

Supplementary Information

Integrated Theranostic Nanoplatfrom Empowers Precision Cancer Care via Radionuclide-Labeled NIR-II Aggregation-Induced Emission Luminogens

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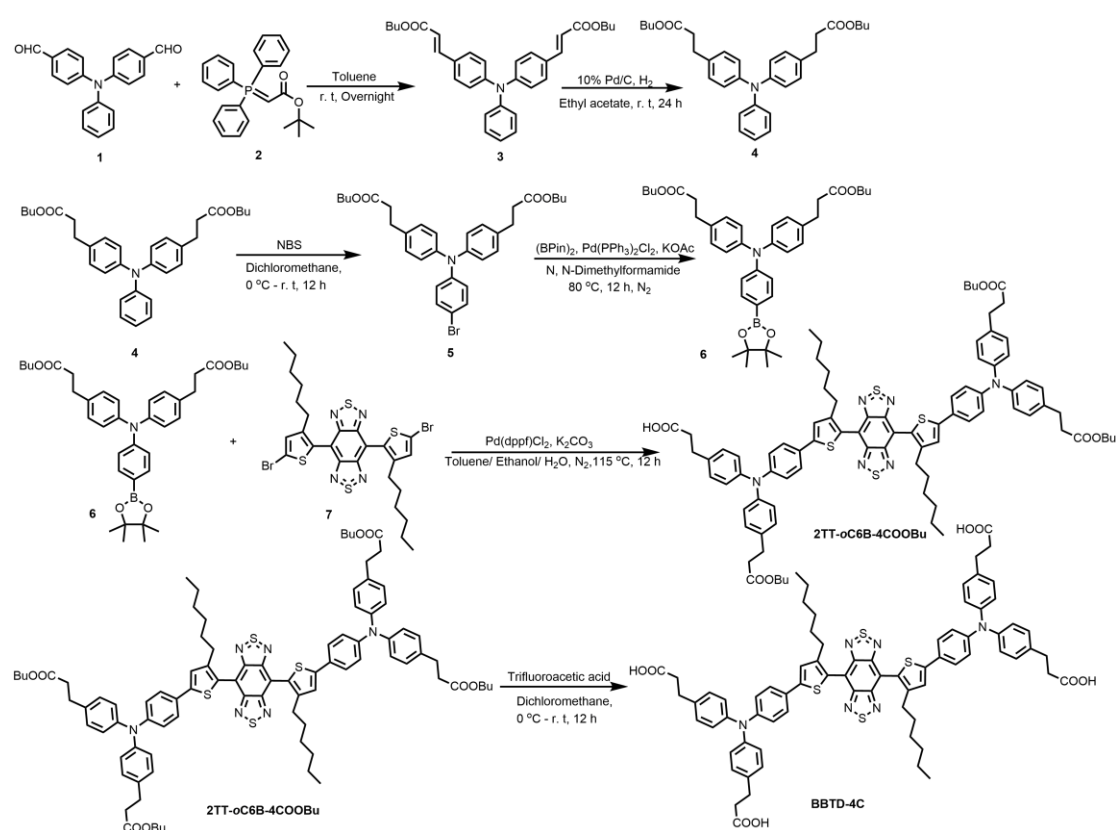
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Synthesis and characterization



Supplementary Fig. 1 The structure and synthetic route of BBTD-4C.

The synthesis procedure of compound 3

(tert-Butoxycarbonylmethylene) triphenylphosphorane (8.3 g, 22.0 mmol) was added to a solution of compound **1** (3.01 g, 10.0 mmol) in anhydrous toluene (100 mL) under an inert atmosphere (N₂). The solution was stirred for 24 h at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 16:1 v/v) to give a bright yellow oil **3** (4.5 g, yield 90%). **¹H NMR** (500 MHz, CDCl₃) δ 7.53 (d, J = 15.9 Hz, 2H), 7.38 (d, J = 8.4 Hz, 4H), 7.31 (t, J = 7.8 Hz, 2H), 7.18 – 7.09 (m, 3H), 7.04 (d, J = 8.4 Hz, 4H), 6.25 (d, J = 15.9 Hz, 2H), 1.53 (s, 18H). **¹³C NMR** (126 MHz, CDCl₃) δ 166.8, 148.8, 146.6, 143.0, 129.8, 129.3, 129.2, 126.0, 124.8, 123.5, 118.4, 80.5, 28.4. **HRMS** (ESI): m/z calculated for: C₃₂H₃₆NO₄⁺ ([M+H]⁺): 498.2644, found: 498.2644.

The synthesis procedure of compound 4

A mixture of compound **3** (4.5 g, 9.05 mmol) and 10% Pd/C (0.45 g) in ethyl acetate (100 mL) was evacuated and back-filled with H₂ (40 atm). After stirring 24 h at room

temperature, the mixture was filtered over a pad of Celite (ethyl acetate as the eluent) and the solvent was evaporated in vacuo. The crude product was further purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 16:1 v/v) to afford a colorless oil (4.5 g, yield 99%). **¹H NMR** (500 MHz, CDCl₃) δ 7.70 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 7.9 Hz, 2H), 7.18 (t, J = 8.2 Hz, 4H), 7.14 (d, J = 8.2 Hz, 4H), 7.07 (t, J = 7.5 Hz, 1H), 2.90 (t, J = 7.8 Hz, 2H), 2.56 (t, J = 7.8 Hz, 2H), 1.43 (s, 18H). **¹³C NMR** (126 MHz, CDCl₃) δ 172.5, 148.1, 146.1, 135.2, 129.2, 129.2, 124.4, 123.5, 122.2, 80.4, 37.2, 30.6, 28.2. **HRMS** (ESI): m/z calculated for: C₃₂H₄₀NO₄⁺ ([M+H]⁺): 502.2957, found: 502.2962 and C₃₂H₄₀NO₄Na⁺ ([M+Na]⁺): 524.2771, found: 502.2962.

The synthesis procedure of compound 5

To 150 mL of dichloromethane in 500 mL round bottomed flask was added compound 4 (2.505 g, 5.0 mmol). After stirring at 0-5 °C for 5 min, NBS (934 mg, 5.25 mmol) was added in 5 portions. The reaction mixture was warmed to room temperature and stirred for additional 16 h. The reaction was then completed and filtered through a pad of Celite and concentrated in vacuo, to give a brown oil. Purification of the crude product by flash chromatography (Petroleum ether/Ethyl acetate = 20:1 v/v) afforded a colorless oil 4 (2.75 g, yield 95%). **¹H NMR** (500 MHz, CDCl₃) δ 7.27 (d, J = 8.9 Hz, 2H), 7.07 (d, J = 8.5 Hz, 4H), 6.97 (d, J = 8.5 Hz, 4H), 6.88 (d, J = 8.8 Hz, 2H), 2.86 (t, J = 7.7 Hz, 4H), 2.53 (t, J = 7.7 Hz, 4H), 1.43 (s, 18H). **¹³C NMR** (126 MHz, CDCl₃) δ 172.3, 147.2, 145.5, 135.8, 132.1, 129.4, 124.6, 124.4, 114.1, 80.4, 37.1, 30.6, 28.2. **HRMS** (ESI): m/z calculated for: C₃₂H₃₉BrNO₄⁺ ([M+H]⁺): 580.2062, found: 580.2055.

The synthesis procedure of compound 6

To a solution of Pd(PPh₃)₂Cl₂ (140 mg, 0.2 mmol), KOAc (470 mg, 4.8 mmol), and (Bpin)₂ (609 mg, 2.4 mmol) in N, N-Dimethylformamide (20 mL) was added compound 5 (1.158 g, 2.0 mmol) under an nitrogen atmosphere. The reaction mixture was heated in an oil bath at 80 °C for 24 h. The solution was cooled, dilute with H₂O (40 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over anhydrous Na₂SO₄ and evaporated in vacuo. The residue was purified by column chromatography (Petroleum ether/Ethyl acetate = 8:1 v/v) to give compound 6 (892 mg, yield 71%) as a colorless oil. **¹H NMR** (500 MHz, CDCl₃) δ 7.62 (d, J = 7.8 Hz, 2H), 7.08 (d, J = 8.0 Hz, 4H), 7.00 (d, J = 8.0 Hz, 4H), 6.96 (d, J = 7.8 Hz, 2H), 2.86 (t, J = 7.1 Hz, 4H), 2.53 (t, J = 8.3 Hz, 4H), 1.42 (s, 18H),

1.32 (s, 12H). **¹³C NMR** (126 MHz, CDCl₃) δ 172.5, 150.9, 145.6, 136.0, 135.9, 129.4, 125.2, 121.1, 83.6, 80.5, 37.2, 30.7, 28.2, 25.0. **HRMS** (ESI) m/z calculated for: C₃₈H₅₁BNO₆⁺ ([M+H]⁺): 628.3809. found: 628.3826.

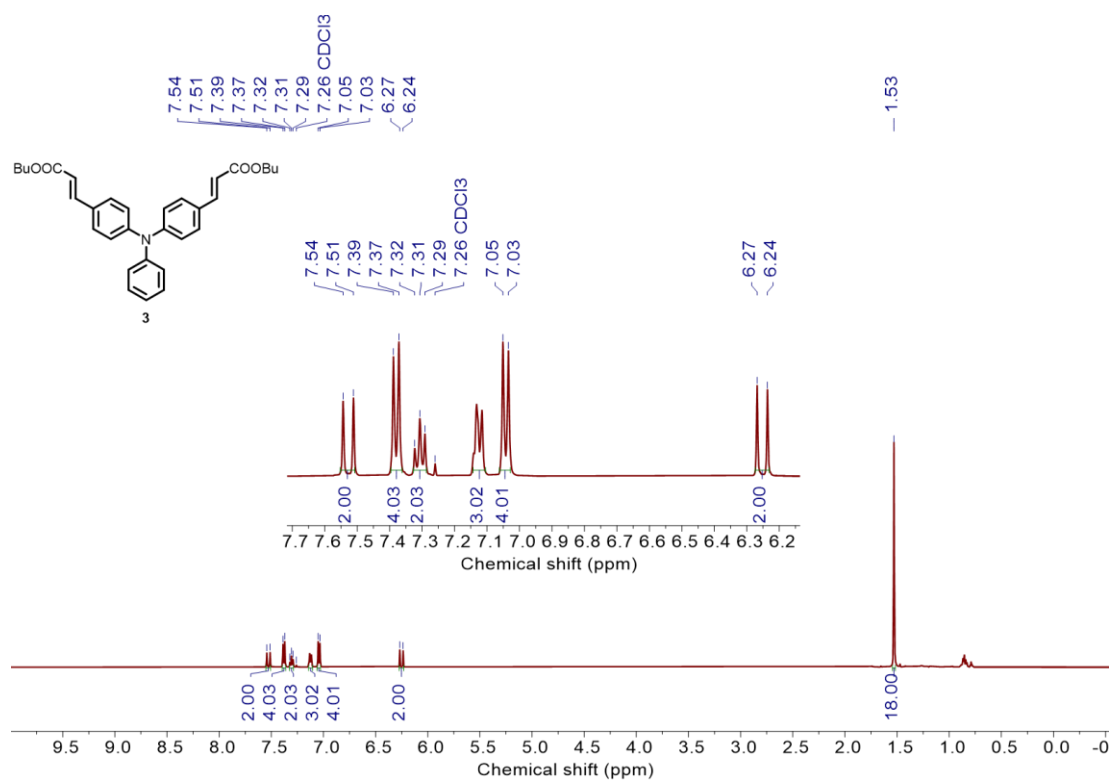
The synthesis procedure of 2TT-*o*C6B-COOBu

A 50 mL two-necked flask was charged with compound **6** (1.254 g, 2.0 mmol), compound **7** (500 mg, 0.73 mmol), and Pd(dppf)Cl₂ (106 mg, 0.145 mmol), aqueous K₂CO₃ (1 M, 5 mL) in toluene (25 mL) and ethyl alcohol (10 mL) under an inert atmosphere (N₂). The resulting mixture was further degassed with an N₂ stream for 20 min and heated in an oil bath at 115 °C and stirred until TLC showed the complete consumption of **6**. The reaction was allowed to cool to room temperature and extracted with ethyl acetate (2×20 mL). The combined organic layers were washed with water (50 mL) and sat. brine (100 mL). After drying over anhydrous Na₂SO₄ and removal of the solvents under reduced pressure, the residue was purified by column chromatography on silica gel (Petroleum ether/Ethyl acetate = 8:1 v/v) to yield the product as a dark green solid (219 mg, yield 21%). **¹H NMR** (500 MHz, CDCl₃) δ 7.42 (d, J = 8.7 Hz, 4H), 7.10 – 6.94 (m, 20H), 6.80 (s, 2H), 2.87 (t, J = 7.7 Hz, 8H), 2.59 (t, J = 7.6 Hz, 4H), 2.54 (t, J = 7.7 Hz, 8H), 1.64 (m, 4H), 1.44 (s, 36H), 1.38 – 1.26 (m, 12H), 0.90 (t, J = 6.5 Hz, 6H). **¹³C NMR** (126 MHz, CDCl₃) δ 172.5, 147.3, 145.7, 145.0, 144.3, 143.9, 143.0, 135.5, 130.7, 129.3, 128.5, 126.5, 124.6, 123.7, 123.2, 118.6, 105.5, 80.5, 37.2, 31.8, 30.7, 30.6, 30.5, 29.1, 28.2, 22.7, 14.2. **HRMS** (ESI) m/z calculated for: C₉₀H₁₀₄N₆O₈S₄Na⁺ ([M+Na]⁺): 1547.6696. found: 1547.6697.

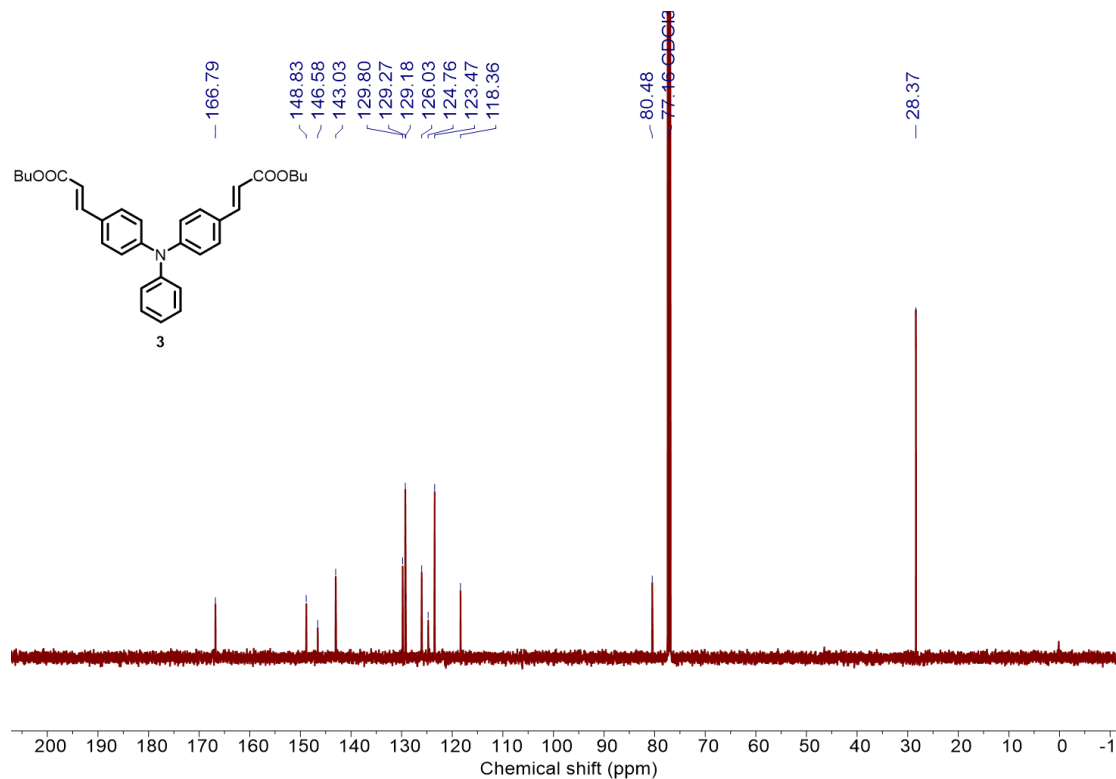
The synthesis procedure of BBTD-4C

To a solution of compound 2TT-*o*C6B-4COOBu (50 mg, 0.0073 mg) in DCM (5 mL) was added TFA (2 mL) at 0 °C. The reaction mixture was slowly warmed to ambient temperature. The reaction was completed in 60 min by TLC analysis. The solvent was removed in vacuo and the crude product was washed by acetonitrile (5×5 mL) to yield the desired product **BBTD-4C** as a green solid (42.2 mg, yield 99%). **¹H NMR** (500 MHz, MeOD) δ 7.56 (d, J = 8.1 Hz, 4H), 7.40 (s, 2H), 7.19 (d, J = 8.1 Hz, 8H), 7.00 (d, J = 7.5 Hz, 12H), 2.89 (t, J = 7.9 Hz, 8H), 2.59 (m, 4H), 2.46 (t, J = 8.1 Hz, 8H), 1.53 (m, 4H), 1.12 – 0.79 (m, 12H), 0.65 (t, J = 7.0 Hz, 6H). **¹³C NMR** (126 MHz, CDCl₃) δ 179.1, 154.9, 152.8, 150.9, 147.1, 145.8, 144.0, 135.1, 132.2, 131.1, 129.4, 124.7, 121.5, 114.5, 111.7, 106.4, 35.7, 31.8, 30.7, 30.3, 30.1, 29.0, 22.7, 14.2. **HRMS** (ESI)

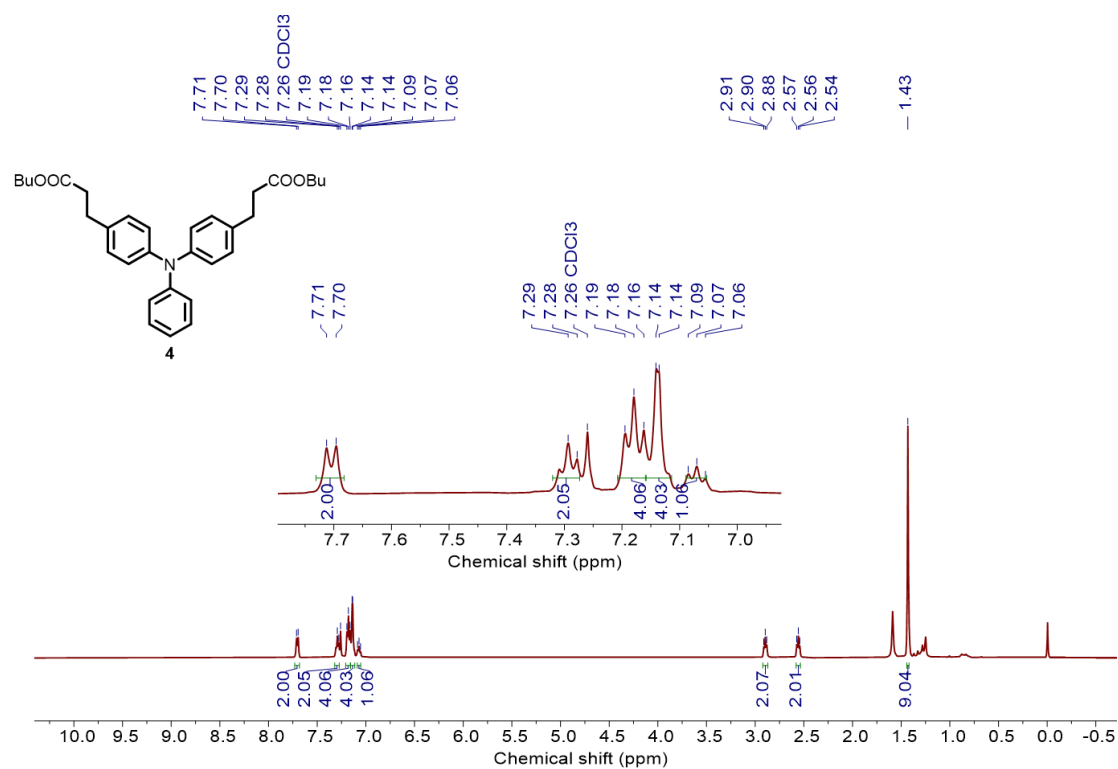
calculated for: $C_{74}H_{73}N_6O_8S_4^+$ ($[M+H]^+$): 1301.4367. found: 1301.4325.



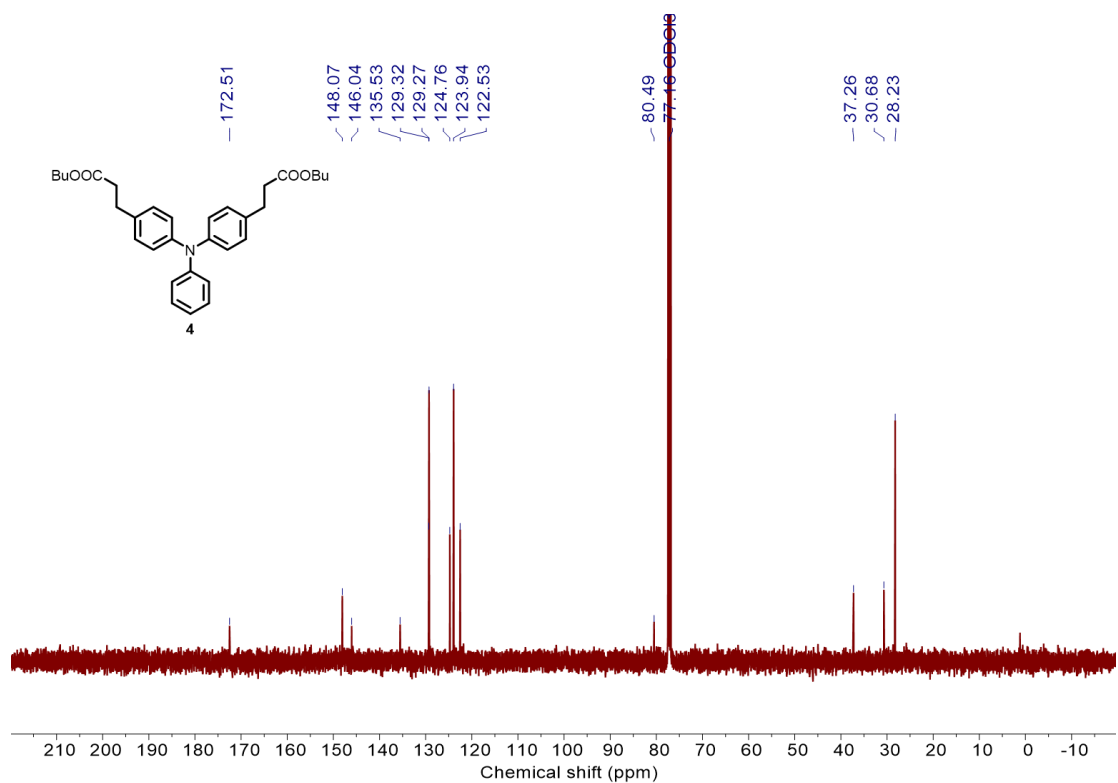
Supplementary Fig. 2 ¹H NMR spectrum of compound 3.



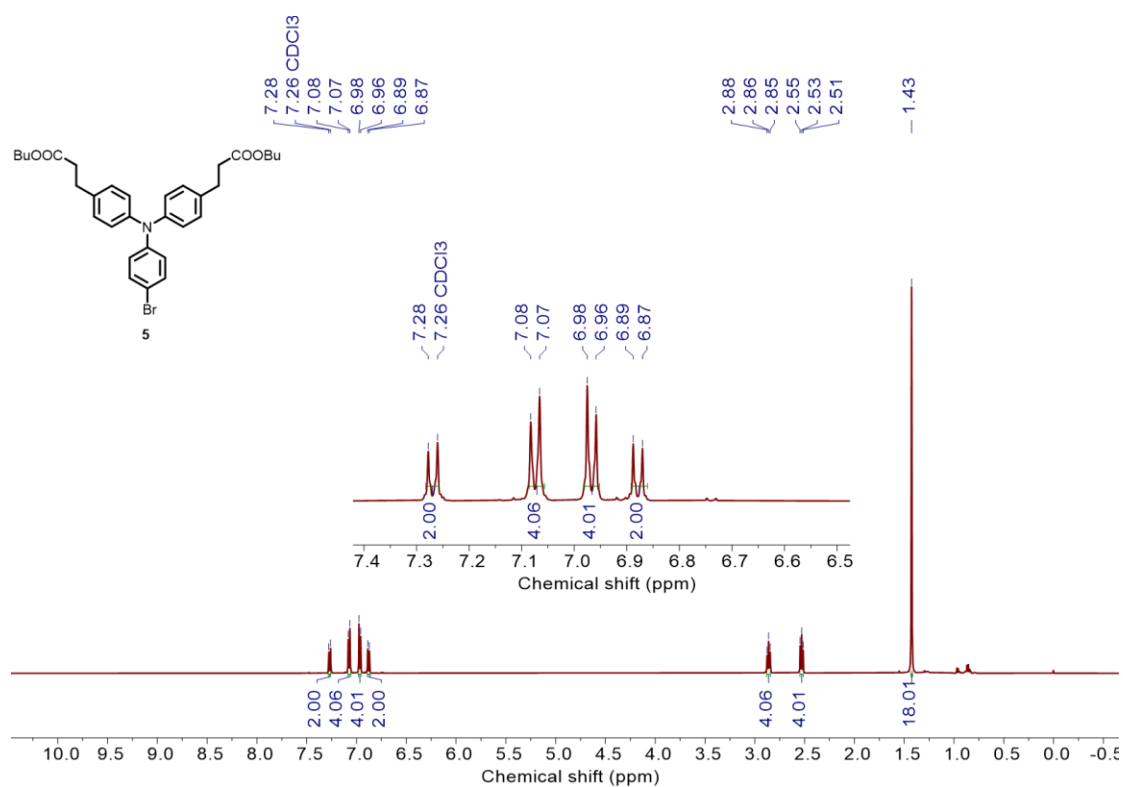
Supplementary Fig. 3 ¹³C NMR spectrum of compound 3.



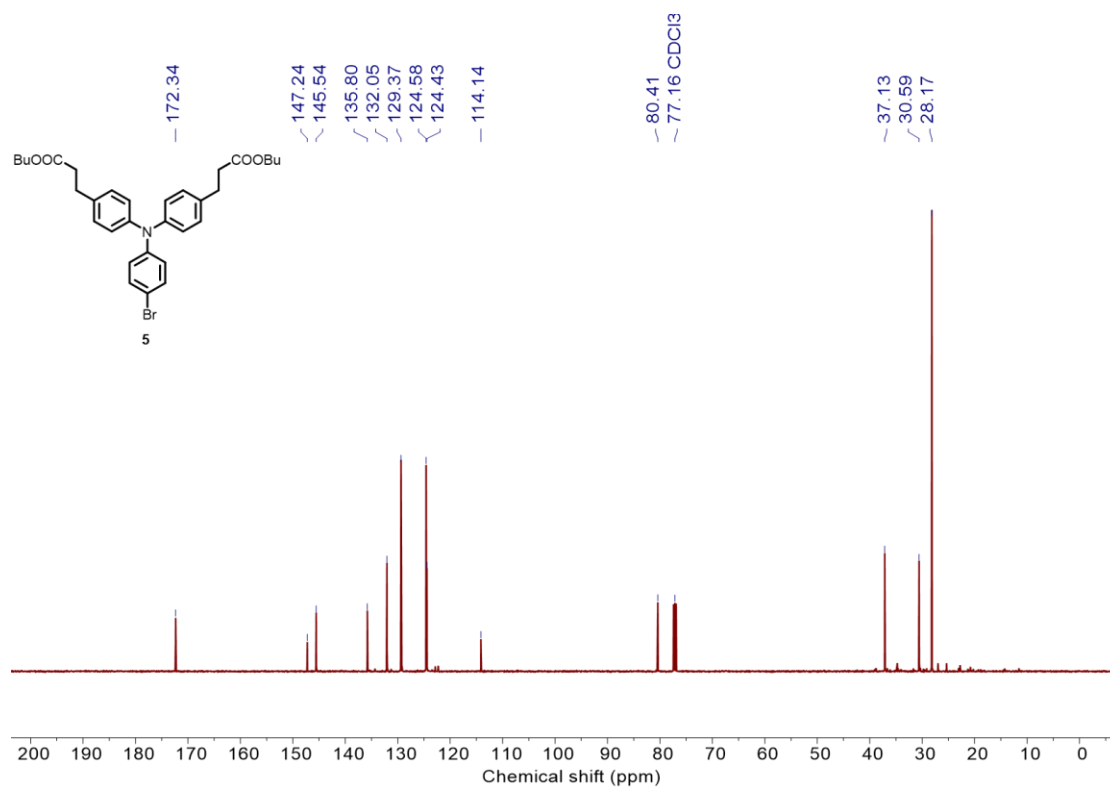
Supplementary Fig. 4 ^1H NMR spectrum of compound 4.



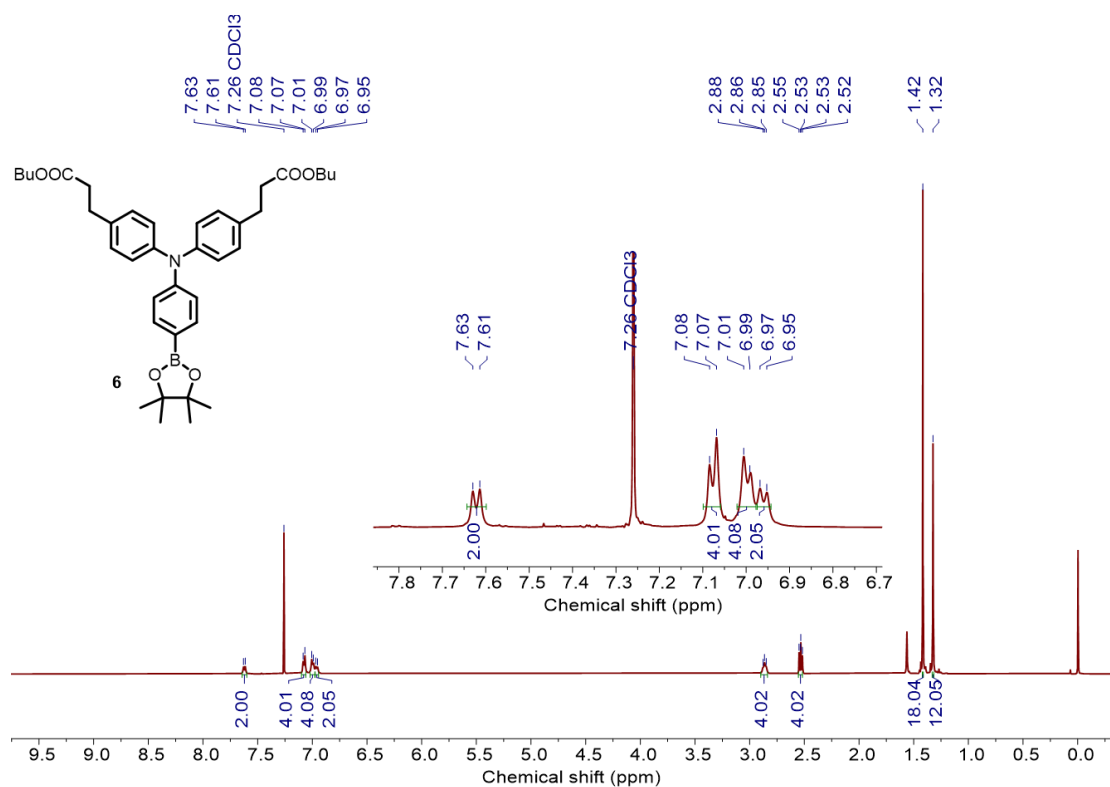
Supplementary Fig. 5 ^{13}C NMR spectrum of compound 4.



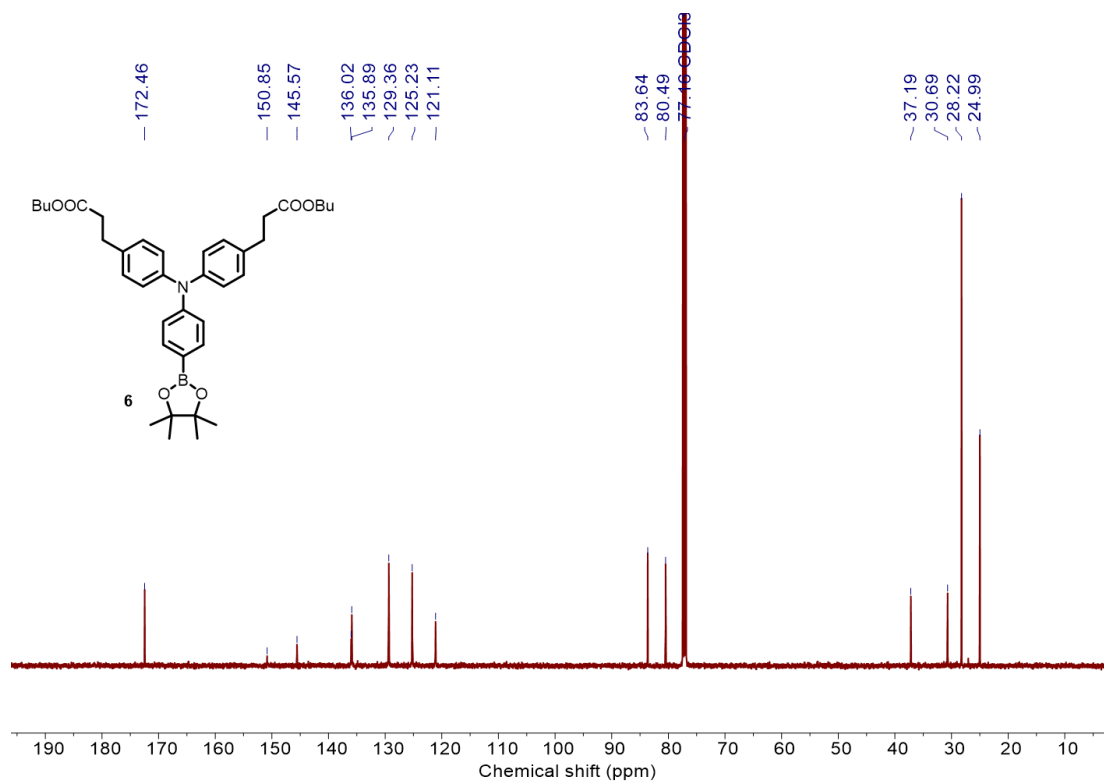
Supplementary Fig. 6 ¹H NMR spectrum of compound **5**.



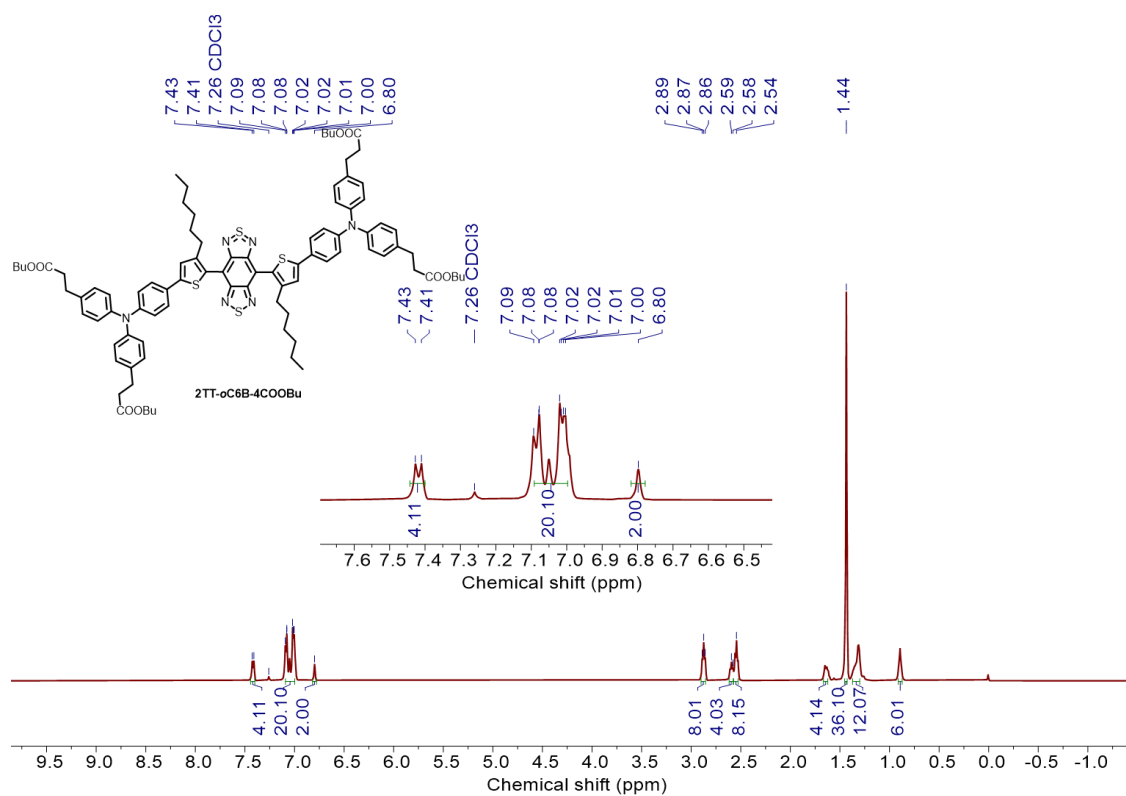
Supplementary Fig. 7 ¹³C NMR spectrum of compound **5**.



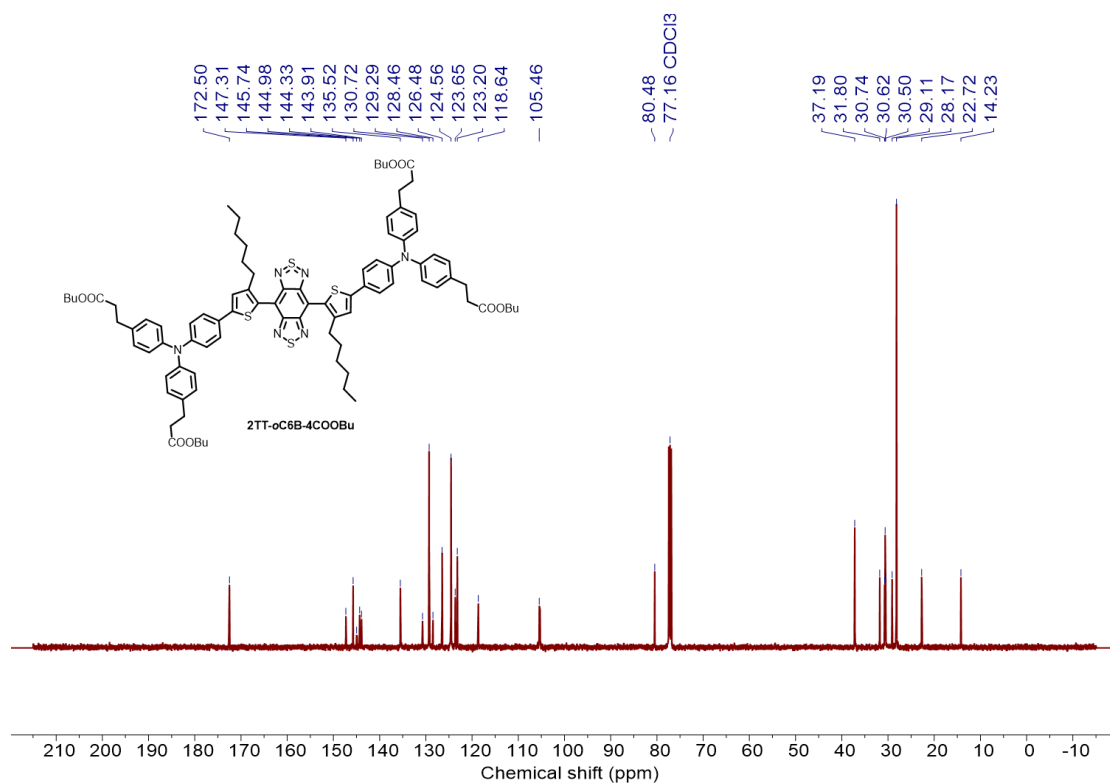
Supplementary Fig. 8 ¹H NMR spectrum of compound 6.



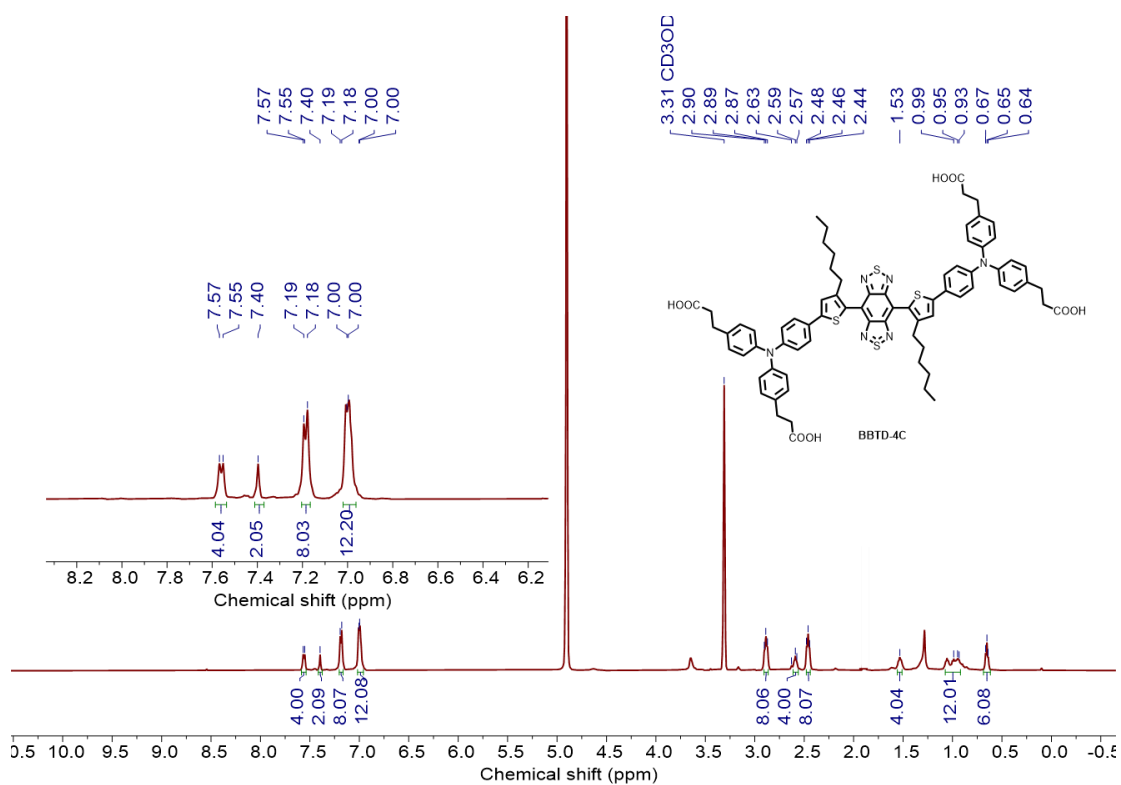
Supplementary Fig. 9 ¹³C NMR spectrum of compound 6.



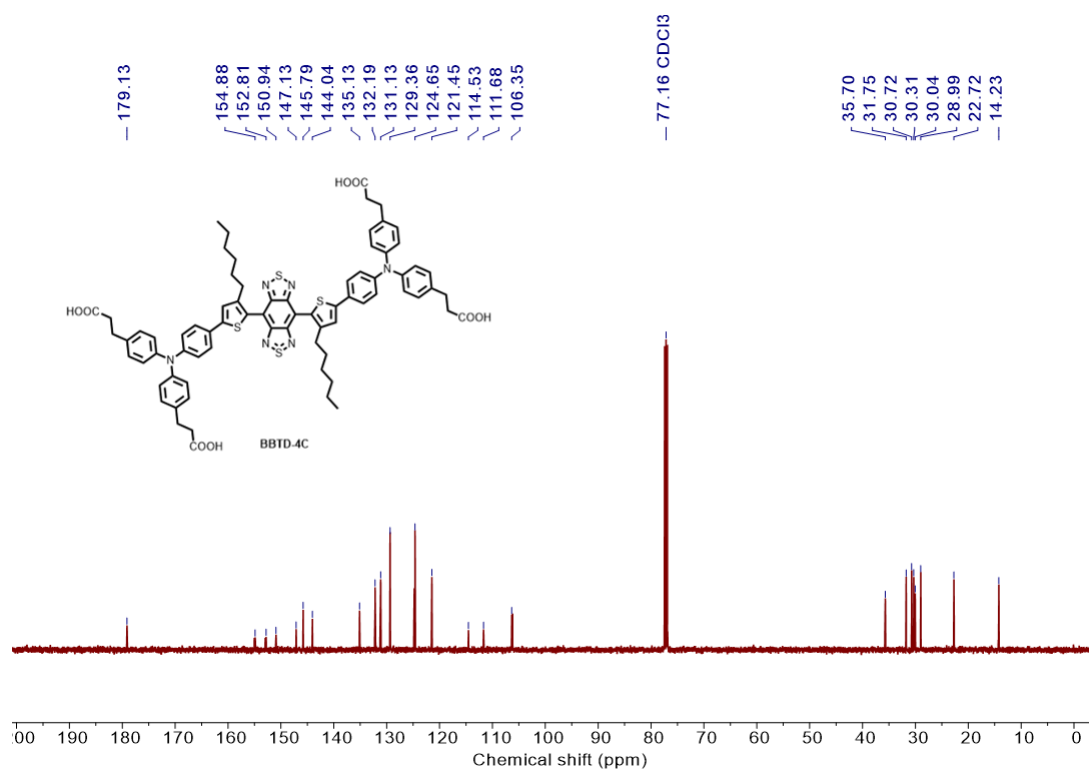
Supplementary Fig. 10 ¹H NMR spectrum of compound 2TT-oC6B-COOBu.



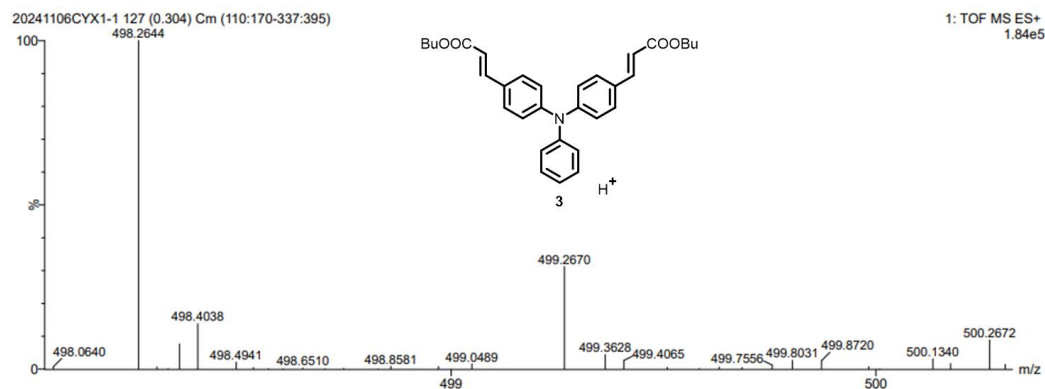
Supplementary Fig. 11 ¹³C NMR spectrum of compound 2TT-oC6B-COOBu.



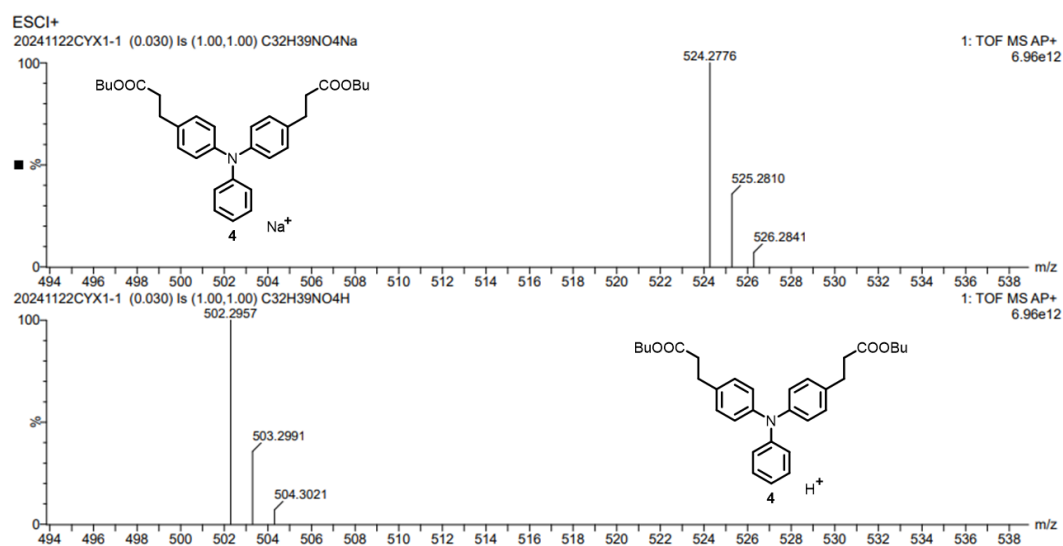
Supplementary Fig. 12 ¹H NMR spectrum of compound **BBTD-4C**.



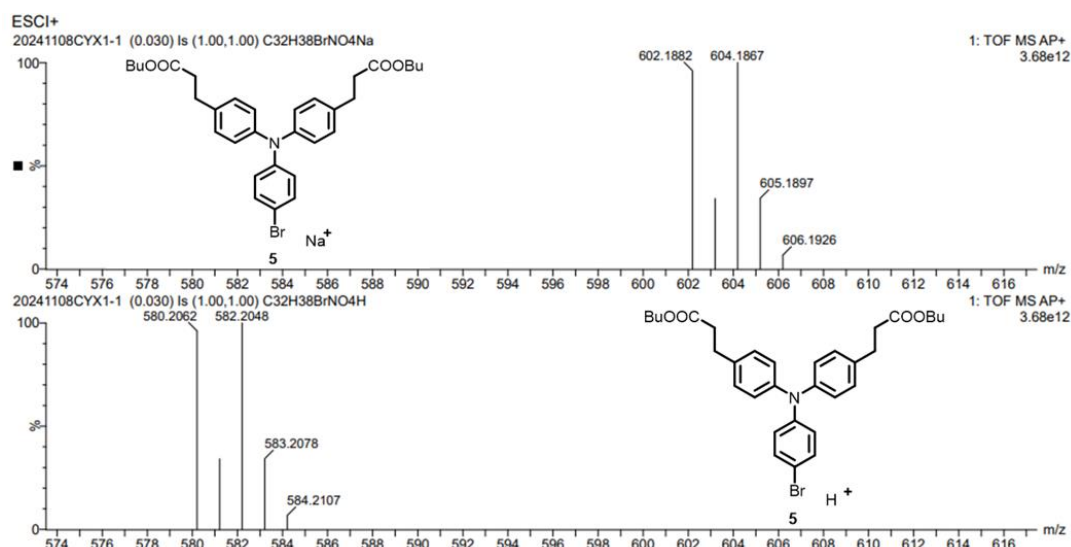
Supplementary Fig. 13 ¹³C NMR spectrum of compound **BBTD-4C**.



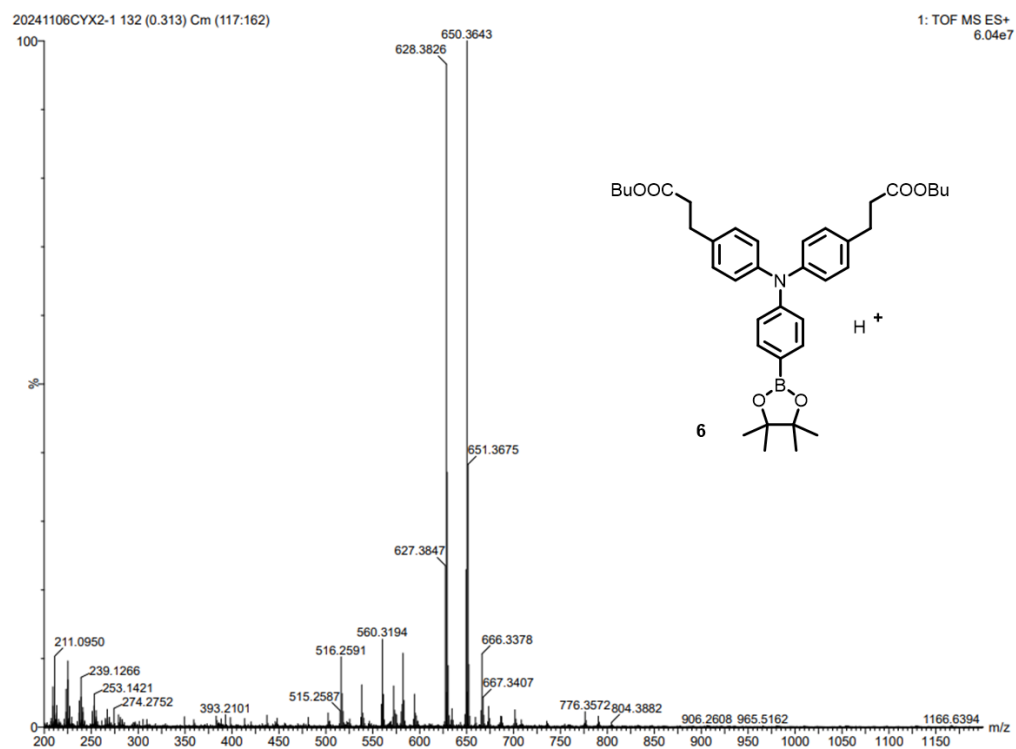
Supplementary Fig. 14 HRMS spectrum of compound 3.



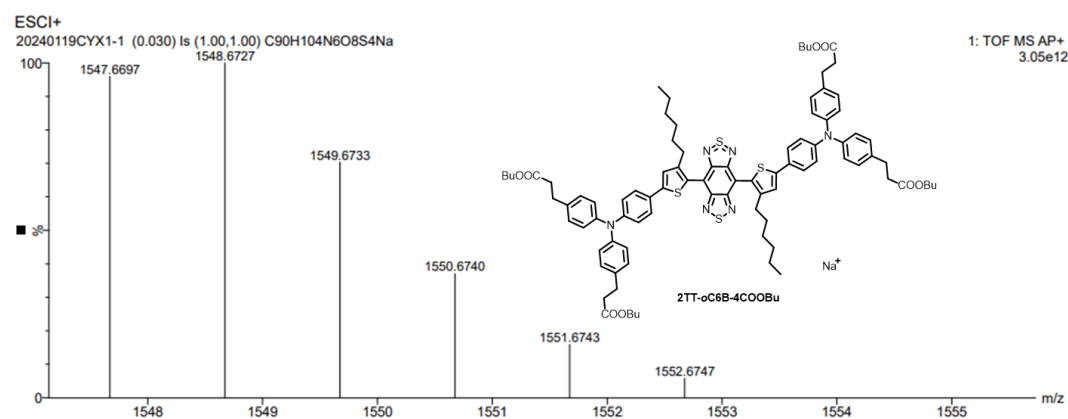
Supplementary Fig. 15 HRMS spectrum of compound 4.



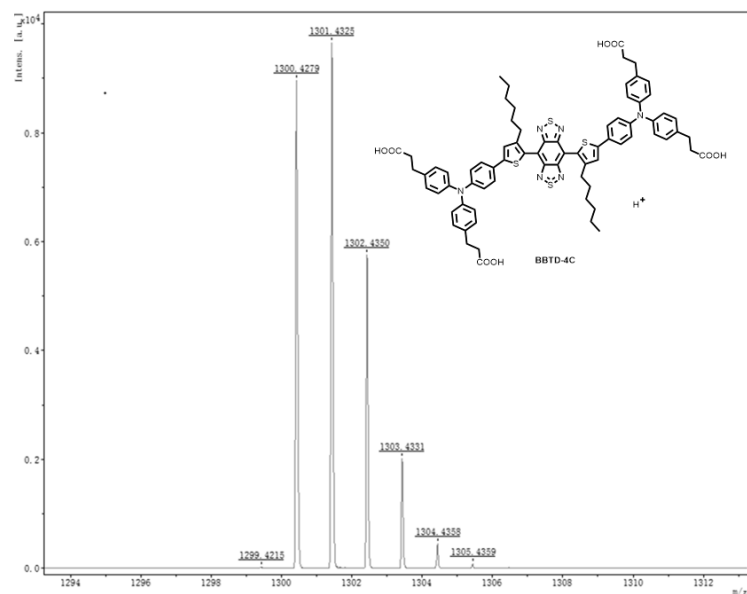
Supplementary Fig. 16 HRMS spectrum of compound 5.



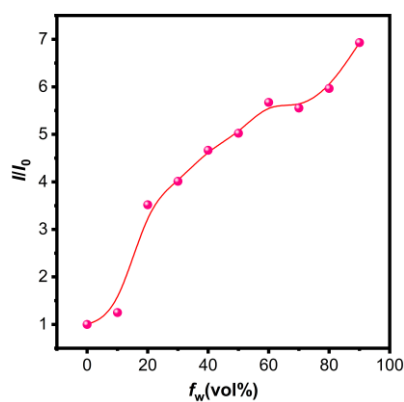
Supplementary Fig. 17 HRMS spectrum of compound 6.



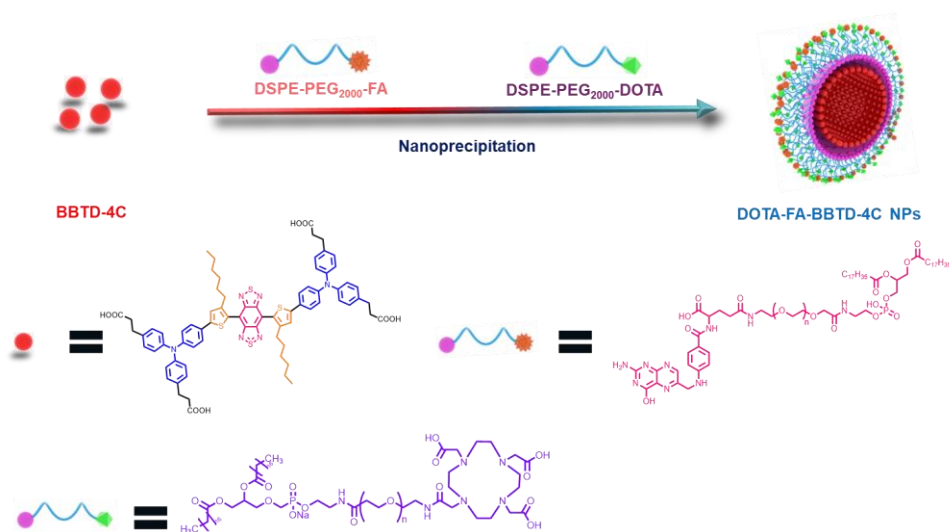
Supplementary Fig. 18 HRMS spectrum of compound 2TT-αC6B-COOBu.



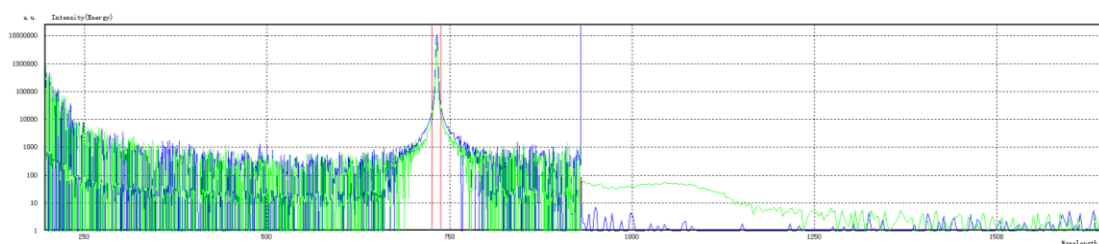
Supplementary Fig. 19 HRMS spectrum of compound **BBTD-4C**.



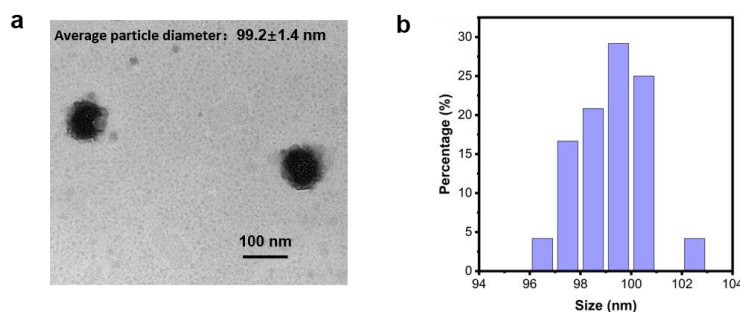
Supplementary Fig. 20 Plots of relative PL intensity (I/I_0) versus f_W of BBTD-4C at 1007 nm. I_0 = PL intensity of BBTD-4C in pure DMSO. [BBTD-4C] = 1.0×10^{-5} M.



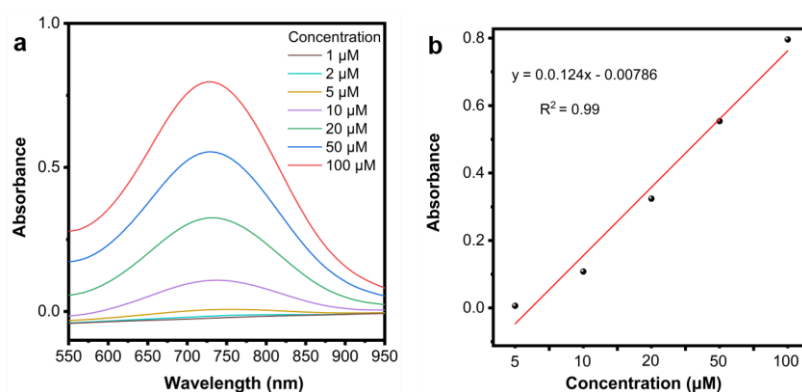
Supplementary Fig. 21 Preparation process of DOTA-FA-BBTD-4C NPs.



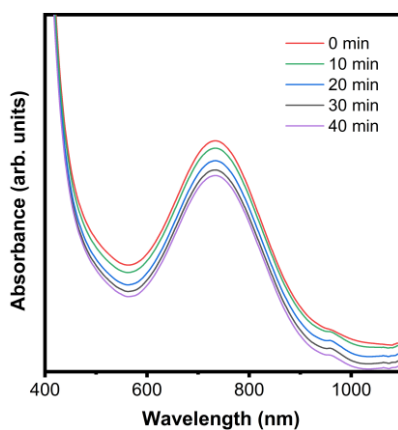
Supplementary Fig. 22 Absolute Quantum Yield of Fluorescence in DOTA-FA-BBTD-4C NPs. Concentration: [DOTA-FA-BBTD-4C NPs] = 10 μ M. fluorescence quantum yield = 0.12%.



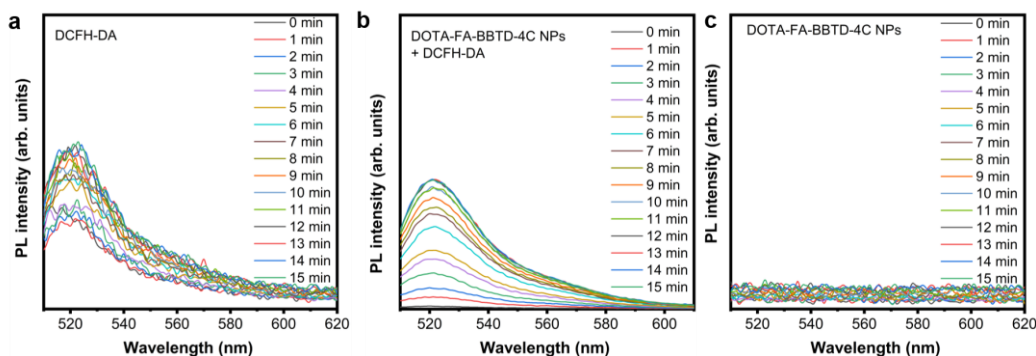
Supplementary Fig. 23 **a** The TEM of DOTA-FA-BBTD-4C NPs. Results are representative of at least three independent experiments. **b** The particle size distribution histogram of DOTA-FA-BBTD-4C NPs.



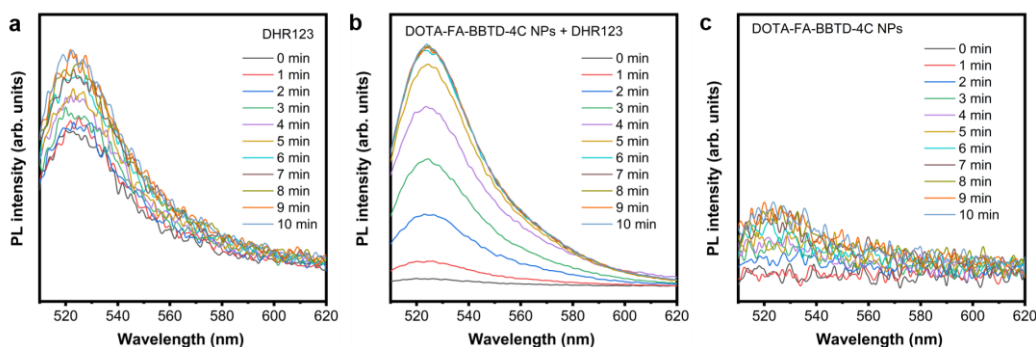
Supplementary Fig. 24 **a** Absorbance spectra of DOTA-FA-BBTD-4C NPs at different concentrations, and **b** Linear regression analysis showing the correlation between nanoparticle concentration and absorbance.



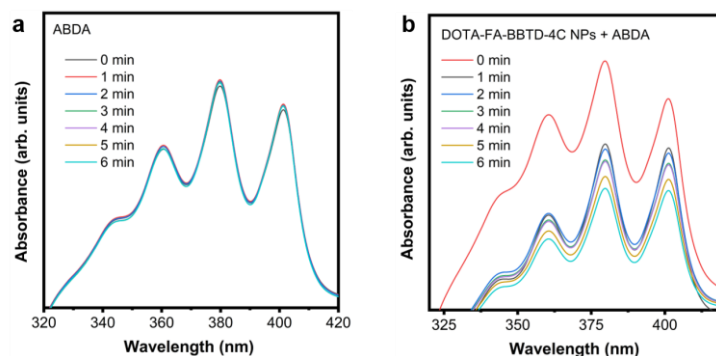
Supplementary Fig. 25 Relative absorbance of DOTA-FA-BBTD-4C NPs before and after 40 min of laser irradiation. (660 nm, 1.2 W/cm²).



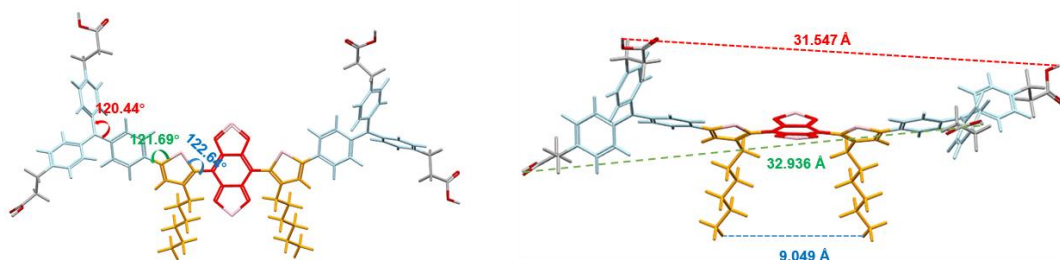
Supplementary Fig. 26 The fluorescence spectra changes of 2',7'-Dichlorofluorescein diacetate (DCFH-DA) for ROS detection. **a** DCFH-DA, **b** DOTA-FA-BBTD-4C NPs + DCFH-DA, **c** DOTA-FA-BBTD-4C NPs alone under laser with different irradiation time. Concentration: [DOTA-FA-BBTD-4C NPs] = 10 μ M, [DCFH-DA] = 5 μ M; Light power: (660 nm, 0.8 W/cm²).



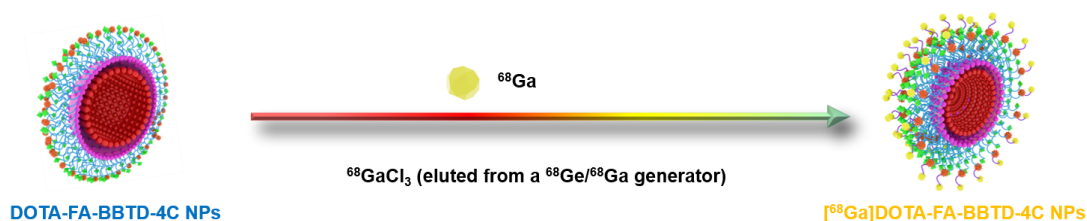
Supplementary Fig. 27 The fluorescence spectra changes of Dihydrorhodamine 123 (DHR123). **a** DHR123, **b** DOTA-FA-BBTD-4C NPs + DHR123, **c** DOTA-FA-BBTD-4C NPs alone under laser with different irradiation time. Concentration: [DOTA-FA-BBTD-4C NPs] = 10 μ M, [DHR123] = 10 μ M; Light power: (660 nm, 0.8 W/cm²).



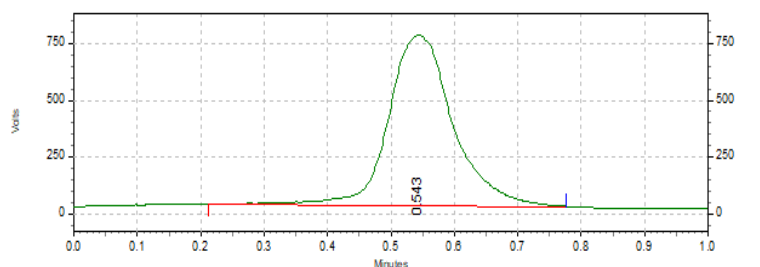
Supplementary Fig. 28 UV-vis spectra changes of 9,10-Anthracenediyl-bis (methylene)dimalonic acid (ABDA) in PBS. **a** ABDA, **b** DOTA-FA-BBTD-4C NPs + ABDA under laser with different irradiation time. Concentration: [DOTA-FA-BBTD-4C NPs] = 10 μ M, [ABDA] = 50 μ M, Light power: (660 nm, 0.8 W/cm²).



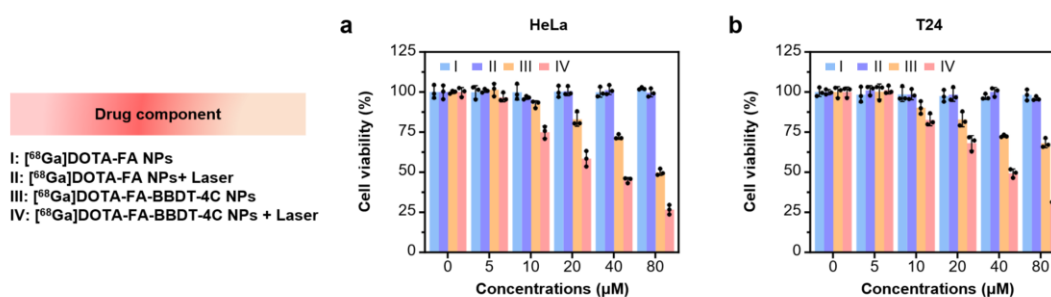
Supplementary Fig. 29 Optimized chemical structures of BBTD-4C.



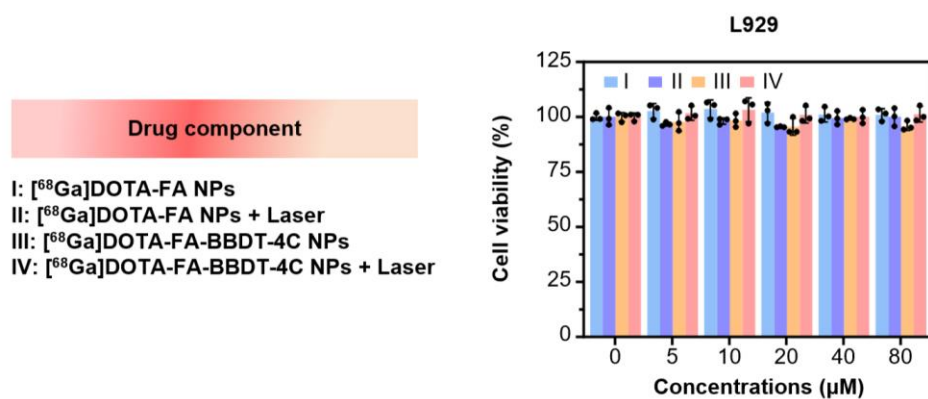
Supplementary Fig. 30 Preparation process of $[^{68}\text{Ga}]\text{DOTA-FA-BBTD-4C NPs}$.



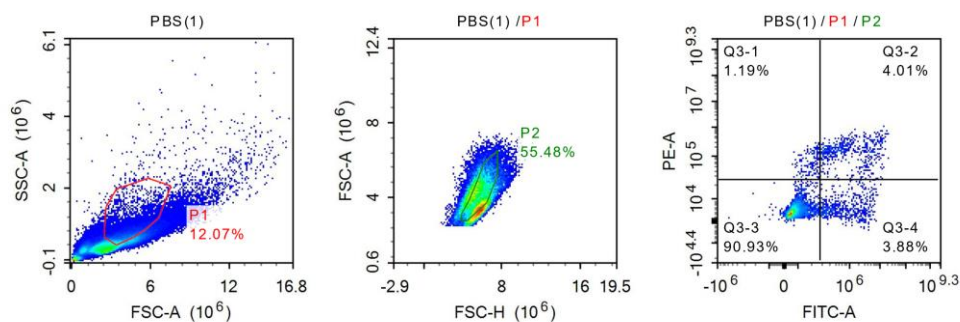
Supplementary Fig. 31 Thin-Layer Chromatography (TLC) analysis of $[^{68}\text{Ga}]\text{DOTA-FA-BBTD-4C NPs}$.



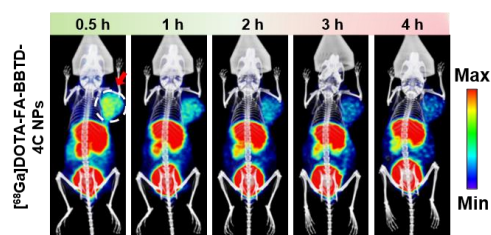
Supplementary Fig. 32 Cell viability of **a** HeLa and **b** T24 cells after laser treatment with different concentrations of DOTA-FA-BBTD-4C NPs (n = 3 technical replicates).



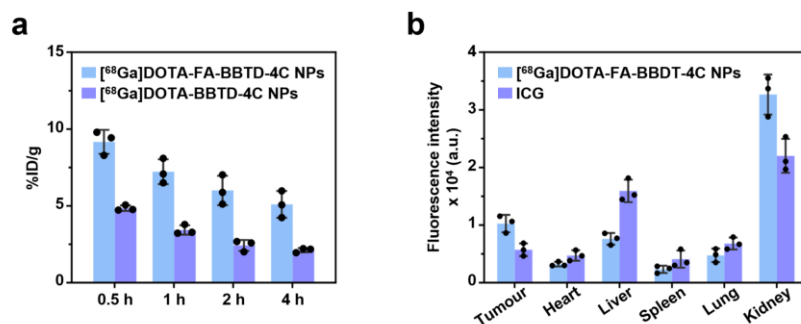
Supplementary Fig. 33 Cell viability of NCTC clone 929 (L929) after laser treatment with different concentrations of DOTA-FA-BBTD-4C NPs (n = 3 technical replicates).



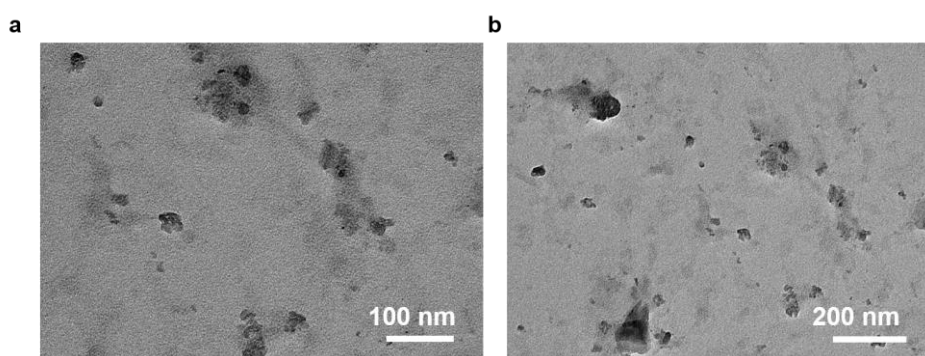
Supplementary Fig. 34 Gating strategy for flow cytometric analysis in Fig. 4i.



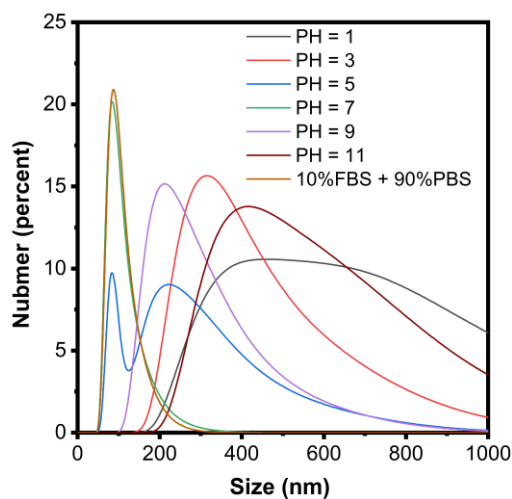
Supplementary Fig. 35 Representative PET/CT images of [^{68}Ga]DOTA-FA-BBTD-4C NPs in Hela tumor-bearing mice (n = 3 mice, Dashed white circle: tumor).



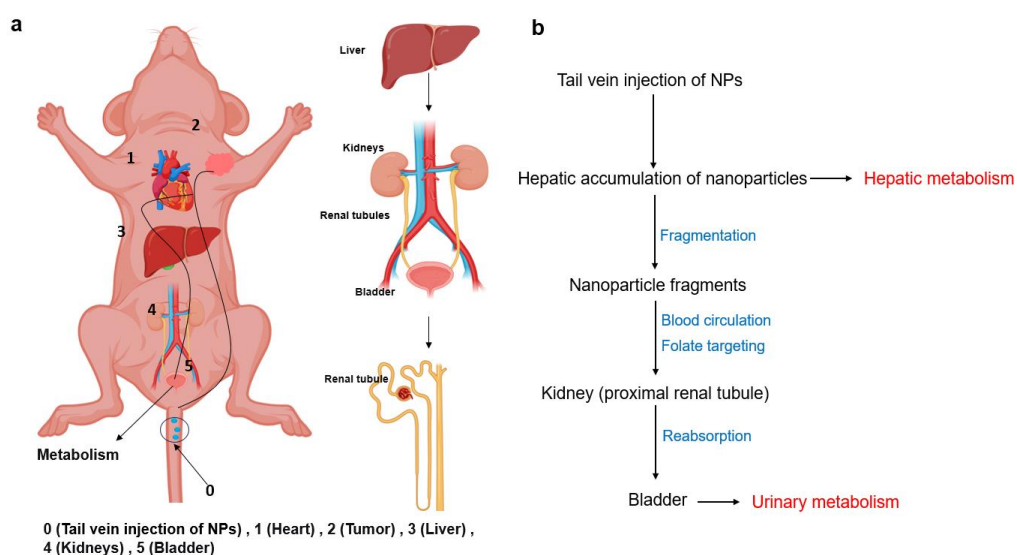
Supplementary Fig. 36 **a** Quantitative analysis of different materials intensity in mouse kidney tissue (n = 3 mice). **b** Quantitative analysis of fluorescence intensity in various organs following tumor resection for $[^{68}\text{Ga}]\text{DOTA-FA-BBDT-4C NPs}$ vs. ICG (n = 3 mice).



Supplementary Fig. 37 Transmission electron microscopy images of urine collected from mice treated with **a** $[^{68}\text{Ga}]\text{DOTA-BBDT-4C NPs}$ and **b** $[^{68}\text{Ga}]\text{DOTA-FA-BBDT-4C NPs}$. Results are representative of at least three independent experiments.



Supplementary Fig. 38 Particle size of $[^{68}\text{Ga}]\text{DOTA-FA-BBDT-4C NPs}$ under different pH conditions.



Supplementary Fig. 39 **a** Schematic diagram of the biological clearance mechanism. **b** Flowchart of the biological clearance process. Created in BioRender. Guoling, Z. (2026) <https://BioRender.com/9ni604q>.

Supplementary Table 1. The composition of each nanoparticle.

Name	Folic acid targeting	Chelating agent	AI Egen	Radionuclide
DOTA-FA NPs	DSPE-PEG ₂₀₀₀ -FA	DSPE-PEG ₂₀₀₀ -DOTA	/	/
DOTA-FA-BBTD-4C NPs	DSPE-PEG ₂₀₀₀ -FA	DSPE-PEG ₂₀₀₀ -DOTA	BBTD-4C	/
[⁶⁸ Ga]DOTA-FA NPs	DSPE-PEG ₂₀₀₀ -FA	DSPE-PEG ₂₀₀₀ -DOTA	/	⁶⁸ Ga
[⁶⁸ Ga]DOTA-BBTD-4C NPs	/	DSPE-PEG ₂₀₀₀ -DOTA	BBTD-4C	⁶⁸ Ga
[⁶⁸ Ga]DOTA-FA-BBTD-4C NPs	DSPE-PEG ₂₀₀₀ -FA	DSPE-PEG ₂₀₀₀ -DOTA	BBTD-4C	⁶⁸ Ga