

Highly Sensitive and Selective Chemosensor for Hg^{2+} Based on the Rhodamine Fluorophore

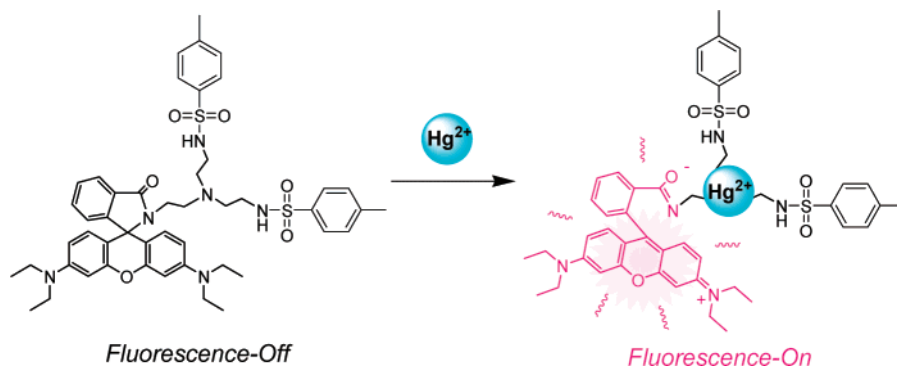
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ABSTRACT



A novel tren-based tripodal chemosensor **1** bearing a rhodamine and two tosyl groups was synthesized and its sensing behavior toward metal ions was investigated by UV/vis and fluorescence spectroscopies. Addition of a Hg^{2+} ion to a CH_3CN solution of **1** gave a visual color change as well as significantly enhanced fluorescence, while other ions including Pb^{2+} , Zn^{2+} , Cu^{2+} , Ca^{2+} , Ba^{2+} , Cd^{2+} , Co^{2+} , Mg^{2+} , Ag^+ , Cs^+ , Li^+ , and Na^+ induced no or much smaller color/spectral changes, which constituted a Hg^{2+} -selective fluorescent chemosensor (OFF–ON).

Currently, considerable attention has been focused on fluorescent chemosensors for the selective and rapid determination of the toxic heavy metal ions, such as the Pb^{2+} , Cd^{2+} , and Hg^{2+} ions.^{1–4} Especially in this regard, the Hg^{2+} ion is considered highly dangerous because both elemental and ionic mercury can be converted into methyl mercury by bacteria in the environment, which subsequently bioaccumulates through the food chain.⁵ Therefore, there is a high

demand for the determination of the Hg^{2+} ion both in environmental analysis and in industrial waste treatment.

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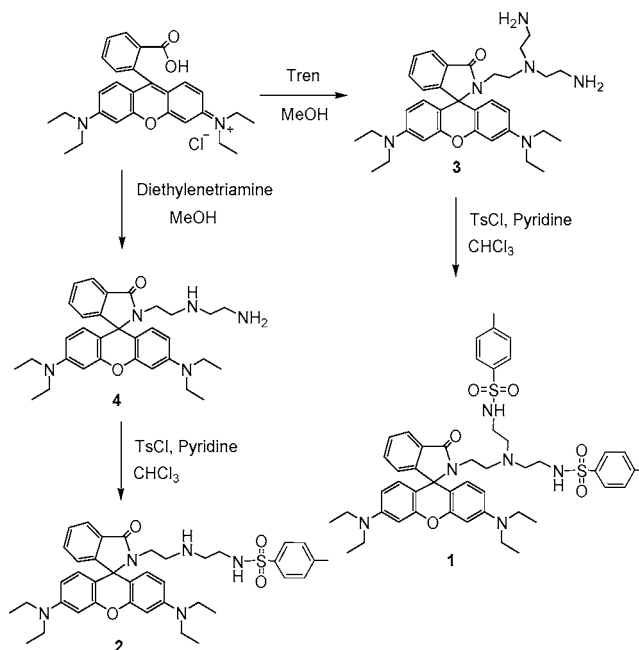
The rhodamine framework is an ideal mode to construct OFF–ON fluorescent chemosensors due to its particular structural property. As is well-known, rhodamine derivatives with spirolactam structure are nonfluorescent, whereas ring-opening of the spirolactam gives rise to a strong fluorescence emission.⁶ Moreover, the rhodamine fluorophore has a longer emission wavelength (over 550 nm), which is often preferred to serve as a reporting group for analyte to avoid the influence of background fluorescence (below 500 nm).⁷ To date, several rhodamine-modified chemosensors for Hg²⁺, Cu²⁺, Pb²⁺, and Fe³⁺ ions have been developed.⁸ For example, two rhodamine B molecules were linked through a diethylenetriamine spacer to afford a Fe³⁺-selective sensor.^{8a} Xiang et al. used a rhodamine-based hydrazine bearing a salicylaldehyde binding site as a Cu²⁺ amplified sensor.^{8b} Yang et al. reported a Hg²⁺ chemodosimeter based on the rhodamine-hydrazine framework.^{8c} In addition, Kwon et al. exploited ethylenediamine to link a rhodamine and a DPA moiety to yield a fluorescent chemosensor for the Pb²⁺ ion.^{8d} Besides hydrazine, diethylenetriamine, and ethylenediamine as the usual linking groups, the tris(2-aminoethyl)amine moiety (tren) can also serve as a good binding group due to its tripodal steric structure for some special metals, and several tren-based sensors have been reported so far.⁹ However, as we noticed, the tren moiety has not been applied for the rhodamine-based chemosensors until now.

Herein, we report a tren-based rhodamine derivative (**1**) as a sensitive and selective chemosensor for Hg²⁺. In the absence of Hg²⁺ ion, the solution of **1** is colorless and nonfluorescent, while addition of the Hg²⁺ ion can lead to a visual color change as well as a fluorescent OFF–ON observation, which constitutes a Hg²⁺-selective fluorescent chemosensor.

Chemosensor **1** is a *N*-tripodal structural compound consisting of a rhodamine moiety (spirolactam form) and two branched tosyl groups. Initial considerations for **1** serving as a Hg²⁺-selective fluorescent chemosensor are as follows: (a) the structure of tripodal tren gives a good chelating ability for some specific metal ions; (b) the spirolactam-structured rhodamine moiety is directly linked to tren, and when the binding event takes place, it also leads to a ring-opening of spirolactam along with an obvious OFF–ON optical signal; and (c) the strong electron acceptor—two tosyl groups were introduced to strengthen the acidity of the amide and further produce a stronger binding ability with cations. In addition,

we use diethylenetriamine instead of tren to synthesize a similar compound **2** (with only one tosyl group) for comparative investigation to explore which structure is preferable to the Hg²⁺ ion chelating. In our present work, **1** exhibits a more sensitive and selective binding ability toward the Hg²⁺ ion than **2**, implying that the tripodal structure (tren) is superior to the dipodal group (diethylenetriamine) for Hg²⁺ binding in this case.

Scheme 1. Synthetic Pathways of **1** and **2**



As depicted in Scheme 1, compounds **1** and **2** were synthesized from the reaction of rhodamine B with tren or diethylenetriamine, followed by the amidation reaction with tosyl chloride and pyridine as a base refluxing in CHCl₃ for 12 h to afford **1** or **2** in 68% and 58% overall yields, respectively. The final products (**1** and **2**) were well characterized by ¹H NMR, ¹³C NMR, FT-IR, and FAB-MS. The spirolactam forms of **1** and **2** were confirmed by the presence of a peak at 65 ppm in their ¹³C NMR spectra.

Figure 1 shows spectral changes of **1** in CH₃CN upon addition of various competitive metal ions, such as Hg²⁺, Pb²⁺, Zn²⁺, Cu²⁺, Ca²⁺, Cd²⁺, Co²⁺, Ba²⁺, Mg²⁺, Cs⁺, Li⁺, Na⁺, and Ag⁺. From UV/vis spectra of **1** (10 μM) (Figure 1a), we can clearly observe a new absorption band centered at 556 nm in the presence of 10 equiv of Hg²⁺ ion. In contrast, other ions lead to much smaller spectral changes (Cu²⁺ and Pb²⁺), or almost no spectral changes (Zn²⁺, Ca²⁺, Cd²⁺, Co²⁺, Ba²⁺, Mg²⁺, Cs⁺, Li⁺, Na⁺, and Ag⁺). On the other hand, fluorescence spectra (Figure 1b) also show a similar result, which is consistent with that of UV/vis spectra. Addition of 10 equiv of Hg²⁺ ion results in an obviously enhanced fluorescence at 575 nm (OFF–ON), while other ions induce much smaller (Ca²⁺, Ba²⁺, and Cu²⁺) or no fluorescence increases. Further experiments for Hg²⁺-selective sensing were performed with 1 μM **1** in CH₃CN in the

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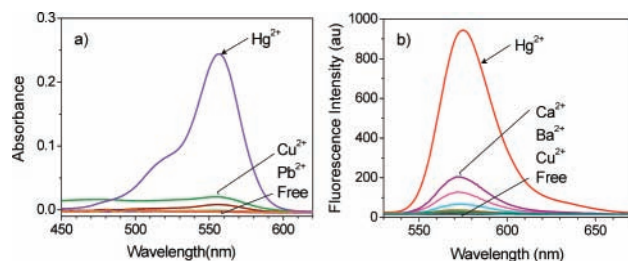


Figure 1. (a) Absorption spectra of **1** (10 μM) and (b) fluorescence spectra of **1** (1 μM) with addition of ClO₄[−] salts of Hg²⁺, Pb²⁺, Zn²⁺, Cu²⁺, Ca²⁺, Ba²⁺, Cd²⁺, Co²⁺, Mg²⁺, Ag⁺, Cs⁺, Li⁺, and Na⁺ (10 equiv, respectively) in CH₃CN with an excitation at 520 nm.

presence of multifarious cations including Pb²⁺, Zn²⁺, Cu²⁺, Ca²⁺, Cd²⁺, Co²⁺, Ba²⁺, Mg²⁺, Cs⁺, Li⁺, Na⁺, and Ag⁺ (1 equiv, respectively, Figure S1 in the Supporting Information). Upon addition of 1 equiv of Hg²⁺ ion, the solution above still displays a distinctly enhanced fluorescence. Both UV/vis and fluorescence results indicate that **1** shows a good selectivity and sensitivity toward Hg²⁺ over other competitive cations.

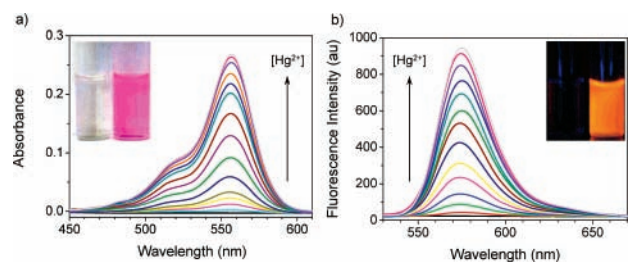


Figure 2. (a) Absorption spectra of **1** (10 μM) and (b) fluorescence spectra of **1** (1 μM) with addition of various concentrations of Hg(ClO₄)₂ [(a) 0, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μM, respectively; (b) 0, 1.0, 1.6, 1.8, 1.9, 2.1, 2.8, 3.0, 4.0, 4.2, 4.5, 4.6, 4.8, 4.9, 8.0, and 10 μM, respectively] in CH₃CN with an excitation at 520 nm. Inset: Visual color and fluorescence changes of **1** upon addition of Hg(ClO₄)₂ in CH₃CN.

Figure 2 gives detailed absorption and fluorescence changes of **1** upon gradual titration of Hg²⁺ ion. As expected, the addition of a small amount of Hg²⁺ ion induces a new absorption band as well as a visual color change from colorless to pink. The fluorescence titration of Hg²⁺ ion was conducted with 1.0 μM **1** in CH₃CN. Upon addition of increasing concentrations of Hg²⁺ ion, a new emission band peaked at 575 nm appears with increasing intensity. The visual color and fluorescence changes in the presence of Hg²⁺ ion are also shown in Figure 2. To confirm the stoichiometry between **1** and Hg²⁺ ion, FAB-MS analysis was conducted (Figure 3). Mass peaks at *m/z* 1079 and 879 corresponding to [1 + Hg]⁺ and free **1** are clearly observed, which gave solid evidence for the formation of a 1:1 complex. On the

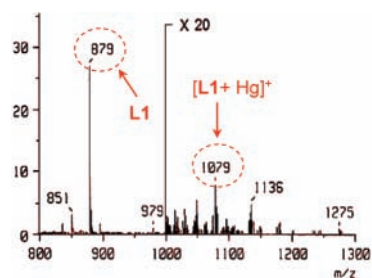


Figure 3. FAB-MS of **1** in the presence of 10 equiv of Hg(ClO₄)₂ in CH₃CN.

basis of 1:1 stoichiometry and UV/vis titration data in Figure 2, the association constant of **1** with Hg²⁺ ion in CH₃CN was found to be $1.59 \times 10^6 \text{ M}^{-1}$.¹⁰

It is explicit that the binding interaction between tren and Hg²⁺ ion induces the ring-opening of the spirolactam in **1**, which is responsible for the above dual color and fluorescence changes. To get insight into the roles that the two tosyl groups of **1** play, intermediate **3** was used separately for spectral tests (Figure S2, Supporting Information). At the same conditions, **3** does not give distinct absorption and fluorescence spectral changes upon addition of Hg²⁺ ion (Figure 4), which implies that two tosyl groups also play an

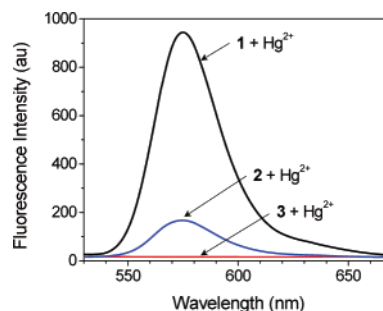


Figure 4. Fluorescence spectra of **1–3** (1 μM, respectively) with addition of Hg(ClO₄)₂ (10 μM) in CH₃CN with an excitation at 520 nm.

important role for Hg²⁺ sensing. On the other hand, we also studied the spectral properties of diethylenetriamine-bridged rhodamine derivative **2**, which only has one tosyl group. Upon addition of Hg²⁺ ion, **2** only gave rise to much smaller absorption and fluorescent spectral changes (Figures S3–S6, Supporting Information). In addition, as shown in Figure 4, in the presence of 10 equiv of Hg²⁺ ion, **1** exhibits a 6-fold enhanced fluorescence more than that of **2**. From the titration experiment, the association constant of **2** with the Hg²⁺ ion

(10) (a) Association constants were calculated with the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom. (b) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.

in CH₃CN was calculated to be $5.2 \times 10^5 \text{ M}^{-1}$ on the basis of 1:1 stoichiometry.¹⁰ The above results indicate that **1** exhibited a superior binding ability toward the Hg²⁺ ion than **2** and **3**.

To further look into the nature of the interaction between **1** and the Hg²⁺ ion, ¹H NMR experiments were carried out in CD₃CN (Figure 5). For free **1**, the chemical shift of tosyl

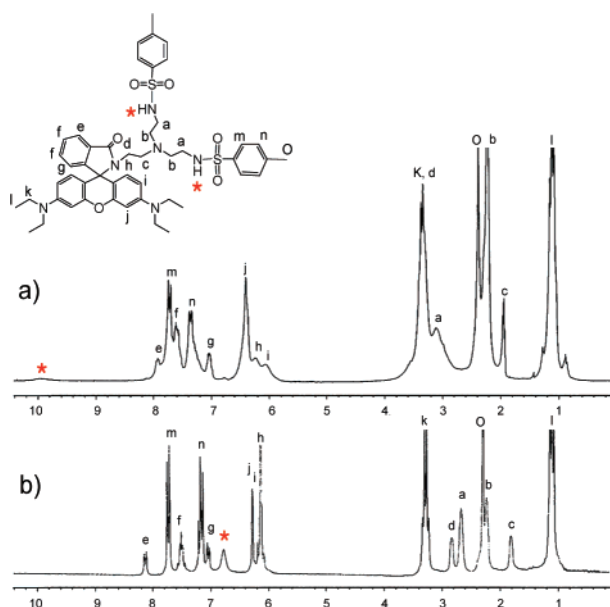


Figure 5. ¹H NMR data of **1** (22 mM) in CD₃CN in the presence (a) and absence (b) of 10 equiv of Hg(ClO₄)₂.

NH is about 6.85 ppm, whereas in the presence of 10 equiv of Hg(ClO₄)₂, it was broadened and shifted downfield (9.98 ppm). In addition, the chemical shifts of tren protons (H_a, H_c, H_d) also show distinct downfield changes. On the other hand, infrared spectra (Figure S7, Supporting Information) were also conducted to confirm the binding of the carbonyl group of **1** with the Hg²⁺ ion. It was clearly observed that upon addition of the Hg²⁺ ion, the carbonyl stretching band of **1** at 1685 cm⁻¹ was changed to a lower wavenumber (1670 cm⁻¹). Both ¹H NMR and IR results firmly support that tren and the carbonyl group of spirolactam are involved in the Hg²⁺ binding, thus inducing a ring-opening of the spirolactam in **1**.

For practical applicability, the experimental conditions for the Hg²⁺ detection were optimized in aqueous solution. In the presence of the Hg²⁺ ion, variation of the fluorescence

intensity of **1** with various CH₃CN/H₂O ratios is shown in Figure S8 (Supporting Information). With the increase of H₂O content, the fluorescence intensity of the solution is decreased. Here, to get a better sensitivity, we select the mixed solvent (CH₃CN:H₂O 9:1) for the pH investigation. Figure 6 shows variations of fluorescence intensity of **1** with

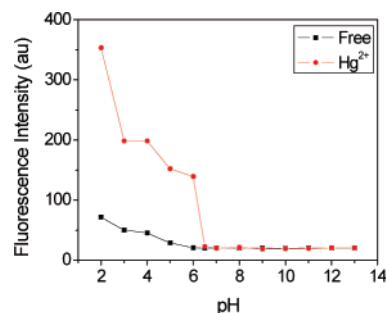


Figure 6. Variation of fluorescence intensity at 575 nm of **1** (1 μM) in aqueous solution (CH₃CN:H₂O 9:1) with and without Hg²⁺ ion (10 μM) as a function of pH.

pH values in the absence and presence of the Hg²⁺ ion in aqueous solution (CH₃CN:H₂O 9:1). It is found that a suitable pH span for Hg²⁺ determination is between pH 3 and 6 because in this region free **1** is a weak fluorescent, while addition of the Hg²⁺ ion can lead to a remarkable increase of fluorescence, that is to say, complexation between **1** and the Hg²⁺ ion takes place more efficiently.

In conclusion, we report the tren-structured rhodamine derivative **1** serves as a sensitive chemosensor, which can show a good selectivity toward Hg²⁺ over other competitive ions in CH₃CN. For the practical Hg²⁺ detection, the experimental conditions were optimized in 90% CH₃CN aqueous solution with a pH span of 3–6. In addition, **2** with similar structure as **1** was also prepared to illustrate the excellent binding ability of the tren structure toward the Hg²⁺ ion. To the best of our knowledge, this is the first case to use the tren-based rhodamine chemosensor for Hg²⁺ detection.

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Supporting Information Available: Synthetic details, NMR spectra, and additional spectral data. This material is available free of charge via the Internet at <http://pubs.acs.org>. OL0708931