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Diametrically Disubstituted Cyclam Derivative Having Hg^{2+}-Selective Fluoroionophoric Behaviors

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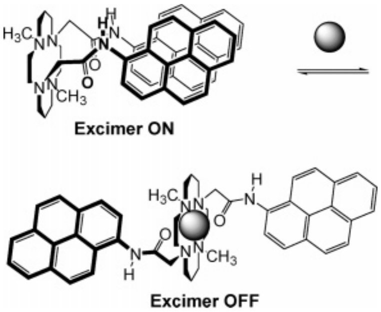

A new fluoroionophore has been synthesized by appending two signaling pyrenylacetamide subunits on the binding motif of 1,8-dimethylcyclam. The designed compound exhibited highly selective and sensitive fluoroionophoric behavior toward Hg^{2+} ions of excimer emission in aqueous dioxane (dioxane/H_2O = 1:9, v/v) solution with a detection limit of 1.3×10^{-6} M. The "ON−OFF" type signaling behavior of the fluoroionophore is due to the metal ion induced conformational changes from folded to open-winged conformations by exploiting the two nearby appended pyrenyl fluorophores.

In recent years, the developments of selective and sensitive chemosensors for the important chemical species have been the focus of much attention.[1] Mercury is a metal of great environmental concern owing to the highly toxic nature of many of its compounds.[2] A number of Hg^{2+}-selective sensors[3] has been devised by utilizing chromogenic and fluorogenic behavior of the designed compounds. For the design of sensitive chemosensors having fluorescence responses, pyrene and anthracene subunits are particularly useful as signaling handles.[4] Cyclam (1,4,8,11-tetraazacyclotetradecane) is one of the most attractive molecular framework for the design of transition metal ion selective functional chemosensor systems.[5] We attempted to exploit the unique excimer behavior of pyrene,[6] which has been ingeniously utilized in the design of supramolecular systems, by introducing two nearby placed pyrene subunits into the cyclam platform. Direct introduction of two pyrene moieties into cyclam might be synthetically demanding due to the difficulties in the control of regioselectivity and degree of alkylation;[7] therefore, the compound **1** seems to be suitable for the synthesis of diametrically disubstituted products. We have reported PET (photoinduced electron transfer) based Hg^{2+}-selective fluoroionophoric properties of bis(anthrylmethyl) derivative of **1** by utilizing the structural properties of dimethyl protected cyclam framework.[3j] In this paper, we report the synthesis and Hg^{2+}-selective fluoroionophoric properties of the 1,8-bis(pyrenylacetamide) substituted cyclam derivative. The compound was designed by conjugating the two well-known molecular motifs of pyrenylacetamide as signaling handle and basic molecular framework of cyclam as recognition subunit in the hope for the utilization of excimer emissions. The designed compound exhibited a pronounced Hg^{2+}-selectivity among the representative transition metal ions sufficient for the determination of toxic Hg^{2+} ions in micromolar concentration range of environmental and biological samples in aqueous media.

Diametrically disubstituted cyclam derivative **2** was prepared from the 1,8-dimethylcyclam (1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane)[8] **1** readily by the reac-

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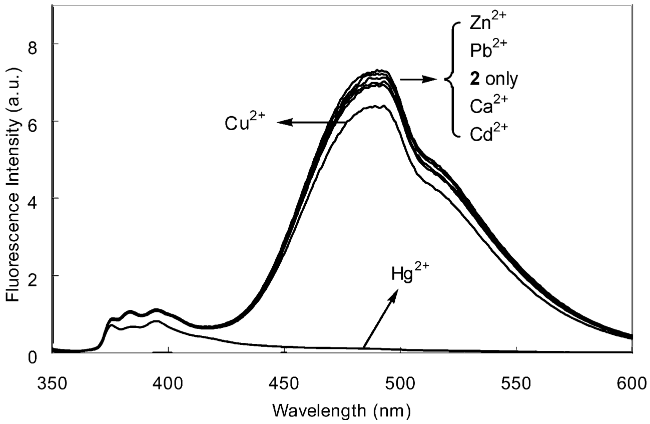
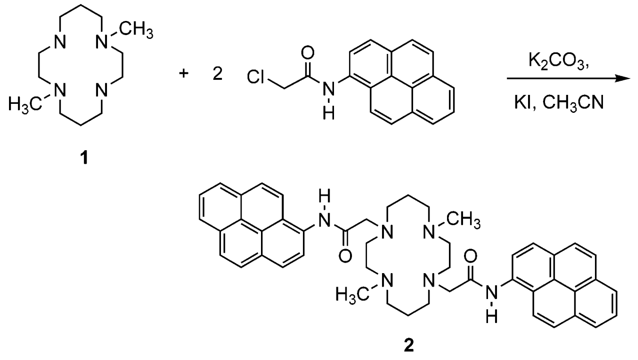


FIGURE 1. Fluorescence spectra of **2** in the presence of varying metal ions. [**2**] = 1.0×10^{-5} M. [M^{2+}] = 1.0×10^{-4} M. In dioxane−H_2O (1:9, v/v). [Acetate buffer] = 1.0×10^{-2} M (pH = 4.8). λ_{ex} = 340 nm.

SCHEME 1



tion with 2-chloro-*N*-pyren-1-ylacetamide[9] (K_2CO_3, KI, CH_3CN), which was obtained by the reaction of 1-aminopyrene with chloroacetyl chloride, in good yield (60%) (Scheme 1).

The chemosensor behavior of the ionophore **2** was investigated by the fluorescence measurements. The fluorescence characteristics of **2** were found to be strongly dependent on the nature of the employed medium. In relatively nonpolar solvents of chloroform, THF, and dioxane the characteristic monomer emissions of pyrene fluorophore were observed around 370−400 nm. On the other hand, in aqueous organic solution excimer emissions became prominent and the intensity was enhanced as the composition of water increased. For example, the compound revealed a strong excimer[10] fluorescence around 490 nm in dioxane−H_2O (1:9, v/v) solution (Figure 1), while it showed mainly monomeric emission at 370−400 nm with almost no excimer emissions in dioxane−H_2O (9:1, v/v) solution. In this case, the fluorescence in the pyrene excimer region increased significantly as the water content increased, particularly in the vicinity of 40% water composition then the changes were not so significant up to 90% aqueous solution. So, we have measured the excimer fluorescence characteristics of **2** in dioxane−H_2O (1:9, v/v, buffered at pH 4.8 with 10 mM acetate buffer) where the fluorescence behavior of free ionophore was relatively unaffected by the changes in water composition of the medium.

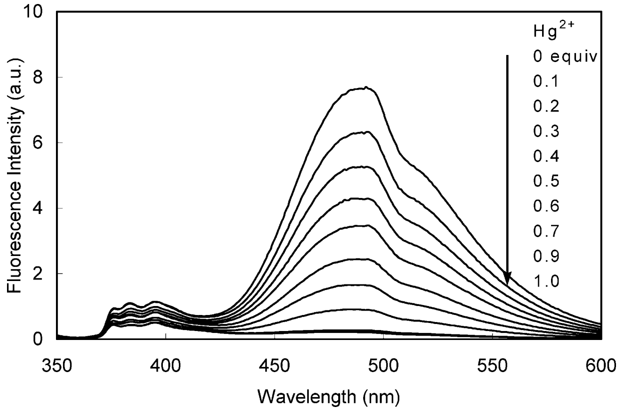


FIGURE 2. Fluorescence changes as a function of [Hg^{2+}] in excimer region of **2**. [**2**] = 1.0×10^{-5} M. In dioxane/H_2O = 1:9, v/v. [Acetate buffer] = 1.0×10^{-2} M (pH = 4.8). λ_{ex} = 340 nm.

Since the compound has basic amino groups in the molecular framework which might influence the fluorescence behavior of the ionophore, we tried to find the optimum pH for the selective responses of the fluorescence behavior of **2** in excimer region. The compound exhibited a strong fluorescence in acidic pH region which starts to decrease around pH 5 down to the baseline and then no further changes were observed at higher pH above 7. However, in the presence of Hg^{2+} ions, the fluorescence of **2** starts to decrease from more acidic region of pH 3 and was almost completely quenched around pH 4 (Supporting Information). Therefore, we tried to investigate the selective fluorescence quenching behavior of **2** in excimer region under the condition of acetate buffered solution (10 mM) at pH 4.8 in dioxane−H_2O (1:9, v/v).

Based on these observations, we investigated the fluorescence behavior of **2** in aqueous 90% dioxane solution where the excimer emissions around 490 nm were predominant (Figure 1). The emission spectra of **2** were not so significantly affected by the addition of 10 equiv of representative transition metal ions of Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} ions as well as alkali and alkaline earth metal ions. However, with 10 equiv of Hg^{2+} ions the pyrene excimer emissions centered around 490 nm were almost completely quenched. In this case, the fluorescence quenching was very efficient such that upon interaction with 1 equiv of Hg^{2+} ions, the excimer emission was almost completely quenched down to less than 2% of its original intensity. The fluorescence changes of **2** can be visually discernible readily by the disappearance of the intense greenish blue fluorescence. In contrast to this, monomeric fluorescences of **2** around 370−400 nm were not so pronouncedly affected. The selective fluorescence quenching efficiencies toward Hg^{2+} and second most responding Cu^{2+} ions expressed as the ratio of the fluorescence intensity (I_o/I) at 490 nm, in the presence (I) and the absence of metal ions (I_o), respectively, were 68.9 for Hg^{2+} and 1.12 for Cu^{2+} ions.

The stoichiometry of the **2**−Hg^{2+} complex was found to be 1:1 in the present system by Job's plot.[11] From the

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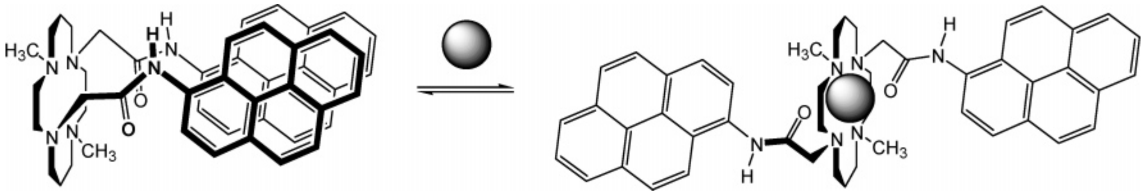
SCHEME 2



fluorescence titration results the association constant (K_{assoc}) was evaluated by nonlinear curve fitting procedure[12] and was found to be very large ($> 10^6$ M^{-1}) (Figure 2). Detection limit[13] for the analysis of Hg^{2+} ions was also estimated from the plot of fluorescence intensity changes versus concentration of added metal ions and was found to be 1.3×10^{-6} M that is sufficient enough for the analysis of micromolar concentration of Hg^{2+} ions encountered in chemical and environmental samples. The selectivity toward Hg^{2+} ions was further ascertained by the competition experiments with commonly encountered interfering physiologically or environmentally important metal ions. The fluorescence quenching efficiency of Hg^{2+} was not affected in the presence of 100 equiv of surveyed alkali, alkaline earth, and representative transition metal ions as background.

The selective quenching behavior might be due to the intrinsic quenching nature of the Hg^{2+} ions[14] which were strongly and selectively complexed by the cyclam backbone and also by the appending carboxamide functions.[15] The strong peak of $[\mathbf{2} + Hg-H]^+$ at $m/z = 943.4$ (100% intensity) observed in MALDI-TOF spectrum also supported the Hg^{2+}-selectivity of **2** in ion-sensing behavior. In fact, Hg^{2+} ion binding properties of cyclam has been utilized in the design of functional supramolecular systems.[16]

The plausible quenching mechanism of **2** in the present system is schematically depicted in Scheme 2. The strong excimer emission of the compound **2** in dioxane–H_2O (1:9, v/v) solution suggests that the free ionophore **2** exists on the average in conformations of the two pyrene moieties situated in close proximity.[17] This folded conformation resulted in a strong excimer emissions in employed solvents of high water contents. The intramolecular nature of excimer formation of **2** was confirmed by the observation of the relatively concentration independent behavior of the excimer/monomer ratio[18] in the range of 1×10^{-4}–5×10^{-6} M under the same experimental conditions. We also tried to obtain evidences for the proposed Hg^{2+}-ion induced conformational changes by NMR spectroscopy but failed due to the limited solubility or extensive broadening of the resonances under the relevant experimental conditions. By complexation with Hg^{2+} ions the molecule took relatively extended conformation which resulted in almost complete disappearance of the strong pyrene excimer emissions. The transformation into extended conformation might be partly due to the participation of the carbonyl group of the amide function of the appending side chain in the complex formation with the guest metal ions, which forces the two pyrene moieties in the relatively extended and far separated orientations. In this extended conformation the pyrene fluorescences in monomer emission region were not revived as expected for the extended conformation of the ligand and also quenched by the complexed Hg^{2+} ions having intrinsic quenching characteristics. The metal ion induced conformational changes[19] effected in response to the added metal ion observed in the present system are reminiscence of a butterfly-like motion of the molecule by folding and extending the two large wings of pyrene moiety around cyclam backbone as a pivot in response to the presence of Hg^{2+} ions.

In summary, a new Hg^{2+}-selective fluoroionophore was designed by the conjugation of the well-known molecular motifs of cyclam ionophore and pyrene fluorophore. The designed compound exhibited highly selective and sensitive fluorescence quenching efficiency in excimer emission by a metal ion induced molecular motion in response to the added chemical input of Hg^{2+} ions. The ON–OFF type fluorescence responding behavior of the compound could be utilized as a new signaling or switching component for the design of functional supramolecular systems employing the toxic and important chemical species of Hg^{2+} ions.

Experimental Section

Synthesis. 1,8-Dimethyl-1,4,8,11-tetraazacyclotetradecane **1** and 1-aminopyrene were purchased. 2-Chloro-*N*-pyren-1-ylacetamide was prepared following the reported procedure.[9]

4,11-Bis(*N*-pyren-1-yl-acetamide)-1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane 2. A mixture of 1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane (228 mg, 1 mmol) and 2-chloro-*N*-pyren-1-yl-acetamide (730 mg, 2.5 mmol) was heated under reflux in the presence of K_2CO_3 (552 mg, 4 mmol) and KI (166 mg, 1 mmol) in acetonitrile (50 mL). After 24 h the reaction mixture was evaporated and the residue was partitioned between water and CH_2Cl_2. The organic layer was separated and washed with water and the solvent was evaporated. The crude mixture was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9:1) followed by the crystallization from hexane–CH_2Cl_2 to yield light amber colored **2**: yield 447 mg (60%); ^{1}H

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NMR ($CDCl_3$, 300 MHz) δ 10.99 (s, 2H), 8.51 (d, $J = 8.4$ Hz, 2H), 8.27 (d, $J = 9.3$ Hz, 2H), 8.21−7.95 (m, 14H), 3.39 (s, 4H), 2.82 (br m, 4H), 2.76 (br m, 4H), 2.64 (br m, 8H), 2.13 (s, 6H), 1.73 (m, $J = 6.3$ Hz, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.8, 131.6, 131.5, 131.1, 129.1, 127.6, 127.5, 126.9, 126.3, 125.7, 125.6, 125.5, 125.1, 123.8, 121.9, 121.3, 60.1, 55.7, 54.0, 53.9, 53.1, 43.7, 26.0; HR-MS (FAB, *m*-NBA) found 743.404, calcd for $C_{48}H_{51}N_6O_2$ 743.407.

General Procedure for Fluorescence Measurements. All the solvents used for the fluorescence measurements were spectroscopic grade. For a given measurement condition, aliquots of stock solutions of ligand (dioxane solution), metal ions (aqueous solution), and buffer (aqueous acetate buffer solution) were added and diluted with calculated amounts of water or organic solvents to make the required ionophore and metal ion concentrations as well as the solvent compositions.

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Supporting Information Available: ^{1}H NMR spectra, pictures of fluorescence of **2**, and effects of water composition, pH, and solvents on the fluorescence behavior of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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