Electronic Supplementary Material (ESI) for ChemComm.

A nanoplatform for mild-temperature photothermal and type I & II photodynamic therapy in NIR-II Biowindow

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Materials

Unless stated otherwise, all the chemical reagents and solvents were obtained commercially and used without further purification. Compound 1, Compound 2, and 2-(5,6-difluoro-3-oxo-2,3-dihydro-1H-inden-1-ylidene)malononitrile (2FIC) were purchased from SunaTech Inc. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from PAA Laboratories (Austria). Other organic reagents were purchased from Meryer Chemical Inc and TCI Chemical Inc. Human cervical cancer cells (HeLa cells) were ordered from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). The Balb/c nude mice were obtained from the Chinese Academy of Sciences (Shanghai, China).

Instruments

The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AVANCE 400 MHz spectrometer. Scanning electron microscopy (SEM) investigations were carried out on a HitachiS-3400 SEM instrument. Dynamic light scattering measurements were performed on a goniometer ALV/CGS-3 using a UNIPHASE He-Ne laser operating at 632.8 nm. UV-Vis spectra were recorded in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer. Fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Varian Cary Eclipse. The output power of the laser was controlled by a fiber coupled laser system (LR-MFJ-980/1000mW, Changchun Lei Rui Optoelectronics Technology). The intracellular fluorescence imaging was observed using fluorescence microscopy (Nexcope, nib610-fl).

DFT calculation

All calculations were carried out with the Gaussian 16 software.[S1] The ground state geometry optimization was performed at B3LYP/Def2-SVP theoretical level. TD-DFT method was used for S1 and T1 states calculation.

Cell experiments

Intracellular ROS detection HeLa cells were incubated with DPTTIC NPs (100 μg/mL) for 6 h followed by incubation with ROS probes for 30 min. After being washed by PBS buffer for three times, cells were irradiated with 980 nm laser at a power density of 1.0 W/cm^2 for 10 min. Then, the fluorescence was immediately observed using confocal laser scanning microscopy. In order to investigate the effects of Vc, the cells were incubated with Vitamin C (Vc, 4 μM) at 37 ^{o}C for 2 h, followed by incubation with DPTTIC NPs (100 μg/mL) and then DCFH-DA (10 μM) for 30 min. All the other experimental procedures were the same to the experiment mentioned above.

Cytotoxicity experiments HeLa cells were incubated in DMEM. The medium was supplemented with 10% FBS and 1% Penicillin-Streptomycin. HeLa cells were seeded in 96-well plates (5×10^4 cell mL^{-1}, 0.1 mL per well) for 24 h at 37 ^{o}C in 5% CO_2. Then DMEM containing different concentrations of DPTTIC NPs was introduced to replace the original medium. Four hours later, the cells were treated with or without an 980 nm laser (1.0 W cm^{-2}). After 10 min irradiation, HeLa cells were cultured for the next 24 h. The relative cellular viability was determined by the MTT assay. In order to investigate the only PTT activity, the cells were incubated with Vitamin C (Vc, 4 μM) at 37 ^{o}C for 2 h, followed by incubation with DPTTIC

NPs. All the other experimental procedures were the same to the experiment mentioned above.

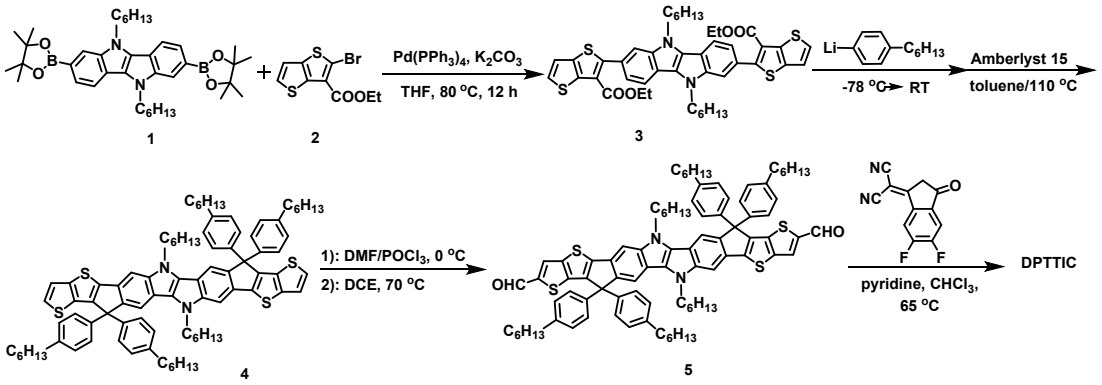
Live-Dead Cell Staining The same density of HeLa cells (3×10^5 cell mL^{-1}) were distributed into three confocal dishes (35 mm) for 12 h. Then the 2-plate cells were cultured with new DMEM containing DPTTIC NPs (200 μg/mL). After 5 h, the cells were subjected to dark or laser irradiation (980 nm, 1.0 W/cm^2, 10 min). After 48 h, the cells were stained with a calcein AM/propidium iodide mixture for 30 min and washed twice using PBS. The fluorescence images were eventually acquired via a confocal laser scanning microscope.

In vivo antitumor

All animal experiments were approved by the Laboratory Animal Ethics Committee of the Nantong University.

After being acclimated and tested for infectious diseases for 1 week, 4-week-old Balb/c mice were subcutaneously injected with HeLa cells (1×10^7 cells each mouse) at the flank region. When the tumor volume reached approximately 100 mm^3, the HeLa tumor-bearing Balb/c nude mice were stochastically assigned to four groups (n = 4) for different treatments. DPTTIC NPs (1 mg mL^{-1}, 100 μL) were administered into the mice via tail vein. At 24 h postinjection, the tumor sites were irradiated by 980 nm laser (1 W/cm^2). The body weight and tumor volumes of mice from different groups were continually measured and recorded up to 18 days. After 18 days, the tumors were dissected for H&E staining.

Synthesis



Scheme S1 The synthetic route for DPTTIC

Compound 3 Compound **1** (626 mg, 1.00 mmol), compound **2** (699 mg, 2.40 mmol), $Pd(PPh_3)_4$ (173 mg, 0.15 mmol), K_2CO_3 (11.04 g, 80.00 mmol) and THF/H_2O (120 mL, v/v, 2/1) were added to a three-neck round-bottom flask under argon. The mixture was refluxed for 12 h and then cooled down to room temperature. The crude product was obtained through filtration. The crude product can be used for the next step without further purification. The crude product was further purified by column chromatography on silica gel using dichloromethane as the eluent yielding a yellow solid (700 mg, 88%). ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.80 (d, J = 8.2 Hz, 2H), 7.64 (s, 2H), 7.41 (d, J = 5.2 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 5.2 Hz, 2H), 4.45 (t, J = 7.2 Hz, 4H), 4.22 (q, J = 7.0 Hz, 4H), 1.98 – 1.88 (m, 4H), 1.22 – 1.17 (m, 12H), 0.77 (t, J = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ.162.3, 155.4, 140.8, 140.3, 135.8, 128.1, 127.2 (2C), 120.6, 120.2, 118.9, 117.1, 114.4, 111.6, 60.9, 45.5, 31.6, 30.4, 26.9, 22.5, 14.2, 14.0. MALDI-TOF-MS: *m/z* 794.07 (M^+).

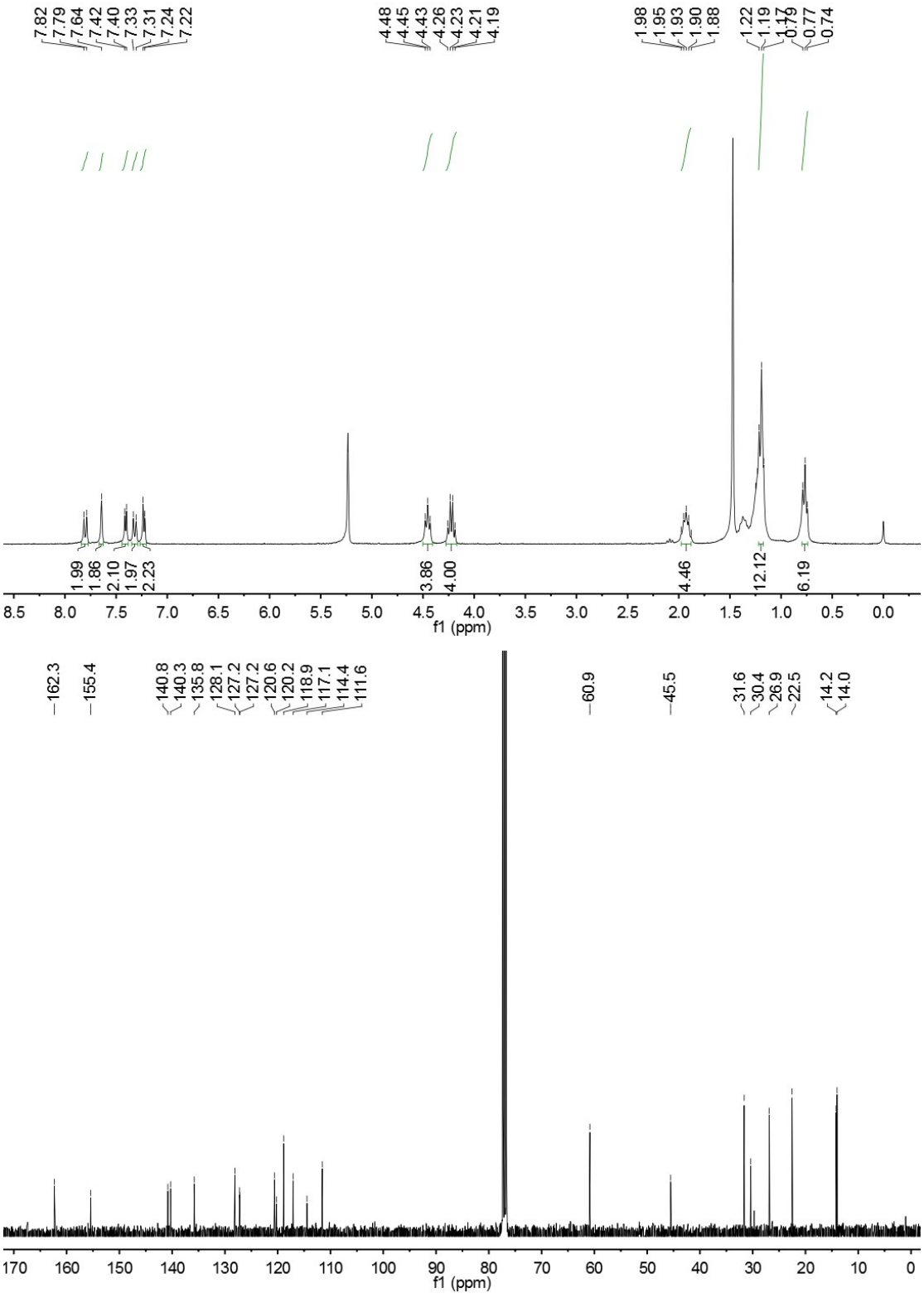


Fig. S1 The ^{1}H NMR and ^{13}C NMR spectra for compound 3

Compound 4 To a solution of (4-bromophenyl)hexane (579 mg, 2.4 mmol) in dry THF (20 mL) was added ^{n}BuLi (1.00 mL, 2.4 M in hexane) dropwise at -78 °C

under argon. The reaction mixture was stirred at the same temperature for 2 h, then an anhydrous THF (10 mL) solution of compound **3** (239 mg, 0.30 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight. Saturated aq. NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed by a rotating evaporator and the residue was used for next step without further purification. The residue was dissolved in toluene (30 mL) and Amberlyst 15 (250 mg) was added. The reaction mixture was heated to 110 °C and stirred for 3 h. The mixture was cooled down to room temperature and filtered. After removing the solvent from filtrate, the rude product was obtained by column chromatography on silica gel using petroleum ether/dichloromethane (20 : 1) as eluent. The rude product can be used for the next step without further purification.

Compound 5 A Vilsmeier reagent, which was prepared with $POCl_3$ (0.4 mL) in DMF (2 mL), was added to a solution of compound **4** (132 mg, 0.10 mmol) in 1,2-dichloroethane (20 mL) under the protection of argon. The mixture was stirred at reflux for 20 h. After cooling down to room temperature, the mixture was poured into ice water (50 mL) and then extracted with dichloromethane (2 × 50 mL). After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using petroleum ether/dichloromethane (1 : 1) as eluent to give a red solid (128 mg, 93%). ^{1}H NMR (400 MHz, CD_2Cl_2): δ 9.80 (s, 2H), 7.90 (s, 2H), 7.62 (s, 2H), 7.23 (s, 2H), 7.14 (d, J = 8.2 Hz, 8H), 7.06 (d, J = 8.1 Hz, 8H), 4.04 – 3.71 (m, 4H), 2.52 – 2.47 (m, 8H), 1.62 – 1.59 (m, 4H), 1.54 – 1.48 (m, 8H),

1.25 – 1.18 (m, 36H), 0.77 (d, J = 6.9 Hz, 12H), 0.66 (t, J = 6.8 Hz, 6H). The ^{13}C NMR spectrum was not obtained due to its poor solubility. MALDI-TOF-MS: *m/z* 1370.88 (M^+).

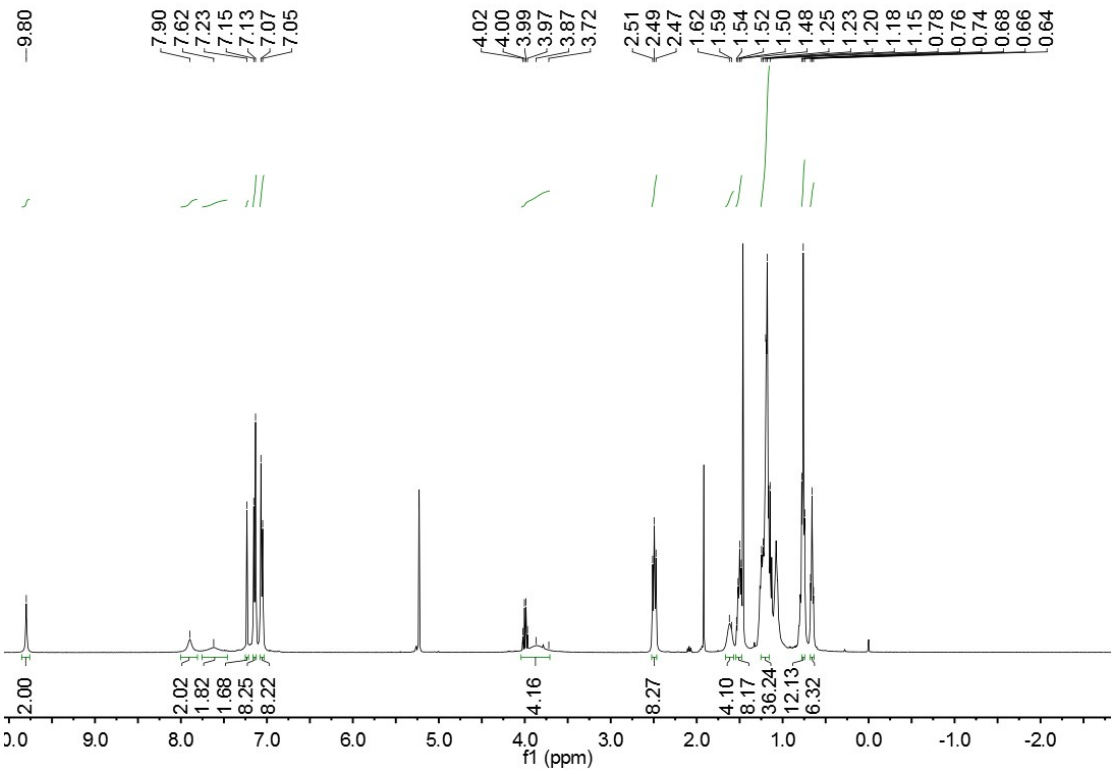
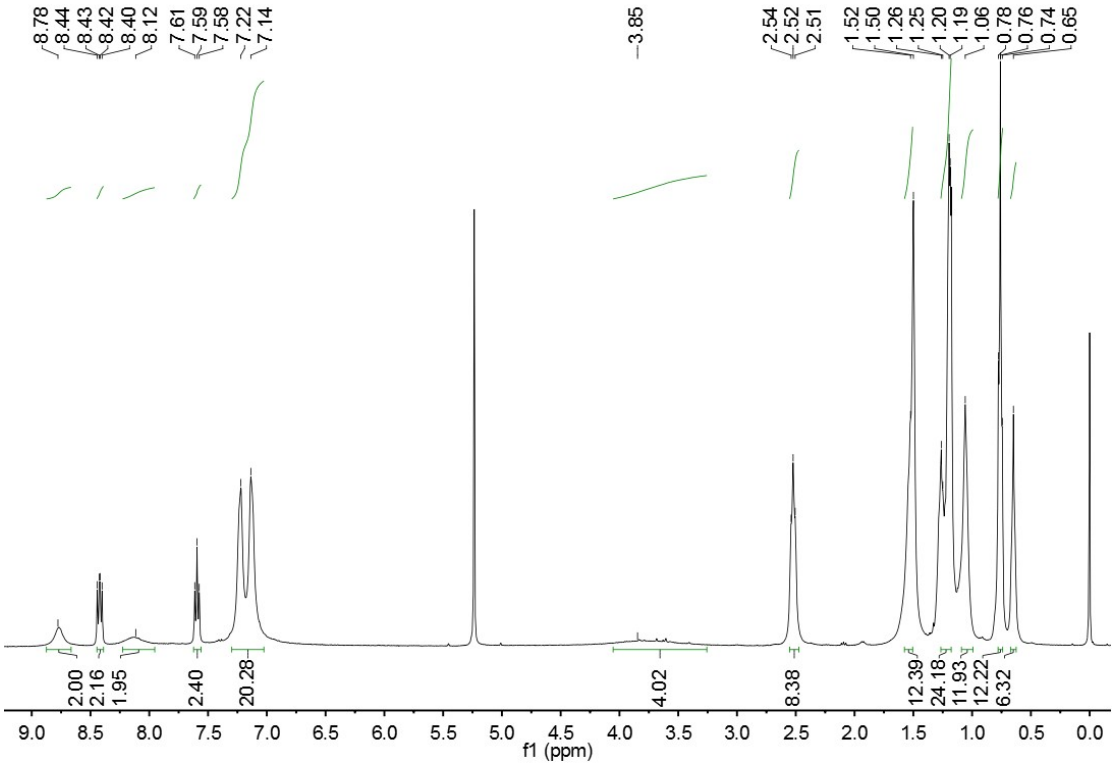


Fig. S2 The ^1H NMR spectrum for compound 5

DPTTIC To a three-necked round bottom flask were added compound **5** (137 mg, 0.10 mmol), 2FIC (92 mg, 0.40 mmol), pyridine (0.3 mL) and chloroform (15 mL). The mixture was stirred at reflux for 20 h. The mixture was washed by methanol (100 mL) and filtered. The residue was purified by column chromatography on silica gel using dichloromethane as eluent yielding a blue solid (117 mg, 65%). ^1H NMR (400 MHz, CD_2Cl_2) δ 8.78 (s, 2H), 8.42 (dd, J = 10.0, 6.6 Hz, 2H), 8.12 (bs, 2H), 7.59 (t, J = 7.6 Hz, 2H), 7.22 – 7.14 (m, 20H), 3.85 (bs, 4H), 2.54 – 2.51 (m, 8H), 1.52 – 1.50 (m, 12H), 1.26 – 1.19 (m, 24H), 1.06 (s, 12H), 0.76 (t, J = 6.7 Hz, 12H), 0.65 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.8, 154.7, 154.6, 154.5, 152.1, 152.0, 151.9

(2C), 141.1, 140.1, 139.9, 139.8, 139.6, 139.4, 135.7, 135.6, 133.6, 133.4 (2C), 127.7, 126.9, 123.8, 113.9, 113.7, 113.5 (2C), 113.4 (2C), 111.5, 111.4, 61.4, 43.8, 34.6, 30.7, 30.6, 30.3, 28.7, 28.2, 25.6, 21.6, 21.4, 13.1, 12.9. MALDI-TOF-MS: *m/z* 1796.84 (M^+).



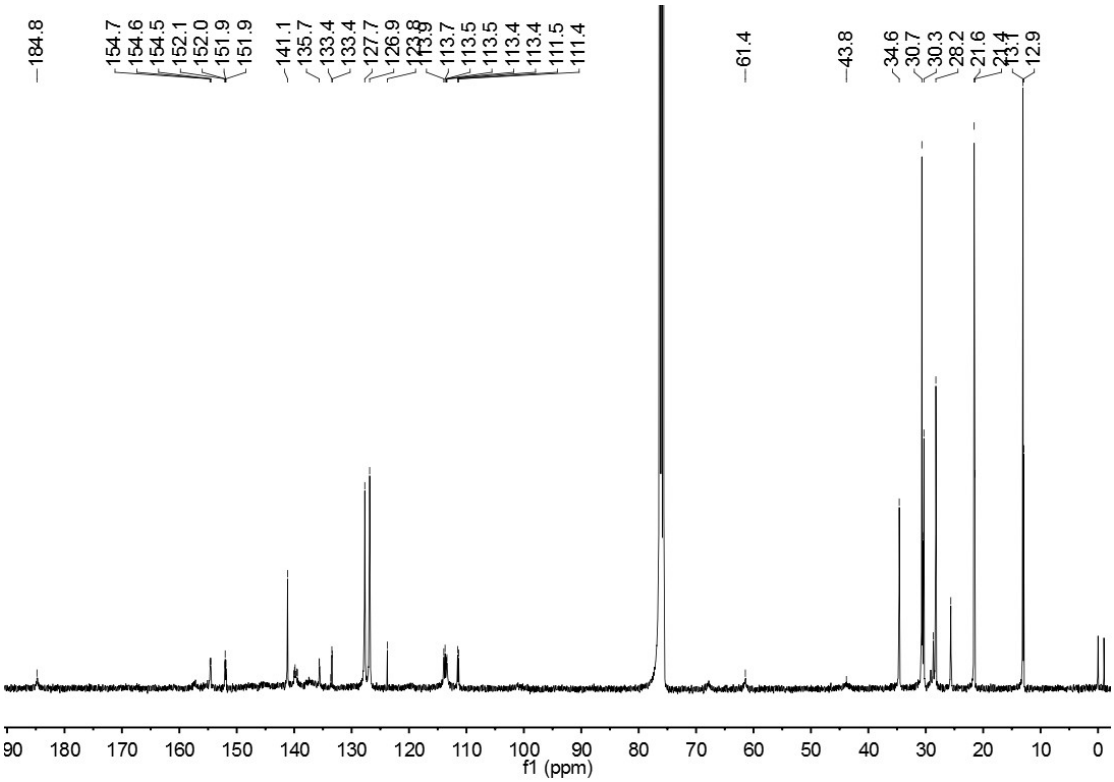
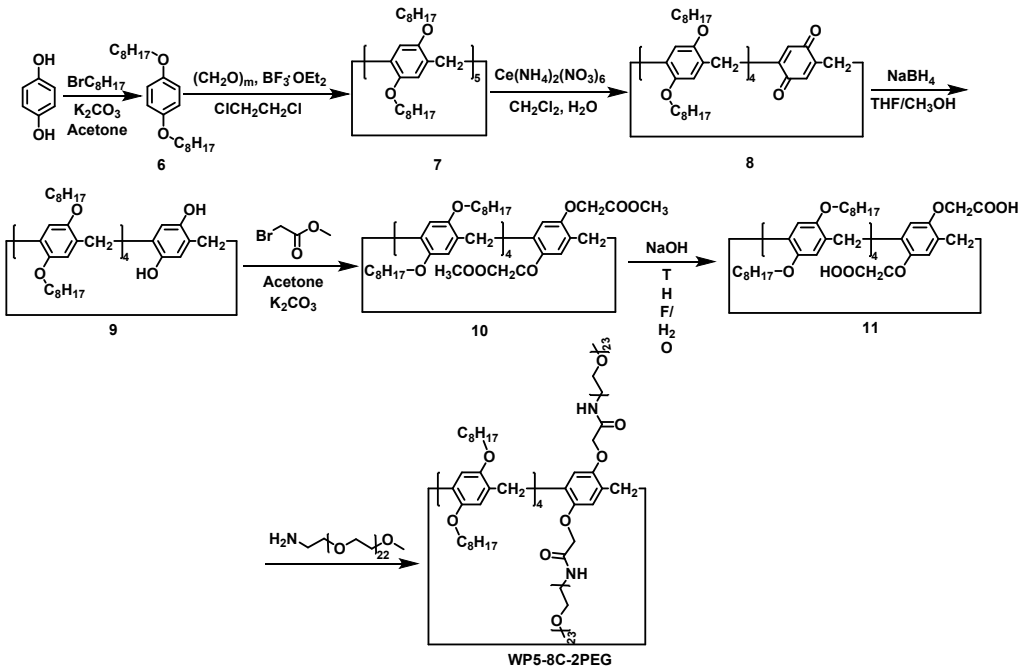


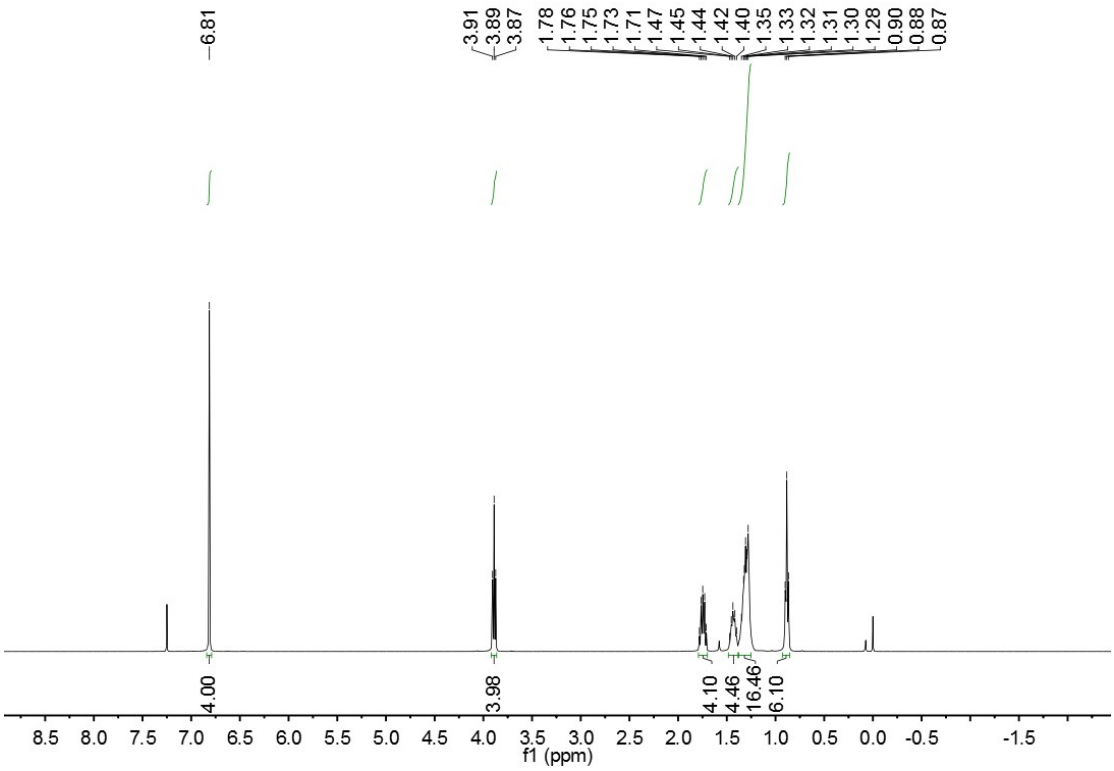
Fig. S3 The ^{1}H NMR and ^{13}C NMR spectra for DPTTIC



Scheme S2 The synthetic route for WP5-8C-2PEG

Compound 6 [S2] To an one-necked round bottom flask were added hydroquinone (11 g, 100 mmol), 1-bromooctane (58 g, 300 mmol), K_2CO_3 (36.1 g, 260 mmol) and

acetone (240 mL). The mixture was stirred at 60 °C overnight. The cooled reaction mixture was filtered. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using petroleum ether/dichloromethane (10 : 1) as eluent to give a white solid (23 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (s, 4H), 3.89 (t, *J* = 6.6 Hz, 4H), 1.78 – 1.71 (m, 4H), 1.47 – 1.40 (m, 4H), 1.35 – 1.28 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.2, 115.4, 68.6, 31.8, 29.4 (2C), 29.3, 26.1, 22.7, 24.1.



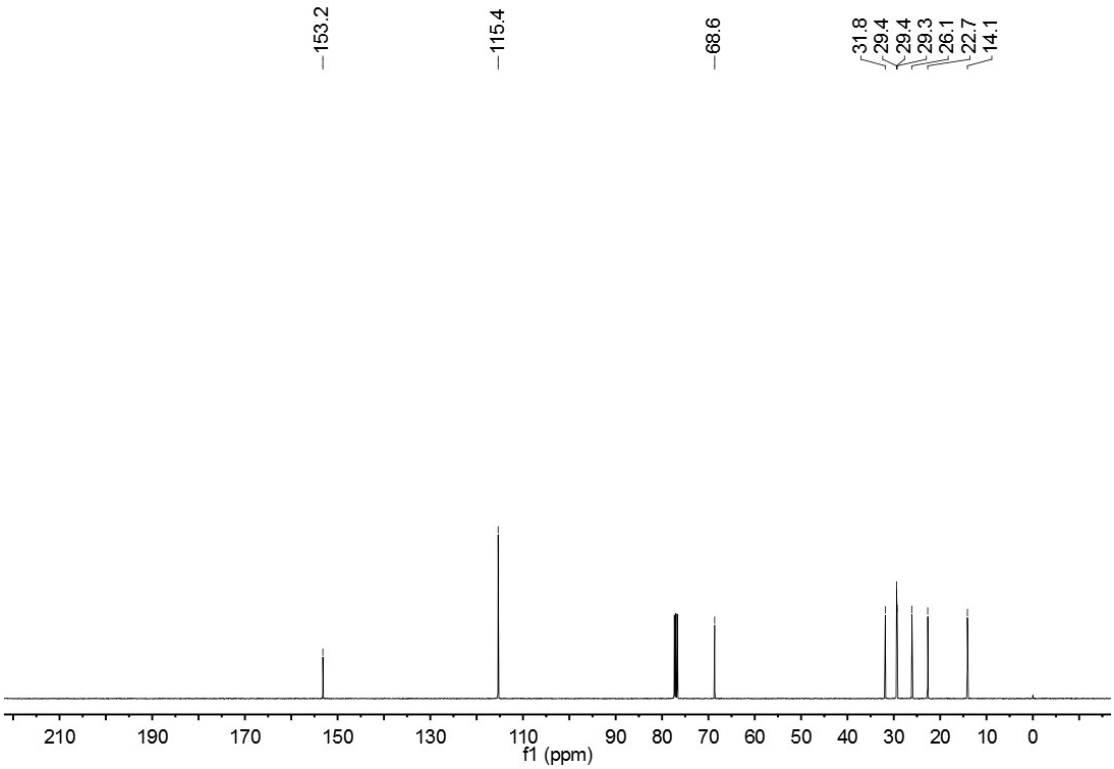


Fig. S4 The ^{1}H NMR and ^{13}C NMR spectra for compound 6

Compound 7 [S3] To an one-necked round bottom flask were added 1,4-dioctoxybenzene (669 mg, 2.00 mmol), polyoxymethylene (186 mg) and 1,2-dichloroethane (20 mL). The mixture was stirred for 10 min at room temperature, and then Boron fluoride diethyl ether (285 mg, 2 mmol) was added. The mixture was stirred at room temperature for an additional 30 min. Saturated aq. $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (2 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using petroleum ether/dichloromethane (5 : 1) as eluent to give a white solid (284 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 10H), 3.85 (s, 20H), 3.75 (s, 10H), 1.86 – 1.78 (m, 20H), 1.53 – 1.47 (m, 20H), 1.37 – 1.15 (m, 80H), 0.86 (d, J = 5.4 Hz, 30H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 128.1, 114.6, 68.2, 31.8, 29.9, 29.6, 29.3, 26.4, 22.6,

14.1.

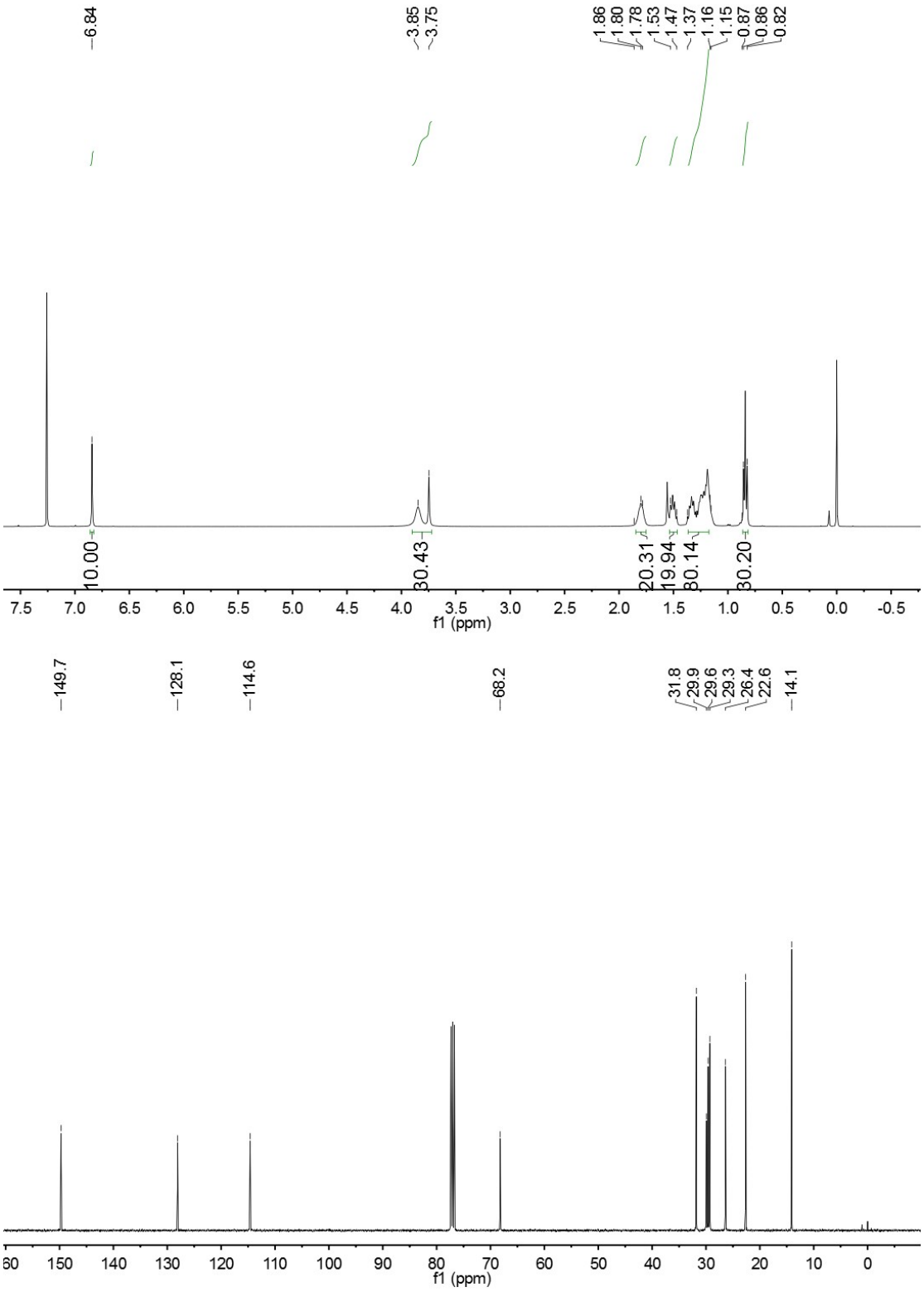
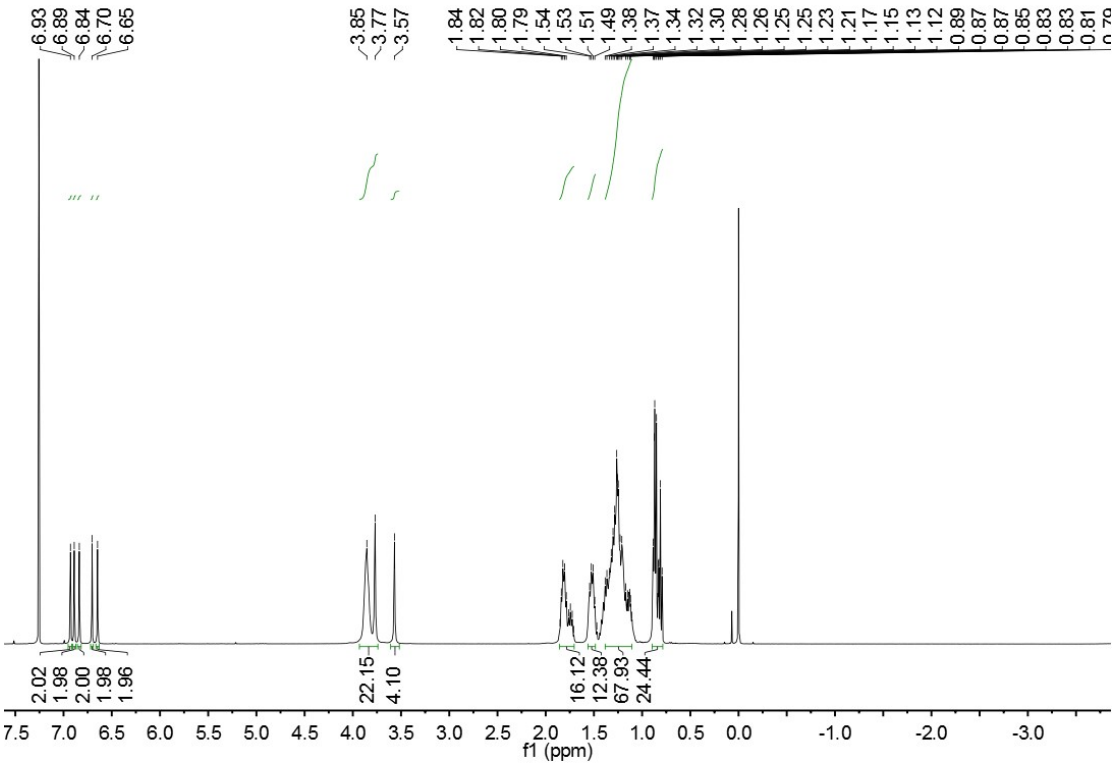


Fig. S5 The ^{1}H NMR and ^{13}C NMR spectra for compound 7

Compound 8 To an one-necked round bottom flask were added compound 8 (866

mg，0.5 mmol), Ce $(NH_4)_2(NO_3)_6$ (822 mg，1.50 mmol), CH_2Cl_2 (20 mL) and H_2O (2 mL). The mixture was stirred at room temperature for 12 h. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using petroleum ether/dichloromethane (5 : 1) as eluent to give a red solid (467 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 2H), 6.89 (s, 2H), 6.84 (s, 2H), 6.70 (s, 2H), 6.65 (s, 2H), 3.85 – 3.77 (m, 22H), 3.57 (s, 4H), 1.85 – 1.71 (m, 16H), 1.54 – 1.47 (m, 12H), 1.38 – 1.11 (m, 68H), 0.89 – 0.79 (m, 24H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.7, 150.2, 149.8, 149.7, 146.2, 133.4, 129.6, 128.4, 127.8, 123.1, 114.9, 114.8, 114.5, 114.3, 68.6, 68.4, 68.2, 68.1, 31.9 (2C), 31.8, 31.7, 30.0 (2C), 29.9, 29.7, 29.6, 29.4, 29.3 (3C), 29.2, 27.8, 26.4 (2C), 26.3, 26.1, 22.7 (2C), 22.6, 14.1 (2C). MALDI-TOF-MS: *m/z* 1505.83 (M^+).



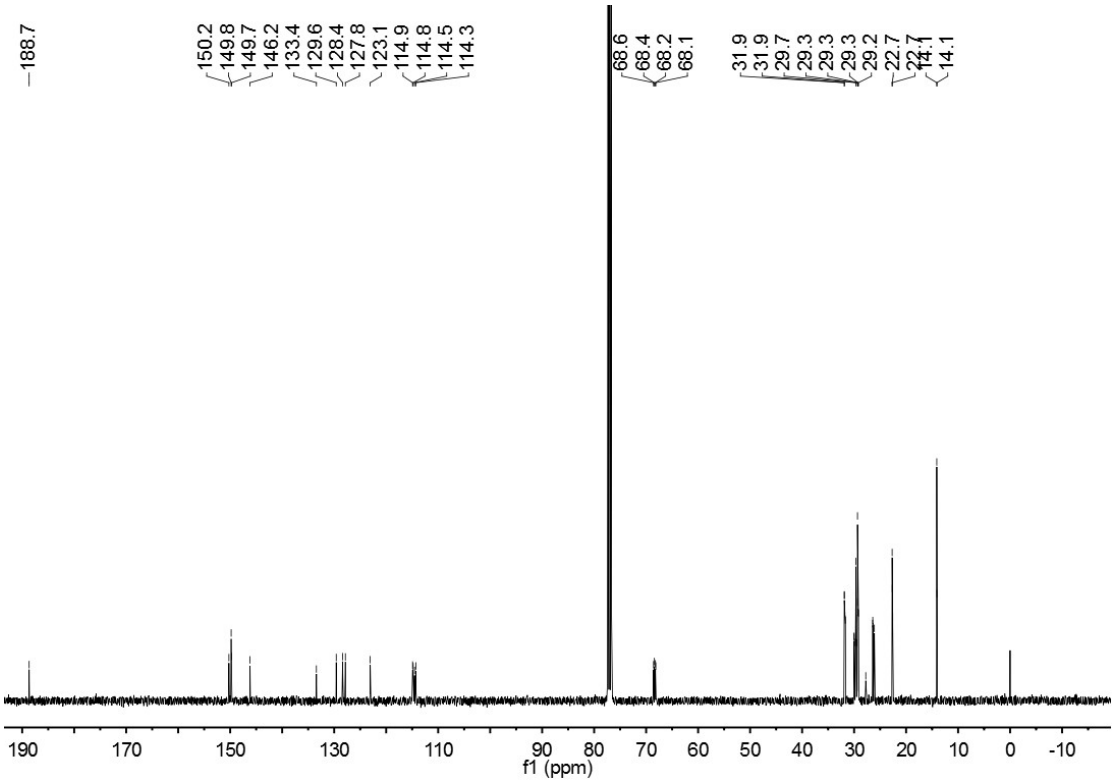
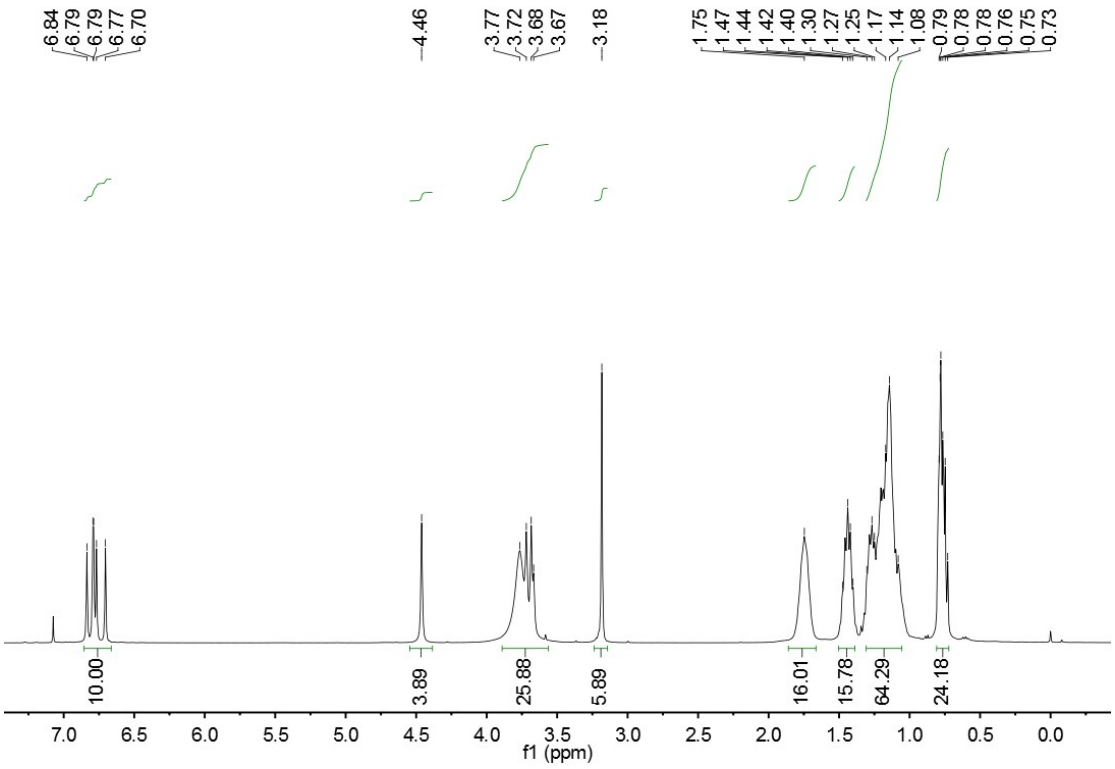


Fig. S6 The ^{1}H NMR and ^{13}C NMR spectra for compound 8

Compound 9 To an one-necked round bottom flask were added compound 8 (1.05 g, 0.70 mmol), $NaBH_4$ (132 mg，3.50 mmol) , methanol (10 mL) and THF (30 mL). The mixture was stirred at room temperature for 1 h. Then HCl (2 M) was added and the mixture was extracted with CH_2Cl_2 (2 × 20 mL). The crude product was obtained by the removal of the solvent under reduced pressure and can be used for the next step without further purification.

Compound 10 Methyl bromoacetate (3.06 g, 20.00 mmol) and K_2CO_3 (2.76 g, 20.00 mmol) were added to a solution of compound 9 (301 mg, 0.20 mmol) in acetone (20 mL).The mixture was heated under nitrogen at reflux for 12 h. The cooled reaction mixture was filtered. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using petroleum ether/dichloromethane (2 : 1) as eluent to give a white solid (142 mg, 43%). ^{1}H NMR

(400 MHz, $CDCl_3$) δ 6.84 – 6.70 (m, 10H), 4.46 (s, 4H), 3.77 – 3.67 (m, 26H), 3.18 (s, 6H), 1.75 (s, 16H), 1.47 – 1.40 (m, 16H), 1.30 – 1.08 (m, 64H), 0.79 – 0.73 (m, 24H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 149.9, 149.8, 149.7 (2C), 149.2, 128.7, 128.6, 128.4, 128.3, 127.5, 115.2, 114.4, 114.3 (2C), 68.5, 68.3, 65.5, 51.6, 32.0 (3C), 31.9, 30.2, 29.8 (2C), 29.7 (2C), 29.6, 29.5, 29.4, 29.3 (2C), 26.6, 26.5, 22.8 (2C), 22.7, 14.2 (2C). MALDI-TOF-MS: *m/z* 1651.49 (M^+).



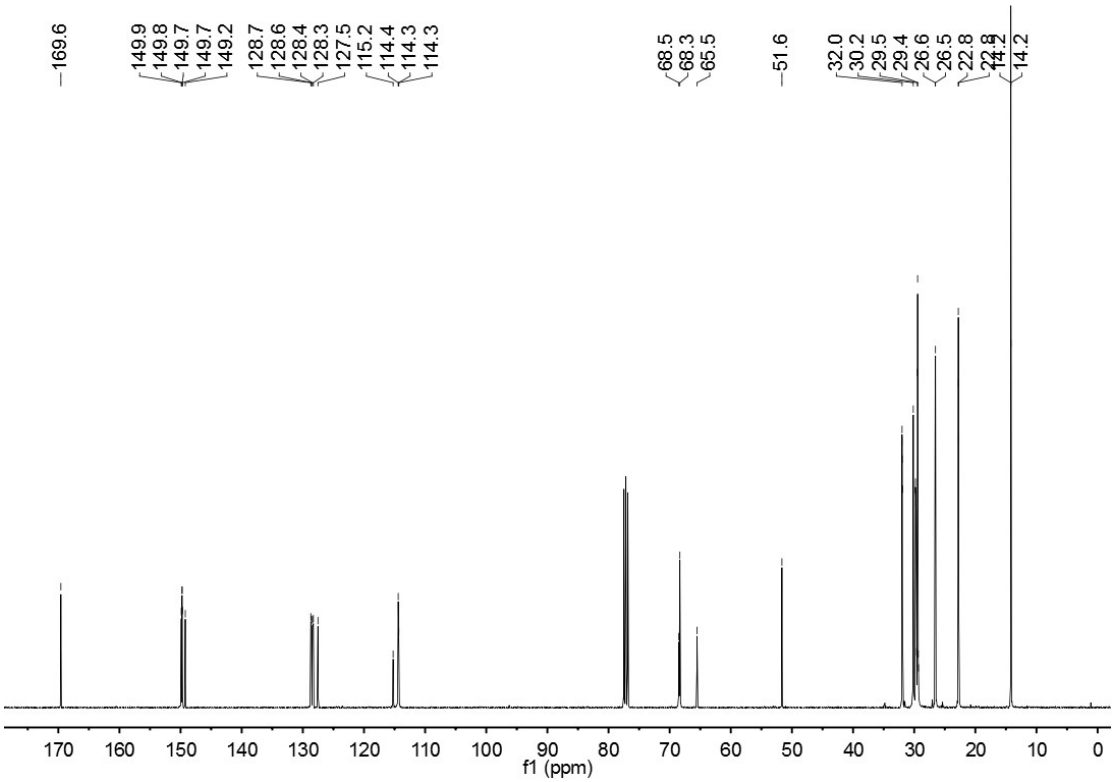


Fig. S7 The ^{1}H NMR and ^{13}C NMR spectra for compound 10

Compound 11 To a solution of compound 9 (496 mg, 0.30 mmol) in THF (20 mL) was added aq. NaOH (0.75 mol/mL, 20 mL). The mixture was refluxed overnight. After the reaction mixture was cooled, THF was removed under reduced pressure. Then HCl (1 M) was added into the mixture to adjust PH to 1 and the mixture was extracted with $CHCl_3$ (2 × 30 mL). The solvent was removed to give compound 9 (463 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 2H), 6.86 – 6.81 (m, 8H), 4.54 (s, 4H), 3.87 – 3.76 (m, 26H), 1.84 – 1.81 (m, 16H), 1.59 – 1.26 (m, 80H), 0.88 – 0.85 (m, 24H). ^{13}C NMR (100 MHz, DMSO) δ 173.2, 149.1, 148.9, 148.6, 147.8, 127.8, 127.7, 127.6, 127.0, 126.2, 114.0, 113.8, 113.4, 113.2, 113.0, 67.6, 67.5, 67.1, 64.2, 30.9 (2C), 30.8, 30.1, 29.8, 29.3, 29.1, 28.9, 28.8, 28.7, 28.5, 28.4 (2C), 28.1, 25.6, 25.5, 25.4, 21.7, 21.6 (2C), 20.5, 13.1, 12.7. MALDI-TOF-MS: *m/z* 1623.55 (M^{+}).

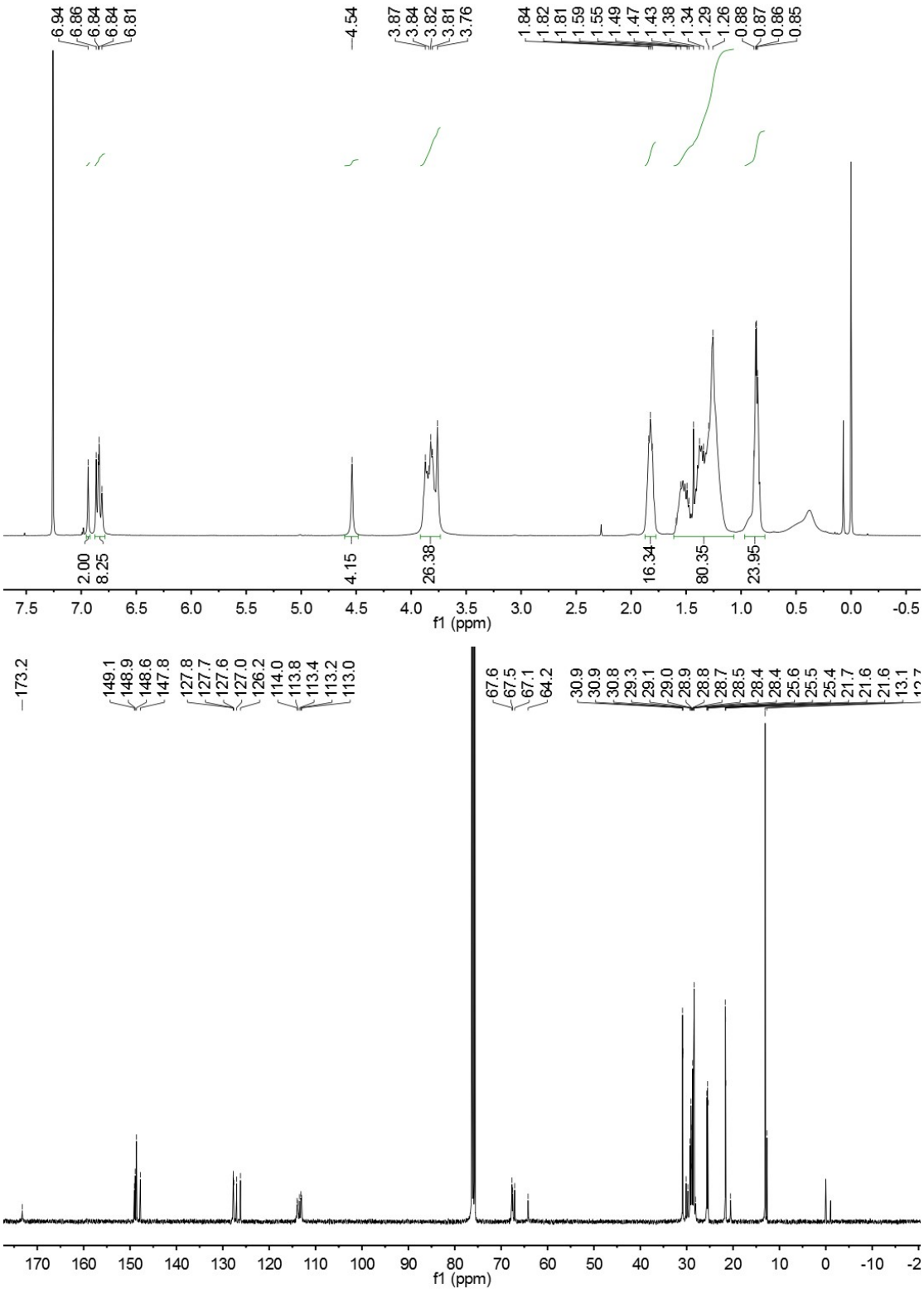


Fig. S8 The ^{1}H NMR and ^{13}C NMR spectra for compound 11

WP5-8C-2PEG To an one-necked round bottom flask were added compound 11 (162 mg, 0.10 mmol), mPEG-NH_2 (Mn = 1000 g/mol, 300 mg), DIPEA (45 mg, 0.30

mmol), HATU (76 mg, 0.20 mmol) and DMF (3 mL). The mixture was stirred at room temperature overnight. After the reaction was completed, the reaction mixture was put into the dialysis bag (MWCO: 3500). WP5-8C-2PEG was purified by dialysis in distilled water for 48 h.

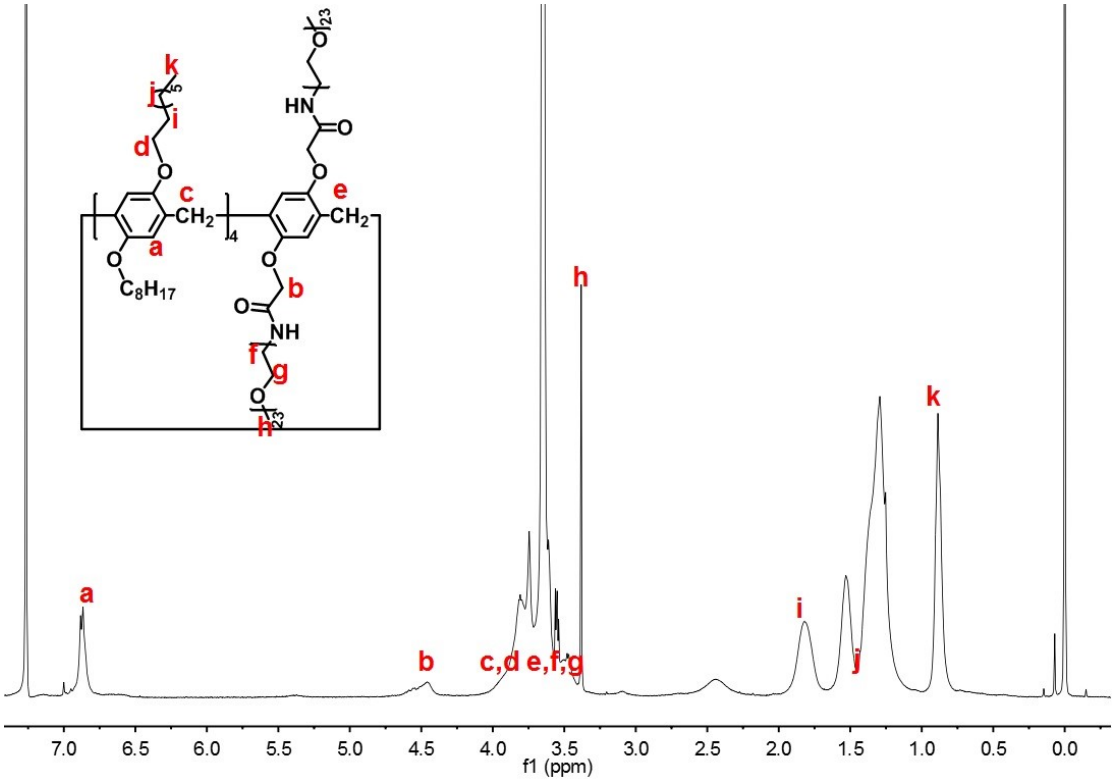


Fig. S9 The ^{1}H NMR spectrum for WP5-8C-2PEG

DPTTIC NPs 1 mg of DPTTIC and 2.5 mg of WP5-8C-2PEG were dissolved into 1 mL of THF. The obtained solution was dropwise injected into 10 mL of deionized water under vigorous stirring for 2 h. The prepared nanoparticles were purified by dialysis (molecular weight cutoff 10000) in distilled water for 48 h.

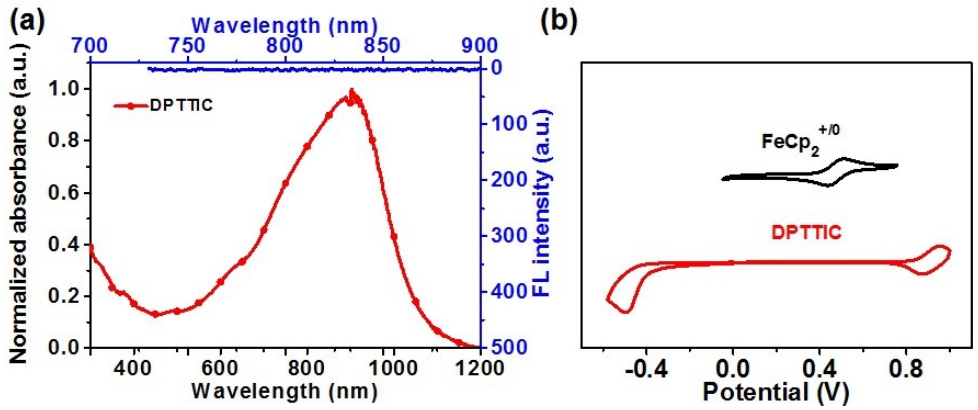


Fig. S10 (a) The absorption and fluorescence spectra of DPTTIC in solid; (b) cyclic voltammogram curve of DPTTIC measured in 0.1 M Bu_4NPF_6 acetonitrile solution.

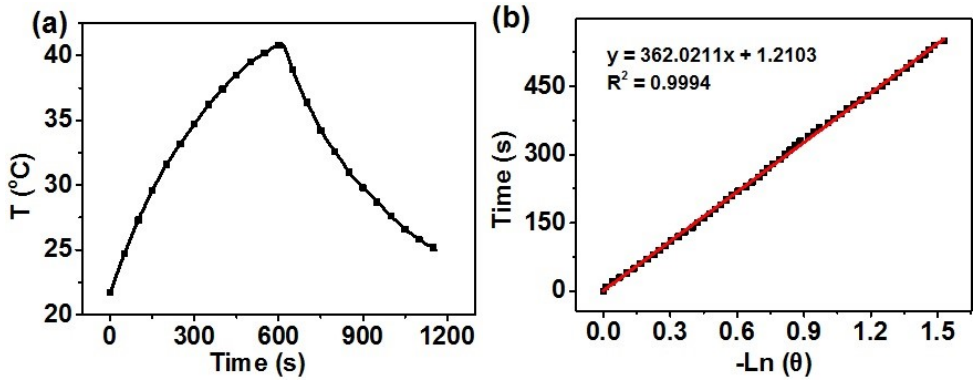


Fig. S11 (a) The temperature change curve of DPTTIC NPs aqueous solution with and without laser irradiation; (b) the fitting line of the cooling period versus the negative natural logarithm of the temperature decrease.

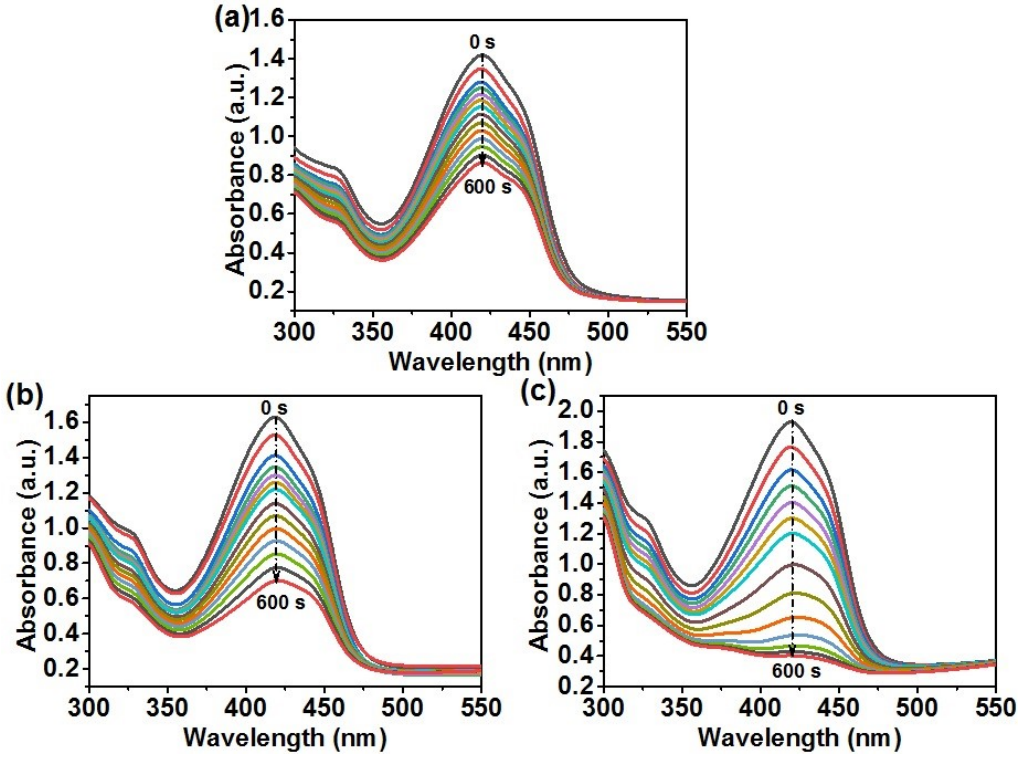


Fig. S12 The absorption changes of DPTTIC NPs aqueous solution containing DPBF with different concentrations under laser irradiation (980 nm, 1.0 W/cm^2): (a) 50 μg/mL; (b) 100 μg/ mL; (c) 200 μg/mL.

The singlet oxygen quantum yield was calculated according to eq (1)

$$\Phi_{DPTTIC} = \Phi_{ICG} \times (S_{DPTTIC} / S_{ICG}) \times (F_{ICG} / F_{DPTTIC}) \quad (1)$$

Where S is the slope of a plot of the fluorescence intensity of SOSG at 530 nm versus irradiation time, and F is calculated by $F = 1\text{-}10^{-OD}$, where OD represents the absorbance of DPTTIC NPs and ICG aqueous solution at 980 nm and 808 nm respectively. Therefore the singlet oxygen quantum yield was calculated to be 3.6% using ICG as reference (Φ_{ICG} = 0.2%).

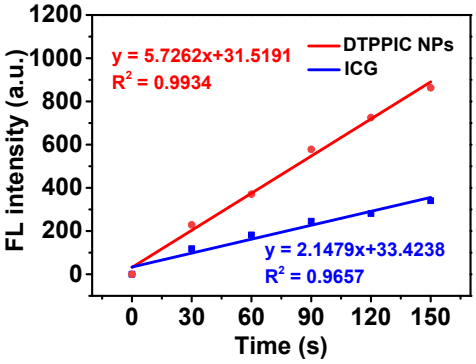


Fig. S13 The FL changes of SOSG in DPTTIC NPs and ICG aqueous solution under laser irradiation (DPTTIC NPs: 980 nm; ICG: 808 nm)

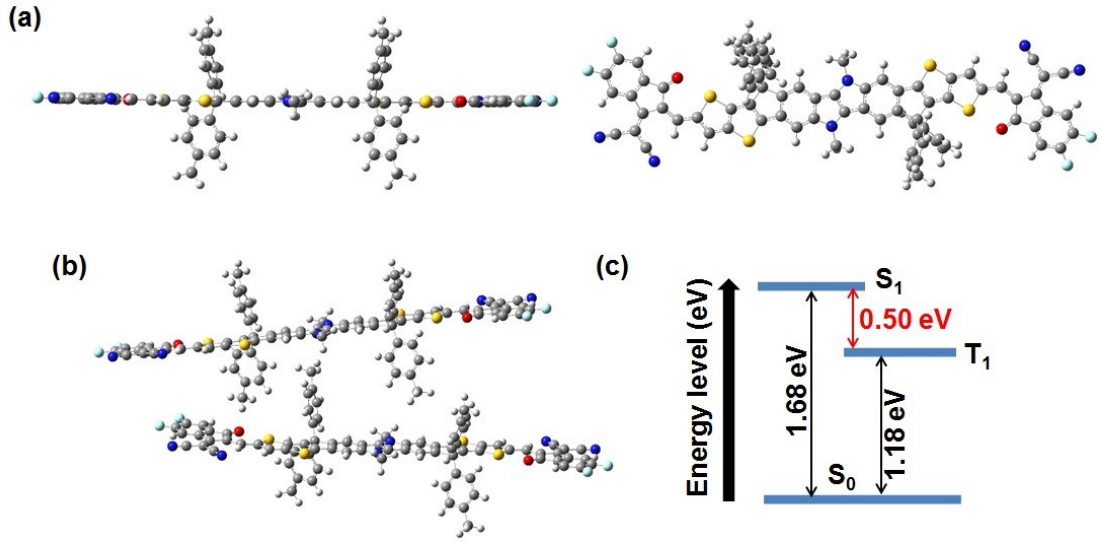


Fig. S14 a) Optimized geometries of DPTTIC; b) optimized dimer geometries of DPTTIC; c) the energy levels of ground state (S_0), singlet state (S_1) and triplet state (T_1), and ΔE_{ST} values of DPTTIC

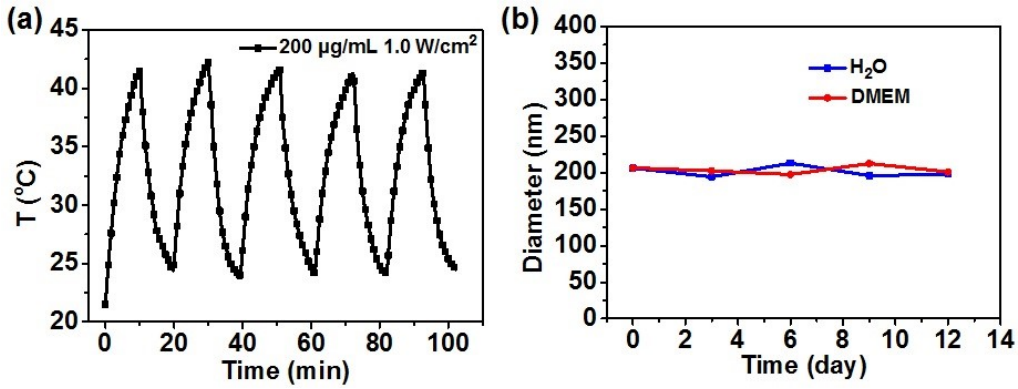


Fig. S15 (a) The photostability of DPTTIC NPs; (b) the diameter changes of DPTTIC NPs in DMEM and water respectively.

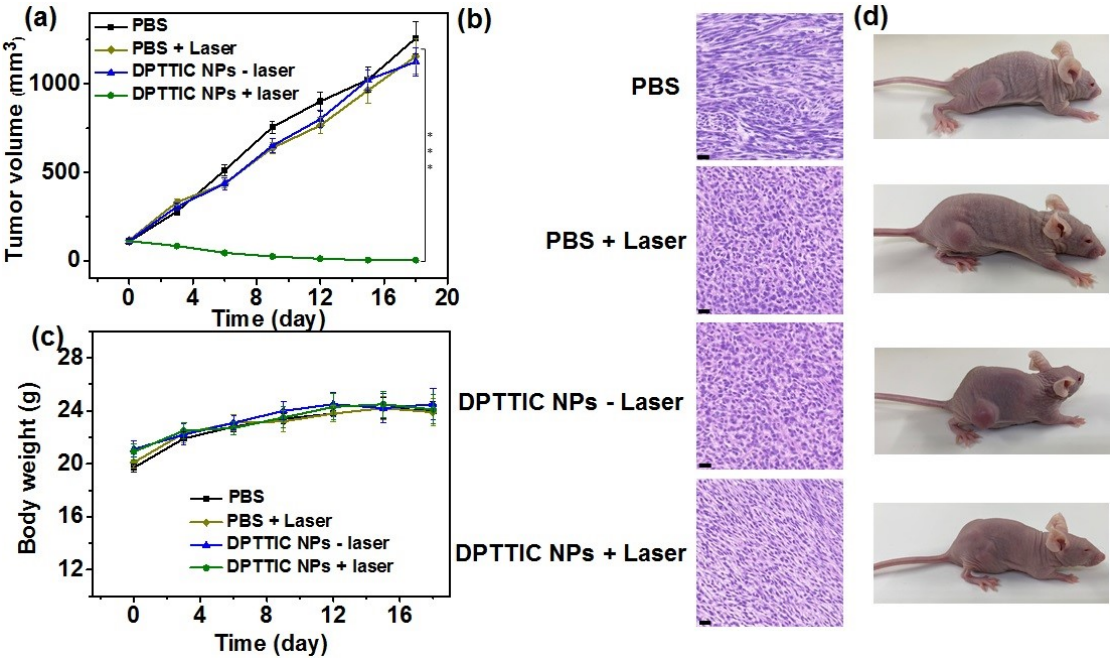


Fig. S16 (a) The record of the tumor volumes in different mice groups during different treatments. ***P < 0.001; (b) the H&E stained slices of tumors in different mice groups after different treatments (Scal bar: 20 μm); (c) the record of the body weight in different mice groups during different treatments. (d) Tumor images

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