Chinese Chemical Letters 27 (2016) 205–210

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

A selective and sensitive off–on probe for palladium and its application for living cell imaging

Ying Long, Jia Zhou, Mei-Pan Yang, Bing-Qin Yang *

Key Laboratory of Synthetic and Natural Functional Molecule Chemistry, Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi'an 710069, China

ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form 10 June 2015
Accepted 15 June 2015
Available online 16 September 2015

Keywords:
Rhodamine B
Palladium
Fluorescence probe
Living cell imaging

ABSTRACT

A new rhodamine B derivative **T1** has been rationally synthesized and displayed selective Pd(II)-amplified absorbance and fluorescence emission above 540 nm in methanol–water. Upon the addition of Pd(II), the spirolactam ring was unfolded and a 1:1 metal-ligand complex formed, which can be used for "naked-eyes" detection. In addition, fluorescence imaging experiments of Pd^{2+} in HepG2 living cells showed its valuable application in biological systems.

1. Introduction

Fluorescence probe has become a widely used and important tool for monitoring metal ion concentration in biological samples. Most sensors reported have a metal chelating site linked to a fluorophore, and the metal binding causes a change in fluorescence intensity [1–6]. The development of synthetic receptors enabled the recognition of important metal ions, especially transition-metal ions, in biologically and environmentally relevant samples. This has attracted widespread interest from biologists, chemists, environmentalists and clinical biochemists in recent years [7–12]. The metal ions, like platinum, mercury, zinc and palladium have environmental and biological importance.

Palladium belongs to the platinum-group elements (PGEs; consist of Pt, Pd, Ru, Rh, Ir and Os,). It is widely used in various materials such as catalysts, dental crowns, jewelry and fuel cells [13–15]. Pd-catalyzed reactions such as the Heck, Sonogashira, Buchwald–Hartwig and Suzuki–Miyaura reactions represent powerful transformations for the synthesis of complex molecules, which played an important role in pharmaceuticals [16–23]. However, their fruitful and frequent use can also result in the contamination of soil and water systems [23] and therefore could cause health hazards [24–26]. The restrictions of government on the residual heavy metals in products are very critical. Therefore, palladium detection both in living systems and in environment has attracted tremendous attention.

Detecting the content of palladium was usually carried out by atomic absorption/emission spectroscopy, ion-coupled plasma emission-mass spectroscopy, solid-phase microextraction/high performance liquid chromatography, and X-ray fluorescence spectroscopy [27,28]. Although these conventional methods provide an extremely sensitive and rapid analysis, they need sophisticated sample-pretreatment procedures, complicated instrumentation and rigorous experimental conditions.

In recent years, the fluorescence method has been developed for palladium analysis and the colorimetric technique has frequently been applied [29–31]. For example, palladium could be detected by fluorescent ligands *via* fluorescence quenching [32–38]. Schwarze *et al. designed the first chemical sensors for* Pd^{2+} *detection through increasing fluorescence [39].*

Rhodamines are dyes widely employed in the study of complicated biological systems as fluorescence probes due to their high fluorescence quantum yields, high absorption coefficients, long-wavelength emissions and absorptions [40]. Owing to the spirolactam scaffold in rhodamines, which undergoes a conformational transformation from the spirolactam (nonfluorescent and

* Corresponding author.
E-mail address: yangbq@nwu.edu.cn (B.-Q. Yang).

http://dx.doi.org/10.1016/j.cclet.2015.09.003

colorless) to an open-ring structure (fluorescent and colored), they have been widely applied in the design of chemical sensors in recent years, and these new designed chemosensors have been reviewed recently [41–44]. In this spirocyclic form, the sensing event occurs in its five-membered lactam moiety. Obviously, these properties still present abundant opportunities for the design of new fluorescent probes.

Our research work encompasses the design, synthesis, spectroscopy and biological applications of fluorescent chemosensors for selective sensing of Pd^{2+}. Here we are reporting a novel rhodamine based chemosensor **T1**, which was prepared by a three-step synthesis using inexpensive materials. Particularly, the chemosensor showed highly selective and sensitive fluorescence "turn-on" behaviors toward Pd^{2+} followed the remarkable color changes from colorless to pink, which can be used for "naked eyes" detection. Moreover, the probe can give highly selective spectroscopic responses to Pd^{2+} over other metal ions in living cells, which showed **T1** can penetrate cell membranes and react with Pd^{2+} within living cells.

2. Experimental

2.1. Materials

All the solvents and reagents were of analytic grade and they were used without further purification unless for special needs. The **T1** was dissolved in DMF-MeOH in concentration of 1 mmol/L as stock solution. And then quantificational **T1** was used for different testing systems. We used the HITACHI F-4500 fluorescence spectrophotometer to measure the fluorescence spectra. And the absorbance spectra measurements were also performed on a Shimadzu UV-1700 spectrophotometer. IR spectra were collected on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on a Varian INOVA −400 MHz spectrometer (at 100 MHz for ^{13}C NMR and 400 MHz for ^{1}H NMR). A Bruker micro TOF-Q II ESI-TOF LC/MS/MS Spectroscopy was used to perform mass spectra. And the living cells imaging was performed on an Olympus FV1000 confocal microscope. We set the excitation wavelength as 540 nm. All reagents used for synthesis were of analytical-reagent grade and commercially available from Aldrich. Thin-layer chromatography (TLC) and column flash chromatography were performed using silica gel GF254 and Merck silica gel (250–400 mesh ASTM), respectively. And the twice-distilled water was used throughout the experiment.

2.2. Synthesis

Synthesis of compound **2**: We synthesized the compound 2 from Rhodamine B using the procedures published in literature [45–48].

Synthesis of compound **3**: In a 100 mL flask, an excess of isophthalaldehyde (0.268 g, 0.002 mol) was dissolved in 20 mL of methanol, cooled in a cold water bath. Then Rhodamine hydrazide (0.461 g, 0.001 mol) was dissolved in 30 mL of methanol and added dropwise to the above solution with vigorous stirring, and the stirred mixture was allowed to stand in the cold water bath for about 4 h, then the solvent was removed under reduced pressure, the crude was purified by silica gel column chromatography to give 3 (white powder) in 78.8% yield. MALDI-TOF MS calcd. for ($C_{36}H_{36}N_4O_3$) m/z = 573.2860 (M^+ + 1), Found: 573.2802. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (t, 12H, J = 8 Hz), 3.24–3.29 (m, 8H), 6.41–6.43 (m, 2H), 6.63–6.67 (m, 4H), 7.11 (d, 1H, J = 8 Hz), 7.37 (t, 1H, J = 8 Hz), 7.53–7.57 (m, 3H), 7.73 (d, 1H, J = 8 Hz), 7.98 (d, 1H, J = 8 Hz), 8.16 (d, 1H, J = 8 Hz), 9.18 (s, 1*H*), 9.64 (s, 1*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.2, 165.2, 153.1, 151.9, 149.0, 144.8, 136.6, 136.4, 133.6, 132.8, 129.9, 129.4, 129.0, 128.9, 128.4, 127.9, 123.9, 123.5, 108.0, 105.8, 97.9, 44.3, 12.6.

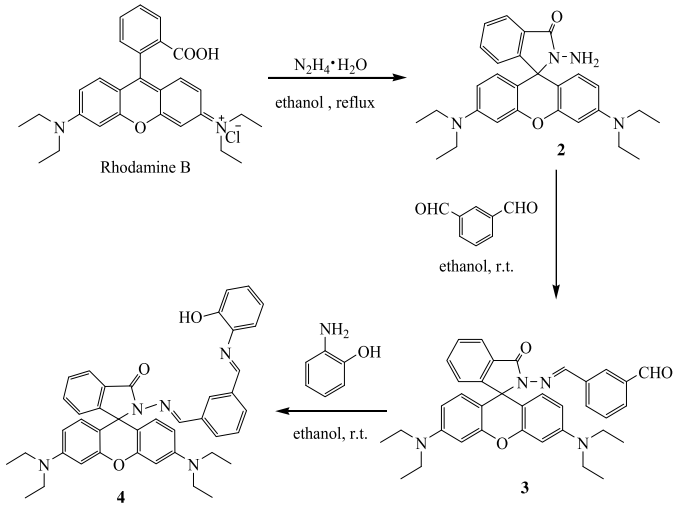
Synthesis of Compound **4**: Compound **3** (0.572 g, 0.001 mol) was dissolved in 20 mL of methanol. Then a solution of 2-aminophenol (1.2 g, a little excess) in methanol (20 mL) was added and the mixture was stirred for 2 h at 60 °C. Then the precipitates were collected and washed 3 times with 10 mL of cold ethanol. After drying under reduced pressure, the crude product was purified by recrystallization in CH_3CN/H_2O to give compound **4** (white solid) in 82.6% yield. IR (KBr/cm^{-1}): υ 3444, 2967, 2925, 1694, 1615, 1550, 1512, 1460, 1426, 1356, 1307, 1270, 1223, 1120, 1072, 1011, 980, 946, 873, 820, 786, 755, 688. MALDI-TOF MS calcd. for ($C_{42}H_{41}N_5O_3$) m/z = 663.3282, Found: 663.3298. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.15 (t, 12H, J = 8 Hz), 3.30–3.35 (m, 8H), 6.23–6.27 (m, 2H),6.49–6.54 (m, 4H), 6.92 (t, 1H, J = 4 Hz), 7.02 (d, 1H, J = 4 Hz), 7.12–7.17 (m, 2H), 7.28–7.31 (m, 1H), 7.37–7.49 (m, 3H), 7.77–7.82 (m, 2H), 7.97–8.03 (m, 2H), 8.64 (s, 1*H*), 8.82 (s, 1H), 9.96 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.6, 44.1, 66.2, 97.9, 105.8, 115.1, 120.1, 123.9, 127.9,129.0, 135.9, 145.9, 151.5, 152.4, 165.0. (Scheme 1).

2.3. Preparation of the test solution

The 10 μmol/L stock solution of probe **T1** was prepared in methanol and water (4/6, v/v). The solutions of various testing cation species were prepared from $CaCl_2$, $AgNO_3$, $MgCl_2$, $CoCl_2{\cdot}6H_2O$, $CdCl_2$, $ZnCl_2$, $CuCl_2{\cdot}2H_2O$, $MnSO_4{\cdot}H_2O$, $HgCl_2$, $NiCl_2{\cdot}6H_2O$, $Pb(NO_3)_2$, $CrCl_3{\cdot}6H_2O$, $PdCl_2$, $BaCl_2{\cdot}2H_2O$, NaCl, $AlCl_3{\cdot}6H_2O$ and $FeCl_3{\cdot}6H_2O$ in the doubly distilled water. Before spectroscopic measurements, the corresponding solutions of **4** were freshly prepared by diluting the high concentration stock solution. All the measurements were made according to the procedures as follows. Placing 1 mL of the probe solution and an appropriate aliquot of each metal stock into a 10 mL glass tube, and diluting the solution to 10 mL with methanol–water (4/6, v/v). The absorbance was at 550 nm and the fluorescence emission appeared at 583 nm. Both the excitation and emission wavelength band passes were set as 5.0 nm and the excitation wavelength was set at 540 nm.

2.4. Cell culture and fluorescence imaging

The human cancer cell line HepG2 (liver cells) were cultured in RPMI 1640 replenished with 10% FBS. Before the experiments, cells were preprocessed with probe **T1** (10 μmol/L) for 1 h at 37 °C in



Scheme 1. Synthesis of **4**.