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# New Fluorescent Rhodamine Hydrazone Chemosensor for Cu(II) with High Selectivity and Sensitivity

**Yu Xiang, Aijun Tong,* Peiyuan Jin, and Yong Ju**

*Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China*

*tongaj@mail.tsinghua.edu.cn*

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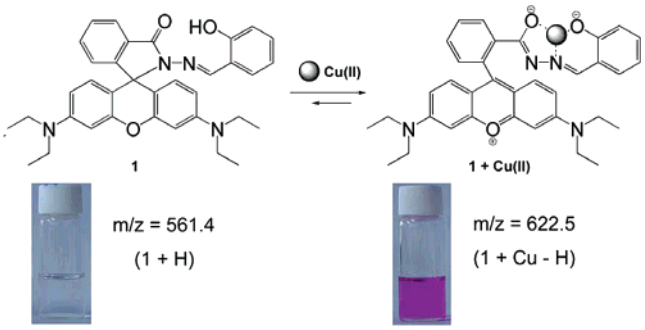
## ABSTRACT




**A new fluorescent probe, salicylaldehyde rhodamine B hydrazone (1), was synthesized and displayed selective Cu(II)-amplified absorbance and fluorescence emission above 500 nm in neutral buffered media. Upon the addition of Cu(II), the spirolactam ring of 1 was opened and a 1:1 metal–ligand complex was formed. The detection of Cu(II) by 1 at a lower micromolar level was successful even in buffered water.**

The development of artificial receptors for the sensing and recognition of environmentally and biologically important ionic species, especially transition-metal ions, is currently of great interest.[1] Because copper is a widely used pollutant and an essential trace element in biological systems, much attention has been drawn to the design of fluorescent probes for the detection of copper ions due to the nondestructive, quick, and sensitive advantages of emission signals.[2] Most of the classic and early-reported cation sensors, however, generally undergo fluorescence quenching upon the binding of Cu(II),[3] which is not as sensitive as a fluorescence enhancement response; moreover, the selectivity for Cu(II) over other ions, such as Fe(III) and Pb(II), is not very satisfactory for some of the probes. To overcome these disadvantages, fluoroionophores which show a selective response to Cu(II) by a copper-amplified fluorescence emission have been well developed in recent years.[4] Among these sensors, unfortunately, those that can be applied in aqueous solutions at neutral pH are still rare mainly because of the strong hydration ability of Cu(II) in water. Actually, sensors of this kind are always considered to be much more attractive and efficient in the respect that most copper-containing samples are near-neutral aqueous systems.[1,2]

On the other hand, fluorophores of long-wavelength emissions are often preferred to serve as the reporting group for analyte to avoid the influence of background fluorescence

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(<400 nm). The introduction of the rhodamine moiety to construct probes of the "off−on" type is a reliable method because of the well-known spirolactam (fluorescence "off") to ring-opened amide (fluorescence "on") equilibrium (Figure 1) of rhodamine derivatives.[6] We have successfully designed

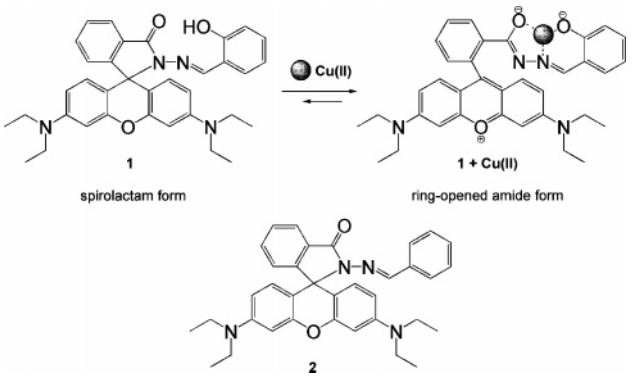


**Figure 1.** Chemical structures of compound **1**, **1**−Cu(II),[5] and **2**.

a fluorescence chemosensor for Fe(III) utilizing rhodamine B as the fluorophore in our previous work.[6d] Herein, we describe a new rhodamine-based chemosensor **1** (Figure 1), which shows a reversible, selective, and sensitive fluorescence enhancement response to Cu(II) in neutral buffered media.

Compound **1** was facilely synthesized from rhodamine B by a two-step reaction (Supporting Information, S-Figure 1). This Shiff base was stable in neutral water solutions for at least 2 days. It was designed to chelate with metal ions via its carbonyl O, imino N, and phenol O atoms.[7] The spirolactam moiety of the rhodamine group acted as a signal switcher, which was envisioned to turn on when the cation was bound. A solution of **1** in Tris−HCl buffer (5 mM, pH = 7.0) or organic media is colorless and weakly fluorescent, indicating that the spirolactam form of **1** exists predominantly. The characteristic peak of the 9-carbon of **1** near 66 ppm in the $^{13}$C NMR spectrum (Supporting Information, S-Figure 2d) also supports this consideration.[8] Besides, no obvious characteristic color or fluorescence of rhodamine could be observed for **1** between pH 5.0 and 10.0 (Supporting Information, S-Figure 3), suggesting that **1** is insensitive to pH and that the spirolactam form is still preferred in this range. As we expected, addition of Cu(II) to a solution of **1** in either $CH_3CN$ or water caused a significant enhancement of absorbance and fluorescence intensity in the 500−650 nm range immediately as a result of the Cu(II)-induced ring opening of the spirolactam form. We also found that a solution of 10 $\mu$M **1** in 50% (v/v) buffered (10 mM Tris−HCl, pH = 7.0) water/$CH_3CN$ could display an obvious purple color in the presence of Cu(II) at the micromolar level, and other ions of our interest showed little interference (Figure 2). These results suggested that **1** could serve as a

**Figure 2.** Pictures of 10 $\mu$M **1** as a selective naked-eye chemosensor for Cu(II) in 50% (v/v) water/$CH_3CN$ (10 mM Tris−HCl, pH = 7.0). Top (from left to right): 0, 2.5, 5.0, 10.0 $\mu$M Cu(II). Bottom (from left to right): 50 $\mu$M Fe(III), Fe(II), Zn(II), Pb(II), Hg(II).

"naked-eye" chemosensor selective for Cu(II) in neutral buffered media.

The absorption spectra of **1** (10 $\mu$M) in 50% (v/v) water/$CH_3CN$ (10 mM Tris−HCl buffer, pH = 7.0) exhibited only a very weak band above 500 nm, which was ascribed to the trace ring-opened form of molecules of **1**. Upon the addition of up to 5 equiv of Cu(II), the absorbance was significantly enhanced (> 500-fold) and a new peak at 558 nm was observed (Figure 3a), suggesting the clear formation of the ring-opened amide form of **1**. Other metal ions had little interference. Only Fe(II) displayed a 65-fold enhancement at the same concentration, but a much longer response time was required (Supporting Information, S-Figure 4a−d). The nonlinear fitting of the titration curve assumed a 1:1 stoichiometry for the **1**−Cu(II) complex (Figure 1) with an association constant $K_a$ value of far more than $10^6$,[9] showing the high affinity of **1** to Cu(II). This binding mode was also supported by the data of Job's plots[10] evaluated from the absorption spectra of **1** and Cu(II) with a total concentration of 3.3 $\mu$M (Supporting Information, S-Figure 5). A more direct evidence was obtained by comparing the ESI mass spectra of **1** and **1**−Cu(II) (Figure 4). The unique peak at $m/z$ = 622.5 (calcd = 622.3) corresponding to [**1** + Cu−H]$^+$ was clearly observed when 1.2 equiv of Cu(II) was

(5) The copper ion may be chelated by the counteranion or solvent oxygen to satisfy the need for four-coordination. They are omitted for clarity.

(6) For rhodamine-based chemosensors for metal ions, see: (a) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386. (b) Yang, Y.-K.; Yook, K.-J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127*, 16760. (c) 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. (d) Xiang, Y.; Tong, A. *Org. Lett.* **2006**, *8*, 1549.

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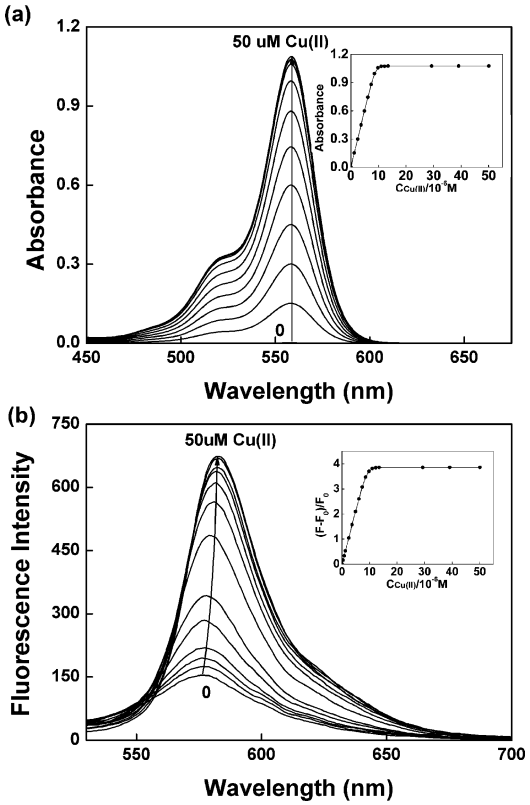


**Figure 3.** (a) Absorption spectra of **1** (10 $\mu$M) in Tris−HCl (10 mM, pH = 7.0) buffer containing 50% (v/v) water/$CH_3CN$ in the presence of different amounts of Cu(II). Inset: absorbance at 558 nm as a function of Cu(II) concentration, indicating a 1:1 metal−ligand ratio. (b) Fluorescence spectra of **1** (10 $\mu$M) under the same conditions. Excitation was performed at 520 nm. Inset: fluorescence enhancement factor $(F - F_0)/F_0$ at 583 nm as a function of Cu(II) concentration.

added to **1**, whereas **1** without Cu(II) exhibited peaks only at $m/z$ = 561.4 (calcd = 561.3) and 583.3 (calcd = 583.3) which corresponded to $[\mathbf{1} + H]^+$ and $[\mathbf{1} + Na]^+$, respectively. To achieve this 1:1 stoichiometry, carbonyl O, imino N, and phenol O atoms of **1** are the most possible binding sites for Cu(II).[7] In fact, the phenol group of **1** which undergoes deprotonation during the binding plays an indispensable role in the affinity of **1** to Cu(II) because the contrast compound **2** (no phenol group compared with **1**, Figure 1) displayed nearly no response to Cu(II) in either absorption or ESI mass spectra when treated with excess (10 equiv) Cu(II) (Supporting Information, S-Figure 6a−c). It was also confirmed that the response of **1** to Cu(II) was reversible rather than a cation-catalyzed reaction:[11] (i) the color and fluorescence of **1**−Cu(II) disappeared instantly upon the addition of EDTA, whereas excess Cu(II) would recover the signal; (ii) the TLC results and the mass spectra data of a buffered solution containing 10 $\mu$M **1** and 12 $\mu$M Cu(II) after standing at room temperature for 2 days indicated no other chemical species except for **1** and **1**−Cu(II).

(11) For sensors of this type, see ref 6a,b.

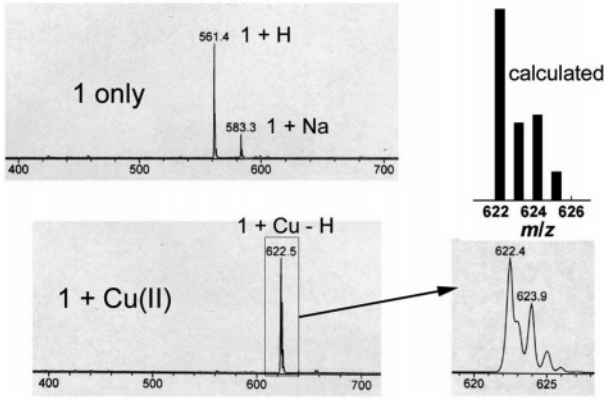


**Figure 4.** ESI mass spectra (positive) of **1** (10 $\mu$M) in the absence and presence of Cu(II) (1.2 equiv). Right: calculated (top) and observed (bottom) isotopic patterns for the $[Cu(\mathbf{1}-H)]^+$ cation, indicating the formation of a 1:1 metal−ligand complex.

The high enhancement factor (>500) of absorbance of **1** upon the binding of Cu(II) and the large association constant ($K_a > 10^6$) of the **1**−Cu(II) complex implied the possibility of copper sensing at a very low concentration level by **1**. Indeed, the absorbance of **1** (10 $\mu$M) at 558 nm was found to increase linearly with the concentration of Cu(II) in the range of 25 nM−3.3 $\mu$M (Supporting Information, S-Figure 7a−d). However, signals acquired from absorption spectra are generally not as efficient as those evaluated from fluorescence spectra. The fluorescence titration[12] was also carried out using 10 $\mu$M **1** in 50% (v/v) buffered water/$CH_3CN$ at pH 7.0. Although the emission spectra of **1** underwent a red shift from 576 to 583 nm upon the addition of 5 equiv of Cu(II), the fluorescence intensity at the new peak (583 nm) had only a 3.8-fold enhancement (Figure 3b), which was much weaker compared to that of absorbance. Nevertheless, the selective sensing of Cu(II) at the 100 nM level was still available when using 1.0 $\mu$M **1** under the same conditions (Supporting Information, S-Figure 8).

To get a practical application view, the fluorescence sensing behavior of **1** for Cu(II) in buffered water solution (with no more than 2% $CH_3CN$) was also investigated. The titration was performed using 10 $\mu$M solutions of **1** in Tris−HCl (5 mM, pH = 7.0) aqueous buffer (Figure 5). A 9.4-fold enhancement of fluorescence and a continuous red shift of the emission peak from 573 to 585 nm were observed upon the addition of 20 equiv of Cu(II) compared to that of only **1** in solution. The nonlinear fitting of the titration curve and the data of Job's plots from absorption spectra (Supporting Information, S-Figures 9 and 10) also assumed a 1:1 stoichiometry for the **1**−Cu(II) complex with an association constant around $K_a$ = 69 110. The response was proved to be revisable, and the selectivity of **1** to Cu(II) over other cations was still satisfactory. We found that alkali and alkaline earth metal ions had hardly any effect on the

(12) All the fluoresence spectra were measured by excitation at 520 nm to obtain a full view of emissions from 530 to 700 nm.

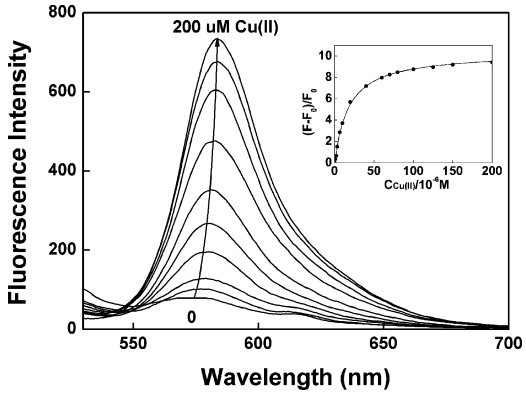


**Figure 5.** Fluorescence spectra of **1** (10 $\mu$M) in Tris−HCl (5 mM, pH = 7.0) aqueous buffer solutions in the presence of different amounts of Cu(II). Excitation was performed at 520 nm. Inset: fluorescence enhancement factor at 585 nm as a function of Cu(II) concentration.

fluorescence spectra of **1** (10 $\mu$M) in buffered water even at the millimolar level. Other transition-metal ions, such as Fe(III), Co(II), Ni(II), Zn(II), Mn(II), Ag(I), Cd(II), and Hg(II), gave a much weaker response compared to Cu(II) at the same concentration (100 $\mu$M) (Supporting Information, S-Figure 11), and the fluorescence signal of **1**−Cu(II) in the presence or absence of these contrast ions also exhibited only a mild difference (Supporting Information, S-Figure 12). Furthermore, the detection of Cu(II) from 0.25 to 2.0 $\mu$M was successful when utilizing 1.0 $\mu$M **1** in 1 mM Tris−HCl aqueous buffers at pH 7.0 (Supporting Information, S-Figure 13), indicating the high sensitivity of **1** for Cu(II) even in aqueous media.

With careful investigation, one may note that when Cu(II) was added to **1**, the enhancement of absorbance was much more significant than that of fluorescence intensity. However, the ring opening of the spirolactam form of rhodamine derivatives generally results in comparable amplifications of absorption and fluorescence signals.[5c,d] In the case of **1**, binding of Cu(II) does open the spirolactam ring, but at the same time, the fluorescence of the ring-opened amide form is probably partially quenched by Cu(II). The quenching mechanism may be similar to that of some Cu(II) probes displaying fluorescence quenching for the paramagnetic nature of the copper ion.[1,13] From a sensitivity viewpoint, it is preferable to inhibit this quenching effect to generate a more notable fluorescence enhancement. Chemical modification on the phenol group may be a good improvement,[14] but the affinity of **1** to Cu(II) could probably decrease for the important role of the phenol moiety. Works related to this topic are now under our investigation.

In conclusion, we have synthesized a new rhodamine-based fluoroionophore **1**, which displayed a reversible absorption and fluorescence enhancement response to Cu(II) via a 1:1 binding mode. Its selectivity toward Cu(II) is very high because little interference was observed for other commonly coexistent metal ions. Furthermore, the sensitivity of **1** for Cu(II) can be lower than 25 nM in 50% (v/v) buffered water/$CH_3CN$ by the absorption spectra method (still at the 0.1 $\mu$M level for the fluorescence method under this condition). Even in neutral buffered aqueous solutions, the fluorescent sensing of Cu(II) at a lower micromolar level was successful.

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**Supporting Information Available:** Experimental procedures, characterization data for the compounds described, and selected spectroscopic data of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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