MS (FAB) (m/z, relative intensity) 633 ($M^+$+1, 1), 141 (100). Anal. Calcd for $C_{38}H_{48}O_8$: C, 72.13; H, 7.65. Found: C, 71.87; H, 7.47.

**(2*R*,12*R*)-2,12-Dimethyl-2,12-bis[(1-pyrenylmethoxy)methyl]-1,4,7,10,13,16-hexaoxacyclooctadecane [(*R*,*R*)-5].**

To a suspension of NaH (0.194 g, $4.84 \times 10^{-3}$ mol) and (*R*,*R*)-**1** (0.284 g, $8.06 \times 10^{-4}$ mol) in THF (20 mL) was added dropwise a solution of 1-bromomethylpyrene (0.951 g, $3.22 \times 10^{-3}$ mol) in THF (20 mL) and the resulting mixture was stirred for 36 h

at refluxing temperature. After cooling to room temperature, a small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. Water (100 mL) was added to the residue, and then extracted with dichloromethane (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and the dichloromethane was evaporated off. The residue was purified by chromatography over alumina (benzene:ethyl acetate = 90:10) to give (*R*,*R*)-**5** (0.317 g, $4.06 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 50%. $[\alpha]_D^{26}$= +19.5 (c 0.30, $CHCl_3$). $^1$H NMR ($CDCl_3$) δ 1.11 (s, 6H), 3.38-3.63 (m, 24H), 5.18 (d, 2H, *J* = 11.7 Hz), 5.23 (d, 2H, *J* = 11.7 Hz), 7.93-8.39 (m, 18H). IR (neat, $cm^{-1}$) 3050, 2850, 1710, 1630, 1450, 1360, 1240, 1100, 840, 710. MS (FAB) (m/z, relative intensity) 780 ($M^+$, 3), 215 (100). Anal. Calcd for $C_{50}H_{52}O_8$: C, 76.90; H, 6.71. Found: C, 77.17; H, 6.44.

**2-Methyl-1,2,3-propanetriol (13).**

β-Methallyl alcohol (50.0 g, 0.693 mol) and $H_2WO_4$ (1.32 g) were dissolved in distilled $H_2O$ (50 mL) by heating 70 ˚C. The oil bath was replaced by an ice bath and the temperature was maintained at 70-77 ˚C by the controlled addition of 30% $H_2O_2$. After a consumption of 93.9 g, the reaction mixture was kept for 1.5 h at 75 ˚C and in order to destroy residual $H_2O_2$, for another 3 h at 93 ˚C. The reaction mixture was cooled to room temperature, filtered, and passed through a Dowex 1-X4 column (1000 mL, OH-form in distilled $H_2O$) in order to remove $H_2WO_4$. The crude compound was distilled under reduced pressure (0.06 torr, 110 ˚C) to give **13** (48.6 g, 0.457 mol) as a slightly yellowish liquid. Yield 66%. $^1$H NMR ($CDCl_3$) δ 1.17 (s, 3H), 2.26 (t, 2H, *J* = 5.1 Hz, OH), 2.73 (s, 1H, OH), 3.45-3.71 (m, 4H). MS (FAB) (m/z, relative intensity) 107 ($M^+$+1, 46), 89 (100).

**2,2,4-Trimethyl-1,3-dioxolane-4-methanol (12).**

**13** (25.0 g, 0.236 mol), *p*-toluenesulfonic acid monohydrate (2.25 g, $1.18 \times 10^{-2}$ mol), and acetone (93.2 mL) were stirred in pentane (100 mL) at the refluxing temperature for 21 h and water produced by reaction was successively excluded by Dean-Stark trap. For neutralization the reaction mixture was stirred with $Na_2CO_3$ (4.89 g) for 2 h and the filtrate was concentrated, and the residue was distilled under reduced pressure (10 torr, 95 ˚C) to give **12** (24.6 g, 0.168 mol) as a slightly yellowish liquid. Yield 71%. $^1$H NMR ($CDCl_3$) δ 1.29 (s, 3H), 1.41 (s, 3H), 1.43 (s, 3H), 1.92 (t, 1H, *J* = 6.6 Hz, OH), 3.44-3.54 (m, 2H), 3.73 (d, 1H, *J* = 8.4 Hz), 3.97 (d, 1H, *J* = 8.4 Hz). MS (CI) (m/z, relative intensity) 147 ($M^+$+1, 21), 89 (100).

**[(4*S*)-2,2,4-Trimethyl-1,3-dioxolane-4-yl]methyl acetate [(*R*)-14].**

To a solution of *rac*-**12** (24.5 g, 0.168 mol) and lipase QL (0.600 g) in isopropyl ether (100 mL) was added isopropenyl acetate (16.8 g, 0.168 mol). The resulting mixture was stirred at 35˚C. The reaction was pursued by GC and was stopped when the conversion to acetate reached 40%. After filtration isopropyl ether was evaporated off. Then the residue was purified by chromatography over silica (hexane:ethyl acetate = 90:10) to give (*R*)-**14** (11.0 g, $5.84 \times 10^{-2}$ mol) as a slightly yellowish liquid. Yield 35%. $^1$H NMR ($CDCl_3$) δ 1.32 (s, 3H), 1.40 (s, 6H), 2.10 (s, 3H), 3.72 (d, 1H, *J* = 8.8 Hz), 3.94-4.05 (m, 3H). MS (CI) (m/z, relative intensity) 189 ($M^+$+1, 10), 131 (100).

**(4*S*)-2,2,4-Trimethyl-1,3-dioxolane-4-methanol [(*S*)-12].**

After sodium hydroxide (5.16 g, $1.29 \times 10^{-3}$ mol) was dissolved in a mixed solvent of water/ethanol (2:1 v/v, 30 mL), (*R*)-**14** (8.11 g, $4.31 \times 10^{-2}$ mol) was added to the mixture followed by stirring at room temperature for 12 h. The mixture was filtered and concentrated in vacuo. The residue was purified by chromatography over alumina (chloroform) to give (*S*)-**12** (4.82 g, $3.30 \times 10^{-2}$ mol) as a slightly yellowish

liquid. Yield 77%. The enantiomeric excess of (*S*)-**12** was determined by GC (>97% ee). $[\alpha]_D^{25}$= -21.5 (c 0.30, $CHCl_3$). $^1$H NMR ($CDCl_3$) δ 1.29 (s, 3H), 1.41 (s, 3H), 1.43 (s, 3H), 1.92 (t, 1H, *J* = 6.6 Hz, OH), 3.44-3.54 (m, 2H), 3.73 (d, 1H, *J* = 8.4 Hz), 3.97 (d, 1H, *J* = 8.4 Hz). MS (CI) (m/z, relative intensity) 147 ($M^+$+1, 21), 89 (100).

**(4*S*)-4-Benzyloxymethyl-2,2,4-trimethyl-1,3-dioxolane [(*S*)-15].**

To a suspension of NaH (1.92 g, $8.00 \times 10^{-2}$ mol) and (*S*)-**12** (5.89 g, $4.03 \times 10^{-2}$ mol) in THF (60 mL) was added dropwise a solution of benzyl bromide (27.5 g, $1.61 \times 10^{-2}$ mol) over a period of 30 min at room temperature. The resulting mixture was stirred for 20 h at room temperature. A small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. Water (100 mL) was added to the residue, and then extracted with diethyl ether (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and diethyl ether was evaporated off. The low-boiling impurities were excluded by distillation under reduced pressure (0.06 torr, 50 ˚C) to a 11.6 g of slightly yellowish liquid. The crude compound was used for the next step without further purification.

**(3*R*)-3-Benzyloxy-2-methylpropane-1,2-diol [(*R*)-16].**

After (*S*)-**15** (11.6 g, $4.91 \times 10^{-2}$ mol) was dissolved in a mixed solvent of methanol/water (2:1 v/v, 120 mL), Dowex 50-X2 ($H^+$ form) (2.00 g) was added to the mixture followed by stirring at room temperature for 20 h. The mixture was filtered and concentrated in vacuo. The residue was purified by chromatography over silica (hexane:ethyl acetate = 90:10) to give (*R*)-**16** (6.70 g, $3.41 \times 10^{-2}$ mol) as a slightly yellowish liquid. Yield 70% [based on (*S*)-**12**]. $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.13 (s, 3H), 3.38-3.64 (m, 4H), 4.54 (d, 2H, *J* = 2.2 Hz), 7.26-7.37 (m, 5H). IR (neat, $cm^{-1}$) 3550-3210, 2860, 1450, 1370, 1280, 1060, 920, 700, 610. MS (FAB) (m/z, relative intensity) 197 ($M^+$+1, 38), 91 (100). Anal. Calcd for $C_{11}H_{16}O_3 \cdot 1/8H_2O$: C, 66.56; H,

8.25. Found: C, 66.34; H, 8.13.

**(4*S*,14*S*)-4,14-Dimethyl-1,17-diphenyl-2,6,9,12,16-pentaoxaheptadecane-4,14-diol [(*S*,*S*)-17].**

To a suspension of NaH (0.370 g, $1.53 \times 10^{-2}$ mol) and (*R*)-**16** (3.00 g, $1.55 \times 10^{-2}$ mol) in DMF (15 mL) was added a solution of diethyleneglycol-di-*p*-tosylate (2.11 g, $5.10 \times 10^{-3}$ mol) in DMF (5 mL). The resulting mixture was stirred for 122 h at 40˚C. After cooling to room temperature, the mixture was filtered and concentrated in vacuo. The crude product was purified by chromatography over silica (hexane:acetone = 90:10) to give (*S*,*S*)-**17** (1.92 g, $4.15 \times 10^{-3}$ mol) as a slightly yellowish viscous liquid. Yield 81%. $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.17 (s, 6H), 3.51 (s, 4H), 3.55-3.68 (m, 12H), 4.51 (s, 4H), 7.28-7.32 (m, 10H). IR (neat, $cm^{-1}$) 3480-3370, 3090, 3060, 3030, 2850, 1120, 740, 700. MS (FAB) (m/z, relative intensity) 463 ($M^+$+1, 32), 91 (100). Anal. Calcd for $C_{26}H_{38}O_7 \cdot 1/4H_2O$: C, 66.86; H, 8.31. Found: C, 66.68; H, 8.18.

**(2*S*,12*S*)-2,12-bis(Benzyloxymethyl)-2,12-dimethyl-1,4,7,10,13,16-hexaoxacyclooctadecane [(*S*,*S*)-18].**

To a suspension of NaH (0.127 g, $5.30 \times 10^{-3}$ mol) and potassium tetrafluoroborate (0.668 g, $5.30 \times 10^{-3}$ mol) in diglyme (50 mL) was added dropwise a solution of diethyleneglycol-di-p-tosylate (0.879 g, $2.12 \times 10^{-3}$ mol) and (*S*,*S*)-**17** (0.491 g, $1.06 \times 10^{-3}$ mol) in diglyme (50 mL) over a period of 8 h and the resulting mixture was stirred for 122 h at 100˚C. After cooling to room temperature, a small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. The residue was purified by chromatography over silica (benzene:ethyl acetate = 70:30) to give (*S*,*S*)-**18** (0.221 g, $4.15 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 39%. $[\alpha]_D^{26}$= -0.88 (c 0.27, $CHCl_3$). $^1$H NMR ($CDCl_3$) δ 1.17 (s, 6H), 3.33-3.74 (m, 24H), 4.52 (s, 4H), 7.27-7.34 (m, 10H).

IR (neat, $cm^{-1}$) 3090, 3060, 3030, 2860, 1100, 740, 700. MS (FAB) (m/z, relative intensity) 555 ($M^+$+Na), 91 (100). Anal. Calcd for $C_{30}H_{44}O_8 \cdot H_2O$: C, 65.43; H, 8.42. Found: C, 65.72; H, 8.04.

**(2*S*,12*S*)- 2,12-Dimethyl-2,12-bis(hydroxymethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane [(*S*,*S*)-1].**

Suspension of (*S*,*S*)-**18** (0.638 g, $1.20 \times 10^{-3}$ mol) and 20% Pd/C (0.100 g) in a mixed solvent of dioxane/acetic acid (1:1 v/v, 20 mL) was hydrogenated for 48 h at room temperature. The mixture was filtered and concentrated in vacuo. The residue was purified by chromatography over alumina (hexane:chloroform = 50:50) to give (*S*,*S*)-**1** (0.333 g, $9.45 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 79%. $[\alpha]_D^{25}$= +3.56 (c 0.30, $CHCl_3$). $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.09 (s, 6H), 3.46-3.75 (m, 24H). IR (neat, $cm^{-1}$) 3550-3250, 2920, 1650, 1470, 1360, 1300, 1090, 960. MS (FAB) (m/z, relative intensity) 353 ($M^+$+1, 48), 154 (100). Anal. Calcd for $C_{16}H_{32}O_8 \cdot H_2O$: C, 51.88; H, 9.25. Found: C, 51.99; H, 8.95.

**[[(4*S*)-2,2,4-Trimethyl-1,3-dioxolane-4-yl]methoxy]acetic Acid Methyl Ester [(*S*)-19].**

To a suspension of NaH (3.71 g, $9.27 \times 10^{-2}$ mol) and (*S*)-**12** (4.52 g, $3.09 \times 10^{-2}$ mol) in THF (20 mL) was added dropwise a solution of bromoacetic acid methyl ester (14.8 g, $9.27 \times 10^{-2}$ mol) in THF (10 mL) and the resulting mixture was stirred for 37 h at refluxing temperature. After cooling to room temperature, a small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. Water (100 mL) was added to the residue, and then extracted with dichloromethane (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and dichloromethane was evaporated off. The crude compound was distilled under reduced pressure (0.06 torr, 120 ˚C) to give (*S*)-**19** (6.44 g, $2.95 \times 10^{-2}$

mol) as a slightly yellowish liquid. Yield 96%. $^1$H NMR ($CDCl_3$) δ 1.34 (s, 3H), 1.39 (s, 6H), 3.70 (d, 1H, *J* = 8.4 Hz), 3.74 (s, 3H), 4.08 (d, 1H, *J* = 8.4 Hz), 4.15 (s, 2H). MS (FAB) (m/z, relative intensity) 219 ($M^+$+1, 83), 115 (100).

**2-[[(4*S*)-2,2,4-Trimethyl-1,3-dioxolane-4-yl]methoxy]ethanol [(*S*)-20].**

To a suspension of $LiAlH_4$ (3.76 g, $7.94 \times 10^{-2}$ mol) in THF (10 mL) was added dropwise a solution of (*S*)-**19** (5.78 g, $2.65 \times 10^{-2}$ mol) in THF (10 mL) at 0°C. The resulting mixture was stirred for 41 h at refluxing temperature. After cooling to room temperature, successively a small portion of ethanol and 5% NaOH aqueous solution were added to the mixture in order to deactivate the excess $LiAlH_4$. The mixture was filtered and concentrated in vacuo to give (*S*)-**20** (3.67 g, $1.93 \times 10^{-2}$ mol) as slightly yellowish viscous liquid. The crude compound was used for the next step without further purification.

**[[[(4*S*)-2,2,4-Trimethyl-1,3-dioxolane-4-yl]methoxy]ethoxy] *p*-Toluenesulfonate [(*S*)-21].**

After sodium hydroxide (6.48 g, 0.162 mol) and (*S*)-**20** (3.54 g, $1.86 \times 10^{-2}$ mol) was dissolved in water (10 mL), *p*-tosyl chloride (10.6 g, $5.58 \times 10^{-2}$ mol) was added to the mixture followed by stirring at room temperature for 40 h. The mixture was filtered concentrated in vacuo. Water (100 mL) was added to the residue, and then extracted with dichloromethane (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and dichloromethane was evaporated off. The residue was purified by chromatography over silica (chloroform) to give (*S*)-**21** (6.39 g, $1.86 \times 10^{-2}$ mol) as a slightly yellowish viscous liquid. Yield 72% [based on (*S*)-**19**]. $^1$H NMR ($CDCl_3$) δ 1.23 (s, 3H), 1.34 (s, 3H), 1.37 (s, 3H), 2.44 (s, 3H), 3.26 (d, 1H, *J* = 9.2 Hz), 3.35 (d, 1H, *J* = 9.2 Hz), 3.61 (d, 1H, *J* = 8.8 Hz), 3.66-3.68 (m, 2H), 3.90 (d, 1H, *J* = 8.8 Hz), 4.14-4.16 (m, 2H), 7.34 (d, 2H, *J* = 8.4 Hz), 7.80 (d, 2H, *J* = 8.4 Hz). IR (neat, $cm^{-1}$)

3030, 2870, 1740, 1600, 1450, 1370, 1120, 990, 820. MS (FAB) (m/z, relative intensity) 345 ($M^+$+1, 41), 199 (100).

**1,6-bis[(4*S*)-2,2,4-Trimethyl-1,3-dioxolane-4-yl]-2,5-dioxahexane [(*S*,*S*)-22].**

To a suspension of NaH (2.36 g, $5.91 \times 10^{-2}$ mol) and (*S*)-**12** (2.14 g, $1.46 \times 10^{-2}$ mol) in THF (20 mL) was added dropwise a solution of (*S*)-**21** (5.04 g, $1.46 \times 10^{-2}$ mol) in THF (10 mL) at 0˚C and the resulting mixture was stirred for 45 h at refluxing temperature. After cooling to room temperature, a small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. Water (100 mL) was added to the residue, and then extracted with dichloromethane (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and the dichloromethane was evaporated off. The crude product was purified by chromatography over silica (hexane:ethyl acetate = 90:10) to give (*S*,*S*)-**22** (3.61 g, $1.13 \times 10^{-2}$ mol) as a slightly yellowish viscous liquid. Yield 78%. $^1$H NMR ($CDCl_3$) δ 1.31 (s, 6H), 1.39 (s, 12H), 3.33 (d, 2H, *J* = 9.2 Hz), 3.42 (d, 2H, *J* = 9.2 Hz), 3.42-3.50 (m, 2H), 3.63 (s, 4H), 3.67 (d, 2H, *J* = 8.4 Hz), 4.00 (d, 2H, *J* = 8.4 Hz). IR (neat, $cm^{-1}$) 2870, 1740, 1450, 1370, 1290, 1210, 1120, 1060. MS (FAB) (m/z, relative intensity) 357 ($M^+$+K, 32), 115 (110). Anal. Calcd for $C_{16}H_{30}O_6$: C, 60.35; H, 9.50. Found: C, 60.73; H, 9.46.

**(2*R*,9*R*)-2,9-Dimethyl-4,7-dioxadecane-1,2,9,10-tetraol [(*R*,*R*)-23].**

After (*S*,*S*)-**22** (2.48 g, $7.79 \times 10^{-3}$ mol) was dissolved in a mixed solvent of methanol/water (2:1 v/v, 45 mL), Dowex 50-X2 ($H^+$ form) (1.00 g) was added to the mixture followed by stirring at room temperature for 20 h. The mixture was filtered and concentrated in vacuo. The crude compound was used for the next step without further purification.

**(4*R*,11*R*)-4,11-Dimethyl-1,14-diphenyl-2,6,9,13-tetraoxatetradecane-4,11-diol [(*R*,*R*)-24].**

To a suspension of NaH (0.857 g, $2.14 \times 10^{-2}$ mol) and (*R*,*R*)-**23** (2.42 g, $1.02 \times 10^{-2}$ mol) in THF (25 mL) was added dropwise a solution of benzyl bromide (3.66 g, $2.14 \times 10^{-2}$ mol) over a period of 30 min at room temperature. The resulting mixture was stirred for 43 h at room temperature. A small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. The crude product was purified by chromatography over silica (hexane:ethyl acetate = 90:10) to give (*R*,*R*)-**24** (2.05 g, $4.90 \times 10^{-3}$ mol) as a slightly yellowish viscous liquid. Yield 48% [based on (*S*,*S*)-**22**]. $^1$H NMR ($CDCl_3 + D_2O$) δ 1.18 (s, 6H), 2.70 (s, 2H, OH), 3.38 (d, 2H, *J* = 9.2 Hz), 3.39 (s, 4H), 3.48 (d, 2H, *J* = 9.2 Hz), 3.63 (s, 4H), 4.54 (s, 4H), 7.27-7.38 (m, 10H). IR (neat, $cm^{-1}$) 3640-3200, 3060, 3030, 2850, 1740, 1210, 740, 700. MS (FAB) (m/z, relative intensity) 419 ($M^+$+1, 22), 91 (100). Anal. Calcd for $C_{24}H_{34}O_6 \cdot 1/4H_2O$: C, 68.14; H, 8.22. Found: C, 68.27; H, 8.09.

**(2*R*,9*R*)-2,9-bis(Benzyloxymethyl)-2,9-dimethyl-1,4,7,10,13,16-hexaoxacyclooctadecane [(*R*,*R*)-25].**

To a suspension of NaH (0.420 g, $1.05 \times 10^{-2}$ mol) and potassium tetrafluoroborate (1.42 g, $1.05 \times 10^{-2}$ mol) in diglyme (50 mL) was added dropwise a solution of triethyleneglycol-di-*p*-tosylate (1.93 g, $4.20 \times 10^{-3}$ mol) and (*R*,*R*)-**24** (0.888 g, $2.10 \times 10^{-3}$ mol) in diglyme (50 mL) over a period of 4 h and the resulting mixture was stirred for 62 h at 100°C. After cooling to room temperature, a small portion of ethanol was added to the mixture in order to deactivate the excess NaH and the mixture was filtered and concentrated in vacuo. The residue was purified by chromatography over silica (benzene:ethyl acetate = 95:5) to give (*R*,*R*)-**25** (0.395 g, $7.42 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 35%. $^1$H NMR ($CDCl_3$) δ 1.18 (s, 6H),

3.36-3.76 (m, 24H), 4.54 (s, 4H), 7.27-7.36 (m, 10H). IR (neat, $cm^{-1}$) 3060, 3030, 2870, 1720, 1450, 1370, 1100, 740, 700. MS (FAB) (m/z, relative intensity) 571 ($M^+$+K, 100), 555 ($M^+$+Na, 43). Anal. Calcd for $C_{30}H_{44}O_8$: C, 67.65; H, 8.33. Found: C, 67.38; H, 8.27.

**(2*R*,9*R*)-2,9-Dimethyl-2,9-bis(hydroxymethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane [(*R*,*R*)-2].**

Suspension of (*R*,*R*)-**25** (0.310 g, $5.82 \times 10^{-4}$ mol) and 20% Pd/C (0.050 g) in a mixed solvent of dioxane/acetic acid (1:1 v/v, 10 mL) was hydrogenated for 47 h at room temperature. The mixture was filtered and concentrated in vacuo. The residue was purified by chromatography over alumina (hexane:chloroform = 50:50) to give (*R*,*R*)-**2** (0.148 g, $4.20 \times 10^{-4}$ mol) as a slightly yellowish viscous liquid. Yield 72%. $[\alpha]_D^{15}$= +37.7 (c 0.30, $CHCl_3$). $^1$H NMR ($CDCl_3$ + $D_2O$) δ 1.12 (s, 6H), 3.49-3.75 (m, 24H). IR (neat, $cm^{-1}$) 3700-3060, 2920, 1730, 1460, 1370, 1290, 1100, 960. MS (FAB) (m/z, relative intensity) 353 ($M^+$+1, 32), 154 (100). Anal. Calcd for $C_{16}H_{32}O_8$: C, 54.53; H, 9.15. Found: C, 54.53; H, 9.04.

**Ammonium Salts (6-10).**

**6** and **7** were prepared according to the literature.[19] **8** and **9** were obtained from Aldrich. **10** were prepared according to the following literature.[20]

**Enantiomeric Separation in the $^1$H NMR Spectra.**

The chiral crown ether ($2.50 \times 10^{-3}$ M) and the racemic ammonium salts ($1.25 \times 10^{-3}$ M) were dissolved in 0.6 mL of $CDCl_3/CD_3OD$ (9/1 v/v), and were assessed by NMR at 400 MHz. Differences in the chemical shifts between the enantiomers were then recorded. All chemical shift values were referenced to the internal tetramethylsilane standard.

**Titration Method for the Determination of the Stability Constants.**

As an example, the titration experiment for the complexation of crown ether (*R*,*R*)-**4** with the ammonium salt (*R*)-**6** is described. A 2.63 mM solution of (*R*,*R*)-**4** in 0.6 mL of $CDCl_3/CD_3OD$ (3/7 v/v) was prepared, and an initial NMR measurement of this solution was recorded. A 119 mM solution of (*R*)-**6** in 0.4 mL of $CDCl_3/CD_3OD$ (3/7 v/v) was separately prepared. Samples were then made by adding various amounts of the guest solution into the host solution. The spectra of these eight different solutions were recorded. The stability constants were calculated from the chemical shift changes using an iterative nonlinear least squares curve-fitting program.[21]

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# Conclusion

This thesis deals with the discrimination of metal ions and ammonium ions by crown ether analogues with polycyclic aromatic rings. The results obtained through this work are summarized as follows.

In **Chapter 1**, two types of double-armed lariat ethers with pyrene moieties at each end of two sidearms were synthesized and their complexation properties toward alkali metal and alkaline earth metal cations were examined by fluorescence spectroscopy. Pyrene excimer emission decreased accompanied by an increase in monomer emission upon metal ion complexation. This finding is ascribed to the change of the spatial distance of two pyrene rings by movement suppression of both the crown ring and one of the two sidearms based on complexation with the metal cation. The selectivity for alkaline earth metal cations was highly dependent on the fitness of the host cavity and the guest size.

In **Chapter 2**, new type of monoazacryptand-type fluorescent indicators with a pyrene ring were described and their fluorescent characteristics in the presence of alkali metal and alkaline earth metal cations in water were examined. The detection of metal cations was performed by the change of the fluorescence intensity of host compounds based on PET mechanism. In aqueous solution, the fluorophore showed little fluorescence upon the addition of $Ba^{2+}$ because of the very weak complexation ability with $Ba^{2+}$, but the coexistence of Triton X-100 micelles enabled the fluorophore to detect $Ba^{2+}$ with very high sensitivity and selectivity. Moreover, the complexation behavior between a monoazacryptand-type fluorophore and $Ba^{2+}$ was utilized as a novel pyrene-functionalized probe for the CMC determination. Based on a comparison of the CMC values obtained by the PET method versus the CMC values obtained by conventional fluorescent methods utilizing DPH, ANS and pyrene probes, as well as the

values reported in the literature, one can conclude that the PET method was useful for the CMC determination of various nonionic and anionic surfactants. In particular, the PET method was more effective for the CMC determination of nonionic surfactants with very low CMC values ($10^{-5}$ M) than any other fluorescent method. This notable achievement may be due to the high sensitivity of the pyrene ring, the higher solubility of the probe (due to the presence of the cryptand functionality) over pyrene, and the drastic change in the fluorescence intensity based on the PET mechanism.

In **Chapter 3**, new types of $C_2$-symmetric chiral double-armed crown ethers were prepared, and their enantiomeric recognition abilities as chiral NMR discriminating agents towards primary ammonium salts were examined. Among these chiral crown ethers, the most effective enantiomeric discrimination of ammonium salts in the $^1$H NMR spectra was attained in the presence of the chiral crown ether with two pyrenylmethyl sidearms. The pyrene rings linked to the oxygen atom of the sidearm through the methylene spacer could strongly affect the chiral recognition of ammonium salts. This result clearly shows that a subtle change of the structure of sidearms remarkably affects the chiral recognition ability.

In this study, the fluorescent sensing of metal ions with high selectivity and sensitivity and the asymmetric discrimination of ammonium ions were achieved by crown ether analogues with polycyclic aromatic rings. It is hopeful that a range of molecular recognition sensors will be designed based on information from this work in the future.

# List of Publications

1. Fluorometric Sensing of Alkaline Earth Metal Cations by New Lariat Ethers Having Plural Pyrenylmethyl Groups on the Electron-Donating Sidearms
   Yoshio Nakahara, Yasuo Matsumi, Wanbin Zhang, Toshiyuki Kida, Yohji Nakatsuji, and Isao Ikeda
   *Org. Lett.* **2002**, *4*, 2641-2644. (Chapter 1)

2. A Novel Fluorescent Indicator for $Ba^{2+}$ in Aqueous Micellar Solutions
   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi
   *Chem. Commun.* **2004**, 224-225. (Chapter 2)

3. Synthesis of Double-Armed Lariat Ethers with Pyrene Moieties at Each End of Two Sidearms and Their Fluorescence Properties in the Presence of Alkali Metal and Alkaline Earth Metal Cations
   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi
   *J. Org. Chem.* **2004**, *69*, 4403-4411. (Chapter 1)

4. A New Fluorescence Method for the Determination of the Critical Micelle Concentration by Photosensitive Monoazacryptand Derivatives
   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi
   *Langmuir*, submitted. (Chapter 2)

5. Fluorometric Sensing of Alkali Metal and Alkaline Earth Metal Cations
   by Novel Type of Photosensitive Monoazacryptand Derivatives
   in Aqueous Micellar Solutions
   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi
   *Org. Biomol. Chem.*, submitted. (Chapter 2)

6. Novel $C_2$-Symmetric Chiral 18-Crown-6 Derivatives with Two Aromatic Sidearms
   as Chiral NMR Discriminating Agents
   Yohji Nakatsuji, Yoshio Nakahara, Akiko Muramatsu, Toshiyuki Kida,
   and Mitsuru Akashi
   *Tetrahedron Letters*, submitted. (Chapter 3)

7. Synthesis of New Types of $C_2$-Symmetric Chiral Double-Armed Crown Ethers
   Yohji Nakatsuji, Yoshio Nakahara, Masahiro Muraoka, Katsumori Nagamiya,
   Yuki Itoh, Kentaro Uesugi, Toshiyuki Kida, and Mitsuru Akashi
   in preparation. (Chapter 3)

# Other Publications

8. The First Study on the Mobility of Surface-Adsorbed Molecules Using $^{1}$H NMR Spin-Lattice Relaxation Time
   Taizo Mouri, Yoshio Nakahara, Wanbin Zhang, Yohji Nakatsuji, Toshiko Muneishi, Yoko Miyaji, and Isao Ikeda
   *Chem. Lett.* **2000**, 988-989.

9. Study on the Adsorption Behavior of Dispersants onto Pigment Using $^{1}$H NMR Spin-Lattice Relaxation Time
   Taizo Mouri, Yoshio Nakahara, Wanbin Zhang, Toshiyuki Kida, Yohji Nakatsuji, and Isao Ikeda
   *J. Oleo Sci.* **2001**, *50*, 623-631.

10. Adsorption Mechanism of Poly (Ammonium Carboxylate) onto Phthalocyanine Blue Pigment Deduced by $^{1}$H NMR Spin-Lattice Relaxation Time
    Taizo Mouri, Yoshio Nakahara, Wanbin Zhang, Toshiyuki Kida, Yohji Nakatsuji, and Isao Ikeda
    *J. Oleo Sci.* **2001**, *50*, 857-863.

# Presentations at International Conferences

1. Fluorescent Characteristics of Monoazacryptand Derivatives Containing a Pyrenylmethyl Group

   Yoshio Nakahara, Toshiyuki Kida, and Yohji Nakatsuji

   The 1st International 21Century COE Symposium on Integrated EcoChemistry, Osaka, Japan, February, 2002.

2. Fluorescent Characteristics of Crown Ether Derivatives Containing Plural Pyrenylmethyl Groups

   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi

   The 2nd International 21Century COE Symposium on Integrated EcoChemistry, Hyogo, Japan, July, 2003.

3. Synthesis and Molecular Recognition Ability of $C_2$-Symmetric Chiral 18-Crown-6 Derivatives

   Yoshio Nakahara, Akiko Muramatsu, Toshiyuki Kida, Yohji Nakatsuji,

   and Mitsuru Akashi

   The 3rd International 21Century COE Symposium on Integrated EcoChemistry, Osaka, Japan, January, 2004.

4. A Novel Fluorescent Indicator for $Ba^{2+}$ in Aqueous Micellar Solutions

   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi

   227th American Chemical Society National Meeting, Anaheim, USA, March, 2004.

5. Synthesis of Double-Armed Lariat Ethers with Pyrene Moieties at Each End of Two Sidearms and Their Fluorescence Properties in the Presence of Alkali Metal and Alkaline Earth Metal Cations
   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi
   40th International Symposium on Macromolecules, Paris, France, July, 2004.

6. A New Fluorescence Method for Determination of Critical Micelle Concentrations by Photosensitive Monoazacryptand Derivatives
   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi
   The 4th International 21Century COE Symposium on Integrated EcoChemistry, Osaka, Japan, August, 2004.

7. Fluorometric Detection of Alkali Metal and Alkaline Earth Metal Cations by Photoresponsive Monoazacryptand Derivatives in Aqueous Micellar Solutions
   Yoshio Nakahara, Toshiyuki Kida, Yohji Nakatsuji, and Mitsuru Akashi
   The 5th International 21Century COE Symposium on Integrated EcoChemistry, Osaka, Japan, January, 2005.

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