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Rhodamine 6G hydrazone bearing pyrrole unit: Ratiometric and selective fluorescent sensor for Cu^{2+} based on two different approaches

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ABSTRACT

A new rhodamine-based derivative bearing pyrrole unit, **R1** 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 **R1** and ratiometric fluorescent signal output when Cu^{2+} displaces the bound Cd^{2+} in the **R1**-Cd^{2+} complex can be observed. The application of fluorescent **R1** to biological imaging was also demonstrated.

1. Introduction

The development of new artificial chemosensors for the recognition of physiologically and environmentally important heavy and transition metal ions, such as Hg^{2+} [1–9], Zn^{2+} [10–12], Cd^{2+} [13,14], Cr^{3+} [15–17], Fe^{3+} [18–24] and Cu^{2+} [25–51], has attracted considerable attention. Especially in this regard, 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 [28–32]. However, under overloading conditions, Cu^{2+} can be toxic and cause oxidative stress and disorders associated with neurodegenerative diseases, including Menkes and Wilson diseases, familial amyotropic lateral sclerosis, Alzheimer's disease, and prion diseases [25–27]. Therefore, it is so imperative to develop analytic and detective methods for sensitive sensing of Cu^{2+} ion.

Up to now, UV–vis and fluorescence spectroscopy still remain the most frequently used modes due to their high sensitivity and easy operational use [52]. It is well known that the rhodamine framework is an ideal mode to construct off-on fluorescent chemosensors due to its particular structural and excellent photophysical properties [52]. As a result, a large amount of rhodamine-modified chemosensors for Cu^{2+} ion have been developed [28–51]. Particularlly, several successful attempts have resulted in the development of selective chemiluminescence, colorimetric and fluorescence sensors for Cu^{2+} ion based on rhodamine hydrazone using substituted salicylaldehyde [28], coumarin-2-aldehyde [43], pyrrolealdehyde [31,33,38] and furfural [36] and so on. However, only a few examples can display ratiometric fluorescent changes in emission spectra, which are desirable for analytical purposes by the fluorescence changes in the ratio of the intensities of the emission at two wavelengths [29]. Generally, Förster Resonance Energy Transfer (FRET) is the most exploited sensing mechanism for design of ratiometric fluorescent probes. Some FRET-based ratiometric fluorescent probes for Cu^{2+} containing rhodamine and coumarin [29] (indole [41], cyanine [44] or pyrene [51], etc.) groups have been reported. In any case, a simple approach to the ratiometric detection of Cu^{2+} ions still remains a challenge.

Recently, it has been highlighted that introducing suitable 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 [28]. Fortunately, pyrrole bound rhodamine derivatives is well suited for such purpose. (i) Some pyrrol-2-yl-methyleneamine compounds have been proved to be promising fluorescent materi-

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als for Zn^{2+} sensors. The co-existence of Cu^{2+} and Zn^{2+} with sensor gave no fluorescence because Cu^{2+} could form more stable complexes with the sensors than Zn^{2+} [10,11]. (ii) In fact, our previous work has shown that Cd^{2+} ion could also enhance the fluorescence emission intensity of some pyrrol-2-yl-methyleneamine sensors in solution. (iii) In addition, several pyrrole bound rhodamine derivatives were reported as Cu^{2+} selective chemosensors [31,33,38]. Herein, we report here the syntheses of a novel rhodamine derivative **R1** (Scheme 1) via condension of rhodamine 6G hydrazide with ethyl 5-formyl-3,4-dimethyl-pyrrole-2-carboxylate. As expected, sensor **R1** displays a selective colorimetric change and fluorescence "turn-on" change at 550 nm via rhodamine ring-opening approach toward Cu^{2+} among the other examined metal ions. **R1** also showed a remarkable fluorescence enhancement toward Cd^{2+} at 500 nm by a typical ICT response [13,28]. Moreover, another ratiometric fluorescent signal output for Cu^{2+} can be observed when Cd^{2+} in the **R1**-Cd^{2+} complex was displaced with Cu^{2+}. These results demonstrated that **R1** could act as a dual mode Cu^{2+}-selective sensor via two mechanisms: the rhodamine ring-opening mechanism and ratiometric displacement from **R1**-Cd^{2+} complex. Furthermore, potential application of **R1** imaging intracellular Cu^{2+} in human cancer (HeLa) cells was examined by confocal fluorescence microscopy.

2. Experimental

2.1. Materials and instrumentation

Solvents and starting materials for syntheses were purchased commercially and used as received. Rhodamine 6G hydrazide [33] and ethyl 5-formyl-3,4-dimethyl-pyrrole-2-carboxylate [53] were synthesized according to the literature mothod.

The melting point of the sensor is determined on an XT4-100X microscopic melting point apparatus (made in Beijing, China). Elemental analyses were carried out on an Elemental Vario EL analyzer. ^1H NMR spectra of **R1** were recorded on a Bruker AV400 NMR spectrometer in DMSO-d_6 solution. The IR spectra ($\nu = 4000–400\ cm^{-1}$) were determined by the KBr pressed disc method on a Bruker V70 FT-IR spectrophotometer. The UV spectra were recorded on a Purkinje General TU-1800 spectrophotometer. Fluorescence spectra were determined on a Varian CARY Eclipse spectrophotometer, in the measurements of emission and excitation spectra the pass width is 5 nm. ESI-MS spectra were obtained on a Bruker Daltonics Esquire 6000 mass spectrometer. Fluorescent images were taken on Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope. The X-ray diffraction measurement for **R1** was performed on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromatized Mo$K\alpha$ radiation ($\lambda = 0.71073$ Å) by using ϕ-ω scan mode. Semi-empirical absorption correction was applied to theintensity data using the SADABS program [54]. The structures were solved by direct methods and refined by fullmatrixleast-square on F^2 using the SHELXTL-97 program [55]. All non-hydrogen atoms were refined anisotropically. All H atoms were positioned geometrically and refined using a riding model.

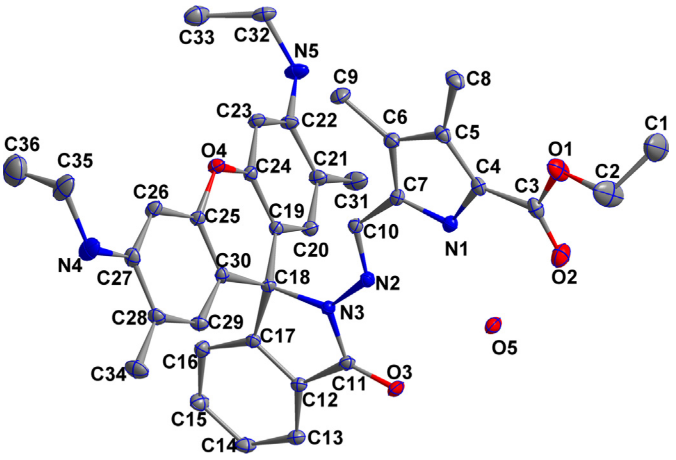


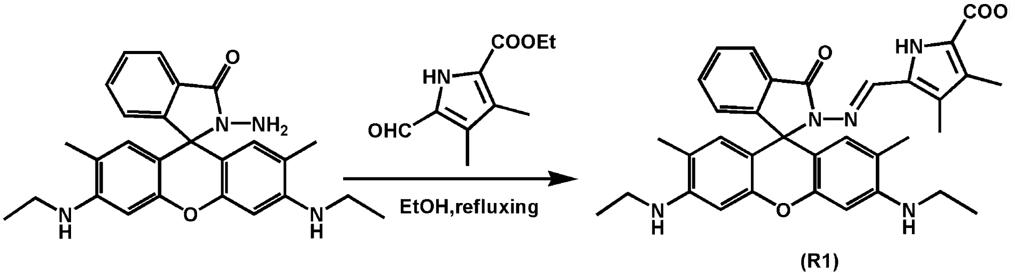
Fig. 1. The crystal structrue of **R1**·H_2O.

2.2. Synthesis of **R1**

A quantity of rhodamine 6G hydrazide (428 mg, 1 mmol) was added to a CH_3CN solution (20 ml) containing ethyl 5-formyl-3,4-dimethyl-pyrrole-2-carboxylate (195 mg, 1 mmol). The mixture was refluxed for 3 h, and then cooled to room temperature. The separated solid was filtered and washed with CH_3CN. Yield 75.37%. M.p. 288-292 °C. Anal. Calc. for $C_{22}H_{19}N_2O_5$: C, 71.38; H, 6.49; N, 11.56. Found: C, 71.09; H, 6.68; N, 11.34%. ^1H NMR (400 MHz, DMSO-d_6), δ (ppm): 11.481 (s, 1H,NH), 9.032 (s, 1H, CH=N), 7.847–7.867 (m, 1H, Ar-H), 7.540-7.585 (m, 2H, Ar-H), 7.041-7.059 (d, 1H, Ar-H), 6.262 (s, 2H, Ar-H), 6.106 (s, 2H, Ar-H), 5.030-5.057 (t, 2H, NH-CH_2), 4.142–4.194 (d, 2H, CH_2-O), 3.069-3.135 (m, 4H, CH_2-NH), 2.098 (d, 3H, CH_3), 1.819 (s, 6H,2CH_3), 1.746–1.766 (d, 3H, CH_3), 1.217–1.253(t, 3H, CH_3), 1.155–1.190 (t, 6H, 2CH_3). IR (KBr, cm^{-1}): $\upsilon(C{=}O)_{carboxylate}$ 1688; $\upsilon(C{=}O)_{acylhydrazone}$ 1638; $\upsilon(C{=}N)$ 1623. ESI-MS: $m/z = 605.98$ for $[\mathbf{R1}]^+$. Crystals of **R1**·H_2O suitable for X-ray diffraction analysis were obtained by recrystallization of **R1** from CH_3CN solution. Crystal data for $C_{36}H_{41}N_5O_5$: crystal size: 0.25 × 0.22 × 0.20 mm, monoclinic, space group C2/c. $a = 17.676(7)$ Å, $b = 16.681(7)$ Å, $c = 23.600(9)$ Å, $\beta = 94.701(9)°$, $V = 6935(5)$ Å^3, $Z = 8$, $T = 296(2)$ K, $\theta = 1.68–25.00°$, 18107 reflections measured, 6116 unique ($R_{int} = 0.0678$). Final residual for 422 parameters and 6116 reflections with $I > 2\sigma(I)$: $R_1 = 0.0727$, $wR_2 = 0.2000$ and GOF = 1.037 (Fig. 1). CCDC: 1424259.

2.3. General UV–vis and fluorescence spectra measurements

The spectral analyses were accomplished in EtOH or Tris–HCl buffered (10 mM, pH 7.20, 2/8, v/v) EtOH solution at room temperature. The concentration of the sensor **R1** for UV–vis and



Scheme 1. Synthesis route of the sensor **R1**.