A mitochondrial-targeted dual-functional aggregation-induced emission luminogen for intracellular mitochondria imaging and photodynamic therapy

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Materials and Methods

Materials

All chemicals for synthesis were purchased from Energy Chemical (Shanghai, China) Co., Ltd unless specified. Hydrochloric acid, thionyl chloride, dichloromethane, methanol, ethyl acetate and petroleum ether (60-90) were purchased from Sinopharm Chemical Reagent Co.,Ltd (Shanghai, China). Hoechst 33342 and Propidium Iodide (PI) were purchased from Sigma-Aldrich (St. Louise, MO, USA). Mito Tracker Red and CellROX® Deep Red Reagent were purchased from Invitrogen (Carlsbad, USA). Fetal bovine serum, RPMI 1640 media, PBS buffer, trypsin/EDTA, and

penicillin-streptomycin were supplied by Hyclone Laboratories, Inc. (Logan, Utah, USA); N-acetylcysteine (NAC) was purchased from Beyotime Biotechnology (Shanghai, China).

Preparation and characterization of TPP-TPEDCH

Reactions were monitored by analytical thin layer chromatography (TLC) silica gel plate (SGF254, Yucheng Chemical (shanghai) Co., Ltd). Flash chromatography purification was performed on Isolera One (Biotage, normal phase) and the Prep HPLC was conducted on LC-20AR (Shimadzu, C18, reverse phase). ^{1}H and ^{13}C NMR spectra were measured on Bruker Avance 500 MHz or 400 MHz. Electrospray ionization high resolution mass spectra (HR-MS) were obtained on an Orbitrap Thermo Q Exactive Plus spectrometer.

Photophysical properties

A 10 mL (1 mM) DMSO solution of TPP-TPEDCH was prepared for use in advance. It was diluted to 20 μM in various solvents, pH, interferences and water fractions prior to measurement. If not specified, the phosphate buffer saline (PBS, pH = 7.4) was used to replace water. UV-visible absorption spectra were recorded on a Shimadzu UV 2600 spectrometer. Fluorescence spectra in solutions were measured on Hitachi F-7100 spectrofluorometer using cells of 1 cm optical pathway. Spectroscopic measurements in solutions were conducted at 20°C in a temperature-controlled cell and all fluorescence spectra were corrected.

Photostability

MCF-7 cells (1×10^5 cells per dish) were cultured in 35 mm glass-bottom culture dishes. The cells were incubated with TPP-TPEDCH (20 μM) or commercial Mito-Tracker-Red-CMXRos-kit (Beyotime Co., Ltd., Shanghai, China), and then irradiated at the power dose of 2.3 W/cm^2 for 0, 2, 5, and 10 min, respectively. The fluorescence images were captured with an inverse fluorescent

microscope (Axio Observer 5, Zeiss, Germany), and was further processed with Image J software. The semi-quantitative analysis results of fluorescence intensity were also provided.

Mitochondria targeting specificity

MCF-7 cells were seeded on 35 mm glass-bottom culture dishes at a density of 1×10^6 cells per dish and incubated in a CO_2 incubator. After incubation for 12 h, the cells were incubated with TPP-TPEDCH (20 μM), and the fluorescent images at a series of incubation time points (0, 3, 6, 9 and 12 min) were acquired from a live-cell imaging workstation.

To study the intracellular distribution of TPP-TPEDCH, cellular imaging co-localization analysis was used. MCF-7 cells were cultured and seeded as described above. All MCF-7 cells were incubated with TPP-TPEDCH (20 μM) for 0.5 h firstly, then Mito-Tracker-Red-CMXRos-kit were used to label mitochondria, TPEDCH as control. After that, the medium was removed, and cells were fixed with methanol at -20°C, and then sealed. For the commercial probes, the emission channel was chosen in accord with the manufactures' instructions. The pictures of cellular imaging were obtained, and the co-localization analysis was further performed.

Biosafety

Cell survival after treated with TPP-TPEDCH was investigated via CCK-8 assay. Briefly, MCF-7, HepG2 and A549 cells (5×10^3 cells per well) were seeded into 96-well plates and incubated with various concentrations of TPP-TPEDCH (0, 5, 10, 20, 40, 60, and 100 μM). After a further incubation of 24 h, cytotoxicity was measured using a CCK-8 assay as our previous study.

The hemolysis assay was performed to assess the safety of TPP-TPEDCH intravenous administration. Blood from healthy rabbits was collected in ethylenediamine tetraacetic acid (EDTA) coated tubes. The red blood cells (RBCs) were collected and washed with sterile isotonic 0.9% saline by centrifugation at 1500 rpm for 5 min for three times. The purified 2% RBCs suspension (1 mL) was mixed with dd water, sterile saline, or TPP-TPEDCH solution at various concentrations (10, 40 and 100 μg/mL), respectively. Deionized water and sterile isotonic saline

were used as positive control and negative control, respectively. All samples were incubated at 37°C for 2 h, respectively, and then centrifuged at 4000 rpm for 2 min. The absorbance values of the supernatants were measured at 545 nm using an ultraviolet (UV)-visible spectroscopy (UV-2910, Hitachi Ltd., Tokyo, Japan). The percent hemolysis of RBCs was calculated using the following formula:

$$\text{Hemolysis ratio (\%)} = ([A_{sample} - A_{negative\ control}]/[A_{positive\ control} - A_{negative\ control}])\times 100\%$$

Healthy mice were intravenously injected with TPP-TPEDCH at the dose of 10 mg/kg every three days, saline-treated mice as control. At day 21 after intravenous administration, blood samples were collected for testing. The major organs, including heart, liver, kidney, lung and spleen, were harvested for H&E staining.

ROS detection *in vitro*

The intracellular ROS generation profile was evaluated under different conditions: including with or without TPP-TPEDCH (20 μM), and with or without laser irradiation. Acetylcysteine, as the ROS scavenger, was also investigated here at a pretreatment concentration of 50 μM to explore the ROS generation profile. The details of the incubation parameters are listed in Figure 4a. After treatment, the cells were incubated with CellROX® Deep Red Reagent (Invitrogen®) to detect ROS level, and then observed under an inverted fluorescent microscope and the fluorescence intensities were further calculated by the Image J software.

Cytotoxicity

MCF-7, HepG2, or A549 cells were incubated with TPP-TPEDCH at different concentrations (0, 1, 5 or 20 μM), followed by laser irradiation (808 nm, 3 min, 2.3 W/cm^2). Cells did not receive any treatments were used as controls. After irradiation, cell viability was measured using CCK-8 method according to the manufactures' instructions. The effect of laser irradiation duration on the PDT effect was also studied. The MCF-7, HepG2, or A549 cells were incubated with TPP-TPEDCH

(20 μM), followed by laser irradiation (808 nm, 2.3 W/cm^2) for different times (0, 1, 2, 3 or 4 min).

The tumor cell killing effect of the PDT effect mediated by TPP-TPEDCH was further explored by PI cell staining and Hoechst 33342/PI cell staining (Solarbio technology Co. Limited, Beijing, China). The MCF-7 cells were incubated with TPP-TPEDCH at different concentrations (0, 1, 5 or 20 μM), and then irradiated with laser (808 nm, 3 min, 2.3 W/cm^2). The cells were further stained by Hoechst 33342/PI (352/488 nm) to observe the cellular apoptosis or necrosis situation, and observed by an inverted fluorescent microscope.

***In vivo* antitumor activity**

All animal experiments were conducted in accordance with the ARRIVE Guideline and the guidelines of the US National Institutes of Health for the care and use of laboratory animals, and all animal experiments were approved by the Animal Ethics Committee of Ningbo University. A tumor model in BALB/c nude mice were established by orthotopically injecting MCF-7/Luc cells (1×10^5) into the breast pads. The course of treatment was shown in Figure 4a, and saline-treated mice was used as control. The growth of breast tumor was monitored using an IVIS spectrum imaging system (Caliper, PerkinElmer) after the intraperitoneal injection of D-luciferin (10 mg/mL, 200 μL). After the orthotopical tumor formed, TPP-TPEDCH was intratumorally injected and further irradiated by NIR laser (2.3 W/cm^2 for 3 min), saline-treated mice as control. After treatment, the mice were sacrificed, and the tumors were harvested, weighted, fixed in 4% paraformaldehyde, and sectioned for hematoxylin and eosin (H&E) staining. In addition to the H&E staining, laser-triggered intratumoral ROS generation was evaluated using CellROX® Deep Red Reagent. Collected tumors tissues were embedded, cut into slices, incubated with CellROX® Deep Red Reagent and Hoechst 33342, and then observed immediately by an inverted fluorescent microscope. Adjacent tumor slices were also collected from the mice in the two groups, stained with Ki67 and TUNEL to further study the PDT effect *in vivo*.

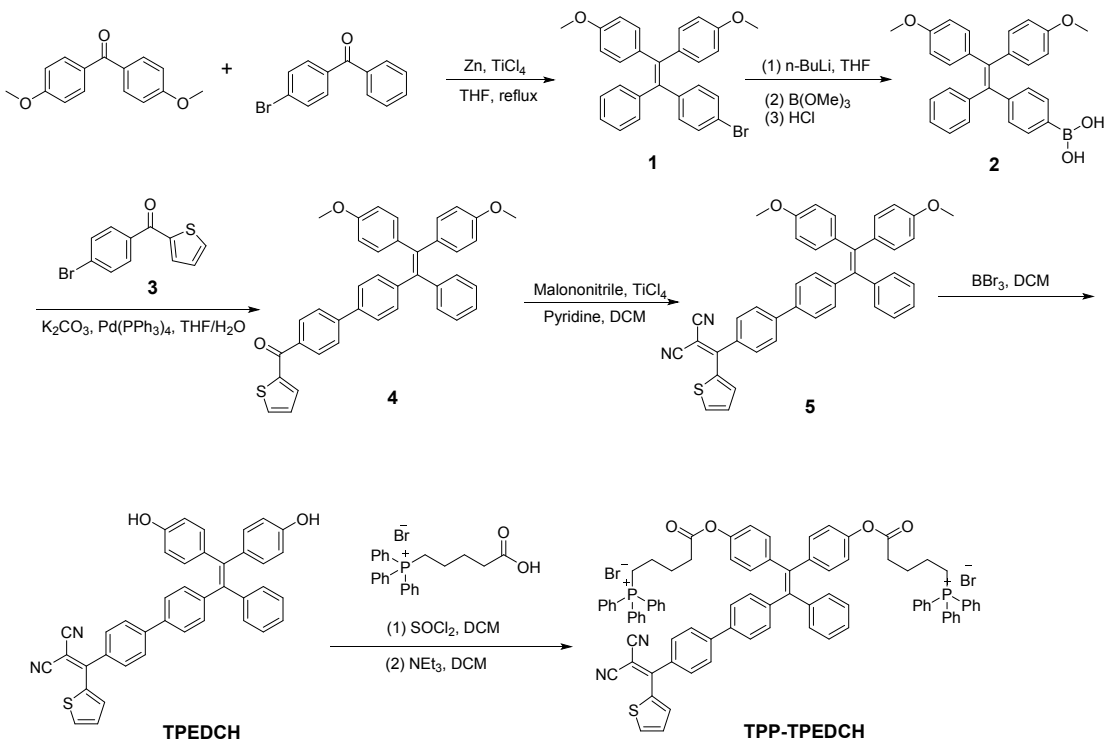
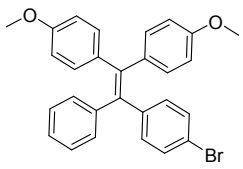


Figure S1. The synthesis route of TPP-TPEDCH.

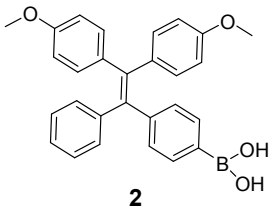
Experimental Procedure：



1

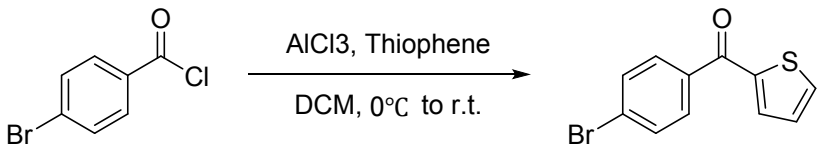
To a solution of 4,4'-dimethoxybenzophenone (1.96 g, 8.1 mmol) in dry THF (40 mL) was added 4-bromobenzophenone (2.74 g, 10.5 mmol) and zinc powder (3.05 g, 47 mmol). The mixture was stirred under Ar atmosphere at -78℃ with the addition of titanium tetrachloride (2.5 mL) drop-wisely. Then the mixture was warmed up to room temperature and refluxed for 16 h. The reaction was monitored by thin layer chromatography (TLC, EA/PE = 1/20, v/v). The mixture was cooled to room temperature until the consumption of 4,4'-dimethoxybenzophenone. Then a saturated $NaHCO_3$ solution (50 mL) was added slowly under ice-water bath. The resulting mixture was extracted with ethyl acetate (80 mL × 3). All the organic layers were combined, washed with water (100 mL × 2) and brine (60 mL × 3), dried over anhydrous Na_2SO_4 and concentrated to dry. The crude product was purified by flash column chromatography on a silica gel (EA/PE = 1/50-1/10)

to yield compound 1 as a light yellow solid (0.99 g, 26.1%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.24 – 7.18 (m, 2H), 7.13 – 7.07 (m, 3H), 7.04 – 6.97 (m, 2H), 6.91 (dddd, *J* = 18.2, 10.0, 5.7, 1.8 Hz, 6H), 6.64 (ddd, *J* = 18.0, 8.7, 1.7 Hz, 4H), 3.76 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 158.27, 158.18, 143.78, 143.30, 140.77, 137.90, 136.01, 135.92, 133.04, 132.55, 132.51, 131.33, 130.84, 127.80, 126.29, 120.01, 113.20, 113.02, 77.22, 55.11, 55.08. HR-MS (ESI): m/z $C_{28}H_{23}O_2Br$, calcd for $[M+H]^+$ 471.0954, found 471.0953.



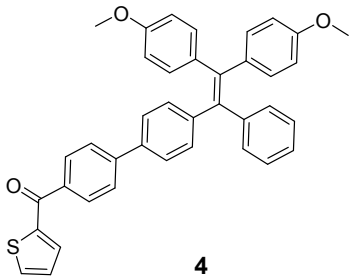
2

To a solution of compound 1 (0.94 g, 2.0 mmol) in dry THF (20 mL) was added n-butyllithium (2.5 M in hexane, 1.30 mL, 3.2 mmol) at -78℃ under Ar atmosphere. The mixture was stirred at at -78℃ for 3 h followed by the addition of trimethyl borate (0.45 mL, 4.0 mmol) at the same temperature. Then the mixture was warmed up to room temperature and stirred for another 4 h. The reaction was quenched by HCl solution (3 M, 10 mL) and the resulting mixture was stirred for 8 h. Then the mixture was partitioned between ethyl acetate (50 mL) and brine (100 mL). The organic phase was separated, washed with water (50 mL × 2) and brine (80 mL × 2), dried over anhydrous Na_2SO_4 and concentrated to dry. The residue was purified by flash column chromatography on a silica gel (EA/PE = 1/10-1/2) to yield compound 2 as a white solid (0.34 g, 39%).^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.89 (d, *J* = 7.8 Hz, 1H), 7.21 – 7.05 (m, 6H), 7.04 – 6.99 (m, 2H), 6.98 – 6.90 (m, 4H), 6.64 (t, *J* = 7.5 Hz, 4H), 3.74 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 158.18, 144.01, 136.15, 136.09, 134.98, 132.87, 132.62, 132.58, 131.39, 131.37, 131.06, 130.97, 127.75, 126.19, 113.09, 113.01, 77.22, 55.10, 55.08.



3

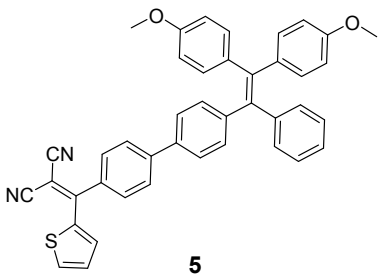
compound 5 is prepared according to literature with a slight modification[1]. In a three-neck round bottom flask was added 4-bromobenzoyl chloride (1.32 g, 6 mmol) and $AlCl_3$ (1.04 g, 7.8 mmol) in dry CH_2Cl_2 (10 ml). The reaction was stirred at 0°C under Ar atmosphere. Then a solution of thiophene (0.50 g, 6 mmol) in dry CH_2Cl_2 (10 ml) was added dropwise and continued to stir at room temperature for 3 h. After the reaction, crushed iced was added to quench the reaction. The resulting mixture was acidified with 37% HCl (0.6 ml) and extracted with CH_2Cl_2 (40 ml × 2). All the organic layers were combined and wash washed with water (40 mL × 2) and brine (50 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was purified by flash column chromatography on a silica gel (EA/PE = 1/30-1/10) to yield compound 2 as a yellow solid (1.48 g, 93%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.78 – 7.69 (m, 3H), 7.68 – 7.58 (m, 3H), 7.18 (dd, *J* = 5.0, 3.8 Hz, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 187.06, 143.18, 136.86, 134.79, 134.58, 131.75, 130.71, 128.07, 127.27.



4

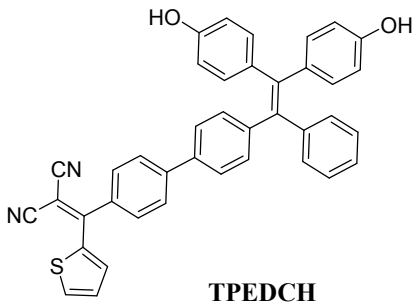
To the solution of compound **2** (160 mg, 0.37 mmol) and compound **3** (197 mg, 0.74 mmol) in THF/H_2O (v/v = 3/1, 8 mL) was added anhydrous K_2CO_3 (511 mg, 3.7 mmol) and tetrakis(triphenylphosphine) palladium(0) (21 mg, 0.0185 mmol). The mixture was stirred at 62°C and refluxed for 24 h until the consumption of compound **2**. Then ethyl acetate (30 mL) and water (30 mL) were added and the organic phase was separated, washed with water (50 mL) and brine (30 mL × 2), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on a silica gel (EA/PE = 1/10-1/2) to yield compound 4 as a light yellow solid (130 mg, 61%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.95 – 7.89 (m, 2H), 7.72 (dd, *J* = 4.9, 1.1 Hz, 1H), 7.71 – 7.66 (m, 3H), 7.45 – 7.38 (m, 2H), 7.17 (dd, *J* = 5.0, 3.8 Hz, 1H), 7.16 – 7.09 (m, 5H), 7.07 (dd, *J* = 8.0, 1.7 Hz, 2H), 7.01 – 6.93 (m, 4H), 6.72 – 6.61 (m, 4H), 3.75 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 158.24, 158.15, 144.68, 144.49, 140.72, 138.52, 137.10, 136.54, 136.23, 134.58, 134.04, 132.63, 132.61, 132.02, 131.45, 129.82, 127.94,

127.79, 126.73, 126.41, 126.24, 113.15, 113.02, 55.12, 55.10. HR-MS (ESI): m/z $C_{39}H_{30}O_3S$, $[M+H]^+$ calcd 579.1988, found 579.1981; $[M+Na]^+$ calcd 601.1808, found 601.1806.



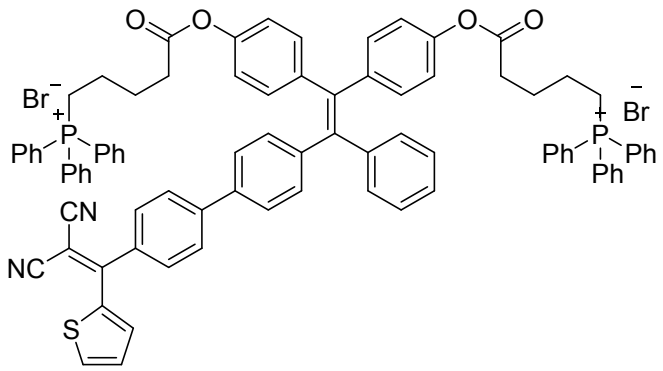
5

To a solution of compound **4** (130 mg, 0.22 mmol) and malononitrile (44 mg, 0.66 mmol) in dry CH_2Cl_2 (5 mL) was added titanium tetrachloride (85 μL, 0.77 mmol) dropwise at 0℃ under Ar atmosphere. After the reaction mixture was stirred for 30 min, pyridine (62 μL, 0.77 mmol) was injected and stirred for another 30 min. The mixture was refluxed at 40℃ for 5 h and then cooled to room temperature. Water (10 mL) was added to quench the reaction and the solution changed from dark to brown, then the mixture was extracted with CH_2Cl_2 (15 mL × 2). All the organic layers were combined and washed with water (20 mL) and brine (20 mL × 2), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on a silica gel (EA/PE = 1/10-1/2) to yield compound **5** as an orange solid (120 mg, 87%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.81 (dd, *J* = 10.6, 4.5 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.51 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 4.6 Hz, 1H), 7.16 – 7.09 (m, 5H), 7.08 – 7.04 (m, 2H), 6.97 (dd, *J* = 15.5, 8.4 Hz, 4H), 6.65 (t, *J* = 9.3 Hz, 4H), 3.75 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 158.27, 144.79, 144.07, 140.85, 138.69, 138.44, 136.60, 136.40, 136.18, 136.00, 132.64, 132.61, 132.09, 131.44, 130.31, 128.95, 127.81, 126.94, 126.38, 126.27, 113.16, 113.03, 55.13, 55.10.



TPEDCH

To the solution of compound **5** (115 mg, 0.18 mmol) in dry CH_2Cl_2 (6 mL) was added boron tribromide (1.0 M in dry CH_2Cl_2, 0.54 mmol) under ice bath. Then the reaction mixture was warmed to room temperature and stirred for 5 h. The reaction was quenched by water (10 mL) under ice bath and extracted with CH_2Cl_2 (10 mL × 2). The organic layers were collected and washed with water (20 mL) and brine (10 mL × 2), dried over anhydrous $MgSO_4$ and concentrated in vacuo to obtain an orange-red solid (105 mg, 95%). The product **TPEDCH** was used in the next step without further purification. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.85 – 7.77 (m, 2H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.24 (t, *J* = 4.5 Hz, 1H), 7.12 (dt, *J* = 7.5, 3.5 Hz, 5H), 7.07 – 7.03 (m, 2H), 6.92 (dd, *J* = 14.6, 8.6 Hz, 4H), 6.58 (dd, *J* = 9.8, 8.5 Hz, 4H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 154.31, 154.21, 144.68, 144.48, 143.97, 140.71, 138.67, 138.57, 136.45, 136.28, 136.07, 132.82, 132.79, 132.07, 131.41, 130.32, 128.97, 127.81, 126.95, 126.40, 126.32, 114.74, 114.60. HR-MS (ESI): m/z $C_{40}H_{26}N_2O_2S$, M calcd 598.1715, found 598.1711.



TPP-TPEDCH

To a solution of (4-carboxybutyl)triphenylphosphonium bromide (222 mg, 0.5 mmol) in dry CH_2Cl_2 (10 mL) was added thionyl chloride (0.5 mL) under Ar atmosphere. The reaction mixture was stirred at 40℃ and refluxed for 3 h. Then the mixture was cooled to room temperature and evaporated to dryness. The resulting mixture was dissolved in dry CH_2Cl_2 (5 mL), followed by adding CH_2Cl_2 solution of compound **TPEDCH** (60 mg, 0.1 mmol) and triethylamine (0.5 mL) dropwise. The mixture was raised to room temperature and reacted for another 8 h until the consumption of **TPEDCH**. The resulting mixture was quenched by water and extracted with CH_2Cl_2 (20 mL x 3). All the organic layers were combined, washed with water (20 mL × 3) and brine (20 mL × 2), dried over anhydrous $NaSO_4$ and concentrated to dry. The residue was purified by flash

column chromatography on a silica gel (CH_3OH/ CH_2Cl_2 = 1/99-1/10) to obtain a red solid. The product was further purified by HPLC (C18 column, gradient from 50% to 90% acetonitrile/water in 25 min) to yield compound **TPP-TPEDCH** as a red solid (33 mg, 23%).^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.90 – 7.81 (m, 14H), 7.74 – 7.68 (m, 21H), 7.56 – 7.43 (m, 5H), 7.29 – 7.27 (d, *J* = 4.4 Hz, 1H), 7.20 – 7.12 (m, 4H), 7.11 – 6.95 (m, 6H), 6.82 – 6.61 (m, 4H), 2.70 – 2.64 (m, 4H), 2.26 (t, *J* = 7.6 Hz, 4H), 1.82 – 1.76 (m, 4H), 1.41 – 1.15 (m, 4H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.60, 136.32, 135.17, 135.03, 135.02, 133.72, 133.71, 133.64, 133.53, 133.48, 133.40, 132.26, 132.06, 132.01, 132.00, 131.33, 130.64, 130.57, 130.55, 130.47, 130.45, 130.32, 128.58, 128.48, 127.03, 117.95, 77.26, 58.37, 21.85, 18.43. ^{31}P NMR (162 MHz, Chloroform-d) δ 24.2. HR-MS (ESI): $[C_{86}H_{70}N_2O_4SP_2]^{2+}[Br_2]^{2-}$, z = 2, m/z calcd 644.2260, found 644.2251.

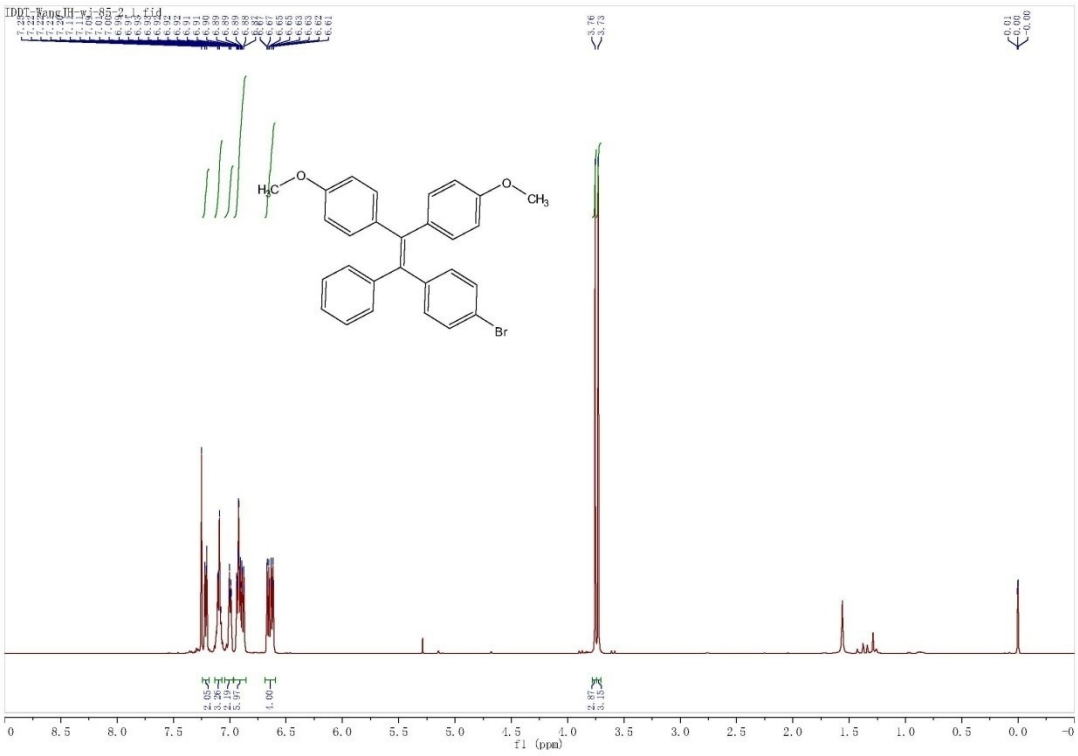


Figure S2. ^{1}H NMR spectrum of compound 1 (500 MHz, Chloroform-d).

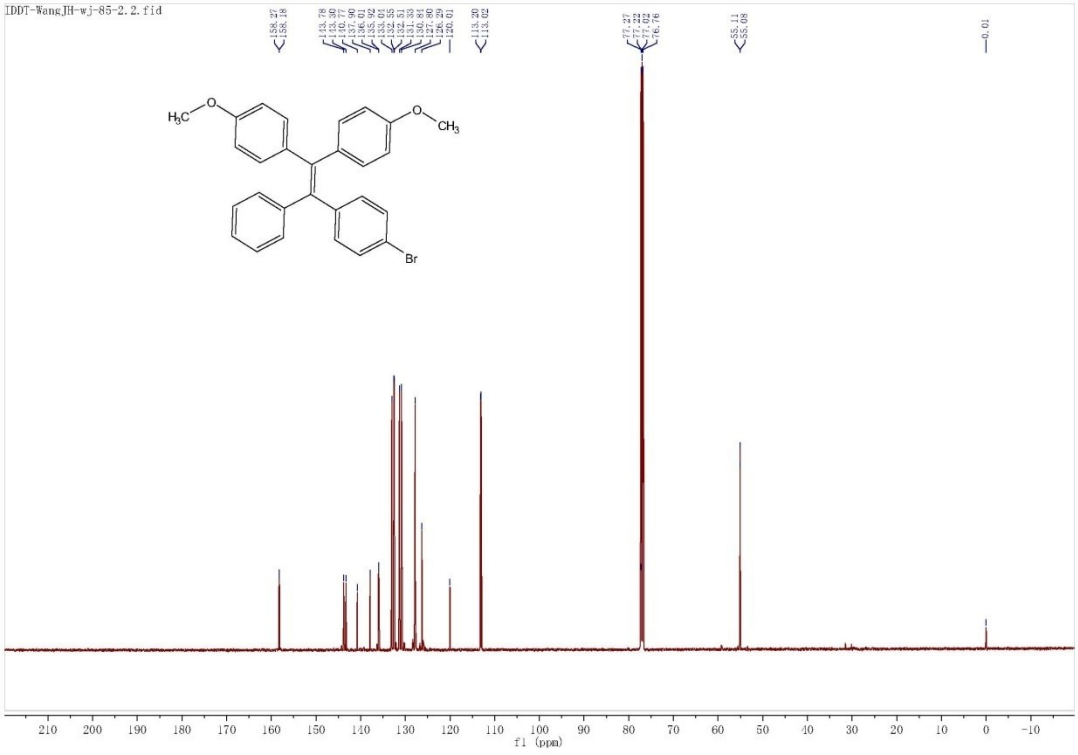


Figure S3. ^{13}C NMR spectrum of compound 1 (126 MHz, Chloroform-d).

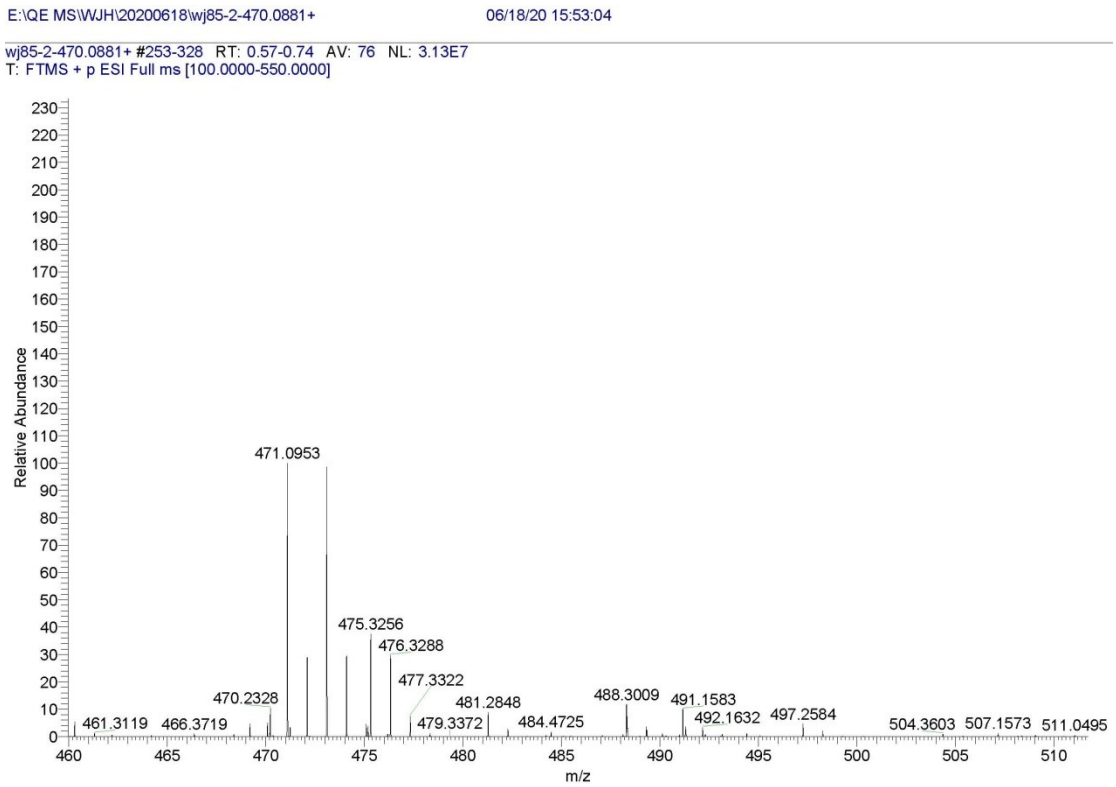


Figure S4. High resolution mass spectrum (HR-MS) of compound 1.

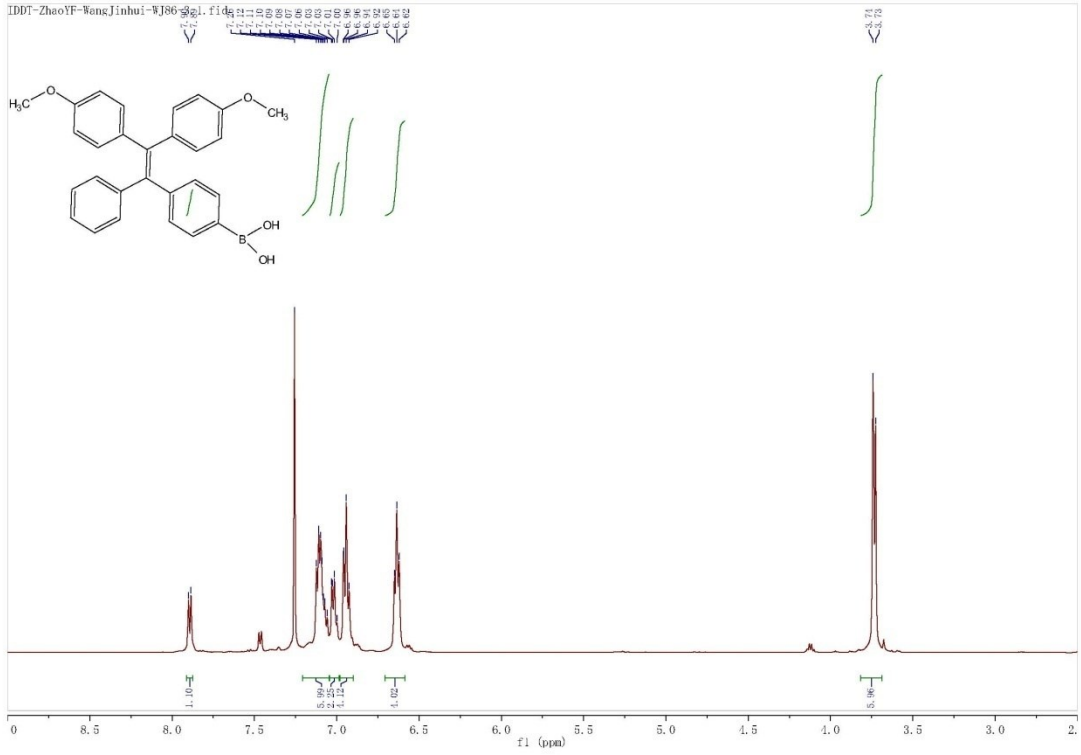


Figure S5. ^{1}H NMR spectrum of compound 1 (500 MHz, Chloroform-d).

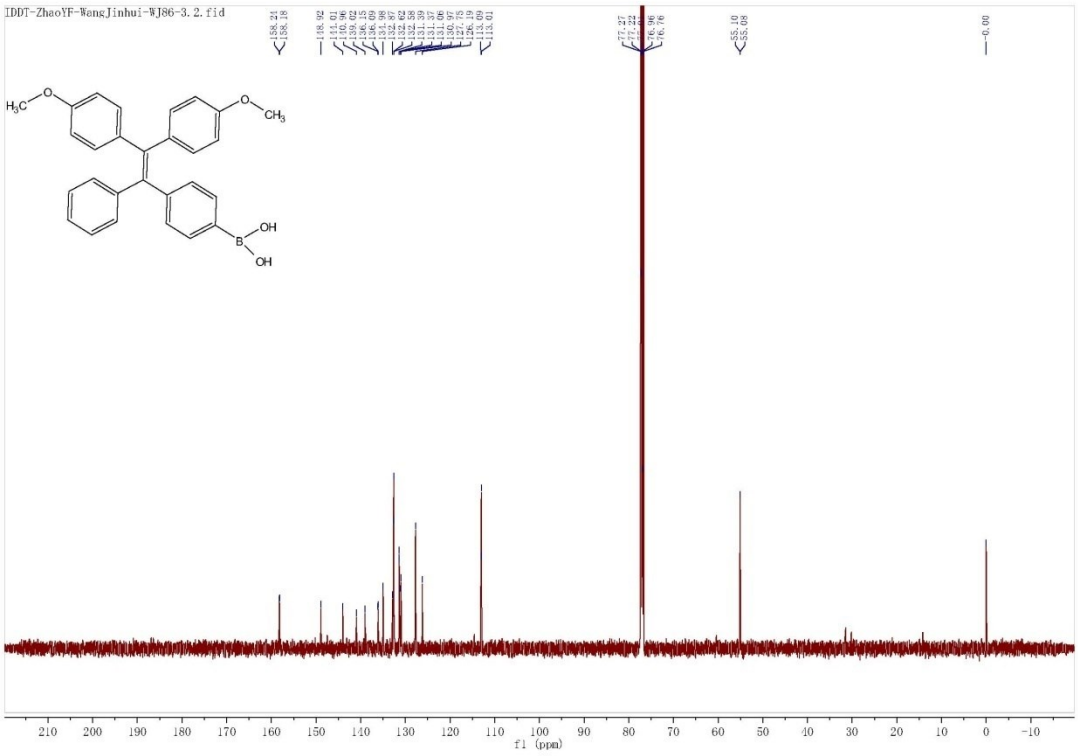


Figure S6. ^{13}C NMR spectrum of compound 2 (126 MHz, Chloroform-d).

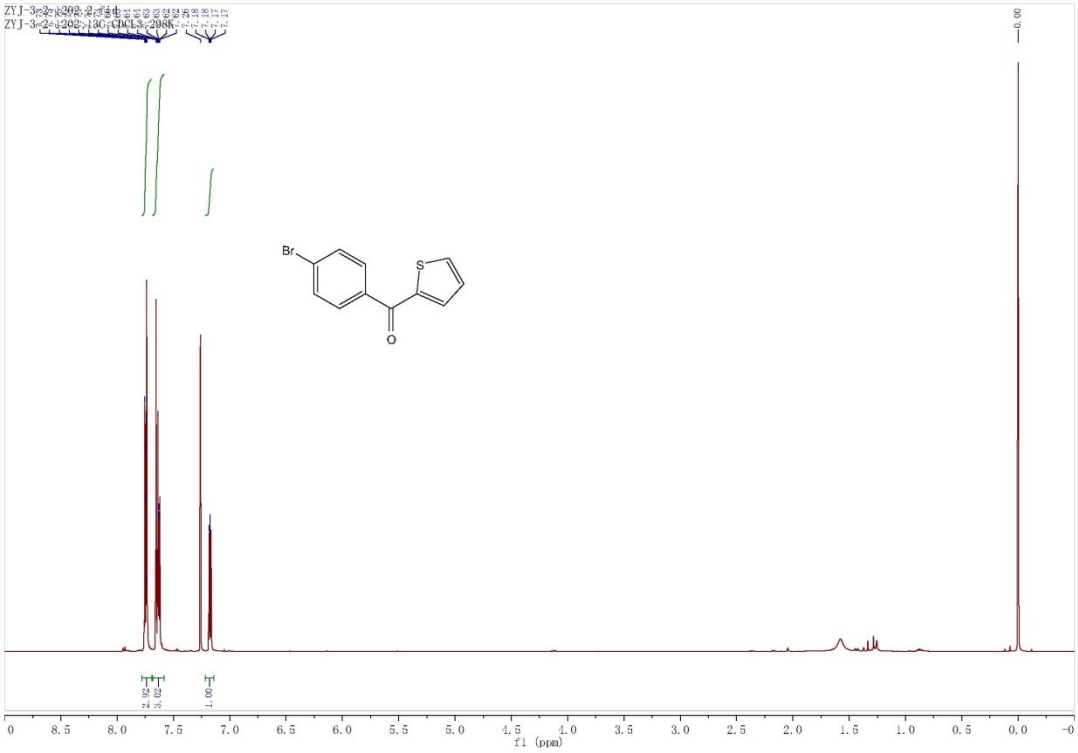


Figure S7. ^{1}H NMR spectrum of compound 3 (500 MHz, Chloroform-d).

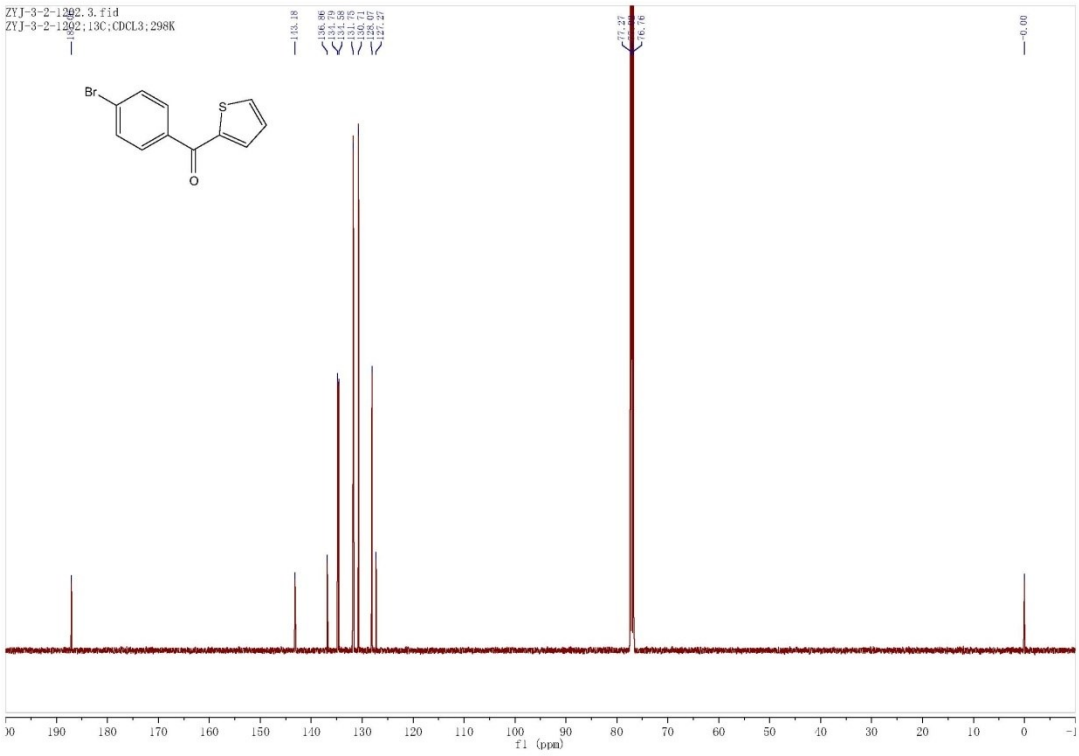


Figure S8. ^{13}C NMR spectrum of compound 3 (126 MHz, Chloroform-d).

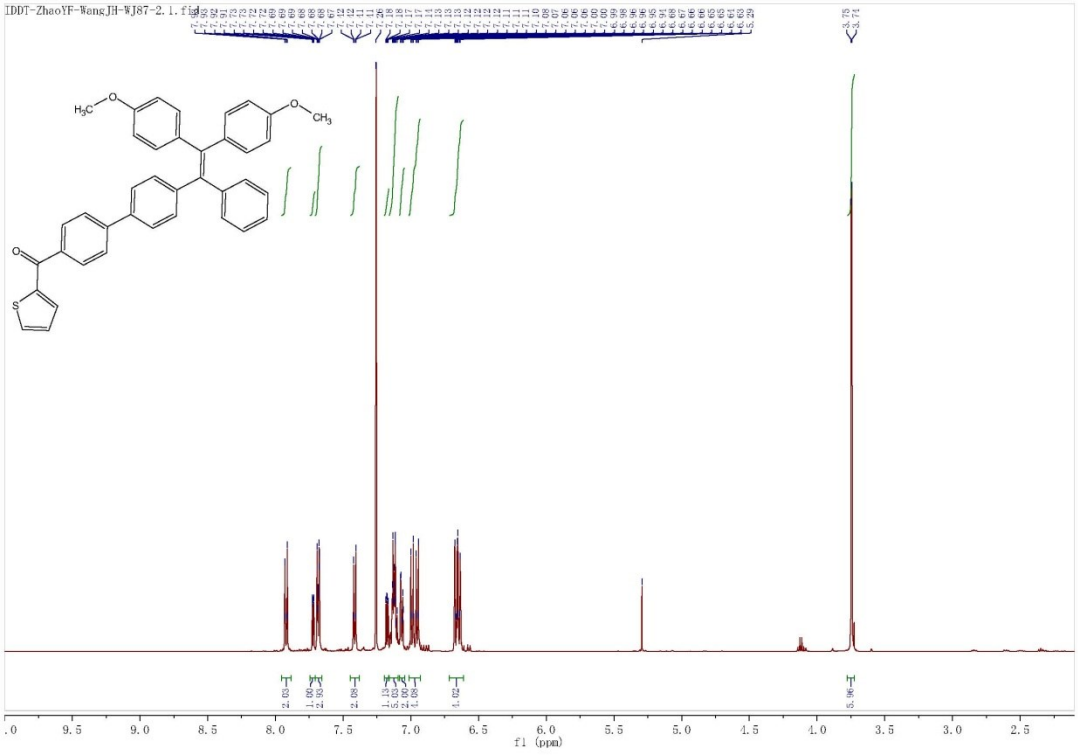


Figure S9. ^{1}H NMR spectrum of compound 4 (500 MHz, Chloroform-d).

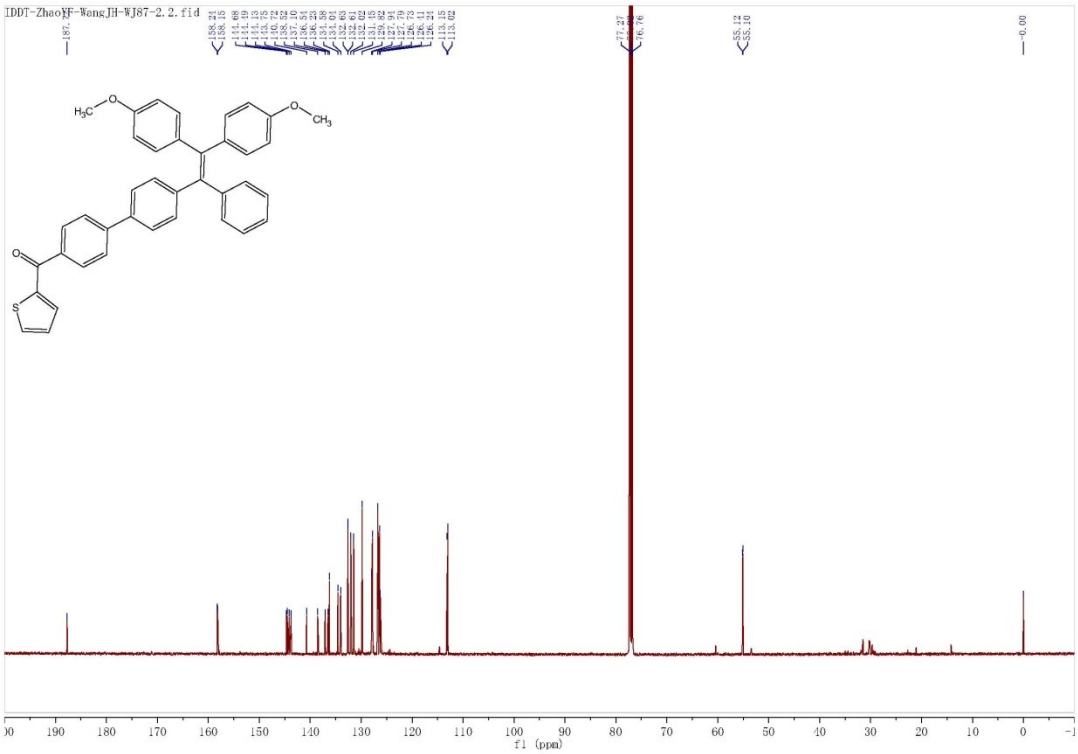


Figure S10. ^{13}C NMR spectrum of compound 4 (126 MHz, Chloroform-d).

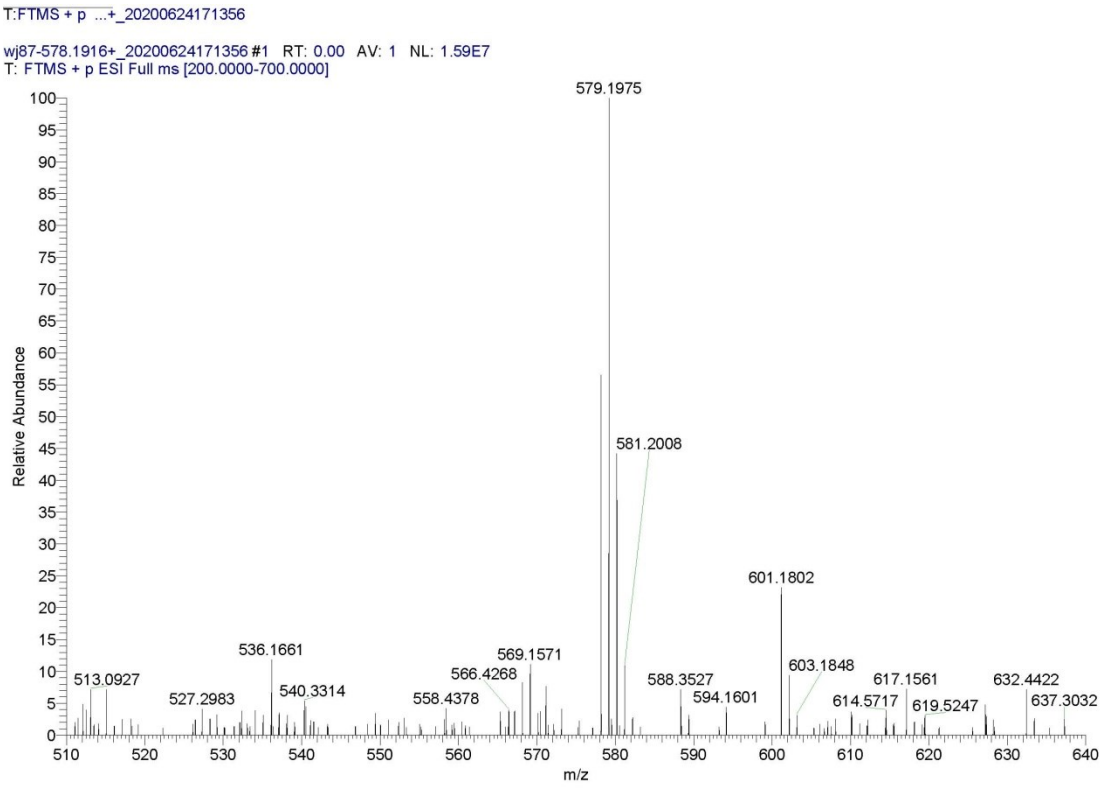


Figure S11 . High resolution mass spectrum (HR-MS) of compound 4.

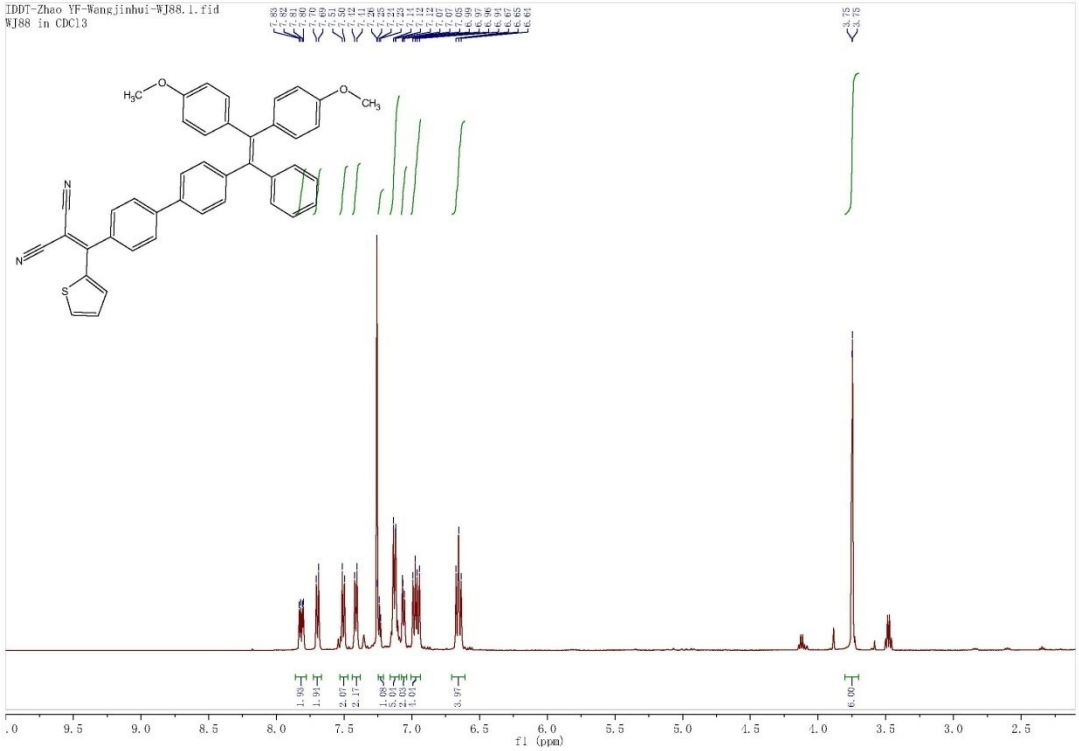


Figure S12. ^{1}H NMR spectrum of compound 5 (500 MHz, Chloroform-d).

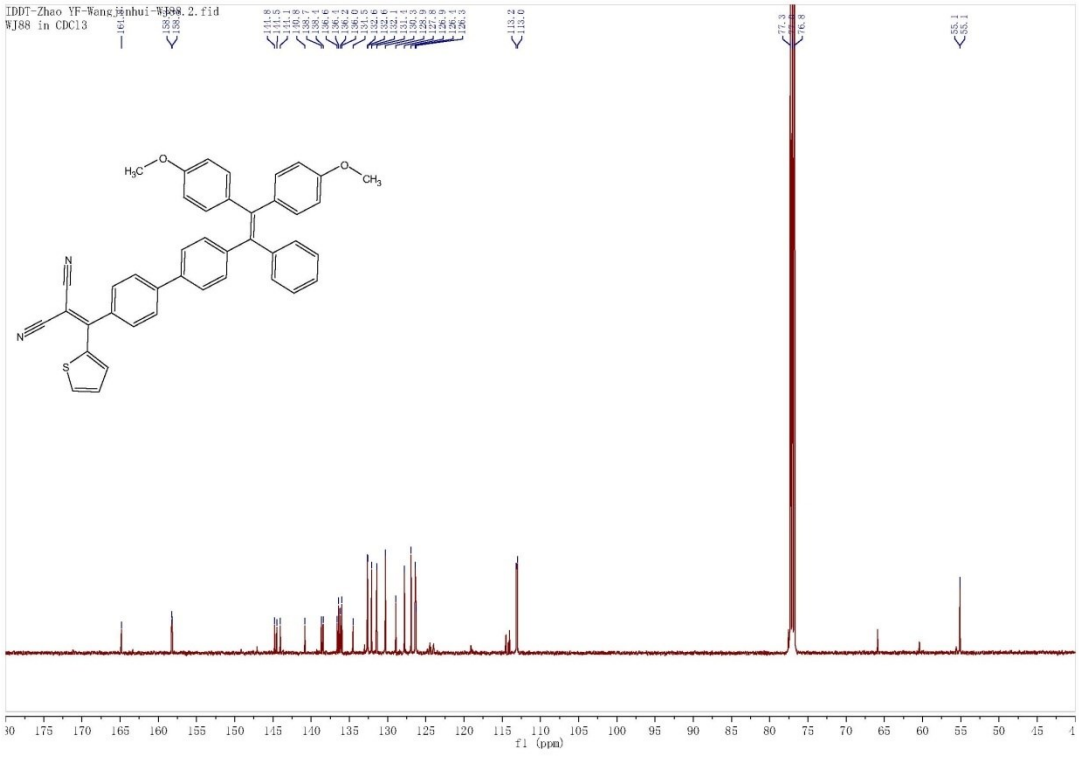


Figure S13. ^{13}C NMR spectrum of compound 5 (126 MHz, Chloroform-d).

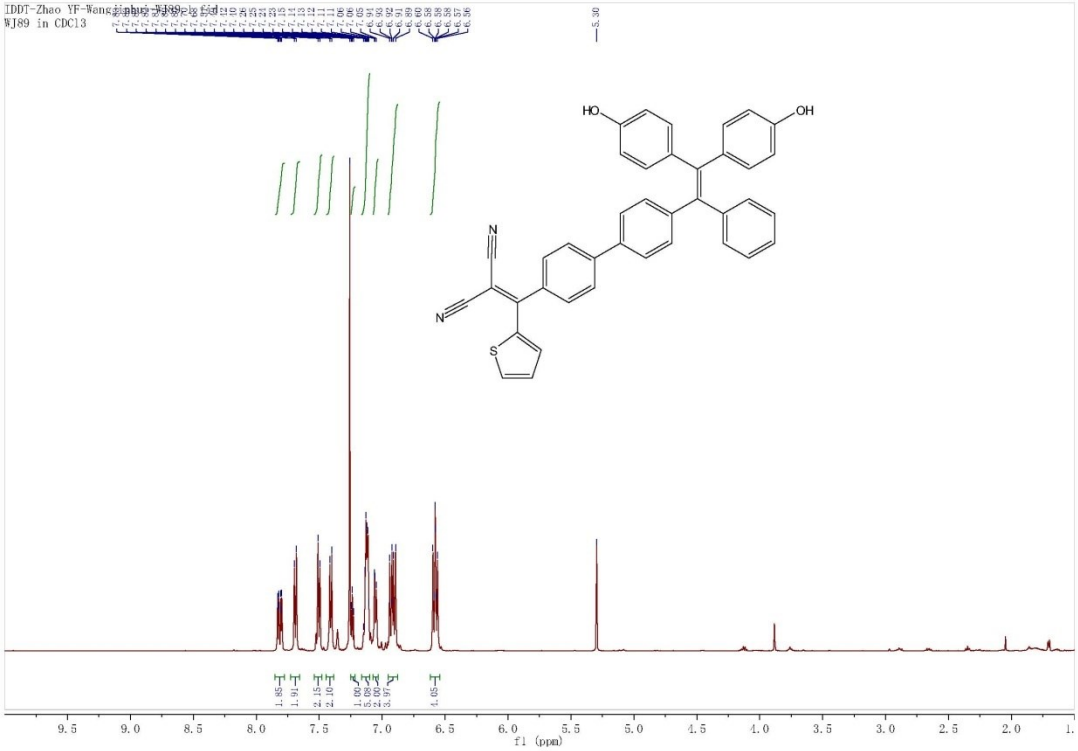


Figure S14. ^{1}H NMR spectrum of compound TPEDCH (500 MHz, Chloroform-d).

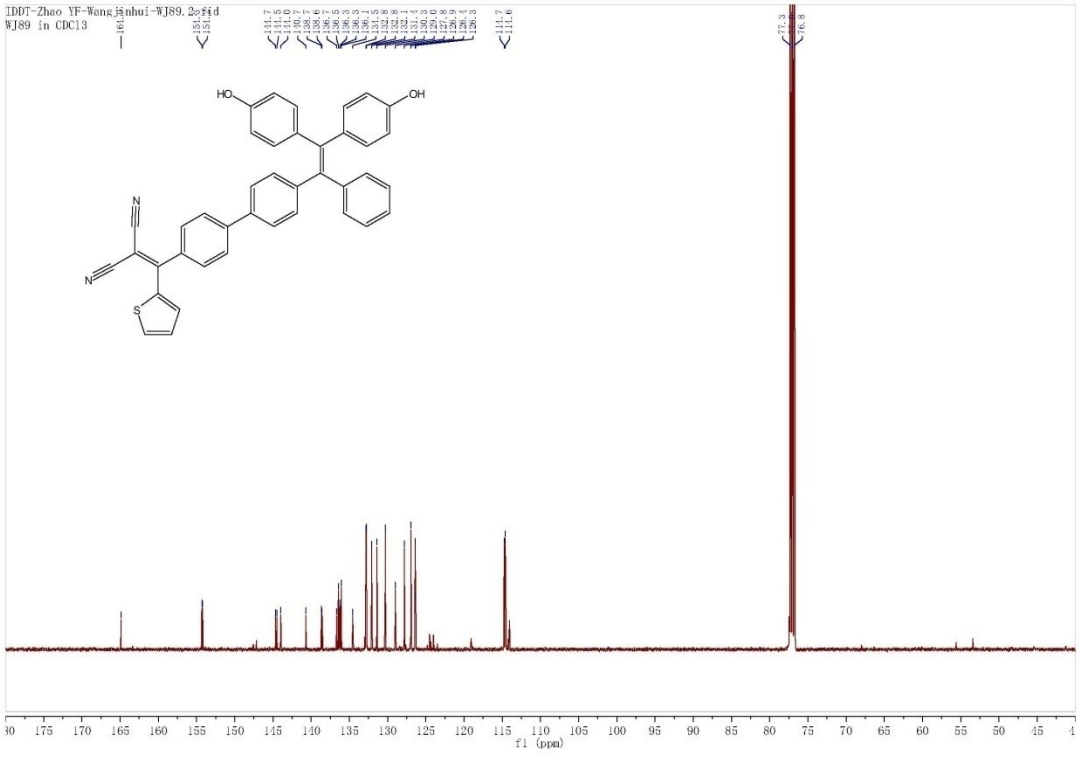


Figure S15. ^{13}C NMR spectrum of compound TPEDCH (126 MHz, Chloroform-d).

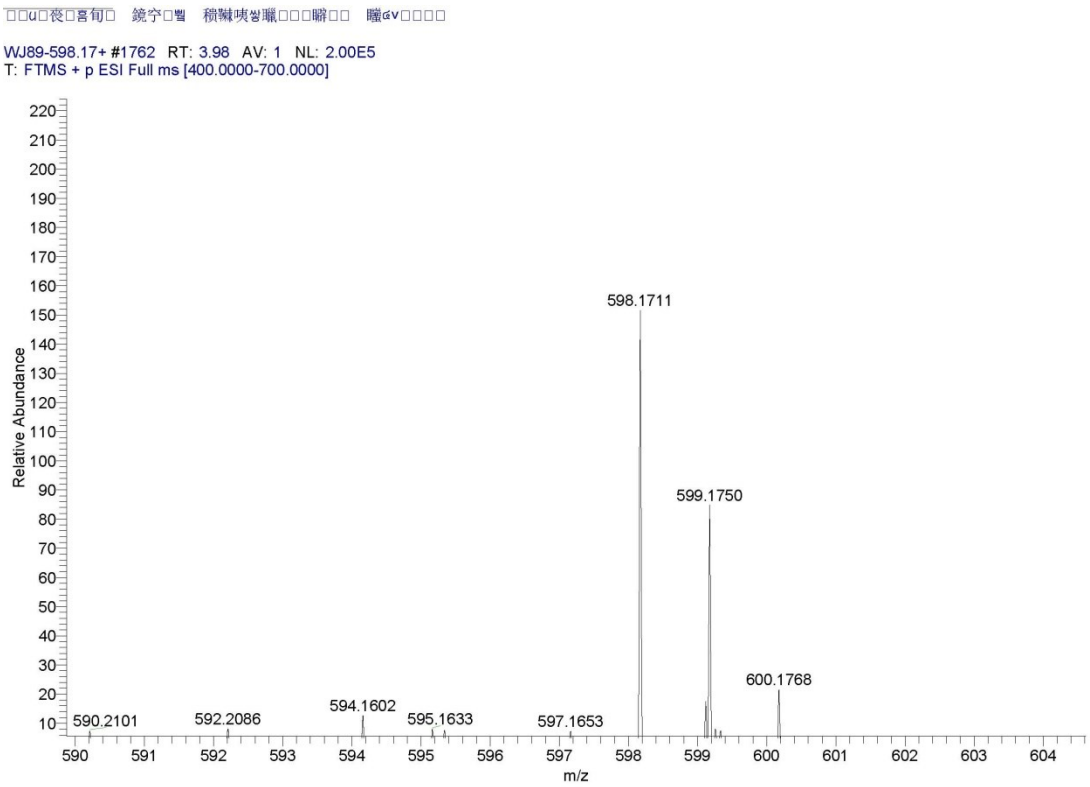


Figure S16. High resolution mass spectrum (HR-MS) of compound TPEDCH.

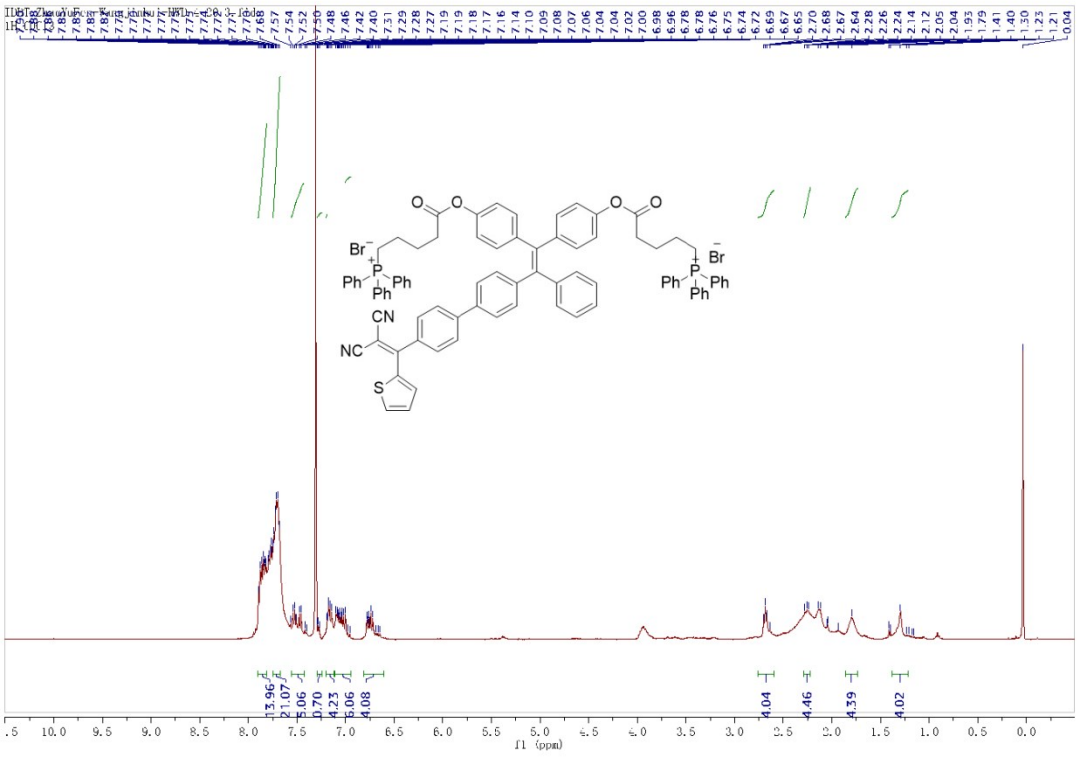


Figure S17. ^{1}H NMR spectrum of compound TPP-TPEDCH (500 MHz, Chloroform-d).

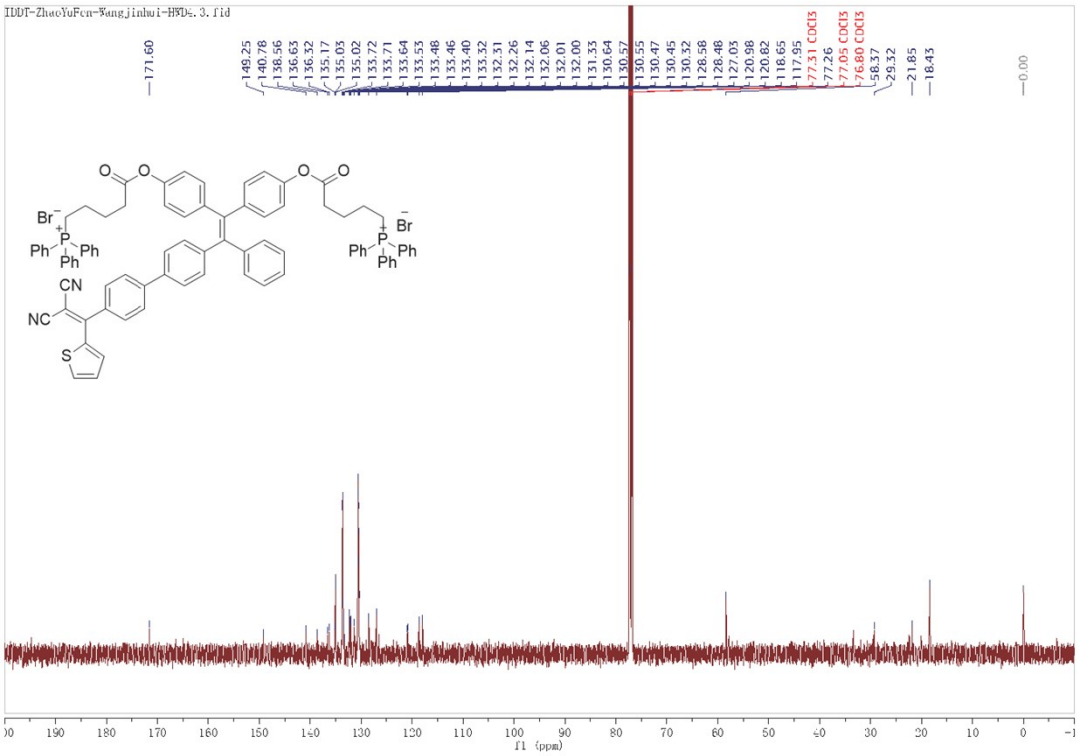


Figure S18. ^{13}C NMR spectrum of compound TPP-TPEDCH (126 MHz, Chloroform-d).

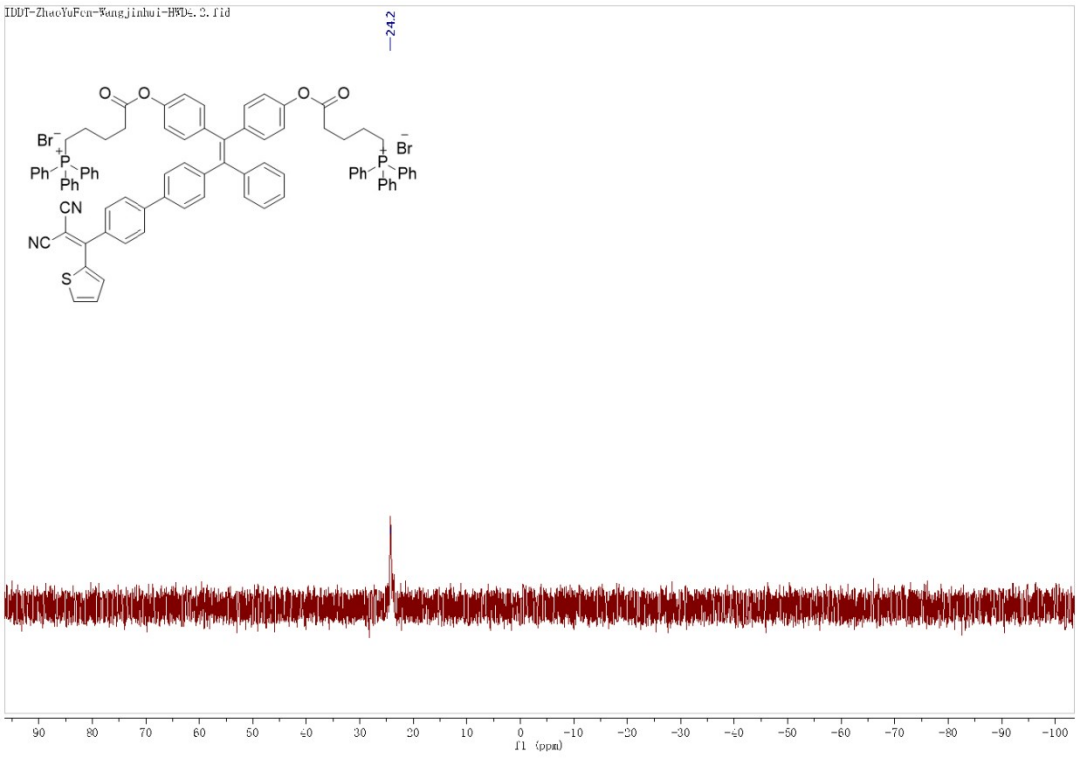


Figure S19. ^{31}P NMR spectrum of compound TPP-TPEDCH (162 MHz, Chloroform-d).

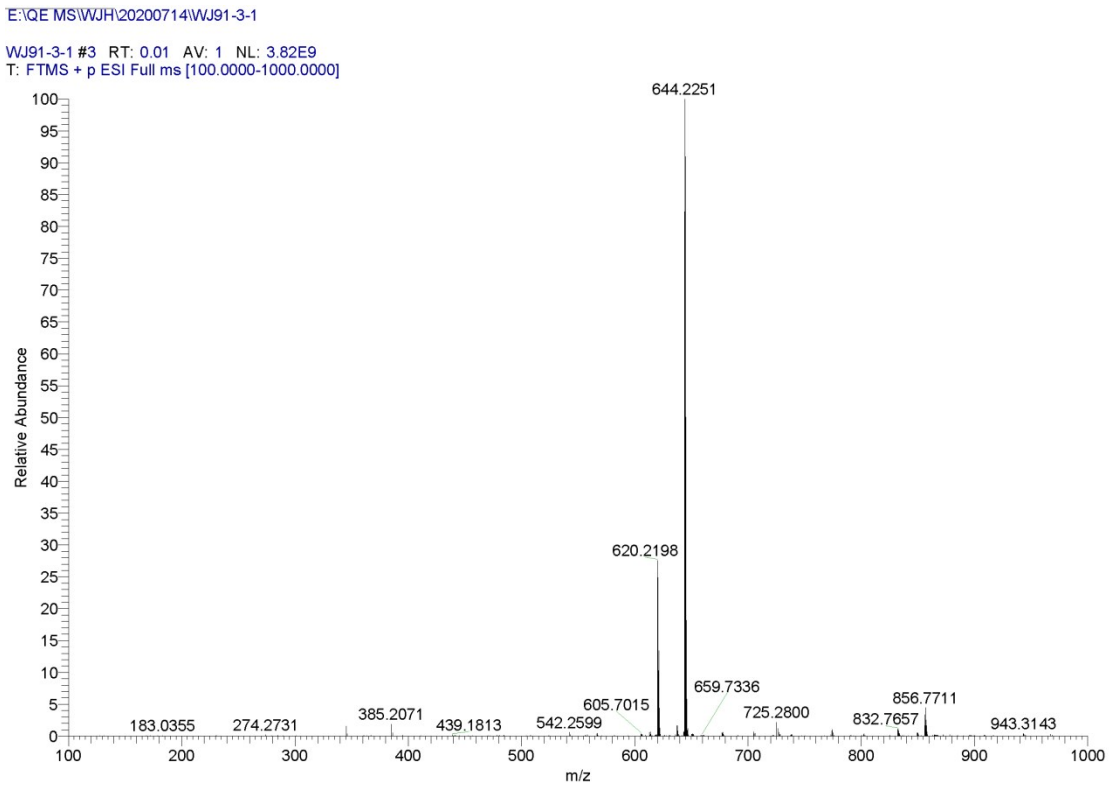


Figure S20. High resolution mass spectrum (HR-MS) of compound TPP-TPEDCH.

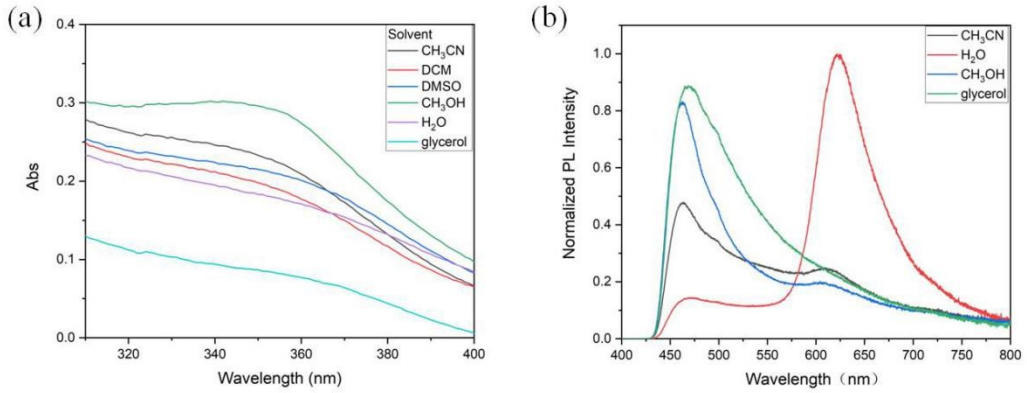


Figure S21. UV-vis absorption (a) and emission spectra (b) of 20 μM TPP-TPEDCH in various solvents. The emission spectrum is measured upon excitation at 380 nm.

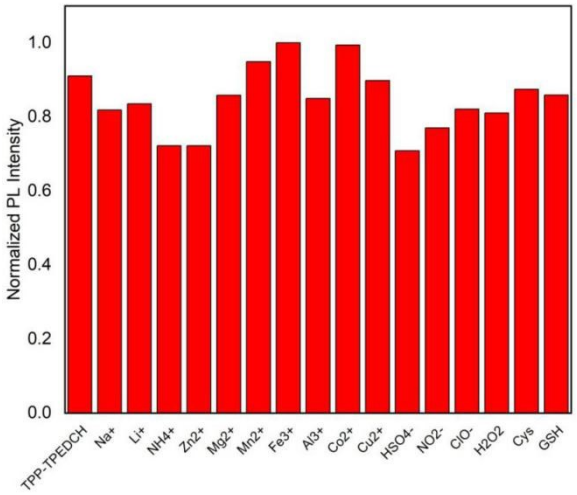


Figure S22. Fluorescence intensity (at 626 nm) of TPP-TPEDCH upon addition of 10 eq. various analytes in 99% PBS/DMSO solution. Compound concentration: 20 μM. Analytes concentration: 200 μM.

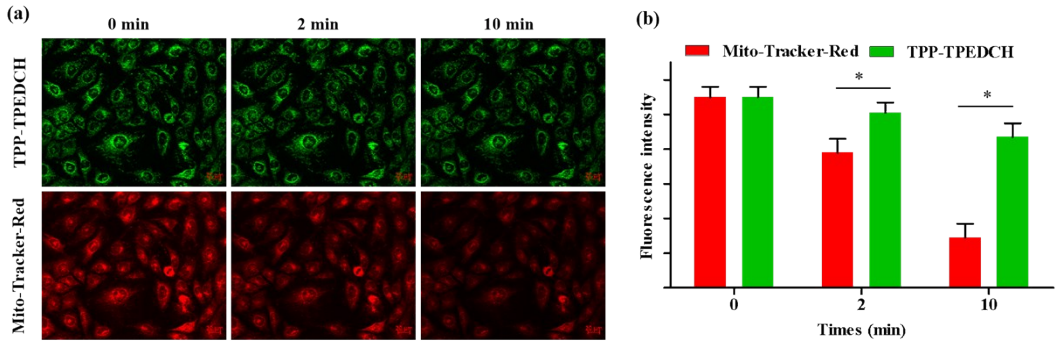


Figure S23. (a) Intracellular photostability of TPP-TPEDCH after irradiation for 0, 2 and 10 min, commercial Lyso Tracker Red fluorescent dyes as control. Scale bars, 20 μm. (b) Semi-quantitative analysis of (a). The data are the means ± SD, n = 3, **P*<0.05.

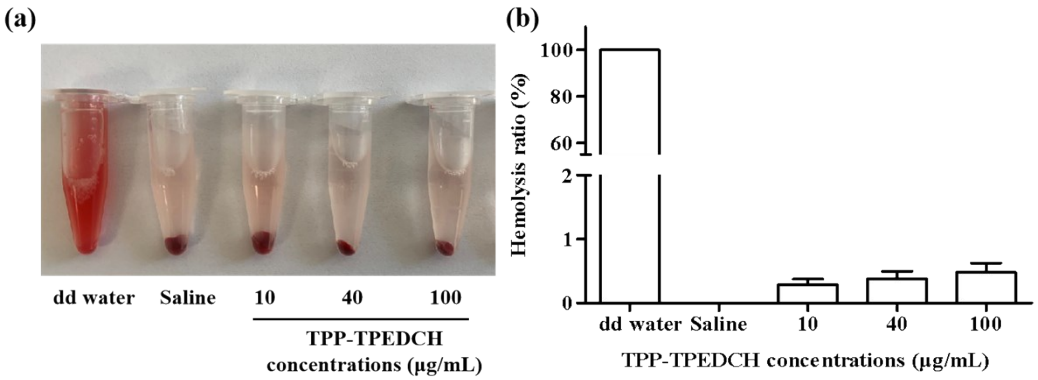


Figure S24. Hemocompatibility of TPP-TPEDCH (a) Representative images of red blood cells

incubated with dd water, saline and TPP-TPEDCH (10, 40 and 100 μg/mL) for 2 h, respectively. (b) Hemolysis ratio of red blood cells.

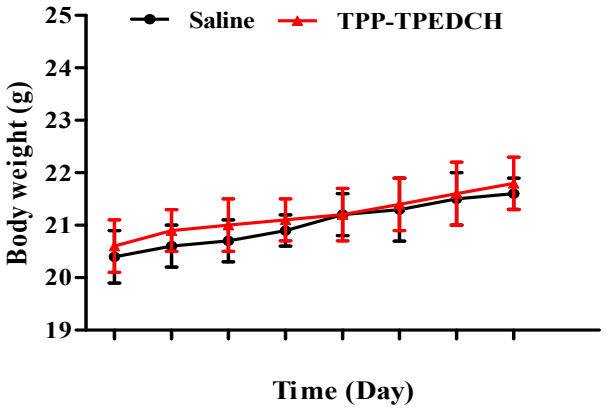


Figure S25. The changes in body weight of mice treated with TPP-TPEDCH, saline-treated mice as control. The data are the means ± SD, n = 6.

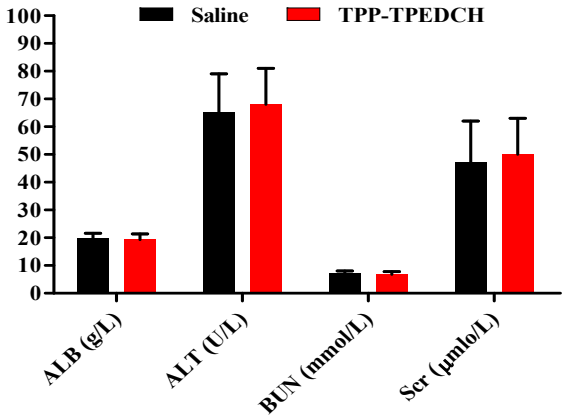


Figure S26. The changes in biochemical indexes mice treated with TPP-TPEDCH, saline-treated mice as control. The data are the means ± SD, n = 6.

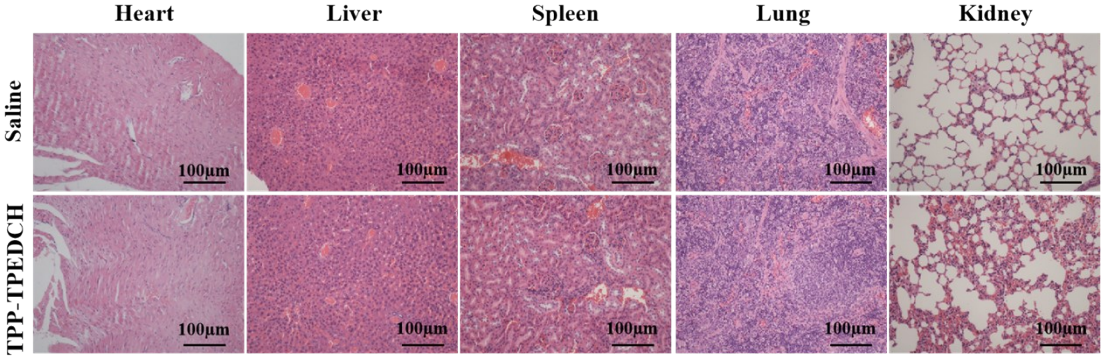


Figure S27. Hematoxylin and eosin (H&E) staining results of heart, liver, spleen, lung, and kidney from mice. Scale bars, 100 μm.

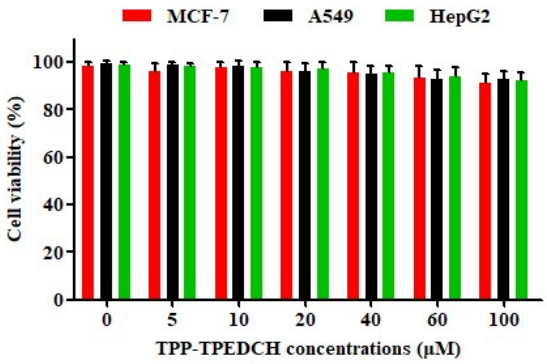


Figure S28. Cell viability of MCF-7, A549, and HepG2 cells incubated with various concentrations of TPP-TPEDCH for 24 h (0 to 100 μM). The data are the means ± SD, n = 3.

References

1. Xu, S.; Wu, W.; Cai, X.; Zhang, C. J.; Yuan, Y.; Liang, J.; Feng, G.; Manghnani, P.; Liu, B., Highly efficient photosensitizers with aggregation-induced emission characteristics obtained through precise molecular design. *Chem Commun (Camb)* **2017,** *53* (62), 8727-8730.

2. Tambade, P. J.; Patil, Y. P.; Panda, A. G.; Bhanage, B. M., Phosphane-Free Palladium-Catalyzed Carbonylative Suzuki Coupling Reaction of Aryl and Heteroaryl Iodides. *European Journal of Organic Chemistry* **2009,** *2009* (18), 3022-3025.