Synthesis of mannosidase-stable Man_3 and Man_4 glycans containing S-linked Manα1→2Man termini

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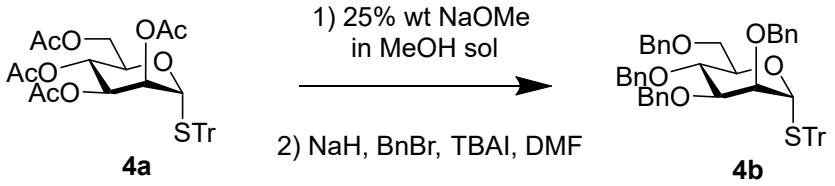
Table of Contents

General Experimental Methods

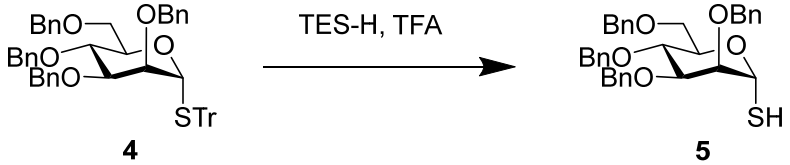
Reactions were carried out in oven-dried glassware under a nitrogen atmosphere and were stirred magnetically. All reagents were purchased from Sigma-Aldrich, Acros Organics, Fluka, Alfa Aesar or TCI, and used without further purification unless otherwise noted. Molecular sieves were crushed and freshly activated prior to use by heating on gas flame in quartz round bottom flask under < 1 Torr on a vacuum manifold attached to a Welch 1400 vacuum pump. The word "concentrated" refers to removal of solvents by means of rotary evaporator attached to a Welch 1400 oil pump (bled to 5-300 mm Hg as needed) followed by removal of residual solvents at < 1 Torr on a vacuum manifold attached to a Welch 1400 vacuum pump. SiliaFlash® F60 (230-400 mesh) from Silicycle® was used for flash column chromatography unless specifically indicated. Analytical thin layer chromatography (TLC) was performed using silica gel F-354 pre-coated glass plates (0.25 mm). TLC plates were analyzed by short wave UV illumination or dipping in cerium -ammonium-molybdate (CAM) stain (40g of ammonium pentamolybdate, 1.6g of Cerium (IV) sulfate, 800 mL of diluted sulfuric acid (1:9, with water, v/v) and heating on a hot plate. Tetrahydrofuran (THF), dichloromethane (DCM) and toluene, were purified by degassing with argon and passage through activated alumina columns. ^{1}H, ^{13}C, ^{19}F and HSQC NMR were recorded on a Varian Innova 400 MHz and Bruker 800 MHz in deuterated solvents at ambient temperature unless otherwise noted. Spectra acquired in $CDCl_3$ were internally referenced to TMS (0 ppm) or residual $CDCl_3$ (7.26 for ^{1}H and 77.06 for ^{13}C). ^{19}F Fluorine spectra were referenced to trifluorotoluene (-63.72 ppm), internally or by substituting a tube containing standard. Spectra acquired in D_2O were referenced to sodium 3-(trimethylsilyl) propane sulfonate by the substitution method, or referenced to the HDO peak using temperature-specific values. Chemical shifts are reported in parts per million (ppm). Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplate), app (apparent) and br (broad). High Resolution mass spectra (HRMS) were performed by Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign using electron impact (EI) or electrospray (ESI) ionization and a TOF analyzer. LC/MS analysis was performed at Brandeis University on a Waters Acquity UPLC chromatograph with reverse phase C18 and C4 column, and Waters Photodiode Array and Micro mass ZQ4000 mass detectors. IR spectra were recorded on a Varian 640-IR FT-IR spectrometer and are reported in wavenumbers (cm^{-1}). Optical rotation was measured on JASCO-polarimeter.

Experimental Section



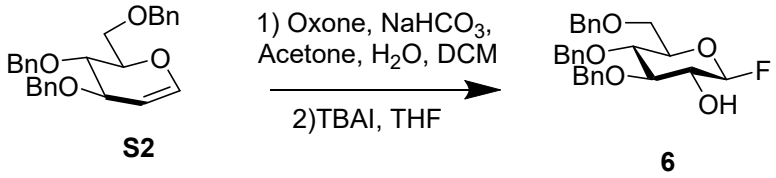
Triphenylmethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-mannopyranoside (4b)

NaOMe in methanol (25% wt; 565 µL; 2.47 mmol; 0.3 equiv.) was added to a solution of mannoside **4a**[1] (5 g; 8.24 mmol; 1 equiv.) dissolved in dry dichloromethane and methanol (1:1; 50 mL) at rt with stirring for 4h. The solution was adjusted to pH 6-7 with Amberlite IR-120 resin (H^+ form), filtered, and the filtrate evaporated to dryness under reduced pressure to give the tetraol, which was coevaporated with toluene two times and used without purification for next step. Crude tetraol (3.6 g; 8.21 mmol; 1 equiv.) was dissolved in dry DMF (30 ml) and cooled in ice bath. Sodium hydride (1.97 g; 49.25 mmol; 6 equiv; 60%) was added to reaction mixture and stirred for 30 minutes at 0 °C, followed by dropwise addition of benzyl bromide (5.85 mL; 49.25 mmol; 6 equiv.) and TBAI (2.1 g; 2.21 mmol; 1 equiv.). The reaction mixture was stirred for 6 hours at rt and quenched with 2 mL of methanol at 0 °C. The reaction mixture was then diluted with EtOAc and washed with brine, then dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude was purified by column chromatography (1:5; EtOAc: hexane) to give compound **4b** as a yellow syrup (4.4 g; 5.58 mmol) in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.10 (multiple signals, 35H + residual $CHCl_3$), 4.87 (d, J = 10.8 Hz, 1H), 4.83 (d, J = 1.6 Hz, 1H), 4.69 (d, J = 12.1 Hz, 1H), 4.58 – 4.41 (m, 4H), 4.25 – 4.17 (m, 1H), 4.15 (d, J = 12.6 Hz, 1H), 3.99 (t, J = 9.6 Hz, 1H), 3.89 – 3.70 (m, 4H), 3.61 (app. t, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, Selected signals): δ 144.7, 138.6, 138.4, 138.3, 138.2, 130.1, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8, 127.6, 127.5, 127.4, 126.9, 81.8, 80.4, 78.1, 75.1, 74.6, 74.5, 71.9, 70.6, 69.8, 69.3. $[\alpha]_D^{25}$: +76.1 (c1.0, $CHCl_3$). IR (cm^{-1}):3028, 2863, 1949, 1451, 1087. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for $C_{53}H_{50}O_5SNa$: 821.3277, found 821.3294.



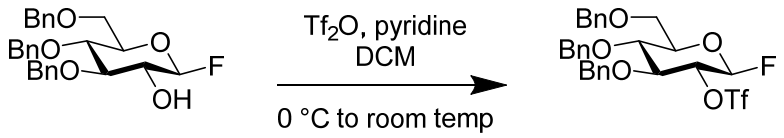
2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl thiol (5)

Triethyl silane (1.5 mL; 9.39 mmol; 5 equiv.) and TFA (3 mL, 30.04 mmol; 16 equiv.) were added to a solution of mannosyl trityl thiol **4** (1.5g; 1.88 mmol; 1 equiv.) dissolved in dry dichloromethane (30 mL) under a N_2 atm. After 30 minutes the reaction had reached completion and the solvents were evaporated followed by co-evaporation with toluene and kept for high vacuum for 3 hours to afford crude of compound **5** as yellow oil, which was used in next steps without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 6.76 (multiple signals, 20H + residual $CHCl_3$+ triphenylmethane), 5.66 (dd, *J* = 7.0, 1.8 Hz, 1H), 4.87 (d, *J* = 10.7 Hz, 1H), 4.71 (d, *J* = 12.5 Hz, 1H), 4.67 – 4.45 (multiple signals, 6H), 4.11 (ddd, *J* = 9.4, 5.0, 1.8 Hz, 1H), 4.01 (app t, *J* = 9.4 Hz, 1H), 3.86 (dd, *J* = 9.4, 3.0 Hz, 1H), 3.81 (dd, *J* = 3.0, 1.8 Hz, 1H), 1H), 3.68 (dd, *J* = 10.8, 1.8 Hz, 1H), 2.02 (d, *J* = 7.0 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$, Selected Signals) δ 138.2, 138.1, 138.0, 137.8, 128.3, 128.3, 128.3, 128.2, 128.2, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 79.0, 75.1, 76.2, 74.8, 73.3, 72.5, 72.1, 72.0, 68.9, 67.9, 56.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{34}H_{36}O_5SNa$: 579.2181, found 579.2173.



3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl fluoride (6)

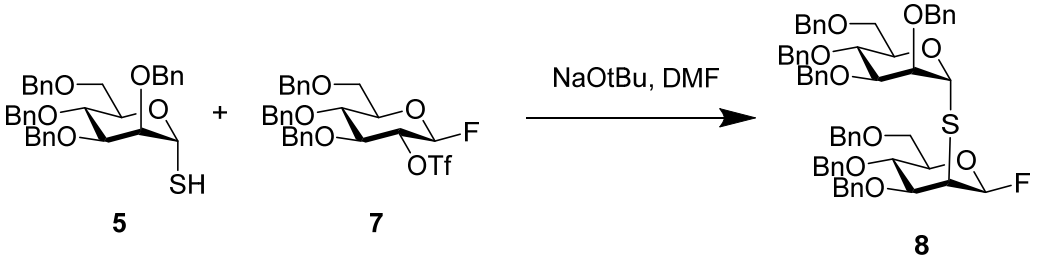
To a vigorously stirred, cooled biphasic solution of 3,4,6-tri-*O*-benzyl-D-glucal **S2**[2] (6 g, 14.42 mmol; 1 equiv.) in dichloromethane (60 mL), acetone (6 mL) and sat. aq. $NaHCO_3$ (100 mL), a solution of oxone (17.74 g; 57.68 mmol; 4 equiv.) dissolved in water (36 mL) was added dropwise over 20 minutes. The reaction mixture was stirred vigorously for 2 hours at room temperature. The organic layer was separated, and aqueous layer was extracted twice with 140 mL of dichloromethane. The combined organic layers were dried by $MgSO_4$ and concentrated under reduced pressure to afford white solid 1,2-anhydro-3,4,6-tri-*O*-benzyl-α/β-D-glucopyranose, which was used for next reaction without purification and dissolved in anhydrous THF (60 mL) and cooled to 0 °C. 1M Tetrabutylammonium fluoride in THF[3] (28.2 mL; 28.21 mmol; 2 equiv.) was added dropwise and stirred for 6 hours. The reaction mixture was extracted with dichloromethane 150 mL two times and combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (1:4; EtOAc: hexane) to afford compound **6** (1.8 g; 8.32 mmol) as yellow amorphous solid in 29% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.12 (multiple signals, 15H + residual $CHCl_3$), 5.14 (dd, $^1J_{FH}$ = 53.1, J_{H1H2} = 6.4 Hz, 1H), 4.81 (app s, 2H), 4.76 (d, *J* = 10.9 Hz, 1H), 4.59 (d, *J* = 12.1 Hz, 1H), 4.56 (d, *J* = ~11 Hz, 1H), 4.53 (d, *J* = 12.1 Hz, 1H), 3.80 – 3.63 (m, 5H), 3.58 (app t, *J* = 8.2 Hz, 1H), 2.56 (d, *J* = 3.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.1, 137.8, 137.6, 128.5, 128.4, 128.4, (127.92, 127.91, 127.84, 127.82, 127.71, 6 resonances with an overlap), 109.0 (C-F, d, $^1J_{CF}$ = 215.1Hz), 81.1 (C-F d, $^3J_{CF}$ = 9.3Hz), 76.3, 74.86, 74.82, 74.5, 73.5, 73.0 (C-F, d, $^2J_{CF}$ = 23.7Hz), 68.48. ^{19}F NMR (376 MHz, $CDCl_3$) δ -140.13 (dd, J_{HF} = 53.2, 11.7). $[\alpha]_D^{25}$: +59.76 (c1.0, $CHCl_3$). IR (cm^{-1}):3431.3, 2872.1, 2362.2, 2019.1, 1736.4, 1080.1, 1027.7. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{27}H_{29}FO_5Na$: 475.1897, found 475.1898.



6 **7**

3,4,6-tri-*O*-benzyl-2-*O*-trifluoromethylsulfonyl-β-D-glucopyranosyl fluoride (7)

To a solution of compound **6** (1 g; 2.21 mmol; 1 equiv.) and pyridine (2.19 mL, 22.1 mmol, 10 equiv.) in 15 mL of CH_2Cl_2 was added Tf_2O (408 µL; 2.43 mmol; 1.1 equiv.) at 0 °C under N_2 atm. After 0.5 hour at 0 °C and 1 h at ambient temperature, the solution was diluted with 30 mL of CH_2Cl_2 and washed with ice-cold sat aq. $NaHCO_3$, ice-cold solution of $CuSO_4$, and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated in vacuo to afford 1.21 g (2.06 mmol; 93%) of compound **7** as yellow liquid which was used for next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.12 (multiple signals, 15H + residual $CHCl_3$), 5.301 (dd, $^1J_{FH}$ = 52.4 Hz, J_{H1H2} = 7.0 Hz, 1H), 4.87 – 4.70 (multiple signals, 4H), 4.60 – 4.48 (multiple signals, 3H), 3.84 (app t, J = 9.1 Hz, 1H), 3.77 (app t, J = 9.1 Hz, 1H), 3.739 – 3.668 (m, 2H), 3.61 (ddd, J = 9.4 Hz, 2.9 Hz, 2.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.27, 136.98, 136.56, (128.31, 128.26, 127.89, 127.67, 127.65, 127.62; 9 resonances with overlaps), 118.17 (C-F, q, $^1J_{CF}$ = 319.54Hz), 105.15 (C-F, d, $^1J_{CF}$ = 219.07 Hz), 83.90 (C-F, d, $^2J_{CF}$ = 24.7 Hz), 80.32 (C-F, d, $^3J_{CF}$ = 8.4Hz), 75.56, 75.14 (C-F, d, long range J_{CF} = 4.3 Hz), 74.89, 73.40, 67.38. ^{19}F NMR (376 MHz, $CDCl_3$) δ -74.38 (d, $^6J_{FF}$ = 9.7 Hz), -140.11 (ddq, $^1J_{HF}$ = 52.4, $^2J_{HF}$ = 9.7 Hz, $^6J_{FF}$ = 9.7 Hz). LRMS (ESI-single quad) m/z: $[M+NH_4]^+$: Calcd. for $C_{28}H_{32}F_4O_7NS$: 602.1836, Found: 602.15 (this compound was too unstable to send for HRMS).

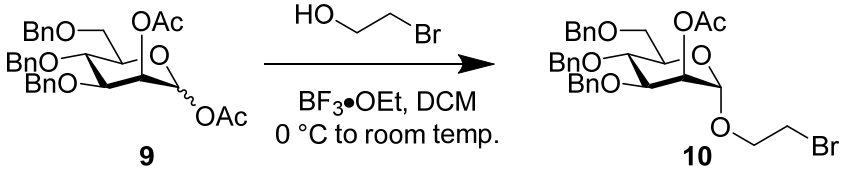


5 **7** **8**

3,4,6-tri-*O*-benzyl-2-*S*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→2))-β-D-mannopyranosyl fluoride (8)

To a solution of freshly prepared crude compound **5** and compound **7** (1.21 g; 2.07 mmol; 1.1 equiv.) in 20 mL of dry DMF was added NaO^{t}Bu (181 mg; 1.89 mmol; 1 equiv.) under N_2 atm. After 30 min, the reaction mixture was diluted with 50 mL of EtOAc and washed with ice-cold brine solution. Combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure maintaining water bath temperature 30 °C. The crude was purified by column chromatography by using triethyl amine quenched silica gel (1:5; EtOAc: hexane) to afford compound **8** (1.18 g; 1.19 mmol) as yellow amorphous solid in 63% yield. ^{1}H NMR (400 MHz,

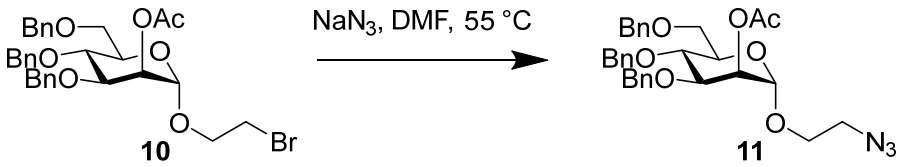
$CDCl_3$) δ 7.33 – 7.16 (multiple signals, 35H + residual $CHCl_3$), 5.48 (dd, $J_{F,H}$ = 53.15 Hz, $J_{H,H}$ = 2.2 Hz, 1H), 5.50 (s, 1H), 4.88 (d, J = 11.0 Hz, 1H), 4.72-4.63 (multiple signals, 2H), 4.62 – 4.43 (multiple signals, 12H), 4.27 (dd, J = 9.6, 4.1 Hz, 1H), 4.03 (app t, J = 9.5 Hz, 1H), 3.92 (dd, J = 9.3, 3.1 Hz, 1H), 3.88 – 3.79 (multiple signals, 5H), 3.77 – 3.65 (multiple signals, 3H), 3.60 (app dt, J = 16.8, 2-3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.3, 138.2, 138.1, 138.0, 137.7, 137.3, 128.41 (2C), 128.38 (2C), 128.29 (2C), 128.26 (2C), 128.21 (2C), 128.19 (4C), 127.86 (2C), 127.85, 127.84, 127.79 (2C), 127.69 (2C), 127.67 (2C), 127.66 (4C), 127.56 (2C), 127.54, 127.52, 127.49, 127.4, 127.3, 106.1 (C-F, d, $^1J_{CF}$ = 221.9 Hz), 82.1, 80.2, 77.6 (C-F, d, $^3J_{CF}$ = 3.32 Hz), 76.2, 74.9 (2C), 74.2, 73.31, 73.30, 73.1, (72.7, 72.5, 72.2, 71.9, 71.8, 6C with 2 overlapping signals), 69.4, 69.0, 46.79 (C-F, d, $^2J_{CF}$ = 22.10 Hz). ^{19}F NMR (376 MHz, $CDCl_3$) δ -129.83 (dd, J = 53, 16.8 Hz). $[\alpha]_D^{25}$: +12.65 (c1.0, $CHCl_3$). IR (cm^{-1}): 3029, 2866, 2251, 1973, 1363, 1087. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{61}H_{63}FO_9SNa$: 1013.4075, found 1013.4073.



9 **10**

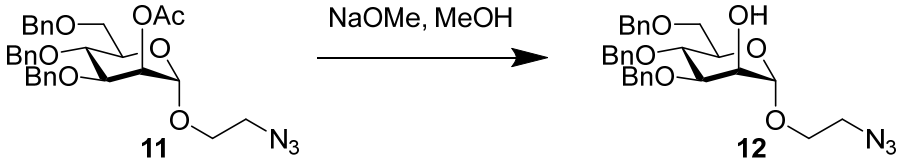
2-Bromoethyl-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (10)

A solution of 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranose **9**[4](1.1g; 2.06 mmol; 1 equiv.) and bromoethanol (365 µL; 5.14 mmol; 2.5 equiv.) in dry dichloromethane (11 mL) was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (1.41 mL; 10.29 mmol; 5 equiv.) was added dropwise. The ice bath was allowed to melt and warm to room temperature as the stirred overnight. The reaction mixture was diluted with 40 mL of dichloromethane and washed with cold aq. sat. $NaHCO_3$ and brine solution. The organic layers were dried over $MgSO_4$, concentrated and crude was purified by column chromatography (ethyl acetate: hexane; 3:1) to afford compound **10** (925 mg; 1.54 mmol) as a syrup in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.21 (multiple signals, 13H + residual $CHCl_3$), 7.18 – 7.13 (m, 2H), 5.38 (dd, J = 3.4, 1.8 Hz, 1H), 4.89 (d, J = 1.8 Hz, 1H), 4.86 (d, J = 10.8 Hz, 1H), 4.71 (d, J = 11.2 Hz, 1H), 4.67 (d, J = 12.4 Hz, 1H), 4.55 (d, J = 11.2 Hz, 1H), 4.51 (d, J = 12.4 Hz, 1H), 4.47 (d, J = 10.8 Hz, 1H),, 4.02 – 3.91 (m, 2H), 3.91 – 3.84 (m, 2H), 3.84 – 3.74 (m, 2H), 3.71 (d, J = 10.7 Hz, 1H), 3.47 (t, J = 6.1 Hz, 2H), 2.15 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ (carbonyl C not observed), 138.3, 138.1, 137.9, 128.4 (2C), 128.3 (4C), 128.1 (2C), 127.9 (2C), 127.74 (2C), 127.73 127.63, 127.58, 98.0, 78.1, 75.2, 74.2, 73.4, 71.9, 71.8, 68.8, 68.6, 67.9, 29.9, 21.1. $[\alpha]_D^{25}$: +3.2 (c1.0, $CHCl_3$). IR (cm^{-1}): 2915, 2015, 1745, 1237, 1095. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{31}H_{35}BrO_7Na$: 621.1464, found 621.1453.



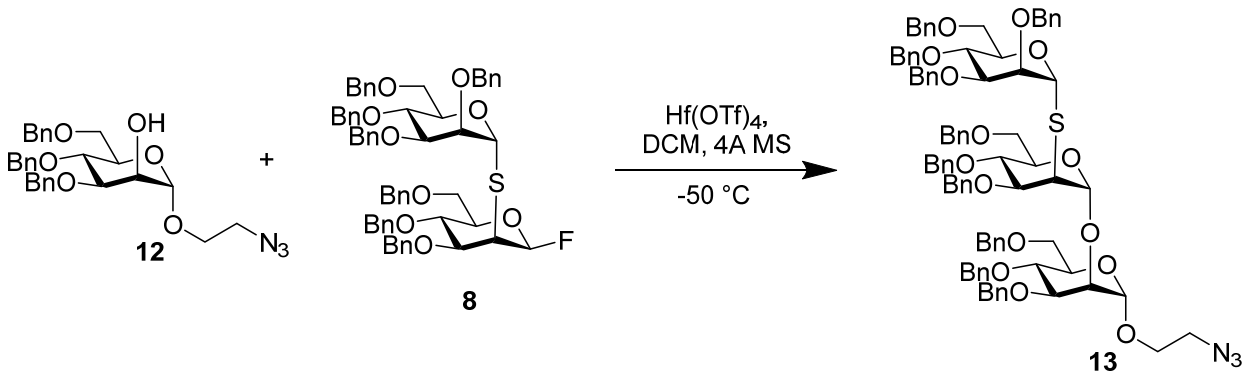
2-azidoethyl-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (11)

A solution of the compound **10** (910 mg; 1.52 mmol; 1 equiv.) and sodium azide (493 mg; 7.59 mmol; 5 equiv.) in dry DMF (25 mL) was stirred at 55 °C for 6 hours. After completion of the reaction, solvent was co-evaporated with toluene under reduced pressure, and the residue was dissolved in the mixture of water and ethyl acetate and extracted with ethyl acetate. The organic layers were dried over $MgSO_4$, concentrated and residue was purified by column chromatography (ethyl acetate and hexane; 2:1) to afford compound **11** as colorless syrup (826 mg; 1.47 mmol) in 97% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (multiple signals + residual $CHCl_3$, 13H), 7.15 (dd, *J* = 7.3, 2.2 Hz, 2H), 5.39 (app t, *J* = 1.9 Hz, 1H), 4.89 (d, *J* = 1.8 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.68 (dd, *J* = 11.6, 9.6 Hz, 2H), 4.01 (dd, *J* = 8.9, 3.3 Hz, 1H), 3.94 – 3.75 (m, 4H), 3.71 (dd, *J* = 10.4, 1.5 Hz, 1H), 3.65 – 3.57 (m, 1H), 3.44 – 3.31 (m, 2H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 138.3, 138.1, 137.8, 128.4, 128.3, 128.3, 128.1, 127.8, 127.7, 98.0, 78.0, 75.1, 74.1, 73.4, 71.9, 71.7, 68.8, 68.6, 66.7, 50.4, 21.1. $[\alpha]_D^{25}$: +1.9 (c1.0, $CHCl_3$). IR (cm^{-1}):3030, 2866, 2103, 1743, 1233, 1088. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{31}H_{35}N_3O_7Na$: 584.2373, found 584.2372.



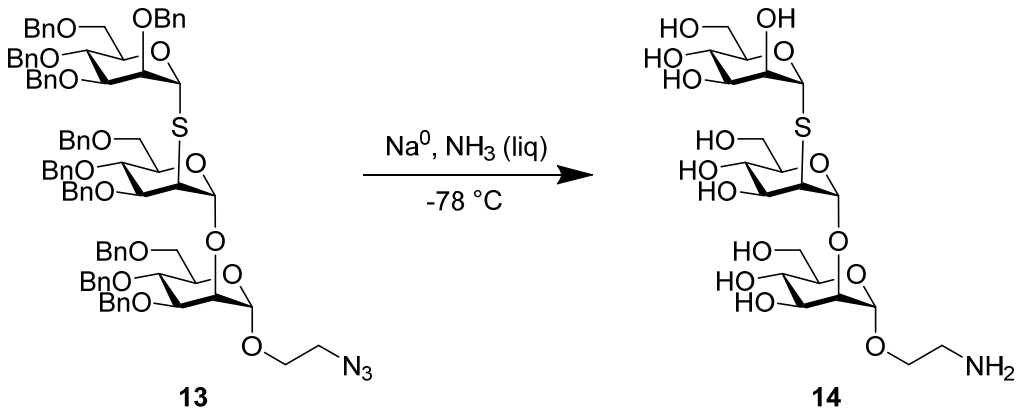
2-azidoethyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (12)

Compound **11** (810 mg; 1.44 mmol; 1 equiv.) was dissolved in methanol (4 mL) and dichloromethane (4 mL) and 65 µL (289 µmol; 0.2 equiv.) 25% sodium methoxide in methanol solution was added and stirred reaction mixture for 2 hours at room temperature. Reaction was neutralized with Amberlite IR-120 H^+ form ion exchange resin. Resin was filtered off and the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography to afford compound **12** (704 mg; 1.36 mmol) as colorless syrup in 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.23 (multiple signals, 13H + residual $CHCl_3$), 7.21–7.14 (m, 2H), 4.94 (d, *J* = 1.7 Hz, 1H), 4.82 (d, *J* = 10.8 Hz, 1H), 4.68 (app s, 2H), 4.63 (d, *J* = 12.2 Hz, 1H), 4.52 (d, *J* = 12.2 Hz, 1H), 4.50 (d, *J* = 10.8 Hz, 1H), 4.05 (br s, 1H), 3.92 – 3.66 (multiple signals, 6H), 3.61 (ddd, *J* = 10.6, 6.7, 3.7 Hz, 1H), 3.44 – 3.27 (m, 2H), 2.54 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 138.2, 137.8, 128.5, 128.4, 128.3, 127.95, 127.93, 127.92, 127.8, 127.7, 127.6, 99.4, 80.0, 75.1, 74.1, 73.5, 72.1, 71.4, 68.9, 68.1, 66.6, 50.5. $[\alpha]_D^{25}$: +22.3 (c1.0, $CHCl_3$). IR (cm^{-1}): 3436, 3030, 2914, 2101, 1960, 1495, 1060. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{29}H_{33}N_3O_7Na$: 542.2267, found 542.2265.



2-azidoethyl-3,4,6-tri-*O*-benzyl-2-*O*-[3,4,6-tri-*O*-benzyl-2-*S*--α-D-mannopyranosyl-(1→2)]-α-D-mannopyranoside (13)

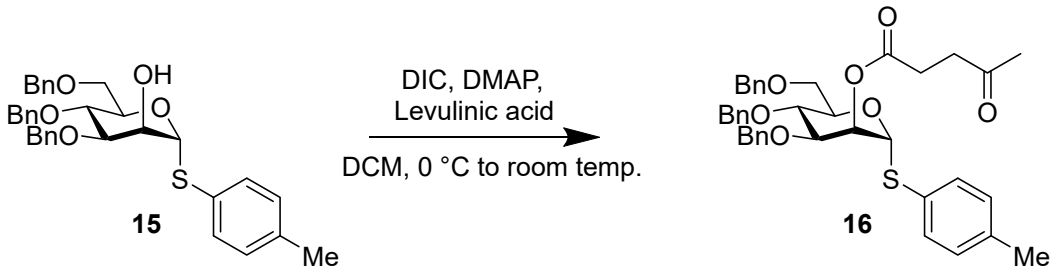
Disaccharide donor **8** (300 mg; 303 µmol; 1 equiv.) and acceptor **12** (235 mg; 454 µmol; 1.5 equiv.) were dissolved in of anhydrous dichloromethane (3 mL), and freshly crushed and flame dried 4 Å molecular sieves were added and stirred for 15 min at room temperature. The mixture was cooled at -50 °C, then $Hf(OTf)_4$ (234 mg; 303 µmol; 1 equiv.) was added and allowed to stir for next 1 h. The reaction was neutralized with a few drops of triethylamine, diluted with CH_2Cl_2, filtered through a celite pad and concentrated. Purification by column chromatography (EtOAc:Hexane, 1:5) afforded trisaccharide **13** as white foam in 64% yield (287 mg, 194 µmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.08 (multiple signals, 50H + residual $CHCl_3$), 5.59 (s, 1H), 5.33 (s, 1H), 4.97 (d, *J* = 1.8 Hz, 1H), 4.94 – 4.80 (multiple signals, 3H), 4.71 – 4.45 (multiple signals, 15H), 4.40-4.32 (multiple signals, 2H), 4.23 (dd, *J* = 9.3, 4.3 Hz, 1H), 4.18 – 4.10 (m, 1H), 4.09 – 4.02 (m, 2H), 4.01 – 3.95 (m, 1H), 3.94 – 3.89 (m, 2H), 3.88 – 3.64 (multiple signals, 11H), 3.59 (dd, *J* = 11.0, 1.7 Hz, 1H), 3.32 (ddd, *J* = 10.5, 6.0, 4.1 Hz, 1H), 3.18 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7, 138.6, 138.50, 138.47, 138.45, 138.39, 138.37, 138.3, 138.1 (two resonances), 128.5, 128.4, 128.38 (multiple resonances), 128.37, 128.35, 128.30 (multiple resonances), 128.26, 128.00, 127.95, 127.93, 127.92, 127.90, 127.83, 127.79, 127.77, 127.72, 127.68, 127.67, 127.66, 127.63, 127.57, 127.55, 127.54, 127.50, 127.44, 127.41, 102.7, 98.9, 83.1, 80.5, 79.4, 78.9, 75.8, 75.7, 75.17, 75.14, 74.96, 74.90, 74.7, 73.5, 73.3, 73.1, 72.45, 72.40, 72.1, 72.00, 71.98, 71.4, 69.6, 69.3, 69.0, 66.4, 50.4, 49.5. $[\alpha]_D^{25}$: +5.1 (c1.0, $CHCl_3$). IR (cm^{-1}): 3035, 2358, 2167, 1981, 1219; HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{90}H_{95}N_3O_{15}SNa$: 1512.6382, found 1512.6356.



13 **14**

2-aminoethyl-[α-D-mannopyranosyl-(1→2)-2-*S*-{α-D-mannopyranosyl-(1→2)}]-α-D-mannopyranoside (14)

Into an oven dried 50 mL 3-necked flask, ~10 mL ammonia was condensed under stream of nitrogen at -78 °C. Next, 23 mg of (1.0 mmol, 100 equiv.) Na° was added, and the bright blue reaction was allowed to stir for 1 hour to ensure that the color stably persisted. 15 mg (10 µmol; 1equiv.) of trisaccharide **13** in 1 mL of dry THF was added by syringe, and the reaction was allowed to stir for 4 hours. When reaction was finished (monitored by direct-infusion LC-MS), solid NH_4Cl was added portionwise until the disappearance of blue color and the reaction mixture was warmed to room temperature. The crude product was desalted on a Biogel P-2 size exclusion gel column followed by lyophilization, affording 3.5 mg (6.2 µmol) of deprotected trisaccharide **14** as a glassy white solid in 62% yield. ^{1}H NMR (400 MHz, D_2O) δ 5.41 (s, 1H), 5.27 (s, 1H), 5.12 (s, 1H), 4.26 (dd, *J* = 9.6, 4.6 Hz, 1H), 4.12 (dd, *J* = 3.4, 1.5 Hz, 1H), 4.06 – 3.59 (m, 17H), 3.51 (t, *J* = 9.7 Hz, 1H), 3.33 – 3.20 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 103.0, 98.1, 86.8, 78.4, 73.4 (multiple resonances), 72.9, 71.3, 70.8, 69.9, 69.0, 67.8, 67.0, 66.8, 63.7, 60.8 (multiple resonances), 52.2, 39.0. $[\alpha]_D^{25}$: -1133.9 (c1.0, H_2O) IR (cm^{-1}): 3422(b), 3016, 2360, 2051, 1625, 1437; HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{20}H_{37}NO_{15}SNa$: 586.1782, found 586.1770.

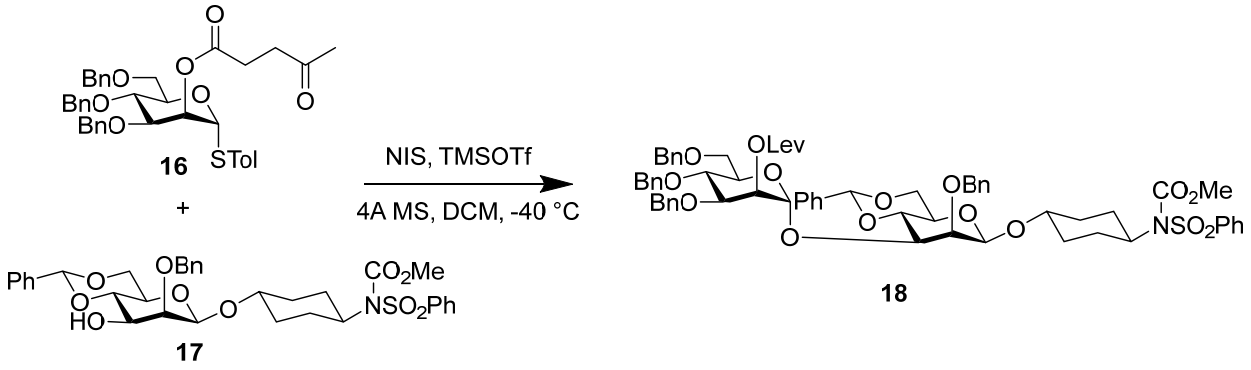


15 **16**

***p*-Tolyl-3,4,6-tri-*O*-benzyl-2-*O*-(4-oxopentanoyl)-1-thio-α-D-mannopyranoside (16)**

To a solution of alcohol **15**[5] (600 mg; 1.077 mmol; 1 equiv.) in anhydrous CH_2Cl_2 (10 mL), DMAP (26 mg; 0.215 mmol; 0.2 equiv.), levulinic acid (0.21 mL; 2.1 mmol; 2 equiv.) and N,N'-diisopropylcarbodiimide (0.33 mL; 2.1 mmol; 2 equiv.) were added sequentially at 0 °C. The reaction mixture was gradually warmed up to ambient temperature and stirred for 5 hours. After

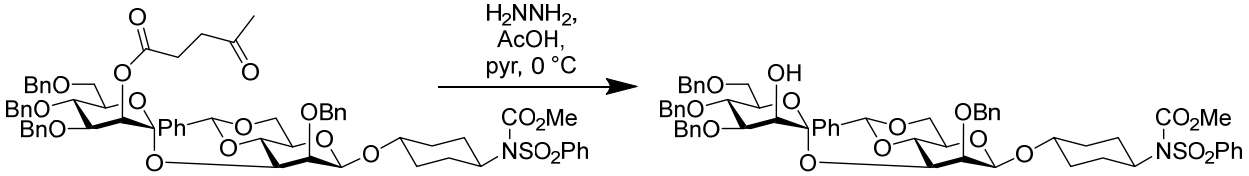
complete consumption of the starting alcohol, the reaction was concentrated in vacuo and purified by flash chromatography (30% ethyl acetate in hexane) to afford corresponding levulinoate ester **16** (648 mg; 0.99 mmol; 92%) as white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.22 (multiple signals, 15H + residual $CHCl_3$), 7.20 (m, 2H), 7.04 (d, *J* = 7.9 Hz, 2H), 5.58 (dd, *J* = 2.8, 1.7 Hz, 1H), 5.44 (d, *J* = 1.7 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.63 (d, *J* = 11.9 Hz, 1H), 4.54 (d, *J* = 11.2 Hz, 1H), 4.52 (d, *J* = 10.8 Hz, 1H), 4.46 (d, *J* = 11.9 Hz, 1H), 4.34 (m, 1H), 3.94 (dd, *J* = 10.7, 2.8 Hz, 1H), 3.91 (app t, 9 Hz, 1H), 3.84 (dd, *J* = 10.9, 4.6 Hz, 1H), 3.71 (dd, *J* = 10.9, 1.9 Hz, 1H), 2.82 – 2.62 (m, 4H), 2.29 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.2, 171.9, 138.3, 138.2, 137.9, 137.7, 132.4, 129.8, 129.7, 128.4, 128.3, 128.3, 128.1, 127.9, 127.8, 127.7, 127.7, 127.5, 86.4, 78.4, 75.2, 74.5, 73.3, 72.3, 71.7, 70.4, 68.9, 37.9, 29.7, 28.1, 21.1. $[\alpha]_D^{25}$: +99.4 (c1.0, $CHCl_3$). IR (cm^{-1}): 2908, 2861, 2660, 1739, 1718, 1101. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{39}H_{42}O_7SNa$: 677.2549, found: 677.2542.



2-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(4-oxopentanoyl)-α-D-mannopyranosyl)-4,6-*O*-benzylidine-β-D-mannopyranoside (18)

500 mg (0.763 mmol; 1 equiv.) of **16** and 598 mg (0.915 mmol; 1.2 equiv.) of **17**[6] in a 50 mL flask were dissolved in toluene and cooled to -78 °C. Vacuum was applied and the cooling bath was removed and allowed to warm to room temperature as the toluene evaporated. This procedure was repeated twice. The dry residue was dissolved in 14 mL of CH_2Cl_2 and activated 4Å molecular sieves were added stirred for 20 minutes at room temperature. The reaction mixture was cooled to -40 °C and stirred another 15 minutes more. 429 mg of NIS (1.91 mmol; 2.5 equiv.) was added, and after 15 minutes, 27 µL of TMSOTf (0.15 mmol; 0.2 equiv.) dissolved in 2 mL of CH_2Cl_2 was added dropwise. The reaction was stirred at -40 °C for 2 h and quenched with aq. sat. $NaHCO_3$ and solid $Na_2S_2O_3$. The reaction mixture was filtered, and the filtrate was washed with aq. sat. $Na_2S_2O_3$, aq. sat. $NaHCO_3$ and brine, then the organic layer was dried ($MgSO_4$) and concentrated prior to flash column chromatography (25% ethyl acetate and hexane). Compound **18** (741 mg; 0.626 mmol; 82%) was obtained as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (app d, *J* = 8 Hz, 2H), 7.62 (app t, *J* = 7.2 Hz, 1H), 7.53 (app t, *J* = 8 Hz, 2H), 7.5 (m, 2H), 7.41 – 7.10 (multiple signals, 23H), 5.60 (s, 1H), 5.58 (dd, *J* = 2.8, 1.3 Hz,1H), 5.23 (d, *J* = 1.3 Hz, 1H), 4.92 – 4.83 (m, 2H), 4.78 (d, *J* = 12.3 Hz, 1H), 4.66 (d, *J* = 11.3 Hz, 1H), 4.57 – 4.38 (multiple signals, 6H), 4.32 (dd, *J* = 10.4, 4.9 Hz, 1H), 4.18 (app t, J = 9.7 Hz, 1H), 3.90 (multiple signals,

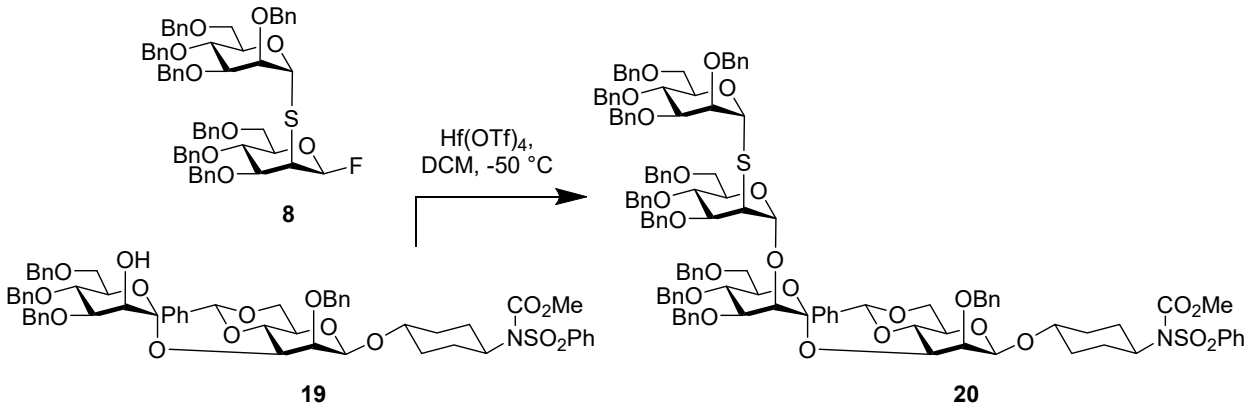
3H), 3.82 – 3.54 (multiple signals, 6H), 3.65 (s, 3H), 3.31 (app td, *J* = 9.7, 4.9 Hz, 1H), 2.73 – 2.58 (m, 4H), 2.37-2.13 (m, 3H), 2.10 (s, 3H), 2.0 – 1.91 (m, 1H), 1.89 – 1.79 (m, 2H), 1.59 – 1.48 (m, 1H), 1.40 – 1.28 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.1, 202.2 171.7, 152.6, 140.3, 138.6, 138.3, 138.1, 137.9, 137.3, 133.4, 128.8, 128.7, 128.36 (two resonances), 128.34, 128.3, 128.2, 128.13, 128.10, 127.9, 127.75, 127.71, 127.67, 127.63, 127.61, 127.56, 126.0, 101.2, 100.1, 98.7, 78.6, 77.81, 77.75, 76.4, 75.2, 75.0, 74.9, 74.3, 73.4, 72.0, 71.5, 69.2, 68.5, 68.4, 67.4, 58.5, 53.5, 38.1, 33.2, 31.5, 29.8, 28.46, 28.44, 28.2. $[\alpha]_D^{25}$: -3.6 (c1.0, $CHCl_3$). IR (cm^{-1}):3030, 2924, 2861, 1962, 1732, 1496, 1356, 1088. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{66}H_{73}NO_{17}SNa$: 1206.4497, found 1206.4469.



18 **19**

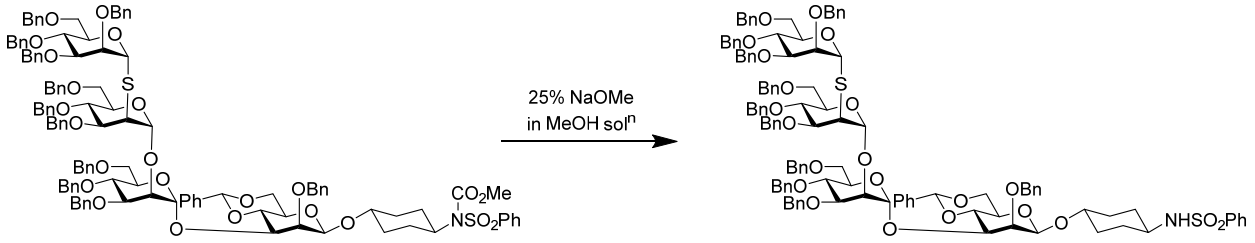
2-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-*O*-benzylidine-β-D-mannopyranoside (19)

Compound **18** (700 mg; 0.591 mmol; 1 equiv.) was dissolved in 10 mL of CH_2Cl_2 and cooled to 0 °C. Pyridine (190 µL; 2.364 mmol; 4 equiv.) and acetic acid (34 µL; 0.591 mmol; 1 equiv.) was mixed in a 1 mL ice cooled vial and added to reaction mixture dropwise, followed by addition of hydrazine (91µL; 2.955 mmol; 5 equiv.) to reaction mixture. The reaction was stirred for 2 h at 0 °C and quenched with 2 mL of acetone. The reaction mixture was concentrated under reduced pressure and the crude was purified by column chromatography in 30% ethyl acetate and hexane to afford compound **19** (430 mg; 0.395 mmol; 67%) as white glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (app d, *J* = 8 Hz, 2H), 7.62 (app t, *J* = 7.4 Hz, 1H), 7.53 (app t, *J* = 7.8 Hz, 2H), 7.47 – 7.12 (multiple signals, 25H + residual $CHCl_3$), 5.56 (s, 1H), 5.33 (d, *J* = 1.7 Hz, 1H), 4.91 (d, *J* = 12.4 Hz, 1H), 4.85 (d, *J* = 11.1 Hz, 1H), 4.80 (d, *J* = 12.4 Hz, 1H), 4.67 (d, *J* = 11.4 Hz, 1H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.54 – 4.46 (m, 4H), 4.41 (tt, *J* = 12.1, 3.8 Hz, 1H), 4.30 (dd, *J* = 10.4, 4.8 Hz, 1H), 4.18 (dd, *J* = 2.8, 1.7 Hz, 1H), 4.13 (app t, *J* = 9.7 Hz, 1H), 4.00 (dd, *J* = 10.1, 3.1 Hz, 1H), 3.91 (app t, *J* = 10.3 Hz, 1H), 3.87 – 3.80 (m, 2H), 3.79 – 3.52 (m, 5H), 3.66 (s, 3H), 3.33 (app td, *J* = 9.7, 4.9 Hz, 1H), 2.40 (br s, 1H), 2.35 – 2.10 (m, 3H), 2.00 – 1.9 (m, 1H), 1.9 – 1.8 (m, 2H), 1.59 – 1.45 (m, 1H + H_2O), 1.39 – 1.26 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.5, 140.3, 138.5, 138.33, 138.28, 137.8, 137.4, 133.4, 128.95, 128.8, 128.5, 128.4, 128.33, 128.30, 128.28, 128.2, 127.94, 127.90, 127.88, 127.72, 127.71, 127.59, 127.58, 127.56, 126.0, 101.5, 100.3, 100.1, 79.9, 78.7, 78.1, 76.4, 75.3, 74.93, 74.90, 74.3, 73.4, 71.9, 71.8, 69.3, 68.6, 68.0, 67.4, 58.4, 53.5, 33.2, 31.5, 30.9, 28.4. $[\alpha]_D^{25}$: -2.01 (c1.0, $CHCl_3$). IR (cm^{-1}): 3540, 2919, 1732, 1452, 1666, 1359, 1086. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{61}H_{67}NO_{15}SNa$: 1108.4129, found 1108.4065.



***Trans*-(*N*-methylcarbonate-*N*-1-phenylsulfonyl)-cyclohexyl-2-*O*-benzyl-4,6-*O*-benzylidine-3-*O*-[{3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)}-2-*O*-{3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)}-2-*S*-{3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)}]-β-D-mannopyranoside (20)**

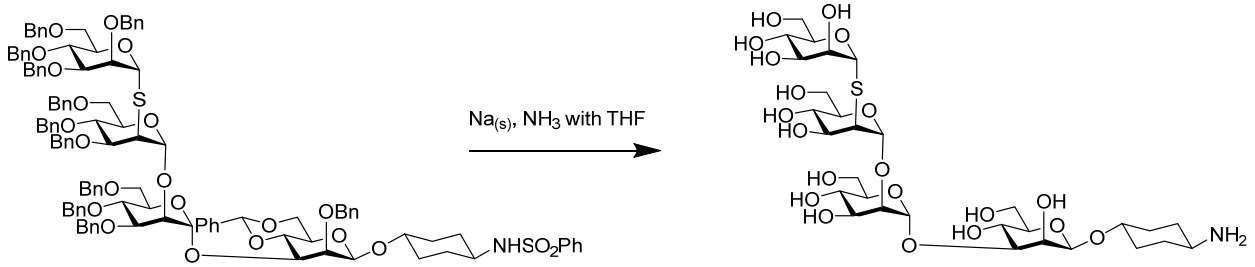
Disaccharide donor **8** (280 mg; 282 µmol; 1 equiv.) and acceptor **19** (230 mg; 212 µmol; 0.75 equiv.) were dissolved in anhydrous dichloromethane (5 mL), and freshly crushed and flame dried 4 Å molecular sieves was added and stirred for 15 min at room temperature. The mixture was cooled at -50 °C, then $Hg(OTf)_4$ (219 mg; 282 µmol; 1 equiv.) was added and allowed to stir for 1 h. The reaction was neutralized with a few drops of triethylamine, diluted with CH_2Cl_2 and filtered through celite pad and concentrated, and then purification by column chromatography by 35% of EtOAc in Hexane, afforded trisaccharide **20** as white foam in 77% yield based on acceptor **19** (335 mg, 163.84 µmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (app d, J = 7.4 Hz, 2H), 7.61 (app t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.7 Hz, 2H), 7.44 – 6.98 (multiple signals, 60H + residual $CHCl_3$), 5.57 (s, 1H), 5.36 (s, 2H), 5.33 (s, 1H), 5.01 – 4.73 (multiple signals, 5H), 4.62 – 4.30 (multiple signals, 17H), 4.29 – 4.15 (multiple signals, 5H), 4.08 – 3.97 (multiple signals, 3H), 3.96 – 3.39 (m, 20H), 3.30 (d, J = 11.0 Hz, 1H), 3.12 (app td, J = 9.6, 4.9 Hz, 1H), 2.34 – 2.16 (m, 2H), 2.16 – 2.08 (m, 1H), 2.00 – 1.69 (m, 3H), 1.57 – 1.43 (m, 1H), 1.34 – 1.19 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (selected signals) 152.5, 140.3, 138.9, 138.76, 138.63, 138.52, 138.50, 138.32, 138.28, 138.22 (two resonances), 138.1 (two resonances), 137.3, 133.4, 128.8, 128.46, 128.40, 128.38, 128.34 (multiple resonances), 128.31, 128.28 (multiple resonances), 128.23, 128.21, 128.17, 128.12, 128.0, 128.0, 127.9, 127.8, 127.75, 127.71, 127.66, 127.64, 127.59, 127.54, 127.51, 127.49, 127.47, 127.40, 127.3, 127.1, 125.95, 102.1, 101.3, 99.9, 99.8, 83.0, 80.4, 78.9, 78.3, 78.1, 76.3, 75.7, 75.5, 75.19, 75.14, 75.0, 74.9, 74.8, 74.0, 73.2, 73.1, 72.9, 72.5, 72.41, 72.46, 72.2, 71.9, 71.3, 69.7, 68.9, 68.8, 68.5, 67.3, 58.5, 53.4, 49.4, 33.1, 31.4, 28.4. $[\alpha]_D^{25}$: +12.0 (c1.0, $CHCl_3$). IR (cm-^{1}): 3030, 2853, 1732, 1495, 1260. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{122}H_{129}NO_{25}S_2Na$: 2078.8244, found 2078.8289.



20 **S3**

***Trans*-(*N*-1-phenylsulfonyl)-cyclohexyl-2-*O*-benzyl-4,6-*O*-benzylidine-3-*O*-[{3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)}-2-*O*-{3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)}-2-*S*-{3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)}]-β-D-mannopyranoside (S3)**

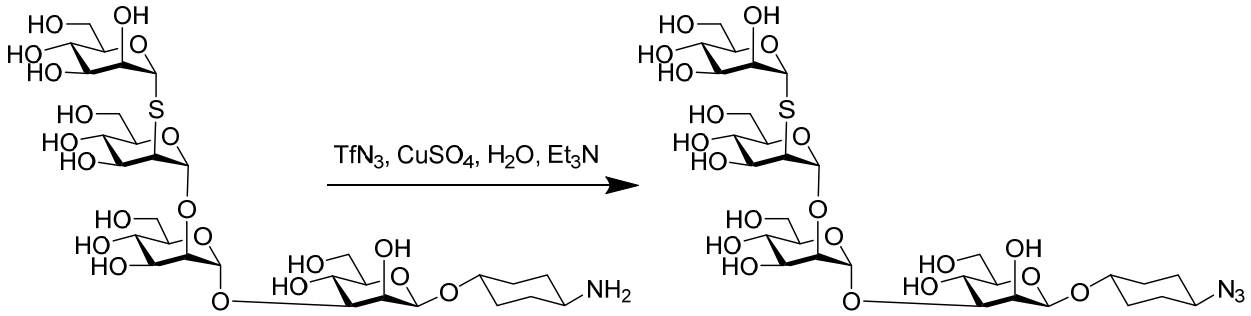
Compound **20** (280 mg; 136 µmol; 1 equiv.) was dissolved in methanol (2 mL) and dichloromethane (2 mL) and 10 µL (40.8 µmol; 0.3 equiv.) 25% sodium methoxide in methanol solution was added. After 3 hours at room temperature, the reaction was neutralized with Amberlite IR-120 H^+ form ion exchange resin with caution to avoid acidifying below pH 6. The resin was filtered off and the filtrate was concentrated under reduced pressure. The concentrate was purified by column chromatography to afford compound **S3** (244 mg; 122 µmol) as colorless syrup in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (app d, *J* = 7.1 Hz, 2H), 7.59 (app t, *J* = 7 Hz, 1H), 7.52 (app t, *J* = 7 Hz, 2H), 7.47 – 7.09 (multiple signals, 58H + residual $CHCl_3$), 7.08 – 6.98 (m, 2H), 5.56 (s, 1H), 5.33 (app s, 2H), 5.31 (s, 1H), 4.95 – 4.77 (multiple signals, 4H), 4.71 (d, *J* = 12.3 Hz, 1H), 4.62 – 4.39 (multiple signals, 13H), 4.39 – 4.29 (multiple signals, 4H), 4.27 – 4.11 (multiple signals, 5H), 4.08 – 3.97 (m, 3H), 3.93 – 3.54 (multiple signals, 14H), 3.50 – 3.36 (m, 3H), 3.28 (d, *J* = 10.6 Hz, 1H), 3.20 – 3.00 (m, 2H), 2.25 – 1.62 (m, 4H), 1.41 – 1.13 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (selected signals) 141.1, 139.0, 138.8, 138.7, 138.56, 138.54, 138.36, 138.34, 138.25, 138.23, 138.16, 138.13, 137.3, 132.7, 129.2, 129.0 128.48, 128.43, 128.41, 128.35, 128.33, 128.31, 128.29, 128.25, 128.22, 128.19, 128.12, 128.05, 127.9, 127.8, 127.75, 127.74, 127.71, 127.69, 127.61, 127.59, 127.57, 127.50, 127.45, 127.40, 127.3, 127.2, 126.9, 126.0, 101.3, 99.84, 99.82, 83.0, 80.5, 78.9, 78.3, 78.1, 75.8, 75.7, 75.5, 75.2, 75.1, 75.0, 74.95, 74.85, 74.0, 73.2, 73.15, 73.0, 72.54, 72.47, 72.37, 72.30, 71.9, 71.3, 69.7, 69.0, 68.9, 68.5, 67.4, 51.7, 49.4, 31.0 (multiple signals), 29.3. $[\alpha]_D^{25}$: +8.0 (c1.0, $CHCl_3$). IR (cm^{-1}): 3029, 2862, 1731, 1496, 1452, 1090. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{120}H_{127}NO_{25}S_2Na$: 2020.8189, found 2020.8182.



S3 **S4**

***Trans*-aminocyclohexyl-3-*O*-[{α-D-mannopyranosyl-(1→2)}-2-*O*-{α-D-mannopyranosyl-(1→2)}-2-*S*-{α-D-mannopyranosyl-(1→2)}]-β-D-mannopyranoside (S4)**

Into an oven dried 500 mL 3-necked flask, ~200 mL ammonia was condensed under stream of nitrogen at -78 °C. 471 mg of (20.5 mmol) Na° was added, and the bright blue reaction was allowed to stir for 1 hour to observe persistence of the blue color. Next, 205 mg (103 μmol; 1 equiv.) of tetrasaccharide **S3** in 2 mL of dry THF was added by syringe, and the reaction was allowed to stir for 2 hours. When reaction was finished (monitored by direct-infusion LCMS), solid NH_4Cl (548 mg; 10.3 mmol) was added portionwise until the disappearance of blue color and the ice bath was removed, allowing the mixture to warm to room temperature. The crude product was desalted on a Biogel P-2 size exclusion gel column and lyophilization afforded ~105 mg of deprotected trisaccharide **S4** (including some residual salt) as a hazy glassy white solid, which was used for the next step without further purification. ^{1}H NMR (400 MHz, D_2O) δ 5.13 (s, 1H), 5.06 (s, 1H), 5.00 (s, 1H), 4.54 (s, 1H), 3.99 (dd, *J* = 9.7, 4.5 Hz, 1H), 3.84 (d, *J* = 2.8 Hz, 1H), 3.81 – 3.77 (m, 2H), 3.76 – 3.68 (m, 2H), 3.68 – 3.33 (m, 17H), 3.28 (app t, *J* = 9.5 Hz, 1H), 3.15 – 3.08 (m, 1H), 2.97 – 2.86 (m, 1H), 1.92 – 1.73 (m, 4H), 1.35 – 1.05 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ 103.0, 100.4, 97.7, 86.7, 80.4, 78.4, 75.9, 73.4, 73.3, 73.2, 71.3, 70.8, 70.6, 69.9, 69.1, 67.5, 67.0, 66.8, 66.1, 60.82, 60.78, 60.6, 52.1, 49.0, 46.6, 30.2, 28.9, 28.1, 28.0, 8.1. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{30}H_{53}NO_{20}SNa$: 802.2779, found 802.2761.



S4 **21**

***Trans*-azidocyclohexyl-3-*O*-[{α-D-mannopyranosyl-(1→2)}-2-*O*-{α-D-mannopyranosyl-(1→2)}-2-*S*-{α-D-mannopyranosyl-(1→2)}]-β-D-mannopyranoside (21)**

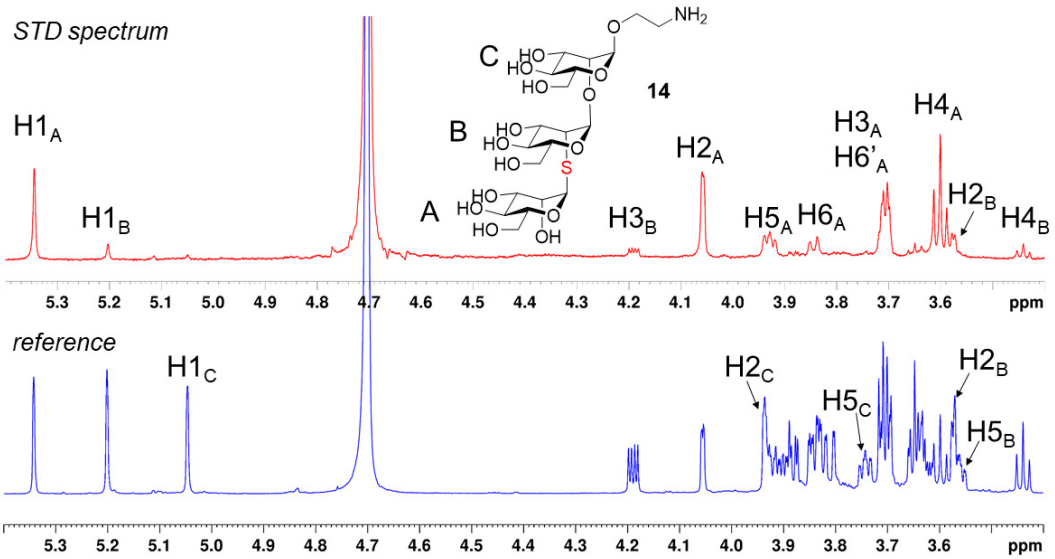
In a 5 ml oven-dried reaction vial with magnetic stir bar, 105 mg (1.62 mmol; 1 equiv.) of sodium azide was cooled to 0 °C and dissolved in 1.3 mL of pyridine, followed by treatment with 327 μL triflic anhydride (1.94 mmol; 1.2 equiv.). While this stirred for 2 hours at 0 °C, in other 5 mL RB, ~105 mg of crude tetrasaccharide amine **S4** (used directly from previous step) was dissolved in 1.4 mL of water and cooled to 0 °C. 5.1 mg of cupric sulphate (20.5 mmol; 0.2 equiv.) dissolved in 1 mL of H_2O was added to reaction mixture followed addition of 142 μL (143 mmol; 10 equiv.) of triethyl amine. The triflyl azide solution prepared above was added dropwise to tetrasaccharide reaction mixture at 0 °C and stirred for 1 hour. The crude material was desalted on a Biogel P-2 size exclusion gel column to yield 70 mg of crude product. The crude material was then purified

by reverse phase HPLC (Column: Waters Xbridge Prep, C_{18}, 5µm, 19 x 250 mm, 150Å pore diameter. Method: 16 mL/ min flow rate, A = H_2O; B = Acetonitrile without buffer; 1 % B for 10 minutes, then 1-20% B over 55 minutes. Product was detected by UV at 220 nm. Product containing fractions from 3 injections were lyophilized to yield 33 mg (41.04 µmol) in 40% yield based on **S3** as a colorless glass. ^{1}H NMR (800 MHz, D_2O, 293 K) δ 5.40 (s, 1H), 5.33 (s, 1H), 5.28 (s, 1H), 4.80 (s, 1H), 4.26 (dd, *J* = 9.7, 4.6 Hz, 1H), 4.12 (dd, *J* = 3.4, 1.5 Hz, 1H), 4.08 – 4.06 (m, 2H), 4.02 – 3.97 (m, 2H), 3.94 – 3.86 (m, 4H), 3.85 – 3.65 (m, 12H), 3.63 (dd, *J* = 4.7, 1.5 Hz, 1H), 3.55 (app t, *J* = 9.5 Hz, 1H), 3.53 – 3.48 (m, 1H), 3.39 (ddd, *J* = 9.7, 6.2, 2.3 Hz, 1H), 2.12 – 1.94 (m, 4H), 1.52 – 1.30 (m, 4H). ^{13}C NMR (200 MHz, D_2O) δ 103.1, 100.5, 97.7, 86.8, 80.5, 78.5, 76.0, 75.9, 73.41, 73.36, 73.26, 71.4, 70.9, 70.8, 70.0, 69.1, 67.6, 67.1, 66.9, 66.1, 60.9 (two resonances), 60.8, 60.6, 58.8, 52.2, 30.0, 28.6, 28.3, 28.1. $[\alpha]_D^{25}$: -1533 (c1.0, H_2O).IR (cm^{-1}):3370, 3266, 2236, 2173, 1970, 1063. HRMS (ESI-TOF) m/z: $[M+Na]^+$: Calcd. for $C_{30}H_{51}N_3O_{20}SNa$: 828.2684, found 828.2676.

STD NMR experiments of trisaccharide 14 and tetrasaccharide 21

STD NMR experiments were carried out with oligosaccharides **14** and **21** in the presence of the IgG antibody 2G12 (200:1 sugar:2G12, with 2G12 at 25 µM) in pH 6.7 deuterated phosphate buffered saline. The temperature was 298 K for trisaccharide **14** and 293 K for tetrasaccharide **21** in order to move the HDO peak to avoid covering an anomeric resonance. The experiments were performed without suppression of the residual HDO signal. On-resonance frequencies of 0.86 ppm δ (protein aliphatic) and 7 ppm δ (protein aromatic) were applied for trisaccharides **14** and tetrasaccharide **21** respectively, in both cases with a saturation time of 3s and an off-resonance frequency of 40 ppm δ.

Figure S1. STD for S-Man$_3$ (**14**) with HIV IgG antibody 2G12. For S-Man$_4$ (**21**), see Main Text, Figure 1.



Mannosidase experiments

SPAAC labeling with LC-MS/UV tag: To facilitate separation and detection of degradation products via LC/MS, Man_4-cyclohexyl derivatives **21** and **22** were labeled by strain-promoted azide/alkyne cycloaddition (SPAAC) with DBCO amine linker **23**. Man_4-cyclohexyl-N_3 **22** or the thioether derivative **21** were dissolved in degassed H_2O to afford a 25 mM stock, while the DBCO-NH_2 SPAAC reagent **23** was dissolved in acetonitrile (29 mM). Under a N_2 atmosphere, 1.1 equiv. of **23** was added to the Man_4 derivative, followed by heating to 37°C and reaction monitoring by LC/MS. At 30 minutes, both SPAAC reactions were complete by LC/MS, showing two regioisomers, separable by LC/MS. Crude reactions were analyzed with an analytical C4 column (ACQUITY UPLC Protein BEH C4, 2.1mm X 150 mm, 300Å, 1.7μm) using the method in the table below with mobile phases A: H_2O (0.7% formic acid) and B: MeCN (0.1% formic acid).

Time (minutes)	Flow (mL/min)	%A	%B
0	0.3	90	10
1	0.3	90	10
7	0.3	70	30
7.05	0.3	5	95
10	0.3	5	95
10.05	0.3	90	10
13	0.3	90	10

Mannosidase Digestion: 100μg/mL DBCO-NH_2-labeled Man_4-derivative was digested with an α1-2,3 Mannosidase cloned from the pathogenic bacterium *Xanthomonas manihotis* (NEB: P0729S) according to manufacturer's instructions. Briefly, either Man_4-derivative was diluted into 1X glycobuffer I (provided with the enzyme) supplemented with 100μg/mL BSA and finally 80 units of α1-2,3 Mannosidase. Reactions were monitored by LC/MS by the same method detailed above, over the course of 48 hr (see Figures S2 and S3).

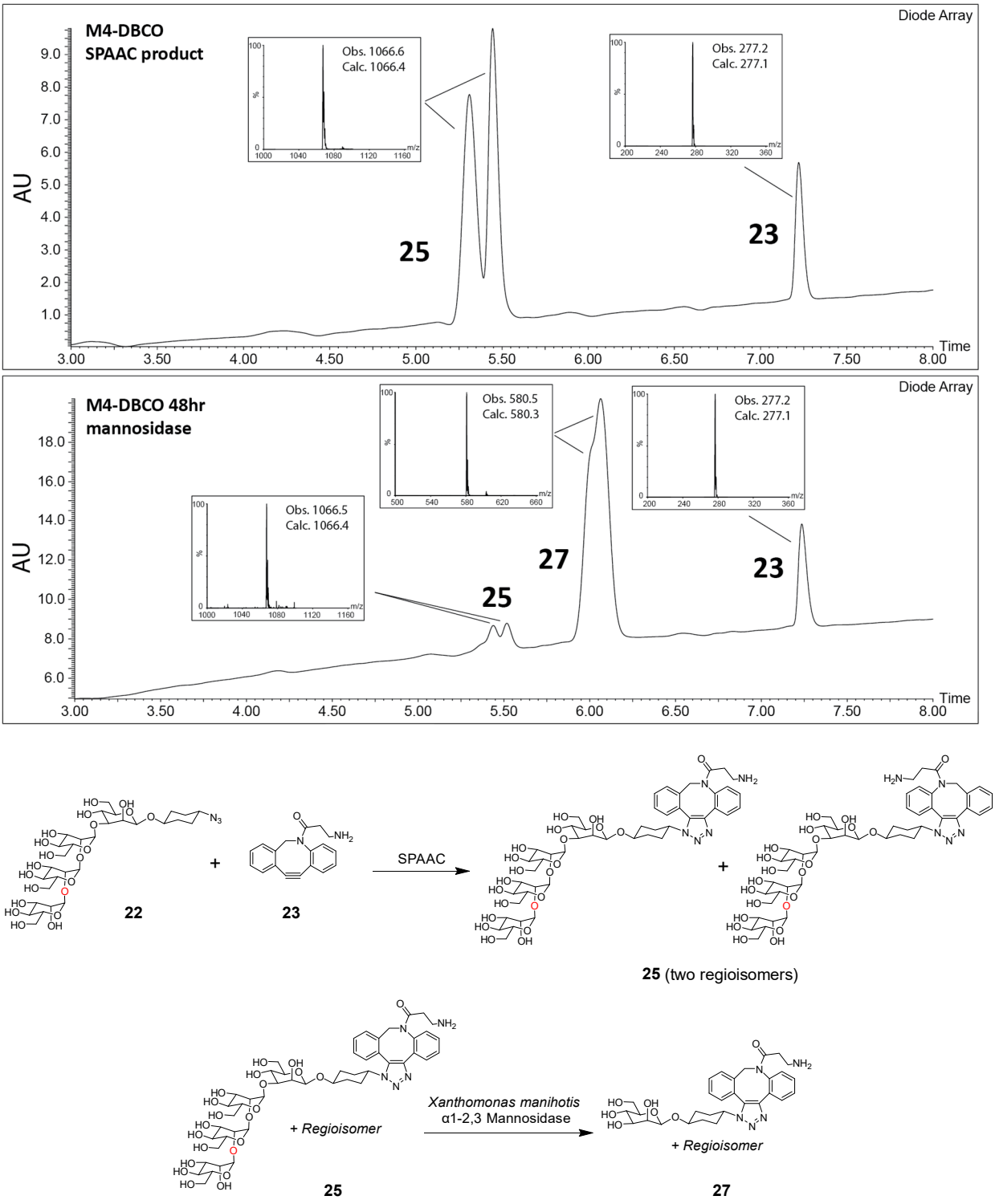


Figure S2. SPAAC labeling and mannosidase digestion of Man_4 derivative 22.

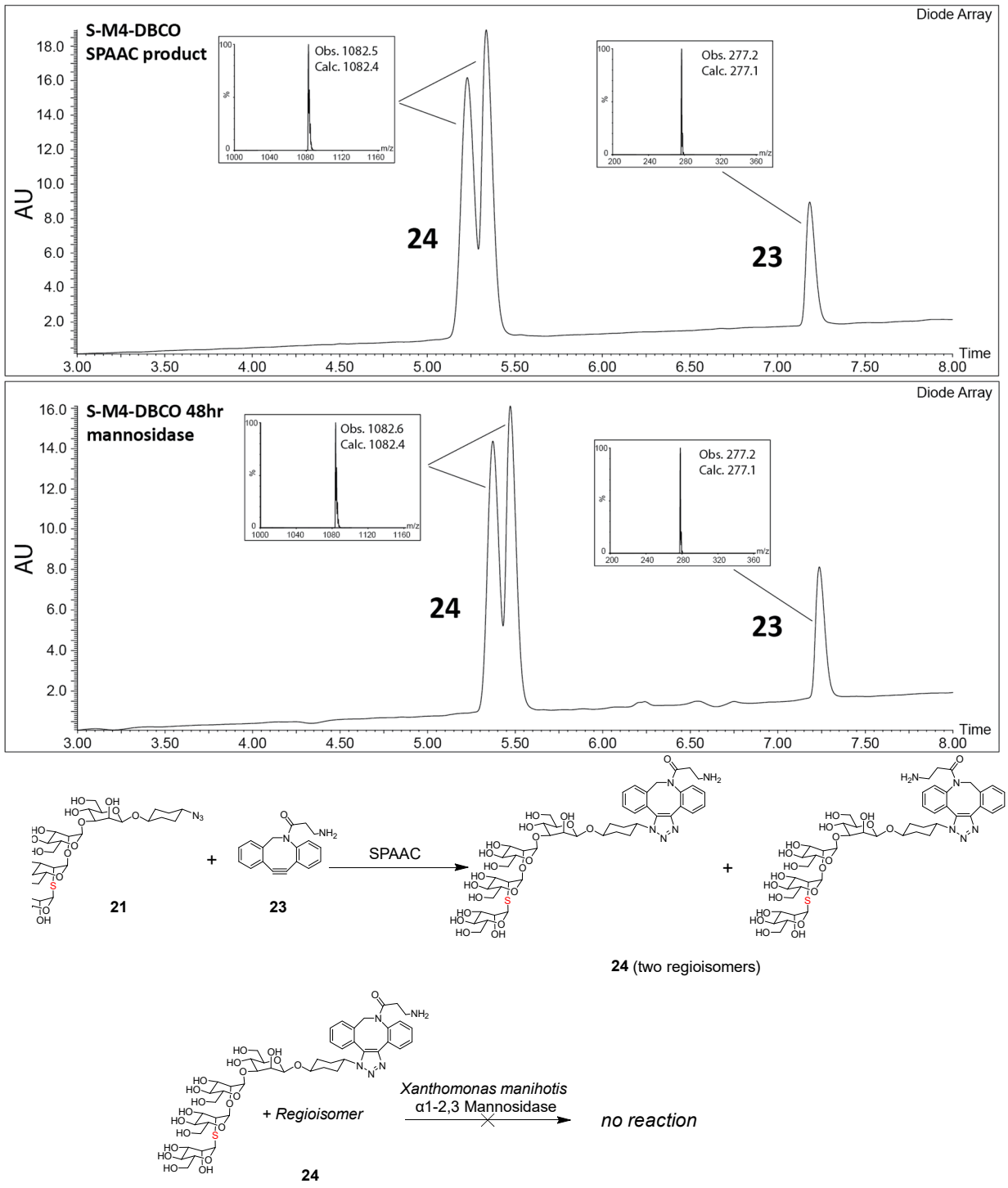


Figure S3. SPAAC labeling and mannosidase digestion of Man_4 derivative 21.

References:

1. Matta, K. L.; Girotra, R. N.; Barlow, J. J., Synthesis of p-nitrobenzyl and p-nitrophenyl 1-thioglycopyranosides. *Carbohydr Res* **1975,** *43*, 101-9.
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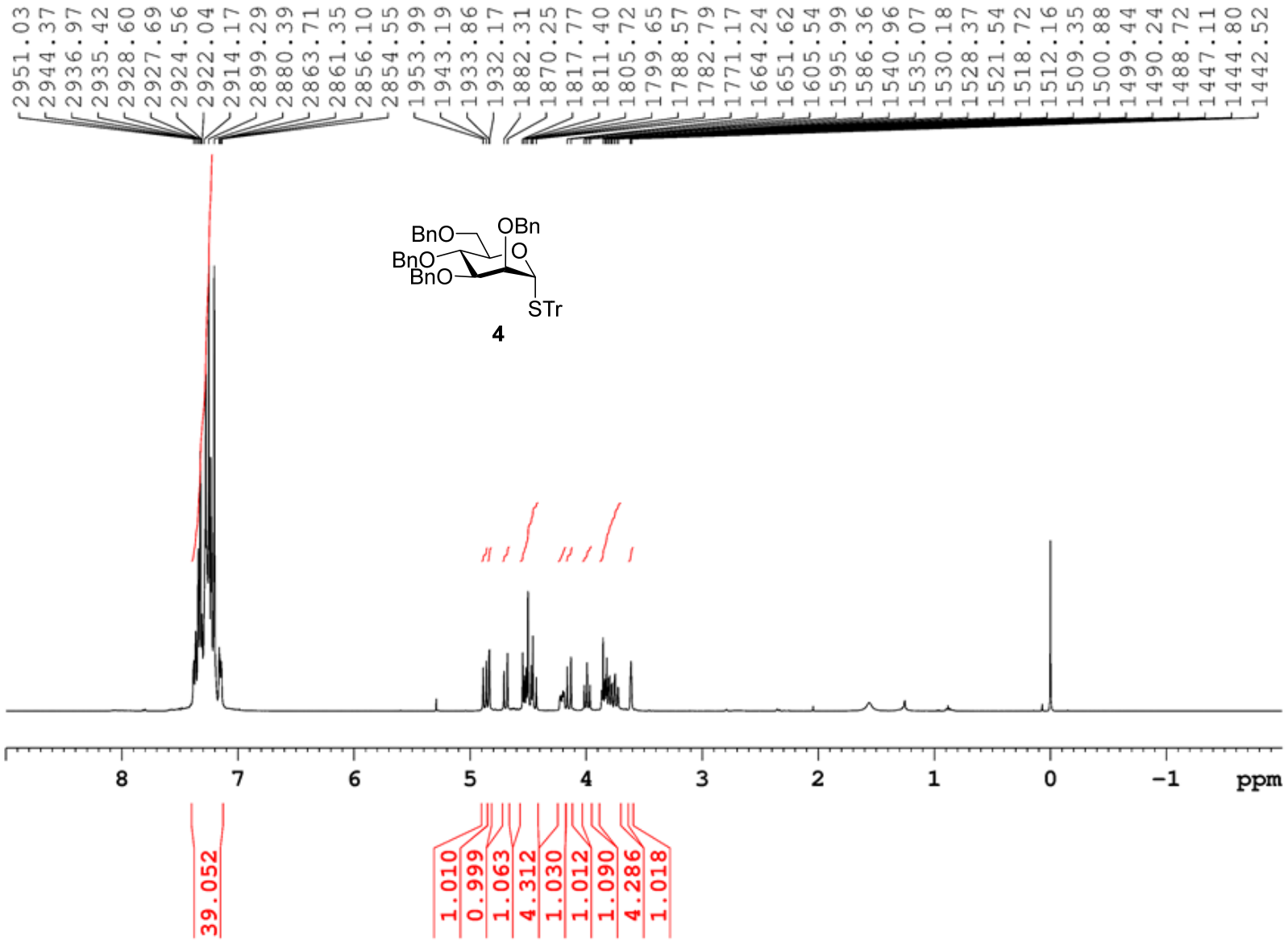
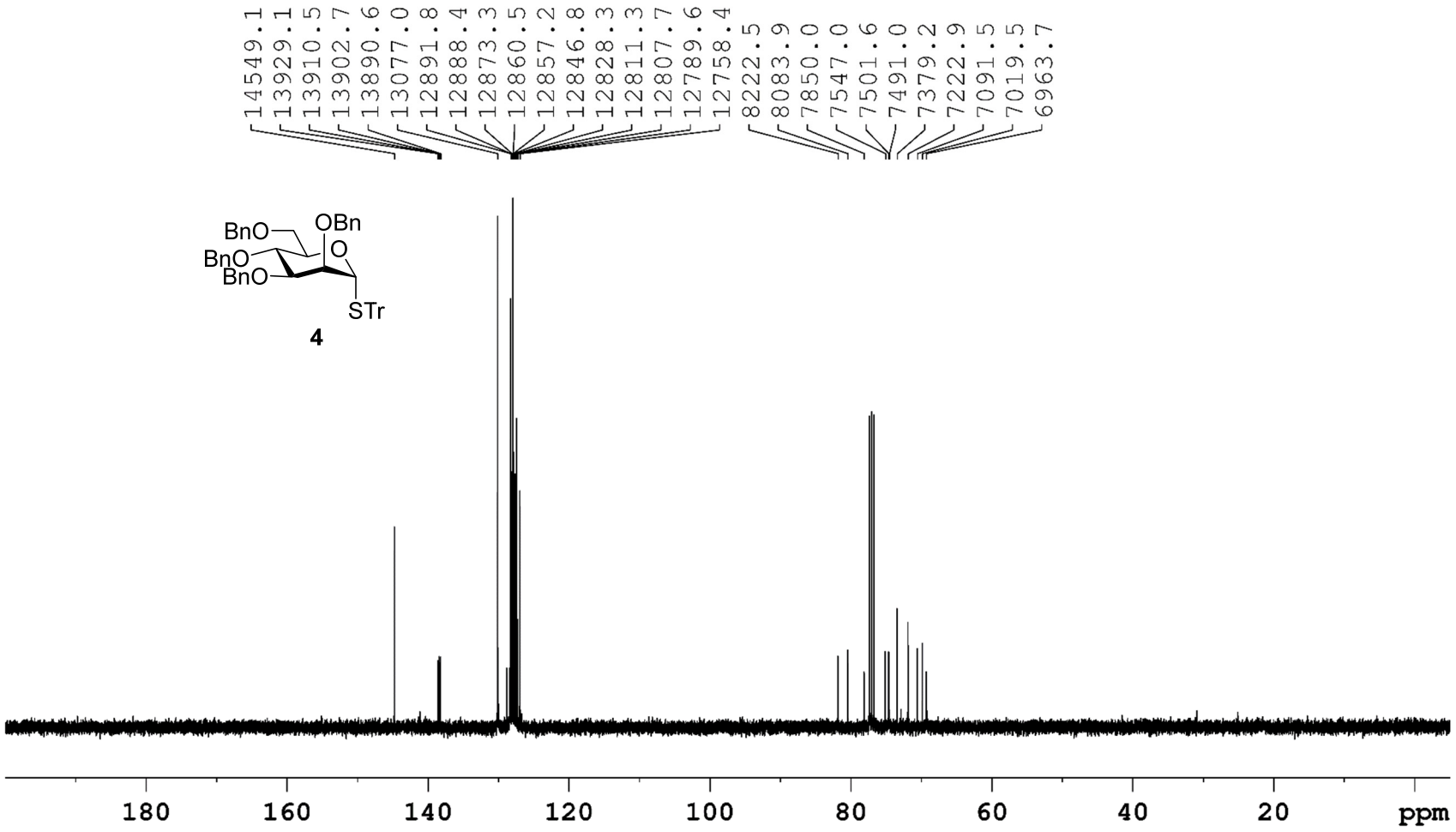
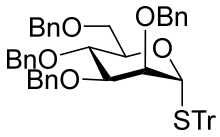
^{1}H NMR (400 MHz) of **4** in $CDCl_3$



^{13}C NMR (100 MHz) of **4** in $CDCl_3$



DEPT NMR (100 MHz) of **4** in $CDCl_3$



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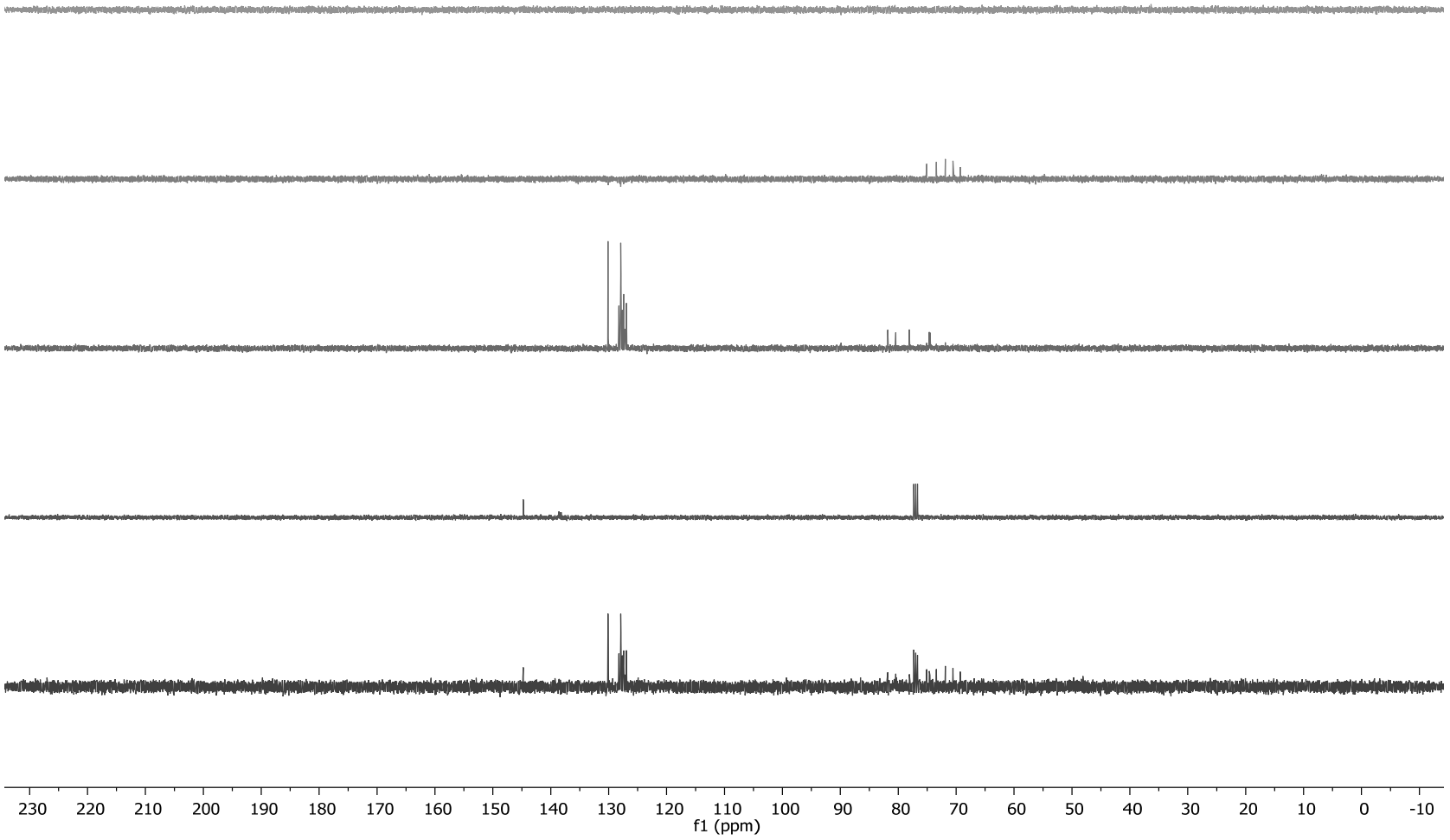
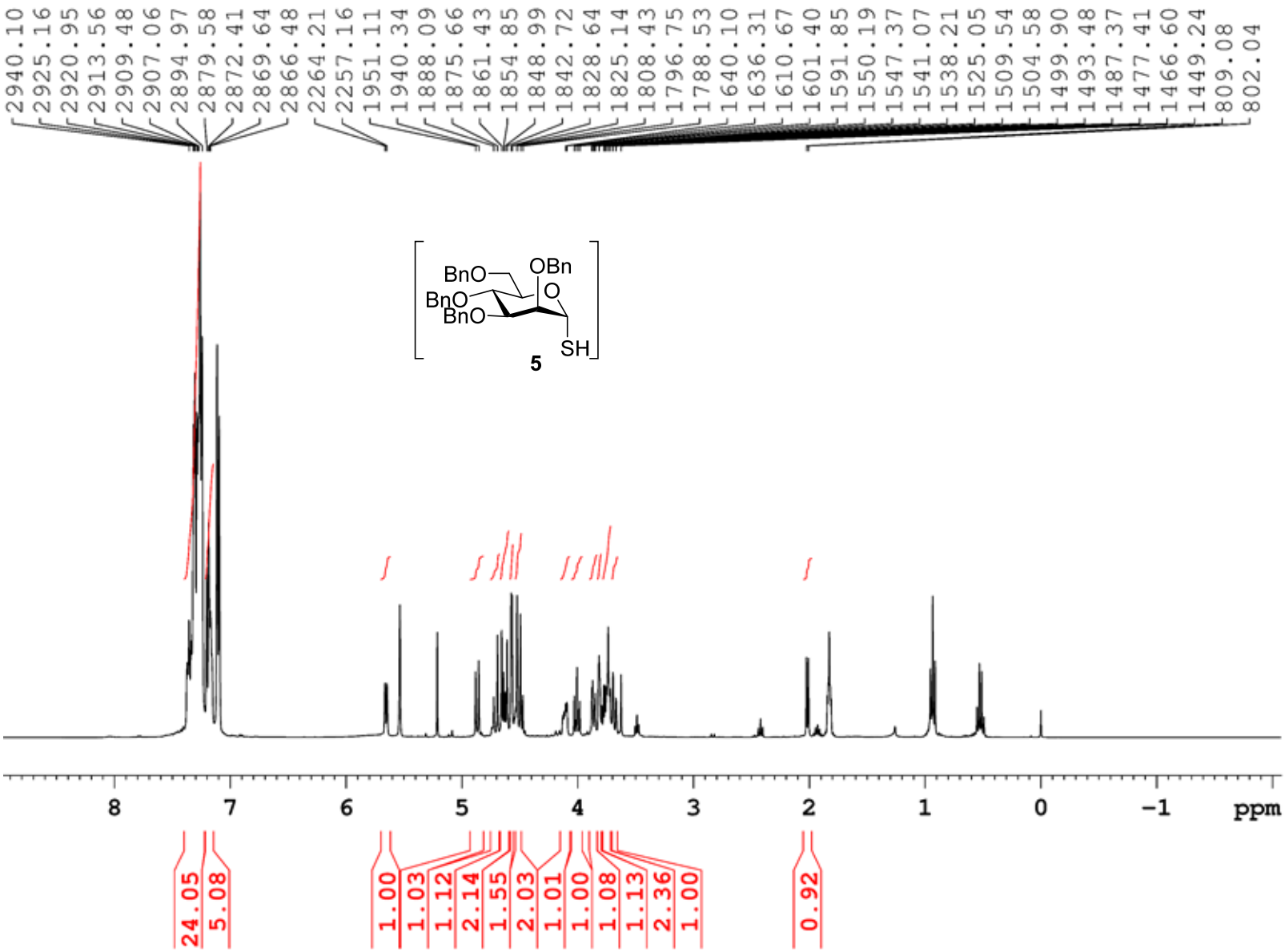
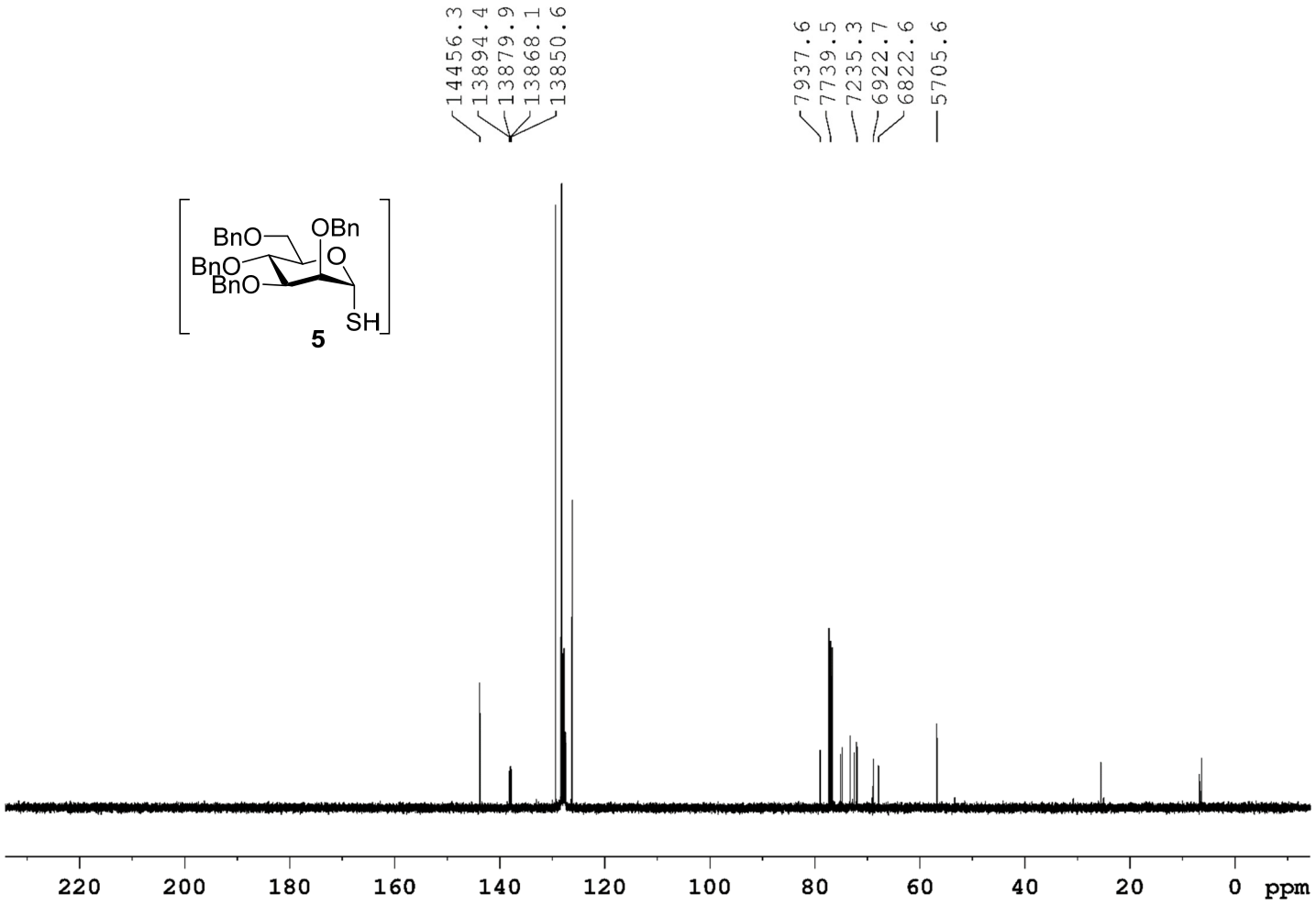


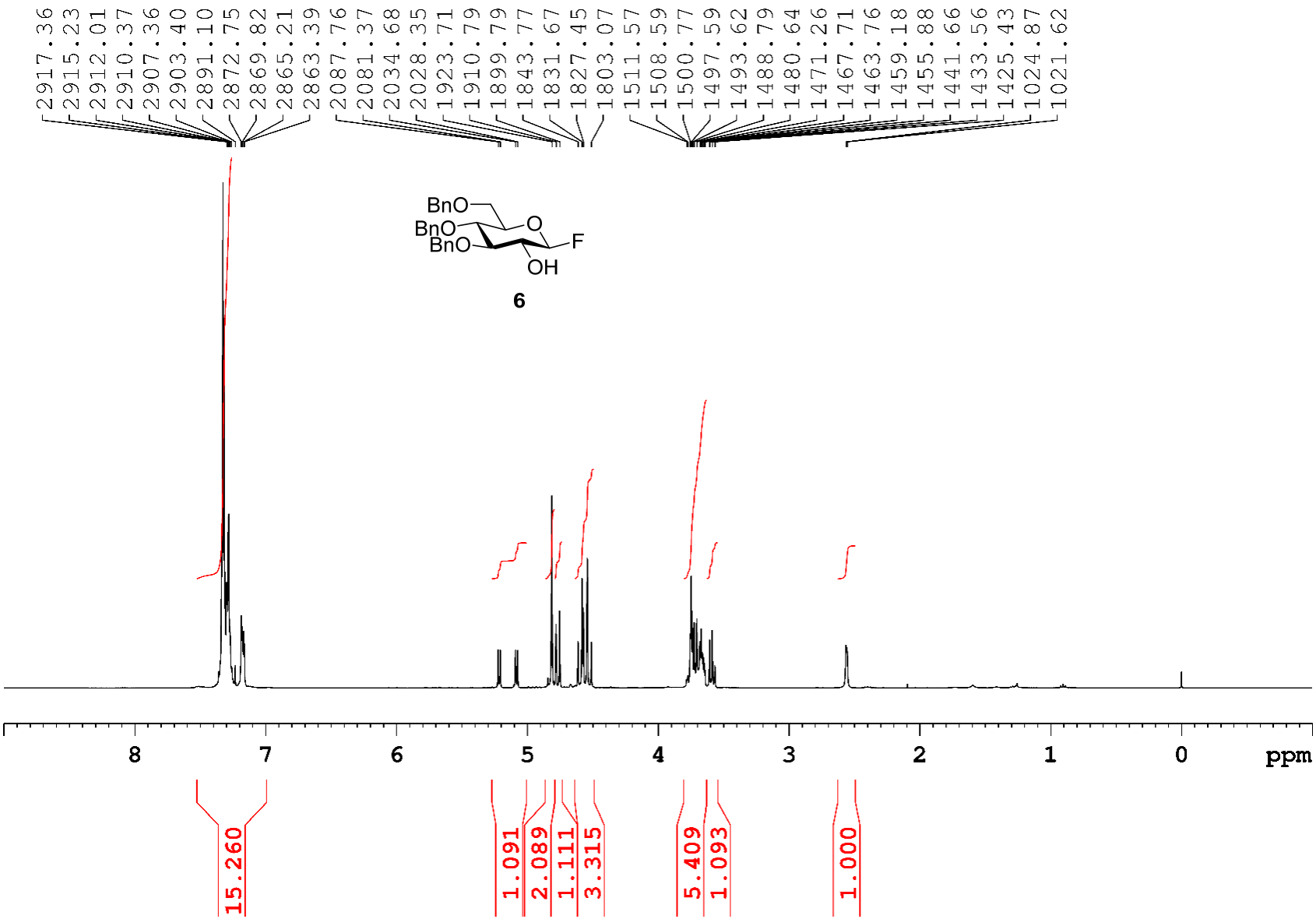
^{1}H NMR (400 MHz) of crude **5** in $CDCl_3$



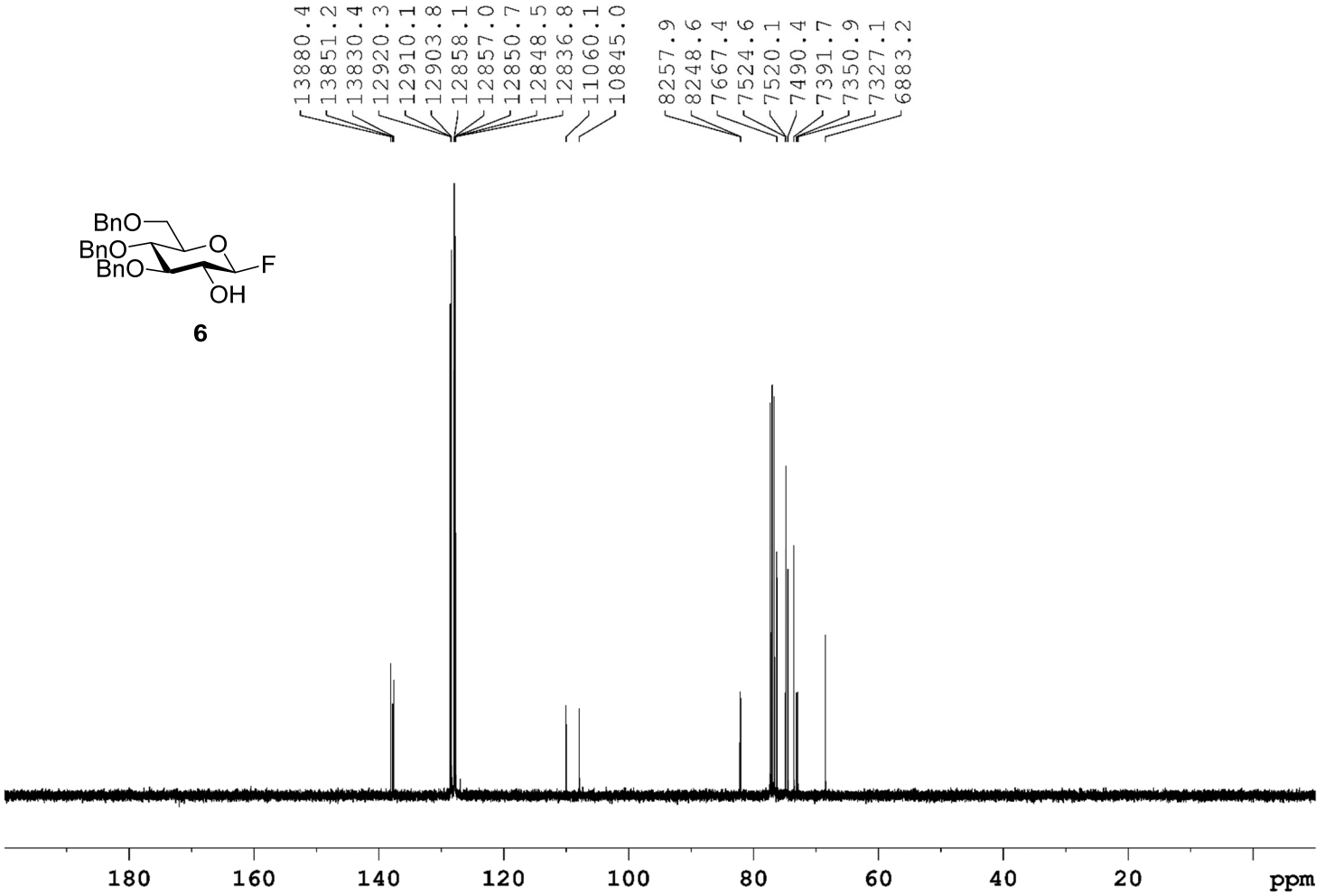
^{13}C NMR (100 MHz) of crude **5** in $CDCl_3$



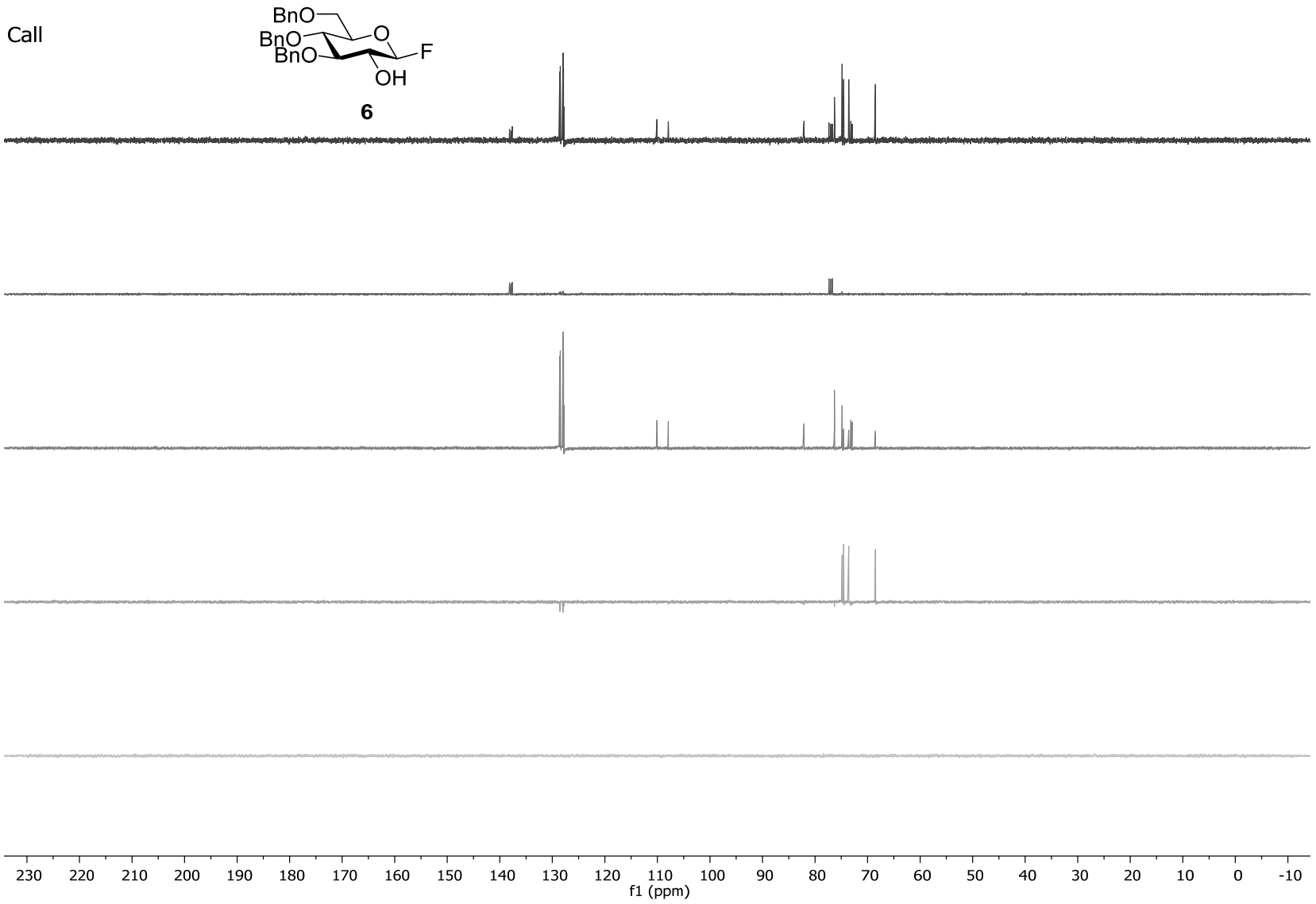
^{1}H NMR (400 MHz) of **6** in $CDCl_3$



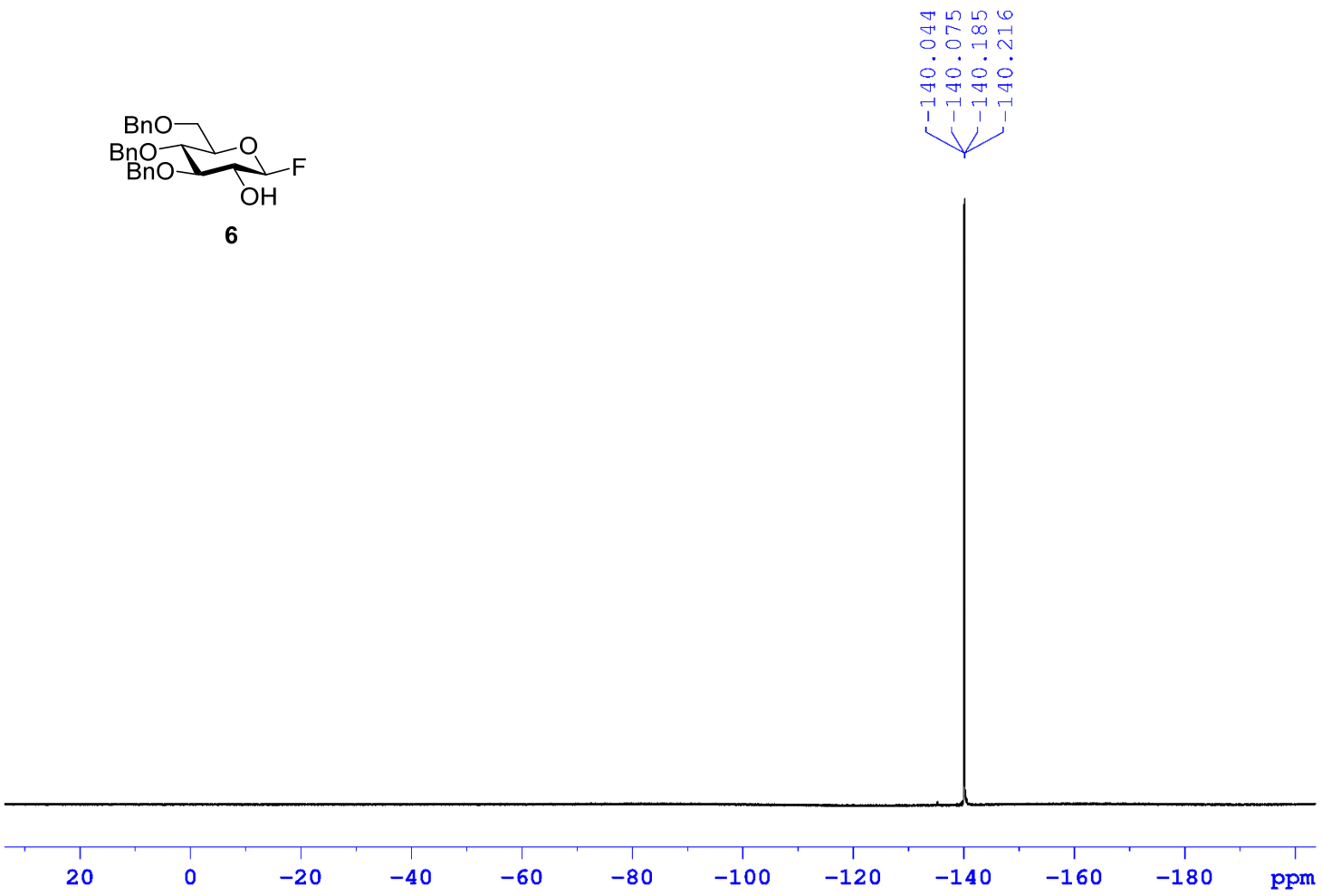
^{13}C NMR (100 MHz) of **6** in $CDCl_3$



DEPT NMR (100 MHz) of **6** in $CDCl_3$

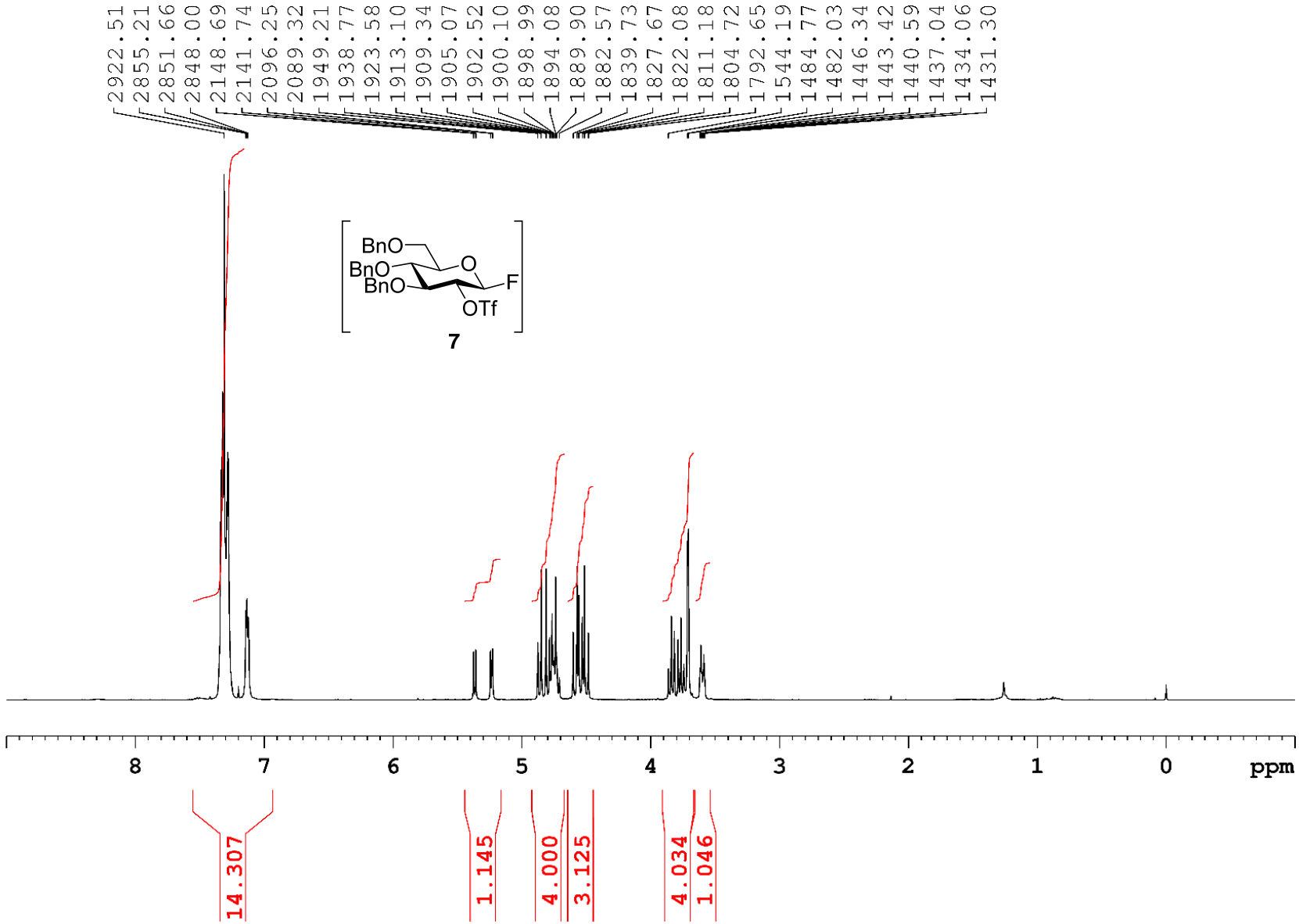


^{19}F NMR (376 MHz) of **6** in $CDCl_3$

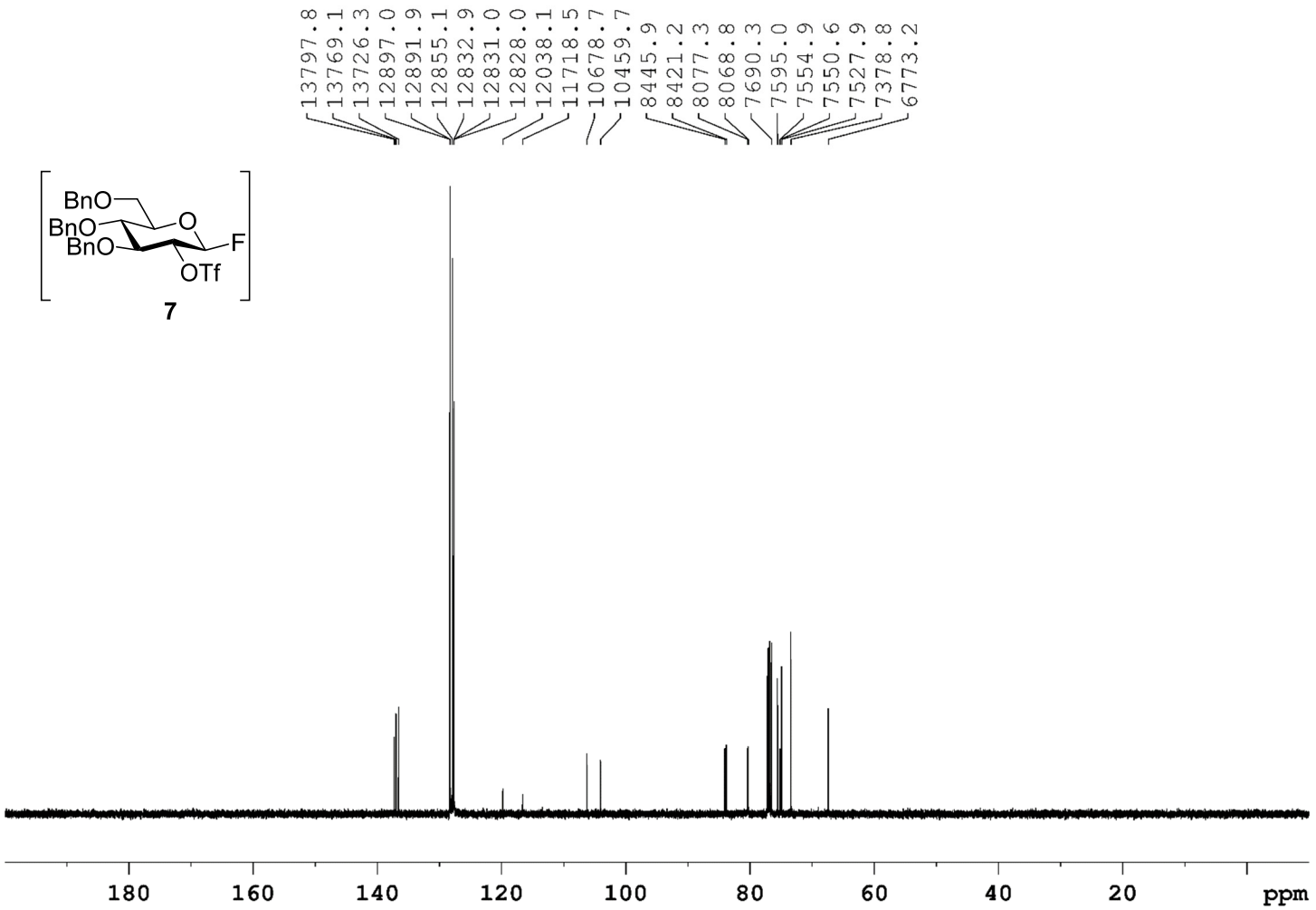


6

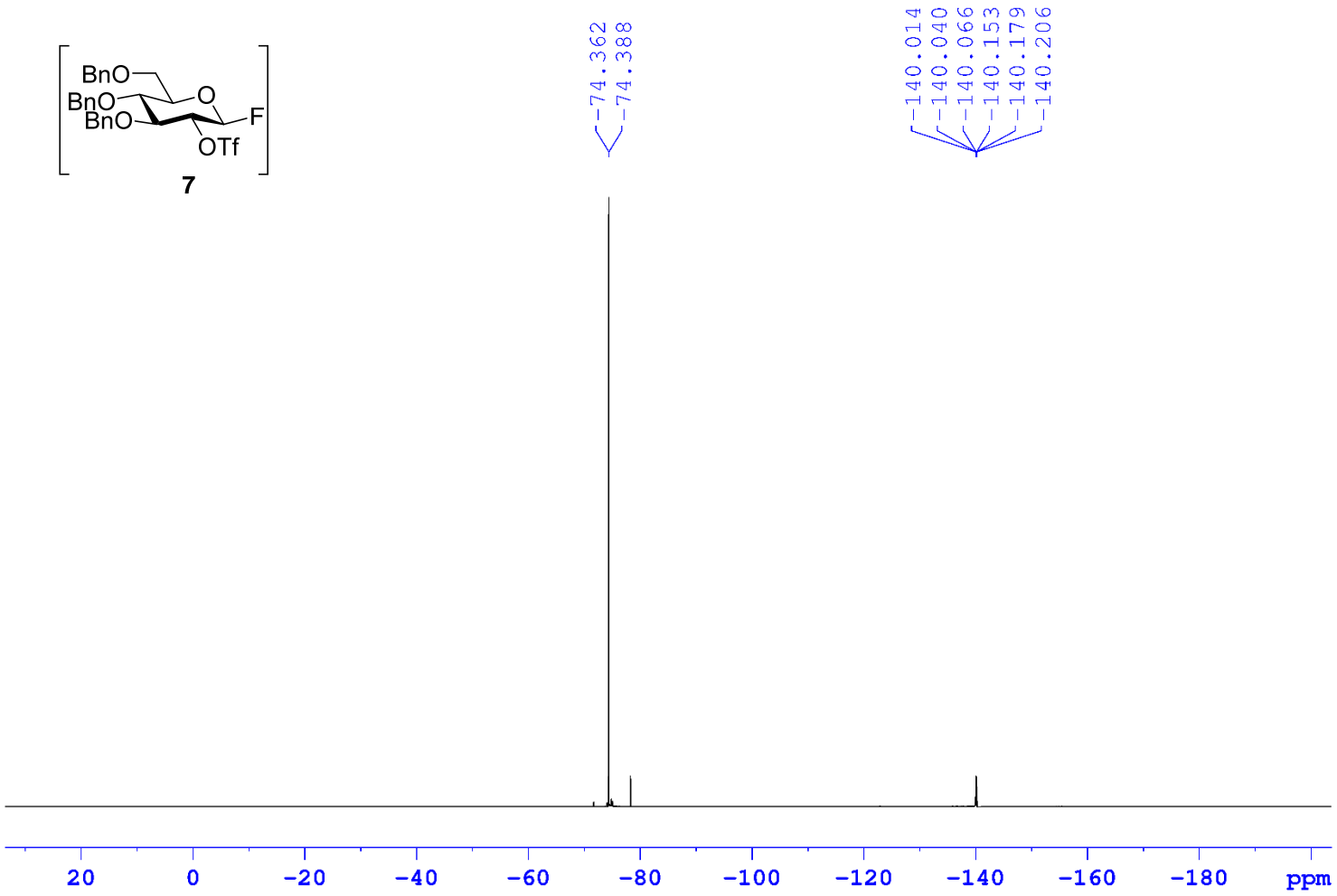
^{1}H NMR (400 MHz) of **7** in $CDCl_3$



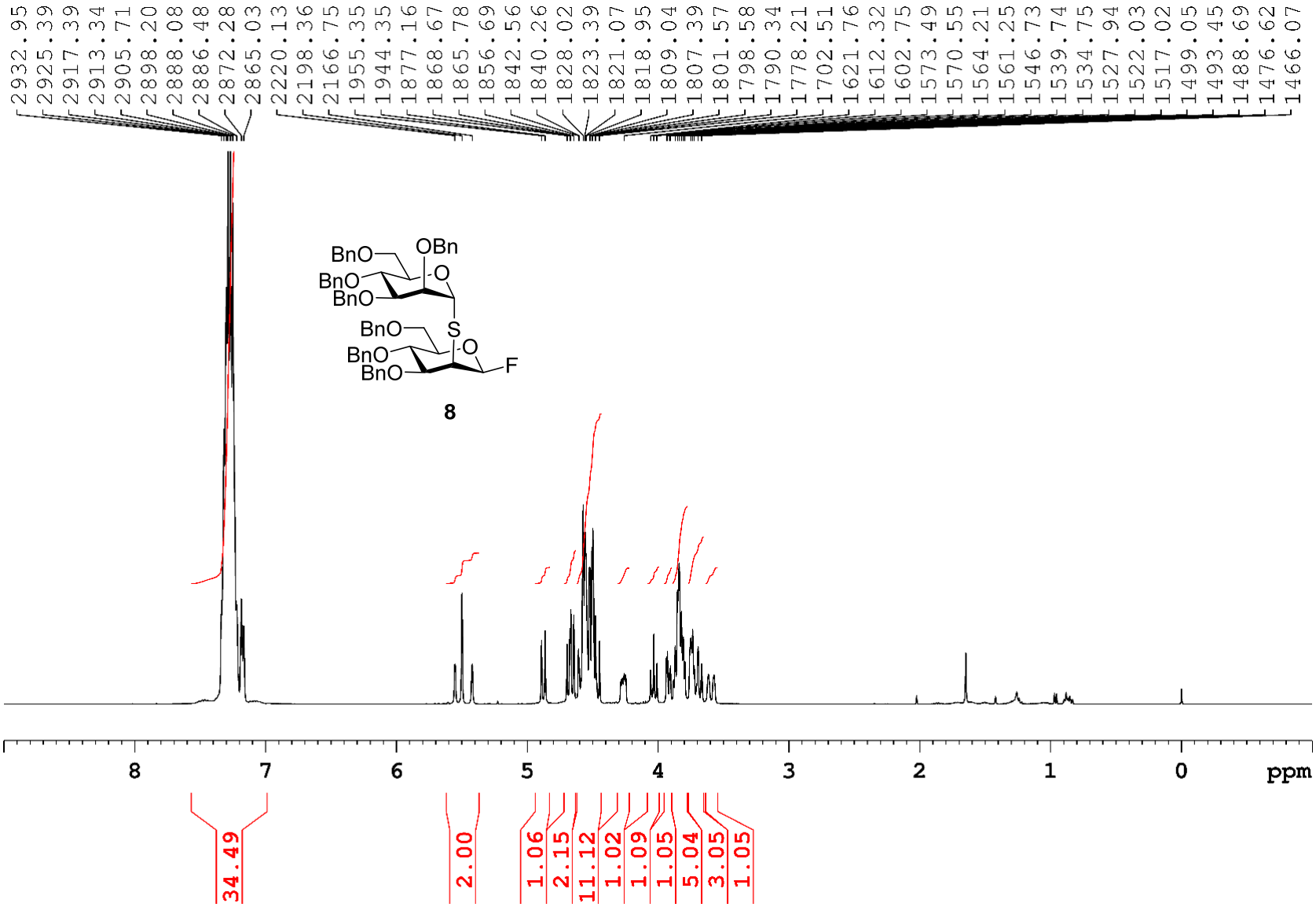
^{13}C NMR (100 MHz) of **7** in $CDCl_3$



^{19}F NMR (376 MHz) of **7** in $CDCl_3$



^{1}H NMR (400 MHz) of **8** in $CDCl_3$



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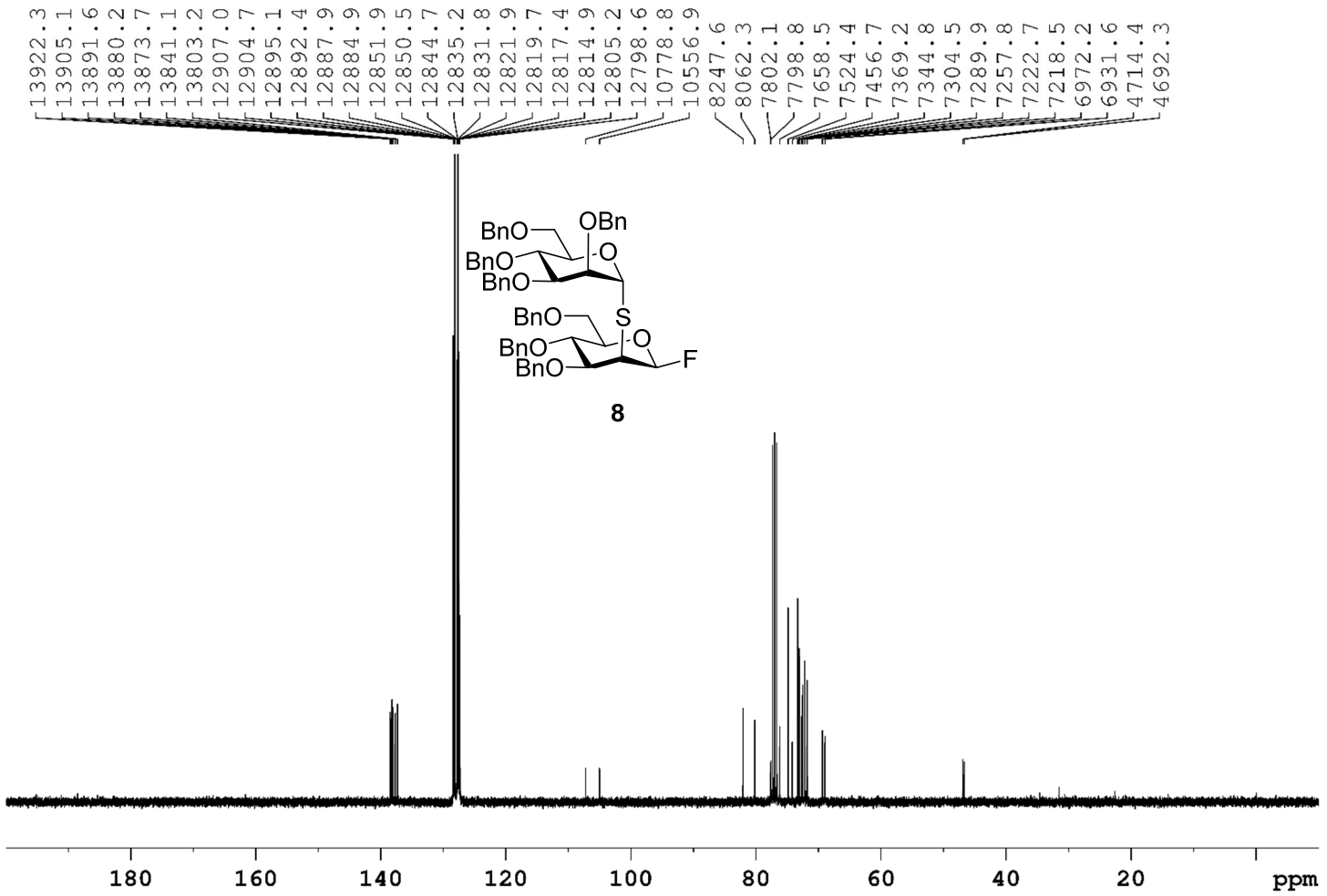
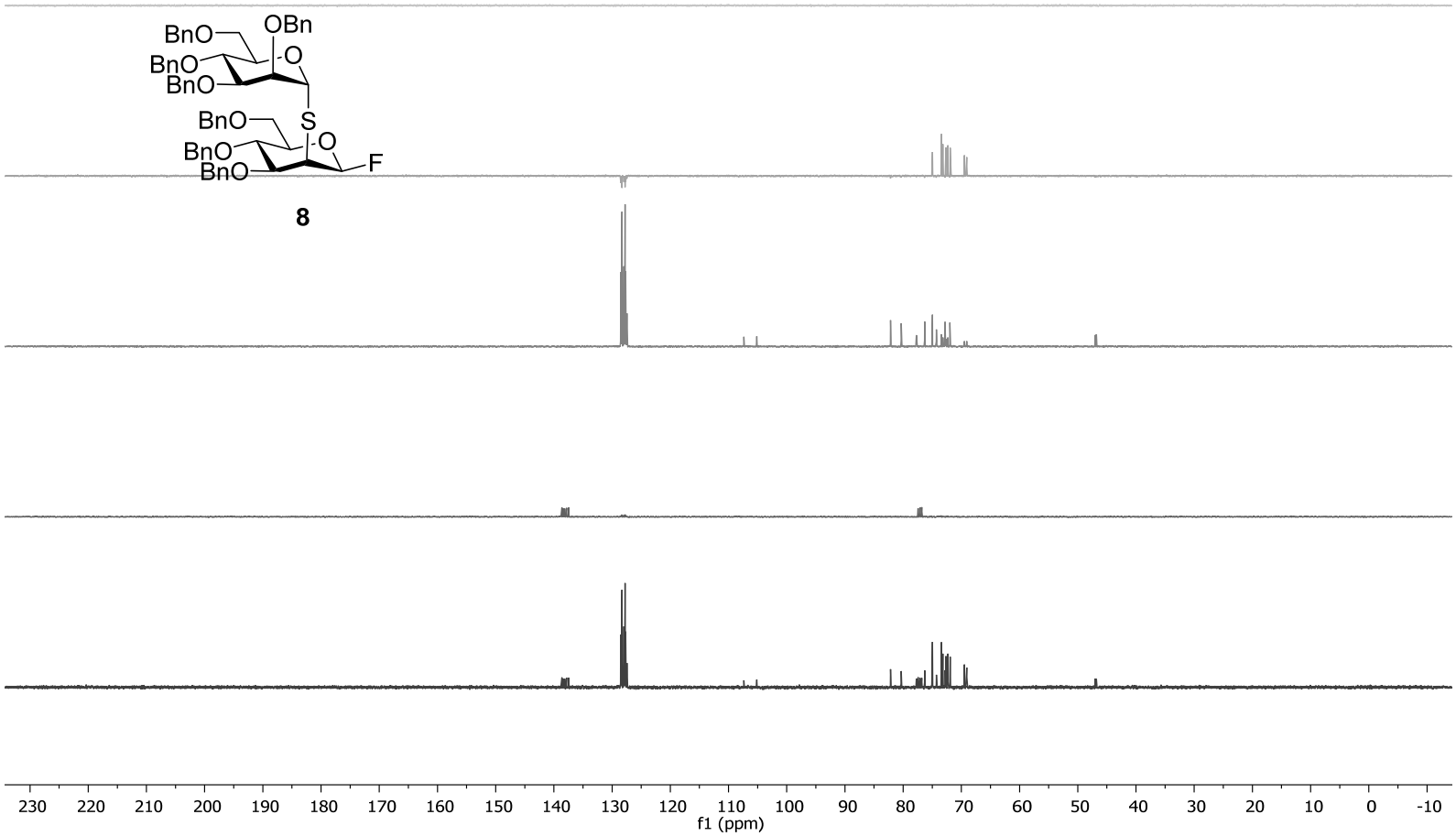
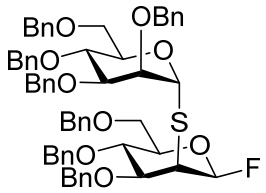


DEPT NMR (100 MHz) of **8** in $CDCl_3$



^{19}F NMR (376 MHz) of **8** in $CDCl_3$



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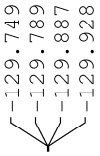
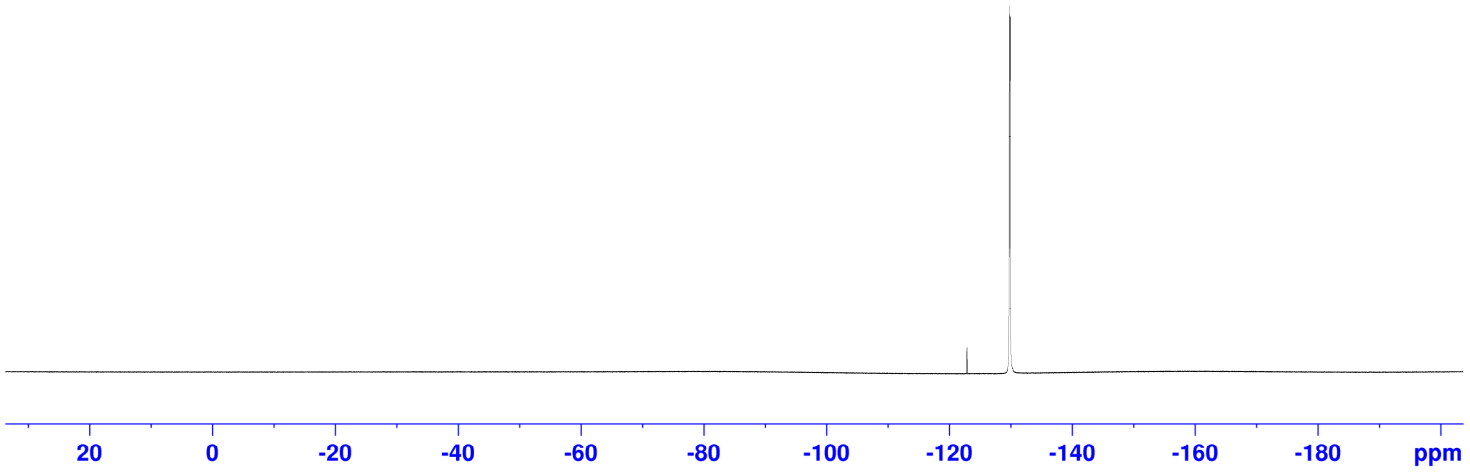
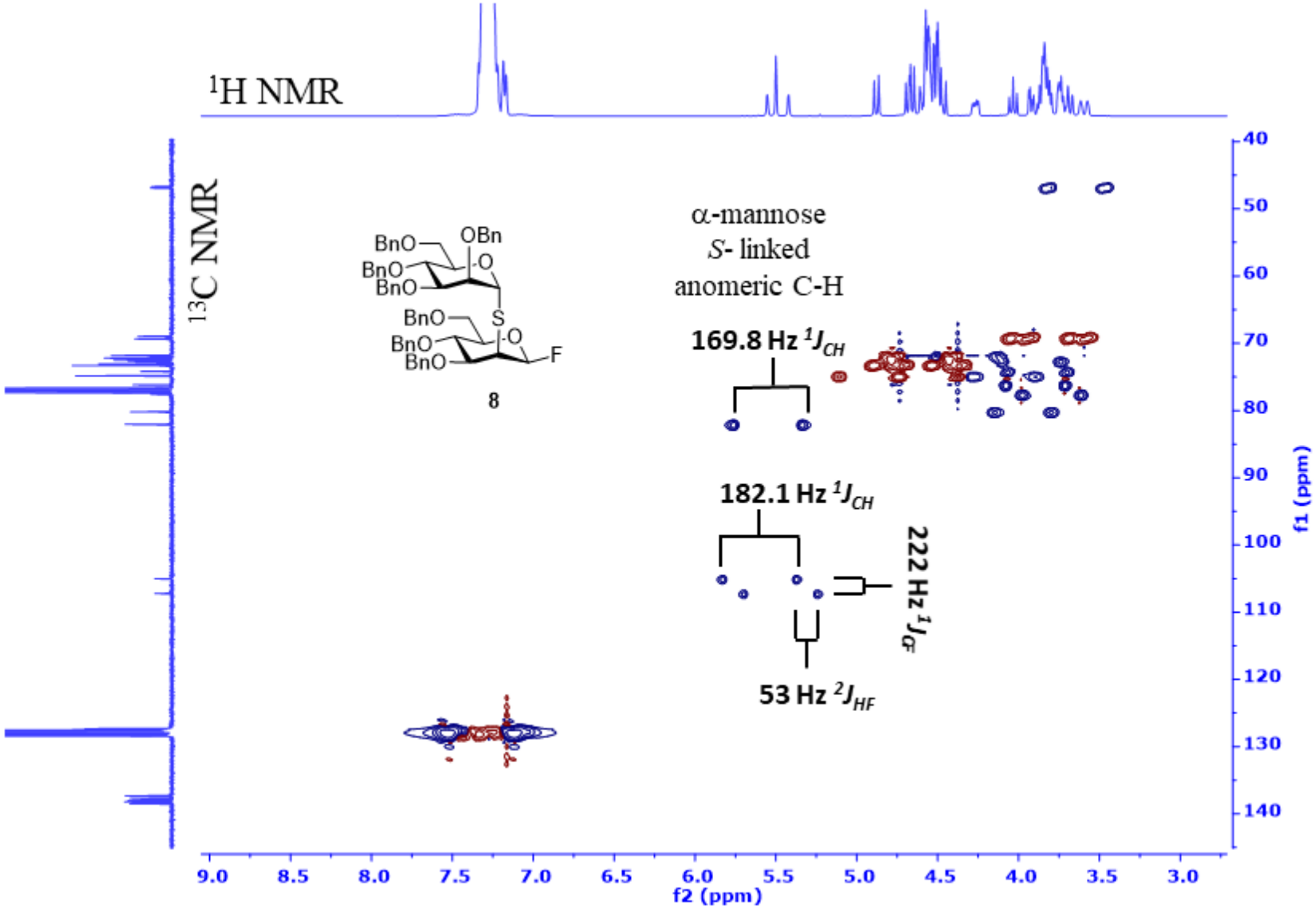
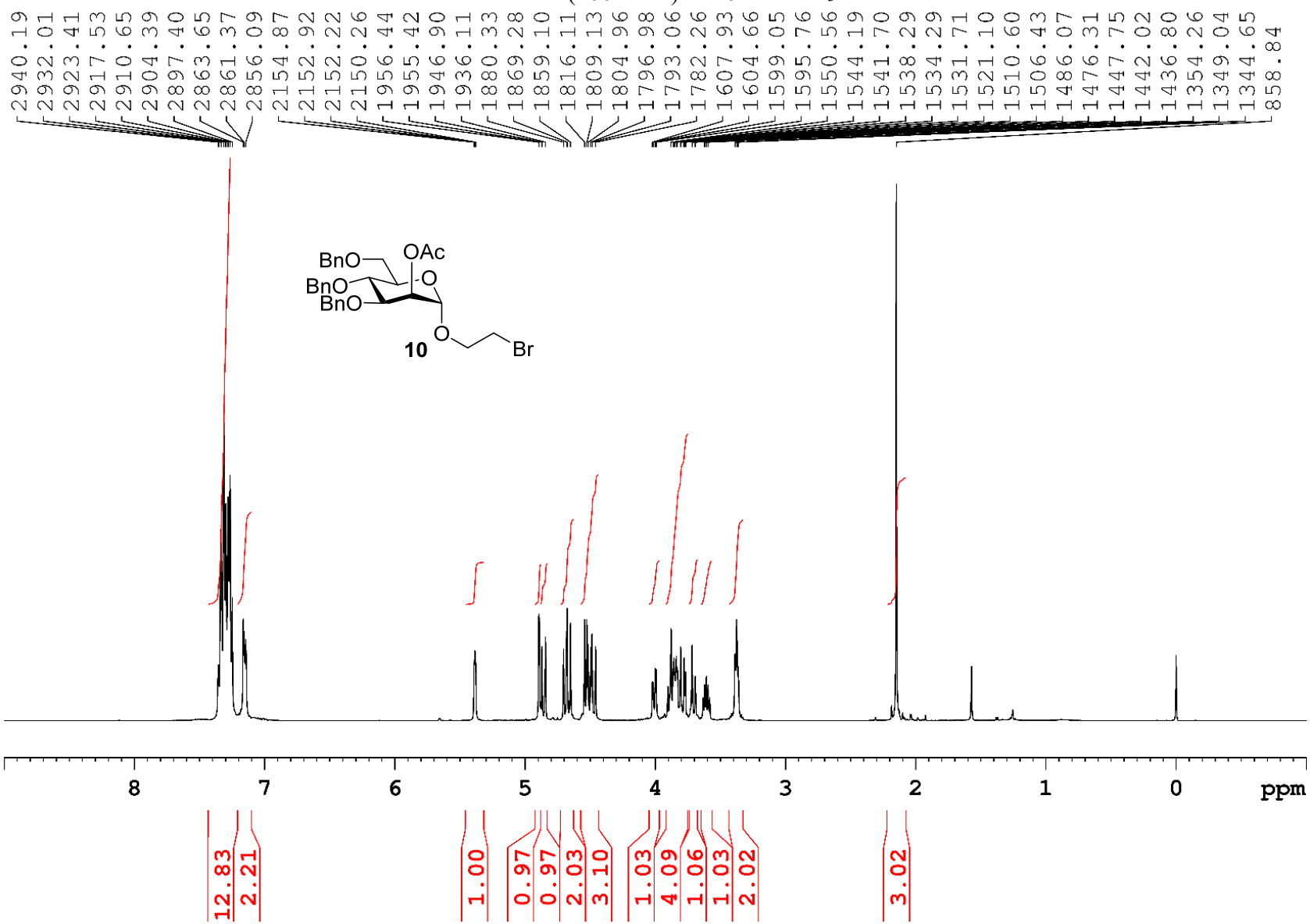




^{1}H - ^{13}C coupled HSQC NMR (400 MHz) of **8** in $CDCl_3$



^{1}H NMR (400 MHz) of **10** in $CDCl_3$



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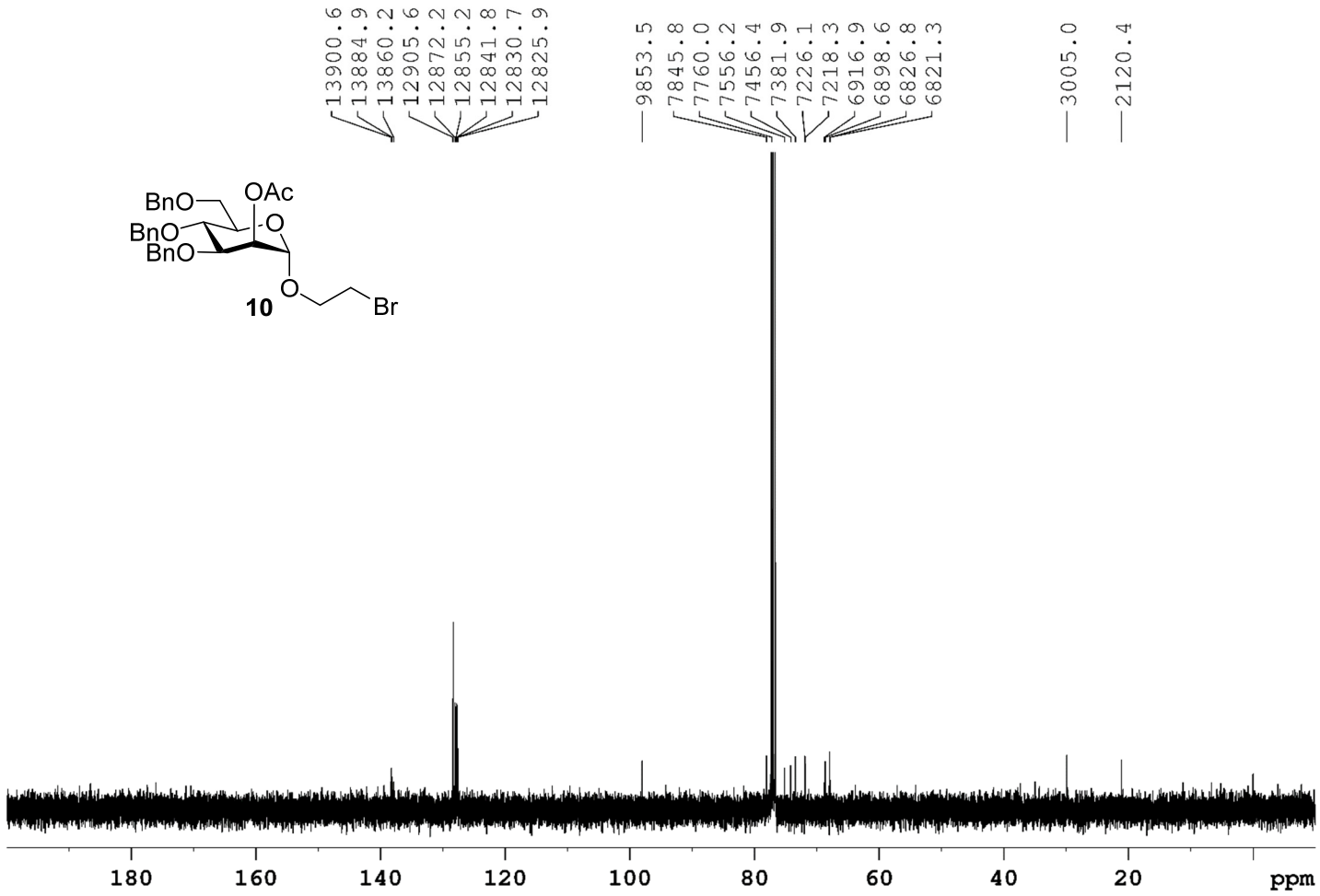
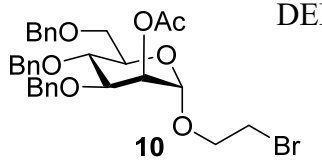


DEPT NMR (100 MHz) of **10** in $CDCl_3$



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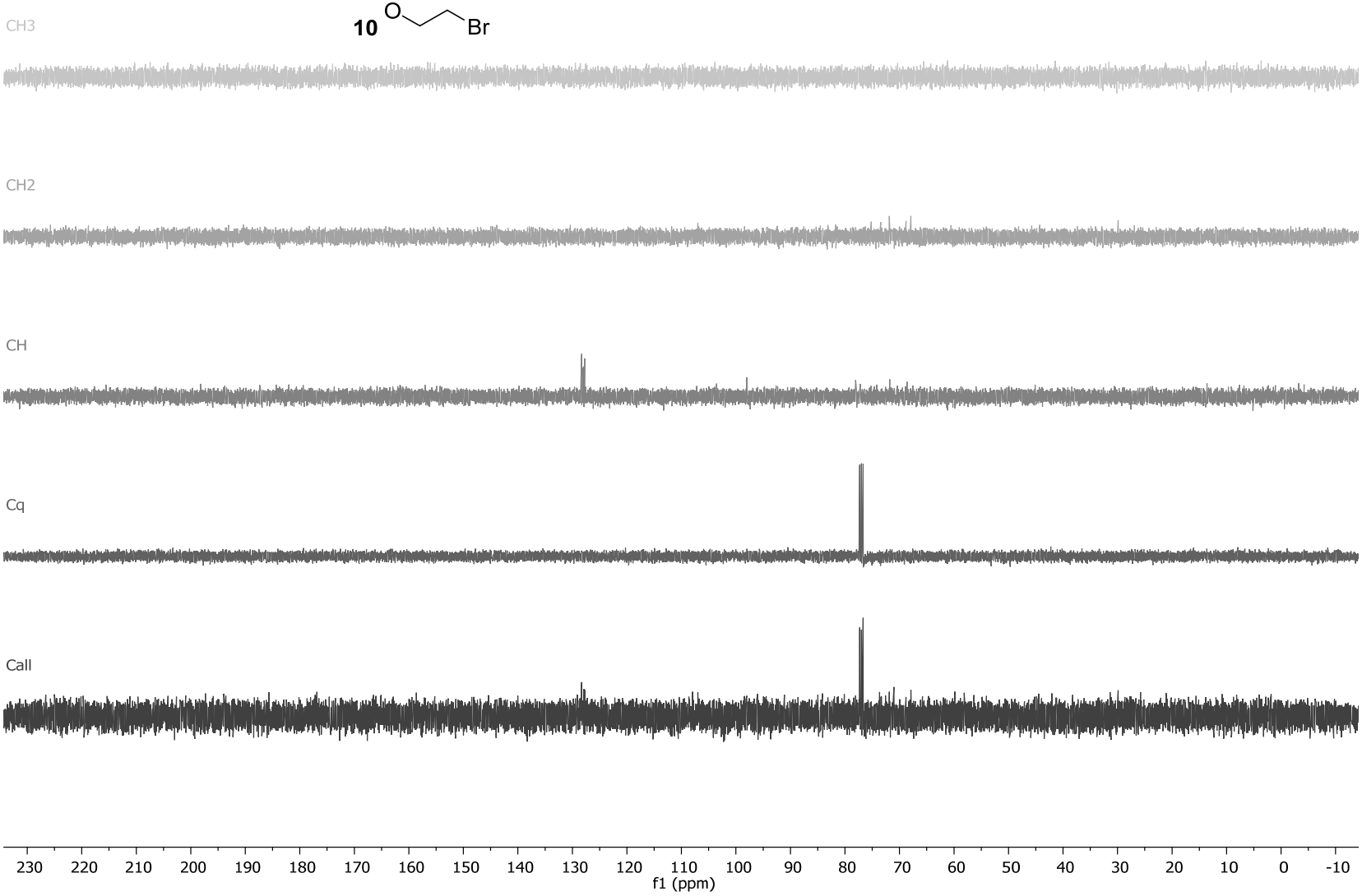
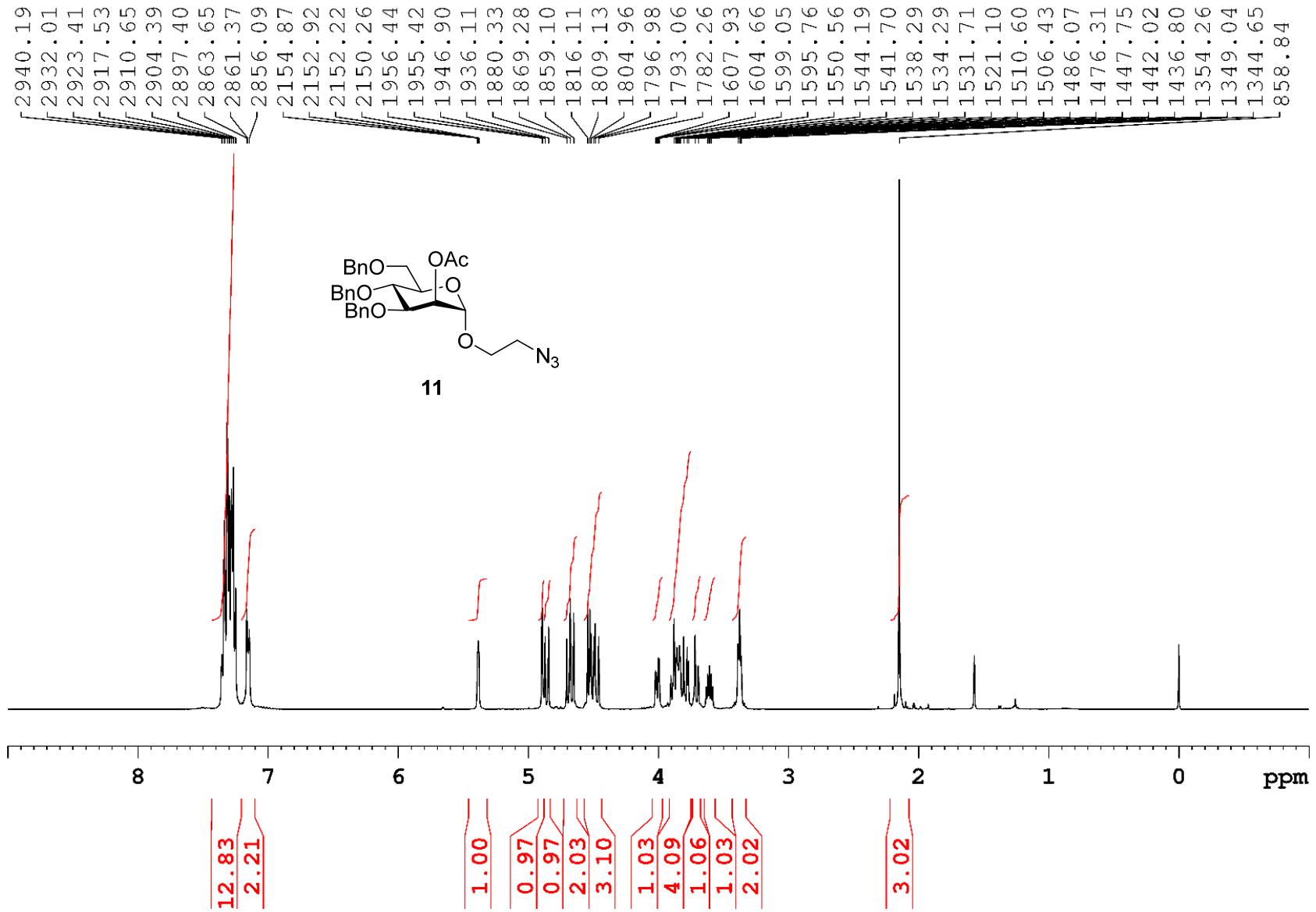
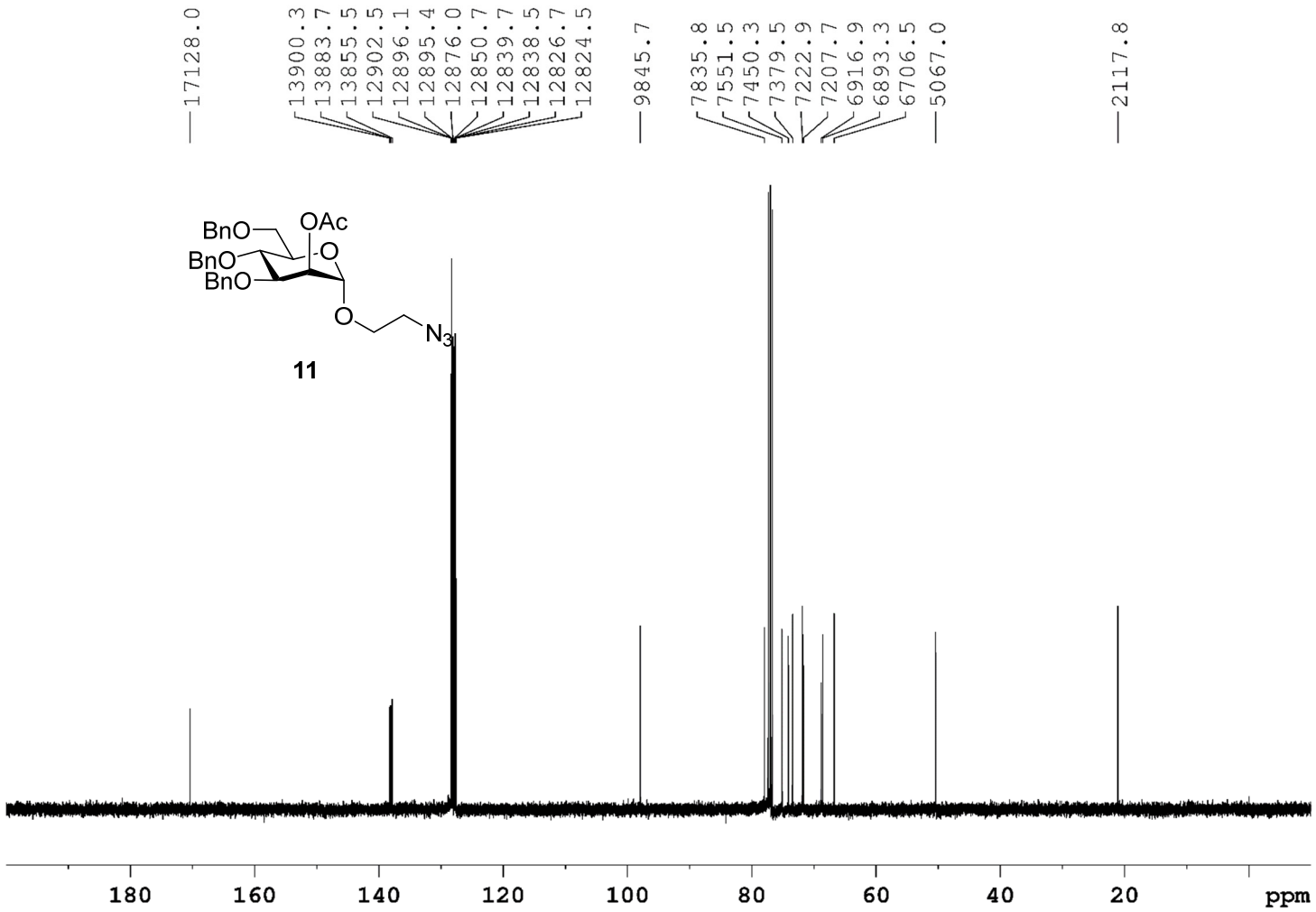


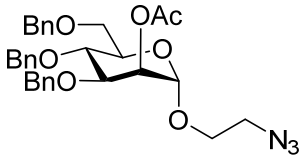
^{1}H NMR (400 MHz) of **11** in $CDCl_3$



^{13}C NMR (100 MHz) of **11** in $CDCl_3$



DEPT NMR (100 MHz) of **11** in $CDCl_3$



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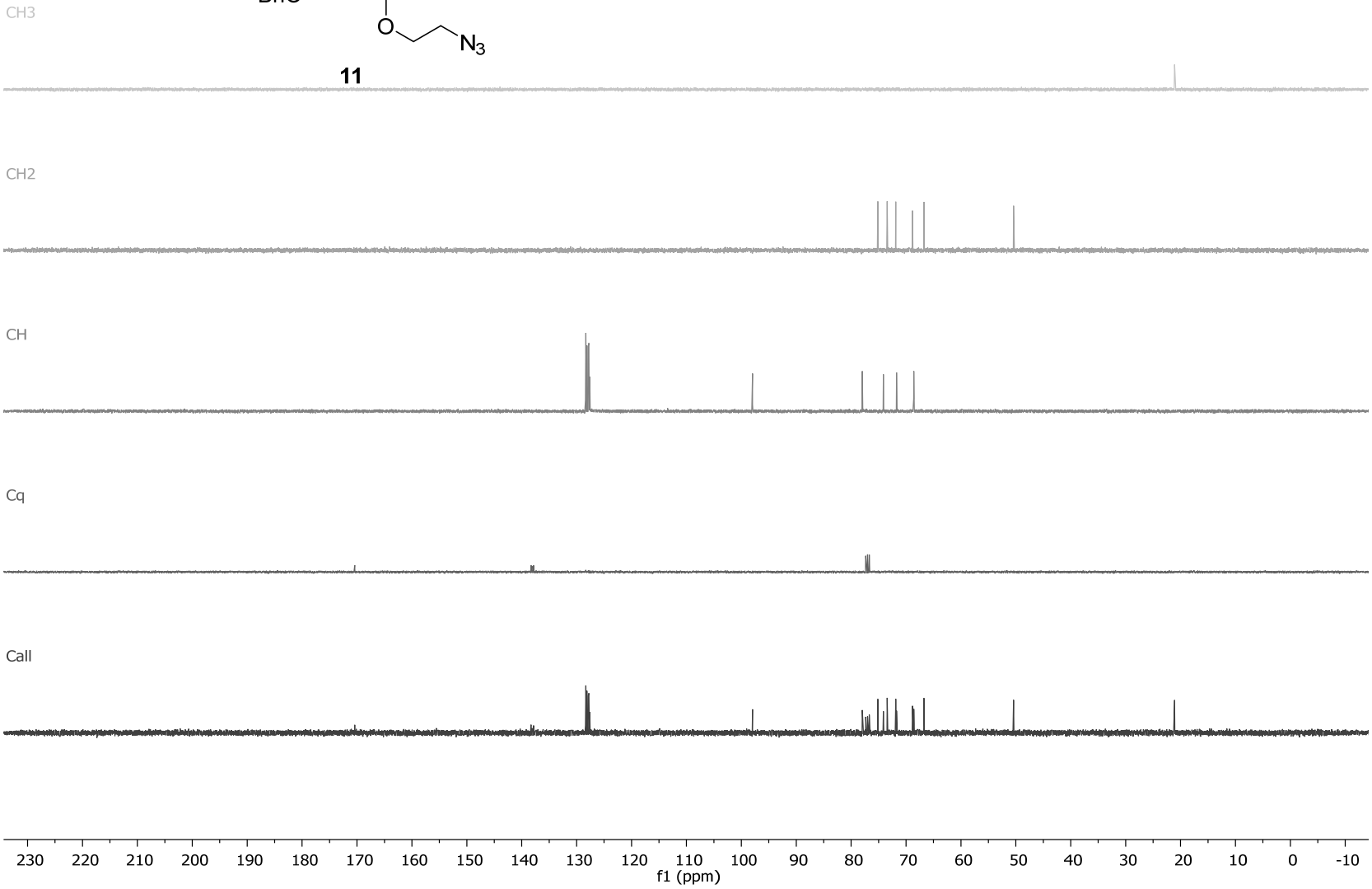
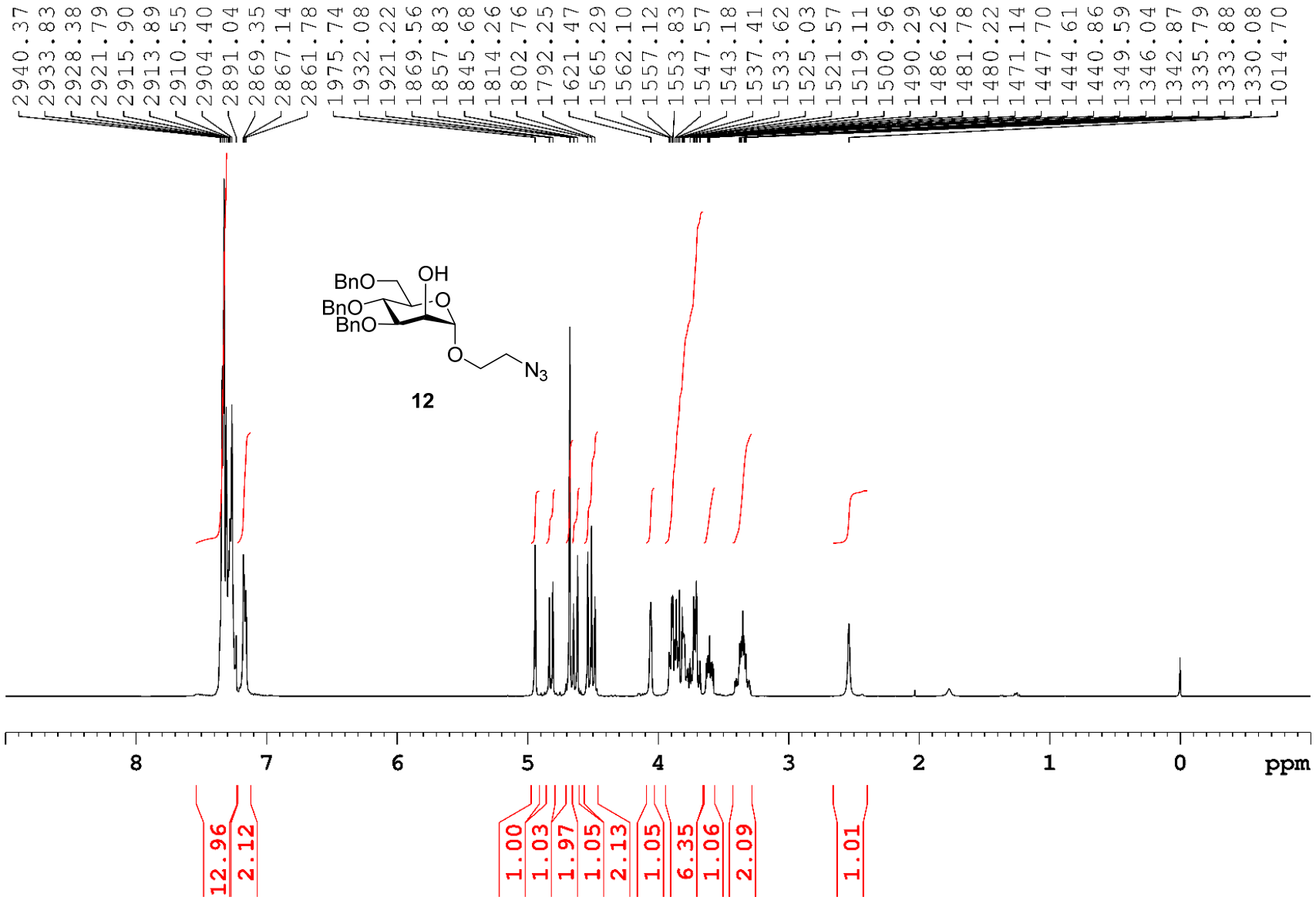
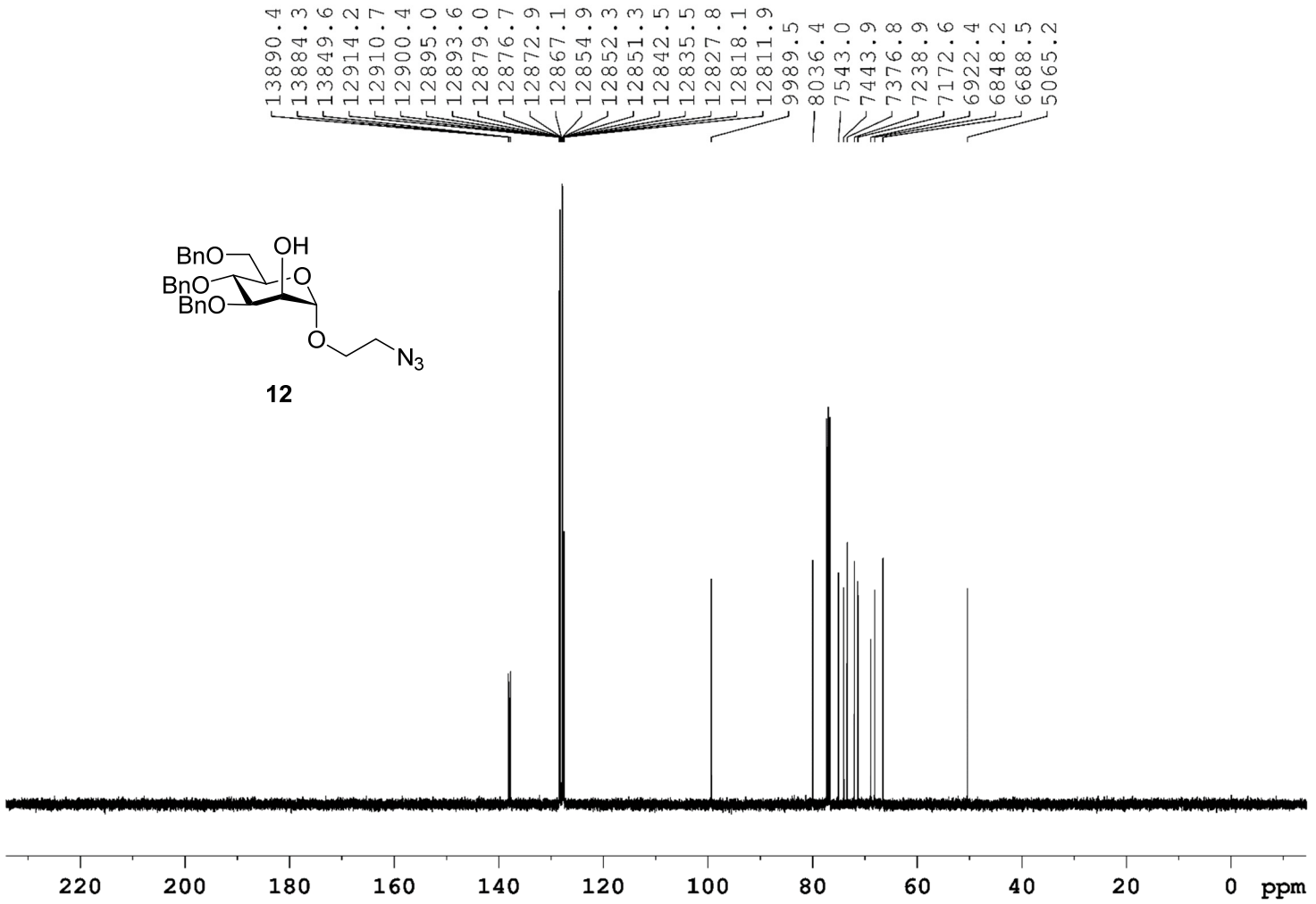


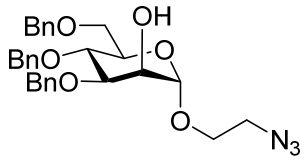
^{1}H NMR (400 MHz) of **12** in $CDCl_3$



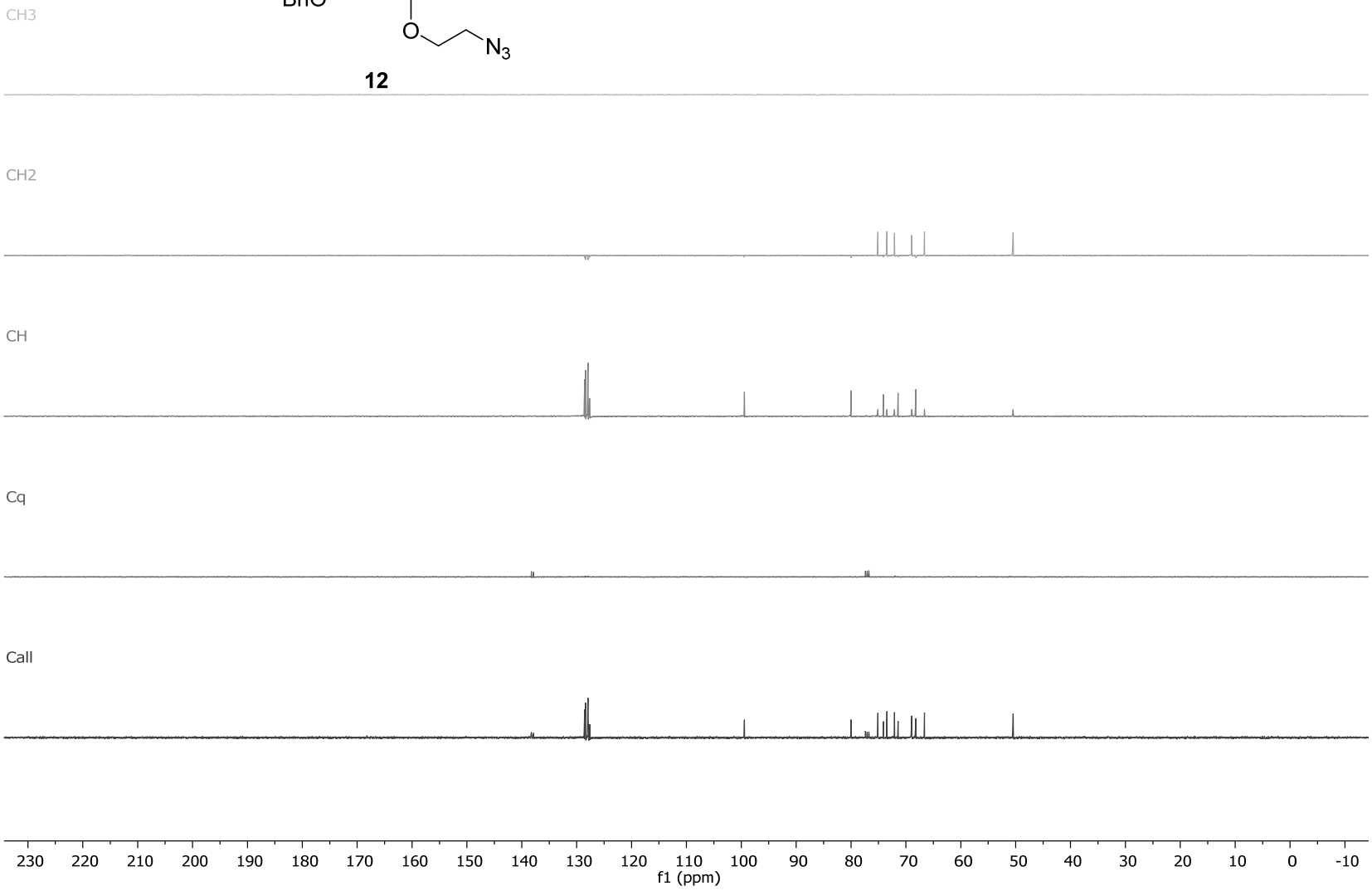
^{13}C NMR (100 MHz) of **12** in $CDCl_3$



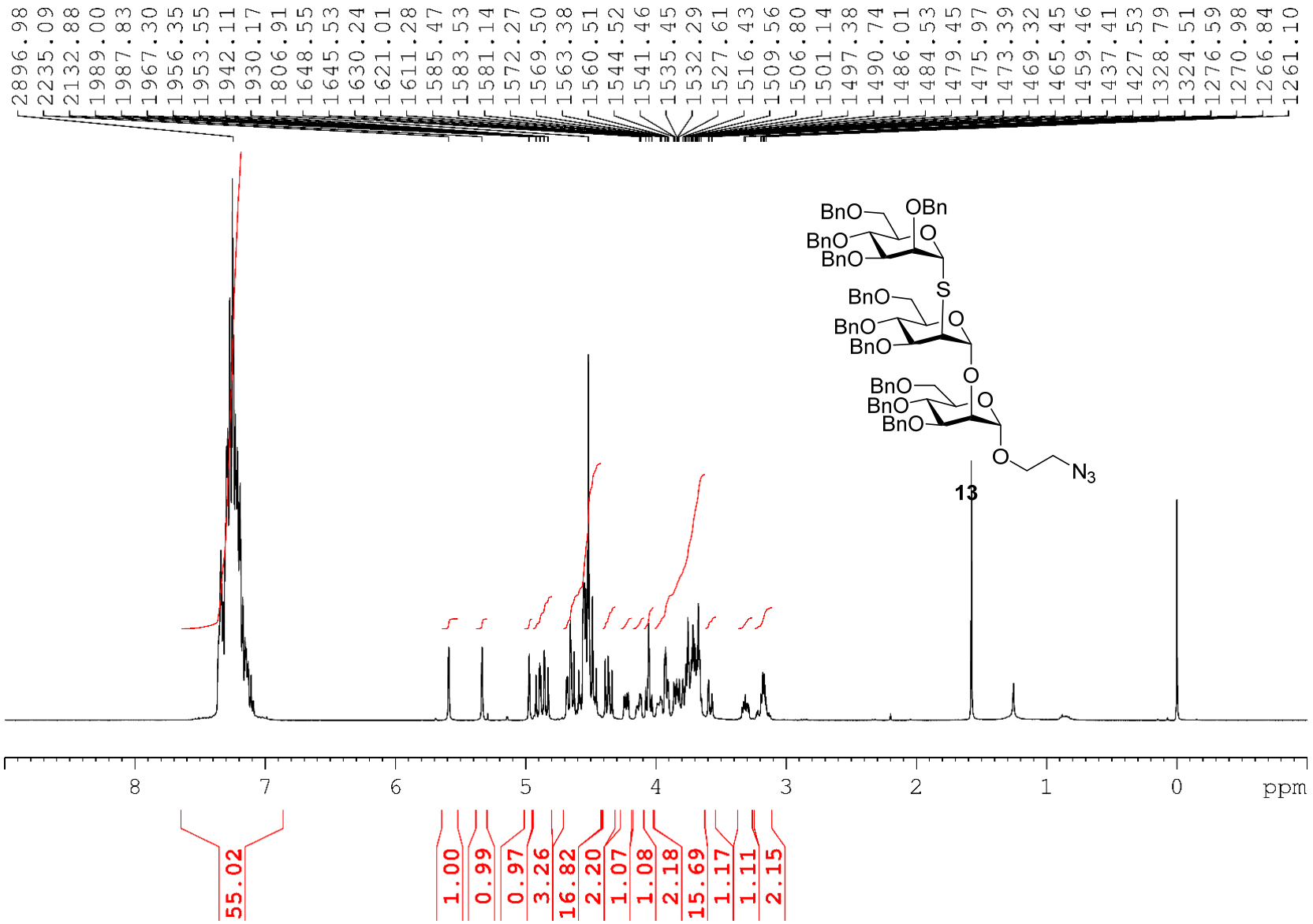
DEPT NMR (100 MHz) of **12** in $CDCl_3$



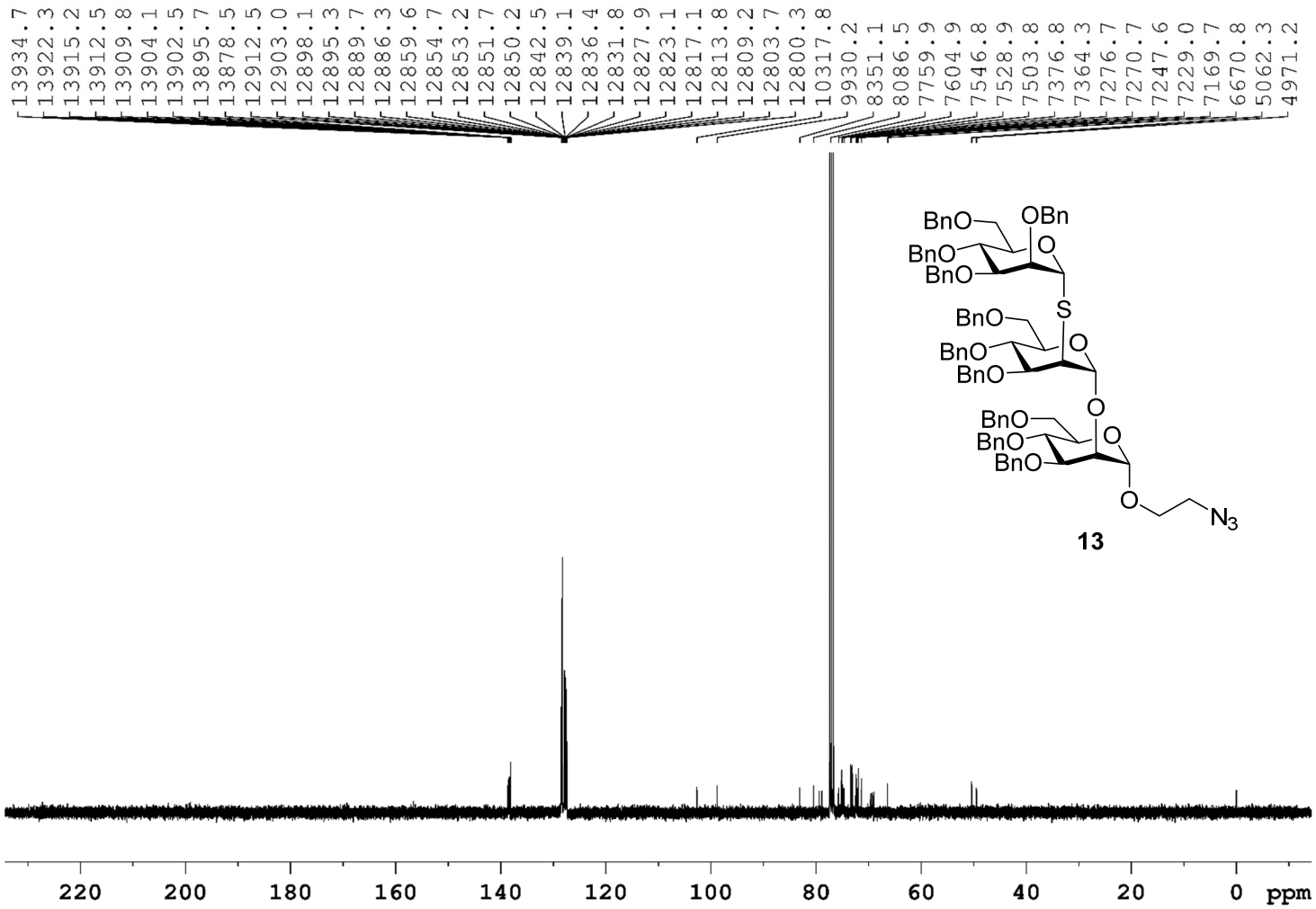
12



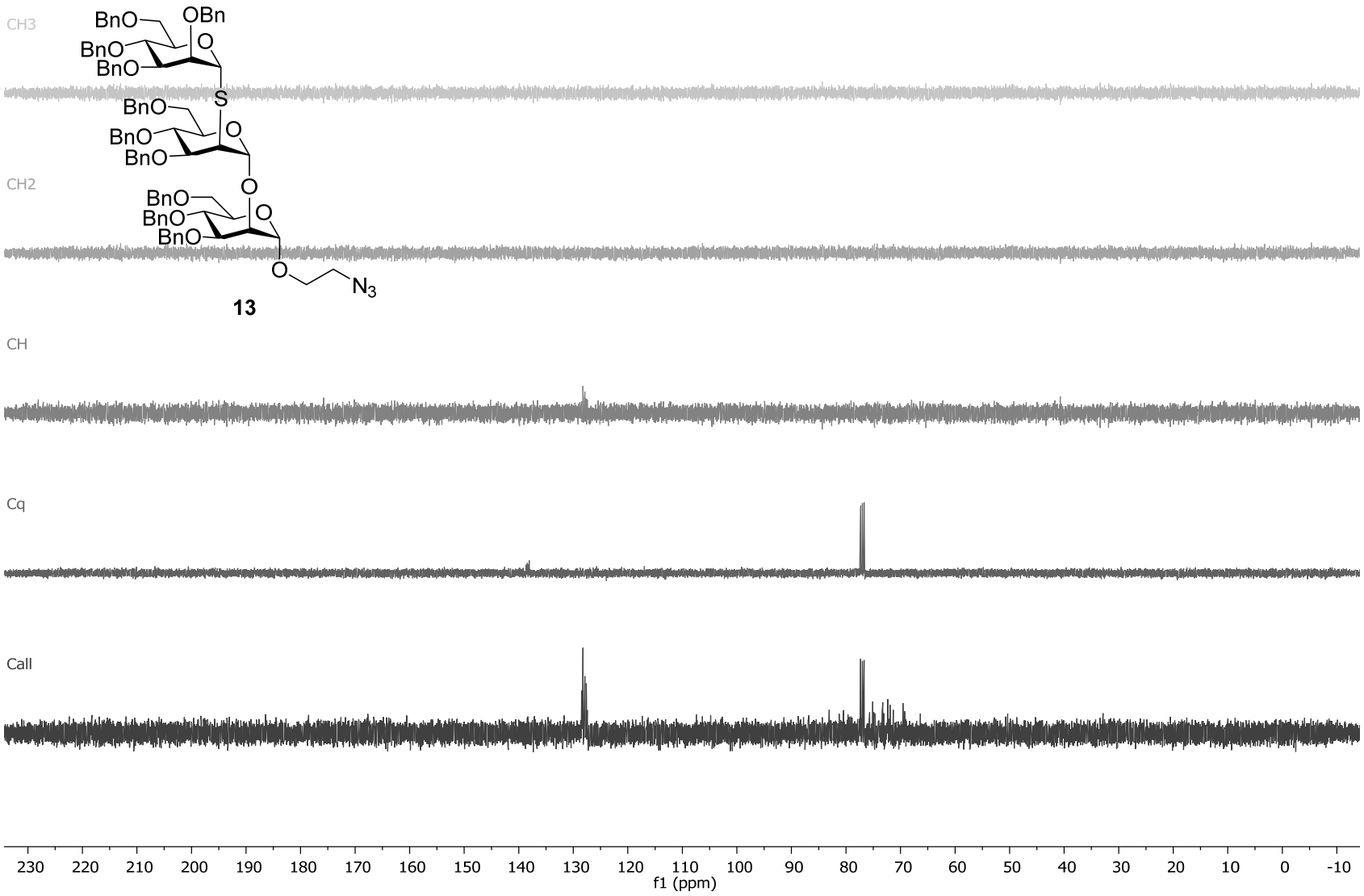
^{1}H NMR (400 MHz) of **13** in $CDCl_3$



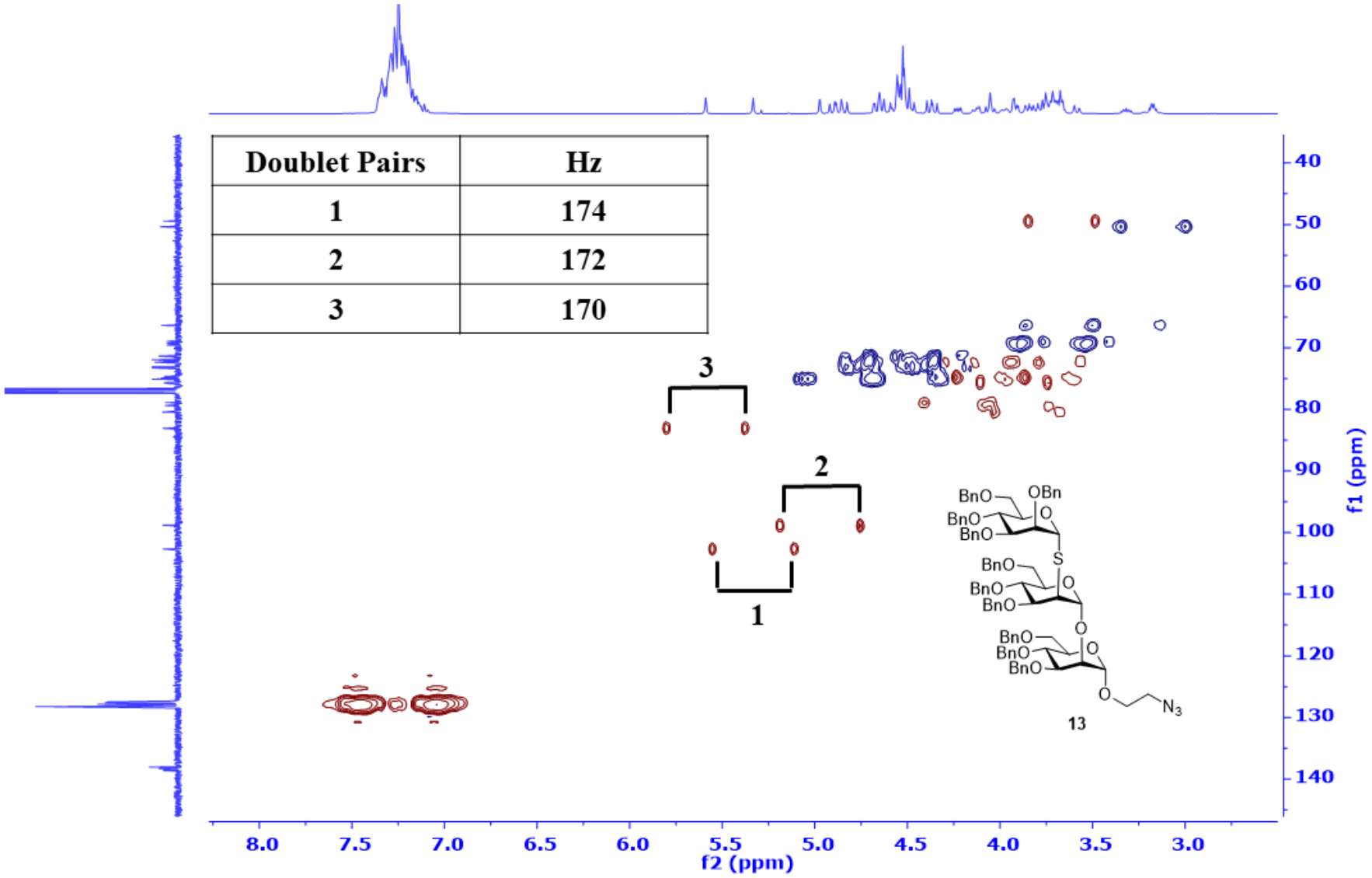
^{13}C NMR (100 MHz) of **13** in $CDCl_3$



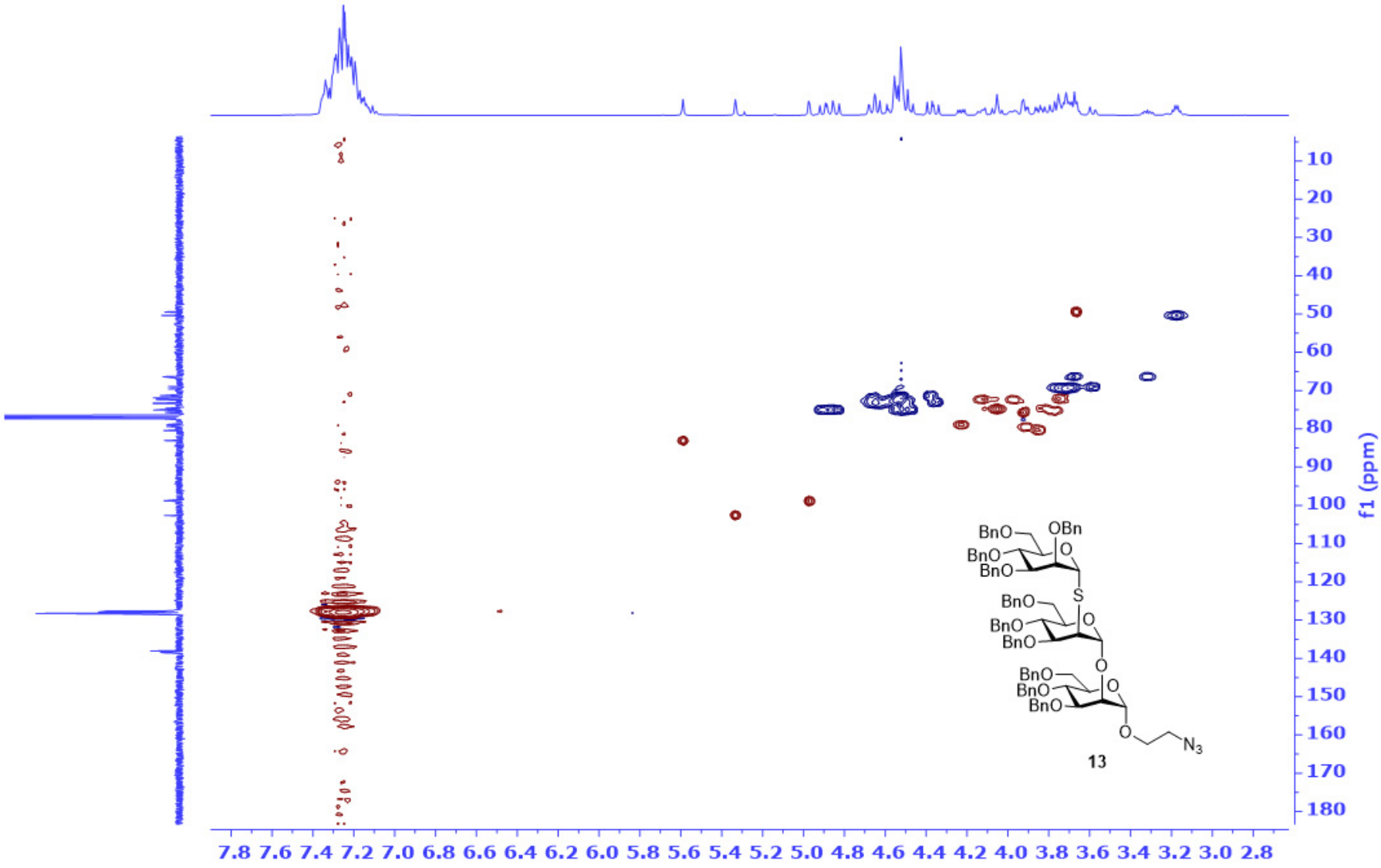
DEPT NMR (100 MHz) of **13** in $CDCl_3$



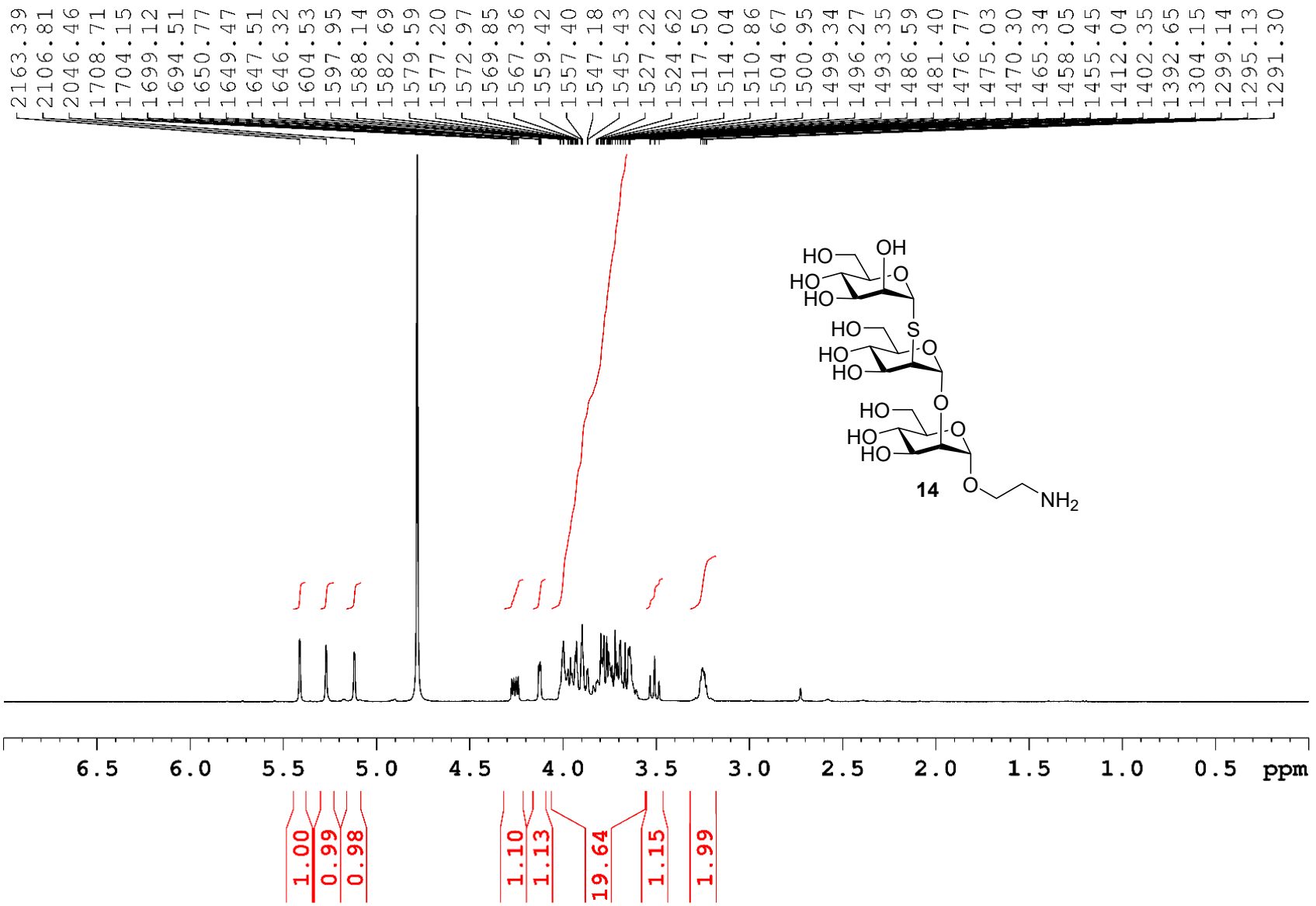
^{1}H - ^{13}C coupled HSQC NMR (400 MHz) of **13** in $CDCl_3$



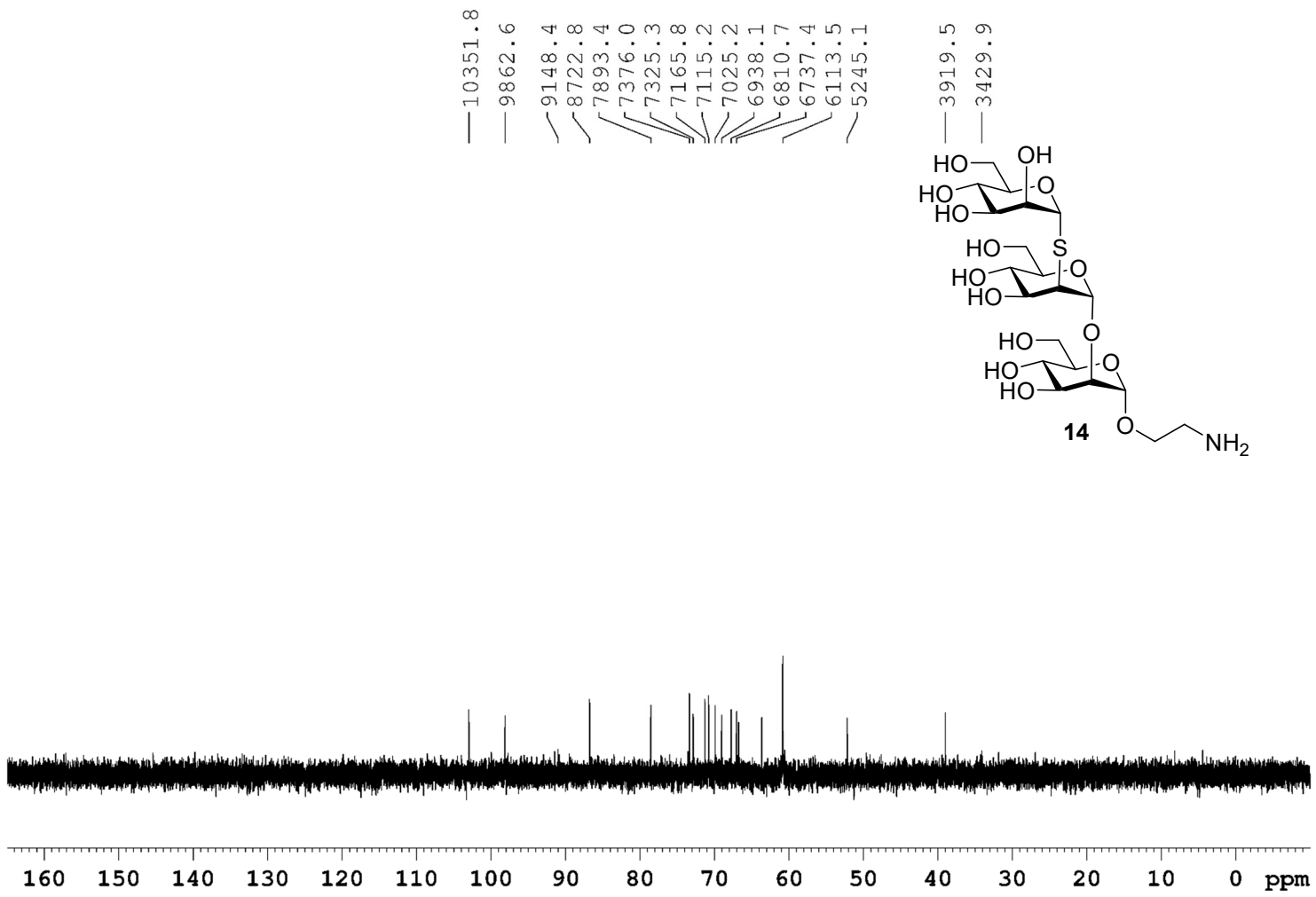
^{1}H - ^{13}C HSQC NMR (400 MHz) of **13** in $CDCl_3$



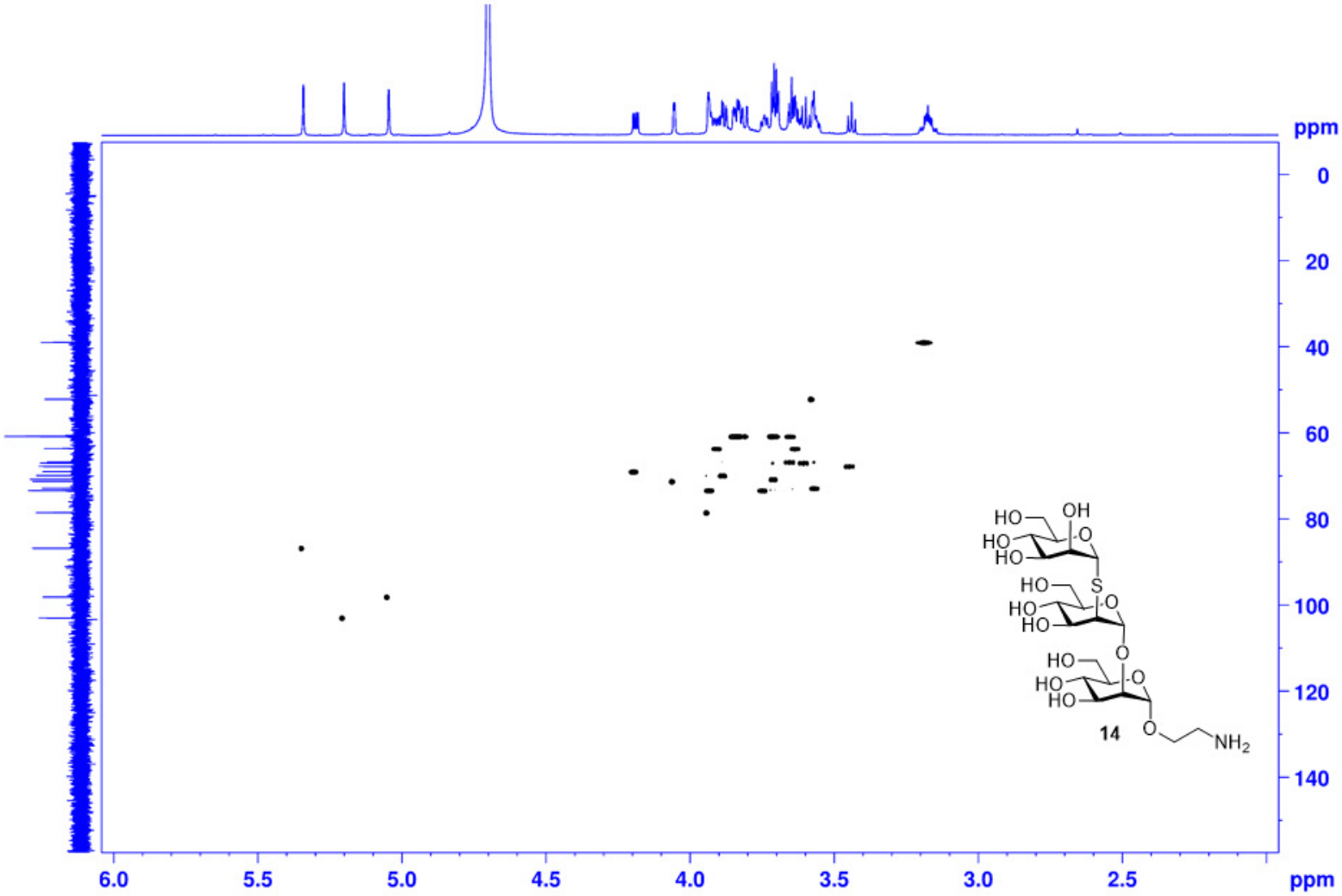
^{1}H NMR (400 MHz) of **14** in D_2O



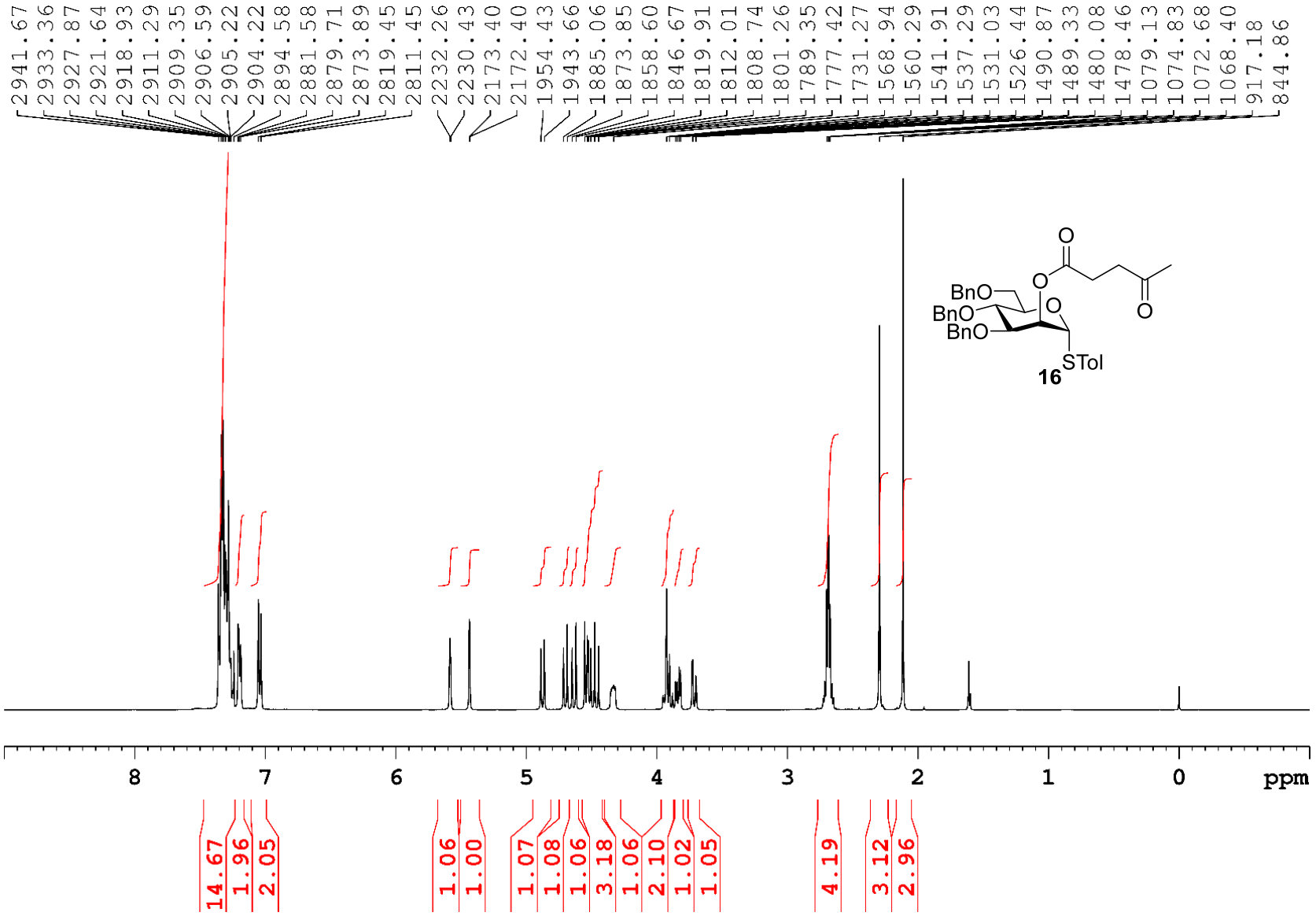
^{13}C NMR (100 MHz) of **14** in D_2O



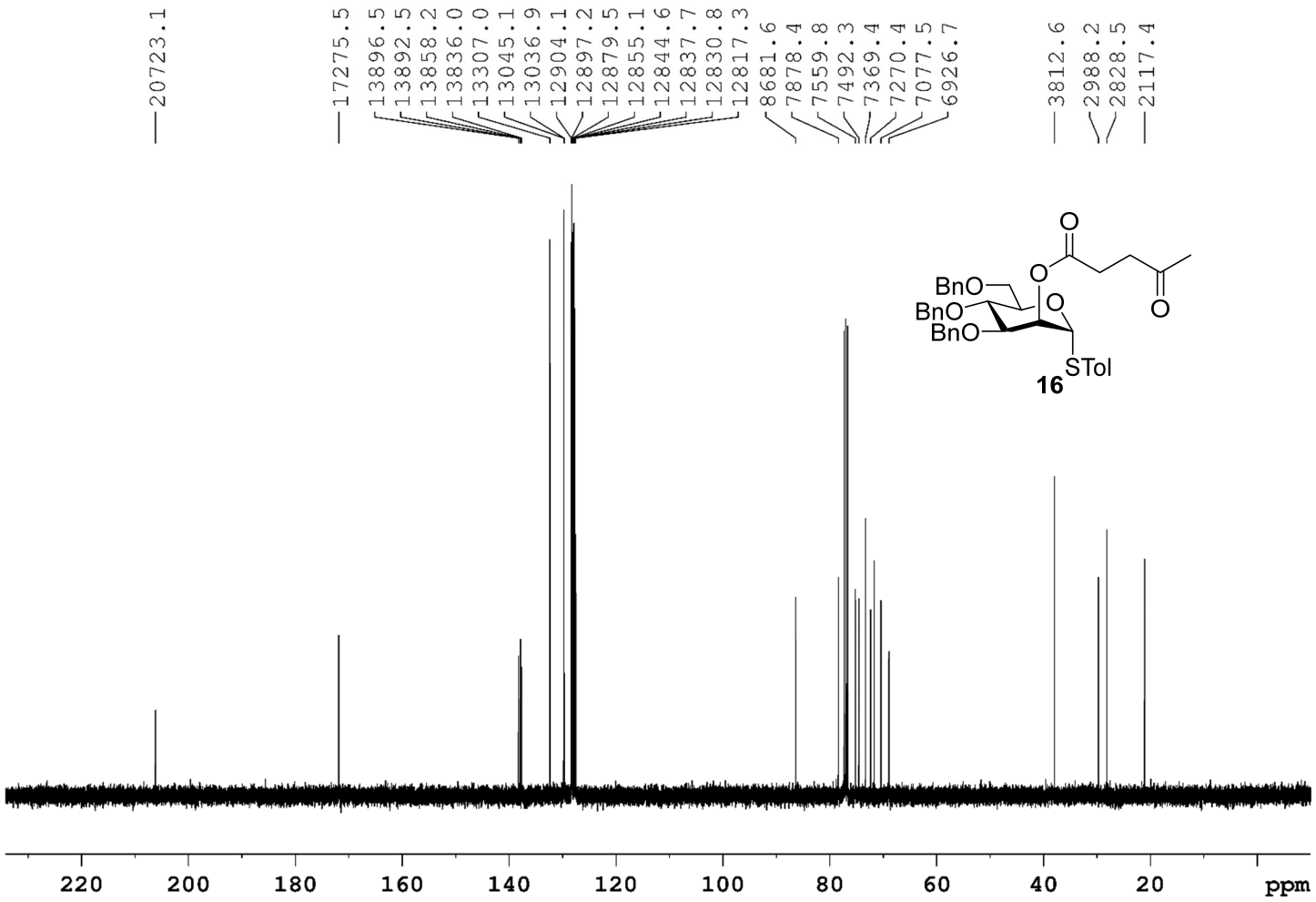
^{1}H - ^{13}C HSQC NMR (800 MHz) of **14** in D_2O



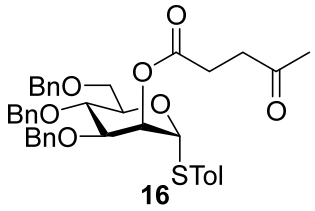
^{1}H NMR (400 MHz) of **16** in $CDCl_3$



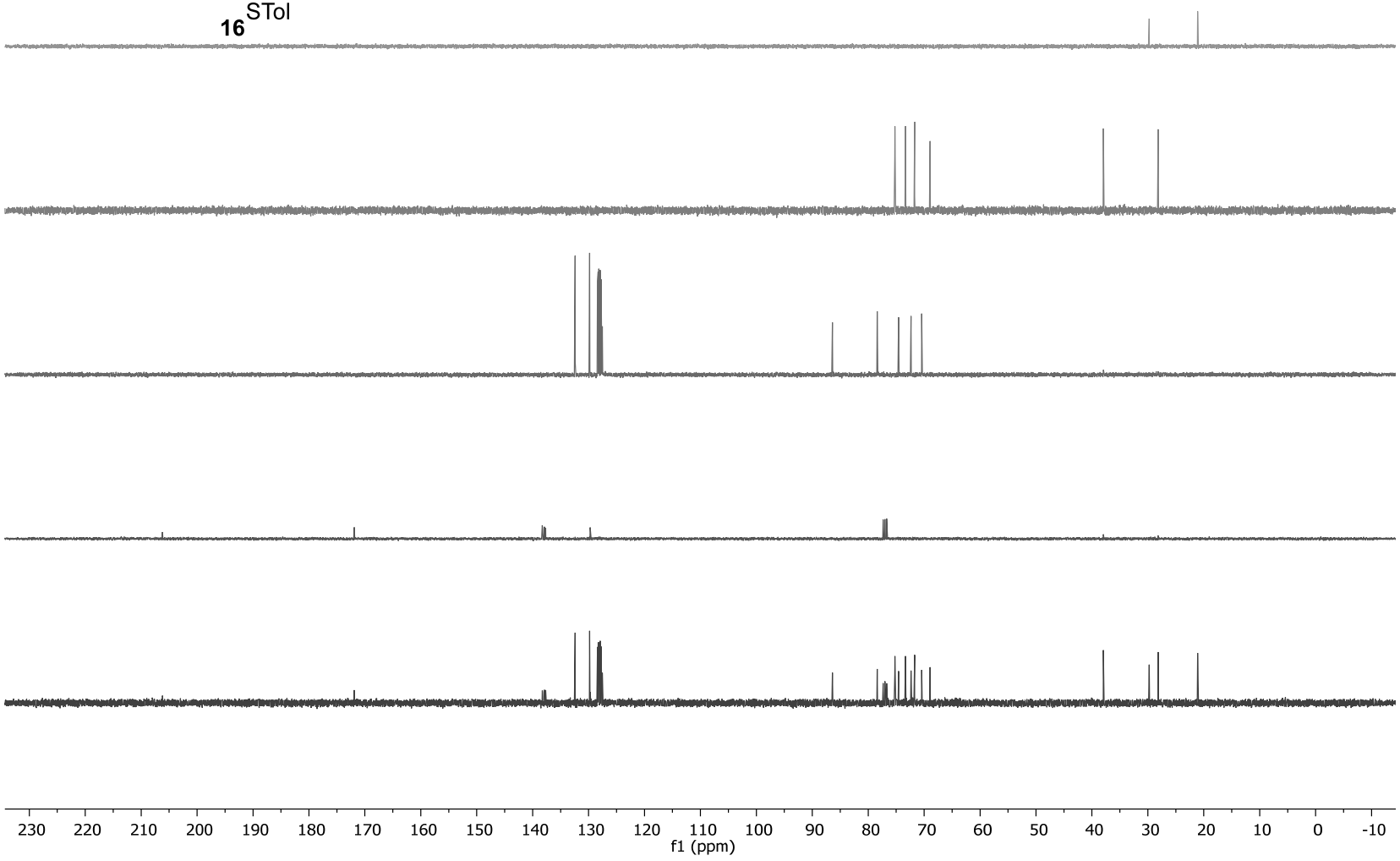
^{13}C NMR (100 MHz) of **16** in $CDCl_3$



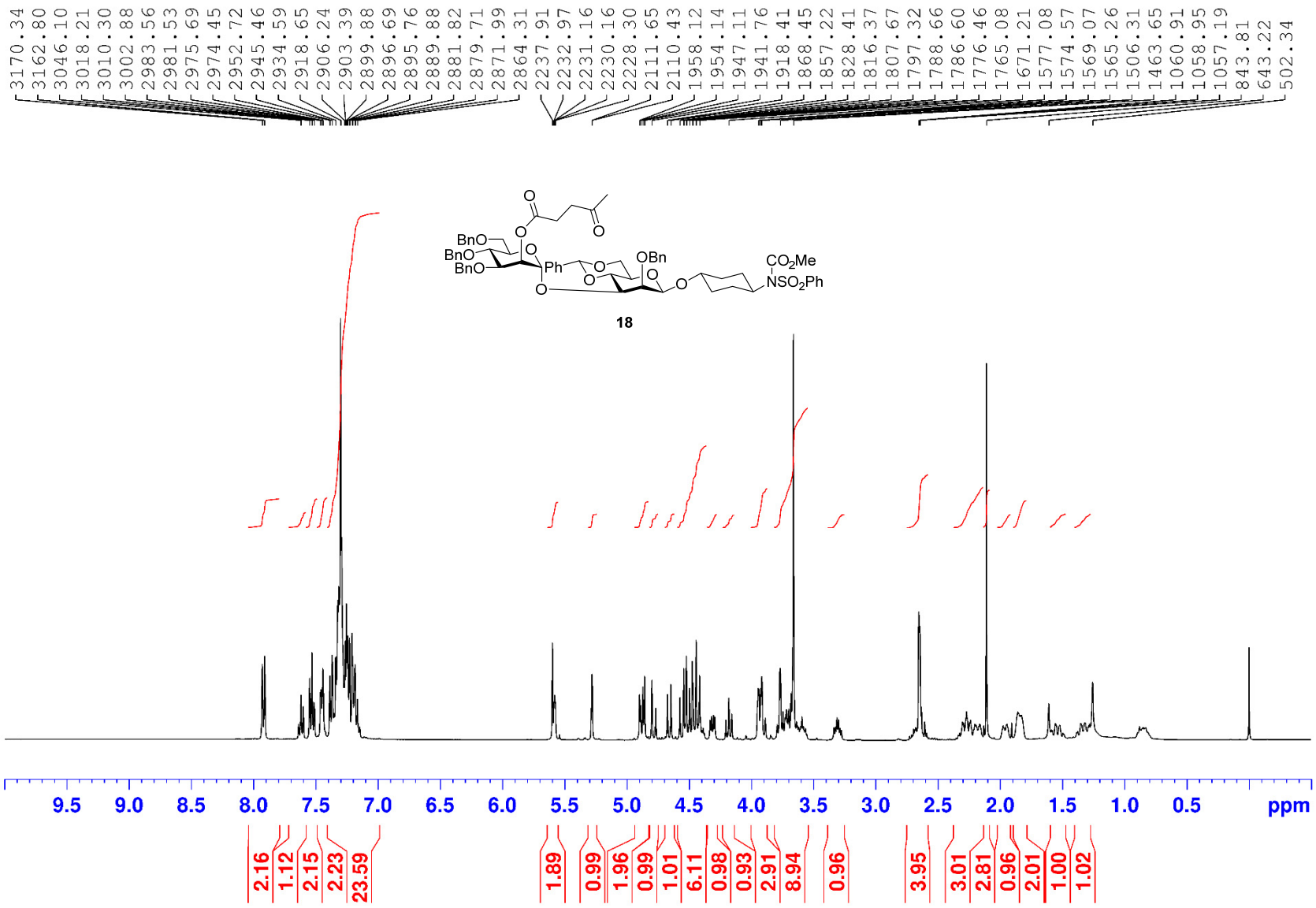
DEPT NMR (100 MHz) of **16** in $CDCl_3$



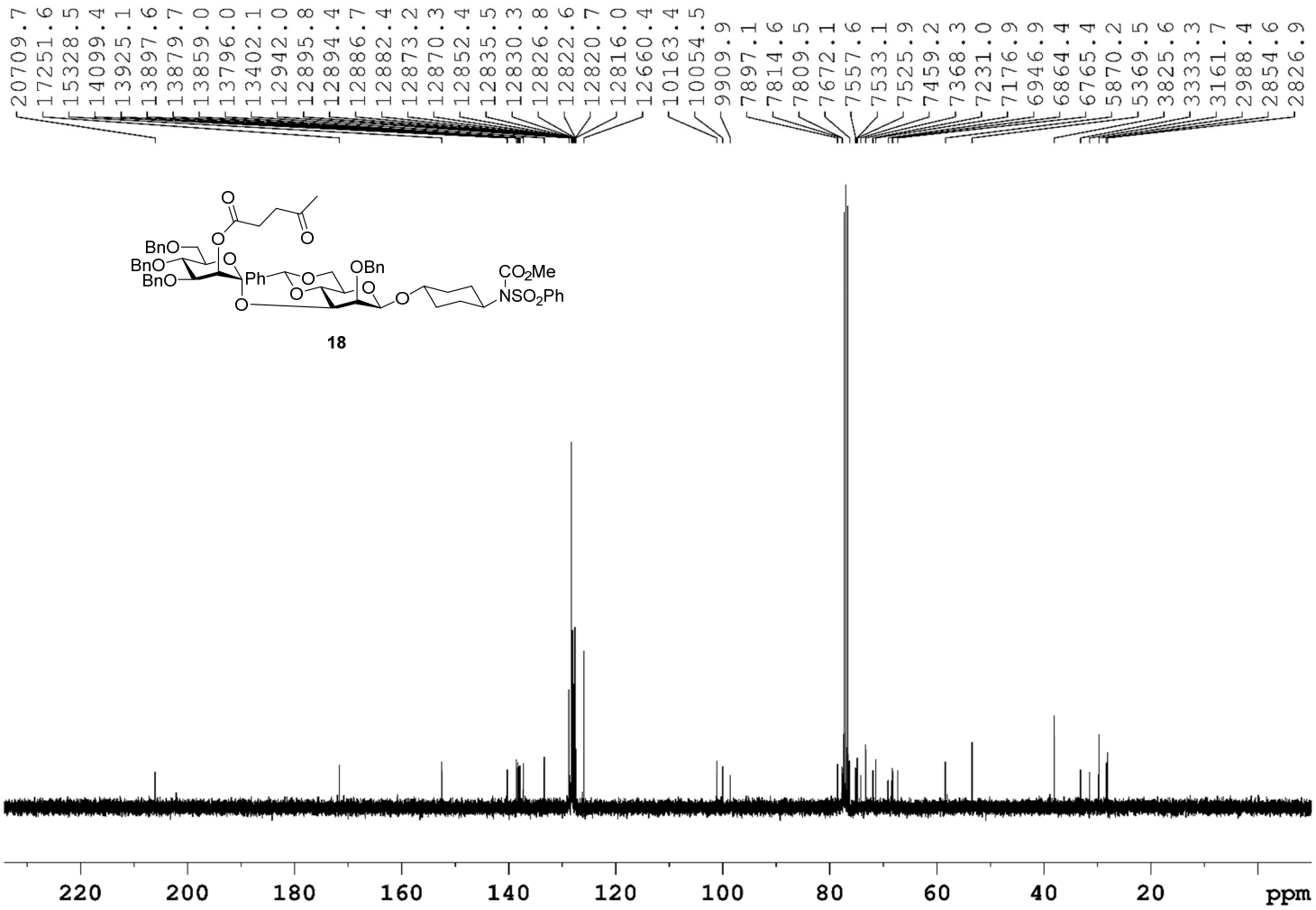
16



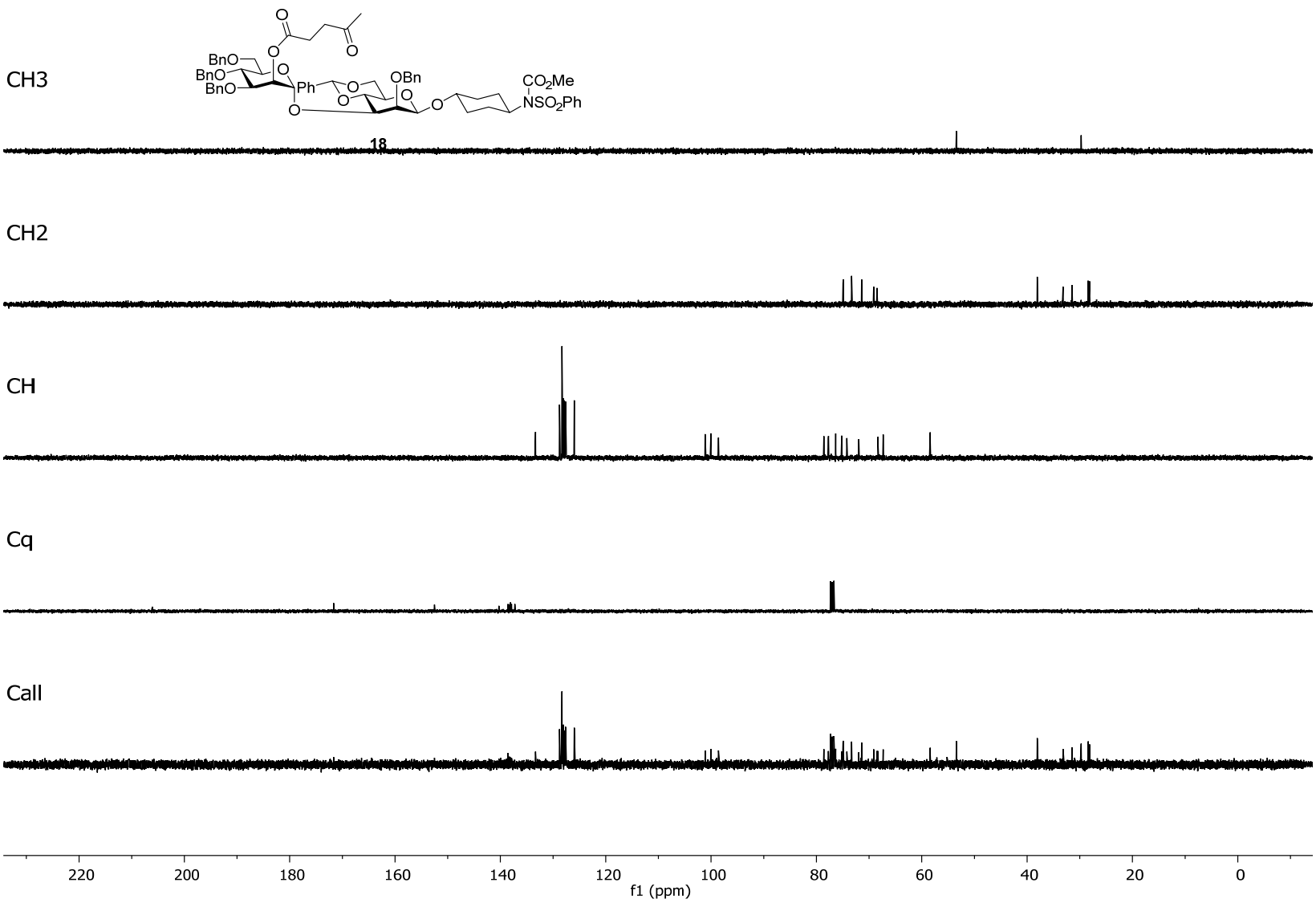
^{1}H NMR (400 MHz) of **18** in $CDCl_3$



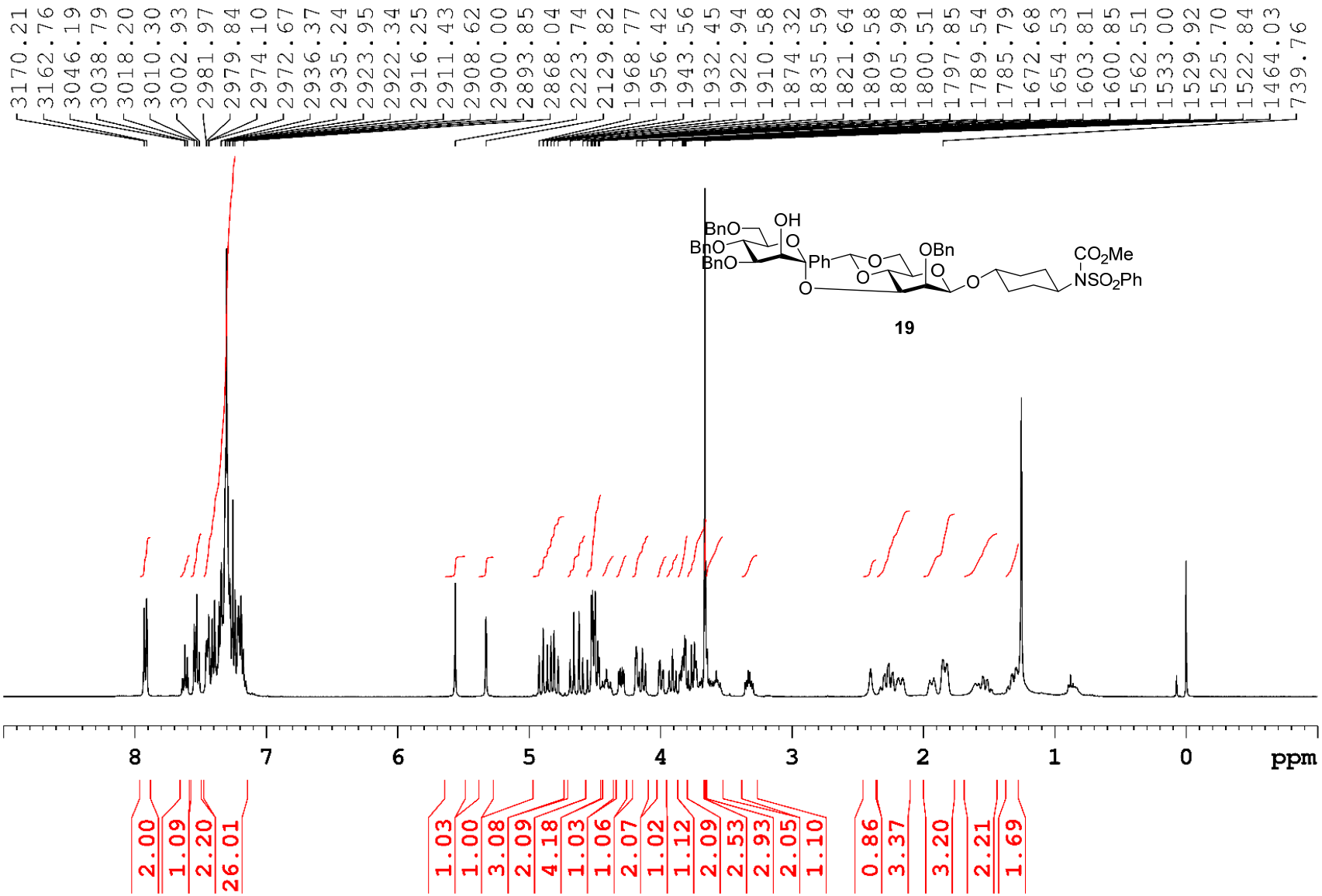
^{13}C NMR (100 MHz) of **18** in $CDCl_3$



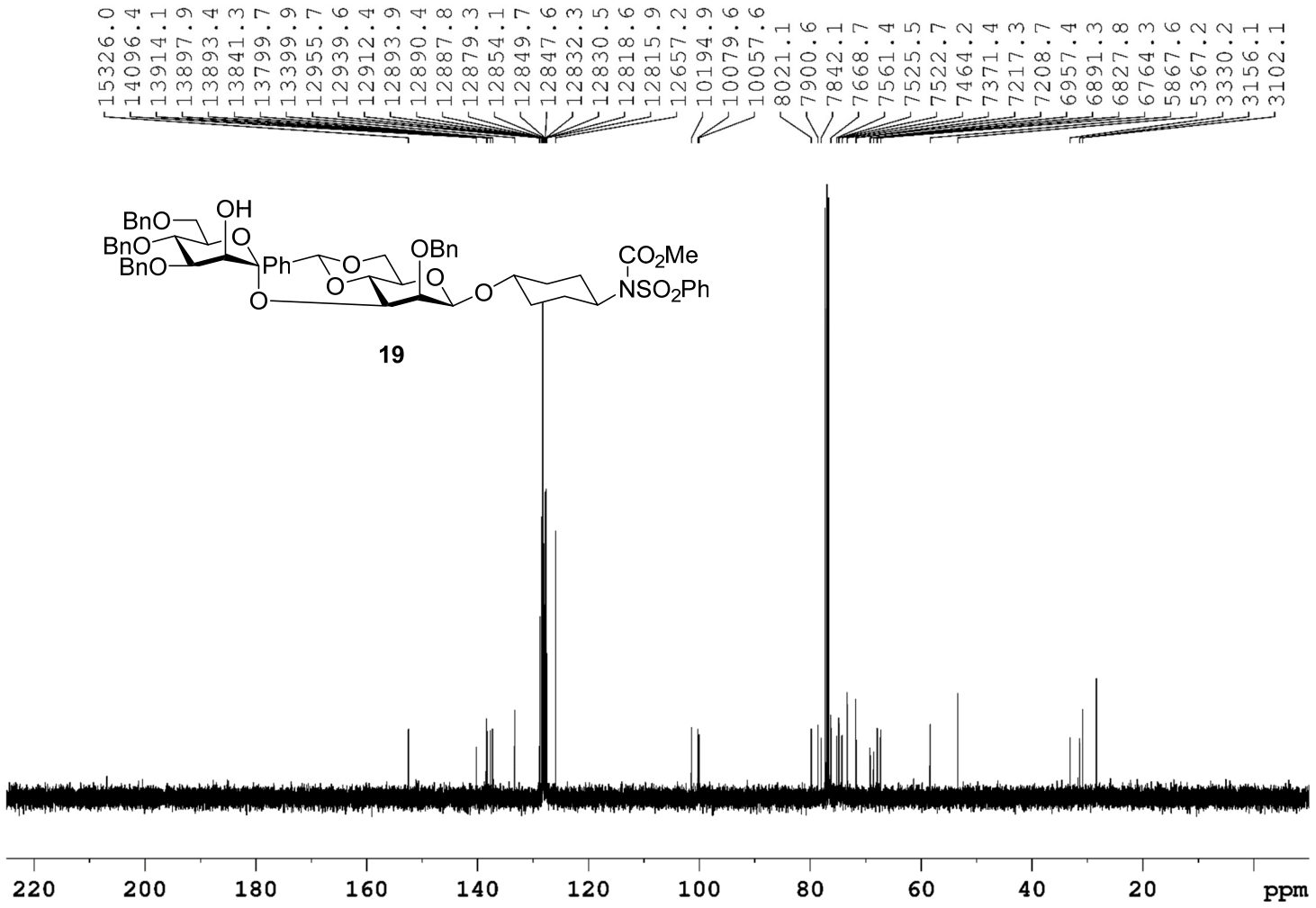
DEPT NMR (100 MHz) of **18** in $CDCl_3$



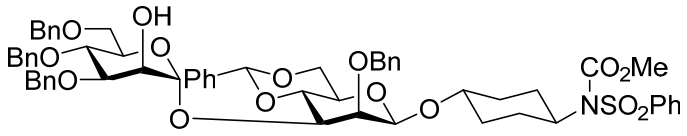
^{1}H NMR (400 MHz) of **19** in $CDCl_3$



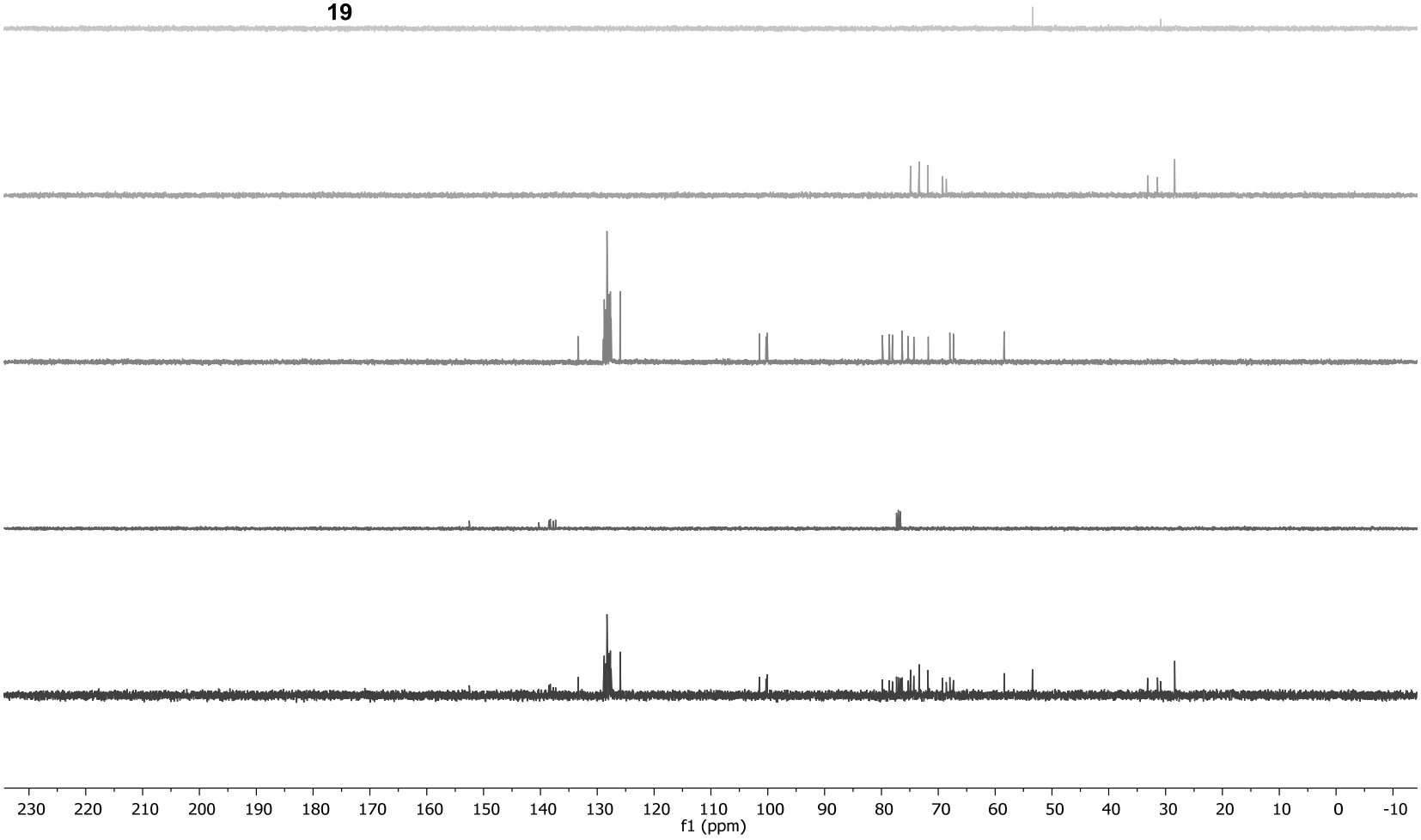
^{13}C NMR (100 MHz) of **19** in $CDCl_3$



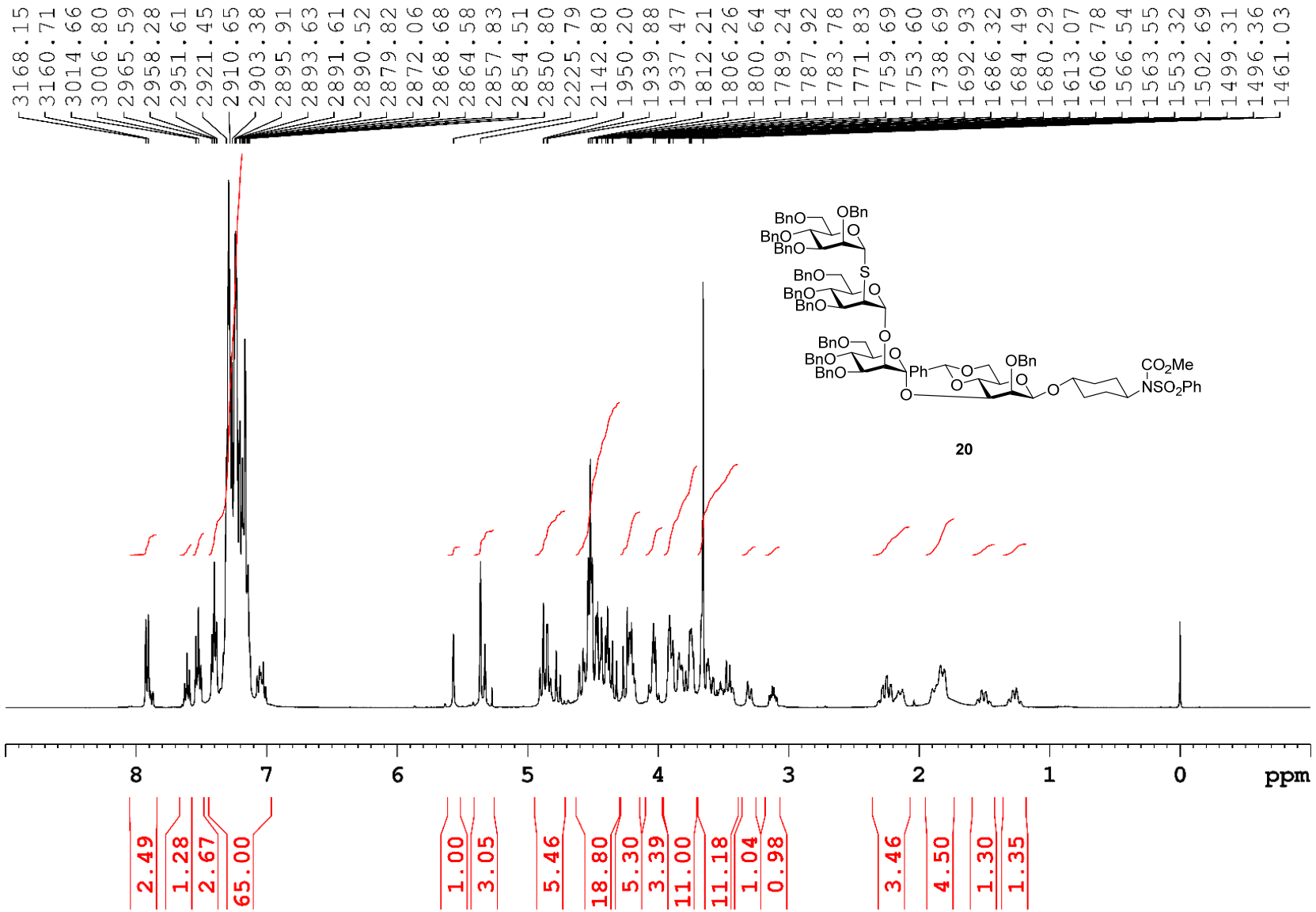
DEPT NMR (100 MHz) of **19** in $CDCl_3$



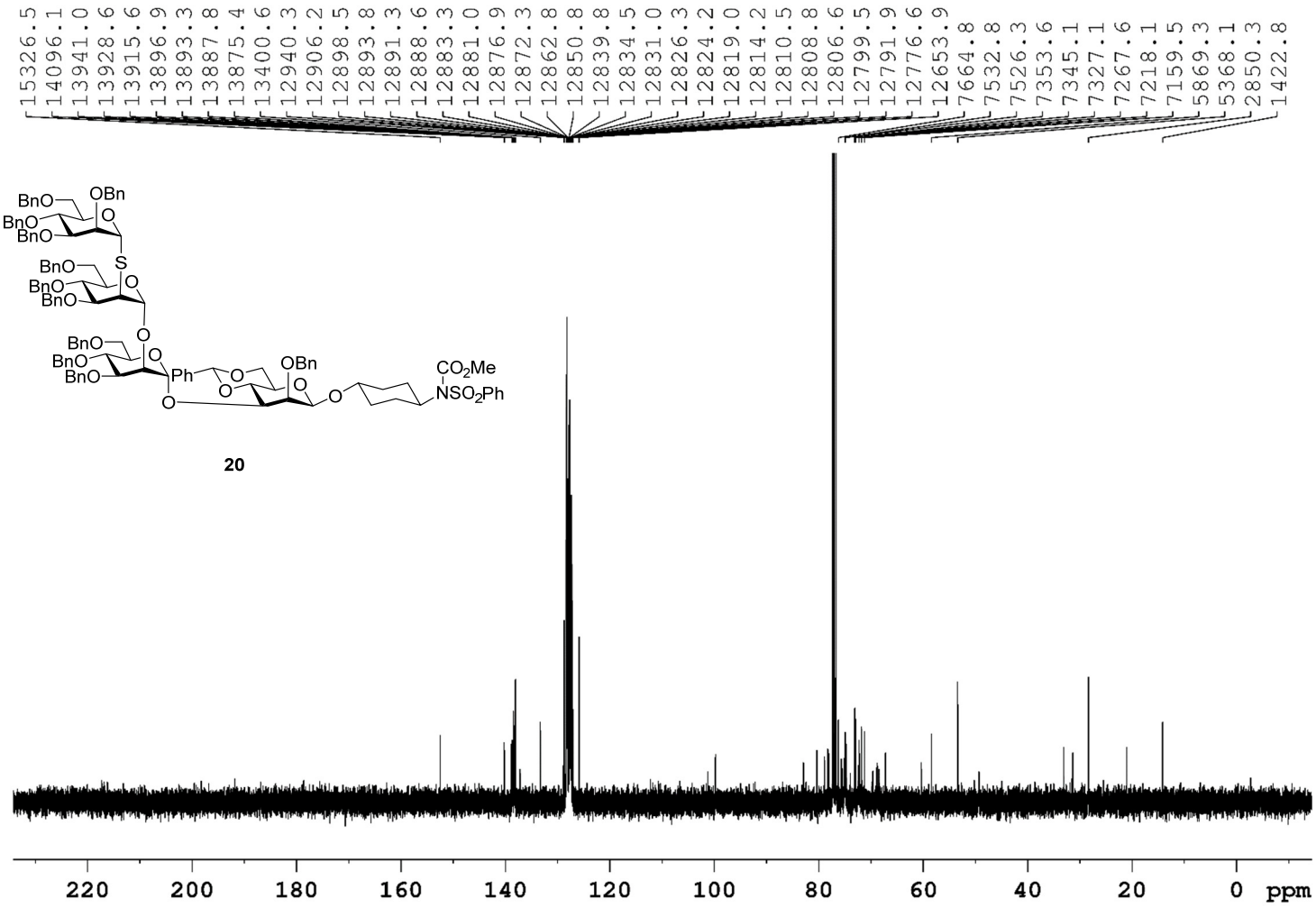
19



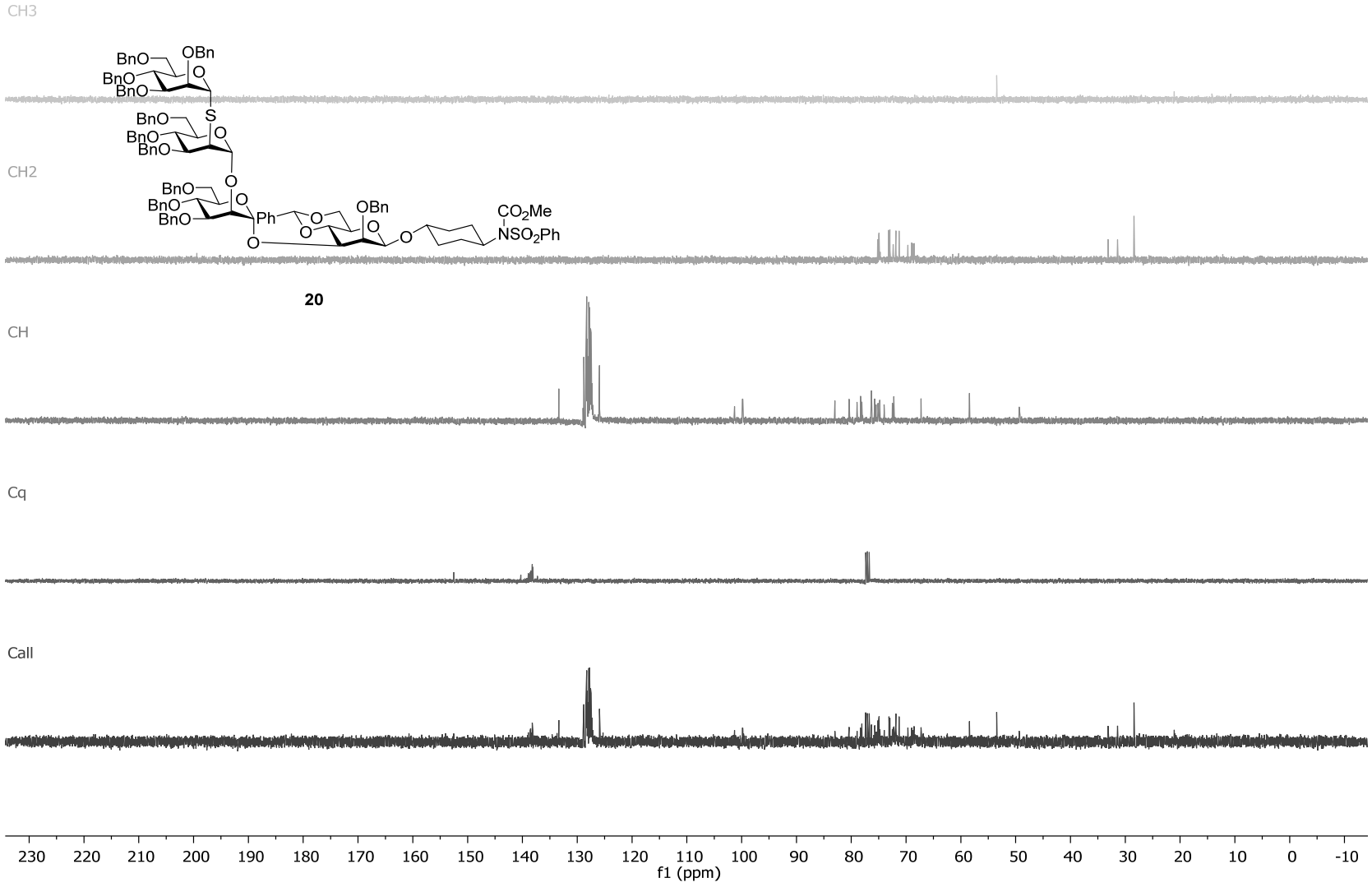
^{1}H NMR (400 MHz) of **20** in $CDCl_3$



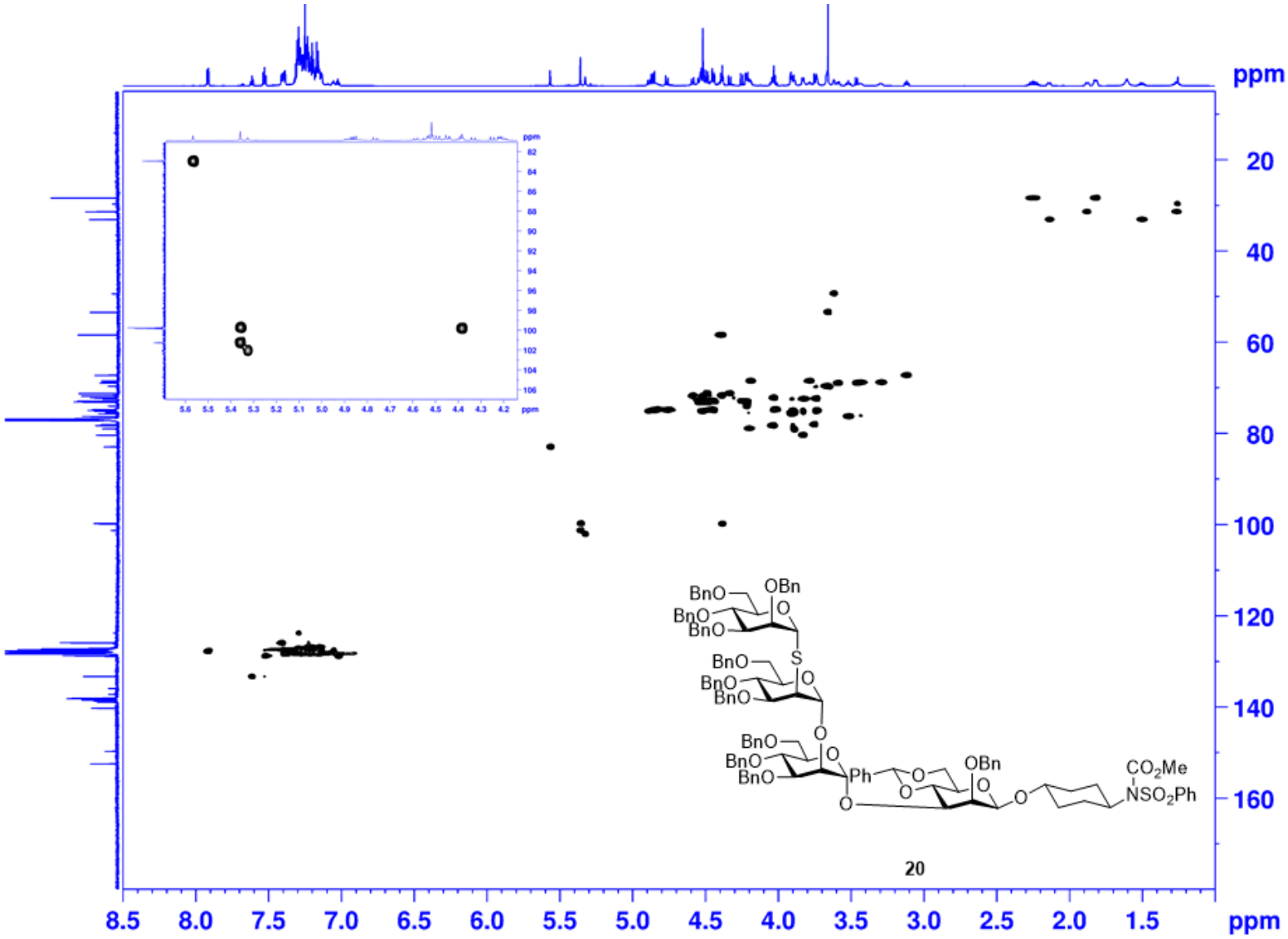
^{13}C NMR (100 MHz) of **20** in $CDCl_3$



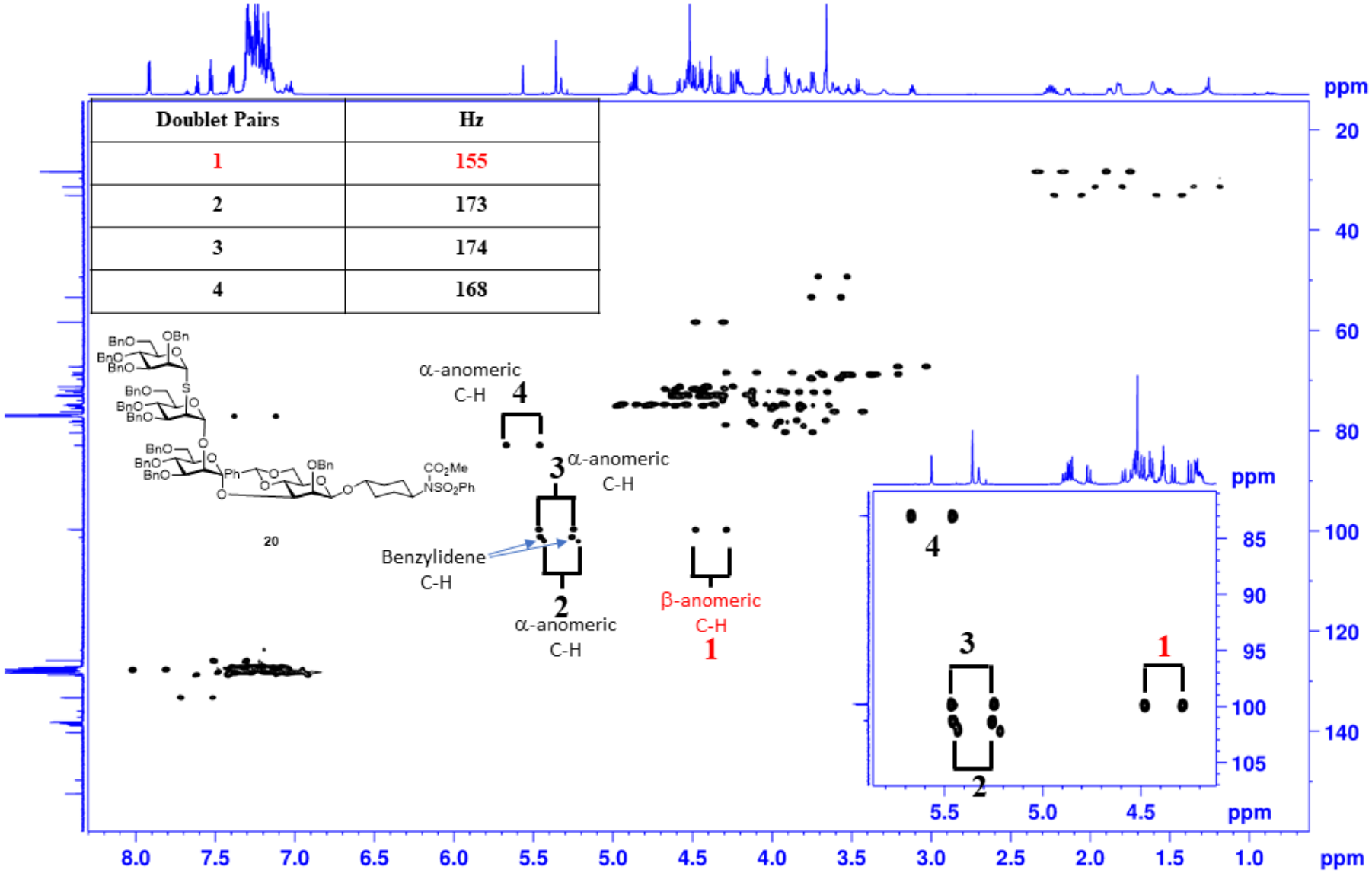
DEPT NMR (100 MHz) of **20** in $CDCl_3$



^{1}H - ^{13}C HSQC NMR (800 MHz) of **20** in $CDCl_3$

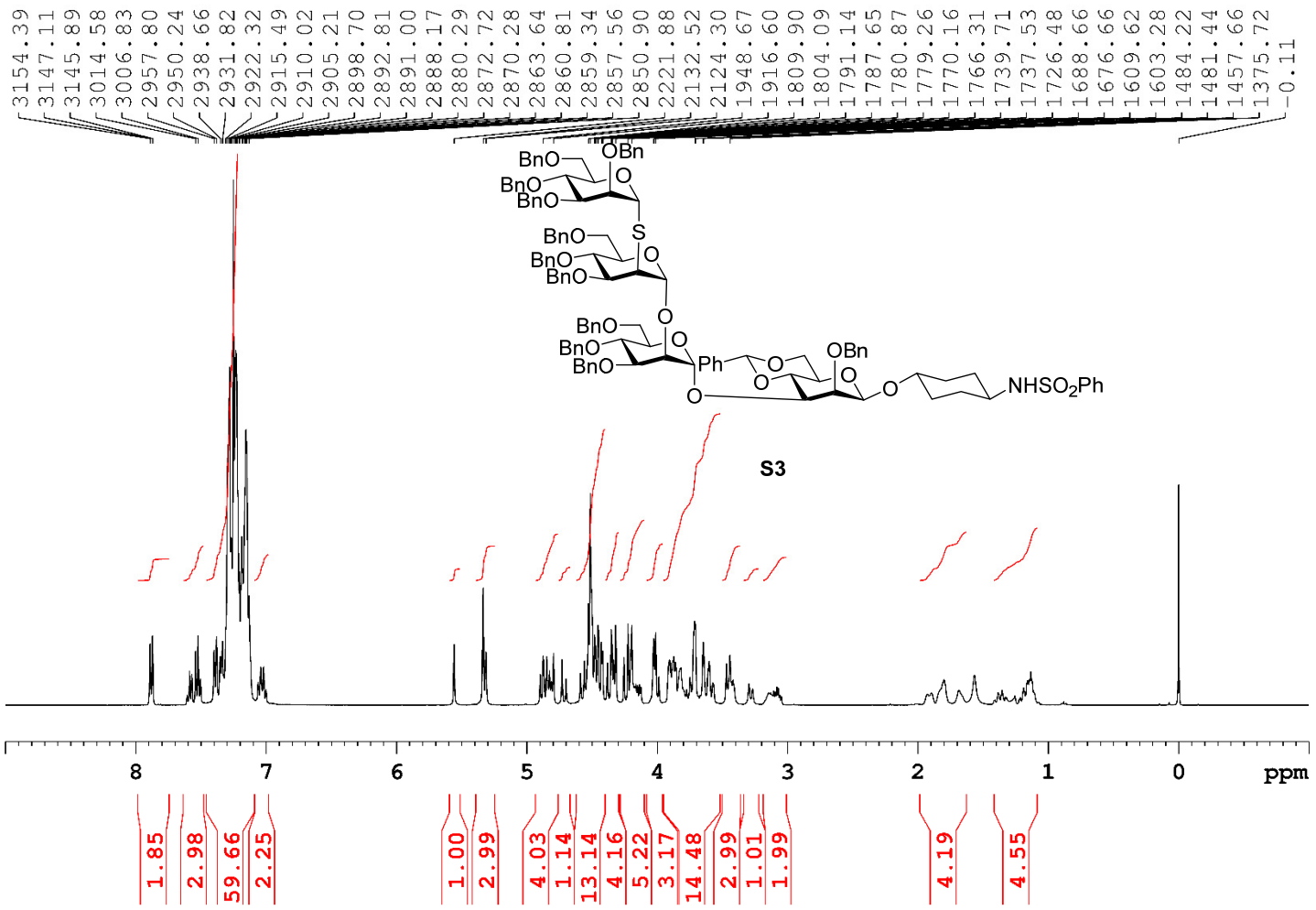


^{1}H - ^{13}C coupled HSQC NMR (800 MHz) of **20** in $CDCl_3$

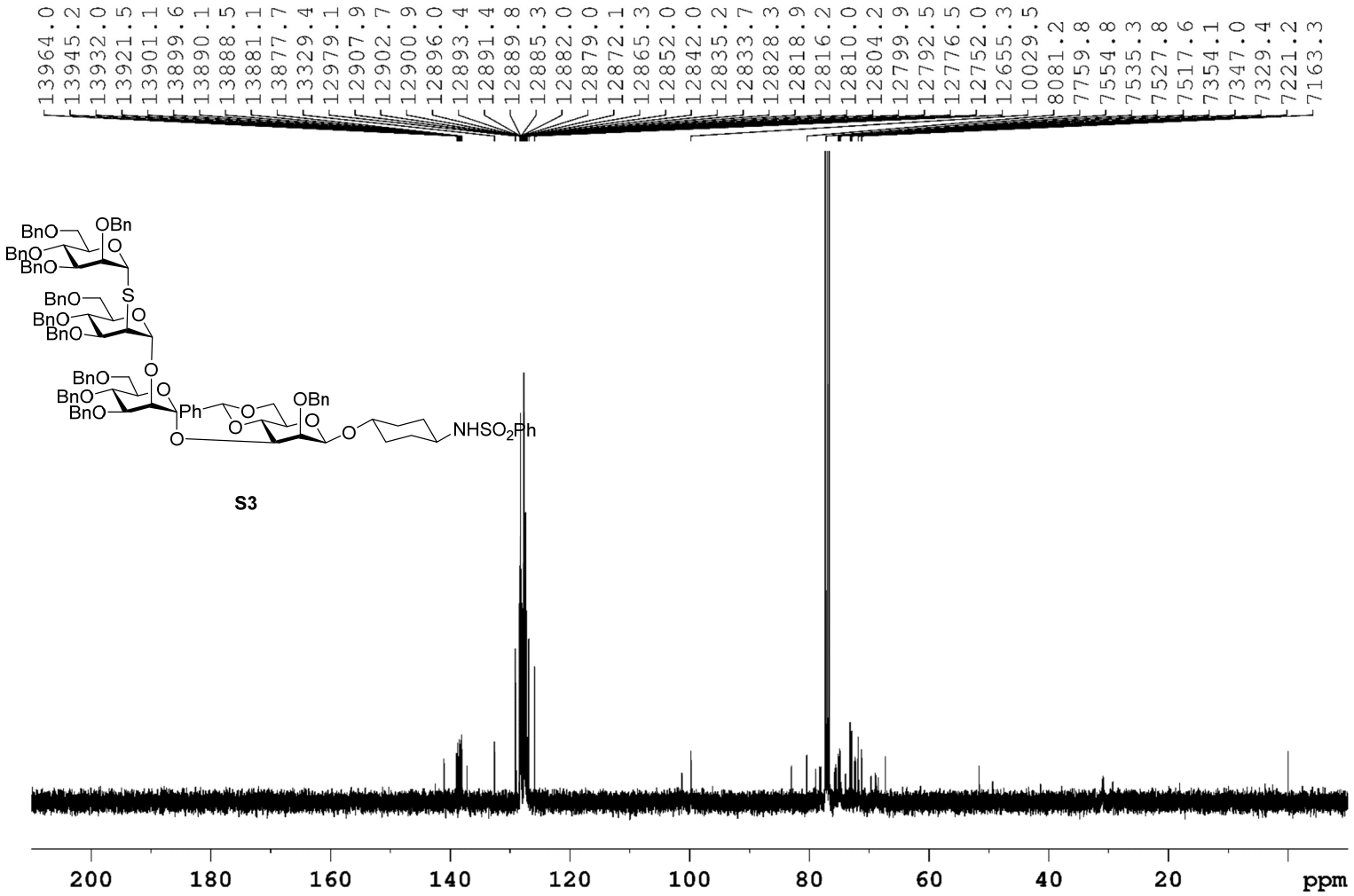


Doublet Pairs	Hz
1	155
2	173
3	174
4	168

^{1}H NMR (400 MHz) of **S3** in $CDCl_3$

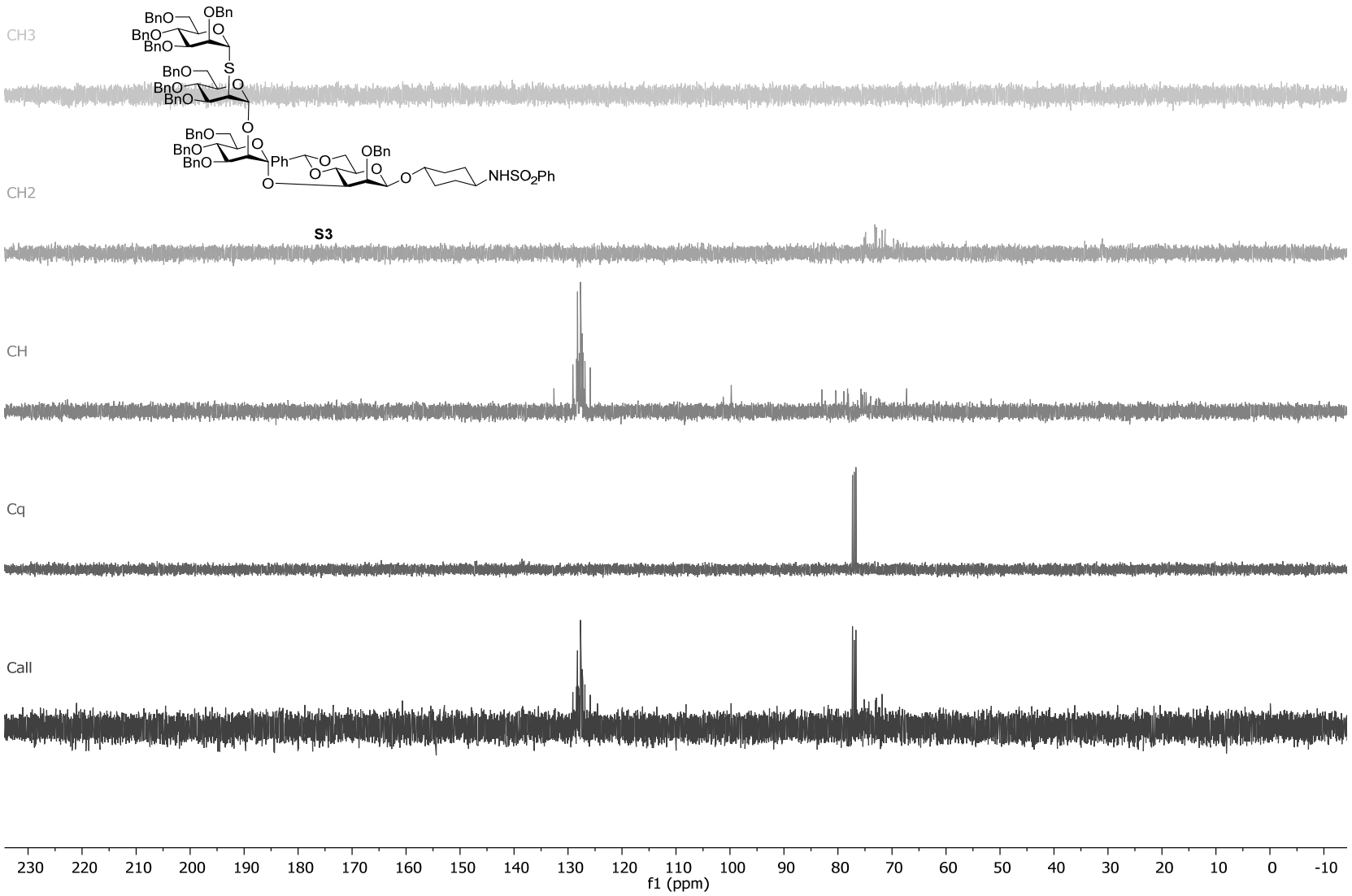


^{13}C NMR (100 MHz) of **S3** in $CDCl_3$

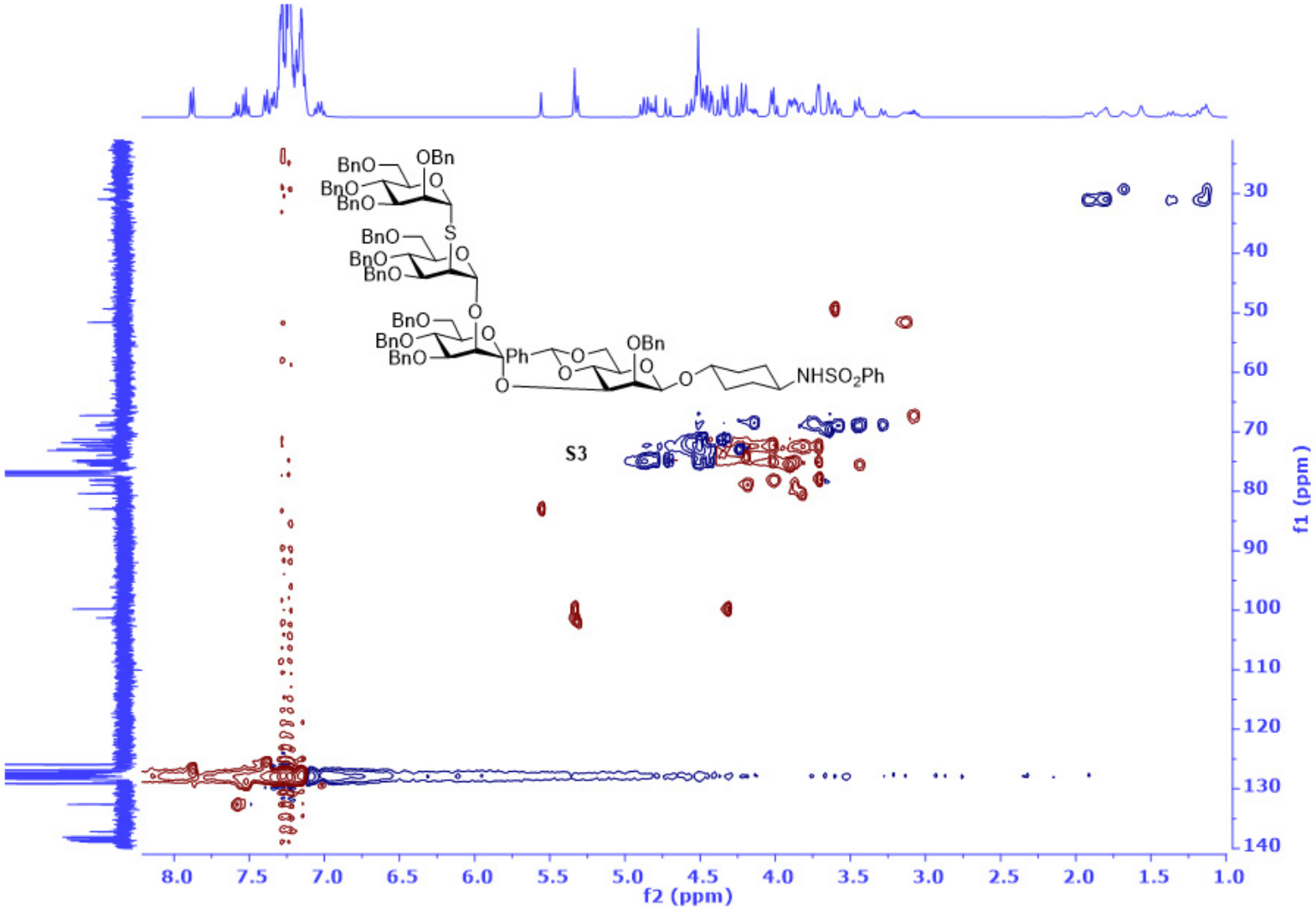


S3

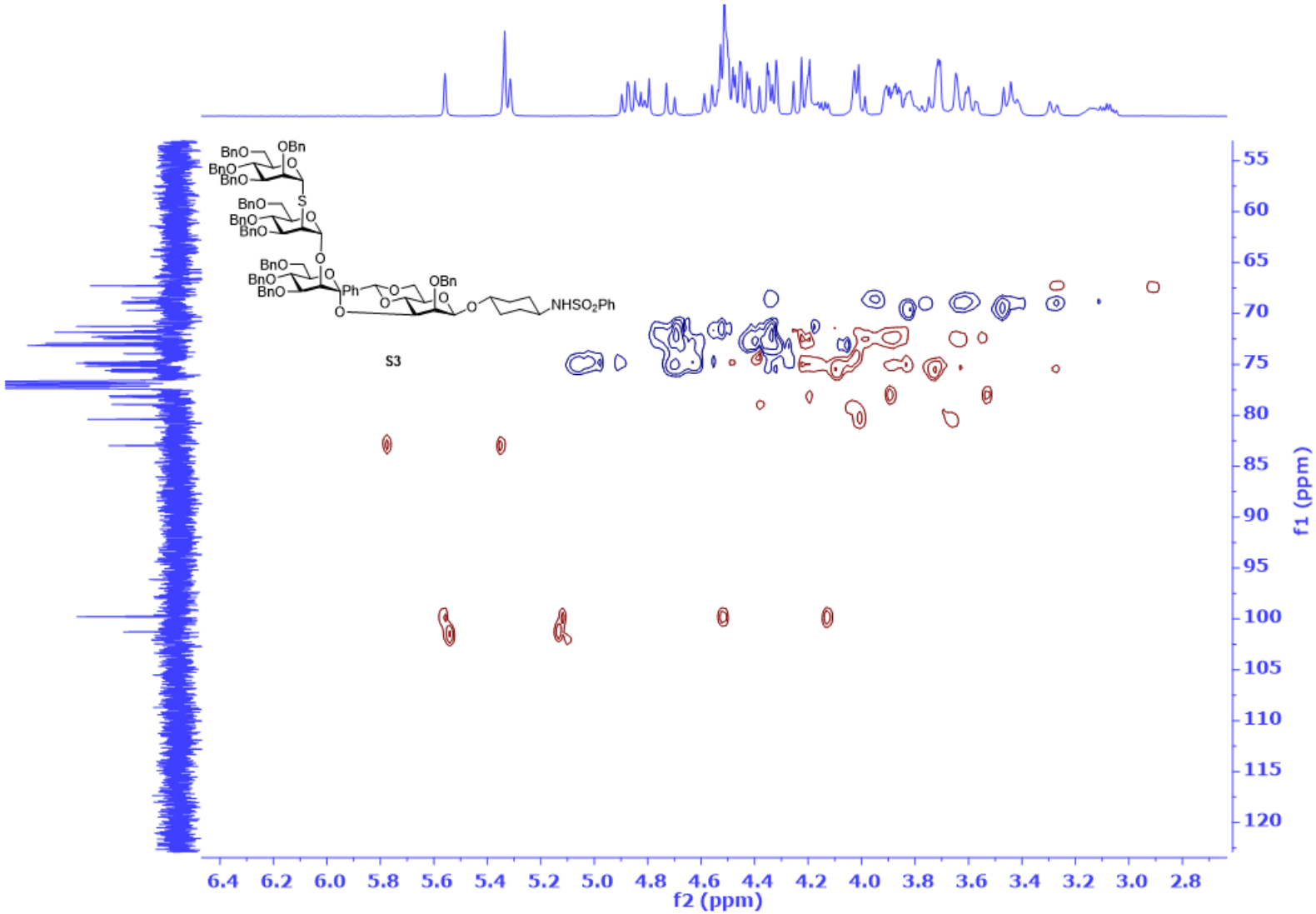
DEPT NMR (100 MHz) of **S3** in $CDCl_3$



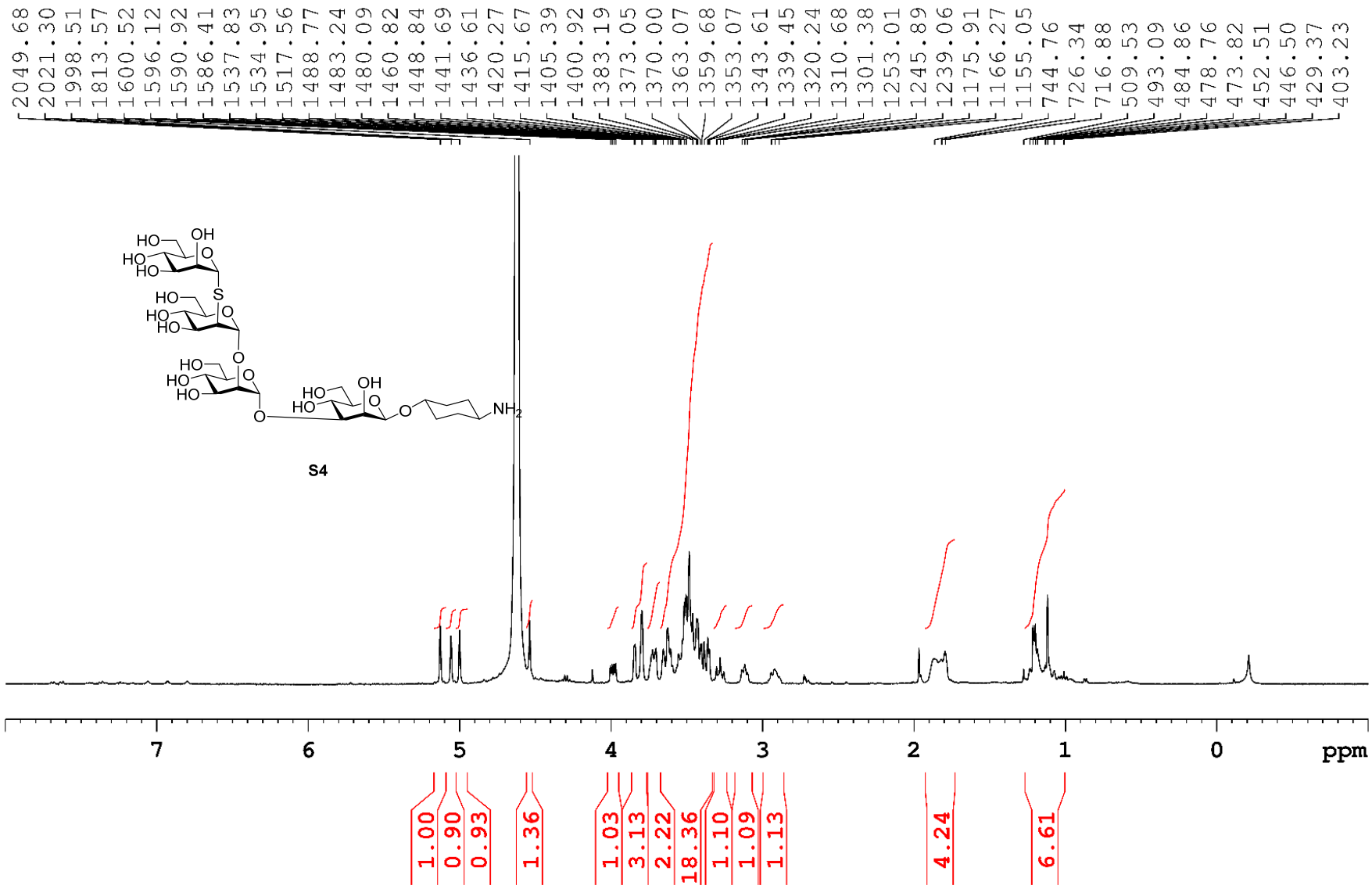
^{1}H - ^{13}C HSQC NMR (400 MHz) of **S3** in $CDCl_3$



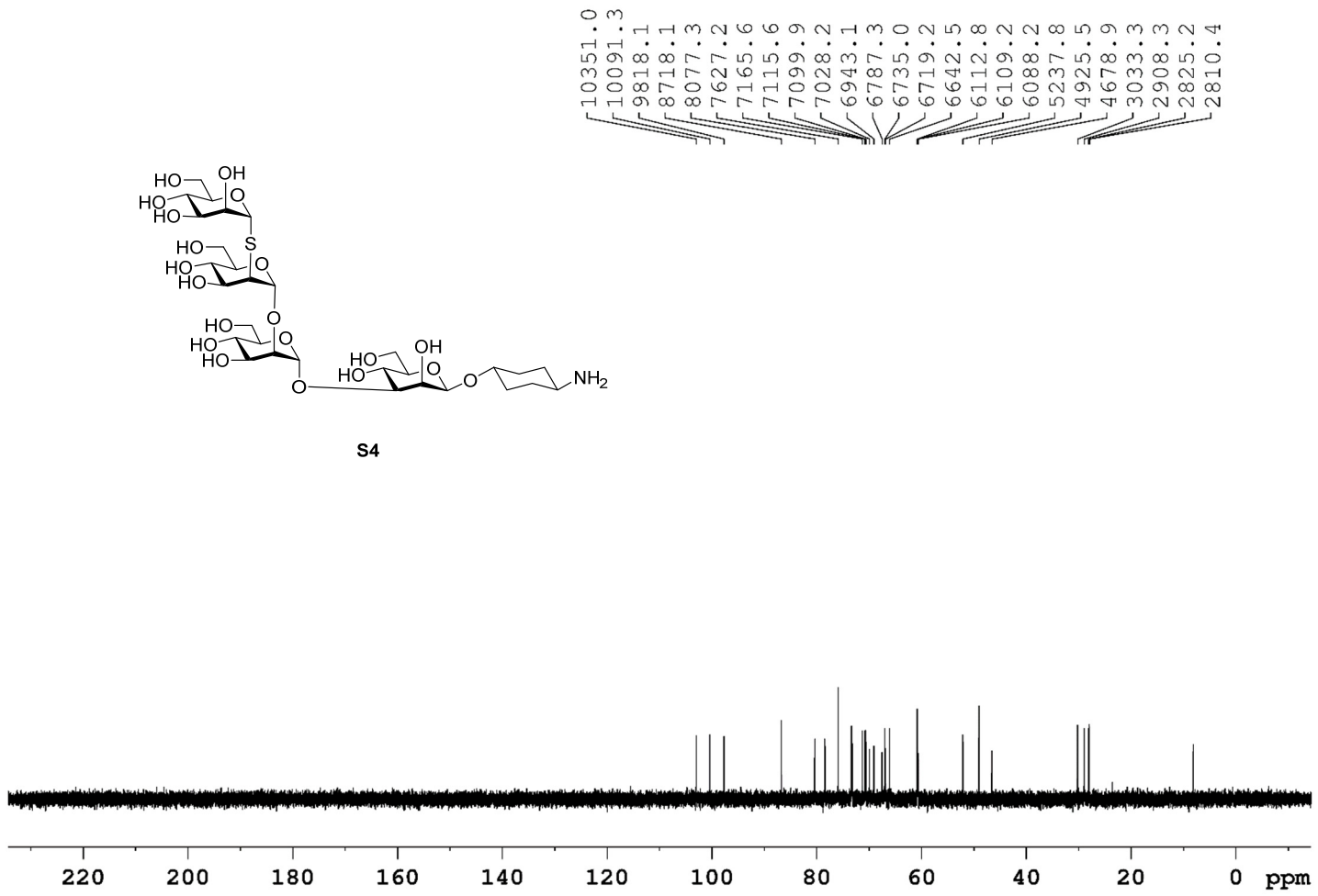
^{1}H - ^{13}C coupled HSQC NMR (400 MHz) of **S3** in $CDCl_3$



^{1}H NMR (400 MHz) of **S4** in D_2O

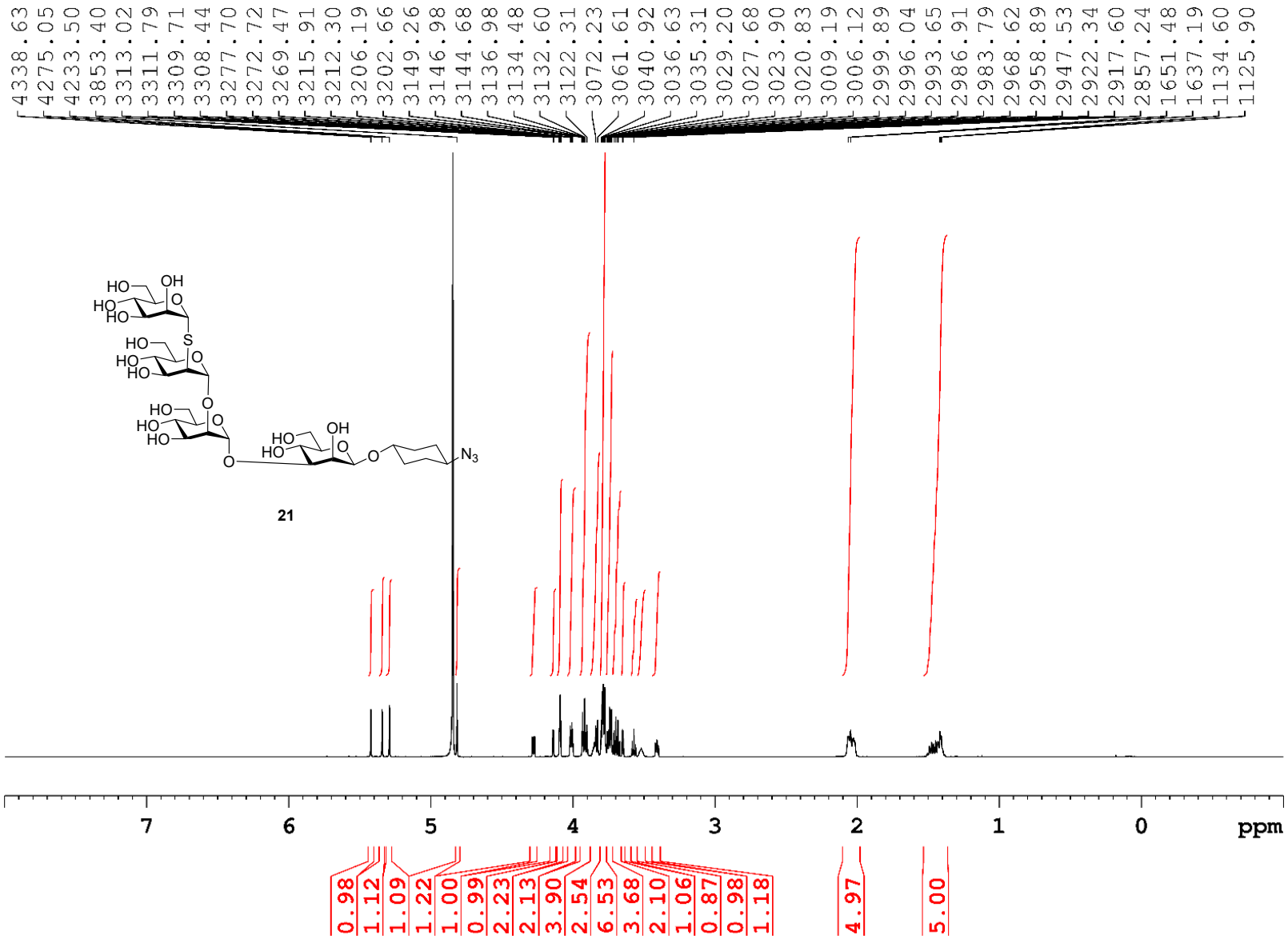


^{13}C NMR (100 MHz) of **S4** in D_2O

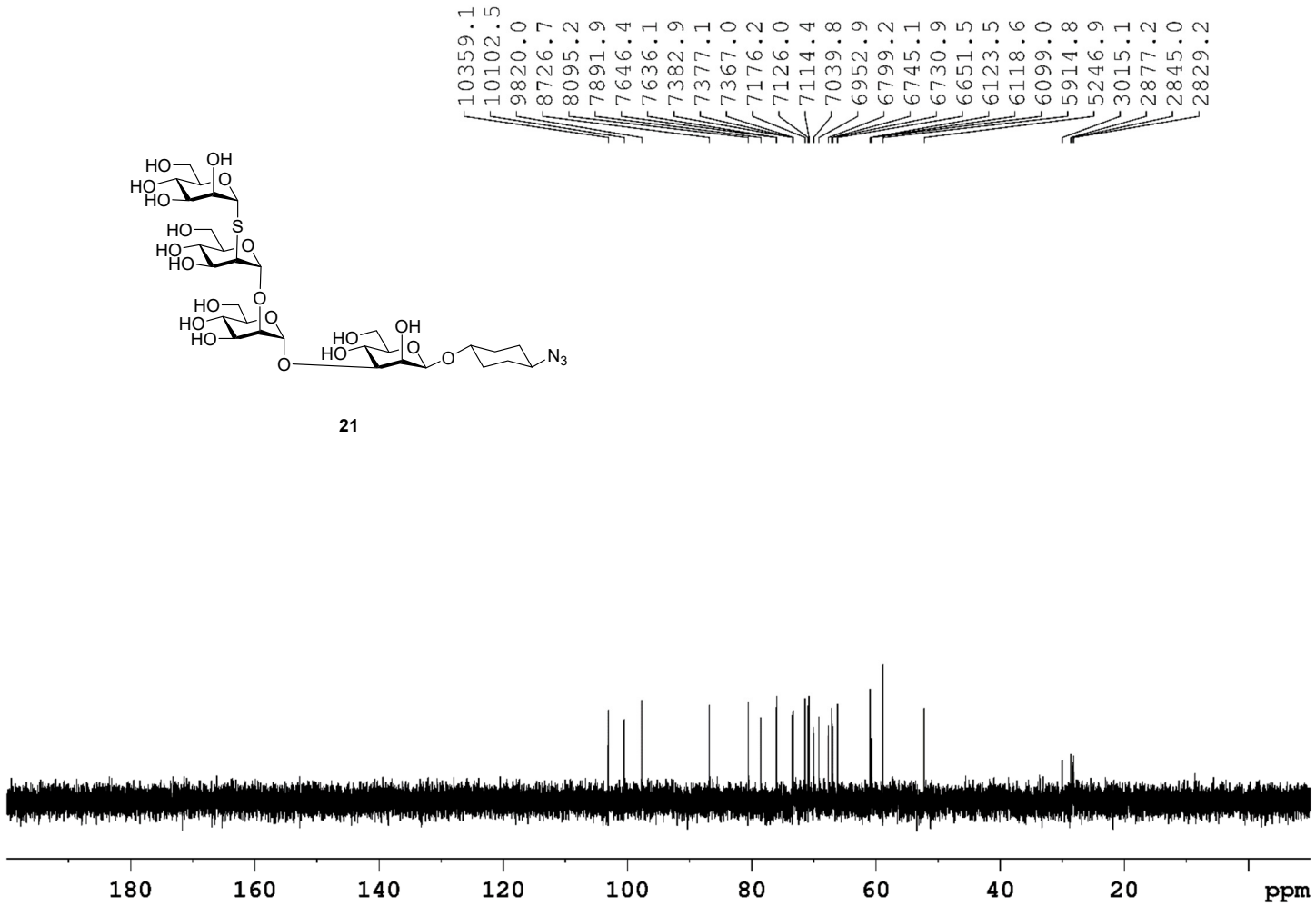


S4

^{1}H NMR (800 MHz) of **21** in D_2O

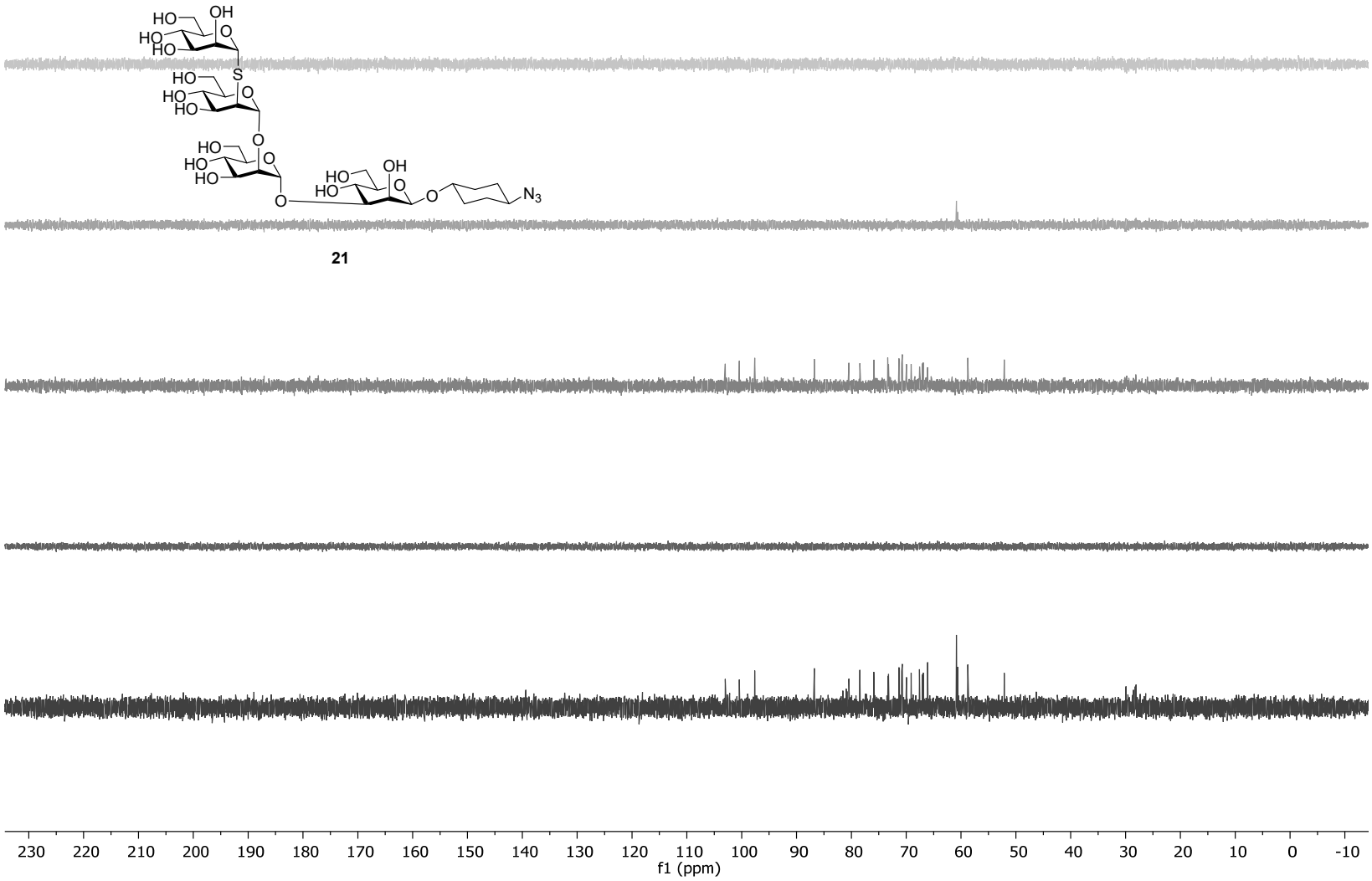


^{13}C NMR (200 MHz) of **21** in D_2O

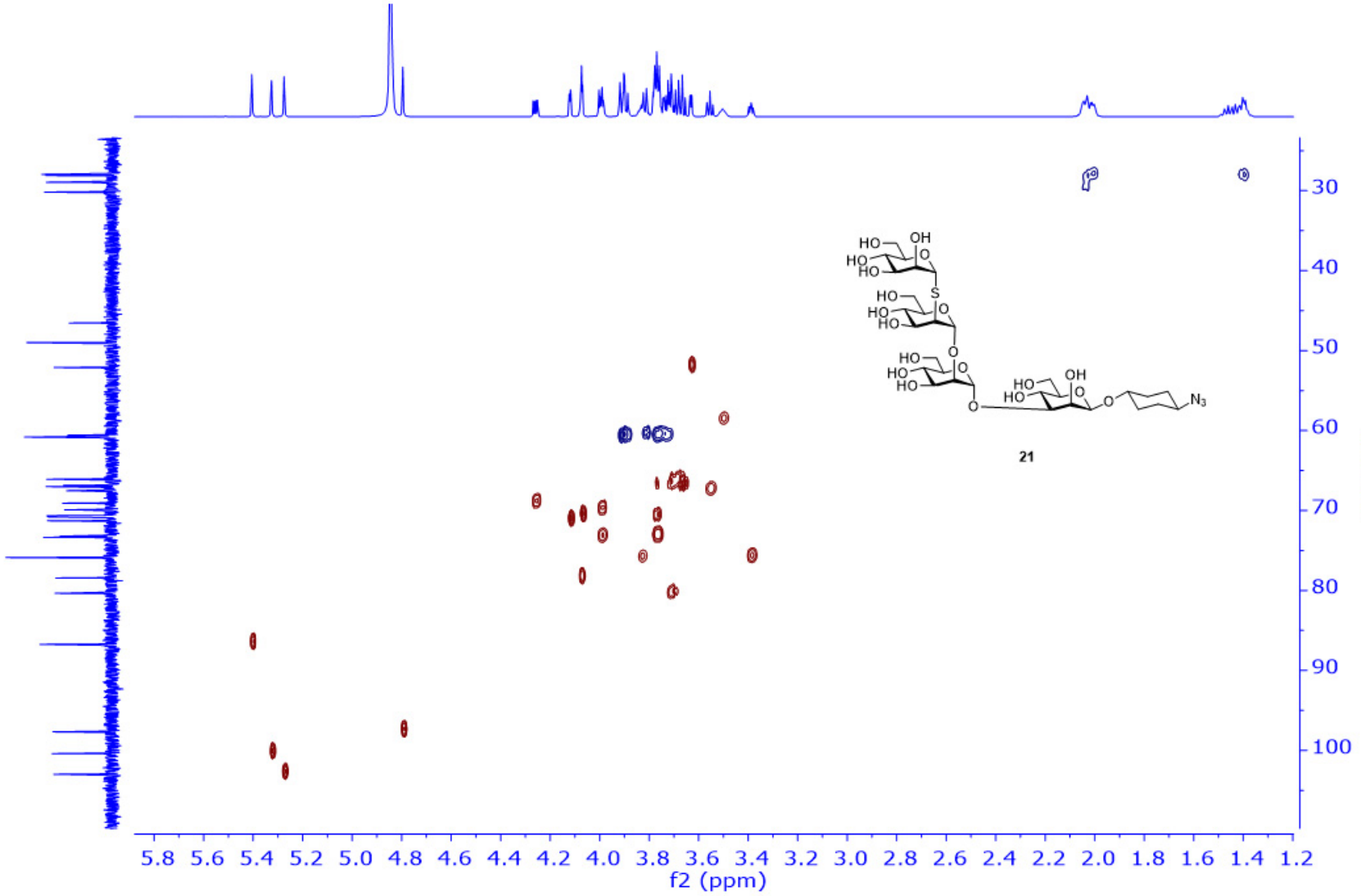


21

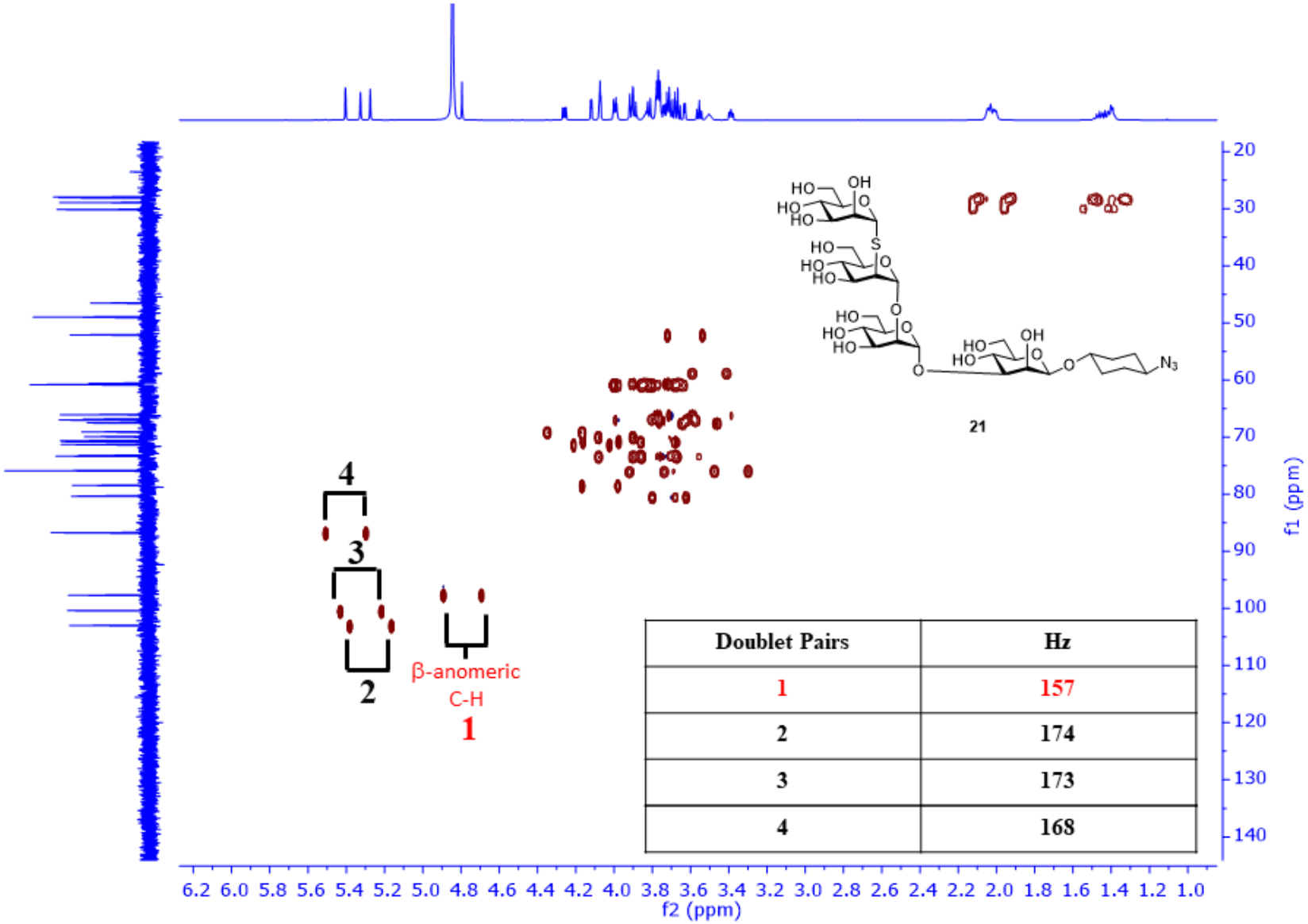
DEPT NMR (100 MHz) of **21** in D_2O



^{1}H - ^{13}C HSQC NMR (800 MHz) of **21** in D_2O



^{1}H - ^{13}C coupled HSQC NMR (800 MHz) of **21** in D_2O



Doublet Pairs	Hz
1	157
2	174
3	173
4	168