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A novel colorimetric and turn-on fluorescent chemosensor for iron(III) ion detection and its application to cellular imaging

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ABSTRACT

A novel rhodamine-based dual probe **Rh-2** for trivalent ferric ions (Fe^{3+}) was successfully designed and synthesized, which exhibited a highly sensitive and selective recognition towards Fe^{3+} with an enhanced fluorescence emission in methanol-water media (v/v = 7/3, pH = 7.2). The probe **Rh-2** could be applied to the determination of Fe^{3+} with a linear range covering from 3.0×10^{-7} to 1.4×10^{-5} M and a detection limit of 1.24×10^{-8} M. Meanwhile, the binding ratio of **Rh-2** and Fe^{3+} was found to be 1:1. Most importantly, the fluorescence and color signal changes of the **Rh-2** solution were specific to Fe^{3+} over other commonly coexistent metal ions. Moreover, the probe **Rh-2** has been used to image Fe^{3+} in living cells with satisfying results.

1. Introduction

Iron is one of the most important trace elements in living cells, being indispensable for all living systems due to the crucial role in many biological processes [1,2]. However, both its deficiency and excess from the normal permissible limit would lead to the physiological disorders and health issues such as Alzheimer's disease [3], anemia and hemochromatosis [4]. In addition, iron ions have severe impacts on the production and quality of crops and water bodies via various industrial and agricultural activities [5,6]. Therefore, the effective monitoring and rapid detection of iron ions that are in biological, environmental and also in different industrial samples have turned to be currently of great significance. To date, several analytical methods such as inductively coupled plasma mass spectrometry [7], voltammetry [8], atomic absorption spectroscopy [9] and spectrophotometry [10] have been adopted for the detection of iron ions. However, because of the complicated pretreatment procedures, time-consuming analysis and the sophisticated instrumentations, the widespread use of these methods is largely restricted. Obviously, as a highly efficient and convenient analysis method, fluorometric and chromogenic analysis have received considerable interest and great efforts have been devoted to the design of novel optical probe nowadays [11,12].

At present, several cost-effective fluorescent probes for iron ions detection have been reported by some research groups [13–15]. Nevertheless, most of them undergo a fluorescence quenching (turn-off) response. These "on-off" fluorescent probes may suffer from unsatisfactory selectivity towards a specific analyte and offer limited spatial resolution. Therefore, the design of turn-on fluorescent probes for iron ions detection tends to be much more valuable and superior. In recent years, rhodamine derivatives are employed widely in designing fluorescent probes for the selective detection of various metal ions [16–20] as the rhodamine fluorophore has excellent photophysical properties such as long wavelength absorption and emission, high fluorescence quantum yield, large extinction coefficient, and high photobleaching threshold [21]. Moreover, the rhodamine derivatives with spirolactam structure (closed-ring) are basically non-fluorescent and colorless, whereas the addition of specific metal ions enables the corresponding spirolactam to be opened via the coordination reaction, which also results in strong fluorescence emission and noticeable color change for naked-eye [22,23]. Therefore, the rhodamine framework is an ideal template to construct "turn-on" fluorescent probes for recognizing metal ions. However, most of the reported rhodamine based probes for iron are based on mono-rhodamine and still remain some shortcomings in practical application, such as cross-sensitivity towards other coexistent metal ions, poor sensitivity [24–26]. Currently, some bis-(rhodamine) derivatives have been developed to turn-on response to metal ions [27–29] and it is considered that a bis-(rhodamine) derivative with an appropriate ligand on spirolactam ring capable of binding with metal ions would improve the selectivity and sensitivity for recognizing metal ions compared with the conventional mono-rhodamine derivatives [30,31].

Herein, a new turn-on probe **Rh-2** based on bis-(rhodamine) has been reported (Scheme 1), which was used as colorimetric and fluorescent indicator for selective detection of Fe^{3+} ions. As expected, the **Rh-2** displayed a remarkable chromogenic and fluorescent change towards

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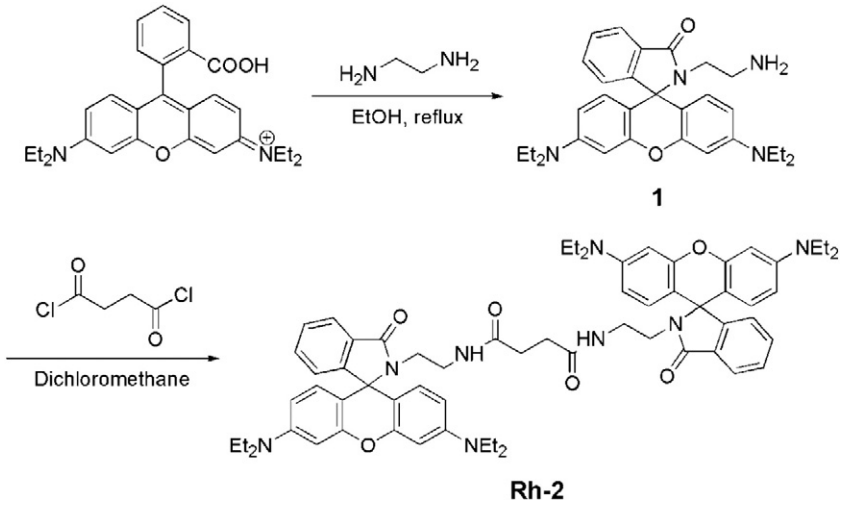

Scheme 1. The synthetic route for the probe **Rh-2**.

Fe^{3+} over other common interfering metal ions. Furthermore, the probe has been successfully employed in fluorescence imaging of Fe^{3+} ions in living cells.

2. Experimental

2.1. Materials and instruments

All the materials were analytical reagent grade and used without further purification unless specified. All the solutions of metal ions were prepared from their nitrate or chloride salts. Double-distilled water was used in all experiments.

Absorption spectra were measured on a Hitachi U-3900 spectrophotometer (Tokyo, Japan). Fluorescence spectra measurements were performed on a Hitachi F-7000 spectrofluorometer (Tokyo, Japan). The pH was recorded with a Model pHs-3C meter (Shanghai, China). The melting points were determined on X-4 microscopic melting point apparatus with a digital thermometer (Shanghai, China). NMR spectra were acquired on a Bruker 400 MHz spectrophotometer in $CDCl_3$. High resolution mass spectra (HRMS) were obtained from Thermo Fisher Scientific LTQ FT Ultra mass spectrometer. Nikon TE2000 inverted fluorescence microscope was used for the fluorescence images experiments.

2.2. Synthesis of probe *Rh-2*

Compound **1** was facilely synthesized according to the procedure as published in the literature [32].

A mixed solution of compound **1** (0.68 g, 1.4 mmol) and triethylamine (400 μL, 3.0 mmol) in dry dichloromethane (25 mL) was cooled with ice bath. Then succinyl chloride (0.1 g, 0.65 mmol) was dissolved in the dry dichloromethane (10 mL) and added dropwise into the solution with vigorous stirring. After the addition, the reaction was kept for 4 h under nitrogen atmosphere in ice bath. Then, the solution was washed with water for three times and the organic layer was dried over anhydrous sodium sulfate. After the solvent was removed under reduced pressure, the crude solid was purified by recrystallization from methanol to obtain a white powder at 69% yield (0.47 g), m.p. 189.5 °C–191 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.87–7.92 (m, 2H), 7.42–7.47 (m, 4H), 7.06–7.09 (m, 2H), 6.74 (t, 2H), 6.43 (d, 4H), 6.38 (d, 4H), 6.27–6.30 (dd, 4H), 3.31–3.36 (q, 16H), 3.26–3.29 (m, 4H), 3.01–3.05 (m, 4H), 2.35 (s, 4H), 1.17 (t, 24H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 171.78, 169.57, 153.76, 153.27, 148.91,

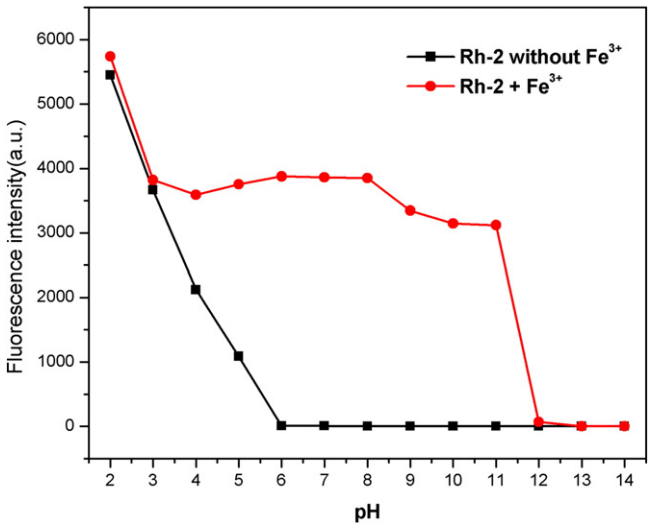

Fig. 1. Variation of fluorescent intensity of **Rh-2** (10 μM) in the absence and presence of Fe^{3+} (2.0 equiv.) in methanol-water media (v/v = 7/3) as a function of pH.

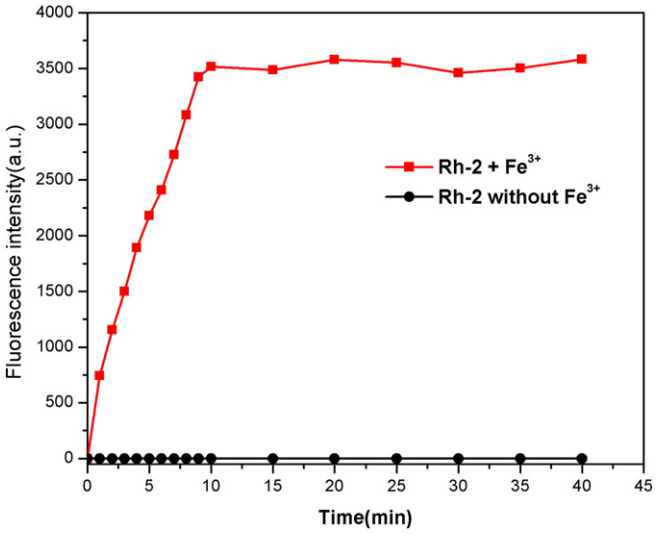

Fig. 2. Effect of reaction time on the fluorescent intensity of **Rh-2** (10 μM) in the absence and presence of Fe^{3+} (2.0 equiv.) in methanol-water media (v/v = 7/3, pH = 7.2).