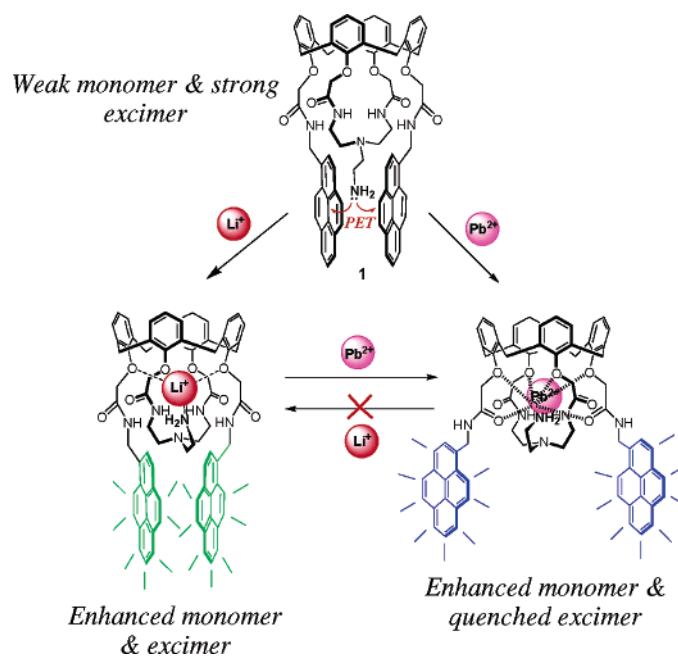# Fluorescence Ratiometry of Monomer/Excimer Emissions in a Space-Through PET System

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Fluorogenic calix[4]arenes (**1** and **2**) bearing a pendent ethyleneamine on their triazacrown rings, respectively, were synthesized in the cone conformation. Compared with **4**, free **1** and **2** display a relatively weak emission, reflecting that a PET process from the pendent amine group ($-CH_2CH_2-NH_2$) to the fluorogenic pyrenes is mainly operated. Addition of various metal ions or anions to the solution of **1** or **2** reduces the PET because the pendent alkylamine takes part in the complexation, causing their fluorescence spectra to be changed. When Pb^{2+}, a quenching metal ion, is added to **1** or **2**, their pyrene monomer emission is enhanced with their excimer emission quenched, which is due to conformational changes of the facing carbonyl groups as well as to the participation of the ethyleneamine into the three-dimensional Pb^{2+} ion encapsulation. In contrast, upon addition of alkali metal ions to the **1** and **2**, both monomer and excimer emissions are observed to increase, which is attributable to the CHEF effect and the retained conformations. For anion sensing, both **1** and **2** show a high selectivity for F^- ions over other anions tested. When the F^- ion is bound to **1** or **2** by hydrogen bonding between the amide N*H* of the triazacrown ring and F^-, both their monomer and excimer emissions are weakened due to PET from the bound F^- to the pyrene units.

Introduction

As cations and anions play crucial roles in vivo or in vitro, considerable efforts have been devoted to the development of fluorescent chemosensors capable of selectively recognizing specific chemical species in potential analytical applications of many different fields including chemistry, biology, and medicine.[1–4] For example, Pb^{2+} ion can affect almost every organ and system

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in the human body, causing various symptoms such as anemia, kidney damage, a disorder of the blood, memory loss, muscle paralysis, and mental retardation by lead poisoning.[5] On the other hand, among biologically important anions, F^{−} ion is known to prevent dental caries[6] and has also been extensively investigated as a treatment for osteoporosis.[7] Accordingly, selective signaling has been very important topics for the detection and treatment of toxic metal ions and anions.

Fluorescent chemosensors for ions typically consist of an ion recognition unit (ionophore) and a fluorogenic unit (fluorophore) linked to the ionophore through a proper spacer.[3] As recognition moieties for cations and anions, calixarenes with appropriate appended groups have been good candidates because of their high selectivity toward specific cations and anions,[8−12] and their potential applications as fluorescence sensing agents have received increasing interest. Calix[4]arenes have two reactive sites: (1) phenolic OHs (lower rim) and (2) para positions (upper rim) to the hydroxy groups which can be easily functionalized with various cation-ligating groups such as carboxylic acid, amides, crown ether, and azacrown ether.[13,14] In particular, amide groups as a functional group are known to capture not only cations through oxygen atoms[15] but also anions through hydrogen bonding between anions and amide N−H.[16] Most such calixarene-based fluorescence sensors were reported to utilize
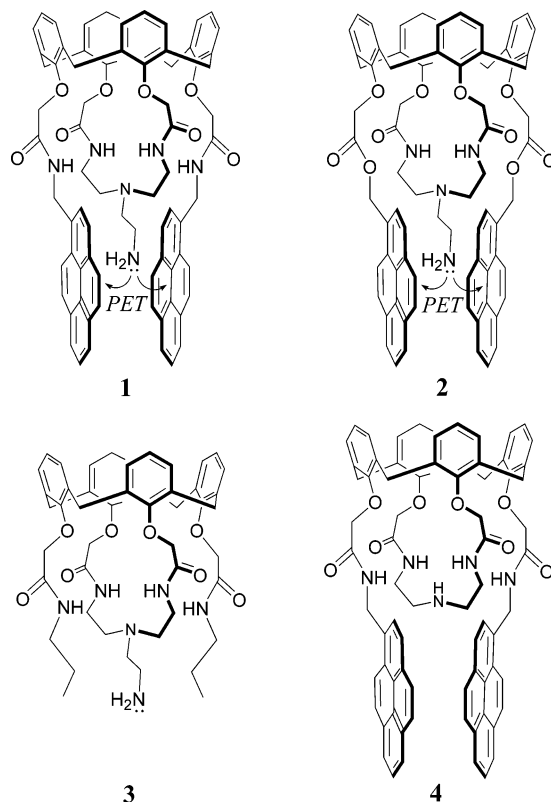

FIGURE 1. Structures of compounds **1**−**4**.

photophysical changes produced by cation binding: PET (photoinduced electron transfer);[10−13,17] PCT (photoinduced charge transfer);[8] excimer/exciplex formation and extinction;[18] or energy transfer.[19]

Among fluorophores, pyrenes are known as one of the most useful fluorogenic units because they display not only a well-defined monomer emission at 370−430 nm but also an efficient excimer emission at around 480 nm.[20,21] With an intensity ratio of excimer to monomer emission (I_E/I_M) being sensitive to the conformational changes of the pyrene-appended receptors, the I_E/I_M changes upon the metal ion complexation can be an informative parameter in various sensing systems.[1,21−23] For the formation of an excimer emission of pyrenes, it was reported that the calixarene containing pyrenyl esters form a strong intramolecular excimer by a strong $\pi-\pi$ interaction between two pyrenes and its ratio of excimer to monomer is changed dependent on concentrations of the existing Na^{+} ions.[23] Recently, we reported that **4** in Figure 1 is able to selectively sense Pb^{2+} ion in

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SCHEME 1. Synthetic Routes for Ionophores 1−3
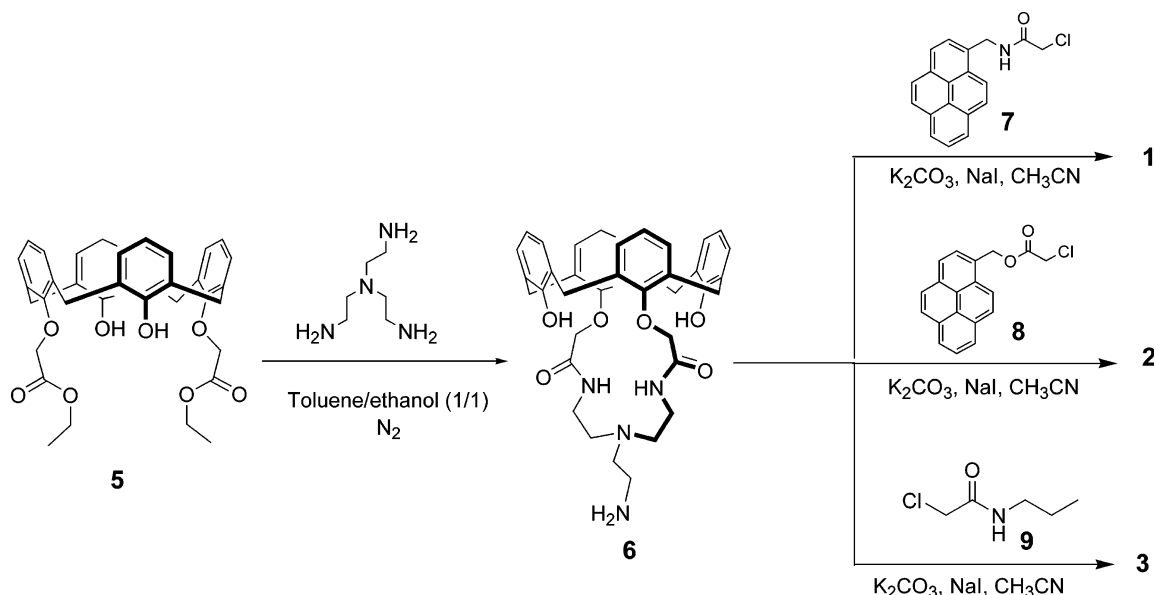
such a manner that the Pb²⁺ ion is encapsulated by two pyrene−amide oxygen atoms with aid of nitrogen atoms of the triazacrown loop of **4**.[24] Eventually, binding of the Pb²⁺ ion gave rise to declining fluorescence intensity of **4** in both monomer and excimer bands. In addition, selective complexation of F⁻ with **4** was reported to cause the excimer to be weakened via a conventional PET from F⁻ to pyrene units.

In a continuation of research on the pyrene−pyrene excimer formation which can be one of the potential indicators in an ion-sensing system, compound **1,** in which a pendent primary alkylamine capable of influencing on excimer formation and extinction is attached on the nitrogen atom of the triazacrown of **4**, has been newly synthesized. We also synthesized **2** and **3** as reference materials to elucidate the binding mechanisms of **1** to cations and anions. The key roles of the pendent primary alkylamine onto the fluorescence changes upon cation and anion complexation have been fully investigated and are reported herein.

Results and Discussion

For the synthesis of **1**, we encountered problems with an attachment of the primary alkylamine such as ethylenamine on the nitrogen atom of the triazacrown loop of **4**; thus, we changed its synthetic pathway to Scheme 1. Under a nitrogen atmosphere, treatment of **5**[25] with tris(2-aminoethyl)amine in a mixture of ethanol and toluene (1:1) as a solvent led to calix[4]triazacrown **6** in 72% yield. Reaction of **6** with *N*-(1-pyrenylmethyl)-chloroacetamide (**7**)[15a] using K₂CO₃ as a base in CH₃CN with a catalytic amount of NaI gave **1** in 51% yield. Compounds **2** and **3** could be also synthesized by using pyrenemethyl chloroacetate (**8**)[26] and *N*-propylchloro-acetoamide (**9**), respectively. ¹H NMR spectra of **1−3**
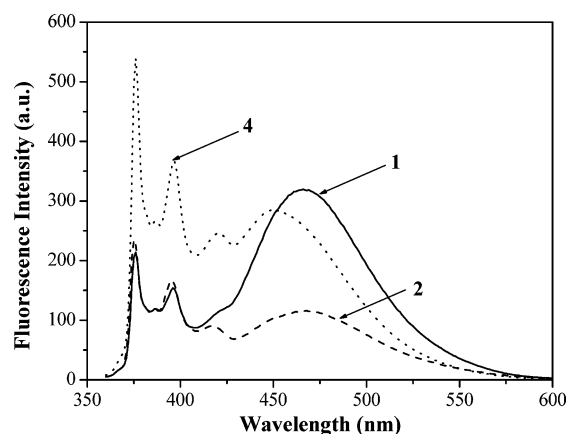
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FIGURE 2. Fluorescence spectra of free **1**, **2**, and **4** (6.0 μM) in CH₃CN. The excitation wavelength is 343 nm.

exhibit two doublets at around 4.0 and 3.0 ppm in the AB splitting pattern, corresponding to protons of the methylene bridge of the calix[4]arene, which suggests that **1−3** be all in the cone conformation. In addition, the resonances at 31 ppm in the ¹³C NMR spectra for Ar*C*H₂Ar provide additional concrete evidence for their cone conformation.

Excited at 343 nm, the maximum absorption wavelength of the pyrene, compounds **1** and **2** both display monomer and excimer emissions at 396 and 467 nm, respectively, whereas compound **4** emits its excimer at 448 nm (Figure 2). This is different from that observed in the compound having a simple crown-5 or -6 instead of a triazacrown ring where the two pyrene pendants form neither intramolecular excimer nor intermolecular excimer because of the crown ether ring crossing the two pyrene groups.[15b] Despite the existence of the proximal triazacrown ring, the two pyrenes in **1** and **2** are partially overlapped, emitting excimer probably because there exist hydrogen bonds between the amide groups or the ester groups linked to pyrenes and the amide groups of the triazacrown ring. In both **1** and **2**, the intensity ratio of excimer to monomer is rarely changed in the concen-
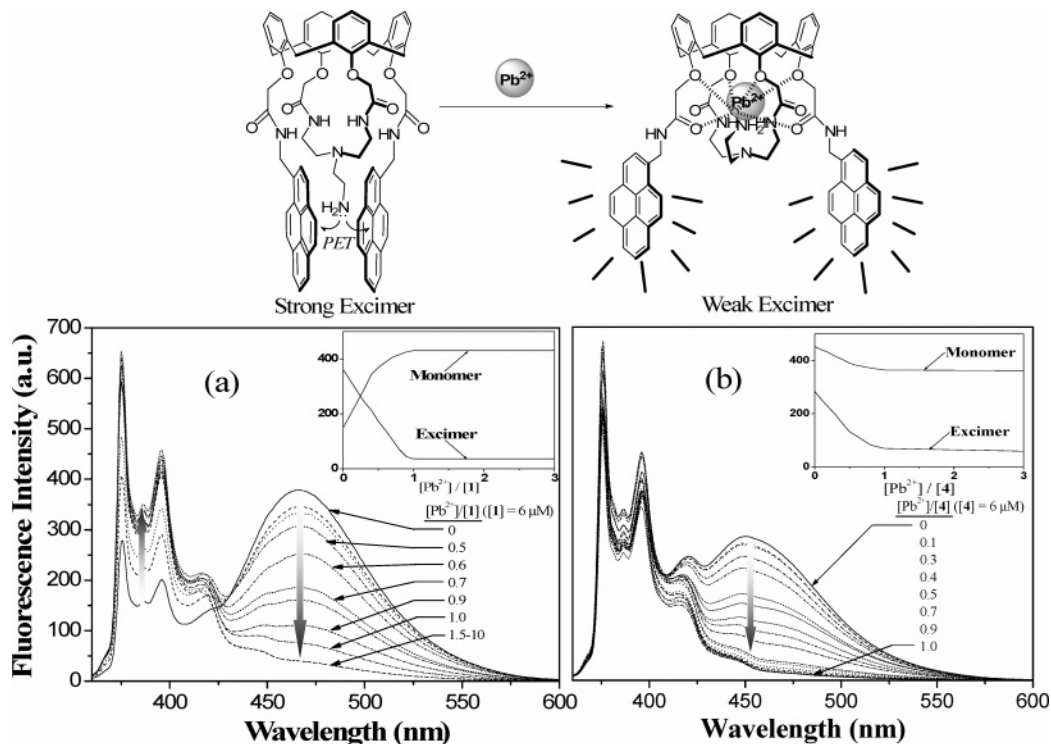
FIGURE 3. Fluorescence emission spectra of (a) **1** and (b) **4** (6.0 μM) with Pb^{2+} complexation in CH_3CN. Inset: changes of fluorescence intensity in function of $[Pb^{2+}]$. The excitation wavelength is 343 nm.

tration range of 10^{-7}–10^{-4} M, indicating that their excimer emissions result from an intramolecular excimer but not from an intermolecular excimer. On the other hand, compared with that of **4**, relatively weak emissions of free **1** and **2** are attributable to the fact that the pendent amine group ($-CH_2CH_2NH_2$) takes part in a PET (photoinduced electron transfer) process: an electron transfer from a lone-pair electron of the nitrogen atom to two pyrene units of **1** (see Figure 1). We also found that the relative ratios of excimer to monomer ($I_{\text{excimer}}/I_{\text{monomer}}$) for **1** and **2** are 1.49 and 0.49, respectively. The larger ratio of **1** is presumably because the hydrogen bonds between amide groups in **1** are stronger than those between an ester group and an amide group in **2**, resulting in a stronger π–π interaction between two pyrenes. For the red-shifted excimer emission of **1** and **2**, it is conceivable for two pyrenyl groups of **1** to be more overlapped compared with that of **4**.[27]

To obtain insight into the binding properties of **1**, **2**, and **4** toward metal ions, we first investigated fluorescence changes upon addition of the perchlorate salt of Li^+, Na^+, K^+, Cs^+, Ag^+, Mg^{2+}, Ca^{2+}, Pb^{2+}, and Zn^{2+} to the acetonitrile solutions of **1**, **2**, and **4**. As presented in Table 1, **1** and **2** show mutually similar optical responses to metal ions, suggesting that they have almost the same binding properties for the metal ions. For both **1** and **2**, divalent cations among metal ions tested made the monomer emission enhanced (+ value) but excimer emission diminished (- value) at the same time with 425 nm of the isoemissive point. This finding is consistent with the facts reported by Shinkai et al. and Jin et al.[26,28] By contrast, addition of monovalent metal ions enhanced

TABLE 1. Fluorescence Changes ($I - I_0$) of 1, 2, and 4 upon the Addition of Various Metal Cations[a]

ligand	λ_{em} (nm)	Li^+	Na^+	K^+	Cs^+	Ag^+	Mg^{2+}	Ca^{2+}	Pb^{2+}	Zn^{2+}
1	396	146	50	14	27	142	573	379	266	533
	467	214	29	35	40	21	−269	−101	−284	−216
2	396	209	97	58	55	147	318	359	318	380
	467	45	58	61	61	83	−8	−30	−75	−53
4	396	76	7	1	−3	128	132	180	−17	182
	448	−74	26	23	23	−114	−91	−120	−223	−103

[a] Conditions: **1**, **2**, and **4**: 6.0 μM in CH_3CN; excitation at 343 nm; metal ions, 500 equiv in CH_3CN. I_0: fluorescence emission intensity of free **1**, **2**, and **4**. I: fluorescence emission intensity of metal-ion-complexed **1**, **2**, and **4**.

both the monomer and the excimer emission, indicating that the binding modes of the monovalent cations to **1** or **2** are different from those of divalent cations. In regard to the extent of the fluorescence intensity changes in both monomer and excimer emission, **1** shows more significant change than does **4**, except the case for the Pb^{2+} ion complexation in which the binding mode of **1** is quite different from that of **4** (vide infra).

Figure 3 shows the fluorescence changes of **1** and **4** with a function of $[Pb^{2+}]$. The fluorescence change pattern of **1** upon the titration of Pb^{2+} ion differs from that of **4**. With the Pb^{2+} ion, the monomer emission of **1** increases as its excimer emission decreases, whereas both monomer and excimer emission of **4** decrease. In the case of **4**·Pb^{2+}, the fluorescence change is ascribed to the fact that complexation of Pb^{2+} ion induces a conformational change of two amide groups, giving a $C=O\cdots Pb^{2+}$ coordination

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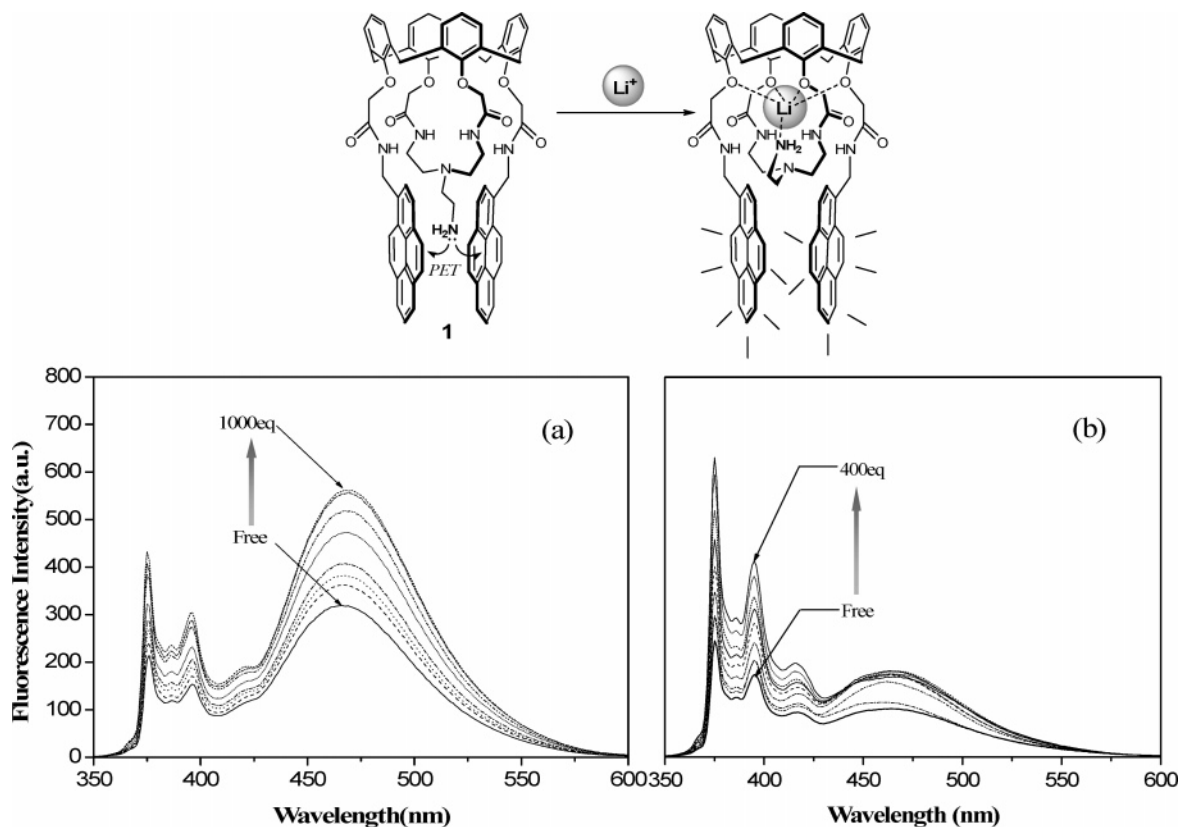
FIGURE 4. Fluorescence emission spectra of **1** and **2** (6.0 μM) upon addition of Li$^+$ in CH$_3$CN. The excitation wavelength is 343 nm.

mode, resulting in a quenched excimer emission.[24,29] For the weakened monomer, it is due to a reverse-PET and heavy metal ion effect.[15b] On the contrary, in the case of **1**·Pb^{2+}, the monomer emission is found to increase, implicating those of reverse-PET and heavy metal ion effects are somewhat excluded. This is obviously because the PET process from the primary alkylamine to two facing pyrenyl units which is mainly considered in initial stage becomes quite diminished upon Pb^{2+} ion complexation.

Not only those fluorescence changes in monomer and excimer, but also ^{1}H NMR spectra of **1**·Pb^{2+}, gave us concrete evidence for the participation of the pendent alkylamine into the metal ion complexation in a three-dimensional way. In the ^{1}H NMR spectra of **1**·Pb^{2+} (Figure S1(c), Supporting Information), protons (H$_d$, H$_e$, and H$_f$) in the ethylene moiety of ethylenamine ($-$NCH_2-CH_2NH$_2$) and ($-$CONHCH_2CH_2N$-$) of the triazacrown loop were found to shift downfield. In particular, upon Pb^{2+} ion complexation, the hydrogen atoms on the amide nitrogens (H$_a$ and H$_b$) were found to shift downfield. In addition, Hs adjacent to the pyrenes are split by chemically different environments induced by metal binding. Those combining observations of the chemical shift changes and fluorescence changes upon metal ion complexation led us to conclude that Pb^{2+} ion complexation is performed not only by the three-dimmensional caging of the primary alkylamine with the aid of the triazacrown loop but also by the free rotated amide carbonyl groups.

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This is the first example that Pb^{2+} ion known as a quenching metal ion enhances the fluorescence intensity when it is encapsulated in the fluorogenic macrocyclic compounds.

Compound **1** also responds to divalent cations such as Mg^{2+} ion exhibiting a fluorescence behavior similar to that of **1**·Pb^{2+} as seen in Figure S2 (Supporting Information). However, enhancement of the monomer emission induced by the alkaline earth metal ions, e.g., Mg^{2+} are much greater than that by Pb^{2+}, which is simply because in the alkaline earth metal ion complexation the heavy metal ion effect is ruled out. Instead, the CHEF (CHelating Enhanced Fluorescence) effect plays an intrinsic role on enhancing the fluorescence intensity.

Fluorescence changes of **1** for alkali metal ion complexation are found to be different from those for heavy metal ions and divalent ions. In particular, in the case of Li$^+$ ion complexation, both monomer and excimer bands of **1** were markedly enhanced (Figure 4). This is obviously because unlike the case of Pb^{2+} ion complexation the major factor making effects on the fluorescence changes is the CHEF (CHelation Enhanced Fluorescence) effect induced by metal ion complexation with the nitrogen lone pair electrons of the pendant amine, but not reverse PET or the heavy metal effect. That is, in a similar manner with **1**·Pb^{2+}, the nitrogen atom of free $-$CH$_2$CH$_2$NH_2 no longer takes part in the PET process toward two pyrenes, but, instead, it participates in the Li$^+$ complexation with aid of triazacrown loop of the calixarene, providing an increased monomer intensity. More interestingly, we found that the carbonyl oxygen
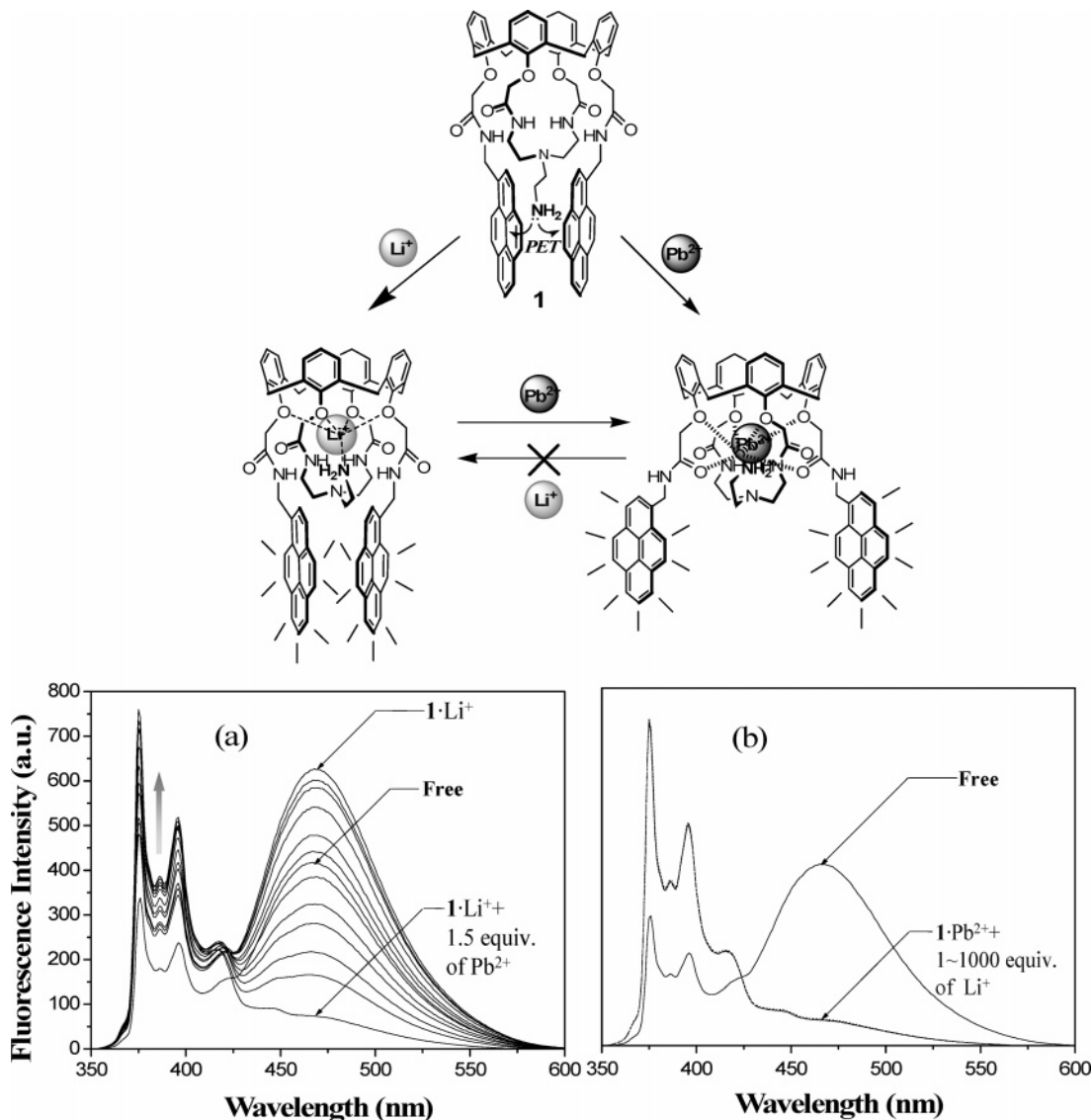
FIGURE 5. (a) Fluorescence changes of **1** (6.0 μM) with Pb^{2+} titration in the presence of Li$^+$. (b) Fluorescence changes of **1** (6.0 μM) with Li$^+$ titration in the presence of Pb^{2+}. The excitation wavelength is 343 nm.

atom of the pyrene-amide are hardly involved in the lithium ion complexation with their conformation retained, providing an enhanced excimer emission, which is in good agreement with the facts reported by Reinhoudt et al.[15a] The retained conformation of the pyrene-amide groups is also evidenced by the ^{1}H NMR spectroscopy as seen in Figure S1(b) (Supporting Information). Phenomena similar to those for **1** are also observed in **2**, suggesting that Li$^+$ ion forms a complexation with **1** and **2** through the same binding manner.

In addition, to further compare the binding ability of **1** for Li$^+$ and Pb^{2+}, a competitive metal ion exchange experiment was carried out. When Pb^{2+} is titrated into a solution of **1**·Li$^+$ complex, the excimer emission is completely quenched with the monomer emission increased with only 1.5 equiv of Pb^{2+} ion (Figure 5a). In the reverse of this metal ion exchange process, the addition of even more than 1000 equiv of Li$^+$ ion to a solution of **1**·Pb^{2+} gave no fluorescence change (Figure 5b), implying that the metal ion exchange does not occur.

Accordingly, one can assert that the binding ability of **1** for Pb^{2+} ion is much greater than that for Li$^+$ ion.

We also investigated binding behaviors of **1** and **2** toward various anions (F$^-$, Cl$^-$, Br$^-$, I$^-$, CH$_3$COO$^-$, HSO$_4^-$, and H$_2$PO$_4^-$) using their tetrabutylammonium salts in CH$_3$CN. The results are listed in Table 2. Similar to the case of **4**, both **1** and **2** also show remarkable fluorescence changes for F$^-$ ion, indicating that they have high selectivities for F$^-$ over other anions tested. When the F$^-$ ion is bound to **1** and **2** both the monomer and the excimer emission are quenched, which is due to the PET effect from the F$^-$ to pyrene units. On the contrary, the changes of fluorescence upon addition of I$^-$ are ascribable to heavy anion effects.

Figure 6 shows the fluorescence spectra of **1** and **2** with increasing amount of F$^-$. In **1**, the fluorescence changes are saturated with more than 1000 equiv of F$^-$, whereas in the case of **2** they are with only 1.0 equiv of F$^-$, reflecting that **2** forms a complexation with F$^-$ more readily than **1** and **4** do. This is probably because,
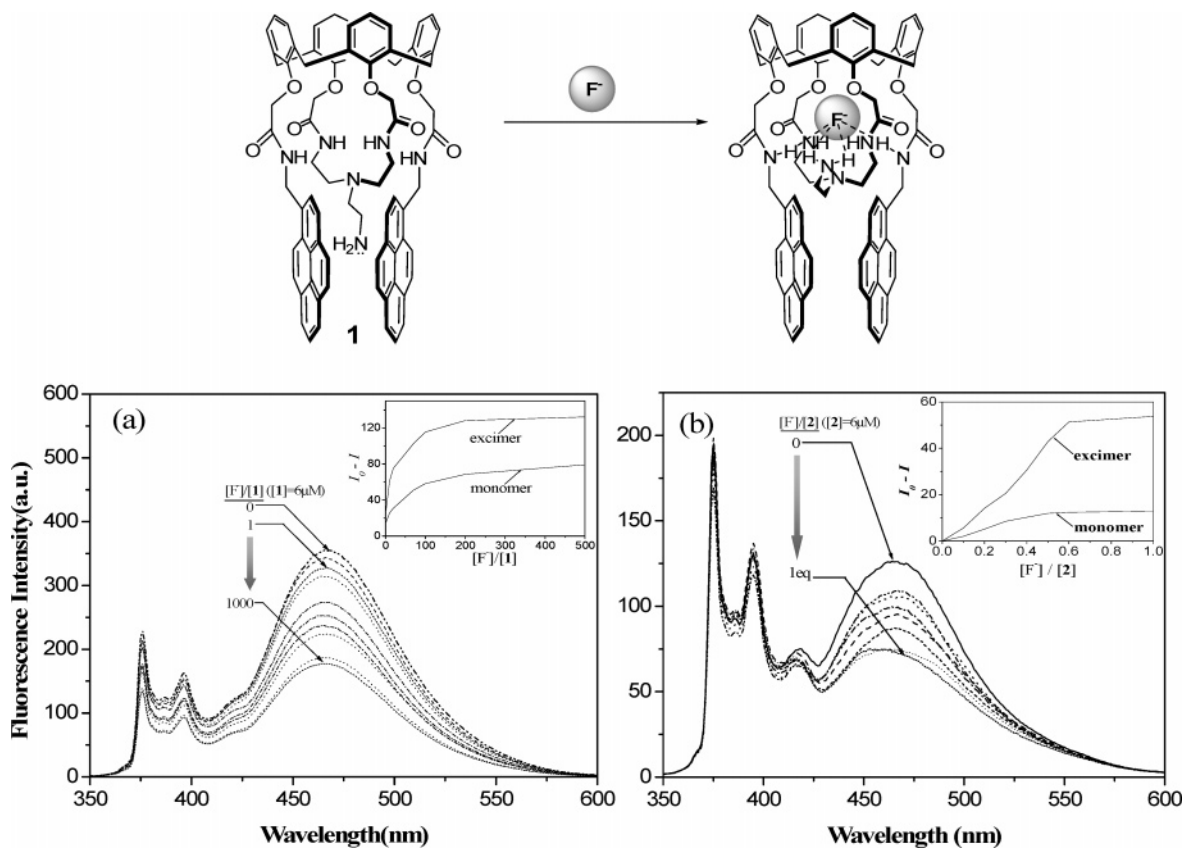
FIGURE 6. Fluorescence spectra of (a) **1** and (b) **2** (6.0 μM) upon addition with F$^-$ in CH$_3$CN. Inset: Changes of fluorescence intensity with a function of [F$^-$]. The excitation wavelength is 343 nm.

TABLE 2. Fluorescence Changes ($I - I_0$) of 1, 2 and 4 upon the Addition of Various Metal Cations[a]

ligand	λ_{em} (nm)	F$^-$	Cl$^-$	Br$^-$	I$^-$	HSO$_4^-$	CH$_3$COO$^-$	H$_2$PO$_4^-$
1	396	−50	−13	−7	−41	−12	−28	−10
	467	−121	−13	−4	−43	−7	−56	−16
2	396	−1	8	20	−47	10	−8	17
	467	−89	−34	−27	−53	0	−12	−17
4	396	−308	−13	−11	−96	−19	−69	−35
	448	−75	−38	−28	−54	−35	−64	−47

[a] Conditions: **1**, **2**, and **4**, 6.0 μM in CH$_3$CN, excitation at 343 nm; anions, 500 equiv in CH$_3$CN. I_0: fluorescence emission intensity of free **1**, **2**, and **4**. I: fluorescence emission intensity of anion-complexed **1**, **2**, and **4**.

compared with those in **2**, the relatively strong hydrogen bondings between the four amide groups of **1** and **4** prevent F$^-$ from being bound to them. From this titration experiment the association constants of **1** and **2** (K_a) are estimated to be 5.86 × 10^3 and 3.7 × 10^6 M^{-1}, respectively.[30] Noticeably, for **1**, the relative ratio of excimer to monomer is constant without being influenced by F$^-$, which is due to the hydrogen bonding between F$^-$ and the amide groups linked to the fluorophores keeping the two pyrene units overlapping (see Figure 6).

To further elucidate the exact binding mechanism of **1** to anions we also synthesized compound **3** bearing propyl groups instead of pyrene units. Figure S4 (Sup-

porting Information) shows the chemical shift changes in the ^{1}H NMR spectra of **1** and **3** upon addition of 1.0 equiv of F$^-$. When F$^-$ is added to **3**, the peaks of the pendant amine (NH_2) as well as amides (NH_a and NH_b) disappear, which evidently indicates that not only the amide protons but also the amine protons participate in the binding with F$^-$.

Conclusions

We newly synthesized fluorogenic calix[4]triazacrowns (**1** and **2**) bearing a pendent primary alkylamine capable of influencing excimer formation and extinction. In contrast to **4**, compounds **1** and **2** reveal a quenched monomer emission because the pendent amine group ($-$CH$_2$CH$_2$NH$_2$) takes part in a PET process: an electron transfer from a lone-pair electron of the nitrogen atom to two pyrene units. When Pb^{2+} is added to a CH$_3$CN solution of **1**, the monomer emission increases with an excimer emission quenched due to conformational changes of the carbonyl bonds (flipping over) as well as to the participation of the primary amine into the three-dimensional encapsulation, which is supported by the Mg^{2+} complexation in **1**. In contrast, the addition of Li$^+$ to the ligand **1** causes both monomer and excimer emission to increase, which is mainly attributed to the CHEF effect without conformational changes of the carbonyl groups. This is the first case that Pb^{2+} ion known as a quenching metal ion enhances the fluorescence intensity when it is encapsulated in the fluorogenic macrocyclic compounds. In addition, both **1** and **2** show a high selectivity for F$^-$ ions over other anions tested.

(30) (a) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom. (b) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.

When F$^-$ ions are bound to **1** or **2** via hydrogen bonding between the amide N*H* of the triazacrown ring and F$^-$, both their monomer and excimer emissions are weakened due to PET from the bound F$^-$ to the pyrene units.

Experimental Section

Synthesis. Compounds **4**,[24] **5**,[25] **7**,[15a] and **8**[26] were prepared following procedures reported in the literature.

Calix[4]amidocrown-5, Cone (6). To a mixture of 1.0 g (1.67 mmol) of **5**[25] in absolute ethanol (60 mL) and toluene (60 mL) was added 0.49 g (3.35 mmol) of tris(2-aminoethyl)-amine. The reaction mixture was refluxed for 24 h. After removal of the solvent in vacuo, the resulting solid was dissolved in CH$_2$Cl$_2$ (100 mL), and the organic layer was washed three times with water. The organic layer was dried over anhydrous MgSO$_4$ and evaporated in vacuo to give the crude product, which was purified by recrystallization from diethyl ether to give 0.81 g (72% yield) of the product **6** as a white solid: mp 198–206 °C; IR (KBr pellet, cm^{-1}) 3334, 1740; FAB MS *m/z* (M$^+$) calcd 672.8, found 672.8. Anal. Calcd for C$_{39}$H$_{52}$N$_4$O$_6$: C, 69.62; H, 8.33. Found: C, 69.63; H, 8.37.

Fluorogenic Calix[4]triazacrown (1). To a mixture of 0.3 g (0.45 mmol) of calix[4]triazacrown (**6**) and 0.27 g (0.87 mmol) of *N*-(1-pyrenylmethyl)chloroacetamide (**5**)[15a] in 100 mL of dry CH$_3$CN were added anhydrous K$_2$CO$_3$ (0.18 g, 1.30 mmol) and a catalytic amount of NaI under nitrogen atmosphere. The reaction mixture was refluxed for 24 h. After removal of the solvent in vacuo, to the resulting solid were added aqueous NaHCO$_3$ solution (100 mL) and CH$_2$Cl$_2$ (100 mL), and the organic layer was separated and then washed two times with 50 mL of water. The organic layer was dried over anhydrous MgSO$_4$, and the solvent was evaporated in vacuo to give a crude solid, which was purified by recrystallization from diethyl ether to give 0.26 g (51% yield) of **1** as a white solid: mp 225–232 °C; IR (KBr pellet, cm^{-1}) 3322, 1780; FAB MS *m/z* (M$^+$) calcd 1051.2, found 1051.0. Anal. Calcd for C$_{72}$H$_{62}$N$_2$O$_6$: C, 82.26; H, 5.94. Found: C, 82.29; H, 5.96.

Fluorogenic Calix[4]triazacrown (2). Compound **2** was prepared by modification of the procedure given above for **1**. The crude product was purified by recrystallization from diethyl ether to give 0.21 g (40% yield) of **2** as a white solid: mp 205–217 °C; IR (KBr pellet, cm^{-1}) 3320, 1730; FAB MS *m/z* (M$^+$) calcd 1195.3, found 1195.2. Anal. Calcd for C$_{76}$H$_{66}$N$_4$O$_6$: C, 76.36; H, 5.57. Found: C, 76.35; H, 5.56.

25,27-Bis[*N*-(1-propyl)aminocarbonylmethoxy]calix[4]-triazacrown-5 (3). Compound **3** was synthesized by following the procedure reported above for **1**: white solid (78% yield); mp 164–172 °C; IR (KBr pellet, cm^{-1}) 3322, 1780; FAB MS *m/z* (M$^+$) calcd 871.1, found 871.4. Anal. Calcd for C$_{49}$H$_{70}$N$_6$O$_8$: C, 67.56; H, 8.10. Found: C, 67.55; H, 8.12.

General Procedures for Fluorescence Study. Fluorescence spectra were recorded with a RF-5301PC spectrofluorophotometer. Stock solutions (1.00 mM) of the metal perchlorate salts were prepared in MeCN. Stock solutions of **1**, **2**, and **4** (0.06 mM) were prepared in MeCN. For all measurements, excitation was made at 343 nm and emission slit widths was 3 nm. Titration experiments were performed using 6.0 μM solutions of **1**, **2**, and **4** in MeCN and various concentrations of metal perchlorate in MeCN.