

Supplementary Information

Negatively charged yellow-emitting 1-aminopyrene dyes for reductive amination and fluorescence detection of glycans

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General experimental information and synthesis

Solvents (p.a. or HPLC grade) were from VWR international (Merck). Oligosaccharides were purchased from Biosynth [2-O-(α -D-mannopyranosyl)-D-mannose, 3-O-(α -D-Mannopyranosyl)-D-mannose, 6'-sialyllactose sodium salt, 3'-sialyllactose sodium salt], Sigma-Aldrich (glucose, mannose, maltotriose, maltoheptaose) and Megazyme [4-O-(α -D-mannopyranosyl)-D-mannose, mannotriose, mannotetraose]. All commercially available substances were used without further purification.

Chromatographic Methods

- *Thin Layer Chromatography*

Normal phase TLC was performed on regular *silica gel 60 F₂₅₄* (Merck Millipore). For TLC on reversed phase (RP), *silica gel 60 RP-18 F_{254s}* (Merck Millipore) was used. Spots of compounds were detected by exposing TLC plates to UV-light (254 or 366 nm).

- *Column Chromatography*

Flash chromatography (normal phase; regular silica gel) was performed on an automated *Isolera™ One* system (Biotage GmbH) or Interchim puriFlash™ flash purification system with commercially available cartridges. For isolation of the phosphorylated dyes, the preparative reversed phase column chromatography was applied (on *POLYGOPREP® 60-50 C₁₈*; Macherey Nagel). For isolation of the dye – sugar conjugates (see Methods A and B below), flash chromatography (RP C₁₈, 15C18AQ-F0025 cartridge, ACN – 20 mM TEAB, pH 8, 0–5% ACN, 15 column volumes) was applied.

- *High-Performance Liquid Chromatography (HPLC)*

Analytical HPLC system: Azura (Knauer) with 10 mL pump-heads and diodes array detection (DAD 6.1L). Analytical column: *Eurospher-100 C₁₈*, 5 μ m, 150x4 mm, 1.2 mL/min (if not stated otherwise), *Kinetex C₁₈ 100*, 5 μ m, 250x4 mm, 1.2 mL/min, or *Interchim Uptisphere C₁₈-HQ*, 10 μ m, 250x4.6 mm, 1.2 mL/min. Solvent A: water + 0.1% v/v trifluoroacetic acid (TFA); solvent B: MeCN + 0.1% v/v TFA (if not stated otherwise). Preparative columns: *Kinetex C₁₈ 100*, 5 μ m, 250x20 mm; Interchim Uptisphere Strategy C₁₈-HQ, 10 μ m, 250x21 mm. For isolation and purification of phosphorylated dyes and

their conjugates, acetonitrile – aqueous systems containing 0.05 – 0.1 M of $\text{Et}_3\text{N}^*\text{H}_2\text{CO}_3$ buffer (pH = 8, self-prepared from 1 M aq. Et_3N and CO_2 gas obtained by evaporation of solid CO_2) were used.

Analytical instruments

- *Absorption Spectroscopy*

Absorption spectra were recorded with a double-beam UV–vis spectrophotometer (*Varian 4000*) in quartz cuvettes with a 1 cm path length. Emission spectra were recorded on a Cary Eclipse fluorescence spectrometer (*Varian*). Fluorescence quantum yields (absolute values) were obtained on a *Quantaaurus-QY Absolute PL* quantum yield spectrometer C11347 (*Quantaaurus QY*). Excited states lifetimes were measured with *Quantaaurus-Tau* device with TDC Unit M12977-01 (Hamamatsu).

- *Nuclear Magnetic Resonance (NMR)*

NMR spectra were recorded at 25 °C with an Agilent 400-MR spectrometer at 400.1 MHz (^1H), 376.4 MHz (^{19}F), 161.9 MHz (^{31}P) and 100.6 MHz (^{13}C) and are reported in ppm. All ^1H spectra are referenced to tetramethylsilane ($\delta = 0$ ppm) using the signals of the residual protons of HDO (4.79 ppm) in D_2O , CHD_2OD (3.31 ppm) in CD_3OD , $\text{CHD}_2\text{COCD}_3$ (2.05 ppm) in $(\text{CD}_3)_2\text{CO}$, CHD_2CN (1.94 ppm) in CD_3CN , DMSO- d_5 (2.50 ppm) in DMSO- d_6 , CHCl_3 (7.26 ppm) in CDCl_3 . Multiplicities of the signals are described as follows: s = singlet, br. s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. Coupling constants (J , $^nJ_{x,y}$) are given in Hz, where n is the number of bonds between the coupled nuclei x and y . ^{13}C NMR spectra were also acquired with an INOVA 500 spectrometer (Bruker). ^{13}C spectra are referenced to tetramethylsilane ($\delta = 0$ ppm) using the signals of the solvent: CD_3CN (1.32 ppm), CD_3OD (49.00 ppm), $(\text{CD}_3)_2\text{CO}$ (29.84 ppm), DMSO- d_6 (39.52 ppm), CDCl_3 (77.36 ppm). If ^{13}C -signals were revealed by indirect detection by HSQC (*Agilent 400MR DD2* spectrometer), only resonances of the protonated carbon atoms were visible.

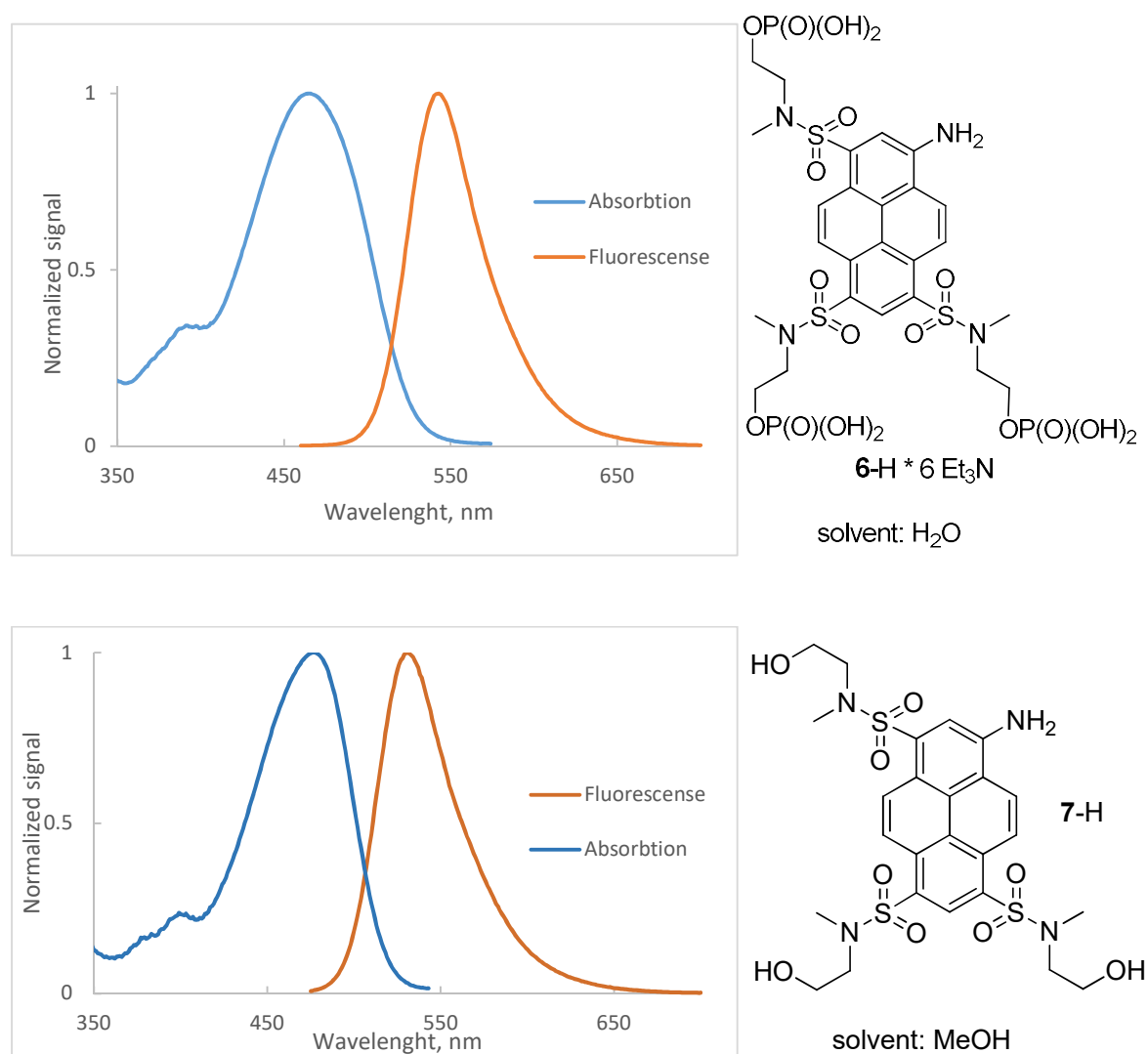
- *Mass-Spectrometry (MS)*

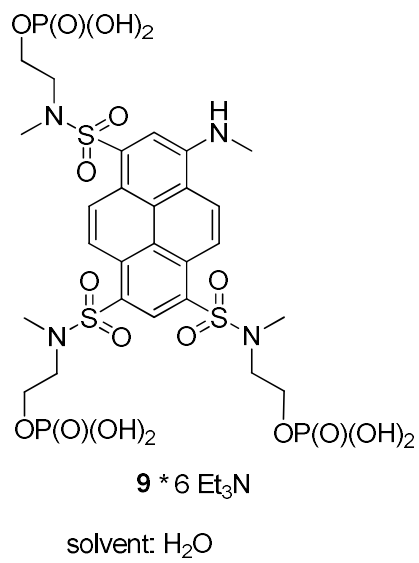
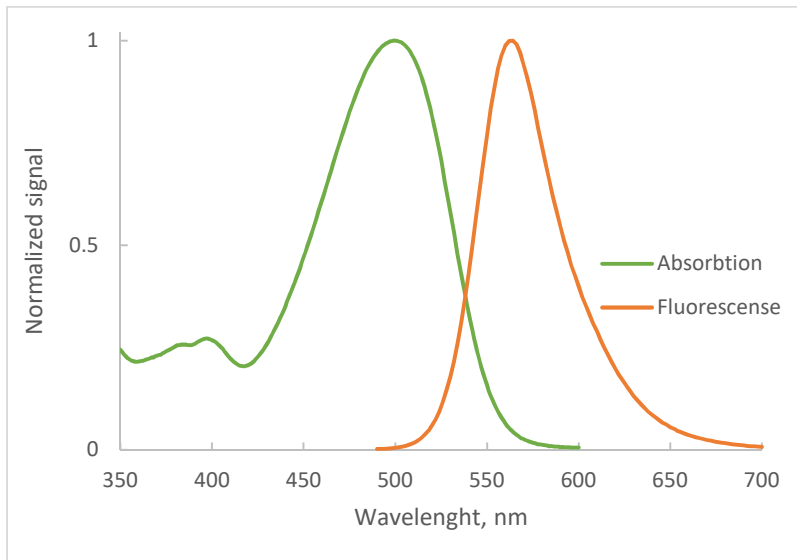
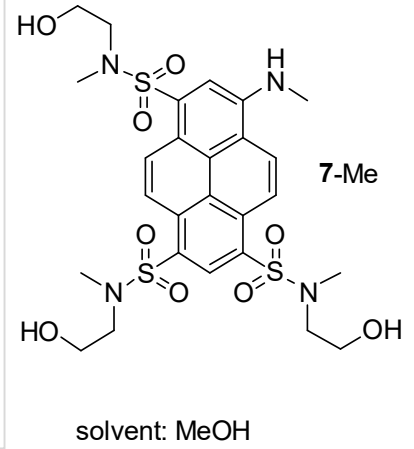
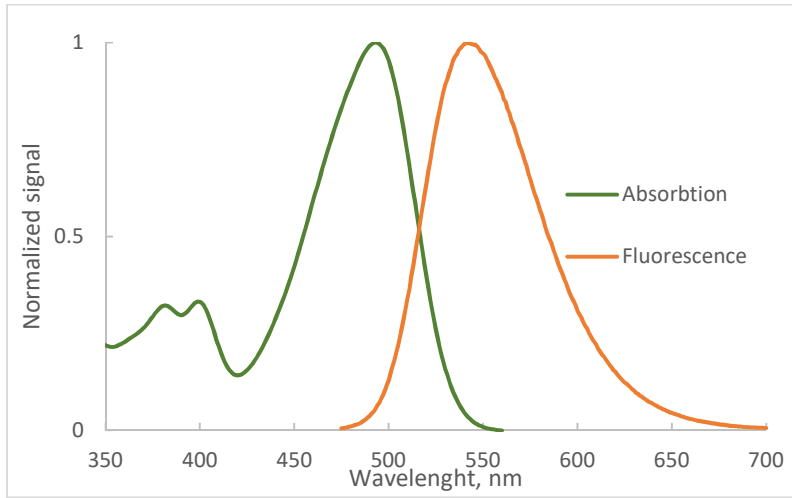
Low resolution mass spectra (50 - 3500 m/z) with electro-spray ionization (ESI) were obtained on a *Varian 500-MS* spectrometer. High resolution mass spectra (ESI-HRMS) were obtained on a *Bruker micro TOF (ESI-TOF-MS)* spectrometer.

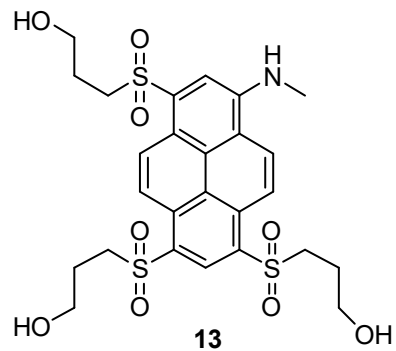
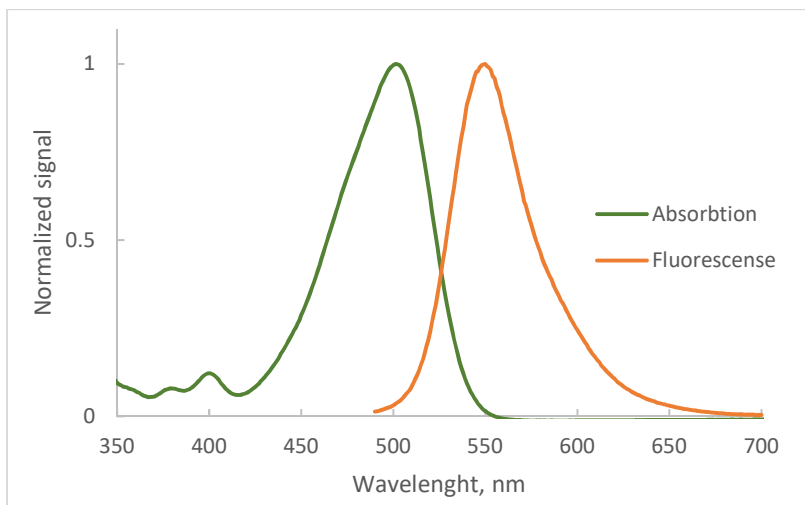
Electrophoresis (general conditions)

Ca. 2 nmol of each dye and its conjugates (1 nmol for mannobioses' isomers) was dissolved in 15 μ L of ultrapure water, mixed with an equal volume of loading solution (80% formamide, 52 mM EDTA, 89 mM Tris, 89 mM boric acid) and separated on a denaturated 20% polyacrylamide gel (7 M urea). The dyes and sugar-dye-conjugates were detected under UV-light of 366 nm. For further details, see section "Reductive amination of sugars".

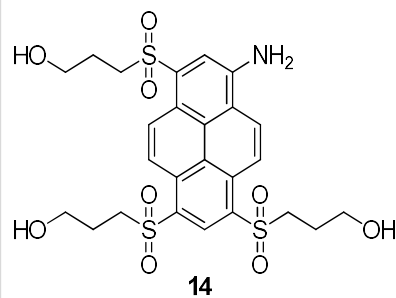
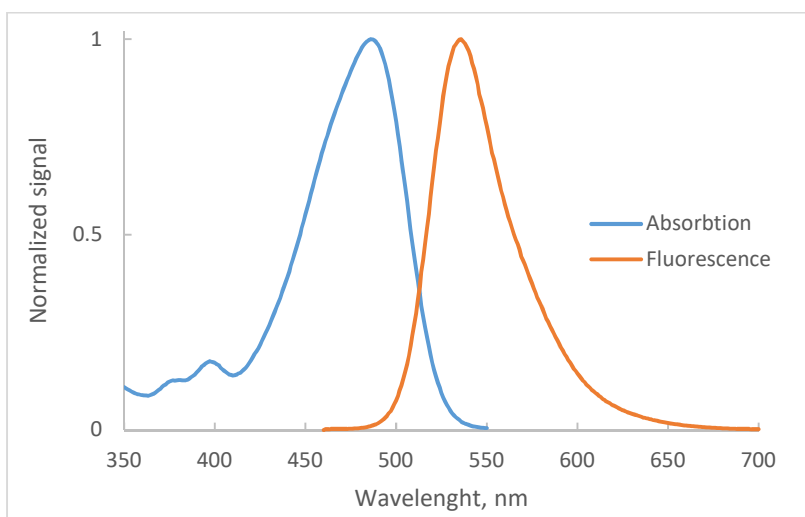
Figure S1. Absorption and emission spectra of pyrene dyes (see also Table 1 in the main text)



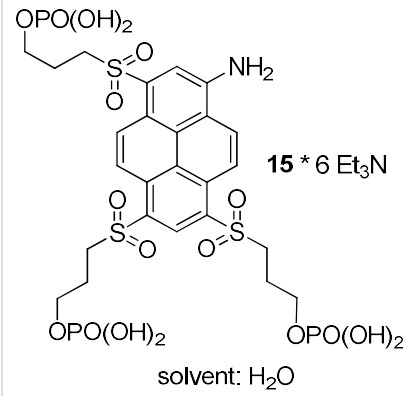
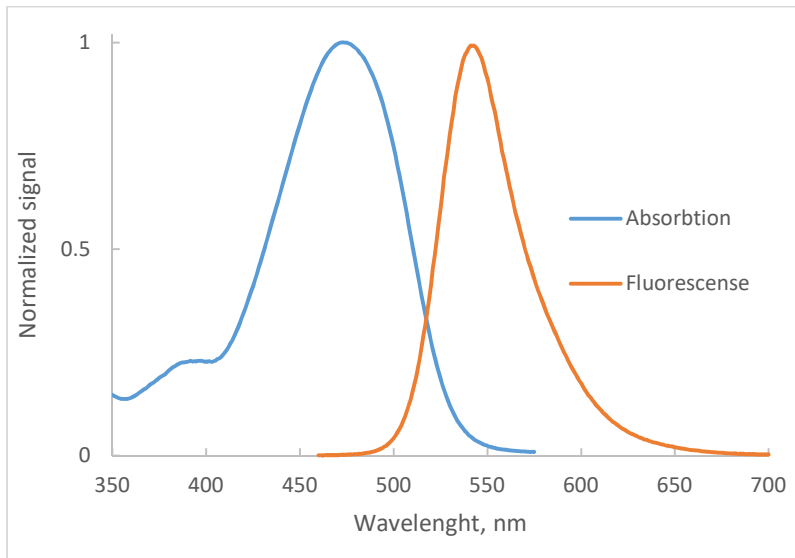




solvent: MeOH

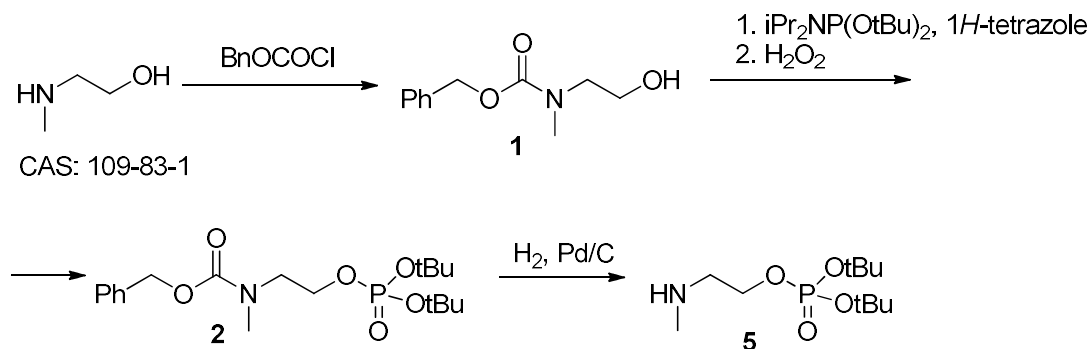


solvent: MeOH



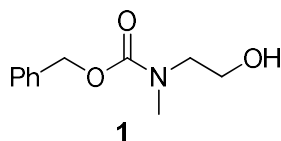
Synthesis of new pyrene dyes

Sulfonamides 6-9



Scheme S1. Synthesis of 2-(methylamino)ethyl di(t-butyl)phosphate

Benzyl N-(2-hydroxyethyl)methylcarbamate¹ (1)



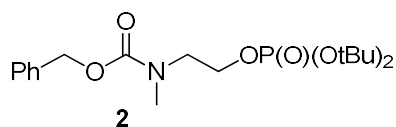
Benzylchloroformate (1.2 eq, 13.7 g, 81 mmol) and triethylamine (11 mL, 81 mmol) were sequentially and dropwise added at 0 °C to a solution of 2-(methylamino)ethanol (5.0 g, 67 mmol) in dry DCM (150 mL). The cooling bath was removed, and the reaction mixture was stirred overnight at r. t. The reaction mixture was washed with water (2 × 50 mL), brine, and the organic solution was dried over MgSO_4 and concentrated in vacuo. TLC (SiO_2): $R_f = 0.25$ (EtOAc/hexane = 1:1). The title compound was isolated by column chromatography on SiO_2 , eluting with 50% EtOAc–hexane to provide 11.2 g of **1** as a white-yellow solid (yield 80%).

^1H NMR (400 MHz, CDCl_3): δ 7.39 – 7.28 (m, 5H), 5.12 (s, 2H), 3.74 (br. s, 2H), 3.44 (t, $J = 5.4$ Hz, 2H), 2.99 (s, 3H) ppm.

^{13}C NMR (101 MHz, CDCl_3): δ 136.7, 128.6, 128.1, 127.9, 67.4, 61.2, 52.0, 35.4 ppm.

¹ D. L. Mohler, G. Shen, *Org. Biomol. Chem.* **2006**, 4, 2082–2087

Benzyl *N*-[[di(*t*-butoxy)phosphoryl]oxy]ethylmethylcarbamate² (**2**)



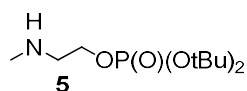
Tetrazole (0.45 M solution in CH₃CN, 45 mL, 20 mmol) and di-*t*-butyl *N,N*-diisopropylphosphoramidite (5.3 mL, 15 mmol) were added to a solution of **1** (2.1 g, 10.0 mmol) in THF (50 mL) under Ar, and the mixture was stirred at r. t. for 3 h. HPLC indicated that the starting material was consumed. The mixture was cooled to 0 °C, and H₂O₂ (70% aqueous, 8.0 mL) was added. After 30 min, an aqueous solution of Na₂SO₃ (10%, 50 mL) was added with cooling in an ice bath. Organic solvents were removed under reduced pressure, and the residue was extracted with EtOAc (3 × 50 mL). The combined organic solutions were washed with brine, dried over MgSO₄, and evaporated. The residue was subjected to column chromatography on SiO₂ (50 g); elution with 50% EtOAc–petroleum ether provided 2.36 g of **2** as a colorless oil (yield 59%).

¹H NMR (400 MHz, CD₃CN) δ 7.41 – 7.26 (m, 4H), 5.09 (s, 2H), 4.04 – 3.93 (m, 2H), 3.54 – 3.45 (m, 2H), 2.94 (d, J = 10.2 Hz, 3H), 1.42 (s, 18H) ppm.

¹³C NMR (101 MHz, CD₃CN) δ 138.3, 129.4, 128.8, 128.7, 83.0, 82.9, 67.6, 67.5, 30.1, 30.0, 29.7, 19.4 ppm.

³¹P NMR (162 MHz, CD₃CN) δ -9.97 ppm.

2-(methylamino)ethyl di(*t*-butyl)phosphate² (**3**)



A Schlenk flask was charged with a stirring bar, Pd/C (10% wt, 0.12 g, VWR International, oxidized form) was added followed by MeOH (10 mL). The flask was closed with a septum, flushed with argon (outlet through the septum via cannula) and then filled with hydrogen supplied from a balloon attached to the side arm of the flask. After stirring for 30 min, a solution of **2** (1.2 g, 3.0 mmol) in MeOH (10 mL) was added to a Schlenk-flask charged with hydrogen-activated Pd/C (10% wt, 0.12 g) suspended in MeOH. The reaction mixture was stirred at r. t. overnight under hydrogen. The reaction mixture was

² R. B. Diebold, T. Gero, P. Grover, S. Huang, S. Ioannidis, C. A. Ogoe, J. C. Saeh, J. G. Varnes, J. Gilbert, PCT Int. Appl. (2012), WO 2012017251 A1 20120209.

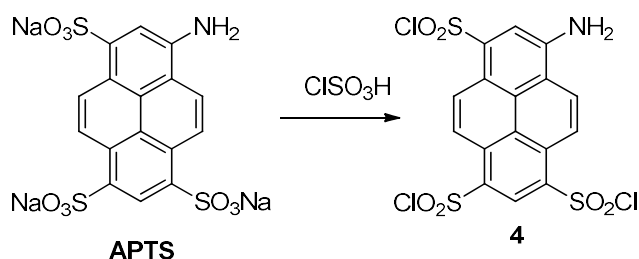
thoroughly flushed with argon (with stirring) and transferred into centrifugation tubes. The catalyst was removed by centrifugation, washed with MeOH, and the combined organic solutions were evaporated in vacuo to afford 800 mg of **5** (84% yield) as a colorless oil.

^1H NMR (400 MHz, CD_3CN) δ 4.03 – 3.95 (m, 2H), 2.83 – 2.77 (m, 2H), 2.40 (s, 3H), 1.45 (s, 18H) ppm.

^{13}C NMR (101 MHz, CD_3CN) δ 83.1, 83.0, 66.3, 66.2, 51.6, 51.5, 35.8, 30.10, 30.06 ppm.

^{31}P NMR (162 MHz, CD_3CN) δ -9.74 ppm.

8-Amino-1,3,6-pyrenetrisulfonyl trichloride (**4**)



Trisodium salt of 8-aminopyrene-1,3,6-trisulfonic acid (**APTS**)³ (446 mg, 0.98 mmol) was introduced into a 10 mL flask, cooled to 0 °C in an ice bath, and then chlorosulfonic acid (8.5 mL, 0.12 mol) was added dropwise with stirring. The reaction mixture was stirred at r. t. for 4 h, cooled to 0 °C, and carefully transferred onto crushed ice (100 g). The red precipitate of trisulfonyl chloride was washed with ice water (3 × 50 mL) and centrifuged. The crude compound was freeze-dried to afford 437 mg of **4** (87% yield) as a dark-red powder. The flask was purged with Ar and kept in the freezer (-20 °C).

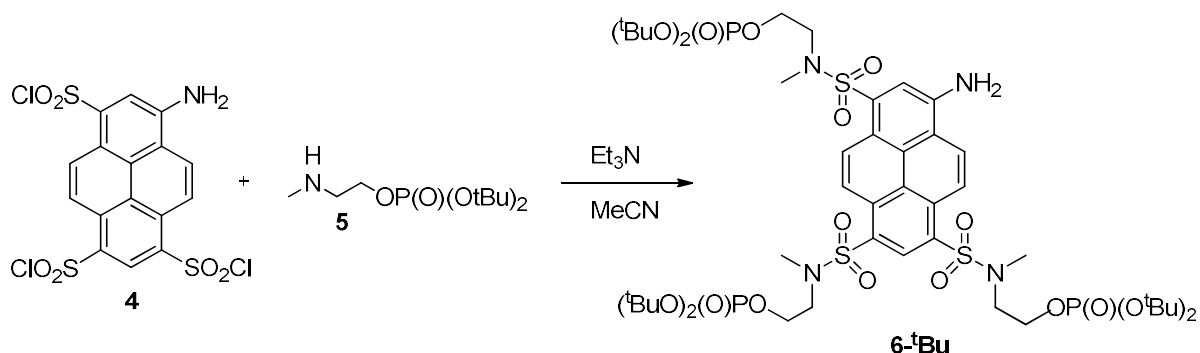
^1H NMR (400 MHz, CD_3CN) δ 9.26 (d, J = 9.8 Hz, 1H), 9.20 (s, 1H), 8.85 (d, J = 9.7, 2.7 Hz, 2H), 8.60 (d, J = 9.7 Hz, 1H), 8.30 (s, 1H) ppm.

^{13}C NMR (101 MHz, CD_3CN) δ 148.9, 142.0, 134.5, 132.8, 132.2, 131.7, 130.0, 129.0, 128.9, 125.6, 121.7, 119.4, 117.5, 116.7 ppm.

$\text{C}_{16}\text{H}_8\text{Cl}_3\text{NO}_6\text{S}_3$, M = 510.9 for $^{35}\text{Cl} \times 3$. EI-MS: m/z = 510.9 [M^+]

³ Z. Sharett, S. Gamsey, L. Hirayama, B. Vilozny, J. T. Suri, R. A. Wessling, B. Singaram, *Org. Biomol. Chem.* **2009**, *7*, 1461–1470.

8-Amino-[[N,N',N''-tris(2-hydroxyethyl)-N,N',N''-trimethyl]pyrene-1,3,6-trisulfonamide]-O,O',O''-triphosphate hexakis *tert*-butyl ester (6-*t*Bu)



8-aminopyrene-1,3,6-trisulfonyl trichloride **4** (52 mg, 0.10 mmol) was added to a solution of 2-(methylamino)ethyl di(*t*-butyl)phosphate **5** (0.6 mmol, 160 mg) and triethylamine (0.14 mL, 1.0 mmol) in acetonitrile (2 mL) under Ar. The reaction mixture was stirred at r. t. overnight and concentrated in vacuo. The title compound was isolated by flash chromatography on SiO₂ (SNAP “Ultra” cartridge with 50 g SiO₂, DCM/MeOH with 2-20% MeOH gradient over 13 CV) to afford 96 mg of **6-*t*Bu** (80% yield) as a brown-orange solid.

¹H NMR (400 MHz, CD₃OD) δ 9.18 (d, J = 9.8 Hz, 1H), 9.06 (s, 1H), 8.95 (d, J = 9.7 Hz, 1H), 8.79 (d, J = 9.8 Hz, 1H), 8.68 (d, J = 9.7 Hz, 1H), 8.13 (s, 1H), 4.13 – 4.08 (m, 6H), 3.63 – 3.55 (m, 6H), 3.05 (s, 3H), 3.01 (s, 3H), 3.00 (s, 3H), 1.51 (d, J = 0.6 Hz, 18H).

¹³C NMR (101 MHz, CD₃OD) δ 149.1, 138.1, 134.8, 133.8, 131.1, 130.2, 129.3, 128.8, 128.7, 128.0, 127.5, 123.5, 120.6, 118.3, 117.6, 117.0, 85.0, 84.9, 84.8, 84.7, 66.5, 66.4, 66.3, 66.2, 66.1, 51.1, 51.0, 50.9, 50.8, 50.7, 36.5, 36.3, 36.2, 30.7, 30.6, 30.2, 30.1.

³¹P NMR (162 MHz, CD₃OD) δ -10.22, -10.29, -10.37.

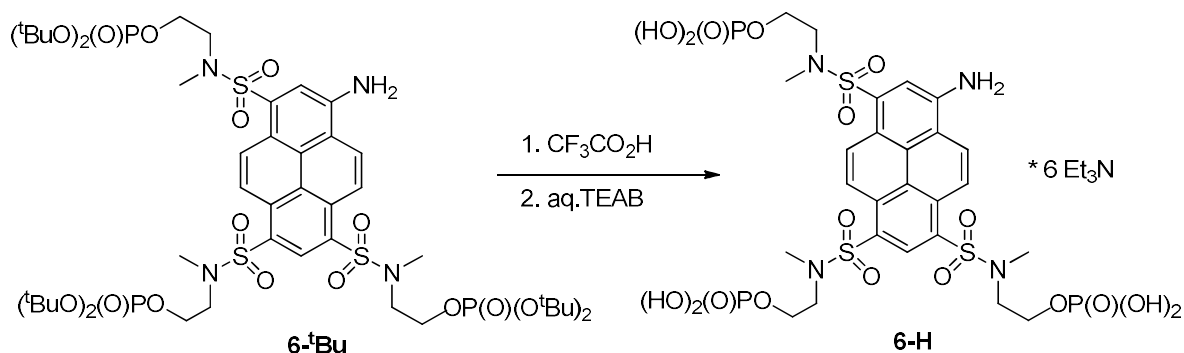
ESI-HRMS: found 1227.3970 [M+Na]⁺, calculated 1227.3979.

8-Amino-[N,N',N''-tris(2-hydroxyethyl)-N,N',N''-trimethylpyrene-1,3,6-trisulfonamide]-O,O',O''-triphosphate triethylammonium salt (6-H).

Method C:

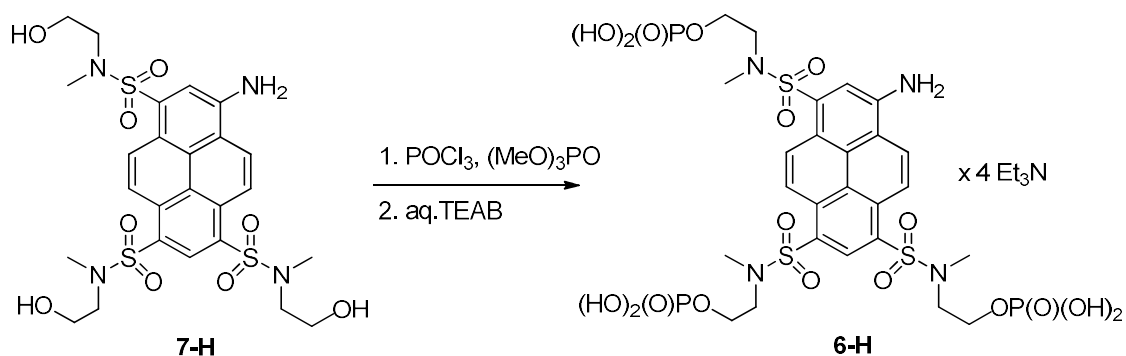
Ester **6-*t*Bu** (96 mg, 80 μmol) was dissolved in dichloromethane (1.0 mL), the solution cooled to +5°C in an ice bath, and then TFA (1.0 mL) was added slowly with stirring. The reaction mixture was allowed to warm-up to r. t. and stirred for 1 h. The volatile materials were removed in vacuo, the residue was co-evaporated with dichloromethane (3 times)

and kept in vacuo (0.1 mbar) for 2 h. The residue was treated and stirred with 1 M aq. $\text{Et}_3\text{N}\cdot\text{H}_2\text{CO}_3$ buffer (TEAB; pH = 8-9), until the pH stabilized at 8-9. The title compound was isolated by preparative HPLC (see below in Method D). Yield – 89 mg (82%) of the title compound (6-H) as hexakis triethylammonium salt.



Method D:

Dye 7-H (19 mg, 30 μmol) was dissolved in $(\text{MeO})_3\text{PO}$ (0.1 mL), and freshly distilled POCl_3 (131 μL , 1.4 mmol) in $(\text{MeO})_3\text{PO}$ (0.1 mL) was added with stirring at r. t. A weak exothermic reaction was observed, and the solution turned orange-brown. The reaction mixture was stirred for 3 h at r. t. All volatile components were removed in vacuo, and the residue was further dried by in vacuum (0.02 mbar). The residue was treated and stirred with 1 M aq. TEAB buffer (pH = 8-9). Fresh portions of the TEAB buffer were added, when the solution became acidic, until the pH stabilized at 8-9. The title compound was isolated by preparative HPLC using 0.1 M TEAB buffer (pH 8.5–9) as an eluent and a Kinetex column. Analytical HPLC: Kinetex, 5 μm C18 100, 25 cm, 4.6 mm, ACN/ H_2O : 10/90 – 30/70 in 20 min, 1.2 mL/min; t_R = 10.8 min. Freeze-drying of the eluate gave 5 mg of tetra-triethylammonium salt of the title compound as orange crystals. Yield 16%.



^1H NMR (400 MHz, D_2O) δ 8.76 (s, 1H), 8.69 (d, J = 9.7 Hz, 1H), 8.36 (d, J = 9.6 Hz, 1H), 8.30 (d, J = 9.4 Hz, 1H), 8.07 (d, J = 9.6 Hz, 1H), 7.85 (s, 1H), 4.05 – 3.95 (m, 6H), 3.62 – 3.48 (m, 6H), 3.15 (q, J = 7.3 Hz, 24H, Et_3N), 2.99 (s, 3H), 2.96 (s, 3H), 2.92 (s, 3H), 1.24 (t, J = 7.3 Hz, 36H, Et_3N) ppm.

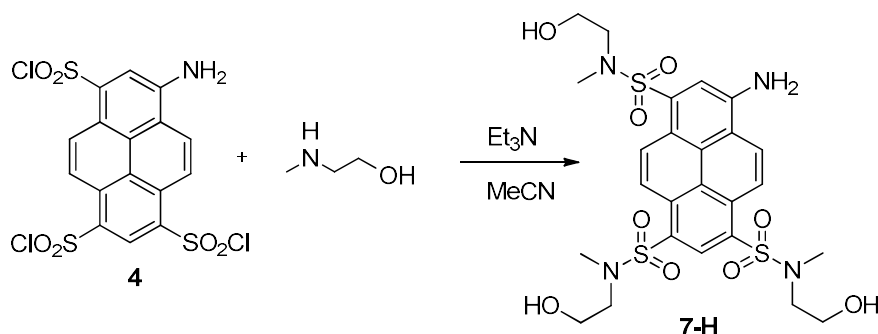
^{13}C NMR (101 MHz, D_2O) δ = 146.6, 135.6, 133.0, 132.0, 129.3, 128.7, 126.9, 126.4, 126.3, 126.2, 125.5, 122.1, 119.6, 117.0, 116.5, 115.6, 62.2, 62.0, 50.7, 50.4, 46.6, 35.6, 35.3, 35.1, 8.2 ppm.

^{31}P NMR (162 MHz, D_2O) δ = 1.1, 0.96, 0.91 ppm.

ESI-HRMS: found 867.0246 [M-H] $^-$; calculated 867.0249.

λ_{max} (absorption) = 465 nm (H_2O), λ_{max} (emission) = 544 (H_2O); fluorescence lifetime 5.9 ns (in H_2O ; excitation at 430 nm); fluorescence quantum yield: 0.88 (H_2O , standard: Coumarin 153 with emission efficiency of 0.54 in ethanol, excitation at 400 nm), 0.88 (absolute value in 0.05 M aq $\text{Et}_3\text{N}^+\text{H}_2\text{CO}_3$ buffer, pH 8; excitation at 460 nm).

8-Amino-[N,N',N'' -tris(2-hydroxyethyl)- N,N',N'' -trimethyl]pyrene-1,3,6-trisulfonamide (7-H).



8-aminopyrene-1,3,6-trisulfonyl trichloride **4** (462 mg, 0.90 mmol) was added to a stirred solution of *N*-(methylamino)ethanolamine (1.0 g, 13 mmol) in aqueous acetonitrile (1:1, 25 mL) at 0 °C. The reaction mixture was vigorously stirred at room temperature, until it became homogeneous, and then lyophilized. The title compound was isolated by chromatography on SiO_2 (100 g) with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (80:18:2) mixture as an eluent. Yield – 252 mg (45%) of a brown-orange solid obtained after two purification steps by chromatography. HPLC: t_{R} = 15.8 min, ACN/ H_2O : 20/80 – 50/50 in 25 min, 1.2 mL/min, 254 nm.

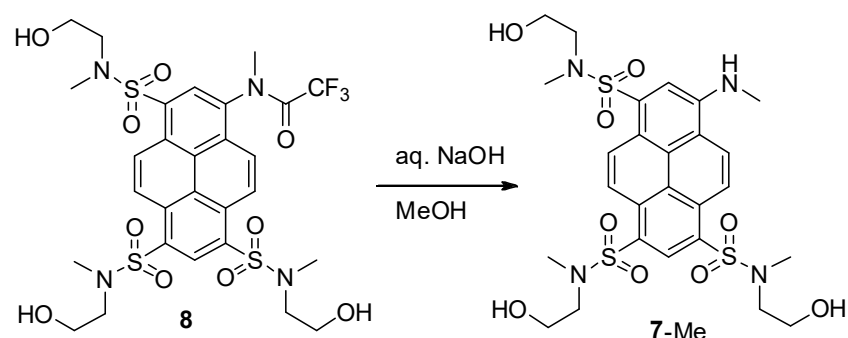
^1H NMR (400 MHz, DMSO-*d*6) δ 9.01 (d, J = 9.7 Hz, 1H), 8.86 (s, 1H), 8.79 (d, J = 9.7 Hz, 1H), 8.75 (d, J = 9.7 Hz, 1H), 8.64 (d, J = 9.7 Hz, 1H), 8.06 (s, 1H), 7.58 (br. s, 2H, NH₂), 4.76 (s, 3H, OH), 3.55 – 3.45 (m, 6H), 3.29 – 3.21 (m, 6H), 2.90 (s, 3H), 2.86 (s, 3H), 2.85 (s, 3H) ppm.

^{13}C NMR (101 MHz, DMSO-*d*6) δ 147.9, 136.5, 132.9, 131.8, 129.4, 128.6, 127.6, 126.9, 126.8, 126.7, 126.2, 121.5, 118.9, 115.9, 115.6, 115.4, 59.2, 59.0, 58.9, 51.7, 51.5, 51.4, 35.5, 35.2, 35.1 ppm.

ESI-HRMS: found 651.1227 [M+Na]⁺, calculated 651.1224.

λ_{max} (absorption) = 477 nm (ϵ = 22 400 M⁻¹ cm⁻¹, MeOH), λ_{max} (emission) = 535 nm (MeOH, excitation at 470 nm); fluorescence lifetime 5.6 ns (MeOH); fluorescence quantum yield (0.96; absolute value in MeOH).

8-Methylamino-*N,N',N''*-tris(2-hydroxyethyl)-*N,N',N''*-trimethylpyrene-1,3,6-trisulfonamide (7-Me)



The title compound was obtained from trifluoroacetate **8** (25 mg, 34 μmol) upon treatment with 1 M aq. NaOH diluted with methanol (ca. 1:1), so that the reaction mixture remained homogeneous. The course of the reaction was monitored by TLC on regular SiO₂ (for solvent systems, see below). The product was isolated as an orange solid (20 mg, 95% yield) by chromatography on SiO₂ using a 15:1 mixture of DCM and methanol as an eluent.

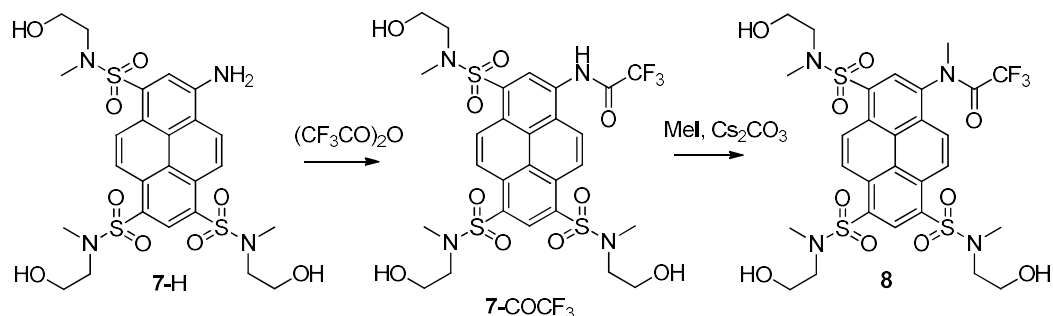
^1H NMR (400 MHz, CD₃CN) δ = 9.17 (d, J = 9.8 Hz, 1H), 9.01 (d, 2H), 9.00 (s, 1H), 8.80 (d, J = 9.8 Hz, 1H), 8.52 (d, J = 9.7 Hz, 1H), 7.92 (s, 1H), 3.68 – 3.56 (m, 6H), 3.40 – 3.31 (m, 6H), 3.19 (d, J = 4.8 Hz, 3H), 2.97 (s, 3H), 2.94 (s, 3H), 2.94 (s, 2H).

^{13}C NMR (126 MHz, DMSO-*d*6) δ = 147.0, 137.0, 132.4, 132.0, 128.8, 128.6, 127.7, 127.2, 126.8, 125.9, 125.8, 122.0, 119.0, 116.5, 115.7, 110.0, 59.1, 59.0, 58.9, 51.6, 51.5, 51.4, 35.5, 35.2, 35.2, 30.0 ppm.

ESI-HRMS: found $[\text{M}+\text{Na}]^+$ 665.1366, calculated 665.1380.

λ_{max} (absorption) = 493 nm (ϵ = 23000 $\text{M}^{-1} \text{cm}^{-1}$, MeOH), λ_{max} (emission) = 549 nm (MeOH; excitation at 480 nm); fluorescence lifetime 5.9 ns (MeOH), fluorescence quantum yield: 0.97 (absolute value in MeOH); 0.83 (relative value obtained in MeOH using Rhodamine 6G as a standard (QY = 0.94 in ethanol), excitation at 480 nm).

8-[*N*-methyl-*N*-(trifluoroacetyl)]amino-*N,N',N''*-tris(2-hydroxyethyl)-*N,N',N''*-trimethylpyrene-1,3,6-trisulfonamide (8)



Compound 7-H (177 mg, 0.28 mmol) was suspended in 10 mL of anhydrous DCM, and a 10% solution of trifluoroacetic anhydride in DCM ($d = 1.33 \text{ g/mL}$, 1.8 mL, $\sim 1.2 \text{ mmol}$) followed by Et₃N (250 μL , $d = 0.73 \text{ g/mL}$, 1.8 mmol) was added at r. t. The reaction mixture was stirred for 30 min (with control by TLC). All volatile materials were evaporated under reduced pressure, the residue was co-evaporated with acetonitrile (3 times), dissolved in methanol (50 mL), and NaHCO₃ (50 mg) was added. These operations remove trifluoroacetate groups from hydroxyl groups. After stirring at r. t. for 30 min, TLC displayed the full conversion into intermediate 7-COCF₃. The reaction mixture was neutralized with acetic acid, and all volatile materials removed *in vacuo*. The title compound was isolated by chromatography on SiO₂ (50 g), using CH₂Cl₂/acetone (2:1) as an eluent. Yield – 165 mg (82%) of 7-COCF₃ as a yellow solid. HPLC: $t_{\text{R}} = 10.8 \text{ min}$, ACN/H₂O: 20/80 – 100/0 in 25 min, 254 nm.

^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) δ 9.50 (d, $J = 9.9$ Hz, 1H), 9.40 (dd, $J = 9.9, 1.9$ Hz, 2H), 9.27 (s, 1H), 9.03 (s, 1H), 8.76 (d, $J = 9.8$ Hz, 1H), 3.75 – 3.68 (m, 6H), 3.51 – 3.42 (m, 6H), 3.09 (s, 3H), 3.07 (s, 3H), 3.07 (s, 3H) ppm.

^{19}F NMR (376 MHz, $(\text{CD}_3)_2\text{CO}$) $\delta = -75.7$ ppm.

ESI-MS, negative mode: m/z (rel. int., %) = 723 (100) $[\text{M}-\text{H}]^-$.

To a solution of compound **7**- COCF_3 (120 mg, 0.17 mmol) in anhydrous DMF (2 mL), Cs_2CO_3 (42 mg, 0.13 mmol) and CH_3I (960 mg, 6.7 mmol) were added under argon. The reaction mixture was stirred at 70°C for 40 min (TLC control), and the solvent was evaporated under reduced pressure. Compound **8** was isolated by chromatography on regular SiO_2 (50 g) using a 15:1 mixture of DCM and methanol as an eluent; yield – 110 mg (88%) of a yellow solid. HPLC: $t_{\text{R}} = 12.2$ min, ACN/ H_2O : 20/80 – 100/0 in 25 min, 254 nm.

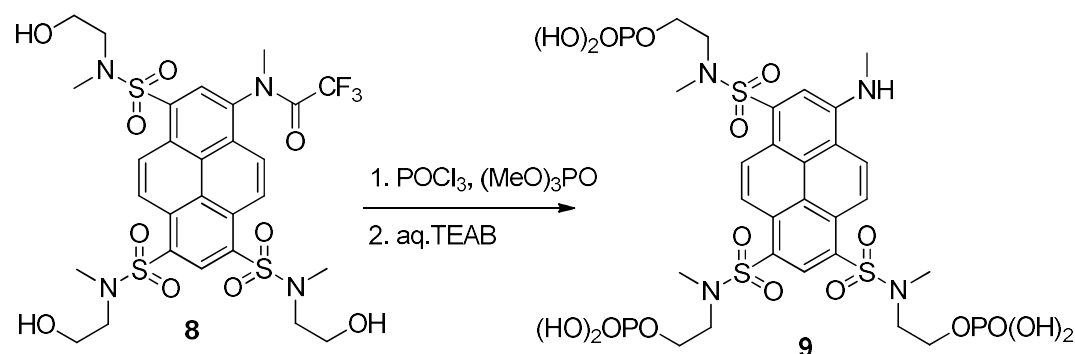
^1H NMR (400 MHz, CD_3OD) δ 9.16 (d, $J = 9.8$ Hz, 1H), 9.04 (s, 1H), 8.99 (d, $J = 9.7$ Hz, 1H), 8.77 (d, $J = 9.8$ Hz, 1H), 8.63 (d, $J = 9.7$ Hz, 1H), 7.94 (s, 1H), 3.73 – 3.67 (m, 6H), 3.44 – 3.37 (m, 6H), 3.21 (s, 3H), 2.99 (s, 3H), 2.99 (s, 6H) ppm.

^{13}C NMR (126 MHz, CD_3OD) δ 148.4, 138.2, 134.3, 133.9, 130.9, 130.1, 129.5, 129.2, 128.6, 127.6, 126.2, 124.0, 120.7, 118.5, 118.0, 111.6, 60.9, 60.8, 53.0, 52.9, 36.1, 36.0, 35.9, 30.6 ppm. CF_3 and CO signals were not detected due to low intensities.

^{19}F NMR (376 MHz, CD_3OD) $\delta -77.0$ ppm.

ESI-HRMS: found $[\text{M}+\text{H}]^+ 739.1366$, calculated 739.1389

8-Methylamino-[N,N',N'' -tris(2-hydroxyethyl)- N,N',N'' -trimethylpyrene-1,3,6-trisulfonamide]- O,O',O'' -triphosphate triethylammonium salt (9**)**



To a solution of POCl₃ (74 mg, 0.48 mmol) in 0.1 mL of (MeO)₃PO, the solution of compound **8** (40 mg, 54 μmol) in 0.5 mL of (MeO)₃PO was added dropwise at 0 °C. Then the reaction mixture was stirred for 1.5 h at r. t. All volatile materials (excess of POCl₃ and most of trimethyl phosphate) were removed in vacuum (using rotary evaporator and then an oil pump; 0.5 mbar, 60 °C, cold trap cooled with dry ice for collecting distillate). The residue was treated and stirred with 1 M Et₃N*H₂CO₃ buffer (TEAB; initial pH = 8-9) and pH-value was controlled. Fresh portions of the TEAB buffer were added when the solution became acidic, until the pH-value stabilized at about 6–7. The cleavage of trifluoroacetyl protecting group takes place under basic conditions in aq. TEAB. Then the reaction was complete (HPLC indicated no changes), the mixture was loaded on RP-18 (ca. 30 g) and the title compound was eluted using 1:4 mixture of MeCN and aqueous 0.1 M Et₃N*H₂CO₃ buffer (pH 8) to afford 30 mg of **9** as a red solid (51% yield). HPLC: *t*_R = 6.9 min H₂O/ACN (+0.1% TFA): 80/20 → 0/100 in 25 min, 254 nm.

¹H NMR (400 MHz, CD₃OD) δ 9.17 (d, *J* = 9.8 Hz, 1H), 9.06 (s, 1H), 9.01 (d, *J* = 10.2 Hz, 1H), 8.78 (d, *J* = 9.8 Hz, 1H), 8.68 (d, *J* = 9.6 Hz, 1H), 7.91 (s, 1H), 4.06 – 3.94 (m, 6H), 3.60 – 3.50 (m, 6H), 3.23 (s, 3H), 3.16 (q, *J* = 7.3 Hz, 14H, Et₃N), 3.05 (s, 3H), 3.04 (s, 3H), 2.97 (s, 3H), 1.29 (t, *J* = 7.3 Hz, 23H, Et₃N) ppm.

¹³C NMR (126 MHz, CD₃OD) δ = 148.6, 138.2, 134.3, 134.1, 131.2, 130.2, 129.3, 128.8, 128.6, 127.6, 126.6, 124.1, 120.6, 118.6, 117.9, 111.5, 64.7, 64.5, 64.3, 59.5, 51.5, 51.4, 51.1, 47.6, 36.6, 36.4, 36.2, 30.6, 9.1 ppm.

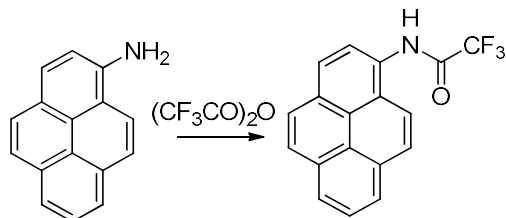
³¹P NMR (162 MHz, CD₃OD) δ = 0.7 ppm.

ESI-HRMS found [M-H]⁻ 881.0387, calculated 881.0405.

λ_{\max} (absorption) = 502 nm (H₂O), λ_{\max} (emission) = 563 nm (H₂O; excitation at 490 nm); fluorescence lifetime 3.6 ns (H₂O) fluorescence quantum yield 0.85 (H₂O, standard: Rhodamine 6G with emission efficiency of 0.94 in ethanol, excitation at 500 nm).

Alkylsulfonyl derivatives 10-16

2,2,2-Trifluoro-N-(pyren-1-yl)acetamide



1-Aminopyrene (1.00 g, 4.60 mmol) was dissolved in anhydrous DCM (ca. 30 mL) under stirring (and by using an ultrasonic bath for a short time). Trifluoroacetic anhydride ($d=1.51$, 772 μL , 1.17 g, 5.55 mmol, 1.21 eq.) was added dropwise over a period of 10 min. The obtained suspension was stirred for 30 min at r.t. The precipitate was removed by filtration and washed with cyclohexane (3×15 mL). A white precipitate formed in the filtrate was also filtered off. The combined solids (a light grey powder) were dried in vacuo and provided 1.13 g (79%) of the title compound.

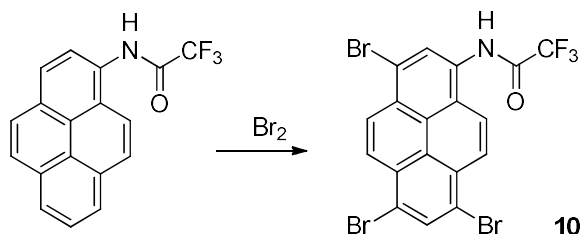
^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) δ 8.37 – 8.32 (m, 3H), 8.30 – 8.19 (m, 5H), 8.11 (t, $J = 7.7$ Hz, 1H).

^{13}C NMR (101 MHz, $(\text{CD}_3)_2\text{CO}$) δ 209.9, 132.1, 131.7, 131.5, 129.1, 128.7, 128.0, 127.5, 126.8, 126.7, 126.5, 125.9, 125.8, 125.4, 125.3, 125.1, 122.4, 110.9 ppm. CF_3 signal was not detected due to low intensity.

^{19}F NMR (376 MHz, $(\text{CD}_3)_2\text{CO}$): δ -75.7 ppm.

ESI-HRMS: 314.0783 found $[\text{M}+\text{H}]^+$; calculated 314.0787.

2,2,2-Trifluoro-N-(3,6,8-trisbromopyren-1-yl)acetamide (10)



N-Trifluoroacetyl-1-aminopyrene (870 mg, 2.76 mmol) was suspended in nitrobenzene (30 mL) and stirred at 50 °C for 30 min. Bromine (500 μL , 19.5 mmol, 7.07 eq.) was dissolved in nitrobenzene (5 mL) and added to the solution of pyrene. The

reaction mixture was stirred in a closed vessel at 80 °C for 30 min, allowed to reach r.t. and diluted with cyclohexane (20 mL). The precipitate was removed by filtration, washed with cyclohexane (2 × 25 mL) and dried in vacuo. The title product was obtained as a light yellow powder (1.22 g, 81%).

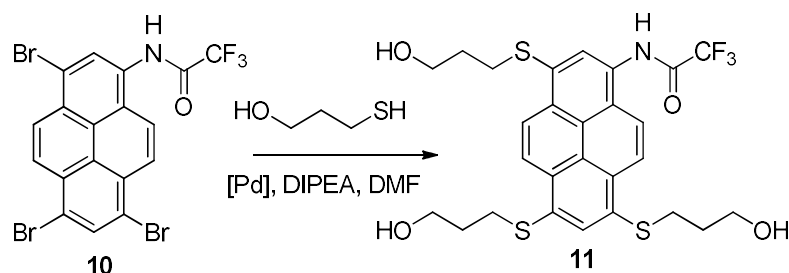
^1H NMR (400 MHz, DMSO-*d*₆) δ 8.73 (d, *J* = 1.1 Hz, 1H), 8.58 (s, 1H), 8.49 (d, *J* = 9.5 Hz, 1H), 8.44 – 8.39 (m, 2H), 8.26 (d, *J* = 9.5 Hz, 1H) ppm.

^{13}C resonances were very weak due to very low solubility.

^{19}F NMR (376 MHz, DMSO-*d*₆): δ = -73.5 ppm.

ESI-HRMS: found 547.7930 [M+H]⁺; calculated 547.7937.

2,2,2-Trifluoro-N-[3,6,8-tris(3-hydroxypropylthio)pyrene-1-yl]-acetamide (**11**)



DIPEA (116 μL , 0.66 mmol) and tribromide **10** (110 mg, 0.2 mol) were suspended in anhydrous DMF (4 mL) and flushed with argon for 15 min. 3-Mercapto-1-propanol (60 μL , 65 mg, 0.66 mmol), and dry DMF (1 mL) were added, and a gentle argon stream was bubbled through the solution for 20 min. Afterwards, Pd₂(dba)₃ (9.2 mg, 10 μmol , 0.05 eq) and Xantphos (12 mg, 20 μmol , 0.1 eq) were added. The mixture was stirred at 100 °C under argon for 18 h. The degree of conversion was controlled by HPLC (*t*_R = 8.3 min (MeCN/H₂O 50:50 → 100:0 + 0.1% TFA in 25 min detected at 254 nm). Upon completion of the reaction, solvents were removed in vacuo, the residue was taken-up in MeOH, applied to Celite®, dried in the rotary evaporator and submitted to flash chromatography (SNAP Ultra cartridge with 25 g SiO₂, DCM/MeOH with 2-18% MeOH-gradient over 15 CV) to provide the title compound **11** (100 mg, 86%) as a pale-yellow solid.

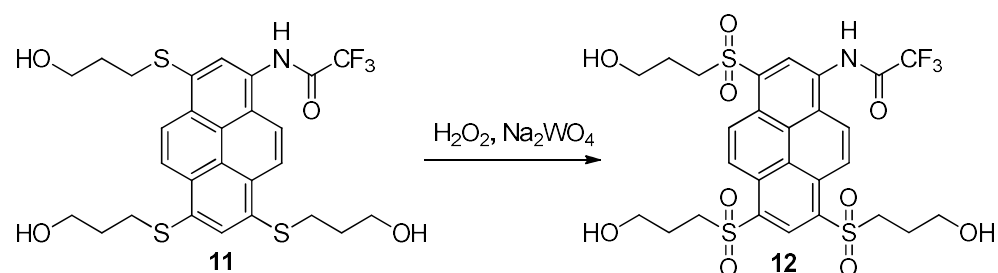
^1H NMR (400 MHz, DMSO-*d*₆) δ 11.85 (s, 1H, NH), 8.57 (d, *J* = 9.5 Hz, 1H), 8.53 (d, *J* = 9.5 Hz, 1H), 8.50 (d, *J* = 9.5 Hz, 1H), 8.17 (s, 2H), 8.06 (d, *J* = 9.5 Hz, 1H), 4.67 – 4.58 (m, 3H, OH), 3.61 – 3.48 (m, 6H), 3.32 – 3.21 (m, 6H), 1.87 – 1.72 (m, 6H) ppm.

^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 132.9, 132.8, 132.3, 128.6, 127.6, 127.4, 127.2, 125.5, 124.7, 124.6, 124.4, 124.2, 123.7, 123.2, 121.8, 117.7, 59.2, 32.0, 31.7, 30.0, 29.9 ppm. CF_3 and CO signals were not detected due to low intensities.

^{19}F NMR (376 MHz, $\text{DMSO-}d_6$): $\delta = -73.5$ ppm.

ESI-HRMS: found 582.1049 $[\text{M-H}]^-$, calculated 582.1060.

8-(Trifluoroacetyl)amino-1,3,6-tris[(3-hydroxypropyl)sulfonyl]-pyrene (12)



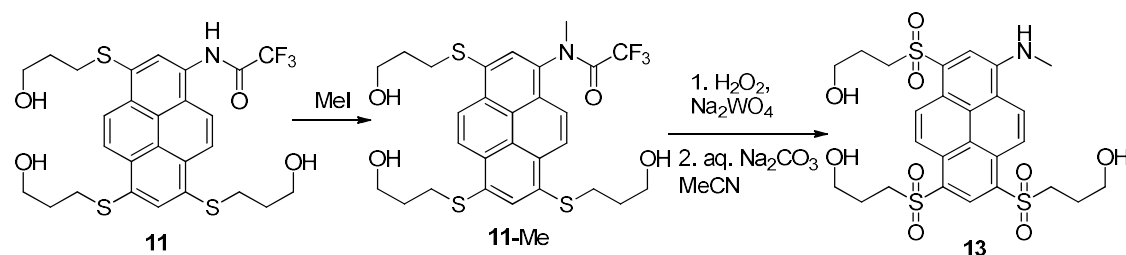
Compound **11** (100 mg, 0.17 mmol) was suspended in AcOH (10 mL). Then sodium tungstate dihydrate (17 mg, 50 μmol , 0.25 eq) was added, the solution was cooled in an ice bath, and aq. H_2O_2 (50%, 3 mL) was added over a period of 10 min. The solution was stirred for 30 min at $+4^\circ\text{C}$, and then at r.t. overnight. The solvents were removed by freeze-drying, and the residue dissolved in minimal amount of aq. MeCN. Celite® was added and, after removing all volatiles in vacuo (rotary evaporator), the residue was submitted to flash chromatography (SNAP Ultra cartridge, 25 g SiO_2 , DCM/MeOH with 2-20% MeOH-gradient over 15 CV) to provide compound **12** (88 mg, 77%) as a pale-yellow solid.

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.35 (s, 1H, NH), 9.49 (d, $J = 9.8$ Hz, 1H), 9.39 – 9.30 (m, 3H), 9.27 (s, 1H), 8.99 (s, 1H), 8.79 (d, $J = 9.7$ Hz, 1H), 4.59 (s, 3H, OH), 3.93 – 3.63 (m, 6H), 3.46 – 3.38 (m, 6H), 1.86 – 1.71 (m, 6H) ppm.

^{19}F NMR (376 MHz, $\text{DMSO-}d_6$): $\delta = -73.5$ ppm.

ESI-HRMS: found 678.0756 $[\text{M-H}]^-$, calculated 678.0755.

3,6,8-Tris[(3-hydroxypropyl)sulfonyl]pyrene-1-(methylamine) (13)



Compound **11** (75 mg, 0.13 mmol) was suspended in dry DMF (0.1 mL) under argon, Cs₂CO₃ (55 mg, 0.17 mmol) was added followed by MeI (0.15 mL). The reaction mixture was stirred for 1 h in a screw-cap tube at 50 °C. HPLC indicated that the reaction was complete. HPLC: starting material $t_R = 13.7$ min, product $t_R = 15.8$ min (MeCN/H₂O 30:70 → 100:0 + 0.1% TFA in 25 min detected at 254 nm). DMF was removed in vacuum, and the residue was taken up in dichloromethane – water mixture. The organic layer was separated, washed with brine, dried over sodium sulfate, filtered and evaporated in vacuo. The *N*-methylated product (56 mg, 72 %) was isolated by chromatography on SiO₂ (25 g) in the course of elution with 0 – 5% MeOH in ethyl acetate.

¹H NMR (400 MHz, CD₃OD): $\delta = 8.71$ (dd, $J = 9.5, 5.2$ Hz, 2 H), 8.65 (d, $J = 9.6$ Hz, 1H), 8.29 (s, 1H), 8.18 (d, $J = 0.9$ Hz, 1H), 8.00 (d, $J = 9.4$ Hz, 1H), 3.70 (m, 6H), 3.58 (s, 3H), 3.29 (m, 6H), 1.89 (m, 6H) ppm.

For oxidation, *N*-methylated compound **11** was dissolved in acetic acid (5 mL), water was added (3 mL), followed by Na₂WO₄·2H₂O (12 mg, catalyst), and the mixture was cooled to 0°C. Hydrogen peroxide (1.5 mL of a ca. 80% solution) was added at 0 °C. The solution was stirred for 30 min in an ice bath, and then at r.t. overnight. The solvents were removed by freeze-drying, and the residue was dissolved in minimal amount of aq. MeCN. The cleavage of the CF₃CO group (deprotection) in the intermediate compound was performed as follows: a saturated aqueous solution of Na₂CO₃ (ca. 20 wt.-%, 1.5 mL) was added. The reaction mixture turns to be bright orange in several minutes; it was stirred overnight at room temperature. HPLC indicated complete conversion to a new substance (title compound) with $t_R = 6.4$ min (MeCN/H₂O 30:70 → 100:0 + 0.1% TFA in 25 min detected at 254 nm). Sodium carbonate was neutralized by addition of glacial AcOH, and the frozen reaction mixture was lyophilized. The residue was submitted to flash

chromatography (SNAP Ultra cartridge, 25 g SiO₂, DCM/MeOH with 2-20% MeOH-gradient over 15 CV) to provide the title compound **13** (50 mg, 77%) as a red solid.

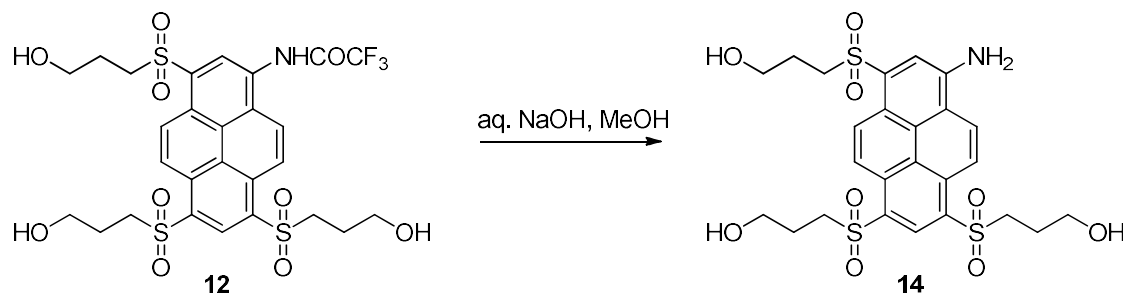
¹H NMR (400 MHz, DMSO-d₆) δ 9.19 (d, J = 9.7 Hz, 1H), 9.04 (s, 1H), 8.97 (d, J = 9.8 Hz, 1H), 8.94 (d, J = 9.6 Hz, 1H), 8.77 (d, J = 9.7 Hz, 1H), 8.39 – 8.32 (m, 1H, NH), 7.96 (s, 1H), 4.64 – 4.53 (m, 3H, OH), 3.68 – 3.59 (m, 6H), 3.44 – 3.36 (m, 6H), 3.17 (d, J = 4.4 Hz, 3H), 1.81 – 1.70 (m, 6H) ppm.

¹³C NMR (101 MHz, DMSO-d₆) δ 147.6, 137.3, 133.5, 133.1, 130.0, 129.0, 128.1, 127.6, 126.9, 126.4, 125.8, 121.5, 118.7, 116.9, 115.8, 111.1, 58.6, 58.5, 53.5, 52.5, 52.3, 30.0, 26.0, 25.9 ppm.

ESI-HRMS: calculated 596.1088 [M-H]⁻, found 596.1078.

λ_{max} (absorption) = 502 nm (MeOH, ε = 23000 M⁻¹ cm⁻¹); 509 nm (H₂O, ε = 19500 M⁻¹ cm⁻¹), λ_{max} (emission) = 550 nm (MeOH; excitation at 480 nm); 563 nm (H₂O; excitation at 490 nm); fluorescence lifetime 6.3 ns (MeOH); 6.4 ns (H₂O), fluorescence quantum yield 0.88 (MeOH); 0.67 (H₂O).

8-amino-1,3,6-tris(3-hydroxypropyl)-pyrene sulfone (**14**)



Trifluoroacetate **12** (20 mg, 29 μmol) was suspended in MeOH (6 mL). Aq. NaOH (750 μL of the saturated aq. solution) was added, and the reaction mixture was stirred for 30 min at r.t. Celite® and MeOH were added, and the solvents were removed under reduced pressure. HPLC: *t*_R = 13.1 min (MeCN/H₂O 10:90 → 100:0 + 0.1% TFA in 25 min detected at 225 nm). The product was isolated by flash chromatography (SNAP Ultra cartridge with 10 g SiO₂, 2-20% of MeOH in DCM over 12 CV) as an orange solid (14 mg, 24 μmol, 81%).

¹H NMR (400 MHz, DMSO-d₆) δ 9.11 (d, J = 9.6 Hz, 1H), 8.99 (s, 1H), 8.90 (d, J = 9.7 Hz, 1H), 8.84 (d, J = 9.6 Hz, 1H), 8.73 (d, J = 9.6 Hz, 1H), 8.21 (s, 1H), 7.84 (s, 2H,

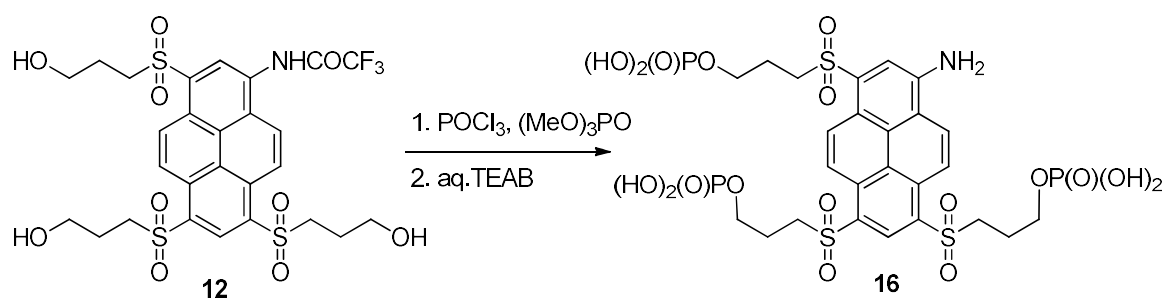
NH₂), 4.58 – 4.49 (m, 3H, OH), 3.62 – 3.52 (m, 6H), 3.41 – 3.30 (m, 6H), 1.76 – 1.63 (m, 6H) ppm.

¹³C NMR (101 MHz, DMSO-*d*₆) δ 148.5, 136.8, 134.1, 132.8, 129.9, 128.8, 128.0, 127.9, 127.3, 126.4, 126.1, 121.0, 118.5, 116.8, 115.9, 115.8, 58.6, 58.5, 53.4, 52.5, 52.5, 26.1, 26.0, 25.9 ppm.

ESI-HRMS: found 582.0927 [M-H]⁻, calculated 582.0932.

λ_{max} (absorption) = 486 nm (ϵ = 21 000 M⁻¹ cm⁻¹, MeOH), λ_{max} (emission) = 534 nm (MeOH, excitation at 470 nm); fluorescence lifetime 4.9 ns (MeOH); fluorescence quantum yield (0.80; absolute value in MeOH).

3,8,10-Tris[(3-hydroxypropyl)sulfonyl]pyrene-1-amine *O,O',O''*-triphosphate triethylammonium salt (**16**)



A solution of compound **12** (68 mg, 0.1 mmol) in trimethylphosphate (0.1 mL) was added dropwise to the freshly distilled and ice-cooled POCl₃ (0.15 mL, 1.67 mmol) under argon atmosphere. The mixture was stirred at 0 °C for 10 min and 4 h at r. t. All volatile components were removed in vacuo, and the residue was further dried by lyophilization (0.02 mbar). An aqueous Et₃N-H₂CO₃ buffer (1 M, 8 mL) was added gradually to the residue, until the pH stabilized at 8 (gas evolution). Analytical HPLC: t_R = 5.5 min (MeCN/H₂O + 0.05 M TEAB, 10:90 → 100:0 in 20 min, detected at 254 nm). The reaction mixture was concentrated to ca. 3 mL by freeze-drying and purified by preparative HPLC (Kinetex 5 μm EVO C18 100A 250×21 mm column, MeCN/water + 0.05 M TEAB, 10 mL/min, 5-30% MeCN over 20 min) to afford 23 mg of **16** as red solid (16% yield).

¹H NMR (400 MHz, D₂O) δ 8.94 (s, 1H), 8.72 (d, J = 9.6 Hz, 1H), 8.35 (t, J = 9.7 Hz, 2H), 8.07 (d, J = 9.6 Hz, 1H), 7.91 (s, 1H), 3.91 – 3.75 (m, 6H), 3.73 – 3.53 (m, 6H), 3.15 (q, J = 7.3 Hz, 24H, Et₃N), 2.04 – 1.88 (m, 6H), 1.23 (t, J = 7.3 Hz, 36H, Et₃N) ppm.

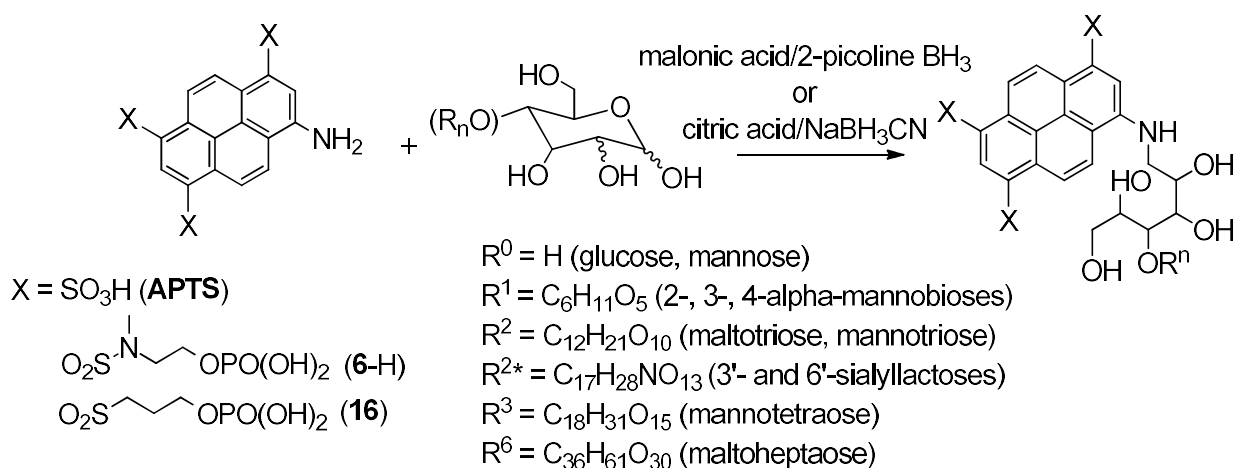
^{13}C NMR (101 MHz, D_2O) δ 146.7, 134.5, 133.6, 132.4, 130.5, 128.4, 126.8, 126.4, 125.8, 125.1, 124.5, 121.1, 118.8, 117.4, 116.4, 116.0, 62.9, 62.8, 62.7, 53.5, 52.7, 52.4, 46.6, 23.8, 23.7, 23.6, 8.2 ppm.

^{31}P NMR (162 MHz, D_2O) δ 0.95, 0.93, 0.85 ppm.

ESI-HRMS: found 821.9913 $[\text{M}-\text{H}]^-$, calculated 821.9922.

λ_{max} (absorption) = 477 nm ($\epsilon = 19\,600\ \text{M}^{-1}\ \text{cm}^{-1}$, 0.05 M aq. $\text{Et}_3\text{N}^*\text{H}_2\text{CO}_3$ buffer, pH 8.5), λ_{max} (emission) = 542 nm (0.05 M aq. $\text{Et}_3\text{N}^*\text{H}_2\text{CO}_3$ buffer, pH 8.5, excitation at 470 nm); fluorescence lifetime 5.8 ns; fluorescence quantum yield 0.92 (absolute value in a TEAB buffer, pH 8.5, measured in Hamamatsu device C11347-12 with an integration sphere).

Reductive amination of sugars



Scheme S2. APTS (a reference dye), aminopyrenes **6-H** and **16** react with various sugars in a two-step procedure with malonic acid and 2-picoline borane (*Method A*). Conjugates of APTS and dye **16** with 3'- and 6'-sialyllactoses were prepared using $\text{Na}(\text{CN})\text{BH}_3$ in THF and aqueous citric acid (*Method B*).

Method A:

1.5 mL Eppendor tube was charged with dye (10 μL of 0.1 M solution in water), and then sugar (5 eq, 50 μL of 0.1 M solution in water) and malonic acid (10 eq, 10 μL of 1 M

solution in DMSO) were added. The samples were shaken at 40 °C for 1 h in an Eppendorf ThermoMixer®, and solvents were completely removed in a freeze-dryer (Martin Christ, residual pressure <0.1 mbar, temp. of the cooling coil –80°C: step 1). A solution of 2-picoline-borane complex (10 eq, 10 µL of 1 M solution in DMSO) was added, and the samples were stirred at 40°C for 16 h in an Eppendorf ThermoMixer® (step 2). The products were isolated either by flash chromatography (RP C18, 15C18AQ-F0025 cartridge, ACN – 20 mM TEAB, pH 8, 0–5% ACN, 15 column volumes, Interchim puriFlash™), or by preparative HPLC with UV-VIS detection (MeCN (A)/TEAB 0.05 M in water (B), 5:95 → 30:70 in 20 min detected at 500 nm; columns: *Kinetex C18 100*, 5 µm, 250x20 mm, or Interchim Uptisphere Strategy C18-HQ, 10 µm, 250x21 mm, flow rate 20 mL/min). The constitutions of the products (see structures below) were confirmed by ESI-HRMS.

Method A worked fine for all saccharides, except 3'- and 6'-sialyllactoses. In these two cases, the reductive amination of was only possible by using Na(CN)BH₃ in THF and aqueous citric acid (see *Method B* below).⁴

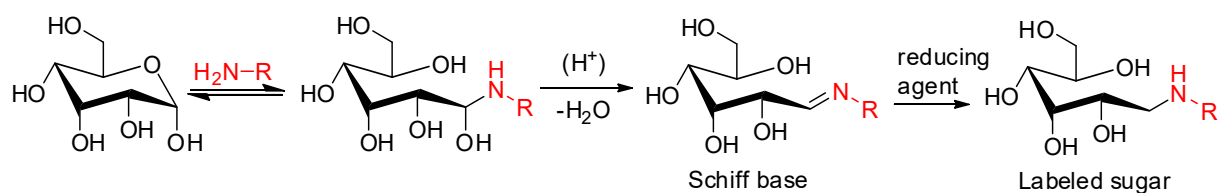
In Method A, the Schiff's base was formed in the presence of malonic acid (pK_{a1} 2.83)⁵ and then reduced by 2-picoline borane complex⁶.

The formation of the Schiff's base (Scheme S3) was observed by HPLC analysis only after complete drying of the reaction mixture (step 1); when 2-picoline-borane complex was not yet added (Figure S2). The absorption maximum of the Schiff's base is blue-shifted to 459 nm (dye **6-H**: 472 nm, conjugate with glucose 496 nm). In this experiment, the Schiff base was isolated and its constitution confirmed by ESI-MS. These observations explain the necessity of complete removal of water by drying in vacuo, before addition of reducing agent.

⁴F.-T. A. Chen, T. S. Dobashi, R. A. Evangelista, *Glycobiology* **1998**, *8*, 1045–1052

⁵R. A. Evangelista, A. Guttman, F.-T. A. Chen, *Electrophoresis* **1996**, *17*, 347–351.

⁶L. R. Ruhaak, E. Steenvoorden, C. A. M. Koeleman, A. M. Deelder, M. Wuhrer, *Proteomics* **2010**, *10*, 2330-2336.



Scheme S3. Reductive amination of glucose

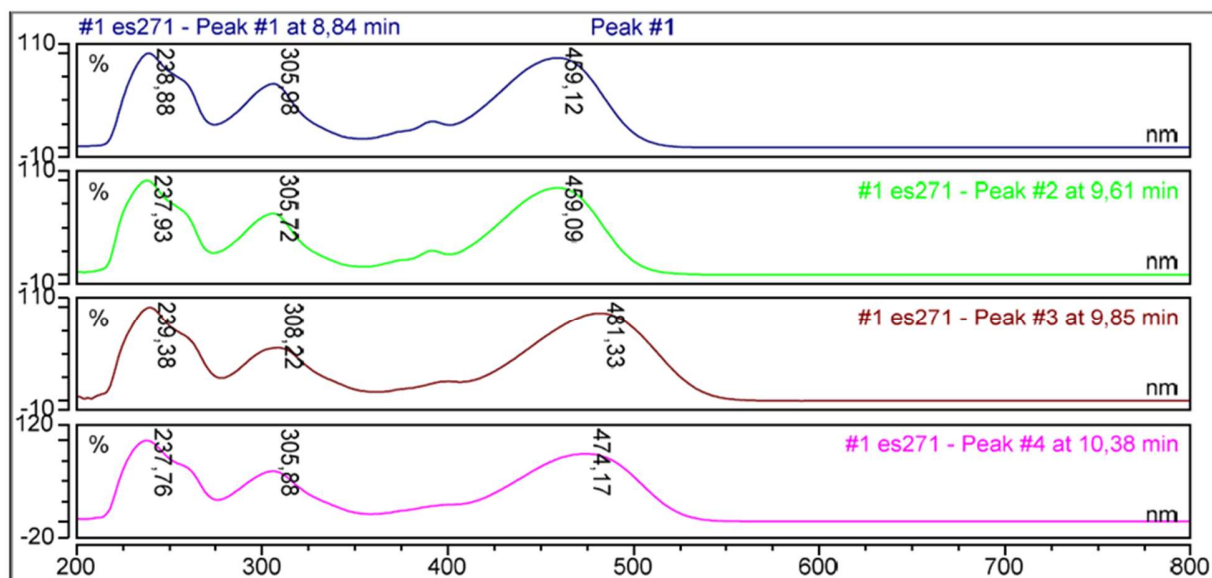
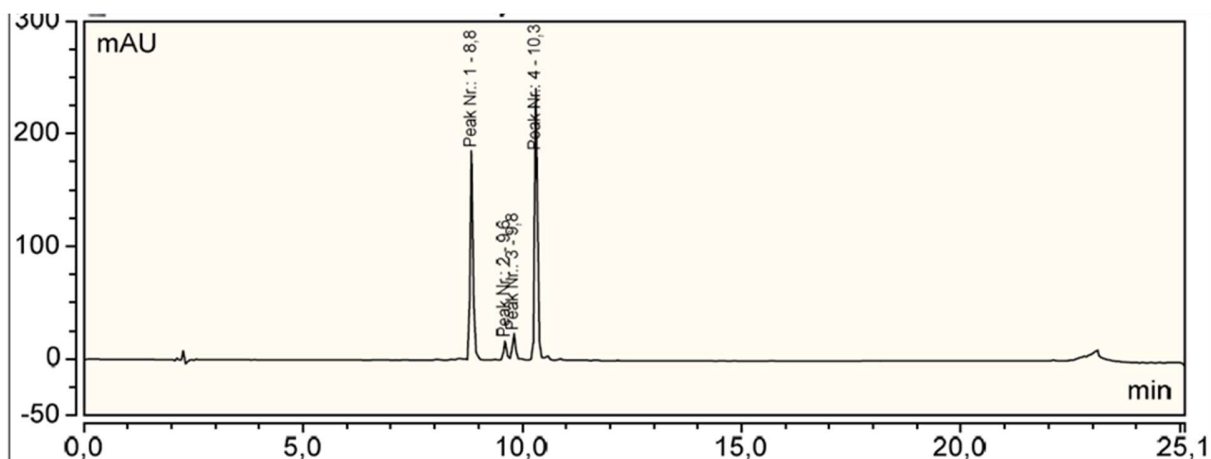


Figure S2. Step 1: dye **6-H**, glucose and malonic acid were mixed and shaken at 40 °C for 1 h. Then the reaction mixture was dried. Starting material ($t_R = 10.3$, $\lambda_{\text{max}} = 474$ nm, magenta line, last spectrum) and Schiff base ($t_R = 8.8$, $\lambda_{\text{max}} = 459$ nm, dark blue line, first spectrum) were observed.

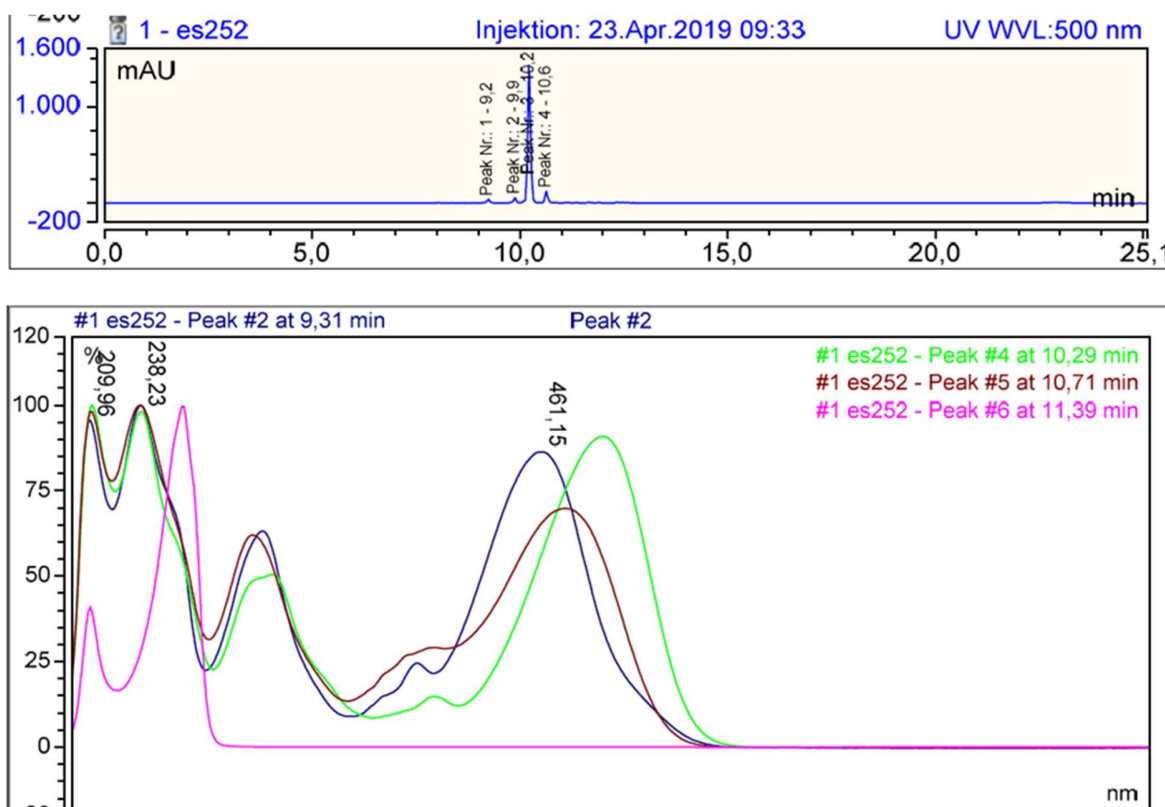


Figure S3. Step 2: 2-picoline-borane complex was added and the reaction mixture was shaken at 40 °C for 16 hours. Only traces of the starting material ($t_R = 10.7$ min, $\lambda_{max} = 471$ nm, brown line) and Schiff base ($t_R = 9.3$ min, $\lambda_{max} = 461$ nm, dark blue line) were observed. The main peak with $t_R = 10.2$ min represents the desired product ($\lambda_{max} = 496$ nm, green line).

Method B:

1.5 mL Eppendorf tube was charged with dye (10 μ L of 0.1 M solution in water), sugar (5 eq, 50 μ L of 0.1 M solution in water), citric acid (10 eq, 10 μ L of 1 M solution in water) and NaBH_3CN (10 eq, 10 μ L of 1 M solution in THF) were added. The samples were shaken at 60 °C overnight. The products were isolated by RP chromatography (see *Method A* above), and their constitutions (see structures below) were confirmed by ESI-HRMS.

Determination of the yields

Sugars: G = glucose, G₃ = maltotriose, G₇ = maltoheptaose;

M = mannose, M₂ = mannobioses (2O bond, 3O bond, 4O bond), M₃ = mannotriose, M₄ = mannotetraose.

Table S1. Yields in reductive amination (HPLC with a diode array detector)

Sugar / Dye	APTS	16	6-H
G	100 ^a	93 ^a	100 ^a
G ₃	94 ^a	90 ^a	63 ^a
G ₇	93 ^a	73 ^a	90 ^a
M	100 ^a	92 ^a	-
M ₂ -2O	87 ^a	35 ^a	-
M ₂ -3O	77 ^a	54 ^a	-
M ₂ -4O	93 ^a	69 ^a	-
M ₃	80 ^a	23 ^a	-
M ₄	78 ^a	30 ^a	-
3'-sialyllactose	22 ^b	21 ^b	-
6'-sialyllactose	24 ^b	24 ^b	-

^aMethod A; ^bMethod B (for conditions, see the previous section)

The product yields were determined by HPLC, by measuring peak areas of the residual dyes and products in the reaction mixtures at isosbestic points (APTS/APTS+G – 440 nm, **6-H/6-H+G** – 484 nm, **16/16+G** – 487 nm), as exemplified in Figure S4.

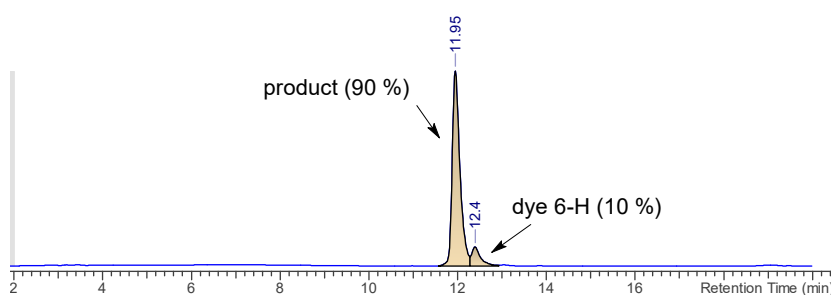


Figure S4. HPLC analysis of the reductive amination of **6-H** with maltoheptaose (G₇) showed the conversion to glycoconjugate (detection at the isosbestic wavelength of 484 nm).

Characterization of conjugates

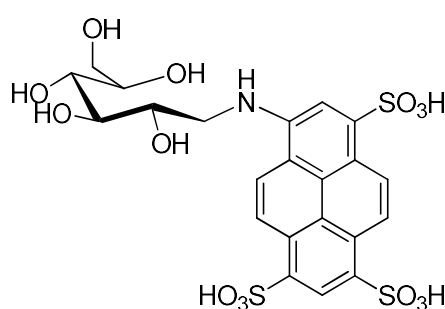
HPLC conditions are given for each conjugate;

Column A: Kinetex C18 100, 5 μ m, 250x4 mm, 1.2 mL/min

Column B: Interchim Uptisphere C18-HQ, 10 μ m, 250x4.6 mm, 1.2 mL/min

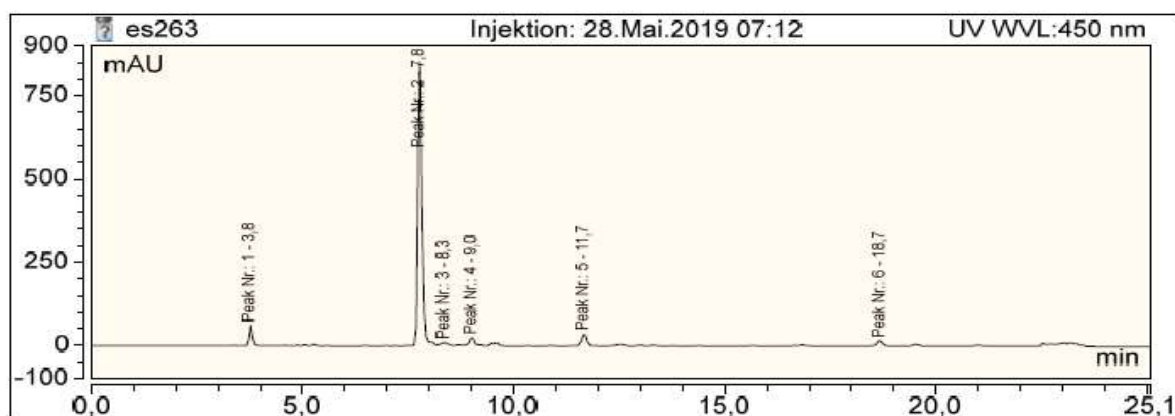
APTS-derived sugars

APTS + G



Chemical Formula: C₂₂H₂₃NO₁₄S₃
Exact Mass: 621.03

Gradient : A 2,0 % B 98,0 % ----> A 10,0 % B 90,0 % T = 20 Min.



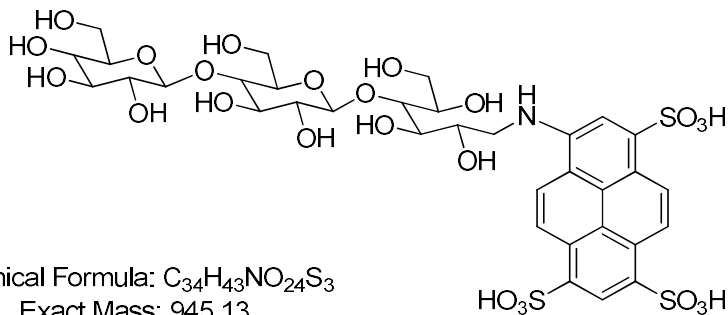
HPLC: t_R = 7.8 min (Column A; MeCN (A)/TEAB 0.05 M in water (B)), 2:98 \rightarrow 10:90
in 20 min detected at 450 nm

Yield 100 %

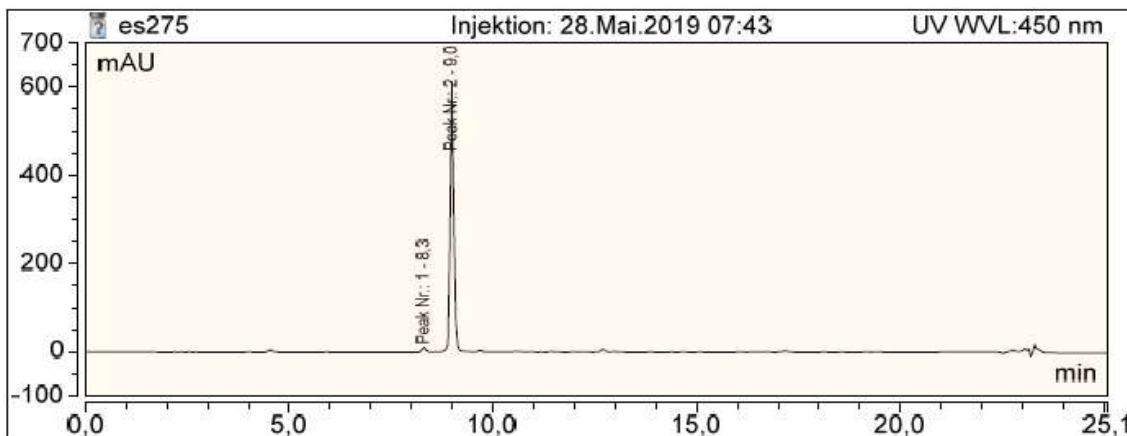
ESI-HRMS: found 620.0206 [M-H]⁻, calculated 620.0208; found 309.5063 [M-2H]²⁻,
calculated 309.5068

λ_{max} (absorption) = 454 nm

APTS + G₃



Gradient : A 2,0 % B 98,0 % ----> A 10,0 % B 90,0 % T = 20 Min.



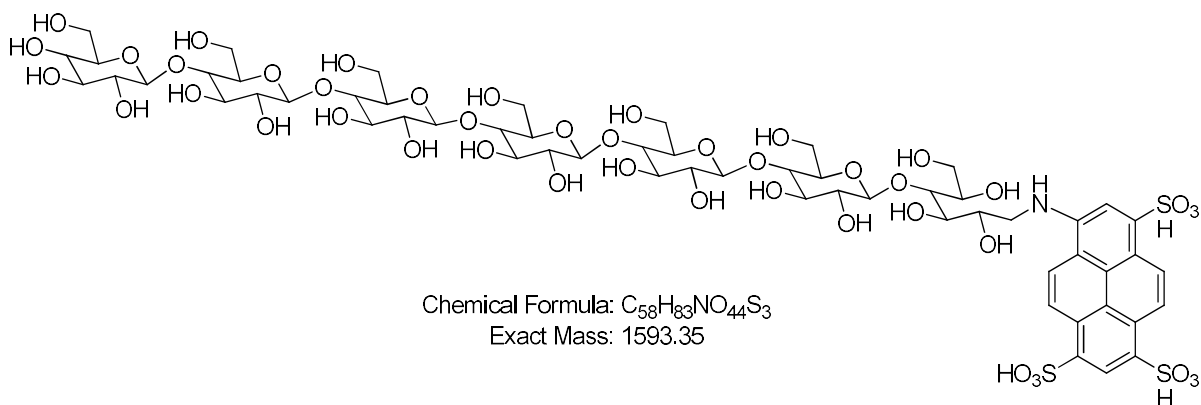
HPLC: $t_R = 9.0$ min (Column A; MeCN (A)/TEAB 0.05 M in water (B)), 2:98 → 10:90
in 20 min detected at 450 nm

Yield 94 %

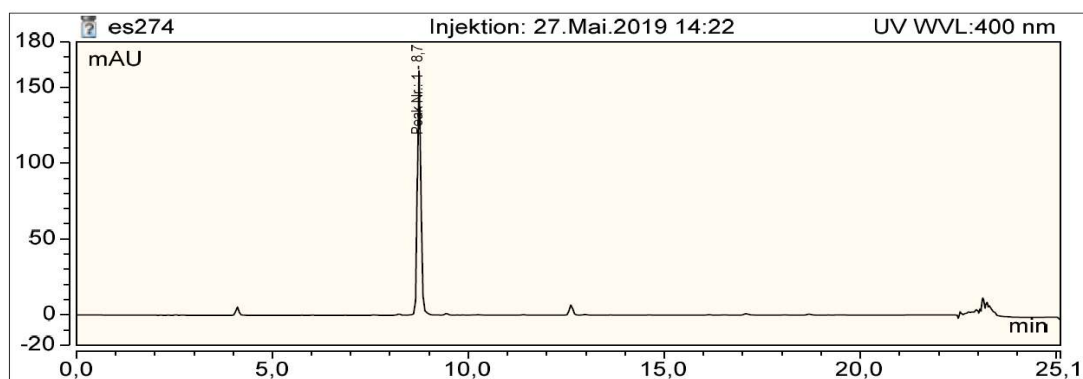
ESI-HRMS: found 944.1261 [M-H]⁻, calculated 944.1297; found 471.5594 [M-2H]²⁻,
calculated 471.5596

λ_{max} (absorption) = 454 nm

APTS + G₇



Gradient : A 2,0 % B 98,0 % ----> A 10,0 % B 90,0 % T = 20 Min.



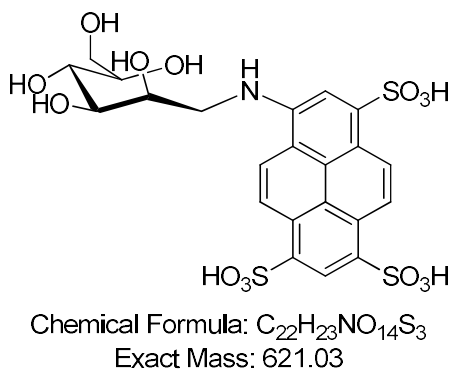
HPLC: $t_R = 8.7$ min (Column A; MeCN (A)/TEAB 0.05 M in water (B)), 2:98 → 10:90
in 20 min detected at 400 nm

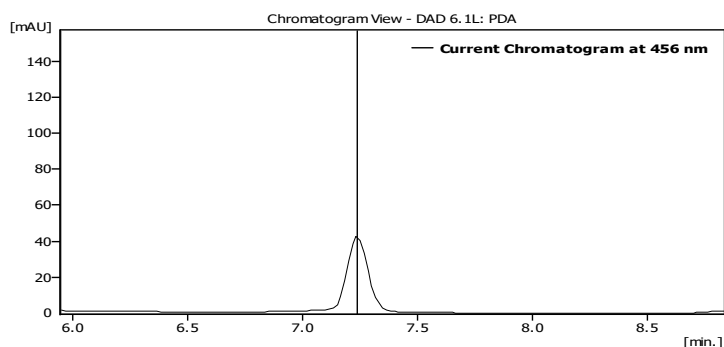
Yield 93 %

ESI-HRMS: found 795.6624 [M-2H]²⁻, calculated 795.6652

λ_{max} (absorption) = 454 nm

APTS + M





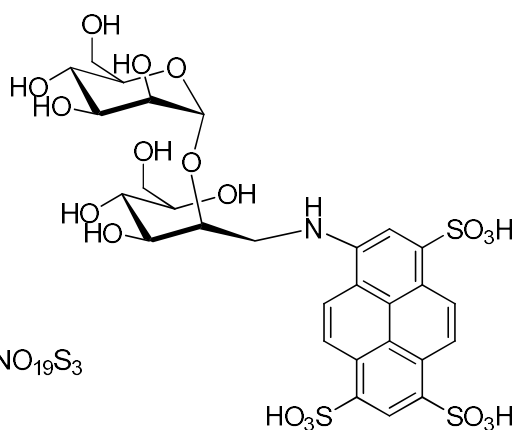
HPLC: $t_R = 7.2$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 30:70
in 20 min detected at 450 nm

Yield 100 %.

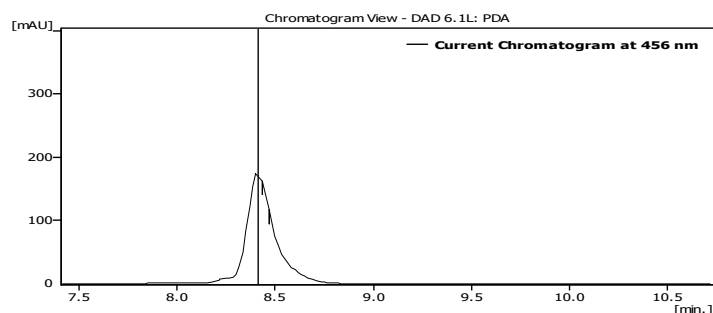
ESI-HRMS: found 309.5065 $[M-2H]^{2-}$, calculated 309.5068; found 206.0022 $[M-3H]^{3-}$,
calculated 206.0021

λ_{max} (absorption) = 456 nm

APTS + M₂ (2-O)



Chemical Formula: $C_{28}H_{33}NO_{19}S_3$
Exact Mass: 783.08



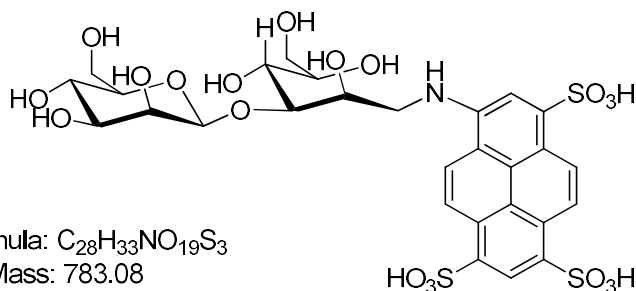
HPLC: $t_R = 8.4$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 450 nm

Yield 87 %

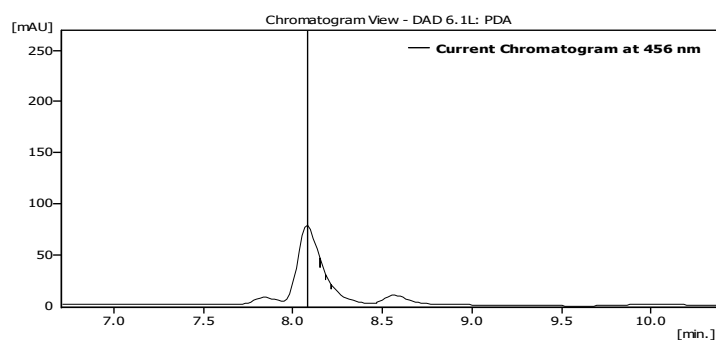
ESI-HRMS: found 782.0741 [M-H]⁻, calculated 782.0736; found 390.5335 [M-2H]²⁻, calculated 390.5332

λ_{\max} (absorption) = 456 nm

APTS + M₂ (3-O)



Chemical Formula: C₂₈H₃₃NO₁₉S₃
Exact Mass: 783.08



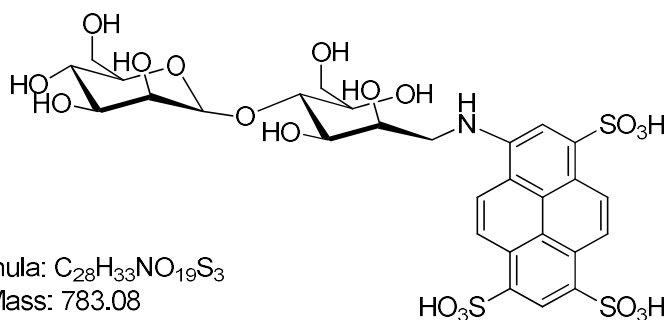
HPLC: t_R = 8.1 min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75 in 20 min detected at 450 nm

Yield 77 %

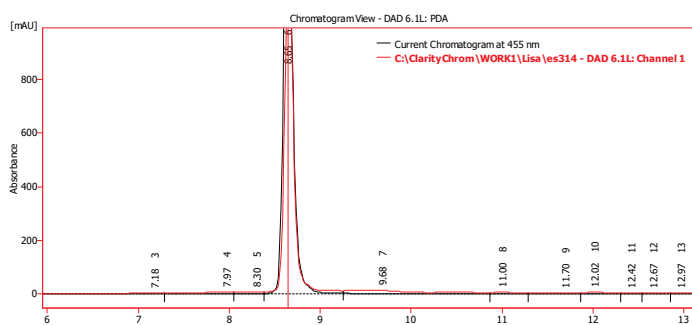
ESI-HRMS: found 782.0734 [M-H]⁻, calculated 782.0736; found 390.5332 [M-2H]²⁻, calculated 390.5332

λ_{\max} (absorption) = 456 nm

APTS + M₂ (4-O)



Chemical Formula: C₂₈H₃₃NO₁₉S₃
Exact Mass: 783.08

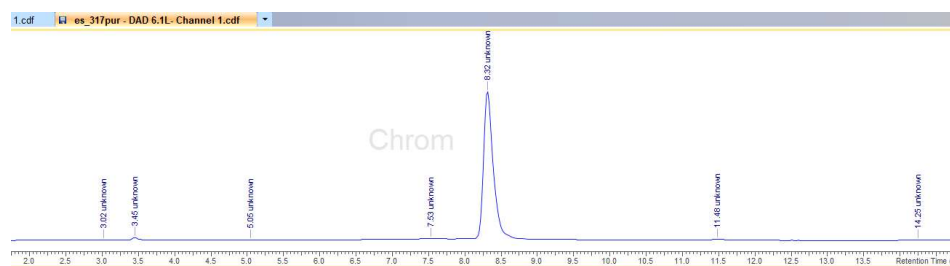
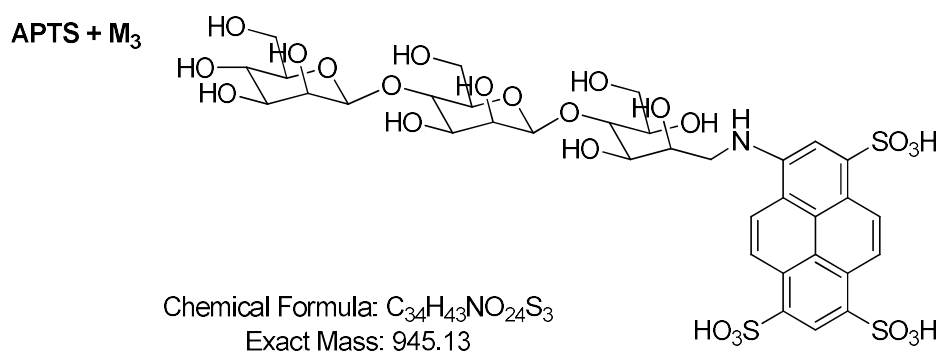


HPLC: $t_R = 8.6$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 450 nm

Yield 93 %

ESI-HRMS: found 782.0720 $[M-H]^-$, calculated 782.0736; found 390.5332 $[M-2H]^{2-}$,
calculated 390.5332

λ_{max} (absorption) = 455 nm



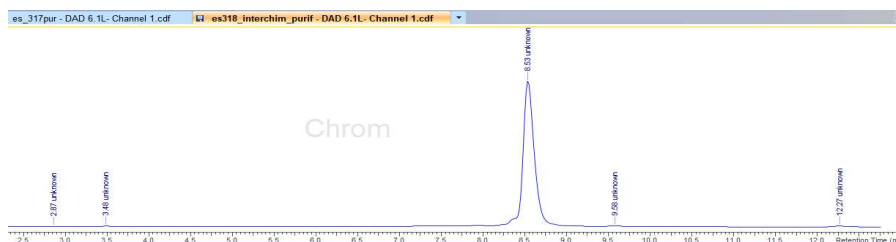
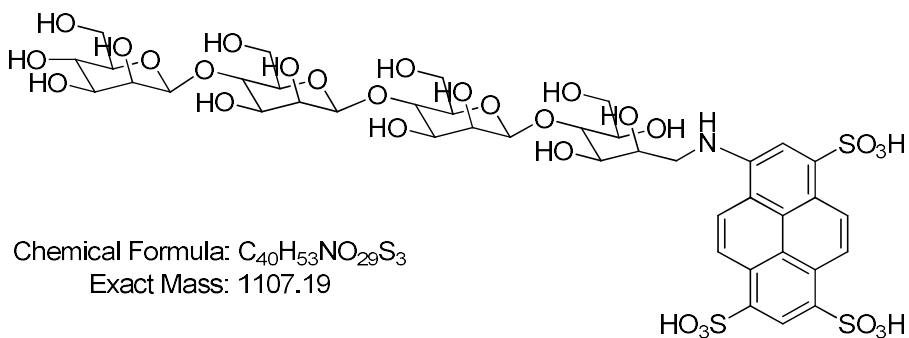
HPLC: $t_R = 8.3$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 450 nm

Yield 80 %

ESI-HRMS: found 944.1251 $[M-H]^-$, calculated 944.1264; found 471.5595 $[M-2H]^{2-}$,
calculated 471.5596

λ_{max} (absorption) = 454 nm

APTS + M₄



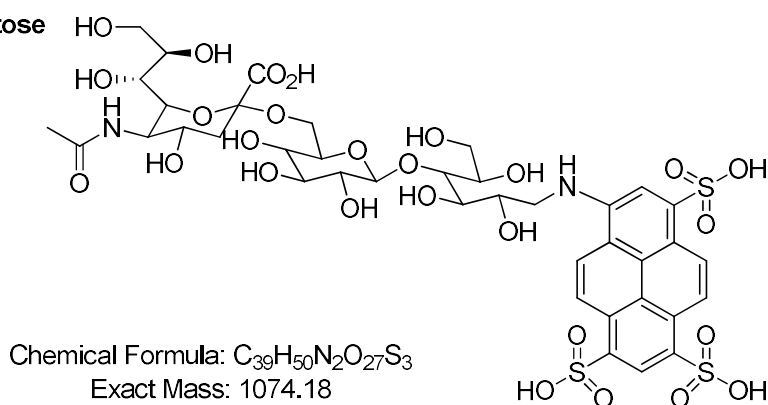
HPLC: $t_R = 8.5$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75
in 20 min detected at 450 nm

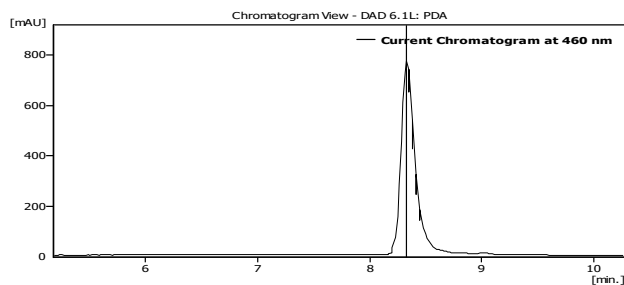
Yield 78 %

ESI-HRMS: found 1106.1776 [M-H]⁻; calculated 1106.1793; found 552.5864 [M-2H]²⁻,
calculated 552.5860

λ_{max} (absorption) = 454 nm

APTS + 6'-sialyllactose





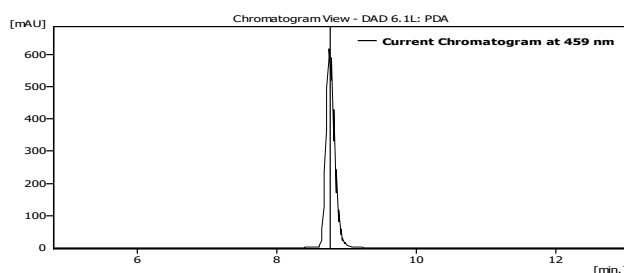
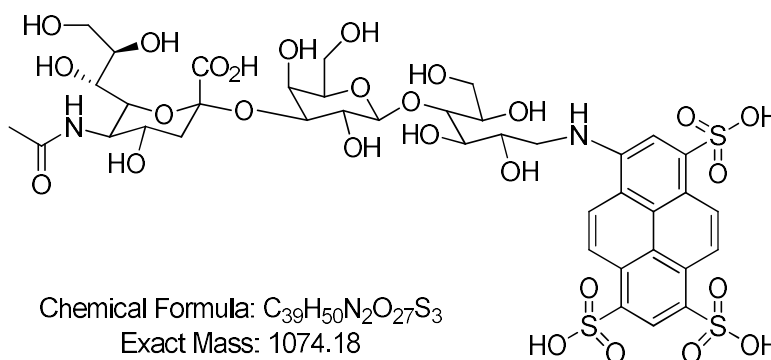
HPLC: $t_R = 8,3$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 450 nm

Yield 24 %

ESI-HRMS: found 536.0817 $[M-2H]^{2-}$, calculated 536.0809; found 357.0512 $[M-3H]^{3-}$,
calculated 357.0515

λ_{max} (absorption) = 460 nm

APTS + 3'-sialyllactose



HPLC: $t_R = 8.7$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 450 nm

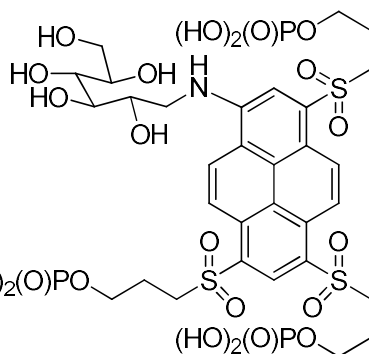
Yield 22 %

ESI-HRMS: found 536.0813 $[M-2H]^{2-}$, calculated 536.0809; found 357.0507 $[M-3H]^{3-}$,
calculated 357.0515

λ_{max} (absorption) = 459 nm

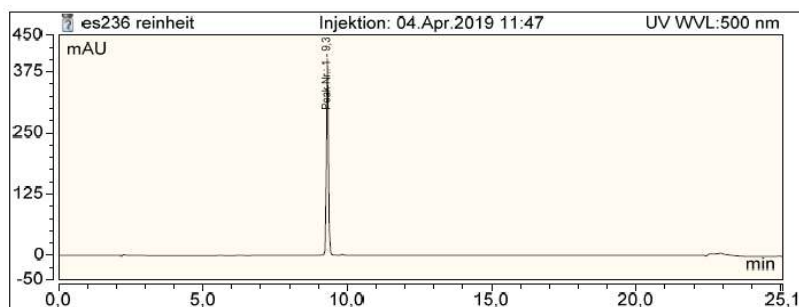
Conjugates of dye 16

16 + G



Chemical Formula: $C_{31}H_{44}NO_{23}P_3S_3$
Exact Mass: 987.07

Gradient : A 5,0 % B 95,0 % ----> A 30,0 % B 70,0 % T = 20 Min.



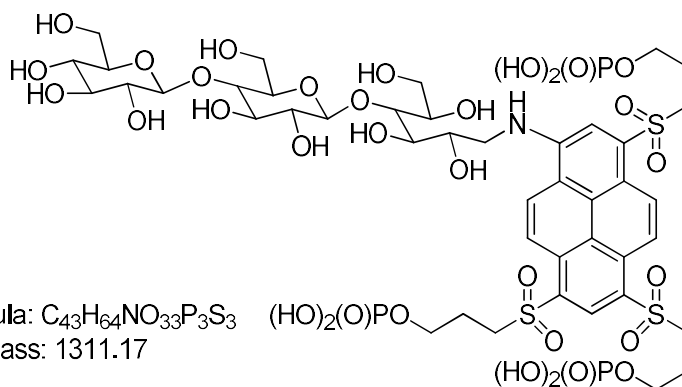
HPLC: $t_R = 9.3$ min (Column A; MeCN (A)/TEAB 0.05 M in water (B)), 5:95 \rightarrow 30:70
in 20 min detected at 500 nm

Yield 93 %

ESI-HRMS: found 986.0612 $[M-H]^-$, calculated 986.0606; found 492.5271 $[M-2H]^{2-}$,
calculated 492.5267

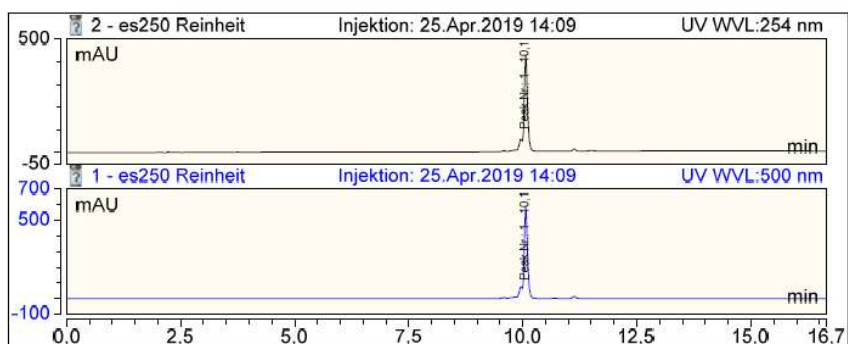
λ_{max} (absorption) = 502 nm

16 + G₃



Chemical Formula: $C_{43}H_{64}NO_{33}P_3S_3$
Exact Mass: 1311.17

Gradient : A 5,0 % B 95,0 % ----> A 30,0 % B 70,0 % T = 20 Min.



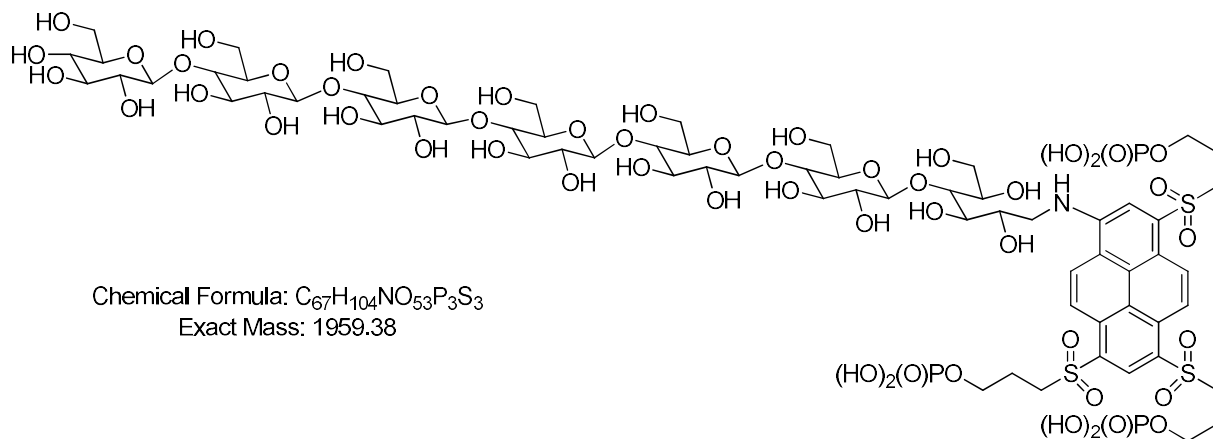
HPLC: $t_R = 10.1$ min (Column A; MeCN (A)/TEAB 0.05 M in water (B)), 5:95 \rightarrow 30:70 in 20 min detected at 500 nm

Yield 90 %

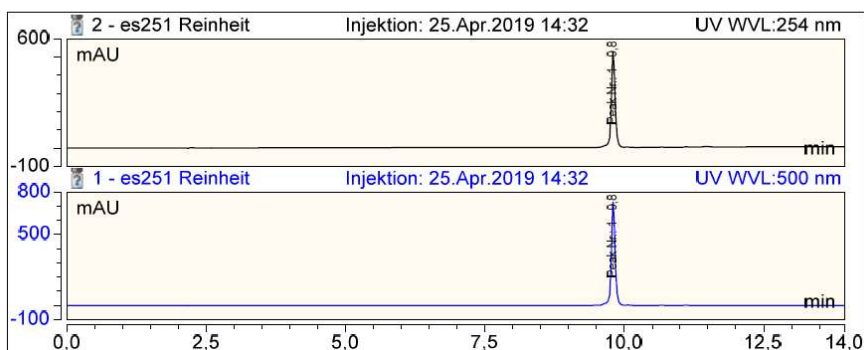
ESI-HRMS: found 1334.1639 $[M+Na]^+$, calculated 1334.1628; found 654.5797 $[M-2H]^{2-}$, calculated 654.5795

λ_{max} (absorption) = 504 nm

16 + G₇



Gradient : A 5,0 % B 95,0 % ----> A 30,0 % B 70,0 % T = 20 Min.



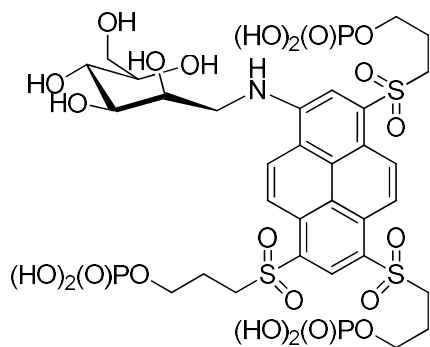
HPLC: $t_R = 9.8$ min (Column A; MeCN (A)/TEAB 0.05 M in water (B)), 5:95 → 30:70
in 20 min detected at 500 nm

Yield 73 %

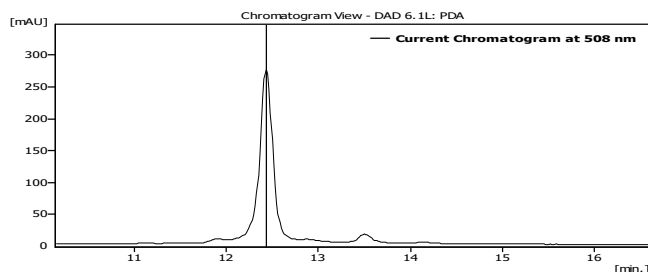
ESI-HRMS: found 2004.6557 $[M-2Na+H]^+$, calculated 2004.3560; found 1958.3786 $[M-H]^-$, calculated 1958.3776; found 978.6857 $[M-2H]^{2-}$, calculated 978.6851

λ_{max} (absorption) = 504 nm

16 + M



Chemical Formula: $C_{31}H_{44}NO_{23}P_3S_3$
Exact Mass: 987.07



HPLC: $t_R = 12.4$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75
in 20 min detected at 500 nm

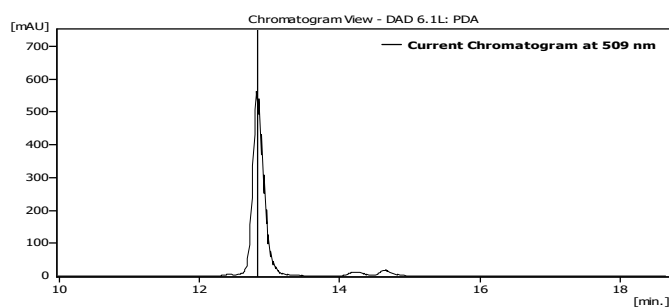
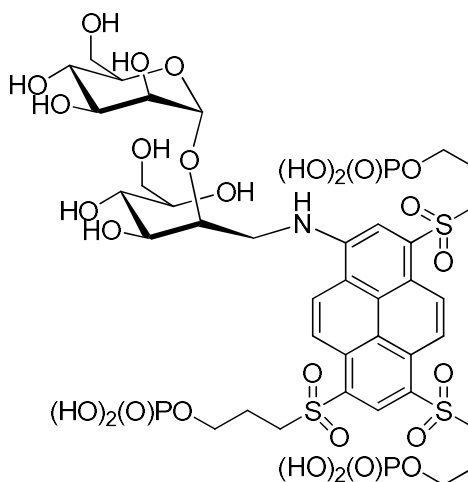
Yield 92 %

ESI-HRMS: found 492.5263 [M-2H]²⁻, calculated 492.5267; found 328.0154 [M-3H]³⁻,
calculated 328.0154

λ_{\max} (absorption) = 508 nm

16 + M₂ (2-O)

Chemical Formula: C₃₇H₅₄NO₂₈P₃S₃
Exact Mass: 1149.12



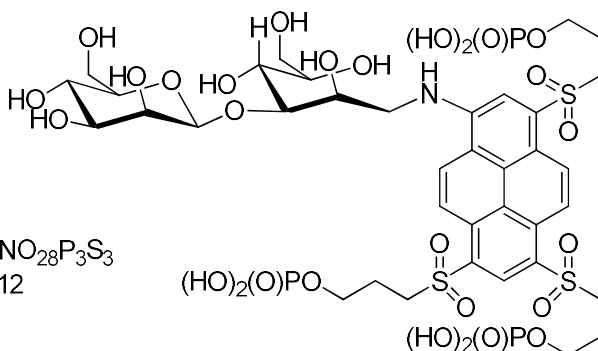
HPLC: t_R = 12.8 min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75
in 20 min detected at 500 nm

Yield 35 %

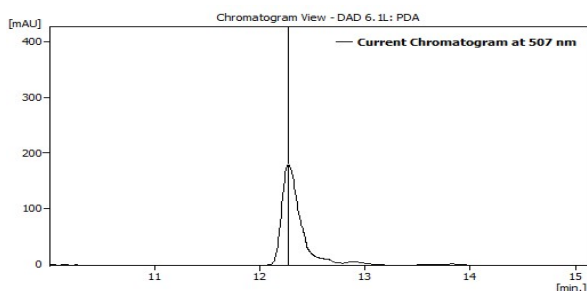
ESI-HRMS: found 1148.1135 [M-H]⁻, calculated 1148.1135; found 573.5535 [M-2H]²⁻,
calculated 573.5531

λ_{\max} (absorption) = 509 nm

16 + M₂ (3-O)



Chemical Formula: C₃₇H₅₄NO₂₈P₃S₃
Exact Mass: 1149.12



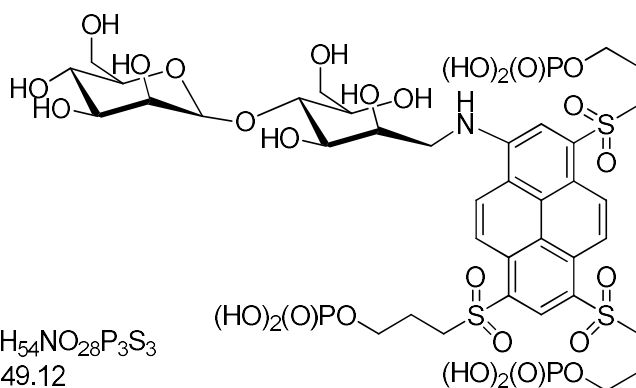
HPLC: $t_R = 12.3$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75
in 20 min detected at 500 nm

Yield 54 %

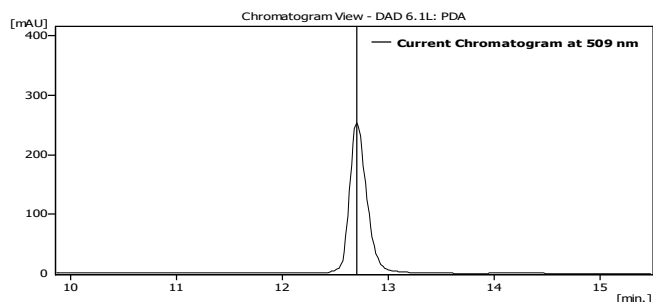
ESI-HRMS: found 1148.1135 [M-H]⁻, calculated 1148.1135; found 573.5537 [M-2H]²⁻,
calculated 573.5531

λ_{max} (absorption) = 507 nm

16 + M₂ (4-O)



Chemical Formula: C₃₇H₅₄NO₂₈P₃S₃
Exact Mass: 1149.12



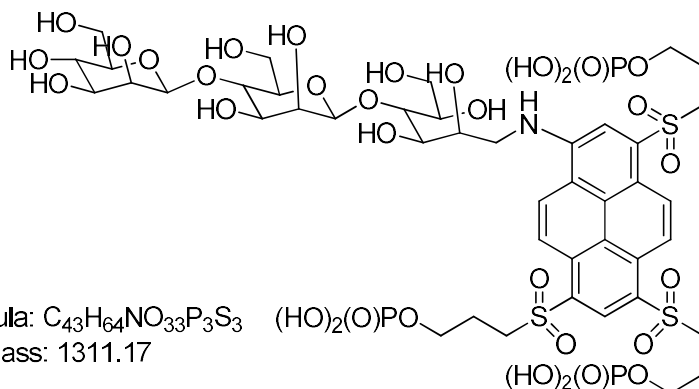
HPLC: $t_R = 12.7$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75
in 20 min detected at 500 nm

Yield 69 %

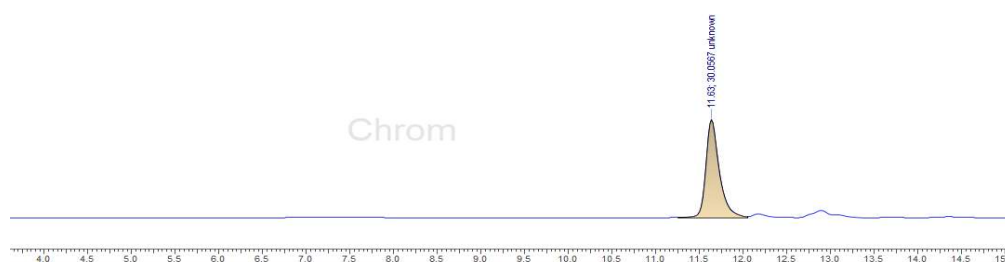
ESI-HRMS: found 1148.1133 [M-H]⁻, calculated 1148.1135; found 573.5539 [M-2H]²⁻,
calculated 573.5531

λ_{max} (absorption) = 509 nm

16 + M₃



Chemical Formula: C₄₃H₆₄NO₃₃P₃S₃
Exact Mass: 1311.17



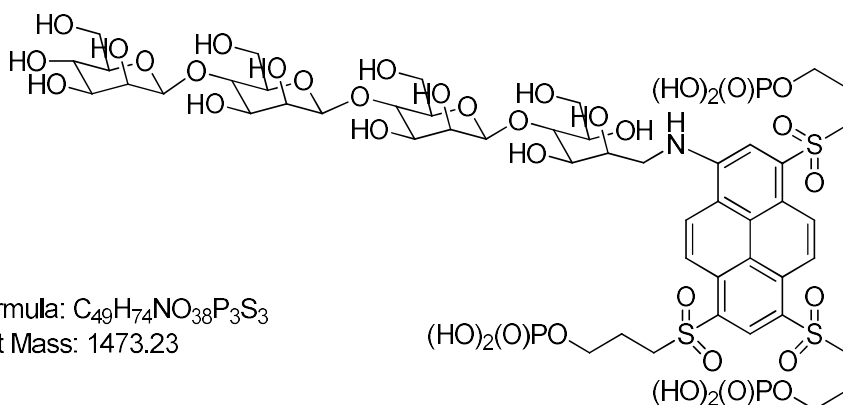
HPLC: $t_R = 11.6$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75
in 20 min detected at 500 nm

Yield 23 %

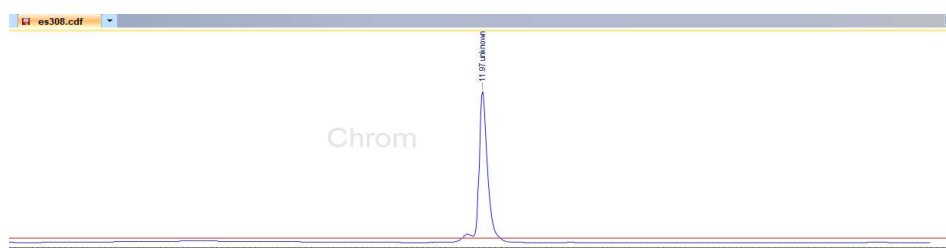
ESI-HRMS: found 654.5794 [M-2H]²⁻, calculated 654.5795; found 436.0511 [M-3H]³⁻,
calculated 436.0506

λ_{max} (absorption) = 509 nm

16 + M₄



Chemical Formula: C₄₉H₇₄NO₃₈P₃S₃
Exact Mass: 1473.23



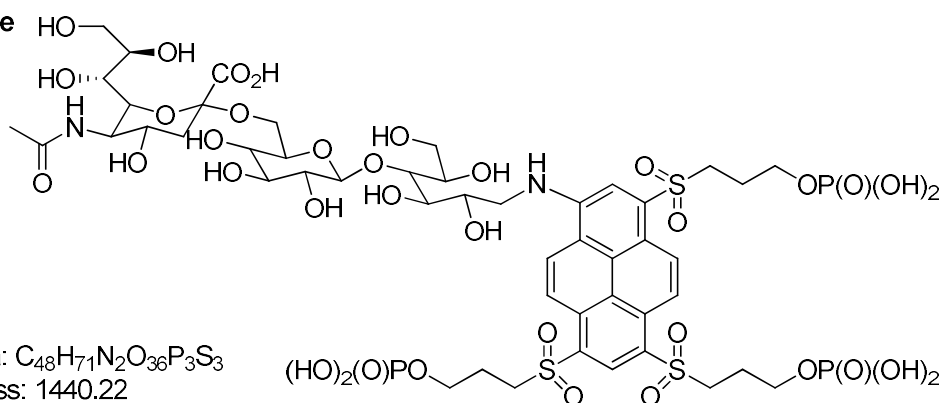
HPLC: $t_R = 12.0$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 → 25:75
in 20 min detected at 500 nm

Yield 30 %

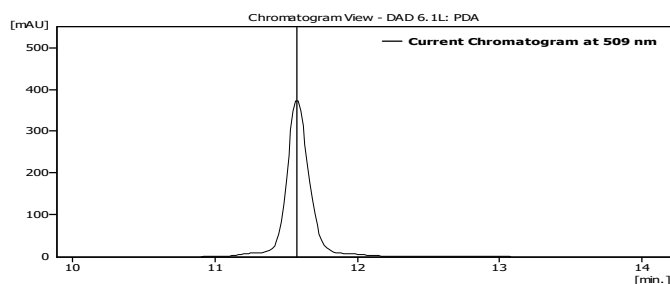
ESI-HRMS: found 735.6080 [M-2H]²⁻, calculated 735.6059; found 490.0685 [M-3H]³⁻,
calculated 490.0682

λ_{max} (absorption) = 505 nm

16 + 6'-sialyllactose



Chemical Formula: C₄₈H₇₁N₂O₃₆P₃S₃
Exact Mass: 1440.22



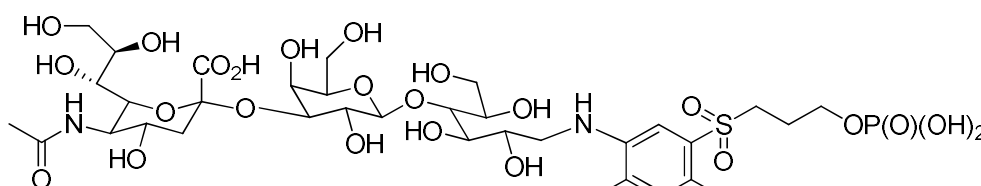
HPLC: $t_R = 11.6$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 500 nm

Yield 24 %

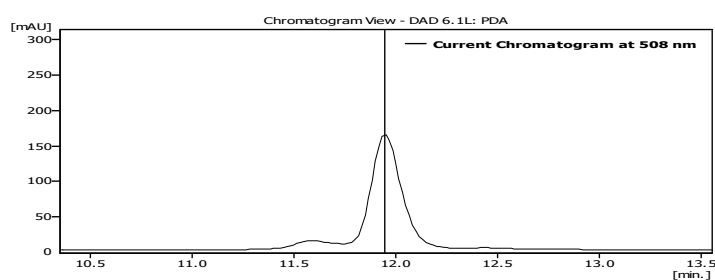
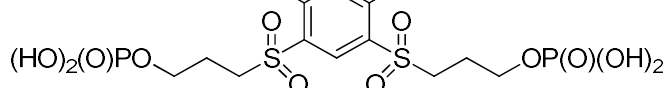
ESI-HRMS: found 719.1016 $[M-2H]^{2-}$, calculated 719.1008; found 479.0647 $[M-3H]^{3-}$,
calculated 479.0648

λ_{max} (absorption) = 509 nm

16 + 3'-sialyllactose



Chemical Formula: $C_{48}H_{71}N_2O_{36}P_3S_3$
Exact Mass: 1440.22



HPLC: $t_R = 11.9$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 500 nm

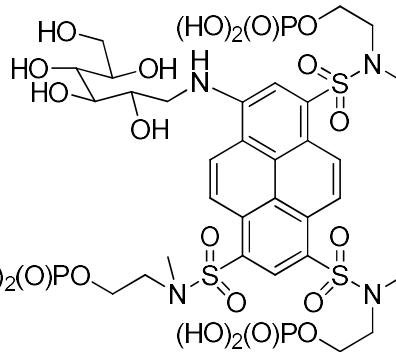
Yield 21 %

ESI-HRMS: found 359.0464 $[M-4H]^{4-}$, calculated 359.0468.

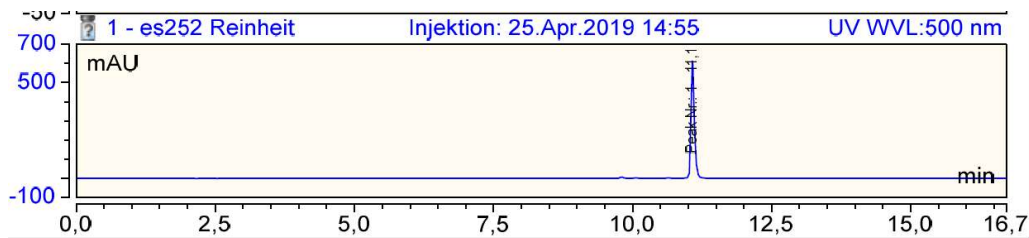
λ_{max} (absorption) = 508 nm

6-H-derived sugars

6-H + G



Chemical Formula: $C_{31}H_{47}N_4O_{23}P_3S_3$
Exact Mass: 1032.10



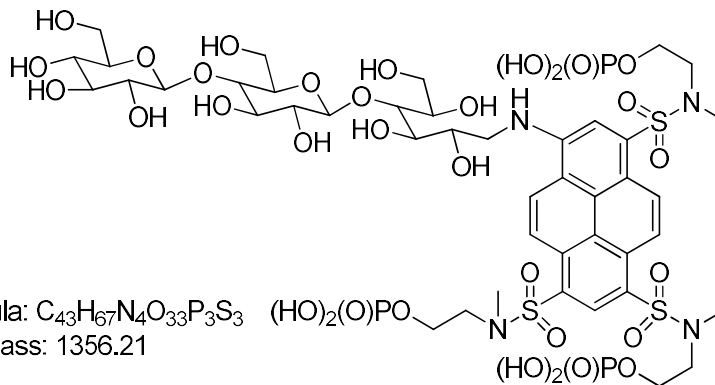
HPLC: $t_R = 11.1$ min (Column A; MeCN (A)/TEAB 0.05 M in water (B)), 5:95 → 30:70
in 20 min detected at 500 nm

Yield 100 %

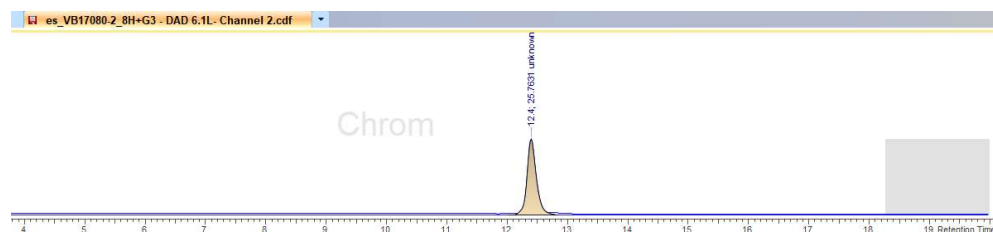
ESI-HRMS: found 515.0452 $[M-2H]^{2-}$, calculated 515.0430

λ_{max} (absorption) = 496 nm

6-H + G₃



Chemical Formula: $C_{43}H_{67}N_4O_{33}P_3S_3$
Exact Mass: 1356.21



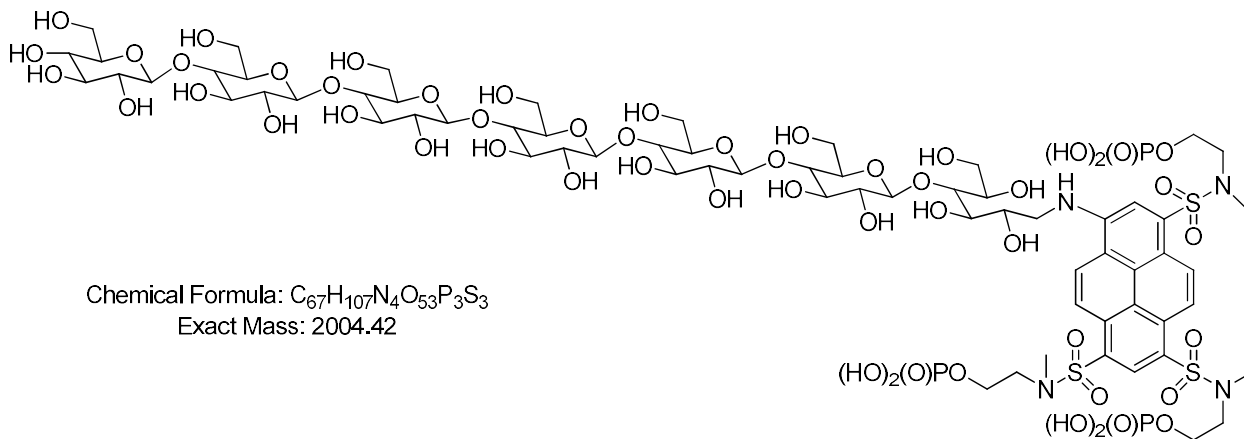
HPLC: $t_R = 12.4$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 500 nm

Yield 63 %

ESI-HRMS: found 677.0971 $[M-2H]^{2-}$, calculated 677.0958

λ_{max} (absorption) = 496 nm

6-H + G₇



HPLC: $t_R = 12.3$ min (Column B; MeCN (A)/TEAB 0.05 M in water (B)), 1:99 \rightarrow 25:75
in 20 min detected at 500 nm

Yield 90 %

ESI-HRMS: found 1001.2026 $[M-2H]^{2-}$, calculated 1001.2015

λ_{max} (absorption) = 496 nm

Polyacrylamide Gel Electrophoresis (PAGE)

Free dyes (APTS, **6-H** and **16** in Scheme S1) and their conjugates with sugars were analyzed by PAGE. Samples were applied to a 20% (w/v) polyacrylamide slab gel (0.7 mm x 230 mm x 300 mm) in TBE buffer (pH 8). The electrophoresis was performed at ambient temperature and constant power (35 W; 1750 - 2200 V) for 90 min. The bands were visualized by emission (excitation with 366 nm UV-lamp), and the pictures (Figure 2 in the main text, and Figures S5-S6) were taken by using a digital camera.

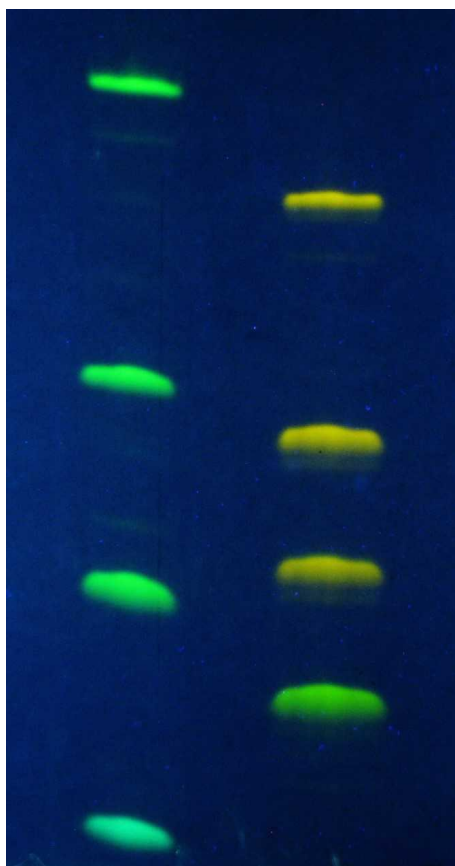


Figure S5. Gel electrophoresis results (migration from “north” to “south”, pH 8.3). For structures, see Scheme S1 and the previous section (*Characterization of Conjugates*). Left (reference) lane (from bottom to top): APTS (lowest bluish band), APTS+G, APTS+G₃ and APTS+G₇; right lane (from bottom to top): dye **6-H**, **6-H+G**, **6-H+G₃**, **6-H+G₇** (yellow bands). Spots were detected by emission (excitation at 365 nm).

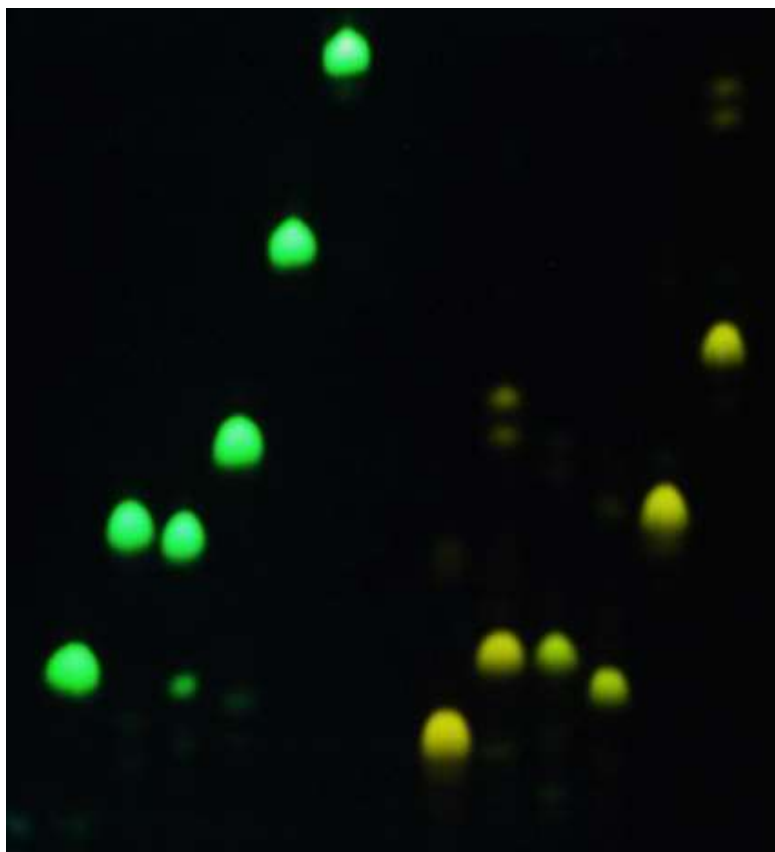
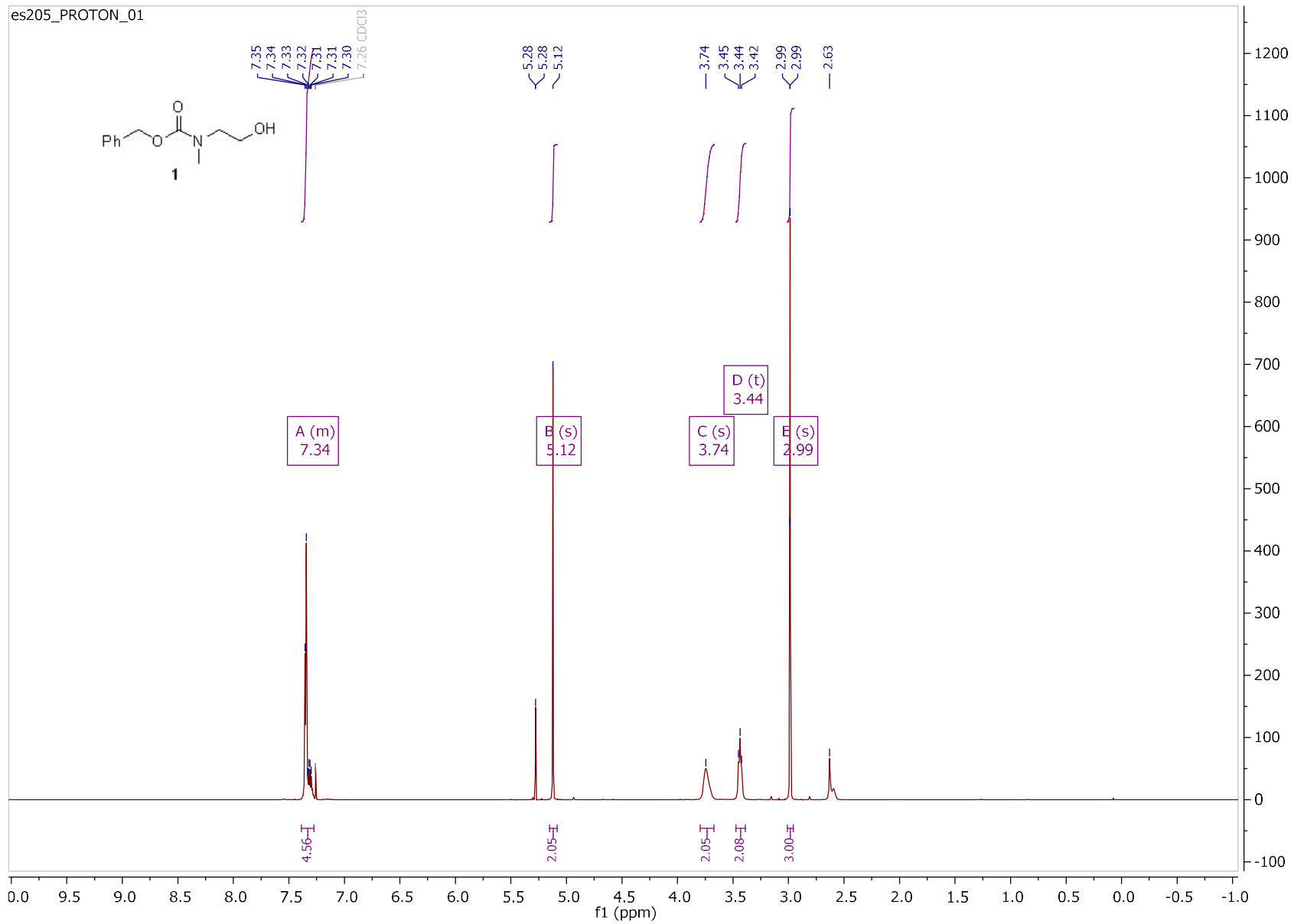
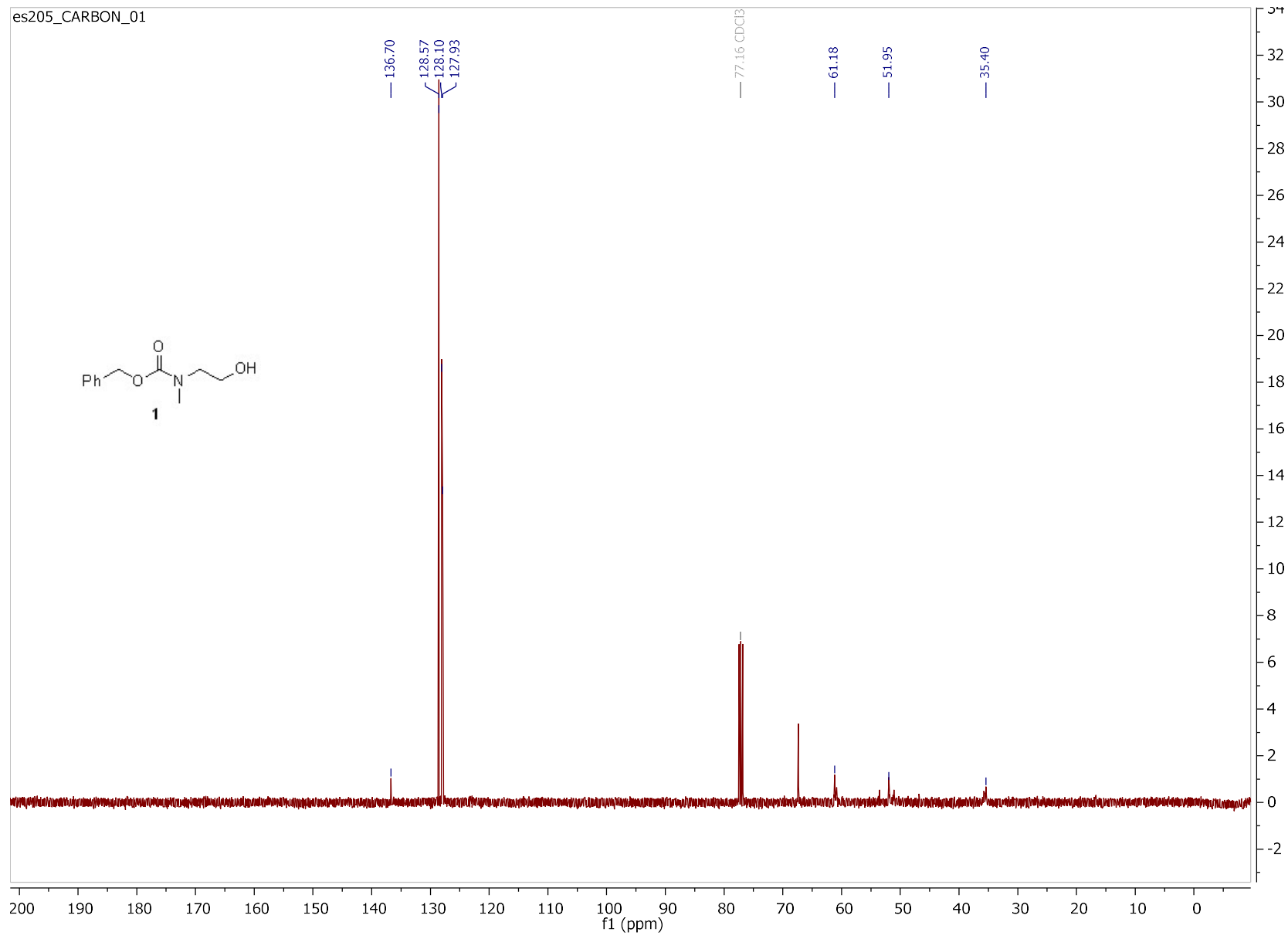
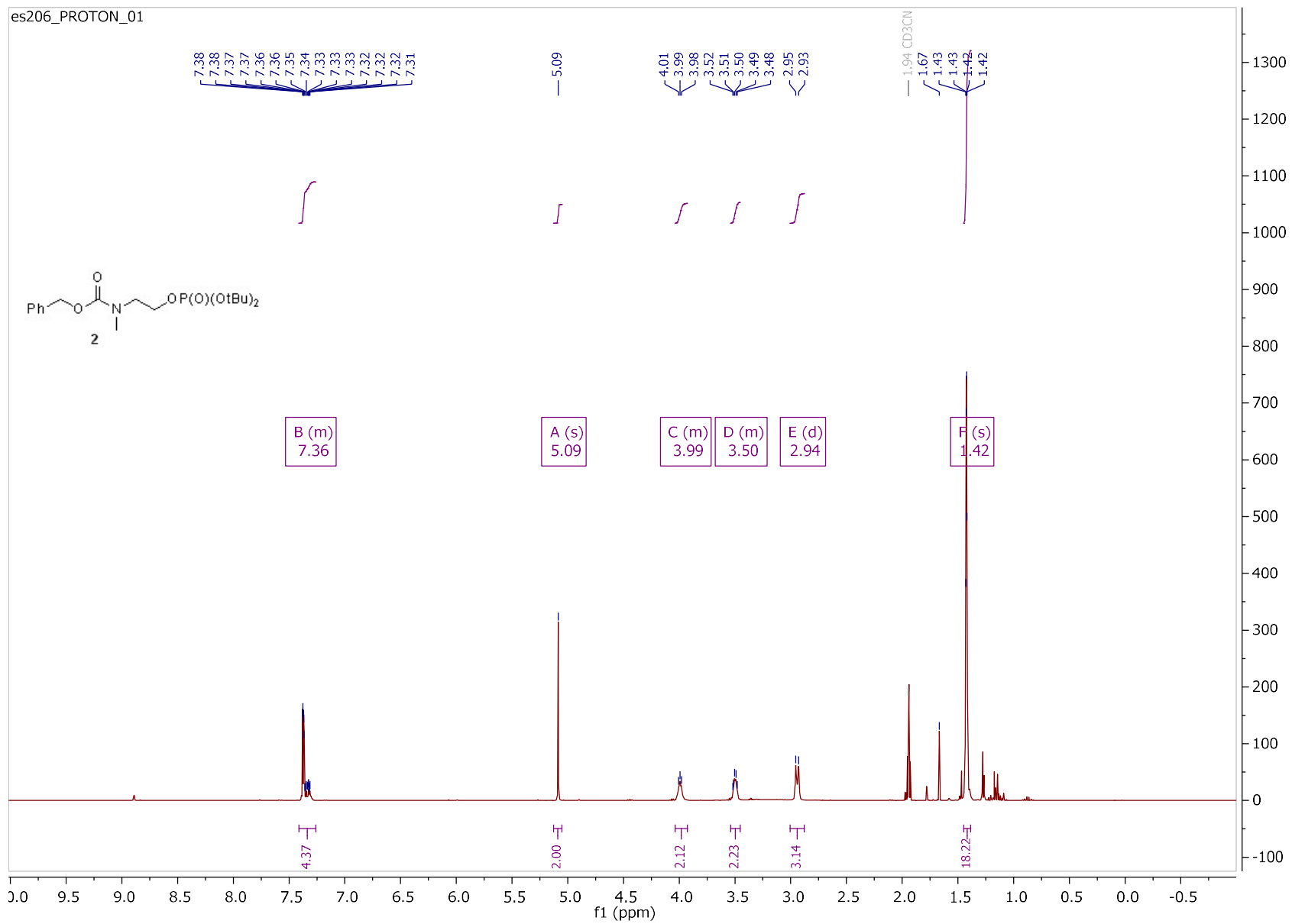


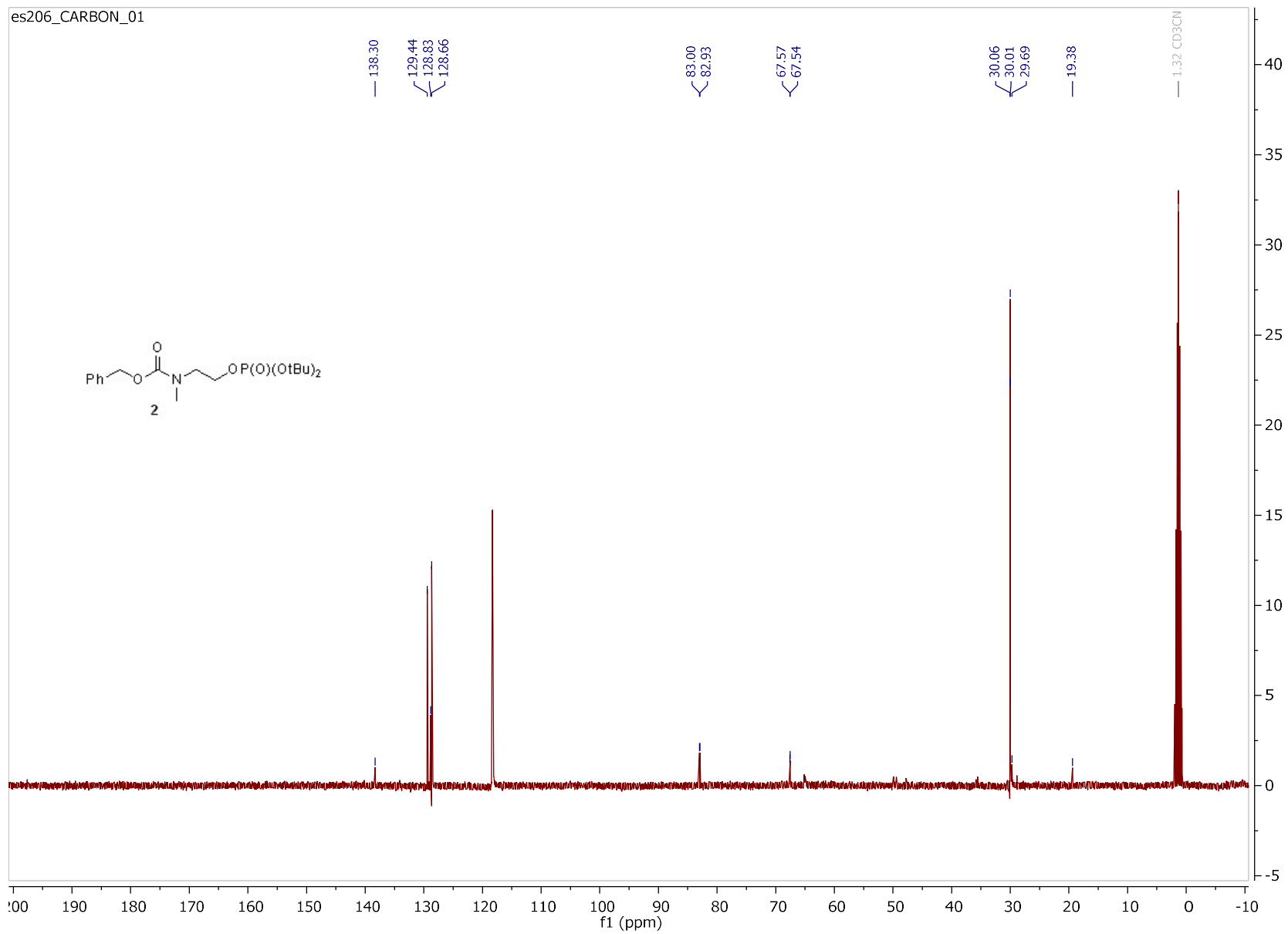
Figure S6. Gel electrophoresis of APTS conjugates with mannose and its oligomers (left part, green spots) and conjugates of dye **16** with mannose and its oligomers (right part, yellow spots); migration from “north” to “south”, pH 8.3. For structures, see Scheme S1 and the previous section (*Characterization of Conjugates*). Left part, green spots, from left to center: APTS+M, APTS+M₂-2O, APTS+M₂-3O, APTS+M₂-4O, APTS+M₃ and APTS+M₄. Right part, yellow spots, from center to right: **16**+M, **16**+M₂-4O, **16**+M₂-2O, **16**+M₂-3O, **16**+M₃ and **16**+M₄. Bands were detected by emission (excitation at 365 nm). Note that APTS+M₂-2O moves a bit slower than APTS+M₂-3O, **16**+M₂-2O and **16**+M₂-4O are undistinguishable, APTS+M₂-4O moves much slower than other dimers, **16**+M₂-3O moves faster than **16**+M₂-2O or **16**+M₂-4O.

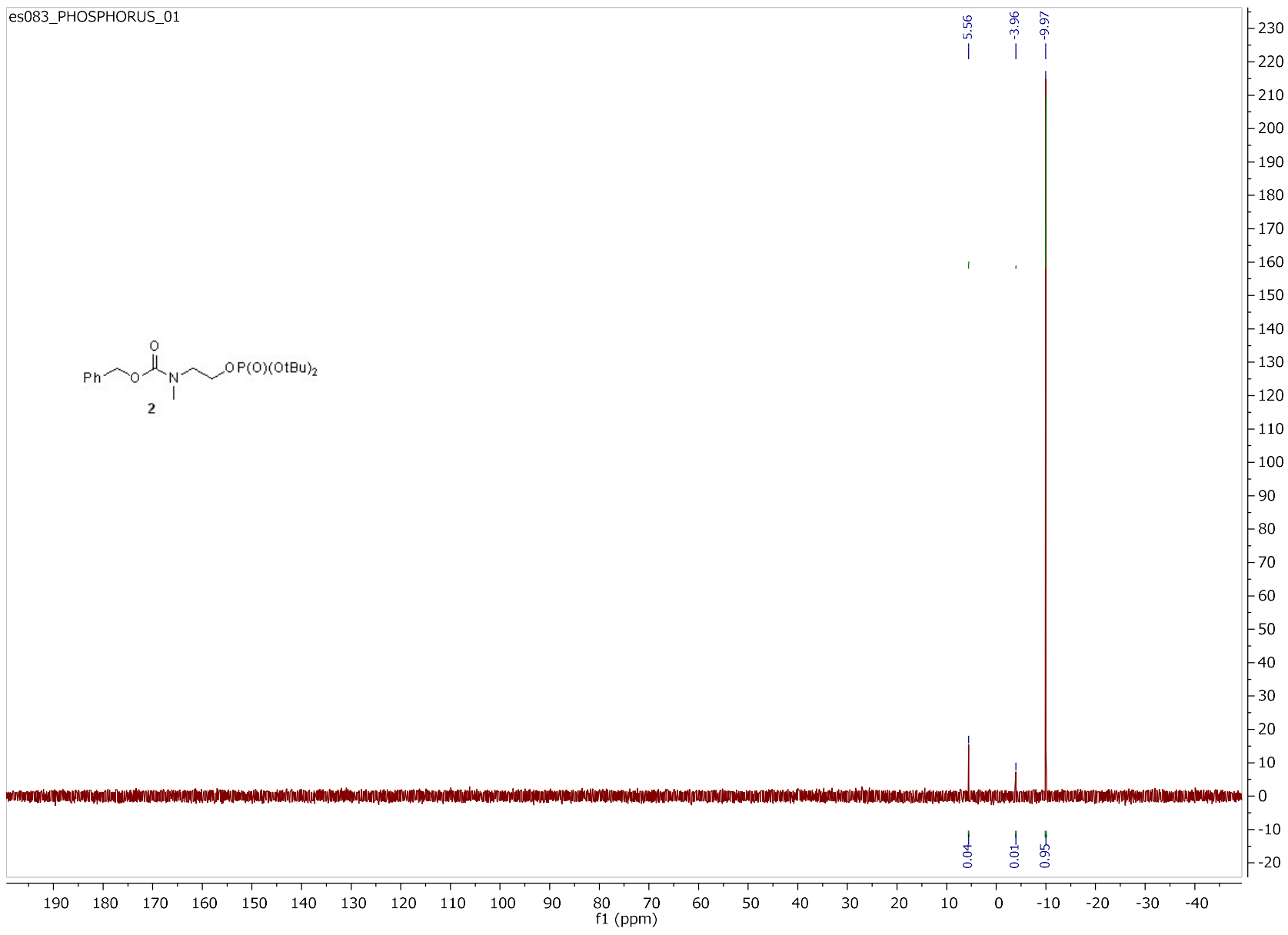
NMR spectra of new compounds

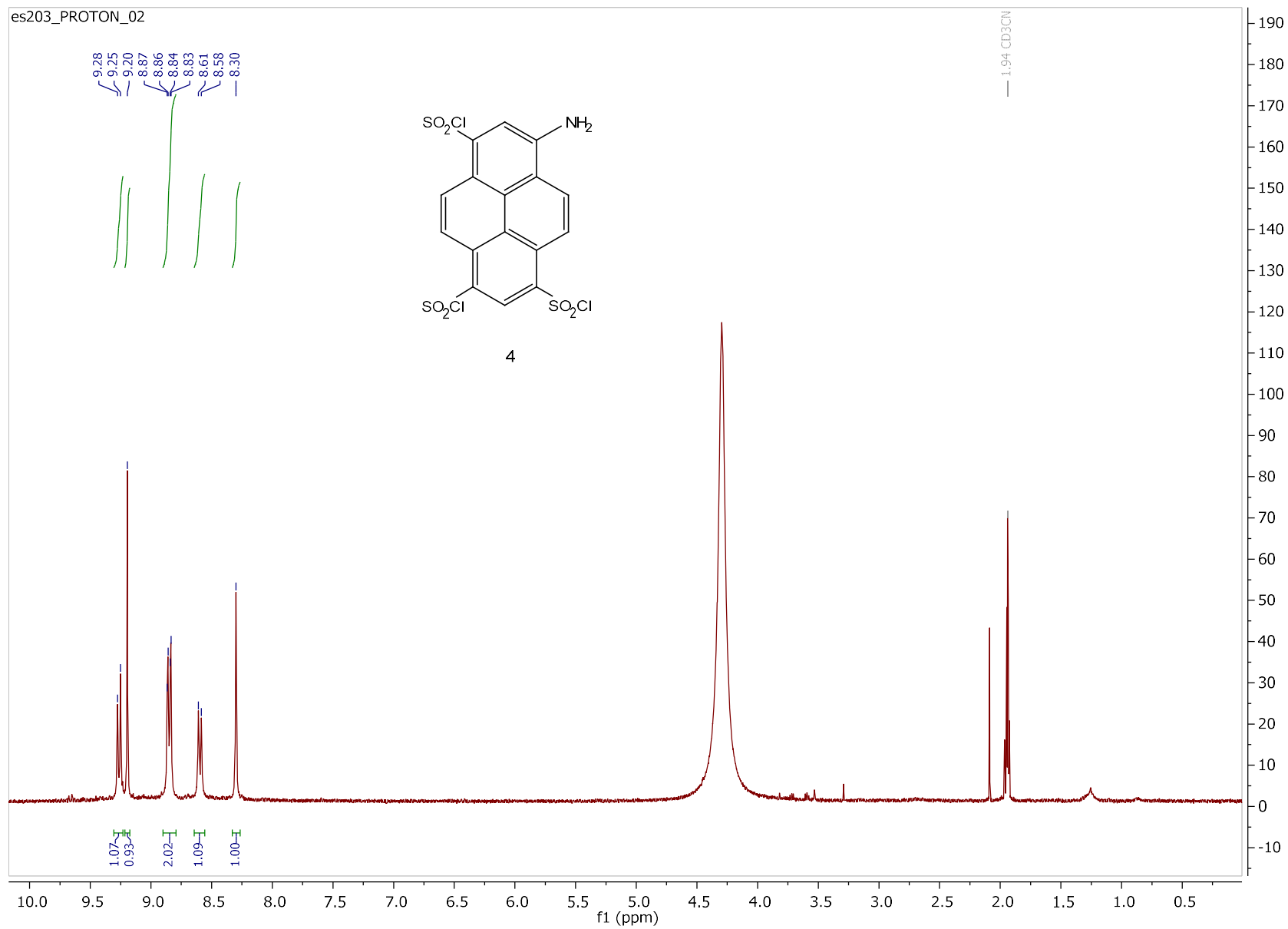


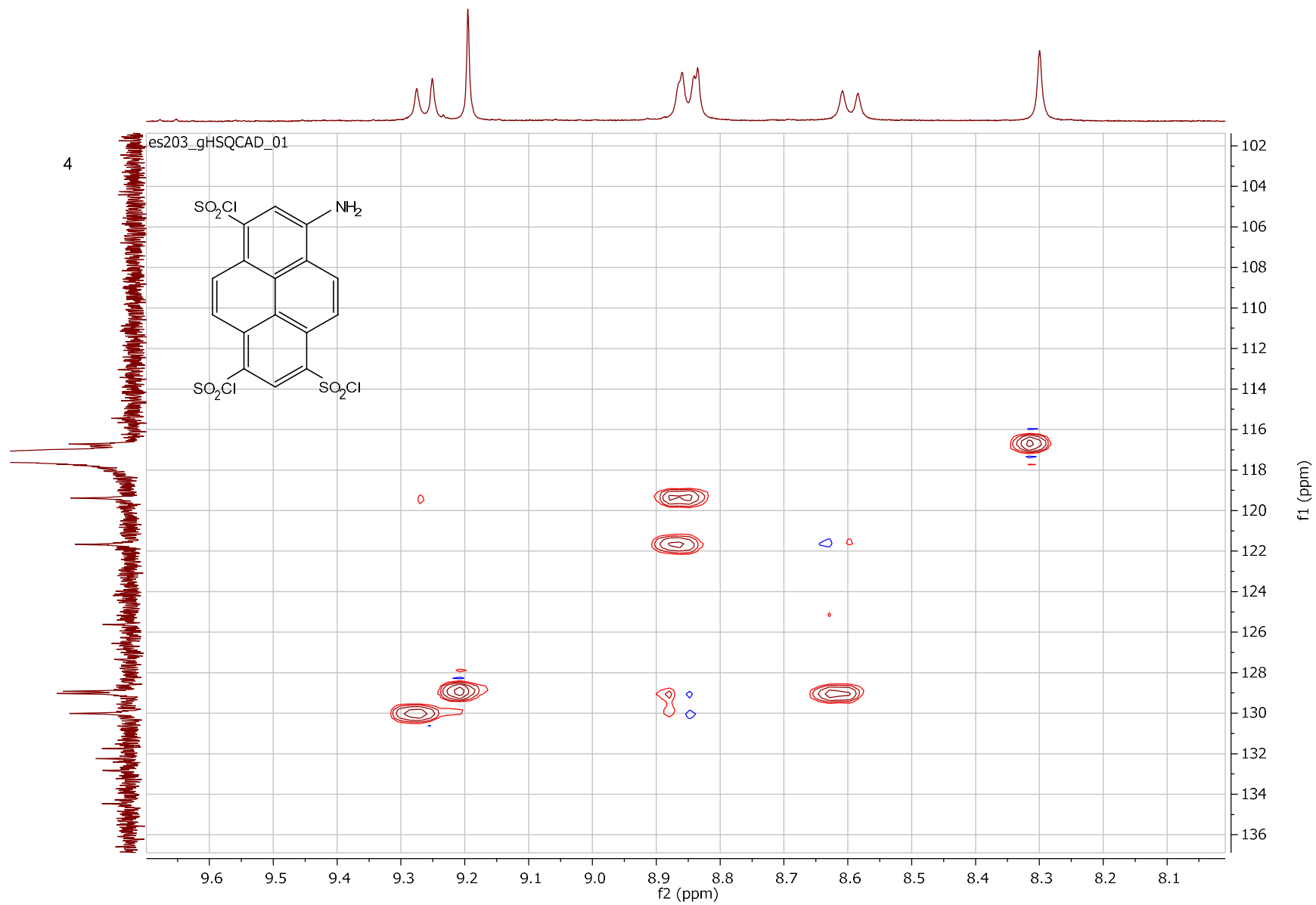


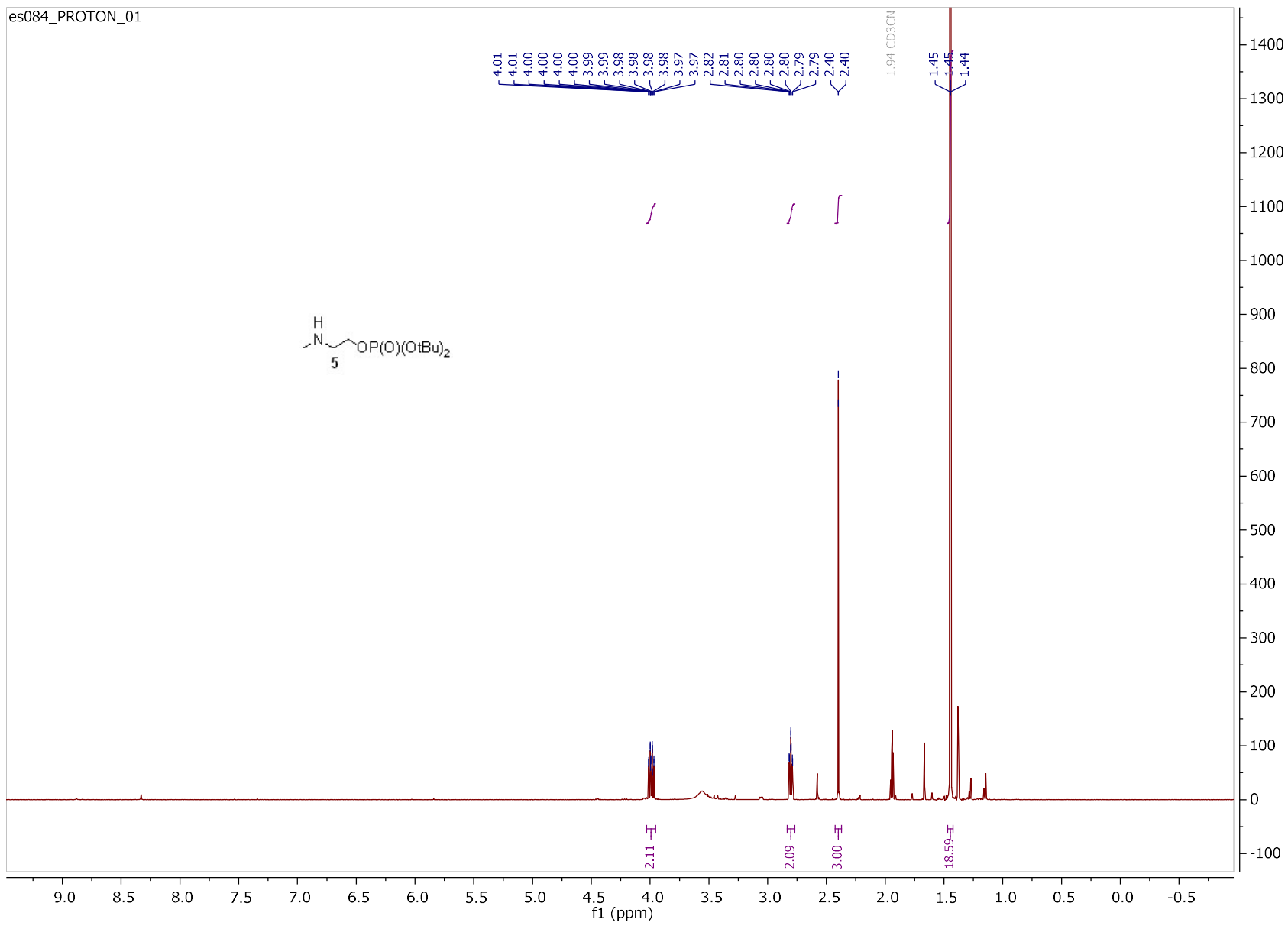


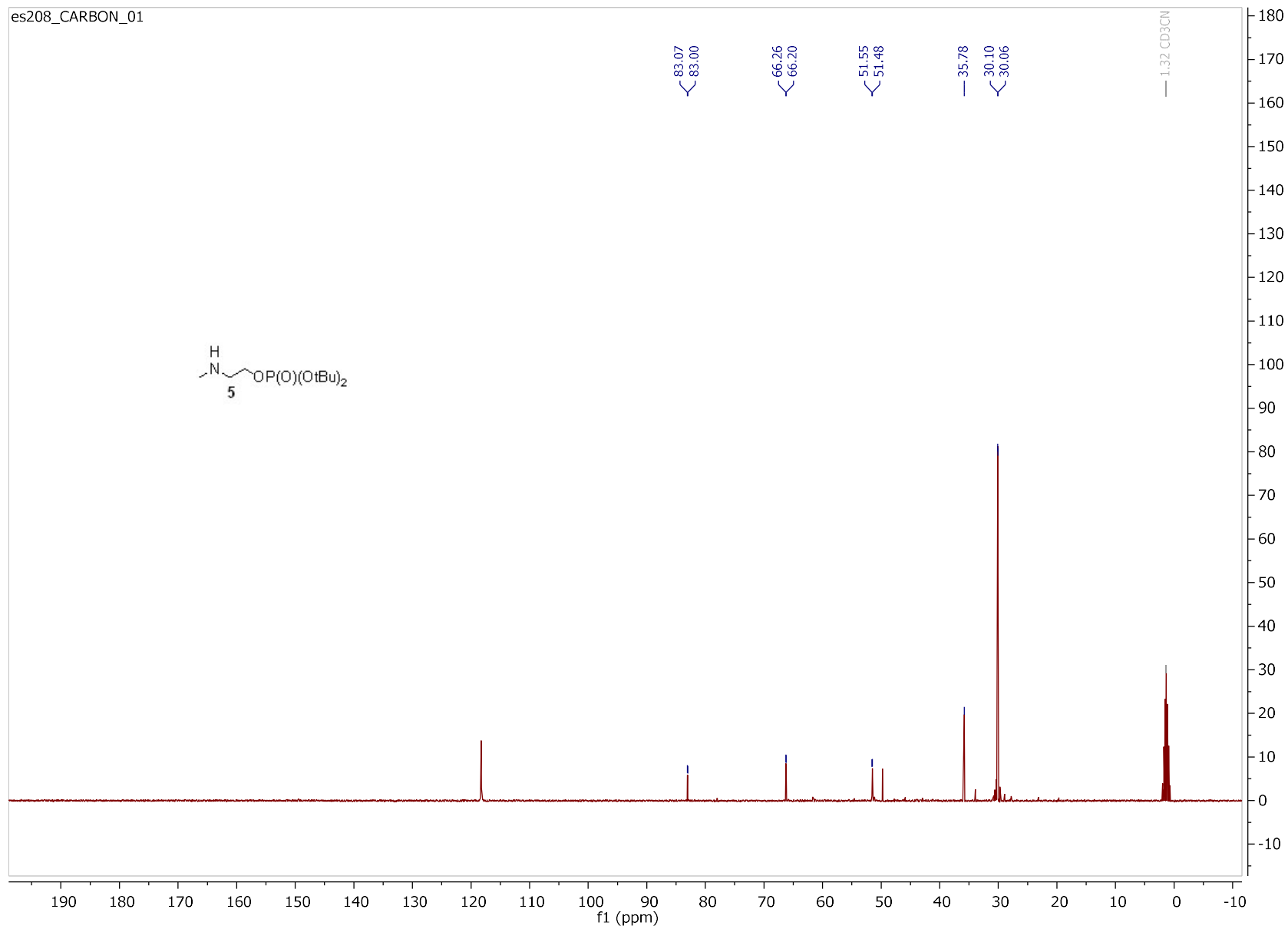


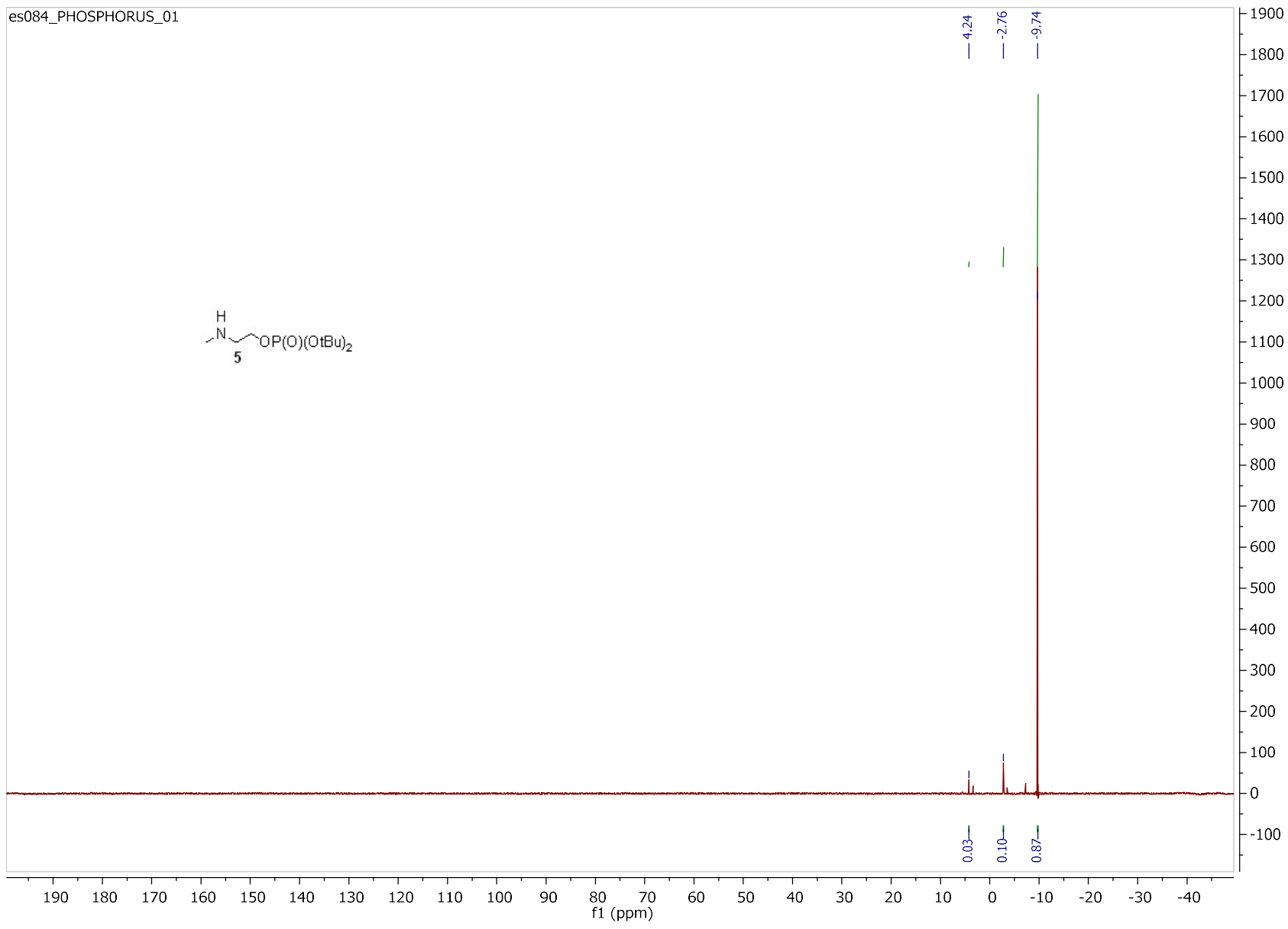


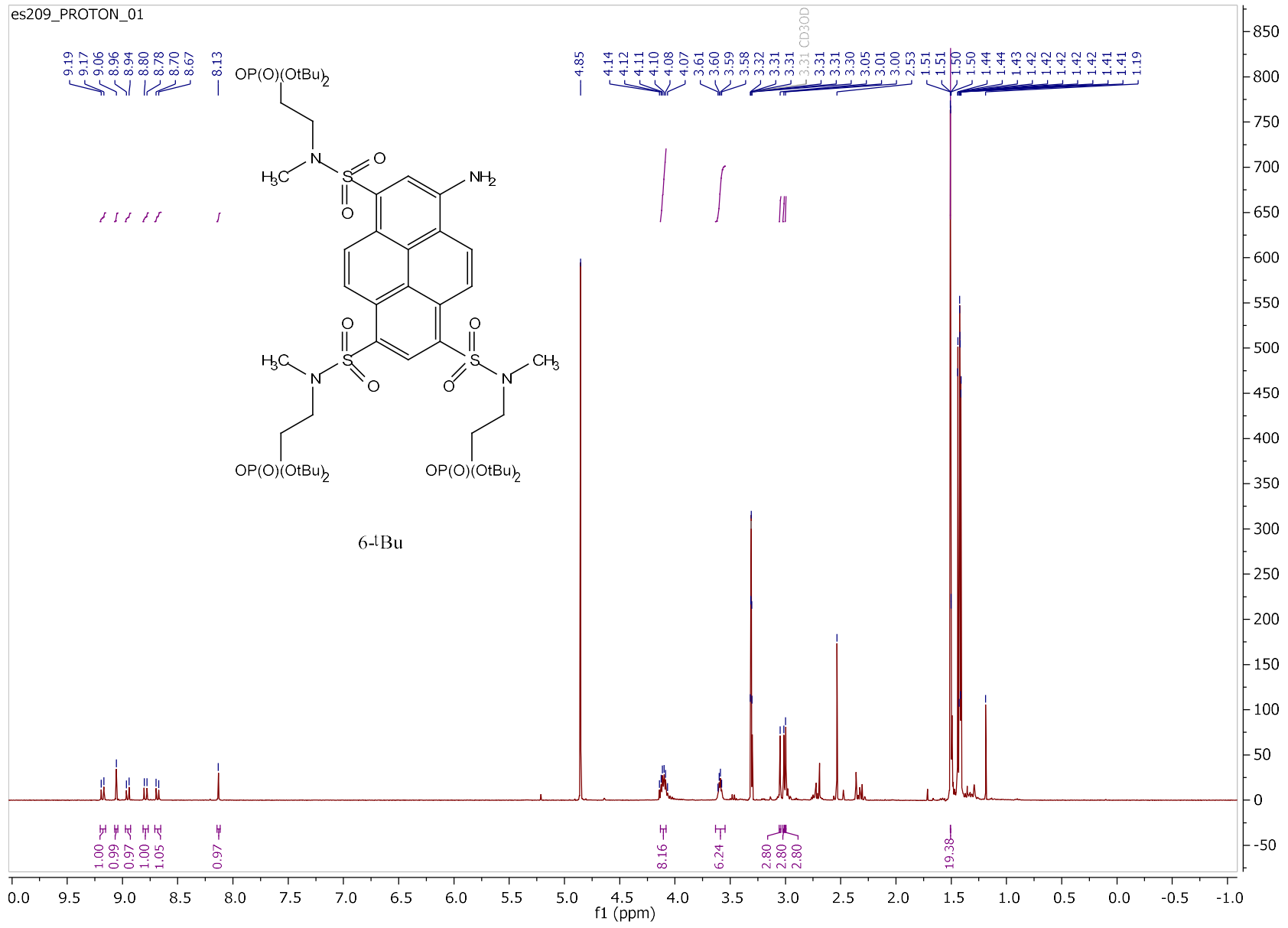




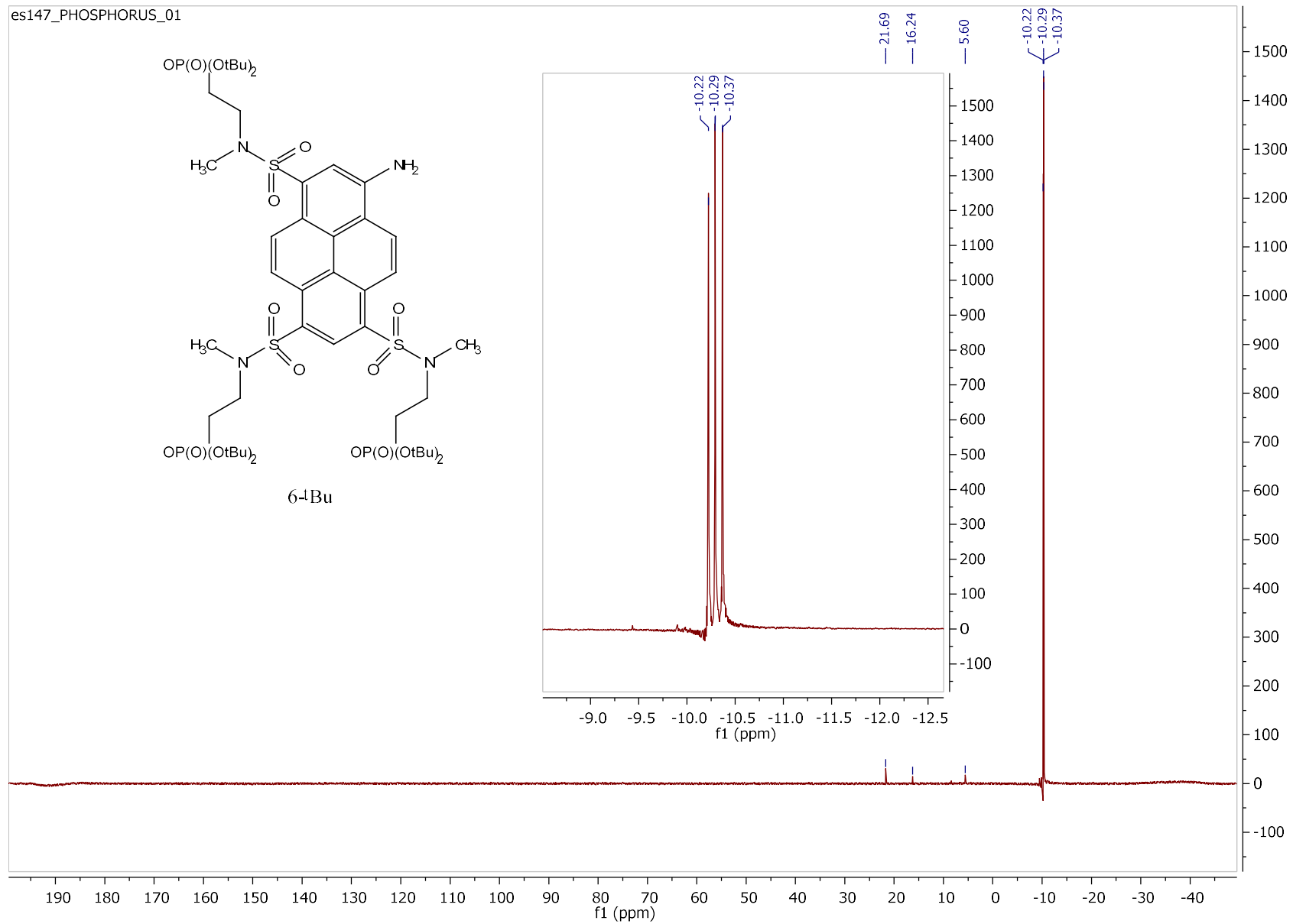
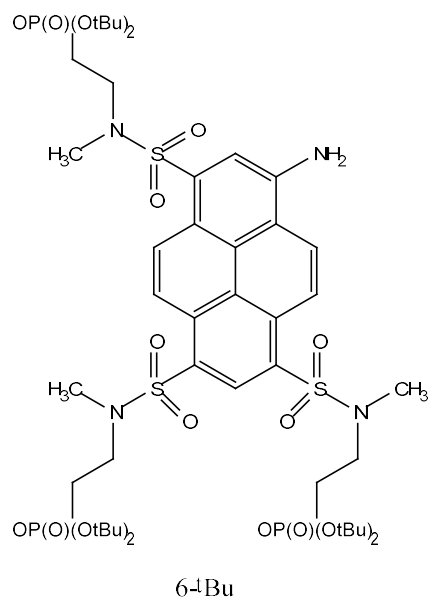




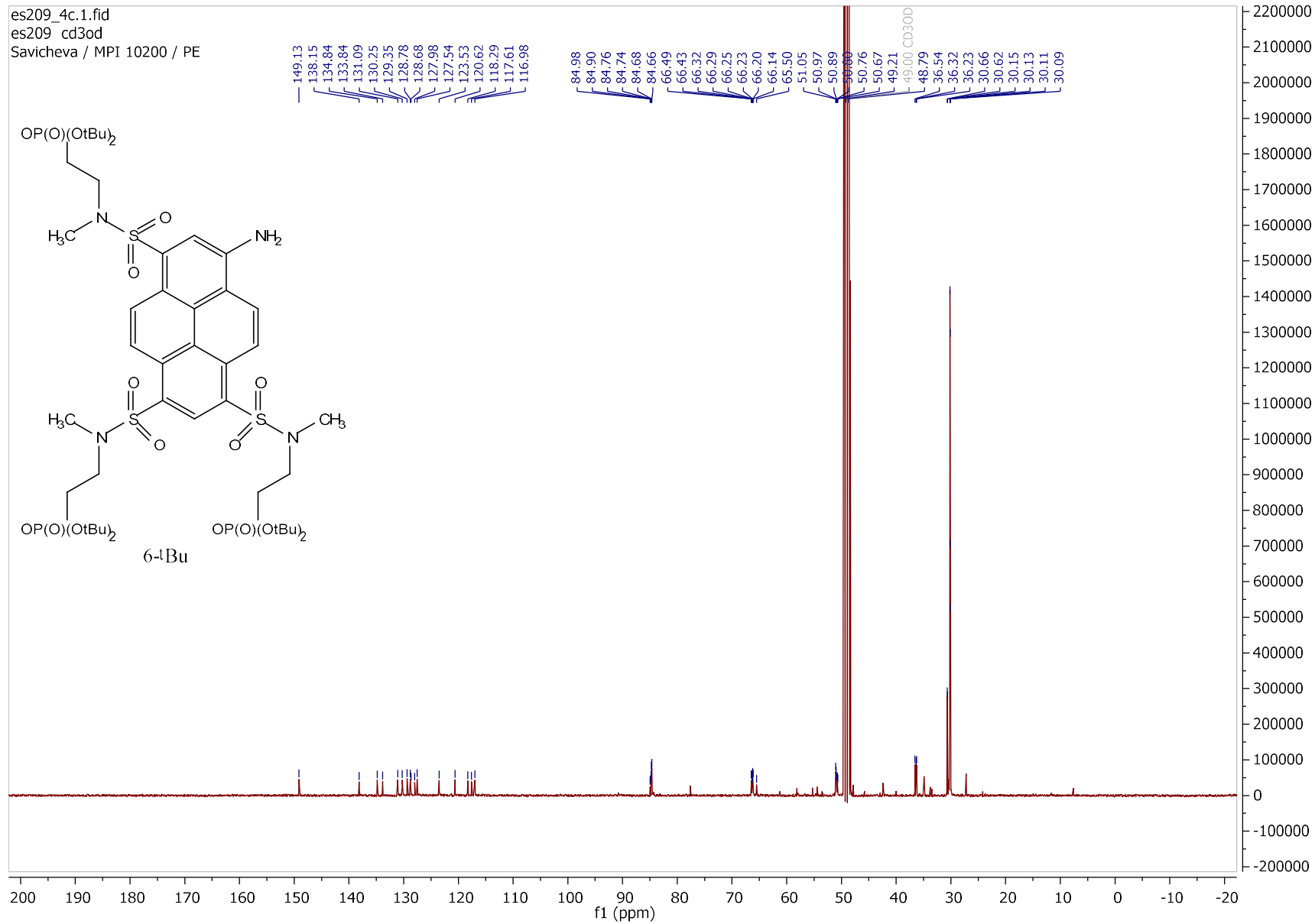


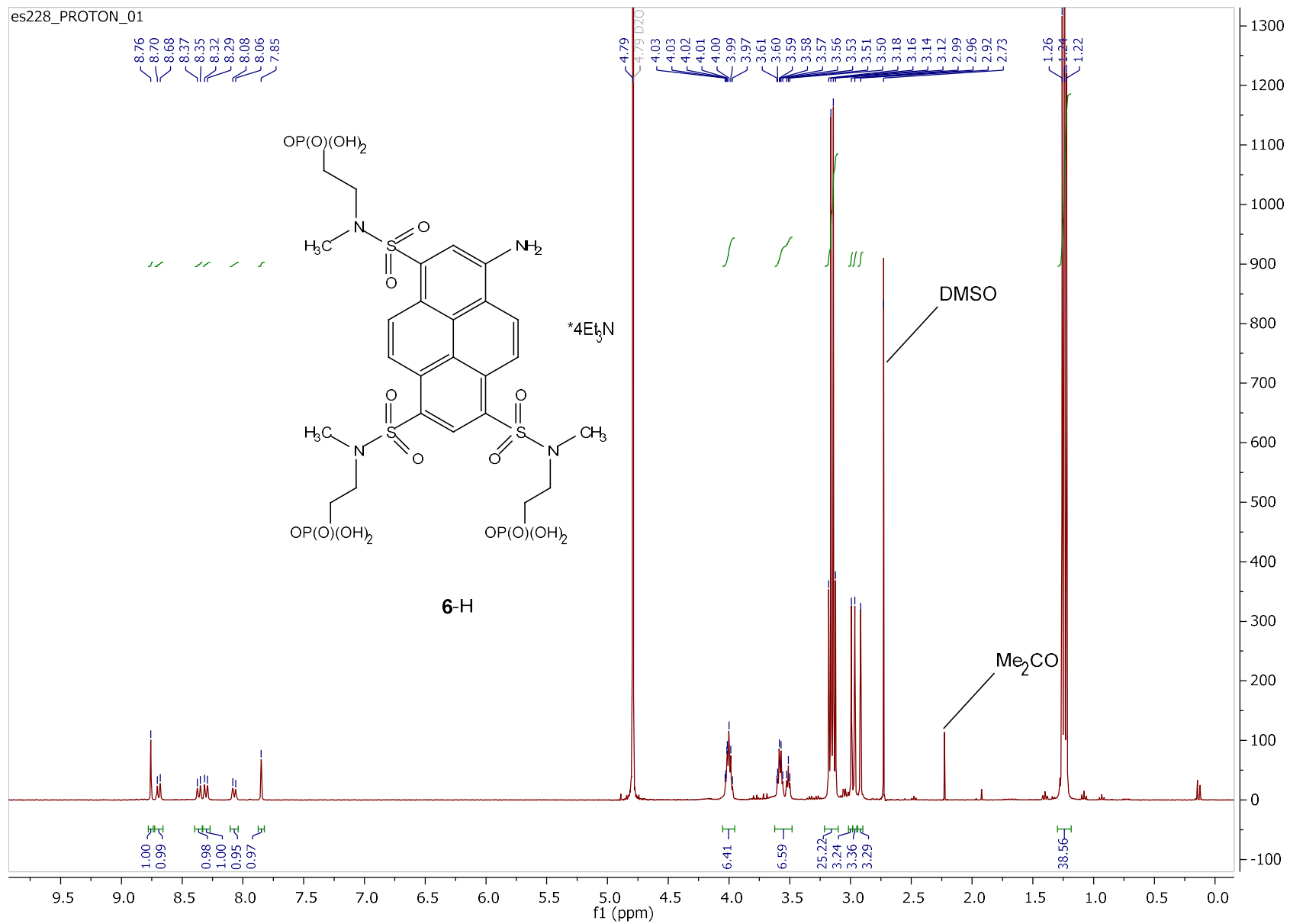


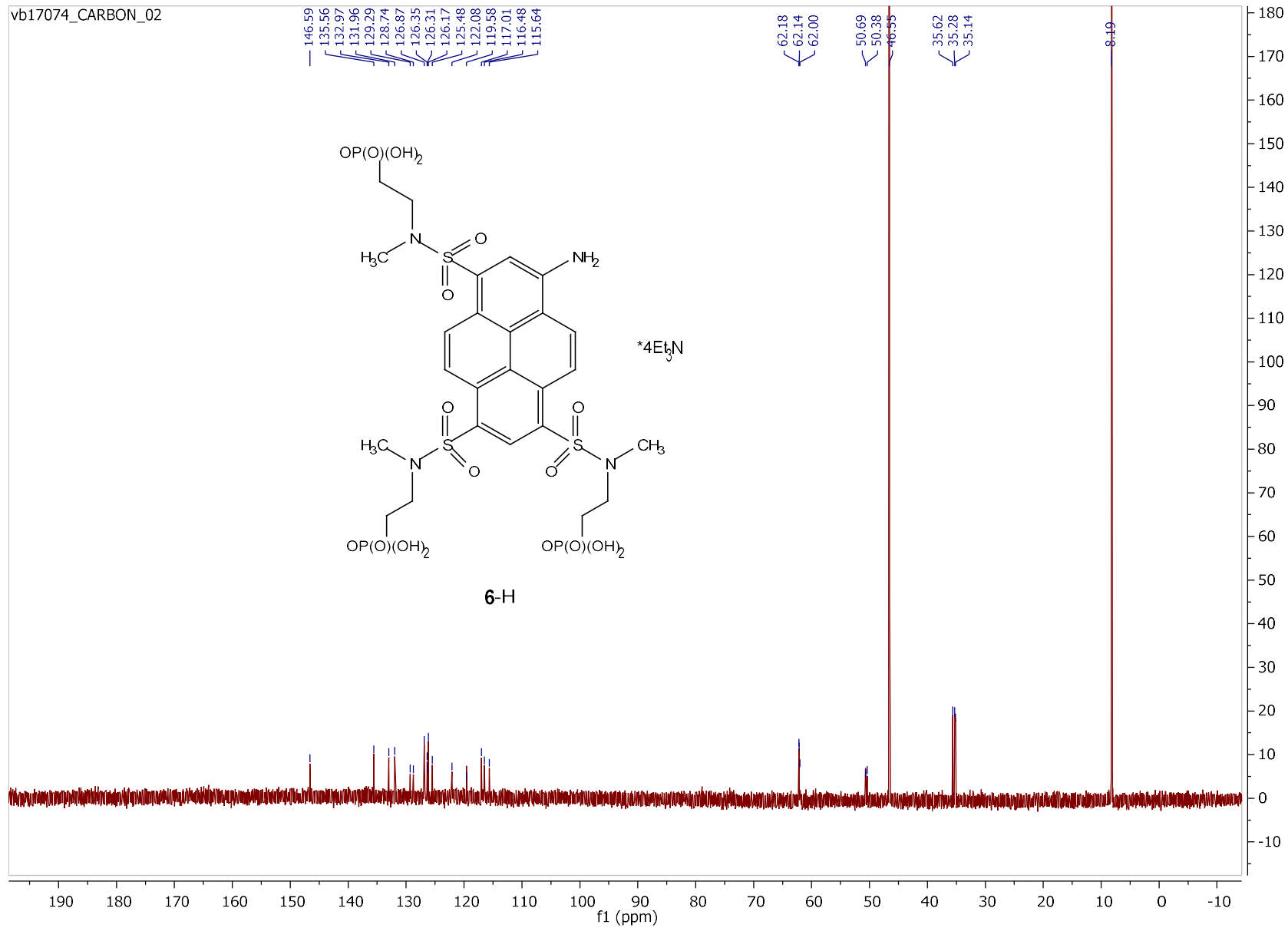
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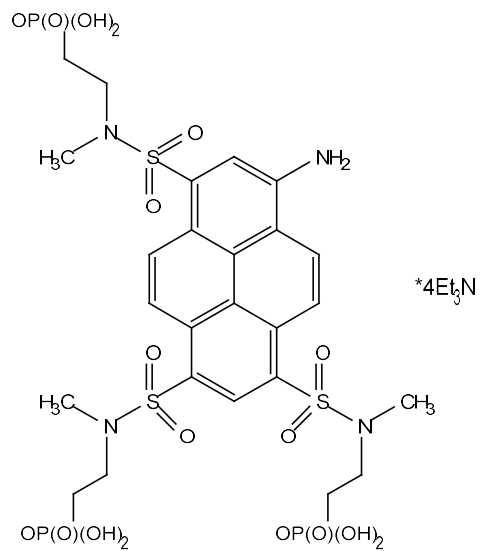
es209_4c.1.fid
es209 cd30d
Savicheva / MPI 10200 / PE



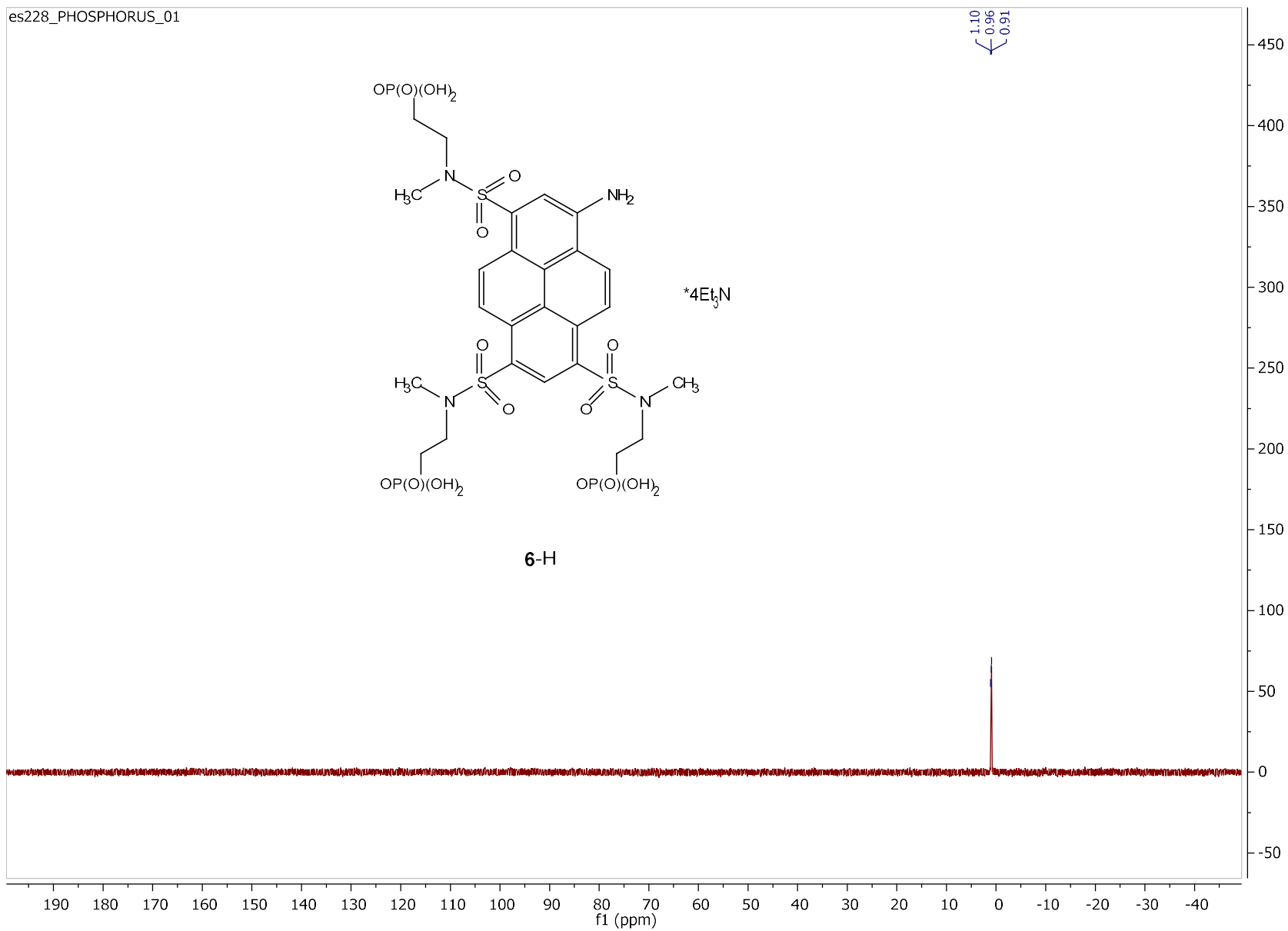


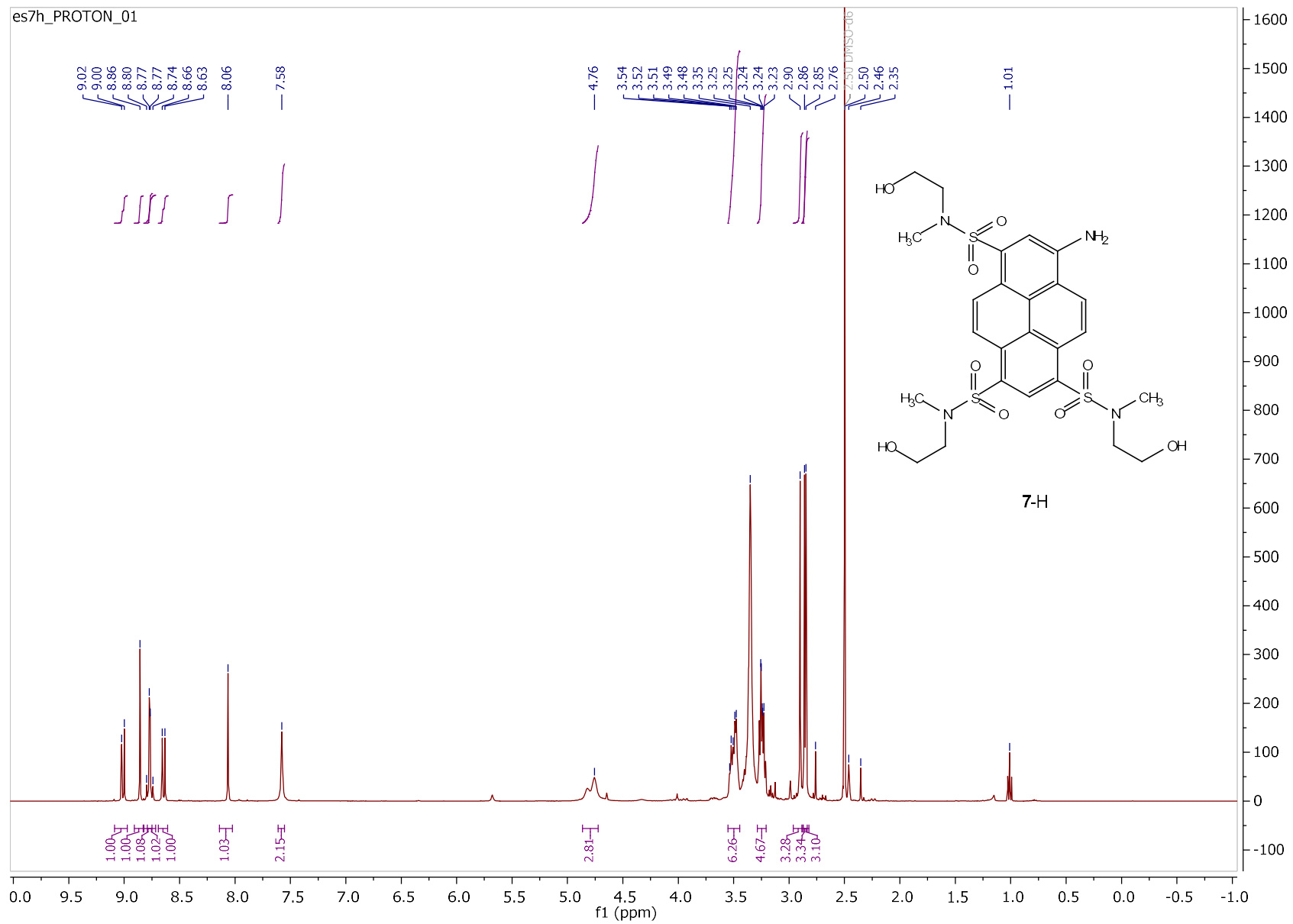


es228_PHOSPHORUS_01

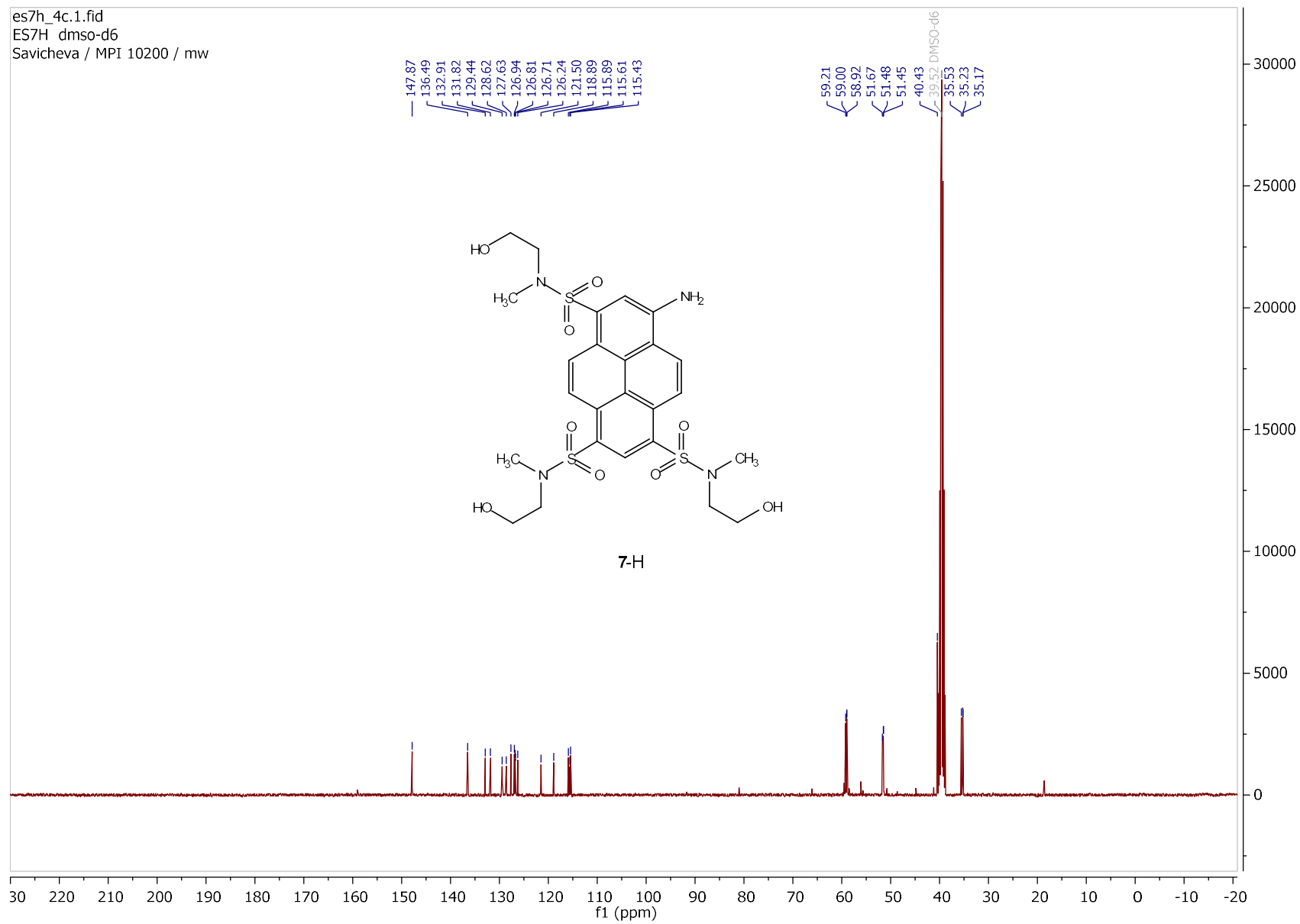


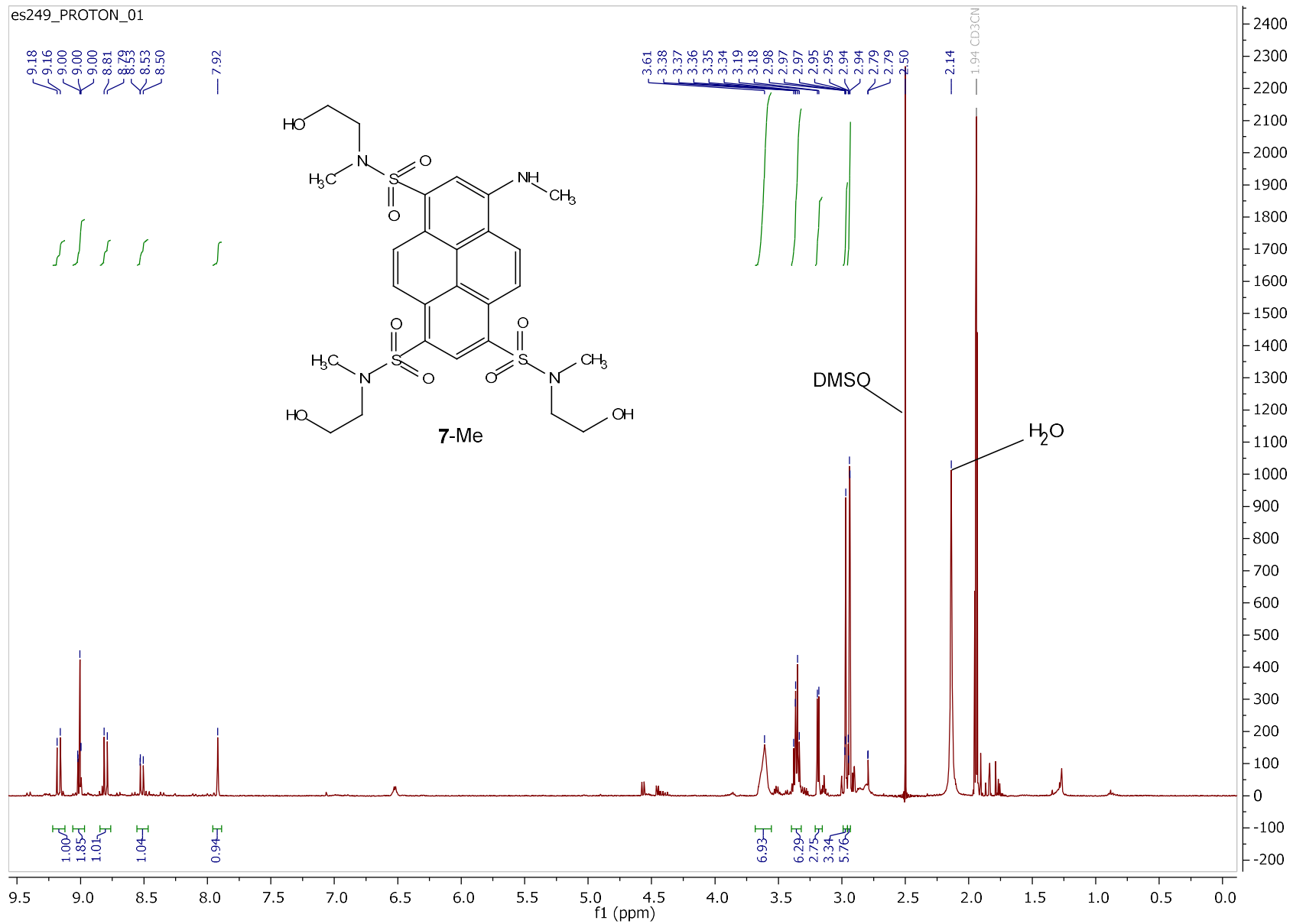
6-H

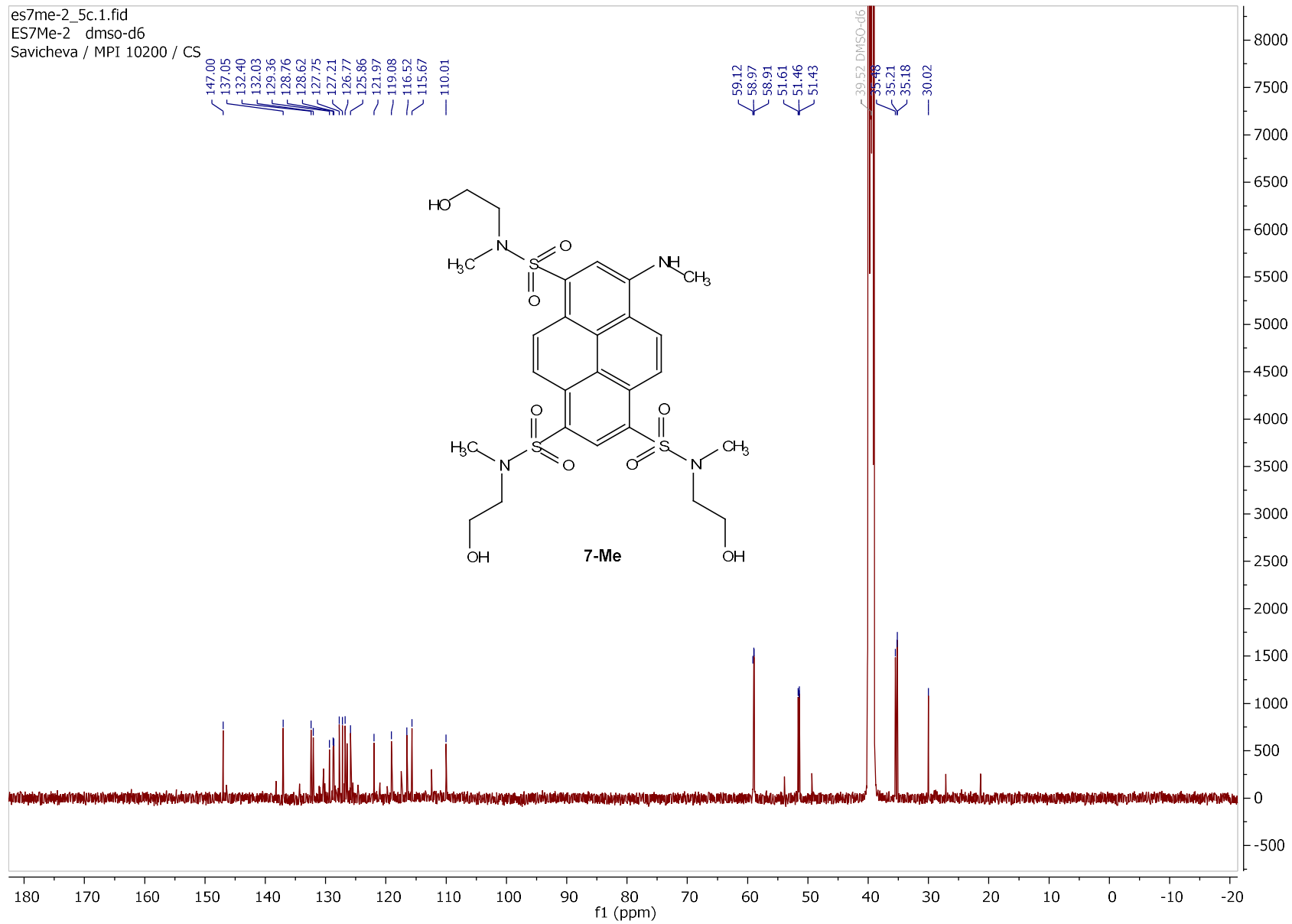


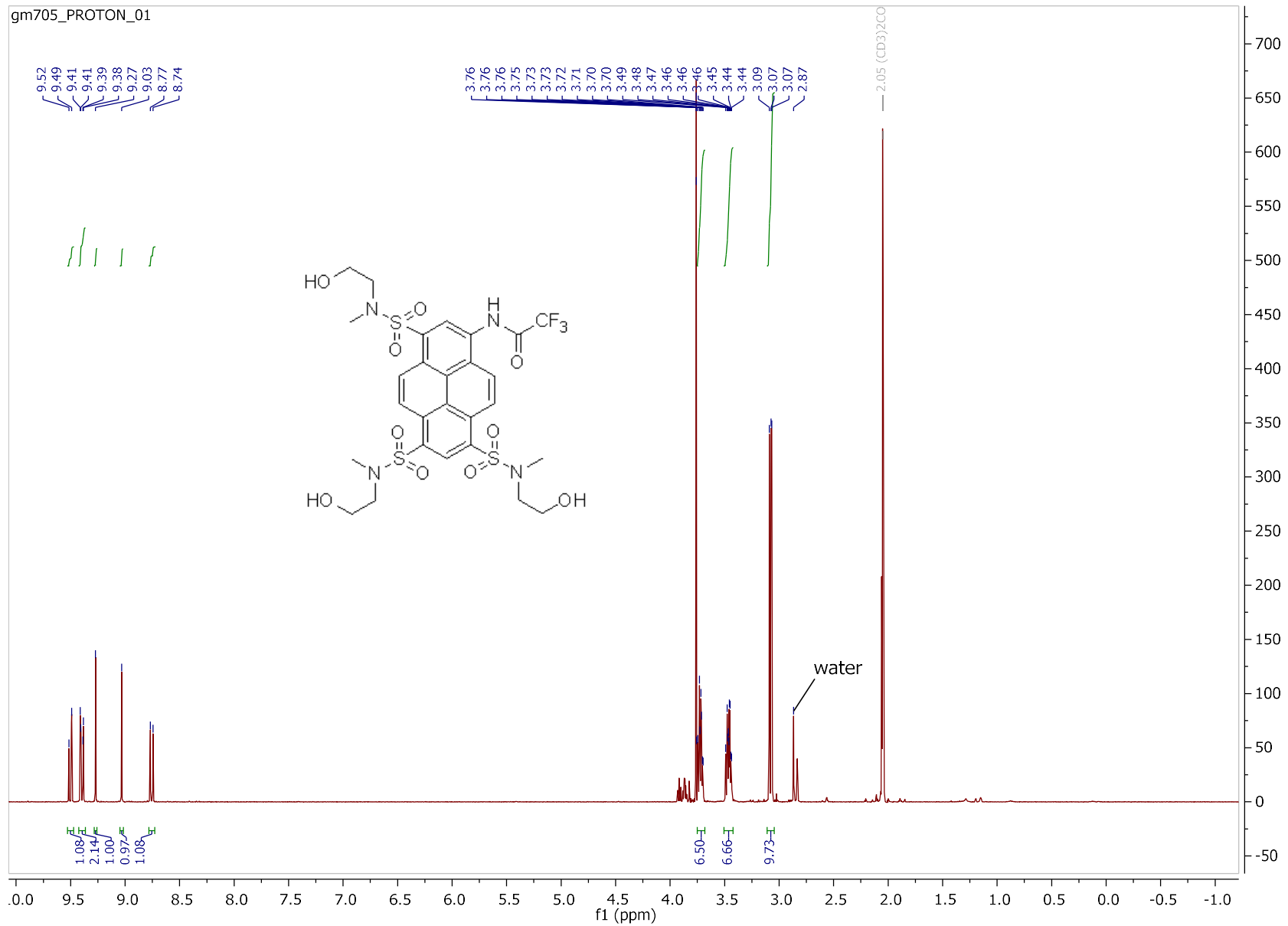


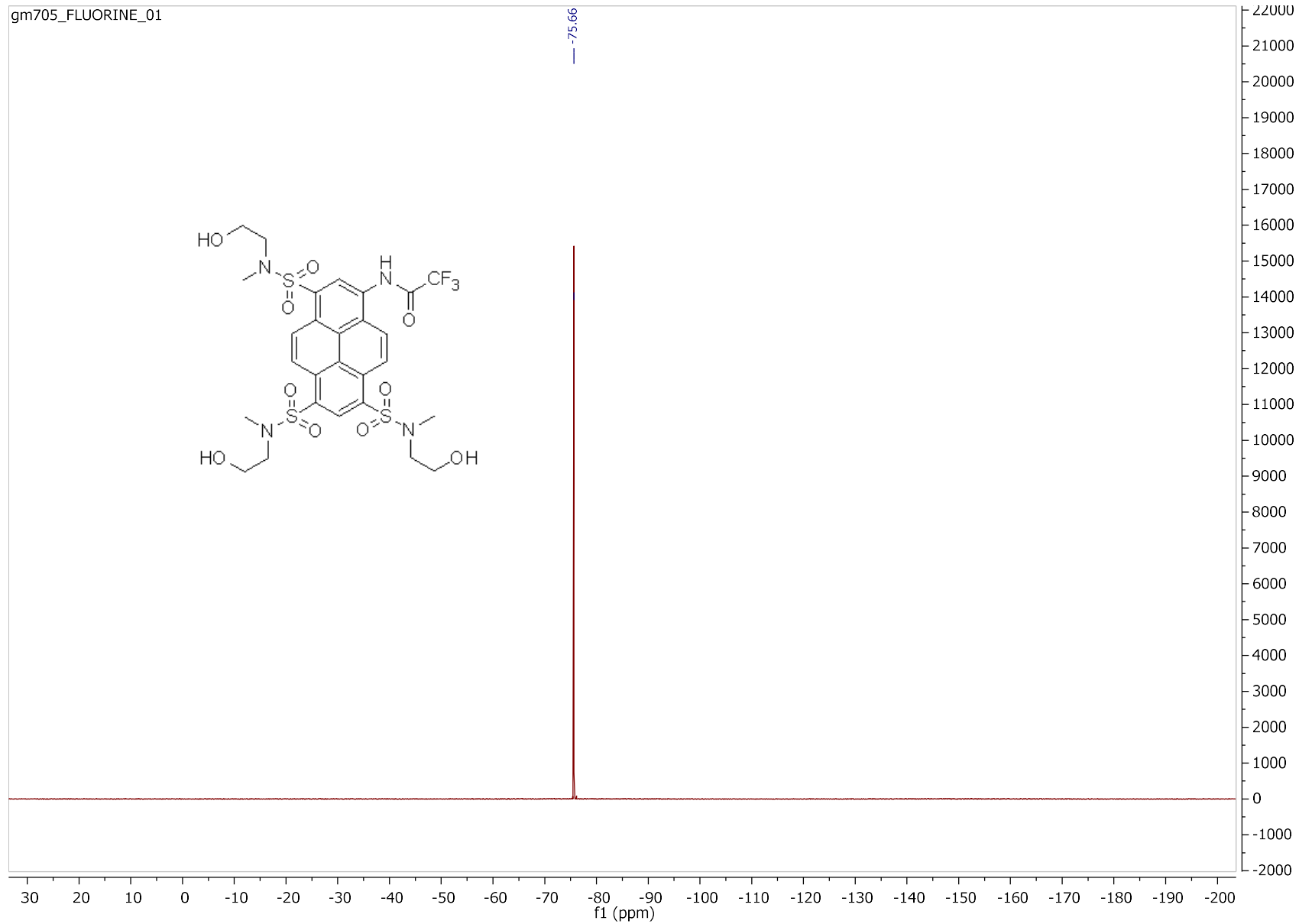
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ES7H dms0-d6
Savicheva / MPI 10200 / mw

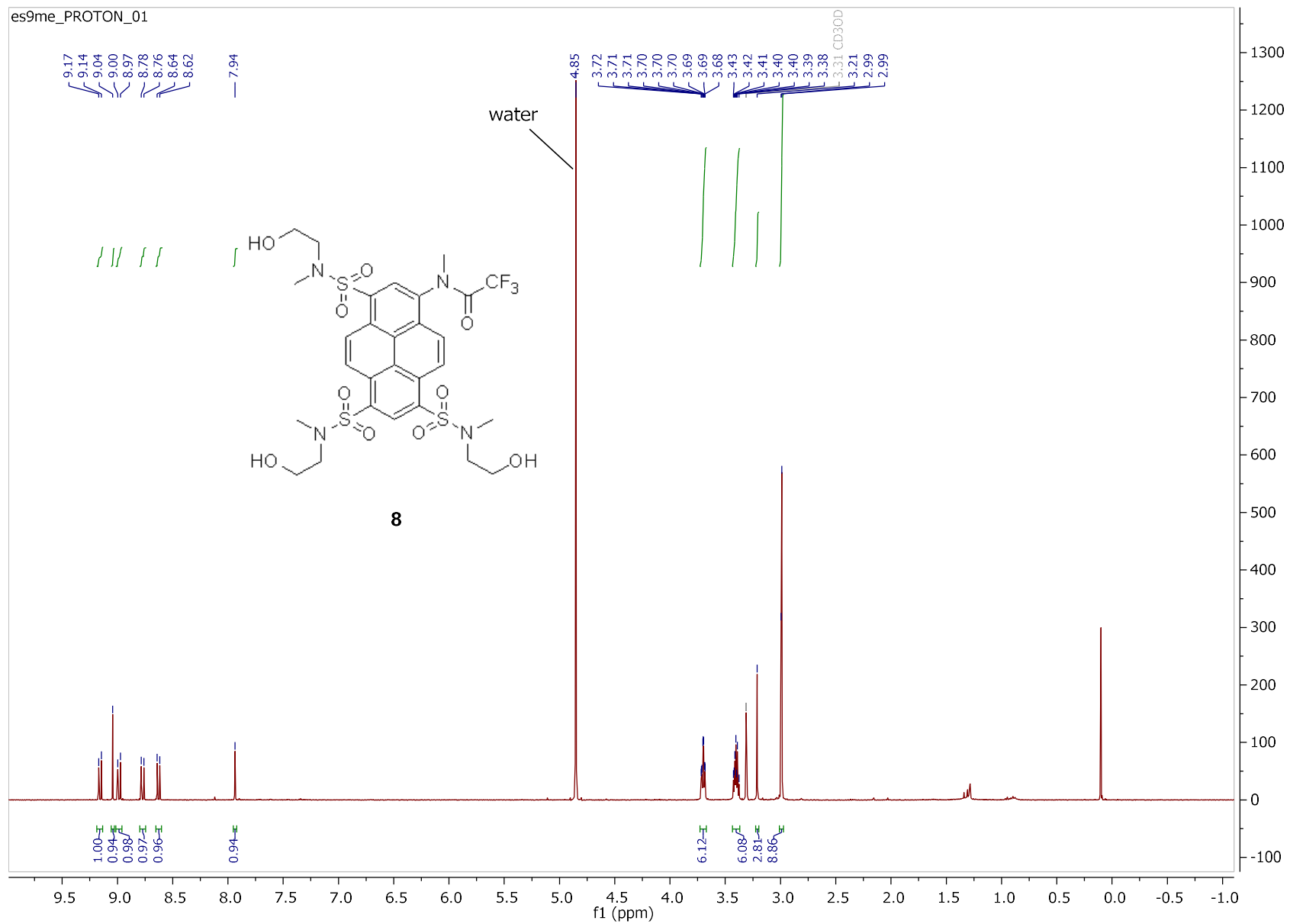




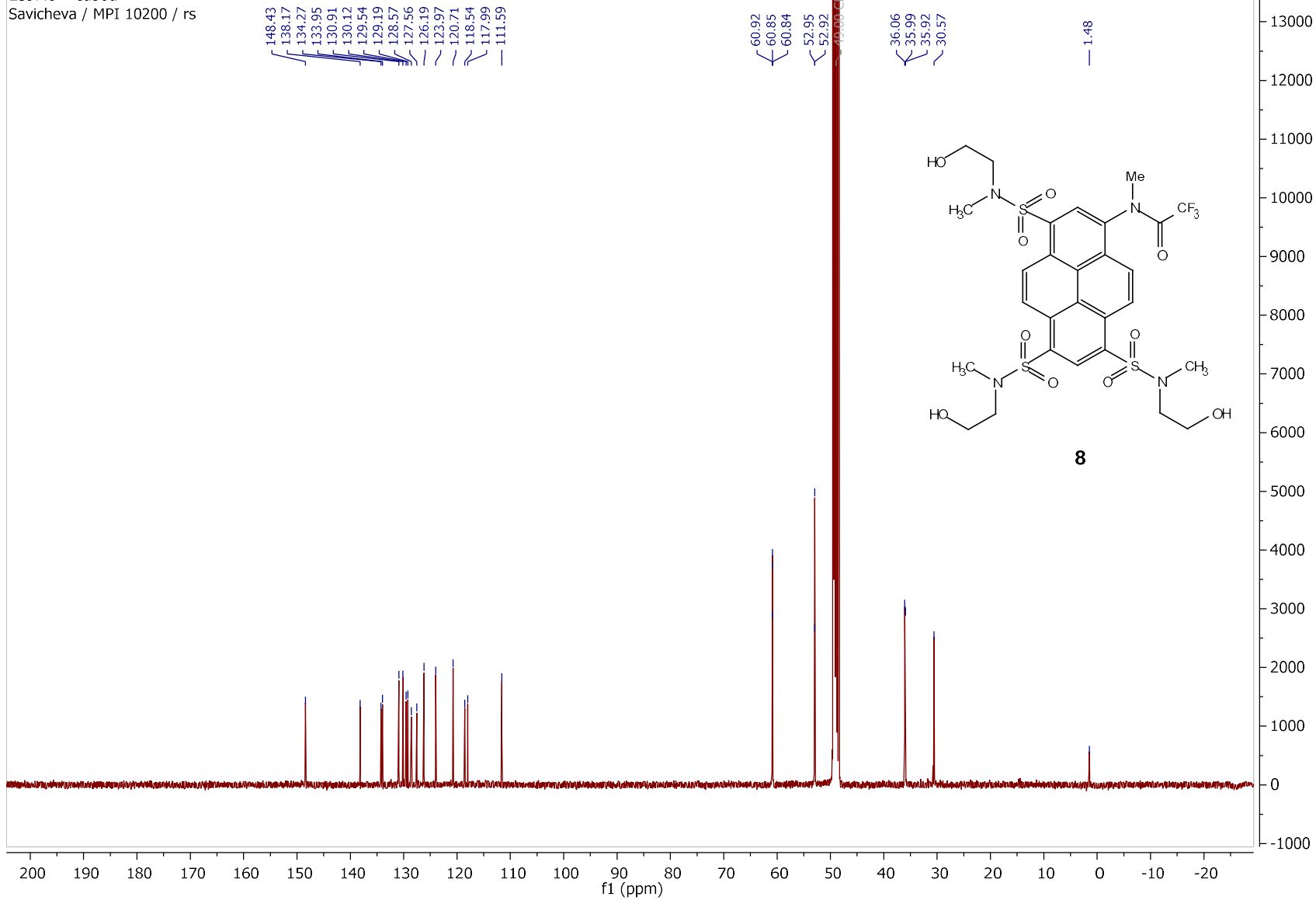


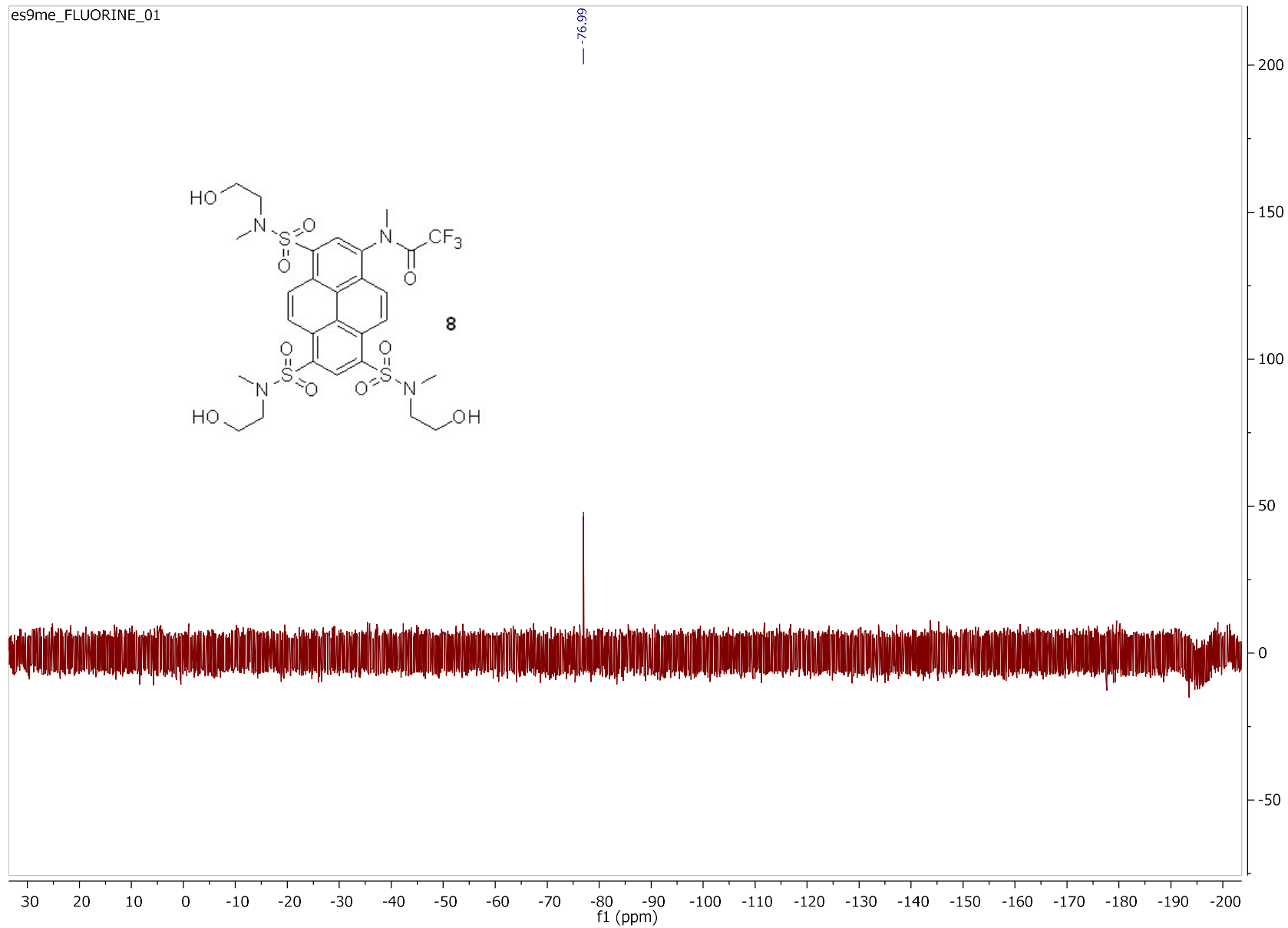


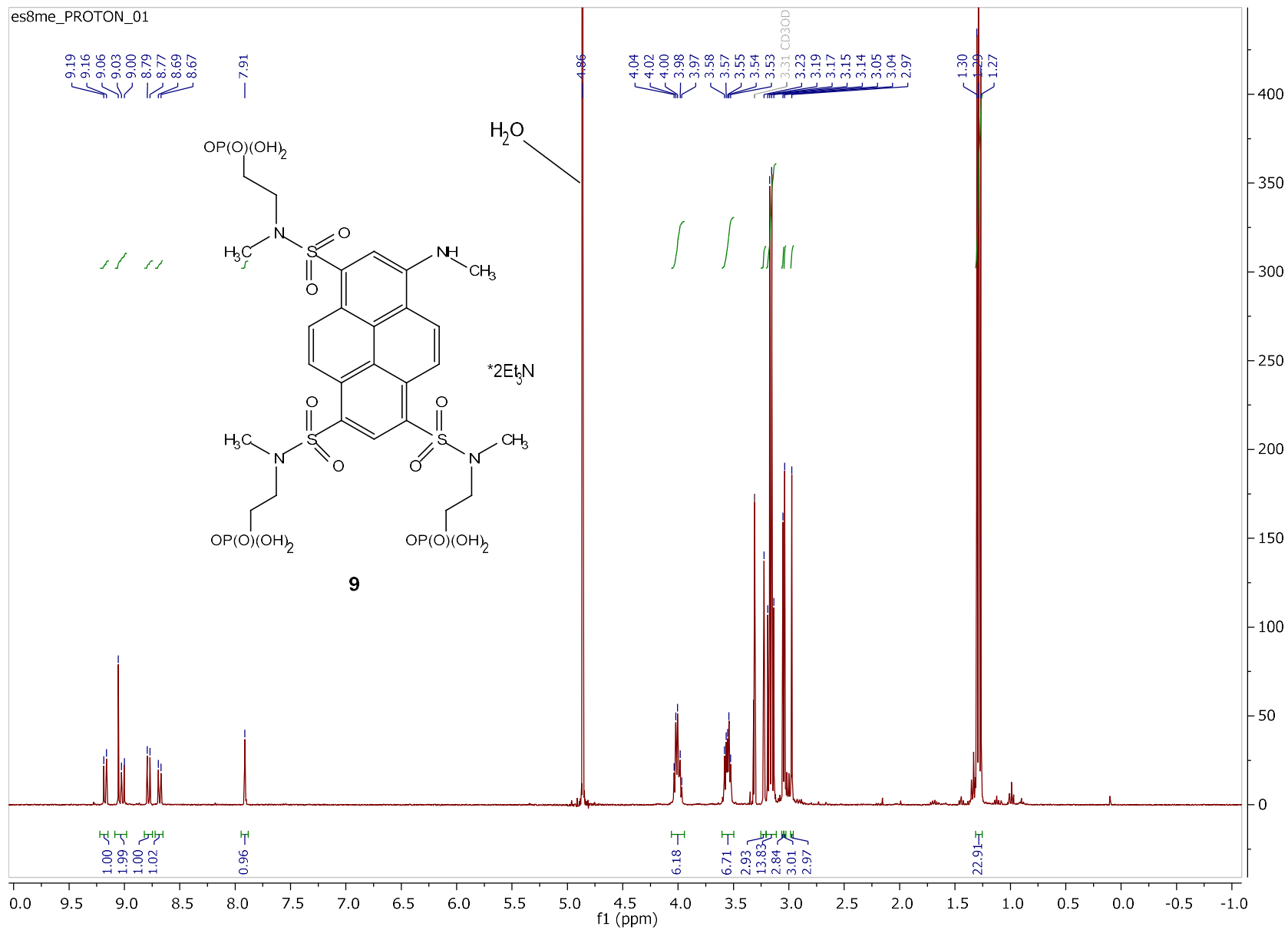




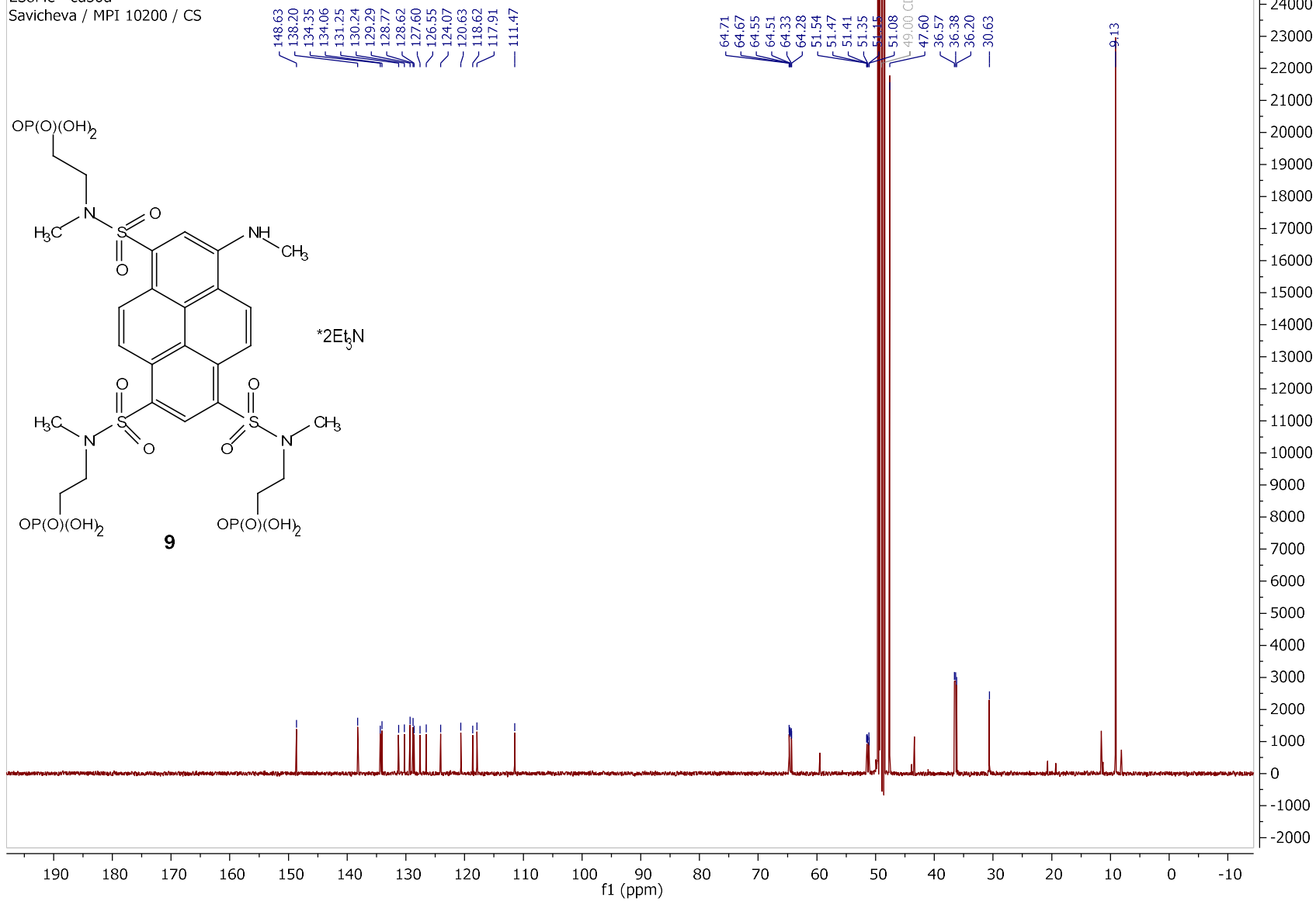
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ES9Me cd3od
Savicheva / MPI 10200 / rs



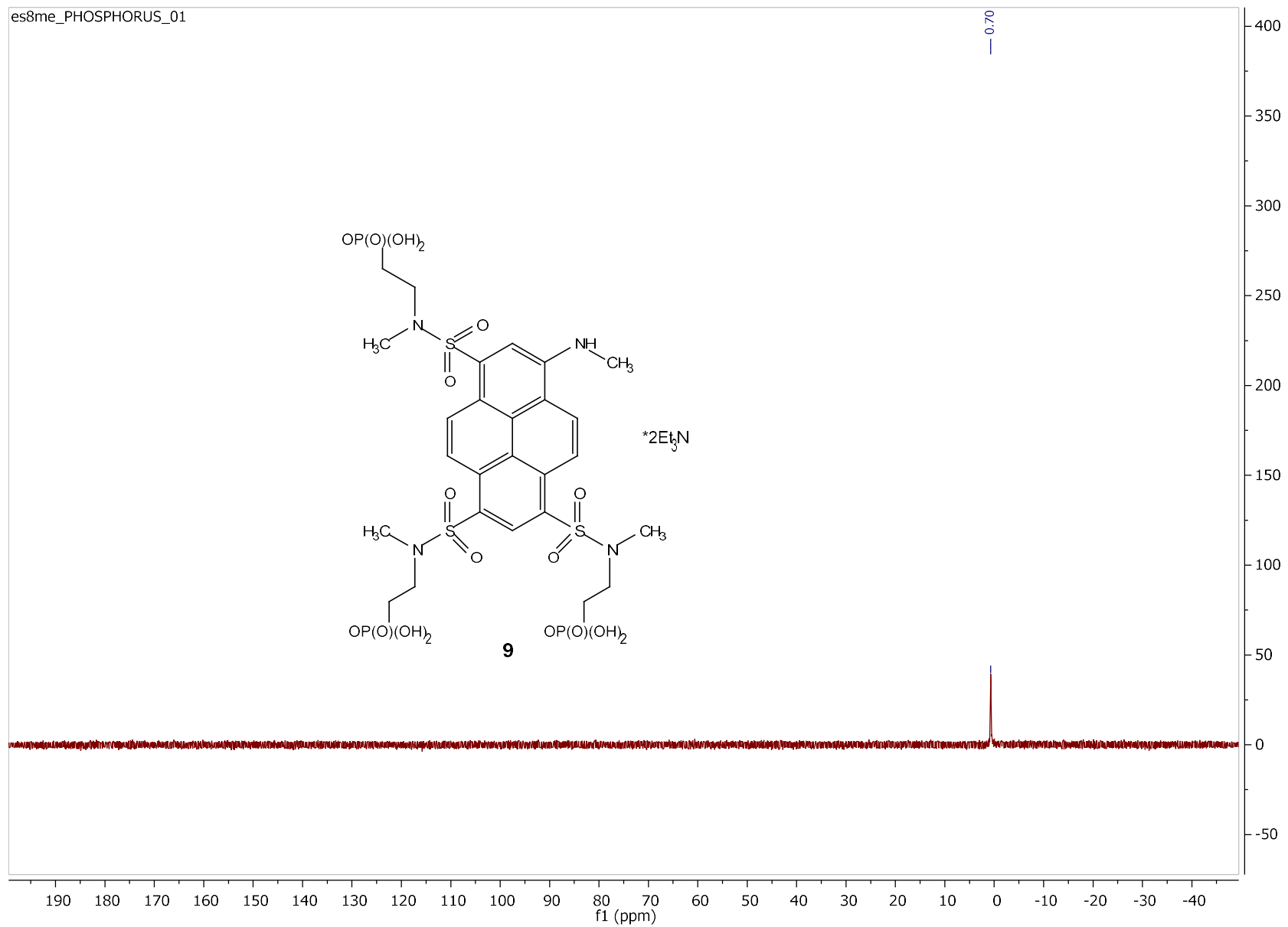


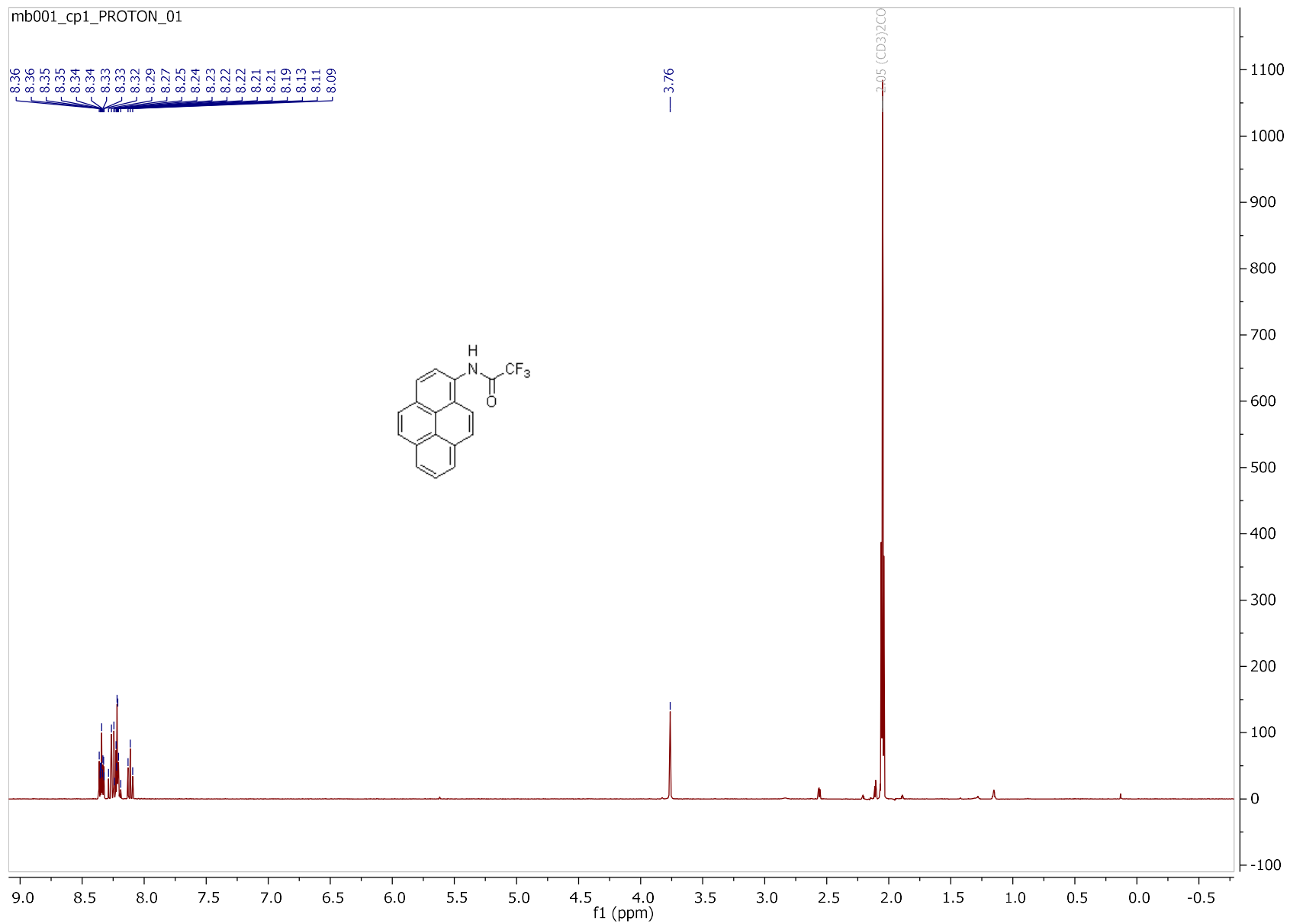


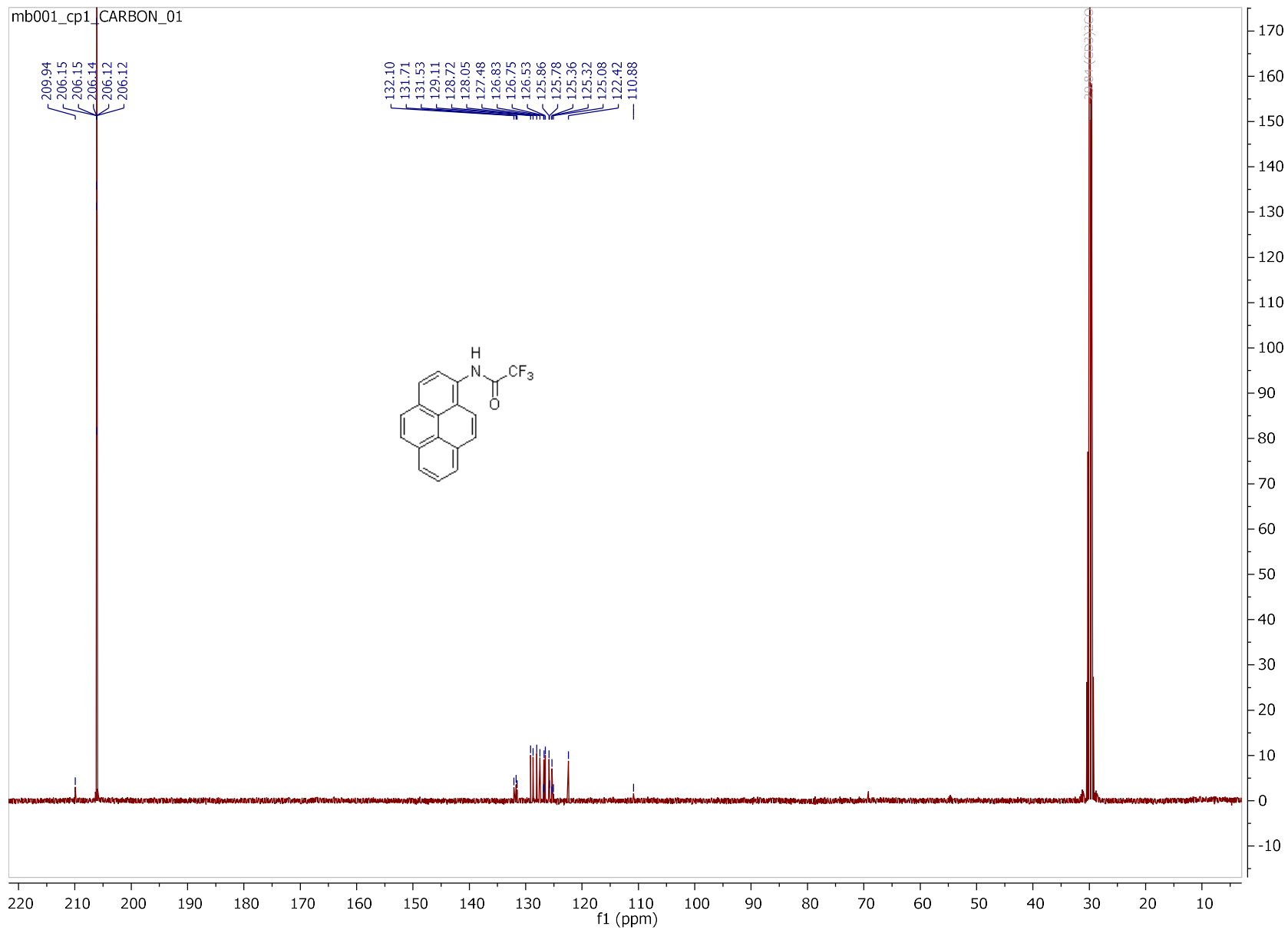
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ES8Me cd30d
Savicheva / MPI 10200 / CS

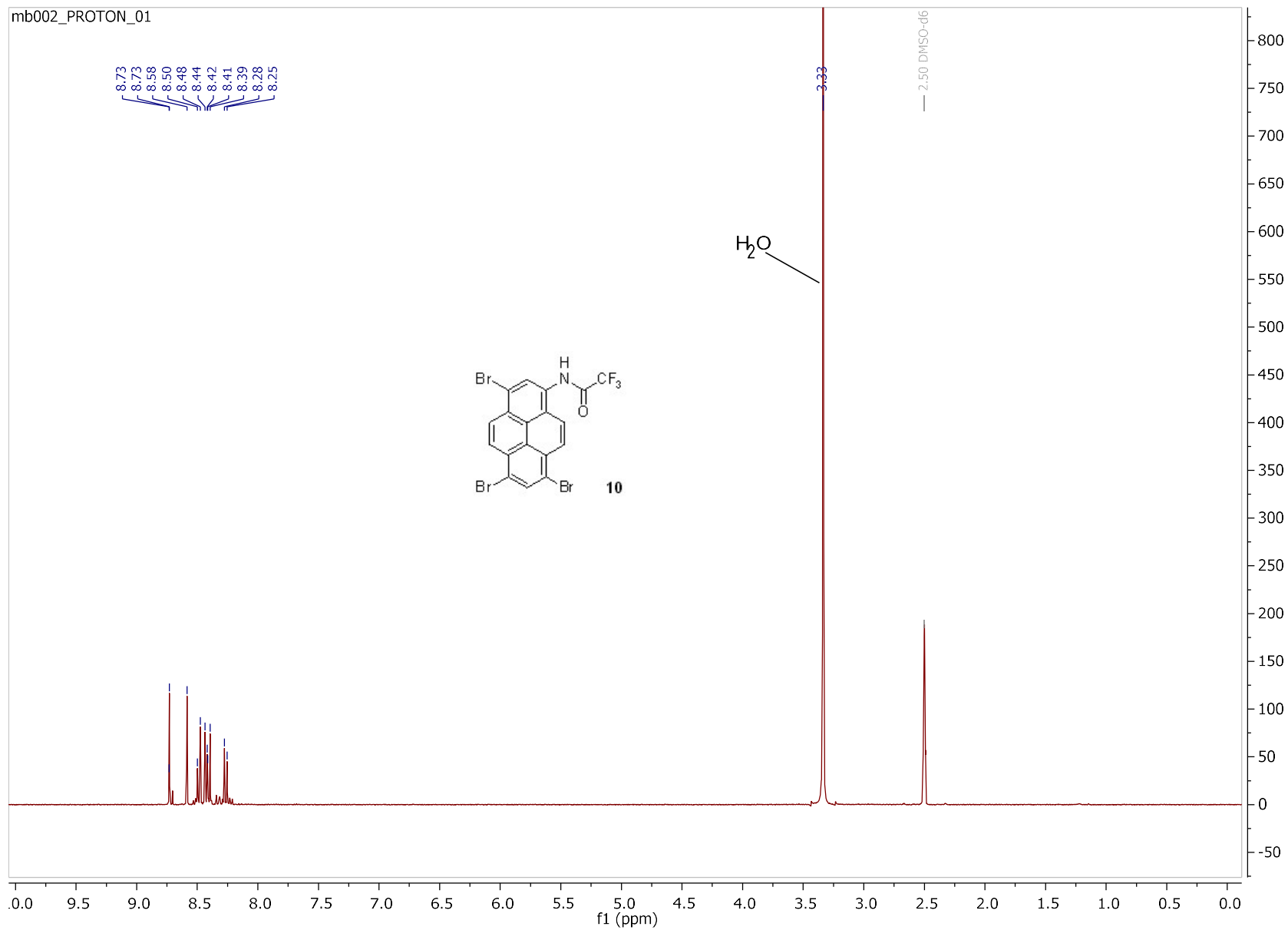


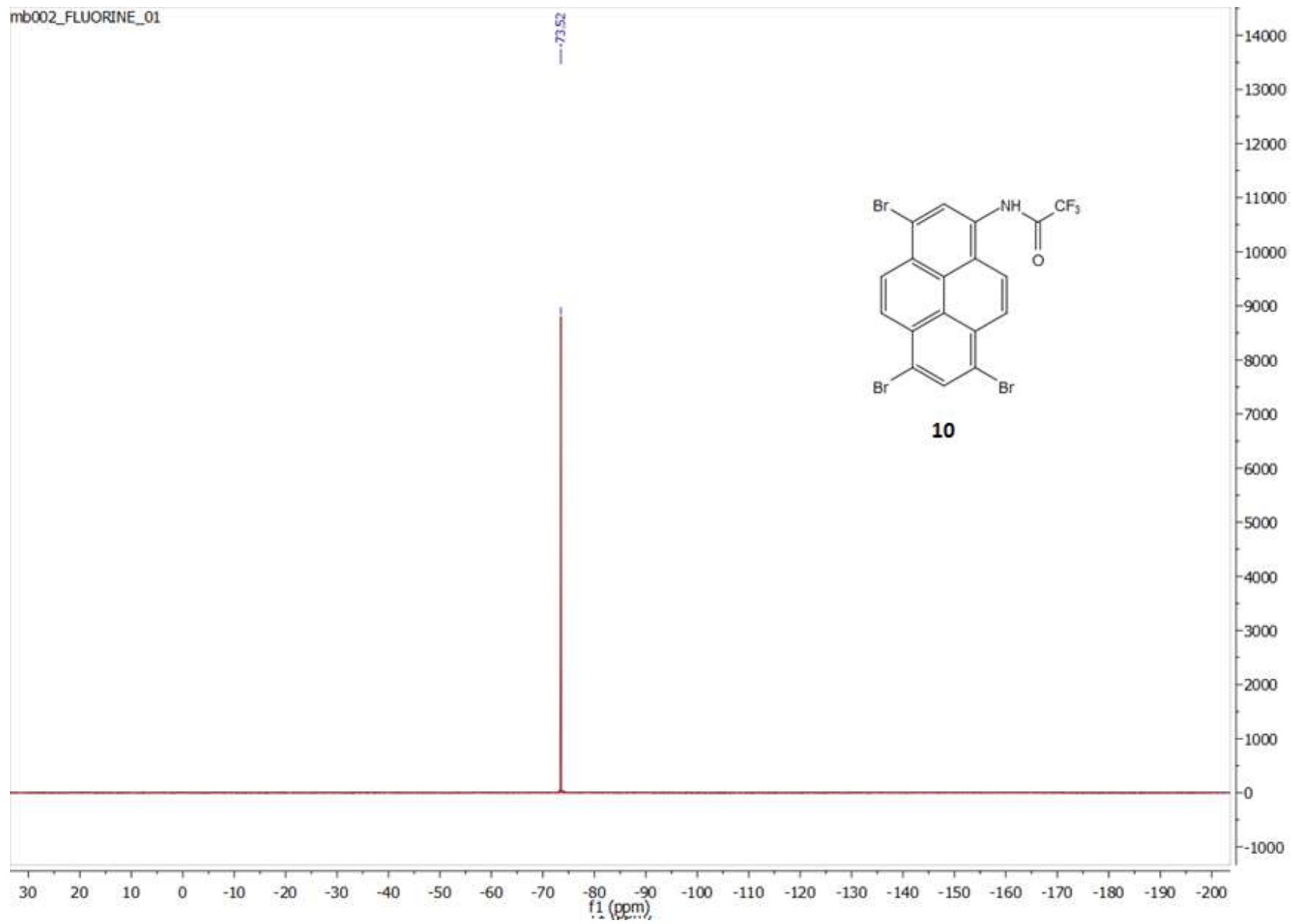
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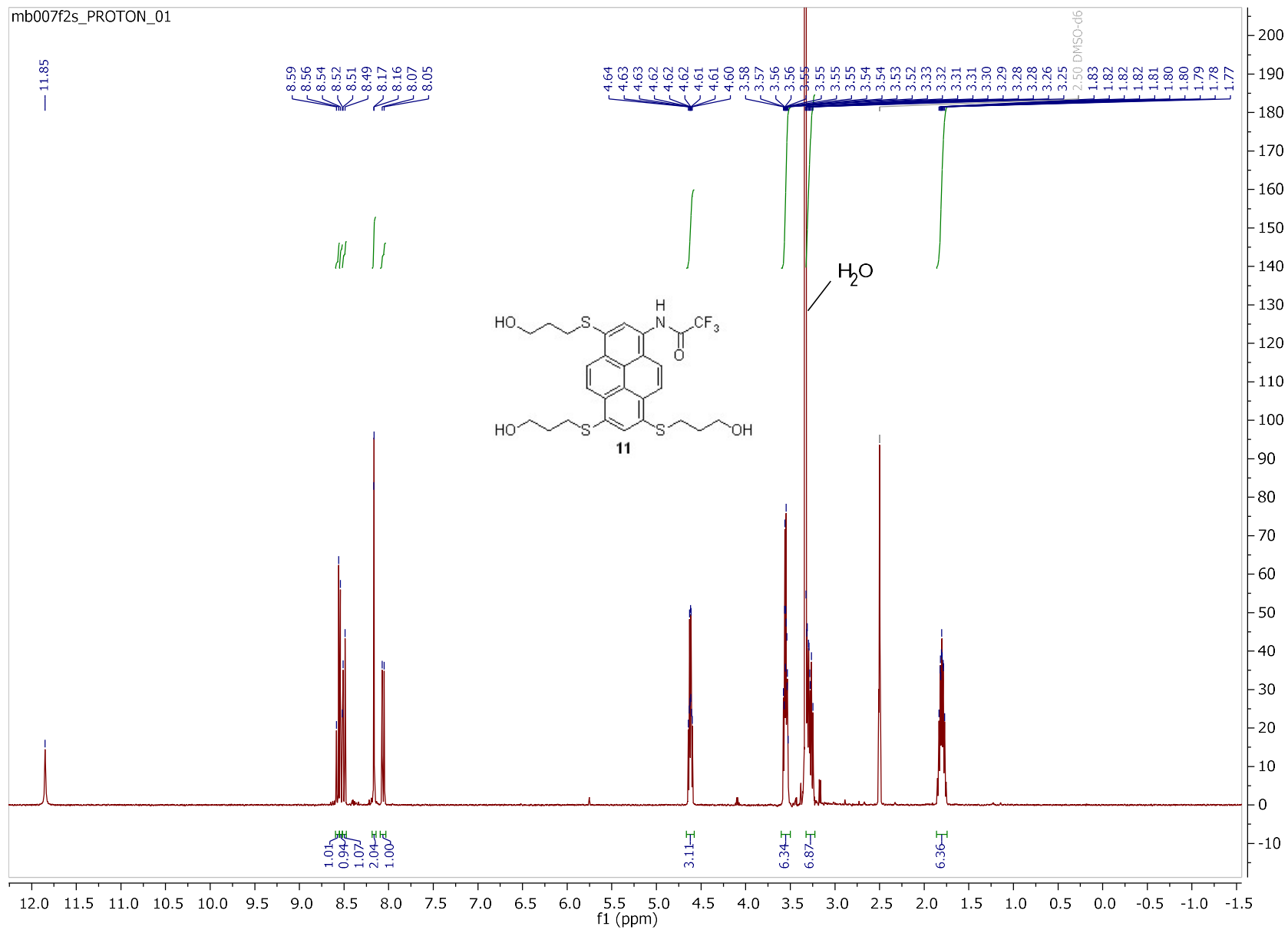


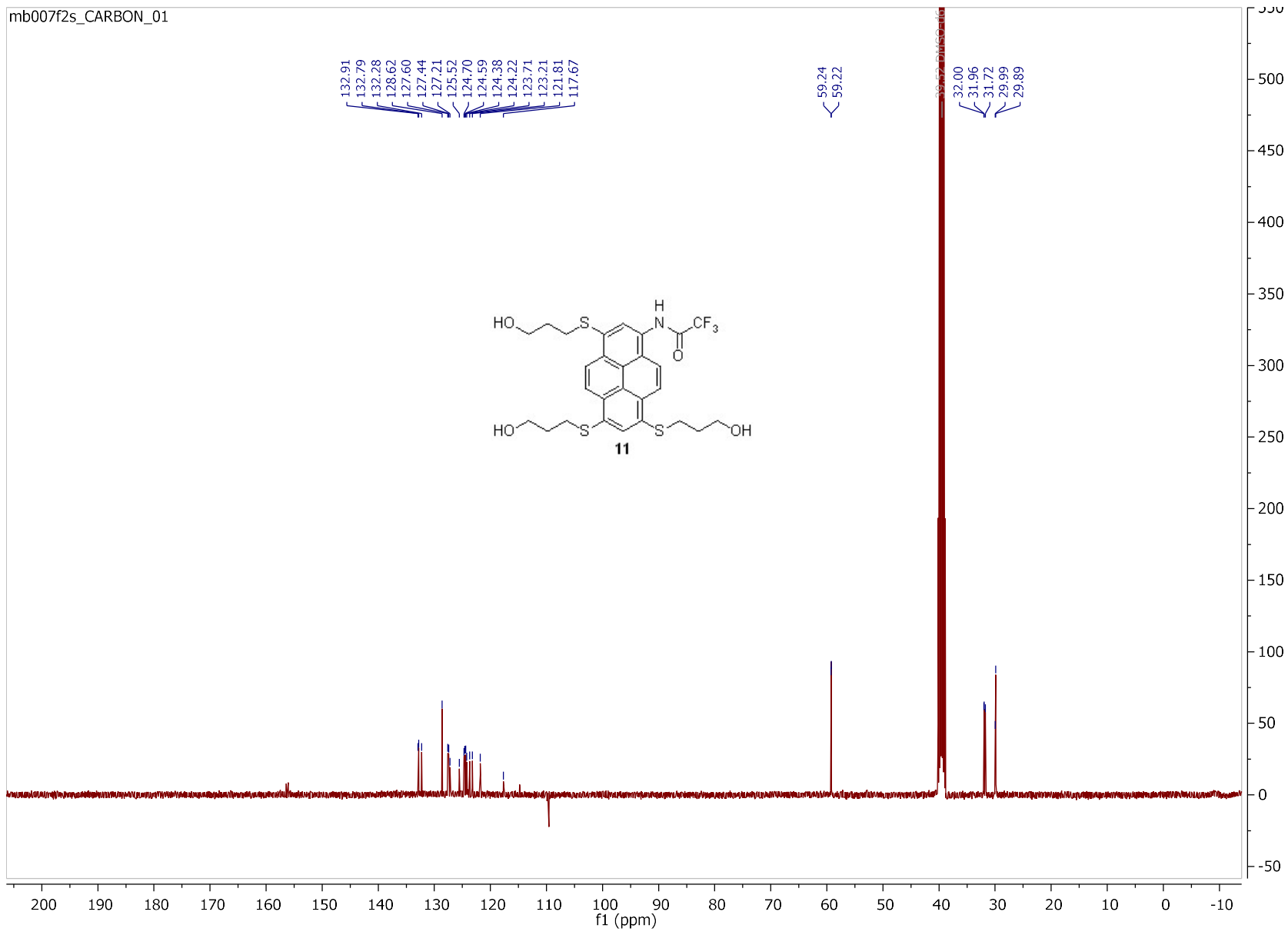




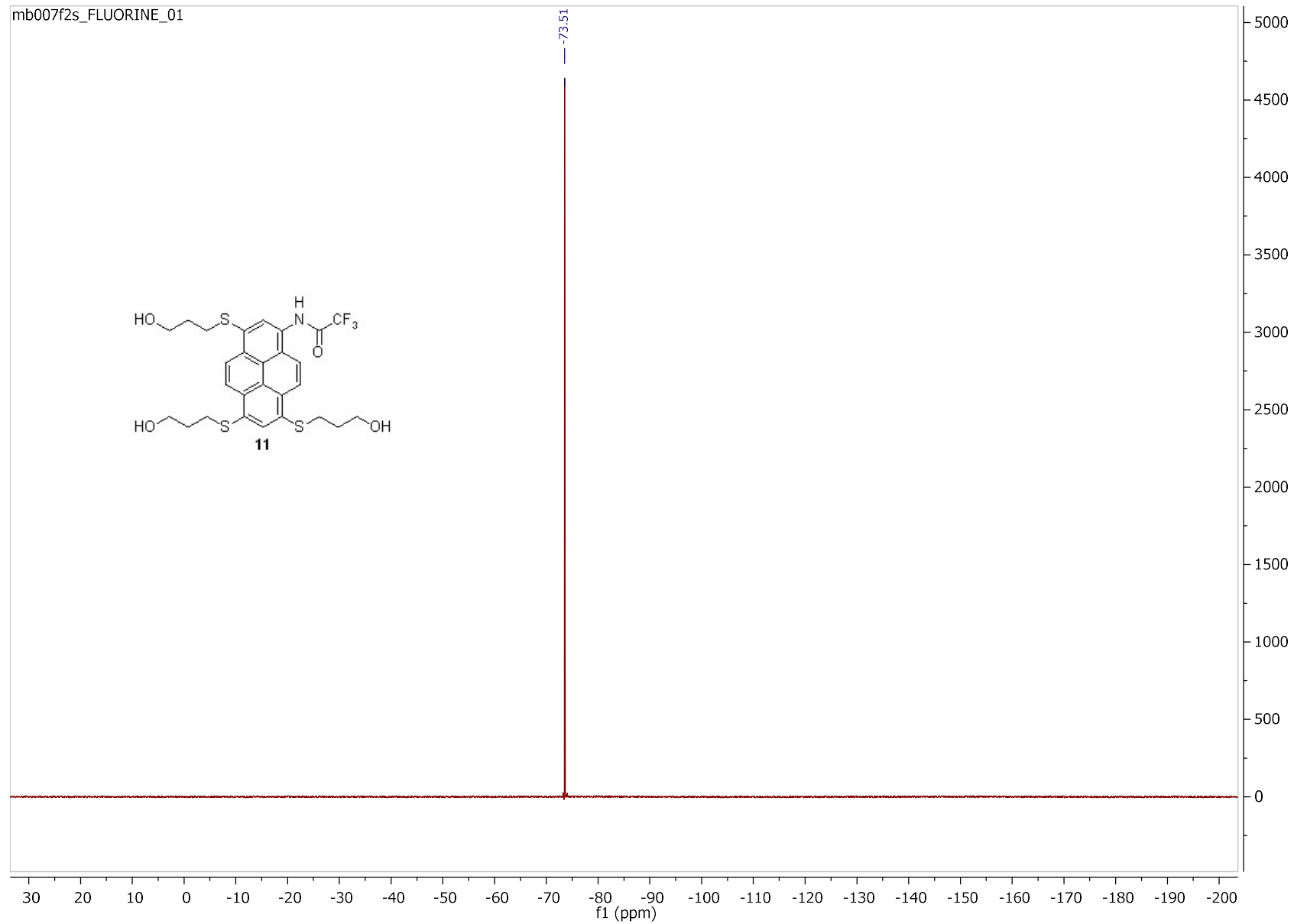
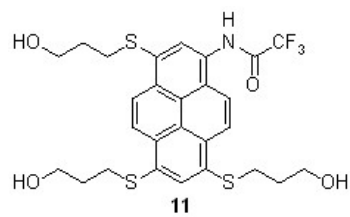


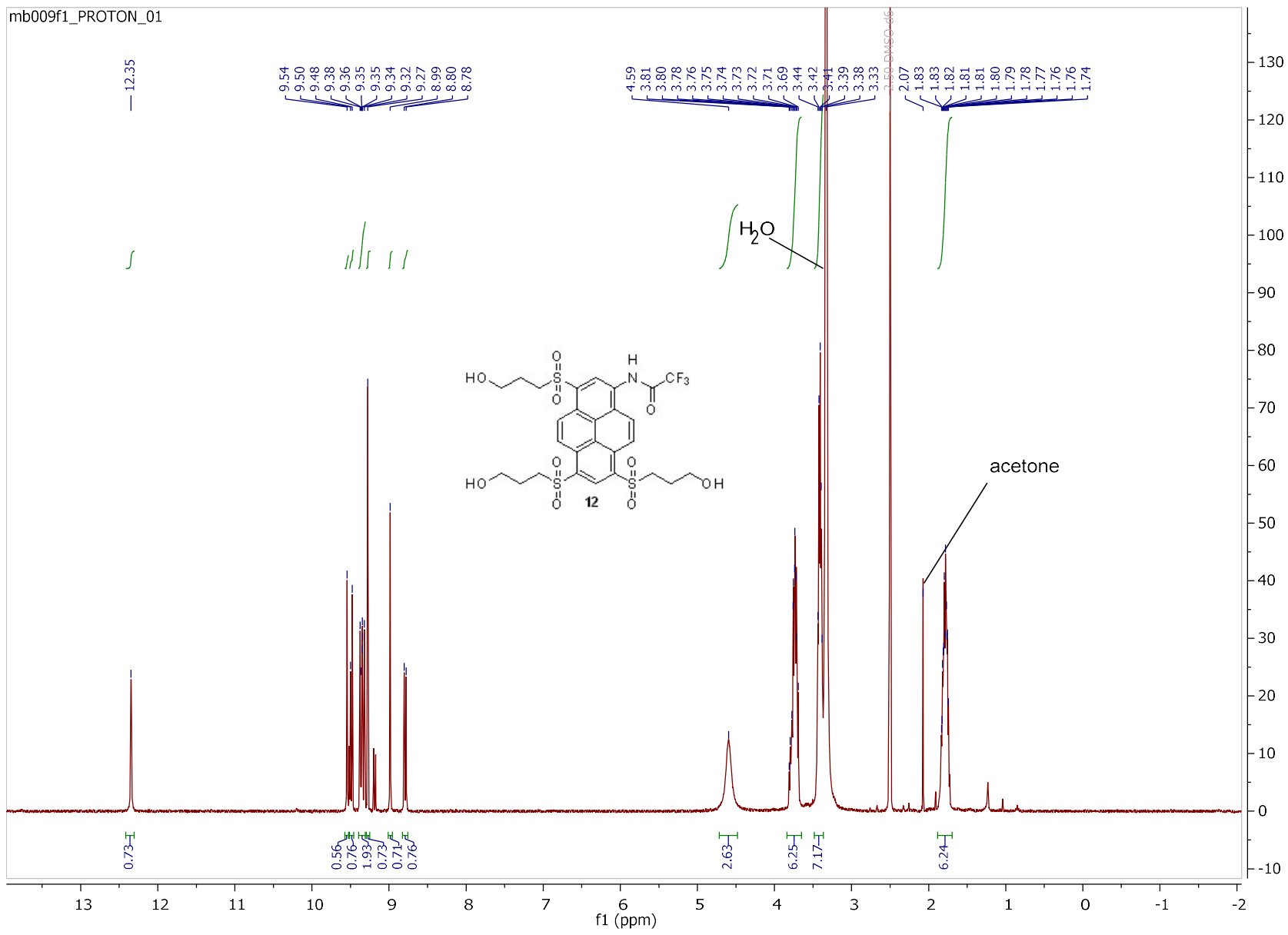


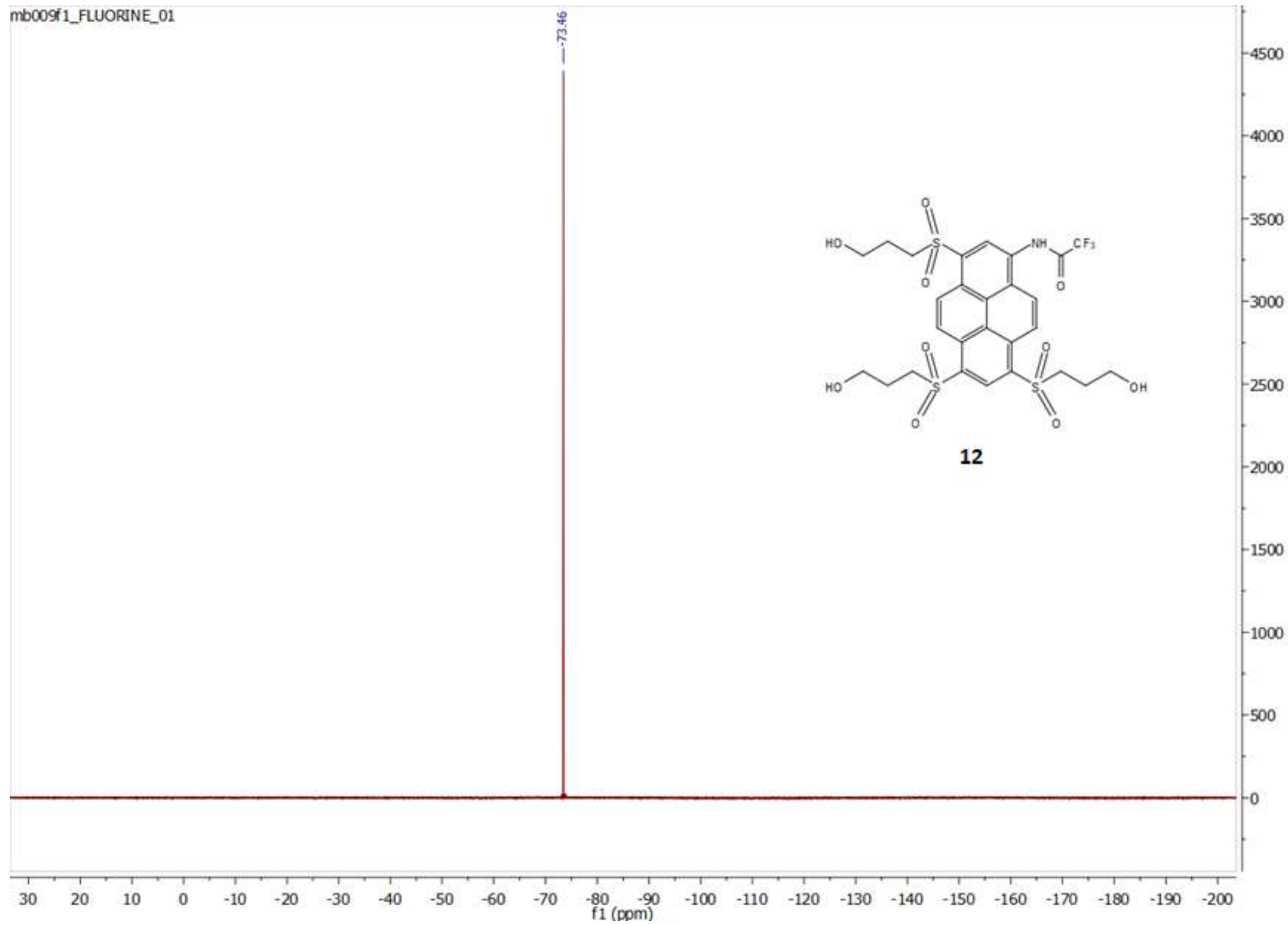


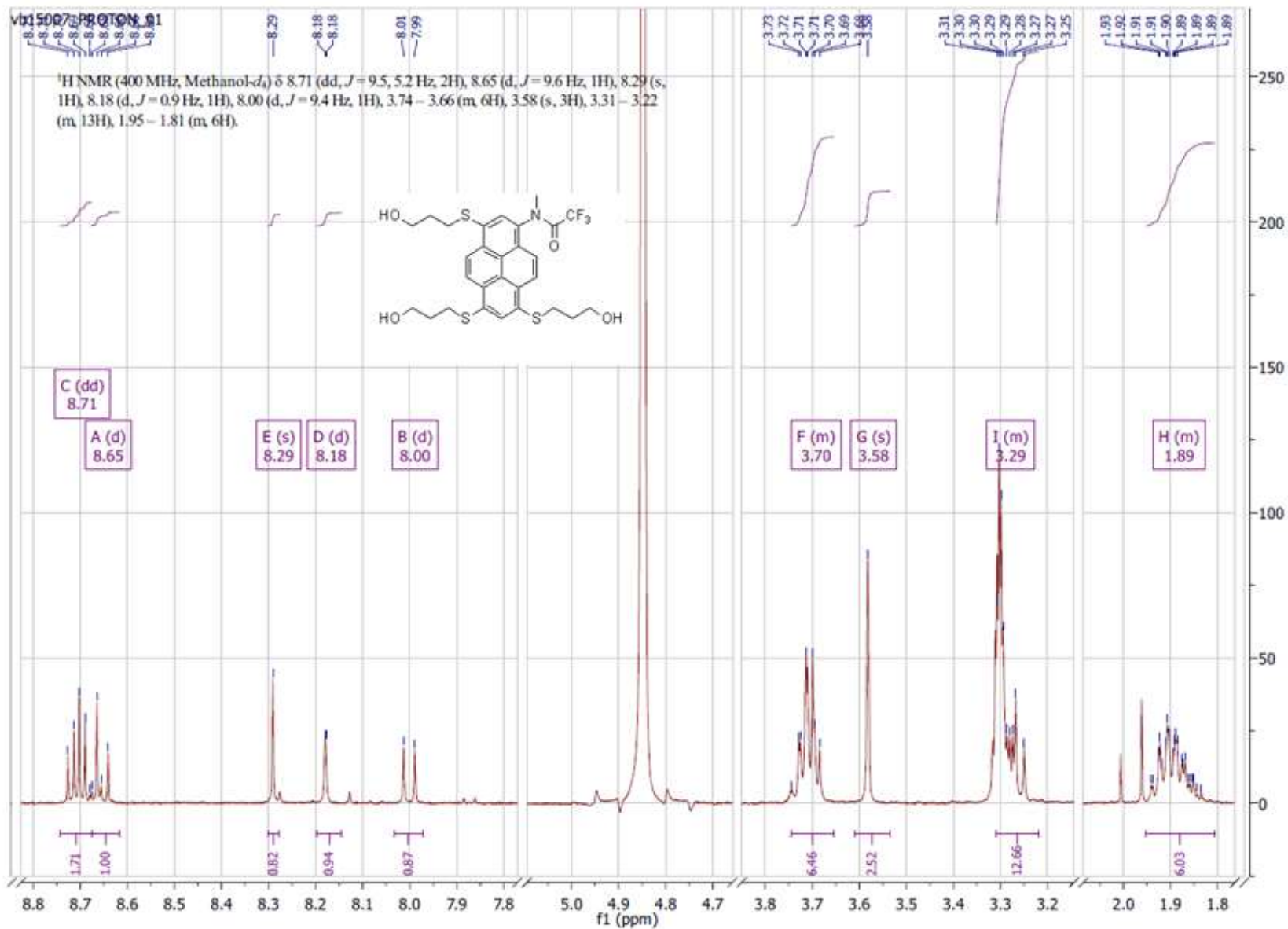


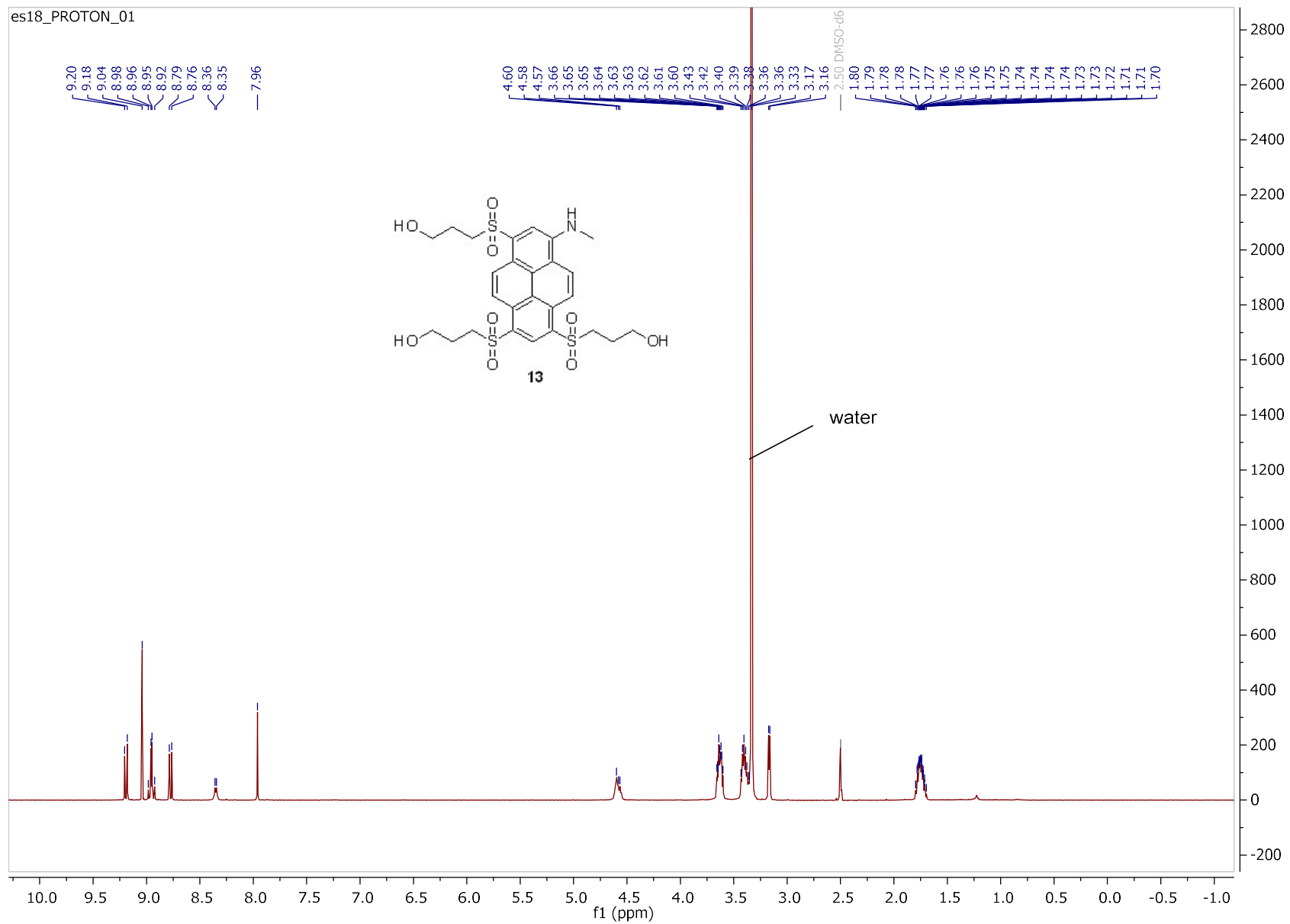
mb007f2s_FLUORINE_01



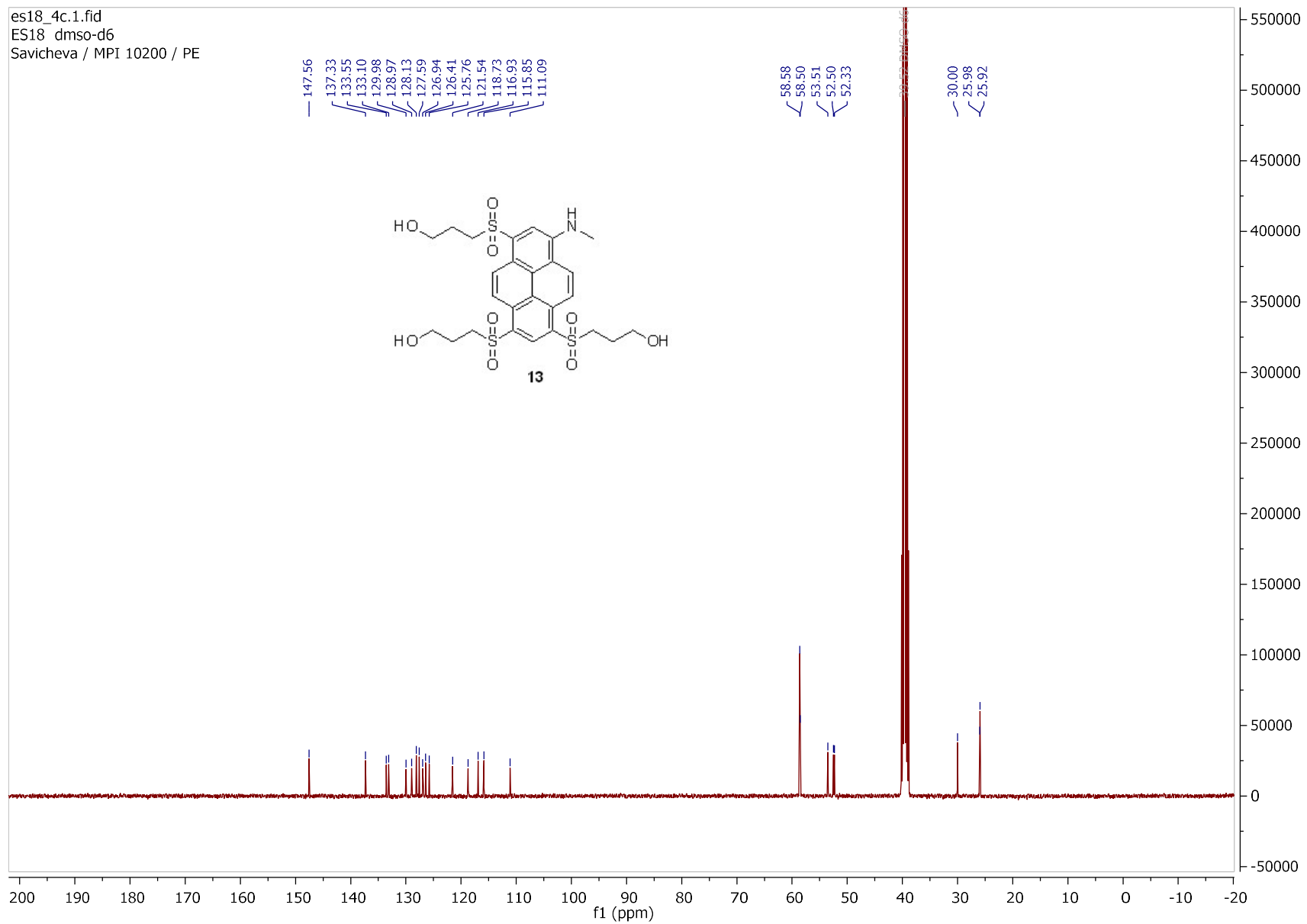


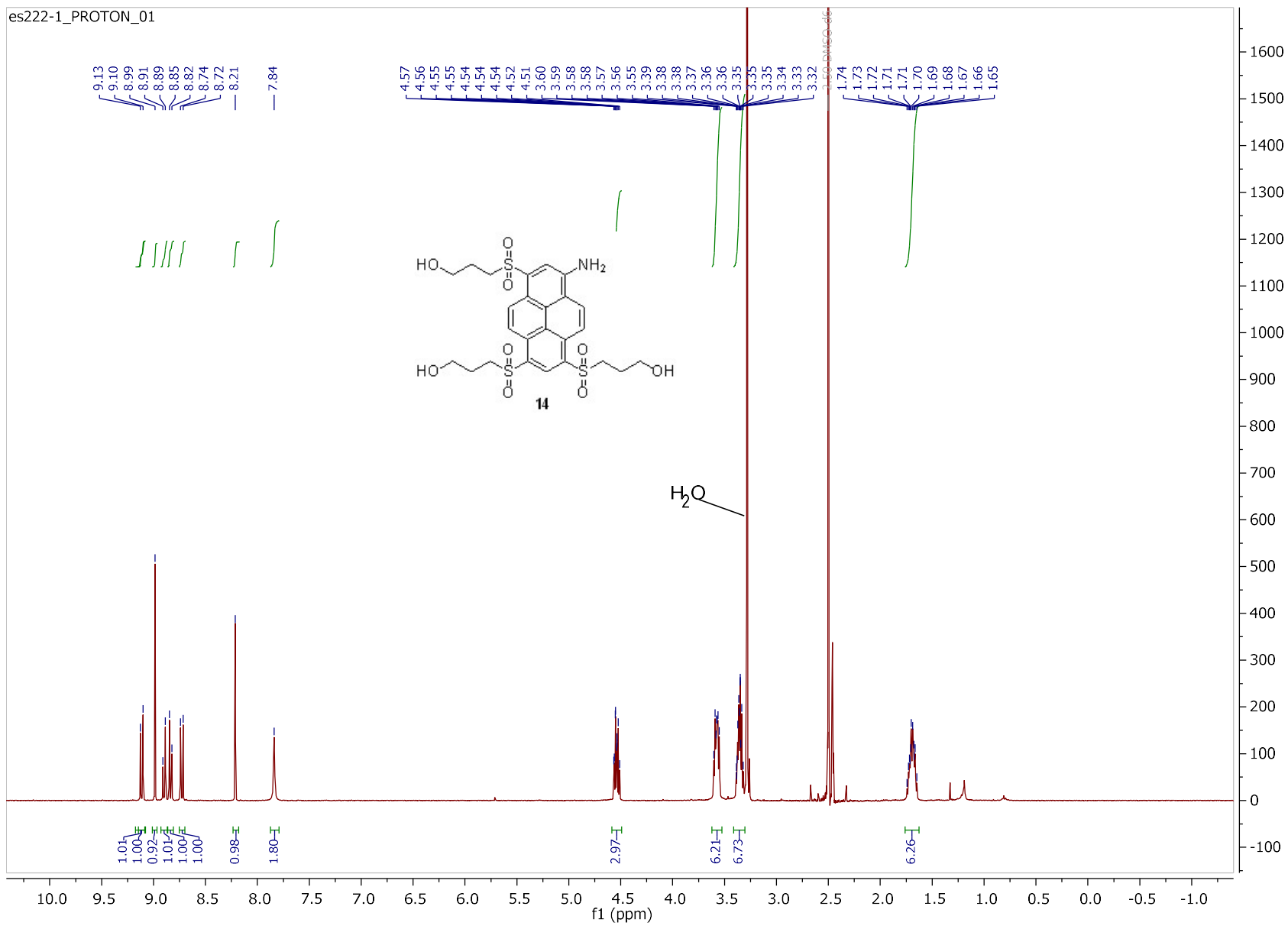


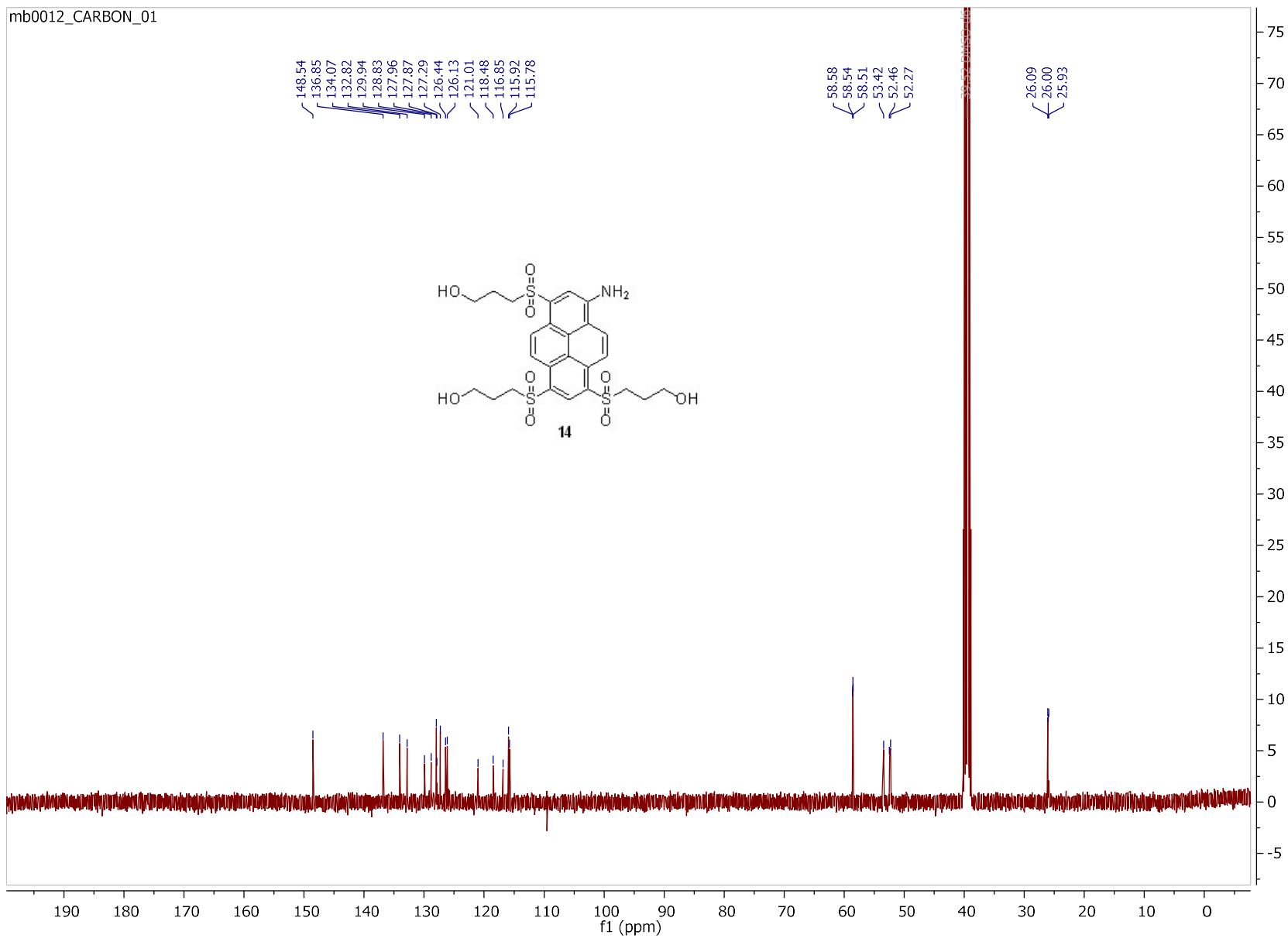


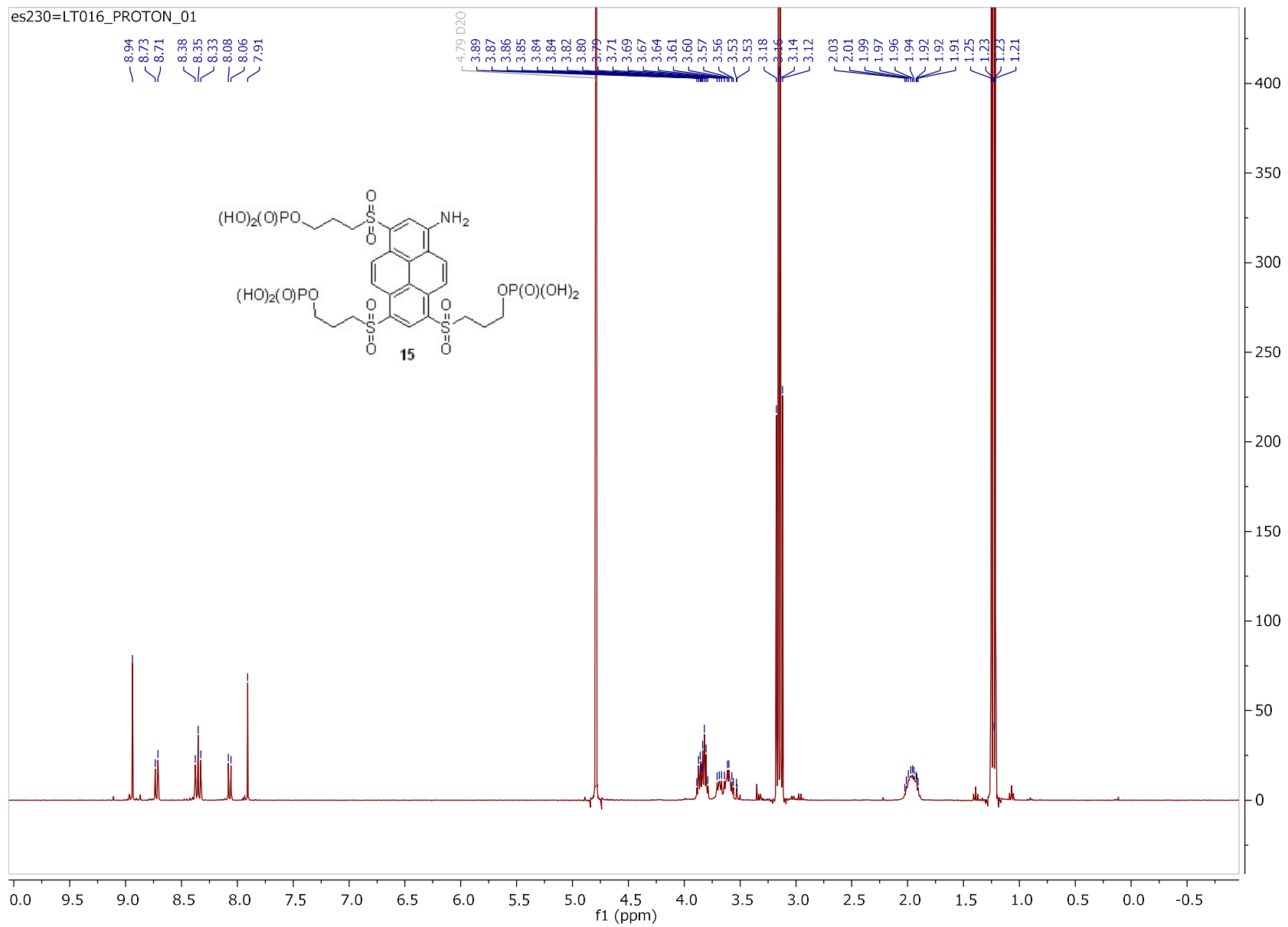


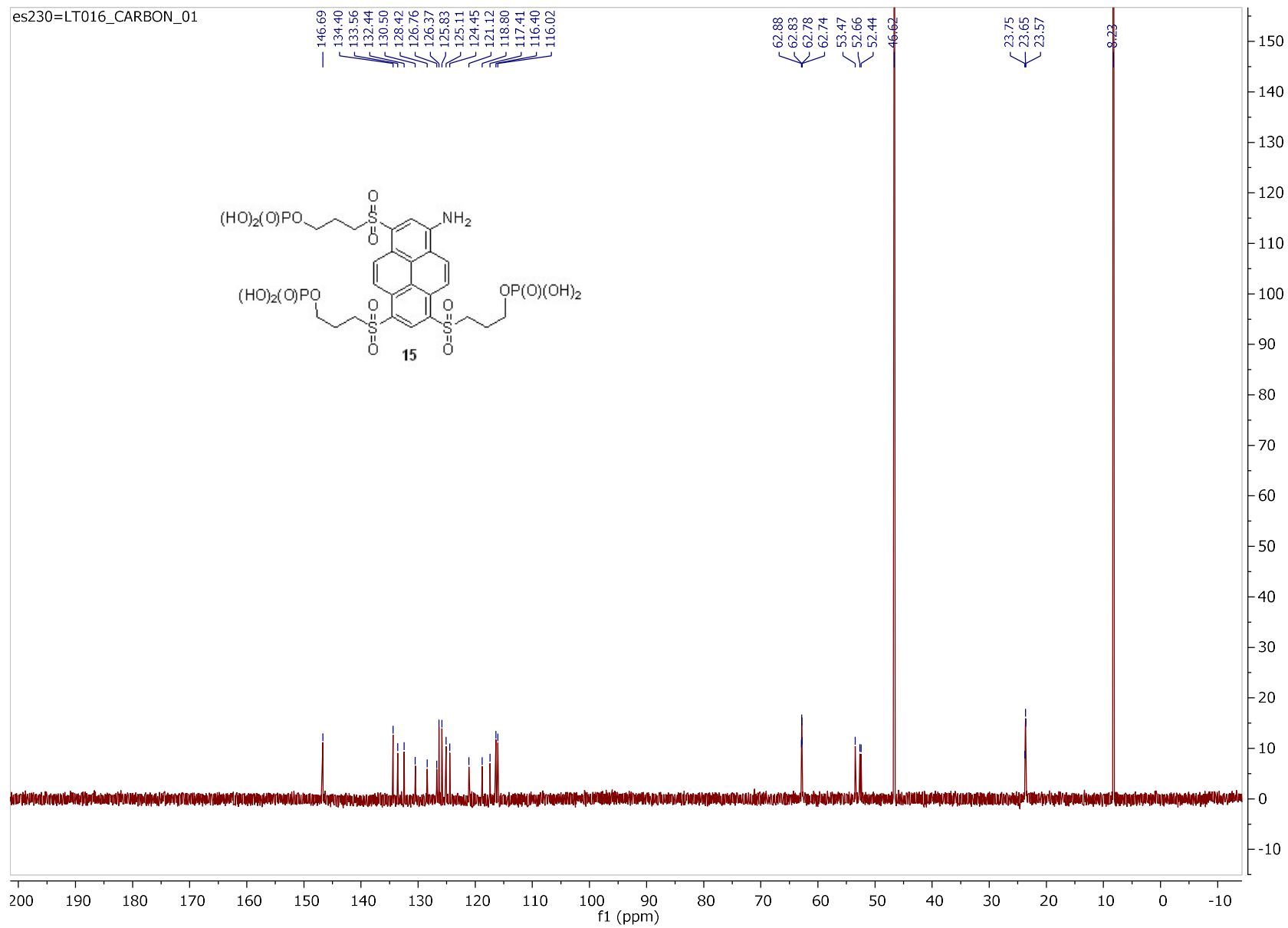
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Savicheva / MPI 10200 / PE











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