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Hg^{2+}-Selective OFF–ON and Cu^{2+}-Selective ON–OFF Type Fluoroionophore Based upon Cyclam

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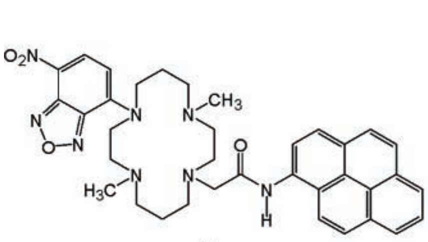
ABSTRACT



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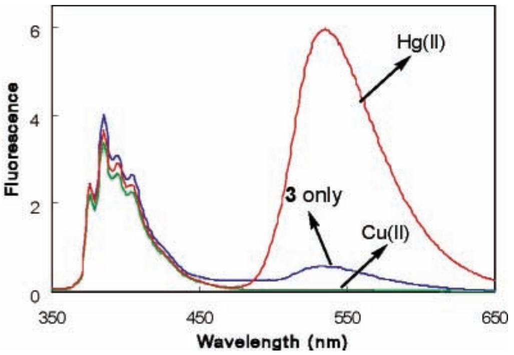

A new cyclam derivative having two different fluorophores of pyrene and NBD subunits was prepared, and its Hg^{2+}- and Cu^{2+}-selective signaling behaviors were investigated. The detection limits for the analysis of Hg^{2+} and Cu^{2+} ions were found to be 7.9×10^{-6} and 2.6×10^{-7} M in aqueous acetonitrile solution ($H_2O–CH_3CN$ = 10:90, v/v), respectively. The compound also exhibited a selective Hg^{2+}/Cu^{2+}-induced OFF–ON–OFF type of signaling pattern that can be utilized for the construction of functional supramolecular switching systems.

Selective signaling of chemically and biologically important metal ions has attracted active research interest in supramolecular chemistry.[1] Of particular interest is the development of chemosensors for the heavy and transition metal ions because of their toxic impacts on our environments[2] and many important roles in living systems.[3] Cyclams have very attractive structural properties for the design of transition metal ion selective chemosensors and many elaborately designed systems have been reported.[4] Among various structures of cyclams, dimethylcyclam **1** has ideal structural properties particularly for the preparation of diametrically disubstituted cyclam derivatives.[5] On the other hand, NBD (7-nitrobenzo-2-oxa-1,3-diazolyl) moiety has been widely used as a signaling handle for the design of functional supramolecular systems.[6] Particularly, cyclam derivatives having NBD functions are developed for the selective recognition of Cu^{2+} and Ni^{2+} ions.[7,8] In this paper we report

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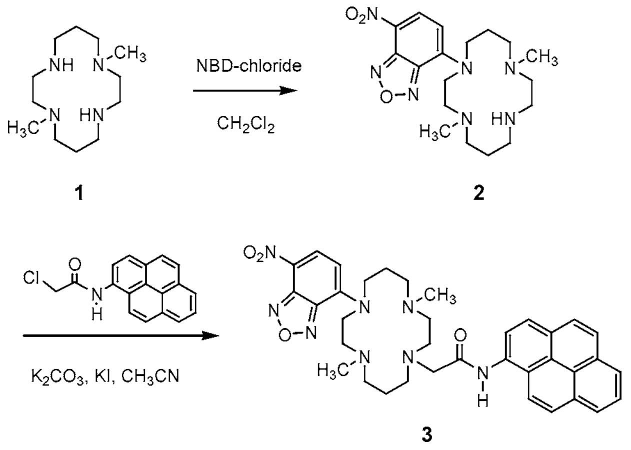
the synthesis and fluoroionophoric properties of a new cyclam derivative appended with two different fluorophores of pyrene and NBD moieties aiming for the selective recognition of transition metal ions. The prepared compound exhibited pronounced Hg^{2+}-selective OFF−ON and Cu^{2+}-selective ON−OFF type signaling behaviors that can be utilized for the analysis of Hg^{2+} and Cu^{2+} ions.[9,10] Furthermore, a unique competitive cation-induced OFF−ON−OFF type of fluorescent signal control behavior with Hg^{2+} and Cu^{2+} was also observed, which could be utilized as a novel switching system for the construction of new molecular level devices.[11]

Scheme 1. Synthesis of Mixed Functionalized Cyclam



Mixed functionalized cyclam derivative **3** was prepared from dimethylcyclam by succesive introduction of two fluorophores following Scheme 1. Monoalkylation of 1,8-dimethylcyclam **1** with 0.3 equiv of NBD chloride (CH_2Cl_2, reflux) afforded cyclam-NBD derivative **2** in moderate yield (64%) with dialkylated byproduct (<3%). Cyclam-NBD **2** was subsequently reacted with 2-chloro-*N*-pyren-1-ylacetamide[12] (K_2CO_3, KI, CH_3CN) to yield mixed functionalized NBD-cyclam-pyrene derivative **3** (76%).

Compound **3** was designed in the hope for the possible utilization of dual signaling behaviors of two unique fluorophores of pyrene and NBD moieties. The UV−vis spectrum of **3** showed two broad absorption bands around 342 and 484 nm typical of pyrene and NBD subunits, respectively. Upon interaction with Hg^{2+} ions a slight blue shift ($\Delta\lambda_{max} = -11$ nm) in the absorption band of the NBD region was observed with some splitting of the pyrene absorption bands (Figure 1). This shift resulted in the color change from light orange to yellow. Cu^{2+} ions exhibited somewhat increased absorbance in the pyrene region with a pronouncedly reduced absorbance with a blue shift ($\Delta\lambda_{max} = -11$ nm) in the NBD region reminiscent to the reported behavior of NBD-functionalized cyclam.[7] Other tested metal ions of representative alkali, alkaline earth, and transition metal ions showed almost insignificant changes.

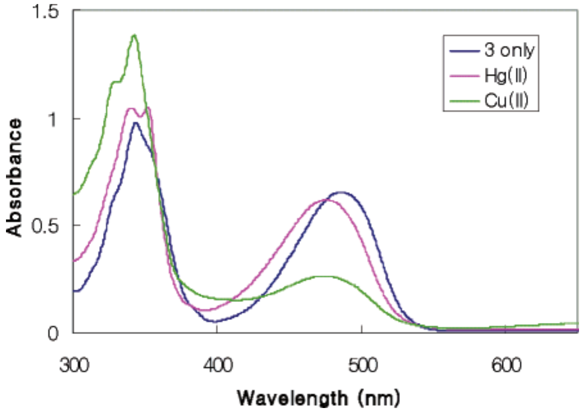


Figure 1. UV−Vis spectral behaviors of **3** in the presence of Hg^{2+} and Cu^{2+} ions. [**3**] = 2.5×10^{-5} M, [M^{2+}] = 2.5×10^{-3} M, in H_2O−MeCN (10:90, v/v). Buffered at pH 4.8 with acetate buffer (10 mM).

The fluorescent spectral properties of **3** were surveyed in typical organic solvent systems including their aqueous solutions. Along with this, to have a more optimized condition for the realization of peak selectivity for the targeting metal ions, the effects of water on the fluorescence of **3** in the absence and presence of metal ions were systematically investigated (Figure S9, Supporting Information).

On the basis of these optimizations, the fluoroionophoric behaviors of **3** toward metal ions were investigated in 90% aqueous acetonitrile (H_2O−CH_3CN, 10:90, v/v) solution ([**3**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M, buffered with 10

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mM acetate buffer at pH 4.8). Surveyed metal ions were representative alkali (Na^+, K^+), alkaline earth (Ca^{2+}, Mg^{2+}), and transition metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}). Free ionophore **3** showed characteristic emission bands around 385 and 538 nm for pyrene and NBD moieties, respectively (Figure 2). The fluorescence behavior of **3** in pyrene region was not so discernibly affected by the addition of varying metal ions. On the other hand, a sizable fluorescence intensity enhancement was observed in the NBD emission region upon interaction with Hg^{2+} ions, as has been reported earlier for an NBD-functionalized acyclic azathia system.[6a] The increase in fluorescence intensity of **3** induced by Hg^{2+} ions at 538 nm was about 10-fold. Other metal ions, except for Cu^{2+}, revealed relatively insignificant responses in this region manifesting the pronounced OFF−ON type of Hg^{2+}-selectivity of **3**. With Cu^{2+} ions, however, a marked quenching of the NBD region was observed, again consistent with the earlier reported mono-NBD-functionalized cyclam,[7] and the fluorescence intensity reduced almost completely to the baseline; quenching efficiency = 90% with 100 equiv (5.0×10^{-4} M) of Cu^{2+} ions. The discrimination between two oppositely responding Hg^{2+} and Cu^{2+} ions could be realized ratiometrically[13] by using the large changes in NBD emission with reference to the relatively invariant pyrene emissions as internal fluorescence reference. The selectivities toward surveyed metal ions expressed by the ratio (I_{NBD}/I_{pyrene}) of the fluorescence intensity at 538 and 385 nm were found to be 1.60 (Hg^{2+}) and 0.016 (Cu^{2+}). The rest of metal ions revealed relatively constant I_{NBD}/I_{pyrene} values of 0.12−0.15 (Figure S10).

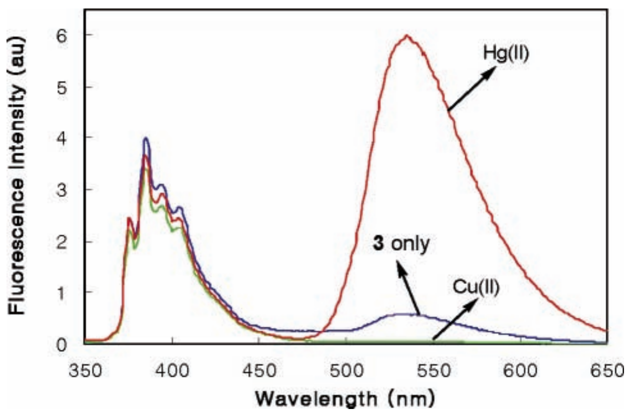

Figure 2. Fluorescence spectra of **3** in response to the presence of Hg^{2+} and Cu^{2+} ions. [**3**] = 5.0×10^{-6} M, [M^{2+}] = 5.0×10^{-4} M, in H_2O−MeCN (10:90, v/v). λ_{ex} = 340 nm. Buffered at pH 4.8 with acetate buffer (10 mM).

Titration of **3** with Hg^{2+} or Cu^{2+} ions yielded progressively enhanced or quenched fluorescence of NBD moiety at 538 nm, respectively (Figure S11). From the titration data, association constants (K_{assoc}) for the formation of **3**−Hg^{2+} and **3**−Cu^{2+} complexes were estimated by the nonlinear curve fitting procedure[14] and found to be 2.5×10^4 and 8.3×10^6 M^{-1}, respectively. The relatively low K_{assoc} values of **3** compared to those of other classical tetraalkylated cyclams might be due to the transformation of one nitrogen atom of the cyclam into part of a very weak ligating NBD substituent.[6d] The K_{assoc} values obtained suggest that the Cu^{2+} ions formed more stable complexes with compound **3** than Hg^{2+} ions did. This fact was also confirmed by the competition experiments carried out for the complexation of the ionophore between Cu^{2+} and Hg^{2+} ions. That is, upon treatment with Cu^{2+} ions (100 equiv) the fluorescence profile of the **3**−Hg^{2+} system (obtained by treating **3** with 100 equiv of Hg^{2+} ions) was transformed into almost that of the **3**−Cu^{2+} system, whereas upon treatment of Hg^{2+} ions with the **3**−Cu^{2+} system the fluorescence profile was not affected at all. From the fluorescence titration results, the detection limits[15] for the analysis of Hg^{2+} and Cu^{2+} ions in 90% aqueous acetonitrile were calculated to be 7.9×10^{-6} and 2.6×10^{-7} M, respectively.

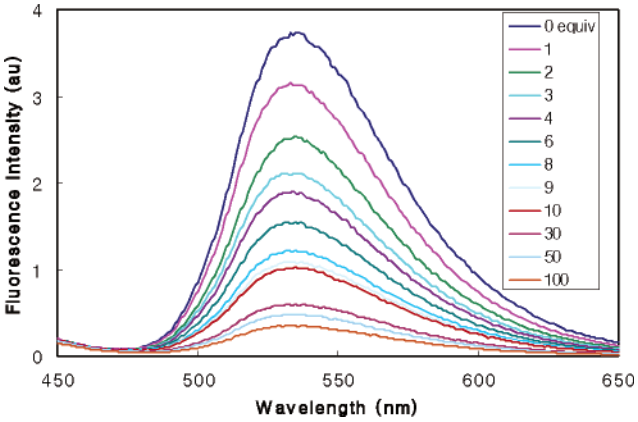

Figure 3. Titration of **3** with Cu^{2+} ions in the presence of 10 equiv of Hg^{2+}. [**3**] = 5.0×10^{-6} M, [Hg^{2+}] = 5.0×10^{-5} M, in H_2O−MeCN (10:90, v/v). λ_{ex} = 340 nm. Buffered at pH 4.8 with acetate buffer (10 mM). Inserts denote the equivalent of Cu^{2+} added.

To take possible advantage of the fluorescence enhancement induced by Hg^{2+} ions of fluoroionophore **3** in the ON−OFF type analysis of Cu^{2+} ions, the effects of diverse metal ions on the fluorescence behaviors of the **3**−Hg^{2+} system were tested. First the solution of **3** (5.0×10^{-6} M) was treated with 10 equiv of Hg^{2+} ions to obtain significantly enhanced fluorescence of the **3**−Hg^{2+} system in the NBD region. Upon interaction with varying metal ions, the enhanced fluorescence signal of the **3**−Hg^{2+} system was effectively quenched only with Cu^{2+} ions. By the treatment with an incremental amount of Cu^{2+} ions, the emission profile of the **3**−Hg^{2+} system was progressively transformed into that of the **3**−Cu^{2+} system (Figure 3). The competitive Hg^{2+}/Cu^{2+}

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cation-induced OFF−ON−OFF type of fluorescent signal control behavior is reminiscent of the result for Ca^{2+}/K^+-induced chromogenic behavior of a calix[4]-crown-5 derivative having an indoaniline chromophore.[16]

The Hg^{2+}- and Cu^{2+}-selective responses of **3** were found to be not affected by the presence of physiologically important metal ions ($[Na^+] = 145$ mM, $[K^+] = 5$ mM, $[Mg^{2+}] = 2$ mM, and $[Ca^{2+}] = 5$ mM),[17] which suggests the possibility of **3** for the determination of Hg^{2+} and Cu^{2+} concentrations in physiologically relevant samples (Figure S12). The reversibility of the compound **3** for the sensing of Hg^{2+} and Cu^{2+} ions was evidenced by the treatment with EDTA (Figure S13). For example, the enhanced fluorescence of **3** (5.0×10^{-6} M) induced by 10 equiv of Hg^{2+} ions was transformed into that of free **3** by the treatment with 100 equiv of EDTA. Subsequent treatment with Hg^{2+} ions (200 equiv) again resulted in largely enhanced fluorescence of the **3**−Hg^{2+} system. Similar demetalation with EDTA and subsequent fluorescence chemosensing behavior was also observed for the **3**−Cu^{2+} system.

The OFF−ON and ON−OFF type signalings of **3** in the NBD region by Hg^{2+} ions[6a] and Cu^{2+} ions[7] were consistent with the results obtained by other chemosensors having NBD moiety. The complexation of **3** with Hg^{2+} ions was further evidenced by the ^{1}H NMR measurements (CD_3CN−CD_3OD, 1:1). In the NMR spectrum the addition of Hg^{2+} ions resulted in significant broadening particularly for the resonances of the cyclam moiety of **3**. However, the resonances for NBD protons remained discernible and experienced a significant shift resulting in narrowing of the separation between the two protons upon complexation with Hg^{2+} ions (Figure S14). Because of the extensive broadening, the NMR measurement for the **3**−Cu^{2+} system does not provide any useful information. Complexation of Cu^{2+} ions could be further evidenced by the MALDI measurements. Compound **3** exhibited an intense peak for the **3**−Cu^{2+} species at $m/z = 711.26$ (100% intensity) upon treatment with $Cu(ClO_4)_2$.

In summary, a new cyclam derivative having two different fluorophores of pyrene and NBD subunits was prepared, and the Hg^{2+}-selective OFF−ON type and Cu^{2+}-selective ON−OFF type signaling behaviors were investigated. The pronounced fluorogenic signaling behavior of the prepared compound could be utilized as a probe for monitoring Hg^{2+} and Cu^{2+} levels in extracellular physiological systems. The compound also exhibited a Hg^{2+}/Cu^{2+}-induced OFF−ON−OFF type of fluorescent signaling pattern that can be utilized for the construction of more elaborate supramolecular switching systems.

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Supporting Information Available: Experimental details and characterization for new compounds, NMR spectra, and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

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