Supporting Information

for

A supramolecular near-Infrared nanophotosensitizer from host-guest complex of lactose-capped pillar[5]arene with aza-BODIPY derivative for tumor eradication

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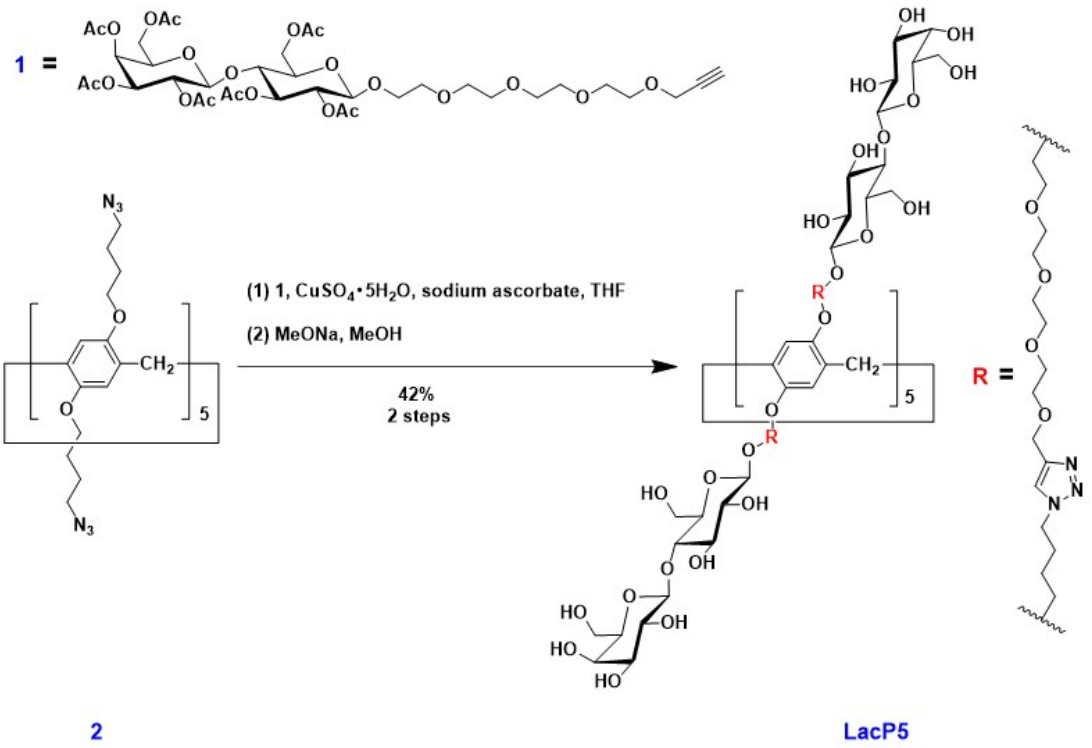
Contents

General information

All reagents were purchased from commercial suppliers and used without further purification unless specified. Water used in this work was ultrapure water. ^{1}H NMR spectra were recorded on a Bruker 400 or 500 MHz Spectrometer, with working frequencies of 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C nuclear magnetic resonance (NMR), respectively. UV-Vis spectra were recorded with Shimadzu 1750 UV-Visible spectrophotometer (Japan) at 298 K. HRMS (High Resolution Mass Spectrometer) analysis was performed with an AB SCIEX LC-30A-Triple TOF 5600^{+}. DLS measurements were performed on a Malvern Zen3600 instrument (Malvern Instruments Limited, U.K.). SEM images were obtained using a S-4800 instrument (Hitachi Ltd.) with an accelerating voltage of 10.0 kV. Negative-stained TEM images were taken on a HT7700 instrument (Hitachi Ltd., 80 kV). CLSM images were obtained by a confocal laser scanning microscope (Lecia Stellaris 8).

Synthesis and characterization of the compounds

Synthesis of LacP5



Scheme S1. Synthetic route of LacP5.

*Synthesis of compound **1***[S1]

Compound **1** was synthesized according to the previously reported method.[S1] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.34 (d, J = 3.2 Hz, 1H), 5.18 (t, J = 9.3 Hz, 1H), 5.09 (dd, J_1 = 10.2 Hz, J_2 = 8.0 Hz, 1H), 4.94 (dd, J_1 = 10.4 Hz, J_2 = 3.4 Hz, 1H), 4.88 (t, J = 8.8 Hz, 1H), 4.55 (d, J = 7.9 Hz, 1H), 4.49-4.46 (m, 2H), 4.20 (d, J = 2.4 Hz, 2H), 4.13-4.06 (m, 3H), 3.92-3.84 (m, 2H), 3.78 (t, J = 9.4 Hz, 1H), 3.70-3.61 (m, 16H), 2.43 (t, J = 2.1 Hz, 1H), 2.14 (s, 3H), 2.11 (s, 3H), 2.05-2.03 (4×s, 12H), 1.96 (s, 3H).

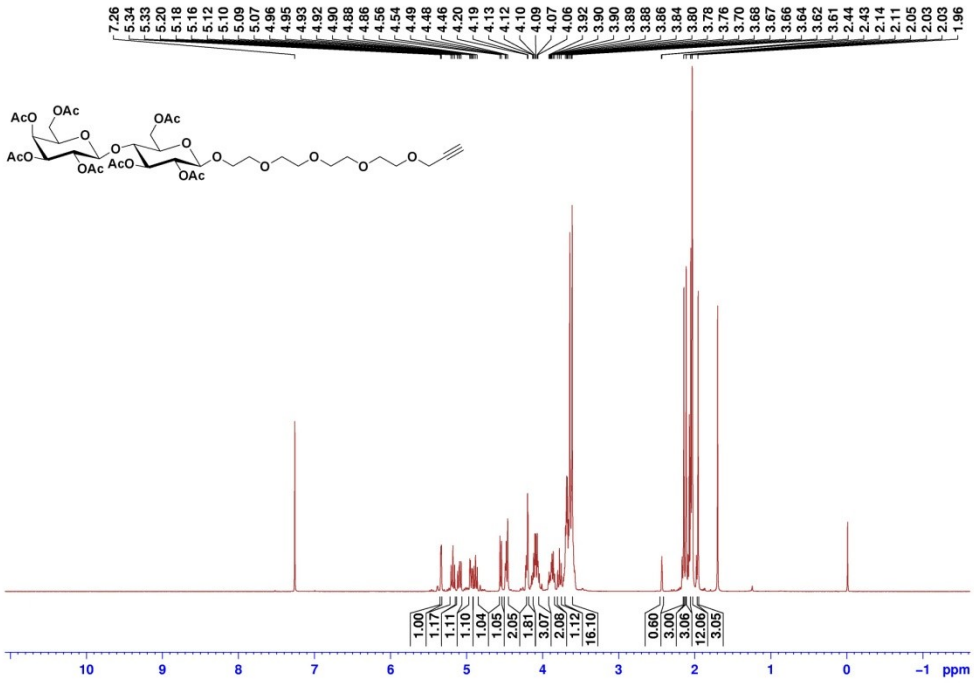


Figure S1. ^{1}H NMR spectrum of **1** in $CDCl_3$.

Synthesis of compound ***2***[S2]

Compound **2** was synthesized according to the previously reported method.[S2] ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.79 (s, 10H), 3.90 (t, J = 5.8 Hz, 20H), 3.76 (s, 10H), 3.29 (t, J = 6.6 Hz, 20H), 1.87-1.81 (m, 20H), 1.78-1.73 (m, 20H).

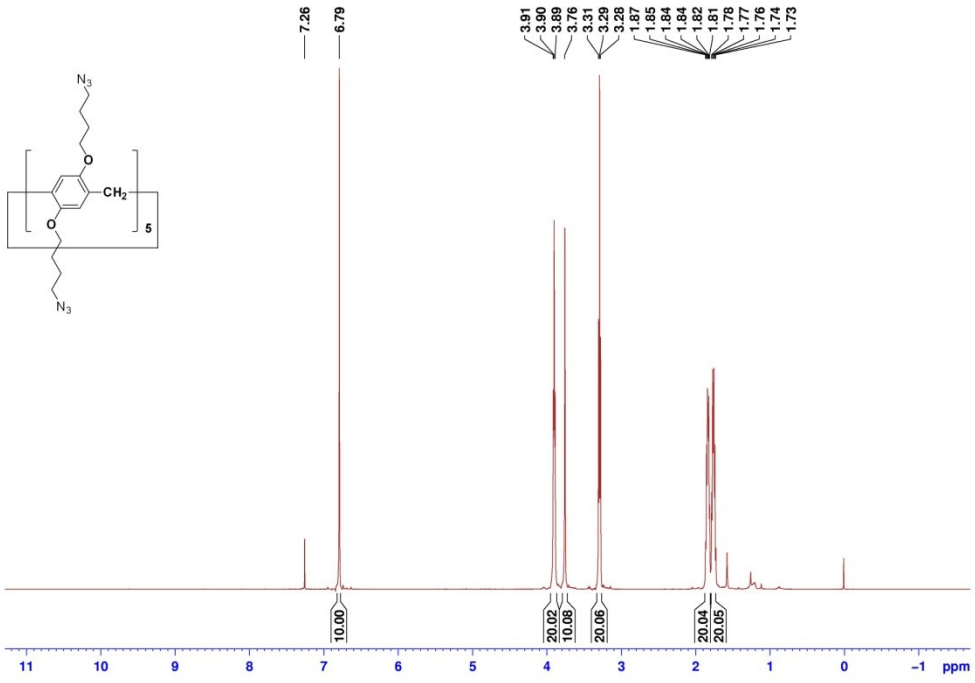


Figure S2. ^{1}H NMR spectrum of **2** in $CDCl_3$.

Synthesis of LacP5

Compound **1** (1.072 g, 1.26 mmol) and **2** (0.1 g, 0.063 mmol) were dissolved in 20 mL of THF under a nitrogen atmosphere. $CuSO_4•5H_2O$ (0.629 g, 2.52 mmol) in 5 mL of H_2O and sodium ascorbate (0.998 g, 5.04 mmol) in 5 mL of H_2O were added to the mixture, respectively. After stirring overnight at room temperature, 20 mL of H_2O was added. The mixture was extracted with ethyl acetate for 3 times, the organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by chromatography on a silica gel column to afford the desired product (0.3 g, yield, 47%) as a colorless oil. The oil (0.267 g, 0.027 mmol) and MeONa (0.15 g, 2.777 mmol) were dissolved in 10 mL of MeOH. The mixture was stirred at room temperature for 12 h, then neutralized by the addition of ion-exchange resin (Amberlite IR 120 H^+) until pH 7, filtered, and the solvent was removed under reduced pressure to afford the desired host molecule LacP5 as a colorless oil (0.161 g, yield, 89%). ^{1}H NMR (400 MHz, D_2O/DMSO-d_6, 1:4, v/v) δ (ppm): 8.05 (s, 10H), 6.61 (s, 10H), 4.46 (s, 20H), 4.38 (s, 20H), 4.20-4.18 (m, 20H), 3.82-3.80 (m, 20H), 3.71 (d, J = 11.2 Hz, 10H), 3.61-3.58 (m, 20H), 3.56-3.49 (m, 80H), 3.45-3.43 (m, 120H), 3.31-3.27 (m, 50H), 3.02 (t, J = 8.1 Hz, 10H), 1.98 (s, 20H), 1.62 (s, 20H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 149.1, 144.0, 128.0, 123.9, 103.9, 102.7, 80.8, 75.6, 75.1, 74.9, 73.3, 73.2, 70.6, 69.8, 69.7, 69.0, 68.2, 68.1, 63.6, 60.6, 60.5, 49.4, 26.9, 26.5. MALDI-TOF-MS: m/z calculated for $[M + Na]^+$ $C_{305}H_{500}N_{30}O_{160}Na^+$, 7169.19, found 7169.58. m/z calculated for $[M + K]^+$ $C_{305}H_{500}N_{30}O_{160}K^+$, 7185.16, found 7185.72.

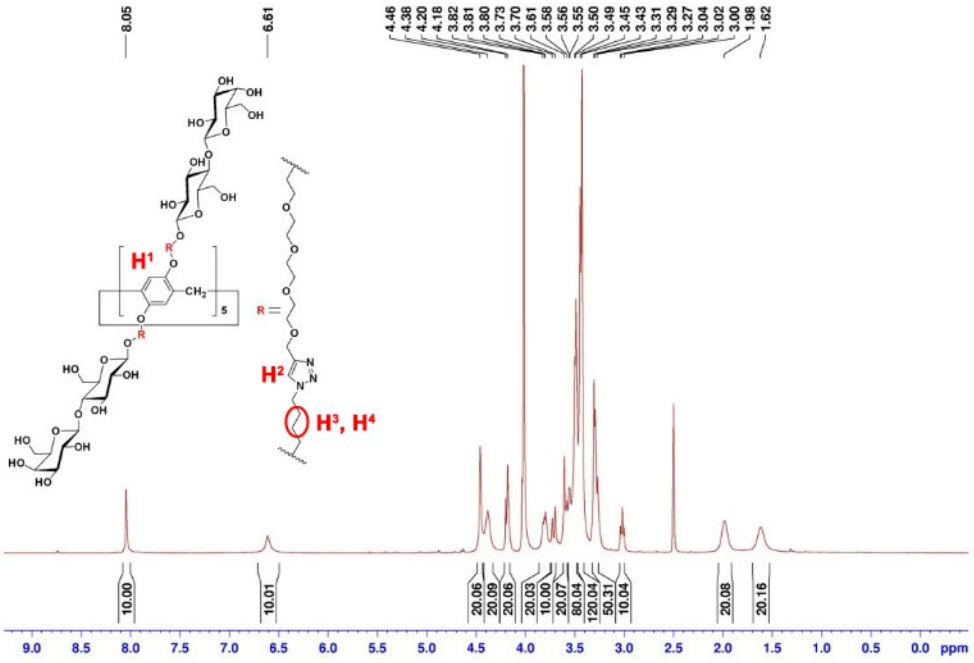


Figure S3. ^{1}H NMR spectrum of LacP5 in D_2O/DMSO-d_6 (1:4, v/v).

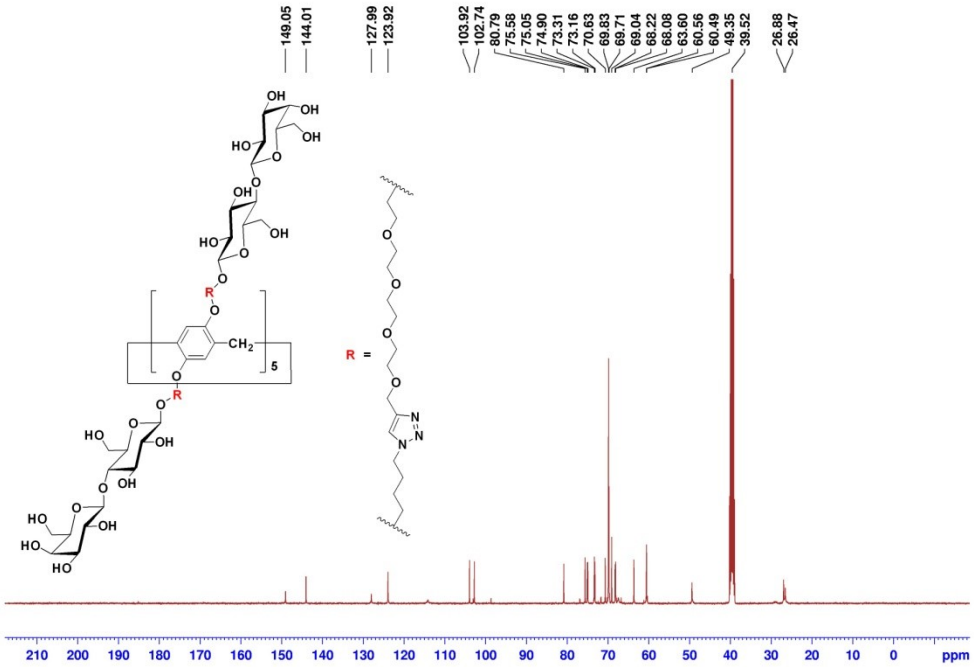


Figure S4. ^{13}C NMR spectrum of LacP5 in DMSO-d_6.

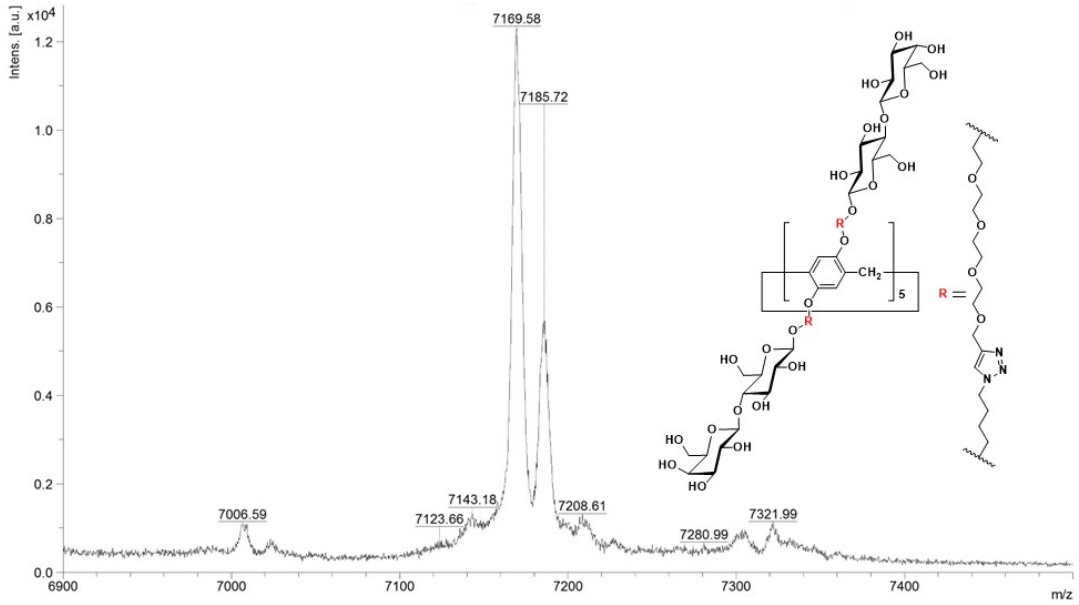
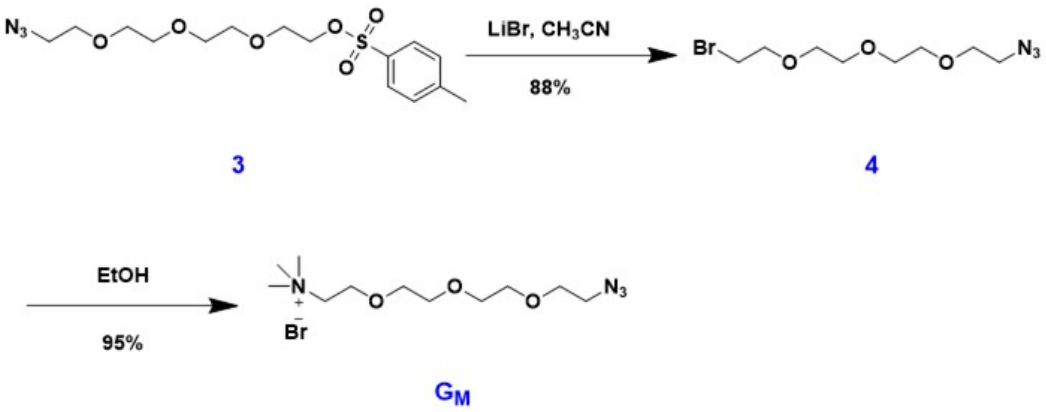


Figure S5. MALDI-TOF-MS spectrum of LacP5 in positive ion mode.

Synthesis of compound G_M



Scheme S2. Synthetic route of $G_{M.}$

*Synthesis of compound **3***[S3]

Compound **3** was synthesized according to the previously reported method.[S3] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.79 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 4.15 (t, J = 4.8 Hz, 2H), 3.70-3.59 (m, 12H), 3.38 (t, J = 5.0 Hz, 2H), 2.44 (s, 3H).

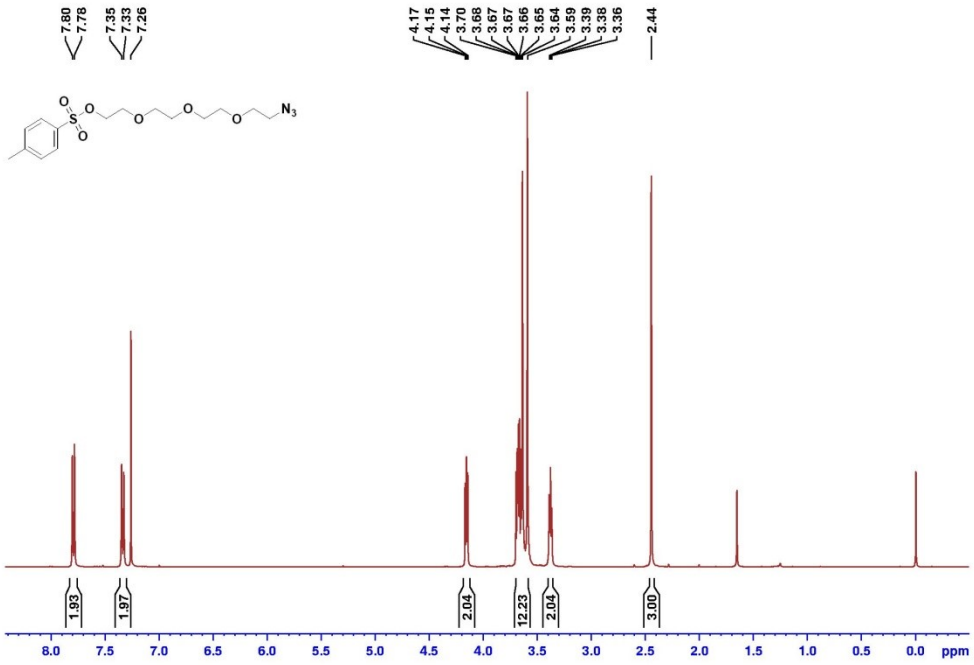


Figure S6. ^{1}H NMR spectrum of **3** in $CDCl_3$.

Synthesis of compound ***4***[S4, S5]

Compound **3** (200 mg, 0.536 mmol) and LiBr (93 mg, 1.072 mmol) were suspended in 10 mL of CH_3CN. The mixture was stirred and heated at 85 °C for 12 h. After filtration, the solvent was removed under reduced pressure and the oily residue was purified by chromatography on silica gel to afford the desired product (0.133 g, yield, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.81 (t, J = 6.3 Hz, 2H), 3.69-3.67 (m, 10H), 3.47 (t, J = 6.3 Hz, 2H), 3.39 (t, J = 5.0 Hz, 2H).

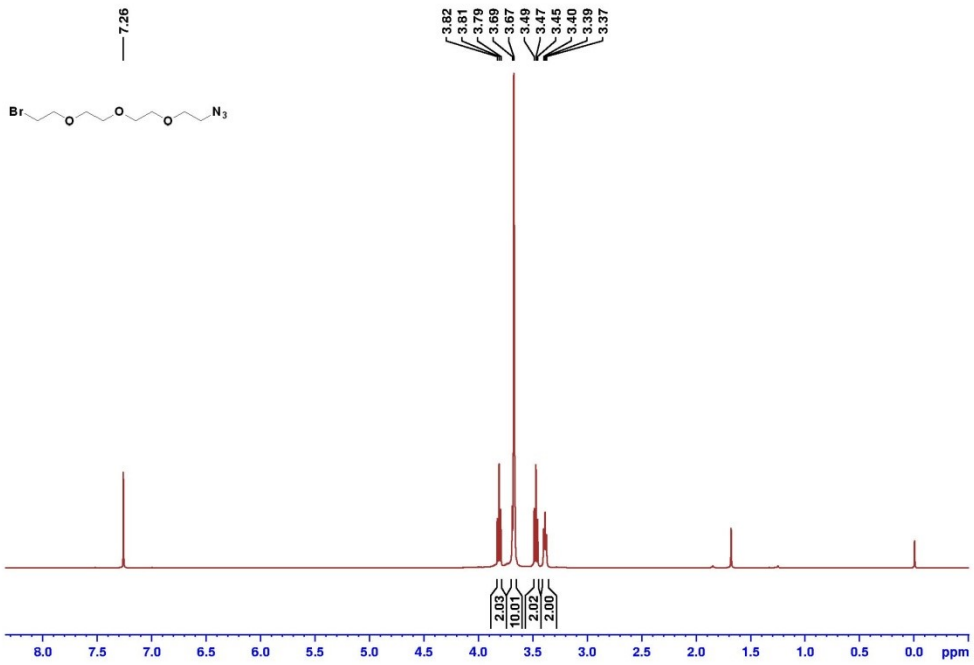


Figure S7. ^{1}H NMR spectrum of **4** in $CDCl_3$.

Synthesis of compound G_M[S6, S7]

To a solution of compound **4** (300 mg, 1.063 mmol) in EtOH (5 mL), trimethylamine (7 mL, 33% in methanol) was added. The mixture was stirred and heated at 75 °C for 12 h. The resulting solution was removed under vacuum to afford the desired product (0.345 g, yield, 95%) as a colorless oil. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 4.01-3.98 (m, 2H), 3.74-3.72 (m, 10H), 3.61 (t, J = 4.7 Hz, 2H), 3.51 (t, J = 4.9 Hz, 2H), 3.21 (s, 9H). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 68.9, 68.9, 68.9, 68.9, 68.6, 64.7, 63.7, 58.9, 53.4, 53.3, 53.3. ESI-HRMS: calculated for $[M - Br]^+$ $C_{11}H_{25}N_4O_3^+$, 261.1927, found 261.1926.

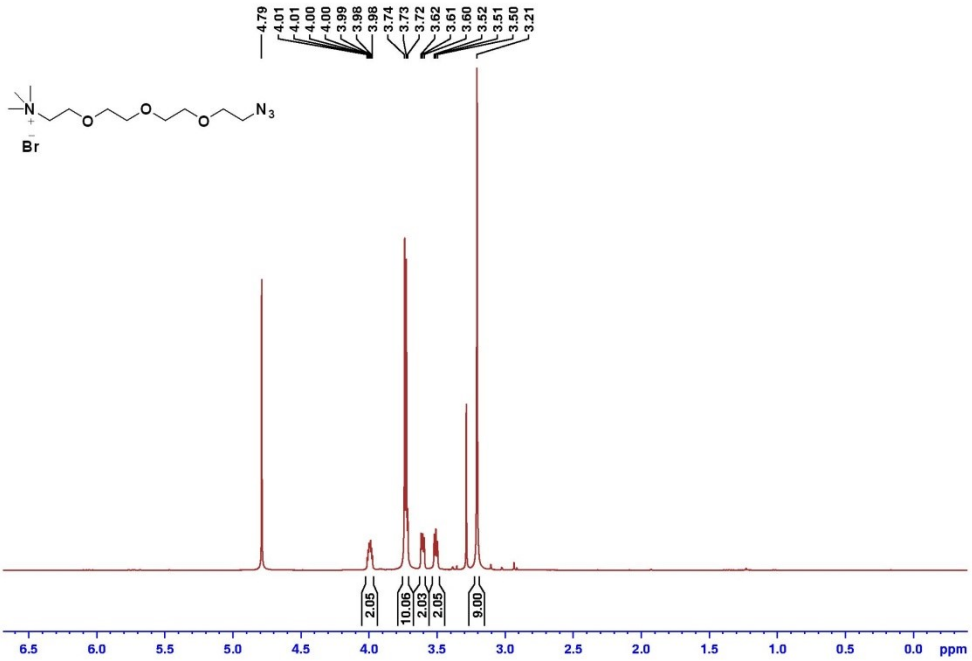


Figure S8. ^{1}H NMR spectrum of G_M in D_2O.

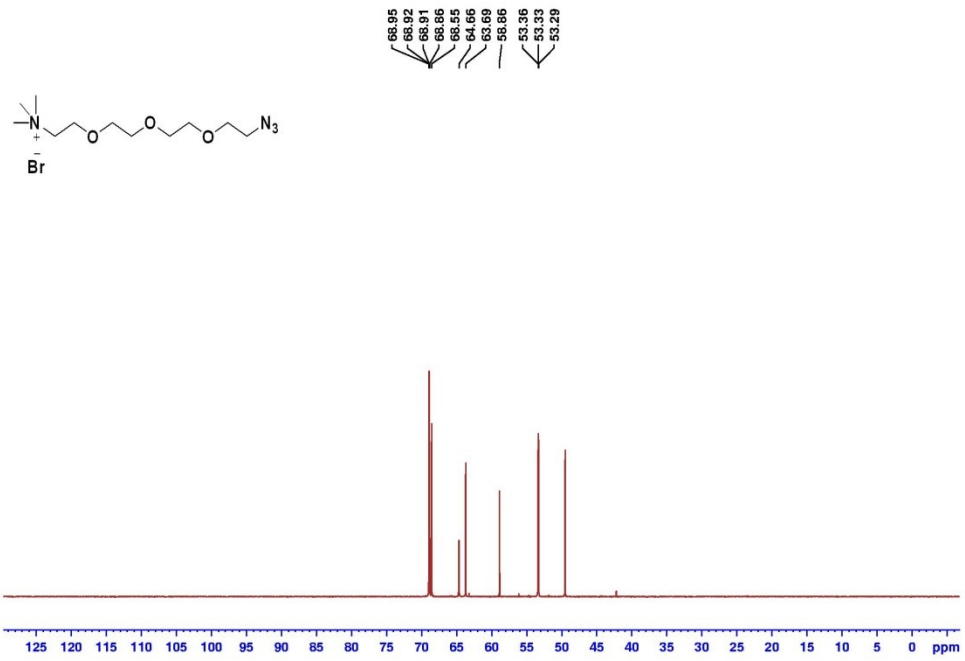


Figure S9. ^{13}C NMR spectrum of G_M in D_2O.

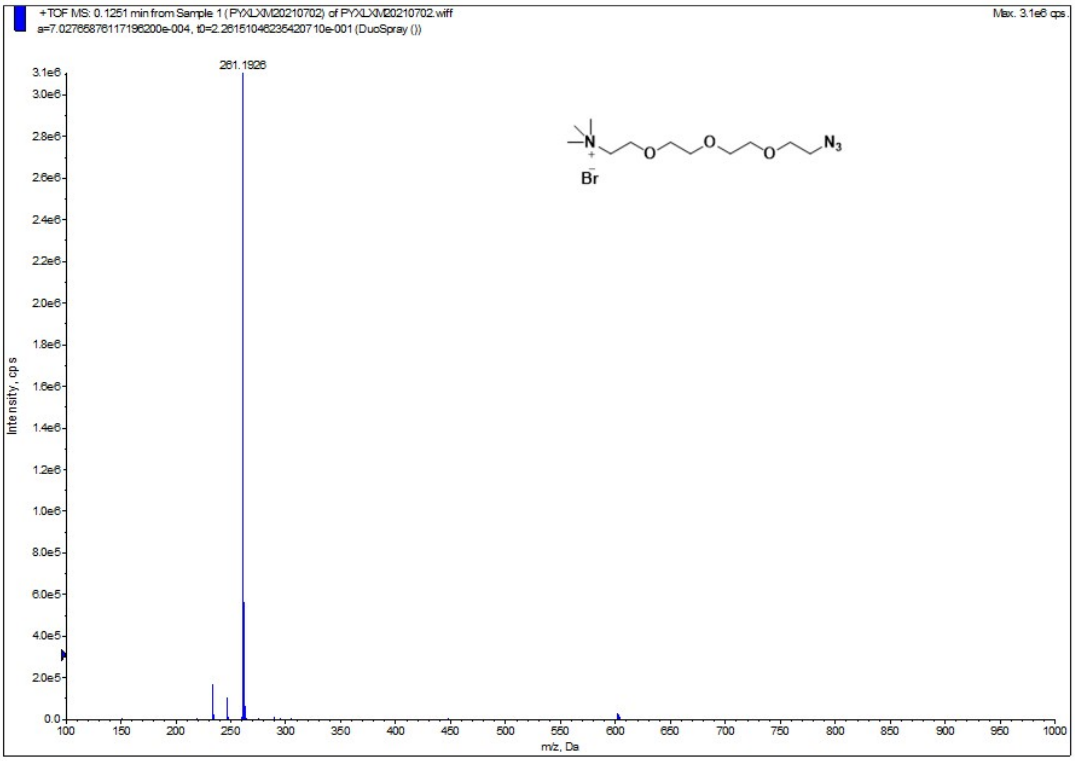
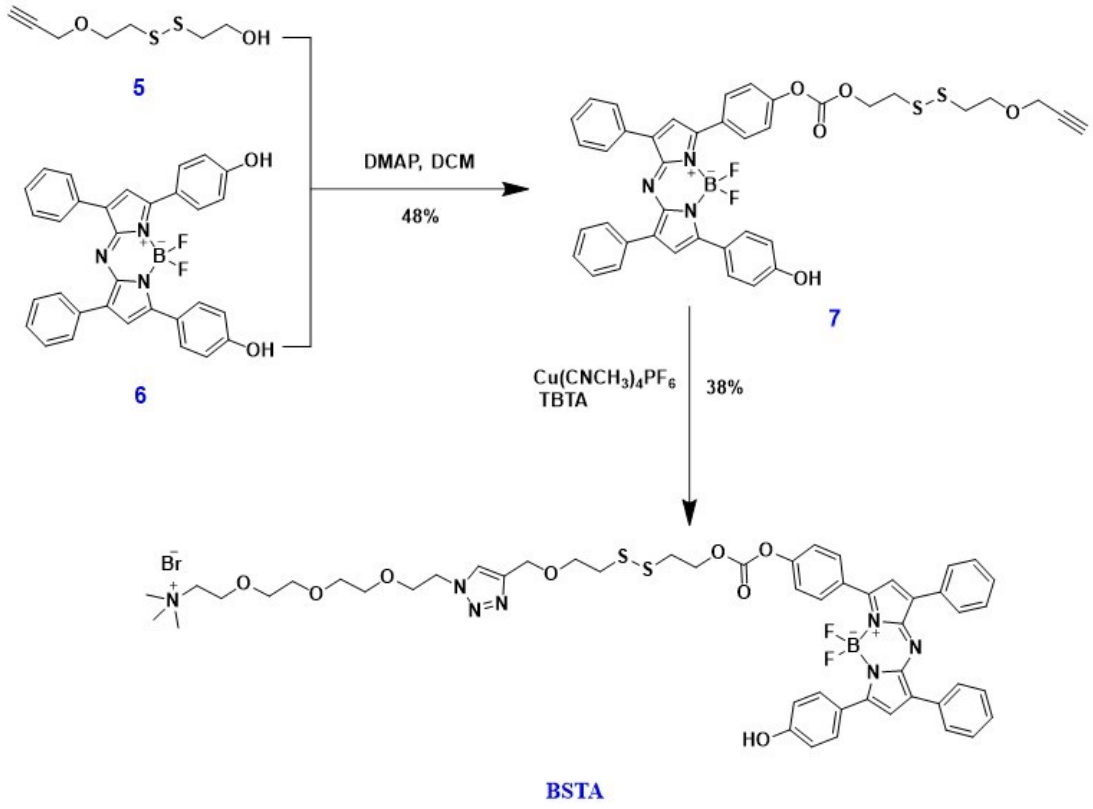


Figure S10. ESI-HRMS of G_M in positive ion mode.

Synthesis of BSTA



Scheme S3. Synthetic route of BSTA.

Synthesis of compound ***5***[S8]

Compound **5** was synthesized according to the previously reported method.[S8] ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.20 (d, J = 2.4 Hz, 2H), 3.90 (dd, J_1 = 11.5 Hz, J_2 = 5.8 Hz, 2H), 3.81 (t, J = 6.4 Hz, 2H), 2.93 (t, J = 6.4 Hz, 2H), 2.88 (t, J = 5.8 Hz, 2H), 2.46 (t, J = 2.4 Hz, 1H), 2.07 (t, J = 6.1 Hz, 1H).

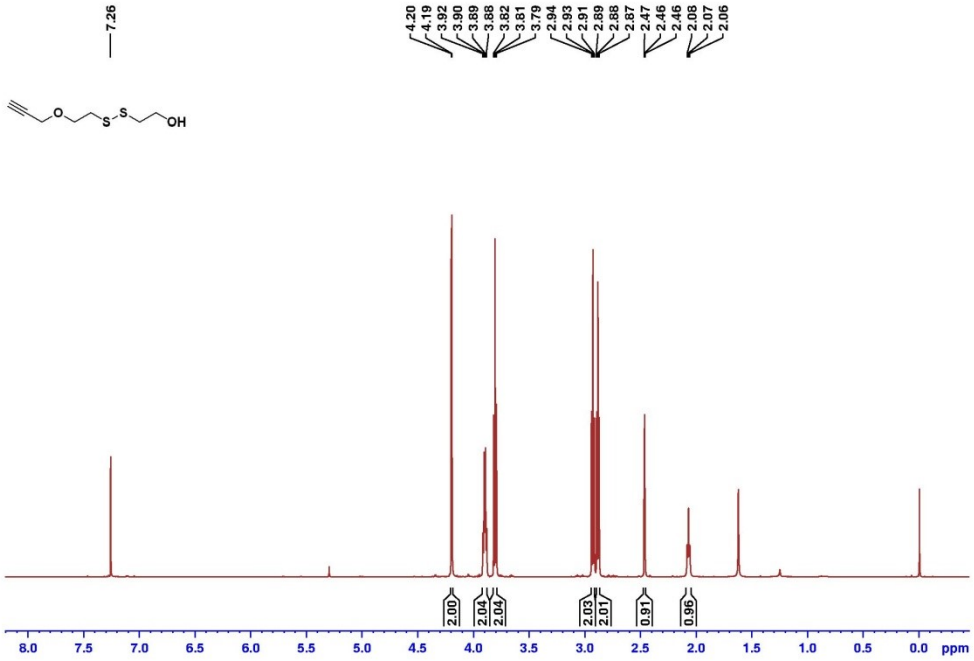


Figure S11. ^{1}H NMR spectrum of **5** in $CDCl_3$.

Synthesis of compound ***6***[S9]

Compound **6** was synthesized according to the previously reported method.[S9] ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.48 (s, 2H), 8.15 (d, J = 7.4 Hz, 4H), 8.10 (d, J = 8.8 Hz, 4H), 7.56 (s, 2H), 7.53 (dd, J_1 = 7.3 Hz, J_2 =7.6 Hz, 4H), 7.46 (t, J = 7.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 4H).

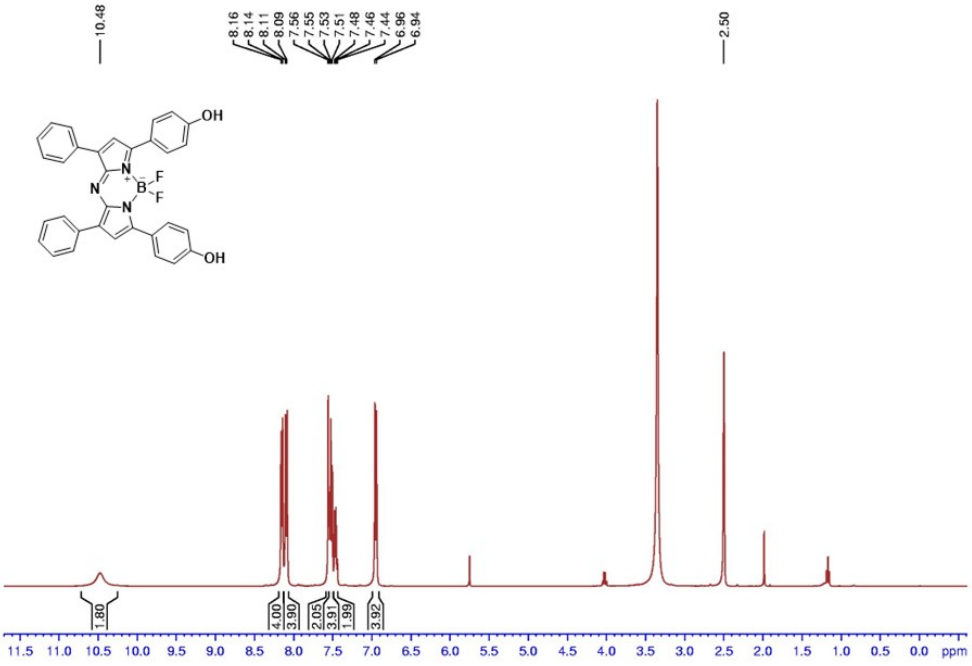


Figure S12. ^{1}H NMR spectrum of **6** in DMSO-d_6.

Synthesis of compound 7[S10]

Compound **7** was synthesized according to a modified literature procedure.[S10] Compound **6** (59 mg, 0.111 mmol) and triphosgene (12 mg, 0.04 mmol) were dissolved in 5 mL of dry DCM under nitrogen atmosphere. The mixture was stirred at 0 °C for 1 h. 4-Dimethylaminopyridine (271 mg, 2.218 mmol) in 4 mL of dry DCM was added. After stirring 1 h at 0 °C, compound **5** (32 mg, 0.166 mmol) in 2 mL of dry DCM was added. The mixture was stirred at room temperature under nitrogen atmosphere for 24 h. After which 20 mL of 2 M hydrochloric acid solution was added to acidify the reaction solution, then 20 mL of water and 100 mL of ethyl acetate were added. The organic layer was separated and evaporated to dryness. The residue was purified by chromatography on silica gel to afford the desired product (0.04 g, yield, 48%) as a dark green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.07-8.04 (m, 8H), 7.47-7.41 (m, 6H), 7.32 (d, J = 8.7 Hz, 2H), 7.09 (s, 1H), 6.96 (s, 1H), 6.91 (d, J = 8.7 Hz, 2H), 5.79 (s, 1H), 4.55 (t, J = 6.6 Hz, 2H), 4.21 (d, J = 2.3 Hz, 2H), 3.82 (t, J = 6.3 Hz, 2H), 3.06 (t, J = 6.6 Hz, 2H), 2.97 (t, J = 6.3 Hz, 2H), 2.47 (t, J = 2.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.0, 159.6, 155.3, 153.3, 152.4, 146.5, 144.9, 144.5, 142.3, 132.7, 132.4, 132.1, 131.0, 130.2, 129.8, 129.5, 129.3, 129.2, 128.7, 123.3, 121.2, 119.8, 118.2, 116.2, 79.4, 75.2, 68.1, 66.7, 58.4, 38.5, 36.9. ESI-HRMS: calculated for $[M + Na]^+$ $C_{40}H_{32}BF_2N_3O_5S_2Na^+$, 770.1742, found 770.1745.

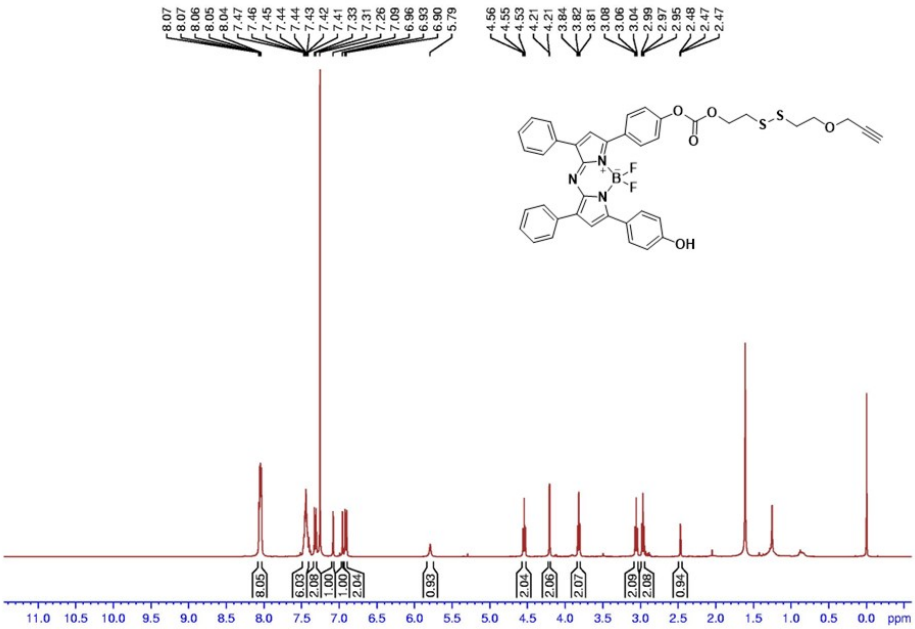


Figure S13. ^{1}H NMR spectrum of **7** in $CDCl_3$.

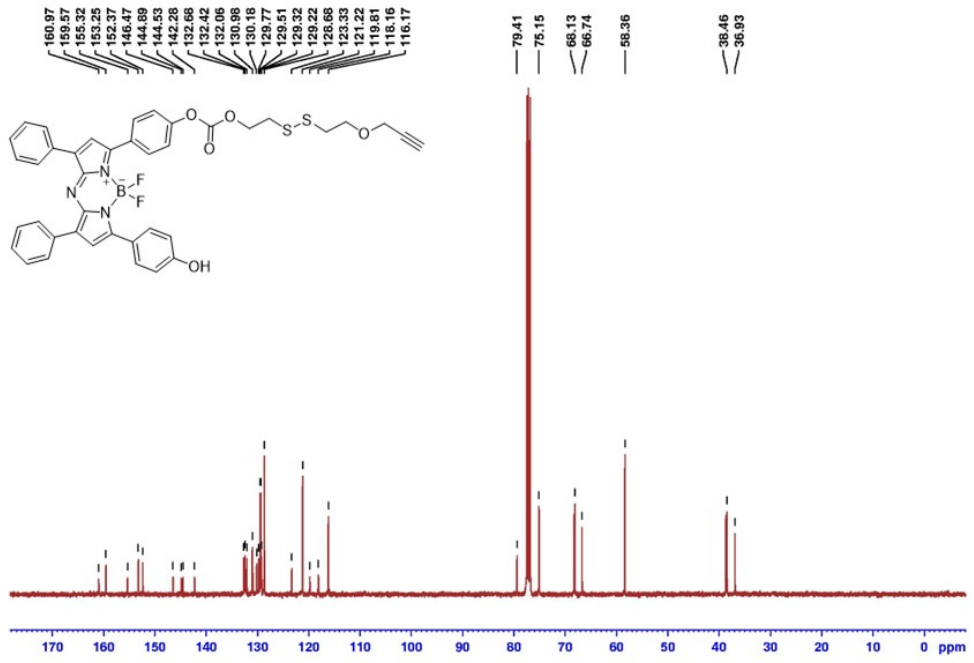


Figure S14. ^{13}C NMR spectrum of **7** in $CDCl_3$.

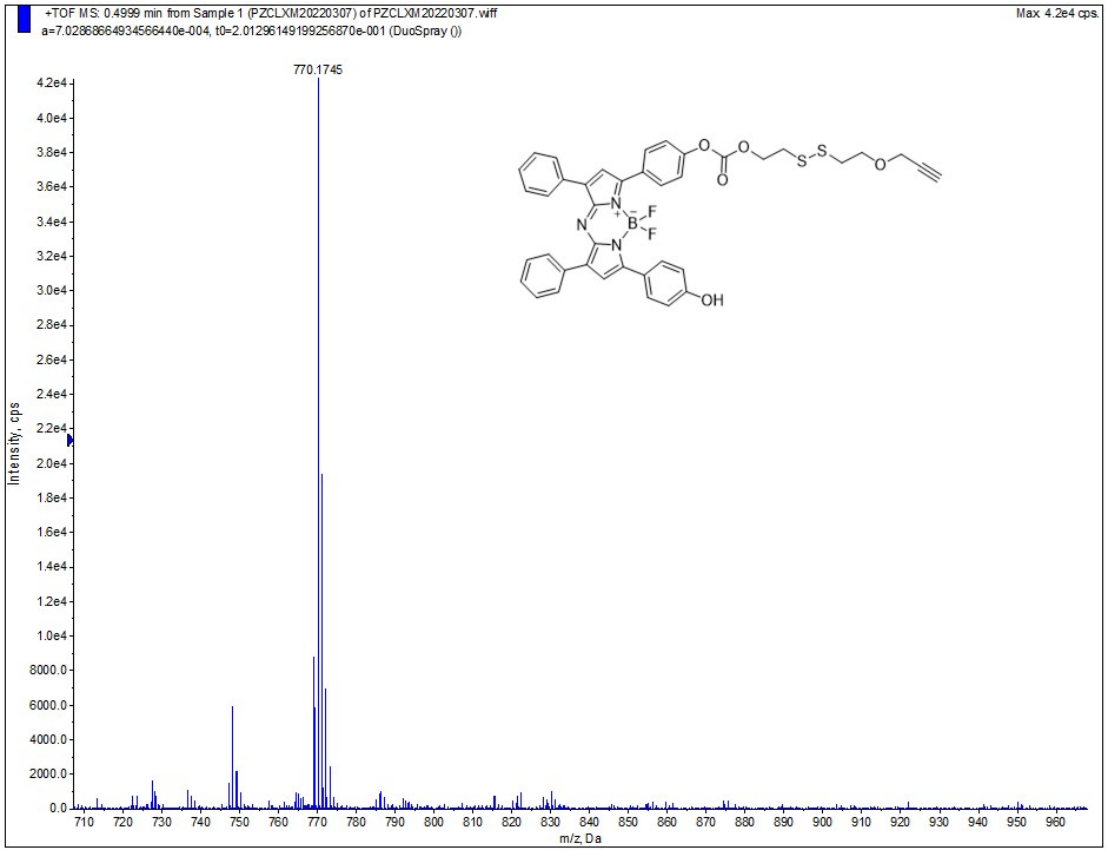


Figure S15. ESI-HRMS of **7** in positive ion mode.

Synthesis of compound BSTA

A mixture of G_M (8 mg, 0.023 mmol), $Cu(CNCH_3)_4PF_6$ (2 mg, 0.005 mmol) and TBTA (2 mg, 0.004 mmol) in 4 mL of dry MeOH was stirred under nitrogen atmosphere for 1 h at room temperature. After which compound **7** (18 mg, 0.024 mmol) in 1 mL of dry DCM was added. The resulting mixture was stirred at room temperature under nitrogen atmosphere for another 5 h. The mixture was directly purified by chromatography on silica gel to afford the desired product (10 mg, yield, 38%) as a dark green solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.23-8.19 (m, 4H), 8.14-8.09 (m, 5H), 7.83 (s, 1H), 7.58-7.51 (m, 5H), 7.47-7.42 (m, 4H), 7.00 (s, 1H), 6.98 (s, 1H), 4.57 (s, 2H), 4.51 (t, J = 5.3 Hz, 2H), 4.48 (t, J = 6.2 Hz, 2H), 3.80 (t, J = 5.1 Hz, 4H), 3.72 (t, J = 6.2 Hz, 2H), 3.55-3.48 (m, 10H), 3.10 (t, J = 6.2 Hz, 2H), 3.08 (s, 9H), 2.98 (t, J = 6.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 156.2, 153.1, 152.3, 152.0, 145.7, 144.1, 143.4, 140.7, 132.5, 131.8, 131.2, 131.1, 130.2, 129.8, 129.4, 129.2, 128.2, 127.1, 126.7, 125.5, 125.2, 124.8, 124.6, 122.0, 116.9, 116.9, 70.0, 70.0, 69.9, 69.8, 69.2, 68.2, 66.8, 64.9, 64.5, 63.7, 53.6, 49.1, 38.3, 36.6. ESI-HRMS: calculated for $[M - Br]^+$ $C_{51}H_{57}BF_2N_7O_8S_2^+$, 1008.3771, found 1008.3771.

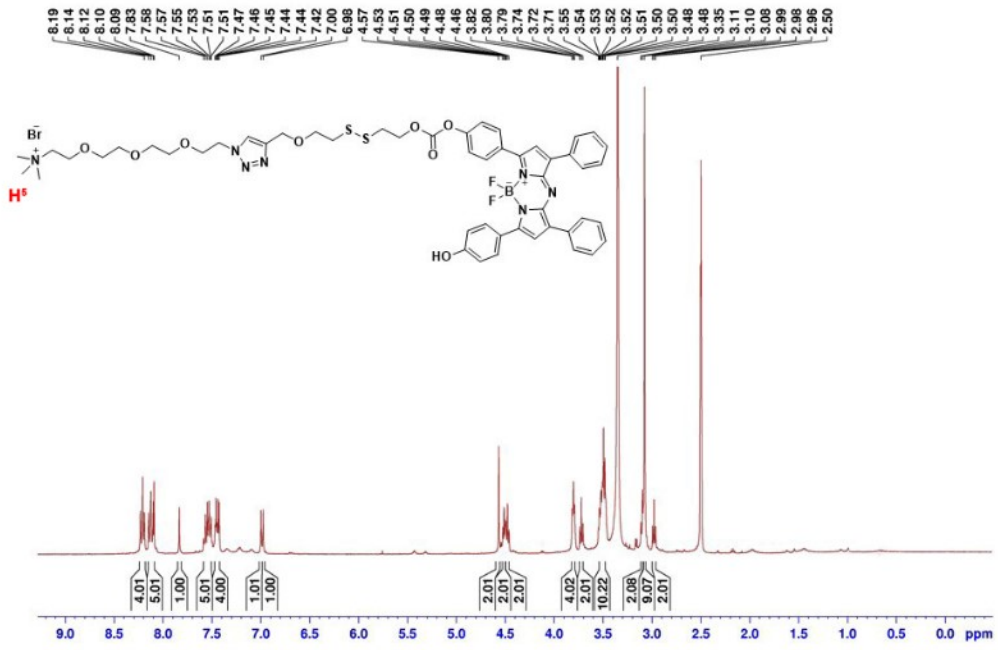


Figure S16. ^{1}H NMR spectrum of BSTA in DMSO-d_6.

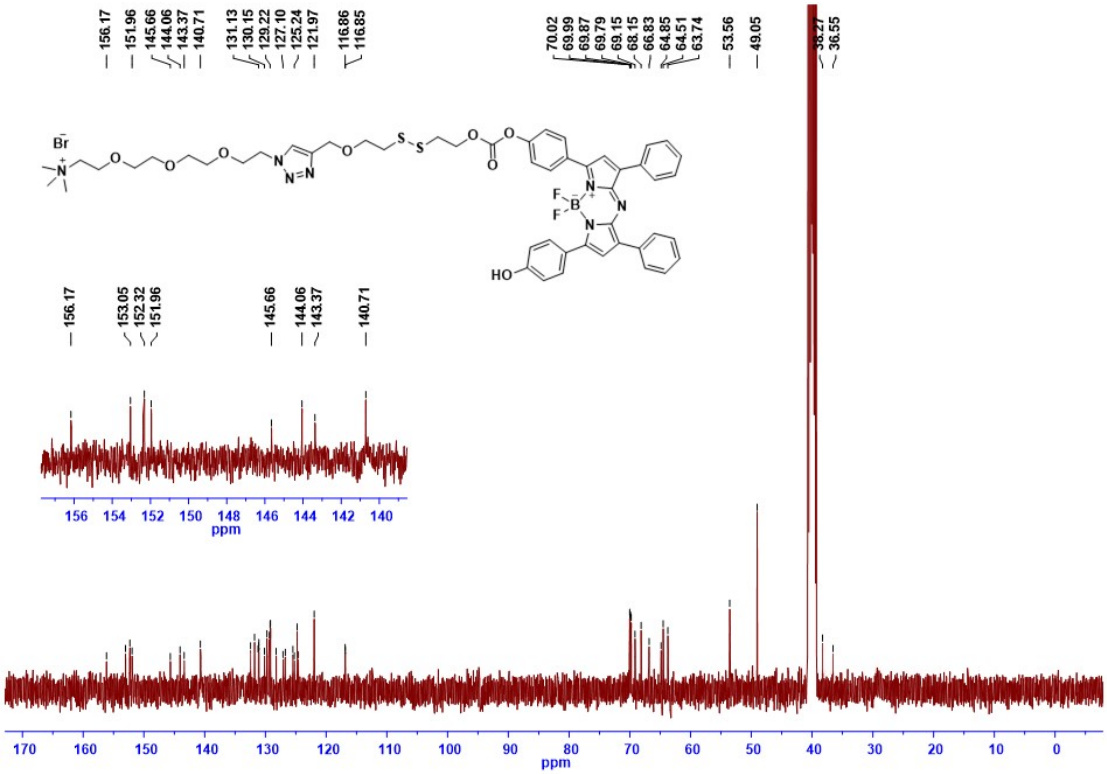


Figure S17. ^{13}C NMR spectrum of BSTA in DMSO-d_6.

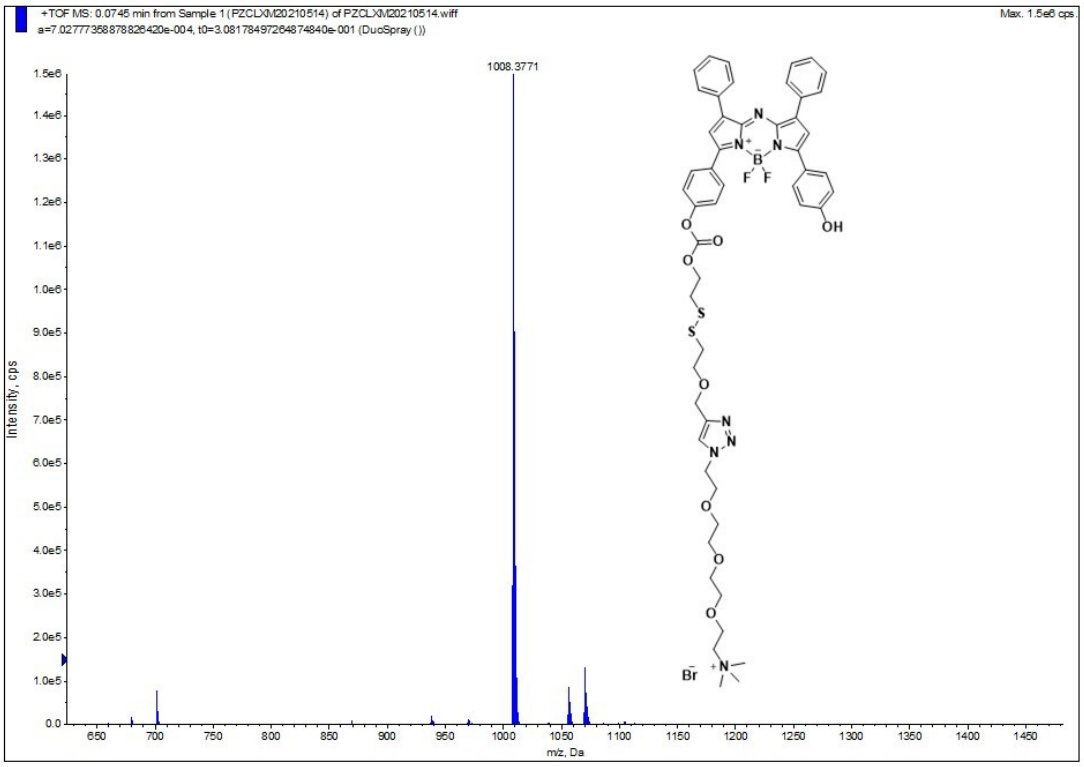


Figure S18. ESI-HRMS of BSTA in positive ion mode.

Fabrication of LacP5 ⊃ BSTA supramolecular cationic vesicles

Compound BSTA (2.2 mg, 0.002 mmol) was added to a solution of LacP5 (14.3 mg, 0.002 mmol) in 4 mL of sterile water. The mixture was subjected to ultrasonication for 1 h, and then left to stand still overnight to obtain the vesicles, which were characterized by DLS, SEM and TEM, respectively.

Preparation of LacP5 ⊃ BSTA@DSF NPs

The aqueous solution of DSF (89 μL, 2248 μM) and LacP5⊃BSTA (870 μL, 230 μM) was added to 1.041 mL of ultrapure water, then agitated the mixture for 24 h. After that, the unloaded DSF was removed by dialysis (molecular weight cut-off (MWCO) = 2,000 Da) against distilled water for 24 h until water outside the dialysis tube exhibited negligible DSF by HPLC.

Determination of the binding constant (Ka) for LacP5 ⊃ BSTA

To determine the binding constant between LacP5 and BSTA, UV-Vis titrations were carried out in solutions, which had a constant concentration of LacP5 (36 μM) and varying concentrations of BSTA. By a linear curve-fitting method, the binding constant between LacP5 and BSTA was calculated.

The linear curve-fitting was based on the following equation:

$$1/(A_0 - A) = 1/(a \times K_a \times C_0) + 1/a$$

where A_0 is the initial summary of the respective absorbance of LacP5 and BSTA at 490 nm, A is the absorbance of the complexation of LacP5 and BSTA at 490 nm, C_0 is the varying concentrations of BSTA and a is a constant.

Detection of singlet oxygen ($^1O_2$) production

1,3-Diphenylisobenzof-uran (DPBF) was used as a singlet oxygen probe for measuring singlet oxygen production. Briefly, 10.0 μM **6** and LacP5⊃BSTA were each mixed with 30.0 μM DPBF in water and then irradiated for 60 s (685 nm light, 0.08 W/cm^2). The absorbance of DPBF at 425 nm was then monitored every 10 seconds. The singlet oxygen quantum yield is calculated according to the following formula:

$$\phi_\Delta = \phi_\Delta^{MB} \frac{W \times I_{abs}^{MB}}{W^{MB} \times I_{abs}} \qquad (1)$$

$$I_{abs} = \sum_\lambda (1 - 10^{-abs_\lambda}) I_\lambda \qquad (2)$$

ϕ_Δ: The singlet oxygen quantum yield of sample; ϕ_Δ(MB): The singlet oxygen quantum yield of Methylene blue, that has been used previously with a 0.52 in water[S11]; W: photobleaching rates of the DPBF in the presence of the sample; W^{MB}: photobleaching rates of the DPBF in the presence of MB; I_λ: corrected light source intensity at the wavelength λ; abs_λ: the absorbance of the photosensitizer at the wavelength λ[S12].

Photothermal conversion efficiency of LacP5⊃BSTA and 6

To evaluate the photothermal property of our compounds, 3 mL of ultrapure water, 20 μM LacP5⊃BSTA, and 20 μM **6** in quartz dishes were irradiated using a 685 nm laser (2 W/cm^2) for 30 min, respectively. We measured the photothermal conversion efficiency (η) of LacP5⊃BSTA and **6** according to previous reports. The η value was calculated as follows:

$\eta = (hA\Delta T_{max} - Q_s)/I(1 - 10\text{^}(-A_\lambda))$

h: heat transfer coefficient; A: surface area of the container; ΔT_{max}: temperature change at the maximum steady-state temperature; I: laser power; A_λ: absorbance of LacP5⊃BSTA and **6** at 685 nm; Q_s: the heat associated with the light absorbance of the solvent; η: photothermal conversion efficiency.

DSF standard curve

DSF (50 mg) were diluted to 250 mL volumetric flask to obtain DSF mother liquor. Take 0.25 mL, 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, and 2.5 mL dilute to 10 mL volumetric flasks to obtain DSF solutions with different concentrations. The above solution was detected by Shimadzu high performance liquid chromatography, detector: SPD-M20A, pump: LC-20AT, column: Topsil C18 4.6 x 250 mm, 5 μm, mobile phase: methanol/water = 80/20 (v/v). 1.0 mL/min. Ret. Time (major): 6.991 min.

Cell culture

Human liver hepatocellular carcinoma HepG2, human cervical cancer HeLa, human liver HL7702 cell lines were obtained from KeyGEN BioTECH Co. (Nanjing, China). HepG2, and HL7702 cell lines were cultured in 1% (v/v) antibiotics and 10% (v/v) FBS supplemented Roswell Park Memorial Institute medium (RPMI 1640, Gibco) under 5% CO_2 at 37 °C. HeLa cells were cultured at the same conditions except Dulbecco's Modified Eagle Medium (DMEM, Gibco) was used.

Cellular uptake and targetability of LacP5⊃BSTA

HepG2 cells were seeded onto confocal dish with 1 x 10^5 cells per dish. After 24 h culturing, cells were transferred to fresh medium containing 2 μM LacP5⊃BSTA and continue culture for different time. Cells were washed with PBS before using confocal laser scanning microscope (CLSM) to observe cell uptake. Targetability of LacP5⊃BSTA was analyzed by adding nanoparticles to different cells (1 x 10^5 cells per dish) and incubating at 37 °C for 4 h. For lactobionic acid (LBA) blocking experiments, cells were treated with LBA (2 mg/mL) for 4 h before LacP5⊃BSTA NPs were added. Cells were washed with PBS and fixed by 4% paraformaldehyde (PFA, Yuanye Bio-Technology Co., Ltd, Shanghai, China). Before using confocal laser scanning microscope (CLSM) to observe cell uptake, the nucleus is stained with Hoechst 33258 (Solarbio Science & Technology Co., Ltd.). HL7702 human liver normal cells, Hela human cervical cancer cells and HepG2 human liver cancer cells were utilized. Compound **6**, Ex/Em: 685/750 nm; Hoechst, Ex/Em: 405/461 nm.

Intracellular ROS levels

The intracellular ROS levels were monitored using DCFH-DA (Beyotime, Shanghai, China) according to the manufacturer's instructions. In brief, HepG2 cells were seeded onto confocal dish with 1 x 10^5 cells per dish. After overnight culturing, the cells were incubated with 2 μM LacP5⊃BSTA for 24 h. The cells were washed with PBS before photo-irradiation (685 nm, 80 mW/cm^2, 20 min). Thereafter the cells were washed with PBS and transferred to the fresh medium containing 10 μM DCFH-DA for 20 min, the cells were washed with PBS and then observed under CLSM.

Intracellular GSH

HepG2 cells were cultured in 6-well plate for 24 h before the cells were transferred to fresh medium containing DSF or LacP5⊃BSTA@DSF (dose of DSF: 5 μM) to continue culturing for 4 and 8 h. After that the cells were collected to detect the intracellular GSH content according to the operation of the GSH detection kit (Solarbio Science & Technology Co., Ltd.).

Cell apoptosis detection

Apoptosis was detected by flow cytometry with Annexin V-FITC/PI kit. In brief, HepG2 cells were cultured in a 6-well plate at a density of 1 x 10^5 per well until they adhered to the wall. Then the cells were transferred to fresh culture medium containing 2 μM LacP5⊃BSTA for continuous cultivation 24 h. After that the cells were washed with PBS three times, and then the cells were irradiated with light (685 nm, 0.08

W/cm^2) for 20 min. Staining and testing according to the manufacturer's instructions at 0 h and 24 h after irradiation.

Biocompatibility of LacP5⊃BSTA

HL7702 cells were seeded onto 96-well plate (5 × 10^3 cells/well). After overnight culture, cells were treated with LacP5⊃BSTA at the doses of 0, 10, 20, 30 ,40, 50 μM in medium for 24 h, respectively. Thereafter, the cells were washed with PBS and cultured with fresh medium. After culturing for 24 h, the cell viability was evaluated by MTT assay.

Cell cytotoxicity of LacP5⊃BSTA

HepG2 Cells were seeded onto 96-well plate (5 × 10^3 cells/well). After overnight culture, cells were treated with LacP5⊃BSTA or LacP5⊃BSTA@DSF (doses of LacP5⊃BSTA: 0, 0.5, 1, 1.5, 2, 5 μM in medium) for 24 h, respectively. Cell was washed with PBS and transferred to fresh medium before receiving photo-activation (685 nm, 80 mW/cm^2, 20 min). After photo irradiation, cells were cultured for another 24 h, and the MTT assay was used to detect cell viability.

RBC hemolysis of LacP5⊃BSTA

The experiment was modified with reference to the previous reports[S13]. In brief, mice whole blood was collected in tubes containing Na-heparin. Add 9 mL of PBS (pH 7.4) to 1 mL blood and mix gently, then centrifuge (1500 rpm, 5 min) to discard the supernatant, and repeat three times. The experiments were divided into four groups (n =3) as follows: 0.2 mL blood + 1.8 mL H_2O; 0.2 mL blood + 1.8 mL LacP5⊃BSTA (final concentration: 0.5 mg/mL); 0.2 mL blood + 1.8 mL LacP5⊃BSTA (final concentration: 1 mg/mL); 0.2 mL blood + 1.8 mL PBS. Place the samples in a water bath set at 37 °C and incubate for 3 h mixing the samples every 30 min. The supernatant was collected by centrifugation (2500 rpm, 5min), and the absorbance of the supernatant at 540 nm was detected using UV-Vis spectrophotometer. The hemolysis rate is calculated according to the following formula:

Hemolysis rate = $(A_{test} - A_{negative}) / (A_{positive} - A_{negative})$ *100%

A: Absorbance test: blood + LacP5⊃BSTA negative: blood + PBS positive: blood + H_2O

In vivo imaging of LacP5⊃BSTA

Female BALB/C mice were purchased from TengXin Biotechnology Co., Ltd. Xi'an Branch (Xi'an, China)**.** All animal procedures complied with all relevant ethical

regulations and were approved by the Northwest A&F University Animal Care Committee (NWAFU-314020038). 6-week-old BALB/c female mice were subcutaneously injected with 5 x 10^5 mouse hepatoma (H22) cells in the right hindlimb to build a H22 solid tumor-bearing mice model. After tumor volume reached ~200 mm^3, LacP5⊃BSTA was injected through the tail vein, and IVIS Lumina II was used to detect the fluorescence of compound **6** after different times (Ex/Em = 675 nm/Cy 5.5).

Antitumor effect in vivo

6-week-old BALB/c female mice were subcutaneously injected with 5 x 10^5 H22 cells in the right hindlimb to build a H22 solid tumor-bearing mice model. After tumor volume reached ~200 mm^3, mice were randomly divided into five treatment groups (n = 5): PBS, Free DSF, LacP5⊃BSTA@DSF, LacP5⊃BSTA + Light, LacP5⊃BSTA@DSF + Light. Different drugs were injected into the corresponding mice via the tail vein. (The dose of free DSF: 1 mg/kg. While the others were treated with an equivalent dose of LacP5⊃BSTA: 20 mg/kg). After 24 h intravenous (*i.v.*) administration, tumors in mice in the + Light group were irradiated by using 685 nm (2 W/cm^2, 3 min) laser. PBS and free DSF groups were administrated every other day. The others were administrated two times every seven days. Tumor size and mice body weight were recorded every two days. To assess the necrosis of tumor tissue, mice were sacrificed to collect tumor for H&E staining. Major organs of mice also were collected for biosafety analysis.

Job's Plot for LacP5 ⊃ G_M

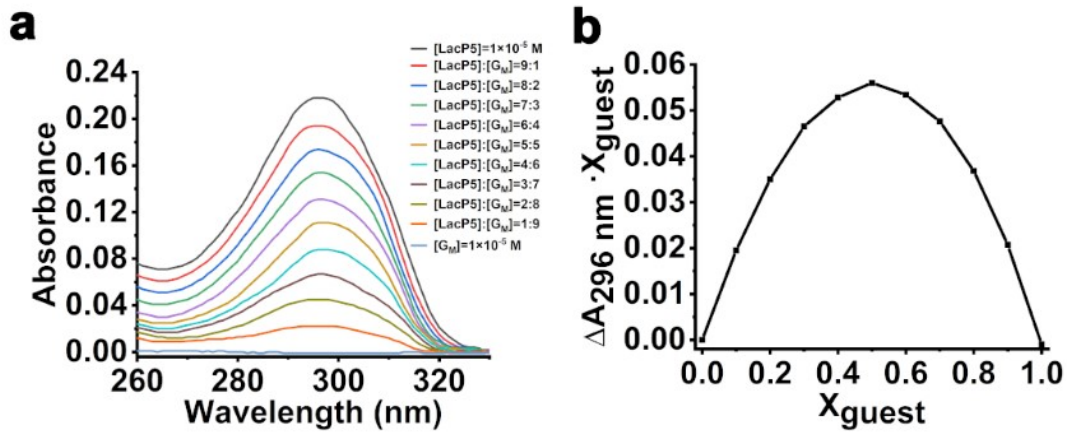


Figure S19. (a) UV-Vis absorption spectra of the mixture of LacP5 and G_M in water at different molar ratios while [LacP5] + [G_M] = 10 μM. (b) Job's Plot shows 1:1 stoichiometry of the complex between LacP5 and G_M by plotting the absorbance difference at 296 nm (a characteristic absorption peak of LacP5).

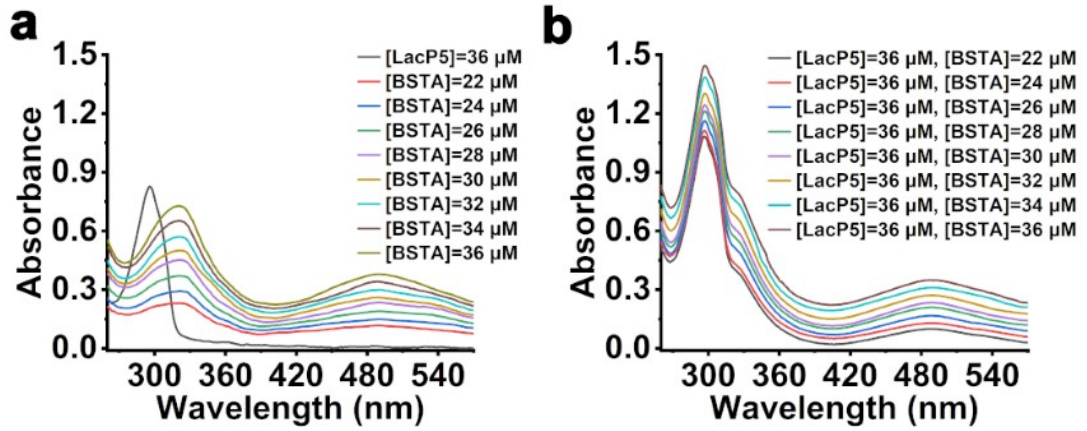


Figure S20. (a) UV-Vis spectra of LacP5 at the concentration of 36 μM, and BSTA at the varying concentrations of 22, 24, 26, 28, 30, 32, 34 and 36 μM, respectively. (b) UV-Vis spectra of the complexation of LacP5 and BSTA, the concentration of LacP5 is 36 μM, and the varying concentrations of BSTA are 22, 24, 26, 28, 30, 32, 34 and 36 μM, respectively.

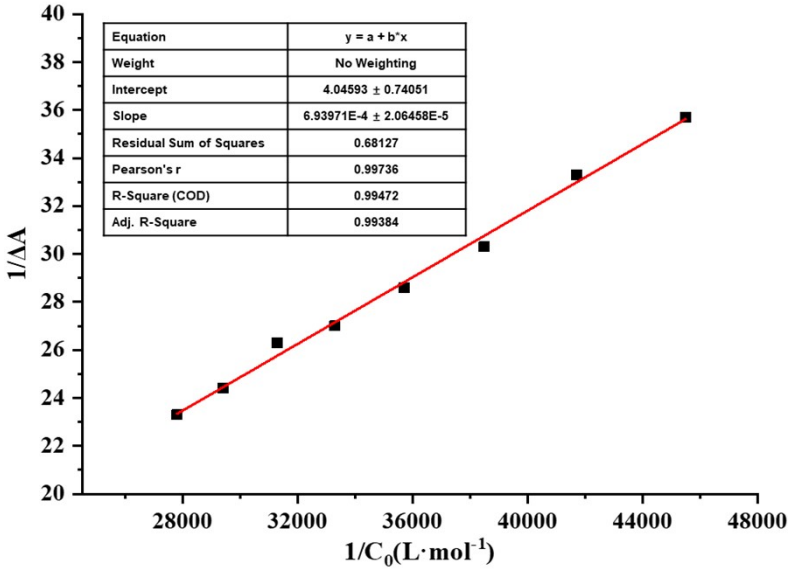


Figure S21. The plot of $^{1}/(A_0 - A)$ vs $^{1}/C_0$, the binding constant (Ka) of LacP5 and BSTA was estimated to be about 5.8×10^{3} M^{-1}.

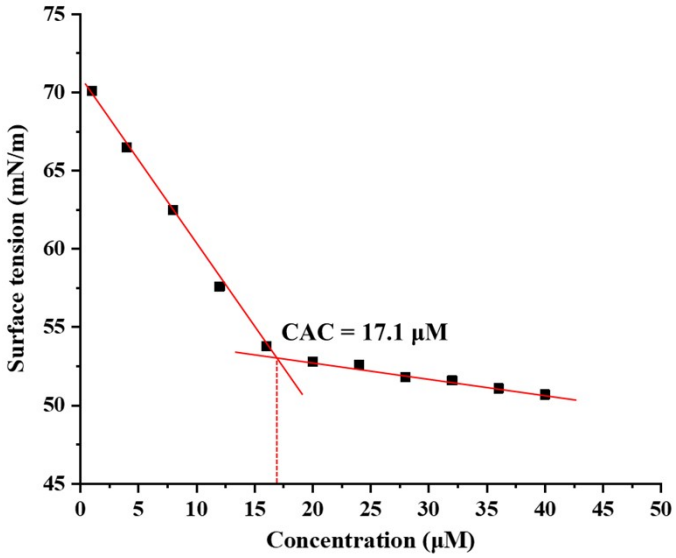


Figure S22. The critical aggregation concentration (CAC) of LacP5 ⊃ BSTA in water. Surface tension of water as a function of LacP5 ⊃ BSTA concentration. The crossing point of two tangent lines in the curve implies that the CAC of LacP5 ⊃ BSTA is approximately 17.1 μM.

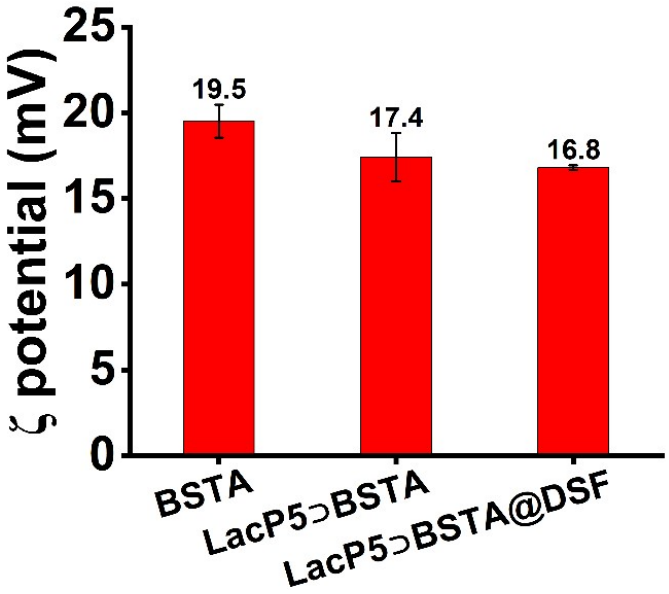


Figure S23. The zeta potential of BSTA, LacP5 ⊃ BSTA and LacP5 ⊃ BSTA@DSF.

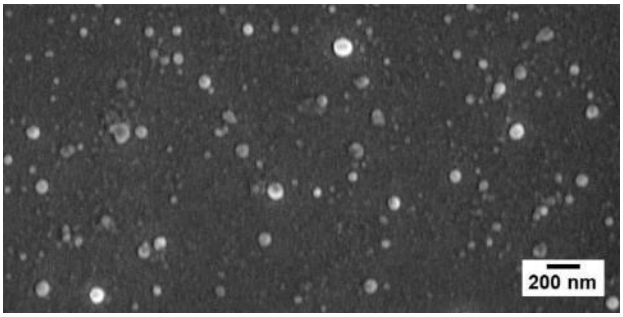


Figure S24. SEM image of LacP5⊃BSTA nanoparticles obtained by freeze drying (average size about 94 nm). Scale bar, 200 nm.

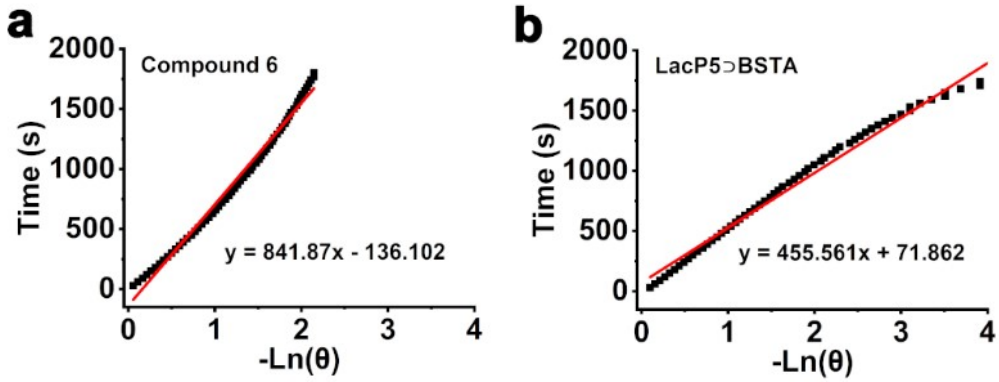


Figure S25. Fitting curve between cooling coefficient and time of (a) compound **6** and (b) LacP5 ⊃ BSTA.

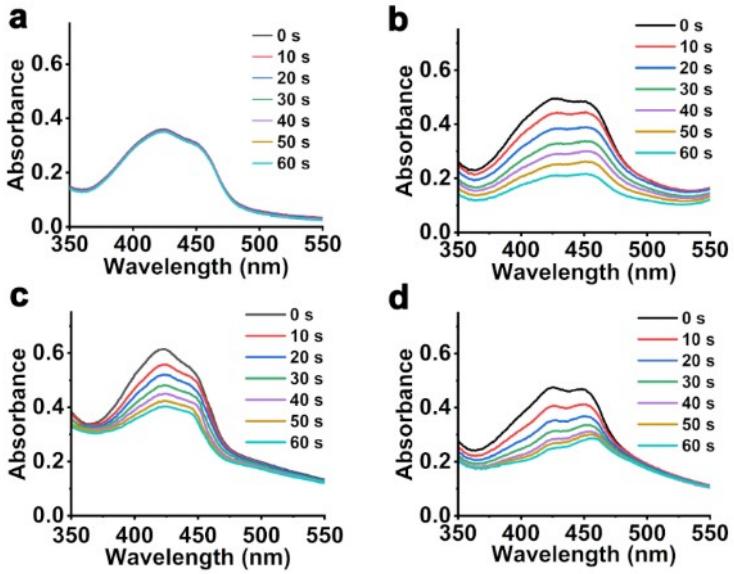


Figure S26. The UV-Vis of DPBF (30 μM) with (a) water, (b) MB, (c) LacP5 ⊃ BSTA and (d) compound **6** under irradiation.

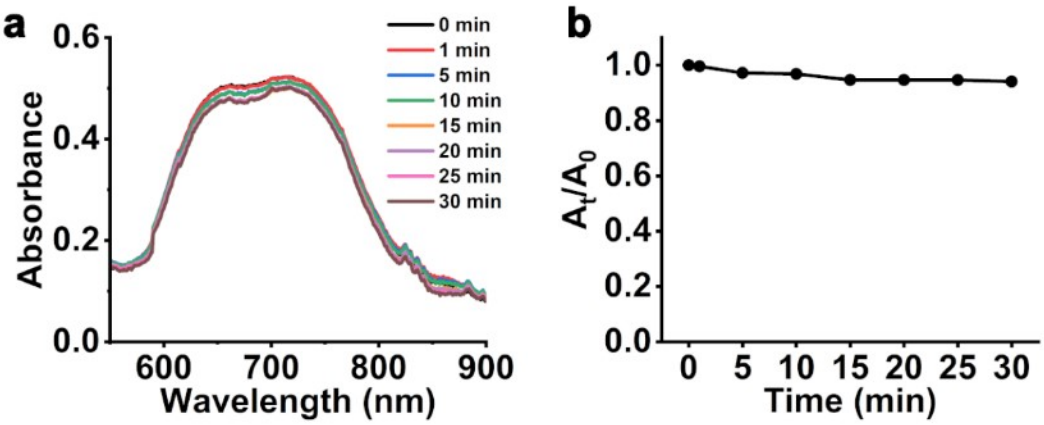


Figure S27. The Photostability of compound **6**. (a) The UV-Vis absorption spectra of compound **6** after different time of irradiation (685 nm, 2 W/cm^2). (b) A_t/A_0 of compound **6** solution after different time of irradiation (685 nm, 2.0 W/cm^2, 30 min).

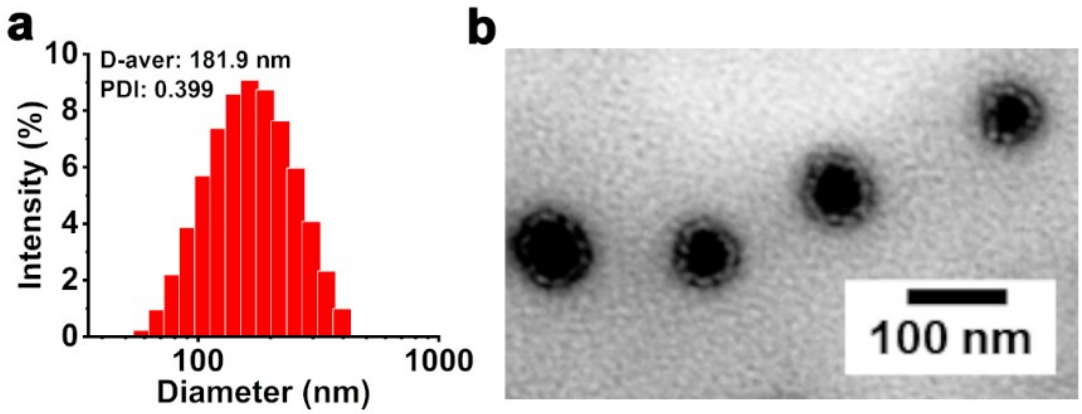


Figure S28. The (a) DLS data and (b) TEM of LacP5⊃BSTA@DSF.

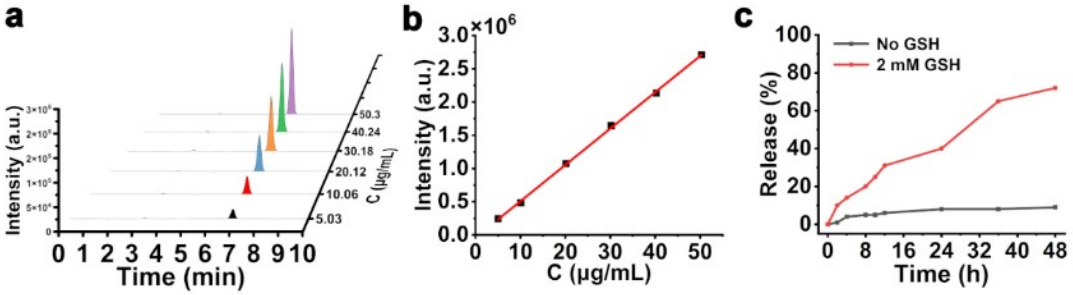


Figure S29. (a) HPLC Spectra of DSF with different concentrations. (b) The standard curve of DSF. (c) The release behavior of compound **6** in the presence and absence of GSH.

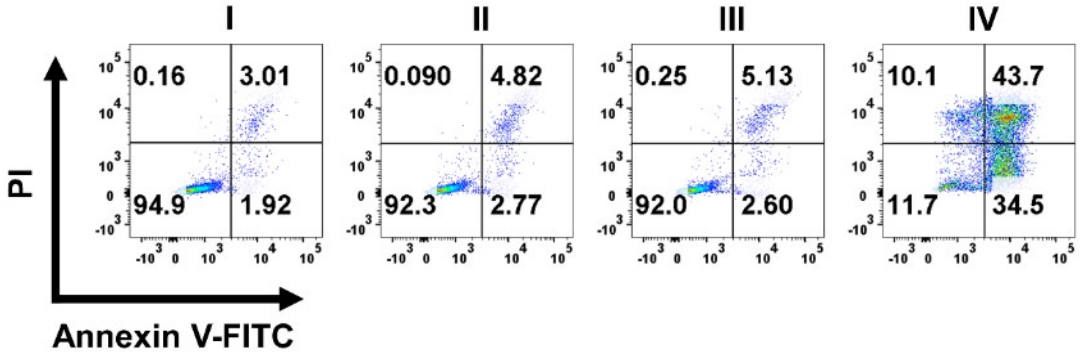


Figure S30. The Annexian V-FITC/PI assay by flow cytometry of HepG2 cell after different treatments. I: PBS; II: Incubate with LacP5⊃BSTA for 24 h without irradiation; III: Incubate with LacP5⊃BSTA for 24 h and then continue to incubate for 0 h after irradiation; IV: Incubate with LacP5⊃BSTA for 24 h and then continue to incubate for another 24 h after irradiation. (Light: 685 nm, 0.08 W/cm^2, 20 min)

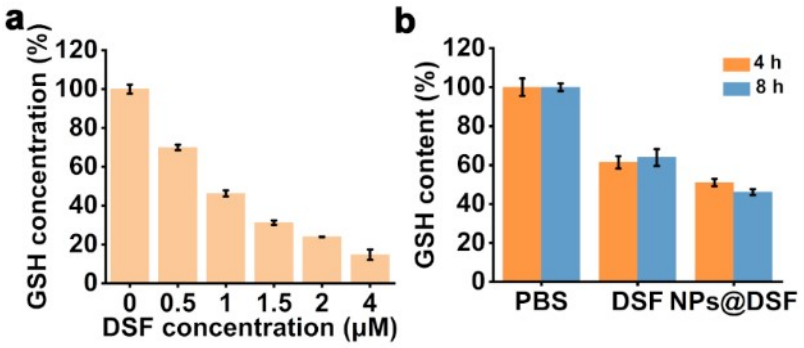


Figure S31. **(a)** Consumption of GSH solution (0.4 mg/mL) by different concentrations of DSF. (b) Consumption of intracellular GSH in HepG2 cells after incubation with DSF or NPs@DSF for 4 h and 8 h.

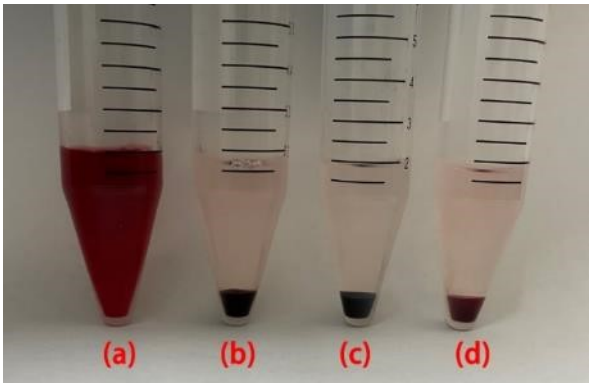


Figure S32. The photograph of red blood cell hemolysis. (a) H_2O (b) 0.5 mg/mL LacP5⊃BSTA (c) 1 mg/mL LacP5⊃BSTA (d) PBS.

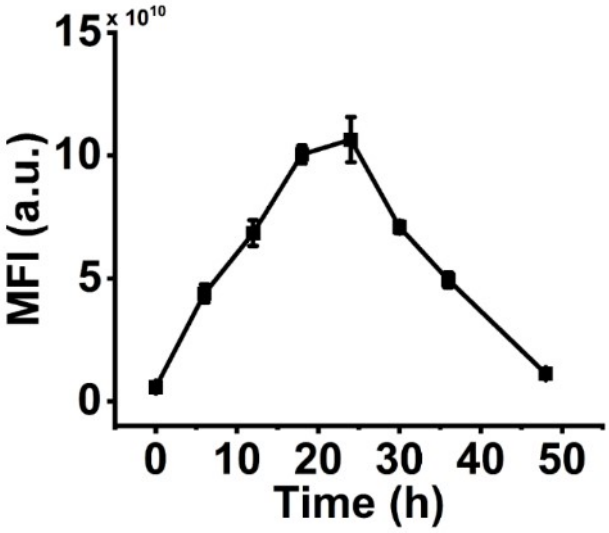


Figure S33. The mean fluorescence intensity of mice tumor sites at different time points (n = 3).

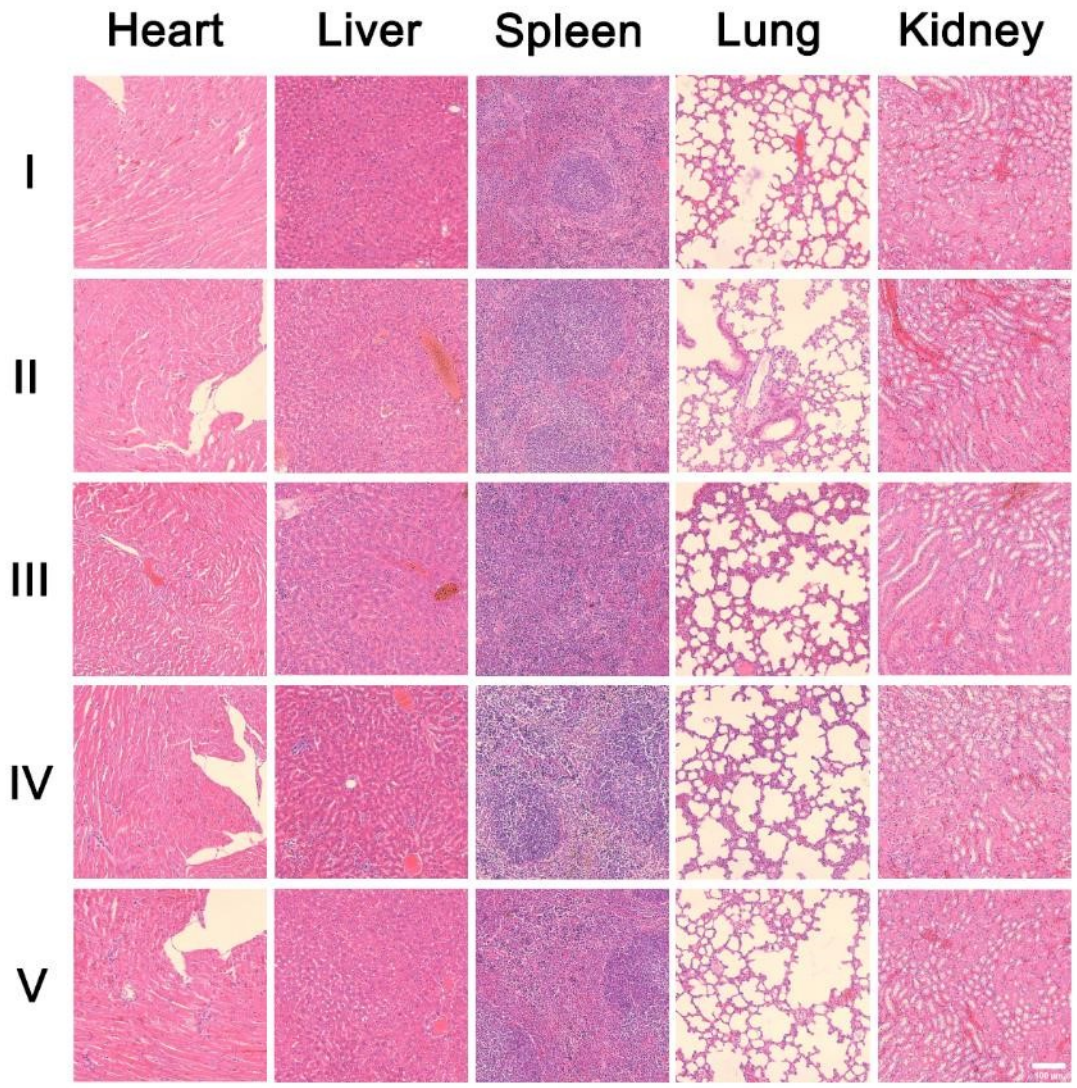


Figure S34. After treatment, the main organs of BALB/c mice bearing H22 tumor were examined histologically. Paraffin section thickness, 5 μm; Scale bar: 100 μm. I: PBS; II: Free DSF; III: NPs@DSF; IV: NPs + light; V: NPs@DSF + light.

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