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Hg²⁺- and Cu²⁺-selective fluoroionophoric behaviors of a dioxocyclam derivative bearing anthrylacetamide moieties

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Abstract—New dioxocyclam derivatives bearing two anthracene fluorophores were prepared, and their fluoroionophoric properties toward transition metal ions were investigated. Chemosensor **2** having anthrylacetamide moieties exhibited pronounced Hg²⁺- and Cu²⁺-selective fluoroionophoric properties in aqueous acetonitrile solution over other representative transition metal ions, as well as alkali and alkaline earth metal ions. Chemosensor **2** also exhibited Hg²⁺ and Cu²⁺ selectivity under competitive conditions in the presence of physiologically and environmentally important metal ions. The detection limits for the sensing of Hg²⁺ and Cu²⁺ ions were 7.8×10^{-6} and 1.5×10^{-6} M, respectively, in aqueous 95% acetonitrile solution.
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Dioxocyclams are a subclass of cyclam in which two of the four amine groups are replaced by two amide groups.[1] They are intermediate in their metal complexation properties between cyclic peptides and cyclic polyamines.[2] Due to their great affinity toward transition metal ions, dioxocyclam and its analogues are one of the most attractive molecular frameworks for the design of functional ionophores for the selective recognition of transition metal ions. The secondary amine groups of dioxocyclam can act as efficient ligands for the binding of transition metal ions, and can also be further functionalized with suitable ligating groups or signaling handles to yield various functional ionophores. In this line, dioxocyclams having two appending methylfuran or methylquinoline moieties have been synthesized and the structures of their corresponding Ni²⁺ or Cu²⁺ complexes have been elucidated.[3] On the other hand, similarly structured isomers of dioxocyclam, also 14-membered tetraazamacrocycles having two alternating amines and amides as part of the ring have been synthesized and the ability of their Ni²⁺ complexes to cleave DNA has been reported.[4] Other interesting mono- and bis-dioxocyclams derivatized with pyridine cap and polyethylene glycol linkages for the complexation of Cu²⁺ and Gd³⁺ have also been reported.[2,5]

More recently, a naphthylimide appended macrocyclic dioxotetraamine exhibiting Cu²⁺- and Hg²⁺-selective colorimetric and fluorescent sensing behaviors was reported.[6] However, relatively few examples of macrocyclic dioxotetraamines having signaling functional groups have been reported in comparison with their widely investigated cyclam-type macrocyclic tetraamine analogues.[7]

In recent decades, a large number of fluorogenic ionophores of various structures based upon many versatile molecular platforms have been designed,[8] and anthracene fluorophores have emerged as one of the most effective functional groups for the purpose of fluorescence signaling.[9] We have reported that bis(9-anthrylmethyl) derivative of 1,8-dimethylcyclam exhibits significant OFF–ON type fluorescence responses upon interaction with Hg²⁺ and Cd²⁺ ions.[10] We attempted to develop new fluorogenic ionophores based on the structural characteristics of the dioxocyclam binding site by appending two anthracene functions, with the intent to selectively sense transition metal ions. In this Letter, we report the synthesis and fluoroionophoric properties of two new dioxocyclam derivatives bearing anthracene fluorophores for the recognition of transition metal ions. In semi-aqueous media, the anthrylacetamide derivative **2** exhibited a markedly selective fluorescence signaling suitable for the selective sensing of Hg²⁺ or Cu²⁺ ions[11,12] in the presence of other competing transition metal ions, as well as alkali and alkaline earth metal ions (see Scheme 1).
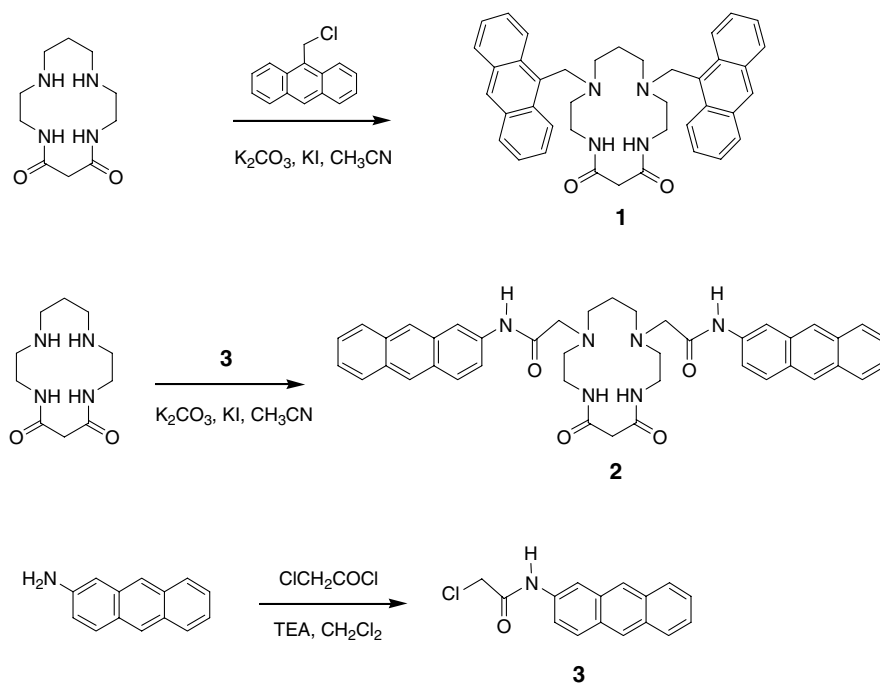
Scheme 1.

Designed chemosensors **1** and **2** were easily prepared by the alkylation of dioxocyclam (1,4,8,11-tetraazacyclo-tetradecane-5,7-dione) with 9-(chloromethyl)anthracene or *N*-anthracen-2-yl-2-chloroacetamide (K$_2$CO$_3$, KI, CH$_3$CN), respectively.[13] *N*-Anthracen-2-yl-2-chloroacet-amide **3** was prepared by the reaction of 2-amino-anthracene with chloroacetyl chloride.[14] The bis-(anthrylmethyl) derivative **1** is a simple conjugation of the two well-known molecular motifs of dioxocyclam and anthracene, and the complexation events would be signaled by the control of the photoinduced electron transfer (PET) process[15] operating between the amino group of the dioxocyclam and the anthracene fluoro-phore. On the other hand, bis(anthrylacetamide) deriva-tive **2** was prepared with the goal of enhancing binding strength and possibly improving selectivity through the introduction of strongly ligating carboxamide functions in the side arm as additional binding sites.

First, the chemosensing properties of **1** were studied by measuring fluorescence responses toward various metal ions. Despite attempts to optimize conditions for the selective signaling of the metal ions through a systematic survey, utilizing a series of solvents, buffers, and water compositions, compound **1** exhibited relatively poor sig-naling behavior toward a specific targeted metal ion. Compound **1** showed some fluorescence quenching behavior in response to Cu^{2+} ions, but other metal ions also responded in varying degrees (Fig. S1, Supplemen-tary data). We next surveyed fluoroionophoric behav-iors of compound **2** in common organic solvents as well as their aqueous solutions. Preliminary fluorescence measurements showed that the acetate buffered (pH 4.8) aqueous acetonitrile solution revealed a relatively opti-mized selectivity. For example, in aqueous 90% acetoni-trile solution, compound **2** revealed a strong emission of

the anthryl moiety around 428 nm, which was effectively quenched in the presence of 100 equiv of Hg^{2+} or Cu^{2+} ions (Fig. 1). On the other hand, compound **2** exhibited less pronounced chemosensing behavior in aqueous dioxane and methanol solution (Fig. S2, Supplementary data), or some precipitates formed in Hepes (pH 7.0, Pb^{2+}, Hg^{2+}, and Zn^{2+}) or Tris (pH 8.1, Pb^{2+} and Cu^{2+}) buffered aqueous 95% acetonitrile solution. These observations imply that the selective signaling of Hg^{2+} or Cu^{2+} ions could be realized in an aqueous acetonitrile solution with water content less than 10%.

Based on this observation, we focused on the fluores-cence behaviors of **2** in response to the various metal ions in aqueous 95% acetonitrile solution at pH 4.8 (acetate buffer). In this solvent system, compound **2**
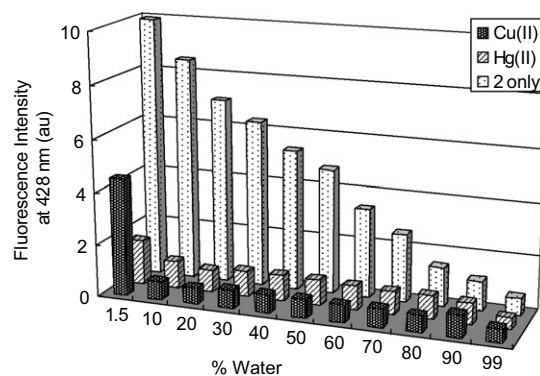

Figure 1. Changes in fluorescence intensity at 428 nm of **2** in the absence and the presence of Hg^{2+} or Cu^{2+} ions as a function of water content. [**2**] = 5.0×10^{-6} M, [M^{2+}] = 5.0×10^{-4} M in acetate-buffered aqueous acetonitrile (10 mM, at pH 4.8). λ_{ex} = 340 nm. The pH value refers to the water portion before mixing with acetonitrile.
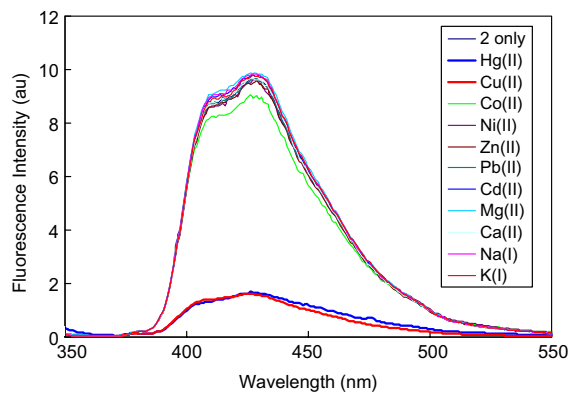
Figure 2. Fluorescence spectra of **2** in the presence of various metal ions. $[2] = 5.0 \times 10^{-6}$ M and $[M^{n+}] = 5.0 \times 10^{-4}$ M in acetate buffered (10 mM, at pH 4.8) acetonitrile–water solution (95:5, v/v). $\lambda_{ex} = 340$ nm.

Figure 3. Fluorescence titration of **2** with Hg^{2+} ions. $[2] = 5.0 \times 10^{-6}$ M in acetate buffered (10 mM, at pH 4.8) acetonitrile–water solution (95:5, v/v). $\lambda_{ex} = 340$ nm.

exhibited a strong emission band of the anthracene moiety, centered at 410 and 428 nm. Upon interaction with representative alkali, alkaline earth, and transition metal ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Cd^{2+}, and Hg^{2+} in perchlorate), the emission spectra of **2** were considerably affected only by Hg^{2+} and Cu^{2+} ions (Fig. 2). In the presence of Hg^{2+} and Cu^{2+} ions, the fluorescence at 428 nm was effectively quenched and the intensity was reduced to 17% and 16%, respectively, of the fluorescence intensity for free **2**. Other metal ions had relatively insignificant effects on the fluorescence profile; their I/I_o values, which are the ratios of the fluorescence intensities in the presence and the absence of each metal ion, varied from 0.93 for Co^{2+} to 1.02 for Mg^{2+}, demonstrating the selective chemosensing behaviors of **2** for Hg^{2+} and Cu^{2+} ions. The efficient quenching of the fluorescence emission is due to the intrinsic quenching nature of the complexed Hg^{2+} and Cu^{2+} ions.[16] In this case, upon complexation, the fluorescence quenching effects of Hg^{2+} and Cu^{2+} ions overpowered the possible enhancing effect of the metal ions induced by the suppression of the PET process between nitrogen atoms and the anthracene fluorophore.[17] The complex formation with Cu^{2+} and Hg^{2+} ions was also confirmed by the measurements of FAB-MS spectra: upon treatment with 5 equiv of metal ions in *m*-NBA matrix, relevant peaks were observed at $m/z = 756.20$ for $[M+H+Cu]^+$ and 893.19 for $[M-H+Hg]^+$, respectively.

In the relatively optimized solvent of aqueous 95% acetonitrile, the association constants (K_{assoc}) were determined by metal-ion-dependent fluorescence changes using a nonlinear curve-fitting procedure.[18] The formation of 1:1 complexes (**2**-Hg^{2+} and **2**-Cu^{2+}) was independently confirmed by Job's plot, which shows maximums at mole fraction of around 0.5 (Fig. S3, Supplementary data).[19] From the fluorescence titration results, the K_{assoc} values were estimated to be 4.8×10^4 M^{-1} and 8.2×10^4 M^{-1} for **2**-Hg^{2+} (Fig. 3) and **2**-Cu^{2+} (Fig. S4, Supplementary data), respectively. Although the measurement conditions are not identical, these association constants are significantly smaller compared
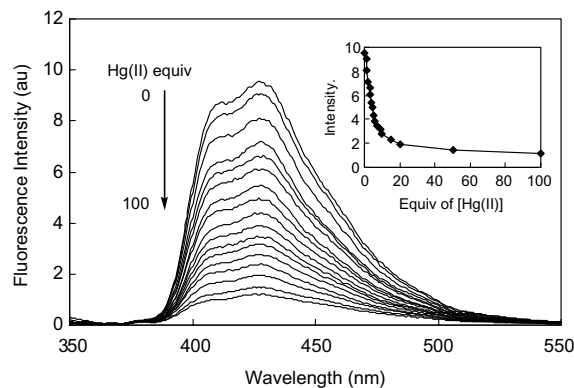
with the analogous cyclam derivative, which was similarly functionalized with two additional acetamide groups.[20] The chemosensing behavior of dioxocyclam binding unit consisting of two amide and two amine groups seems to be somewhat inferior to that of the cyclam molecular framework having four amine ligating groups. However, the attractive structural characteristics of dioxocyclam, such as the possibility of pH switching of the complexation process and controlling of the redox properties, could be utilized in the design of other advanced functional chemosensor systems.[1b,21] By using the above mentioned fluorescence titration results, the detection limits[22] of **2** for the analysis of Hg^{2+} and Cu^{2+} ions were determined as 7.8×10^{-6} and 1.5×10^{-6} M, respectively.

Finally, the possible applicability of chemosensor **2** for the analysis of Hg^{2+} or Cu^{2+} ions in practical samples was tested. For example, the competitive signaling behaviors of **2** with 10 equiv of Cu^{2+} ions were investigated in the presence of representative transition metal ions and environmentally important metal ions (100 equiv of Na^+, K^+, Mg^{2+}, Ca^{2+}, and 10 equiv of Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Pb^{2+}, and Hg^{2+}) as background. The degree of changes in fluorescence intensity of **2** upon interaction with Cu^{2+} ions in the presence of various metal ions ($I - I_o$) was assessed in reference to the changes induced by the Cu^{2+} ions alone ($I_{Cu(II)} - I_o$) (Fig. 4). Although some variation in the fluorescence of **2** due to the presence of the background competing metal ions was observed, the $(I - I_o)/(I_{Cu(II)} - I_o)$ values were found to remain between 0.95 and 1.08; that is, a relatively consistent Cu^{2+}-induced fluorescence quenching was observed. The **2**-Hg^{2+} system also displayed similar behavior in the presence of the aforementioned background metal ions (Fig. S5, Supplementary data). However, precise discrimination of the individual Hg^{2+} and Cu^{2+} ions using compound **2** is not possible, because **2** exhibited similar responses toward these two metal ions both in binding strength as well as spectroscopic changes. The compound used for the sensing of Hg^{2+} or Cu^{2+} ions was found to be easily regenerated by treatment with EDTA solution. For example, quenched fluorescence of **2** in the presence of 10 equiv
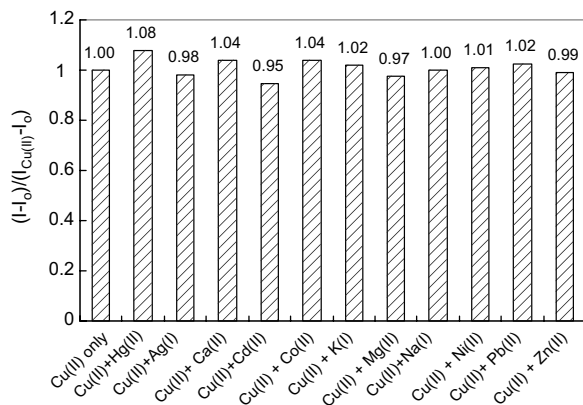
Figure 4. Competitive experiments in the **2**-Cu^{2+} system with common interfering metal ions. [**2**] $= 5.0 \times 10^{-6}$ M, [Cu^{2+}] $= 5.0 \times 10^{-5}$ M, and [M^{n+}] $= 5.0 \times 10^{-5}$ M or 5.0×10^{-4} M in acetate buffered (10 mM, at pH 4.8) acetonitrile–water solution (95:5, v/v). $\lambda_{ex} = 340$ nm.

of Cu^{2+} ions was almost completely revived upon treatment with 50 equiv of EDTA. Regenerated compound **2** responded to the subsequent addition of Cu^{2+} ions (100 equiv) and the resulting fluorescence spectrum was almost equal to that of the **2**-Cu^{2+} system obtained with **2** in the presence of 100 equiv of Cu^{2+} ions.

In summary, we have developed a new Hg^{2+}- and Cu^{2+}-selective fluoroionophore by combining the simple molecular motifs of dioxocyclam and anthracene. A large Hg^{2+}- and Cu^{2+}-selective fluorescence quenching was observed in the presence of other environmentally abundant metal ions in an aqueous acetonitrile solution. The compound could be easily regenerated by treatment with EDTA, and the selective signaling in semi-aqueous media has potential application in the analysis of Hg^{2+} or Cu^{2+} ions in environmental and biological samples.

Acknowledgment

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.08.070.

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13. *Selected data for* **1**: Yield: 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (s, 2H), 8.38 (d, *J* = 8.7 Hz, 4H), 8.02 (d, *J* = 8.4 Hz, 4H), 7.61 (m, 4H), 7.48 (m, 4H), 6.33 (br m, 2H), 4.43 (s, 4H), 3.10 (m, 4H), 2.98 (s, 2H), 2.49 (br t, 4H), 2.16 (br t, 4H), 1.80 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3, 134.3, 131.6, 131.4, 129.5, 128.0, 126.6, 125.2, 124.5, 52.1, 51.5, 50.7, 46.0, 37.3, 27.3. HRMS (FAB, *m*-NBA) calcd for [M+H]$^+$ $C_{40}H_{41}N_4O_2$ 609.3230, found 609.3212. For **2**: Yield: 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.62 (s, 2H), 8.62 (s, 2H), 8.36 (s, 2H), 8.24 (s, 2H), 7.93 (m, 4H), 7.85 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J* = 9.3 Hz, 2H), 7.43 (m, 4H), 6.77 (m, 2H), 3.45 (m, 4H), 3.39 (s, 2H), 3.27 (s, 4H), 2.56 (m, 8H), 1.50 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 167.2, 135.7, 131.64,

131.60, 130.4, 128.7, 128.5, 128.0, 127.7, 125.7, 125.5, 125.0, 124.9, 121.4, 113.9, 60.2, 55.0, 54.0, 45.5, 38.6, 26.5. HRMS (FAB, *m*-NBA) calcd for $[M+H]^+$ $C_{42}H_{43}N_6O_4$ 695.3346, found 695.3016.

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