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Hg^{2+}-selective fluoroionophore of *p-tert*-butylcalix[4]arene-diaza-crown ether having pyrenylacetamide subunits

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Abstract—A new ionophore having two pyrenylacetamide moieties based on the *p-tert*-butylcalix[4]arene-diaza-crown ether has been prepared and its fluoroionophoric properties were investigated. Bis(pyrenyl) derivative was found to exhibit selective ON–OFF type sensing behavior toward Hg^{2+} ions over other representative transition and heavy metal ions. The fluorescence quenching efficiency of larger than 20-fold was observed with 100 equiv of Hg^{2+} ions and the association constant was found to be $4.5 \times 10^4\,M^{-1}$ in methanol. The ionophore also exhibited a very efficient quenching of excimer fluorescence selectively upon treatment with Hg^{2+} ions in 50% aqueous methanol solution. The observed Hg^{2+}-selective ON–OFF type fluorescence behavior could be utilized as efficient sensing and switching devices for the design of other supramolecular systems.

The toxic effects of mercury species on the environment and our living systems are well known[1] and the developments of efficient detection[2] and treatment techniques[3] are of increasingly importance. A variety of interesting molecules having pyrene moiety as basic signaling functions in fluorescent probes have been reported.[4] With pyrene fluorophores, generally the changes in fluorescence intensity at monomer emissions have been utilized in sensing various target metal ions.[5] In some cases, however, excimer emissions of pyrene rings are more informative in view of the possible utilization of excimer-to-monomer ratio and changes in the maximum and the intensity of excimer emission, which are sensitive to the stimuli around the pyrene molecular probes.[6] Utilizing the characteristics of excimer emissions a variety of molecular probes for the signal transduction of alkaline earth metal ions,[7] Cu^{2+} ions,[8] phosphate,[9] and molecular logic circuits[10] are successfully devised. We have reported that the calix[4]arene-diaza-crown ether itself and its dinitrophenylazo and anthryl appended derivatives exhibit selective ionophoric,[11] chromogenic,[12] and fluorogenic[13] behavior toward mercury ions. In this paper, we report the synthesis of a new fluorogenic ionophore by conjugating the binding site of calix[4]arene-diaza-crown ether with the signaling unit of two fluorogenic pyrenylacetamide moieties. The designed ionophore exhibited a highly Hg^{2+}-selective ON–OFF type fluorescence quenching effect among the tested transition and heavy metal ions in both monomer and excimer emission regions.

Pyrenylacetamide derivative **2** was prepared by the alkylation of *p-tert*-butylcalix[4]arene-diaza-crown ether **1**[13] with 2-chloro-*N*-pyren-1-yl-acetamide **3** (K_2CO_3, KI, CH_3CN, 60 °C), which was prepared by the reaction of 1-aminopyrene with chloroacetyl chloride (NEt_3/CH_2Cl_2), as shown in Scheme 1.[14] The design concept for compound **2** is based on the introduction of two nearby situated pyrenyl groups into the binding site of calix-diaza-crown ether that might act as a sensitive probe by exhibiting changes in either monomer or excimer emissions depending on the state of the ionophore upon the interaction with targeting metal ions or the relative proximity of the two pyrene moieties. The compound **2** can be further functionalized as occasion demands by utilizing extensively investigated functionalization on the upper rim of calix[4]arene[15] into electroactive quinones[16] or by attaching other spectroscopic signaling subunits to yield more information rich dual or multi channel chemosensors.[17] Furthermore, the binding strength toward guest metal ions might be considerably enhanced by the presence of strongly ligating

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Keywords: Calix-aza-crown ether; Fluoroionophore; Pyrene; Hg^{2+}-selectivity; ON–OFF type switching.

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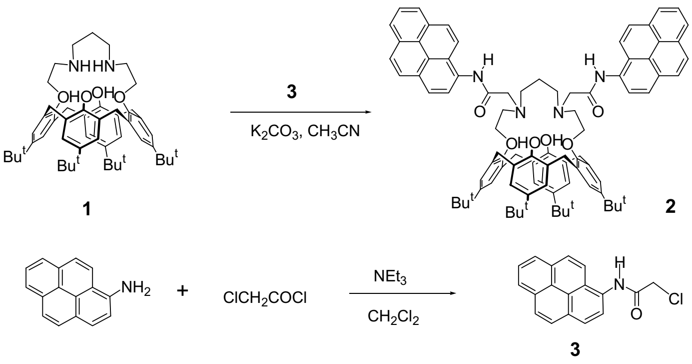


Scheme 1.

amide carbonyl functions around the binding site of calix-aza-crown ether compared with parent compound **1**.

The introduction of pyrenylacetamide groups into the calix-diaza-crown compound resulted in some broadening of the ^{1}H NMR resonances for the protons of diazacrown moiety. In addition to this, aromatic protons of calix[4]arene framework showed significant upfield shifts from δ 7.06 and 6.91 to δ 6.40 and 6.24 due to the presence of pyrene moieties. Such substantial upfield shifts were also observed for the bridging methylene protons (from δ 4.33 and 3.36 to δ 3.87 and 2.65) and in some lesser degree for *tert*-butyl protons (from δ 1.27 and 1.03 to δ 0.89 and 0.67) on the upper rim of the calixarene, too. Similar phenomena were observed for the previously reported anthrylmethyl derivatives of calix[4]arene-aza-crown ethers.[13]

Compound **2** showed an absorption spectrum typical of pyrene derivatives in MeOH. Treatment of the solution ([**2**] = 1.0×10^{-5} M) with 100 equiv of alkaline earth and transition metal ions in perchlorate resulted in no significant changes in its absorption spectral behavior. With Hg^{2+} ions, some broadening of the absorption bands were observed but the changes were insignificant and not so useful for the practical purpose of ion sensing.

In the fluorescence spectrum of pyrene derivative **2**, a series of characteristic monomer emission bands of pyrene moiety were observed around 384 and 404 nm along with a weak band originated from pyrene excimer around 475 nm in MeOH. Interestingly, the fluorescence behavior of **2** was found to be strongly dependent upon the composition of water in the aqueous methanol medium (Fig. 1). The excimer emissions were dominant in MeOH–H_2O (1:1, v/v) solution while monomer emissions were prominent in 100% methanol solution. That might be due to the fact that the increases in water content forced the two pyrene moieties to convert from relatively extended conformation to rather associated one that are responsible for the monomer and excimer emissions, respectively.[18]

Based on these observations, we first investigated the fluoroionophoric properties of **2** in 100% MeOH solution, where the compound **2** showed a fluorescence spectrum consists mainly of the monomeric emission bands with negligible excimer emissions. The surveyed metal ions are alkali, alkaline earth, and representative transition and heavy metal ions (Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+}, and Cd^{2+}). The ionophore showed a very high Hg^{2+}-selectivity and the emission intensity of **2** (1.0×10^{-5} M) was efficiently quenched upon addition of Hg^{2+} ions (Fig. 2). The band intensity at 384 nm was reduced about 96% of its free form in the presence of 100 equiv of Hg^{2+} ions. On the other hand, Cu^{2+} ions showed much lesser responses with the quenching efficiency of only 9% in the presence of 100 equiv of ions and the rest of metal ions showed almost insignificant responses.

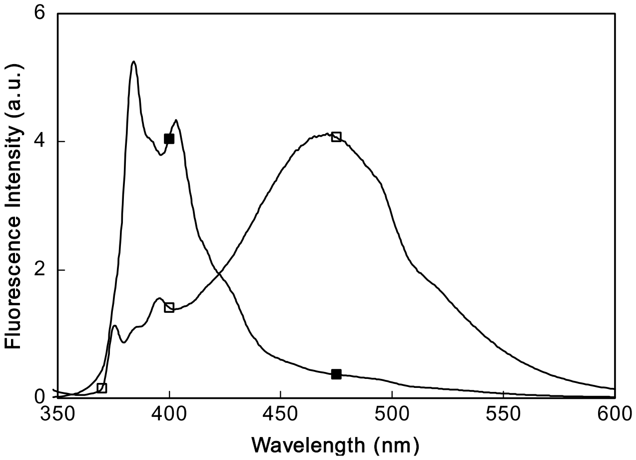


Figure 1. Fluorescence of **2** in pure MeOH (■) and aqueous MeOH (□, MeOH–H_2O = 1:1, v/v). [**2**] = 1.0×10^{-5} M, λ_{ex} = 340 nm.

In order to have more insight into the chemosensor properties of **2** for the Hg^{2+} ion determination, we next performed the fluorescence titration of **2** with Hg^{2+} ions in 100% MeOH solution. The fluorescence intensity decreased continuously as the concentration of Hg^{2+} ions increased (Fig. 3). Particularly, the fluorescence changes are most pronounced in the concentration region of 10^{-5} M to 10^{-4} M of Hg^{2+} ions, where about 80% of the total fluorescence changes of **2** were observed. From the fluorescence titration results the association constants (K_{assoc}) were determined by nonlinear curve fitting procedure[19] and found to be 4.5×10^4 and 3.6×10^2 M^{-1} for Hg^{2+} and Cu^{2+} ions, respectively, which is

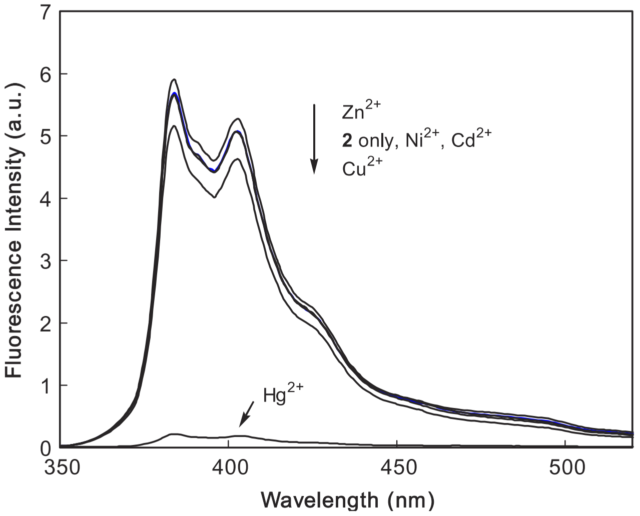


Figure 2. Fluorescence spectra of **2** in MeOH in the absence and presence of metal ions. [**2**] = 1.0×10^{-5} M. [M^{2+}] = 1.0×10^{-3} M. λ_{ex} = 340 nm.

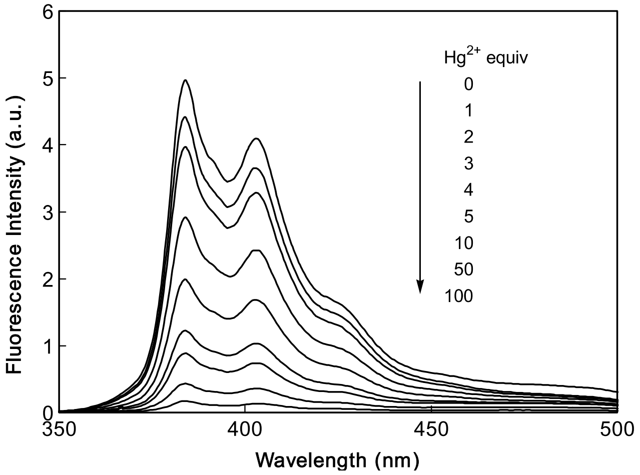


Figure 3. Changes in fluorescence intensity of **2** as a function of Hg^{2+} ions. [**2**] = 1.0×10^{-5} M in MeOH. λ_{ex} = 340 nm.

suitable for the selective analysis of Hg^{2+} ions in submillimolar concentration ranges in environmental and biological samples. Other metal ions revealed no appreciable changes in emission behavior and the binding constants could not be estimated with reasonable accuracy.

On the other hand, in 50% aqueous methanol solution, the intensity of the excimer emission band of **2** around 471 nm increased significantly while those of monomeric bands around 375 and 392 nm decreased about 5-fold when compared with 100% methanol system. The fluorescence intensity of **2** was measured again in the presence of representative transition and heavy metal ions. Treatment of **2** with 100 equiv of Hg^{2+} ions resulted in almost complete disappearance of the excimer band in addition to the significant quenching of the monomer bands, too. In this case, Cu^{2+} also exhibited some responses (35% quenching in the presence of 100 equiv of Cu^{2+} ions) while the other metal ions showed almost no responses.

The practical applicability of the pyrene excimer emission of **2** for the analysis of Hg^{2+} in physiological samples was tested by the experiment performed in the presence of common background metal ions of alkali, alkaline earth, and some representative transition metal ions. In MeOH–water (1:1, v/v) solution, the ionophore revealed a selective and Hg^{2+}-concentration dependent fluorescence quenching behavior in the presence of physiologically important background metal ions ([Na^+] = 138, [K^+] = 4, [Mg^{2+}] = 1, [Ca^{2+}] = 3, [Fe^{2+}] = 0.02, [Co^{2+}] = 0.002, [Cu^{2+}] = 0.015, and [Zn^{2+}] = 0.02 mM)[20] (Fig. 4). Even in the presence of these background metal ions, the ionophore **2** exhibited a selective and sensitive fluorescence quenching behavior for the sensing of Hg^{2+} ions with an association constant of $K_{assoc} = 1.2 \times 10^4 M^{-1}$.

The observed fluorescent behaviors of **2** in response to Hg^{2+} ions in monomer and excimer regions are outlined in Scheme 2. Generally, Hg^{2+} ions have been well recognized as heavy metal ion quenchers.[21] In monomer emission region, the intense pyrene fluorescence of free ionophore **2** was quenched by proximal approach of Hg^{2+} ions upon the complexation, which resulted in ON–OFF type quenching of the compound **2**. Meanwhile, the efficient disappearance of the excimer band might be due to the changes in the orientation of the two pyrene moieties in metal ion complexed state compared with free ionophore in 1:1 MeOH–H_2O solution. The appearance of a strong excimer in this medium suggests that the two pyrene moieties take relatively associated conformation in free ionophore state. On the other hand, the two pyrene moieties of the ionophore adopt relatively divergent orientation in complexed state. That might be due to the presence of amide carbonyl functions, which participate strongly in the complex formation. Through this interaction with rigid *trans* carboxamide function, the two pyrene moieties could not take associated conformation to yield intramolecular stacked excimer emissions. The high selectivity of **2**

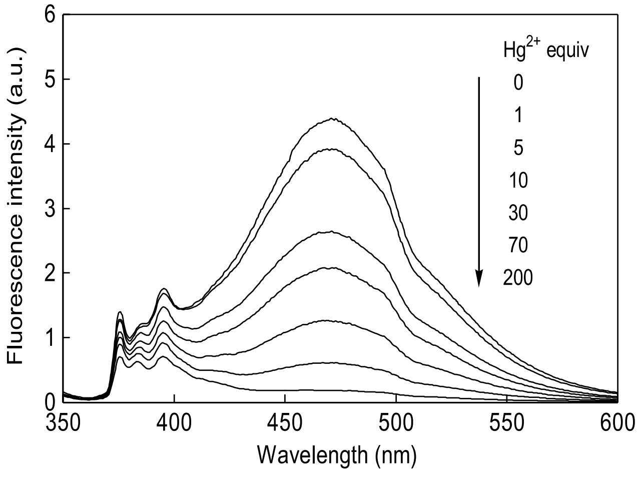
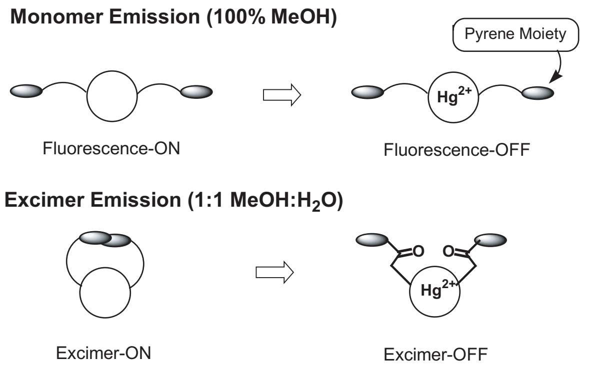


Figure 4. Fluorescence spectra of **2** upon interaction with Hg^{2+} ions in MeOH–H_2O (1:1, v/v). [**2**] = 1.0×10^{-5} M. In the presence of physiologically important metal ions as background ([Na^+] = 138, [K^+] = 4, [Mg^{2+}] = 1, [Ca^{2+}] = 3, [Fe^{2+}] = 0.02, [Co^{2+}] = 0.002, [Cu^{2+}] = 0.015, and [Zn^{2+}] = 0.02 mM).



Scheme 2.

toward Hg^{2+} ions is not completely unexpected since the selective recognition of Hg^{2+} by the analogous calix-aza-crown ether derivatives have been reported previously.[11–13] However, attempts to carry out detailed binding studies for the sensing of Hg^{2+} ions using ^1H NMR spectroscopy failed due to the extensive broadening of the resonances of **2** by the complexation with Hg^{2+} ions in common organic solvent systems including $CDCl_3$–CD_3CN.

In conclusion, the pyrenyl derivative of *p*-*tert*-butylcalix[4]-diaza-crown ether exhibited pronouncedly selective and sensitive ON–OFF type fluoroionophoric properties toward Hg^{2+} ions. The designed compound **2** seems to be relatively well-optimized for the signaling of Hg^{2+} ions over other common interfering transition and heavy metal ions in aqueous media and can be used as a novel sensor material for the detection of Hg^{2+} ions in various biological and environmental samples. The compound also can be used as efficient ON–OFF type switches[22] for the construction of functional supramolecular devices by utilizing monomer or excimer emissions of pyrene moieties.

Acknowledgements

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14. *p*-*tert*-Butylcalix[4]arene-diaza-crown ether **1** was prepared as previously described.[13]
   Synthesis of 2-chloro-*N*-pyren-1-yl-acetamide (**3**). 1-Aminopyrene (1.09 g, 5 mmol) and triethylamine (1.1 mL, 8 mmol) was dissolved in 100 mL of CH_2Cl_2 and chloroacetyl chloride (0.56 mL, 7 mmol) was added dropwise via a syringe. The reaction mixture was stirred for 12 h under N_2 and partitioned between water and CH_2Cl_2. The organic phase was separated and washed with water twice and evaporated. The residue was purified by the column chromatography (silica gel, CH_2Cl_2/EtOAc = 9:1) to yield the product as light gray powder. Yield: 90%, ^1H NMR ($CDCl_3$, 300 MHz) δ 9.03 (br s, 1H), 8.46 (d, $J = 8.3$ Hz, 1H), 8.21 (m, 3H), 8.17 (d, $J = 9.2$ Hz, 1H), 8.06 (m, 3H), 8.03 (t, $J = 7.6$ Hz, 1H), 4.44 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.78, 131.52, 130.96, 129.87, 129.19, 128.72, 127.51, 127.48, 127.18, 126.55, 125.99, 125.57, 125.45, 124.90, 123.84, 121.99, 119.91, 43.63. HR-MS (FAB, *m*-NBA) calcd for $C_{18}H_{12}ClNO$ 293.0607; found: 293.0695.
   Synthesis of pyrenylacetamide derivative of calix-aza-crown ether (**2**). A mixture of calix-aza-crown ether **1** (155 mg, 0.2 mmol), 2-chloro-*N*-pyren-1-yl-acetamide **3** (147 mg, 0.5 mmol), KI (17 mg, 0.1 mmol), and K_2CO_3 (277 mg, 2 mmol) in CH_3CN (100 mL) was refluxed under N_2 atmosphere for 24 h. After filtration of the reaction mixture and evaporation under reduced pressure, the residue was partitioned between water and CH_2Cl_2. The organic phase was separated and washed with water. Evaporation of the solvent and purification by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) yielded the desired product as white powder. Yield 24%. ^1H NMR (600 MHz, $CDCl_3$) δ 10.34 (s, 2H), 8.18–7.84 (m, 9H), 7.35 (s, 2H), 6.40 and 6.24 (s, 4H each), 4.01 (br m, 4H), 3.87 (d, $J = 12.9$ Hz, 4H), 3.76 (s, 4H), 3.53 (br m, 4H), 3.21 (br m, 4H), 3.03 (br m, 2H), 2.65 (d, $J = 13.2$ Hz, 4H), 0.89

and 0.67 (s, 18H each). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.78, 149.08, 148.50, 146.96, 141.17, 132.30, 131.11, 130.74, 130.09, 129.31, 127.46, 127.31, 127.09, 126.63, 125.78, 125.19, 125.15, 124.85, 124.68, 123.93, 123.75, 121.48, 73.71, 60.59, 55.64, 55.39, 33.65, 33.23, 31.30, 30.81, 30.65, 29.99. HR-MS (FAB, PEG 1000): calcd for $C_{87}H_{93}N_4O_6$ 1289.710; found 1289.705.

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