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A two-photon fluorescent turn-on probe for palladium imaging in living tissues

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ABSTRACT

We have rationally constructed the first two-photon fluorescence palladium probe based on the **GCTPOC** two-photon fluorescent dye and deprotection of aryl propargyl ethers by palladium. The probe **GCTPOC-Pd** displayed a 100-fold enhancement turn-on to palladium species with high sensitivity and selectivity. Additionally, the novel **GCTPOC-Pd** probe is suitable for fluorescence imaging of palladium in living cells and tissues.

1. Introduction

Detection and removal of toxic metals have received intense attention because of their significance in chemistry, biology, and environmental science [1]. In particular, palladium, can have adverse effects on our health and the environment, because it can bind to thiol containing amino acids, proteins, DNA, and other biomolecules and disturb a variety of cellular processes [2]. In addition, palladium is also often used to prepare dental materials, jewelry, and electric equipment, and automobile exhaust catalysts [2]. And palladium, a rare inner transition metal, is the subject of intense research in light of its powerful catalytic ability. Hence, it is very critical to detect the presence of palladium species both in the living and the environmental setting.

So far, several analytical techniques including colorimetric and electrochemical methods, chromatography and sulfide precipitation have been developed for the detection of palladium [3,4]. Although these methods are sensitive toward palladium, they require complicated sample preparation and destruction of tissues or cells; and therefore, they are not suitable for living biosystems. In recent years, fluorescent probes, as excellent detection tools, have attracted increasing attention for high selectivity, high sensitivity, as well as real-time imaging, and they have been widely applied for the detection of biological molecules [5–29]. On the other hand, two-photon microscope (TPM) provides an attractive technique to study biomolecules in live cells and tissues. TPM, where fluorescence is triggered by two-photon excitation, shows a variety of advantages over conventional one-photon fluorescence microscopy. It can facilitate three-dimensional imaging of living tissues, reduce photodamage to biosamples, increase tissue penetration, and lower background fluorescence [30–35].

So far, some research groups have actively engaged in the development of colorimetric and fluorometric detection methods for palladium [36–52]. Although the dection of palladium in living cells has been demonstrated, the detection of palladium in much thicker biosamples, for instance, living tissues, has not been realized. It is known that two-photon fluorescent probes are favorable for tracking biomolecules in living tissues. Thus, it is of great interest to design two photon fluorescent probes which are suitable for monitoring palladium not only in living cells but also in living tissues.

Recently, our group have reported a two-photon fluorescent dye platform **GCTPOC** [53], which contains a rigid oxygen-bridge and a hydroxyl group at the 2-position (para-position). **GCTPOC** exhibits excellent two-photon properties with a two-photon cross-section (s) above 810 GM and a two-photon excitation action cross-section above 270 GM, indicating that this two-photon dye

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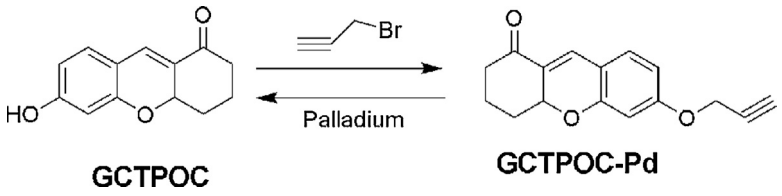

Scheme 1. The design and synthesis of two-photon fluorescent turn-on probe **GCTPOC-Pd**.

is potentially useful for bioimaging applications. **GCTPOC** has been demonstrated to be an efficient two-photon platform for designing two-photon probes with its tunable two-photon properties at the hydroxyl group. Herein, we set out to design the **GCTPOC-Pd** as the two-photon fluorescent turn-on probe for palladium based on the **GCTPOC** two-photon platform (Scheme 1). The two-photon probe **GCTPOC-Pd** might have a large fluorescence enhancement, which renders it attractive for imaging palladium in living cells and tissues with deep tissue penetration.

2. Experimental

2.1. Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. The fluorescence imaging of cells was performed with OLYMPUS FV1000 (TY1318) confocal microscopy. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

2.2. Synthesis

The detail synthetic approaches are shown in Supporting Information (SI). All the chemical structures of compounds were verified by Mass, ^{1}H NMR, and ^{13}C NMR spectroscopy, seen in SI.

2.3. Preparation of the test solution

The stock solution of the **GCTPOC-Pd** probe was prepared at 0.5 mM in ethanol. The solutions of various testing species were prepared from $AlCl_3{\cdot}6H_2O$, $CaCl_2$, $CdCl_2{\cdot}1/2H_2O$, $CoCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $HgCl_2$, KCl, $MgCl_2$, $MnSO_4{\cdot}H_2O$, NaF, $Pb(NO_3)_2$, $ZnCl_2$, $Pd(OAc)_2$, $PdCl_2$, $Pd(PPh_3)_4$, K_2PdCl_6, K_2PtCl_4, and K_2PtCl_6, twice-distilled water. The test solution of the **GCTPOC-Pd** probe (5 μM) in 3 mL aqueous buffer (pH 7.4, 25 mM PBS buffer with 10% ethanol) was prepared by placing 0.030 mL of the **GCTPOC-Pd** probe stock solution and 0.27 mL ethanol in 2.7 mL of 25 mM PBS buffer (pH = 7.4). The test solution was shaken well at room temperature before recording the spectra. Unless otherwise noted, for all measurements, the excitation wavelength was 410 nm, the excitation slit widths were 5 nm, and emission slit widths were 5 nm.

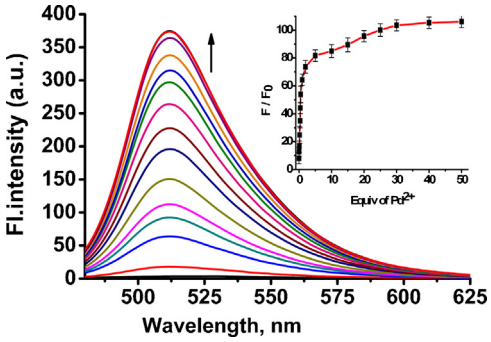

Fig. 1. Fluorescence spectra of **GCTPOC-Pd** (5.0 μM) in pH 7.4 PBS buffer-ethanol (9:1, v/v) in the absence or presence of Pd(II) (Na_2PdCl_4) (0–50 equiv.). Inset: fluorescence intensity ratio (F/F_0) changes at 512 nm of **GCTPOC-Pd** (5.0 μM) with the amount of Pd(II) (Na_2PdCl_4). The spectra were recorded after incubation of the probe with Pd(II) for 40 min. Excitation at 450 nm.

2.4. Cell culture and fluorescence imaging

SMMC7721 cells were grown in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. Immediately before the experiments, the cells were washed with PBS buffer. SMMC7721 cells incubated with **GCTPOC-Pd** (5.0 μM) for 20 min, then with Na_2PdCl_4 (50.0 μM) for 30 min at 37 °C. The fluorescence images were acquired with an Olympus FV1000 equipped with a CCD camera.

2.5. Preparation of fresh mouse liver slices and two-photon fluorescence imaging

Slices were prepared from the liver of 14-day-old mice. Slices were cut to 400 mm thickness by using a vibrating-blade microtome in 25 mM PBS (pH 7.4). For the control experiments, slices

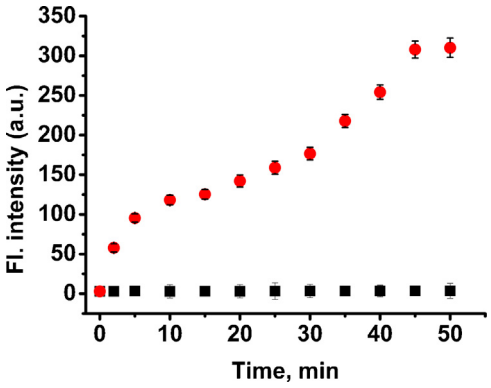

Fig. 2. Reaction-time profiles of **GCTPOC-Pd** (5.0 μM) in the absence [■] or presence of Na_2PdCl_4 (10 equiv.) [●]. The fluorescence intensities at 512 nm were continuously monitored at time intervals in pH 7.4, 25 mM PBS buffer-ethanol (9:1, v/v). Time points represent 0, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 55 min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)