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# Naphthalimide Modified Rhodamine Derivative: Ratiometric and Selective Fluorescent Sensor for $Cu^{2+}$ Based on Two Different Approaches

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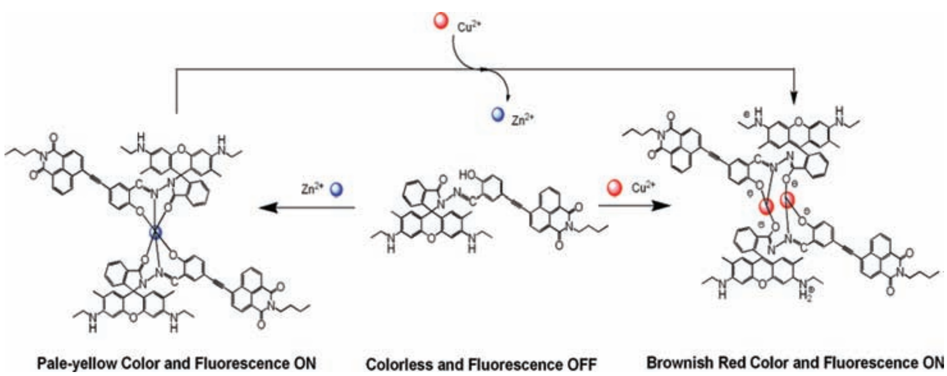
## ABSTRACT




**A new rhodamine-based derivative bearing a 1,8-naphthalimide group (1) was synthesized as a dual-mode $Cu^{2+}$-selective sensor *via* the rhodamine ring-opening approach and ratiometric displacement. A colorimetric and "off−on" signal for $Cu^{2+}$ through rhodamine ring opening in 1 and ratiometric fluorescent signal output when $Cu^{2+}$ displaces the bound $Zn^{2+}$ in the 1-$Zn^{2+}$ complex can be observed.**

Fluorescent sensors for the detection and measurement of transition-metal ions are widely investigated because of their simplicity and high sensitivity of response.[1] In particular, the development of a fluorescent probe for copper ions in the presence of a variety of other metal ions has received great attention. As is well-known, $Cu^{2+}$ plays an important role in living systems such as those occurring in the human nervous system, gene expression, and the functional and structural enhancement of proteins.[2] However, under overloading conditions, copper can be toxic and can cause oxidative stress and disorders associated with neurodegenerative diseases, including Menkes and Wilson diseases, familial amyotropic lateral sclerosis, Alzheimer's disease, and prion diseases.[3]

Even though some examples of selective recognition sensors for $Cu^{2+}$ have been reported,[4] most of these sensors

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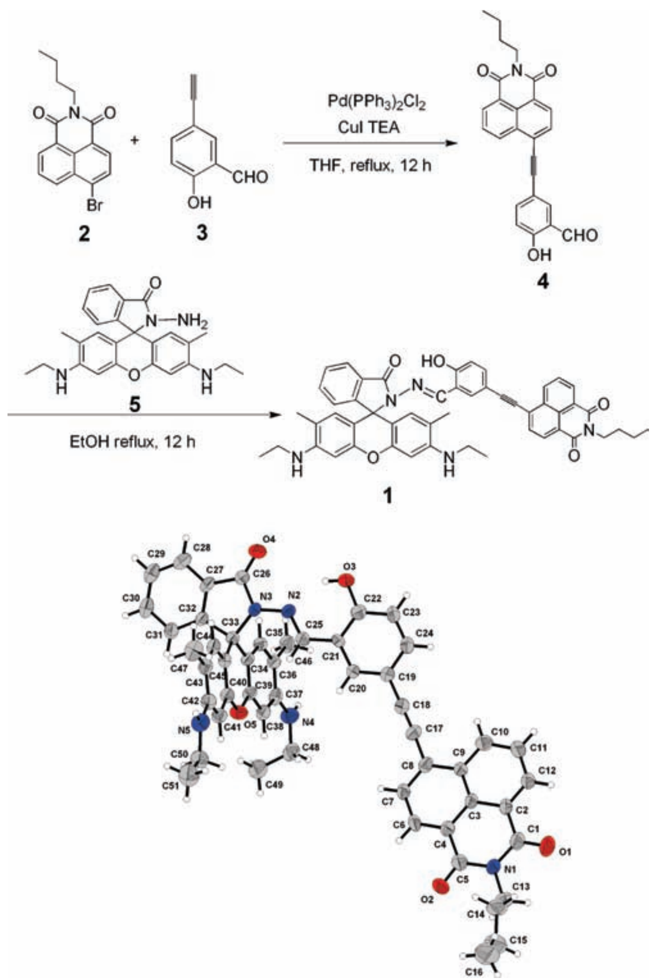
show "turn-off" manner in emission spectra upon $Cu^{2+}$ binding due to the fluorescence-quenching nature of paramagnetic $Cu^{2+}$.[5] Furthermore, only a few examples can display "turn-on" or ratiometric fluorescent changes in emission spectra, which are desirable for analytical purposes by the enhancement of fluorescence or changes in the ratio of the intensities of the emission at two wavelengths.[6]

Recently a rhodamine-based derivative bearing a pyrene group as a chemosensor for $Cu^{2+}$ was reported in our work, in which the rhodamine ring-opening process was introduced to give a colorimetric change and "turn-on" fluorescence signal toward $Cu^{2+}$.[7,8] Since the fluorescence of pyrene was quenched upon addition of $Cu^{2+}$, the signal is not observed obviously. Introducing another fluorophore to give a strong fluorescence first upon binding with some other ions and show the typical rhodamine fluorescence later by the replacement of $Cu^{2+}$ could ensure the ratiometric signal output. Therefore, 1,8-naphthalimide, as a typical ICT fluorophore, was introduced to form the ratiometric displacement system in **1**.[9]

This paper reports design and synthesis of a new rhodamine-based derivative bearing a *N*-butyl-1,8-naphthalimide group (**1**), which displays a selective colorimetric change and fluorescence "turn-on" changes at 550 nm *via* rhodamine ring-opening approach toward $Cu^{2+}$ among the other examined metal ions. Compound **1** also showed a remarkable ratiometric fluorescence enhancement toward $Zn^{2+}$ with 100 nm red-shift by a typical ICT response. As expected, the naphthalimide moiety served successfully as a source of these ratiometric changes. Moreover, another ratiometric fluorescent signal output for $Cu^{2+}$ can be observed when $Zn^{2+}$ in the **1**-$Zn^{2+}$ complex was displaced with $Cu^{2+}$. These results demonstrated that **1** could act as a dual-mode $Cu^{2+}$-selective sensor *via* two mechanisms: the rhodamine ring-opening mechanism and ratiometric displacement from **1**-$Zn^{2+}$ complex. As far as we are aware, **1** is the first rhodamine-based dual-mode ratiometric sensor for $Cu^{2+}$ ion utilizing two different mechanisms.

**Scheme 1.** Synthesis of Chemosensors **1** and Its Crystal Structure




As shown in Scheme 1, 4-(5′-ethynylsalicylaldehyde)-*N*-butyl-1,8-naphthalimides (**4**) was first synthesized by modifying the reported procedure with an improved yield of 82%.[10] Compound **4** was then reacted with rhodamine 6G hydrazide (**5**) to give the hydrazone **1** in 56% yield. The detailed experimental procedures and the characterization of the new compounds are described in Supporting Information. Sensor **1** was further confirmed by X-ray analysis (Scheme 1). The single crystal of **1** suitable for X-ray diffraction studies was grown by the vapor diffusion of diethyl ether into a $CH_3CN$ solution of **1**.

To get further insight into the binding of $Cu^{2+}$ with **1**, the absorption spectra of **1** upon titration with $Cu^{2+}$ were recorded (Figure 1). Upon addition of $Cu^{2+}$, three new

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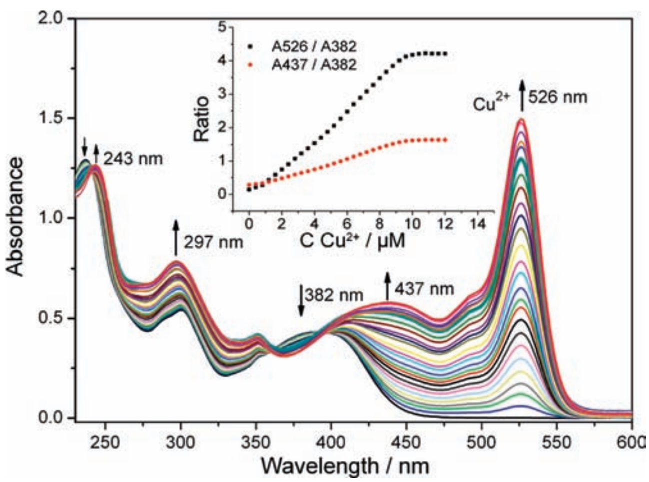


**Figure 1.** Absorbance spectra of **1** (10 $\mu$M) in $CH_3CN$−HEPES buffer (0.02 M, pH = 7.4) (5:5, v/v) in the presence of different amounts of $Cu^{2+}$. Inset: the ratio of absorbance at 526 nm and absorbance at 382 nm (black dot line) and the ratio of absorbance at 437 nm and absorbance at 382 nm (red dot line) as a function of $Cu^{2+}$ concentration.

absorption band centered at 297, 437, and 526 nm appeared with increasing intensity, which induced a clear color change from pale yellow to brownish red. Meanwhile, the absorption band centered at 382 nm decreased gradually with two isosbestic points at 359 and 395 nm was observed. A linear dependence of the ratio of absorbance at 526 nm and absorbance at 382 nm (black line) and the ratio of absorbance at 437 nm and absorbance at 382 nm (red line) as a function of $Cu^{2+}$ concentration were observed. At the same time, the fluorescence intensity at 548 nm was obviously enhanced because of the rhodamine ring-opening process (Figure 2). The linear dependence of the intensity ratio within the equivalent range of $Cu^{2+}$ ion testified that **1** forms a 2:2 complex with $Cu^{2+}$, whose association constant ($K_a$) was determined to be about 0.52 $\times$ $10^4$ from the titration experiments. Moreover, the Job's plot and FAB mass confirms the 2:2 stoichiometry for the **1**-$Cu^{2+}$ complex, which also strongly supports the above conclusion (Figures S6 and S20 in Supporting Information).

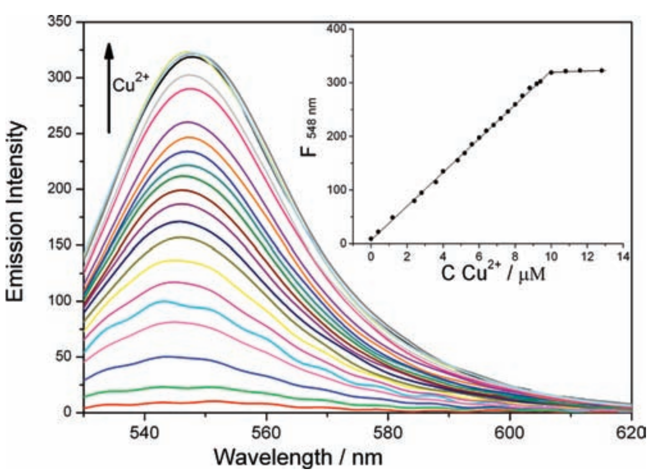


**Figure 2.** Fluorescence spectra of **1** (10 $\mu$M) in $CH_3CN$−HEPES buffer (0.02 M, pH = 7.4) (5:5, v/v) in the presence of different amounts of $Cu^{2+}$. Inset: the fluorescent intensity at 548 nm as a function of $Cu^{2+}$ concentration. Excitation wavelength was 510 nm.

To evaluate the selectivity of **1** for $Cu^{2+}$, absorption and fluorescence intensity changes upon addition of excess amount of various metal ions were measured. The unique absorption change with appearance of the brownish red of **1** was observed only by the addition of $Cu^{2+}$, which can be ascribed to the spirolactam bond cleavage of the rhodamine group. The other metal ions such as $Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$, $Ag^+$, $Mg^{2+}$, $Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$, $Cd^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Al^{3+}$, $In^{3+}$, $Co^{2+}$, and $Ni^{2+}$ did not cause any significant changes in the UV spectra as shown in Figure S1 in Supporting Information. Free **1** showed weak fluorescence emission around 493 nm upon excitation at 400 nm in buffer solution because of the efficient PET quenching from amide of rhodamine 6G to the naphthalic amine fluorophore (Figure S2 in Supporting Information).

Addition of $Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$, $Ag^+$, $Mg^{2+}$, $Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$, $Cd^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Al^{3+}$, $In^{3+}$, $Co^{2+}$, and $Ni^{2+}$ exerted little or no effect on the emission of **1**, whereas $Cu^{2+}$ gave a new weak emission peak centered at 550 nm (Figure S2 in Supporting Information). The efficient FRET was not observed since the fluorescent of naphthalic amine was quenched upon addition of $Cu^{2+}$. Remarkable ratiometric fluorescence enhancements and red-shift of about 100 nm were detected upon binding $Zn^{2+}$, showing a typical response of ICT sensors (Figure S3 in Supporting Information). The $K_a$ was determined to be about 0.34 $\times$ $10^3$ from the titration experiments. The ratio of emission intensities ($F_{595\ nm}/F_{493\ nm}$) varies from 0.12 to 3.97 and is saturated up to a molar ratio (**1**/$Zn^{2+}$) of 2:1 (Figure S3 inset in Supporting Information). To confirm the stoichiometry between **1** and $Zn^{2+}$, the Job's plot and FAB mass spectrometry were undertaken, and the results supported this experimental finding (Figures S10 and S21 in Supporting Information).

Probe **1** exhibited some difference spectra changes upon addition of these metal ions in buffer solution by excitation of the rhodamine fluorophore at 510 nm (Figure 3). Remarkable fluorescence enhancements were detected upon the addition of $Zn^{2+}$ and $Cu^{2+}$, respectively. Upon binding $Zn^{2+}$, **1** showed a broad emission band centered at 595 nm, which is similar as excitation at 400 nm (Figure S3 in Supporting Information), implicating that a promoted ICT process occurred. In contrast, upon binding $Cu^{2+}$, only enhanced fluorescent intensity at 550 nm were observed, suggesting that sensor **1** recognizes $Cu^{2+}$ principally based on the rhodamine ring-opening mechanism. Furthermore, in the competition experiment, the absorption and fluorescence properties of **1** toward other metal ions, including $Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$, $Ag^+$, $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Fe^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Fe^{3+}$, $Al^{3+}$, and $In^{3+}$ were also measured (Figures S4 and S5 in Supporting Information). The increases of absorbance and fluorescence intensity

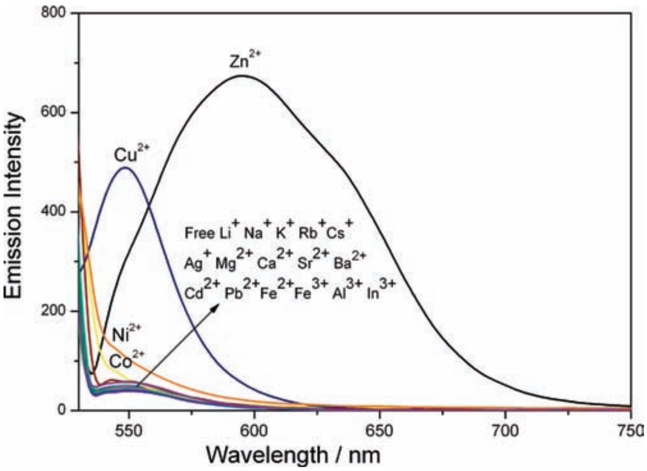


**Figure 3.** Fluorescence spectra of **1** (10 $\mu$M) and **1** in the presence of various metal ions (5 equiv) in $CH_3CN$−HEPES buffer (0.02 M, pH = 7.4) (5:5, v/v). Excitation wavelength was 510 nm.

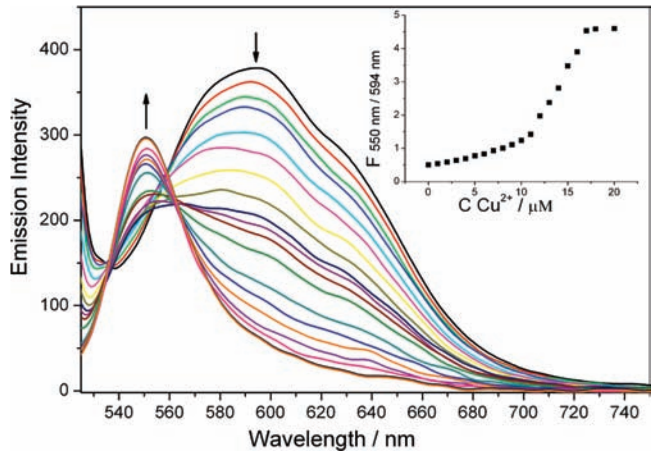


**Figure 4.** Fluorescence spectra of **1**-$Zn^{2+}$ (20 $\mu$M **1** addition of 100 $\mu$M $Zn^{2+}$) in $CH_3CN$-HEPES buffer (0.02 M, pH = 7.4) (5:5, v/v) in the presence of different amounts of $Cu^{2+}$. Inset: the ratio of the fluorescent intensity at 550 nm and intensity at 594 nm as a function of $Cu^{2+}$ concentration. Excitation wavelength was 510 nm.

resulting from the addition of the $Cu^{2+}$ ion were not influenced by the subsequent addition of miscellaneous cations. All of these indicate that the selectivity of **1** for the $Cu^{2+}$ ion over other competitive cations in the water medium is remarkably high.

On the basis of the competition experiments, we realized that **1** had higher binding affinity for $Cu^{2+}$ than for $Zn^{2+}$. As expected, the addition of $Cu^{2+}$ into a solution of **1**-$Zn^{2+}$ resulted in immediate absorption (Figure S7 in Supporting Information) and fluorescence (Figure 4) changes. The emission maximum of **1**-$Zn^{2+}$ underwent a gradual blue-shift from 594 to 550 nm, implying that $Cu^{2+}$ can displace $Zn^{2+}$ to form the **1**-$Cu^{2+}$ complex. The ratio of **1**-$Cu^{2+}$to **1**-$Zn^{2+}$ emission intensities ($F_{550\ \mathrm{nm}}/F_{594\ \mathrm{nm}}$) varied from 0.5 to 4.6. Further more, in the competition experiment, only $Cu^{2+}$ ion exhibited obvious influence of the absorbance and fluorescence of **1**-$Zn^{2+}$ complex (Figures S8 and S9 in Supporting Information). These data indicate that **1**-$Zn^{2+}$ could be used for sensing $Cu^{2+}$ in micromolar range. Thus, **1** could be a dual-mode $Cu^{2+}$-selective sensor *via* the rhodamine ring-opening mechanism and ratiometric displacement in addition to ring-opening approach.

In conclusion, we have developed a novel fluorescent sensor **1**, which showed a ratiometric and "off−on" response to $Cu^{2+}$ based on rhodamine ring-opening approach. Moreover, the **1**-$Zn^{2+}$ can be displaced by $Cu^{2+}$, which resulted in another ratiometric sensing signal output. Thus, **1** can be a dual mode $Cu^{2+}$-selective sensor *via* the rhodamine ring-opening mechanism and ratiometric displacement mechanism.

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

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