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Electronic Supplementary Information

A novel distyryl boron dipyrromethene with two functional tags for site-specific bioorthogonal photosensitisation towards targeted photodynamic therapy

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Experimental Section

General

All the reactions were performed under an atmosphere of nitrogen. Toluene and tetrahydrofuran (THF) were dried over sodium and distilled under atmospheric pressure. *N*,*N*-dimethylformamide (DMF) was dried over barium oxide and distilled under reduced pressure. All other solvents and reagents were of reagent grade and used as received. Chromatographic purification was performed using silica gel (Macherey-Nagel, 230-400 mesh) with the indicated eluents. Size-exclusion chromatography was carried out on Bio-Beads S-X1 beads (200-400 mesh) with THF as eluent. Reverse-phase analytical and semi-preparative HPLC was performed on a XBridge-C18 analytic column (3.5 µm, 4.6 mm x 150 mm) and a XBridge-C18 column (5 µm, 10 mm x 250 mm) respectively using a Waters 1525 binary HPLC pump with a Waters 2998 photodiode array detector. Acetonitrile and deionised water used for HPLC analysis were of HPLC grade. Compounds **1**,[R1] **3**[R2] and **5**[R3] were prepared as described.

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker AVANCE III 300 or 400 spectrometer (^{1}H, 300 or 400 MHz; ^{13}C, 100.6 MHz) in $CDCl_3$ or CD_2Cl_2/CD_3OD. Spectra were referenced internally by using the residual solvent (^{1}H, δ 7.26 for $CDCl_3$) or solvent (^{13}C, δ 77.2 for $CDCl_3$; δ 53.7 for CD_2Cl_2) resonances relative to $SiMe_4$. Electrospray ionisation (ESI) mass spectra were recorded on a Thermo Q Exactive Focus Orbitrap mass spectrometer. UV-Vis and steady-state fluorescence spectra were taken on a Hitachi UH5300 UV-Vis-NIR spectrophotometer and a Hitachi F-7000 spectrofluorometer respectively. The fluorescence quantum yield (Φ_F) of the sample was determined by the equation: $\Phi_{F(sample)} = (F_{sample}/F_{ref})(A_{ref}/A_{sample})(n_{sample}^2/n_{ref}^2)\Phi_{F(ref)}$, where F, A and n are the measured fluorescence (area under the emission peak), the absorbance at the excitation position and the refractive index of the solvent respectively.[R4] Zinc(II) phthalocyanine (ZnPc) in DMF was used as the

reference [$\Phi_{F(ref)} = 0.28$].[R5] The singlet oxygen generation was measured in DMF by using the method of chemical quenching of 1,3-diphenylisobenzofuran with ZnPc as the reference. The light source consisted of red light from a 300 W halogen lamp after passing through a water tank for cooling and a colour glass filter (Newport) cut-on at 610 nm.

Preparation of compound 2

A mixture of compound **1** (113 mg, 0.27 mmol) and *N*-bromosuccinimide (192 mg, 1.08 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 2 h. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography using *n*-hexane/EtOAc (3:1, v/v) as eluent, followed by precipitation with $CHCl_3$ (1 mL) and *n*-hexane (5 mL) (130 mg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (d, *J* = 8.7 Hz, 2 H, ArH), 7.23 (d, *J* = 8.7 Hz, 2 H, ArH), 3.11 (s, 3 H, tetrazine-CH_3), 2.63 (s, 6 H, CH_3), 2.10 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 167.2, 163.3, 160.5, 154.9, 150.3, 137.9, 130.5, 127.3, 126.3, 115.6, 111.0, 21.2, 13.8, 13.4. HRMS (ESI) calculated for $C_{22}H_{18}BBr_2F_2N_6$ [M-H]$^-$ 575.0011, found 574.9995.

Preparation of compound 4

A mixture of compound **2** (130 mg, 0.23 mmol), benzaldehyde **3** (42 mg, 0.26 mmol), piperidine (0.50 mL) and acetic acid (0.40 mL) in toluene (15 mL) was stirred at 85 °C for 1 h. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography using *n*-hexane/EtOAc (2:1, v/v) as eluent, followed by precipitation with $CHCl_3$ (1 mL) and *n*-hexane (5 mL) (33 mg, 20%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79 (d, *J* = 8.1 Hz, 2 H, ArH), 8.11 (d, *J* = 16.5 Hz, 1 H, C=CH), 7.61 (d, *J* = 8.7 Hz, 2 H, ArH), 7.54-7.57 (m, 3 H, ArH and C=CH), 7.01 (d, *J* = 8.7 Hz, 2 H, ArH), 4.75 (d, *J* = 2.4 Hz, 2 H, CH_2), 3.15 (s, 3 H, tetrazine-CH_3), 2.67 (s, 3 H, CH_3), 2.55 (t, *J* = 2.4 Hz, 1 H, C≡CH), 1.49 (s, 3 H,

CH_3), 1.45 (s, 3 H, CH_3). $^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): 167.8, 163.5, 158.7, 154.5, 149.1, 141.3, 139.8, 139.0, 133.1, 131.1, 131.0, 130.4, 129.4, 129.2, 128.8, 116.2, 115.3, 112.4, 110.5, 78.2, 75.7, 65.8, 55.9, 53.4, 31.0, 21.2, 15.2, 14.0. HRMS (ESI) calculated for $C_{32}H_{26}BBr_2F_2N_6O$ $[M+H]^+$ 719.0578, found 719.0572.

Preparation of compound 6

A mixture of compound **4** (33 mg, 0.05 mmol), benzaldehyde **5** (39 mg, 0.09 mmol), piperidine (0.18 mL) and acetic acid (0.15 mL) in toluene (10 mL) was stirred at 85 °C for 1 h. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography using CH_2Cl_2/MeOH (100:1, v/v) as eluent, followed by precipitation with $CHCl_3$ (1 mL) and *n*-hexane (5 mL) (10 mg, 20%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.82 (d, *J* = 8.4 Hz, 2 H, ArH), 8.15 (d, *J* = 16.4 Hz, 1 H, C=CH), 8.09 (d, *J* = 16.4 Hz, 1 H, C=CH), 7.59-7.68 (m, 6 H, ArH and C=CH), 7.31 (d, *J* = 8.8 Hz, 1 H, ArH), 7.21 (s, 1 H, ArH), 7.06 (d, *J* = 8.8 Hz, 2 H, ArH), 6.99 (d, *J* = 8.8 Hz, 1 H, ArH), 4.78 (d, *J* = 2.4 Hz, 2 H, CH_2), 4.24-4.30 (m, 4 H, OCH_2), 3.92-3.94 (m, 4 H, OCH_2), 3.79-3.80 (m, 4 H, OCH_2), 3.67-3.72 (m, 8 H, OCH_2), 3.53-3.60 (m, 4 H, OCH_2), 3.41 (s, 3 H, OCH_3), 3.38 (s, 3 H, OCH_3), 3.18 (s, 3 H, tetrazine-CH_3), 2.60 (t, *J* = 2.4 Hz, 1 H, C≡CH), 1.52 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR (100.6 MHz, CD_2Cl_2/CD_3OD): 167.9, 163.7, 158.9, 150.5, 148.9, 148.7, 148.6, 143.1, 141.2, 139.3, 139.1, 138.9, 137.8, 133.3, 131.8, 130.4, 130.3, 129.7, 129.2, 128.8, 123.7, 123.5, 121.7, 116.3, 115.4, 113.9, 113.5, 110.5, 110.4, 78.3, 77.7, 77.5, 77.2, 75.8, 71.8, 70.7, 70.5, 70.3, 69.7, 69.5, 68.9, 68.6, 60.0, 58.6, 58.0, 55.9, 29.7, 21.0, 13.8. HRMS (ESI) calculated for $C_{53}H_{57}BBr_2F_2N_6NaO_9$ $[M+Na]^+$ 1153.2502, found 1153.2507.

Preparation of TCO-GE11

The GE11 peptide-resin [Fmoc-Tyr(tBu)-His(Trt)-Trp(Boc)-Tyr(tBu)-Gly-Tyr(tBu)-Thr(Trt)-Pro-Gln(Trt)-Asn(Trt)-Val-Ile-resin] was prepared manually according to the standard 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis (SPPS) protocol with commercially available *N*-α-Fmoc-protected amino acids. Sieber amide resin was used as solid support, and 1-hydroxybenzotriazole (HOBt) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU) were used as the carboxyl group activating agents. An excess amount (3 equiv.) of Fmoc-protected amino acids and activating agents were used for each coupling. The Fmoc protecting group of tyrosine was removed from the GE11 peptide-resin with piperidine (20%) in DMF, and then the peptide-resin was washed with DMF (5 mL × 2) and CH_2Cl_2 (5 mL × 2). Finally, all the remaining protecting groups on the peptide and the resin were removed with a solution of trifluoroacetic acid (TFA, 88%), phenol (5%), triisopropylsilane (TIS, 2%) and water (5%). The peptide was purified by repeated precipitation using dimethylsulfoxide (DMSO) and diethyl ether. The Fmoc-deprotected GE11 peptide (5.8 mg, 3.76 μmol) and (*E*)-cyclooct-4-enyl-2,5-dioxo-1-pyrrolidinyl carbonate (TCO-NHS, 1.0 mg, 3.74 μmol) were dissolved in DMSO (300 μL) and triethylamine (TEA, 4 μL). The mixture was stirred at room temperature for 12 h, and then diethyl ether (1 mL) was added to the mixture. The crude product was washed with diethyl ether (1 mL x 3) and further purified using semi-preparative HPLC. The conditions were set as follows: solvent A = 0.1% TFA in acetonitrile; solvent B = 0.1% TFA in distilled water; gradient: maintained 100% B in the first 3 min, and then changed to 65% A + 35% B in 18 min and maintained this condition for 3 min, and then changed back to 100% B in 3 min and kept this condition for 3 min. The flow rate was fixed at 1 mL min^{-1} for analysis and 3.0 mL min^{-1} for purification. The absorbance of TCO-GE11 at 280 nm was monitored. HRMS (ESI) calculated for $C_{84}H_{111}N_{18}O_{20}$ $[M+H]^+$ 1691.8217, found 1691.8223.

Preparation of RB-GE11

The Fmoc protecting group of tyrosine was first removed from the GE11 peptide-resin (50 mg) with piperidine (20%) in DMF. The peptide resin was then washed with DMF (5 mL × 2) and CH_2Cl_2 (5 mL × 2). Rhodamine B (25 mg, 52.2 μmol) was conjugated to the Fmoc-deprotected GE11 peptide-resin by the SPPS protocol using HOBt and HBTU as the carboxyl group activating agents. The rhodamine B labelled peptide-resin was then treated with a solution of TFA (88%), phenol (5%), TIS (2%) and water (5%) for 1 h to remove all the protecting groups on the peptide and the resin. The RB-GE11 was precipitated with diethyl ether (2 mL) and washed with diethyl ether (1 mL x 3). The crude product was further purified using semi-preparative HPLC. The conditions were set as follows: solvent A = 0.1% TFA in acetonitrile; solvent B = 0.1% TFA in distilled water; gradient: maintained 20% A + 80% B in the first 5 min, and then changed to 65% A + 35% B in 10 min and maintained this condition for 5 min, and then changed back to 20% A + 80% B in 5 min and kept this condition for 5 min. The flow rate was fixed at 1 mL min^{-1} for analysis and 3.0 mL min^{-1} for purification. The absorbance of RB-GE11 at 560 nm was monitored. HRMS (ESI) calculated for $C_{103}H_{127}N_{20}O_{20}$ $[M]^+$ 1964.9560, found 1964.9570.

Cell line and culture conditions

The A431 human epidermoid carcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM, ThermoFisher, cat. no. 11965092) supplemented with fetal calf serum (10%), penicillin-streptomycin (100 units mL^{-1} and 100 μg mL^{-1} respectively) and sodium pyruvate (1 mM). The cells were grown at 37 °C in a humidified 5% CO_2 atmosphere.

Fluorescence microscopic study with RB-GE11

Approximately 3×10^5 A431 cells in DMEM (2 mL) were seeded on a 35 mm glass bottom dish (MatTek Corporation P35G-0-14-C) and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. After removal of the medium, the cells were incubated with RB-GE11 in DMEM (16 μM, 2 mL) for different periods of time (10, 20, 30 or 40 min). After rinsing the cells with PBS (1 mL) twice, the cells were examined with a Zeiss laser scanning microscope (LSM 880 NLO with Airyscan) equipped with a 561 nm laser. The emission signals at 570-620 nm were collected and the images were digitised and analysed using the Zen software.

Fluorescence microscopic study of 6 with or without pre-treatment of the cells with TCO-GE11

Approximately 3×10^5 A431 cells in DMEM (2 mL) were seeded on a 35 mm glass bottom dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. Compound **6** (64 nmol) was dissolved in DMF (50 μL) and the solution was diluted with the culture medium (0.95 mL) to a concentration of 64 μM, which was further diluted to 16 μM with the medium. After removal of the medium, the cells were incubated with TCO-GE11 (16 μM) in the medium (2 mL) or the neat medium for 20 min. The medium was removed and then the cells were incubated with **6** in the medium (16 μM, 2 mL) for 4 h. After rinsing the cells with phosphate buffered saline (PBS) (1 mL x 2), the cells were examined with a Zeiss laser scanning microscope equipped with a 633 nm laser. The emission signals at 640-735 nm were collected and the images were digitised and analysed using the Zen software.

Study of intracellular reactive oxygen species (ROS) generation with or without pre-treatment of the cells with TCO-GE11

The intracellular ROS generation was examined using a commercially available probe, namely 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA). Approximately 3×10^5

A431 cells in DMEM (2 mL) were seeded on a 35 mm glass bottom dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. After removal of the medium, the cells were incubated with TCO-GE11 in the medium (16 μM, 2 mL) or the neat medium for 20 min. The medium was removed and then the cells were incubated with **6** in the medium (16 μM, 2 mL) for 4 h. After rinsing with PBS (1 mL), the cells were incubated with a solution of H_2DCFDA in PBS (10 μM, 2 mL) at 37 °C for 30 min. The cells were rinsed and refilled with PBS (1 mL) 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 610 nm. The fluence rate ($\lambda > 610$ nm) was 18 mW cm^{-2}. Illumination of 20 min led to a total fluence of 21.6 J cm^{-2}. The cells were examined with a Zeiss laser scanning microscope equipped with a 488 nm laser. The emission signals of DCFDA at 490-630 nm were collected and the images were digitised and analysed using the Zen software.

Optimisation of the pre-incubation time for Ac_4ManNAz

Approximately 3×10^5 A431 cells in DMEM (2 mL) were seeded on a 35 mm glass bottom dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. After removal of the medium, the cells were incubated with Ac_4ManNAz in the medium (50 μM, 2 mL) for 24 h or 48 h. The cells were rinsed with PBS (1 mL x 2), and then they were incubated with DBCO-Cy 5.5 in the medium (10 μM, 2 mL) for 10 min. After rinsing with PBS (1 mL x 2), the cells were examined with a Zeiss laser scanning microscope equipped with a 633 nm laser. The emission signals at 640-720 nm were collected and the images were digitised and analysed using the Zen software.

Fluorescence microscopic study of 6 with or without pre-treatment of the cells with Ac_4ManNAz

Approximately 3×10^5 A431 cells in DMEM (2 mL) were seeded on a 35 mm glass bottom dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The medium was removed and the cells were incubated with Ac_4ManNAz in the medium (50 μM, 2 mL) for 24 h. After rinsing with PBS (1 mL x 2), the cells were treated with a mixture of $CuSO_4$ (20 μM), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 50 μM), sodium ascorbate (0.4 mM) and **6** (16 μM) in PBS (2 mL) for 10 min. The control experiment was also performed without the pre-incubation with Ac_4ManNAz and the addition of the coupling reagents. After rinsing the cells with PBS (1 mL x 2), the cells were examined with an Zeiss laser scanning microscope equipped with a 633 nm laser. The emission signals at 640-735 nm were collected and the images were digitised and analysed using the Zen software.

Study of intracellular ROS generation with or without pre-treatment of the cells with Ac_4ManNAz

Approximately 3×10^5 A431 cells in DMEM (2 mL) were seeded on a glass bottom dish and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The medium was removed and the cells were incubated with Ac_4ManNAz in the medium (50 μM, 2 mL) for 24 h. After rinsing with PBS (1 mL x 2), the cells were treated with a mixture of $CuSO_4$ (20 μM), THPTA (50 μM), sodium ascorbate (0.4 mM) and **6** (16 μM) in PBS (2 mL) for 10 min. For the control experiment, the cells were only treated with **6** in PBS (16 μM, 2 mL) for 10 min without the addition of Ac_4ManNAz and the coupling reagents. The cells were rinsed with PBS, and then they were incubated with a solution of H_2DCFDA in PBS (10 μM, 2 mL) at 37 °C for 30 min. The cells were rinsed and refilled with PBS (1 mL) 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 610 nm. The fluence rate ($\lambda > 610$ nm) was 18 mW cm^{-2}. Illumination of 30 min led to a total fluence of 32.4 J cm^{-2}.

The cells were examined with a Zeiss laser scanning microscope equipped with a 488 nm laser. The emission signals of DCFDA at 490-630 nm were collected and the images were digitised and analysed using the Zen software.

Studies of cytotoxicity of 6

Approximately 3×10^4 A431 cells per well in 100 μL cell culture medium were added to 96-well plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The culture medium was removed and the following procedure (i) or (ii) was performed. Compound **6** (64 nmol) was dissolved in DMF (50 μL) and the solution was diluted with the medium (0.95 mL) to a concentration of 64 μM, which was further diluted to 16 μM with the medium.

(i) With or without pre-treatment with TCO-GE11:

The cells were treated with TCO-GE11 in DMEM (16 μM, 100 μL) or the neat medium for 20 min. After removal of the medium, the cells were incubated with different concentrations of **6** in DMEM (100 μL) for 4 h. The medium was removed and the cells were rinsed with PBS (50 μL x 2). Before the cells were illuminated at ambient temperature for 20 min, the wells were refilled with fresh medium (100 μL) to maintain the cellular activity. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on at 610 nm. The fluence rate ($\lambda > 610$ nm) was 18 mW cm^{-2}. Illumination of 20 min led to a total fluence of 21.6 J cm^{-2}.

(ii) With or without pre-treatment with Ac_4ManNAz:

The cells were treated with Ac_4ManNAz in DMEM (50 μM, 100 μL) or the neat medium for 24 h. After rinsing the cells with PBS (50 μL x 2), the cells were treated with a mixture of $CuSO_4$ (20 μM), THPTA (50 μM), sodium ascorbate (0.4 mM) and **6** (16 μM) in PBS (100 μL) for 10 min. For the control experiment, the cells were only treated with different

concentrations of **6** in PBS (100 μL) for 10 min without pre-treatment with Ac_4ManNAz and in the absence of the coupling reagents. The medium was removed and the cells were rinsed with PBS (50 μL x 2). Before the cells were illuminated at ambient temperature for 30 min, the wells were refilled with fresh medium (100 μL) to maintain the cellular activity. Illumination of 30 min led to a total fluence of 32.4 J cm^{-2}.

After illumination, the cells were incubated overnight. Cell viability was determined by means of the colourimetric MTT assay [MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide].[R6] After rinsing the cells with PBS (50 μL x 2), a MTT solution in PBS (3 mg mL^{-1}, 50 μL) and medium (100 μL) were added to each well followed by incubation for 4 h under the same conditions. DMSO (150 μL) was then added to each well to lyse the cells. The plates were placed on a microplate reader (BioTek Synergy™ H1) at ambient temperature and the absorbance at 490 nm of each well was taken. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the reading 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} data (i = 1, 2, …, n), $A_{control}$ is the absorbance of the control wells, in which the drug was absent, and n is the number of data points.

In vivo fluorescence labelling of tumour-bearing nude mice with 6

Female Balb/c nude mice (20-25 g) were obtained from the Laboratory Animal Services Centre at The Chinese University of Hong Kong. All animal experiments had been approved by the Animal Experimentation Ethics Committee of the City University of Hong Kong. The mice were kept under pathogen-free conditions with free access to food and water. A431 cells (5×10^6 cells in 200 μL) suspended in Hank's balanced salt solution (HBSS) were inoculated subcutaneously on the back of the mice. When the size of the tumour reached 60-100 mm^3,

the mice were injected intratumourally with a dose of TCO-GE11 in PBS (50 nmol, 20 μL). After 20 min, a solution of **6** in PBS (100 μL, equivalent to 40 nmol) was injected into the tail vein of the tumour-bearing mice. This solution was prepared by dissolving **6** (320 nmol) in DMF (40 μL) in the presence of Tween 80 (4 μL), followed by dilution with PBS (756 μL) to a concentration of 400 μM. For the control experiments, the mice were not pre-treated with TCO-GE11. The fluorescence images of the mice were captured before and after the injection of **6** at different time points for 48 h with an Odyssey infrared imaging system (excitation wavelength at 680 nm, emission wavelength at ≥ 700 nm). The mice were sacrificed after 48 h and the tumour and organs were harvested. The *ex vivo* biodistribution of the compound was examined based on the fluorescence intensity obtained with the Odyssey infrared imaging system. The images were digitised and analysed using the Odyssey imaging system software (no. 9201-500). Four mice were used for each experiment.

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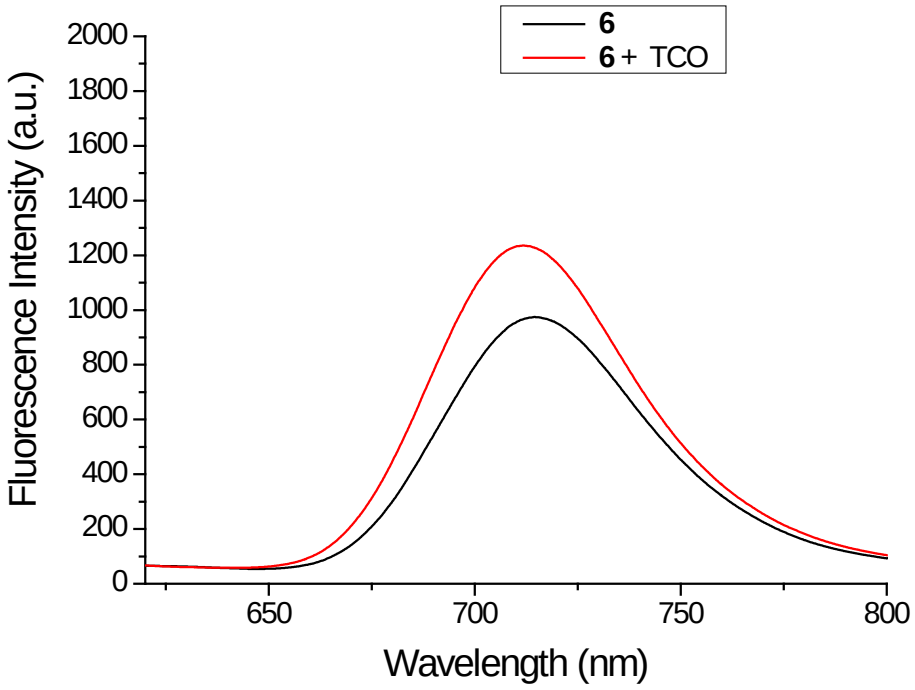


Fig. S1 Fluorescence spectra of **6** (5 μM) before and after cycloaddition reaction with TCO (40 μM) for 30 min in DMF (λ_{ex} = 610 nm).

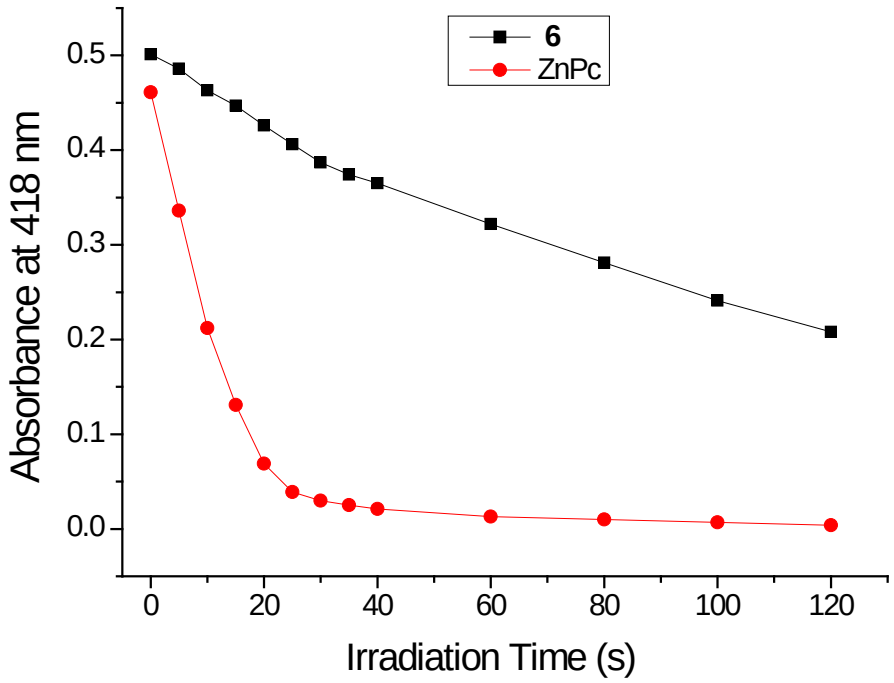
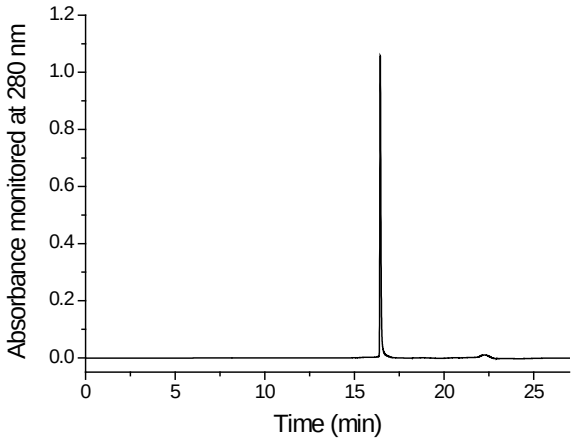


Fig. S2 Comparison of the rates of photo-degradation of DPBF (initial concentration = 25 μM) in DMF, as monitored spectroscopically at 418 nm, using **6** and ZnPc (both at 5 μM) as the photosensitisers.

(a)



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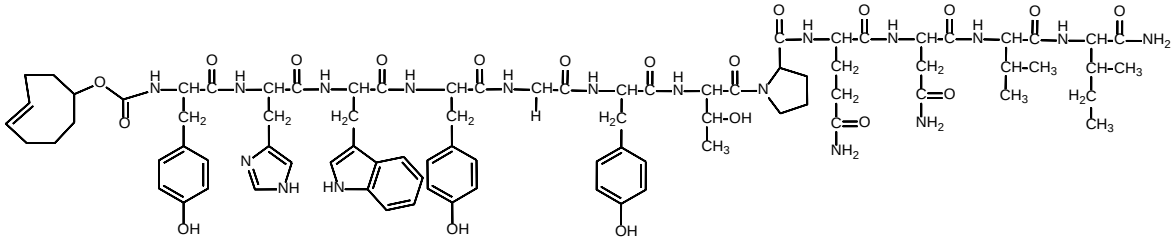


Molecular formula :	$C_{84}H_{110}N_{18}O_{20}$
Experimental Mass $[M+H]^+$, $[M+H+Na]^{2+}$:	1691.82225, 857.40556
Theoretical Mass $[M+H]^+$, $[M+H+Na]^{2+}$:	1691.82165, 857.40544
Error (ppm) :	0.4, 0.1

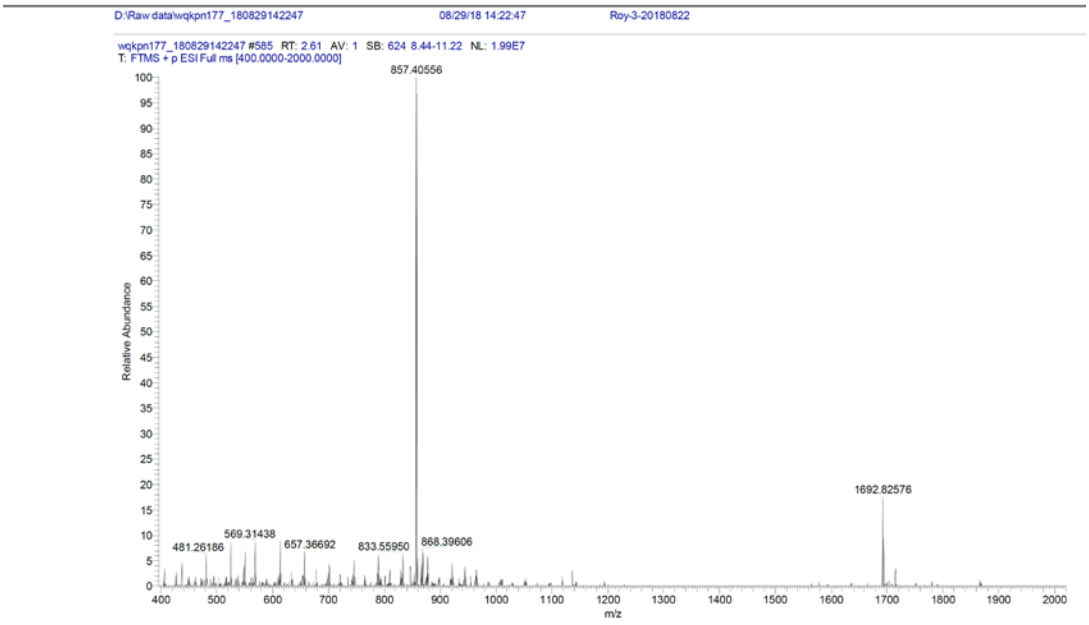
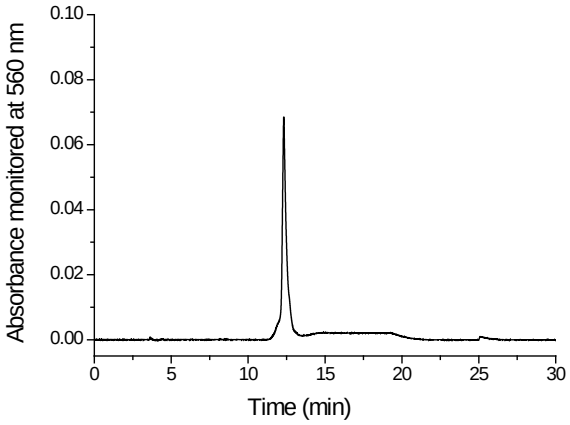
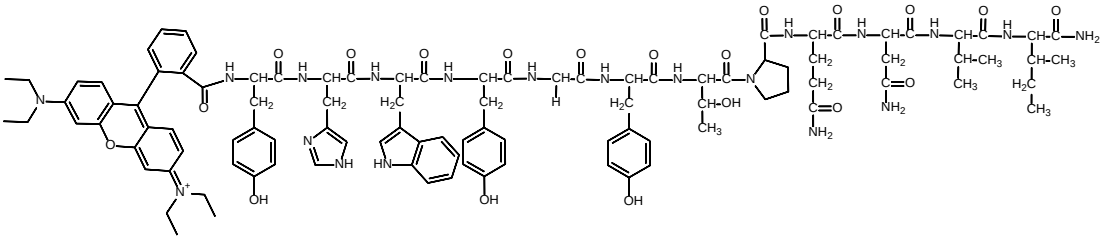


Fig. S3 (a) HPLC chromatograph and (b) ESI mass spectrum of TCO-GE11.

(a)



(b)



Accurate Mass Measurement

Molecular formula :	$C_{103}H_{127}N_{20}O_{20}$
Experimental Mass $[M]^+$,$[M+H]^{2+}$:	1964.95699, 982.98192
Theoretical Mass $[M]^+$,$[M+H]^{2+}$:	1964.95601, 982.98164
Error (ppm) :	0.5, 0.3

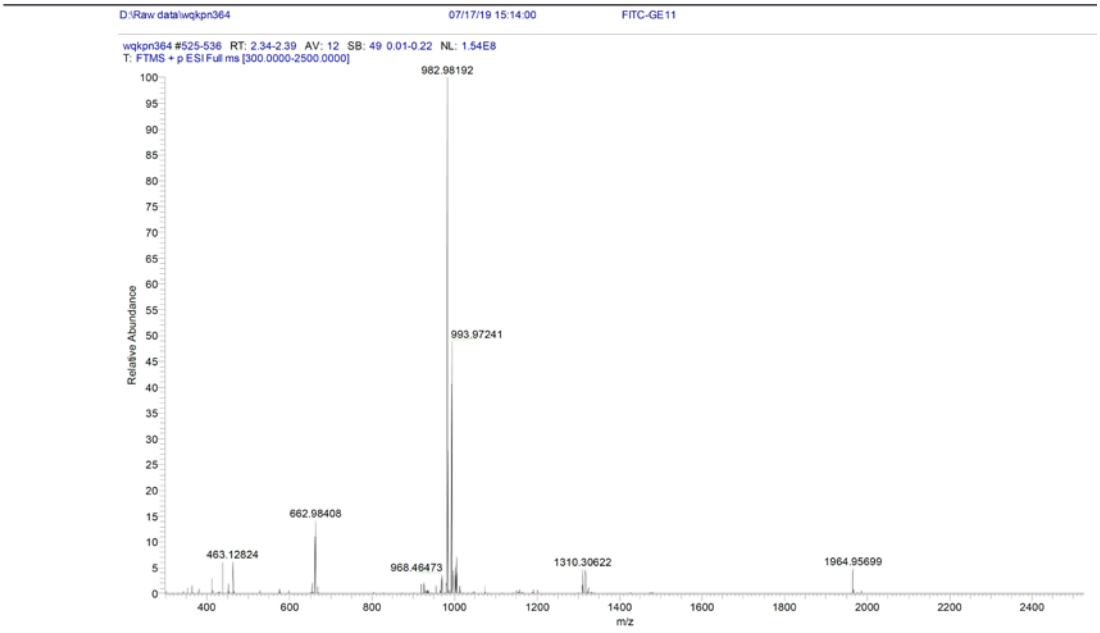


Fig. S4 (a) HPLC chromatograph and (b) ESI mass spectrum of RB-GE11.

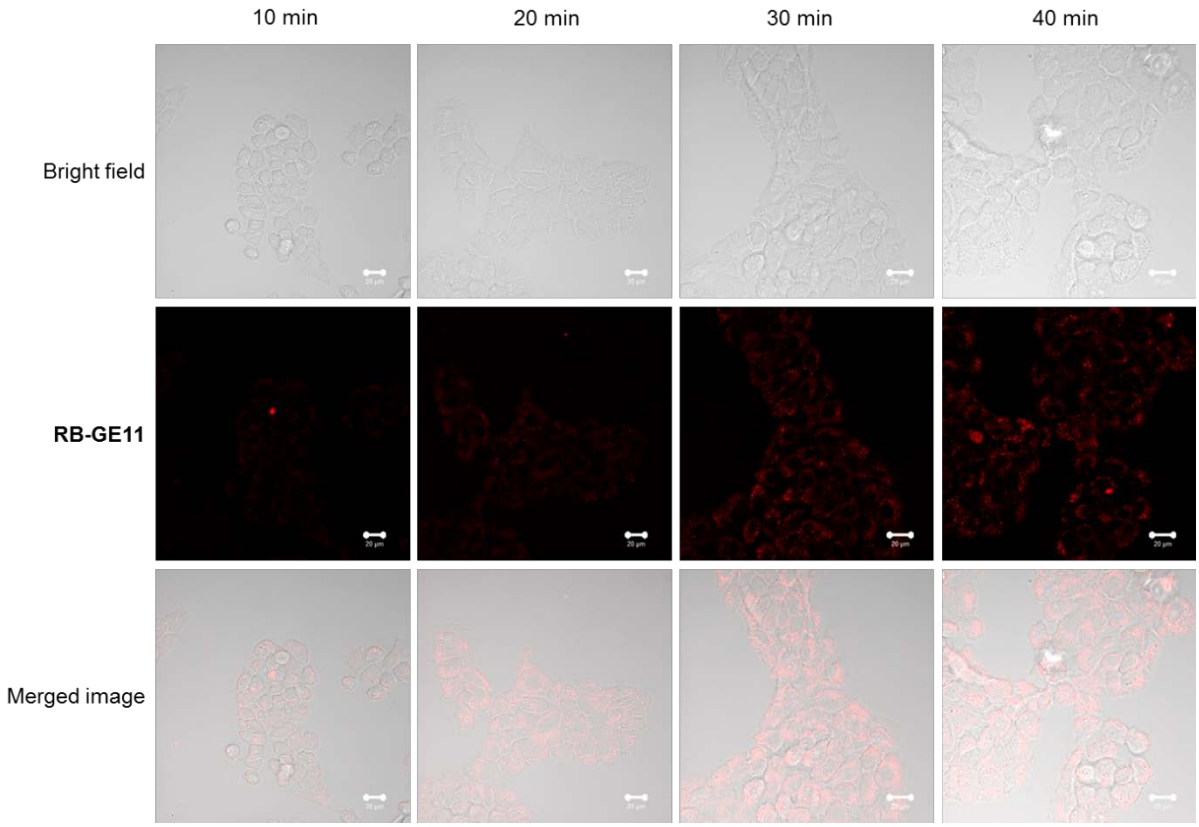


Fig. S5 Bright field, fluorescence and merged images of A431 cells after incubation with RB-GE11 (16 μM) for 10-40 min.

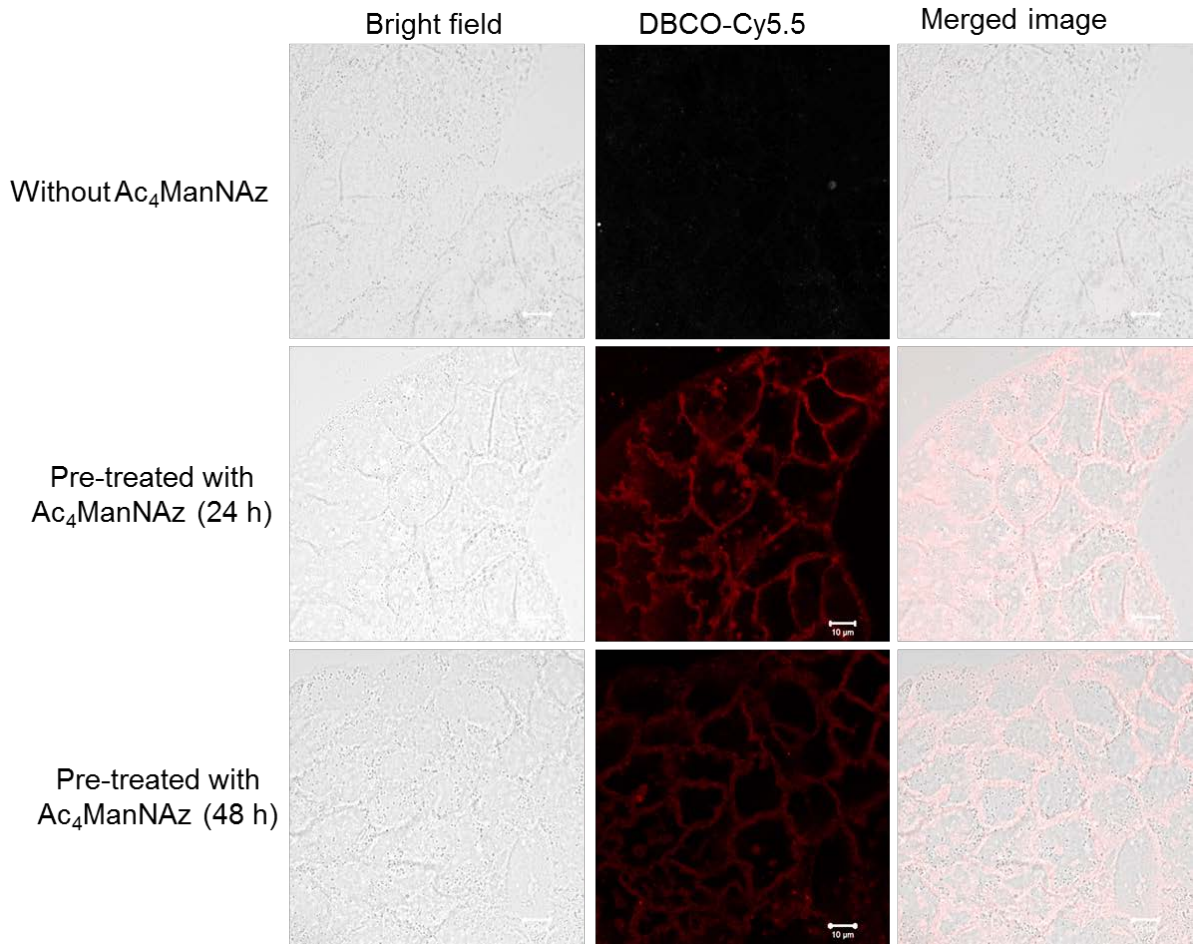


Fig. S6 Bright field, fluorescence and merged images of A431 cells with or without pre-treatment with Ac_4ManNAz (50 μM), followed by incubation with DBCO-Cy5.5 (10 μM) for 10 min.

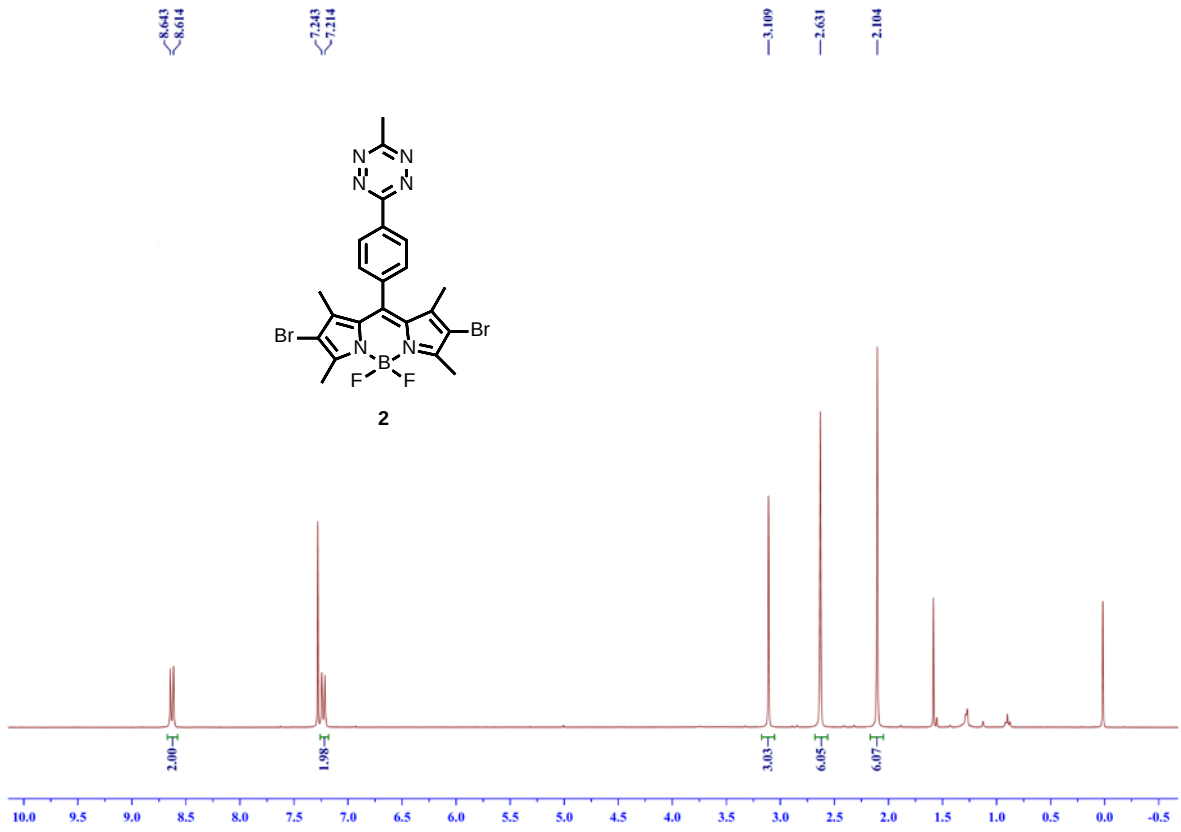


Fig. S7 ^{1}H NMR spectrum of **2** in $CDCl_3$.

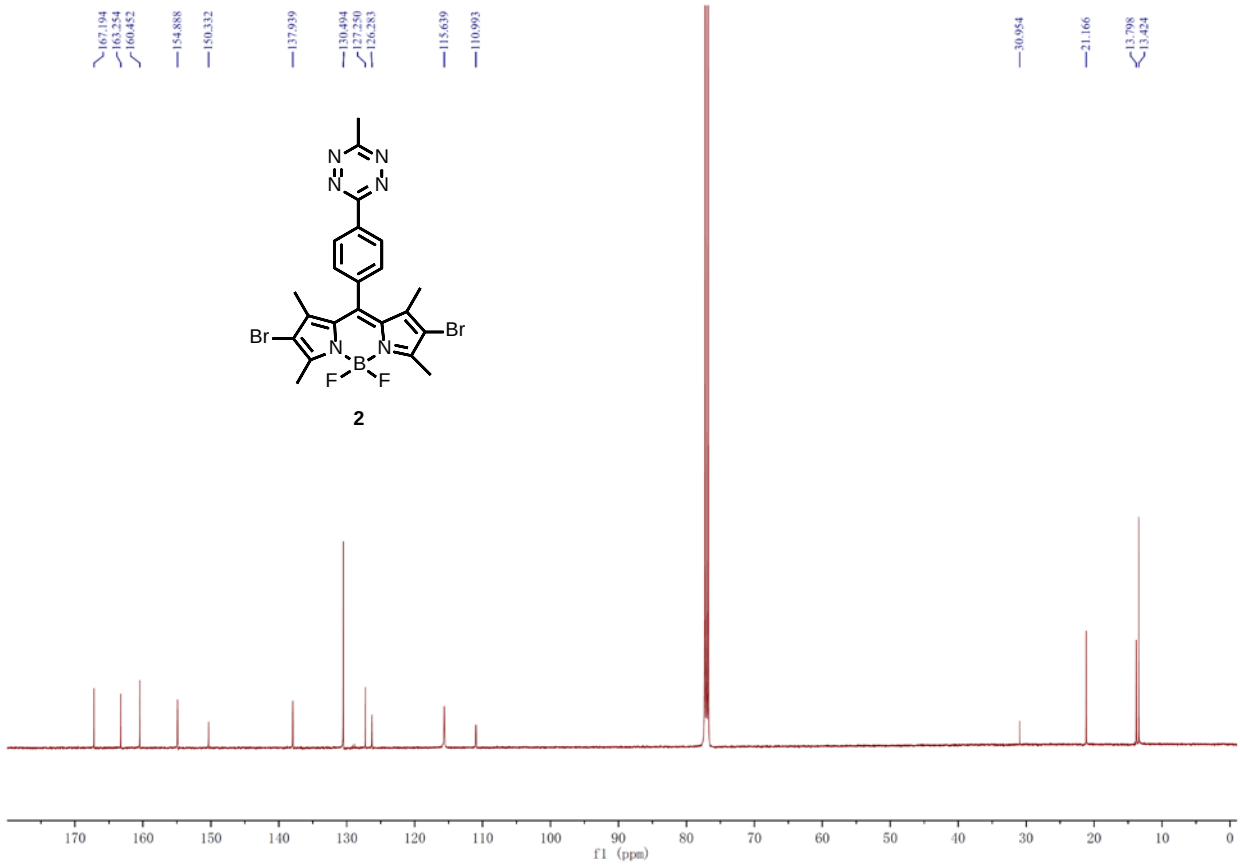


Fig. S8 $^{13}C\{^{1}H\}$ NMR spectrum of **2** in $CDCl_3$.

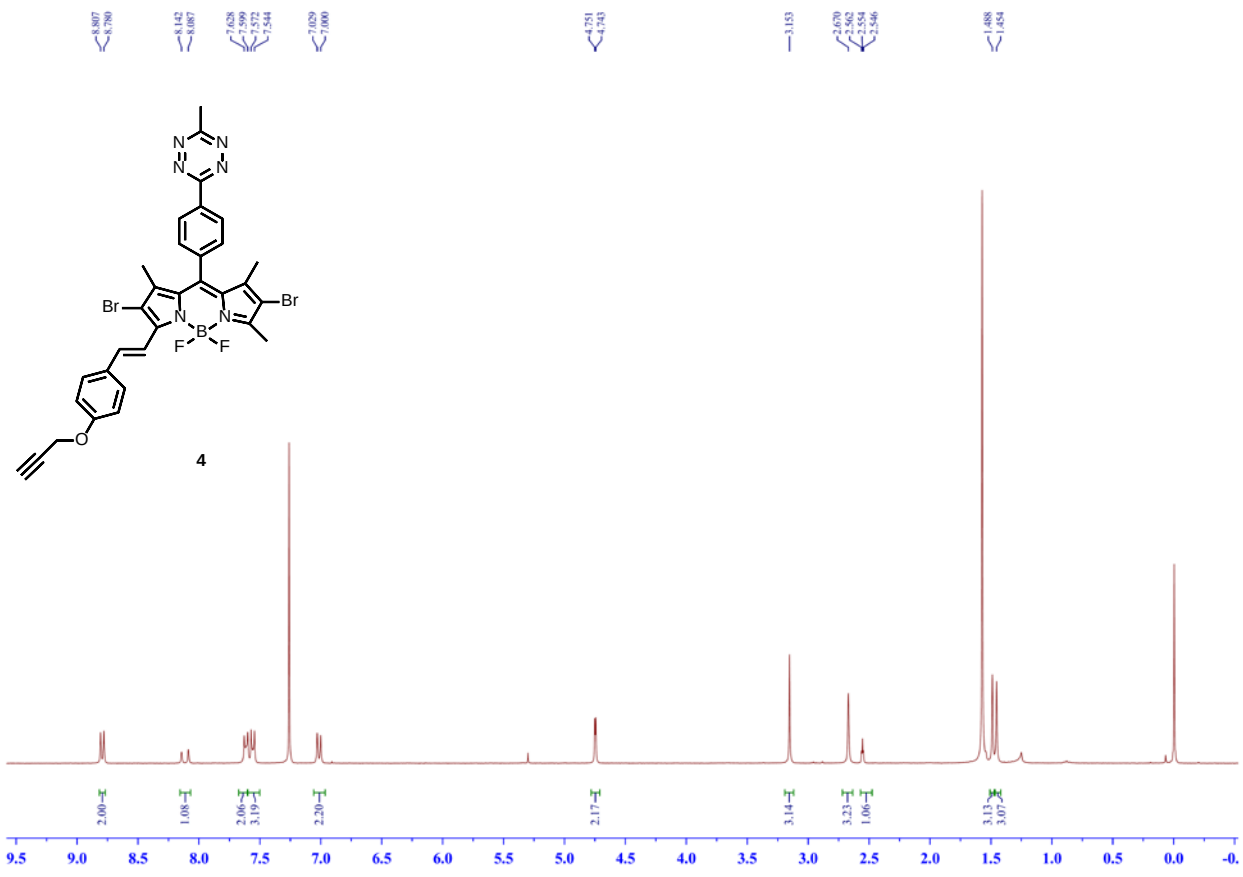


Fig. S9 ^{1}H NMR spectrum of **4** in $CDCl_3$.

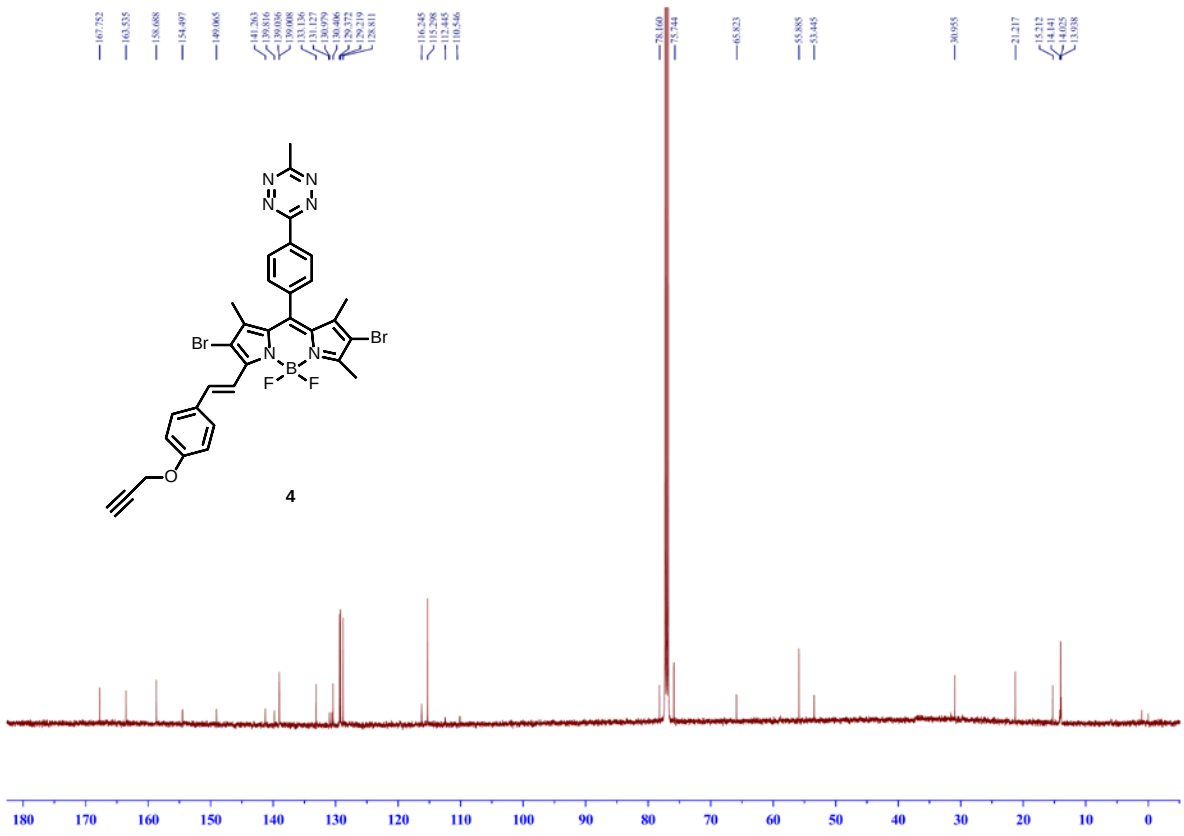


Fig. S10 $^{13}C\{^{1}H\}$ NMR spectrum of **4** in $CDCl_3$.

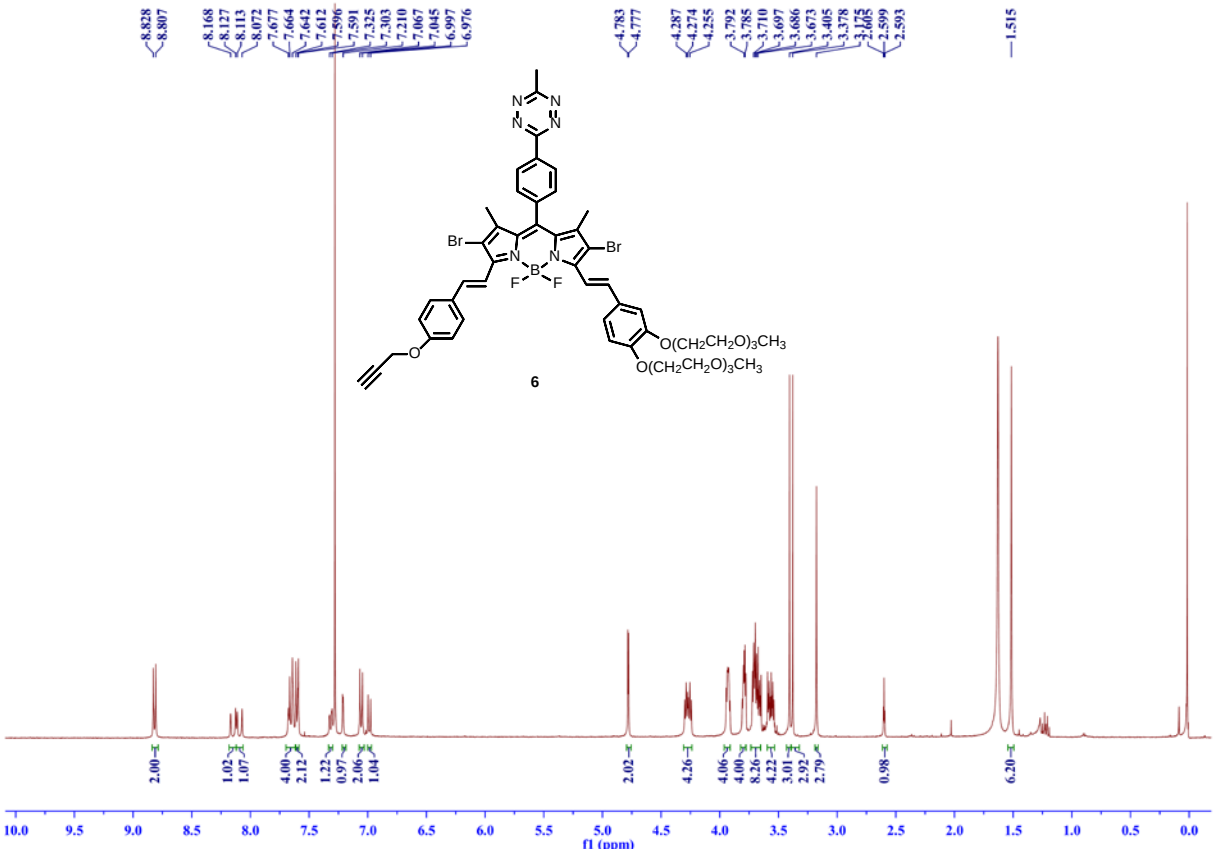


Fig. S11 ^{1}H NMR spectrum of **6** in $CDCl_3$.

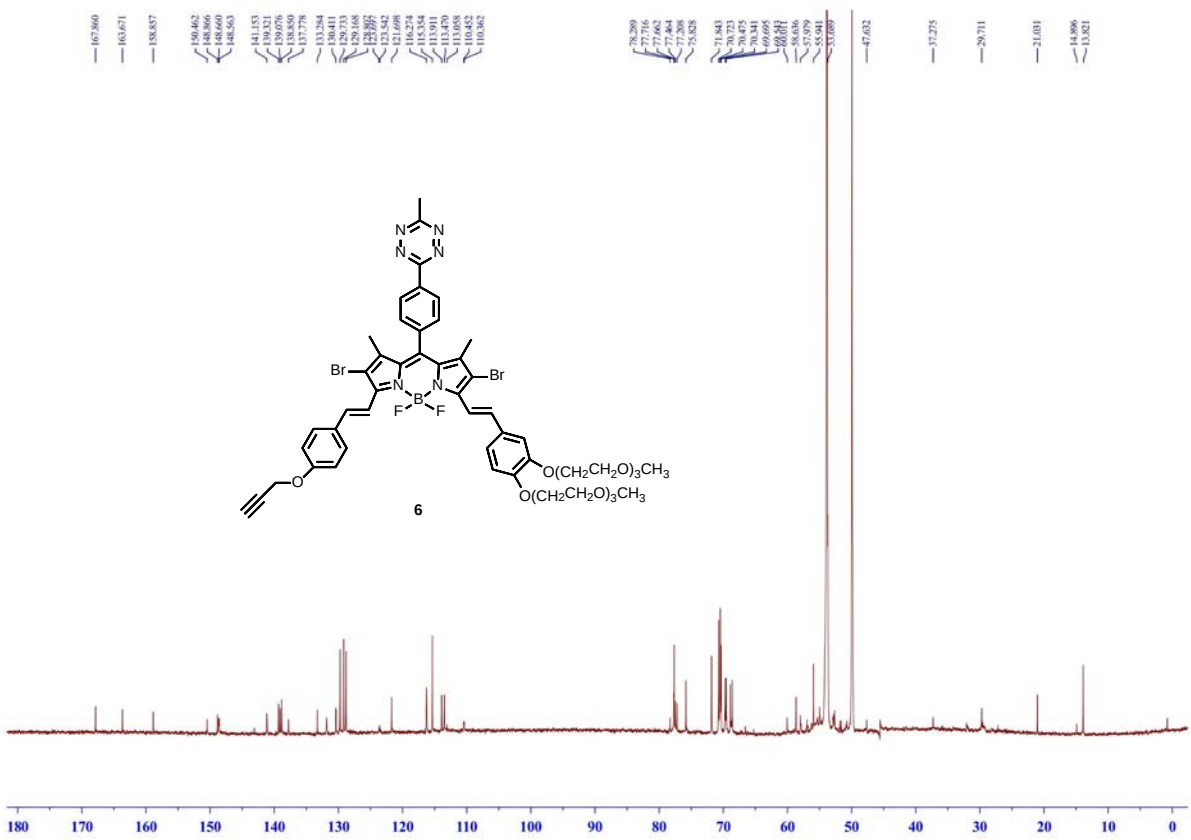


Fig. S12 $^{13}C\{^{1}H\}$ NMR spectrum of **6** in CD_2Cl_2/CD_3OD.