Supporting Information

A Water Soluble ESIPT-Based Fluorescent Chemodosimeter for the Ratiometric Detection of Palladium Ions in Aqueous Solution and Its Application in Live-Cell Imaging

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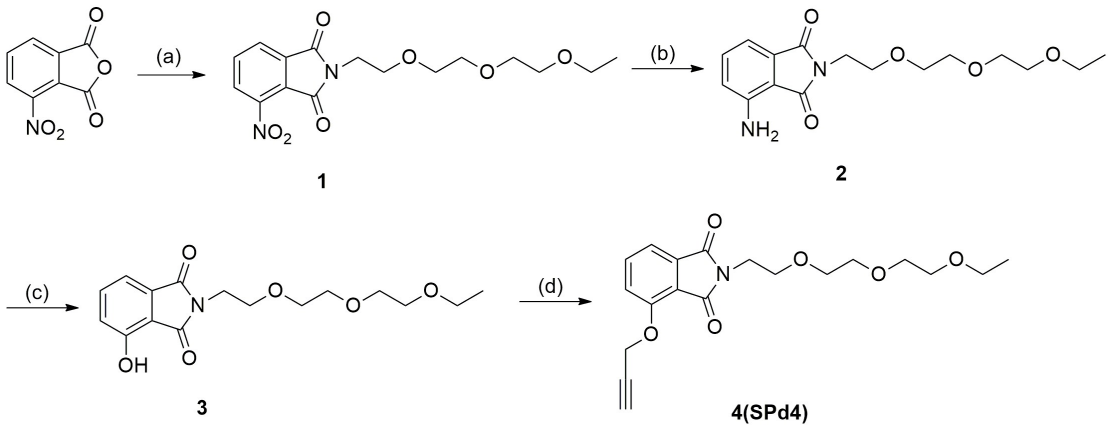
Contents

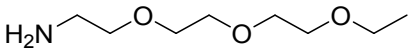
Materials and methods

All the solvents were of analytic grade. NMR experiments were carried out on a Bruker AV-400 NMR spectrometer with chemical shifts reported in ppm (in $CDCl_3$ or TMS as an internal standard). Mass spectra were measured on an Agilent 1290 LC-MS spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-10. Fluorescence spectra were determined on a PerkinElmer LS55 Fluorescence spectrophotometer. Absorption spectra were collected on a Shimadzu UV 2501(PC)S UV-Visible spectrophotometer. All the cation solutions were prepared from NaCl, KCl, CsCl, $MgCl_2$, $CaCl_2$, $NiCl_2$, $FeCl_2$, $CrCl_3$, $CuCl_2$, $AgNO_3$, $ZnCl_2$, $CdCl_2$, $HgCl_2$, and $PdCl_2$ in distilled water, with a concentration of 1 mM, respectively. The excitation and emission widths for **SPd4** were all 3/5.

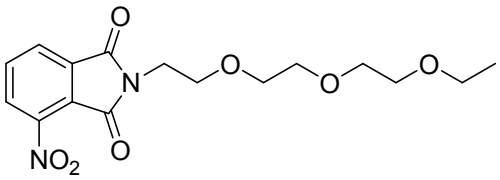
Synthesis and characterization



Scheme S1 Synthesis of **SPd4**: (a) 2-(2-(2-ethoxyethoxy)ethoxy)ethanamine, AcOH, reflux, 2.5 h, 79%; (b) Pd/C, H_2, MeOH, r.t., 12h, 88%; (c) $NaNO_2$, H_2SO_4, 0°C, 30 min then 90°C, 1h, 49%; (d) 3-bromoprop-1-yne, K_2CO_3, Acetone, reflux, 12h, 71%.



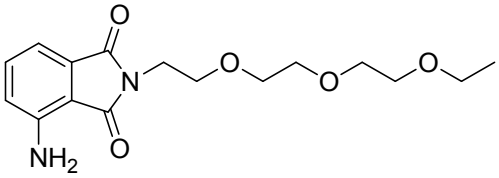
2-(2-(2-ethoxyethoxy)ethoxy)ethanamine was synthesized according to the literature.[1] ^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.62 (m, 8H), 3.54 (m, 2H), 3.48 (m, 2H), 3.34 (m, 2H), 1.16 (t, *J* = 6.6 Hz, 3H).



1

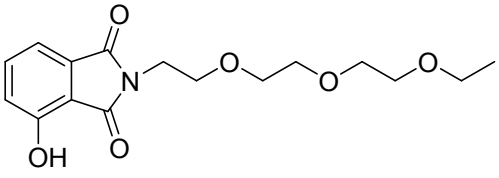
2-(2-(2-(2-ethoxyethoxy)ethoxy)ethyl)-4-nitroisoindoline-1,3-dione (1): 3-nitrophthalic acid anhydride (1.15 g, 5.96 mmol) and 2-(2-(2-ethoxyethoxy)ethoxy)ethanamine (1.58 g, 8.93 mmol) were dissolved in AcOH (15 mL) under nitrogen atmosphere, then reflux for 2.5 h until all starting material got consumed which was monitored by TLC analysis. The mixture solution was then

poured into water (150 mL), extracted by EtOAc (3 × 150 mL). The extract was washed with brine (150 mL), dried over sodium sulfate and then concentrated under vacuum. The product was purified by flash chromatography using petroleum ether/ethyl acetate (2:1, v/v) as eluant to give **1** (1.66 g, 79%) as a pale yellow oil. R_f = 0.40 (petroleum ether/ethyl acetate 1:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 (m, 2H), 7.89 (m, 1H), 3.83 (m, 2H), 3.66 (m, 2H), 3.55 (m, 6H), 3.40 (m, 4H), 1.06 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 145.04, 135.21, 134.14, 128.35, 126.88, 70.57, 70.46, 70.03, 69.66, 67.46, 66.52, 37.93, 29.60, 29.57, 15.00.



2

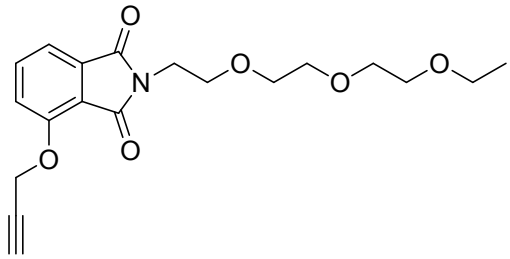
4-amino-2-(2-(2-(2-ethoxyethoxy)ethoxy)ethyl)isoindoline-1,3-dione (2): The mixture of compound **1** (450 mg, 1.28 mmol) and Pd/C 10% (45 mg) in methanol (15 mL) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 12 h. The catalyst was filtered off and rinsed with methanol. The filtrate was concentrated, and the residue was dried under vacuum for 1 h to give **2** (361 mg, 88%) as a yellow oil. R_f = 0.43 (petroleum ether/ethyl acetate 1:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (t, *J* = 7.2 Hz, 1H), 7.09 (d, *J* = 7.0 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 4.89 (brs, 1H), 3.82 (t, *J* = 5.8 Hz, 2H), 3.70 (t, *J* = 5.8 Hz, 2H), 3.61 (m, 6H), 3.50 (m, 4H), 1.17 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 145.29, 134.84, 132.64, 120.88, 112.27, 70.52, 70.38, 70.02, 69.61, 67.90, 66.40, 36.75, 14.98.



3

2-(2-(2-(2-ethoxyethoxy)ethoxy)ethyl)-4-hydroxyisoindoline-1,3-dione (3): To a solution of compound **2** (361 mg, 1.12 mmol) in 50% sulfuric acid (16.5 mL) at -5 ^{0}C was added the solution

of $NaNO_2$ (77 mg, 1.12 mmol) in 2 mL water dropwisely. The reaction mixture was stirred for 30 min at -5 ^{0}C, and then heated to 90 ^{0}C for 1 h. Water (45 mL) was then added to the solution, and the reaction mixture was extracted with ethyl acetate (3 × 20 mL). The extract was washed with brine (60 mL), dried over sodium sulfate and then concentrated under vacuum. The product was purified by flash chromatography using petroleum ether/ethyl acetate (2:1, v/v) as eluant to give **3** (178 mg, 49%) as a yellow oil. R_f = 0.35 (petroleum ether/ethyl acetate 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (brs, 1H), 7.60 (t, J = 7.6 Hz, 1H), 7.27 (d, J = 7.6 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H), 3.67 (t, J = 5.8 Hz, 2H), 3.58 (t, J = 5.8 Hz, 2H), 3.50 (m, 2H), 3.43 (m, 4H), 3.36 (m, 4H), 1.05 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 169.97, 167.70, 154.58, 136.11, 132.18, 122.54, 115.68, 70.57, 70.46, 70.09, 69.66, 67.76, 66.47, 60.26, 37.10, 20.87, 14.98, 14.07.



4(SPd4)

2-(2-(2-(2-ethoxyethoxy)ethoxy)ethyl)-4-(prop-2-yn-1-yloxy)isoindoline-1,3-dione (4, SPd4): Compound **3** (200 mg, 0.62 mmol), K_2CO_3 (85 mg, 0.62 mmol), and KI (10 mg, 0.06 mmol) were dissolved in acetone (20 mL) under nitrogen atmosphere, and stirred at room temperature for 30 min. Then 3-bromoprop-1-yne (60 μL, 0.74 mmol) was added to the mixture, and the reaction mixture was refluxed for 12 h until all starting material got consumed which was monitored by TLC analysis. The mixture solution was then poured into water (150 mL), extracted by DCM (3 × 100 mL). The extract was washed with brine (200 mL), dried over sodium sulfate and then concentrated under vacuum. The product was purified by flash chromatography using petroleum ether/ethyl acetate (2:1, v/v) as eluant to give **4** (158 mg, 71%) as a pale yellow soild. R_f = 0.38 (petroleum ether/ethyl acetate 1:2). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 (t, J = 7.9 Hz, 1H), 7.37 (d, J = 7.3 Hz, 1H), 7.29 (d, J = 8.7 Hz, 1H), 4.87 (d, J = 2.3 Hz, 2H), 3.76 (t, J = 5.9 Hz, 2H), 3.63 (t, J = 6.0 Hz, 2H), 3.53 (m, 6H), 3.41 (m, 4H), 2.60 (t, J = 2.2 Hz, 1H), 1.08 (t, J = 7.6 Hz,

3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.29, 165.85, 154.01, 136.41, 133.72, 119.87, 117.11, 115.71, 79.38, 78.35, 69.82, 69.62, 69.49, 69.16, 66.91, 65.50, 56.35, 36.95, 15.11. HR-MS (TOF-ESI): *Calcd*. for $([M+H])^+$, 362.1604; Found, 362.1671.

Photophysical properties of SPd4

Table S1 Photophysical properties of the probe.

entry	$\lambda_{ab\ (nm)}$	$\lambda_{em\ (nm)}$	Φ^a	ε / M^{-1} cm^{-1}
SPd4	340	405	0.29	6128
SPd4+Pd^{2+}	400	509	0.22[b]	8257

(a) The quantum yield (Φ) of **SPd4** and **SPd4**-Pd^{2+} system were determined according to the literature.[2] (b) Φ was determined in the present of 15.0 equiv. of Pd^{2+}.

$$\Phi_{Sample} = \frac{\Phi_{QS} \cdot A_{QS} \cdot F_{Sample} \cdot \lambda_{exQS} \cdot \eta^2_{Sample}}{A_{Sample} \cdot F_{QS} \cdot \lambda_{exSample} \cdot \eta^2_{QS}}$$

Where Φ is quantum yield; A is absorbance at the excitation wavelength; F is integrated area under the corrected emission spectra; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the Sample and QS refer to the sample and the standard, respectively. We chose quinine sulfate in 0.1N H_2SO_4 as standard, which has the quantum yield of 0.546.[3]

Additional spectroscopic data

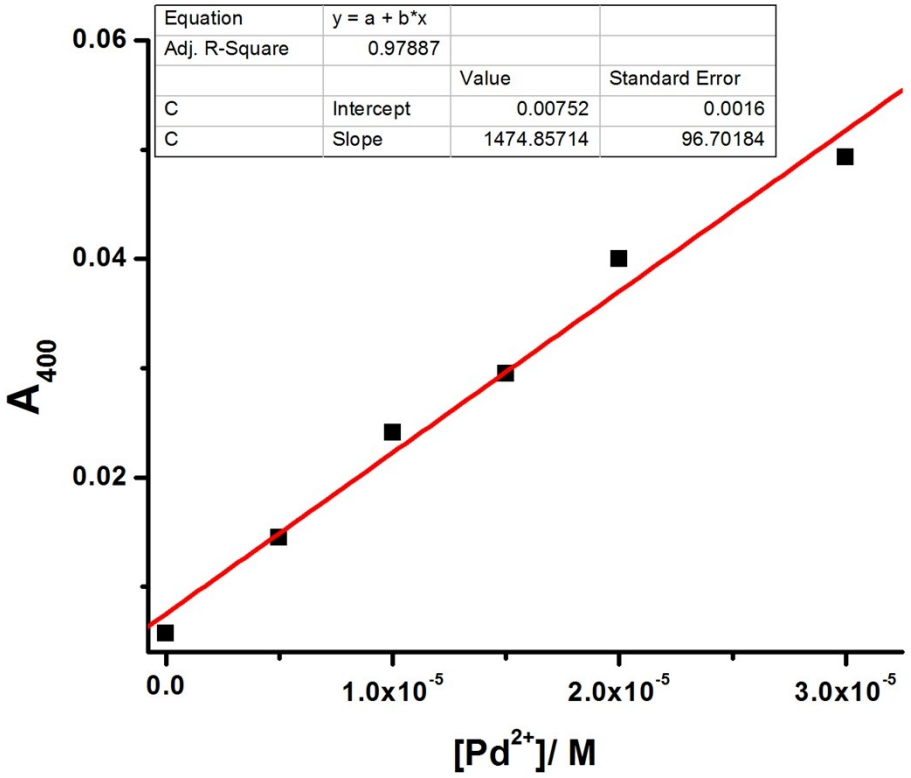


Fig. S1 The UV-vis absorption of **SPd4** (10.0 μM) at 400 nm as a function of Pd^{2+} concentration (0-30.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% EtOH).

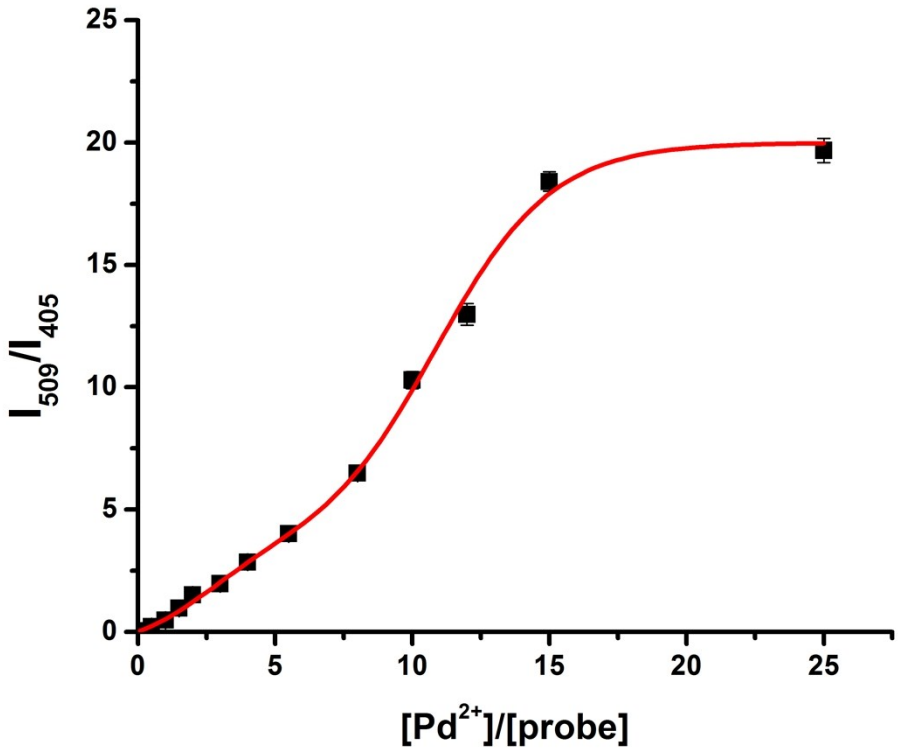


Fig. S2 The ratio of fluorescent intensity of **SPd4** (10.0 μM) at 509 nm and 405 nm (I_{509}/I_{405}) as a function of Pd^{2+} concentration (0-250.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% EtOH) (λ_{ex} = 337 nm).

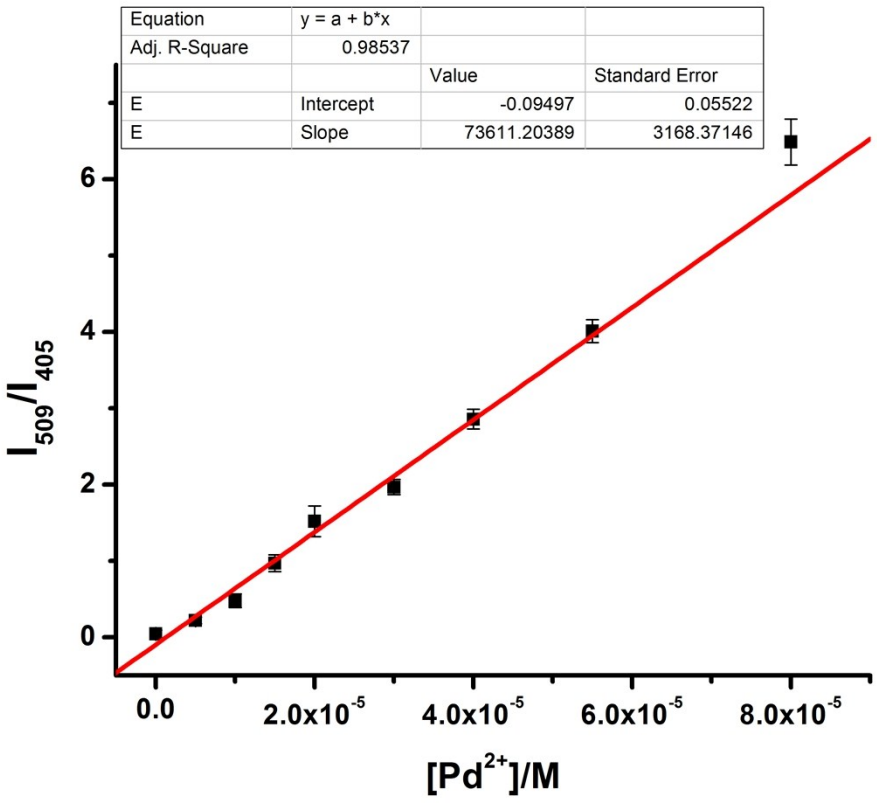


Fig. S3 The changes of the ratio of fluorescent intensity of **SPd4** (10.0 μM) at at 509 nm and 405 nm (I_{509}/I_{405}) as a function of Pd^{2+} concentration (0-80.0 μM) under the same condition as the Pd^{2+} titration.

The detection limit (DL) of Pd^{2+} using **SPd4** was determined from the following equation: [4]

$$DL = 3*\sigma/K$$

Where σ is the standard deviation of the blank solution; K is the slope of the calibration curve.

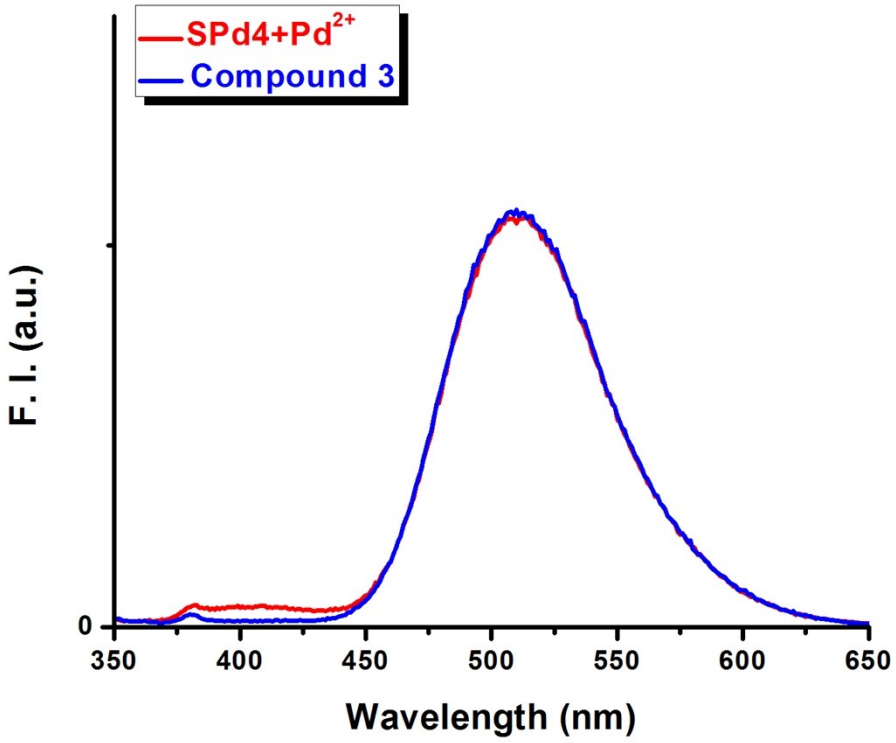


Fig. S4 The comparison of fluorescent spectra of **SPd4**+Pd^{2+} system, and compound **3** (control) in 10 mM PBS buffer solution, pH 7.4, containing 1% EtOH, λ_{ex} = 337 nm.

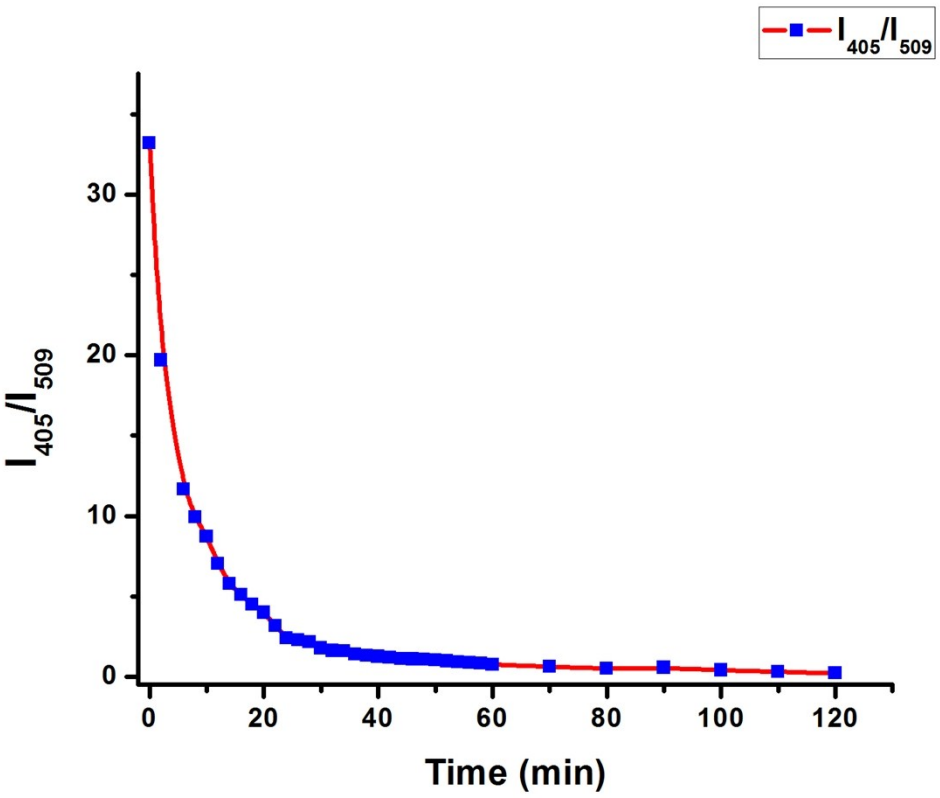


Fig. S5 The changes of the ratio of fluorescent intensity of **SPd4** (10.0 μM) in the present of Pd^{2+} (150.0 μM) at 405 nm and 509 nm (I_{405}/I_{509}) as a function of reaction time (0-120 min) under the same condition as the Pd^{2+} titration.

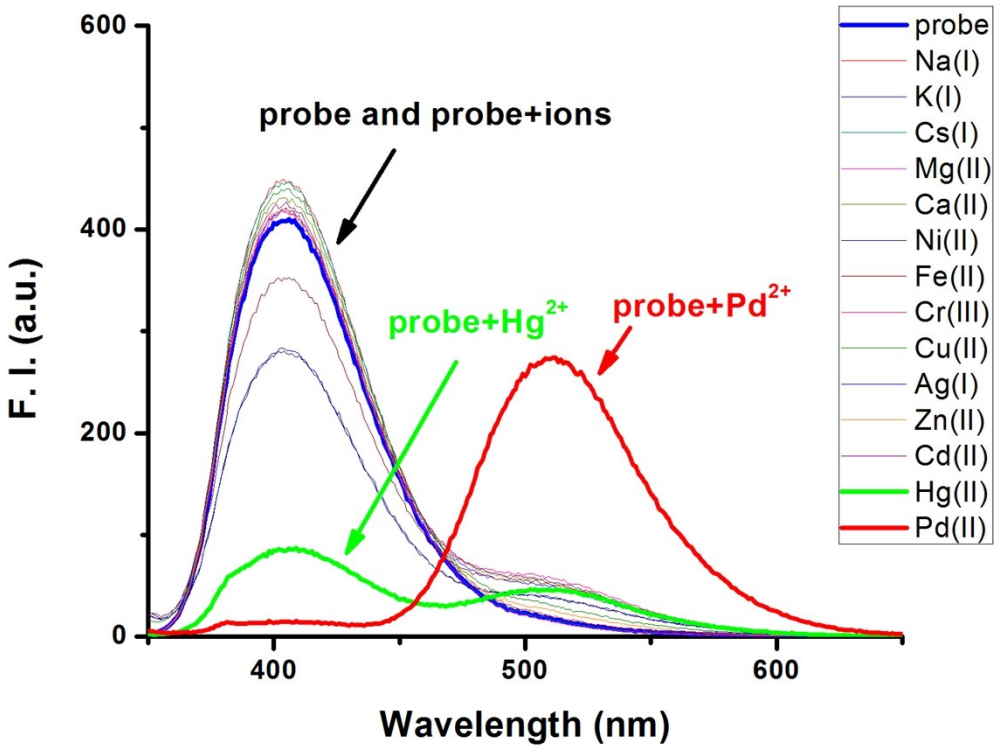


Fig. S6 Fluorescence responses of **SPd4** (10.0 μM) with 15.0 equiv. of metal ions in PBS buffer solution (10 mM, pH 7.4, containing 1% EtOH). Metal ions include Na^{+}, K^{+}, Cs^{+}, Mg^{2+}, Ca^{2+}, Ni^{2+}, Fe^{2+}, Cr^{3+}, Cu^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pd^{2+}, (λ_{ex} = 337 nm).

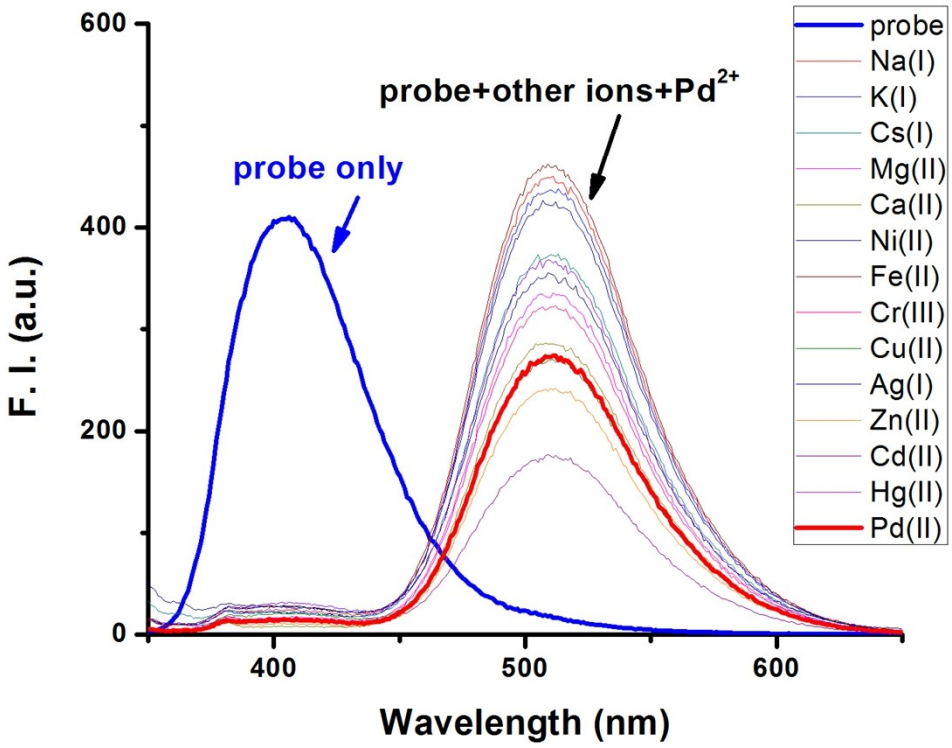


Fig. S7 Fluorescence responses of **SPd4** (10.0 μM) in the presence of 15.0 equiv. of metal ions (Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ni^{2+}, Fe^{2+}, Cr^{3+}, Cu^{2+}, Ag^+, Zn^{2+}, Cd^{2+}, and Hg^{2+}) in PBS buffer solution (10 mM, pH 7.4, containing 1% EtOH), followed by 15.0 equiv. of Pd^{2+} (λ_{ex} = 337 nm).

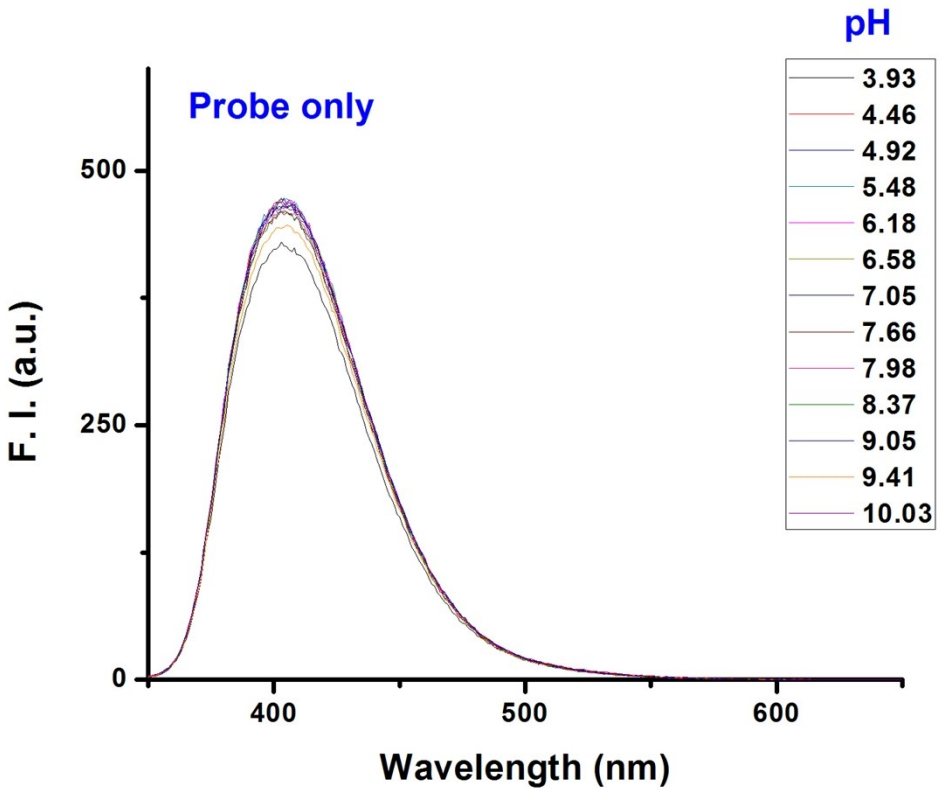


Fig. S8 Effect of the pH on the fluorescence emission of **SPd4** (10.0 μM) (λ_{ex} = 337 nm).

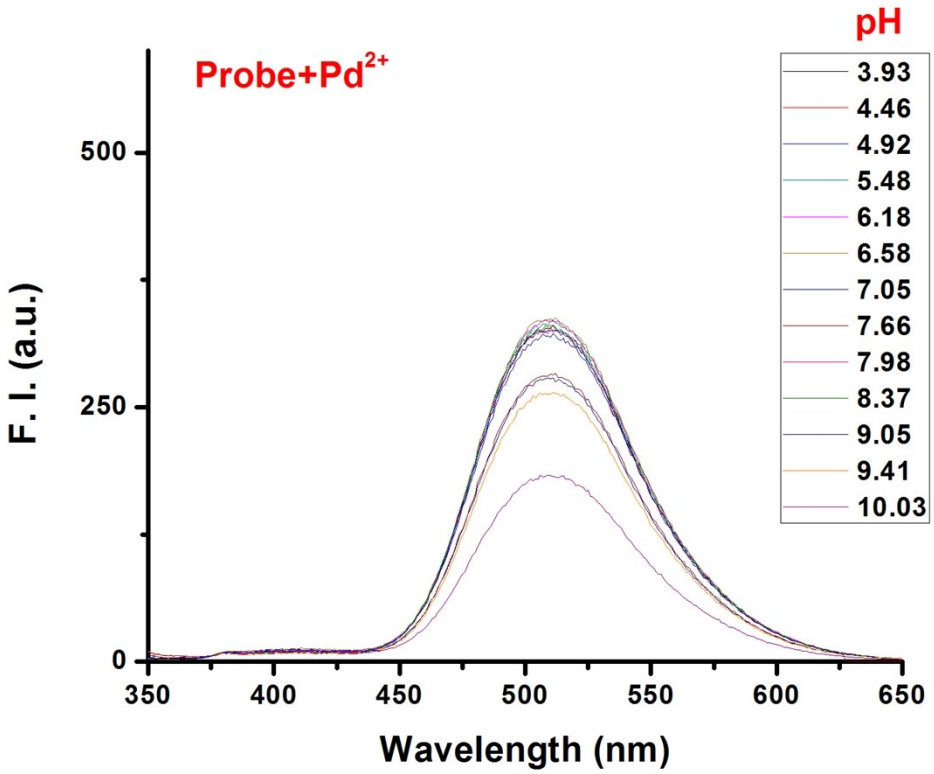


Fig. S9 Effect of the pH on the fluorescence emission of **SPd4**-Pd^{2+} system (10.0 μM of **SPd4** in the present of 15.0 equiv. of Pd^{2+}) (λ_{ex} = 337 nm).

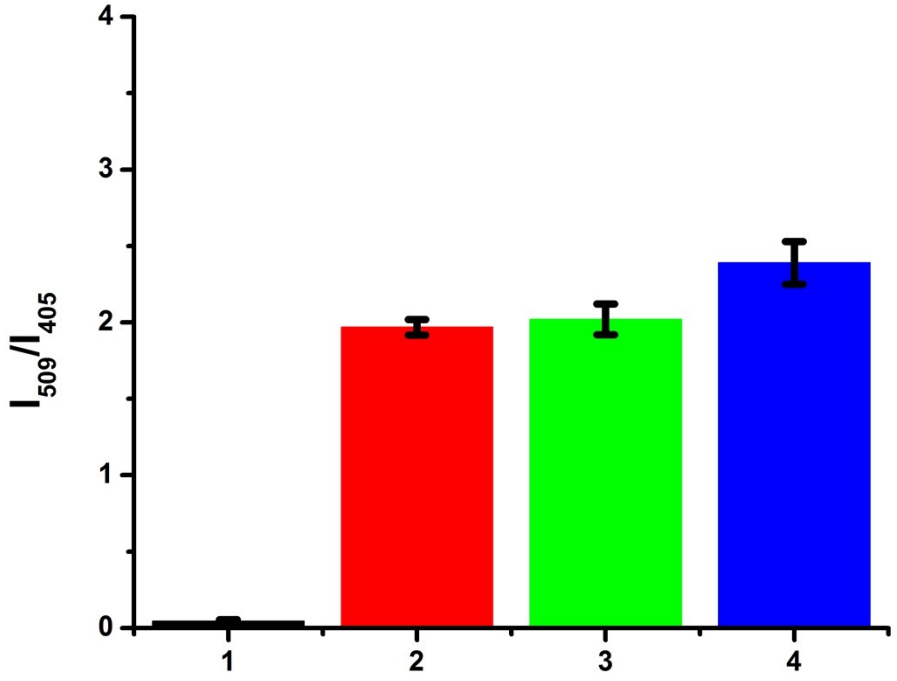


Fig. S10 Fluorescence responses of **SPd4** to various Pd (including 1: probe; 2: $PdCl_2$, 3: K_2PdCl_6, and 4: $Pd(PPh_3)_4$). (The addition of 3.0 equiv. of the appropriate Pd to a 10.0 μM solution of **SPd4**, in PBS buffer solution, 10 mM, pH 7.4, containing 1% EtOH, λ_{ex} = 337 nm).

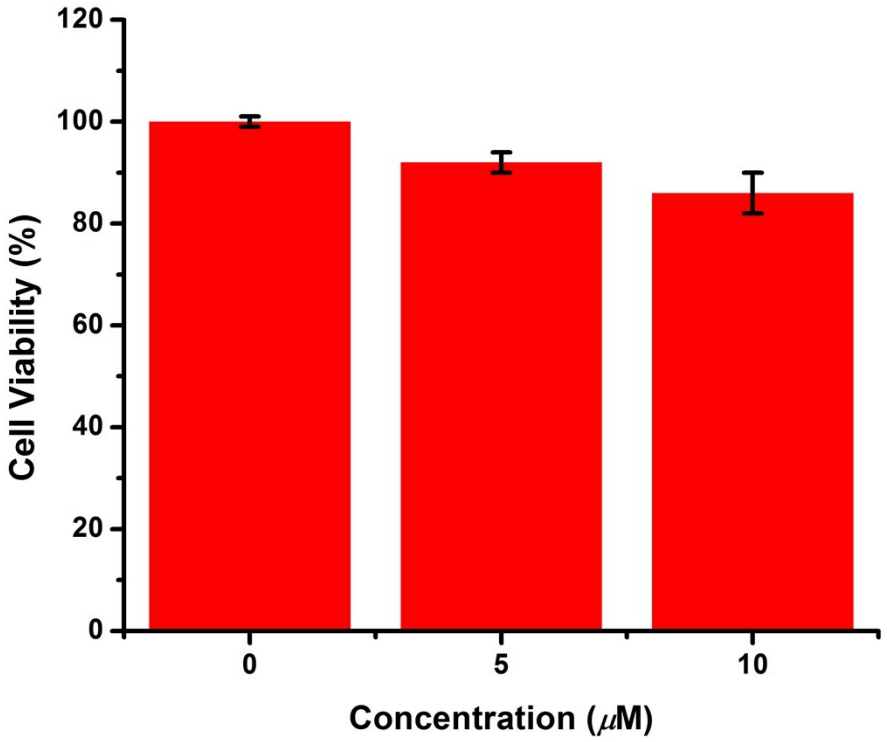
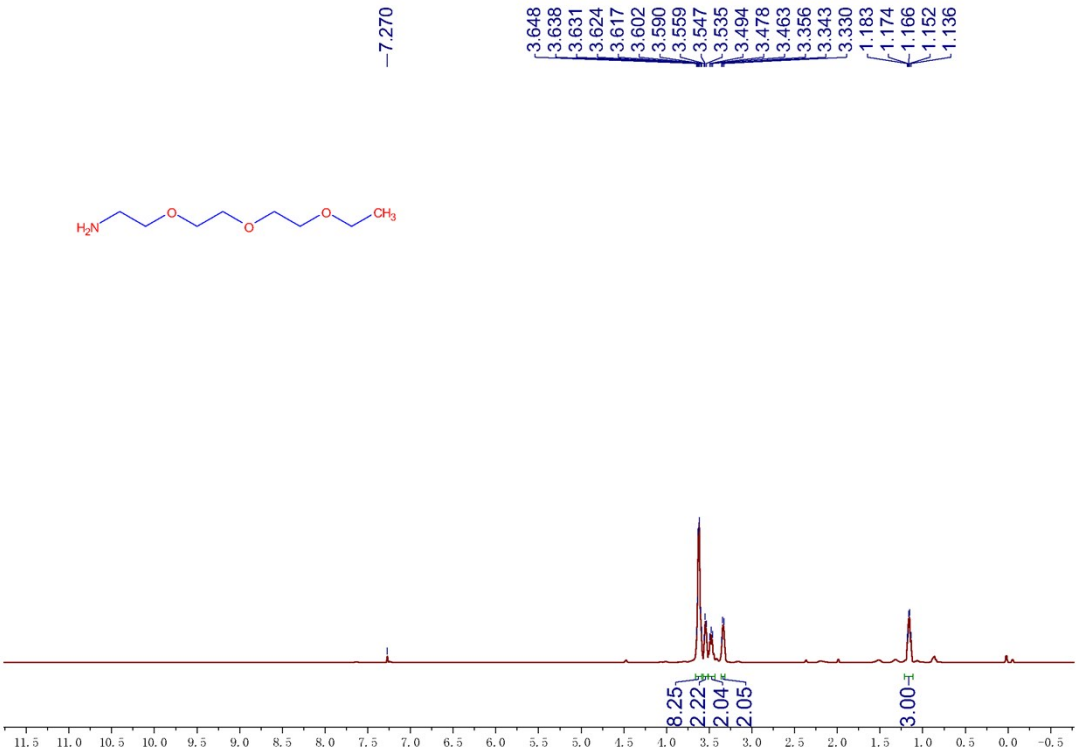


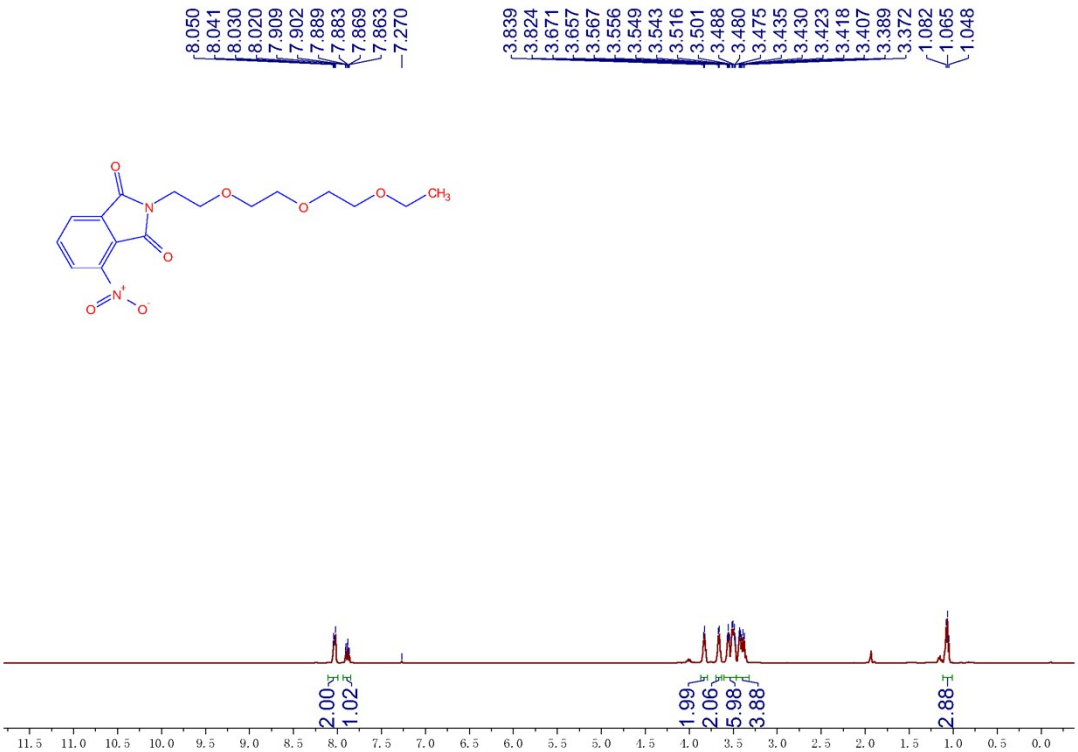
Fig. S11 Cell viability of HeLa cells treated with different concentration of **SPd4** for different time periods. No cytotoxic effect was observed for the cells incubated with **SPd4** at 10 μM even for 24 h.

The characterization data of SPd4

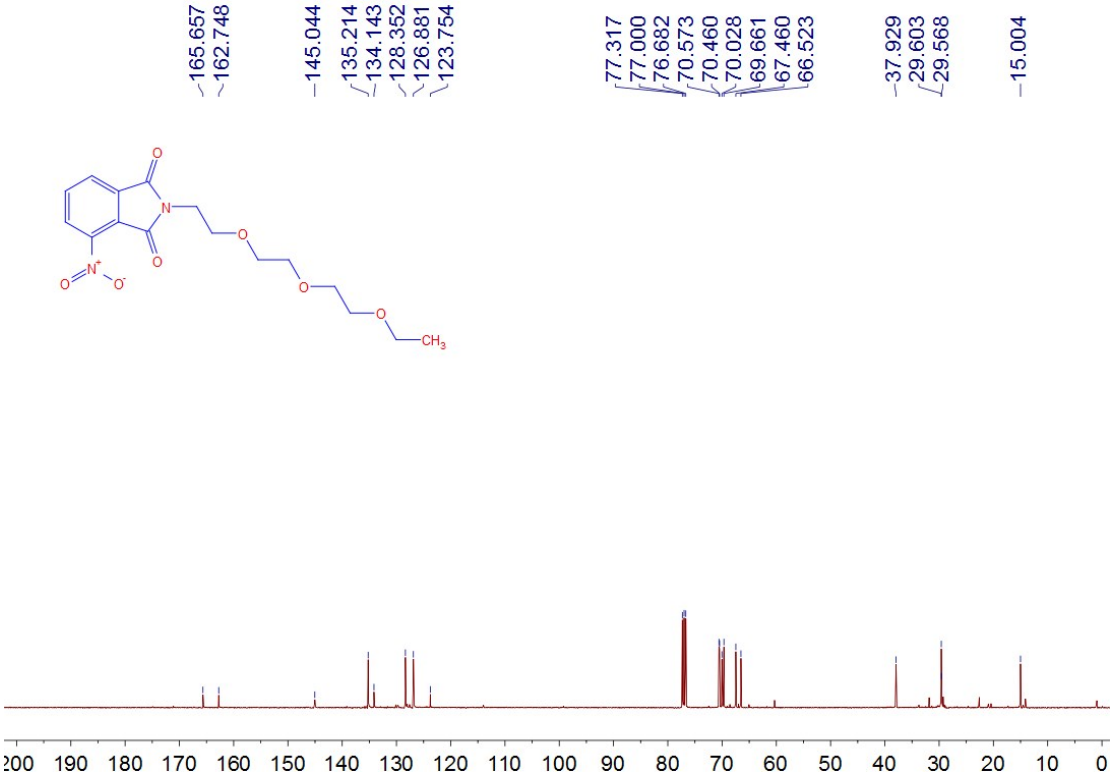
^{1}H NMR of **2-(2-(2-ethoxyethoxy)ethoxy)ethanamine**



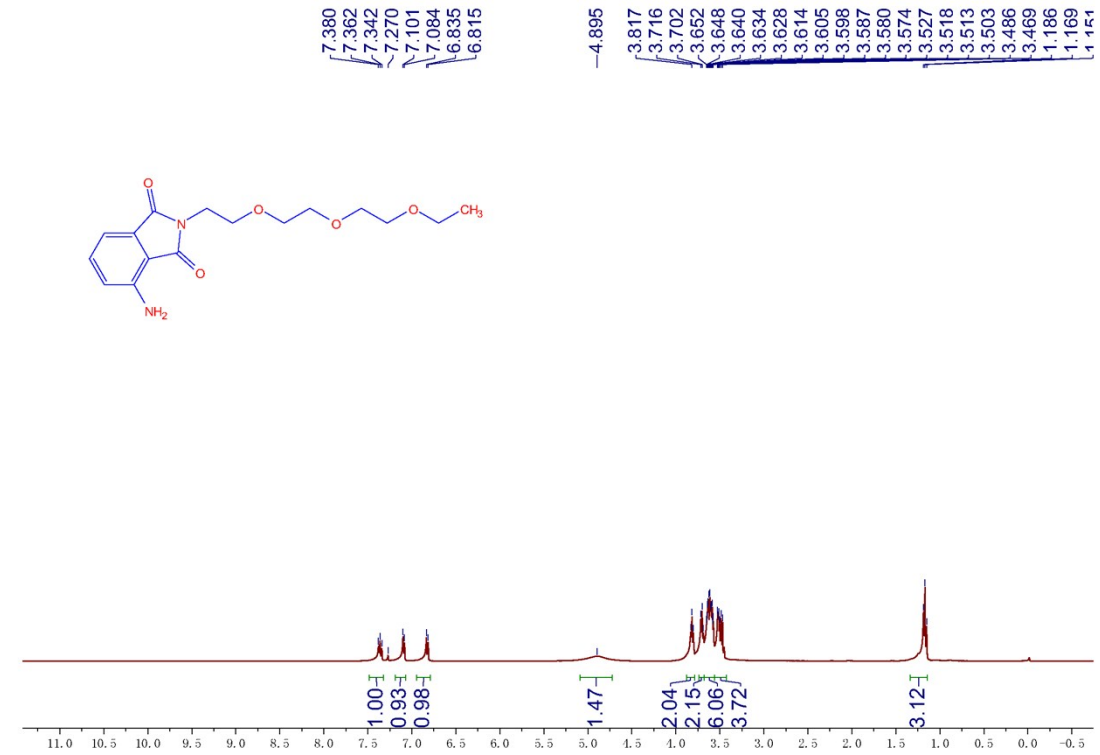
^{1}H NMR of **1**



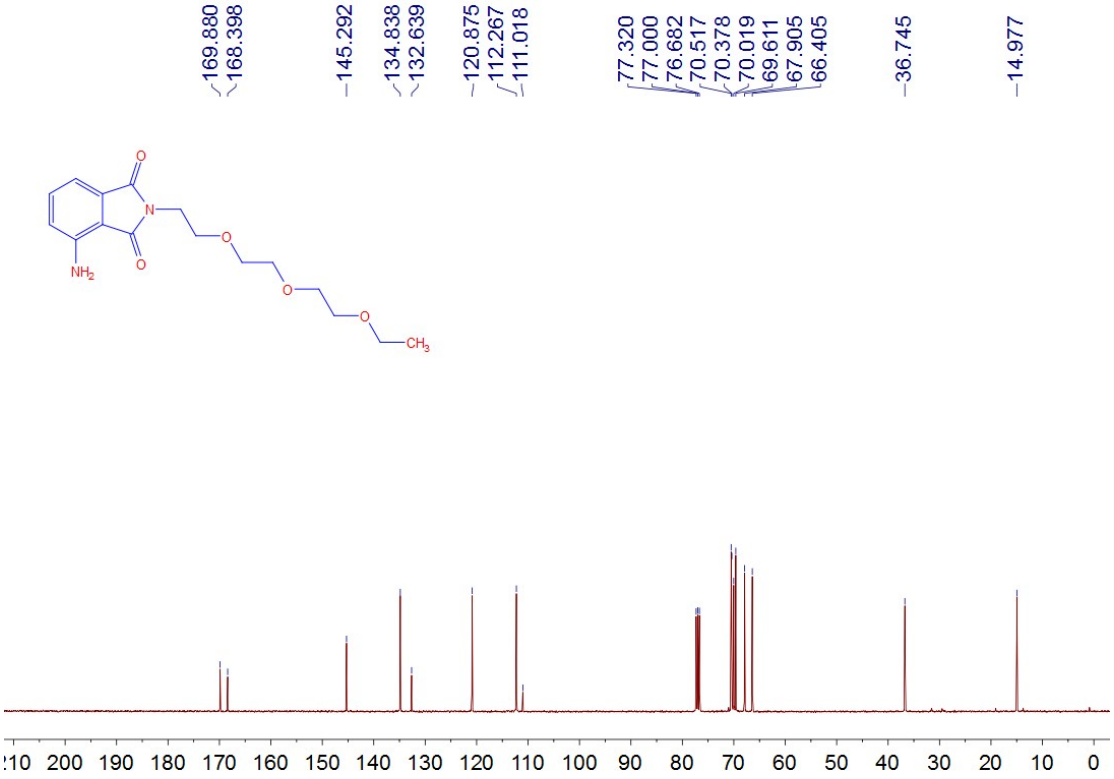
^{13}C NMR of **1**



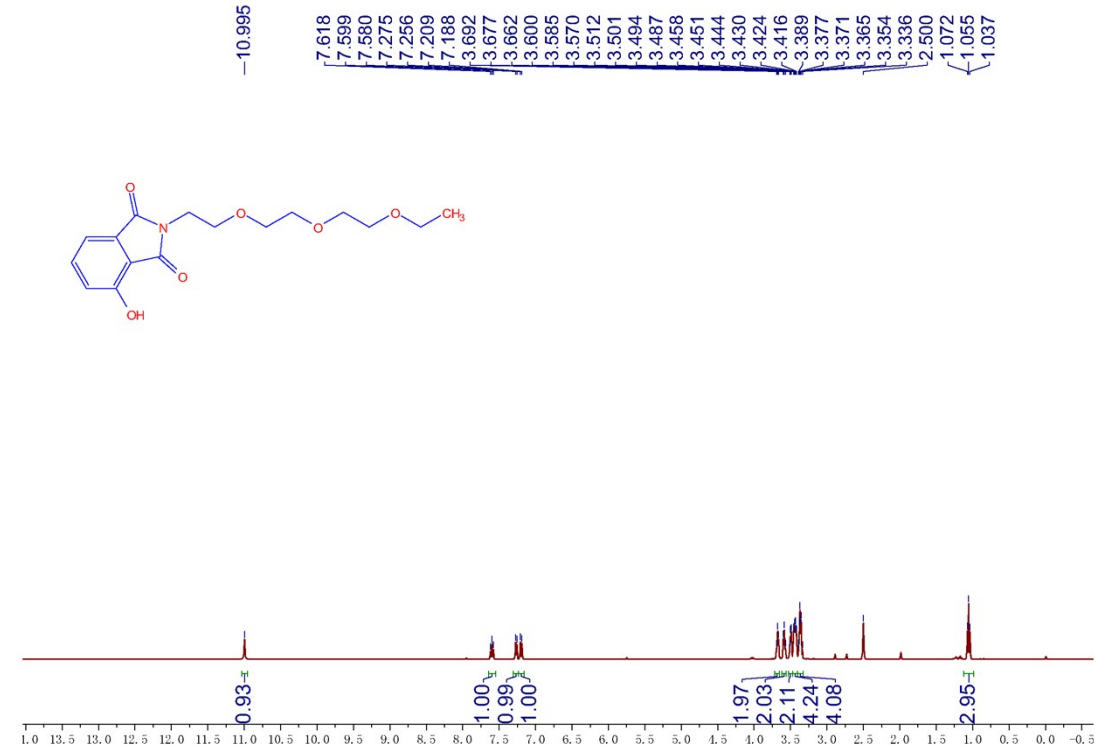
^{1}H NMR of **2**



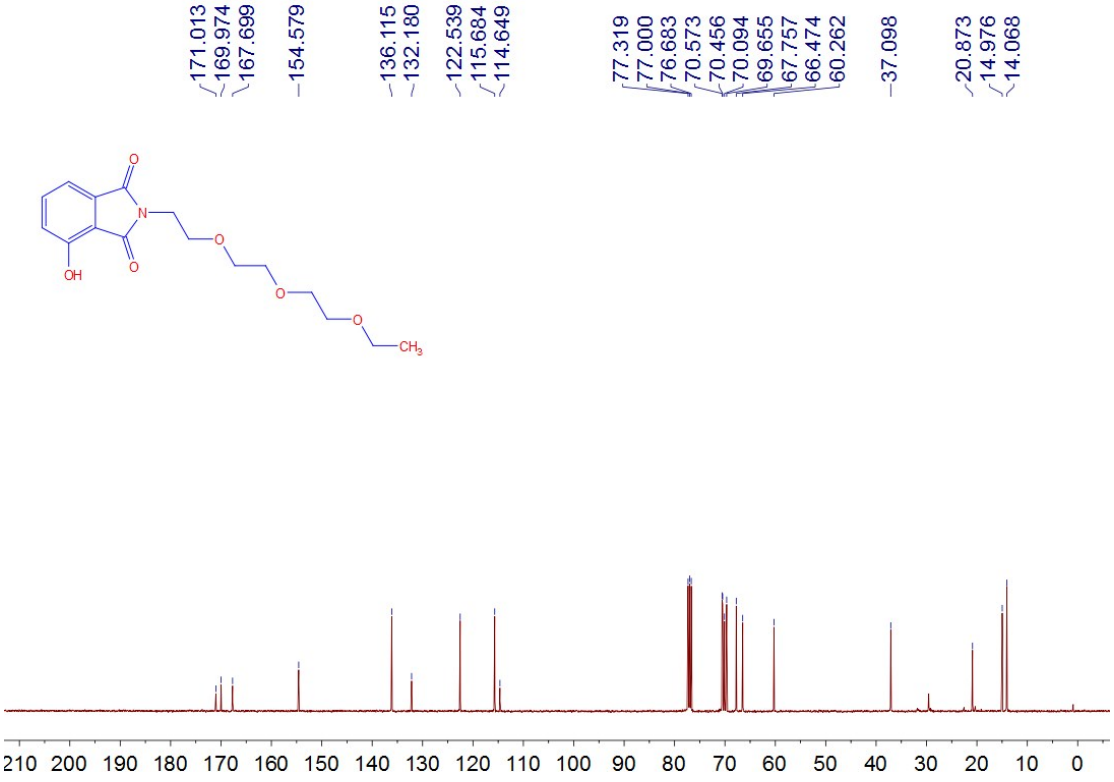
^{13}C NMR of **2**



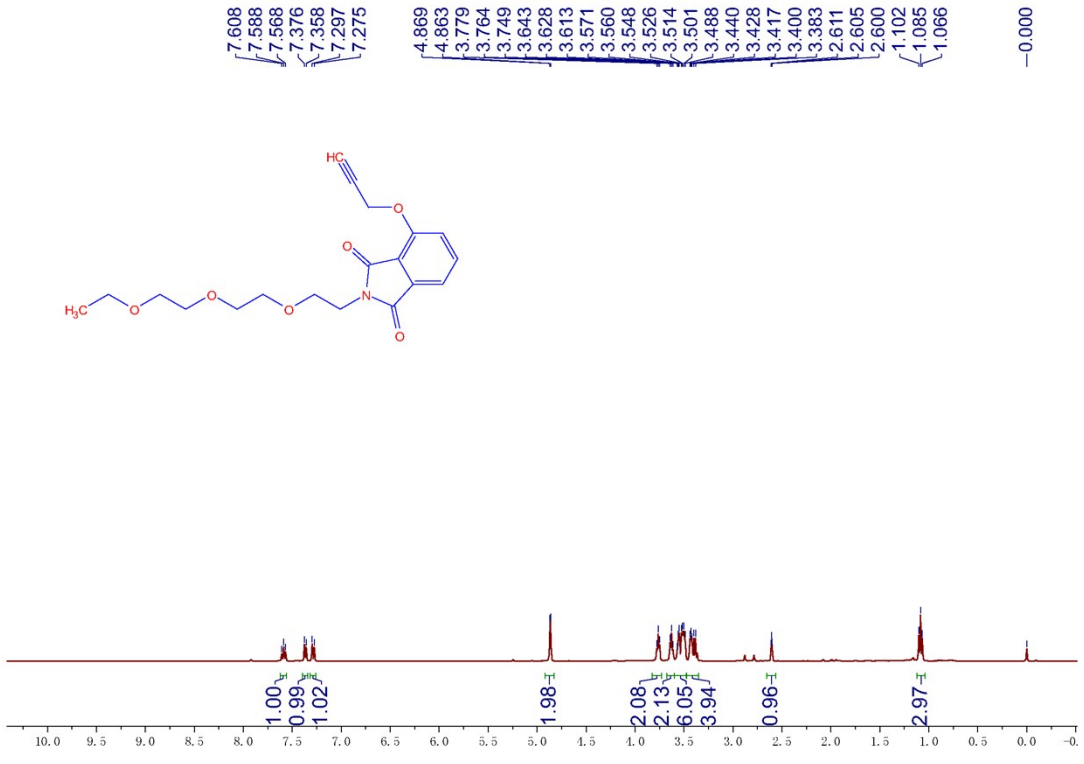
^{1}H NMR of **3**



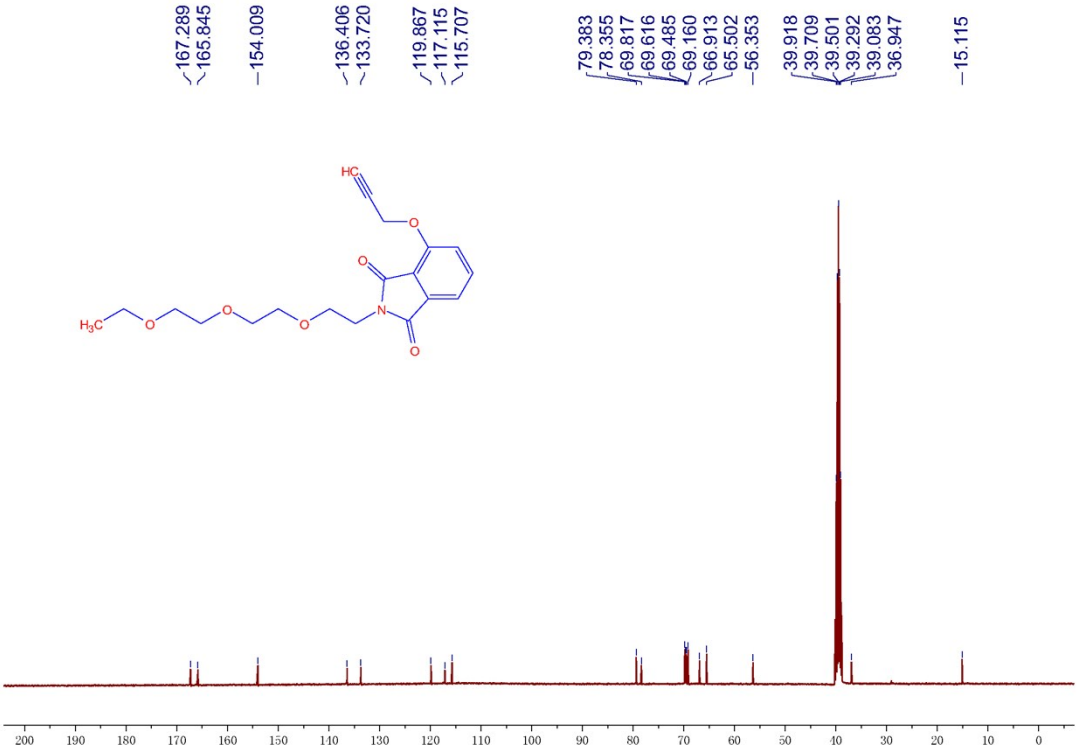
^{13}C NMR of **3**



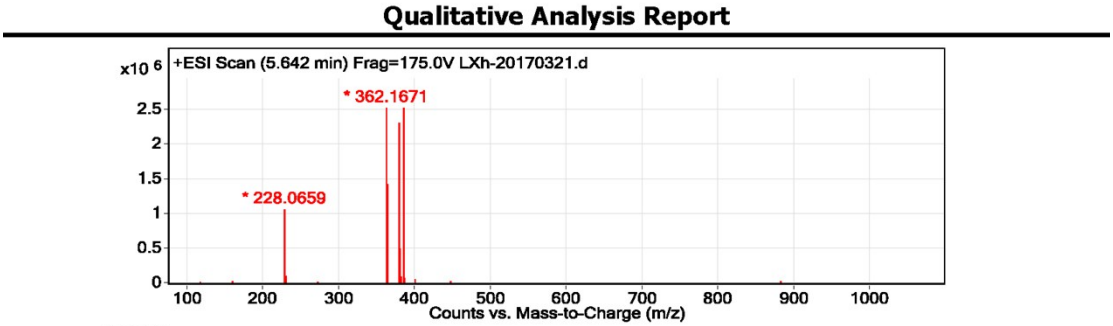
^{1}H NMR of **4 (SPd4)**



^{13}C NMR of **4 (SPd4)**



HR-MS of **4 (SPd4)**



Peak List

m/z	z	Abund
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362.1671		2536766.8
362.3365		390414.1
363.1629	1	1448532
364.1651	1	185506.3
379.1867	1	2323067
380.1888	1	511229.2
384.1437	1	2526671
384.3236		196665.6
385.1446	1	753073.5

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