Inorganic Chemistry Communications 65 (2016) 28–31

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

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

Short communication

A new copper(II) selective fluorescence probe based on naphthalimide: Synthesis, mechanism and application in living cells

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ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form 8 October 2015
Accepted 13 October 2015
Available online 23 October 2015

Keywords:
Fluorescence probe
Copper
Naphthalimide
Cell imaging

ABSTRACT

A new fluorescence probe **L** based on naphthalimide has been synthesized for selective and quantitative detection of Cu^{2+} in CH_3CN:H_2O (4:1, *v*/*v*) solution. **L** exhibited a strong green fluorescence. Upon addition of 2 equiv. of Cu^{2+}, the fluorescence emission shows a steady and smooth decrease until a plateau is reached with a 30-fold quenching of fluorescence intensity. In the presence of Cu^{2+}, the absorbance peak of **L** maximum at 466 nm decreased, and a new absorption band at 600 nm appeared. Under the identical conditions, other physiological and environmental important metal ions induced negligible spectroscopic changes. The 1:2 stoichiometry binding mode of **L** with Cu^{2+} was supported by the Benesi–Hildebrand analysis and ESI-MS spectra studies. The detection limit for Cu^{2+} was estimated to be 64 *ppb*. Fluorescence microscopy experiments showed that **L** has practical application in living cells.

1. Introduction

The development of molecular fluorescence probes for the detection of environmentally and biologically important species has always been of particular importance, and usually involves the design and synthesis of molecules that contain binding sites and signaling subunits able to display selective changes in fluorescence emission upon guest binding [1–4]. Metal-selective fluorescent probes as useful tools have been widely exploited to detect biologically relevant metal ions [5–14]. Especially, the detection of Cu^{2+} is attracting continuous attention, as copper is a significant metal pollutant due to its widespread use, but it is also an essential trace element that plays a pivotal role in a variety of fundamental physiological processes in organisms ranging from bacteria to mammals [15]. For example, the copper ion plays a critical role as a catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome oxidase and tyrosinase [16]. Its concentration in the neuronal cytoplasm may contribute to the etiology of Alzheimer's [17] or Parkinson's disease [18]. However, exposure to a high level of copper can cause gastrointestinal disturbance even liver or kidney damage [19]. The average concentration of blood copper in the normal group is 100–150 μg/L (15.7–23.6 μM) [20]. The U.S. Environmental Protection Agency (EPA) has set the limit of copper in drinking water to be 1.3 *ppm* (20 μM) [21]. By virtue of its highly sensitive and high-speed spatial analysis of cells, fluorescence bioimaging has provided a facile and less cell-damaging means of visualizing analytes of biological interest in living cells [22]. To image intracellular metal ions, highly sensitive and selective probes that exhibit visible fluorescent emission changes in aqueous media need to be developed. Thus, the development of highly selective and sensitive fluorescent probes for monitoring Cu^{2+} in living cells remains highly required [23]. Herein, we would like to report a new naphthalimide-based Cu^{2+}-selective fluorescence probe 4-tert-Butyl-2,6-diformylphenol-6-hydrazino-benzo[de]isoqui noline-1,3-diones (**L**), which could be used for rapid, highly selective and sensitive detection of Cu^{2+}, and the biological application of **L** to monitor Cu^{2+} in cultured cells was also presented.

2. Experimental

The synthetic routes were shown in Scheme 1. Compounds **1**, **2** and **3** were synthesized according to the literatures [24–26]. Compound **L** was synthesized conveniently from the reaction of 6-hydrazino-benzo[de]isoquinoline-1,3-diones with 4-tert-Butyl-2,6-diformylphenol [27,Fig. S1]. **Cu–L** was synthesized from **L** and $Cu(ClO_4)_2 \cdot 6H_2O$ under reflux condition for 3 h.

3. Results and discussions

Free **L** displayed an absorption band with a maximum absorbance peak at about 466 nm ($\log \epsilon = 5.34$) (Fig. 1). Upon addition of $Cu(ClO_4)_2$ in the CH_3CN:H_2O (4:1, *v*/*v*) solution of **L** (20 μM), the absorption band centered around 466 nm decreased, finally remained constant

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http://dx.doi.org/10.1016/j.inoche.2015.10.022
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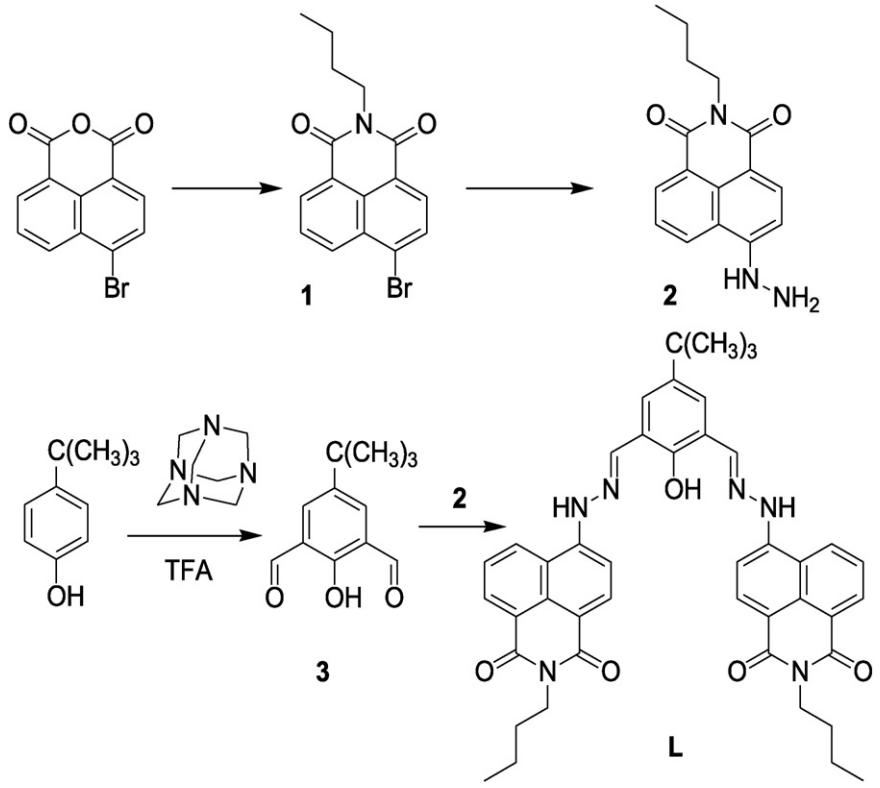

Scheme 1. Synthesis procedure of the probe **L**.

after approximate 2 equivalents of Cu^{2+} were added (Fig. S2). Meanwhile, a new absorption band centered around 600 nm appeared (Fig. 1). The former band should be assigned to the π–π* transition of naphthalimide chromophore, while the latter should be attributed to a metal-to-ligand charge transfer (MLCT) band caused by Cu^{2+}-binding. A Benesi–Hildebrand analysis established a 1:2 stoichiometry for the **L**–Cu^{2+} complexation species, with an association constant (K) being calculated as $K = 1.58 \pm 0.45 \times 10^9\ M^{-2}$ (R = 0.999) (Fig. S3) [28].

However, for **L** (20 μM) solutions, no significant absorbance changes around 466 nm were observed in the presence of 10 equivalents of alkali, alkaline earth and the other transition metal ions (Figs. S4 and S5), and even rare earth metal ions (except radioactive element promethium, Figs. S6 and S7), implying that **L** could have special binding ability toward Cu^{2+}. The high selectivity of the **L** for Cu^{2+} over other metal ions should be in part contributing to the strong coordination ability of Cu^{2+} and its larger association constant.

When excited at 466 nm, free **L** exhibited an emission band centered about 560 nm ($\Phi_f = 0.38$) in CH_3CN:H_2O (4:1, *v*/*v*) solution (Fig. 2) [29]. Upon the addition of Cu^{2+} ions, the fluorescence was quenched significantly. The titration measurements showed a steady and smooth decrease until a plateau was reached ($\Phi_f = 0.021$). The overall effect upon addition of 2 equiv. of Cu^{2+} was a 30-fold quenching of fluorescence at 560 nm (Fig. S8).

To further explore the availability of **L** as a highly selective probe for Cu^{2+}, fluorescent responses of **L** to the other metal ions that probably affect the fluorescence intensity were examined. No significant spectra

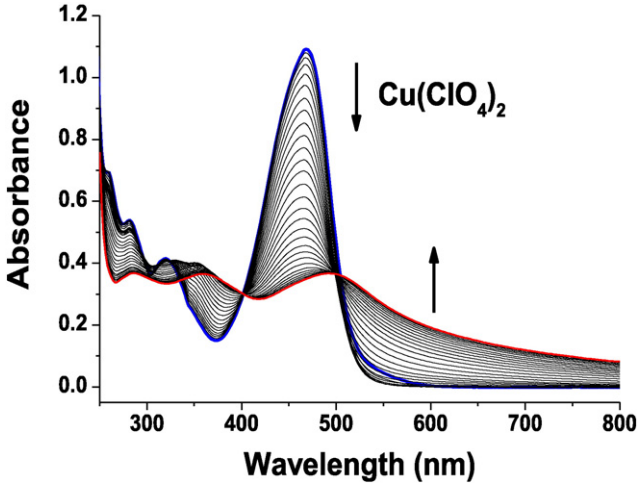

Fig. 1. UV–vis spectra of **L** (20 μM) in CH_3CN:H_2O (4:1, *v*/*v*) solution upon addition of increasing concentrations of $Cu(ClO_4)_2$.

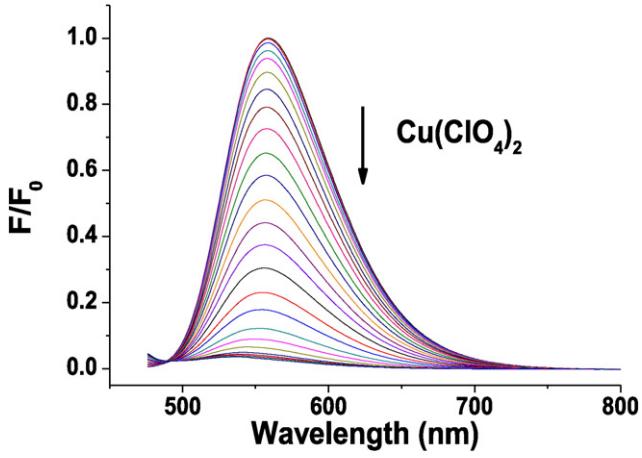

Fig. 2. Fluorescence emission spectra of **L** (20 μM) in CH_3CN:H_2O (4:1, *v*/*v*) solution upon addition of increasing concentrations of $Cu(ClO_4)_2$ with an excitation wavelength at 466 nm.