

A New bis(rhodamine)-Based Fluorescent Chemosensor for Fe³⁺

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Abstract A new bis(rhodamine)-based fluorescent probe **4** was synthesized, and it exhibited high selectivity for Fe³⁺ over other commonly coexistent metal ions in both 50% ethanol and Tris–HCl buffer. Upon the addition of Fe³⁺, the spirocyclic ring of **4** was opened and a significant enhancement of visible color and fluorescence in the range of 500–600 nm was observed.

Keywords Fluorescent probe · Rhodamine · Fe³⁺

Introduction

During the recent two decades, the design and synthesis of compounds for sensing environmentally and biologically relevant important ionic species, particularly for heavy metal and transition metal cations, is currently

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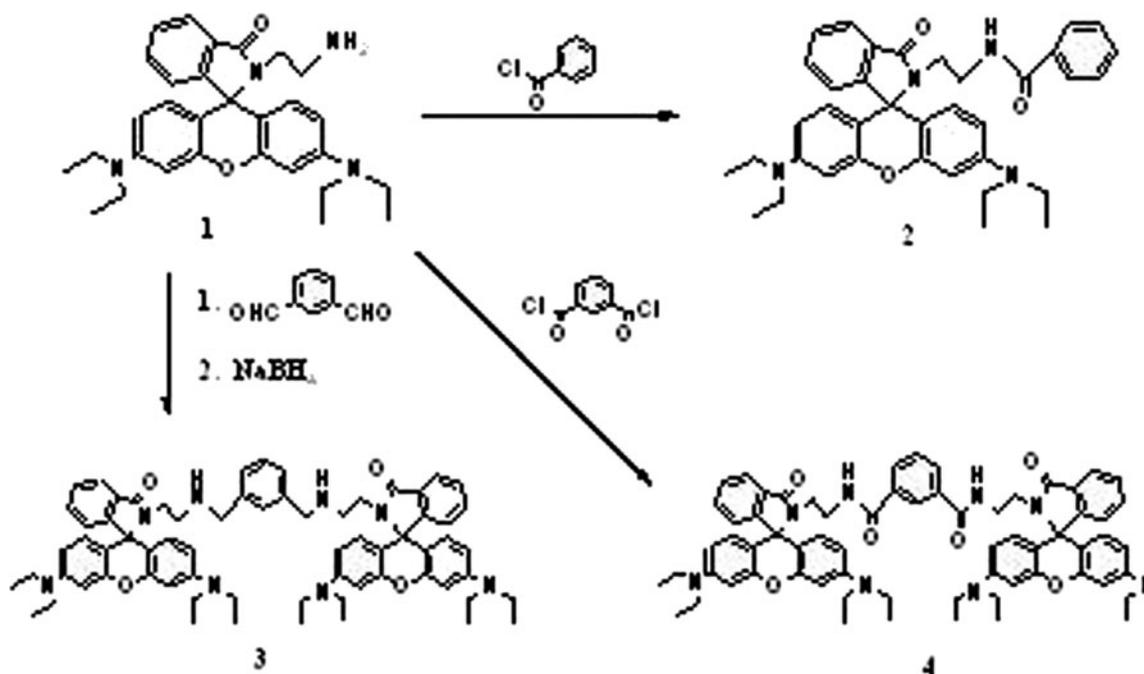
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of great interest [1–5]. Numerous excellent works focus on the selective and sensitive detection of transition metal ions; e.g., detection of Cu²⁺, Pb²⁺, Zn²⁺, and Hg²⁺ have been reported [6–15]. The trivalent form of iron is an essential element in man. It provides the oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain [16–18]. However, the development of new fluorescent Fe³⁺ indicators, especially those that exhibit selective Fe³⁺-amplified emission [19–22], is still a challenge. Therefore, there is an urgent need to develop chemical sensors that are capable of detecting the presence of iron in environmental and biological samples at a physiological pH value. On the other hand, rhodamine derivatives are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and a pink color [23–28]. Recently, some bis(rhodamine)-based fluorescent probe were reported for the detection of Fe³⁺ metal ions [29–31]. In this paper, we report three new rhodamine-based turn-on fluorescent sensors **2–4** (Scheme 1) for Fe³⁺. Sensor **4** shows very high sensitivity and selectivity toward Fe³⁺ over other metal ions.

Experimental Section

All the materials for synthesis were purchased from commercial suppliers and used without further purification. The solutions of metal ions were prepared from their nitrate salts, except for FeCl₂, CrCl₃ and MnCl₂. The use of Fe(NO₃)₃ and FeCl₃ yielded nearly the same results. Tris–HCl buffer solutions (pH=7.15) were prepared using 0.01 M Tris, 0.1 M KNO₃ and proper amount of HCl (about 0.01 M).



Scheme 1 Synthetic route of title compounds

Rhodamine B derivative (**1**) was synthesized according to the literature [1–5]. A Hitachi F-4500 spectrofluorimeter was used for fluorescence measurements. The absorption spectra were recorded with a Techcomp UV-8500 spectrophotometer (Shanghai, China). NMR spectra were measured on a Bruker AMX-400 spectrometer at 400 MHz in CDCl₃. Elemental analyses were carried out with a Flash EA 1112 instrument. Mass spectra were acquired in positive ion mode using a Bruker ESQUIRE 3000 ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to *m/z* 6000.

Synthesis of 2

Benzoyl chloride (0.140 g, 1 mmol) was slowly added into the mixture of **1** (0.484 g, 1 mmol) and triethylamine (0.5 mL) in 20 mL CH₂Cl₂ at 0 °C. After the addition, the mixture was stirred at room temperature for 5 h. The solvent was removed and the residue was purified by silica gel column chromatography with EtOAc/PE (4/1, v/v) as eluent to afford **2**, yield 98.5%; ¹H NMR (400 MHz, CDCl₃, δ ppm): 8.33 (s, 1H), 7.96 (m, 3H), 7.47 (m, 5H), 7.10 (s, 1H), 6.48 (s, 1H),

6.46 (s, 1H), 6.41 (d, 2H, *J*=3 Hz), 6.28 (d, 2H, *J*=3 Hz), 3.47 (m, 2H), 3.23–3.37 (m, 8H), 3.23 (m, 2H), 1.16–1.19 (m, 12H); ¹³C NMR (100 MHz, CDCl₃, δ ppm): 170.3, 166.9, 153.9, 153.3, 148.9, 134.3, 132.8, 131.1, 130.3, 128.4, 128.1, 127.1, 123.9, 122.8, 108.3, 104.5, 97.7, 65.9, 44.3, 41.9, 39.9, 12.6; ESI-MS: *m/z* 589.2 [M+H]⁺; Element analysis (%): found: C 75.50, H 6.88, N 9.54, calcd: C 75.48, H 6.85, N 9.52.

Synthesis of 3

Isophthalaldehyde (0.166 g, 1.24 mmol) was slowly added into the solution of **1** (1.20 g, 2.48 mmol) in 30 mL MeOH under argon. After the addition three drops of AcOH and some 4 Å molecular sieve, the mixture was refluxed for 8 h. NaBH₄ (0.469 g, 12.4 mmol) was added in three portions at 0 °C. Then the reaction was stirred for 12 h at room temperature before quenching with HCl (5 mL) and adjusted to pH 7 by NaOH aqueous solution. After removal of the solvent, the residue was extracted with HCCl₃ (3 × 15 mL). The combined organic layers were washed with brine and dried over Na₂SO₄. Removal of the solvents *in vacuo* and purification by silica gel column chromatography with

Fig. 1 Color change of the neutral buffer solution of **4** in the presence of metal ions



CH₂Cl₂/MeOH (20/1, v/v) yielded the desired product **3** as solid. yield 85.7%, M.p: 121–123 °C. ¹H NMR (400 MHz, CDCl₃, δ ppm):7.89 (m, 2H), 7.42 (m, 4H), 7.06 (m, 5H), 6.38 (m, 8H), 6.22 (m, 4H), 3.56 (br, 4H), 3.29–3.35 (m, 20H), 2.45(br, 4H), 1.14–1.17 (t, 24H, J=6.8 Hz); ¹³C NMR (100 MHz, CDCl₃, δ ppm):168.7, 153.6, 153.2, 148.7, 139.4, 132.4, 130.9, 128.6, 128.2, 128, 126.8, 123.7, 122.7, 108.1, 105.3, 97.7, 65.1, 52.9, 47.5, 44.3, 39.9, 12.6; ESI-MS: *m/z* 1071.7 [M+H]⁺; Element analysis (%): found: C 76.26, H 7.21, N 10.43, calcd: C 76.23, H 7.34, N 10.46

Synthesis of 4

m-Phthaloyl chloride (0.17 g, 0.843 mmol) was slowly added into the mixture of **1** (0.918 g, 1.897 mmol) and triethylamine(1 ml) in 10 mL CH₂Cl₂ at 0 °C. After the addition, the mixture was stirred at room temperature for 5 h. The solvent was removed and the residue was purified by silica gel column chromatography with EtOAc/PE (2/1, v/v) as eluent to afford **4**, yield 95.8%, M.p : 166–168 °C; ¹H NMR (400 MHz, CDCl₃, δ ppm):8.39 (s, 1H), 8.21 (s, 1H), 8.02 (s, 1H), 8.0 (s, 1H), 7.95 (m, 2H), 7.53 (t, 1H, J=8 Hz), 7.45 (m, 4H), 7.1 (m, 2H), 6.47 (d, 4H, J=8.8 Hz), 6.39 (m, 4H), 6.29 (d, 2H, J=2.4 Hz), 6.27 (d, 2H, J=2.4 Hz), 3.46 (m, 4H), 3.28–3.44 (m, 16H), 3.26 (m, 4H), 1.16 (t, 24H, J=6.8 Hz); ¹³C NMR (100 MHz, CDCl₃, δ ppm):170.2, 166.4, 153.8, 153.2, 148.9, 134.6, 132.7, 130.4, 129.7, 128.7, 128.4, 128.1, 126.1, 123.8, 123, 108.3, 104.6, 97.7, 65.3, 44.3, 41.5, 40, 12.6; ESI-MS: *m/z* 1098.6 [M+H]⁺, *m/z* 1121.6 [M+Na]⁺; Element analysis (%): found: C 74.26, H 6.76, N 10.21, calcd: C 74.29, H 6.78, N 10.19.

Results and Discussion

Compound **1** was facily synthesized from rhodamine B and ethylenediamine according to procedures in the

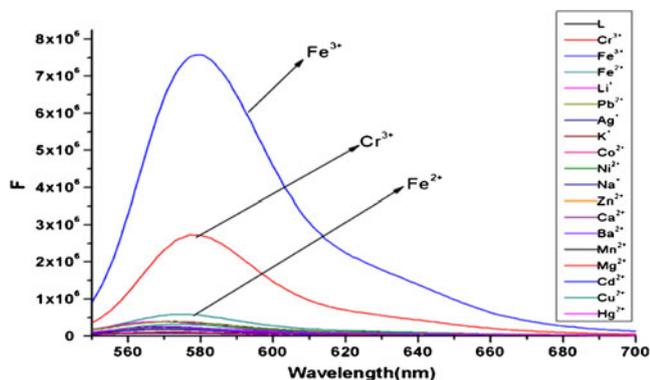


Fig. 2 Fluorescence intensity of **4** (10 μM) and 16 eq of various other metal ions in ethanol–water (1: 1,v/v) Solution Ex:548 nm, Em: 575 nm slit: 5

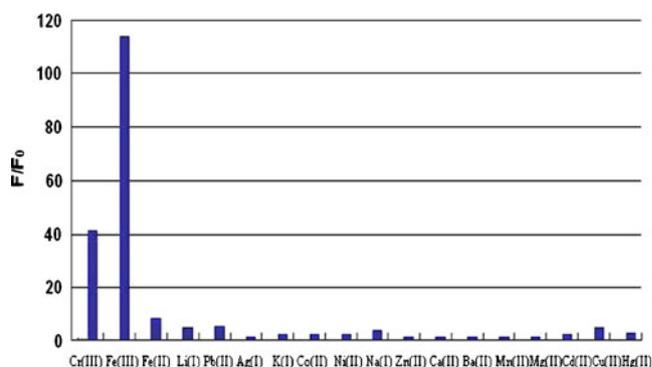


Fig. 3 Changes of fluorescence intensity (F/F_0) of compound **4** (10 μM) upon the addition of different metal ions in ethanol–water solution. F and F_0 are the fluorescence intensities of **4** with and without adding the interference

literature and obtained as light orange crystals. As shown in Scheme 1, compounds **2** and **4** were prepared in high yield by reacting **1** with benzoyl chloride and *m*-phthaloyl chloride in the presence of triethylamine respectively. Compound **3** was synthesized in two steps: production of Schiff's bases from **1** and isophthalaldehyde, followed by reduction by NaBH₄ in MeOH. Their structures have been confirmed using ¹H NMR, ¹³C NMR, ESI mass spectrometry, and elemental analysis (see Supporting Information). Although **4** is a derivative of rhodamine B, it forms a nearly colorless solution in either Tris–HCl aqueous buffer (pH 3–9) or absolute ethanol, indicating that the spirocyclic form exists predominantly. The characteristic peak near 65.0 ppm (9-carbon) in the ¹³C NMR spectrum of **4** also supports this consideration [32]. Besides, neither the color nor the fluorescence (excited at 530 nm) characteristics of rhodamine could be observed for **4** between pH 3.0 and 9.0 in water, suggesting that the spirocyclic form was still preferred in this range (S-Figure 10 and 11).

The free probes **2–3** are also colorless and nonfluorescent because they are both in “ring-closed” states. After addition of Fe³⁺ to **2–3** solutions, the complexation induced the three

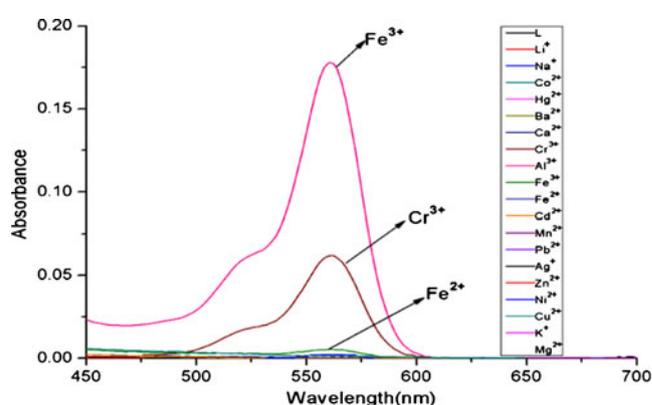


Fig. 4 Changes in the absorption spectra of **4** in the presence of different metal ions in ethanol in ethanol-water

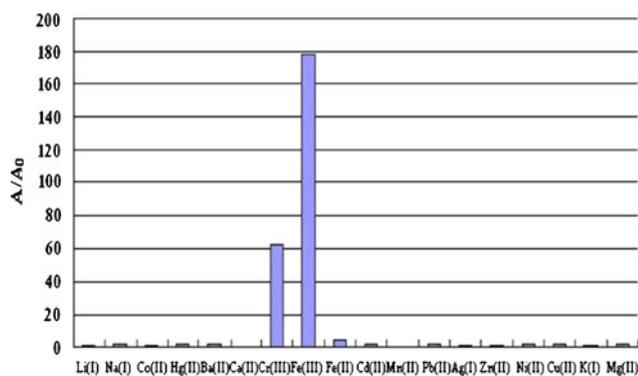


Fig. 5 Change in the absorbance at 560 nm of **4** (10 μ M) in presence of 16 eq of various different metal ions in ethanol–water (1 1,v/v)

probes to their “ring-open” states, thus leading to evident color change (from colorless to brilliant pink) and emission of a strong fluorescence. The fluorescence enhancement of **2** to Fe^{3+} , Cr^{3+} and Fe^{2+} is as high as 33, 20 and 6-fold, respectively (S-Figure 6). The fluorescence enhancement of **3** (10 μ M) to Fe^{3+} (16 equiv) is as high as 53-fold (S-Figure 8). The colorimetric and fluorometric responses between the probes and Fe^{3+} can also be conveniently detected by the naked eye (S-Figure 9).

Interestingly, the addition of Fe^{3+} into the colorless solutions (in both neutral buffer and ethanol) of **4** also generated a purple color and orange fluorescence rapidly, while other ions, such as Li^+ , Pb^{2+} , Ag^+ , K^+ , Co^{2+} , Ni^{2+} , Na^+ , Zn^{2+} , Ca^{2+} , Ba^{2+} , Mn^{2+} , Mg^{2+} , Cd^{2+} , Cu^{2+} and Hg^{2+} , gave no visible change except for Cr^{3+} and Fe^{2+} , which caused a mild effect compared to Fe^{3+} in ethanol-water (1:1). This interesting feature reveals that **4** can serve as a selective “naked-eye” chemosensor for Fe^{3+} (Fig. 1).

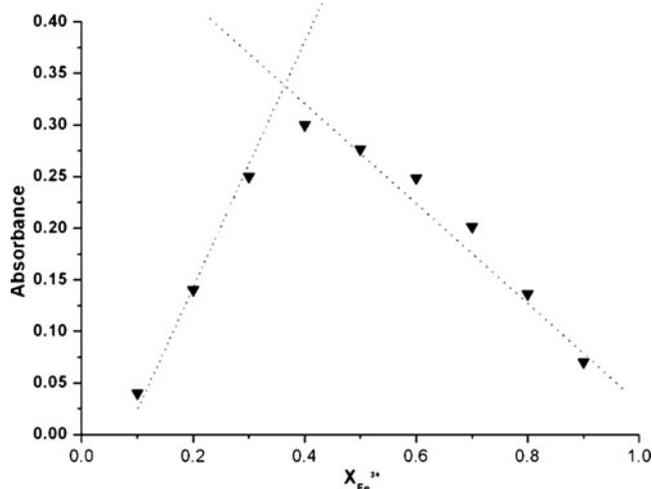


Fig. 6 Plots according to the method for continuous variations, indicating the 1:2 stoichiometry for **4**- Fe^{3+} (the total concentration of **4** and Fe^{3+} is 100 μ M). $X_{\text{Fe}^{3+}} = C_{\text{Fe}^{3+}} / (C_{\text{Fe}^{3+}} + C_4)$

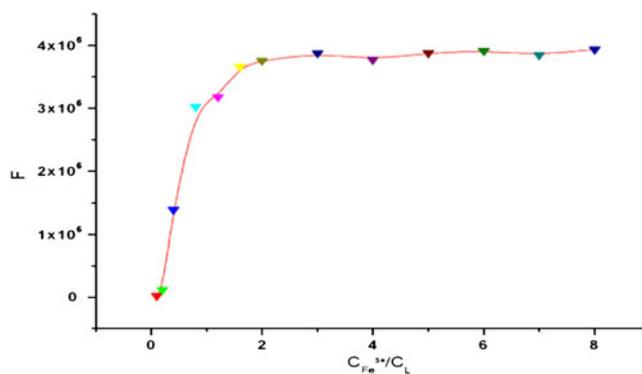


Fig. 7 Fluorescence intensities of **4** (80 μ M) with gradual addition of different amounts of (from bottom 0–8 eq Fe^{3+})

The fluorescence enhancement effects of various metal ions on **4** were investigated under excitation at 548 nm (Figs. 2 and 3). In the absence of metal ions, **4** exhibited a very weak fluorescence peak near 580 nm, which was probably the emission of trace open-ring molecules of **4**. When Fe^{3+} was introduced to a 10 μ M solution of **4** in ethanol-water, a bit red shift (~ 5 nm) and obvious enhancement of fluorescence spectra were observed, whereas other ions of interest displayed much weaker response. In ethanol-water, **4** (10 μ M) exhibited a 117-fold enhancement of fluorescence intensity at peak wavelength $\lambda_{\text{max}} = 575$ nm in the presence of 16 equiv Fe^{3+} . A mild fluorescence enhancement factors (FEF) were also detected for Cr^{3+} (40-fold), Fe^{2+} (6-fold), and Li^+ , Pb^{2+} , Ag^+ , K^+ , Co^{2+} , Ni^{2+} , Na^+ , Zn^{2+} , Ca^{2+} , Ba^{2+} , Mn^{2+} , Mg^{2+} , Cd^{2+} , Cu^{2+} or Hg^{2+} showed nearly no response (Fig. 2). **4** displays better selectivity and sensitivity than **2** and **3** maybe due to it has more amides to binding the Fe^{3+} .

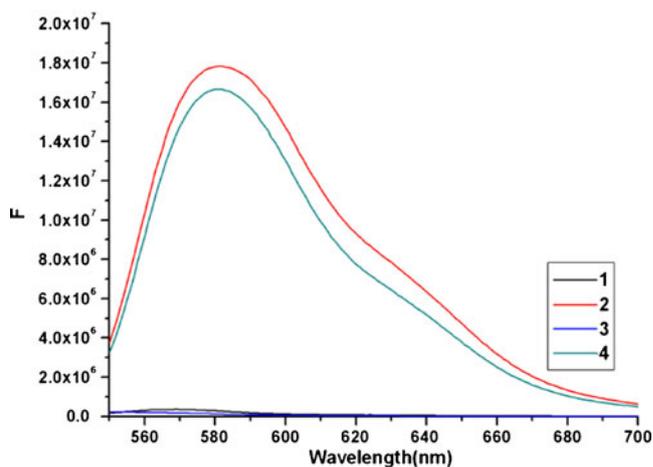


Fig. 8 Fluorescence intensity of **4** (10 μ M) to Fe^{3+} in ethanol–water (1: 1,v/v) solution. (1) baseline: 10 μ M **4** only; (2) 10 μ M **4** with 16 eq Fe^{3+} ; (3) **4** with 16 eq Fe^{3+} and then addition of 64 eq PO_4^{3-} (K salt); (4) **4** with 16 eq Fe^{3+} and 64 eq PO_4^{3-} , then addition of 16 eq Fe^{3+} ; excitation wavelength: 543 nm, slit: 5

Figure 4 shows the absorption spectra of **4** in the presence of various metal ions in ethanol-water. When no metal ion was added to the solution of **4** (10 μM), almost no absorption above 570 nm could be observed, whereas a significant enhancement of the characteristic absorption of rhodamine B emerged soon after Fe^{3+} was injected into the solution. There was a large enhancement factor (178-fold) of absorbance at $\lambda_{\text{max}}=560$ nm upon the addition of 16 equiv of Fe^{3+} . A mild increase of absorbance at 560 nm was also detected when the same amount (160 μM) of Cr^{3+} (causing 61-fold absorption enhancement) was added due to their low binding affinity to **4**. Other cations of interest gave no response (Fig. 5).

The Fe^{3+} binding stoichiometry of **4** can be determined from Fe^{3+} titration and the Job plot [33]. Detailed kinetic analyses and the mechanisms are discussed later. The “activity Job plots [34, 35]” with a maximum at $X_{\text{Fe}} \sim 0.36$ are best fitted to $\text{Fe}^{3+}:\mathbf{4} = 2:1$ (Fig. 6), indicating that the complex $(\text{Fe}^{3+})_2\text{-}\mathbf{4}$ is the predominant active species.

To further investigate the binding stoichiometry of **4** and Fe^{3+} ion, a fluorescence titration experiment was carried out. An increase of fluorescence intensity of **4** could be observed with gradual addition of Fe^{3+} ion. The Fig. 7 indicates that a 1:2 stoichiometry is most possible for the binding mode of Fe^{3+} and **4** in ethanol-water (1:1). The stability constant (K) of **4** with Fe^{3+} ion was calculated according to the 1:2 model ($K=2.25 \times 10^4$). The moderate stability constant of the 4- Fe^{3+} complex is mainly because the need of Fe^{3+} for six-coordination is not satisfied, and moreover, the strong hydration ability of iron in water. As with many reported rhodamine-based spirolactam chemosensors, the Fe^{3+} induced fluorescence enhancement of chemosensor **4** is most likely the result of the spiro ring-opening mechanism. That is, the chelation of Fe^{3+} with the oxygen atoms of the amide groups of **4** results in the formation of the open-ring form.

Furthermore, since the color and fluorescence of 4- Fe^{3+} disappeared immediately when excess EDTA or diethylenetriamine was added, the sensing process was considered to be reversible rather than an ion-catalyzed reaction. Moreover, upon the addition of 64 eq K_3PO_4 to the solution of 10 μM **4** with Fe^{3+} (16 eq), the fluorescence intensity at 580 nm was quenched (Fig. 8 blue line) due to the competitive binding of Fe^{3+} from **4** by K_3PO_4 , and further addition of 40 eq Fe^{3+} could recover the strong fluorescence again (green line).

Conclusion

In summary, we synthesized a new bis(rhodamine)-based fluorescent probe for Fe^{3+} . This spirolactam compound showed excellent selectivity for Fe^{3+} in ethanol-water and aqueous Tris-HCl buffer over other common cations, such

as Hg^{2+} , Pb^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Ca^{2+} , Ba^{2+} , Mn^{2+} , Mg^{2+} , Cd^{2+} , Cu^{2+} , Na^+ , Ag^+ , K^+ or Li^+ . The colorimetric and fluorescent response to Fe^{3+} can be conveniently detected even by the naked eye, which provides a facile method for visual detection of Fe^{3+} . The main limitation of this probe is probably its moderate binding capacity to Fe^{3+} in aqueous media, which hinders its usefulness in biochemical applications. However, its selectivity is excellent, and the detection of Fe^{3+} at 10^{-5} M level is still available. The modification of **4** to develop new fluorescent probes for Fe^{3+} with stronger binding ability is now under investigation.

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