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Electronic Supplementary Information

Facile One-Pot Synthesis of Cyclic Peptide-Conjugated Photosensitisers 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 **L1**,[R1] **6**,[R2] **10**,[R3] **12**[R4] and **15**[R3] 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 $CDCl_3$), 3.31 (for CD_3OD), 2.50 ppm (for DMSO-d_6)] or solvent [^{13}C: δ = 77.2 (for $CDCl_3$), 49.0 (for CD_3OD), 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 DPBF as the singlet oxygen scavenger and ZnPc as the reference [$\Phi_\Delta(\text{ref}) = 0.56$ in DMF].[R7] A solution of 1,3-diphenylisobenzofuran (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 rates and the rates 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

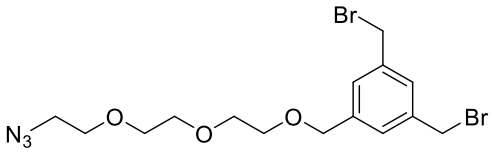
a Waters 2998 photodiode array detector. The condition used for the analysis was set as follows: solvent A = 0.1% trifluoroacetic acid (TFA) in acetonitrile and solvent B = 0.1% TFA in deionised water; gradient: 5% A + 95% B in the first 5 min, then changed to 15% A + 85% B in 10 min, further changed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

Preparation of RGD, RPM and SFITGv6

These three peptides were synthesised manually by using a modified 9-fluorenylmethoxycarbonyl (Fmoc) solid phase peptide synthesis (SPPS) 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 Fmoc-protected amino acids (4 equiv), HATU (4 equiv) and diisopropylethylamine (DIPEA) (8 equiv) in DMF were used for each coupling at room temperature. After the final coupling and Fmoc deprotection, the resin was treated with a solution containing 95% TFA, 2.5% triisopropylsilane (TIPS) and 2.5% CH_2Cl_2 for 1 h to cleave 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 dimethylsulfoxide (DMSO) and then precipitated again by the addition of diethyl ether. Lyophilisation of the precipitated peptide afforded the crude peptide which was purified by reverse-phase HPLC. HRMS (MALDI-TOF) of **RGD**: *m*/*z* calcd for $C_{27}H_{43}N_{10}O_8S_2$ $[M+H]^+$: 699.2701, found: 699.2684. HRMS (MALDI-TOF) of **RPM**: *m*/*z* calcd for $C_{42}H_{72}N_{13}O_{13}S_3$ $[M+H]^+$: 1062.4529, found: 1062.4469. HRMS (MALDI-TOF) of **SFITGv6**: *m*/*z* calcd for $C_{79}H_{125}N_{24}O_{23}S_3$ $[M+H]^+$: 1873.8506, found: 1873.8553.

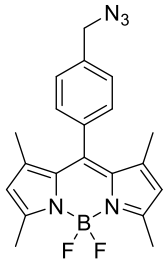
Preparation of L2



A mixture of 1,3,5-tris(bromomethyl)benzene (1.5 g, 4.2 mmol) and sodium hydride (60% suspension in mineral oil, 0.14 g, 3.5 mmol) in THF (10 mL) was stirred at 0 °C. A solution of 1-azido-8-hydroxy-3,6-dioxaoctane[R9] (0.3 g, 1.7 mmol) in THF (10 mL) was added to the suspension dropwise. The mixture was kept stirring at 0 °C for 1 h and then allowed to warm to room temperature for overnight. The solvent was evaporated under reduced pressure. After addition of water (50 mL), the mixture 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 hexane/ethyl acetate (3:2 v/v) as the eluent to afford **L2** (0.4 g, 52%). ^{1}H NMR (400 MHz,

$CDCl_3$): δ 7.33 (s, 1 H, ArH), 7.31 (s, 2 H, ArH), 4.55 (s, 2 H, OCH_2), 4.47 (s, 4 H, CH_2Br), 3.65-3.72 (m, 10 H, OCH_2), 3.39 (t, *J* = 4.8 Hz, 2 H, CH_2N_3). $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): δ 139.9, 138.5, 128.8, 128.2, 72.5, 70.8, 70.7, 70.1, 69.9, 50.7, 32.8. HRMS (ESI): *m*/*z* calcd for $C_{15}H_{21}Br_2N_3NaO_3$ $[M+Na]^+$, 473.9822; found, 473.9828.

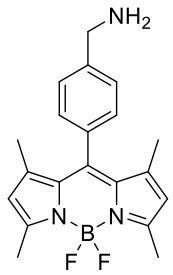
Preparation of azido BODIPY 8



A mixture of azido benzaldehyde **6** (1.0 g, 6.2 mmol) and 2,4-dimethylpyrrole (**7**) (1.4 mL, 13.6 mmol) in CH_2Cl_2 (200 mL) was stirred at room temperature. After addition of a few drops of TFA, the mixture was stirred at room temperature overnight. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.4 g, 6.2 mmol) was added and the mixture was stirred for further 5 h. Et_3N (8.5 mL, 60.9 mmol) and $BF_3{\cdot}Et_2O$ (11.5 mL, 93.2 mmol) were then added slowly. After stirring for 1 h, the mixture was washed with water and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (5:1 v/v) as the eluent (0.8 g, 34%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, *J* = 8.0 Hz, 2 H, ArH), 7.32 (d, *J* = 8.0 Hz, 2 H, ArH), 5.98 (s, 2 H, pyrrole-H), 4.44 (s, 2 H, CH_2), 2.56 (s, 6 H, CH_3), 1.37 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR

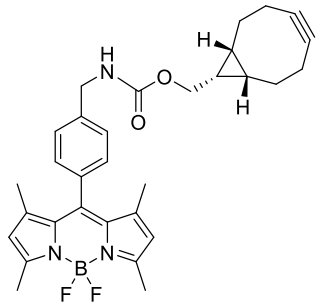
(125.8 MHz, $CDCl_3$): δ 155.7, 143.1, 140.9, 136.0, 135.2, 131.4, 129.1, 128.6, 121.4, 54.3, 14.6, 14.4. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{20}BF_2N_5Na$ $[M+Na]^+$, 402.1672; found, 402.1667.

Preparation of amino BODIPY 9



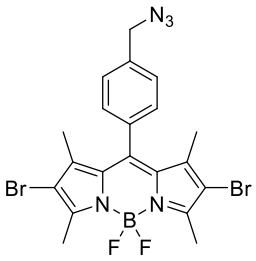
A mixture of BODIPY **8** (80 mg, 0.21 mmol) and PPh_3 (83 mg, 0.32 mmol) in THF (10 mL) was stirred at room temperature. Water (1 mL) was then added and the mixture was stirred overnight. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with $CHCl_3/CH_3OH/Et_3N$ (60:1:0.5 v/v/v) as the eluent (55 mg, 74%). ^1H NMR (400 MHz, CD_3OD): δ 7.50 (d, *J* = 8.0 Hz, 2 H, ArH), 7.23 (d, *J* = 8.0 Hz, 2 H, ArH), 6.03 (s, 2 H, pyrrole-H), 3.88 (s, 2 H, CH_2), 2.47 (s, 6 H, CH_3), 1.38 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR (125.8 MHz, CD_3OD): δ 156.6, 145.1, 144.6, 143.5, 134.7, 132.7, 129.4, 129.3, 122.2, 46.3, 14.7, 14.6 (some of the signals were overlapped). HRMS (ESI): *m*/*z* calcd for $C_{20}H_{23}BF_2N_3$ $[M+H]^+$, 354.19476; found, 354.19395.

Preparation of BDP1



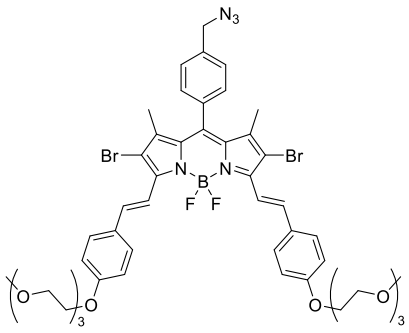
A mixture of BODIPY **9** (100 mg, 0.28 mmol), bicyclo[6.1.0]non-4-yne (BCN) derivative **10** (90 mg, 0.28 mmol) and Et_3N (80 μL, 0.6 mmol) in DMF (10 mL) was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (5:1 v/v) as the eluent to afford **BDP1** (110 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (d, *J* = 8.0 Hz, 2 H, ArH), 7.24 (d, *J* = 8.0 Hz, 2 H, ArH), 5.97 (s, 2 H, pyrrole-H), 5.09 (br s, 1 H, NH), 4.47 (d, *J* = 6.0 Hz, 2 H, CH_2), 4.05 (d, *J* = 6.8 Hz, 2 H, CH_2), 2.54 (s, 6 H, CH_3), 2.38-2.42 (m, 2 H, CH_2), 2.25-2.32 (m, 2 H, CH_2), 2.13-2.16 (m, 2 H, CH_2), 1.36 (s, 6 H, CH_3), 1.24-1.25 (m, 2 H, CH_2), 0.69-0.78 (m, 3 H, CH). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 156.4, 155.1, 142.6, 140.9, 139.3, 133.7, 131.0, 127.9, 127.5, 120.8, 98.3, 69.0, 44.2, 32.8, 23.3, 22.5, 21.0, 14.2, 14.1. HRMS (ESI): *m*/*z* calcd for $C_{31}H_{34}BF_2N_3NaO_2$ $[M+Na]^+$, 552.2604; found, 552.2599.

Preparation of dibromo BODIPY 11



A mixture of azido BODIPY **8** (200 mg, 0.5 mmol) and *N*-bromosuccinimide (NBS) (185 mg, 1.0 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/ethyl acetate (6:1 v/v) as the eluent to afford **11** (250 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49 (d, J = 8.0 Hz, 2 H, ArH), 7.30 (d, J = 8.0 Hz, 2 H, ArH), 4.47 (s, 2 H, CH_2), 2.61 (s, 6 H, CH_3), 1.37 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 154.2, 141.3, 140.5, 136.7, 134.5, 130.3, 129.3, 128.5, 111.9, 54.2, 13.8, 13.7. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{18}BBr_2F_2N_5Na$ $[M+Na]^+$, 559.9862; found, 559.9859.

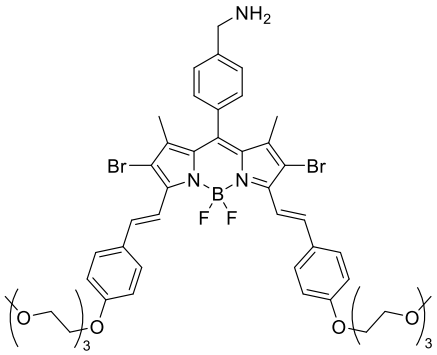
Preparation of distyryl BODIPY 13



Dibromo BODIPY **11** (50 mg, 0.09 mmol) and triethylene glycol-substituted benzaldehyde **12** (150 mg, 0.56 mmol) were dissolved in toluene (30 mL). Acetic acid (0.5 mL), piperidine (0.5 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 complete consumption of **11** 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 resulting mixture 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 residue was purified by column chromatography on silica gel with $CHCl_3/CH_3OH$ (60:1 v/v) as the eluent to afford **13** (60 mg, 64%). ^1H NMR (500 MHz, $CDCl_3$): δ 8.11 (d, *J* = 16.5 Hz, 2 H, C=CH), 7.60-7.63 (m, 6 H, C=CH and ArH), 7.49 (d, *J* = 8.0 Hz, 2 H, ArH), 7.34 (d, *J* = 8.0 Hz, 2 H, ArH), 6.97 (d, *J* = 8.5 Hz, 4 H, ArH), 4.47 (s, 2 H, CH_2), 4.19 (t, *J* = 5.0 Hz, 4 H, OCH_2), 3.89 (t, *J* = 5.0 Hz, 4 H, OCH_2), 3.75-3.77 (m, 4 H, OCH_2), 3.69-3.71 (m, 4 H, OCH_2), 3.66-3.68 (m, 4 H, OCH_2), 3.55-3.57 (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 160.1, 148.6, 140.8, 139.0, 137.8, 136.6, 135.1, 131.9, 129.9, 129.3, 129.2, 129.1, 116.1, 115.0, 110.3, 72.0, 70.9, 70.7, 70.6, 69.7, 67.6, 59.1, 54.3, 13.8. HRMS (ESI): *m*/*z* calcd for $C_{48}H_{54}BBr_2F_2N_5NaO_8$ $[M+Na]^+$, 1060.2272; found, 1060.2276.

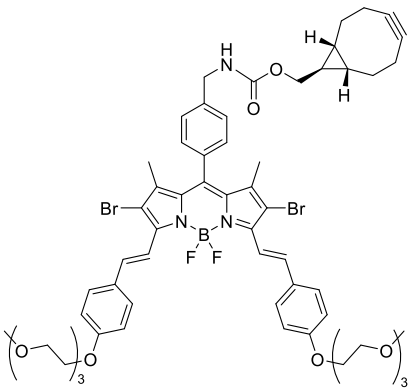
Preparation of distyryl BODIPY 14



A mixture of distyryl BODIPY **13** (50 mg, 0.05 mmol) and PPh_3 (19 mg, 0.07 mmol) in THF (10 mL) was stirred at room temperature. Water (1 mL) was then added and the mixture was

stirred continuously overnight. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3/CH_3OH/Et_3N$ (30:1:0.5 v/v/v) as the eluent to afford **14** (35 mg, 72%). ^1H NMR (500 MHz, DMSO-d_6): δ 8.07 (d, *J* = 16.5 Hz, 2 H, C=CH), 7.68 (d, *J* = 8.0 Hz, 2 H, ArH), 7.61 (d, *J* = 8.5 Hz, 4 H, ArH), 7.55 (d, *J* = 8.0 Hz, 2 H, ArH), 7.47 (d, *J* = 16.5 Hz, 2 H, C=CH), 7.08 (d, *J* = 8.5 Hz, 4 H, ArH), 4.20 (s, 2 H, CH_2), 4.17 (t, *J* = 4.5 Hz, 4 H, OCH_2), 3.77 (t, *J* = 4.5 Hz, 4 H, OCH_2), 3.59-3.61 (m, 4 H, OCH_2), 3.51-3.55 (m, 8 H, OCH_2), 3.43 (t, *J* = 4.5 Hz, 4 H, OCH_2), 3.24 (s, 6 H, OCH_3), 1.40 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR (125.8 MHz, DMSO-d_6): δ 160.2, 147.7, 140.9, 139.2, 138.8, 135.7, 133.8, 131.5, 129.7, 129.1, 128.7, 128.6, 115.4, 115.0, 109.7, 71.3, 70.0, 69.8, 69.6, 68.9, 67.5, 58.1, 42.0, 13.7. HRMS (ESI): *m*/*z* calcd for $C_{48}H_{56}BBr_2F_2N_3NaO_8$ $[M+Na]^+$, 1034.2381; found, 1034.2384.

Preparation of BDP2



A mixture of distyryl BODIPY **14** (30 mg, 0.03 mmol), BCN derivative **15** (10 mg, 0.03 mmol) and Et_3N (8 μL, 0.06 mmol) in DMF (5 mL) was stirred at room temperature for 2 h. The

solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3/CH_3OH$ (30:1 v/v) as the eluent (22 mg, 62%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, *J* = 16.4 Hz, 2 H, C=CH), 7.59-7.63 (m, 6 H, C=CH and ArH), 7.45 (d, *J* = 8.0 Hz, 2 H, ArH), 7.26 (d, *J* = 7.6 Hz, 2 H, ArH), 6.96 (d, *J* = 8.8 Hz, 4 H, ArH), 5.14 (br s, 1 H, NH), 4.51 (d, *J* = 5.6 Hz, 2 H, CH_2), 4.19 (t, *J* = 4.8 Hz, 4 H, OCH_2), 4.06 (d, *J* = 6.8 Hz, 2 H, CH_2), 3.89 (t, *J* = 4.8 Hz, 4 H, OCH_2), 3.75-3.77 (m, 4 H, OCH_2), 3.65-3.71 (m, 8 H, OCH_2), 3.55-3.57 (m, 4 H, OCH_2), 3.38 (s, 6 H, OCH_3), 2.30-2.43 (m, 2 H, CH_2), 2.26-2.33 (m, 2 H, CH_2), 2.15-2.19 (m, 2 H, CH_2), 1.39 (s, 6 H, CH_3), 1.34-1.35 (m, 2 H, CH_2), 0.71-0.80 (m, 3 H, CH). $^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 160.1, 148.6, 140.9, 140.5, 139.0, 134.1, 132.1, 129.9, 129.4, 128.8, 128.2, 116.1, 115.0, 110.3, 98.9, 72.0, 70.9, 70.7, 70.6, 69.8, 69.6, 67.6, 59.1, 44.7, 33.3, 23.8, 23.0, 21.5, 13.9. HRMS (ESI): *m*/*z* calcd for $C_{59}H_{68}BBr_2F_2N_3NaO_{10}$ $[M+Na]^+$, 1210.3222; found, 1210.3219.

General procedure for the one-pot peptide cyclisation and BODIPY conjugation

The linear peptide (**RGD**, **RPM** or **SFITGv6**) (1 mM) and the bifunctional linker (**L1** or **L2**) (1 mM) were dissolved in a mixture of DMF and NH_4HCO_3 buffer (pH 8.0) (2:1 v/v). After complete consumption of the linear peptide as revealed by HPLC, a solution of BCN-substituted BODIPY (**BDP1** or **BDP2**) in DMF (2 mM) was added. After completion, the product was purified by reversed-phase HPLC and dried by lyophilisation.

Study of stability in serum

Conjugate **1** or **RGD** was incubated in Dulbecco's modified Eagle medium (DMEM) (ThermoFisher Scientific, cat. no. 12100-046) with fetal bovine serum (FBS) (ThermoFisher Scientific, cat. no. 10270-106) (20% v/v) and DMSO (5% v/v) at 37 ºC. An aliquot of the solution was drawn at different time points and precipitated with an equal volume of cold methanol. The mixture was centrifuged and the supernatant was collected for HPLC analysis. The peak areas of **1** or **RGD** at different time points were compared.

Cell lines and culture conditions

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) were maintained in DMEM supplemented with FBS (10%) and penicillin-streptomycin (100 units mL^{-1} and 100 μg mL^{-1} respectively). All the cells were grown at 37 ºC in a humidified 5% CO_2 atmosphere.

Confocal fluorescence microscopic studies

Approximately 2×10^5 cells in MEM or DMEM (2 mL) were seeded on a confocal dish and

incubated overnight at 37 ºC in a humidified 5% CO_2 atmosphere. Conjugate **1** and **BDP2** (20 nmol) were first dissolved in DMSO (25 μL) to prepare stock solutions of 800 μM. The solutions were further diluted with a serum-free medium to 2 μM. The cells, after being rinsed with PBS, were incubated with these solutions (2 μM) at 37 ºC for 2 h. The solutions were then removed, and the cells were rinsed with phosphate buffered saline (PBS) twice before being viewed with a Leica TCS SP8 high speed confocal microscope equipped with a solid-state 638 nm laser. The compound was excited at 638 nm and its fluorescence was monitored at 650–750nm. The images were digitised and analysed using a Leica Application Suite X software.

Flow cytometric studies

Approximately 2×10^5 cells per well in MEM or DMEM were seeded on a 12-multiwell plate and incubated overnight at 37 ºC in a humidified 5% CO_2 atmosphere. Conjugate **1** and **BDP2** (20 nmol) were first dissolved in DMSO (25 μL) to prepare stock solutions of 800 μM. The solutions were further diluted with a serum-free medium to 2 μM. The cells, after being rinsed with PBS, were incubated with these solutions (2 μM) at 37 ºC for 2 h. 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 suspended in PBS (1.0 mL) and the intracellular 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 640 nm and the emitted fluorescence was monitored at 720–840 nm. The data collected were analysed by using the BD FAC-Suite. All experiments were performed in triplicate.

Photocytotoxicity assay

Approximately 2×10^4 cells per well in MEM or DMEM were inoculated in 96-multiwell plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. A stock solution of **1** (800 μM) was prepared as described above, which was then diluted with a serum-free medium to respective concentrations. The cells, after being rinsed with PBS twice, were incubated with 100 μL of these BODIPY solutions for 2 h 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 $\lambda = 610$ nm. The fluence rate ($\lambda > 610$ 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.[R10] 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 the 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.

In vivo imaging

Female Balb/c nude mice (20–25 g) were obtained from the Laboratory Animal Services Centre of The Chinese University of Hong Kong. All animal experiments were approved by the Animal Experimentation Ethics Committee of the University. The mice were kept under pathogen-free conditions with free access to food and water. A549 cells (1×10^7 cells in 200 μL) were inoculated subcutaneously at the back of the mice. Once the tumours had grown to a size of 80–100 mm^3, conjugate **1** or **BDP2** (20 nmol) dissolved in 200 μL of distilled water containing 2.5% DMSO and 0.1% Tween 80 was injected to the tumour-bearing mice through tail intravenous injection. *In vivo* fluorescence imaging was performed before and after the injection at different time points up to 48 h with an Odyssey infrared imaging system

(excitation wavelength = 680 nm, emission wavelength ≥ 700 nm). The images were digitised and analysed by the Odyssey imaging system software (no. 9201-500). After the *in vivo* imaging studies, the animals were euthanised at 48 h post-injection. The tumour and various organs were then harvested and their fluorescence intensities were measured with the Odyssey infrared imaging system again. Three mice were used for each compound.

In vivo photodynamic therapy

A549 tumour-bearing nude mice were prepared as described above. The length and width of the tumour were measured by a micrometer digital caliper (SCITOP Systems). The tumour volume (mm^3) was calculated by the formula: tumour volume = (length × $width^2$)/2. Once the tumours had grown to a size of 80–100 mm^3, conjugate **1** (40 nmol) dissolved in 200 μL of distilled water containing 2.5% DMSO and 0.1% Tween 80 was injected to the tumour-bearing mice through tail intravenous injection. Four mice were used for this group. After 24 h post-injection, the tumour was illuminated with a diode laser (Biolitec Ceralas) at 680 nm operated at 0.47 W. Illumination on a circular spot with 1.0 cm diameter for 10 min led to a total fluence of 360 J cm^{-2}. The tumour sizes of the nude mice were monitored periodically for the next 20 days. The tumour volumes were compared with a PBS control group of mice without light treatment. Three mice were used for the control.

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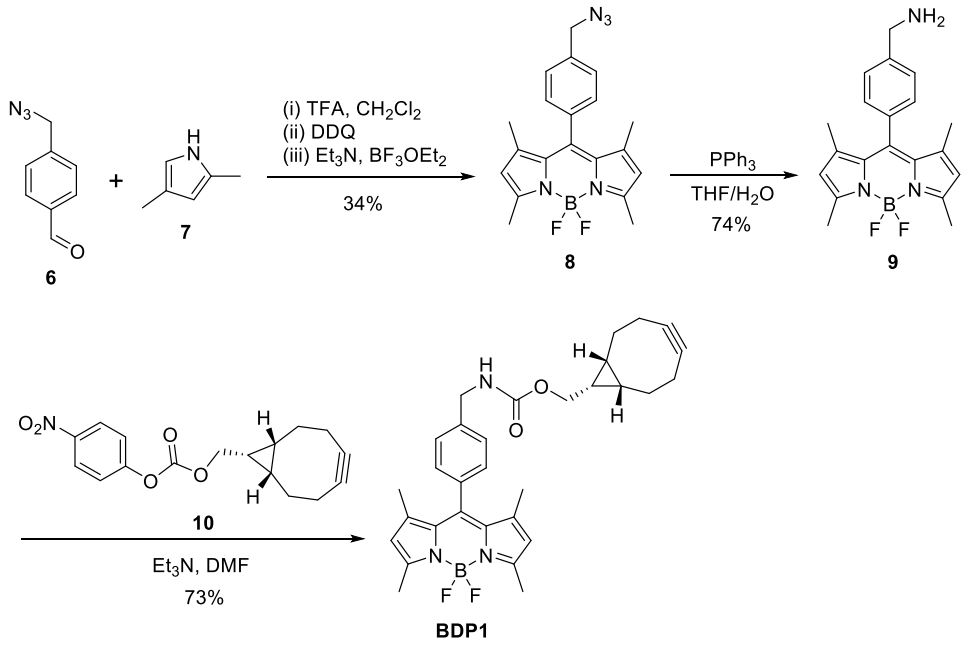
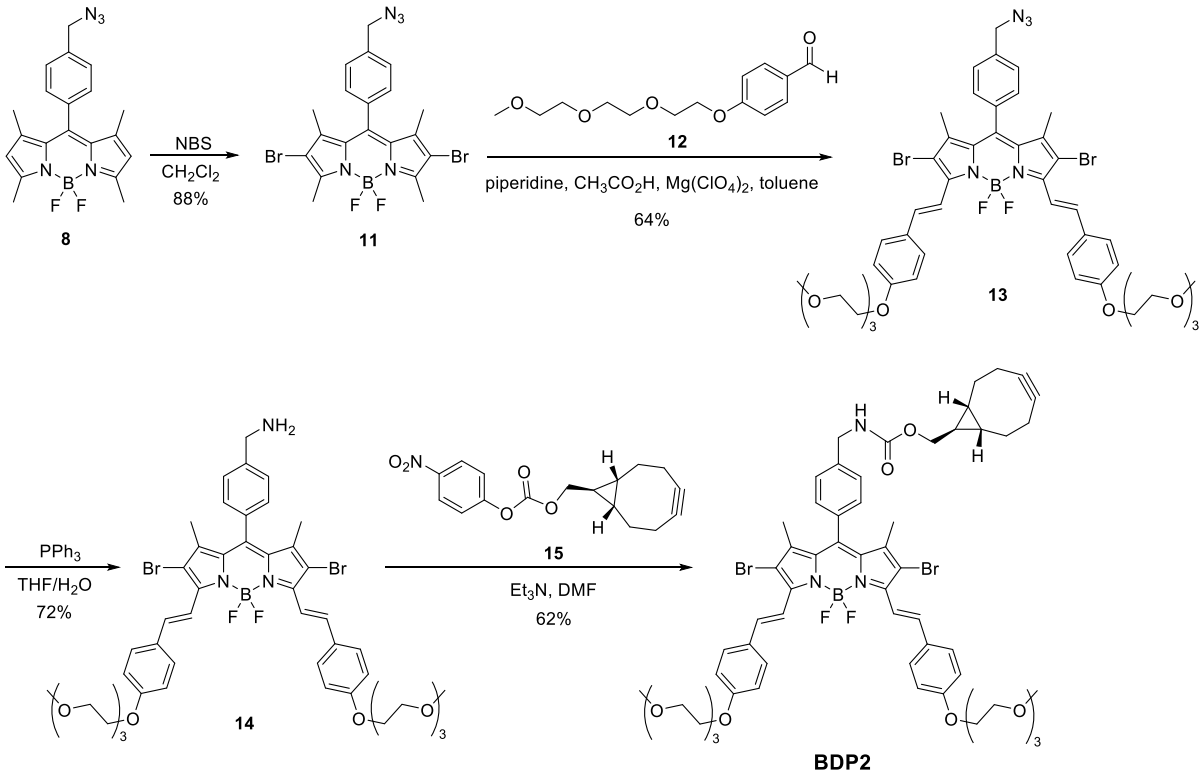
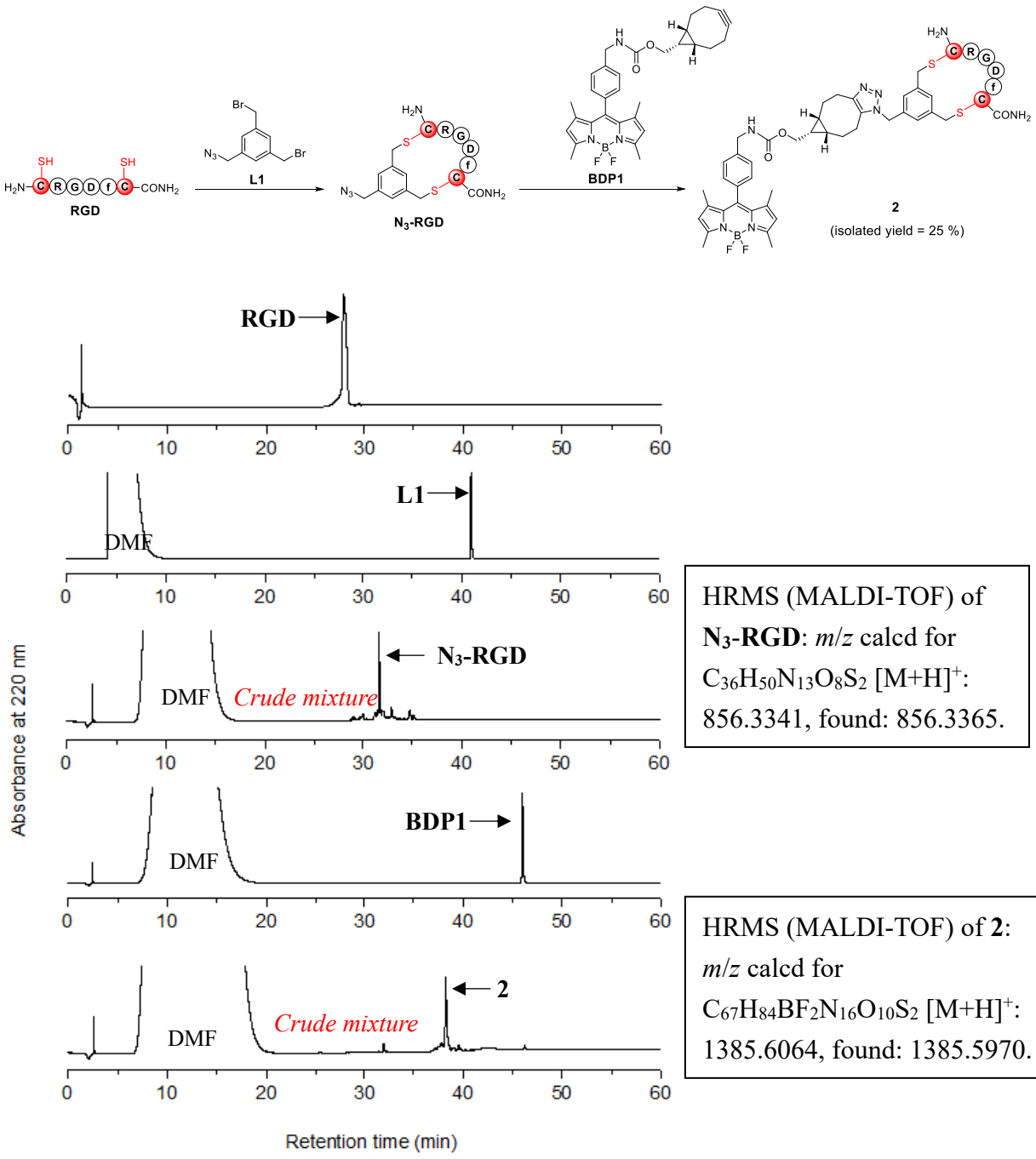


Scheme S1 Synthetic route of **BDP1**.



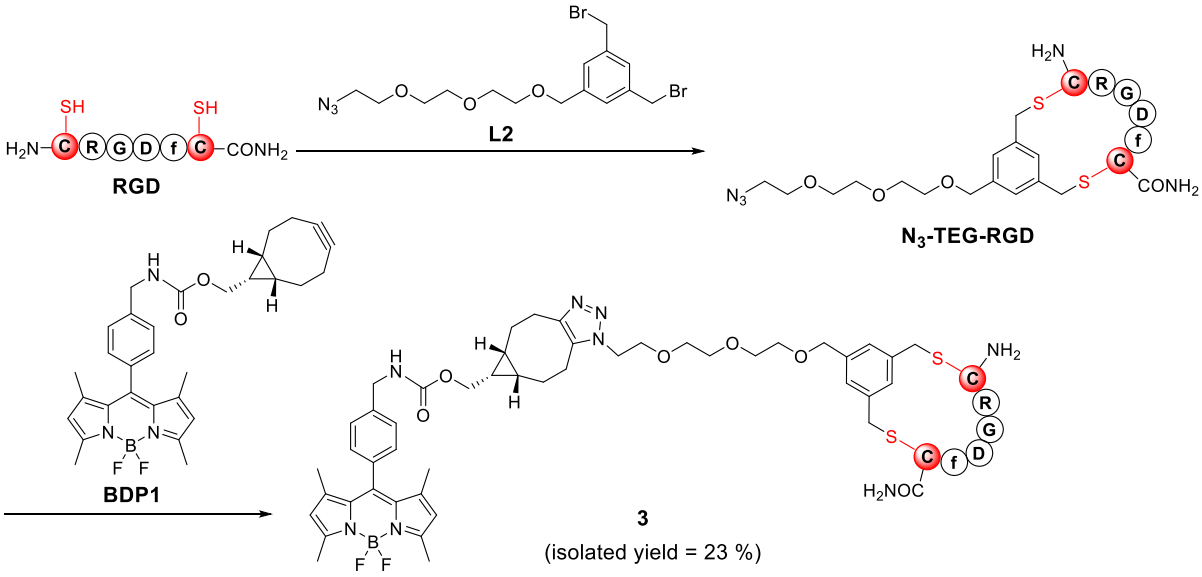
Scheme S2 Synthetic route of **BDP2**.

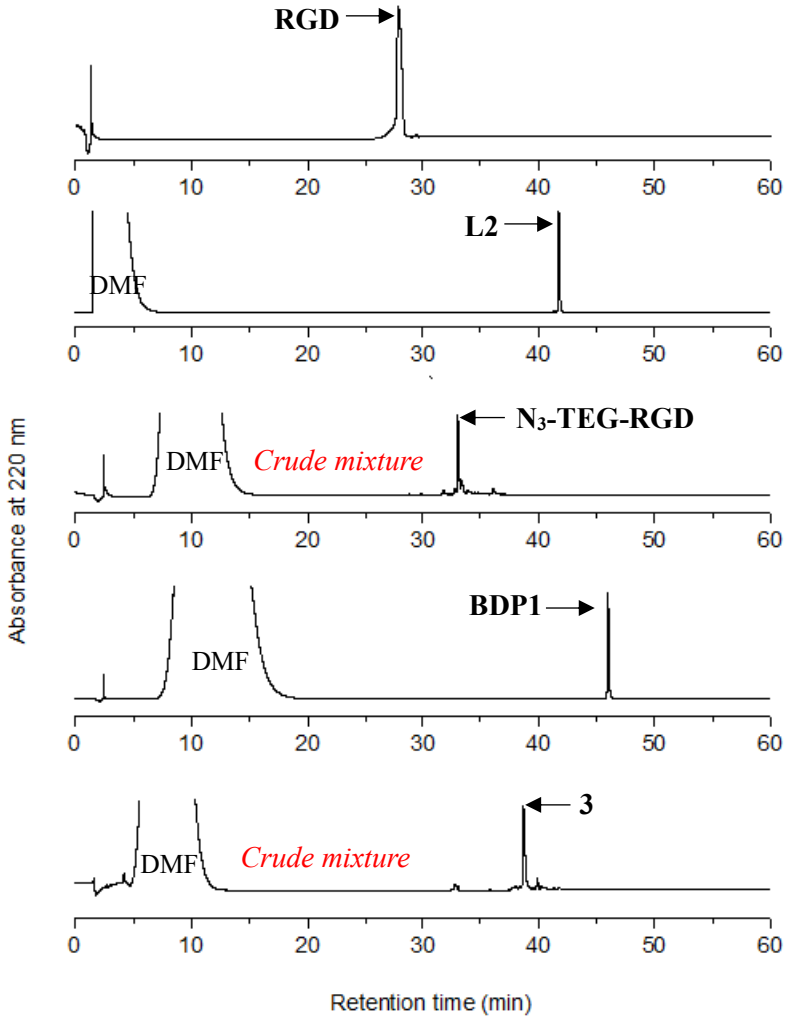


HRMS (MALDI-TOF) of **N_3-RGD**: *m/z* calcd for $C_{36}H_{50}N_{13}O_8S_2$ $[M+H]^+$: 856.3341, found: 856.3365.

HRMS (MALDI-TOF) of **2**: *m/z* calcd for $C_{67}H_{84}BF_2N_{16}O_{10}S_2$ $[M+H]^+$: 1385.6064, found: 1385.5970.

Fig. S1 Monitoring the course of the formation of conjugate **2** using HPLC.

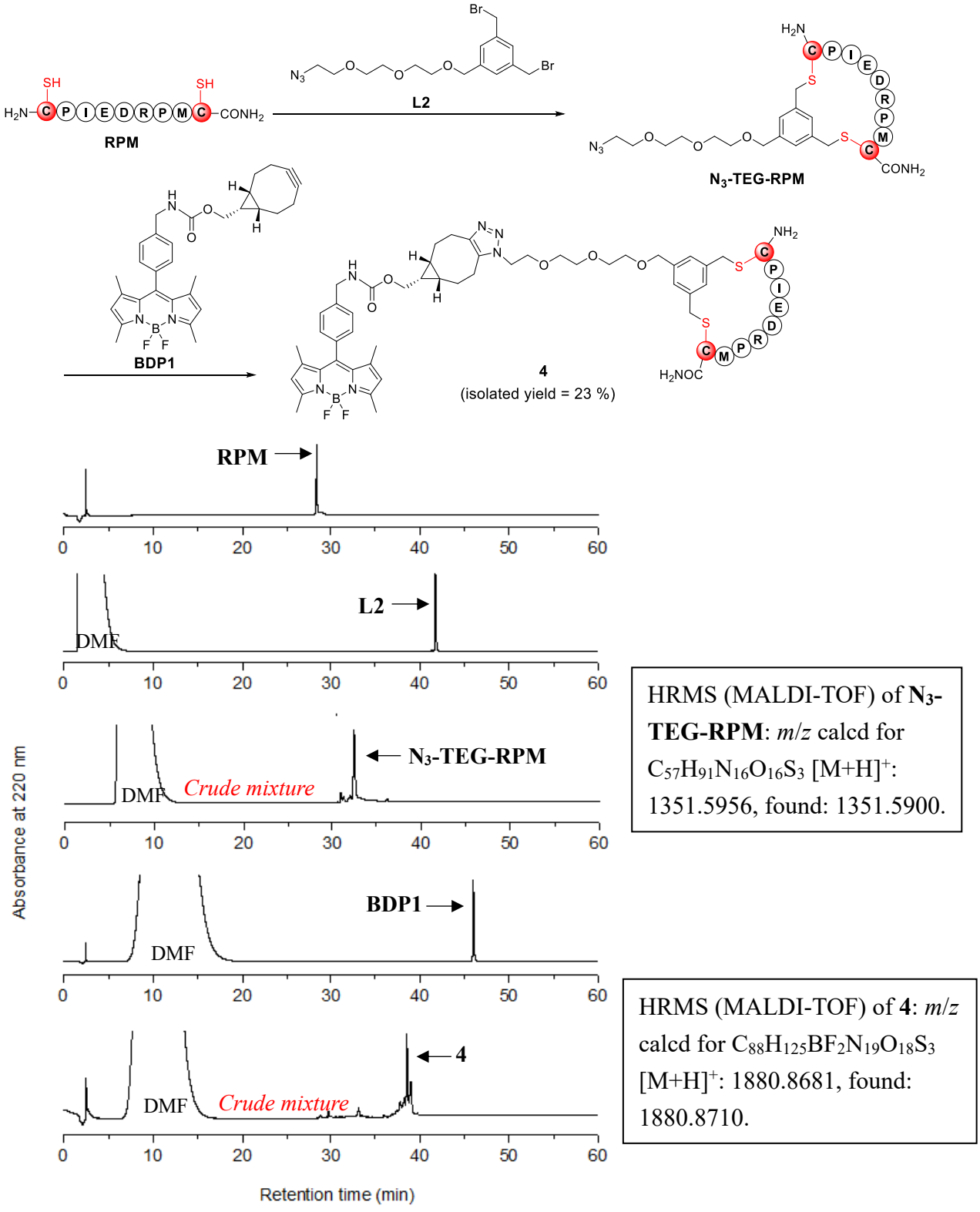




HRMS (MALDI-TOF) of **N_3-TEG-RGD**: *m/z* calcd for $C_{42}H_{62}N_{13}O_{11}S_2$ $[M+H]^+$: 988.4128, found: 988.4150.

HRMS (MALDI-TOF) of **3**: *m/z* calcd for $C_{73}H_{96}BF_2N_{16}O_{13}S_2$ $[M+H]^+$: 1517.6852, found:1517.6878.

Fig. S2 Monitoring the course of the formation of conjugate **3** using HPLC.



HRMS (MALDI-TOF) of **N_3-TEG-RPM**: *m/z* calcd for $C_{57}H_{91}N_{16}O_{16}S_3$ $[M+H]^+$: 1351.5956, found: 1351.5900.

HRMS (MALDI-TOF) of **4**: *m/z* calcd for $C_{88}H_{125}BF_2N_{19}O_{18}S_3$ $[M+H]^+$: 1880.8681, found: 1880.8710.

Fig. S3 Monitoring the course of the formation of conjugate **4** using HPLC.

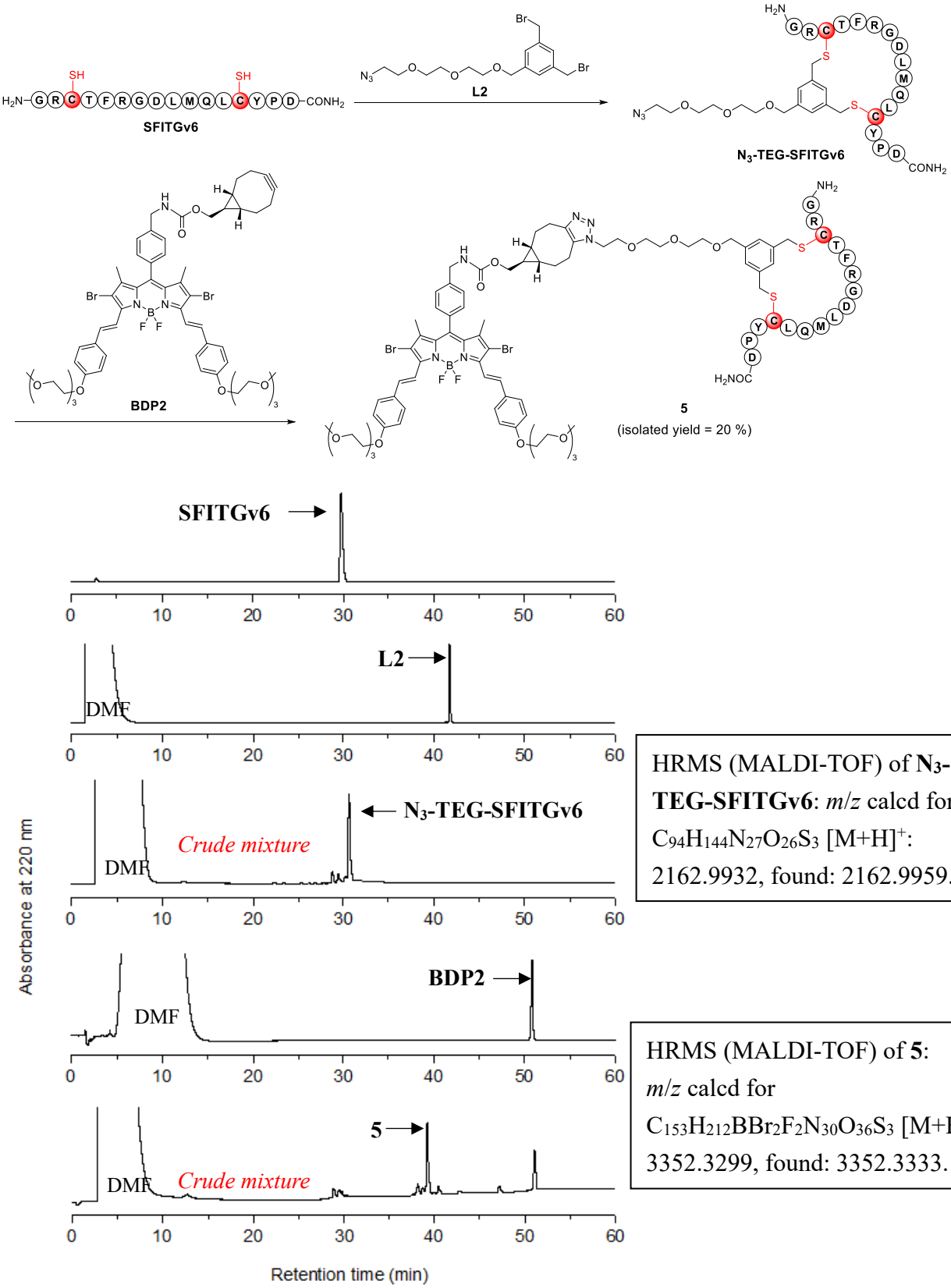


Fig. S4 Monitoring the course of the formation of conjugate **5** using HPLC.

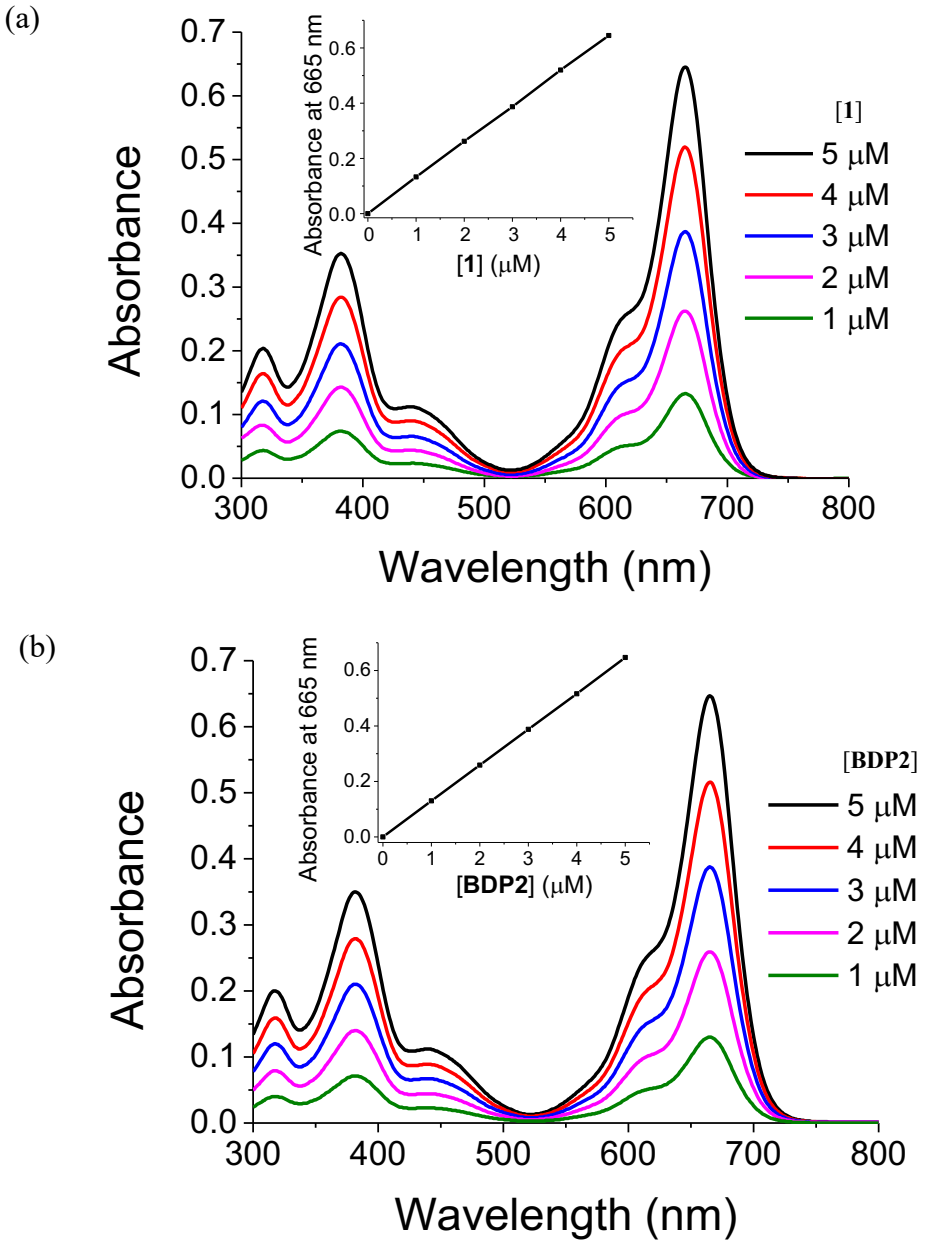
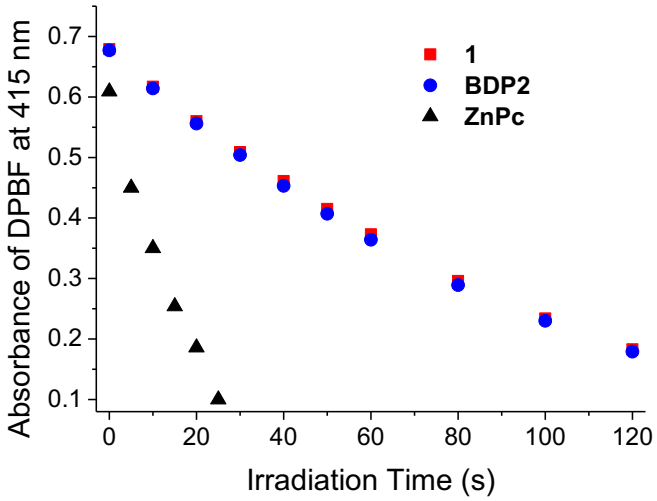


Fig. S5 UV-Vis spectra of (a) **1** and (b) **BDP1** at different concentrations in DMF. The inset of each spectrum plots the absorbance of the longest-wavelength absorption versus the concentration of BODIPY and the line represents the best-fitted straight line.

(a)



(b)

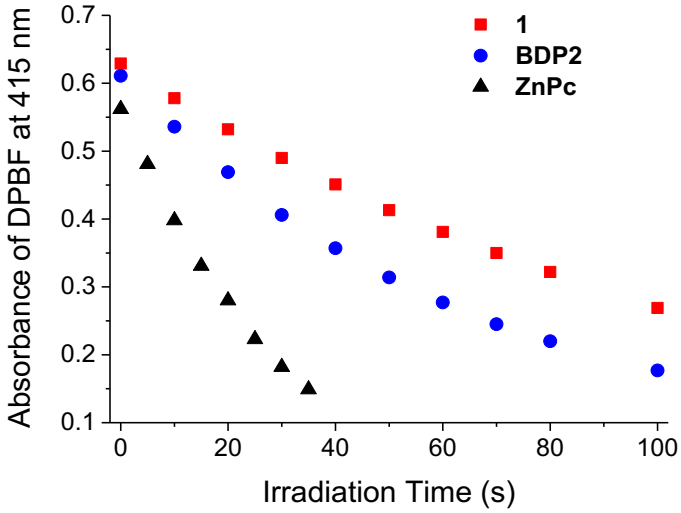


Fig. S6 Comparison of the rate of decay of DPBF (initial concentration = 30 μM) sensitised by **1**, **BDP2** and **ZnPc** (all at 2 μM) in (a) DMF and (b) PBS with Tween 80 (0.1% v/v).

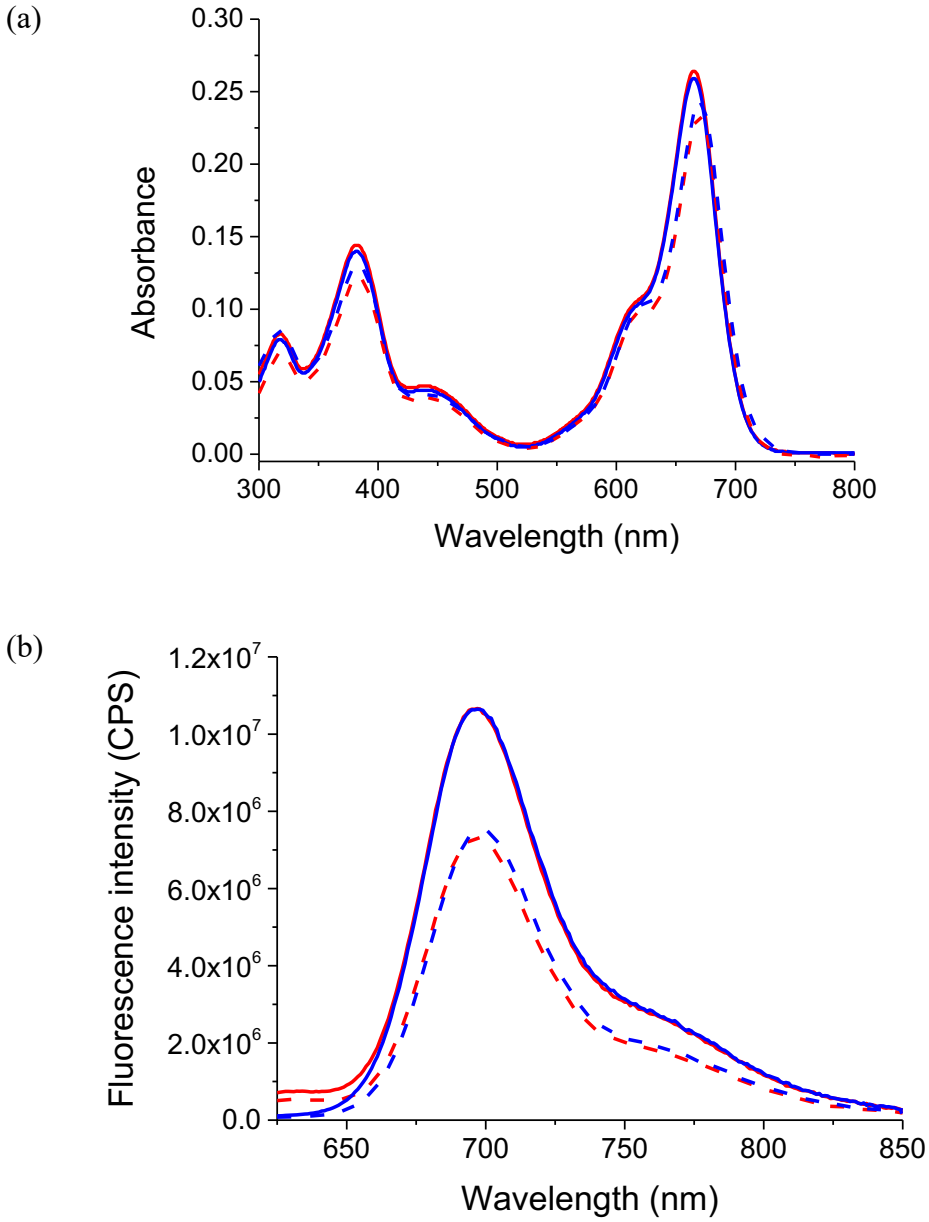
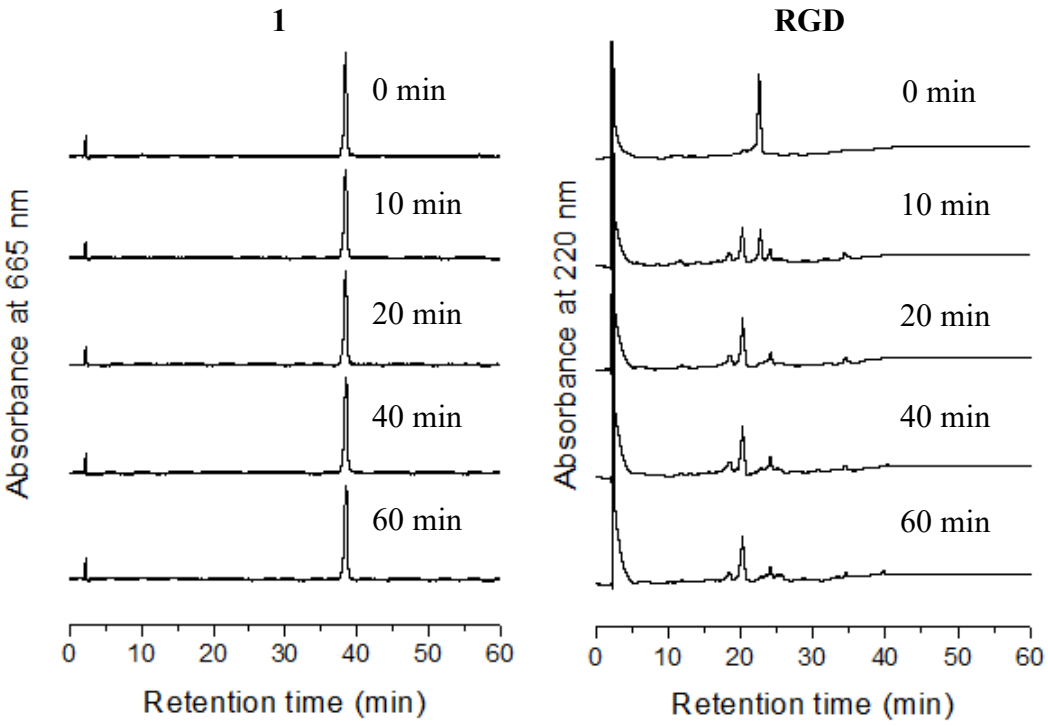


Fig. S7 (a) UV-Vis and (b) fluorescence (excited at 610 nm) spectra of **1** (red) and **BDP2** (blue) (both at 2 μM) in DMF (solid) and PBS with Tween 80 (0.1% v/v) (dotted).

(a)



(b)

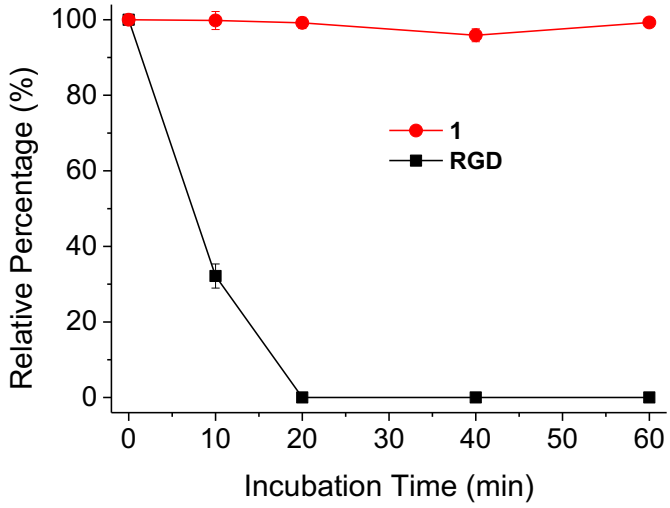


Fig. S8 (a) Stability of **1** (left) and **RGD** (right) in DMEM with FBS (20% v/v) as studied by HPLC analysis. (b) The corresponding relative percentages at different time points as determined by integrating the peak areas in the HPLC chromatograms.

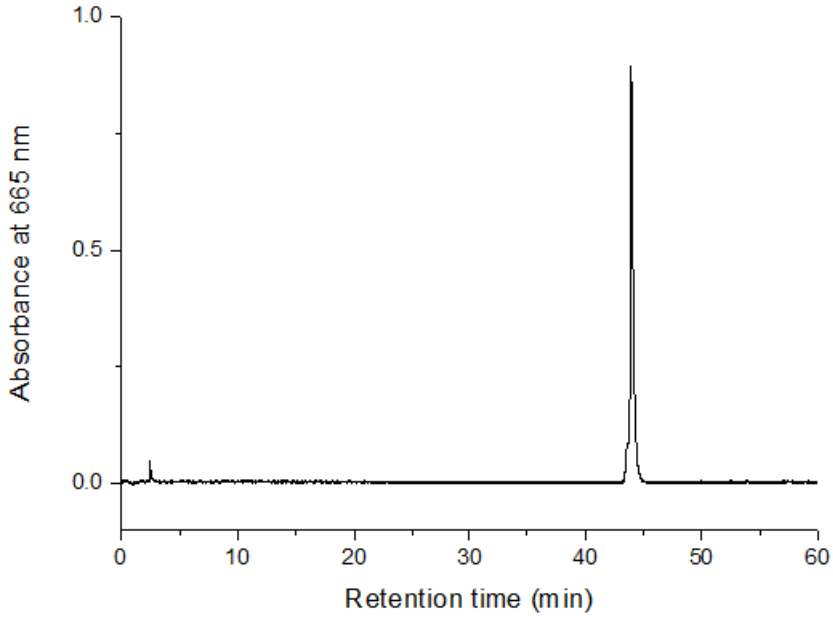


Fig. S9 HPLC chromatogram of conjugate **1**.

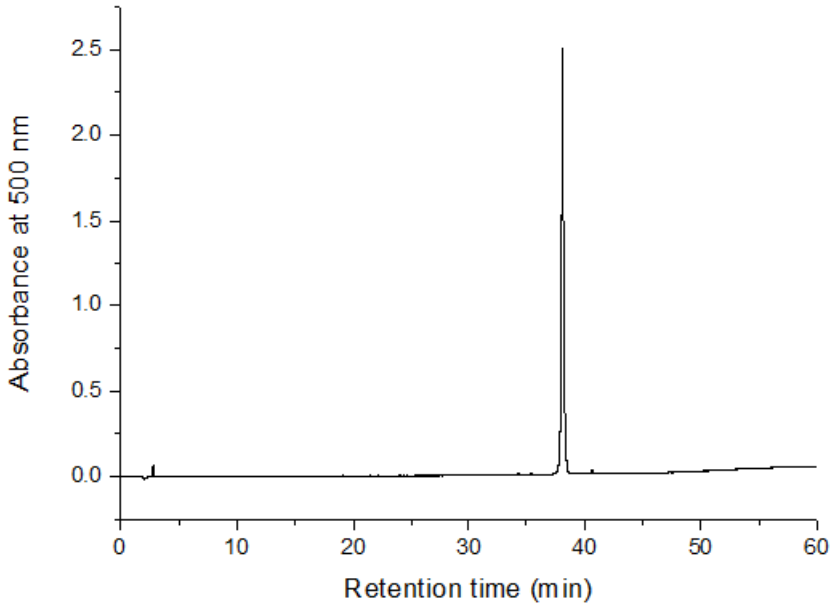


Fig. S10 HPLC chromatogram of conjugate **2**.

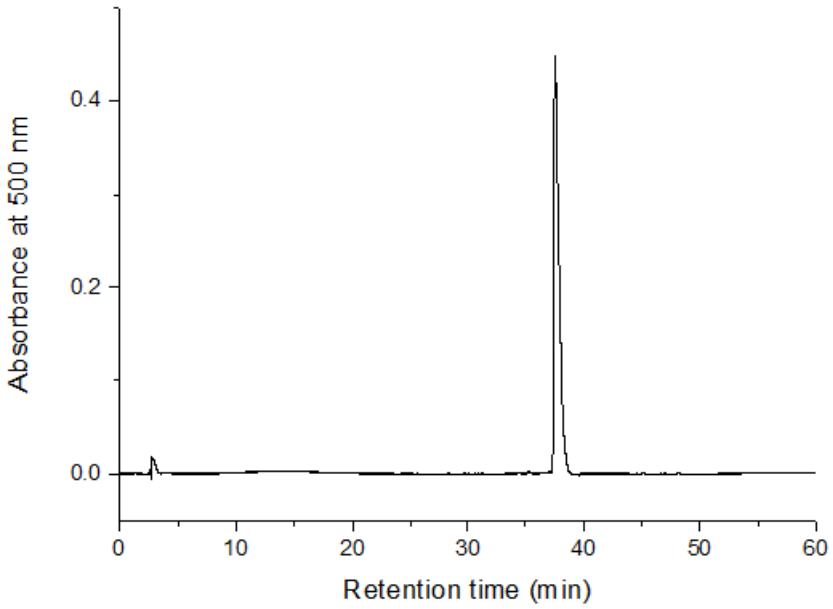


Fig. S11 HPLC chromatogram of conjugate **3**.

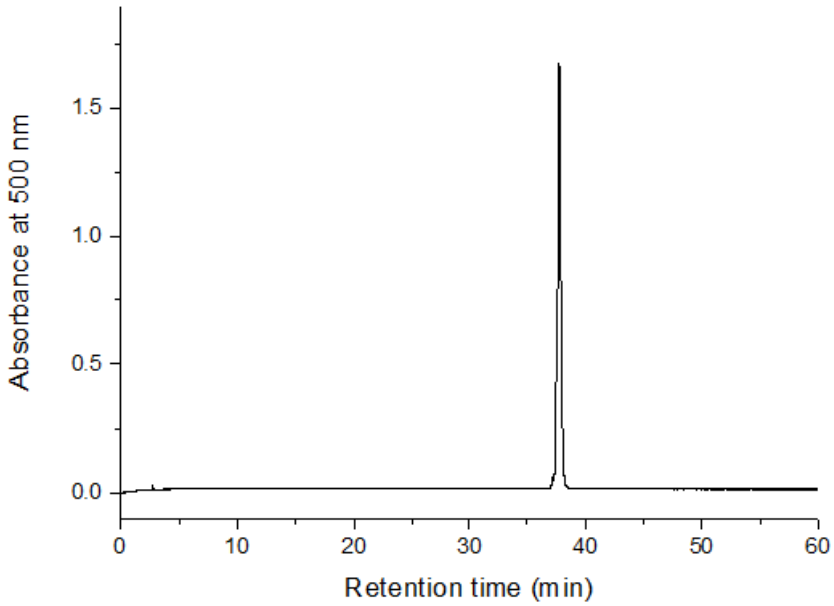


Fig. S12 HPLC chromatogram of conjugate **4**.

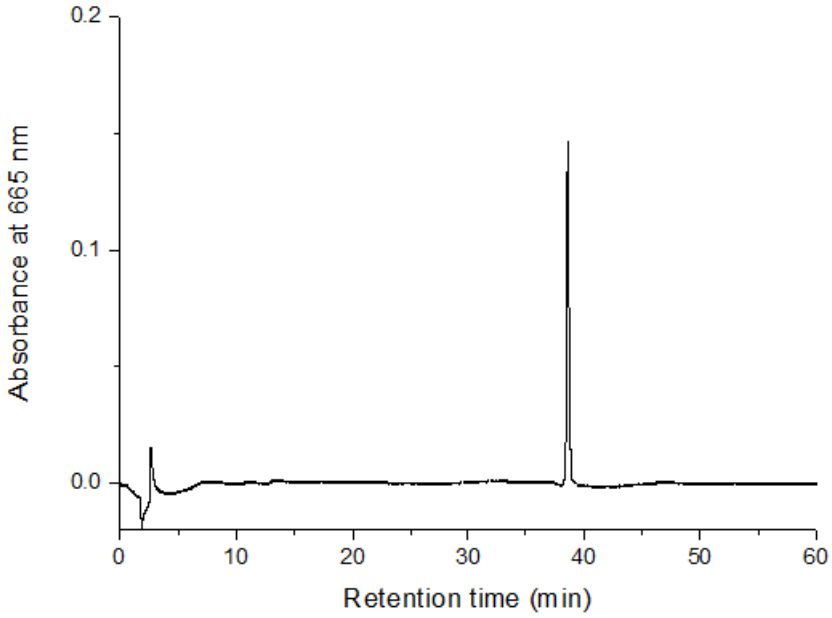


Fig. S13 HPLC chromatogram of conjugate **5**.

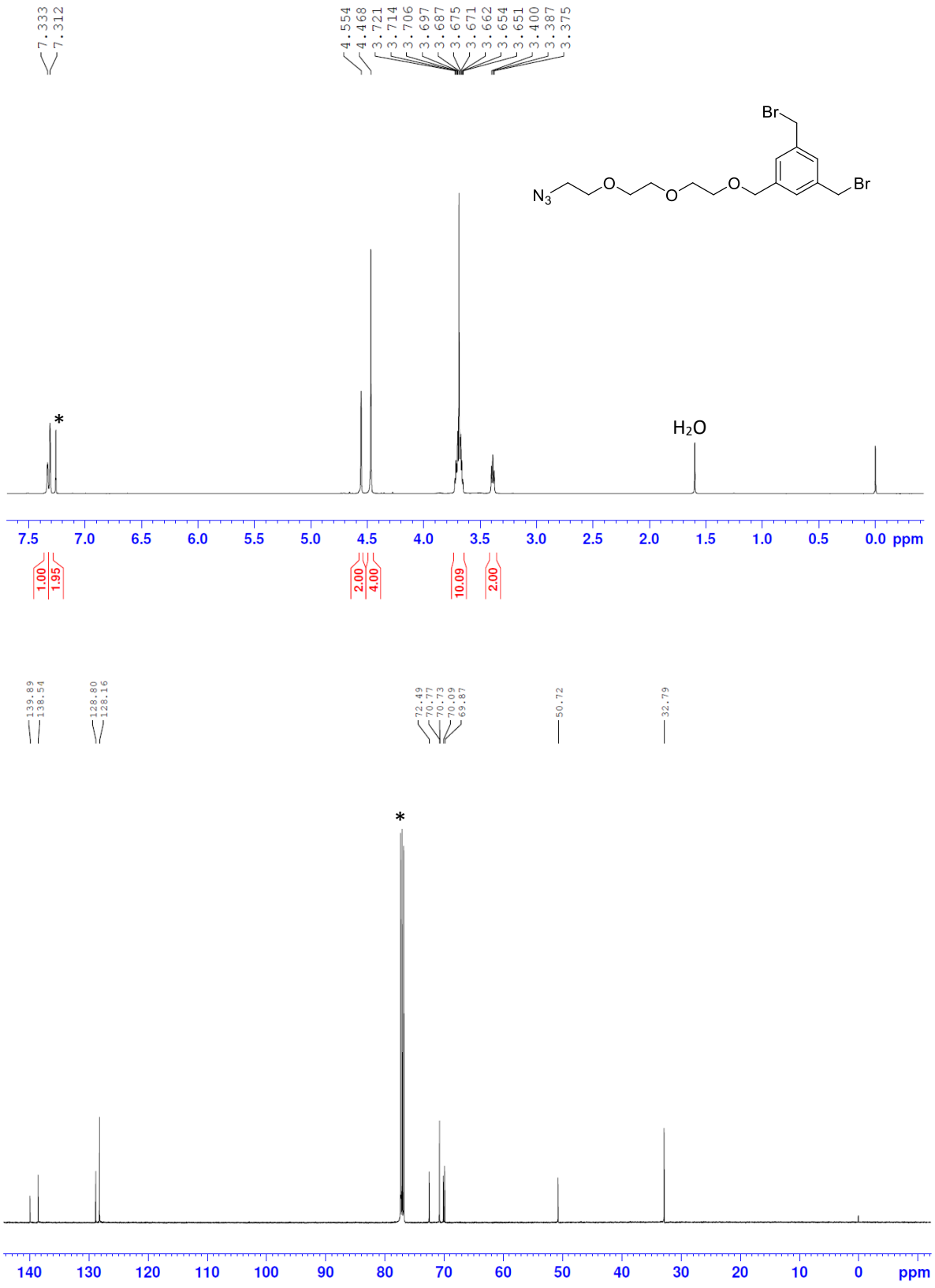


Fig. S14 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **L2** in $CDCl_3$.

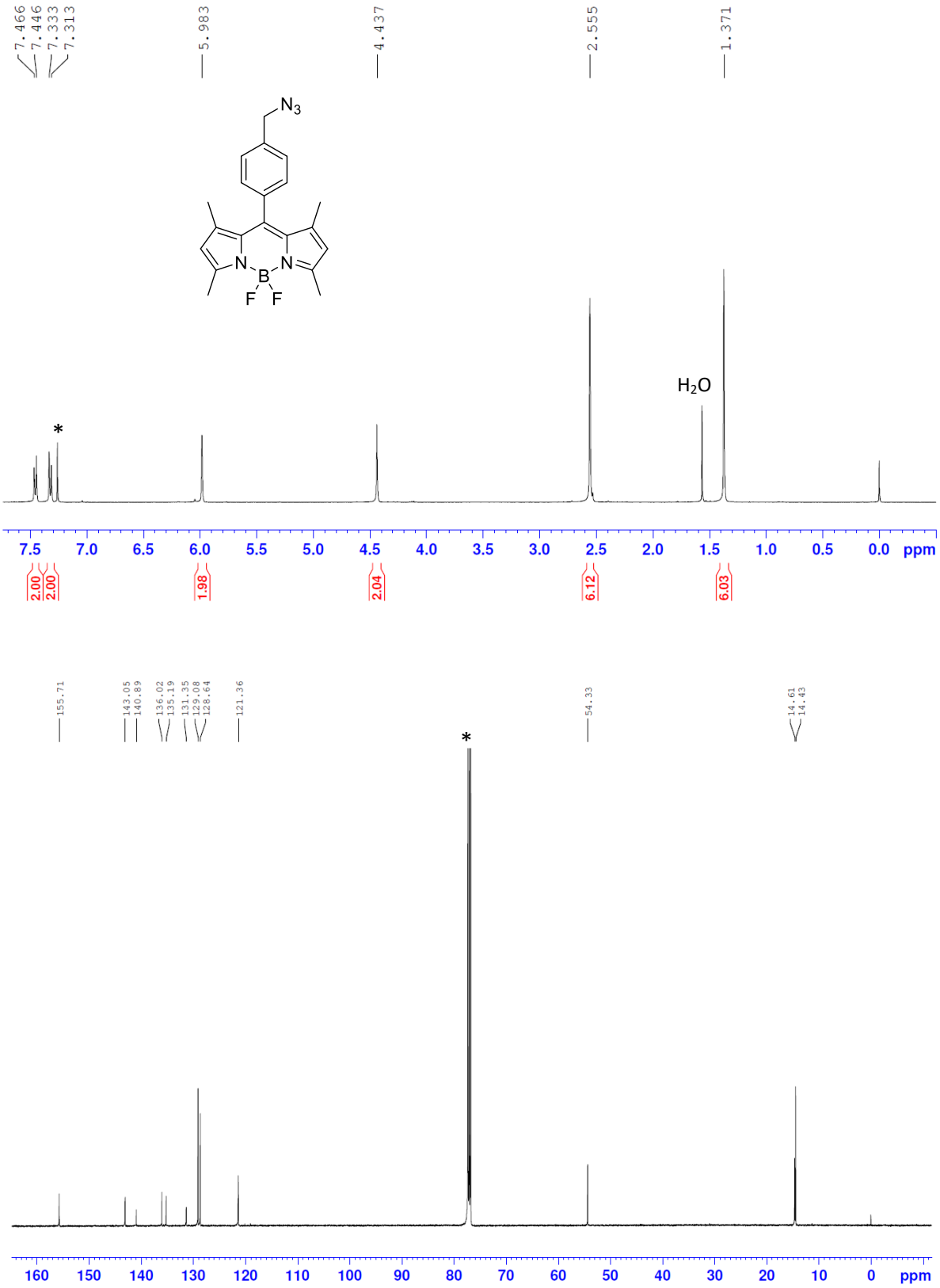


Fig. S15 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **8** in $CDCl_3$.

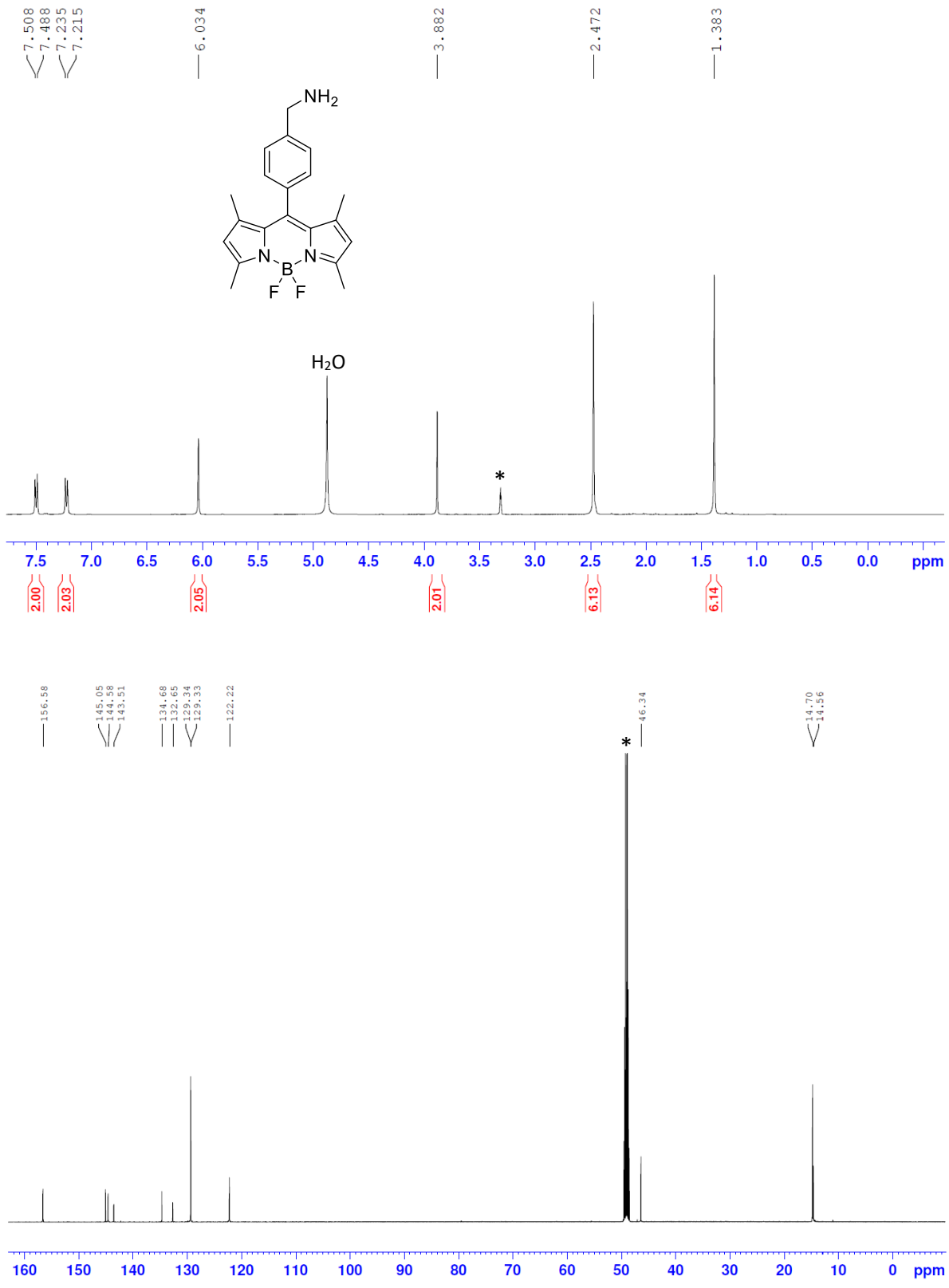


Fig. S16 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **9** in CD_3OD.

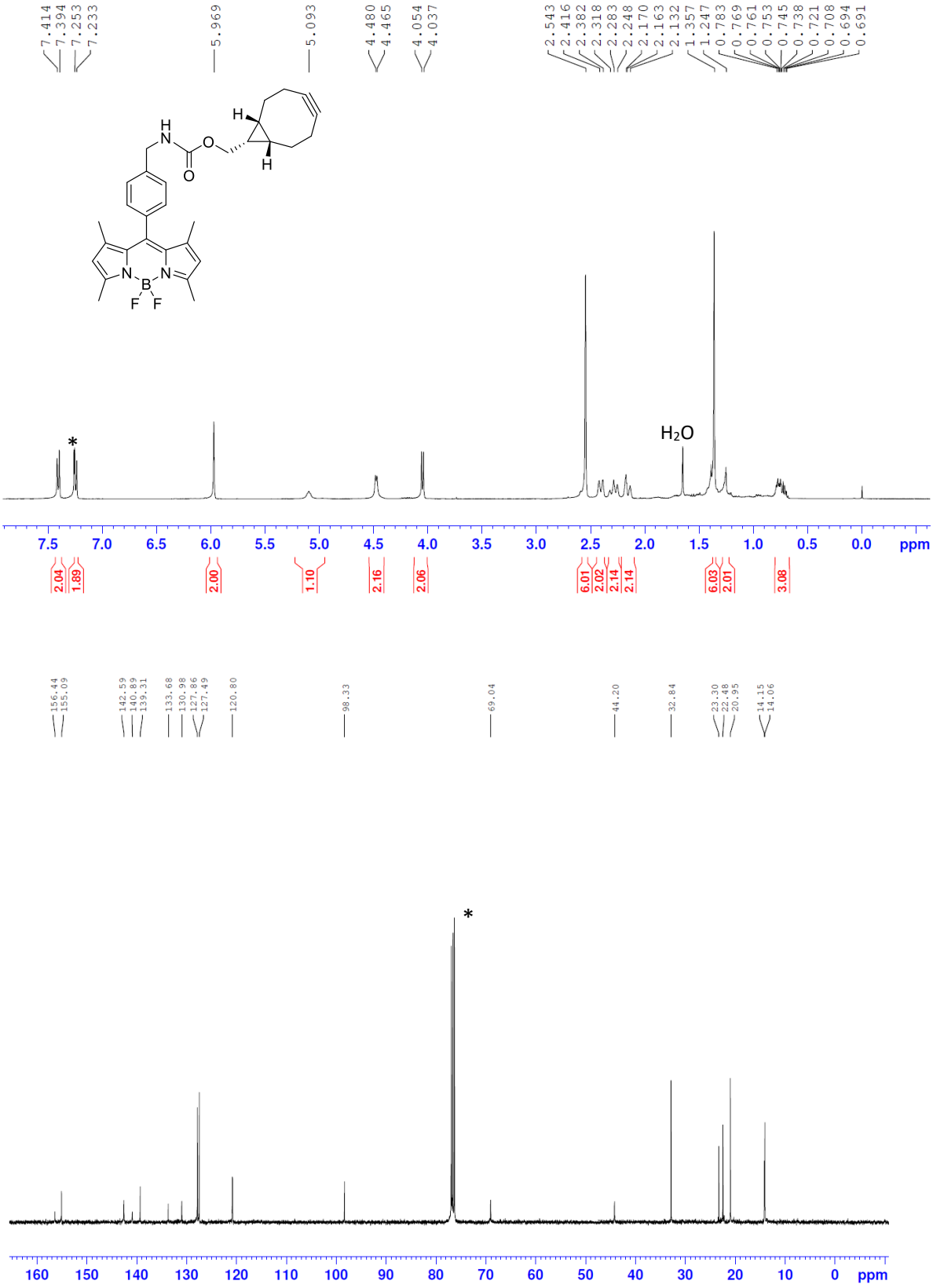


Fig. S17 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **BDP1** in $CDCl_3$.

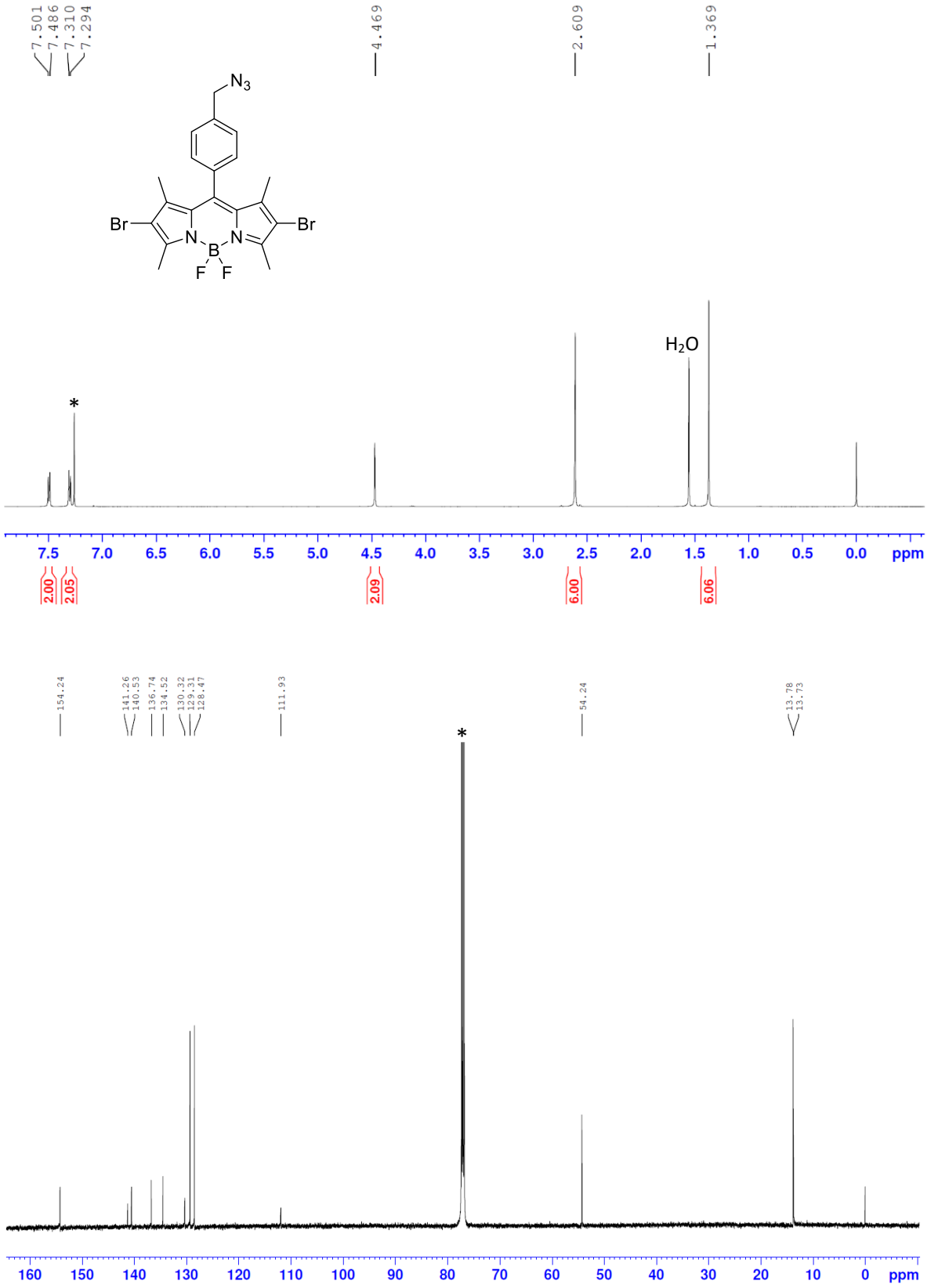


Fig. S18 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **11** in $CDCl_3$.

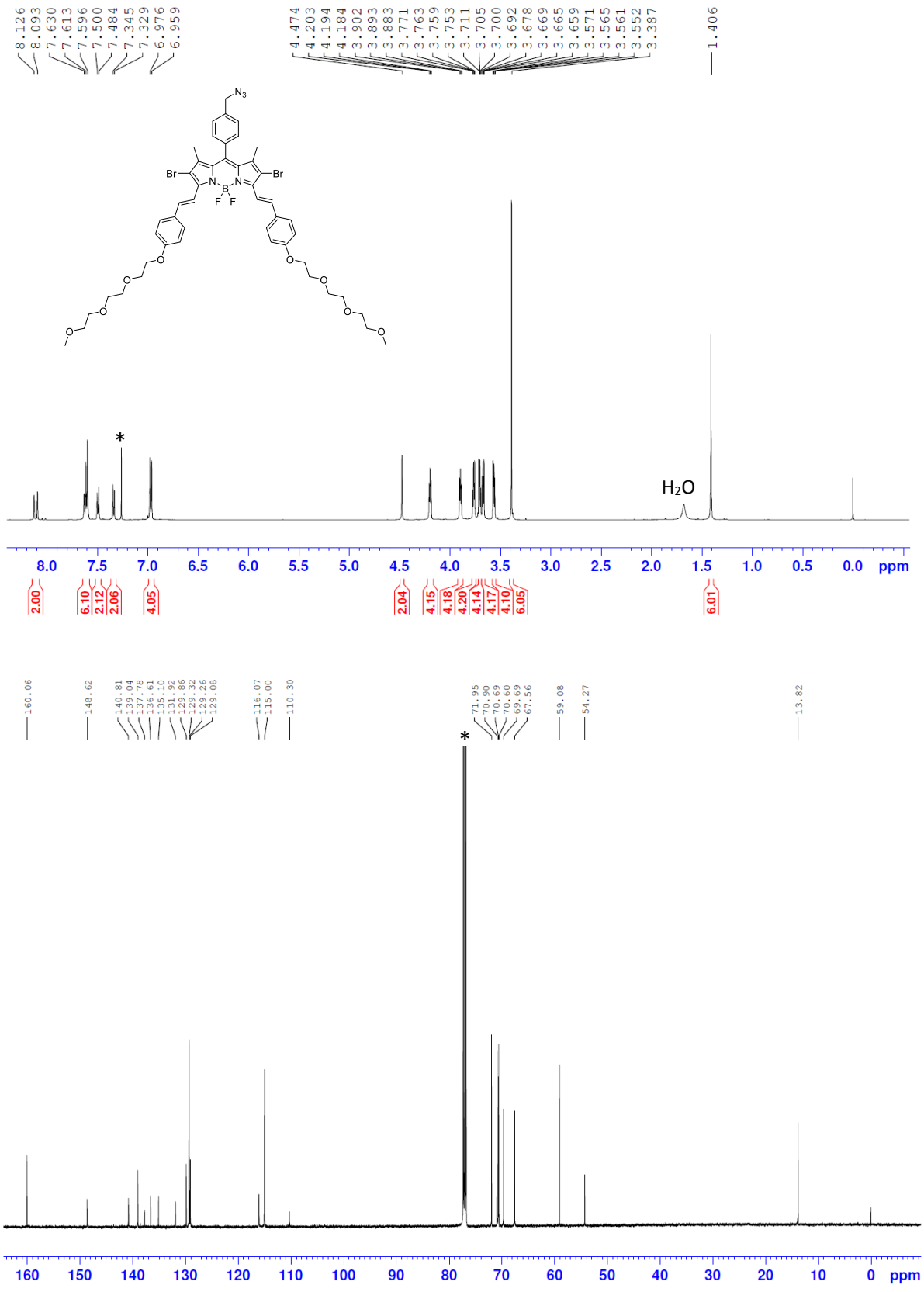


Fig. S19 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **13** in $CDCl_3$.

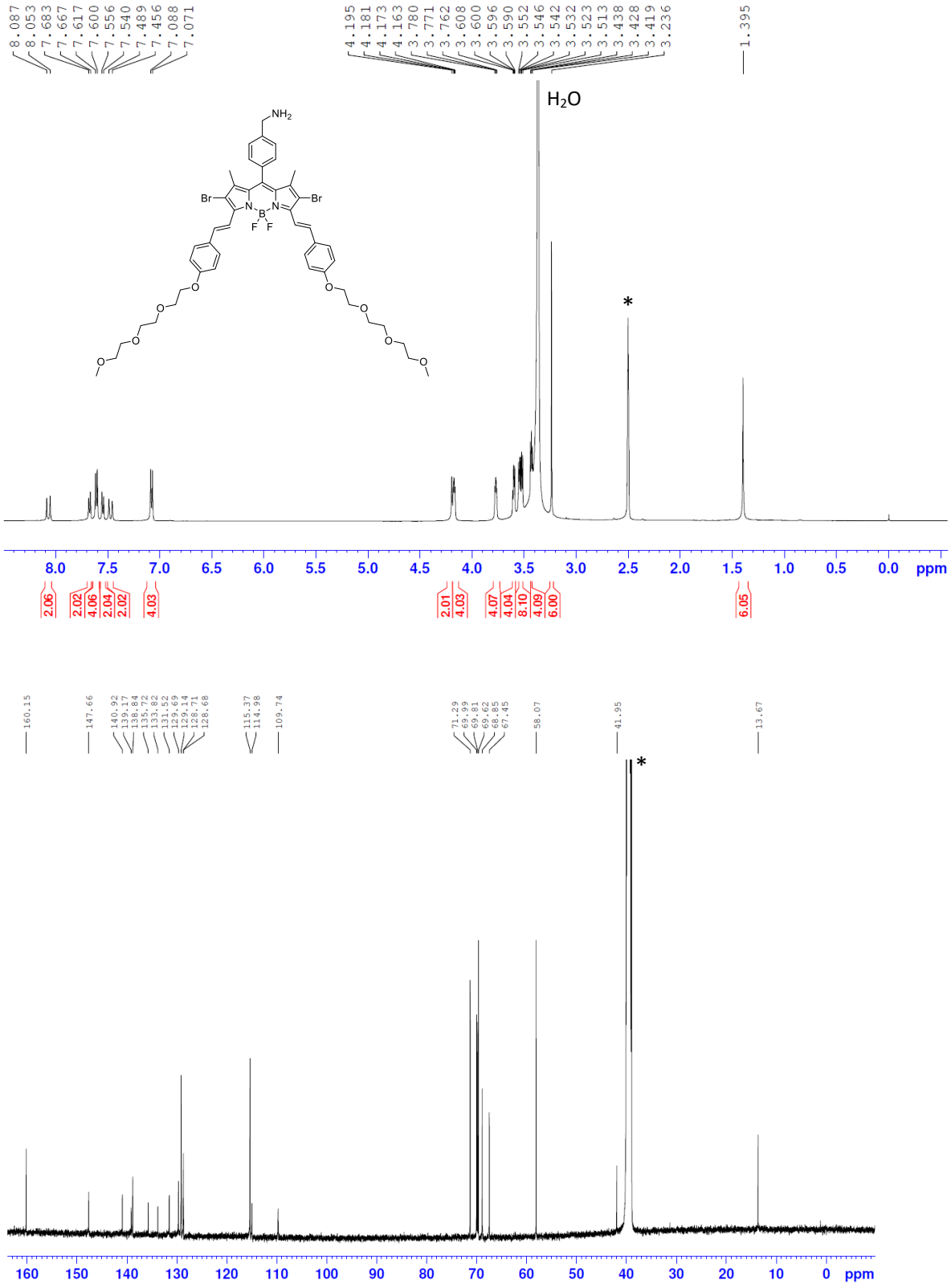


Fig. S20 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **14** in DMSO-d_6.

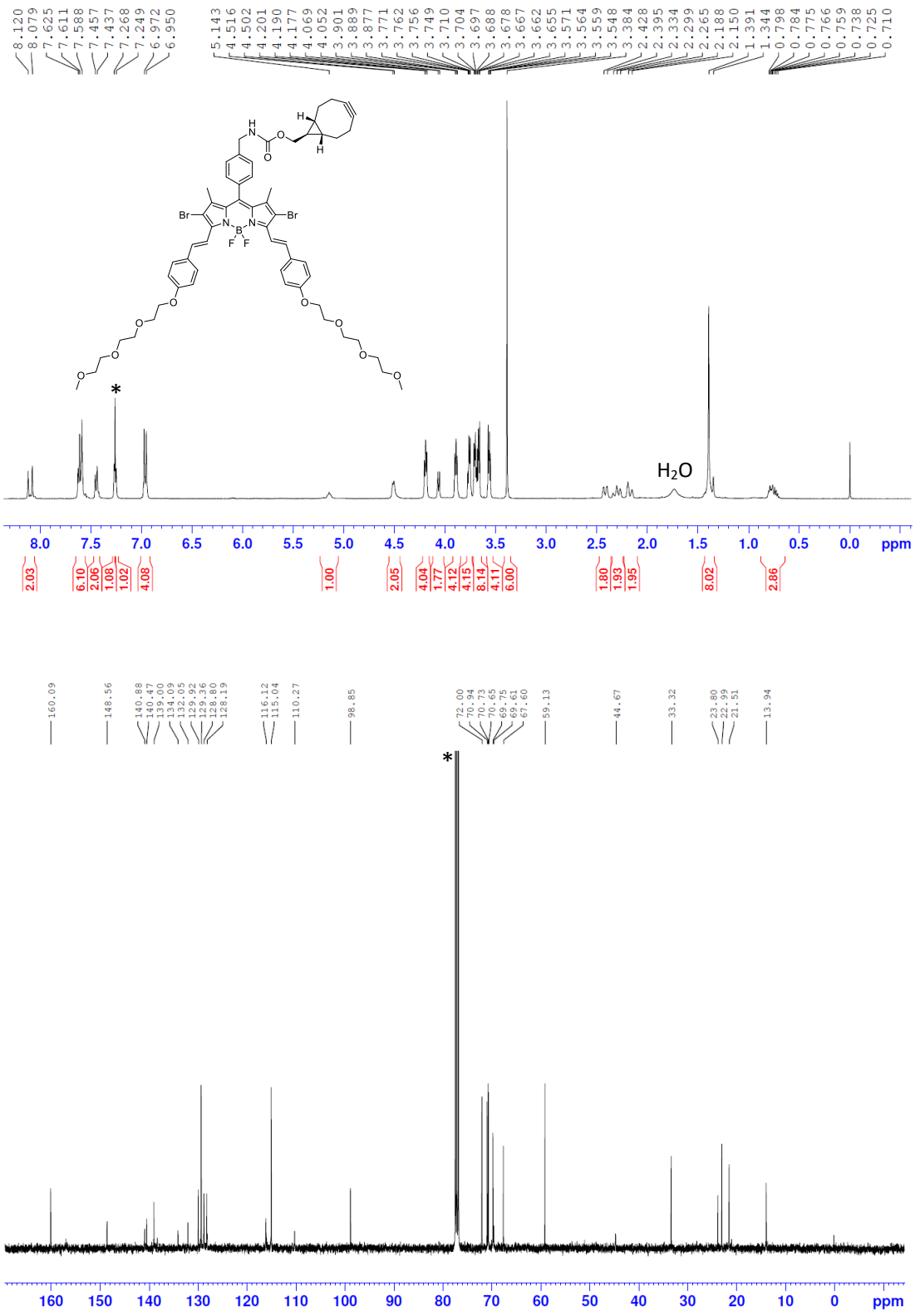


Fig. S21 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **BDP2** in $CDCl_3$.

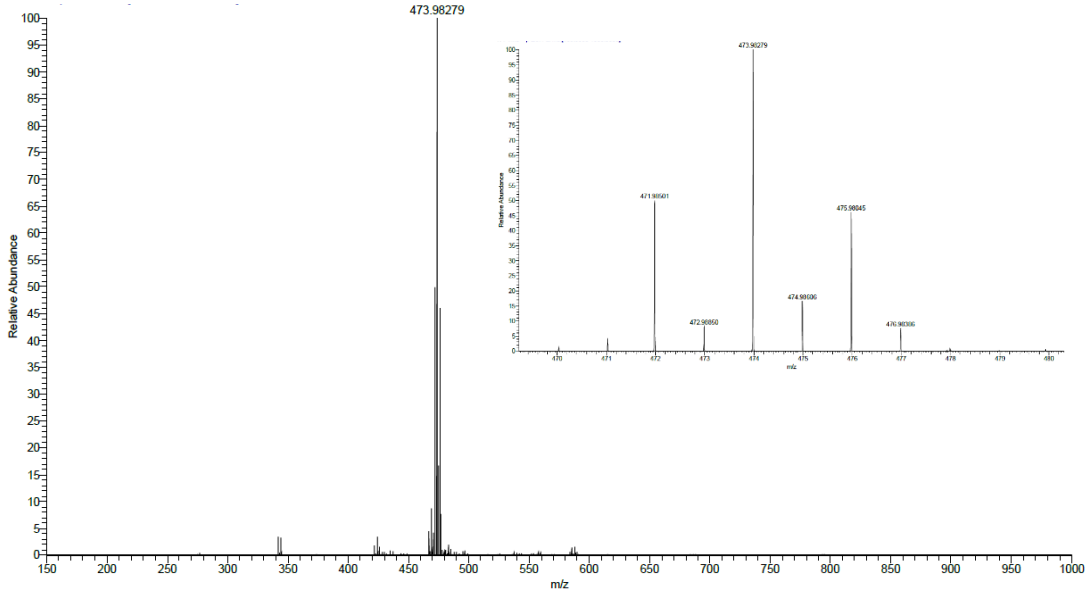


Fig. S22 ESI mass spectrum of **L2**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

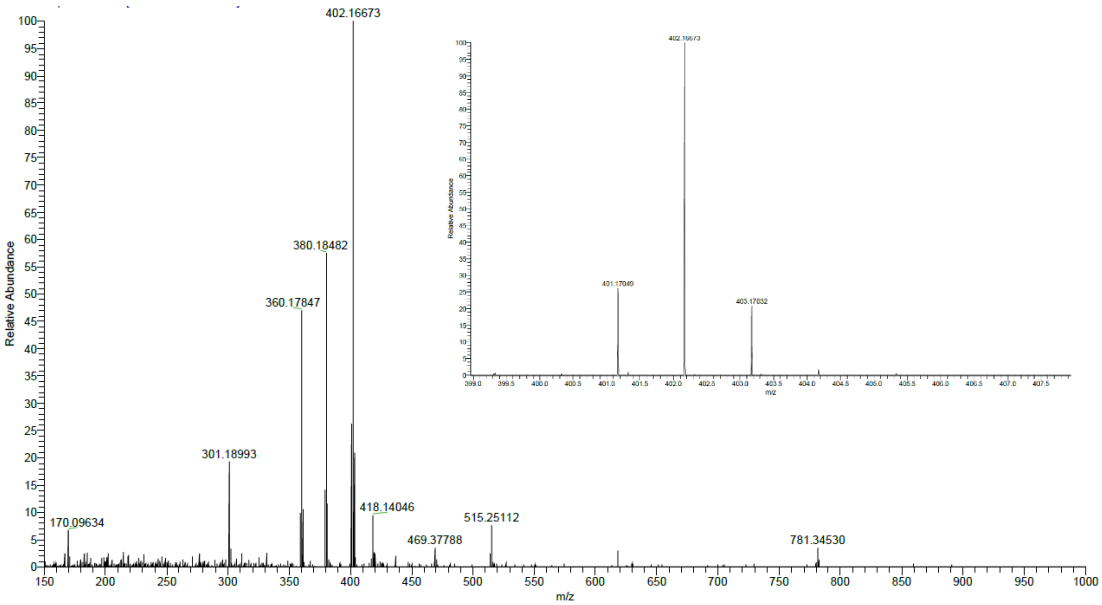


Fig. S23 ESI mass spectrum of **8**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

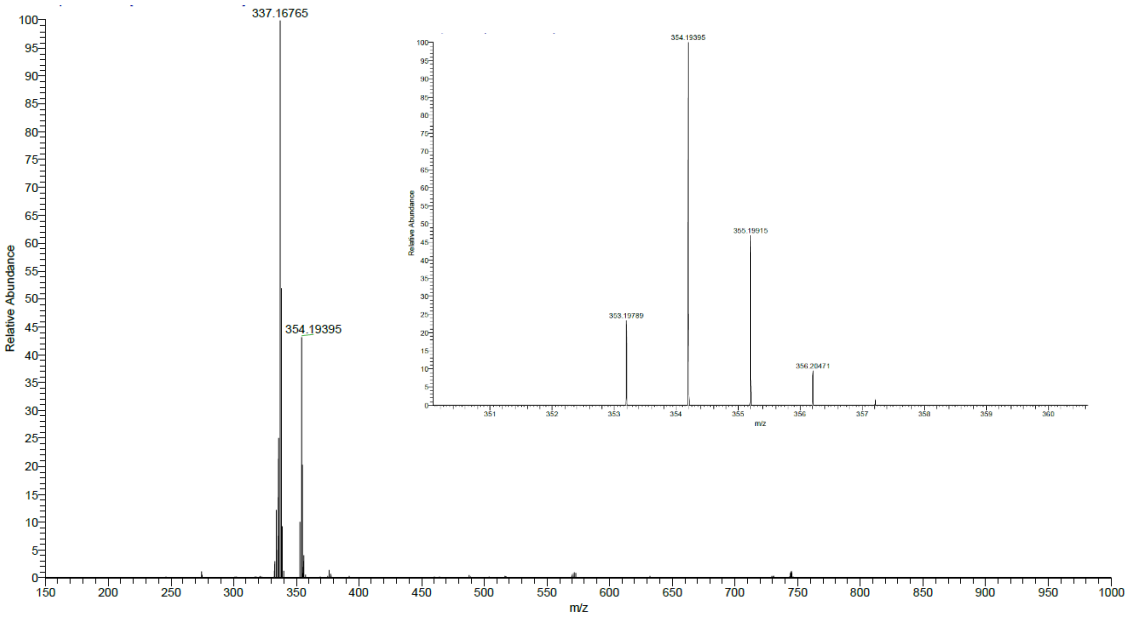


Fig. S24 ESI mass spectrum of **9**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

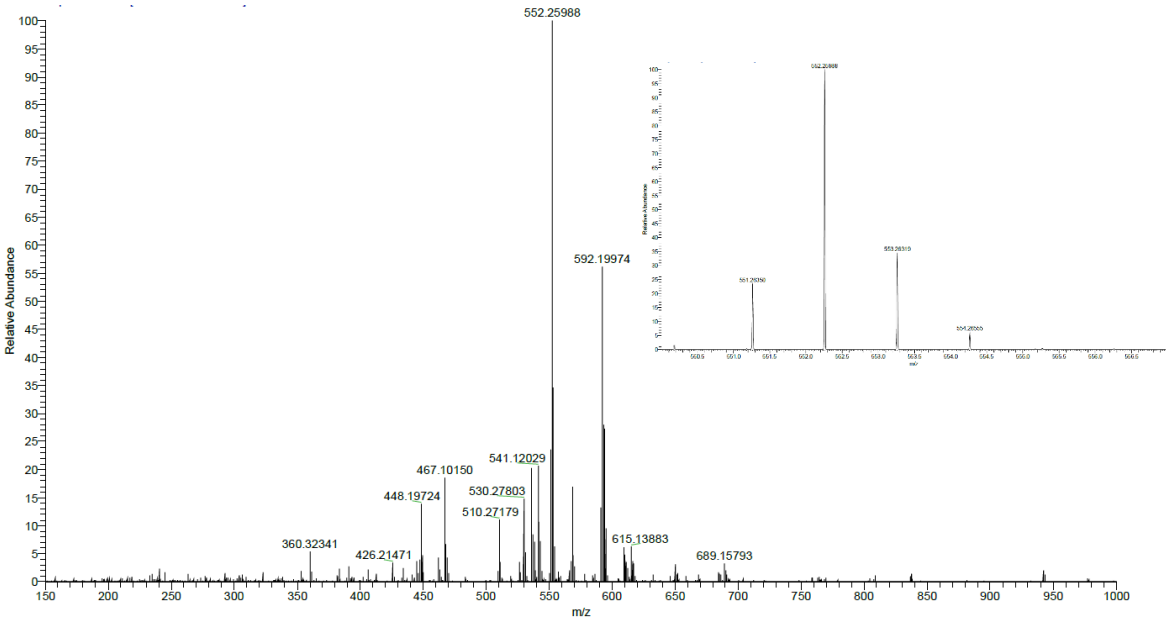


Fig. S25 ESI mass spectrum of **BDP1**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

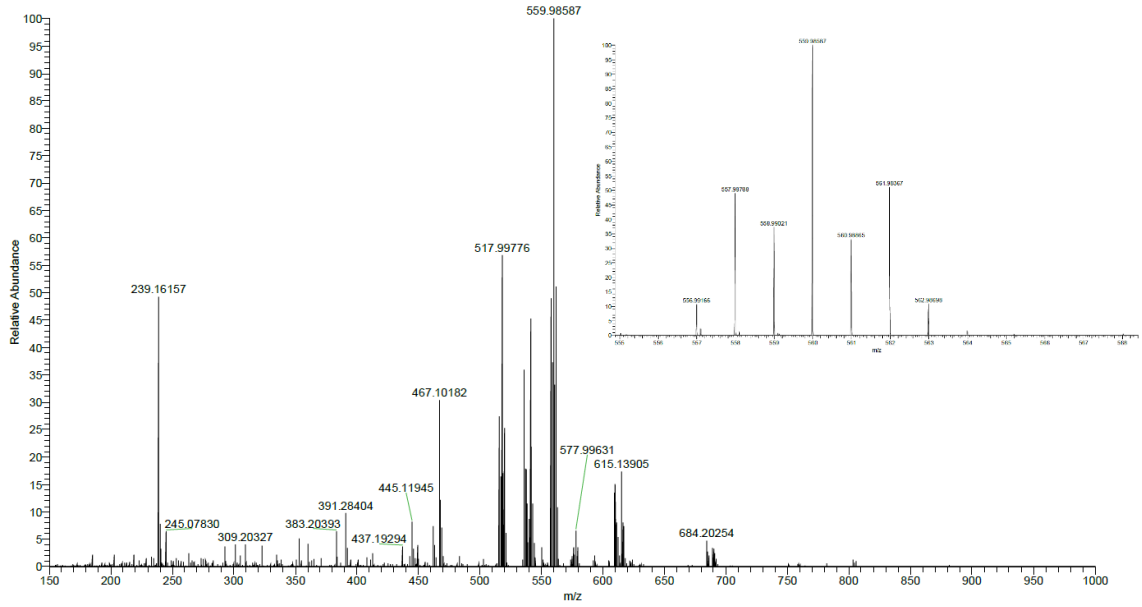


Fig. S26 ESI mass spectrum of **11**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

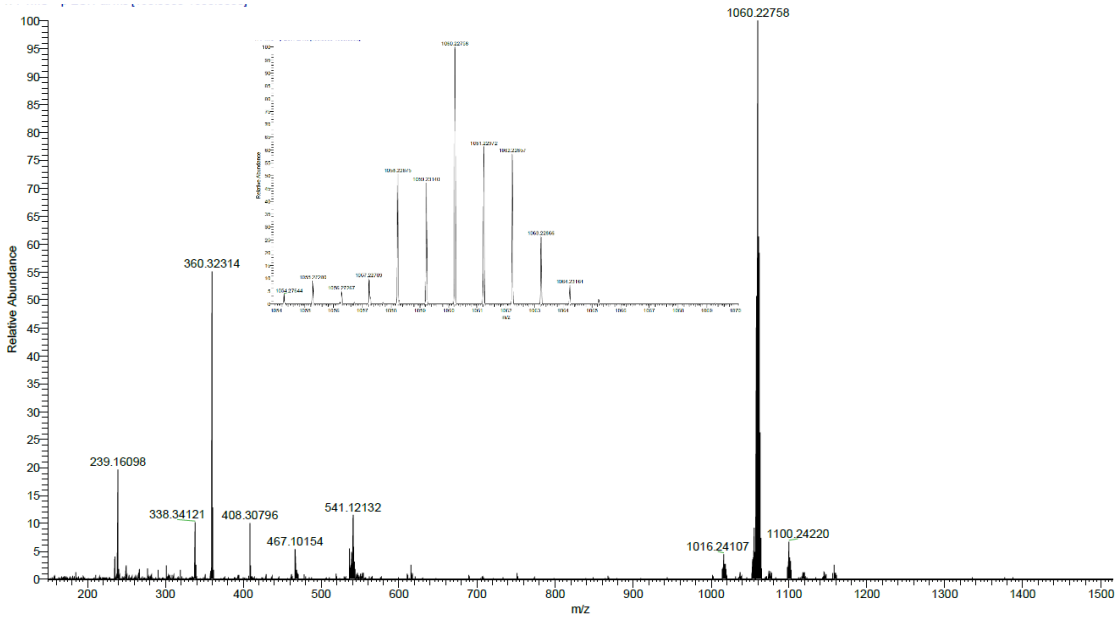


Fig. S27 ESI mass spectrum of **13**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

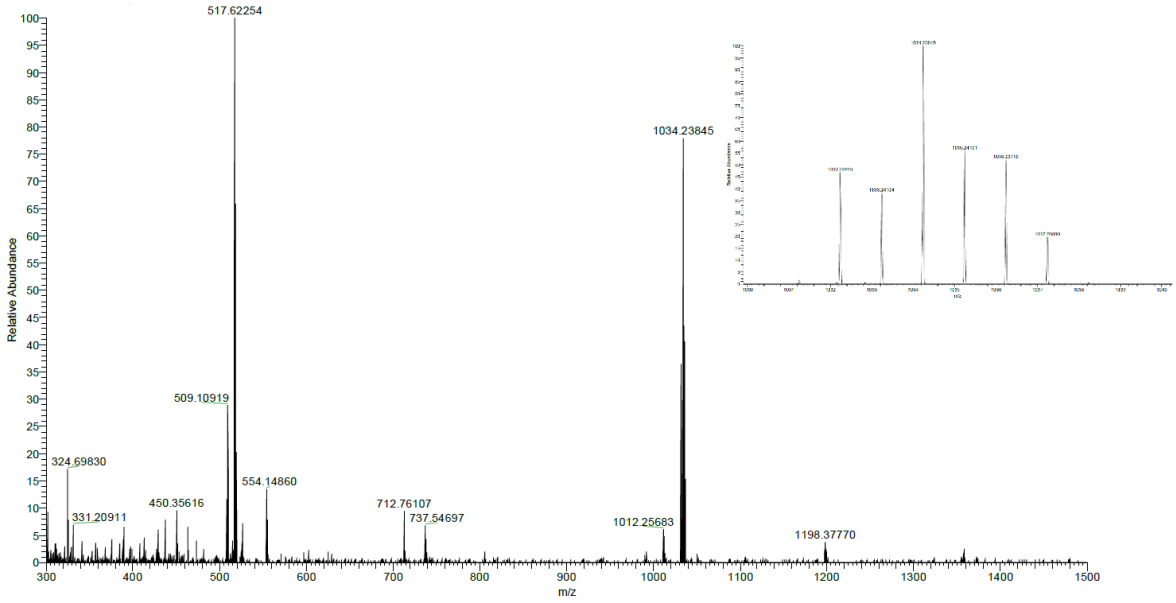


Fig. S28 ESI mass spectrum of **14**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

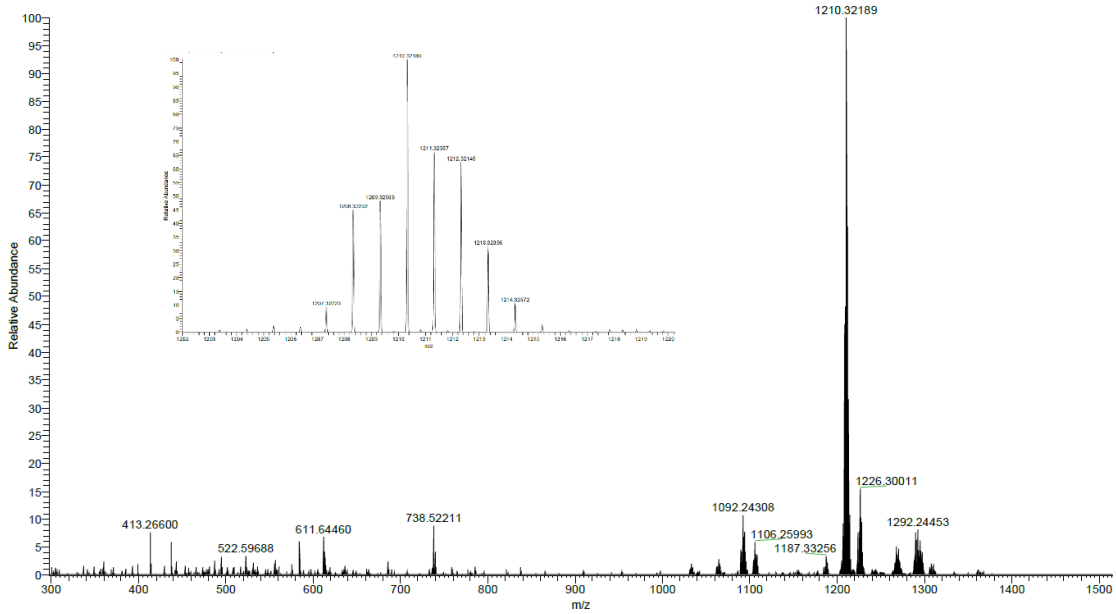


Fig. S29 ESI mass spectrum of **BDP2**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

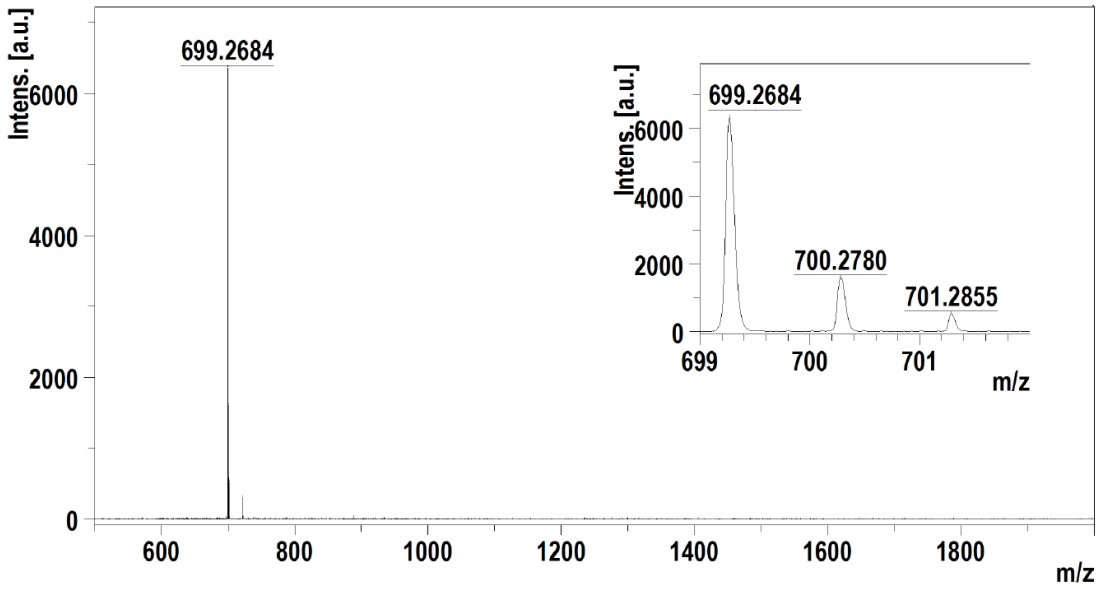


Fig. S30 MALDI-TOF mass spectrum of **RGD**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

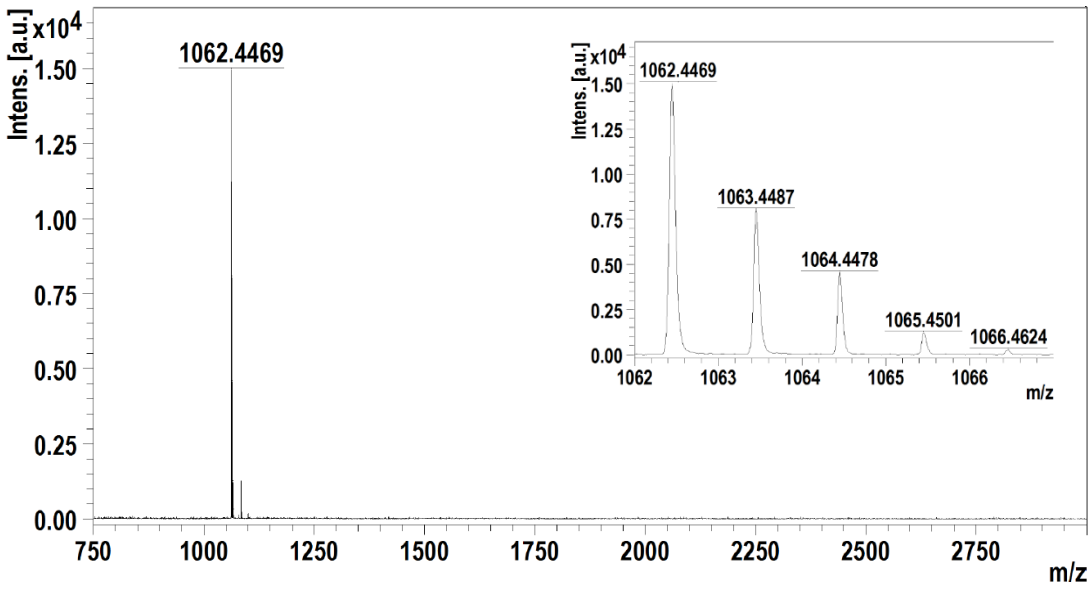


Fig. S31 MALDI-TOF mass spectrum of **RPM**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

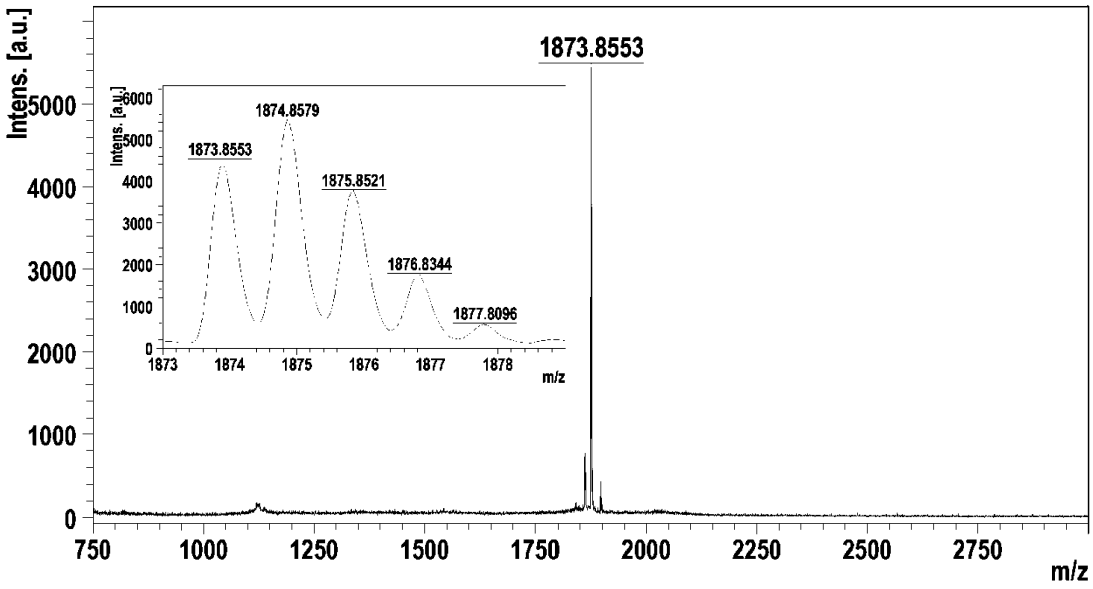


Fig. S32 MALDI-TOF mass spectrum of **SFITGv6**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

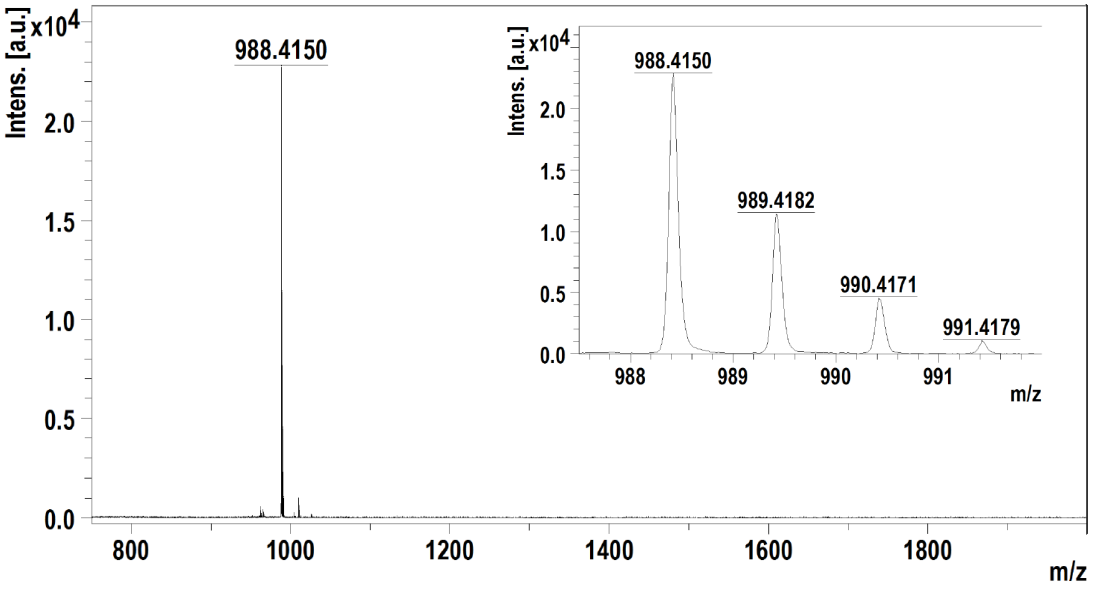


Fig. S33 MALDI-TOF mass spectrum of **N_3-TEG-RGD**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

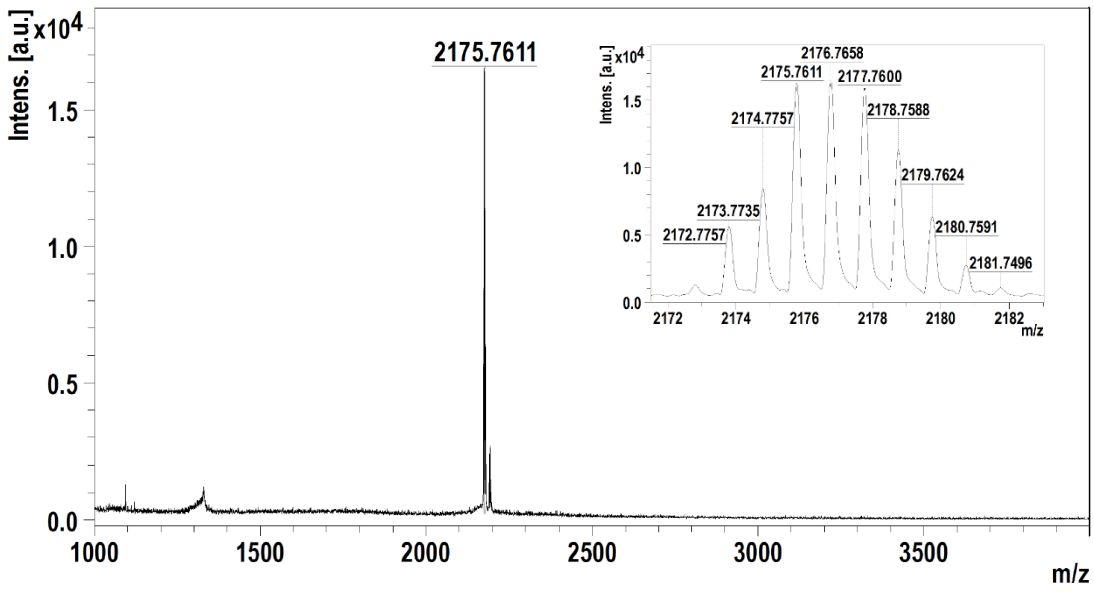


Fig. S34 MALDI-TOF mass spectrum of conjugate **1**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

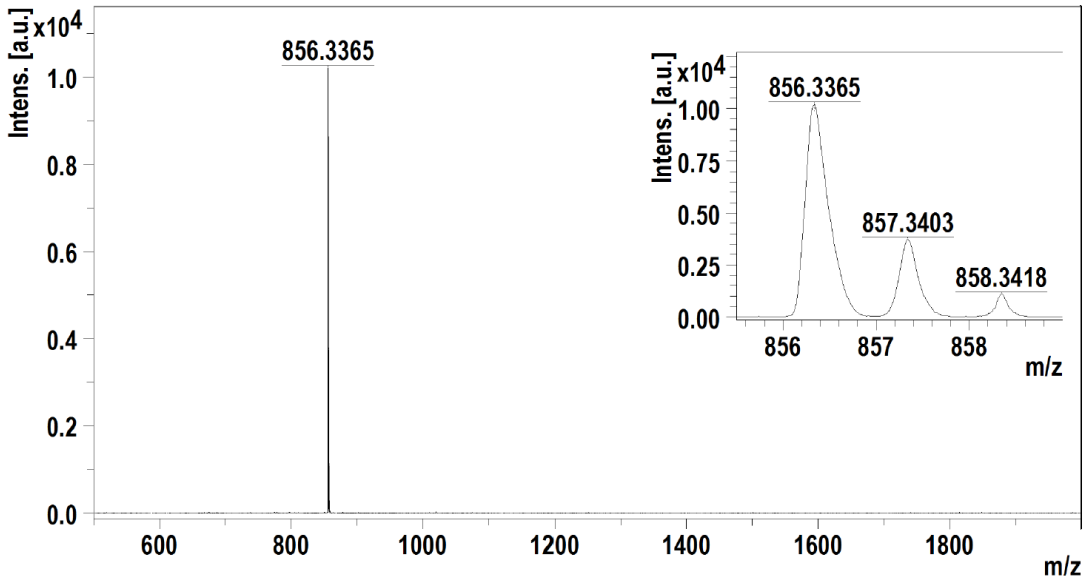


Fig. S35 MALDI-TOF mass spectrum of **N_3-RGD**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

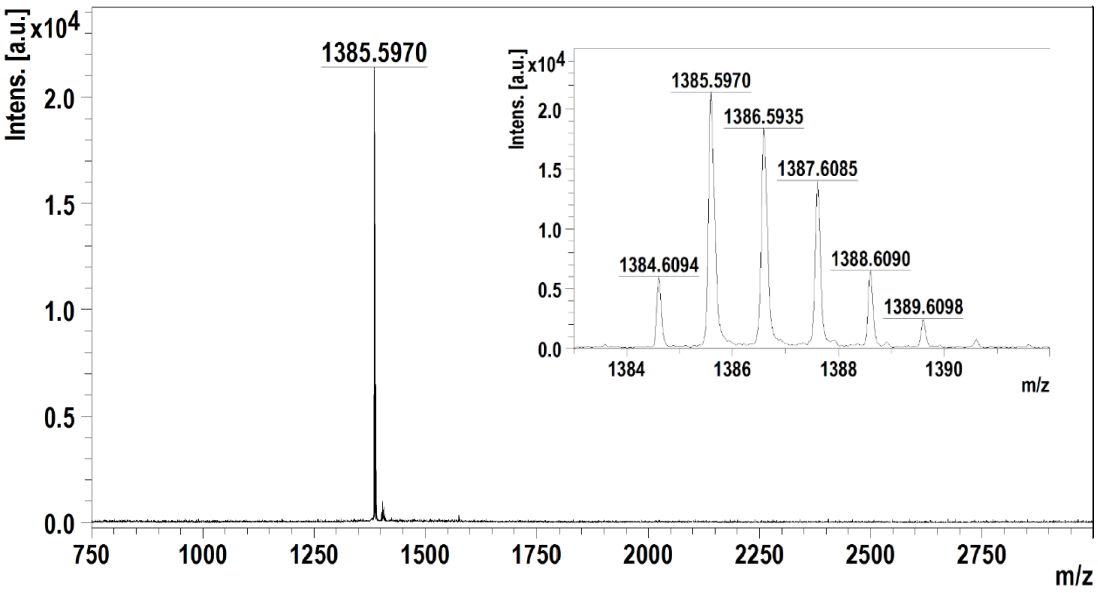


Fig. S36 MALDI-TOF mass spectrum of conjugate **2**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

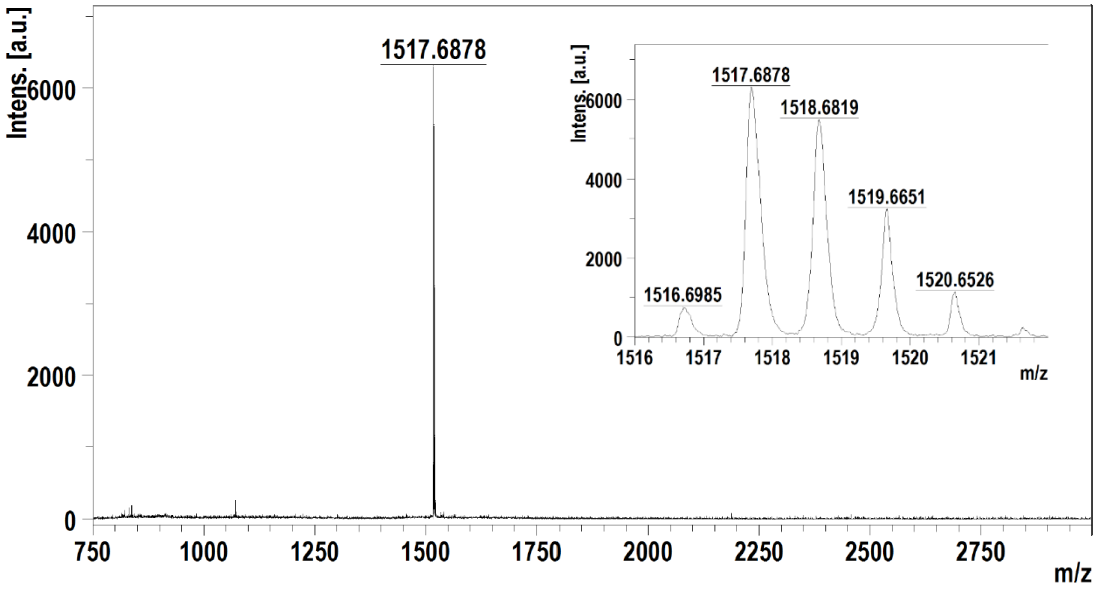


Fig. S37 MALDI-TOF mass spectrum of conjugate **3**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

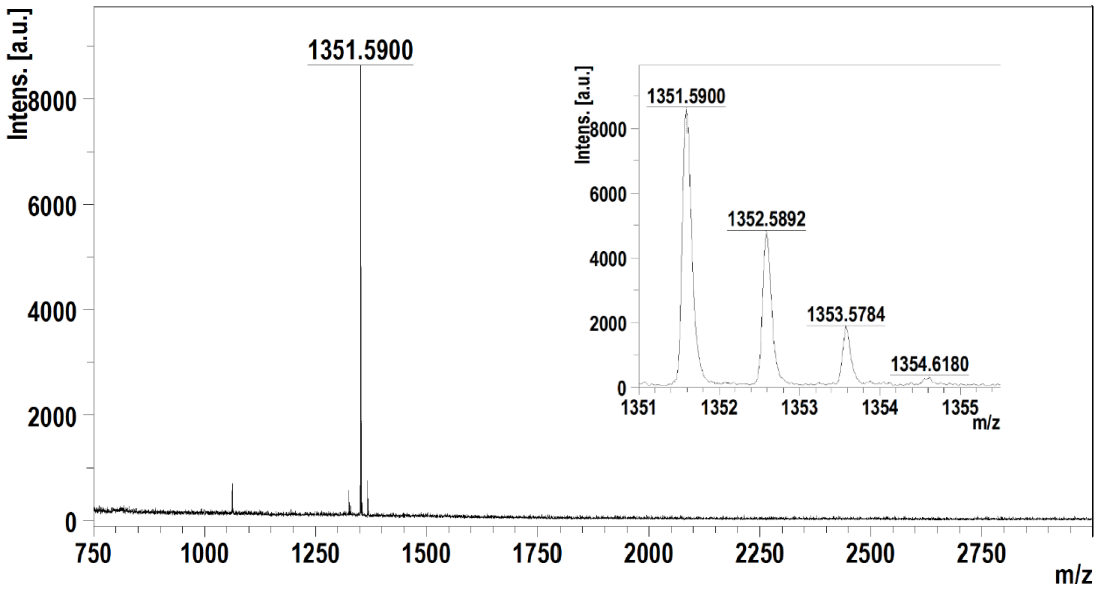


Fig. S38 MALDI-TOF mass spectrum of **N_3-TEG-RPM**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

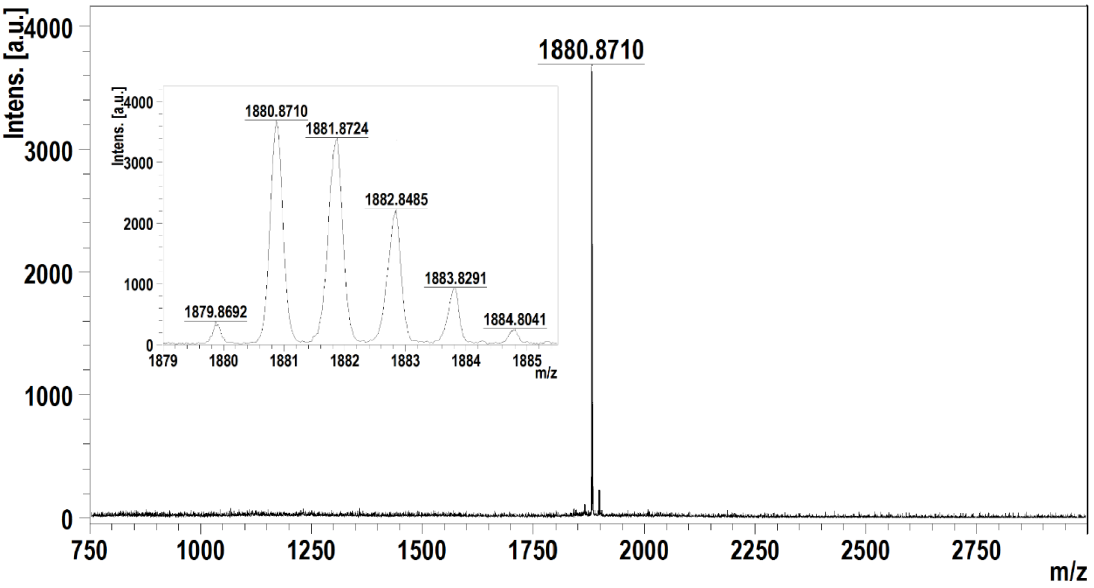


Fig. S39 MALDI-TOF mass spectrum of conjugate **4**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

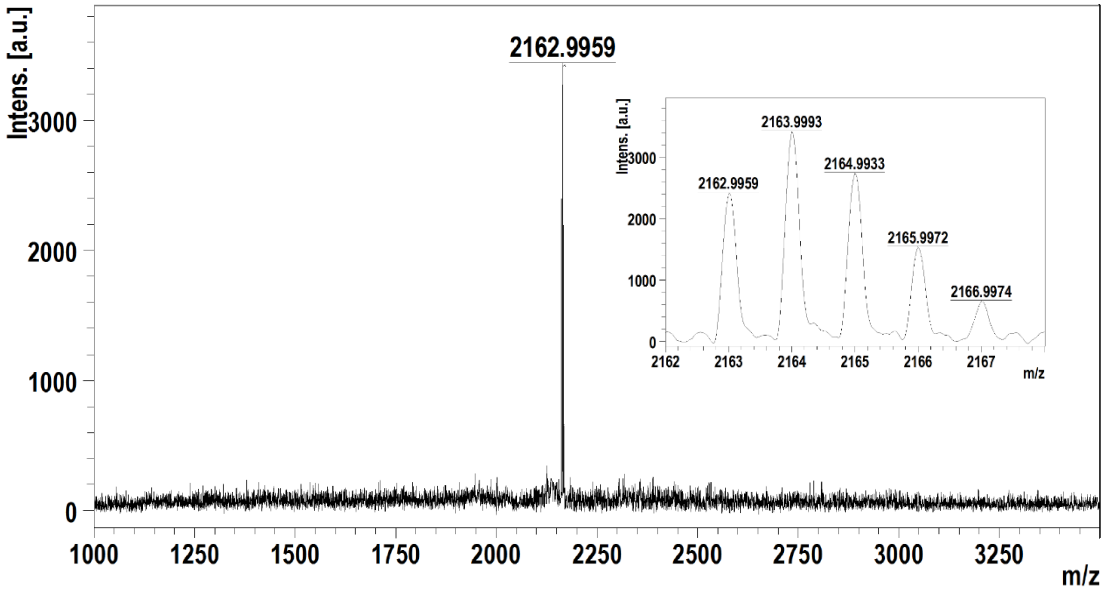


Fig. S40 MALDI-TOF mass spectrum of **N_3-TEG-SFITGv6**. The inset shows the enlarged isotopic envelop of the molecular ion peak.

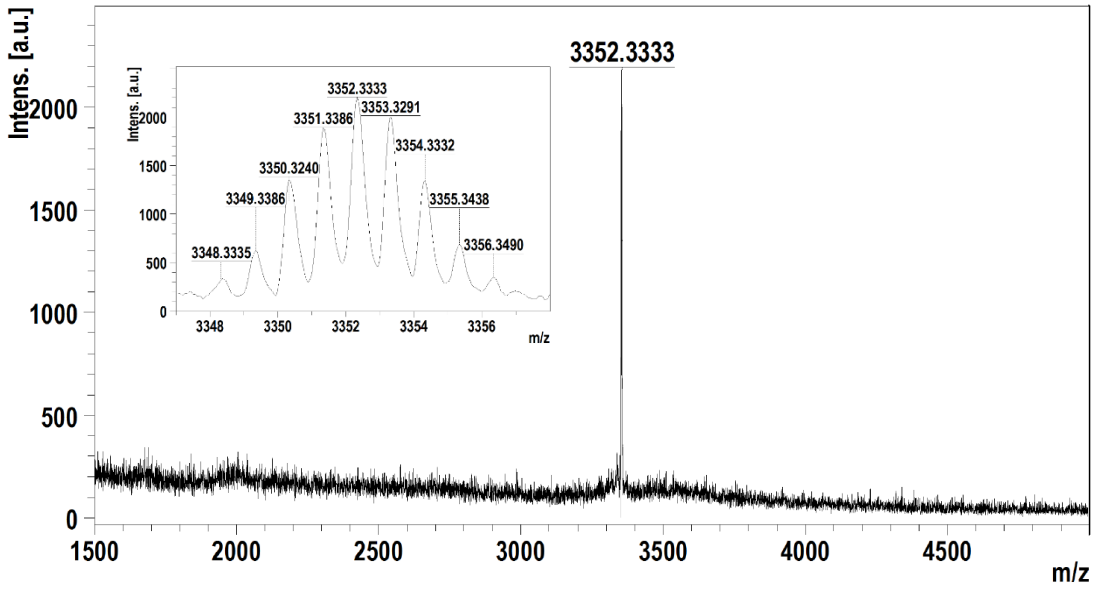


Fig. S41 MALDI-TOF mass spectrum of conjugate **5**. The inset shows the enlarged isotopic envelop of the molecular ion peak.