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Electronic Supplementary Information

A camptothecin prodrug induces mitochondria-mediated apoptosis in cancer cells with cascade activations

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Materials. *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC•HCl), 4-dimethylaminopyridine (DMAP) and N-hydroxysuccinimide (NHS) were purchased from Aladdin. *N*, *N*-Diisopropylethylamine, 3-mercaptopropionic acid, ethylenediamine, 3-bromopropylamine hydrobromide and camptothecin (CPT) were purchased from Energy Chemical in Shanghai. All chemicals were used as received without further purification.

Synthesis and characterization. All compounds used in this study were synthesized according to Supplementary Scheme 1. Molecular masses of the synthesized compounds were measured on an Esquire 3000 Plus with electrospray ionization (ESI) and an ICR analyzer. ^{1}H NMR spectra were acquired on a Bruker Avance III 400 MHz NMR spectrometer. ^{13}C NMR spectra were acquired on a Bruker AVANCE III HD Ascend 600 MHz spectrometer.

Analysis by high performance liquid chromatography (HPLC). HPLC analysis on ROS mediated thioketal cleavage and consequent cascaded release of the drug (CPT) and photosensitizer (TPP-NIR) was performed with a reverse-phase (RP) C18 HPLC column (250 × 4.6 mm, 5 micrometers) using gradient elution. Eluent A and Eluent B were 0.05% TFA in deionized water and acetonitrile, respectively.

Singlet Oxygen Detection. 1,3-Diphenylisobenzofuran (DPBF) was used to detect the generation of singlet oxygen because DPBF could react with singlet oxygen, resulting in the decline of its absorption at 410 nm. In a typical experiment, 10 μL of DPBF (1 mg/mL in acetonitrile) was added to a dispersion of TPP-NIR-ss-CPT (1.6 mg/mL, 1 mL). Then the UV-visible absorption spectra of the resulting mixture were acquired after laser irradiation (660 nm, 0.1 W/cm^{2}) for different periods of time.

Cell culturing. All cell lines were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences and cultured in Dulbecco's Modified Eagle's Media (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone). All cells were maintained in a humidified atmosphere containing 5% CO_2.

Cytotoxicity evaluation. A549 cells were seeded in 96-well plates at a density of 5 × 10^{4} cells per well and incubated in culturing media for 12 h. After washed twice with phosphate-buffered saline (PBS), the cells were treated with different formulas as indicated for 4 h. After exposure to laser (660 nm, 0.1 W/cm^{2}) for 10 min, the cells were further incubated for 20 h. After two time washing with PBS, the media were replaced with DMEM containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the cells were incubated for another 4 h. 100 μL of DMSO

was added to dissolve the precipitates after the media containing MTT were discarded. The absorbance of the resulting solution in each well at 492 nm was measured on a MultiSkan FC microplate reader (Thermoscientific).

Intracellular localization of TPP-NIR-ss-CPT. A549 cells were seeded in 35 mm glass bottom dishes at a density of 10^5 cells per dish and cultured for 12 h. After 4 h incubation with TPP-NIR-ss-CPT (8 μM) in 2 mL DMEM (containing 10% FBS and 1% DMSO), the cells were washed with PBS (400 μL × 3). Mitotracker Green was used to stain mitochondria. Then the cells were subjected to confocal laser scanning microscopy (CLSM) on a Leica TCS SP5 (excitation/emission: 405/430 nm for CPT, 490/516nm for Mitotracker Green, and 620/740nm for TPP-NIR).

ROS levels in A549 cells: Intracellular ROS levels in A549 cells were detected using 2′,7′-dichlorofluorescein diacetate (DCFH-DA). A549 cells were seeded in 35 mm glass bottom dishes at a density of 10^5 cells per dish and cultured for 12 h. After 4 h incubation with PBS (blank), CPT (8 μM), TPP-NIR-ss-CPT (8 μM) or TPP-NIR (8 μM) and subsequent treatment with/without laser irradiation (0.1 W/ cm^{-2} for 10 min) as indicated, the cells were further incubated for 4 h and treated with DCFH-DA (2 μM). Then the cells were subjected to flow cytometry on a BD FACS Aria II and CLSM on a Leica TCS SP5 (excitation/emission: 495/515 nm).

Evaluation of mitochondrial depolarization. JC-10 was used to evaluate mitochondrial depolarization in A549 cells. A549 cells were seeded in 35 mm glass bottom dishes at a density of 10^5 cells per dish and cultured for 12 h. After 4 h incubation with PBS (blank), CPT (8 μM), TPP-NIR-ss-CPT (8 μM) or TPP-NIR (8 μM) and subsequent treatment with/without laser irradiation (0.1 W/cm^2 for 10 min) as indicated, the cells were further incubated for 4 h and treated with JC-10 dye (5 mg/L, 1 mL) at 37 °C for 20 min. Then the cells were rinsed twice with PBS, stored in fresh serum-free media, and subjected to CLSM on a Leica TCS SP5 (excitation/emission: 515/529 nm for green JC-10 monomers, 585/590 nm for red JC-10 aggregates).

Assessment of cell apoptosis by flow cytometry. A549 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and cultured for 12 h. After 4 h incubation with PBS (blank), CPT (8 μM), TPP-NIR (8 μM), or TPP-NIR-ss-CPT (8 μM) and subsequent treatment with/without laser irradiation (0.1 W/cm^2 for 10 min) as indicated, the cells were further incubated for 20 h, treated with an Annexin V/7-AAD apoptosis detection kit according to the manufacture's protocols, and subjected to flow cytometry on a BD FACS Aria II. Data plots were generated and analyzed with Flowjo V.

Western blot analysis. A549 cells were seeded in 6-cm petri dishes at a density of 10^6 cells per dish and cultured for 24 h. After 4 h incubation with PBS (blank), CPT (8 μM), TPP-NIR (8 μM), or TPP-NIR-ss-CPT (8 μM) and subsequent treatment with/without laser irradiation (0.1 W/cm^2 for 10 min) as indicated, the cells were further incubated for 20 h and washed with precooled PBS for three times. Then the cells were collected and lysed with radioimmunoprecipitation assay (RIPA) buffer containing 1% protease inhibitor cocktail at 4 °C for 30 min. After centrifugation, the supernatant was added to an equal volume of loading buffer and the resulting mixture was heated at 100 °C for 5 min. The proteins were loaded on polyacrylamide gel, subjected to electrophoresis, and transferred to a PDVF membrane. Protein bands were visualized using Western Bright ECL (Advanta), the images of which were captured on a GE Healthcare Bio-sciences AB. The expression level of GADPH was used as a control to normalize the expression levels of target proteins.

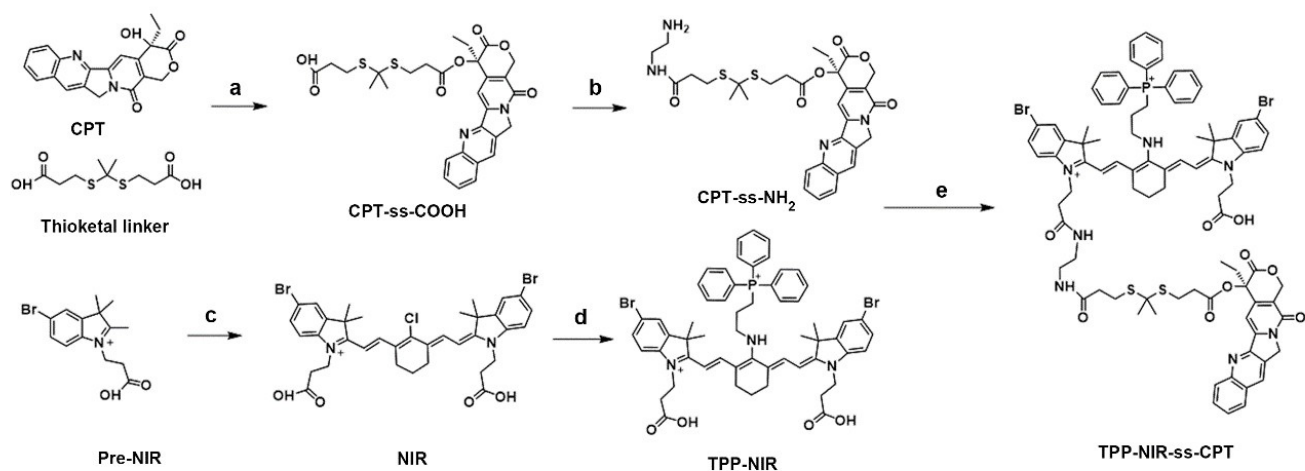
Animal ethics. Animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of Xiamen University.

***In vivo* treatment.** About 10^8 A549 cells suspended in 80 μL PBS were subcutaneously injected into the right leg of a female nude mouse for inoculation. The tumor was allowed to grow for 2 weeks. On Day 1, the A549 tumor-bearing nude mice were randomly separated into four groups, intravenously injected with TPP-NIR (10.4 mg/kg), free CPT (3.5 mg/kg), TPP-NIR-ss-CPT (16.5 mg/kg) or PBS, and subjected to laser irradiation (660 nm, 0.1 W/cm^2) for 10 min if indicated. The treatment was repeated on Day 3, 5, 7, and 9. The therapeutic results of each group were evaluated by measuring the length and width of each tumor every other day. Tumor volume (V) was calculated according to the equation: $V = 1/2 \times \text{length} \times \text{width}^2$. Relative tumor volume was calculated as V/V_0 (V_0 was the corresponding tumor volume when the treatment was initiated). The weights of the mice in each group were measured every other day during the treatment.

Biocompatibility evaluation. Healthy BALB/c mice of about 20 g were divided into three groups (n=3/group) and injected with PBS (blank), free CPT (3.5 mg/kg), or TPP-NIR-ss-CPT (16.5 mg/kg) via tail vein on Day 1. The injections were repeated on Days 3, 5, 7, and 9. On Day 15, blood samples were collected from eyelids of the mice for blood biochemical analysis.

Statistical analysis. Statistical analysis was performed using Student's t-test for unpaired data and a p value of less than 0.05 were accepted as an indicator of a statistically significant difference compared to controls.

Synthetic protocols



Scheme S1. Synthesis of TPP-NIR-ss-CPT. Reagents: (a) EDC•HCl, DMAP; (b) EDC•HCl, NHS, $FmocNHCH_2CH_2NH_2$, then piperidine; (c) Vilsmeier–Haack reagent, NaOAc, *N*-[(3-(anilinomethylene)-2-chloro-1-cyclohexen-1-yl) methylene] aniline monohydrochloride; (d) triethylamine, TPP-NH_2; (e) EDC•HCl, NHS, DIPEA.

Synthesis of the thioketal linker. The ROS-sensitive thioketal linker was prepared according to a previous report.[1, 2] A mixture of anhydrous 3-mercaptopropionic acid (2.65 g, 25.0 mmol), anhydrous acetone (2.95 g, 50.0 mmol) and TFA (0.05 mL) was stirred at room temperature for 6 h before cooled in an ice-salt bath. The precipitates were collected by filtration, washed with hexane and ice-cold water, and dried *in vacuo* to give the thioketal linker a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (t, 4H, *J* = 7.6), 2.70 (t, 4H, *J* = 7.4), 1.62 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.07 (2C), 56.42, 34.02 (2C), 30.74 (2C), 24.83 (2C).

Synthesis of CPT-ss-COOH. To a solution of the thioketal linker (490.0 mg, 3.0 mmol) in CH_2Cl_2 (20 mL), a solution of CPT (500.0 mg, 1.5 mmol), EDC•HCl (383.4 mg, 2 mmol), and DMAP (125.0 mg, 1.5 mmol) in CH_2Cl_2 (20 mL) was added dropwise. The resulting mixture was stirred overnight at room temperature and concentrated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 20:1 v/v) to give CPT-ss-COOH as a pale-yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.20 (d, 2H, *J* = 8.4), 7.82 (t, 1H, *J* = 10.0), 7.71 (t, 1H, *J* = 13.2), 7.25 (s, 1H), 5.50 (s, 2H), 5.32 (d, 2H, *J* = 7.9), 2.83-2.78 (m, 4H), 2.74-2.72 (m, 2H), 2.44-2.41 (m, 2H), 2.20-2.18 (m, 2H),1.59 (s, 3H), 1.57 (s, 3H), 0.9 (t, 3H, *J* = 11.2); ^{13}C NMR (151 MHz,

DMSO-d_6) δ 171.0 (2C), 167.6, 157.0, 152.9, 148.4, 146.4, 145.9, 132.0, 130.9, 130.3, 129.3, 129.0, 128.5, 128.2, 119.3, 95.9, 76.7, 66.7, 56.5, 50.7, 34.7, 34.3, 31.1 (2C), 30.7, 25.5, 25.2, 8.1; ESI-MS (m/z) calcd for $C_{29}H_{30}N_2O_7S_2Na$ $[M + Na]^+$ 605.2, found 605.0.

Synthesis of CPT-ss-NH_2. To a solution of $FmocNHCH_2CH_2NH_2$ (477.0 mg,1.5 mmol) in CH_2Cl_2 (10 mL), a solution of CPT-ss-COOH (900.0 mg, 1.5 mmol), EDC•HCl (300.0 mg, 2 mmol), and NHS (228.0 mg, 2 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The resulting mixture was stirred for 24 h at room temperature and concentrated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 50:1 v/v) to give CPT-ss-NH-Fmoc as a white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.23 (d, 2H, J = 6.0), 7.93 (d, 2H, J = 5.7), 7.81-7.79 (m, 1H), 7.76 (d, 2H, J = 10.0), 7.72-7.70 (m, 1H), 7.63 (d, 2H, J = 11.7), 7.42-7.35 (m, 5H), 6.28 (s, 1H), 5.74 (d, 2H, J = 12.4), 5.28 (s, 2H), 4.42 (d, 2H, J = 10.0), 3.54-3.38 (m, 4H), 2.94-2.75 (m, 4H), 2.48-2.42 (m, 2H), 2.25-2.22 (m, 2H), 2.20-2.17 (m, 2H), 1.59 (s, 3H), 1.56 (s, 3H), 1.0 (t, 3H, J = 10.0); ESI-MS (m/z) calcd for $C_{46}H_{47}N_4O_8S_2$ $[M + H]^+$ 847.3, found 847.1. To a solution of CPT-ss-NH_2-Fmoc (846 mg, 1.0 mmol) in dry $CHCl_2$ (5 mL), 5.0 mL of piperidine was added. The mixture was stirred for 1 h and concentrated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 15:1 v/v) to give CPT-ss-NH_2 as a pale-yellow solid: ^1H NMR (400 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.20 (d, 2H, J = 8.4), 7.82 (t, 1H, J = 10.3), 7.71 (t, 1H, J = 10.0), 7.25 (s, 1H), 5.50 (s, 2H), 5.32 (d, 2H, J = 6.4), 2.80-2.68 (m, 8H), 2.74-2.72 (m, 2H), 2.35-2.30 (m, 2H), 2.20-2.18 (m, 2H),1.59 (s, 3H), 1.57 (s, 3H), 0.9 (t, 3H, J = 12.0); ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.5, 171.1, 167.6, 157.0, 152.8, 148.4, 146.4, 145.9, 132.1, 130.8, 130.3, 129.3, 129.0, 128.5, 128.3, 119.2, 95.9, 76.7, 66.7, 56.5, 50.7, 43.9, 38.9, 36.8, 35.6, 25.7, 25.2, 22.7, 22.7, 8.0; ESI-MS (m/z) calcd for $C_{31}H_{37}N_4O_6S_2$ $[M + H]^+$ 625.2, found 625.1.

Synthesis of TPP-NIR. TPP-NIR was prepared according to a previous report.[3] Firstly, Pre-NIR was synthesized. To a solution of 5-bromo-2,3,3-trimethyl-3*H*-indole (1.1 g, 4.8 mmol) in MeCN (20 mL), a mixture of Na_2CO_3 (530.0 mg, 5 mmol) and 3-bromopropionic acid (695.0 mg, 5.0 mmol) was added. The resulting mixture was stirred at room temperature for 24 h and filtered. The filtrate was concentrated *in vacuo* before 10 mL diethyl ether was added to precipitate Pre-NIR, which was obtained as a red powder: ^1H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 1H), 8.10 (t, 1H, J =10.5), 7.84

(t, 1H, J = 6.9), 4.62-4.60 (m, 2H), 3.02-2.98 (m, 2H), 2.80 (s, 3H), 1.57 (s, 6H); ESI-MS (m/z) calcd for $C_{14}H_{17}BrNO_2$ $[M]^+$ 311.2, found 311.5. A solution of Pre-NIR (311.0 mg, 1.0 mmol), Vilsmeier-Haack reagent (0.5 equiv with respect to Pre-NIR), sodium acetate (350 mg, 4.2 mmol), and *N*-[(3-(anilinomethylene)-2-chloro-1-cyclohexen-1-yl) methylene] aniline monohydrochloride (360.0 mg, 1.0 mmol) in ethanol (10.0 mL) was stirred at 70 °C for 12 h before cooled to room temperature. Diethyl ether was used to precipitate crude NIR, which was purified with preparative HPLC on a C18 column (0.05% TFA in water (Eluant A)/0.05% TFA in acetonitrile (Eluant B)). A gradient elution was used (0-5-20-40-50 min, 0-10-80-100-0% Eluant B). ^{1}H NMR (400 MHz, MeOD) δ 8.47 (d, 2H, J = 7.0), 7.75 (s, 2H), 7.64 (d, 2H, J = 12.4), 7.35 (d, 2H, J = 13.3), 6.49 (d, 2H, J = 7.4), 4.38-4.33 (m, 4H), 2.85-2.79 (m, 4H), 2.62-2.59 (m, 4H), 1.89-1.92 (m, 2H), 1.67-1.75 (m, 12H); ESI-MS (m/z) calcd for $C_{36}H_{38}Br_2ClN_2O_4^+$ $[M]^+$ 757.1, found 757.0. A solution of NIR (76.0 mg, 0.1 mmol), (3-amino-propyl) triphenylphosphonium (TPP-NH_2, 1.5 equiv. with respect to NIR) and triethylamine (3 equiv. with respect to NIR) in DMSO (1.0 mL) was stirred at 85 °C for 3 h. Diethyl ether was used to precipitate crude TPP-NIR, which was purified using preparative HPLC on a C18 column (0.05% TFA in water (Eluant A)/0.05% TFA in acetonitrile (Eluant B)). A gradient elution was used during the analysis (0-5-20-40-50 min, 0-10-80-100-0% Eluant B). ^{1}H NMR (400 MHz, CD_3OD) δ 8.40 (br-s, 2H), 7.88-7.79 (m, 15H), 7.68 (d, 2H, J = 7.6), 7.55 (d, 2H, J = 8.5), 7.32 (d, 2H, J = 8.5 Hz), 6.45 (d, 2H, J = 14.1 Hz), 4.37-4.34 (m, 4H), 3.61-3.57 (m, 2H), 3.11-3.09 (m, 4H), 2.76-2.74 (m, 4H), 2.59-2.57 (m, 2H), 1.99-1.94 (m, 4H), 1.73-1.69 (m, 12H); ^{13}C NMR (151 MHz, CD_3OD) δ 176.4 (2C), 172.3 (2C), 149.9, 144.2 (2C), 143.5 (2C), 141.4 (2C), 135.2 (3C), 133.6 (6C), 131.5, 130.3 (6C), 127.8 (2C), 125.5 (2C), 118.2, 117.9, 117.5, 112.7 (2C), 101.9 (2C), 49.4 (2C), 41.8, 40.0, 35.0 (2C), 26.9 (6C), 25.9, 21.9, 21.6, 20.8, 19.5; ESI-MS (m/z) calcd for $C_{57}H_{59}Br_2N_3O_4P^+$ $[M]^+$ 1040.3, found 1040.1.[3]

Synthesis of TPP-NIR-ss-CPT. To a solution of TPP-NIR (28.0 mg, 0.2 mmol) in dry CH_2Cl_2 (5 mL), EDC•HCl (30.0 mg, 0.22 mmol) and NHS (22.8mg, 0.2mmol) were added. The resulting mixture was stirred for 24 h at room temperature before DIPEA (2.0 μL) and CPT-ss-NH_2 (12.5 mg 0.2 mmol) were added. The resulting mixture was further stirred for 24 h. Diethyl ether was added to precipitate the product, which was purified with preparative HPLC on a C18 column (0.05% TFA in water (Eluant A)/0.05% TFA in acetonitrile (Eluant B)). A gradient elution was used (0-5-20-40-50 min, 0-10-60-

100-0% Eluant B). ^{1}H NMR (400 MHz, CD_3OD) δ 8.68 (s, 1H), 8.40 (br-s, 2H), 8.12 (m, 2H), 7.90-7.60 (m, 16H), 7.55 (d, 2H, *J* = 7.9), 7.36 (s, 1H),7.33 (d, 2H, *J* = 12.4 Hz), 6.93 (d, 1H, *J* = 4.5 Hz), 6.45 (d, 2H, *J* = 13.3 Hz), 5.48 (m, 2H), 5.32 (m, 2H), 4.39-4.35 (m, 4H), 3.85-3.77 (m, 2H), 3.55-3.47 (m, 2H), 3.11-3.09 (m, 2H), 2.88-2.79 (m, 4H), 2.78-2.72 (m, 4H), 2.64-2.56 (m, 6H), 2.48-2.44 (m, 4H), 2.20-2.18 (m, 2H), 1.98-1.90 (m, 4H), 1.75-1.68 (m, 12H). 1.60-1.55 (m, 6H), 0.89 (t, 3H, *J* = 12.0); ^{13}C NMR (151 MHz, CD_3OD) δ 177.6 (2C), 173.8, 172.9, 169.8 (2C), 159.1, 153.5, 151.5, 149.6, 148.3, 147.7, 145.7 (2C), 144.9 (2C), 142.8 (2C), 136.4 (4C), 134.8 (6C), 133.6, 132.8, 132.1, 131.6, 131.5 (6C), 130.1, 129.9, 129.7, 129.4, 129.2, 126.9, 120.5, 119.7, 119.3, 114.2 (2C), 108.2, 103.3 (2C), 97.7, 78.3, 67.9, 56.6, 51.7, 50.8, 43.3, 40.0, 37.7 (2C), 36.1 (2C), 34.6, 31.9, 31.4 (4C), 28.3 (8C), 27.4 (3C), 22.2, 19.4, 17.2, 8.1; ESI-MS (m/z) calcd for $C_{88}H_{93}Br_2N_7O_9PS_2$ $[M]^+$ 1646.5, found 1646.3. HRMS calcd for $C_{88}H_{94}Br_2N_7O_9PS_2$ $[M+H]^{2+}$: 823.7322, found: 823.7297.

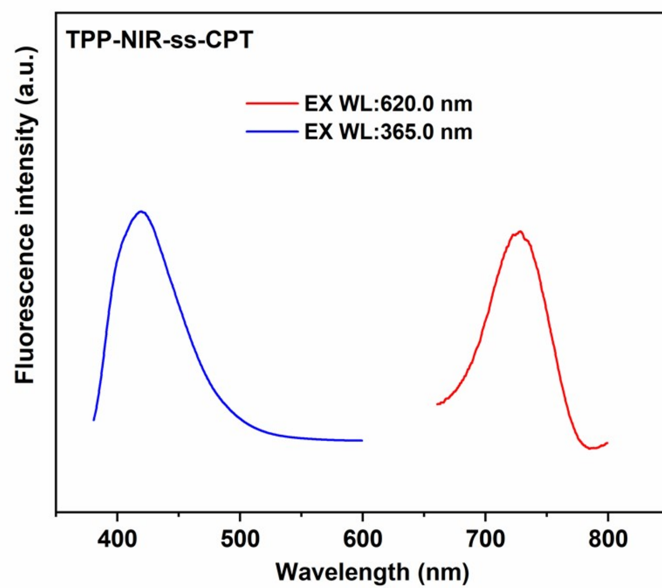


Figure S1. Fluorescence spectra of TPP-NIR-ss-CPT with excitation at 365 nm (blue) or 620 nm (red).

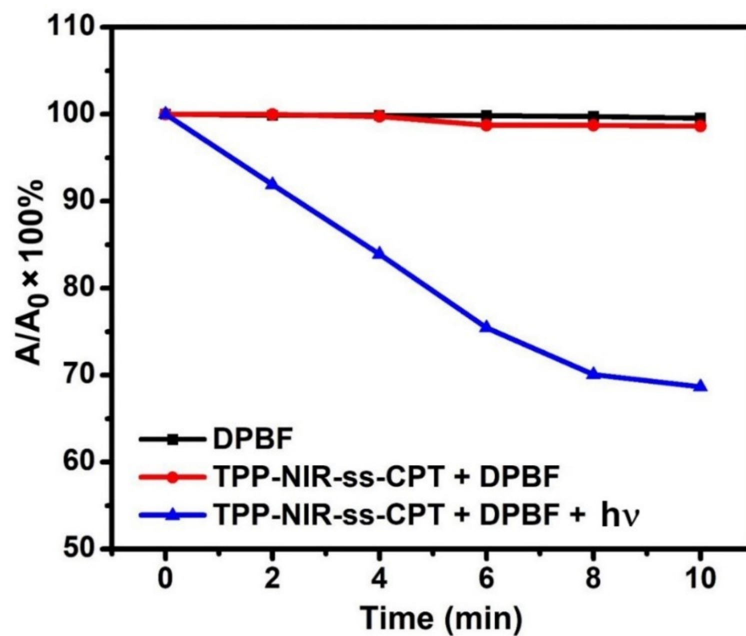


Figure S2. The changes in absorbance (at 410 nm) of a DPBF solution and TPP-NIR-ss-CPT + DPBF solutions with/without light irradiation (660 nm, 0.1 W/cm^2) over time. A_0 and A are the absorbances of a sample solution at the beginning and indicated time point during light irradiation, respectively.

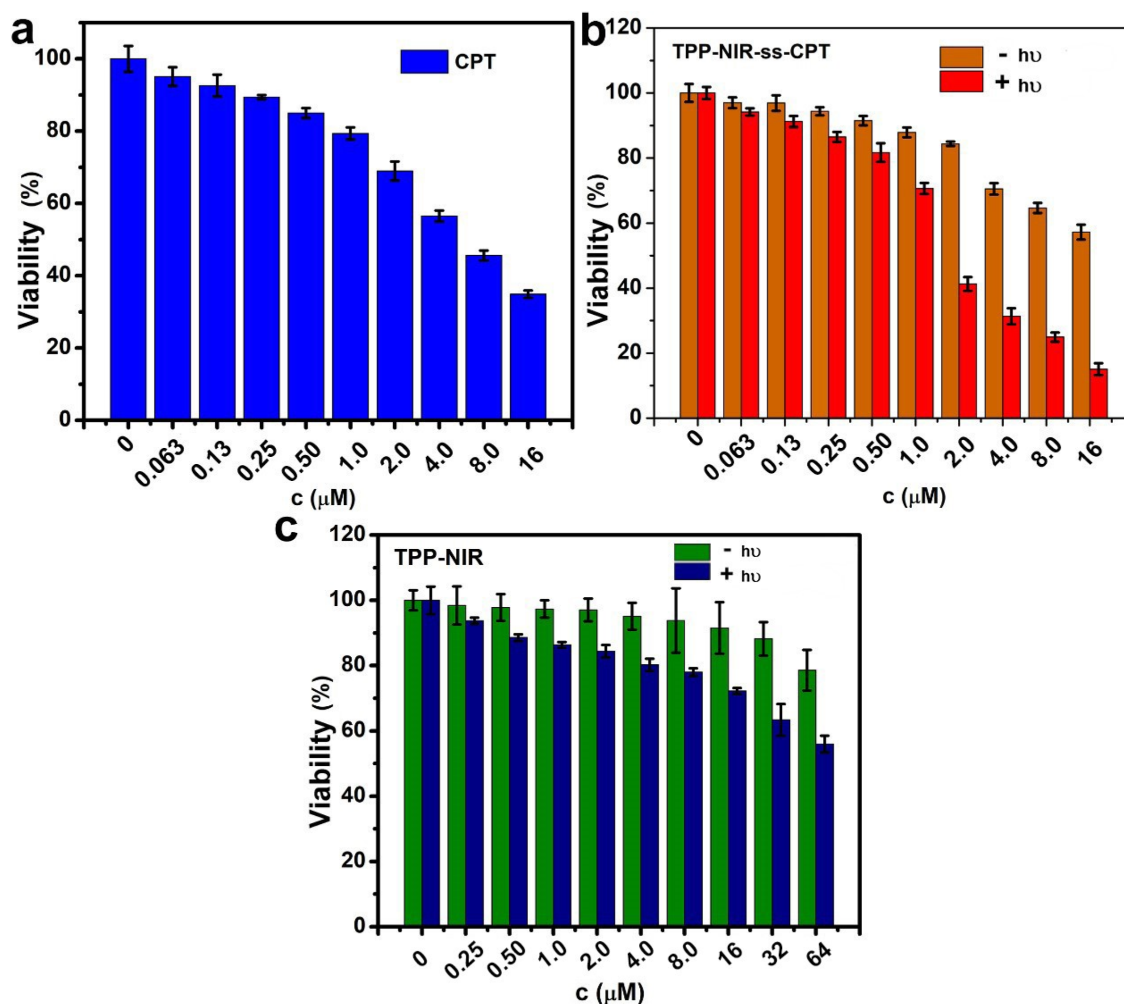


Figure S3. *In vitro* cytotoxicity evaluation with MTT assays. After 4 h incubation with (a) CPT (8 μM), (b) TPP-NIR-ss-CPT (8 μM) or (c) TPP-NIR (8 μM) and subsequent treatment with/without subsequent light irradiation (660 nm, 0.1W/cm^2 for 10 min) as indicated, the cells were further incubated for 20 h before subjected to MTT assays.

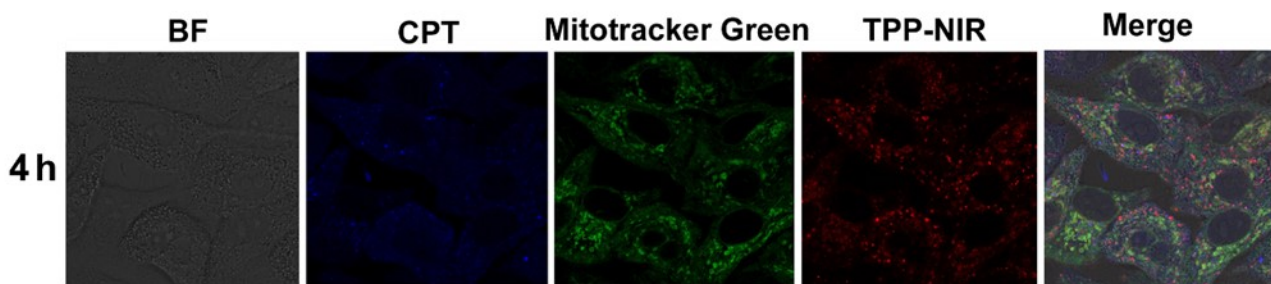


Figure S4. Intracellular localization of TPP-NIR-ss-CPT. A549 cells were treated with TPP-NIR-ss-CPT (8 μM) for 4 h. Mitotracker (green channel) was used to stain mitochondria. The Pearson correlation coefficients for CPT/Mitotracker Green channels and Mitotracker Green/TPP-NIR channels were calculated to be 0.72 and 0.82, respectively.

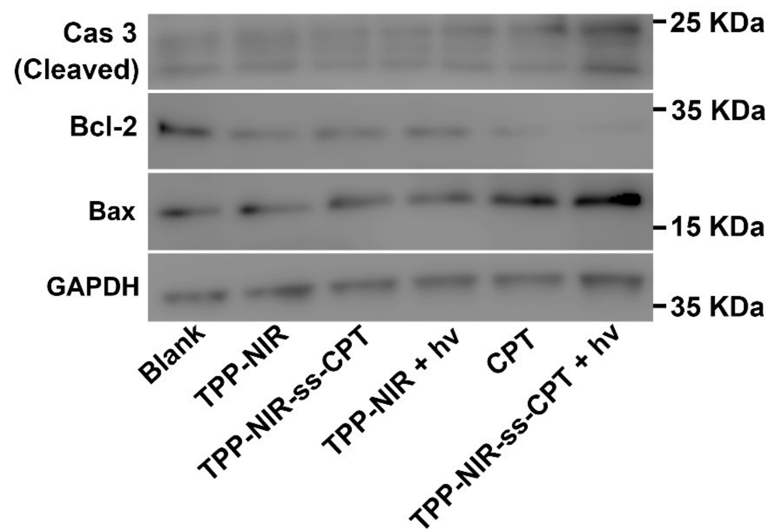


Figure S5. Representative western blotting analysis on the expression levels of cleaved caspase 3, Bcl-2, and Bax in A549 cells treated as indicated. GAPDH was used as a loading control.

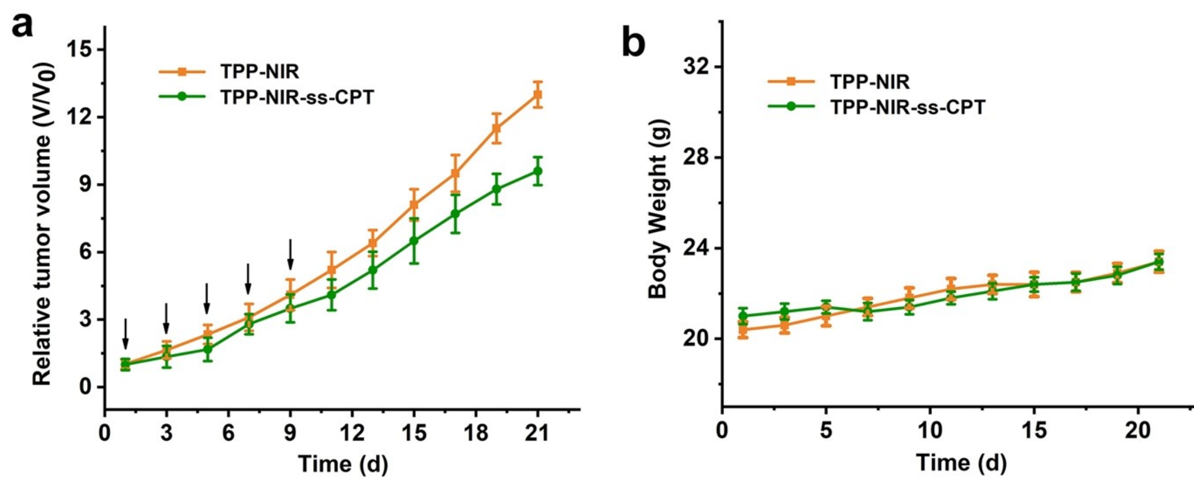


Figure S6. (a) Tumor volume changes of A549-tumor-bearing mice treated with TPP-NIR and TPP-NIR-ss-CPT (without light irradiation). (b) Body weight changes of A549-tumor-bearing mice treated as indicated.

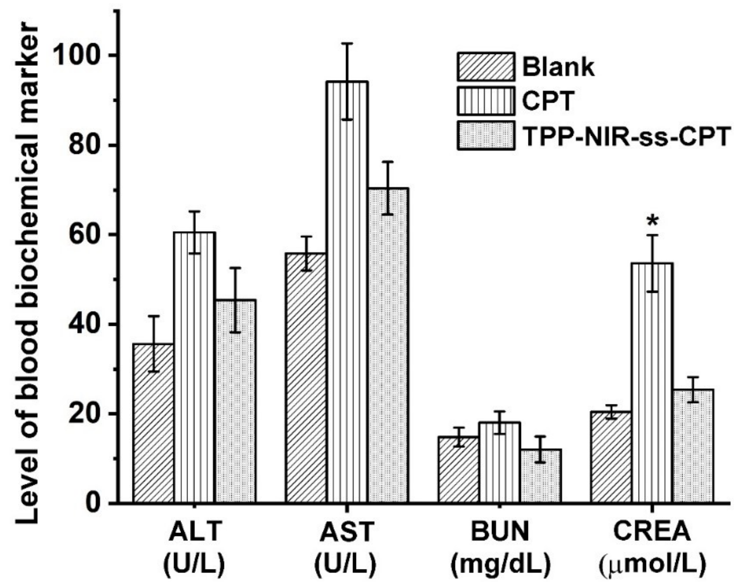


Figure S7. Blood biochemical analysis. Major biochemical indexes for hepatic and renal functions, including alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and creatinine (CREA) are shown for the mice treated as indicated. See **Biocompatibility evaluation** section for experimental details. *$p < 0.05$, compared to the mice treated with saline (blank).

References

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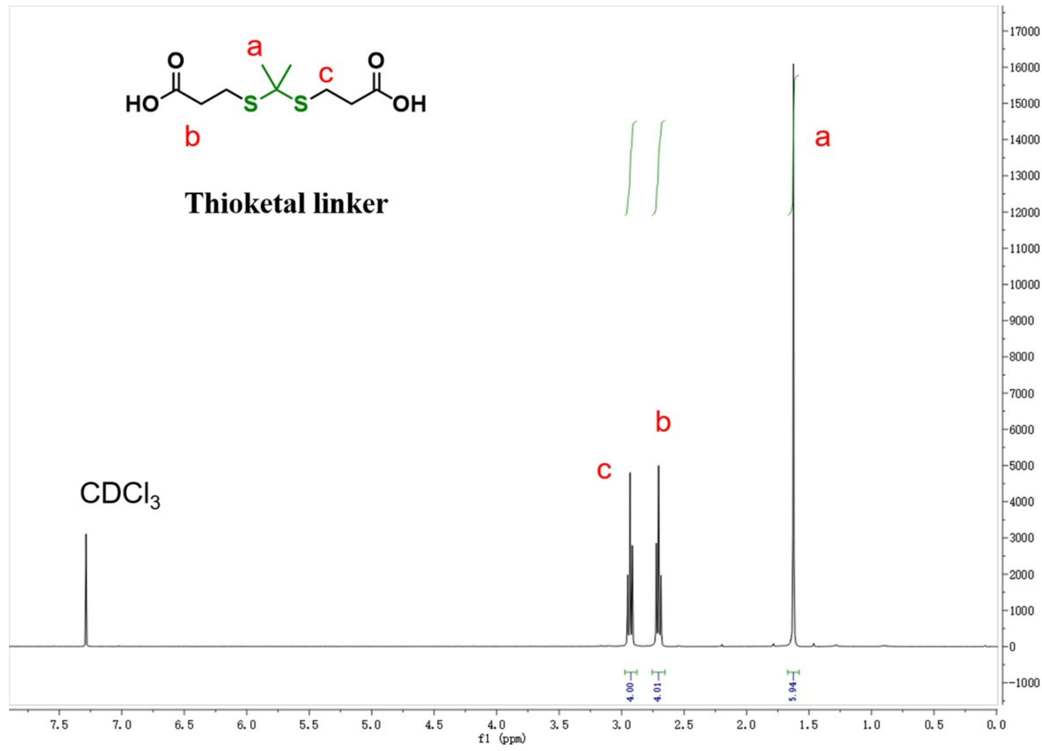
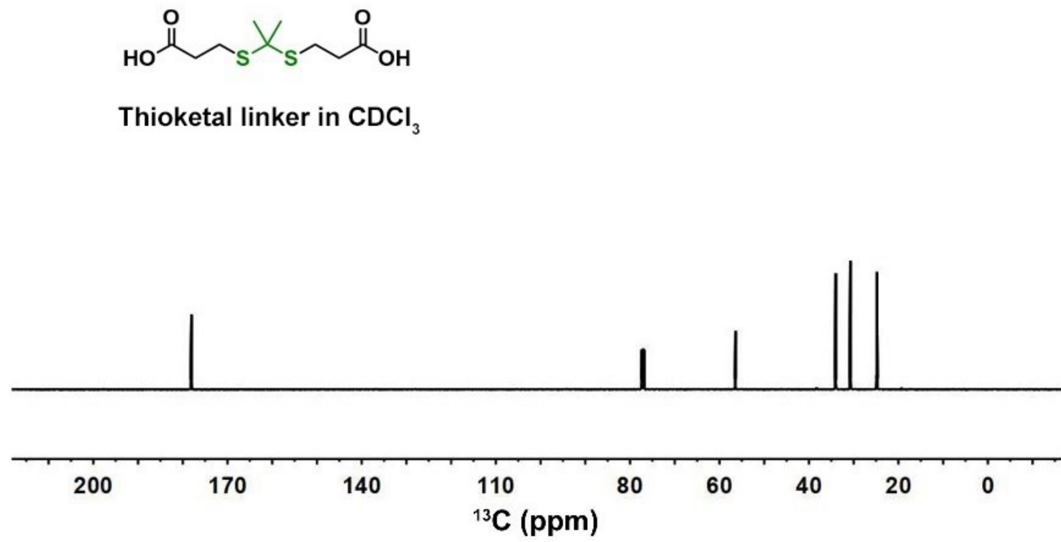
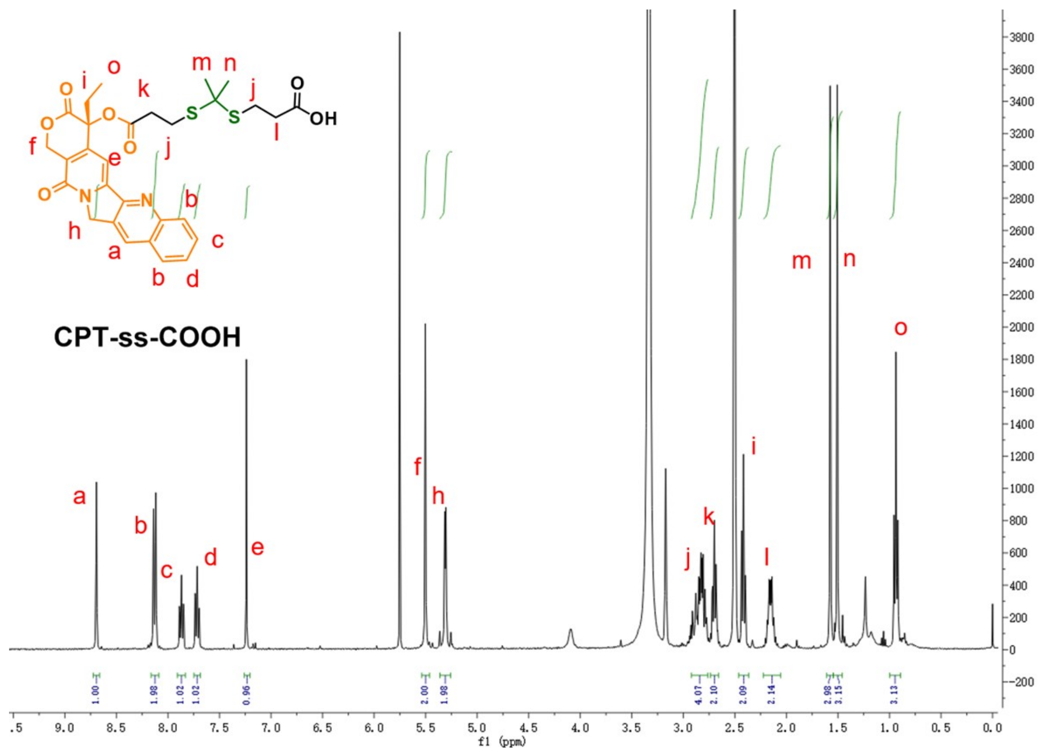
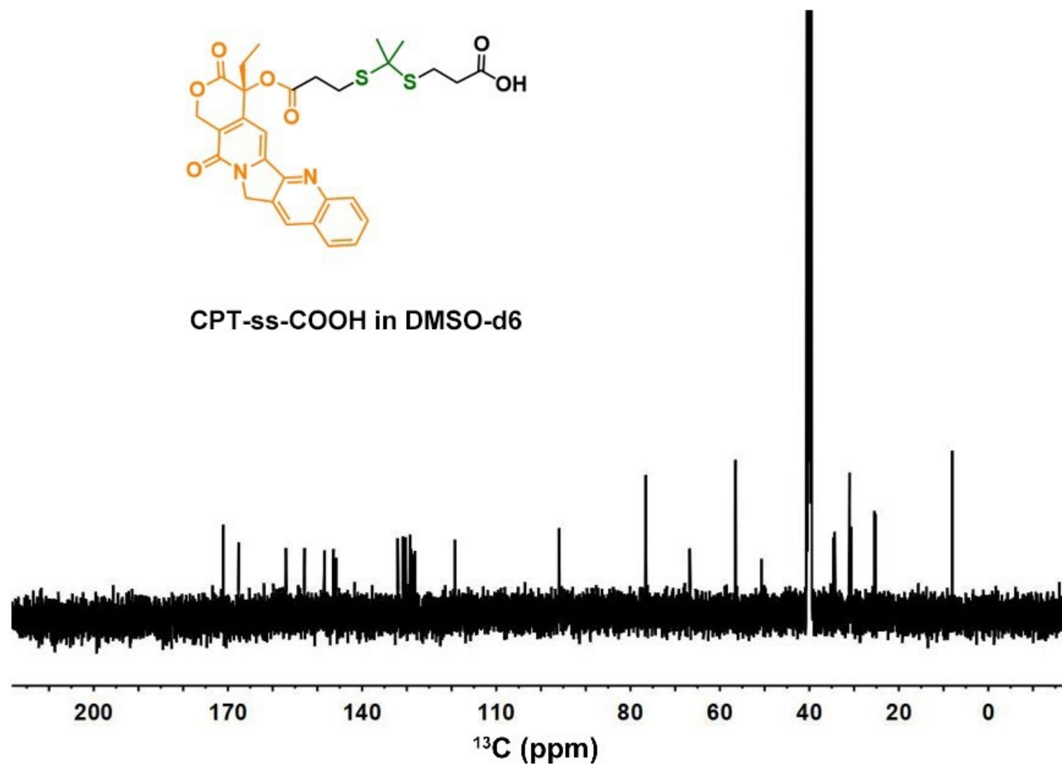
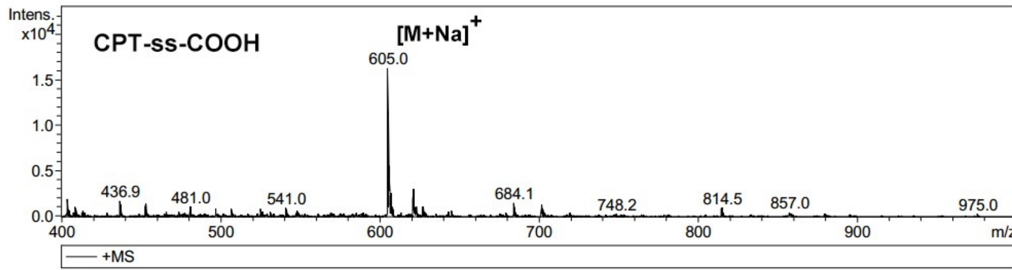
^{1}H-NMR, ^{13}C-NMR and ESI-MS

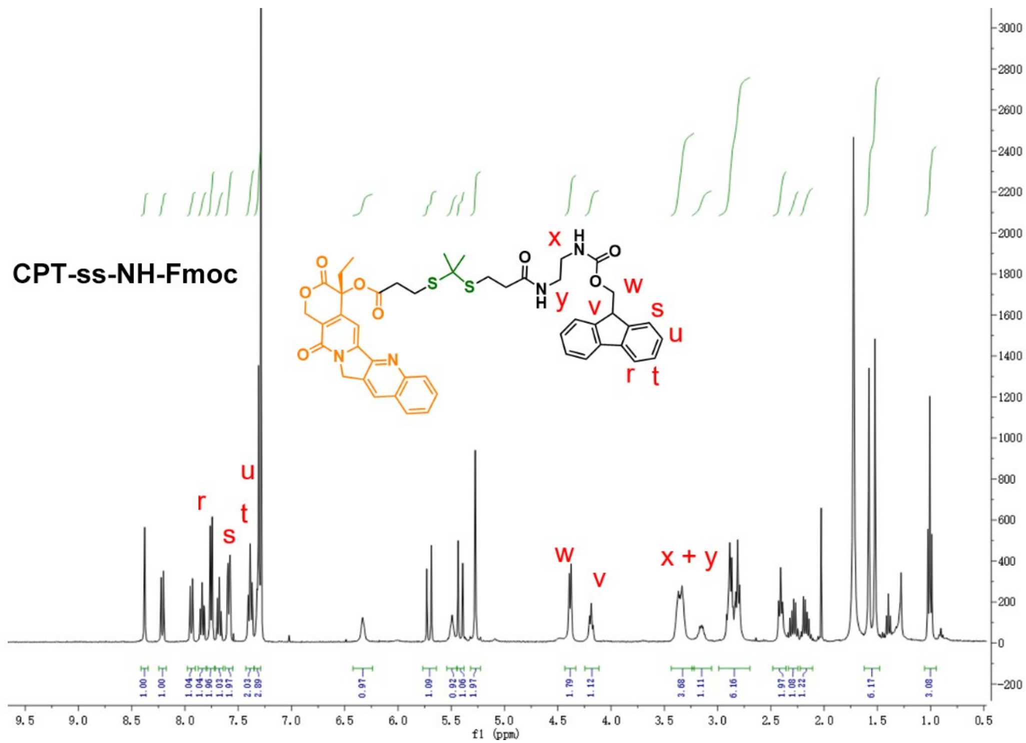


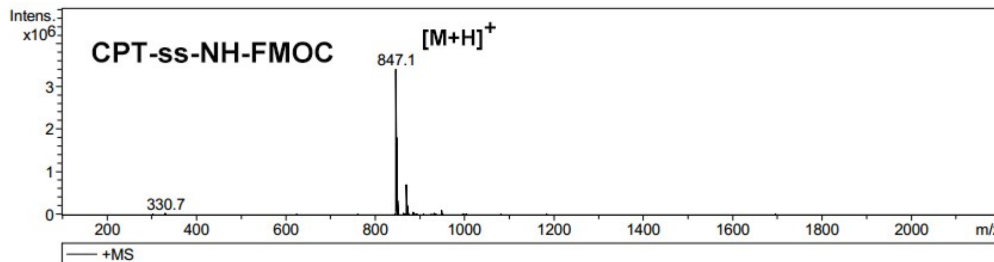


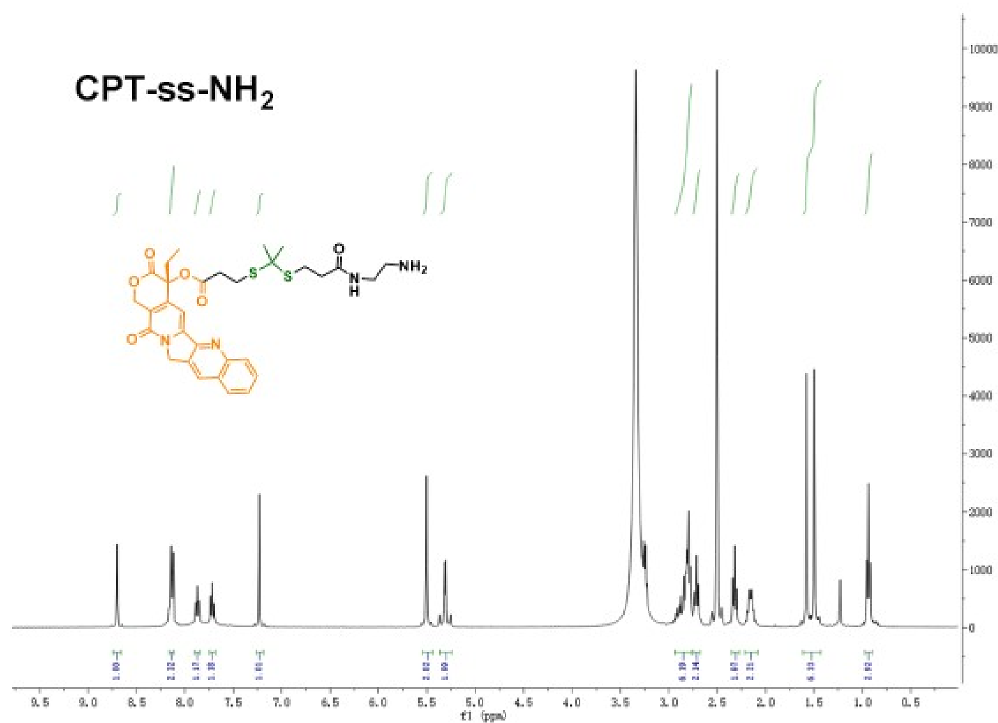

CPT-ss-COOH
f1 (ppm)

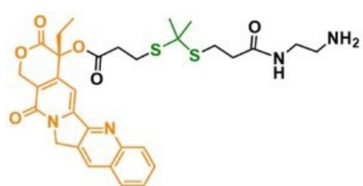

CPT-ss-COOH in DMSO-d6
^{13}C (ppm)

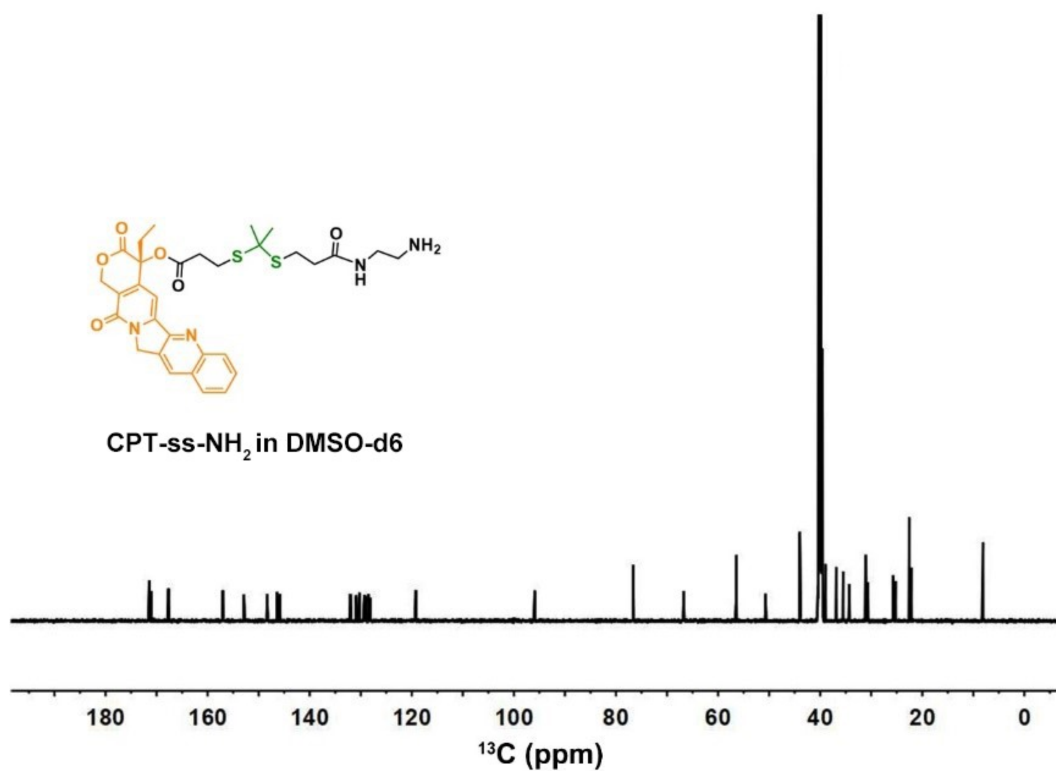

Intens.
x10^4
CPT-ss-COOH
[M+Na]+
605.0
436.9
481.0
541.0
684.1
748.2
814.5
857.0
975.0
m/z
+MS

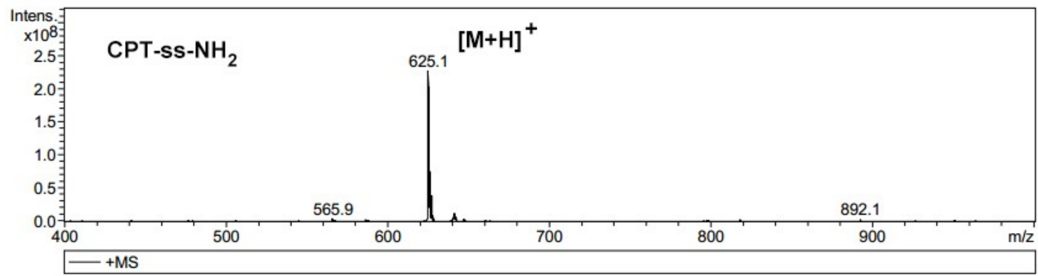

CPT-ss-NH-Fmoc
r
s
t
u
w
v
x + y
f1 (ppm)

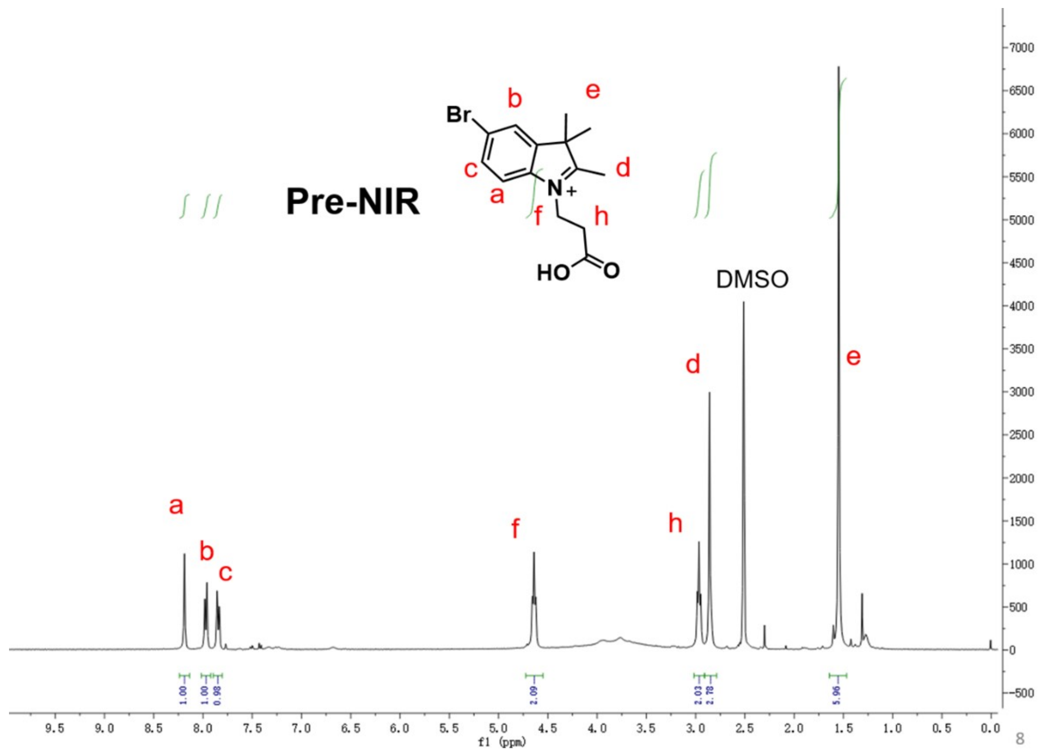

Intens.
x10^6
CPT-ss-NH-FMOC
[M+H]+
847.1
330.7
m/z
+MS


CPT-ss-NH2
f1 (ppm)

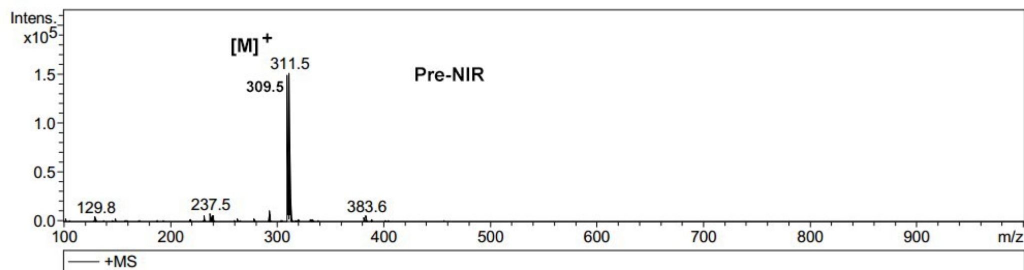


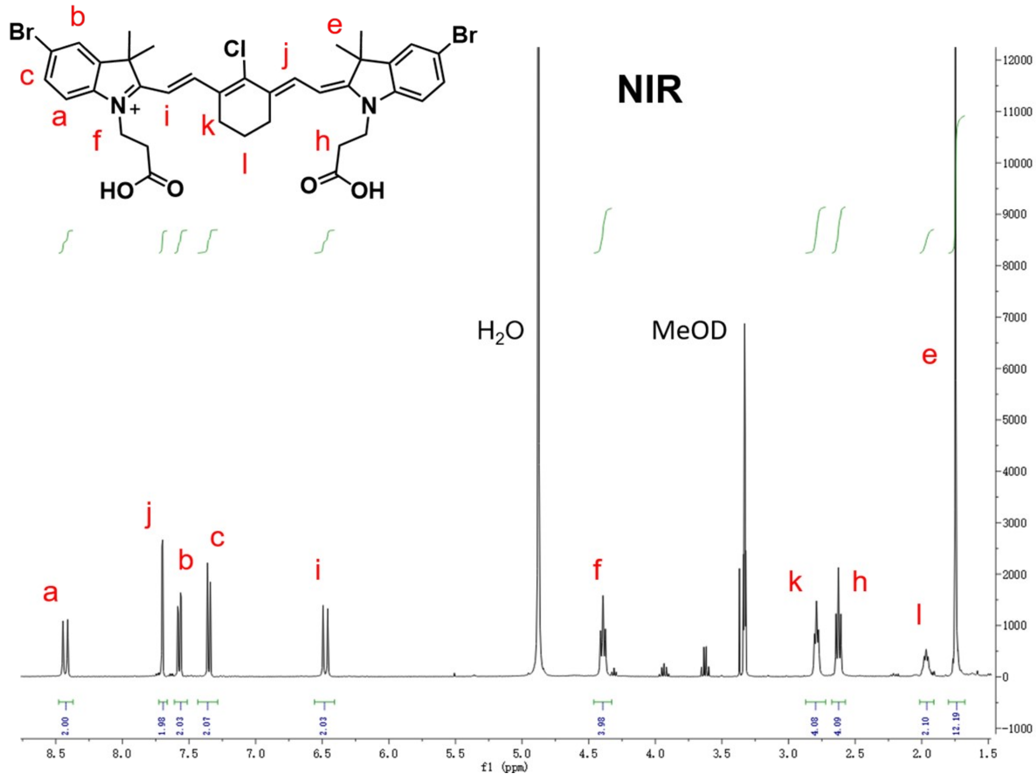

CPT-ss-NH2 in DMSO-d6
13C (ppm)

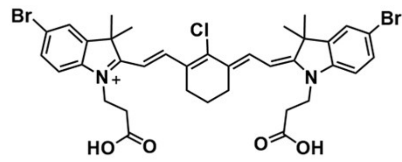




8

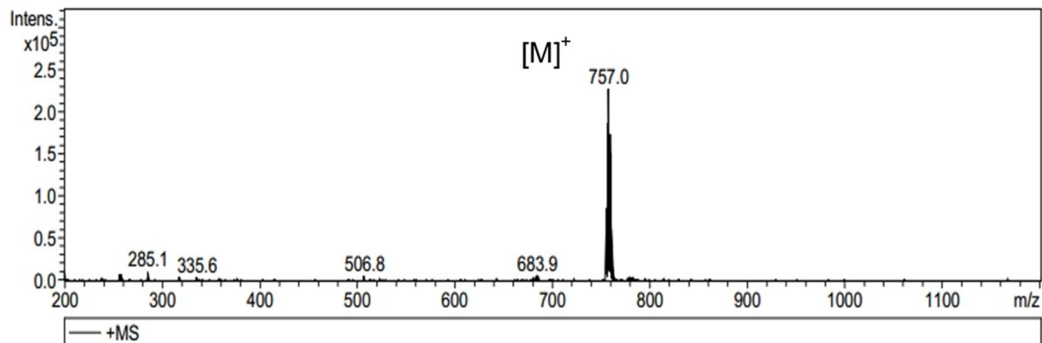


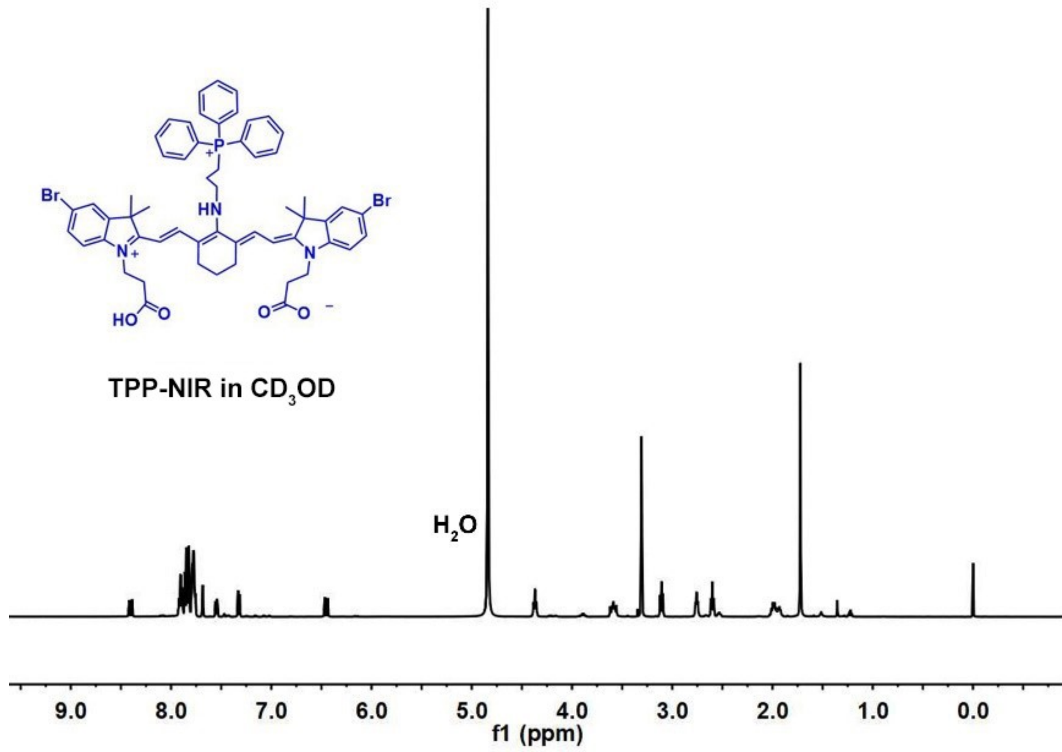


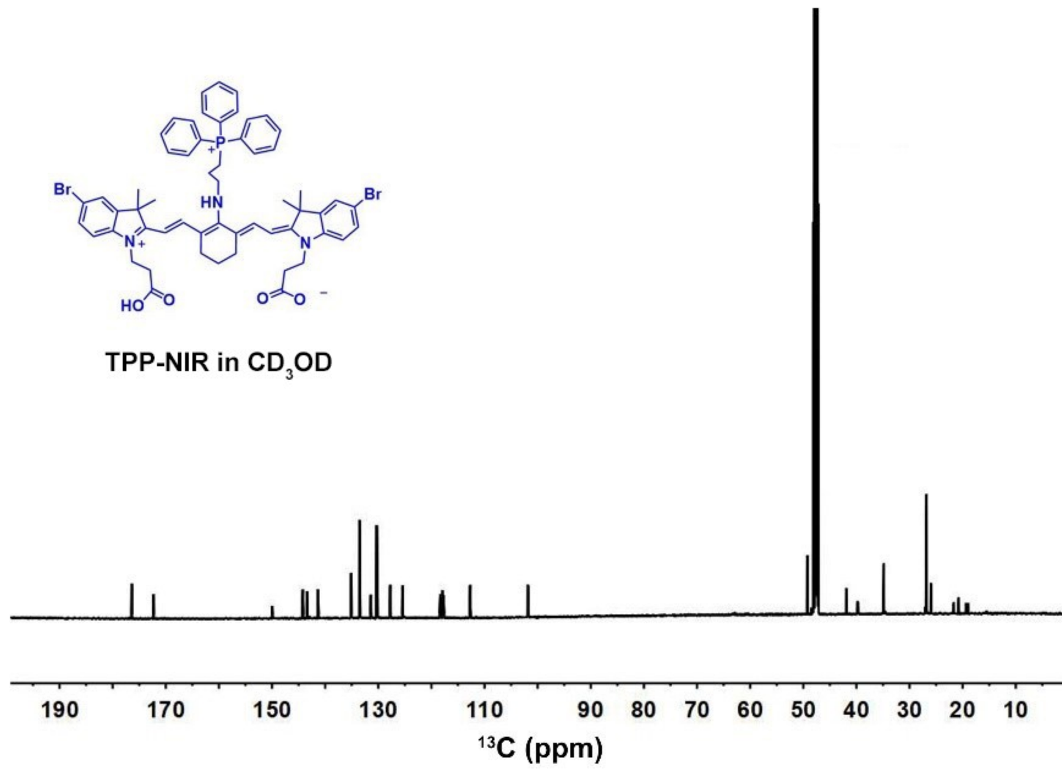


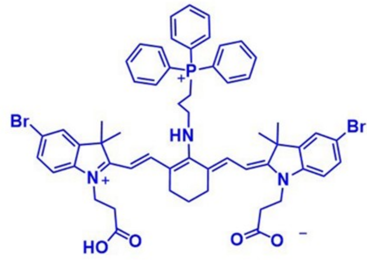
NIR

m/z:757.1



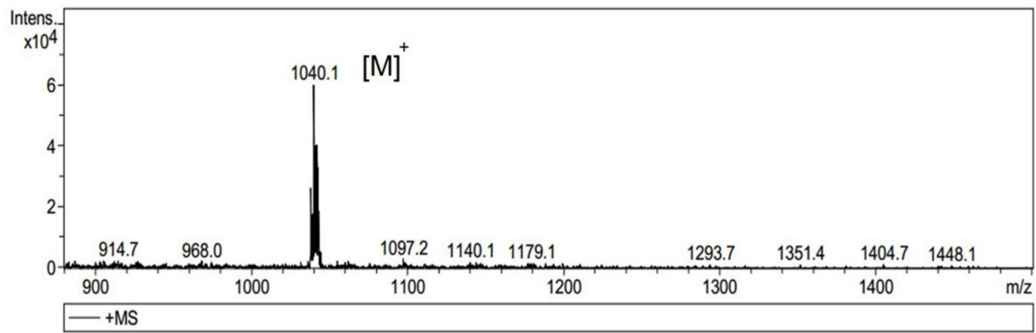

Br
HN
P
Br
N
N
HO
O
O
O
TPP-NIR in CD_3OD
H_2O
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

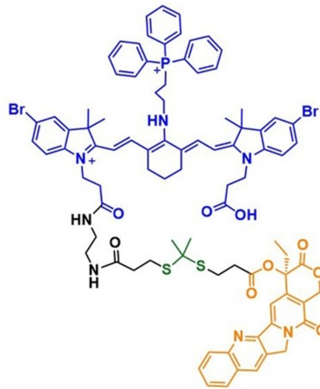

Br
HN
P
Br
N
N
HO
O
O
O
TPP-NIR in CD_3OD
190
170
150
130
110
90
80
70
60
50
40
30
20
10
^{13}C (ppm)



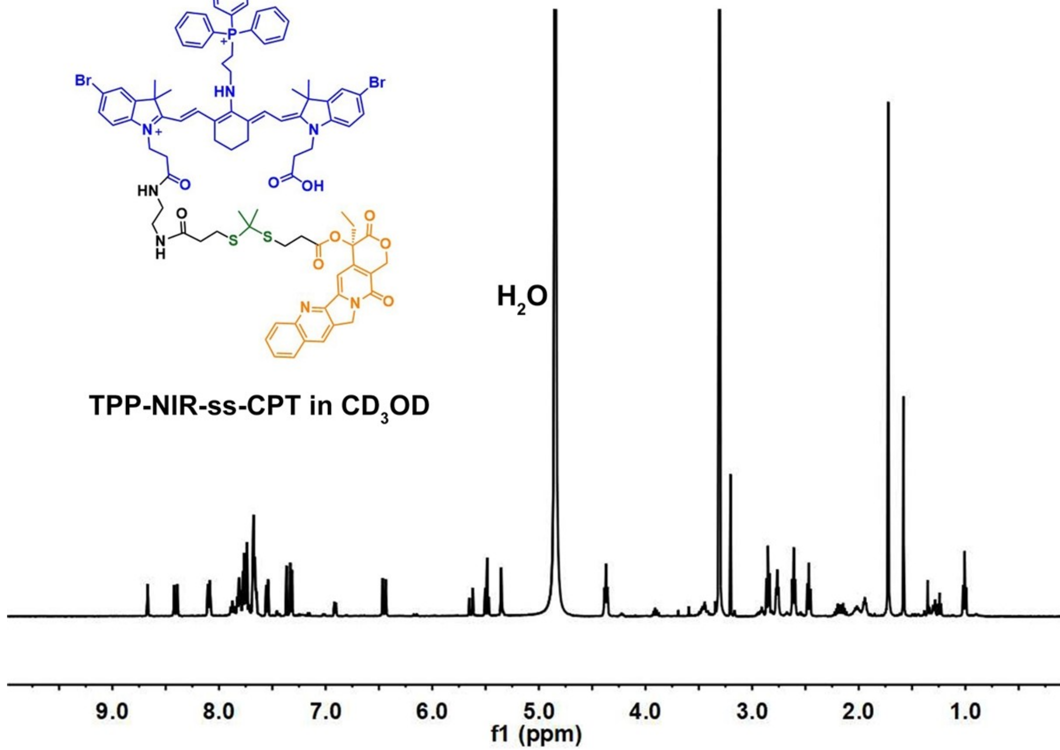
TPP-NIR

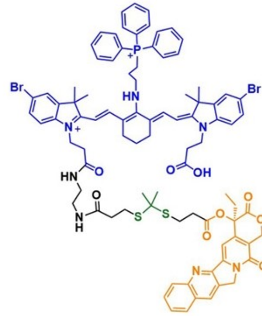
m/z: 1040.26

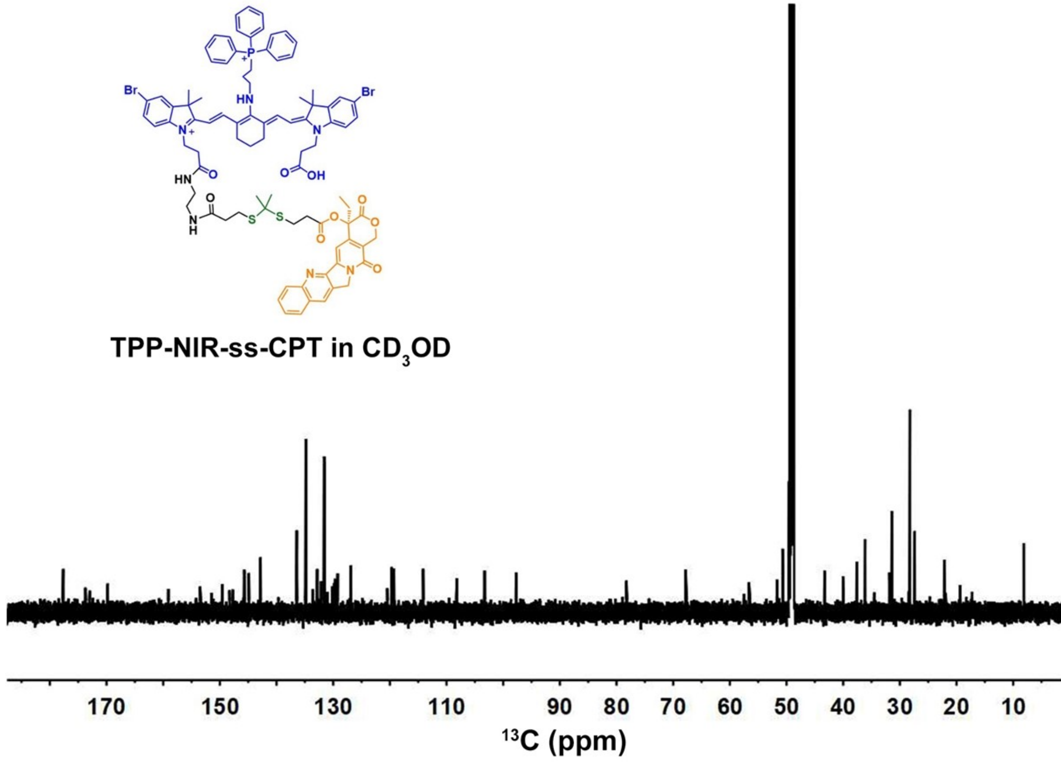


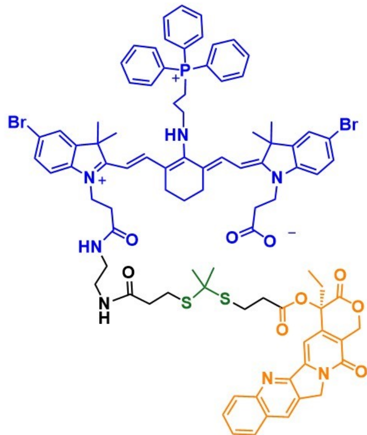


TPP-NIR-ss-CPT in CD_3OD



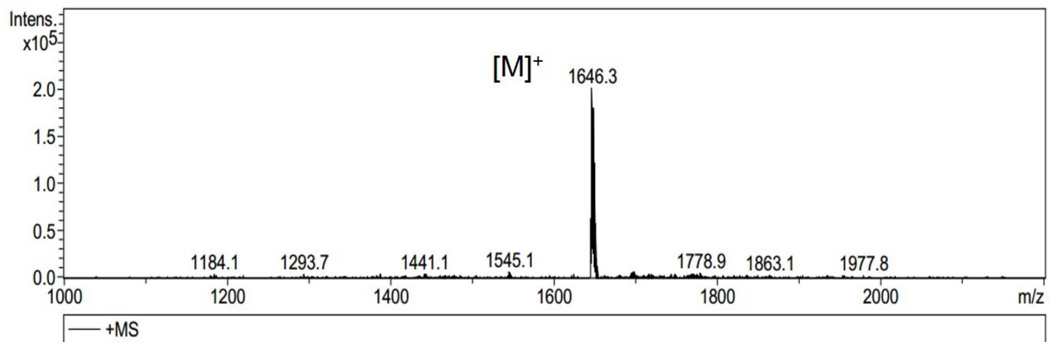


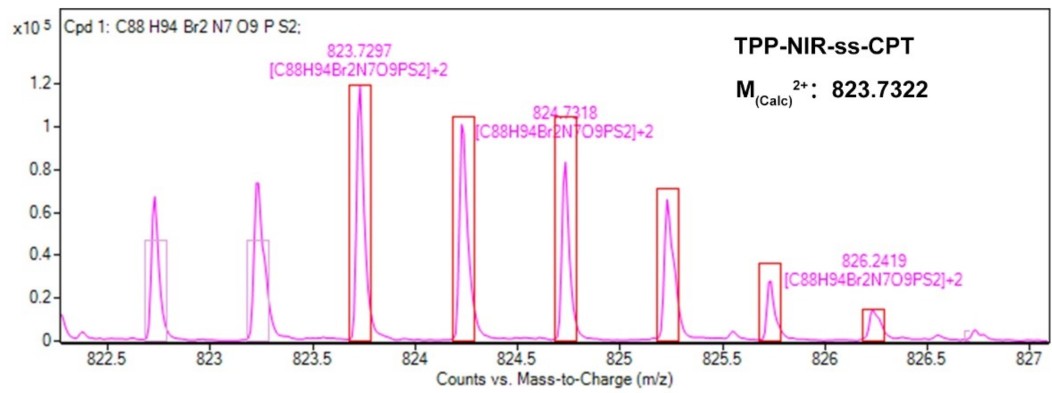




TPP-NIR-ss-CPT

m/z：1646.5




x10 5
Cpd 1: C88 H94 Br2 N7 O9 P S2;
823.7297
[C88H94Br2N7O9PS2]+2
824.7318
[C88H94Br2N7O9PS2]+2
826.2419
[C88H94Br2N7O9PS2]+2
TPP-NIR-ss-CPT
M(Calc)2+: 823.7322
1.2
1
0.8
0.6
0.4
0.2
0
822.5
823
823.5
824
824.5
825
825.5
826
826.5
827
Counts vs. Mass-to-Charge (m/z)