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A BODIPY-based "turn-on" fluorescent and colorimetric sensor for selective detection of Cu^{2+} in aqueous media and its application in cell imaging

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ABSTRACT

We report a turn-on fluorescent and colorimetric sensor based on an easily prepared BODIPY derivative for selective detection of Cu^{2+} in aqueous media. The sensor showed effective and selective detection of Cu^{2+} at a low detection limit. These advantages allow for the application of sensor **1** to detect trace amounts of Cu^{2+} in real water samples. The sensor was successfully applied to fluorescence imaging of Cu^{2+} in living cells.

1. Introduction

Much attention has been given in recent years to the development of receptors and sensors for heavy metal ions due to their environmental harm and biological importance [1–5]. Copper(II) ion, as a heavy metal and the third most abundant transition metal in human bodies, plays vital roles in many fundamental physiological processes such as bone formation, cellular respiration, and connective tissue development and serves as a significant catalytic co-factor for several metalloenzymes [6–10]. However, unregulated overloading of copper can induce severe neurodegenerative diseases such as Alzheimer's, Parkinson's and prion diseases [11–13]. On the other hand, high concentration of copper is toxic and harm to environment. Thus, it is necessary to design and develop a specific copper ion sensor for the selective and rapid detection, especially for the trace amounts of copper ion in biological system.

Currently, 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY) derivatives are extensively used as chemosensors for heavy metal ions for their high excitation coefficients, high fluorescence quantum yields, and high stability against light and chemical reactions [14–16]. Although there are various BODIPY-based fluorescent sensors designed for Cu^{2+}, most reported probes exhibited fluorescence "on–off" behavior due to the intrinsic fluorescence quenching property of Cu^{2+} [17–20], this kind of probes could be affected by other quenchers in practical applications. Although some Cu^{2+} selective fluorescence "off–on" probes have been developed in recent years [21–28], many of them could not detect the trace amounts of Cu^{2+} in biological system. So, it is still challenge to develop "off–on" probes for Cu^{2+} recognition in biological system and environment.

Herein, we report a simple method to prepare a new BODIPY derivative (**1**) as an "off–on" fluorescent sensor and colorimetric sensor for selective detection of Cu^{2+} with a low detection limit. Compound **1** displayed selective colorimetric sensing and fluorescence turn-on responses with Cu^{2+} in aqueous media, and could be applied to detect trace amounts of Cu^{2+} in real water samples. Finally, probe **1** was further applied to the cell-imaging of Cu^{2+} using HepG2 cells.

2. Experimental

2.1. General

General methods were used unless otherwise noted; materials were obtained from commercial suppliers and were used

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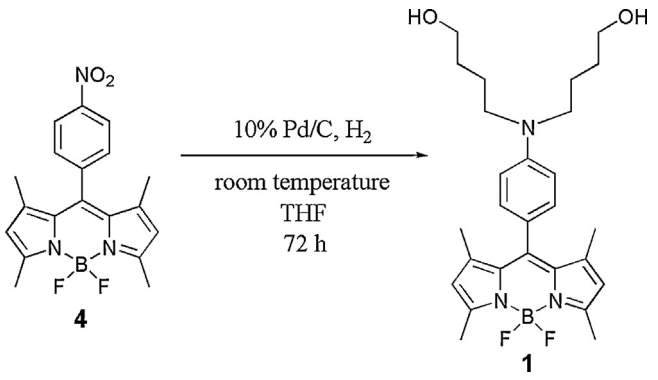

Scheme 1. Synthesis of sensor **1**.

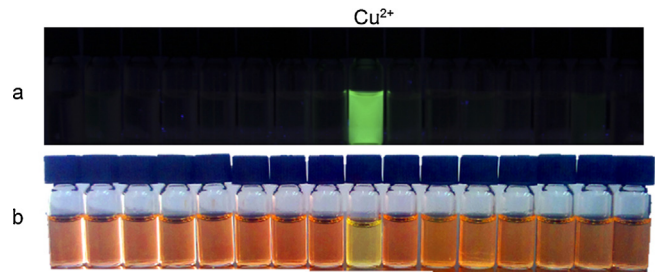

Fig. 1. (a) Color changes of sensor **1** with various cations under UV excitation (365 nm), and (b) color changes of sensor **1** with various cations under light in PBS buffer (20 mM, pH 7.4, 10% CH_3CN). [**1**] = 10 μM, [cations] = 30 μM. Cations from left to right: none, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ag^+, Co^{2+}, Cu^{2+}, Ba^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+} and Cd^{2+}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

without further purification. Column chromatography was performed using silica gel (300–400 mesh). NMR spectra were measured using Bruker AV-400 Nuclear Magnetic Resonance spectroscopy. Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. HRMS data were measured using Bruker APEX IV Fourier Transform Ion Cyclotron Resonance Mass spectrometer. UV–vis spectra were recorded on an UV–vis spectrophotometer at 25 °C which is controlled by the thermostated compartment of the spectrophotometer. Fluorescent data were measured using Hitachi F-7000 Fluorescence spectrophotometer. Standard quartz cuvettes with a 10 mm lightpath were used for all UV–vis spectra and fluorescent spectra measurements. All the fluorescent and UV–vis experiments were repeated three times.

2.2. Synthesis of sensor *1*

As shown in Scheme 1, the target compound **1** was synthesized in 43% yield by a one-pot reaction with **4** as the starting materials (Scheme 1). Firstly, compound **4** was synthesized according to the literature [29]. Briefly, a mixture of **4** (369 mg, 1.0 mmol) and 10% Pd/C (110 mg, 0.10 mmol) in THF (20 mL) under H_2 was stirred for 72 h at room temperature. After filtration to remove Pd/C, the solvent was removed under reduced pressure. The crude product was purified by column chromatography over silica gel eluting with chloroform/methanol (20:1, v/v) to give the desired product as a purple solid (207 mg, 43% yield): ^{1}H NMR (400 MHz, DMSO) δ 7.03 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 6.14 (s, 2H), 4.44 (t, J = 5.1 Hz, 2H), 3.43 (q, J = 5.8 Hz, 4H), 3.32 (t, J = 8.1 Hz, 4H), 2.43 (s, 6H), 1.62–1.55 (m, 4H), 1.48–1.43 (m, 10H); ^{13}C NMR (100 MHz, DMSO) δ 153.9, 148.4, 143.6, 142.6, 131.5, 128.6, 120.9, 111.6, 111.5, 60.5, 50.0, 29.8, 23.3, 14.3, 14.1; HRMS (ESI) calcd. for $C_{27}H_{37}BF_2N_3O_2$ $[M+H]^+$: m/z 484.2947, found: m/z 484.2938.

The proposed process of the synthesis of **1** was shown in Fig. S1. Firstly, the nitro group of **4** was converted to an amino group, and then reacted with 1 equiv. of THF to give **2** by Pd-catalyst. The secondary amine group of **2** further reacted with one equiv. of THF to give the desired compound **1**. These intermediates were well separated and then characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. These spectra are shown in the supporting information.

2.3. General UV–vis and fluorescence spectra measurements

Stock solutions (10 mM) of the perchlorate salts of Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ag^+, Co^{2+}, Cu^{2+}, Ba^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+} and Cd^{2+} in distilled water were prepared. Stock solution of sensor **1** (1 mM) was prepared in acetonitrile. Test solutions were prepared by placing 30 μL of the sensor stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 3 mL with PBS buffer (20 mM, pH 7.4, 10% CH_3CN). The fluorescence spectra were obtained by excitation of the sensor at 494 nm, and both the excitation and emission slits were 2.5 nm.

2.4. Application in real water samples

The river water samples were obtained from the Beixiao River (Beijing, China). Tap water samples were obtained from our laboratory and waste water samples were obtained from the sewer of our schoolyard. All the collected samples were simply filtered and showed that no Cu^{2+} was present. To reduce the influence of pH values during the detection, 1 mL of PBS buffer solution was added to 2 mL water samples containing **1** (10 μM, final concentration) to keep the pH value at 7.4 and then its fluorescence intensity change was detected before and after being spiked with stock solution of Cu^{2+} (10 mM).

2.5. Cell culture and confocal imaging

Living human hepatoma HepG2 cells were maintained by following the protocols provided by the American Type Culture Collection. Cells were seeded at a density of 1×10^6 cells mL^{-1} for confocal imaging in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2 g L^{-1}), and 1% antibiotics (penicillin/streptomycin, 100 U mL^{-1}). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2. Confocal fluorescence imaging studies were performed on a LSM 710 confocal laser-scanning microscope (Carl Zeiss Co., Ltd.). Cell imaging was carried out after washing the cells three times with PBS. The samples were excited with 488 nm, under observation between 490 and 650 nm.

3. Results and discussion

3.1. Colorimetric study

Colorimetric sensing by naked eye is the simplest way to observe the selectivity of **1** to the various metal ions. As shown in Fig. 1a, color change of **1** was observable under 365 nm UV irradiation. Upon the addition of 3.0 equiv. of various metal ions, including Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ag^+, Co^{2+}, Cu^{2+}, Ba^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+} and Cd^{2+}, sensor **1** displayed a dramatic color change with Cu^{2+} and almost no color change with others ions in PBS buffer (20 mM, pH 7.4, 10% CH_3CN). Moreover, as shown in Fig. 1b, upon the addition of various metal ions to **1** in PBS buffer (20 mM, pH 7.4, 10% CH_3CN), it was found that only Cu^{2+} caused an obvious color change from orange to green, whereas no significant changes were found with other tested cations. This selective color change can be used for the "naked eye" detection of Cu^{2+} in aqueous solution.