Electronic Supplementary Information

One-Pot Peptide Cyclisation and Surface Modification of Photosensitiser-Loaded Red Blood Cells for Targeted Photodynamic Therapy

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Contents

Experimental Section

General

All the reactions were performed under an atmosphere of nitrogen. *N*,*N*-Dimethylformamide (DMF), tetrahydrofuran (THF) and toluene were purified by using an INERT solvent purification system. All other solvents and reagents were of HPLC or reagent grade and used as received. All the reactions were monitored by thin layer chromatography (TLC) performed on Merck pre-coated silica gel 60 F254 plates. Chromatographic purification was performed on silica gel (Macherey-Nagel, 230−400 mesh). Compounds **1**,[R1] **6**,[R2] **7**[R3] and **8**[R4] were prepared as described.

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) or a Bruker AVANCE III 500 MHz spectrometer (^{1}H, 500 MHz; ^{13}C, 125.8 MHz) in deuterated solvents. Spectra were referenced internally by using the residual solvent {^{1}H: δ = 7.26 (for $CDCl_3$), 2.50 ppm [for dimethyl sulfoxide (DMSO)-d_6]} or solvent [^{13}C: δ = 77.2 (for $CDCl_3$), 39.5 ppm (for DMSO-d_6)] resonances relative to $SiMe_4$. Electrospray ionisation (ESI) mass spectra were recorded on a Thermo Finnigan MAT 95 XL mass spectrometer. Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectra were recorded on a Bruker Autoflex Speed MALDI-TOF mass spectrometer.

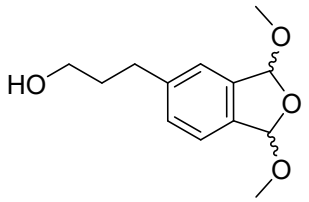
Electronic absorption and steady-state fluorescence spectra were taken on a Cary 5G UV-

Vis-NIR spectrophotometer and a HORIBA FluoroMax-4 spectrofluorometer respectively. To minimise reabsorption of the radiation by the ground-state species, the fluorescence spectra were recorded in very dilute solutions (2 μM in DMF). The fluorescence quantum yields (Φ_F) of the samples in DMF were determined by the equation $\Phi_F(\text{sample}) = (F_{\text{sample}}/F_{\text{ref}})(A_{\text{ref}}/A_{\text{sample}})(n^2_{\text{sample}}/n^2_{\text{ref}})\Phi_F(\text{ref})$,[R5] in which F, A and n are the measured fluorescence (area under the emission band), the absorbance at the excitation wavelength (610 nm) and the refractive index of the solvent respectively. Unsubstituted zinc(II) phthalocyanine (ZnPc) in DMF was used as the reference [$\Phi_F(\text{ref}) = 0.28$].[R6] The singlet oxygen quantum yields (Φ_Δ) were calculated by using 1,3-diphenylisobenzofuran (DPBF) as the singlet oxygen scavenger and ZnPc as the reference [$\Phi_\Delta(\text{ref}) = 0.56$ in DMF].[R7] A solution of DPBF (30 μM) and the photosensitiser (2 μM) in DMF was irradiated with red light from a 100 W halogen lamp after passing through a water tank for cooling and a colour filter with a cut-on wavelength at 610 nm (Newport). The absorption maximum of DPBF at 417 nm was monitored along with the irradiation time. The Φ_Δ values were calculated according to the equation $\Phi_\Delta(\text{sample}) = \Phi_\Delta(\text{ref})[W_{\text{sample}} I_{\text{abs}}(\text{ref})]/[W_{\text{ref}} I_{\text{abs}}(\text{sample})]$, in which W and I are the DPBF photobleaching rate and the rate of light absorption respectively.[R8]

Reverse-phase HPLC separation was performed on a XBridge BEH300 C18 column (5 μm, 4.6 mm × 150 mm) at a flow rate of 1 mL min^{-1} for analytical purpose or on a XBridge BEH300 Prep C18 column (5 μm, 10 mm × 250 mm) at a flow rate of 3 mL min^{-1} for

preparative purpose by using a Waters system equipped with a Waters 1525 binary pump and a Waters 2998 photodiode array detector. The condition used for the analysis was set as follows: solvent A = 0.1% trifluoroacetic acid (TFA) in acetonitrile and solvent B = 0.1% TFA in deionised water; gradient: 5% A + 95% B in the first 5 min, then changed to 15% A + 85% B in 10 min, further changed to 100% A + 0% B in 30 min, maintained under this condition for 5 min, changed to 0% A + 100% B in 5 min, and maintained under this condition for further 5 min.

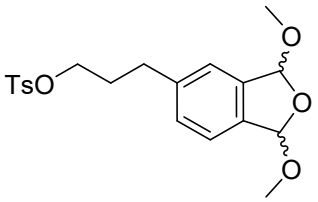
Preparation of compound 2



Methyl-ester acetal-protected phthalaldehyde **1**[R1] (0.5 g, 1.9 mmol) was treated with $LiAlH_4$ (75 mg, 2.0 mmol) in THF (20 mL) at 0 °C. The reaction mixture was kept stirring for 3 h and then quenched by the addition of methanol. The mixture was filtered through celite and the filtrate was evaporated under reduced pressure. Water (20 mL) was then added to the residue, and the crude product was extracted with ethyl acetate (20 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (1:1 v/v) as eluent to afford **2** (0.42 g, 94 %). ^{1}H NMR (400 MHz, $CDCl_3$):

δ 7.27-7.32 (m, 2 H, ArH), 7.23 (s, 1 H, ArH), 6.29-6.31 (m, 1 H, CH), 6.04 (d, J = 8.4 Hz, 1 H, CH), 3.67 (q, J = 5.6 Hz, 2 H, OCH_2), 3.42-3.46 (m, 6 H, CH_3), 2.77 (t, J = 7.6 Hz, 2 H, CH_2), 1.89 (quintet, J = 6.4 Hz, 2 H, CH_2), 1.31 (t, J = 4.8 Hz, 1 H, OH). $^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 144.0, 143.9, 138.8, 138.7, 136.1, 136.0, 130.2, 130.1, 122.8, 122.7, 106.5, 105.4, 61.9, 54.3, 54.1, 34.1, 31.9. HRMS (ESI): *m/z* calcd for $C_{13}H_{18}NaO_4$ $[M+Na]^+$, 261.1097; found, 261.1096.

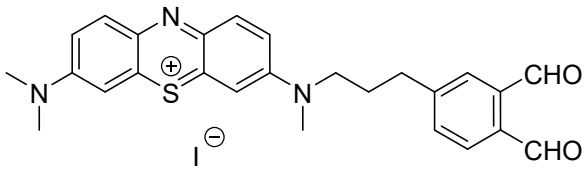
Preparation of compound 3



A mixture of **2** (0.4 g, 1.7 mmol) and Et_3N (0.5 mL, 3.6 mmol) in CH_2Cl_2 (20 mL) was stirred at 0 °C. *p*-Toluenesulfonyl chloride (0.4 g, 2.1 mmol) was then slowly added to the reaction mixture. After stirring overnight, the mixture was evaporated under reduced pressure. Water (20 mL) was then added to the residue, and the crude product was extracted with ethyl acetate (20 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (3:1 v/v) as eluent to afford **3** (0.56 g, 85 %). ^1H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, J = 8.4 Hz, 2 H, ArH), 7.35 (d, J = 8.4 Hz, 2 H, ArH), 7.28 (s, 1 H, ArH), 7.13-7.15 (m, 2 H, ArH), 6.25-6.28 (m, 1 H, CH), 6.01 (d, J = 13.6

Hz, 1 H, CH), 4.02 (t, J = 6.4 Hz, 2 H, OCH_2), 3.42-3.44 (m, 6 H, CH_3), 2.71 (t, J = 7.6 Hz, 2 H, CH_2), 2.46 (s, 3 H, CH_3), 1.96 (quintet, J = 7.2 Hz , 2 H, CH_2). $^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 144.9, 142.7, 142.5, 139.1, 139.0, 136.7, 136.6, 133.2, 130.3, 130.2, 130.0, 128.0, 123.1, 122.8, 106.6, 105.5, 69.5, 54.5, 54.4, 54.3, 31.5, 30.6, 21.8. HRMS (ESI): *m/z* calcd for $C_{20}H_{24}NaO_6S$ $[M+Na]^+$, 415.1186; found, 415.1186.

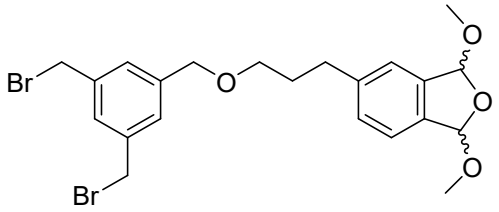
Preparation of MB-OPA



A mixture of **3** (0.5 g, 1.3 mmol) and methylamine (40% in H_2O, 0.2 mL, 1.8 mmol) in methanol (5 mL) and water (5 mL) was stirred at room temperature overnight. After complete consumption of **3** as indicated by TLC, the solvent was evaporated under reduced pressure. The residue was further treated with methylene blue (**4**) (0.5 g, 1.4 mmol) in methanol (10 mL) at room temperature overnight. The solvent was evaporated under reduced pressure. The residue was treated with a mixture of TFA (3 mL) and CH_2Cl_2 (3 mL) at room temperature for 3 h, followed by evaporation under reduced pressure. The residue was dissolved in acetonitrile (5 mL). After filtration, the crude product was purified by HPLC to afford **MB-OPA** (90 mg, 12%) after lyophilisation. ^1H NMR (500 MHz, DMSO-d_6): δ 10.46 (s, 1 H, CHO), 10.43 (s, 1 H, CHO), 7.90-7.96 (m, 3 H, ArH), 7.84 (s, 1 H, ArH), 7.76 (d, J = 7.5 Hz, 1 H, ArH), 7.50-

7.51 (m, 4 H, ArH), 3.80 (t, J = 8.0 Hz, 2 H, CH_2), 3.37 (s, 6 H, CH_3), 3.32 (s, 3 H, CH_3), 2.87 (t, J = 8.0 Hz, 2 H, CH_2), 2.03 (t, J = 8.0 Hz, 2 H, CH_2). $^{13}C\{^1H\}$ NMR (125.8 MHz, DMSO-d_6): δ 193.1, 192.8, 158.0, 157.7, 154.0, 153.4, 148.2, 137.9, 136.5, 135.2, 135.0, 134.4, 133.7, 133.6, 130.8, 129.6, 119.3, 119.0, 106.8, 52.4, 41.2, 32.0, 30.7. HRMS (ESI): *m/z* calcd for $C_{27}H_{30}N_3O_3S$ $[M+CH_3OH]^+$, 476.2002; found, 476.2001.

Preparation of compound 5



A mixture of 1,3,5-tris(bromomethyl)benzene (0.28 g, 0.8 mmol) and sodium hydride (60% suspension in mineral oil, 25.2 mg, 0.6 mmol) in THF (5 mL) was stirred at 0 °C. A solution of compound **6** (75.0 mg, 0.3 mmol) in THF (5 mL) was added to the suspension in dropwise. The reaction mixture was kept stirring at 0 °C for 1 h and then slowly warmed to room temperature for overnight. The solvent was evaporated under reduced pressure. Water (20 mL) was then added to the residue, and the crude product was extracted with CH_2Cl_2 (20 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4. After evaporating the solvent under reduced pressure, the residue was purified by column chromatography on silica gel with hexane/ethyl acetate (3:1 v/v) as eluent to afford **6** (56.0 mg, 35 %). ^1H NMR (400 MHz, $CDCl_3$): δ 7.22-7.34 (m, 6 H, ArH), 6.29-6.30 (m, 1 H, CH), 6.04 (d, J = 6.8 Hz, 1 H, CH),

4.48 (s, 6 H, CH_2), 3.49 (t, J = 6.4 Hz, 2 H, OCH_2), 3.43-3.45 (m, 6 H, CH_3), 2.78 (t, J = 7.6 Hz, 2 H, CH_2), 1.92-1.99 (m, 2 H, CH_2). $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): δ 144.0, 143.9, 140.1, 138.9, 138.8, 138.6, 136.2, 136.1, 130.3, 130.2, 128.8, 128.1, 122.9, 122.8, 122.8, 122.7, 106.6, 105.5, 72.2, 69.7, 54.3, 54.2, 32.8, 32.3, 31.3, 31.0. HRMS (ESI): *m/z* calcd for $C_{22}H_{26}Br_2NaO_4$ $[M+Na]^+$, 537.0071; found, 537.0081.

Preparation of RGD

RGD (AcNH-CRGDfC-$CONH_2$) peptide was synthesised manually by using a modified 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis protocol with the commercially available *N*-α-Fmoc-protected amino acids. The rink amide resin was used as the solid support. A solution of 20% piperidine in DMF was used to remove the Fmoc protecting group, and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) was used as the carboxyl group activating agent. An excess of the Fmoc-protected amino acids (4 equiv), HATU (4 equiv) and *N,N*-diisopropylethylamine (8 equiv) in DMF were used for each coupling at room temperature. For *N*-terminal acetylation, CH_2Cl_2, pyridine and acetic anhydride (2:1:1 v/v/v) were added and the mixture was stirred at room temperature for 30 min. After the final coupling and Fmoc deprotection, the resin was treated with a solution containing 95% TFA, 2.5% triisopropylsilane and 2.5% CH_2Cl_2 for 1 h to detach the peptide from the resin and remove the protecting groups. The resin was removed

by filtration and the filtrate was precipitated by the addition of diethyl ether. After centrifugation, the supernatant was removed. The solid was redissolved in DMSO and then precipitated again by the addition of diethyl ether. The crude peptide was further purified with HPLC. HRMS (MALDI-TOF): *m/z* calcd for $C_{29}H_{45}N_{10}O_9S_2$ $[M+H]^+$: 741.2807, found: 741.2610.

Preparation of cRGD-OPA

Compound **5** was treated with 50% TFA in H_2O for 1 h at room temperature. After the *in situ* deprotection, it was treated with RGD peptide (1 mM) in borate buffer (pH 8.5) for 2 h at room temperature. The product was purified by HPLC. HRMS (MALDI-TOF): *m/z* calcd for $C_{49}H_{63}N_{10}O_{12}S_2$ $[M+H]^+$: 1047.4063, found: 1047.4093.

Preparation of photosensitiser-loaded RBCs

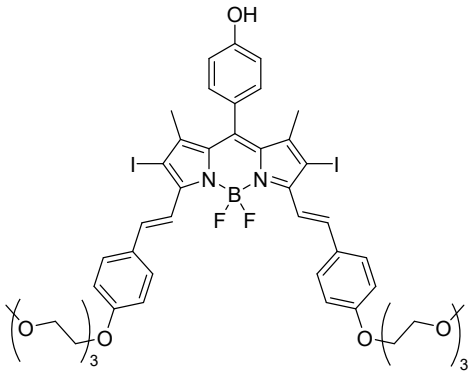
Rabbit RBCs were obtained from the Laboratory Animal Services Centre of The Chinese University of Hong Kong. The blood was centrifuged at 8000 rpm for 10 min and the supernatant was discarded. The remaining red pellet was further washed with phosphate-buffered saline (PBS) for 3 times. The RBCs were swollen in a hypotonic NaCl solution (0.65% w/v) at 4 ^oC for 5 min to loosen the membrane. After centrifugation at 8000 rpm for 5 min, different concentrations of ZnPc, **dsBDP(TEG)$_2$** or **dsBDP(COOH)$_2$** in DMSO (10 μL) were

added into the recovered RBC suspension (0.9 mL) at 4 °C for 10 min with an orbital shaker (50 rpm). Afterwards, upon treatment with a hypertonic NaCl solution (9% w/v) (0.1 mL) for 30 min at room temperature, the RBC membrane was resealed. The suspension was subjected to centrifugation at 8000 rpm for 5 min. The residue was washed further with Hank's Balanced Salt Solution (HBSS) (ThermoFisher Scientific, cat. no. 14025092) twice to afford the photosensitiser-loaded RBCs.

Conjugation of cRGD-OPA with RBC

A solution of **cRGD-OPA** in borate buffer was added in dropwise into the RBC suspension in HBSS. The mixture was gently shaken for 30 min at room temperature with an orbital shaker (50 rpm). The peptide-modified RBCs were then washed by HBSS for 3 times.

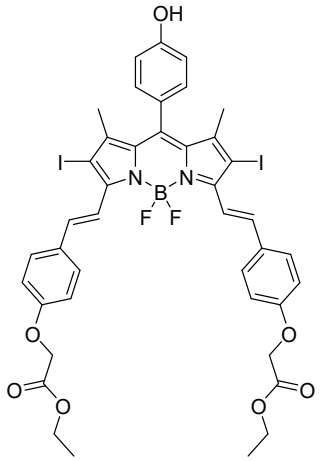
Preparation of dsBDP(TEG)$_2$



Diiodo BODIPY **6**^{R2} (35 mg, 0.06 mmol) and benzaldehyde **7**^{R3} (95 mg, 0.36 mmol) were dissolved in toluene (30 mL). Acetic acid (0.35 mL), piperidine (0.42 mL) and a small amount

of $Mg(ClO_4)_2$ were then added. The mixture was heated under reflux with a Dean−Stark trap. After consumption of **6** as indicated by TLC, the mixture was cooled to room temperature, and the solvent was evaporated under reduced pressure. Water was then added to the residue, and the crude product was extracted with CH_2Cl_2 (50 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with $CHCl_3/CH_3OH$ (60:1 v/v) as eluent to afford **dsBDP(TEG)$_2$** (44 mg, 68 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, *J* = 16.4 Hz, 2 H, C=CH), 7.51-7.58 (m, 6 H, C=CH and ArH), 7.05 (d, *J* = 8.4 Hz, 2 H, ArH), 6.92-6.96 (m, 6 H, ArH), 4.19 (t, *J* = 4.8 Hz, 4 H, OCH_2), 3.89 (t, *J* = 4.8 Hz, 4 H, OCH_2), 3.75-3.78 (m, 4 H, OCH_2), 3.66-3.72 (m, 8 H, OCH_2), 3.56-3.59 (m, 4 H, OCH_2), 3.39 (s, 6 H, OCH_3), 1.41 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): δ 159.9, 157.5, 157.4, 150.3, 145.8, 139.0, 138.8, 133.5, 130.0, 129.9, 129.8, 129.3, 117.0, 116.5, 115.1, 72.0, 71.0, 70.8, 70.7, 69.8, 67.6, 59.2, 17.8. HRMS (ESI): *m/z* calcd for $C_{47}H_{53}BF_2I_2N_2NaO_9$ $[M+Na]^+$, 1115.1802; found, 1115.1806.

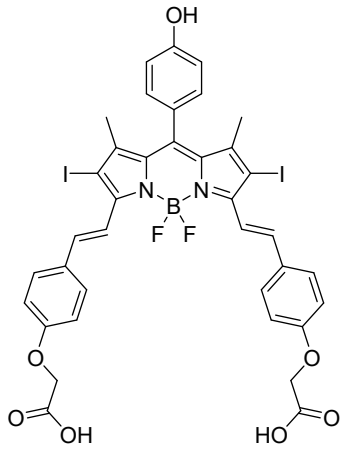
Preparation of BODIPY 9



Diiodo BODIPY **6**^{R2} (35 mg, 0.06 mmol) and benzaldehyde **8**^{R4} (74 mg, 0.36 mmol) were dissolved in toluene (30 mL). Acetic acid (0.35 mL), piperidine (0.42 mL) and a small amount of $Mg(ClO_4)_2$ were then added. The mixture was heated under reflux with a Dean−Stark trap. After consumption of **6** as indicated by TLC, the mixture was cooled to room temperature, and the solvent was evaporated under reduced pressure. Water was then added to the residue, and the crude product was extracted with CH_2Cl_2 (50 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with $CHCl_3/CH_3OH$ (80:1 v/v) as eluent to afford BODIPY **9** (42 mg, 73 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, J = 16.8 Hz, 2 H, C=CH), 7.54-7.62 (m, 6 H, C=CH and ArH), 7.11 (d, J = 8.0 Hz, 2 H, ArH), 6.92-6.97 (m, 6 H, ArH), 4.69 (s, 4 H, OCH_2COO), 4.30 (q, J = 7.2 Hz, 4 H, OCH_2), 1.50 (s, 6 H, CH_3), 1.33 (t, J = 7.2 Hz, 6 H, CH_3). ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 168.8, 158.8, 156.8, 150.3, 145.8, 138.9, 138.7, 133.3, 130.6, 129.8, 129.3, 127.4, 117.3, 116.4, 115.0, 114.3,

82.7, 65.4, 61.6, 17.7, 14.2. HRMS (ESI): *m/z* calcd for $C_{41}H_{37}BF_2I_2N_2NaO_7$ $[M+Na]^+$, 995.0651; found, 995.0651.

Preparation of dsBDP(COOH)$_2$



BODIPY **9** (40 mg, 0.04 mmol) was dissolved in THF (10 mL). 4 M NaOH (aq) (10 mL) was then added and the mixture was stirred at room temperature for 4 h. The pH of the mixture was then adjusted to 5 by adding 4 M HCl solution slowly. The volatiles were evaporated under reduced pressure. The product was extracted with $CHCl_3$ (20 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure to afford **dsBDP(COOH)$_2$** (30 mg, 80 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.04 (d, *J* = 16.4 Hz, 2 H, C=CH), 7.57 (d, *J* = 8.4 Hz, 4 H, ArH), 7.42 (d, *J* = 16.4 Hz, 2 H, C=CH), 7.20 (d, *J* = 8.4 Hz, 2 H, ArH), 7.02 (d, *J* = 8.4 Hz, 4 H, ArH), 6.96 (d, *J* = 8.4 Hz, 2 H, ArH), 4.73 (s, 4 H, OCH_2), 1.50 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (125.8 MHz, DMSO-d_6): δ 170.4, 159.7, 159.1, 150.1, 146.1, 140.6, 138.9, 133.5, 130.0, 129.5, 129.3, 124.9, 116.9, 116.8, 115.8,

84.5, 65.2, 17.7. HRMS (ESI): *m/z* calcd for $C_{37}H_{27}BF_2I_2N_2O_7$ $[M-2H]^{2-}$, 456.9993; found, 456.9995.

Scanning electron microscopic studies

Glass coverslips (12-mm) were first treated with 10% poly-D-lysine solution in PBS at room temperature for 30 min and then rinsed with water twice. The RBCs, after being washed with PBS, were immediately fixed with 1% glutaraldehyde in PBS at 4 ^{o}C for 30 min. The cells were centrifuged at 8000 rpm for 5 min. After removing the supernatant, the cells were washed with PBS twice and then added on poly-D-lysine-coated coverslips for 1 h. The coverslips were rinsed with PBS twice to remove the non-adhered RBCs. The cells were then incubated in 0.1 M cacodylate buffer at room temperature for 10 min and post-fixed with 1% osmium tetroxide in PBS at room temperature for 1 h. After being rinsed with water twice, the samples were dehydrated in an alcohol series and incubated twice in hexamethyldisilazane for 30 min. The samples were then dried under the hood and coated with a layer of gold/palladium using an Edwards S150B sputter coater before being viewed with a FEI Prisma E scanning electron microscope.

Cell lines and culture conditions

AsPC-1 human pancreatic carcinoma cells (ATCC, no. CRL-1682) were maintained in Roswell

Park Memorial Institute (RPMI) 1640 medium (Invitrogen, cat. no. 23400-021) supplemented with fetal bovine serum (FBS) (10%; ThermoFisher Scientific, cat. no. 10270-106) and penicillin-streptomycin (100 units mL^{-1} and 100 μg mL^{-1} respectively). U87-MG human glioblastoma cells (ATCC, no. HTB-14) and MCF-7 human breast adenocarcinoma cells (ATCC, no. HTB-22) were maintained in minimum essential medium (MEM) (Sigma-Aldrich, no. M5650) supplemented with FBS (10%) and penicillin-streptomycin (100 units mL^{-1} and 100 μg mL^{-1} respectively). A549 human lung carcinoma cells (ATCC, no. CCL-185) and HEK-293 human embryonic kidney cells (ATCC, no. CRL-1573) were maintained in Dulbecco's modified Eagle medium (DMEM) (ThermoFisher Scientific, cat. no. 12100-046) supplemented with FBS (10%) and penicillin-streptomycin (100 units mL^{-1} and 100 μg mL^{-1} respectively). All the cells were grown at 37 °C in a humidified 5% CO_2 atmosphere.

Haemolysis assay

A suspension of **BDP@cRGD-RBC** in PBS (100 μL) was added to each well of 96-multiwell plates and irradiated by light for different periods of time. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on at $\lambda = 610$ nm. The fluence rate ($\lambda > 610$ nm) was 23 mW cm^{-2}. The RBCs suspension was centrifuged at 1000 rpm for 5 min. Aliquots (50 μL) of the supernatant were transferred to other 96-multiwell plates. The haemoglobin released was monitored at 450 nm using a microplate reader (Tecan

Spark 10M). The native RBC suspension in PBS was used as a negative control. The absorbance of the wells with RBCs lysed with 0.5% Triton X-100 was taken as 100% haemolysis. The percentage of haemolysis was calculated using the following equation:

$$\text{Haemolysis } \% = \frac{A_{450}\, in\ solution\ - A_{450}\, in\ PBS}{A_{450}\, in\ 0.5\ \%\ Triton\ X-100\ - A_{450}\, in\ PBS} \times\ 100$$

Confocal fluorescence microscopic studies

Approximately 2×10^5 cells in the culture medium (2 mL) were seeded on a confocal dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. For the cell surface conjugation, **MB-OPA** was first dissolved in DMSO and further diluted with a serum-free medium to 2 μM. The AsPC-1 cells, after being rinsed with PBS, were incubated with **MB-OPA** at 37 °C for 15 min. After the incubation, the solution was then removed, and the cells were rinsed with PBS twice. For the binding study of RBCs, approximately 3×10^6 cells of **BDP@cRGD-RBC** or **BDP@RBC** were incubated with the U87-MG, A549, MCF-7 or HEK-293 cells in a serum-free medium at 37 °C for 30 min. After the incubation, the solution was removed and the cells were rinsed with PBS twice. For the double staining experiment, U87-MG and A549 cells were pre-stained with CellTracker Green BODIPY Dye (3 μM; ThermoFisher Scientific, cat. no. C2102) at 37 °C for 30 min in a serum-free medium. For the study of photo-induced RBC lysis and photosensitiser release, A549 cells were treated with **BDP@cRGD-RBC** followed by irradiation at ambient temperature. The light source consisted

of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on at λ = 610 nm. The fluence rate (λ > 610 nm) was 23 mW cm^{-2}. The cells were irradiated for different period of time. The confocal images were taken with a Leica TCS SP8 high speed confocal microscope equipped with a solid-state 488 nm and a 638 nm laser. The BODIPY was excited at 638 nm and its fluorescence was monitored at 650–750 nm. The CellTracker Green was excited at 488 nm and its fluorescence was monitored at 510–550 nm. The images were digitised and analysed using a Leica Application Suite X software.

Intracellular ROS generation studies

Approximately 2 × 10^5 cells in the culture medium (2 mL) were seeded on a confocal dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The cells, after being rinsed with PBS, were incubated with approximately 3 × 10^6 cells of **BDP@cRGD-RBC** or **BDP@RBC** in a serum-free medium at 37 °C for 30 min. After the incubation, the solution was removed and the cells were rinsed with PBS twice and then incubated with H_2DCFDA in PBS (10 μM, 1 mL) at 37 °C for 30 min. The cells were rinsed and refilled with PBS before being illuminated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on at λ = 610 nm. The fluence rate (λ > 610 nm) was 23 mW cm^{-2}. Illumination for 10 min led to a total fluence of 14 J cm^{-2}. After illumination, the cells were examined with a Leica TCS SP8 high speed confocal

microscope equipped with a Leica TCS SP8 high speed confocal microscope equipped with a 488 nm laser. The fluorescent product after the oxidation of H_2DCFDA by ROS, namely DCF, was excited at 488 nm and its fluorescence was monitored at 500–550 nm. The images were digitised and analysed using a Leica Application Suite X software.

Live/dead double staining assay

Approximately 2×10^5 cells in the culture medium (2 mL) were seeded on a confocal dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The cells, after being rinsed with PBS, were incubated with approximately 3×10^6 cells of **BDP@cRGD-RBC** or **BDP@RBC** in a serum-free medium at 37 °C for 30 min. After the incubation, the solution was removed and the cells were irradiated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on at $\lambda = 610$ nm. The fluence rate ($\lambda > 610$ nm) was 23 mW cm^{-2}. Illumination for 10 min led to a total fluence of 14 J cm^{-2}. After illumination, the cells were stained with Calcein AM (1 μM) and propidium iodide (PI) (1 μg mL^{-1}) for 30 min in HBSS at 37 °C. The cells were then examined with a Leica TCS SP8 high speed confocal microscope equipped with a 488 nm and a 552 nm laser. The Calcein AM was excited at 488 nm and its fluorescence was monitored at 500–550 nm, while PI was excited at 552 nm and its fluorescence was monitored at 590–630 nm. The images were digitised and analysed using a Leica Application Suite X software.

Photocytotoxicity assay

Approximately 2 × 10^4 U87-MG or MCF-7 cells per well in MEM were inoculated in 96-multiwell plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The cells, after being rinsed with PBS twice, were incubated with 100 μL of different cell numbers of **BDP@cRGD-RBC** for 30 min at 37 °C under 5% CO_2. The cells were then rinsed again with PBS and refed with 100 μL of the culture medium before being illuminated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on at λ = 610 nm. The fluence rate (λ > 610 nm) was 23 mW cm^{-2}. Illumination for 20 min led to a total fluence of 28 J cm^{-2}. Cell viability was determined by means of a colourimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[R9] After illumination, the cells were incubated at 37 °C under 5% CO_2 overnight. A MTT (Sigma) solution in PBS (3 mg mL^{-1}, 50 μL) was added to each well followed by incubation for 4 h under the same environment. DMSO (100 μL) was then added to each well. Solutions in all wells were mixed until homogenous. The absorbance at 490 nm of each well on the plate was taken by a microplate reader (Tecan Spark 10M) at ambient temperature. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of the other wells. The cell viability was then determined by the equation: % viability = $[\Sigma(A_i/A_{control} \times 100)]/n$, where A_i is the absorbance of the i^{th} datum (i = 1, 2, …, n), $A_{control}$ is

the average absorbance of the control wells in which the compound was absent, and *n* (=4) is the number of data points.

Annexin V-GFP/PI co-staining assay

Approximately 2 × 10^5 U87-MG or MCF-7 cells in MEM (2 mL) were seeded on a 6-multiwell plate and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The cells, after being rinsed with PBS, were incubated with 3 × 10^6 cells of **BDP@cRGD-RBC** or **BDP@RBC** in a serum-free medium at 37 °C for 30 min. After the incubation, the cells were rinsed with PBS and refed with 2 mL of the culture medium before being illuminated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on at λ = 610 nm. The fluence rate (λ > 610 nm) was 23 mW cm^{-2}. Illumination for 20 min led to a total fluence of 28 J cm^{-2}. The solutions were then removed, and the cells were rinsed with PBS twice and harvested by 0.25% trypsin-ethylenediaminetetraacetic acid (Invitrogen, 0.2 mL) for 5 min. The activity of trypsin was quenched with a serum-containing medium (0.5 mL), and the mixture was centrifuged at 1500 rpm for 3 min at room temperature. The pellet was then washed with PBS (1.0 mL) and then subjected to centrifugation. The cells were stained with Annexin V-GFP and PI in binding buffer for 15 min and the fluorescence intensities were measured by using a BD FACSVerse flow cytometer (Becton Dickinson) with 10^4 cells counted in each sample. The compounds

were excited by an argon laser at 488 nm and the emitted fluorescence was monitored at 500–530 nm (for Annexin V-GFP) and 570–630 nm (for PI). The data collected were analysed by using the BD FAC-Suite. All experiments were performed in triplicate.

Statistical analysis

Data shown on figures were presented as the means with the SEM or SD. The data were analysed using the Student's t-test with p values < 0.05 considered as significant; *p <0.05; **p < 0.01; and ***p < 0.001. Statistical calculations were performed using a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA).

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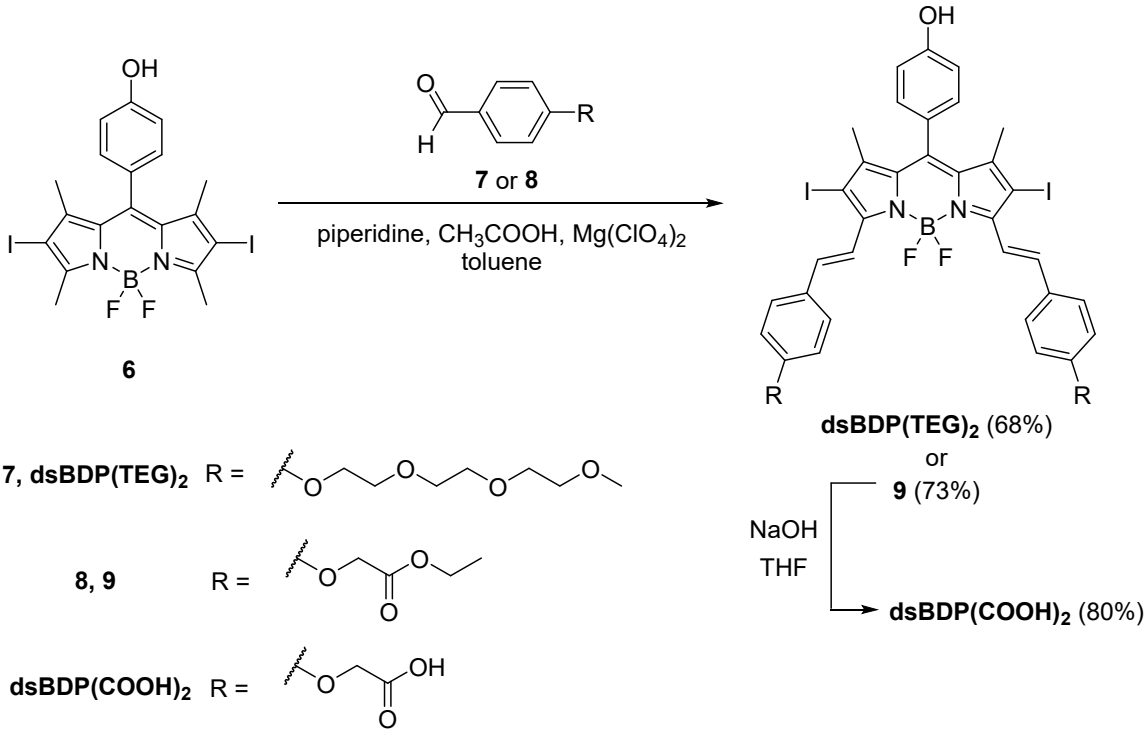

Scheme S1 Synthetic routes for **dsBDP(TEG)$_2$** and **dsBDP(COOH)$_2$**.

Table S1. Electronic absorption and photophysical properties of ZnPc, **dsBDP(TEG)$_2$** and **dsBDP(COOH)$_2$** in DMF.

Compound	λ_{abs} (nm) (log ε)	λ_{em}[a] (nm)	Φ_F	Φ_Δ
ZnPc	341 (4.64), 607 (4.45), 670 (5.30)	676	0.28	0.56
dsBDP(TEG)$_2$	378 (4.53), 450 (4.10), 611 (4.42), 660 (4.81)	691	0.22[b]	0.54[c]
dsBDP(COOH)$_2$	321 (4.40), 382 (4.53), 458 (4.17), 616 (4.45), 665 (4.79)	700	0.20[b]	0.52[c]

[a] Excited at 610 nm. [b] Using ZnPc as the reference (Φ_F = 0.28 in DMF). [c] Using ZnPc as the reference (Φ_Δ = 0.56 in DMF).

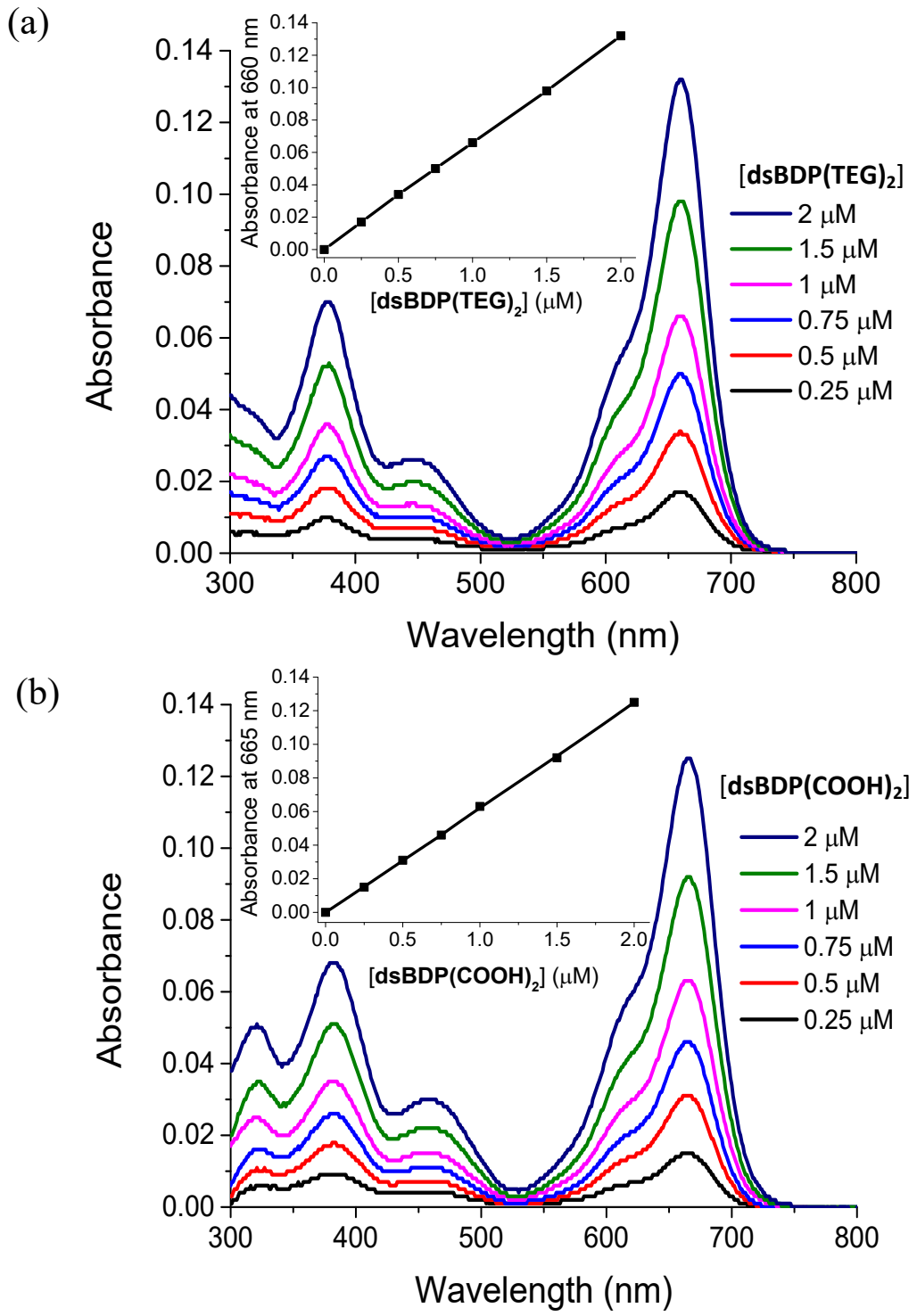

Fig. S1 UV-Vis spectra of (a) **dsBDP(TEG)$_2$** and (b) **dsBDP(COOH)$_2$** at different concentrations in DMF. The inset of each figure plots the absorbance of the longest-wavelength absorption versus the concentration of BODIPY and the line represents the best-fitted straight line.

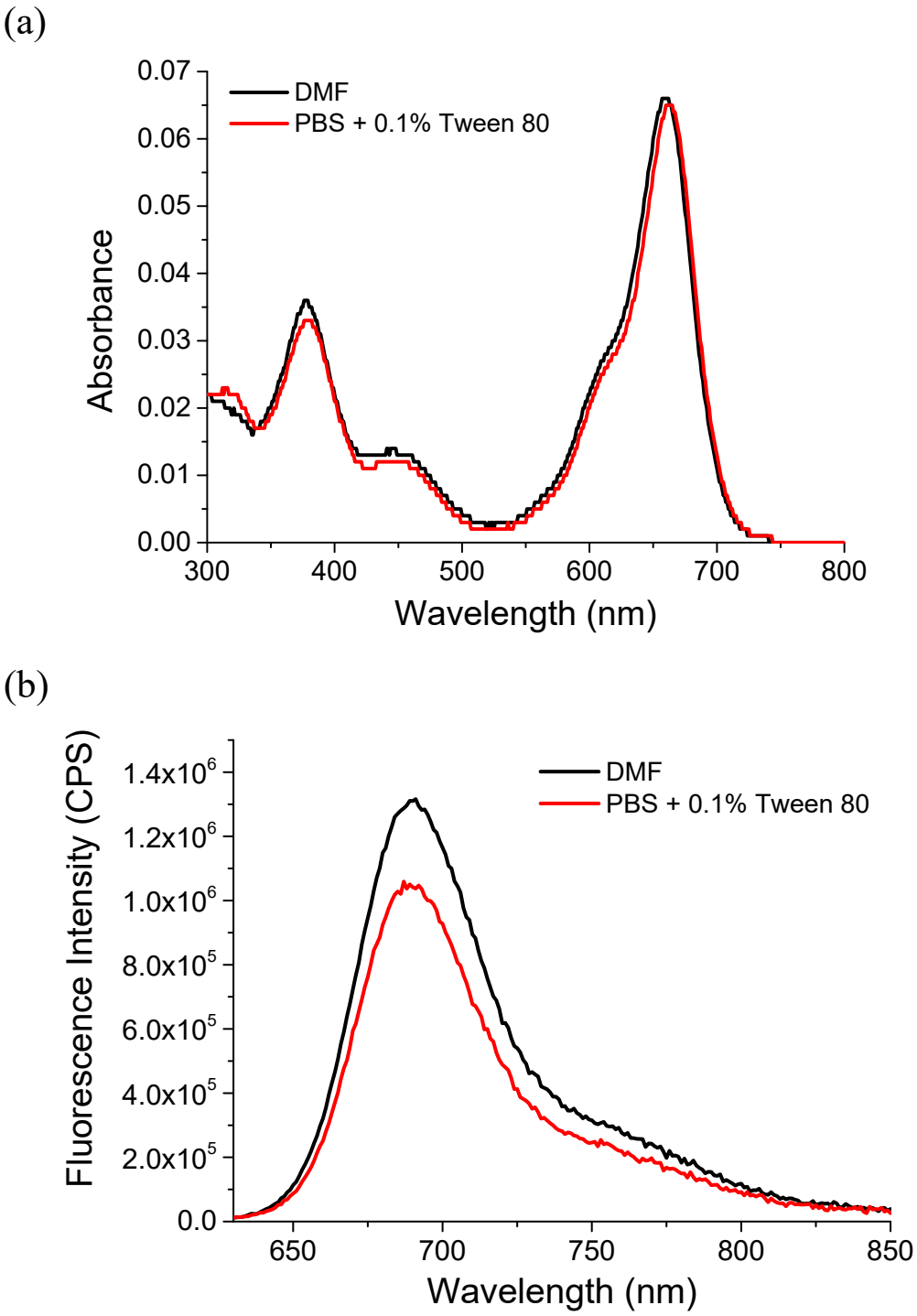

Fig. S2 (a) UV-Vis and (b) fluorescence (excited at 610 nm) spectra of **dsBDP(TEG)$_2$** (1 μM) in DMF and PBS with Tween 80 (0.1% v/v).

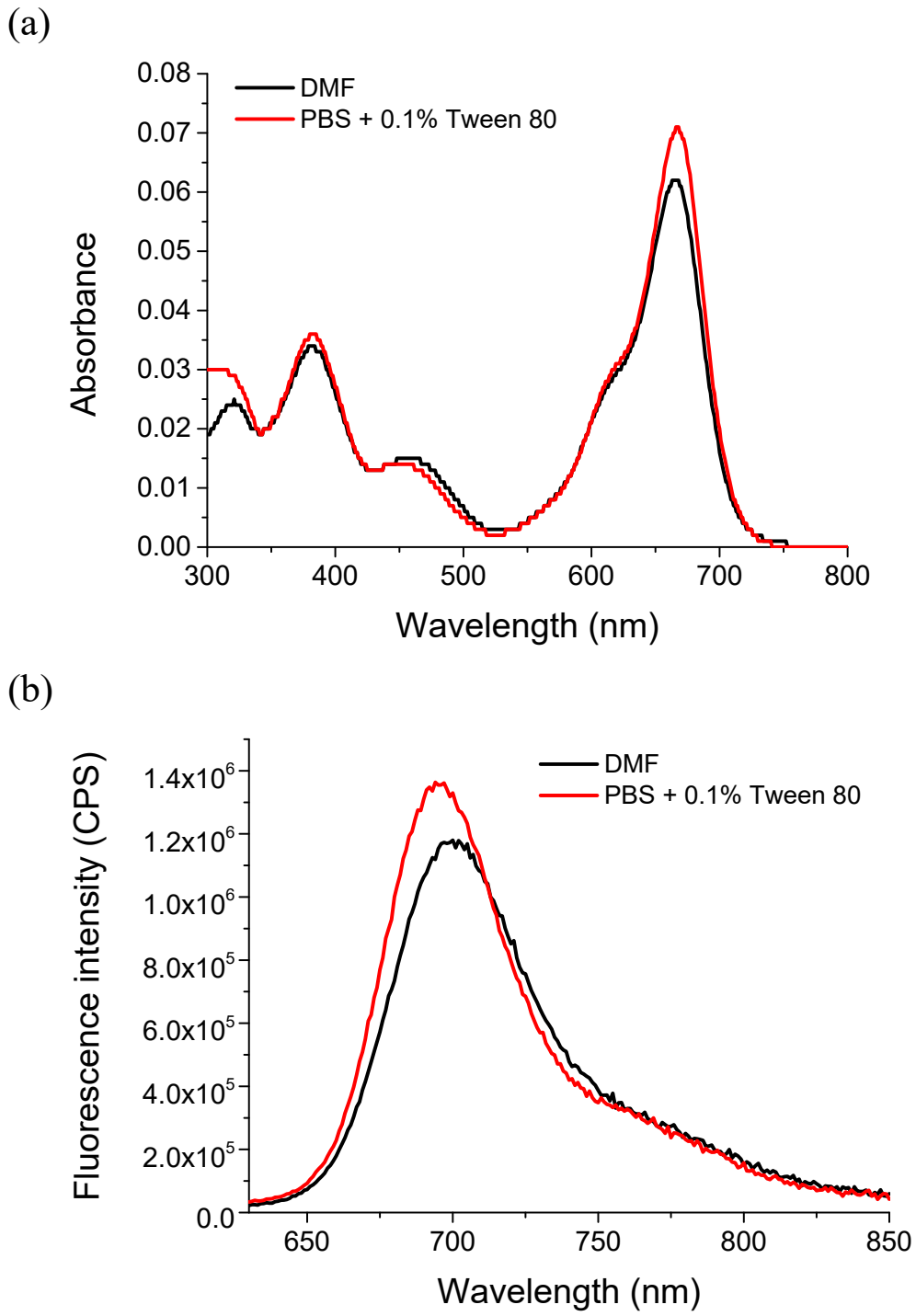

Fig. S3 (a) UV-Vis and (b) fluorescence (excited at 610 nm) spectra of **dsBDP(COOH)$_2$** (1 μM) in DMF and PBS with Tween 80 (0.1% v/v).

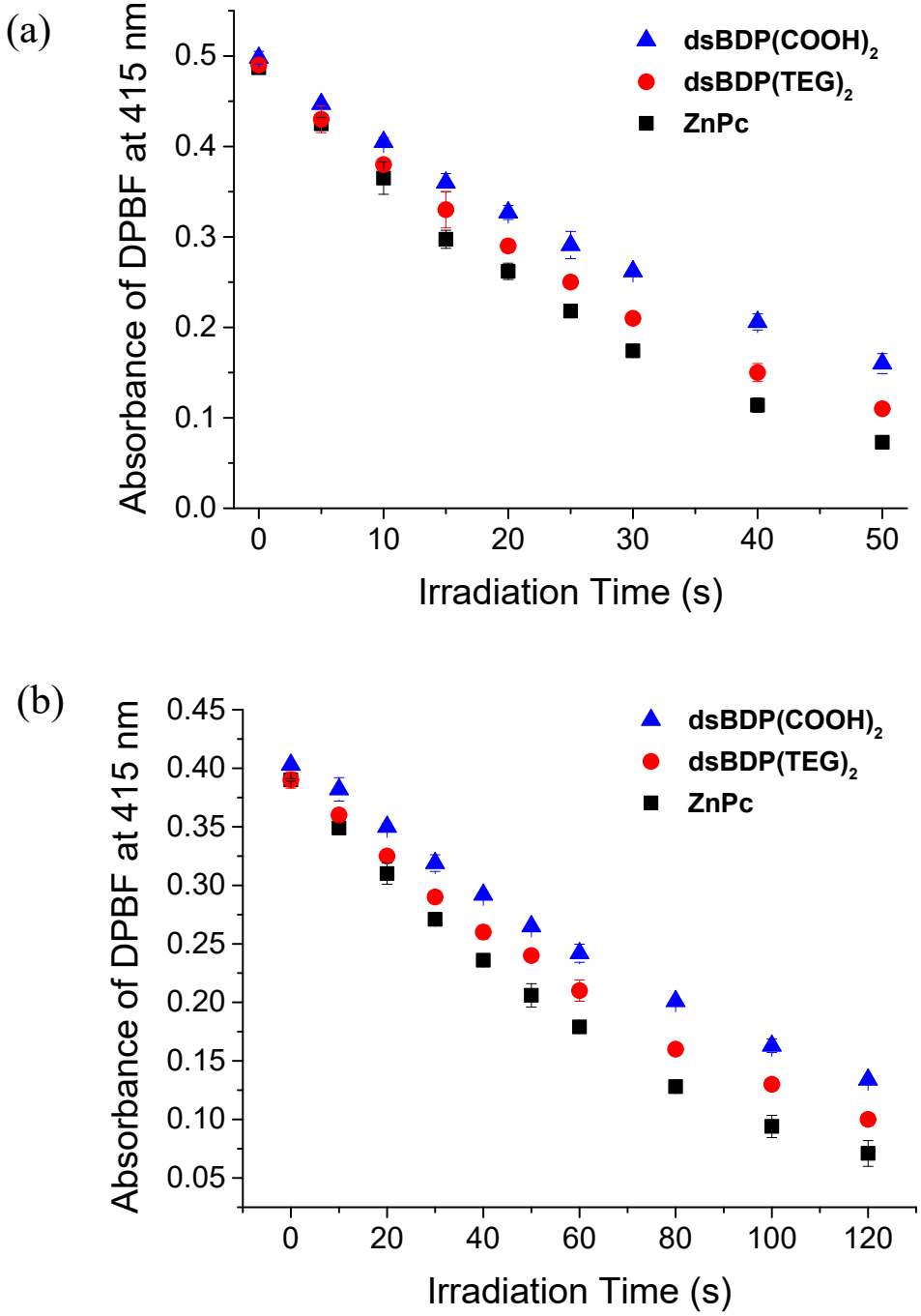

Fig. S4 Comparison of the rate of decay of DPBF (initial concentration = 30 μM) sensitised by **dsBDP(TEG)$_2$**, **dsBDP(COOH)$_2$** and ZnPc (all at 1 μM) in (a) DMF and (b) PBS with Tween 80 (0.1% v/v). Data are expressed as the mean value ± standard deviation of three independent experiments.

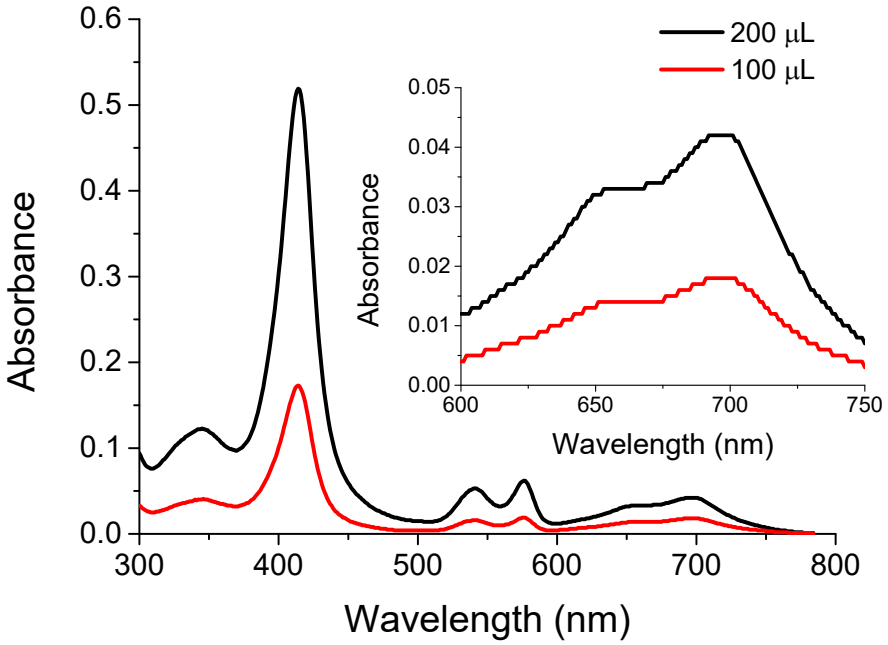

Fig. S5 UV-Vis spectra of different amounts of **BDP@cRGD-RBC** suspension (100 and 200 μL) in PBS (1 mL). The inset shows the enlarged spectra in the region of 600-750 nm.

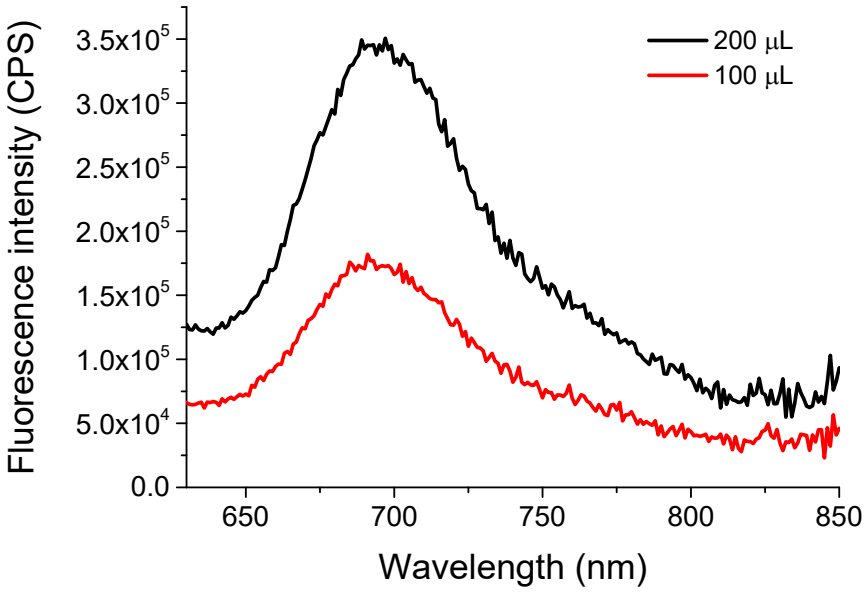

Fig. S6 Fluorescence spectra of different amounts of **BDP@cRGD-RBC** suspension (100 and 200 μL) in PBS (1 mL) (λ_{ex} = 610 nm).

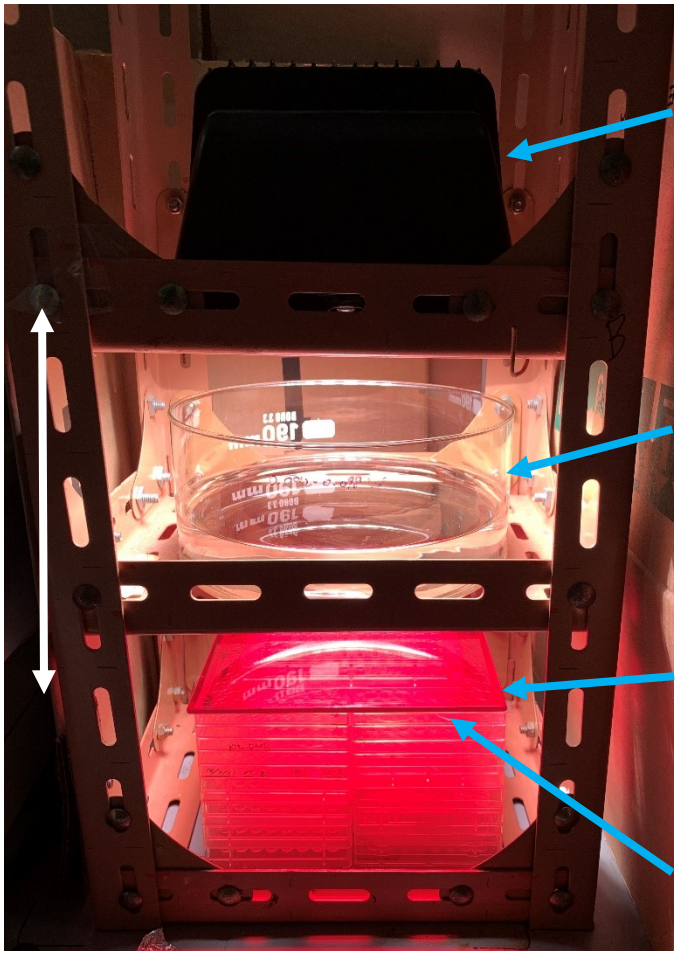

Fig. S7 The set-up of red-light irradiation.

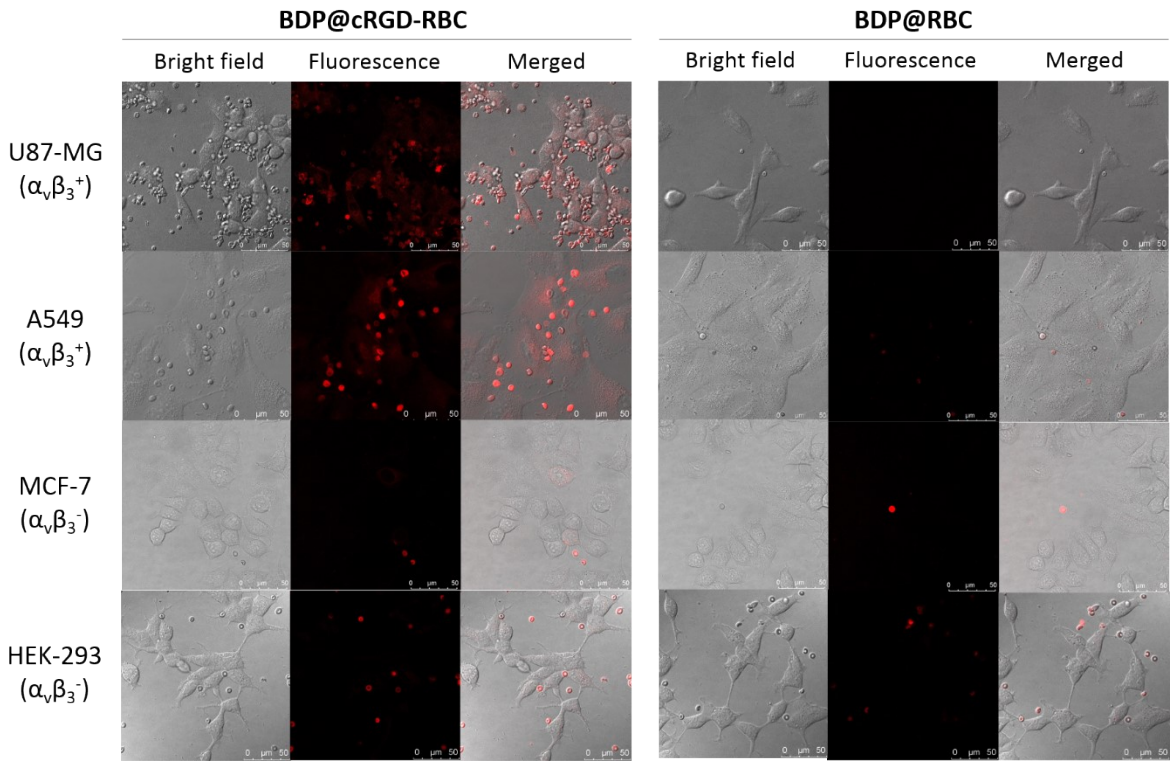

Fig. S8 Bright field, fluorescence and the merged images of U87-MG, A549, MCF-7 and HEK-293 cells after incubation with **BDP@cRGD-RBC** or **BDP@RBC** for 30 min.

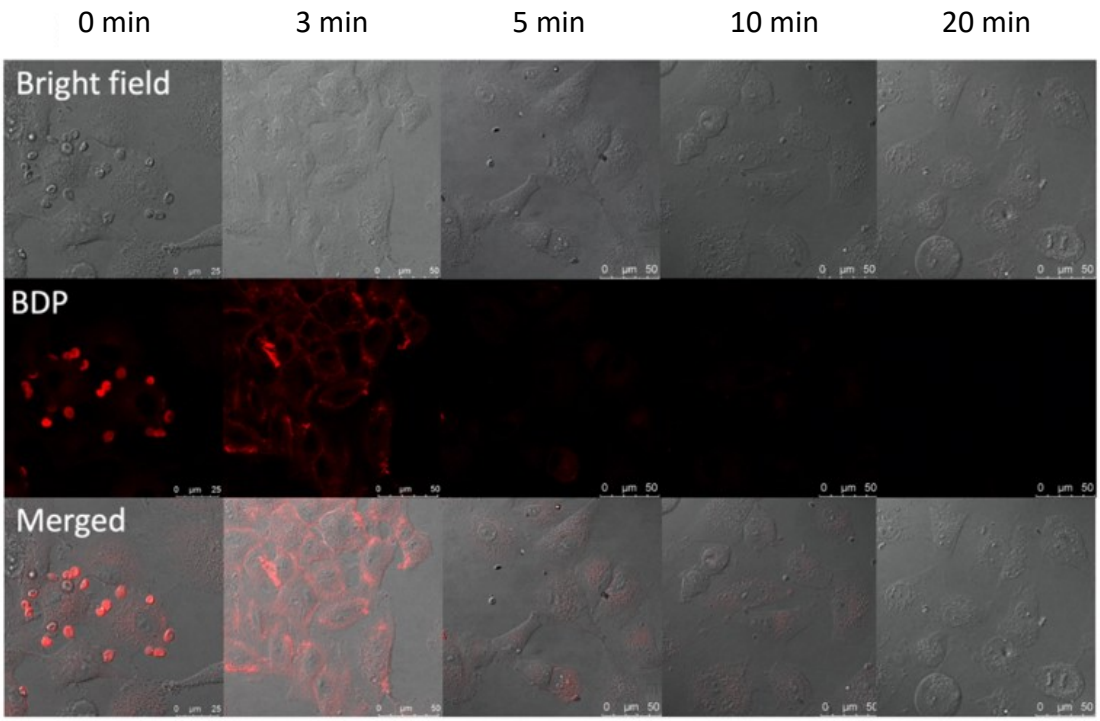

Fig. S9 Confocal images of A549 cells after incubation with **BDP@cRGD-RBC** for 30 min, followed by light irradiation ($\lambda > 610$ nm, 23 mW cm^{-2}) for different periods of time.

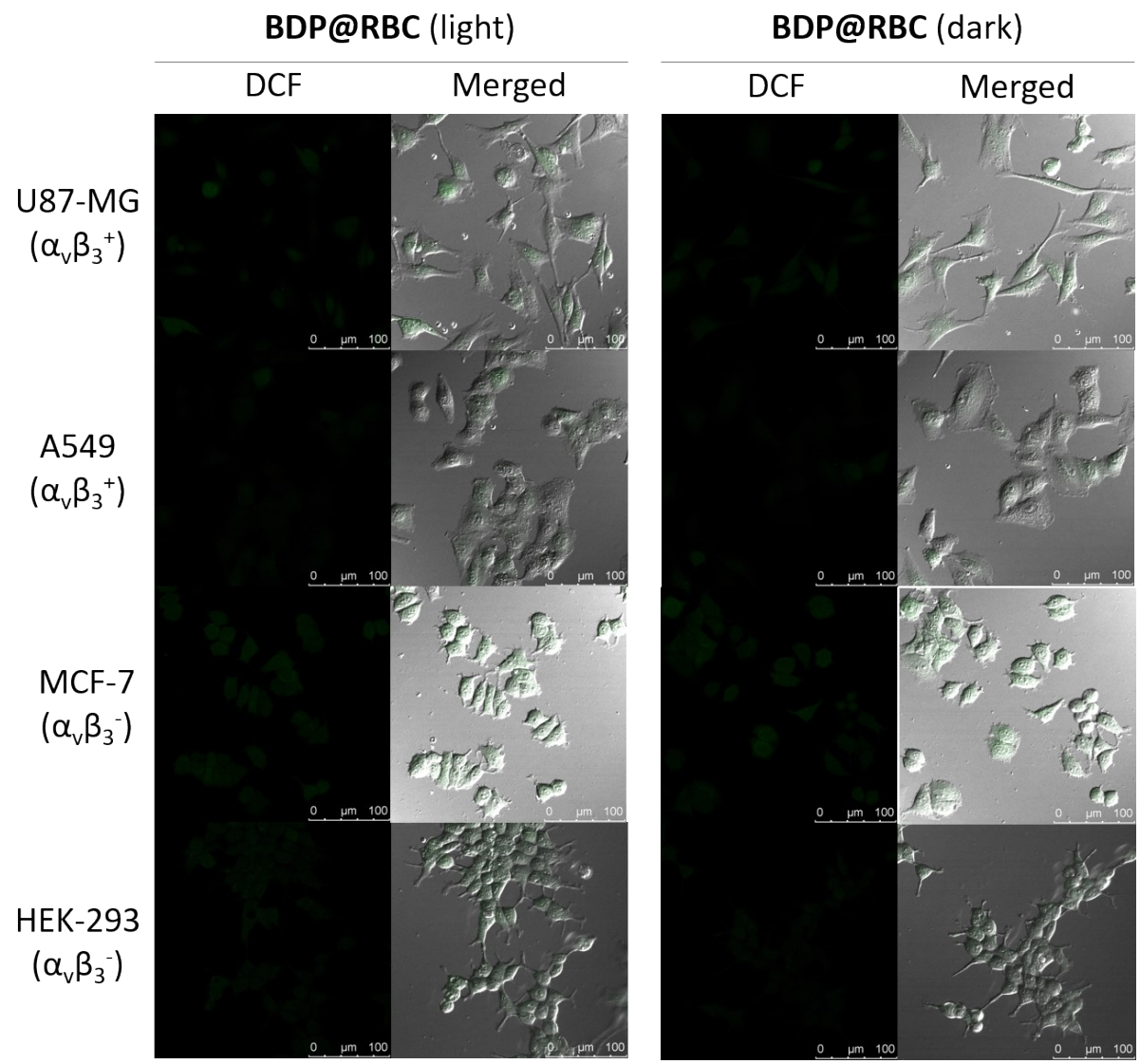

Fig. S10 Intracellular ROS production as reflected by the intracellular fluorescence of DCF in U87-MG, A549, MCF-7 and HEK-293 cells after incubation with **BDP@RBC** for 30 min, followed by light irradiation for 10 min ($\lambda > 610$ nm, 23 mW cm^{-2}, 14 J cm^{-2}).

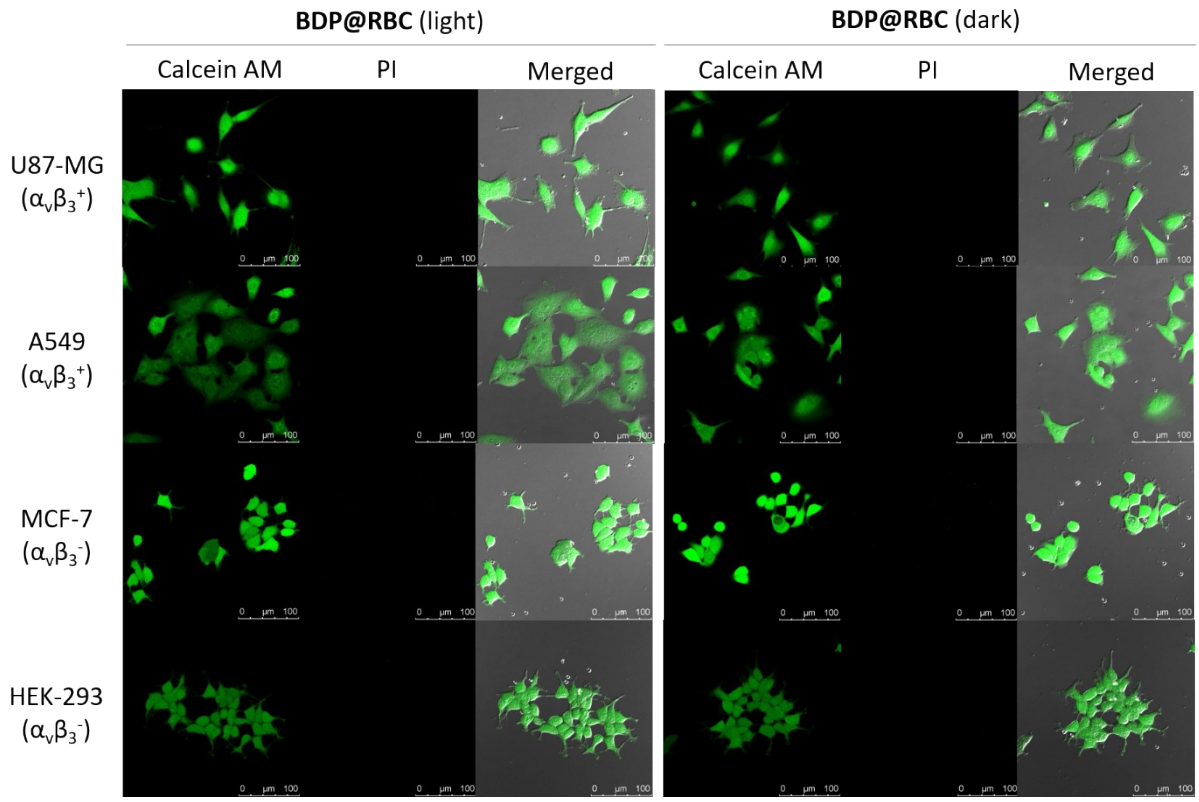

Fig. S11 Live/dead double staining of different cell lines upon treatment with **BDP@RBC** with or without light irradiation for 10 min ($\lambda > 610$ nm, 23 mW cm^{-2}, 14 J cm^{-2}).

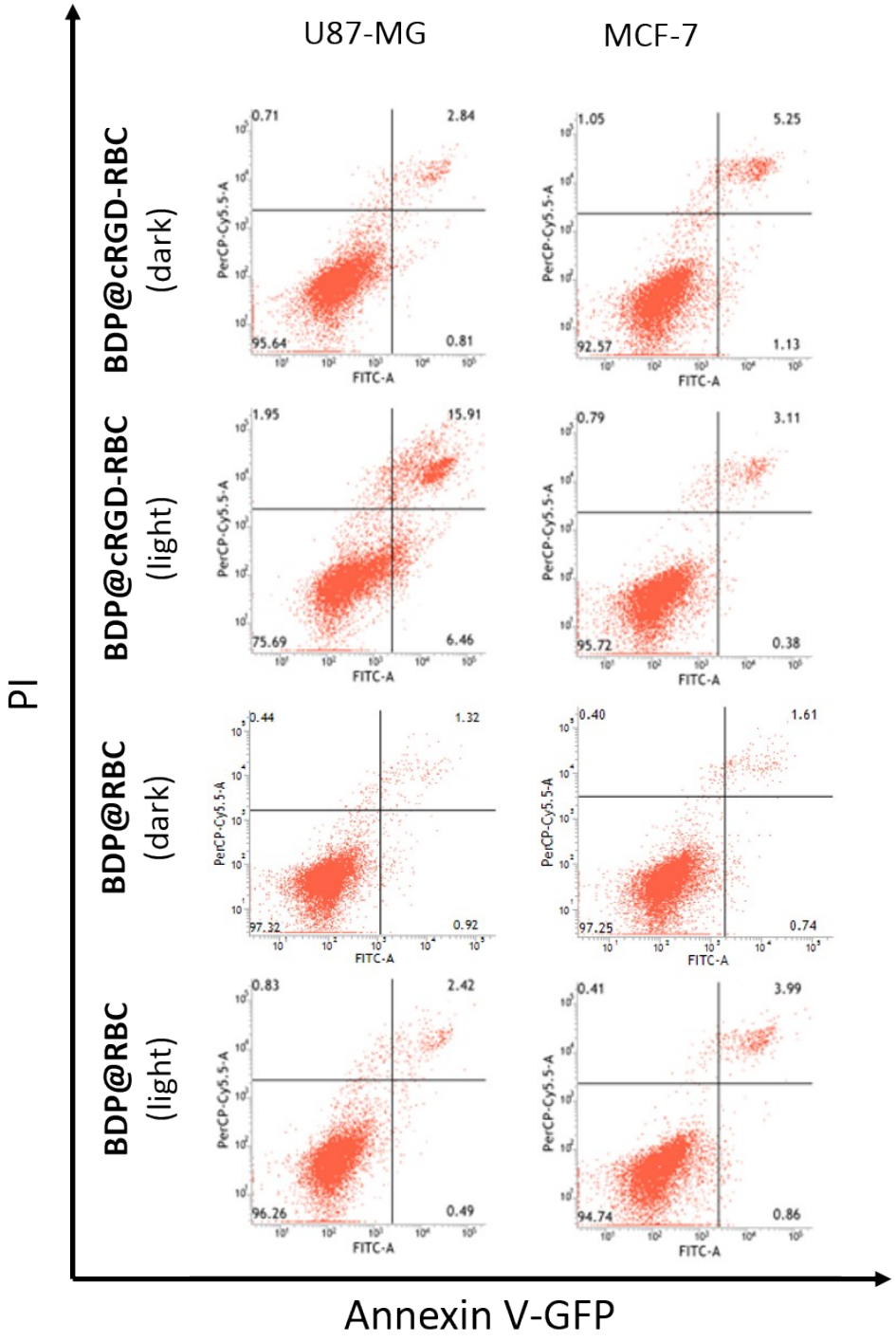

Fig. S12 Flow cytometry results of annexin V-GFP and PI staining of U87-MG and MCF-7 cells after the treatment with **BDP@cRGD-RBC** or **BDP@RBC** in the absence and presence of light irradiation ($\lambda > 610$ nm, 23 mW cm^{-2}, 28 J cm^{-2}).

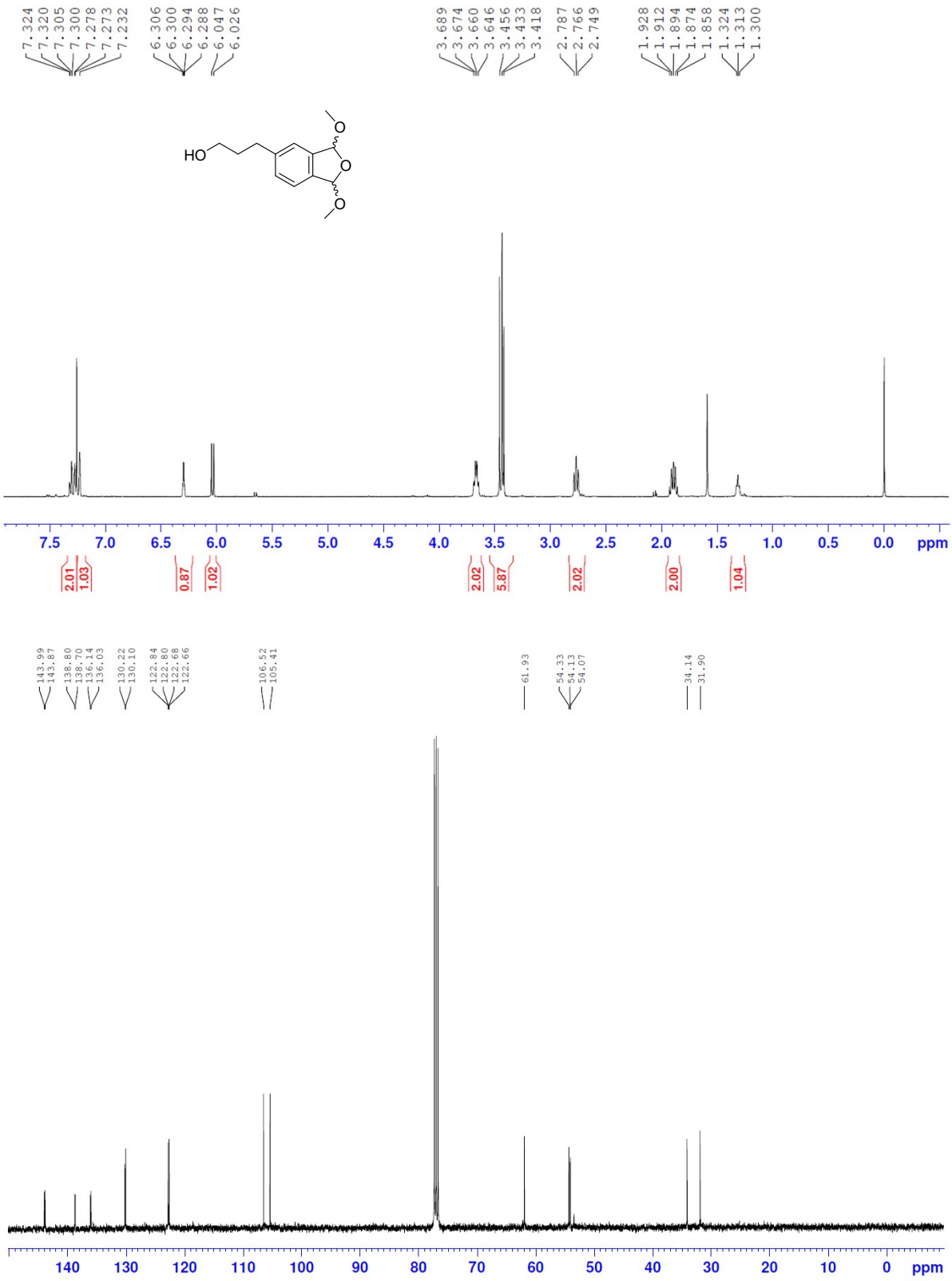

Fig. S13 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **2** in $CDCl_3$.

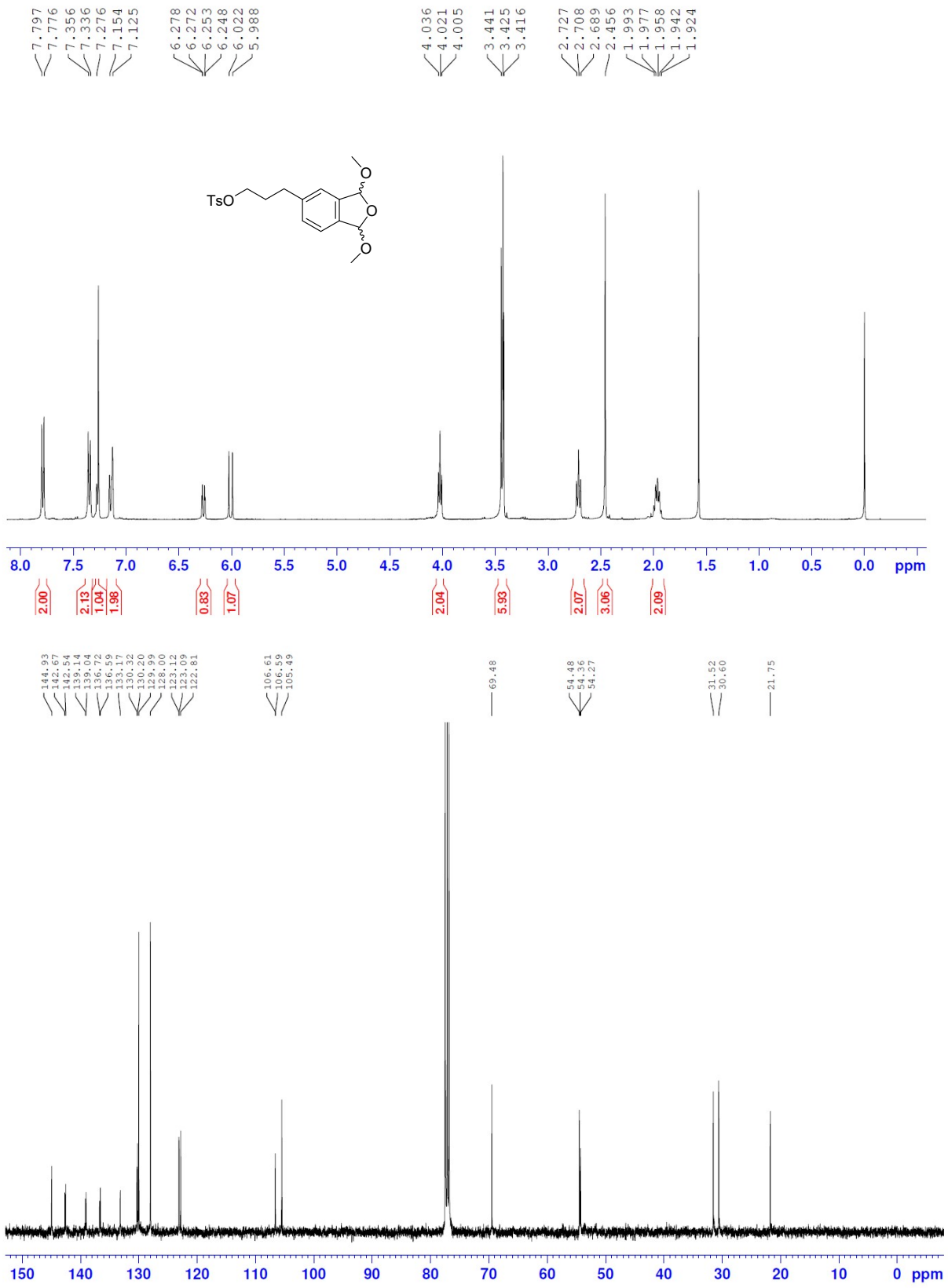

Fig. S14 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **3** in $CDCl_3$.

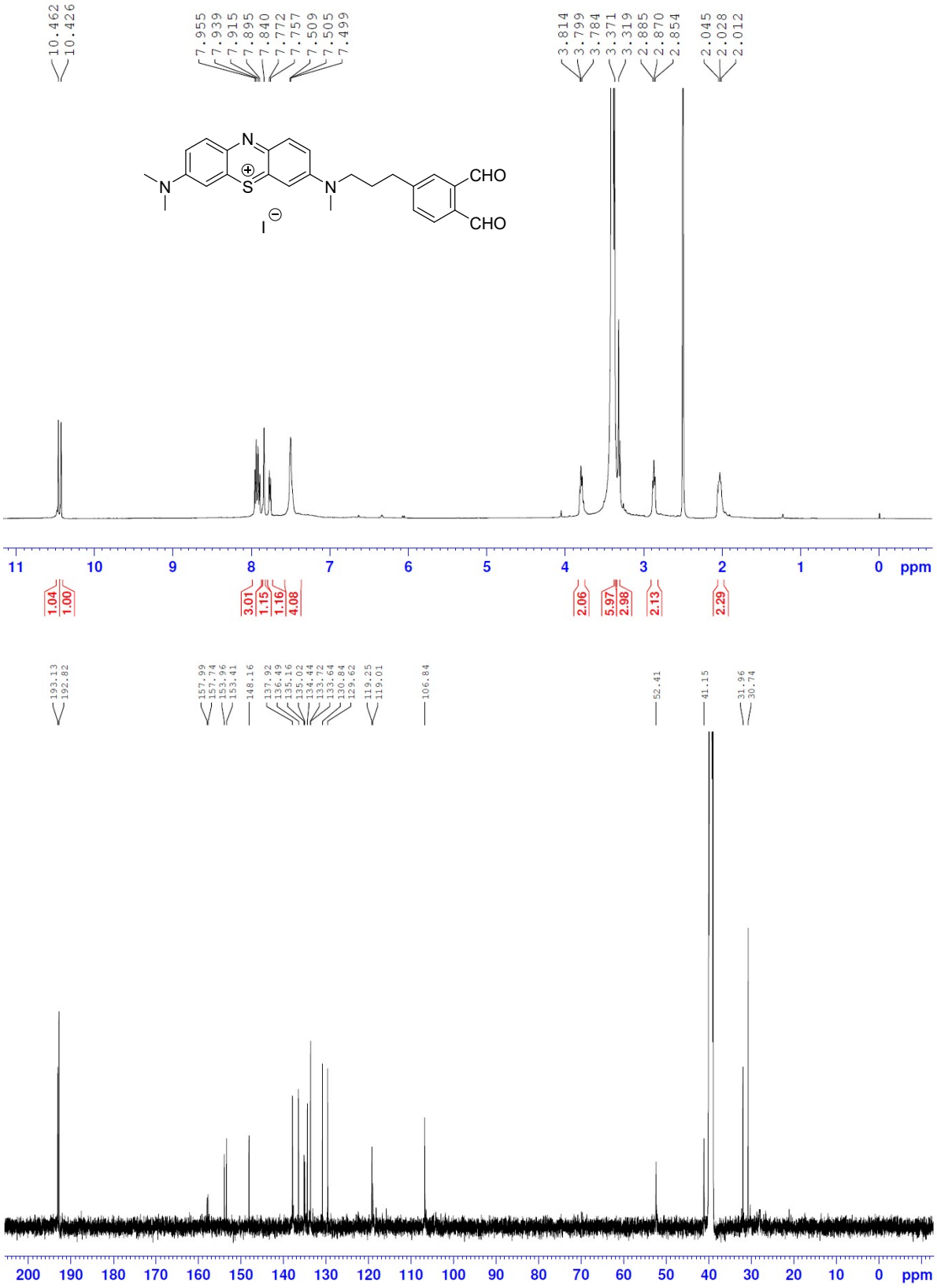

Fig. S15 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **MB-OPA** in DMSO-d_6.

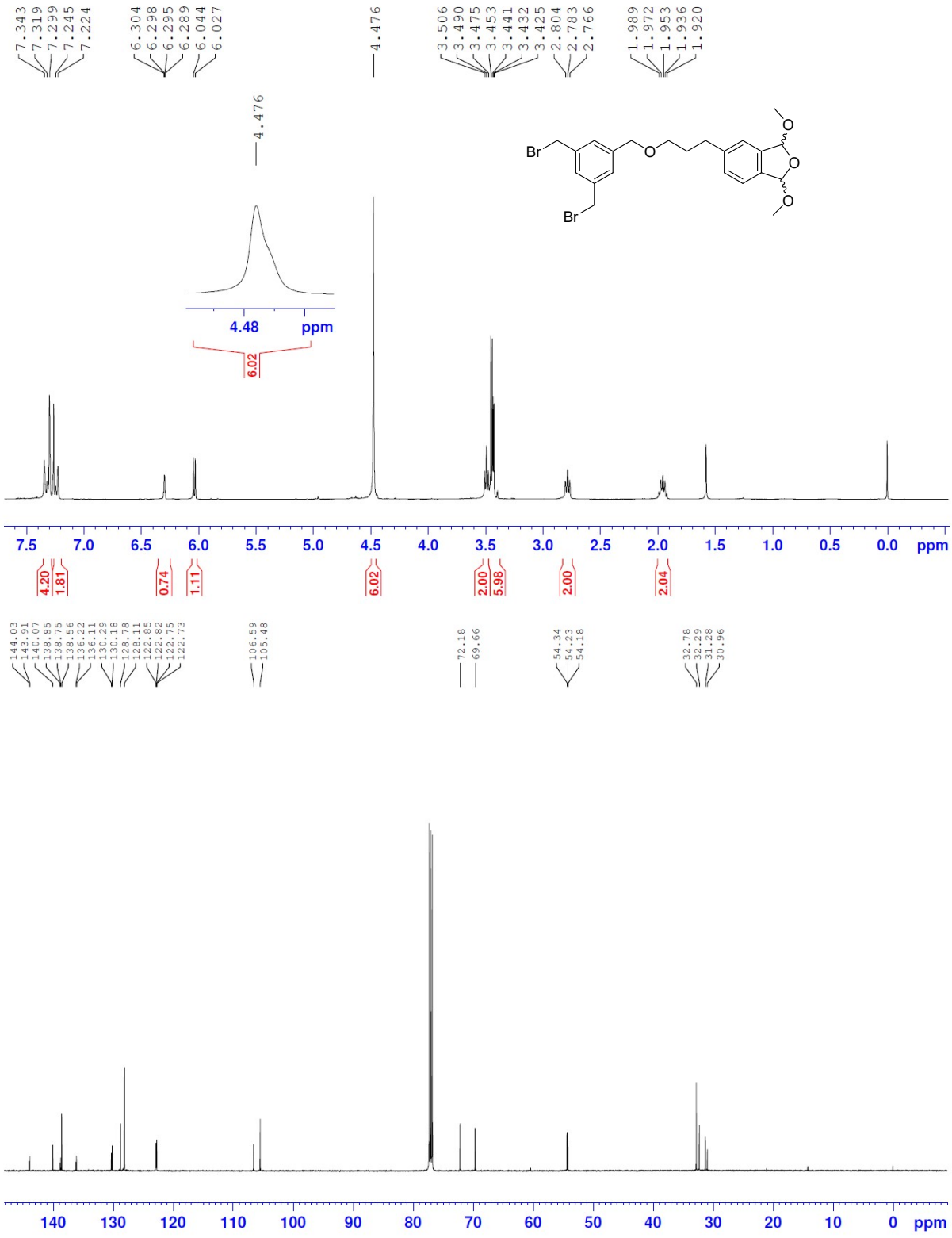

Fig. S16 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **5** in $CDCl_3$.

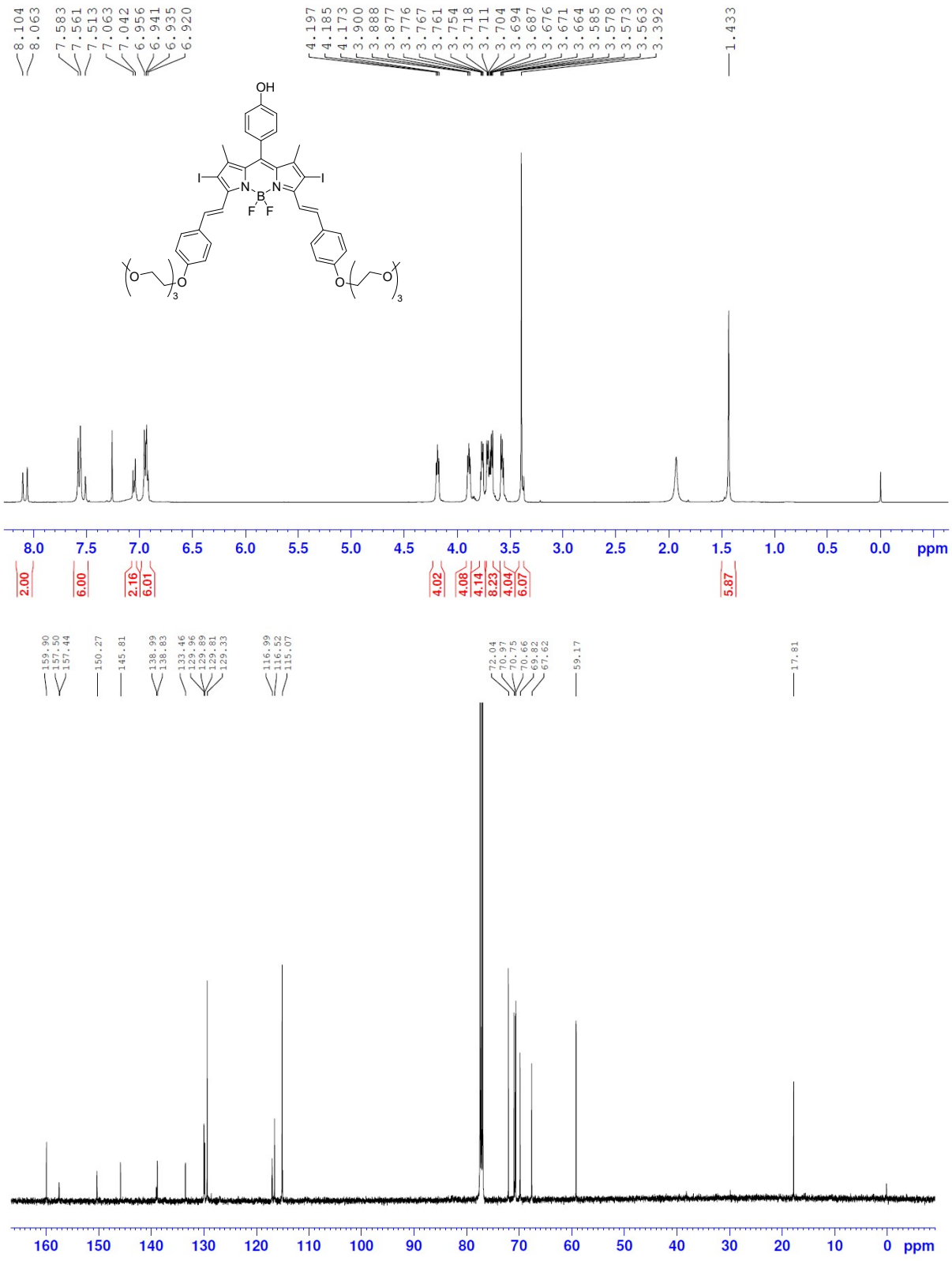

Fig. S17 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **dsBDP(TEG)$_2$** in $CDCl_3$.

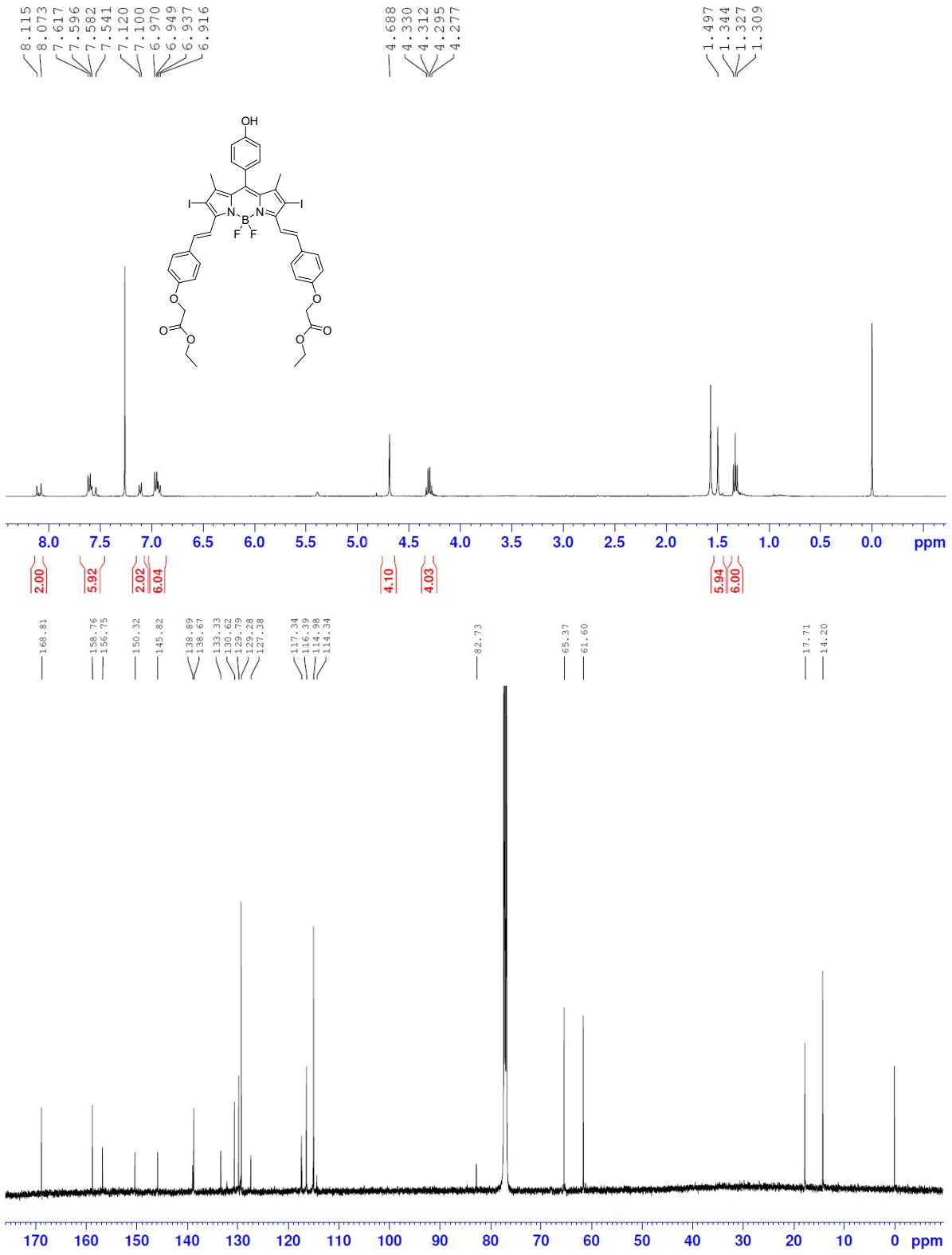

Fig. S18 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **9** in $CDCl_3$.

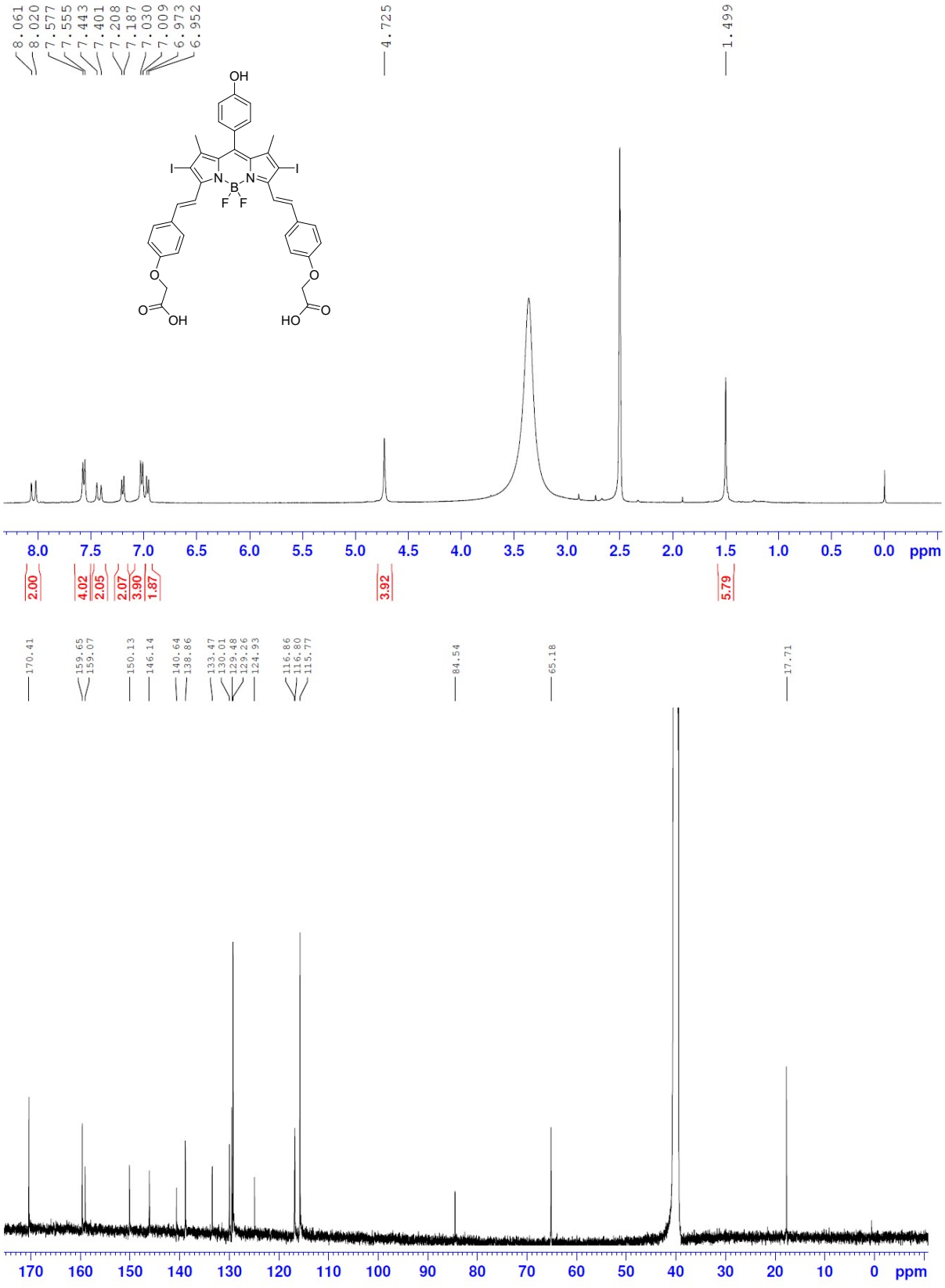

Fig. S19 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **dsBDP(COOH)$_2$** in DMSO-d_6.

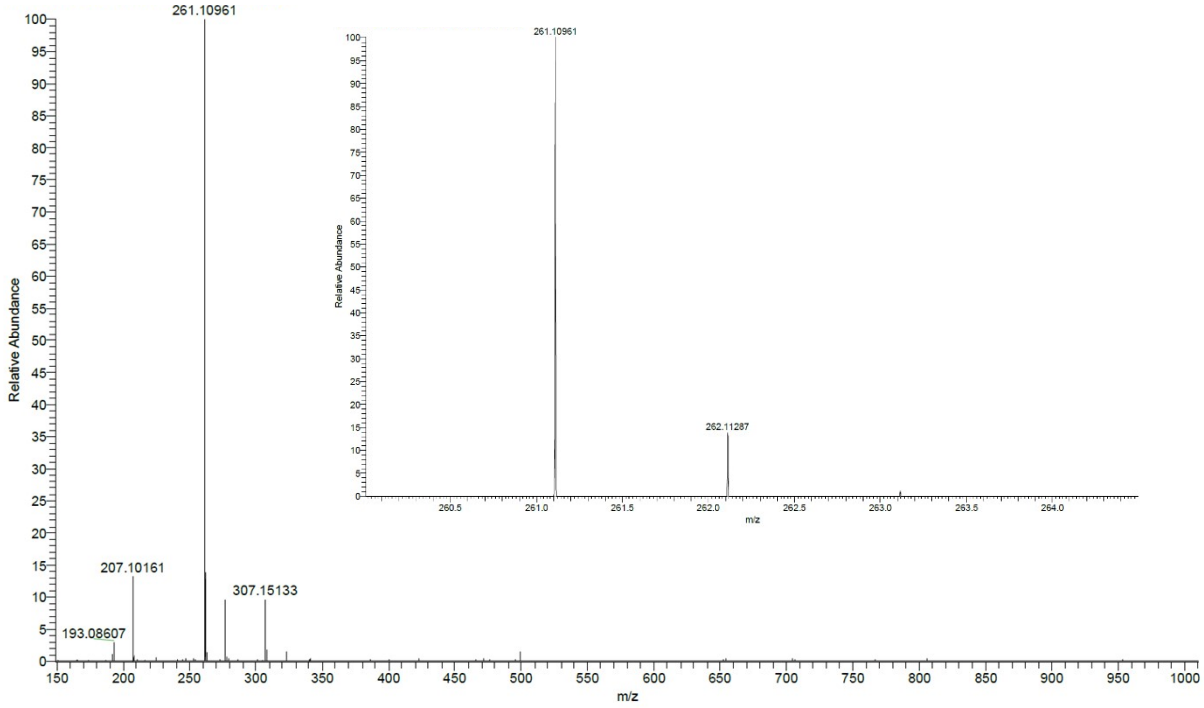

Fig. S20 ESI mass spectrum of **2**. The inset shows the enlarged isotopic envelop for the $[M+Na]^+$ species.

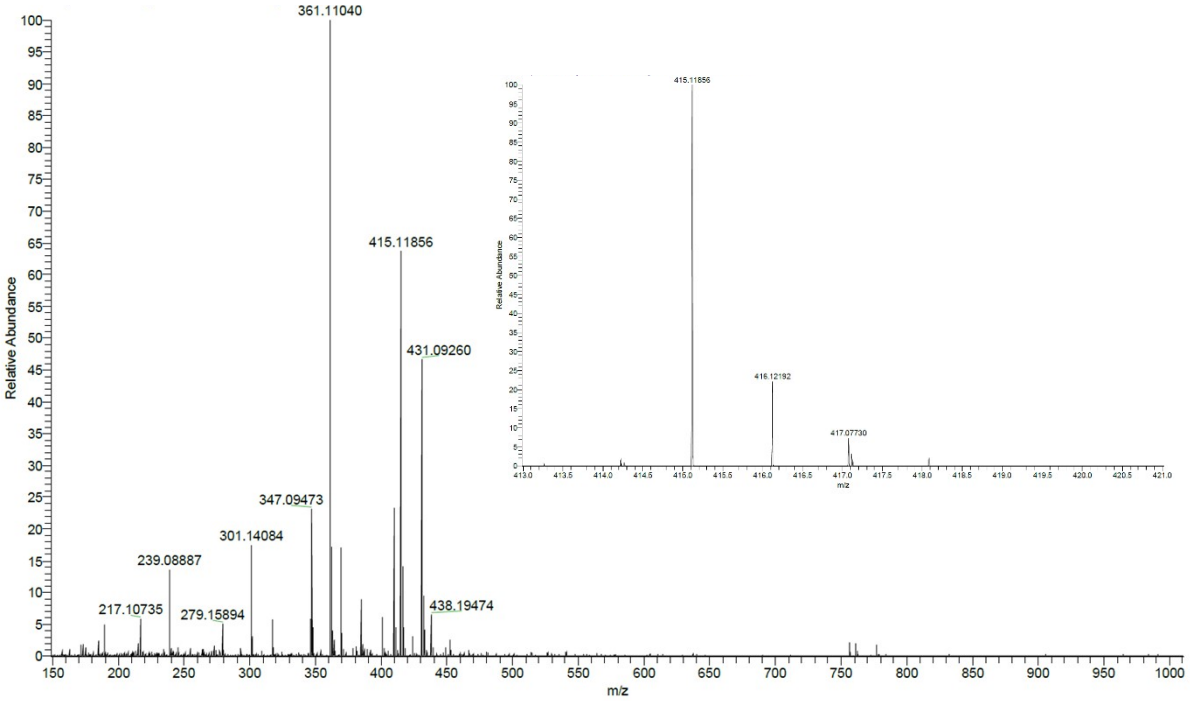

Fig. S21 ESI mass spectrum of **3**. The inset shows the enlarged isotopic envelop for the $[M+Na]^+$ species.

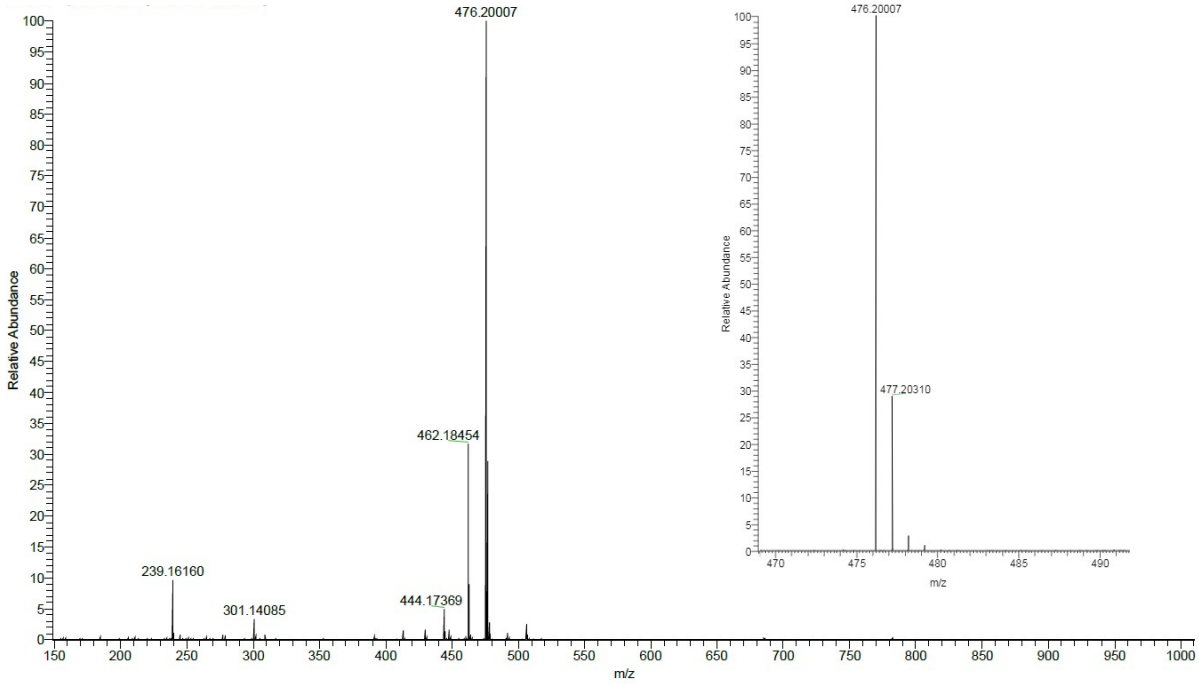

Fig. S22 ESI mass spectrum of **MB-OPA**. The inset shows the enlarged isotopic envelop envelop for the $[M+CH_3OH]^+$ species.

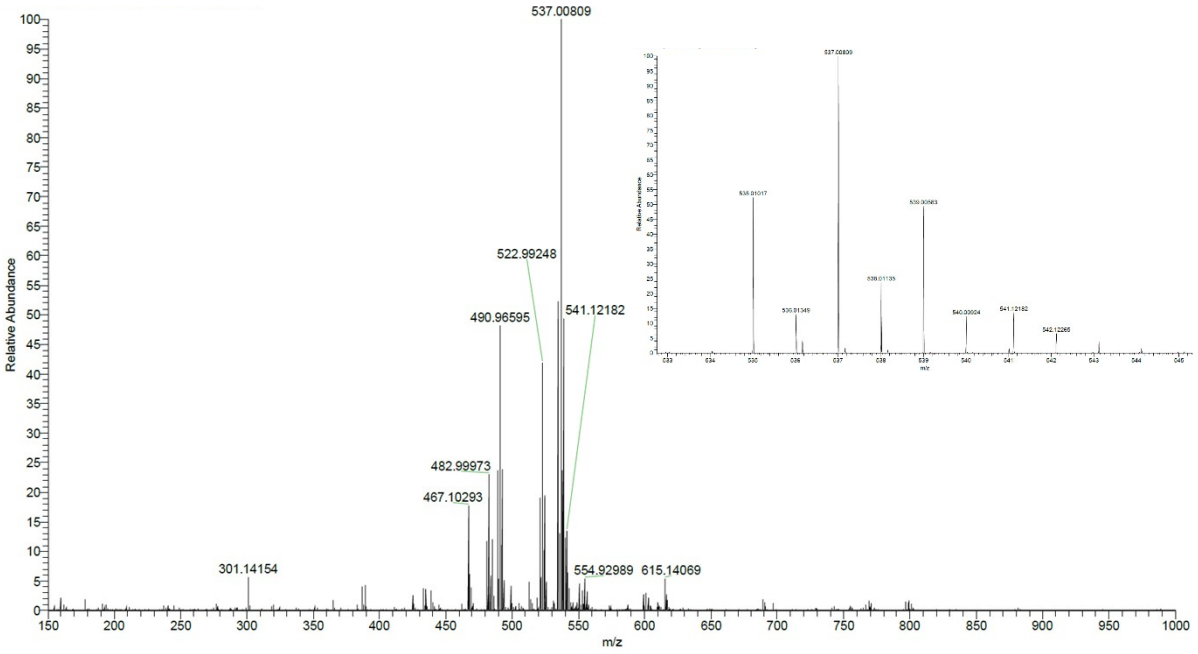

Fig. S23 ESI mass spectrum of **5**. The inset shows the enlarged isotopic envelop for the $[M+Na]^+$ species.

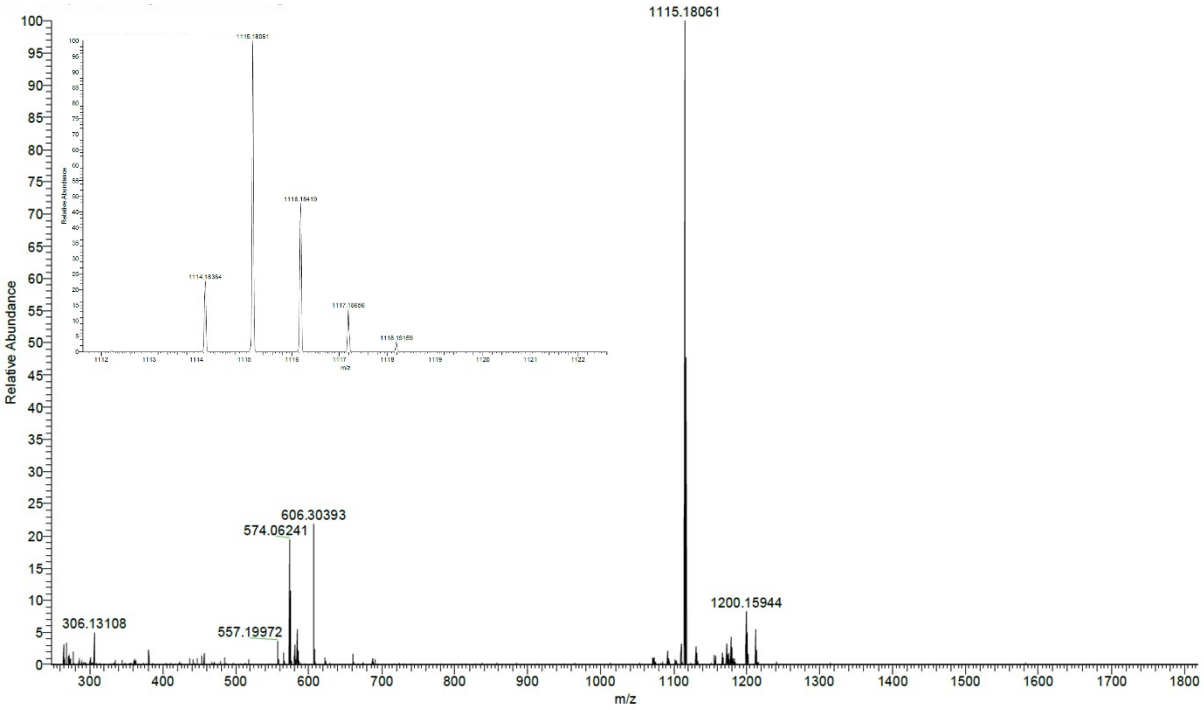

Fig. S24 ESI mass spectrum of **dsBDP(TEG)$_2$**. The inset shows the enlarged isotopic envelop for the $[M+Na]^+$ species.

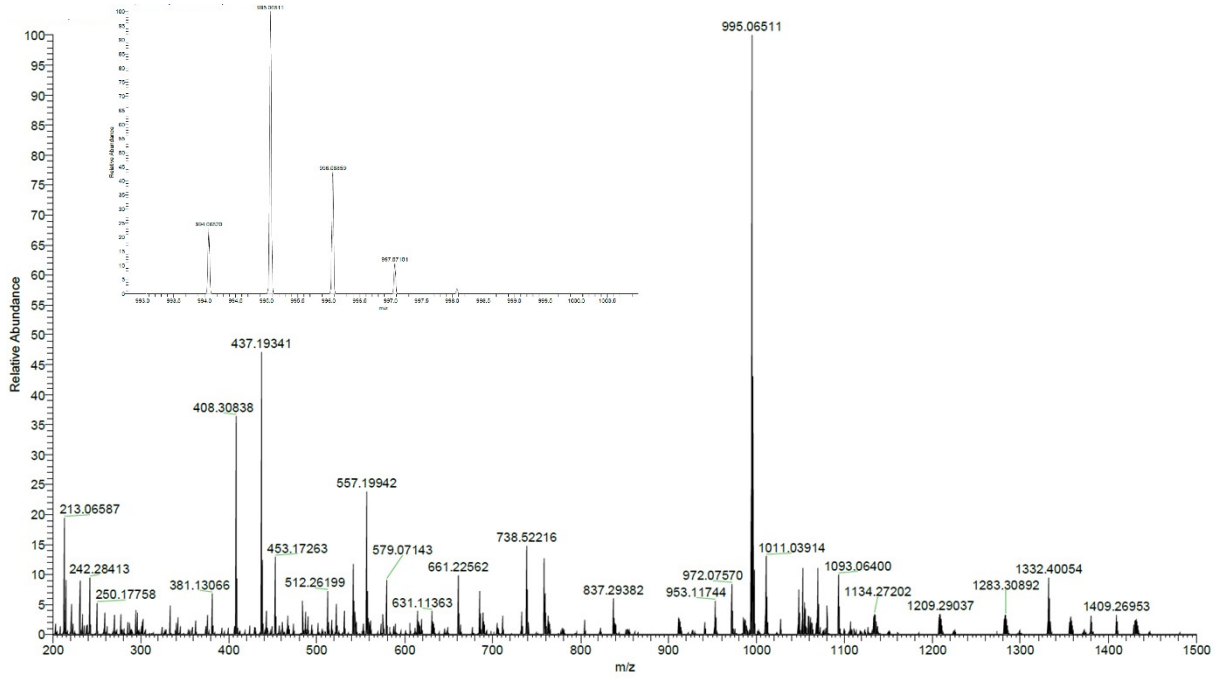

Fig. S25 ESI mass spectrum of **9**. The inset shows the enlarged isotopic envelop for the $[M+Na]^+$ species.

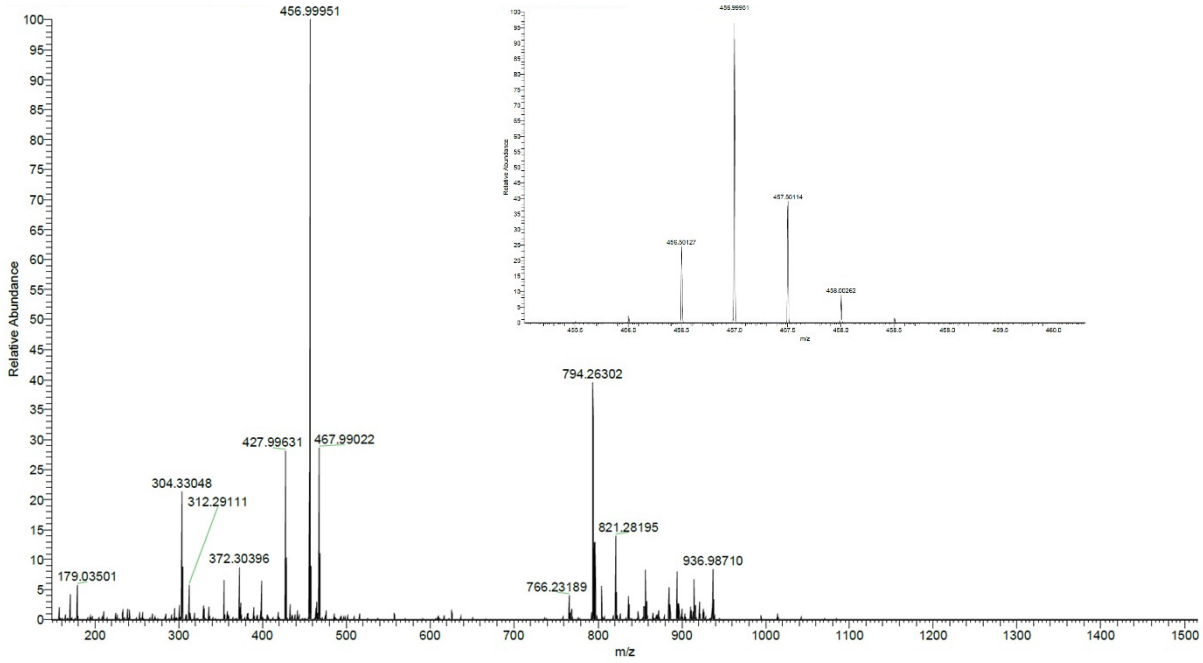

Fig. S26 ESI mass spectrum of **dsBDP(COOH)$_2$**. The inset shows the enlarged isotopic envelop for the $[M-2H]^{2-}$ species.

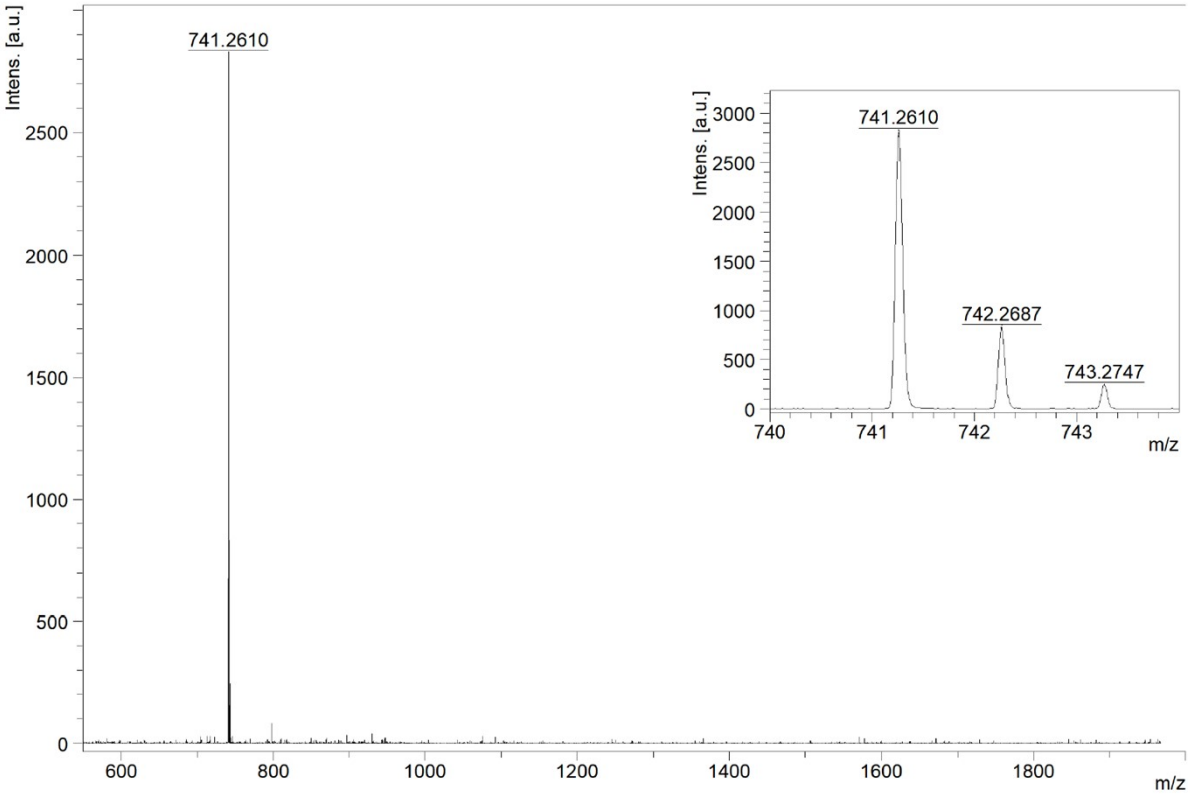

Fig. S27 MALDI-TOF mass spectrum of RGD. The inset shows the enlarged isotopic envelop for the $[M+H]^+$ species.

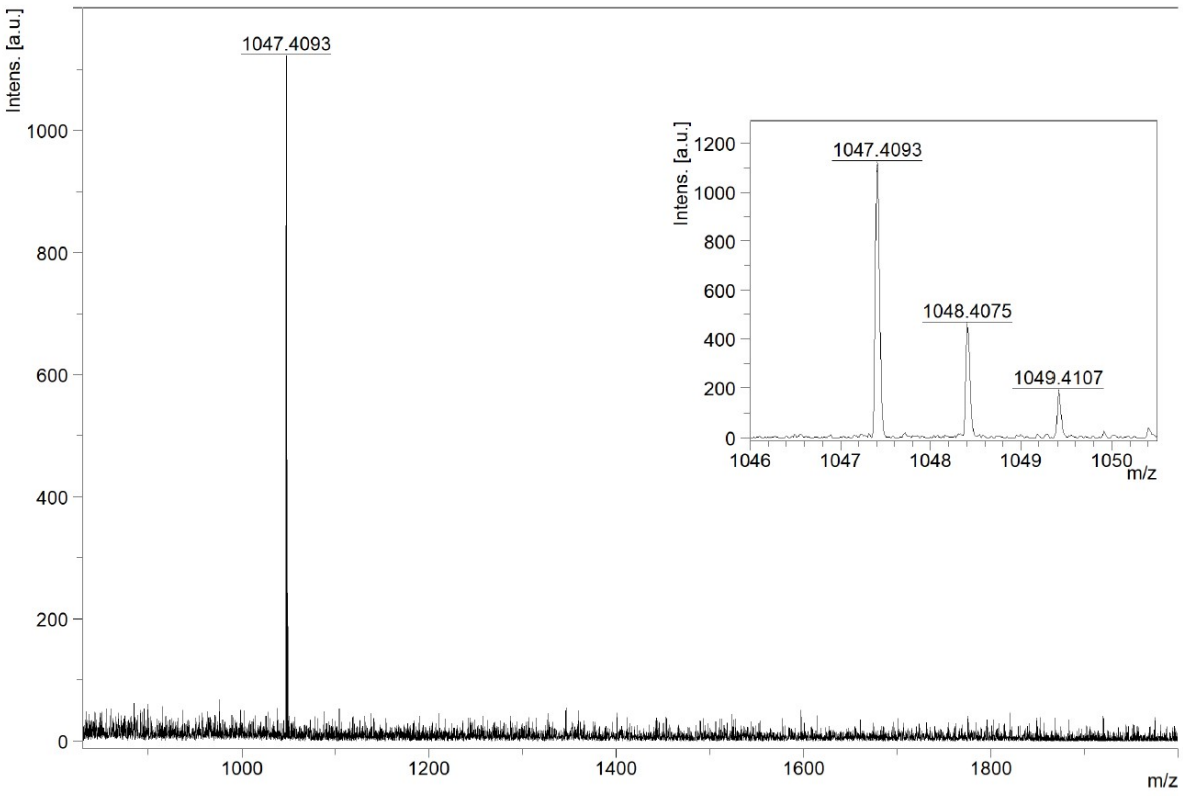

Fig. S28 MALDI-TOF mass spectrum of **cRGD-OPA**. The inset shows the enlarged isotopic envelop for the $[M+H]^+$ species.