Sensors and Actuators B 202 (2014) 388–394

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A novel acridine-based fluorescent probe for the cascade recognition of Cr^{3+} and PO_4^{3-}

Xiu-Juan Jiang [a], Yan Fu [a], Lin-Hua Xu [a], Hong-Lin Lu [a], Shuang-Quan Zang [a,*], Ming-Sheng Tang [a], Thomas C.W. Mak [a,b]

[a] College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001, PR China
[b] Department of Chemistry and Center of Novel Functional Molecules, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, PR China

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 22 May 2014
Accepted 25 May 2014
Available online 2 June 2014

Keywords:
Fluorescent probe
Cr^{3+}
PO_4^{3-}
Water analysis
MGC-803 cells

ABSTRACT

A novel fluorescent probe (**L**) based on acridine was rationally designed and synthesized. The fluorescent probe exhibited good selectivity and sensitivity toward Cr^{3+} in methanol. Meanwhile, the **L**-Cr^{3+} solution also displayed excellent selectivity to PO_4^{3-} by a displacement approach. Moreover, the probe **L** could be used in neutral methanol aqueous medium, which had potential applications in biological and environmental systems. More importantly, both **L** and the **L**-Cr^{3+} complex had cell permeability and were successfully demonstrated in living MGC-803 cells for the detection of Cr^{3+} and PO_4^{3-}, respectively.

1. Introduction

In recent years, the fluorometric method has been paid much attention owing to their instantaneous response, visual simplicity and high sensitivity compared to other detection methods [1–6]. Especially, more and more fluorescent probes for common heavy and transition metal ions and anions have been described because of their vital roles in a wide range of chemical, biological and environmental processes [1–9]. Trivalent chromium (Cr^{3+}) is not only an essential component of a balanced human and animal diet in amounts of 50–200 μg per day (WHO 1988), but also plays an important role in the metabolism of carbohydrates, lipids, proteins, and nucleic acids [10]. On the other hand, chromium is an environmental pollutant due to various industrial and agricultural activities [11]. Moreover, high levels of Cr^{3+} can negatively affect cellular structures [12]. As is known, inorganic phosphate in environmental and biological systems is becoming more and more important with technological developments [13]. Among various anions, phosphate is an integral part of nucleotides that make up genes. Furthermore, phosphate anions are present in extracellular fluids at 1–3 mM concentrations, and they play important roles in the formation of extracellular matrix and other biological processes [14–16]. However, inorganic phosphate is one of several major environmental contaminants. The determination of the amount of inorganic phosphate in drinking water ($<1 \times 10^{-4}$ M) is of utmost importance in situations where most of the waste-water needs to be recycled for later consumption purposes [13,17]. In addition, deficiency of phosphate can result in muscle weakness, impaired leukocyte function, and irregularity in bone mineralization causing rickets or osteomalaciaphosphate [18]. On the contrary, an elevated level of phosphate indicates abnormal renal function [19]. Recently, there is a report that serum phosphate level can even predict early mortality in HIV-positive adults starting antiretroviral therapy [20]. Therefore, there is an urgent need to develop fluorescent probes for chromium ions and phosphate anions in environmental and biological samples.

However, paramagnetic Cr^{3+} is described as one of the most efficient fluorescence quenchers via the electron transfer and intersystem crossing processes among the transition metal ions [21,22], which renders it difficult to develop a Cr^{3+} turn-on probe. Up to now, several examples of Cr^{3+} turn-on fluorescent probes have been reported [12,21–32]. Whereas PO_4^{3-} fluorescent probes remain extremely scarce. Several probes of phosphate anions that rely on hydrogen bonding via the amide, urea, and thiourea subunits have been reported [33–36]. But most of them cannot sense anions in aqueous solvents and even cellular medium because of

* Corresponding author. Tel.: +86 371 67780136; fax: +86 371 67780136.
E-mail address: zangsqzg@zzu.edu.cn (S.-Q. Zang).

http://dx.doi.org/10.1016/j.snb.2014.05.098
0925-4005/

hydrogen bonding sites [37–39]. To overcome this problem, metal-based complexes have been successfully developed for sensing anions, such as PPi, S^{2-}, CN^-, F^- and even HSO_4^- [40–50]. In this context, chromium complex could be utilized for sensing phosphate anion through sequestrating Cr^{3+} from the complex by means of formation of $CrPO_4$ precipitates.

Besides, the luminescence and sensitivity properties of acridine and its derivatives make them promising candidates of new fluorescent probes [51–53]. However, they are sparsely used as the signaling part in probes for metal ions [52]. Until recently, a few acridine derivatives have been developed as fluorescent probes for metal ions [51–54]. Generally, fluorescent probes are developed either by a fluorescence enhancement or a fluorescence reduction of a predesigned ligand upon interaction with a metal ion [1,2,55]. Based on the above ideas, herein we have designed and synthesized a novel acridine-based ligand (**L**), which was a successful fluorescent probe because it could bind Cr^{3+} in methanol as well as neutral methanol aqueous medium and give rise to variations in its fluorescence intensity. Moreover, its resultant complex (**L**-Cr^{3+}) could sense PO_4^{3-} by the displacement approach. More importantly, both **L** and the **L**-Cr^{3+} complex could be used to detect Cr^{3+} and PO_4^{3-} in MGC-803 cells. According to the reported literatures, only few fluorescent probes for Cr^{3+} in living cells have been reported up to now [21–24], but the fluorescent probes for detecting intracellar PO_4^{3-} have not been found. Therefore, our present work may provide good strategies for developing new fluorescent probes which can recognize intracellar Cr^{3+} and PO_4^{3-}, respectively.

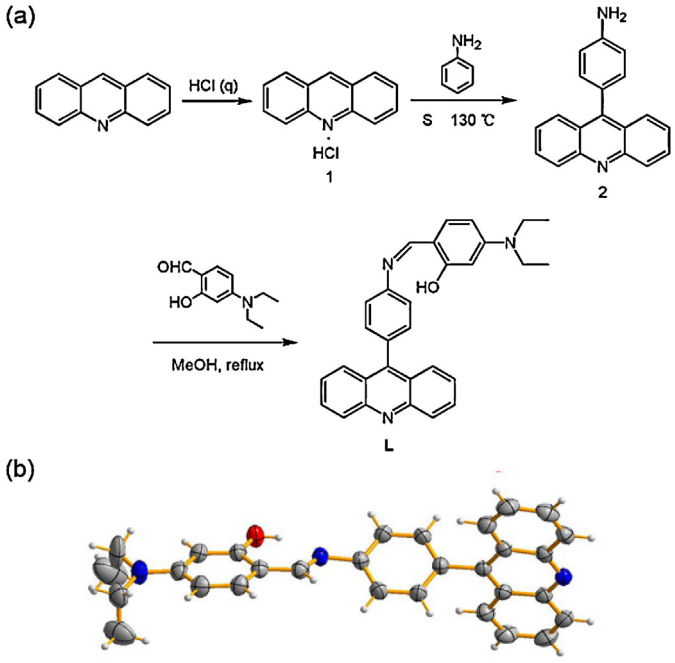
2. Experimental

2.1. Apparatus

^{1}H NMR and ^{13}C NMR were carried out on a Bruker 400 spectrometer. Mass spectra were recorded on a LC-MSD-Trap-XCT instrument by electrospray ionization (ESI) or a Bruker MALDI-TOF/TOF by matrix (HCCA). UV–visible absorption spectra were recorded on a TU-1900 double-beam UV–vis spectrophotometer. Fluorescence emission spectra were recorded on a FluoroMax-4 spectrofluorometer with 5 nm slit for both excitation and emission. The Fourier transform infrared (FT-IR) spectra were obtained in the range of 400–4000 cm^{-1} as KBr pellets on a Bruker VECTOR 22 spectrometer. Melting point was determined on an XT4A microscope electron thermal apparatus. X-ray single crystal data was collected using Mo-Kα (λ = 0.7107 Å) radiation on a SMART APEX II diffractometer equipped with CCD area detector. Data collection, data reduction, structure solution/refinement were carried out using the software package of SMART APEX II.

2.2. Materials

All of the materials for synthesis were purchased from commercial suppliers and used without further purification. All the solvents were of analytical reagent grade. The stock solutions of NaCl, KCl, $CaCl_2$ and $MgCl_2$ (12.5 mM) were prepared in distilled water. The stock solutions of heavy and transition metal ions (2.5 mM) were prepared from chloride or nitrate salts in distilled water. A stock solution of **L** (100 μM) was prepared in freshly distilled CH_3OH. When studying the fluorescence selectivity of **L** toward various metal ions, 75 μL stock solution of **L** was put into a quartz cuvette containing 3 mL distilled CH_3OH, then 20 or 100 equiv various metal salts were added to the above solutions, finally they were mixed and measured. Fluorescence quantum yields of **L** and **L**-Cr^{3+} were determined according to the reported literatures [56–58]. We chose acridine in ethanol as the standard (Φ = 0.98). The samples and standard were excited at the same wavelength.



Scheme 1. (a) Synthesis of **L** and (b) X-ray single crystal structure for **L**.

2.3. Synthesis of *L*

A solution of 9-(4-aminophenyl)acridine [59,60] (270 mg, 1 mmol) in absolute methanol was added to a solution containing 2-hydroxy-4-diethylaminobenzaldehyde (193 mg, 1 mmol) in absolute methanol. The mixture was refluxed for 24 h. After reaction, the solution was cooled to room temperature and yellowish green precipitate was obtained. The crude product was filtered off, washed with diethyl ether several times and dried under vacuum. The target product was recrystallized in methanol to give 334 mg of **L** in 75% yield, m.p. 240–241 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.61 (s, 1H), 8.46 (d, 2H), 7.85 (m, 4H), 7.51 (m, 6H), 7.27 (t, 1H), 6.31 (s, 2H), 6.27 (s, 1H), 3.46 (m, 4H), 1.26 (t, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm): δ 164.18, 161.07, 152.10, 149.21, 148.46, 147.44, 134.00, 132.75, 131.55, 130.23, 129.31, 126.90, 125.74, 125.26, 120.95, 109.18, 104.01, 97.81, 44.66, 12.74. FT-IR (KBr plate, cm^{-1}): 3444, 1636, 1584, 1558, 1518, 1474, 1456, 1421, 1376, 1348, 1297, 1248, 1204, 1172, 1129, 1077, 1015, 901, 866, 824, 783, 759, 703. Mass (ESI-MS): m/z 446.2230 $[M+H]^+$.

2.4. Cell culture

MGC-803 cells were provided by the Institute of Biochemistry and Cell Biology (China). The cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, incubated under 5% CO_2 at 37 °C. Then these cells were treated with the relative compounds using fluorescence microscopic imaging.

3. Results and discussion

3.1. Synthesis

The synthesis of **L** was shown in Scheme 1 according to the reported method [59,60]. New compound **L** was fully characterized in supporting information (Fig. S1–S4) and its single crystal was also obtained by solvent evaporation method in CH_3OH (Scheme 1, Table S1).