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Electronic Supplementary Information

A bioorthogonally activatable photosensitiser for site-specific photodynamic therapy

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Experimental Section

General. All the reactions were performed under an atmosphere of nitrogen. Dichloromethane and DMF were purified with an INERT solvent purification system. All other solvents and reagents were of reagent grade and used as received. All the reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica gel 60 F254 plates. Chromatographic purification was performed on silica gel (Macherey-Nagel, 230-400 mesh) with the indicated eluents. Compounds **1**,[R1] **3**[R2] and **4**[R3] were prepared as described.

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker AVANCE III 700 spectrometer (^{1}H, 700 MHz; ^{13}C, 176.0 MHz) or a Bruker AVANCE III 400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) in $CDCl_3$ or CD_3OD. Spectra were referenced internally by using the residual solvent [^{1}H, δ = 7.26 (for $CDCl_3$), δ = 3.27 (for CD_3OD)] or solvent [^{13}C, δ = 77.2 (for $CDCl_3$), δ = 49.0 (for CD_3OD)] resonances relative to $SiMe_4$. Electrospray ionisation (ESI) and matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectra were recorded on a Thermo QEF MS mass spectrometer and a Bruker Autoflex speed MALDI-TOF mass spectrometer respectively. Electronic absorption and steady-state fluorescence spectra were taken on a Shimazu UV-1800 UV-Vis spectrophotometer and a Horiba FluoroMax spectrofluorometer respectively.

Preparation of 2. A mixture of **1** (104 mg, 0.25 mmol) and NBS (89 mg, 0.50 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 2 h. The solvent was then evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/CH_2Cl_2 (1:1 v/v) as eluent to afford **2** (102 mg, 71%). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.79 (d, J = 8.4 Hz, 2 H, ArH), 7.54 (d, J = 8.4 Hz, 2 H, ArH), 3.15 (s, 3 H, CH_3), 2.63 (s, 6 H, CH_3), 1.45 (s, 6 H, CH_3). $^{13}C\{^{1}H\}$ NMR (176.0 MHz, $CDCl_3$): δ 167.8, 163.5, 154.6, 140.5,

140.4, 138.7, 133.1, 130.0, 129.1, 128.8, 112.1, 21.3, 14.0, 13.8. HRMS (ESI): *m*/*z* calcd for $C_{22}H_{18}BBr_2F_2N_6$ $[M-H]^-$: 575.0011, found: 575.0003.

Preparation of 5. Compound **3** (48.7 mg, 0.17 mmol) and DIPEA (300 μL, 1.7 mmol) were added to a solution of **4** (50 mg, 0.17 mmol) in DMF (5 mL) with stirring at room temperature. When the reaction was completed as monitored by TLC (about 20 h), the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (18:1 v/v) as eluent to give the product as a pale-yellow solid (47 mg, 63%). ^{1}H NMR (700 MHz, CD_3OD): δ 5.53-5.57 (m, 1 H, C=CH), 5.41-5.46 (m, 1 H, C=CH), 4.45 (dd, *J* = 4.9, 7.7 Hz, 1 H, biotin-NCH), 4.25-4.28 (m, 2 H, biotin-NCH and OCH), 3.11-3.20 (m, 5 H, NCH_2CH_2N and biotin-SCH), 2.89 (dd, *J* = 4.9, 12.6 Hz, 1 H, biotin-SCH), 2.66 (d, *J* = 12.6 Hz, 1 H, biotin-SCH), 2.27-2.31 (m, 2 H, CH_2), 2.14-2.17 (m, 2 H, CH_2), 1.86-1.94 (m, 4 H, CH_2O), 1.52-1.72 (m, 8 H, CH_2), 1.38-1.40 (m, 2 H, CH_2). ^{13}C{^{1}H} NMR (176.0 MHz, CD_3OD): δ 176.4, 166.1, 158.8, 136.1, 133.8, 81.8, 63.3, 61.6, 56.9, 42.2, 41.2, 41.1, 40.4, 39.6, 36.8, 35.2, 33.5, 32.1, 29.7, 29.4, 26.8. HRMS (ESI): *m*/*z* calcd for $C_{21}H_{34}N_4NaO_4S$ $[M+Na]^+$: 461.2193, found: 461.2193.

Preparation of 6. Compound **5** (8.8 mg, 0.02 mmol) was added to a solution of **2** (5.7 mg, 0.01 mmol) in DMF (2 mL). After stirring at room temperature for 10 h, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (10:1 v/v) as eluent to give the product as a dark-red solid (6.8 mg, 69%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.92-7.94 (m, 1 H, ArH), 7.63-7.67 (m, 1 H, ArH), 7.47-7.49 (m, 1 H, ArH), 7.24-7.26 (m, 1 H, ArH), 4.59-4.66 (m, 1 H, OCH), 4.41-4.45 (m, 1 H, biotin-NCH), 4.22-4.28 (m, 1 H, biotin-NCH), 3.27 (s, 3 H, CH_3), 3.14-3.19 (m, 5 H, NCH_2CH_2N and biotin-SCH), 2.82-2.96 (m, 2 H, CH_2), 2.73-2.75 (m, 1 H, biotin-SCH), 2.65-2.68 (m, 1 H, biotin-SCH), 2.53 (s, 3 H, CH_3), 2.51 (s, 3 H, CH_3), 2.12-2.16 (m, 4 H, CH_2), 1.85-2.01 (m, 4 H, CH_2), 1.56-1.70 (m, 8 H, CH_2), 1.47 (s, 3 H, CH_3), 1.42 (s, 3 H, CH_3), 1.35-

1.41 (m, 2 H, CH_2). $^{13}C\{^1H\}$ NMR (100.6 MHz, CD_3OD): δ 176.4, 166.0, 155.3, 154.9, 143.4, 141.9, 140.5, 140.2, 136.1, 131.5, 130.8, 129.5, 129.3, 127.8, 112.6, 112.3, 77.2, 64.3, 63.3, 61.6, 56.9, 54.8, 41.3, 41.0, 40.4, 36.8, 35.2, 35.0, 30.8, 29.7, 29.5, 26.8, 26.6, 26.0, 24.6, 23.2, 20.1, 14.3, 13.8. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{43}H_{51}BBr_2FN_8O_4S$ $[M\text{-}F]^+$: 965.2183, found: 965.2188.

Inverse-electron-demand Diels-Alder reaction of 2 and TCOH. This bioorthogonal reaction was performed in a 1 cm × 1 cm quartz cuvette. A stock solution of **2** was prepared in DMF (2 mM), which was used to prepare a solution of **2** in PBS (pH 7.4) (1 μM). Another stock solution of TCOH in DMF (20 mM) was also prepared, which was then added to the PBS solution of **2** prepared above to make the final concentrations of TCOH ranging from 5 μM to 50 μM. The emission spectra were recorded from 520 nm to 700 nm along with time upon excitation at 510 nm.

Determination of Φ_f. The values of Φ_f were determined by the equation $\Phi_{f(s)} = (F_s/F_{ref})(A_{ref}/A_s)(n_s^2/n_{ref}^2)\Phi_{f(ref)}$,[R4] where subscript s refers to the sample solution while ref stands for the reference. F, A and n are the measured fluorescence (area under the emission peak), the absorbance at the excitation position (510 nm) and the refractive index of the solvent respectively. Fluorescein in NaOH(aq) (0.1 M, pH 13) ($\Phi_F = 0.925$) was used as the reference.[R1] To minimise reabsorption of radiation by the ground-state species, the emission spectra were obtained in very dilute solutions of which the absorbance at 510 nm was about 0.03.

Measurement of singlet oxygen generation. DPBF was used as the singlet oxygen scavenger. A solution of DPBF (30 μM) and BODIPY **2** (2 μM) in PBS with or without TCOH (40 μM) was irradiated with red light from a 100 W halogen lamp after passing through a water tank for cooling and a colour filter with cut-on wavelength at 515 nm (Newport). The absorption maximum of DPBF at 415 nm was monitored along with time.

Cell lines and culture conditions. All cell culture reagents were purchased from ThermoFisher Scientific Inc., Carlsbad, CA, USA unless otherwise specified. HeLa cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum, 100 unit mL^{-1} penicillin and 100 μg mL^{-1} streptomycin in a humidified incubator with 5% CO_2 at 37 °C. HCT116 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, 100 unit mL^{-1} penicillin and 100 μg mL^{-1} streptomycin in a humidified incubator with 5% CO_2 at 37 °C.

Study of photocytotoxicity. A stock solution of **2** or **6** (2 mM) was prepared by dissolving the compound (40 nmol) in DMF (20 μL). The stock solution was diluted to various concentrations with the serum-free medium for various *in vitro* assays. A stock solution of **5** (8 mM) was prepared by dissolving the compound (80 nmol) in DMF (10 μL). The stock solution was then diluted to 20 μM with the culture medium for *in vitro* assays. Photodynamic treatment was carried out on cells seeded on 96-well plates (2 x 10^4 cells per well) 24 h prior to the assays. The cells were incubated with or without **5** for 12 h. After being washed twice with PBS, the cells were further incubated with **2** at different concentrations (100 μL) (1.0, 0.50, 0.25, 0.13 and 0.063 μM) in the dark for 1 h. To study the photocytotoxicity of **6**, the cells were incubated directly with **6** under the same conditions. The cells were then rinsed with PBS (100 μL x 2) and refed with the culture medium, and then they were irradiated with a halogen lamp (300 W) with a yellow glass filter (Newport, cut-on at λ = 515 nm) for 20 min at room temperature. The fluence rate used was 25.5 mW cm^{-2}, giving a total fluence of 30.6 J cm^{-2}. After irradiation, 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].[R5] After rinsing the cells with PBS (100 μL), a MTT solution (3 mg mL^{-1} in PBS, 50 μL) was added to the cells, which were incubated at 37 °C for 4 h followed

by the addition of dimethyl sulfoxide (70 μL). Absorbance at 490 nm was measured using a plate reader (Tecan Spark 10M Microplate Reader).

Intracellular fluorescence imaging. Approximately 2 x 10^5 HeLa cells or HCT116 cells in 2 mL of the culture medium were incubated on 35 cm^2 glass-bottom confocal dishes overnight at 37 °C with 5% CO_2. The medium was then removed. The cells, after being rinsed with PBS, were pre-incubated in the medium for 12 h with or without **5** (20 μM). After removal of the medium and being washed with PBS, the cells were incubated with **2** (2 μM) in a serum-free medium for 1 h, and then rinsed with PBS (1 mL x 2). For comparison, the cells were also incubated directly with **6** (2 mM) for 1 h. For the competitive assays, the HeLa cells were pre-incubated in the medium for 12 h with **5** (20 μM) and free biotin at different concentrations (0, 2 and 10 mM), followed by the incubation with **2** (2 μM). The cells were then washed with PBS once before being examined with a Leica TCS SP8 high-speed confocal microscope equipped with a 488 nm argon laser. The BODIPY was excited at 488 nm and its fluorescence was monitored at 515-610 nm. The images were digitised and analysed using a Leica Application Suite X software. The average intracellular fluorescence intensities (for a total of 20 cells x 3 times in each experiment) were also determined.

References

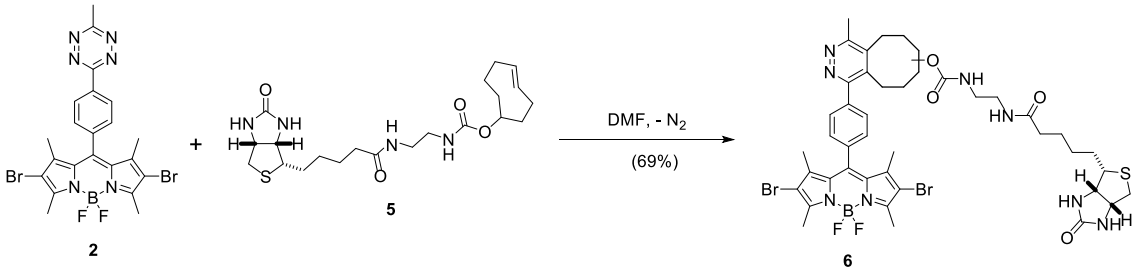
R1 J. C. T. Carlson, L. G. Meimetis, S. A. Hilderbrand and R. Weissleder, *Angew. Chem. Int. Ed.*, 2013, **52**, 6917-6920.

R2 Y. A. Povrozin, O. S. Kolosova, O. M. Obukhova, A. L. Tatarets, V. I. Sidorov, E. A. Terpetschnig and L. D. Patsenker, *Bioconjugate Chem.*, 2009, **20**, 1807-1812.

R3 D. S. Liu, A. Tangpeerachaikul, R. Selvaraj, M. T. Taylor, J. M. Fox and A. Y. Ting, *J. Am. Chem. Soc.*, 2012, **134**, 792-795.

R4 D. F. Eaton, *Pure Appl. Chem.*, 1988, **60**, 1107-1114.

R5 H. Tada, O. Shiho, K. Kuroshima, M. Koyama and K. Tsukamoto, *J. Immunol. Methods*, 1986, **93**, 157-165.



Scheme S1 Synthesis of conjugate **6**.

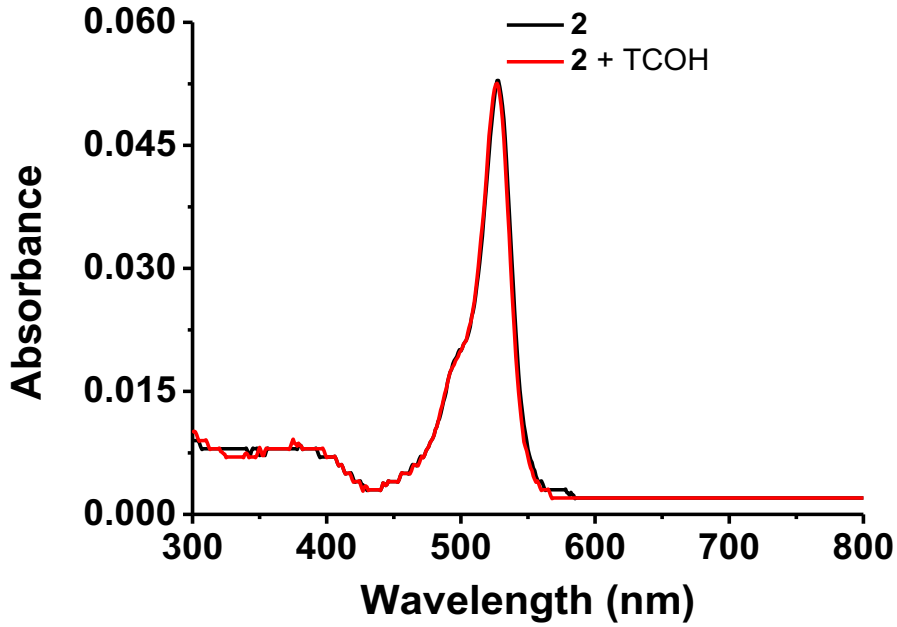


Fig. S1 Electronic absorption spectra of **2** (1 μM) in the absence and presence of TCOH (20 μM) in PBS (pH 7.4).

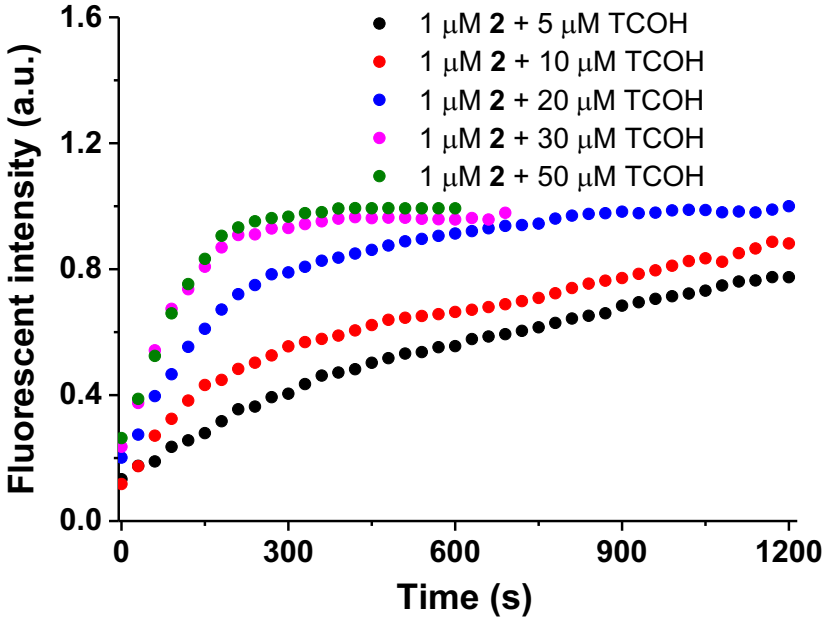


Fig. S2 Change in fluorescence intensity of **2** (1 μM) in PBS (pH 7.4) in the presence of different concentrations of TCOH (5 to 50 μM).

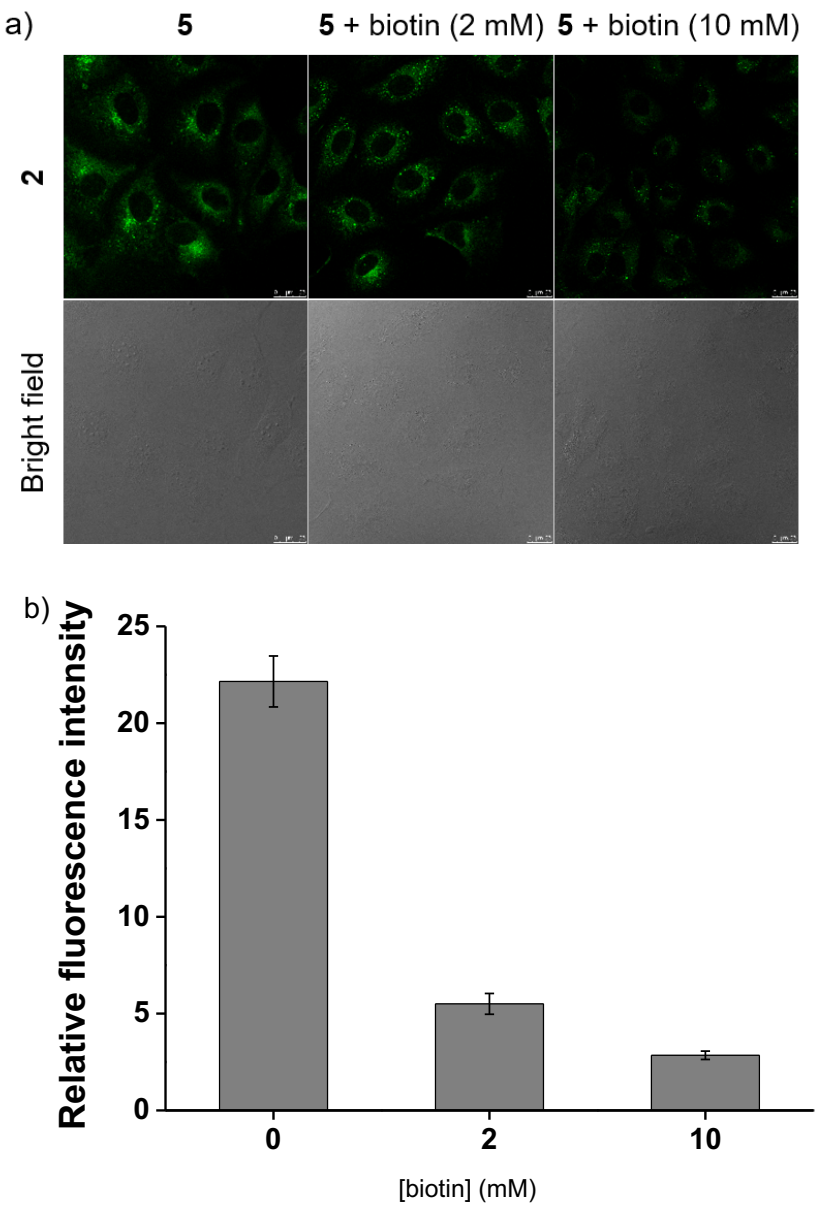


Fig. S3 (a) Bright field and fluorescence images of HeLa cells after pre-treatment with **5** (20 μM) and free biotin (0, 2 and 10 mM) for 12 h, followed by incubation with **2** (2 μM) for 1 h. (b) Comparison of the average intracellular fluorescence intensity of **2** in HeLa cells. Data are expressed as the mean ± SEM of three independent experiments, each counted with 20 cells.

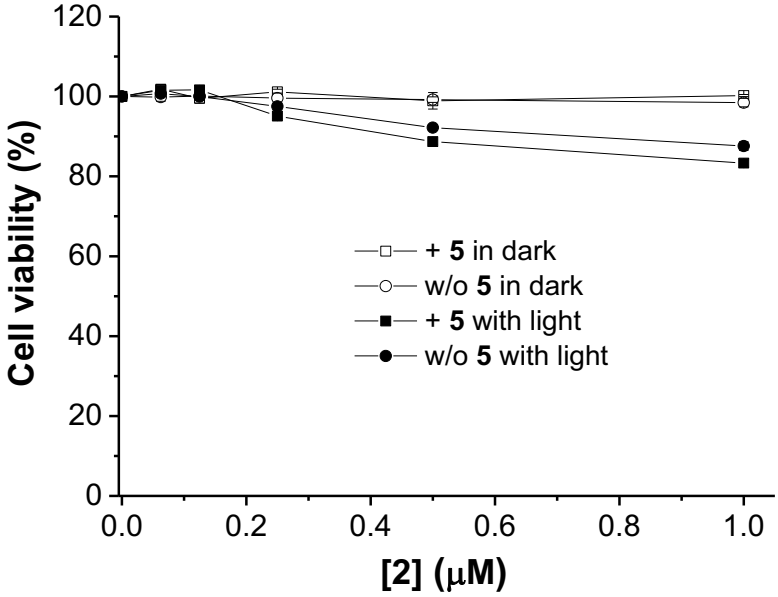


Fig. S4 Cytotoxicities of **2** against HCT116 cells in the absence (open symbols) and presence (closed symbols) of light ($\lambda > 515$ nm, 25.5 mW cm^{-2}, 30.6 J cm^{-2}) with (square symbols) and without (circle symbols) pre-treatment with **5**. Data are expressed as the mean ± SEM of three independent experiments, each performed in quadruplicate.

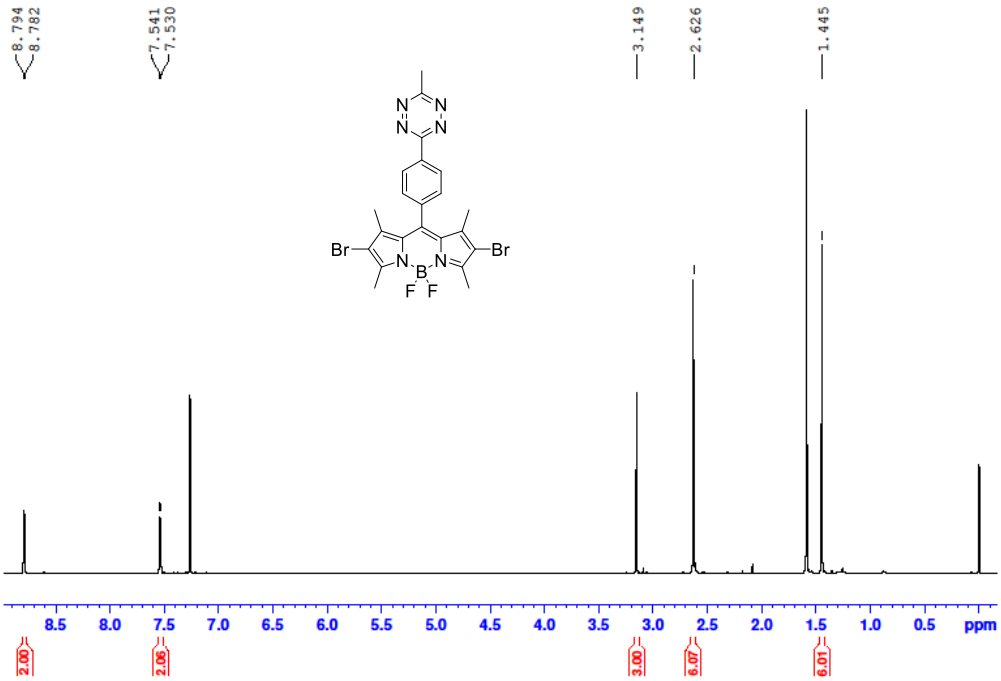


Fig. S5 ^{1}H NMR spectrum of **2** in $CDCl_3$.

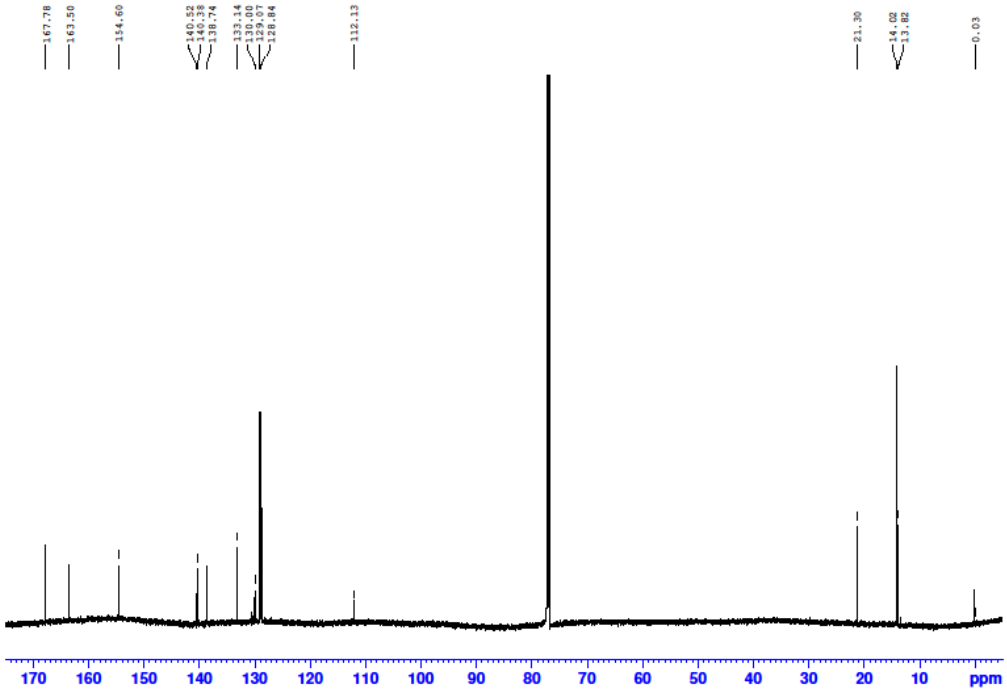


Fig. S6 $^{13}C\{^{1}H\}$ NMR spectrum of **2** in $CDCl_3$.

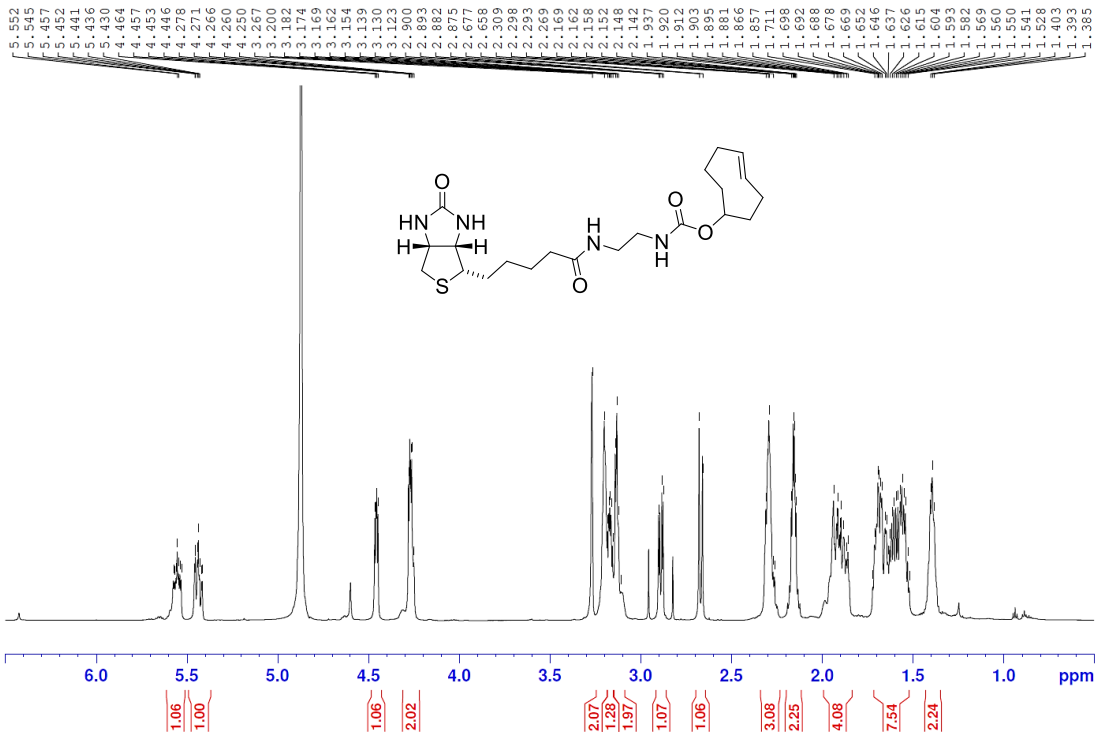


Fig. S7 ^{1}H NMR spectrum of **5** in CD_3OD.

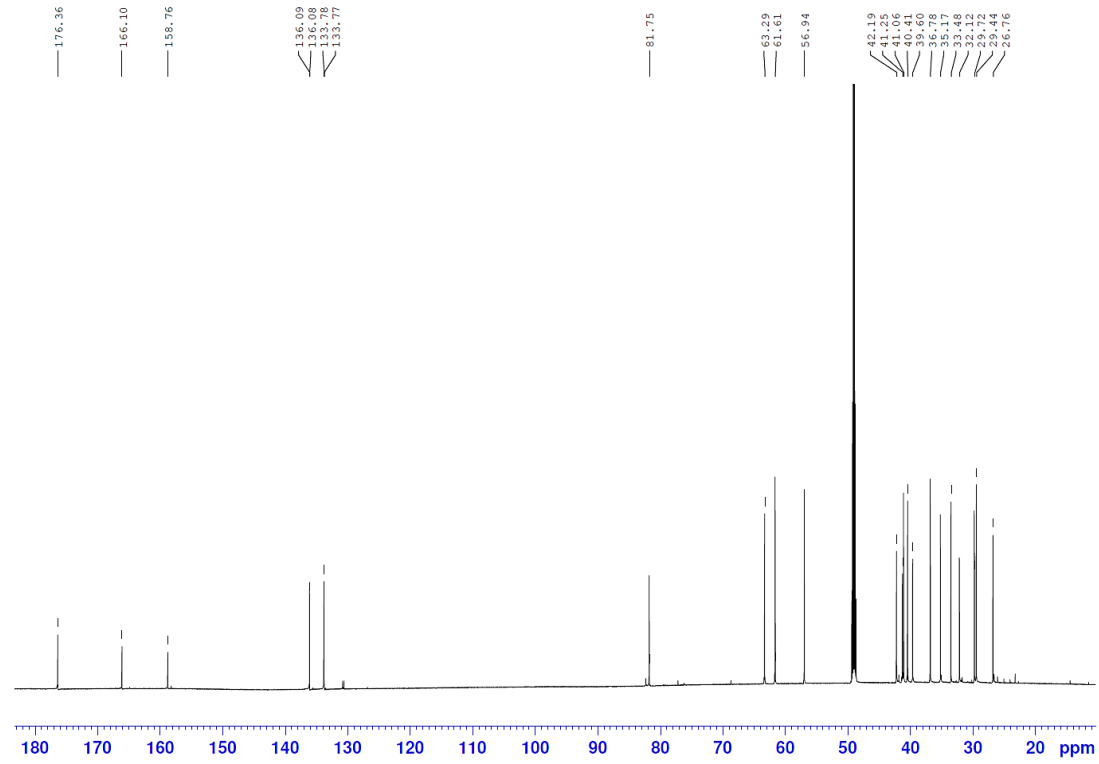


Fig. S8 $^{13}C\{^{1}H\}$ NMR spectrum of **5** in CD_3OD.

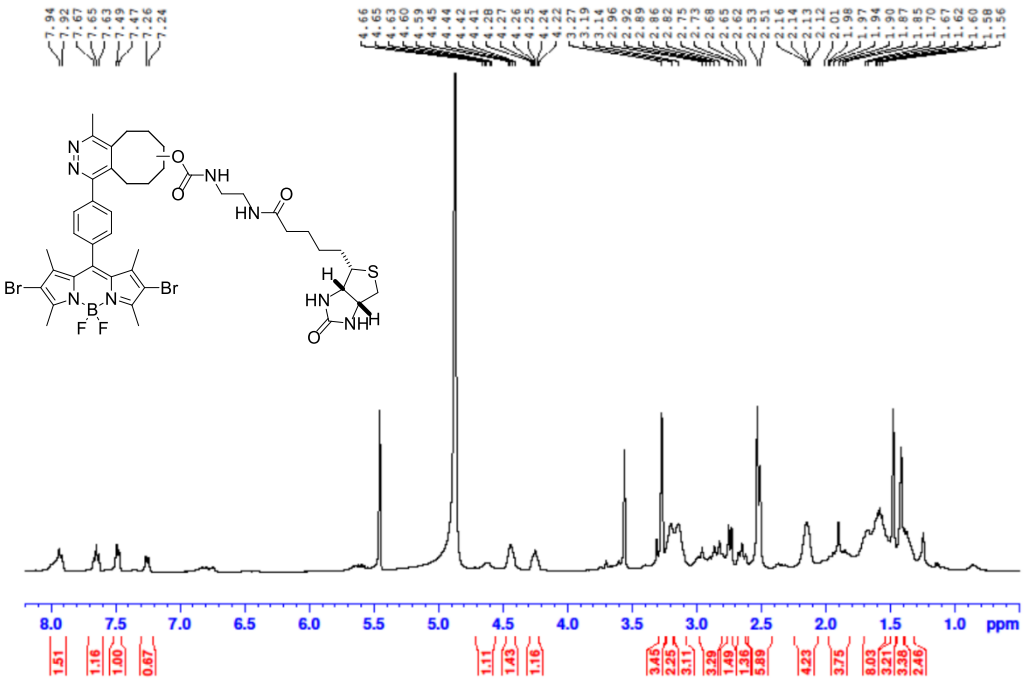


Fig. S9 ^{1}H NMR spectrum of **6** in CD_3OD.

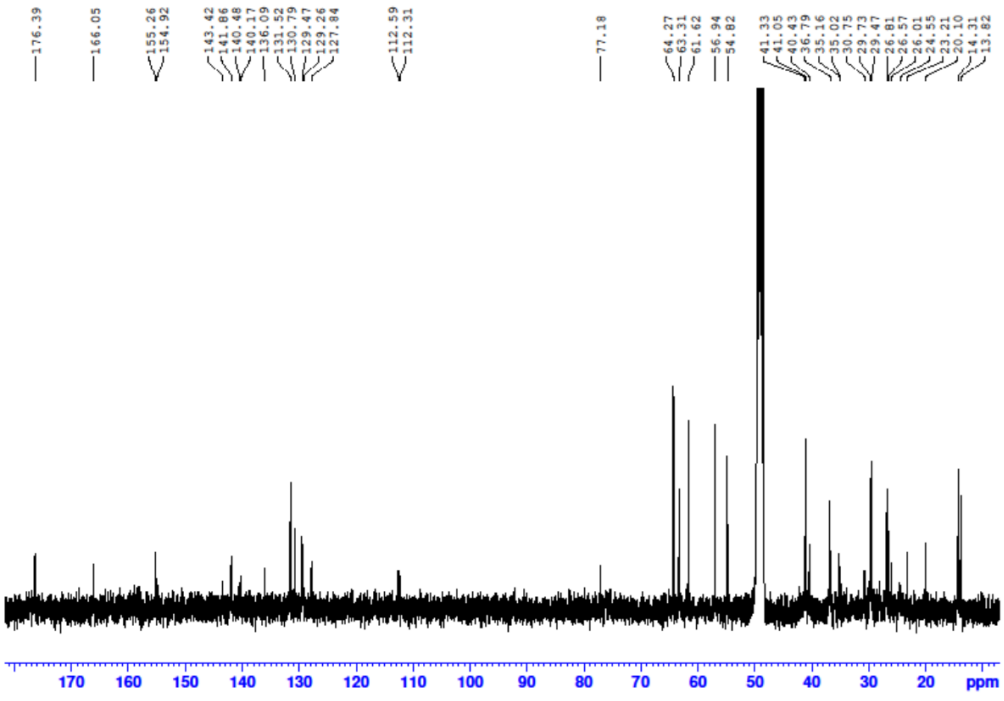


Fig. S10 $^{13}C\{^{1}H\}$ NMR spectrum of **6** in CD_3OD.

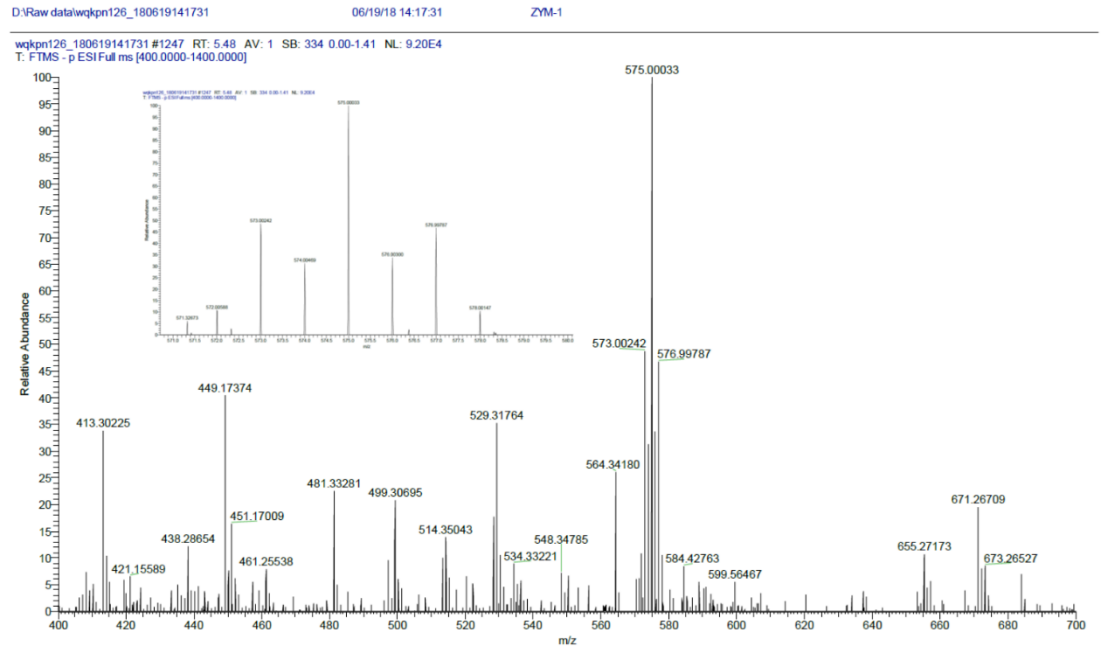


Fig. S11 Negative-ion mode ESI mass spectrum of **2**. The inset shows the enlarged isotopic envelop for the $[M-H]^-$ species.

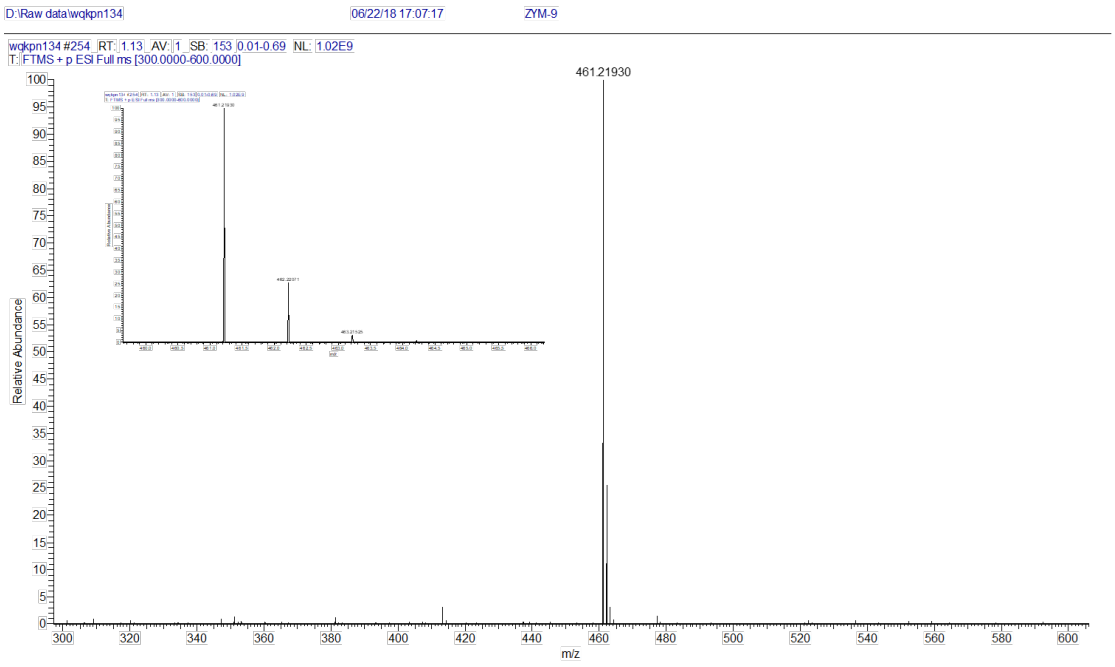


Fig. S12 Positive-ion mode ESI mass spectrum of **5**. The inset shows the enlarged isotopic envelop for the $[M+Na]^+$ species.

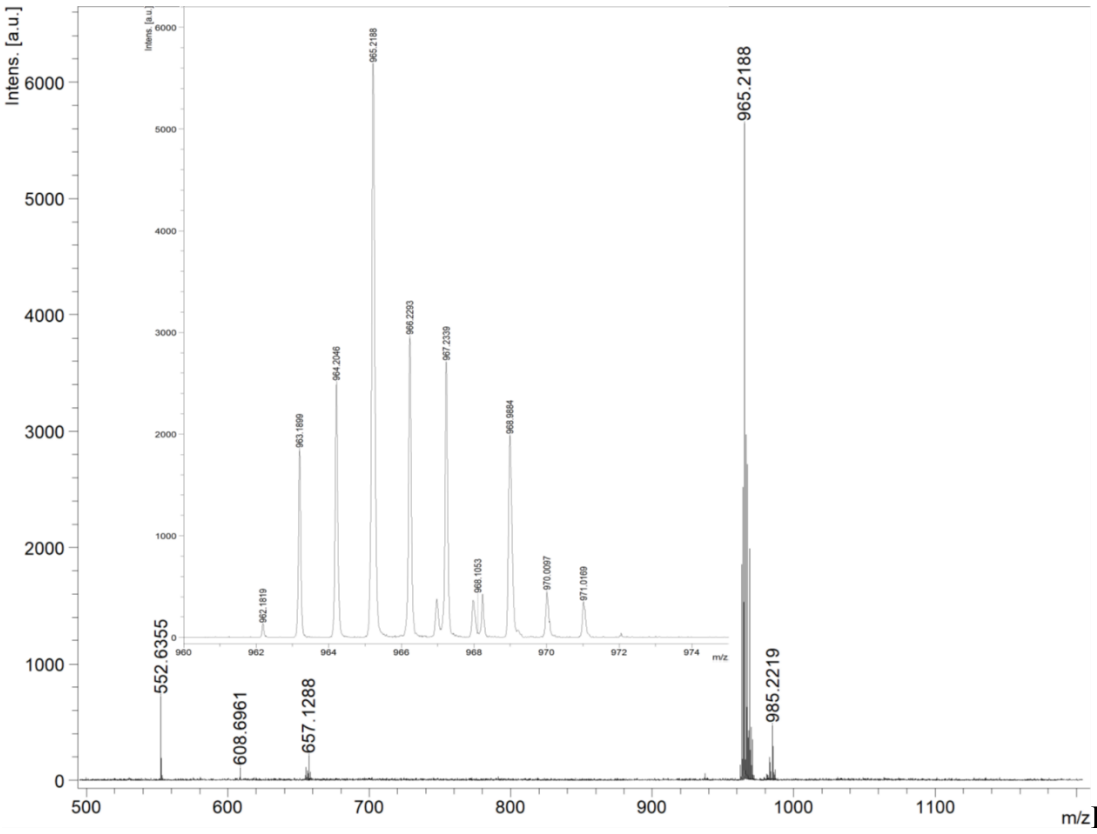


Fig. S13

Fig. S13 MALDI-TOF mass spectrum of **6**. The inset shows the enlarged isotopic envelop for the $[M\text{-}F]^+$ species.