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Detecting Hg^{2+} Ions with an ICT Fluorescent Sensor Molecule: Remarkable Emission Spectra Shift and Unique Selectivity

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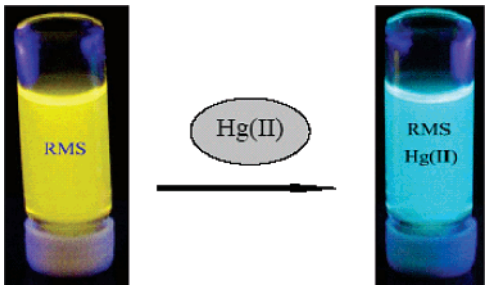

A fluorescent ratiometric Hg^{2+} ion sensor RMS, based on a coumarin platform coupled with a tetraamide receptor, is presented. This sensor, employing the ICT mechanism, could be used to specifically detect Hg^{2+} ions in a neutral buffered water solution with an ~100-nm blue shift in emission spectra.

It is appealing to selectively detect Hg^{2+} ions in water solution with a highly sensitive fluorescent sensor molecule.[1] Since the analyte, as one of the most prevalent components in the mercury family compounds (elemental, inorganic, and organic mercury), plays an important role in mercury biogeochemical cycling and mercury toxicology,[2] closer monitoring of the Hg^{2+} ions will help us to attain a thorough evaluation of the character and process of mercury distribution and transformation.[3]

Herein, we report a novel fluorescent ratiometric[4] Hg^{2+} ions sensor (RMS) that could be used to selectively detect Hg^{2+} ions in a neutral buffered water solution with a significant emission wavelength shift. We chose coumarin as the fluorophore of RMS in consideration of its desirable photophysical properties such as large Stokes shift and visible excitation and emission wavelength.[5,6] Another important qualification is the tunability of photophysical properties of the coumarin family compounds. In RMS, the fluorophore is a strong "push−pull" π-electron system, with the two aniline nitrogen atoms as the electron donor, and two electron-deficient groups, namely, one carbonyl and one benzothiazolyl group, as the electron acceptor. The fluorophore will undergo an intramolecular charge transfer (ICT)[7] from the donor to the acceptor upon excitation by light. This excited state shows a long emission wavelength because of the large extent of π-electron conjugation. However, when the tetraamide receptor[8] has caught a Hg^{2+} ion, the electron-donating ability of the 6-, 7-nitrogen will be decreased, and thus a blue shift in both absorption and emission spectra should be observed, resulting from a reduction in the π-electron conjugation. RMS and the tetraester control sensor (CS) were prepared by a series of steps starting from 4-amino-3-nitrophenol. The tetraamide Hg^{2+} ion receptor (MR) was prepared starting from *o*-phenylenediamine (Scheme 1).

UV−visible absorption spectra and emission spectra of RMS did not exhibit any detectable change under pH ranging from 3.0 to 8.0 ($\epsilon = 24\,000\ M^{-1}\ cm^{-1}$, $\phi = 0.051$).[9] When pH was further decreased, a band centered at 490 nm formed and developed. The solution color changed from yellow to orange. A pK_a value of 1.2 was derived from the pH titration curve. Red shift of the absorption spectra and the presence of an isosbestic point at 452 nm together indicated a new species corresponding to the protonation of the benzothiazole nitrogen on the coumarin fluorophore (Figure 1).[10] This interpretation was also supported by the gradual fluorescence quenching at lower pH (see Supporting Information).

The Hg^{2+} ion titration experiment revealed several intriguing features of RMS. The unbound RMS exhibited a maximum absorption at 430 nm, which gradually shifted to short wavelength with the sequential addition of Hg^{2+} ions (Figure 2), illustrating that Hg^{2+} ion was caught by the tetraamide chelating site of RMS and resulted in a reduction in the aniline nitrogens'

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(1) For recent examples, see: (a) Ros-Lis, J. V.; Marcos, M. D.; Martínez-máñez, R.; Rurack, K.; Soto, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 4405. (b) Coronado, E.; Galán-Mascaros, J. R.; Martí-Gastaldo, C.; Palomares, E.; Durrant, J. R.; Vilar, R.; Gratzel, M.; Nazeeruddin, Md. K. *J. Am. Chem. Soc.* **2005**, *127*, 12351. (c) Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272. (d) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300. (e) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270. (f) Descalzo, A. B.; Martínez-máñez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 3418.

(2) Tchounwou, P. B.; Ayensu, W. K.; Ninashvili, N.; Sutton, D. *Environ. Toxicol.* **2003**, *18*, 149.

(3) Zhang, Z.; Guo, X.; Qian, X.; Lv, Z.; Liu, F. *Kidney Int.* **2004**, *66*, 2279.

(4) Ratiometric fluorescent sensors have many inherent properties suitable for detecting analytes. See: (a) Grynkiewicz G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440. (b) Mello, J. V.; Finney, N. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 1536. (c) Choi, K.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2001**, *40*, 3912. (d) Takakusa, H.; Kikuchi, K.; Urano, Y.; Sakamoto, S.; Yamaguchi, K.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 1653.

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(6) Lim, N. C.; Schuster, J. V.; Porto, M. C.; Tanudra, M. A.; Yao, L.; Freake, H. C.; Brückner, C. *Inorg. Chem.* **2005**, *44*, 2018.

(7) (a) Valeur, B. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH: Weinheim, 2001; pp 337−350. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.

(8) Wang, J.; Qian, X. *Chem. Commun.* **2006**, 109. Recently, we have disclosed that *o*-phenylenediamine-based triamide receptor could be used to selectively bind Hg^{2+} ions in water solution.

(9) Determined relative to quinine sulfate. Meech, S. R.; Phillips, D. C. *Photochemistry* **1983**, *23*, 193.

(10) ^1H NMR studies also indicate that protonation occurs at the benzothiazole nitrogen since proton H_a (Figure 1, inset) near the ring nitrogen undergoes a significant downfield shift (0.4 ppm) upon addition of hydrochloric acid (see Supporting Information). Corrent, S.; Hahn, P.; Pohlers, G.; Connolly, T. J.; Scaiano, J. C.; Fornés, V.; García, H. *J. Phys. Chem. B* **1998**, *102*, 5852.

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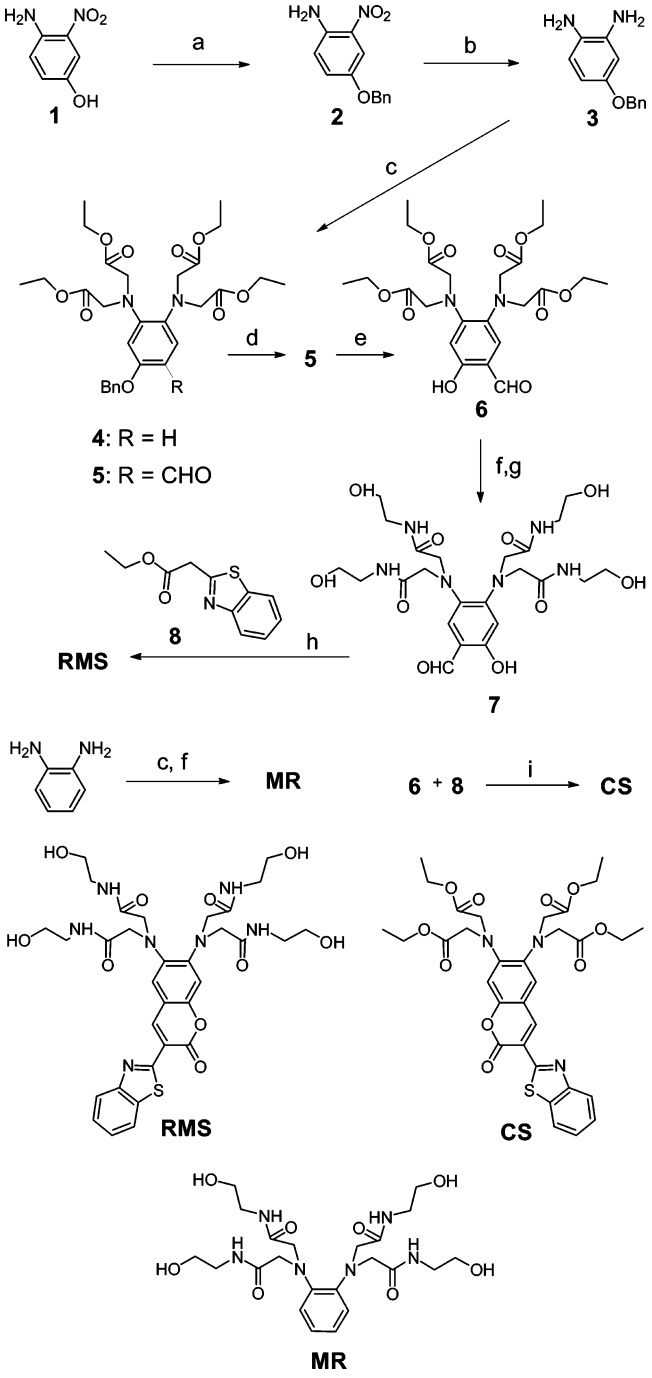
SCHEME 1. Synthesis of Compounds RMS, MR, and CS[a]



[a] Reagent: (a) benzyl bromide, NaOH, tetrabutylammonium bromide, $H_2O-CH_2Cl_2$; (b) $SnCl_2 \cdot 2H_2O$, acetonitrile ethanol; (c) ethyl bromoacetate, *N*,*N*-diisopropylethylamine, acetonitrile; (d) DMF, $POCl_3$; (e) Pd/C (10%), cyclohexene, tetrahydrofuran; (f) 2-aminoethanol, acetonitrile; (g) CF_3COOH, methanol; (h) piperidine, methanol; (i) piperidine, ethanol.

ability to participate in π-electron conjugation. The solution color changed from yellow to almost colorless in the presence of 40 equiv of Hg^{2+} ions. A clear isosbestic point at 390 nm indicated the coexistence of the free RMS and the $Hg^{2+}-$RMS complex.

A Job's plot (see Supporting Information) indicated that RMS formed a 1:1 complex with Hg^{2+} ion in water solution.[11] The association constant log K_s, derived from the titration curve, was 4.41 ± 0.02.

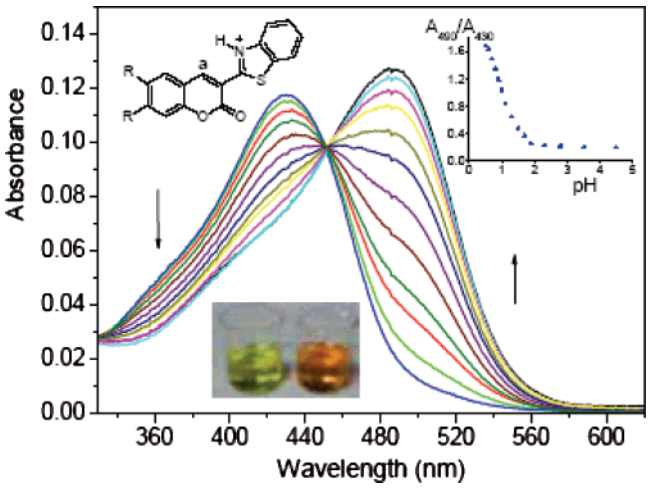


FIGURE 1. pH effect on the UV−visible absorption of RMS. Inset: structure of the protonated fluorophore, A_{490}/A_{430} as a function of solution pH, and the visualized solution color change.

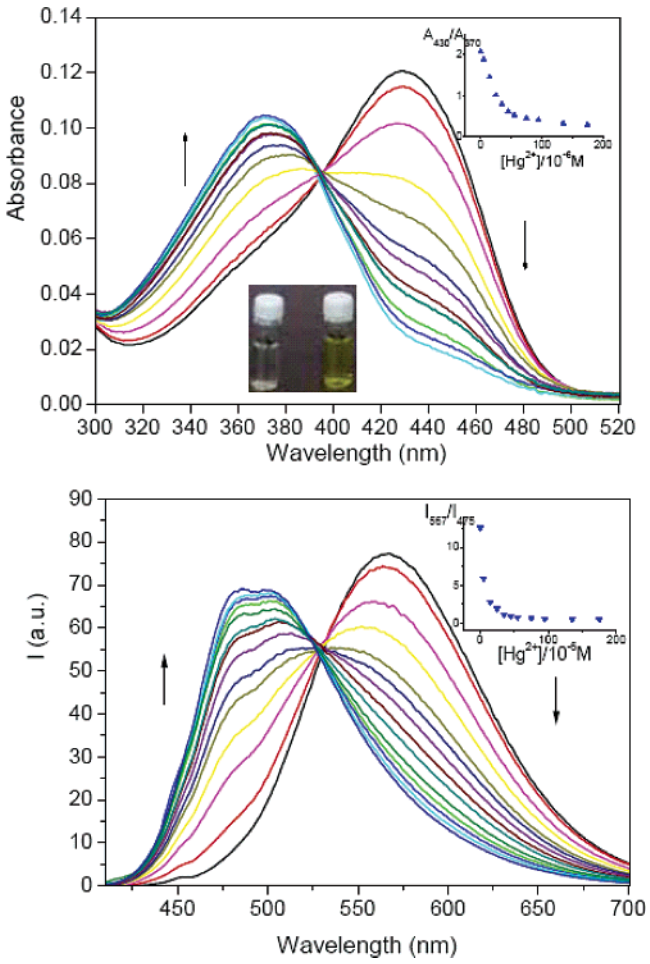


FIGURE 2. Absorption (top, inset: A_{430}/A_{370} as a function of Hg^{2+} ion concentration and the visualized solution color change) and emission (bottom, inset: I_{567}/I_{475} as a function of Hg^{2+} ion concentration, λ_{ex} = 390 nm) spectra of RMS in the presence of different concentrations of Hg^{2+} ions. Condition: 5 μM RMS in 0.05 M phosphate-buffered water solution (pH = 7.5).

When excitation was at 390 nm, emission spectra revealed a significant blue shift with the gradual increasing of Hg^{2+} ion

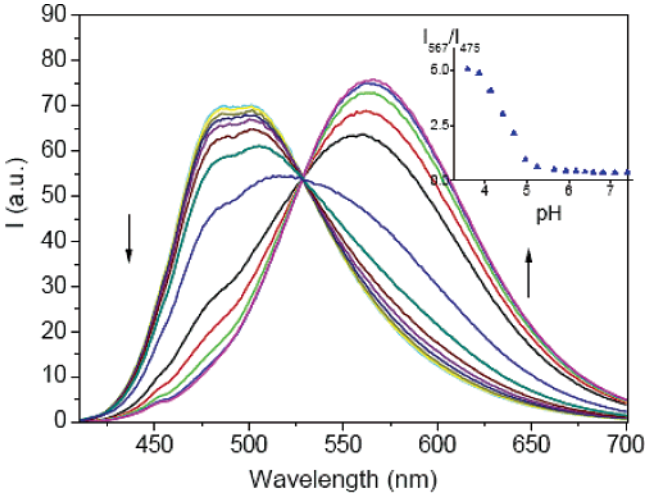


FIGURE 3. Modulating the fluorescence of RMS−Hg^{2+} complex by adjusting solution pH. Condition: 40 equiv of Hg^{2+} ions in water solution containing 5 μM RMS. pH was adjusted with $HClO_4$ and NaOH. Inset: emission intensity ratio of RMS at 567 to 475 nm as a function of solution pH.

concentration. An ~100-nm emission band shift from 567 to 475 nm was achieved in the presence of 40 equiv of Hg^{2+} ions, and the fluorescence intensity ratio I_{567}/I_{475} changed significantly from 11.9 to 0.4. The fluorescence color changed from orange to cyan. Such a remarkable fluorescence color change was rarely seen in the ever-reported ICT sensors, although a prominent absorption wavelength shift or a weaker emission wavelength shift was common. We would like to attribute such a significant emission change to the coexistence of two electron-rich aniline nitrogen atoms in the electron-donating receptor moiety, which precluded Hg^{2+} ion ejection[7,12] from them simultaneously in the excited ICT fluorophore. Another attractive feature, compared with sensor molecules whose emission decreased in the presence of analytes, was the almost constant quantum yield (0.051) of RMS at different Hg^{2+} ion concentrations.

Consistent with our early work,[8] the amide arms in RMS chelated the Hg^{2+} ion through an amide deprotonation process (Figures 3 and 6), which was hampered at lower pH. This was reflected on emission spectra by the vanishing of the peak centered at 475 nm, assigned to the RMS−Hg^{2+} complex, and the reformulation of the unbound RMS's emission at 567 nm with the decreasing of solution pH. A pK_{a1} value of 4.5 was derived, different from that (pK_a = 1.2) of the free RMS. A simultaneous absorption spectra shift was also observed (see Supporting Information).

RMS has a notable selectivity toward the Hg^{2+} ion over other heavy or transition metal ions. Only the Hg^{2+} ion can modulate the fluorescence of RMS in a neutral buffered water solution

FIGURE 4. Fluorescence response of RMS in the presence of 20 equiv of different metal ions. Conditions: 10 μM RMS in 0.05 M HEPES[13]-buffered water solution (pH = 7.5). 0, control. 1, Cd^{2+}. 2, Hg^{2+}. 3, Fe^{3+}. 4, Zn^{2+}. 5, Ag^{+}. 6, Co^{2+}. 7, Cu^{2+}. 8, Ni^{2+}. 9, Pb^{2+}. The salts employed are $Cd(NO_3)_2 \cdot 4H_2O$, $HgCl_2$, $Fe(NO_3)_3$, $Zn(NO_3)_2$, $AgClO_4 \cdot H_2O$, $CoCl_2 \cdot 6H_2O$, $Cu(ClO_4)_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, and $Pb(ClO_4)_2 \cdot 3H_2O$, respectively.

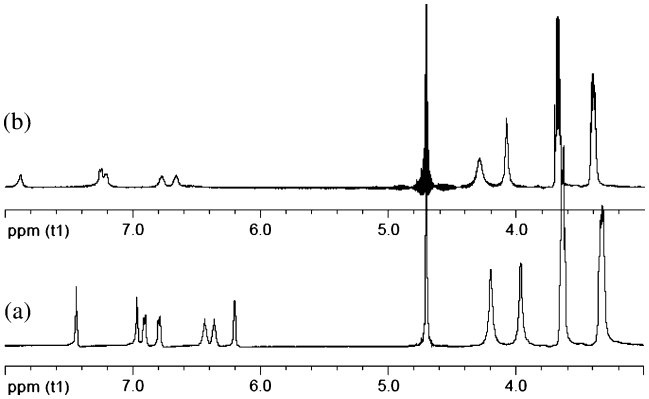


FIGURE 5. ^1H NMR spectra of free RMS (a) and RMS−Hg^{2+} complex (b) in D_2O (20 equiv of Hg^{2+} ions were added to 5 mM RMS).

(Figure 4). The presence of other background metal ions does not show any obvious disturbance with the signal response induced by RMS−Hg^{2+} complexation (see Supporting Information). Several factors might cooperate to achieve the unique selectivity of RMS, such as the structural rigidity of the *o*-phenylenediamine-based tetraamide receptor, the larger radius of the Hg^{2+} ion, and the amide deprotonation ability of the Hg^{2+} ion in neat water solution.

Polysulfur receptors have been extensively used[1e,f] in Hg^{2+}-selective sensor molecules for their well-known Hg^{2+} affinity. However, the O,S chelate formed by the thiazol-S and the coumarin-carbonyl-O could not catch a Hg^{2+} ion in the RMS sensor system. This is clearly seen from a CS in which the tetraamide receptor is replaced by a tetraester one. If the O,S chelate in the electron-withdrawing moiety caught a Hg^{2+} ion, a red shift in both absorption and emission spectra should have been observed. In fact, CS keeps silent in the presence of 40 equiv of Hg^{2+} ions in an acetonitrile−water solution (6:4, v/v). The absence of Hg^{2+}−S complexation is reasonable: (1) a sulfur atom infused into an electron-deficient aromatic benzothiazol ring possesses a lower Hg^{2+} affinity compared with that of sulfur ether; (2) to fulfill the linear S−Hg^{2+}−S or to realize the usual tetrahedral[14] Hg^{2+}−ligand coordination structure may involve an intermolecular assembly process that has a high entropic barrier.

NMR studies of RMS (Figure 5) provide independent evidence on Hg^{2+}−RMS interactions. When 20 equiv of Hg^{2+} ions are added, the peaks, assigned to the seven aromatic protons, experience clear (0.3−0.4 ppm) downfield shifts and

(11) A 1:1 Job's plot does not necessarily indicate a 1:1 binding mode: in a recently reported Pb^{2+} sensor molecule based on coumarin fluorophore, a 2:2 sensor Pb^{2+} binding chemistry was observed, where the coumarin carbonyl oxygen participated in sensor Pb^{2+} complexation. However, for RMS, the 2:2 binding mode could be easily excluded, because metal−ion binding at the positive pole of the coumarin fluorophore will result in a blue-shifted spectra, while at the negative pole, it will produce a red-shifted one; if both poles participate in complexation, the opposite effects will counterbalance each other and result in a moderate spectra shift, instead of the large one (60 nm) detected for the RMS−Hg^{2+} complex. Chen, C.-T.; Huang, W.-P. *J. Am. Chem. Soc.* **2002**, *124*, 6246.

(12) If metal ion ejection takes place on an excited ICT sensor, no clear emission spectra change could be observed.

(13) We used HEPES instead of phosphate here because some metal ions formed precipitate in phosphate-buffered water solution.

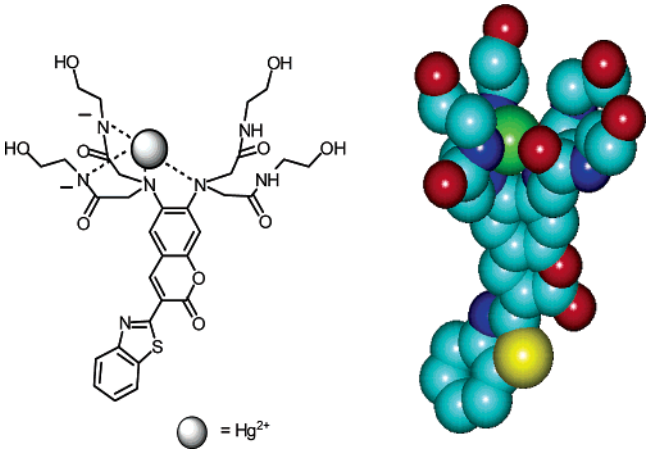


FIGURE 6. Proposed RMS−Hg^{2+} complex structure and the energy-minimized conformation by using Hyperchem software with the molecular mechanics subroutine.[17]

get broadened. This could be attributed to a deshielding effect, arising from the decrease of the electron density in the coumarin fluorophore caused by N−Hg^{2+} (N, 6-, 7-nitrogen) complexation, while the aliphatic protons, around the amide groups, display only small downfield shifts. This could be attributed to the shielding effect, arising from the negatively charged amide groups (introduced by $^{-}$N−Hg^{2+} complexation, $^{-}$N-deprotonated amide nitrogen), which, to some extent, counterbalances the deshielding effect. In addition, ligand exchanges between the four amide arms, on the NMR time scale, might occur since only one set of aliphatic proton signals is observed. ^{1}H NMR spectra of the tetraamide receptor MR, in the presence of different concentrations of Hg^{2+} ions, are also recorded (see Supporting Information). Despite similar spectral shifting trends, MR exhibits a higher Hg^{2+} ion binding strength[15] since 2 equiv of Hg^{2+} ions could almost bring about the maximum spectra changes. This is reasonable in consideration of the presence of two electron-deficient substituent groups in RMS, which decreases the electron density on the 6-, 7-nitrogen and results in a weaker N−Hg^{2+} binding strength.

Accordingly, on the basis of the evidence mentioned above, Figure 6 presents a proposed Hg^{2+}−RMS complexation structure, in which two deprotonated amide groups cooperate with the two *o*-phenylenediamine nitrogen atoms to form a tetrahedral ligand atmosphere for a Hg^{2+} ion. The other two unbound amide arms may exert steric effects, which restrict the free rotation of the amide arms and favor the Hg^{2+}−RMS complexation.

In summary, we have developed a ratiometric ICT fluorescent Hg^{2+} ion sensor RMS by incorporating an *o*-phenylenediamine-derived tetraamide receptor into a coumarin platform. RMS has several desirable sensor properties such as remarkable emission wavelength shift, absolute selectivity, and almost constant quantum yield for the detecting of Hg^{2+} ions in a neutral buffered water solution. Unfortunately, RMS−Hg^{2+} binding strength, with a K_s value in the range of 10^4 M^{-1}, is weak, indicating that RMS is only effective at high Hg^{2+} ion concentrations (in the present condition, Hg^{2+} ions could be detected down to the micromolar range; clear emission spectra shift was observed when Hg^{2+} ion concentration was down to 5 μM). Nonetheless, this work undoubtedly has paved the way toward a highly sensitive ratiometric Hg^{2+} ion sensor molecule. It is envisioned that sensor−Hg^{2+} binding strength will be enhanced if the tetraamide receptor is incorporated into a "push−pull" π-electron system, where the electron deficiency in the electron-acceptor moiety, compared with that of RMS, is reduced to some extent.

Experimental Section

RMS. 7 (150 mg) and **8** (90 mg, 0.408 mmol)[16] were dissolved in 15 mL of dry methanol containing five drops of piperidine. The mixture was refluxed under nitrogen for 2.5 h. Then the solution was cooled and concentrated under vacuum. The residue was purified by flash chromatography using methanol−dichloromethane (20:100, v/v) as eluant, affording 33 mg (0.046 mmol, 17%) of RMS as a yellow solid. Mp: 195−197 °C; IR (KBr): 3368, 2937, 1655, 1615, 1549, 1056 cm^{-1}. ^{1}H NMR (500 MHz, D_2O): δ 7.44 (s, 1 H), 6.97 (s, 1H), 6.90 (d, J = 6.9 Hz, 3 H), 6.79 (d, J = 7.2 Hz, 1 H), 6.44 (t, J = 7.1 Hz, 1H), 6.36 (t, J = 7.1 Hz, 1H), 6.20 (s, 1 H), 4.20 (s, 4 H), 3.96 (s, 4 H), 3.62−3.66 (m, 8 H), 3.31−3.35 (m, 8 H); ^{13}C NMR (500 MHz, D_2O) δ 172.7, 172.6, 160.4, 150.9, 150.6, 149.2, 141.1, 139.0, 135.2, 126.5, 125.2, 122.9, 121.2, 113.9, 113.1, 107.1, 60.8, 55.9, 55.7, 42.3, 29.7, 14.2; HRMS (ES+) Calcd for $([M + Na])^+$, 736.2377; Found, 736.2373.

CS was similarly prepared from RMS (94%) as a yellow solid. Note: in this reaction, we used *ethanol* as the solvent instead of *methanol*. Mp: 186−188 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.98 (s, 1 H), 8.06 (d, J = 8.2 Hz, 1 H), 7.95 (d, J = 7.9 Hz, 1 H), 7.51 (t, J = 8.1 Hz, 1 H), 7.38−7.40 (overlapped, 2 H), 6.99 (s, 1H), 4.46 (s, 4 H), 4.24 (s, 4 H), 4.10−4.19 (m, 8 H), 1.21−1.26 (m, 12 H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 170.2, 170.1, 160.7, 160.3, 152.5, 151.5, 148.2, 141.5, 138.6, 136.6, 126.3, 125.1, 122.7, 121.9, 121.7, 117.3, 113.7, 108.0, 61.1, 60.9, 52.7, 29.7, 14.2; MS (ES+) Calcd for $([M + H])^+$, 654; Found, 654.

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Note Added after ASAP Publication. The emission intensity ratio in Figure 3 was shown as 567 to 485 nm, instead of 475 nm, in the version published ASAP April 29, 2006; the corrected version was published May 4, 2006.

Supporting Information Available: Synthesis and characterization of compounds **2**−**7**, MR, NMR spectra, and spectrascopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) (a) Su, C.-Y.; Goforth, A. M.; Smith, M. D.; Loye, H.-C. Z. *Inorg. Chem.* **2003**, *42*, 5685. (b) Li, G.; Song, Y.; Hou, H.; Li, L.; Fan, Y.; Zhu, Y.; Meng, X.; Mi, L. *Inorg. Chem.* **2003**, *42*, 913.

(15) The difference in the Hg^{2+} ion-binding stoichiometry also occurs between MR and RMS. Job's plot using ^{1}H NMR data indicates that MR binds Hg^{2+} ion with a 1:2 stoichiometry (see Supporting Information). While it is difficult to determine the high association constant between MR and the Hg^{2+} ion using NMR techniques, a photoinduced electron transfer (PET) fluorescent sensor molecule, based on BODIPY (boron dipyrromethene) fluorophore and the tetraamide receptor MR, shows that the association constant of the 1:2 MR−Hg^{2+} complexation is larger than 10^{10} M^{-2}. This result will be published soon in a separate paper.

(16) Compound **8** was synthesized following the literature method. Abbotto, A.; Bradamante, S.; Facchetti, A.; Pagani, G. A. *J. Org. Chem.* **2002**, *67*, 5753.

(17) Detailed information on the calculation methods and results is provided in Supporting Information.