Supporting Information

Bright "D-A-D" Semiconducting Small Molecule Aggregates for NIR-II Fluorescence Bioimaging Guiding Photothermal Therapy

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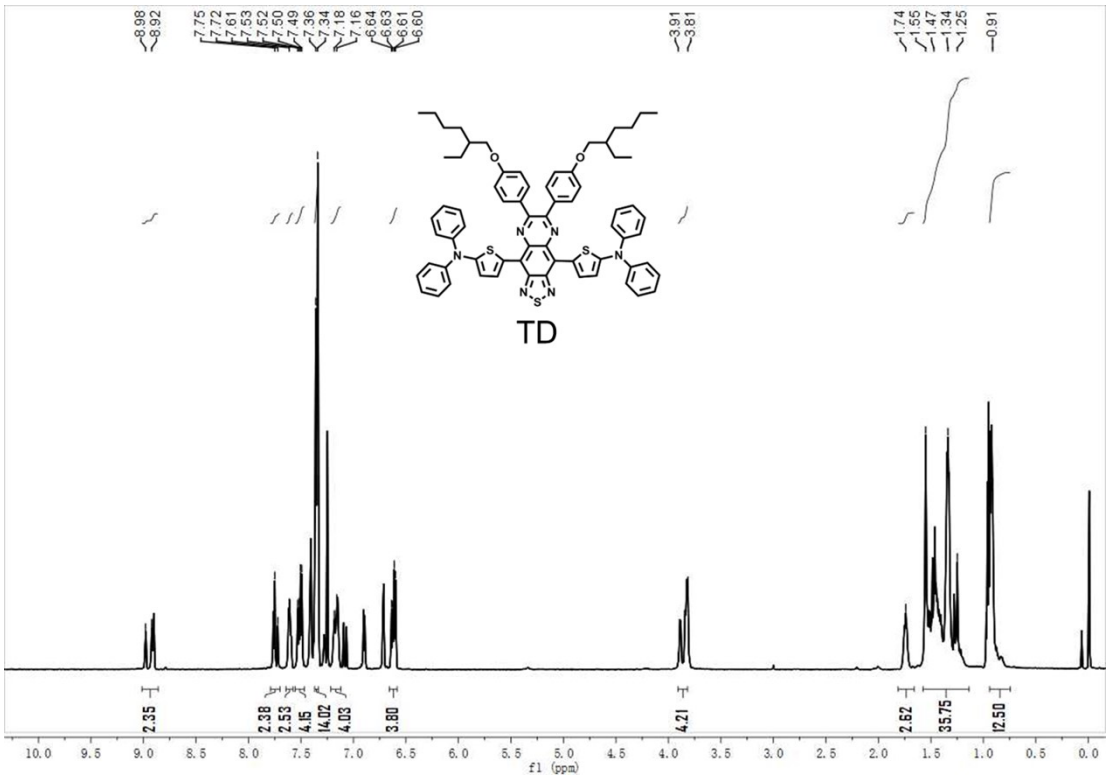


Fig. S1 ^{1}H NMR Spectrum of TD in $CDCl_3$.

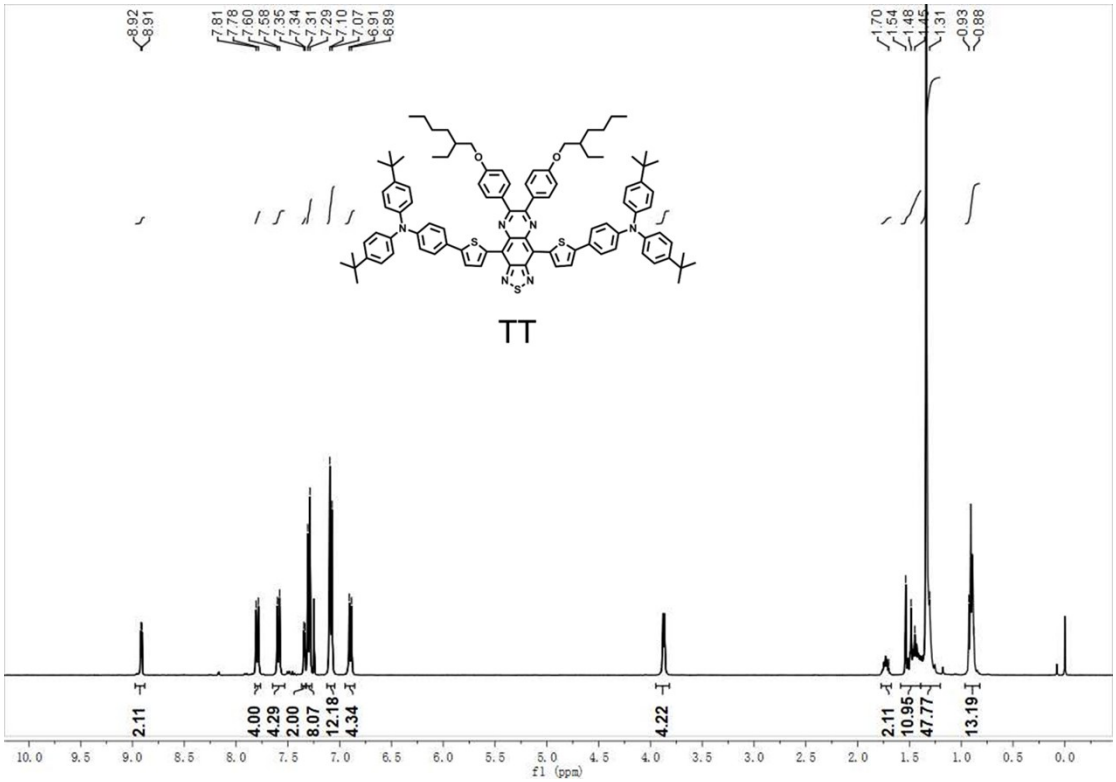


Fig. S2 ^{1}H NMR Spectrum of TT in $CDCl_3$.

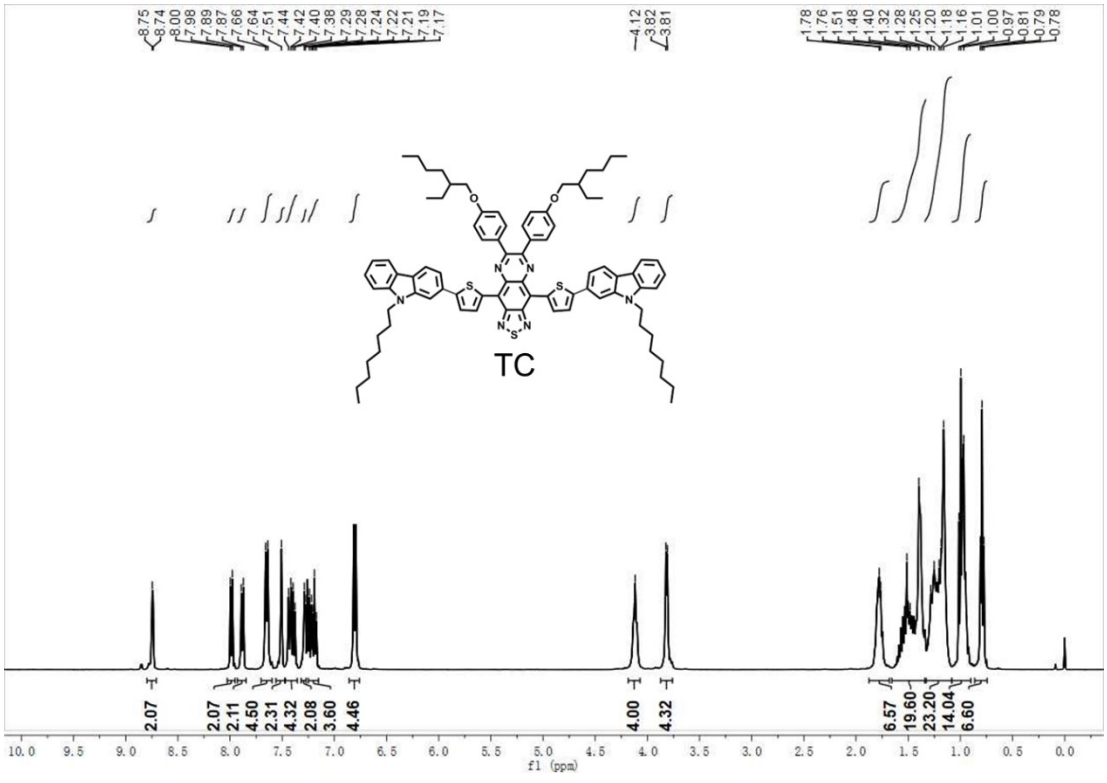


Fig.S3 ^{1}H NMR Spectrum of TC in $CDCl_3$.

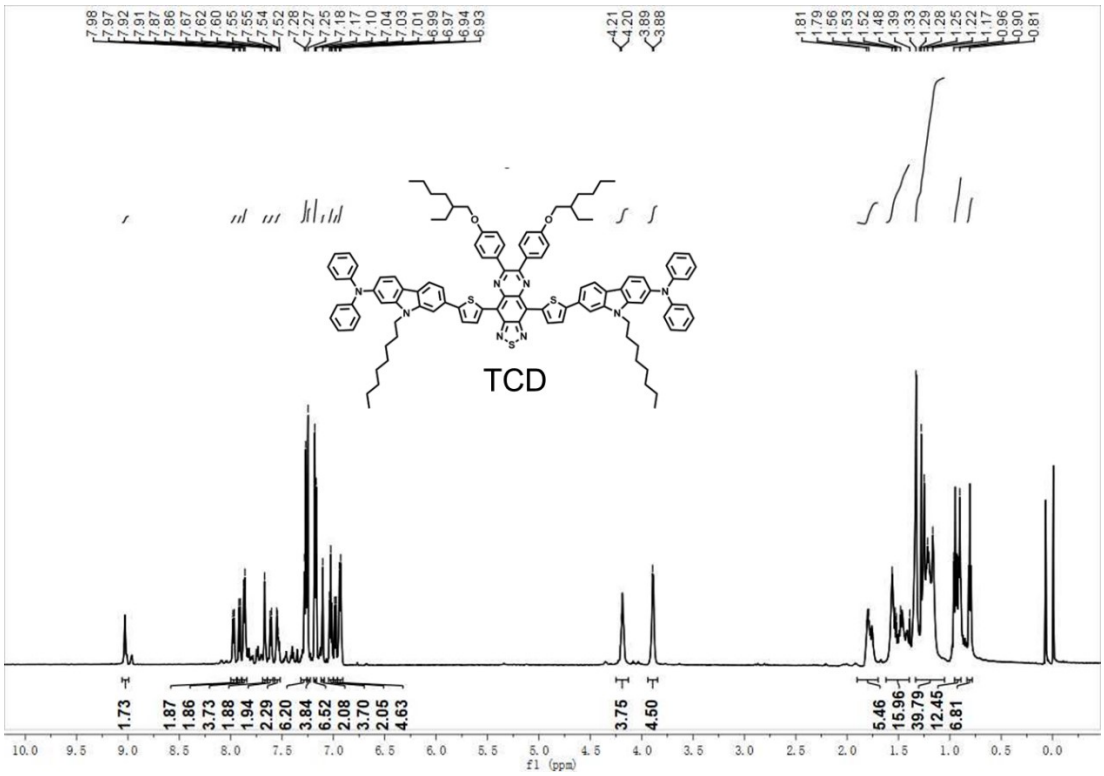


Fig.S4 ^{1}H NMR Spectrum of TCD in $CDCl_3$.

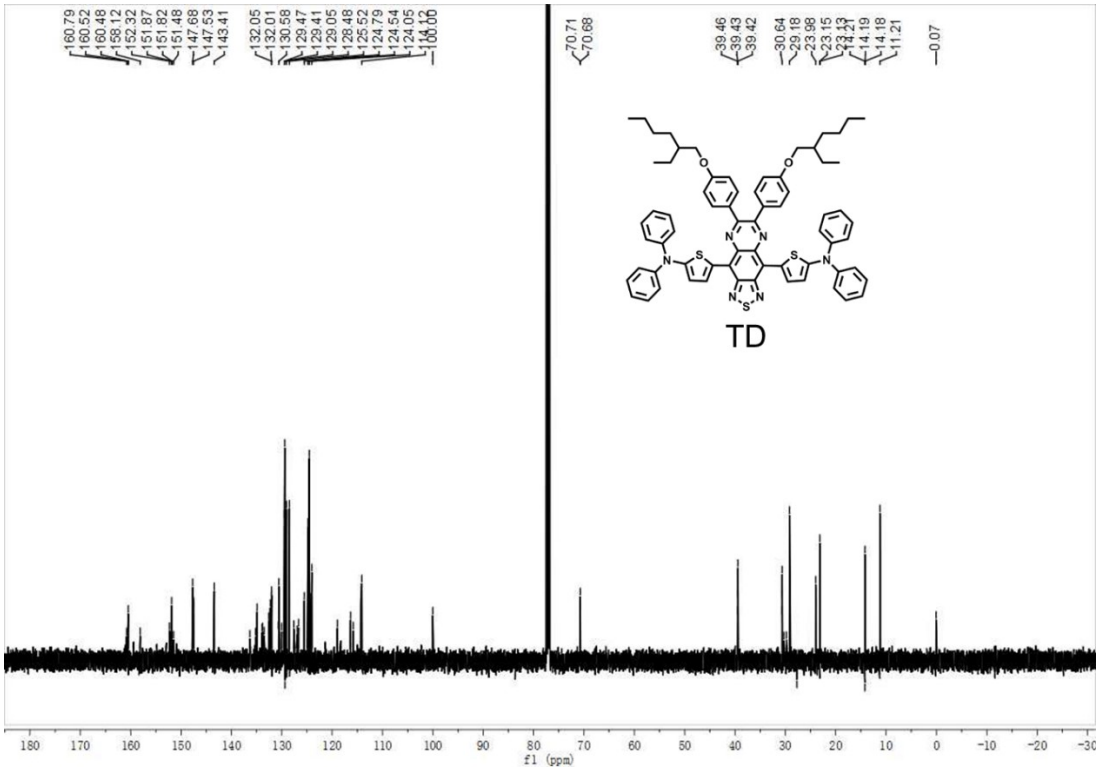


Fig.S5 ^{13}C NMR Spectrum of TD in $CDCl_3$.

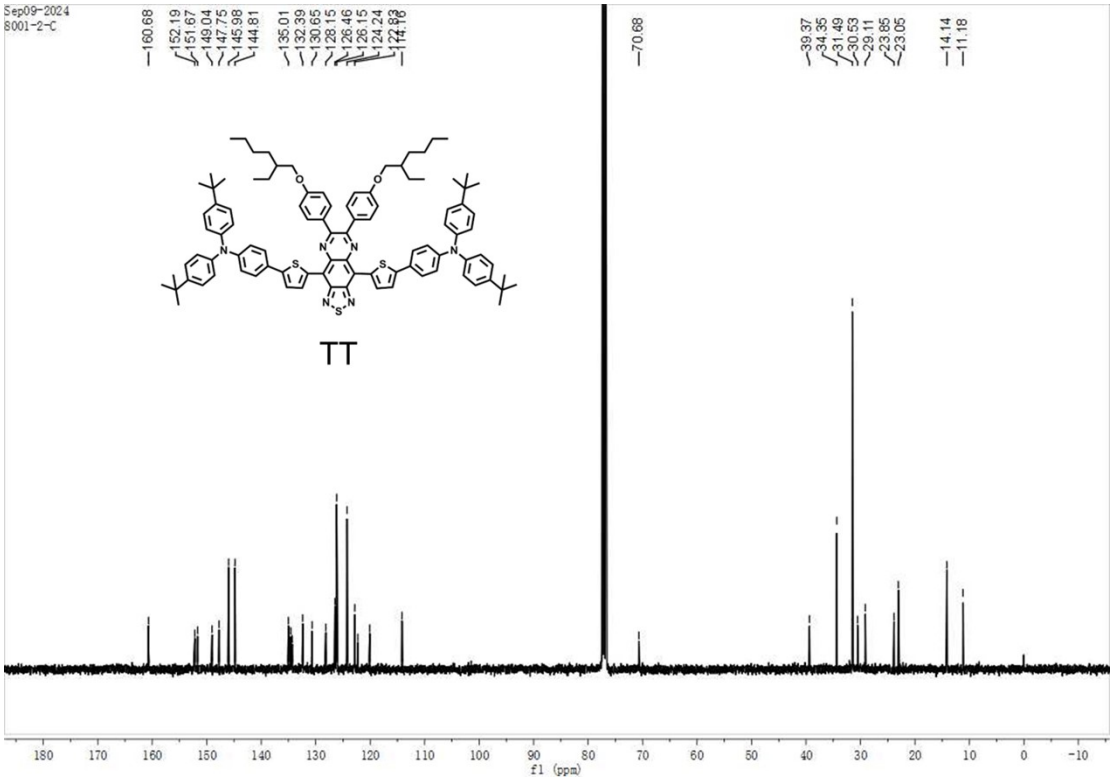


Fig.S6 ^{13}C NMR Spectrum of TT in $CDCl_3$.

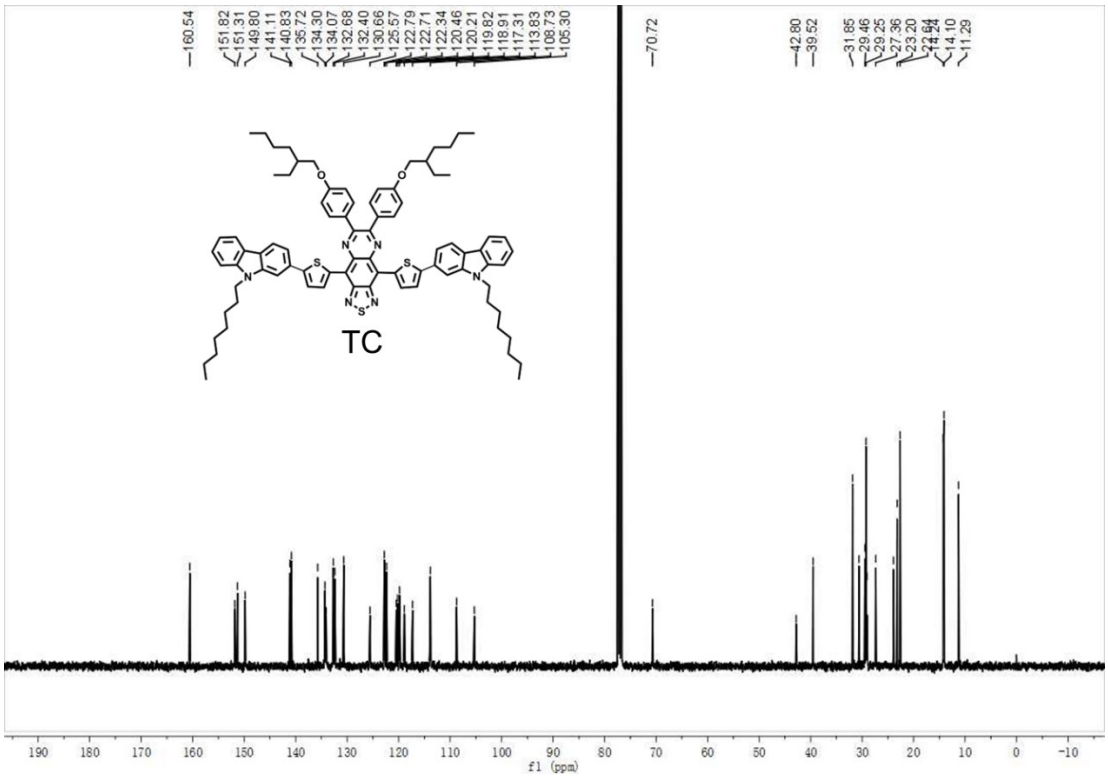


Fig.S7 ^{13}C NMR Spectrum of TC in $CDCl_3$.

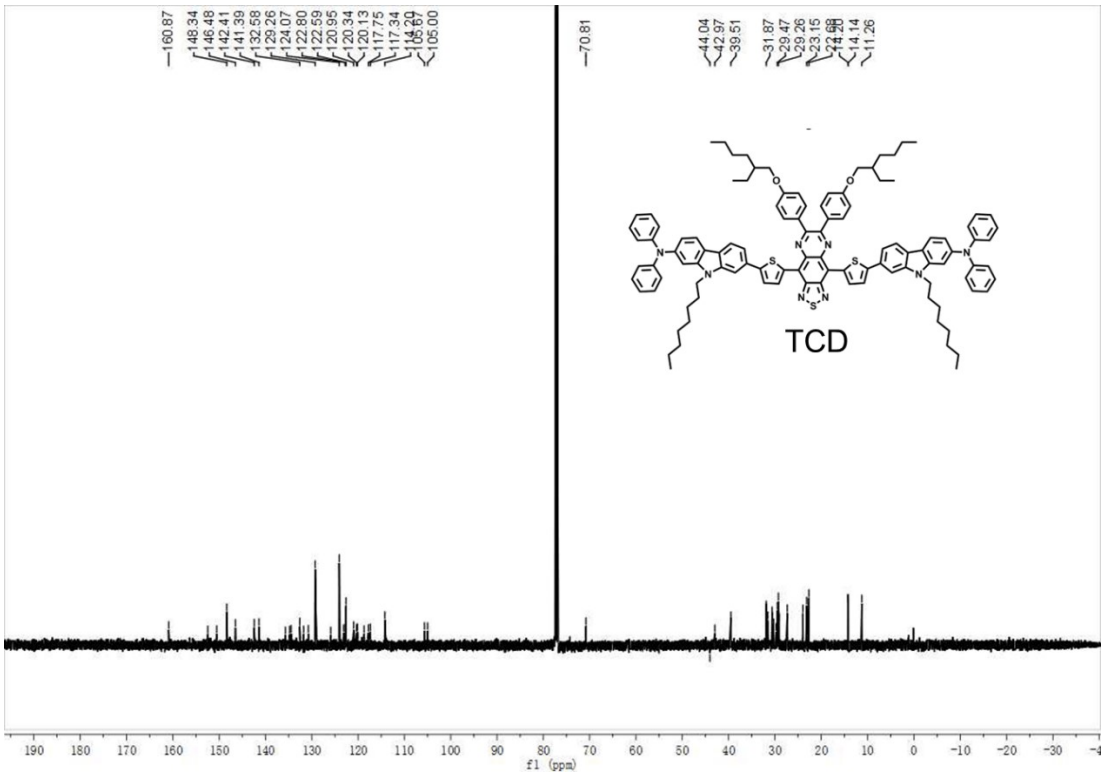


Fig.S8 ^{13}C NMR Spectrum of TCD in $CDCl_3$.

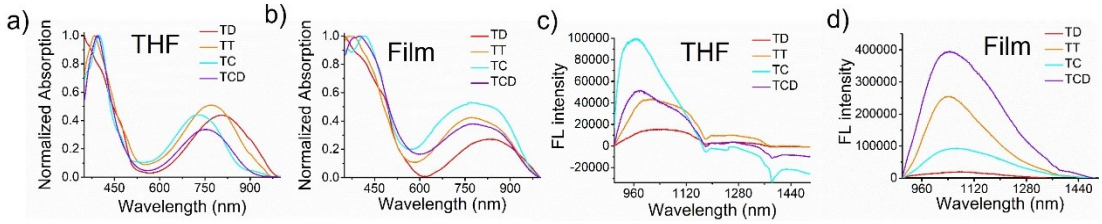


Fig. S9 (a) Absorption spectra of TD, TT, TC, and TCD in THF solution. (b) Absorption spectra of TD, TT, TC, and TCD films. (c) NIR-II fluorescence spectra of TD, TT, TC, and TCD in THF solution. (d) NIR-II fluorescence spectra of TD, TT, TC, and TCD films. Absorption spectra of SPNs1-3 in water at different concentrations.

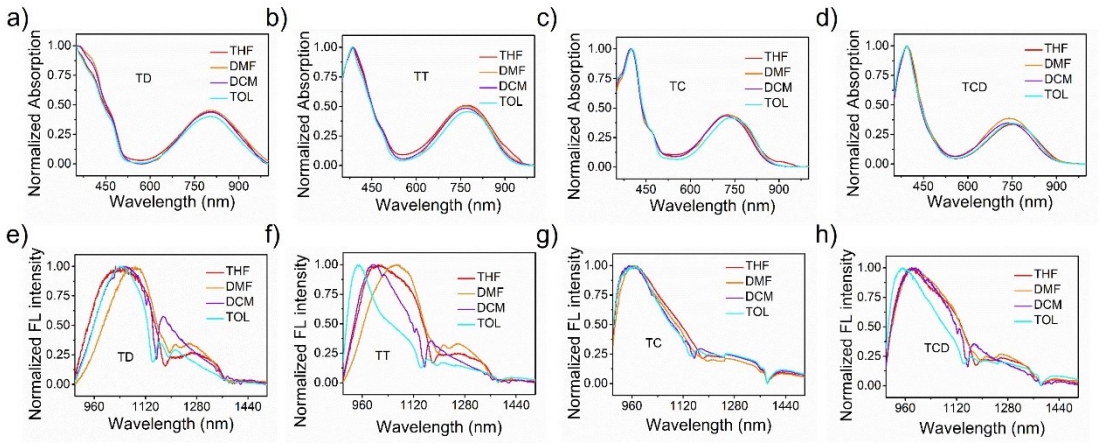


Fig. S10 (a-d) Absorption spectra of TD, TT, TC, and TCD in THF, DMF, DCM, and TOL solutions. (e-h) NIR-II fluorescence spectra of TD, TT, TC, and TCD in THF, DMF, DCM, and TOL solutions.

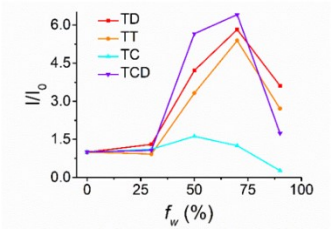


Fig. S11 Plots of the relative emission intensity (I/I_0) versus f_w (volume factions of water), I_0 and I are the PL peak values of TD, TT, TC, and TCD in the THF and THF/water mixture, respectively.

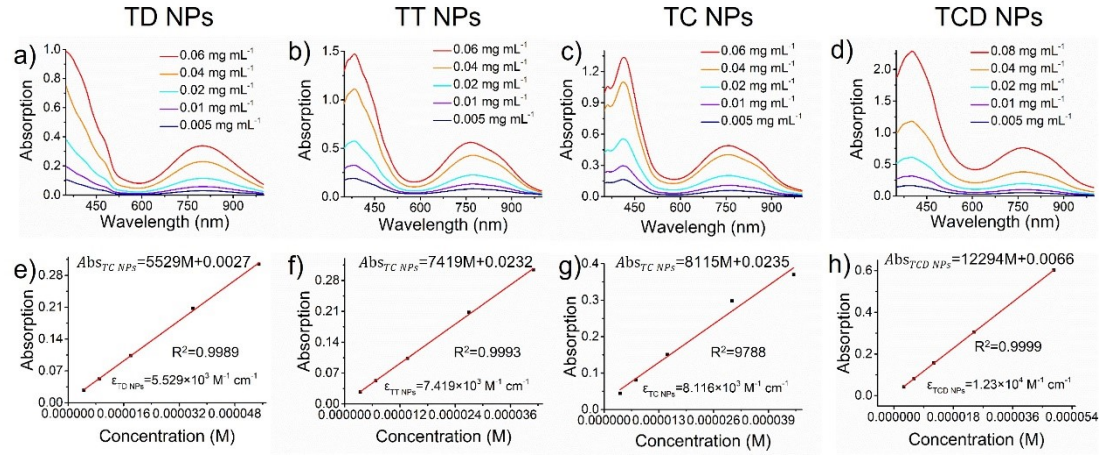


Fig. S12 (a-d) Absorption of the aqueous solutions of TD NPs, TT NPs, TC NPs, and TCD NPs in different concentration. (e-h) The fitting linear curve of absorption at 850 nm and TD NPs, TT NPs, TC NPs, and TCD NPs concentration.

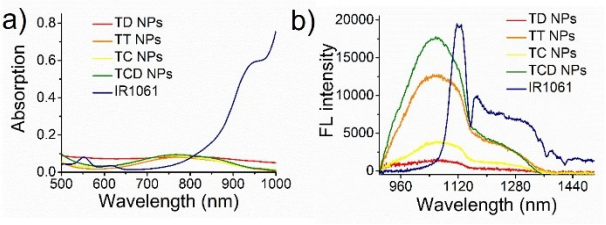


Fig. S13 Determination of NIR-II QYs. (a) Absorption spectra of TD NPs, TT NPs, TC NPs, TCD NPs, and IR1061. (b) NIR-II fluorescence spectra of TD NPs, TT NPs, TC NPs, TCD NPs, and IR1061. (TD NPs, TT NPs, TC NPs, and TCD NPs were dissolved in aqueous solution. IR1061 were dissolved in DCM.)

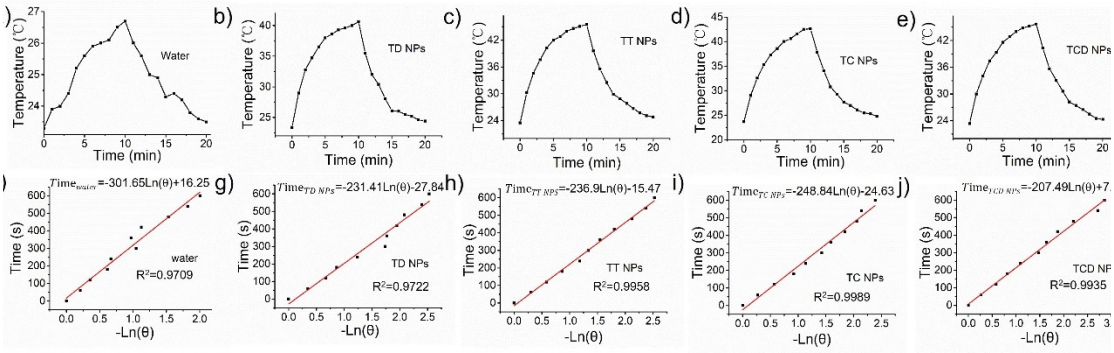


Fig. S14 Determination of photothermal conversion efficiency. (a-e) Photothermal heating and cooling curves of water and TD NPs, TT NPs, TC NPs, and TCD NPs aqueous solutions under 850 nm laser irradiation (400 mW cm^{-2}). Time constant (τ_s) of water (f), TD NPs (g), TT NPs (h), TC NPs (i), and TCD NPs (j) for the heat transfer from the system determined by applying the linear time data from the cooling period.

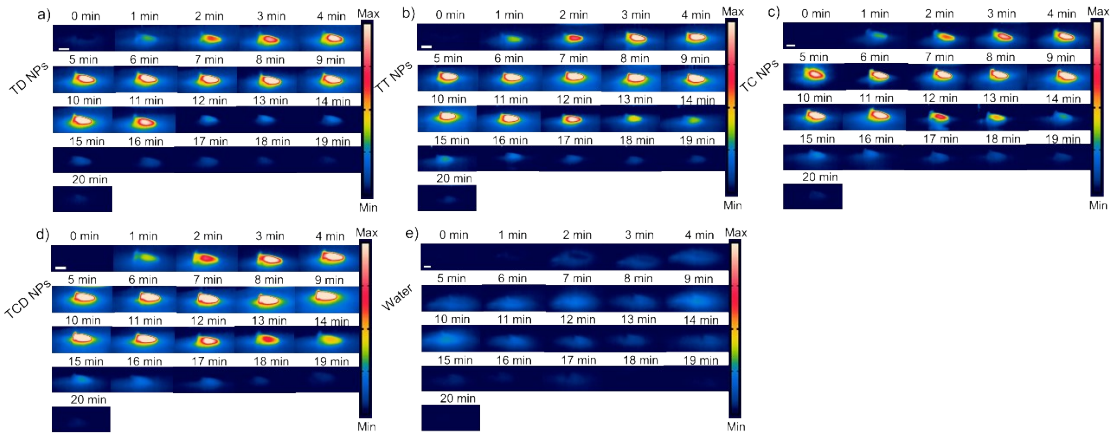


Fig. S15 The thermal images of TD NPs, TT NPs, TC NPs, and TCD NPs aqueous solution which were exposured under 850 nm irradiation (400 mW cm^{-2}) for 10 min

and then cooled to room temperature for 10 min. (Scale bar = 0.35 cm)

Table S1 Photophysical data of TD, TT, TC, and TCD.

Molecule	TD	TT	TC	TCD
$Bandgap_{monomer}$ (eV)	1.67	1.72	1.55	1.76
$Bandgap_{trimer}$ (eV)	1.40	1.51	1.14	1.40
$\lambda_{abs}{}^{a}$ (nm)	805	772	752	727
$\lambda_{abs}{}^{b}$ (nm)	825	774	764	778
$\lambda_{abs}{}^{c}$ (nm)	800	772	762	765
$\lambda_{em}{}^{a}$ (nm)	1027	1012	968	972
$\lambda_{em}{}^{b}$ (nm)	1069	1042	1068	1043
$\lambda_{em}{}^{c}$ (nm)	1052	1027	1044	1050
ε^{d} (M^{-1} cm^{-1})	5.529×10^3	7.419×10^3	8.116×10^3	1.23×10^4
QYs^{c} (%)	0.44	1.71	0.14	1.85
η^{c} (%)	40.1	35.8	33.3	41.1

[a] In THF solutions; [b] In film states; [c] Nanoparticles in aqueous solutions; [d] Molar extinction coefficients of Nanoparticles in aqueous solutions.

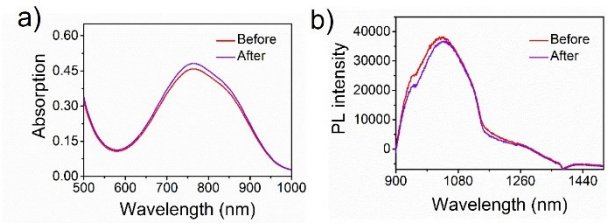


Fig. S16 Absorption spectra (a) and NIR-II emission spectra (b) of TCD NPs under 850 nm irradiation (400 mW cm^{-2}) for 50 min.

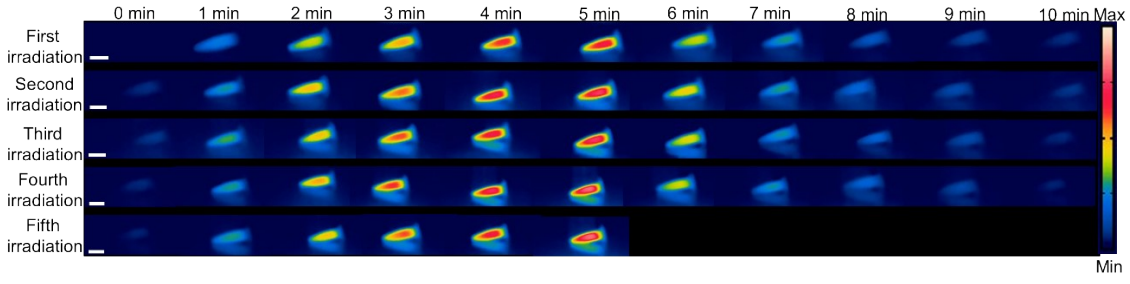


Fig. S17 The thermal images of TCD NPs aqueous solution (40 μg mL^{-1}) after five cycles of on/off 850 nm irradiation at a power density of 400 mW cm^{-2}. (Scale bar = 0.3 cm)

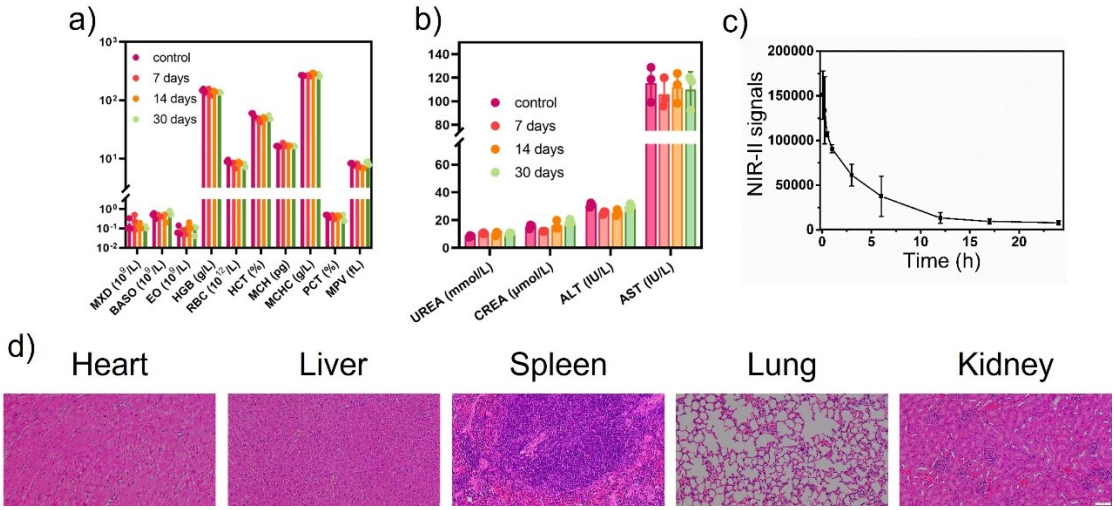


Fig. S18 (a) Hematological index of the mice (n = 3) with intravenous administration of TCD NPs during 30 days. The terms include MXD, BASO, EO, HGB, RBC, HCT, MCH, MCHC, PCT, and MPV. (b) Biochemical blood analysis of the different treated mice at 7, 14 and 30 days postinjection. The results show the mean and SD of UREA, CREA, ALT, and AST. (c) Blood circulation time curves of the TCD NPs based on the NIR-II fluorescent intensity excited by 808 nm at various time points at 24-h post-injection. (d) H&E staining analysis of the main organ tissues extracted from TCD NPs-treated mice after the therapy experiment (Scale bar = 50 μm).

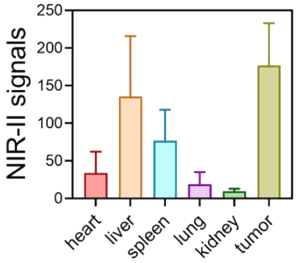


Fig. S19 NIR-II signals of *ex vivo* main organs.

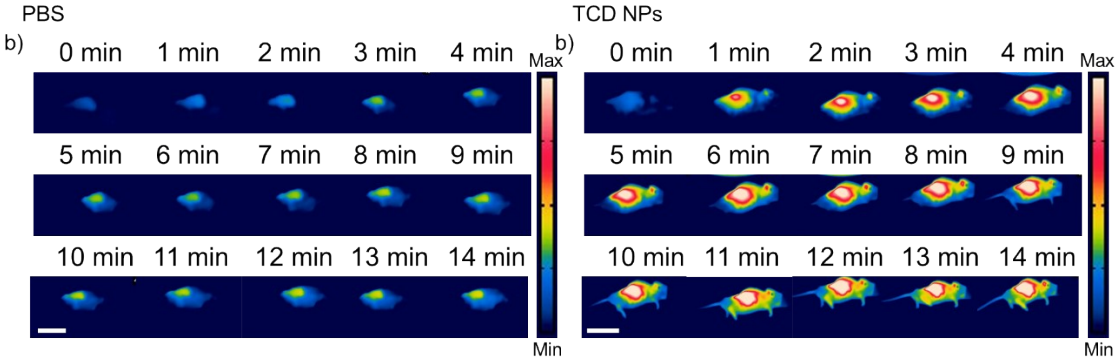


Fig. S20 The thermal images of PBS or TCD NPs-treated mice under 850 nm irradiation

(400 mW cm^{-2}) for 14 min. (Scale bar = 3 cm)

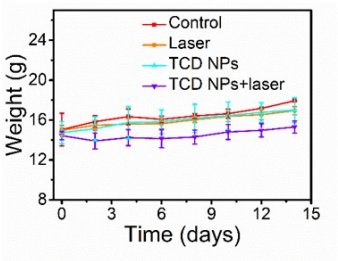


Fig. S21 Body weights of 4T1 tumor-bearing mice in different treatment groups.

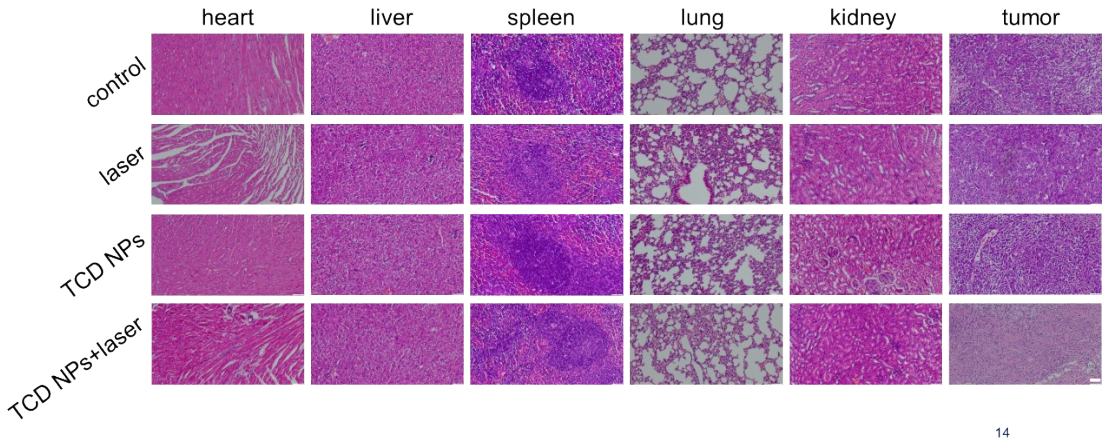


Fig. S22 H&E staining analysis of the main organ tissues extracted from different groups of mice after the therapy experiment (magnification: 20×).

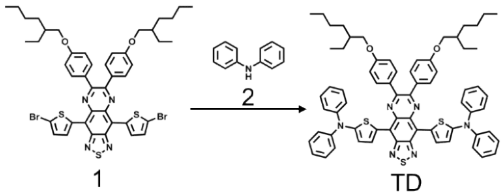
EXPERIMENTAL SECTION

Materials

All organic agents were commercial products, which used as received without any purification. 4,9-bis(5-bromothiophen-2-yl)-6,7-bis(4-((2-ethylhexyl)oxy)phenyl)-[1,2,5]thiadiazolo[3,4-g]quinoxaline (TTQ) (1) were purchased from SunaTech Inc. Diphenylamine (2), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3) and tetrakis(triphenylphosphine)palladium (0) (4), Sodium tert-butoxide (5), and Xantphos (6) were gotten from Macklin Biochemical Technology Co., Ltd. 4-(tert-butyl)-N-(4-(tert-butyl)phenyl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)aniline (7), 9-octyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9H-carbazole (8), 2,7-dibromo-9-octyl-9H-carbazole (9), tris(dibenzylideneacetone)dipalladium (10)， and tetrabutylammonium bromide (TBAB) were obtained by Bide Pharmatech Co.,Ltd.

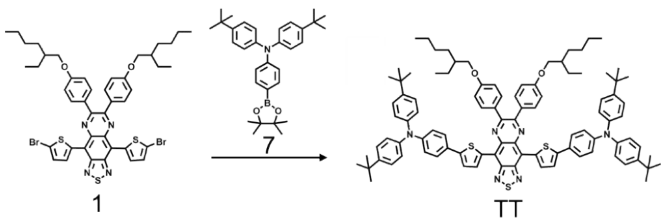
[1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (11) was purchased from Sigma-Aldrich Chemical Co. Potassium carbonate, toluene, potassium acetate, 1,4-dioxane, tetrahydrofuran (THF) dichloromethane (DCM), and petroleum ether 60~90 (PE) were obtained from Chongqing Chuandong Chemical (Group) Co., Ltd. Pluronic F-127 was obtained from Sigma-Aldrich Chemical Co. Toluene were dried and distilled under a nitrogen atmosphere before synthesis.

Synthesis of TD



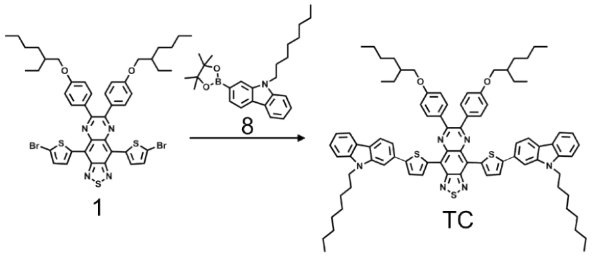
Compound 1 (0.1 g, 0.11 mmol), compound 2 (0.076 g, 0.45 mmol), tris(dibenzylideneacetone)dipalladium (compound 10, 0.015 g, 0.017 mmol), sodium tert-butoxide (compound 5, 0.174 g, 1.82 mmol), and Xantphos (compound 6, 0.023 g, 0.039 mmol) were dissolved in toluene (4 mL) together. This solution was degassed by N_2. The mixture was heated to 120 ℃ and stirred overnight under a N_2 atmosphere. After removal of the solvent, the crude product was purified via column chromatography (silica gel) by using DCM and PE as eluent to give compound TD (0.042 g, 35% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.95 (d, 2H), 7.73 (d, 2H), 7.61 (s, 2H), 7.51 (dd, 4H), 7.36-7.31 (m, 14H), 7.17 (d, 4H), 6.62 (dd, 4H), 3.86 (d, 4H), 1.74 (s, 2H), 1.40 (dd, 36H), 0.91 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.50, 151.85, 147.68, 143.41, 133.87, 132.58, 132.03, 130.60, 129.44, 128.48, 125.52, 124.79, 124.05, 116.32, 114.14, 77.30, 77.09, 76.88, 70.70, 39.53-39.35, 30.64, 29.18, 24.06-23.77, 23.14, 14.18, 11.21. MALDI-TOF m/z [M + H]$^+$: Calcd for $C_{68}H_{66}N_6O_2S_3$: 1094.44; found: 1095.294.

Synthesis of TT



Compound 1 (0.1 g, 0.11 mmol), compound 7 (0.193 g, 0.4 mmol), tetrakis(triphenylphosphine)palladium(0) , (compound 4, 0.02 g, 0.017 mmol), and TBAB (0.015 g, 0.046 mmol) were dissolved in toluene (3 mL) and degassed by N_2. This solution was heated to 80 ℃ and added potassium carbonate aqueous solution (1 M, 1.5 mL) under a N_2 atmosphere. Then the mixture was heated to 100 ℃ and stirred overnight under a N_2 atmosphere. After removal of the solvent, the crude product was purified via column chromatography (silica gel) by using DCM and PE as eluent to give compound TT (0.097 g, 51% yield). ^{1}H NMR (400 MHz, CDCl3) δ 8.91 (d, 2H), 7.80 (d,4H), 7.59 (d, 4H), 7.34 (d, 2H), 7.30 (d, 8H), 7.08 (d, 12H), 6.90 (d, 4H), 3.87 (d, 4H), 1.70 (s, 2H), 1.55-1.39 (m, 10H), 1.31 (s, 46H), 0.90 (d, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.68, 152.19, 151.67, 149.04, 147.75, 145.98, 144.81, 135.01, 134.56, 132.39, 130.65, 128.15, 126.46, 124.24, 122.83, 122.27, 120.08, 114.16, 70.68, 39.37, 34.35, 31.49, 30.53, 29.11, 23.85, 23.05, 14.14, 11.18. MALDI-TOF m/z [M + H]$^+$: Calcd for $C_{96}H_{106}N_6O_2S_3$: 1470.75; found: 1471.554.

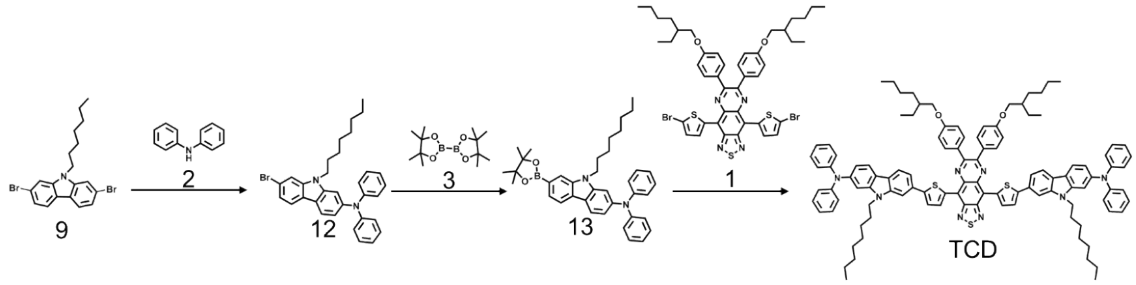
Synthesis of TC



Compound 1 (0.1 g, 0.11 mmol), compound 8 (0.121 g, 0.3 mmol), tetrakis(triphenylphosphine)palladium(0) , (compound 4, 0.02 g, 0.017 mmol), and TBAB (0.015 g, 0.046 mmol) were dissolved in toluene (3 mL) and degassed by N_2. This solution was heated to 80 ℃ and added potassium carbonate aqueous solution (1 M, 1.5 mL) under a N_2 atmosphere. Then the mixture was heated to 100 ℃ and stirred overnight under a N_2 atmosphere. After removal of the solvent, the crude product was purified via column chromatography (silica gel) by using DCM and PE as eluent to give compound TC (0.064 g, 44% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, 2H), 7.99 (d, 2H), 7.88 (d, 2H), 7.65 (d, 4H), 7.51 (s, 2H), 7.41 (dd, 4H), 7.28 (d, 2H), 7.25-7.15

(m, 4H), 6.79 (t, 4H), 4.12 (s, 4H), 3.82 (d, 4H), 1.77 (d, 6H), 1.63-1.34 (m, 18H), 1.29-1.10 (m, 20H), 0.99 (t, 14H), 0.79 (t, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.54, 151.82, 149.80, 141.11, 140.83, 135.72, 134.30, 132.54, 132.38-132.22, 130.66, 125.57, 122.75, 120.33, 120.17-120.01, 119.82, 118.91, 117.31, 113.83, 108.73, 105.30, 70.72, 42.80, 39.52, 31.85, 30.61, 29.46, 29.15, 27.36, 23.91, 23.20, 22.64, 14.17, 11.29. MALDI-TOF m/z $[M + H]^+$: Calcd for $C_{84}H_{94}N_6O_2S_3$: 1314.66; found: 1315.324.

Synthesis of TCD



Compound 9 (0.6 g, 1.3 mmol), compound 2(0.152 g, 0.8 mmol), tris(dibenzylideneacetone)dipalladium (compound 10, 0.03 g, 0.03 mmol), sodium tert-butoxide (compound 5, 0.31 mg, 3.23 mmol), and Xantphos (compound 6, 0.04 g, 0.069 mmol) were dissolved in toluene (10 mL) together. This solution was degassed by N_2. The mixture was heated to 120 ℃ and stirred overnight under a N_2 atmosphere. After removal of the solvent, the crude product was purified via column chromatography (silica gel) by using DCM and PE as eluent to give compound 12 (0.2 g, 37.5% yield).

Compound 12 (0.2 g, 0.3 mmol), compound 3 (0.25 g, 1 mmol), bis(diphenylphosphino)ferrocene]dichloropalladium(II) (compound 11, 0.038 g, 0.05 mmol), and potassium acetate (0.08 g, 0.82 mmol) were dissolved in 1,4-dioxane (17 mL). This mixture was degassed by N_2 and then heated to 90 ℃. This reaction was stirred overnight under a N_2 atmosphere. After removal of the solvent, the crude product was purified via column chromatography (silica gel) by using DCM and PE as eluent to give compound 13 (0.082 g, 47% yield).

Compound 1 (0.1 g, 0.11 mmol), compound 13 (0.143 g, 0.25 mmol), tetrakis(triphenylphosphine)palladium(0), (compound 4, 0.02 g, 0.017 mmol), and TBAB (0.015 g, 0.046 mmol) were dissolved in toluene (3 mL) and degassed by N_2.

This solution was heated to 80 ℃ and added potassium carbonate aqueous solution (1 M, 1.5 mL) under a N_2 atmosphere. Then the mixture was heated to 100 ℃ and stirred overnight under a N_2 atmosphere. After removal of the solvent, the crude product was purified via column chromatography (silica gel) by using DCM and PE as eluent to give compound TCD (0.074 g, 41% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.02 (dd, 2H), 7.97 (d, 2H), 7.91 (d, 2H), 7.87 (d, 4H), 7.67 (s, 2H), 7.61 (d, 2H), 7.54 (dd, 2H), 7.28 (d, 6H), 7.25 (s, 4H), 7.17 (d, 6H), 7.10 (s, 2H), 7.03 (t, 4H), 6.98 (d, 2H), 6.94 (d, 4H), 4.20 (d, 4H), 3.89 (d, 4H), 1.80 (d, 6H), 1.52 (dd, 16H), 1.32-1.09 (m, 40H), 0.96 (s, 12H), 0.81 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.87, 152.43, 150.51, 148.34, 146.48, 142.41, 141.39, 135.69, 134.79, 132.58, 131.76, 130.74, 129.26, 124.07, 123.11, 122.80, 122.59, 120.95, 120.13, 118.69, 117.75, 114.20, 105.67, 70.81, 42.97, 39.51, 31.87, 31.52, 30.62, 30.28, 29.78, 29.47, 29.33-28.96, 27.34, 23.96, 23.15, 22.68, 14.17, 11.26. MALDI-TOF m/z $[M + H]^+$: Calcd for $C_{108}H_{112}N_8O_2S_3$: 1648.81; found: 1649.534.

Preparation of nanoparticles

The four water-soluble "D-A-D" type semiconducting molecules nanoparticles (NPs) were prepared through classical nanoprecipitation methods. Firstly, the "D-A-D" type molecule (0.2 mg) was dissolved in THF (1 ml), and amphiphilic polymer (Pluronic F-127, 8 mg) and deionized water (10 ml), respectively. Then the THF solution was rapidly injected into deionized water by continues ultrasonication. After nanoprecipitation, THF was fully evaporated at room temperature overnight. To purity and modulate the size of particles, aqueous solutions were filtered through a polyethersulfone (PES) syringe driven filter (0.22 μm) (Jin Teng). Then monodisperse solution was centrifuged and washed with deionized water for three times using a 50 K centrifugal filter unit (Millipore) under centrifugation at 3,400 rpm for 15 min. The final solutions were collected and stored in 4 ℃ environment.

Characterizations

Nuclear magnetic resonance (NMR) spectra of the four semiconducting molecules were recorded on JEOL-JNM-ECZ600R 600 MHz spectrometer or JNM-ECZ400S/II 400MHz spectrometer, which used tetramethylsilane (TMS) and $CDCl_3$ as the internal

reference and the deuterated solvent, respectively. The UV-VIS-NIR absorption spectra were recorded on a INESA L6S UV-VIS-NIR spectrophotometer (INESA (Group) Co., Ltd) at room Temperature. Dynamic light scattering (DLS), a commercial particle size analyzer (Nicomp 380 Z3000, PSS, USA) at a scattering angle of 90°, was used to characterize the average size and size distribution of nanoparticles. When preparing the samples, the 0.45 μm Millipore filters were used to ensure the samples pure. All experiments were operating at room temperature and repeated for three times. Transmission electron microscopy (TEM) images were obtained from transmission electron microscope (HT7700, Hitachi, Japan) with an operational voltage of 100 kV. NIR-II fluorescence spectra of the four nanoparticles were detected by a commercial photoluminescence spectrometer (FLS1000, Edinburgh Instruments). The CCK-8 assay was carried out by a Power Wave XS/XS2 microplate spectrophotometer (BioTek, Winooski, VT). The Olympus Fluoview FV1000 laser scanning confocal (Tokyo, Japan) was used to acquire confocal laser scanning microscopy (CLSM) cells images. Flow cytometry experiments were performed by Flow Sight Imaging Flow Cytometer (Merck Millipore, Darmstadt, Germany). The photothermal performance of the four nanoparticles were studied by Fotric 225 (Infrared thermoviewer) diwas, purchased from Fotric. (Shanghai, China). The NIR-II bioimaging experiments were carried out using the NIR-II small animal imaging instrument (In-vivo master, Wuhan Grand-imaging Technology Co., Ltd) equipped with a thermoelectrically cooled two-dimensional InGaAs camera and 1000 nm long-pass filter.

Cell Lines and Cell Culture

Procell Life Science &Technology (Wuhan, China) sold us the Murine breast cell line 4T1. 4T1 cells were incubated in complete Dulbecco's Modified Eagle Medium (DMEM) cell culture medium containing 10% fetal bovine serum (FBS) under the conventional environment (37 °C, 5% CO_2).

Animal Model

All animal experiments were in accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication no. 85-23 Rev. 1985) and approved by the Laboratory Animal Welfare and Ethics Committee of the Army Medical University

(AMUWEC20234909). Female BALB/c mice (5 ~ 6 weeks old) were purchased from Beijing Hfk. Bioscience Co. Ltd. To establish a tumor model, 4T1 cells suspended in 50 μL PBS (1×10^6) were subcutaneous injected into the right armpit or right hind leg of mice. Tumor volume was about 60 ~ 300 mm^3 before being used for NIR-II bioimaging and photothermal treatment.

Calculation of the relative NIR-II quantum yields

The relative NIR-II quantum yields of TD NPs, TT NPs, TC NPs, and TCD NPs were measured according to the previous reports.[1] The fluorescence spectra of them in the range from 900 to 1,500 nm were collected under the 808 nm excitation. This study selected a reference NIR-II fluorophore IR1061 with a known NIR-II quantum yield of 1.70% (Φ_{st}) in DCM.[2] The optical densities (OD) of all fluorophores were below 0.1 at 808 nm in solutions. η is the refractive index of solvent. F_{st} was the integrated NIR-II fluorescence emission intensities of IR1061 in the 1000-1400 nm region. F_x was the integrated NIR-II fluorescence emission intensities of the four nanoparticles in the 1000-1400 nm region. The quantum yield (Φ_x) of these four nanoparticles in NIR-II window was determined by following equation (1),

$$\Phi_x = \Phi_{st} \times \frac{F_x}{F_{st}} \times \frac{1 - 10^{-OD_{st}}}{1 - 10^{-OD_x}} \times \frac{\eta_x^2}{\eta_{st}^2} \quad (1)$$

***In Vitro* Photothermal Performance of the four nanoparticles**

The four nanoparticles in aqueous solution with 40 μg mL^{-1} were irradiated by an 850 nm LED lamp for 10 min at 0.4 W cm^{-2} and then stewing 10 min at dark atmosphere. The thermal images and temperature changes were recorded every 1min by a commercial infrared camera in the whole process.

The photostability of TCD NPs was studied by monitoring photothermal performance after five cycles of on/off 850 nm irradiation at a power density of 0.4 W cm^{-2}. Briefly, the TCD NPs solution was irradiated by laser for 5 min, followed cooling to room temperature for 5 min, which were repeated for 5 times.

Calculation of the photothermal conversion efficiency. The photothermal conversion efficiency (η) was calculated according to Equation (2), according to

published studies.[3,4]

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{diss}}{I(1 - 10^{-A_\lambda})} \quad (2)$$

Where h is the heat transfer coefficient, S is the irradiated area, T_{max} is the maximum temperature of the sample, and T_{surr} is the temperature of the surroundings. Q_{diss} is heat dissipation from the system to the surroundings, which was calculated to be 10.3 mW, I is the laser incident power, A_λ is the absorbance of samples at a wavelength of 850 nm. Here, hS can be obtained by Equation (3).

$$hS = \frac{m \cdot C_p}{\tau_s} \quad (3)$$

In which m and C_p are the mass and thermal capacity of the sample, respectively, and τ_s is the system time constant, calculated by Equation (4).

$$t = -\tau_s ln\theta = -\tau_s ln(\frac{T - T_{surr}}{T_{max} - T_{surr}}) \quad (4)$$

t is the time of the cooling process after irradiation.

***In Vitro* Cytotoxicity Assay**

The *in vitro* cytotoxicity of TCD NPs was studied by CCK-8 assay in 4T1 cells. The cells were seeded in 96-well plates (Costar, IL, USA) at a density of 5 × 10^3 cells/well and incubated for 24 h under standard conditions (37 ℃, 5% CO_2). Different concentrations of TCD NPs (100 μL) were diluted in DMEM and added to wells. The cells were incubated containing SPNs solutions for 18 h without light. After that, CCK-8 solution was added to each well at a ratio of 1:10 relative to the volume of the culture test kit. After another 4 h incubation, a PowerWave XS/XS2 microplate spectrophotometer was carried out to determine the absorption of 450 nm to evaluate relative cell viability.

***In Vitro* Photothermal Ablation of Cells**

4T1 cells were seeded in 96-wells plates at a concentration of 5 × 10^3 cells mL^{-1}. After 24 h incubation at 37 ℃, the incomplete DMEM containing different concentration of TCD NPs were added to wells and the cells were incubated for another 4 h. Then, the cells were exposed to 850 nm LED lamp light for 10 min with a low

power density (400 mW cm^{-2}). After the cells were cultured for an additional 12 h, the CCK-8 assay was used to evaluate the viability of cells

Cell Apoptosis Assay by Flow Cytometry

4T1 cells were seeded in 6-well plates (2×10^5 cells $well^{-1}$) and incubated with complete DMEM (containing 10% FBS) for 24 h. Then the culture medium was replaced by fresh incomplete DMEM containing of TCD NPs (100 μg mL^{-1}) or not. The "control" and "laser" groups were just incubated with fresh incomplete DMEM. And the "TCD NPs" and "TCD NPs+laser" groups were cultured with incomplete DMEM containing of TCD NPs (100 μg mL^{-1}). These cells continuously cultured under standard condition. After 4 h incubated, the treating cells in the "laser" and "TCD NPs+laser" groups were irradiated with 850 nm LED lamp light at 400 mW cm^{-2} for 10 min. After cultured for an additional 12 h, the culture medium was removed. The cells were washed softly with fresh PBS and then harvested by trypsin treatment. Next, the cells were washed by PBS three times gently followed by staining with Annexin V-FITC/ propidium iodide (PI). Flow cytometry assay was performed via BD Fortessa, and the data were analyzed with FlowJo (v10.8.1).

Photothermal Effect *In Vitro* by Confocal Imaging

4T1 cells were randomly divided into four groups (namely, "control", "laser", "TCD NPs", and "TCD NPs+laser") and seeded in four single-well confocal dishes at a concentration of 8×10^4 cells mL^{-1}. After a 24 h incubation under 37 ℃ in a humidified atmosphere of 5% CO_2, the cells were washed by PBS and cultured with incomplete DMEM medium containing TCD NPs (100 μg mL^{-1}) or not for 5 h without 850 nm light. Then the cells in "laser" and "TCD NPs+laser" groups were exposed to 850 nm LED lamp light (400 mW cm^{-2}) for 10 min. The cells were cultured for another 12 h and then stained with Calcein-AM/propidium iodide (PI) solution for 15 min. The cells were washed by PBS and cultured by fresh incomplete DMEM medium. Finally, the cells were imaged by CLSM (ZEISS 880).

Blood Hematology and Biochemistry Analysis

Blood samples were collected from the fundus artery after injection of 100 μL of PBS and TCD NPs (2 mg mL^{-1}) solutions at 7, 14 and 30 days. The collected blood

samples were centrifuged at 4000 rpm for 15 min and washed several times with PBS. Then, hepatic function markers (ALT and AST) and renal function markers (CREA and UREA) were measured. The uncentrified blood samples were added with diluent for routine blood tests. The routine blood tests were performed to red blood cells (RBC), monocyte (MXD) haemoglobin (HGB), Eosinophils (EO), basophil (BASO) mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), haematocrit (HCT), latelet crit (PCT) and mean platelets volume (MPV).

***In Vitro* and *In Vivo* NIR-II Bioimaging**

For *in vitro* NIR-II fluorescence bioimaging, 4T1 cells were used as a model cell line and seeded in four petri dishes at a concentration of 3×10^6 cells per dish. Incomplete DMEM culture medium with the same concentration of TCD NPs (200 μg mL^{-1}) were co-incubated with three of these four dishes of 4T1 cells for 1, 10 and 24 h. The rest one dish of 4T1 cells was set to be the control group which was co-incubated with incomplete DMEM culture medium without TCD NPs for 24 h. After that, 4T1 cells in the four dishes were collected in four tubes and washed by PBS. Then the four tubes of 4T1 cells were transported to four wells of 96-wells plate and confirmed their NIR-II fluorescent signals by a commercial NIR-II bioimaging system. The related NIR-II fluorescence intensity was evaluated by ImageJ software.

For NIR-II bioimaging in live mice, 4T1-tumor-bearing Balb/c mice were firstly injected intravenously with TCD NPs (120 μL, 2000 μg mL^{-1}), and the NIR-II fluorescence images of the whole mice were obtained under 808 nm laser excitation. For the duration of bioimaging, the treated mice were anesthetized using 2−3% isoflurane in oxygen. The *vivo* NIR-II imaging system was set as 1000 ms exposure time and equipped with a 1000 nm long-pass filter. The NIR-II images of the whole 4T1-tumor-bearing mice were acquired via NIR-II bioimaging instrument at different time points (0, 4, 8, 12, 24, and 48 h). At 48 h after injection of TCD NPs, the main organs (heart, liver, spleen, lung kidneys and tumor) were collected and imaged under 808 nm laser. The related NIR-II fluorescence intensity and signal-to-background ratio (SBR) was evaluated by ImageJ software

***In Vivo* PTT**

To assess *in vivo* photothermal therapy efficiency, the BALB/c mice bearing 4T1 tumor on the right hind leg were injected with 150 μL TCD NPs solutions (2000 μg mL^{-1}) or PBS through tail vein. After 12 h, we used continuous 850 nm LED lamp (400 mW cm^{-2}) to inspire PTT effect in tumor for 15 min. Under the LED lamp excitation, the temperature on tumor section was determined and collected by a commercial infrared camera. The treated mice were anesthetized throughout the entire process of recording the *vivo* PTT effect.

To confirm tumor suppression of PTT, the 4T1 tumor-bearing mice were randomly divided into four groups ("control", "laser", "TCD NPs", and "TCD NPs+laser" groups, 3 mice per group). When tumors had grown up to 60 ~ 150 mm^3, mice in the "laser" and "TCD NPs+laser" groups were firstly intravenously injected by 150 μL PBS or TCD NPs solutions (2000 μg mL^{-1}) and tumors of mice were explored by continuous 850 nm LED lamp (400 mW cm^{-2}) after 12 h postinjection. Additionally, mice in the "control" and "TCD NPs" groups were intravenously injected by 120 μL PBS or TCD NPs solutions (3000 μg mL^{-1}) without any further treatments. After the above-mentioned treatments, the volumes of the tumors in every group were conducted by a vernier caliper every 2 days during 14 days and evaluated by the equation: tumor volume = (tumor length) × $(\text{tumor width})^2$ / 2. Simultaneously, the body weight of the mice were measured to evaluate the health of every mouse. After 14 days, tumor tissues and other major organs (heart, liver, spleen, lung and kidney) were dissected and (H&E) staining analysis of them was conducted. The dissected tumor tissues in all groups were weighed and photographed finally.

Histopathological analysis

For H&E staining, all tissues were fixed with 4% paraformaldehyde, dehydrated in ethanol solution, embedded in paraffin, and cut into sections with a thickness of 10 μm. The sections were washed with xylene and ethanol and then immersed in hematoxylin working solution for 5 min and eosin working solution for 3 min, followed by washing with distilled water. The stained sections were examined using an inverted fluorescence microscope (IX83, Olympus) and analyzed with ImageJ.

Statistics

All the graph bars represent mean ± standard deviation and their statistical analyses were performed using an unpaired t-test (GraphPad Prism). Comparisons with p-values less than 0.05 were considered to be statistically significant. All the micrographs are representative of at least three independent experiments.

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