Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

A Fluorescent Electrophile for CLIPS: Self Indicating TrkB Binders

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A. General Experimental Procedures

All the reagents have been purchased from commercial sources and used without further purification. ^{1}H and TOCSY-NMR spectra were recorded on Bruker Avance III at 400 MHz at room temperature in a solvent of 90 % H_2O + 10 % D_2O, $CDCl_3$, D_2O, and DMSO-d_6. Chemical shifts of ^{1}H NMR spectra were reported in ppm as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd =doublet of doublet), coupling constants, and number of protons. Mass spectra (MS) were measured under High-resolution electrospray ionization mass spectrometry (ESI-MS) in negative mode.

Prep HPLC was performed on Agilent 1260 Infinity in 30 – 95 % CH_3CN/water gradient with 0.1% TFA over 20 mins. Graphpad Prism version 9.0 or later (Graphpad Software) carried out all statistical analyses.

B. Supporting Data

Table S1: BODIPY Substitution Conditions

Table S1: BODIPY substitution conditions; conversions as measured by analytical HPLC. Reactions were conducted in 1 mL of the listed solvent with 100 mM nucleophile and 10 mM BODIPY **1**.

nucleophile	solvent	base	temperature	time (h)	product	conversion (%)
N-acetyl cysteine	DMF	DIPEA	r.t.	24	**2a**	44
	bicarb	bicarb	r.t.	1	**3a**	100
	1:1 bicarb/MeCN	bicarb	r.t.	19	**3a**	46
	3:2 bicarb/TFE	bicarb	r.t.	19	**3a**	53
	bicarb	bicarb	75 °C	1	**3a**	100
	1:1 bicarb/MeCN	bicarb	75 °C	4	**3a**	100
	3:2 bicarb/TFE	bicarb	75 °C	4	**3a**	100
N-α-acetyl lysine	DMF	DIPEA	r.t.	24	**2b**	15
	bicarb	bicarb	r.t.	1	**2b**	99
	1:1 bicarb/MeCN	bicarb	r.t.	1	**2b**	99
	3:2 bicarb/TFE	bicarb	r.t.	1	**2b**	100
	bicarb	bicarb	75 °C	1	**2b**	98
	1:1 bicarb/MeCN	bicarb	75 °C	1	**2b**	98
	3:2 bicarb/TFE	bicarb	75 °C	1	**2b**	99
	bicarb	bicarb	75 °C	25	**2b**	96
	1:1 bicarb/MeCN	bicarb	75 °C	25	**2b**	95
	3:2 bicarb/TFE	bicarb	75 °C	25	**2b**	96
N-ε-acetyl lysine	DMF	DIPEA	r.t.	24	**2c**	21
	bicarb	bicarb	r.t.	22	**2c**	98
proline	DMF	DIPEA	r.t.	24	**2d**	23
	bicarb	bicarb	r.t.	3	**2d**	96
phenol	DMF	DIPEA	r.t.	24	**2e**	27
ethanol	DMF	DIPEA	r.t.	24	**2f**	23
piperidine	bicarb	bicarb	r.t.	3	**2g**	100

Figure S1: Absorbance and Fluorescence Spectra of **5a-g**

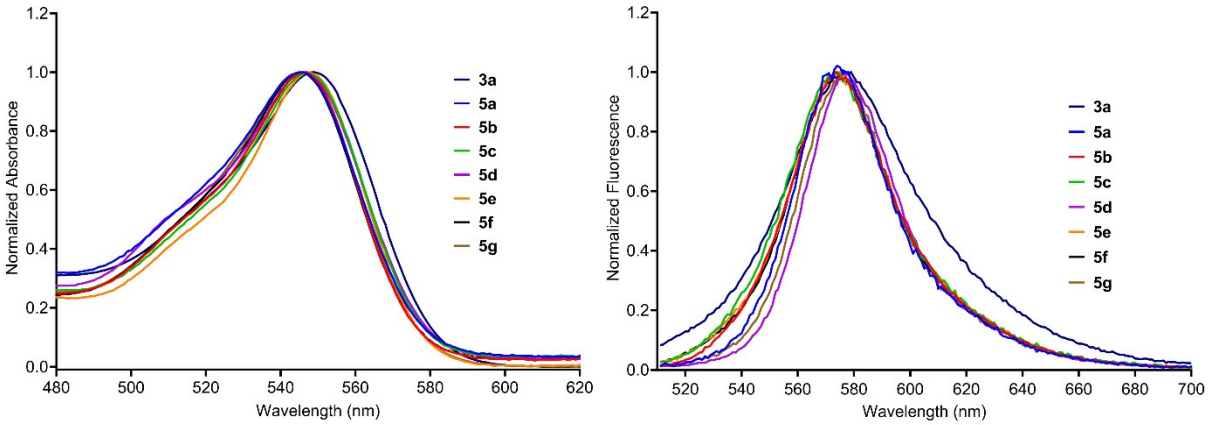


Figure S1: Normalized absorbance and emission spectra of **3a** and fluorescent peptide loops **5a** – **5g** taken at 2 μM in water.

Figure S2: Calibration curve using UV absorbance

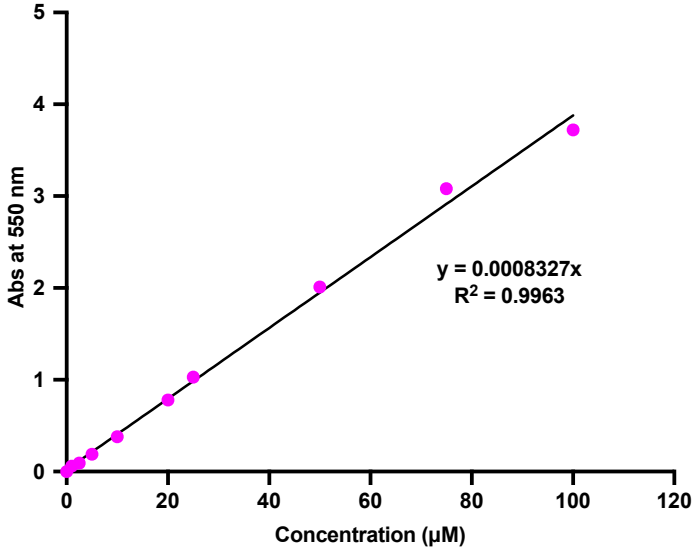
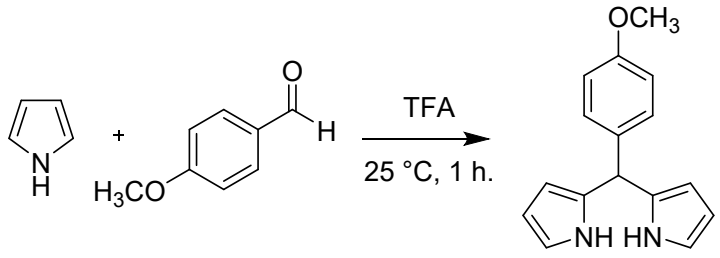


Figure S2: Calibration curve using UV absorbance at 550 nm in water.

C. Synthesis and Characterization

1. BODIPY Synthesis

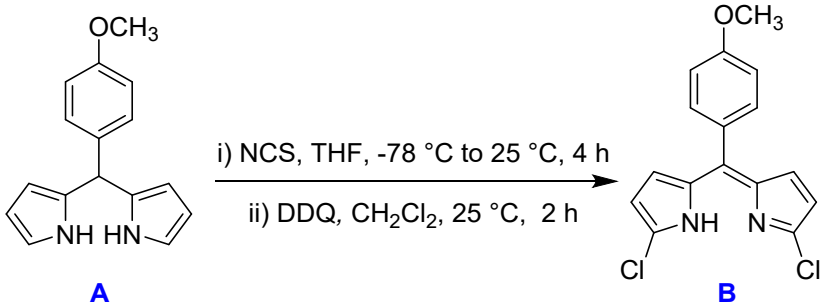
Synthesis of 5-(4-methoxyphenyl)dipyrromethene



A

Pyrrole (142 mL, 2.6 mol, 25 eq) and 4-methoxybenzaldehyde (10 mL, 82.2 mmol, 1 eq) were added to a 500 mL round-bottomed flask and degassed with a stream of Ar gas for 5 min. Trifluoroacetic acid (0.63 mL, 8.2 mmol, 0.1 eq) was added to the reaction mixture. The reaction was stirred under argon at 25 °C for 1 h. The excess pyrrole was removed under reduced pressure. The residue was purified via silica column chromatography using dichloromethane to give a yellow solid **A** (7.74 g, 38 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.90 (br, 2H), 7.17 (d, *J* = 8.56 Hz, 2H), 6.90 (d, *J* = 8.68 Hz, 2H), 6.71-6.69 (m, 2 H), 6.21-6.19 (m, 2H), 5.96-5.95 (m, 2H), 5.44 (s, 1H), 3.83 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 158.9, 134.3, 132.9, 129.4, 117.1, 114.0, 108.4, 107.0, 55.3, 43.2 ppm. HRMS(ESI+) calcd. for $C_{16}H_{15}N_2O^+$ {M-H}$^+$ 251.1179, found 251.1175.

Synthesis of 1,1'-dichloro-5-(4-methoxyphenyl)dipyrromethene

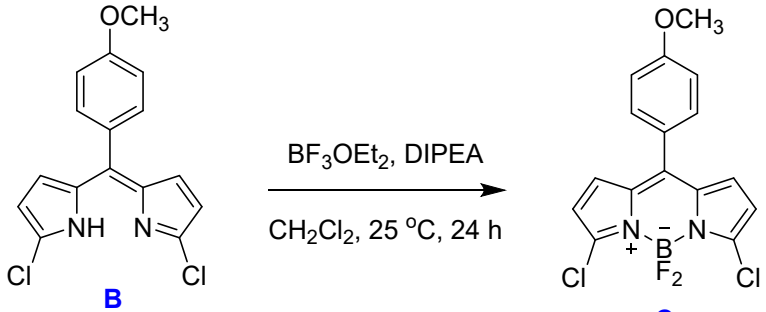


A **B**

A solution of **A** (7.74 g, 30.6 mmol, 1 eq) in 220 mL of tetrahydrofuran was purged with Ar gas and cooled to -78 °C. A suspension of *N*-chlorosuccinimide (8.2 g, 61.3 mmol, 2 eq) in 80 mL of tetrahydrofuran was added to the cooled solution. The reaction mixture was stirred at -78 °C for 1 h and warmed to 25 °C, then stirred for an additional 3 h. Water (100 mL) was added to the mixture. After extraction with CH_2Cl_2 (3 X 100 mL), the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and the solution was evaporated to dryness. The residue was immediately used without further purification.

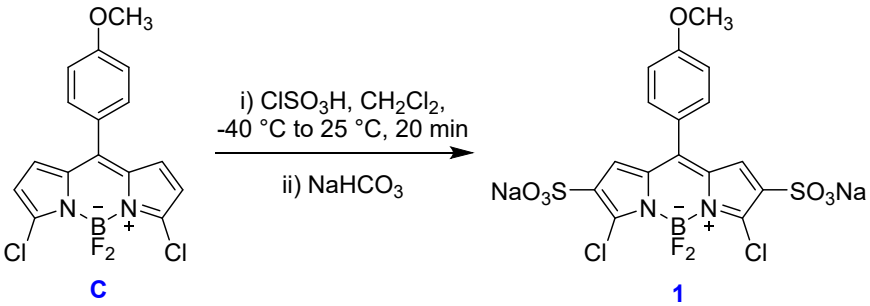
DDQ (8.0 g, 35.2 mmol, 1.2 eq) was added to the solution of the intermediate dichloro-dipyrromethane in 350 mL of dichloromethane. The mixture was stirred at 25 °C for 2 h. After evaporation of the solvent, the residue was purified by silica column chromatography using 5 % EtOAc in CH_2Cl_2 to afford a red solid **B** (7.0 g, 63 % for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.29, (d, *J* = 8.64 Hz, 2H), 6.88 (d, *J* = 8.68 Hz, 2H), 6.49, (d, *J* = 4.24 Hz, 2H), 6.17 (d, *J* = 4.24 Hz, 2H), 3.80 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 160.8, 141.4, 140.1, 138.5, 132.5, 130.1, 127.8, 116.7, 113.5, 55.4 ppm. HRMS(ESI+) calcd. for $C_{16}H_{13}Cl_2N_2O^+$ {M-H}$^+$ 319.0399, found 319.0396.

Synthesis of 3,5-dichloro-8-(4'-methoxyphenyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacence

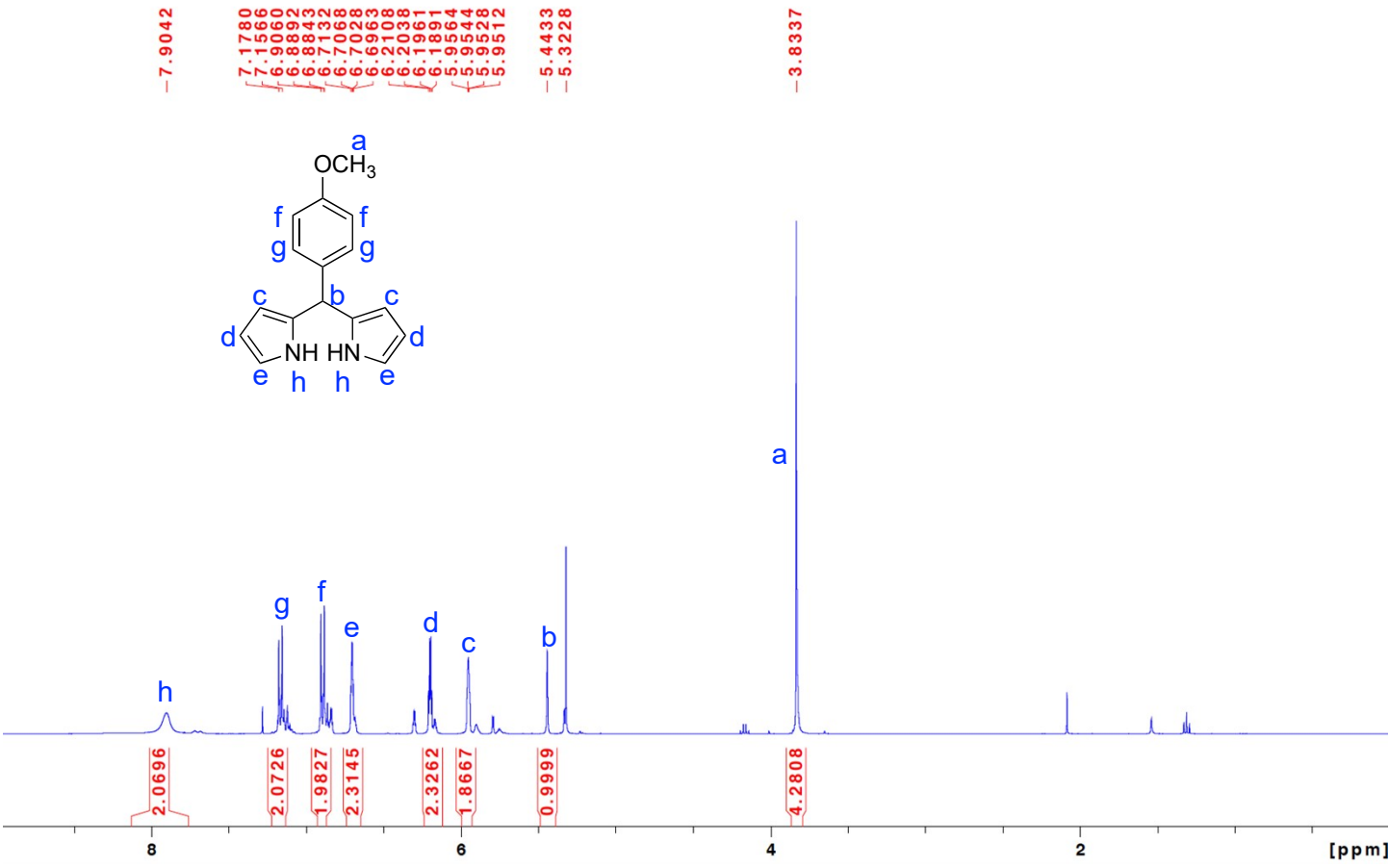


A solution of compound **B** (7 g, 22.0 mmol, 1 eq) and *N*,*N*-diisopropylethylamine (22.9 mL, 132 mmol, 6 eq) in 200 mL of dry dichloromethane was stirred under argon atmosphere at 25 °C for 10 min. Then boron trifluoride diethyletherate (27.6 mL, 219 mmol, 10 eq) was added slowly over 10 min. The resulting solution was stirred at 25 °C for 24 h. Then the solution was washed with water (3 x 100 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by silica column chromatography using 1 % EtOAc in CH_2Cl_2 to afford a brown solid **C** (3.2 g, 40 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.37 (d, *J* = 8.7 Hz, 2H), 6.96 (d, *J* = 8.7 Hz, 2H), 6.80 (d, *J* = 4.3 Hz, 2H), 6.36 (d, *J* = 4.3 Hz, 2H), 3.83 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 162.2, 144.2, 144.1, 133.7, 132.3, 131.4, 124.8, 118.6, 114.2, 55.6 ppm. HRMS(ESI+): *m/z* calcd. for $C_{16}H_{12}BCl_2F_2N_2O^+$ {M-H}$^+$ 367.0382, found 367.0378.

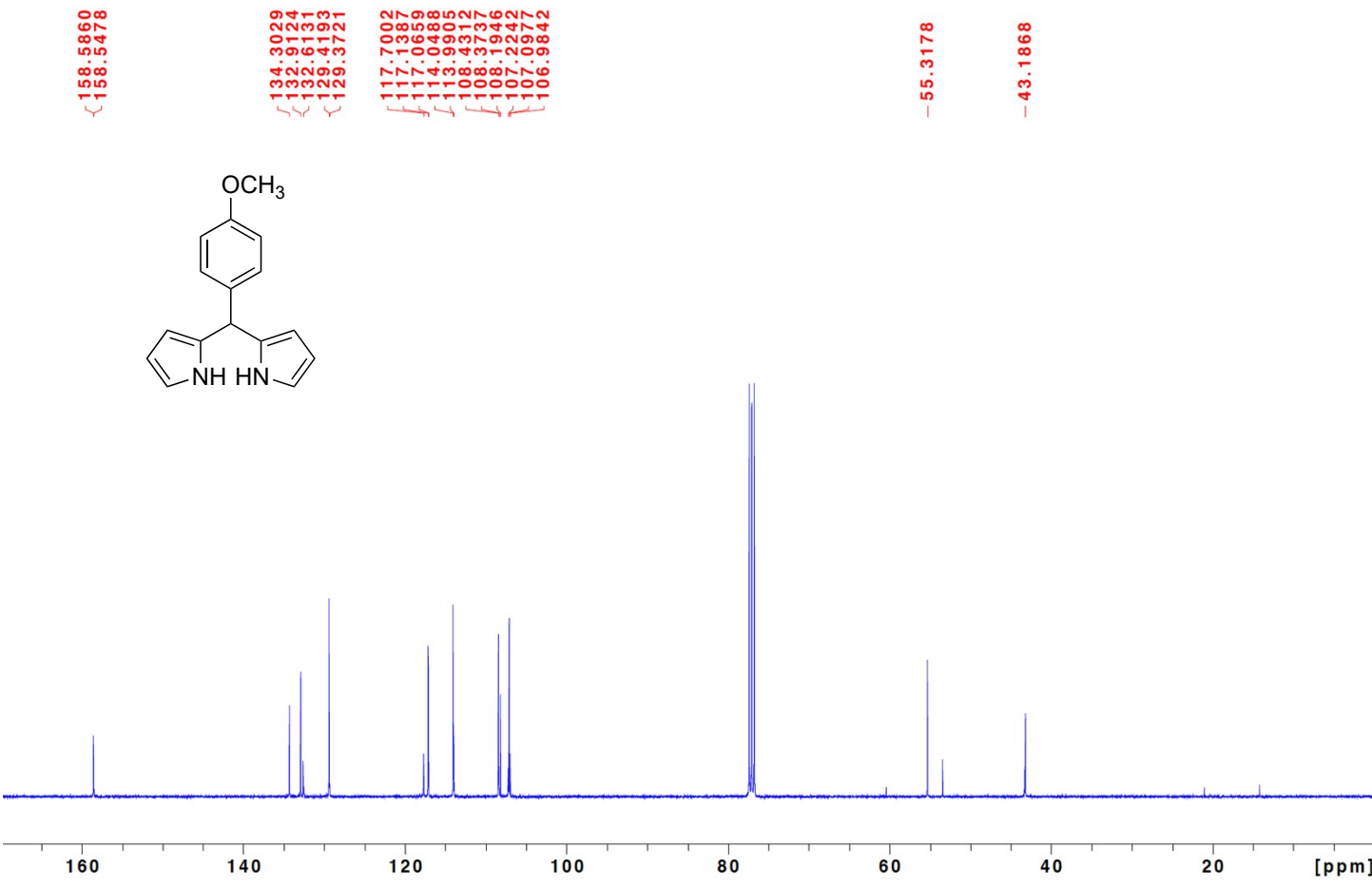
*Synthesis of disodium 2,6-disulfonate-3,5-dichloro-8-(4'-methoxyphenyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacence (BODIPY-Cl$_2$, **1**)*



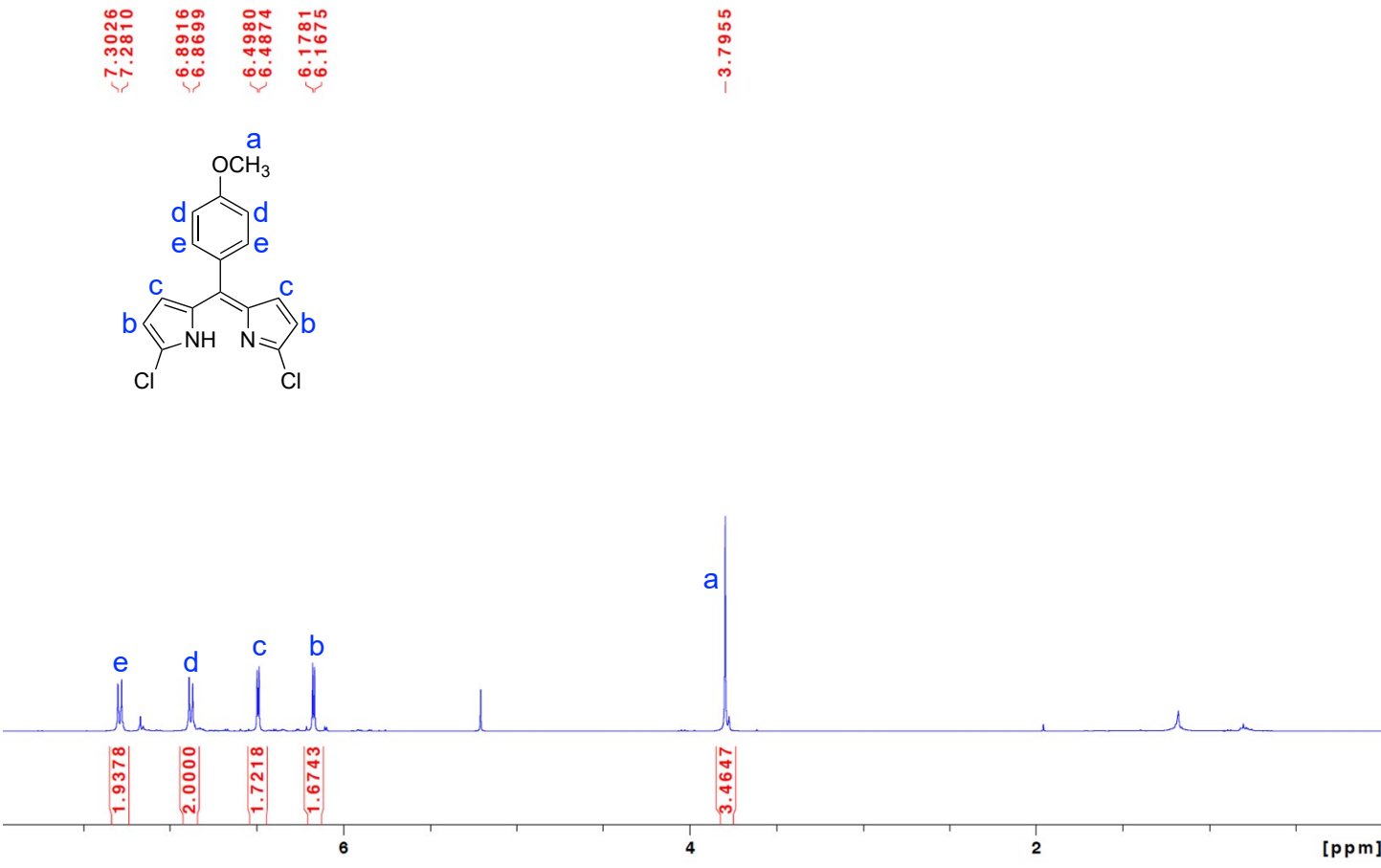
A solution of chlorosulfonic acid (0.4 mL, 5.99 mmol, 2.2 eq) in 5 mL of dichloromethane was added dropwise to a solution of compound **C** (1g, 2.73 mmol, 1 eq) in 45 mL of dichloromethane over 10 min under argon at -40 °C. The solution was then warmed slowly to 25 °C. The product precipitated from the reaction mixture and was isolated via filtration. The solids were then dissolved in water and neutralized with $NaHCO_3$ (0.51g, 6.0 mmol, 2 eq). The aqueous solution was lyophilized to afford an orange solid **1** (800 mg, 53 %). The dye is used as is or further purified via preparative HPLC if necessary. ^{1}H NMR (400 MHz, D_2O) δ (ppm) = 7.53 (d, J = 8.7 Hz, 2H), 7.31 (s, 2H), 7.13 (d, J = 8.7 Hz, 2H), 3.92 (s, 3H) ppm. ^{13}C NMR (100 MHz, D_2O) δ (ppm) = 162.9, 148.1, 140.2, 133.2, 131.0, 130.8, 123.4, 114.5, 114.4, 55.4 ppm. HRMS(ESI-) calcd. for $C_{16}H_9BCl_2F_2N_2O_7S_2^{2-}${M-2Na}$^{2-}$ 261.9639, found 261.9652.



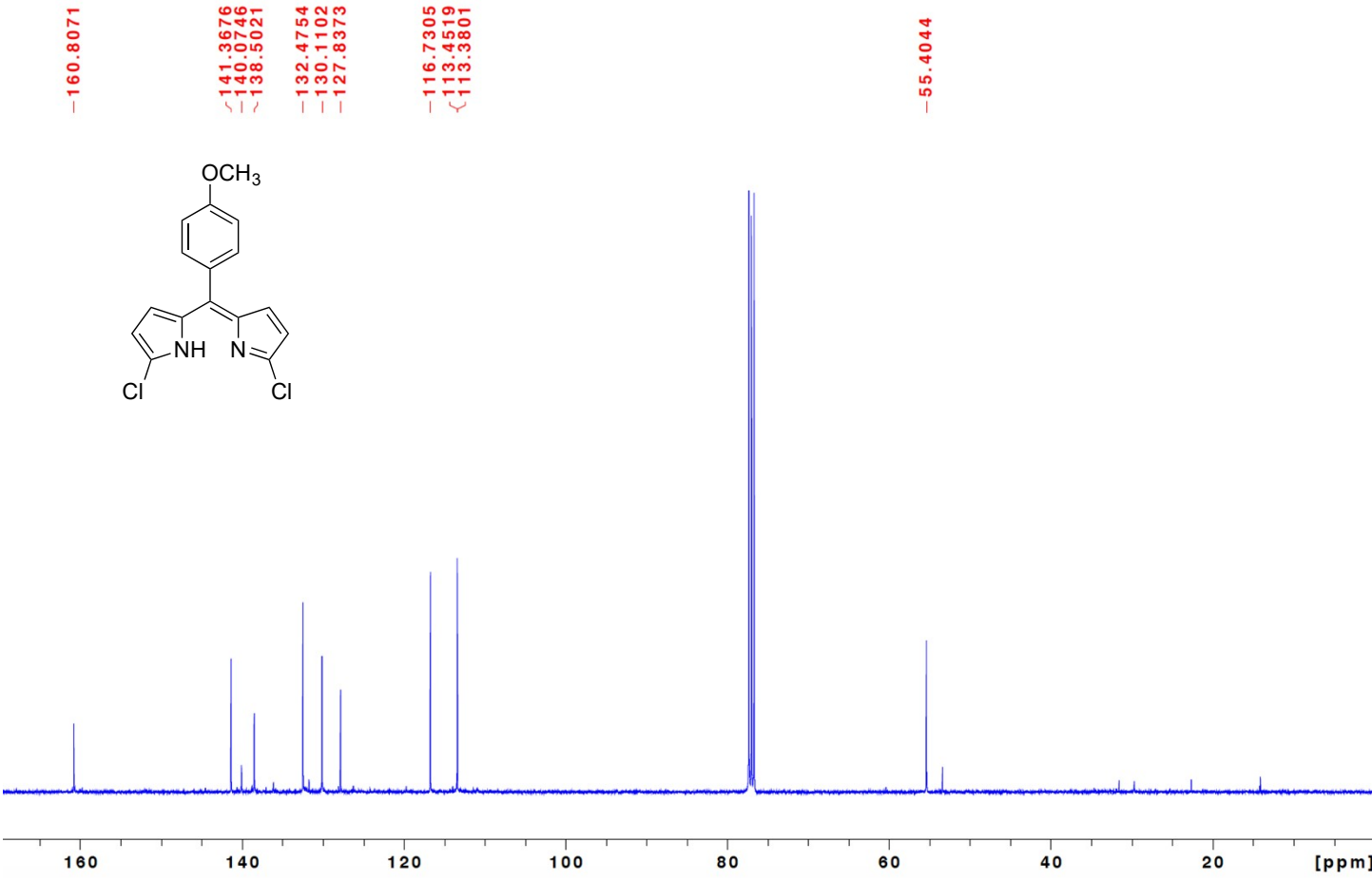
^{1}H-NMR of compound A



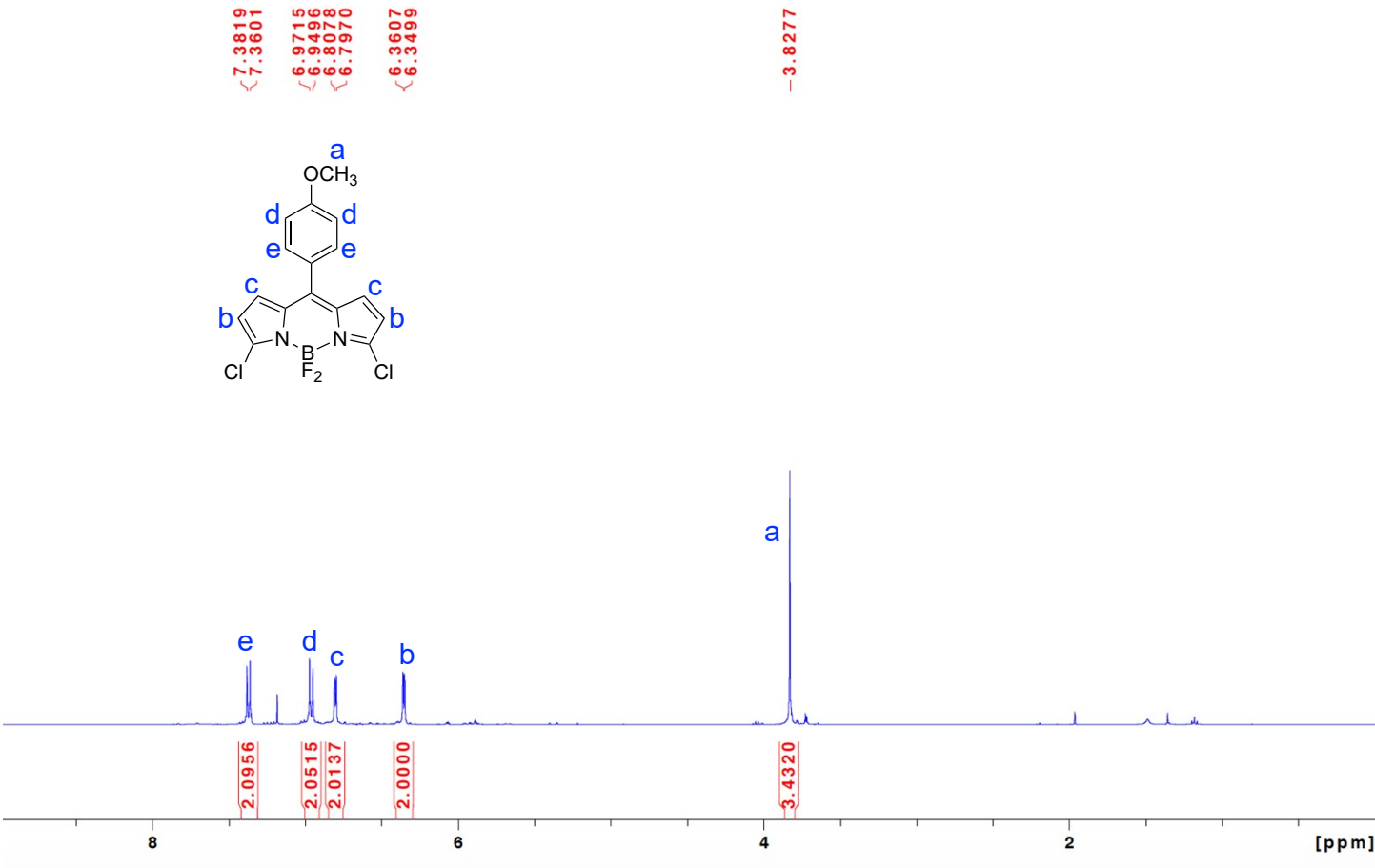
^{13}C-NMR of compound A



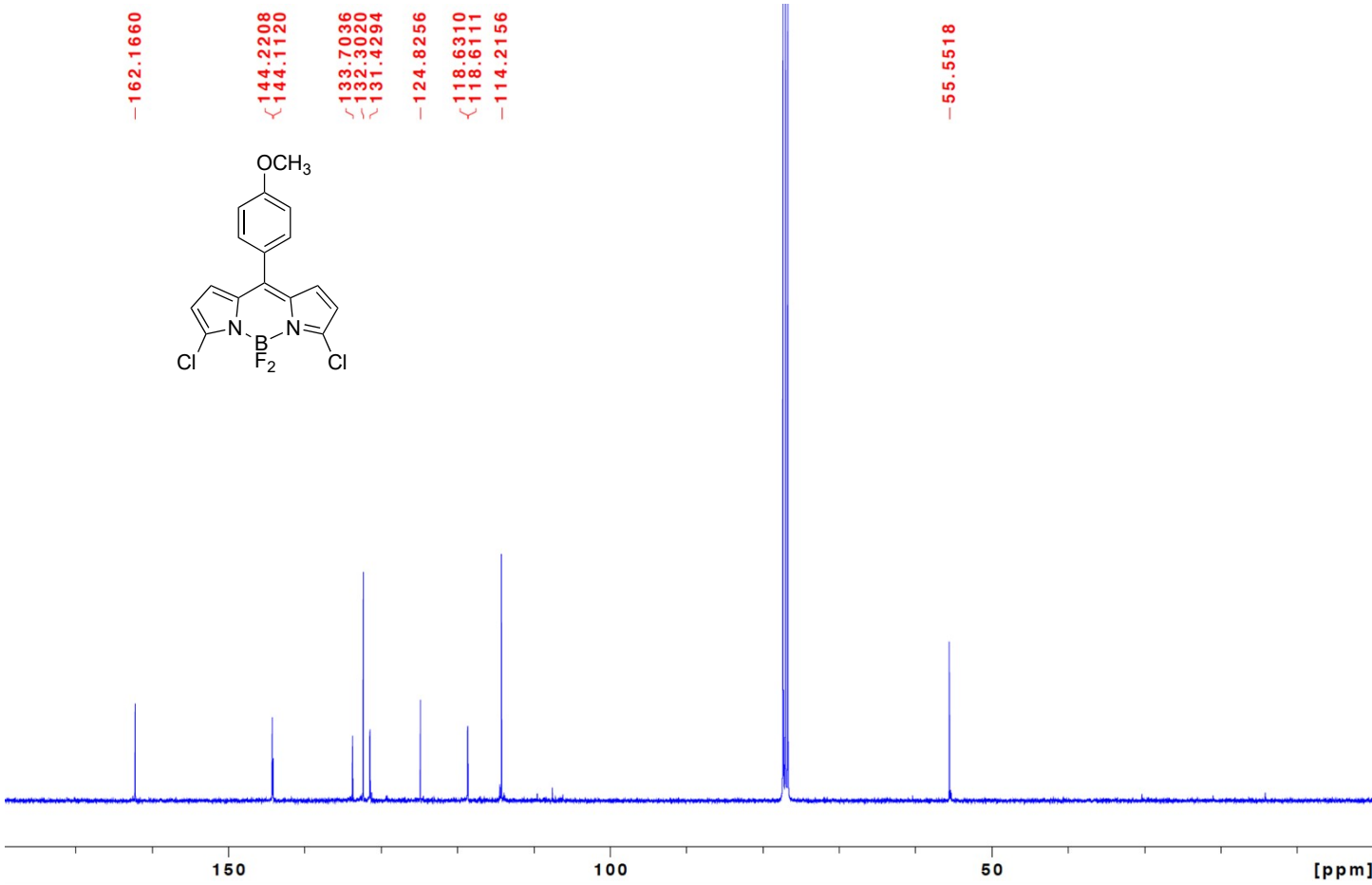
^{1}H-NMR of compound B



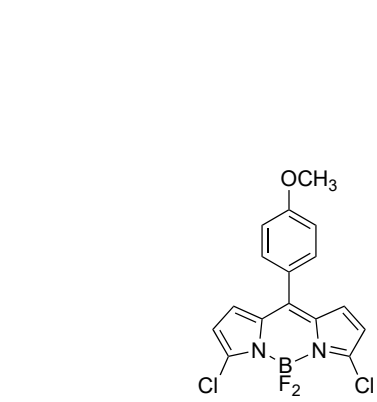
^{13}C-NMR of compound B



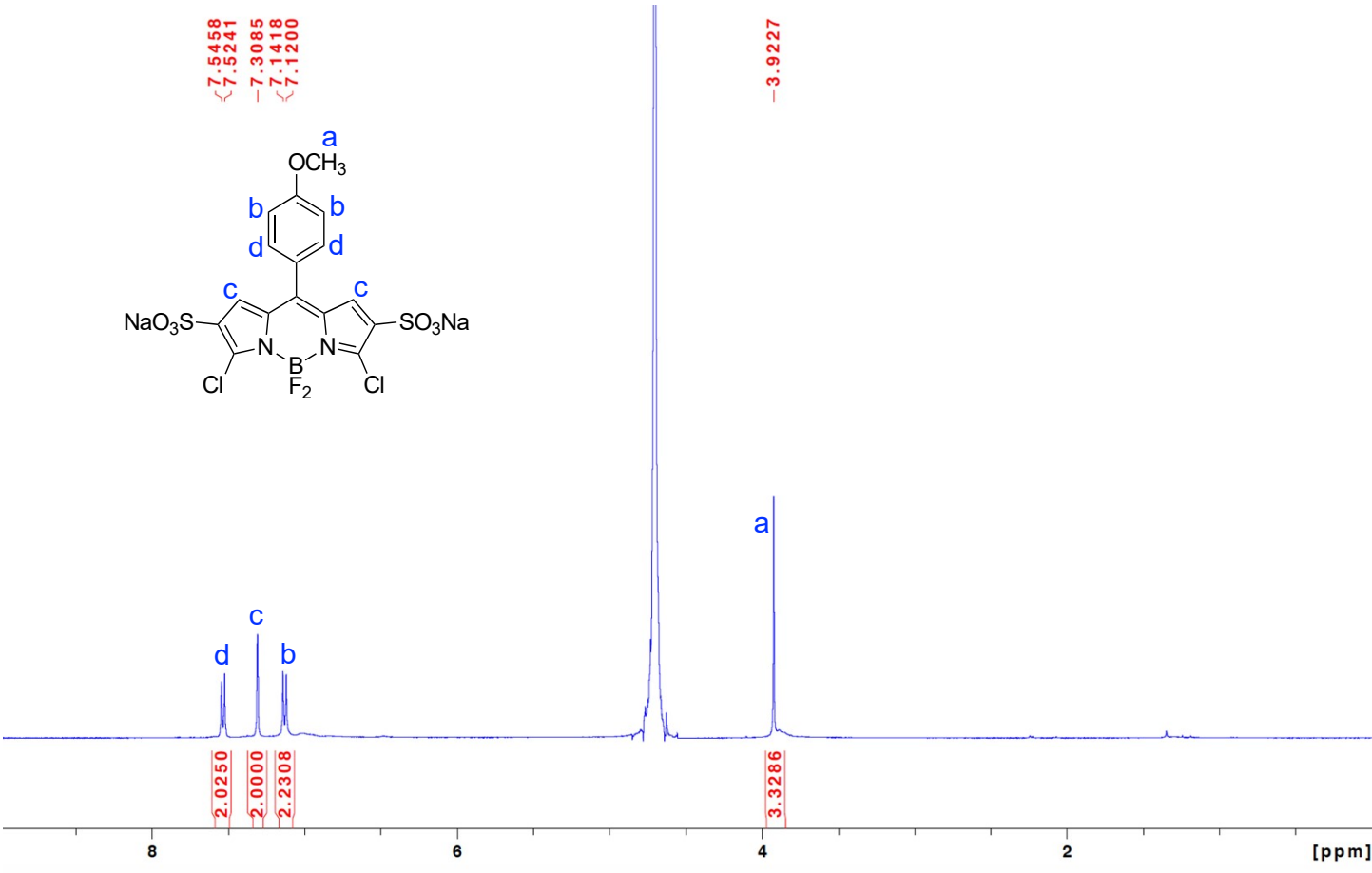
^{1}H-NMR of compound C



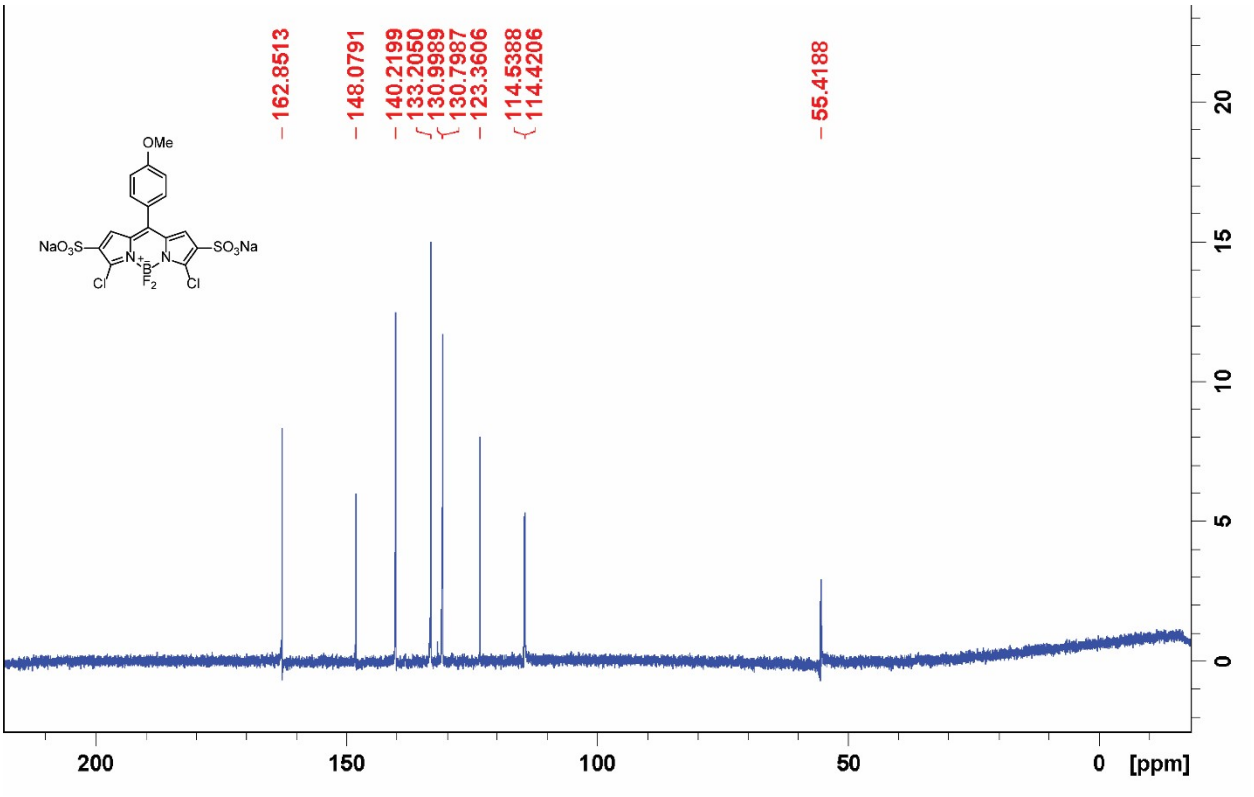
^{13}C-NMR of compound C



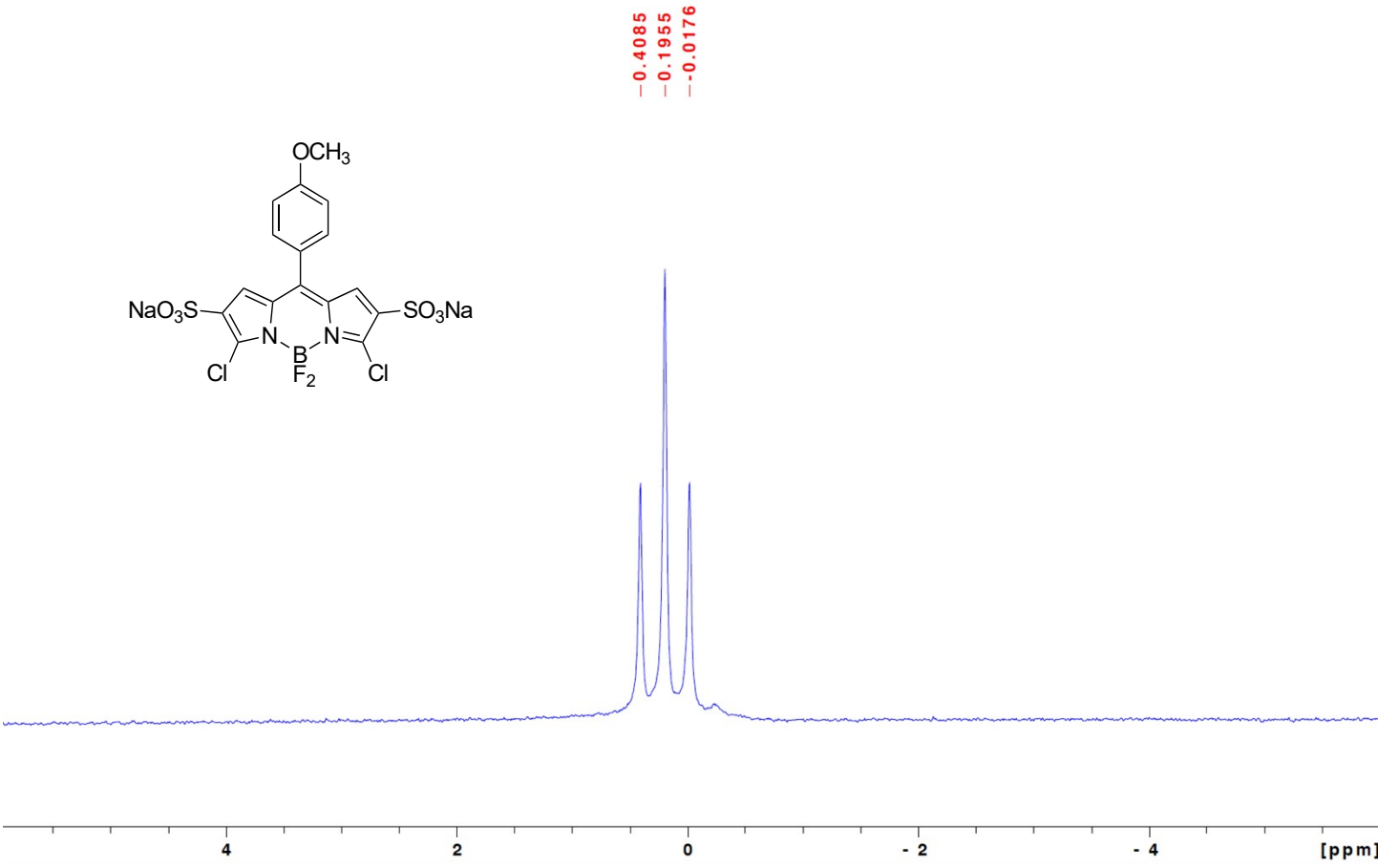
^{11}B-NMR of compound C



^{1}H-NMR of compound 1



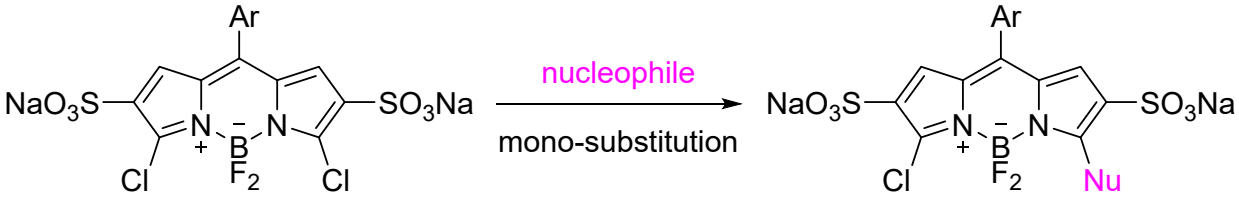
^{13}C-NMR of compound 1



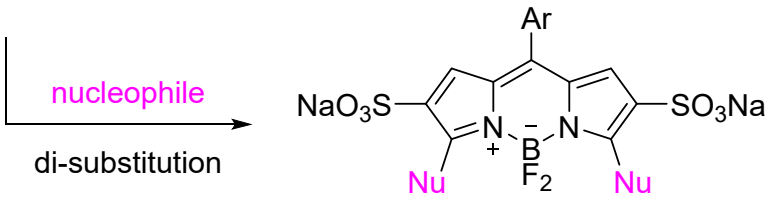
^{11}B-NMR of compound 1

2. BODIPY Substitution

General Reaction Procedure



1 **2a-g**

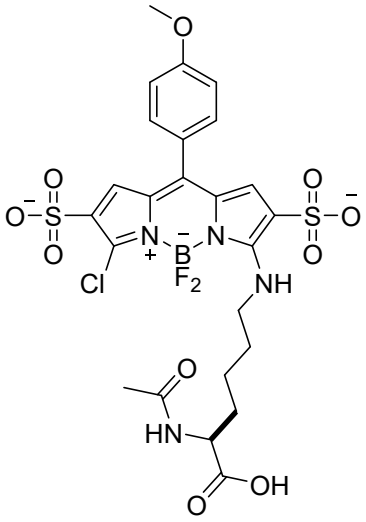


3a-g

nucleophiles:
a: *N*-acetyl cysteine
b: *N*-α-acetyl lysine
c: *N*-ε-acetyl lysine
d: proline
e: phenol
f: ethanol
g: piperidine

A 10X stock solution (100 mM) of BODIPY dye **1** was prepared in the selected solvent system. 0.1 mL of the BODIPY stock was added to 0.9 mL of a solution of nucleophile (10 equivalents) in the same solvent and/or base (see Table S1 for specifics). The reaction was run either to completion or until no change in product concentration could be detected by analytical HPLC. Products formed in appreciable amounts (**2b**, **2c**, **2d**, **2g**, **3a**) were isolated by preparative HPLC and characterized via analytical HPLC (Agilent Eclipse XDB-C18 column, 4.6 x 250 mm, 5 μm particle size, 5 – 95 % MeCN gradient over 15 min at 1.2 mL/min), ^{1}H NMR (Bruker Avance III 400 MHz in DMSO-D_6), and ESI-MS (high-resolution electrospray ionization mass spectrometry in negative mode).

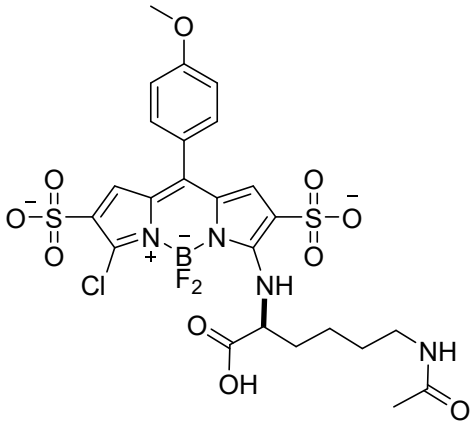
Characterization of BODIPY-(N-α-acetyl lysine)$_1$Cl$_1$ ***2b***



2b

The purity was found to be 94 % by HPLC at 280 nm detection, retention time of 6.03 min. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) = 7.38 (d, *J* = 9.4 Hz, 2H), 7.11 (d, *J* = 9.4 Hz, 2H), 6.78 (s, 1H), 6.23 (s, 1H), 4.17 (m, 1H), 3.85 (s, 3H), 1.87 (s, 3H), 1.68 (m, 4H), 1.43 (m, 2H). Two protons on the ε-carbon of lysine were obscured by the water peak at 3.33 ppm and not detected. HRMS (ESI$^-$) calcd. for $[C_{24}H_{24}BClF_2N_4O_{10}S_2 + H]^-$ 677.0756, found 677.0758.

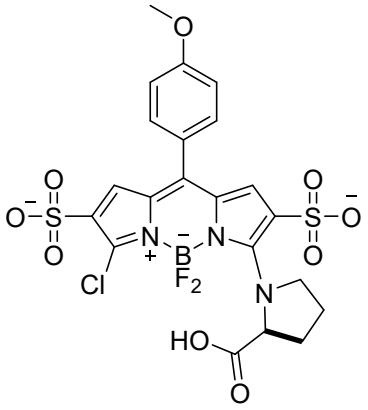
Characterization of BODIPY-(N-ε-acetyl lysine)$_1$Cl$_1$ ***2c***



2c

The purity was found to be 92 % by HPLC at 280 nm detection, retention time of 5.80 min. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) = 7.78 (s, 1H), 7.41 (d, *J* = 9.1 Hz, 2H), 7.12 (d, *J* = 9.1 Hz, 2H), 6.25 (s, 1H), 3.85 (s, 3H), 3.01 (m, 2H), 1.96 (m, 2H), 1.78 (s, 3H), 1.43 (m, 4H). The proton on the α-carbon of lysine was obscured by the broad water peak at ~3.5-4.0 ppm and was not observed. HRMS (ESI$^-$) calcd. for $[C_{24}H_{24}BClF_2N_4O_{10}S_2 + H]^-$ 677.0756, found 677.0779.

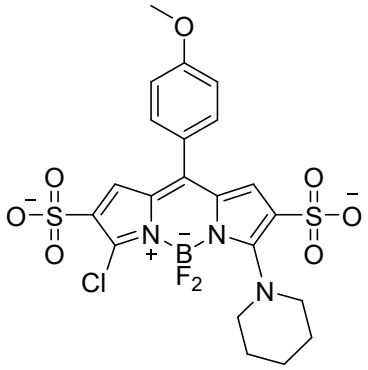
Characterization of BODIPY-(proline)$_1$Cl$_1$ ***2d***



2d

The purity was found to be 92 % by HPLC at 280 nm detection, retention time of 5.72 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 7.38 (d, *J* = 8.9 Hz, 2H), 7.11 (d, *J* = 8.9 Hz, 2H), 7.07 (s, 1H), 6.21 (s, 1H), 4.29 (t, *J* = 7.6 Hz, 1H), 3.86 (s, 3H), 2.25 (m, 1H), 2.12 (m, 1H), 1.91 (m, 2H). Two protons on the δ-carbon of proline were obscured by the broad water peak at 3.32 ppm and not detected. HRMS (ESI$^-$) calcd. for $[C_{21}H_{17}BClF_2N_3O_9S_2 + H]^-$ 604.0229, found 604.0250.

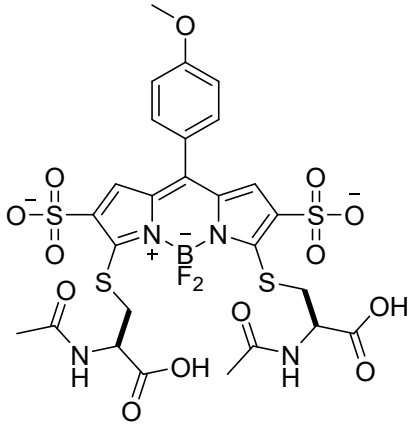
Characterization of BODIPY-(piperidine)$_1$Cl$_1$ ***2g***



2g

The purity was found to be 94 % by HPLC at 280 nm detection, retention time of 6.42 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 7.38 (d, *J* = 8.9 Hz, 2H), 7.11 (d, *J* = 8.9 Hz, 2H), 7.03 (s, 1H), 6.25 (s, 1H), 4.06 (m, 4H), 3.86 (s, 3H), 1.73 (m, 4H), 1.64 (m, 2H). HRMS (ESI$^-$) calcd. for $[C_{21}H_{19}BClF_2N_3O_7S_2 + H]^-$ 574.0487, found 574.0501.

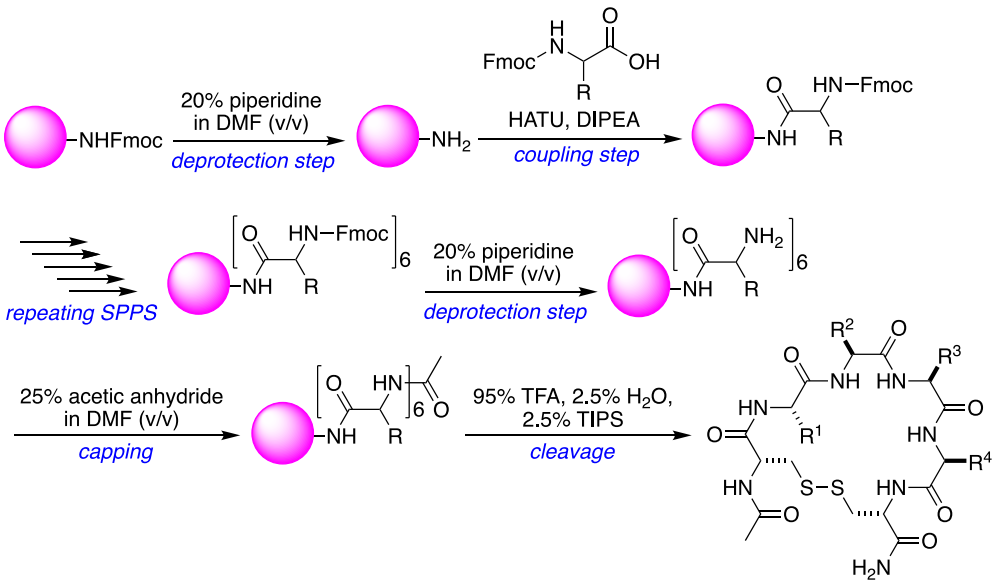
Characterization of BODIPY-(N-acetyl cysteine)$_2$ ***3a***



3a

The purity was found to be 100 % by HPLC at 280 nm detection, retention time of 5.29 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 7.53 (d, *J* = 9.2 Hz, 2H), 7.19 (d, *J* = 9.2 Hz, 2H), 6.90 (s, 2H), 4.43 (m, 2H), 3.89 (s, 3H), 1.80 (s, 6H). Four protons on the β-carbon of cysteine were obscured by the broad water peak at 3.39 ppm and not detected. HRMS (ESI$^-$) calcd. for $[C_{26}H_{25}BF_2N_4O_{13}S_4 + H]^-$ 779.0435, found 779.0443.

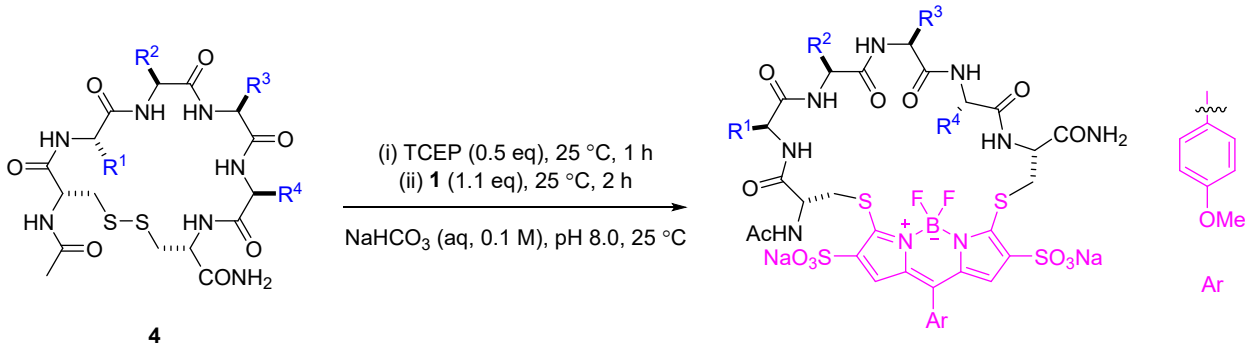
3. Peptide Synthesis



The peptides were synthesized according to SPPS using Fmoc/tBu strategy. Resin beads (TentaGel® S RAM, 0.23 mmol) were swelled in DMF in 10 mL- fritted syringe for 30 min. DMF was removed by vacuum filtration. For Fmoc deprotection, 20% piperidine in DMF (v/v) was added to the resin and shaken for 10 min 2x, then the resin was washed with DMF 5x. For amino acid coupling, a mixed solution of amino acid (4 eq, 0.92 mmol), HATU (3 eq, 0.69 mmol) and DIPEA (6 eq, 1.38 mmol) in DMF was shaken with the resin for 30 min and removed by vacuum filtration. This deprotection-coupling cycle was repeated until the requisite peptides **4** were made. The following Fmoc-amino acid derivatives were utilized for these syntheses: Fmoc-Ile-OH (I), Fmoc-Glu(OtBu)-OH (E), Fmoc-Met-OH (M), Fmoc-Leu-OH (L), Fmoc-Lys(Boc)-OH (K), Fmoc-Arg(Pbf)-OH (R), Fmoc-Asn(Trt)-OH (N), Fmoc-Ser(tBu)-OH (S), Fmoc-Ala-OH (A), Fmoc-Gln(Trt)-OH (Q), Fmoc-Val-OH (V), Fmoc-Thr(tBu)-OH (T), Fmoc-Asp(OtBu)-OH (D), and Fmoc-Cys(Trt)-OH (C). After the peptide chain was complete, the deprotection step was performed to remove Fmoc, and the N-terminus of the peptide was acetylated with a solution of 25 % acetic anhydride in DMF (v/v) for 15 min, then washed with DMF 5x. The peptide was then deprotected and cleaved from the resin using the acid cleavage cocktail (95 % TFA, 2.5 % H_2O, and 2.5 % TIPS) for 2 h, with the solution being drained and refreshed one time in the middle. The collected peptide was purged by N_2 gas to remove TFA and precipitated using cold diethyl ether. To isolate peptide solution, the crude peptide was spun down by centrifugation (2400 rpm, 5 min) and washed with cold diethyl ether twice. The crude peptide was dissolved in 10 % ACN in H_2O (v/v) and lyophilized. The crude peptide was dissolved in 0.5 mL MeCN and 2.0 mL of 0.1 % aqueous TFA, filtered through a 13 μm syringe filter, and purified via preparative HPLC. The product is checked for purity with analytical HPLC and LCMS, then lyophilized.

4. Fluorescent Loop Synthesis

General Reaction Procedure



4 **5**

The peptide **4** (0.02 mmol) was dissolved in 19.6 mL of 0.1 M $NaHCO_3$ (pH 8.0) and purged with N_2 gas for 30 min to remove oxygen. A solution of TCEP (0.5 eq, 0.01 mmol) in water (0.5 mL) was added and stirred for 1 h to reduce disulfide to thiols. Then, a solution of **1** (1.1 eq, 0.022 mmol) in water (0.5 mL) was added to the mixture and stirred at 25 °C for 2 h. The crude product was lyophilized and purified via preparative HPLC with a 30 % MeCN gradient in water over 20 min. Product **5** is obtained as a red solid and characterized by analytical HPLC (30 – 95 % MeCN in water gradient over 25 min), ^{1}H NMR, TOCSY-NMR and high-resolution mass spectrometry (HRMS). ^{1}H and TOCSY-NMR spectra were recorded on a Bruker Avance III at 400 MHz at room temperature in 90 % H_2O + 10 % D_2O. Chemical shifts of ^{1}H NMR spectra were reported in ppm as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd =doublet of doublet), coupling constants, and number of protons. Mass spectra (MS) were measured by high-resolution electrospray ionization mass spectrometry (ESI-MS) in negative mode.

Compound Characterization Data

5a: The purity was found to be 91 % by HPLC analysis at 280 and 550 nm detection, retention time 7.438 min. ^{1}H NMR (400 MHz, 90 % H_2O + 10 % D_2O) δ 8.55 (d, *J* = 7.10 Hz, 1H), 8.45 (d, *J* = 5.76 Hz, 1H), 8.33 (s, 1H), 8.31 (s, 1H), 8.14 (d, *J* = 12.66 Hz, 1H), 7.86 (d, *J* = 7.89 Hz, 2H), 7.65 (d, *J* = 9.37 Hz, 2H), 7.46 (s, 1H), 7.36 (s, 1H), 7.22 (d, *J* = 9.37 Hz, 2H), 7.08 (s, 2H), 4.29-4.33 (m, 1H), 4.07-4.17 (m, 1H), 3.94 (s, 3H), 3.92 (s, 1H), 3.91 (s, 1H), 3.90 (s, 2H), 3.88 (s, 1H), 3.86 (s, 1H), 3.82 (d, *J* = 5.64 Hz, 2H), 3.95 (d, *J* = 5.09 Hz, 1H), 3.71 (d, *J* = 5.13 Hz, 1H), 3.58 (d, *J* = 3.63 Hz, 1H), 3.54 (d, *J* = 5.38 Hz, 1H), 2.74-2.85 (m, 2H), 2.60-2.71 (m, 2H), 2.12-2.21 (m, 2H), 2.04 (s, 3H), 1.99-2.06 (m, 3H) High Resolution ESI$^-$: m/z calcd for $[C_{38}H_{44}BF_2N_9O_{17}S_5]^{2-}$ 553.5754 found 553.5773.

5b: The purity was found to be 96 % by HPLC analysis at 280 and 550 nm detection, retention time 7.619 min. ^{1}H NMR (400 MHz, 90 % H_2O + 10 % D_2O) δ 8.43 (d, *J* = 6.54 Hz, 1H), 8.38 (d, *J* = 6.83 Hz, 1H), 8.16, (d, *J* = 7.07 Hz, 1H), 8.12, (d, *J* = 7.97 Hz, 1H),

7.88, (d, *J* = 5.96 Hz, 1H), 7.77, (d, *J* = 7.11 Hz, 1H), 7.65 (d, *J* = 9.33 Hz, 2H), 7.52 (s, 1H), 7.47 (s, 1H), 7.33, (s, 1H), 7.21 (d, *J* = 9.44 Hz, 2H), 7.10 (s, 1H), 4.18-4.23 (m, 1H), 4.02 (s, 1H), 3.97 (s, 1H), 3.94 (s, 3H), 3.88 (s, 2H), 3.86 (s, 2H), 3.67-3.69 (m, 1H), 3.63-3.66 (m, 2H), 3.58-3.60 (m, 2H), 3.55-3.56 (m, 1H), 2.82-2.85 (m, 2H), 2.15 (m, 1H), 2.06 (s, 3H), 1.61-1.70 (m, 2H), 1.44-1.54 (m, 2H), 1.22-1.26 (m, 2H), 0.96 (t, *J* = 8.63 Hz, 6H). High Resolution ESI$^-$: m/z calcd for $[C_{40}H_{51}BF_2N_{10}O_{15}S_4]^{2-}$ 544.1234 found 544.1248.

5c: The purity was found to be 99 % by HPLC analysis at 280 and 550 nm detection, retention time 8.897 min. ^{1}H NMR (400 MHz, 90 % H_2O + 10 % D_2O) δ 8.88 (d, *J* = 6.79 Hz, 1H), 8.39 (d, *J* = 6.02 Hz, 1H), 7.91 (d, *J* = 5.40 Hz, 1H), 7.72 (d, *J* = 7.18 Hz, 1H), 7.64 (d, *J* = 9.27 Hz, 2H), 7.58 (t, *J* = 17.35 Hz, 1H), 7.45 (s, 1H), 7.40 (s, 1H), 7.31 (s, 1H), 7.21 (d, *J* = 9.32 Hz, 2H), 7.10 (s, 2H), 4.18 (d, *J* = 4.05 Hz, 1H), 4.15 (d, *J* = 3.52 Hz, 1H), 4.06 (s, 1H), 4.04 (s, 1H), 3.94 (s, 3H), 3.75 (t, *J* = 10.59 Hz, 2H), 3.37-3.40 (m, 1H), 3.40-3.37 (m, 1H), 3.05 (d, *J* = 7.31 Hz, 1H), 3.01 (s, 1H), 2.97 (s, 2H), 2.85 (s, 2H), 2.80 (d, *J* = 6.74 Hz, 1H), 2.75 (d, *J* = 6.60 Hz, 1H), 2.03 (s, 3H), 1.98-2.04 (m, 2H), 1.81-1.87 (m, 2H), 1.70-1.81 (m, 2H), 1.64-1.66 (m, 2H), 1.59-1.62 (m, 2H), 1.48-1.55 (m, 2H), 1.36-1.46 (m, 2H), 1.26-1.28 (m, 2H), 1.22-1.24 (m, 2H) High Resolution ESI$^-$: m/z calcd for $[C_{43}H_{56}BF_2N_{11}O_{17}S_4]^{2-}$ 587.6394 found 587.6413.

5d: The purity was found to be 91 % by HPLC analysis at 280 and 550 nm detection, retention time 6.861 min. ^{1}H NMR (400 MHz, 90 % H_2O + 10 % D_2O) δ 8.56 (d, *J* = 6.31 Hz, 1H), 8.41 (d, *J* = 6.21 Hz, 1H), 8.08 (d, *J* = 6.56 Hz, 1H), 8.04 (s, 1H), 8.02 (s, 1H), 8.01 (s, 1H), 7.65 (d, *J* = 9.35 Hz, 2H), 7.48 (s, 1H), 7.45 (s, 1H), 7.35 (s, 1H), 7.21 (d, *J* = 9.40 Hz, 2H), 7.09 (s, 2H), 4.31-4.34 (m, 1H), 4.19-4.24 (m,1H), 4.09-4.10 (m, 2H), 4.05 (d, *J* = 7.33 Hz, 1H), 3.87-3.91 (m, 2H), 3.84 (d, *J* = 5.12, 1H), 3.58-3.75 (m, 2H), 3.45-3.52 (m, 1H), 3.03-3.08 (m, 2H), 2.65-2.83 (m, 2H), 2.06 (s, 3H), 1.72-1.79 (m, 2H), 1.60-1.68 (m, 2H), 1.53-1.59 (m, 2H), 1.22 (s, 1H), 0.82-0.89 (m, 6H) High Resolution ESI$^-$: m/z calcd for $[C_{42}H_{53}BF_2N_{12}O_{16}S_4]^{2-}$ 579.1318 found 579.1332.

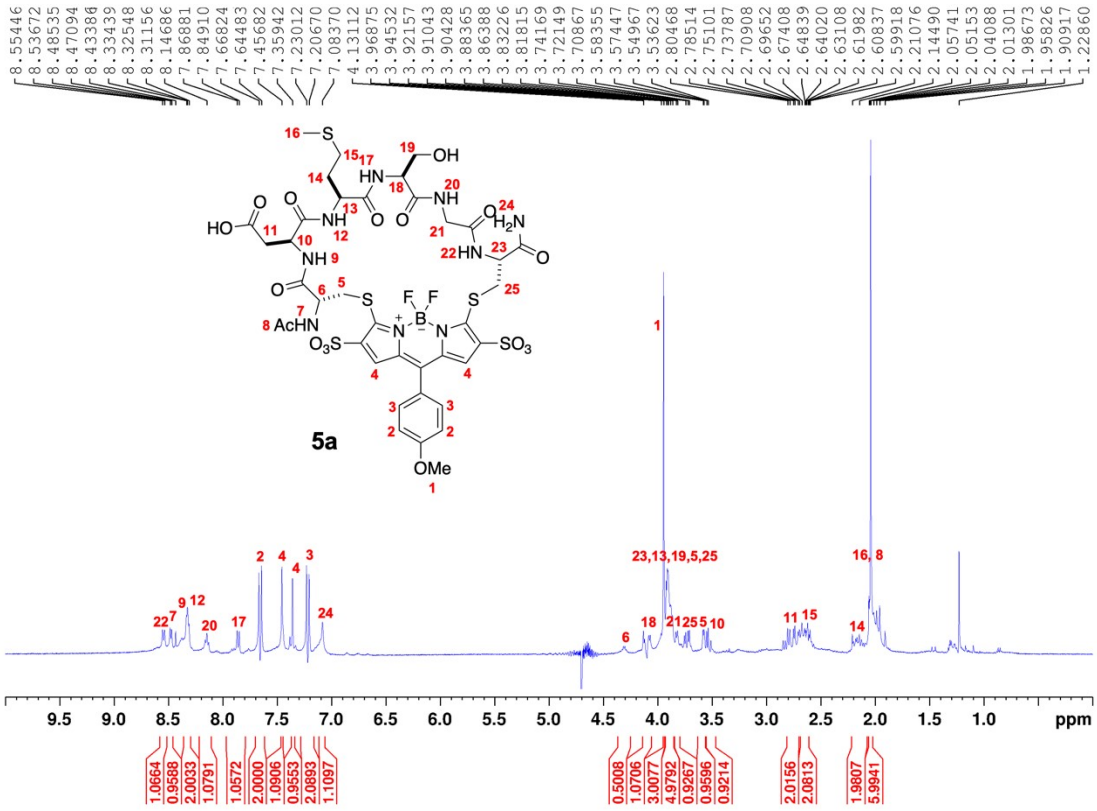
5e: The purity was found to be 92 % by HPLC analysis at 280 and 550 nm detection, retention time 6.624 min. ^{1}H NMR (400 MHz, 90 % H_2O + 10 % D_2O) δ 8.65 (d, *J* = 3.91 Hz, 1H), 8.36 (d, *J* = 6.09 Hz, 1H), 8.32 (d, *J* = 7.58 Hz, 1H), 8.26 (s, 1H), 8.23 (d, *J* = 6.73 Hz, 1H), 7.67 (d, *J* = 10.03 Hz, 2H), 7.44 (s, 1H), 7.41 (s, 1H), 7.22 (d, *J* = 10.03 Hz, 2H), 7.07 (s, 1H), 4.24-4.28 (m, 1H), 4.08-4.14 (m, 1H), 4.05 (s, 1H), 3.98 (d, *J* = 5.06, 2H), 3.92 (d, *J* = 5.40 Hz, 2H), 3.81-3.82 (m, 1H), 3.74-3.78 (m, 2H), 3.66-3.70 (m, 2H), 3.49-3.55 (m, 2H), 1.98 (s, 3H), 1.41 (d, *J* = 7.76 Hz, 3H). High Resolution ESI$^-$: m/z calcd for $[C_{34}H_{38}BF_2N_9O_{15}S_4]^{2-}$ 494.5710 found 494.5721.

5f: The purity was found to be 99 % by HPLC analysis at 280 and 550 nm detection, retention time 7.078 min. ^{1}H NMR (400 MHz, 90 % H_2O + 10 % D_2O) δ 8.55 (t, *J* = 7.58 Hz, 1H), 8.30-8.32 (m, 1H), 8.26-8.28 (m, 1H), 8.09-8.11 (m, 1H), 8.00-8.01 (m, 1H), 7.89-7.99 (m, 1H), 7.65 (d, *J* = 9.33 Hz, 2H), 7.46 (s, 1H), 7.21 (d, *J* = 9.33 Hz, 2H), 7.10 (s, 1H), 7.05 (s, 1H), 6.92 (s, 1H), 4.21-4.25 (m, 1H), 4.04-4.06 (m, 1H), 3.97-4.00 (m, 1H), 3.94 (s, 3H), 3.88-3.91 (m, 2H), 3.84-3.85 (m, 1H), 3.78-3.80 (m, 1H), 3.75-3.76 (m, 1H), 3.57-3.61 (m, 1H), 3.52-3.55 (m, 1H), 2.86-2.34 (m, 2H), 2.46 (t, *J* = 7.83

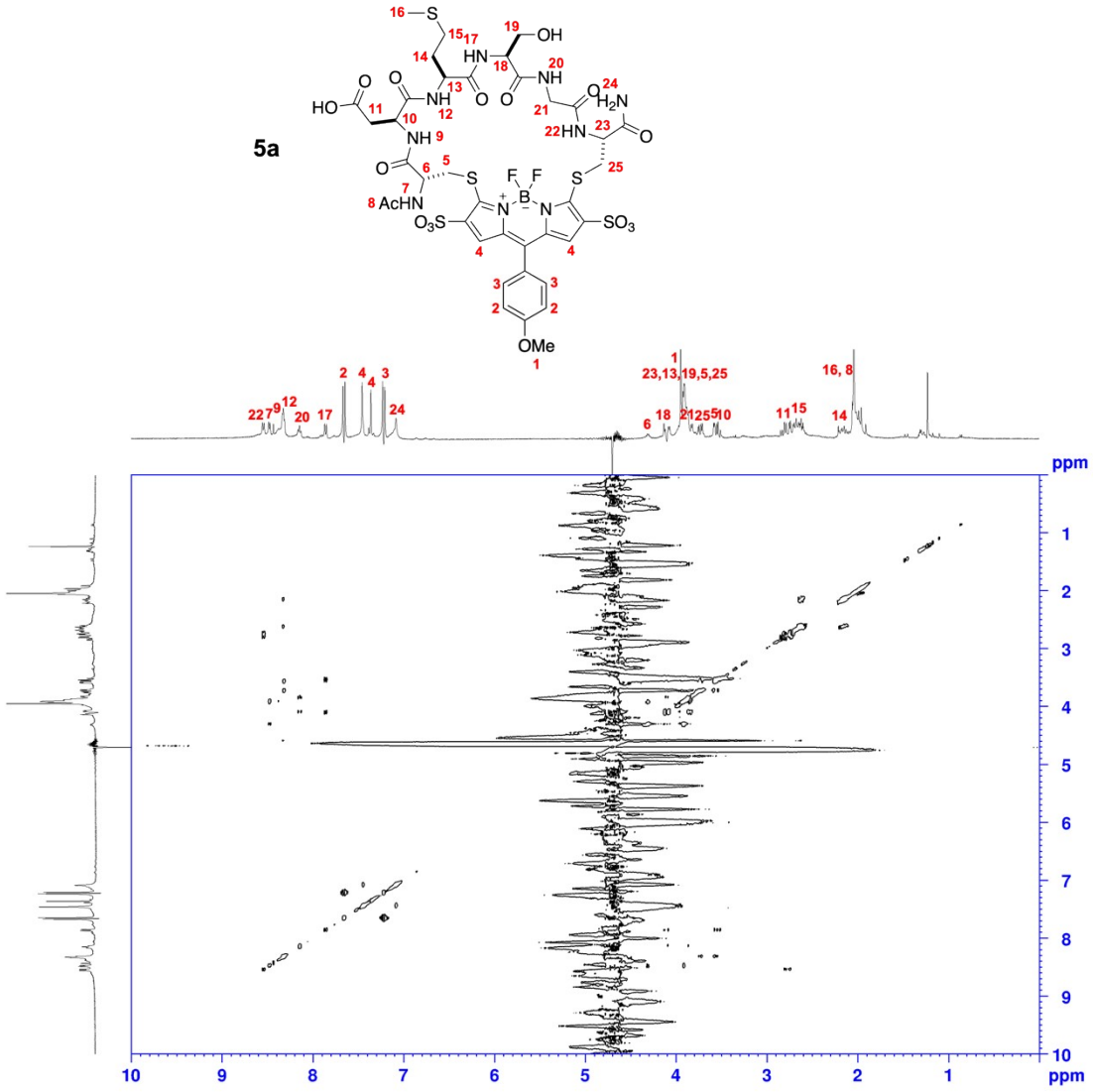
Hz, 1H), 2.33 (t, *J* = 7.85 Hz, 1H), 2.07-2.14 (m, 2H), 2.02 (s, 1H), 1.42 (dd, *J* = 7.13, 3.80 Hz, 3H). High Resolution ESI$^-$: m/z calcd for $[C_{38}H_{43}BF_2N_{10}O_{17}S_4]^{2-}$ 544.0870 found 544.0884.

5g: The purity was found to be 98 % by HPLC analysis at 280 and 550 nm detection, retention time 8.662 min. ^{1}H NMR (400 MHz, 90 % H_2O + 10 % D_2O) δ 8.46 (d, *J* = 7.29 Hz,1H), 8.41 (d, *J* = 6.45 Hz, 1H), 8.18 (d, *J* = 5.87 Hz, 1H), 8.10 (t, *J* = 6.38 Hz, 1H), 7.98 (d ,*J* = 7.91 Hz, 1H), 7.72 (d, *J* = 6.63 Hz,1H), 7.65 (d, *J* = 9.23 Hz, 2H), 7.57 (s, 3H), 7.45 (s, 1H), 7.37 (s, 1H), 7.22 (d, *J* = 9.25 Hz, 2H), 7.14 (s, 2H), 4.14-4.15 (m, 1H), 4.10-4.12 (m, 1H), 3.91-3.97 (m, 2H), 3.94 (s, 3H), 3.74 (dd, *J* = 13.86, 6.27 Hz, 2H), 3.58-3.60 (m, 1H), 3.55-3.57 (m, 1H), 2.90-2.96 (m, 2H), 2.79-2.86 (m, 2H), 2.04-2.06 (m, 2H), 1.77-1.82 (m, 1H), 1.72-1.96 (m, 1H), 1.59-1.68 (m, 2H), 1.38-1.44 (m, 2H), 1.28-1.36 (m, 1H), 1.08-1.18 (m, 1H), 0.83 (t, *J* = 7.96 Hz, 3H), 0.71 (t, *J* = 7.96 Hz, 3H) High Resolution ESI$^-$: m/z calcd for $[C_{42}H_{53}BF_2N_{10}O_{16}S_4]^{2-}$ 565.1287 found 565.1305.

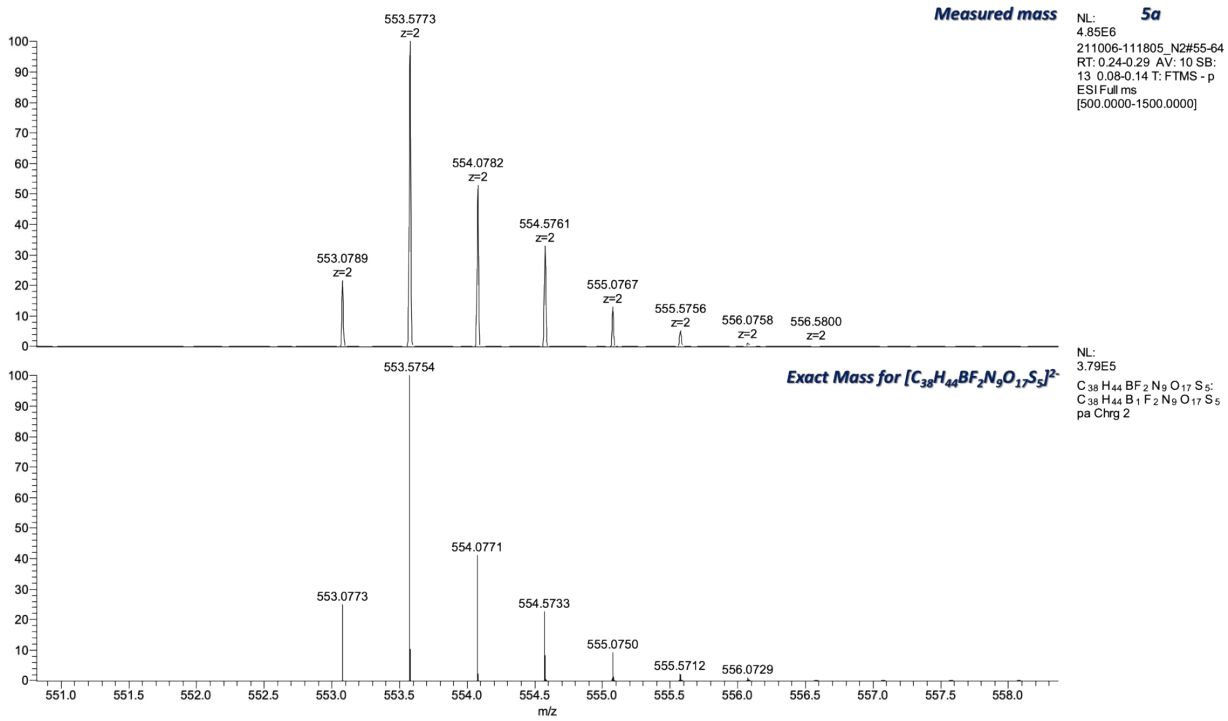
NMR and Mass Spectra



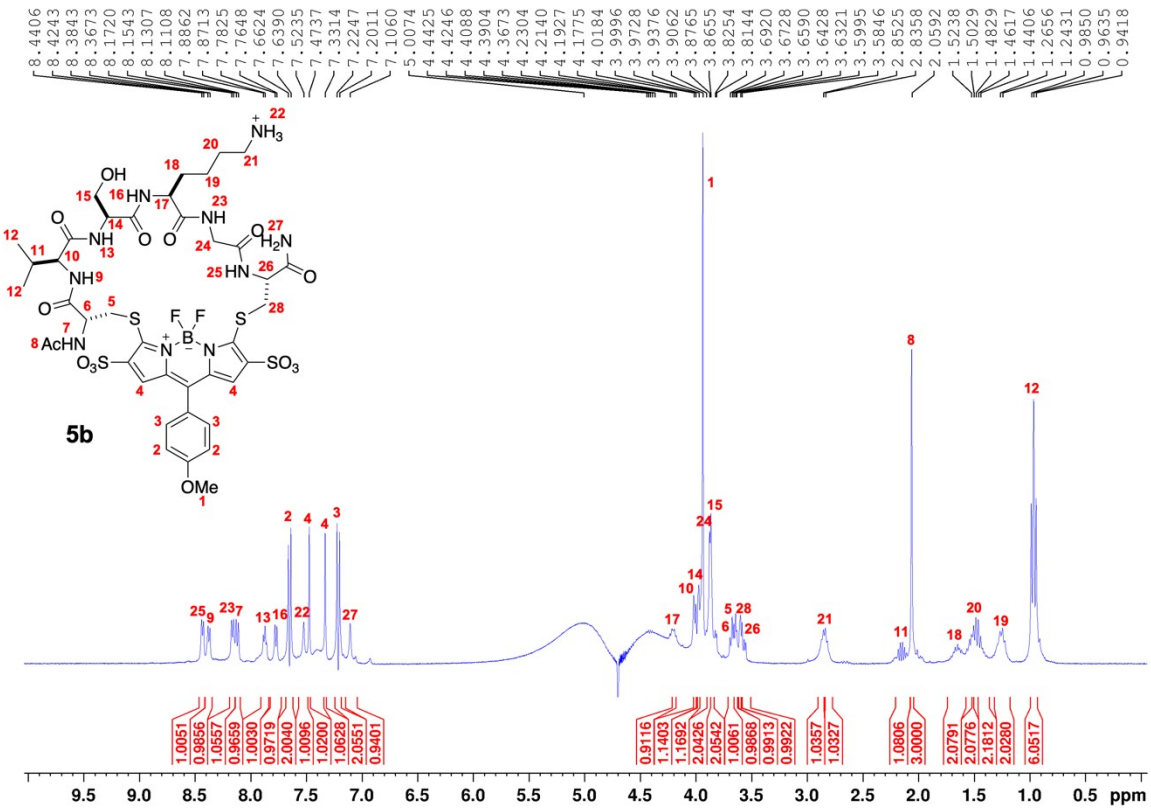
^{1}H NMR of **5a**



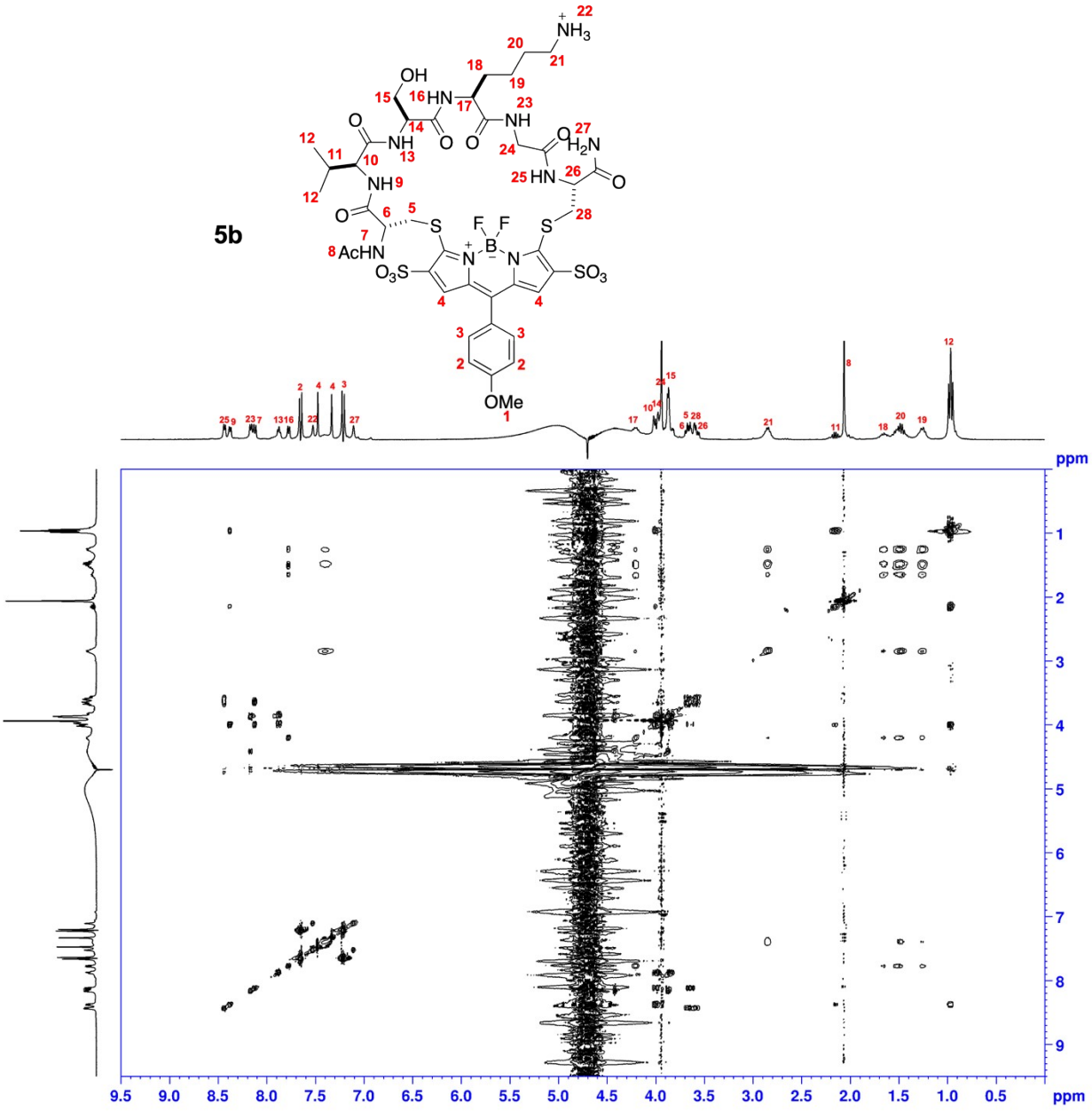
TOCSY of **5a**



HRMS of **5a**



^{1}H NMR of **5b**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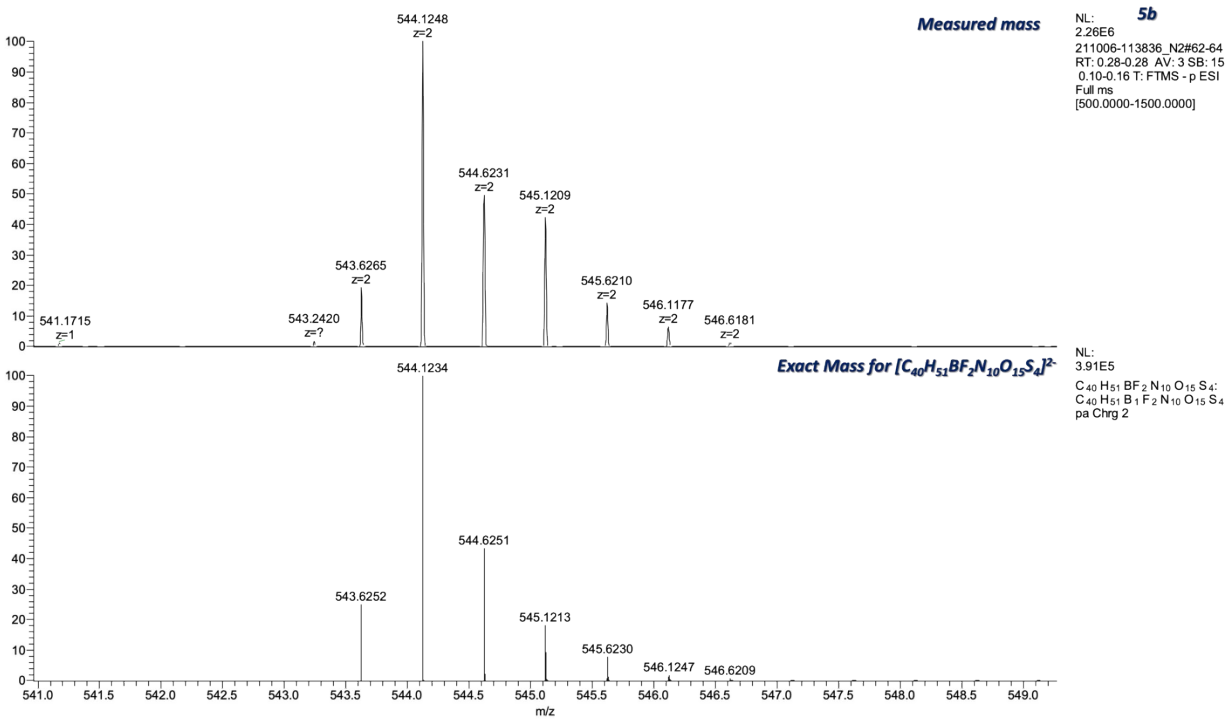
1 2 3 4 5 6 7 8 9

TOCSY of **5b**



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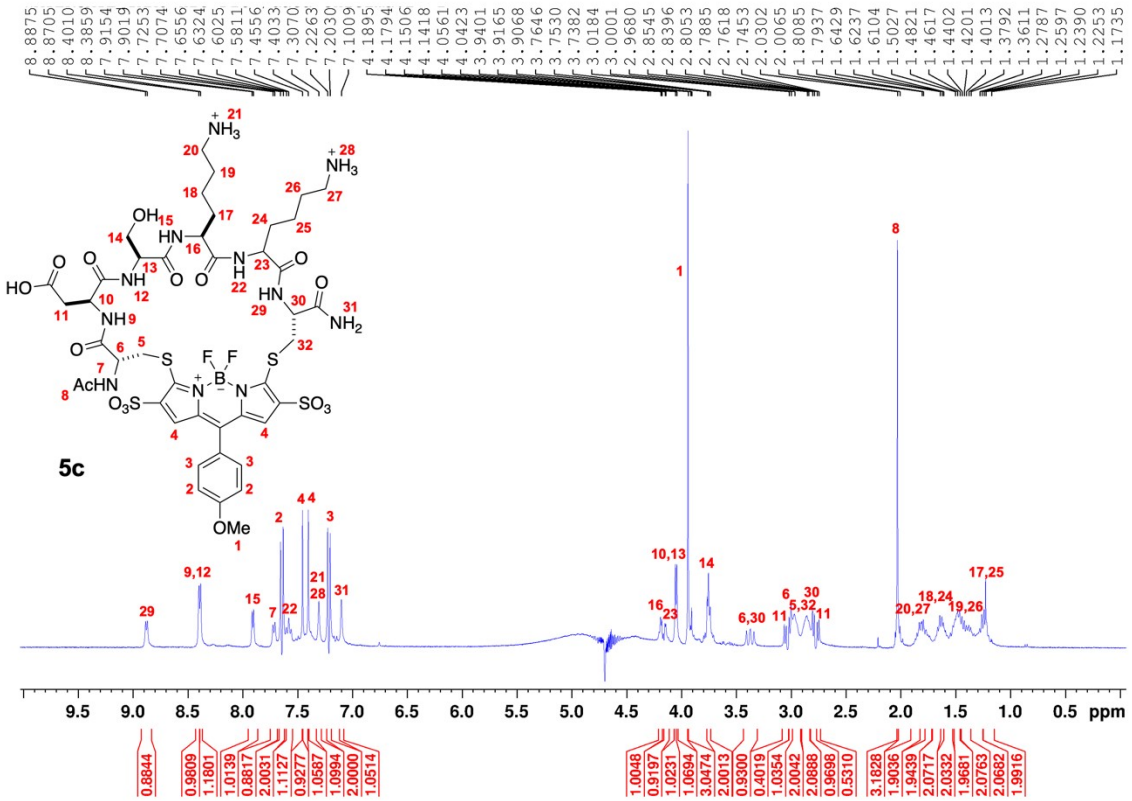
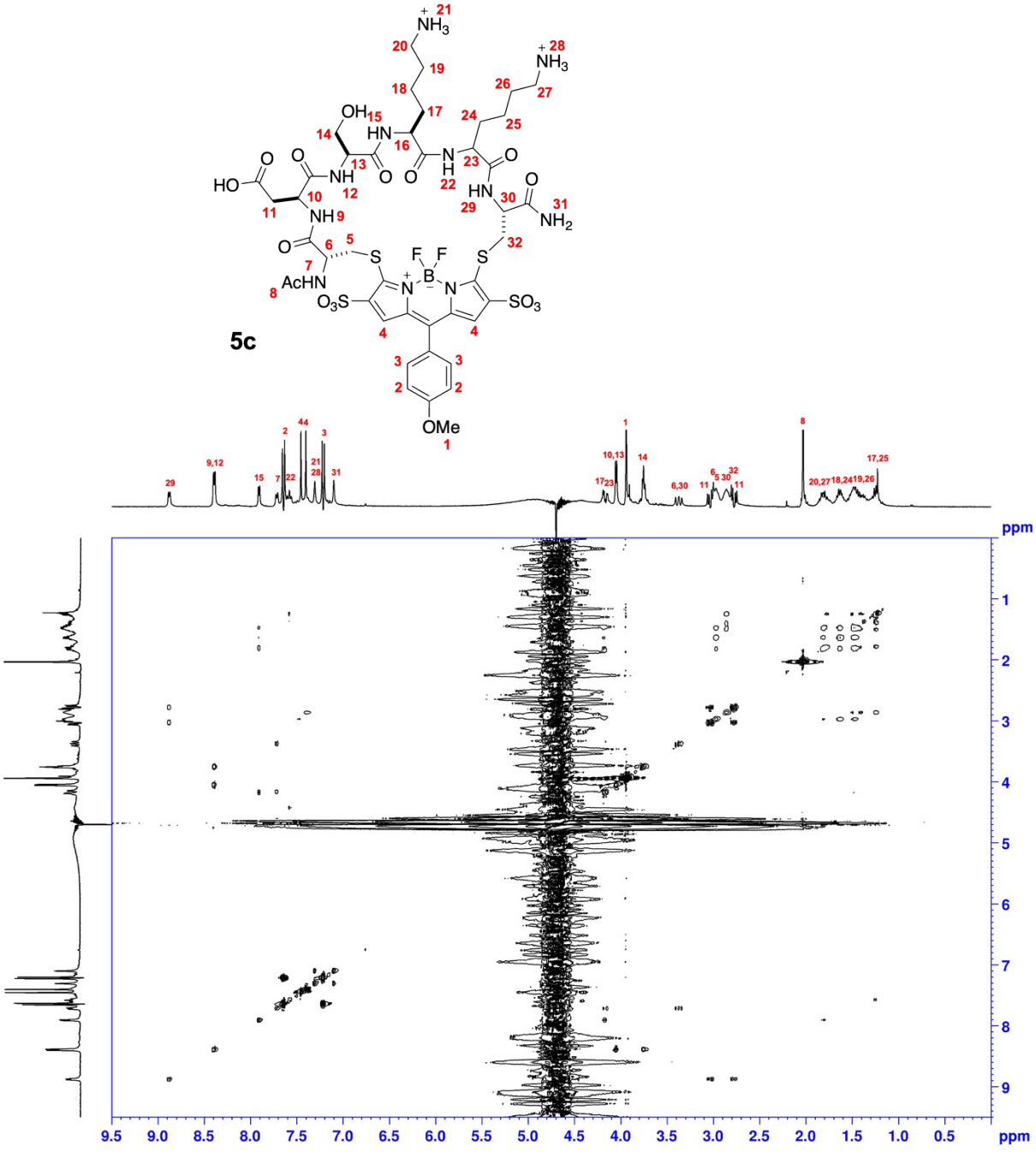


^{1}H NMR of **5c**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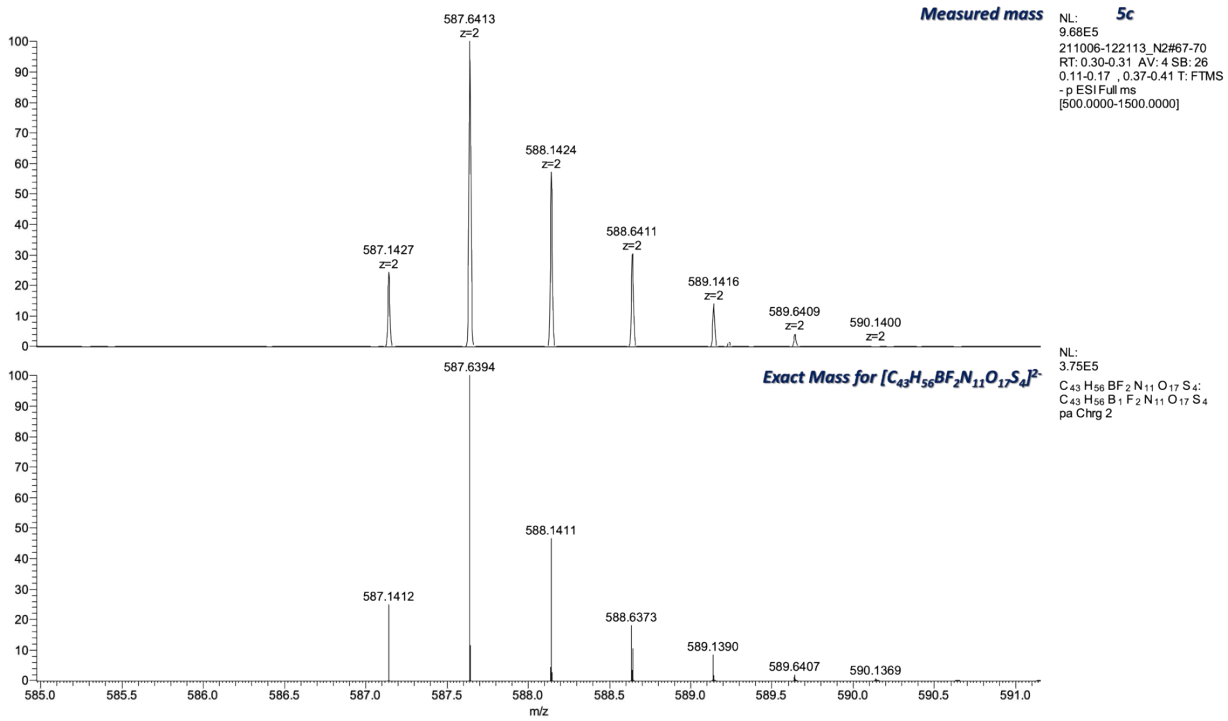
TOCSY of **5c**



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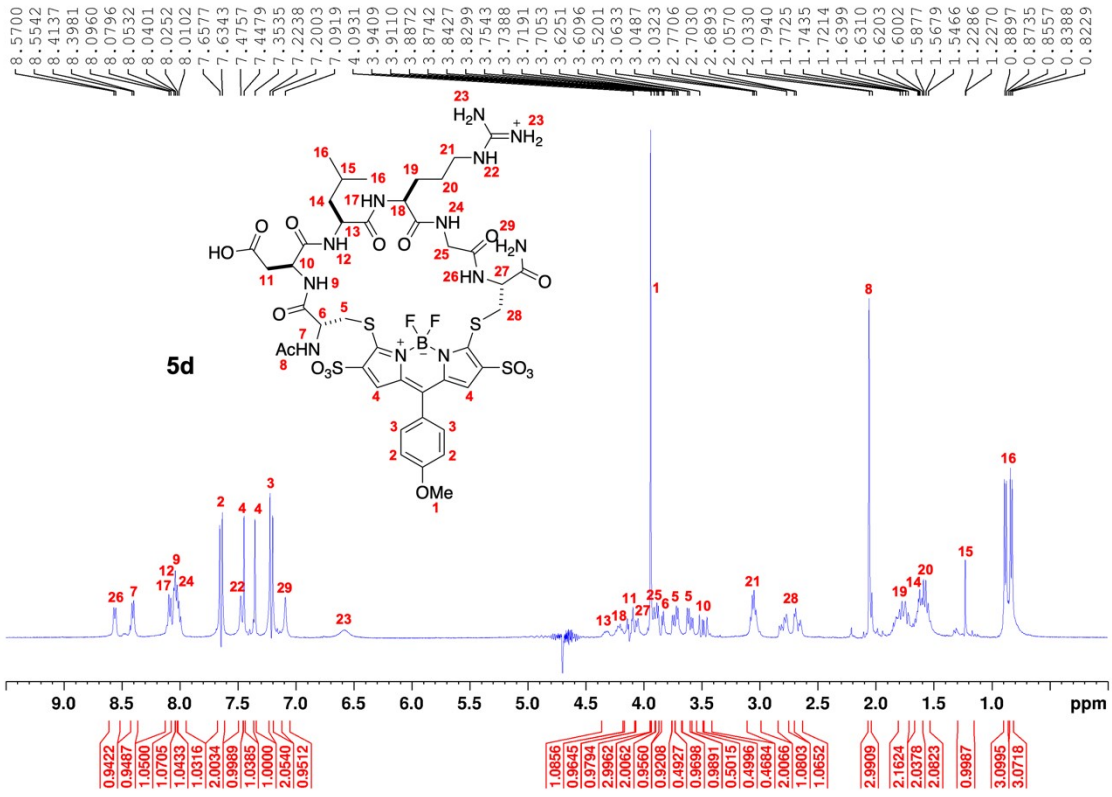
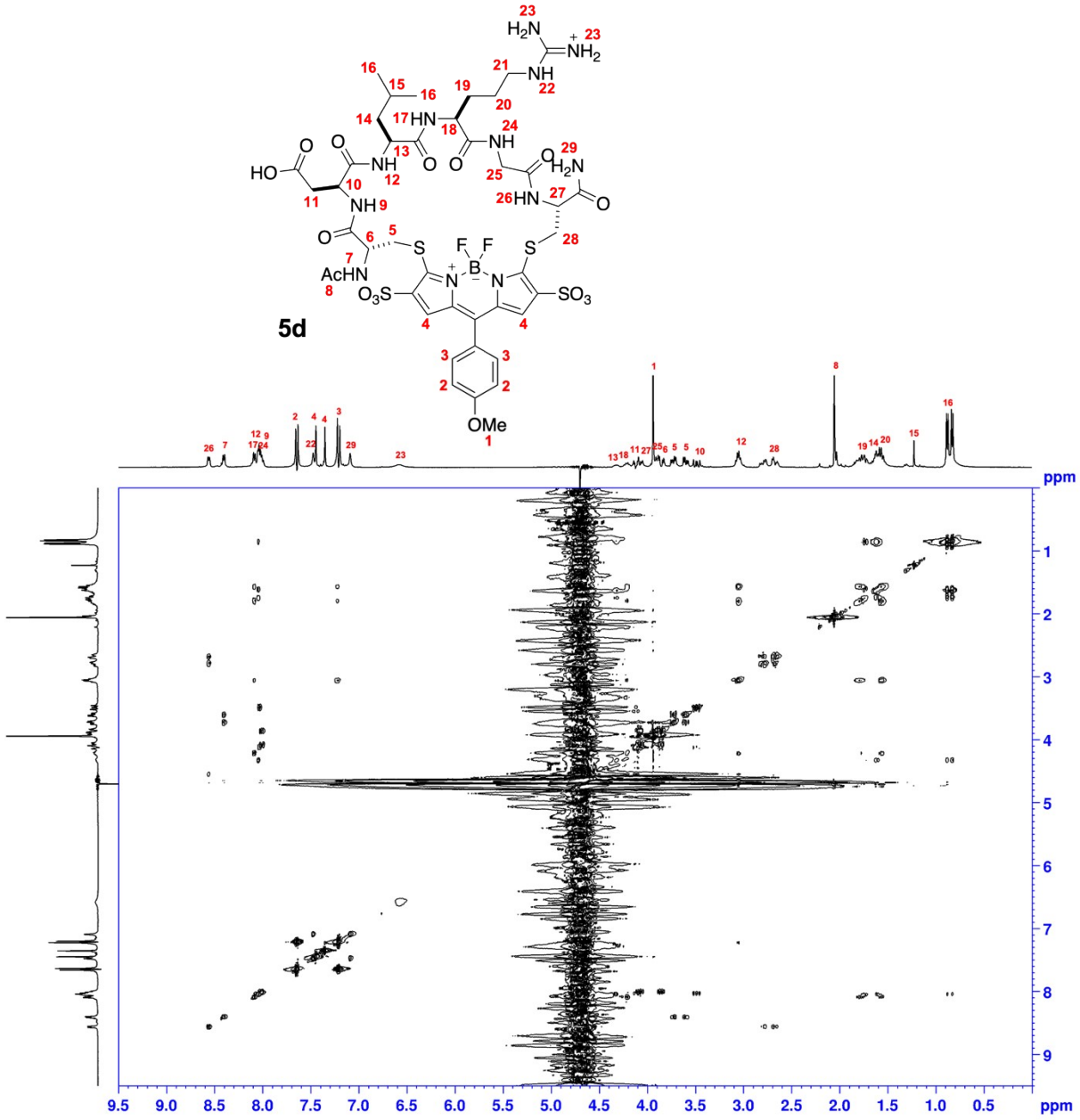


^{1}H NMR of **5d**



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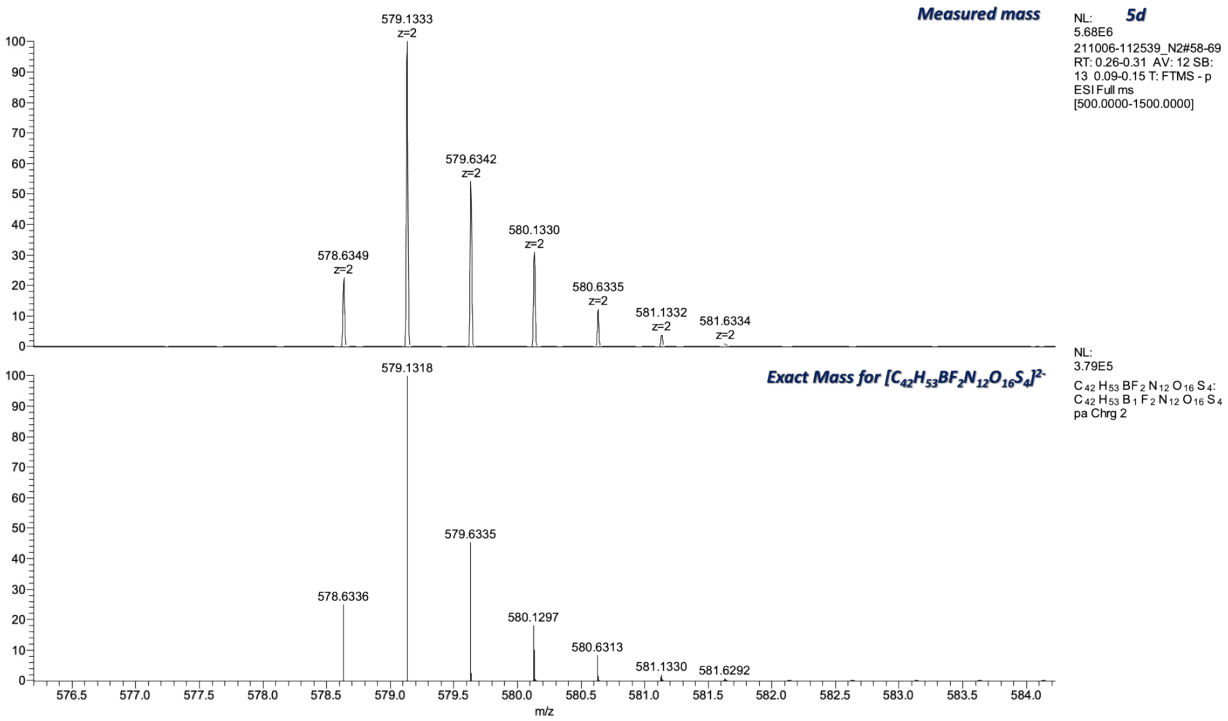
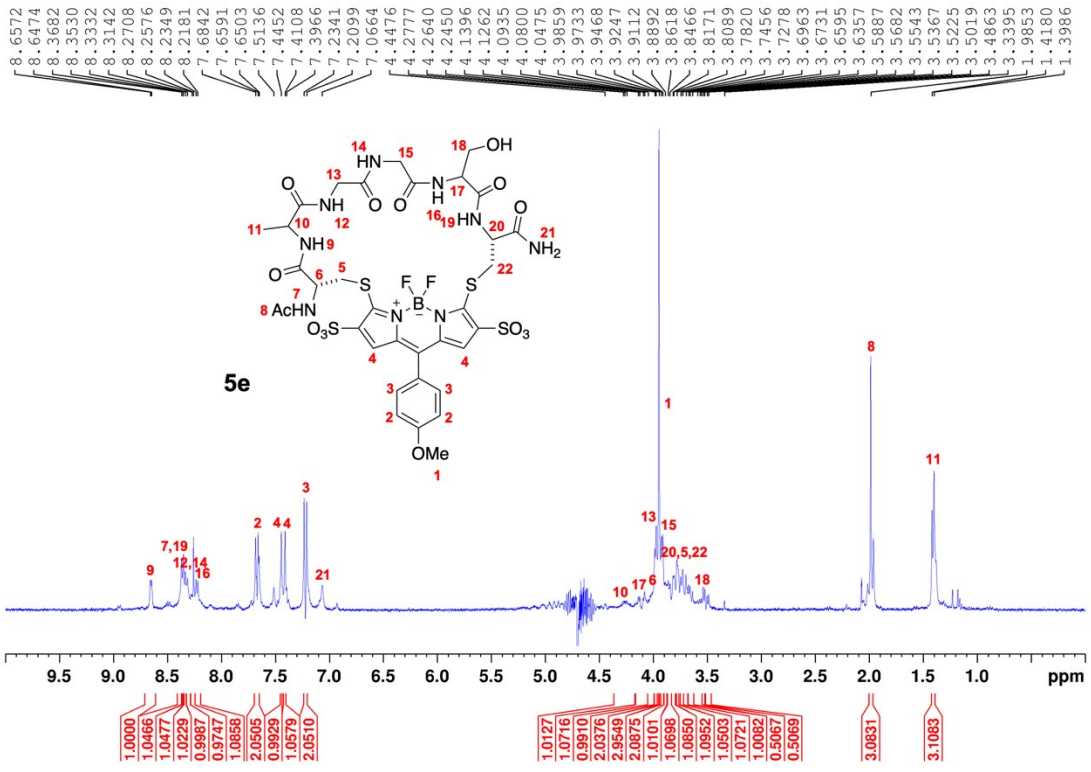
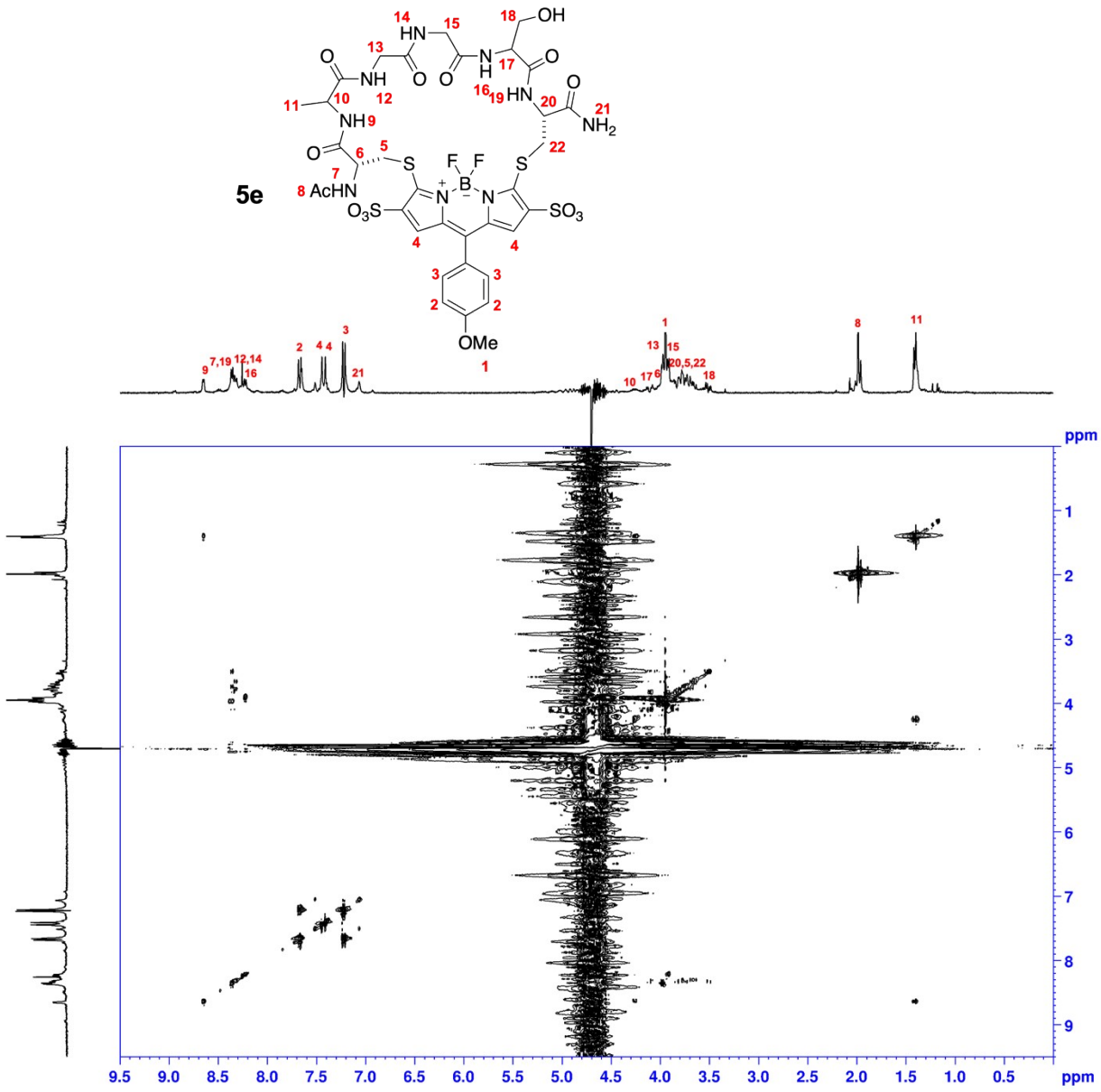


HRMS of **5d**



^{1}H NMR of **5e**


5e
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O_3S
SO_3
OMe
NH_2
ppm
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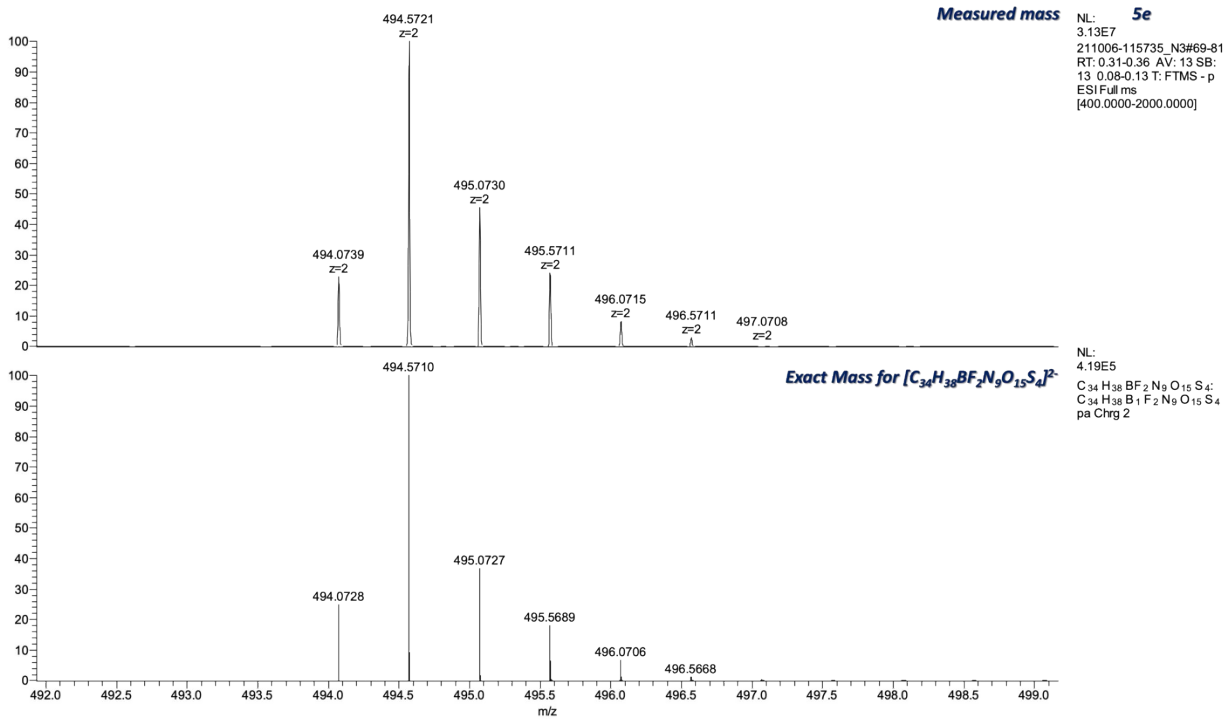
TOCSY of **5e**



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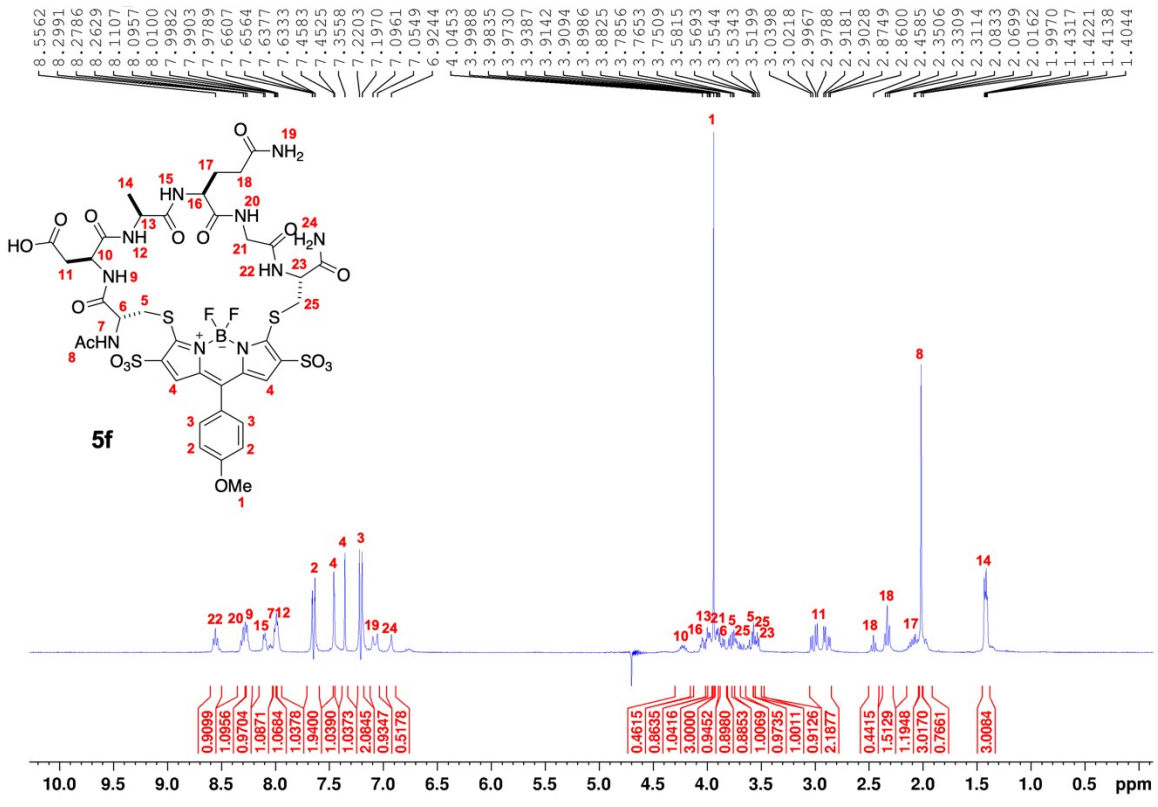
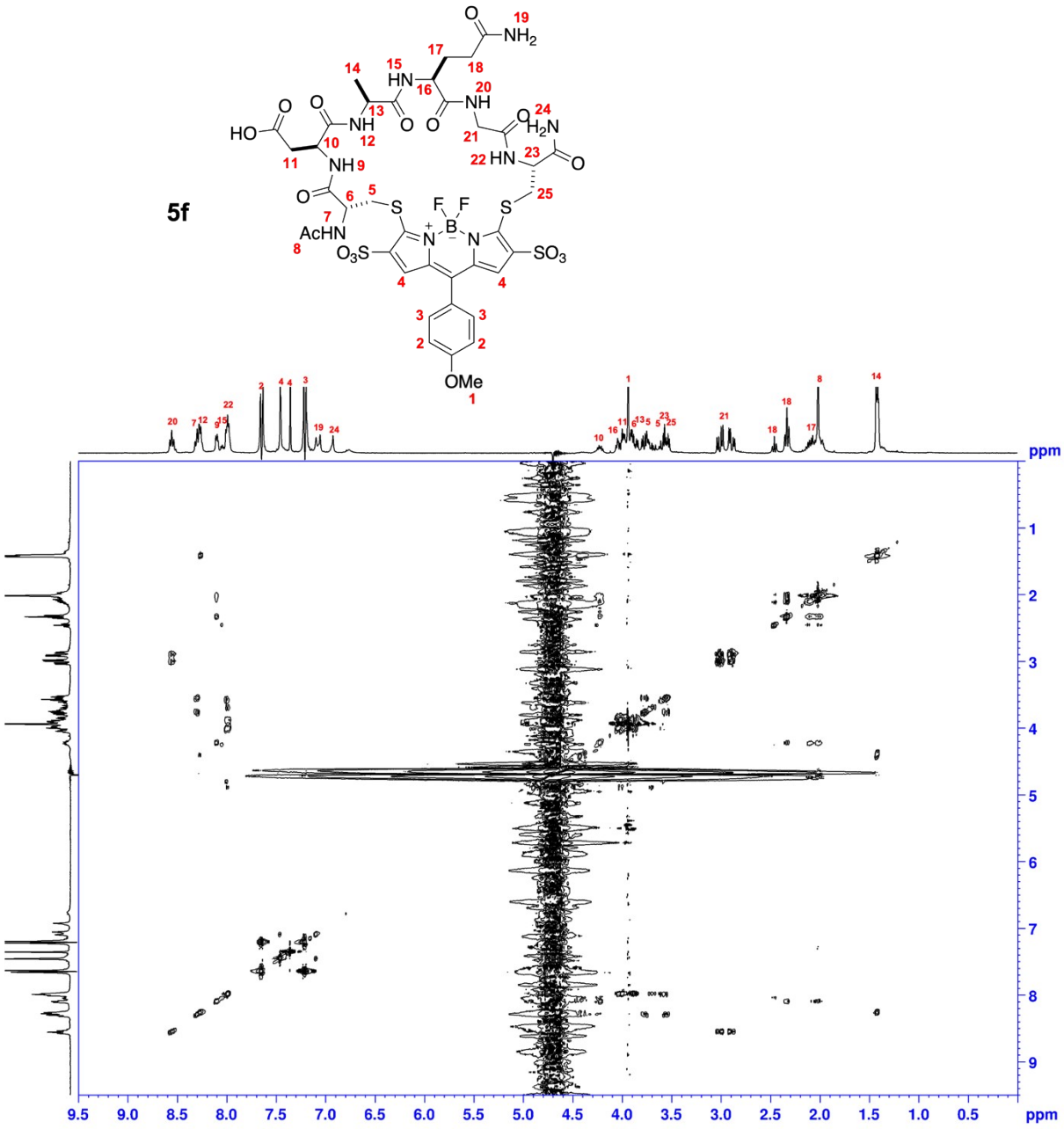


^{1}H NMR of **5f**

5f



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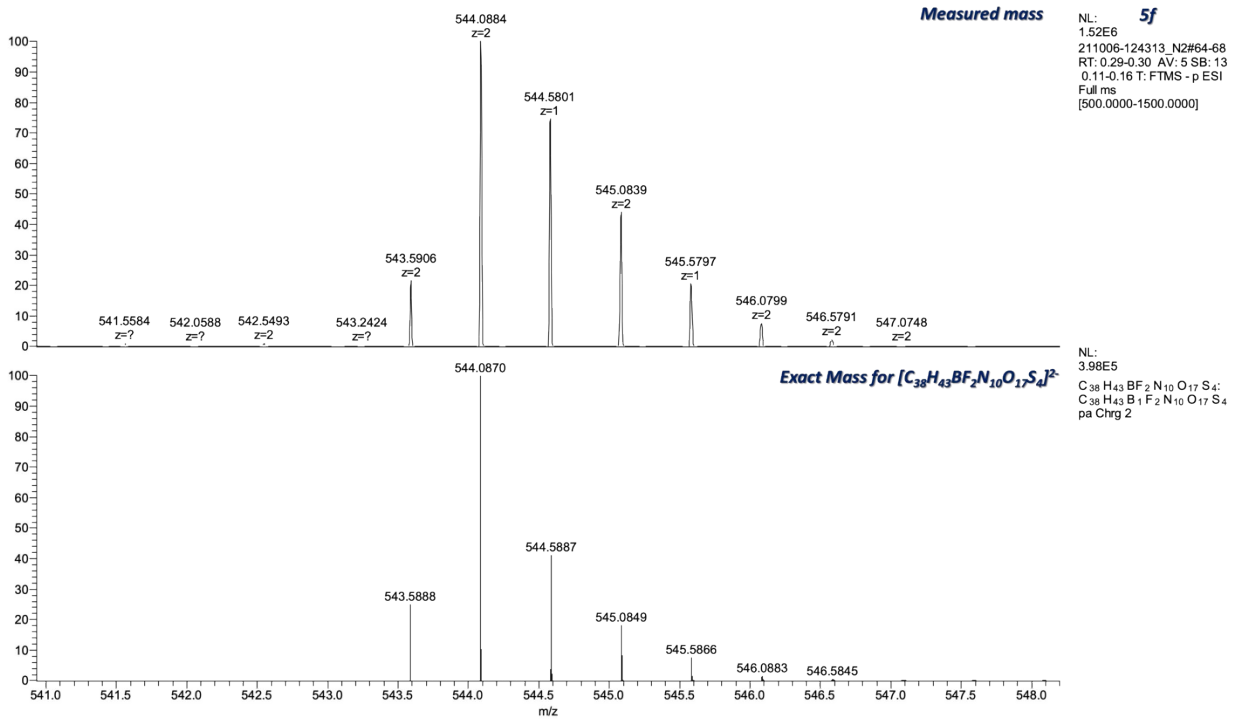

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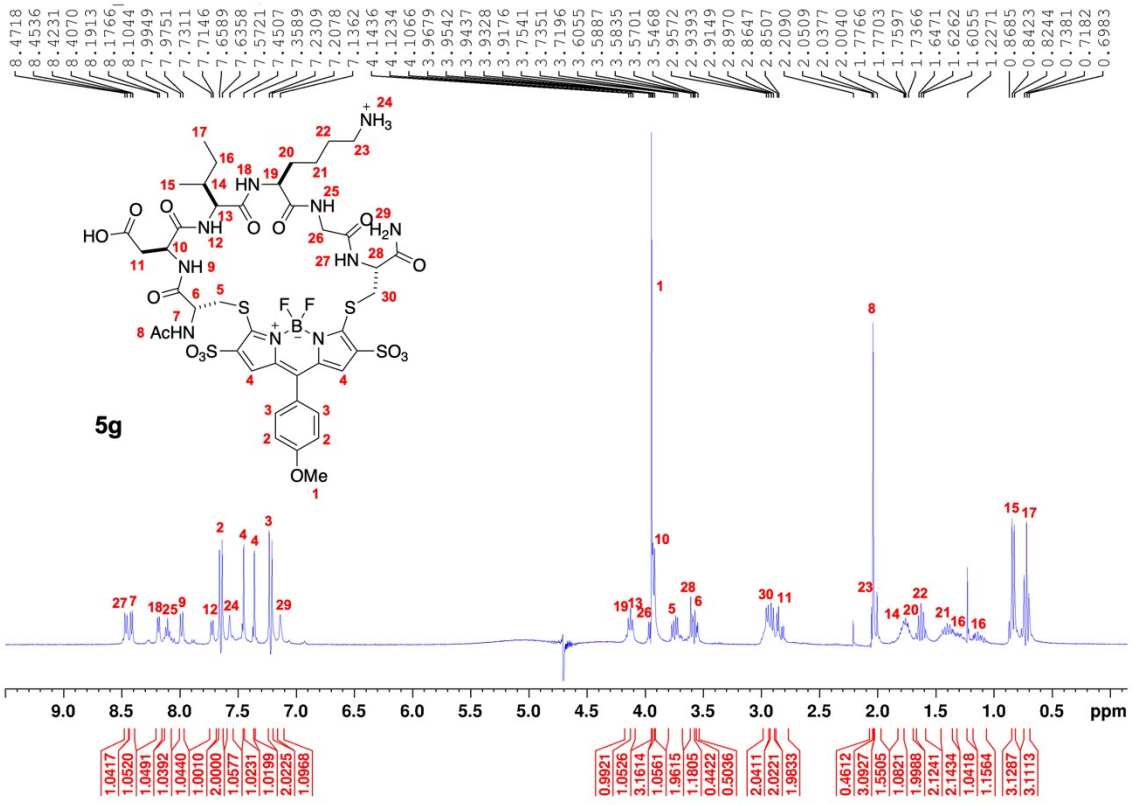
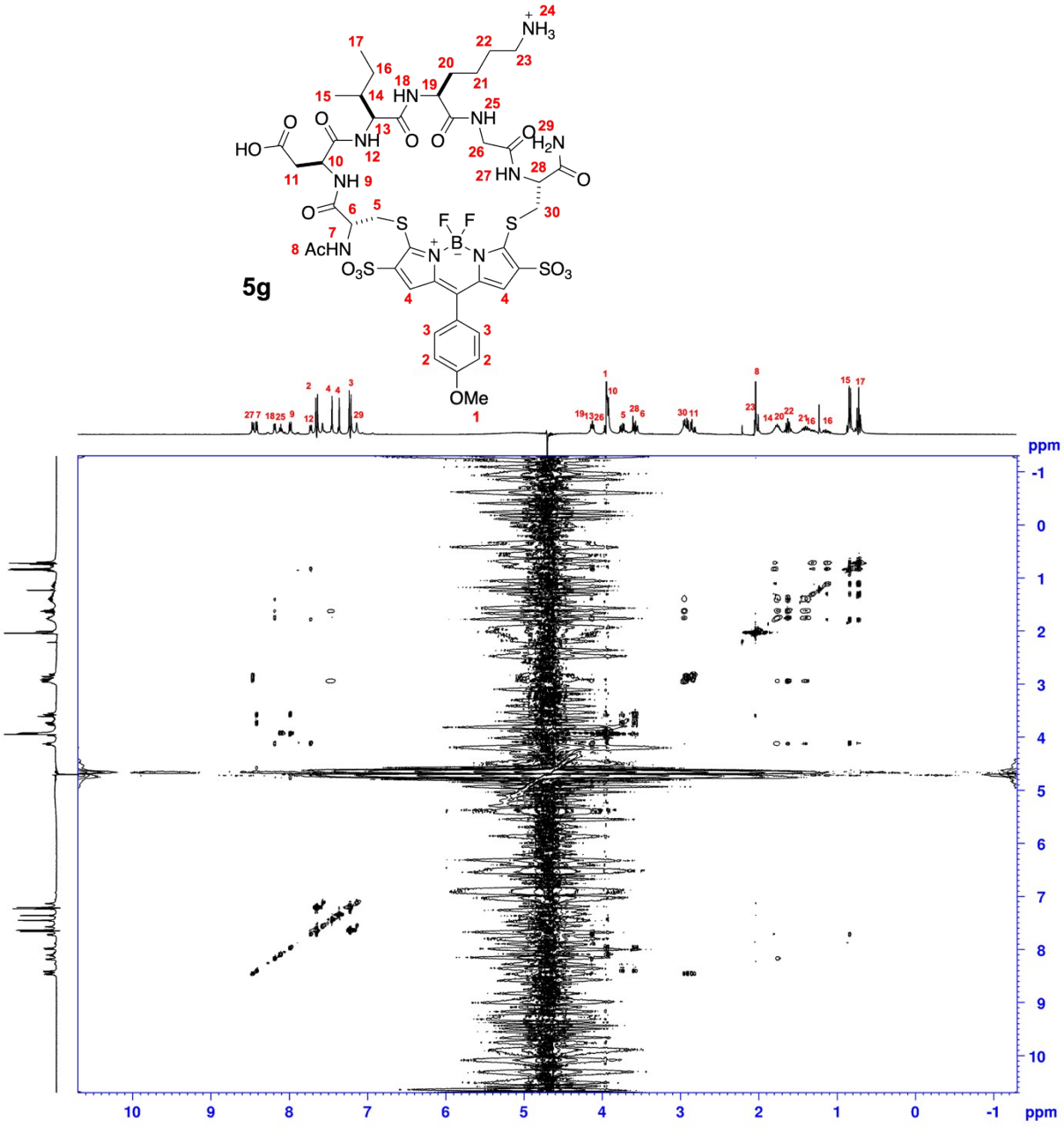
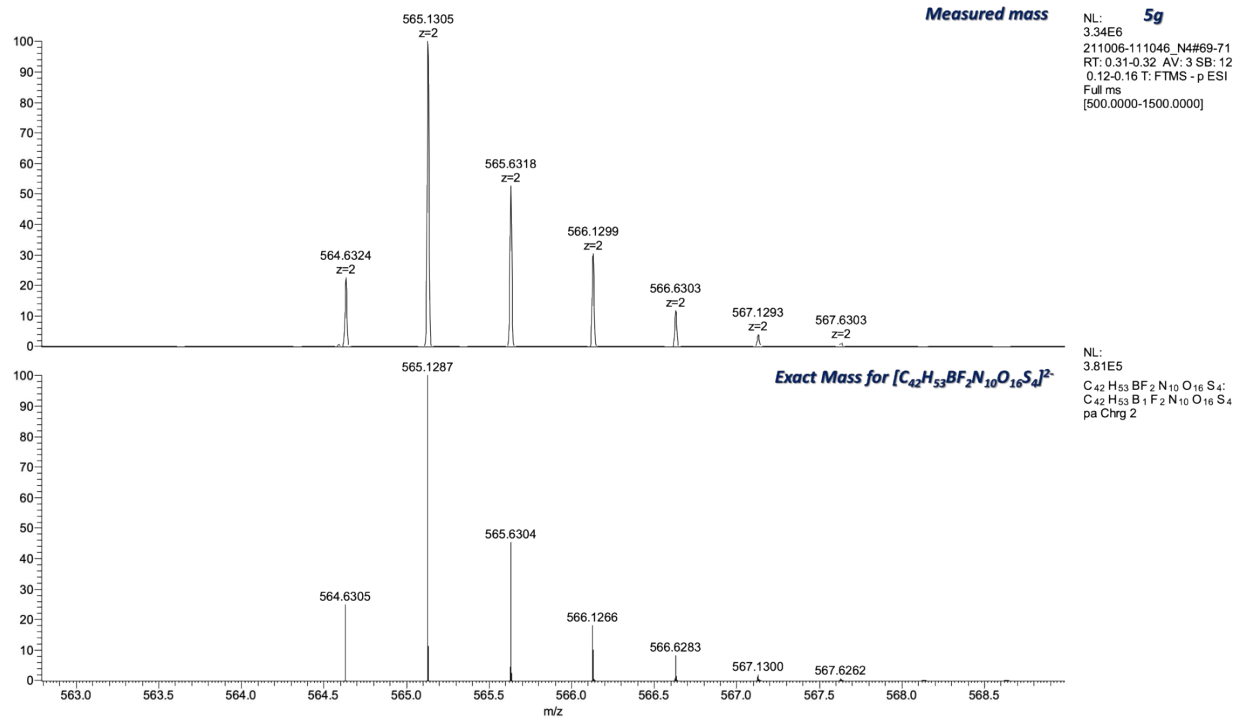


^{1}H NMR of **5g**



TOCSY of **5g**



HRMS of **5g**

Absorbance and Emission Spectra

UV/vis absorption spectra and fluorescence were recorded on a Cary 100 Bio UV-Visible Spectrophotometer and a Cary Eclipse Fluorescence Spectrophotometer using a quartz cuvette with a 1 cm path length. In both experiments, stock solutions of compounds were prepared in DMSO. 2 μM solutions of compound in DI water were prepared for working solutions. Absorption of all compounds have a maximum absorption wavelength about 543-550 nm. For fluorescence experiments, the emission spectra were recorded at excitation wavelength of 500 nm and maximum emission wavelength of all compounds are 572-577 nm.

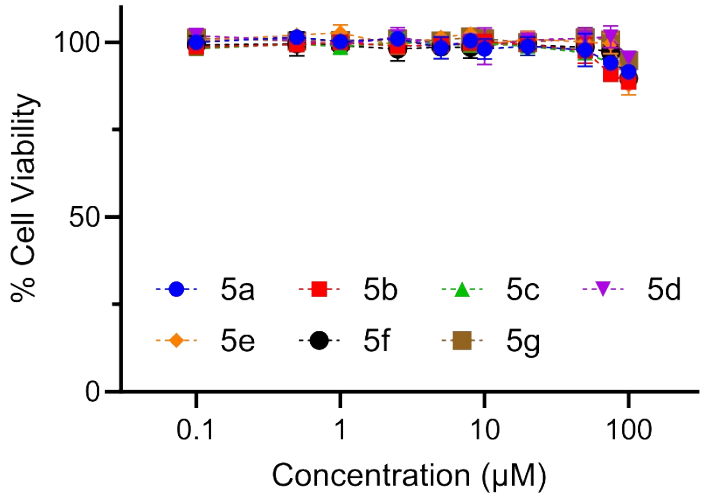
D. Biological Assays

1. General Procedures and Cell Lines

The transfected cell lines HEK293-TrkB and HEK293-WT were cultured in T-75 flasks in complete medium (Dulbecco's Modified Eagle's Medium-high glucose (Sigma Aldrich) supplemented with 10% fetal bovine serum (FBS, Corning) and 1% penicillin–streptomycin (Corning)). The transfectant contained a G-418 resistance gene to select for TrkB expressing cells. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The HEK293-TrkB cell line was given to us by Dr. Moses Chao.

2. Cell Cytotoxicity

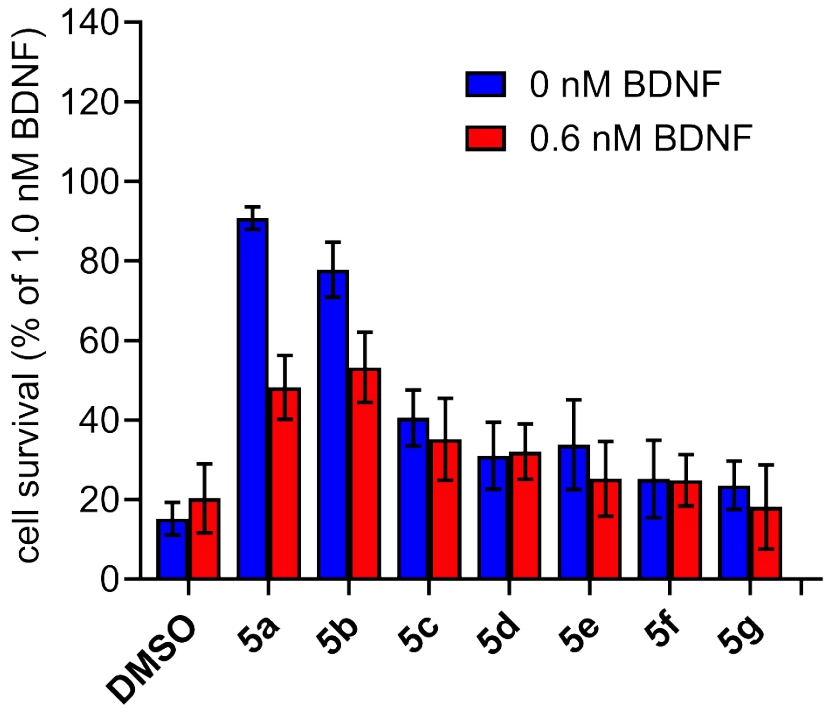
Cells were seeded at approximately $1x10^4$ cells/well in 96-well plate and incubated for 24 h. Once adhered, the cells were treated with the compounds increasing in concentration (0, 0.1, 0.5, 1, 2.5, 5, 8, 10, 20, 50, 75, and 100 μM) in complete media for 24 h. After that, the cells were washed with PBS buffer twice and detached from the bottom of the plate with 50 μL of trypsin for 3 min in 37°C. The trypsinization was quenched with 100 μL of complete media and suspended before investigating cell count by flow cytometry (CytoFLEX LX).



Cell cytotoxicity of all compounds in HEK293-TrkB cells for 24 h. No significant cytotoxicity was observed up 50 μM (the concentration used for compound screening) relative to untreated cells.

3. Cell Survival

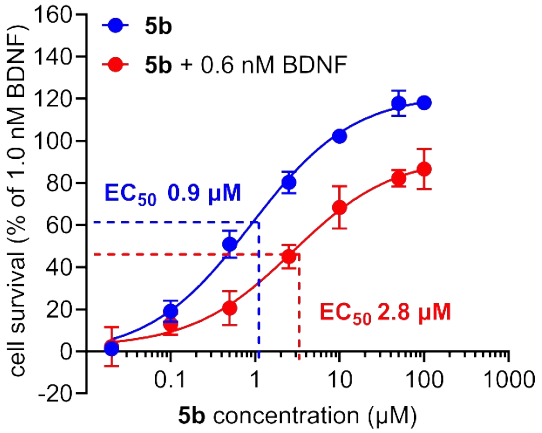
All cells were seeded at approximately $2x10^3$ cells/well in 96-well plate and incubated for 24 h. The cultures were untreated or supplemented with suboptimal BDNF (0.6 nM) in conjunction with 50 µM of each compound in serum-free media for 48 h. Survival was normalized to 100% observed at optimal concentrations of BDNF (1.0 nM). After treatment, the cells were washed with PBS buffer twice and detached from the bottom of the plate with 50 µL of trypsin for 3 min at 37 °C. The trypsinization was quenched with 100 µL of complete media and the cells were suspended before investigating cell count by flow cytometry (CytoFLEX LX). Cell survival was standardized from cell count (CC) readings relative to optimal BDNF = 100%. The CC of untreated cells were subtracted and cell survival (%) was calculated following the formula: % survival= $[(CC_{test} – CC_{untreated})/(CC_{NFT} – CC_{treated})]$ x100.

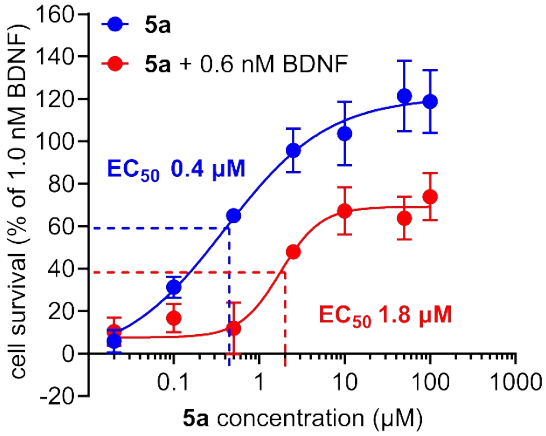


Cell survival of 50 µM all compounds in *TrkB*-HEK293 for 48 h

4. Cell Survival Dose Response

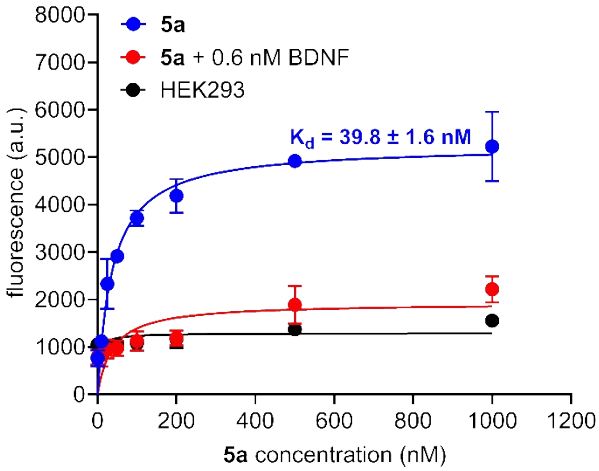
All cells were seeded at approximately $2x10^3$ cells/well in 96-well plate and incubated for 24 h. The cultures were untreated or supplemented with suboptimal BDNF (0.6 nM) in conjunction with a serial dilution (0, 0.02, 0.1, 0.5, 2.5, 10, 50, 100 µM) of each compound in serum-free media for 48 h. Survival was normalized to 100% observed at optimal concentrations of BDNF (1.0 nM). After treatment, the cells were washed with PBS buffer twice and detached from the bottom of the plate with 50 µL of trypsin for 3 min at 37 °C. The trypsinization was quenched with 100 µL of complete media and the cells were suspended before investigating cell count by flow cytometry (CytoFLEX LX). Cell survival was standardized from cell count (CC) readings relative to optimal BDNF = 100%. The CC of untreated cells were subtracted and cell survival (%) was calculated following the formula: % survival= $[(CC_{test} – CC_{untreated})/(CC_{NFT} – CC_{treated})]$ x100. EC_{50} was calculated by GraphPad Prism9 ([Agonist] vs response – Variable slope (four parameter)).

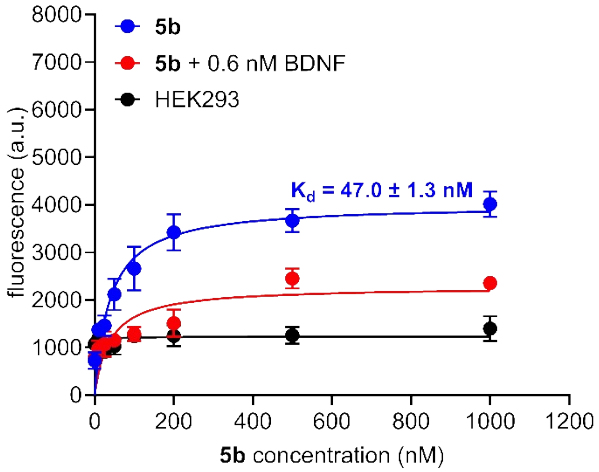




5. Binding to Trk Receptors in Live Cells

HEK293-TrkB and HEK293-WT were seeded at approximately $2x10^3$ cells/well in 96-well plates and incubated for 24 h. The cells were treated with a serial concentration of compound at 0, 10, 25, 50, 100, 200, 500, 1000 nM in serum-free media with and without the suboptimal concentration of 0.6 nM BDNF for 2.5 h. After that, the cells were washed with PBS once to remove unbound fluorescent compound, then dissolved in 1% (w/v) aqueous sodium dodecyl sulfate. Cell-associated fluorescence was measured with a BioTek Synergy H4 Hybrid Reader in fluorescence mode using the filter set $\boldsymbol{\lambda}_{ex}$ (540/25 nm) and $\boldsymbol{\lambda}_{em}$ (620/40 nm). K_d was calculated by GraphPad Prism9 (Binding saturation (One site – Specific binding)).





K_d of 5a and 5b (the two compounds with the highest cell survival) on HEK293-TrkB and HEK293-WT cells.