Electronic Supporting Information for:

PEGylated AIEgen Molecular Probe for Hypoxia-Mediated Tumor Imaging and Photodynamic Therapy

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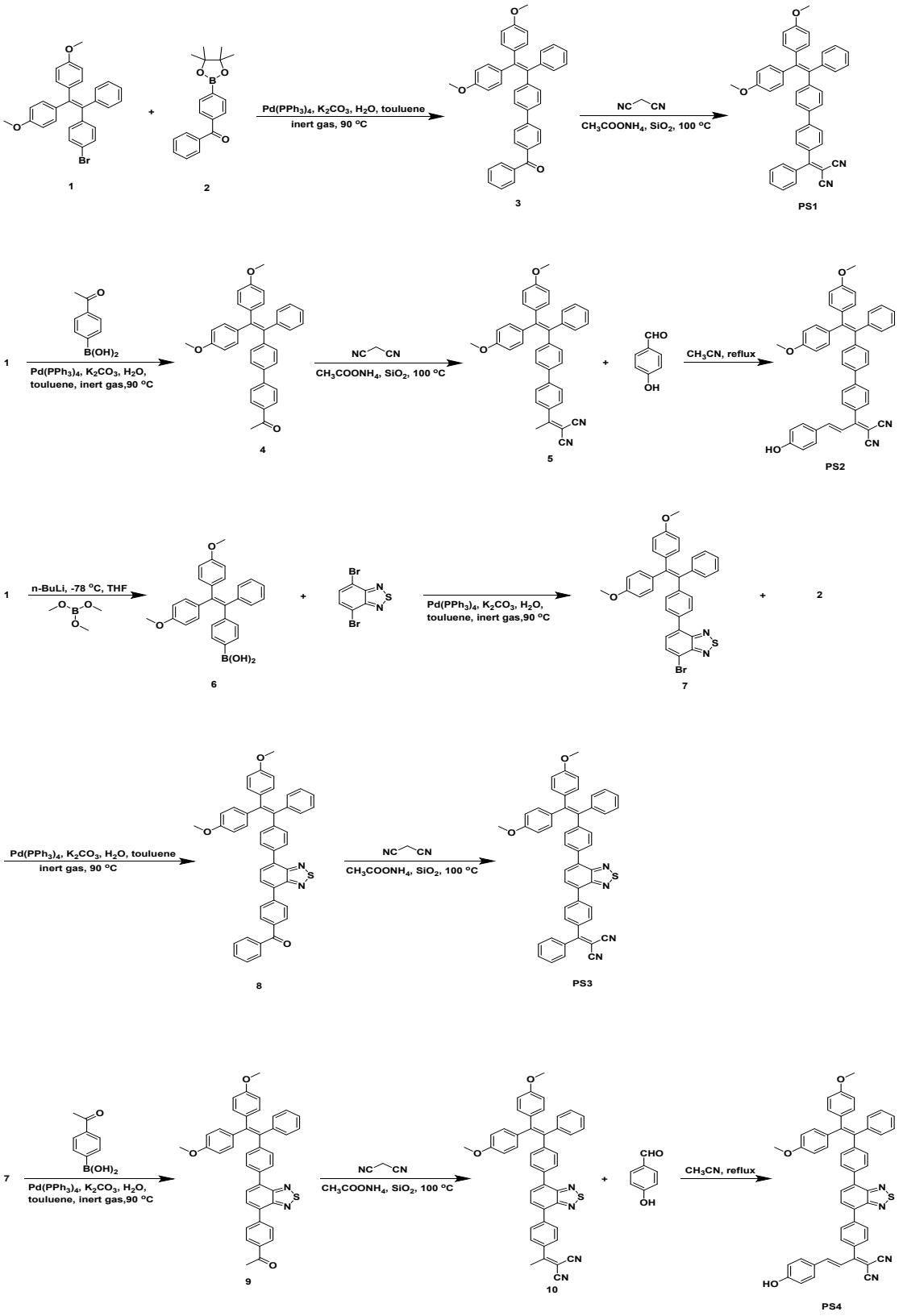
Table of Content

EXPERIMENTAL SECTION

Materials and Methods. 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), p-phthalic acid, propidium iodide (PI), calcein acetoxymethyl (calcein AM), dichlorofluorescein diacetate (DCFDA), liver microsomes and NADPH were purchased from Sigma-Aldrich Co., Ltd. (Saint Louis, MO, USA). All other reagents were purchased from commercial suppliers and used without further purification. Water (18 M Ω) used in all experiments was purified via a Milli-Q water system (Millipore, Burlington, MA, USA). HepG-2 cells and Rat models were provided by the Biomedical Engineering Center of Hunan University (China). The study was approved by the Ethics Committee of the College of Biology, Hunan University, Changsha, China.

Dynamic light scattering (DLS) experiments were performed at room temperature using a Malvern Zeta Sizer Nanoseries (Nano ZS90). UV-vis absorption spectra were obtained on a Hitachi U-4100 UV/Vis spectrometer (Hitachi, Ltd., Tokyo, Japa) using a quartz cuvette having 1 cm path length. Fluorescence spectra were recorded on a PTI QM4 Fluorescence System (Photo Technology International Inc., Birmingham, NJ, USA). MTT assay was carried out by using a Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA). 1H and 13C NMR spectra were recorded on a Bruker 400MHZ Advance spectrometer. Mass spectra were measured with a JEOL JMS-T100LC mass spectrometer (ESI+) or MALDI-TOF Mass Spectrometry (Bruker, ultraflExtreme). Fluorescence images of cells were captured using a Nikon A1R confocal microscope system (Nikon Corp., Tokyo, Japan). Fluorescence images of mice were captured using a IVIS Lumina XR in vivo imaging system (PerkinElmer).

Synthesis of the AIEgen photosensitizers (PS1-PS4).



Scheme S1. The synthetic routes of PS1-PS4.

Synthesis of PS1.[1] Compound 3 (935 mg, 1.6 mmol), malononitrile (345 mg, 5.2 mmol), ammonium acetate (420 mg, 5.4 mmol) was added to a solvent of 10 mL methanol and 10 mL dichloromethane. The reaction mixture was ultra-sounded until the solid was completely dissolved. Then silica gel (2.0 g) was added to the reaction mixture. The solvent was evaporated under reduced pressure. The reaction was stirred at 100 ^{o}C for 10 h. Upon completion of the reaction monitored by TLC, the reaction mixture was cooled to room temperature followed by purification on silica gel column chromatography with DCM/Hexane (1/3) as the eluent to afford PS1 as a red solid **(**541 mg, 0.87 mmol) resulting in a yield of 55%. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.66 (d, J = 8.3 Hz, 2H), 7.62 – 7.56 (m, 1H), 7.47 (m, 6H), 7.39 (d, J = 8.2 Hz, 2H), 7.16 – 7.09 (m, 5H), 7.06 (d, J = 7.7 Hz, 2H), 7.01 – 6.91 (m, 4H), 6.70 – 6.61 (m, 4H), 3.75 (s, 6H). MALDI-TOF MS (DHB, linear mode, m/z): 620.198 [M+] 620.246 calc. for $C_{44}H_{32}N_2O_2$. (Figure S1-S2)

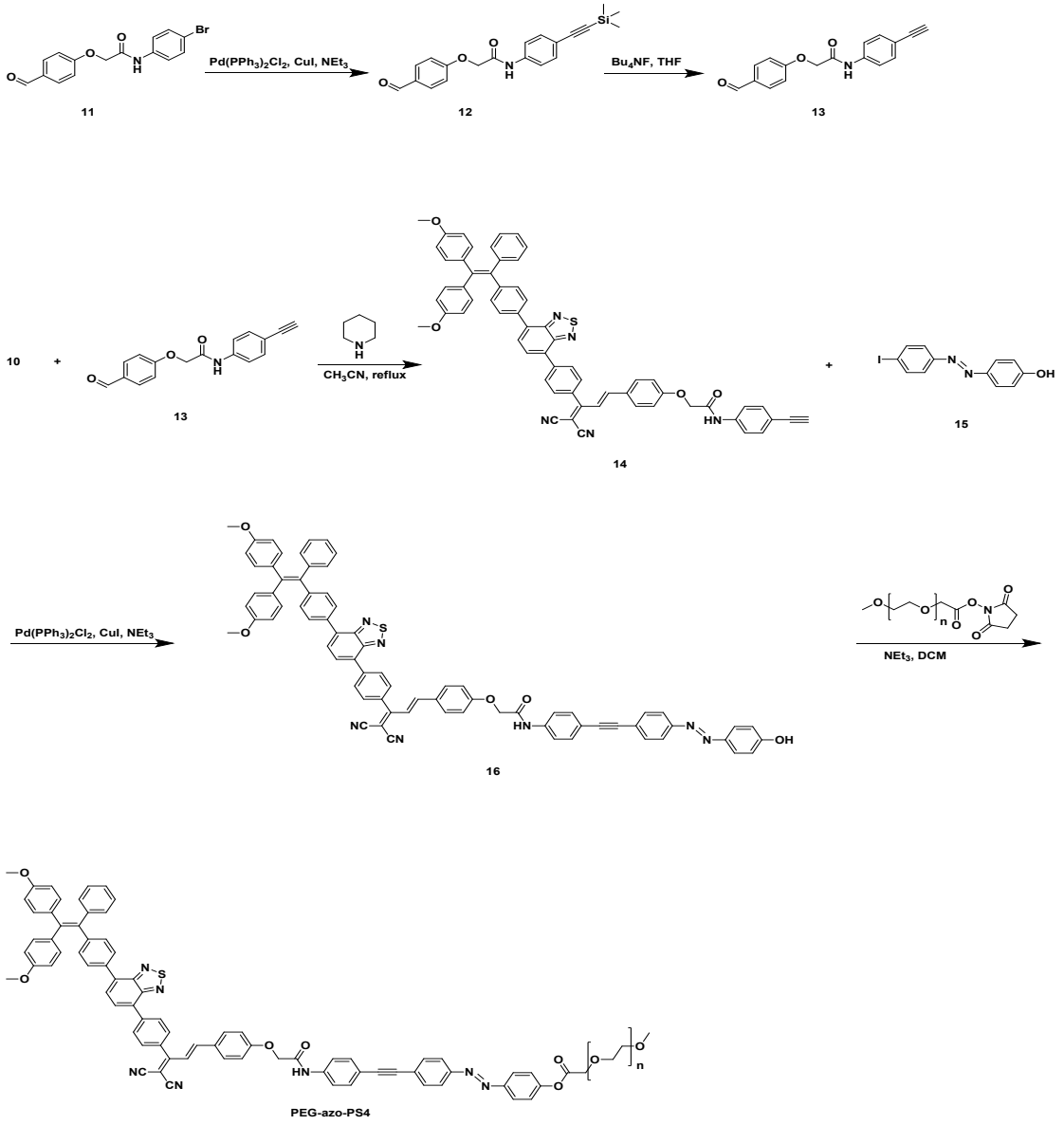
Synthesis of PS2. Compound 5 (558 mg, 1.0 mmol) and 4-hydroxybenzaldehyde (122 mg, 1.0 mmol) was dissolved in 20 mL acetonitrile, then two drops of piperidine were added to the solution. The reaction mixture was heat to reflux for 6 h. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel with DCM as the eluent to afford PS2 as a dark red solid with a yield of 52% (427 mg, 0.52 mmol). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.72 (d, J = 8.2 Hz, 2H), 7.44 (m, 7H), 7.16 – 7.10 (m, 5H), 7.07 (d, J = 7.9 Hz, 2H), 6.97 (dd, J = 12.7, 8.7 Hz, 4H), 6.86 (d, J = 8.9 Hz, 3H), 6.66 (t, J = 8.2 Hz, 4H), 3.75 (d, J = 1.8 Hz, 6H). MALDI-TOF MS (DHB, linear mode, m/z): 662.165 [M+] 662.257 calc. for $C_{46}H_{34}N_2O_3$. (Figure S3-S4)

Synthesis of PS3.[1] Compound 8 (821 mg, 1.2 mmol), malononitrile (541 mg, 8.2 mmol), ammonium acetate (247 mg, 3.2 mmol) was added to a solvent of 20 mL methanol and 10 mL dichloromethane. The reaction mixture was ultra-sounded until the solid was completely dissolved. Then silica gel (5.0 g) was added to the reaction mixture. The solvent was evaporated under reduced pressure. The reaction was stirred

at 100 °C for 8 h. Upon completion of the reaction monitored by TLC, the reaction mixture was cooled to room temperature followed by purification on silica gel column chromatography with DCM/Hexane (1/2) as the eluent to afford PS3 as a red solid (441 mg, 0.58 mmol) resulting in a yield of 49%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.12 (d, *J* = 7.6 Hz, 2H), 7.81 (m, 4H), 7.68 – 7.47 (m, 7H), 7.20 (d, *J* = 7.5 Hz, 2H), 7.12 (d, *J* = 5.3 Hz, 5H), 7.03 (d, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 7.4 Hz, 2H), 6.65 (m, 4H), 3.75 (s, 6H). MALDI-TOF MS (DHB, linear mode, m/z): 754.162 [M+] 754.240 calc. for $C_{50}H_{34}N_4O_2S$. (Figure S5-S6)

Synthesis of PS4. Compound 10 (692 mg, 1.0 mmol) and 4-hydroxybenzaldehyde (122 mg, 1.0 mmol) was dissolved in 20 mL acetonitrile, then two drops of piperidine were added to the solution. The reaction mixture was heat to reflux for 6 h. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel with DCM as the eluent to afford PS4 as a dark red solid with a yield of 48% (382 mg, 0.48 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 (d, *J* = 6.9 Hz, 2H), 7.88 – 7.79 (m, 4H), 7.57 – 7.47 (m, 5H), 7.21 (d, *J* = 6.8 Hz, 2H), 7.16 – 7.09 (m, 5H), 7.06 – 6.95 (m, 5H), 6.87 (d, *J* = 7.8 Hz, 2H), 6.71 – 6.63 (m, 4H), 3.75 (s, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.15, 159.18, 158.27, 158.16, 153.86, 149.14, 144.83, 144.16, 140.85, 140.24, 138.71, 136.32, 136.31, 132.98, 132.70, 132.65, 131.75, 131.54, 131.24, 131.17, 129.65, 129.36, 128.87, 128.51, 127.81, 127.37, 126.24, 122.23, 116.38, 113.84, 113.21, 113.03, 55.14, 55.12. MALDI-TOF MS (DHB, linear mode, m/z): 796.156 [M+] 796.251 calc. for $C_{52}H_{36}N_4O_3S$. (Figure S7-S9)

Synthesis of the PEGylated AIEgen molecular probe (PEG-azo-PS4).



Scheme S2. Synthetic route of the PEG-azo-PS4 probe.

Synthesis of compound 9. Compounds 5, 7, 13, and 15 (Figure S10-S12) were synthesized according to the reported literature.[1-4] Compound 7 (3.2 g, 5.3 mmol), (4-acetylphenyl) boronic acid (950 mg, 5.79 mmol), K_2CO_3 (2.4 g, 17.2 mmol) and Pd $(PPh_3)_4$ (3 %) were all carefully degassed and charged with argon, respectively. The reaction was stirred at 80 ^oC overnight. Upon completion of the reaction monitored by TLC, the reaction mixture was extracted with dichloromethane for three times. The

combined organic phase was dried with anhydrous magnesium sulfate for 30 min. Then the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with DCM/Hexane (1/1) as the eluent to afford compound 9 as a red solid (3.2 g, 5.0 mmol) resulting in a yield of 95%. ^{1}H NMR (400 MHz, Chloroform-d, 298 K) δ (TMS, ppm): 8.15-8.02 (m, 4H), 7.76 (m, J = 19.1 Hz, 4H), 7.20 (d, J = 8.2 Hz, 2H), 7.12 (m, 5H), 7.04 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 6.66 (dd, J = 12.6, 8.7 Hz, 4H), 3.73 (s, 3H), 3.72 (s, 3H), 2.66 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d, 298K) δ (ppm): 197.70, 158.32, 158.20, 144.80, 144.20, 140.88, 138.73, 136.32, 134.62, 133.89, 132.72, 132.67, 131.74, 131.56, 129.39, 128.69, 128.63, 128.52, 127.85, 127.67, 126.27, 113.24, 113.07, 55.14, 55.12, 26.76. MALDI-TOF MS (DHB, linear mode, m/z): 644.21 [M^+] 644.59 calc. for $C_{42}H_{32}N_2O_3S$. (Figure S13-S15)

Synthesis of compound 10. Compound 9 (2.3 g, 3.6 mmol), malononitrile (472 mg, 7.1 mmol), ammonium acetate (547 mg, 7.1 mmol) was added to a solvent of 20 mL methanol and 20 mL dichloromethane. The reaction mixture was ultra-sounded until the solid was completely dissolved. Then silica gel (6.0 g) was added to the reaction mixture. The solvent was evaporated under reduced pressure. The reaction was stirred at 100 ^{o}C for 10 h. Upon completion of the reaction monitored by TLC, the reaction mixture was cooled to room temperature followed by purification on silica gel column chromatography with DCM/Hexane (1/1) as the eluent to afford compound 10 as a red solid (996.48 mg, 1.44 mmol) resulting in a yield of 40%. ^{1}H NMR (400 MHz, Chloroform-d, 298 K) δ (TMS, ppm): 8.17-8.04 (m, 4H), 7.84-7.75 (m, 4H), 7.20 (d, J = 8.4 Hz, 2H), 7.12 (m, 5H), 7.04 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.7 Hz, 2H), 6.67 (dd, J = 12.3, 8.8 Hz, 4H), 3.75 (s, 3H), 3.74 (s, 3H), 2.68 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d, 298K) δ (ppm): 197.70, 158.30, 158.19, 153.95, 144.78, 144.18, 140.85, 136.55, 136.32, 134.62, 133.95, 132.69, 132.64, 131.72, 131.54, 129.39, 128.67, 128.63, 128.49, 127.81, 127.68, 126.24, 113.21, 113.05, 55.13, 26.72. MALDI-TOF MS (DHB, linear mode, m/z): 692.31 [M^+] 692.22 calc.

for $C_{45}H_{32}N_4O_2S$. (Figure S16-S18)

Synthesis of compound 13. Compound 11 (1.2 g, 3.6 mmol), triethyl amine (20 mL), Pd $(PPh_3)_2Cl_2$ (1 %), CuI (1 %) was carefully degassed and charged with argon followed by the addition of Ethynyltrimethylsilane (424 mg, 4.32 mmol). The reaction mixture was stirred at room temperature overnight. Upon completion of the reaction monitored by TLC, tetra butyl ammonium fluoride (2 M in THF, 4 mL, 8 mmol) was added to the reaction mixture. After completion of the reaction monitored by TLC, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with DCM as the eluent to afford compound 13 as a yellow solid (893 mg 3.2 mmol) resulting in a yield of 89%. ^{1}H NMR (400 MHz, Chloroform-d, 298 K) δ (TMS, ppm): 9.97-9.90 (m, 1H), 8.24 (s, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.61-7.46 (m, 4H), 7.12 (d, J = 8.5 Hz, 2H), 4.70 (s, 2H), 3.07 (s, 1H). ^{13}C NMR (101 MHz, Chloroform-d, 298K) δ (ppm): 190.52, 165.15, 161.41, 137.01, 133.08, 132.23, 131.47, 119.76, 118.67, 115.15, 83.11, 77.24, 67.47. (Figure S19-S20)

Synthesis of compound 14. Compound 10 **(**990 mg, 1.43 mmol) and compound 13 **(**400 mg, 1.43 mmol) was dissolved in 40 mL ultra-dry acetonitrile, then two drops of piperidine were added to the solution. The reaction mixture was heat to reflux for 6 h. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel with DCM as the eluent to afford compound 14 as a dark red solid with a yield of 60% (812 mg, 0.85 mmol). ^{1}H NMR (400 MHz, Chloroform-d, 298 K) δ (TMS, ppm): 8.24 (s, 1H), 8.18 (d, J = 8.1 Hz, 2H), 7.87-7.78 (m, 4H), 7.62-7.52 (m, 7H), 7.47 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 7.16-7.09 (m, 5H), 7.06-6.95 (m, 7H), 6.67 (dd, J = 13.9, 8.6 Hz, 4H), 4.64 (s, 2H), 3.74 (s, 6H), 3.06 (s, 1H). ^{13}C NMR (101 MHz, Chloroform-d, 298K) δ (ppm): 165.33, 159.46, 158.30, 154.04, 148.23, 144.87, 144.17, 140.89, 140.39, 138.70, 137.07, 136.31, 133.08, 132.76, 132.71, 132.66, 131.76, 131.55, 131.12, 131.06, 129.71, 129.35, 129.00, 128.89, 128.53, 127.83, 127.78, 126.27, 123.41, 119.76, 118.61, 115.57, 113.23, 113.06, 83.14, 67.49, 55.15, 55.13. MALDI-TOF MS (DHB,

linear mode, m/z): 953.523 [M^+] 953.00 calc. for $C_{62}H_{43}N_5O_4S$. (Figure S21-S23)

Synthesis of compound 16. Compound 14 (805 mg, 0.84 mmol), compound 15 (355 mg, 1.1 mmol) triethyl amine (20 mL), Pd $(PPh_3)_2Cl_2$ (1 %), CuI (1 %) was carefully degassed and charged with argon. The reaction mixture was stirred overnight. The crude product after the evaporation of the solvent was purified by column chromatography on silica gel with DCM: ethyl acetate (5:1) as the eluent to afford compound 16 as a dark red solid with a yield of 4% (40 mg, 0.034 mmol). ^1H NMR (400 MHz, Chloroform-d, 298 K) δ (TMS, ppm): 8.26 (s, 1H), 8.19 (d, J = 7.6 Hz, 2H), 7.85 (m, 8H), 7.66-7.53 (m, 12H), 7.22 (d, J = 8.4 Hz, 2H), 7.13 (m, 5H), 7.05 (t, J = 6.5 Hz, 5H), 6.96 (t, J = 8.2 Hz, 4H), 6.67 (dd, J = 13.9, 9.0 Hz, 4H), 4.68 (s, 2H), 3.75 (s, 6H). MALDI-TOF MS (DHB, linear mode, m/z): 1149.300 [M^+] 1149.37 calc. for $C_{74}H_{51}N_7O_5S$. (Figure S24-S25)

Synthesis of PEG-azo-PS4. PEG-NHS 2000 (60 mg) and compound 16 (35 mg, 0.03 mmol) were dissolved in 10 mL anhydrous dichloromethane, then one drop of triethylamine was added to the solution, the reaction mixture was stirred for 2 h. The solvent was evaporated under reduced pressure. Then the residue was dissolved into water and filtered through syringe. Then the stock solution was freeze-dried and mass spectrometry analysis showed the successful link of PEG chain and compound 16.

Evaluation of the Hypoxia-Responsible Ability of the PEG-azo-PS4 Probes. Exactly, 19 mg PEG-azo-PS4 and 50 μL of rat liver microsomes were first dispersed in 2.95 mL of PBS (pH 7.4), respectively, and the final concentration of PEG-azo-PS4 is 2 μM and rat liver microsomes is 50 μL/3 mL. Then, the hypoxic or normoxic solution was obtained by introducing high purity of nitrogen or not into the above mixture solution for 20 min. Subsequently, NADPH was added (the final concentration is 50 μM) and the time-based change of the fluorescence emission intensity at 650 nm of the obtained mixture solution was recorded immediately on a PTI QM4 Fluorescence System (λ_{ex} = 420 nm).

Evaluation of $^1O_2$-Generating Ability of the PS1-PS4 and PEG-azo-PS4 Molecules. To investigate the $^1O_2$-generating ability of the synthesized photosensitizers, a mixture solution of PS1-PS4 (10 μM) and ABDA (50 μM) in DMSO/H_2O (0.1%) was exposed to white light irradiation (50 mW/cm^2) for different times, and after each interval irradiation, Uv-vis spectrum of each mixture solution was recorded on a Hitachi U-4100 UV/Vis spectrometer. For investigating the $^1O_2$-generating ability of the PEG-azo-PS4 probe, the probe was directly dispersed in H_2O instead of the DMSO-H_2O mixture solution. Then, ABDA was added into the reaction solution (at a final ABDA concentration of 50 μM), and the subsequent experimental process was as that described above.

Cell Culture. HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin streptomycin, and cultured in a humidified incubator at 37 ^{o}C with an atmosphere of 5% CO_2.

Hypoxia Imaging Assay in Living Cells with the PEG-azo-PS4 Probe. HepG-2 cells were first seeded into culture dishes for 24 h (37°C, 5% CO_2). (1) For the hypoxia imaging assay, HepG-2 cells were then first co-incubated with the PEG-azo-PS4 probe (5 μM) for 2 h at 37 ^{o}C under different O_2 concentrations (i.e., 20, 8, 5, 1 and 0.1%), and then washed several times with PBS (pH 7.4) to remove free PEG-azo-PS4 probes. Subsequently, cells were imaged using a Nikon A1R confocal microscope system with 405 nm excitation. (2) For the intracellular localization imaging experiments, cells were then first incubated with the PEG-azo-PS4 probes (5 μM) for 2 h at 37 ^{o}C under 0.1% O_2 concentrations, then treated with DAPI (1.0 μM) and MitoTracker Green (1.0 μM) for 30 min in the incubator at 37 °C with 5 % CO_2, and were then rinsed three times with PBS. Subsequently, cells were imaged using a Nikon A1R confocal microscope system.

$^1O_2$-Generation Assay of the PEG-azo-PS4 Probes in Living Cells. HepG-2 cells were first seeded into culture dishes for 24 h (37 ^{o}C, 5% CO_2). Afterwards, the cells

were incubated with the PEG-azo-PS4 probes (5 μM) for 2 h at 37 °C under 0.1% O_2 concentrations, then further incubated with DCFH-DA (10 μM) for 30 min under normoxia condition, and then irradiated by a white light (20 mW cm^{-2}) for different durations, and were then rinsed three times with PBS. Eventually, the green fluorescence emission of DCFH-DA in the cells was measured by a Nikon A1R confocal microscope system with 488 nm excitation.

Evaluation of the Cell-Killing Ability of the PEG-azo-PS4 Probes. For the cell viability assay, HepG-2 cells were first seeded in a 96-well plate and allowed to adhere. Then, the cells were incubated with the PEG-azo-PS4 probes (5 μM) at 37 °C for 2 h under normoxia (20% O_2) or hypoxia (0.1% O_2) conditions. Subsequently, the cells were subjected to be irradiated by a white light with different power density for 2 min or with a power density of 50 mW cm^{-2} for different time. Thereafter, the cells were washed three times with PBS and incubated for another 24 h in the dark. Next, the post-reaction culture medium was replaced by MTT solution (1 mg/mL, 40 μL/well) and further incubated for 3 h in dark. Afterwards, the remaining MTT solution was removed and 100 μL of DMSO was added into each well and shaken for 10 min. Finally, the absorbance at 550 nm was measured using a RT 6000 microplate reader.

For the live/dead cell staining assay, HepG-2 cells were first seeded in a 35-mm confocal dish and allowed to adhere. Then, the cells were incubated with the PEG-azo-PS4 probes (5 μM) at 37 °C for 2 h under normoxia (20% O_2) or hypoxia (0.1% O_2) conditions. Subsequently, the cells were subjected to be irradiated by a white light with a power density of 50 mW cm^{-2} for 10 min. Thereafter, the cells were washed three times with PBS, then phenol-red-free DMEM was added and further incubated for another 8 h in the dark. Next, the medium was replaced with DMEM containing 1 μM of Calcein AM and 1 μM of PI, and further incubated for 15 min. Finally, the cells were washed with DPBS and observed using confocal microscopy.

Construction of the tumor models. Female nude Balb/c mice (18-20 g) were purchased from Hunan SJA Laboratory Animal Co. Ltd. (Changsha, China). All the animal experiments were conducted in compliance with the guide for the care and use of laboratory animals. The study was approved by the Ethics Committee of the College of Biology, Hunan University, Changsha, China. Afterwards, the tumor model was established by injecting HepG2 cells (1.0×10^7 cells) into the buttock of each female Balb/c mouse. Tumors were allowed to grow for 14 days before use.

***In Vivo* Hypoxia Imaging with the PEG-azo-PS4 Probes.** (1) A female nude Balb/c mouse (18 g) bearing the HepG-2 cell-derived tumors was injected in the tumor site or the same place of the healthy mouse with the PEG-azo-PS4 probes (50 μL, 20 μM in PBS) and the fluorescence images was recorded after injection for different times (0.5 h, 1 h, 3 h, 5 h, 7 h, 24 h, 48 h); (2) Both the tumor-bearing mouse and the healthy mouse was injected in the tail vein with the PEG-azo-PS4 probes (50 μL, 20 μM in PBS) and the fluorescence images was recorded after injection for different times (0.5 h, 1 h, 3 h, 5 h, 7 h, 24 h, 48 h).

Tumor Therapy with the PEG-azo-PS4 Probes. Nine female nude Balb/c mice (18-20 g) bearing the HepG-2 cell-derived tumors (~100 mm^3) were randomly divided into three groups (n = 3 in each group), and vehicle (50 μL, PBS), or PEG-azo-PS4 (50 μL, 20 μM in PBS) was administered by intratumoral injection. Three hours later after the injection, 10 min of tumor site irradiation with a white light (400 mW cm^{-2}) was carried out for both the PBS-injected group and one of the probe-injected groups, while the another probe-injected group was keeping in dark throughout the whole time. These above treatments were repeated once every two days for a total of eight doses (0, 2, 4, 6, 8, 10, 12, 14, 16 d). The size of the tumors was measured every other day and calculated using the following formula: volume = (length) × $(width)^2/2$. Similarly, the mice were weighed every other day. After 16 days of treatment, the mice were sacrificed and the tumors were removed for H&E staining assay.

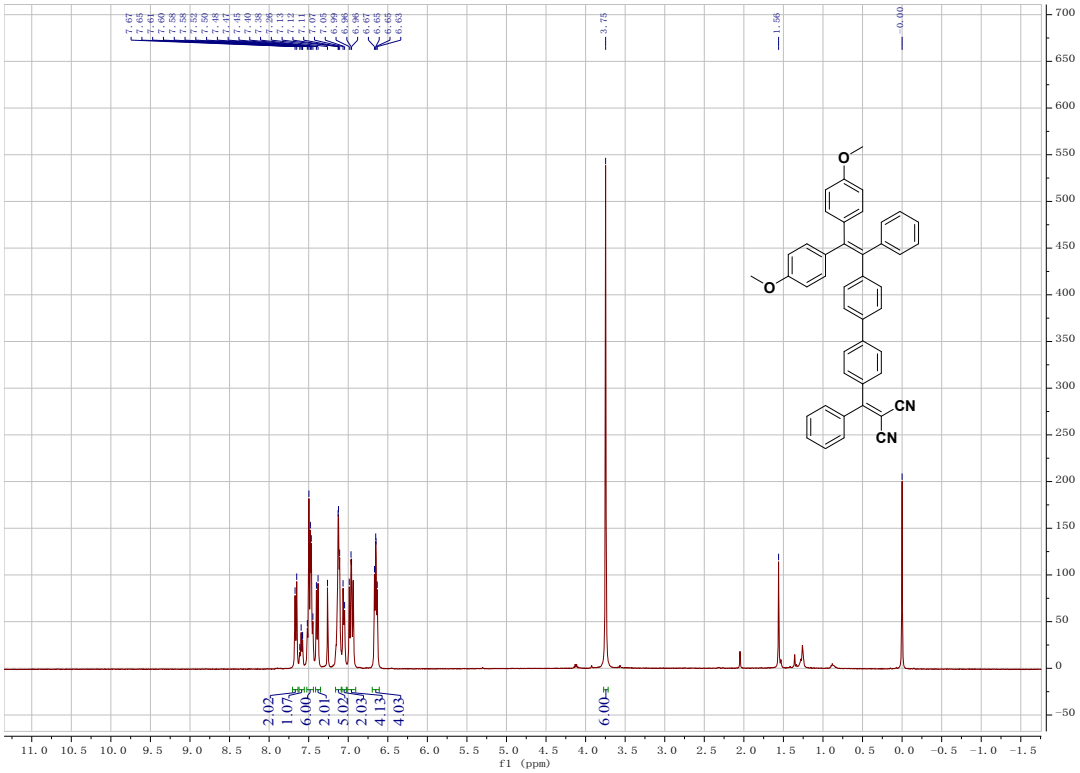


Figure S1. ^{1}H NMR spectrum of PS1 in $CDCl_3$.

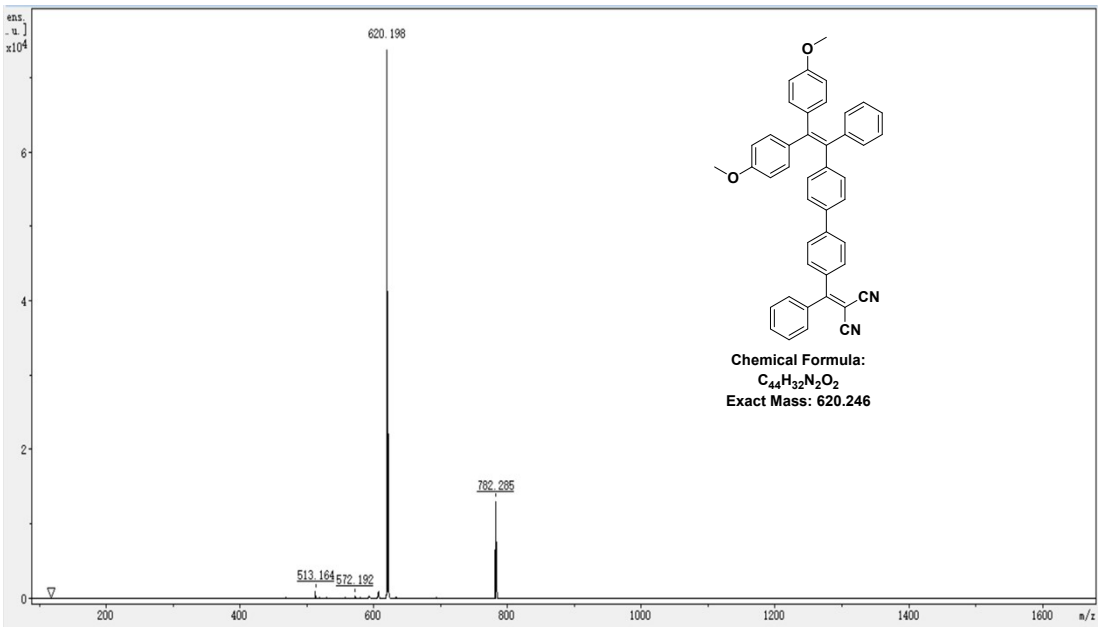


Figure S2. Mass spectrum of PS1 in CH_3CN.

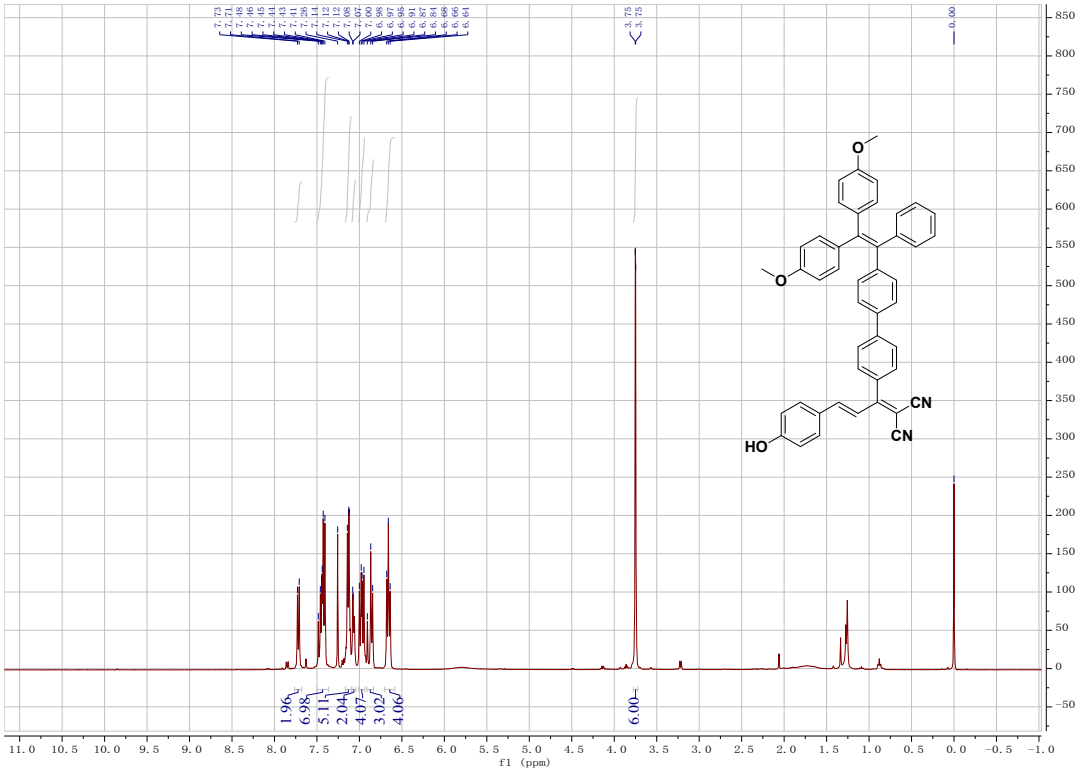


Figure S3. ^{1}H NMR spectrum of PS2 in $CDCl_3$.

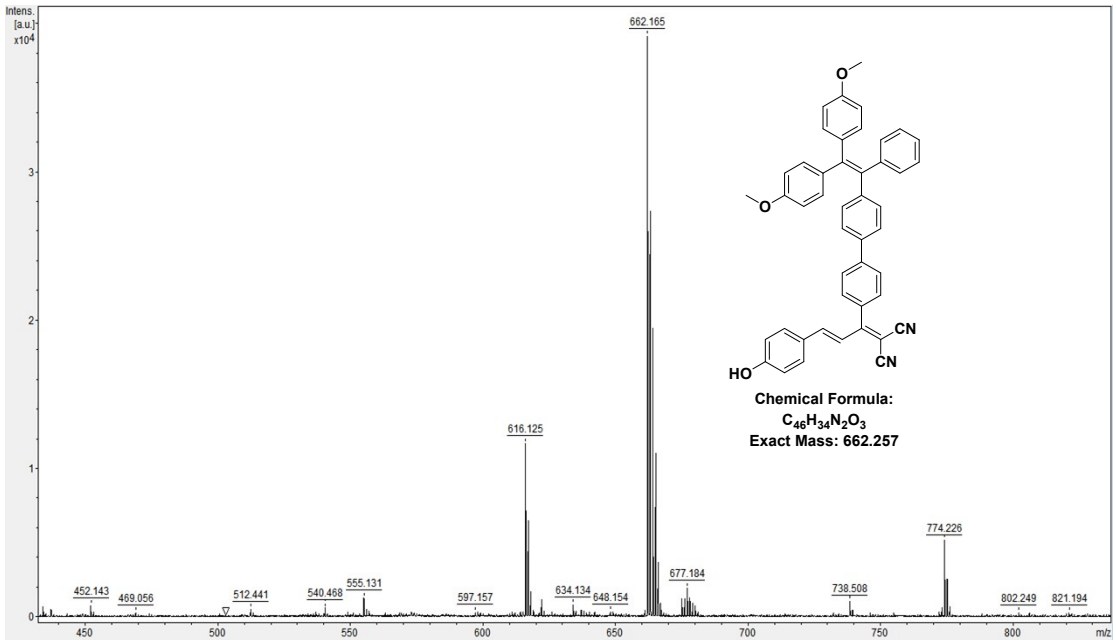


Figure S4. Mass spectrum of compound PS2 in MeCN.

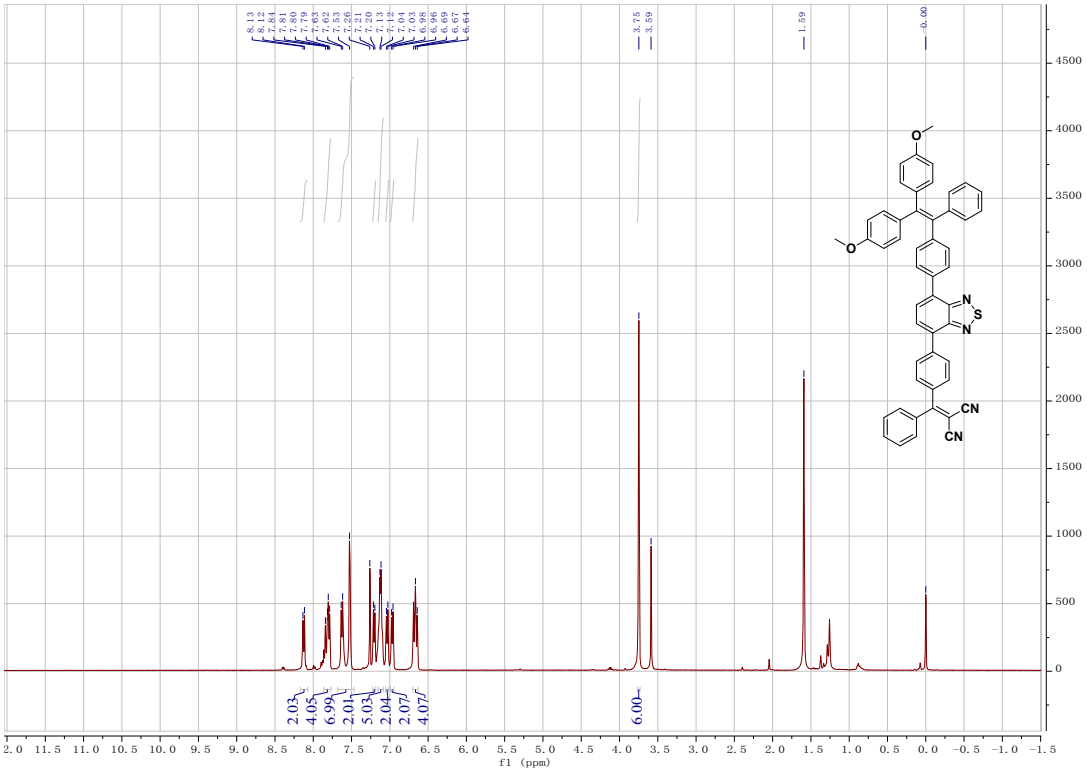


Figure S5. ^{1}H NMR spectrum of PS3 in $CDCl_3$.

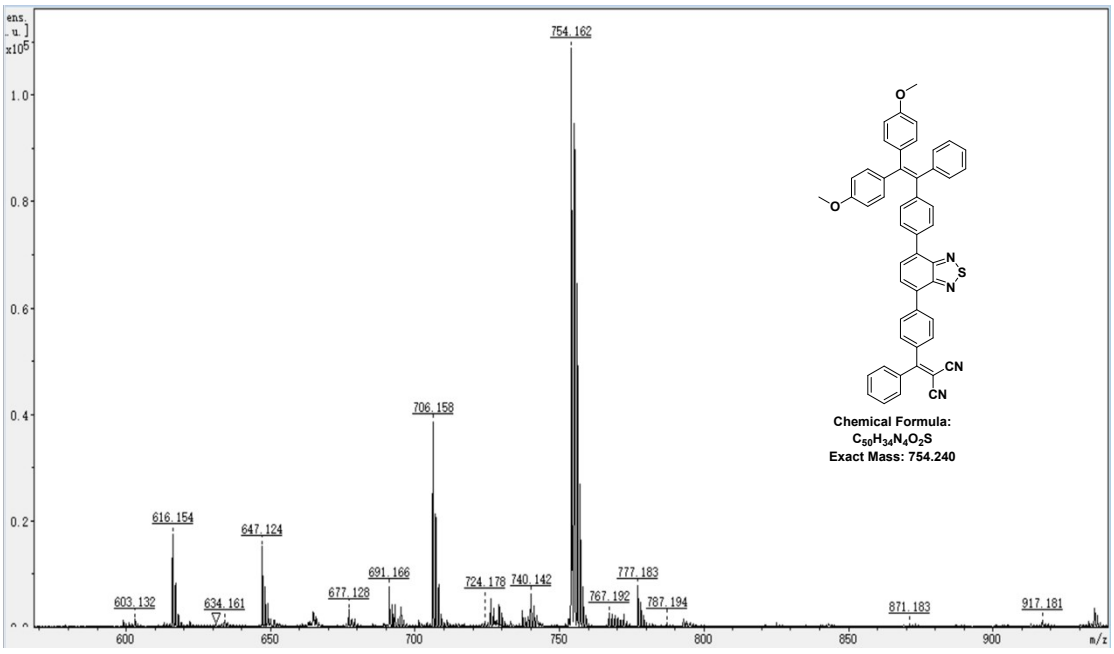


Figure S6. Mass spectrum of compound PS3 in MeCN.

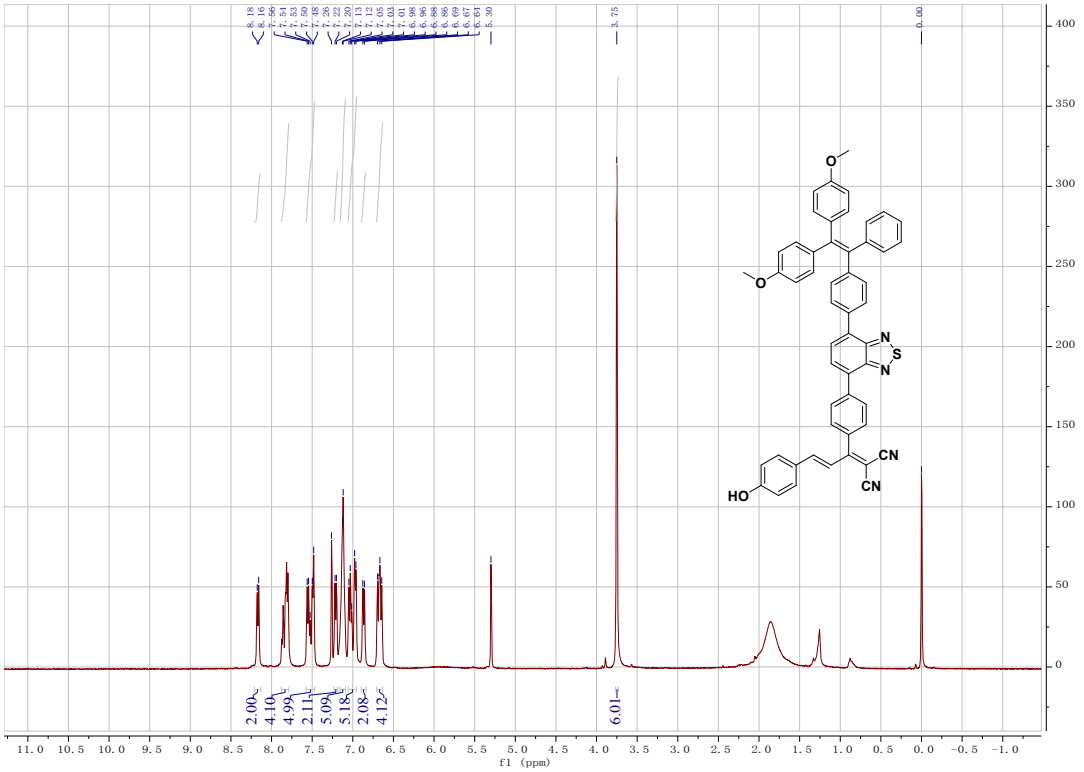


Figure S7. ^{1}H NMR spectrum of PS4 in $CDCl_3$.

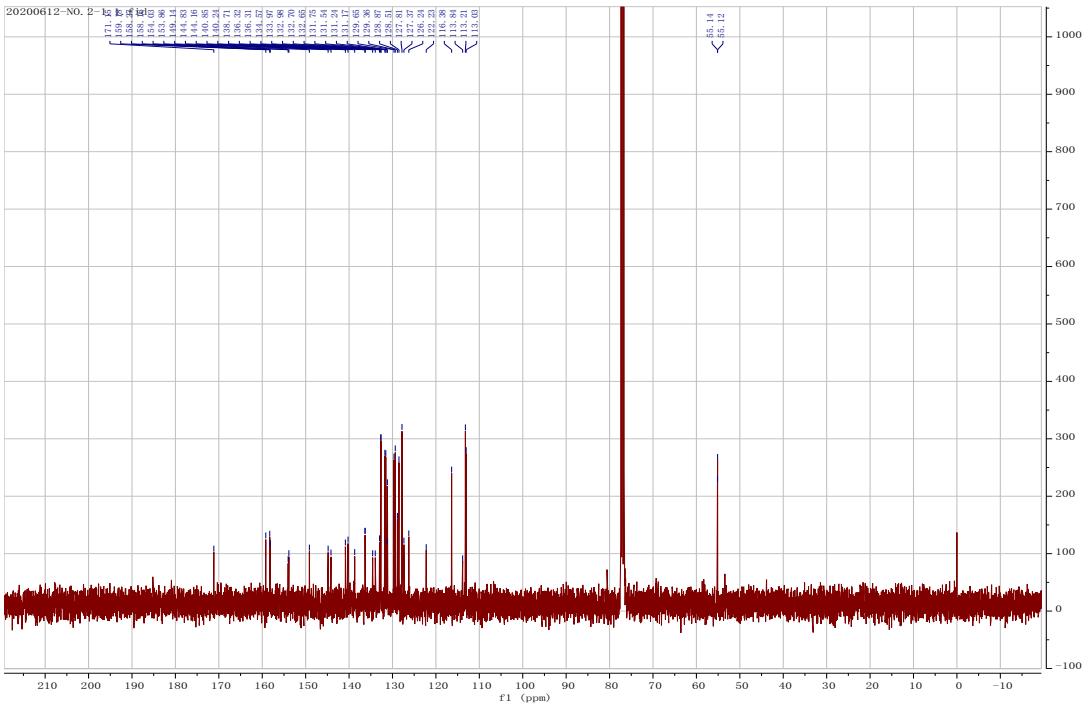


Figure S8. ^{13}C NMR spectrum of PS4 in $CDCl_3$.

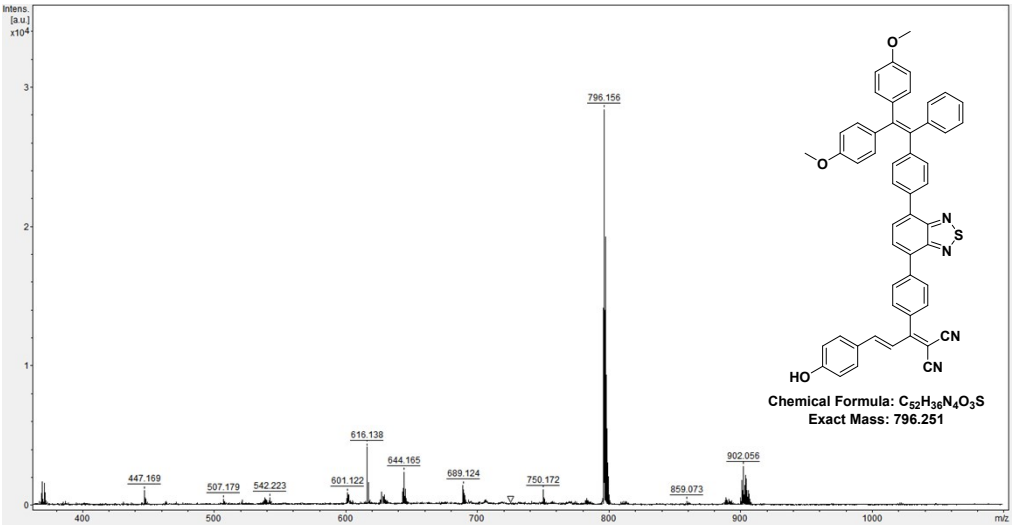


Figure S9. Mass spectrum of compound PS4 in MeCN.

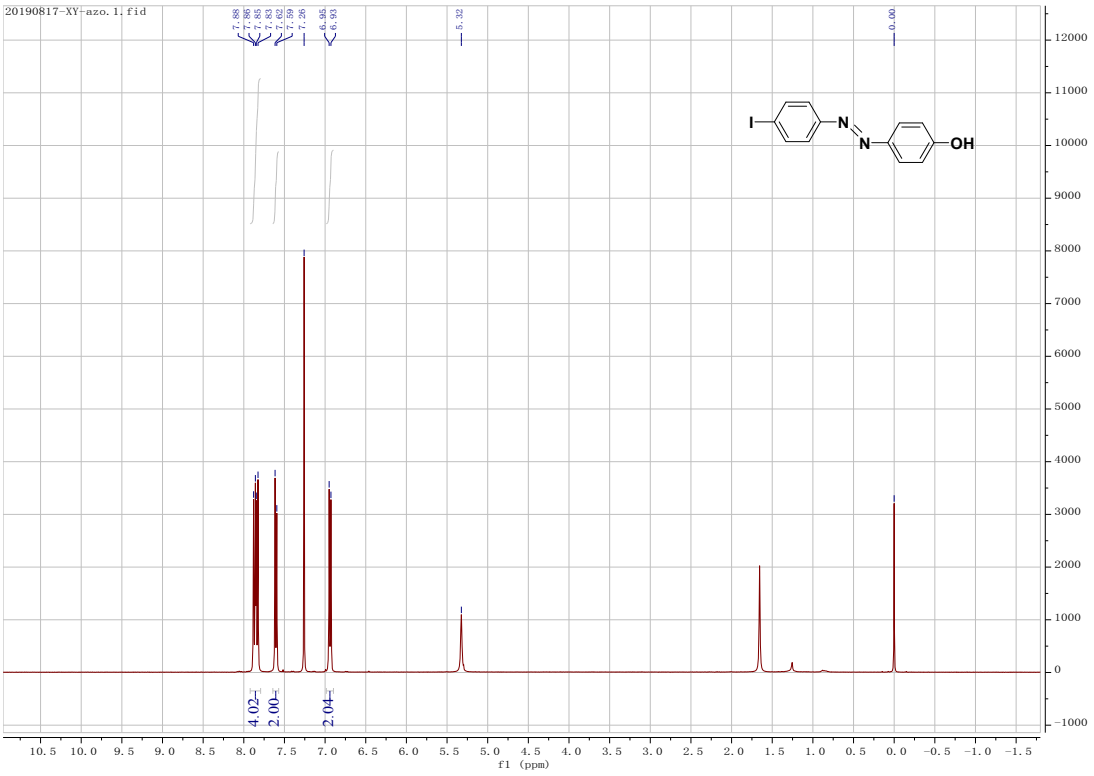


Figure S10. ^{1}H NMR spectrum of compound 15 in $CDCl_3$.

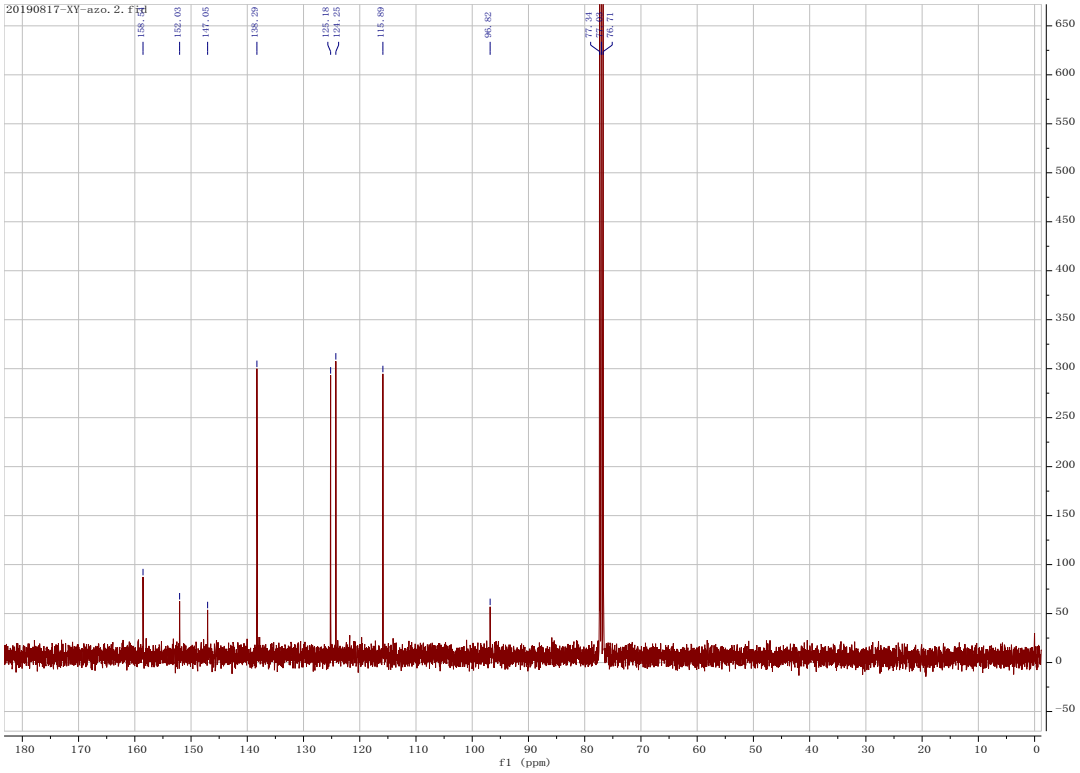


Figure S11. ^{13}C NMR spectrum of compound 15 in $CDCl_3$.

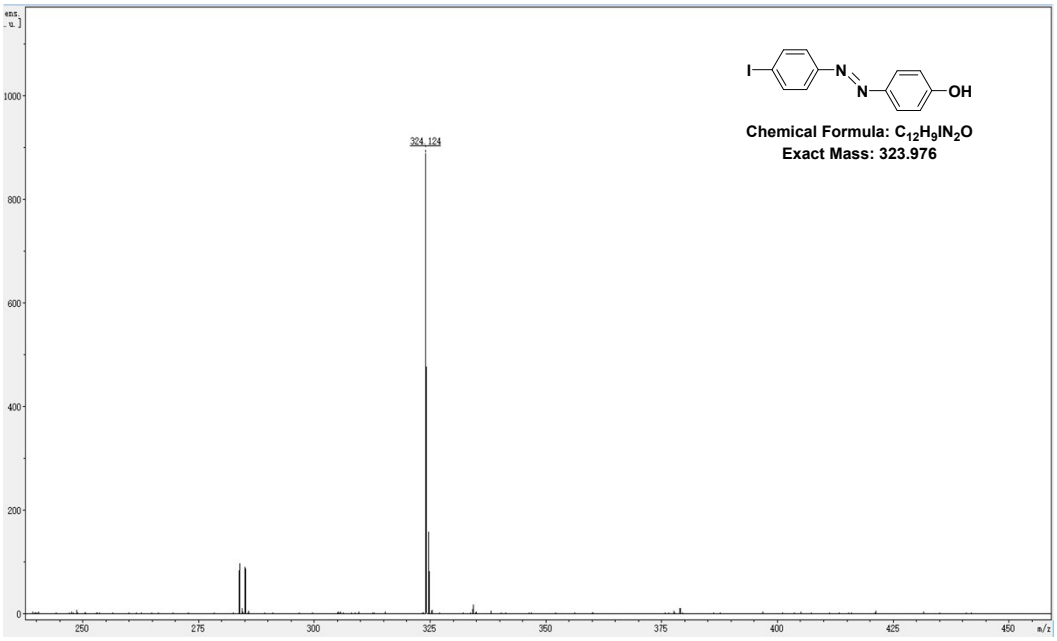


Figure S12. Mass spectrum of compound 15 in CH_3CN.

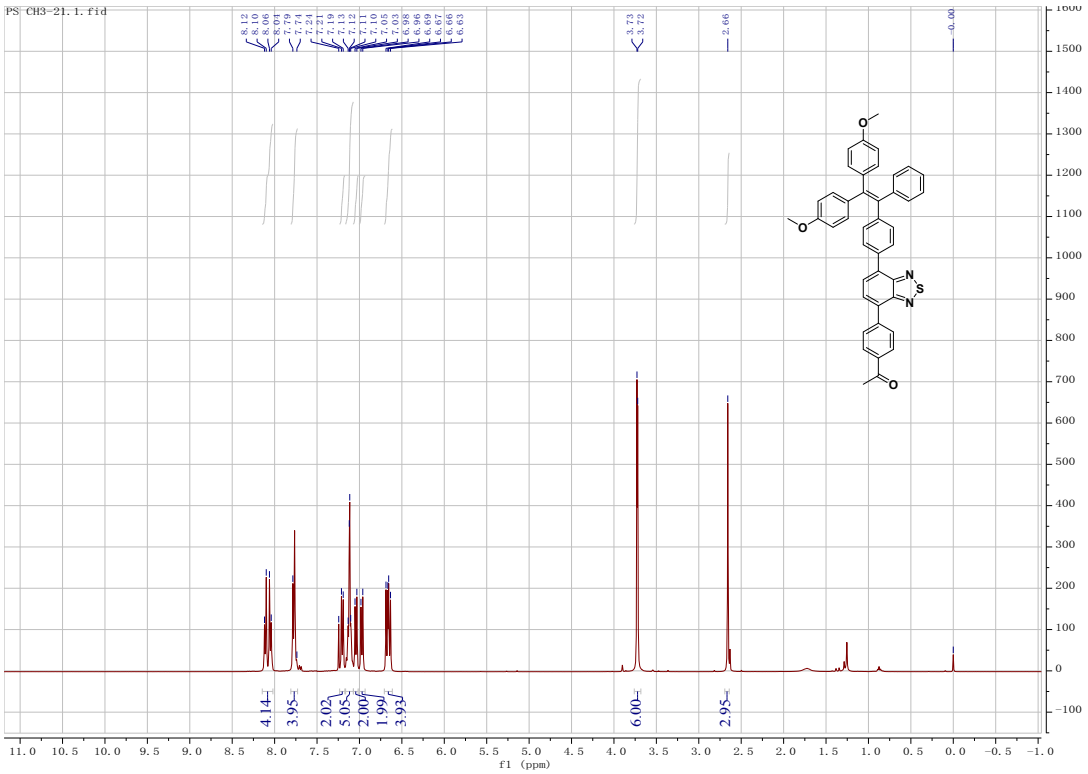


Figure S13. ^{1}H NMR spectrum of compound 9 in $CDCl_3$

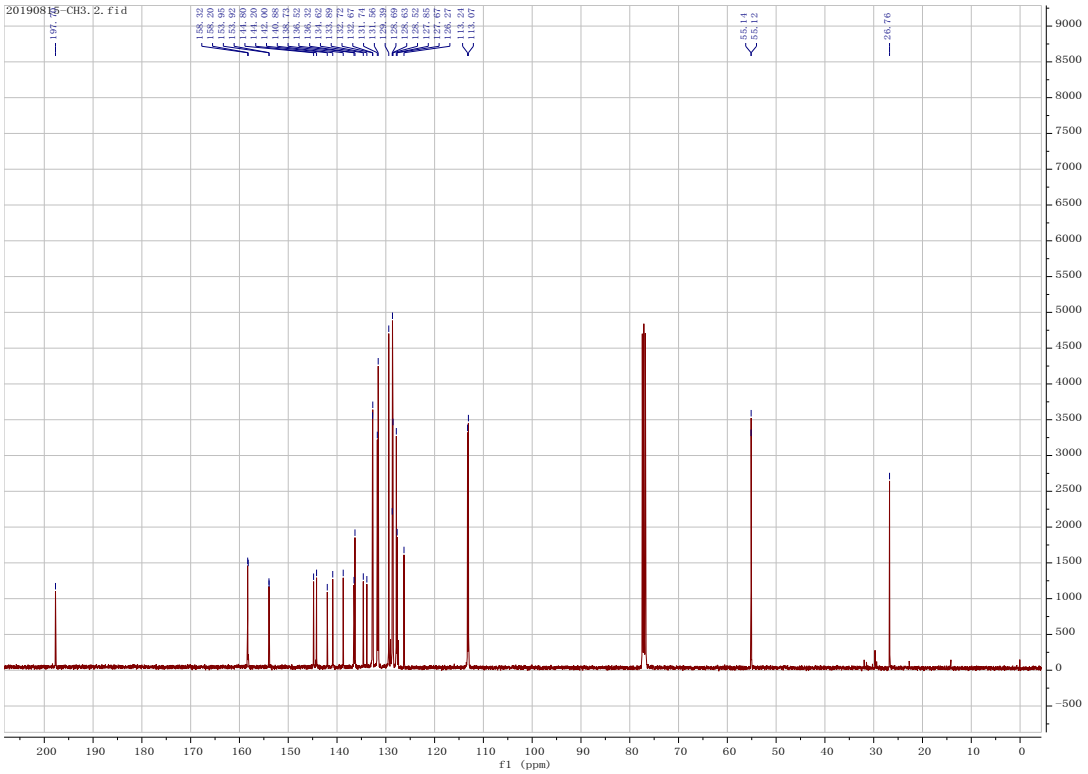


Figure S14. ^{13}C NMR spectrum of compound 9 in $CDCl_3$.

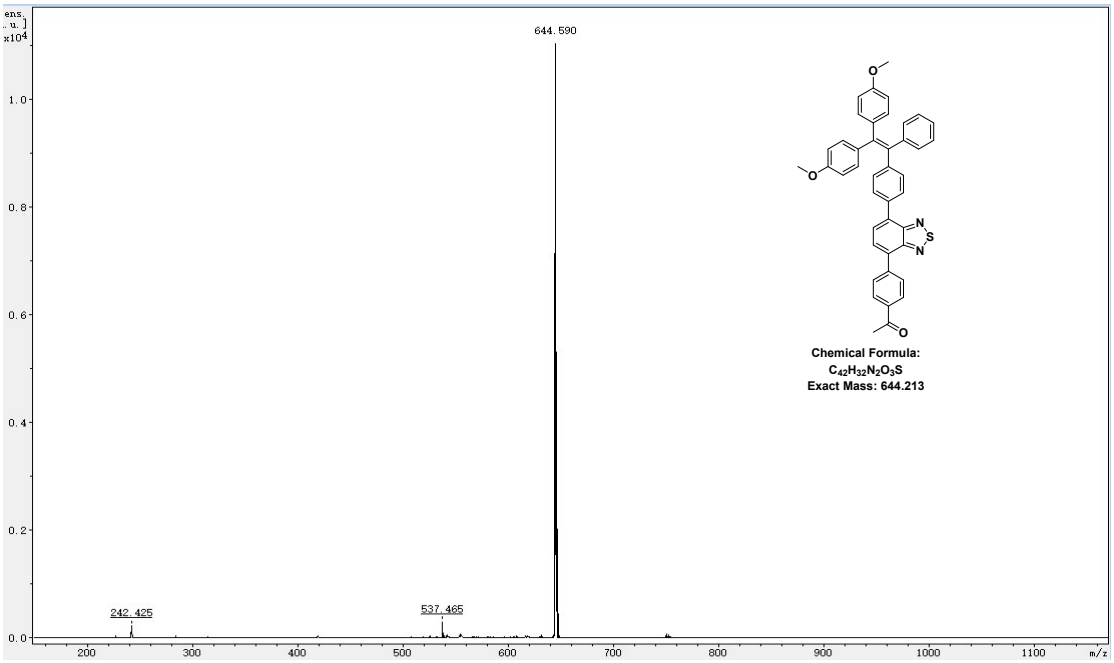


Figure S15. Mass spectrum of compound 9 in $CDCl_3$.

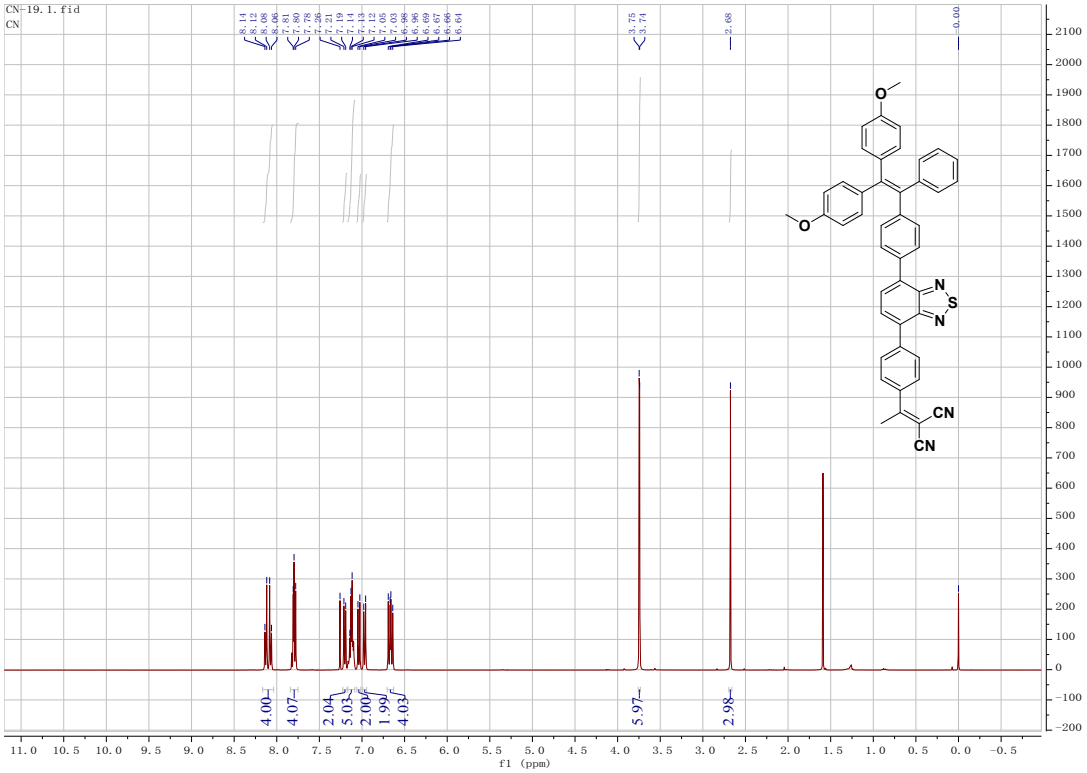


Figure S16. ^1H NMR spectrum of compound 10 in $CDCl_3$.

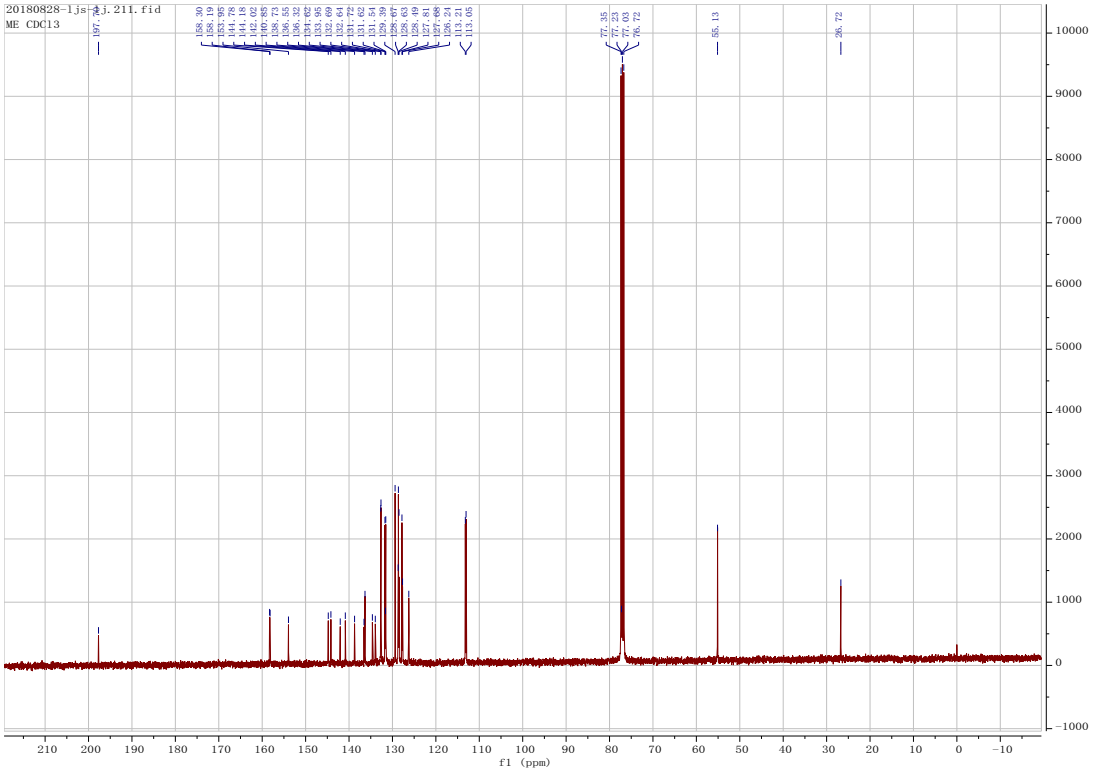


Figure S17. ^{13}C NMR spectrum of compound 10 in $CDCl_3$

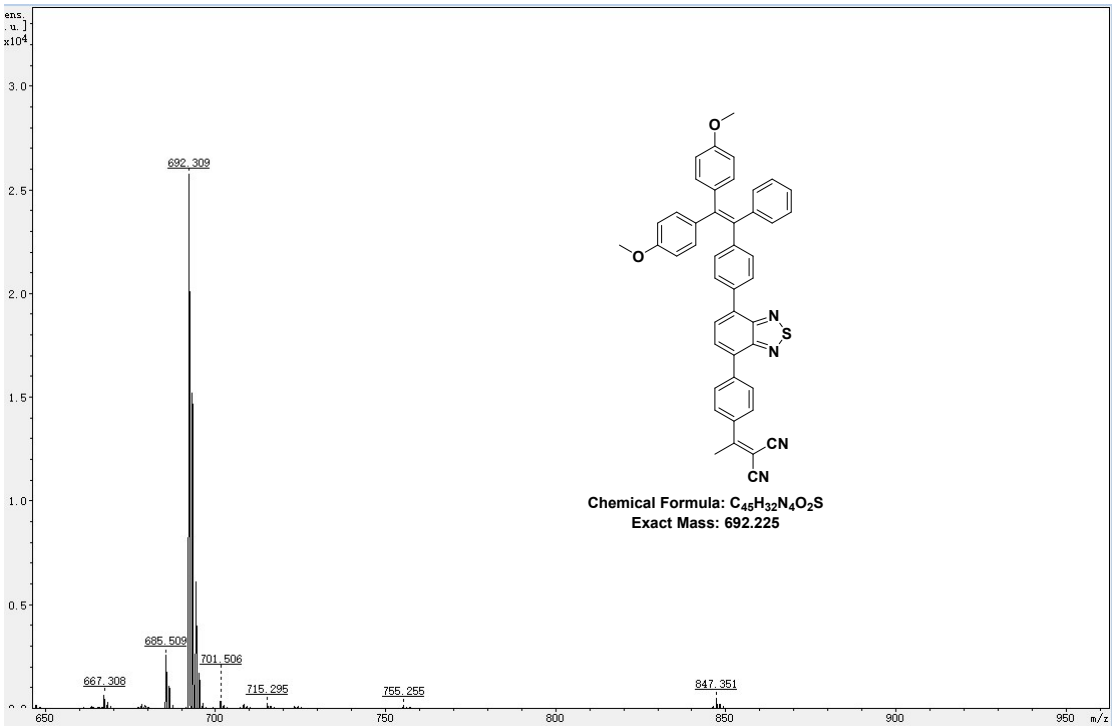


Figure S18. Mass spectrum of compound 10 in MeCN.

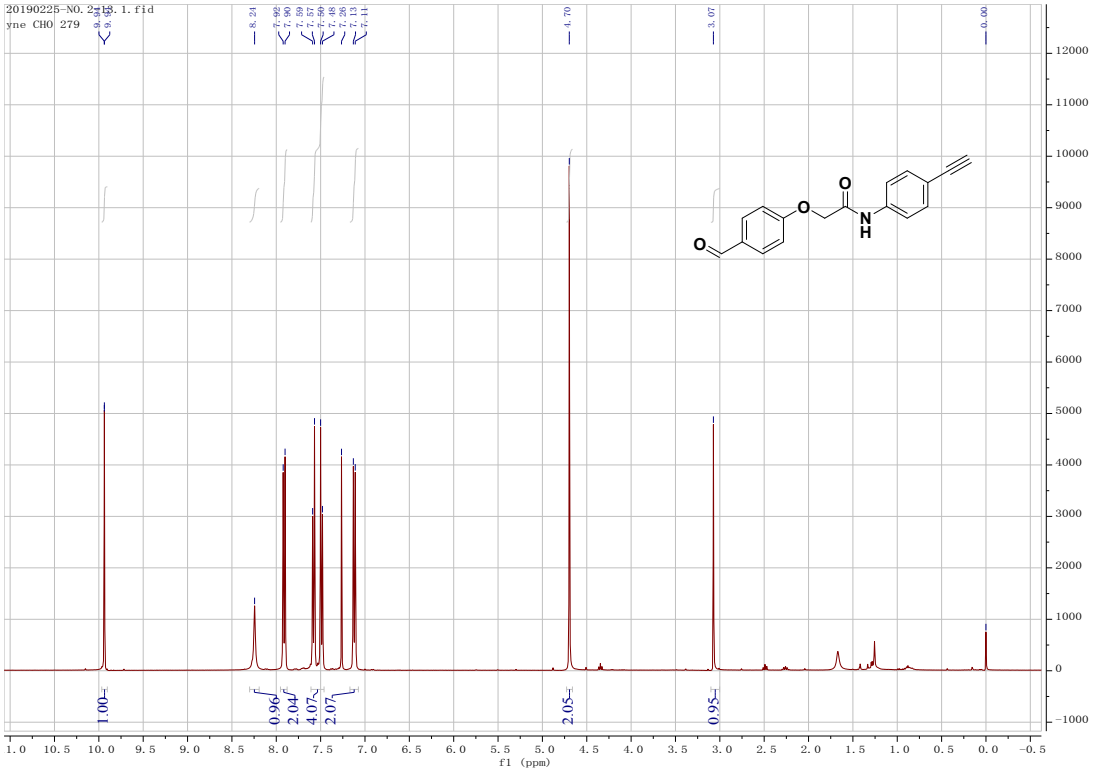


Figure S19. ^{1}H NMR spectrum of compound 13 in $CDCl_3$.

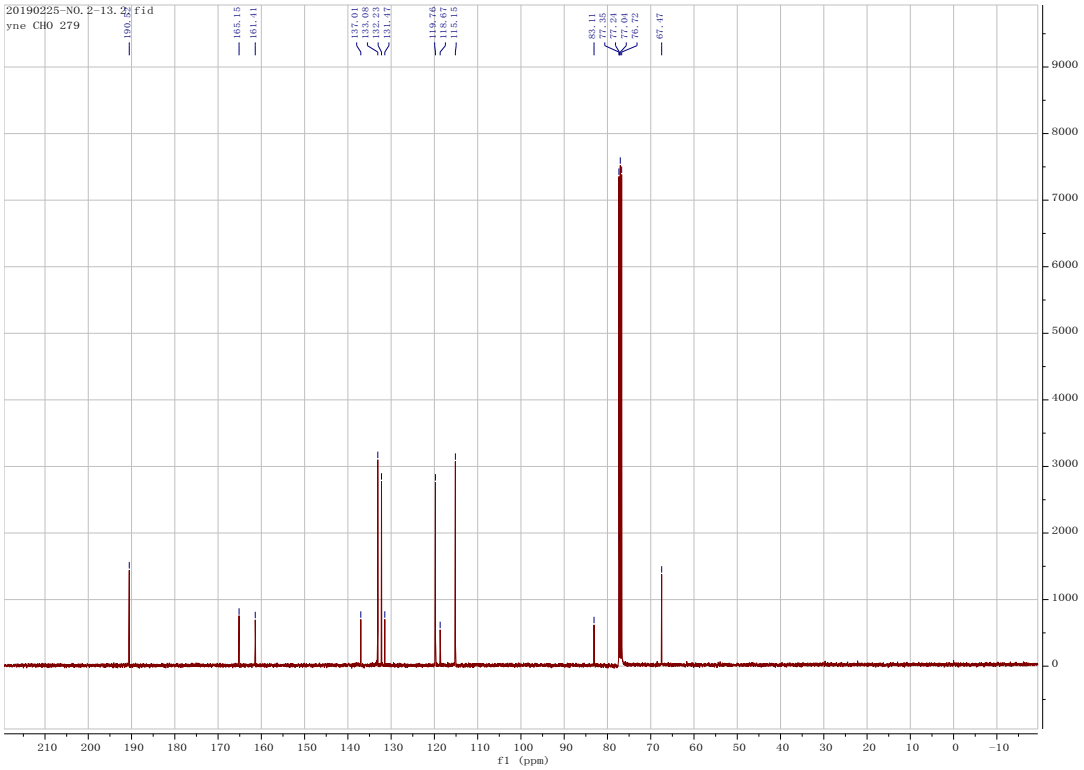


Figure S20. ^{13}C NMR spectrum of compound 13 in $CDCl_3$.

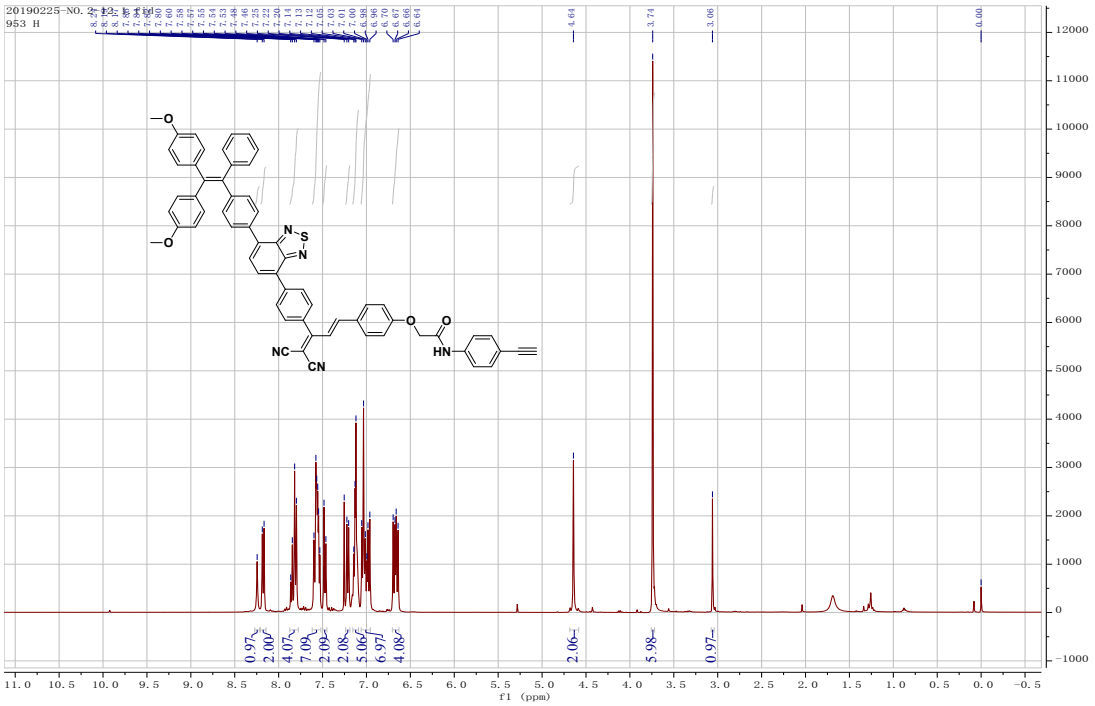


Figure S21. ^{1}H NMR spectrum of compound 14 in $CDCl_3$.

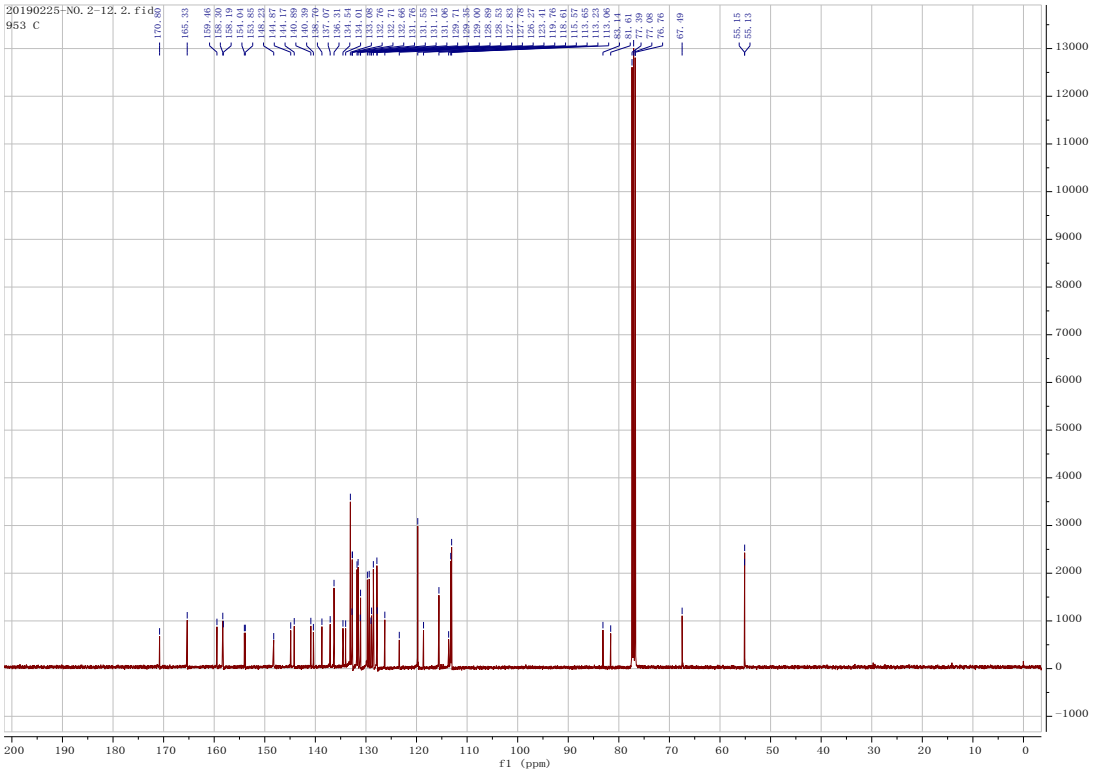


Figure S22. ^{13}C NMR spectrum of compound 14 in $CDCl_3$.

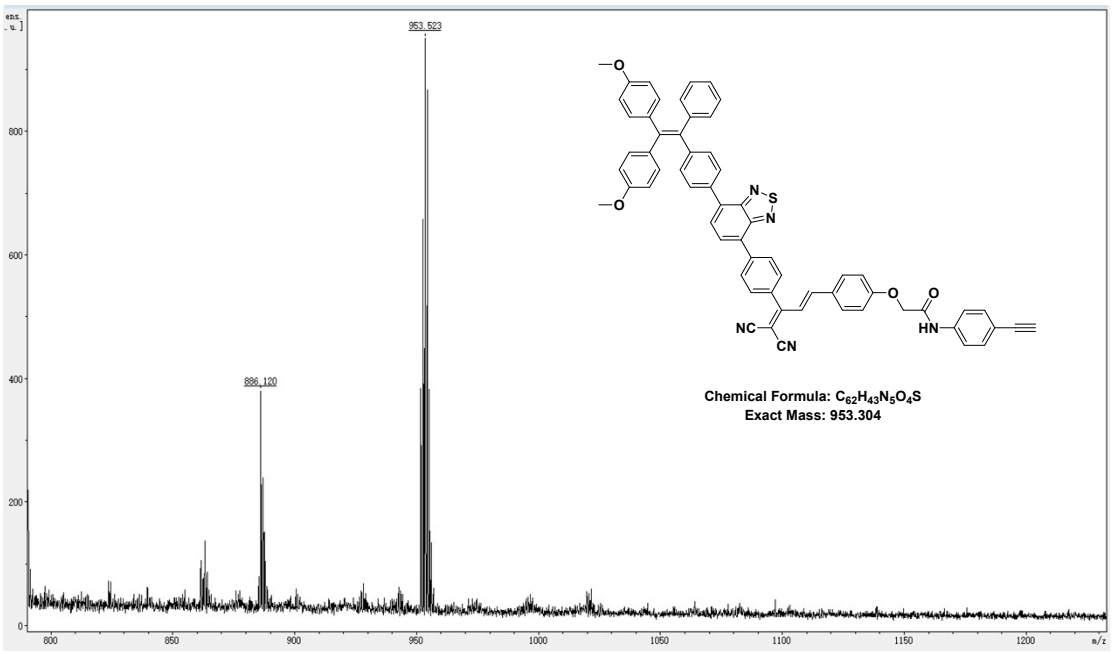


Figure S23. Mass spectrum of compound 14 in MeCN.

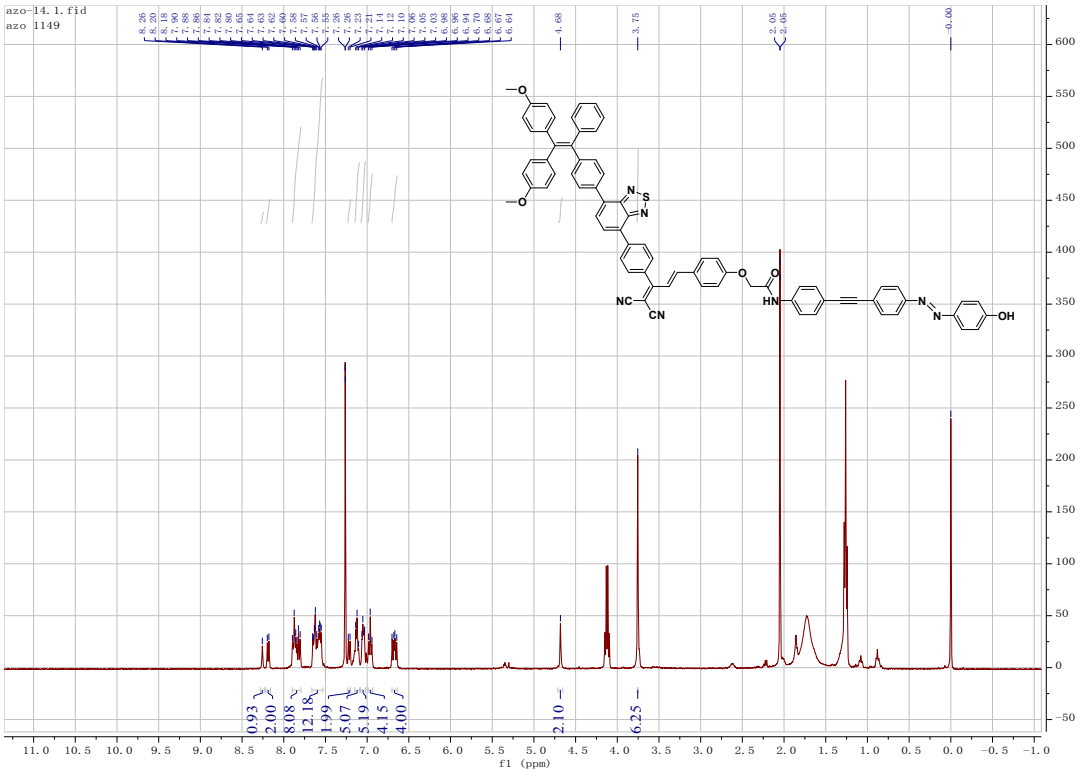


Figure S24. ^{1}H NMR spectrum of co mpound 16 in $CDCl_3$.

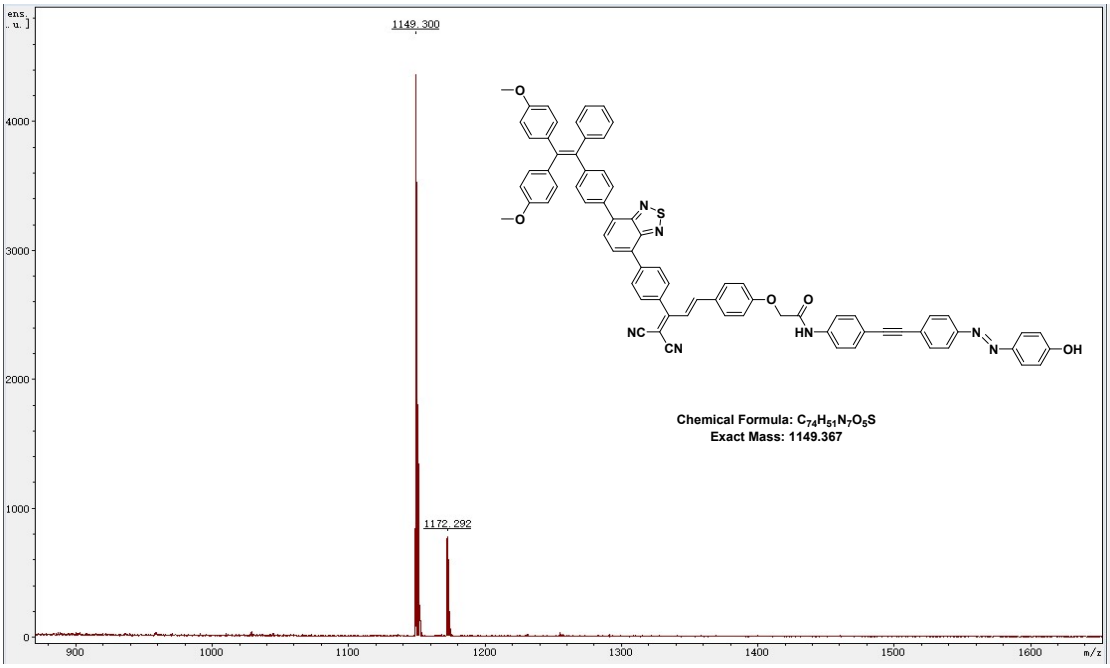


Figure S25. Mass spectrum of compound 16 in MeCN.

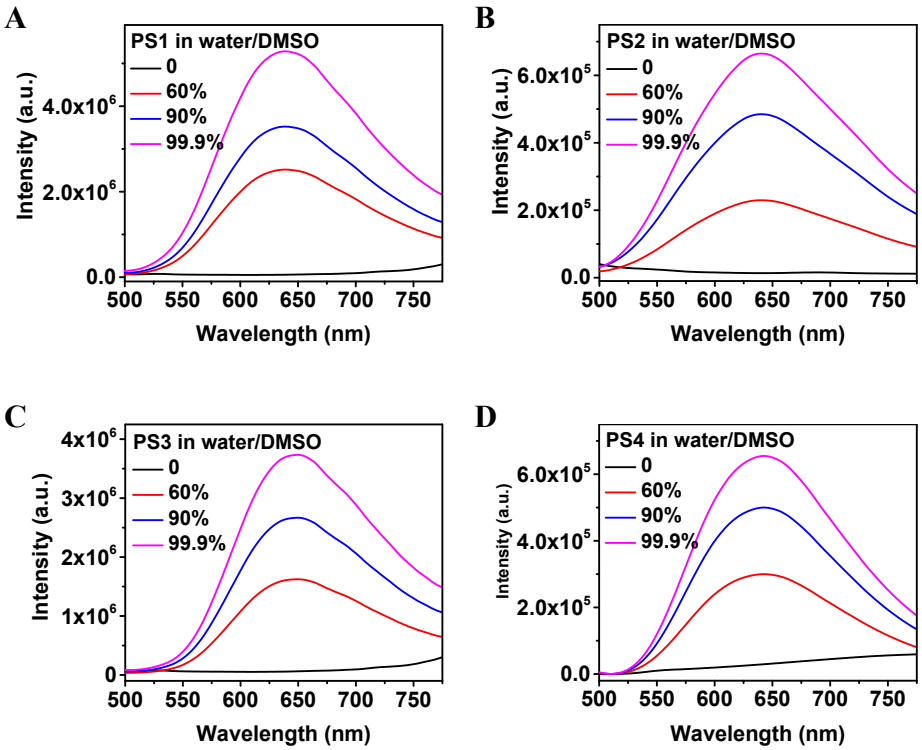


Figure S26. (A-D) The fluorescence emission spectra of PS1 (A), PS2 (B), PS3 (C) and PS4 (D) in the H_2O/DMSO mixture solution with different ratios. The concentration of PS1-PS4 is 10 μM. λ_{ex} = 410 nm for PS1 and PS2; λ_{ex} = 450 nm for PS3; λ_{ex} = 420 nm for PS4.

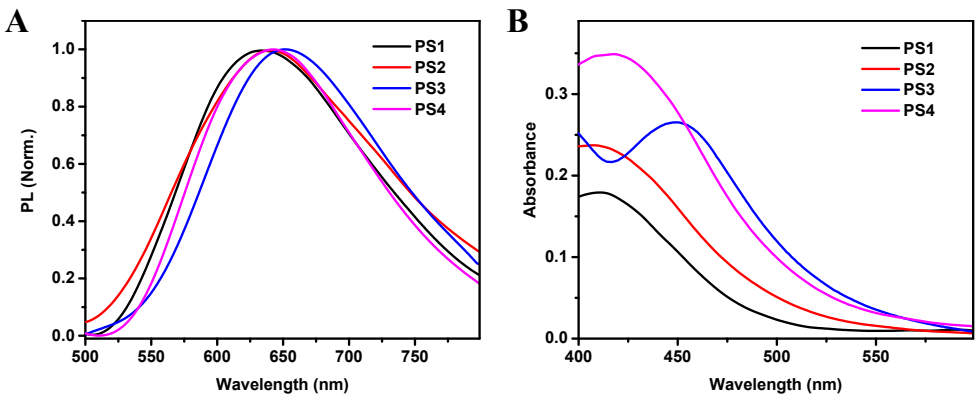


Figure S27. (A-B) Normalized fluorescence spectra (A) and absorption spectra (B) of the PS1-PS4 molecules (20 μM) in DMSO: water (v: v, 0.1: 99.9).

Table S1. Summary of photophysical properties of the PS1-PS4 molecules.

photosensitizer	λ_{max} (nm) absorption	λ_{max} (nm) emission	quantum yield (φ)	ε_{max} (10^3 $M^{-1}cm^{-1}$)
PS1	411	638	0.15	9.0
PS2	410	639	0.04	12
PS3	450	650	0.11	13
PS4	418	642	0.03	17

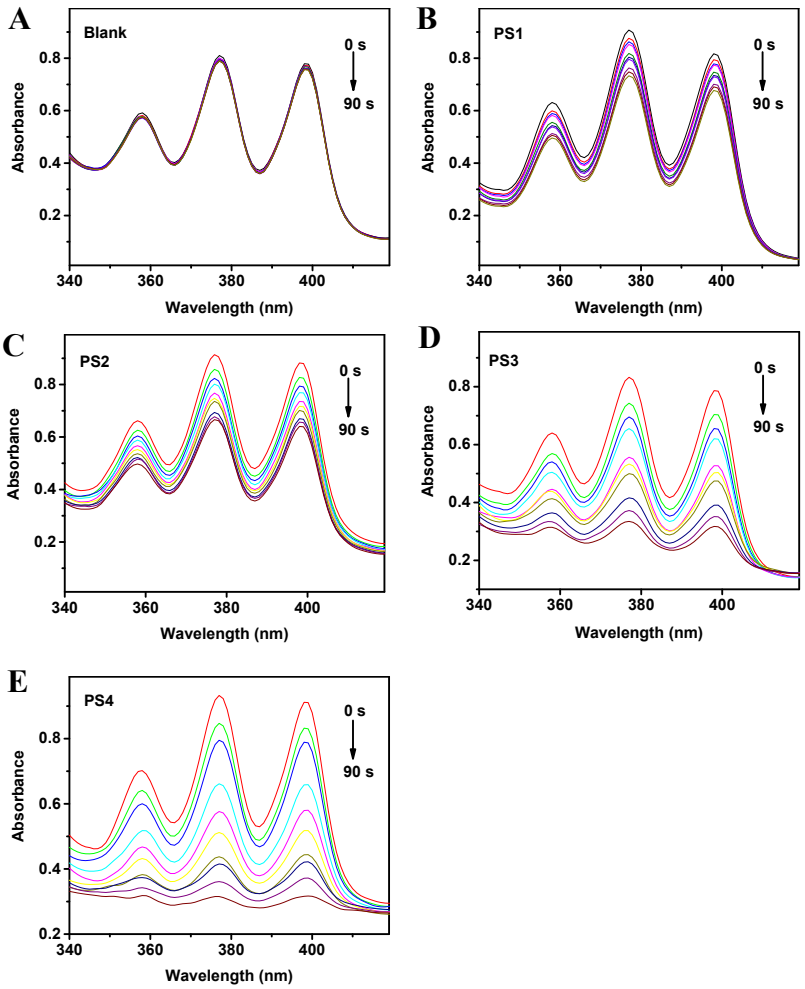


Figure S28. (A-E) Uv-vis spectra of the mixture solutions of ABDA (50 μM) and PS1-PS4 (10 μM) after being irradiated by using a white light source (50 mW cm^{-2}) for different time (A: ABDA; B: ABDA+PS1; C: ABDA+PS2; D: ABDA+PS3; E: ABDA+PS4).

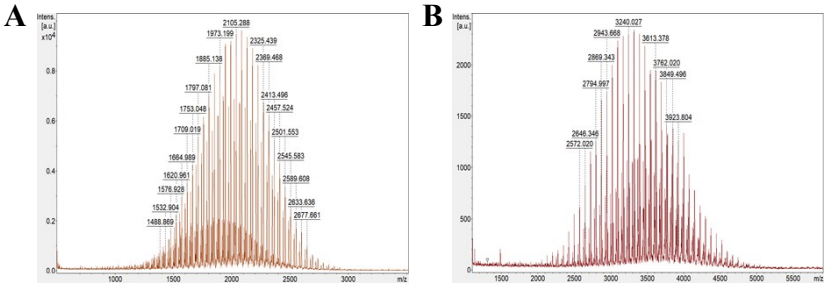


Figure S29. (A-B) MALDI spectra of (A) PEG-NHS and (B) PEG-azo-PS4 using DHB as the matrix.

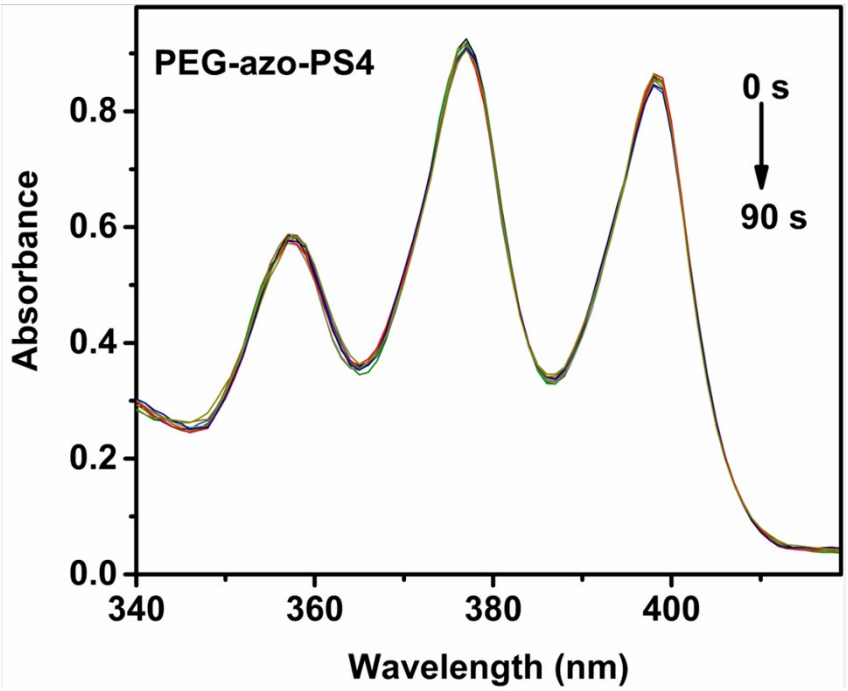


Figure S30. Uv-vis spectra of the mixture solution of ABDA (50 μM) and PEG-azo-PS4 (10 μM) after being irradiated by using a white light source (50 mW cm^{-2}) for different time.

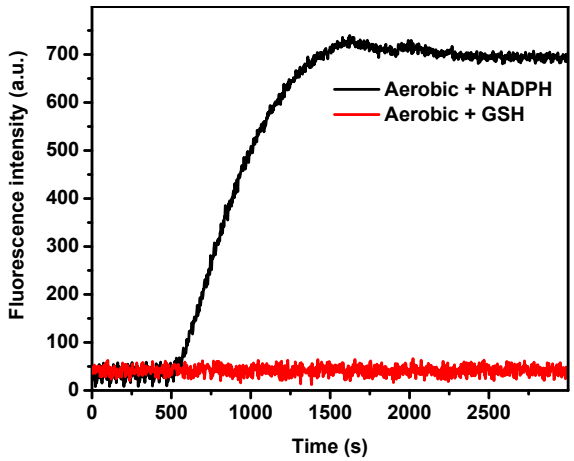


Figure S31. Time-based change of the fluorescence emission intensity at 650 nm of the mixture solution of PEG-azo-PS4 (2 μM) and rat liver microsomes (50 μL/3 mL) in PBS (pH 7.4) upon addition of NADPH (the final concentration is 50 μM, black curve) or GSH (the final concentration is 10 mM, red curve) under hypoxic conditions (λ_{ex} = 420 nm, λ_{em} = 650 nm).

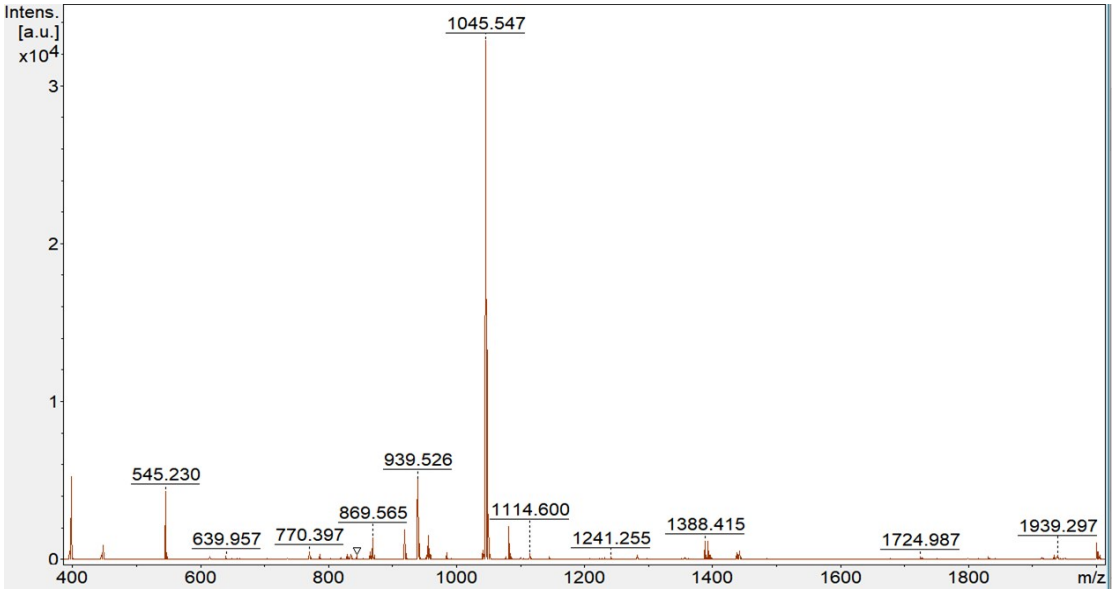


Figure S32. MALDI-TOF analysis of the cleaved product (AAPS in Scheme 1) of the PEG-azo-PS4 probes. To obtain AAPS, the hypoxia-treated mixture solution of PEG-azo-PS4 (2 μM), rat liver microsomes (50 μL/3 mL), and NADPH (the final concentration is 50 μM) in PBS (pH 7.4) was extracted with dichloromethane and characterized with MALDI-TOF.

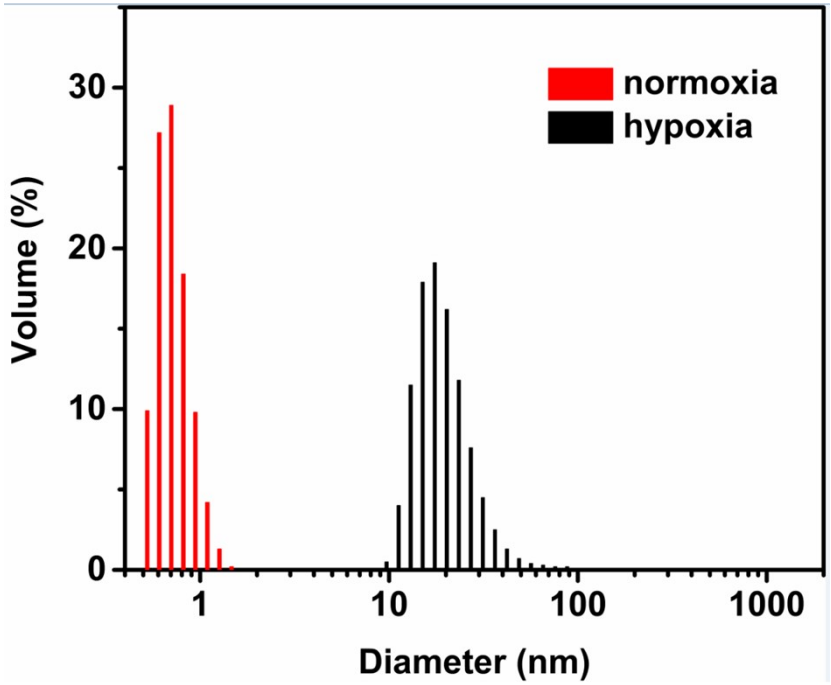


Figure S33. Size distribution for the "Fig. 1D" samples.

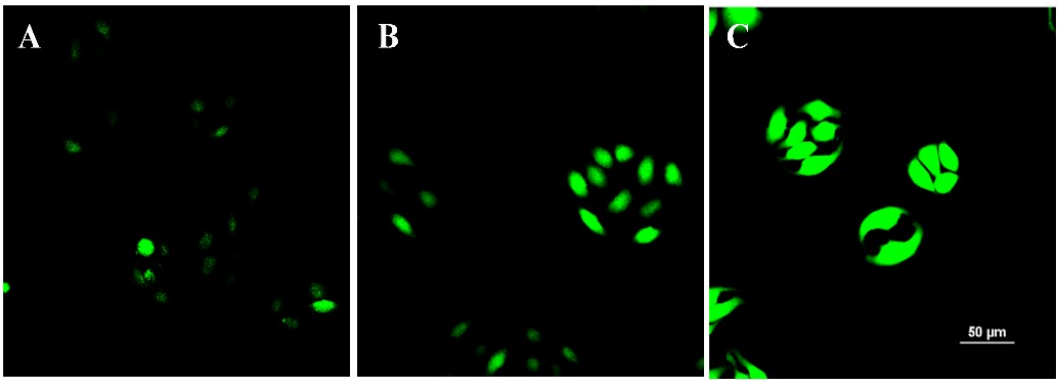


Figure S34. $^1O_2$-generation assay of the PEG-azo-PS4 probes in living cells. HepG-2 cells were first incubated with the PEG-azo-PS4 probes (5 μM) for 4 h at 37 °C under 0.1% O_2 concentrations, then incubated with DCFH-DA (10 μM) for 30 min under normoxia condition, and then irradiated by a white light (20 mW cm^{-2}) for 0 min (A), 2 min (B), and 5 min (C), respectively, and were then rinsed three times with PBS. The green fluorescence emission (505-525 nm) of DCFH in the cells was measured by a Nikon A1R confocal microscope system with 488 nm excitation.

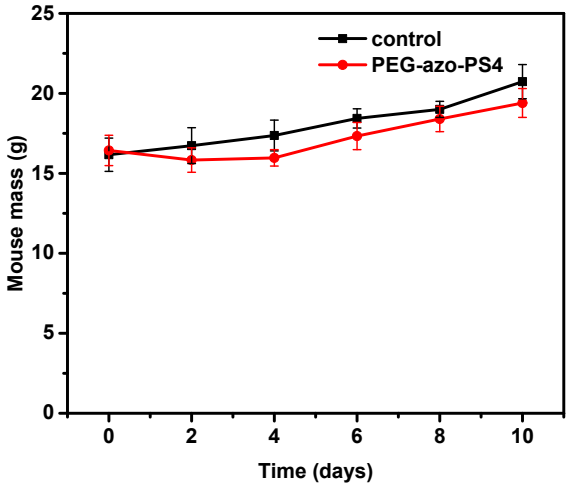


Figure S35. Body weight changes of mice for different groups. The mouse was injected in the tail vein with (a) the PEG-azo-PS4 (50 μL, 20 μM in PBS) or (b) PBS (50 μL) every two days.

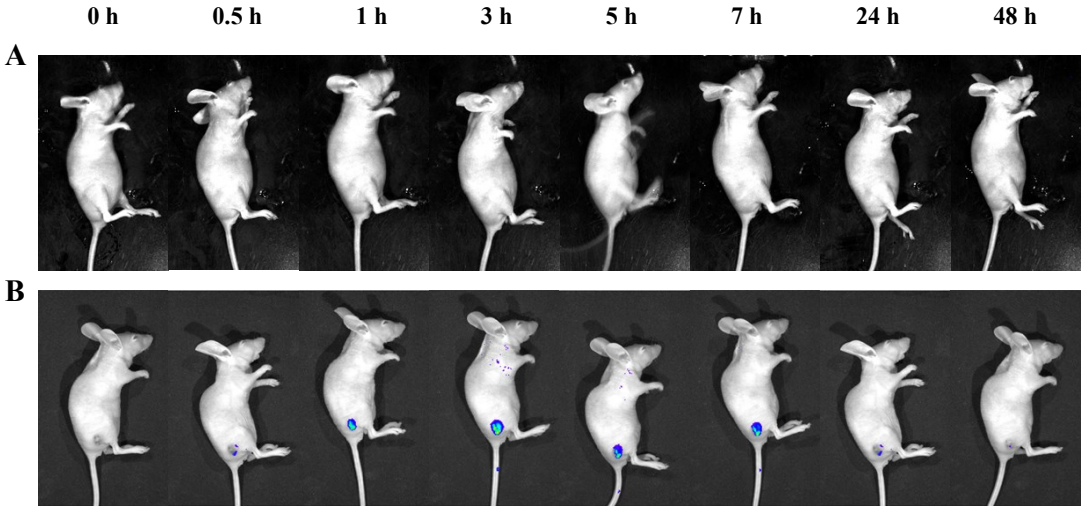


Figure S36. Time-dependent *in vivo* fluorescence images of (A) the healthy mice and (B) the tumor-bearing mice after being injected in the tail vein with the PEG-azo-PS4 probes (20 μM, 50 μL).

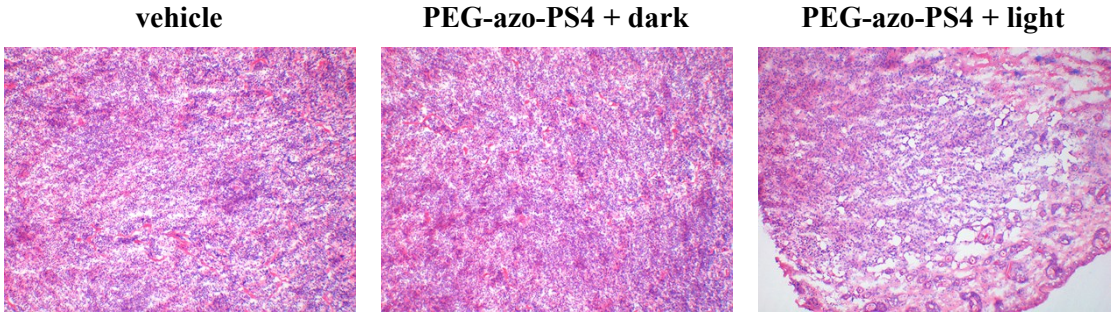


Figure S37. Hematoxylin and eosin (H&E) staining of the tumors' sections for different groups after 14 days treatment.

References

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3. D. Kumar, G. Khare, Beena, S. Kidwai, A. K. Tyagi, R. Singh and D. S. Rawat, *Medchemcomm*, 2015, **6**, 131-137.
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