Tetrahedron 69 (2013) 4743–4748

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Coumarin-based novel fluorescent zinc ion probe in aqueous solution

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ARTICLE INFO

Article history:
Received 23 December 2012
Received in revised form 26 February 2013
Accepted 8 March 2013
Available online 14 March 2013

Keywords:
Coumarin
Zn^{2+}
Fluorescent probe

ABSTRACT

A novel coumarin-derived fluorescent probe, **FCP**, was designed for Zn^{2+} quantification based on the photo-induced electron transfer (PET) mechanism. This probe selectively and sensitively detects Zn^{2+} in aqueous solution with wide pH range. Large fluorescence enhancement (13-fold) was observed upon the addition of Zn^{2+}. **FCP** also displays excellent cell permeability in HeLa cell model and very low cytotoxicity to HEK-293 cell model.

1. Introduction

As the second most abundant transition metal essential for human health, zinc ion (Zn^{2+}) has drawn considerable interest due to its broad biological functions, including modulation of catalytic activity of hundreds of specific enzymes, regulation of gene transcription, and involvement in brain pathology.[1,2] Abnormal concentrations of Zn^{2+} are known to be closely related to several diseases, including chronic diarrhea, growth failure, immune deficiency, Alzheimer's disease.[3,4] In addition, with the development of modern industry, the environment pollution caused by transition metals like Zn^{2+} has become more serious and pose a threat to human heath and the environment.[5] Therefore, the accurate measurement of Zn^{2+} concentrations in either the clinical setting or for environmental monitoring is of great importance.

Fluorescence emission spectrometry has emerged as one of the most popular methods for specific measurement because of its high sensitivity, simplicity, and real time monitoring without complicated pretreatment.[6–9] Numerous fluorescent probes for Zn^{2+} detection have been developed by conjugating different kinds of fluorophores.[10–18] However, many of the existing Zn^{2+} probes have poor water solubility, which prevents their applications in biological systems. Other probes can not be applied to living systems due to poor cell permeability. Thus, efforts directed toward the development of novel Zn^{2+} probes are ongoing.

A fluorescent probe usually consists of two structural units: the receptor unit for specifically recognizing the target molecule or ion, and the fluorophore unit for translating the host–guest recognition into fluorescence signal.[19,20] Coumarins are widely used as effective fluorophores due to their high fluorescence quantum yield.[13,21,22] Furthermore, coumarin derivatives are usually easily synthesized and possess good water solubility, low cytotoxicity, and good cell permeability,[23,24] making them powerful chemical tools for studying biological systems. As one of the most commonly used chelators, di-2-picolylamine (DPA) is a classical membrane-permeable chelator with high selectivity for Zn^{2+} over other alkali and alkaline-earth metal ions.[25–27] Usually, electronegative atoms, such as N,[28] O,[29] S,[30] were introduced to the fluorophore part of molecular probes as additional electron donors with the aim to improve the chelator's affinity toward metal ions. To the best of our knowledge, few coumarin-based DPA derivatives as fluorescent Zn^{2+} chemical probes have been described until recently.[31,32]

Herein, based on the mechanism of photo-induced electron transfer (PET),[20] we designed and synthesized a coumarin-based DPA fluorescent probe (**FCP**) with high sensitivity and selectivity for zinc ion in aqueous solution (Scheme 1). We envisioned that fluorescence intensity would be greatly enhanced when **FCP**'s PET pathway was blocked upon the binding with Zn^{2+}, which might be utilized to quantify Zn^{2+} levels conveniently for healthcare and environmental monitoring.

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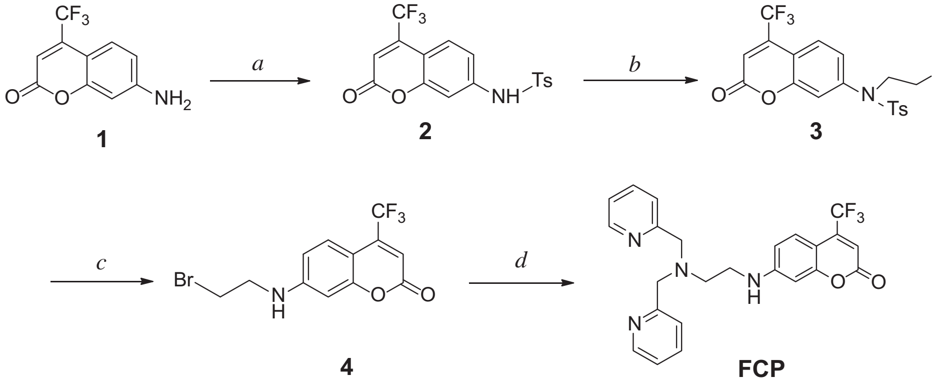
http://dx.doi.org/10.1016/j.tet.2013.03.032



Reagents and conditions: *a*, TsCl, pyridine, CH_2Cl_2, *r.t.*, 4 h; *b*, $Br(CH_2)_2Br$, Cs_2CO_3, CH_3CN, reflux, 12 h; *c*, H_2SO_4 (conc.), 90 °C; *d*, DPA, K_2CO_3, KI, CH_3CN, reflux, 12 h

Scheme 1. Synthesis of the new fluorescent probe **FCP**.

2. Experimental

2.1. Reagents and instruments

Unless otherwise noted, all chemical reagents were commercially available and treated with standard methods before use. Silica gel column chromatography (CC): silica gel (200–300 mesh); Qingdao Makall Group Co., Ltd; Qingdao; China. Solvents were dried in a routine way and redistilled. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3CN-d_3 on a Varian Mercury 600 or 400 spectrometer and resonances (δ) are given in parts per million relative to tetramethylsilane (TMS). The following abbreviations were used to designate chemical shift multiplicities: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. High resolution mass spectra (HRMS) were acquired in positive mode on a WATERS MALDI SYNAPT G2 HDMS (MA, USA).

2.2. Synthesis and characterization of coumarin derivatives

2.2.1. Synthesis of 7-[N-(p-toluenesulfonyl)amino]-4-trifluoromethylcoumarin. 7-Amino-4-trifluoromethylcoumarin 3.71 g (16.2 mmol) was dissolved in dichloromethane (20 mL). After slow addition of the distilled pyridine (20 mL) and *p*-toluenesulfonyl chloride (6.80 g, 35.7 mmol), the mixture was then stirred for 4 h at room temperature. Then ethyl acetate 150 mL was added, followed by wash of citric acid solution for three times and brine once. The resulting solution was dried with sodium sulfate and evaporated, washed with a small portion of cooled dichloromethane and dried in vacuo to give the compound **2** as white solid.[33] Yield: 5.64 g (91%). Mp 193–194 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.81 (d, J=8.0 Hz, 2H), 7.57 (d, J=8.8 Hz, 1H), 7.30 (d, J=8.0 Hz, 2H), 7.21 (d, J=2.4 Hz, 1H), 7.08 (dd, J_1=2.0 Hz, J_2=2.0 Hz, 1H), 6.67 (s, 1H), 2.40 (s, 3H). EI-MS: *m*/*z*, calcd 383.34, found 383.02.

2.2.2. Synthesis of 7-[N-(2-bromoethyl)-N-(p-toluenesulfonyl)amino]-4-trifluoro -methyl coumarin. A mixture of 7-Tosylamino-4-trifluoromethylcoumarin (3.2 g, 8.35 mmol), distilled acetonitrile (30 mL), cesium carbonate (4.06 g, 12.50 mmol), and 1, 2-dibromoethane (15.6 g, 83.5 mmol) was stirred for 12 h at 80 °C. After filtration, the solution was evaporated and purified by silica gel column chromatography to afford the compound **3** as white solid.[33] Yield: 2.28 g (81%). Mp 80–81 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.71 (d, J=7.8 Hz, 1H), 7.51–7.50 (m, 2H), 7.31–7.24 (m, 3H), 7.10 (s, 1H), 6.82 (s, 1H), 3.94 (t, J=7.2 Hz, 2H), 3.43 (t, J=7.2 Hz, 2H), 2.45 (s, 3H). EI-MS: *m*/*z*, calcd 490.29, found 490.91.

2.2.3. Synthesis of 7-[N-(2-bromoethyl)amino]-4-trifluoromethylcoumarin. 7-(*N*-(2-Bromoethyl)-*N*-tosylamino)-4-trifluoromethylcoumarin (0.75 g, 1.53 mmol) was added to concentrated sulfuric acid 5 mL and the solution was stirred for 4 h at 90 °C. The reaction mixture was cooled and carefully poured into water. The blue solid was precipitated, followed by filtration and silica gel column chromatography to obtain the compound **4** as yellow solid.[33] Yield: 0.3 g (58%). Mp 151–152 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50 (d, J=7.2 Hz, 1H), 6.61 (d, J=8.8 Hz, 1H), 6.53 (s, 1H), 6.47 (s, 1H), 3.76 (t, J=5.6 Hz, 2H), 3.60 (t, J=5.6 Hz, 2H). EI-MS: *m*/*z*, calcd 336.10, found 336.96.

*2.2.4. Synthesis of probe **FCP**.* DPA (199 mg, 1 mmol), K_2CO_3 (138 mg, 1 mmol), KI (166 mg, 1 mmol), intermediate **4** (168 mg, 0.5 mmol) were dissolved in distilled CH_3CN, and the reaction mixture was stirred under reflux for 12 h. After removing the solvent under reduced pressure, the residue was then purified by silica column chromatography using CH_2Cl_2/CH_3OH (100/1, v/v) to afford the final product as yellow oil. Yield: 91 mg (40%). IR (neat): 3392, 2921, 2851, 1643, 1466, 1260, 1094 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.60 (d, J=4.4 Hz, 2H), 7.66–7.62 (m, 2H), 7.44 (d, J=8.8 Hz, 1H), 7.37 (d, J=8.0 Hz, 2H), 7.20–7.18 (m, 2H), 6.78 (s, 1H), 6.62 (dd, J_1=2.4 Hz, J_2=2.4 Hz, 1H), 6.41 (d, J=2.4 Hz, 1H), 6.37 (s, 1H), 3.96 (s, 4H), 3.22 (t, J=5.6HZ, 2H), 2.96 (t, J=5.6HZ, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.55, 158.59, 157.10, 152.75, 149.03, 141.98, 141.64, 136.61, 125.82, 123.32, 122.28, 111.42, 107.41, 103.03, 97.47, 59.92, 51.84, 40.74. HRMS (ESI+) calcd for $[FCP+H]^+$: calcd 455.1695, found: 455.1690.

2.3. Determination of quantum yield

The quantum yields of fluorescence were determined by comparison of the integrated area of the corrected emission spectrum of samples with a reference. Specifically, using fluorescein (Φ=0.98, 0.1 M H_2SO_4) as reference, **FCP** (10 μM) were prepared in 0.1 M phosphate (pH 7.0) buffer and diluted to certain solution to make their absorption less than 0.05. Then their UV–vis absorption spectrum was studied and the corresponding emission at relevant wavelength of excitation was measured as well. After correction of the refractive index of the different solvents determined by Abbe's refractometer, the quantum yields were calculated with the expression in following equation.