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A sugar-aza-crown ether-based fluorescent sensor for Cu²⁺ and Hg²⁺ ions

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ABSTRACT

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Keywords: Fluorescent sensor Chemosensor Pyrene A sugar-aza-crown ether (SAC)-based fluorescent sensor **4** was prepared. It contains a pyrene as the fluorophore and its fluoroionophoric properties toward transition metal ions were investigated. Chemosensor **4** exhibits highly selective recognition toward Cu^{2+} and Hg^{2+} ions among a series of tested metal ions in methanol solution. The association constants for **4**×Cu²⁺ and **4**×Hg²⁺ in methanol solution were calculated to be 7.4 × 10¹ M⁻¹ and 4.4 × 10³ M⁻¹, respectively. Chemosensor **4** formed complexes with the Cu²⁺ or Hg²⁺ ion at a 1:1 ligand-to-metal ratio with a detection limit of 1.3×10^{-4} M Cu²⁺ and 1.26×10^{-5} M Hg²⁺, respectively.

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The design and synthesis of new chemosensors for the efficient detection of trace metal ions is one of the most important research topics in environmental chemistry and biology.¹ Among these metal ions, the copper ion is a significant environmental pollutant and also plays a critical role in various biological processes.^{2–4} The mercury ion is also a significant environmental pollutant which is accumulated in plants, soil, and water. Some prokaryotes convert this inorganic mercury to methyl mercury, which is a lipophilic compound that is readily absorbed and poorly excreted. When it enters the food chain it biomagnifies in higher organisms, especially in the muscles of large predatory fish, including tuna, sword-fish, and whales, and when it is subsequently ingested by humans leads to DNA and nervous system damage.^{5–9}

Fluorescence quenching by heavy metal ions is a well-known phenomenon and occurs by a number of pathways, which include spin-orbit coupling, energy transfer, and electron transfer.¹⁰⁻¹⁴ Because Cu^{2+} and Hg^{2+} ions have the propensity to quench fluorophore emission, turn-off detection is a facile approach for monitoring these metal ions. For fluorescence detection of Cu^{2+} and Hg^{2+} ions, enhancement ('turn-on') is preferable to fluorescence quenching ('turn-off') because it lessens the chance of false positives and is more amenable to multiplexing, the simultaneous use of several detectors that uniquely respond to different analytes. However, to date, few turn-on type Cu^{2+} and Hg^{2+} fluorescent sensors have been found in the literature.^{10,11} Therefore, designing fluorescent sensors that provide a turn-on response following Cu^{2+} or Hg^{2+} recognition is a considerable challenge. Recently, modified pyranoid-based SAC (sugar-aza-crown ether) with bispyrenyl moieties was synthesized to form a new fluorescent molecular sensor

for $Cu^{2+,15}$ In addition, we have reported that a SAC-based cavitand as a turn-on type sensor for Cu^{2+} ion in methanol solution¹⁶ and SAC-based sensor with two anthracenetriazolymethyl groups exhibit turn-off type fluorescence responses¹⁷ upon interaction with Cu^{2+} and Hg^{2+} ions in water solution. Herein, we report a SACbased structure with two pyrenylmethyl groups as a fluorescent sensor for metal ions. The designed chemosensor **4** exhibits a turn-on type response and highly selective fluorescence behavior for Cu^{2+} and Hg^{2+} ions in methanol solution.

Fluorescent sensor **4** can be easily prepared from 1-pyrenemethanol **1** in three steps as shown in Scheme 1. 1-Pyrenemethanol **1** was treated with thionyl chloride to obtain 1-chloropyrenemethane **2** (98%). Compound **2** was further coupled with SAC **3** using K₂CO₃ as a base in CH₃CN–KI–*n*-Bu₄NI solvent under reflux conditions¹⁸ afforded sensor **4** (48%). The structures of **4** were confirmed by NMR spectroscopy and mass spectra (see Supplementary data, Figs. S1–S4).

We examined the effect of different solvents (CH₂Cl₂, methanol, THF, CH₃CN, and DMSO) on the fluorescence spectrum of **4** (144 μ M). As shown in Figure 1, the monomer–excimer ratio of **4** is not affected by the polarity of the solvents, only the monomer is dominant over the excimer, in which the excimer emission is quenched completely owing to strong solvation. This indicates that the two pyrenes of **4** are not present in the parallel conformation. The fluorescence of **4** (11 μ M) revealed three strong emission bands of the pyrene moiety at 377, 396, and 418 nm in methanol. We examined the chemosensing behavior of the fluorescent sensor **4** by fluorescence measurements in the presence of various metal ions in methanol by comparing the fluorescence intensities of the solutions before and after addition of 100 equiv of the following 12 metal ions as their perchlorate salts: Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, Hg²⁺, Co²⁺, Ni²⁺, Pb²⁺, Cu²⁺, Cd²⁺, and Zn²⁺. In the presence of Cu²⁺ and Hg²⁺ ions, sensor **4** showed modest and large fluorescence



Note

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Scheme 1. Synthesis of fluorescent sensor 4.



Figure 1. Fluorescence spectra of $4 (144 \,\mu\text{M})$ in different solvents.

enhancement, respectively, while the other metal ions had only small effects (Fig. 2). The enhancement efficiency $(I - I_0/I_0 \times 100\%)$ observed at 377 nm was ~128% and 51% greater than the control in the absence of Hg²⁺ and Cu²⁺ ions, respectively



Figure 2. Fluorescent spectrum of **4** (11 μ M) upon the addition of Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, Hg²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, and Pb²⁺ (100 equiv) in MeOH (excitation at 343 nm).

(Fig. 3). The fluorescence enhancement can be attributed to the photoinduced electron transfer (PET) that occurs upon complexation of the nitrogen atoms by metal ion. These results suggest that Cu^{2+} or Hg^{2+} ion can be recognized by the two nitrogen atoms of the linker of **4**.

To further investigate the interaction between sensor 4 and metal ions, we performed fluorescence titrations of 4 with Cu²⁺ and Hg²⁺ ions in methanol. As the concentration for Cu²⁺ and Hg²⁺ ions was gradually increased, the fluorescence intensity of 4 is also increased (Fig. 4). From the fluorescence titration profiles, the association constants for **4***Cu²⁺ and **4***Hg²⁺ in methanol were calculated as 7.4×10^1 and 4.4×10^3 M⁻¹, respectively, by a Stern–Volmer plot (Figs. S5 and S6). By the Job plot (Fig. S7), a maximum fluorescence change was observed when the molar fraction of the sensor [**4**] versus [Cu²⁺]+[**4**] and [**4**] versus [Hg²⁺]+[**4**] was 0.5, indicating that a 1:1 adduct was formed between **4** and Cu^{2+} or Hg^{2+} ion. By using the above-mentioned fluorescence titration results, the detection limits of $\mathbf{4}$ for the analysis of Cu^{2+} and Hg^{2+} ions were determined as 1.3×10^{-4} and 1.26×10^{-5} M, respectively. We also tried to obtain evidence for the proposed Cu²⁺ and Hg²⁺ ions induced conformational changes by NMR spectroscopy but failed due to the extensive broadening of the resonances under the relevant experimental conditions. ESI-MS was conducted using a 1 mM solution of **4** in methanol with an excess of $Cu(ClO_4)_2$ to give the result of m/z = 927.7 (**4**+Cu²⁺+2H₂O+1H⁺, Fig. S8), which gave fur-



Figure 3. Fluorescence intensity changes ($(I - I_0)/I_0 \times 100\%$) of 4 (11 µM) with various metal ions (11 mM) in MeOH at 377 nm (excitation at 343 nm).



Figure 4. (a) Fluorescence spectra of **4** (13 μ M) in MeOH upon addition of increasing concentrations of Hg(ClO₄)₂; λ_{ex} = 343 nm. (b) Fluorescence spectra of **4** (13 μ M) in MeOH upon addition of increasing concentrations of Cu(ClO₄)₂; λ_{ex} = 343 nm.

ther evidence of the formation of a 1:1 complex between ${\bf 4}$ and $Cu^{2\ast}$ ion.

The selectivity toward Hg^{2+} or Cu^{2+} ions was further ascertained by the competitive experiment employed with addition of the same amount (100 equiv) of competing metal ions to the $4*Cu^{2+}$ or $4*Hg^{2+}$ mixtures. The fluorescence quenching efficiency of Cu^{2+} and Hg^{2+} ions was not affected in the presence of surveyed other interfering metal ions as background (Fig. S9), which means that the ionophore can signal the presence of Cu^{2+} and Hg^{2+} ions in common analytes having various metal ions as background.

To further investigate the coordinating mode of the $4-Hg^{2+}$ complex, computations for $4-Hg^{2+}$ were conducted at the B3LYP/ LanL2DZ theory level with the GAUSSIAN 03 package. On the basis of these calculations of $4-Hg^{2+}$ complex, we found three stationary points that could be verified as genuine minima via vibrational frequency analyses. The optimized structure for the most stable isomer of $4-Hg^{2+}$ is illustrated in Figure 5. It is clear that the Hg^{2+} ion occupies the coordination center of 4, surrounded by two nitrogen atoms of the linker and two oxygen atoms of the ribosyl unit. The average bond length of Hg–N and Hg–O was estimated as 2.52 Å and 2.53 Å, respectively.

In conclusion, we synthesized a SAC-based fluorescent sensor **4**, which shows recognition toward Cu^{2+} and Hg^{2+} ions in methanol among 12 metal ions tested. Upon addition of Cu^{2+} and Hg^{2+} ions into a methanol solution of **4**, the fluorescence intensity was enhanced because of the PET process operating between the amino group of the SAC ether and the pyrene fluorophore.



Figure 5. Conformation of 4/Hg²⁺ optimized by density functional theory.

1. Experimental

1.1. General methods

All reagents were obtained from commercial suppliers and were used without further purification. CH₃CN was distilled over CaH₂. Analytical thin-layer chromatography was performed using Silica Gel 60 F254 plates (Merck). The ¹H and ¹³C NMR spectra were recorded with Bruker AM 300 (300 MHz) spectrometers. Chemical shifts are expressed in ppm with residual CHCl₃ as reference. Low- and high-resolution mass spectra were recorded under fast atom bombardment (FAB) conditions. UV–vis spectra were recorded by using an HP-8453 spectrophotometer with a diode array detector, and the resolution was set at 1 nm. Fluorescence spectra were recorded on a Cary Eclipse Fluorescence spectrophotometer. Microwave reactions were carried out in a Milestone Start S with a maximum power of 300 W.

1.2. 2-(Chloromethyl)-3,6-dihydropyrene (2)

To a solution of 1-pyrenemethanol **1** (1.52 g, 6.55 mmol) in dry CH₂Cl₂ (40 mL) was added SOCl₂ (1.18 mL, 16.32 mmol) at 0 °C. The mixture was stirred for 1 h, then the mixture was concentrated to give **2** (1.60 g, 98%) as a green solid; mp 149.9 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.34 (d, *J* = 9.3 Hz, 1H), 8.21–8.15 (m, 3H), 8.10 (d, *J* = 7.8 Hz, 1H), 8.07–7.964 (m, 4H), 5.29 (s, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 131.9, 131.1, 130.6, 130.2, 129.1, 128.3, 128.0, 127.6, 127.2, 126.1, 125.6, 125.0, 124.7, 124.6, 122.7, 44.8; HRMS (FAB): calcd for C₁₇H₁₁Cl (M⁺), *m/z* 250.0549, found *m/z* 250.0551.

1.3. Chemosensors 4

To a solution of SAC **3** (0.26 g, 0.65 mmol) in acetonitrile were added compound **2** (2.5 equiv, 0.41 g), *t*-BuNI (1 equiv, 0.24 g), KI (5 equiv, 0.54 g), and K₂CO₃ (4.0 equiv, 0.36 g). After the solution was stirred overnight at reflux, the mixture was concentrated, and then dissolved in CH₂Cl₂–H₂O containing a drop of NaOH (1 M). The solution was extracted by CH₂Cl₂, filtered, concentrated, and purified by chromatography (EtOAc–MeOH 1:1 to EtOAc–MeOH 20:1) to give **4** (0.26 g, 48%) as a yellow solid; mp 136.7 °C; R_f = 0.22 (EtOAc–MeOH 8:1); ¹H NMR (300 MHz, CDCl₃)

δ: 8.51 (d, *J* = 9.3 Hz, 2H), 8.18–7.96 (m,16H), 4.46 (d, *J* = 13.2 Hz, 2H), 4.39–4.37 (m, 4H), 4.12–4.10 (m, 2H), 4.06–4.02 (m, 2H), 3.86–3.85 (m, 2H), 3.12–3.08 (m, 2H), 2.87–2.83 (m, 2H), 2.70–2.67 (m, 4H), 1.92 (br, 4H), 1.33 (s, 6H), 0.88 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ: 133.0, 131.3, 130.8, 130.7, 129.7, 128.2, 127.4, 127.1, 125.9, 125.0, 124.8, 124.6, 123.9, 112.7, 83.8, 82.6, 81.9, 77.8, 58.0, 56.2, 52.7, 27.5, 26.2, 24.3. HRMS (FAB): calcd for C₅₄H₅₄N₂O₆ [M+H]⁺, *m*/*z* 826.3982, found *m*/*z* 827.4058.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.03.010.

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