Rhodamine thiospirolactone. Highly selective and sensitive reversible sensing of Hg(II)†

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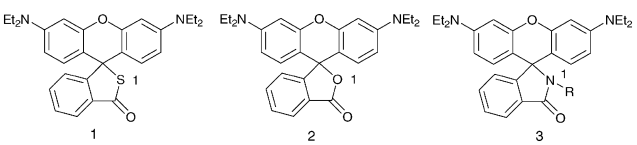
A novel rhodamine thiospirolactone chemosensor 1 was found to develop prominent absorbance and fluorescence enhancements in the presence of Hg^{2+} in aqueous solution and this was suggested to result from the thiospiro ring opening induced by Hg^{2+} binding.

The design and synthesis of chemosensors for environmentally and biologically relevant species in aqueous solutions is currently of great interest.[1] In this regard, chemosensors that can highly sensitively and selectively monitor heavy metal ions such as Hg^{2+}, Cu^{2+} and Pb^{2+} are especially important.[2] Hg^{2+}, widely distributed in the environment due to human activities, is considered to be toxic in biological activities. Highly selective and sensitive chemosensors for Hg^{2+} are hence demanded. Examples of chromogenic and fluorogenic chemosensors have been reported,[3] many of them, however, have limitations in terms of heavy synthetic efforts and/or lack of practical applicability in aqueous solutions.

Rhodamines are dyes extensively employed[4] in bio-labeling and material sciences because of their high absorption coefficient, high fluorescence quantum yield and long-wavelength absorption and emission. Among the rhodamines with spirocyclic form (for example, **2** and **3** in Scheme 1), rhodamine lactone **2**, *i.e.* rhodamine B base, is generally accepted to be in its colored and fluorescent zwitterion form (rhodamine B) in protic solvents.[5] Rhodamine spirolactam **3** has recently received increasing attention in designing chemosensors for metal ions. This was realized *via* a metal-induced structural change from colorless and nonfluorescent spirocyclic form to colored and fluorescent open form. Several excellent chemosensors of this kind have been reported[6] for transition metal ions such as Hg^{2+}, Cu^{2+}, Pb^{2+}, and Fe^{3+}. As for sensing Hg^{2+}, an irreversible rhodamine chemosensor was reported by Tae[6e,f] employing thiosemicarbazide as a binding site. Thiohydrazide,[6g] carbohydrazone,[6h] phenylthioureaethylenediamine[6i] and tren[6j] were also reported to serve as Hg^{2+} binding sites. All those aforementioned examples of rhodamine spirolactam chemosensor showed that, compared with its counterpart rhodamine spirolactone, rhodamine spirolactam remained its spirocyclic form in protic solvents and, therefore, provided the feasibility for constructing chemosensors applicable in aqueous solutions. This character of rhodamine spirolactam can be explained by the fact that the amine group of rhodamine spirolactam is more nucleophilic than the hydroxy group of rhodamine spirolactone, which favors a spirocyclic form strongly. Thus, by regulating the nucleophilicity of the functional group in the 1-position, the stability of the spirocyclic structure can be improved. It was noted that these spirolactams, however, might undergo ring opening reaction under acidic condition even in the absence of a metal ion.[6c,i]

It was hence wondered what will result in if the lactone O atom in the rhodamine base is replaced by an S atom. Enlightened by the facts that Hg(II) is a sulfurphilic ion[7] and that the thiol group is more nucleophilic than the hydroxy group and even than the amine group, we thus synthesised **1**, bearing a monothiospirolactone group in rhodamine architecture. It was observed that **1** showed extremely good selectivity and high sensitivity toward Hg^{2+} in aqueous solutions. Compared with its counterpart rhodamine lactone and lactam, compound **1** revealed three advantages: first, the probe showed high tolerance to pH, existing in a spirocyclic form within a pH range of 1–11; secondly, the thiol atom served not only as an enhancer for cyclization but also as a center for the direct attack of thiophilic Hg^{2+}, thus attaining a high molecular sensitivity; finally, the probe could be easily synthesized by a one step or "one pot" reaction.

Compound **1** was facilely synthesized from commercially available rhodamine B base **2** by a one-step reaction or "one pot synthesis" with moderate to good yield (Scheme 2).‡ **1** was characterized by X-ray crystallography, ^1H and ^{13}C NMR and MS (Fig. S1–3 in ESI†). Single crystals of **1** grown from CH_2Cl_2–CH_3CN were suitable for X-ray crystallography, and the determined crystal structure (Fig. 1) confirmed the unique spirothiolactone structure.§



Scheme 1 Chemical structures of rhodamine B derivatives.

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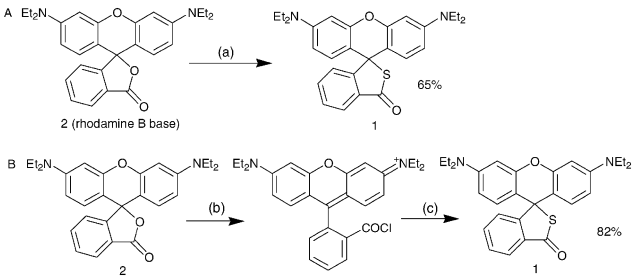
† Electronic supplementary information (ESI) available: ^1H, ^{13}C NMR and ESI-MS spectra of **1**, absorption and fluorescence responses of **1** at different pH, Job plots of the complexation. CCDC reference number 671476. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b719473k



Scheme 2 *Synthesis* of **1**. (a) 1 eq. Lawesson's regent, benzene, reflux, N_2, 4 h; (b) $ClCH_2CH_2Cl$, $POCl_3$, reflux, 4 h; (c) excess Na_2S saturated aqueous solution.

Aqueous solutions of **1** within pH 1–11 were found to be colorless and nonfluorescent at visible range of wavelength >400 nm (Fig. S4–5 in ESI†). Those observations suggest that **1**, differing from their lactone and lactam counterparts, is stable not only in acidic but alkaline conditions as well. Addition of Hg^{2+} into a solution of **1** in H_2O–CH_3CN (99 : 1, v/v) immediately resulted in a significant enhancement of absorbance in the visible range of 500–650 nm at room temperature. This suggests the delocalization of the xanthene moiety of rhodamine as a result of Hg^{2+} binding at the thiospirolactone moiety. Fig. 2(a) shows the absorption spectra of **1** in the presence of Hg^{2+}. With increasing Hg^{2+} concentration, a new peak at 559 nm was observed together with a shoulder at 538 nm. These variations are characteristic of rhodamine dyes. The absorbance of **1** at 559 nm was proportional to Hg^{2+} concentration over the range of 10 nM–4.5 μM (Fig. 2(b)), with a detection limit of 2.1×10^{-9} M. This dramatic change of color in the presence of Hg^{2+} suggests that **1** would be a practical 'naked-eye' chemosensor of Hg^{2+} in aqueous solutions.

The nice nonlinear fitting of the absorbance of **1** against Hg^{2+} concentration assumed a 1 : 2 binding ratio, suggesting a 1 : 2 binding stoichiometry (Fig. 2(b)), which was further supported by Job plots[8] (Fig. S6, ESI†). The binding constant (K_a) of the complex was calculated to be 9.8×10^{13} M^{-2}. Solid evidence of the binding mode comes from comparing the ESI-MS spectra of both the free probe **1** and the complex of probe

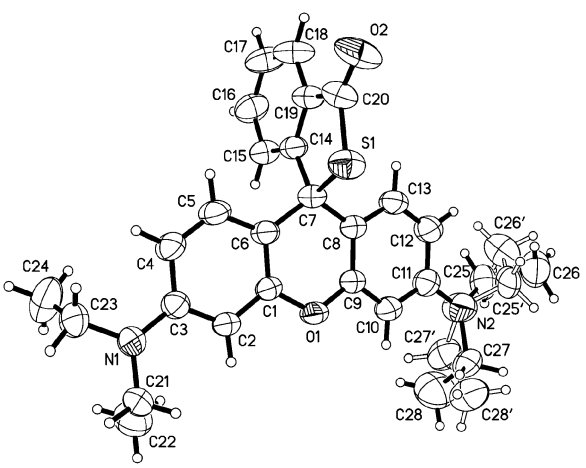


Fig. 1 Crystal structure of **1** shown at 50% probability. All hydrogen atoms are omitted for clarity.

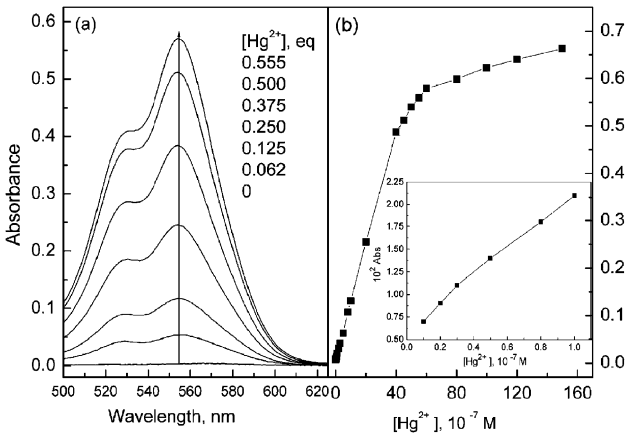


Fig. 2 (a) Changes in the absorption spectra of **1** in 99 : 1 H_2O–CH_3CN solutions at pH 4.0 (in a 10 mM NaOAc–HOAc buffer) upon addition of different amounts of Hg^{2+}. (b) Absorbance of **1** (10 μM) at 559 nm *vs.* Hg^{2+} concentration under the same condition. Inset: enlarged region of (1–10) $\times$ 10^{-8} M of Hg^{2+}.

1 with Hg^{2+}. The peak at *m*/*z* 558.7 corresponding to $[Hg(\mathbf{1})_2]^{2+}$ (Fig. S7, ESI†) was clearly observed when 1.0 eq. of Hg^{2+} was added to **1**, whereas free probe **1** exhibited only a peak at *m*/*z* 459.2 corresponding to $[\mathbf{1} + H]^+$. The chromogenic behavior of **1** toward Hg^{2+} and related heavy transition metal ions was investigated. As shown in Fig. 3 and Fig. S8, ESI,† the absorption response of **1** displays an excellent selectivity to Hg^{2+} over all the other tested ions. Competitive experiments also showed high tolerance of the assay system toward foreign transition ions (Fig. S9, ESI†).

Furthermore, the presence of Hg^{2+} also induced significant enhancement of the fluorescence intensity of **1** at 585 nm with excitation at 530 nm (Fig. 4(a)). The response of **1** toward all the tested metal ions was also examined and the assays revealed that the enhancement in the fluorescence emission took place only in the case of Hg^{2+} (Fig. 4(b)), indicating high selective sensing towards Hg^{2+}.

It is important to indicate that the spectral sensing is reversible.[6g,9] The fluorescence and color of the **1**–Hg^{2+} complex disappeared immediately upon addition of KI (4 eq. to Hg^{2+}) and was restored after the treatment with excess amounts of Hg^{2+}. This regeneration capability makes the current thiospirolactone based chemosensor, **1**, much more practical.[3e] Based on the description above, the binding mode between probe **1** and Hg^{2+} is proposed in Scheme 3.

In conclusion, a new thiospirolactone based rhodamine-B base chemosensor for Hg^{2+} has been developed, which exhibited prominent absorption and fluorescence enhancements to Hg^{2+} with a particular selectivity and excellent sensitivity and could be used for naked-eye detection in aqueous

Fig. 3 Color changes observed for **1** (10 μM) in 99 : 1 H_2O–CH_3CN solutions at pH 4.0 (in a 10 mM NaOAc–HOAc buffer) upon addition of 5 μM metal ions. From left to right: blank, Ba^{2+}, Cr^{3+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Hg^{2+}, Ag^+, Fe^{3+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Mn^{2+}.

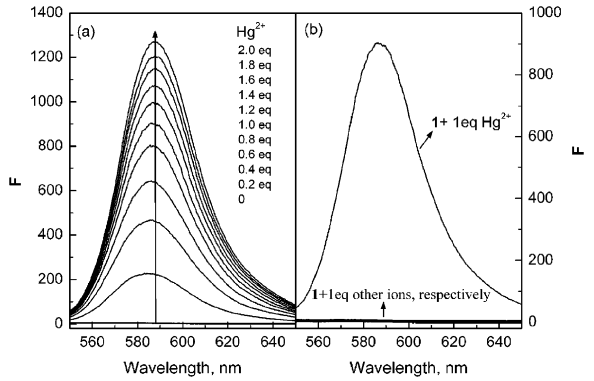
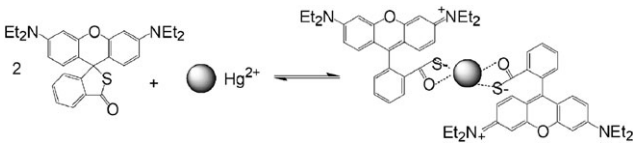


Fig. 4 (a) Fluorescence spectra of **1** (10 μM) in 99 : 1 (v/v) H_2O–MeCN solutions at pH 4.0 (in a 10 mM NaOAc–OHAc buffer) in the presence of different amounts of Hg^{2+}. (b) Fluorescence spectra of **1** in the presence of 1 eq. different metal ions under the same condition. Other ions: Ca^{2+}, Mg^{2+}, Ba^{2+}, Cr^{3+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Ag^{+}, Fe^{3+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Mn^{2+}. λ_{ex} = 530 nm. Slit: 5 nm/5 nm.



Scheme 3 The proposed binding mode between **1** and Hg^{2+}.

solutions. This chemosensor was easily prepared and found to be stable in both alkaline and acidic solutions. The spectral response toward Hg^{2+} was established to be reversible. All the aforementioned characteristics of **1** implies that **1** has a potential to be applied to an optical fiber chemical sensor. The thiolactone framework was also concluded to be a promising structural element for Hg^{2+}-selective chemosensor. Our following work will focus on using thiolactone as a recognition unit for designing other chemosensors.

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Notes and references

‡ Rhodamine B base (1.00 g, 2.26 mmol) and Lawesson's reagent (0.92 g, 2.26 mmol) were dissolved in dry benzene, and the reaction mixture was refluxed for 4 h under N_2 atmosphere. After removal of benzene, the residue was purified by flash chromatography with CH_2Cl_2–petroleum = 1 : 1 as the eluent to afford compound **1** as a pale yellow solid in 65% yield. Compound **1** can also be prepared by a "one pot synthesis" as follows: A solution of rhodanine B base (0.5 g, 1.3 mmol) in 1,2-dichloromethane (8 mL) was stirred at room temperature, then phosphorus oxychloride (0.4 mL) was added dropwise over a period of 5 min and the mixed solution was refluxed for 4 h. After cooling, the reaction mixture was concentrated under vacuum to obtain crude rhodanine B acid chloride. 3 mL of saturated Na_2S aqueous solution (25 mmol) was then added to the crude rhodanine B acid chloride and the mixture was stirred for 3 h at room temperature. By extraction with MeCOOEt, the extraction product was purified by flash chromatography with CH_2Cl_2–petroleum (1 : 1) as the eluent to afford the pale yellow solid **1** in 82% yield (0.41 g). ^{1}H NMR ($CDCl_3$) δ 1.147 (t, J = 7.2 Hz, 12H, NCH_2CH_3), 3.316 (q, J = 7.2 Hz, 8H, NCH_2CH_3), 6.292 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H, xanthene H), 6.337 (d, J = 2.4 Hz, 2H, xanthene H), 6.703 (d, J = 9.6 Hz, 2H, xanthene H), 7.196 (d, J = 8 Hz, 1H, ArH), 7.433 (dt, J_1 = 7.2 Hz, J_2 = 0.4 Hz, 1H, ArH), 7.505 (dt, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H, ArH), 8.848 (d, J = 7.2 Hz, 1H, ArH). ^{13}C NMR ($CDCl_3$) δ 12.53, 44.27, 62.69, 97.46, 108.13, 108.41, 122.43, 127.18, 128.06, 129.71, 134.13, 135.48, 148.34, 152.21, 157.86, 197.65. ESI mass spectrometry, m/z 459.3 $(M + 1)^+$.

§ *Crystal data* for **1**: $C_{28}H_{30}N_2O_2S$, M = 458.60, monoclinic, space group $P2_1/n$, a = 10.9203(5), b = 15.6081(7), c = 14.5574(7) Å, β = 97.6870(10), V = 2458.9(2) Å^3, T = 297(2) K, Z = 4, D_c = 1.239 g cm^{-3}, 5934 reflections measured, $R1$ [$I > 2\sigma(I)$] = 0.0528, $wR2$ (all data) = 0.1562.

1 For recent examples, see: M. M. Baruah, W. W. Qin and N. Boens, *Org. Lett.*, 2005, **7**, 4377; Y. K. Yang and J. S. Tae, *Org. Lett.*, 2006, **8**, 5721; A. Buryak and K. Severin, *J. Am. Chem. Soc.*, 2005, **127**, 3700; S. L. Wang and Y. T. Chang, *J. Am. Chem. Soc.*, 2006, **128**, 10380; A. Ojida, H. Nonaka, Y. Miyahara, S. Tamaur, K. Sada and I. Hamachi, *Angew. Chem., Int. Ed.*, 2006, **45**, 5518; C. L. Chen, Y. H. Chan, C. Y. Chen and S. S. Shun, *Org. Lett.*, 2006, **8**, 5053.

2 A. Coskun and E. U. Akkaya, *J. Am. Chem. Soc.*, 2005, **127**, 10464; P. G. Jiang, L. Z. Chen, J. Lin, Q. Liu, J. Ding, X. Gao and Z. J. Guo, *Chem. Commun.*, 2002, 1424; Q. He, E. W. Miller, A. P. Wong and C. J. Chang, *J. Am. Chem. Soc.*, 2006, **128**, 9316; R. Martínez, F. Zapata, A. Caballero, A. Espinosa, A. Tárraga and P. Molina, *Org. Lett.*, 2006, **8**, 3235.

3 J. S. Kim, M. G. Choi, K. C. Song, K. T. No, S. Ahn and S. K. Chang, *Org. Lett.*, 2007, **9**, 1129–1132; A. Coskun, M. D. Yilmaz and E. U. Akkaya, *Org. Lett.*, 2007, **9**, 607–610; S. Tatay, P. Favina, E. Coronado and E. Palomares, *Org. Lett.*, 2006, **8**, 3857; Y. F. Cheng, D. T. Zhao and C. H. Huang, *Tetrahedron Lett.*, 2006, **47**, 6413; E. M. Nolan, M. E. Racine and S. J. Lippard, *Inorg. Chem.*, 2006, **45**, 2742–2745; S. Y. Moon, N. R. Cha, Y. H. Kim and S. K. Chang, *J. Org. Chem.*, 2004, **69**, 181–184; J. B. Wang, X. H. Qian and J. G. Cui, *J. Org. Chem.*, 2006, **71**, 4308.

4 J. Bujdá and N. Iyi, *J. Phys. Chem. B*, 2006, **110**, 2180; S. Jeon, J. Turner and S. J. Granick, *J. Am. Chem. Soc.*, 2003, **125**, 9908; R. Bandichhor, A. D. Petrescu and K. C. Burgess, *J. Am. Chem. Soc.*, 2006, **128**, 10688; M. P. Okoh, J. L. Hunter and M. R. Webb, *Biochemistry*, 2006, **45**, 14764.

5 A. A. El-Rayyes, A. Al-Betar, T. Htun and U. K. A. Klein, *Chem. Phys. Lett.*, 2005, **414**, 287.

6 (*a*) V. Dujols, F. Ford and A. W. Czarnik, *J. Am. Chem. Soc.*, 1997, **119**, 7386; (*b*) J. Y. Kwon, J. Y. Jang, Y. J. Lee, W. Nam and J. Y. Yoon, *J. Am. Chem. Soc.*, 2005, **127**, 10107; (*c*) Y. Xiang, A. J. Tong and Y. Ju, *Org. Lett.*, 2006, **8**, 2863; (*d*) Y. Xiang and A. J. Tong, *Org. Lett.*, 2006, **8**, 1549; (*e*) Y. K. Yang, K. J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760; (*f*) S. K. Ko, Y. K. Yang, J. Tae and I. Shin, *J. Am. Chem. Soc.*, 2005, **128**, 14150; (*g*) H. Zheng, Z. H. Qian, L. Xu and J. G. Xu, *Org. Lett.*, 2006, **8**, 859; (*h*) D. Y. Wu, W. Huang, C. Y. Duan, Z. H. Lin and Q. J. Meng, *Inorg. Chem.*, 2007, **46**, 1538–1541; (*i*) J. S. Wu, I. C. Hwang, K. S. Kim and J. S. Kim, *Org. Lett.*, 2007, **9**, 907; (*j*) M. H. Lee, J. S. Wu, J. W. Lee, J. H Jung and J. S. Kim, *Org. Lett.*, 2007, **9**, 2501.

7 J. V. Ros-lis, M. D. Marcos, R. Martinez-Manez, K. Rurack and J. Soto, *Angew. Chem., Int. Ed.*, 2005, **44**, 4405; B. Liu and H. Tian, *Chem. Commun.*, 2005, **25**, 3156; G. Hennrich, W. Walther, Ute Resch-Genger and H. Sonnenschein, *Inorg. Chem.*, 2001, **40**, 641; K. C. Song, J. S. Kim, S. M. Park, K. C. Chung, S. Ahn and S. K. Chang, *Org. Lett.*, 2006, **8**, 3413.

8 K. A. Connors, *Binding Constants, The Measurement of Molecular Complex Stability*, John Wiley & Sons, New York, 1987, pp. 24–28.

9 E. Coronado, M. J. R. Galán, C. M. Gastaldo and E. Palomares, *J. Am. Chem. Soc.*, 2005, **127**, 12351.