Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

A Two-Photon Ratiometric ESIPT Probe for Discrimination of Different Palladium Species and Its Application in Bioimaging

Weifang Luo and Weisheng Liu*

Key Laboratory of Nonferrous Metals Chemistry and Resources Utilization of Gansu Province and State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China

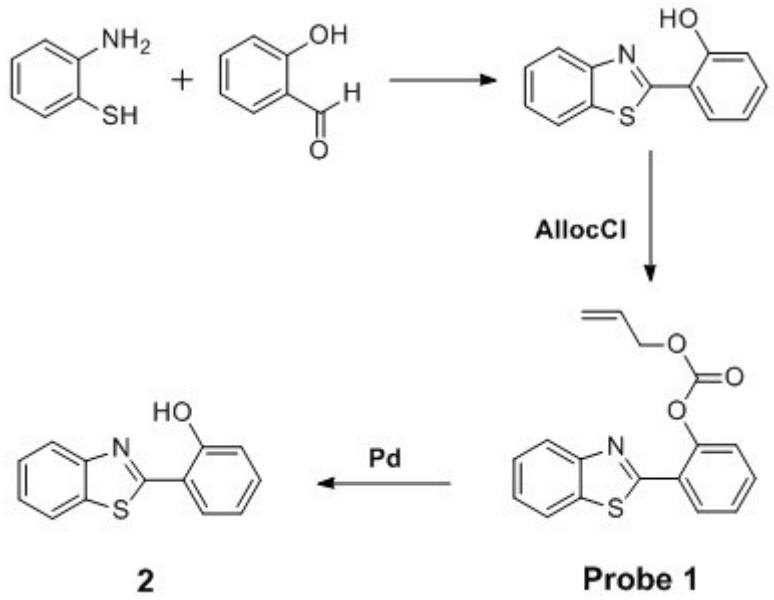
*Corresponding author. Tel: +86/931/8915151

Fax number: +86/931/8912582

E/mail: liuws@lzu.edu.cn

Contents

1. Scheme caption and Fluorescence spectra



Scheme S1. Synthetic route of **1** and its cleavage to form **2**.

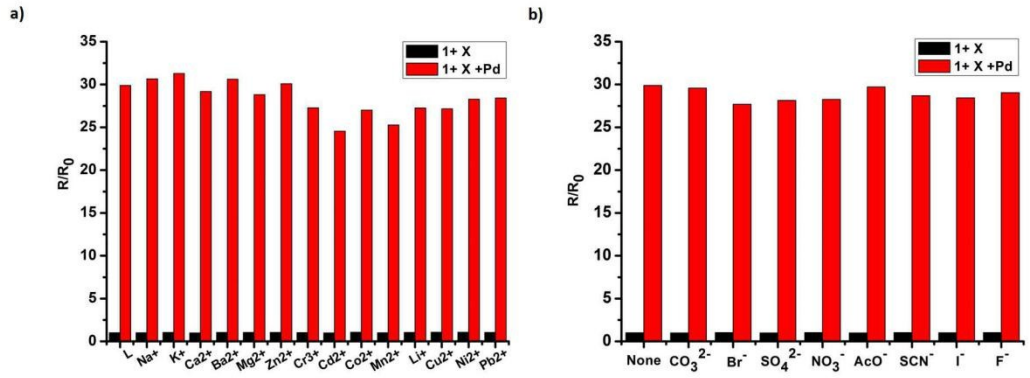


Fig. S1. a) Changes in fluorescence of **1** (5 uM) upon addition of Pd^0 (5 equiv.) with various mental ions (5 equiv.) Na^+, K^+, Ca^{2+}, Ba^{2+}, Mg^{2+}, Zn^{2+}, Cr^{3+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Li^+, Cu^{2+}, Ni^{2+} and Pb^{2+}; b) Fluorescence responses of **1**, **1**-Pd^0 (5 uM) with various anions (5 equiv.): CO_3^{2-}, Br^-, SO_4^{2-}, NO_3^-, AcO^-, SCN^-, I^- and F^- in PBS buffer solution (pH = 7.4, containing 10% CH_3CN).

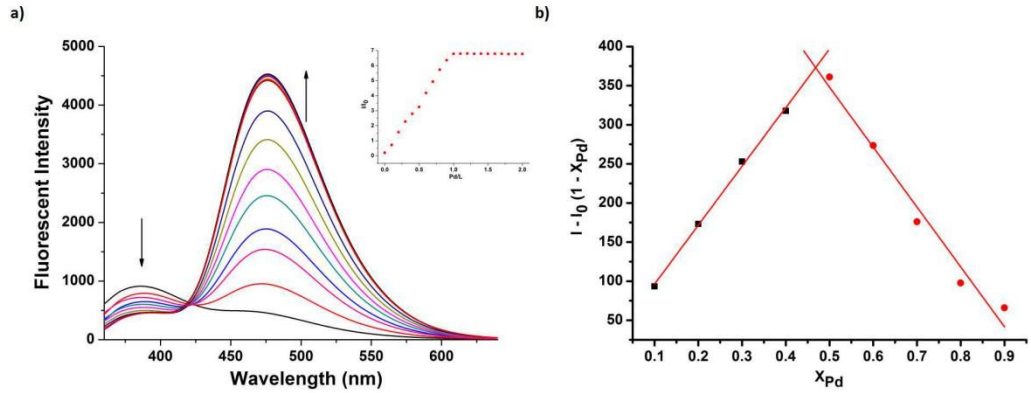


Fig. S2. a) Fluorescence spectra of **1** (5 uM) upon titration with Pd^0 (0-10 uM) in PBS buffer solution (pH = 7.4, containing 10% CH_3CN), inset: the fluorescence intensity ratio (I_{476nm}/I_{420nm}) changes of **1** upon addition of Pd^0; b) Job's plot for determining the stoichiometry of **1** and Pd^0, the total concentration of **1** and Pd^0 was 5 uM, X_{Pd} = $[Pd^0]/([Pd^0] + [\mathbf{1}]$.

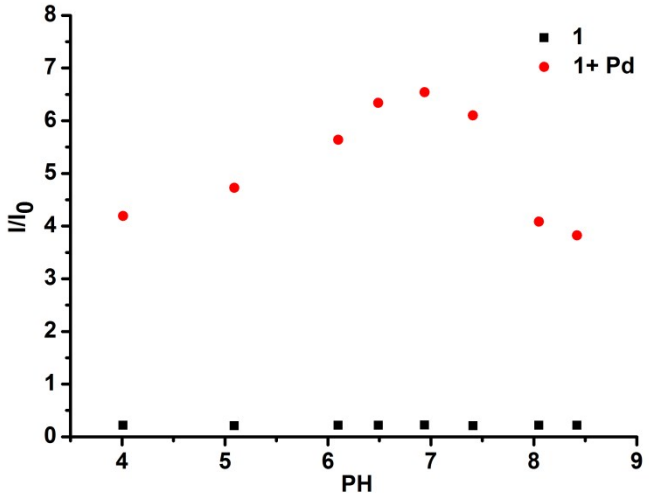


Fig. S3. The fluorescence intensity ratios (I_{476nm}/I_{420nm}) of 5 uM **1**, **1**- Pd^0 as a function of pH in PBS buffer solution (pH = 7.4, containing 10% CH_3CN).

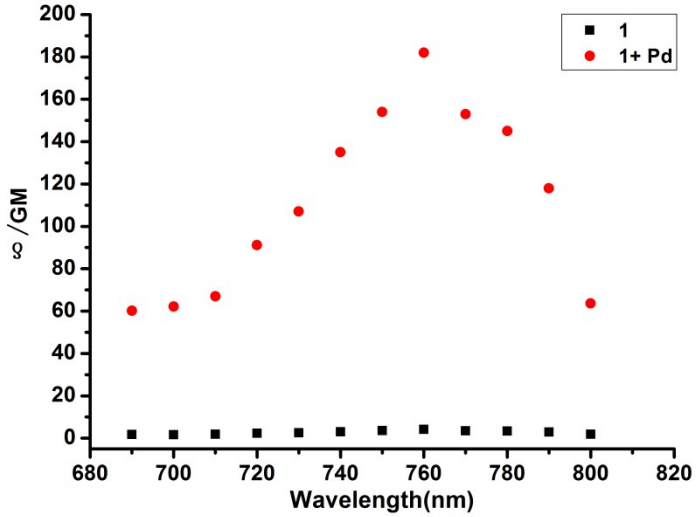


Fig. S4. Two-photon excitation spectra of **1** and **1**- Pd^0 (5 uM) in PBS buffer solution (pH = 7.4, containing 10% CH_3CN).

2. OP and TP Bioimaging

2.1. Cell cytotoxicity

The cytotoxic effect of **1** and **1**-Pd^0 was determined by an MTT assay following the manufacturer instruction (Sigma-Aldrich, MO). HeLa cells were initially propagated in a 25 cm^2 tissue culture flask in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 ug/mL), and streptomycin (100 ug/mL) in a CO_2 incubator. For cytotoxicity assay, cells were seeded into 96-well plates (approximately 104 cells per well), and various concentrations of **1** and **1**-Pd^0 (5, 10, 20, 30, 50, 75 and 100 uM) made in DMEM were added to the cells and incubated for 24 h. Solvent control samples (cells treated with CH_3CN alone) and cells treated with Pd^0 (100 uM) alone were also included in parallel sets. Following incubation, the growth media was removed, and fresh DMEM containing MTT solution was added. The plate was incubated for 2-3 h at 37 °C. Subsequently, the supernatant was removed, the insoluble colored formazan product was solubilized in DMSO, and its absorbance was measured in a microtiter plate reader (Infinite M200, TECAN, Switzerland) at 550 nm. The assay was performed in six sets for each concentration of **1** and **1**−Pd^0. Data analysis and calculation of standard deviation was performed with origin 8.0. For statistical analysis, a one way analysis of variance (ANOVA) was performed using Sigma plot.

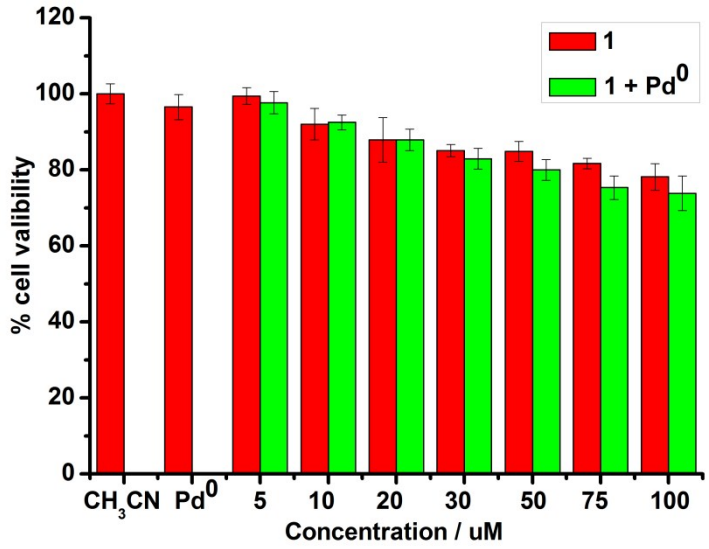


Fig. S5. MTT assay to determine the cytotoxic effect of **1** and **1**−Pd^0 on HeLa cells. Statistically significant values derived by ANOVA are indicated by bar marks.

2.2. Cell culture and tissue culture

HeLa cells were procured from the biomedical engineering center of Lanzhou University (Lanzhou, China). The cells were propagated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin (100 ug/mL), and streptomycin (100 ug/mL). Cells were maintained under a humidified atmosphere of 5% CO_2 and at 37 °C incubator as mentioned before. For cell imaging studies, cells were seeded into a confocal dish and incubated at 37 °C in a CO_2 incubator for one day. Then the cells were washed three times with PBS buffer (pH 7.4) and divided into several groups. For group one cells were washed three times with phosphate buffered saline (pH = 7.4) and incubated with 10 uM **1** in DMEM at 37 ˚C for 30 minutes in a CO_2 incubator and observed under Olympus FV1000 laser confocal microscope IX81. For group two cells incubated with 10 uM **1** were again washed thrice with PBS (pH = 7.4) to remove the free probe, and then incubated with 5 uM Pd^0 for 60 minutes. For group three cells incubated with 10 uM **1** were again washed thrice with PBS (pH = 7.4) to remove the free probe, and then incubated with 10 uM Pd^0 for 60 minutes. Again, images were taken using Olympus FV1000 laser confocal microscope IX81. the cells were washed with PBS three times to remove free compound before analysis. Then, fluorescence microscopic images were acquired. Also the concentration based imaging detected as the previous procedure.

Tissue slices were prepared from Hela cancer cells. A total of 2×106 Hela cancer cells diluted in 100 uL of serum-free PBS medium were injected subcutaneously into the right flank of 6- to 8-week-old BALB/c-nude mice to inoculate tumors. After Hela cancer cells inoculation was for 15 days, mice were sacrificed. Tumors were transferred and embedded with O.C.T (Sakura Finetek, USA, Torrance, CA) for frozen sections. The tissues were cut into 400 um- thick slices using a vibrating-blade microtome. Slices were incubated with 50 uM of **1** and Pd^0 for 12 h at 4 °C. After washing with PBS for three times, the slices were mounted with 10% glycerol and sealed with nail varnish on a glass substrate.

2.3. Fluorescence imaging

One-photon fluorescence images of dye labeled cells and tissues were obtained with exciting wavelength at 405 nm with Olympus FV1000 laser confocal microscope IX81 with 60 objective, numerical aperture (NA)=0.4. The images signals at 420–550 nm range were collected by internal PMTs in an 12 bit unsigned 1024*1024 pixels at 40 Hz scan speed.

Two-photon fluorescence images of dye labeled cells and tissues were obtained by exciting the probes with a modelocked titanium-sapphire laser source (Mai Tai DeepSee, 80 MHz, 90 fs) set at wavelength 760 nm with Olympus FV1000 laser confocal microscope IX81 with 60 objective, numerical aperture (NA)=0.4. The images signals at 420–550 nm range were collected by internal PMTs in an 12 bit unsigned 1024*1024 pixels at 40 Hz scan speed.

3. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of **1** was measured by ten times and the standard deviation of blank measurement was achieved. To gain the slope, the fluorescence intensity was plotted as a concentration of Pd^0. So the detection limit of **1** was calculated with the following equation:

Detection limit = $3\sigma/k$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus Pd^0.

4. NMR and ESI-mass Data

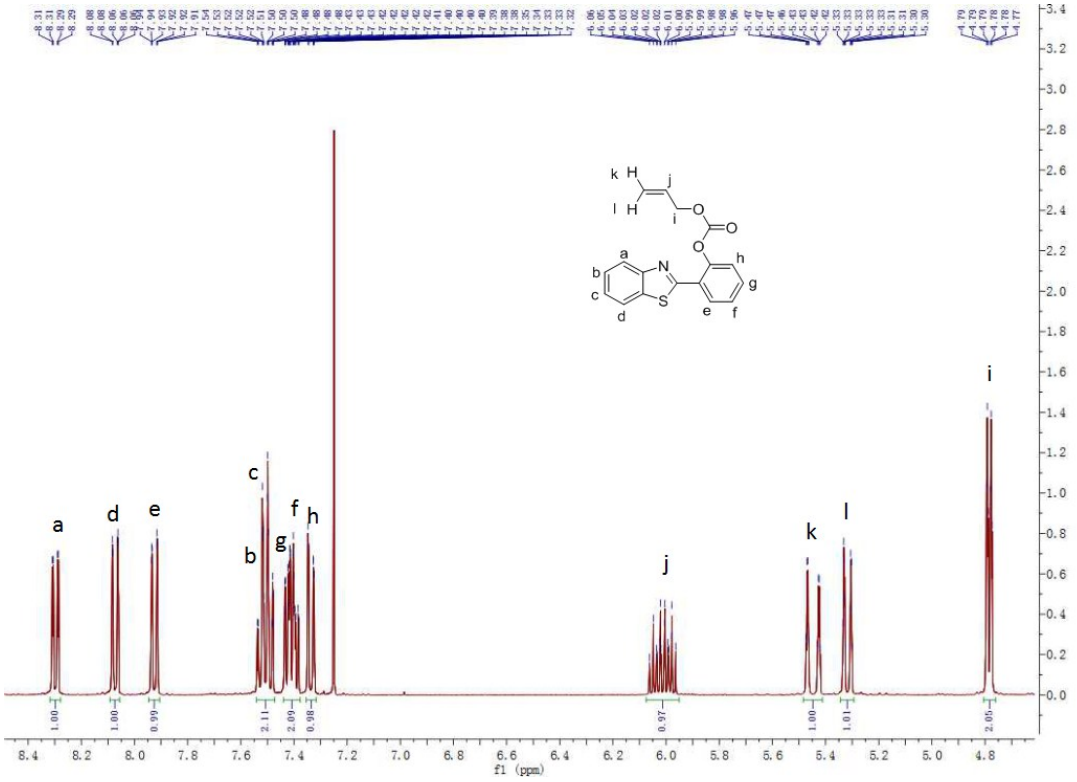


Fig. S6. ^{1}H NMR spectrum of **1** ($CDCl_3$)

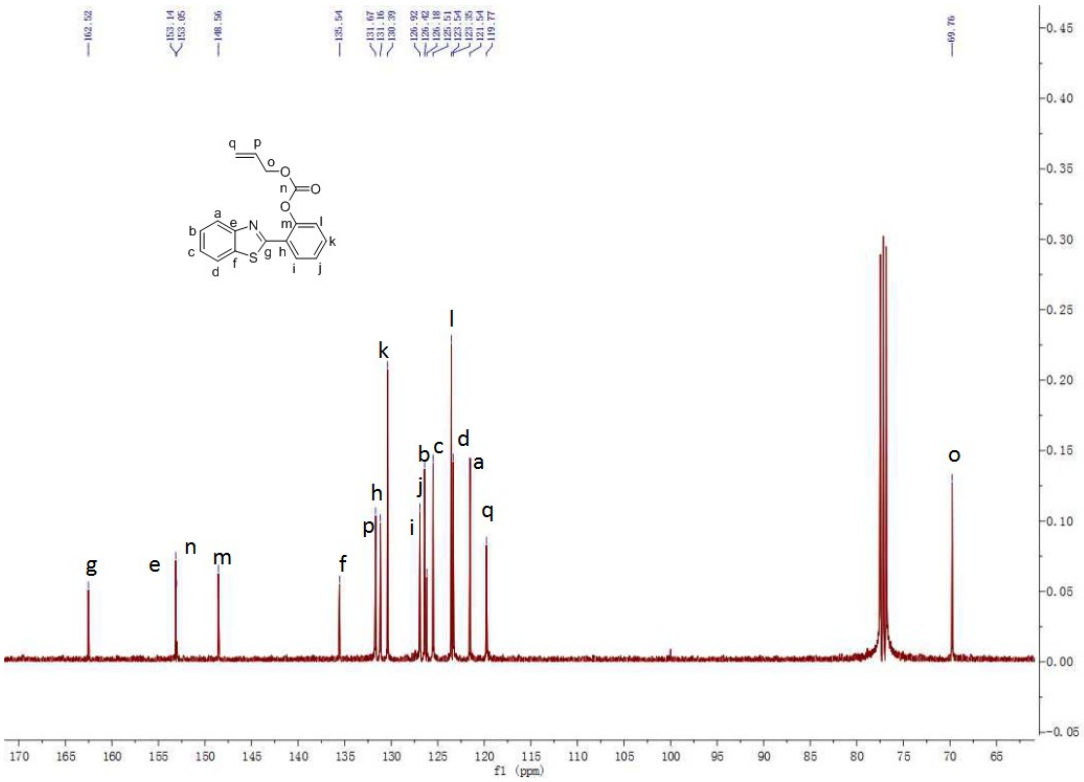
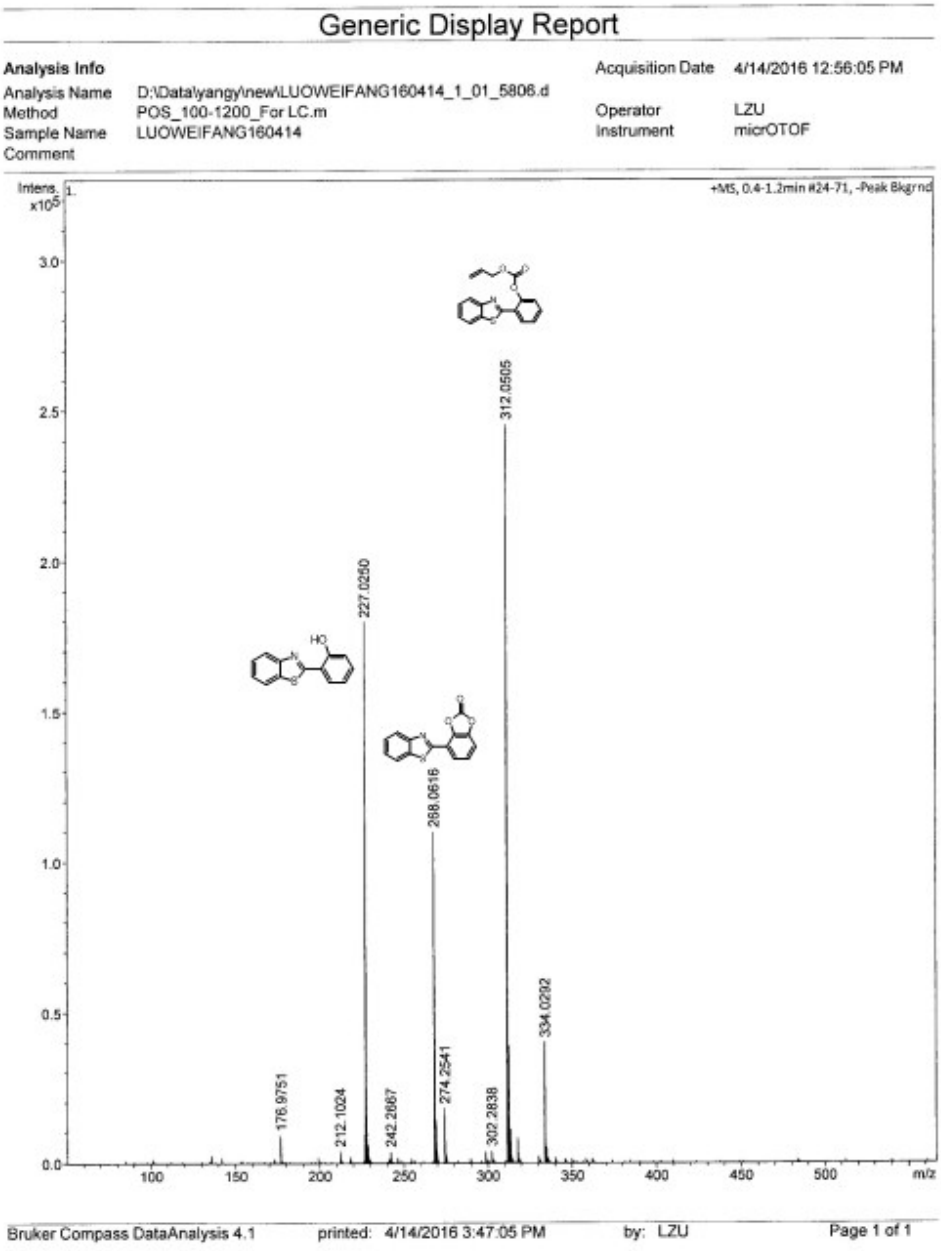


Fig. S7. ^{13}C NMR spectrum of **1** ($CDCl_3$)



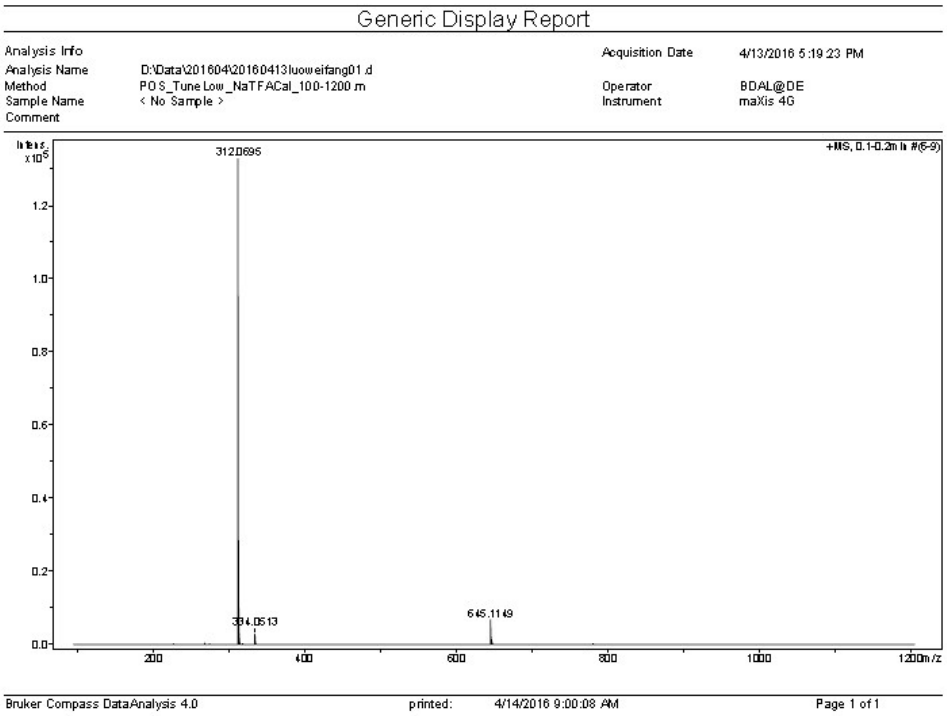


Fig. S8. ESI-mass spectrum of **1**.

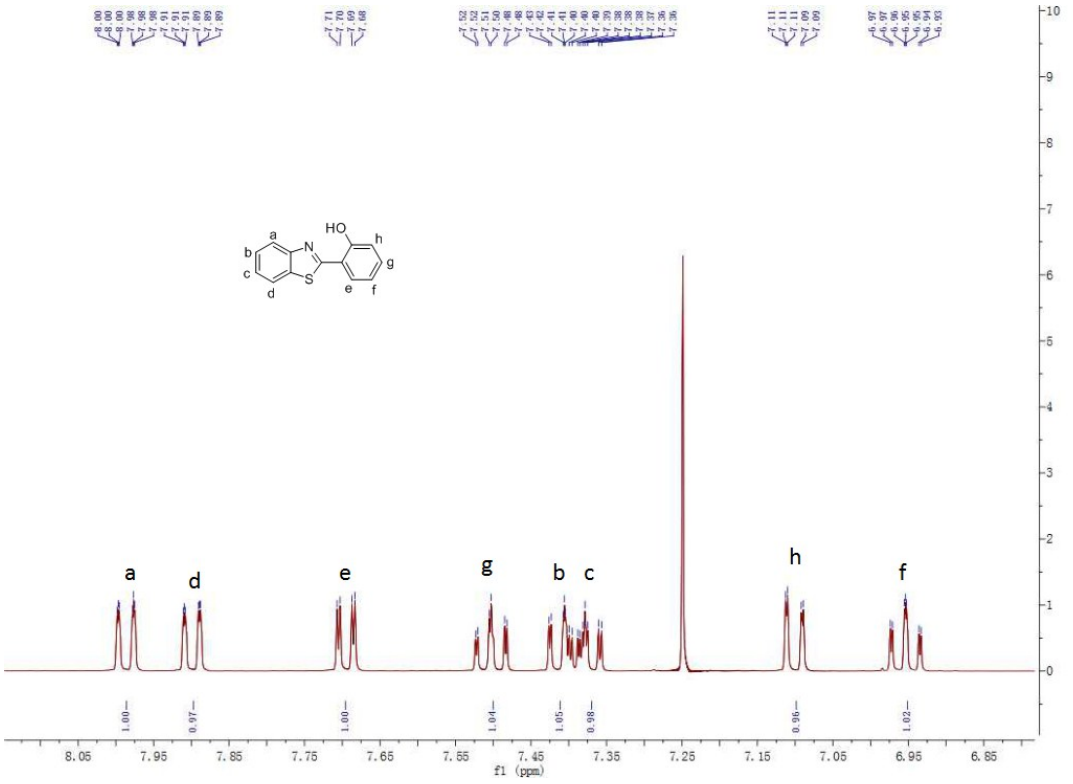


Fig. S9. ^{1}H NMR spectrum of **1-Pd (2)** ($CDCl_3$)

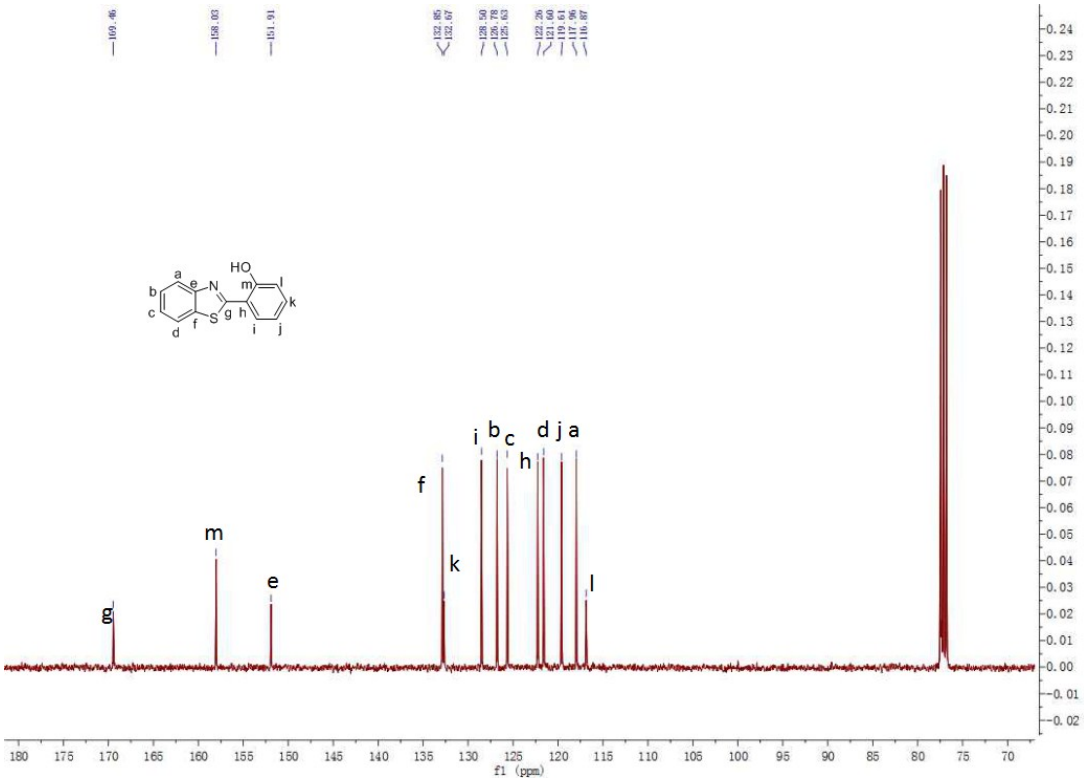


Fig. S10. ^{13}C NMR spectrum of **1-Pd (2)** ($CDCl_3$)

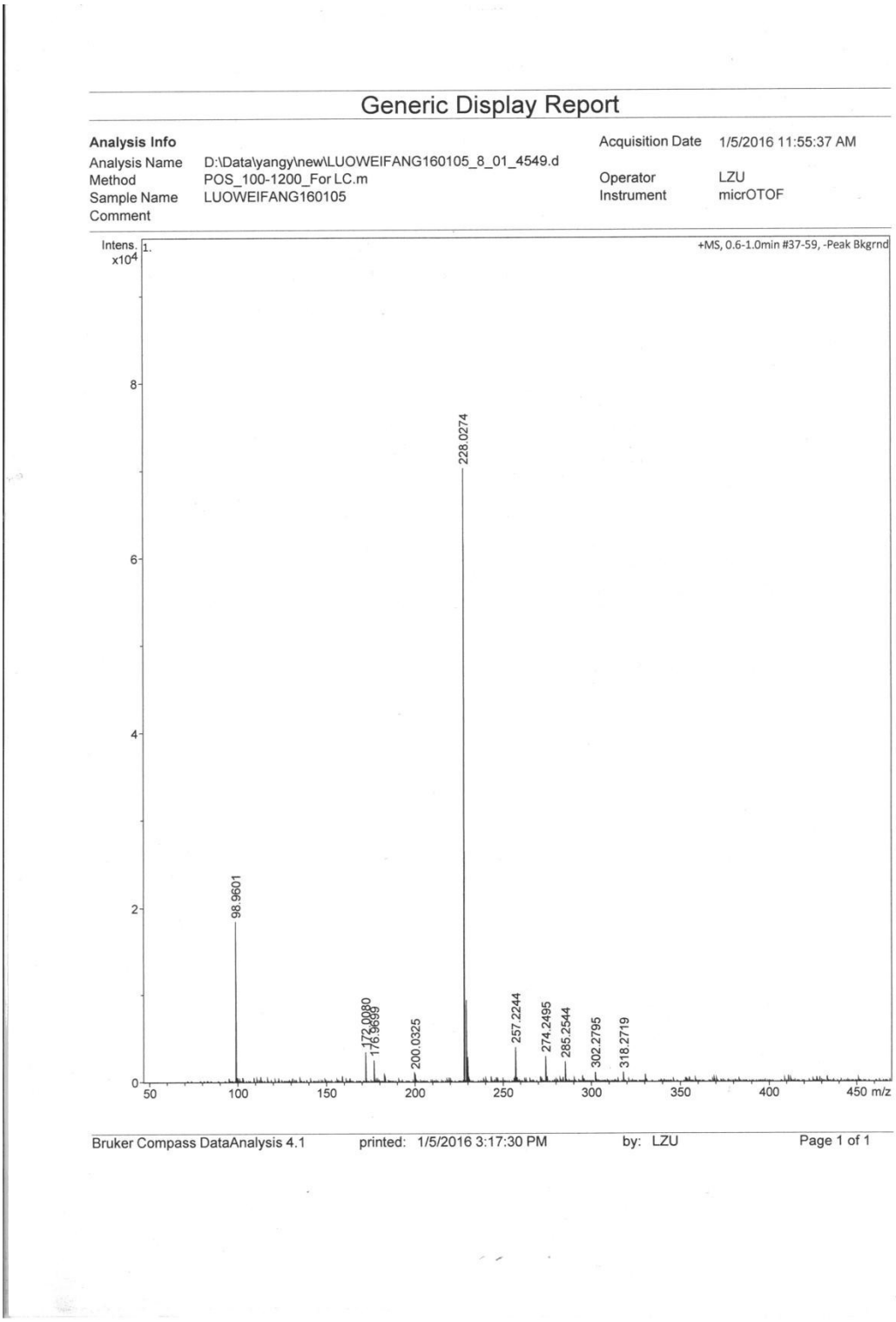


Fig. S11. ESI-mass spectrum of **1**-Pd (**2**).