

A Fluorescent Chemosensor Based on 7-Nitrobenz-2-oxa-1,3-diazole (NBD) for the Selective Detection of Hg(II)

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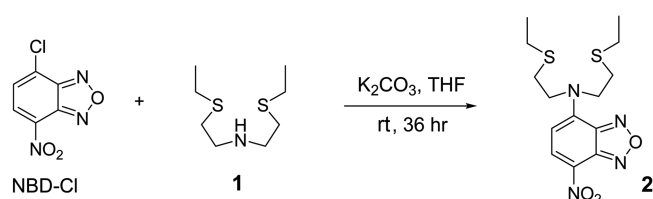
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Highly sensitive chemosensors for the detection of the mercuric ion (Hg^{2+}) have attracted considerable attention, because Hg^{2+} is one of the most serious environmental and health threats originating from natural and industrial sources.^{1,2} Especially, fluorescent chemosensors for the selective detection of Hg^{2+} are quite attractive, because of their high sensitivity and selectivity. A large number of fluorescent chemosensors for Hg^{2+} have been developed by combining an appropriate fluorophore as a signaling moiety with an appropriate ionophore or receptor as a metal-ion recognition moiety. Various fluorophores including, for example, rhodamine,³ fluorescein,⁴ boron-dipyrromethene (BODIPY),⁵ dansyl,⁶ 7-nitrobenz-2-oxa-1,3-diazole (NBD)⁷ and others,⁸ have been utilized for the development of fluorescent chemosensors for the selective detection of Hg^{2+} . In the case of NBD-based fluorescent chemosensors, cyclic ionophores such as cyclam,^{7b} diazatetraphia crown ether,^{7c} thiaoxaaza-macrocyclic,^{7c} large acyclic ionophores such as 3,6,12,15-tetrathia-9-azaheptadecane^{7a} and U-shaped 2,6-bis(aminomethyl) pyridine receptor^{7d} and simple triazole binding receptor^{7f} have been successfully utilized for the selective detection of Hg^{2+} . However, the relatively small acyclic ionophore, 3,9-dithia-6-azaundecane (**1**), combined with NBD (compound **2**) was reported to show no fluorescence change in the presence of various metal ions.^{7a} However, **1** combined with a fluorescein-based fluorophore was reported to show a significant fluorescence enhancement in the presence of Hg^{2+} .^{4a} In this instance, it is hard to accept that compound **2** exhibits no fluorescence change in the presence of Hg^{2+} .

In this study, we prepared **2** by treating NBD chloride (4-chloro-7-nitrobenz-2-oxa-1,3-diazole) with **1** at room temperature in the presence of potassium carbonate in tetrahydrofuran as shown in Scheme 1 and investigated the absorption and emission behaviors of **2** in the presence of



Scheme 1. Synthesis of **2**.

various metal ions.

The UV-vis absorption behaviors of **2** (100 μM) in 50% acetonitrile in water in the presence of various metal ions (100 equiv) are shown in Figure 1. In the absence of metal ions, **2** shows an intense absorption at 484 nm, which is assignable to the intramolecular charge transfer (ICT) transition from the N atom of the 3,9-dithia-6-azaundecane moiety to the nitro group of the NBD moiety of **2**.⁹ No significant change in the absorption of **2** was observed upon the addition of various metal ions including Na^+ , K^+ , Rb^+ , Cs^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} , Cd^{2+} and Cr^{3+} . However, its absorption intensity was decreased and its absorption maximum (λ_{max}) was changed from 484 nm to 467 nm upon the addition of Hg^{2+} . Consequently, the orange color of **2** in 50% acetonitrile in water changed to a yellow color upon the addition of Hg^{2+} (Figure 1, inset). The complexation of **2** with Hg^{2+} is expected to induce a blue-shift (17 nm) of the ICT transition by tying up the lone pair electrons of the N atom.

The fluorescence emission behaviors of **2** (100 μM) in 50% acetonitrile in water in the presence of various metal ions (100 equiv) are shown in Figure 2. Without metal ions, **2** shows a weak fluorescence band with a maximum wave-

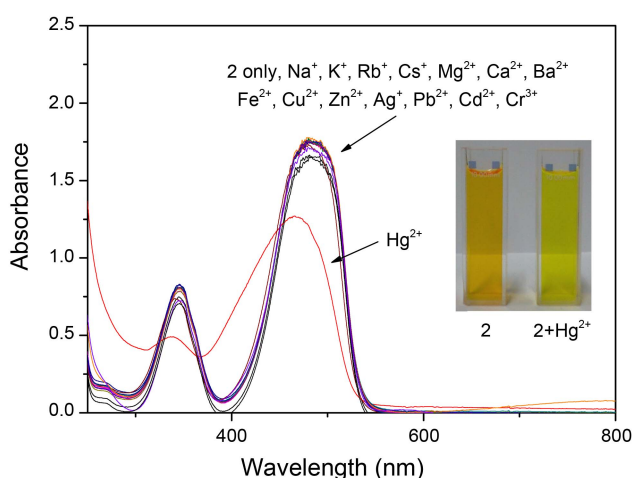


Figure 1. UV-Visible spectra of **2** (100 μM) in 50% acetonitrile in water upon the addition of each of perchlorate salts of various metal ions (100 equiv) including Na^+ , K^+ , Rb^+ , Cs^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} , Cd^{2+} , Cr^{3+} and Hg^{2+} .

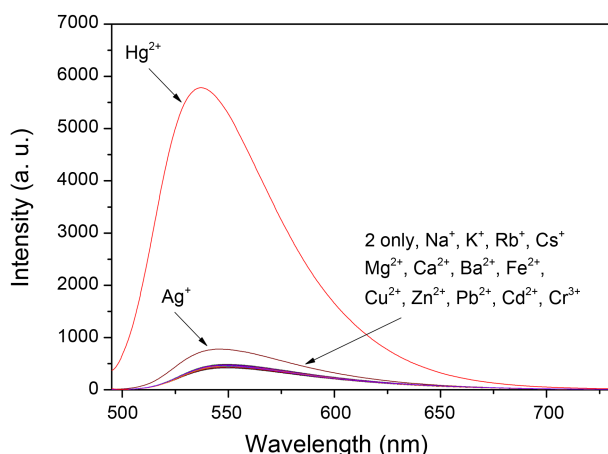


Figure 2. Fluorescence spectra of **2** (100 μM) upon the addition of each of perchlorate salts of various metal ions (100 equiv) including Na^+ , K^+ , Rb^+ , Cs^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} , Cd^{2+} , Cr^{3+} and Hg^{2+} in 50% acetonitrile in water (excitation: 484 nm).

length at 550 nm upon its excitation at 484 nm. The photoinduced electron transfer (PET) process from the HOMO of the N atom to the excited fluorophoric moiety of **2** is expected to lower the fluorescence intensity.⁹ The addition of various metal ions such as Na^+ , K^+ , Rb^+ , Cs^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} , Cd^{2+} and Cr^{3+} was found to lead to almost no fluorescence change. When Ag^+ was added, a slight enhancement of the fluorescence was observed. However, when Hg^{2+} was added, a significant enhancement of fluorescence was observed. The significant fluorescence enhancement of **2** upon the addition of Hg^{2+} is visualized in Figure 3. The tying up of the lone pair electrons of the N atom by the effective complexation of **2** with Hg^{2+} is expected to suppress the PET process and, consequently, to induce a large CHEF (chelation enhanced fluorescence).

The selectivity of **2** for various metal ions was evaluated by determining the relative emission intensities, I/I_0 , where I and I_0 are the maximum emission intensities of **2** in the presence and absence of metal ion at 550 nm, respectively. The ratio, I/I_0 , was 13.60 for Hg^{2+} and 1.80 for Ag^+ , while it was in the range of 1.10–1.21 for other metal ions. The selectivity of **2** for various metal ions evaluated by the ratio, I/I_0 , is graphically presented in Figure 4. From Figure 4, it is easily recognized that **2** shows a prominent selectivity for Hg^{2+} over other metal ions.

The fluorescence titration spectra of **2** upon the addition of

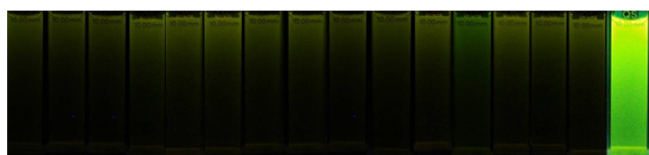


Figure 3. Visualized fluorescent emission change of **2** (100 μM) upon the addition of various metal ions (100 equiv) in 50% acetonitrile in water.

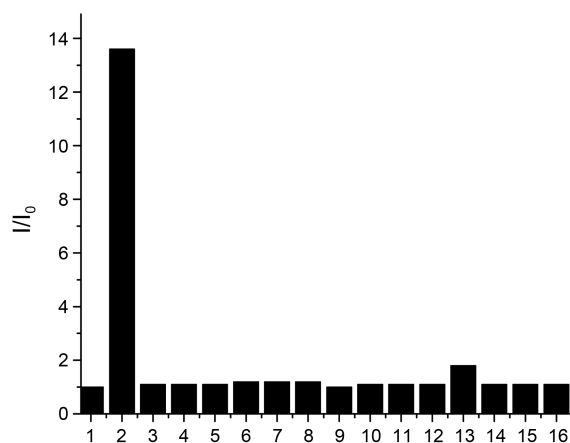


Figure 4. Relative emission intensities of **2** (100 μM), given as the ratio, I/I_0 , upon the addition of different metal ion (100 equiv) in 50% acetonitrile in water: **2** only (1), Hg^{2+} (2), Na^+ (3), K^+ (4), Rb^+ (5), Cs^+ (6), Mg^{2+} (7), Ca^{2+} (8), Ba^{2+} (9), Fe^{2+} (10), Cu^{2+} (11), Zn^{2+} (12), Ag^+ (13), Pb^{2+} (14), Cd^{2+} (15), Cr^{3+} (16).

Hg^{2+} in 50% acetonitrile in water are shown in Figure 5. As the concentration of Hg^{2+} was increased from 0 to 100 equiv, the fluorescence maximum shifted from 550 nm to 538 nm and the fluorescence intensity increased. From the fluorescence titration spectra, the association constant of **2** for Hg^{2+} was calculated to be $1.22 \times 10^2 \text{ M}^{-1}$ according to the Benesi-Hildebrand expression.^{10,11} The detection limit of Hg^{2+} was calculated to be $6.5 \times 10^{-6} \text{ mol/L}$.

When the pH was changed from neutral to 1.02 by adding HNO_3 to 50% acetonitrile in water, the fluorescence of **2** was found to increase slightly upon its excitation at 484 nm. The protonation of the N atom of **2** might suppress the PET process and enhance the fluorescence. When Hg^{2+} was added, a significant fluorescence enhancement was also observed while the addition of other metal ions was found not to enhance the fluorescence. The association constant of **2** for Hg^{2+} ($6.67 \times 10^2 \text{ M}^{-1}$) under the acidic condition (pH = 1.02)

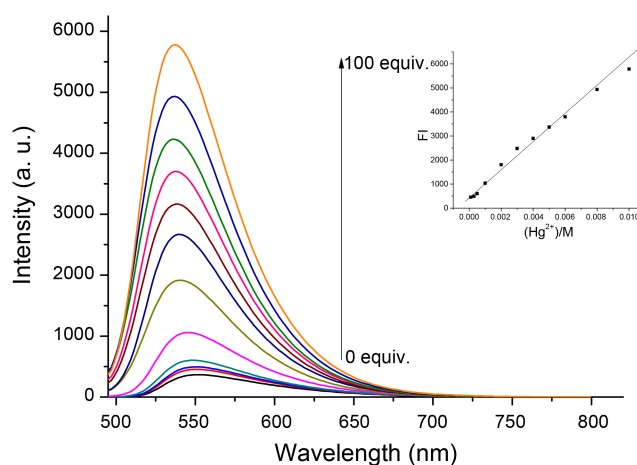


Figure 5. Fluorescence titration spectra of **2** (100 μM) upon the addition of Hg^{2+} (100 μM , 300 μM , 500 μM , 1000 μM , 2000 μM , 3000 μM , 4000 μM , 5000 μM , 6000 μM , 8000 μM , 10000 μM) in 50% acetonitrile in water (excitation: 484 nm). (Inset: the fluorescent intensity vs the Hg^{2+} concentration.)

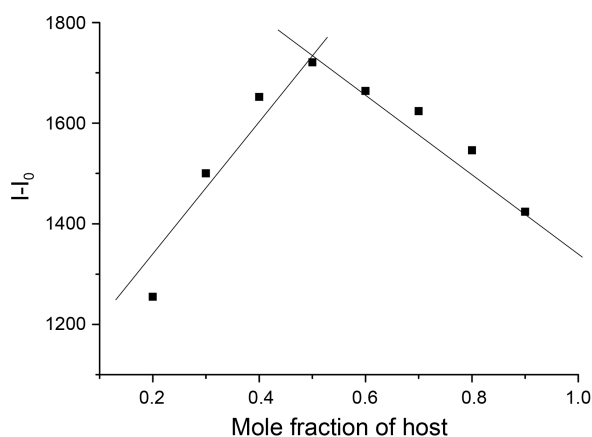


Figure 6. Job's plot of **2** and Hg^{2+} in 50% acetonitrile in water.

was greater than that ($1.22 \times 10^2 \text{ M}^{-1}$) under the neutral condition, but the reason is not clear yet.

The Job's plot experiment performed by varying the concentration of **2** and Hg^{2+} shows a maximum point at a mole fraction of 0.5, as shown in Figure 6 and, consequently, the formation of the typical 1:1 complex between **2** and Hg^{2+} is to be expected.

In summary, a fluorescent chemosensor, **2**, was prepared by combining the NBD fluorophore with a simple metal ion binding ionophore, 3,9-dithia-6-azaundecane. The fluorescent chemosensor was found to be very useful for the selective detection of Hg^{2+} in 50% acetonitrile in water. The association constant of the fluorescent chemosensor for Hg^{2+} was $4.00 \times 10^5 \text{ M}^{-1}$. From these results, it is concluded that **2**, which was reported previously to show no fluorescence change in the presence of Hg^{2+} , can be used as an effective fluorescent chemosensor for the selective detection of Hg^{2+} .

Experimental Section

Reagents and Instruments. 4-Chloro-7-nitrobenzofurazan was purchased from Aldrich. Bis(2-chloroethyl)amine hydrochloride and ethanethiol were purchased from TCI. All glass wares, syringes, and magnetic stirring bars were completely dried. The reactions were monitored with thin layer chromatography (TLC). Commercial TLC plates (Silica gel 60 F_{254} , Merck Co.) were developed and the spots were observed under UV light at a short wavelength of 254 nm and long wavelength of 365 nm. Silica column chromatography was performed with silica gel 60 G (Merck Co.) The ^1H NMR and ^{13}C NMR spectra were measured on a Varian Mercury Plus 300 MHz spectrometer. The high-resolution mass spectral data were obtained from the Korea Basic Science Institute Daegu Center (HR-FAB Mass) on a Jeol JMS 700 high resolution mass spectrometer. The fluorescence spectra were measured on HITACHI model F-7000 fluorescence spectrophotometer. The UV-visible spectra were measured in the range of 190–1100 nm on a SIWCO S-3100 spectrophotometer.

Synthesis of 2. In this study, **2** was prepared via a slightly

different procedure from that reported previously,^{7a} as shown in Scheme 1. Compound **2** prepared in this study was found to show different melting point and color compared to those previously reported.^{7a} In a 50 mL one-necked round bottom flask were placed 3,9-dithia-6-azaundecane (0.29 g, 1.5 mmol), which was prepared from bis(2-chloroethyl)amine hydrochloride and ethanethiol via the reported procedure,⁹ potassium carbonate (0.691 g, 5.0 mmol) and tetrahydrofuran (THF) (10 mL). The mixture was stirred under an argon atmosphere at room temperature. NBD chloride (0.2 g, 1 mmol) dissolved in THF was added to the reaction mixture. The reaction mixture was stirred for 36 hr under an argon atmosphere at room temperature in the dark. The solvent was removed by a rotary evaporator. The residue was dissolved in dichloromethane. The organic solution was washed with water and dried over Na_2SO_4 . The solvent was removed by a rotary evaporator. The dark green residue was purified by chromatography on silica gel (ethyl acetate/*n*-hexane, 1/5, v/v) to afford **2** as an orange-red solid. Yield: 0.25 g (70%). mp 82–83 °C. ^1H NMR (300 MHz, CDCl_3) δ (ppm) 1.31 (6H, t, $J = 7.4$ Hz), 2.68 (4H, q, $J = 7.5$ Hz), 2.89 (4H, t, $J = 7.6$ Hz), 4.13 (4H, broad s), 6.19 (1H, d, $J = 9.1$ Hz), 8.46 (1H, d, $J = 9.1$ Hz); ^{13}C NMR (300 MHz, CDCl_3) δ (ppm) 15.1, 26.7, 28.9, 54.4, 101.7, 123.2, 135.4, 144.21, 144.6, 145.0; HR FAB-MS m/z [M⁺] calcd. for $\text{C}_{14}\text{H}_{20}\text{N}_4\text{O}_3\text{S}_2$ 356.0977. Found: 356.0966; IR (KBr pellet) cm^{-1} 2923, 1609, 1549, 1489.

Fluorescent Spectra Measurement. The stock solutions of **2** and metal ions (perchlorate salts) were prepared in 50% acetonitrile in water. For the fluorescent measurements, the sample solutions were prepared by mixing a measured amount of the stock solution of **2** with a measured amount of the stock solution containing metal perchlorate and, finally, by diluting it with 50% acetonitrile in water to get the desired concentrations of **2** and metal ions. The fluorescent measurements were carried out by exciting the sample at 484 nm with an excitation band width of 5 nm and an emission band width of 10 nm.

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