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Fe(III)- and Hg(II)-selective dual channel fluorescence of a rhodamine–azacrown ether conjugate

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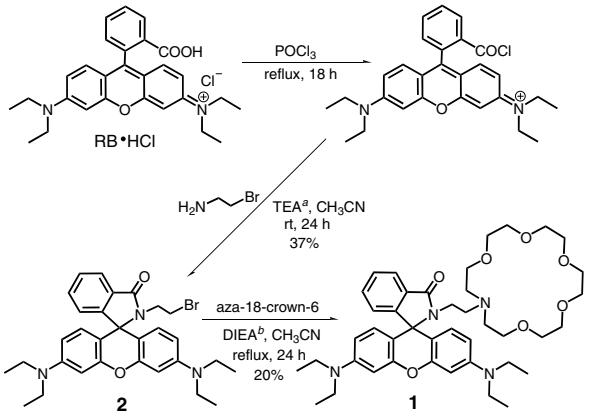
Abstract

A rhodamine–azacrown ether conjugate (**1**) demonstrates Fe(III)-selective green fluorescence, while showing Hg(II)-selective orange fluorescence. This is the first example of rhodamine-based fluorescent probe that shows dual channel fluorescence for two different metal cations.

Fluorometric detection of ionic species has attracted a great deal of attention.[1] Of particular interest is the development of fluorescent probes for heavy and transition metal cations, such as Hg^{2+} and Fe^{3+}, due to their biological and environmental importance.[2] Hg^{2+} is one of the most hazardous components in the environment,[3] and Fe^{3+} plays a pivotal role in many biochemical processes in a cellular level.[4] A number of fluorescent probes for the detection of Hg^{2+} and Fe^{3+} have been proposed so far.[5,6] However, most of these probes show fluorescence quenching (turn-off) response,[5] and fluorescent probes that show fluorescence enhancement (turn-on) response are still rare.[6]

Rhodamine is a dye used extensively as a fluorescent labeling reagent due to its excellent photophysical properties, such as long absorption and emission wavelengths elongated to visible region, high absorption coefficient, and high fluorescence quantum yield.[7] Recently, various rhodamine-based turn-on fluorescent probes for Hg^{2+} or Fe^{3+} have been proposed.[8,9] The cation sensing mechanism of these probes is based on the change in structure between the spirocyclic and open-cycle forms. Without cations, these probes exist in a non-emissive spirocyclic form. Addition of metal cation leads to spirocycle opening via a reversible coordination or an irreversible chemical reaction with the probe, resulting in an appearance of orange fluorescence (550–650 nm). These probes show this single channel fluorescence against Hg^{2+} or Fe^{3+} and, hence, can detect either Hg^{2+} or Fe^{3+}.

Herein, we report that a new rhodamine derivative (**1**) containing an aza-18-crown-6 moiety (Scheme 1, synthesis[10]) behaves as a dual channel fluorescent probe for Hg^{2+} and Fe^{3+}. Compound **1** shows Hg^{2+}-selective ordinary orange fluorescence (550–650 nm), while showing



Scheme 1. Synthesis of the probe **1**. (a) Triethylamine. (b) *N*,*N*-Diisopropylethylamine.

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Fe^{3+}-selective green fluorescence (490–600 nm). To the best of our knowledge, this is the first example of the rhodamine-based fluorescent probe that shows dual channel fluorescence for two different metal cations.

Figure 1 shows fluorescence spectra ($\lambda_{ex} = 480$ nm) of **1** (5 μM) measured in CH_3CN with respective metal cations (90 equiv). Without cations, **1** is non-fluorescent. Addition of Hg^{2+}, however, leads to an appearance of orange fluorescence at 578 nm (fluorescence enhancement: 45-fold, Fig. S1[10]). In contrast, addition of Fe^{3+} creates a remarkably enhanced green fluorescence at 525 nm (fluorescence enhancement: 378-fold, Fig. S1[10]). These data clearly indicate that probe **1** detects Hg^{2+} and Fe^{3+} with two different fluorescence channels.

Figure 2 shows the results of fluorescence titration of **1** with Hg^{2+} and Fe^{3+}. Hg^{2+} addition (Fig. 2a) leads to a monotonous increase in the orange fluorescence (578 nm), where the increase is saturated with 12 equiv of Hg^{2+}.[11] This emission behavior is similar to that for early-reported rhodamine-based Hg^{2+} probes.[8] As shown in Figure 2b, addition of <10 equiv of Fe^{3+} creates similar emission at ca. 575 nm. Further Fe^{3+} addition, however, leads to blue-shift of the emission, along with a drastic intensity increase, where the emission color changes from pale orange to green (Fig. S2[10]). The blue-shift and enhancement of the emission stop upon the addition of 90 equiv of Fe^{3+} (Fig. 2b).[11] It must be noted that the Fe^{3+}-selective 'green' emission is the first example among the rhodamine-based probes.[9]

As shown in Figure 3, without cations, **1** scarcely shows an absorption at 500–600 nm, indicating that **1** exists as a spirocycle-closed form.[8,9] This is confirmed by a distinctive spirocycle carbon shift at 64.81 ppm in the ^{13}C NMR spectrum of **1**.[10] Hg^{2+} addition leads to an appearance of strong 556 nm absorption, along with a clear color change from colorless to pink, as is observed for ordinary rhodamine-based probes.[8,9] Fe^{2+}, Cu^{2+}, and Pb^{2+} show minor absorption increase, whereas other metal cations show negligible increase. In contrast, Fe^{3+} shows a blue-shifted absorption at 502 nm.

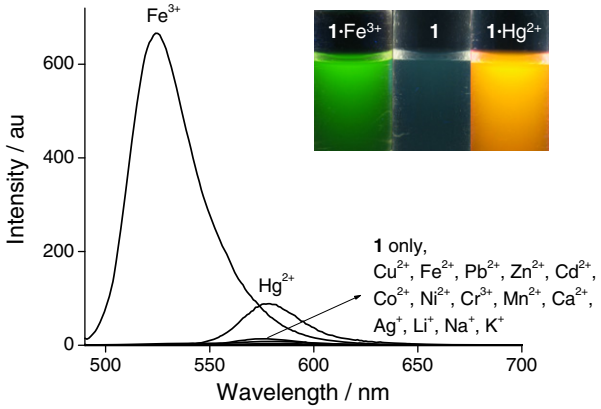

Fig. 1. Fluorescence spectra of **1** (5 μM) measured in CH_3CN with 90 equiv of various metal cations ($\lambda_{ex} = 480$ nm). Change in fluorescence color (inset).

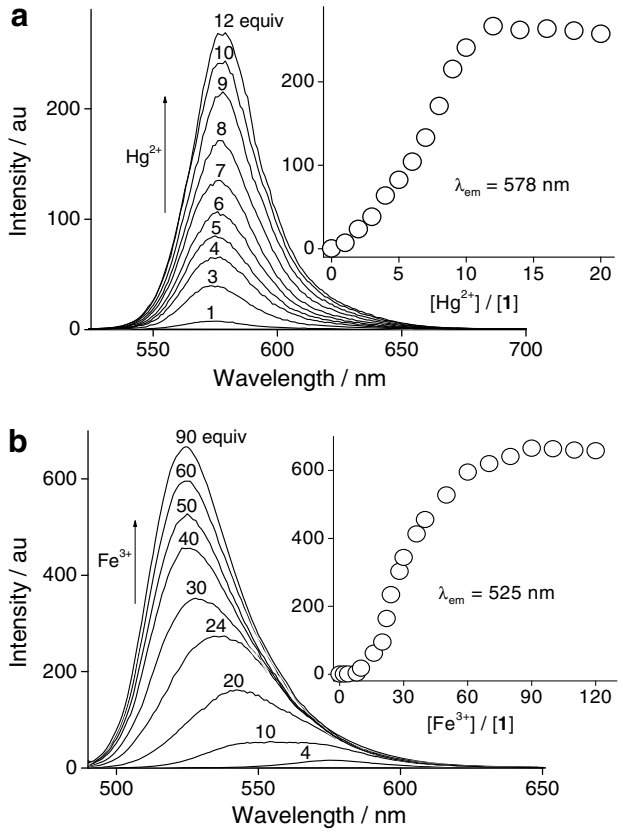

Fig. 2. Fluorescence titration of **1** (5 μM) in CH_3CN with (a) Hg^{2+} ($\lambda_{ex} = 510$ nm) and (b) Fe^{3+} ($\lambda_{ex} = 480$ nm). Intensity change (inset).

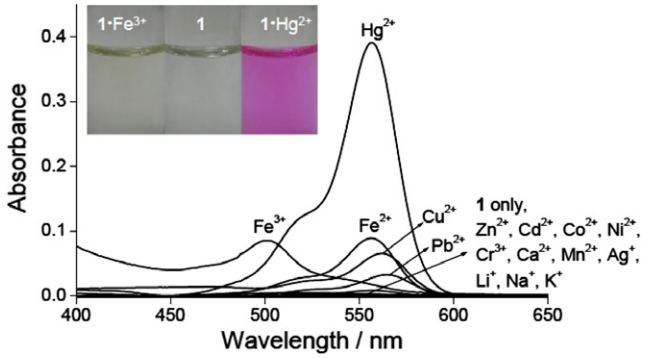

Fig. 3. Absorption spectra of **1** (5 μM) measured in CH_3CN with respective metal cations (90 equiv). Change in solution color (inset).

Figure 4 shows the results of absorption titration of **1**. Hg^{2+} addition leads to monotonous increase in the 556 nm absorption, which is saturated upon the addition of 12 equiv of Hg^{2+}. With <10 equiv of Fe^{3+}, 556 nm absorption also increases. However, with >10 equiv of Fe^{3+}, the absorption decreases and the 502 nm absorption then increases. This increase is saturated with >90 equiv of Fe^{3+}.

Excitation spectra of **1** with Hg^{2+}, monitored at 580 nm (orange emission), appear at 559 nm (Fig. S3[10]), which are similar to the absorption spectra (Fig. 4a). With <10 equiv of Fe^{3+}, similar excitation spectra appear at 559 nm (Fig.

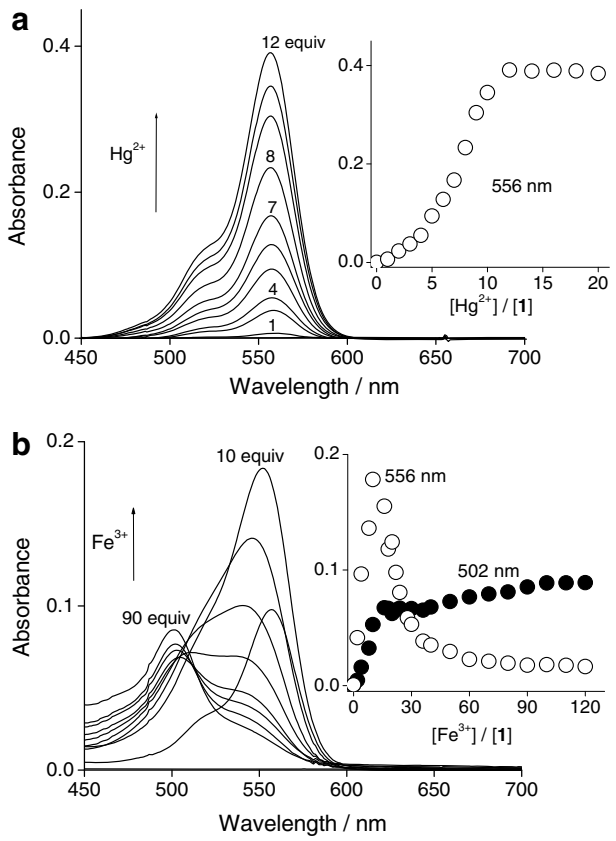

Fig. 4. Absorption titration of **1** (5 μM) in CH_3CN with (a) Hg^{2+} and (b) Fe^{3+}. Change in absorbance (inset).

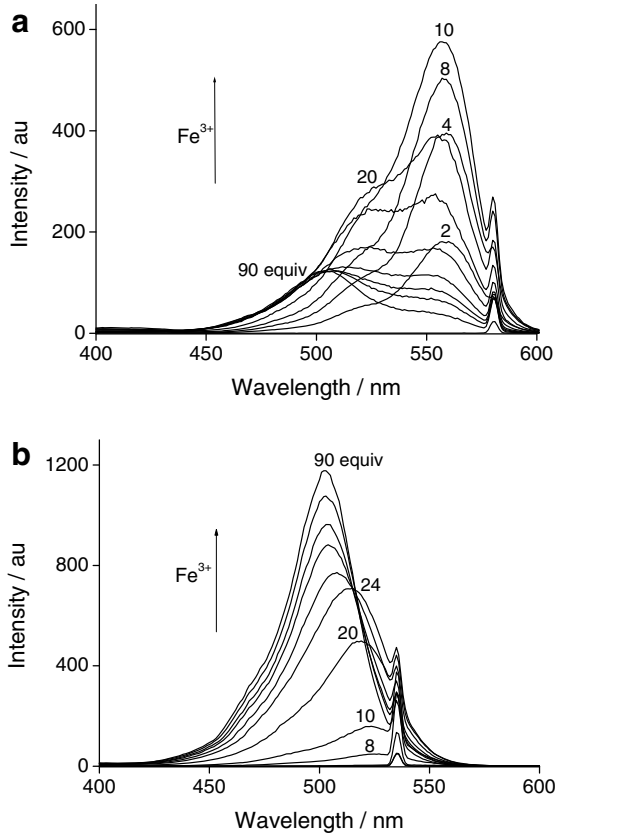

Fig. 5. Excitation spectra of **1** (5 μM) in CH_3CN with Fe^{3+} monitored at (a) 580 nm and (b) 530 nm.

5a). However, with >10 equiv of Fe^{3+}, this band decreases and a blue-shifted band appears at 450–550 nm; with 90 equiv of Fe^{3+}, only the 505 nm band remains. The appearance of the blue-shifted band is more apparent when the spectra are monitored at 530 nm (green emission; Fig. 5b): excitation band blue-shifts continuously upon Fe^{3+} addition. These suggest that the respective orange and green emissions originate from different ground state species.

Hill analysis of the fluorescence titration data (578 nm) obtained with Hg^{2+} (Fig. S4[10]) provides a Hill coefficient $n = 2.0$ with association constant log $K_a = 8.7$, indicative of the formation of $\mathbf{1}\cdot(Hg^{2+})_2$ complex.[12] In contrast, analysis of the titration data obtained with Fe^{3+} (525 nm; Figure S4[10]) provides an unresolved coefficient $n = 3.3$ (log $K_a = 12.6$). This indicates that **1** associates with Fe^{3+} in a complicated stoichiometry.

IR analysis of **1** in CH_3CN (Fig. S5[10]) reveals that both amide carbonyl (C=O) and ether (C–O) absorptions of **1** at 1684.5 and 1120.5 cm^{-1} shift to lower frequency upon the addition of Hg^{2+} (1632.9 and 1100.2 cm^{-1}) and Fe^{3+} (1635.8 and 1101.6 cm^{-1}). This indicates that both carbonyl and azacrown ether moiety are involved in metal cation coordination.[9d,13] ^1H NMR titration in CD_3CN (Fig. S6[10]) shows that both aromatic and azacrown ether protons of **1** shift downfield and become broader upon the addition of Fe^{3+} or Hg^{2+}. This is due to the decrease in electron density of these moieties, indicating that **1** actually coordinates with Hg^{2+} or Fe^{3+}.[9d,13] In addition, upon addition of Hg^{2+} or Fe^{3+}, CH_3 proton of **1** (1.12 ppm) decreases, and new protons appear downfield, indicating that the coordination of **1** with Hg^{2+} or Fe^{3+} leads to spirocycle opening.[9d] Addition of ethylenediamine to the solution of **1** containing either Hg^{2+} or Fe^{3+} leads to the disappearance of both absorption and emission spectra, indicating that **1** coordinates reversibly with these cations.[8,9,13] The emission behaviors of **1** are therefore explained by the ordinary spirocycle opening mechanism:[8,9,13] coordination of metal cations with the amide carbonyl and azacrown ether moieties of **1** leads to the formation of spirocycle-opened emitting species (orange emitter). The formation of the 'green' emitter of **1** upon Fe^{3+} addition involves a different mechanism in addition to the spirocycle opening mechanism.

Recently, we found that a rhodamine derivative containing an ethylenediamine-*N*,*N*-diacetic acid moiety shows blue-shifted absorption and emission spectra upon Cu^{2+} addition.[14] Inherent aggregation properties of rhodamine in solution[15] and the spectral data imply that the emitting species for the blue-shifted emission is an 'aggregate' of multiple molecules formed by coordination association with multiple Cu^{2+} ions. The absorption and emission behaviors of the rhodamine derivative are similar to those of **1** with Fe^{3+}; therefore, the green emission of **1** upon

Fe^{3+} addition may probably be explained by Fe^{3+}-induced aggregation mechanism. This assumption is supported by some spectral data: Hill analysis of the fluorescence titration data shows unresolved stoichiometry ($n = 3.3$; Fig. S4[10]); absorption spectra do not show clear isosbestic point (Fig. 4b). These indicate that **1** aggregates via coordination association with multiple Fe^{3+} ions. The appearances of the blue-shifted excitation spectra (Fig. 5) clearly indicate that the green emission is formed via direct photoexcitation of the ground state aggregates. In addition, saturation of the green emission increase after Fe^{3+} addition (296 K) requires >5 h, while the orange emission increase after Hg^{2+} addition saturates relatively faster (<1 h) (Fig. S7[10]). As reported,[16] rhodamine aggregation is enhanced at higher temperature due to the decrease in solvation interaction. The green emission increase after Fe^{3+} addition occurs rapidly at higher temperature (Fig. S7[10]). These findings clearly indicate that the aggregation interaction is involved in the formation of the green emitter.

In conclusion, we found that a new rhodamine derivative (**1**) containing an azacrown ether moiety shows Fe^{3+}- and Hg^{2+}-selective dual channel fluorescence in CH_3CN.[17] This is the first rhodamine-based probe showing dual channel fluorescence for different metal cations. Although the detailed mechanism for the Fe^{3+}-selective green emission is unclear, the results presented here may contribute to the design of more useful rhodamine-based fluorescent probes for heavy and transition metal cations.

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Supplementary data

Supplementary data (materials, synthesis, methods, and Figures) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.102.

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17. The probe **1** does not work in aqueous medium: the fluorescence of **1** with Fe^{3+} or Hg^{2+} is completely quenched by an addition of 5% water to CH_3CN.