Supporting Information for

A Bioluminescent Probe for Imaging Endogenous Hydrogen Polysulfides in Live Cells and Murine Model of Bacterial Infection

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Reagents and Instruments.

All reagents were obtained from Bidepharm, Aladdin or Promega. Luminescence spectra were obtained on F-7000 fluorescence spectrofluorometer (HITACHI) with the excitation slits shut down. Absorption spectra were acquired on a measurement of UV-2450 spectrophotometer (Shimadzu). Silica gel and silica gel plates were obtained from Jiangyou Silica Gel (China), using in thin layer chromatography analysis, chromatography purification, respectively. NMR spectra, Mass spectra were acquired using DRX-400 spectrometer (Bruker) and LCQ-Advantage mass spectrometer (Thermo Finnigan), respectively. Analyses of HPLC were conducted on LC-20AR (Shimadzu) using a C18 column with water and CH_3CN as the mobile phase, both containing 0.1% of trifluoroacetic acid. The bioluminescence images were captured with open filter using the IVIS Lumina XR in vivo imaging system (PerkinElmer). The intensities of bioluminescence were measured using IVIS living image 4.3 software.

Synthetic procedures

Synthesis of 1. Using a modification of the literature procedure, A mixture of $NaHCO_3$ (2.4 g, 28 mmol) and mercaptoacetic acid (1.54 g, 10 mmol) was added in H_2O (50 mL). After the mixture cooled to 0 °C, benzoyl chloride (1.4 g, 10 mmol) was added. The mixture was stirred for 15 minutes at 0 °C, and then stirred at RT for another 1 h. Aqueous HCl was added to the solution and white precipitate was obtained, washed with cold H_2O, and dried to provide **1** (2.0 g, 78% yield) as a pure white powder. ^1H NMR (DMSO-d6, 400 MHz) δ 13.20 (s, 1H), 7.98 (d, J = 7.5 Hz, 2H), 7.95 (d, J = 7.6 Hz, 1H), 7.74 (t, J = 7.4 Hz, 1H), 7.66 (d, J = 3.3 Hz, 2H), 7.63 –

7.58 (m, 3H). ^{13}C NMR (DMSO-d6, 101 MHz) δ 189.13, 167.84, 137.46, 136.55, 136.47, 134.66, 132.19, 130.88, 130.31, 129.71, 127.55, 127.00.

Synthesis of 2. A mixture of **1** (258 mg, 1.0 mmol), EDCI (230 mg, 1.2 mmol) and DMAP (146 mg, 1.2 mmol) was added in CH_2Cl_2 (25 mL) at RT. After stirring for 30 min, 6-hydroxy-benzothiazole-2-carbonitrile (176 mg, 1.0 mmol) was added. The mixture was stirred for 6 h and solution was evaporated. **Compound 2** (250 mg, 60% yield) was obtained after silica gel chromatography purification as a yellow powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (d, J = 7.6 Hz, 1H), 8.12 (d, J = 9.0 Hz, 1H), 7.94 (d, J = 7.6 Hz, 2H), 7.77 (s, 1H), 7.68 – 7.48 (m, 4H), 7.39 (t, J = 7.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 189.23, 164.30, 150.87, 150.08, 137.32, 136.73, 136.34, 136.12, 133.98, 133.22, 133.00, 131.65, 129.77, 129.09, 128.87, 127.56, 125.94, 122.93, 114.66, 112.74.

Synthesis of BP-PS. **2** (42 mg, 0.1 mmol) was dissolved in a 1:1 mixture of H_2O and MeOH (4 mL). A solution of K_2CO_3 (21 mg, 0.15 mmol) and D-cysteine hydrochloride (26 mg, 0.15 mmol) in a 1:1 mixture of H_2O and MeOH (4 mL) were added. The mixture was stirred for 15 min. The solution was concentrated *in vacuo*, and then treated with aqueous HCl. The precipitate was collected. **BP-PS** (26 mg, 50 % yield) was obtained after silica gel chromatography purification as a yellow powder. ^{1}H NMR (DMSO-d6, 400 MHz) δ 8.28 (d, J = 7.0 Hz, 1H), 8.21 (d, J = 8.9 Hz, 1H), 8.13 (s, 1H), 8.01 (d, J = 7.7 Hz, 2H), 7.83 – 7.70 (m, 4H), 7.59 (t, J = 7.6 Hz, 2H), 7.48 (d, J = 9.1 Hz, 1H), 5.45 (t, J = 9.0 Hz, 1H), 3.82 – 3.69 (m, 2H). ^{13}C NMR (DMSO-d6, 101 MHz) δ 189.04, 171.49, 164.80, 164.76, 161.77, 151.08, 149.57, 137.69, 136.54, 136.11, 134.93, 133.90, 133.68, 131.92, 130.80, 129.80, 127.90, 127.66, 125.30, 122.32, 116.30, 78.67, 35.34. MS (ESI): $[M+H]^+$ calcd for $C_{25}H_{17}N_2O_5S_3^+$, 521.0; found, 520.8.

Spectrophotometric Measurements.

Both the UV-vis absorption and luminescence spectra were conducted in 10 mM PBS with 10 mM $MgCl_2$ at pH 7.4. 1.0 mM **BP-PS** was made in DMSO and diluted to 20 μM final concentration in PBS in a tube. Na_2S_4 (100 μM, as a H_2S_n donor)

solution was added into the tube. After 30 min incubation, the UV-vis absorption was conducted and the luminescence spectrum (λ_{em} = 450-700 nm) was collected after the addition of fLuc solution (10 μg/mL fLuc, 2 mM ATP).

In Vitro Bioluminescence Measurements.

20 μM **BP-PS** and various concentrations of Na_2S_4 (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 50, 100, 200 μM) were mixed and incubated for 30 min in PBS. After the addition of fLuc solution (10 μg/mL fLuc, 2 mM ATP), bioluminescence images were captured immediately with the IVIS Lumina XR in vivo imaging system. Wells of 96-well plates were drawn with regions of interest (ROIs) and bioluminescence intensity was quantified by living image 4.3 software.

Cell Viability Studies.

The toxicity of **BP-PS** towards MDA-MB-231 cells was assessed by MTT assays. MDA-MB-231 cells were seeded into 96-well plates (1×10^4 cells per well) and incubated for 24 h. Different concentrations of **BP-PS** (0, 12.5, 25.0, 50.0, 100.0, 200.0 μM) was added. The cells were incubated for 24 or 48 h. To determine toxicity, each well was added with 20 μL of MTT and the cells were incubated for 4 h. After discarding the medium, each well was treated with DMSO (100 μL) to fully dissolve the formazan. The absorbance (570 nm) was measured and the cell viability was calculated: viability (%) = (average absorbance values of **BP-PS** treated cells / average absorbance values of untreated cells) × 100.

Live Cell Bioluminescence Imaging.

The MDA-MB-231-luc cells (fLuc-transfected MDA-MB-231 cells) were seeded into 96-well plates (4×10^5 cells per well) and incubated for 24 h. After discarding the medium, the cells were pretreated with different concentrations of DL-propargylglycine (PAG, CSE inhibitor) for 30 min or Na_2S_4 (100 μM). Then, cells were treated with the same doses of **BP-PS** (20 μM). Bioluminescence images were performed at different times.

Live Mice Bioluminescence Imaging.

All animal experiments were performed according to the Chinese Regulations for the Administration of Affairs Concerning Experimental Animals and approved by the Institutional Animal Care and Use Committee at the Laboratory Animal Center of Hunan. For inflamed BALB/c mice, the right hind leg of each mouse was subcutaneously injected with lipopolysaccharide (LPS, 20 μL of 10 mg/mL). 12 h later, the left and right hind leg of each mouse were subcutaneously injected with 20 μL fLuc solution (0.3 mg/mL fLuc, 2.5 mM Mg^{2+} and 1 mM ATP) and 20 μL **BP-PS** (100 μM). For bacterial infected mice, the right hind leg of each mouse was subcutaneously injected with 100 μL of *Escherichia coli* (*E. coli*, 5×10^6 CFU mL^{-1}). At 12 h post-infection, the left and right hind leg of each mouse were subcutaneously injected with 20 μL fLuc solution (0.3 mg/mL fLuc, 2.5 mM Mg^{2+} and 1 mM ATP) and 20 μL **BP-PS** (100 μM). For negative control, inflammation tissues were first treated with NEM (2 mM, N-ethylmaleimide, a RSS scavenger) for 20 min before the same treatment as above. The anesthetized mice were transferred to an IVIS imaging system for image acquisition. ROIs were drawn in the inflamed and normal areas and the bioluminescence intensity was quantified using living image software.

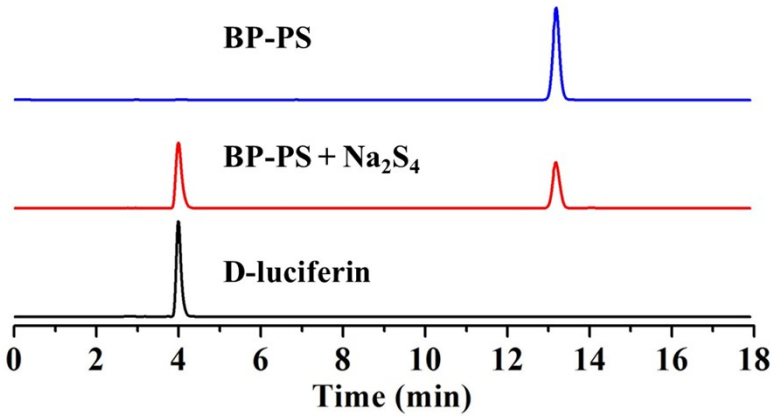

Fig. S1 HPLC traces of **BP-PS**, D-luciferin, and reaction product of **BP-PS** with H_2S_n after incubation of them for 30 min in PBS (10 mM, pH 7.4) solution. The blue, black, and red lines represent **BP-PS**, D-luciferin, and the reaction product of **BP-PS** with H_2S_n, respectively. Wavelength for detection: 320 nm.

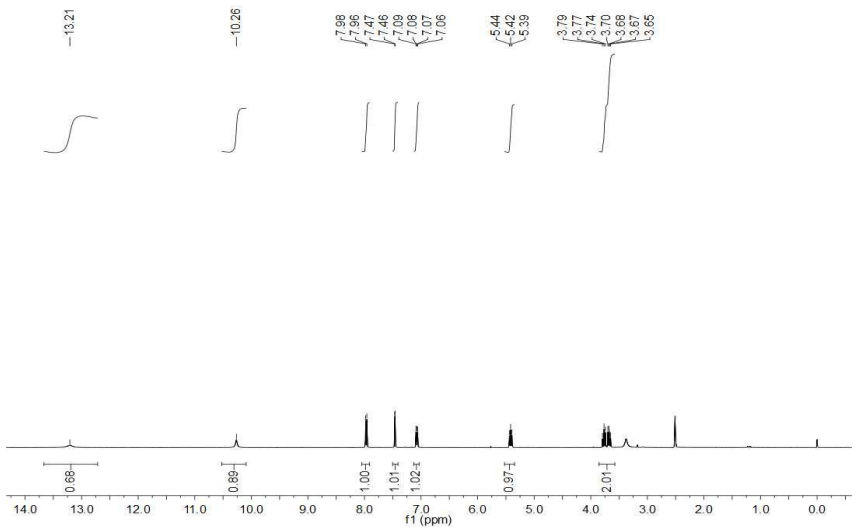

Fig. S2 ^{1}H NMR spectrum of **BP-PS** with H_2S_n. ^{1}H NMR spectrum showing D-luciferin was produced by the reaction of **BP-PS** with H_2S_n. ^{1}H NMR (400 MHz, DMSO) δ 13.21 (s, 1H), 10.26 (s, 1H), 7.97 (d, *J* = 8.9 Hz, 1H), 7.46 (d, *J* = 2.3 Hz, 1H), 7.08 (dd, *J* = 8.9, 2.3 Hz, 1H), 5.53 – 5.35 (m, 1H), 3.85 – 3.57 (m, 2H).

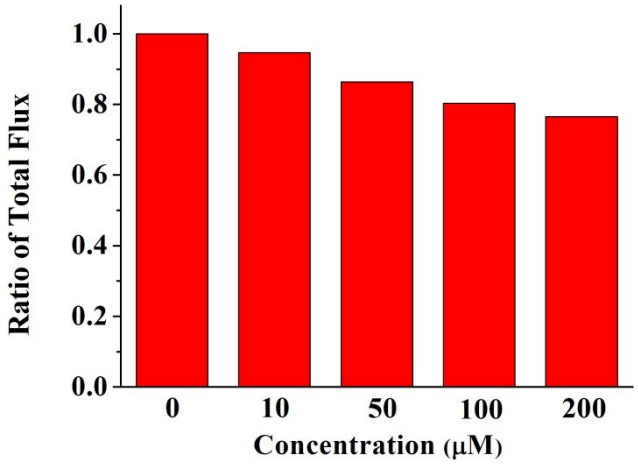

Fig. S3 Ratio of the total flux of D-luciferin in the presence of 0.1 mg/mL fLuc and 2 mM ATP after incubation with different concentrations of Na_2S_4 (0, 10, 50, 100, 200 μM) in PBS.

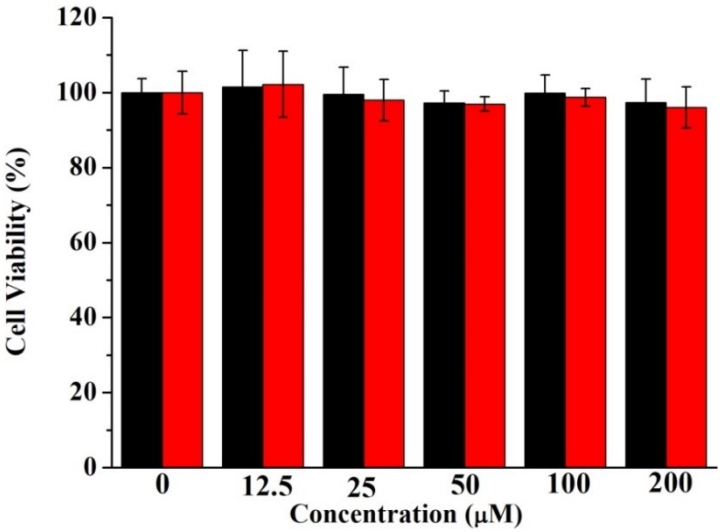

Fig. S4 MTT assay of probe **BP-PS** on MDA-MB-231 cells (non-luciferase transfected). The cells were incubated for 24 h (black bar) or 48 h (red bar). The results are the mean ± standard deviation of five separate measurements.

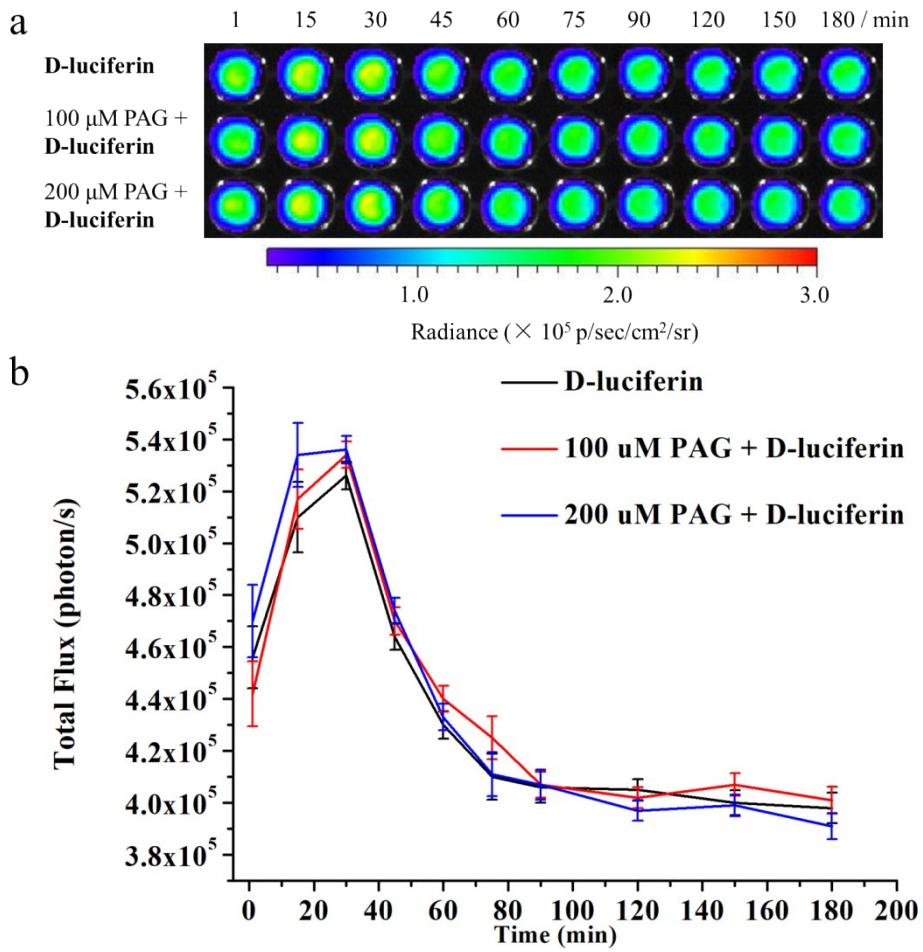

Fig. S5 (a) Time-course BL imaging of fLuc-transfected MDA-MB-231 cells preincubated with PAG (0, 100, 200 μM) for 30 min and then with 20 μM D-luciferin acquired at 0, 15, 30, 45, 60, 75, 90, 120, 150, and 180 min in serum-free culture medium at 37 °C. (b) Quantification of the total flux (photon/s) for the cells BL images. All assays were performed in triplicate and expressed as the mean ± SD.

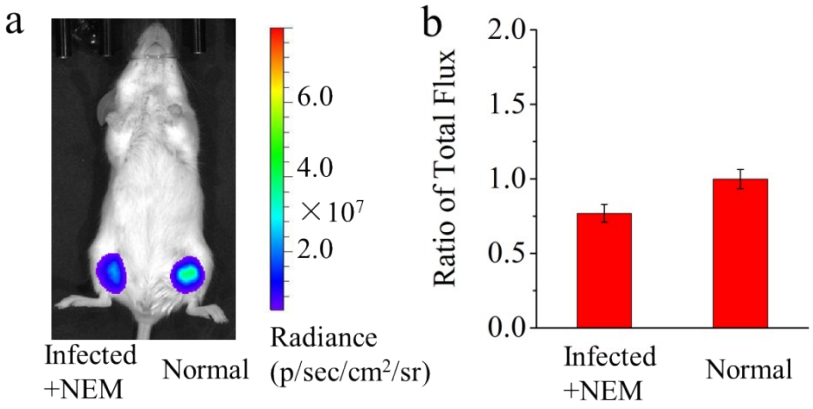

Fig. S6 100 μL of *E. coli* was subcutaneously injected into right legs of mice. After 12 h, inflammation tissues were first treated with NEM for 20 min. Then, the mice were locally injected with **BP-PS** and fLuc. (a) BL imaging of mice after injection of **BP-PS** and fLuc. (b) Ratio of the total flux in panel a. All assays were performed in triplicate and data represent the mean ± SD.

Table S1. HPLC condition.

Time (min)	Flow (mL/min)	H_2O % (0.1 % TFA)	CH_3CN % (0.1 % TFA)
0	1.0	50	50
3	1.0	50	50
35	1.0	10	90
37	1.0	10	90
38	1.0	50	50
40	1.0	50	50

NMR spectrum and MS of all the new compounds

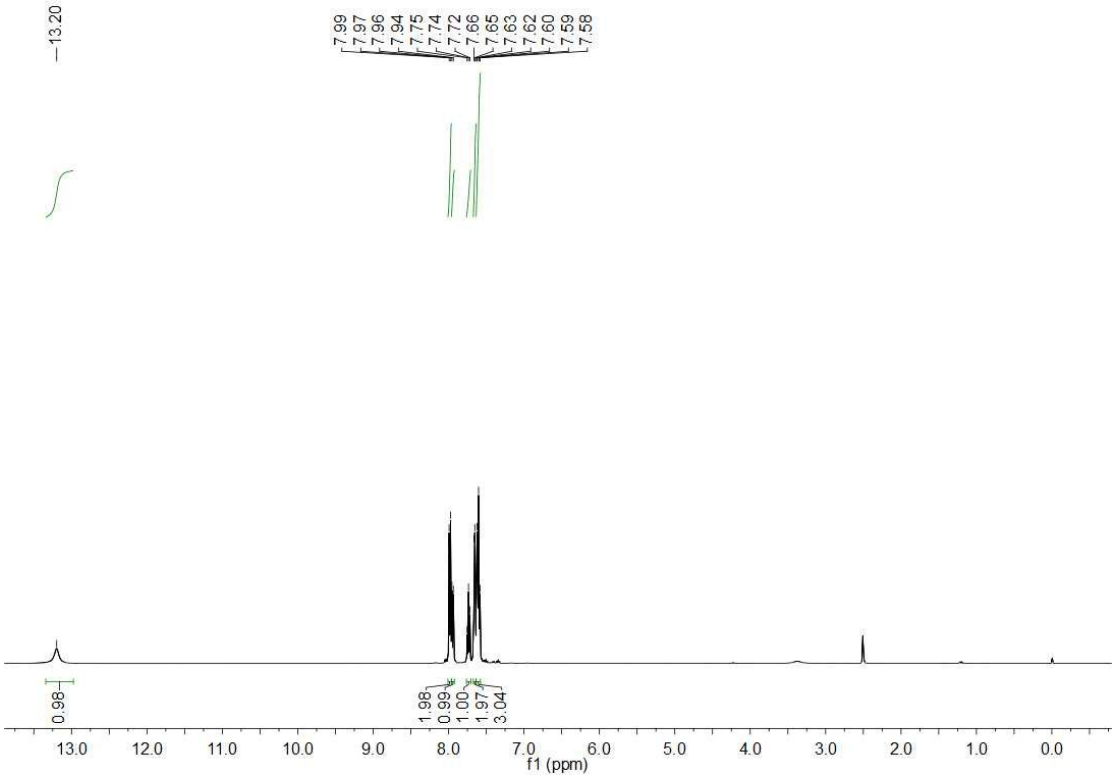

Fig. S7 ^{1}H NMR spectrum of the compound **1**

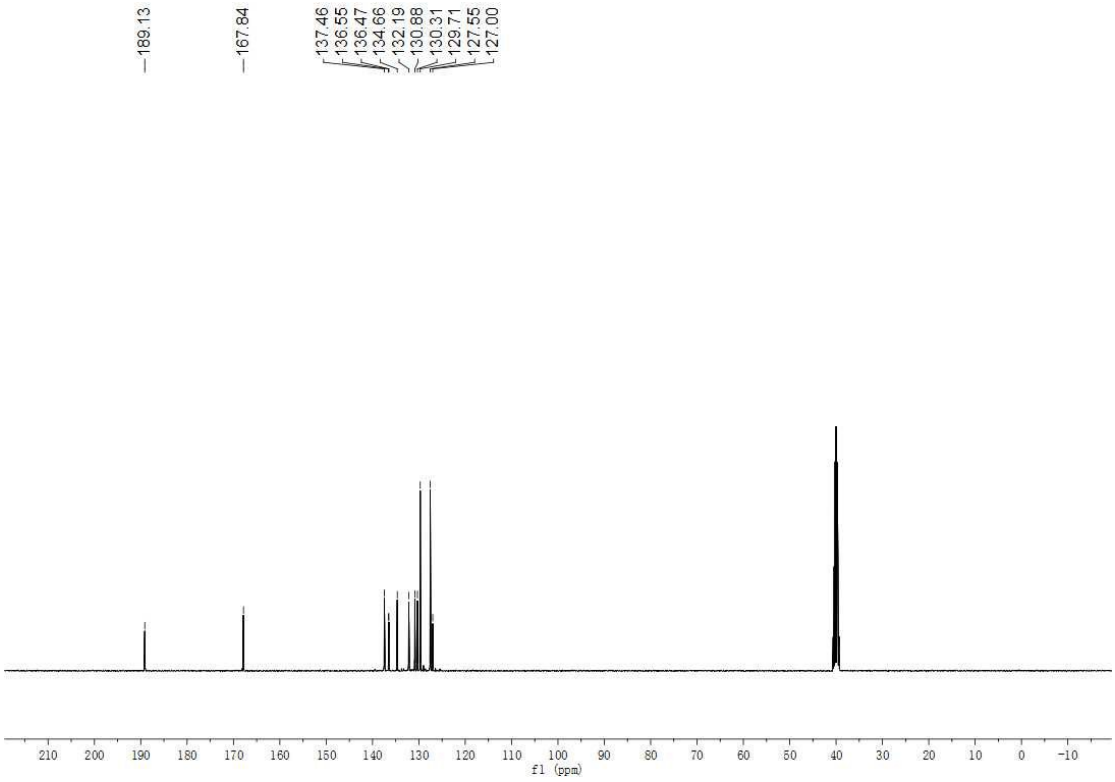

Fig. S8 ^{13}C NMR spectrum of the compound **1**

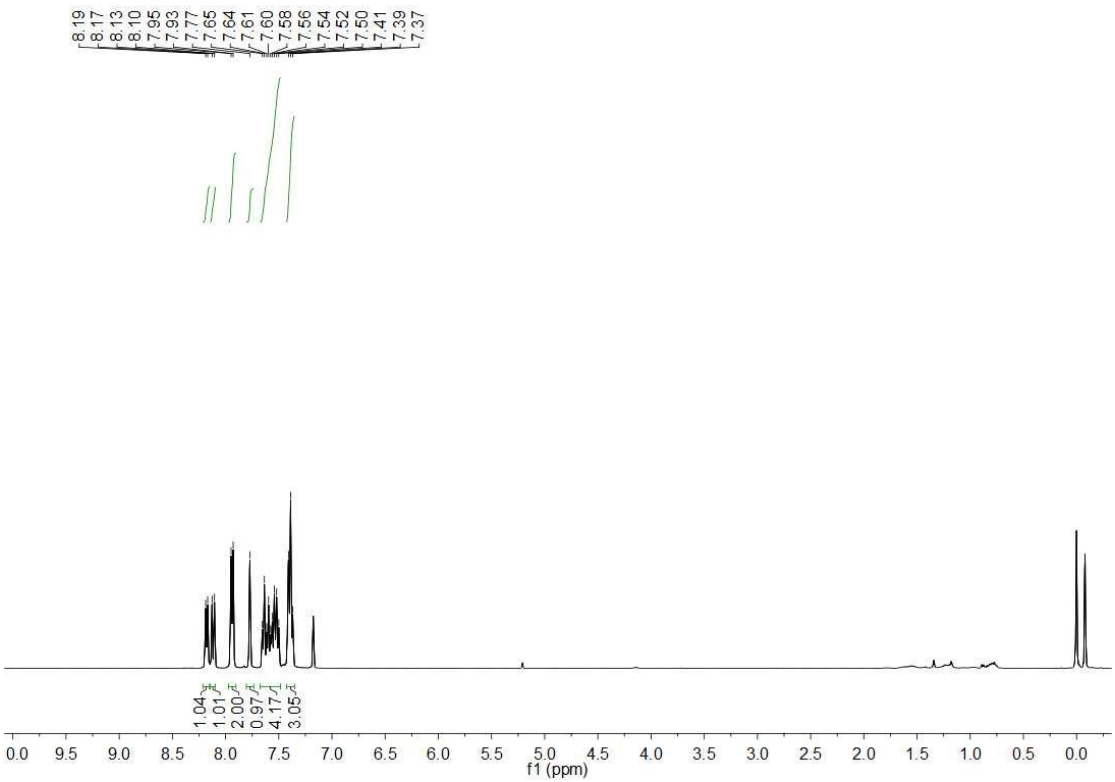

Fig. S9 ^{1}H NMR spectrum of the compound **2**

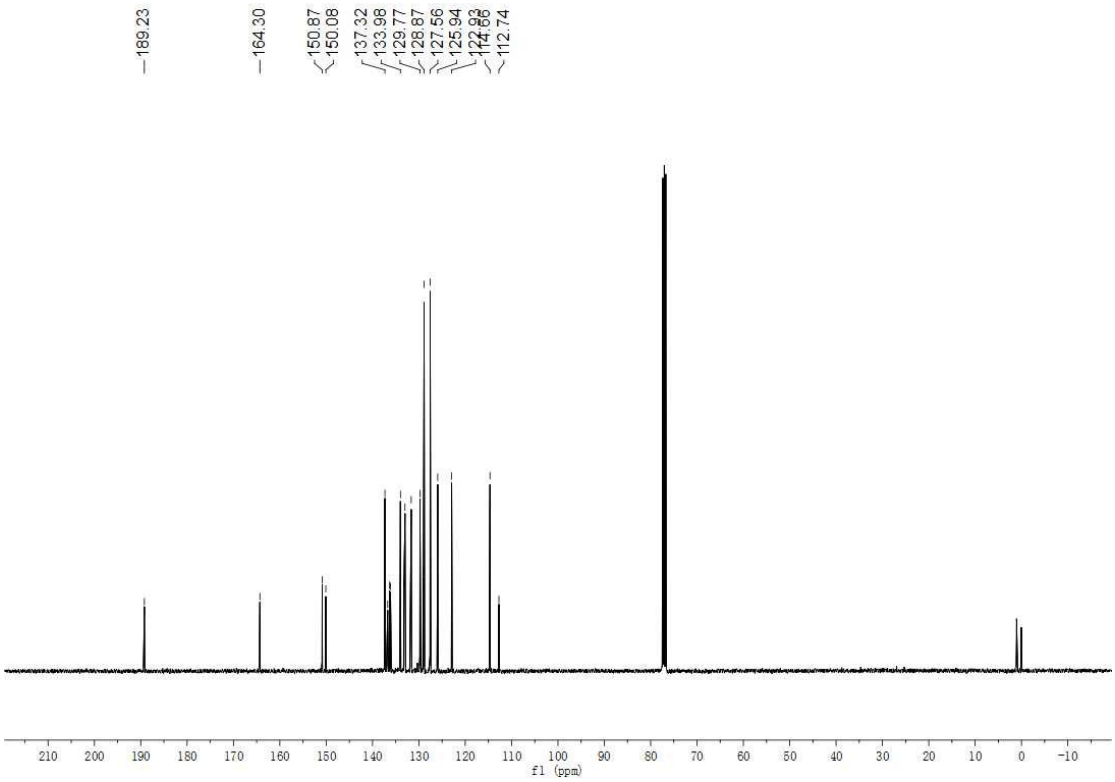

Fig. S10 ^{13}C NMR spectrum of the compound **2**

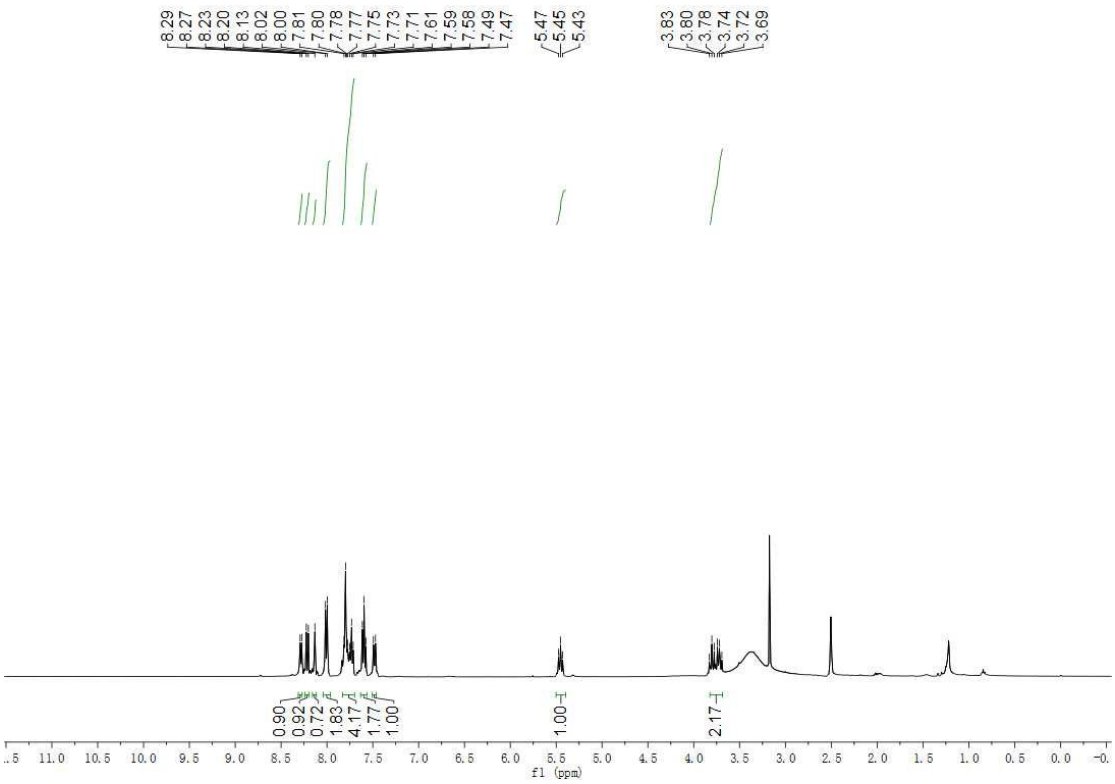

Fig. S11 ^{1}H NMR spectrum of the compound **BP-PS**

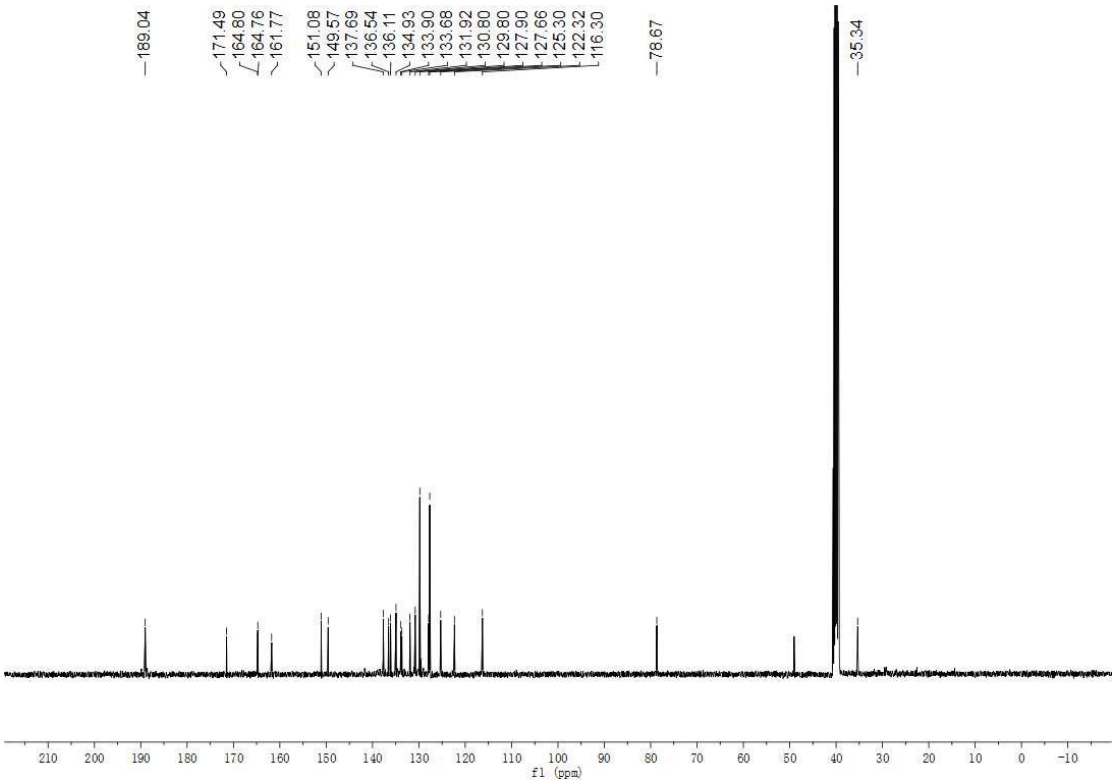

Fig. S12 ^{13}C NMR spectrum of the compound **BP-PS**

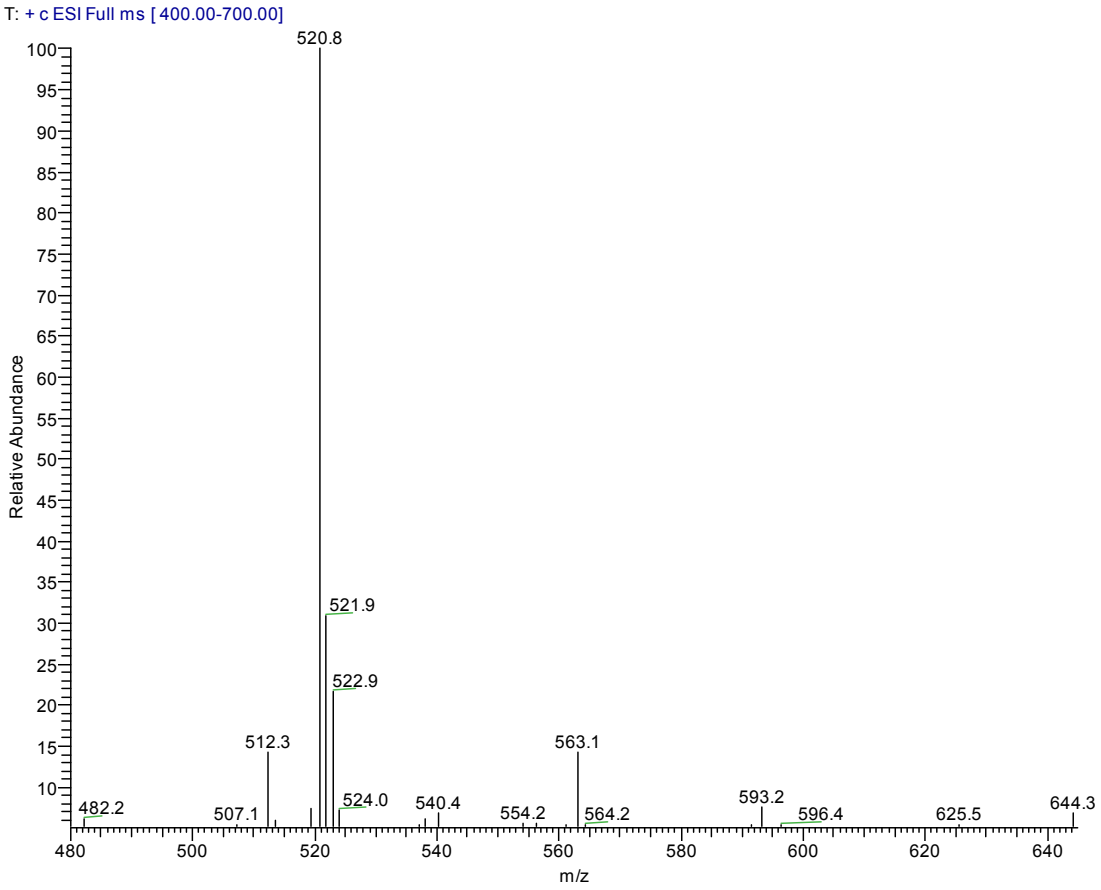

Fig. S13 ESI mass spectrum of the compound **BP-PS**