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Cu(II)-Selective Green Fluorescence of a Rhodamine–Diacetic Acid Conjugate

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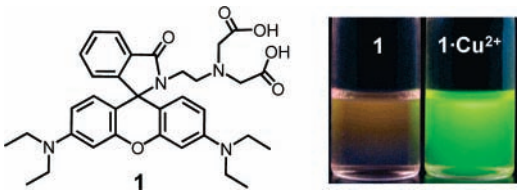
ABSTRACT



A new rhodamine derivative (1) containing an ethylenediamine-*N*,*N*-diacetic acid moiety exhibits Cu(II)-selective strong green fluorescence, while showing very weak orange fluorescence with other metal cations.

Design of fluorescent chemosensors for heavy and transition metal (HTM) cations is an area of intense research activity.[1] Much effort has been devoted to the development of Cu^{2+}-selective fluorescent chemosensors because of biological and environmental importance of Cu^{2+}.[2] So far, various Cu^{2+} sensors have been proposed; however, most of these show a turn-off (fluorescence quenching) response,[3] because Cu^{2+} usually acts as a quencher via an energy or electron-transfer process.[4] "Turn-on" type Cu^{2+} sensors have also been proposed;[5] however, most of these are photoexcited by UV light. There are only five reports of the turn-on type Cu^{2+}-selective sensors driven by visible light excitation.[5c−e,i,j]

Rhodamine is a molecule used extensively as a fluorescent labeling reagent and a dye laser source because of its excellent photophysical properties, such as long absorption and emission wavelengths elongated to visible region, high fluorescence quantum yield, and large absorption coefficient.[6] Recently, rhodamine-based fluorescent chemosensors[5i,7] or chemodosimeters,[5j,8] which are driven by visible light excitation and show turn-on response to the targeted HTM cation, such as Pb^{2+},[7a] Cu^{2+},[5i,j,8a] Hg^{2+},[7b−e,8b−d] Fe^{3+},[7f−i] and Cr^{6+},[8e] have been proposed. The cation-sensing mechanism of these

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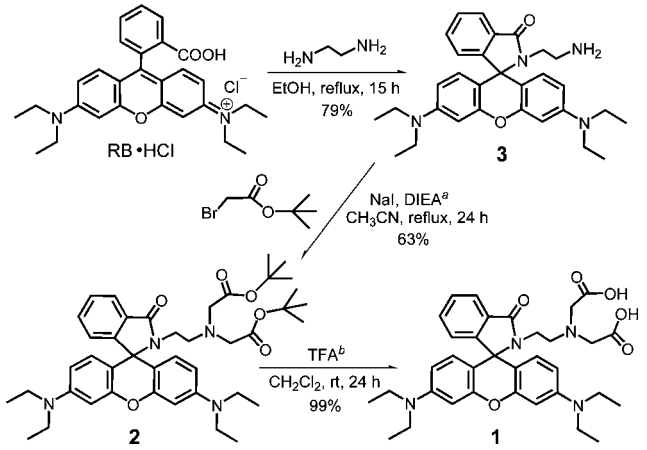
probes is based on the change in structure between spirocyclic and open-cycle forms. Without cations, these probes exist in a spirocyclic form, which is colorless and nonfluorescent. Addition of metal cation leads to a spirocycle opening via coordination[5i,7] or irreversible chemical reaction,[5j,8] resulting in an appearance of pink color and orange fluorescence.

Here we report that a new rhodamine derivative (**1**), containing an ethylenediamine-*N*,*N*-diacetic acid moiety, an analogue of a classical chelator EDTA (Scheme 1; Synthesis[9]), acts as a Cu^{2+}-selective fluorescent sensor. This demonstrates a strong "green" fluorescence upon binding with Cu^{2+}, while showing a very weak ordinary orange fluorescence with other metal cations. To the best of our knowledge, this is the first rhodamine-based fluorescent chemosensor showing green fluorescence.

Scheme 1. Synthesis of **1**



[a] *N*,*N*-Diisopropylethylamine. [b]Trifluoroacetic acid.

Figure 1 shows fluorescence spectra (λ_{ex} = 480 nm) of **1** (25 μM) measured in CH_3CN with respective metal cations (6 equiv). Without cations, **1** shows a very weak fluorescence at 575 nm; however, Cu^{2+} addition creates a remarkably enhanced (49-fold) and blue-shifted (45 nm) green fluorescence at 530 nm. Hg^{2+} also induces an emission enhancement, but the enhancement is very small (2.8-fold) and the emission appears at 570−580 nm (orange fluorescence), which is similar to that obtained with the already-reported rhodamine-based probes.[5i,j,7,8]

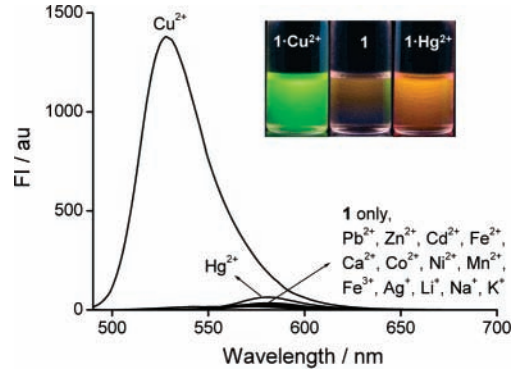


Figure 1. Fluorescence spectra (λ_{ex} = 480 nm) of **1** (25 μM) measured in CH_3CN with respective metal cations (6 equiv). For detailed intensity and spectra, see Figures S1 and S2.[9]

Figure 2 shows the results of fluorescence titration of **1** with Cu^{2+}. Upon addition of <2 equiv of Cu^{2+}, 575 nm emission increases. However, addition of >2 equiv of Cu^{2+} leads to a drastic increase in the shorter wavelength emission, along with a continuous blue-shift of the emission band. As shown in Figure 3 (bottom), the emission color continuously changes from orange to green with increasing Cu^{2+} amount. As shown in Figure 2 (inset), the emission intensity increases with increasing Cu^{2+} amount up to 6 equiv, but decreases at >6 equiv.[10] The blue-shift of the emission band also stops with >6 equiv of Cu^{2+} (Figure S3[9]). In contrast, Hg^{2+}

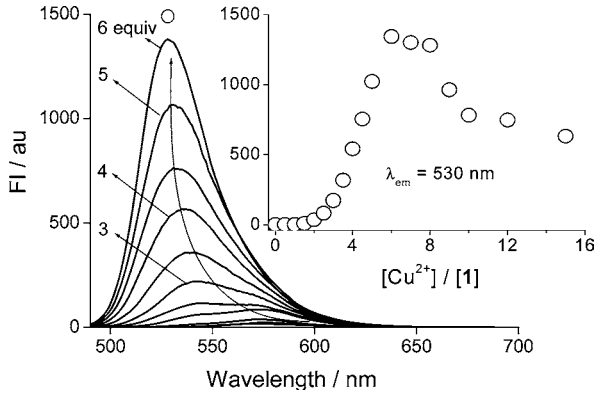


Figure 2. Change in fluorescence spectra of **1** (25 μM) in CH_3CN with the Cu^{2+} amount added (λ_{ex} = 480 nm). Inset: Change in the emission intensity monitored at 530 nm. For detailed change in spectra, see Figure S3.[9]

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(9) See the Supporting Information.

(10) Fluorescence quantum yield of **1** obtained with 6 equiv of Cu^{2+} is 0.32, where fluorescein (Φ_F = 0.85 in 0.1 M NaOH) is used as a standard: Parker, C. A.; Rees, W. T. *Analyst* **1960**, *85*, 587−600.

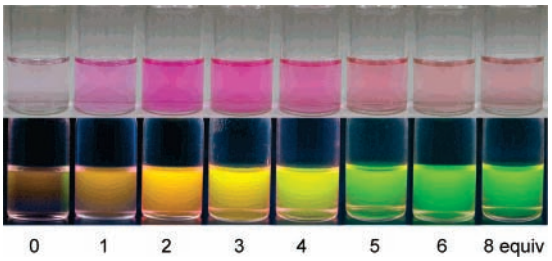

Figure 3. Change in (top) color and (bottom) fluorescence of **1** (25 μM) in CH_3CN with different Cu^{2+} amounts.

addition leads to a monotonous enhancement of the orange emission at 570−580 nm, where the emission blue-shift does not occur at any Hg^{2+} amount (Figure S4[9]), as is usually observed for the rhodamine-based probes.[5i,j,7,8]

Figure 4 shows absorption spectra of **1** (25 μM). Without

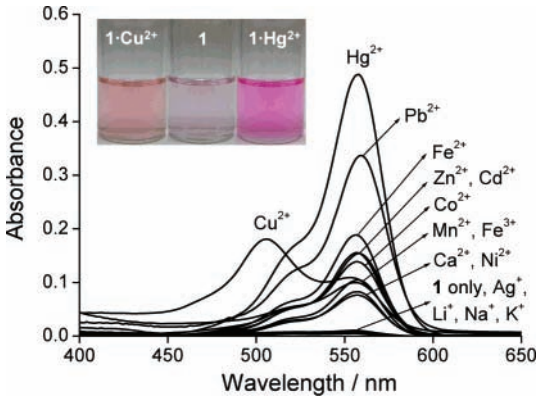

Figure 4. Absorption spectra of **1** (25 μM) upon addition of respective metal cations (6 equiv) in CH_3CN.

cations, **1** scarcely shows absorption at 500−600 nm, indicating that **1** exists as a spirocycle-closed form.[5i,j,7,8] This is confirmed by a distinctive spirocycle carbon shift at 65.84 ppm in the ^{13}C NMR spectrum of **1**.[9] Addition of metal cations (except for Li^+, Na^+, K^+, and Ag^+) leads to an appearance of 556 nm absorbance, as is also the case for the reported rhodamine-based probes.[5i,j,7,8] In contrast, Cu^{2+} addition shows a blue-shifted absorption at 506 nm.

Figure 5 shows the results of absorption titration of **1** with Cu^{2+}. With <2 equiv of Cu^{2+}, 556 nm absorption increases with increasing Cu^{2+} amount, along with an appearance of pink color (Figure 3, top). In contrast, with >2 equiv of Cu^{2+} (Figure 5), the 556 nm absorbance decreases, and 506 nm absorbance increases. As shown in Figure 3 (top), the pink color of the solution fades with >2 equiv of Cu^{2+}. The 506 nm absorbance, however, decreases with >6 equiv of Cu^{2+} (Figure 5, inset, closed circle). In contrast, Hg^{2+} addition leads to a monotonous increase in the 556 nm absorption, where the shorter wavelength absorption does not appear at any Hg^{2+} amount (Figure S4[9]).

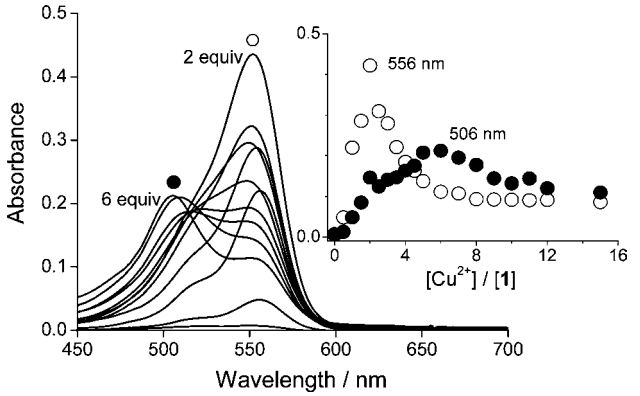

Figure 5. Change in absorption spectra of **1** (25 μM) in CH_3CN with Cu^{2+} addition. Inset: Change in absorbance at (open circle) 556 and (closed circle) 506 nm. For detailed absorption spectra with >6 equiv of Cu^{2+}, see Figure S5.[9]

IR analysis of **1** in CH_3CN (Figure S6[9]) reveals that both carboxylic and amide carbonyl absorption at 1749.1 and 1674.4 cm^{-1} shifts to lower frequency upon addition of Cu^{2+} (1634.9 cm^{-1}) and Hg^{2+} (1661.4 cm^{-1}). This indicates that both carbonyl groups are involved in the coordination with these cations.[7a,i] ^1H NMR titration in CD_3CN (Figure S7[9]) shows that the aromatic protons of **1** shift downfield and become broader upon Cu^{2+} addition. This is due to the decrease in electron density of the aromatic ring, indicating that **1** actually coordinates with Cu^{2+}.[7a,i] Addition of ethylenediamine to the solution of **1** containing either Cu^{2+} or Hg^{2+} leads to disappearance of both absorption and emission spectra, indicative of a reversible coordination between **1** and these cations.[5i,7] The above findings indicate that the emission enhancement of **1** upon addition of Cu^{2+} and Hg^{2+} involves the spirocycle-opening mechanism, as is also the case for related rhodamine-based sensors:[5i,7] coordination of metal cations with carboxylic and amide carbonyl groups of **1** leads to the spirocycle opening, resulting in appearance of the absorption and emission spectra (Figures 3 and S4[9]). The blue-shift of both absorption and emission spectra of **1** upon addition of >2 equiv of Cu^{2+} indicates that the system involves different mechanisms.

Figure 6 shows the contour map of the fluorescence intensity of **1** as a function of excitation/emission wavelengths measured with different Cu^{2+} amounts. With <2 equiv of Cu^{2+} (Figure 6a,b), excitation/emission maximum appears at 560/575 nm (orange emission). In contrast, with >2 equiv of Cu^{2+} (Figure 6c−f), a new maximum appears at 510/530 nm (green emission). With >5 equiv of Cu^{2+} (Figure 6e,f), the latter maximum becomes predominant along with disappearance of the former maximum. In contrast, Hg^{2+} shows a single set of maximum at 560/580 nm (orange emission) (Figure S8[9]), which is similar to that obtained with <2 equiv of Cu^{2+}. This suggests that the spirocycle-opening process is actually involved in the emission enhancement of **1** with Cu^{2+}. The appearance of the 510/530 nm maximun with >2 equiv of Cu^{2+} (Figure 6c−f) indicates that the green emission of **1** originates from the

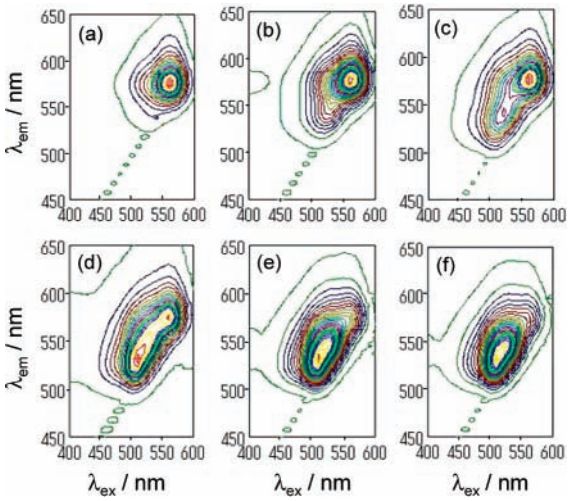

Figure 6. Contour map of the fluorescence intensity of **1** (25 μM) as a function of excitation/emission wavelengths measured with (a) 1, (b) 2, (c) 3, (d) 4, (e) 5, and (f) 6 equiv of Cu^{2+} in CH_3CN. For detailed excitation spectra, see Figure S9.[9]

direct photoexcitation of the ground-state species formed through association between **1** and Cu^{2+} (the green emitting species). This is supported by the appearance of the blue-shifted absorption at 506 nm (Figure 5).

It is well-known that rhodamine dye tends to aggregate in solution.[11] This leads to a bathochromic (J-type) or hypsochromic (H-type) shift of the absorption band. The blue-shifted absorption of **1** obtained with >2 equiv of Cu^{2+} (Figure 5) agrees well with the hypsochromic shift of the H-aggregate of rhodamine dye.[11] It is, however, well-known that the H-aggregate is nonfluorescent on the basis of the exciton theory.[11] A few exceptions have been reported for the fluorescent H-aggregate; however, they show a "red-shifted" emission.[12] The detailed mechanism of the Cu^{2+}-induced green emission of **1** cannot be clarified at this stage; however, some characteristic properties were observed in the preliminary investigation. A Job's plot (A_{556nm} and A_{506nm}) shows unresolved maximum absorption at X (=$[Cu^{2+}]/([Cu^{2+}] + [\mathbf{1}])$) = 0.55−0.65, while the plot with Hg^{2+} (A_{556nm}) shows a clear 1:1 stoichiometry (Figure S10[9]). This means that the green emitting species have a complicated stoichiometry. In addition, as shown in Figure 5, the absorption spectra of **1** do not show a clear isosbestic point. This suggests that multiple species form through association of **1** with different amounts of Cu^{2+}, leading to formation of the green emitting species. It must also be noted that the saturation of the emission increase after Cu^{2+} addition requires >60 min (300 K), whereas Hg^{2+} shows rapid saturation (<5 min) (Figure S11[9]). These findings and the inherent aggregation property of rhodamine suggest that the green emitting species may be a self-assembled "aggregate" containing multiple **1** molecules, formed by coordination association with multiple Cu^{2+} ions.[13] Rhodamine aggregation is enhanced at high temperature due to a decrease in a solvation interaction.[14] As shown in Figure S11,[9] the saturation of the green emission increase after Cu^{2+} addition occurs more rapidly at higher temperature. This means that the aggregation is the crucial factor for the green emitting species formation.[15]

In conclusion, we found that a new rhodamine derivative (**1**) exhibits Cu^{2+}-selective green fluorescence in CH_3CN,[16] while showing very weak orange fluorescence with other metal cations. Although the detailed mechanism remains to be explained, the results presented here may contribute to the development of more useful fluorescent sensors for HTM cations based on the rhodamine platform.

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Supporting Information Available: Materials, synthesis, methods, and supplementary data (Figures S1−S21). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Formation of insoluble material was not detected by dynamic light scattering analysis (detection limit: 3 nm) at any Cu^{2+} amount, indicating that the green emitting species is the "soluble" aggregate.

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(15) Without Cu^{2+}, niether absorption nor fluorescence of **1** blue-shifts even with high concentration of **1** up to 0.33 mM (Figure S12[9]). This indicates that the coordination association of **1** with Cu^{2+} triggers the aggregation, leading to formation of the green emitting species.

(16) The fluorescence intensity of **1** (25 μM), when measured with 6 equiv of Cu^{2+} in CH_3CN containing 0.1% water, is similar to that obtained without water; however, 30% and 100% intensity reductions occur in the presence of 1% and 5% water, respectively.