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Turn-On Fluorescent Sensor for Selective Detection of Zn^{2+}, Cd^{2+}, and Hg^{2+} in Water

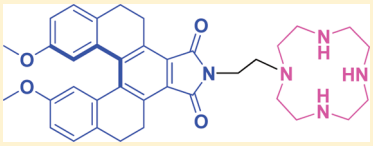
Meng Li,[†,‡] Hai-Yan Lu,*[,‡] Rui-Li Liu,[‡] Jun-Dao Chen,[†] and Chuan-Feng Chen*[,†]

[†]Beijing National Laboratory for Molecular Sciences, CAS Key Laboratory of Molecular Recognition and Function, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

[‡]Graduate University of Chinese Academy of Sciences, Beijing 100049, China

Supporting Information

ABSTRACT: A new fluorescent chemosensor based on a helical imide as fluorophore and a cyclen moiety as ionophore was synthesized, which not only showed enhanced fluorescent responses in the presence of Zn^{2+}, Cd^{2+}, and Hg^{2+} but also could simultaneously and selectively distinguish the three cations in a simulated physiological condition with the help of cysteine as an auxiliary reagent.



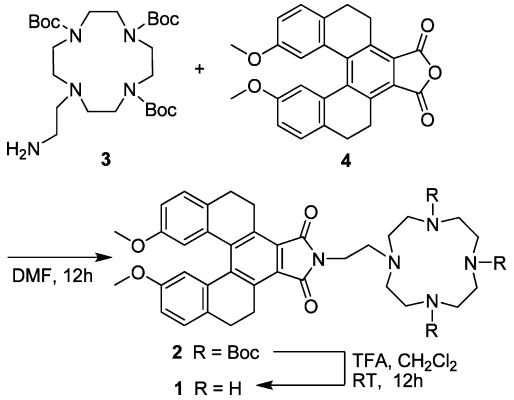
Zinc, cadmium, and mercury, owing to their biological importance or environmental harm, have attracted more and more attention.[1] However, the selective detection of zinc, cadmium, and mercury ions has always been problematic mainly due to their closed-shell d^{10} electronic configurations which cause them to be spectroscopically silent.[2] Fluorescent sensors have thus become the most effective means for detecting the metal ions[3] because of its high sensitivity and simplicity as well as ease of signal transduction.

Although there are various fluorescent sensors designed for zinc(II),[4] cadmium(II),[5] or mercury(II),[6] few sensors that could detect the three cations simultaneously have been reported.[7] On the other hand, there still exist some deficiencies among the known chemosensors: the types of fluorophores used for detecting the cations are limited;[8] most of the sensors show fluorescence quenching; and "turn-on" responses are relatively scarce,[9] and the detections are usually carried out in organic solvents which limited many sensors' practical applications.[10] So, developing new turn-on fluorescent chemosensors for the selective detection of Zn^{2+}, Cd^{2+}, and Hg^{2+} under aqueous conditions is still practical and challenging.

Herein, we report a new receptor **1** based on a helical imide connecting with a cyclen moiety. It is different from many known fluorophores in that the fluorophore in **1** combined the intramolecular push−pull electronic effect, the conjugative effect, and a twist helical structure. The first two effects cause the fluorophore to have long excitation wavelength and long emission wavelength. The twist structure effectively avoids the intermolecular $\pi-\pi$ stacking interaction which can usually cause the fluorescence quenching. Moreover, the cyclen as ionophore can also improve the receptor's water solubility.[11] Consequently, it was found that receptor **1** not only is an enhanced fluorescent probe but also can effectively distinguish Zn^{2+}, Cd^{2+}, and Hg^{2+} in an aqueous condition under an auxiliary reagent of cysteine.

The target compound **1** was conveniently synthesized in 73.5% overall yield by a two-step reaction with **3**[12a] and **4**[12b] as the starting materials (Scheme 1). The target product has good solubility in many organic solvents including CH_3OH, CH_3CN, DMSO, CH_2Cl_2, and $CHCl_3$. Moreover, compound **1** also exhibits very good solubility in water, and this property may make it feasible for practical applications.

Scheme 1. Synthesis of 1



It was found that free **1** exhibited very weak fluorescence due to an intramolecular PET effect between the fluorophore and the cyclen unit,[13] which provided an ideal platform for the excellent signaling output. Considering the Raman peak of water and the weak fluorescence of receptor **1**, the excitation wavelength at 330 nm was chosen. To investigate the impact of pH on the fluorescence intensity of **1**, a wide pH range was also tested. The results showed that the fluorescence intensity of **1** is stable at around pH = 7.2.[14] Thus, we first investigated the fluorescence responses of **1** to various cations in simulated physiological conditions (10 mM HEPES buffer, pH = 7.2). As shown in Figure 1, upon the addition of 3.0 equiv of various cations, including Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ag^+, Co^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}, to **1** (1.0×10^{-5} M) in HEPES, it was found that Zn^{2+} and Cd^{2+} caused the

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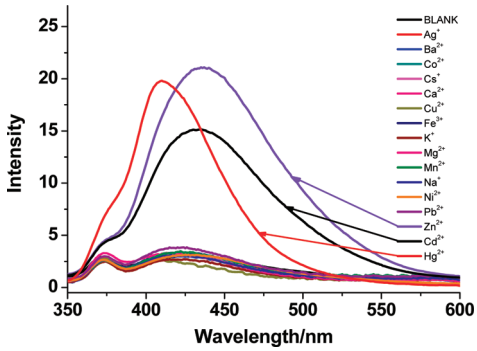

Figure 1. Fluorescence responses of **1** (1.0×10^{-5} M) in HEPES (10 mM, pH = 7.2) to 3 equiv of various metal ions, λ_{ex} = 330 nm.

significant emission enhancement of **1**, while Hg^{2+} caused not only emission enhancement of **1** but also a 25 nm blue shift. For other tested cations, no obvious changes of receptor **1** were found.

To further distinguish Zn^{2+}, Cd^{2+}, and Hg^{2+}, we have chosen a commercial agent cysteine as the masking agent.[15] As shown in Figure 2, it was found that, upon the addition of 3.0 equiv of

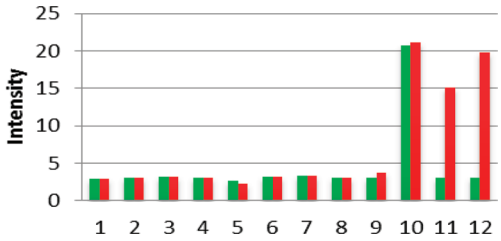

Figure 2. Fluorescence intensity of **1** (1.0×10^{-5} M) and 3.0 equiv of metal ions in HEPES (10 mM, pH = 7.2) (red bars), and the fluorescence responses followed by adding 3.0 equiv of cysteine (green bars): 1 blank; 2 G^{n+} = Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}; 3 Ag^+; 4 Co^{2+}; 5 Cu^{2+}; 6 Fe^{3+}; 7 Mn^{2+}; 8 Ni^{2+}; 9 Pb^{2+}; 10 Zn^{2+}; 11 Cd^{2+}; 12 Hg^{2+} (λ_{ex} = 330 nm).

cysteine to the mixture of receptor **1** and various cations in HEPES, only the solution of **1** and Zn^{2+} maintained the enhanced emission, while the systems containing other tested cations including Cd^{2+} and Hg^{2+} ion showed almost the same fluorescence intensity of free receptor **1**, probably due to the combination of heavy metal ions with mercaptotropone in the amino acid.[16] Considering that Zn^{2+}, Cd^{2+}, and Hg^{2+} caused the different fluorescence changes of **1** (Figure 1), we can draw a conclusion that, with the help of cysteine, Zn^{2+}, Cd^{2+}, and Hg^{2+} could be simultaneously and selectively detected by receptor **1**.

The stoichiometries of the complexes **1**−Zn^{2+}, **1**−Cd^{2+}, and **1**−Hg^{2+} were determined by the Job's plot. The results showed that 1:1 complexes between receptor **1** and Zn^{2+}, Cd^{2+}, and Hg^{2+} were all formed.[14] According to the fluorescence titration experiments (Figure 3), the association constant of the 1:1 complex between receptor **1** and Cd^{2+} was calculated to be $1.32(\pm 0.04) \times 10^4$ M^{-1}.[17] Similarly, for the 1:1 complexes between **1** and Zn^{2+} and Hg^{2+}, the association constants were determined to be 1.14×10^4 and 1.55×10^4 M^{-1}, respectively (errors were less than 5%).[14]

Absorption spectral experiments of **1** in the presence of various cations were also carried out to get insight into the complexation between **1** and the cations in aqueous solution. As shown in Figure 4, Zn^{2+}, Cd^{2+}, and Hg^{2+} caused dramatic

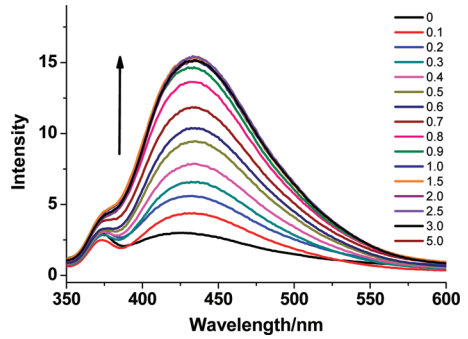

Figure 3. Fluorescence responses of **1** (1.0×10^{-5} M) in HEPES (10 mM, pH = 7.2) to various concentrations of Cd^{2+} (λ_{ex} = 330 nm).

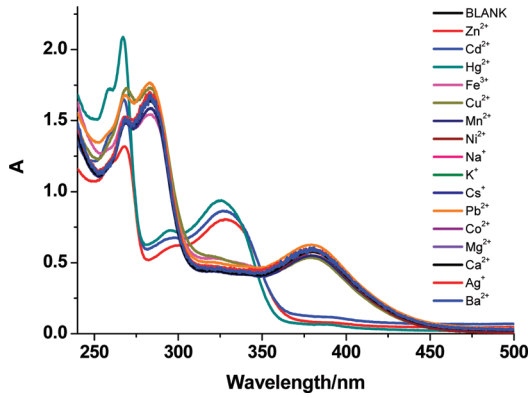

Figure 4. UV−vis spectra of **1** (5×10^{-5} M) in HEPES (10 mM, pH = 7.2) upon the addition of 3.0 equiv of various metal ions.

absorption changes of **1**, while no significant changes were observed in the presence of other tested cations, which are consistent with those results of fluorescence. To detect the binding process of **1** toward Zn^{2+}, Cd^{2+}, and Hg^{2+} in aqueous solution, absorption titrations were further carried out.[14] The results showed that, with increasing amounts of the cations, absorption bands of **1** at 380 and 285 nm gradually decreased, while two new absorption bands at 330 and 268 nm appeared, and three isosbestic points at 271, 299, and 350 nm for Zn^{2+}, 270, 300, and 348 nm for Cd^{2+}, and 270, 298, and 347 nm for Hg^{2+} were observed, indicating the 1:1 equilibrium process of the complexation between **1** and the cations.

Furthermore, ^{1}H NMR titration experiments were also carried out.[14] Consequently, upon the addition of Zn^{2+} to the solution of receptor **1** in D_2O, it was found that the protons of **1** all gradually shifted downfield. When 1 equiv of Zn^{2+} was added, the chemical shifts of **1** achieved the maximum. More Zn^{2+} could not cause the chemical shifts of **1** to be changed obviously, which suggested the formation of the 1:1 complex between **1** and Zn^{2+}. Under the same conditions, formation of the 1:1 complex between **1** and Cd^{2+} was also evidenced by ^{1}H NMR titration experiments.[14]

In conclusion, we have presented a turn-on fluorescent sensor based on a new fluorophore and a cyclen moiety as the ionophore, which showed the effective and selective detection of Zn^{2+}, Cd^{2+}, and Hg^{2+} in a simulated physiological condition with the help of cysteine as an auxiliary reagent. Formation of the 1:1 complexes between **1** and the cations was also evidenced by the absorption and ^{1}H NMR titration experiments. Further studies will focus on developing new fluorescent

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sensors for cations based on the helical imide as fluorophore, which are now in progress.

EXPERIMENTAL SECTION

Synthesis of 2. A mixture of **3** (114 mg, 0.22 mmol) and **4** (82.4 mg, 0.20 mmol) in DMF (15 mL) under Ar was stirred for 12 h at 110 °C. After cooling to room temperature, the reaction mixture was poured into water (30 mL) and followed by addition of CH_2Cl_2 (50 mL). After stirring for 10 min, the organic phase was separated and washed with saturated brine (3 × 25 mL). The CH_2Cl_2 extract was dried over Mg_2SO_4 and then concentrated under reduced pressure to give a yellow residue, which was purified by column chromatography over silica gel eluting with EtOAc/PE (1:3, v/v) to give the desired product as a yellow solid (136 mg, 75% yield): mp 123−126 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 7.20 (d, J = 8.0 Hz, 2H), 6.74 (d, J = 7.4 Hz, 2H), 6.58 (s, 2H), 3.87 (br s, 2H), 3.62 (br s, 2H), 3.32 (br s, 18H), 2.73 (br s, 10H), 2.26−2.24 (m, 2H), 1.35 (br s, 27H); ^{13}C NMR (75 MHz, CD_3CN) δ 168.1, 157.0, 155.3, 154.9, 138.2, 138.0, 133.5, 131.4, 128.3, 125.8, 115.2, 114.3, 78.7, 78.4, 59.8, 54.3, 53.6, 47.3, 47.0, 27.6, 27.4, 26.8, 23.9; HRMS (ESI) m/z calcd for $C_{51}H_{67}N_5O_{10}$ $[M + H]^+$ 910.4961, found 910.4968.

Synthesis of 1. Compound **2** (91 mg, 0.1 mmol) was dissolved in CH_2Cl_2/TFA (20 mL, 1:1, v/v) and stirred for 12 h at room temperature. The CH_2Cl_2 and excess trifluoroacetic acid were removed under reduced pressure to give a yellow solid, which was dissolved in water (20 mL) and then extracted with CH_2Cl_2 (3 × 10 mL). After phase separation, the solvent was removed in vacuo to give the product (59.7 mg, 98% yield) as a yellow solid without further purification: mp 135−138 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.20 (d, J = 8.4 Hz, 2H), 6.75 (dd, J = 8.4, 2.5 Hz, 2H), 6.41 (s, 2H), 3.61 (br s, 4H), 3.31 (s, 6H), 3.07 (br s, 8H), 2.91−2.66 (m, 12H), 2.54−2.43 (m, 2H), 2.17−2.05 (m, 2H); ^{13}C NMR (75 MHz, CD_3CN) δ 169.0, 157.0, 138.4, 138.4, 133.7, 131.6, 128.3, 125.7, 115.2, 114.6, 54.4, 51.9, 48.1, 44.0, 41.7, 41.3, 34.5, 26.7, 24.0; HRMS (ESI) m/z calcd for $C_{36}H_{43}N_5O_4$ $[M + H]^+$ 610.3388, found 610.3391. Anal. Calcd for $C_{36}H_{43}N_5O_4 \cdot 1.5H_2O$: C, 67.90; H, 7.28; N, 11.00. Found: C, 67.69; H, 7.55; N, 10.82.

ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra for new compounds; Job's plots of **1** and metal ions; ^{1}H NMR titrations, fluorescence titrations, and UV−vis titrations of **1** with metal ions. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: cchen@iccas.ac.cn, haiyanlu@gucas.ac.cn.

Notes

The authors declare no competing financial interest.

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