# $Pb^{2+}$ Sensing Chemo-sensor: Thiacalix[4]crown-based Lumino-ionophore

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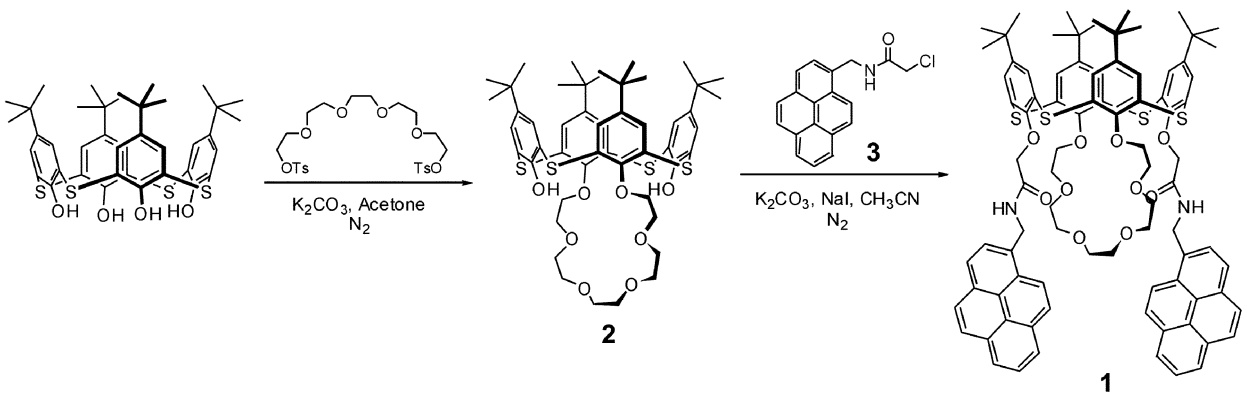
Selective separation of heavy metal ions able to cause adverse environmental and health problems have been much attention to chemists. In particular, $Pb^{2+}$ ion can affect almost every organ and system in human body, causing various symptoms such as anemia, kidney damage, a disorder of the blood, memory loss, muscle paralysis, and mental retardation by lead poisoning.[1] In this point of view, development of $Pb^{2+}$ ion-selective fluorescent chemosensor would be helpful to clarify the cellular role of the lead ions *in vivo* as well as to measure the amount of $Pb^{2+}$ ion in the sources contaminated with lead ion including human body. In fact, although a variety of effective fluorescent chemosensors[2] for alkali, alkaline earth metal ions, $Hg^{2+}$,[3] and $Zn^{2+}$ [4] have been already developed, so far few for $Pb^{2+}$ ion have been reported.[5]

Most fluorescent chemosensors for cations consist of a cation recognition unit (ionophore) along with a fluorogenic unit (fluorophore) and are thus called a fluoroionophore.[6] The best effective fluorescence chemosensor must convert the event of cation recognition by the ionophore into light signals over the fluorophore with a high sensitivity and ease to monitor. In designing sensors, therefore, the recognition moiety linked to the fluorophore should be preliminarily considered because they are responsible for the selectivity and the binding efficiency of the whole chemosensor.

As such recognition moieties, thiacalixarene having proper functional groups would be good candidates for cation probes because of their high selectivity toward specific cations.[7] Since the convenient synthetic methods for thiacalixarenes were reported in 1997 by Kumagai *et al.*,[8] they have been paid much attention as alternatives to the conventional calixarenes[9] by providing sites for functionalization not only on the aromatic rings but also on the bridging sulfide.[10] Thiacalix[4]arene is composed of four benzene rings, linked to each other *via* sulfide bridges, through which, unlike the conventional calix[4]arene, it can form complex with metal ions.[11] Thus far, synthesis and complexation studies with thiacalix[4]arene framework are rarely known[7,10] while many other calixarene-based fluorescent chemosensors have been developed.[12]

Such calixarene-based fluorescence sensors have been designed on the photo-induced mechanism inducing the photophysical changes upon cation binding: PET (photo-induced electron transfer),[12] PCT (photo-induced charge transfer),[13] excimer/exciplex formation and extinction[14] or energy transfer.[15] As a PET-utilizing chemosensor, we reported on the fluorescent calix[4]crowns bearing two facing amide groups,[5b] in which the amide groups took part in complexation with $Pb^{2+}$ ion, accompanying fluorescence quenching by the reverse PET[5b,16] from the pyrene unit to the electron-deficient amide oxygen atom as well as by the heavy metal effect.[5b,17]

In a continuation of the research on the fluorescence changes of the fluoroionophore upon the metal ion complexation, we herein report the first example of $Pb^{2+}$ ion-selective fluorescent chemosensor based on *tert*-butylthia-




**Scheme 1.** Synthetic route for fluorescence chemosensor **1**.

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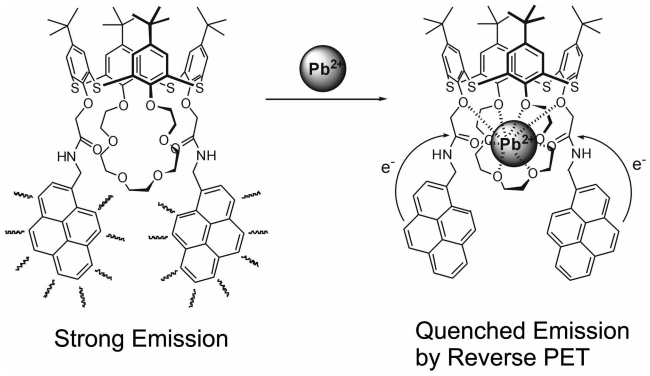
calix[4]arene. In this system, the amide oxygen atoms not only participate in the binding with $Pb^{2+}$ ion but also behave as a PET acceptor from the pyrene units.

Scheme 1 shows the synthetic route for the 1-(methylpyrenyl) amide-bearing *tert*-butylthiacalix[4]crown-6 (**1**). *tert*-Butylthiacalix[4]*mono*crown-6 (**2**) was prepared from the reaction of *tert*-butylthiacalix[4]arene and pentaethyleneglycol ditosylates in the presence of 1 equiv. of $K_2CO_3$ in acetone. The reaction of **2** with 2 equiv. of *N*-(1-pyrenylmethyl)chloroacetamide (**3**)[18] using $K_2CO_3$ as a base in acetonitrile with a catalytic amount of sodium iodide provided the fluorogenic chemosensor **1** in 34% yield. The $^1$H NMR spectrum of **1** shows two singlets at 7.01 and 7.13 ppm attributable to the *meta*-protons of the thiacalixarene framework, suggesting that **1** is in the cone conformation. The cone conformation of the **1** is also evidenced by the fact that there is a monomer emission band at around 400 nm without an excimer emission. This is because the crown-6 ring blocks excimer formation between the facing pyrene groups. For this regard, we have reported that in the 1,3-alternate conformation two facing pyrene amides incorporated with the calix[4]crowns exhibit strong excimer fluorescence around 470 nm, whereas pyrene groups of the cone calix[4]crowns display a monomer emission only.[19]

To obtain insight into the ability of **1** to selectively sense metal ions we first investigated fluorescence changes of **1**



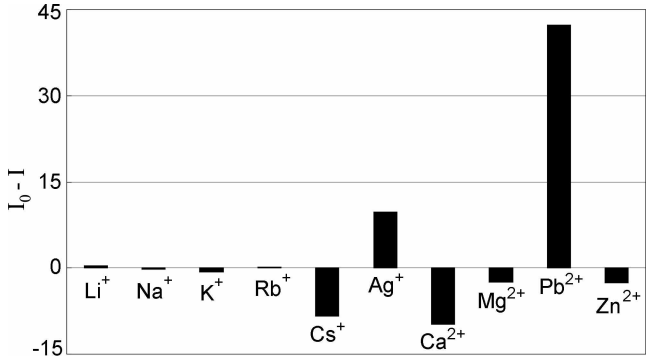




**Figure 1.** Fluorescence changes ($I_0$-$I$) of **1** at 396 nm upon the addition of various metal ions. Conditions: **1**, 6 $\mu$M in $CH_3CN$, excitation at 344 nm; metal ions, 500 equiv. in $CH_3CN$. $I_0$: fluorescence emission intensity of free **1**; $I$: fluorescence emission intensity of metal ion-complexed **1**.

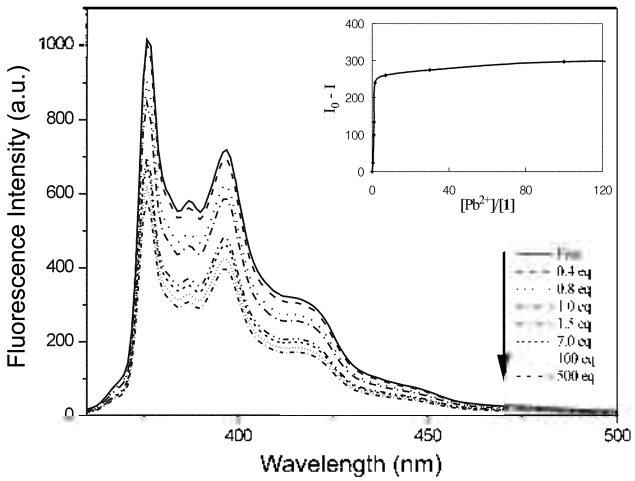



**Figure 2.** Fluorescence emission spectra of compound **1** (6 $\mu$M) upon the addition of various amounts of $Pb^{2+}$ in $CH_3CN/CHCl_3$ (1 : 1, v/v). The excitation wavelength was 344 nm.

upon the addition of various metal ions such as $Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$, $Ag^+$, $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, and $Pb^{2+}$. The results show that $Pb^{2+}$ ions cause a significant change in fluorescence spectra of **1**, whereas upon the addition of $Cs^+$, $Ag^+$, and $Ca^{2+}$, minor emission changes are observed (Figure 1). The remarkable fluorescence quenching induced by $Pb^{2+}$ is ascribed not only to reverse PET from pyrene units to the carbonyl oxygen atoms of which the electron density is diminished by metal ion complexation, but also to heavy metal effect.[5b,17] For **1**, the high selectivity toward $Pb^{2+}$ is probably achieved by the assistance of the crown ring. In contrast, the *N*-methylpyrene amide-appended calix[4]arene derivative having two propyl groups instead of a crown ring exhibited no selectivity for specific metal ions,[20] suggesting that the crown part of the **1** plays an important role in its selective encapsulation of $Pb^{2+}$.

Figure 2 shows the fluorescence changes of **1** with $Pb^{2+}$ ion concentration. The fluorescence intensity was gradually decreased by the addition of the $Pb^{2+}$ ion up to 500 equiv. and then saturated. From this titration experiment, we could obtain the association constant of **1** ($2.57 \times 10^5$ $M^{-1}$) for $Pb^{2+}$ ion complexation in acetonitrile/chloroform (1 : 1, v/v).[21] Structural variation by the crown ether ring size to enhance the metal ion selectivity is now in progress and will be reported elsewhere.

In conclusion, a new *tert*-butylthiacalix[4]arene-framed fluorescent chemo- sensor bearing two facing amide groups and a crown-6 ring as cation recognition sites was synthesized in the cone conformation and its binding properties were investigated *via* fluorescence changes upon metal ion complexation. The free ligand **1** displayed strong emission at around 400 nm by excitation at 344 nm. The fluorescence changes upon the addition of metal ions showed that compound **1** has a high selectivity for $Pb^{2+}$ over other metal ions tested. When $Pb^{2+}$ ions were added to compound **1**, the fluorescence intensity markedly decreased because of the reverse PET from the pyrene unit to the electron-deficient

amide oxygen atom caused by $Pb^{2-}$ ion binding as well as the heavy metal effect.

## Experimental Section

**Synthesis.** Compound **3** was prepared following procedures reported in literature.[18]

**5,11,17,23-Tetra-*tert*-butyl-26,28-dihydroxy-2,8,14,20-tetrathiacalix[4]-25,27-*mono*crown-6, Cone (2).** Under nitrogen, *tert*-butylthiacalix[4]arene (5.00 g, 6.93 mmol), pentaethyleneglycol ditosylates (5.69 g, 10.4 mmol) and $K_2CO_3$ (0.96 g, 6.93 mmol) in 100 mL of acetone were heated to reflux temperature. After refluxed for 24 hours, acetone was removed *in vacuo*. To the resulting white solid, 5% aqueous HCl solution (100 mL) and $CH_2Cl_2$ (50 mL) were added and the organic layer was separated and washed three 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 white solid. Column chromatography on silica gel using a mixture of ethyl acetate/hexane (1/3) as an eluent gave 1.05 g (16% yield) of **2** as a white solid. Mp: 170-178 °C; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 8.04 (s, 2H, ArO*H*), 7.66 (s, 4H, Ar$H_m$), 6.92 (s, 4 H, Ar$H_m$), 4.75 (t, 4H, OC$H_2$CH$_2$O, $J$ = 4.4), 4.13 (t, 4H, OC$H_2$CH$_2$O, $J$ = 4.6), 3.88-3.85 (m, 4H, OC$H_2$CH$_2$O), 3.82-3.79 (m, 4H, OC$H_2$CH$_2$O), 3.74 (s, 4H, OC$H_2$CH$_2$O), 1.33 (s, 18H, CC$H_3$, *tert*-butyl), 0.77 (s, 18H, CC$H_3$, *tert*-butyl); $^{13}$C NMR (100 MHz, $CDCl_3$): 156.0, 147.9, 142.5, 134.6, 132.6, 129.1, 122.2, 73.8, 71.0, 70.9, 70.8, 34.2, 34.0, 31.5, 30.7 ppm; FAB MS $m/z$ ($M^-$): calcd, 923.3. Found, 923.3; Anal. Calcd. for $C_{50}H_{66}O_8S_4$: C, 64.97; H, 7.20; S 13.89. Found: C, 64.97; H, 7.12; S, 13.84.

**5,11,17,23-Tetra-*tert*-butyl-26,28-bis[(*N*-(1-pyrenylmethyl)aminocarbonyl)methoxy]-2,8,14,20-tetrathiacalix[4]-25,27-monocrown-6, Cone (1).** To the mixture of 0.2 g (0.22 mmol) of *tert*-butylthiacalix[4]monocrown-6 (**2**) and 0.13 g (0.433 mmol) of *N*-(1-pyrenylmethyl)chloroacetamide (**3**) in 50 mL of dry acetonitrile, anhydrous potassium carbonate (0.09 g, 0.65 mmol) and a catalytic amount of sodium iodide were added. The reaction mixture was refluxed for 2 days. After removal of the solvent *in vacuo*, the residue was acidified with 10% aqueous HCl solution (50 mL), and then extracted with $CH_2Cl_2$ (50 mL). The organic layer was separated, and washed with 10% HCl solution, and dried over anhydrous $MgSO_4$, and the solvent was evaporated to give the crude product. Column chromatography using a mixture of ethyl acetate/hexane (2 : 1) as an eluent on silica gel gave 0.11 g of **1** in 34% yield. Mp: 267-280 °C; IR (KBr pellet, cm$^{-1}$): 3312 (-NH), 1720 (CO); $^1$H NMR (200 MHz; $CDCl_3$): $\delta$ 8.75 (t, 2H, CONHCH$_2$), 8.36-7.9 (m, 18H, Ar*H*, pyrene), 7.13 (s, 4H, Ar-$H_m$), 7.01 (s, 4H, Ar-$H_m$), 5.32-5.30 (d, 4H, NHC$H_2$pyrene, $J$ = 5.20), 5.11 (s, 4H, ArOC$H_2$CO), 3.99 (t, 4H, OC$H_2$CH$_2$O, $J$ = 5.20 Hz), 3.77 (t, 4H, OC$H_2$CH$_2$O), 3.50-3.40 (m, 12H, OCH$_2$C$H_2$O), 1.01 (s, 18H, CC$H_3$, *tert*-butyl), 0.94 (s, 18H, CC$H_3$, *tert*-butyl); $^{13}$C NMR ($CDCl_3$): $\delta$ 168.9, 159.0, 146.6, 146.2, 134.5, 134.0, 131.7, 131.2, 130.9, 130.8, 129.3, 128.1, 127.5, 125.9, 124.6, 123.5, 74.3, 72.8, 70.6, 70.3, 34.0, 31.0, 30.9 ppm; FAB MS $m/z$ ($M^+$): calcd, 1465.9. Found, 1465.7; Anal. Calcd. for $C_{88}H_{92}N_2O_{10}S_4$: C, 72.10; H, 6.33; S, 8.75. Found: C, 72.41; H, 6.25; S, 8.74.

**General Procedure for Fluorescence Experiment.** Fluorescence spectra were recorded with a RF-5301PC spectrofluorophotometer. Stock solutions (1.00 mM/$CH_3CN$)) of those metal perchlorate salts were prepared. Stock solution of **1** was prepared in acetonitrile (0.06 mM). For all measurements, the excitation was made at 344 nm; excitation and emission slit widths were both 3 nm. Fluorescence titration experiments were performed using 6 $\mu$M of **1** and various amounts of $Pb^{2+}$ perchlorate in a mixture of acetonitrile and chloroform (1 : 1, v/v). After calculating the concentration of the complex forms of **1** and free ligand **1** from the fluorescence titration experiment, the association constant was obtained using the computer program ENZFITTER.[21]

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