Supporting Information

Initial analysis of the arylomycin D antibiotics

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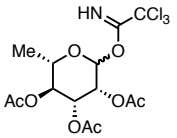
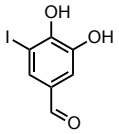
Procedures and characterization

Trichloroacetimidate 3



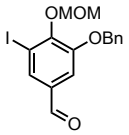
To a stirred suspension of L-rhamnose monohydrate (5 g, 27.4 mmol) in acetic anhydride (25 mL) at 0 °C was added 2-3 drops of conc. sulfuric acid. The suspension was stirred at 0 °C for a further 30 minutes before it was diluted with EtOAc and water. The organic layer was washed sequentially with sat. $NaHCO_3$, water and brine, then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in THF (25 mL) and to it was added 40% methylamine in water (7.1 mL, 3 eq.) at 0 °C. The reaction was stirred at room temperature for a further 2 h before it was diluted with EtOAc and water, and extracted two more times with EtOAc. The organic layers were combined and sequentially washed with sat. NH_4Cl, water and brine, then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in anhydrous CH_2Cl_2 (100 mL). DBU (0.82 mL, 5.48 mmol, 0.2 eq.) and trichloroacetonitrile (3.0 mL, 30.2 mmol, 1.1 eq.) was added and the reaction mixture was stirred for 14 h at room temperature. Solvents were removed under reduced pressure, and the crude product was purified by flash column chromatography (SiO_2, 20% EtOAc in hexanes) to give trichloroacetimidate **3** as a yellow oil (5.5 g, 46% yield). R_f = 0.73 (40% EtOAc in hexanes). The ^{1}H and ^{13}C NMR spectra were identical to that reported previously for this compound.[1]

Compound S1



To a stirred suspension of 5-iodovanillin (10.0 g, 36.0 mmol) in anhydrous dichloromethane (100 mL) at 0 °C was added boron tribromide (5.5 mL, 57.6 mmol, 1.6 eq.). The reaction mixture was allowed to warm to room temperature and stirred for 3 h, before being cooled to 0 °C and quenched by the slow addition of methanol. Solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (SiO_2, 40% EtOAc in hexanes) to give **S1** as a white solid (8.86 g, 93% yield). R_f = 0.40 (50% EtOAc in hexanes). ^{1}H NMR (600 MHz, Methanol-d_4) δ 9.64 (s, 1H), 7.75 (d, J = 1.9 Hz, 1H), 7.27 (d, J = 1.9 Hz, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 191.69, 153.76, 146.28, 135.50, 131.93, 114.46, 83.41. ESI HRMS calcd for $C_7H_5IO_3$ $[M-H]^-$ 262.9211, found 262.9219.

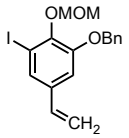
Compound S2



S1 (8.27 g, 31.3 mmol) was dissolved in anhydrous DMF (70 mL) and cooled to 0 °C before the addition of 60% NaH dispersion in mineral oil (2.88 g, 72.0 mmol, 2.3 eq.). [**CAUTION**: *potentially hazardous combination of reagents,*[2] *this reaction should not be run without the use of proper precautions such as careful cooling and quenching, and the use of a blast shield.*] The reaction mixture was stirred at 0 °C for 30 minutes before the slow dropwise addition of benzyl bromide (3.7 mL, 31.3 mmol, 1.0 eq.), then stirred for a further 1 h at 0 °C. Chloromethyl methyl ether (3.6 mL, 47.0 mmol, 1.5 eq.) was added and the reaction was allowed to warm to room temperature and stirred for 2 h. Water was added to quench the reaction and the mixture was extracted with EtOAc (3×). The organic layers were combined and sequentially washed with water and brine, then dried over Na_2SO_4 and concentrated

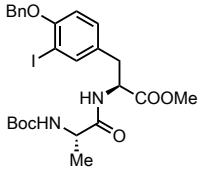
under reduced pressure. The crude material was purified by flash column chromatography (SiO_2, 10% EtOAc in hexanes) to give **S2** as a pale yellow oil (10.86 g, 88% yield). R_f = 0.52 (20% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.81 (s, 1H), 7.90 (d, *J* = 1.8 Hz, 1H), 7.49 (d, *J* = 1.8 Hz, 1H), 7.44 – 7.38 (m, 4H), 7.36 – 7.33 (m, 1H), 5.31 (s, 2H), 5.14 (s, 2H), 3.60 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 189.67, 151.67, 151.54, 135.64, 135.41, 133.91, 128.90, 128.63, 127.85, 112.72, 99.08, 92.52, 71.41, 58.71. ESI HRMS calcd for $C_{16}H_{15}IO_4$ $[M-H]^-$ 396.9942, found 396.9951.

Styrene 8



To a stirred suspension of aldehyde **S2** (10.86 g, 27.3 mmol) and methyltriphenylphosphonium bromide (14.6 g, 40.9 mmol, 1.5 eq.) in anhydrous THF (100 mL) at 0 °C was added 60% NaH dispersion in mineral oil (1.64 g, 40.9 mmol, 1.5 eq.). The reaction mixture was allowed to warm to room temperature and stirred for 14 h. The mixture was partitioned between EtOAc and sat. NH_4Cl and extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (SiO_2, 5% EtOAc in hexanes) to give styrene **8** as a colorless oil (9.29 g, 86% yield). R_f = 0.50 (10% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.45 (d, *J* = 1.9 Hz, 1H), 7.44 – 7.37 (m, 4H), 7.35 – 7.33 (m, 1H), 6.99 (d, *J* = 1.9 Hz, 1H), 6.55 (dd, *J* = 17.5, 10.9 Hz, 1H), 5.62 (d, *J* = 17.5, 1H), 5.22 (d, *J* = 10.9 Hz, 1H), 5.19 (s, 2H), 5.09 (s, 2H), 3.60 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 151.27, 146.15, 136.39, 135.78, 135.08, 129.60, 128.77, 128.34, 127.71, 114.54, 112.28, 99.01, 93.01, 71.29, 58.55. ESI HRMS calcd for $C_{17}H_{17}IO_3$ $[M+H]^+$ 397.0295, found 397.0293.

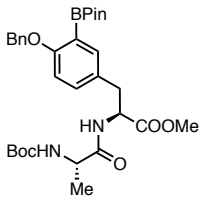
Dipeptide 12



To a solution of 3-iodo-L-tyrosine (6 g, 19.5 mmol) in MeOH (75 mL) was slowly added $SOCl_2$ (3.0 mL, 41.0 mmol, 2.1 eq.) at 0 °C. The solution was refluxed for 2 h, and then the solvent was removed under reduced pressure. The crude material was dissolved in anhydrous DMF (100 mL) and treated sequentially with triethylamine (13.6 mL, 97.5 mmol, 5.0 eq.), 1-Hydroxybenzotriazole hydrate (HOBt) (3.0 g, 19.5 mmol, 1.0 eq.), and Boc-L-Ala-OH (4.06 g, 21.5 mmol, 1.1 eq.). The mixture was cooled to 0 °C and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC) (5.60 g, 29.2 mmol, 1.5 eq.) was added in one portion. The reaction was allowed to warm to room temperature and stirred for 14 h. The mixture was partitioned between EtOAc and water, and extracted twice with EtOAc. The organic layers were combined and washed with water and brine, dried over Na_2SO_4 and concentrated to a white foam under reduced pressure. The crude material was dissolved in acetone (100 mL) and treated sequentially with K_2CO_3 (13.5 g, 97.5 mmol, 5.0 eq.) and benzyl bromide (2.5 mL, 21.4 mmol, 1.1 eq.), then refluxed for 14 h. The solvent was removed under reduced pressure and the residue was partitioned between EtOAc and water, and extracted twice with EtOAc. The organic layers were combined and washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 40% EtOAc in hexanes) to give **12** as a white solid (9.36 g, 82% yield). R_f = 0.26 (40% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.53 (d, *J* = 2.1 Hz, 1H), 7.48 (d, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.02 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.60 (br s, 1H), 5.12 (s, 2H), 4.91 (br s, 1H), 4.77 (dt, *J* = 7.7, 5.8 Hz, 1H), 4.19 – 4.08 (m, 1H), 3.72 (s, 3H), 3.08 (dd, *J* = 14.0, 5.8 Hz, 1H), 2.9 (dd, *J* = 14.0, 5.8 Hz, 1H), 1.44 (s, 9H), 1.33 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.36,

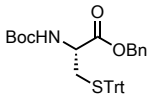
171.64, 156.52, 155.50, 140.40, 136.56, 130.41, 130.33, 128.69, 128.04, 127.13, 112.66, 86.84, 80.39, 71.01, 53.39, 52.56, 50.38, 36.62, 28.43, 18.28. ESI HRMS calcd for $C_{25}H_{31}IN_2O_6$ $[M+H]^+$ 583.1300, found 583.1299.

Dipeptide 13



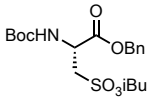
Bis(pinacolato)diboron (914 mg, 3.6 mmol, 1.5 eq), **12** (1.4 g, 2.4 mmol), KOAc (706 mg, 7.2 mmol, 3.0 eq) and $PdCl_2$(dppf) (196 mg, 0.24 mmol, 0.1 eq) were suspended in anhydrous DMSO (6.2 mL, degassed by sparging with Ar), and heated to 90 ºC for 14 h under Ar. The mixture was cooled to room temperature and partitioned between EtOAc and water, and extracted twice with EtOAc. The organic layers were combined and washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 45% EtOAc in hexanes) to give compound **13** as a white foam (1.3 g, 93% yield). R_f = 0.29 (40% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.60 (d, *J* = 7.6 Hz, 2H), 7.39 – 7.32 (m, 3H), 7.27 (t, *J* = 16.5 Hz, 1H), 7.10 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.74 – 6.69 (br s, 1H), 5.58 – 5.51 (br s, 1H), 5.09 (ABq, *J* = 12.2 Hz, 2H), 4.79 (q, *J* = 6.1 Hz, 1H), 4.27 – 4.21 (m, 1H), 3.72 (s, 3H), 3.21 (dd, *J* = 13.9, 5.0 Hz, 1H), 3.01 (dd, *J* = 13.9, 5.0 Hz, 1H), 1.46 – 1.29 (m, 24H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.35, 171.60, 162.52, 155.89, 138.46, 137.60, 133.34, 128.23, 127.47, 127.40, 126.70, 115.03, 112.03, 83.82, 75.11, 69.84, 53.41, 52.38, 50.03, 36.38, 28.35, 25.27, 24.89. ESI HRMS calcd for $C_{31}H_{43}BN_2O_8$ $[M+H]^+$ 583.3191, found 583.3183.

Compound S3a



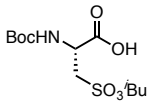
To a stirred suspension of S-Trityl-L-cysteine (500mg, 1.38 mmol) and $NaHCO_3$ (1.73 mg, 2.06 mmol, 1.5 eq.) in a 1:1 mixture of acetone and water (6 mL) was added Boc_2O (330 mg, 1.51 mmol, 1.1 eq.) at 0 °C. The reaction was allowed to warm to room temperature and stirred for 14 h. The mixture was treated with 1 N HCl until pH 4, and extracted with EtOAc (3×). The organic layers were combined and washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure to a white foam. The crude material was dissolved in CH_2Cl_2 (10 mL), and treated sequentially with DMAP (17mg, 0.14 mmol, 0.1 eq.), benzyl alcohol (0.16 mL, 1.52 mmol, 1.1 eq.), and DCC (313 mg, 1.52 mmol, 1.1 eq.), and stirred for 14 h at room temperature. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 15% EtOAc in hexanes) to give **S3a** as a white solid (685mg, 90% yield). R_f = 0.61 (20% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.37 (d, *J* = 7.8 Hz, 6H), 7.35 – 7.30 (m, 5H), 7.26 (t, *J* = 7.6 Hz, 6H), 7.20 (t, *J* = 7.2 Hz, 3H), 5.17 (d, *J* = 11.9 Hz, 1H), 5.12 (d, *J* = 11.9 Hz, 1H), 5.09 – 5.05 (m, 1H), 4.37 – 4.31 (m, 1H), 2.63 (dd, *J* = 12.2, 6.1 Hz, 1H), 2.56 (dd, *J* = 12.2, 4.5 Hz, 1H), 1.44 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.83, 155.13, 144.40, 135.37, 129.61, 128.65, 128.47, 128.37, 128.12, 126.97, 80.15, 67.40, 66.90, 52.72, 34.37, 28.45. ESI HRMS calcd for $C_{34}H_{35}NO_4S$ $[M+Na]^+$ 576.2179, found 576.2185.

Compound S4a



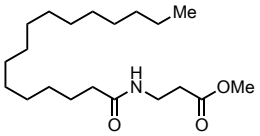
To a mixture of **S3a** (554 mg, 1.0 mmol) and H_2O (54 μL, 3.0 mmol, 3.0 eq.) in a 1:1 mixture of CH_3CN and THF (10 mL) was added *t*-BuOCl[3] (326 mg, 3.0 mmol, 3.0 eq.) at 0°C. The reaction was stirred at 0°C for 15 minutes before solvents were removed under reduced pressure. The crude material was dissolved in CH_2Cl_2 (5 mL) and to it was added NEt_3 (0.7 mL. 5.0 mmol, 5.0 eq.) and isobutyl alcohol (0.46 mL, 5.0 mmol, 5.0 eq.) at 0°C. The reaction was allowed to warm to room temperature and stirred for 3h before solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 10% to 20% EtOAc in hexanes) to give **S4a** as a colorless oil (253 mg, 61% yield). R_f = 0.44 (20% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.38 – 7.34 (m, 5H), 5.58 (d, *J* = 7.9 Hz, 1H), 5.24 (d, *J* = 12.1 Hz, 1H), 5.19 (d, *J* = 12.1 Hz, 1H), 4.69 (dt, *J* = 7.9, 4.9 Hz, 1H), 3.99 – 3.92 (m, 2H), 3.80 – 3.70 (m, 2H), 2.00 (m, 1H), 1.43 (s, 9H), 0.96 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.90, 155.11, 134.88, 128.79, 128.69, 80.89, 76.44, 68.38, 51.00, 50.23, 28.37, 28.36, 18.76. ESI HRMS calcd for $C_{19}H_{29}NO_7S$ $[M+Na]^+$ 438.1557, found 438.1565.

Compound 16a



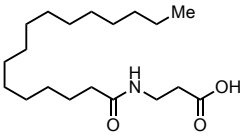
A mixture of **S4a** (250 mg, 0.60 mmol) and 10% Pd/C (128 mg, 0.12 mmol, 0.2 eq.) in MeOH (9 mL) was stirred at room temperature for 6 h under a hydrogen atmosphere. The mixture was filtered through celite and concentrated under reduced pressure to give **16a** as a white solid which was used without further purification (196 mg, quantitative yield). ^{1}H NMR (600 MHz, Methanol-d_4) δ 4.57 (dd, *J* = 8.5, 3.5 Hz, 1H), 4.02 (d, *J* = 6.5 Hz, 2H), 3.77 (dd, *J* = 14.9, 3.5, 1H), 3.66 (dd, *J* = 14.9, 8.5 Hz, 1H), 2.05 – 1.98 (m, 1H), 1.45 (s, 9H), 0.99 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (151 MHz, MeOD) δ 172.47, 157.42, 80.99, 77.65, 51.46, 51.04, 29.51, 28.70, 18.97. ESI HRMS calcd for $C_{12}H_{23}NO_7S$ $[M+Na]^+$ 348.1087, found 348.1097.

Compound S5



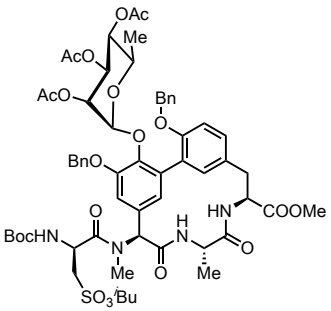
To a solution of β-Ala-OMe.HCl (500 mg, 3.6 mmol) and NEt_3 (1.0 mL, 7.2 mmol, 2.0 eq.) in DMF (20 mL) was added palmitoyl chloride (1.1 mL, 3.6 mmol. 1.0 eq.) dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 14 h. The mixture was partitioned between EtOAc and water, and extracted two more times with EtOAc. The organic layers were combined and washed sequentially with sat. NH_4Cl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 33% to 50% EtOAc in hexanes) to give **S5** as a white solid (1.0 g, 81% yield). R_f = 0.31 (50% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.01 (s, 1H), 3.70 (s, 3H), 3.52 (td, *J* = 6.1 Hz, 6.1 Hz, 2H), 2.59 – 2.46 (m, 2H), 2.20 – 2.07 (m, 2H), 1.64 – 1.55 (m, 2H), 1.35 – 1.20 (m, 24H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.35, 173.29, 51.89, 36.91, 34.84, 33.99, 32.04, 29.82, 29.81, 29.80, 29.77, 29.73, 29.62, 29.48, 29.47, 29.39, 25.83, 22.81, 14.24. ESI HRMS calcd for $C_{20}H_{39}NO_3$ $[M+H]^+$ 342.3003, found 342.3008.

Compound 17



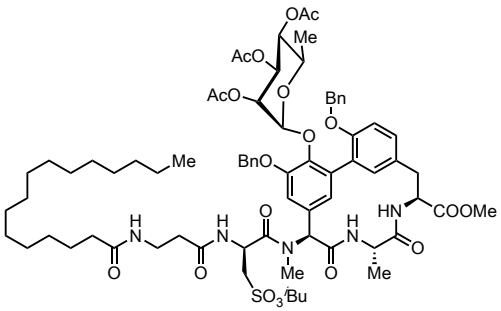
S5 (342 mg, 1 mmol) was dissolved in THF (2 mL) and treated with 1 N LiOH (2 mL, 2 mmol, 2 eq.) and stirred for 14 h at room temperature. THF was removed under reduced pressure. The residue was acidified with 1 N HCl and extracted three times with CH_2Cl_2. The organic layers were combined and washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give compound **17** as a white solid which was used without further purification. (327 mg, quantitative yield). ^{1}H NMR (600 MHz, Methanol-*d*$_4$) δ 3.41 (t, *J* = 6.8 Hz, 2H), 2.49 (t, *J* = 6.8 Hz, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.59 (quint, *J* = 7.3 Hz, 2H), 1.34 – 1.27 (m, 24H), 0.90 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 176.40, 37.05, 36.47, 33.08, 30.79, 30.76, 30.73, 30.61, 30.48, 30.45, 30.26, 27.01, 23.74, 14.44. ESI HRMS calcd for $C_{19}H_{37}NO_3$ $[M+H]^+$ 328.2846, found 328.2852.

Compound 2b



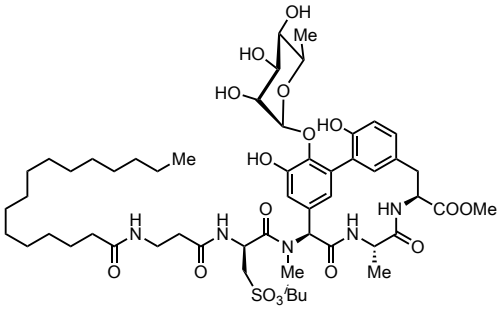
Compound **2b** was prepared in the same manner as compound **2a** from compounds **15** and **16b**. R_f = 0.42 (67% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, $CDCl_3$) multiple isomers, see spectrum. ESI HRMS calcd for $C_{60}H_{74}N_4O_{20}S$ $[M+H]^+$ 1203.4690, found 1203.4667.

Compound 18b



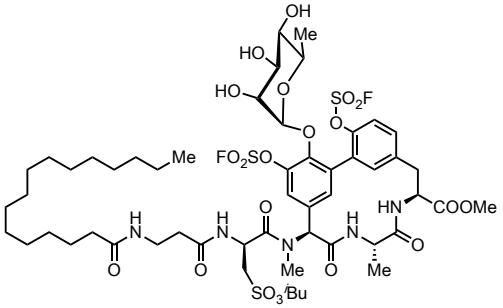
Compound **18b** was prepared in the same manner as compound **18a** from compounds **17** and **2b**. R_f = 0.16 (80% EtOAc in hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, $CDCl_3$) multiple isomers, see spectrum. ESI HRMS calcd for $C_{74}H_{101}N_5O_{20}S$ $[M+H]^+$ 1412.6833, found 1412.6843.

Compound 19b



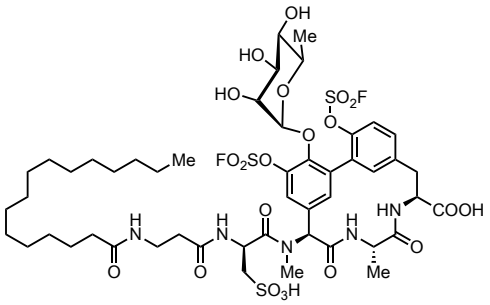
Compound **19b** was prepared in the same manner as compound **19a** from compound **18b**. ^{1}H NMR (600 MHz, Methanol-d_4) multiple isomers, see spectrum .^{13}C NMR (151 MHz, MeOD) multiple isomers, see spectrum. ESI HRMS calcd for $C_{54}H_{83}N_5O_{17}S$ $[M+H]^+$ 1106.5577, found 1106.5576.

Compound 20b



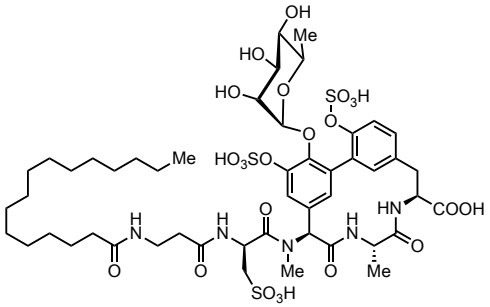
Compound **20b** was prepared in the same manner as compound **20a** from compound **19b**. ^{1}H NMR (600 MHz, Methanol-d_4) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, MeOD) multiple isomers, see spectrum. ESI HRMS calcd for $C_{54}H_{81}F_2N_5O_{21}S_3$ $[M+H]^+$ 1270.4627, found 1270.4637.

Compound S6b



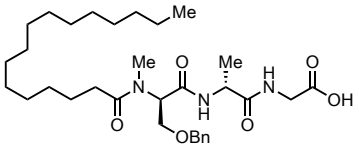
Compound **S6b** was prepared in the same manner as compound **S6a** from compound **20b**. ^{1}H NMR (600 MHz, Methanol-d_4) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, MeOD) multiple isomers, see spectrum. ESI HRMS calcd for $C_{49}H_{71}F_2N_5O_{21}S_3$ $[M+H]^+$ 1200.3844, found 1200.3844.

Arylomycin derivative 1b



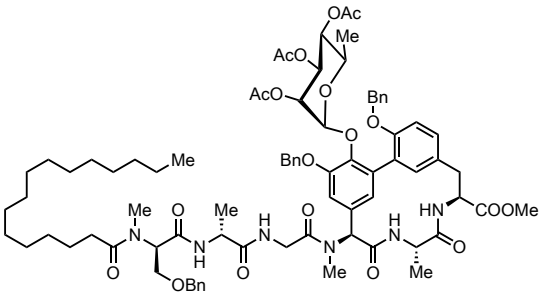
Derivative **1b** was prepared in the same manner as derivative **1a** from compound **S6b**. ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.56 (d, J = 8.6 Hz, 1H), 7.54 (d, J = 2.3 Hz, 1H), 7.20 (dd, J = 8.6, 2.3 Hz, 1H), 6.90 (d, J = 2.3 Hz, 1H), 6.77 (d, J = 2.3 Hz, 1H), 6.21 (s, 1H), 5.45 (dd, J = 9.1, 3.6 Hz, 1H), 5.26 (d, J = 1.9 Hz, 1H), 4.79 – 4.70 (m, 2H), 4.34 (dd, J = 3.4, 1.9 Hz, 1H), 3.93 (dd, J = 9.2, 3.4 Hz, 1H), 3.47 (dd, J = 19.3, 6.3 Hz, 2H), 3.29 – 3.11 (m, 6H), 3.04 (s, 3H), 2.45 – 2.38 (m, 2H), 2.25 – 2.17 (m, 2H), 1.63 – 1.53 (m, 2H), 1.37 – 1.22 (m, 26H), 0.90 (t, J = 7.1 Hz, 3H), 0.62 (d, J = 5.8 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 176.45, 173.61, 173.07, 171.47, 171.06, 163.29, 149.57, 147.87, 146.71, 136.41, 134.38, 133.96, 132.43, 131.90, 130.35, 123.51, 120.92, 105.38, 74.05, 72.02, 71.68, 71.16, 64.14, 54.25, 53.01, 50.47, 49.57, 47.91, 37.12, 36.81, 36.70, 35.18, 33.08, 30.80, 30.77, 30.67, 30.55, 30.48, 30.40, 26.99, 23.74, 19.23, 17.48, 14.44. ESI HRMS calcd for $C_{49}H_{73}N_5O_{23}S_3$ $[M-2H]^{2-}$ 596.6856, found 596.6848.

Compound 21



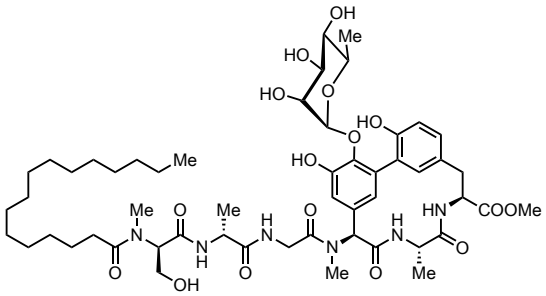
Compound **21** was synthesized as described in a previous literature procedure.[4] The ^{1}H and ^{13}C NMR spectra were identical to that reported previously for this compound.

Compound S7



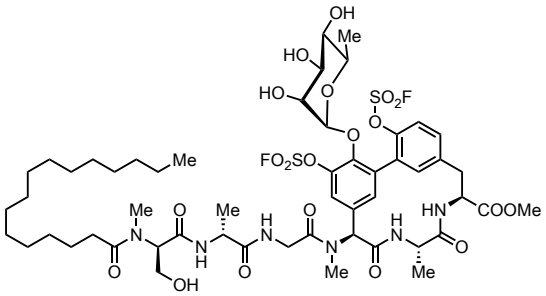
A solution of glycosylated macrocycle **15** (65 mg, 0.073 mmol) in DMF (2 mL) was treated sequentially with **21** (71 mg, 0.22 mmol mmol, 3.0 eq.), $NaHCO_3$ (61 mg, 0.73 mmol, 10.0 eq.), and DEPBT (65 mg, 0.22 mmol, 3.0 eq.), and stirred at room temperature for 16 h. The reaction was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution, and extracted twice with EtOAc. The organic layers were combined and washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash column chromatography (SiO_2, 3% MeOH in CH_2Cl_2) to give compound **S7** as a white solid (80 mg, 76% yield). R_f = 0.36 (5% MeOH in CH_2Cl_2). ^{1}H NMR (600 MHz, Chloroform-d) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, $CDCl_3$) multiple isomers, see spectrum. ESI HRMS calcd for $C_{80}H_{104}N_6O_{19}$ $[M+H]^+$ 1453.7429, found 1453.7439.

Compound S8



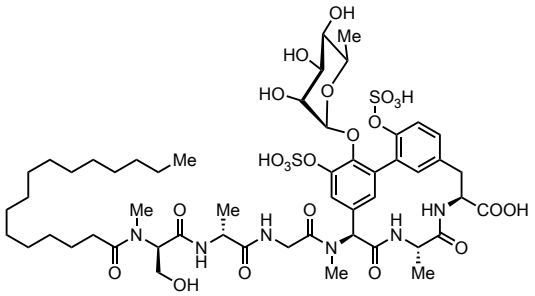
Compound **S7** (63 mg, 0.043 mmol) was dissolved in anhydrous MeOH (2.5 mL), and to it was added 30% NaOMe in MeOH (24 μL, 0.086 mmol, 3.0 eq.) at 0 °C and stirred for 2 h at the same temperature. Amberlyst-15 ion exchange resin was added to the reaction and the mixture was stirred for a further 30 minutes. The resin was filtered off and solvents were removed under reduced pressure. The residue and 10% Pd/C (46 mg, 0.043 mmol, 1.0 eq.) were suspended in MeOH (2.5 mL) and stirred for 3 h at room temperature under a hydrogen atmosphere. The Pd/C was filtered off through celite, and solvents were removed under reduced pressure to yield **S8** (33 mg, 72% yield) as a white solid which was used without further purification. ^{1}H NMR (600 MHz, Methanol-d_4) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, MeOD) multiple isomers, see spectrum. ESI HRMS calcd for $C_{53}H_{80}N_6O_{16}$ $[M+H]^+$ 1057.5703, found 1057.5691.

Compound 22



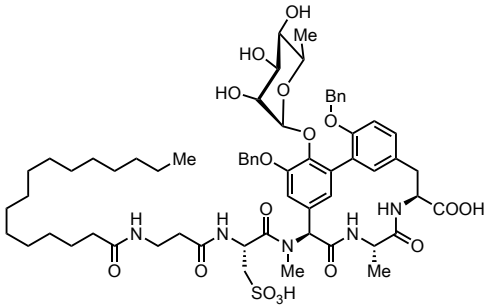
S8 (12 mg, 0.011 mmol) and NEt_3 (16 μL, 0.11 mmol, 10 eq.) were dissolved in a 1:1 mixture of DMF and H_2O (1.0 mL) and stirred for 24 h at room temperature under a SO_2F_2 atmosphere. The reaction mixture was partitioned between EtOAc and 0.5 N HCl, and the aqueous layer was extracted twice with EtOAc. The organic layers were combined and washed sequentially with 5% aqueous LiCl solution and brine, dried over Na_2SO_4, and concentrated under reduced pressure to give **22** as a white solid (10 mg, 72% yield) which was used without further purification. ^{1}H NMR (600 MHz, Methanol-d_4) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, MeOD) multiple isomers, see spectrum. ESI HRMS calcd for $C_{53}H_{78}F_2N_6O_{20}S_2$ $[M+H]^+$ 1221.4753, found 1221.4748.

Arylomycin derivative 23



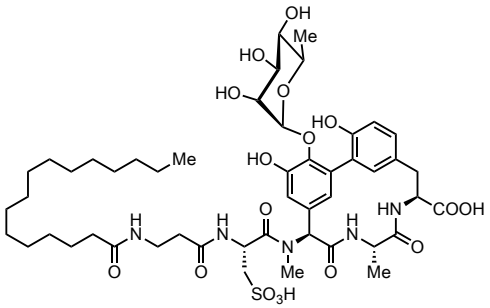
Compound **22** (5 mg, 4.1 μmol), dried cesium carbonate (6 mg, 0.018 mmol, 4.5 eq.), and activated 3 Å molecular sieves were suspended in anhydrous ethylene glycol (0.1 mL) in a flame-dried flask and heated to 50 ºC for 20 minutes under Ar. The reaction was cooled to room temperature and water (0.1 mL) was added to it. The mixture was stirred for a further 2 h at room temperature, then purified by reverse phase preparatory HPLC (NH_4OAc buffered conditions) to yield derivative **23** as a white solid (1.1 mg, 22% yield) after lyophilization. ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.56 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 6.80 (d, J = 2.1 Hz, 1H), 5.25 (d, J = 1.8 Hz, 1H), 4.98 (dd, J = 7.6, 5.9 Hz, 1H), 4.76 (q, J = 7.0 Hz, 1H), 4.46 (d, J = 4.9 Hz, 1H), 4.37 – 4.30 (m, 1H), 4.24 (d, J = 17.1 Hz, 1H), 4.18 – 4.10 (m, 1H), 4.03 (dd, J = 11.3, 6.0 Hz, 1H), 3.98 – 3.85 (m, 2H), 3.69 – 3.62 (m, 1H), 3.47 (d, J = 15.4 Hz, 1H), 3.24 – 3.14 (m, 3H), 3.10 (s, 3H), 2.88 (d, J = 21.7 Hz, 2H), 2.48 – 2.41 (m, 2H), 1.90 (s, 3H), 1.68 – 1.55 (m, 2H), 1.47 – 1.25 (m, 29H), 0.90 (t, J = 7.0 Hz, 3H), 0.61 (d, J = 5.8 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 177.03, 174.90, 171.81, 171.54, 163.30, 163.07, 162.84, 149.51, 147.83, 146.78, 136.43, 135.29, 134.53, 132.65, 132.37, 131.55, 130.51, 123.06, 120.54, 105.38, 74.08, 72.01, 71.65, 71.15, 64.31, 60.71, 56.25, 50.84, 50.37, 49.85, 42.53, 35.83, 34.59, 34.22, 33.67, 33.08, 30.79, 30.76, 30.69, 30.61, 30.48, 26.09, 23.74, 19.21, 17.94, 17.48, 14.44. ESI HRMS calcd for $C_{52}H_{78}N_6O_{22}S_2$ $[M-2H]^{2-}$ 600.2227, found 600.2228.

Compound S9



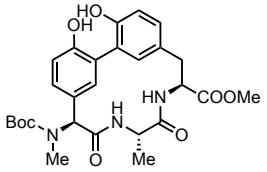
18a (40 mg, 0.028 mmol) was dissolved in anhydrous MeOH (2.0 mL), and to it was added 30% NaOMe in MeOH (15 μL, 0.085 mmol, 3.0 eq.) at 0 °C and stirred for 2 h at the same temperature. Amberlyst-15 ion exchange resin was added to the reaction and the mixture was stirred for a further 30 minutes. The resin was filtered off and solvents were removed under reduced pressure. A mixture of the residue and trimethyltin hydroxide (31 mg, 0.17 mmol, 6 eq.) in anhydrous DCE (2 mL) was heated to 70 °C for 16 h, then cooled to room temperature. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and 0.5 N HCl, and the aqueous layer was extracted twice with EtOAc. The organic layers were combined and washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue and sodium iodide (42 mg, 0.28 mmol, 10 eq.) were suspended in acetone (2 mL) and heated to 50 °C for 4 h, then concentrated under reduced pressure. The crude material was taken up in MeOH and purified by reverse phase preparatory HPLC (0.1% TFA conditions) to yield compound **S9** as a white solid (13 mg, 38% yield) after lyophilization. ^{1}H NMR (600 MHz, Methanol-d_4) multiple isomers, see spectrum. ^{13}C NMR (151 MHz, MeOD) multiple isomers, see spectrum. ESI HRMS calcd for $C_{63}H_{85}N_5O_{17}S$ $[M+H]^+$ 1216.5734, found 1216.5733.

Arylomycin derivative 24



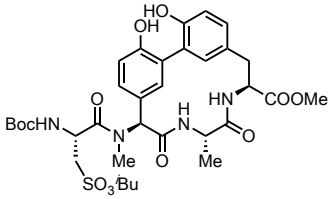
S9 (10 mg, 8.22 μmol) and 10% Pd/C (8.7 mg, 8.22 μmol, 1.0 eq.) were suspended in MeOH (5.0 mL) and stirred for 3 h at room temperature under a hydrogen atmosphere. The Pd/C was filtered off through celite, and solvents were removed under reduced pressure. The crude material was taken up in MeOH and purified by reverse phase preparatory HPLC (NH_4OAc buffered conditions) to yield derivative **24** as a white solid (5.8 mg, 68% yield) after lyophilization. ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.07 (d, J = 9.2 Hz, 1H), 6.88 (s, 1H), 6.82 (d, J = 9.2 Hz, 1H), 6.78 (s, 1H), 6.44 (s, 1H), 6.28 (s, 1H), 5.42 (s, 1H), 5.18 (d, J = 11.4 Hz, 1H), 4.26 (s, 1H), 3.80 – 3.68 (m, 1H), 3.52 (q, J = 6.4 Hz, 2H), 3.45 – 3.38 (m, 1H), 3.21 – 3.04 (m, 4H), 2.98 (d, J = 10.1 Hz, 2H), 2.49 (s, 2H), 2.23 (t, J = 7.5 Hz, 2H), 1.66 – 1.50 (m, 2H), 1.43 – 1.23 (d, J = 21.8 Hz, 34H), 0.90 (t, J = 7.0 Hz, 3H), 0.75 – 0.63 (m, 3H). ^{13}C NMR (151 MHz, MeOD) δ 176.72, 174.71, 174.61, 174.21, 174.14, 173.73, 153.06, 151.55, 147.94, 130.71, 130.45, 127.95, 126.64, 117.73, 117.11, 115.37, 103.98, 102.66, 95.85, 73.15, 72.23, 72.05, 71.46, 61.94, 53.33, 50.23, 50.00, 49.84, 40.42, 37.06, 36.57, 33.53, 33.08, 30.80, 30.76, 30.67, 30.51, 30.48, 30.39, 27.02, 23.74, 19.19, 17.94, 17.35, 14.44. For the same compound in DMSO-d_6, see spectrum. ESI HRMS calcd for $C_{49}H_{73}N_5O_{17}S$ $[M+Na]^+$ 1058.4614, found 1058.4613.

Macrocycle 25



Macrocycle **25** was synthesized as described in a previous literature procedure.[5] The ^{1}H and ^{13}C NMR spectra were identical to that reported previously for this compound.

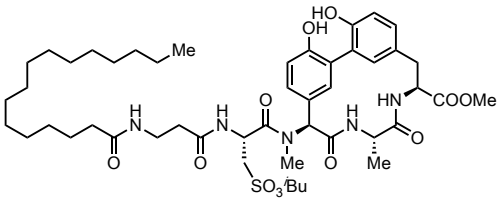
Compound S10



To a solution of macrocycle **25** (80 mg, 0.15 mmol) in CH_2Cl_2 (3.2 mL) was slowly added TFA (0.8 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 1.5 h. The solvents were removed under reduced pressure to yield the free amine used in the next step without purification. A solution of **16a** (148 mg, 0.45 mmol, 3 eq.) in THF (2 mL) was treated sequentially with $NaHCO_3$ (96 mg, 1.5 mmol, 10 eq.) and DEPBT (136 mg, 0.45 mmol, 3 eq.) and stirred at room temperature for 30 minutes. The above amine (dissolved in 2 mL of THF) was added and the reaction stirred at room temperature for a further 16 h. The reaction was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution, and extracted twice with EtOAc. The organic layers were combined and washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash column chromatography (SiO_2, 67% EtOAc in

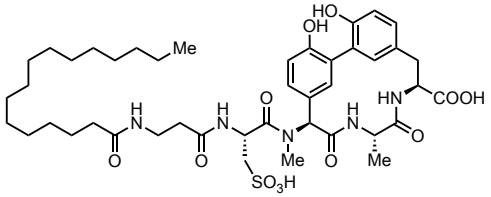
hexanes) to give **S10** as a white solid (50 mg, 45% yield). R_f = 0.26 (67% EtOAc in hexanes). ^{1}H NMR (600 MHz, Methanol-d_4) multiple conformational isomers, major isomer annotated in spectrum below, see spectrum below. ^{13}C NMR (151 MHz, MeOD) multiple conformational isomers, major isomer annotated in spectrum below, see spectrum below. ESI HRMS calcd for $C_{34}H_{46}N_4O_{12}S$ $[M+Na]^+$ 757.2725, found 757.2724.

Compound 26



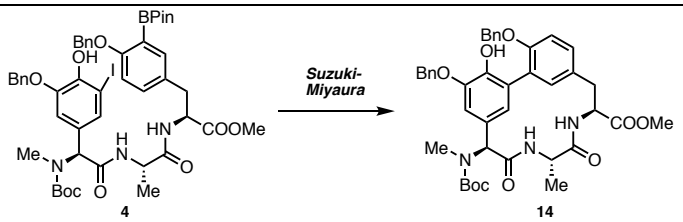
To a solution of **S10** (50 mg, 0.068 mmol) in CH_2Cl_2 (2.7 mL) was slowly added TFA (0.3 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 1.5 h. The solvents were removed under reduced pressure to yield the free amine used in the next step without purification. A solution of **17** (67 mg, 0.204 mmol, 3 eq.) in THF (3 mL) was treated sequentially with $NaHCO_3$ (29 mg, 0.34 mmol, 5 eq.) and DEPBT (61 mg, 0.204 mmol, 3 eq.) and stirred at room temperature for 30 minutes. The above free amine (dissolved in 2 mL of THF) was added and the reaction stirred at room temperature for a further 16 h. The reaction was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution, and extracted twice with EtOAc. The organic layers were combined and washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash column chromatography (SiO_2, 5% MeOH in CH_2Cl_2) to give **26** as a white solid (20 mg, 31% yield). R_f = 0.10 (80% EtOAc in hexanes). ^{1}H NMR (600 MHz, Methanol-d_4) multiple conformational isomers, major isomer annotated in spectrum below, see spectrum below. ^{13}C NMR (151 MHz, MeOD) multiple conformational isomers, major isomer annotated in spectrum below, see spectrum below.. ESI HRMS calcd for $C_{48}H_{73}N_5O_{12}S$ $[M+H]^+$ 944.5049, found 944.5043.

Arylomycin derivative 27



A mixture of compound **26** (15 mg, 0.0159 mmol) and trimethyltin hydroxide (14 mg, 0.0794 mmol, 5 eq.) in anhydrous DCE (1.5 mL) was heated to 70 °C for 16 h, then cooled to room temperature. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and 0.5 N HCl, and the aqueous layer was extracted twice with EtOAc. The organic layers were combined and washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue and sodium iodide (24 mg, 0.159 mmol, 10 eq.) were suspended in acetone (1.5 mL) and heated to 50 °C for 4 h, then concentrated under reduced pressure. The crude material was taken up in MeOH and purified by reverse phase preparatory HPLC (0.1% TFA conditions) to yield derivative **27** as a white solid (4.6 mg, 33% yield) after lyophilization. ^{1}H NMR (600 MHz, Methanol-d_4) multiple conformational isomers, see spectrum below. ^{13}C NMR (151 MHz, MeOD) multiple conformational isomers, see spectrum below. ESI HRMS calcd for $C_{43}H_{63}N_5O_{12}S$ $[M+H]^+$ 874.4266, found 874.4267.

Table S1: Additional optimization of the Suzuki-Miyaura reaction.



Entry	Pd Catalyst	Base	Solvent	Yield [b]
1	$PdCl_2$dppf (0.2 eq.)	K_2CO_3	DMSO	21%
2	$PdCl_2$dppf (0.2 eq.)	K_2CO_3	DMSO [c]	<10%
3	Pd(0)[(P(*t*Bu)$_3$]$_2$ (0.2 eq.)	K_2CO_3	DMSO	<10%
4	$PdCl_2(PPh_3)_2$ (0.2 eq.)	K_2CO_3	DMSO	unreacted SM
5	PEPPSI-IPr (0.2 eq.)	K_2CO_3	DMSO	unreacted SM
6	$PdCl_2$ (0.2 eq.)+ SPhos	K_2CO_3	DMSO	<10%
7	$PdCl_2$dppf (0.2 eq.)	$NaHCO_3$	DMSO	<10%
8	$PdCl_2$dppf (0.2 eq.)	Cs_2CO_3	DMSO	<10%
9	$PdCl_2$dppf (0.2 eq.)	K_3PO_4	DMSO	<10%
10	$PdCl_2$dppf (0.2 eq.)	K_2CO_3	30:1 Tol:H_2O	unreacted SM
11	$PdCl_2$dppf (0.2 eq.)	K_2CO_3	CH_3CN [c]	19%
12	$PdCl_2$dppf (0.2 eq.)	K_2CO_3	DMF	15%
13	$PdCl_2$dppf (0.4 eq.)	K_2CO_3	DMSO	29%
14	$PdCl_2$dppf (1.0 eq.)	K_2CO_3	DMSO	27%

[a] Reactions were treated with 10 equiv. of base and heated to 90°C for 18 h under argon. [b] Isolated yield. [c] These reactions were heated to 80°C for 18 h.

Fig. S1: Mass fragmentation analysis of actinocarbasin (positive mode).

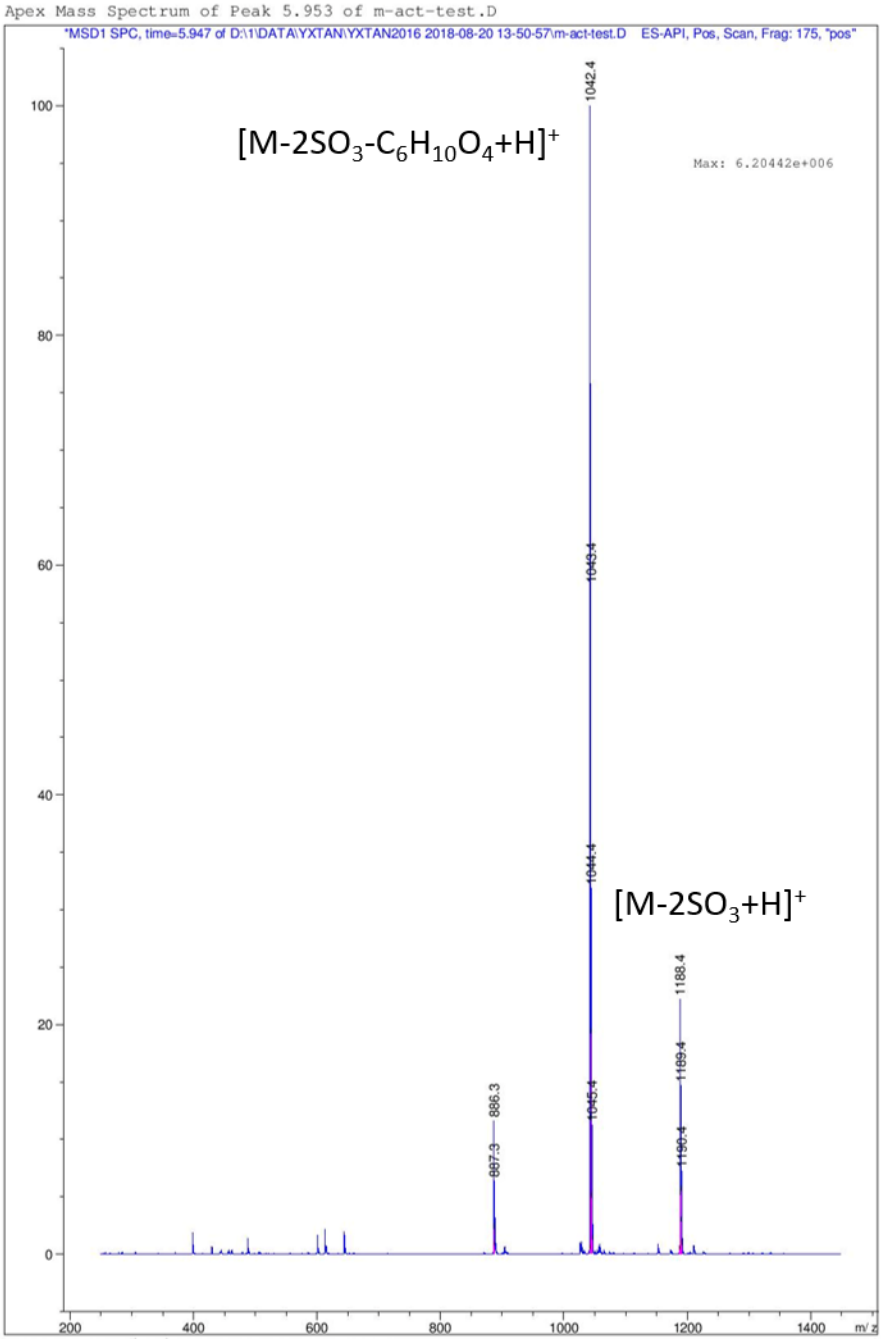
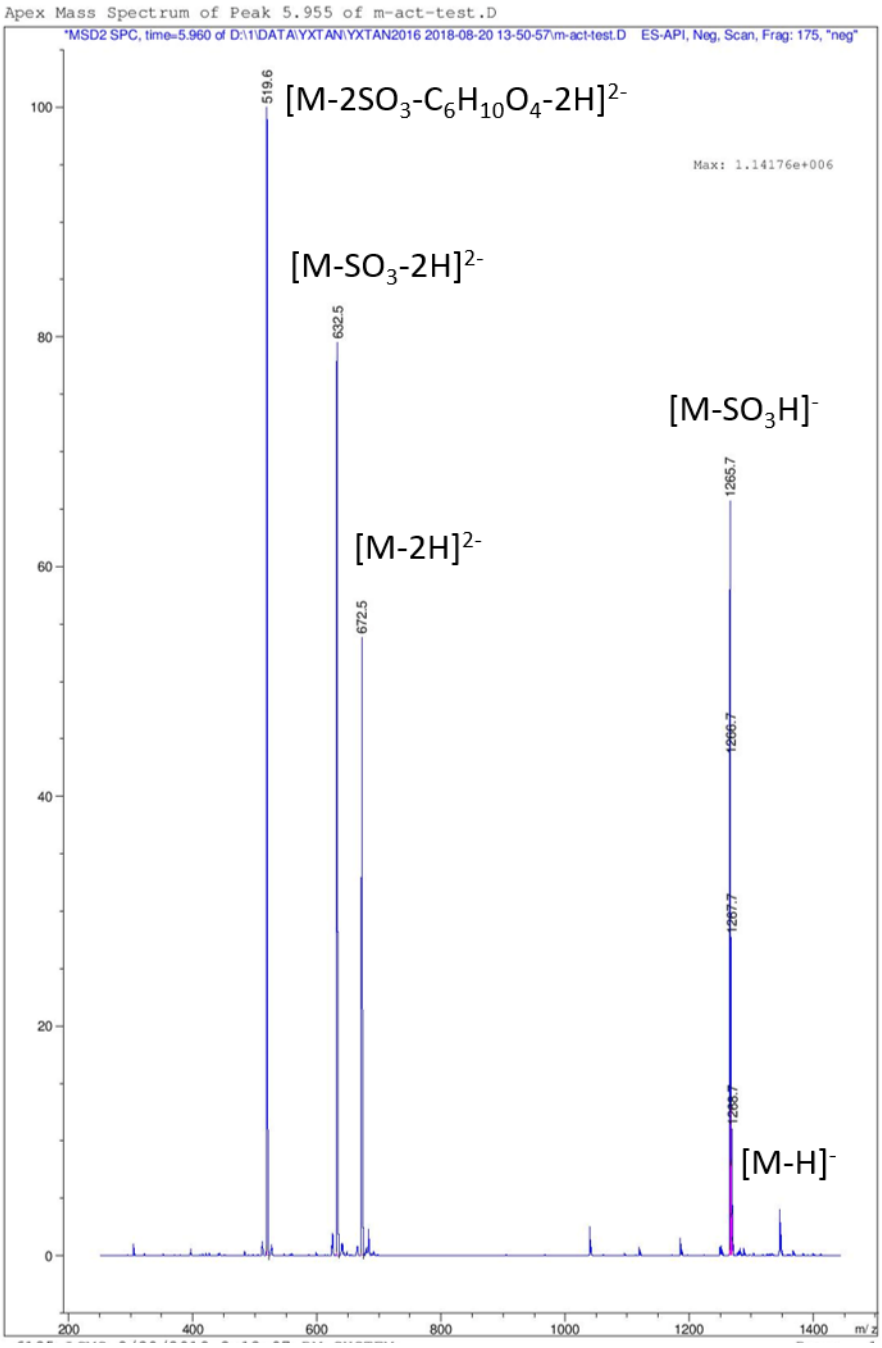


Fig. S2: Mass fragmentation analysis of actinocarbasin (negative mode).



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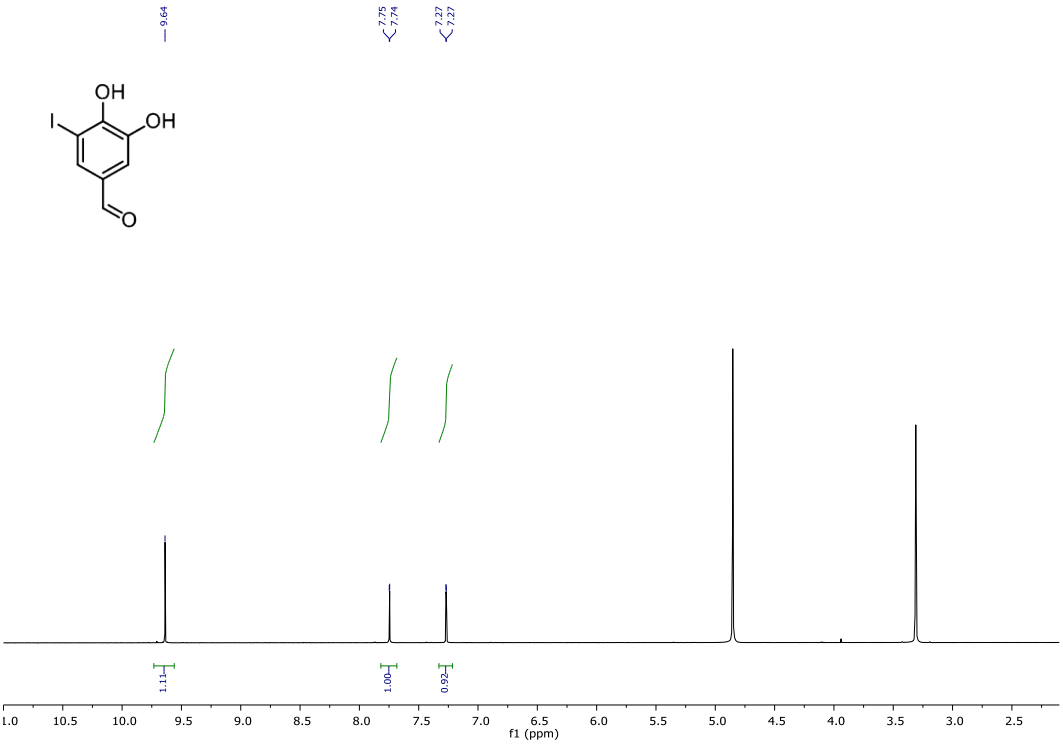
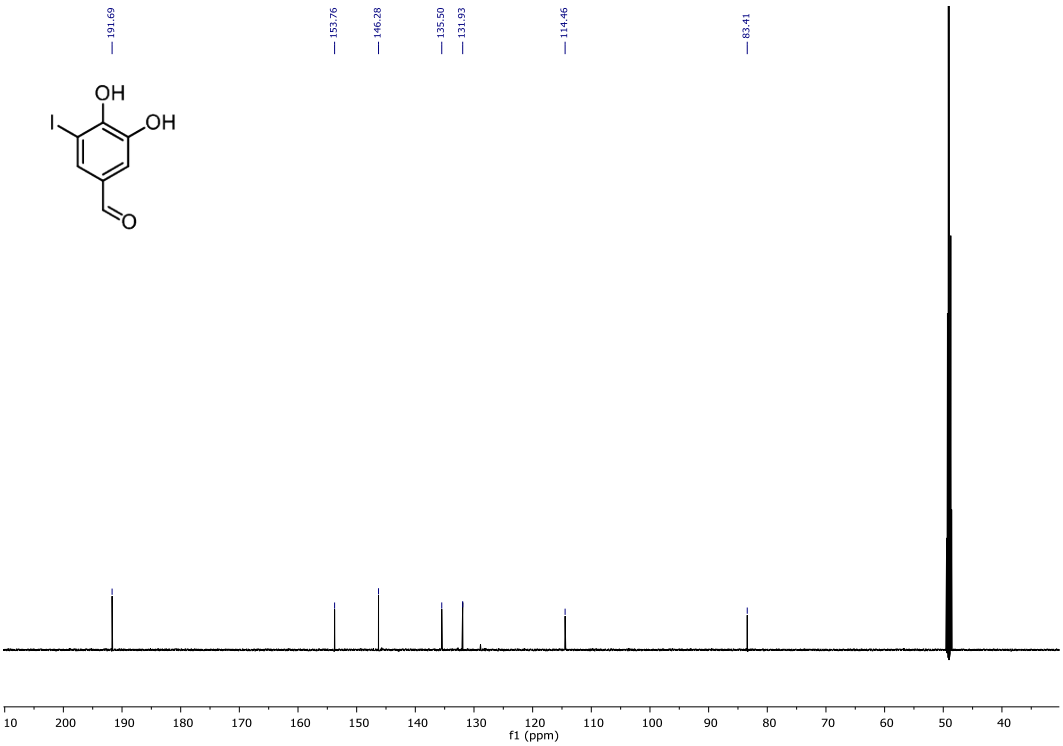
5. Peters, D. S.; Romesberg, F. E.; Baran, P. S. *J. Am. Chem. Soc.* **2018**, *140*, 2072.

NMR spectra

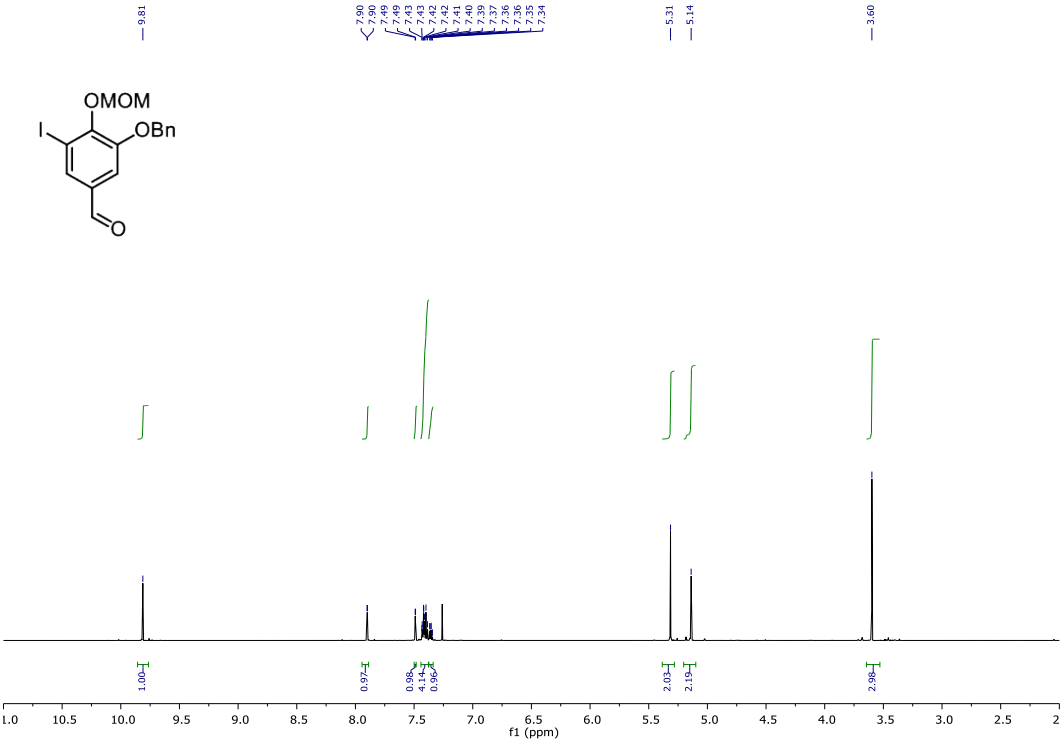
Compound S1 ^{1}H NMR (Methanol-d_4, 600 MHz)



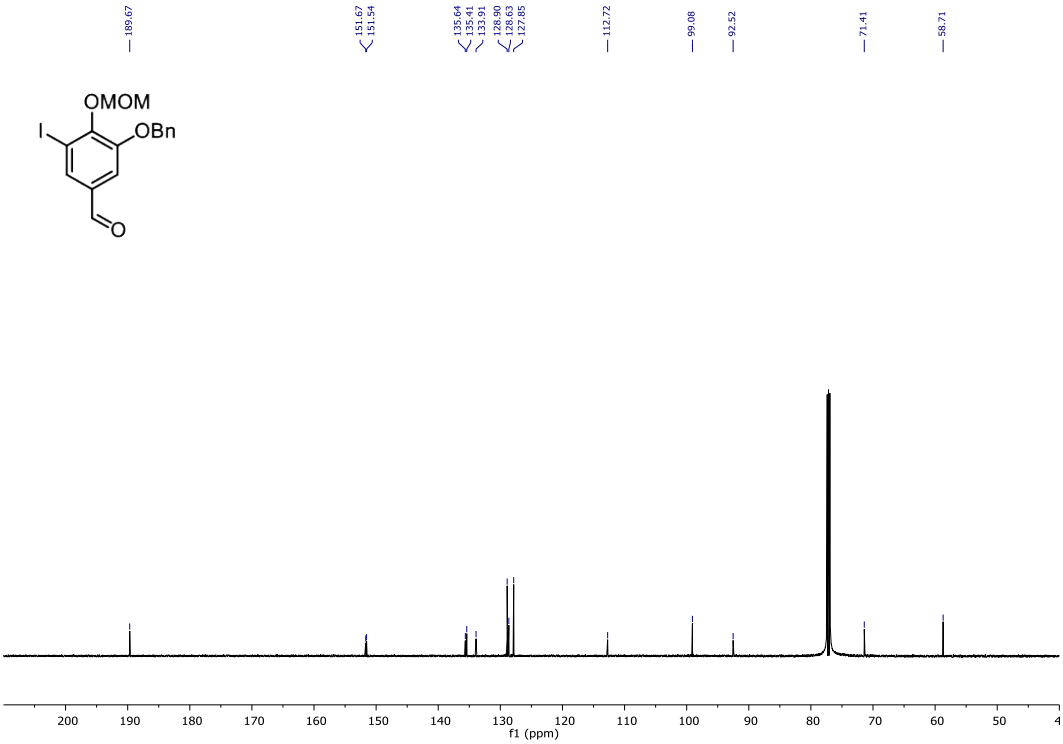
Compound S1 ^{13}C NMR (Methanol-d_4, 151 MHz)



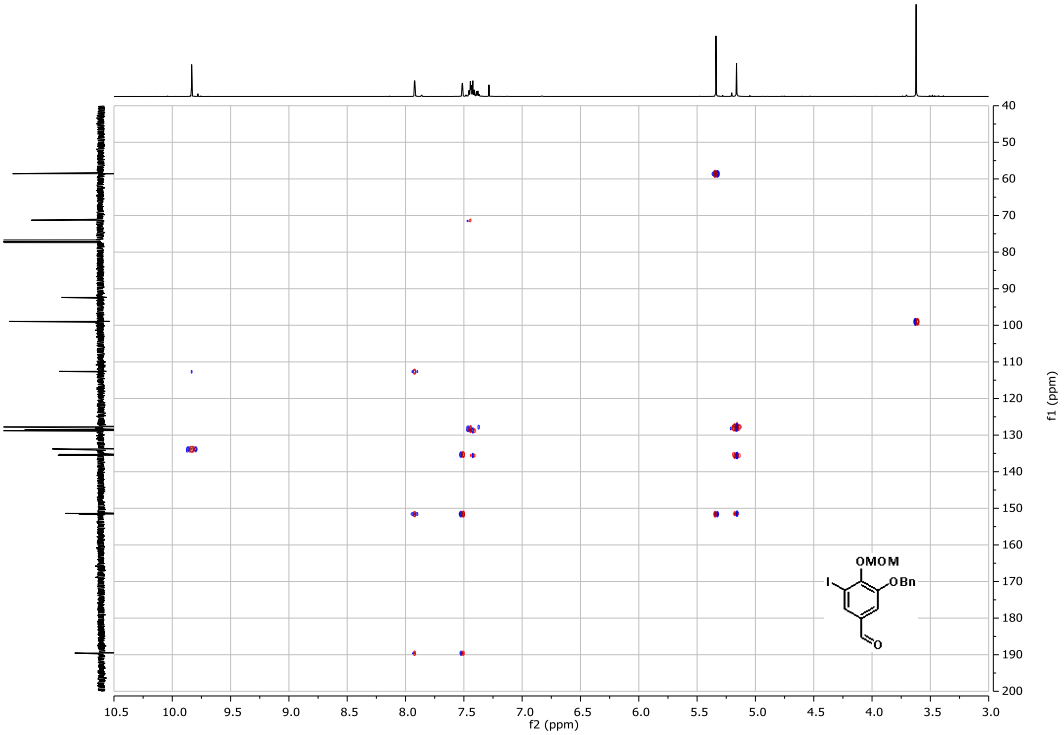
Compound S2 ^{1}H NMR ($CDCl_3$, 600 MHz)



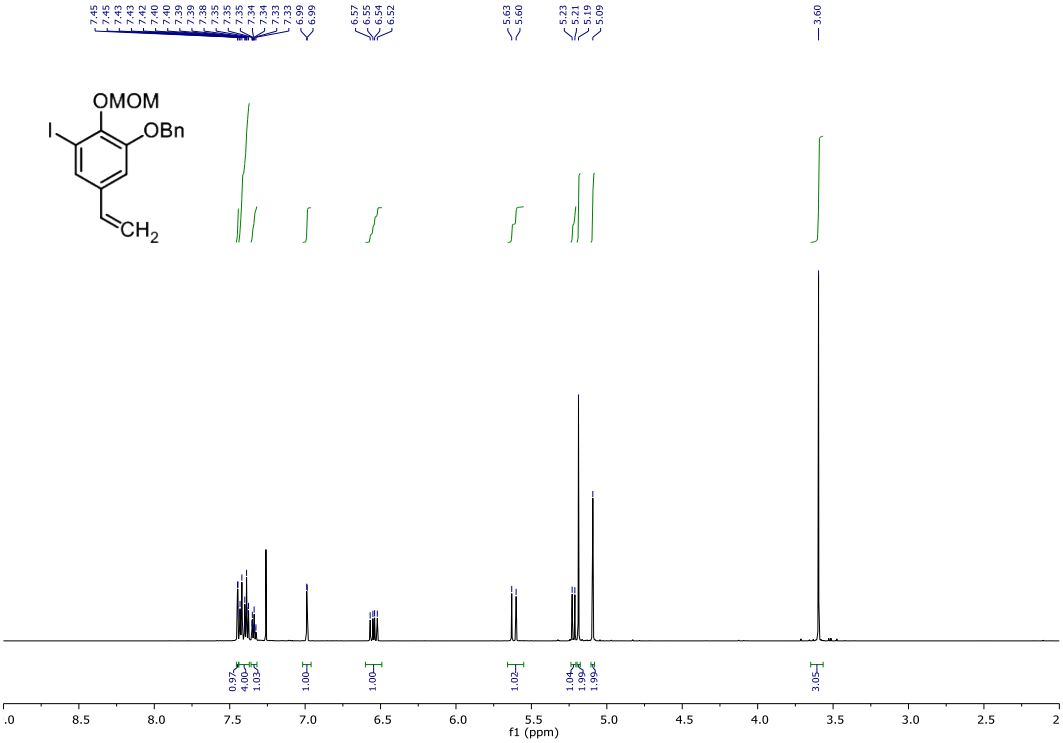
Compound S2 ^{13}C NMR ($CDCl_3$, 151 MHz)



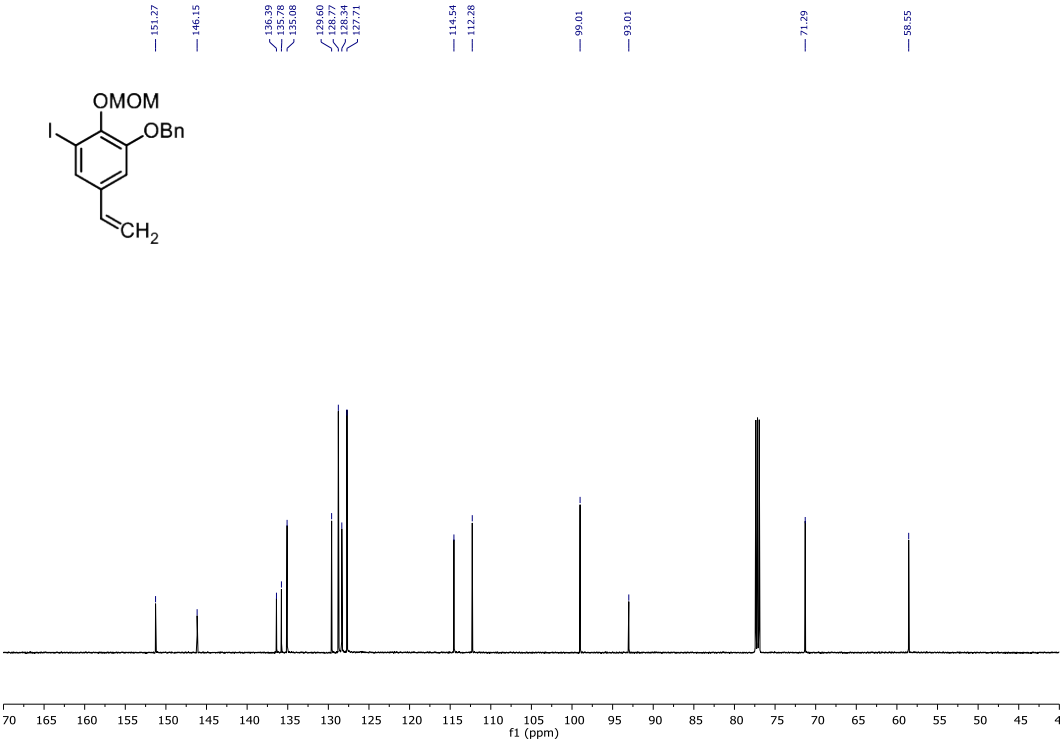
Compound S2 HSQC NMR ($CDCl_3$)



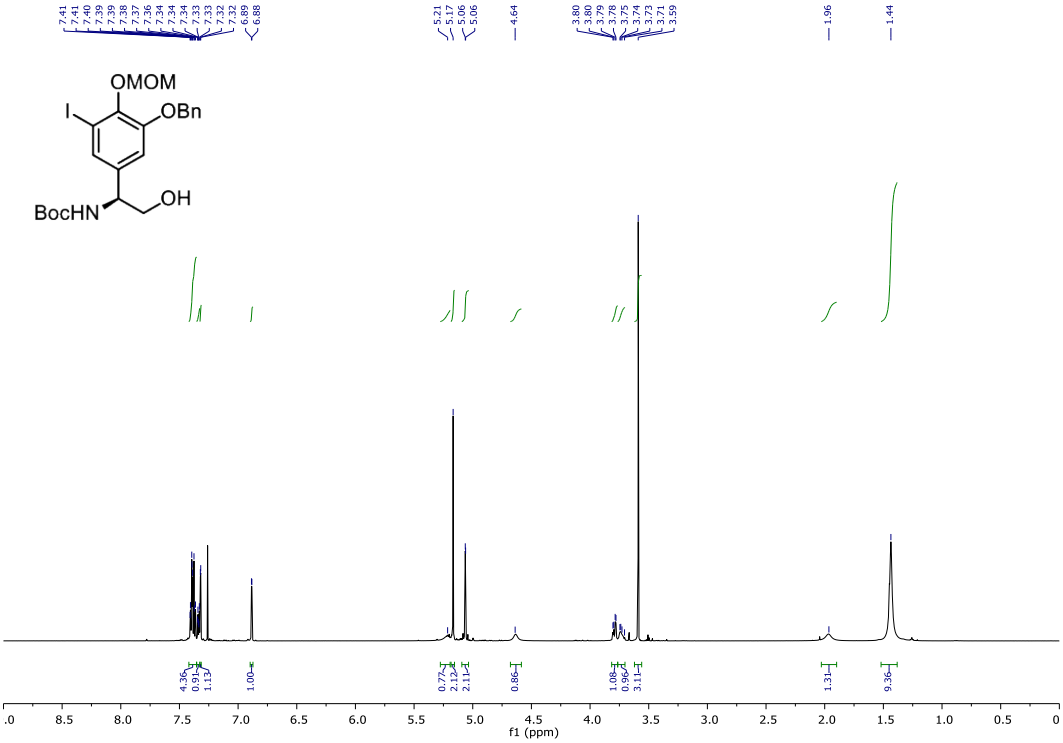
Compound 8 ^{1}H NMR ($CDCl_3$, 600 MHz)



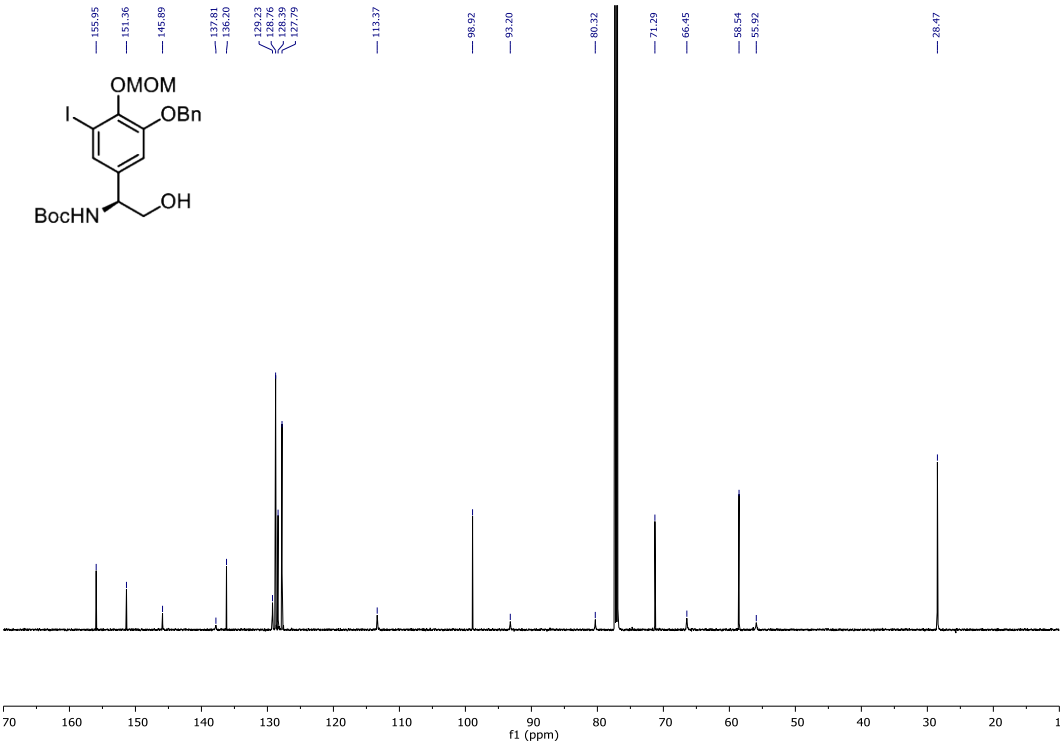
Compound 8 ^{13}C NMR ($CDCl_3$, 151 MHz)



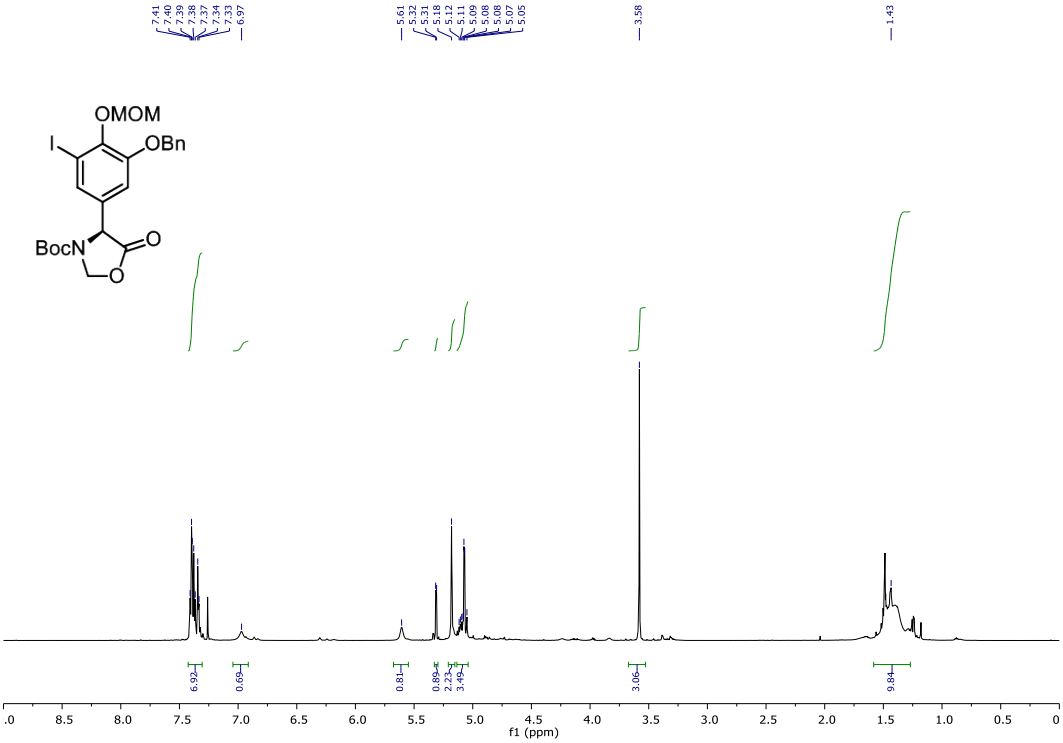
Compound 9 ^{1}H NMR ($CDCl_3$, 600 MHz)



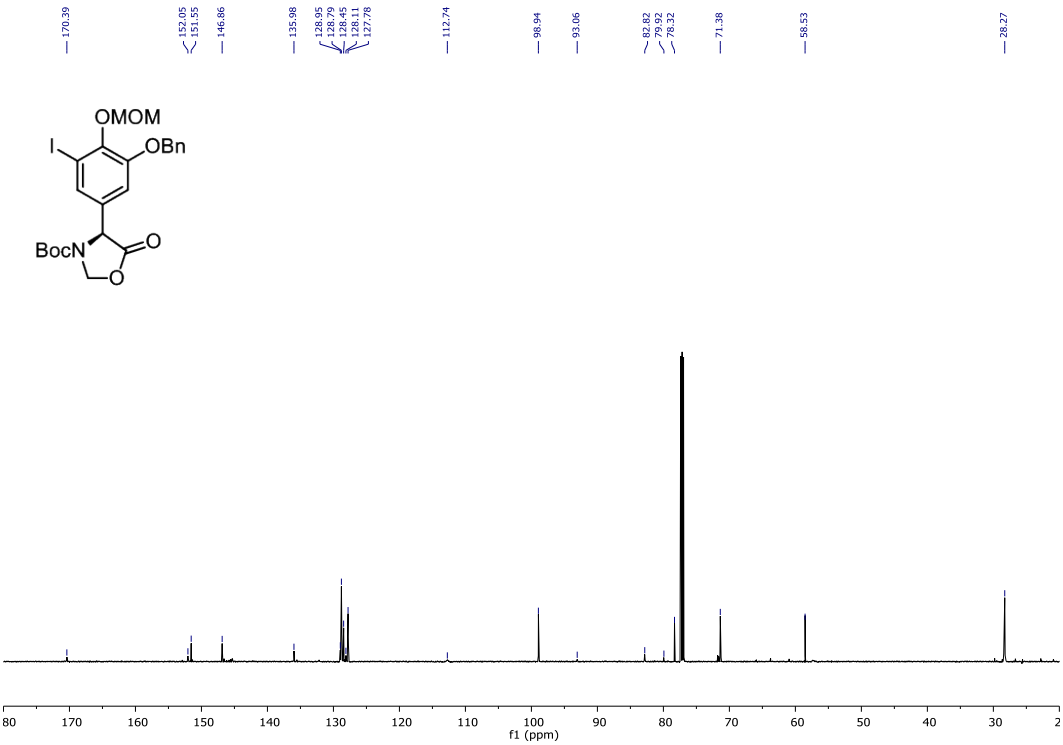
Compound 9 ^{13}C NMR ($CDCl_3$, 151 MHz)



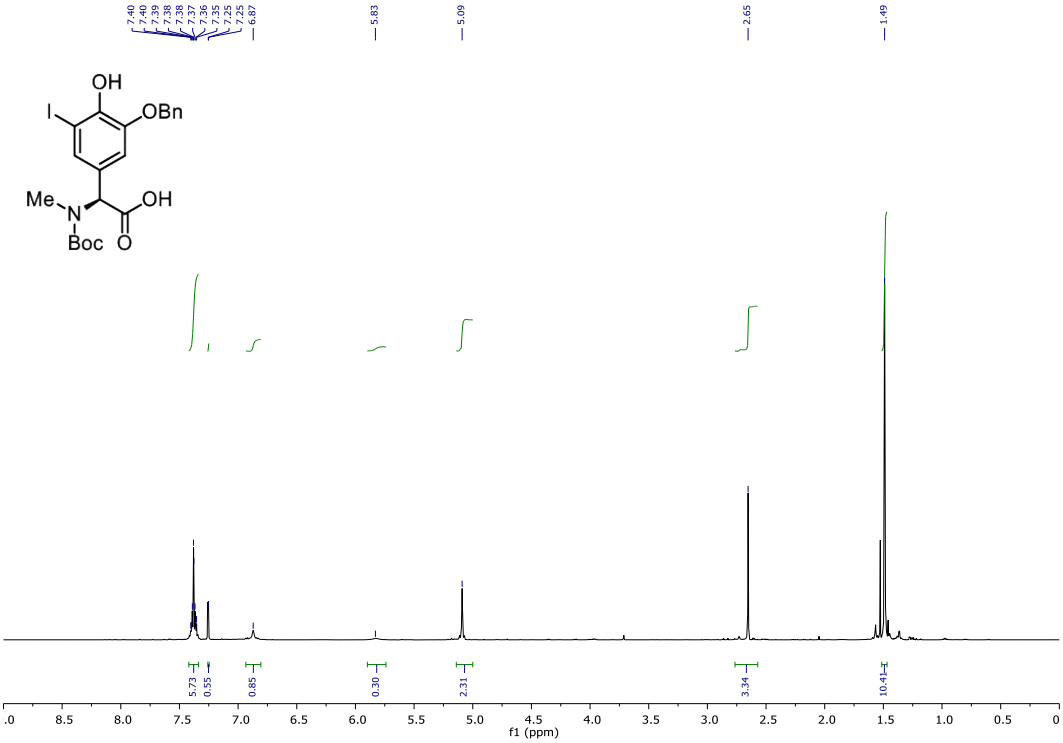
Compound 11 ^{1}H NMR ($CDCl_3$, 600 MHz)



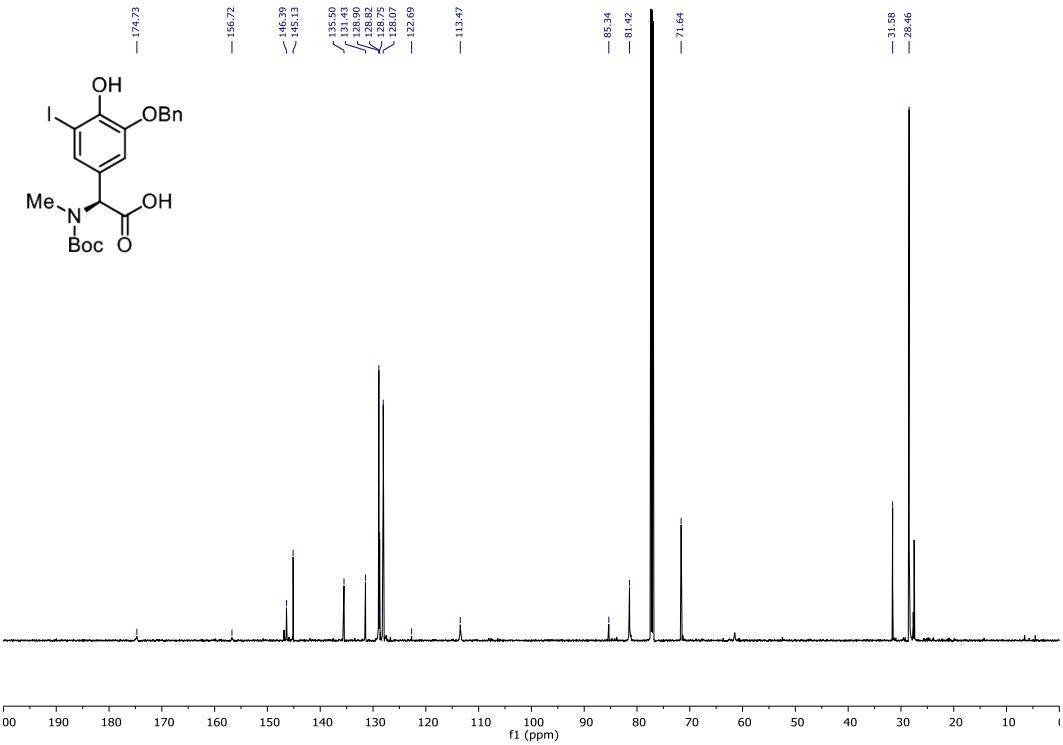
Compound 11 ^{13}C NMR ($CDCl_3$, 151 MHz)



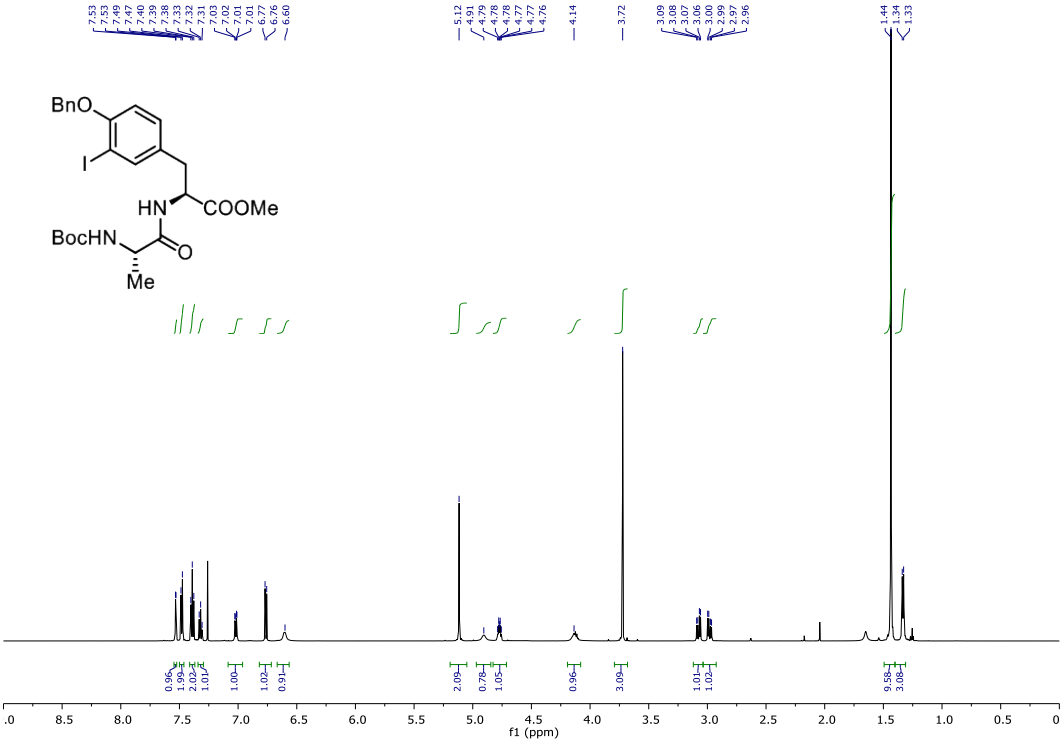
Compound 6 ^{1}H NMR ($CDCl_3$, 600 MHz)



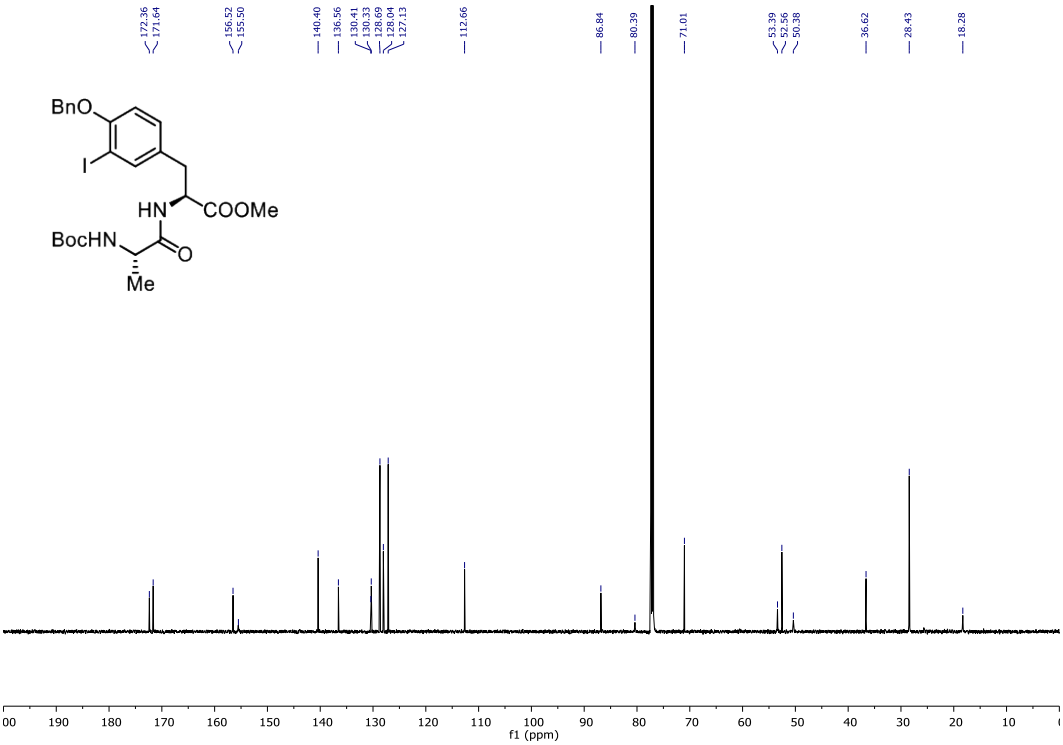
Compound 6 ^{13}C NMR ($CDCl_3$, 151 MHz)



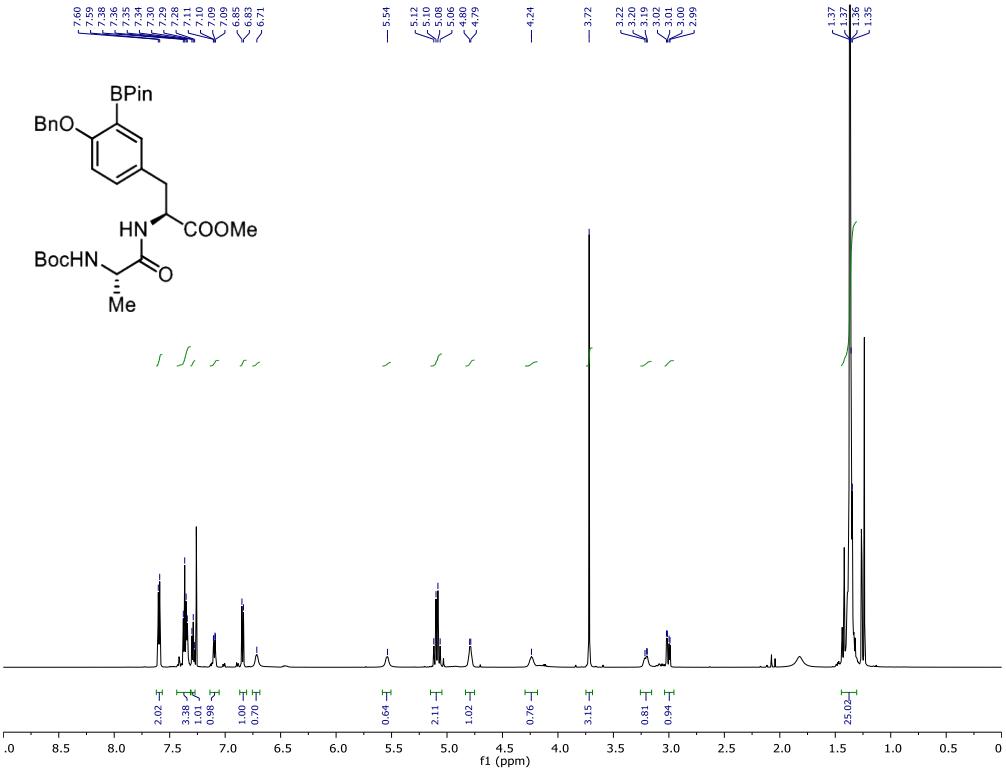
Compound 12 ^{1}H NMR ($CDCl_3$, 600 MHz)



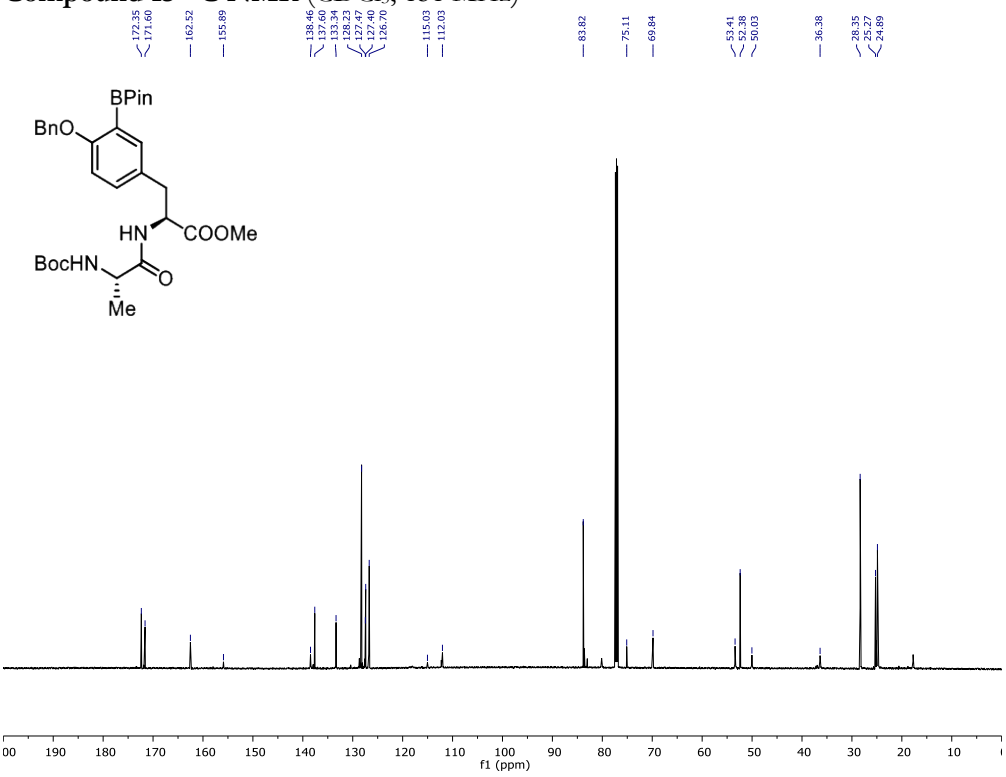
Compound 12 ^{13}C NMR ($CDCl_3$, 151 MHz)



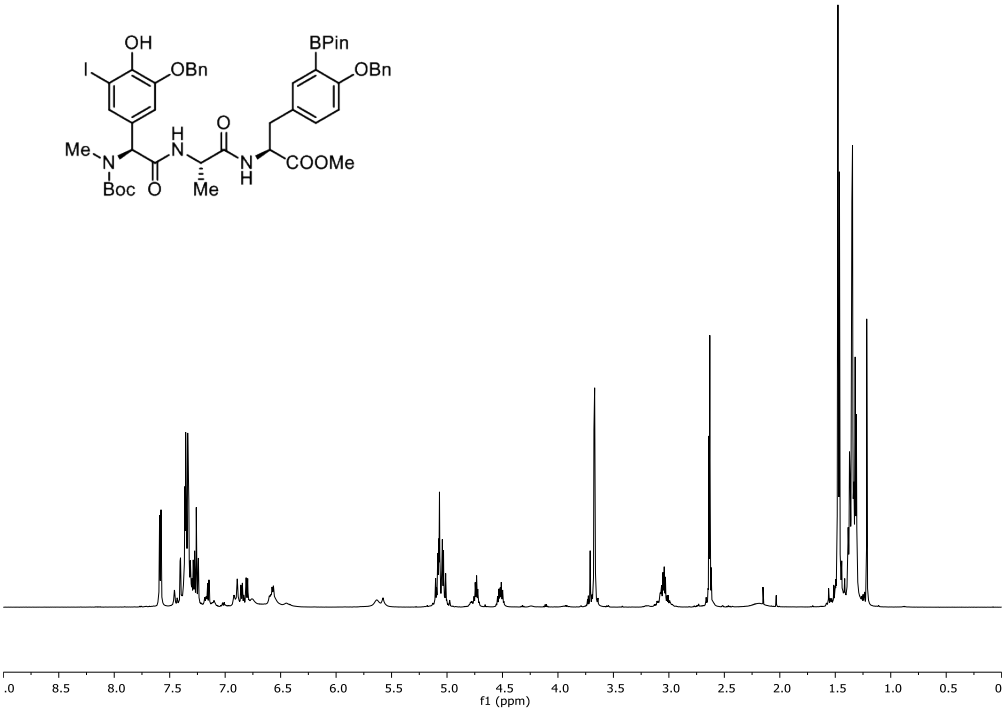
Compound 13 ^{1}H NMR ($CDCl_3$, 600 MHz)



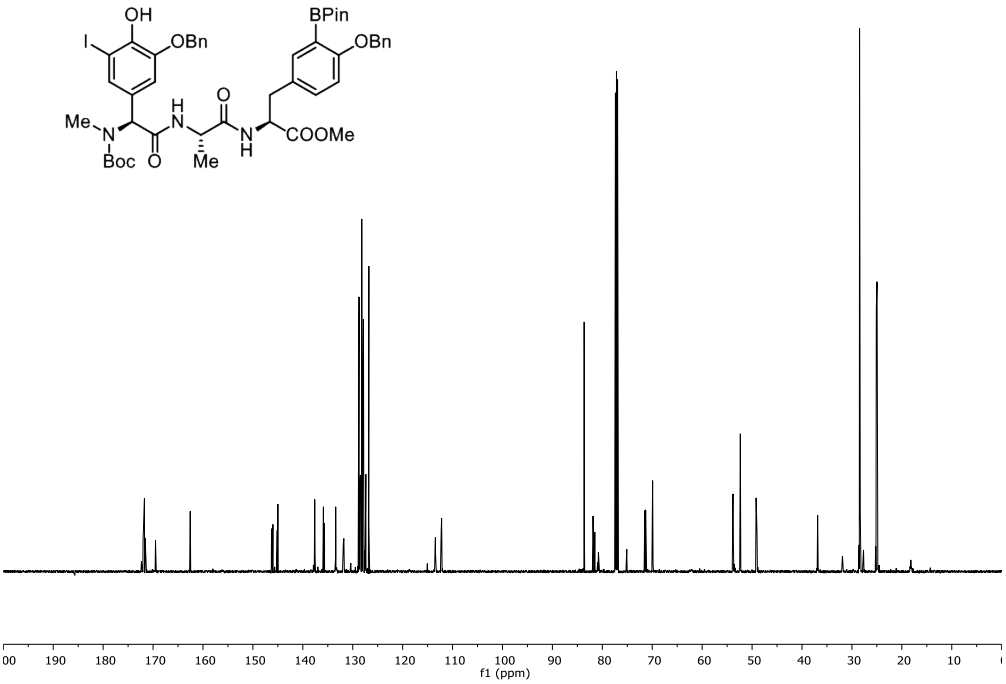
Compound 13 ^{13}C NMR ($CDCl_3$, 151 MHz)



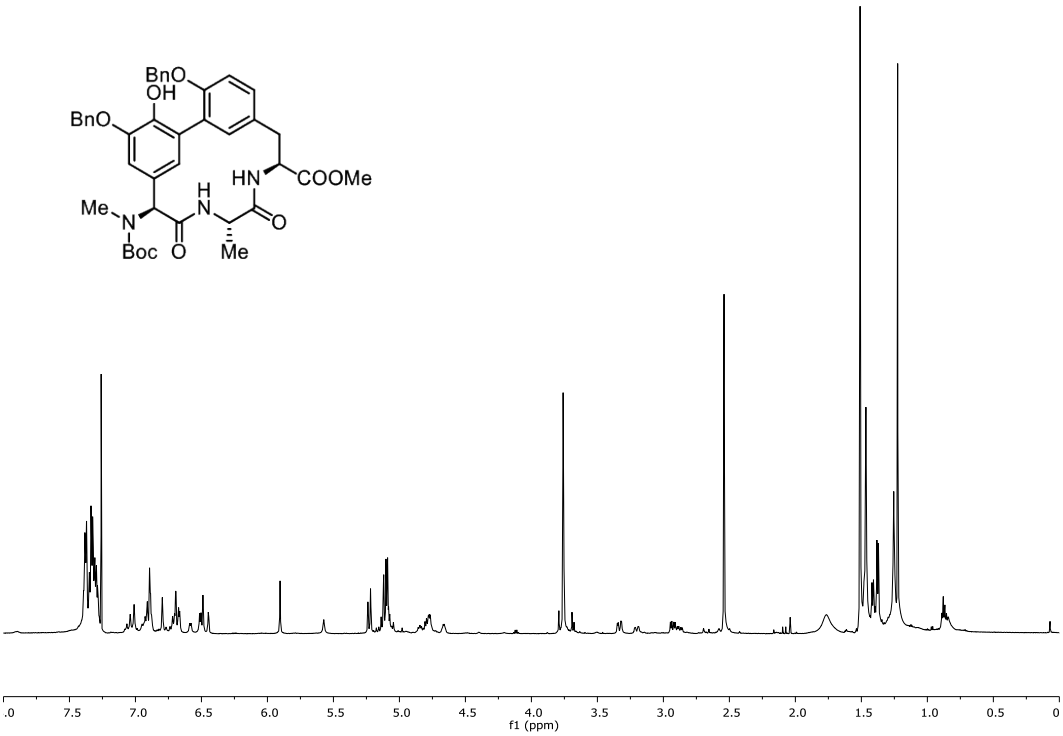
Compound 4 ^{1}H NMR ($CDCl_3$, 600 MHz)



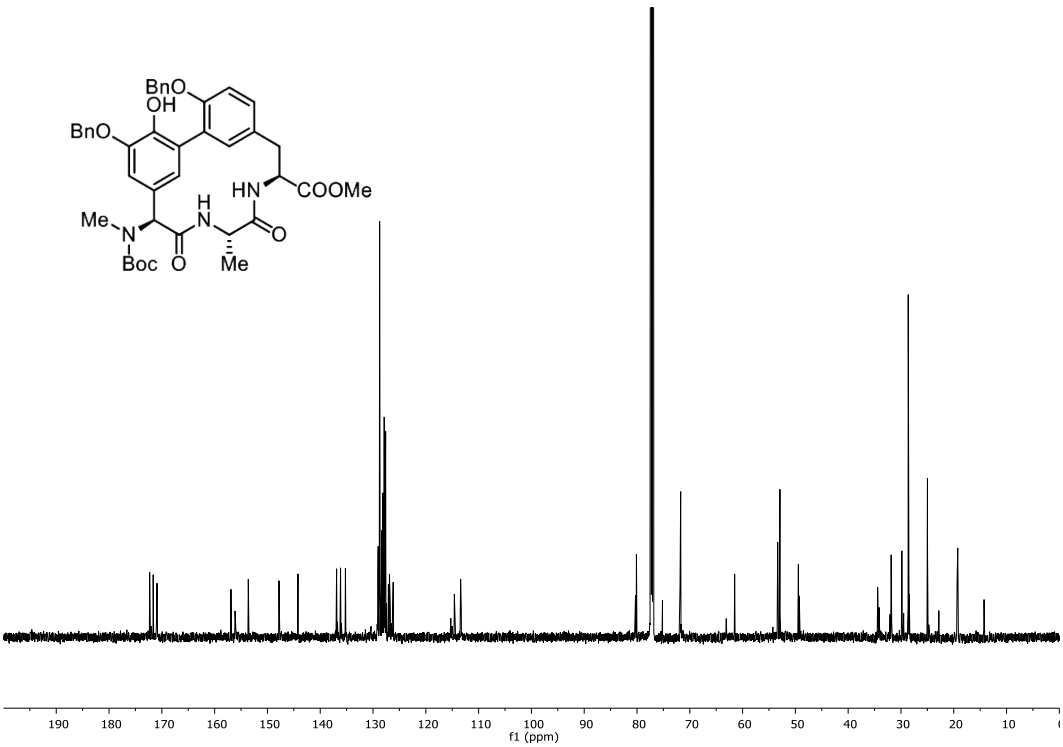
Compound 4 ^{13}C NMR ($CDCl_3$, 151 MHz)



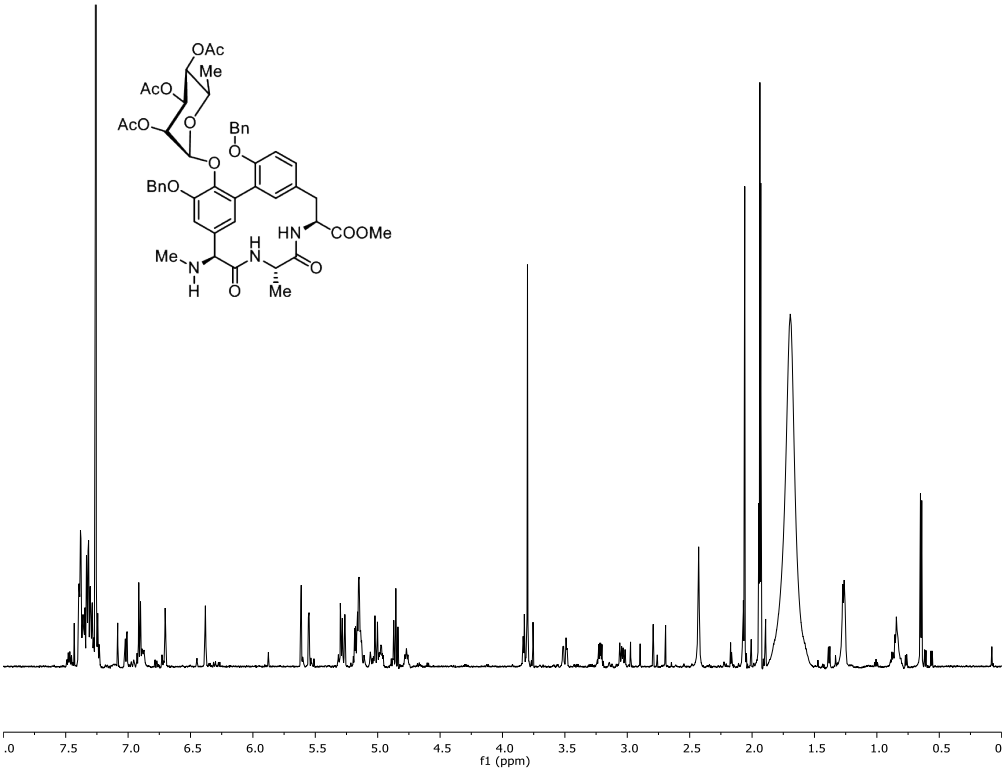
Compound 14 ^{1}H NMR ($CDCl_3$, 600 MHz)



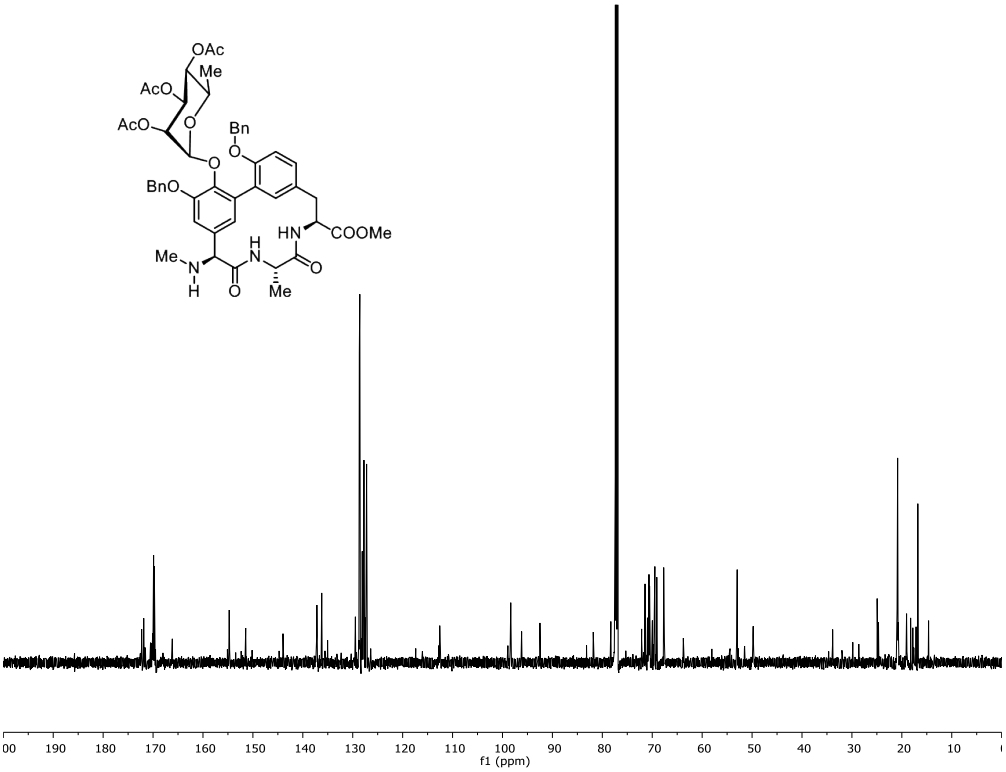
Compound 14 ^{13}C NMR ($CDCl_3$, 151 MHz)



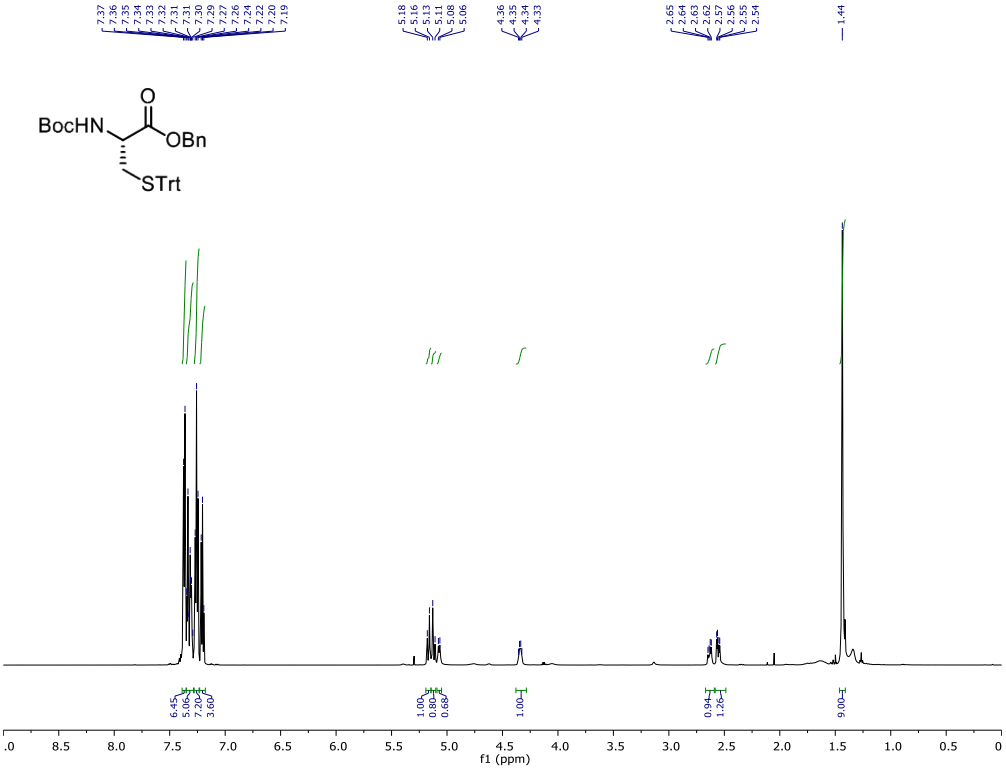
Compound 15 ^{1}H NMR ($CDCl_3$, 600 MHz)



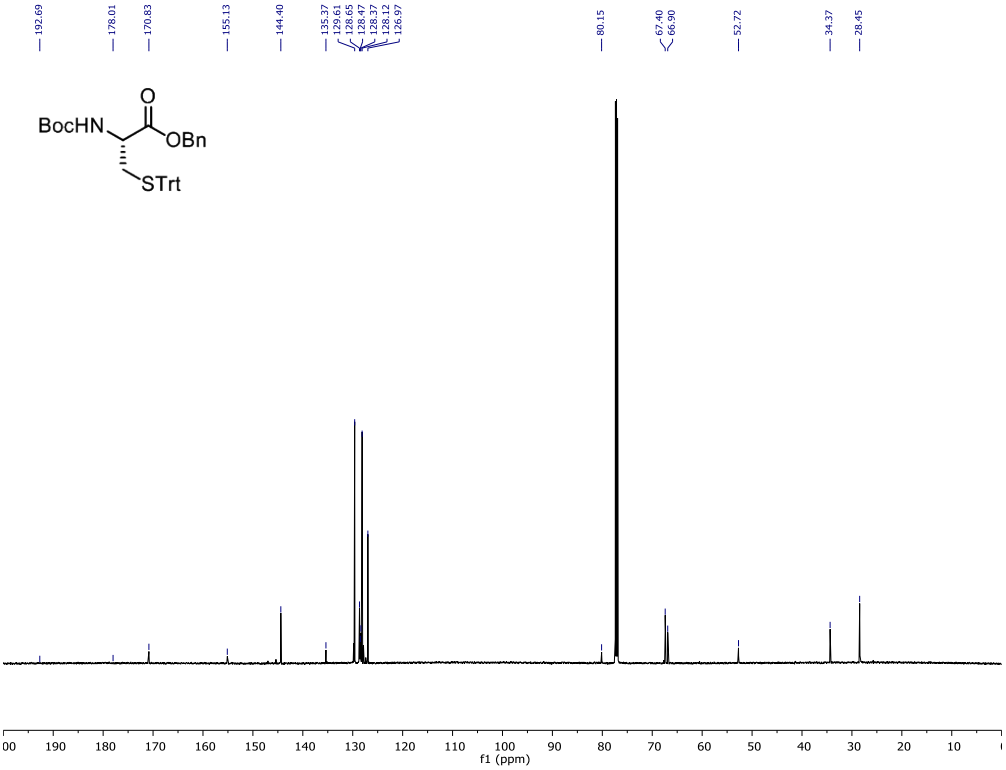
Compound 15 ^{13}C NMR ($CDCl_3$, 151 MHz)



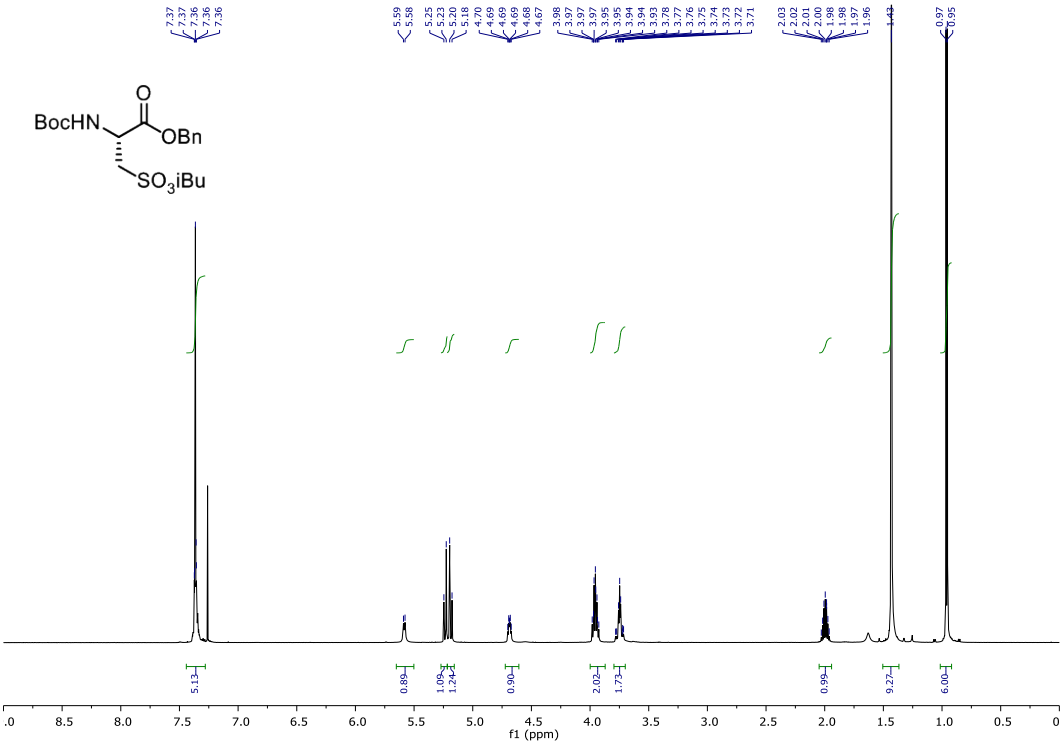
Compound S3a ^{1}H NMR ($CDCl_3$, 600 MHz)



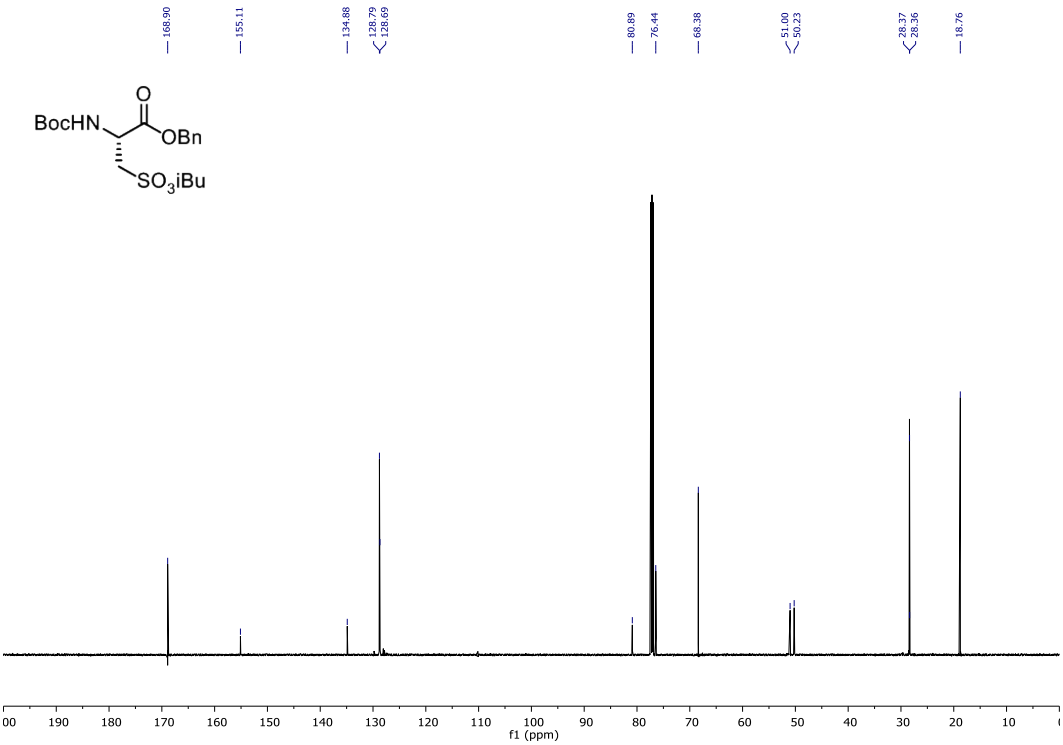
Compound S3a ^{13}C NMR ($CDCl_3$, 151 MHz)



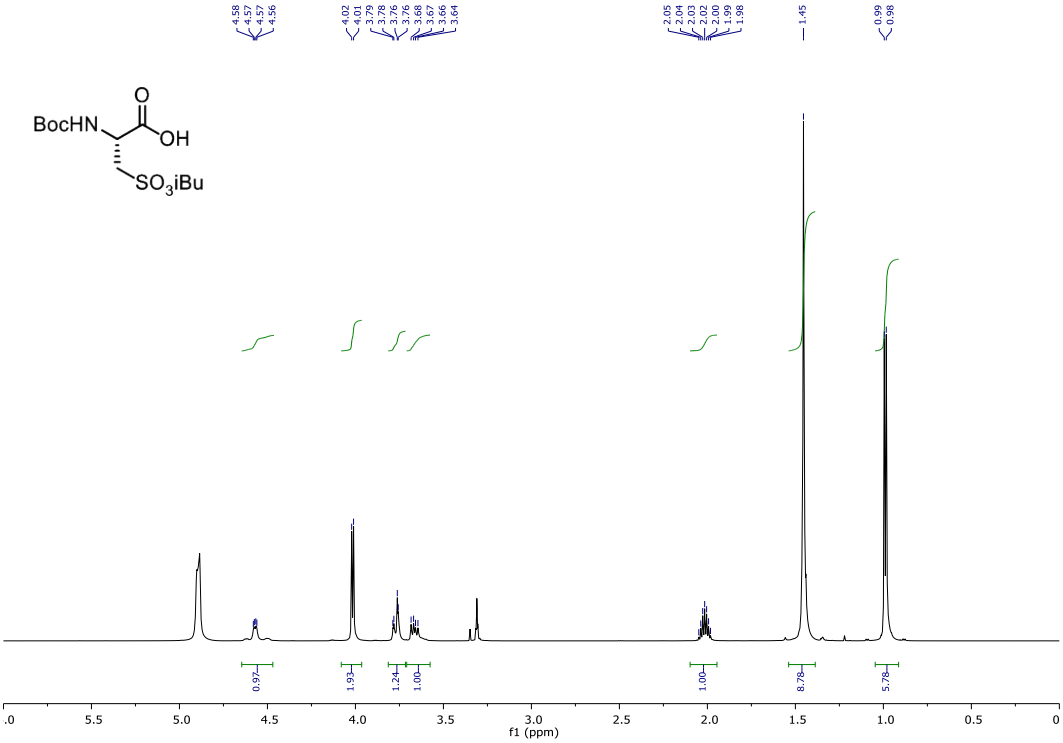
Compound S4a ^{1}H NMR ($CDCl_3$, 600 MHz)



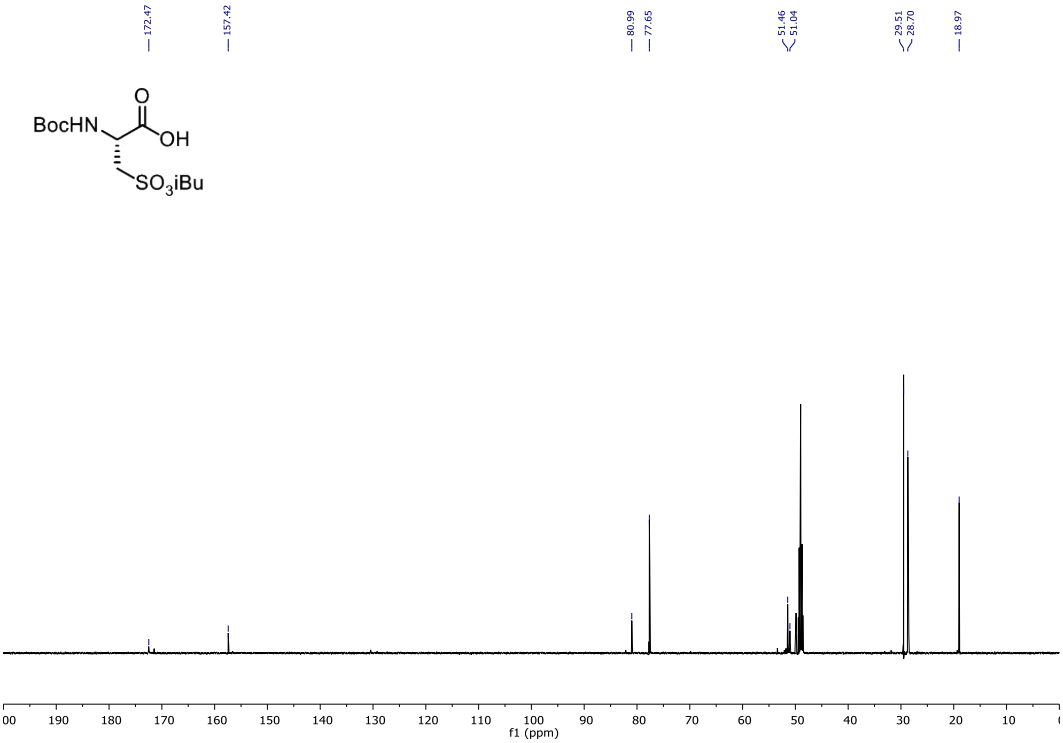
Compound S4a ^{13}C NMR ($CDCl_3$, 151 MHz)



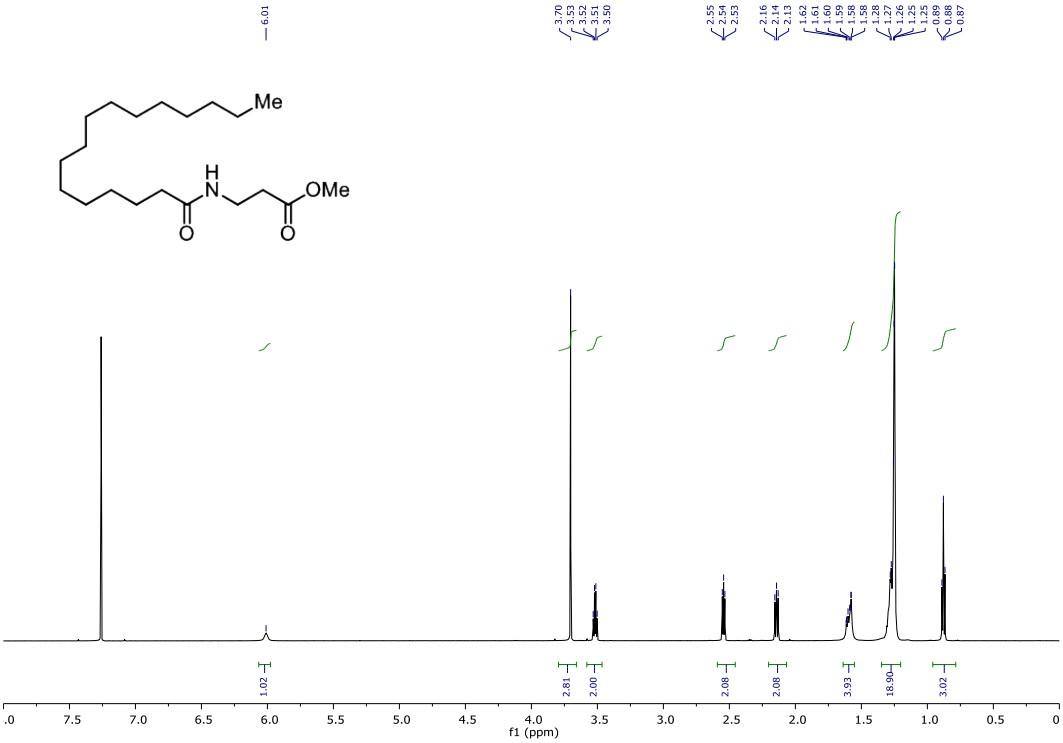
Compound 16a ^{1}H NMR (Methanol-d_4, 600 MHz)



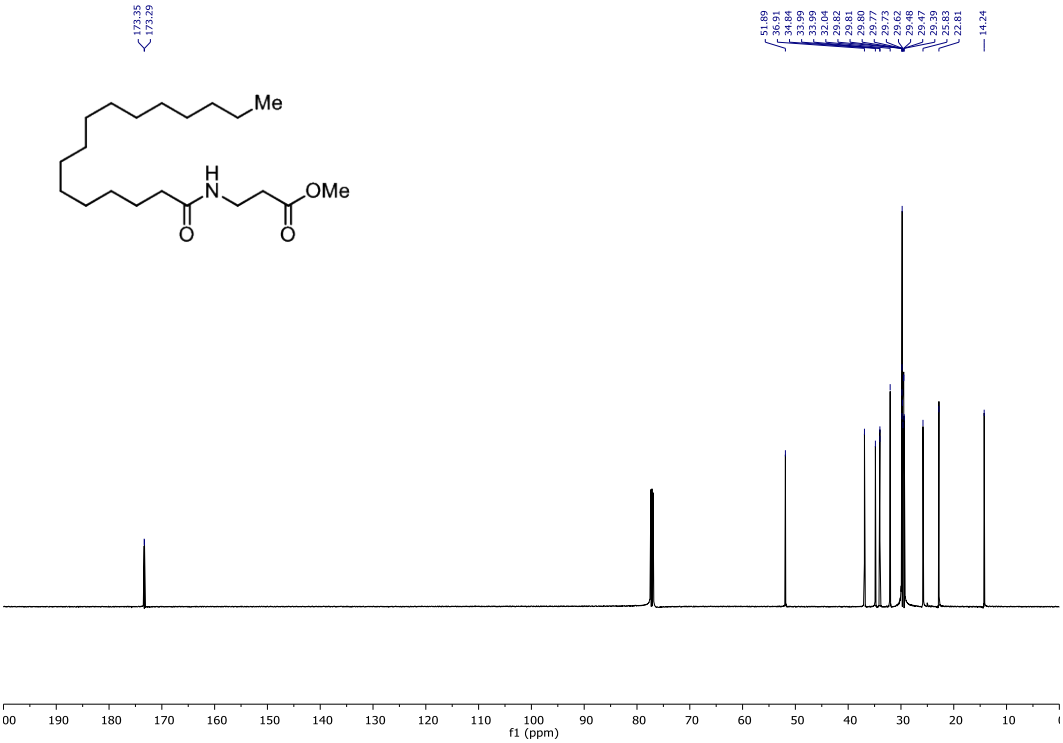
Compound 16a ^{13}C NMR (Methanol-d_4, 151 MHz)



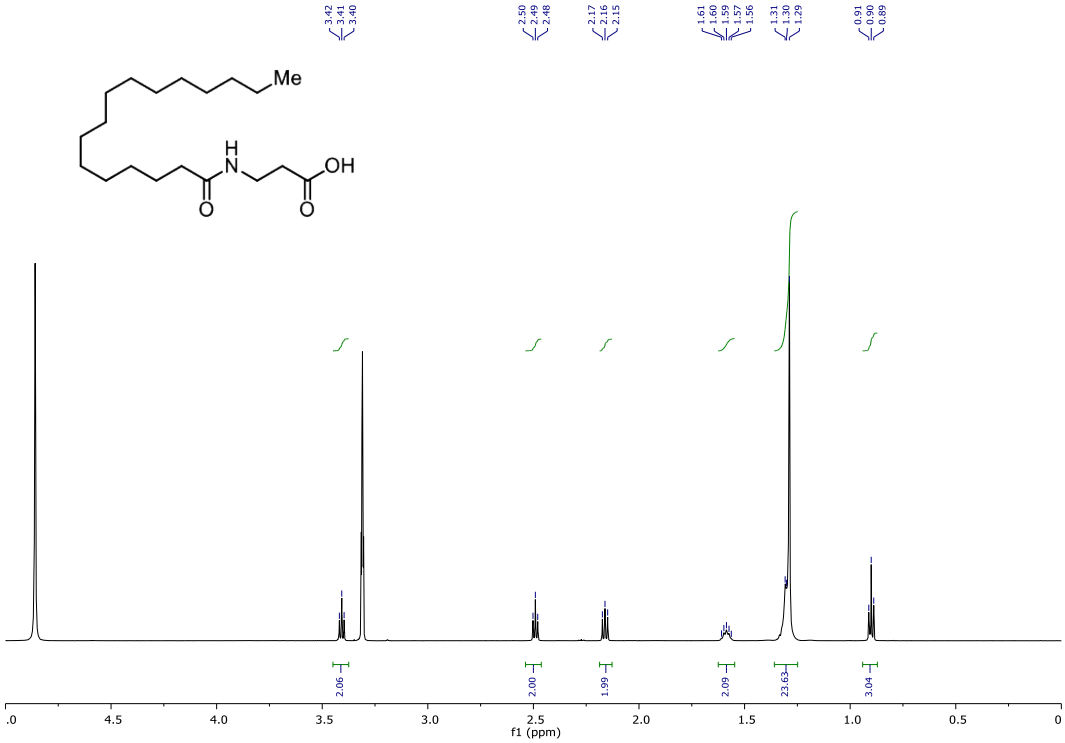
Compound S5 ^{1}H NMR ($CDCl_3$, 600 MHz)



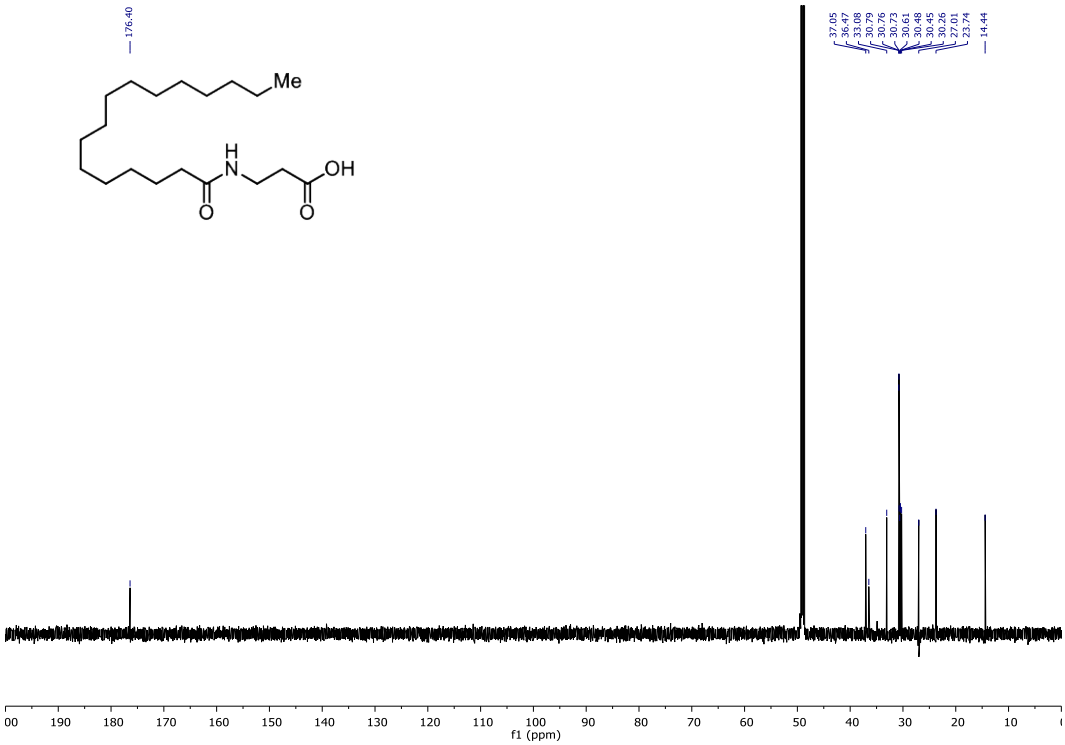
Compound S5 ^{13}C NMR ($CDCl_3$, 151 MHz)



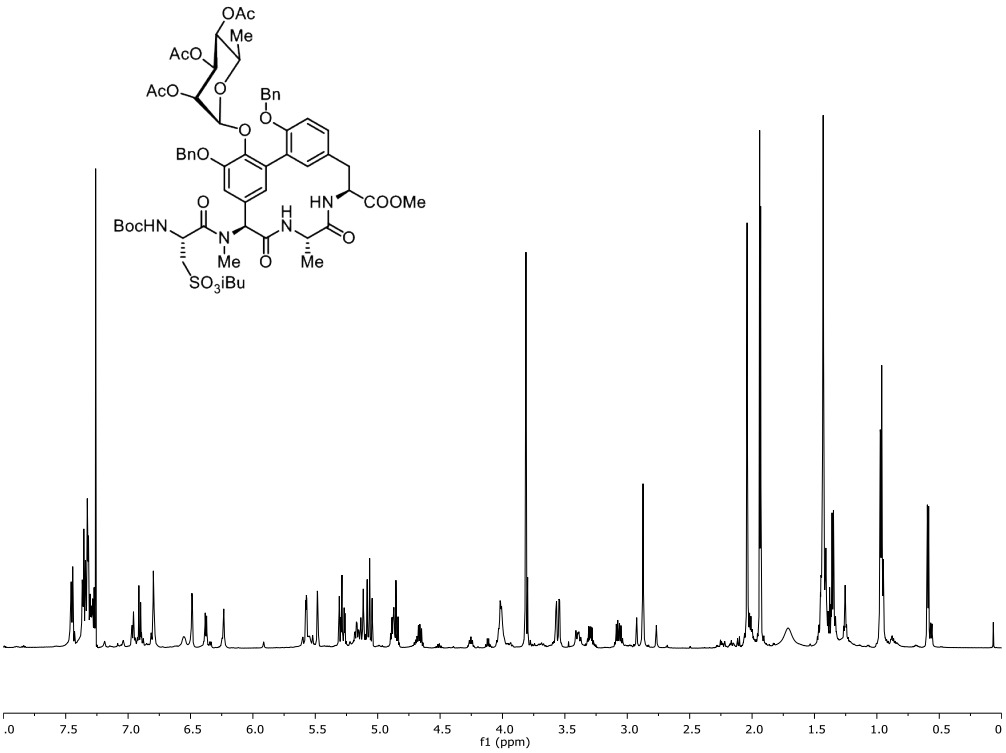
Compound 17 ^{1}H NMR (Methanol-d_4, 600 MHz)



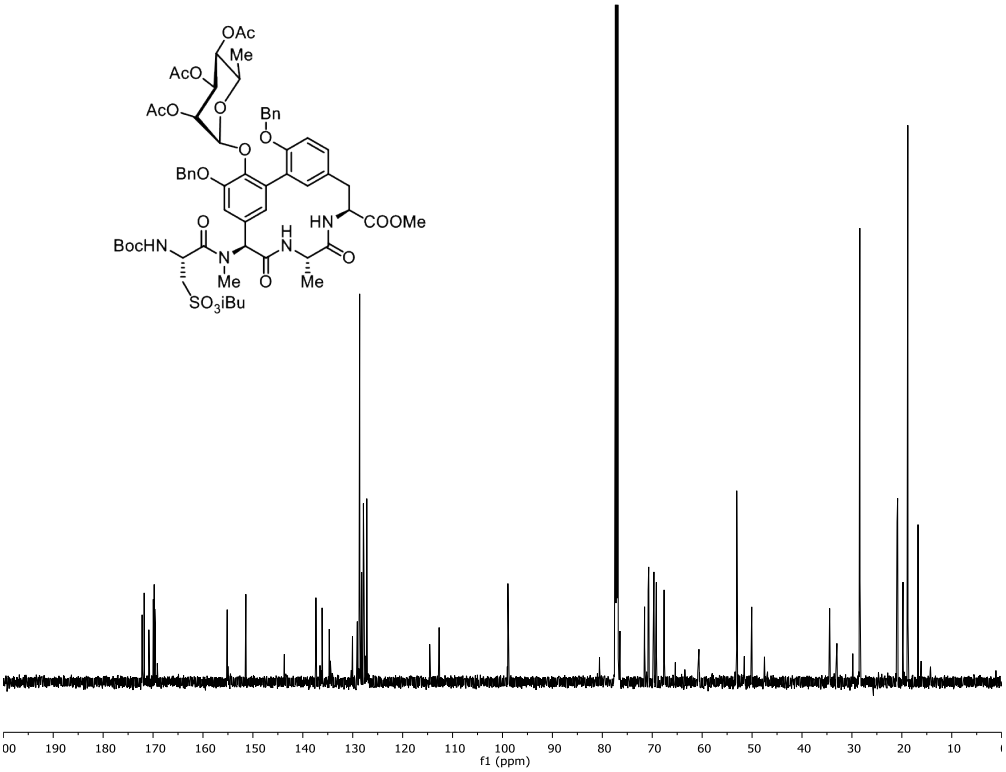
Compound 17 ^{13}C NMR (Methanol-d_4, 151 MHz)



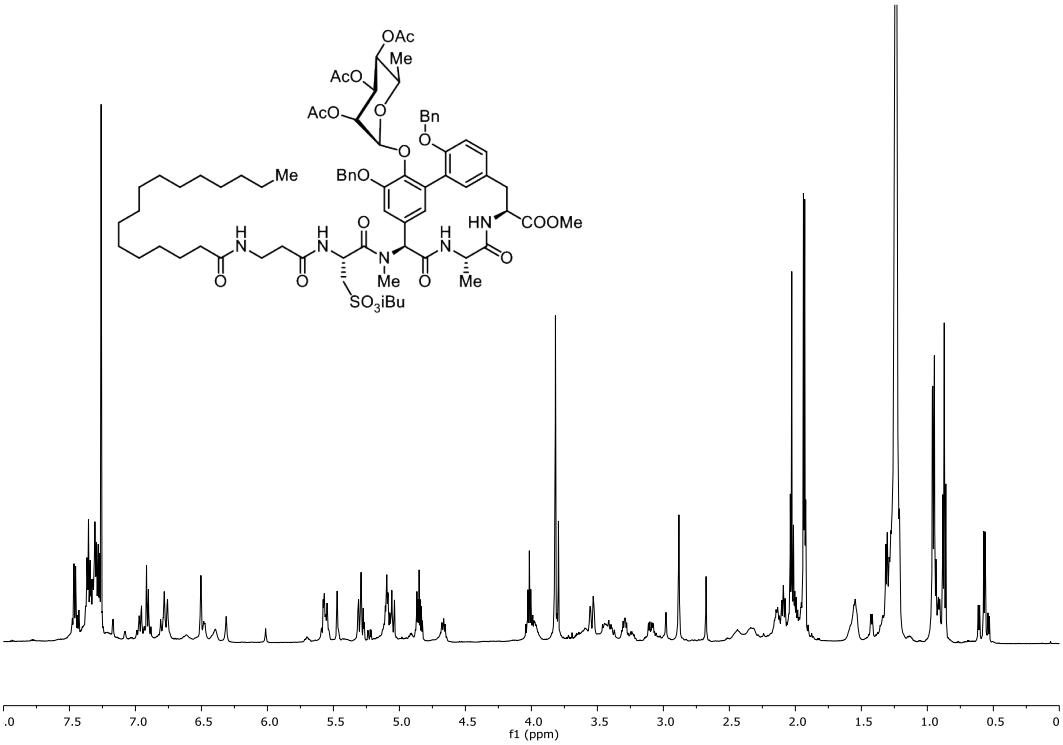
Compound 2a ^{1}H NMR ($CDCl_3$, 600 MHz)



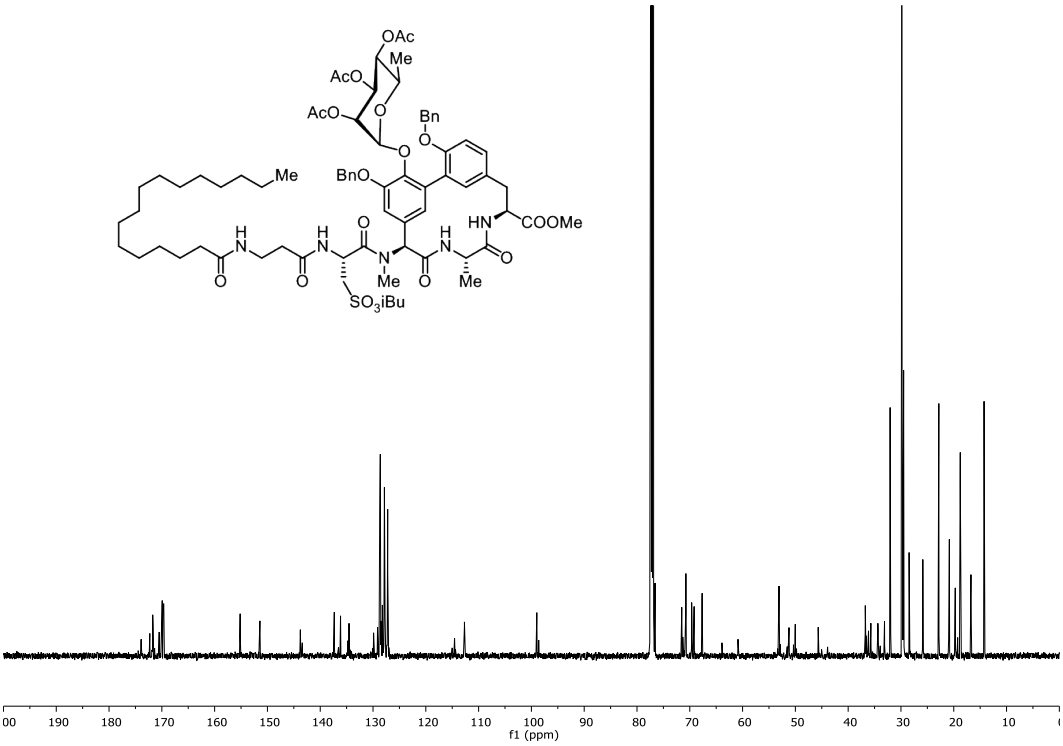
Compound 2a ^{13}C NMR ($CDCl_3$, 151 MHz)



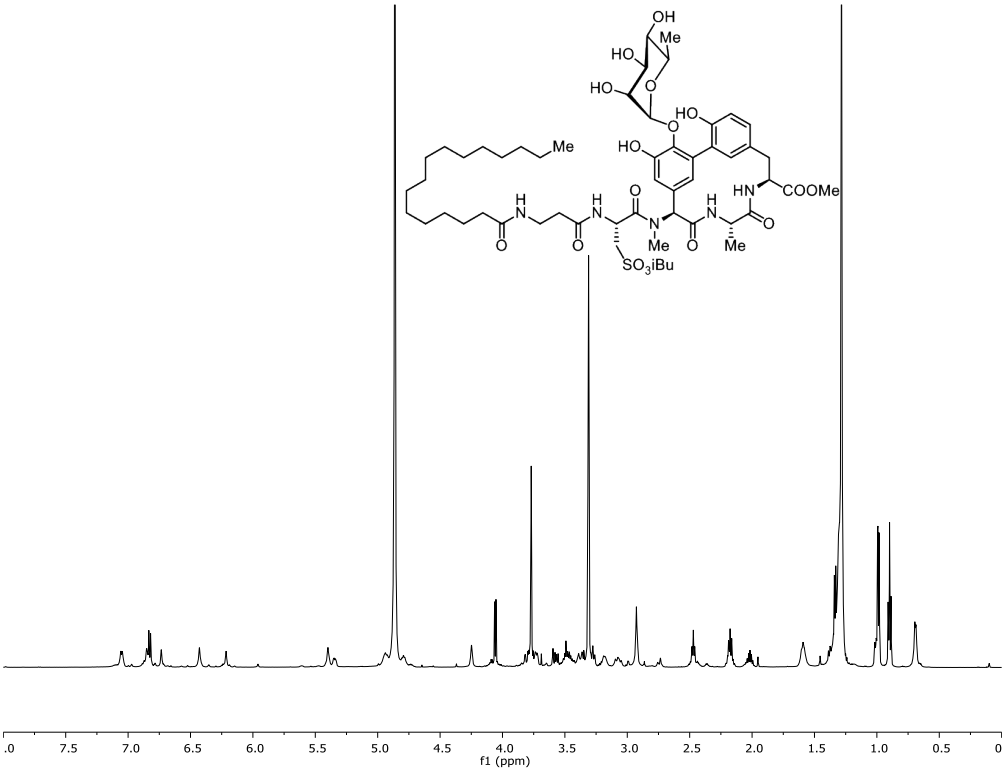
Compound 18a ^{1}H NMR ($CDCl_3$, 600 MHz)



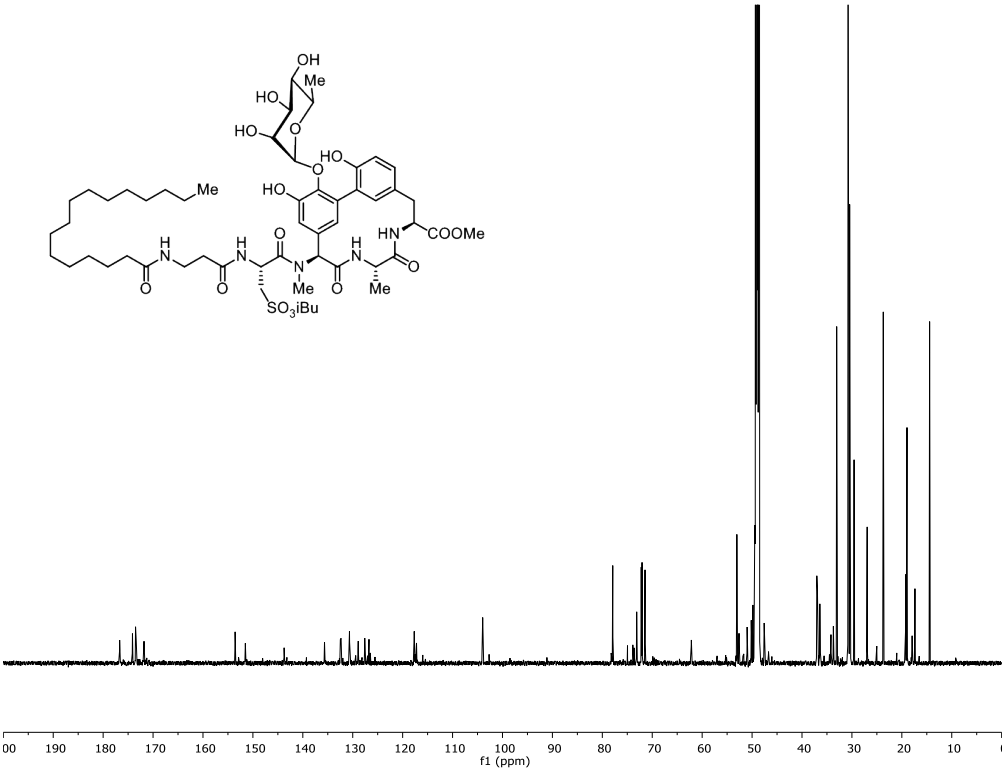
Compound 18a ^{13}C NMR ($CDCl_3$, 151 MHz)



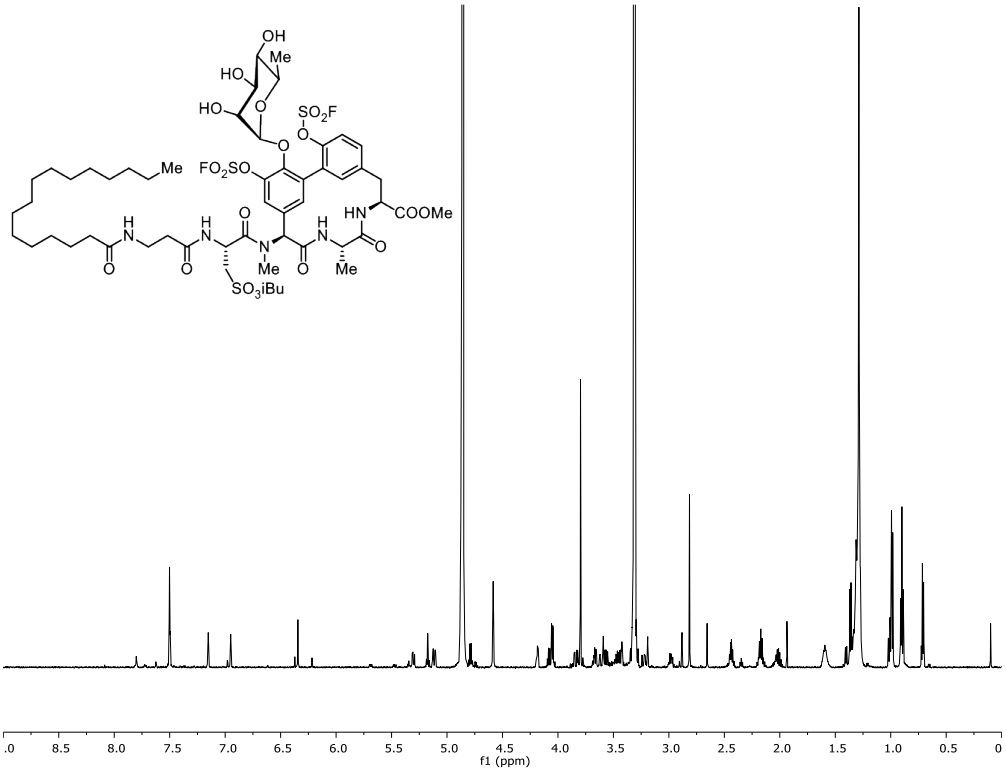
Compound 19a ^{1}H NMR (Methanol-d_4, 600 MHz)



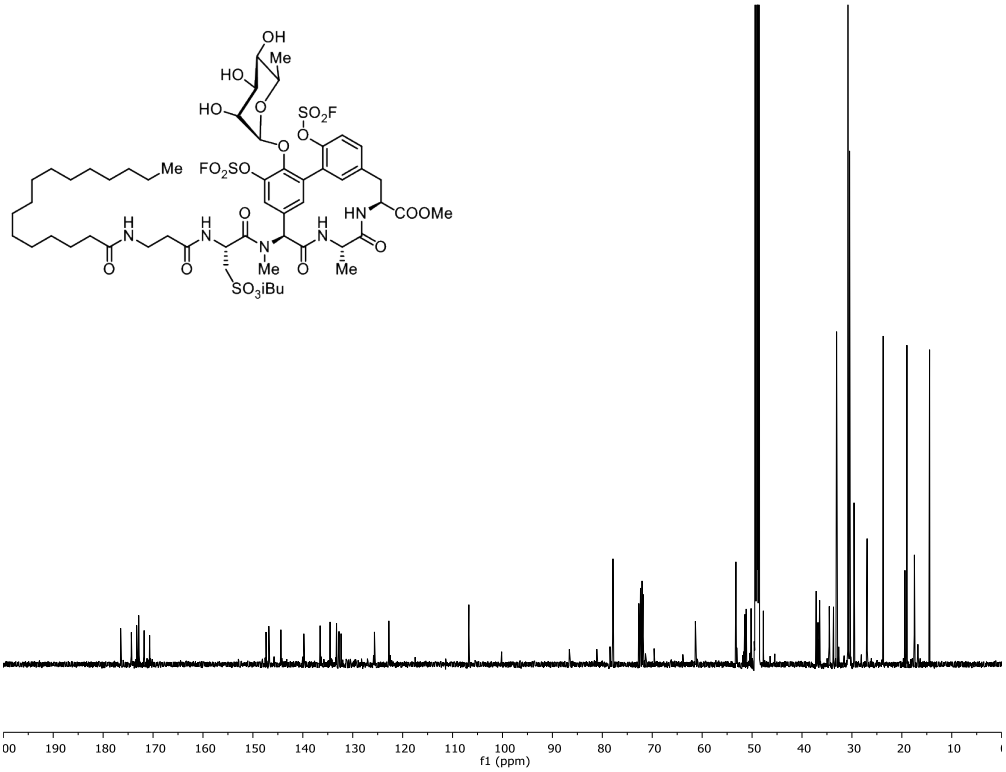
Compound 19a ^{13}C NMR (Methanol-d_4, 151 MHz)



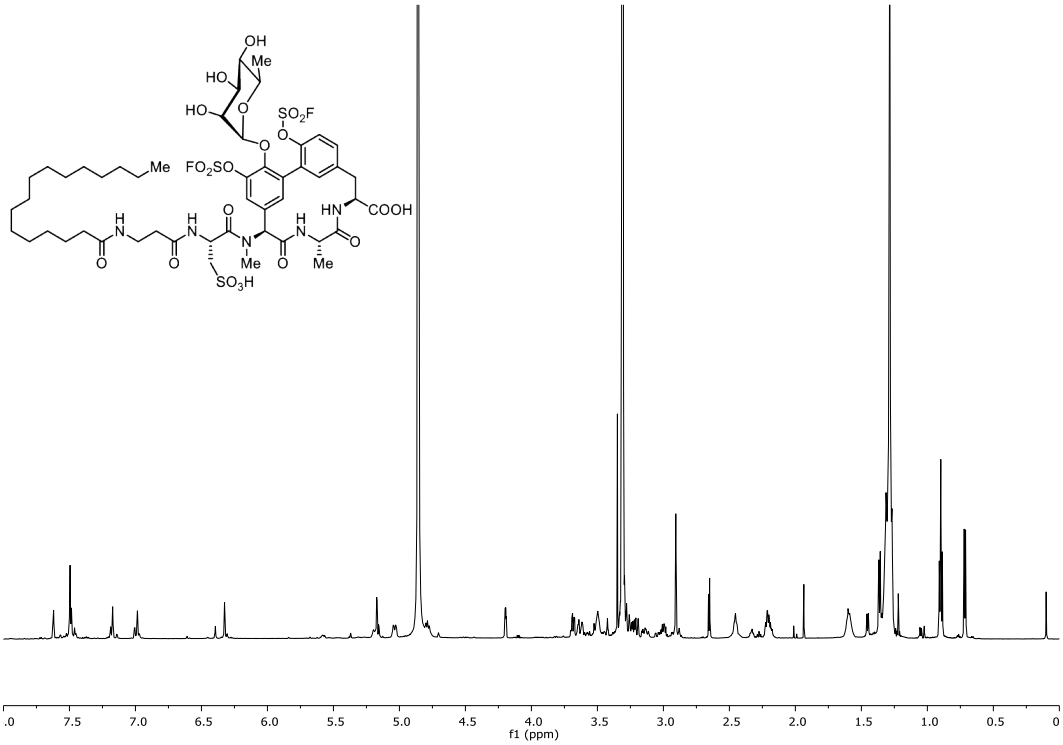
Compound 20a ^{1}H NMR (Methanol-d_4, 600 MHz)



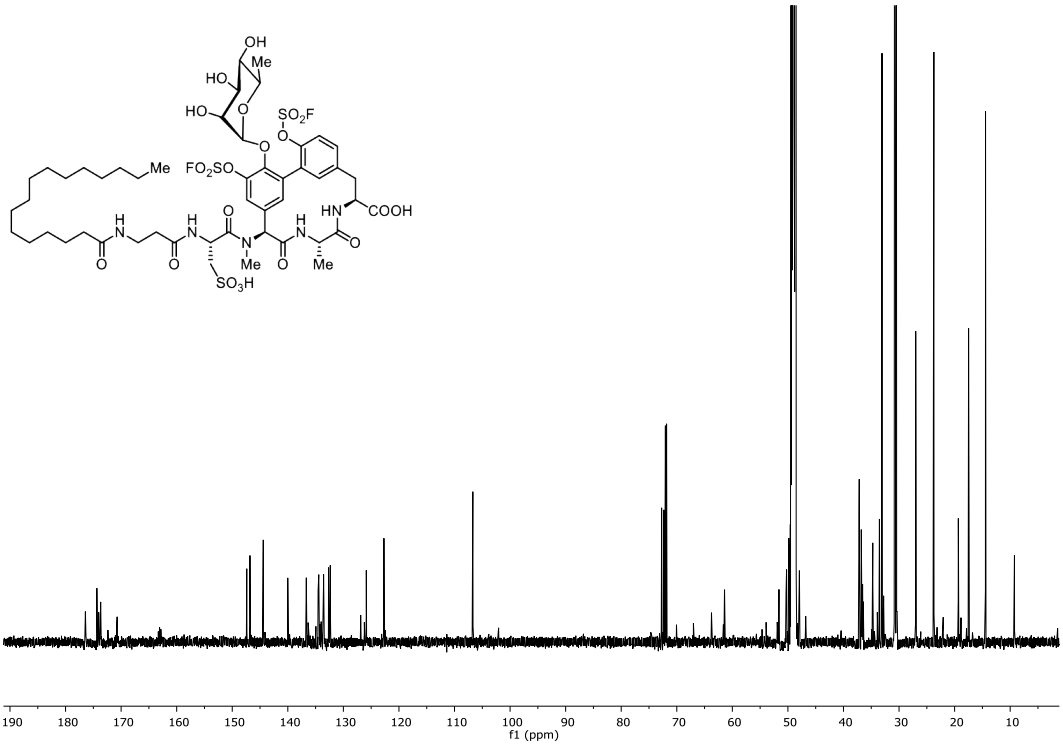
Compound 20a ^{13}C NMR (Methanol-d_4, 151 MHz)



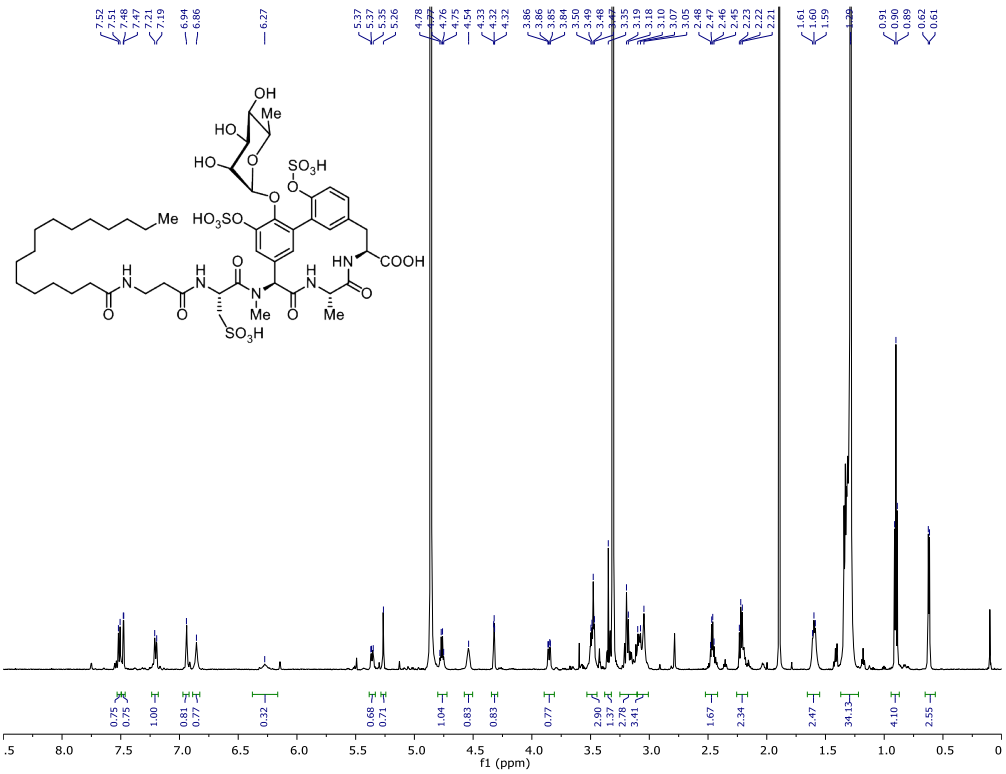
Compound S6a ^{1}H NMR (Methanol-d_4, 600 MHz)



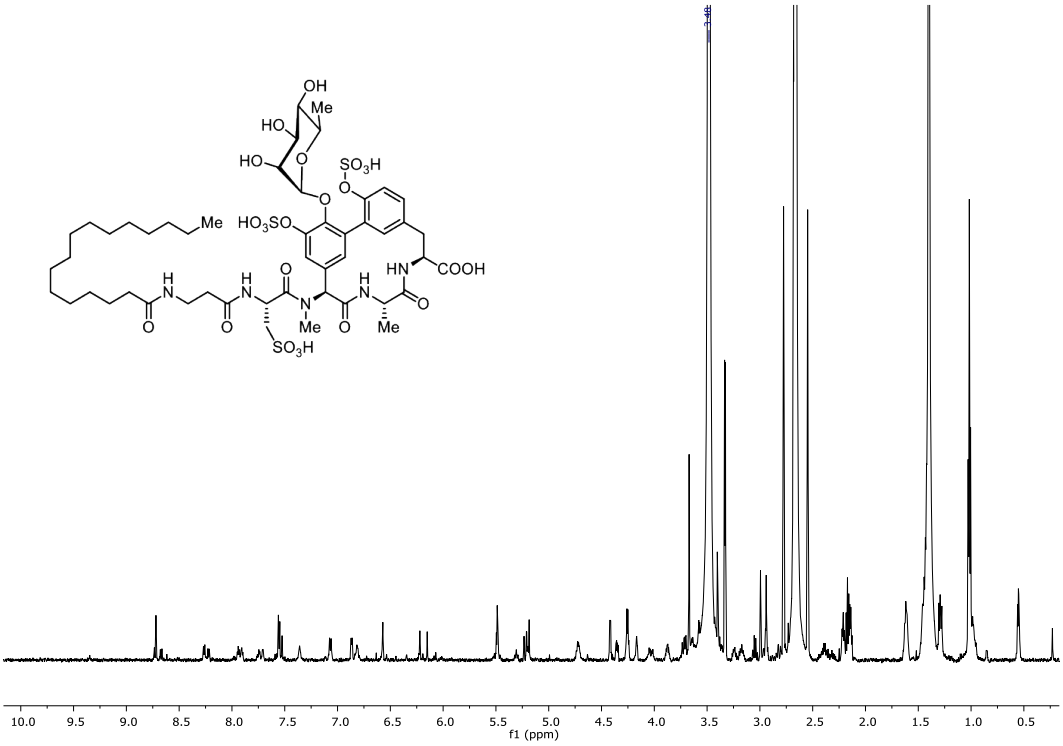
Compound S6a ^{13}C NMR (Methanol-d_4, 151 MHz)



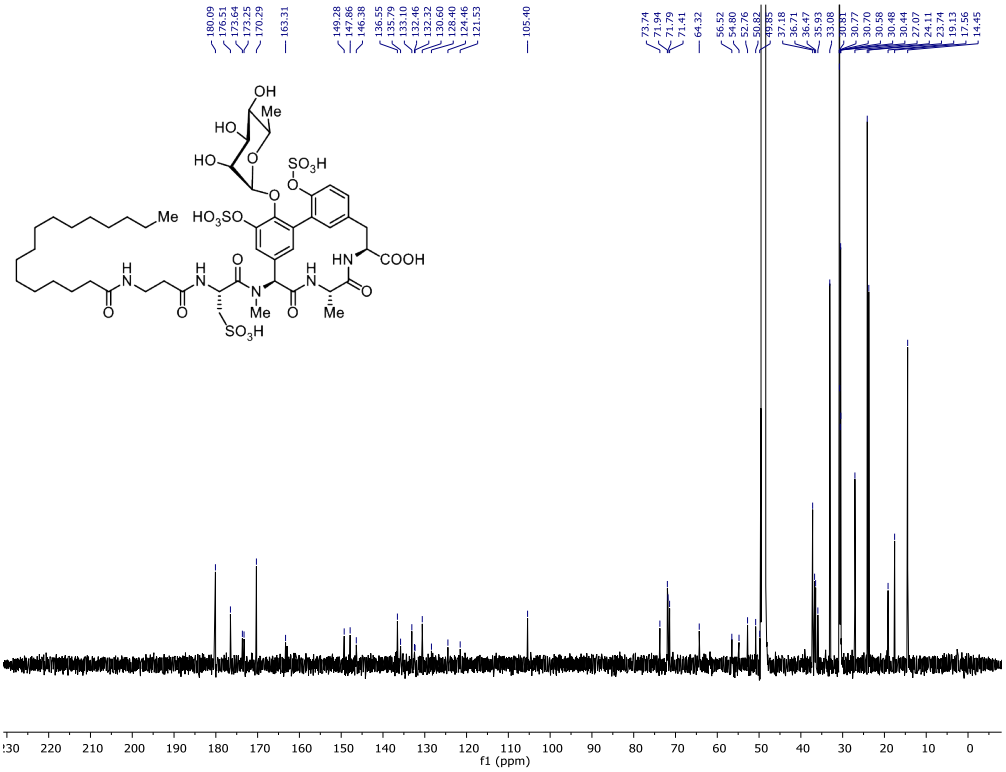
Compound 1a ^{1}H NMR (Methanol-d_4, 600 MHz)



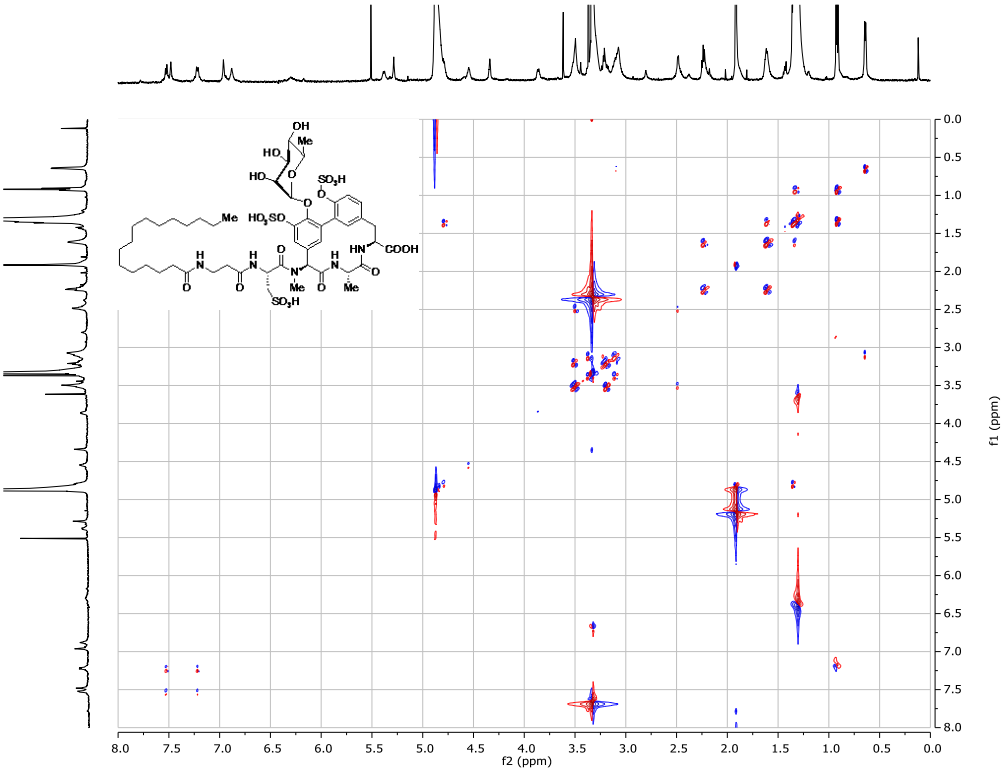
Compound 1a ^{1}H NMR (DMSO-d_6, 600 MHz)



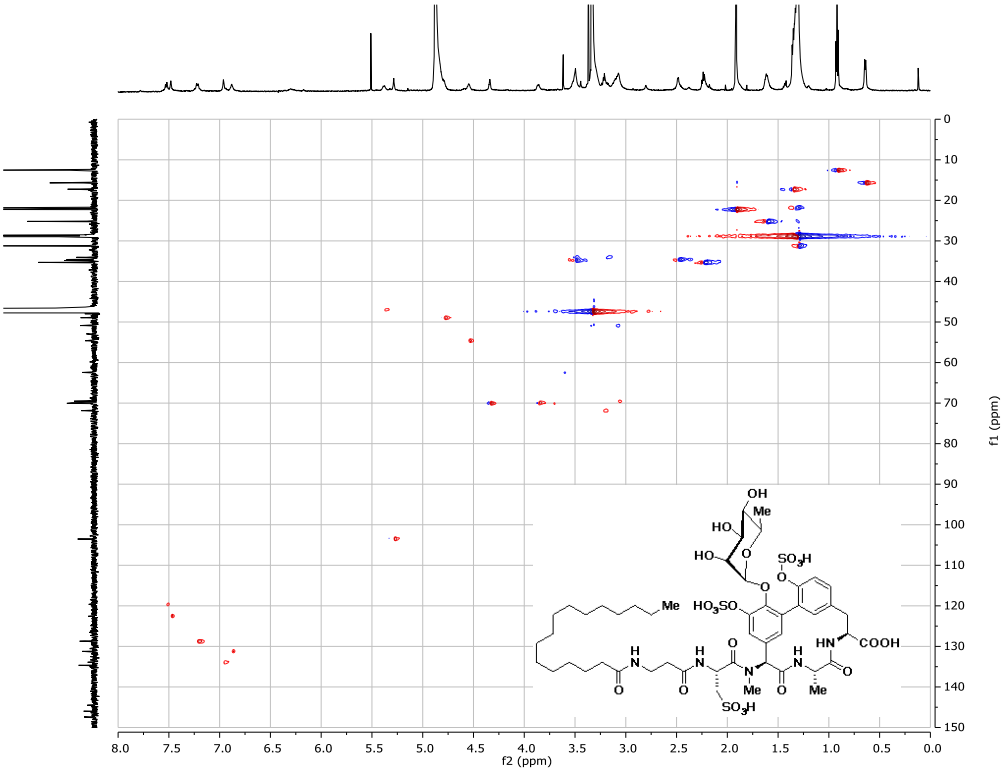
Compound 1a ^{13}C NMR (Methanol-d_4, 151 MHz)



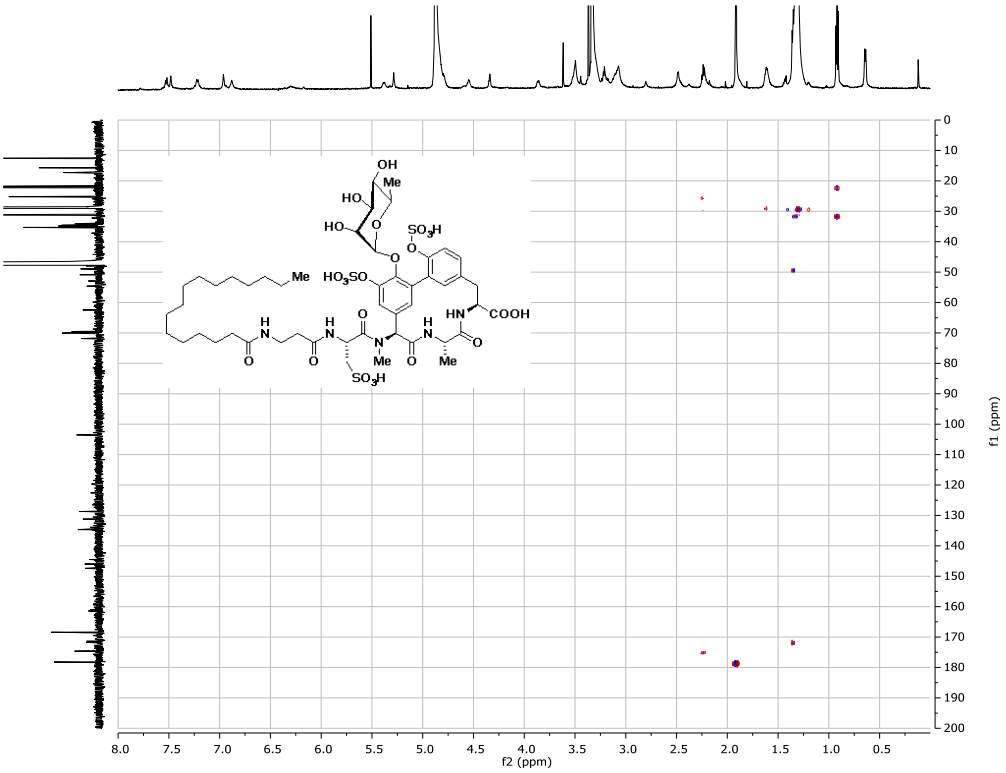
Compound 1a COSY NMR (Methanol-d_4)



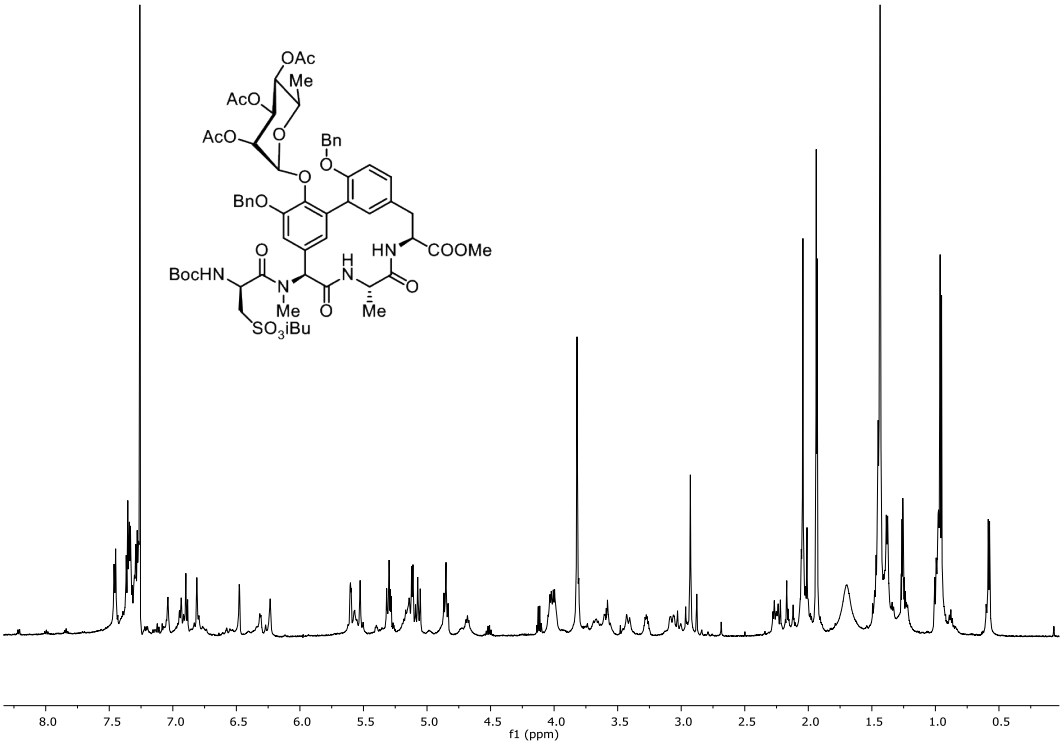
Compound 1a HSQC NMR (Methanol-d_4)



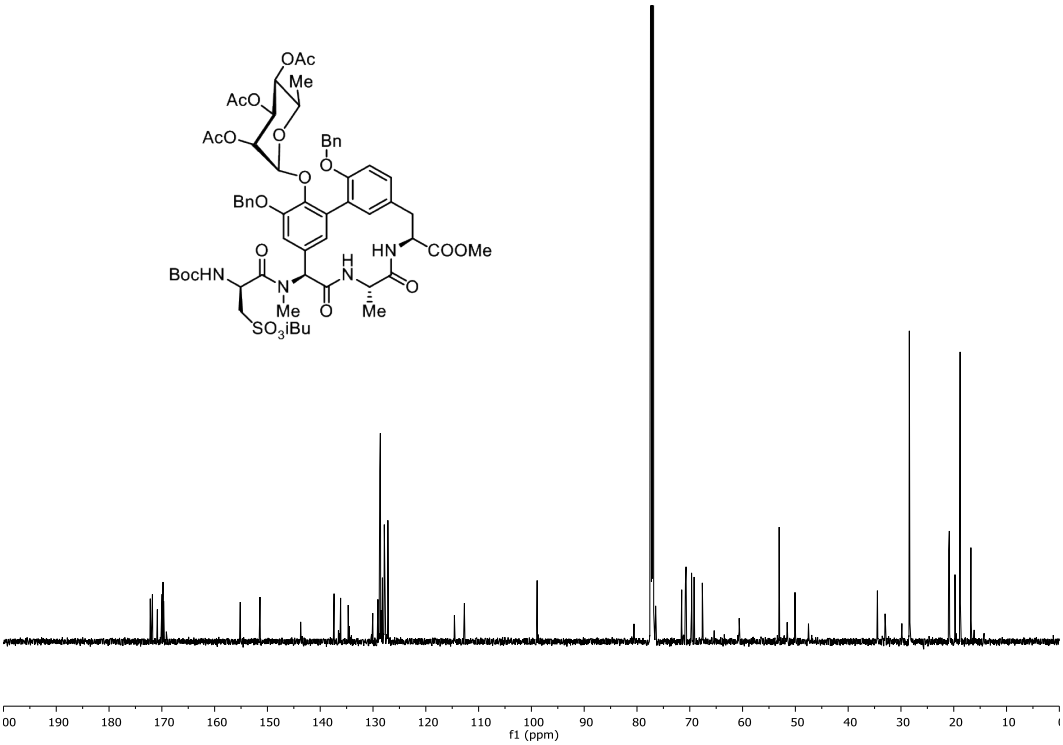
Compound 1a HMBC NMR (Methanol-d_4)



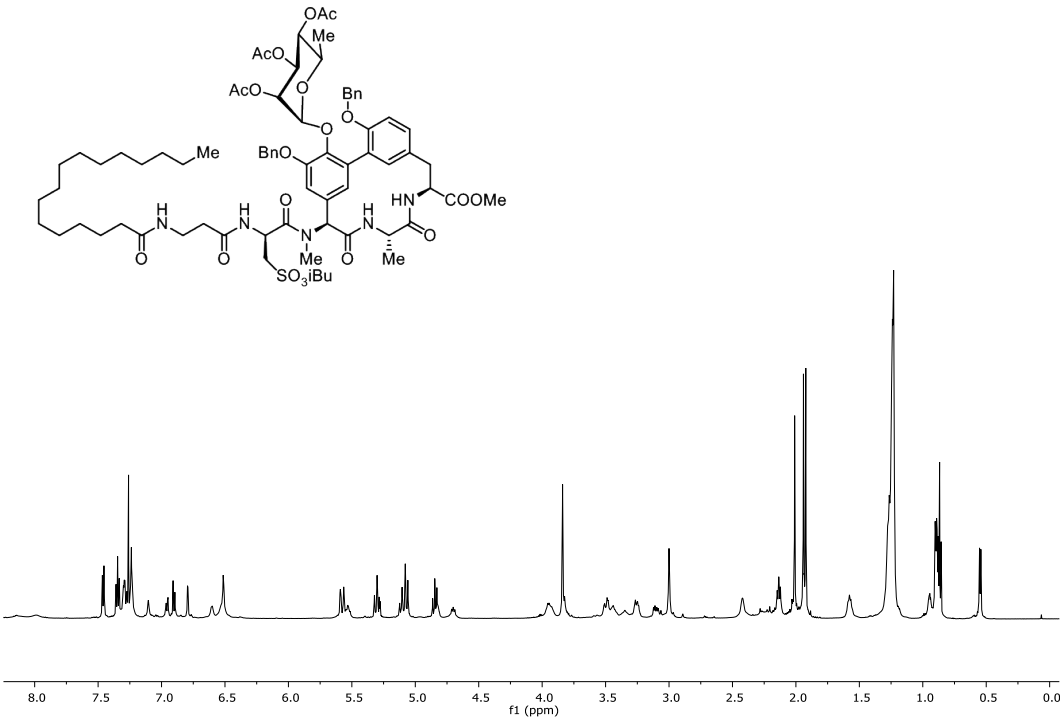
Compound 2b ^{1}H NMR ($CDCl_3$, 600 MHz)



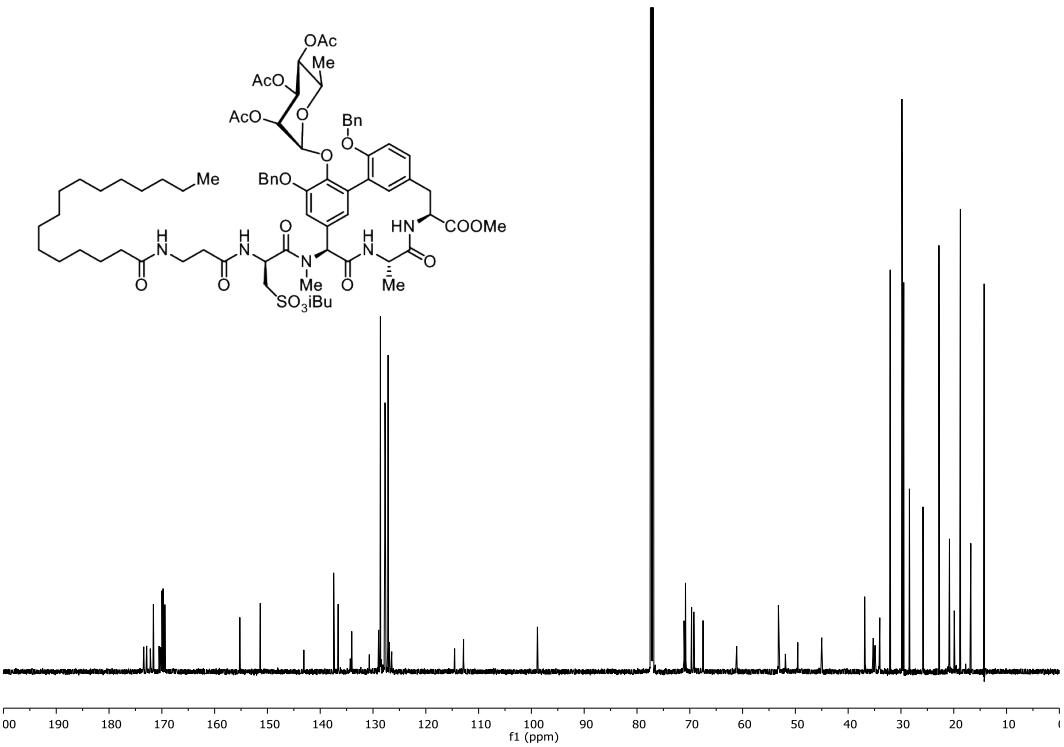
Compound 2b ^{13}C NMR ($CDCl_3$, 151 MHz)



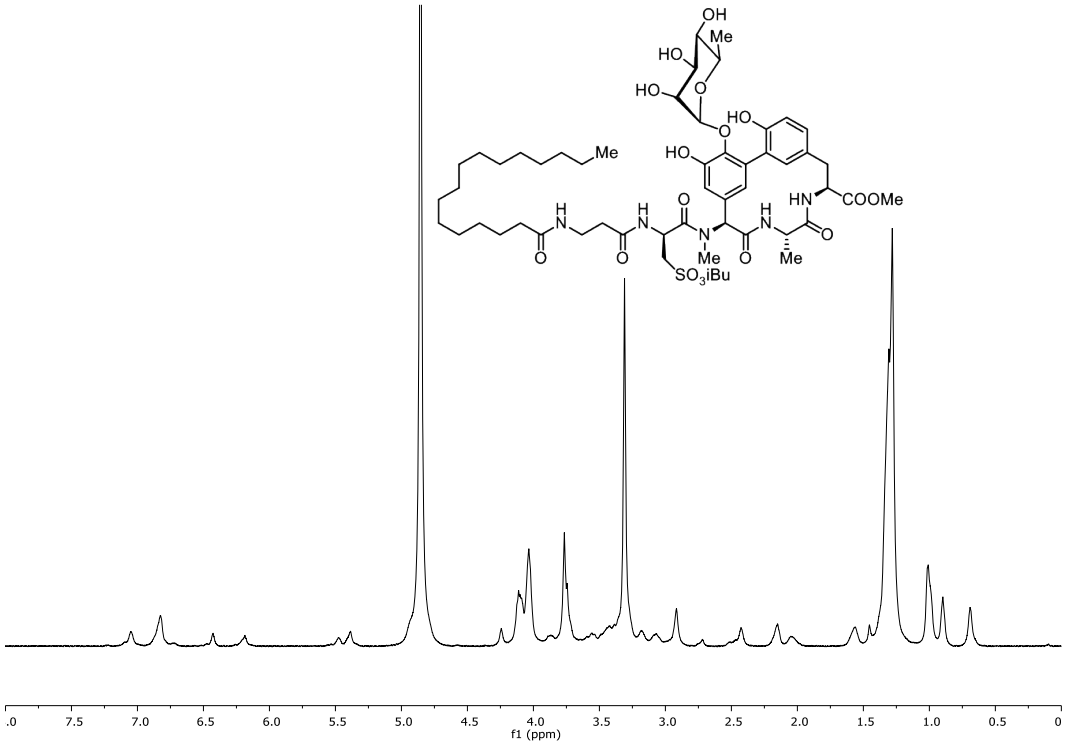
Compound 18b ^{1}H NMR ($CDCl_3$, 600 MHz)



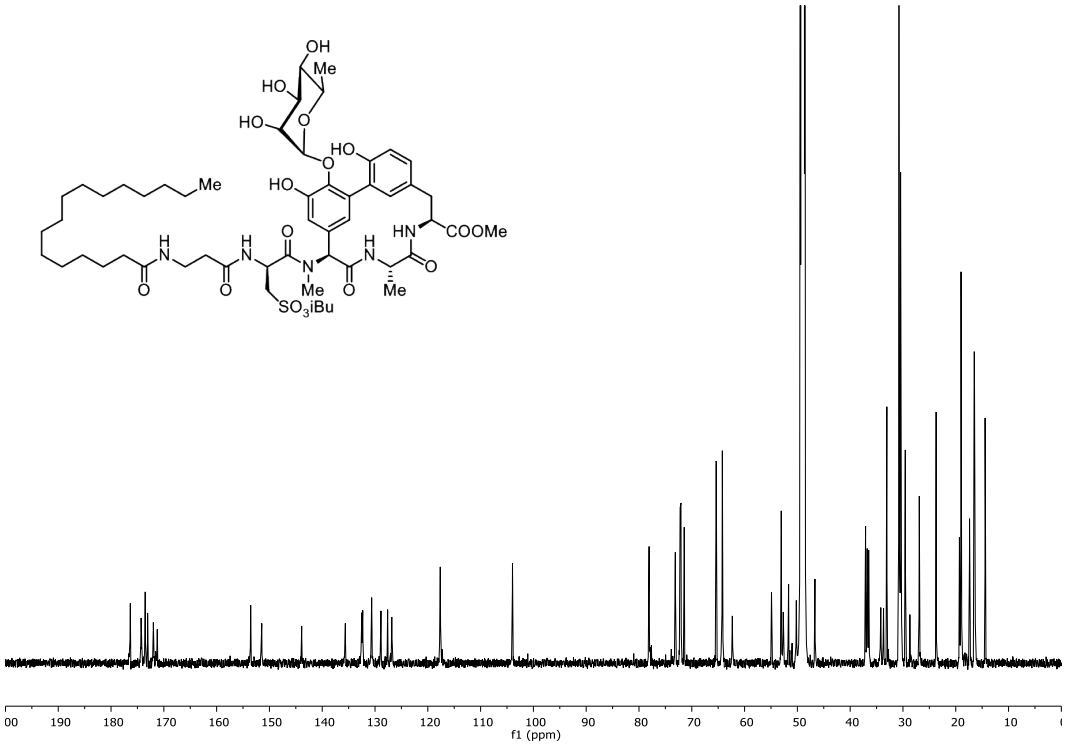
Compound 18b ^{13}C NMR ($CDCl_3$, 151 MHz)



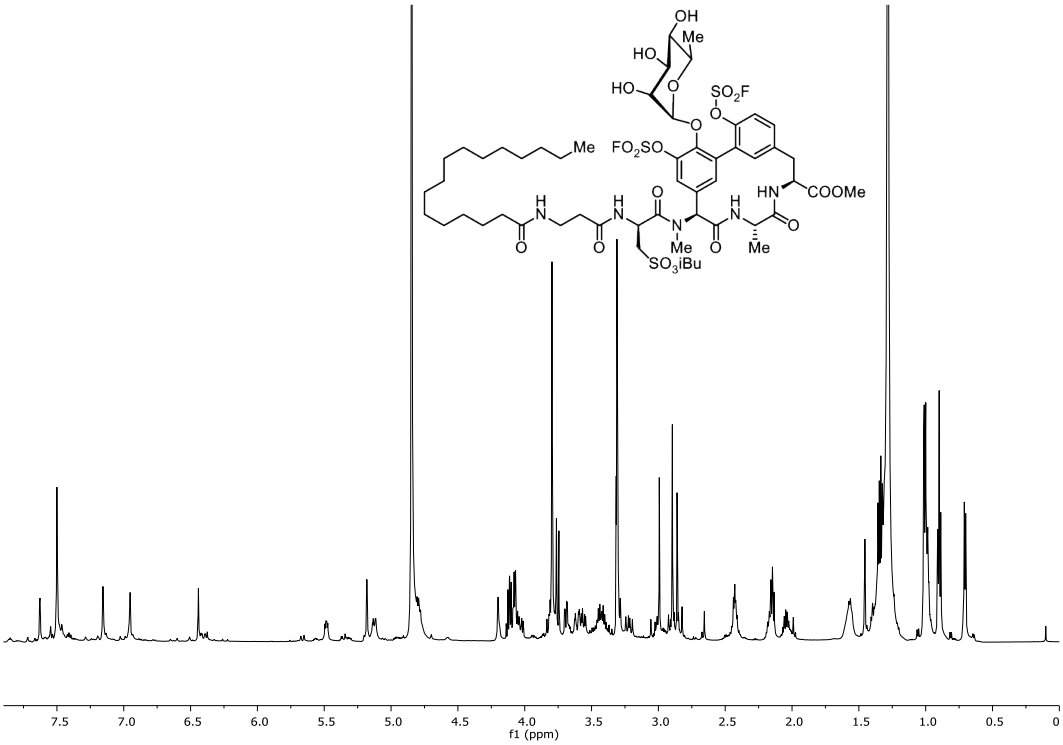
Compound 19b ^{1}H NMR (Methanol-d_4, 600 MHz)



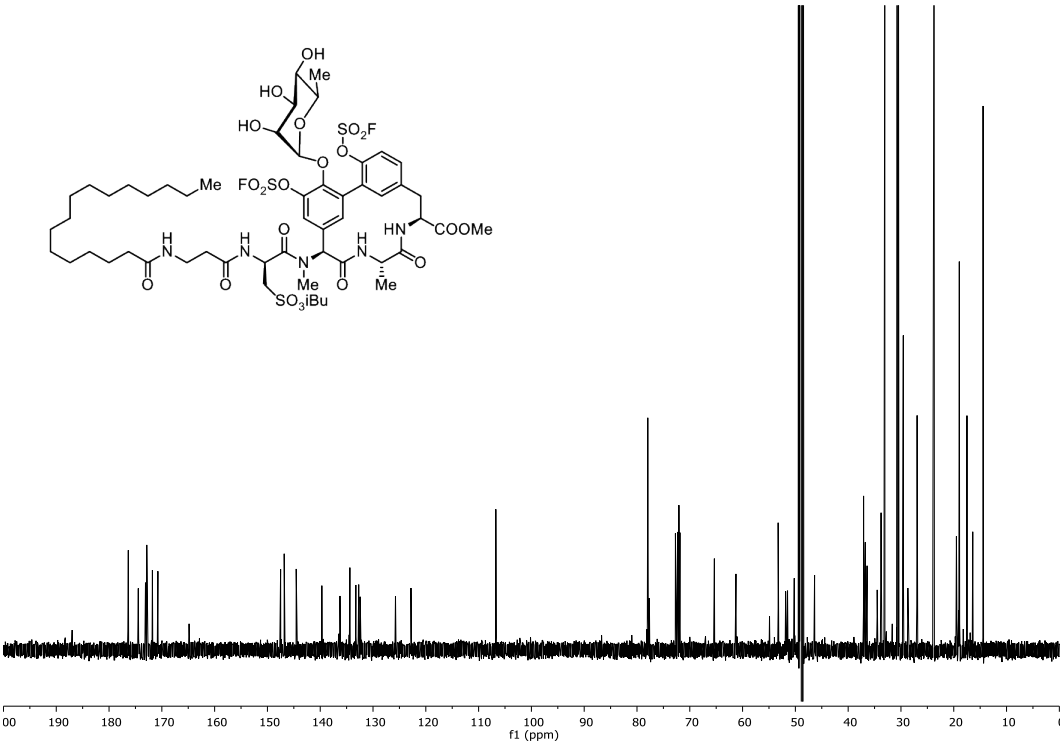
Compound 19b ^{13}C NMR (Methanol-d_4, 151 MHz)



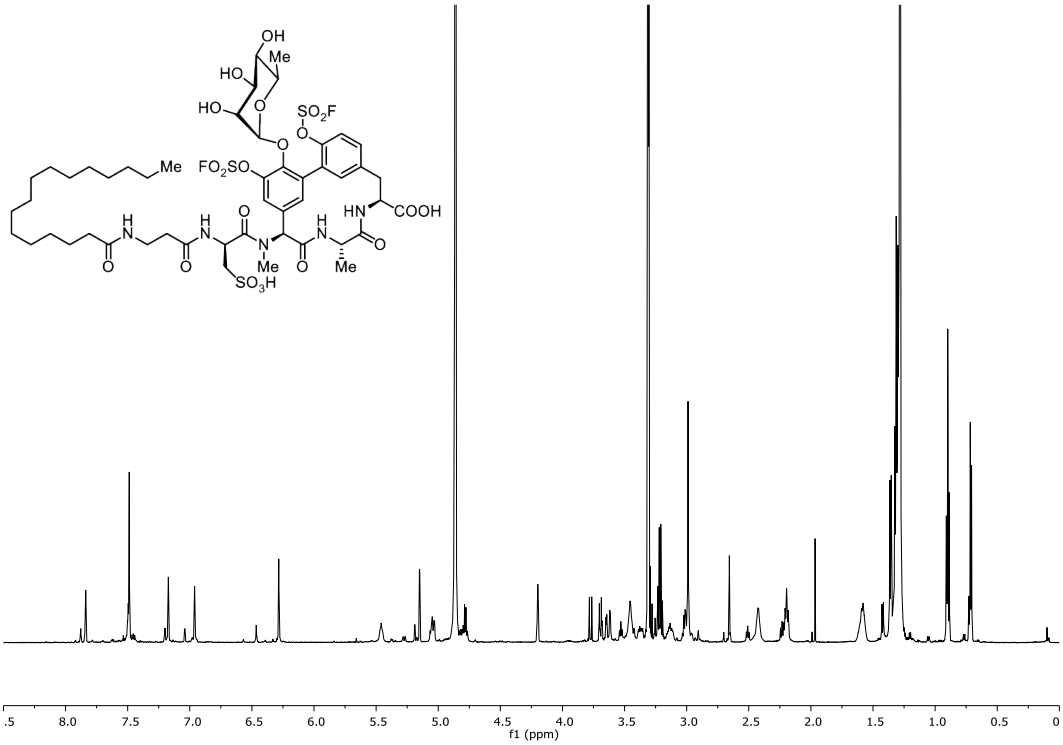
Compound 20b ^{1}H NMR (Methanol-d_4, 600 MHz)



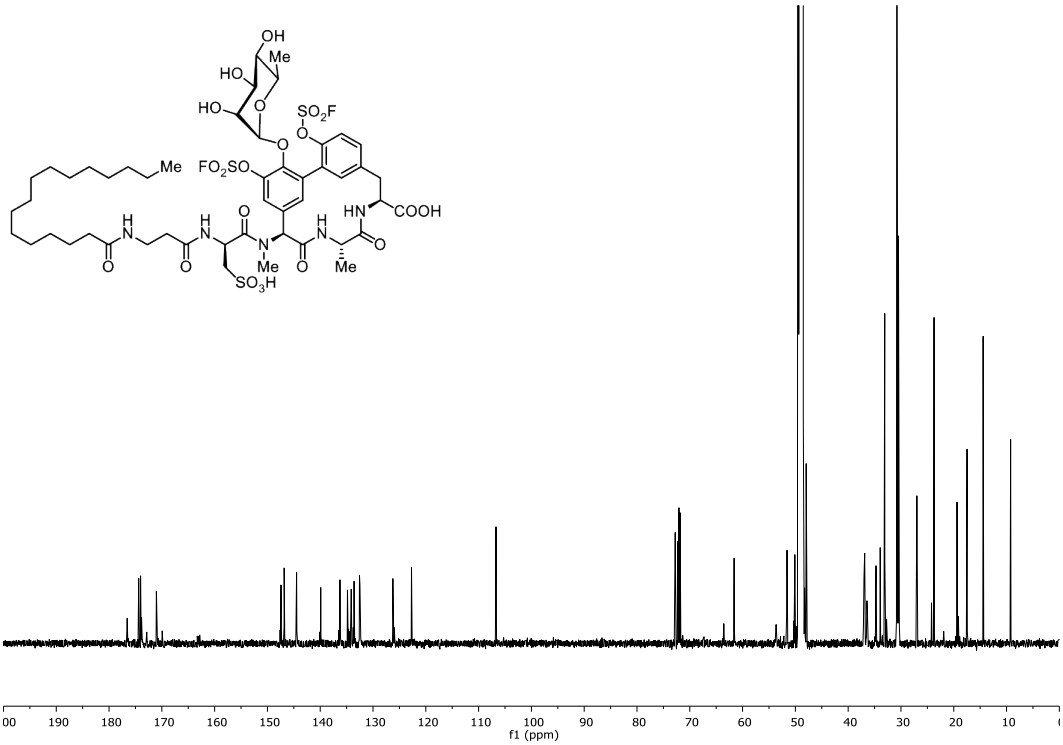
Compound 20b ^{13}C NMR (Methanol-d_4, 151 MHz)



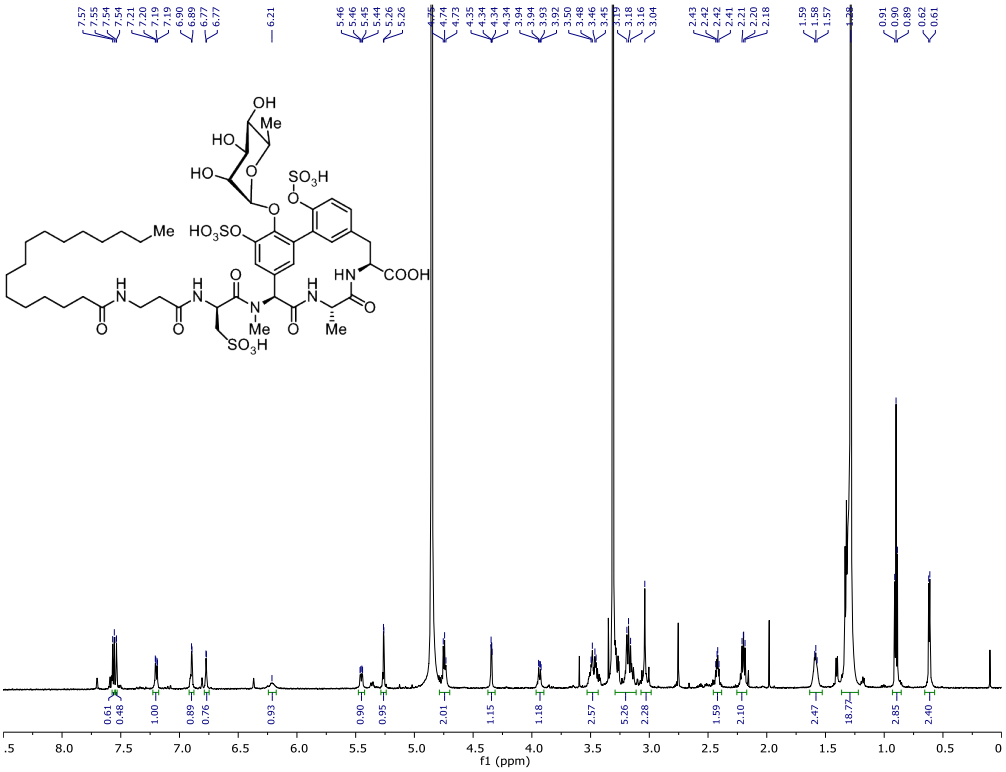
Compound S6b ^{1}H NMR (Methanol-d_4, 600 MHz)



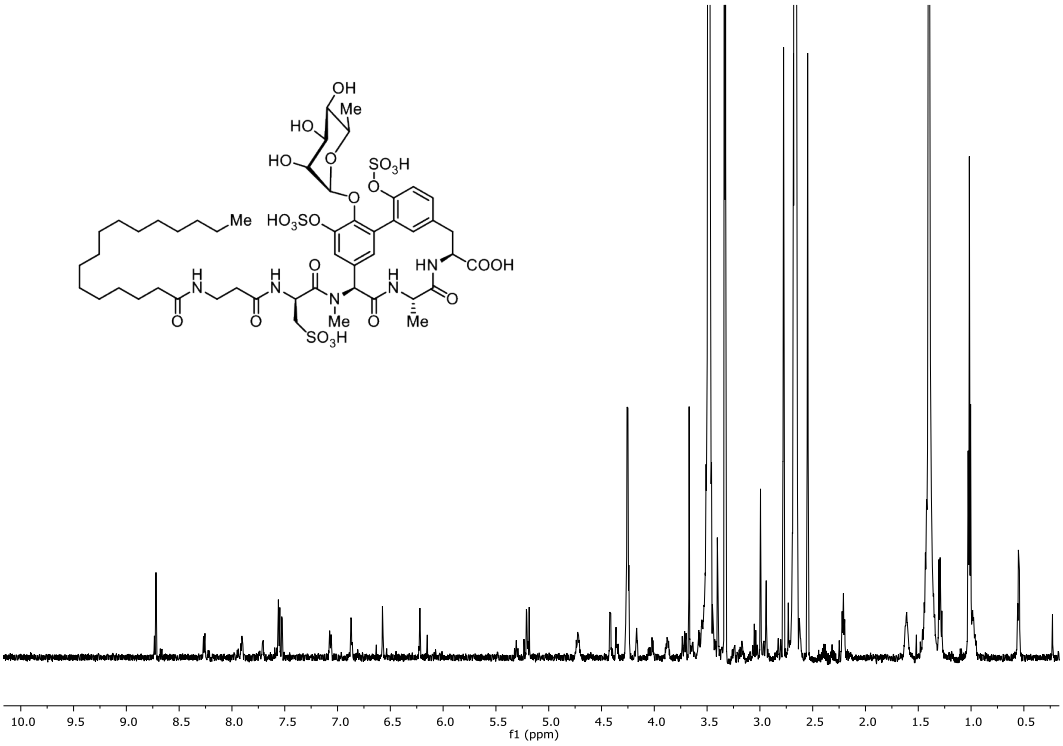
Compound S6b ^{13}C NMR (Methanol-d_4, 151 MHz)



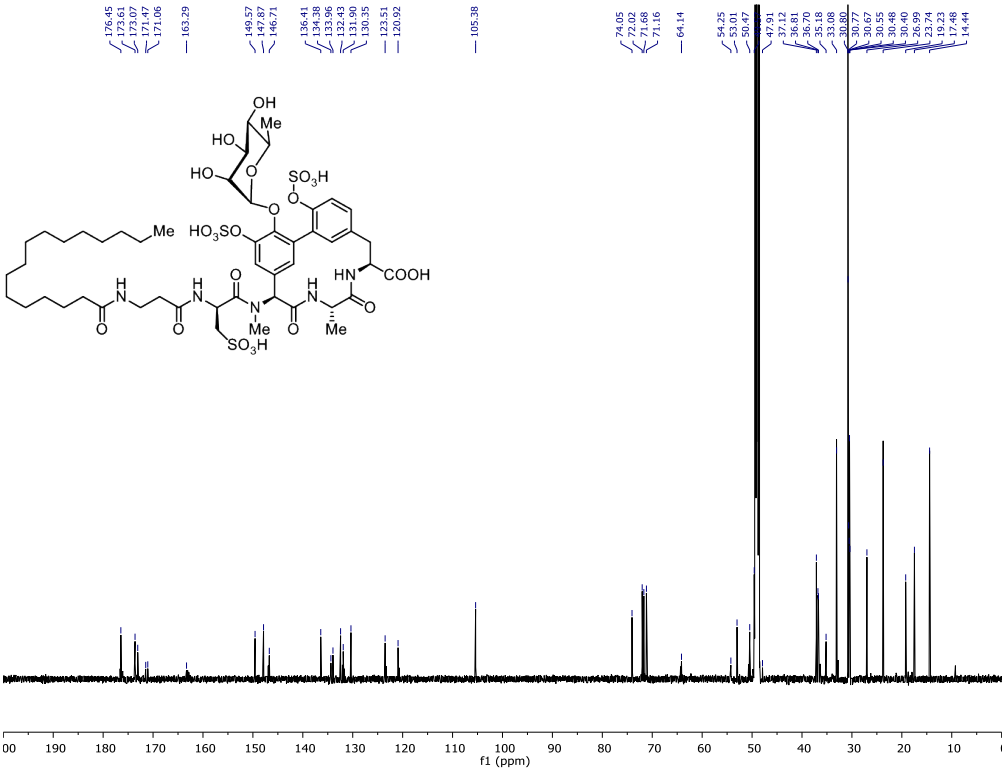
Compound 1b ^{1}H NMR (Methanol-d_4, 600 MHz)



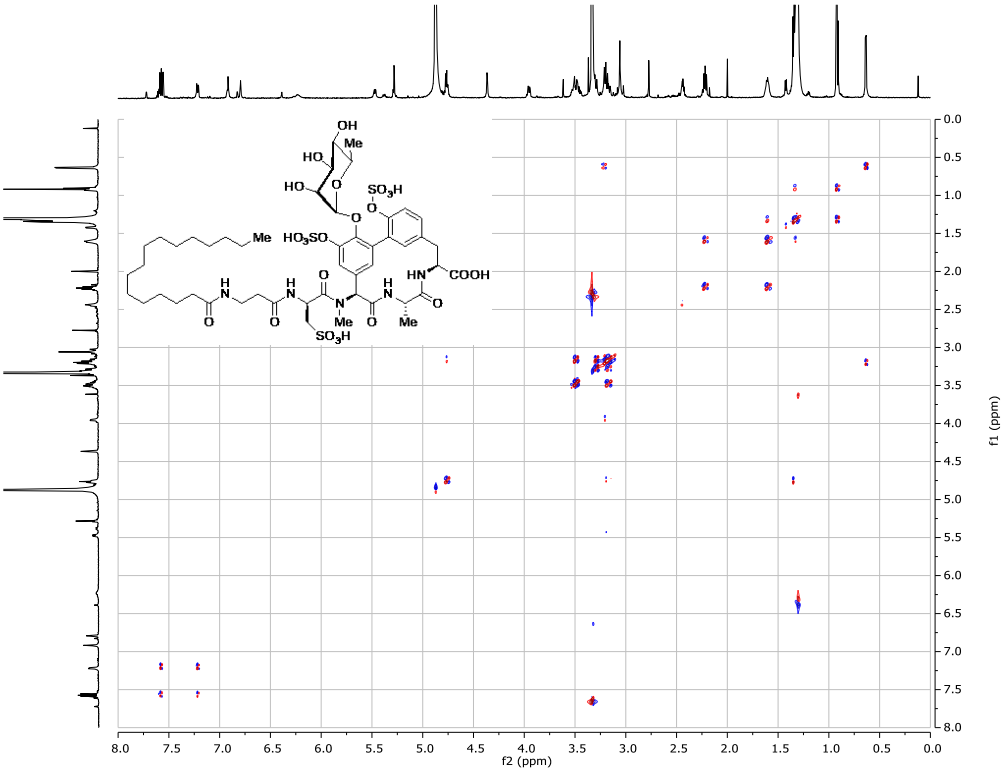
Compound 1b ^{1}H NMR (DMSO-d_6, 600 MHz)



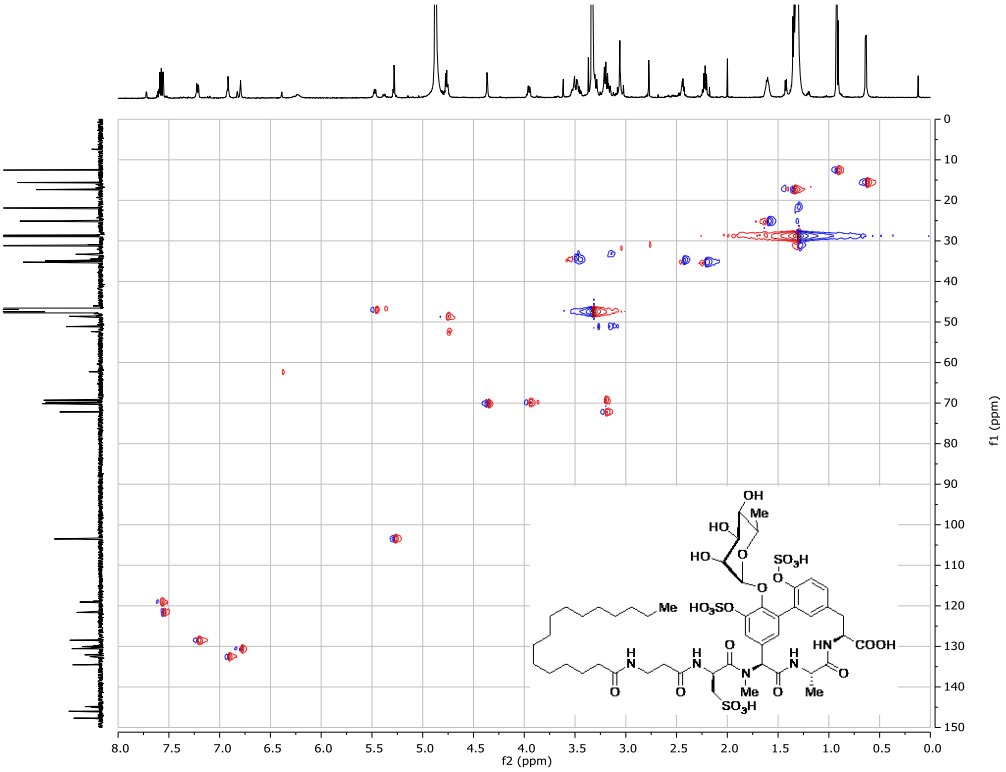
Compound 1b ^{13}C NMR (Methanol-d_4, 151 MHz)



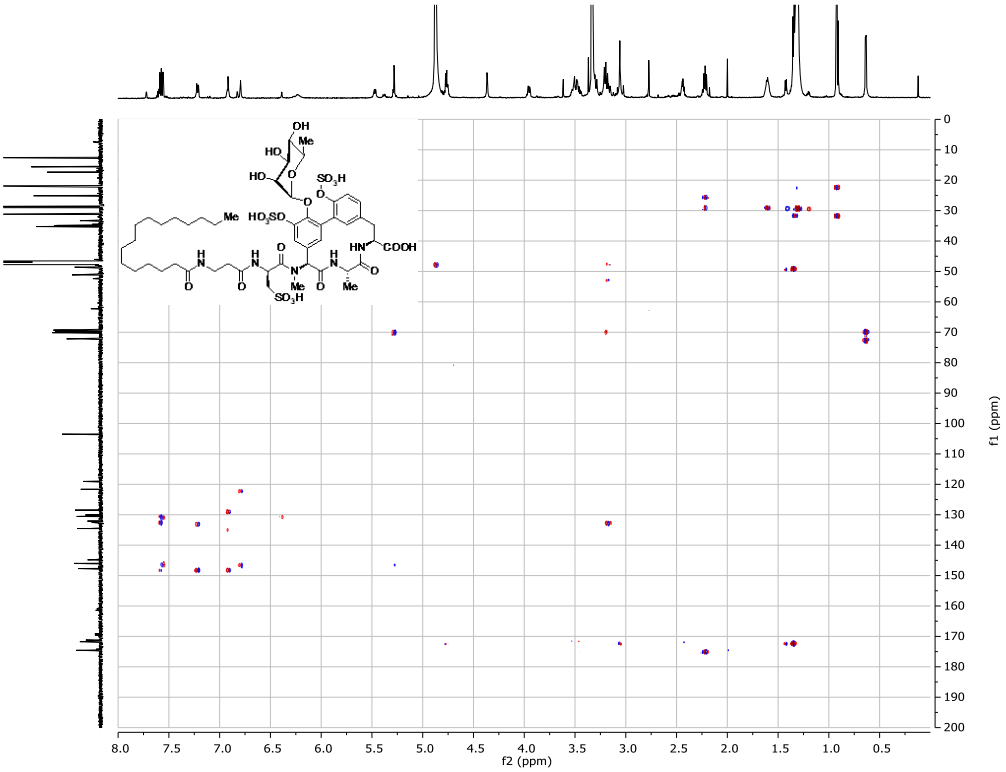
Compound 1b COSY NMR (Methanol-d_4)



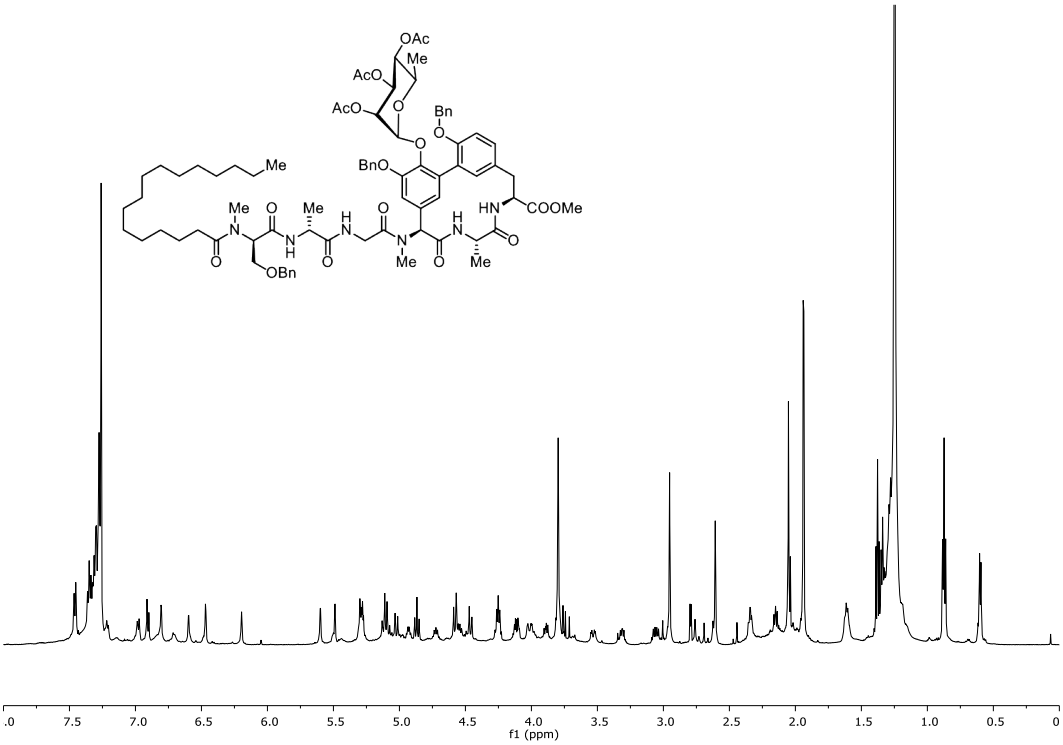
Compound 1b HSQC NMR (Methanol-*d*4)



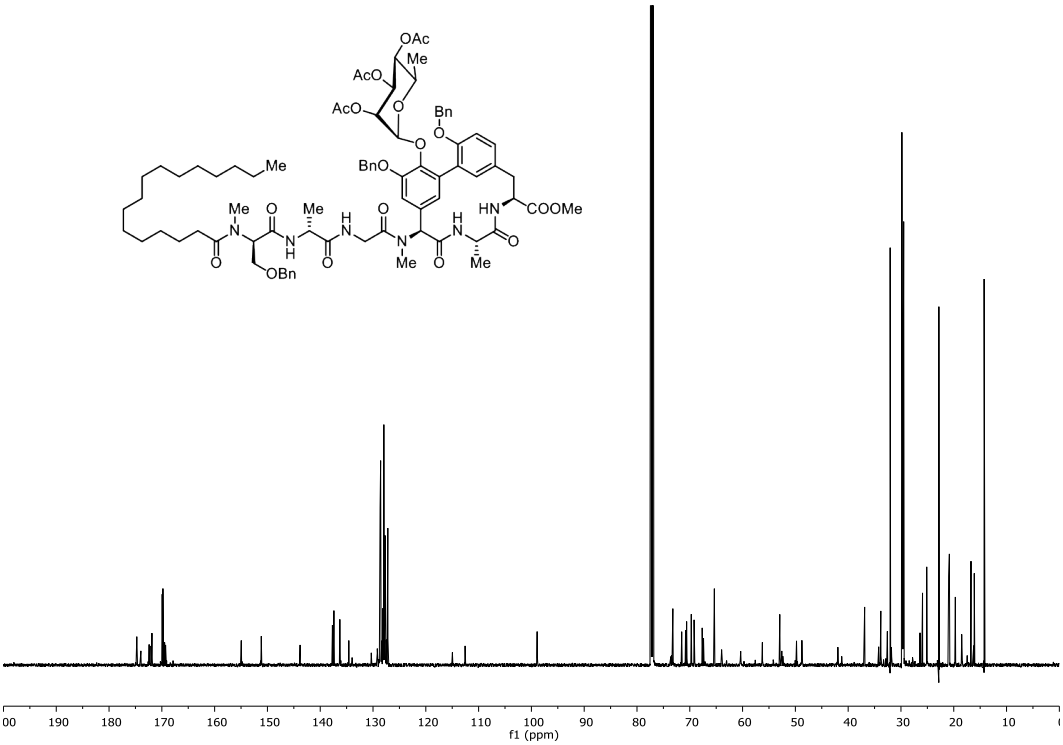
Compound 1b HMBC NMR (Methanol-*d*4)



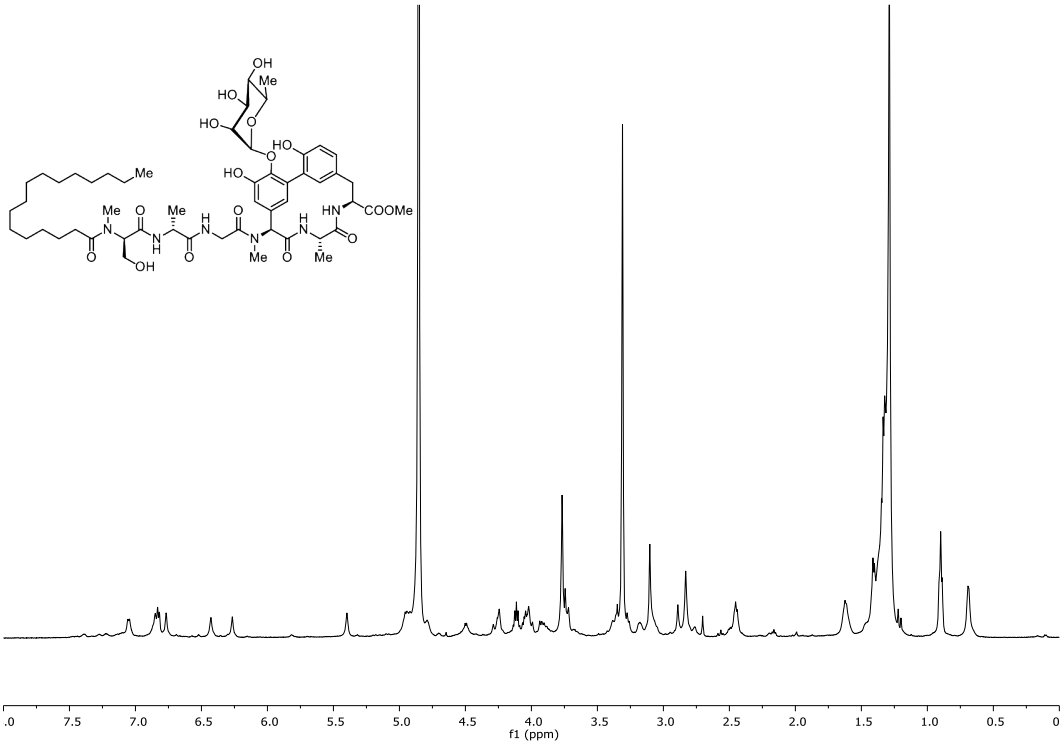
Compound S7 ^{1}H NMR ($CDCl_3$, 600 MHz)



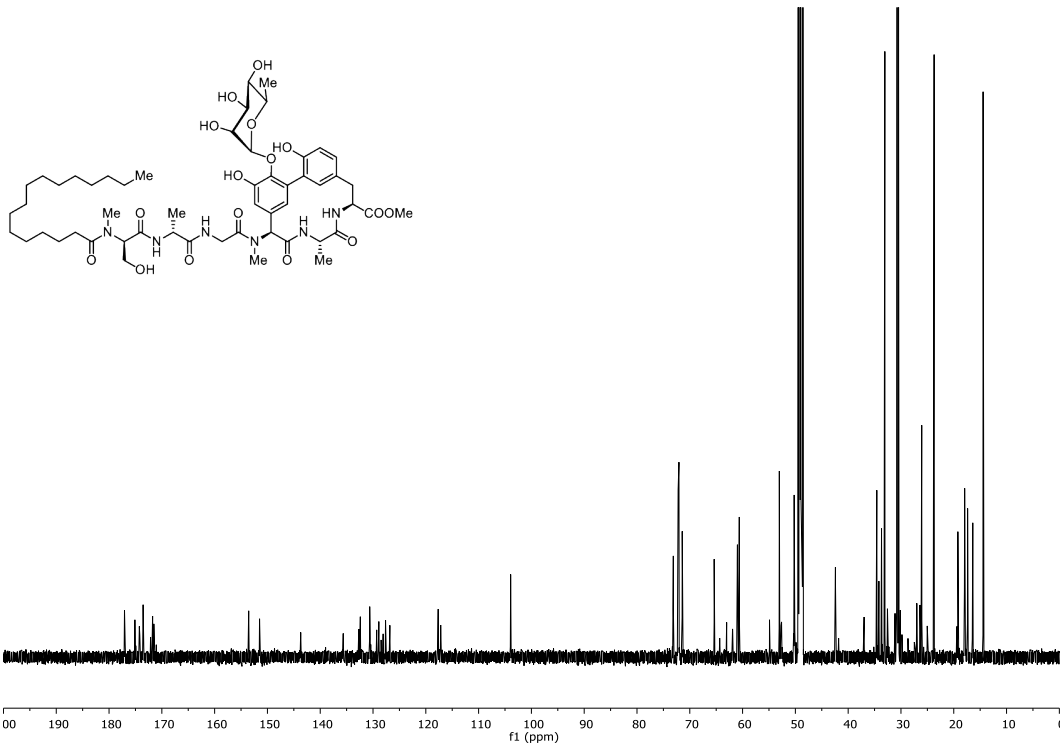
Compound S7 ^{13}C NMR ($CDCl_3$, 151 MHz)



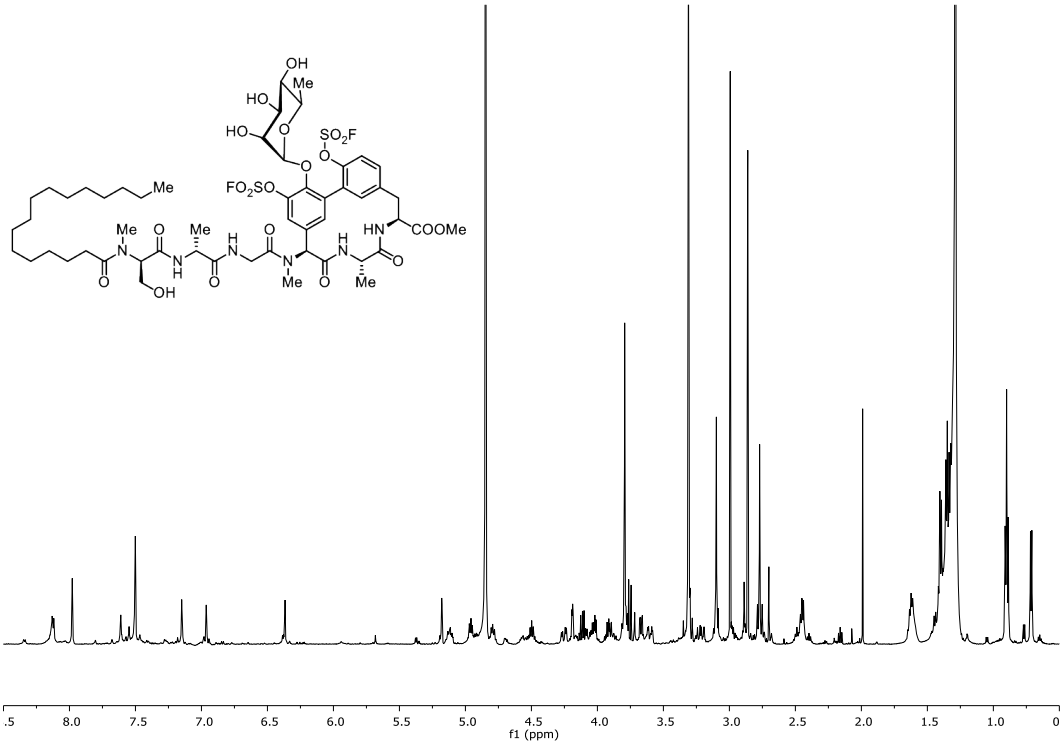
Compound S8 ^{1}H NMR (Methanol-d_4, 600 MHz)



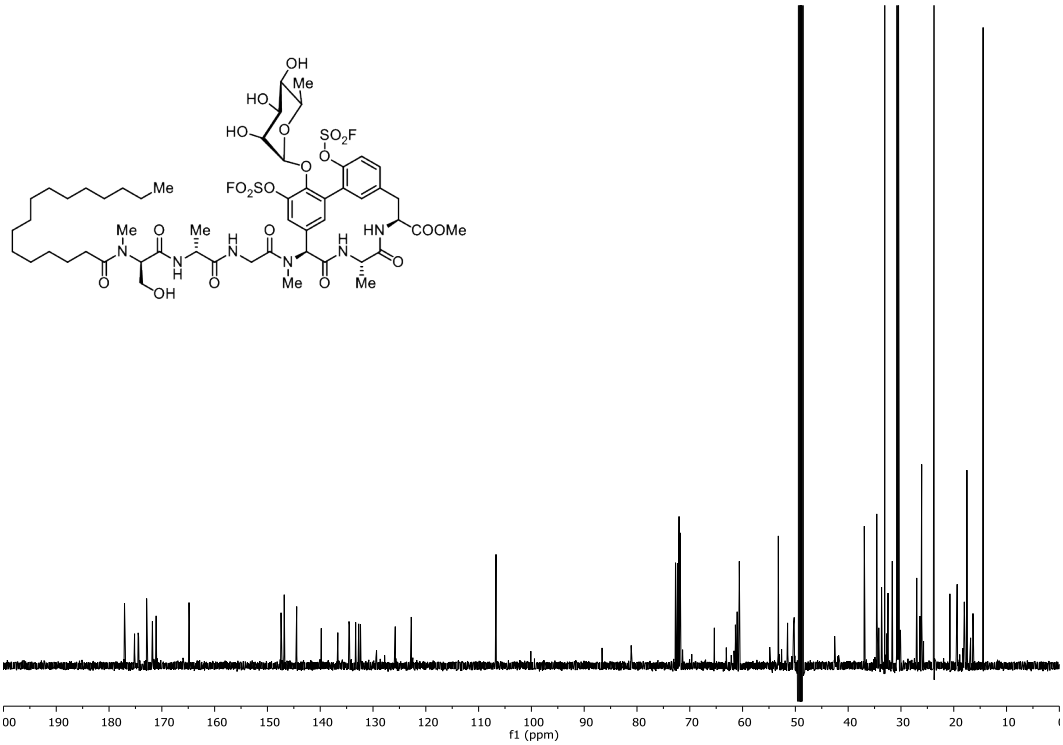
Compound S8 ^{13}C NMR (Methanol-d_4, 151 MHz)



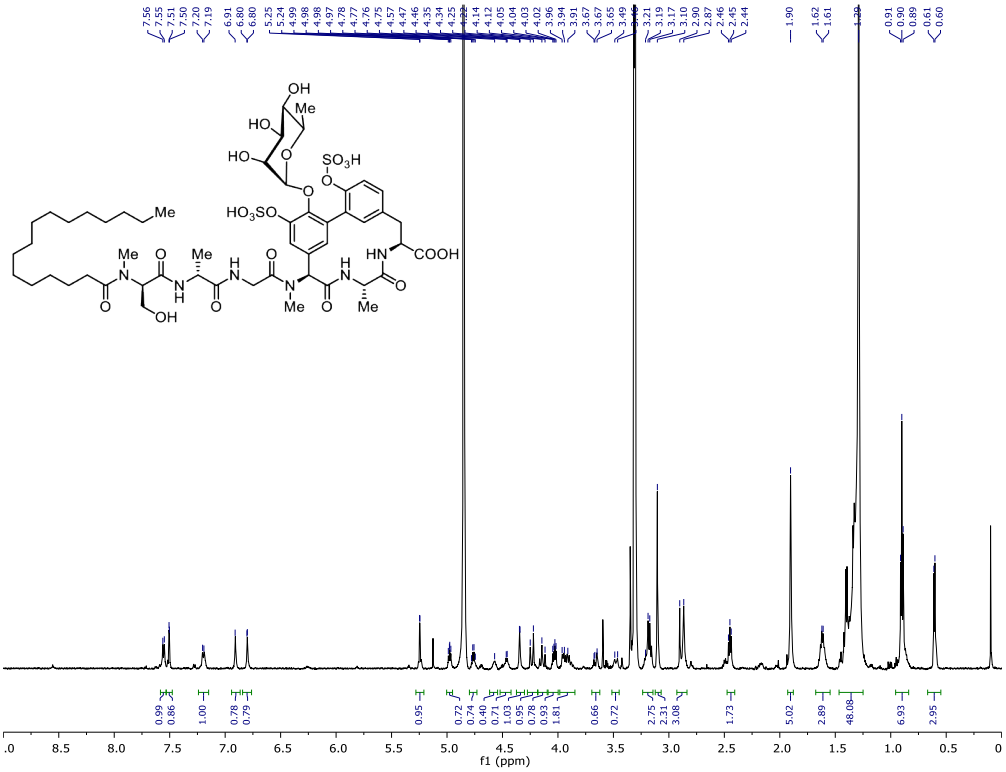
Compound 22 ^{1}H NMR (Methanol-d_4, 600 MHz)



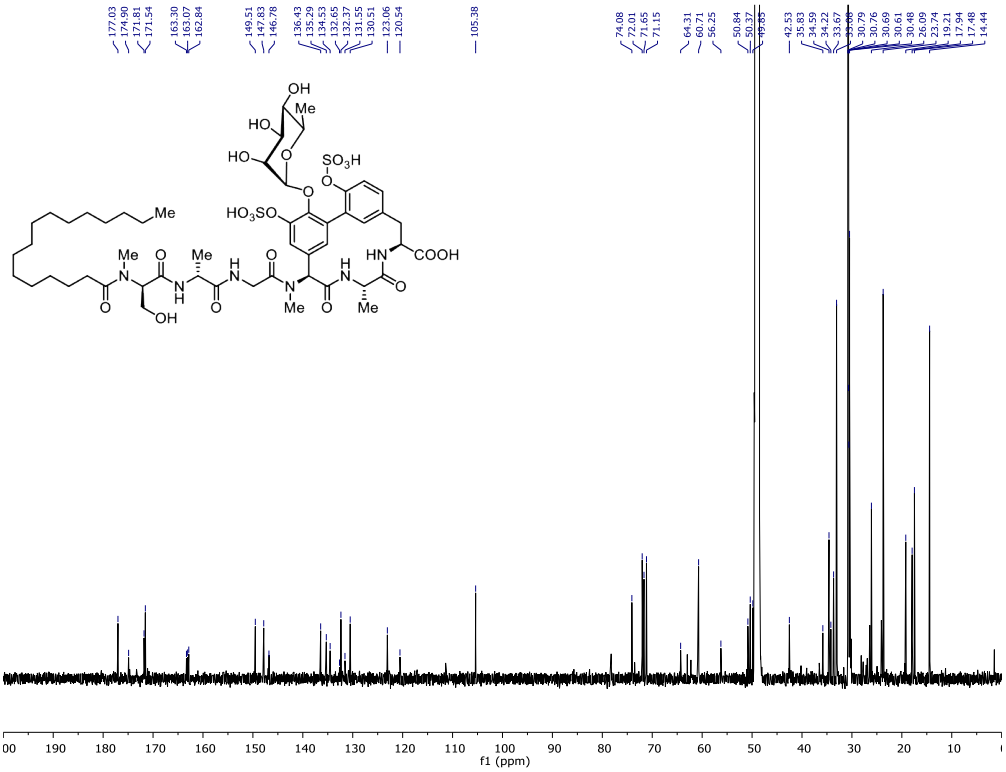
Compound 22 ^{13}C NMR (Methanol-d_4, 151 MHz)



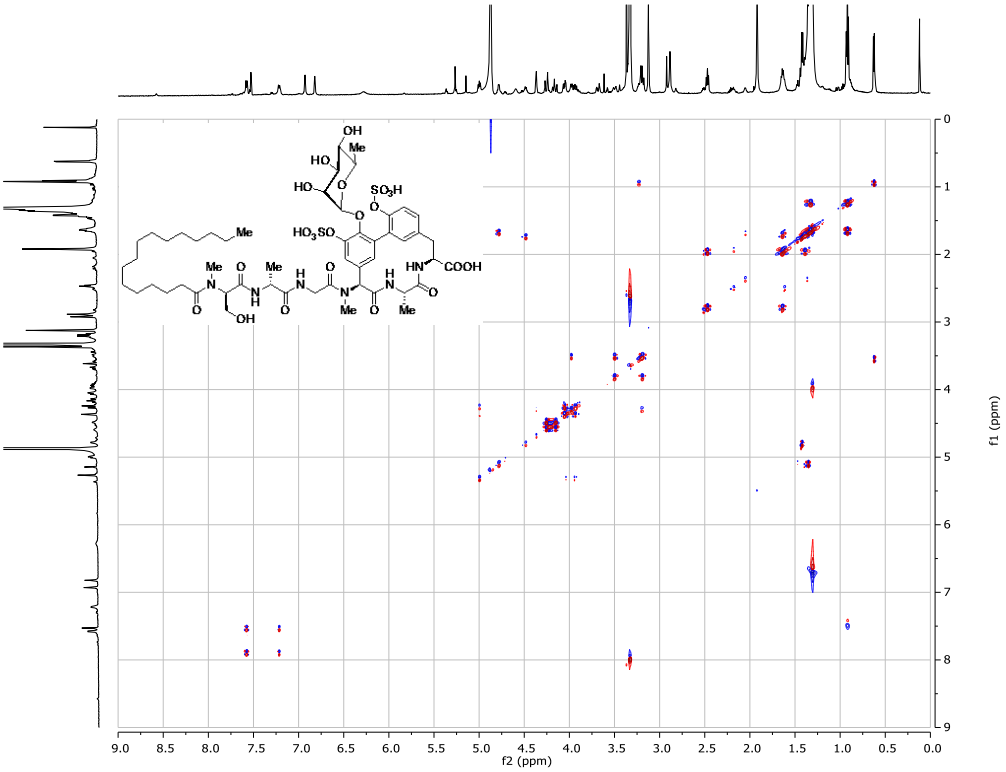
Compound 23 ^{1}H NMR (Methanol-d_4, 600 MHz)



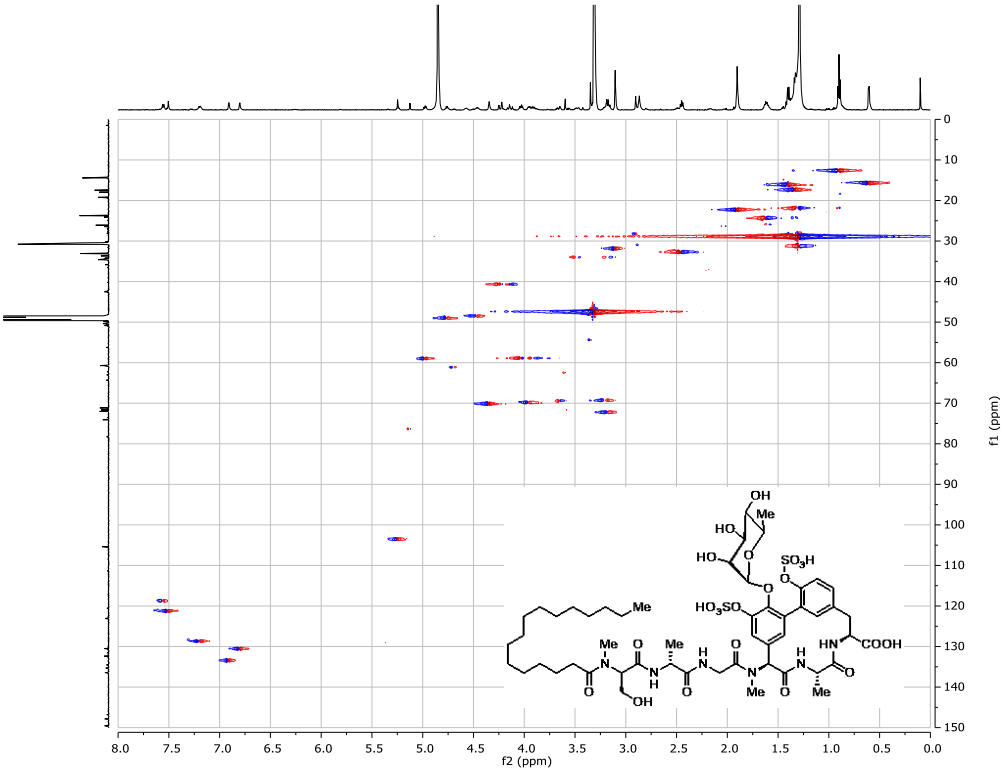
Compound 23 ^{13}C NMR (Methanol-d_4, 151 MHz)



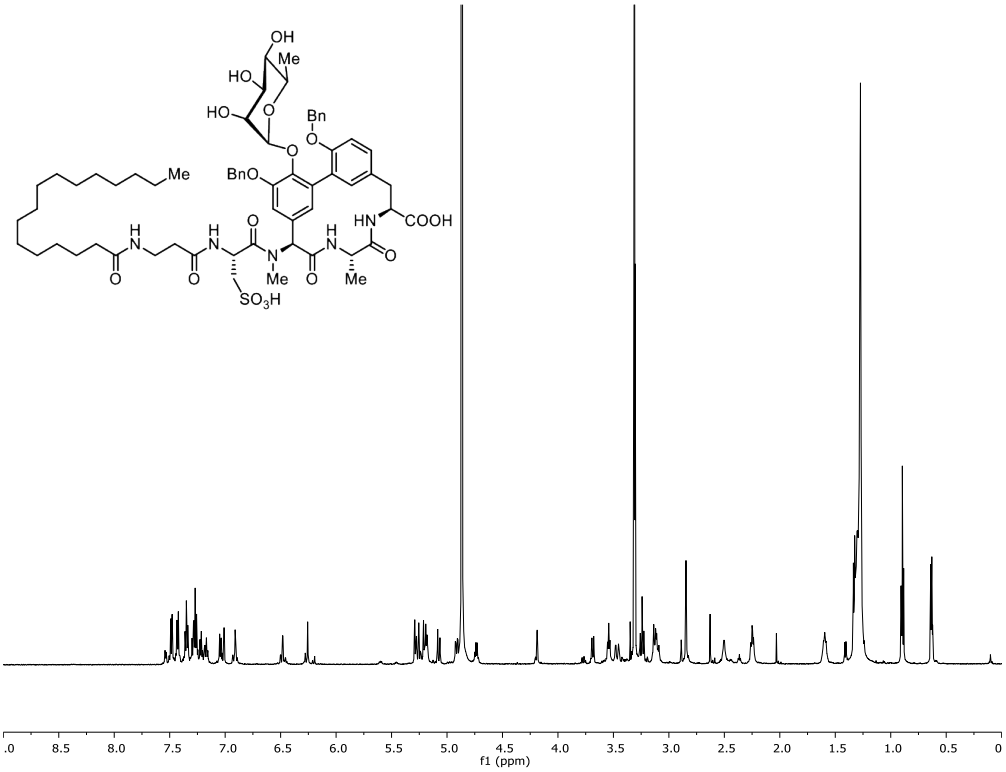
Compound 23 COSY NMR (Methanol-d_4)



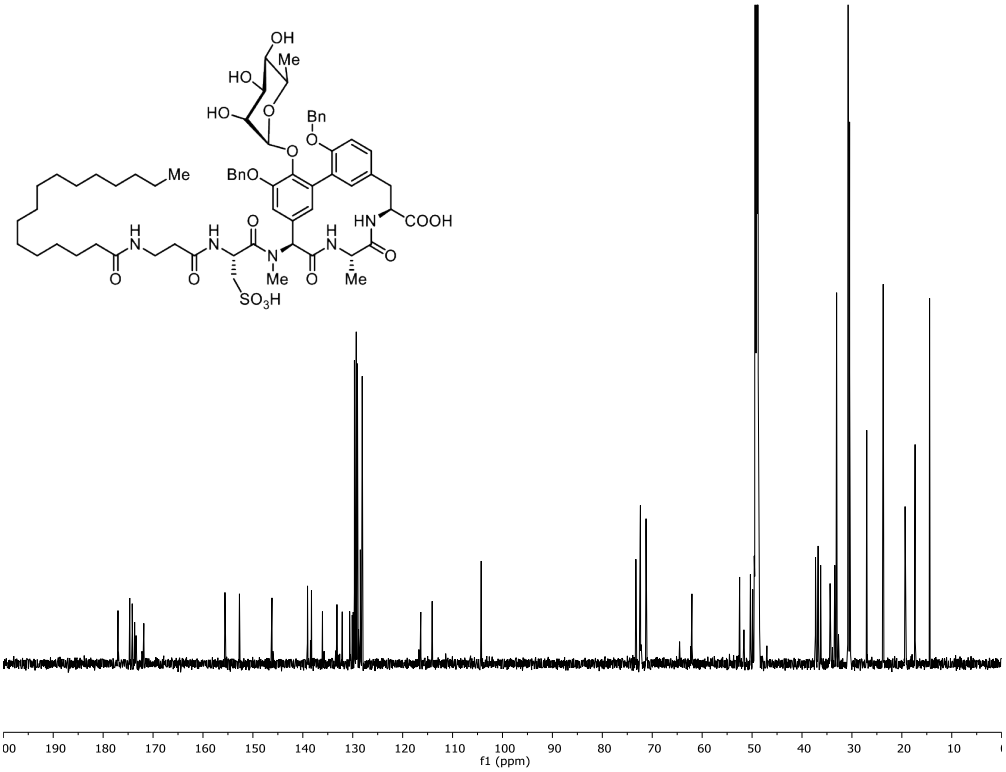
Compound 23 HSQC NMR (Methanol-d_4)



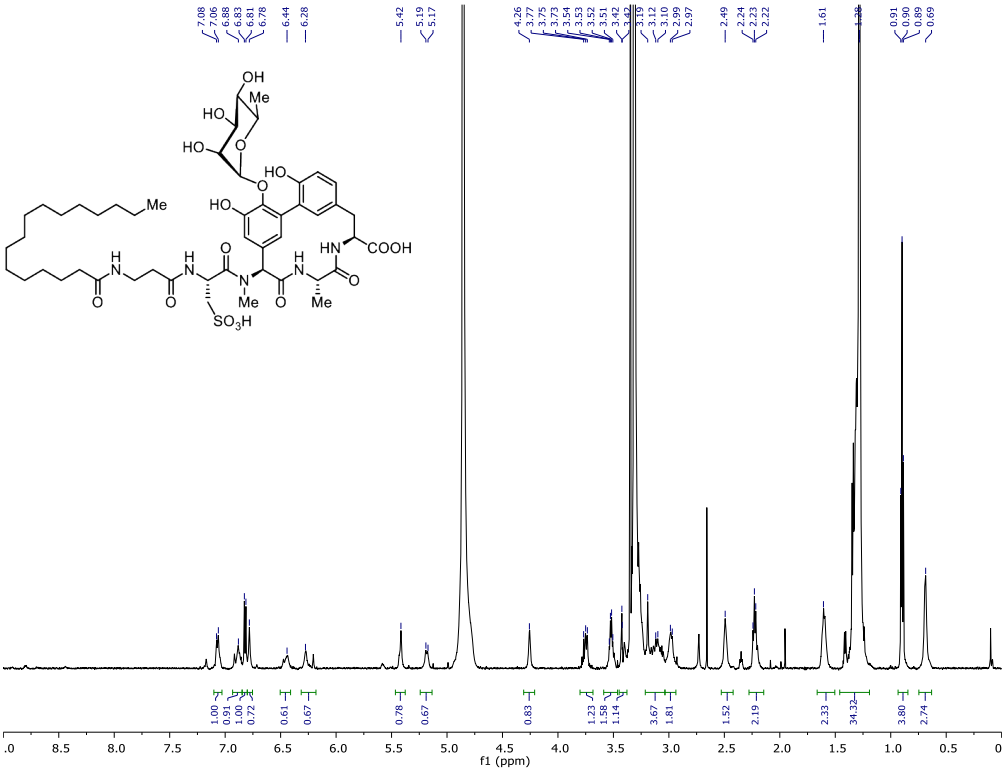
Compound S9 ^{1}H NMR (Methanol-d_4, 600 MHz)



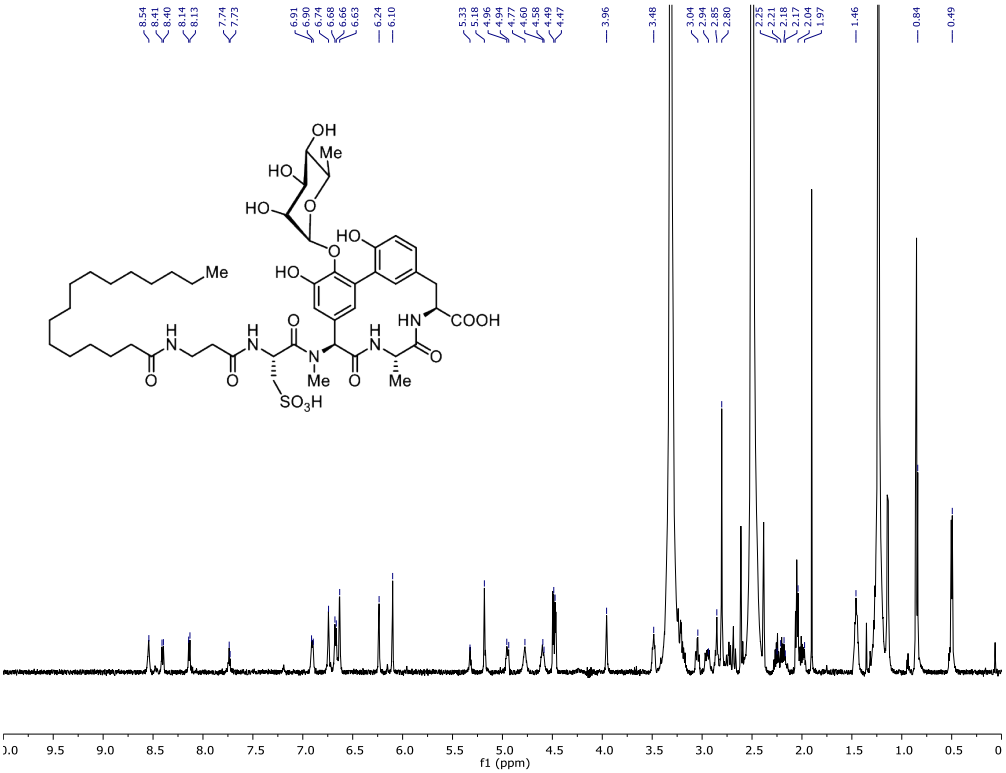
Compound S9 ^{13}C NMR (Methanol-d_4, 151 MHz)



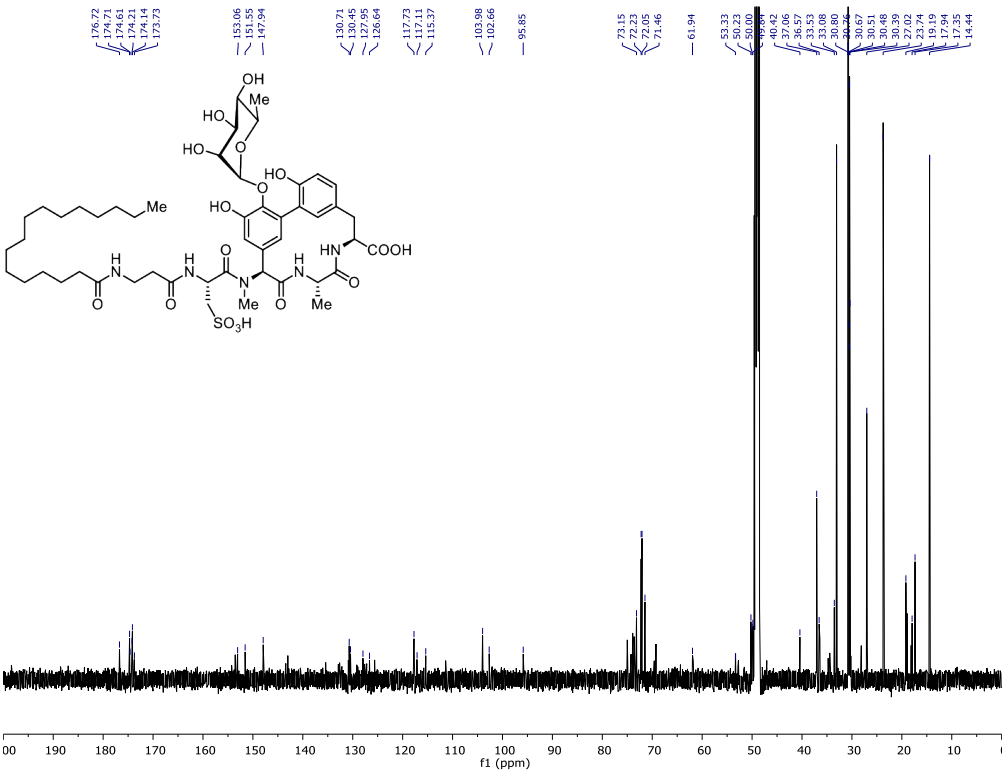
Compound 24 ^{1}H NMR (Methanol-d_4, 600 MHz)



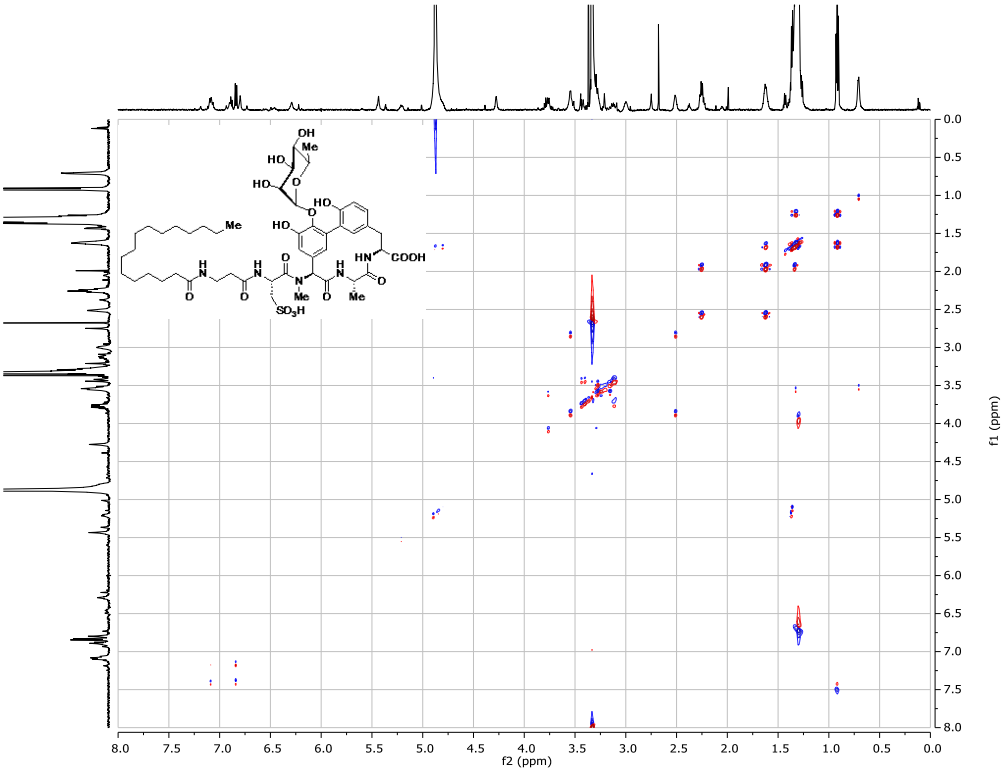
Compound 24 ^{1}H NMR (DMSO-d_6, 600 MHz)



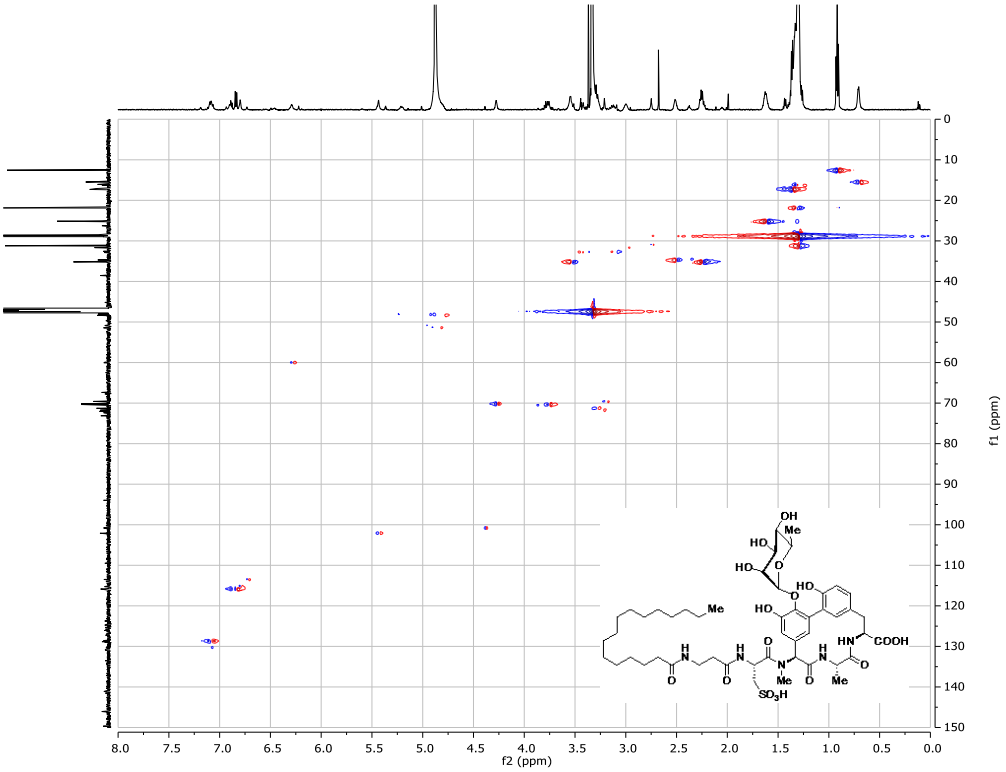
Compound 24 ^{13}C NMR (Methanol-d_4, 151 MHz)



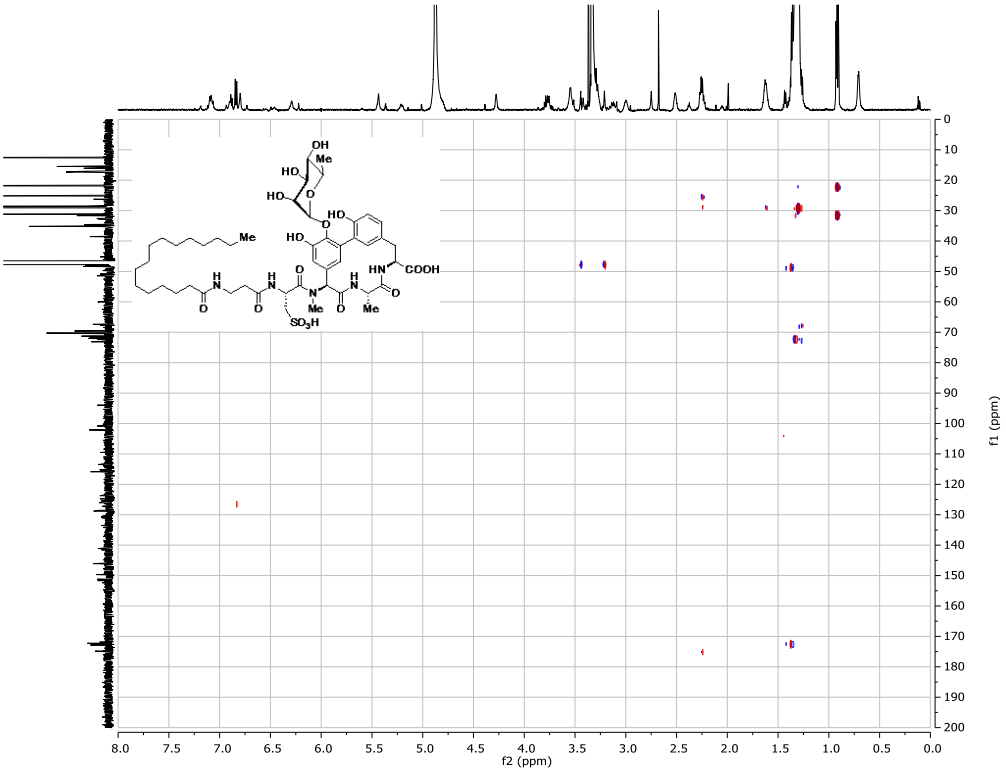
Compound 24 COSY NMR (Methanol-d_4)



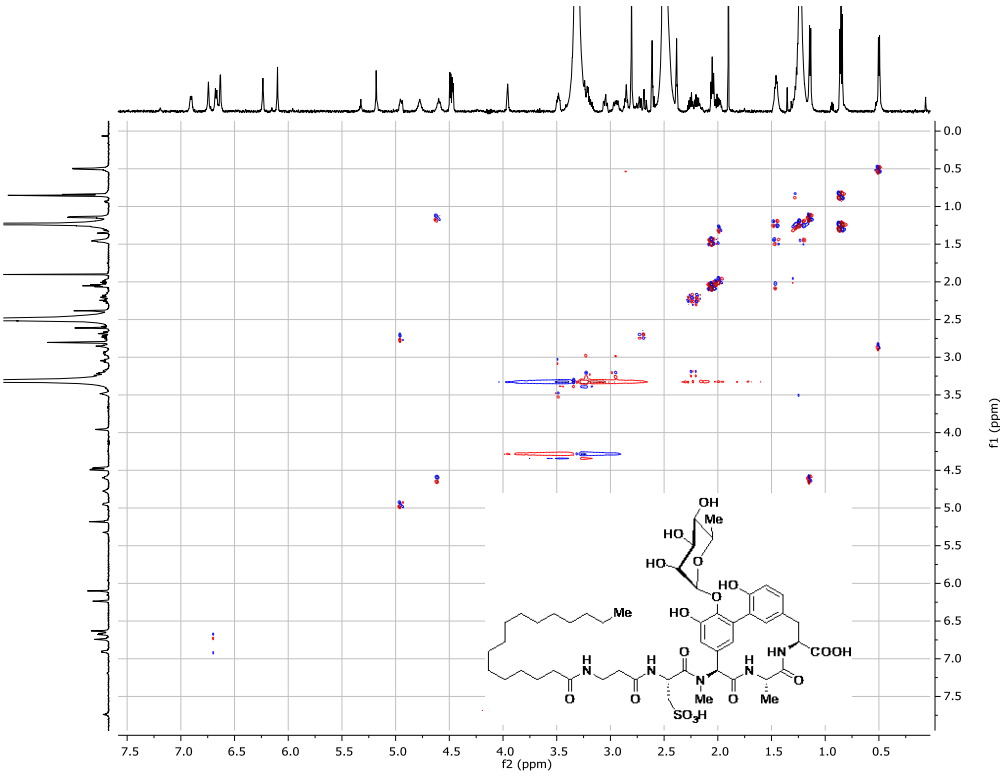
Compound 24 HSQC NMR (Methanol-d_4)



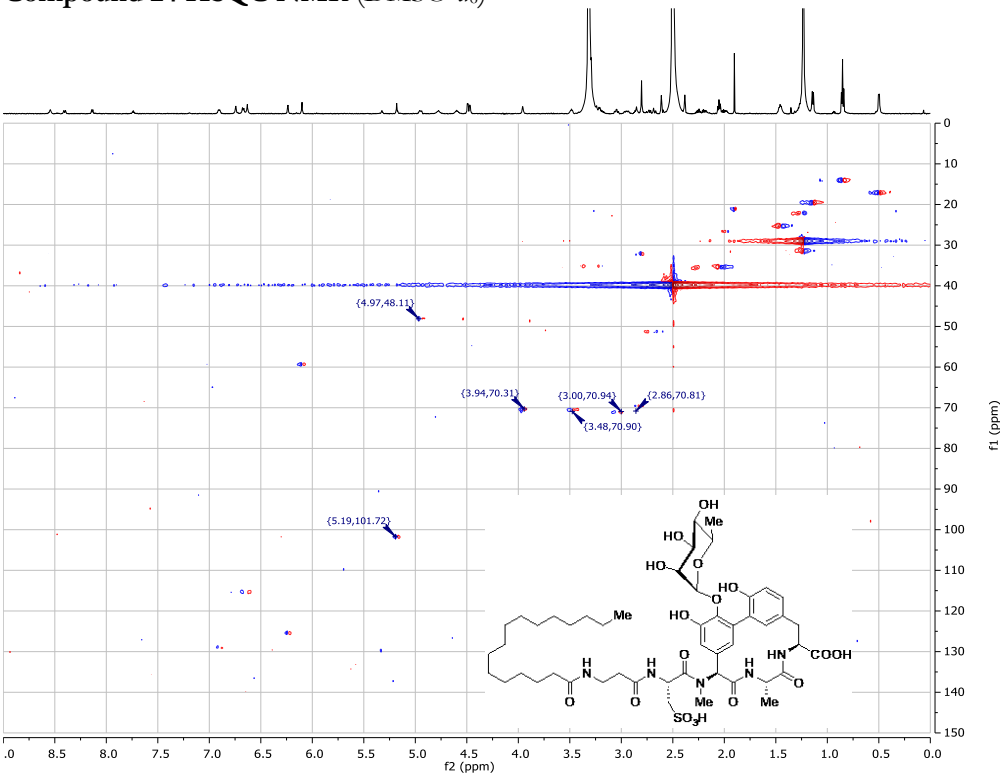
Compound 24 HMBC NMR (Methanol-d_4)



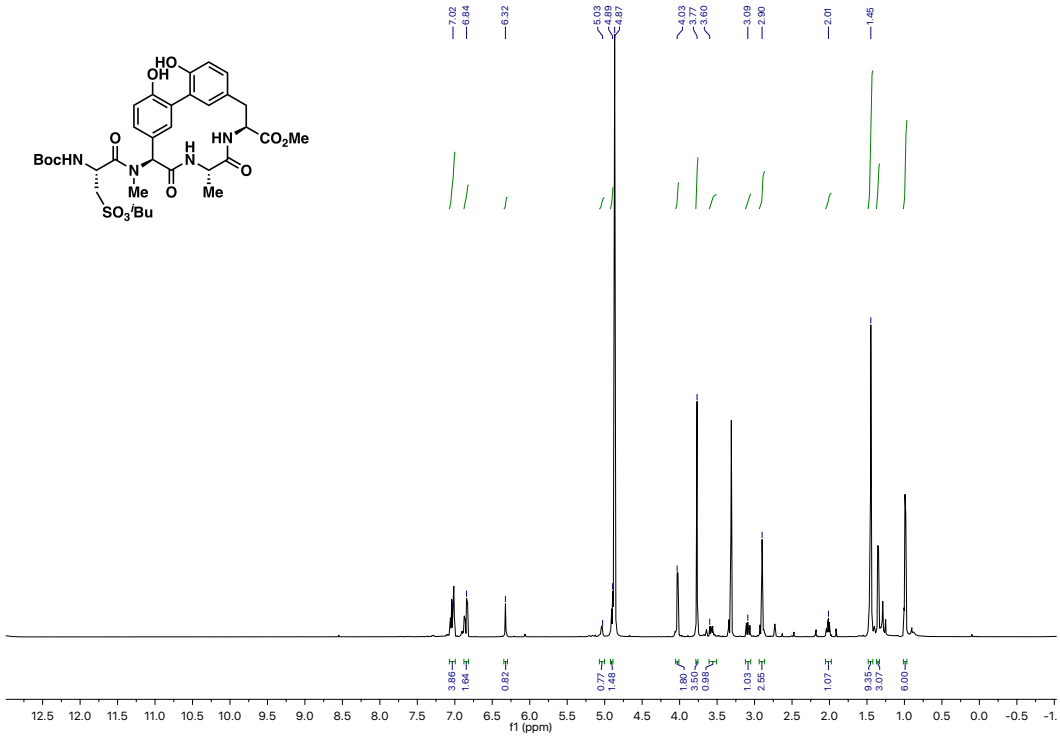
Compound 24 COSY NMR (DMSO-d_6)



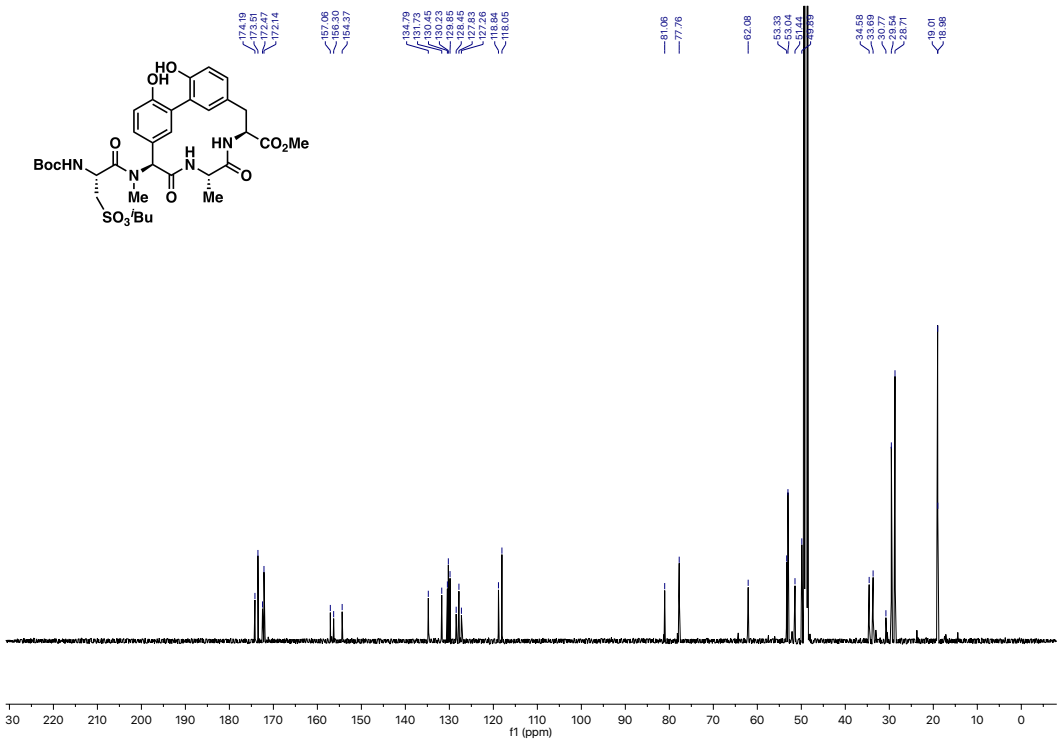
Compound 24 HSQC NMR (DMSO-d_6)



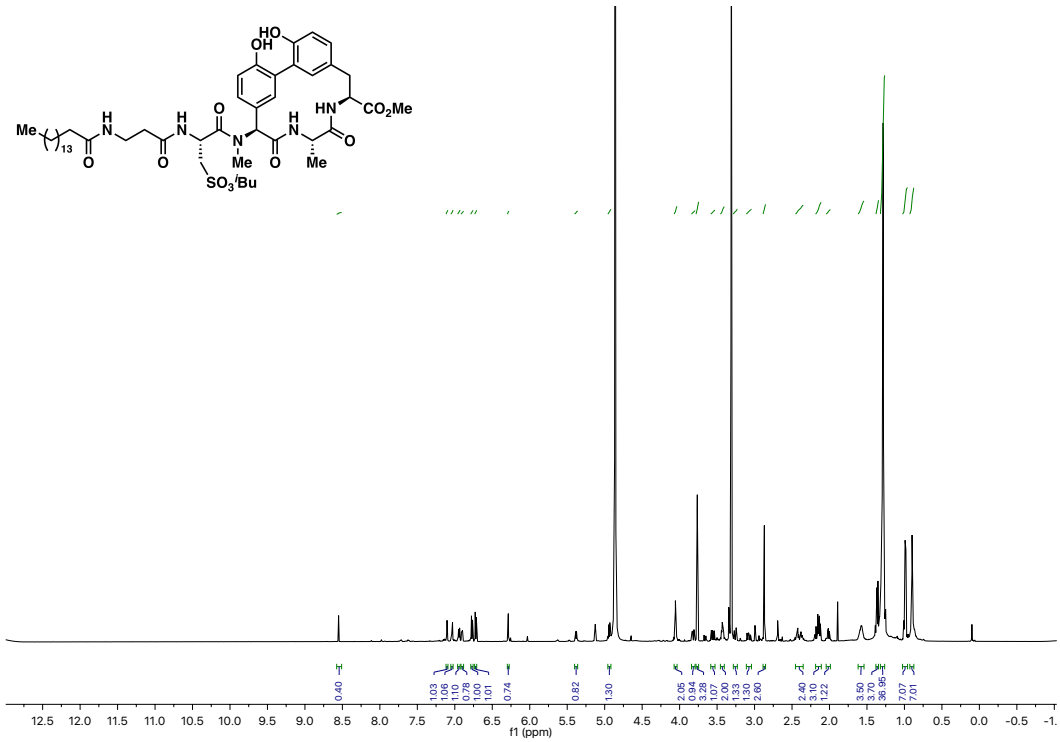
Compound S10 ^{1}H NMR (Methanol-d_4, 600 MHz)



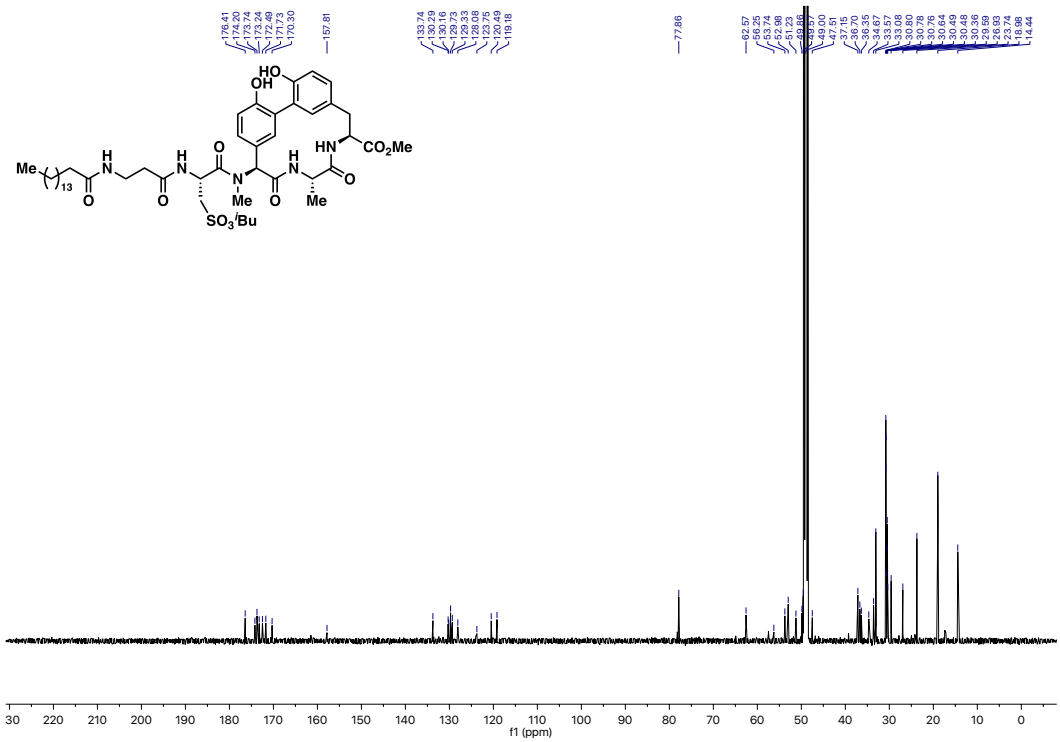
Compound S10 ^{13}C NMR (Methanol-d_4, 151 MHz)



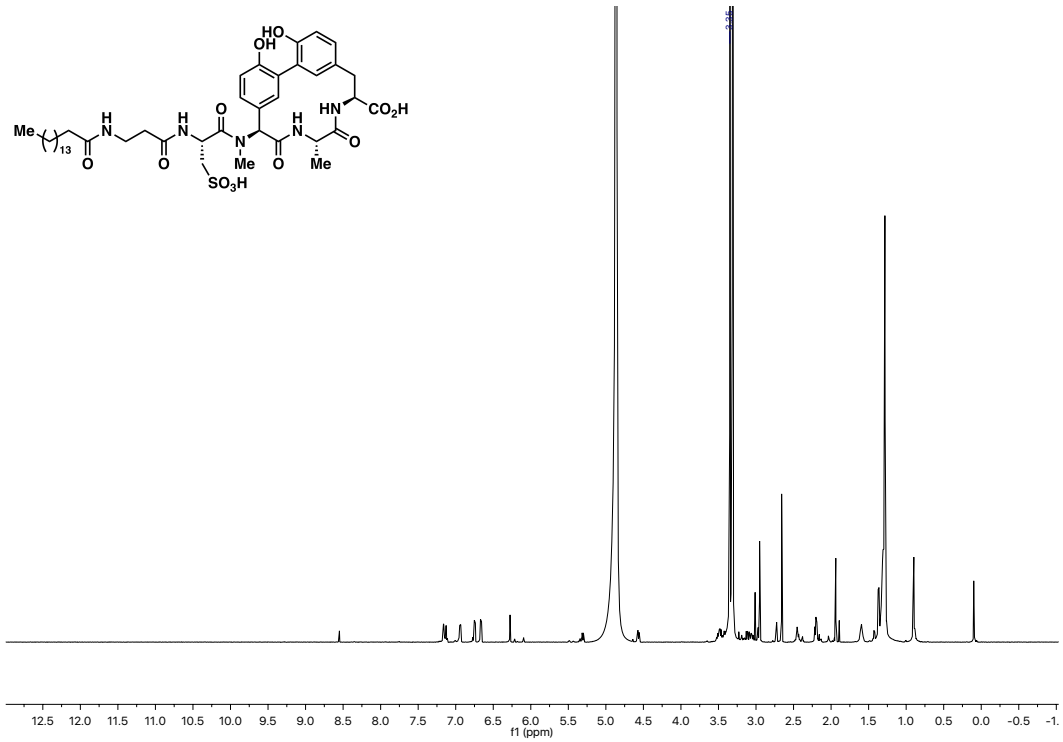
Compound 26 ^{1}H NMR (Methanol-d_4, 600 MHz)



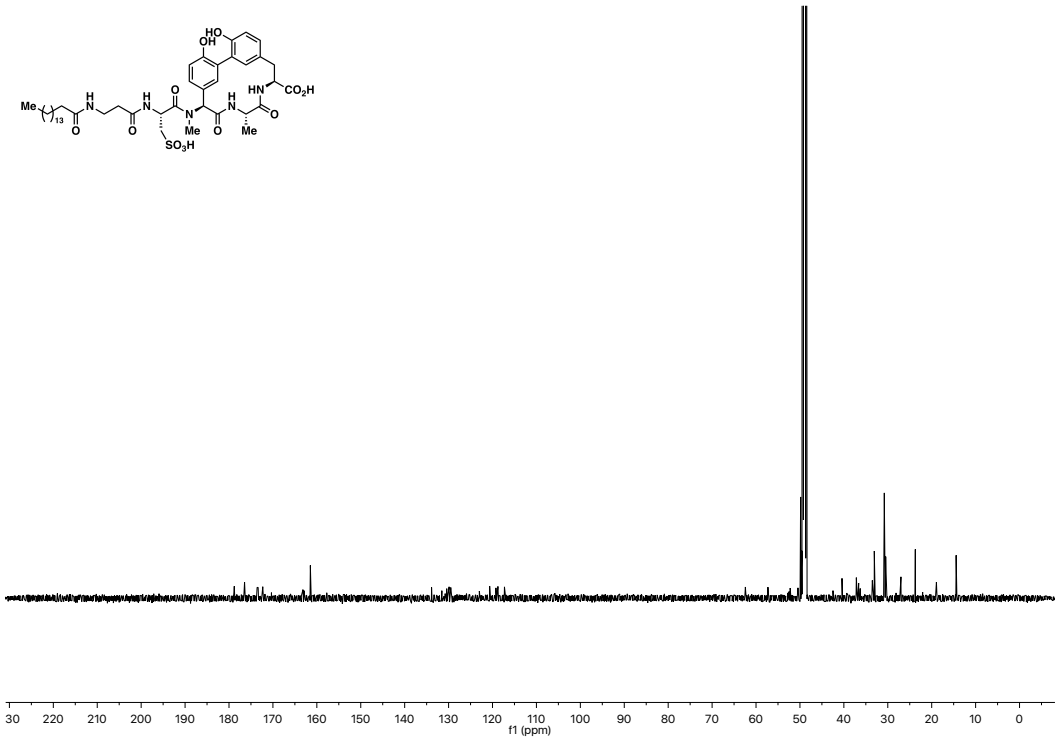
Compound 26 ^{13}C NMR (Methanol-d_4, 151 MHz)



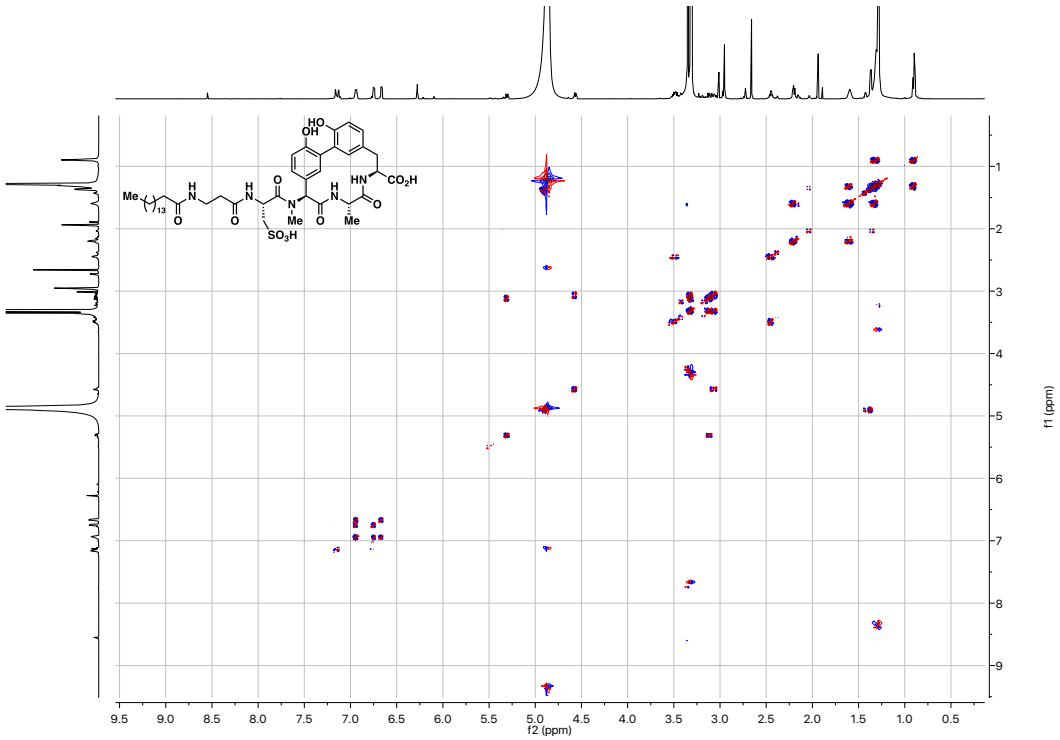
Compound 27 ^{1}H NMR (Methanol-d_4, 600 MHz)



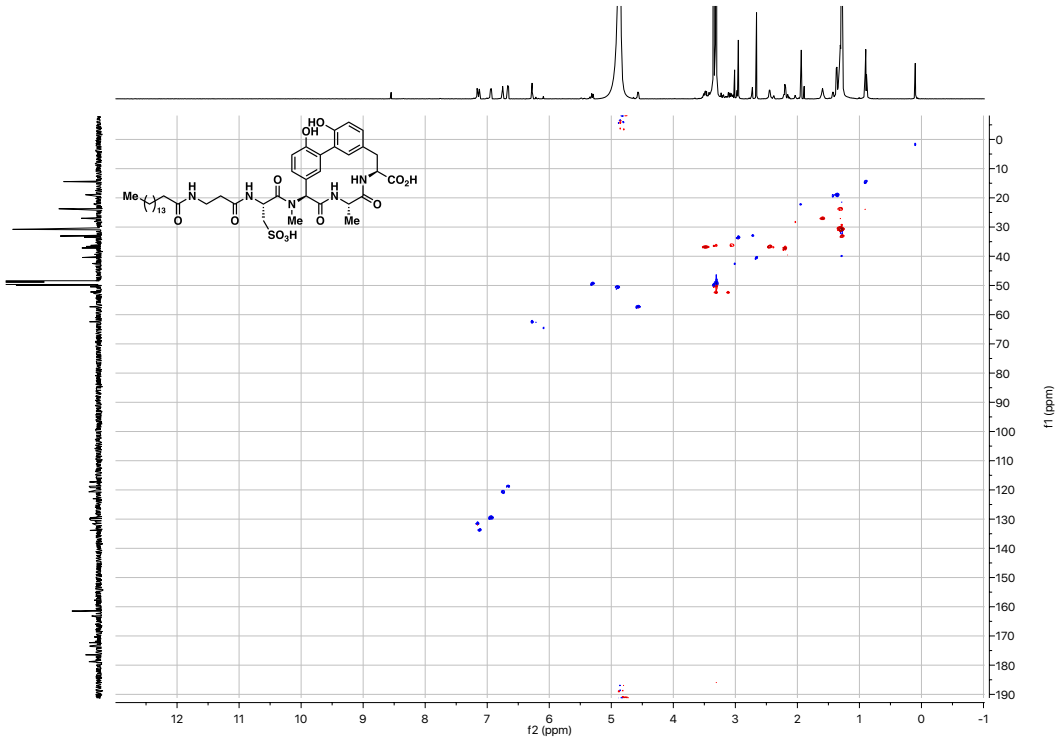
Compound 27 ^{13}C NMR (Methanol-d_4, 151 MHz)



Compound 27 COSY NMR (Methanol-d_4)



Compound 27 HSQC NMR (Methanol-d_4)



Compound 27 HMBC NMR (Methanol-d_4)