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Highly Sensitive Fluorescent Probe for Selective Detection of Hg^{2+} in DMF Aqueous Media

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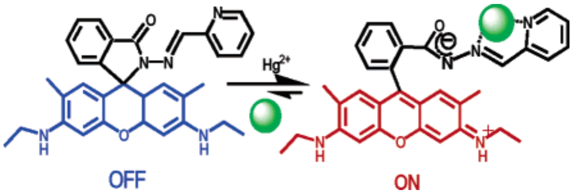
A highly sensitive fluorescent probe **1** for selective detection of Hg ion in mixed *N*,*N*-dimethylformamide aqueous media was designed and prepared by incorporating the well-known Rhodamine 6G fluorophore and a carbohydrazone binding unit into one molecule. The fluorescent probe **1** can detect the parts per billion level of Hg^{II} in a mixed aqueous environment and displays a highly selective response of fluorescence enhancement toward Hg^{II}.

Mercury is a highly toxic element, and its contamination is widespread and arises from a variety of natural and anthropogenic sources.[1] The toxicity of Hg^{2+}, even at very low concentration, has long been recognized as a problem of primary concern.[2] The Environmental Protection Agency (EPA) standard for the maximum allowable level of inorganic Hg^{II} in drinking water is 2 ppb.[3] Recently, Chang and coworkers demonstrated the use of fluorescent sensors for toxic mercury detection in fish at EPA levels.[4] Accordingly, mercury-indicating methodologies, which are developed to provide critical information for mercury hazard assessment and mercury pollution management, are in high demand. An ideal probe should thus display a very low detection limit but should retain its selectivity toward Hg^{2+}. Fluorescent molecular sensing, which translates molecular recognition into tangible fluorescence signals, has received much attention in this field.[5,6] Known by their excellent spectroscopic properties of large molar extinction coefficient (ϵ) and high fluorescence quantum yield (Φ), rhodamine-based dyes are an excellent candidate for fluorescent sensors that can detect Hg^{2+} ion below 2 ppb in aqueous media.[7] By coupling the carbohydrazone block with Rhodamine 6G, we report here a fluorescent probe that exhibits high sensitivity for selective detection of Hg^{2+} with a linear response toward Hg^{2+} at the parts per billion level in *N*,*N*-dimethylformamide (DMF) aqueous media.

Scheme 1. Proposed Mechanism of Fluorescent Enhancement of **1** upon the Addition of Hg^{II} with the Second Ligand To Meet the Coordination Requirement for Hg^{II} Omitted for Clarity



Compound **1** (Scheme 1) was facilely synthesized from Rhodamine 6G by a two-step reaction (Supporting Information, Figure S1).[8] The characteristic peak of the 10-carbon of **1** near 66 ppm in the ^{13}C NMR spectrum suggests that the spirolactam form of **1** exists predominantly in solution[9] (Supporting Information, Figure S4). Such a special conformation of the rhodamine group makes compound **1** in solution colorless and fluorescence inactive.[10] Thus, compound **1** is expected to act as a signal switcher, which is envisioned to turn on when the target cation is bound.

An optimized DMF/H_2O (1:1, v/v) solution of **1** was selected for the spectral investigation. The acid titration control experiments revealed that the titration solution did not emit any obvious and characteristic fluorescence (excita-

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(1) (a) Renzoni, A.; Zino, F.; Franchi, E. *Environ. Res.* **1998**, *77*, 68. (b) Benoit, J. M.; Fitzgerald, W. F.; Damman, A. W. *Environ. Res.* **1998**, *78*, 118.

(2) (a) Boening, D. W. *Chemosphere* **2000**, *40*, 1335. (b) Harris, H. H.; Pickering, I. J.; George, G. N. *Science* **2003**, *301*, 1203. (c) Tchounwou, P. B.; Ayensu, W. K.; Ninashvili, N.; Sutton, D. *Environ. Toxicol.* **2003**, *18*, 149.

(3) Mercury Update: Impact on Fish Advisories. EPA Fact Sheet EPA-823-F-01-011; EPA, Office of Water: Washington, DC, 2001.

(4) Yoon, S.; Albers, A. E.; Wong, A. P.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16030.

(5) (a) Caballero, A.; Martinez, R.; Lloveras, V.; Ratera, I.; Vidal-Gancedo, J.; Wurst, K.; Tarraga, A.; Molina, P.; Veciana, J. *J. Am. Chem. Soc.* **2005**, *127*, 15666. (b) Guo, X. F.; Qian, X. H.; Jia, L. H. *J. Am. Chem. Soc.* **2004**, *126*, 2272. (c) Descalzo, A. B.; Martinez-Manez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 3418. (d) Mello, J. V.; Finney, N. S. *J. Am. Chem. Soc.* **2005**, *127*, 10124.

(6) (a) Nolan, E. M.; Lippard, S. J. *J. Am Chem. Soc.* **2003**, *125*, 14270. (b) Nolan, E. M.; Racine, M. E.; Lippard, S. J. *Inorg. Chem.* **2006**, *45*, 2742. (c) Caballero, A.; Martinez, R.; Lloveras, V.; Ratera, I.; Vidal-Gancedo, J.; Wurst, K.; Tarraga, A.; Molina, P.; Veciana, J. *J. Am. Chem. Soc.* **2005**, *127*, 15666. (d) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2006**, *128*, 14474. (e) Ros-lis, J. V.; Marcos, M. D.; Martinez-manez, R.; Rurack, K.; Soto, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 4405. (f) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300.

(7) Yang, Y.-K.; Yook, K.-J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127*, 16760.

(8) For details on the preparation and characterization of compound **1**, see the Supporting Information.

(9) Anthoni, U.; Christophersen, C.; Nielsen, P.; Puschl, A.; Schaumburg, K. *Struct. Chem.* **1995**, *3*, 161.

(10) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. *Org. Lett.* **2006**, *8*, 859.

10.1021/ic062274e CCC: $37.00

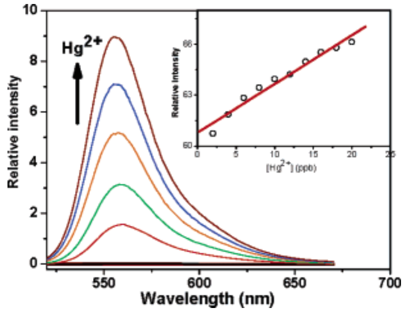

Figure 1. Fluorescence emission changes of **1** (10 μM) in a DMF/H_2O (1:1) solution upon the addition of Hg^{2+} (1−150 μM). Inset: Emission intensities at 560 nm of compound **1** (5 μM) as a function of the mercury concentration (2−20 ppb). Excitation was at 500 nm.

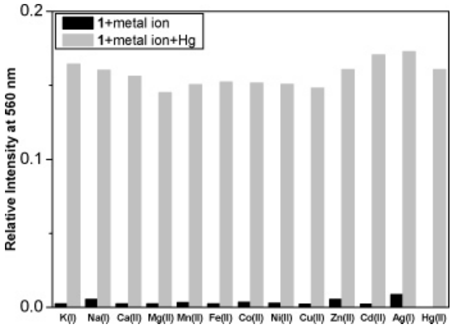

Figure 2. Fluorescence response of **1** to variuos cations and selectivity of **1** for Hg^{2+} in the prescence of other metal ions in a DMF aqueous solution. The dark bars respresent the emission of **1** in the presence of 25 equiv of the cation of interest. The light bars respresent the change of the emission that occurs upon the subsequent addition of 2.5 equiv of Hg^{2+} to a solution containing **1** and 25 equiv of the cation of interest. [**1**] = 10 μM; λ_{ex} = 500 nm. The emission intensities were recorded at 560 nm.

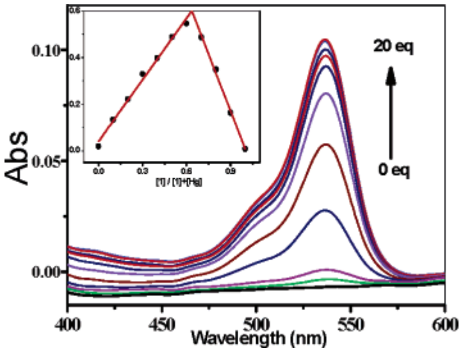

Figure 3. UV−vis spectra of **1** (10^{-5} M) in a DMF aqueous solution (50:50, v/v) in the presence of different amounts of Hg^{2+}.

tion at 500 nm) in the pH range from 5.0 to 10.0, suggesting that **1** was insensitive to the pH (Supporting Information, Figure S5). Upon the addition of Hg^{II}, a new emission band with the maximum emission wavelength at 560 nm appeared and developed (quantum yield, Φ, 0.42)[11] (Figure 1), which can be ascribed to the delocalized xanthene moiety of the rhodamine group. The fluorescent titration profile of **1** with Hg^{2+} (inset in Figure 1) demonstrated that the detection of Hg^{2+} was at the parts per billion level when **1** was employed at 5 μM. Meanwhile, under the optimized conditions, the fluorescence intensity of the solution of **1** was nearly proportional to the amount of Hg^{2+} added (Supporting Information, Figures S6 and S7).[12]

As is well-known, a highly selective probe for Hg^{2+} that gives a positive response rather than fluorescent quenching upon analyte binding is usually preferred to promote the sensitivity. The fluorescence enhancement effects of various metal ions on **1** in 50% (v/v) water/DMF solution were investigated under excitation at λ_{ex} = 500 nm. The fluorescence response of **1** to various cations and its selectivity for Hg^{2+} are illustrated in Figure 2; no significant spectral changes of **1** occurred in the presence of alkali or alkaline-earth metals, such as Na^+, K^+, Mg^{2+}, and Ca^{2+}, and the first-row transition metals Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}, respectively. The presence of 25 equiv excess of its group 12 congeners Zn^{2+} and Cd^{2+}, respectively, in addition to Pb^{2+} and Ag^+ does not induce any obvious fluorescence response, which is probably due to several combined influences cooperating to achieve the unique selectivity for the Hg^{2+} ion, such as the suitable coordination geometry conformation of the bischelating Schiff-based receptor, the larger radius of the Hg^{2+} ion, the nitrogen-affinity character of the Hg^{2+} ion, and the amide deprotonation ability of the Hg^{2+} ion.[13] The competition experiments revealed that the Hg-induced luminescence response is unaffected in the background of 100 equiv of environmentally relevant alkali or alkaline-earth metals, such as Na^+, K^+, Mg^{2+}, and Ca^{2+}. In addition, the first-row transition-metal ions, including Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}, do not interfere with the Hg^{2+}-induced fluorescence increase, indicating that probe **1** has a remarkable selectivity for Hg^{2+}.

The absorption spectra of **1** (10 μM) in 50% (v/v) water/DMF exhibited only a very weak band above 500 nm, which was ascribed to the spirolactam form of molecule **1**. Upon the addition of 2.5 equiv of Hg^{2+}, the absorbance was significantly enhanced with a new peak appearing at 538 nm (log ϵ = 4.33; Figure 3), clearly suggesting the formation of the ring-opened amide form of **1** upon Hg^{II} binding.[14] Accordingly, the titration solution exhibited an obvious and characteristic color change from colorless to pink, indicating that probe **1** can serve as a "naked-eye" indicator for Hg^{2+} in DMF aqueous media. The nonlinear fitting of the titration curve assuming a 2:1 stoichiometry for the **1**/Hg^{2+} complex yields an association constant K_a of ca. 2.4×10^9 M^{-2} [15] (Supporting Information, Figure S8). This binding mode was also supported by the data of Job's plots evaluated from the absorption spectra of **1** and Hg^{2+} with a total concentration of 100 μM (inset in Figure 3).[16]

Solid evidence comes from the comparison between the electrospray ionization mass spectrometry (ESI-MS) spectra

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(11) (a) $Ru(2,2\text{-bpy})_3(ClO_4)_2$ was selected as the reference in spectroscopic-grade CH_3CN (Φ = 0.059) at an excitation wavelength of 450 nm. (b) Juris, A.; Balzani, V. *Coord. Chem. Rev.* **1988**, *84*, 85.

(12) The solutions of Hg^{II} were prepared using $Hg(NO_3)_2$ in distilled water. Various Hg^{II} solutions (10^{-5}, 10^{-4}, and 10^{-3} M) were prepared separately in volumetric flasks. Solutions of probe **1** were prepared in DMF/water (1:1, v/v) by serial dilution at the final concentrations of 10 and 5 μM, respectively. Aliquots of Hg^{II} were added into the 2 mL solution of probe **1** every 2 min, and the fluorescence was recorded.

(13) Wang, J. B.; Qian, X. H. *Org. Lett.* **2006**, *8*, 3721.

(14) (a) Kwon, J. Y.; Jang, Y. J.; Lee, Y. J.; Kim, K. M.; Seo, M. S.; Nam, W.; Yoon, J. *J. Am. Chem. Soc.* **2005**, *127*, 10107. (b) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386.

(15) Connors, K. A. *Binding Constants, the Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987; pp 24−28.

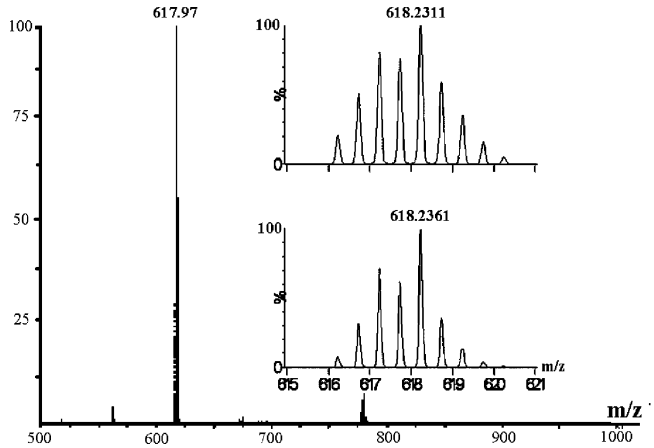

Figure 4. ESI-MS (positive) of **1** in the presence of 1.2 equiv of Hg^{2+}. Inset: Calculated (top) and observed (bottom) isotopic patterns for the $[Hg(\mathbf{1})_2]^{2+}$ cation.

of a Hg^{2+} titration solution and that of Hg-free compound **1** (Figure 4). The unique peak at *m*/*z* 617.97 corresponding to $[Hg(\mathbf{1})_2]^{2+}$ was clearly observed when 1.2 equiv of Hg^{2+} was added to **1**, whereas Hg-free probe **1** exhibited only a peak at *m*/*z* 518.3 corresponding to $[\mathbf{1} + H]^+$ (Supporting Information, Figure S10).

Compound **2** was synthesized by reacting equivalent $Hg(NO_3)_2{\cdot}0.5H_2O$ with ligand **1** in a CH_3OH/CH_3CN combined solution in a high yield of 92.5% (based on molecule **1**).[17] X-ray structural analysis[18] of compound **2** confirms the formation of a 1:2 metal/ligand complex. As shown in Figure 5, the Hg^{2+} center is tightly coordinated to two pyridylimine bidentate units from two ligands, with the average Hg−N(py) and Hg−N(imine) separations being ca. 2.20 and 2.44 Å, respectively. In addition, one nitrate anion and one carboxyl O atom very weakly coordinate to the Hg^{2+} ion, with the Hg−O separations ranging from 2.74 to 2.86 Å. The rhodamine groups were present in the form of a ring-opened amide conformation to indicate the delocalized xanthene moiety, which shows long wavelength absorption and fluorescence enhancement.

With further investigations, using a high concentration (i.e., millimolar level) of other transition-metal ions, such as Mn^{II},

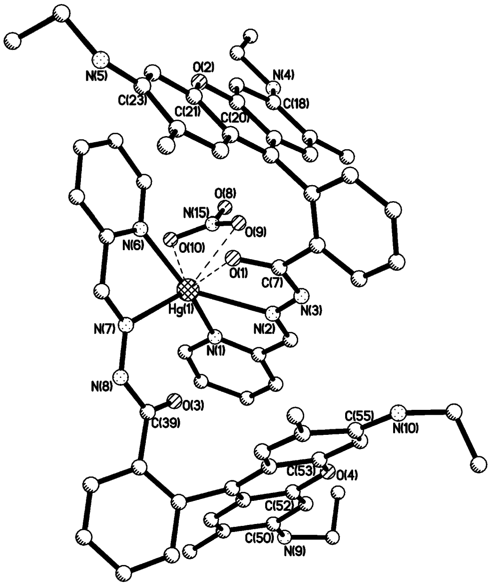

Figure 5. Molecular structure of complex **2** showing the amide conformation of the rhodamine group. H atoms, the uncoordinated nitrate anions, and the solvent molecules are omitted for clarity.

Fe^{II}, Co^{II}, and Ni^{II}, neither a color change nor fluorescent enhancement was observed, suggesting that the molecule **1** simply does not bind Mn^{II}, Fe^{II}, Co^{II}, and Ni^{II}, though the parent metal-binding functionality pyridyl benzoylhydrazone is known to bind not only Hg^{II} but also $Fe^{II/III}$, Ni^{II}, Mn^{II}, and Co^{II}. Only the addition of Cu^{2+} into the solution of probe **1** (10^{-5} mol/L), under the same conditions, can induce the enhancement of absorbance at ca. 540 nm and also show the 1:2 metal/ligand titration curve with the binding constant of 8.2×10^7 M^{-2} (Supporting Information, Figure S9). Meanwhile, in the fluorescence titration, no significant emission is observed even when the concentration of added Cu^{2+} is up to the millimolar level; furthermore, the excessive Cu^{2+} ion (50 equiv) will not compromise the Hg-induced fluorescence (2.5 equiv of Hg). Obviously, the remarkable selectivity for fluorescent detection of Hg^{II} can be available using probe **1** even in the presence of a high concentration of coexisting metal ions.

In summary, a simple and easy-to-prepare rhodamine-based Hg^{II} probe that contains the carbohydrazone unit was prepared and structurally characterized. The current probe can afford Hg^{II}-selective fluorescence enhancement and shows detection limits as low as the 2 ppb level in a DMF aqueous solution. Probe **1** may be useful for application in toxicology and environmental science.

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Supporting Information Available: Detailed experimental procedures, the acid titration control experiments, ^1H and ^{13}C NMR and UV−vis spectra, the titration curve, MALDI-TOF mass spectra of compound **1**, and X-ray crystallographic data in CIF format of crystal structure **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Vosburgh, W. C.; Cooper, G. R. *J. Am. Chem. Soc.* **1941**, *63*, 437.

(17) The Hg complex **2** is obtained as follows: $Hg(NO_3)_2{\cdot}0.5H_2O$ (0.1 mmol, 0.034 g) dissolved in 15 mL of methanol/acetonitrile (8:2, v/v) was added to a suspension of ligand **1** (0.1 mmol, 0.052 g) in 10 mL of methanol. The solution was stirred at boiling temperature for 20 min to obtain a clear red solution and allowed to stand at room temperature. The red cubic crystal suitable for single-crystal X-ray diffraction was obtained in a high yield (0.075 g, 92.5% based on ligand **1**) by evaporating the CH_3OH/CH_3CN combined solution after several days. Anal. Calcd for $Hg(C_{32}H_{30}N_5O_2)_2(NO_3)_4(CH_3CN)_2(H_2O)_3$: H, 4.49; C, 50.48; N, 13.85. Found: H, 4.52; C, 50.81; N, 13.75. ESI-MS: *m*/*z* 617.97 (calcd for $[Hg(\mathbf{1})_2]^{2+}$, 617.92).

(18) Crystal structure of compound **2**: $[(C_{32}H_{30}N_5O_2)_2Hg](NO_3)_4(CH_3CN)_2(H_2O)_3$; $M_r = 1618.02$ g mol^{-1}; triclinic; $P\bar{1}$; $a = 15.057(6)$ Å; $b = 15.887(6)$ Å; $c = 18.534(7)$ Å; $\alpha = 74.932(5)°$; $\beta = 70.676(6)°$; $\gamma = 71.248(5)°$; $V = 3903(3)$ Å^3; $Z = 2$; $\mu_{calcd} = 1.37$ g cm^{-3}; $T = 293(2)$ K. Data were collected on a Siemens SMART-CCD diffractometer using a graphite monochromator with Mo Kα radiation ($\lambda = 0.71073$ Å) using ω scans with a scan range of 1° and an exposure time of 8 s per frame. The structure was solved by direct methods and refined by full-matrix least squares using *SHELXL-97*.

(19) (a) Varnes, A. W.; Dodson, R. B.; Wehry, E. L. *J. Am. Chem. Soc.* **1972**, *94*, 946. (b) Torrado, A.; Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1998**, *120*, 609. (c) Li, Y.; Yang, C. M. *Chem. Commun.* **2003**, 2884.