Inorganic Chemistry Communications 89 (2018) 46–50

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

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

Short communication

Fluorescein-based fluorescent sensor with high selectivity for mercury and its imaging in living cells

Daying Liu [a,b,*], Yajie Wang [a], Ruina Wang [c], Bangchen Wang [c], Hexi Chang [c], Jiatong Chen [b], Guangming Yang [b], Huarui He [c,**]

[a] Department of Applied Chemistry, College of Basic Science, Chemistry Experiment Teaching Center, Tianjin Agricultural University, Tianjin, China
[b] Department of Chemistry, Department of Biochemistry and Molecular Biology, Key Laboratory of Advanced Energy Materials Chemistry (Ministry of Education), Nankai University, Tianjin, China
[c] Heowns Biochem Technologies LLC, Tianjin, China

ARTICLE INFO

Article history:
Received 7 November 2017
Received in revised form 15 January 2018
Accepted 17 January 2018

Keywords:
Fluorescein
Photoinduced electron transfer (PET)
Mercury
Fluorescent sensor

ABSTRACT

A novel fluorescein-based fluorescent Hg^{2+} sensor (Sensor-Hg) with N-Ethylthioethyl-N-[N′,N′-(2′-Diethylthioethylamino)-5′-methyl-Phenoxyethyl]-2-Methoxy Aniline (EDPMA) as receptor, was developed and applied successfully to image Hg^{2+} in living cells. It demonstrates high selectivity and sensitivity for sensing Hg^{2+} with about 51-fold enhancement in aqueous solution, with a characteristic emission band of fluorescein at 539 nm.

1. Introduction

Mercury is well known as one of the most toxic metals and is widespread in air, water, and soil [1]. As it can cause strong damage to the central nervous system, accumulation of mercury in the human body can lead to various cognitive and motor disorders, and Minamata disease [2]. Mercury pollution becomes a global problem [3]. Therefore, there is a great need for methods of detecting and monitoring mercury levels in the food, environment and water. Current techniques for mercury screening, including atomic absorption/emission spectroscopy and inductively coupled plasma mass spectrometry, often require expensive and sophisticated instrumentation or sample preparation [4]. Fluorescent detection of Hg^{2+} offers a promising approach for simple and rapid tracking of mercury ion in biological, toxicological and environmental monitoring. Fluorescent sensors are useful and powerful tools in the detection of metal ions, because of their simplicity, high sensitivity, good selectivity and high response speed [5]. The most applied mechanisms of fluorescence signal transduction in the design of fluorescent chemosensors are photoinduced electron transfer (PET) and intermolecular charge transfer (ICT) [6]. For a PET chemosensor, a fluorophore is usually connected via a spacer to a receptor containing a relatively high-energy non-bonding electron pair, such as nitrogen atom, which can transfer an electron to the excited fluorophore and as a result quench the fluorescence. Fluorescent PET (Photoinduced Electron Transfer) sensors are the potent analytical tools for detection of metal ions [7]. On the other hand, the ICT mechanism has been widely used in the design of ratiometric fluorescent chemosensors. When a fluorophore, without a spacer, is directly connected to a receptor (usually an amino group) to form a p-electron conjugation system with electron rich and electron poor terminals, then ICT from the electron donor to receptor would be enhanced upon excitation by light. When a receptor (playing the role of an electron donor within the fluorophore) interacts with a cation, it reduces the electrondonating character of the receptor and a blue shift of the emission spectrum is expected. In the same way, if a cation receptor plays the role of an electron receptor, the interaction between the receptor and the cation would further strengthen the push–pull effects. Then a red shift in emission would be observed. Most ratiomeric fluorescent sensors based on ICT mechanism are reported [8]. Therefore, highly selective and sensitive, PET-based fluorescent Hg^{2+} sensors are reported more and more [9]. And some examples of fluorescent sensors for mercury have been reported available in living cells [10]. Moreover, for the selective recognition of such a soft heavy metal ion, a sulfur-based functional group should be considered and introduced [11].

* Correspondence to: D.Y. Liu, Department of Applied Chemistry, College of Basic Science, Chemistry Experiment Teaching Center, Tianjin Agricultural University, Tianjin, China.
** Corresponding author.
E-mail addresses: dyxyliu@mail.nankai.edu.cn (D. Liu), huarui.he@heowns.com (H. He).

https://doi.org/10.1016/j.inoche.2018.01.016
1387-7003/

Based on those in mind, herein, a novel, highly selective and sensitive, fluorescein-PET-based fluorescent Hg^{2+} sensor, with N-Ethylthioethyl-N-[N′,N′-(2′- Diethylthioethylamino)-5′-methyl-Phenoxyethyl]-2-Methoxy Aniline (EDPMA) as receptor, was developed and applied successfully to image Hg^{2+} in living cells.

Photo-induced electron transfer (PET) is an electron transfer which occurs when certain photoactive materials interact with light. The general design of a PET-type fluoro-ionophore is the "fluorophore–spacer–receptor (ionophore)" format. A fluorescent moiety (fluorophore) is covalently linked to an ion receptor by means of a non-π-electron-conjugating spacer group, e.g. arkyl group with one to four carbons.

Typically, the ionophore will contain a tertiary amine the electrons of which can ligate the cation. The selection of a suitable ionophore was driven by several design criteria. First, the ionophore must contain tertiary nitrogen that can act as an electron donor and will also interact with a bound mercury cation. Additionally, the ionophore's binding properties should be insensitive to pH changes so as to minimize undesirable pH interference to the measurement of mercury. Finally, the ionophore should preferentially bind mercury in the aqueous medium, while also in the presence of other cations. Anilines have been proven to be efficient ionophores. In order to enhance the binding strength, another tertiary amine is introduced by phenolic hydroxyl group. Therefore, we chose to use fluorescein as the fluorophore reporter in designing **Sensor Hg**, and the introduction of multiple sulfur-based functional groups greatly increased the affinity of the sensor for Hg^{2+}.

2. Experimental section

2.1. Material, measurements and methods

Unless otherwise noted, all materials were obtained from Heowns Biochem Technologies LLC and were used without further purification. Flash chromatography was carried out on silica gel (300–400 mesh). ^{1}H NMR spectra were recorded using Varian 300 MHz. Absorption spectra and fluorescence spectra were measured on the Gangdong A-230 photometer and F-280 fluorometer, respectively.

2.2. Synthesis

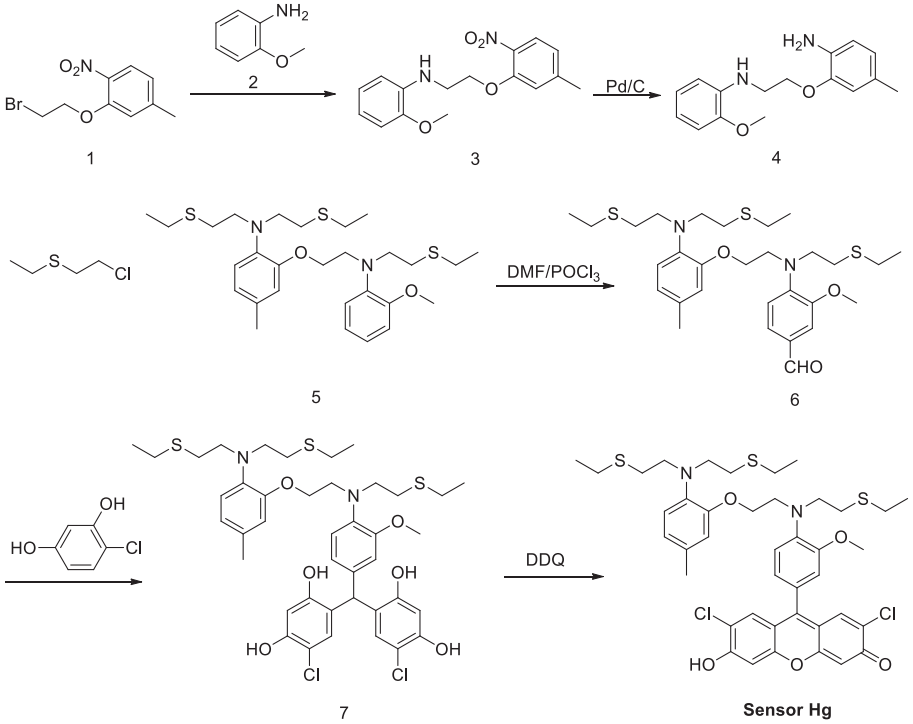
Now, we present our design and synthesis of a new fluorescein-based fluorescent sensor with N-**E**thylthioethyl-N-[N′, N′-(2′-**D**iethylthioethylamino)-5′-methyl- **P**hen oxyethyl]-2-**M**ethoxy **A**niline (EDPMA) as receptor. Scheme 1 explains the synthetic route of **Sensor Hg**. The detailed characterization of the new compounds are described in the Supporting Information.

The compounds **1**, **3** and **4** were synthesized according to a previously reported procedure [7c].

Synthesis of 3 A suspension of 80 g (307.59 mmol) compound **1**, 25 g (205.06 mmol) compound **2**, 56 g (410.12 mol) K_2CO_3 and 33.5 g (205.06 mol) KI in 300 mL acetonitrile was heated under reflux for 20 h. The progress was monitored by TLC (PE: EA = 5:1). After the reaction was completed, the mixture was cooled and solvent was evaporated. The residue was purified by flash column chromatography, to obtain product 44 g. ^{1}HNMR ($CDCl_3$) δ 7.80 (d, J = 8.2 Hz, 1H), 6.86 (ddd, J = 22.3, 12.4, 4.9 Hz, 4H), 6.75–6.66 (m, 2H), 4.30 (t, J = 5.3 Hz, 2H), 3.85 (s, 3H), 3.62 (t, J = 5.3 Hz. 2H), 2.38 (s, 3H).

Synthesis of 4:10.0 g (33.08 mmol) compound **3** was dissolved in 50 mL mixed solvent (DCM: MeOH = 1:5), 1 g 10% palladium on activated carbon was added. This suspension was hydrogenated at 2.2 atm. For 18 h, till no more hydrogen uptake was observed. The progress was monitored by TLC (PE: EA = 4:1).The catalyst was filtered off and the solvent was evaporated, to obtain product 7.7 g. ^{1}HNMR ($CDCl_3$) δ 2.17(s, 3H), 3.51(m, 2H), 3.54(s, 2H), 3.76(s, 3H), 4.12(t, 2H), 4.24(s, 1H), 6.5–7.17(m, 7H).

Synthesis of 5: A suspension of 0.5 g (1.84 mmol) of compound **4**, 3.2 mL (27.54 mmol) of 2-chloroethyl ethyl sulfide, 9.1 mL (55.08 mmol) N, N-diisopropylethyl -amine and 4.57 g (27.54 mmol) of KI in DMF (10 mL) was heated at 110 °C for 20 h under nitrogen atmosphere. The progress was monitored by TLC (PE: EA = 4:1). After the reaction was complete, the mixture was cooled and poured into water. The resultant precipitate was filtered, dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4, filtered and evaporated to get 0.75 g crude product, which was purified by flash column chromatography, to afford product 295 mg. ^{1}HNMR ($CDCl_3$) δ 7.06 (dd, J = 8.2, 1.6



Scheme 1. The synthetic route of **Sensor Hg.**