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A "Turn-on" Fluorescent Hg^{2+} Chemosensor Based on Ferrier Carbocyclization

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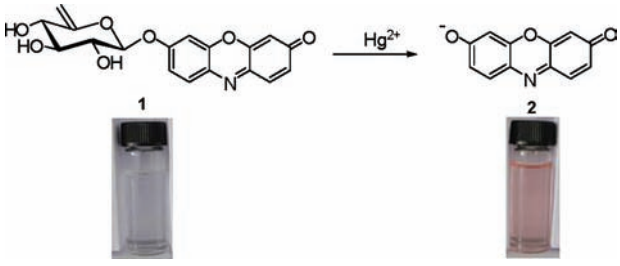
ABSTRACT



A "turn-on" fluorescent chemosensor with excellent selectivity and satisfactory sensitivity on Hg^{2+} detection in 100% water media has been established employing a carbohydrate based Ferrier carbocyclization reaction. The probe has also presented satisfactory results for the imaging of Hg^{2+} ions in cells and organisms.

Mercury is one of the most hazardous species in nature, which may cause prenatal brain damage, cognitive and motion disorders, vision and hearing loss, and even death.[1] Development of a facile and selective method for Hg^{2+} detection has long been demanded in the area of food safety and environmental protection. A number of fluorescent chemosensors for the selective detection of Hg^{2+} ions have been exploited.[2] Most early sensors showed a fluorescence quenching (turn-off) response due to the spin−orbit coupling effect of the Hg^{2+} ion,[3] while examples of "turn-on" Hg^{2+} sensors have also been reported in recent years.[4] Coordination of Hg^{2+} to S-atom-based receptors[5] and utilizing diverse mercury-mediated reactions such as mercury-deselenation reactions,[6] mercury-catalyzed hydrolysis,[7] and the mercuration reaction[8] are major ideas in the development of Hg^{2+} fluorescent probes so far. However, only a few successful examples of fluorescent probes for detecting Hg^{2+} ions in aqueous solutions or pure water have been established,[2n,9] thus hampering

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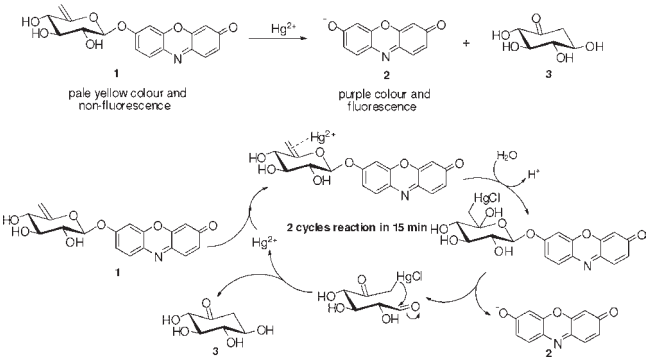
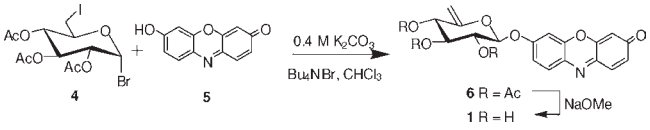
analytical application in real water samples. Herein, we designed a sugar functionalized fluorescent probe **1** (Scheme 1). Our strategy for the fluorescent detection of mercury ion relies on the highly selective mercury-mediated Ferrier carbocyclization, resulting in the formation of a fluorescent intermediate and leading to a dramatic increase in fluorescence intensity. The introduction of a sugar residue in fluorescent probe **1** can greatly improve its water solubility and biocompatibility, as well as the colorimetric and fluorescent "turn-on" selectivity and sensitivity toward the mercury ion in 100% water.

Scheme 1. Mercury-Mediated Ferrier Carbocyclization of **1**



Phase-transfer-catalyzed (PTC) coupling reaction of **4** and resorufin **5** was conducted in a 0.4 M aqueous $K_2CO_3-CHCl_3-Bu_4NBr$ (tetrabutylammonium bromide) system at 50 °C, and compound **6** was obtained in one pot through consecutive glycosylation and HI elimination. After removal of the acetyl groups with MeONa/MeOH (keeping pH at 9), the desired water-soluble fluorescent probe **1** was obtained as a pale yellow solid in 28% overall yield (Scheme 2). Probe **1** was characterized by ^1H NMR (Figure S1), ^{13}C NMR (Figure S2), and mass spectra.

Scheme 2. Synthesis of Probe **1**



As expected, synthetic probe **1** has good water solubility and can completely dissolve in 100% pure water at 1 mg/mL without adding any toxic organic cosolvent. The pH value variation from 3.4 to 9.3 does not cause any significant changes in fluorescence intensity (Figure S3), implying the stability and potential application of probe **1** in an aqueous and biological system. The aqueous solution of probe **1** (20 μM in pure water) itself is colorless and nonfluorescent at a wavelength range of 450 to 700 nm (Figure S4). After Hg^{2+} (1.0 μM in pure water) and probe **1** (20 μM) in pure water (pH 6.0) were mixed, a weak fluorescence signal at $\lambda_{ex}/\lambda_{em}$ = 570/594 nm was detected due to the in situ generation of resorufin **5**. The reaction proceeds relatively fast and can be finished within 15 min. Interestingly, when the pH value of the reaction mixture was adjusted to 6.96 (or greater) by a PBS buffer, a strong and stable fluorescence signal (> 25 times) would suddenly appear due to the formation of *O*-anionic compound **2** from resorufin **5** (Figures S5, S6). This is in accordance with the physicochemical property (pK_a) of resorufin **5**.[10] Moreover, a simultaneously purple color could be observed by the "naked eye" under these conditions (Figure 1).

Considering the potential biological application, we set the pH at 7.4 in the following studies to fit the natural physiological requirement. The generation of *O*-anionic compound **2** was confirmed by mass spectrometry analysis (ESI^- mode, m/z 212.0) on the crude reaction solution (namely, **1** + $HgCl_2$) (Figure S7), as well as the reaction marker, carbo-sugar **3** (m/z 160.7, $M-H^+$). The fluorescence titration of probe **1** with an increasing amount of Hg^{2+} (from 0 to 2.0 equiv) showed saturation behavior at 0.5 equiv of Hg^{2+} (Figure S8) in PBS buffer at pH 7.4.

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Figure 1. "Naked-eye" color changes of probe **1** (20 μM in pure water) before and after addition of Hg^{2+} (1.0 μM) at room temperature (reacted 15 min and adjusted pH to 7.4 with PBS buffer before measurement).

The result suggested that the mercury ion ran 2 cycles in this Ferrier carbocyclization reaction (Scheme 1).[7a,11]

Figure 2a shows the Hg^{2+} concentration dependent emission fluorescence spectra of probe **1** (20 μM). When excited at 570 nm, the emission fluorescence intensity at 594 nm increased about 25-fold upon increasing the concentration of Hg^{2+} from 0 to 1.0 μM. A satisfactory linear relationship between fluorescence intensity and Hg^{2+} concentration was observed with a correlation coefficient as high as 0.9935 (Figure 2b). According to fluorometric method, the detection limit of probe **1** for Hg^{2+} was found to be 0.15 μM (ca. 30 μg/L) at a signal-to-noise (S/N) ratio of 3. This result is comparable to those reported previously and shows potential application in emerging environmental monitoring.[2o,5f,5h]

To evaluate the selectivity of probe **1**, 12 typical cations (Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Ag^{+}, Pb^{2+}, Fe^{3+}, Fe^{2+}, Ni^{2+}) were investigated in parallel under the same testing conditions. As shown in Figure 3, the reaction of probe **1** with Hg^{2+} produces a strong fluorescence response, while the other cation ions do not show this behavior. Adding Hg^{2+} into other cation ions solutions, respectively, presented similar emission spectra at $\lambda_{ex}/\lambda_{em}$ = 570/594 nm (Figure 3a) and fluorescence intensity at λ_{em} = 594 nm (Figure 3b) as those of the Hg^{2+} ion alone. This selectivity indicated that probe **1** has satisfactory specificity toward Hg^{2+} species.

Carbohydrate-modified probe **1** was expected to have good cell permeability and a rapid fluorescence response and, thus, is applicable to Hg^{2+} monitoring in cells and organisms. For this purpose, absorption of mercury ions into cells and organisms was determined by using probe **1** preincubated in living things according to a literature reported method.[12] The A549 cell line (human lung adenocarcinoma epithelial cell line) and 5-day-old zebrafish

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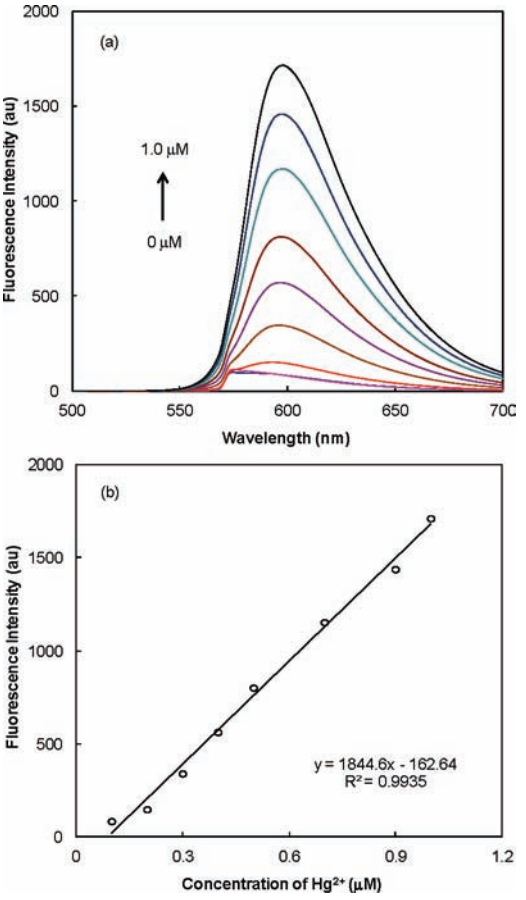

Figure 2. (a) Fluorescence titration spectra (λ_{ex} = 570 nm, with slit widths of 2.5 nm) of probe **1** (20 μM in pure water) with Hg^{2+} from 0 to 1.0 μM at room temperature (reacted 15 min and adjusted pH to 7.4 with PBS before determination). (b) The plot of fluorescence intensity changes ($\lambda_{ex}/\lambda_{em}$ = 570/594 nm, slit widths of 2.5 nm) of probe **1** (20 μM in pure water) against varied concentration of Hg^{2+} from 0.1 to 1.0 μM at room temperature (reacted 15 min and adjusted pH to 7.4 with PBS before determination).

were incubated with probe **1** (20, 20, and 100 μM at 24 h for cells incubation, 30, 30, and 60 μM at 2 h for zebrafish incubation, respectively), washed with PBS, and then treated with a $HgCl_2$ solution (10, 50 μM at 1 h for cells incubation, 15, 30 μM at 0.5 h for zebrafish incubation). As shown in Figure 4, significant fluorescence signals were detected when mercury ions enter the cells and organisms, and the fluorescence intensity showed in a concentration-dependent way. The current experiment suggested that probe **1** can be applied to detect Hg^{2+} ions in cellular systems with satisfactory sensitivity and high selectivity, which is valuable for studying the uptake, bioaccumulation, and bioavailability of Hg^{2+} in living organisms.

In conclusion, an excellently selective and satisfactorily sensitive "turn-on" fluorescent probe for the detection of Hg^{2+} in 100% water media has been designed and synthesized. The probe, presenting a rapid (less than 15 min) "turn-on" fluorescence response and good water solubility

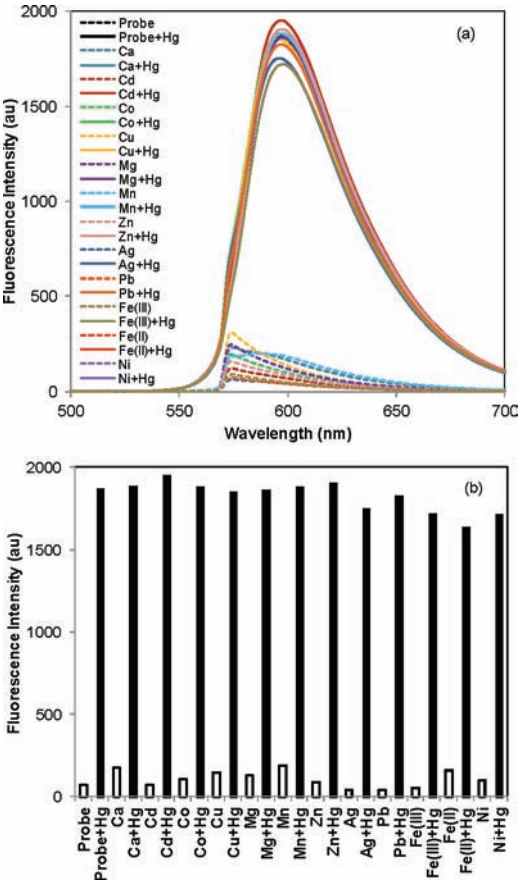

Figure 3. (a) Fluorescence spectra (λ_{ex} = 570 nm, with slit widths of 2.5 nm) of probe **1** (20 μM in pure water) upon addition of various metal ions (1.0 mM in pure water). Dot line: probe **1** + metal cation, solid line: probe **1** + metal cation + Hg^{2+} (reacted 15 min and adjusted pH to 7.4 with PBS before determination). (b) Fluorescence response ($\lambda_{ex}/\lambda_{em}$ = 570/594 nm, with slit widths of 2.5 nm) of probe **1** (20 μM in pure water) upon addition of various metal cations (1.0 mM in pure water). White column: probe **1** + metal cation, black column: probe **1** + metal cation + Hg^{2+} (reacted 15 min and adjusted pH to 7.4 with PBS before determination).

and biocompatibility, is cleverly designed based on a unique Hg^{2+}-mediated carbohydrate Ferrier carbocyclization reaction. The probe was also successfully applied to the imaging of Hg^{2+} ions in A549 cells and zebrafish, which

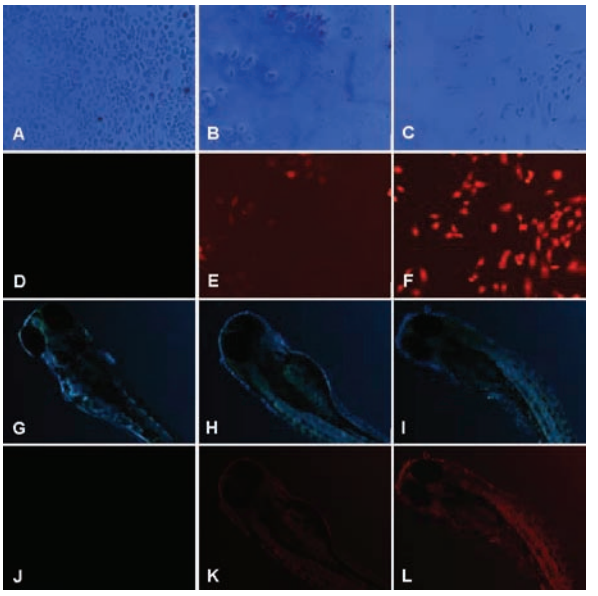

Figure 4. Fluorescence images of Hg^{2+} ions in A549 cells and zebrafish with probe **1**. Bright-field transmission image (A−C) and fluorescence image (D−F) of A549 cells pretreated with 20, 20, and 100 μM of probe **1** for 24 h followed by incubation with 0, 10, and 50 μM of Hg^{2+} ions for 1 h, respectively (excited with green light). Bright-field transmission image (G−I) and florescence image (J−L) of zebrafish pretreated with 30, 30, and 60 μM of probe **1** for 2 h followed by incubation with 0, 15, and 30 μM of Hg^{2+} ions for 0.5 h, respectively (excited with green light).

is valuable for studying Hg^{2+} toxicity in living organisms. The current work demonstrates a new type of carbohydrate assisted fluorescence sensor.

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Supporting Information Available. Experimental procedures for the synthesis and copies of ^{1}HNMR and ^{13}C NMR of probe **1**, data for fluorescence of probe **1**, and other data.This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.