Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 204 (2018) 446–451

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Review Article

Two-photon fluorescent probe with enhanced absorption cross section for relay recognition of Zn^{2+}/$P_2O_7^{4-}$ and *in vivo* imaging

Ying Xia [a], Huihui Zhang [a], Xiaojiao Zhu [a], Qiong Zhang [a], Min Fang [a], Xiaowu Li [a], Hongping Zhou [a,*], Xingyuan Yang [b], Xuanjun Zhang [c], Yupeng Tian [a]

[a] College of Chemistry and Chemical Engineering, Anhui University and Key Laboratory of Functional Inorganic Materials Chemistry of Anhui Province, Anhui Province Key Laboratory of Chemistry for Inorganic/Organic Hybrid Functionalized Materials, 230601 Hefei, PR China
[b] Institute of Physical Science and Information Technology, Faculty of Health Sciences, Anhui University, Hefei 230601, PR China
[c] Faculty of Health Sciences, University of Macau, Taipa, Macau SAR, PR China

ARTICLE INFO

Article history:
Received 18 April 2018
Received in revised form 15 June 2018
Accepted 17 June 2018
Available online 27 June 2018

Keywords:
Fluorescence probe
Zinc ion
Two-photon
Exogenous

ABSTRACT

A novel multifunctional probe, **L**, based on triphenylamine *o*-hydroxyl Schiff base was constructed for the sequential detection of Zn^{2+}/$P_2O_7^{4-}$. Interestingly, probe **L** also showed two-photon fluorescent "off-on" response to Zn^{2+} along with a large effective two-photon absorption cross-section value of 240 GM at 720 nm, a low cytotoxic and a moderate photostability, which made **L** a good candidate for two-photon fluorescence microscopy imaging and monitoring the fluctuation of exogenous Zn^{2+}.

Contents

1. Introduction

Zinc ion is the one of vital trace elements and widely distributed within the body. The indispensable roles of Zn^{2+} are involved in numerous biological processes including neurotransmission or modulators, enzymatic regulation and cell apoptosis [1]. Disorder of intracellular free Zn^{2+} has been considered to be associated with various diseases such as diabetes, Alzheimer's disease, and immune dysfunction [2, 3]. Moreover, $H_4P_2O_7$ (PPi) plays important roles in a range of biological processes such as energy storage, cellular signal transduction and protein synthesis [5]. In this regard, the development for the design of effective and selective Zn^{2+} probes, especially those with bifunctional recognition, *viz*, sequentially recognizing Zn^{2+} and $H_4P_2O_7$ (PPi) based on the strong affinity of Zn^{2+} toward phosphates [4], is crucially important and necessary [6].

* Corresponding author.
E-mail address: zhpzhp@263.net (H. Zhou).

https://doi.org/10.1016/j.saa.2018.06.065
1386-1425/

Previous research is dedicated to designing probes on the basis of one-photon (OP) fluorescence, to some extent, limiting their biological applications [7, 8]. However, for the sake of overcoming the drawbacks of high tissue auto-fluorescence, low penetration ability and photobleaching in OP fluorescence, two-photon (TP) fluorescence probe [9, 10] were used as an attractive approach for sensing analytes *in vitro* and *in vivo* microscopically. Additionally, most organic molecules with large effective two-photon absorption cross-section value (δ_{eff}) usually had the extended conjugated structures, resulting in poor solubility and cell permeability [11, 12], which hindered their further application. This trade-off between δ_{eff} and cell permeability in fluorescence probe has inspired elegant design of suitable conjugated structures [13, 14].

Herein, we highlighted a Schiff base derivative (**L**) two-photon fluorescent probe with enhanced absorption cross section for relay recognition of $Zn^{2+}/P_2O_7^{4-}$. Given the O atom of —OH and —O— bond, the N atom of —CH═N— bond and pyridine nitrogen atom with strong affinity to Zn^{2+}, **L**-Zn^{2+} extended conjugated system and enlarged the planarity, which blocked the photoinduced electron transfer (PET) process [15], and brought about the enhancement of fluorescent intensity and δ_{eff}. Relay recognition of $Zn^{2+}/P_2O_7^{4-}$ with "off–on–off" fluorescence mode was observed by naked eye. Further, the simple molecule successively served as a two-photon probe for detecting exogenous Zn^{2+} in living cells.

2. Materials and Instrumentation

Intermediate **1** was prepared according to our previous methods [16] (Scheme 1).

2: K_2CO_3 (2.20 g, 15.90 mmol) and 2-bromomethyl pyridine hydrobromide (0.85 g, 4.90 mmol) were added to 25 mL acetonitrile solution of **1** (1.00 g, 2.45 mmol). The mixture was refluxed at 75 °C for 3 h. After the solvent was evaporated under reduced pressure, the crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (10: 1, v/v) as eluent to afford 0.70 g of yellowish solid. Yield: 70%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm) (Fig. S1): 8.60–8.61 (J = 4.00, d, 1H), 7.95–7.97 (J = 8.00, d, 1H), 7.88–7.92 (J = 8.00, t, 1H), 7.63–7.65 (J = 8.00, t, 2H), 7.46–7.56 (m, 3H), 7.36–7.32 (J = 8.00, t, 6H), 6.95–7.19 (m, 9H), 5.45 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm) (Fig. S2): 151.84, 149.08, 147.66, 146.70, 144.38, 137.24, 137.12, 132.58, 130.04, 129.63, 128.21, 126.12, 124.56, 123.63, 123.05, 122.17, 121.23, 118.57, 112.36, 71.23. FT-IR (KBr, cm^{-1}): 3062.44 (w), 3031.93 (w), 1630.08 (w), 1580.80 (s), 1507.85 (w), 1490.85 (m), 1412.74 (w), 1378.74 (w), 1330.10 (w), 1279.75 (s), 1174.09 (s), 1091.45 (m), 1027.95 (m), 751.17 (s), 690.05 (s). MS (ESI) (Fig. S3): calcd for $[M + H]^+$, 500.1975; found, 500.1988.

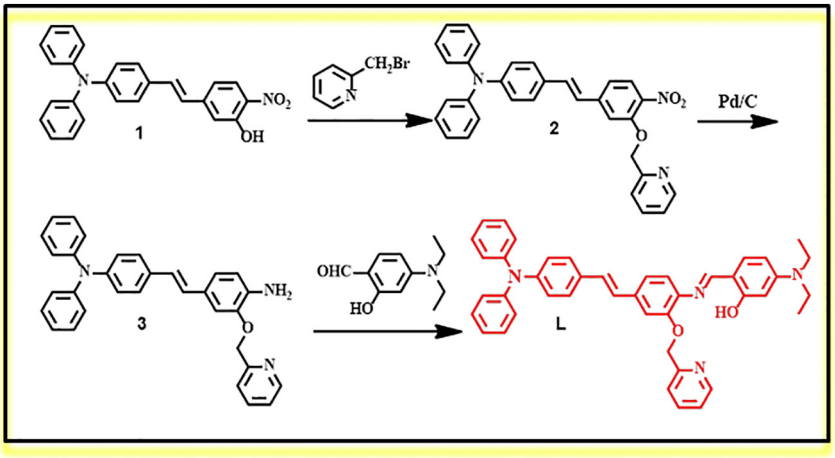
3: 0.05 g of Pd/C catalyst and 1.6 g of hydrazine hydrate were added into 15 mL ethanol solution of (0.40 g, 0.80 mmol) **2** at 75 °C and refluxed for about 0.5 h. The mixture was immediately filtered and 0.08 g of yellowish-white solid was obtained. Yield: 82%. ^{1}HNMR (400 MHz, Acetone-d_6), δ (ppm) (Fig. S4): 8.59–8.60 (J = 4.00, d, 1H), 7.80–7.84 (m, 1H), 7.64–7.66 (J = 8.00, d, 1H), 7.43–7.45 (J = 8.00, d, 2H), 7.28–7.33 (m, 5H), 7.23 (s, 1H), 7.03–7.07 (m, 6H), 6.94–7.00 (m, 5H), 6.73–6.75 (J = 8.00, d, 1H), 6.26 (s, 2H), 4.73–4.76 (J = 12.00, d, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm) (Fig. S5): 157.11, 148.93, 147.09, 145.69, 138.10, 136.90, 132.56, 129.48, 127.93, 126.83, 129.78, 122.92, 122.80, 121.43, 120.85, 113.89, 109.81, 70.50. FT-IR (KBr, cm^{-1}): 3438.86 (m) 3337.89 (s), 3022.85 (s), 1619.41 (m), 1588.42 (s), 1517.15 (w), 1435.03 (m), 1292.83 (m), 1155.97 (m), 1046.48 (w), 956.22 (m), 753.64 (s), 700.322 (s), 620.91 (m), 489.60 (w). MS (ESI) (Fig. S6): calcd for $[M + H]^+$, 470.2233; found, 470.2231.

L: 20 mL ethanol solution of 4-(diethylamino) salicylaldehyde (0.10 g, 0.793 mmol) was added dropwise into 20 mL ethanol solution of **3** (0.10 g, 0.529 mmol). The mixed solution was stirred at room temperature, and orange red solid gradually precipitated after 4 h. Filtrated and recrystallized with ethanol to produce 0.21 g solid. Yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm) (Fig. S7): 14.31 (s, 1H), 8.75 (s, 1H), 8.58–8.59 (J = 4.00, d, 1H), 7.78–7.85 (m, 2H), 7.51–7.53 (J = 8.00, d, 3H), 7.45 (s, 1H), 7.22–7.39 (m, 9H), 7.00–7.17 (m, 9H), 6.33–6.35 (J = 8.00, d, 1H), 5.35 (s, 2H), 3.44–3.48 (m, 4H), 1.18–1.22 (J = 8.00, t, 6H). ^{13}C NMR (100 MHz, Acetone-d_6), δ (ppm) (Fig. S8): 164.70, 159.71, 148.88, 147.51, 133.73, 127.63, 124.30, 122.44, 118.62, 110.76, 103.67, 97.38, 70.89, 44.07, 12.01. FT-IR (KBr, cm^{-1}): 3023.99 (w), 2969.98 (m), 1589.11 (m), 1521.62 (m), 1419.19 (w), 1349.12 (w), 1276.30 (w), 1125.15 (m), 959.19 (w), 819.62 (m), 752.87 (s), 696.19 (s), 619.36 (w). MS (ESI) (Fig. S9): calcd for $[M + H]^+$: 645.3230; found, 645.3245.

3. Results and Discussion

3.1. One-photon Absorption and Emission Properties

Theoretical calculations give us the inspiration that probe **L** delivers high selectivity toward Zn^{2+}, as evidenced by Table S1. In our situation, probe **L** can detect Zn^{2+} in various solvents (Fig. S10). For biological application convenience, probe **L** was dissolved in DMSO, of which optical property was studied by emission spectra. It exhibited a main emission bands at 520 nm when excited at 450 nm. The fluorescence quantum yield of probe **L** (Φ = 6.49%) was very low but increased quickly upon interacting with Zn^{2+} (Φ = 19.56%), and the fluorescence lifetimes also varied from 0.166 ns to 0.222 ns (Fig. S11), imparting a selective fluorescence recognition to Zn^{2+}. Subsequently, to confirm the high



Scheme 1. Synthetic route for probe **L**.