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**Study of novel off-on responsive fluorescent probe
using supramolecular complex formation with
crown ether and cyclodextrin**

A Thesis Presented to Hokkaido University

For

Doctor' Degree



by

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Chapter 1

General Introduction

General Introduction

Fluorescent probes

Fluorescent probes based on supramolecular interaction have been studied extensively in chemical, biological, medical, and environmental fields.¹ They present many benefits for the detection of target species because of their facility, swiftness, and high sensitivity compared to other materials. Chemical analyses used to obtain detailed information have depended on apparatus such as high-performance liquid chromatography (HPLC), gas chromatography (GC), inductive coupled plasma (ICP), mass spectrometry (MS), and nuclear magnetic resonance (NMR). However, those sophisticated apparatuses are limited to indoor use. Moreover, they often need destructive treatment for sampling (*e.g.* living cells). Furthermore, analytical reagents enable the rapid and simplified detection of specific species in a sample solution and monitoring for the time course of the target system. Such reagents are regarded as effective for ascertaining the pollution status from measurements of a degradable sample. Rapid correspondence is sometimes necessary at chemical substance outflow accidents. Numerous analytes are detectable using those probes, which are cations,² anions,³ organic compounds,^{4,5} biomolecules⁶ and even CO₂ gas⁷.

In biological fields, fluorescence imaging probes have been investigated actively for use in a progressive strategy of visualization for the distribution of necessary cations and a change of pH environmental in living cells.⁸ Also, logic circuits of “molecular computers” have been anticipated for future use by replacing circuit voltage to chemical species (*e.g.* cation, H⁺).⁹ Target detection using a fluorescent probe can be observed by changes of the intensity and/or the shape of fluorescence spectra between the absence and presence of guest molecules. Fluorescence switching of the probe is often represented by combinations of ‘Off’ and ‘On’ corresponding to low (Off) and high (On) emissions of fluorescence intensity at a specific wavelength. Definite change of a fluorescence signal is an important performance criterion for a fluorescent probe. In addition, fluorescent probes have been designated as fluorescent sensors, fluoroionophores, fluorescent chemosensors, optical sensors, and others. Although many studies have examined probes and sensors,¹⁰ those terms are often assumed for mechanical devices such as a pH meter. To avoid confusion in this report, it is clearly described herein that fluorescent probes are macrocyclic compounds with fluorescence response to recognition events for small molecules.

Molecular recognition with supramolecular interaction

Some macrocyclic molecules can form an inclusion complex with a small molecule. Crown ether¹¹ synthesized by Pedersen,¹² azacrown,¹³ polyamine,¹⁴ cyclodextrin¹⁵ and calixarene¹⁶ have been used as typical host molecules for metal ions and organic molecules. In 1977, Takagi *et al.* described the first example of chromogenic crown ethers¹⁷, as shown in Fig. 1.1. When K^+ was extracted using chloroform with this compound, the order of the organic layer changed clearly from orange to deep red at pH 11. Many chromeric¹⁸ and fluorimeric¹⁹ crown ethers have been synthesized in subsequent studies. Inclusion phenomena of many probes are caused by one or more interactions such as hydrogen bonding, multi-coordination linkage, electrostatic interaction, van der Waals force, and π - π orbital stacking. The binding ability for target species increases concomitantly with increasing binding sites in the receptor. Cryptands and spherands are well known to have a larger 1 : 1 complex formation constant ($K = 5.62 \times 10^9$ for K^+ , $K \approx 10^{16}$ for Na^+) than crown ether has ($K = 1.26 \times 10^6$ for K^+).²⁰ Consequently, supramolecular components involving multipoint recognition have been studied for the development of more efficient chemosensors. Enantiomer-selective extraction or separation between RS isomers of amino acid ester is achieved by introducing chiral binding site to crown ether.²¹ In conductive polymers, π - π stacking of main chains occurs when guest species are included to the crown ether, which is to the side chain group,²² and which engenders fluorescence quenching. Proteins and DNAs are natural sophisticated systems related to multipoint molecular recognition, which are often emphasized in strategies to assay those biomaterials.²³ Green fluorescent protein (GFP) is known as an extremely representative probe²⁴. Although studies of those materials have slowed because of their extremely complicated structure,²⁵ analytical applications as fluorescent chemosensors have been explored intensively in recent years.^{26, 27}

Fluorescence “Off-On” mechanism

To execute quantitative sensing, it is necessary that the fluorescent probes change the fluorescence intensity and/or the shape after complexation with a target species. Consequently, the following fluorescence switching mechanism is fundamentally incorporated into the fluorescent probes: photoinduced electron transfer (PET),²⁸ twisted intramolecular charge transfer (TICT),²⁹ fluorescence resonance energy transfer

(FRET,³⁰ between a photosensitizer and a rare earth metal ion),³¹ formation of excimer,³² and photodimerization.³³

In our laboratory, many fluorescent probes, including carbonyl group, based on PET³⁴ or TICT,^{35, 36} or both³⁷ have been investigated extensively. They form a supramolecular complex with simple (1 : 1) stoichiometry for alkaline earth metals. PET or TICT mechanisms in the probes act as quenching mechanism that is suppressed by the coordination of alkaline earth metals to a carbonyl group. Consequently, quantitative analysis is possible because of the change of fluorescence intensity.

PET process

In actuality, PET-type fluorescent chemosensors have been studied extensively.²⁷ The pioneer work of Weller provides a kinetic basis of PET.³⁸ Figure 1.2 depicts the concept of PET process. This mechanism is accepted as “Off–On” behavior in many probes. The recognition moiety (*e.g.* benzocrown) acts as an electron donor. The fluorophore (*e.g.* anthracene) plays an electron acceptor. Without guest materials, an electron of the highest occupied molecular orbital (HOMO) in the electron acceptor is excited by light irradiation to the lowest unoccupied molecular orbital (LUMO). Therefore, the transference of the donor electron to the vacant HOMO orbital of the acceptor follows by transfer of electron in acceptor LUMO to the unoccupied HOMO in the donor, which causes fluorescence quenching. Upon complexation with a guest material, the HOMO level of the donor moves to a lower level than that of the acceptor. Consequently, the PET process is prevented and the probe fluorescence is recovered. Many PET probes are based on this strategy.

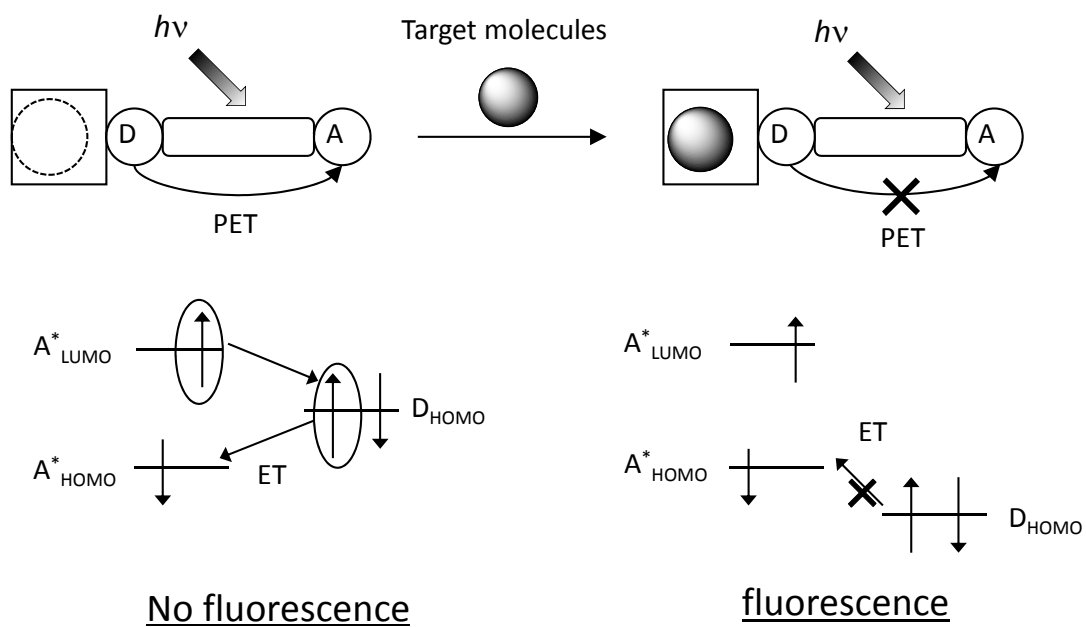
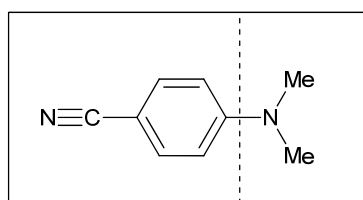
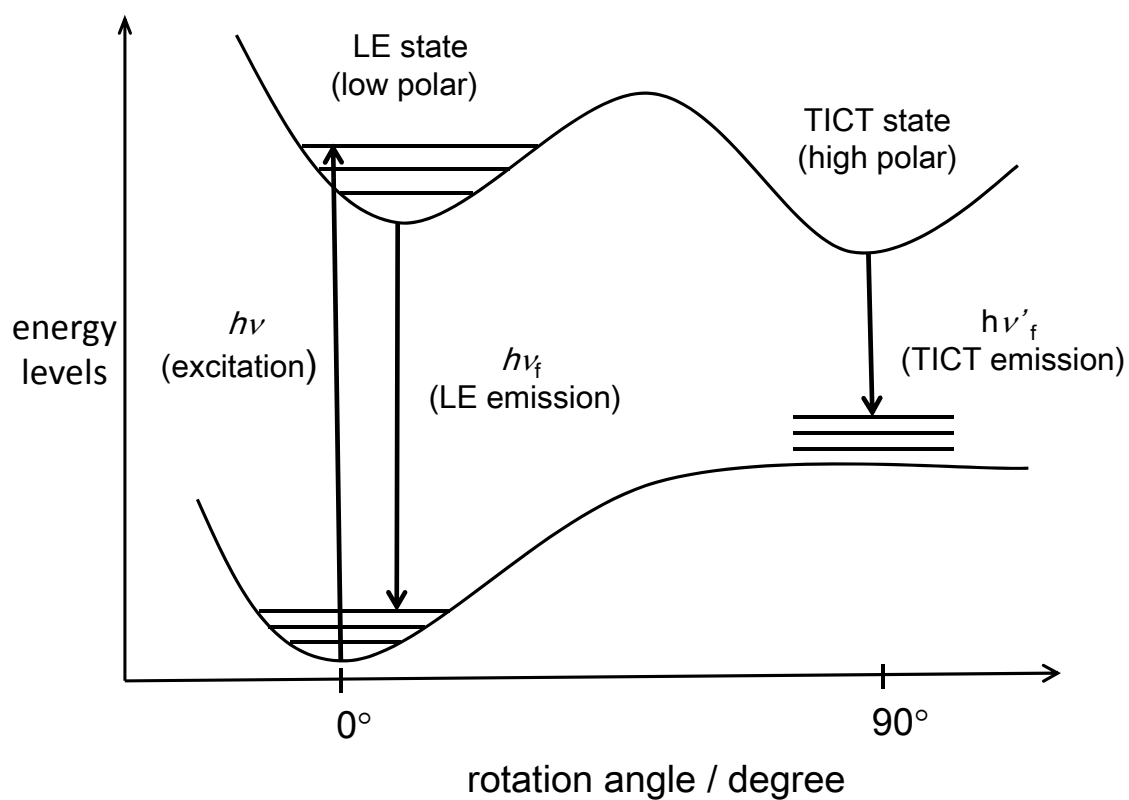


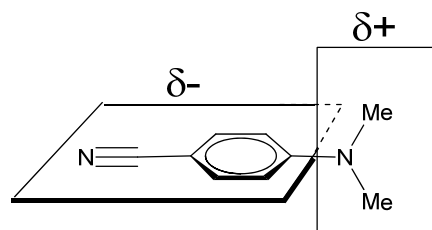
Figure 1.1. Mechanism of photoinduced electron transfer (PET).

TICT mechanism

In pioneer work related to TICT, Rotkiewicz and Grabowski investigated dual fluorescence emission of *p*-(*N,N*-dimethylamino)-benzonitrile (DMABN) in polar and apolar solvents.³⁹ Results of that study have been invoked continually for interpretation of the photophysical properties of fluorophores.⁴⁰ Fluorescence emission of DMABN is observed at 330 nm in less-polar solvents. In contrast, a second emission band at longer wavelength (420–460 nm) arises in polar solvents. The appearance of spectra of two kinds has been explained as TICT,⁴¹ which results from charge separation of π electron in a transient state (Fig. 1.2) of the excited state. In the polar solvent, photoexcited DMABN enables the rotation of C-NMe₂ bond between an electron donor (D: dimethylamino group) and an electron acceptor (A: phenyl ring). This rotation causes rearrangement to the orthogonalization and charge separation of both.⁴² The stability of the charge-separated state depends on the solvent polarity.⁴³ Consequently, in an apolar solution, emission in the short wavelength region occurs compared with that in an apolar solvent. Fluorescent probes based on TICT can reportedly control fluorescent emission by molecular recognition.⁴⁴



LE state (planar structure)



TICT state (charge separation)

Figure 1.2. TICT process of DMANB in photoexcitation.

Application of TICT for sensing

In this study, *N*-phenyl-*x*-anthracene carboxamide (*x*-PAA, *x*=9, 1 and 2) derivatives were used as fluorophores with a TICT function, which has two distinctions with DMABN as follows: 1) three excited states are expected from photoexcitation, 2) if the molecule goes to TICT state, fluorescence emission is quenched.³⁵ The molecular conformation of 9-PAA is orthogonal to minimize the steric barrier between the carbonyl and protons of the anthracene ring in the ground state (Fig. 1.3). The non-planar structure in the ground state differs from the planar DMABN. If 9-PAA remains in the twisted structure at the excited state (excited state I), then fluorescence emission will occur without intramolecular charge separation. Its fluorescence spectrum resembles that of anthracene. Photoexcited 9-PAA rotates around the bond between anthracene and amido moiety approaching coplanar geometry. The amido group is well-known to have a sp^2 hybrid orbital character. Consequently, 9-PAA forms extended π -conjugate system via the bond of that (excited state II), whereas the charge delocalization occurs over the whole molecular plane. In a low polar solvent (*e.g.* cyclohexane), the fluorescence emission was observed with a bathochromic shift by increased π -conjugation, which cancels the “charge”. In contrast, the CO–NH bond in 9-PAA rotates spontaneously to form a charge-separated state with a perpendicular dihedral conformation (excited state III) in a highly polar, low-viscosity solvent.

The charge-separated 9-PAA can be described as a TICT state. Its fluorescence is quenched effectively in acetonitrile and methanol. Fluorescence quantum yield (Φ_f) of fluorophore involving TICT quenching function increases concomitantly with increasing viscosity of the solvent⁴⁵ as follows:

$$\Phi_f = \frac{k_r}{k_{nr}} \left(\frac{\eta}{T} \right)^C$$

Therein, k_r and k_{nr} are the rate constants of emission and non-emission decay paths; η and T respectively denote the viscosity and the absolute temperature of the solvent. C is an experimentally determined constant. Glycerin and diethylene glycol have high viscosity attributable to the formation of a strong hydrogen-bond network at hydroxyl group. The 9-PAA conformation in glycerin is almost fixed in both the ground and excited states. Then, the photo-driven rotation between 9-anthracene and the *N*-phenyl bond is restricted. The anthracene-like fluorescence is emitted from excited state I. The fluorescence intensity of 9-PAA in glycerin was higher than that in diethylene glycol or

and methanol. Although methanol can make a hydrogen-bond network, its viscosity is lowest among those solvents. The order of viscosity is parallel with fluorescence intensity of 9-AA in the emission region. This fact proves that the fluorescence switching of 9-AA is controlled by a TICT mechanism, and that it evokes a 9-PAA chemosensor based on controlled rotation around 9-PAA amide in low-viscosity solvent.^{35, 46}

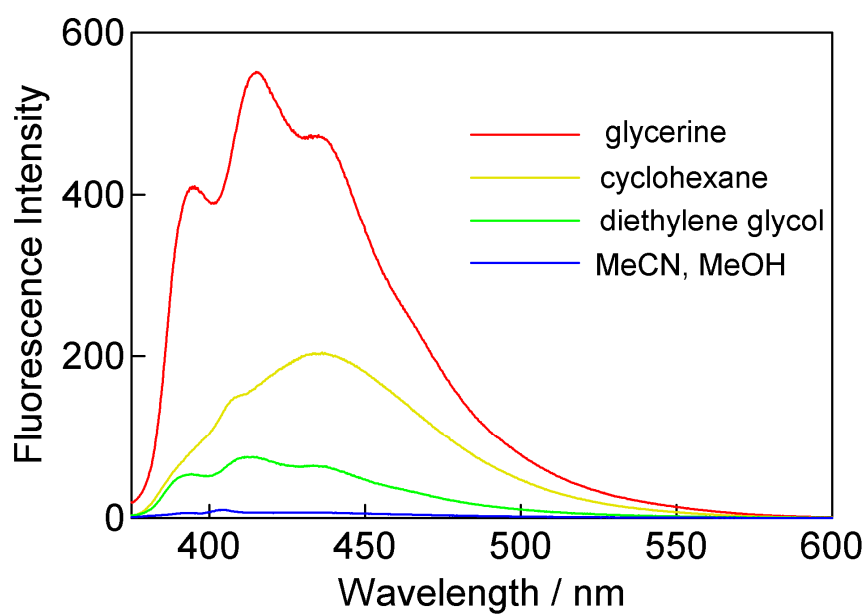
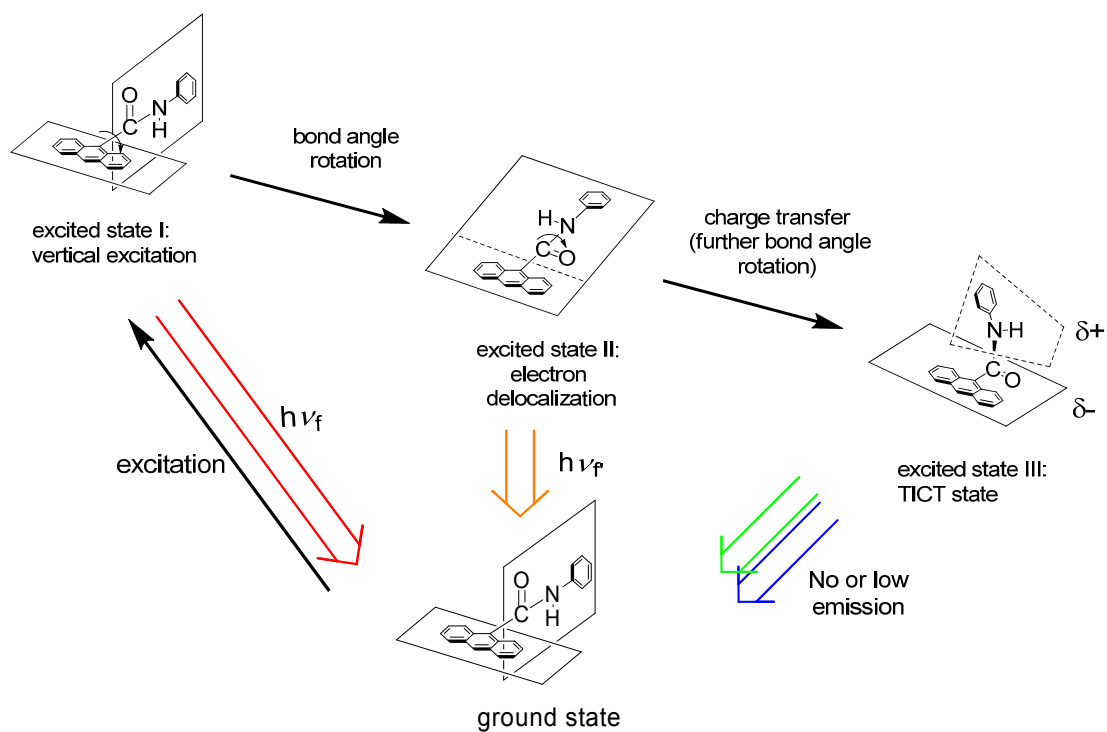


Figure 1.3. TICT process and fluorescence spectra of 9-PAA in various solvents.

Present study

This thesis presents a description of four works. In Chapter 2, a novel fluorescence probe based on crown ether with *N*-phenyl-9-phenanthreneacetamide (PPA) and its improvement strategy for fluorescence “Off–On” switching are described. The PET process is affected directly by the HOMO–LUMO energy level of both the electron donor and acceptor (D–A). Consequently, an appropriate D–A system can improve the PET efficiency by the modification of the electron-releasing substituent. To confirm the ideas presented above, PPA-based fluorescent probes of two types were synthesized: 4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**P1**) and 3'-methyl-4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**P2**). The photochemical behaviors of both those probes were investigated using UV and fluorescence spectra with and without alkaline earth metal ions in acetonitrile solutions.

For the discussion included in chapters 3 and 4, target species of 9-PAA and 1-PAA probes were expanded to organic materials. The PAA unit is bonded covalently to cyclodextrin, which is used for a solubilization in an aqueous medium and for recognition of an organic target in water. Herein, β -cyclodextrin and γ -cyclodextrin based TICT probes are discussed with respect to the sensing of surfactants in water. Then 9-PAA modified β -cyclodextrin (**Ant-CyD**) and its analogues with γ -CyD (**ACs**) were synthesized. Their fluorescence properties were investigated using UV and fluorescence spectroscopy with and without surfactants such as Triton X-100 (TX-100) and sodium dodecyl sulfate (SDS). Moreover, the limit of detection (LOD) of **AC1** (an **AC**) for SDS was estimated to infer the application possibilities for environmental samples.

To extend **Ant-CyD** and **ACs**, 5-(*N,N'*-dimethylaminophenyl)thiophene-2-carboxamide (TH) was introduced as a fluorophore to a novel CyD probe (**TH-CyD**) in chapter 5. In actuality, TH has a strong emission band at long wavelengths despite the small conjugate system, which can be beneficial for the development of high-performance probes. For practical applications, the LOD value of **TH-CyD** for SDS was calculated and compared with the requirements of the United States Environmental Protection Agency (USEPA) and the Ministry of Health, Labour, and Welfare in Japan (MHLW). Moreover, the fluorescence experiment was measured using actual samples obtained from one upstream and two downstream sources in Sapporo, Japan.

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Chapter 2

“Off–On” Responsible Fluoroionophores for Alkaline Earth Metal Ions Based on Benzo-15-crown-5 Bearing 9-Phenanthreneacetamide

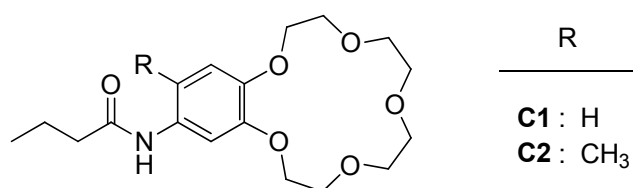
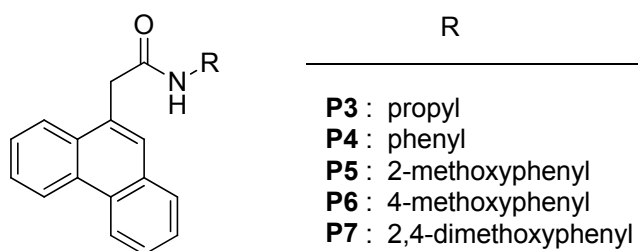
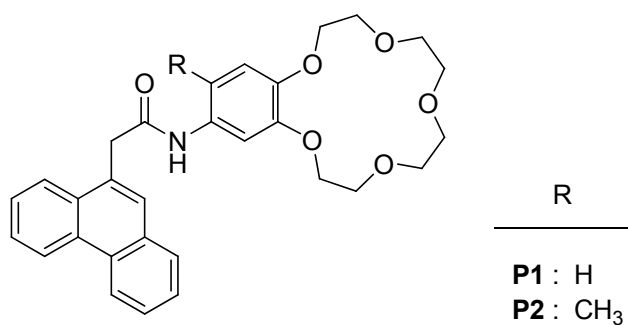
Introduction

Fluoroionophores act as a nanoscale chemical interface between humans and molecules in various analytical fields. Most fluoroionophores consist of a fluorescent group with an accompanying a receptor which recognizes a target substance (*e.g.* a metal cation) through a host–guest interaction with a coordinating moiety (*e.g.* crown ether).¹ To monitor the interactive event between the host and the guest molecules, the fluorescence signal of the fluoroionophore must be changed greatly along with the event.² Consequently, the occurrence of a fluorescence spectra change upon the recognized state is an important factor to evaluate the controllability of fluoroionophores in this research.³ Many fluoroionophores based on a control of the fluorescence quenching, excimer or exciplex emission involving a conformational change have been developed for applications in various fields of biological, medical, and environmental analyses.^{1,5} Various fluoroionophores based on crown ether, azacrown ether, cryptand,^{1,6} cyclodextrin⁶ and calixarene⁷ have been reported. These fluoroionophores were operated by energy transfer⁸ and photoinduced electron transfer (PET)⁹ between a donor and acceptor linked bond.

When a PET process is prevented by complexation, the fluorescence is highly enhanced (fluorescence “On” state). We provided effective control of fluorescence “Off–On” states in previous systems.¹⁰ In earlier papers, we described several fluoroionophores based on linear polyethers having fluorescence molecules at their terminals.^{10, 11} These ionophores gave fluorescence change from weak to strong emission upon metal ion complexation. Recently, we also reported a new mode of PET in a fluorophore connecting *N*-phenylamide derivatives and its applications for chemosensors based on crown ether.¹² It became clear that these photo-excited molecules showed electron transfer (ET) from the *N*-phenyl moiety to fluorophores, resulting in the quenching of fluorescence emission, which were controllable by the breakdown of π –conjugation for PET by bending an amide bond, which is resulted from metal ion complexation showing a fluorescence “Off–On” signal. In our previous study, spectral behaviors of fluoroionophores based on these ET actions were classified as follows. Group 1 anthracene, pyrene: this type ET *via* amide bond occurred when the fluorescent moiety and the donor were connected directly. Group 2 naphthalene, fluorene: this type showed ET over the CH₂ group between the fluorescent ring and the carbonyl group. One difference between group 1 and group 2 is a difference of the UV absorption maximum wavelengths of each fluorescent moiety (*e.g.*, anthracene, 350 nm; naphthalene, 282 nm). Consequently, these ET characteristics will be classified

according to whether the excitation wavelength is longer than 300 nm or not. To study our interpretation for ET *via* an amide bond, we focus phenanthrene as the next target molecule.

Lewis *et al.* reported that the quenching of intramolecular ET of *N*-alkyl-9-phenanthrenecarboxamide derivatives depends on the molecular structures, such as the *Z* and *E* conformer.¹³ In addition, 9-phenanthrenecarboxamide involving *N*-(phenylmethylaminoalkyl) amide exist as a mixture of *Z* and *E* conformers in solution, and a pure *Z* isomer in which the *N*-(aminoalkyl) substituent is folded over the face of the amide group and the phenanthrene and aniline rings adopt an edge-to-face geometry in the solid state, as shown by X-ray crystallography.¹⁴ Rima *et al.* reported that inclusion phenomena of β -cyclodextrin with 9-alkylphenanthrene derivatives was affected by the alkyl chain length using fluorescence spectroscopy.¹⁵ Phenanthrene has also been taken on the dyes of organic electroluminescence devices,¹⁶ and target species for a rapid screening using electrolytic methods in environmental samples.² However, the employment of phenanthrene as a fluoroionophore based on PET is rare. Phenanthrene is structurally similar to anthracene, although its photochemical properties resemble those of naphthalene.¹³ consequently, phenanthrene will show a naphthalene-like photochemical behavior, and thus it is classified into group 2. An introduction of CH₂ group between phenanthrene and the amido moiety will be needed to controll its ET. Herein, we report on 4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**P1**) and 3'-methyl-4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**P2**) as a new PET system, which was tuned for its capability of fluorescence “Off–On”, using fluorescence, UV, and ¹H-NMR spectrometry.



Scheme 2.1. Structures of **P1-P2**, and model compounds **P3-P7** and **C1-C2**.

Experimental

The synthetic pathway of 9-phenanthreneacetamide derivatives **P1** and **P2** is shown in Scheme 2.2. Actually, 9-phenanthrenecarboxylic acid (5.0 g, 0.025 mol), which was prepared according to the literature,¹⁷ was suspended in THF. To this suspension, 1 g (1 eq. for 9-phenanthrenecarboxylic acid) of LiAlH₄ dissolved in 200 mL of THF was added dropwise at room temperature and stirred for 3 h. To this solution, distilled water was added dropwise. A crude product of 9-phenanthrenemethanol was obtained. This compound was dissolved in 50 mL of 1,4-dioxane and refluxed for 2 h with the addition to an excess amount of SOCl₂. The solution was cooled to room temperature. The crude 9-chloromethylphenanthrene was precipitated through the addition of distilled water. The precipitate was collected and dried at room temperature. The precipitate and sodium cyanide (2.45 g) were dissolved in 50 mL of acetonitrile, and was heated at 70°C overnight. The reaction mixture was filtered, and 100 mL of water was added to the filtrate. The resulting precipitate 9-phenanthreneacetonitrile was filtered. The residue was suspended in 150 mL of acetic acid, and was carefully refluxed with addition to 50 mL of concentrated hydrochloric acid. Then, the reflux was continued for 5 h. The solution was cooled and precipitated by addition of water. The crude 9-phenanthreneacetic acid was dissolved in a 20% NaOH aqueous solution, and the precipitate was filtered off. Subsequently, this filtrate was added to 20 mL of concentrated hydrochloric acid. The resulting precipitate was collected by filtration (yields: 89% 5.2 g).

Subsequently, 4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**P1**) was synthesized from 9-phenanthreneacetic acid (1.2 g 0.005 mol) and 4'-aminobenzo-15-crown-5 according to a previous paper.¹⁸ An analogous compound **2** and model compounds **P3-P7** were also synthesized from 0.005 mol of respective amino compounds: 1.64 g of 3'-methyl-4'-aminobenzo-15-crown-5¹⁹ (**P2**), the 0.30 g of propylamine (**P3**), the 0.47 g of aniline (**P4**), the 0.66 g of 2-anisidine (**P5**), the 0.66 g of 4-anisidine (**P6**), and the 0.77 g of 2, 4-dimethoxyaniline (**P7**).

4'-(9-Phenanthreneacetamido)-benzo-15-crown-5 (P1)

Yield 71%, white solid. m.p. 236°C. ¹H-NMR (acetonitrile-*d*₃; δ from TMS) 3.60 (C-CH₂-O-, m, 8H), 3.73 (C-CH₂-O-, m, 4H), 3.99 (C-CH₂-O-, m, 4H), 4.15 (CO-CH₂-phenanthrene-, s, 2H), 6.79 (aromatic, d, 1H), 6.96 (aromatic, dd, 1H), 7.21

(aromatic, s, 1H), 7.65 – 7.70 (aromatic, m, 4H), 7.80 (aromatic, s, 1H), 7.92 (aromatic, d 2H), 8.13 (aromatic, d 2H), 8.28 (CO–NH–, s 1H), 8.74 (aromatic, d 1H), 8.82 (aromatic, d 1H). Found: C, 71.76; H, 6.23; N, 2.67. Calcd. for C₃₀H₃₁NO₆: C, 71.84; H, 6.23; N, 2.79.

3'-Methyl-4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (P2)

Yield 67%, white solid. m.p. 227 – 229°C. ¹H-NMR (acetonitrile-*d*₃; δ from TMS) 3.61 (C–CH₂–O–, m, 8H), 3.74 (C–CH₂–O–, m, 4H), 4.00 (C–CH₂–O–, m, 4H), 4.18 (CO–CH₂–aromatic–, s, 2H), 6.69 (aromatic, s, 1H), 7.00 (aromatic, s, 1H), 7.62 – 7.75 (aromatic, m, 5H), 7.83 (aromatic, s, 1H), 7.93 (aromatic, d 1H), 8.17 (aromatic, d 1H), 8.75 (aromatic, d, 1H), 8.82 (aromatic, d 1H). Found: C, 72.07; H, 6.48; N, 2.69. Calcd. for C₃₁H₃₃NO₆: C, 72.21; H, 6.45; N, 2.72.

N-Propyl-9-phenanthreneacetamide (P3)

Yield 64%, light-yellow solid. m.p. 188 – 190°C. ¹H-NMR (chloroform-*d*₁; δ from TMS), 0.67 (–CH₃, t, 3H), 1.30 (–CH₂–, m, 2H), 3.10 (–N–CH₂–, m, 2H), 4.07 (–phenanthrene–CH₂–CO–, s, 2H), 5.40 (–CONH–, s, 1H), 7.65 (aromatic, m, 5H), 7.87 (aromatic, d, 1H), 8.03 (aromatic, d, 1H), 8.69 (aromatic, d, 1H), 8.74 (aromatic, d, 1H). Found: C, 82.11; H, 6.90; N, 5.05. Calcd. for C₁₉H₁₉NO: C, 82.28; H, 6.90; N, 5.05.

N-Phenyl-9-phenanthreneacetamide (P4)

Yield 73%, white solid. m.p. 235 – 236°C. ¹H-NMR (chloroform-*d*₁; δ from TMS), 4.24 (phenanthrene–CH₂–CO, s, 1H), 7.04 (aromatic, m, 2H), 7.25 (aromatic, m, 5H), 7.69 (aromatic, m, 4H), 7.80 (–CONH–, s, 1H), 7.91 (aromatic, d, 1H), 8.10 (aromatic, d, 1H), 8.71 (aromatic, d, 1H), 8.77 (aromatic, d, 1H). Found: C, 84.90; H, 5.67; N, 4.53. Calcd. for C₂₂H₁₇NO: C, 84.86; H, 5.50; N, 4.50.

N-(2-Methoxyphenyl)-9-phenanthreneacetamide (P5)

Yield 67%, light-yellow solid. m.p. 166.5 – 169°C. ¹H-NMR (chloroform-*d*₁; δ from TMS), 3.36 (–OCH₃, s, 3H), 4.25 (–phenanthrene–CH₂–CO–, s, 2H), 6.66 (aromatic, d, 1H), 6.92 (aromatic, m, 2H), 7.68 (aromatic, m, 5H), 7.80 (–CONH–, s, 1H), 8.12

(aromatic, d, 1H), 8.29 (aromatic, d, 1H), 8.71 (aromatic, d, 1H), 8.76 (aromatic, d, 1H). Found: C, 81.06; H, 5.75; N, 4.15. Calcd. for $C_{23}H_{19}NO$: C, 80.92; H, 5.61; N, 4.10.

N-(4-Methoxyphenyl)-9-phenanthreneacetamide (**P6**)

Yield 70%, white solid. m.p. 252 – 255°C. ^1H -NMR (chloroform- d_1 ; δ from TMS), 3.72 (–OCH₃, s, 3H), 4.22 (phenanthrene–CH₂–CO, s, 2H), 6.74 (aromatic, d, 2H), 6.97 (aromatic, s, 1H), 7.17 (aromatic, d, 2H), 7.70 (aromatic, m, 4H), 7.79 (–CONH–, s, 1H), 7.91 (aromatic, d, 1H), 8.10 (aromatic, d, 1H), 8.71 (aromatic, d, 1H), 8.77 (aromatic, d, 1H). Found: C, 80.97; H, 5.74; N, 4.15. Calcd. for $C_{23}H_{19}NO_2$: C, 80.92; H, 5.61; N, 4.10.

N-(2,4-Dimethoxyphenyl)-9-phenanthreneacetamide (**P7**)

Yield 63%, white solid. m.p. 204 – 207 °C. ^1H -NMR (chloroform- d_1 ; δ from TMS), 3.34 (–O–CH₃, s, 3H), 3.71 (–O–CH₃, s, 3H), 4.21 (CO–CH₂– phenanthrene–, s, 2H), 6.25 (aromatic, d, 1H), 6.39 (aromatic, dd, 1H), 7.64 (aromatic, m, 5H), 7.77 (–CO–NH–, s, 1H), 7.88 (aromatic, d, 1H), 8.11 (aromatic, d, 2H), 8.68 (aromatic, d, 2H), 8.74 (aromatic, d, 2H). Found: C, 77.51; H, 5.86; N, 3.71. Calcd. for $C_{24}H_{21}NO_3$: C, 77.61; H, 5.70; N, 3.77.

Measurement of fluorescence and UV Spectra

Fluorescence spectra were measured using a spectrometer (RF-5300PC; Shimadzu Corp.) in a spectral-grade acetonitrile solution at 25 °C. The excitation wavelength was set to 297 nm, unless described otherwise. Various alkaline earth metal cations were added to a solution of phenanthreneacetamido derivatives as perchlorate salts. The initial concentration of **P1** and **P2** was 5 μ M. Fluorescence spectra of **P3–P7** were measured in a 10 μ M acetonitrile solution at 25°C. All fluorescence spectra were normalized by the absorbance at 297 nm among all compounds while comparing the fluorescence intensity. Because the fluorescence lifetimes of free and metal complexes were less than 10 ns, the sample solutions were used without degassing. The UV spectra were measured (UV-2400PC) under the same conditions as those used for fluorescence measurements.

Measurement of ^1H -NMR

The ^1H -NMR spectra were measured at 30°C (JNM-EX400; JEOL). The phenanthreneacetamido derivatives concentrations were 5 mM in acetonitrile- d_3 . For measurements of alkaline earth metal complexes, excess amounts of metal cations as perchlorates were added to these solutions.

Result and Discussion

Fluorescence properties of model compounds P3-P7

For comparing the electron transfer (ET) effects, a fluorescence study using model compounds **P3-P7** in Scheme 2.1 was conducted; it can be summarized as follows: 1) phenanthrene is an electron acceptor and the *N*-phenyl moiety worked as a donor when phenanthrene was excited; 2) an effective ET could be induced by stronger electron donating groups.¹³ Fluorescence and UV-vis spectra of **P3-P7** are presented in Fig 2.1 and Fig 2.2, respectively. The fluorescence spectrum of model compound **P3**, which is used as a reference, shows the original phenanthrene emission, because no ET was expected between the electron donor and the acceptor. The order of the fluorescence intensity in all model compounds was **P3** > **P4** > **P5** > **P6** >> **P7**, which shows that the efficiency of the ET process corresponds to the electron-donating substituent. In a previous report, the fluorescence intensity of *N*-(2-methoxyphenyl) or (4-methoxyphenyl)-naphthaleneacetamide drastically decreased due to the resulting of ET. However, the fluorescence spectra of **P4 -P6** were similar to those of reference **P3**. The present results suggest that an electron-donating ability of the phenyl group with one methoxy group to phenanthrene was insufficient for ET. The fluorescence intensity of **P7**, which has two methoxy substituent at *ortho*- and *para*- positions in the benzene moiety, decreased to 5% for **P5** and 6% for **P6**, respectively. From these results, to quench the fluorescence emission, this ET system consisting of 9-phenanthreneacetamide (PAA) needs the addition of an electron-releasing substituent on a benzene ring in the electron donor. In consideration of this photochemical property, new fluoroinophores **P1** and **P2** involving a fluorescence “Off–On” ability were synthesized, and the photochemical properties were measured in the presence of various alkaline earth metal ions. On the other hands, the use of viscous solvent can judge that those fluoroinophore occur PET or TICT quenching process. Fluorescence intensity ratio of **P4** in glycerin is merely enhanced to 3.1-folds of the case of acetonitrile (Fig. 2.3). However, other TICT chemosensor is largely intended to the range between 15 to 40-folds. Thus, it can be assumed that attribution of TICT quenching is few.

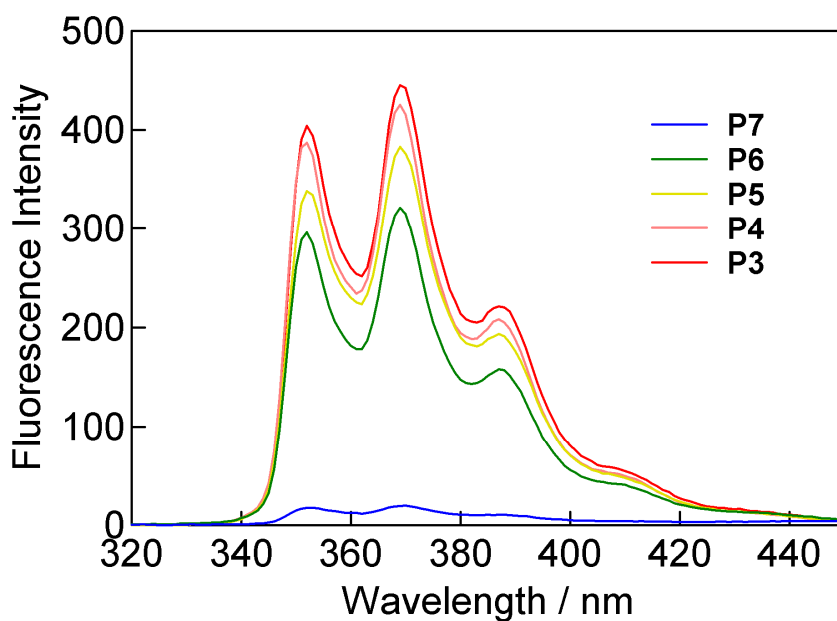


Figure 2.1. Fluorescence spectra of model compounds **P3-P7**. Those intensities were normalized by their absorbances at 297 nm in acetonitrile at 25 °C. The excitation wavelength was 297 nm. The concentrations of all model compounds were 10 μ M.

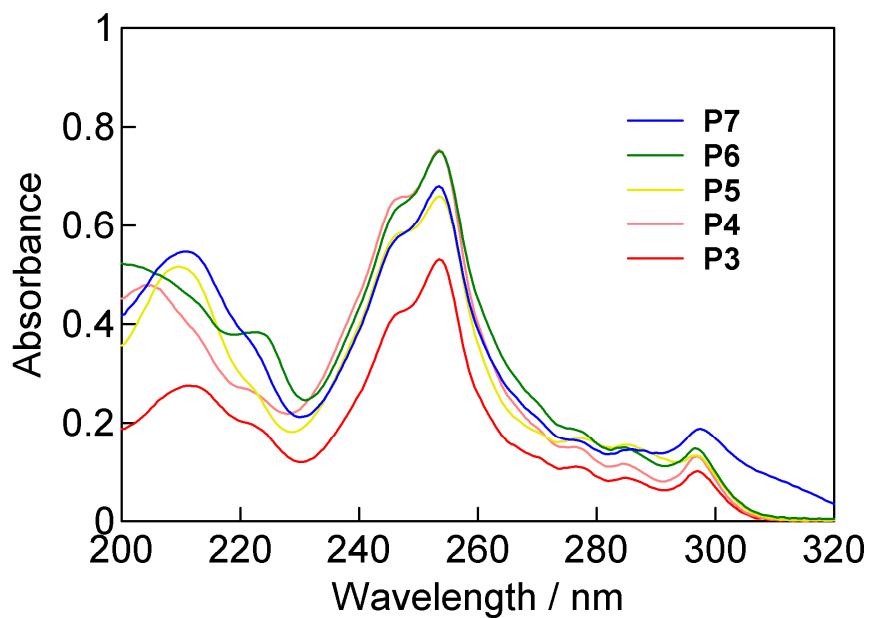


Figure 2.2. UV-vis spectra of model compounds **P3-P7** at 25°C. The concentrations of all model compounds were 10 μ M.

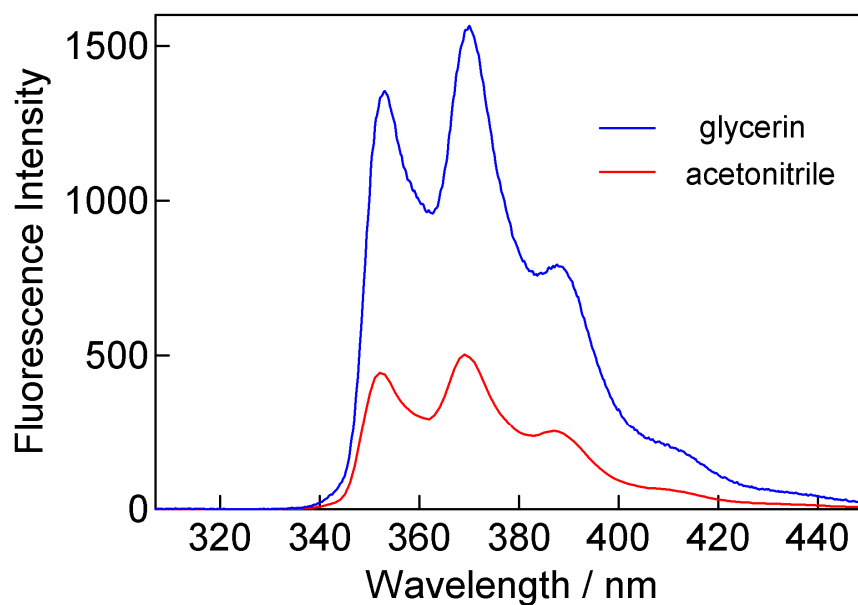


Figure 2.3. Fluorescence spectra of **P4** in glycerin and acetonitrile. Those intensities were normalized by their absorbances at 297 nm in acetonitrile at 25°C. The excitation wavelength was 297 nm. The concentrations of all model compounds were 10 μ M.

Fluorescence spectra of **P1** and **P2**

Figure 2.4 portrays the fluorescence spectra of **P1**, and its Ca^{2+} complex in acetonitrile. Fluorescence emissions from the phenanthrene moiety in the absence of Ca^{2+} were weak compared to those of **P3**. In contrast, dramatically enhanced emissions were observed in the presence of Ca^{2+} . The shapes of these spectra indicate that they are phenanthrene monomer emissions, whose maximum wavelength is *ca.* 370 nm. A similar fluorescence enhancement behavior was also observed in the other alkaline earth metal ions. These “Off–On” behaviors were expressed quantitatively as a fluorescence intensity ratio, I_{max}/I_0 , where I_{max} and I_0 are the fluorescence intensities in the presence (I_{max}) and absence (I_0) of excess amounts of alkaline earth metal ions. These data are presented in Table 2.1 and Fig. 2.5-2.7. A more improved I_{max}/I_0 value for **P2** (16.6) than that of **P1** (4.8) for Ca^{2+} was obtained, suggesting that the electron-donating methyl group in benzocrown moiety can control the fluorescence “Off–On” behavior.

Table 2.1. Fluorescence responses (I_{max}/I_0) of **P1** and **P2** for various metal ions in acetonitrile at 25 °C.

		Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
P1	I_{max}/I_0	4.36	4.76	4.27	4.66
P2	I_{max}/I_0	15.5	16.6	13.2	13.6

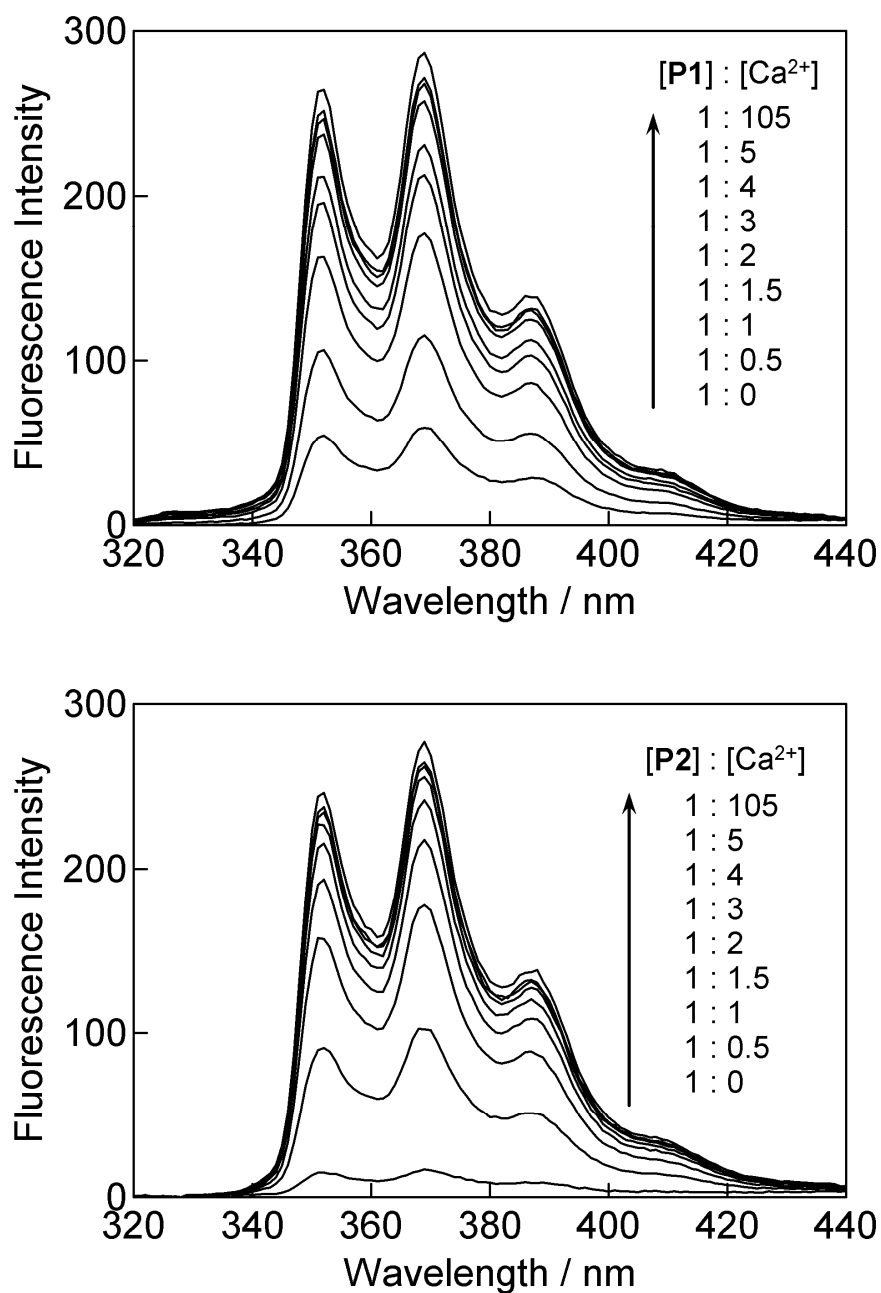


Figure 2.4. Fluorescence spectra of **P1** and its Ca^{2+} complex (upper), and fluorescence spectra of **P2** and its Ca^{2+} complex (lower); they were normalized by the absorbance at 297 nm, in acetonitrile at 25°C. Excitation wavelength, 297 nm. $[\text{P1}]$ and $[\text{P2}] = 5 \mu\text{M}$. The stoichiometric ratios with Ca^{2+} are listed respectively in columns.

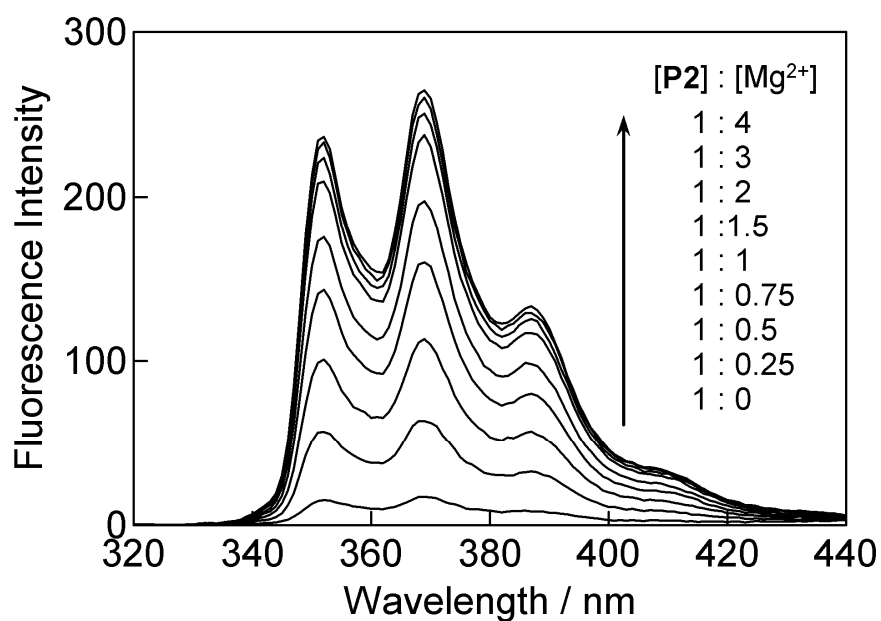
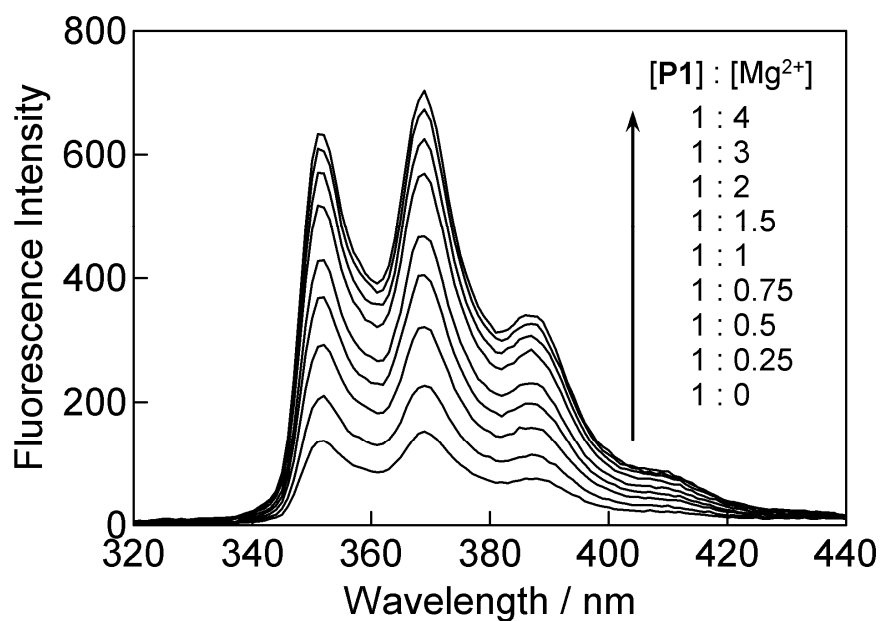


Figure 2.5. Fluorescence spectra of **P1** and its Mg²⁺ complex (upper), and fluorescence spectra of **P2** and its Mg²⁺ complex (lower); they were corrected by the absorbance at 297 nm, in acetonitrile at 25°C. Excitation wavelength, 297 nm. [**P1**] and [**P2**] = 5 μM. The stoichiometric ratios with Mg²⁺ are listed respectively in columns.

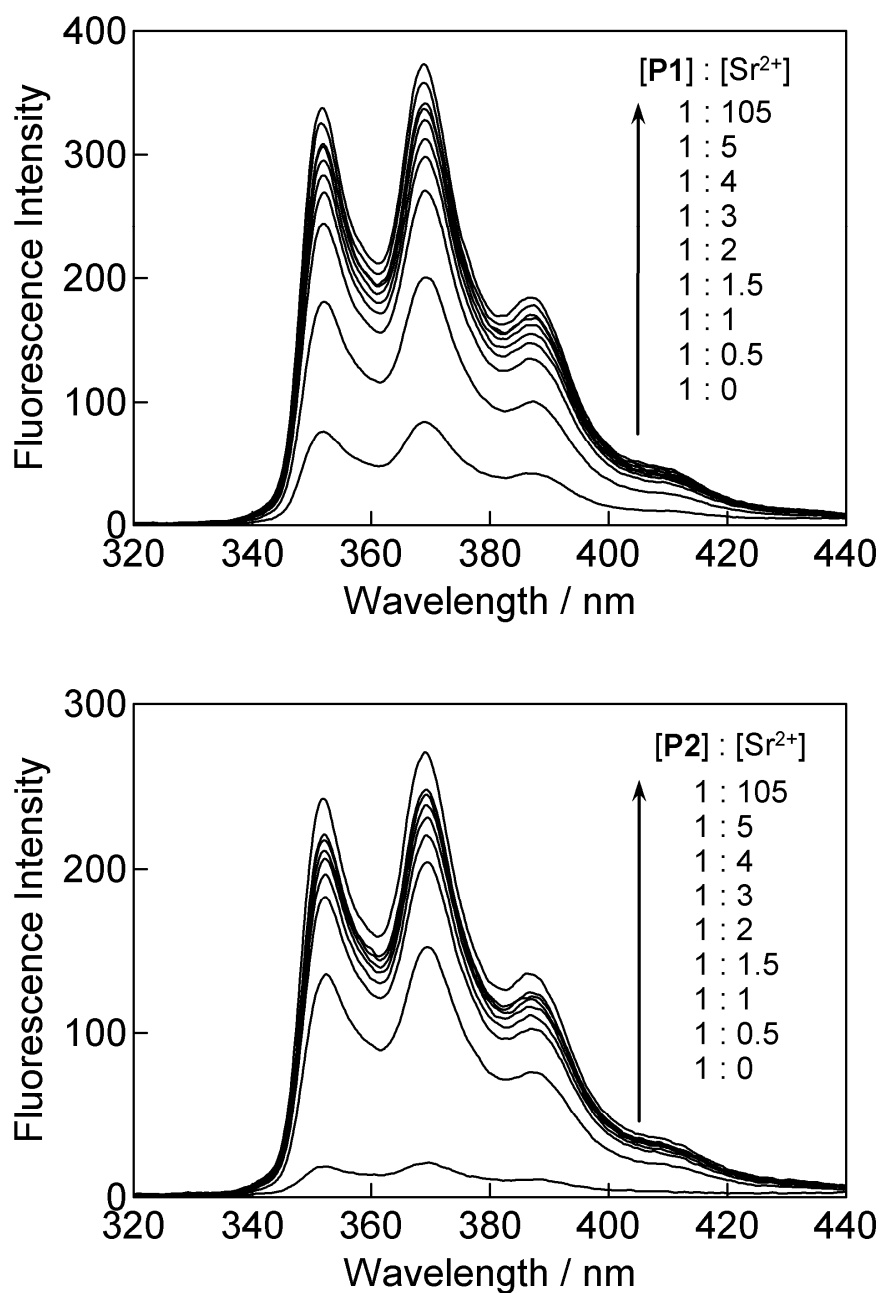


Figure 2.6. Fluorescence spectra of **P1** and its Sr^{2+} complex (upper), and fluorescence spectra of **P2** and its Sr^{2+} complex (lower); they were corrected by the absorbance at 297 nm, in acetonitrile at 25 °C. Excitation wavelength, 297 nm. $[\text{P1}]$ and $[\text{P2}] = 5 \mu\text{M}$. The stoichiometric ratios with Sr^{2+} are listed respectively in columns.

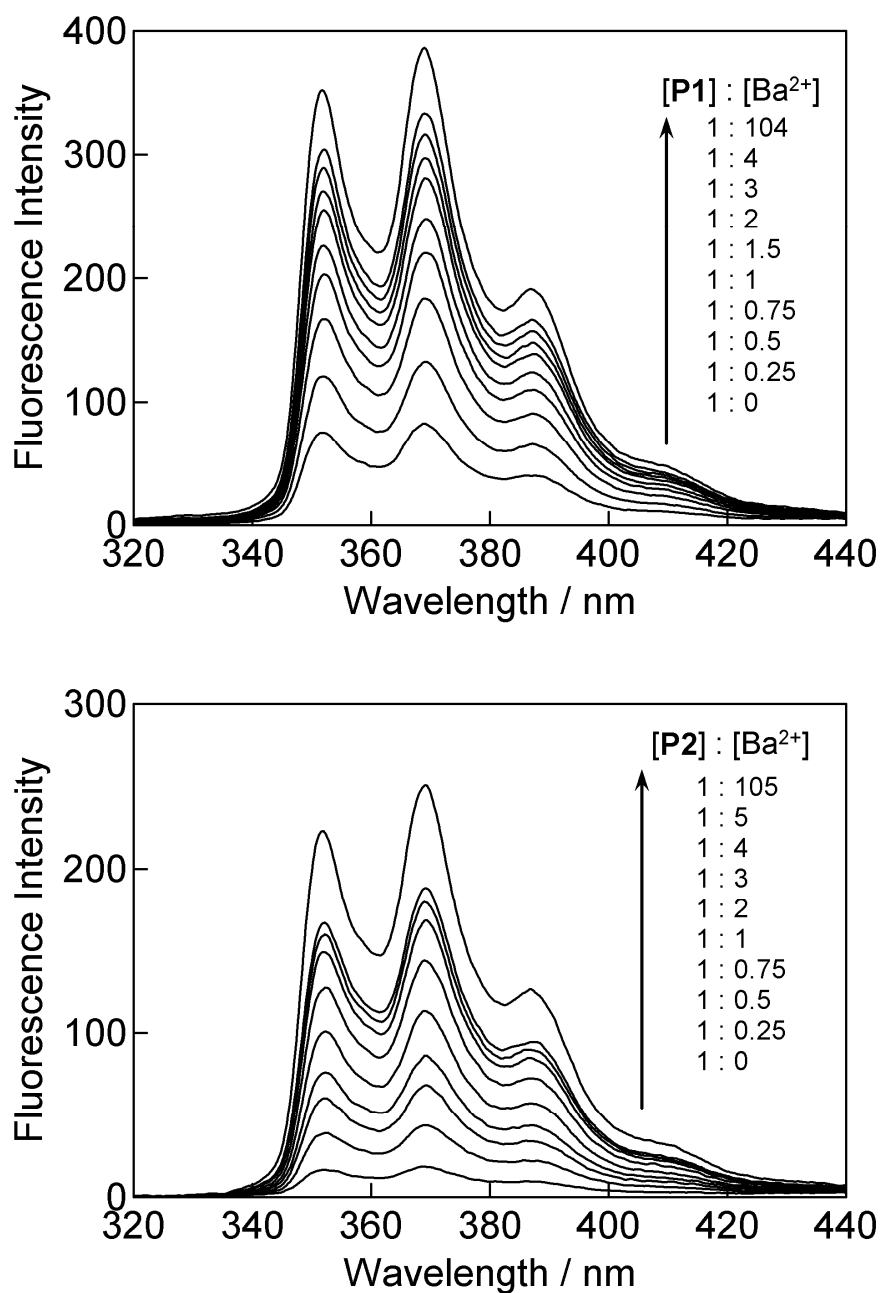


Figure 2.7. Fluorescence spectra of **P1** and its Ba^{2+} complex (upper), and fluorescence spectra of **P2** and its Ba^{2+} complex (lower); they were corrected by the absorbance at 297 nm, in acetonitrile at 25°C. Excitation wavelength, 297 nm. $[\text{P1}]$ and $[\text{P2}] = 5 \mu\text{M}$. The stoichiometric ratios with Ba^{2+} are listed respectively in columns.

*UV-vis and excitation spectra of **P1** and **P2***

Figure 2.8-2.11 shows UV spectra of crown ethers (**P1** and **P2**) with and without alkaline earth ions. Whether Ca^{2+} was present or not, the absorbance and shape of the spectrum between 254 and 297 nm corresponding to the phenanthrene moiety are hardly changed. The UV spectral change of **P1** with Ca^{2+} resembled that of **P2**. These results show that **P1** and **P2** did not have a charge-transfer interaction based on a large conformational change by complexation with Ca^{2+} in the ground state, and a quenching process took place via electron transfer. Model compounds 3'-(butaneamido)benzo-15-crown-5 (**C1**) and 4'-methyl-3'-(butaneamido)benzo-15-crown-5 (**C2**) is also measured as receptor moieties of **P1** and **P2**, were also measured. It was relatively shifted that spectra of **C1** before and after complexation with Ca^{2+} than that of **C2**, and these data was similar for those fluoroionophore in the region of 200 to 260 nm (Fig. 2.12). This result indicates that difference of I_{max} / I_0 between **P1** and **P2** were originated for the distinction of each transition moments (*i.e.* PET efficiency) in benzocrown moieties. Because, PET efficiency is directly impacted to HOMO-LUMO energy levels and a length between donor-acceptor. Figure 2.13 shows fluorescence excitation spectra of **P2** and its Ca^{2+} complex. After the addition of Ca^{2+} , the response of the phenanthrene ring at 297 nm is notably increased without a spectral shift; it is similar to the UV-vis spectra of **P2**. These results support the interpretation obtained from UV measurements.

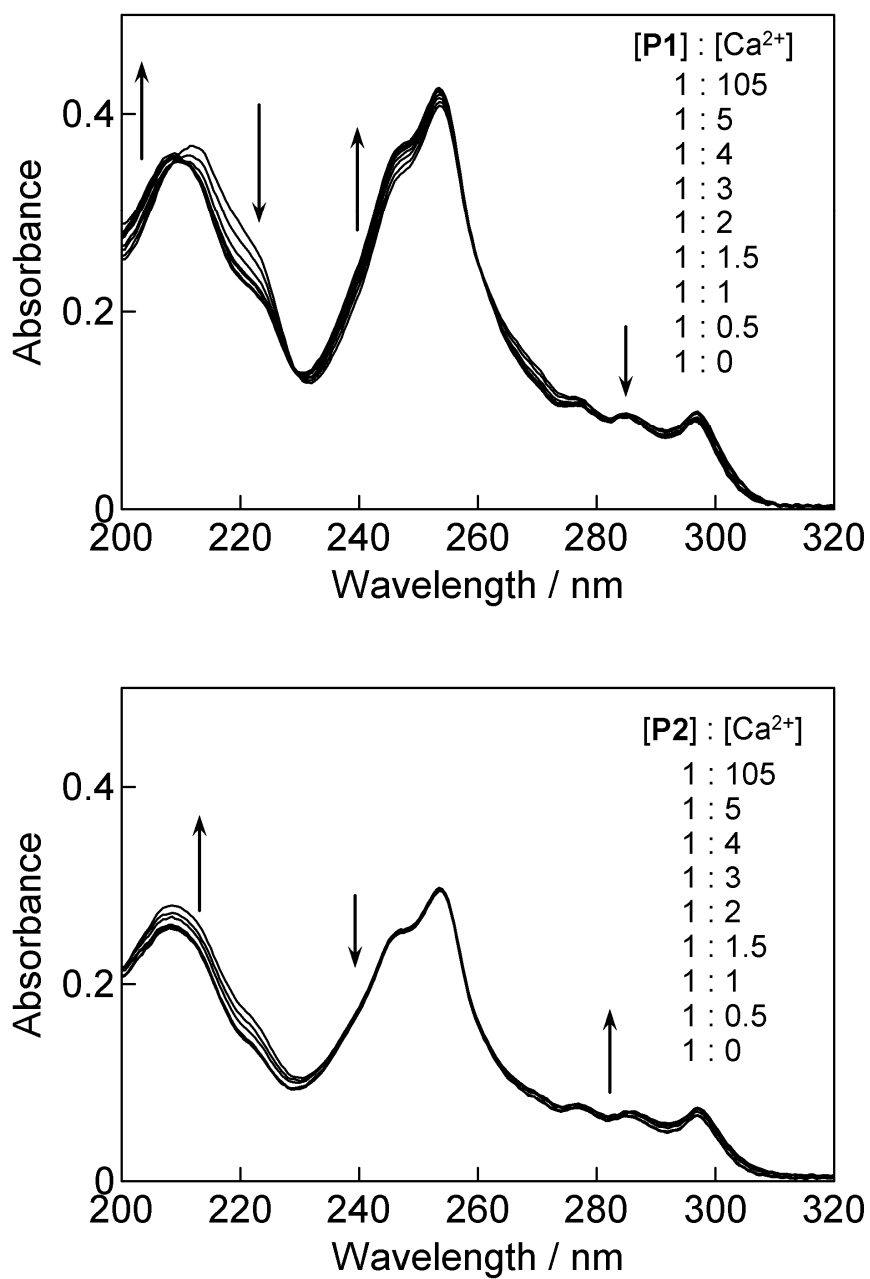


Figure 2.8. UV spectra spectra of **P1** and its Ca^{2+} complex (upper), and fluorescence spectra of **P2** and its Ca^{2+} complex (lower); Stoichiometric ratios of metal ion to those ligand are listed in columns.

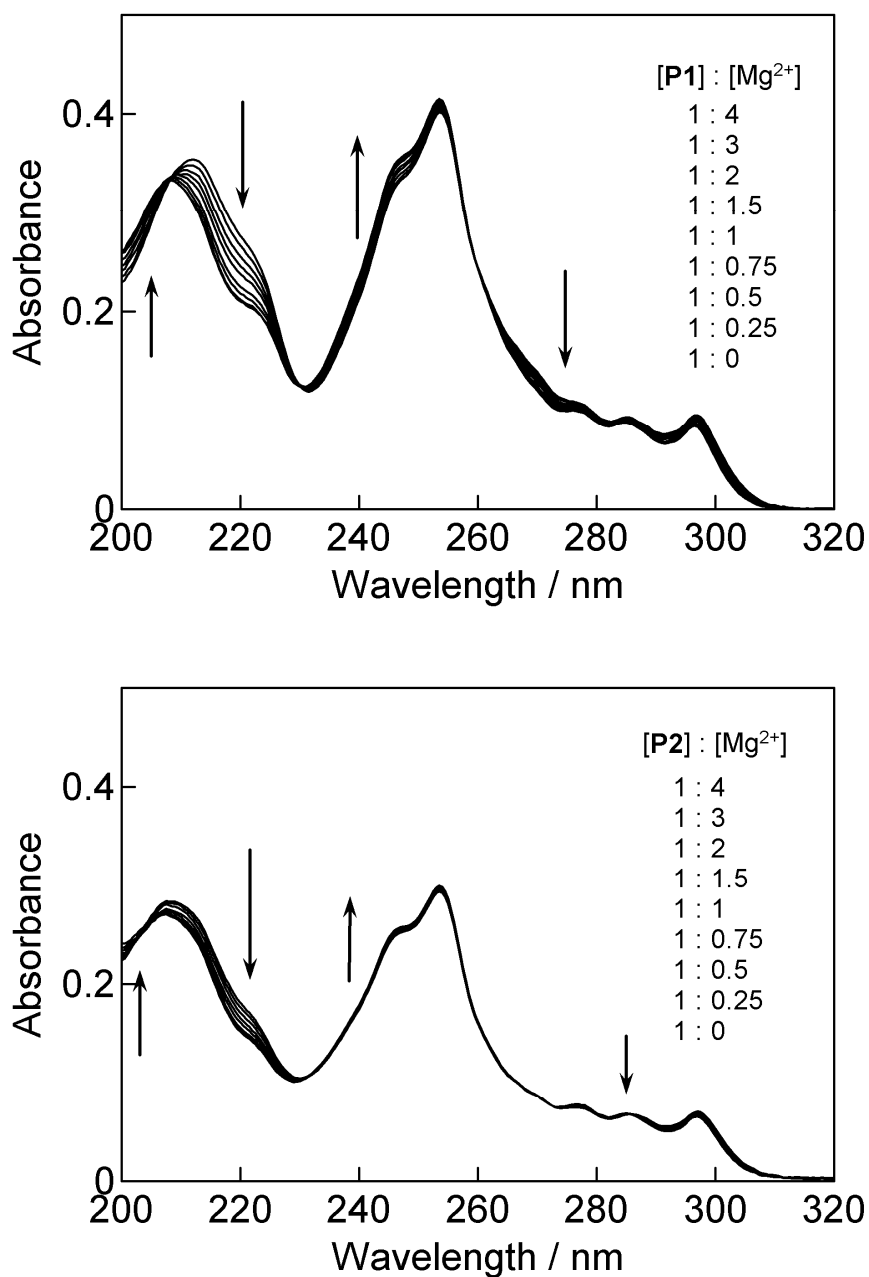


Figure 2.9. UV-vis spectra of **P1** and its Mg^{2+} complex (upper), and UV-vis spectra of **P2** and its Mg^{2+} complex (lower); they were corrected by the absorbance at 297 nm, in acetonitrile at 25°C. **[P1]** and **[P2]** = 5 μM . The stoichiometric ratios with Mg^{2+} are listed respectively in columns.

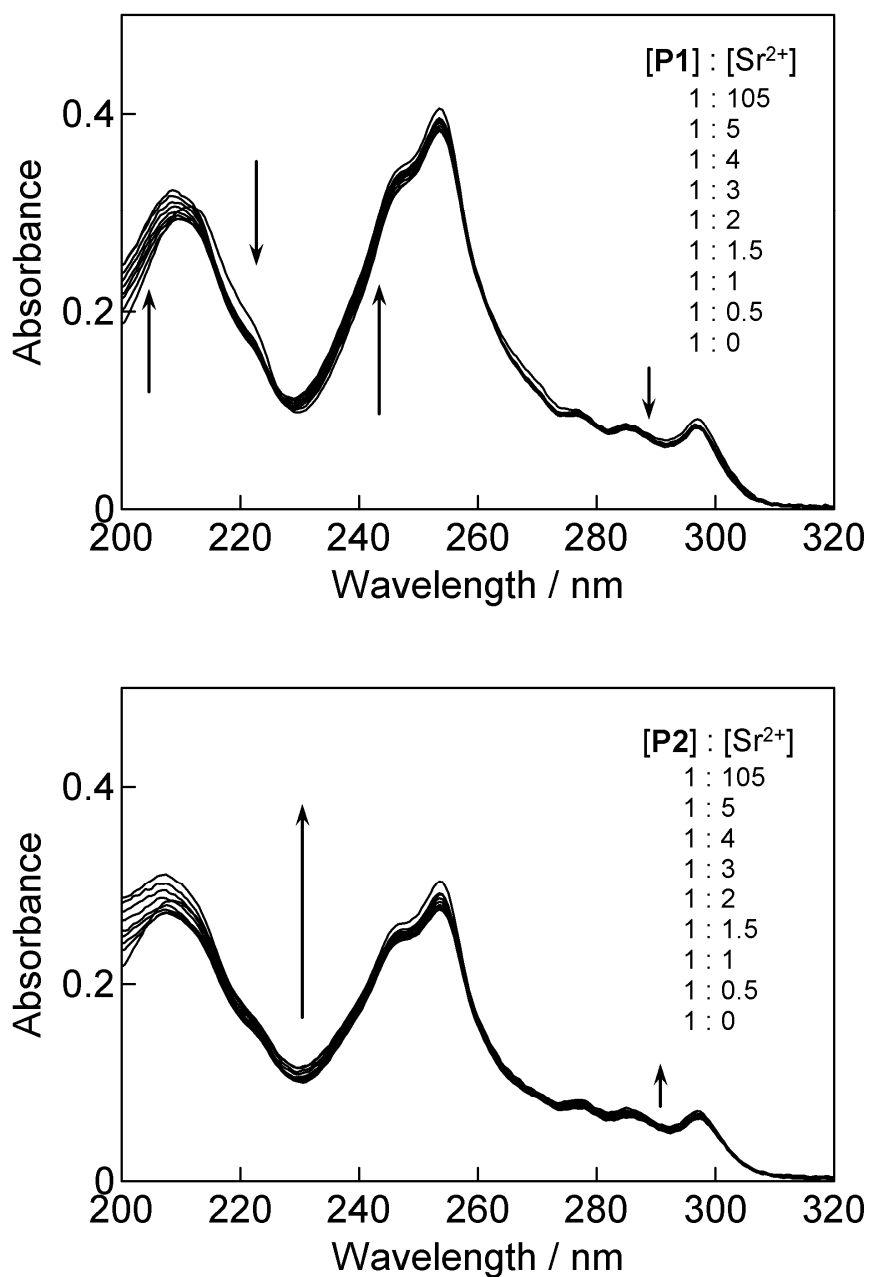


Figure 2.10. UV-vis spectra of **P1** and its Sr^{2+} complex (upper), and UV-vis spectra of **P2** and its Sr^{2+} complex (lower); they were corrected by the absorbance at 297 nm, in acetonitrile at 25 °C. [**P1**] and [**P2**] = 5 μM . The stoichiometric ratios with Sr^{2+} are listed respectively in columns.

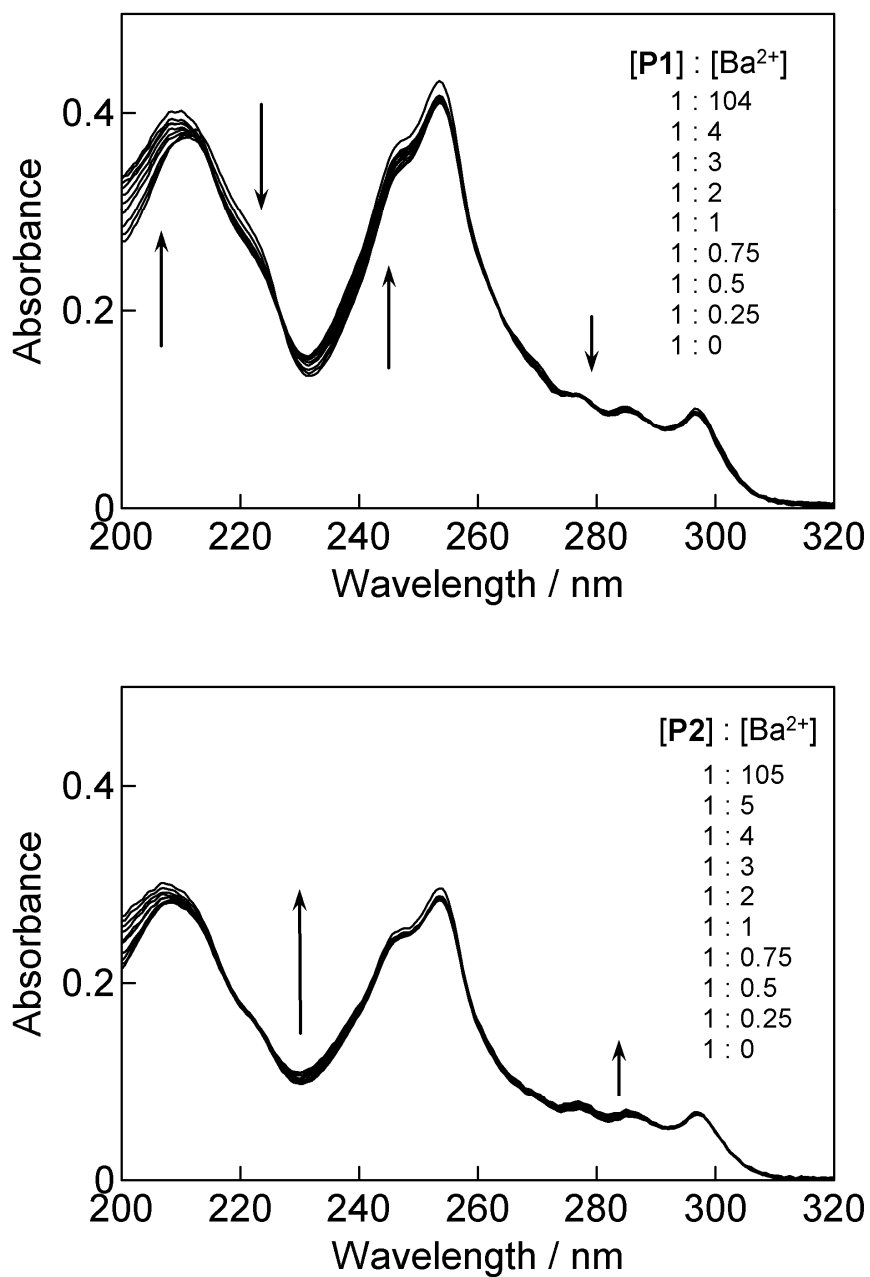


Figure 2.11. UV-vis spectra of **P1** and its Ba²⁺ complex (upper), and UV-vis spectra of **P2** and its Ba²⁺ complex (lower); they were corrected by the absorbance at 297 nm, in acetonitrile at 25 °C. [**P1**] and [**P2**] = 5 μM. The stoichiometric ratios with Ba²⁺ are listed respectively in columns.

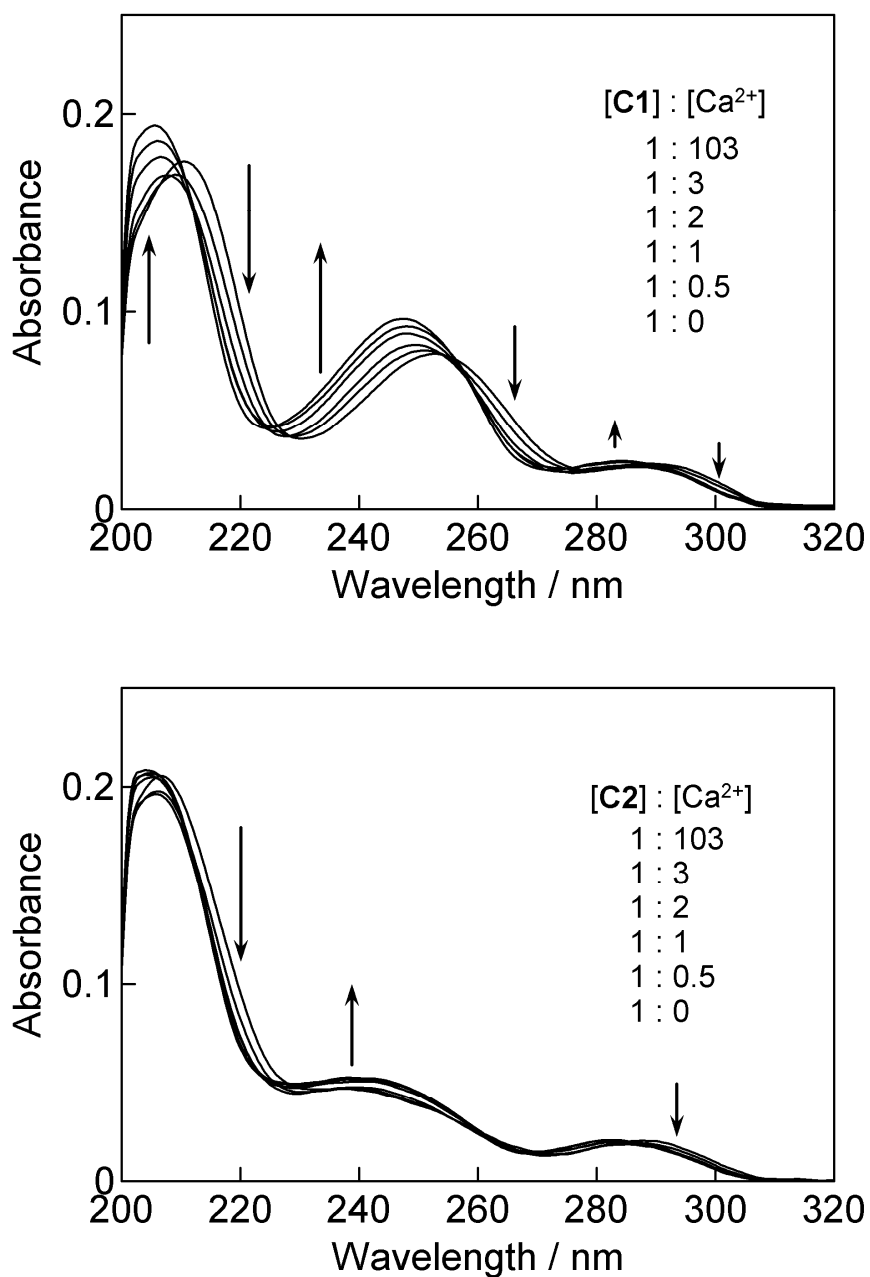


Figure 2.12. UV-vis spectra of **C1** and its Ca^{2+} complex (upper), and fluorescence spectra of **C2** and its Ca^{2+} complex (lower); they were corrected by the absorbance at 297 nm, in acetonitrile at 25 °C. $[\text{C1}]$ and $[\text{C2}] = 5 \mu\text{M}$ and stoichiometric ratios to Ca^{2+} are listed in columns.

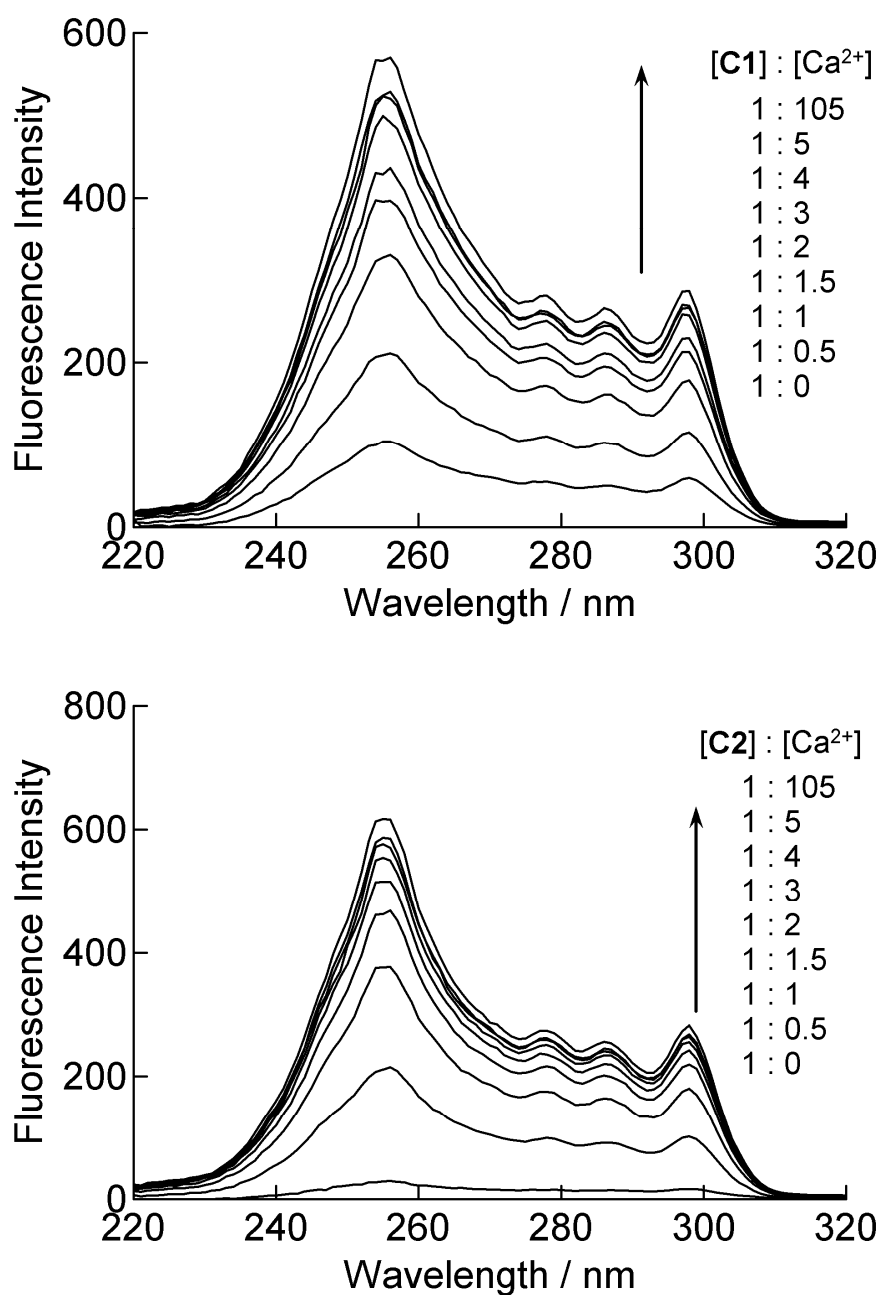


Figure 2.13. Excitation spectra of **C1** and its Ca^{2+} complex (upper), fluorescence spectra of **C2** and its Ca^{2+} complex (lower) in acetonitrile at 25 °C. Emission wavelength: 369 nm. $[\text{C1}]$ and $[\text{C2}] = 5 \mu\text{M}$ and stoichiometric ratios to Ca^{2+} are listed in columns.

¹H-NMR spectra of **P2**

The fluorescence “Off–On” ability of **P2** was enhanced by the modification of a methyl group to **P1**. ¹H-NMR spectra of **P2** were measured to analyze the conformational effect concerning those fluorescence behaviors in the absence and presence of various alkaline earth metal ions in acetonitrile-*d*₃. As the representative result, the comparison between before and after complexation with Ca²⁺ are presented in Fig 2.14; ¹H-¹H COSY and NOESY spectra were measured to respective peaks of **P2** (Fig. 2.15-2.16) and its complex (Fig. 2.17-2.18) for detailed analysis. Remarkable changes of low field chemical shift of **P2** were observed upon complexation with 10 mM of Ca²⁺ as follows: from 3.61 to 3.90 ($\Delta\delta=0.29$, a, b), from 3.74 to 3.97 ($\Delta\delta=0.23$, c), and from 4.00 to 4.30 ($\Delta\delta=0.30$, d) for crown ether moiety, from 6.69 to 6.98 ($\Delta\delta=0.29$, e), and from 7.01 to 7.32 ($\Delta\delta=0.31$, f) for benzene moiety, and from *ca.* 7.7 to 8.1 (*ca.* $\Delta\delta=0.4$, h) for amido proton (Table 2). These changes will be attributed to a coordinating interaction with positive ions which cause a lower field shifts, suggesting that the Ca²⁺ complex was formed cooperatively with the benzocrown moiety and a carbonyl group.

Their chemical shifts are presented in Table 2 for various alkaline earth metal ions. To clear the role of carbonyl group, model compounds **C1** and **C2** were also performed in the presence of four alkaline earth metal ions (Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺). NH protons neighboring to carbonyl group were dramatically altered to the range of 0.23-1.46 ppm as same or more than crown protons involving coordination ability for all ions (Table 3 and Fig 2.19). Thus, it is evident that complexation of any fluoroionophores with any ions rearranged divalent cations to the carbonyl group for causing PET suppressing. This complexation can restrict molecular motion around the amide group and a benzene ring. No protons in the phenanthrene ring indicated a considerable chemical shift change upon complexation with any alkaline earth metal ions. This result demonstrates that these protons did not receive an electron or ring-current effect upon complexation (Fig. 2.20).

On the other hands, when Mg²⁺ and Ba²⁺ were added, detailed ¹H-NMR spectra arrangements of the complex with these ions could not be obtained because of the complicated and poorly resolved spectra (Fig. 2.21). It is assumed that these phenomena resulted from differences of the complexation behaviors based on the size of the crown ether ring and an alkaline earth metal ion radius.

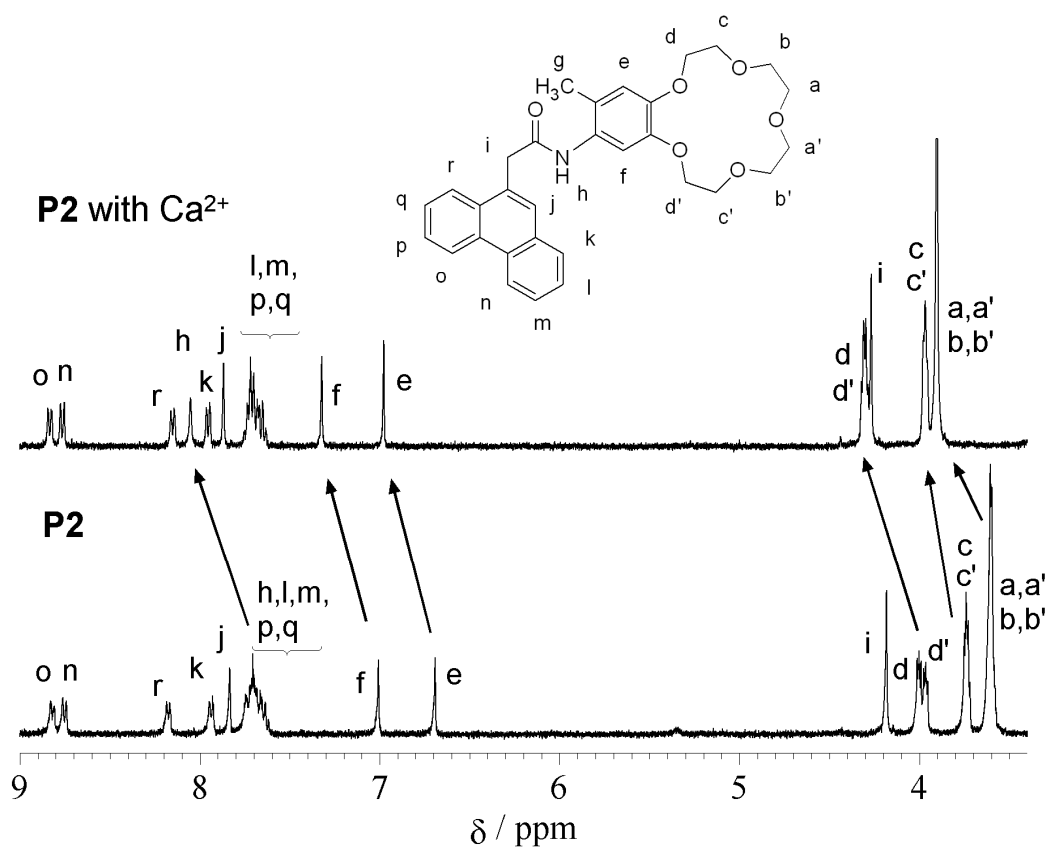


Figure 2.14. ¹H-NMR spectra of **P2** (lower), and its Ca²⁺ complex (upper) in acetonitrile-*d*₃ at 30°C. [**P2**] = 5 mM and [Ca²⁺] = 50 mM.

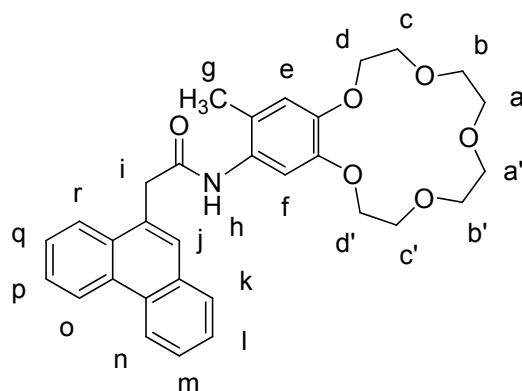
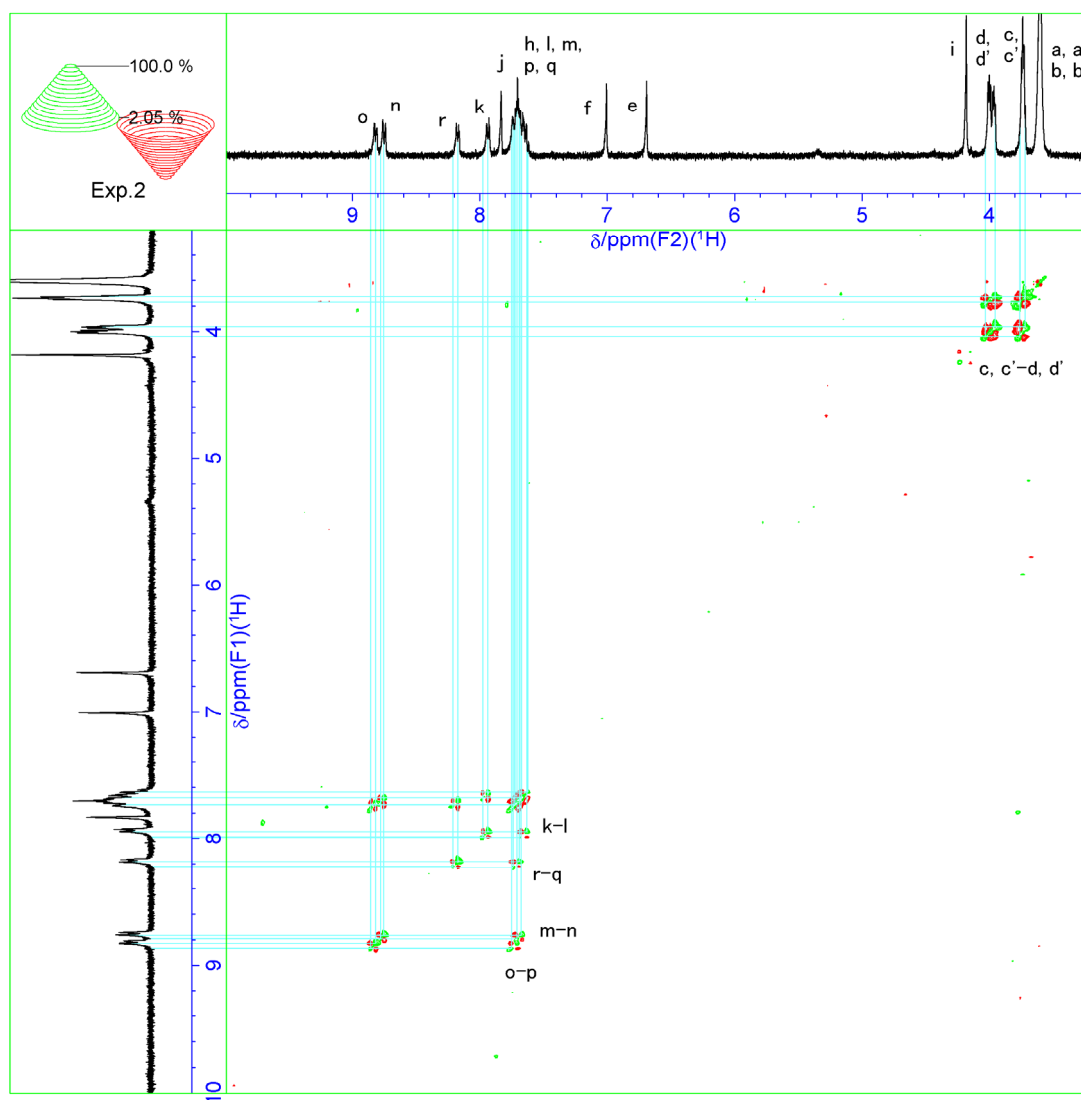


Figure 2.15. ^1H - ^1H COSY spectra of **P2** in acetonitrile- d_3 at 30°C . [**P2**] = 2 mM.

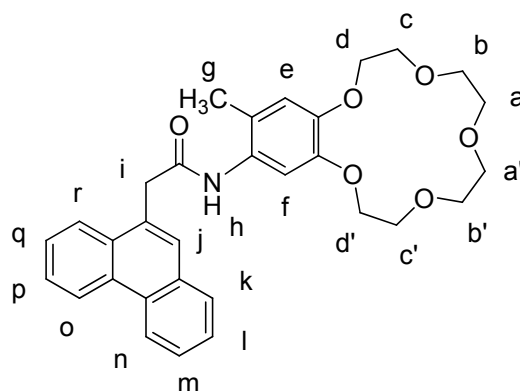
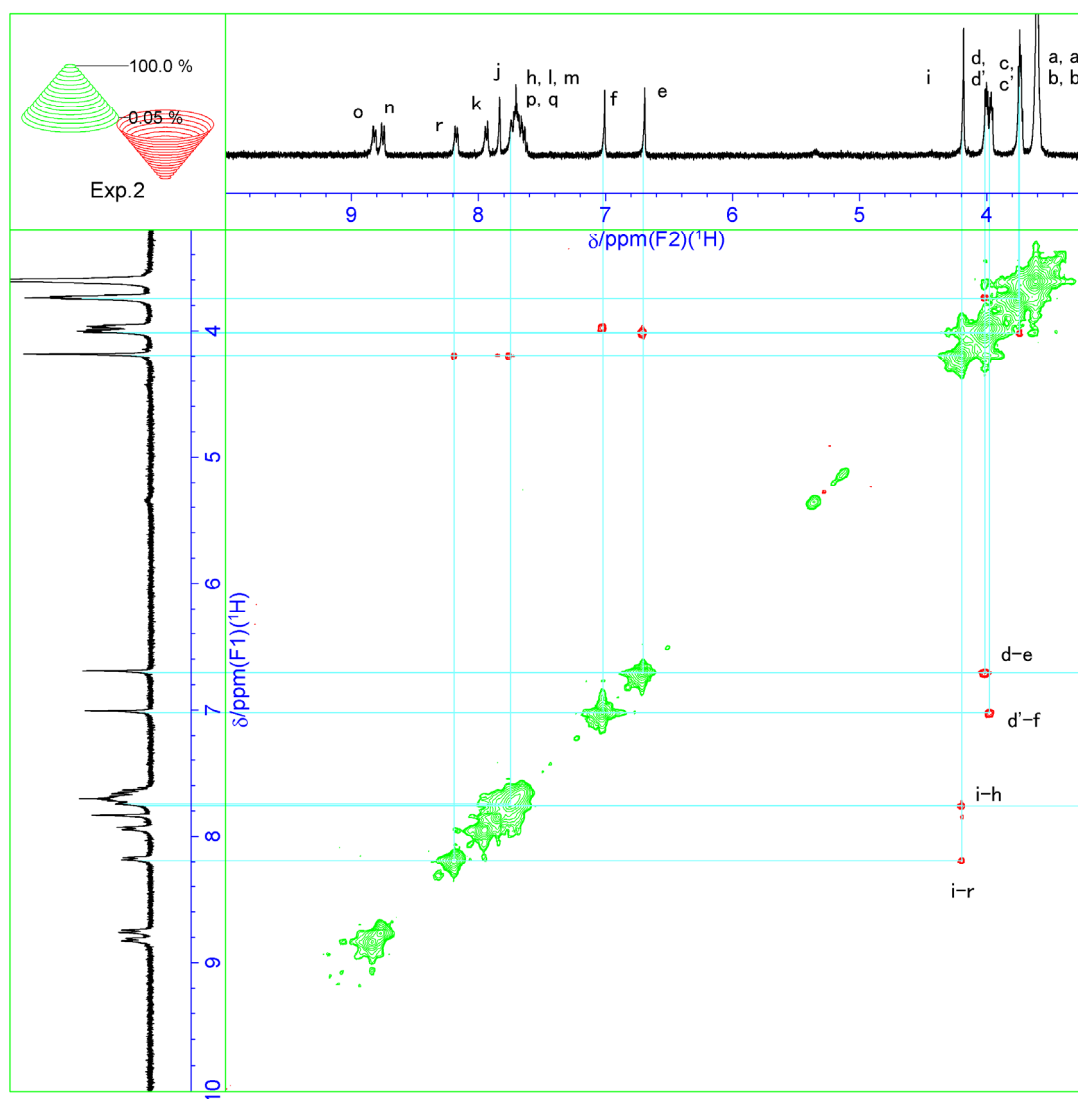


Figure 2.16. ^1H - ^1H NOESY spectra of **P2** in acetonitrile- d_3 at 30°C . [**P2**] = 2 mM.

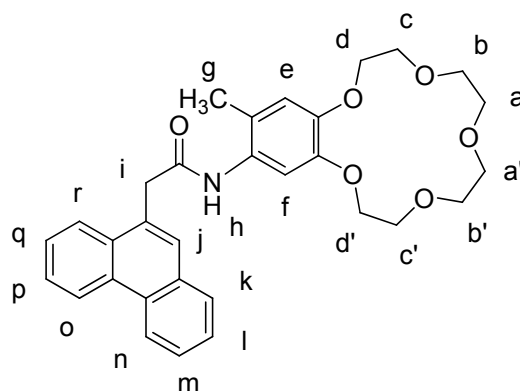
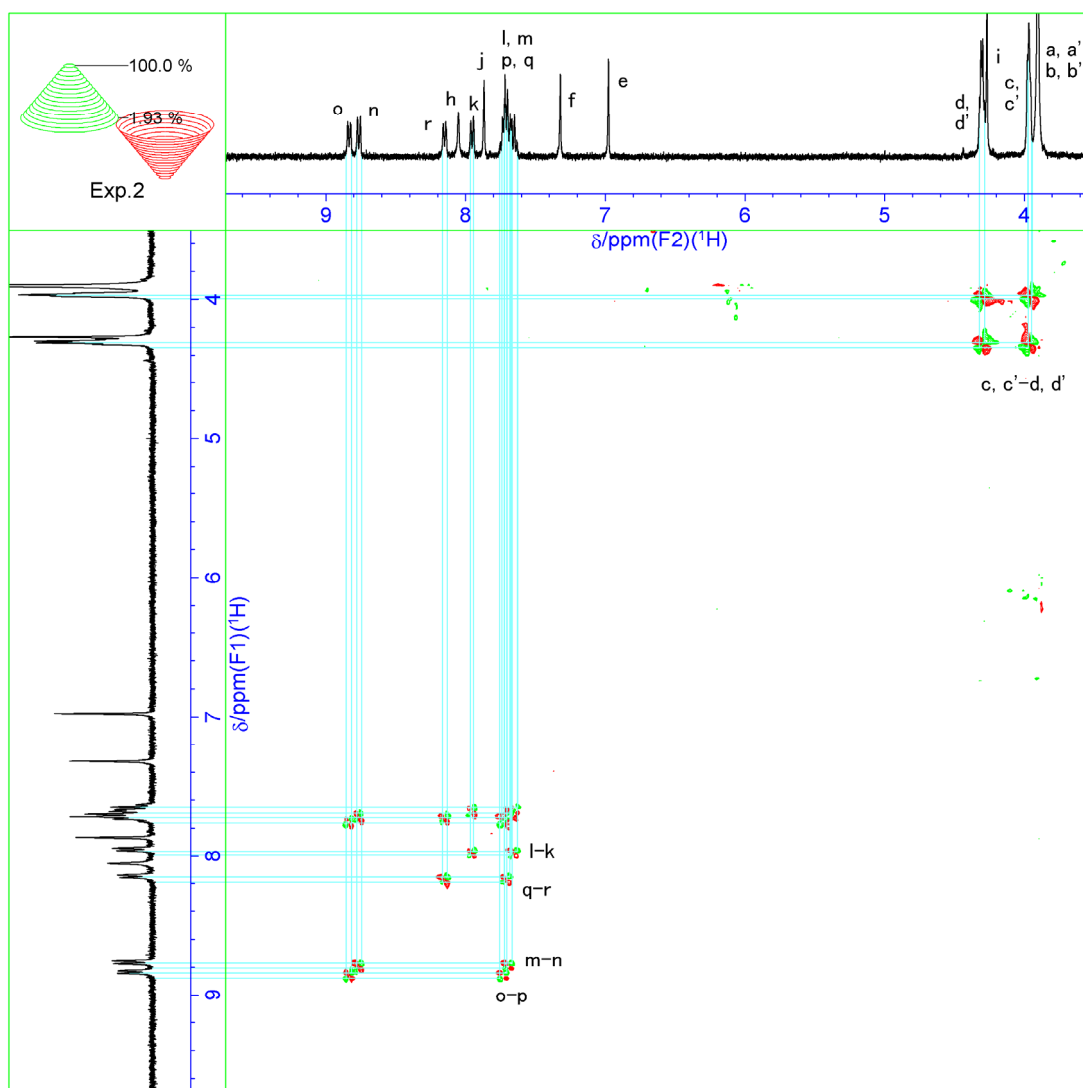


Figure 2.17. ^1H - ^1H COSY spectra of **P2** upon complexation with Ca^{2+} (upper) in acetonitrile- d_3 at 30°C . [**P2**] = 5 mM and [Ca^{2+}] = 50 mM.

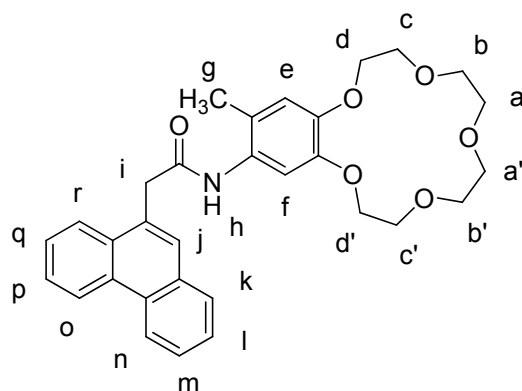
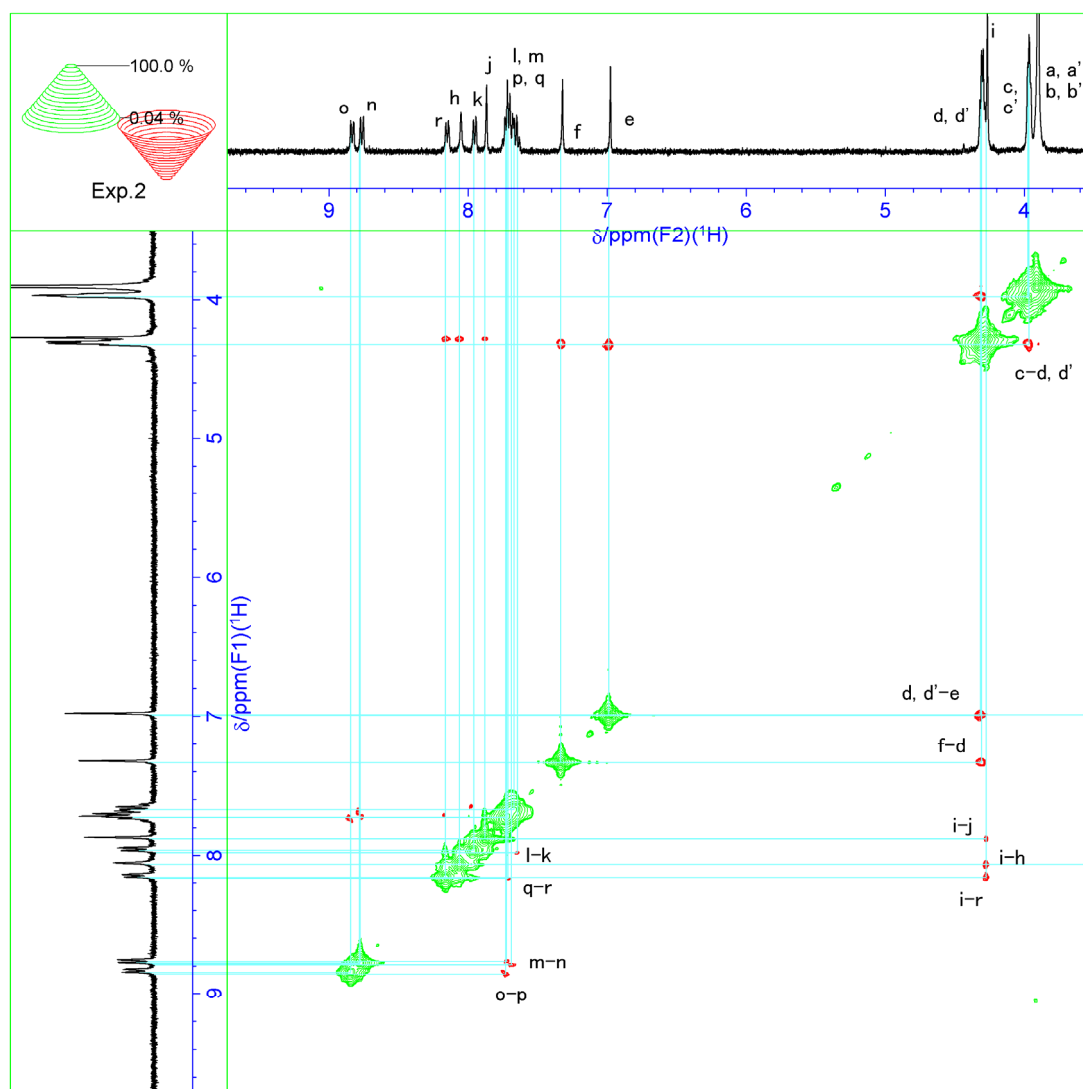


Figure 2.18. ^1H - ^1H COSY spectra of **P2** upon complexation with Ca^{2+} in acetonitrile- d_3 at 30 °C. [**P2**] = 5 mM and [Ca^{2+}] = 50 mM.

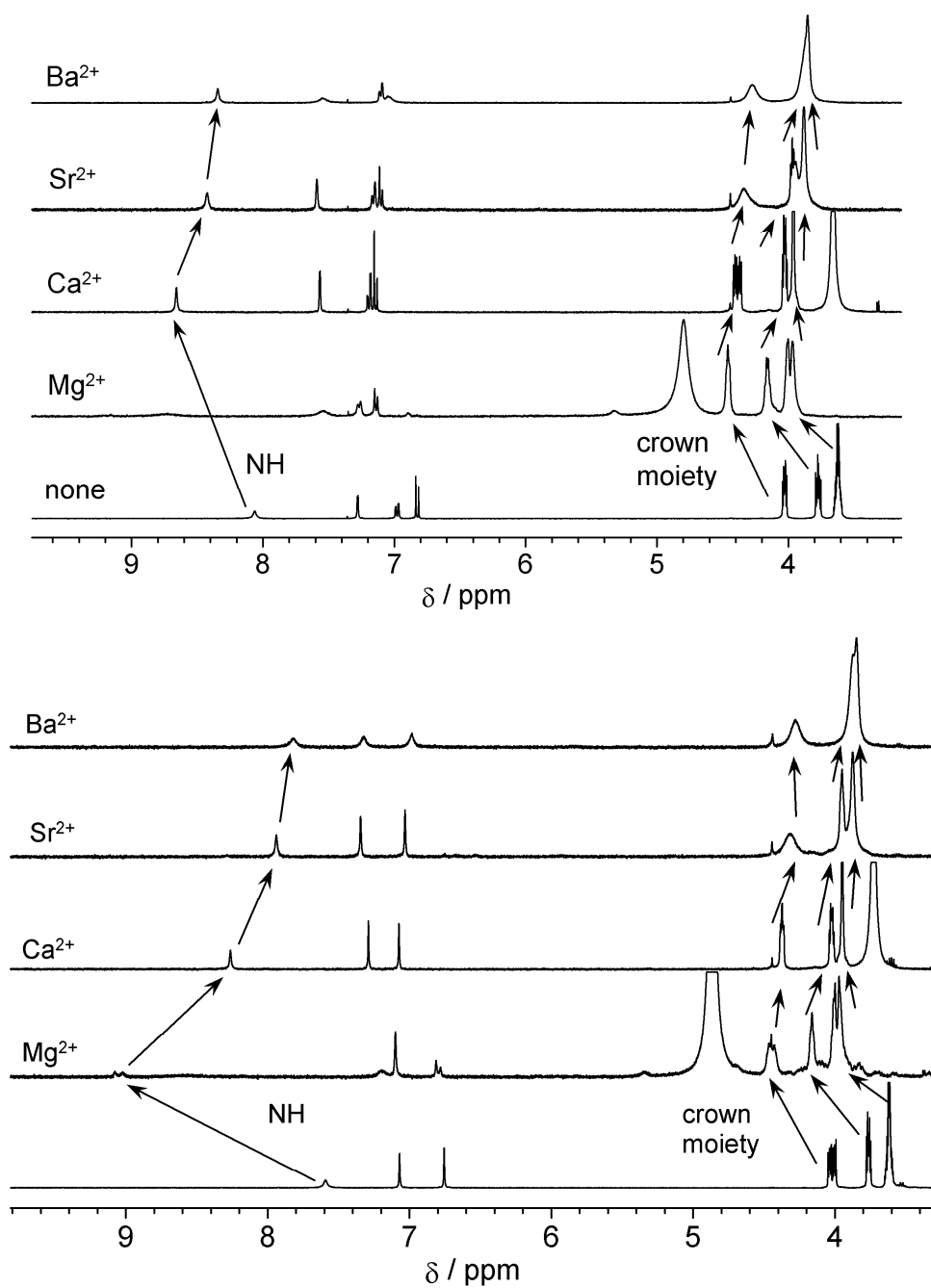


Figure 2.19. ^1H -NMR spectra of **C1** (upper) and **C2** (lower) in the absence and presence of alkaline earth metal ions in acetonitrile- d_3 at 30°C . $[\text{C1}] = [\text{C2}] = 5 \text{ mM}$ and $[\text{Ca}^{2+}] = 50 \text{ mM}$.

Table 2.2. Main chemical shifts (δ ppm) for **P2** and their changes ($\Delta\delta$ ppm) upon complexation with various alkaline earth metal ions^a

	a, a'	b, b'	c, c'	d,	d'	e	f	g ^b	h ^b	i
None	3.61	3.61	3.74	4.00,	3.97	6.69	7.01	1.9	7.7	4.18
Mg ²⁺	0.35	0.35	0.39 ^b	0.39 ^b	0.42 ^b	0.11 ^b	0.0 ^b	0.1	— ^c	0.14 ^b
Ca ²⁺	0.29	0.29	0.23	0.30	0.33	0.29	0.31	0.1	0.4	0.12
Sr ²⁺	0.24	0.24	0.18	0.27 ^b	0.30 ^b	0.25	0.27	0.0	0.3	0.09
Ba ²⁺	0.21	0.21	0.08 ^b	0.22 ^b	0.25 ^b	0.22	0.24	0.0	— ^c	0.04 ^b

^a Alphabets on respective peaks correspond to hydrogens below structure. 2.1. Positive values show lower field shifts. The values in “none” denote chemical shifts (δ ppm from TMS) of protons of **P2** in acetonitrile-*d*₃ at 30°C.

^b This value includes uncertainty because of overlapping with the peak with acetonitrile, water, or other peaks.

^c This value was not assigned because of peak overlapping or broadening.

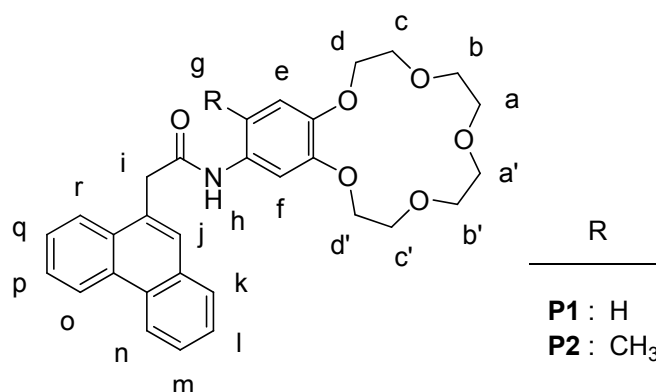


Table 2.3. Lower chemical shift changes of N-H protons in **C1** and **C2** upon complexation with various alkaline earth metal ions.

	none ^a	Mg ²⁺	Ca ²⁺	Sr ²⁺	Ba ²⁺
C1	8.06	— ^b	0.60	0.37	0.28
C2	7.59	1.46	0.67	0.35	0.23

^a Chemical shift values (δ ppm from TMS)

^b This value was not assigned because of peak overlapping or broadening.

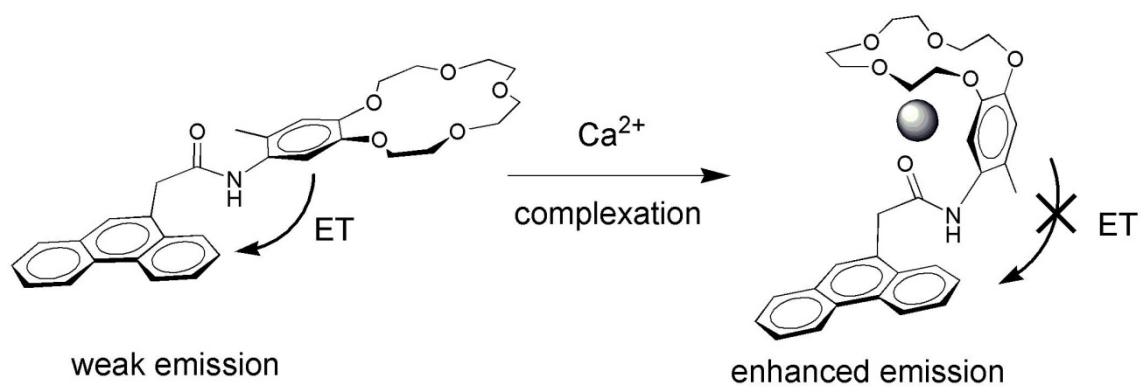


Figure 2.20. Schematic representation of complexation between **P2** and Ca^{2+} involving a conformational change.

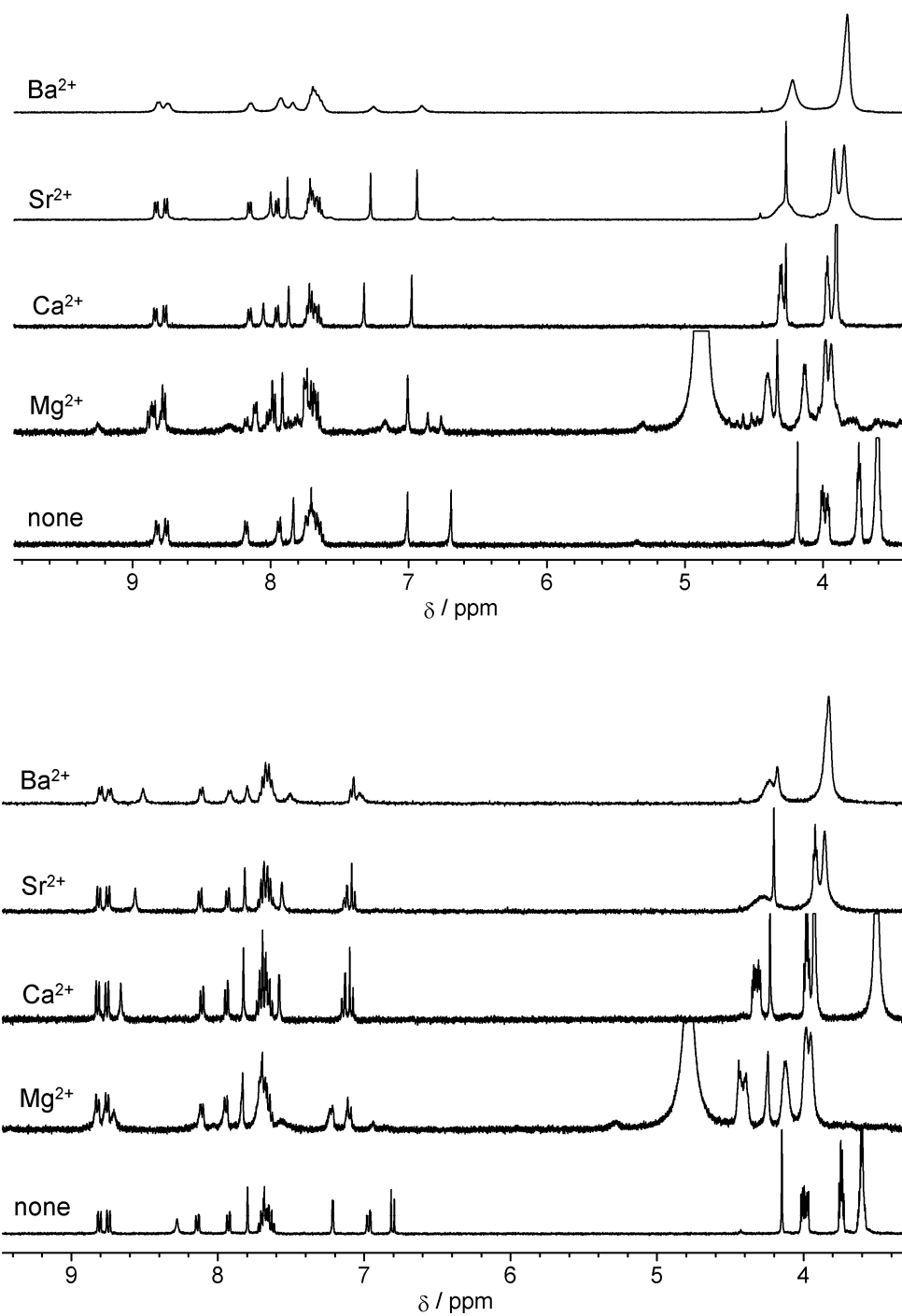


Figure 2.21. ^1H -NMR spectra of **P1** (lower) and **P2** (upper) in the absence and presence of alkaline earth metal ions in acetonitrile- d_3 at 30°C . $[\text{P2}] = 5 \text{ mM}$ and $[\text{Ca}^{2+}] = 50 \text{ mM}$.

Complex formation constants

The complex formation constant (K), which is useful to estimate the binding affinities of the crown ether moiety with various alkaline earth metal ions, is defined as

$$K = \frac{[ML]}{[M][L]}.$$

Therein, $[M]$, $[L]$, and $[ML]$ signify the concentrations of the alkaline earth metal ion, crown ether, and complex, respectively. Then, the values of $\log K$ were determined using a curve-fitting method of a nonlinear least-squares curve fitting (Marquardt's method³⁸). The fluorescence intensities of **P1** and **P2** at 369 nm against the metal to ligand ratio are shown in Fig. 2.22. These plots show that **P1** and **P2** formed a complex with alkaline earth metal ion corresponding to 1 : 1 stoichiometry. Consequently, the obtained complex formation constants for alkaline earth metal ions are presented in Table 4 and Fig. 2.22-2.23. In our previous study, all values of $\log K$ showed a similarity to crown ether compounds.¹² Consequently, all guest species were suitable for fluorescence “Off–On” controlling because of a high response to the benzo-15-crown-5 moiety.

Table 2.4. Complex formation constants ($\log K$)^a of **P1** and **P2** for various metal ions in acetonitrile at 25°C

		Mg ²⁺	Ca ²⁺	Sr ²⁺	Ba ²⁺
log K	P1	6.30	6.36	5.82	5.74
	P2	6.58	6.29	5.68	5.30

^a $K = [\text{complex}] / ([\text{P1 or P2}][\text{guest}])$. The standard deviation of the log K was estimated to be ± 0.05 .

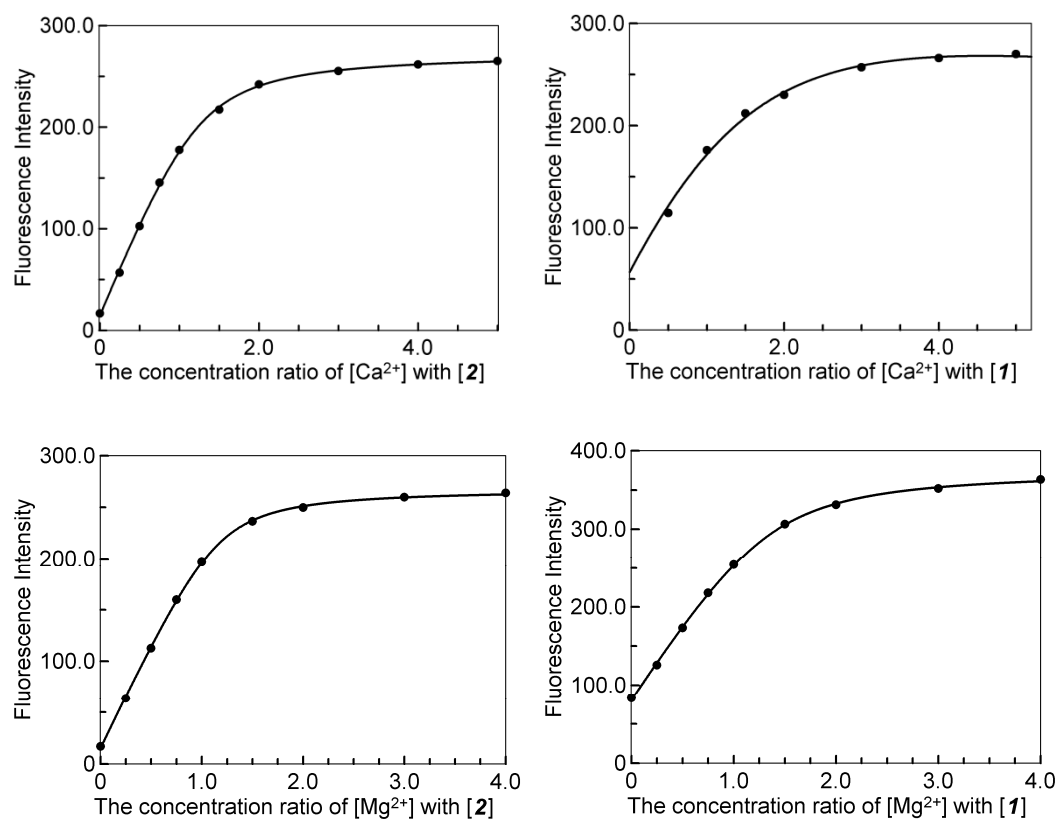


Figure 2.22. Dependence of fluorescence intensity at 369 nm on the concentration of Ca^{2+} and Mg^{2+} , and theoretical curve of stoichiometric 1 : 1 complex. [P1] and [P2] = 5 μM .

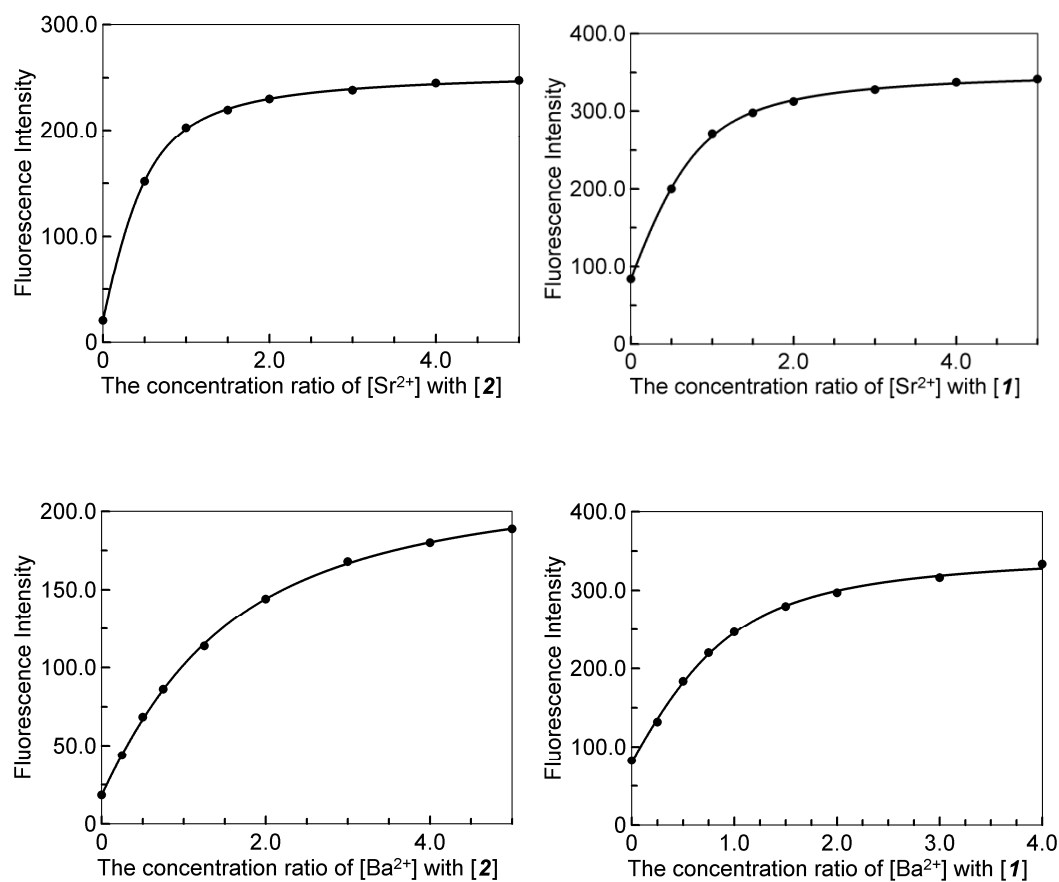


Figure 2.23. Dependence of fluorescence intensity at 369 nm on the concentration of Sr²⁺ and Ba²⁺, and theoretical curve of stoichiometric 1 : 1 complex. [P1] and [P2] = 5 μ M.

Conclusion

Using new fluoroionophores **P1** and **P2**, a fluorescence “Off–On” intramolecular charge-transfer system was produced. The photochemical response (I_{max}/I_0) of **P2**, which has an electron-donating substituent on the phenyl moiety, was increased compared to **P1**. This indicates that the introduction of a methyl group that has an electron-donating character at the 3'-position of the benzocrown ring induced an effective electron transfer (ET) action. On the other hand, a complex formation constant, $\log K$, and the conformations of the complex were similar to those of previous fluoroionophores. Therefore, the fluorescence “Off–On” ability was improved without disturbing the complexation with crown ether. These results indicate that the introduction of a suitable electron-donating group to benzocrown ethers makes a new or improved fluoroionophore available in various chemical situations. This study will be expanded to include possibilities for the development of a new optical analysis method and chemical signal devices for biological materials *in vivo* and *in vitro*.

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Chapter 3

TritonX-100 Selective Chemosensor Based on β -Cyclodextrin Modified by Anthracene Derivative

Introduction

The detection of nonionic surfactant (NS) have been investigated as the most important topics in environmental chemistry. These NS families are regarded as environmental pollutants due to ecological toxicity of those materials¹ and their biodegradation intermediate.² Existing useful analytical methods for NS were combined with extraction,³ HPLC⁴ and mass spectrometry.⁵ However, these analytical techniques include many steps for pretreatment and require expensive instruments. Therefore, simple and rapid detection method for NS in wastewater is desired to establish. Fluorescence detection of environmental material is largely investigated in chemical and biological studies⁶. Especially, cyclodextrin (CyD) modified by a fluorescent substituent can give a useful analytical method for a rapid and reasonable detection.⁷ Ueno *et al.* reported that fluorescence intensity of fluorescent moiety in CyD based chemosensor was decreased with the addition of target materials, which was caused by displacement of fluorescent moiety from the inside to the outside of the CyD cavity.⁸ The fluorescence change was explained by twisted intramolecular charge transfer (TICT) quenching process which is prevented in the CyD cavity.

Recently, we reported that *N*-phenyl-(9-anthracenecarboxamido)phenyl (9-PAA) compounds showed fluorescence quenching by TICT.⁹ We have also developed new chemosensors based on linear polyether and crown ethers bearing those TICT detection moieties and other fluorescence quenching mechanisms such as photoinduced electron transfer (PET).^{10, 11} In the absence of guest ions, these sensors showed weak emission as a result of TICT, although the complexation with guest ions enhanced their emission strongly. These result showed that twisted motion at an excited state can be controlled by the steric hindrance upon a molecular recognition event. To extend our research to new application, we synthesized novel fluorescent cyclodextrin modified by anthracene. It is expected that cyclodextrin and NS will form a pseudoxane type supramolecular structure in which a part of NS protrudes from the CyD cavity. If the protruding part becomes a barrier of the twisting motion at the anthracene ring in the excited state, 9-PAA moiety in the supramolecule will show fluorescence “Off-On” response (Fig. 3.1). The “Off-On” response have an advantage compared with “On-Off” type, since “Off-On” response have capability to improve signal to noise ratio due to no limitation of fluorescence intensity against the silent background, whereas maximum response of “On-Off” type is equal to the initial fluorescence intensity.

In this paper, we report a new “Off-On” probe bearing the TICT moiety, which is a new type of fluorescence-enhancing CyD sensor based on TICT process at 9-PAA

moiety (scheme 3.1). That makes a pseudorotaxane with long structured molecules such as Triton X-100 (TX-100) used as guest molecules. We also consider that fluorescence moiety of this CyD sensor will remain outside CyD cavity in the presence and absence of target materials, the characteristics enables a selective fluorescence response for a specifically guest. These considerations will be clearly different from Ueno's work concept.

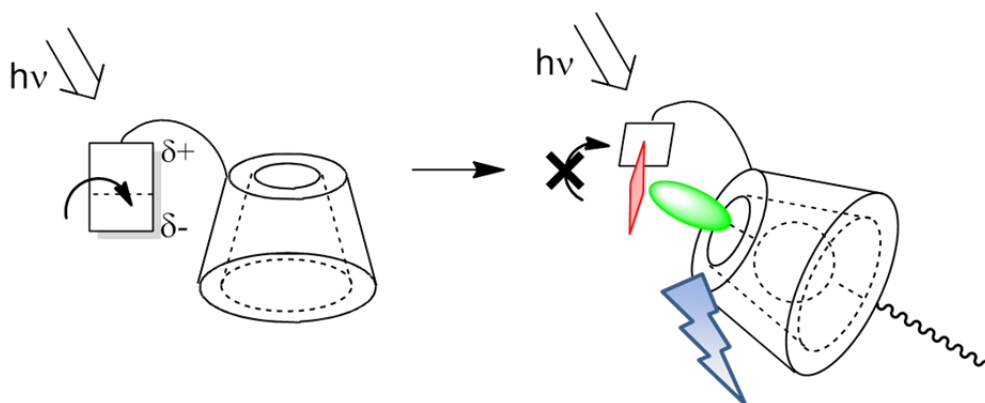
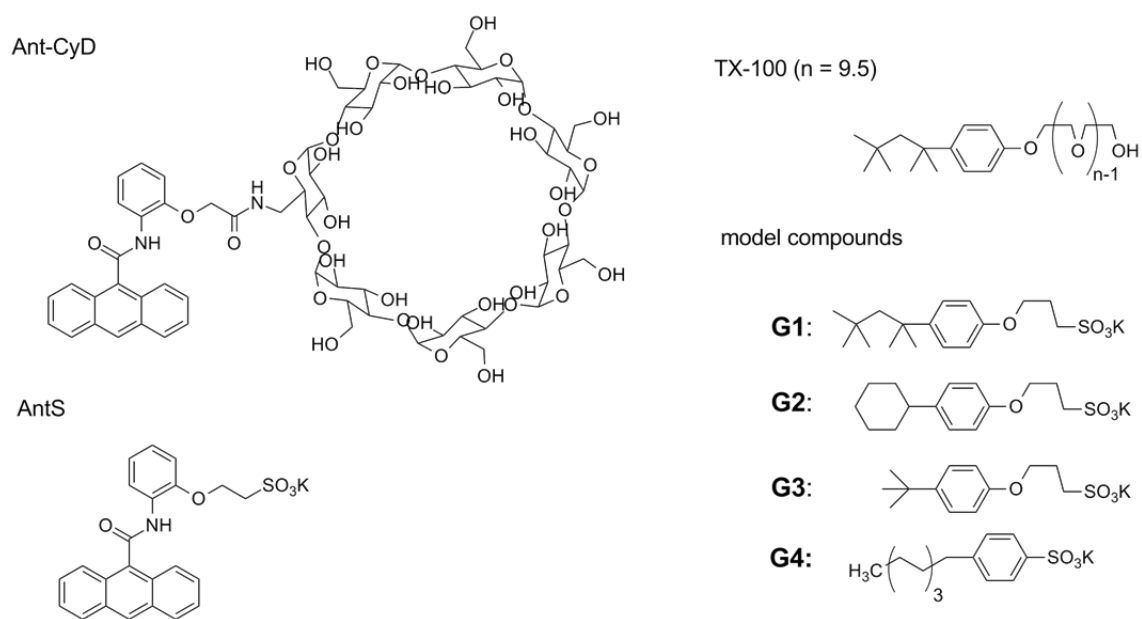


Figure 3.1. Fluorescence switching by TICT inhibiting process outside CyD cavity in the presence of target material.



Scheme 3.1.

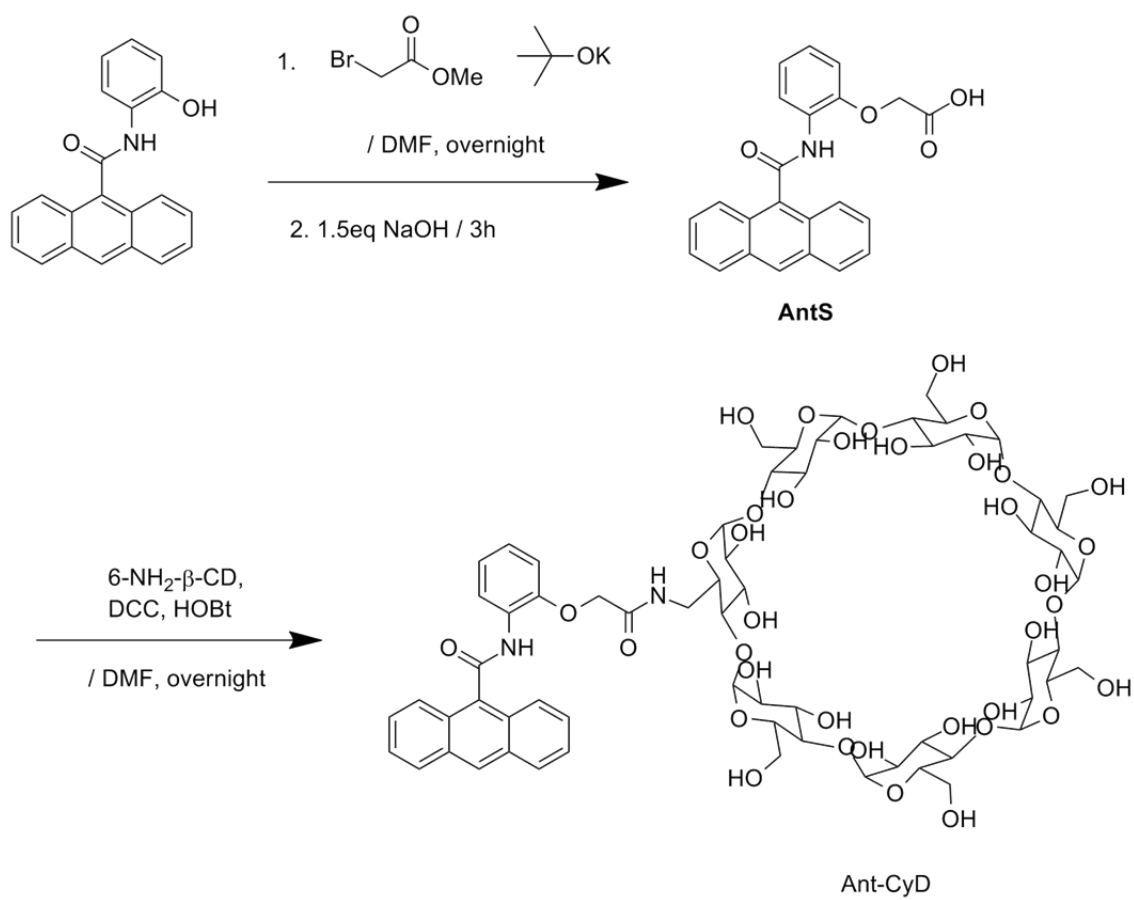
Experimental

Preparation of 2-(9-anthracenecarboxamido)phenoxyacetic acid (AntS)

3.13 g (0.01 mol) of *N*-(2-hydroxyphenyl)-9-anthracenecarboxamide¹¹, 1.67 g (0.01 mol) of ethyl 2-bromoacetate and 1.23 g (0.011 mol) of potassium *tert*-butoxide were dissolved in 60 ml of DMF and stirred overnight at 95 °C. After precipitate was filtered off, the reaction mixture was evaporated under reduced pressure to the half volume. The solution was mixed with 30 mL of EtOH; then added 60 mL of NaOH containing 0.06 g (0.015 mol) aqueous solution and stirred for 3 h. After neutralization, the precipitate was collected by filtration, and recrystallized from EtOH (Scheme 2). Yield: 2.15 g (58%). Yellow solid. ¹H-NMR (DMSO-*d*₆; δ from TMS) 4.74 (-CH₂-, s, 2H), 7.11 (aromatic, m, 2H), 7.24 (aromatic, t, 1H), 7.59 (aromatic, m, 4H), 8.01 (aromatic, dd, 1H), 8.15 (aromatic, d, 2H), 8.20 (aromatic, d, 2H), 8.70 (aromatic, s, 1H), 10.10 (-COOH, s, 1H).

Preparation of Ant-CyD

0.093 g (0.25 mmol) of AntC, 0.28 g (0.25 mmol) of mono-6-deoxy-6-amino- β -cyclodextrin (6-NH₂- β -CyD), 0.038 g (0.28 mmol) of 1-hydroxybenzotriazole (HOBt) and 0.058 g (0.28 mmol) of *N,N'*-dicyclohexylcarbodiimide (DCC) were dissolved in 10 mL DMF and stirred for 1 day at r. t. After the precipitate was removed by filtration, the filtrate was poured into acetone (50 mL) and the precipitate was collected. This crude compound (0.3 g) was purified by HPLC with an ODS column (eluent: MeOH : H₂O = 1 : 1) and dried *in vacuo* for 12 h at 90 °C. Yields: 0.10 g (27%). White solid. ¹H-NMR (DMSO-*d*₆; δ from TMS), 2.80–3.75 (excluded overlap with H₂O region, br, ca. 32H), 4.27–4.33 (m, 2H), 4.41 (m, 4H), 4.57 (m, 2H), 4.71 (m, 2H), 4.79 (m, 2H), 4.82 (m, 3H), 7.09 (aromatic, t, 2H), 5.65 (-OH, m, 14H), 7.22 (aromatic, d, 1H), 7.57 (aromatic, d, 4H), 7.89 (aromatic, d, 1H), 7.94 (-CONH-, s, 1H), 8.15 (aromatic, d, 4H), 8.71 (aromatic, s, 1H), 10.39 (-CONH-, s, 1H). Found: C, 49.80 %; H, 6.04 %; N, 1.75 %; Calcd. for C₈₅H₉₄N₂O₄₁•4H₂O: C, 50.06 %; H, 6.08 %; N, 1.80 %.



Scheme 3.2.

Spectrometric Measurement

TX-100 was obtained from Wako Pure Chemical Industries, Ltd. and used without further purification. Fluorescence spectra were measured using a RF-5300PC (Shimadzu Corp.) spectrometer in distilled water at 25 °C. The UV spectra were measured by UV-2400PC (Shimadzu Corp.). The excitation wavelength was set to 363 nm, unless described otherwise. The initial concentration of **Ant-CyD** derivatives was 5 μ M. TX-100 were added to a solution of **Ant-CyD** as 1 mM of aqueous solution.

The ^1H -NMR spectra for investigation of the complexation behavior between **Ant-CyD** and TX-100 were measured at 30°C by JNM-EX400 (JEOL). The **Ant-CyD** concentrations were 1 mM in D_2O .

Result and Discussion

Fluorescence and UV-vis spectra

Figure 3.2 portrays fluorescence and UV-vis spectra of **Ant-CyD** in the absence and presence of TX-100 in water whose concentration was less than the critical micelle concentration (CMC: 0.2 mM).¹² Fluorescence intensity from the anthracene moiety was weak in the absence of TX-100. In contrast, dramatically enhanced emission was observed by the addition of TX-100. Potassium 3-(2-(anthracene-9-carboxamido)phenoxy)propane-1-sulfonate (AntS), a model compound for **Ant-CyD**, showed slightly enhanced emission (Table 3.1). This enhancement was attributed to the complex formation of **Ant-CyD** with TX-100. The **Ant-CyD** system can take two fluorescence emission states: the fluorescence “Off” state at the free form and the fluorescence “On” state at the complex form with guest compound. This fluorescence “Off–On” switching ability was expressed quantitatively as a fluorescence intensity ratio, $I_{\max}/I_0$, where $I_{\max}$ and I_0 represent fluorescence intensities in the presence ($I_{\max}$) and absence (I_0) of guest materials respectively. The $I_{\max}/I_0$ values were determined for various guests shown in scheme 1 from Fig. 3.3–3.6, and they are listed in Table 3.1. In a previous investigation, this “Off–On” behavior was ascribed to TICT inhibition of the host molecule by the steric repulsion of guest species¹⁰. Guest materials **G1–G4** (**G4**: sodium 4-*n*-octylbenzenesulfonate) were prepared to compare the effects of TICT inhibition based on the steric barrier of the hydrocarbon moiety. The $I_{\max}/I_0$ value of **Ant-CyD** with **G1** and Triton X-405 (TX-405; *n* = 40) was similar to that of TX-100, whereas those of complexes with **G2**, **G3**, and **G4** were ca. 30% compared to TX-100. Moreover, the fluorescence intensity of **Ant-CyD** was changed only slightly by the addition of polyethylene glycol 1000 (PEG; $I_{\max}/I_0$ = 1.1). Those results suggest that the steric barrier of bulky hydrocarbons on the phenyl group dominantly affects the fluorescence enhancement behavior by TICT inhibition, although a hydrophilic moiety such as the polyoxyethylene group does not.

UV-vis spectra of **Ant-CyD** slightly changed before and after complexation except for the region of TX-100 absorption (Fig. 3.7), which the behavior was observed for all other guest compounds. Thus, a electronic perturbation of **Ant-CyD** is vanishingly concerned in π - π^* transition of phenyl parts upon complexation with all guest compounds.

Complex formation constant ($\log K$)

The 1 : 1 complex formation constant $\log K$ was determined by nonlinear least-squares curve fitting method (Marquardt's method¹³) for the fluorescence intensity change (Fig. 3.2). The obtained constants for various guest materials are presented in Table 1. The order of $\log K$ values for guest materials is nearly parallel to those of $I_{\max} / I_0$ values. The good curve fitting (Fig.3.8) suggests that the inclusion phenomenon for this system is dominantly a simple equilibrium as a 1 : 1 complex formation. The difference of the $\log K$ values of **Ant-CyD** with various guest compounds did not have significant difference, which is TX-100 selectivity based on steric repulsion between the substituent moiety in CyD and those guest.

Table 3.1. Fluorescence responses ($I_{\max}/I_0$) and complex formation constants ($\log K$) of **Ant-CyD** for various guest materials in water at 25 °C.

	TX-100	TX-405	G1	G2	G3	G4	SDS	AntS
$I_{\max}/I_0$	9.8	9.5	9.2	2.7	2.0	2.3	2.2	1.1
$\log K$	5.20	5.02	5.24	4.46	3.98	3.84	4.97	-

^a $K = [\text{complex}] / ([\text{Ant-CyD}][\text{guest}])$. The standard deviation of the $\log K$ was estimated to be ± 0.03 .

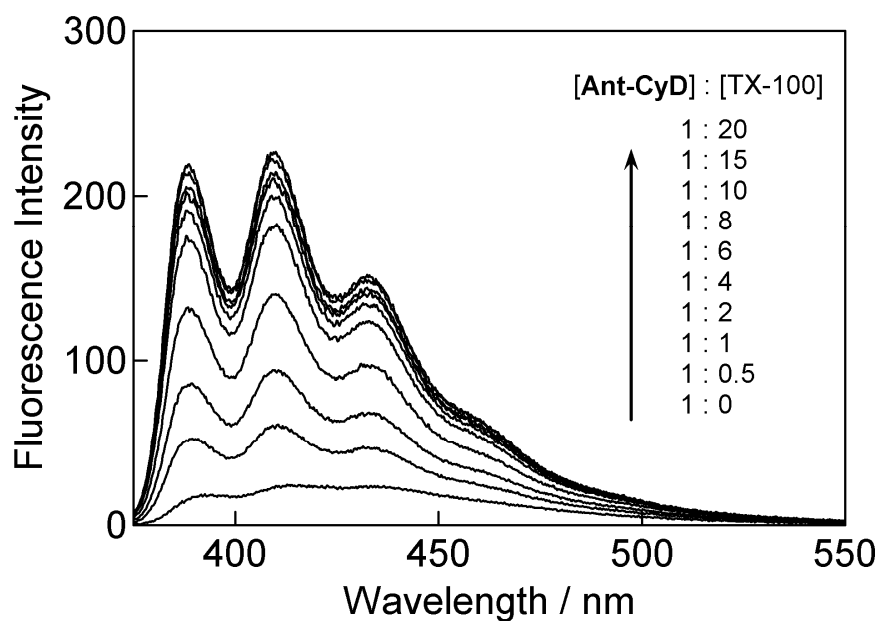


Figure 3.2. Fluorescence of **Ant-CyD** and its TX-100 complex in water at 25 °C. Excitation wavelength: 363 nm. [**Ant-CyD**] = 5 μ M. Molar ratios for TX-100 are listed in columns.

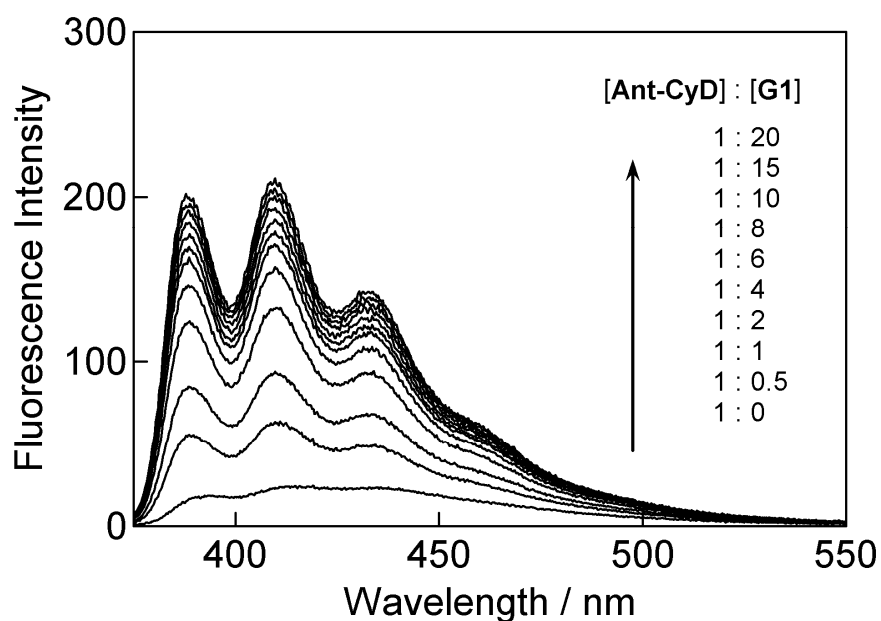


Figure 3.3. Fluorescence of **Ant-CyD** and its **G1** complex in water at 25 °C. Excitation wavelength: 363 nm. [**Ant-CyD**] = 5 μ M. Molar ratios for **G1** are listed in columns.

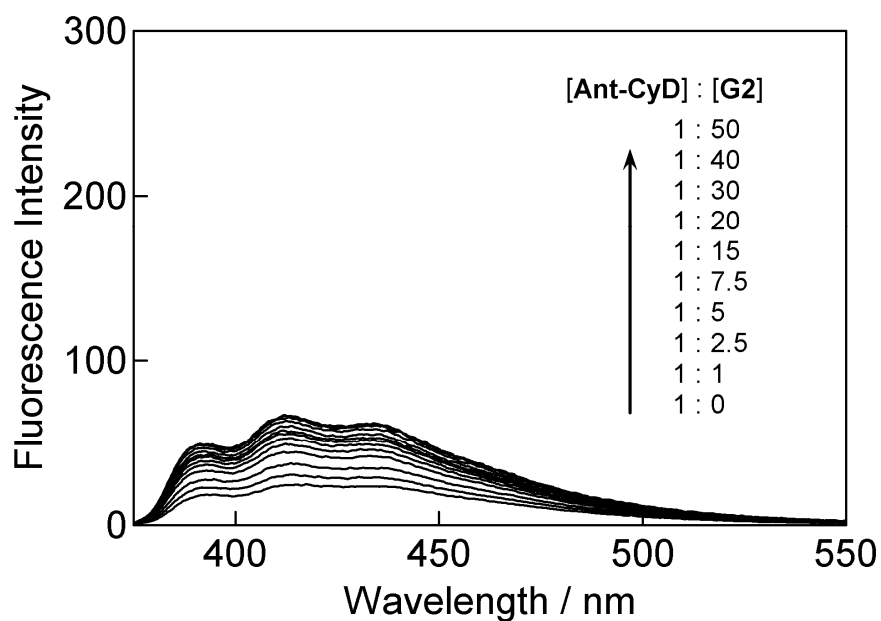


Figure 3.4. Fluorescence of **Ant-CyD** and its **G2** complex in water at 25 °C. Excitation wavelength: 363 nm. [**Ant-CyD**] = 5 μ M. Molar ratios for **G2** are listed in columns.

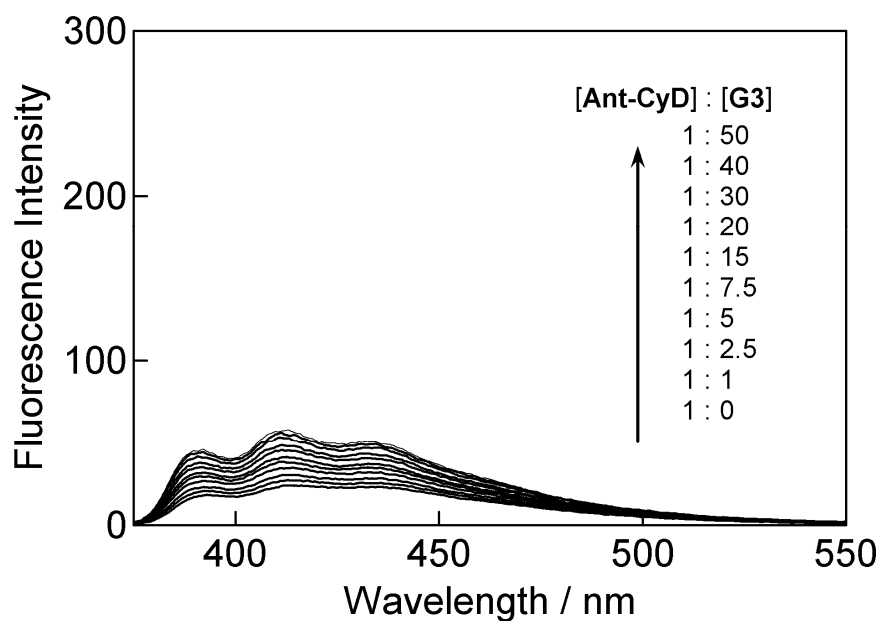


Figure 3.5. Fluorescence of **Ant-CyD** and its **G3** complex in water at 25 °C. Excitation wavelength: 363 nm. [**Ant-CyD**] = 5 μ M. Molar ratios for **G3** are listed in columns.

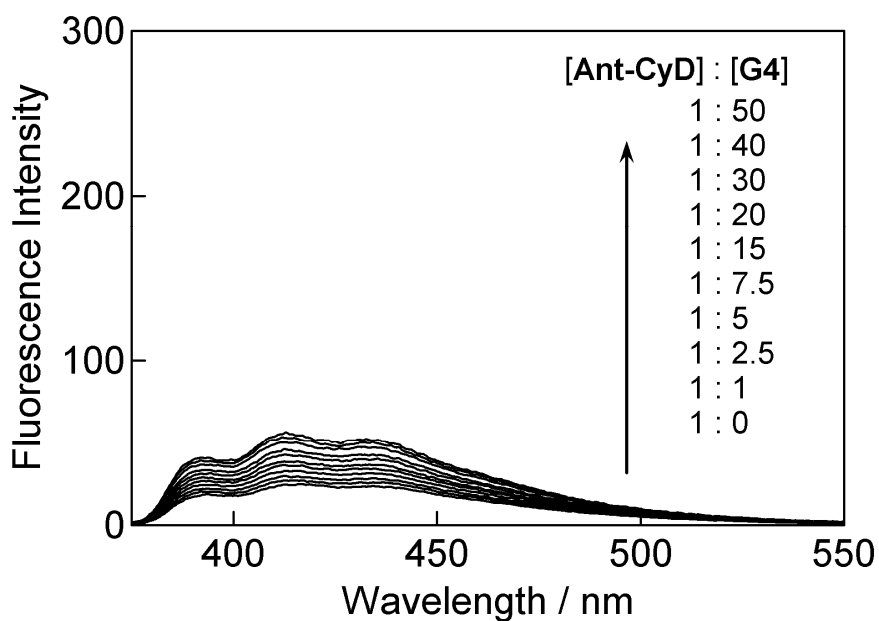


Figure 3.6. Fluorescence of **Ant-CyD** and its **G4** complex in water at 25 °C. Excitation wavelength: 363 nm. [**Ant-CyD**] = 5 μ M. Molar ratios for **G4** are listed in columns.

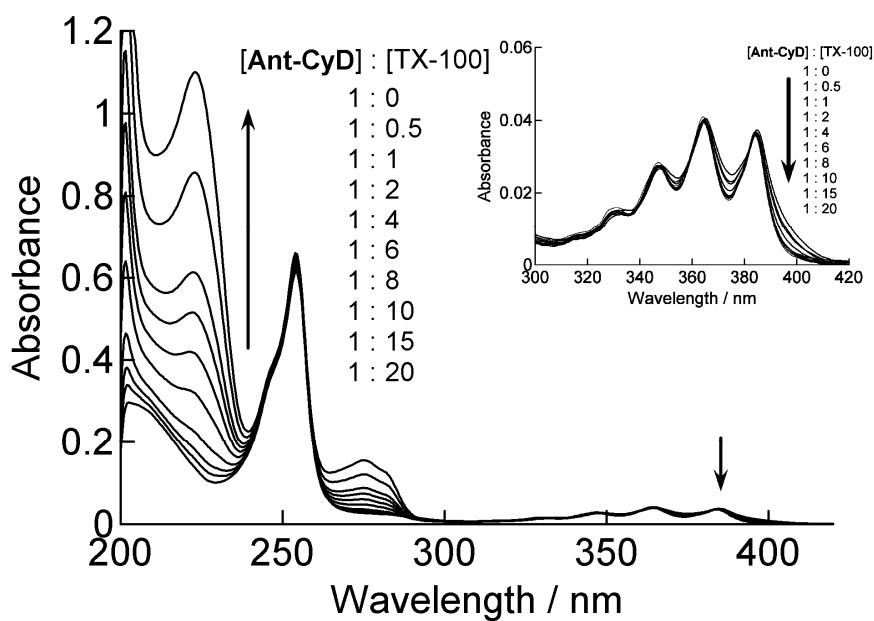


Figure 3.7. UV-vis spectra of **Ant-CyD** and its TX-100 complex in water at 25 °C. Excitation wavelength: 363 nm. [**Ant-CyD**] = 5 μ M. Molar ratios for TX-100 are listed in columns.

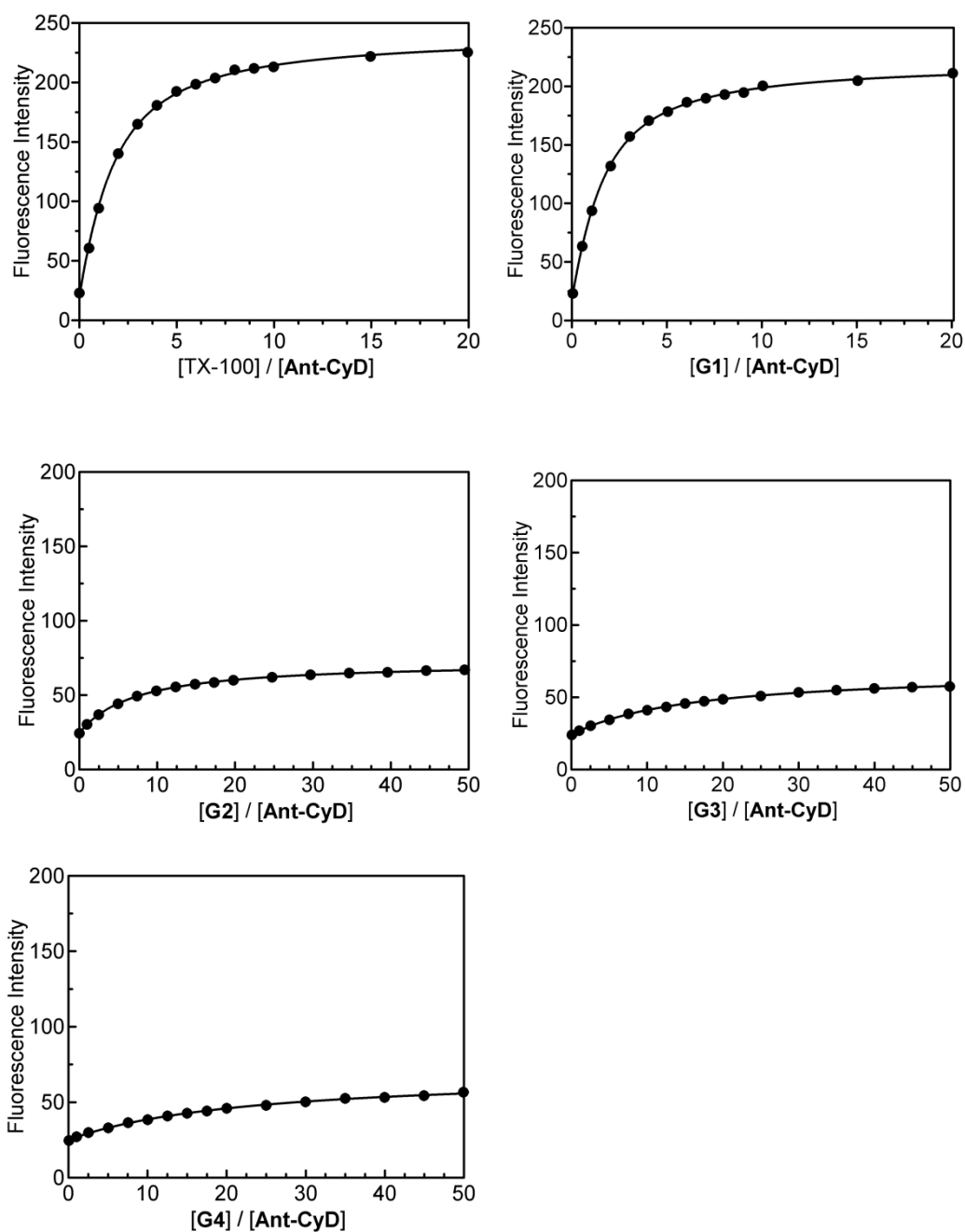


Figure 3.8. dependence of fluorescence intensity at 410 nm on the concentration of TX-100 and guest materials **G1-G4**, and its theoretical curve for formation of stoichiometric 1 : 1 complex.

In view of viscosity and polarity, organic solvent effects on TICT behavior of **Ant-CyD** were investigated by water-glycerin and water-dioxane mixed solvent systems. Fluorescence intensity of **Ant-CyD** was dramatically increased to 40-fold with increase of volume fraction of glycerin (Fig. 3.9). On the other hand, solvent polarity effect on **Ant-CyD** was obtained in presence of dioxane fraction in solution (Fig. 3.10). Although the decrease of the solvent polarity causes fluorescence enhancement by decreasing the stability of the charge separated state, fluorescence intensity of **Ant-CyD** was slightly changed compared to that of viscosity. These results indicate that the major effect on fluorescence enhancement of **Ant-CyD** is the rotational suppression of the anthacenecarboxamido moiety by the viscosity.

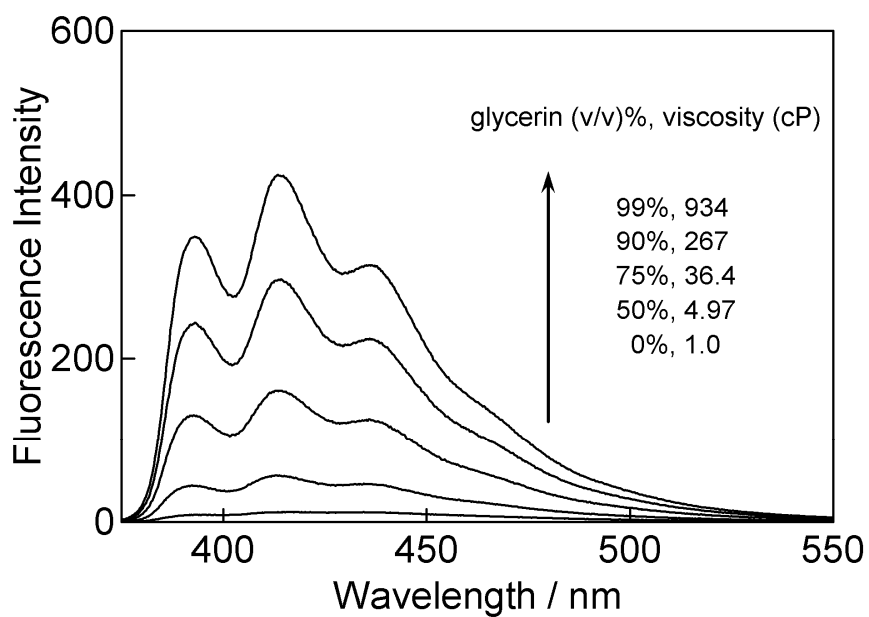


Figure 3.9. Fluorescence spectrum of **Ant-CyD** in various water-glycerin mixed solvent at 25°C. Excited wavelength: 363 nm. [**Ant-CyD**] = 10 μ M.

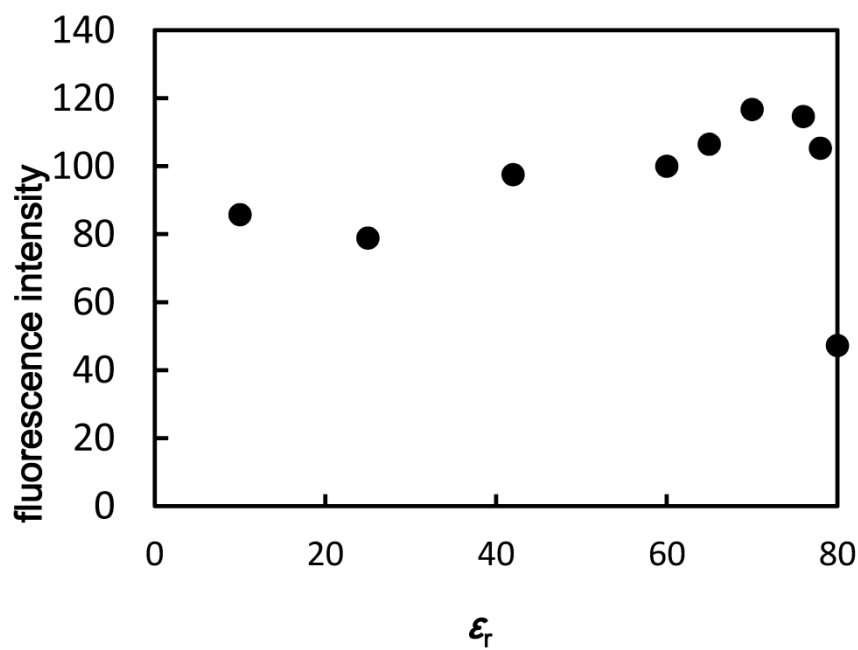
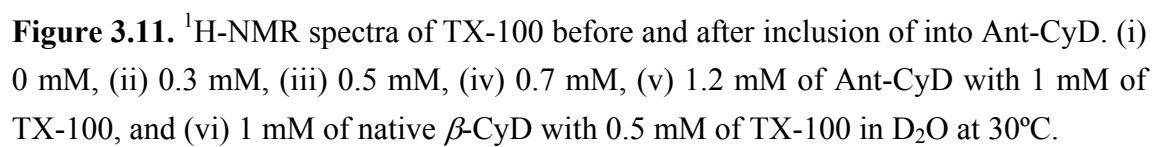


Figure 3.10. Fluorescence intensity of **Ant-CyD** in various water-dioxane mixed solvent. ϵ_r : relative permittivity at 20 °C. Excited wavelength: 363 nm. [**Ant-CyD**] = 10 μ M.

To confirm these considerations, ^1H -NMR measurement was performed. Model compound **1** was used to ^1H - ^1H NOESY measurement because its complex can be prepared in sufficient concentration (10 mM) for 2D measurement. Compound **G1** showed the same fluorescence response for TX-100. Then ^1H -NMR spectral experiments of TX-100 and **G1** were also conducted to consider the effect of micelle association concentrations below (0.1 mM) and over CMC (10 mM) in D_2O . However, no significant spectral difference was found between the two concentrations except for small peak broadenings on *tert*-butyl group in TX-100 ($\Delta\delta < 0.02$ ppm). Thus, the effect on a chemical shift of TX-100 by micelle association is negligible at less than 10 mM. The ^1H -NMR spectra were conducted in 1 mM of TX-100 with various concentrations of **Ant-CyD** in D_2O (Fig. 3.11). Their peak assignment for free Ant-CyD and TX-100 and their complexes were also conducted using ^1H - ^1H COSY, NOESY, along with data referred from the literature²⁰. Large chemical shift changes were observed on an edge moiety (a) of the branched alkyl group in TX-100 (Table 3.2). In contrast, chemical shift changes of TX-100 at complexation with native β -CyD were smaller than those of TX-100 with **Ant-CyD**²⁰. This result demonstrates that a ring current by anthracene ring on CH_3 proton (a) at the complexation event. ^1H - ^1H COSY and NOESY spectra of Ant-CyD were obtained in the presence of **1** (Fig. 3.12-3.13). NOE peaks between the CyD moiety and the branched alkyl and phenyl group in **G1** were observed in Fig. 3.14. This finding strongly suggests that **Ant-CyD** forms a pseudorotaxane type complex as elongate guests stick into CyD cavity. Consequently, the TICT-inhibiting process in Ant-CyD at the excited state is expected to have originated from steric interaction between the edge CH_3 proton (a) of branched alkyl groups in TX-100 and the anthracene ring in Ant-CyD (Fig. 3.15).

Table 3.2. ^1H -NMR chemical shifts (δ ppm) for 1 mM of TX-100 before and after complexation consist of Ant-CyD and 0.5 mM of TX-100 with 1 mM of native β -CyD.

	a	b	c	d	e
TX-100	0.67	1.64	1.26	7.22	6.82
Ant-CyD with TX-100	0.17	1.42	1.27	7.17	6.88
native β -CyD with TX-100	0.80	1.75	1.48	7.37	6.99



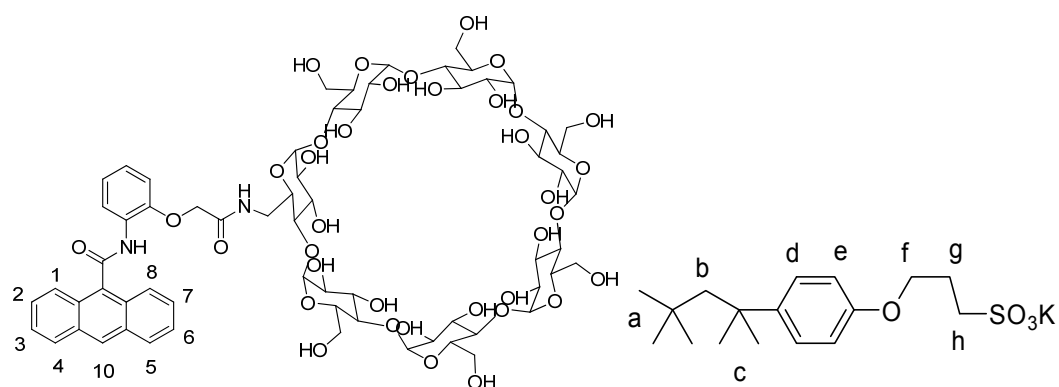
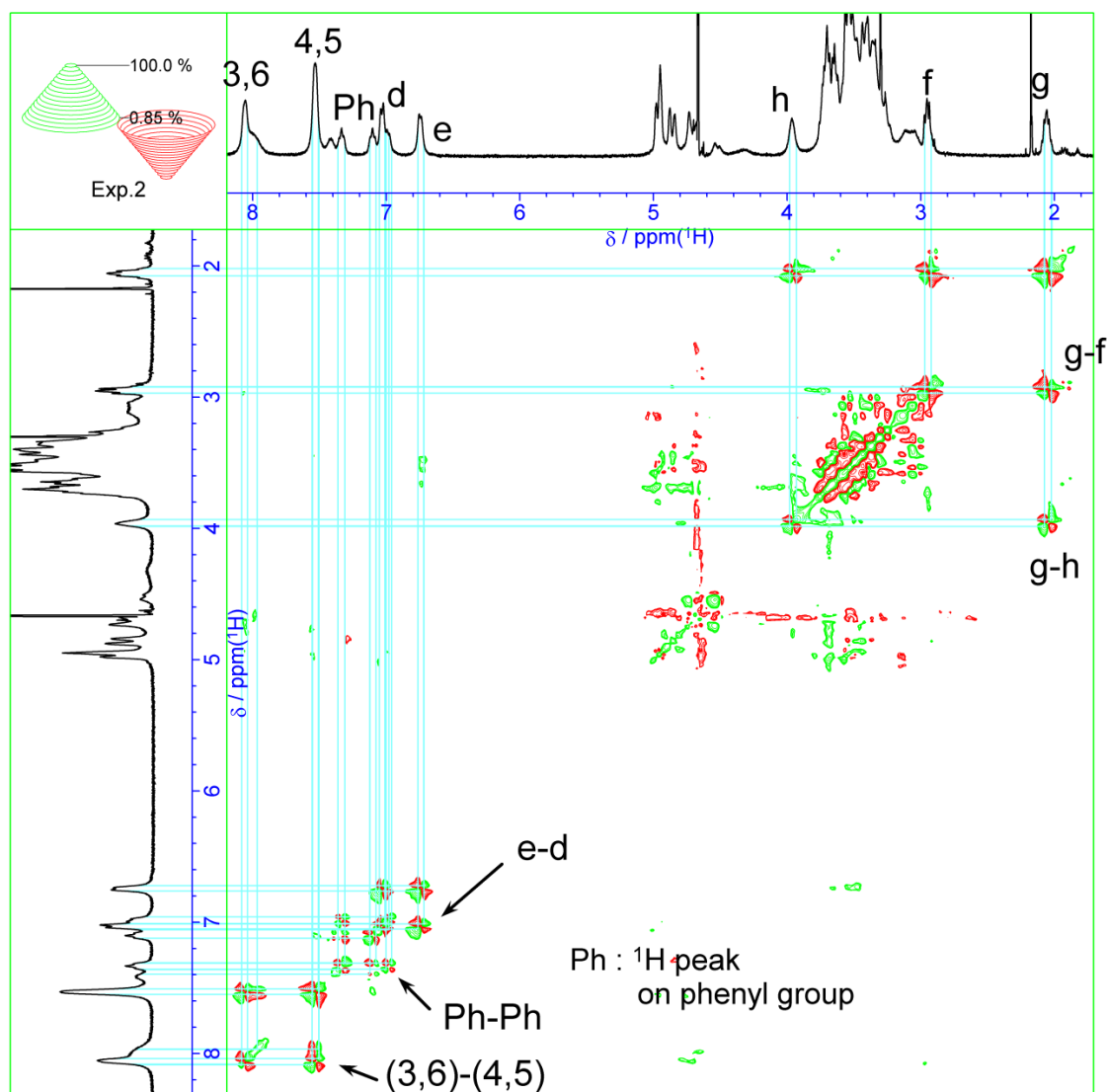


Figure 3.12. 400 MHz ^1H - ^1H COSY spectrum of **Ant-CyD** (10.7 mM) with model compounds **Ant-CyD** (9.7 mM) in D_2O at 30 °C. Alphabets and numbers on the hydrogens correspond to the assignments for ^1H - ^1H COSY spectrum data.

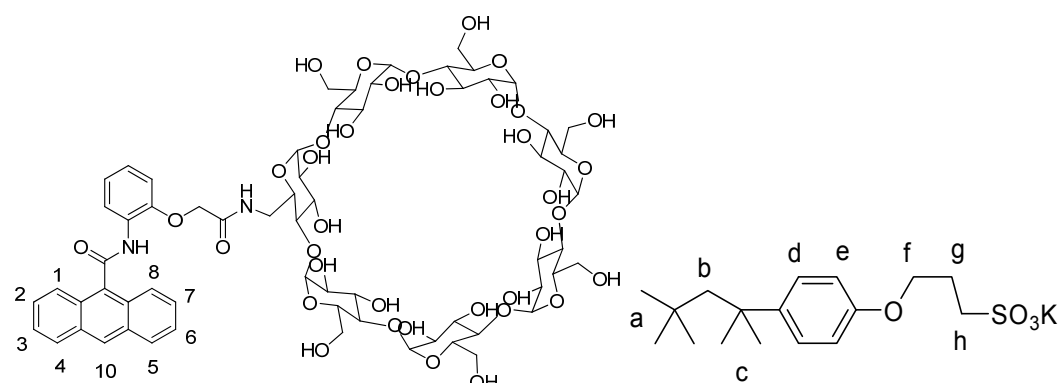
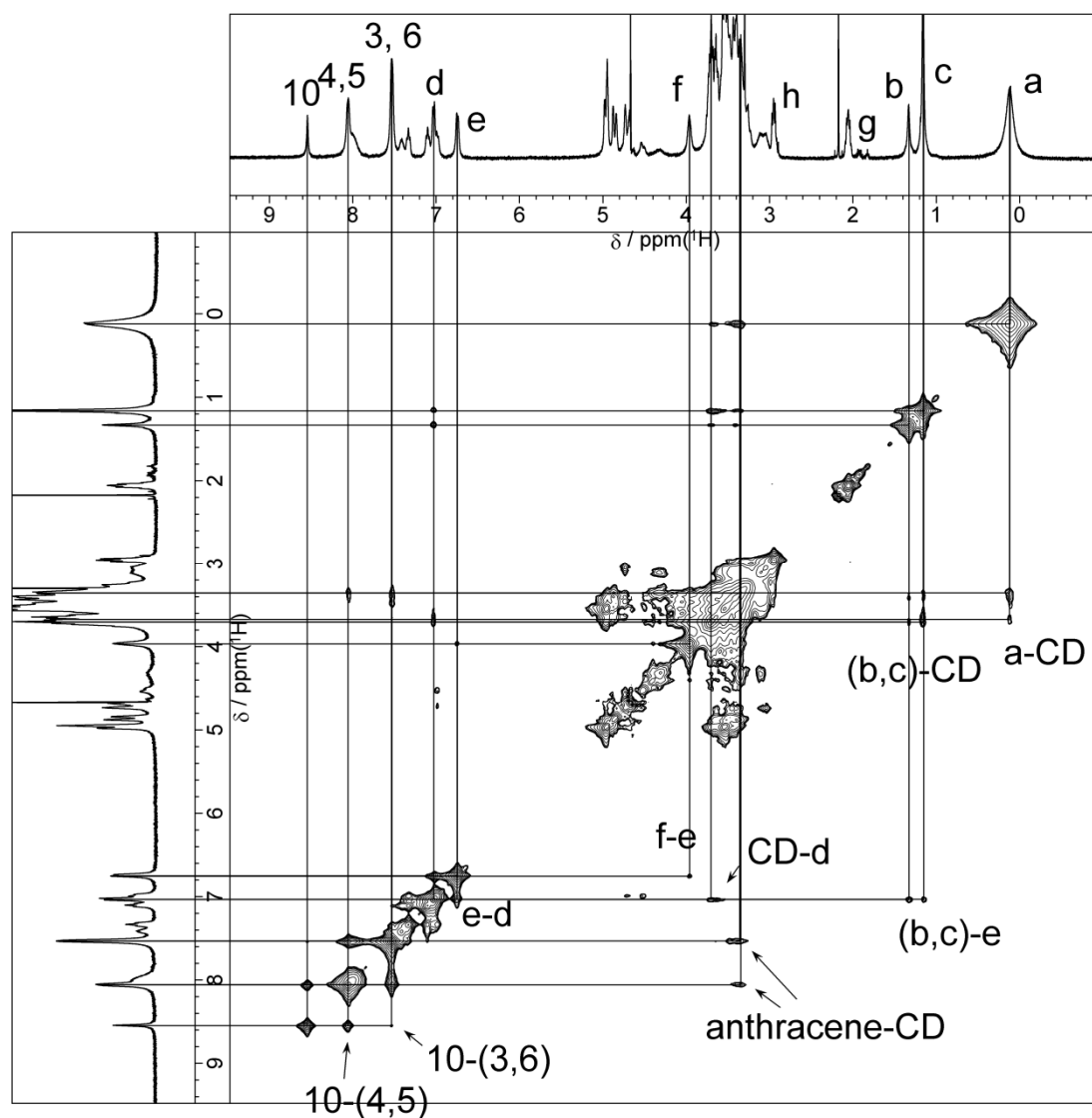


Figure 3.13. 400 MHz ^1H - ^1H NOESY spectrum of Ant-CyD (10.7 mM) with model compounds **Ant-CyD** (9.7 mM) in D_2O at 30 °C. Mixing time: 200 ms. Alphabets and numbers on the hydrogens correspond to the assignments for ^1H - ^1H NOESY spectrum data.

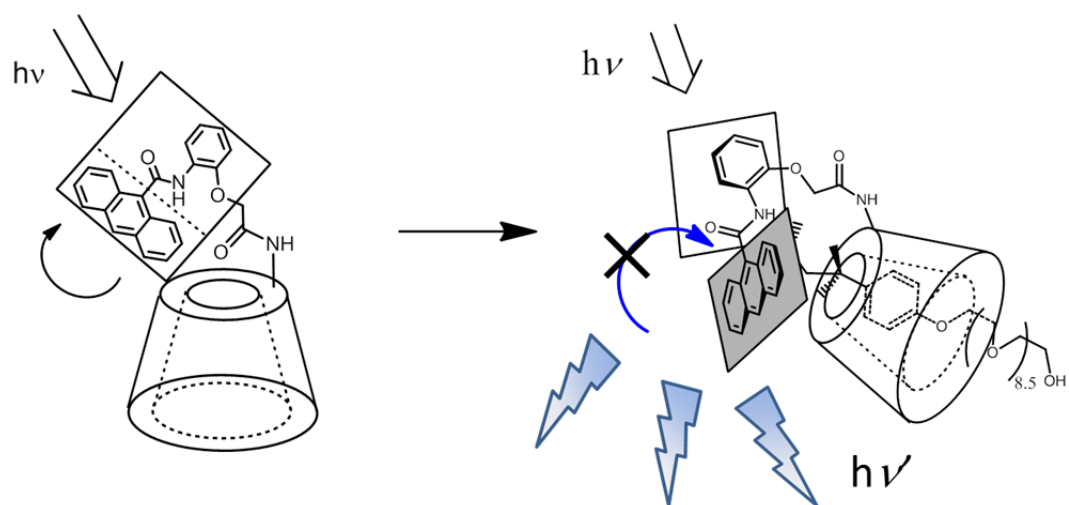


Figure 3.14. Schematic representation of TICT inhibiting for ACP based on the pseudorotaxane type complexation between Ant-CyD with TX-100.

Conclusion

Fluorescence response of Ant-CyD was clearly controlled by TX-100 as a guest molecule below the critical micelle concentration (CMC) in water. Fluorescence intensity of Ant-CyD•TX-100 was dramatically enhanced *c.a.* ten-fold by effective inhibition of TICT. This response was selective for TX-100 compared with materials of linear molecules. The selective response between branched and linear alkyl group was ascribed to the bulkyness of *tert*-buthyl group of TX-100 which caused an effective inhibition of TICT. The TICT controllable Ant-CyD will provide a useful fluorescet cyclodextrin sensor for simple and rapid analysis, which supports a molecular-level insight into analytical applications of environmental materials.

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Chapter 4

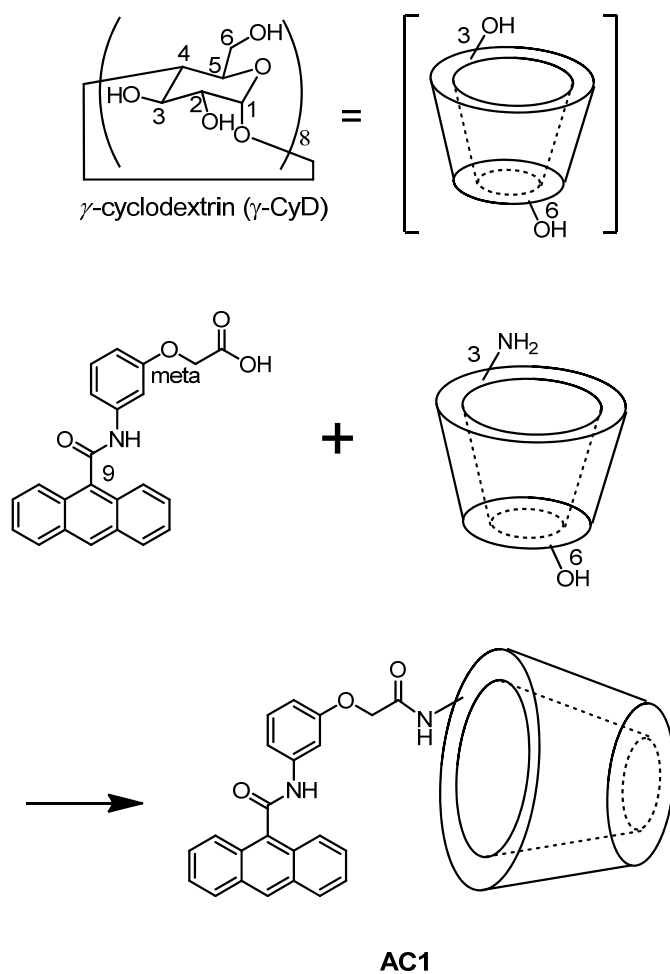
Determination of SDS Using Fluorescent γ -Cyclodextrin Based on TICT in Aqueous Solution

Introduction

Fluorescence detection using chemosensors has received attention for use in analytical fields because of its many advantages.¹ Especially, the simple composition of analytical equipment enables its use outdoors, yielding analytical results on-site.² Fluorescent chemosensors comprise a recognizing moiety for catching target species and a fluorescent moiety that thereby changes its spectral shape;³ various types for target species (cations, anions and ionic organic molecules) have been developed extensively in many laboratories.^{4, 5} To achieve the construction of an analytical system, many environmental samples should be regarded as existing in the aqueous dissolved state. Generally, recognition and fluorescence switching events in many chemosensors are driven by electrostatic interactions between a chemosensor and a target molecule.³ However, a water solvent decreases the complexation capability of an ionic target through competition with hydration. Consequently, this limits the direct use of low-concentration samples in water. Moreover, a strongly reactive functional group in an organic material is demanded for sensing via a chemical reaction.⁵ To date, the extraction of target species to an organic layer has been used as one application for environmental samples.⁶ To address the problem described above, we introduced twisted intramolecular charge transfer (TICT) as a fluorescence-related switching mechanism.^{7, 8} The utilization of the TICT process enables fluorescence switching of a chemosensor by a degree of the steric barrier. It was reported that TICT was controlled by micellar aggregation⁹ and the use of a viscous solvent.¹⁰ Applications using the steric effect were also reported.¹¹ In previous chapter, we described the synthesis of fluorescent β -cyclodextrin (**Ant-CyD**) modified by *N*-phenyl-9-anthracene carboxamidophenyl (9-PAA) derivatives; **Ant-CyD** was demonstrated on a selective determination for Triton X-100 (TX-100) surfactant involving a bulky *tert*-butyl group. Weak fluorescence was emitted from free **Ant-CyD** in water. In contrast, a remarkable enhancement of emission from 9-PAA was observed to be ten-fold in the addition of TX-100 below a critical micellar concentration (CMC: 0.2 mM¹²). In the absence of TX-100, photoexcited 9-PAA in **Ant-CyD** induced free rotation around the amide bond with subsequent quenching of the fluorescence by charge transfer between the anthracene and phenyl moiety via π -conjugation. However, this rotation event, one TICT process, was suppressed upon complexation between **Ant-CyD** with TX-100. This behavior results from the steric effect between 9-PAA and the bulky *tert*-butyl group in TX-100 incorporated to the CyD cavity. Although solubilization of

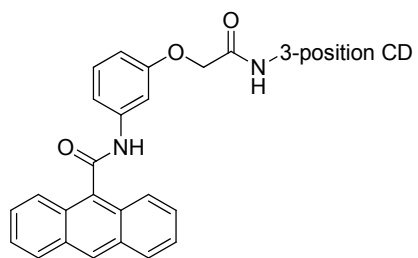
chemosensors in water is one requirement for direct use, hydrophobic chemosensors have provided a useful prototype for the development of practical chemosensors.

This report describes novel **Ant-CyD** analogues (**ACs**) based on γ -CyD modified by 9- and 1-anthracenecarboxamido to detect a more “slender” surfactant than TX-100. As described above, the fluorescence spectral behavior of the **AC** system with surfactants is predisposed to the effects of steric structures of both elements. Consequently, the present study specifically examined alkyl groups in surfactants, substituent positions to CyD and in 9-PAA, and types of CyDs.

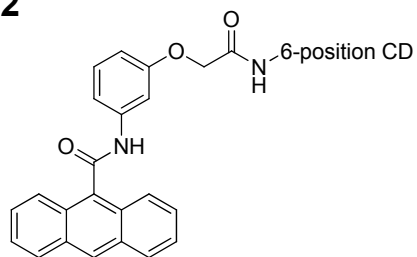


Scheme 4.1. Synthesis pathway of fluorescent γ -cyclodextrin, with **AC1** as a representative example. Table 1 shows the substituents of anthracene, phenyl, and glucopyranose rings (CyD) of **ACs**.

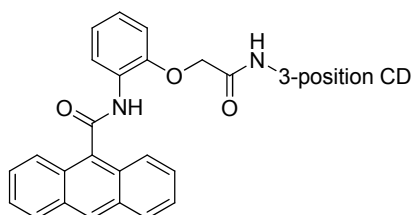
AC1



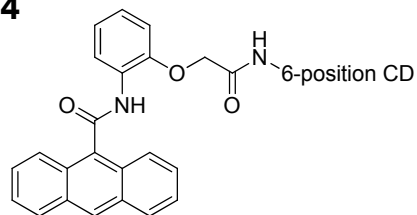
AC2



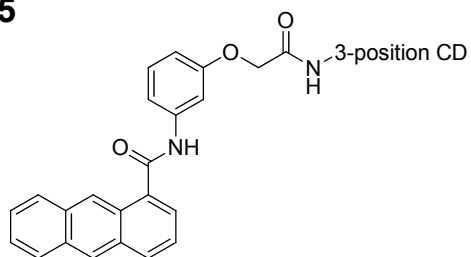
AC3



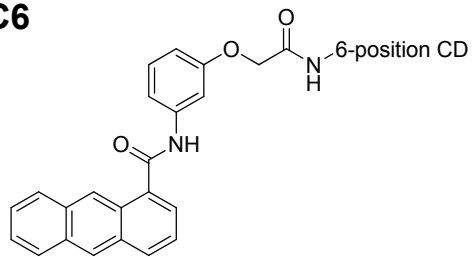
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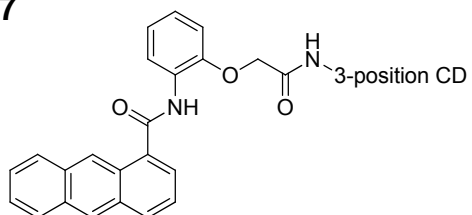
AC5



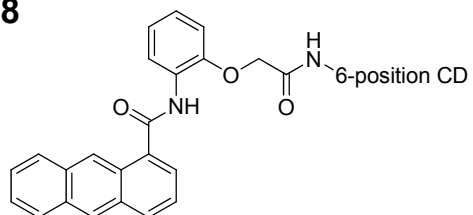
AC6



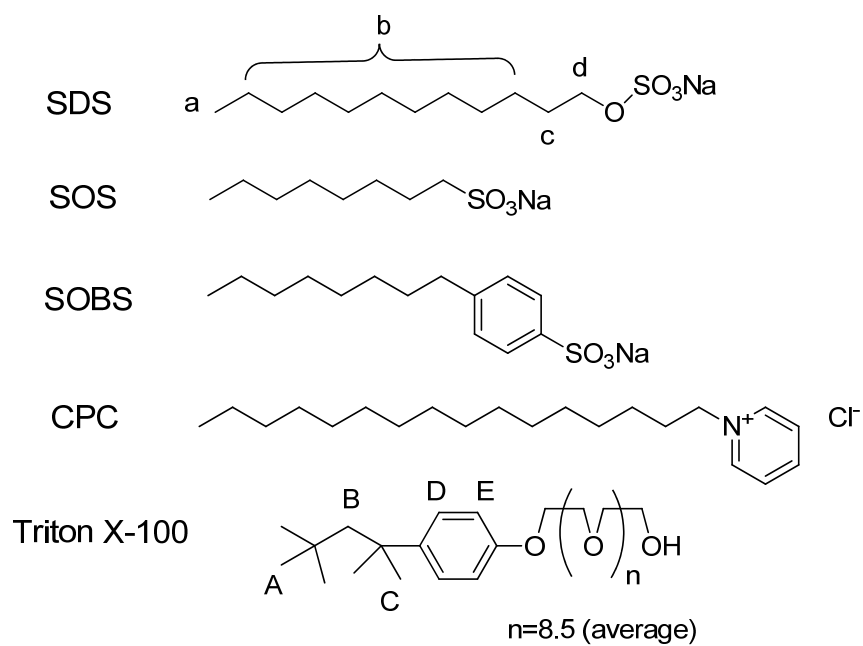
AC7



AC8



Scheme 4.2. Structure details of ACs.



Scheme 4.3. Structures of guest compounds. Alphabets on hydrogen atoms in guest compounds corresponding to assignments for ^1H NMR spectra.

Experimental

Synthesis of AC1 and its analogues

AC1 and its analogues were synthesized by the amide coupling of *x*-deoxy-*x*-monoamino- γ -cyclodextrin (*x*: 3 or 6) and *y*-(*z*-anthracenecarboxamido) phenoxyacetic acid (*y*: 2, 3 ; *z*: 1, 9) according to procedures described in a previous report.⁷

3-Deoxy-3-[3-(9-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC1).

Yield, 27.1%; light-yellow solid; m.p., 273–278 °C; decomp. ¹H NMR (DMSO-*d*₆ δ from TMS) 3.22–3.76 (including overlapping with a H₂O peak br, ca. 36H), 4.40–4.59 (br, 8H), 4.72 (m, 2H), 4.91 (m, 7H), 5.48–5.90 (-OH, m, 16H), 6.81 (aromatic, d, 1H), 7.32 (aromatic, t, 1H), 7.41 (aromatic, d, 1H), 7.58 (aromatic, m, 5H), 8.00 (aromatic, -CONH-, m, 3H), 8.18 (aromatic, d, 2H), 8.74 (aromatic, s, 1H), and 10.83 (-CONH-, s, 1H). Found: C, 49.14; H, 6.07; N, 1.65. Calcd. for C₇₁H₉₆N₂O₄₂•4H₂O: C, 49.53; H, 6.09; N, 1.63.

6-Deoxy-6-[3-(9-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC2).

Yield, 12.5%; light-yellow solid; m.p., 272–276 °C; decomp. ¹H NMR (DMSO-*d*₆ δ from TMS) 3.20–3.90 (including overlapping with a H₂O peak br, ca. 50H), 4.42–4.58 (-OH, C-H, m, 9H), 4.82–4.95 (m, 8H), 5.62–5.90 (-OH, m, 16H), 6.77 (aromatic, d, 1H), 7.32 (aromatic, t, 1H), 7.43 (aromatic, d, 1H), 7.59 (aromatic, m, 5H), 7.87 (-CONH-, t, 1H), 8.00 (aromatic, d, 2H), 8.18 (aromatic, d, 2H), 8.74 (aromatic, s, 1H), and 10.85 (-CONH-, s, 1H). Found: C, 49.45; H, 5.93; N, 1.68. Calcd. for C₇₁H₉₆N₂O₄₂•4H₂O: C, 50.06; H, 6.04; N, 1.64.

3-Deoxy-3-[2-(9-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC3).

Yield, 15.3%; light-yellow solid; m.p., 273–277 °C; decomp. ¹H NMR (DMSO-*d*₆ δ from TMS) 3.20–3.90 (including overlapping with a H₂O peak br, ca. 43H), 4.38–4.99 (-OH and C-H, m, 21H), 5.47 (d, 2H), 5.57–5.85 (-OH, m, 16H), 7.15 (m, 2H), 7.23 (aromatic, m, 1H), 7.59 (aromatic, m, 4H), 8.03 (-CONH-, aromatic, m, 2H), 8.16 (aromatic, t, 4H), 8.70 (aromatic, s, 1H), 10.11 (-CONH-, s, 1H). Found: C, 49.19; H, 5.94; N, 1.67. Calcd. for C₇₁H₉₆N₂O₄₂•4H₂O: C, 50.06; H, 6.04; N, 1.64.

6-Deoxy-6-[2-(9-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC4).

Yield, 21.4%; light-yellow solid; m.p., 273–277 °C; decomp. ^1H NMR (DMSO- d_6 δ from TMS) 2.90–3.72 (including overlapping with a H_2O peak br, ca. 38H), 4.35–4.47 (m, 8H), 4.63 (m, 2H), 4.73 (m, 2H), 4.87 (m, 6H), 5.50–5.92 (-OH, m, 16H), 7.09 (aromatic, t, 2H), 7.25 (aromatic, t, 1H), 7.58 aromatic, (m, 4H), 7.87 (aromatic, d, 1H), 8.03 (aromatic, -CONH-, t, 1H), 8.15 (aromatic, d, 4H), 8.71 (aromatic, s, 1H), and 10.41 (-CONH-, s, 1H). Found: C, 49.86; H, 6.02; N, 1.65. Calcd. for $\text{C}_{71}\text{H}_{96}\text{N}_2\text{O}_{42}\cdot 3\text{H}_2\text{O}$: C, 50.06; H, 6.04; N, 1.64.

3-Deoxy-[3-(1-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC5).

Yield, 20.0%; light-yellow solid; m.p., 273–278 °C; decomp. ^1H NMR (DMSO- d_6 δ from TMS) 3.20–3.98 (including overlapping with a H_2O peak br, ca. 51H), 4.38–5.02 (-OH, C-H, m, 17H), 5.42–5.94 (-OH, m, 16H), 6.79 (aromatic, d, 1H), 7.30 (aromatic, t, 1H), 7.43 (aromatic, br, 1H), 7.58 (aromatic, m, 4H), 7.77 (aromatic, d, 1H), 8.01 (aromatic, d, 1H), 8.13 (aromatic, t, 2H), 8.26 (aromatic, d, 1H), 8.70 (aromatic, s, 1H), 8.86 (aromatic, s, 1H), and 10.61 (-CONH-, s, 1H). Found: C, 49.57; H, 6.10; N, 1.64. Anal. Calcd. For $\text{C}_{71}\text{H}_{96}\text{N}_2\text{O}_{42}\cdot 4\text{H}_2\text{O}$: C, 49.53; H, 6.09; N, 1.63.

6-Deoxy-6-[3-(1-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC6).

Yield, 20.5%; light-yellow solid; m.p., 272–277 °C; decomp. ^1H NMR (DMSO- d_6 δ from TMS) 3.21–3.80 (including overlapping with a H_2O peak br, ca. 51H), 4.40–4.58 (-OH, C-H, 9H), 4.90 (m, 8H), 5.62–5.90 (-OH, 16H), 6.75 (aromatic, d, 1H), 7.30 (aromatic, t, 1H), 7.45 (aromatic, d, 1H), 7.58 (aromatic, m, 4H), 7.77 (aromatic, d, 1H), 7.84 (-CONH-, t, 1H), 8.13 (aromatic, t, 2H), 8.25 (aromatic, d, 1H), 8.69 (aromatic, s, 1H), 8.85 (aromatic, s, 1H), and 10.63 (-CONH-, s, 1H). Found: C, 49.45; H, 5.93; N, 1.68. Calcd. for $\text{C}_{71}\text{H}_{96}\text{N}_2\text{O}_{42}\cdot 3\text{H}_2\text{O}$: C, 50.06; H, 6.04; N, 1.64.

3-Deoxy-3-[2-(1-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC7).

Yield, 13.7%; light-yellow solid; m.p., 272–276 °C; decomp. ^1H NMR (DMSO- d_6 δ from TMS) 3.10–4.10 (including overlapping with a H_2O peak br, ca. 51H), 4.40–4.98 (-OH, C-H, m, 17H), 5.38–5.92 (-OH, m, 17H), 7.16 (aromatic, m, 3H), 7.55 (aromatic,

m, 2H), 7.60 (aromatic, t, 1H), 7.93 (aromatic, d, 1H), 8.03 (aromatic, d, 1H), 8.13 (aromatic, -CONH-, br, 3H), 8.26 (aromatic, d, 1H), 8.69 (aromatic, s, 1H), 9.01 (aromatic, s, 1H), and 9.85 (aromatic, s, 1H). Found: C, 48.87; H, 6.05; N, 1.65. Anal. Calcd. for $C_{71}H_{96}N_2O_{42} \cdot 4H_2O$: C, 49.53; H, 6.09; N, 1.63.

6-Deoxy-6-[2-(1-anthracenecarboxamido)phenoxyacetamido]- γ -cyclodextrin (AC8).

Yields 25.7%; light-yellow solid; m.p., 273–278 °C; decomp. ^1H NMR (DMSO- d_6 δ from TMS) 2.98–4.00 (including overlapping with a H_2O peak br, ca. 52H), 4.46–4.89 (-OH, C-H, m, 17H), 5.64–5.92 (-OH, m, 16H), 7.08 (aromatic, d, 2H), 7.19 (aromatic, t, 1H), 7.56 (aromatic, t, 2H), 7.61 (aromatic, t, 1H), 7.86 (aromatic, d, 1H), 8.04 (aromatic, d, 1H), 8.12 (aromatic, -CONH-, m, 3H), 8.25 (aromatic, d, 1H), 8.68 (aromatic, s, 1H), 8.99 (aromatic, s, 1H), and 10.09 (-CONH-, s, 1H). Found: C, 49.84; H, 6.05; N, 1.68. Calcd. for $C_{71}H_{96}N_2O_{42} \cdot 4H_2O$: C, 49.53; H, 6.09; N, 1.63.

Measurement of fluorescence, UV-vis and circular dichroism spectra

Fluorescence spectra were monitored (RF-5300; Shimadzu Corp.) in distilled water at 25 °C. The excitation wavelength was 363 nm. The sample solution containing guest molecules was dropped sequentially in 5 μM ACs aqueous solution in all cases. UV spectra were also observed (UV-2400PC; Shimadzu Corp.) under the same conditions using fluorescence measurements. Circular dichroism spectra examinations were conducted (J-720; Jasco Corp.) with both 10 μM and 100 μM concentrations of ACs under N_2 gas at r.t.

^1H NMR measurement

The ^1H NMR spectra for guest molecules with ACs were measured at 30 °C (JNM-Ex400; JEOL). ACs concentrations were 0.5 mM in D_2O . Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) used as an external standard.

Results and Discussion

Fluorescence spectra

Figure 4.1 shows fluorescence spectra of **AC1** and **AC5** in the absence and presence of SDS below the critical micellar concentration (CMC: 8.7 mM¹³) in water. Structureless fluorescence of both probe were obtained in the absence of SDS. In contrast, dramatic fluorescence enhancements were observed upon the addition of SDS, which rose 12- and 10.3-folds, respectively. Moreover, CyD lacked precursor for those probes, potassium 3-(3-(9-anthracenecarboxamido)phenoxy)propanesulfonate, which showed only a 1.1-fold fluorescent enhancement with 8 mM of SDS. Consequently, this result clearly indicates that a fluorescence enhancement of **AC1** and **AC5** results from complexation between its CyD cavity and SDS. On the other hand, nonionic surfactant TX-100 (CMC: 0.2 mM) was slightly changed at the same concentration with SDS. To investigate this guest selectivity, structurally different analogues of SDS were used. Sodium octanesulfonate (SOS), sodium octylbenzenesulfonate (SOBS), and cetylpyridinium chloride (CPC) have a straight alkyl group as a hydrophobic moiety. TX-100 involved a branched alkyl group, which selectively induced an enhancement of **Ant-CyD** fluorescence among those guests. The fluorescence response ratio ($I_{\max}/I_0$) was defined by the fluorescence intensity of a free **AC** (I_0), and upon complete complexation ($I_{\max}$) of that with a guest molecule below CMC. For **AC1**, and **AC5**, higher $I_{\max}/I_0$ values were obtained for SDS and SOBS than others (Fig. 4.2). In the β -**AC** system, fluorescence emission of the 9-AA in the addition of TX-100 is enhanced selectively by about four-fold. However, $I_{\max}/I_0$ values of **AC1** with SDS were about five-fold higher than for bulky molecules, such as TX-100. This opposite result to that obtained in our previous study suggests that the complex conformation of **AC** with a guest molecule was different from that of β -**AC**. Moreover, guest selectivity of **ACs** also appeared among linear alkyl compounds, which demonstrates that the **AC** system can exclude structurally similar guests accurately by a steric factor, such as molecular length or bulkiness of alkyl groups.

Other **AC** analogues (**AC2-4**, **AC6-8**) were also investigated to discuss steric effects of host molecules. The type and position of the substituent group on a CyD skeleton are widely known to affect the chemical properties of CyD strongly, such as inclusion capability and selectivity for guest species. From this viewpoint, substituent positions on aromatic rings in the 9-AA, phenyl and hydroxyl group on the CyD skeleton were specifically examined. It is interesting that those **ACs** only weakly responded to any

guest compound. This result confirmed that TICT suppression of AC results from a steric hindrance between 9-AA, and the guest which occurs not only by inclusion events in the CyD cavity.

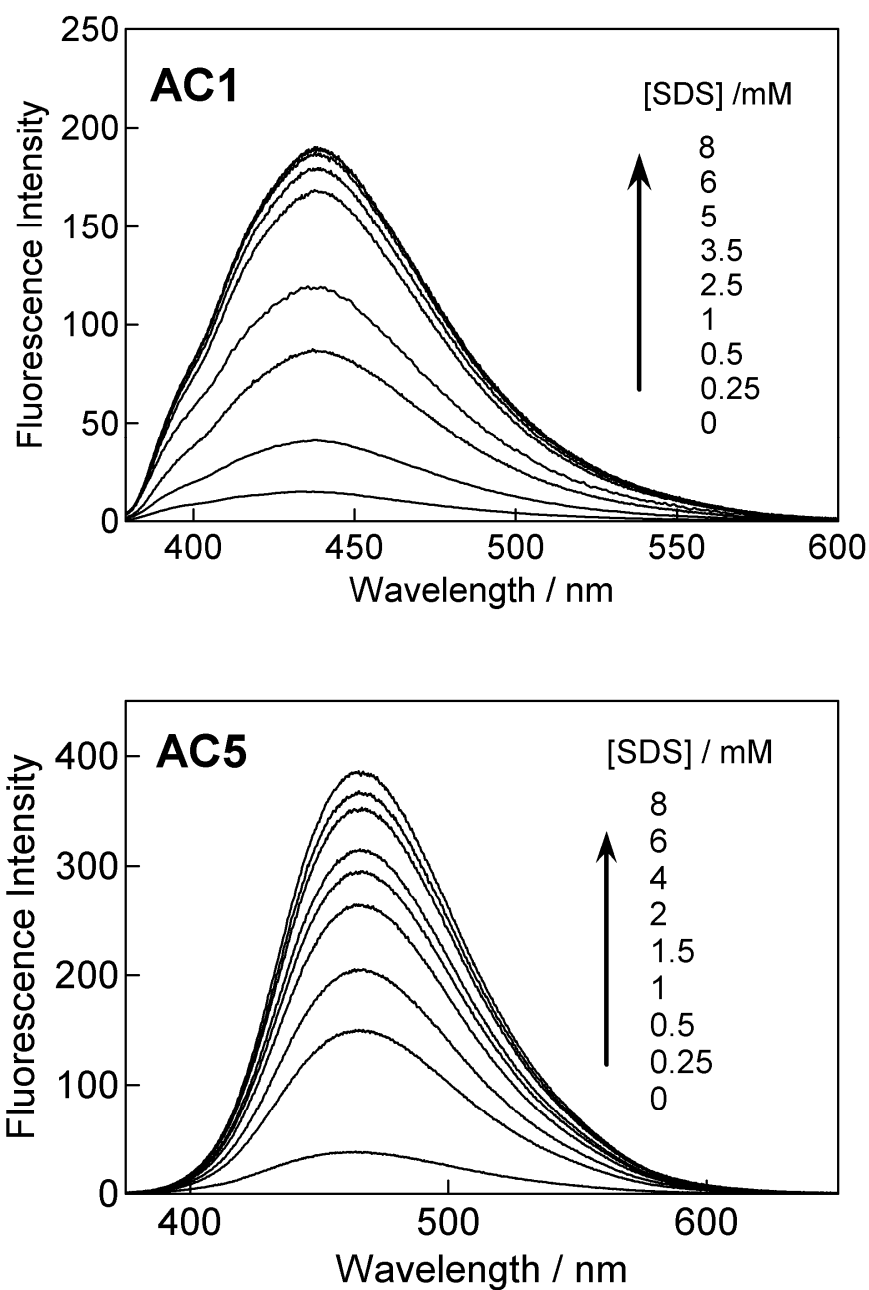


Figure 4.1. Fluorescence spectra of **AC1** (upper) and **AC5** (lower) in the absence and presence of SDS below CMC in water at 25 °C.

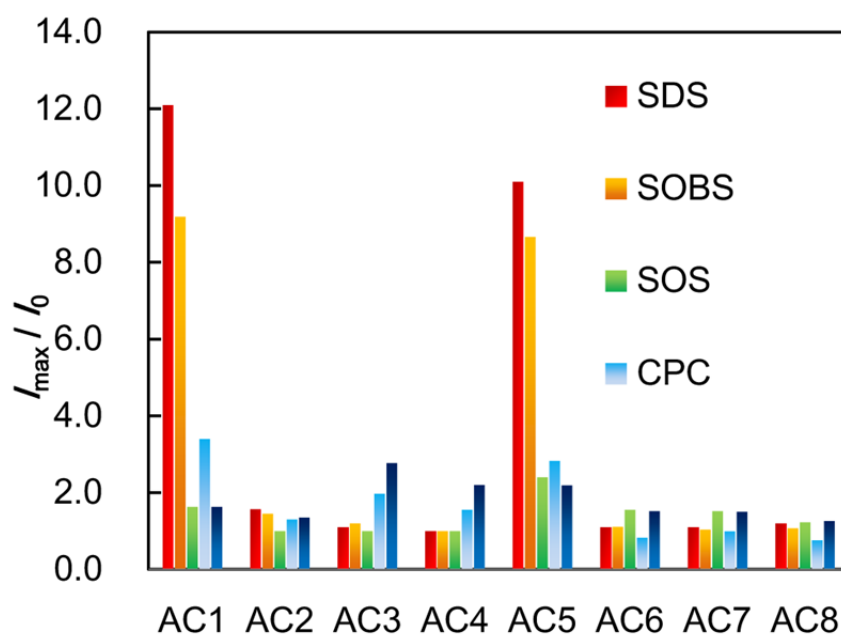


Figure 4.2 Fluorescence response ratio ($I_{\max} / I_0$) of **ACs** and its complexes with guest molecules in water at 25 °C. [**ACs**] = 5 μ M, Excitation: 369 nm. All $I_{\max} / I_0$ values were obtained below CMC of guest compounds to exclude effect of micelle formation.

Complex formation constant (log K) and limit of detection (LOD)

The complexation ability of **ACs** with surfactants is an important parameter to investigate fluorescent reagents. Complex formation constants ($\log K$) of **AC1** and **AC5** were calculated using nonlinear least-squares curve fitting (Marquardt's method¹⁴) below CMC. The obtained $\log K$ values are presented in Table 4.1 and calibration curves are shown in Fig. 4.3 and 4.4. Good fitting of the theoretical curve for experimental data indicates that the process between an **AC** and a guest molecule is dominated strongly by simple equilibrium as the inclusion process of a guest molecule into the cyclodextrin cavity. No **ACs**, except **AC1** and **AC5**, yielded reliable $\log K$ values for any guest, because of the low $I_{\max}/I_0$ values or weak complexation ability. The complexation ability for TX-100 superficially seems inconsistent fluorescence selectivity for SDS of **AC1** and **AC5**. However, a fluorescence efficient enhancement by SDS clearly takes priority of TX-100 at the same concentration. To clearly understand this mention, fluorescence intensities of **AC1** were obtained in high concentration (up to 8mM) of TX-100 (Fig. 4.5), were figurally changed from the range of 0.2 to 8 mM. Thus, the fluorescence selectivity is mainly dominated by the difference of the $I_{\max}/I_0$ values between SDS and TX-100 around CMC.

Consequently, the limit of detection (LOD) value for SDS is estimated to be 0.4 μM (0.12 ppm), as follows: a margin of error for the fluorescence intensity is calculated as a standard deviation (SD) of the background noise obtained from 10 times measurements of a blank solution. The three-folds of the regards LOD and converted into SDS concentration from the calibration curve between **AC1** and SDS (Fig. 4.3). Obtained LOD value was less than environmental standard for drinking water. Thus, there is promising for future investigation such as analysis of environmental sample using TICT type cyclodextrin chemosensor.

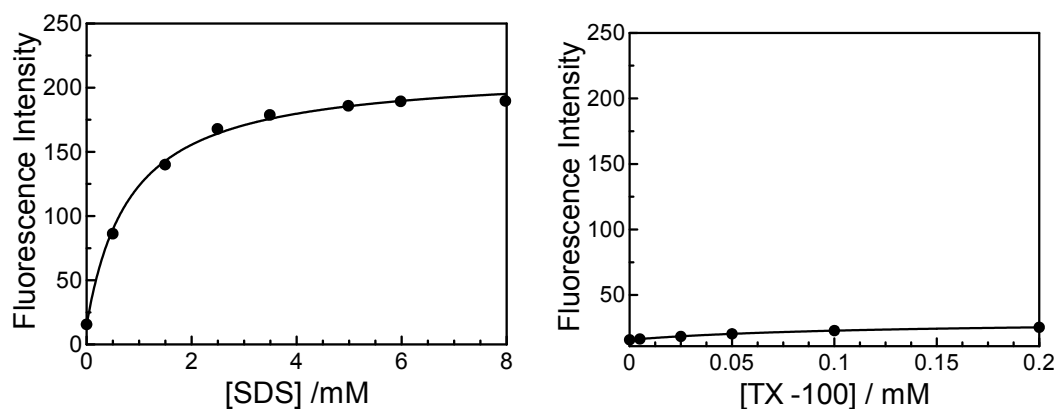


Figure 4.3. Dependence of fluorescence intensity at 438 nm on the concentration of SDS(left) and TX-100 (right), and its theoretical curve for formation of stoichiometric 1:1 complex. [AC1] = 5 μ M at 25°C.

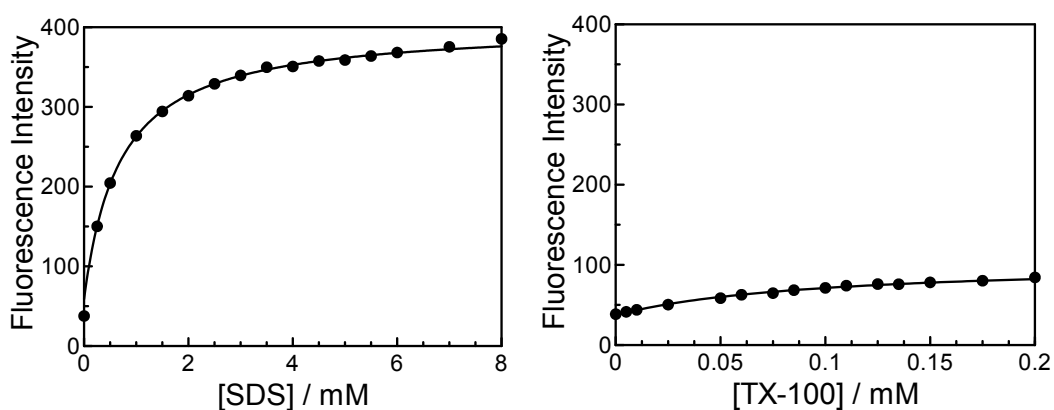


Figure 4.4. Dependence of fluorescence intensity at 438 nm on the concentration of SDS(left) and TX-100 (right), and its theoretical curve for formation of stoichiometric 1:1 complex. [AC5] = 5 μ M at 25°C.

Table 4.2. 1:1 complex formation constants ($\log K^a$) of **ACs** with various guest compounds in water at 25 °C

	SDS	SOS ^a	SOBS	CPC	TX-100
AC1	3.47	-	2.98	3.79	3.85
AC5	3.16	-	3.07	4.05	3.98

^a $K = [\text{complex}] / ([\text{AC1 or AC5}][\text{guest}])$. The standard deviation of the $\log K$ was estimated to be ± 0.04 .

^b This value was not evaluated because of poor change of fluorescence intensity.

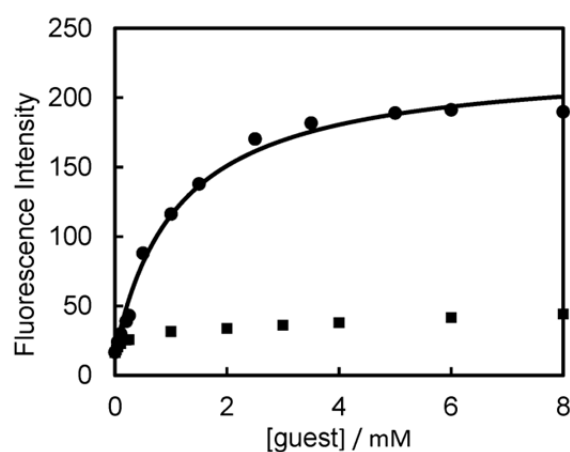


Figure 4.5. Dependence of fluorescence intensity at 438 nm on the concentration of SDS (circle dots) and TX-100 (cubic dots) in water at 25 °C. SDS and TX-100 were added to 5 μM of **AC1** solution. Excitation: 369 nm. Theoretical curve for formation of stoichiometric 1:1 complex under CMC.

Induced circular dichroism (ICD) spectra

An induced circular dichroism (ICD) spectrum is observed for an aromatic chromophore onto the CyD cavity axis through the narrow or wide rim of a CyD.¹⁵ It supports the clarification of those complex structures.¹⁶ Figure 4.6 shows induced circular dichroism (ICD) and UV-vis spectra of **AC5** (10 μ M and 100 μ M) in aqueous solution with 0–8 mM of SDS. An asymmetrically split Cotton effect of the ICD spectrum was observed for free **AC5** in the region of 240–260 nm. This result clearly illustrates the exciton interaction between anthracene and the phenyl moiety existing on the CyD cavity axis. Consequently, their split peaks decreased concomitantly with increasing SDS concentration. This behavior suggests that 9-AA of **AC5** faded out from the CyD cavity axis by a steric repulsion with **AC5**•SDS formation. The signal at the 340–380 nm region, which shows ICD peaks of the anthracene moiety, disappeared with increasing SDS concentration, which supports the consideration described above. For TX-100, as a low-responsive guest, ICD spectra of **AC5** changed similarly, increasing along with the TX-100 concentration (Fig. 4.7). However, the ICD signal did not disappear completely in the region of 240–260 nm. The spectral residue of **AC5** indicates that 9-PAA exists in the position of the ICD effective column on the complex with TX-100.

In ICD spectra of **AC1**, the appearance and disappearance of the asymmetrically split Cotton effect also occurred before and after the addition of SDS in 200–300 nm (Fig. 4.8). However, the ICD peak in the region of long wavelength changed only slightly in any SDS concentration range (0–8 mM), which indicates that the 9-anthracene moiety of **AC1** more closely resembles SDS than it resembles **AC5** upon complexation. This structure of the complex is advantageous for effective TICT suppression by 9-anthracene in **AC1** with a short rotation radius compared to that of 1-anthracene in **AC5**.

For weakly responsive hosts for any guests, the asymmetrically split Cotton effect of the ICD spectra was scarcely changed (**AC7**), or was not observed (**AC2-4**, **AC6** and **AC8**) in the absence and presence of SDS (Fig. 4.9-4.14). ICD spectra behaviors among a series of **ACs** corresponded to low fluorescence response. Consequently, these results showed that the conformation of 9-PAA on the CyD scaffold is not sufficient for the steric repulsion for TICT inhibition upon complexation. As explained above, a suitable structure of **ACs** for the detection of SDS needs that anthracene and benzene rings in 9-PAA are initially oriented on the CyD axis, and that a move to a conformational change will arise during complex formation with SDS.

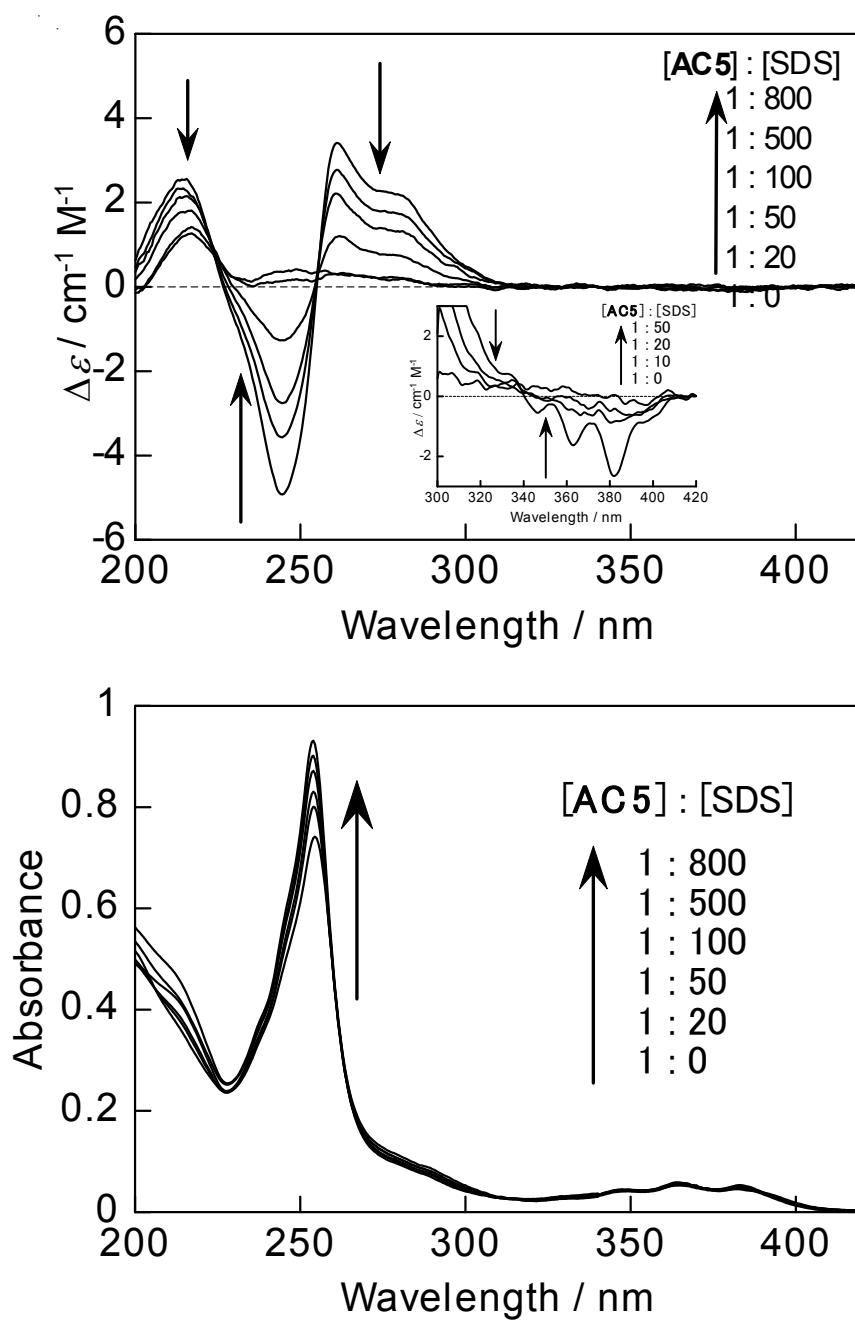


Figure. 4.6. ICD (upper) and UV-vis (lower) spectra of **AC5** and its complex with SDS in water at r.t. SDS is added to 10 μM of **AC5** solution below CMC. Inset: ICD spectra of 100 μM of **AC5** and its complex with SDS in water at r.t.

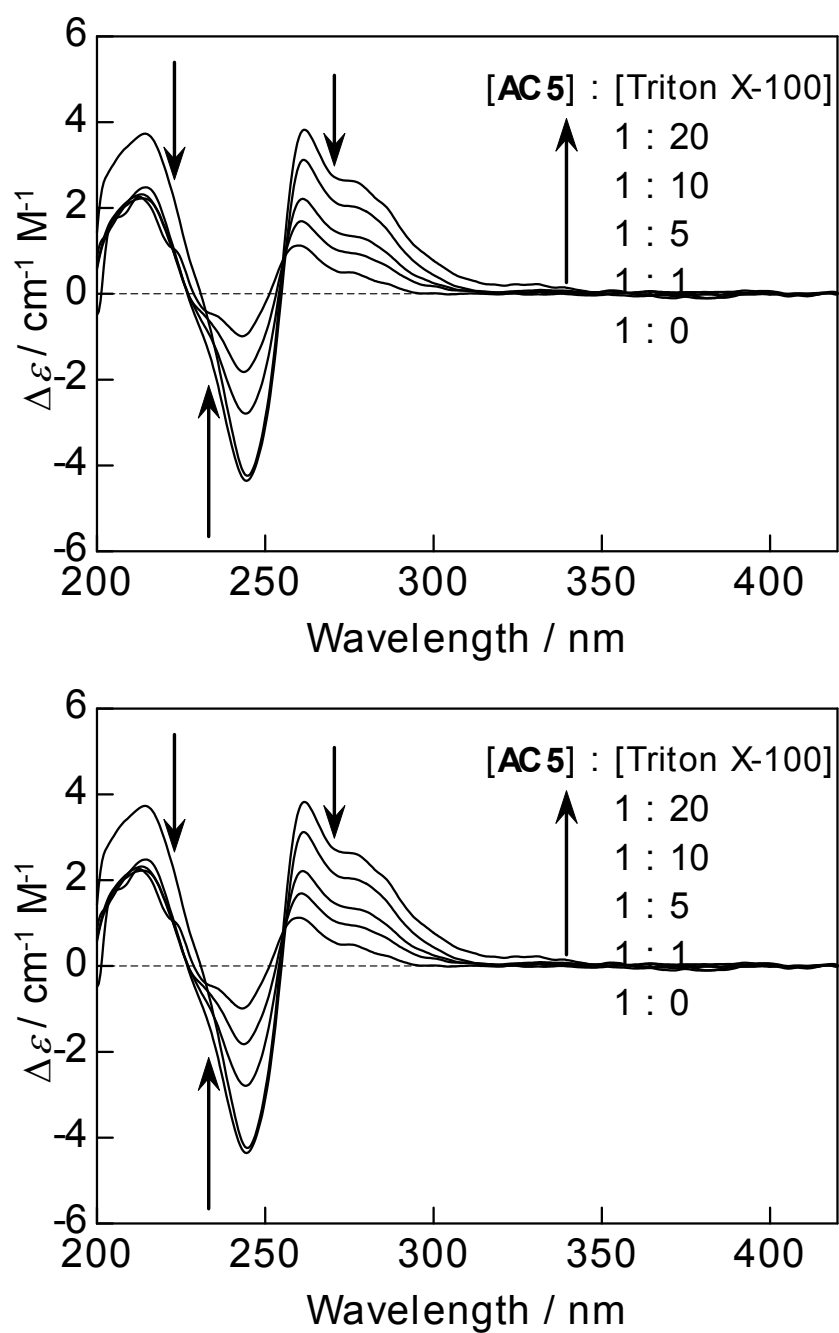


Figure 4.7. ICD (upper) and UV-vis (lower) spectra of **AC5** and its complex with TX-100 in water at r.t. TX-100 is added to 10 μM of **AC5** solution below CMC.

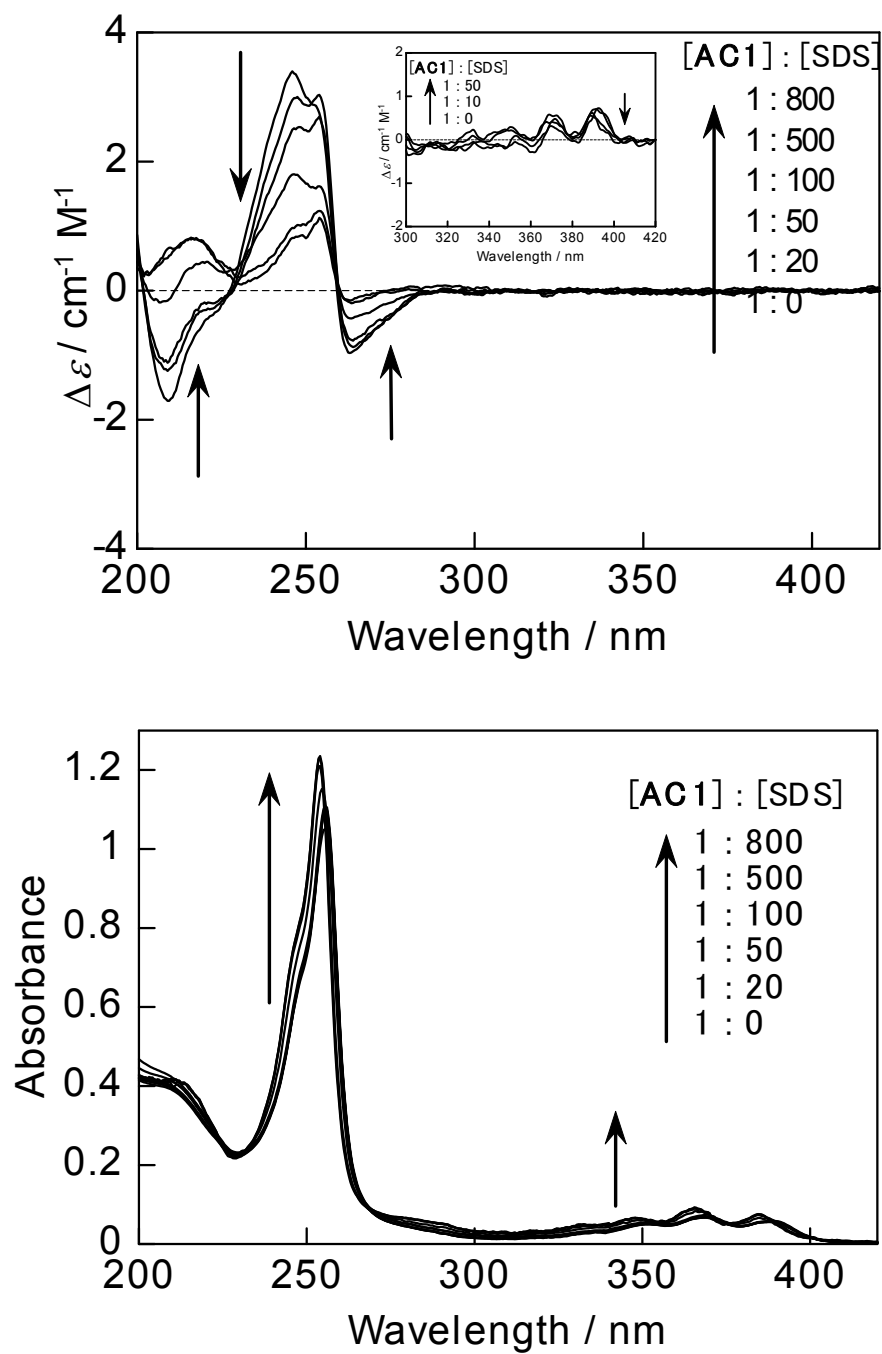


Figure 4.8. ICD (upper) and UV-vis (lower) spectra of **AC1** and its complex with SDS in water at r.t. SDS is added to 10 μM of **AC1** solution below CMC. Inset: ICD spectra of 100 μM of **AC1** and its complex with SDS in water at r.t.

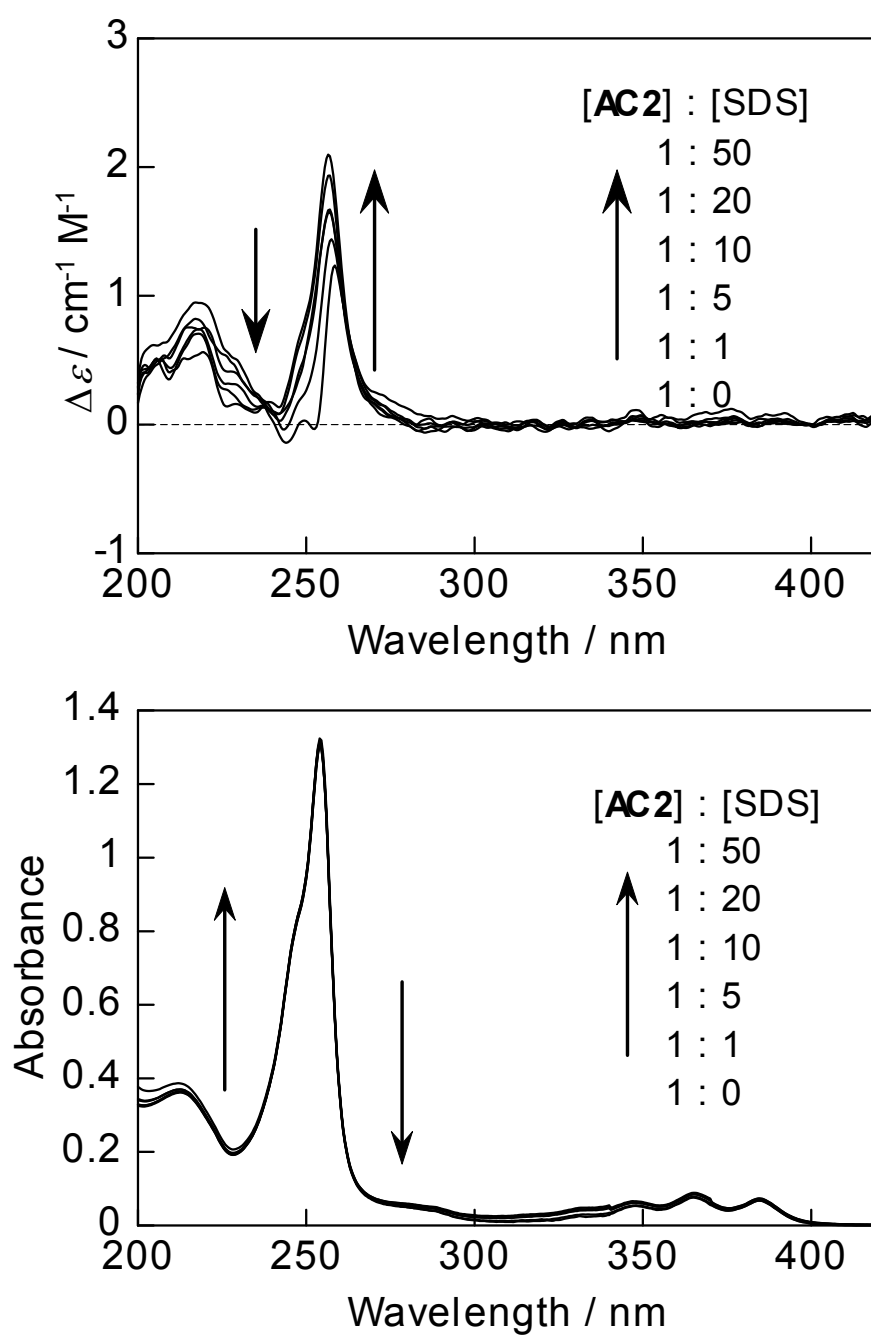


Figure 4.9. ICD (upper) and UV-vis (lower) spectra of **AC2** and its complex with SDS in water at r.t. SDS is added to 10 μM of **AC2** solution below CMC.

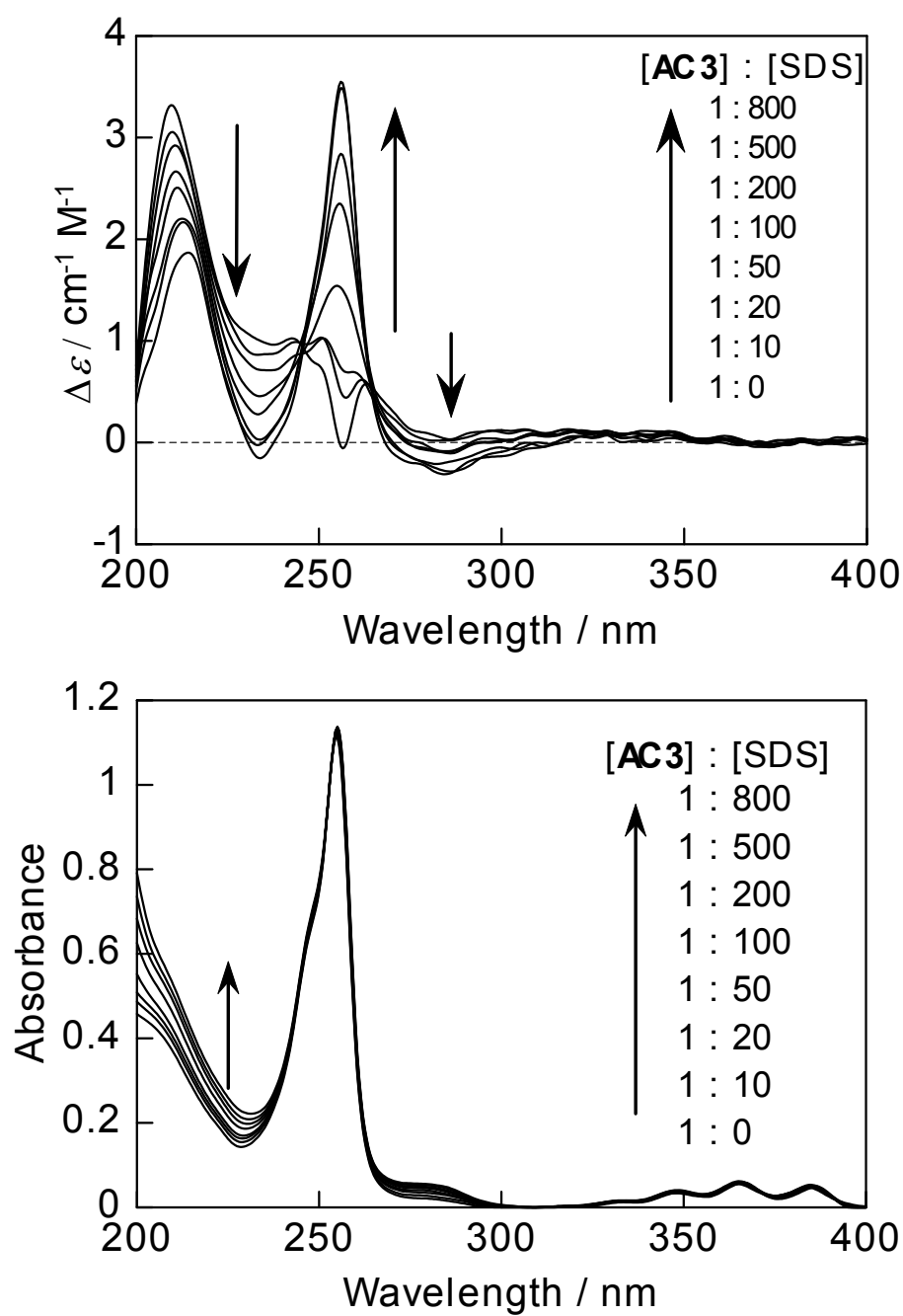


Figure 4.10. ICD (upper) and UV-vis (lower) spectra of **AC3** and its complex with SDS in water at r.t. SDS is added to 10 μM of **AC3** solution below CMC.

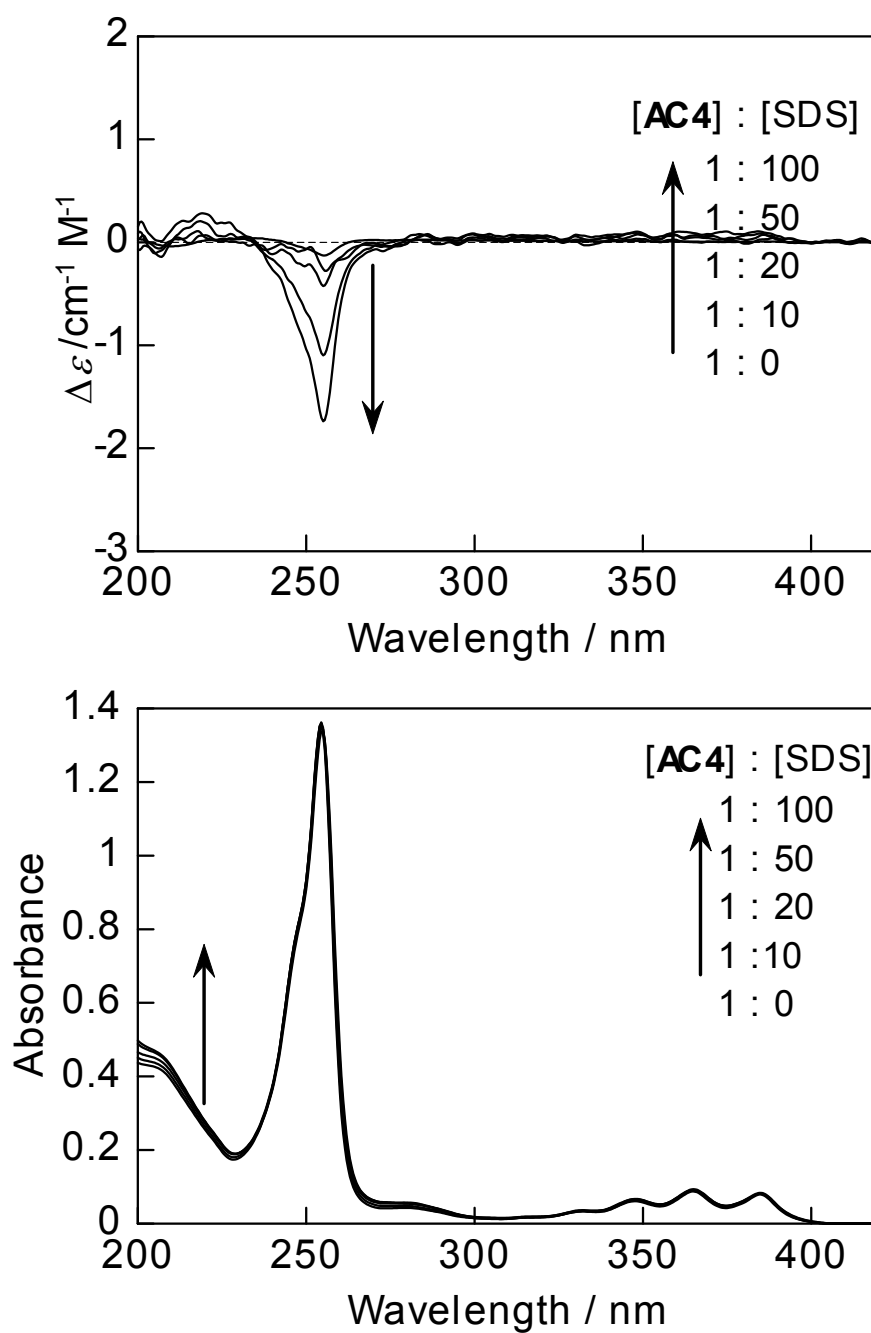


Figure 4.11. ICD (upper) and UV-vis (lower) spectra of **AC4** and its complex with SDS in water at r.t. SDS is added to 10 μM of **AC4** solution below CMC.

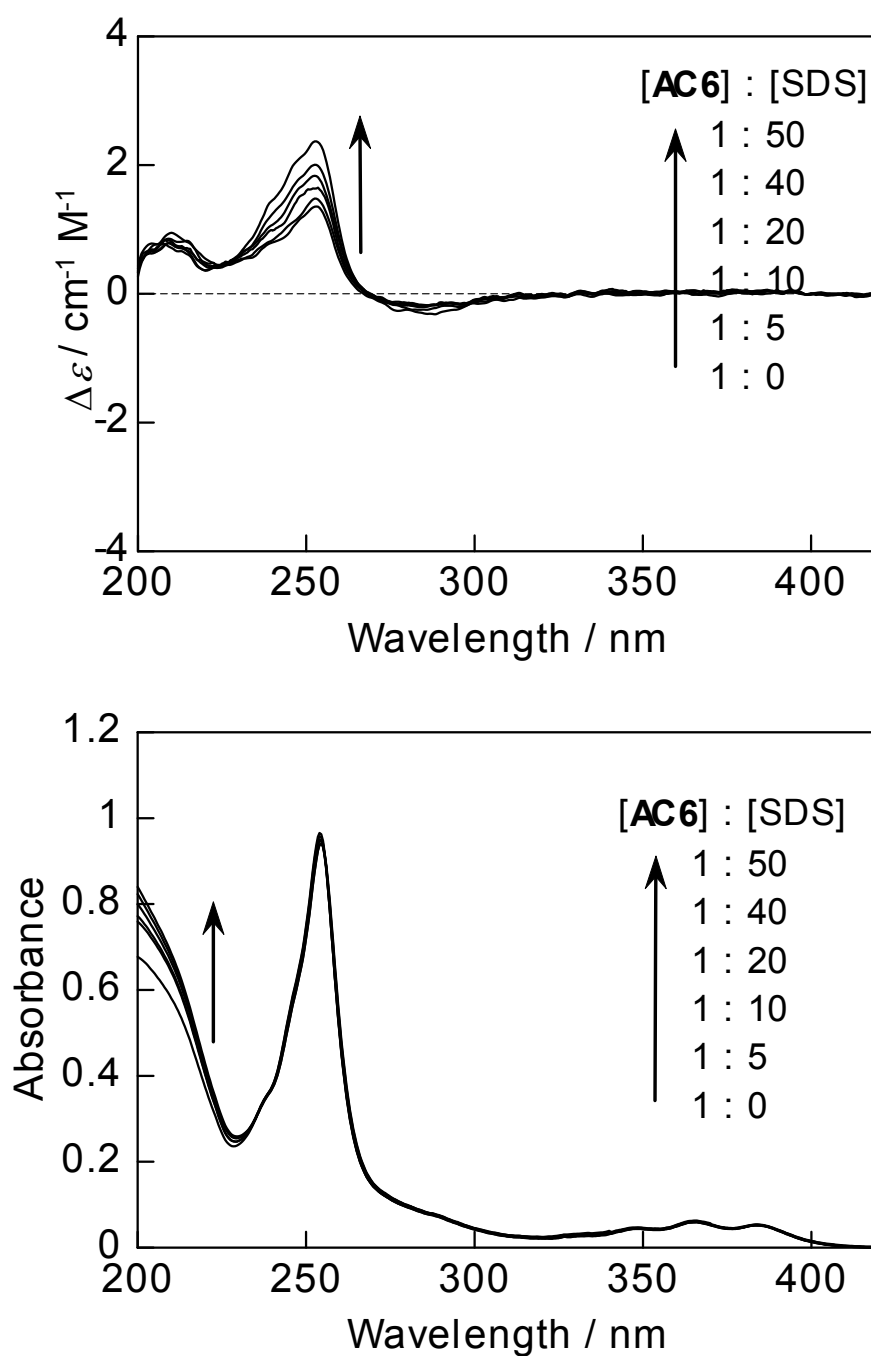


Figure 4.12. ICD (upper) and UV-vis (lower) spectra of **AC6** and its complex with SDS in water at r.t. TX-100 is added to 10 μM of **AC6** solution below CMC.

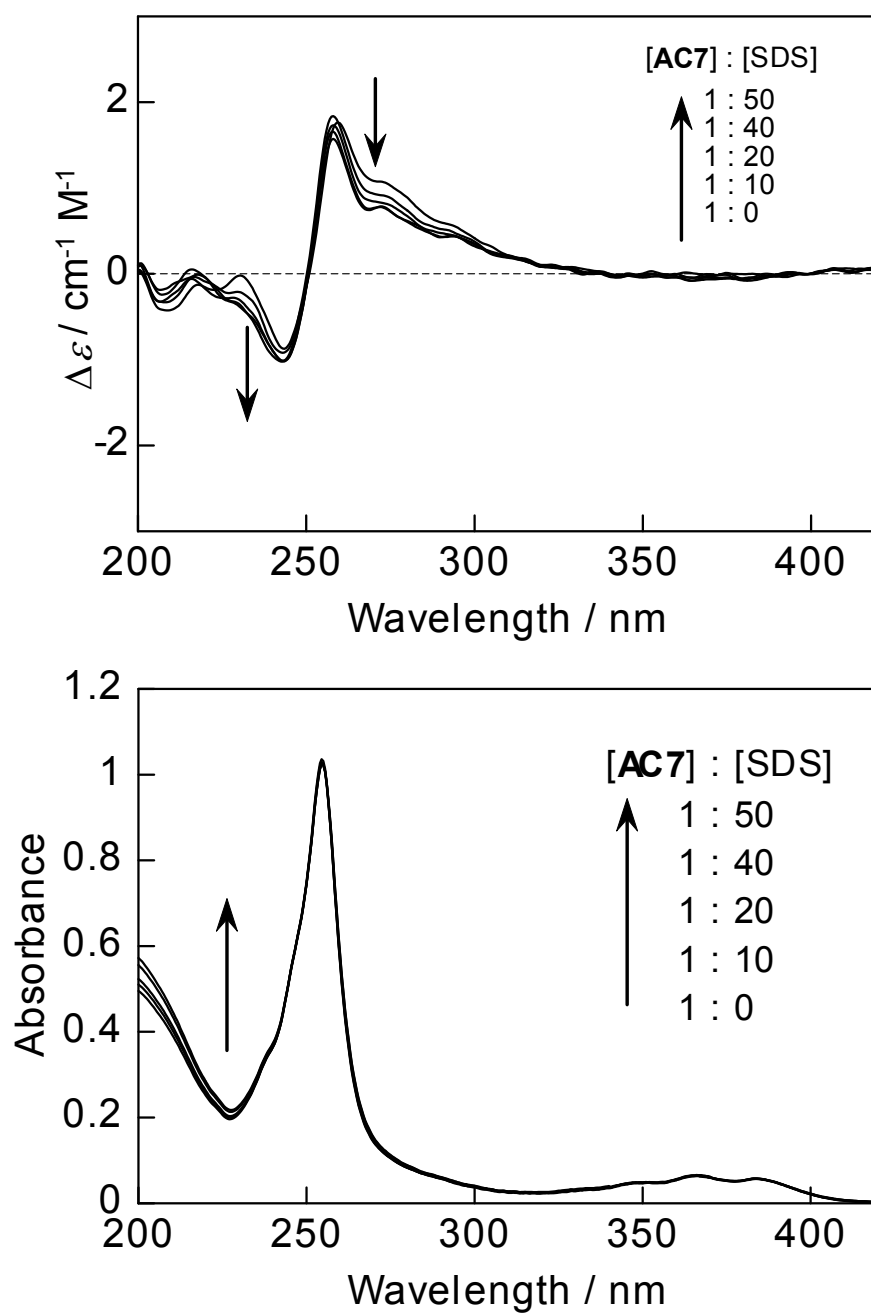


Figure 4.13. ICD (upper) and UV-vis (lower) spectra of **AC7** and its complex with SDS in water at r.t. SDS is added to 10 μM of **AC7** solution below CMC.

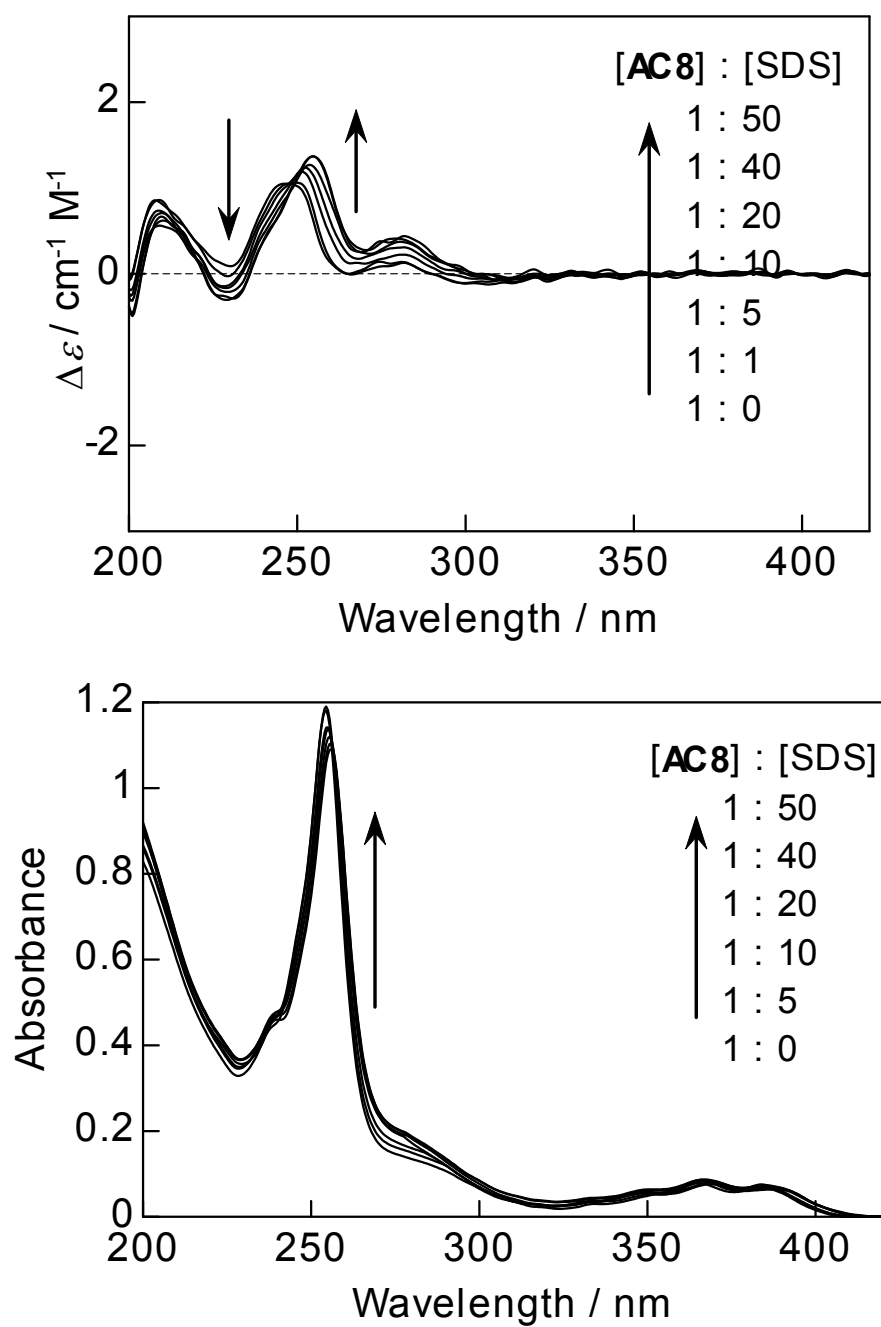


Figure 4.14. ICD (upper) and UV-vis (lower) spectra of **AC8** and its complex with SDS in water at r.t. SDS is added to 10 μM of **AC8** solution below CMC.

¹H NMR measurement

Proton NMR spectroscopy has been used for the investigation of complex conformations between host and guest molecules. The chemical shift changes ($\Delta\delta$) of SDS and TX-100 were measured in both the absence and presence of **AC1** (including 9-PAA) and **AC5** (including 1-PAA) as hosts and were shown in Fig. 4.15 and 4.16 (as ¹H NMR chart). The obtained values are presented in Table 4.2. High field chemical shift changes were observed on all protons of alkyl group (a–c) in SDS ($\Delta\delta = 0.09$ – 0.26) and TX-100 hydrophobic moieties ($\Delta\delta = 0.13$ – 0.21). They are attributed to a ring current effect on the aromatic ring in both **ACs** by approaching 9-PAA and 1-PAA with those protons in guests. In contrast, lower field shifts of alkyl protons in guests were observed for native γ -CyD as a host, which confirms that a high-field shift was induced by the interaction between hydrophobic protons in guests and aromatic rings in **ACs**.

Structural details of the complex were investigated further using the CPK model. The revealed conformations are portrayed in Fig. 4.17. The 9-AA and 1-AA moieties in the hosts were thrown up by the elongated SDS projected from the CyD cavity. Rotations of those excited moieties were inhibited. A hydrogen bond can assist an approach of SO_3^- in SDS to the 9-AA and 1-AA moiety. However, TX-100 incorporated by **ACs** was covered over by the 9-AA moiety on the CyD cavity axis. Free rotation of excited 9-AA remains possible. The interpretation presented above for the complexation behavior is consistent with results obtained from a CD spectral study. Consequently, the fluorescence response selectivity between SDS and TX-100 will reflect the steric difference from the host–guest complex structure.

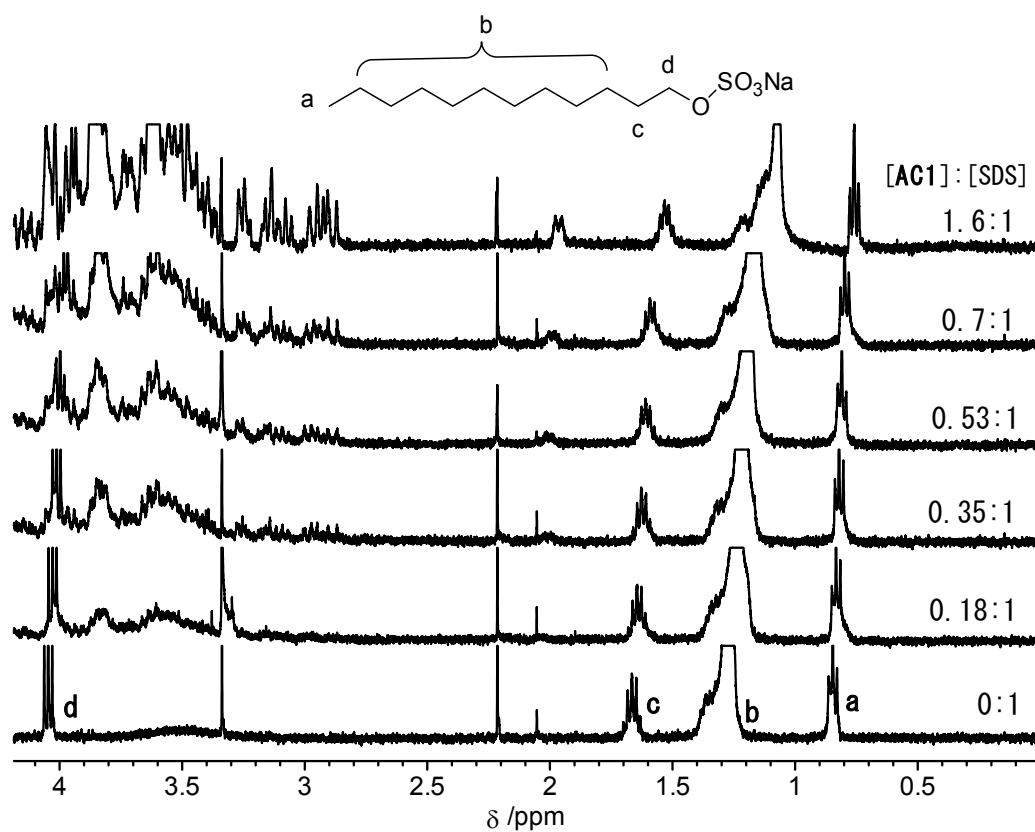


Figure 4.15. ^1H NMR spectra of SDS (0.5 mM) before and after inclusion of into AC1 in D_2O at 30°C .

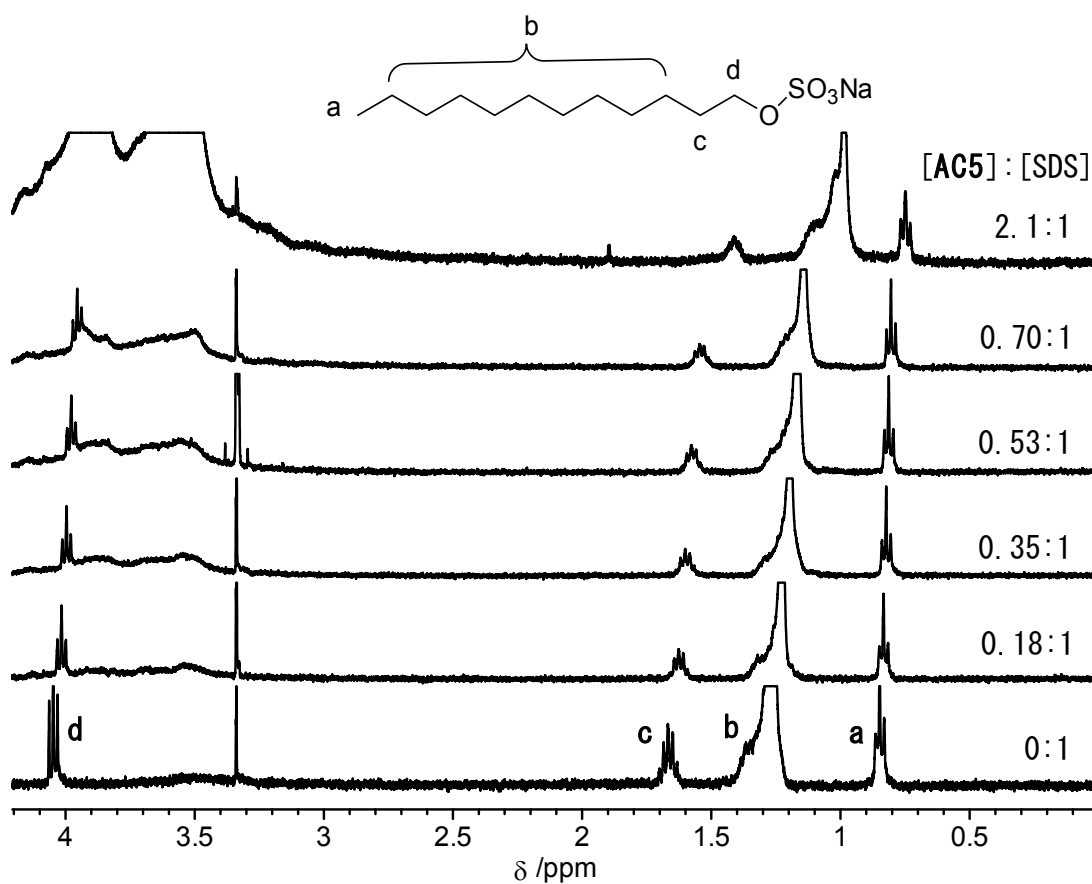


Figure 4.16. ^1H NMR spectra of SDS (0.5 mM) before and after inclusion of into **AC5** in D_2O at 30°C .

Table 4.3. ^1H NMR chemical shift changes^a ($\Delta\delta$ ppm) of guest molecules before and after complexation by **AC1**, **AC5**, and native γ -CyD

		a	b	c	d	
SDS	AC1	0.09	0.2	0.14	0.1	-
	AC5	0.1	0.29	0.26	0.18	-
	native γ -CyD	0.02	0.01	-0.04	-0.04	-
		A	B	C	D	E
T X-100	AC1	0.14	0.13	0.21	0.09	0.17
	AC5	0.06	0.11	0.08	0.24	0.14

a. Positive values show higher field shifts.

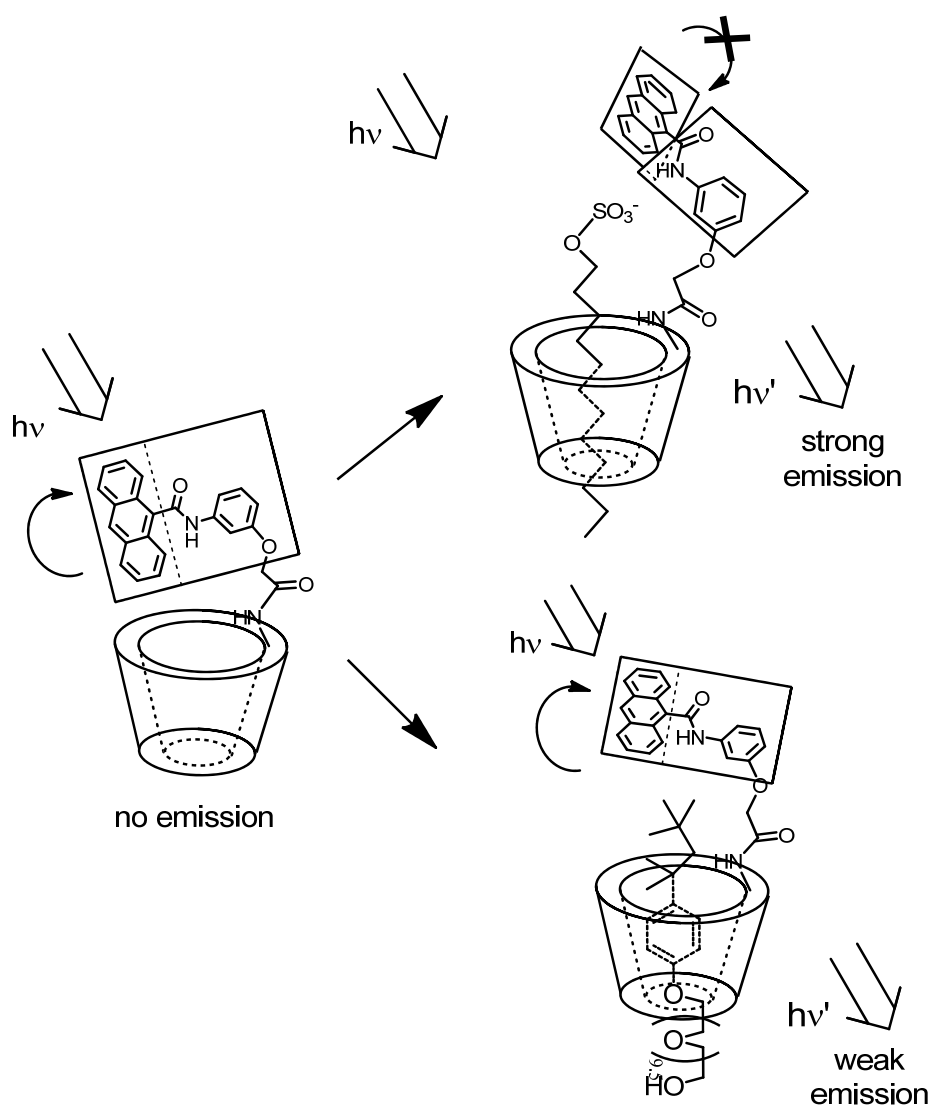


Figure 4.17. Schematic representation of TICT suppression by complexation between AC1 and SDS.

Conclusions

Fluorescence emission of *N*-phenyl-*x*-anthracenecarboxamido- γ -CyD derivatives (**ACs**; $x = 9, 1$) was enhanced selectively and strongly by sodium dodecyl sulfate (SDS) in several surfactants below the critical micellar concentration. From LOD value of **AC1** for SDS, **AC1** indicates that is promising further investigation using those CyD probe. The fluorescence enhancement efficiency was found to be related to substituent positions among **ACs**. An asymmetrically split Cotton effect in ICD spectra was observed in suitable combinations of **AC** substituents. ^1H NMR spectra measurements and CPK model investigation revealed that the fluorescence selectivity between SDS and TX-100 in **AC1** and **AC5** originates from the difference of the host–guest complex structure. Tight selectivity among configuration in all hosts reflects the precision of the supramolecular formation. This interesting result can contribute for the clarification and understanding of fluorescence switching behavior in molecular level. These findings of this study expand the application of the fluorescent chemosensors based on twisted intramolecular charge transfer toward an environmental application.

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Chapter 5

Detection of Ionic Surfactants using fluorescent probe based on Electron “Push-Pull”

Introduction

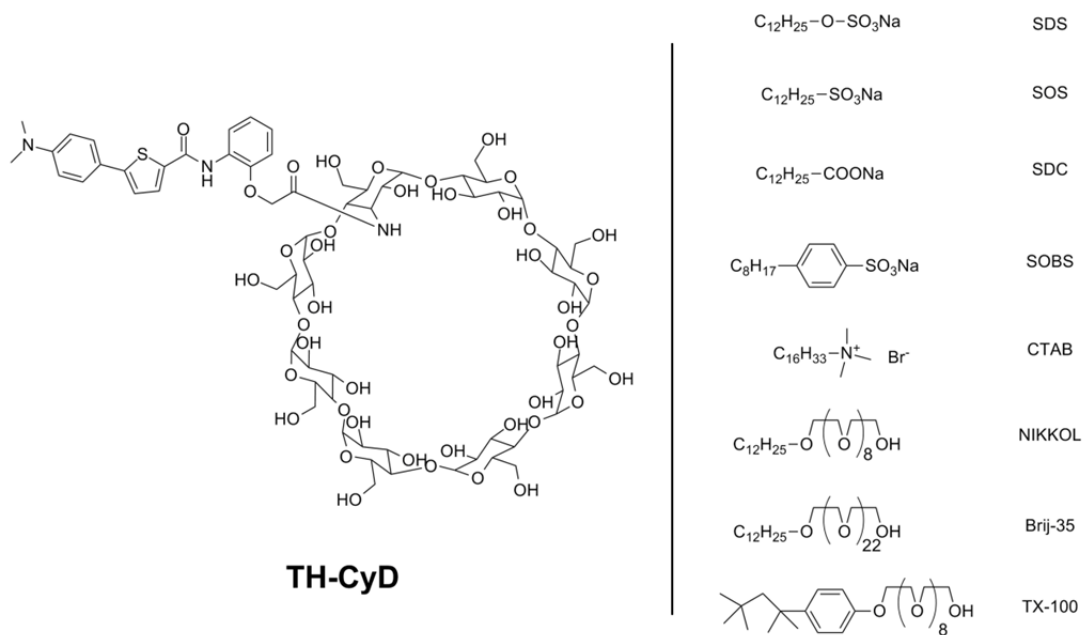
Organic fluorescent probes for chemical and biological species have been developed in various fields,¹ and many have been investigated as convenient methods for the detection of various metal ions² that are toxic or important in living systems. The use of such probes has the advantage of rapid analysis of a target material. Simple analytical systems with probes perform well in assays of degradable materials and enable spills to be dealt with rapidly *in situ*.

A visually detectable fluorescence signal enables easy identification, and does not require an elaborate instrument, so the method is satisfactory for practical applications. However, bioimaging probes have also been developed for characterizations and to enable understanding at the molecular level *in vivo*.³ Strong and visible fluorescence facilitate naked-eye analysis.⁴ However, a fluorophore emitting long wavelength are synthesized via multistage reactions to construct macro π -conjugated systems.⁵ Thus, considerable effort is expended on developing novel probes. Therefore, there is strategically benefit involving large Stokes shifts⁶ and strong “push–pull”⁷ phenomenon to the probe for small π -conjugated systems.

Many probes have macrocyclic compounds as recognition moieties,⁸ and these compounds can form complexes with various metal ions in organic solvents. These systems have been used in practical analysis. Directly detectable probes for metal ions in aqueous or water-rich solutions⁹ and reaction-based probes¹⁰ for organic compounds have recently been reported. Cyclodextrins (CyDs) can include organic compounds in their hydrophobic cavities by host–guest interactions in aqueous media. In an earlier study, CyD-based probes were constructed for cyclic compounds such as alcohols and chloric acids, based simply on the dominant equilibrium.¹¹ We previously reported novel CyD probes for surfactant determination in very low concentrations, below the critical micellar concentration (CMC).^{12,13} The fluorescence enhancement on complexation with a surfactant is explained by a twisted intramolecular charge transfer (TICT) process.¹⁴

In the present paper, we report a novel fluorescent γ -CyD (**1**) modified with *N*-phenyl-5-[4-(*N,N'*-dimethyl amino)phenyl]thiophene-2-carboxamide (DMAPTA) to produce a novel probe, as shown in Scheme 1. The *N,N'*- dimethylamino group has often been used to develop novel fluorescence characteristics because of its π -electron-donating ability.¹⁵ A probe of simple structure has the advantage that a small number of synthetic steps are involved in its development. The naked-eye fluorescence visibility of **1** was investigated below the CMC. The fluorescence selectivity among

various surfactants was studied using circular dichroism (CD). Surfactant detection was studied over a wide concentration range. The fluorescence spectra were obtained at low concentrations, around those specified in environmental standards (0.2–0.5 ppm),¹⁶ to investigate the potential use of the probe in environmental analysis.



Scheme 5.1. The structure of **TH-CyD** and guest surfactants.

Experimental

Fluorescence, UV-vis, and circular dichroism (CD) spectroscopy

Fluorescence spectra were monitored (RF-5300; Shimadzu Corp, Japan) in distilled water at 25 °C. The excitation wavelength was 382 nm. Guest molecules were added to 2.5 μ M **TH-CyD** containing 0.01 M phosphate buffer. UV-vis spectra were also observed (UV-2400PC; Shimadzu Corp.) under the same conditions. CD spectra were recorded (J-720; Jasco Corp, Japan) for 25 μ M **TH-CyD** with a surfactant (0–8 mM) under N₂ gas at room temperature.

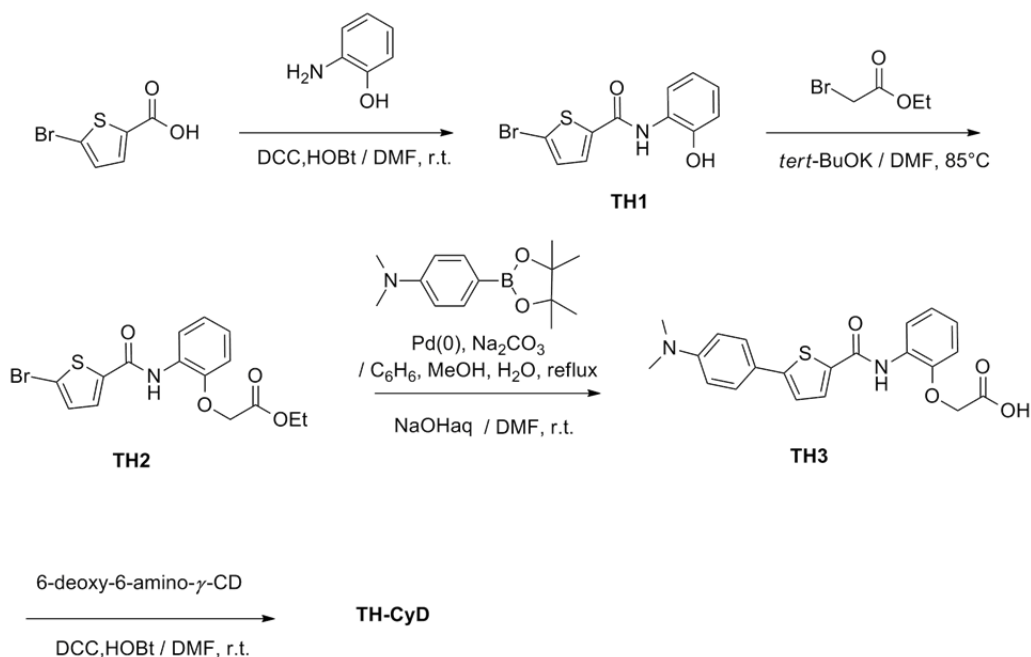
¹H and ¹³C NMR spectroscopy

¹H NMR spectra were recorded in DMSO-*d*₆ using a JNM-Ex-400 JEOL spectrometer, at 30 °C, for identifications of the compounds prepared in this study. The chemical shift values (δ : ppm) are reported relative to tetramethylsilane.

Synthesis of TH-CyD

Compound **TH-CyD** was prepared as shown in Scheme 5.2.

Scheme 5.2.



Synthesis of *N*-(2-hydroxyphenyl)-5-bromothiophene-2-carboxamide (**TH1**)

2-Aminophenol (0.55 g, 5 mmol) and HOBt (0.76 g, 5 mmol) were added to a solution of 5-bromothiophene-2-carboxylic acid (1.04 g, 5 mmol) in 5 mL of DMF. After addition of DCC (1.03 g, 5 mmol) to the solution, the mixture was stirred for 8 h at room temperature. The precipitate was removed by filtration. The crude product was precipitated from the filtrate by addition of distilled water. The crude compound was collected by filtration and purified by flash column chromatography (*n*-hexane:CHCl₃ = 7:3). Compound **TH1** was obtained as a white solid (0.95 g, 64%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.81(t, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 7.05 (t, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 4.0 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 4.0 Hz, 1H), 9.61 (s, 1H), 9.67 (s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 115.7, 117.1, 118.6, 124.4, 125.1, 126.0, 129.5, 131.4, 141.3, 150.0, 158.5. Found: C, 44.21; H, 2.89; N, 4.72; S, 10.75. Calcd. for C₁₁H₈NO₂SBr: C, 44.31; H, 2.70; N, 4.70; S, 10.75. High-resolution ESI-MS 319.9350 ([M + Na]⁺). Calcd. for C₁₁H₈O₂NSBrNa: 319.9351.

Synthesis of ethyl 2-(5-bromothiophene-2-carboxamido)phenoxyacetate (**TH2**)

t-BuOK (0.19 g, 1.7 mmol) and ethyl 2-bromoacetate (0.26 g, 1.7 mmol) were added to a solution of **TH1** (0.48 g, 1.4 mmol) in 10 mL of DMF. The mixture was stirred for 8 h at 85 °C. After filtration, the filtrate was added slowly to distilled water. The crude compound was collected by filtration and purified by flash column chromatography (*n*-hexane:CHCl₃ = 3:7). Compound **S2** was obtained as a light-yellow solid (0.34 g, 63%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.20 (t, *J* = 7.1 Hz, 3H), 4.17 (m, *J* = 7.1 Hz, 2H), 4.85 (s, 2H), 7.02 (t, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 7.16 (dd, *J* = 7.8 Hz, *J* = 1.7 Hz, 1H), 7.37 (d, *J* = 4.0 Hz, 1H), 7.74 (dd, *J* = 7.8 Hz, *J* = 1.5 Hz, 1H), 7.80 (d, *J* = 4.0 Hz, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 13.9, 60.7, 66.3, 113.9, 117.5, 121.4, 124.1, 125.7, 126.7, 129.4, 131.5, 141.3, 149.8, 158.3, 168.8. Found: C, 46.60; H, 3.75; N, 3.62; S, 8.37. Calcd. for C₁₅H₁₄O₄NSBr: C, 46.89; H, 3.67; N, 3.65; S, 8.34. High-resolution ESI-MS 405.9717 (M⁺). Calcd. for C₁₅H₁₄O₄NSBrNa: 405.9719.

Synthesis of 2-[5-(4-*N,N*-dimethylamino)thiophene-2-carboxamido]phenoxyacetic acid (**TH3**)

N,N-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.10 g, 0.40 mmol), **TH2** (0.15 g, 0.39 mmol), Na₂CO₃ (0.17 g, 2.0 mmol), and

tetrakis(triphenylphosphine)Pd(0) (40 mg) were added to an eggplant flask, and the internal air was purged by argon gas for 30 min. The reagents in the eggplant flask were suspended in 12 mL of a mixed solvent (benzene : MeOH : distilled water = 8 : 2 : 2), and refluxed for 12 h in an argon flow. The solution was neutralized with dilute hydrochloric acid and extracted with CHCl₃. The crude product in 10 mL of DMF was added to 10 mL of distilled water containing 0.1 g of NaOH, and the mixture was stirred for 3 h. The mixture solution was neutralized with hydrochloric acid to the low acidic region (pH $\approx$ 4). The precipitate was obtained by filtration and purified by flash column chromatography (*n*-hexane : CHCl₃ = 3 : 7). Compound **S3** was obtained as a light-yellow solid (0.09 g, 58 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.96 (s, 6H), 4.79 (s, 2H), 6.76 (d, *J* = 9.0 Hz, 2H), 7.03 (t, *J* = 7.5 Hz, 1H), 7.10 (m, 2H), 7.37 (d, *J* = 3.9 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.87 (d, *J* = 3.9 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 9.60 (s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 39.8, 67.1, 112.1, 114.7, 120.4, 121.3, 121.7, 122.4, 124.7, 126.5, 127.9, 129.8, 135.3, 149.1, 149.9, 150.3, 159.2, 170.9. Found: C, 62.98; H, 5.14; N, 7.05; S, 7.82. Calcd. for C₂₁H₂₀O₄N₂S: C, 63.62; H, 5.08; N, 7.07; S, 8.09. High-resolution ESI-MS 395.1072 ([M – H][–]). Calcd. for C₂₁H₁₉O₄N₂S: 395.1071.

*Synthesis of 2-[5-(4-*N,N*-dimethylaminophenyl)thiophene-2-carboxamido]phenoxyacetamido-3A-deoxy-(2AS,3AS)- γ -cyclodextrin (**TH-CyD**)*

3A-amino-3A-deoxy-(2AS,3AS)- γ -cyclodextrin (0.13 g, 0.10 mmol) and HOBt (0.02 g, 0.1 mmol) were added to a solution of **TH3** (0.04 g, 0.1 mmol) in 10 mL of DMF. After addition of DCC (0.02 g, 0.1 mmol) to the solution, the mixture was stirred for 8 h at room temperature. The deposit in the solution was removed by filtration. The crude product was precipitated from the filtrate by addition of 50 mL of acetone. The crude compound was purified by HPLC (H₂O:MeOH = 5:5). **TH-CyD** was obtained as a white solid (0.08 g, 48%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.21–3.80 (including overlap with H₂O peak br, ca. 52H), 3.89 (s, 1H), 4.16 (s, 1H), 4.44–5.02 (m, 16H), 5.48–5.80 (m, 16H), 6.75 (d, *J* = 9.0 Hz, 2H), 7.04 (m, 1H), 7.12 (s, 1H), 7.13 (s, 1H), 7.39 (d, *J* = 4.0 Hz, 1H), 7.58 (d, *J* = 9.0 Hz, 2H), 7.90 (d, *J* = 4.1 Hz, 1H), 7.97 (d, *J* = 7.9 Hz, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 9.80 (s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 50.9, 59.6–60.1, 69.0, 70.1, 71.5, 72.1–73.0, 78.8, 79.8, 80.2, 80.4, 80.5, 80.9, 81.3, 101.0, 101.3, 101.6, 101.8, 102.2, 103.8, 112.1, 115.1, 120.6, 121.6, 121.8, 122.6, 124.8, 126.5, 128.0, 130.4, 135.0, 149.23, 149.6, 150.2, 159.4, 168.0. Found: C, 48.58; H, 6.05; N, 2.51; S, 1.85. Calcd. for C₆₉H₉₉O₄₂N₃S•2H₂O: C, 48.45; H, 6.07; N,

2.46; S, 1.87. High-resolution ESI-MS 1672.5351 ($[M - H]^-$). Calcd. for $C_{69}H_{98}O_{42}N_3S$: 1672.5340.

Result and Discussion

Fluorescence spectra

Figure 5.1 shows the fluorescence spectra of **TH-CyD** in the absence and presence of sodium dodecyl sulfate (SDS) at a concentration below the CMC.¹⁷ Free **TH-CyD** showed a slight fluorescence emission at a maximum wavelength ($\lambda_{\max}$) of 505 nm. This solution was almost colorless (Fig. 5.1) in a bright room. However, the fluorescence enhancement of **TH-CyD** was observed in addition of SDS to be 8-folds with a bathochromic shift to $\lambda_{\max} = 525$ nm. The visible green-yellow luminescence from the cell solution was clearly observed by the naked eye (Fig. 5.1). The fluorescence spectra of **TH-CyD** were used to compare fluorescence selectivities among various surfactants. SDS is a typical surfactant, so various SDS analogs were used: sodium dodecylsulfonate (SOS), sodium octylbenzenesulfonate (SOBS), sodium dodecyl carbonate (SDC), cetyltrimethylammonium bromide (CTAB), NIKKOL BL-9EX (NIKKOL), Brij-35, and TX-100 (TX-100).¹⁸ These surfactants, except TX-100, have a linear alkyl group as the hydrophobic moiety. To evaluate the guest selectivity, the enhancement intensity factors ($I_{\max}/I_0$) of the fluorescence emissions were calculated (Fig 5.2), where I_0 and $I_{\max}$ are the minimum and maximum fluorescence intensities, respectively, of **TH-CyD**. The final concentrations of all the surfactants were unified to 8 mM because the CMCs of the surfactants differ. Although the concentrations of CTAB and all the nonionic surfactants greatly exceeded the CMCs, these surfactants slightly enhanced the fluorescence both below and above the CMC. As mentioned above, probe **TH-CyD** selectively detected ionic surfactants, and the signal was visible to the naked eye below CMCs of anionic surfactants.

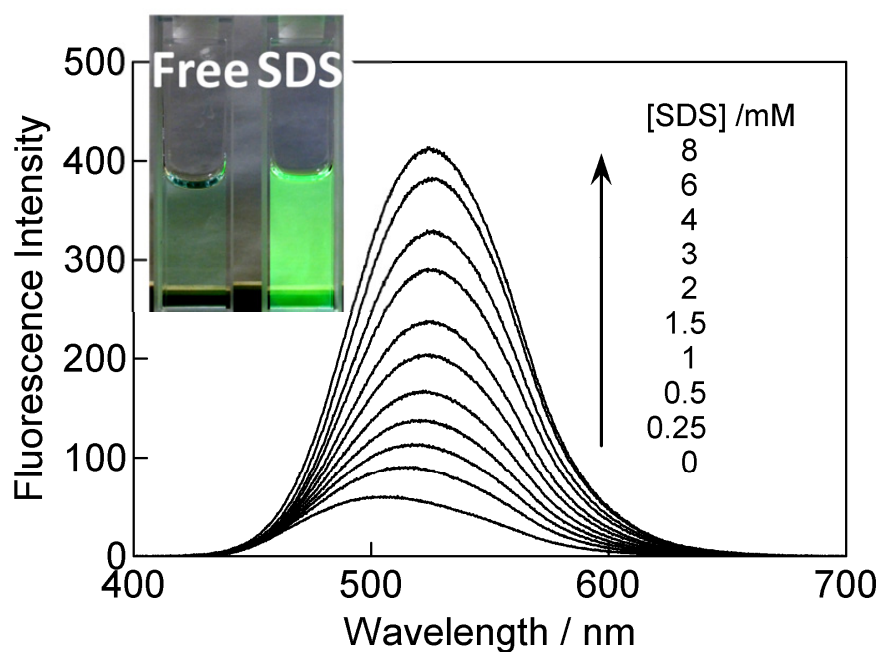


Figure 5.1. Fluorescence spectra of **TH-CyD** with and without SDS in phosphate buffer (0.01 M) at 25 °C. [**TH-CyD**] = 2.5 μ M. Excitation: 382 nm. Photograph: free **TH-CyD** (Free) and **TH-CyD** with 8 mM SDS.

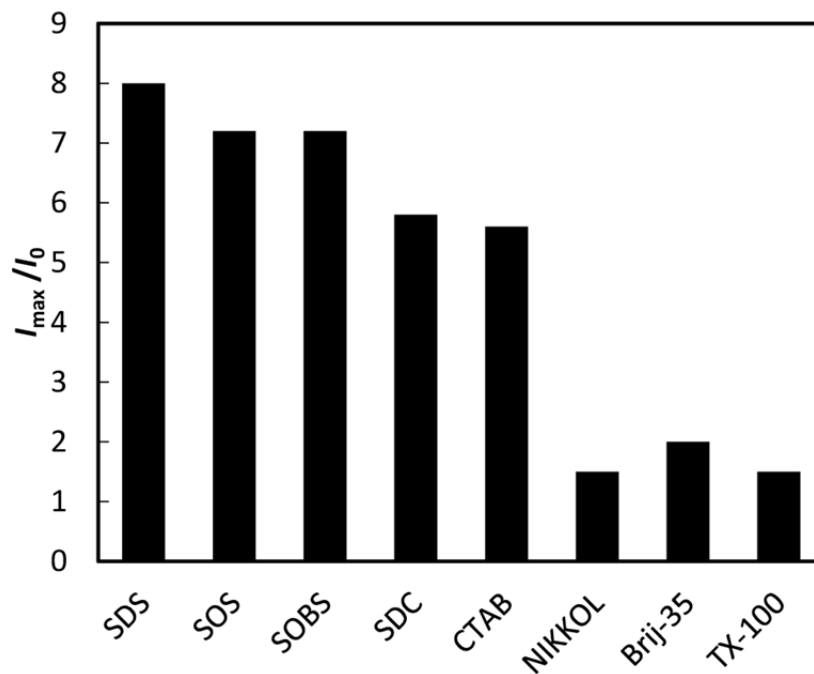


Figure 5.2. Fluorescence response ratios ($I_{\max}/I_0$) of **TH-CyD** without and with 8 mM surfactant in phosphate buffer (0.01 M) at 25 °C. [**TH-CyD**] = 2.5 μ M; excitation 382 nm.

Complex formation constant log K

The obtained 1:1 complex formation constants ($\log K$) were as follows: 2.52 (SDS), 2.09 (SOS), 3.83 (NIKKOL), and 4.15 (TX-100). All the $\log K$ values of the guests, determined using the nonlinear least-squares curve-fitting method, for fluorescence intensity changes below the CMC are listed in Table 5.1. Appropriate curve fitting (Fig. 5.3) indicates that the complexation of **TH-CyD** with surfactants is predominantly a simple 1:1 equilibrium. Interestingly, the $\log K$ values of the nonionic surfactants tend to be higher than those of the ionic surfactants. It is evident that the fluorescence selectivity of **TH-CyD** is mainly determined by $I_{\max}/I_0$. The fluorescence spectrum of precursor **TH3** without CyD (Fig. 5.3 upper) was also obtained under the same conditions as those used for **TH-CyD**. As expected, the fluorescence intensity of **TH3** increased only slightly, with $\lambda_{\max} = 542$ nm, even at the CMC. This result indicates that the visible increase in the fluorescence of **TH-CyD** on addition of SDS can be attributed to complexation in the CyD cavity of **TH-CyD**. Yamada *et al.* reported solvatochromism of DMAPTA analogs, in which the emission maximum wavelength increased with increasing solvent polarity.¹⁹ The polarity of the CyD cavity is nearly equal to that of dioxane. The DMAPT moiety of free **TH-CyD** approached the hydrophobic CyD cavity of **TH-CyD**. TICT control has often been investigated based on solvent viscosity.²⁰ In glycerin, the fluorescence emission of free **TH-CyD** was significantly stronger than that in aqueous solution (Fig. 5.3 lower), as previously reported for TICT chemosensors.¹² These results indicate that the fluorescence enhancement mainly originated from efficient TICT suppression.

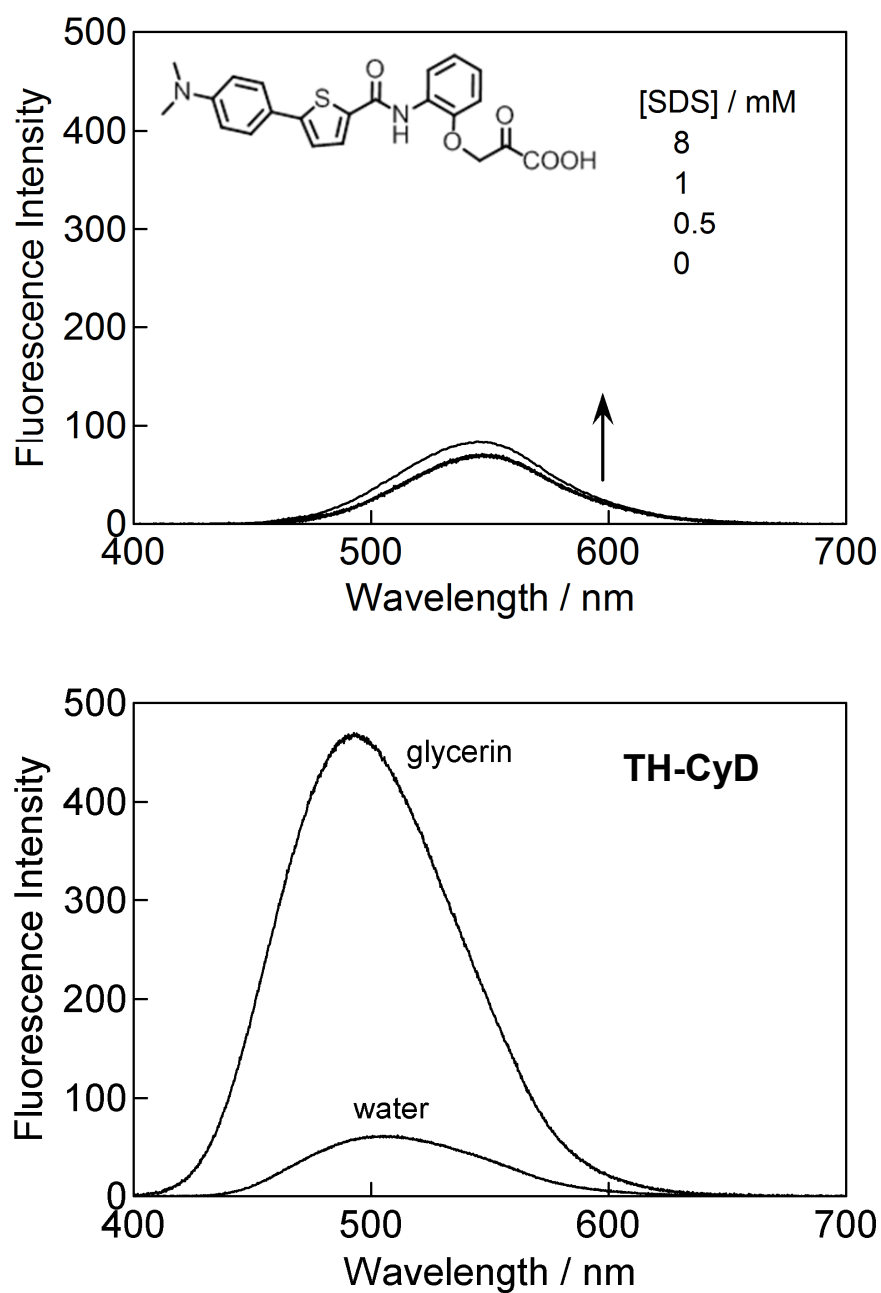


Figure 5.3. Upper: fluorescence spectra of 2.5 μM **TH3** in 0.01 M phosphate buffer, water, and glycerin at 25 $^{\circ}\text{C}$. Lower: fluorescence spectra of 2.5 μM **TH-CyD** in water at 25 $^{\circ}\text{C}$.

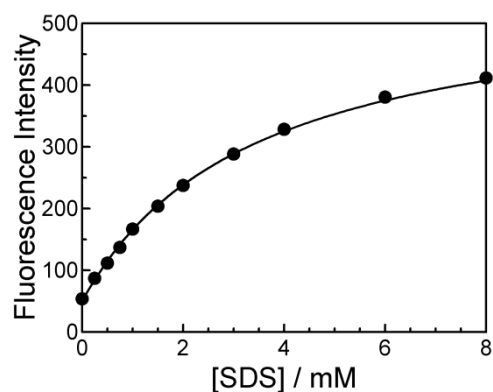


Figure 5.4. Dependence of fluorescence intensity at 525 nm on SDS concentration, and theoretical curve for formation of 1:1 complex. $[\text{TH-CyD}] = 2.5 \mu\text{M}$ at 25°C .

Table 5.1. 1:1 Complex formation constants ($\log K^a$) of **TH-CyD** with various surfactants below CMC in 0.01 M phosphate buffer at 25°C

	SDS	SOS	SOBS	SDC	CTAB	NIKKOL	Brij-35	TX-100
$\log K$	2.52	2.09	2.05	2.1	3.96	3.83	4.00	4.15
$I_{\max}/I_0$	8.0	7.2	7.2	5.8	5.6	1.5	2	1.5

^a $K = [\text{complex}] / ([\text{TH-CyD}][\text{guest}])$. The standard deviation of the $\log K$ was estimated to be ± 0.03

Induced Circular dichroism (ICD) spectra

To investigate the structures of the complexes of **TH-CyD** with surfactants, induced circular dichroism (ICD) spectra were obtained. The ICD of **TH-CyD** with guest compounds could show whether the chromophore position is inside the CyD cavity and whether its axis is through the narrow or wide rim.²⁰ Figure 5.5 shows the ICD spectra of **TH-CyD** in the absence and presence of SDS at concentrations less than the CMC. The ICD signal decreased with increasing SDS concentration; this indicates a conformational change in the TICT moiety as it is released from the hydrophobic CyD cavity to bulk water. It is evident that TICT suppression of the fluorescent moiety is attributable to steric crowding between SDS and the DMATP moiety of **TH-CyD**. In contrast, NIKKOL, whose hydrophilic group is different from that of SDS, did not change the ICD signal of **TH-CyD** at any concentration (0–8 mM, Fig. 5.6). This behavior suggests that NIKKOL was not incorporated into the CyD cavity of **TH-CyD**, so the fluorescent moiety did not cause TICT suppression by photoexcitation.

The structures of the complexes with SDS and NIKKOL are shown in Fig. 5.7. The hydrophobic moiety in SDS is strongly incorporated into the CyD cavity, and the ionic moiety in bulk water forms hydrogen bonds with the amido moiety in **TH-CyD**. As a result, TICT rotation of the amido moiety in **TH-CyD** is suppressed. In the case of CTAB, TICT restriction was assisted by the approach of the trimethylammonium group, which resulted in strong inclusion of the hydrophobic cetyl group in the CyD cavity. However, nonionic surfactants have no attractive interactions between the host and the guest.

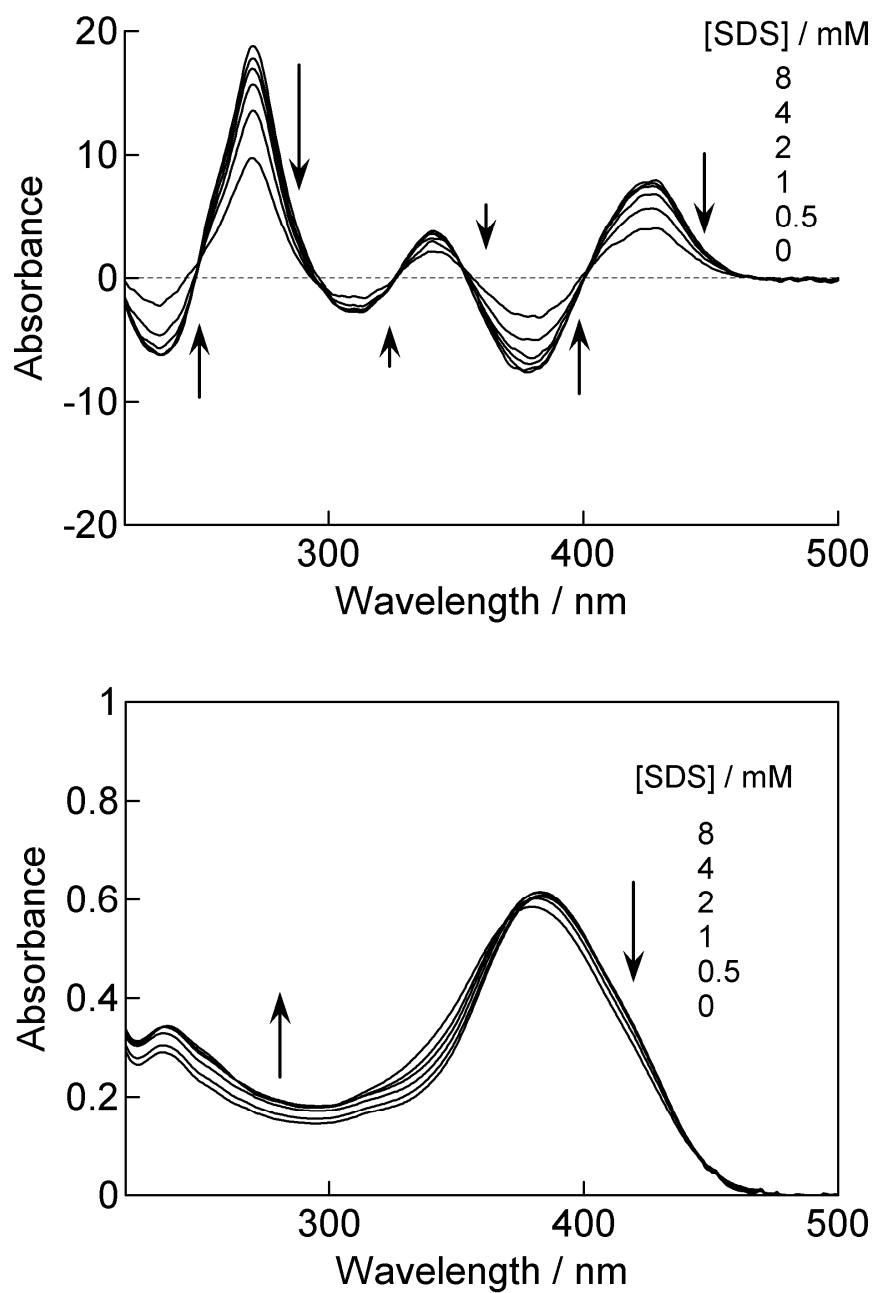


Figure 5.5. ICD (upper) and UV-vis (lower) spectra of **TH-CyD** with 0–8 mM SDS in water at room temperature. [**TH-CyD**] = 25 μ M.

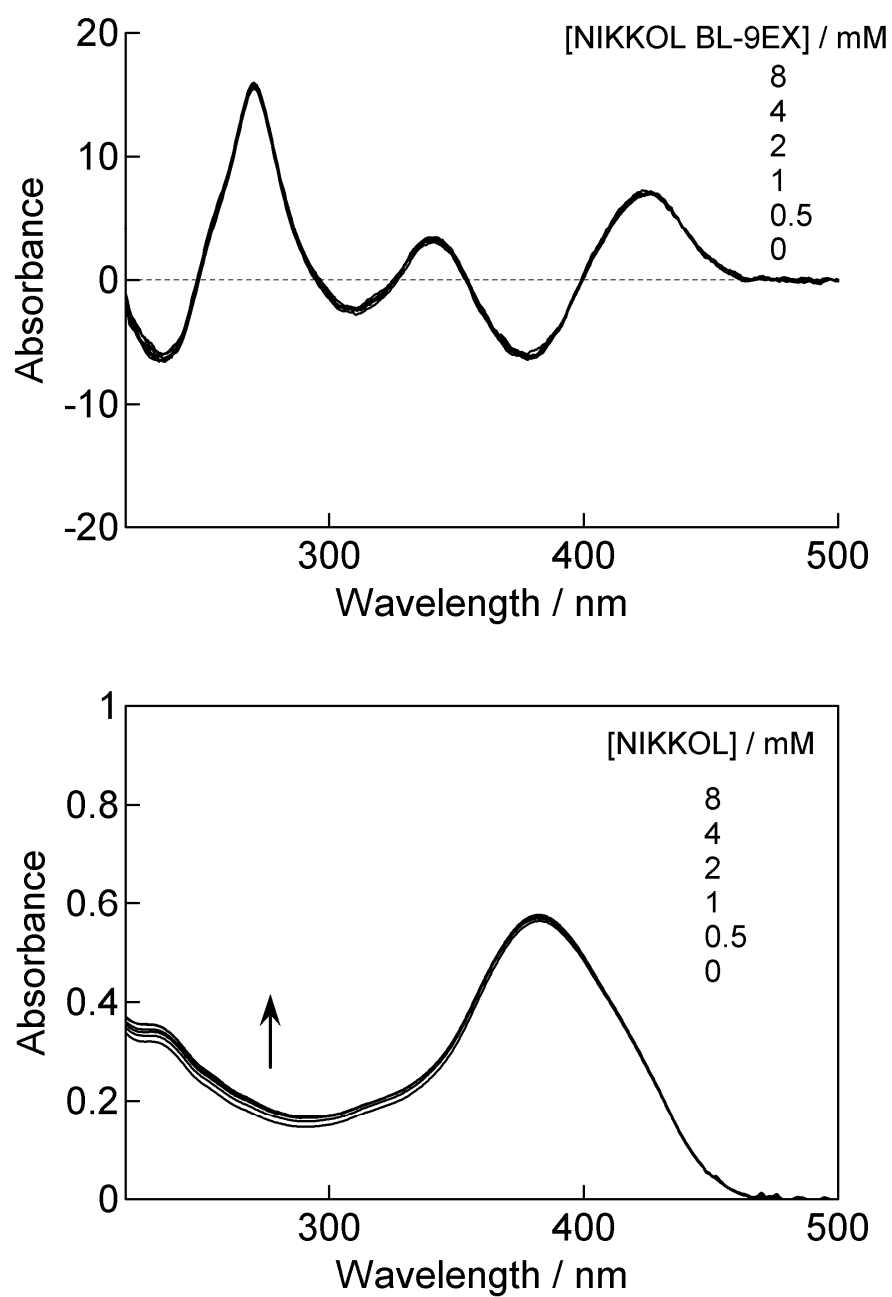


Figure 5.6. ICD (upper) and UV-vis (lower) spectra of **TH-CyD** with 0–8 mM NIKKOL BL-9EX in water at room temperature. [**TH-CyD**] = 25 μ M.

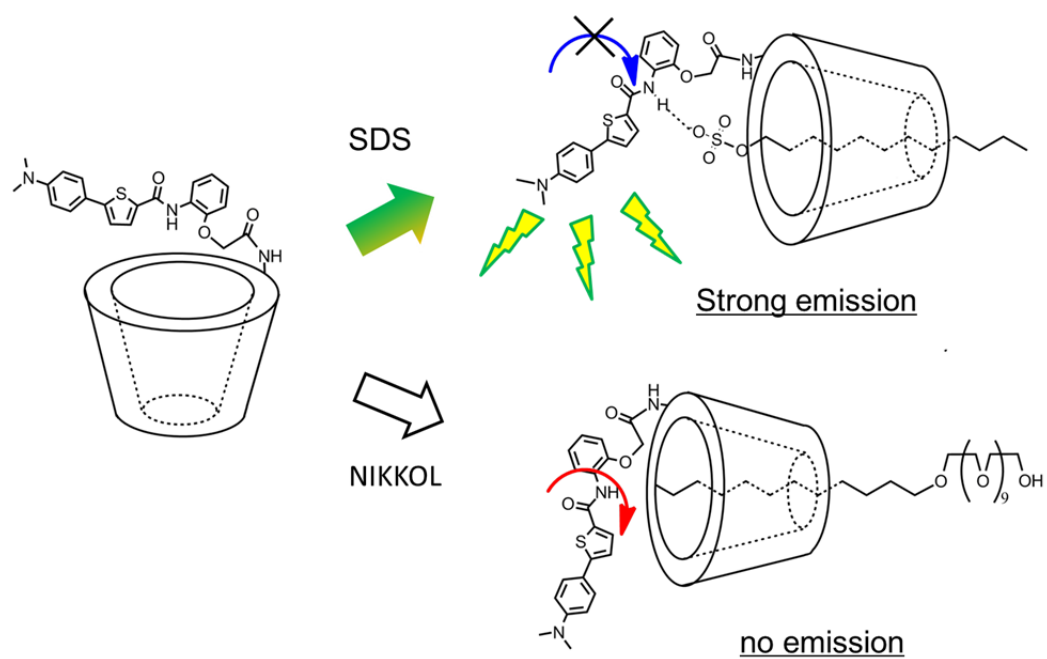


Figure 5.7. Schematic representation of complexation between **TH-CyD** and surfactants (SDS and NIKKOL).

Limit of detection (LOD)

The limit of detection (LOD) of SDS was calculated to be 0.10 ppm from standard curve was well fitted ($R^2 = 0.99$) in low concentration area (0-0.71 ppm) as Fig. 5.8; a margin of error for fluorescence intensity was estimated as three-folds of standard deviation (3σ) for the background noise observed from 10 times measurements. The LOD value amply satisfies the surfactant detection requirements of the United States Environmental Protection Agency (USEPA; 0.5 ppm) and the Ministry of Health, Labour, and Welfare in Japan (MHLW; 0.2 ppm). The lower LOD value for SDS compared with those obtained in previous studies is probably the result of an improved signal/noise ratio, which would increase the fluorescence intensity of free **TH-CyD**. The wide dynamic range of **TH-CyD** makes it suitable for various analytical applications, for instance, as a convenient environmental analysis technique, using a portable device, enabling rapid identification of gross pollution areas after accidents.

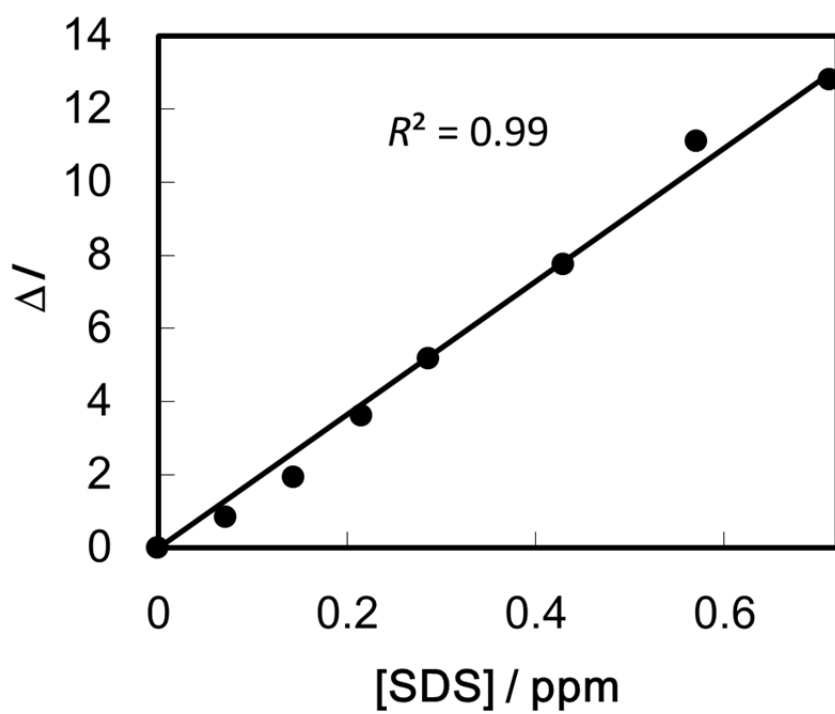


Figure 5.8. Calibration curve for fluorescence intensities of **TH-CyD** with SDS at 505 nm and 25 °C; . 25 μ M **TH-CyD** in 0.01 M phosphate buffer.

pH dependence

Many real samples have neutral pH values between 6 and 8.5. To investigate a potential for an application, pH tolerability of **TH-CyD** was tested on the fluorescence characteristic using five kinds of phosphate buffers with 6 to 8 of pH. For all weak acidic and basic solution, fluorescence intensity and log *K* values of **TH-CyD** were closely similar to that of neutral condition (pH = 6.98) in the absence and presence (8 mM) of SDS (Fig. 5.9 and Table 5.2). The positive outcome indicates that **TH-CyD** probe no affects any slight perturbation for the fluorescence enhancement and supramolecular complexation, and therefore expects the detectable of the surfactant upon the good accuracy. It is noted that basic *N*, *N*-dimethylaminophenyl (DMAP) group in **TH-CyD** is clearly affected by large pH alternative. In fact, low pH (< 4) region showed the dramatically decreasing the fluorescence intensity of **TH-CyD**. The result clearly indicates that **TH-CyD** lost “push-pull” mechanism by the protonation on lone pair in DMAP. Thus, suitable pH value is limited around pKa+1 of DMAP moiety for the scope of an application to a real sample. However, this restriction can be readily resolved because many real samples have neutral pH region of 6-8.5 and can be kept in suitable pH by the using of phosphate buffer. Moreover, the pH of sample solution can be handily confirmed using pH test paper for just in case.

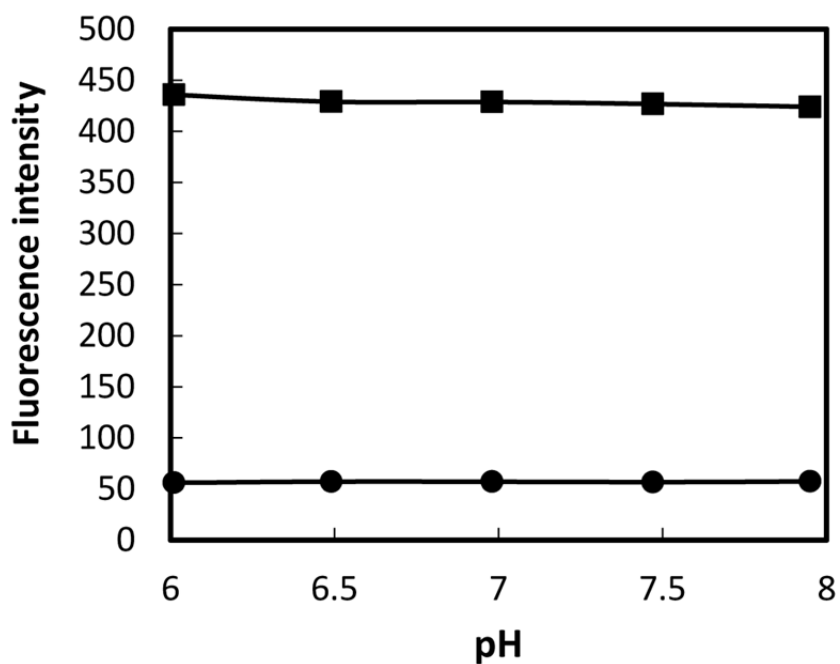


Figure 5.9 Fluorescence intensity of **TH-CyD** without (I_0 : circle dot) and with ($I_{\max}$: cubic dot) SDS in various pH at 25 °C. [**TH-CyD**] = 2.5 μ M. Excitation: 382 nm.

Table 5.2. 1:1 Complex Formation Constant ($\log K^a$) of **TH-CyD** with SDS below CMC in 0.01 M of phosphate buffer at 25 °C

pH	6.01	6.49	6.98	7.47	7.95
$\log K$	2.38	2.39	2.52	2.45	2.45

^a $K = [\text{complex}] / ([\text{TH-CyD}][\text{guest}])$. The standard deviation (σ) of the $\log K$ was estimated to be ± 0.05 .

Real sample measurement

For practical applicability, three river waters were obtained as real samples from one upper stream (Toyohira River, pH = 6.7) and two down streams (Yasuharu River and Sousei River, pH = 6.6, 6.1) in Sapporo city (Fig. 5.10), Japan. All samples were added to 25 μ M of **TH-CyD** buffer solution at 1 : 1 (v/v) ratio had least of the mixed error. Figure 5.11 and 5.12 showed fluorescence spectra of prepared samples by above method using Sousei River water as a representation; when the addition of SDS to the treated sample, the fluorescence of **TH-CyD** was also enhanced. SDS addition was stopped when a precipitate occurred or the concentration of SDS to 8 mM arrived. For the all samples, I_0 , I (4 mM) and $\log K$ values were close to the case of the buffer only solution (Table 5.3). Moreover, in the region of 0-5 ppm, the correlation coefficient between fluorescence intensity of **TH-CyD** and SDS concentration obtained approximately 0.99 as shown in Fig. 5.13. Those results indicate that supramolecular complexation of **TH-CyD** with SDS is no affected by any interfering substance in real samples. The fluorescence intensity of the free **TH-CyD** only showed the slight decreasing despite using the real sample; inaccuracy corresponding to 0.15 ppm seems enough small because of the high potential for improvement by the masking interfering substance. Above results demonstrate that the detection of the surfactant using **TH-CyD** have feasibility is applied with great accuracy in river water. Further progression study is the proceeding.



Figure 5.10. The view of three rivers. Upper: Toyohira Riv. as upper stream, Center: Yasuharu Riv. is discharged as treated waste water. Lower: Sosei Riv. folwed throught urban center.

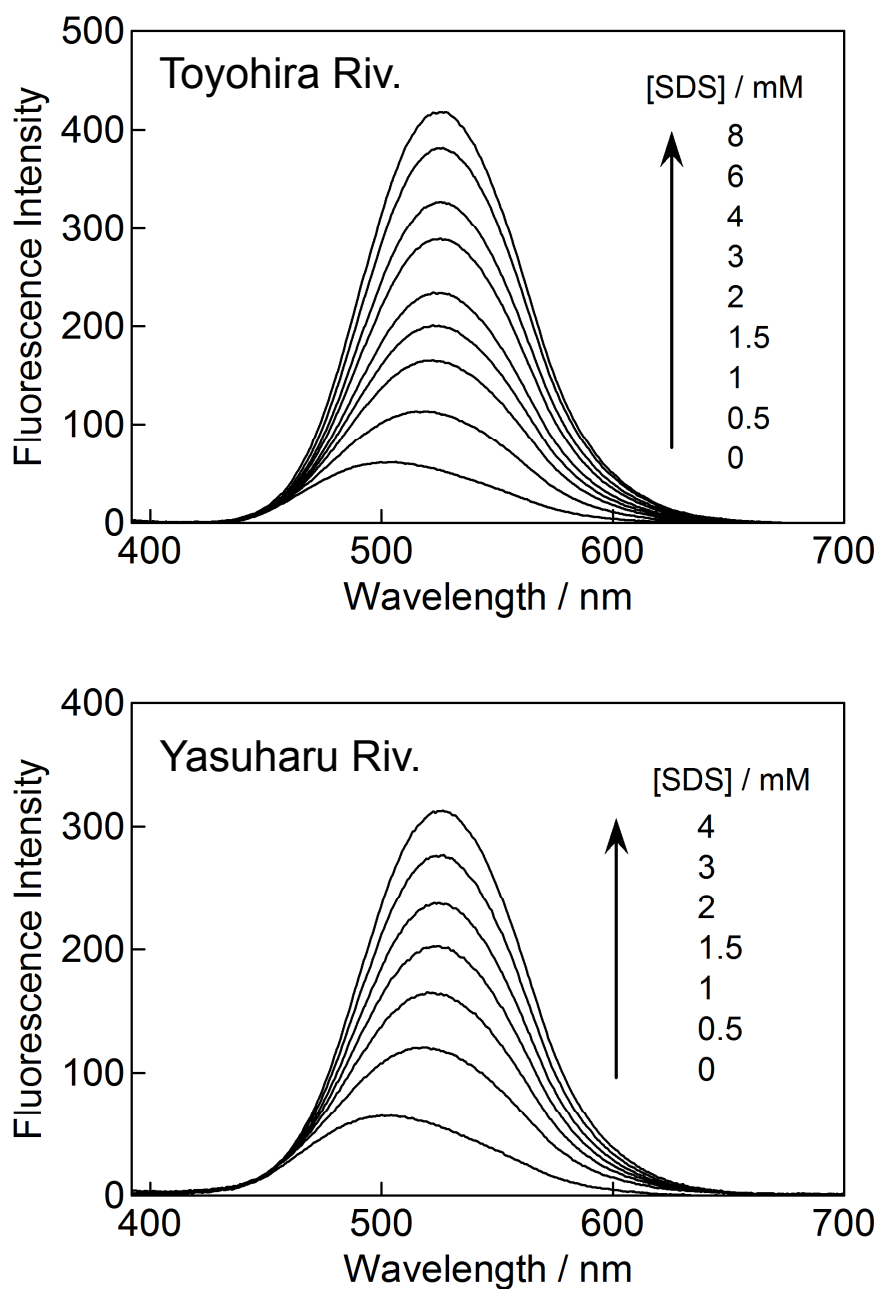


Figure 5.11. Fluorescence spectra of **TH-CyD** with and without SDS in phosphate buffer (0.01 M) involving river water from Toyohira (upper) and Yasuharu (lower) at 25 °C. [TH-CyD] = 2.5 μ M. Excitation: 382 nm.

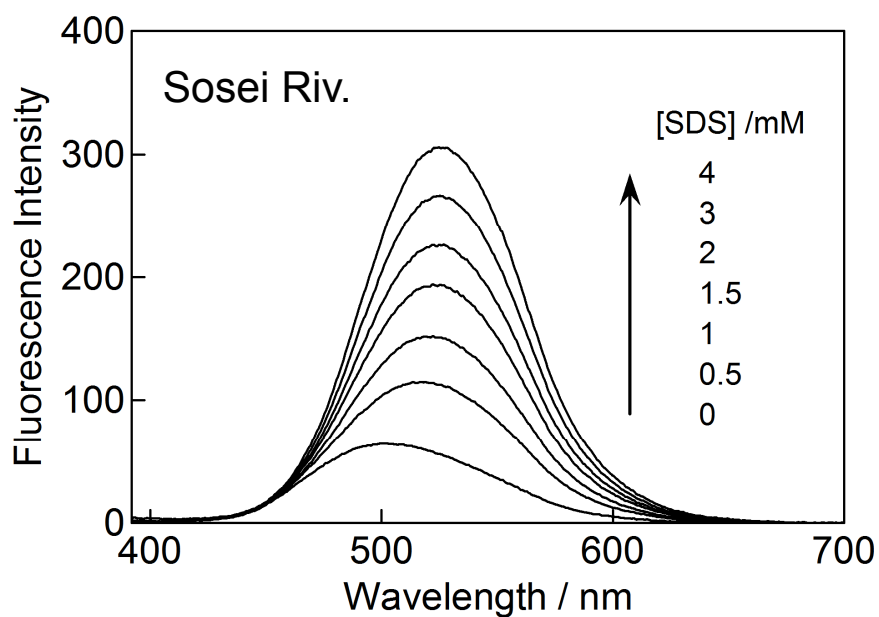


Figure 5.12. Fluorescence spectra of **TH-CyD** with and without SDS in phosphate buffer (0.01 M) involving river water from Sosei river at 25 °C. [TH-CyD] = 2.5 μ M. Excitation: 382 nm.

Table 5.3 1:1 Complex Formation Constant ($\log K^a$) and I/I_0 values of **TH-CyD** with SDS below CMC in 0.01 M of phosphate buffer at 25 °C

	Toyohira	Yasuharu	Sosei	only buffer
I/I_0 (SDS: 4 mM)	5.6	5.6	5.5	6.0
$\log K$	2.36	2.49	2.37	2.52

^a $K = [\text{complex}] / ([\text{TH-CyD}][\text{guest}])$. The standard deviation (σ) of the $\log K$ was estimated to be ± 0.08 .

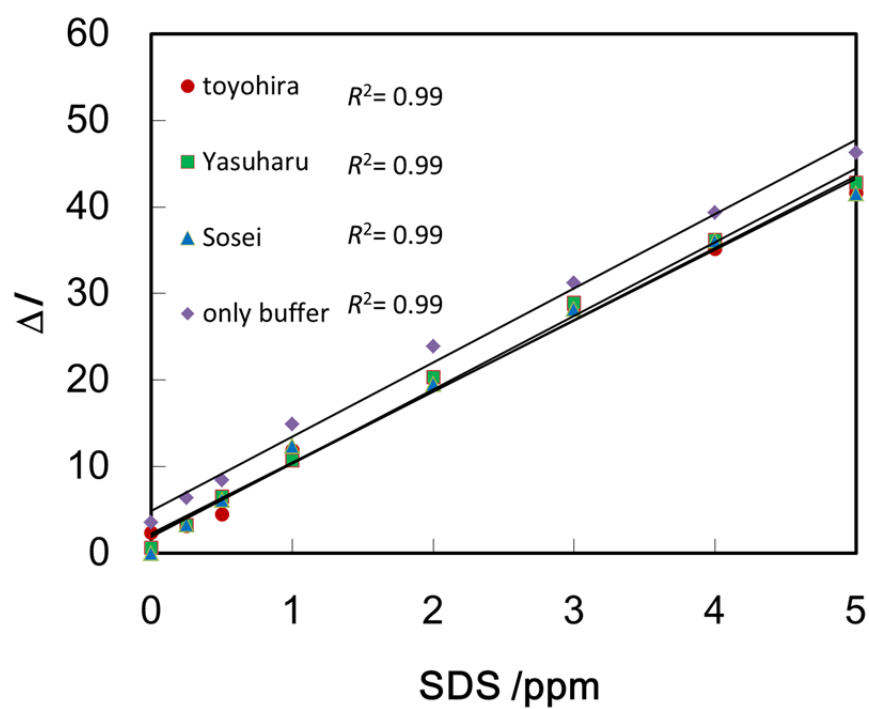


Figure 5.13. Calibration curve for fluorescence intensities of **TH-CyD** with SDS at 505 nm and 25 °C; . 25 μ M **TH-CyD** in 0.01 M phosphate buffer. All samples filtrated by a membrane filter (0.22 μ m).

Conclusion

I prepared a novel TICT probe **TH-CyD**, based on DMAPTA. The fluorescence of **TH-CyD** was enhanced 8-fold by the addition of ionic surfactants at concentrations below the CMC, and was identifiable by the naked eye in bright rooms and outdoors. In contrast, the increase in the fluorescence intensity of **TH-CyD** on addition of a nonionic surfactant was poor, even above the CMC. The fluorescence selectivity for ionic surfactants was attributed to the degree of rotational freedom of **TH-CyD** on complexation with a surfactant. The low LOD value (0.10 ppm) for SDS means that **TH-CyD** can be used for the detection of ionic surfactants at concentrations above those specified in environmental standards, without condensation of water samples. Moreover, the fluorescence spectra of **TH-CyD** is slightly affected involving real samples from three river waters. As mentioned above, probe **TH-CyD** is promising for potential use in simple and rapid analysis of environmental samples.

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General Conclusion

Some novel fluorescent “Off–On” probes (**P2**, **Ant-CyD**, **AC1**, **AC5** and **TH-CyD**) demonstrated via supramolecular interaction. Fluorescence intensity of those probes was enhanced up to around ten-fold in the addition of alkaline earth metal ions, ionic or nonionic surfactants. Especially **TH-CyD** showed the visible-green fluorescence in addition of SDS below critical macular concentration (CMC). **P2** probes were improved. All probes clearly determined those complexation behaviors with target materials by ^1H NMR and/or ICD spectra, which were dominated by 1:1 equilibrium. Complex formation between the host and guest compounds by the simple process presents important benefits. Moreover, the reversible process of the supramolecular formation with a target material has the potential of a consecutive monitoring. It can be suitable for the observation of an ambient change in environmental and biological samples. Cyclodextrin probes (**Ant-CyD**, **AC1**, **AC5**, **TH-CyD**) can directly detect surfactants in water solution without extract to organic solvent or sample condensation. For **AC1** and **TH-CyD**, those limit of detection (LOD) values for sodium dodecylsulfate (SDS) amply satisfied the requirement of the United States Environmental Protection Agency (USEPA; 0.5 ppm) and the Ministry of Health, Labour, and Welfare in Japan (MHLW; 0.2 ppm). Moreover, the application potentiality of **TH-CyD** was demonstrated involving three river water samples.

These findings suggest some attractive benefits for the development of rapid and simple analysis using fluorescent probe both in laboratory and *in situ*. In conclusion, fluorescent probes in this thesis will create a new path to the convenient analytical method for an environmental and a biological material.

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