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A Colorimetric and Fluorometric Dual-Modal Assay for Mercury Ion by a Molecule

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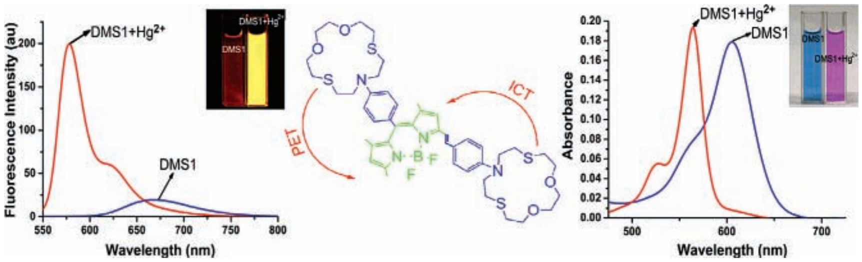
ABSTRACT



The synthesis and sensing characteristics of a new class of colorimetric and fluorometric dual-modal probe for mercury ion are outlined. Judicious placement of two dithia-dioxa-aza macrocycles on the BODIPY chromophore generates this interesting molecule. A highly Hg^{2+}-selective fluorescence enhancing property (>7-fold) in conjunction with a visible colorimetric change from purple to red-pink can be observed, leading to potential fabrication of both "naked-eye" and ratiometric fluorescent detection of Hg^{2+}.

The design and construction of chemosensors with high selectivity and sensitivity for heavy metal cations has received considerable attention, as such metal ions can cause severe risks for human health and the environment.[1,2] Among them, mercury ion (Hg^{2+}) is considered as one of the most dangerous cations for the environment because it is widely distributed in air, water, and soil. Mercury can accumulate in the human body and affects a wide variety of diseases even in a low concentration, such as prenatal brain damage, serious cognitive and motion disorders, and Minamata disease.[3] Therefore, it is very important to develop highly sensitive and selective assays for Hg^{2+} ions. In recent years many efforts have been made to design various chemosensors specific for Hg^{2+} ion detection.[4] Among the reported sensing molecules, fluorophores with a fluorescent switching property driven by photoinduced electron transfer (PET)[2] are often employed to signal metal ion binding. However, the sensitivity and selectivity of the Hg^{2+} detection based on the PET mechanism are still unsatisfactory because of their poor

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fluorescent switching and the interference with other cations, such as Ag^+ and Pb^{2+} ions.[5]

Boradiazaindacenes (BODIPY) are very attractive functional groups for construction of molecular sensors because of their advantageous characteristics, such as sharp absorption and fluorescence bands, high extinction coefficients, high fluorescence quantum yields, and high stability against light and chemical reactions.[7] Recently, many fluorescent chemosensors have been reported based on modified boradiazaindacene fluorophores.[8] However, most of them display only fluorescence intensity changes, although efficient quenching or a large fluorescence enhancement are observed due to the efficient oxidative or reductive PET.[2] Lately, a very interesting modification converting a standard green emitting fluorophore to a longer wavelength absorbing and red emitting intramolecular charge transfer (ICT) fluorophore was reported,[9] where a proton-sensitive amino group (electron-donating) was conjugated to the electron-withdrawing BODIPY group. It demonstrated that the emission properties can be switched on with acid addition, affording possibilities for cation sensing applications with rationally designed chemosensors.[10] To date, there is still a demand for new indicators with improved properties, especially colorimetric probes that can make "naked-eye" detection in the visible wavelength region.[6]

This work is aimed at the design and construction of a new class of colorimetric and fluorometric dual-channel assay to specifically detect the presence of Hg^{2+} over a wide range of other cations. To achieve this goal a modular molecular system is synthesized that is comprised of BODIPY fluorophore and two crown ether ligands with specific recognition ability to the Hg^{2+} ion. Our strategy takes advantage of both PET and ICT processes[2] on a single molecule.

Compound **1** reacted with $POCl_3$ in the presence of DMF to afford compound **2** with 63% yield. Compound **3** was obtained by reaction of compound **2** with 2,4-dimethylpyrrole with added TFA as catalyst followed by oxidization with DDQ and treatment with $BF_3{-}OEt_2$ with 31% yield. Target molecule DMS1 was synthesized by the reaction of compound **3** with compound **2** in toluene, using piperidine, glacial acetic acid, and $Mg(ClO_4)_2$ as catalyst to afford a purple solid.

Figure 1 showed the absorption spectral changes of DMS1 as a function of the Hg^{2+} concentration in a THF−water solution (30:70, v/v, 20 mM HEPES buffer, pH 7.2) at room temperature. The UV/vis spectrum of DMS1 in aqueous solution is characterized by a very intense band centered at 606 nm (ϵ 85 000 M^{-1} cm^{-1}), which is responsible for the purple color of the solution. The absorption maximum of DMS1 has about a 100 nm red shift in comparison to that of the standard BODIPY dye.[7a] This red shift was assigned to an efficient ICT process from the donor nitrogen atom on the dithia-dioxa-aza macrocycle—the one that conjugated to fluorophore to the acceptor BODIPY group.[2,9,10] Upon adding Hg^{2+} the intensity of the absorption maximum of DMS1 at 606 nm gradually decreased following the formation of a new band centered at 564 nm (ϵ 95 000 M^{-1} cm^{-1}), and an isosbestic point at 578 nm was observed. The coordination of the Hg^{2+} to the ligand reduces the electron-donating ability of the nitrogen atom at the dithia-dioxa-aza macrocycle, which conjugated to BODIPY core, thus the ICT process is not possible any more and the red shift in absorption spectra is suppressed. In other words, the blue shift in absorption spectra is observed upon Hg^{2+} binding. More importantly, the Hg^{2+} sensing and the concomitant absorption changes were clearly visible to the naked eye, as can be seen in the photograph, where the purple solution of DMS1 became red-pink upon titration with Hg^{2+} ions.

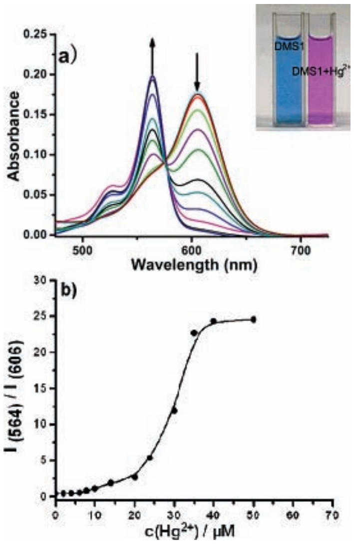

Figure 1. (a) Absorbance spectra of compound DMS1 in the presence of increasing Hg(II) concentrations (0, 2, 4, 6, 8, 10, 14, 20, 24, 30, 35, 40 μM) in a THF−water solution (30:70, v/v, 20 mM HEPES buffer, pH 7.2). The concentration of the DMS1 was 2 μM. (b) In absorption spectra I(564)/I(606) as a function of Hg^{2+} concentration.

To verify specificity, experiments were conducted with other metal cations, such as Fe^{3+}, Co^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Pb^{2+}, Ag^+, and Al^{3+}. As shown in Figure 2, under identical condition to Hg^{2+} ion, no changes were observed in the UV/vis spectra of DMS1 upon addition of 10 equiv of Co^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Pb^{2+}, Ag^+, Al^{3+}, and Fe^{3+} ions or mixed ions, this could probably be attributed to their low affinity with the receptor DMS1.[10,13]

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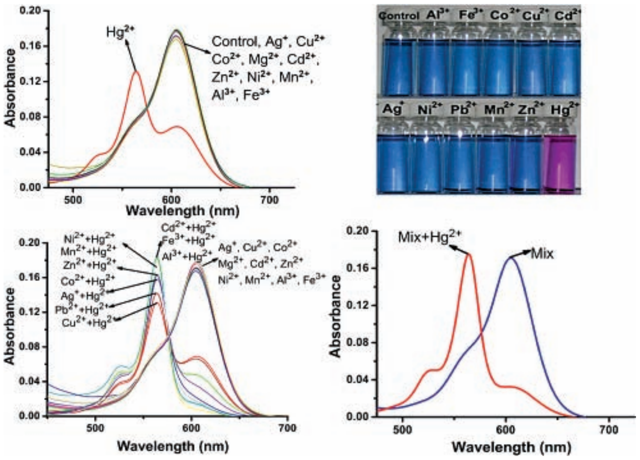

Figure 2. (a) Absorbance spectra change of DMS1 (2 μM) upon addition of different metal cations (10 equiv) in a THF−water solution (30:70, v/v, 20 mM HEPES buffer, pH 7.2). (b) Color change of DMS1 in the presence of different metal cations. From left to right: control, Al^{3+}, Fe^{3+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Ag^{+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Zn^{2+}, and Hg^{2+}. (c) Absorbance spectra change of DMS1 (2 μM) upon addition of different metal cations (10 equiv) and subsequent addition of 30 μM (15 equiv) Hg^{2+} in aqueous solution. (d) Absorbance spectra change of DMS1 (2 μM) upon addition of mixed cations (mix = $Al^{3+} + Fe^{3+} + Co^{2+} + Cu^{2+} + Cd^{2+} + Ag^{+} + Ni^{2+} + Pb^{2+} + Mn^{2+} + Zn^{2+}$); each one is 10 equiv metal cations and subsequent addition of 100 μM (50 equiv) Hg^{2+} in aqueous solution.

The other cations also did not induce any significant color change of DMS1. Therefore, DMS1 can be considered as an effective colorimetric probe for Hg^{2+}. The advantage of this assay is that naked-eye detection of Hg^{2+} becomes possible.

Note that a fluorometric detection of Hg^{2+} is also possible for DMS1. As expect DMS1 is virtually nonfluorescent in apo state ($\phi = 0.04$), which resulted from the efficient photoinduced electron transfer (PET) quenching of the excited state of the BODIPY moiety by the long pair of electrons on the nitrogen atoms in the dithia-dioxa-aza macrocycle.[2,8] As shown in Figure 3, upon adding the Hg^{2+} ion, the fluorescence intensity of DMS increases by over 7-fold ($\phi = 0.33$) accompanied by a large blue shift in the emission spectrum. The emission spectrum of free DMS1 displays a broad band with a maximum at 668 nm. When Hg^{2+} was added to the solution of DMS1, an emission intensity decrease at 668 nm and a significant increase at 578 nm were observed. How could we explain these phenomena? We speculate that the fluorescence intensity enhancement could contribute to two points. First, the capture of Hg^{2+} by the receptor resulted in the reduction of the electron-donating ability of dithia-dioxa-aza macrocycle to directly connect to the BODIPY fluorophores, thus the main quenching process (PET) is partially suppressed and causes an enhancement in the emission. Second, coordination of the Hg^{2+} to the ligand that is conjugated to the BODIPY core suppressed the ICT process, and this process also contributes to the fluorescence intensity enhancement. We suggest that the suppressed PET process is the main reason for >7-fold fluorescence intensity enhancement, but the suppressing of the ICT process we mentioned above is the only reason that gives rise to large changes in the absorption and emission spectra.

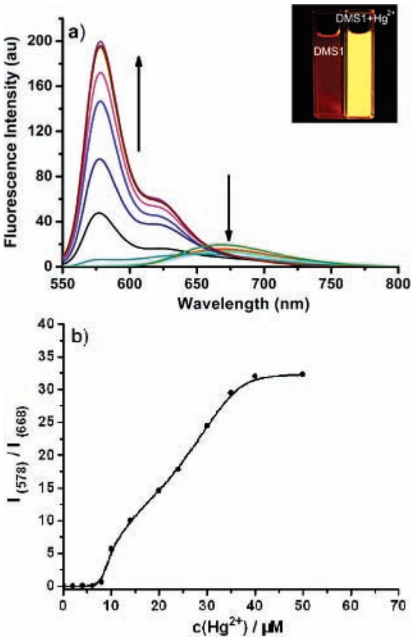

Figure 3. (a) Emission spectra of compound DMS1 in the presence of increasing Hg(II) concentration (0, 2, 4, 6, 8, 10, 14, 20, 24, 30, 35, 40 μM) in a THF−water solution (30:70, v/v, 20 mM HEPES buffer, pH 7.2). Excitation wavelength was 540 nm with 5 nm slit widths. The concentration of the chemosensor was 2 μM. (b) In emission spectra I(578)/I(668) as a function of Hg^{2+} concentration.

To explore further the utility of DMS1 as an ion-selective fluorescence probe for Hg^{2+} ion, the competition experiments were conducted. Figure 4 depicts the fluorescence responses of DMS1 to the presence of 10 equiv of Hg^{2+}, Fe^{3+}, Co^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Pb^{2+}, Ag^{+}, and Al^{3+}. Due to the low affinity with the receptor DMS1,[10,13] the other metal ions showed nearly no changes in emission spectra.

Importantly, the large cation-induced blue shift for Hg^{2+} in emission spectra resulted in a color change from rose to yellow. This visible emission allows DMS1/Hg^{2+} to be readily distinguished by the naked eye under UV light.

To uncover the PET and ICT process reference compound **3** and compound **4** were used to investigate. We noticed that adding Hg^{2+} to solutions of compound **3** did not induce any shifts in emission spectra, only fluorescence intensity enhancement can be observed. Adding Hg^{2+} to solutions of compound **4** induced a large blue shift in both emission and

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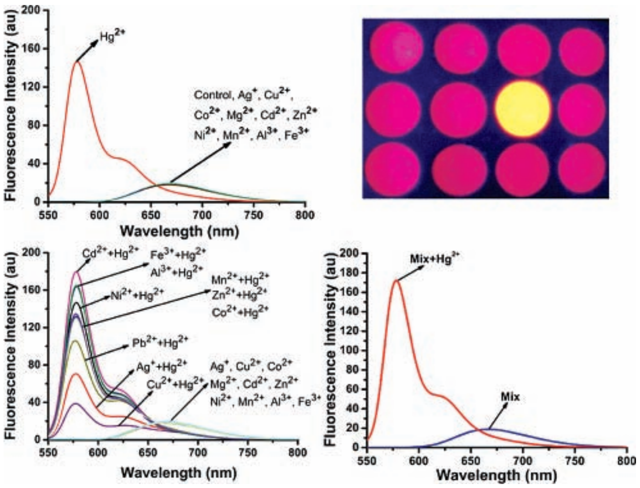


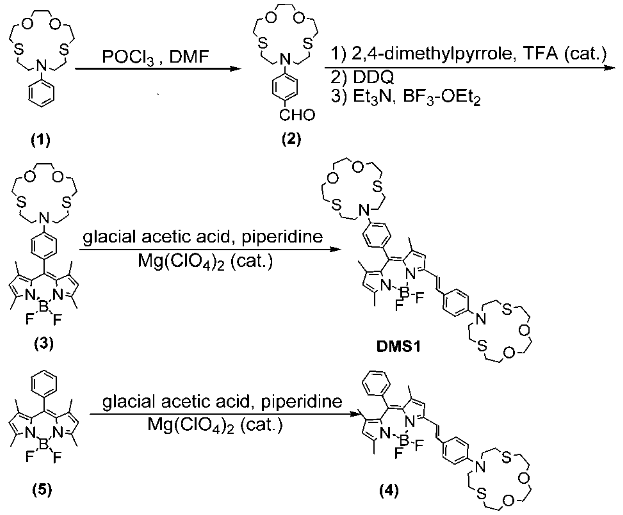
Figure 4. (a) Fluorescence spectra changes of DMS1 (2 μM) upon addition of different metal cations (10 equiv) in a THF−water solution (30:70, v/v, 20 mM HEPES buffer, pH 7.2). (b) Color change of DMS1 in the presence of different metal cations. From left to right and from top to bottom: control, Al^{3+}, Cu^{2+}, Co^{2+}, Fe^{3+}, Cd^{2+}, Ag^{+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Zn^{2+}, Hg^{2+}. (c) Fluorescence spectra changes of DMS1 (2 μM) upon addition of different metal cations (10 equiv) and subsequent addition of 30 μM (15 equiv) Hg^{2+} in aqueous solution (d) Fluorescence spectra changes of DMS1 (2 μM) upon addition of mixed metal cations (mix = Al^{3+} + Fe^{3+} + Co^{2+} + Cu^{2+} + Cd^{2+} + Ag^{+} + Ni^{2+} + Pb^{2+} + Mn^{2+} + Zn^{2+}; each one is 10 equiv) and subsequent addition of 100 μM (50 equiv) Hg^{2+} in aqueous solution

absorbance spectra. These two phenomena proved that the two binding sites underwent different photophysical processes. One is the PET process and another is the ICT process (see the Supporting Information).

The two binding sites one decoupled from the BODIPY chromophore and the one conjugated to the BODIPY system should have different basicities and so have different stoichiometry and binding constants. To better understand the stoichiometry and the binding constant of each ligand, we investigate them respectively through reference compound **3** and compound **4**. Binding analysis by using the method of continuous variation (Job's plot) established that both the receptors formed the 1:1 Hg^{2+}−crown complex. Following a Benesi−Hildebrand-type analysis, the binding constants were determined to be 4.3×10^4 and 2.9×10^4 mol^{-1} dm^3 individually (see the Supporting Information).

In conclusion, we have developed a new class of colorimetric and fluorometric probe for Hg^{2+} detection with high selectivity and sensitivity on the basis of the tuning of PET and ICT processes on a single molecule. A highly Hg^{2+}-selective fluorescence enhancing property (>7-fold) in conjunction with a visible colorimetric change from purple to red-pink can be observed. Significantly, our results make "naked-eye" and ratiometric fluorescent Hg^{2+} detection in a single molecule possible. Because the detection scheme is entirely ligand based, the design strategy is general enough to be readily extended to the development of chemosensors for a variety of different metal ions.

Scheme 1. Synthesis of Dual-Modal Mercurysensor-1 (DMS1)



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Note Added after ASAP Publication. Dual-Model was corrected to Dual-Modal in the title and throughout the paper in the version published May 7, 2007; the incorrect version was published May 3, 2007.

Supporting Information Available: Experimental details including photophysical properties of **3**, **4**, and DMS1, details of Job's plots, and a Benesi−Hildebrand analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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