Supporting Information

Water-Soluble BODIPY Derivatives

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Contents

Experimental Section

1. General Methods

Chemicals and Reagents. Reversed-phase column flash-chromatographies were performed on octadecyl-functionalized silica gel (mean pore size 60 Å) from Aldrich. *N*-Hydroxysulfosuccinimide (Sulfo-NHS) was purchased from Pierce. DMF and NMP were dried by distillation over BaO. DIEA was distilled from CaH_2 and stored over BaO. Disulfonated linker α-sulfo-β-alaninyl-α-sulfo-β-alanine **2** and its *N*-Fmoc protected derivatives **4** were prepared from β-alanine by using synthetic procedures recently reported by us[1]. The HPLC-gradient grade CH_3CN was obtained from Fisher Scientific. Buffers ($NaHCO_3$ and PBS) and aq. mobile-phases for HPLC were prepared using water purified with a Milli-Q system (purified to 18.2 MΩ.cm). Triethylammonium acetate (TEAA, 2.0 M) and triethylammonium bicarbonate (TEAB, 1.0 M) buffers were prepared from distilled triethylamine and glacial acetic acid or CO_2 gas.

Instruments. ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France). Chemical shifts are expressed in parts per million (ppm) from D_2O (δ_H = 4.79) or DMSO-d_6 (δ_H = 2.54, δ_C = 40.45).[2] *J* values are expressed in Hz. UV-visible spectra were obtained on a Varian Cary 50 scan spectrophotometer. Fluorescence

[1] Romieu, A.; Brossard, D.; Hamon, M.; Outaabout, H.; Portal, C.; Renard, P.-Y. *Bioconjugate Chem.* **2008**, *19*, 279-289.
[2] Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.

spectroscopic studies were performed with a Varian Cary Eclipse spectrophotometer. Analytical HPLC was performed on a Thermo Electron Surveyor instrument equipped with a PDA detector. Semi-preparative HPLC was performed on a Finnigan SpectraSYSTEM liquid chromatography system equipped with UV-Visible 2000 detector. Mass spectra were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray source.

HPLC separations. Three chromatographic systems were used for the analytical experiments and the purification steps. Each one of these systems was optimized in order to improve separation conditions.

System A: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5μm, 4.6 x 150 mm) with CH_3CN and 0.1% aq. trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as eluents [100% TFA (5 min), linear gradient from 0 to 80% (40 min) of CH_3CN] at a flow rate of 1.0 mL/min. UV detection was achieved at 260 nm.

System B: RP-HPLC (Varian Kromasil C_{18} column, 10 μm, 21.2 x 250 mm) with CH_3CN and aq. TFA 0.1% as eluents [100% TFA (10 min), linear gradient from 0 to 60% (40 min) of CH_3CN] at a flow rate of 20.0 mL/min. UV detection was achieved at 280 nm.

System C: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5μm, 4.6 x 150 mm) with CH_3CN and 0.1% aq. trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as eluents [80% TFA (5 min), linear gradient from 20 to 40% (5 min) and 40 to 100% (50 min) of CH_3CN] at a flow rate of 1.0 mL/min. Dual UV-visible detection was achieved at 260 and 520 nm.

System D: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5μm, 4.6 x 150 mm) with CH_3CN and TEAA buffer (100 mM, pH 7.0) as eluents [80% TEAA (5 min), linear gradient from 20 to 40% (5 min) and 40 to 100% (50 min) of CH_3CN] at a flow rate of 1.0 mL/min. Triple UV-visible detection was achieved at 260, 520 and 635 nm.

System E: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 μm, 10.0 x 250 mm) with CH_3CN and TEAB buffer (50 mM, pH 7.5) as eluents [90% TEAB (5 min), linear gradient from 10 to 30% (10 min) and 30 to 100% (70 min) of CH_3CN] at a flow rate of 4.0 mL/min. Visible detection was achieved at 635 nm.

2. Synthetic Procedures and Analytical Data

***N*-(9-Fluorenylmethyloxycarbonyl)-α-sulfo-β-alaninyl-α-sulfo-β-alaninyl-α-sulfo-β-alanine (6).**

(a) Preparation of *N*-Hydroxysuccinimidyl ester. *N*-Fmoc disulfonated linker **4** (365 mg, 0.67 mmol) was dissolved in dry DMF (7.5 mL). TSTU reagent (203 mg, 0.67 mmol) and DIEA (350 μL, 2.01 mmol) were added and the resulting reaction mixture was stirred at room temperature for 2 h. The reaction was checked for completion by ESI-MS and the the resulting active ester was used in the coupling reaction without further purification. Yield was assumed to be quantitative. MS (ESI, negative mode), *m/z* 638.00 $[M - H]^-$, calcd mass for $C_{25}H_{25}N_3O_{13}S_2$ 639.62.

(b) Coupling reaction. α-Sulfo-β-alanine (215 mg, 0.80 mmol) was dissolved in 6.8% sodium bicarbonate solution (aq.) (3 mL) and cooled to 4 °C. The crude solution of *N*-hydroxysuccinimidyl ester in DMF was added dropwise to the stirred solution and the mixture was left at room temperature for 5 h. The reaction was checked for completion by RP-HPLC (system A) and the mixture was acidified to pH ~ 2 with 10% HCl (aq.). The reaction mixture was evaporated under reduced pressure. The resulting residue was purified by RP-HPLC (system B, 11 injections, t_R = 25.0-28.5 min). The product-containing fractions were lyophilized to give the tripeptide **6** as a white amorphous powder (241 mg, yield 52%, mixture of 4 racemic diastereomers). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.37-3.64 (m, 9H), 4.15-4.22 (m, 3H), 7.30-7.88 (m, 10H, N*H* & *H*-Fmoc). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 37.9, 40.3, 40.8, 41.7, 46.7, 64.4, 64.5, 64.7, 64.9, 65.3, 65.5, 65.6, 65.8, 66.6, 120.2, 125.6, 127.3, 127.4, 127.7, 140.8, 144.0, 155.9, 156.0, 166.7, 166.8, 167.3, 167.4, 167.8. HPLC (system A): t_R = 19.1, 19.3 and 19.6 min (broad peak, 4 diastereomers), purity > 95%. MS (ESI, positive mode), *m/z* 694.00 $[M + H]^+$, 711.00 $[M + H_2O]^{+\bullet}$. MS (ESI, negative mode), *m/z*: 692.13 $[M - H]^-$, calcd mass for $C_{24}H_{27}N_3O_{15}S_3$ 693.69. Protected tripeptide **6** proved to be too hygroscopic for suitable IR and elemental analysis.

α-Sulfo-β-alaninyl-α-sulfo-β-alaninyl-α-sulfo-β-alanine (7). *N*-Fmoc α-sulfo-β-alanine tripeptide **6** (181 mg, 0.26 mmol) was dissolved in DMF (1.8 mL) and cooled to 4 °C. Et_2NH (163 μL, 1.57 mmol) was added and the resulting reaction mixture was stirred at room temperature for 1 h. The reaction was checked for completion by RP-HPLC (system A) and the mixture was evaporated under reduced pressure. The resulting residue was taken up in deionized water (20 mL) and washed with CH_2Cl_2 (4 x 20 mL). Finally, the aq. solution was lyophilized to give the α-sulfo-β-alanine tripeptidyl linker **7** as a white amorphous powder (165 mg, yield 90%, mixture of 4 racemic diastereomers). ^{1}H NMR (300 MHz, D_2O): δ 1.23 (t, J = 7.3 Hz, 18H, N-CH_2-CH_3, diethylammonium counter ion), 3.03 (q, J = 7.3 Hz, J = 14.7

Hz, 12H, N-C*H*$_2$-CH$_3$, diethylammonium counter ion), 3.48-4.14 (m, 9H). ^{13}C NMR (75.5 MHz, D_2O): δ 10.5, 37.4, 38.0, 38.1, 38.4, 38.5, 38.6, 38.7, 38.8, 42.2, 61.3, 61.4, 63.8, 63.9, 64.1, 64.2, 166.2, 166.3, 166.5, 166.6, 167.5, 167.6, 167.7. HPLC (system A): coeluted with the injection peak. MS (ESI, negative mode), *m/z* 234.80 $[M - 2H]^{2-}$, 470.13 $[M - H]^-$, calcd mass for $C_9H_{17}N_3O_{13}S_3$ 471.44. Tripeptide **7** proved to be too hygroscopic for suitable IR and elemental analyses.

Compound 3.

(a) Preparation of *N*-Hydroxysuccinimidyl ester of BODIPY **1** (15.29 mg, 36.1 μmol, weighed in a 1.5 mL Eppendorf tube) was dissolved in dry NMP (0.6 mL). 300 μL of a solution of TSTU reagent in dry NMP (11.94 mg, 39.7 μmol) and 9.35 μL of DIEA (54.1 μmol) were added and the resulting reaction mixture was protected from light and periodically vortexed for 90 min. The reaction was checked for completion by RP-HPLC (system C) and the resulting *N*-hydroxysuccinimidyl ester was used without further purification. HPLC (system C): t_R = 29.4 min (compared to t_R = 27.0 min for BODIPY carboxylic acid **1**).

(b) Coupling reaction. α-Sulfo-β-alaninyl-α-sulfo-β-alanine **2** (33.43 mg, 71.7 μmol) and solid $NaHCO_3$ (18.74 mg, 222.8 μmol) were dissolved in deionized water (1 mL) and the resulting solution was cooled to 4 °C. The crude solution of *N*-hydroxysuccinimidyl ester in NMP was added dropwise to the stirred solution ; precipitation of this activated ester was immediately observed. Thus, DMF (2 mL) and further amount of bicarbonate buffer (0.5 mL) were added and the reaction mixture was stirred at room temperature for 3 days. The reaction was checked for completion by RP-HPLC (system D). Finally, the reaction mixture was quenched by dilution with aq. TEAB (50 mM, pH 7.5, ~ 5 mL) and purified by RP-HPLC (system E, 2 injections, t_R = 21.5-25.0 min). The product-containing fractions were lyophilized thrice to give the TEA salt of **3** as a red amorphous powder (13.2 mg, yield 38%, mixture of two racemic diastereomers). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.93 (t, *J* = 7.5 Hz, 6H, 2 x C*H*$_3$-Et, BODIPY), 1.14 (t, *J* = 7.1 Hz, 21H, N-CH$_2$-C*H*$_3$, 2.3 x triethylammonium counter ion), 1.17 (s, 6H, 2 x C*H*$_3$, BODIPY), 2.28 (q, *J* = 6.6 Hz, *J* = 14.3 Hz, 4H, 2 x C*H*$_2$-Et, BODIPY), 2.43 (s, 6H, 2 x C*H*$_3$, BODIPY), 3.02 (q, *J* = 7.3 Hz, *J* = 14.4 Hz, 14H, N-C*H*$_2$-CH$_3$, 2.3 x triethylammonium counter ion), 3.15-3.81 (m, 6H, C*H*$_2$-C*H*(SO_3^-)-CO-), 7.45 (m, 2H, Ph, BODIPY), 7.74 (bt, 1H, N*H*), 7.96 (m, 2H, Ph-BODIPY), 8.49 (bm, 1H, N*H*). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 8.9, 11.5, 12.3, 14.5, 16.4, 38.2-39.3 (3C), 45.7, 64.1-64.8

(3C), 127.9, 128.3, 129.7, 132.7, 134.9, 137.5, 138.1, 139.7, 153.4, 165.0, 167.0, 169.3. ^{19}F NMR (282.5 MHz, DMSO-d_6) : δ -142.9 (q, J_{BF} = 20.6 Hz, J = 24.7 Hz, 2F, B*F*). HPLC (system D): t_R = 14.1 min, purity 92%. UV-vis (PBS, pH 7.4, 25 °C) : λ_{max} = 523 nm (57 350 M^{-1} cm^{-1}). Φ_F (PBS, pH 7.4, 25 °C) = 0.62. Φ_F (DMSO, 25 °C) = 0.82. MS (ESI, positive mode): *m/z* 1030.27 $[M + 3TEA + H]^+$. (ESI, negative mode): *m/z* 725.20 $[M - H]^-$, calcd for $C_{30}H_{37}BF_2N_4O_{10}S_2$ (**3**, acid form) 726.57.

Compound 10.

(a) Preparation of *N*-Hydroxysuccinimidyl ester **9** of BODIPY **8** (5.0 mg, 8.3 μmol, weighed in a 1.5 mL Eppendorf tube) was dissolved in dry NMP (75 μL). 75 μL of a solution of TSTU reagent in dry NMP (2.76 mg, 9.2 μmol) and 2.20 μL of DIEA (12.5 μmol) were added and the resulting reaction mixture was protected from light and periodically vortexed for 30 min. Thereafter, this crude reaction mixture was added dropwise to a solution of Sulfo-NHS (4.1 mg, 18.8 μmol) and tetrabutylammonium tetrafluoroborate (7.4 mg, 18.9 μmol) in dry NMP (90 μL) and the resulting solution was protected from light and again periodically vortexed for 1 h. The reaction was checked for completion by ESI-MS and the the resulting active ester was used in the coupling reaction without further purification. Yield was assumed to be quantitative. MS (ESI, negative mode), *m/z* 780.27 $[M - H]^-$, calcd mass for $C_{40}H_{34}BF_2N_3O_9S$ 781.60.

(b) Coupling reaction. α-Sulfo-β-alanine tripeptidyl linker **7** (14.4 mg, 20.8 μmol) and solid $NaHCO_3$ (8.9 mg, 106.5 μmol) were dissolved in deionized water (0.5 mL) and the resulting solution was cooled to 4 °C. The crude solution of *N*-hydroxysulfosuccinimidyl ester in NMP was added dropwise to the stirred solution and the resulting reaction mixture was stirred at room temperature overnight. The reaction was checked for completion by RP-HPLC (system D). Finally, the reaction mixture was quenched by dilution with aq. TEAB (50 mM, pH 7.5, ~ 2 mL) and purified by RP-HPLC (system E, 2 injections, t_R = 24.5-26.0 min). The product-containing fractions were lyophilized thrice to give the TEA salt of **10** as a blue amorphous powder (4.5 mg, yield 41%, mixture of 4 racemic diastereomers). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.15 (t, J = 7.3 Hz, 23H, N-CH_2-CH_3, 2.6 x triethylammonium counter ion), 1.41 (s, 6H, 2 x CH_3, BODIPY), 3.04 (q, J = 7.2 Hz, J = 14.5 Hz, 16H, N-CH_2-CH_3, 2.6 x triethylammonium counter ion), 3.33-3.64 (m, 9H, CH_2-CH(SO_3^-)-CO-), 3.82 (s, 6H, 2 x -OCH_3), 6.95 (s, 2H, pyrrole-BODIPY), 7.05 (d, J = 8.7 Hz, 4H, Ph-BODIPY), 7.40 (d, J = 16.2 Hz, 2H, -C*H*=CH-BODIPY), 7.52-7.60 (m, 8H, -CH=C*H*-BODIPY, Ph-BODIPY), 7.69-

7.83 (bs, 2H, N*H*), 8.02 (bd, *J* = 7.5 Hz, 2H, Ph-BODIPY), 8.51 (bs, 1H, N*H*). ^{13}C NMR (75.5 MHz, DMSO-d_6): quantity isolated was too small for vizualization of all carbon peaks, δ 8.5, 10.8, 14.3, 38.0, 41.2, 45.5, 55.1, 64.2, 64.5, 114.5, 115.7, 118.0, 127.7, 128.4, 128.7, 136.5, 152.0, 160.1. ^{19}F NMR (282.5 MHz, DMSO-d_6) : δ -136.0 (pd, J_{BF} = 52.6 Hz, 2F, B*F*). HPLC (system D): t_R = 17.0 min, purity > 95%. UV-vis (PBS, pH 7.4, 25 °C) : λ_{max} = 370, 609, 654 nm. UV-vis (DMSO, 25 °C) : λ_{max} = 374, 649 nm. Φ_F (PBS, pH 7.4, 25 °C) = n.d. (non fluorescent). Φ_F (DMSO, 25 °C) = 0.40. (ESI, negative mode): *m/z* 351.87 [M - 3H]$^{3-}$, 527.93 [M - 2H]$^{2-}$, 1056.33 [M - H]$^-$, calcd for $C_{45}H_{46}BF_2N_5O_{16}S_3$ (**10**, acid form) 1057.89.

Compound 12

To a degassed solution of 2,6-diiodo-1,3,5,7,8-pentamethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (0.100g, 0.1946 mmol) in benzene (2 ml) and TEA (2 ml), were added [$Pd(PPh_3)_2Cl_2$] (0.008 g, 0.0117 mmol), CuI (0.003 g, 0.0117 mmol), and 1-dimethylamino-2-propyne (0.034 g, 0.486 mmol). The mixture was stirred at 60 °C over night, until the complete consumption of the starting material was observed by TLC. The mixture was evaporated, and then extracted with CH_2Cl_2. The solution was then washed with water, brine and dried. Removal of solvent in vacuum followed by column chromatography of the residue on silica (MeOH / CH_2Cl_2, 1: 9) afforded the compound **12** (0.035 g, 42%)^{1}H NMR (300MHz, $CDCl_3$): δ 3.55 (s, 4H), 2.61-2.58 (m, 9H), δ 2.49(m, 6H), δ 2.38(s, 12H).^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ =156.54, 142.30, 141.99, 131.67, 115.90, 90.99, 53.41, 48.79, 44.07, 16.88, 16.03, 13.51.MS (FAB$^+$, m-NBA): m/z (%) = 424.2 [M + H] $^+$ (100). Anal. Calcd for $C_{24}H_{31}BF_2N_4$: C, 67.93; H, 7.36; N, 13.20. Found: C, 67.72, H, 7.18, N, 12.93.

Compound 13

To a solution of **12** (0.035g, 0.0825 mmol) in dry toluene (3 ml) under argon, the 1,3-propansultone (0.025 g, 0.21 mmol) was added, then the mixture was stirred at 60 °C for 3 days, until the complete consumption of the starting material was observed by TLC. The mixture was centrifugated and washed with toluene (5ml) and pentane (5 ml). The powder was dried in vacuum, the compound **13** (32 mg, 58%).^{1}H NMR (300MHz, D_2O): δ 4.58 (s, 4H), 3.67 (m, 4H), 3.25 (s, 12H), 2.98 (m, 4H), 2.41 (m, 19H). ^{13}C {^{1}H} NMR (75 MHz, D_2O): δ =156.9, 145.1, 131.3, 113.2, 83.6, 62.2, 60.2, 55.1, 50.6, 47.9, 47.4, 42.1, 27.5, 18.5,

16.4, 12.7. MS (FAB^+, m-NBA): m/z (%) = 335.1 (100, doubly charged) $[M + 2H]^+$ (100). ESI-MS positive mode using acetonitrile/H_2O. Anal. Calcd for $C_{30}H_{43}BF_2N_4O_6S_2$+2$H_2O$: C, 51.13; H, 6.72; N, 7.95; Found: C, 51.07, H, 6.50, N, 7.72.

Compound 15

To a solution of 1-dimethylamino-2-propyne (0.136g, 1.976 mmol) in dry THF (5 ml) under argon in a schlenk flask, was added 1.0 M EtMgBr in THF (1.738ml) and the solution was stirred at 60 °C for 2 hour. 2, 6-diethyl-4, 4-difluoro-1, 3, 5, 7-tetramethyl-8-(4'-iodo-phenyl)-4-bora-3a,4a-diaza-s-indacene (0.400g, 0.79 mmol) was dissolved in a separate schlenk flask in dry THF(3 ml) under argon, and then transferred via canula to the schlenk containing the propyne Grignard. The mixture was stirred at 60 °C for about 30 minutes, until complete consumption of the starting material was observed by TLC(MeOH/CH_2Cl_2, 1:9). H_2O (3ml) was added, then extracted with CH_2Cl_2. After evaporation, a column chromatography (silica CH_2Cl_2/ AcOEt, 50: 50, then (MeOH/CH_2Cl_2, 1/9) afforded the compound **15** (0.313 g, 63%).^{1}H NMR ($CDCl_3$ 300 MHz) : 0,97 (t, 6H, ^3J = 7,53 Hz), 1,30 (s, 6H), 2,32 (q, 4H, ^3J= 7,14Hz), 2,38 (s, 12H), 2.72 (s, 6H), 3,30 (s, 4H), 7,06 (AB sys, 2H, J_{AB} = 8,31 Hz), 7,81 (AB sys, 2H, J_{AB} = 8,31 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): = 12,11, 14,12, 14,69, 17,32, 29,36, 43,73, 48,78, 94,29, 128,69, 130,50, 133,03, 135,87, 136,07, 138,10, 138,52, 153,63.MS (FAB^+, m-NBA): m/z (%) = 633.1 $[M + H]^+$ (100). Anal. Calcd for $C_{33}H_{42}BIN_4$. C, 62.67; H, 6.69; N, 8.86. Found: C, 62.52; H, 6.51; N, 8.53.

Compound 16

To a solution of **15** (0.100g, 0.458 mmol) in dry toluene (3 ml) under argon was added 1,3-propansultone (0.077 g, 0.63mmol), then the mixture was stirred at 60 °C over night, until the complete consumption of the starting material was observed by TLC (H_2O/EtOH, 20: 80). The mixture was centrifugated, the residue was washed with ethyl ether and then recrystallized twice from methanol-acetone. The powder was dried in vacuum, the compound **16** (0.086g, 62%). ^{1}H NMR (CD_3OD 300 MHz) : 0,99 (t, 6H, ^3J = 7,53 Hz), 1,37 (s, 6H), 2,20 (m, 4H,), 2.39 (q, 4H, ^3J = 7,53 Hz), 2,72 (s, 6H), 2,82 (t, 4H, ^3J = 6,99 Hz), 3,14 (s, 12H), 3,56 (m, 4H), 4,30 (s, 4H), 7,13 (AB sys, 2H, J_{AB} = 8,28 Hz), 7,93 (AB sys, 2H, J_{AB} = 8,28 Hz); ^{13}C NMR (CD_3OD, 75 MHz): = 10,93, 13,45, 13,59, 16,59, 18,72, 29,27, 49,71, 55,16, 62,68, 94,12, 116,51, 128,66, 130,33, 133,64, 135,09, 137,26, 138,37, 139,59, 153,55. MS (FAB^+, m-NBA): m/z (%) = 876.1 $[M + H]^+$ (100). Anal. Calcd for $C_{39}H_{54}BIN_4O_6S_2$ + H_2O. C, 51.32; H, 6.40; N, 6.14; Found: C, 51.27; H, 6.28; N, 5.82.

Optical properties of water-soluble BODIPY derivatives.

The absorption spectra of water-soluble BODIPYs **3** and **10** were recorded (220-800 nm) in DMSO and PBS (0.1 M phosphate, 0.15 M NaCl, pH 7.4) (concentration: 1.0-10.0 μM) at 25 °C. Emission spectra were recorded under the same conditions after excitation at the corresponding wavelength (excitation and emission slit = 5 nm) : 488 nm for **3**, 605 nm for **10** in PBS and 595 nm for **10** in DMSO. Relative quantum yields were measured in DMSO and PBS at 25 °C by a relative method using either sulfocyanine dye Cy 5.0 (Φ_F = 0.20 in PBS)[3] or rhodamine 6G (Φ_F = 0.76 in water)[4] as a standard.

The following equation was used to determine the relative fluorescence quantum yield:

$$\Phi_F(x) = (A_S/A_X)(F_X/F_S)(n_X/n_S)^2\Phi_F(s)$$

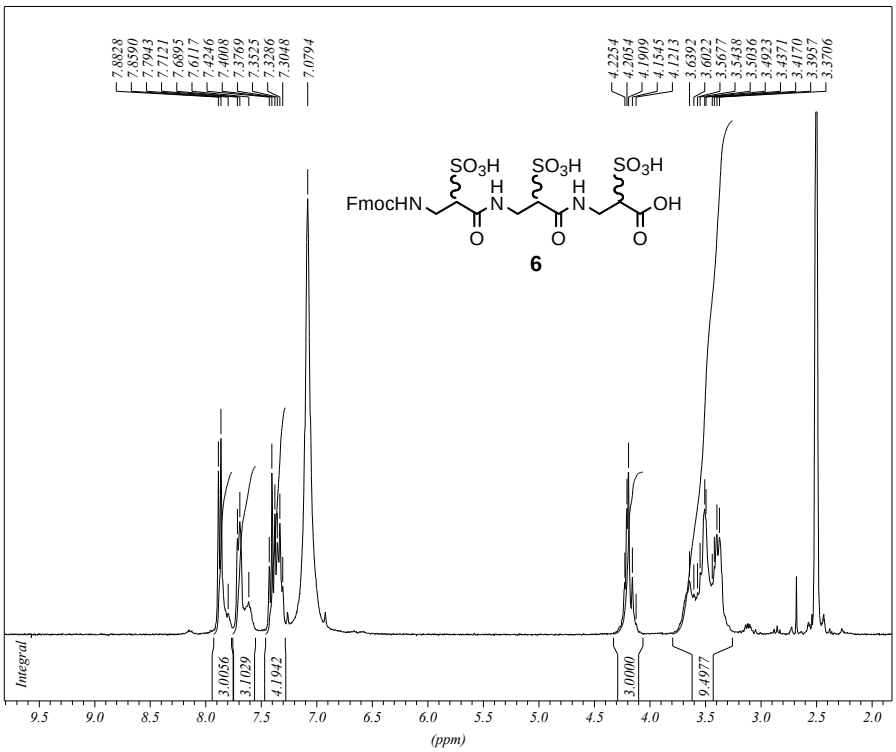
Where A is the absorbance (in the range 0.01-0.1 A.U.), F is the area under the emission curve, n is the refractive index of the solvents (at 25 °C) used in measurements (n = 1.337 for PBS and 1.479 for DMSO), and the subscripts s and x represent standard and unknown, respectively.

[3] Mujumdar, R. B. ; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105-111.

[4] Olmsted III, J. *J. Phys. Chem.* **1979**, *83*, 2581-2584.

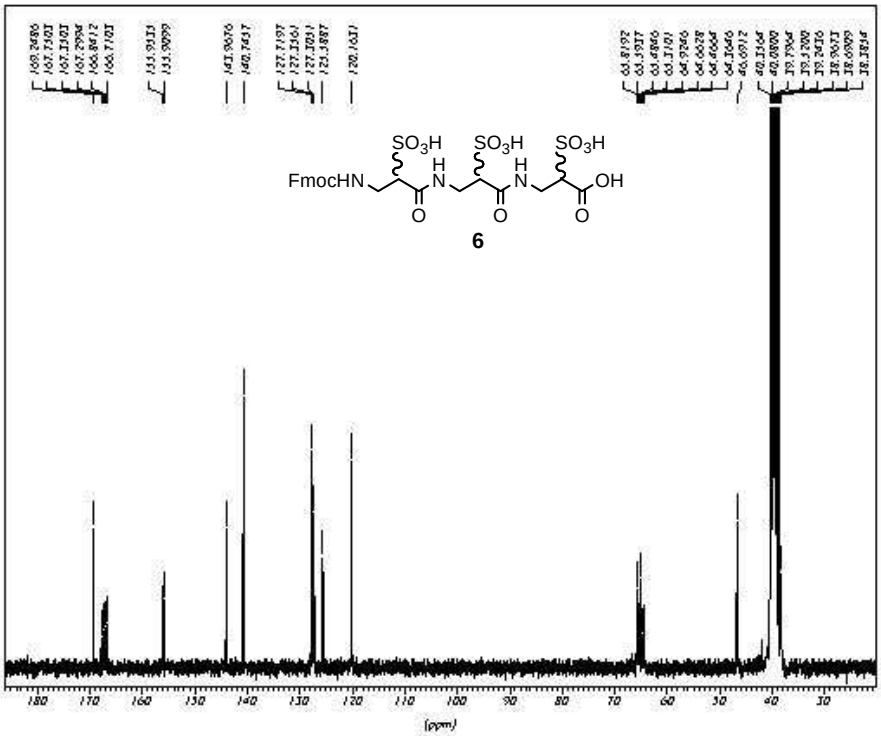
Spectra traces and RP-HPLC

^{1}H NMR spectrum of compound 6 recorded in DMSO-d_6.[a]



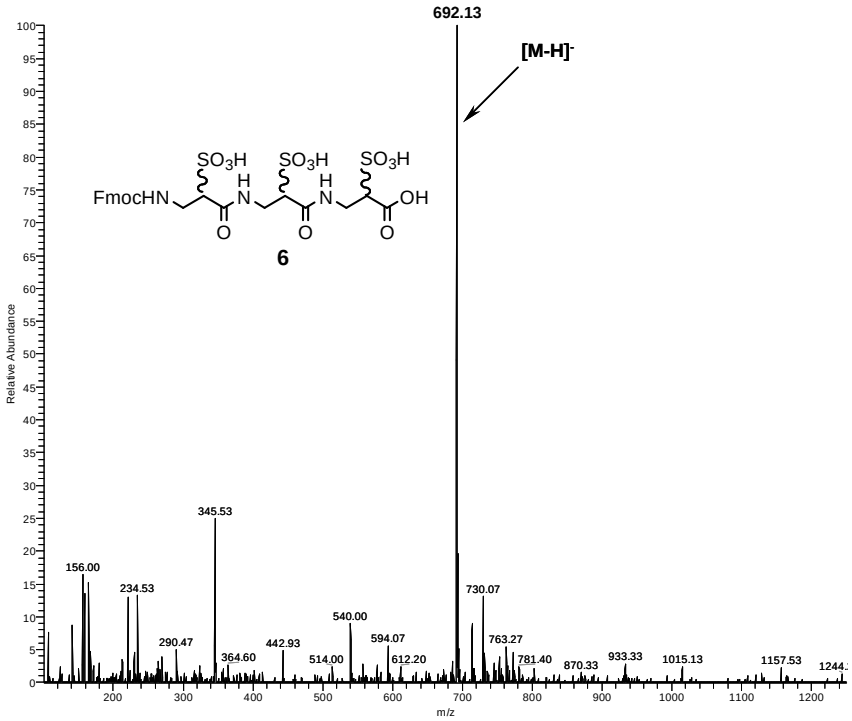
[a]the broad and intense singlet at δ = 7.08 ppm corresponds to trifluoroacetic acid from HPLC eluents not completely removed after lyophilization (confirmed by ^{19}F NMR, singlet at δ = -75.6 ppm (3F, -CF_3)).

^{13}C NMR spectrum of compound 6 recorded in DMSO-d_6.[a]

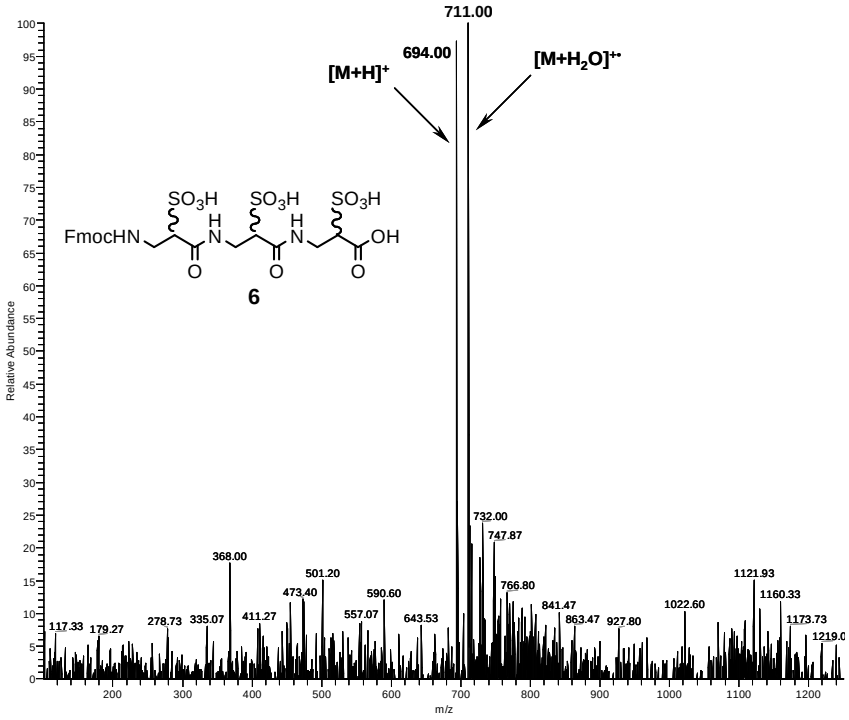


[a]some CH_2 peaks are masked by DMSO and visualized by DEPT135 NMR experiments.

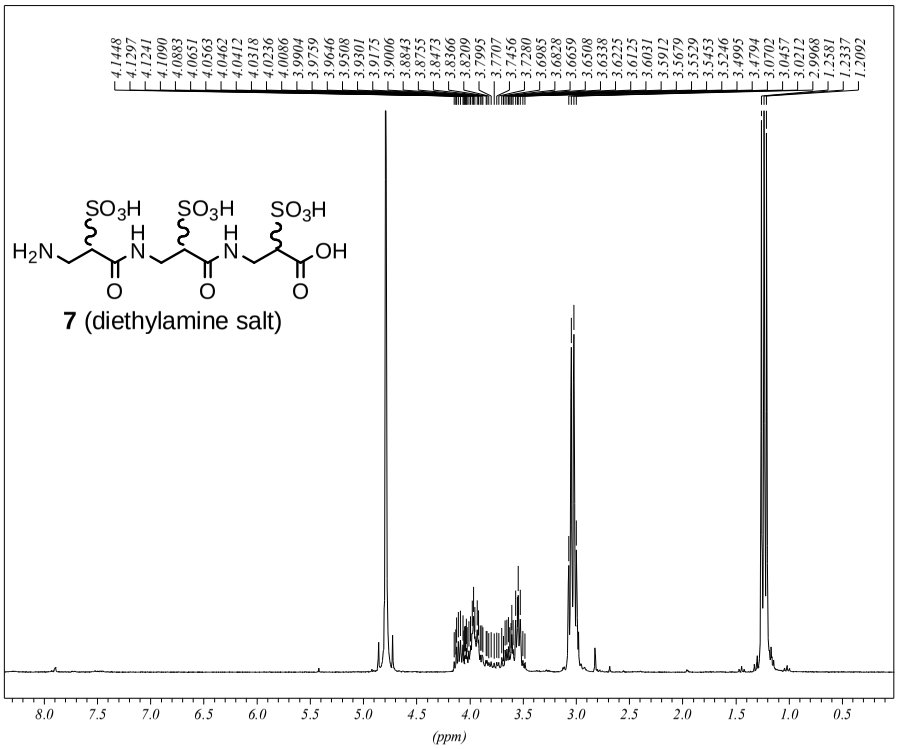
ESI-MS spectrum of compound 6 recorded in the negative mode.



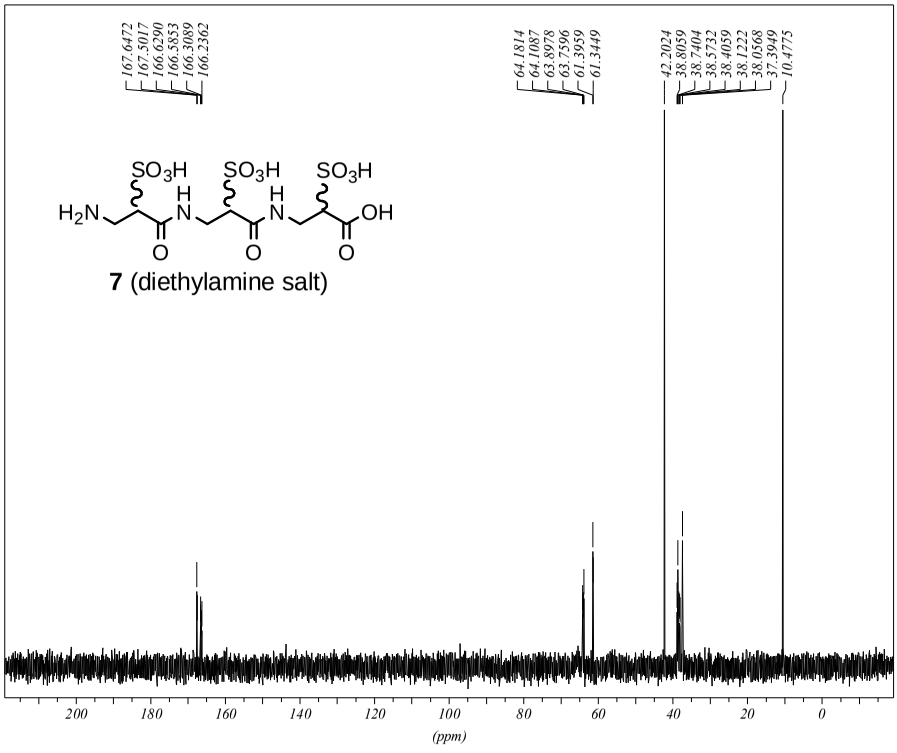
ESI-MS spectrum of compound 6 recorded in the positive mode.



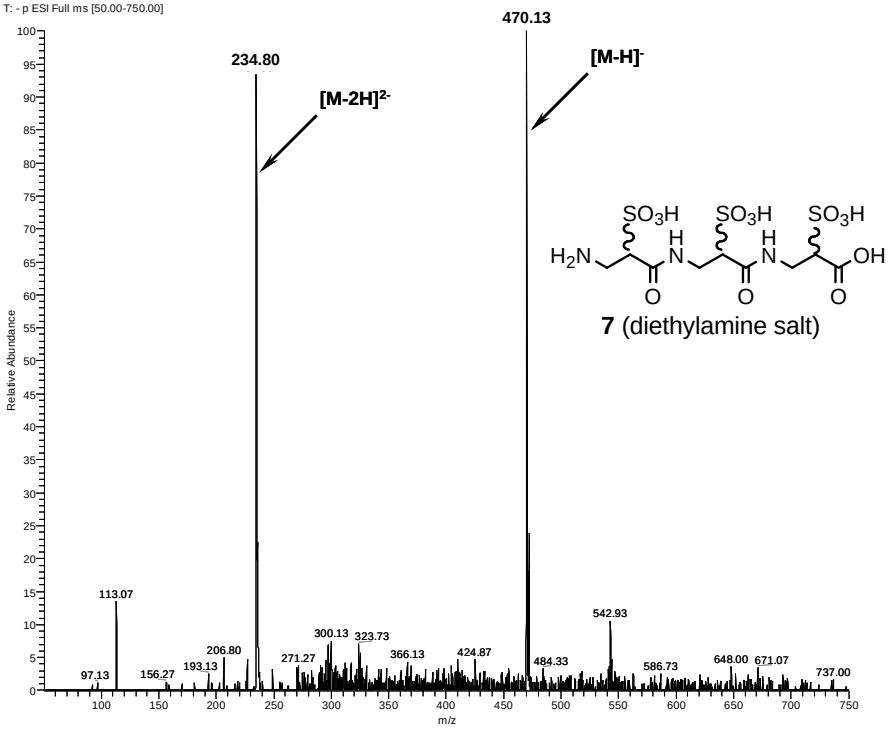
^{1}H NMR spectrum of compound 7 recorded in D_2O.



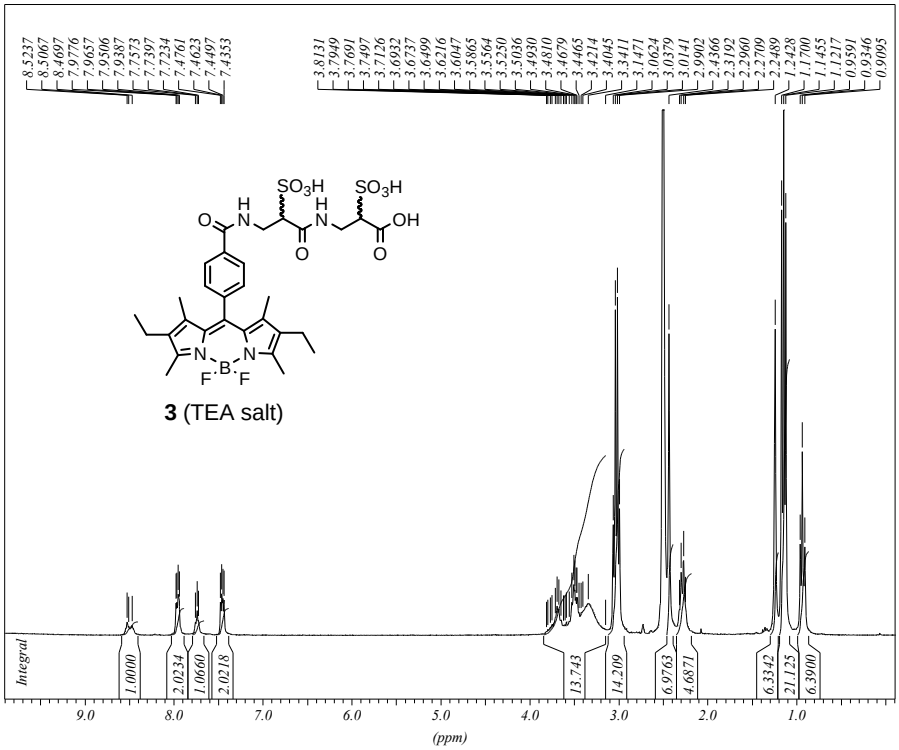
^{13}C NMR spectrum of compound 7 recorded in D_2O.



ESI-MS spectrum of compound 7 recorded in the negative mode.

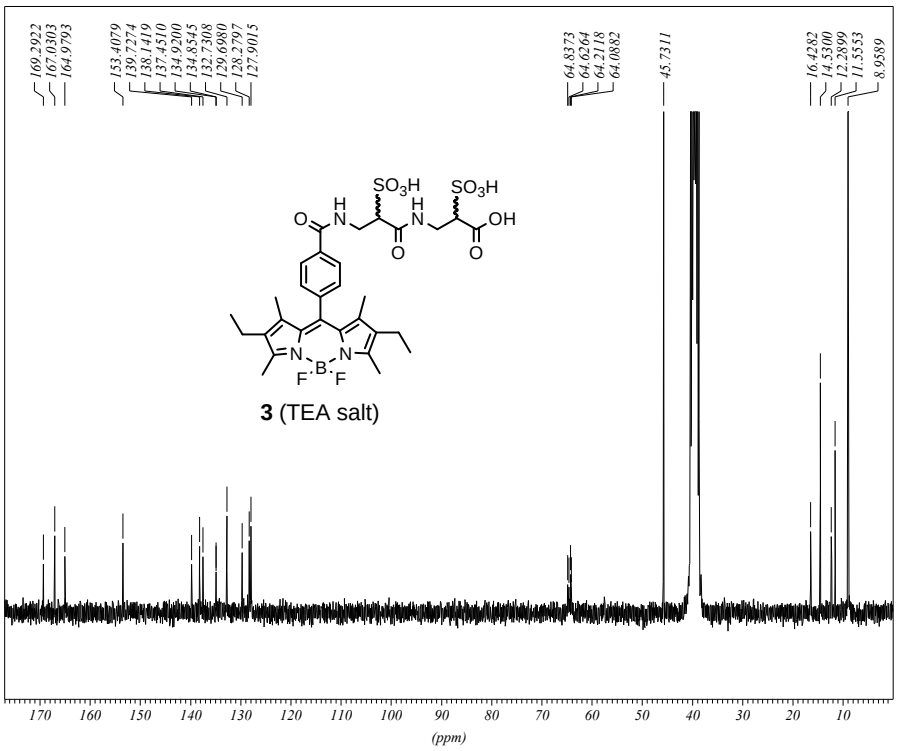


^{1}H NMR spectrum of water-soluble BODIPY 3 recorded in DMSO-d_6.[a]

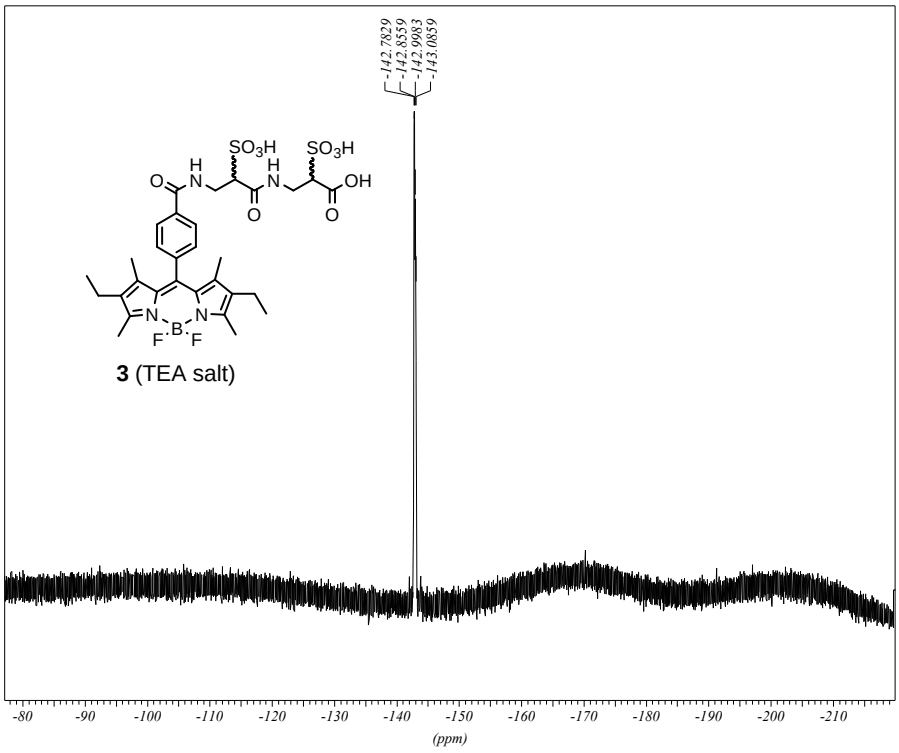


[a]BODIPY dye **3** is completely soluble in D_2O but a bad quality spectrum was obtained (i.e., broad and poor-resolved peaks).

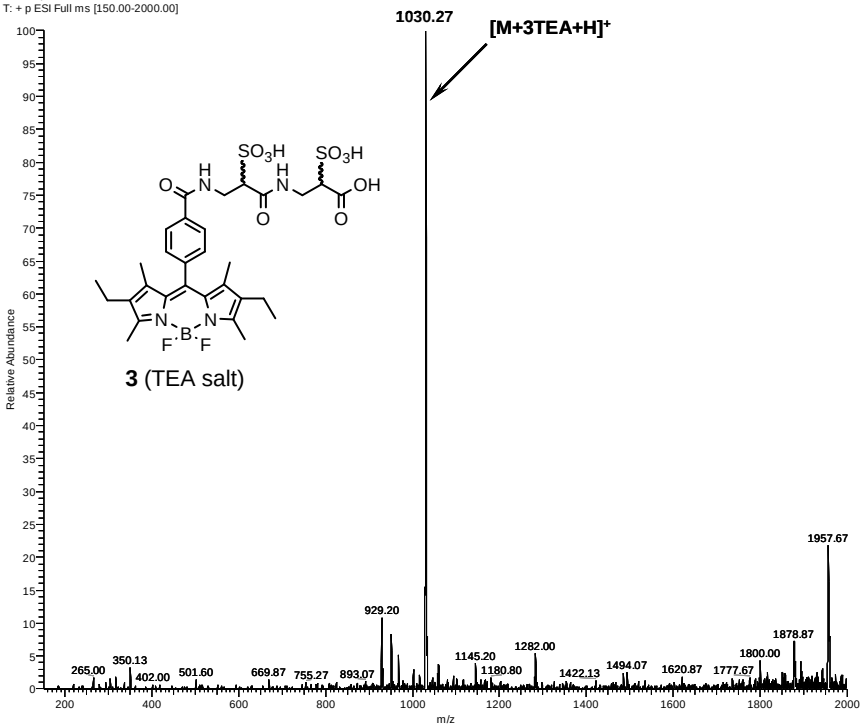
^{13}C NMR spectrum of water-soluble BODIPY 3 recorded in DMSO-d_6.



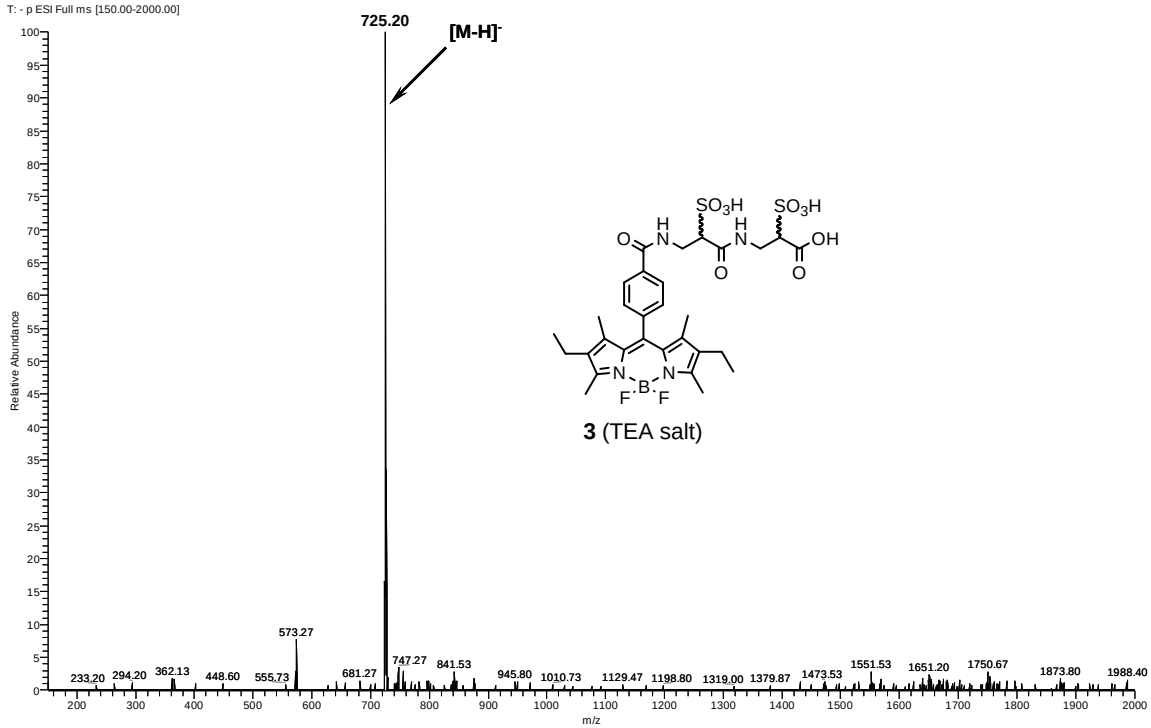
^{19}F NMR spectrum of water-soluble BODIPY 3 recorded in DMSO-d_6.



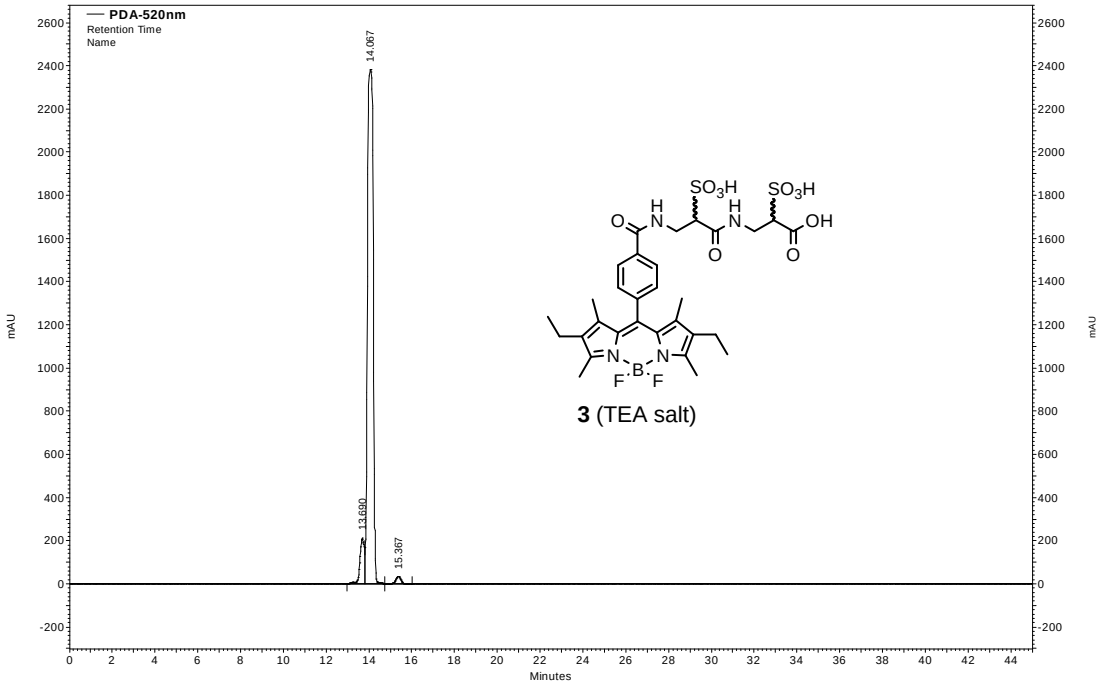
ESI-MS spectrum of water-soluble BODIPY 3 recorded in the positive mode.



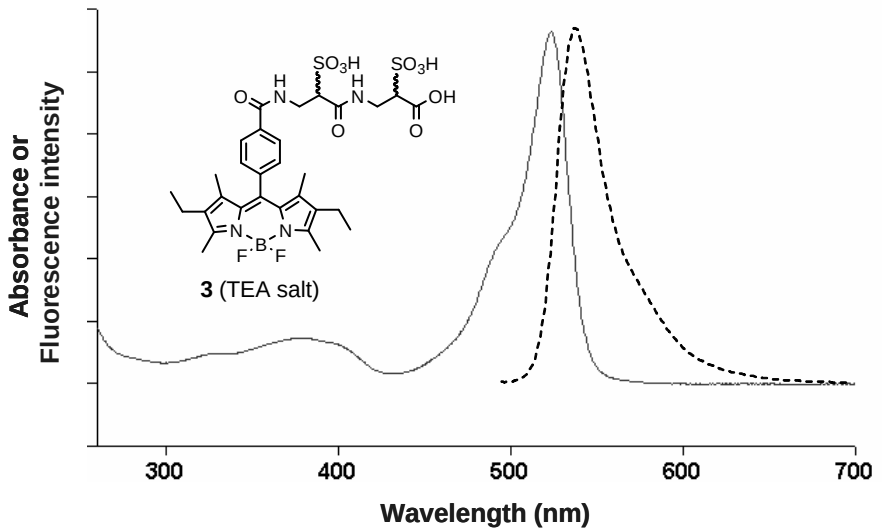
ESI-MS spectrum of water-soluble BODIPY 3 recorded in the negative mode.



RP-HPLC elution profile (system D) of water-soluble BODIPY 3.

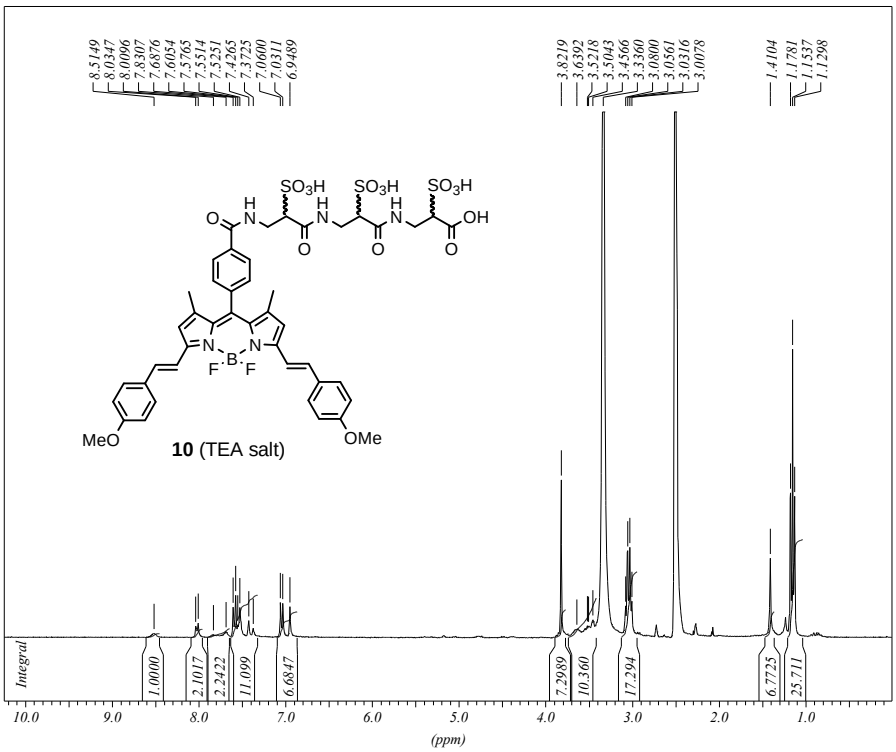


Normalized UV-visible absorption (—) and emission (---) spectra of water-soluble BODIPY 3 (Ex. λ = 488 nm) in PBS at 25 °C.



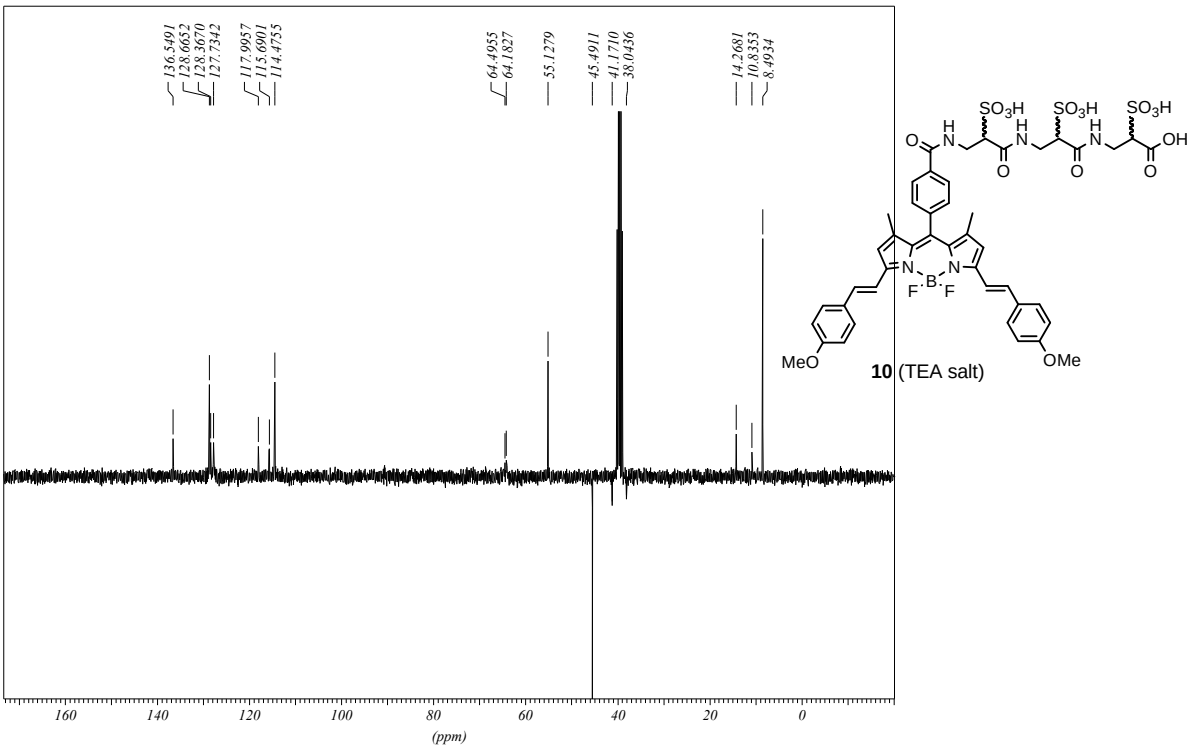
(Abs. λ_{max} = 523 nm & Em. λ_{max} = 539 nm)

^{1}H NMR spectrum of water-soluble BODIPY 10 recorded in DMSO-d_6.[a]

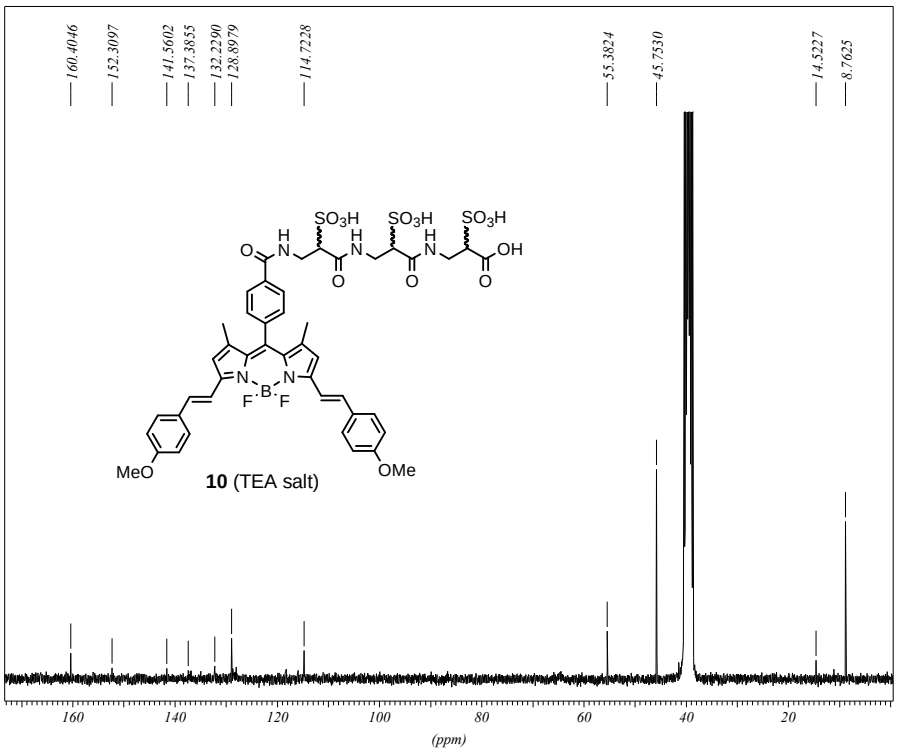


[a]BODIPY dye **10** is completely soluble in D_2O but a bad quality spectrum was obtained (i.e., broad and poor-resolved peaks).

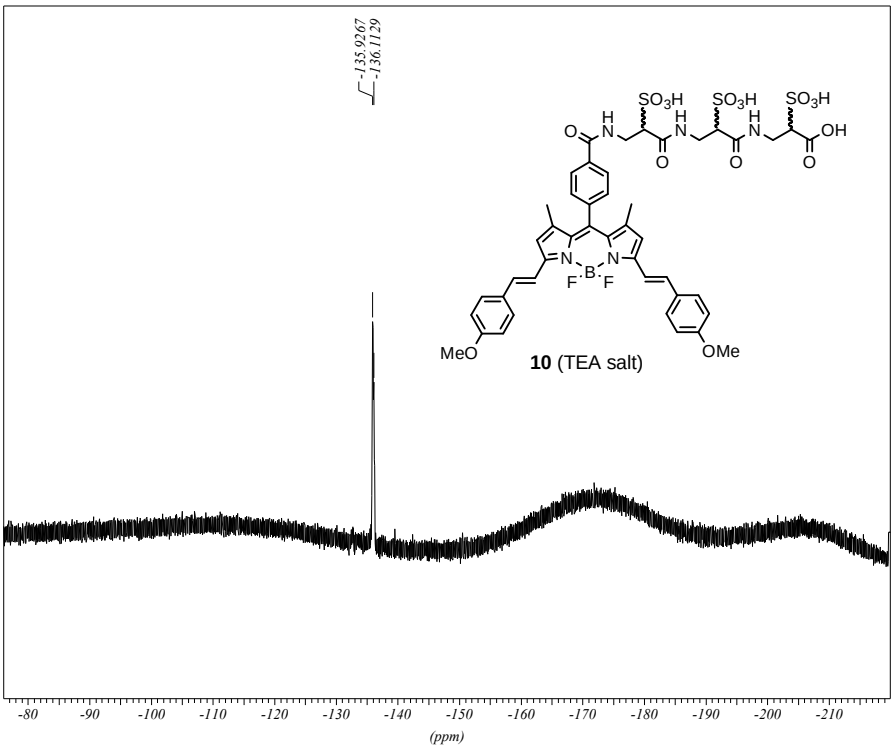
DEPT135 NMR spectrum of water-soluble BODIPY 10 recorded in DMSO-d_6.



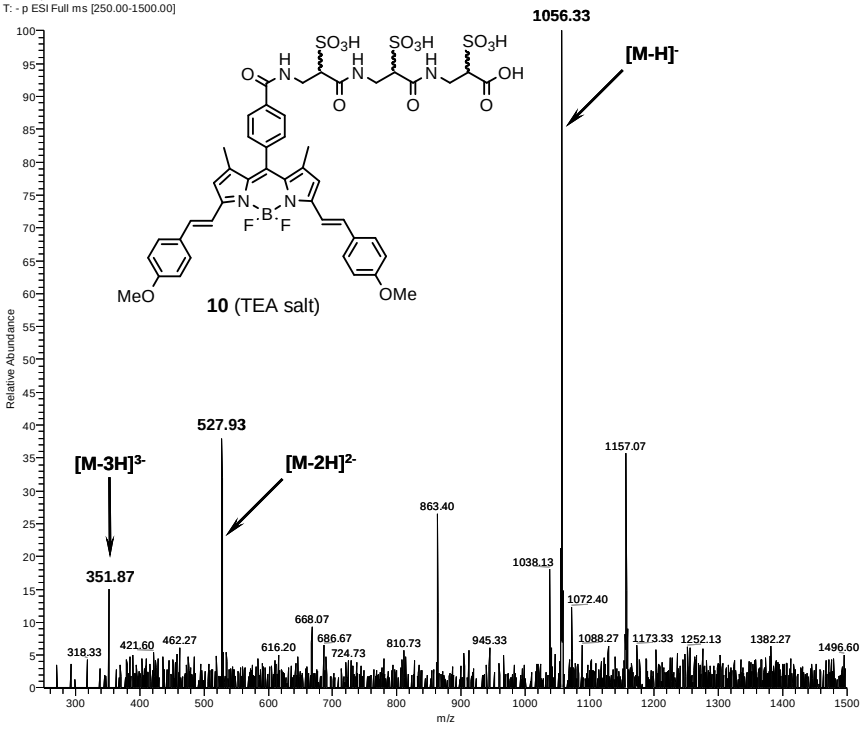
^{13}C NMR spectrum of water-soluble BODIPY 10 recorded in DMSO-d_6.



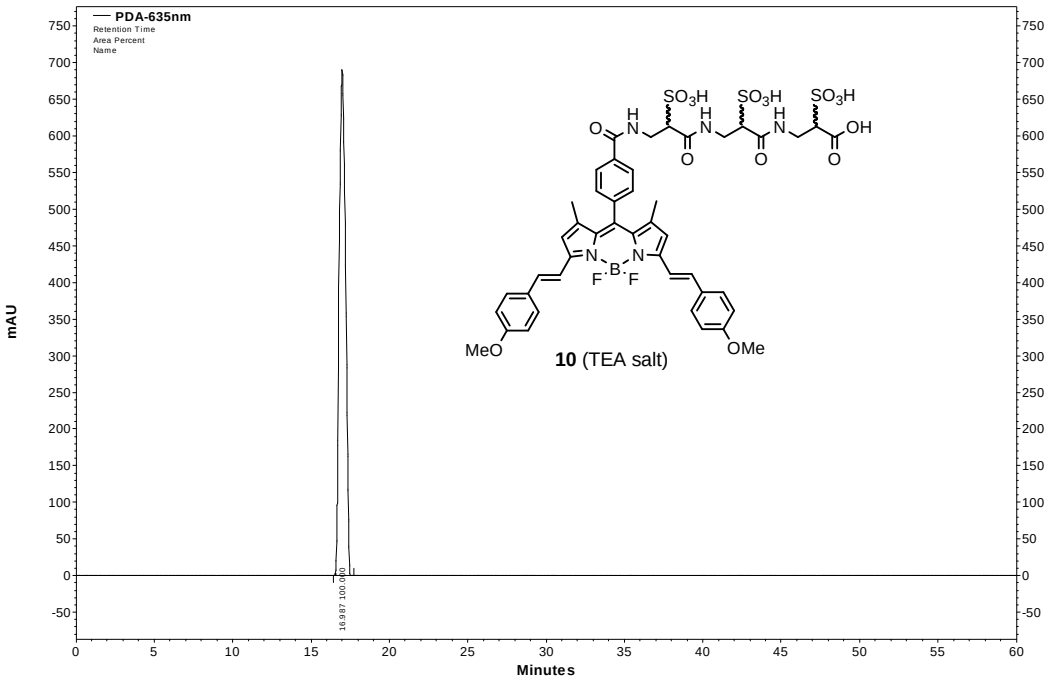
^{19}F NMR spectrum of water-soluble BODIPY 10 recorded in DMSO-d_6.



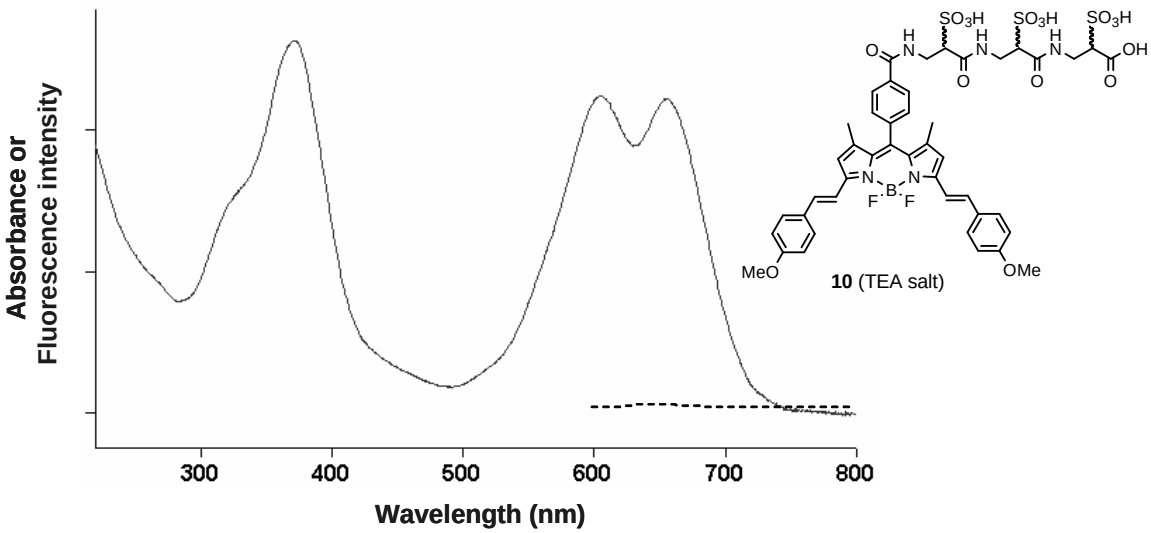
ESI-MS spectrum of water-soluble BODIPY 10 recorded in the negative mode.



RP-HPLC elution profile (system D) of water-soluble BODIPY 10.

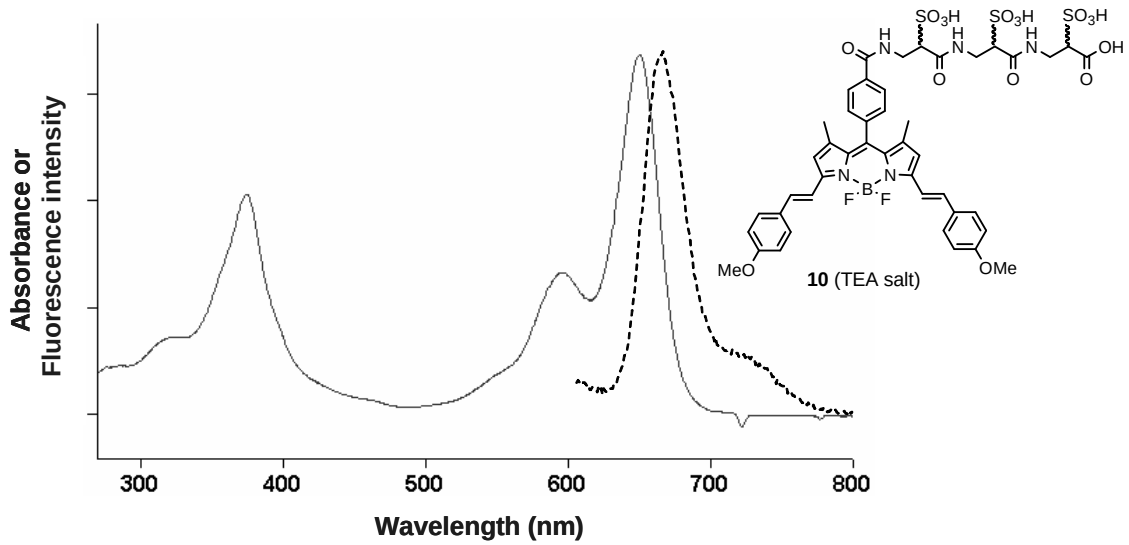


Normalized UV-visible absorption (—) and emission (---) spectra of water-soluble BODIPY 10 (Ex. λ = 605 nm) in PBS at 25 °C.



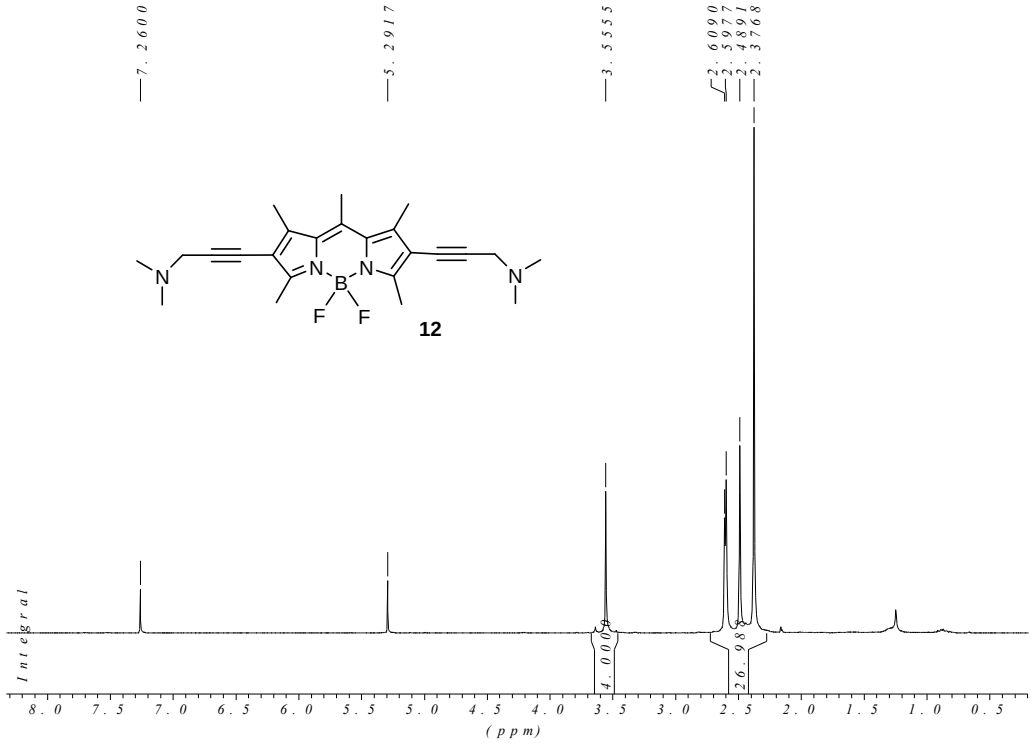
(Abs. λ_{max} = 370, 609, 654 nm & Em. no fluorescence)

Normalized UV-visible absorption (—) and emission (---) spectra of water-soluble BODIPY 10 (Ex. λ = 595 nm) in DMSO at 25 °C.

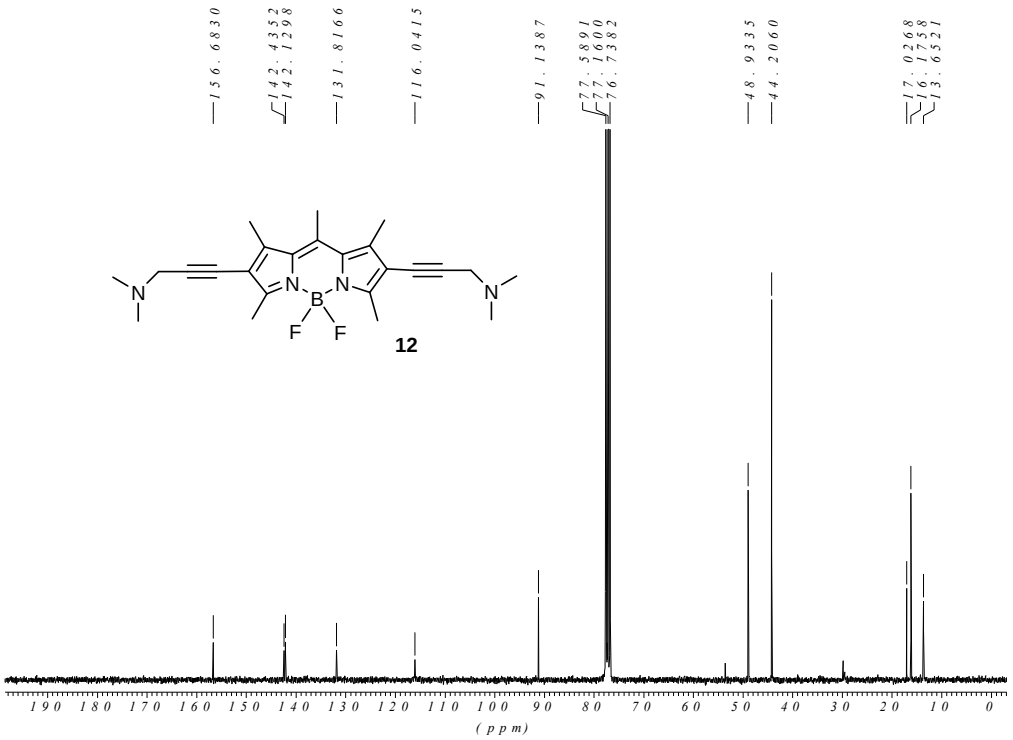


(Abs. λ_{max} = 374, 649 nm & Em. λ_{max} = 666 nm)

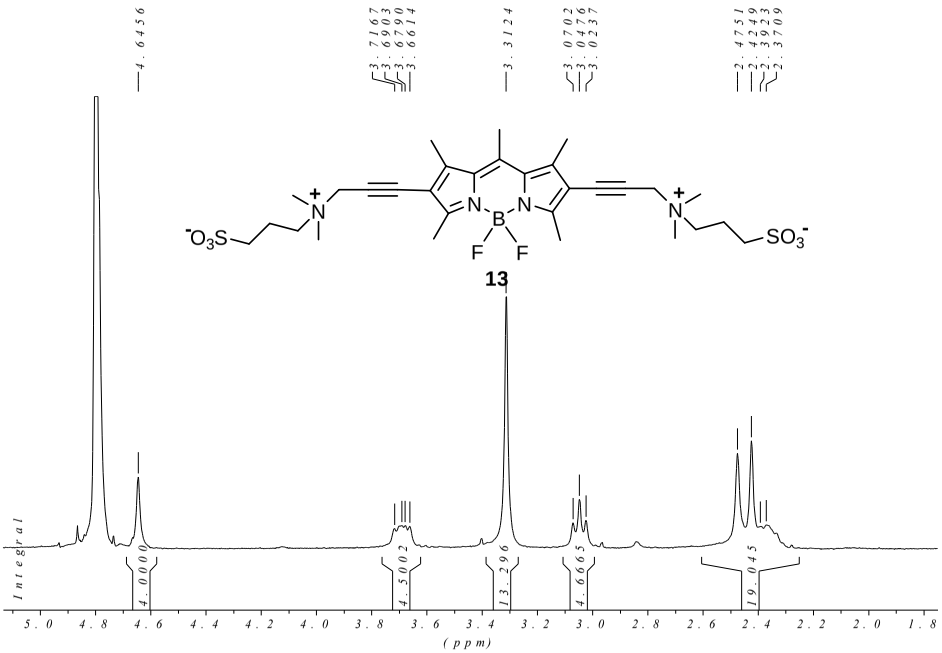
^{1}H NMR spectrum of compound 12 recorded in $CDCl_3$.



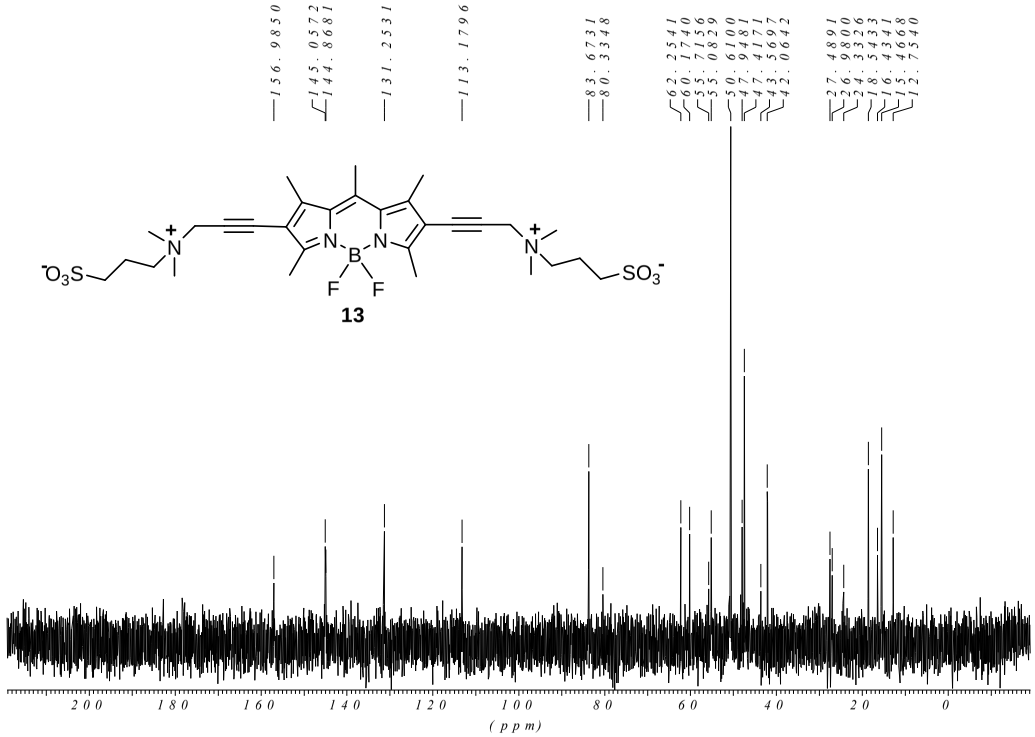
^{13}C NMR spectrum of compound 12 recorded in $CDCl_3$.



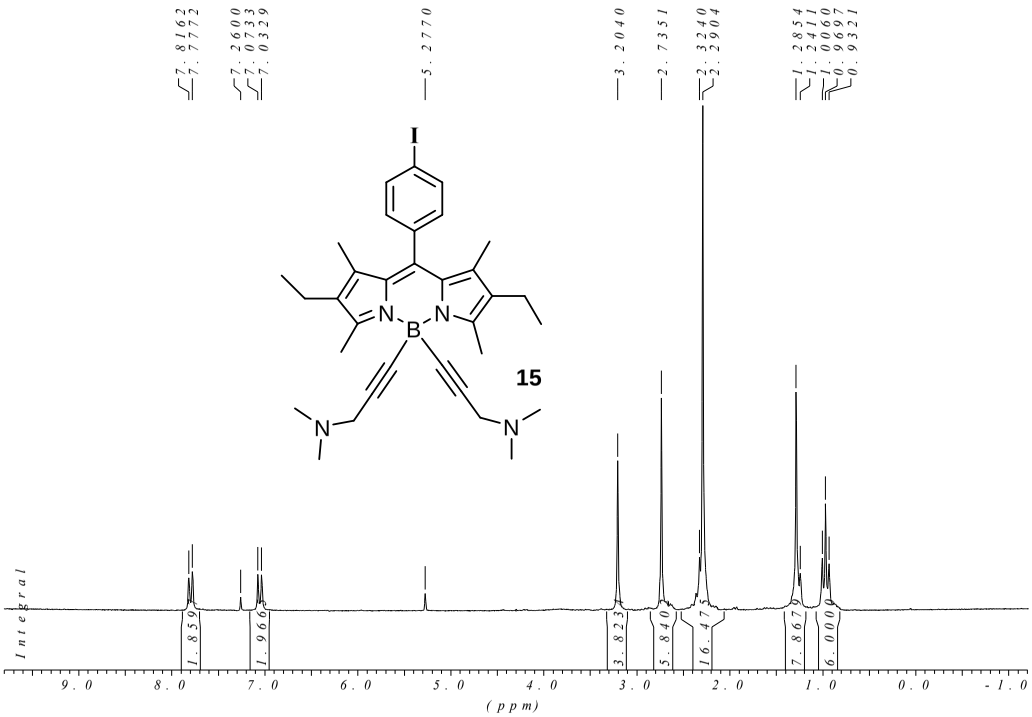
^{1}H NMR spectrum of compound 13 recorded in D_2O.



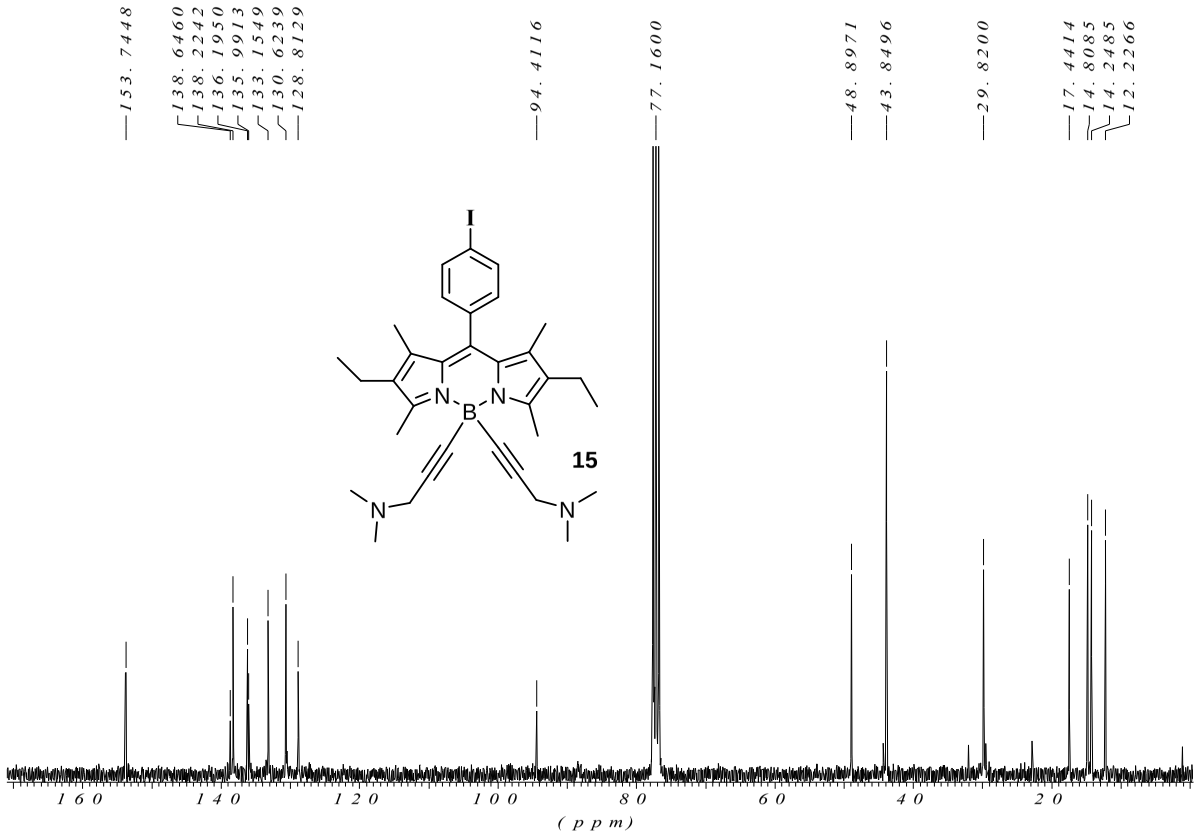
^{13}C NMR spectrum of compound 13 recorded in D_2O.



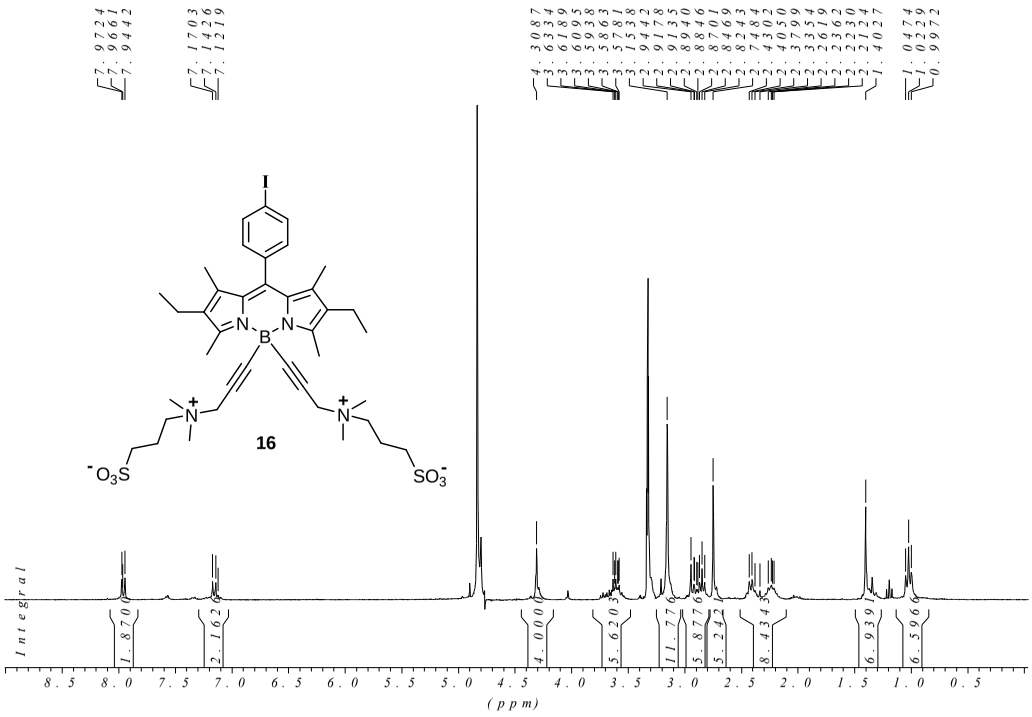
^{1}H NMR spectrum of compound 15 recorded in $CDCl_3$.



^{13}C NMR spectrum of compound 15 recorded in $CDCl_3$.



^{1}H NMR spectrum of compound 16 recorded in CD_3OD.



^{13}C NMR spectrum of compound 16 recorded in CD_3OD.