Supporting information

An Activatable Unimolecular Phototheranostic Agent for Synergistic Chemo-Photodynamic Therapy

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1. Experimental Section

1.1 Materials and Instruments.

All reagents and solvents used were of reagent grade and were obtained from commercial suppliers. Flash column chromatography was performed with silica gel (200–300 mesh) and dichloromethane/methanol or petroleum ether/ethyl acetate was used as eluent. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance II 400 MHz spectrometer. Chemical shift (δ) was reported as ppm in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard. The mass spectrum was recorded on LTQ Orbitrap XLMS instruments. UV-vis spectra and fluorescent spectra were achieved from an Agilent Technologies CARY 60 UV-vis spectrophotometer and Varian CARY Eclipse fluorescence spectrophotometer respectively. The excitation and emission slit widths were adjusted to obtain the fluorescence intensity in the appropriate range. Cell imaging was performed on Olympus FV3000 confocal microscopes (Olympus, Japan).

1.2 Spectral Measurements.

All the optical experiments except pH measurements were performed in PBS buffer (10.0 mM, pH 7.4, containing 30% DMSO). **ICy-Cb**, **ICy** and **ICy-OH** were dissolved in DMSO at a concentration of 5 mM respectively as the stock solution and stored at 20 ℃ for further use. For the titration experiments, a certain amount of NTR or H_2O_2 was added into the solution of a probe (5.0 μM) in a 2.0 mL total volume. The resulting solutions were shaken well and then incubated for 60 min at 25 °C before the spectral measurements.

1.3 Singlet oxygen detection

The singlet oxygen ($^{1}O_2$) capture probe 1,3-diphenylisobenzofuran (DPBF) was used to evaluate the $^{1}O_2$ generation capacity of each test sample in methanol solution. DPBF experiments were conducted in a mixed solvent (PBS/DMSO = 1:1, V/V). Specifically, DPBF (30 μM) was mixed with the compounds (10 μM) in this solvent system, and then exposed to light irradiation (660 nm, 10 mW/cm²). The absorbance changes at 415 nm were recorded at different time points and analyzed using Origin software to evaluate singlet oxygen generation capacity. During the irradiation, the absorbance changes at 415 nm of the solution at different time points were recorded. The variation curve of the absorption intensity of the solution was fitted by Origin to evaluate the ability to generate singlet oxygen. For these experiments, **ICy-Cb** was first dissolved in the same mixed solvent (PBS/DMSO = 1:1, V/V) and treated with

H_2O_2 to activate the probe. Subsequently, this activated solution was used for DPBF experiments following the same protocol described above.

1.4 Cell Culture.

Breast cancer cells (4T1, MCF-7, HeLa and 4T1 cells) were purchased from the Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences. DMEM was added with 10% fetal bovine serum, 1% penicillin and 1% streptomycin as the culture medium, and cells were placed in the medium at 37 °C under a humidified atmosphere containing 5% CO_2 and 95% air. Before imaging experiments, cells were cultured in an 18 mm glass dish.

1.5 Endogenous nitroreductase imaging in living cells.

For hypoxia incubation, 4T1, MCF-7, HeLa and 4T1 cells were seeded in confocal culture dishes at an approximate concentration of 2×10^4 cells per mL and incubated in a cell incubator under normal conditions for 12 h. Then, 4T1, MCF-7, HeLa and 4T1 cells were incubated with 5 μM **ICy-Cb** at 37 ℃ for 60 min and washed with PBS 3 times before scanning under the confocal microscope. The final DMSO concentration was maintained below 1% in all experiments.

For cell culture studies, the final DMSO concentration was maintained below 1% in all experiments. Regarding treatment conditions, cells were exposed to 100 μM H_2O_2 or 1 μg/mL LPS to evaluate H_2O_2-mediated probe activation, while 500 μM NAC was used for 30 min as an ROS scavenger in control experiments.

1.6 Detection of cellular ROS generation within DCFH-DA.

4T1 cells were seeded at confocal culture dishes at a density of 5×10^4 and pre-incubated under hypoxic conditions. Then, various concentrations of **ICy-Cb** and 10 mM DCFH-DA were added and incubated for 60 min. Then, the cells were washed with PBS 3 times and irradiated with a 660 nm laser for 2 min and scanned under a confocal microscope at 488 nm excitation, whereas, the fluorescence channel at 500–550 nm was collected.

1.7 Cytotoxicity Assay.

The MTT assay was employed to evaluate cell viability by measuring the conversion of 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide to formazan crystals by active mitochondrial enzymes. 4T1, MCF-7, HeLa and IOSE80 cells lines were seeded in 96-well plates (1×10^5 cells/mL, 100 μL DMEM with 10% FBS per well). After a 24-hour attachment period in standard culture conditions, the cells were treated with various concentrations of **ICy-Cb** in 100 μL medium for 60 minutes. In

the experimental groups, cells received 660 nm light exposure (10 mW/cm², 10 minutes), followed by continued incubation. Parallel control groups underwent identical procedures but were shielded from light. Untreated cells served as baseline controls, with six replicates established for each experimental condition to ensure robust statistical analysis. Following 12 hours of incubation, the culture medium was replaced with 100 μL of MTT solution (5 mg/mL in DMEM), and cells were incubated for an additional 4 hours at 37°C. The MTT solution was then removed, and the precipitated formazan crystals were dissolved in 100 μL DMSO. After 10 minutes of gentle shaking, absorbance values were measured at wavelengths of 570 nm and 630 nm using a Thermo Fisher Scientific plate reader. Cell viability percentages were determined using the equation: Cell viability (%) = [(test sample absorbance - medium blank absorbance)/(untreated control absorbance - medium blank absorbance)] × 100%.

1.8 Live/Dead cell imaging.

Calcein-AM and propidium iodide (PI) assay kits were used to further test the cytotoxicity of nanoparticles. Cells were seeded in confocal culture dishes at a concentration of 1×10^5 cells mL^{-1} and pre-incubated in normoxia ($air/CO_2 = 95\%/5\%$) for 24 h. For the light groups, cells were exposed to the 660 nm laser (10 mW cm^{-2}, 10 min), followed by further incubation for 4 h. Cells in the dark groups were kept away from light. Next, Calcein-AM (2 μL) and PI (2 μL) were added to the dishes for all cells. After an additional 30 min incubation, cells were rinsed with PBS and placed under LSCM for imaging.

1.9 Construction and testing of tumor sphere models.

In vitro 3D tumor sphere model was cultured according to the reported method. 4T1 cells were seeded at a density of 10^4 per well in a 96-well plate, each well of which was pre-coated with 0.1 mL of agarose gel (2 wt% in serum-free DMEM, low boiling point). The cells were cultured in a normoxic atmosphere at 37°C for 5-7 days, and the medium was changed every 2 days. After macroscopic tumor spheroids (approximately 300-500 μm in diameter) were formed, the intact spheroids were carefully transferred into confocal dishes and replaced with fresh medium. Subsequently, the medium was changed to fresh medium containing **ICy-Cb** (10 μM), **ICy** (10 μM), or **Cb** (10 μM), and tumor spheres were incubated for an additional 4 h under normoxic conditions. Finally, the tumor spheres were placed under the CLSM for 3D-depth scanning.

The cytotoxicity of compounds in tumor spheres was further tested by Calcein-AM and propidium iodide (PI). The construction of the tumor sphere models was the same as described above. After the formation of tumor spheroids visible to the naked eye, the complete tumor spheroids were transferred to confocal dishes. The medium was

then replaced with fresh medium containing **ICy-Cb** (10 μM), **ICy** (10 μM), or **Cb** (10 μM), and the incubation was continued under normoxic conditions for 4 h. For the tumor spheres in the light groups, laser irradiation (660 nm, 10 mW/cm², 20 min) was applied after 4 h of incubation. For the dark groups, the incubation was kept away from light. Finally, all tumor spheres were added Calcein-AM (2 μM) and PI (4 μM), and the incubation was continued for 30 min. All tumor spheres were placed under CLSM for 3D-depth scanning.

1.10 Visualization of nitroreductase and photodynamic therapy in the 4T1 tumor bearing mice model.

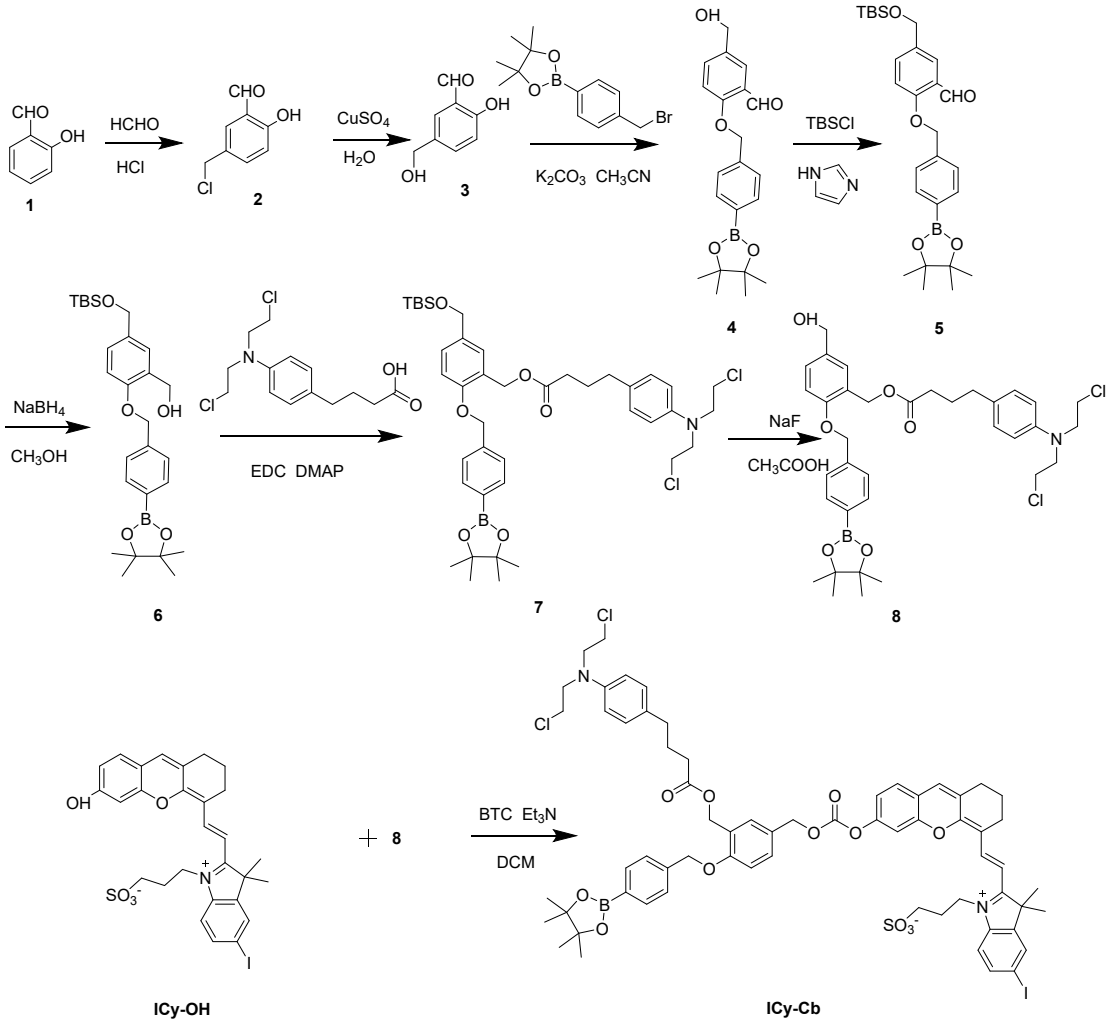
Female BALB/c mice (five weeks old) were acquired from the SPF (Specific Pathogen Free) Experimental Animal Center at Dalian Medical University. All procedures involving animals adhered to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health. The research protocol received approval from the Animal Ethics Committee at Dalian University of Technology (protocol number: 2018-043). For tumor model establishment, female Balb/c mice (4-5 weeks old) received subcutaneous 4T1 cell injections (5×10^6 cells) in the axillary area. When tumors reached approximately 200 mm³ in size, animals were randomly distributed into four treatment groups: Control, Light only, **ICy-Cb** only, and **ICy-Cb** with Light exposure. Tumor measurements were performed using vernier calipers, with tumor volume calculated using the equation: $V = 1/2\times a\times b^2$ where a represents the longest tumor diameter and b corresponds to the shorter perpendicular diameter.

For nitroreductase detection experiments, mice under anesthesia were injected with 50 μM **ICy-Cb** solution and subsequently imaged using a NightOWL II LB983 small animal imaging system fitted with 660 nm excitation source and 700±20 nm emission filters. Photodynamic therapeutic interventions employed light at an intensity of 100 mW/cm².

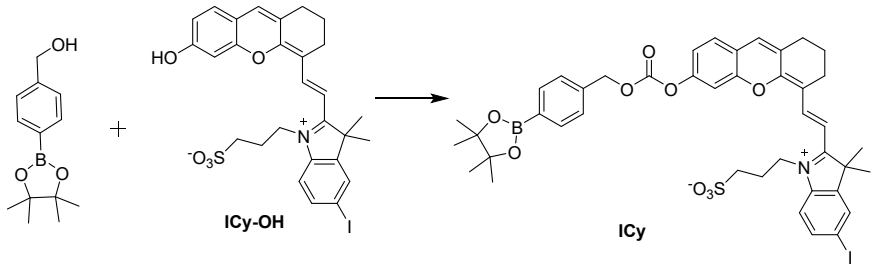
1.11 Statistical Analysis.

All quantitative results are expressed as mean ± standard deviation (SD). Each experimental procedure was performed at least three times to ensure reproducibility. Data processing and visualization were carried out using Origin 2017 software. Statistical comparisons between experimental groups were analyzed using t tests (and nonparametric tests where appropriate). Results were considered statistically significant when $p < 0.05$, with significance levels denoted by asterisks according to the following convention: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

2. Synthesis



Scheme S1. Chemical structures and synthetic approaches to **ICy-Cb**



Scheme S2. Chemical structures and synthetic approaches to **ICy**.

Synthesis of Compounds 2.

Salicylaldehyde (2.132 g, 12.5 mmol) was added to 10 ml of 37% concentrated hydrochloric acid solution containing (20.5 mmol) polyformaldehyde, followed by several drops of concentrated H_2SO_4 in catalytic dose. Stir the mixture at room temperature for 12 hours until a large amount of sediment forms. Water (40 ml) was then added to the reaction system mixture. The product was then extracted three times

using 20 ml of dichloromethane and the organic phase was dried using some anhydrous Na_2SO_4. Finally, CH_2Cl_2 was removed by a rotary evaporator and placed overnight in a vacuum drying oven to obtain a white solid (1.851g, 12.1mmol, yield 86.8%).

Synthesis of Compounds 3.

The compounds **2** (850 mg, 5 mmol, 1.0 eq.) and $CuSO_4$ (795 mg, 5 mmol, 1.0 eq.) were dissolved in a mixture of H_2O (3.5 mL) and DMSO (7 mL), and the reaction mixture was stirred at 110 °C for 2 hours. The reaction mixture is then cooled to room temperature and diluted with just enough water. Compound **3** (807.5mg, 95% yield) was obtained by extracting the reaction mixture with dichloromethane for 3-4 times, drying it with anhydrous sodium sulfate and steaming it with rotary evaporator. ^{1}H NMR (400 MHz, DMSO) δ 10.60 (s, 1H), 10.26 (s, 1H), 7.62 (s, 1H), 7.46 (d, *J* = 10.4 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 5.17 (s, 1H), 4.43 (s, 2H).^{13}C NMR (101 MHz, DMSO) δ 192.31, 160.14, 135.48, 134.09, 127.56, 122.24, 117.48, 62.57. $C_8H_8O_3$： HRMS(ESI, m/z): $[M-H]^-$ calcd for target, 151.05; found .151.1

Synthesis of Compounds 4.

The compound **3** (1.52 g, 10 mmol, 1.0 eq.), Pinacol 4-bromomethyl phenyl borate (2.96 g, 10 mmol, 1.0 eq.), anhydrous K_2CO_3 (2.76 g, 20 mmol, 2.0 eq.) and the catalytic amount of KI (166 mg, 1 mmol, 0.1 eq.) was dissolved in anhydrous acetonitrile solution and the reaction mixture was refluxed at 85 °C for 8 hours. Then the reaction mixture was cooled to room temperature, the solvent was dried by a rotary evaporator, and a mixture of petroleum ether: ethyl acetate = 3:1 (v/v) was used as the development agent. The yellow oily liquid compound **4** (2.912 g, yield 65%) was purified by silica gel column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.57 – 10.48 (m, 1H), 7.85 (d, *J* = 7.8 Hz, 2H), 7.81 (d, *J* = 1.6 Hz, 1H), 7.54 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 2H), 7.01 (d, *J* = 8.6 Hz, 1H), 5.22 (s, 2H), 4.63 (d, *J* = 4.2 Hz, 2H), 1.36 (s, 12H).^{13}C NMR (101 MHz, $CDCl_3$) δ 189.69, 160.44, 139.06, 135.19, 134.90, 134.82, 133.79, 127.00, 126.41, 126.03, 124.93, 113.37, 84.04, 70.54, 64.19, 24.87. $C_{21}H_{25}BO_5$： HRMS(ESI, m/z): $[M+Na]^+$ calcd for target, 391.187; found .391.1688

Synthesis of Compounds 5.

Compound **4** (600 mg, 1.63 mmol, 1.0 eq.) was dissolved in 10 ml dichloromethane for stirring, and then tert-butyldimethylsilane (489 mg, 3.26 mmol, 2.0 eq.) was prepared into 10 ml dichloromethane solution, which was slowly added into the reaction system. After the mixture was evenly mixed, imidazole (221 mg, 3.26 mmol, 2.0 eq.) was added slowly to catalyze the reaction, and the reaction was carried out at room temperature for 1 to 2 hours. Then, appropriate amount of water was added to the reaction mixture, extracted by DCM for several times, dried organic phase using anhydrous Na_2SO_4, added silica gel powder to dry sample, dried solvent by rotary evaporator, purified by silica gel column chromatography, using petroleum ether: ethyl acetate = 10: A mixture of 1 (v/v) was unrolled to obtain a yellow viscous oily liquid compound 13 (980 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.57 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 2.2 Hz, 1H), 7.54 (dt, *J* = 6.9, 3.5 Hz, 1H),

7.46 (t, *J* = 7.7 Hz, 2H), 7.01 (t, *J* = 6.8 Hz, 1H), 5.23 (s, 2H), 4.70 (s, 2H), 1.37 (s, 12H), 0.95 (d, *J* = 2.9 Hz, 9H), 0.12 (d, *J* = 1.8 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 189.69, 160.11, 139.22, 135.19, 134.24, 133.90, 126.38, 126.06, 124.84, 113.15, 83.90, 70.54, 64.14, 25.96, 24.88, 18.40, -5.22. $C_{27}H_{39}BO_5Si$：HRMS(ESI, m/z): $[M+Na]^+$ calcd for target, 505.2552; found .505.2559

Synthesis of Compounds 6.

Compound **5** (600 mg, 1.245mmol, 1.0 eq.) was dissolved in the mixed solution of 10 ml dichloromethanes and 10 ml methanol for stirring. After stirring evenly, $NaBH_4$ solid (94.6 mg, 2.49 mmol, 2.0 eq.) was slowly added to the reaction. The reaction was stirred at room temperature for 30 min to 1 h, and the reaction was basically complete by TLC test. Then, appropriate amount of water was slowly added to the reaction mixture to quenching the remaining $NaBH_4$, and then ethyl acetate was added to extract the organic phase for 3-4 times, saturated NaCl water was used to wash the organic phase, anhydrous Na_2SO_4 was added to the organic phase for drying, silica gel powder was added and the solvent was dried by a rotary evaporator, and then the sample was dry, purified by silica gel column chromatography, and petroleum ether was used: A mixed solution of ethyl acetate = 5:1 (v/v) was developed as a developing agent to obtain a yellow viscous oil-like liquid compound 14 (450mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.7 Hz, 2H), 7.44 (d, *J* = 7.8 Hz, 2H), 7.32 (s, 1H), 7.24 (d, *J* = 8.2 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 5.13 (s, 2H), 4.76 (s, 2H), 4.71 (s, 2H), 2.60 (s, 1H), 1.39 (s, 12H), 0.99 (s, 9H), 0.15 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.42, 140.06, 135.19, 133.94, 129.47, 126.82, 126.57, 126.42, 111.54, 83.88, 70.10, 64.71, 61.76, 26.07, 24.91, 18.48, -5.11. $C_{27}H_{41}BO_5Si$：HRMS(ESI, m/z): $[M+Na]^+$ calcd for target, 507.2708; found .507.2711

Synthesis of Compounds 7.

Nitrobutyrate (200 mg, 0.658 mmol, 1.0 eq.), 1-ethyl - (3-dimethylaminopropyl) carbodiimide hydrochloride (126.2mg, 0.658 mmol, 1.0 eq.), 4-dimethylaminopyridine (160mg, 1.311mmol, 2.0 eq.) dissolved in 10 ml of dichloromethane, stirred at room temperature for 1 h, and then dissolved in 2 ml of dichloromethane (318 mg, 0.658 mmol, 1.0 eq.) when compound 14 was basically transformed into an intermediate detected by TLC spot plate. Methylene chloride solution of compound **6** was added to the reaction system and stirred at room temperature overnight. The reaction system was extracted with water and dichloromethane for 3 to 4 times, and then the organic phase was dried with anhydrous Na_2SO_4. After mixing silica gel, the sample was dried by rotary evaporation apparatus, and purified by silica gel column chromatography. Petroleum ether was used: ethyl acetate = 5: A mixture of 1 (v/v) was eluted as an eluent to obtain a yellowish oily product (285 mg, yield about 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 7.5 Hz, 2H), 7.43 (d, *J* = 7.5 Hz, 2H), 7.31 (s, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 2H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 2H), 5.24 (s, 2H), 5.15 (s, 2H), 4.69 (s, 2H), 3.70 (d, *J* = 6.6 Hz, 4H), 3.65 (d, *J* = 6.6 Hz, 4H), 2.58 (t, *J* = 7.5 Hz, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.94 (p, *J* = 7.4 Hz, 2H), 1.37 (s, 12H), 0.95 (s, 9H), 0.11 (s, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 173.37,

155.60, 143.34, 140.16, 135.03, 133.76, 129.86, 127.90, 127.31, 126.21, 124.53, 113.24, 111.82, 83.84, 70.07, 64.54, 61.88, 54.18, 40.06, 34.05, 33.65, 26.72, 25.99, 24.89, 18.43, -5.19. $C_{41}H_{58}BCl_2NO_6Si$：HRMS(ESI, m/z): $[M+Na]^+$ calcd for target, 792.3396; found .792.3406

Synthesis of Compounds 8.

Compound 7 (220 mg, 0.286 mmol, 1.0 eq.) was dissolved in a mixture of 12 ml acetic acid, 4 ml tetrahydrofuran and 4 ml pure water, and appropriate amount of NaF was added, stirring at room temperature overnight. When the reaction was basically complete through TLC point plate detection, appropriate amount of water was added to the reaction system. The organic phase was dried with anhydrous Na_2SO_4, dried with silica gel in rotary evaporator, and then purified by column chromatography. Petroleum ether: ethyl acetate = 2: A mixture of 1 (v/v) was developed as a developing agent to obtain compound 16, a light yellow solid (170 mg, yield 77.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 7.5 Hz, 2H), 7.43 (d, *J* = 7.5 Hz, 2H), 7.37 (s, 1H), 7.28 (s, 1H), 7.07 (d, *J* = 7.9 Hz, 2H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 2H), 5.25 (s, 2H), 5.16 (s, 2H), 4.63 (s, 2H), 3.70 (d, *J* = 6.5 Hz, 4H), 3.64 (d, *J* = 6.5 Hz, 4H), 2.58 (t, *J* = 7.3 Hz, 2H), 2.39 (t, *J* = 7.2 Hz, 2H), 1.97 – 1.91 (m, 2H), 1.38 (s, 12H).^{13}C NMR (101 MHz, $CDCl_3$) δ 173.50, 156.09, 144.30, 140.00, 135.08, 133.37, 130.77, 129.74, 128.96, 128.45, 126.23, 124.95, 112.34, 112.04, 83.88, 70.07, 64.81, 61.77, 53.68, 40.56, 33.97, 33.64, 26.76, 24.90. $C_{35}H_{44}BCl_2NO_6$：HRMS(ESI, m/z): $[M+Na]^+$ calcd for target,678.2513; found .678.2538.

Synthesis of Compounds ICy.

The compound **ICy-OH** (61.3 mg, 0.1 mmol, 1.0 eq.) and triphosgene (9.9 mg, 0.33 mmol, 0.33 eq.) were dissolved in 15 ml of anhydrous dichloromethane and stirred thoroughly to ensure proper mixing. Subsequently, N, N-diisopropyl ethylamine (42 mg, 0.33 eq.) was slowly added drop by drop (0.326 mmol, 3.26 eq.) and stirred at room temperature for 30 minutes. As acyl chloride intermediates of **ICy-OH** were formed, compound **7** (15.5 mg, 0.1 mmol, 1.0 eq.) was added, followed by a few drops of N, N-diisopropyl ethylamine. The mixture was then stirred at room temperature for 6 hours. Once the reaction was essentially complete, the reaction mixture was directly transferred to a silica gel column using a wet sampling method, and then purified by column chromatography with DCM: MeOH = 20: 1 (v/v) was developed as a developing agent to obtain the final product **ICy**, a bright purple solid (45mg, 43% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.58 (d, *J* = 15.1 Hz, 1H), 8.16 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.69 (s, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 2H), 6.88 (d, *J* = 15.2 Hz, 1H), 6.60 (s, 1H), 5.33 (t, *J* = 4.7 Hz, 1H), 5.17 (d, *J* = 6.6 Hz, 2H), 4.61 (s, 2H), 4.42 (s, 2H), 2.71 (d, *J* = 23.0 Hz, 4H), 1.82 – 1.65 (m, 10H). ^{13}C NMR (101 MHz, DMSO) δ 173.04, 165.87, 159.62, 153.38, 145.46, 138.23, 134.90, 129.13, 127.16, 120.24, 112.81, 110.44, 103.43, 97.67, 82.32, 81.35, 55.70, 52.11, 33.70, 33.38, 27.58, 27.06, 25.14. $C_{36}H_{33}N_2O_9S$：HRMS (ESI, m/z): calcd for $[M+H]^+$：878.2023; found 878.2035

Synthesis of Compound ICy-Cb.

The compounds **ICy-OH** (61.3mg, 0.1 mmol, 1.0 eq.) and triphosgene (9.9 mg, 0.33 mmol, 0.33 eq.) were dissolved in 15 ml of anhydrous dichloromethane, and were continuously stirring to mix well. Later, N, N-diisopropyl ethylamine (42 mg, 0.33 eq.) was slowly added drop by drop. 0.326 mmol, 3.26 eq.), stirring at room temperature for 30 min to 1 h. Then after TLC spot plate detection, when more acyl chloride intermediates of **ICy-OH** were formed, compound **8** (65.5 mg, 0.1 mmol, 1.0 eq.) was added, followed by a few drops of N, N-diisopropyl ethylamine, and the reaction was stirring at room temperature for 6 hours. When the reaction is basically complete after TLC spot plate detection, the reaction liquid is directly added to the silica gel column by wet sampling method, and then purified by column chromatography with DCM: MeOH = 20: 1 (v/v) was developed as a developing agent to obtain the final product **ICy-Cb**, a bright purple solid (53mg, 41.8% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.58 (d, *J* = 15.1 Hz, 1H), 8.16 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.68 (dd, *J* = 13.8, 7.1 Hz, 3H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.49 – 7.45 (m, 3H), 7.43 (s, 2H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 2H), 6.88 (d, *J* = 15.2 Hz, 1H), 6.61 (d, *J* = 8.3 Hz, 2H), 5.35 – 5.23 (m, 4H), 5.17 (d, *J* = 6.6 Hz, 2H), 4.61 (s, 2H), 3.66 (s, 8H), 2.74 (s, 4H), 2.60 (t, *J* = 6.0 Hz, 2H), 2.44 (d, *J* = 7.7 Hz, 2H), 2.32 (d, *J* = 7.5 Hz, 2H), 2.08 (s, 2H), 2.01 – 1.97 (m, 2H), 1.82 (d, *J* = 5.3 Hz, 2H), 1.76 (s, 6H), 1.30 (s, 12H).^{13}C NMR (101 MHz, DMSO) δ 173.10, 159.72, 156.86, 153.05, 152.86, 144.92, 141.65, 140.71, 135.03, 134.98, 130.12, 129.93, 129.73, 127.02, 126.96, 125.03, 112.35, 84.14, 70.26, 69.73, 52.67, 51.20, 47.87, 41.57, 35.60, 33.70, 33.38, 31.75, 29.55, 29.49, 29.29, 29.21, 29.15, 29.05, 27.58, 27.03, 25.58, 25.14, 24.45, 22.56, 14.41. $C_{64}H_{70}BCl_2IN_2O_{12}S$：HRMS(ESI, m/z): $[M+H]^+$ calcd for target,1299.3164; found .1299.3150.

3. Spectroscopic Property.

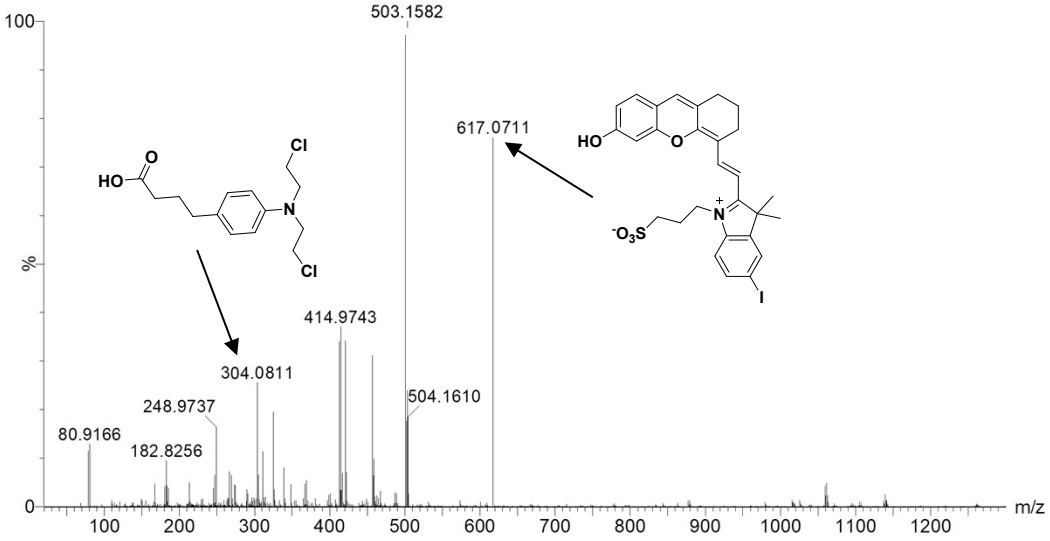


Figure S1. MS (ESI) spectrum of the reaction mixture of probe **ICy-Cb** for H_2O_2.

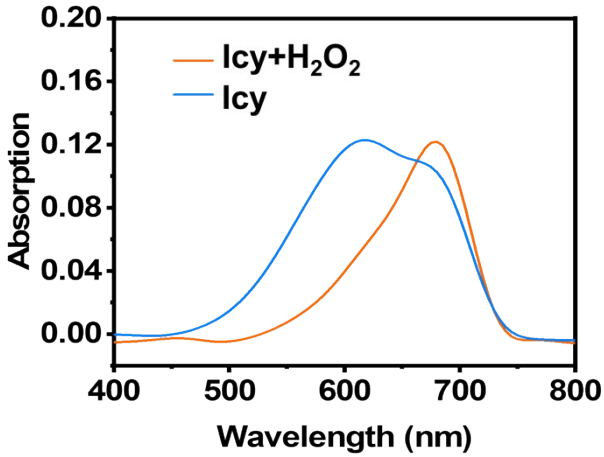


Figure S2. UV-vis spectra absorbance change of **ICy** (5 μM) in PBS buffer (10.0 mM, pH 7.4, containing 30% DMSO), after introduction of H_2O_2.

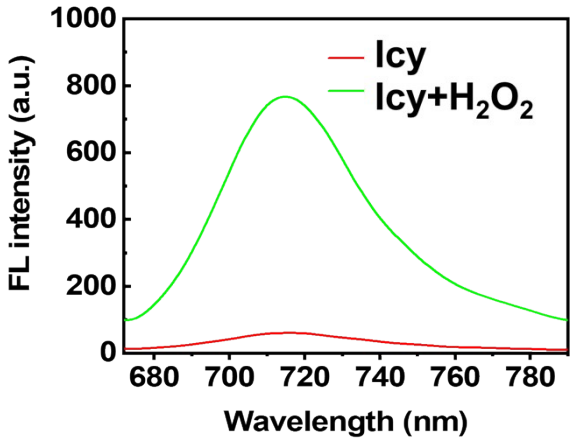


Figure S3. Fluorescence spectra absorbance change of **ICy** (5 μM) in PBS buffer (10.0 mM, pH 7.4, containing 30% DMSO), after introduction of H_2O_2.

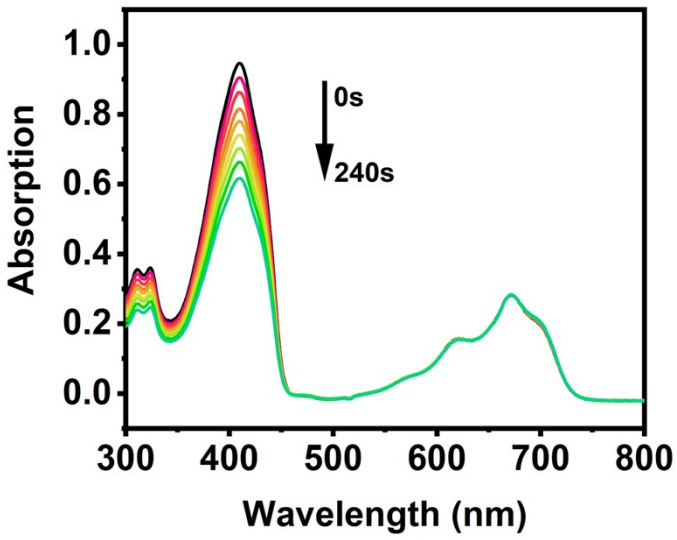


Figure S4. Photodegradation curves of DPBF in the presence of **ICy-OH** were obtained under 660 nm light irradiation for varying durations (0-240 s).

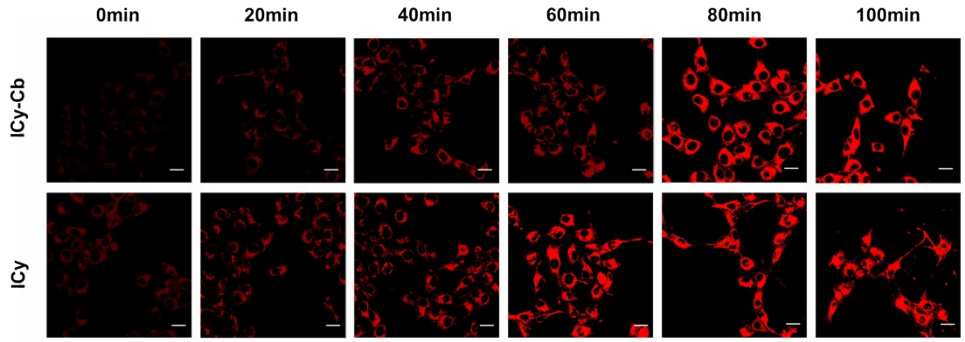


Figure S5. Cellular uptake of **ICy-Cb** and **ICy** in 4T1 cells. Scale bars: 10 μm.

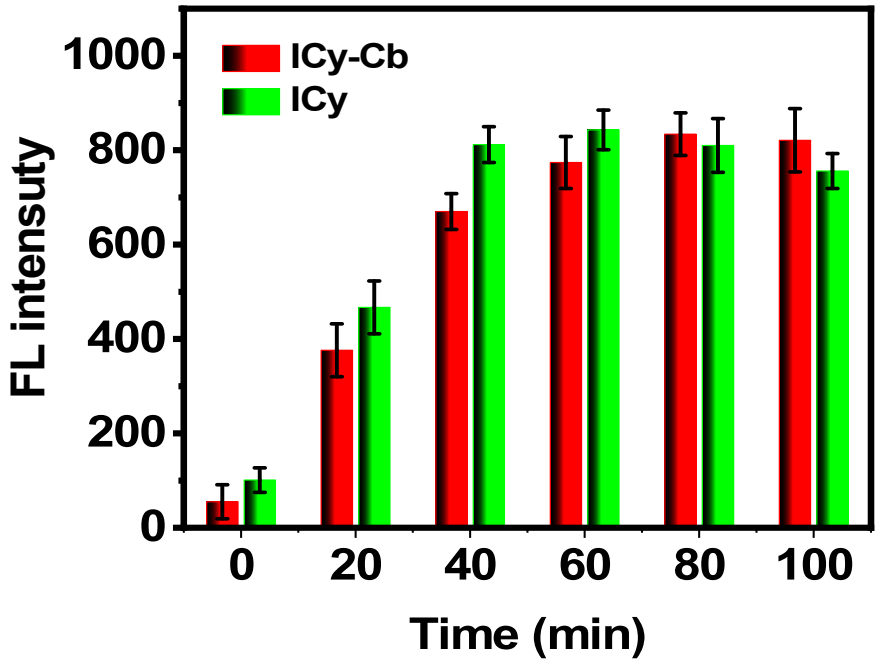


Figure S6. Quantification of fluorescence intensity from **Figure S4**.

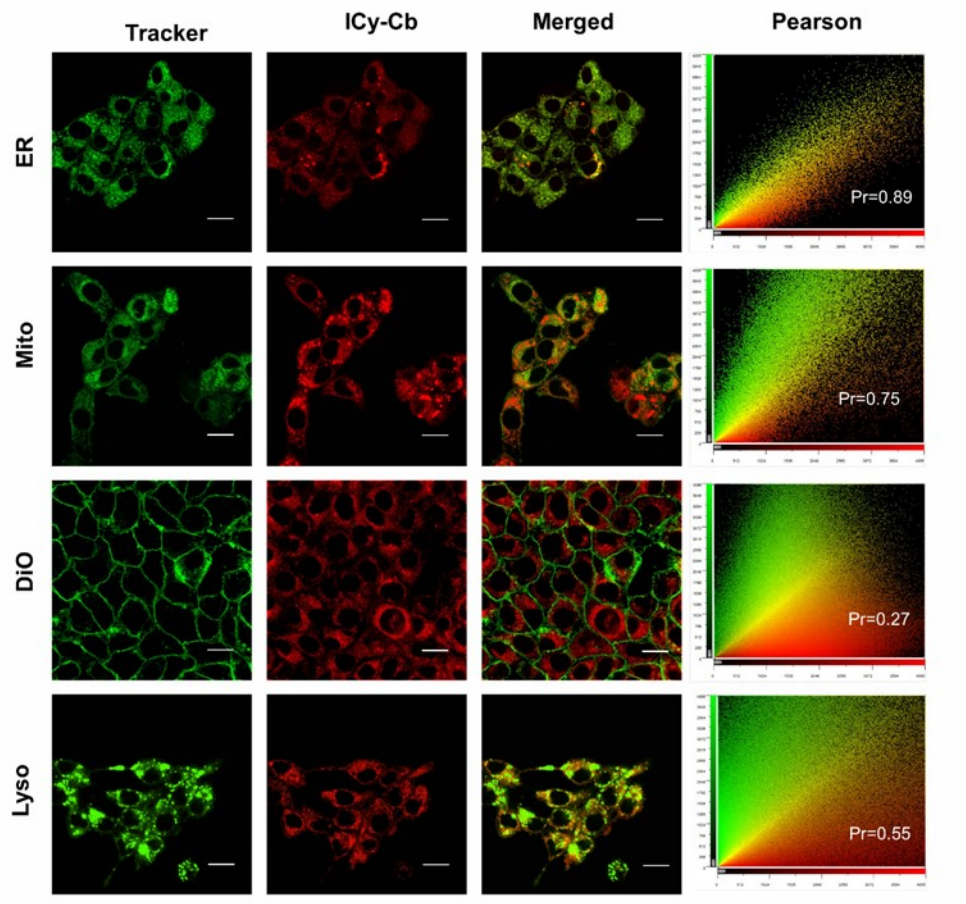


Figure S7. Subcellular colocalization assays of **ICy-Cb** in 4T1 cells. The green channel of ER-Tracker Green, Mito-Tracker Green, DiO and Lyso-Tracker Green was excited at 488 nm and collected at 500-580 nm. The red channel represents the fluorescent signal of **ICy-Cb** (λ_{ex}: 640 nm and λ_{em}: 670-750 nm). Scale bars: 10 μm.

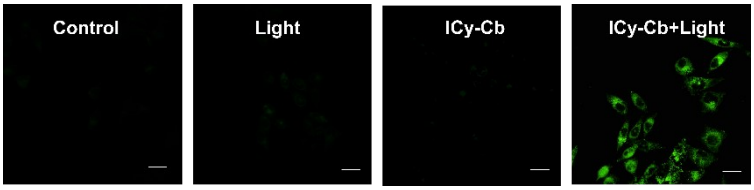


Figure S8. Fluorescence images of 4T1 cells containing **ICy-Cb** (5 μM)/SOSG (10 μM), indicating $^1O_2$ generation of **ICy-Cb** dyes upon 660 nm light irradiation. For SOSG, emissions were collected at 500–580 nm (λ_{ex} = 488 nm), scale bar = 20 μm.

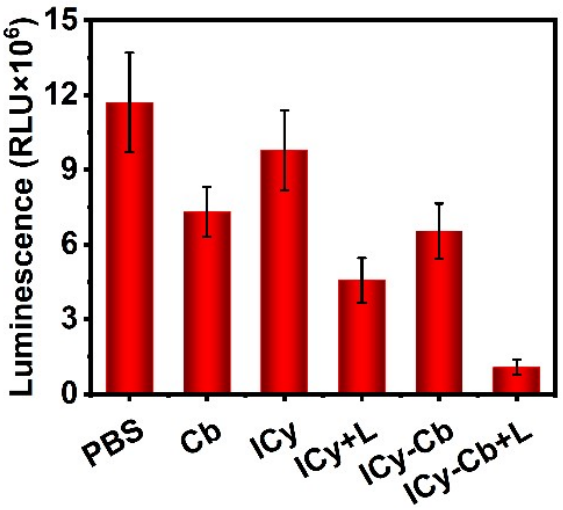


Figure S9. Quantitative assessment of 4T1 tumor spheroid viability using CellTiter-Glo 3D assay following various treatment regimens. Spheroids were treated with PBS (control), Cb (10 μM), ICy (10 μM), ICy (10 μM) + light, ICy-Cb (10 μM), or ICy-Cb (10 μM) + light. Light-treated groups were irradiated with 660 nm light (10 mW/cm², 20 min). The graph displays actual luminescence intensity values (RLU, relative light units) that directly correlate with ATP levels and viable cells in the spheroids.

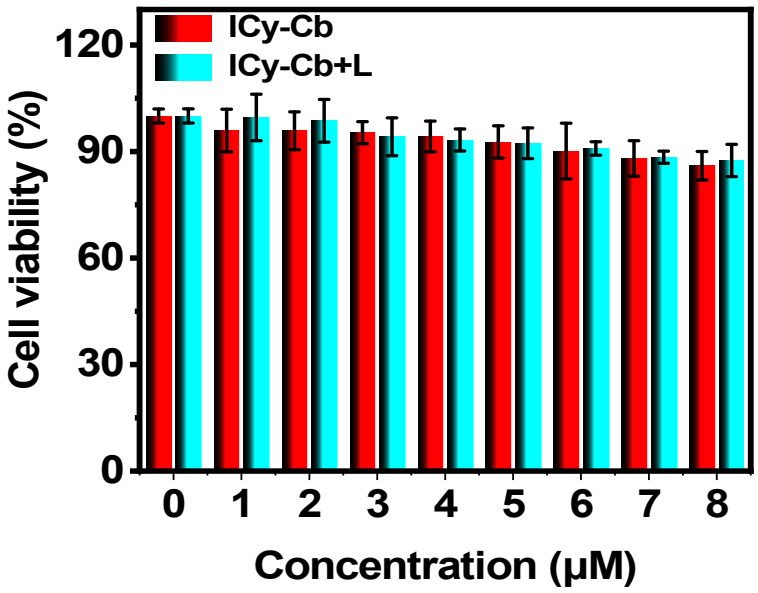


Figure S10. Cytotoxicity of **ICy-Cb** on IOSE80 cells.

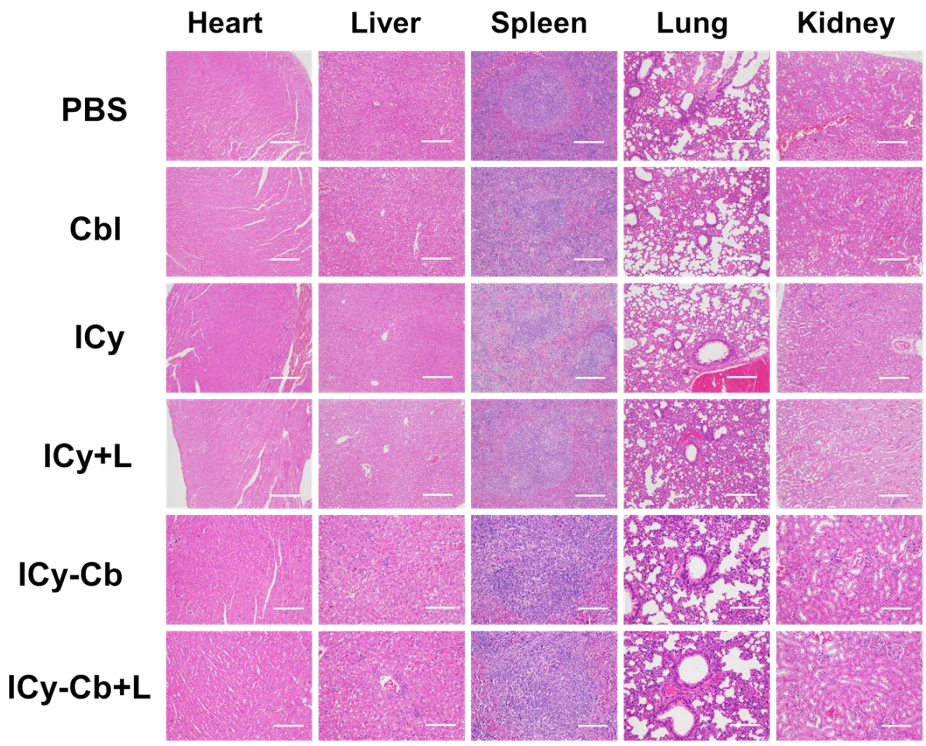


Figure S11. H&E staining of organs from the PBS group and PDT group after 14 days of treatment. Scale bar: 200 μm.

4. ^{1}H NMR, ^{13}C NMR and HRMS spectra.

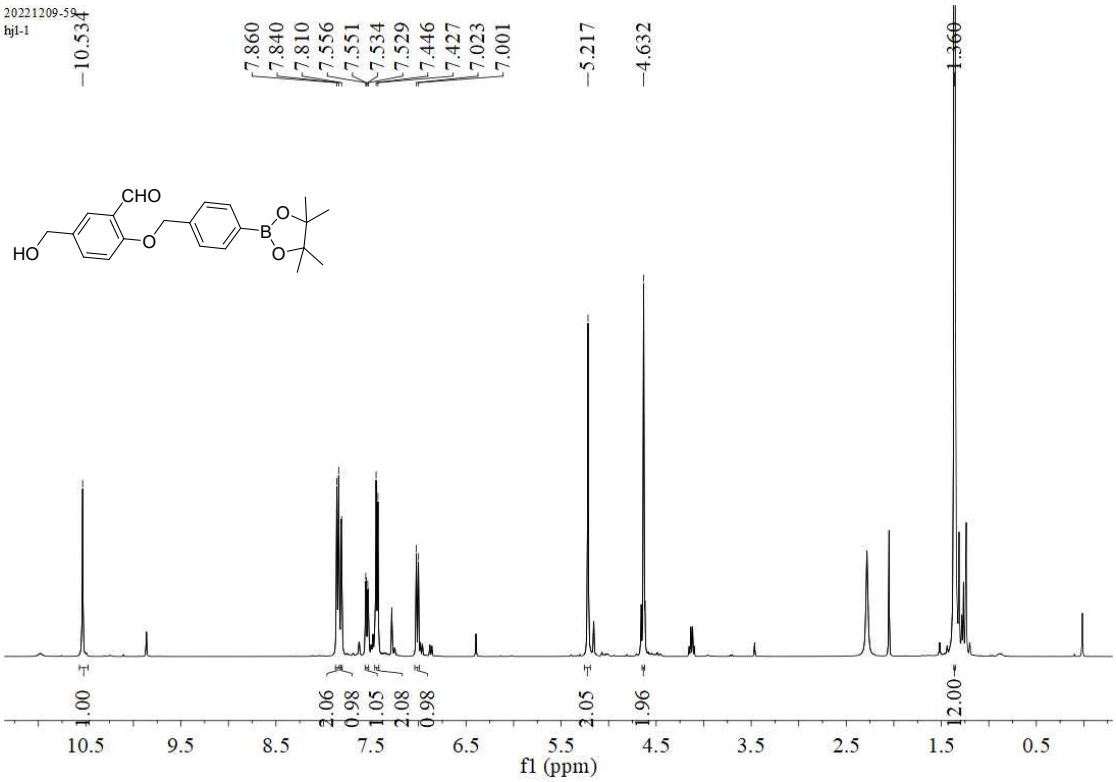


Figure S12. ^{1}H NMR spectrum of Compound **4**.

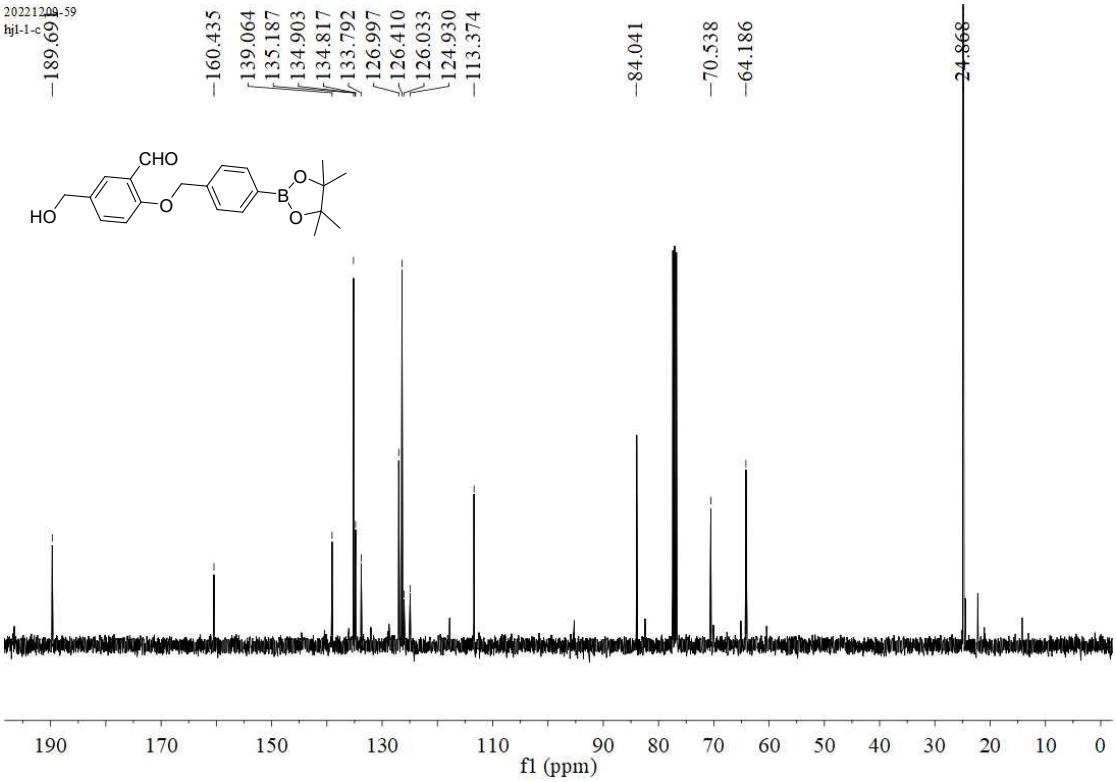


Figure S13. ^{13}C NMR spectrum of Compound **4**.

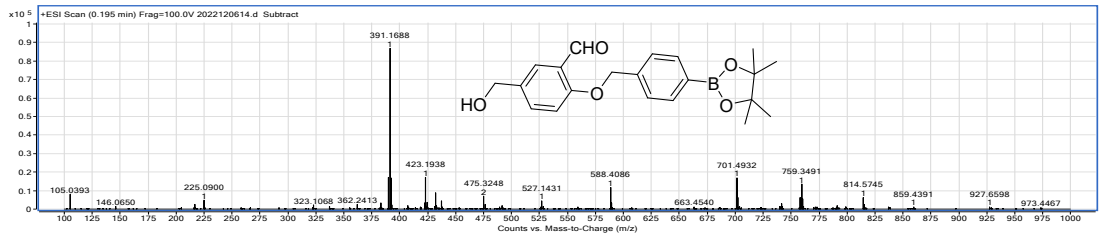


Figure S14. HRMS spectrum of Compound **4**.

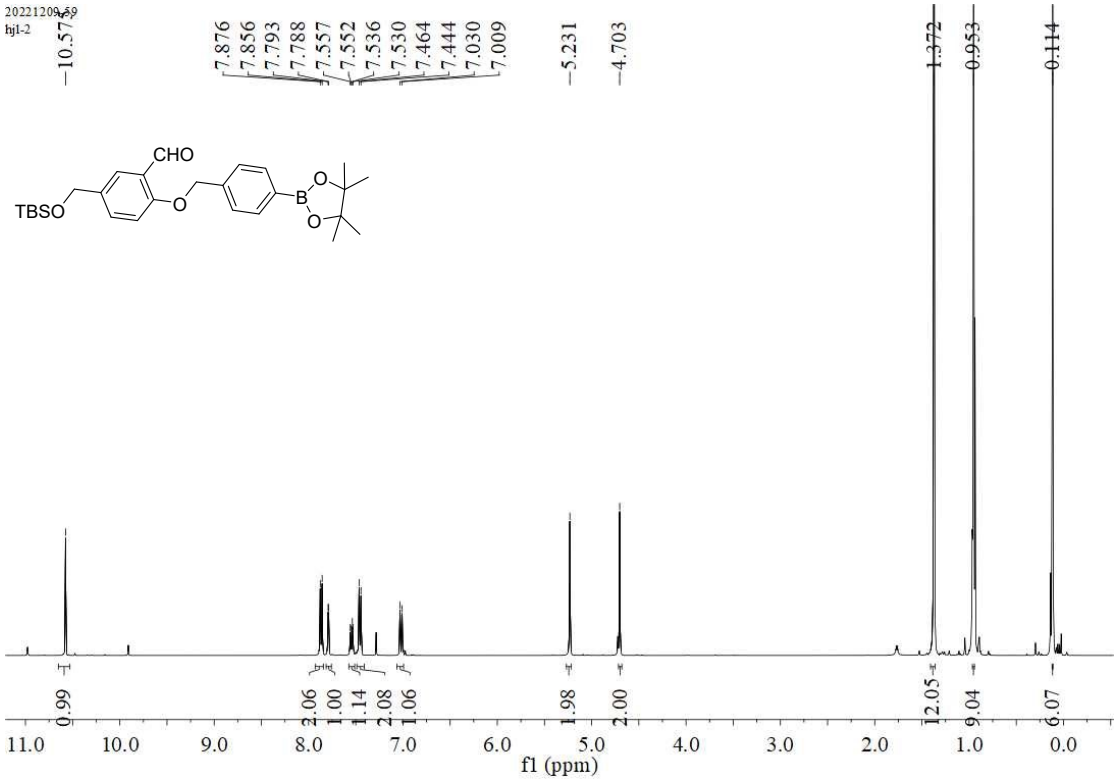


Figure S15. ^{1}H NMR spectrum of Compound **5**.

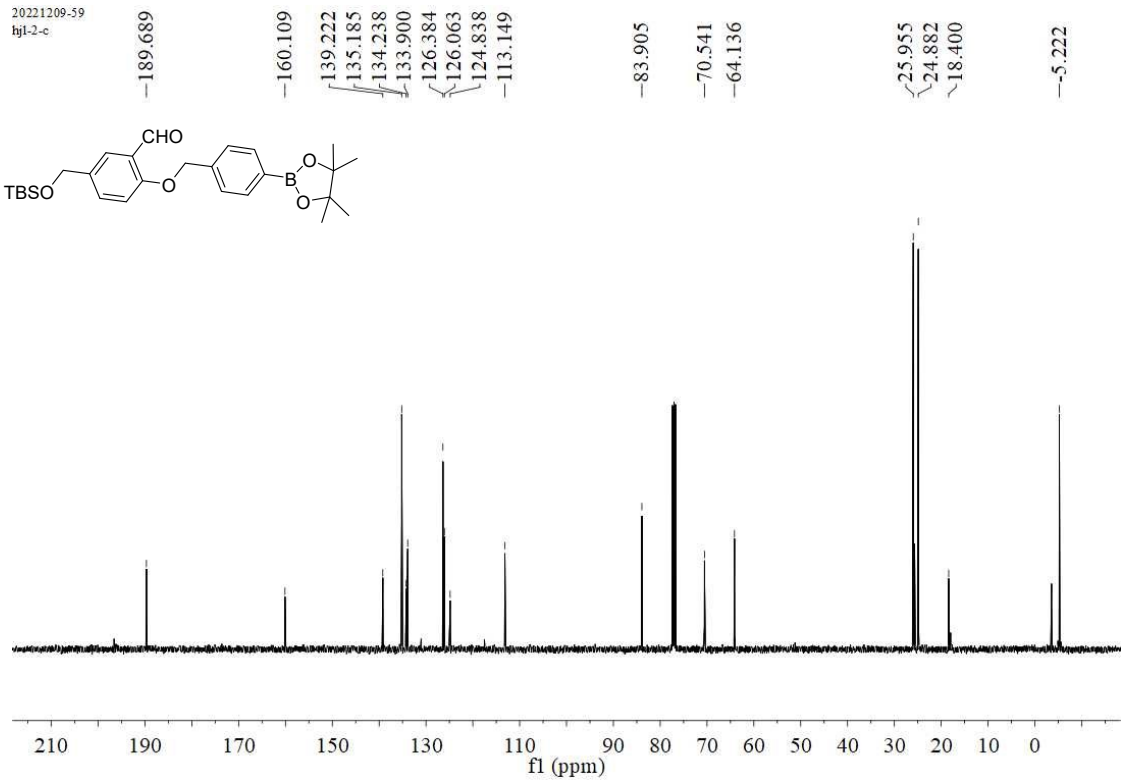


Figure S16. ^{13}C NMR spectrum of Compound **5**.

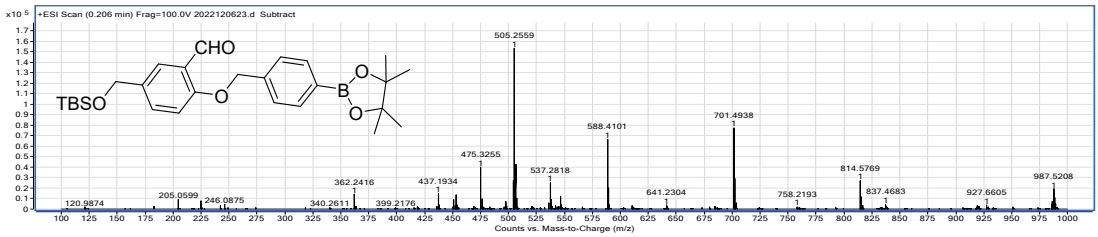


Figure S17. HRMS spectrum of Compound **5**.

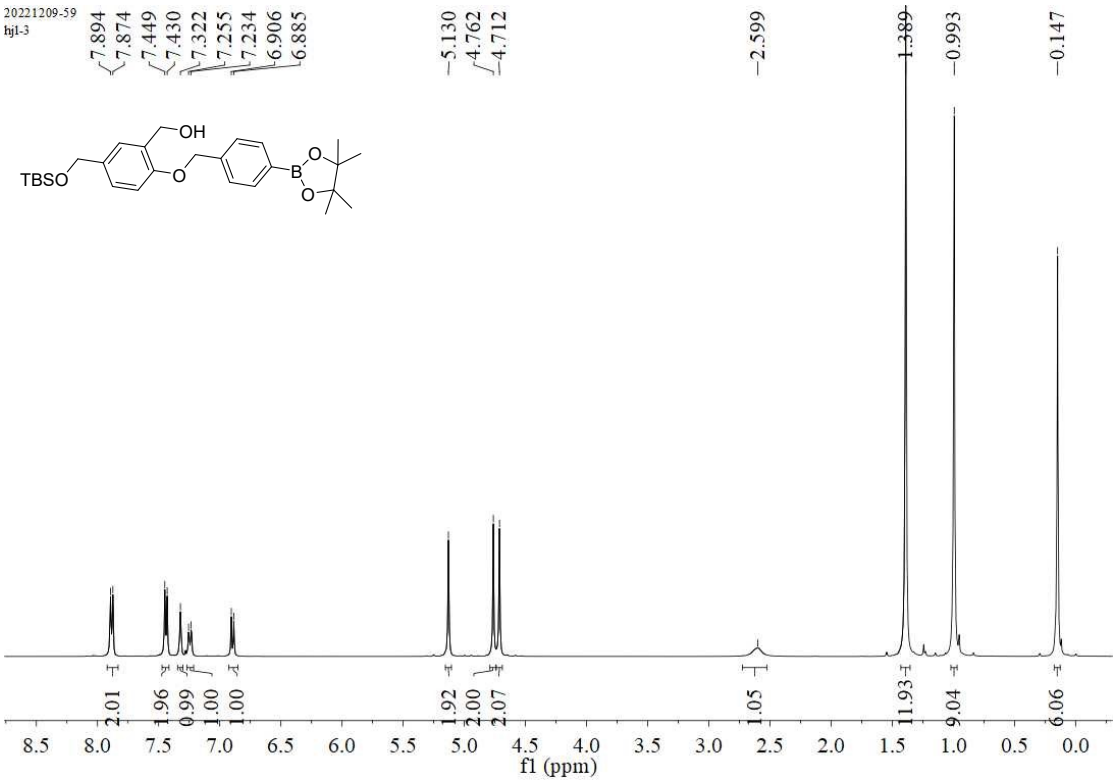


Figure S18. ^{1}H NMR spectrum of Compound **6**.

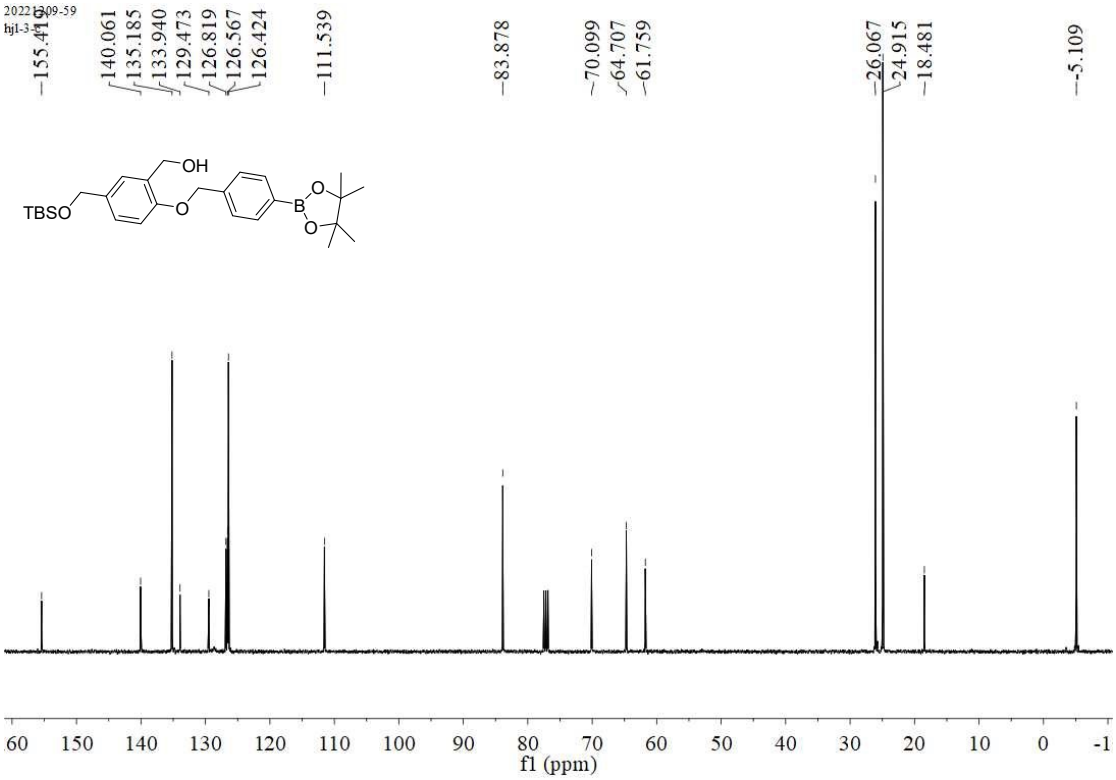


Figure S19. ^{13}C NMR spectrum of Compound **6**.

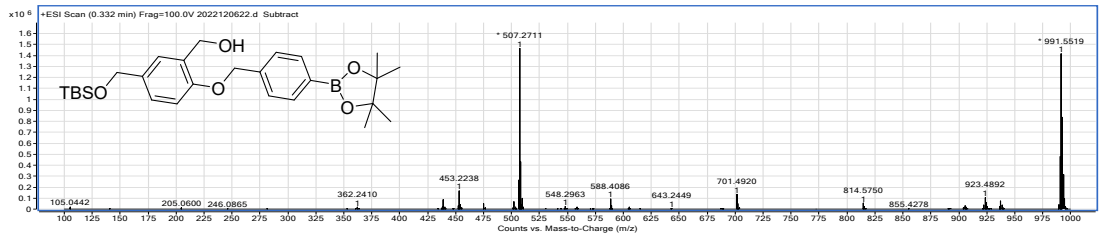


Figure S20. HRMS spectrum of Compound **6**.

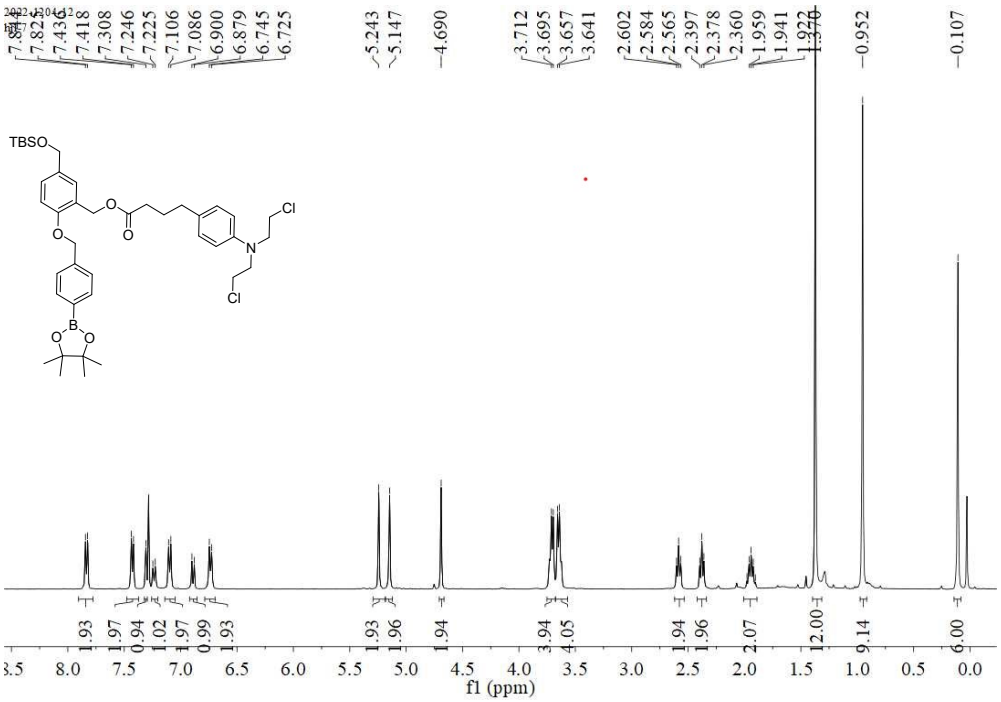


Figure S21. ^{1}H NMR spectrum of Compound **7**.

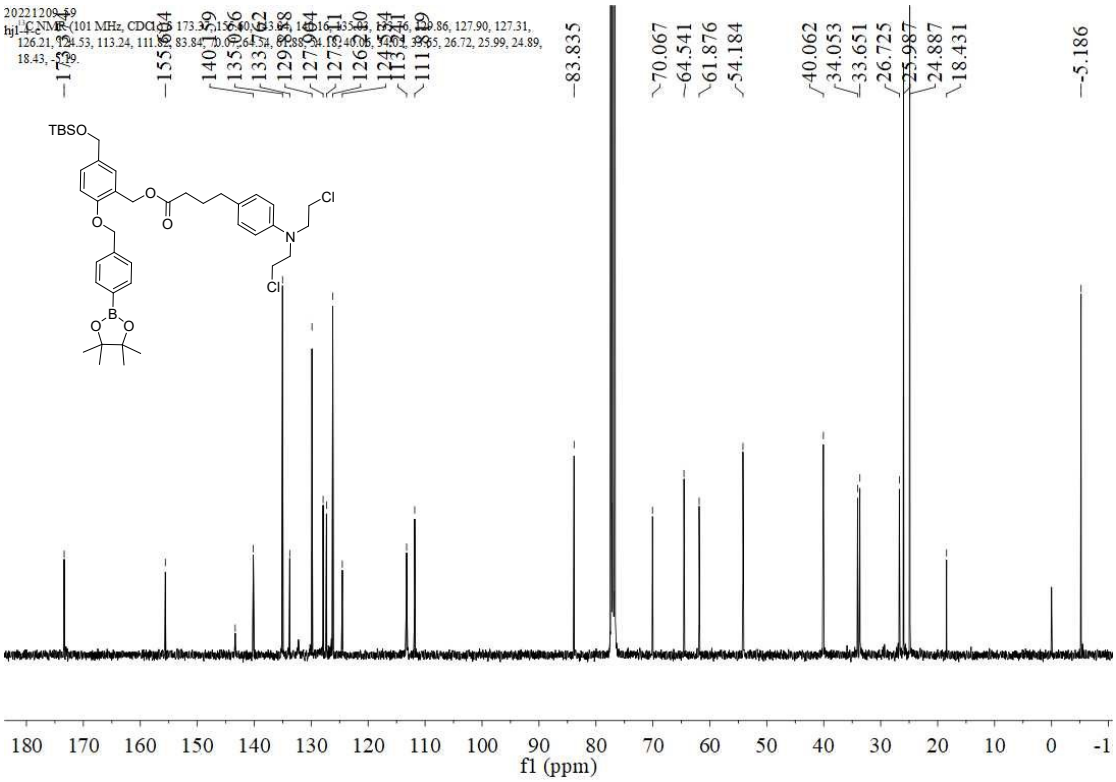


Figure S22. ^{13}C NMR spectrum of Compound **7**.

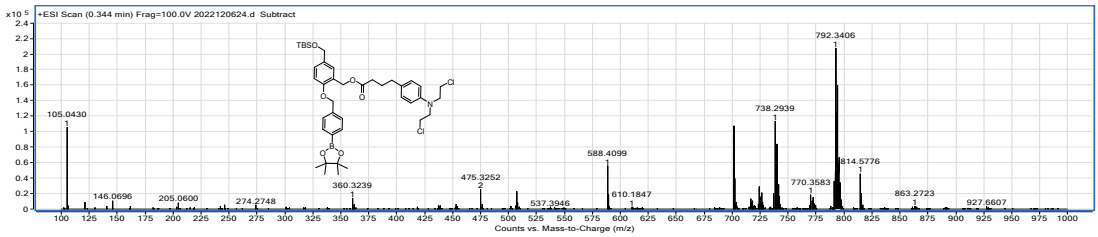


Figure S23. HRMS spectrum of Compound **7**.

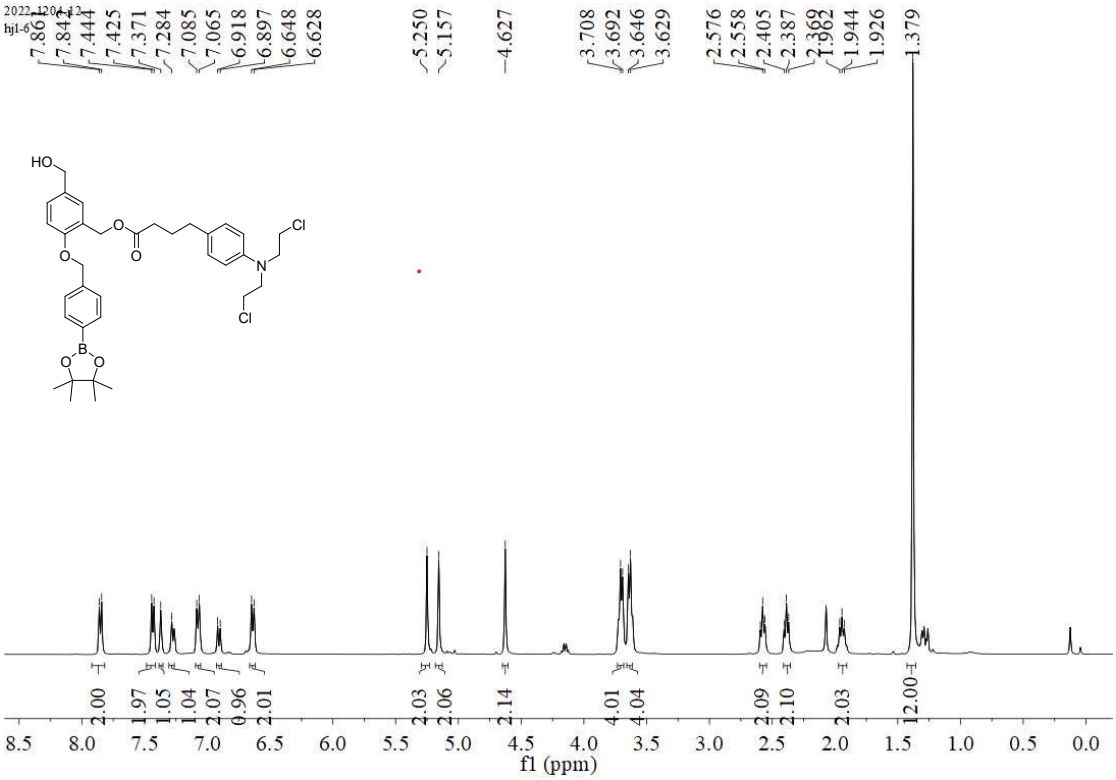


Figure S24. ^{1}H NMR spectrum of Compound **8**.

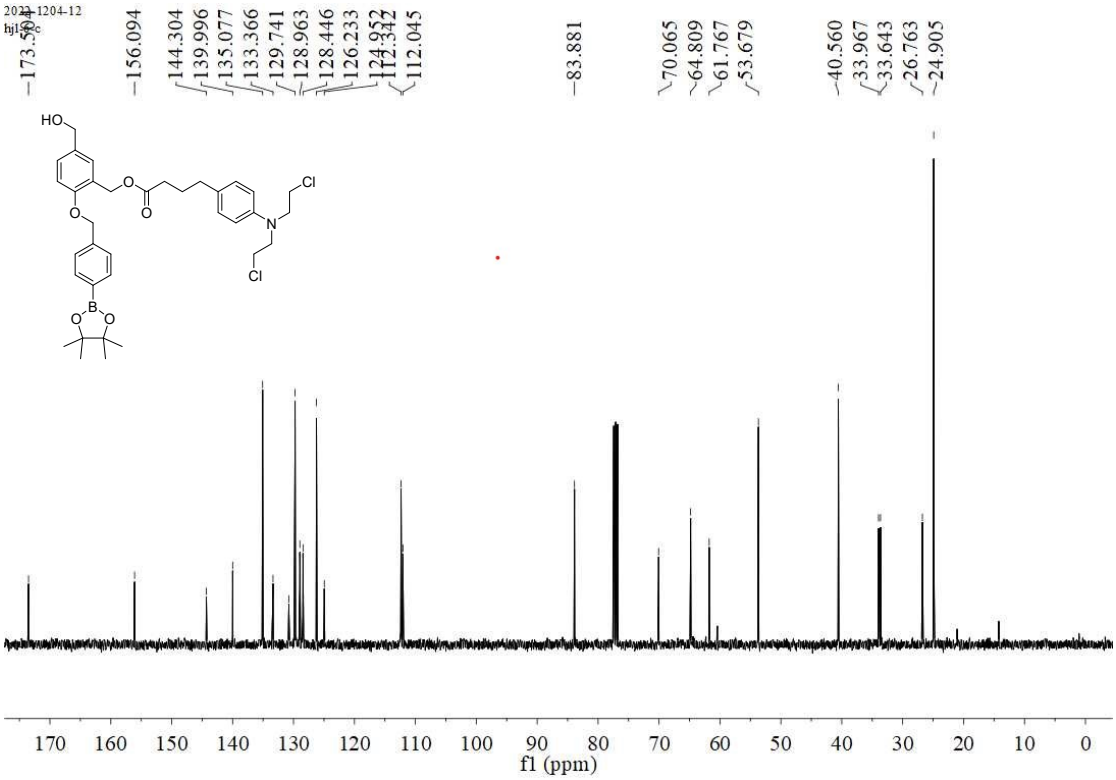


Figure S25. ^{13}C NMR spectrum of Compound **8**.

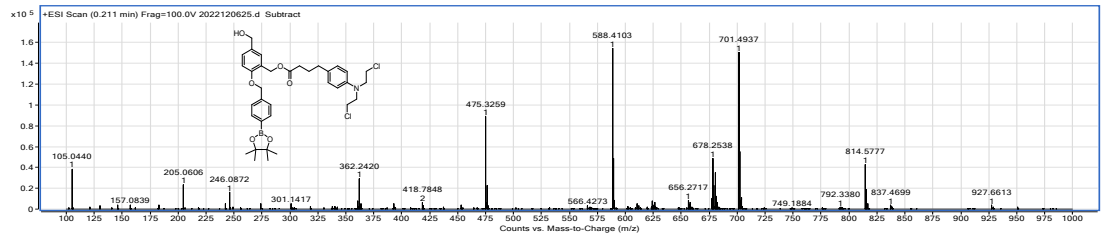


Figure S26. HRMS spectrum of Compound **8**.

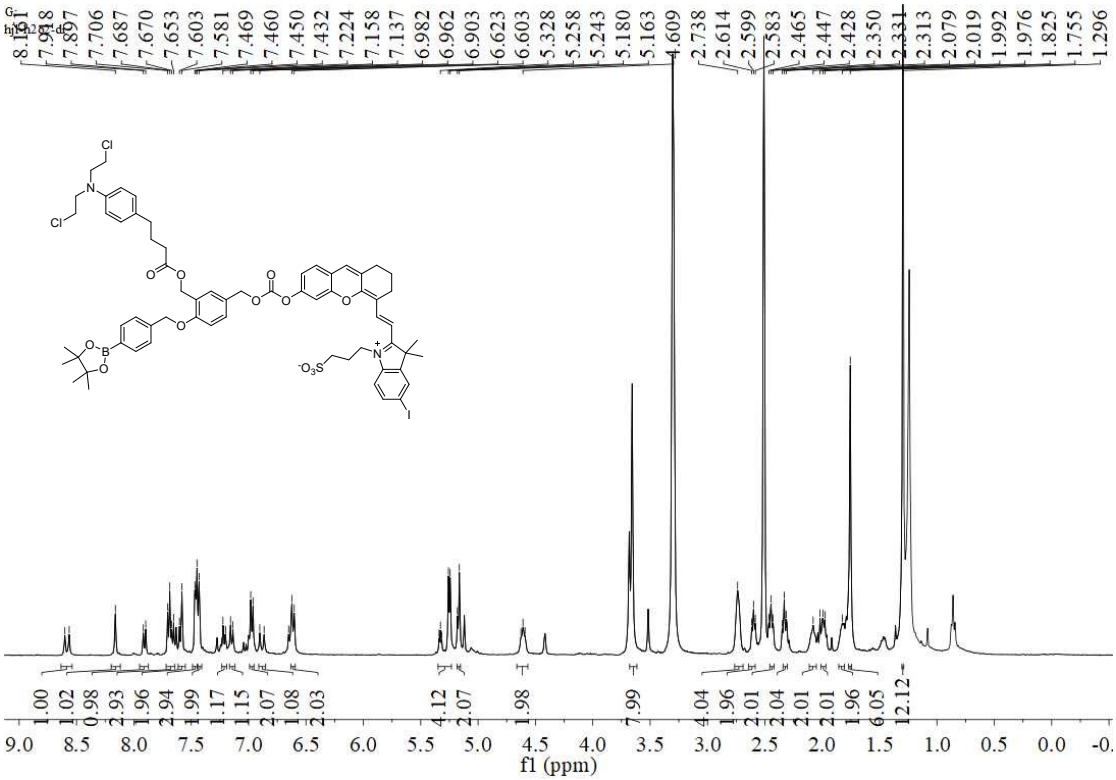


Figure S27. ^{1}H NMR spectrum of Compound **ICy-Cb**.

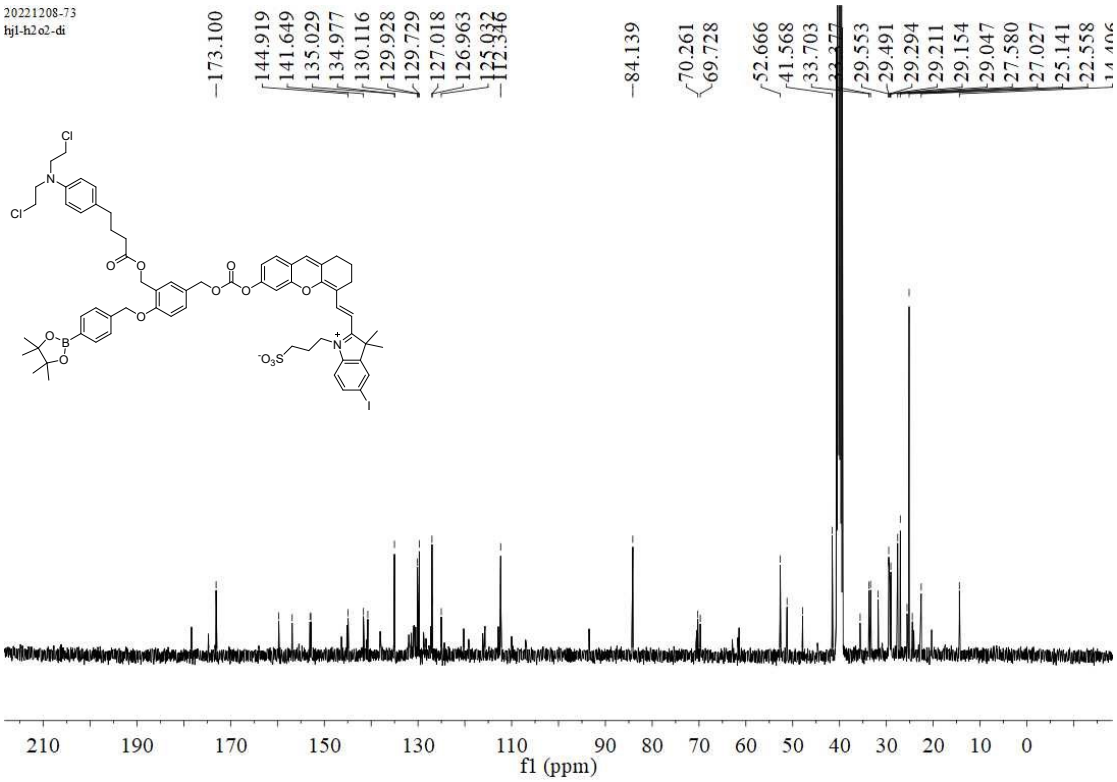


Figure S28. ^{13}C NMR spectrum of Compound **ICy-Cb**.

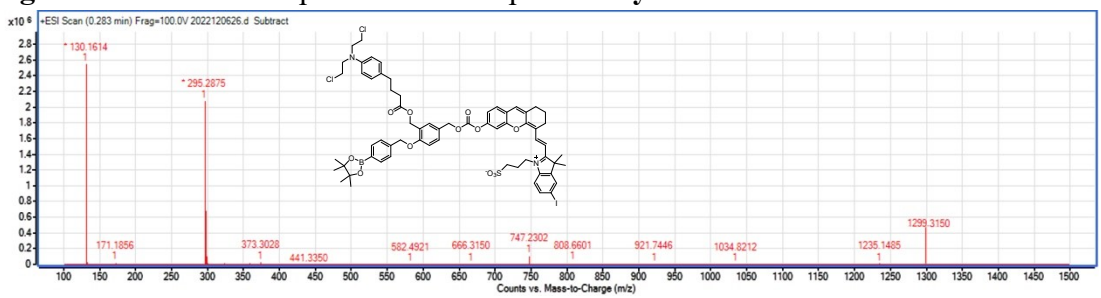


Figure S29. HRMS spectrum of Compound **ICy-Cb**.