Rhodamine B thiolactone: a simple chemosensor for Hg^{2+} in aqueous media†

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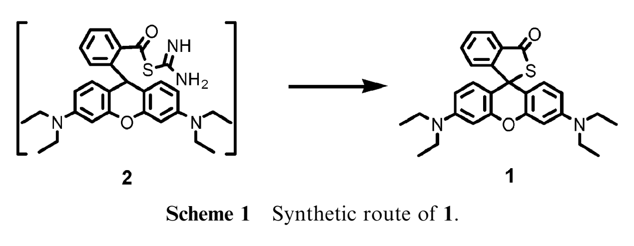
Rhodamine B thiolactone is developed as a simple chemosensor towards Hg^{2+} in neutral aqueous solution with high selectivity.

Mercury contamination has been receiving much attention.[1] Various human activities, such as gold mining and the combustion of solid waste, have caused the fast increase of environmental Hg^{2+} level,[2] and thus the formation of different toxic species such as methylmercury. These poisonous species, which can easily enter the food chain and accumulate to higher concentration, are found to cause some irreversible neurological damages.[3] Therefore, a convenient and rapid method for the analysis of Hg^{2+} is highly desirable. Towards this end, significant contributions to the development of spectroscopic sensing molecules for Hg^{2+} have been made in recent years.[4–8] Unfortunately, most of the reported chemosensors have some disadvantages, such as poor aqueous solubility,[5] cross-sensitivities towards other metal ions,[6] strict reaction condition[7] or complicated synthetic route.[8] Herein, we report rhodamine B thiolactone **1** (Scheme 1) as a simple and novel chemosensor for Hg^{2+}, which can give a highly selective and rapid spectroscopic response to Hg^{2+} in aqueous media.

The design of compound **1** takes advantage of both the high thiophilicity of Hg^{2+} and strong nucleophilicity of sulfur. First, the high thiophilicity of Hg^{2+} has been observed in desulfurization reactions of a variety of compounds such as thioureas, thioethers or thioesters.[9] This property is expected to provide the high selectivity of the chemosensor for Hg^{2+}. Secondly, as shown in Scheme 1, the strong nucleophilicity of sulfur would benefit not only the condensation of thiol with acyl chloride but also the linking of the sulfur atom to the electron-deficient benzyl carbon, leading to the cyclization. Thiourea, usually existing in two forms in solution (Scheme 1), was chosen as a thiol source.[10] Consequently, compound **1** can be readily prepared *via* a possible intermediate **2** from rhodamine B and thiourea in 45% overall yield by two steps (a, phosphorus oxychloride; b, thiourea, triethylamine (TEA)). The structure of **1** was confirmed by ^1H NMR, ^{13}C NMR, MS and X-ray analyses (ESI†). Single crystals of **1** which can be grown as light yellow needles from ethyl acetate/petroleum ether (1/25, v/v) solution, have a unique thiospirocyclic structure (Fig. 1).‡ The two aromatic moieties of the rhodamine framework form vertical planes, which breaks the conjugation of the whole system, thus leading to the non-fluorescence of the molecule.

Although a few chemosensors reported for Hg^{2+} have utilized the spirocyclic structure, all these compounds are spirolactam derivatives and contain an additional benzene ring, which unavoidably decreases the solubility in water.[11] In contrast, **1** is the first chemosensor that possesses a thiospirolactone structure feature, which is relatively a smaller molecule without any unwanted aromatic moiety. This would be favorable to improving solubility. As a result, the chemosensor **1** can be used in aqueous media with no additional cosolvent except the introduction of 0.5% (v/v) of 1,4-dioxane from the stock solution.

Fig. 2 shows the spectroscopic properties of **1** at varied concentrations of Hg^{2+}. As expected, **1** itself displays a colorless solution and emits no fluorescence. This solution remains stable in a wide pH range of 3 to 11 (Fig. S3, ESI†). Addition of Hg^{2+}, however, immediately produced a pink color with an absorption maximum at 560 nm ($\varepsilon = 7.74 \times 10^4\ M^{-1}\ cm^{-1}$) and a strong yellow fluorescence at about 585 nm, which closely resemble the spectroscopic features of rhodamine B. Time course studies reveal that the color reaction is nearly complete in 1 min (Fig. S4, ESI†), and the resulting absorbance and fluorescence signals remain constant for at least 24 h. A change in pH from 5 to 8 causes negligible changes in fluorescence intensity (Fig. S5, ESI†), suggesting that the chemosensor is of potential use in biological systems. Also, UV-Vis absorption and fluorescence titration spectra of **1** with Hg^{2+} from 0 to 100 μM show that these spectroscopic



Scheme 1 Synthetic route of **1**.

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† Electronic supplementary information (ESI) available: Apparatus and reagents, synthesis of **1**, general procedure for Hg^{2+} detection, optimization of experimental conditions, MS evidence for the reaction mechanism. CCDC 667970. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b717718f

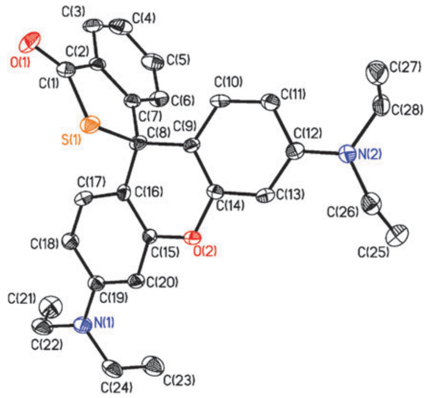


Fig. 1 View of the structure of **1** with displacement atomic ellipsoids drawn at the 20% probability level, excluding H atoms.

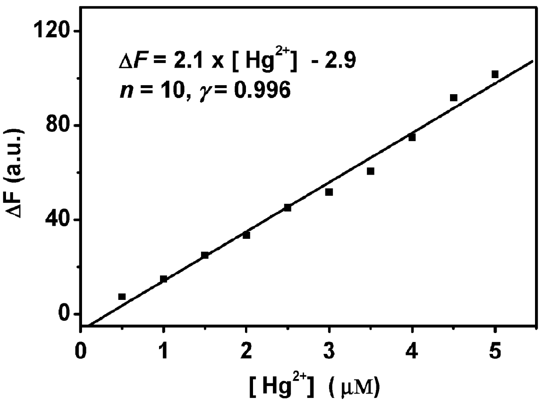


Fig. 3 The plot of fluorescence intensity change of **1** (5 μM) against varied concentration of Hg^{2+} from 0.5 to 5 μM at $\lambda_{ex/em}$ = 530/585 nm.

responses increase with increasing Hg^{2+} concentration, and with the usage of 5 μM of **1** a good linearity is observed in the fluorescence intensity for a concentration range of 0.5–5 μM Hg^{2+} (Fig. 3). The detection limit is 20 nM ($S/N = 3$). Interestingly, a tiny negative deviation from the linearity is repeatedly produced just above 0.5 equiv. of Hg^{2+} (Fig. 3), and the change from 0.5 to 1 equiv. of Hg^{2+} causes a slightly red-shifted fluorescence from 575 to 585 nm (Fig. 2(B)). The reason for this may be rather complicated, possibly resulting from the formation of different fluorescent species (*vide infra*; Scheme 2).

To assess the specificity of the reaction, various ions (including heavy metal ions that not only coexist but also often have a similar reactivity[12]) were examined in parallel under the same condition. As shown in Fig. 4, the reaction of **1** with Hg^{2+} produces both strong absorbance and fluorescence responses; whereas the other ions do not show this behavior, except that Ag^{+} has a small response (error $<5\%$). Therefore, it can be concluded that **1** displays an extremely high selectivity for Hg^{2+} only, instead of the other ions examined. This unique property enables Hg^{2+} to be detected by the naked eye directly even in the presence of the other species (Fig. 5).

In order to explore the reaction mechanism of the present system, the reaction products of **1** with Hg^{2+} were subjected to electrospray ionization mass spectral analyses. Three major ion peaks were detected at m/z 443.4, 559.1 and 695.4, respectively (Fig. S6 and S7, ESI†). The peak at m/z 443.4 $[M + H]^{+}$ was characterized to be rhodamine B, indicating the generation of rhodamine B as a final product. Attempts were made to separate the other two products responsible for m/z 559.1 and 695.4 but were unsuccessful. However, the signal at m/z 559.1 was proven to be a doubly charged ion peak by HRMS, because the mass difference ($\Delta m/z$) between the neighboring isotopic peaks was 0.5 (Fig. S8, ESI†). On the other hand, the introduction of KI[13] into the system can reverse the color reaction only in the presence of less than 0.5 equiv. of Hg^{2+}.§ Based on the above findings, we propose that the reaction in this system may proceed through the route depicted in Scheme 2: the high thiophilicity of Hg^{2+} leads to the formation of two kinds of complexes **3** and **4**, which produce the MS signals at m/z 559.1 and 695.4, respectively; complex **3** is relatively stable in the solution, but the complex **4** can be further degraded to rhodamine B (Fig. S6 and S7, ESI†).

In summary, the first thiospirolactone derivative of rhodamine B has been characterized as a simple chemosensor for Hg^{2+}. The chemosensor exhibits a highly selective

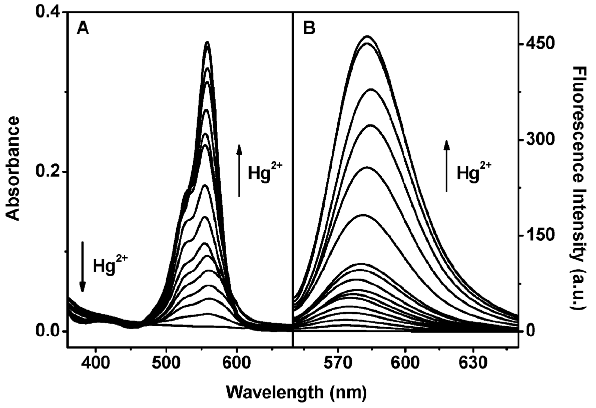
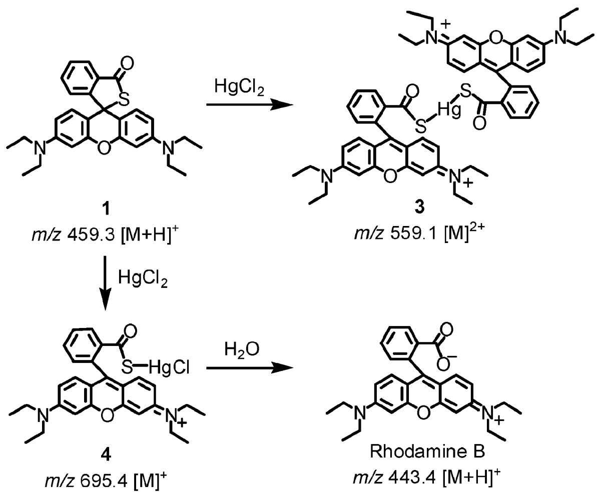


Fig. 2 (A) UV-Vis absorption and (B) fluorescence (λ_{ex} = 530 nm) titration spectra of **1** (5 μM) with Hg^{2+} from 0 to 100 μM in 20 mM Na_2HPO_4–NaH_2PO_4 buffer (pH 7) containing 0.5% (v/v) of 1,4-dioxane.



Scheme 2 Possible reaction mechanism of **1** with $HgCl_2$.

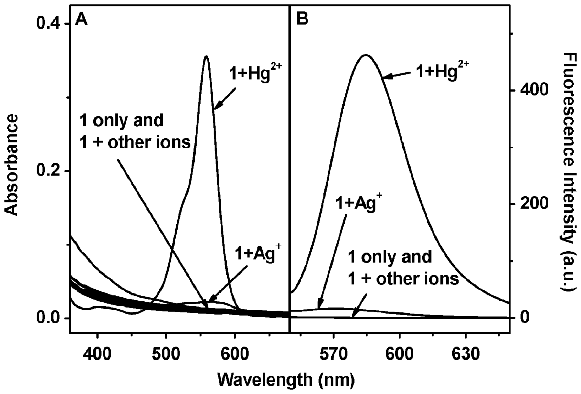


Fig. 4 (A) UV-Vis absorption and (B) fluorescence spectra (λ_{ex} = 530 nm) of **1** (5 μM) in 20 mM Na_2HPO_4–NaH_2PO_4 buffer (pH 7) containing 0.5% (v/v) of 1,4-dioxane with 50 μM metal ion (Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+} or Hg^{2+}).

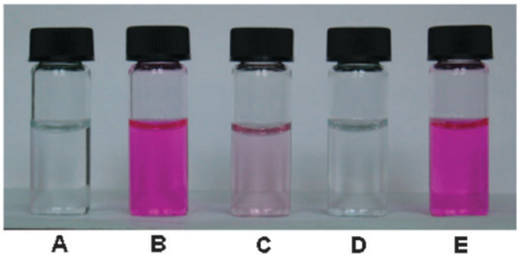


Fig. 5 A photograph of color reactions of **1** (5 μM) with various species. A, **1** only; B, **1** + Hg^{2+} (50 μM); C, **1** + Ag^+ (50 μM); D, **1** + other mixed ions (50 μM of Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, Pb^{2+} and Zn^{2+}); E, the sample D plus Hg^{2+} (50 μM).

spectroscopic response to Hg^{2+} in neutral aqueous media, which makes it of great potential use in the direct detection of Hg^{2+} in the presence of the other species in biosystems.

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Notes and references

‡ *Crystal data* for **1**: $C_{28}H_{30}N_2O_2S$, M = 458.60, orthorhombic, space group $Pna2_1$, a = 16.441(3), b = 12.319(3), c = 12.173(2) Å, V = 2465.4(9) Å^3, T = 294(2) K, Z = 4, D_c = 1.236 g cm^{-3}, 5295 reflections measured, $R1$ [$I > \sigma(I)$] = 0.0468, $wR2$ (all data) = 0.1401, GOF = 0.782.

§ *Investigation of the reversibility of the present system by KI*: When 5 μM of **1** was exposed to no more than 0.5 equiv. of Hg^{2+}, the resulting pink color can fade to colorless by treating with KI (4 equiv. of Hg^{2+}). This is ascribed to the formation of HgI_4^{2-} (the anion at low concentration has no absorption in the visible region) and the decomplexation of complex **3** to release the colorless free **1**. When Hg^{2+} was added to the system again, the pink color can be reproduced. However, when 5 μM of **1** was exposed to more than 0.5 equiv. of Hg^{2+}, the introduction of even excess KI (more than 4 equiv. of Hg^{2+}) can not restore the solution to be colorless, which may be ascribed to the formation of irreversible products **4** and rhodamine B.

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