Novel Solutions for Vaccines and Diagnostics to Combat Brucellosis

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SUPPORTING INFORMATION

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I ADDITIONAL GLYCOCONJUGATES USED IN ELISA SCREENING

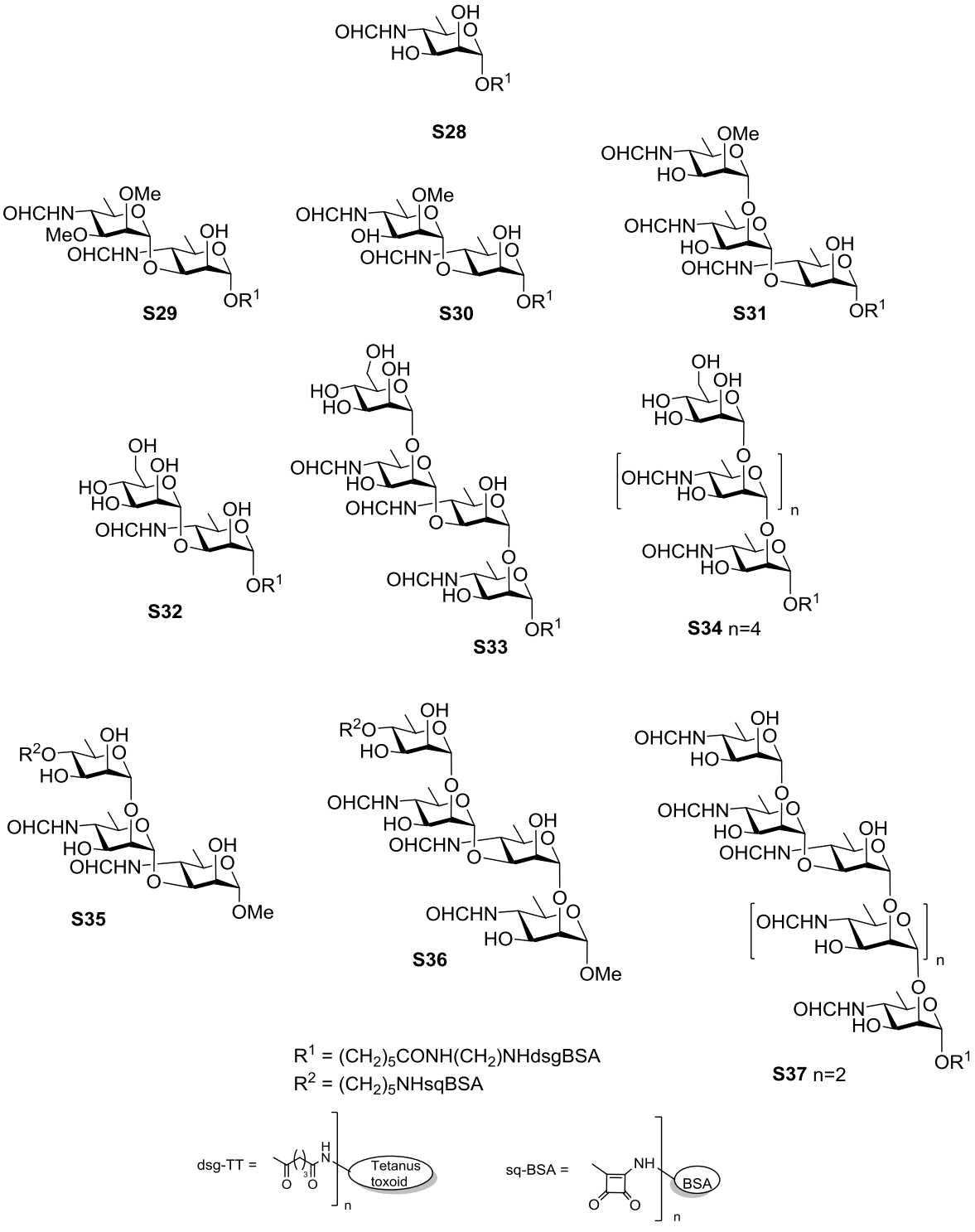


Figure S1 Oligosaccharides available from other studies were activated and conjugated to BSA according to methods reported elsewhere to provide glycoconjugates **S28**-**S37**.[1,2] The number of hapten groups per BSA was 10-15.

II PENTASACCHARIDE INHIBITORS

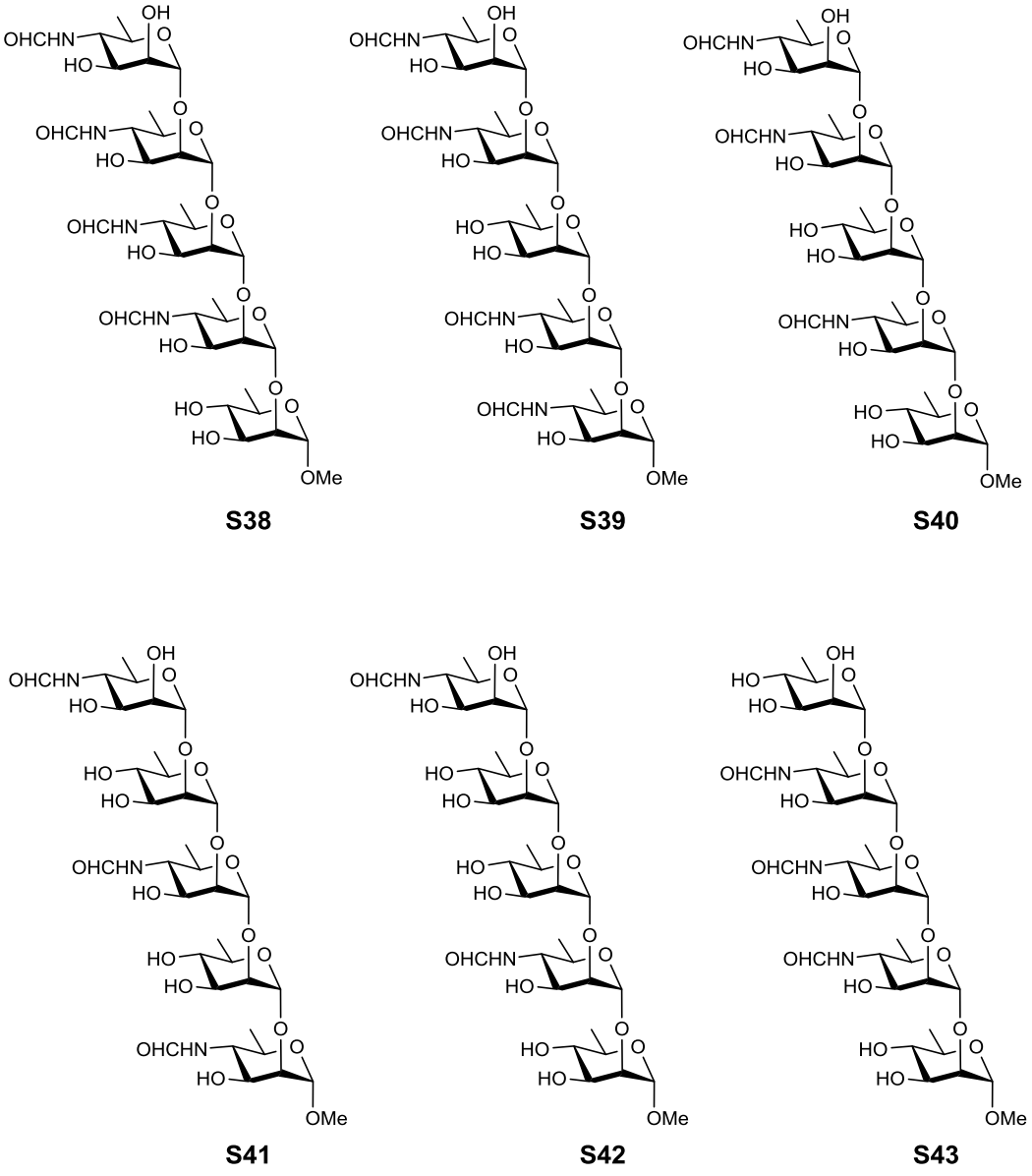


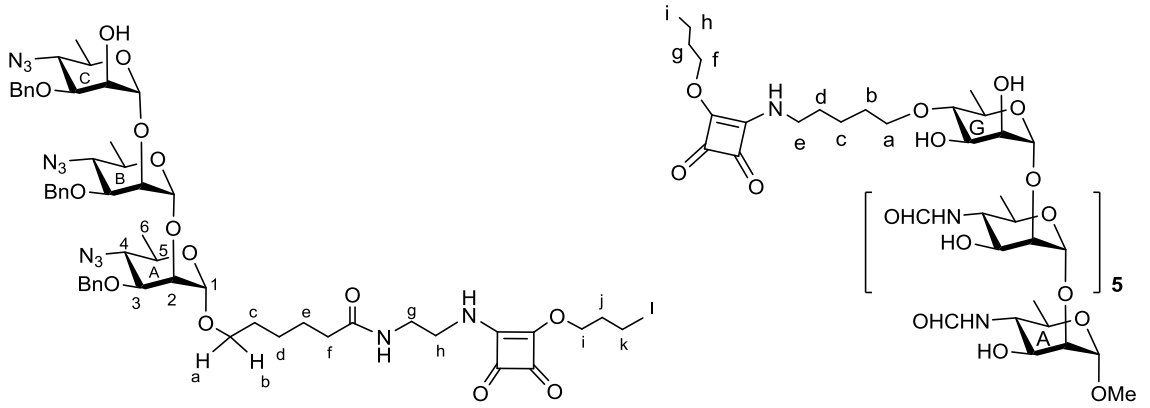
Figure S2 Pentasaccharide methyl glycosides were synthesized as previously described[3,4] and used as inhibitors of sera raised to vaccine **2**.

III. GENERAL INFORMATION

Materials and Methods

Analytical TLC was performed on Silica Gel 60-F_{254} (Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 10% sulfuric acid in ethanol. All commercial reagents were used as supplied. Column chromatography was performed on Silica Gel 230-400 mesh, 60 Å (Silicycle, Ontario) with HPLC quality solvents. Bovine Serum Albumin (purchased from Sigma Aldrich) was used. Molecular sieves (3 Å or 4 Å), were crushed and stored in an oven at 150 °C after activation at 500 °C for 48 h and dried under vacuum before use. Organic solutions were dried with anhydrous $MgSO_4$ prior to concentration under vacuum at <40 ^oC (bath). All final compounds were purified by reverse phase chromatography performed on a Waters 600 HPLC system, using a Beckmann semi preparative C-18 column (10 x 250 mm, 5 μ) with a combination of acetonitrile and water as eluents. Products were detected with a Waters 2487 UV detector. Optical rotations were measured with a Perkin-Elmer 241 polarimeter for samples in a 10 cm cell at 22 ± 2 ^oC. $[\alpha]^D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H NMR spectra were recorded on 500, 600 or 700 MHz spectrometers. First order proton chemical shifts δ_H are referenced to either residual $CHCl_3$ (δ_H 7.26, $CDCl_3$) or CD_2HOD (δ_H 3.30, CD_3OD), or external acetone (δ_H 2.225, D_2O). The assignment of resonances for all compounds was made by two dimensional homonuclear shift correlation and for a limited subset also by heteronuclear chemical shift correlation experiments. Specifically for mono- to trisaccharides: peak assignments were based on 2D-^1H-^1H-gCOSY experiments. Peak assignments for tetra- to hexasaccharides were based on 2D-^1H-^1H-gCOSY, selective 1D-^1H-CSSF-TOCSY (Chemical Shift Selective Filter - TOCSY) experiments and selective 1D-ROESY experiments. Mass analysis was performed by positive-mode electrospray ionization on a hybrid sector-TOF mass spectrometer and for protein glycoconjugates by MALDI mass analysis, employing sinapinic acid as matrix.

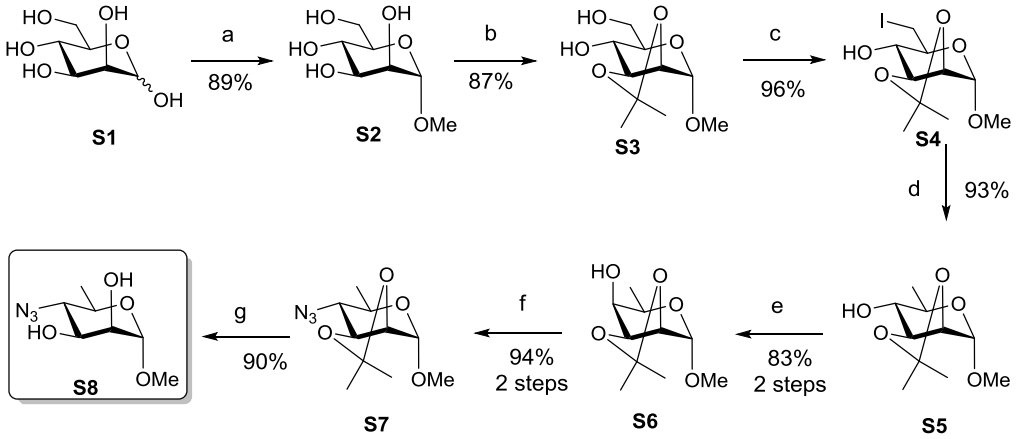
The numbering used for resonance assignments was as follows:



IV. SYNTHESIS OF D RHAMNOSE AND 4-AZIDO-4,6-DIDEOXY-α-D-MANNOPYRANOSE SYNTHONS

Synthesis of Methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (S8).

The key precursor **S8** was prepared according to Scheme S1 and analytical data for the title compound was essentially the same as previously described.[5,6]

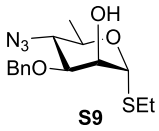


Scheme S1. Conditions: a) AcCl, MeOH, 70 ^{o}C, 6 h; b) DMP, PTSA, rt, H_2O, 4 h; c) PPh_3, Imid, I_2, PhMe, 10 min; d) Pd/C, H_2, rt, EtOH/Et_3N, 16 h; e) i) OxCl, DMSO, DIPEA, CH_2Cl_2, -78 ^{o}C-rt, 16 h; ii) $NaBH_4$, EtOH, rt, 2 h; f) i) MsCl, Py, 0 ^{o}C-rt, 2 h; ii) TFA/H_2O (9:1), CH_2Cl_2, rt, 10 min. g) NaN_3, 15-crown-5, DMF, 100 ^{o}C, 6 h.

V. SYNTHESIS OF GLYCOSYL DONORS

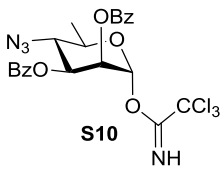
A. <u>Synthesis of glycosyl donor S9 & S10:</u>

Ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside (S9).



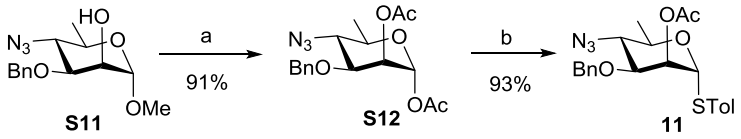
Analytical data for the title compound was essentially the same as previously described.[7-9]

4-Azido-2,3-di-*O*-benzoyl-4,6-dideoxy-α-D-mannopyranosyl trichloroacetimidate (S10).



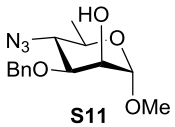
Analytical data for the title compound was essentially the same as previously described.[1]

B. <u>Synthesis of thioglycoside donors 11:</u>



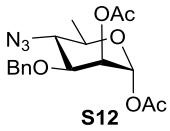
Scheme S2. Conditions: a) Ac_2O, AcOH, H_2SO_4, rt, 6 h; b) BF_3:Et_2O, p-Toluenethiol, CH_2Cl_2, 0^oC to rt, 12 h.

Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (S11).



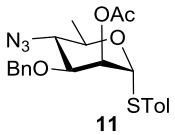
Analytical data for the title compound was essentially the same as previously described.[4]

1,2-di-*O*-acetyl-4-azido-4,6-dideoxy-α-D-mannopyranose (S12).



A solution of **S11** (5 g, 17.05 mmol) in acetic anhydride/acetic acid/sulfuric acid (50:20:0.5, 50 mL) was stirred at 21 ^oC for 3 h, and then poured into ice-cold 1M K_2CO_3 solution (80 mL). The mixture was then diluted with CH_2Cl_2 (~100 mL) and washed with water (2 x 30 mL), sat. aq. $NaHCO_3$ (35 mL), and brine (15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound **S12** (5.6 g, 91%) as a sticky liquid. Analytical data for **S12:** R*f* = 0.35 (ethyl acetate/hexane, 1/4, v/v); [α]D ^{21} = +30.71 (*c* = 1.51, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.33 - 7.38 (m, 4 H, ArH), 7.28 - 7.32 (m, 1 H, ArH), 6.00 (d, *J*=1.8 Hz, 1 H, H-1), 5.32 (dd, *J*=3.3, 2.1 Hz, 1 H, H-2), 4.70 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 4.54 (d, *J*=10.9 Hz, 1 H, -C<u>H</u>Ph), 3.79 (dd, *J*=10.0, 3.3 Hz, 1 H, H-3), 3.59 (dq, *J*=10.0, 6.1 Hz, 1 H, H-5), 3.46 (t, *J*=10.0 Hz, 1 H, H-4), 2.13 (s, 3 H, -COC<u>H</u>$_3$), 2.09 (s, 3 H, -COC<u>H</u>$_3$), 1.32 ppm (d, *J*=6.3 Hz, 3 H, H-6); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 169.8, 168.3, 136.9, 128.5, 128.3, 128.1, 91.0, 75.7, 71.8, 69.3, 66.3, 63.5, 20.8(x2), 18.5 ppm; HRMS (ESI): m/z calcd for $C_{17}H_{21}N_3O_6Na$ [M+Na]+: 386.1323, found: 386.1322.

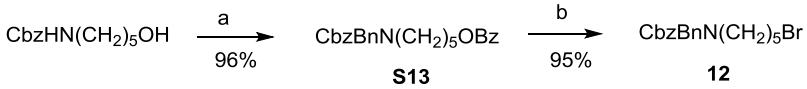
***p*-Tolyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside (11).**



To the stirred solution of **S12** (0.78 g, 2.15 mmol) and *p*-toluenethiol (0.4 g, 3.22 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 ^oC, $BF_3{\cdot}Et_2O$ (0.32 mL, 2.57 mmol) was added drop wise. When TLC showed the reaction was completed, the mixture was then diluted with CH_2Cl_2 (~50 mL) and washed with water (2 x 10 mL), sat. aq. $NaHCO_3$ (15 mL), and brine (10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Ethyl acetate – hexane gradient elution) to give **11** as a sticky liquid (0.854 g, 92.9%). Analytical data for **11:** R*f* = 0.7 (Ethyl acetate /hexane, 1/3, v/v); $[\alpha]D^{21}$ = +135.5 (*c* = 2.25, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$): δ: 7.36 - 7.43 (m, 4 H, ArH), 7.30 - 7.35 (m, 3 H, ArH), 7.12 (d, *J*=8.1 Hz, 2 H, ArH), 5.59 (dd, *J*=3.1, 1.7 Hz, 1 H, H-2), 5.36 (d, *J*=1.5 Hz, 1 H, H-1), 4.72 (d, *J*=11.1 Hz, 1 H, -C<u>H</u>Ph), 4.57 (d, *J*=11.1 Hz, 1 H, -C<u>H</u>Ph), 4.02 - 4.08 (m, 1 H, H-5), 3.80 (dd, *J*=10.0, 3.2 Hz, 1 H, H-3), 3.51 (t, *J*=10.0 Hz, 1 H, H-3), 2.33 (s, 3 H, -C<u>H</u>$_3$), 2.12 (s, 3 H, -COC<u>H</u>$_3$), 1.36 (d, *J*=6.3 Hz, 3 H, H-6); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 170.0, 138.1, 137.0, 132.4, 132.3, 129.9, 129.8, 129.6, 128.5, 128.5, 128.4, 128.1, 86.4, 76.4, 71.7, 69.1, 68.2, 64.2, 21.1, 21.0, 18.4 ppm; HRMS (ESI): m/z calcd for $C_{22}H_{25}N_3O_4SNa$ [M+Na]+: 450.1458, found: 450.1465.

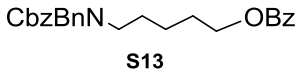
VI SYNTHESIS OF LINKER AND ATTACHMENT TO TERMINAL RHAMNOSE

C. <u>Synthesis of Linker bromoalkane 12</u>:



Scheme S3. Conditions: a) i) BzCl, DMAP, Et_3N, CH_2Cl_2, 0^{o}C to rt, 16 h; ii) BnBr, NaH, DMF, 0^{o}C to rt, 12 h; b) i) NaOMe, CH_3OH, rt, 6 h; ii) CBr_4, TPP, CH_2Cl_2, 0^{o}C to rt, 2 h.

5-(*N*-benzyl((benzyloxy)carbonyl)amino)pentanol benzoate (S13).



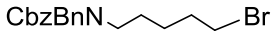
S13

Benzoyl chloride (0.88 mL, 7.59 mmol) was added dropwise to a stirred solution of benzyl (5-hydroxypentyl) carbamate (commercially available) (1.5 g, 6.32 mmol) in anhydrous CH_2Cl_2 (15 mL) containing Et_3N (1.76 mL, 1.26 mmol) at 0 ^oC. After 1 minute DMAP (1.7 g, 13.9 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise to the reaction mixture and stirred at rt overnight. The resulting mixture was diluted with CH_2Cl_2 (~30 mL) and washed with aq. HCl (1M, 1 x 10 mL), water (60 mL), sat. aq. $NaHCO_3$ (30 mL), and brine (30 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was quickly filtered off on silica gel (ethyl acetate – hexane gradient elution) to afford the almost pure compound as oil. This crude material was directly used for benzylation.

To the solution of benzoyl protected compound (0.9 g, 2.63 mmol) dissolved in anhydrous DMF (10 mL) was added NaH (0.12 g, 2.89 mmol) at 0 ^oC. The mixture was stirred at 0 ^oC for 45 min, and then BnBr (0.37 mL, 3.16 mmol) were added. After stirring for another 12 h when TLC showed that the reaction was completed, it was quenched with H_2O at 0 ^oC, and the mixture was diluted with EtOAc. The aqueous layer was extracted with EtOAc (5 × 25 mL), and the organic

phases were combined and dried over Na_2SO_4. The desired product **S13** (1.093 g, 96.1%) was obtained upon flash column chromatography (ethyl acetate – hexane gradient elution) of the condensed product. Analytical data for **S13:** R*f* = 0.6 (ethyl acetate/hexane, 1/3.5, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.99 - 8.05 (m, 2 H, ArH), 7.54 (d, *J*=6.8 Hz, 1 H, ArH), 7.43 (t, *J*=7.6 Hz, 2 H, ArH), 7.24 - 7.38 (m, 9 H, ArH), 7.17 (br. s., 1 H, ArH), 5.17 (d, *J*=17.4 Hz, 2 H, -N$\underline{CH_2}$Ph), 4.50 (d, *J*=16.6 Hz, 2 H, -O$\underline{CH_2}$Ph), 4.18 - 4.33 (m, 2 H, -C$\underline{H_{2a}}$), 3.17 - 3.34 (m, 2 H, -C$\underline{H_{2e}}$), 1.65 - 1.80 (m, 2 H, -C$\underline{H_{2b}}$), 1.52 - 1.64 (m, 2 H, -C$\underline{H_{2d}}$), 1.30 - 1.45 (m, 2 H, -C$\underline{H_{2c}}$); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 166.6, 156.7, 156.2, 137.9, 136.8, 132.8, 130.4, 129.5, 128.5, 128.4, 128.3, 127.8, 127.3, 127.2, 67.2, 64.8, 64.7, 50.5, 50.2, 47.0, 46.0, 28.4, 27.8, 27.4, 23.3 ppm; HRMS (ESI): m/z calcd for $C_{27}H_{29}NO_4Na$ [M+Na]+: 454.1989, found: 454.1986.

benzyl *N*-benzyl(5-bromopentanyl)carbamate (12).

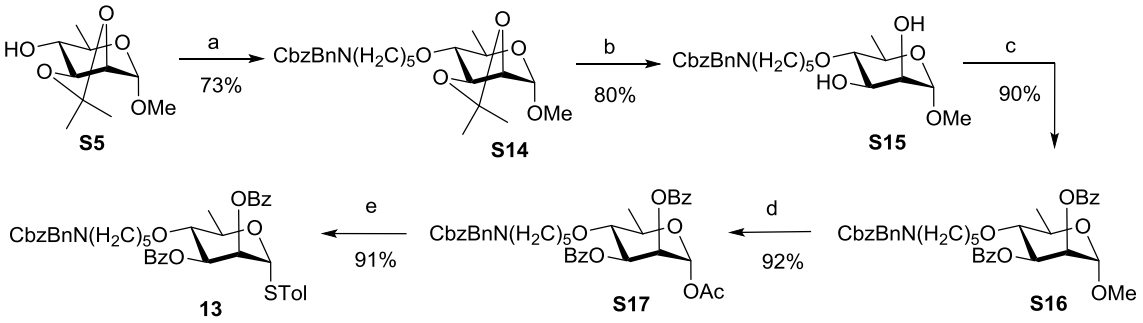


12

Sodium methoxide (~0.8 mL, 0.5 M solution) was added to a solution of **S13** (1.0 g, 2.32 mmol) in CH_3OH (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^{o}C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and this crude material was directly used for bromination.

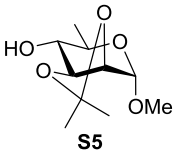
To the solution of deprotected compound (0.96 g, 2.92 mmol) dissolved in anhydrous CH_2Cl_2 (15 mL) were added CBr_4 (1.85 g, 5.55 mmol) and PPh_3 (1.54 g, 5.86 mmol) at 0 ^{o}C. The reaction was allowed to warmup to room temperature and stirring for another 3 h. When TLC showed the reaction was completed, it was quenched with H_2O at 0 ^{o}C, mixture was then diluted with CH_2Cl_2 (~50 mL) and washed with water (2 x 10 mL), sat. aq. $NaHCO_3$ (15 mL), and brine (15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound **12** (1.085 g, 94.8%) as a liquid. Analytical data for **12:** R*f* = 0.85 (ethyl acetate /hexane, 1/4, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.22 - 7.43 (m, 9 H, ArH), 7.18 (br. s., 1 H, ArH), 5.18 (d, *J*=18.8 Hz, 2 H, -N$\underline{CH_2}$Ph), 4.50 (d, *J*=12.9 Hz, 2 H, -O$\underline{CH_2}$Ph), 3.15 - 3.39 (m, 4 H, -C$\underline{H_{2a}}$, -C$\underline{H_{2e}}$), 1.71 - 1.88 (m, 2 H, -C$\underline{H_{2b}}$), 1.45 - 1.60 (m, 2 H, -C$\underline{H_{2d}}$), 1.30 - 1.44 (m, 2 H, -C$\underline{H_{2c}}$); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 156.7, 156.2, 137.8, 136.7, 128.5(x2), 128.4, 128.0, 127.9, 127.4, 127.3, 127.2, 67.3, 67.2, 50.6, 50.3, 46.9, 46.0, 33.6, 33.4, 32.3(x2), 27.2, 26.8, 25.3 ppm; HRMS (ESI): m/z calcd for $C_{20}H_{24}NO_2BrNa$ [M+Na]+: 412.0883, found: 412.0878.

D. <u>Synthesis of the capping *p*-tolyl thioglycoside donor with attached tether 13:</u>



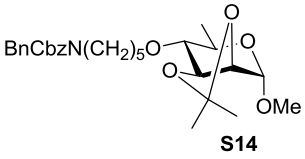
Scheme S4. Conditions: a) CbzBnN$(CH_2)_5$Br, NaH, DMF, 0^{o}C to rt, 18 h; b)TFA/H_2O (9:1), CH_2Cl_2, rt, 10 min.; c) BzCl, DMAP, Et_3N, CH_2Cl_2, 0^{o}C to rt, 12 h; d) Ac_2O, AcOH, H_2SO_4, rt, 4 h; e) BF_3:Et_2O, p-Toluenethiol, CH_2Cl_2, 0^{o}C to rt, 10 h.

Methyl 2,3-*O*-isopropylidene-6-deoxy-α-D-mannopyranoside (S5).



Analytical data for the title compound was essentially the same as previously described.[5]

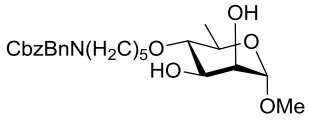
Methyl 4-*O*-(5'-*N*-benzyl-5'-*N*-carboxybenzyl-pentanyl) 2,3-*O*-isopropylidene-6-deoxy-α-D-mannopyranoside (S14).



To the solution of **S5** (2.0 g, 9.17 mmol) dissolved in anhydrous DMF (15 mL) was added NaH (0.4 g, 10.08 mmol) at 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 45 min, and then CbzBnN$(CH_2)_5$Br (4.5 g, 11.01 mmol) were added. After stirring for another 12 h when TLC showed that the reaction was completed, it was quenched with H_2O at 0 ^{o}C, and the mixture was diluted with EtOAc. The aqueous layer was extracted with EtOAc (5 × 25 mL), and the organic phases were combined and dried over Na_2SO_4. The desired product **S14** (3.26 g, 73.2 %) along with eliminated alkene and small amount unreacted starting material **S5** (0.16 g) were obtained upon flash column chromatography (ethyl acetate – hexane gradient elution) of the condensed product. Analytical data for **S14:** R*f* = 0.6 (ethyl acetate/hexane, 1/4, v/v); [α]D ^{21} = +20.48 (*c* = 2.11, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.15 - 7.45 (m, 10 H, ArH), 5.12 - 5.21 (m, 2 H, -N<u>C</u>$\underline{H}_2$Ph), 4.86 (s, 1 H, H-1), 4.45 - 4.52 (m, 2 H, -O<u>C</u>$\underline{H}_2$Ph), 4.10 - 4.14 (m, 2 H, H-2, H-3), 3.81 (br. s., 1 H, -C$\underline{H}_a$), 3.56 - 3.64 (m, 1 H, H-5), 3.39 - 3.48 (m, 1 H, H-4), 3.38 (s, 3 H, -OC$\underline{H}_3$), 3.19 - 3.34 (m, 2 H, -C$\underline{H}_{2e}$), 2.96 - 3.04 (m, 1 H, -C$\underline{H}_a$), 1.49 - 1.61 (m, 4 H, -C$\underline{H}_{2b}$, -C$\underline{H}_{2d}$), 1.51 (s, 3 H, -<u>C</u>$\underline{H}_3$), 1.33 (s, 3 H, -<u>C</u>$\underline{H}_3$), 1.21 - 1.31 (m, 5 H, H-6, -C$\underline{H}_{2c}$); ^{13}C NMR (176

MHz, $CDCl_3$): δ: 156.7, 156.1, 137.9, 136.9, 136.8, 128.5, 128.4, 127.9, 127.8, 127.3, 127.2, 109.0, 98.0, 82.0, 78.5, 75.9, 71.3, 67.1, 64.5, 54.7, 50.4, 50.1, 47.1, 46.1, 29.8, 28.0, 27.9, 27.5, 26.3, 23.4, 17.7 ppm**;** HRMS (ESI): m/z calcd for $C_{30}H_{41}NO_7Na$ [M+Na]+: 550.2775, found: 550.2785.

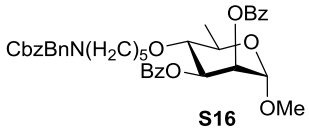
Methyl 4-*O*-(5'-*N*-benzyl-5'-*N*-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranoside S15).



S15

A solution of **S14** (1.0 g, 1.89 mmol) in TFA:H_2O (9:1, 10 mL) was stirred at 21 ^oC for 30 min, and then poured into ice-cold 1M K_2CO_3 solution (50 mL). The mixture was then diluted with CH_2Cl_2 (~50 mL) and washed with water (2 x 30 mL), sat. aq. $NaHCO_3$ (25 mL), and brine (15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound **S15** (0.742 g, 80.3%) as oil. Analytical data for **S15:** R*f* = 0.4 (ethyl acetate/hexane, 1/1, v/v); [α]D ^{21} = +38.31 (*c* = 1.27, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.24 - 7.39 (m, 9 H, ArH), 7.12 - 7.19 (m, 1 H, ArH), 5.12 - 5.20 (m, 2 H, -N$\underline{CH_2}$Ph), 4.64 (s, 1 H, H-1), 4.45 – 4.51 (m, 2 H, -O$\underline{CH_2}$Ph), 3.88 - 3.92 (m, 1 H, H-2), 3.74 - 3.83 (m, 1 H, H-3), 3.49 - 3.70 (m, 3 H, H-5, -C$\underline{H_{2a}}$), 3.33 (s, 3 H, -OC$\underline{H_3}$), 3.23 - 3.27 (m, 1 H, H-4), 3.16 - 3.21 (m, 1 H, -C$\underline{H_e}$), 3.04 - 3.15 (m, 1 H, -C$\underline{H_e}$), 2.30 - 2.52 (2 x br. s., 2 -OH), 1.44 - 1.68 (m, 4 H, -C$\underline{H_{2b}}$, -C$\underline{H_{2d}}$), 1.31 - 1.41 (m, 2 H, -C$\underline{H_{2c}}$), 1.28 (s., 3 H, H-6); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 156.7, 156.3, 137.8, 136.6, 129.6, 128.5, 128.4, 127.9, 127.8, 127.3, 127.2, 100.3, 81.7, 71.4, 71.3, 71.2, 67.2, 67.1, 54.8, 50.5, 50.3, 47.1, 46.1, 30.0, 29.8, 27.9, 27.2, 23.2, 17.9 ppm; HRMS (ESI): m/z calcd for $C_{27}H_{37}NO_7Na$ [M+Na]+: 510.2462, found: 510.2462.

Methyl 2,3-di-*O*-benzoyl-4-*O*-(5'-*N*-benzyl-5'-*N*-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranoside (S16).

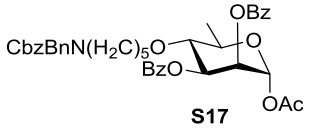


S16

Benzoyl chloride (0.23 mL, 1.97 mmol) was added dropwise to a stirred solution of **S15** (0.4 g, 0.82 mmol) in anhydrous CH_2Cl_2 (10 mL) containing Et_3N (0.46 mL, 3.28 mmol) at 0 ^oC. After 2 minute DMAP (0.451 g, 3.69 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise to the reaction mixture and stirred at rt overnight. The resulting mixture was diluted with CH_2Cl_2 (~20 mL) and washed with aq. HCl (1M, 2 x 5 mL), water (20 mL), sat. aq. $NaHCO_3$ (10 mL), and brine (10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound **S16** (0.513 g, 90%) as oil. Analytical data for **S16**: R*f* = 0.7 (ethyl acetate /hexane, 1/3.5, v/v); [α]D ^{21} = -70.58 (*c* = 1.71, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 8.04 (d, *J*=7.4 Hz, 2 H, ArH), 7.89 (d, *J*=6.7 Hz, 2 H, ArH), 7.58 - 7.61 (m, 1

H, ArH), 7.43 - 7.48 (m, 3 H, ArH), 7.22 - 7.34 (m, 10 H, ArH), 7.08 - 7.20 (m, 2 H, ArH), 5.60 (dd, *J*=9.6, 3.5 Hz, 1 H, H-3), 5.53 - 5.56 (m, 1 H, H-2), 5.13 (d, *J*=7.4 Hz, 2 H, -N$\underline{CH_2}$Ph), 4.78 (d, *J*=1.3 Hz, 1 H, H-1), 4.34 - 4.44 (m, 2 H, -O$\underline{CH_2}$Ph), 3.85 - 3.91 (m, 1 H, H-5), 3.48 - 3.61 (m, 3 H, H-4, -C$\underline{H_{2a}}$), 3.42 (s, 3 H, -OC$\underline{H_3}$), 2.94 - 3.13 (m, 2 H, -C$\underline{H_{2e}}$), 1.24 - 1.46 (m, 7 H, H-6, -C$\underline{H_{2b}}$, -C$\underline{H_{2d}}$), 1.04 - 1.23 (m, 2 H, -C$\underline{H_{2c}}$); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 165.5, 165.2, 156.7, 156.3, 137.9, 133.3, 133.0, 129.9, 129.8, 129.8, 129.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.1, 98.5, 79.5, 73.1, 72.9, 72.1, 71.1, 67.6, 67.1, 55.0, 50.4, 50.1, 47.0, 46.0, 29.9, 27.8, 27.4, 23.3, 18.0 ppm; HRMS (ESI): m/z calcd for $C_{41}H_{45}NO_9Na$ [M+Na]+: 718.2987, found: 718.298.

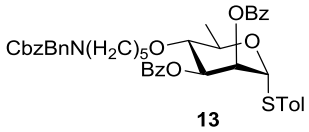
1-*O*-acetyl-2,3-di-*O*-benzoyl-4-*O*-(5'-*N*-benzyl-5'-*N*-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranose (S17).



S17

A solution of **S16** (0.5 g, 0.716 mmol) in acetic anhydride/acetic acid/sulfuric acid (50:20:0.5, 10 mL) was stirred at 21 ^{o}C for 3 h, and then poured into ice-cold 1M K_2CO_3 solution (50 mL). The mixture was then diluted with CH_2Cl_2 (~20 mL) and washed with water (2 x 30 mL), sat. aq. $NaHCO_3$ (15 mL), and brine (10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound **S17** (0.485 g, 92.2%) as a liquid. Analytical data for **S17:** R*f* = 0.55 (ethyl acetate /hexane, 1/4, v/v); [α]D ^{21} = -48.86 (*c* = 1.51, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 8.01 - 8.04 (m, 2 H, ArH), 7.88 (d, *J*=6.8 Hz, 2 H, ArH), 7.59 - 7.62 (m, 1 H, ArH), 7.46 - 7.49 (m, 3 H, ArH), 7.21 - 7.34 (m, 10 H, ArH), 7.08 - 7.20 (m, 2 H, ArH), 6.17 (d, *J*=1.9 Hz, 1 H, H-1), 5.62 (dd, *J*=9.6, 3.5 Hz, 1 H, H-3), 5.56 - 5.58 (m, 1 H, H-2), 5.10 - 5.15 (m, 2 H, -N$\underline{CH_2}$Ph), 4.34 - 4.42 (m, 2 H, -O$\underline{CH_2}$Ph), 3.92 - 3.98 (m, 1 H, H-5), 3.48 - 3.65 (m, 3 H, H-4, -C$\underline{H_{2a}}$), 2.95 - 3.18 (m, 2 H, C$\underline{H_{2e}}$), 2.18 (s, 3 H, -COC$\underline{H_3}$), 1.25 - 1.49 (m, 7 H, H-6, -C$\underline{H_{2b}}$, -C$\underline{H_{2d}}$), 1.03 - 1.23 (m, 2 H, -C$\underline{H_{2c}}$); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 168.6, 165.4, 165.2, 137.8, 136.7, 133.5, 133.2, 129.8, 129.6, 129.4, 129.0, 128.5, 128.5, 128.4, 128.2, 127.9, 127.8, 127.2, 127.1, 90.8, 79.1, 73.4, 71.8, 70.1, 69.9, 67.1, 50.4, 50.1, 46.9, 45.9, 29.9, 27.8, 27.4, 23.3, 21.0, 18.1, ppm; HRMS (ESI): m/z calcd for $C_{42}H_{45}NO_{10}Na$ [M+Na]+: 746.2936, found: 746.2931.

***p*-Tolyl 2,3-di-*O*-benzoyl-4-*O*-(5'-*N*-benzyl-5'-*N*-carboxybenzyl-pentanyl)-6-deoxy-1-thio-α-D-mannopyranoside (13).**



13

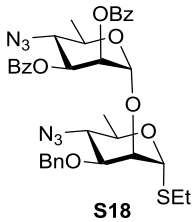
To the stirred solution of **S17** (1.2 g, 1.66 mmol) and *p*-toluenethiol (0.312 g, 2.48 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 ^{o}C, $BF_3{\cdot}Et_2O$ (0.25 mL, 1.99 mmol) was added drop wise. When TLC showed the reaction was completed, the mixture was then diluted with CH_2Cl_2 (~30 mL) and washed with water (2 x 10 mL), sat. aq. $NaHCO_3$ (10 mL), and brine (20 mL). The organic

phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate – hexane gradient elution) to give **13** as a white solid (1.18 g, 90.7%). Analytical data for **13:** R*f* = 0.65 (ethyl acetate /hexane, 1/4, v/v); [α]D ^{21} = -1.02 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ : 8.00 - 8.02 (m, 2 H, ArH), 7.90 (d, *J*=6.5 Hz, 2 H, ArH), 7.56 - 7.60 (m, 1 H, ArH), 7.43 - 7.48 (m, 3 H, ArH), 7.39 - 7.42 (m, 2 H, ArH), 7.21 - 7.34 (m, 11 H, ArH), 7.12 (d, *J*=8.1 Hz, 3 H, ArH), 5.80 (dd, *J*=3.3, 1.5 Hz, 1 H, H-2), 5.61 (dd, *J*=9.6, 3.3 Hz, 1 H, H-3), 5.48 (d, *J*=1.5 Hz, 1 H, H-1), 5.16 - 5.18 (m, 2 H, -N$\underline{CH_2}$Ph), 4.34 - 4.42 (m, 3 H, H-5, -O$\underline{CH_2}$Ph), 3.50 - 3.65 (m, 3 H, H-4, -C$\underline{H_{2a}}$), 2.96 - 3.15 (m, 2 H, -C$\underline{H_{2e}}$), 2.32 (s, 3 H), 1.06 - 1.48 (m, 9 H, H-6, -C$\underline{H_{2b}}$, -C$\underline{H_{2d}}$, -C$\underline{H_{2c}}$); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 165.4, 165.3, 156.7, 156.1, 138.1, 137.9, 136.9, 136.8, 133.4, 133.2, 132.7, 132.3, 130.0, 129.9(x2), 129.8, 129.7(x2), 129.6, 128.5(x2), 128.4(x2), 127.9, 127.8, 127.3(x2), 127.2, 86.2, 79.7, 73.3, 73.1, 72.6, 72.4(x2), 69.2, 67.1, 50.5, 50.2, 47.0, 46.0, 30.0, 27.9, 27.4, 23.3, 21.2, 18.0 ppm; HRMS (ESI): m/z calcd for $C_{47}H_{49}NO_8SNa$ [M+Na]+: 810.3071, found: 810.3069.

VII. SYNTHESIS OF OLIGOSACCHARIDES S20-S21; 14-25

Synthesis of the 1,2 linked trisaccharide S21

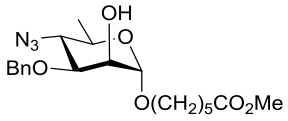
Ethyl 4-azido-2,3-di-*O*-benzoyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside (S18).



S18

Analytical data for the title compound was essentially the same as previously described.[1]

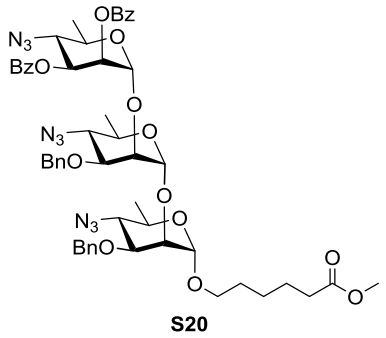
5'-Methoxycarbonylpentyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (S19).



S19

Analytical data for the title compound was essentially the same as previously described.[5]

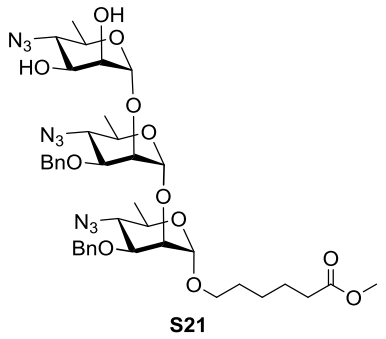
5'-Methoxycarbonylpentyl 4-azido-2,3-*O*-benzoyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (S20).



S20

The glycosyl acceptor compound **S19** (0.2 g, 0.491 mmol), and glycosyl donor compound **S18** (0.414 g, 0.589 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH_2Cl_2 (15 mL), treated with freshly activated 4 Å molecular sieves (1 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.221 g, 0.982 mmol). After cooling to -10 ºC, TMSOTf (19.5 μL, 0.108 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous $NaHCO_3$ (5 mL) and CH_2Cl_2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous $Na_2S_2O_3$ (20%) and water. After extraction of the aqueous layer with CH_2Cl_2 (3 x 5), the combined organic phase was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (ethyl acetate - Hexane gradient elution) to give disaccharide **S20** (0.418 g, 81.3%) as a sticky liquid. Analytical data for **S20**: R*f* = 0.7 (ethyl acetate/Hexane 1:4.5, v/v); $[\alpha]_D^{21}$ = -14.49° (*c* = 1.79, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$): δ 8.02 - 8.05 (m, 2 H, ArH), 7.95 - 7.97 (m, 2 H, ArH), 7.64 - 7.68 (m, 1 H, ArH), 7.50 - 7.57 (m, 3 H, ArH), 7.33 - 7.41 (m, 8 H, ArH), 7.22 - 7.26 (m, 3 H, ArH), 7.13 - 7.17 (m, 1 H, ArH), 5.71 (dd, *J*=3.3, 1.5 Hz, 1 H, H-2_C), 5.59 (dd, *J*=10.3, 3.3 Hz, 1 H, H-3_C), 5.06 (d, *J*=1.8 Hz, 1 H, H-1_B), 5.02 (d, *J*=1.8 Hz, 1 H, H-1_C), 4.76 (d, *J*=11.7 Hz, 1 H, CHPh), 4.62 - 4.69 (m, 4 H, 3 CHPh, H-1_A), 3.95 (dd, *J*=2.2, 0.7 Hz, 1 H, H-2_B), 3.90 (dd, *J*=2.2, 0.7 Hz, 1 H, H-2_A), 3.76 - 3.81 (m, 2 H, H-3_B, H-5_B), 3.74 (dd, *J*=9.9, 2.9 Hz, 1 H, H-3_A), 3.71 (s, 3 H), 3.69 (t, *J* =9.9 Hz, 1H, H-4_C), 3.55 - 3.65 (m, 3 H, H-4_B, H-5_C, -O-CH_{2b}), 3.43 - 3.49 (m, 1 H, H-5_A), 3.38 (dt, *J*=9.7, 6.4 Hz, 1 H, -O-CH_{2a}), 3.27 (t, *J*=9.9 Hz, 1 H, H-4_A), 2.33 - 2.39 (m, 2 H, -CH_{2f}), 1.64 - 1.72 (m, 2 H, -CH_{2e}), 1.56 - 1.64 (m, 2 H, -CH_{2c}), 1.35 - 1.42 (m, 2 H, -CH_{2d}), 1.38 (d, *J*=5.6 Hz, 3 H, H-6_C), 1.32 (d, *J*=5.9 Hz, 3 H, H-6_B), 1.29 (d, *J*=5.9 Hz, 3 H, H-6_A); ^{13}C NMR (126MHz, $CDCl_3$): δ 174.0, 165.2, 164.9, 137.5, 137.3, 133.4, 133.3, 129.8(x2), 129.6, 129.3, 128.5(x2), 128.4, 128.2, 128.1(x2), 128.0, 100.3, 99.0, 98.8, 77.9, 73.9, 73.5, 72.3(x2), 70.9, 69.5, 68.0, 67.5, 67.2, 64.5, 63.9, 63.5, 51.5, 34.0, 29.1, 25.7, 24.7, 18.6(x2), 18.4 ppm; HRMS (ESI): m/z calcd for $C_{53}H_{61}N_9O_{14}Na$ [M+Na]+: 1070.423, found: 1070.4248.

5'-Methoxycarbonylpentyl 4-azido-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (S21).

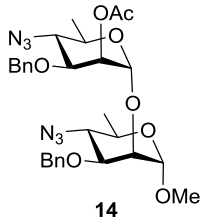


S21

Sodium methoxide (~0.3 mL, 0.5 M solution) was added to a solution of **S20** (0.39 g, 0.372 mmol) in CH_3OH: THF [4:2] (12 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^oC. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (ethyl acetate - Hexane gradient elution) to afford the title compound **S21** (0.299 g, 95.6%) as white solid. Analytical data for **S21**: R*f* = 0.3 (ethyl acetate/Hexane 1:1.5, v/v); $[\alpha]D^{21}$ = +84.18 (*c* = 1.55, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$): δ 7.30 - 7.44 (m, 10 H, ArH), 5.00 (d, *J*=1.8 Hz, 1 H, H-1_B), 4.90 (d, *J*=1.5 Hz, 1 H, H-1_C), 4.72 (d, *J*=11.4 Hz, 1 H, C<u>H</u>Ph), 4.61 - 4.67 (m, 4 H, , 3 C<u>H</u>Ph, H-1_A), 3.93 - 3.97 (m, 2 H, H-2_B, H-2_C), 3.81 - 3.87 (m, 2 H, H-2_A, H-3_A), 3.76 (dd, *J*=9.9, 2.9 Hz, 1 H, H-3_B), 3.73 (dd, *J*=10.0, 2.9 Hz, 1 H, H-3_C), 3.70 (s, 3 H), 3.51 - 3.64 (m, 3 H, H-5_B, H-5_C, -O-C<u>H</u>$_{2b}$), 3.43 - 3.49 (m, 1 H, H-5_A), 3.40 (t, *J* =9.9 Hz, 1H, H-4_C), 3.36 (dt, *J*=9.7, 6.4 Hz, 1 H, -O-C<u>H</u>$_{2a}$), 3.27 (t, *J* =9.9 Hz, 1H, H-4_B), 3.40 (t, *J* =10.2 Hz, 1H, H-4_A), 2.49 (d, *J*=6.9 Hz, 1 OH_{3C},), 2.34 (t, *J*=7.4 Hz, 2 H, -C<u>H</u>$_{2f}$), 2.18 (d, *J*=3.9 Hz, 1 OH_{2C}), 1.63 - 1.70 (m, 2 H, -C<u>H</u>$_{2e}$), 1.54 - 1.61 (m, 2 H, -C<u>H</u>$_{2c}$), 1.33 - 1.40 (m, 2 H, -C<u>H</u>$_{2d}$), 1.30 (d, *J*=6.2 Hz, 6 H, H-6_B, H-6_C), 1.20 (d, *J*=6.2 Hz, 3 H, H-6_A); ^{13}C NMR (126MHz, $CDCl_3$): δ 174.0, 137.4(x2), 128.6(x2), 128.3, 128.2(x2), 128.1, 100.7, 100.4, 98.7, 77.7, 77.2, 77.2, 73.8, 73.2, 72.3, 72.2, 70.2, 69.9, 67.8, 67.5, 67.4, 67.1, 65.8, 64.4, 64.2, 51.6, 33.9, 29.1, 25.7, 24.7, 18.6(x2), 18.2 ppm; HRMS (ESI): m/z calcd for $C_{39}H_{53}N_9O_{12}Na$ [M+Na]+: 862.3706, found: 862.3705.

Synthesis of the heptasaccharide with capping tether 25

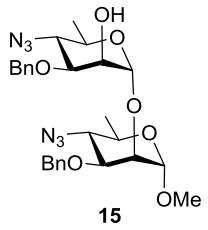
Methyl 4-azido-2-*O*-acetyl-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (14).



14

The glycosyl acceptor compound **S11** (1.42 g, 4.84 mmol), and glycosyl donor compound **11** (2.27 g, 5.33 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH_2Cl_2 (20 mL), treated with freshly activated 4 Å molecular sieves (1.5 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (2.4 g, 9.71 mmol). After cooling to -10 ºC, TMSOTf (0.19 mL, 0.971 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous $NaHCO_3$ (15 mL) and CH_2Cl_2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous $Na_2S_2O_3$ (20%) and water. After extraction of the aqueous layer with CH_2Cl_2 (3x15), the combined organic phase was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate/Hexane gradient elution) to give disaccharide **14** (2.66 g, 92.1%) as a sticky liquid. Analytical data for **14**: R*f* = 0.5 (Ethyl acetate /Hexane 1:4, v/v); $[\alpha]_D^{21}$ = +36.24° (*c* = 1.92, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.32 - 7.38 (m, 8 H, ArH), 7.27 - 7.31 (m, 2 H, ArH), 5.41 (dd, *J*=3.0, 1.9 Hz, 1 H, H-2_B), 4.85 (d, *J*=1.5 Hz, 1 H, H-1_B), 4.71 (d, *J*=11.1 Hz, 1 H, -C<u>H</u>Ph), 4.68 (d, *J*=11.5 Hz, 1 H, -C<u>H</u>Ph), 4.61 (d, *J*=11.6 Hz, 1 H, -C<u>H</u>Ph), 4.57 (d, *J*=1.4 Hz, 1 H, H-1_A), 4.54 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 3.84 - 3.86 (m, 1 H, H-2_A), 3.79 (dd, *J*=9.9, 3.3 Hz, 1 H, H-3_B), 3.72 (dd, *J*=9.9, 3.0 Hz, 1 H, H-3_A), 3.59 (dq, *J*=9.9, 6.1 Hz, 1 H, H-5_B), 3.45 (dq, *J*=10.1, 6.1 Hz, 1 H, H-5_A), 3.39 (t, *J*=9.9 Hz, 1 H, H-4_B), 3.33 (t, *J*=10.1 Hz, 1 H, H-4_A), 3.30 (s, 3 H, -OC<u>H</u>$_3$), 2.08 (s, 3 H, -COC<u>H</u>$_3$), 1.30 (d, *J*=6.3 Hz, 6 H, H-6_A, H-6_B); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 169.7, 137.6, 137.1, 128.5(x2), 128.4, 128.0, 127.9, 127.8, 99.7, 99.4, 77.7, 75.4, 73.7, 72.0, 71.6, 67.6, 67.2, 66.9, 64.1, 63.8, 54.9, 20.9, 18.5(x2) ppm; HRMS (ESI): m/z calcd for $C_{29}H_{36}N_6O_8Na$ [M+Na]+: 619.2487, found: 619.2481.

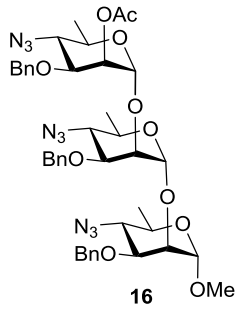
Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (15).



15

Sodium methoxide (~1.2 mL, 0.5 M solution) was added to a solution of **14** (2.6 g, 4.36 mmol) in CH_3OH: THF [4:2] (20 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^oC. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to deprotected disaccharide compound **15** (2.3 g, 95.4%) as white foam. Analytical data for **15**: R*f* = 0.4 (Ethyl acetate /Hexane 1:4.5, v/v); $[\alpha]_D^{21}$ = +28.71 (*c* = 1.56, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.34 - 7.41 (m, 8 H, ArH), 7.29 - 7.34 (m, 2 H, ArH), 4.94 (d, *J*=1.1 Hz, 1 H, H-1_B), 4.71 (d, *J*=11.4 Hz, 1 H, -C<u>H</u>Ph), 4.67 (d, *J*=11.3 Hz, 1 H, -C<u>H</u>Ph), 4.67 (ABq, *J*=11.3 Hz, 1 H, -C<u>H_2</u>Ph), 4.58 (d, *J*=1.5 Hz, 1 H, H-1_A), 3.98 (t, *J*=1.8 Hz, 1 H, H-2_B), 3.90 (t, *J*=2.3 Hz, 1 H, H-2_A), 3.71 (dd, *J*=9.9, 3.1 Hz, 2 H, H-3_B, H-3_A), 3.59 (dq, *J*=10.2, 5.9 Hz, 1 H, H-5_A), 3.45 (dq, *J*=10.1, 6.1 Hz, 1 H, H-5_B), 3.42 (t, *J*=9.9 Hz, 1 H, H-4_A), 3.30 (s, 3 H, -OC<u>H_3</u>), 3.29 (t, *J*=10.0 Hz, 1 H, H-4_B), 2.30 (s, 1 –OH_{2B}), 1.31 (d, *J*=6.1 Hz, 6 H, H-6_A), 1.29 (d, *J*=5.9 Hz, 6 H, H-6_B); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 137.5, 137.1, 128.6, 128.5, 128.3, 128.2(x2), 128.0, 100.8, 99.9, 77.8, 77.6, 73.6, 72.1(x2), 67.3, 67.2, 66.9, 64.3, 63.8, 54.9, 18.6, 18.4 ppm; HRMS (ESI): m/z calcd for $C_{27}H_{34}N_6O_7Na$ [M+Na]+: 577.2381, found: 577.2381.

Methyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (16).

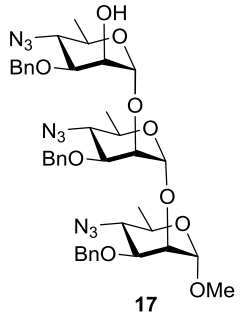


16

The glycosyl acceptor compound **15** (2.25 g, 4.06 mmol), and glycosyl donor compound **11** (1.90 g, 4.46 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH_2Cl_2 (25 mL), treated with freshly activated 4 Å molecular sieves (1.6 g), stirred under an Ar atmosphere at rt for 1 h.

To the mixture was added NIS (1.83 g, 8.11 mmol). After cooling to -10 ºC, TMSOTf (0.16 mL 0.893 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous $NaHCO_3$ (15 mL) and CH_2Cl_2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous $Na_2S_2O_3$ (20%) [30 mL] and water (20 mL). After extraction of the aqueous layer with CH_2Cl_2 (3x15), the combined organic phase was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give trisaccharide **16** (3.09 g, 88.9%) as a sticky liquid. Analytical data for **16**: R*f* = 0.65 (Ethyl acetate /Hexane 1:5, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.32 - 7.39 (m, 12 H, ArH), 7.26 - 7.32 (m, 3 H, ArH), 5.39 (dd, *J*=3.2, 1.9 Hz, 1 H, H-2_C), 4.95 (d, *J*=1.8 Hz, 1 H, H-1_B), 4.82 (d, *J*=1.8 Hz, 1 H, H-1_C), 4.70 (d, *J*=11.5 Hz, 1 H, -C<u>H</u>Ph), 4.69 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 4.63 (d, *J*=11.5 Hz, 1 H, -C<u>H</u>Ph), 4.59 (ABq, *J*=11.5 Hz, 1 H, -C<u>H$_2$</u>Ph), 4.53 (d, *J*=1.8 Hz, 1 H, H-1_A), 4.52 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 3.86 (t, *J*=2.4 Hz, 1 H, H-2_B), 3.83 (t, *J*=2.3 Hz, 1 H, H-2_A), 3.75 (dd, *J*=9.9, 3.3 Hz, 1 H, H-3_C), 3.72 (dd, *J*=9.9, 2.9 Hz, 1 H, H-3_B), 3.67 (dd, *J*=10.0, 2.8 Hz, 1 H, H-3_A), 3.52 (dq, *J*=10.0, 6.1 Hz, 1 H, H-5_B), 3.49 (dq, *J*=10.0, 6.0 Hz, 1 H, H-5_C), 3.42 (dq, *J*=10.1, 6.2 Hz, 1 H, H-5_A), 3.35 (t, *J*=9.9 Hz, 1 H, H-4_C), 3.34 (t, *J*=9.9 Hz, 1 H, H-4_B), 3.29 (s, 3 H, -OC<u>H$_3$</u>), 3.22 (t, *J*=10.0 Hz, 1 H, H-4_A), 2.09 (s, 3 H, -COC<u>H$_3$</u>), 1.29 (d, *J*=6.2 Hz, 3 H, H-6_A), 1.28 (d, *J*=6.3 Hz, 3 H, H-6_B), 1.18 (d, *J*=6.1 Hz, 3 H, H-6_C); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 169.7, 137.4, 137.3, 137.1, 128.5(x2), 128.4, 128.1, 128.0(x3), 100.3, 99.8, 99.1, 77.5, 76.8, 75.4, 73.5, 72.1, 72.0, 71.5, 67.8, 67.6, 67.1, 67.0, 64.4, 64.0, 63.8, 54.9, 21.0, 18.6(x2), 18.3 ppm**;** HRMS (ESI): m/z calcd for $C_{42}H_{51}N_9O_{11}Na$ [M+Na]+: 880.36, found: 880.3607.

Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosid (17).

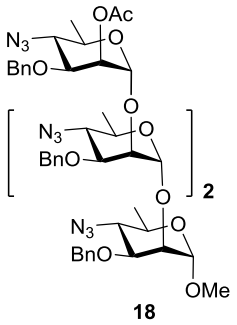


17

Sodium methoxide (~1.5 mL, 0.5 M solution) was added to a solution of **16** (3.0 g, 3.5 mmol) in CH_3OH: THF [4:2] (20 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^{o}C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the deprotected trisaccharide compound **17** (2.6 g, 91.2%) as white solid foam. Analytical data for **17**: R*f* = 0.45 (Ethyl acetate /Hexane 1:5, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.33 - 7.41 (m, 12 H, ArH), 7.28 - 7.32 (m, 3 H, ArH), 4.96 (d, *J*=1.8 Hz, 1 H, H-1_C), 4.95 (d, *J*=1.7 Hz, 1 H, H-1_B), 4.71 (d, *J*=11.3 Hz, 1 H, -C<u>H</u>Ph), 4.67 (d, *J*=11.3 Hz, 1 H, -C<u>H</u>Ph), 4.66 (d, *J*=11.6 Hz, 1 H, -C<u>H</u>Ph), 4.62 (d, *J*=11.1 Hz, 1 H, -C<u>H</u>Ph),

4.61 (d, *J*=11.1 Hz, 1 H, -C<u>H</u>Ph), 4.58 (d, *J*=11.6 Hz, 1 H, -C<u>H</u>Ph), 4.55 (d, *J*=1.8 Hz, 1 H, H-1_A), 3.97 - 3.99 (m, 1 H, H-2_B), 3.92 (t, *J*=2.4 Hz, 1 H, H-2_C), 3.83 (t, *J*=2.3 Hz, 1 H, H-2_A), 3.72 (dd, *J*=9.9, 2.3 Hz, 1 H, H-3_C), 3.69 (dd, *J*=9.8, 3.1 Hz, 1 H, H-3_B), 3.68 (dd, *J*=10.1, 2.8 Hz, 1 H, H-3_A), 3.52 (dq, *J*=10.1, 5.9 Hz, 1 H, H-5_C), 3.50 (dq, *J*=9.9, 6.1 Hz, 1 H, H-5_B), 3.42 (dq, *J*=10.1, 6.0 Hz, 1 H, H-5_A), 3.40 (t, *J*=10.0 Hz, 1 H, H-4_B), 3.31 (t, *J*=9.8 Hz, 1 H, H-4_C), 3.29 (s, 3 H, -OC<u>H</u>$_3$), 3.23 (t, *J*=9.9 Hz, 1 H, H-4_A), 2.29 (s, 1 H, 1 -OH_{2C}), 1.29 (d, *J*=6.0 Hz, 3 H, H-6_A), 1.28 (d, *J*=6.0 Hz, 3 H, H-6_B), 1.18 (d, *J*=6.3 Hz, 3 H, H-6_C); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 137.3(x2), 137.2, 128.6(x3), 128.5, 128.3, 128.3, 128.2(x2), 128.1(x2), 128.0, 100.5, 100.4, 99.8, 77.6, 77.5, 76.8, 73.6, 73.3, 72.2, 72.1(x2), 67.8, 67.3, 67.1, 67.0, 64.4, 64.2, 63.8, 54.9, 18.6(x2), 18.3 ppm; HRMS (ESI): m/z calcd for $C_{40}H_{49}N_9O_{10}Na$ [M+Na]+: 383.3495, found: 838.3501.

Methyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (18).

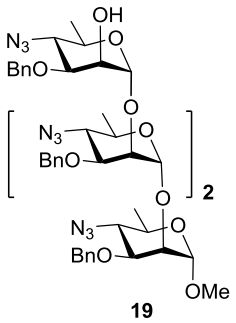


18

The glycosyl acceptor compound **17** (2.05 g, 2.51 mmol), and glycosyl donor compound **11** (1.18 g, 2.76 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH_2Cl_2 (20 mL), treated with freshly activated 4 Å molecular sieves (1.2 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (1.13 g, 5.02 mmol). After cooling to -10 ºC, TMSOTf (0.1 mL, 0.553 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous $NaHCO_3$ (10 mL) and CH_2Cl_2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous $Na_2S_2O_3$ (20%) and water. After extraction of the aqueous layer with CH_2Cl_2 (3 x 10), the combined organic phase was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give tetrasaccharide **18** (2.49 g, 87.8%) as a syrup. Analytical data for **18**: R*f* = 0.5 (Ethyl acetate /Hexane 1:4, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.32 - 7.38 (m, 16 H, ArH), 7.27 - 7.32 (m, 4 H, ArH), 5.39 (dd, *J*=3.1, 1.9 Hz, 1 H, H-2_D), 4.92 (d, *J*=1.8 Hz, 1 H, H-1_C), 4.87 (d, *J*=1.8 Hz, 1 H, H-1_B), 4.83 (d, *J*=1.8 Hz, 1 H, H-1_D), 4.71 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 4.70 (d, *J*=11.7 Hz, 1 H, -C<u>H</u>Ph), 4.62 (d, *J*=11.5 Hz, 1 H, -C<u>H</u>Ph), 4.60 (s, 2 H, -C<u>H</u>$_2$Ph), 4.58 (ABq, *J*=11.7 Hz, 1 H, -C<u>H</u>$_2$Ph), 4.53 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 4.51 (d, *J*=1.8 Hz, 1 H, H-1_A), 3.86 (t, *J*=2.3 Hz, 1 H, H-2_C), 3.82 (t, *J*=2.3 Hz, 1 H, H-2_B), 3.80 (t, *J*=2.3 Hz, 1 H, H-2_A), 3.76 (dd, *J*=9.9, 3.3 Hz, 1 H, H-3_D), 3.64 - 3.69 (m, 3 H, H-3_C, H-3_B, H-3_A), 3.50 (dq, *J*=10.0, 6.1 Hz, 1 H, H-5_D), 3.48 (dq, *J*=9.7, 5.9 Hz, 1 H, H-5_A), 3.42 (dq, *J*=9.7, 6.1 Hz, 1 H, H-

5_C), 3.40 (dq, *J*=9.6, 6.1 Hz, 1 H, H-5_B), 3.36 (t, *J*=10.0 Hz, 1 H, H-4_D), 3.31 (t, *J*=10.0 Hz, 1 H, H-4_C), 3.28 (s, 3 H, -OC$\underline{H}_3$), 3.22 (t, *J*=9.9 Hz, 1 H, H-4_A), 3.18 (t, *J*=9.9 Hz, 1 H, H-4_B), 2.09 (s, 3 H, -COC$\underline{H}_3$), 1.27 (d, *J*=6.1 Hz, 3 H, H-6_B), 1.26 (d, *J*=6.4 Hz, 3 H, H-6_A), 1.19 (d, *J*=6.1 Hz, 3 H, H-6_D), 1.14 (d, *J*=6.1 Hz, 3 H, H-6_C); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 169.8, 137.4, 137.3, 137.1(x2), 128.6(x2), 128.5(x2), 128.4, 128.3, 128.2, 128.1, 128.0(x3), 100.3, 100.1, 99.7, 99.1, 77.4, 76.6, 75.4, 73.6, 73.4(x2), 72.2, 72.1, 72.0, 71.5, 67.8, 67.6, 67.1, 66.9, 64.3, 64.2, 64.0, 63.8, 54.9, 21.0, 18.6(x2), 18.5, 18.4 ppm; HRMS (ESI): m/z calcd for $C_{55}H_{66}N_{12}O_{14}Na$ [M+Na]+: 1141.4714, found: 1141.473.

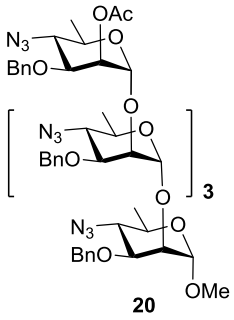
Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (19).



19

Sodium methoxide (~1.2 mL, 0.5 M solution) was added to a solution of **18** (2.2 g, 1.95 mmol) in CH_3OH: THF [4:2] (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^oC. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound **19** (1.86 g, 88.7%) as white solid. Analytical data for **19**: R*f* = 0.4 (Ethyl acetate /Hexane 1:4, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.28 - 7.41 (m, 20 H, ArH), 4.96 (d, *J*=1.5 Hz, 1 H, H-1_D), 4.93 (d, *J*=1.8 Hz, 1 H, H-1_C), 4.88 (d, *J*=1.8 Hz, 1 H, H-1_B), 4.72 (d, *J*=11.5 Hz, 1 H, -C$\underline{H}$Ph), 4.68 (d, *J*=11.3 Hz, 1 H), 4.66 (d, *J*=11.3 Hz, 1 H), 4.62 (d, *J*=11.7 Hz, 1 H, -C$\underline{H}$Ph), 4.60 (s, 2 H, -C$\underline{H}_2$Ph), 4.57 (ABq, *J*=11.5 Hz, 2 H, -C$\underline{H}_2$Ph), 4.51 (d, *J*=1.8 Hz, 1 H, H-1_A), 3.97 - 3.99 (m, 1 H, H-2_D), 3.92 (t, *J*=2.4 Hz, 1 H, H-2_C), 3.81 (t, *J*=2.3 Hz, 1 H, H-2_B), 3.80 (t, *J*=2.3 Hz, 1 H, H-2_A), 3.64 - 3.71 (m, 4 H, H-3_D, H-3_C, H-3_B, H-3_A), 3.51 (dq, *J*=10.1, 6.1 Hz, 1 H, H-5_D), 3.48 (dq, *J*=9.9, 6.1 Hz, 1 H, H-5_B), 3.37 - 3.45 (m, 2 H, H-5_C, H-5_A), 3.40 (t, *J*=9.8 Hz, 1 H, H-4_D), 3.29 (t, *J*=9.5 Hz, 1 H, H-4_C), 3.28 (s, 3 H, -OC$\underline{H}_3$), 3.23 (t, *J*=9.9 Hz, 1 H, H-4_B), 3.18 (t, *J*=10.0 Hz, 1 H, H-4_A), 2.26 (d, *J*=1.8 Hz, 1 H, 1 -OH_{2D}), 1.27 (d, *J*=6.3 Hz, 3 H, H-6_A), 1.25 (d, *J*=6.3 Hz, 3 H, H-6_B), 1.19 (d, *J*=6.1 Hz, 3 H, H-6_D), 1.15 (d, *J*=6.1 Hz, 3 H, H-6_C); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 137.3(x2), 137.1, 128.6(x2), 128.5, 128.4, 128.3(x2), 128.2(x3), 128.1, 128.0, 100.4, 100.3, 100.2, 99.7, 77.7, 77.4, 76.6, 73.6, 73.5, 73.2, 72.2, 72.1(x3), 67.8, 67.3, 67.1, 66.9, 64.3, 64.2(x2), 63.8, 54.9, 18.6(x2), 18.5, 18.3 ppm; HRMS (ESI): m/z calcd for $C_{53}H_{64}N_{12}O_{13}Na$ [M+Na]+: 1099.4608, found: 1099.4625.

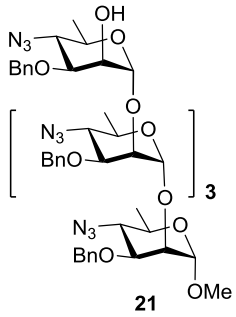
Methyl 2-O-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (20).



20

The glycosyl acceptor compound **19** (1.63 g, 1.51 mmol), and glycosyl donor compound **11** (0.712 g, 1.66 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH_2Cl_2 (15 mL), treated with freshly activated 4 Å molecular sieves (1 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.681 g, 3.03 mmol). After cooling to -10 ºC, TMSOTf (0.06 mL, 0.33 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous $NaHCO_3$ (10 mL) and CH_2Cl_2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous $Na_2S_2O_3$ (20%) [15 mL] and water (15 mL). After extraction of the aqueous layer with CH_2Cl_2 (3 x 10), the combined organic phase was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give pentasaccharide **20** (1.92 g, 91.9%) as a sticky liquid. Analytical data for **20**: R*f* = 0.7 (Ethyl acetate /Hexane 1:4, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.27 - 7.40 (m, 25 H, ArH), 5.39 - 5.41 (m, 1 H, H-2_E), 4.94 (s, 1 H, H-1_D), 4.86 (s, 1 H, H-1_C), 4.86 (s, 1 H, H-1_B), 4.84 (s, 1 H, H-1_E), 4.72 (d, *J*=11.7 Hz, 1 H, -C<u>H</u>Ph), 4.71 (d, *J*=11.1 Hz, 1 H, -C<u>H</u>Ph), 4.64 (d, *J*=11.7 Hz, 1 H, -C<u>H</u>Ph), 4.61 (s, 2 H, -C<u>H$_2$</u>Ph), 4.60 (s, 2 H, -C<u>H$_2$</u>Ph), 4.57 (ABq, *J*=11.7 Hz, 2 H, -C<u>H$_2$</u>Ph), 4.53 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 4.51 (s, 1 H, H-1_A), 3.86 (br. s., 1 H, H-2_D), 3.83 (br. s., 1 H, H-2_C), 3.79 (d, *J*=2.3 Hz, 2 H, H-2_A, H-2_B), 3.77 (dd, *J*=9.9, 2.9 Hz, 1 H, H-3_E), 3.70 (dd, *J*=9.9, 2.3 Hz, 1 H, H-3_D), 3.67 (t, *J*=2.9 Hz, 1 H, H-3_A), 3.66 (t, *J*=3.0 Hz, 1 H, H-3_C), 3.64 (dd, *J*=9.9, 2.0 Hz, 1 H, H-3_B), 3.51 (dq, *J*=10.2, 6.1 Hz, 1 H, H-5_A), 3.47 (dq, *J*=9.5, 6.1 Hz, 1 H, H-5_B), 3.45 (dq, *J*=9.9, 6.1 Hz, 1 H, H-5_D), 3.38 - 3.42 (m, 2 H, H-5_C, H-5_E), 3.37 (t, *J*=10.2 Hz, 1 H, H-4_A), 3.33 (t, *J*=9.9 Hz, 1 H, H-4_D), 3.28 (s, 3 H, -OC<u>H$_3$</u>), 3.16 - 3.23 (m, 3 H, H-4_B, H-4_E, H-4_C), 2.10 (s, 3 H, -COC<u>H$_3$</u>), 1.27 (d, *J*=6.4 Hz, 3 H, H-6_C) 1.24 (d, *J*=6.4 Hz, 3 H, H-6_B), 1.20 (d, *J*=6.1 Hz, 3 H, H-6_A), 1.17 (d, *J*=5.9 Hz, 3 H, H-6_D), 1.14 (d, *J*=6.1 Hz, 3 H, H-6_E); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 169.8, 137.4, 137.3, 137.2, 137.1, 128.6(x3), 128.5(x2), 128.4, 128.3(x2), 128.2, 128.1(x2), 128.0(x3), 100.3, 100.2, 100.0, 99.7, 99.1, 77.4, 76.6, 76.5, 75.4, 73.7, 73.6, 73.4, 73.3, 72.2(x2), 72.1, 72.0, 71.5, 67.8(x2), 67.6, 67.1, 66.9, 64.3, 64.2(x2), 64.1, 63.8, 54.9, 21.0, 18.6(x2), 18.5(x2), 18.4 ppm; HRMS (ESI): m/z calcd for $C_{68}H_{81}N_{15}O_{17}Na$ [M+Na]+: 1402.5827, found: 1402.5856.

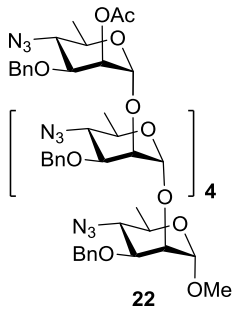
Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (21).



21

Sodium methoxide (~1.2 mL, 0.5 M solution) was added to a solution of **20** (1.8 g, 1.31 mmol) in CH_3OH: THF [4:2] (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^{o}C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound **21** (1.57 g, 89.8%) as white foam. Analytical data for **21**: R*f* = 0.55 (Ethyl acetate /Hexane 1:4, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.27 - 7.43 (m, 24 H, ArH), 7.14 - 7.20 (m, 1 H, ArH), 4.98 (s, 1 H, H-1_E), 4.96 (s, 1 H, H-1_D), 4.88 (d, *J*=1.7 Hz, 2 H, H-1_C, H-1_B), 4.55 - 4.75 (m, 10 H, -C<u>H</u>Ph), 4.52 (s, 1 H, H-1_A), 4.00 (br. s., 1 H, H-2_E), 3.92 - 3.95 (m, 1 H, H-2_D), 3.82 - 3.85 (m, 1 H, H-2_C), 3.81 (br. s., 2 H, H-2_B, H-2_A), 3.71 (dd, *J*=9.8, 2.8 Hz, 2 H, H-3_E, H-3_D), 3.64 - 3.69 (m, 3 H, H-3_A, H-3_C, H-3_B), 3.51 - 3.56 (m, 1 H, H-5_D), 3.44 - 3.51 (m, 2 H, H-5_E, H-5_A), 3.38 - 3.44 (m, 3 H, H-5_C, H-5_B, H-4_D), 3.31 (t, *J*=10.1 Hz, 1 H, H-4_E), 3.29 (s, 3 H, -OC<u>H</u>$_3$), 3.16 - 3.25 (m, 3 H, H-4_B, H-4_C, H-4_A), 2.27 - 2.32 (m, 1 H, 1 -OH_{2E}), 1.28 (dd, *J*=6.0, 2.6 Hz, 3 H, H-6_C), 1.26 (dd, *J*=6.0, 2.7 Hz, 3 H, H-6_A), 1.21 (dd, *J*=6.0, 2.8 Hz, 3 H, H-6_D), 1.19 (dd, *J*=5.9, 2.7 Hz, 3 H, H-6_E), 1.15 (dd, *J*=6.0, 2.7 Hz, 3 H, H-6_B); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 137.3(x2), 137.2, 129.0, 128.6(x4), 128.4, 128.3(x4), 128.2(x2), 128.1, 128.0, 100.5, 100.3, 100.2(x2), 99.7, 77.7, 77.4, 77.0, 76.6, 76.5, 73.7, 73.6, 73.4, 73.2, 72.2, 72.1(x3), 67.8(x2), 67.3, 67.1, 66.9, 64.4, 64.2, 63.8, 54.9, 18.6(x2), 18.5(x2), 18.3 ppm; HRMS (ESI): m/z calcd for $C_{66}H_{79}N_{15}O_{16}Na$ [M+Na]+: 1360.5721, found: 1360.5749.

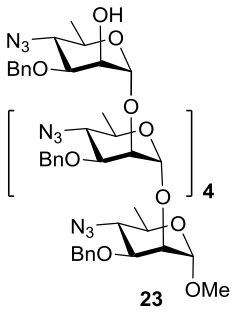
Methyl 2-O-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (22).



22

The glycosyl acceptor compound **21** (1.45 g, 1.08 mmol), and glycosyl donor compound **11** (0.556 g, 1.3 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH_2Cl_2 (15 mL), treated with freshly activated 4 Å molecular sieves (1 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.488 g, 2.16 mmol). After cooling to -10 ºC, TMSOTf (43 μL, 0.24 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous $NaHCO_3$ (10 mL) and CH_2Cl_2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous $Na_2S_2O_3$ (20%) [10 mL] and water (15 mL). After extraction of the aqueous layer with CH_2Cl_2 (3x10), the combined organic phase was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give hexasaccharide **22** (1.601 g, 90.1%) as a sticky liquid. Analytical data for **22**: R*f* = 0.65 (Ethyl acetate /Hexane 1:4, v/v); ^{1}H NMR (700 MHz, $CDCl_3$): δ: 7.26 - 7.40 (m, 30 H, ArH), 5.40 (t, *J*=2.9 Hz, 1 H, H-2_F), 4.95 (d, *J*=1.4 Hz, 1 H, H-1_E), 4.88 (d, *J*=1.5 Hz, 1 H, H-1_D), 4.86 (d, *J*=1.5 Hz, 1 H, H-1_C), 4.85 (s, 2 H, H-1_B, H-1_F), 4.72 (d, *J*=11.4 Hz, 1 H, -CHPh), 4.71 (d, *J*=10.9 Hz, 1 H, -CHPh), 4.64 (d, *J*=11.7 Hz, 1 H, -CHPh), 4.55 - 4.63 (m, 8 H, -CHPh), 4.53 (d, *J*=11.0 Hz, 1 H, -CHPh), 4.51 (d, *J*=1.4 Hz, 1 H, H-1_A), 3.86 - 3.88 (m, 1 H, H-2_E), 3.82 - 3.84 (m, 1 H, H-2_D), 3.76 - 3.81 (m, 4 H, H-2_A, H-2_C, H-2_B, H-3_F), 3.71 (dd, *J*=9.9, 2.9 Hz, 1 H, H-3_E), 3.62 - 3.68 (m, 4 H, H-3_D, H-3_C, H-3_A, H-3_B), 3.52 (dq, *J*=10.0, 6.1 Hz, 1 H, H-5_F), 3.44 - 3.49 (m, 2 H, H-5_A, H-5_E), 3.38 - 3.44 (m, 3 H, H-5_D, H-5_B, H-5_C), 3.37 (t, *J*=10.2 Hz, 1 H, H-4_F), 3.34 (t, *J*=9.9 Hz, 1 H, H-4_E), 3.28 (s, 3 H, -OCH_3), 3.22 (t, *J*=9.9 Hz, 1 H, H-4_D), 3.15 - 3.22 (m, 3 H, H-4_C, H-4_A, H-4_B), 2.10 (s, 3 H, -COCH_3), 1.27 (d, *J*=6.1 Hz, 3 H, H-6_D), 1.25 (d, *J*=6.1 Hz, 3 H, H-6_A), 1.21 (d, *J*=6.0 Hz, 3 H, H-6_F), 1.18 (d, *J*=6.0 Hz, 3 H, H-6_E), 1.16 (d, *J*=6.3 Hz, 3 H, H-6_B), 1.12 (d, *J*=6.1 Hz, 3 H, H-6_C); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 169.8, 137.4, 137.3, 137.2, 137.1(x3), 128.6(x4), 128.5(x2), 128.4, 128.3(x2), 128.2, 128.1(x2), 128.0(x3), 100.3, 100.1(x2), 100.0, 99.7, 99.1, 77.4, 76.7(x2), 76.5, 75.4, 73.6(x2), 73.5, 73.4, 73.3, 72.2, 72.1, 72.0, 71.5, 67.8(x4), 67.6, 67.1, 66.9, 64.3(x2), 64.2(x2), 64.1, 63.8, 54.9, 21.0, 18.6(x2), 18.5(x3), 18.4 ppm**;** HRMS (ESI): m/z calcd for $C_{81}H_{96}N_{18}O_{20}Na$ [M+Na]+: 1663.694, found: 1663.6982.

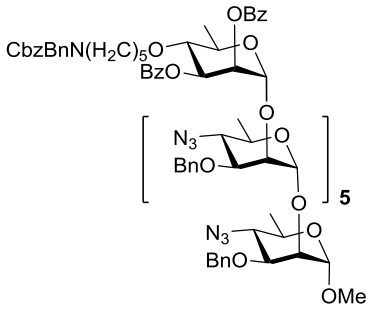
Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (23).



23

Sodium methoxide (~1.0 mL, 0.5 M solution) was added to a solution of **22** (1.3 g, 0.792 mmol) in CH_3OH: THF [4:2] (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^oC. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound **23** (1.17 g, 92.3%) as oil. Analytical data for **23**: R*f* = 0.5 (Ethyl acetate /Hexane 1:4, v/v); ^1H NMR (500 MHz, $CDCl_3$): δ: 7.31 - 7.46 (m, 30 H, ArH), 5.01 (s, 1 H, H-1_F), 4.99 (d, *J*=1.5 Hz, 1 H, H-1_E), 4.92 (d, *J*=1.1 Hz, 1 H, H-1_D), 4.89 (d, *J*=1.1 Hz, 1 H, H-1_C), 4.88 (d, *J*=1.5 Hz, 1 H, H-1_B), 4.76 (d, *J*=11.4 Hz, 1 H, -C<u>H</u>Ph), 4.74 (d, *J*=11.0 Hz, 1 H, -C<u>H</u>Ph), 4.55 - 4.71 (m, 10 H, -C<u>H</u>Ph), 4.54 (d, *J*=1.5 Hz, 1 H, H-1_A), 4.01 - 4.04 (m, 1 H, H-2_F), 3.95 - 3.98 (m, 1 H, H-2_E), 3.85 - 3.87 (m, 1 H, H-2_D), 3.80 - 3.85 (m, 3 H, H-2_B, H-2_C, H-2_A), 3.74 - 3.76 (m, 1 H, H-3_F), 3.72 - 3.74 (m, 1 H, H-3_E), 3.65 - 3.71 (m, 4 H, H-3_D, H-3_C, H-3_B, H-3_A), 3.56 (dq, *J*=10.2, 6.2 Hz, 1 H, H-5_E), 3.38 - 3.53 (m, 6 H, H-5_A, H-5_D, H-5_F, H-4_E, H-5_C, H-5_B), 3.34 (t, *J*=9.9 Hz, 1 H, H-4_F), 3.32 (s, 3 H, -OC<u>H</u>$_3$), 3.27 (t, *J*=9.9 Hz, 1 H, H-4_D), 3.18 - 3.24 (m, 3 H, H-4_B, H-4_C, H-4_A), 2.30 (s, 1 -OH_{2F}), 1.31 (d, *J*=6.2 Hz, 3 H, H-6_E), 1.28 (d, *J*=6.2 Hz, 3 H, H-6_A), 1.24 (d, *J*=6.1 Hz, 3 H, H-6_C), 1.22 (d, *J*=6.3 Hz, 3 H, H-6_F), 1.19 (d, *J*=6.1 Hz, 3 H, H-6_D), 1.16 (d, *J*=6.2 Hz, 3 H, H-6_B); ^{13}C NMR (126 MHz, $CDCl_3$): δ: 137.3, 137.2(x2), 128.7(x3), 128.6(x2), 128.4(x3), 128.3(x2), 128.2, 128.1(x3), 100.5, 100.3, 100.2(x2), 100.1, 99.8, 77.7, 77.5, 76.6(x2), 73.7, 73.6, 73.5(x2), 73.3, 72.2(x2), 72.1, 67.9, 67.8, 67.4, 67.2, 67.0, 64.4, 64.2, 63.9, 54.9, 18.7, 18.6(x2), 18.5(x2), 18.3 ppm; HRMS (ESI): m/z calcd for $C_{79}H_{94}N_{18}O_{19}Na$ [M+Na]+: 1621.6835, found: 1621.688.

Methyl 2,3-di-*O*-benzoyl-4-*O*-(5'-*N*-benzyl-5'-*N*-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (24).

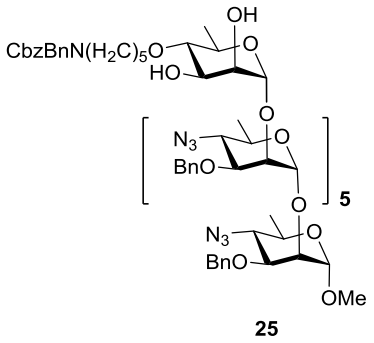


24

The glycosyl acceptor compound **23** (0.270 g, 0.169 mmol), and glycosyl donor compound **13** (0.146 g, 0.186 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH_2Cl_2 (10 mL), treated with freshly activated 4 Å molecular sieves (0.3 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.076 g, 0.337 mmol). After cooling to -10 ºC, TMSOTf (6.4 μL, 0.037 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous $NaHCO_3$ (5 mL) and CH_2Cl_2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous $Na_2S_2O_3$ (20%) [10 mL] and water (10 mL). After extraction of the aqueous layer with CH_2Cl_2 (3 x 5), the combined organic phase was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give heptasaccharide **24** (0.334 g, 87.4%) as a sticky liquid. Analytical data for **24**: R*f* = 0.65 (Ethyl acetate /Hexane 1:4, v/v); $[\alpha]_D^{21}$ = -6.71° (*c* = 1.23, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ : 8.04 - 8.09 (m, 2 H, ArH), 7.93 (d, *J*=7.5 Hz, 2 H, ArH), 7.66 (t, *J*=7.4 Hz, 1 H, ArH), 7.48 - 7.55 (m, 3 H, ArH), 7.23 - 7.44 (m, 38 H, ArH), 7.10 - 7.22 (m, 4 H, ArH), 5.72 (t, *J*=2.9 Hz, 1 H, H-2_G), 5.69 (dd, *J*=9.2, 3.2 Hz, 1 H, H-3_G), 5.16 (s, 2 H, -N$\underline{CH_2}$Ph), 5.06 - 5.09 (br. s., 2 H, H-1_G, H-1_F), 4.94 (d, *J*=1.5 Hz, 1 H, H-1_E), 4.92 (d, *J*=1.4 Hz, 1 H, H-1_D), 4.90 (d, *J*=1.5 Hz, 1 H, H-1_C), 4.89 (d, *J*=1.5 Hz, 1 H, H-1_B), 4.67 (d, *J*=11.7 Hz, 1 H, -C$\underline{H}$Ph), 4.58 - 4.72 (m, 11 H, -C$\underline{H}$Ph), 4.55 (d, *J*=1.5 Hz, 1 H, H-1_A), 4.35 - 4.44 (m, 2 H, -O$\underline{CH_2}$Ph), 4.03 (t, *J*=2.6 Hz, 1 H, H-2_F), 3.95 (dq, *J*=9.2, 6.2 Hz, 1 H, H-5_G), 3.91 (t, *J*=2.9 Hz, 1 H, H-2_E), 3.81 - 3.88 (m, 4 H, H-2_D, H-2_B, H-2_C, H-2_A), 3.77 (dd, *J*=9.7, 2.6 Hz, 1 H, H-3_F), 3.66 - 3.75 (m, 5 H, H-3_D, H-3_A, H-3_D, H-3_B, H-3_C), 3.64 (t, *J*=9.9 Hz, 1 H, H-4_F), 3.38 - 3.59 (m, 9 H, -C$\underline{H}_{2a}$, H-5_F, H-5_A, H-5_E, H-5_D, H-5_C, H-5_B, H-4_G), 3.32 (s, 3 H, -OC$\underline{H}_3$), 3.30 (t, *J*=10.1 Hz, 1 H, H-4_E), 3.18 - 3.27 (m, 4 H, H-4_B, H-4_D, H-4_A, H-4_C), 2.93 - 3.13 (m, 2 H, -C$\underline{H}_{2e}$), 1.36 - 1.51 (m, 4 H, -C$\underline{H}_{2b}$, -C$\underline{H}_{2d}$), 1.27 - 1.36 (m, 14 H, H-6_G, H-6_E, H-6_A, H-6_F, -C$\underline{H}_{2C}$), 1.20 (d, *J*=6.2 Hz, 3 H, H-6_B), 1.20 (d, *J*=5.9 Hz, 3 H, H-6_D), 1.18 (d, *J*=5.9 Hz, 3 H, H-6_C); ^{13}C NMR (126 MHz, $CDCl_3$): δ: 165.3, 165.1, 156.6, 156.1, 137.9, 137.5, 137.4, 137.3, 137.2(x2), 136.8, 133.3, 133.0(x2), 129.9, 129.8, 129.6, 129.1, 128.7(x2), 128.6(x2), 128.5(x2), 128.4(x2),

128.3 (x2), 128.2, 128.1, 127.9, 127.8, 127.3, 125.3, 100.4(x3), 100.2(x2), 99.8, 99.1, 79.8, 77.5, 76.7, 76.6, 73.7, 73.6, 73.5, 73.3, 72.2(x2), 72.1(x2), 71.9, 70.9, 68.5, 68.1, 67.9, 67.8, 67.1, 67.0, 64.4, 64.2, 63.9, 54.9, 50.5, 50.2, 47.0, 46.1, 29.7, 29.4, 27.8, 27.4, 23.3, 18.7, 18.6(x3), 18.5(x2), 18.0 ppm; HRMS (ESI): m/z calcd for $C_{119}H_{135}N_{19}O_{27}Na$ [M+Na]+: 2284.9667, found: 2284.9732.

Methyl 4-*O*-(5'-*N*-benzyl-5'-*N*-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (25).



25

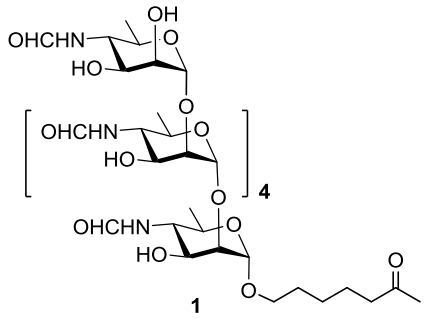
Sodium methoxide (~0.2 mL, 0.5 M solution) was added to a solution of **24** (0.26 g, 0.115 mmol) in CH_3OH: THF [2:3] (10 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 ^oC. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound **25** (0.215 g, 91.2%) as oil. Analytical data for **25**: R*f* = 0.25 (Ethyl acetate /Hexane 1:3.3, v/v); $[\alpha]D^{21}$ = +79.2 (*c* = 2.21, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$): δ : 7.23 - 7.40 (m, 39 H, ArH), 7.13 - 7.19 (m, 1 H, ArH), 5.13 - 5.20 (m, 2 H, -N$\underline{CH_2}$Ph), 4.99 (br. s., 1 H, H-1_F), 4.92 (br. s., 1 H, H-1_G), 4.89 (d, *J*=1.8 Hz, 1 H, H-1_E), 4.86 (d, *J*=1.8 Hz, 1 H, H-1_D), 4.85 (d, *J*=1.9 Hz, 1 H, H-1_C), 4.84 (d, *J*=1.7 Hz, 1 H, H-1_B), 4.54 - 4.72 (m, 12 H, -C$\underline{H}$Ph), 4.51 (d, *J*=1.8 Hz, 1 H, H-1_A), 4.44 - 4.50 (m, 2 H, -O$\underline{CH_2}$Ph), 3.97 - 4.02 (m, 2 H, H-2_F, H-2_G), 3.81 - 3.88 (m, 2 H, H-3_G, H-2_E), 3.77 - 3.81 (m, 4 H, H-2_D, H-2_B, H-2_C, H-2_A), 3.72 (dd, *J*=9.9, 2.8 Hz, 1 H, H-3_F), 3.56 - 3.70 (m, 7 H, H-3_E, H-3_C, H-3_D, H-3_B, H-3_A, -C$\underline{H_{2a}}$), 3.34 - 3.54 (m, 8 H, H-5_G, H-5_F, H-5_A, H-5_E, H-5_D, H-5_C, H-5_B, H-4_G), 3.28 (s, 3 H, -OC$\underline{H_3}$), 3.25 (t, *J*=10.0 Hz, 1 H, H-4_E), 3.07 - 3.21 (m, 7 H, H-4_C, H-4_B, H-4_F, H-4_D, H-4_A, -C$\underline{H_{2e}}$), 2.30 (br. s., 2 OH, -OH_{2G}, -OH_{3G}), 1.42 - 1.72 (m, 4 H, -C$\underline{H_{2b}}$, -C$\underline{H_{2d}}$), 1.27 (d, *J*=6.1 Hz, 3 H, H-6_F), 1.23 - 1.26 (m, 6 H, H-6_G, H-6_E), 1.21 (br. s., 2 H, -C$\underline{H_{2c}}$), 1.18 (d, *J*=6.1 Hz, 3 H, H-6_B), 1.16 (d, *J*=6.2 Hz, 3 H, H-6_A), 1.15 (d, *J*=6.1 Hz, 3 H, H-6_D), 1.12 (d, *J*=6.1 Hz, 3 H, H-6_C); ^{13}C NMR (176 MHz, $CDCl_3$): δ: 156.7, 156.3, 137.8, 137.4, 137.3, 137.2(x2), 137.1(x2), 136.7, 129.0, 128.6(x2), 128.5, 128.4, 128.3(x2), 128.2, 128.1, 128.0, 127.9, 127.8, 127.3, 100.8, 100.4, 100.3, 100.2, 100.1(x2), 99.7, 81.6, 77.4, 76.5, 73.6, 73.6, 73.5, 73.5, 72.9, 72.2, 72.1, 72.1, 72.0, 71.7, 71.1, 68.2, 67.8, 67.7, 67.2, 66.9, 64.3, 64.2, 54.9, 50.5, 50.3, 47.1,

46.1, 29.7, 29.4, 27.9, 27.2, 23.3, 18.6(x2), 18.5(x4), 17.9 ppm; HRMS (ESI): m/z calcd for $C_{105}H_{131}N_{20}O_{25}$ $[M+NH_4]+$: 2071.9589, found: 2071.9639.

VIII. PREPARATION OF OLIGOSACCHARIDE GLYCOCONJUGATES

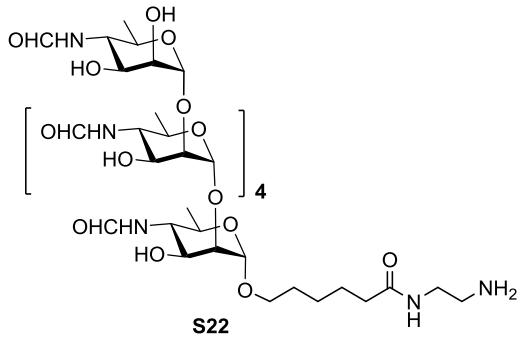
ACTIVATION FOR CONJUGATION OF THE 1,2 HEXASACCHARIDE 1

5'-Methoxycarbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (1).



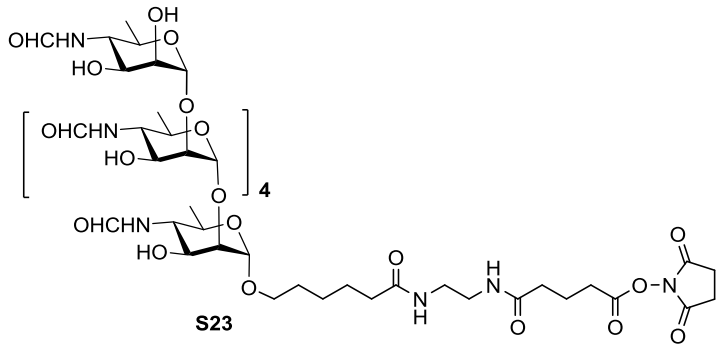
Analytical data for the title compound was essentially the same as previously described.[6]

(2'-Aminoethylamido)carbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (S22).



Analytical data for the title compound was essentially the same as previously described.[6]

(2'-[*N*-succinimidyl]glutarylamidoethylamido)carbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (S23).

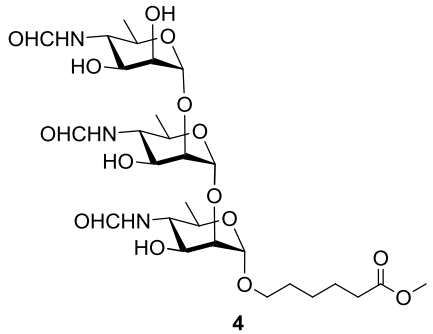


S23

A mixture of hexasaccharide **S22** (4 mg) and disuccinimidal glutarate (15 eq.) in DMF and 0.1 M PBS buffer (4 : 1, 0.5 mL) was stirred at rt for 6 h. The reaction mixture was concentrated under vacuum and the residue was washed with EtOAc 10 times to remove the excess disuccinimidal glutarate. The resultant solid was dried under vacuum for 1 h to obtain activated oligosaccharide **S23** that was directly used for conjugation with BSA and tetanus toxoid. MALDI TOF MS (positive mode): calcd for $C_{59}H_{93}N_9O_{31}Na$ [M + Na]+ m/z, 1446.5977; found, 1446.8936.

ACTIVATION FOR CONJUGATION OF THE 1,2 TRISACCHARIDE 4

5'-Methoxycarbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (4).



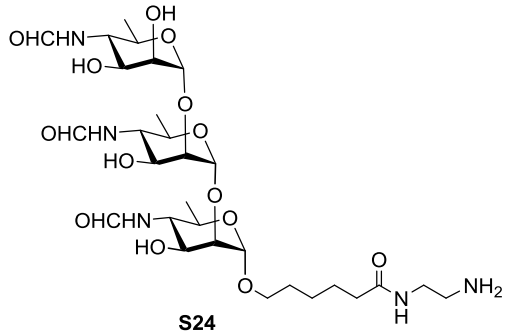
4

To a stirred solution of **S21** (0.2 g, 0.239 mmol), in pyridine (5 mL) and water (2 mL) mixture, H_2S was bubbled for 0.5 h at 40 ^oC, and continued stirring for 16 h. After that, argon was bubbled for 10 min, solvents were removed in vacuo, and the residue was co-evaporated with toluene (3 x 10 mL) and dried. The mass spectrometry analysis showed completion of reaction to corresponding amine compound and no products arising from incomplete reduction.

This crude material was directly used for formylation. Amine compound in CH_3OH (5 mL) at -20 ^oC was added a freshly prepared formic anhydride (5 mL, ethereal solution) and stirred for 3 h, then slowly allowed to warm to 21 ^oC. After that, solvents were evaporated and the residue was passed through column chromatography on silica gel (methanol – dichloromethane gradient elution) to afford trisaccharide. The high resolution mass spectrometry analysis showed completion of formylation reaction. HRMS (ESI): m/z calcd for $C_{42}H_{59}N_3O_{15}Na$ [M+Na]+: 868.3838, found: 868.3837.

Formylated compound was dissolved in CH_3OH/H_2O (2:1, 15 mL), $Pd(OH)_2$ on carbon (20%, 0.090 g) was added. Then it was stirred under a pressure of hydrogen gas at 21 ^oC for 16 h. After filtration through celite pad and washed with CH_3OH (3 x 10 mL), and solvents were removed in vacuo. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound **4** (0.094 g, 59.3%, over 3 steps) as white foam. Analytical data for **4:** $[\alpha]D^{21}$ = +31.58 (*c* = 1.16, H_2O); ^1H NMR (700MHz, D_2O): δ 8.20 - 8.24 (*Z*) and 8.03 - 8.06 07 (E) (m, 3H, NC<u>H</u>O), 5.16 - 5.22 (m, 1 H, H-1_B), 5.05 - 5.08 (m, 1 H, H-1_C), 4.89 - 4.93 (m, 1 H, H-1_A), 4.13 - 4.19 (m, 1 H, H-2_B), 4.06 - 4.13 (m, 2 H, H-2_C, H-3_C), 3.92 - 4.03 (m, 6 H, H-2_A, H-3_A, H-3_B, H-4_C, H-4_B, H-4_A), 3.87 - 3.92 (m, 2 H, H-5_A, H-5_C), 3.80 - 3.84 (m, 1 H, H-5_B), 3.71 - 3.75 (m, 1 H, -O-C<u>H</u>$_{2b}$), 3.71 (s, 3 H), 3.56 (dt, *J*=9.9, 5.9 Hz, 1 H, -O-C<u>H</u>$_{2a}$), 2.42 (t, *J*=7.4 Hz, 2 H, -C<u>H</u>$_{2f}$), 1.60 - 1.68 (m, 4 H, -C<u>H</u>$_{2e}$, -C<u>H</u>$_{2c}$), 1.40 (dq, *J*=14.8, 7.3 Hz, 2 H, -C<u>H</u>$_{2d}$), 1.20 - 1.30 (m, 9 H, 3 x H-6); ^{13}C NMR (176MHz, D_2O): δ 178.4, 168.6(x2), 165.7, 165.7(x2), 102.9, 102.8, 101.5, 99.1, 78.5, 78.4, 78.2, 78.1, 78.0, 69.8, 69.1, 68.8, 68.7(x2), 68.6, 68.5(x2), 68.3(x2), 67.9, 57.8, 52.9, 52.8, 52.7(x2), 52.5, 34.4(x2), 28.9, 25.7, 24.8, 17.8(x2), 17.7(x2), 17.6, 17.5(x2) ppm. HRMS (ESI): m/z calcd for $C_{28}H_{47}N_3O_{15}Na$ [M+Na]+: 688.2899, found: 688.2908.

(2'-Aminoethylamido)carbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (S24)

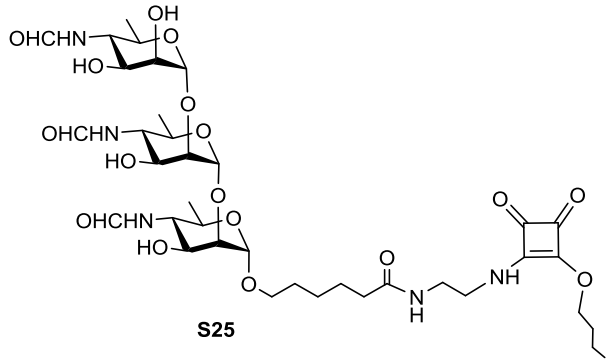


S24

A solution of **4** (0.06 g, 0.09 mmol) in freshly distilled 1,2-diaminoethane (3.0 mL) was stirred at 65 °C for 48 h. After that, excess reagent was removed *in vacuo*, and the residue was co-evaporated with CH_3OH (3 x 10 mL) and dried. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound **S24** (0.052 g, 83.15%) as white foam. Analytical data for **S24**: $[\alpha]D^{21}$ = +37.05 (*c* = 1.14, H_2O); ^1H NMR (500MHz, D_2O): δ 8.24 - 8.33 (*Z*) and 8.05 - 8.12 (E) (m, 3H, NC<u>H</u>O), 5.23 - 5.26 (m, 1 H, H-1_B), 5.12 (s, 1 H, H-1_C), 4.93 - 4.97 (m, 1 H, H-1_A), 4.19 - 4.24 (m, 1 H, H-2_B), 4.10 - 4.18 (m, 2 H, H-2_C, H-3_C), 3.96 - 4.08 (m, 6 H, H-2_A, H-3_A, H-3_B, H-4_C, H-4_B, H-4_A), 3.91 - 3.96

(m, 2 H, H-5_A, H-5_C), 3.84 - 3.89 (m, 1 H, H-5_B), 3.77 (dt, *J*=9.7, 6.8 Hz, 1 H, -O-C$\underline{H}_{2b}$), 3.57 - 3.63 (m, 1 H, , -O-C$\underline{H}_{2a}$), 3.33 (t, *J*=6.2 Hz, 2 H, -C$\underline{H}_{2g}$), 2.82 (t, *J*=6.2 Hz, 2 H, -C$\underline{H}_{2h}$), 2.33 (t, *J*=7.4 Hz, 2 H, -C$\underline{H}_{2f}$), 1.64 - 1.74 (m, 4 H, -C$\underline{H}_{2e}$, -C$\underline{H}_{2c}$), 1.39 - 1.49 (m, 2 H, -C$\underline{H}_{2d}$), 1.25 - 1.35 (m, 9 H, 3 x H-6); ^{13}C NMR (126MHz, D_2O): δ 178.3, 168.8(x2), 165.8(x2), 103.0, 102.9, 101.6, 99.3, 78.6, 78.3, 78.2, 78.1, 69.9, 69.2, 69.0, 68.9, 68.8(x2), 68.6(x2), 68.5, 68.4, 57.7, 53.0, 52.8(x2), 52.7, 42.1, 42.1, 40.7, 36.7, 29.1, 26.0, 25.9, 17.9(x2), 17.8(x2), 17.7(x2), 17.6 ppm; HRMS (ESI): m/z calcd for $C_{29}H_{51}N_5O_{14}Na$ [M+Na]+: 716.3325, found: 716.333.

1-[(2'-Aminoethylamido)carbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside] 2-butoxycyclobutene-3,4-dione (S25).

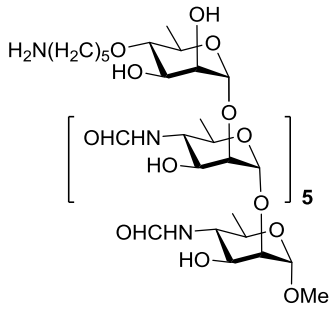


S25

To a stirred solution of **S24** (0.015 g, 0.022 mmol) in water (0.5 mL) and EtOH (0.5 mL), a solution of 3,4-dibutoxy-3-cyclobutene-1,2-dione (20% in ethanol, 70 μL) was added and pH was adjusted to 8 by careful addition of aq.$NaHCO_3$ (1%) solution. After 1 h, mass spectrometry showed the reaction was complete; the reaction mixture was neutralized using CH_3COOH (10%) and concentrated *in vacuo*. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound **S25** (0.0133 g, 73.2%) as white foam. Analytical data for **S25**: ^{1}H NMR (700MHz, D_2O): δ 8.21 - 8.23 (*Z*) and 8.05 (E) (m, 3H, NC$\underline{H}$O), 5.19 (s, 1 H, H-1_B), 5.07 (s, 1 H, H-1_C), 4.90 - 4.92 (m, 1 H, H-1_A), 4.68 - 4.75 (m, 2 H, -C$\underline{H}_{2i}$), 4.14 - 4.19 (m, 1 H, H-2_B), 4.07 - 4.13 (m, 2 H, H-2_C, H-3_C), 3.92 - 4.02 (m, 6 H, H-2_A, H-3_A, H-3_B, H-4_C, H-4_B, H-4_A), 3.89 (m, 2 H, H-5_A, H-5_C), 3.79 - 3.85 (m, 1 H, H-5_B), 3.73 (t, *J*=5.0 Hz, 1 H, -C$\underline{H}_{2g}$), 3.65 - 3.71 (m, 1 H, -O-C$\underline{H}_{2b}$), 3.62 (t, *J*=5.0 Hz, 1 H, -C$\underline{H}_{2g}$), 3.51 (dd, *J*=9.6, 6.5 Hz, 1 H, -O-C$\underline{H}_{2a}$), 3.40 - 3.45 (m, 2 H, -C$\underline{H}_{2h}$), 2.19 - 2.27 (m, 2 H, -C$\underline{H}_{2f}$), 1.77 - 1.84 (m, 2 H, -C$\underline{H}_{2j}$), 1.51 - 1.64 (m, 4 H, -C$\underline{H}_{2e}$, -C$\underline{H}_{2c}$), 1.46 (dt, *J*=15.5, 7.9 Hz, 2 H, -C$\underline{H}_{2k}$), 1.30 - 1.36 (m, 2 H, -C$\underline{H}_{2d}$), 1.20 - 1.30 (m, 9 H, 3 x H-6), 0.94 - 0.98 (m, 3 H, -C$\underline{H}_{2l}$); ^{13}C NMR (176MHz, D_2O): δ 189.7, 184.1, 178.4, 177.8, 174.5, 168.6, 165.7, 165.7, 102.8, 101.5, 99.1, 98.9, 78.4, 78.1, 75.2, 75.1, 69.8, 69.1, 68.8, 68.7, 68.6, 68.4, 68.3(x2), 57.8, 52.9, 52.7, 52.5, 45.0, 44.9, 40.2, 40.0, 36.6, 32.3, 29.1, 26.0, 25.9, 25.8, 25.7, 19.0, 18.9, 17.8(x2), 17.7(x2), 17.6, 17.5, 13.8 ppm; HRMS (ESI): m/z calcd for $C_{37}H_{59}N_5O_{17}Na$ [M+Na]+: 868.3798, found: 868.3808.

ACTIVATION FOR CONJUGATION OF HEPTASACCHARIDE 8

Methyl 4-*O*-(5'-aminopentanyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (8).



8

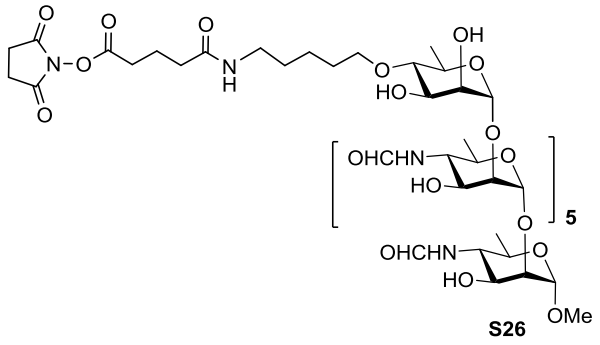
To a stirred solution of **25** (0.11 g, 0.054 mmol), in pyridine (5 mL) and water (2 mL) mixture, H_2S was bubbled for 0.5 h at 40 ^oC, and continued stirring for 16 h. After that, argon was bubbled for 10 min, solvents were removed in vacuo, and the residue was co-evaporated with toluene (3 x 10 mL) and dried. The mass spectrometry analysis showed completion of reaction to corresponding amine compound and no products arising from incomplete reduction. HRMS (ESI): m/z calcd for $C_{105}H_{140}N_7O_{25}$ [M+H]+: 1898.9893, found: 1898.99.

This crude material was directly used for formylation. Amine compound in CH_3OH (5 mL) at -20 ^oC was added a freshly prepared formic anhydride (5 mL, ethereal solution) and stirred for 3 h, then slowly allowed to warm to 21 ^oC. After that, solvents were evaporated and the residue was passed through column chromatography on silica gel (methanol – dichloromethane gradient elution) to afford heptasaccharide. The high resolution mass spectrometry analysis showed completion of formylation reaction. HRMS (ESI): m/z calcd for $C_{111}H_{139}N_7O_{31}Na$ [M+Na]+: 2088.9408, found: 2088.9405.

Formylated compound was dissolved in CH_3OH/H_2O (2:1, 10 mL), $Pd(OH)_2$ on carbon (20%, 0.060 g) was added. Then it was stirred under a pressure of hydrogen gas at 21 ^oC for 16 h. After filtration through celite pad and washed with CH_3OH (3 x 10 mL), and solvents were removed in vacuo. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound **8** (0.0427 g, 61.2%, over 3 steps) as white foam. Analytical data for **8:** $[\alpha]D^{21}$ = +42.44 (*c* = 1.02, H_2O); ^1H NMR (500MHz, D_2O): δ : 8.21 - 8.32 (*Z*) and 8.06 - 8.14 (E) (m, 6H, NC<u>H</u>O), 5.21 - 5.30 (m, 5 H, H-1_G, H-1_F, H-1_E, H-1_D, H-1_C), 5.05 (s, 1 H, H-1_B), 4.86 - 4.89 (m, 1 H, H-1_A), 4.11 - 4.31 (m, 11 H, H-2_F, H-2_E, H-3_A, H-3_B, H-2_D, H-3_C, H-3_D, H-2_B, H-2_A, H-3_F, H-2_C), 3.75 - 4.10 (m, 17 H, H-3_E, H-3_G, H-4_A, H-2_G, H-4_C, H-4_D, H-5_G, H-5_E, H-5_C, H-4_E, H-4_F, H-5_F, H-5_B, H-5_D, H-5_A, H-4_B, -C<u>H</u>$_a$), 3.49 - 3.54 (m, 1 H, -C<u>H</u>$_a$), 3.48 (s, 3 H, -OC<u>H</u>$_3$), 3.36 (t, *J*=9.7 Hz, 1 H, H-4_G), 3.08 (t, *J*=7.5 Hz, 2 H, -C<u>H</u>$_{2e}$), 1.66 - 1.82 (m, 4 H, -C<u>H</u>$_{2d}$, -C<u>H</u>$_{2b}$), 1.46 - 1.59 (m, 2 H, -C<u>H</u>$_{2c}$), 1.23 - 1.43 (m, 21 H, 7 x H-6); ^{13}C NMR (126 MHz, $CDCl_3$): δ: 168.8(x2), 168.6, 165.9(x4), 165.7, 103.2, 103.1(x4), 102.7, 102.5, 101.5(x2), 100.4, 100.3, 81.8, 78.2, 78.1(x3), 78.0(x2), 77.9, 73.5(x2), 71.3, 70.8(x2), 69.2, 68.7(x2), 68.5, 68.4, 67.8, 57.9, 56.4, 55.9, 55.8(x2), 52.9(x2), 52.7(x2), 40.4,

29.7, 27.5, 23.2, 17.9(x2), 17.8(x2), 17.7, 17.6(x4) ppm; HRMS (ESI): m/z calcd for $C_{54}H_{92}N_7O_{29}$ [M+H]+: 1302.5934, found: 1302.5928.

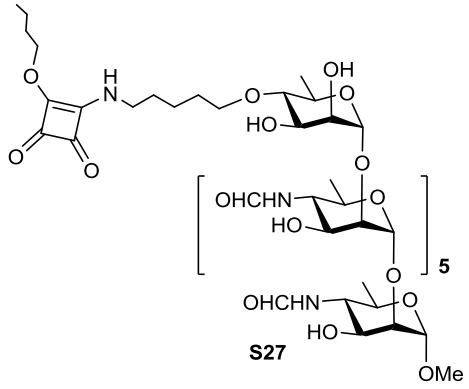
Methyl 4-*O*-(5'-[*N*-succinimidyl]glutarylamidopentanyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (S26).



S26

A mixture of heptasaccharide **8** (9 mg) and disuccinimidal glutarate (15 eq.) in DMF and 0.1 M PBS buffer (4 : 1, 1.5 mL) was stirred at rt for 6 h. The reaction mixture was concentrated under vacuum and the residue was washed with EtOAc 10 times to remove the excess disuccinimidal glutarate. The resultant solid was dried under vacuum for 1 h to obtain activated oligosaccharide **S26** that was directly used for conjugation with BSA & tetanus toxoid. MALDI TOF MS (positive mode): calcd for $C_{63}H_{100}N_8O_{34}Na$ [M + Na]+ m/z, 1535.6342; found, 1535.9996.

1-[(2'-Aminoethylamido)carbonylpentyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside] 2-butoxycyclobutene-3,4-dione (S27).



S27

To a stirred solution of heptasaccharide **8** (0.006 g, 0.005 mmol) in water (0.5 mL) and EtOH (0.5 mL), a solution of 3,4-dibutoxy-3-cyclobutene-1,2-dione (20% in ethanol, 50 μL) was added and pH was adjusted to 8 by careful addition of aq.$NaHCO_3$ (1%) solution. After 1 h, mass spectrometry showed the reaction was complete; the reaction mixture was neutralized using CH_3COOH (10%) and concentrated *in vacuo*. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound **S27** (0.005 g, 72.6%) as white foam. Analytical data for **S27**: ^{1}H NMR (700MHz, D_2O): δ : 8.218 - 8.21 (*Z*) and 8.00 - 8.04 (E) (m, 6H, NC<u>H</u>O), 5.14 - 5.21 (m, 5 H, H-1_G, H-1_F, H-1_E, H-1_D, H-1_C), 4.95 (s, 1 H, H-1_B), 4.80 (s, 1 H, H-1_A), 4.66 - 4.74 (m, 2 H, -C<u>H</u>$_{2f}$), 4.02 - 4.19 (m, 11 H, H-2_F, H-2_E, H-3_A, H-3_B, H-2_D, H-3_C, H-3_D, H-2_B, H-2_A, H-3_F, H-2_C), 3.75 - 4.00 (m, 15 H, H-3_E, H-3_G, H-4_A, H-2_G, H-4_C, H-4_D, H-5_G, H-5_E, H-4_E, H-4_F, H-5_F, H-5_B, H-5_D, H-5_A, -C<u>H</u>$_a$), 3.59 - 3.68 (m, 2 H, H-5_C, H-4_B), 3.40 - 3.45 (m, 1 H, -C<u>H</u>$_a$), 3.39 (s, 3 H, -OC<u>H</u>$_3$), 3.27 (t, *J*=9.7 Hz, 1 H, H-4_G), 1.82 - 1.75 (m, 2 H, -C<u>H</u>$_{2g}$), 1.56 - 1.67 (m, 4 H, -C<u>H</u>$_{2d}$, -C<u>H</u>$_{2b}$), 1.36 - 1.48 (m, 4 H, -C<u>H</u>$_{2c}$, -C<u>H</u>$_{2h}$), 1.17 - 1.29 (m, 21 H, 7 x H-6), 0.90 - 0.96 (m, 3 H, -C<u>H</u>$_{2i}$); ^{13}C NMR (126 MHz, $CDCl_3$): δ: 190.3, 184.2, 183.8, 178.4, 178.0, 174.3, 168.8, 168.7, 168.6, 165.9, 165.7, 103.2, 103.2(x4), 102.8, 102.6, 101.6, 100.4, 100.3, 81.9, 78.2, 78.1(x2), 78.0(x2), 77.9, 73.4, 71.3, 70.7, 69.8, 69.2, 69.1, 68.8, 68.6, 68.5, 68.4, 57.9, 56.4, 55.9, 55.8, 52.9(x2), 52.7, 40.4, 32.4, 30.7, 30.5, 29.7, 27.5, 23.3, 23.2, 19.2, 19.0, 17.9, 17.8(x2), 17.7(x4), 17.6, 13.9 ppm; HRMS (ESI): m/z calcd for $C_{62}H_{99}N_7O_{32}Na$ [M+Na]+: 1476.6335, found: 1476.6406.

OLIGOSACCHARIDE PROTEIN CONJUGATION:

<u>Preparation of tetanus toxoid conjugate **2**</u>: Activated hexasaccharide **S23** (1 mg, 0.518 μmol) was added to the solution of tetanus toxoid (4 mg, 0.025 μmol) in 0.5 M borate buffer pH 9 (1 mL) and stirred slowly at 21 ^oC for 3 days. Then the reaction mixture was washed with PBS buffer, filtered through a millipore filtration tube (10,000 MWCO, 4 x 10 mL) and the resulting tetanus toxoid-conjugate **2** was stored in PBS buffer. The MALDI-TOF mass spectrometry analysis indicated the conjugate **2** had an average of 11.7 hexasaccharides per tetanus toxoid.

<u>Preparation of BSA conjugate **3**</u>: BSA (5.5 mg) and activated hexasaccharide **S23** (2.0 mg) were dissolved in 0.1 M PBS buffer pH 9 (600 μL) and stirred slowly at 21 ^oC for 3 days. Then the reaction mixture was diluted with Mili-Q water, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL), lyophilized and the BSA-conjugate **3** was obtained as a white foam (6.2 mg). The MALDI-TOF mass spectrometry analysis indicated the conjugate **3** had an average of 8.9 hexasaccharides per BSA.

<u>Preparations of BSA conjugate **5**</u>: BSA (15 mg) and trisaccharide squarate **S25** (3.8 mg, 6.77 μmol) were dissolved in 0.1 M PBS buffer pH 9 (600 μL) and stirred slowly at 21 ^oC for 3 days. Then the reaction mixture was diluted with Mili-Q water, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL), lyophilized and the BSA-conjugate **5** was obtained as a white foam (17.6 mg). The MALDI-TOF mass spectrometry analysis indicated the conjugate **5** had an average of 16.2 disaccharides per BSA.

<u>Preparation of tetanus toxoid conjugate **9**</u>: Activated heptasaccharide **S27** (0.8 mg, 0.518 μmol) was added to the solution of tetanus toxoid (4 mg, 0.026 μmol) in 0.5 M borate buffer pH 9 (1 mL) and stirred slowly at 21 ^oC for 3 days. Then the reaction mixture was washed with PBS

buffer, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL) and the resulting tetanus toxoid-conjugate **7** was stored in PBS buffer. The MALDI-TOF mass spectrometry analysis indicated the conjugate **7** had an average of 10.0 heptasaccharide per tetanus toxoid.

Preparation of BSA conjugate **10**: BSA (10 mg) and activated heptasaccharide **S27** (4.5 mg) were dissolved in 0.1 M PBS buffer pH 9 (1.2 mL) and stirred slowly at 21 ^{o}C for 3 days. Then the reaction mixture was diluted with Mili-Q water, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL), lyophilized and the BSA-conjugate **8** was obtained as a white foam (12.2 mg). The MALDI-TOF mass spectrometry analysis indicated the conjugate **8** had an average of 10.3 heptasaccharide per BSA.

IX. PREPARATION OF OPS-TETANUS TOXOID GLYCOCONJUGATE

The sLPS from *B. abortus* S99 and *B. suis* biovar 2 (strain Thomsen) was purified by hot-phenol extraction[10](11) and the OPS was liberated from this product my mild acid hydrolysis.[11] The precipitated Lipid A was removed as the pellet following centrifugation at 17,000 g for 30 mins. The supernatant was buffer exchanged into water by size exclusion chromatography using sephadex G-25 which also removed low molecular weight impurities. The purified OPS, at 2 mg/ml, was oxidised by incubation in 10 mM sodium metaperiodate in 50 mM sodium acetate buffer at pH 5.5 at 4ºc for 1h in the dark. The OPS was then desalted using sephadex G-25 (PD-10 column, GE Healthcare) to buffer exchange into water, removing residual sodium metaperiodate. Oxidised OPS, 5 mg/ml, was incubated in PBS with 0.5 M ammonium chloride and 0.1 M sodium cyanoborohydride at 37ºc for 24h after which the OPS was desalted into water using sephadex-G25 and freeze dried.

The oxidised and aminated OPS was reconstituted to 5 mg/ml with a 10% concentration of DMSO in PBS containing 5 mg/ml DSG (disuccinimidal glutarate) and incubated for 45 mins at room temperature on a rotary shaker then desalted back into PBS using a Zeba 40 kDa column according to the manufacturer's instructions (Pierce) to remove unconjugated DSG. The OPS-DSG conjugate was added to tetanus toxoid (TT) at final concentrations of 2.5 and 0.5 mg/ml respectively. This solution was incubated for 2h at room temperature on a rotary shaker after which a final concentration of 2 mg/ml glycine was added and this was further incubated for 15 mins.

The OPS-TT conjugate was separated from the non-conjugated OPS and glycine by SEC-HPLC using an Agilent Infinity Bioinert HPLC system fitted with a 30 cm Tosoh TSK-gel G3000 PWxl, bore size 7 mm (cat: 05762) size exclusion chromatography column plus guard column comprised of the same matrix. The mobile phase was PBS pH 7.4 (50 mM sodium phosphate, 150 mM sodium chloride) and flow rate was 0.8 ml/min. The fraction eluting at 6.5 to 8.8 mins was collected as this contained the OPS conjugated tetanus toxoid only. The unconjugated OPS eluted from 8.8 to 11.0 mins whereas 75% of the OPS-TT conjugate eluted between 6.5 and 8.8

mins. The effect of the HPLC separation is visible in the SDS-PAGE silver stain image (Figure S3) as the TT light chain fragment is lost from the final preparation used for immunisation.
The *B. abortus* and *B. suis* OPS-TT conjugates were evaluated by SDS-PAGE silver staining and Western blotting to verify their anti-OPS antibody reactivity (Figure S3).
For SDS-PAGE TT and glycoconjugates were diluted to 0.2mg/ml in sample buffer (Invitrogen, Life Technologies) then heated for 5 minutes at 80 °C in a water bath. The antigens were loaded into NuPAGE® Novex Tris-Acetate Gels (Invitrogen, Life technologies) 10 µl per well. HiMark™ Unstained Protein Standard (Invitrogen, Life technologies) was also loaded into the gels for silver staining and HiMark™ Prestained Protein Standard (Invitrogen, Life technologies) for the Western blot gels, 7.5 µl per well. The gels were run at 110 volts in an electrophoresis tank with MOPS running buffer (Invitrogen, Life Technologies) for 90 minutes.
After gel electrophoresis, the gels were stained using a silver staining kit (Biorad). Initially the gels were fixed with in-house 40% methanol, 10% acetic acid fixative for 30 minutes. Then oxidising concentrate (Biorad) was diluted 1 in 10 in deionised water and added to the gels, the gels were incubated for 5 minutes on a rocker. The gels were then washed with deionised water for 15 minutes with frequent changes of water. Silver reagent (Biorad) was diluted 1 in 10 in deionised water and incubated with the gels for 20 minutes on a rocker. The gels were rinsed for 30 seconds then incubated with developing concentrate (Biorad) for 15 minutes until bands were visualised. The gels were then incubated with 5% acetic acid stopper solution (in-house preparation) for 30 minutes and then scanned.
For Western blot the gels were run then they were placed on a nitrocellulose membrane (Invitrogen) and the antigens were transferred to the membrane using the iBlot™ dry transfer system (Invitrogen). The nitrocellulose membranes were blocked overnight at 4-8 °C with blocking buffer (Candor). The membranes were then incubated with mouse anti-*Brucella* OPS monoclonal antibody clones BrG11 (Fzmb, Germany), specific to A epitopes, or BM40[12] (APHA, Weybridge), specific to M epitopes, at 20 µg/ml in Low-cross buffer (Candor) for 90 minutes at room temperature. Then the membranes were washed three times, for fifteen minutes with washing buffer (Candor), on a rocker at room temperature. The membranes were then incubated with anti-mouse Ig:alkaline phosphatase at 1/1000 in Low-cross buffer (Candor) for 90 minutes then washed three times, for 15 minutes. The membranes were incubated with BCIP/NBT tablets (Sigma) until bands were visualised. Then the membranes were allowed to dry and scanned. The same method was used for Western blot with bovine polyclonal sera which was applied at a 1/100 dilution and developed with Protein G:alkaline phosphatase.
The silver stain images (lanes 2-6) show the increase in size of the main TT protein when conjugated to *B. abortus* S99 OPS although the minimum size remains the same. There is a small increase in size visible due to conjugation with *B. suis* OPS. Size exclusion fractionation by HPLC leads to the elimination of the light chain TT fragment in both the OPS-TT preparations. The Western blots confirm that the TT has been conjugated with OPS. Polyclonal anti-*Brucella* sera, derived from a *B. abortus* infected cow binds to both glycoconjugates, although more to the *B. abortus* OPS-TT, with a very low background response to the unconjugated TT. A

monoclonal antibody specific to 'A' OPS epitopes (a series of 5 or more α1,2 linked D-Rha4NFo units) binds to both glycoconjugates, again more so to the *B. abortus* OPS-TT. A monoclonal antibody specific to 'M' epitopes (a short series of D-Rha4NFo units incorporating a single α1,3 link) bound only to *B. abortus* OPS-TT (with a weak background reaction to TT only). This is consistent with the known structure of the two OPS antigens in which the *B. abortus* S99 antigen contains low proportion (2%) of α1,3 links, the remainder α1,2 linked whereas the *B. suis* biovar 2 OPS is exclusively α1,2 linked. The lack of α1,3 links is considered to be unique to *B. suis* biovar 2 and means that the D-RhaN4Fo polymer is identical to that of the unrelated bacteria *Y. enterocolitica* O:9.

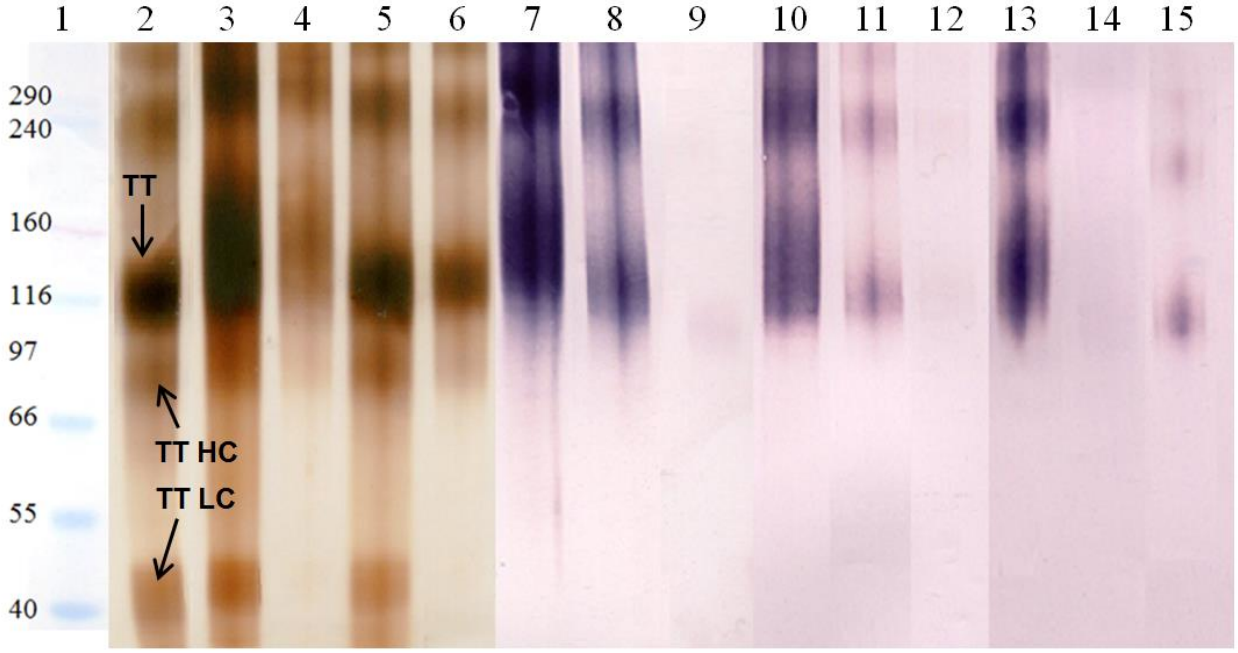


Figure S3: SDS-PAGE of TT and *Brucella* OPS-TT conjugates (post-HPLC fractionation unless stated): Protein molecular weight marker (lane 1), lanes 2-6 silver stains of TT (lane 2), *B. abortus* OPS-TT pre-HPLC (lane 3), *B. abortus* OPS-TT (lane 4), *B. suis* OPS-TT pre-HPLC (lane 5), *B. suis* OPS-TT (lane 6). Lanes 7-15 Western blot of OPS-TT conjugates, lanes 7, 10, and 13, *B. abortus* OPS-TT; lanes 8, 11 & 14, *B. suis* OPS-TT; lanes 9, 12 & 15, TT. Lanes 7-9 incubated with bovine polyclonal anti-*Brucella* sera; lanes 10-12 incubated with anti-OPS A specific MAb BrG11; lanes 13-15 incubated with anti-OPS M specific MAb BM40. TT = tetanus toxoid (~150 kDa), TT HC = tetanus toxoid heavy chain (~100 kDa), TT LC (~50 kDa). Stains greater than 200 kDa attributable to TT fragments cross linked during the TT detoxification process.

The conjugation of tetanus toxoid with OPS was also evaluated by MALDI-ToF using an Applied biosystems/MDS SCIEX 4800 MALDI TOF/TOF analyser. Sample was added in 0.5 µl to the plate and allowed to dry then covered with 0.5 µl of 10 mg/ml sinapic acid. Data was collected in linear mode. The peak mass of unconjugated TT was 152,375 m/z (figure S4). The peak mass for the *B. abortus* S99 OPS-tetanus conjugate was 156,320 m/z (Figure S5) and for *B. suis* biovar 2 OPS-TT conjugate it was 154,114 m/z (Figure S6), although peak broadening

towards higher masses were evident, especially with the *B. abortus* OPS-TT conjugate. Based on this, the OPS content for each conjugate was therefore 2.5% for *B. abortus* OPS-TT and 1.1% for *B. suis* OPS-TT, equivalent to an average of approximately 13 and 20 D-Rha4NFo units per TT respectively.

However, given that the average length of an OPS polymer is 96-100 units[13] it is probable that the conjugation of TT has been poor and that the distribution of the number of OPS molecules that have been conjugated per TT is in the range of 0-4. It is likely that conjugation has favoured shorter OPS molecules as these would more rapidly diffuse within a reaction mixture. Enrichment of longer OPS molecules prior to conjugation would increase the glycan content of the conjugate, as might adoption of a different conjugation technique. For example, the use of a squarate linker such as 3,4-dibutoxy-3-cyclobutene-1,2-dione would reduce the likelihood of any intramolecular linking of the two aldehydes on the oxidised terminal D-Rha4NFo due to the reduced activity of the linker once the first active site has conjugated. However, this may necessitate the use of DSG as the linker for the diagnostic antigens.

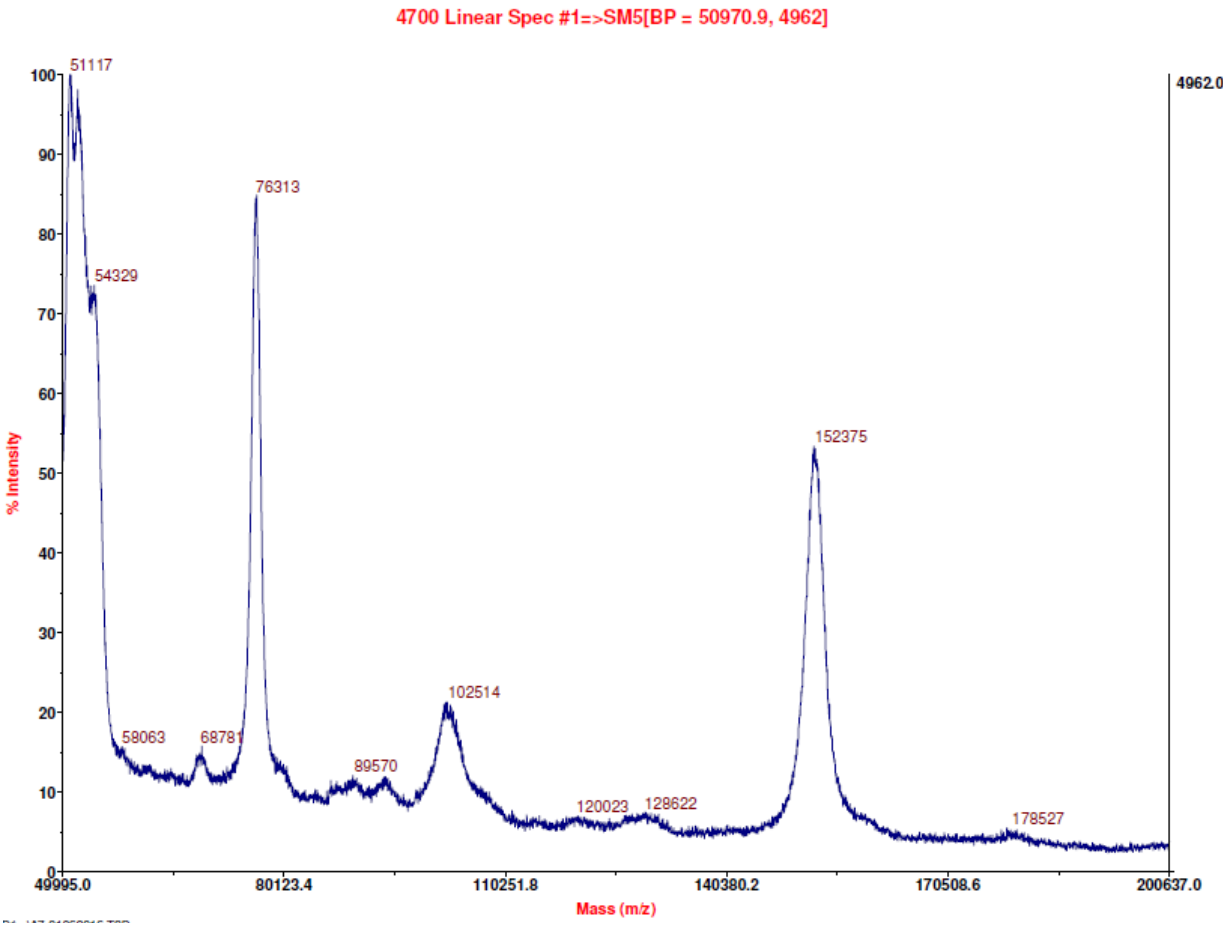


Figure S4: MALDI-ToF spectrum of unconjugated tetanus toxoid (TT)

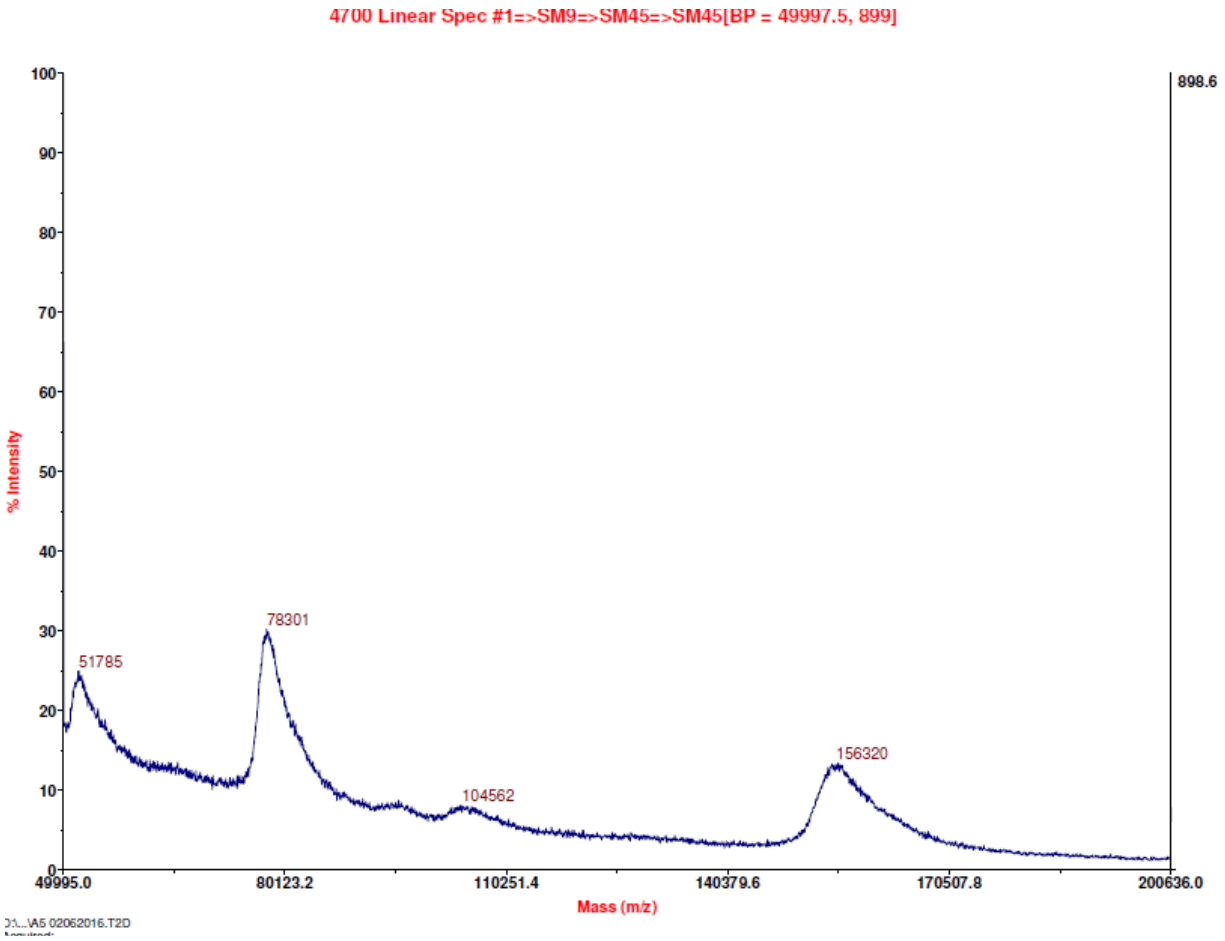


Figure S5: MALDI-ToF spectrum of *B. abortus* S99 OPS tetanus toxoid conjugate (OPS-TTS99)

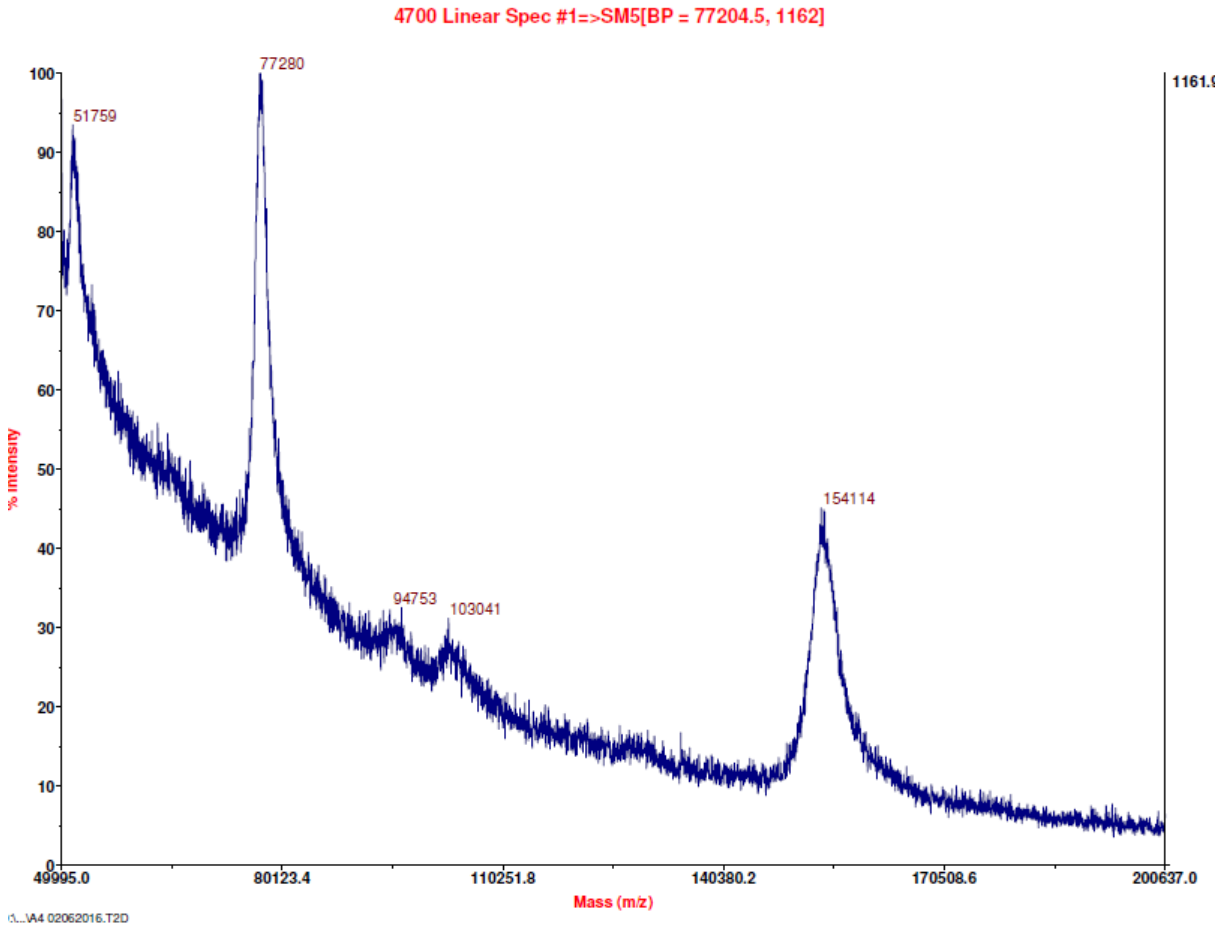


Figure S6 MALDI-ToF spectrum of *B. suis* biovar 2 OPS tetanus toxoid conjugate (OPS-TT2).

X. IMMUNIZATION OF MICE

Vaccine formulation: Alum was suspended in PBS at 50 mg/mL concentration and thimerosal (0.01% w/v) was added and stored at 4°C. Conjugate solutions were prepared at 1 mg/mL of PBS. Alum (14 µL) was mixed with the tetanus toxoid conjugates (144 mL) in 5:1 weight ratio, diluted with 2.85 mL of PBS and the mixture was allowed to rock overnight before administering to animals.[14]

Immunization with glycoconjugates 2 and 9 : Female CD1 mice (Charles River, Canada) age 6-8 weeks in groups of 10 were immunised three times at 21 day intervals. Each mouse received 250 µl distributed 150 µl intraperitoneally and 100 µl subcutaneously. Pre bleeds were collected prior to immunisation and mice were euthanized at day 10 after the final injection and final bleeds were collected. Blood was incubated at 37° C for one hour then spun at 1500 g for 10 min. Clear serum was collected and stored at -20°C until use.

Immunisation with OPS-Tetanus toxoid conjugate

Two groups of 8 female CD1 mice of 7 weeks of age were immunized on days 1, 21 and 35 with 5 µg each of conjugate administered subcutaneously in a 100 µl volume of PBS without

adjuvant. Prebleeds were collected prior to immunization and post vaccination bleeds were taken on days 19, 33 and 49.

XI. ELISA DATA

INDIRECT ELISA

Immunoassays: Antibody titres against glycoconjugate coated plates and plates coated with purified LPS were determined according to a published protocol[15] with minor modification. Briefly, polystyrene microtiter plates were incubated with the coating glycoconjugate antigen (1 µg/mL, 100 µL/well) at 4°C overnight, then washed (5×) with PBST (0.05% Tween-20 in phosphate buffer saline, PBS). LPS 1µg/mL in sodium carbonate buffer.

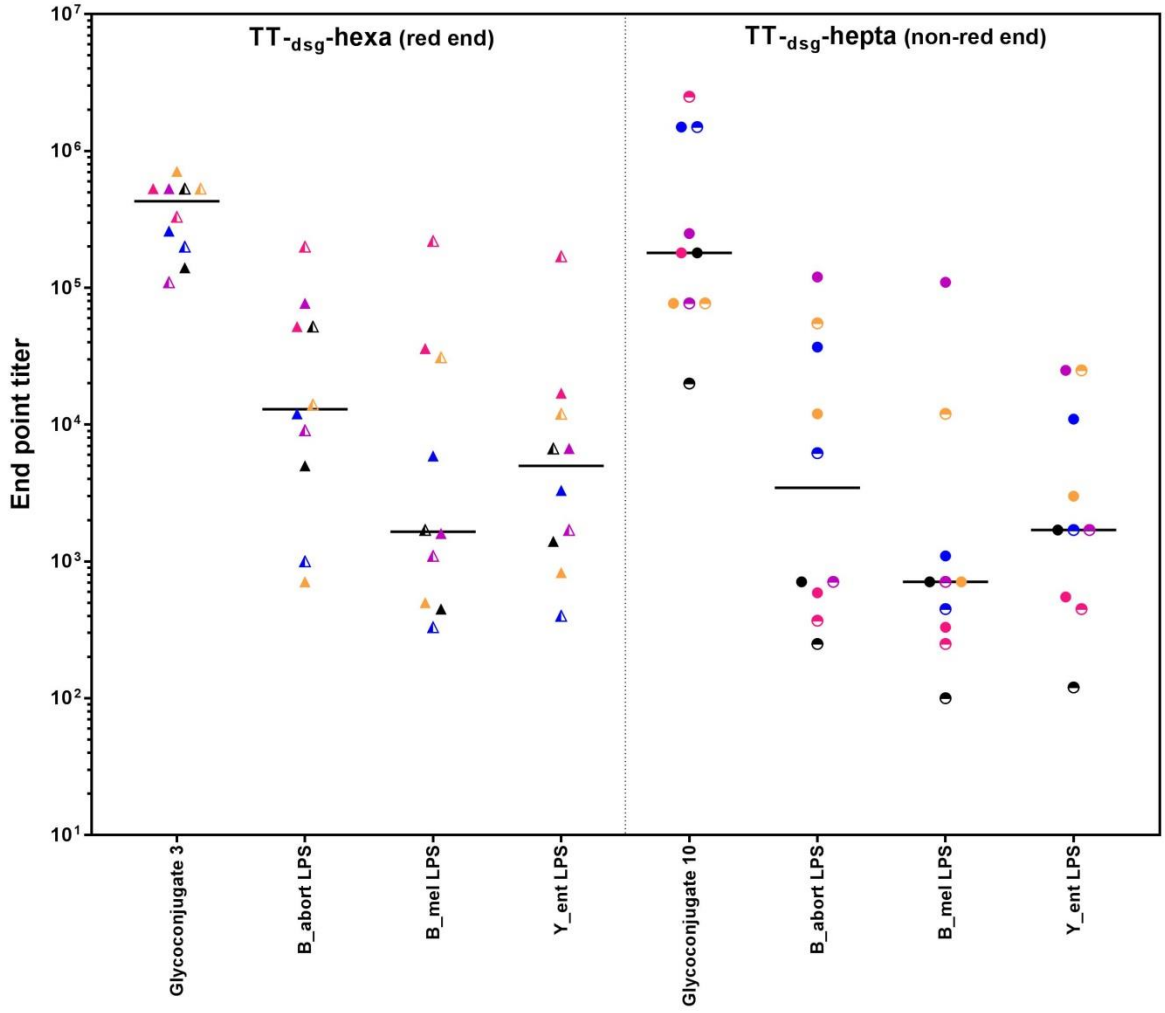


Figure S7 Antibody titres of sera raised to vaccines **2** and **9** titred against the immunizing hapten conjugated to BSA and the three sLPS of *B. abortus*, *B. melitensis* and *Yersenia enterocolitica* O:9.

Mouse sera was diluted 1:100 murine sera in 0.1% BSA in PBST added to the coated well (100 µL/well) at serial √10 dilutions in the same buffer. After incubation at room temperature for 2 h, the plates were washed (5×) with PBST. Then the plate was incubated with 100 µL/well of HRPO labelled goat anti-mouse IgG antibody (KPL) (1:5000 dilution of a 1.0 mg/mL stock) for 30 min at room temperature, then washed (5×) with PBST. Peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB) with H_2O_2, was added. After 15 min the reaction was quenched by addition of phosphoric acid (1M, 100 µL/well). Plates were read at 450 nm and the data were processed using Origin software. End point dilution (x_0) was recorded as the serum dilution giving an absorbance 0.2 above background and serum titer was calculated as the reciprocal of x_0. All the data were processed using Origin 9 and Graphpad Prism software.

Table S1 ELISA Inhibition with pentasaccharides **S38-S43**

Mouse #	**Inhibition as µmole IC_{50}**					
	S38 **P_4R**	**S39** **P_2RP_2**	**S40** **P_2RPR**	**S41** **PRPRP**	**S42** **PR_2PR**	**S43** **RP_3R**
2	40	50	170	<500	NA	NA
3	30	4	9	NA	NA	100
5	3	20	210	NA	NA	NA
6	4	8	30	NA	NA	NA
8	1	6	7	NA	NA	NA
10	100	60	300	NA	NA	NA

NA – not active at 1mM

Immunoassays for OPS-TT immunised mice

The smooth LPS antigens *B. abortus* S99 and *B. melitensis* 16M were diluted to 0.6 µg/ml and detoxified tetanus toxin (TT or dTT) (Statens Serum Institute) was diluted to 2.5 µg/ml in carbonate buffer (Sigma). The whole cell antigens *B. abortus* S99, *B. melitensis* 16M and *B. suis* biovar 2 (strain Thomsen) were diluted 15.6 µg/ml in carbonate buffer (Sigma). The synthetic antigens Disaccharide, Tetrasaccharide, 1,2-Hexasaccharide and 1,3-Hexasaccharide were diluted 2.5µg/ml in carbonate buffer (Sigma). Then 100 µl per well was added to standard bind

ELISA plates (Nunc). The plates were incubated overnight at 4-8 °C then washed four times with PBS-Tween, 200 µl per well and tapped dry on blotting paper.

Mouse sera were diluted in log dilutions (√10) at 1/100, 1/316.22, 1/1000, 1/3162.27, 1/10000, 1/31622.7, 1/100000, 1/316227, 1/1000000 and 1/3162270 in casein buffer and 100 µl per well was added to the antigen coated plates. For the synthetic antigens the sera were also diluted at 1/31.62. Monoclonal antibody BM40, specific to M epitopes, was diluted to 5 µg/ml in casein buffer (Sigma) and added to the plates, 100 µl per well, as the positive control. A positive serum control, mouse sera from a mouse immunised with 1,2-Hexasaccharide, and a negative serum control from a normal (non-immunised) mouse were also included, 100 µl per well, as controls.

The plates were incubated for 30 minutes at room temperature, on a rotator at 120 rpm, then washed four times with PBS-Tween, 200 µl per well and tapped dry on blotting paper. Anti-mouse immunoglobulins HRP conjugate (Dako) was diluted 1 in 1000 in casein buffer and 100 µl/well was added to the plates. The plates were incubated for 60 minutes for the synthetic antigens and tetanus toxoid and 30 minutes for sLPS and whole cell antigens at room temperature, on a rotator at 120 rpm, then washed four times with PBS-Tween, 200 µl per well and tapped dry on blotting paper. Substrate buffer (pH4.0) (Fluka) with 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma) and 3% hydrogen peroxide (Sigma) was added to the plates, 100 µl per well, and incubated at room temperature for 20 minutes. The reaction was slowed with 0.1M sodium azide, 100 µl per well, and the plates were read at 405 nm absorbance. Data was calculated as the blanked mean of duplicate wells as a percentage of the BM40 positive control wells tested with Disaccharide as this was added to every test plate.

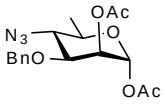
The optical densities (ODs) for each sample and dilution were blanked by subtracting the OD for control wells to which no sera had been added but were otherwise processed as described above. The quantitative data for the samples were then normalised by expressing the ODs as a percentage of the positive control. The end titres were calculated (using GraphPad Prism 6) as the dilution at which the signal (expressed as a percentage of the positive control) was equal to the positive/negative threshold. This threshold was calculated as the mean of the pre-bleed samples plus 1.96 times the standard deviation of the pre-bleed samples.

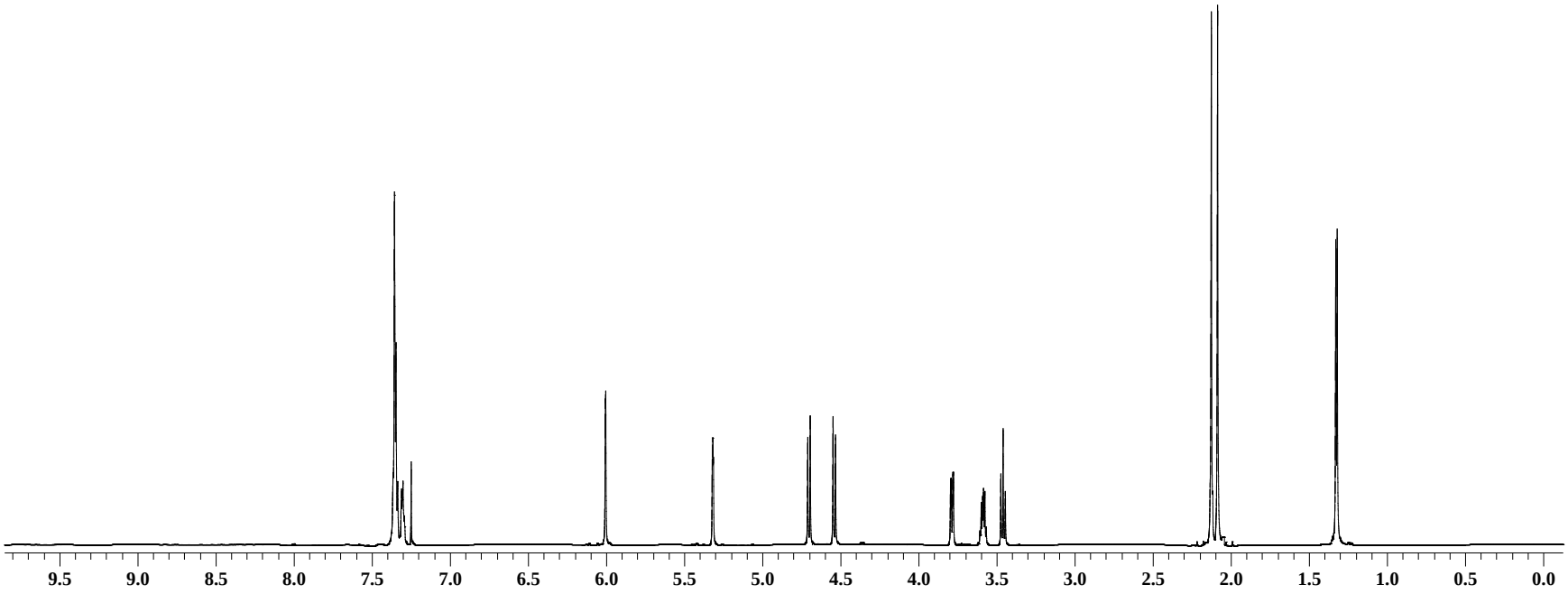
XII. REFERENCES

1) Ganesh, V. N.; Sadowska, J. M.; Sarkar, S.; Howells, L.; McGiven, J.; Bundle, D. R. Molecular Recognition of Brucella A and M Antigens Dissected by Synthetic Oligosaccharide Glycoconjugates Leads to a Disaccharide Diagnostic for Brucellosis. *J. Amer. Chem. Soc*., **2014**, *136*,16260-16269.
2) Mandal, S. S.; Ganesh, V. N.; Sadowska, J. M.; Bundle, D. R. Characterization of the Fine Specificity of Brucella A and M Monoclonal Antibodies. *Org Biomol. Chem.* submitted

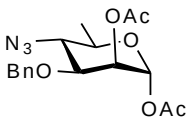
3) Kihlberg, J.; Eichler, E.; Bundle, D. R. The design and synthesis of antibody binding site probes: three pentasaccharide analogues of the *Brucella* A antigen prepared by *in situ* activation of thioglycosides with bromine. *Carbohydr. Res*., **1991**, *211*, 59-75.
4) Kihlberg, J.; Bundle, D. R. The synthesis of antibody binding site probes: A hexasaccharide and two pentasaccharides related to the *Brucella* A antigen prepared by *in situ* activation of thioglycosides with bromine. *Carbohydr. Res*., **1991**, *216*, 67-78.
5) Bundle, D. R.; Gerken, M.; Peters, T. Synthesis of antigenic determinants of the *Brucella* A antigen utilizing methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside efficiently derived from D-mannose. *Carbohydr. Res*. **1988**, *174*, 239−251.
6) Eis, M. J.; Ganem, B. An improved synthesis of D-perosamine and some derivatives *Carbohydr. Res*., **1988**, *176*, 316−323.
7) Peters, T.; Bundle, D. R. Synthetic antigenic determinants of the *Brucella* A polysaccharide; a disaccharide thioglycoside for block synthesis of pentasaccharide and lower homologues of α1,2-linked 4,6-dideoxy-4-formamido-α-D-mannose. *Can. J. Chem*., **1989**, *67*, 491-496.
8) Peters, T.; Bundle, D. R. Synthesis of 4,6-dideoxy-4-formamido-α-D- mannose containing tri- tetra- and pentasaccharides, antigenic determinants of the *Brucella* A and M antigens. *J. Chem. Soc. Chem. Comm*., **1987**, 1648-1650.
9) Saksena, R., Zhang, J. & Kovać , P. Immunogens from a synthetic hexasaccharide fragment of the O-SP of *Vibrio cholerae* O:1, serotype Ogawa. *Tetrahedron: Asymmetry*., **2005**, *16*, 187−197.
10) Westphal, O., Luderitz, O. & Bister, F. Über die Extraktion von Bakterien mit Phenol/Wasser. *Z. Naturforsch*. **1952**, *7*,148-155.
11) Meikle, P. J.; Perry, M. B.; Cherwonogrodzky, J. W.; Bundle, D. R. Fine structure of A and M antigens from Brucella biovars. *Infect. Immun*. **1989**, *57*, 2820-2828.
12) Greiser-Wilke, I.; Moennig, V. Monoclonal antibodies and characterization of epitopes of smooth *Brucella* lipopolysaccharides. *Ann. Inst. Pasteur Microbiol.* **1987**, *138*, 549-560.
13) Cardoso, P., Macedo, G. Azevedo, V. & Oliveira, S. *Brucella spp* noncanonical LPS: structure, biosynthesis, and interaction with host immune system. *Microbial Cell Factories* **2006**, *5*, 13-24.
14) Lipinski, T., Wu, X., Sadowska, J., Kreiter, E., Yasui, Y., Cheriaparambil, S., Rennie, R. and Bundle, D. R. A trisaccharide conjugate vaccine induces high titer β-mannan specific antibodies that aid clearance of *Candida albicans* in immunocompromised rabbits. *Vaccine*, **2012**, *30*, 6263-6269.
15) Bundle, D. R.; Tam, P.-H.; Tran, H.-A.; Paszkiewicz, E.; Cartmell, J.; Sadowska, J. M.; Sarkar, S.; Joe, M.; Kitov, P. I. Oligosaccharides and peptide displayed on an amphiphilic polymer enable solid phase assay of hapten specific antibodies. *Bioconjug. Chem.*, **2014**, *25*, 685-697.

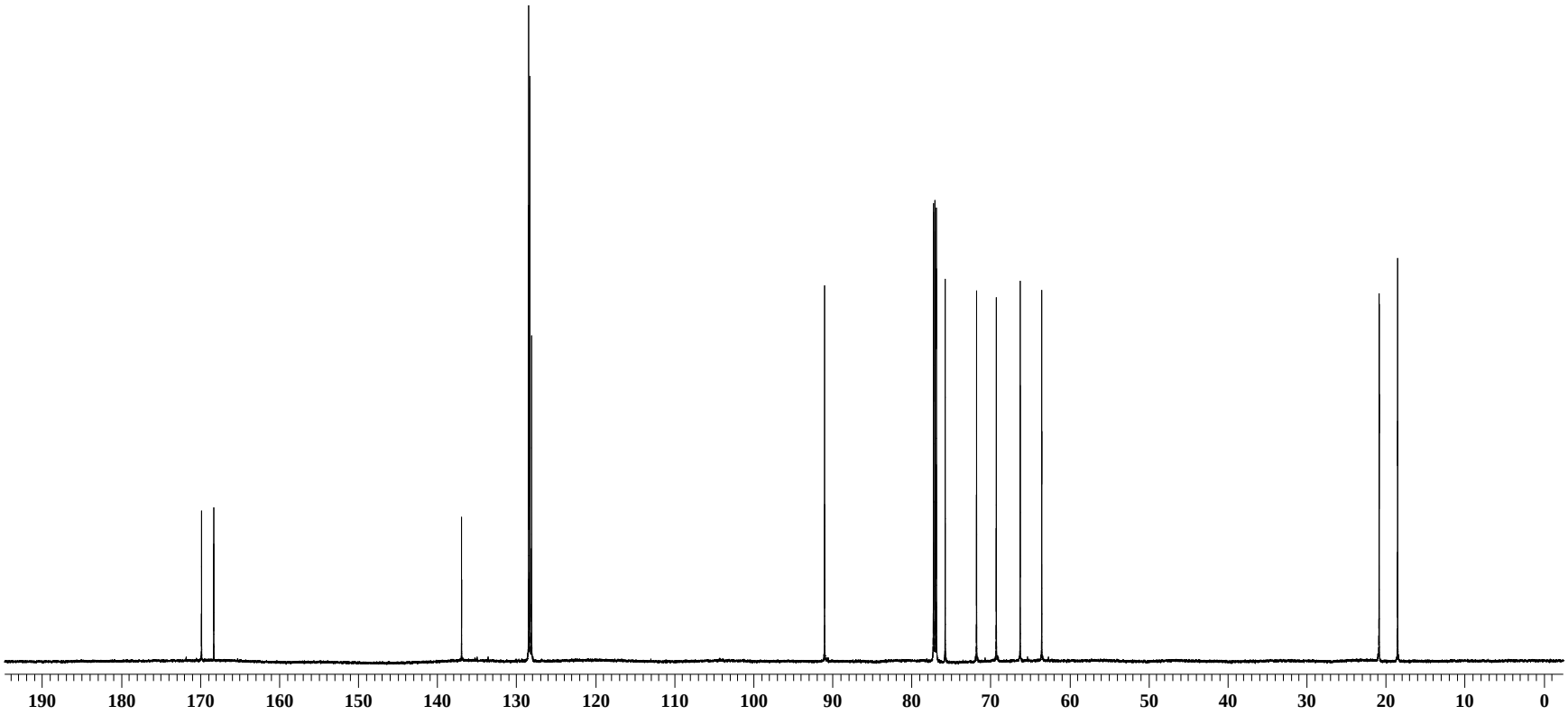
XIII. ^{1}H AND ^{13}C NMR SPECTRA



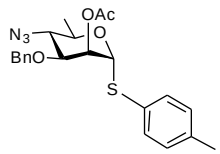


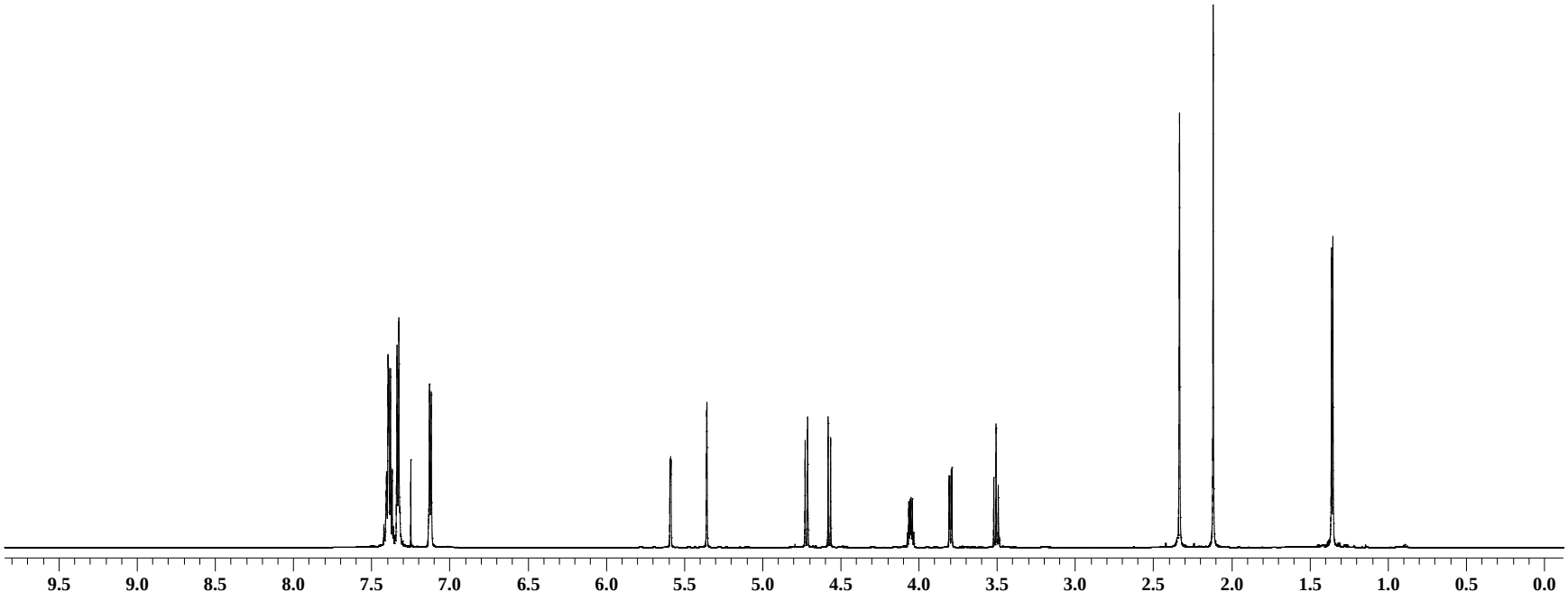
^{1}H NMR Spectrum of compound S12 ($CDCl_3$, 700 MHz).



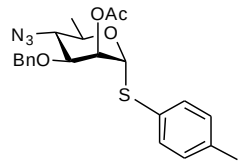


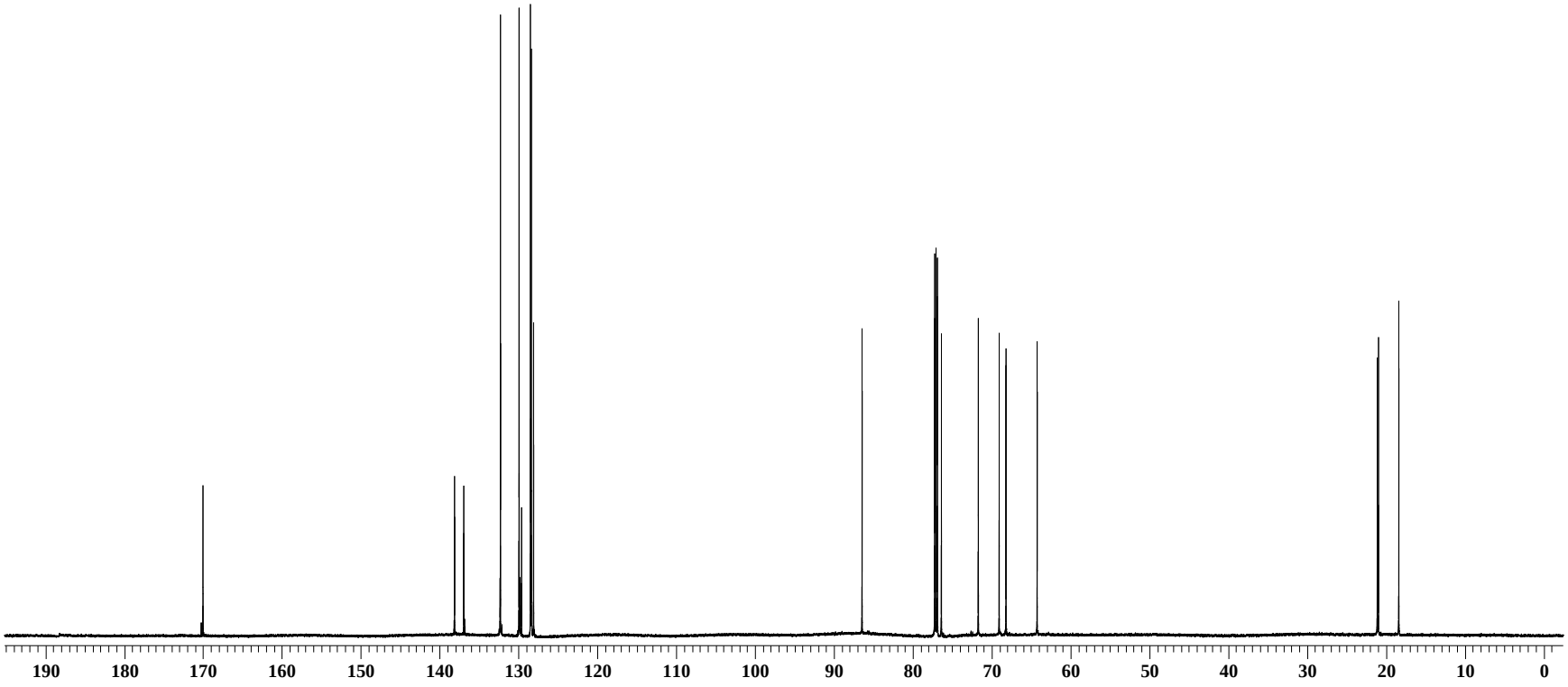
^{13}C NMR Spectrum of compound S12 ($CDCl_3$, 176 MHz).



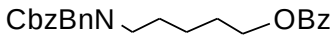


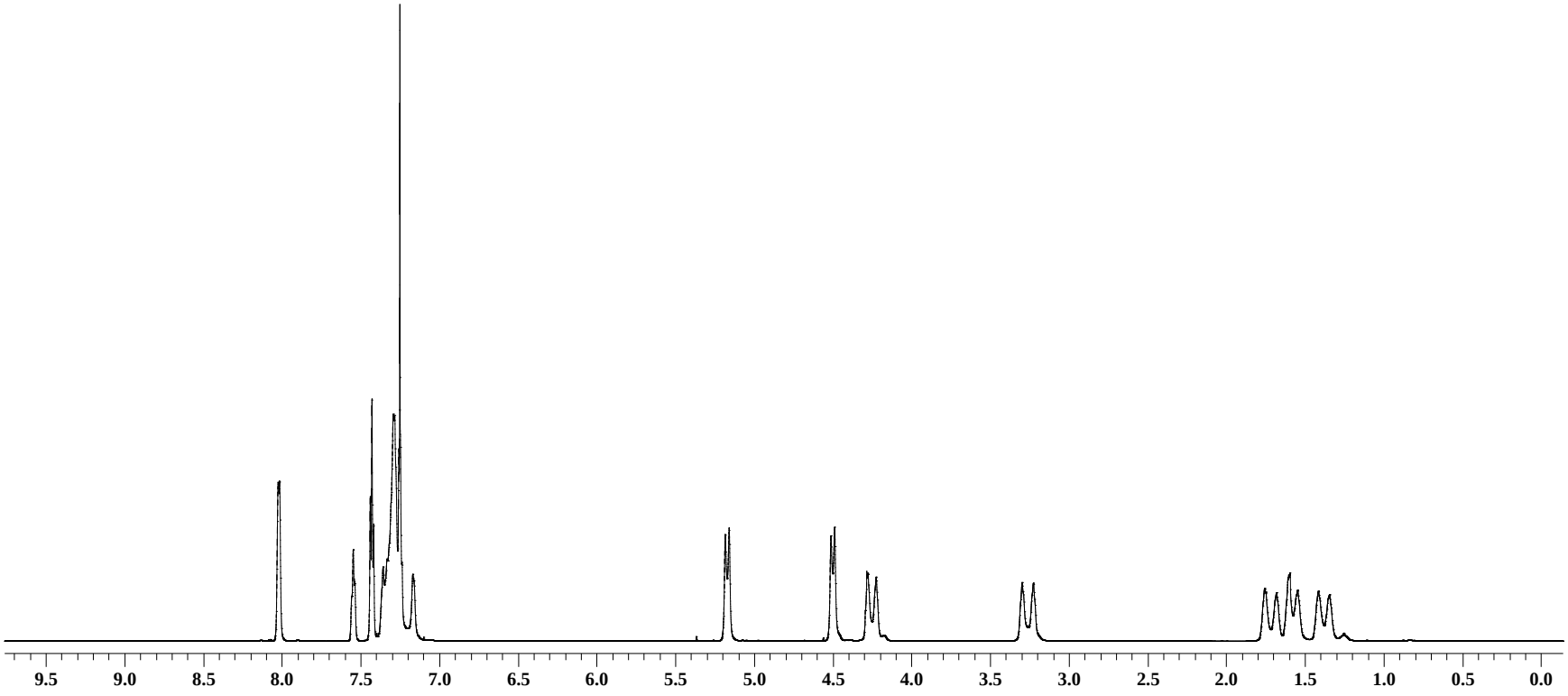
^{1}H NMR Spectrum of compound 11 ($CDCl_3$, 700 MHz).



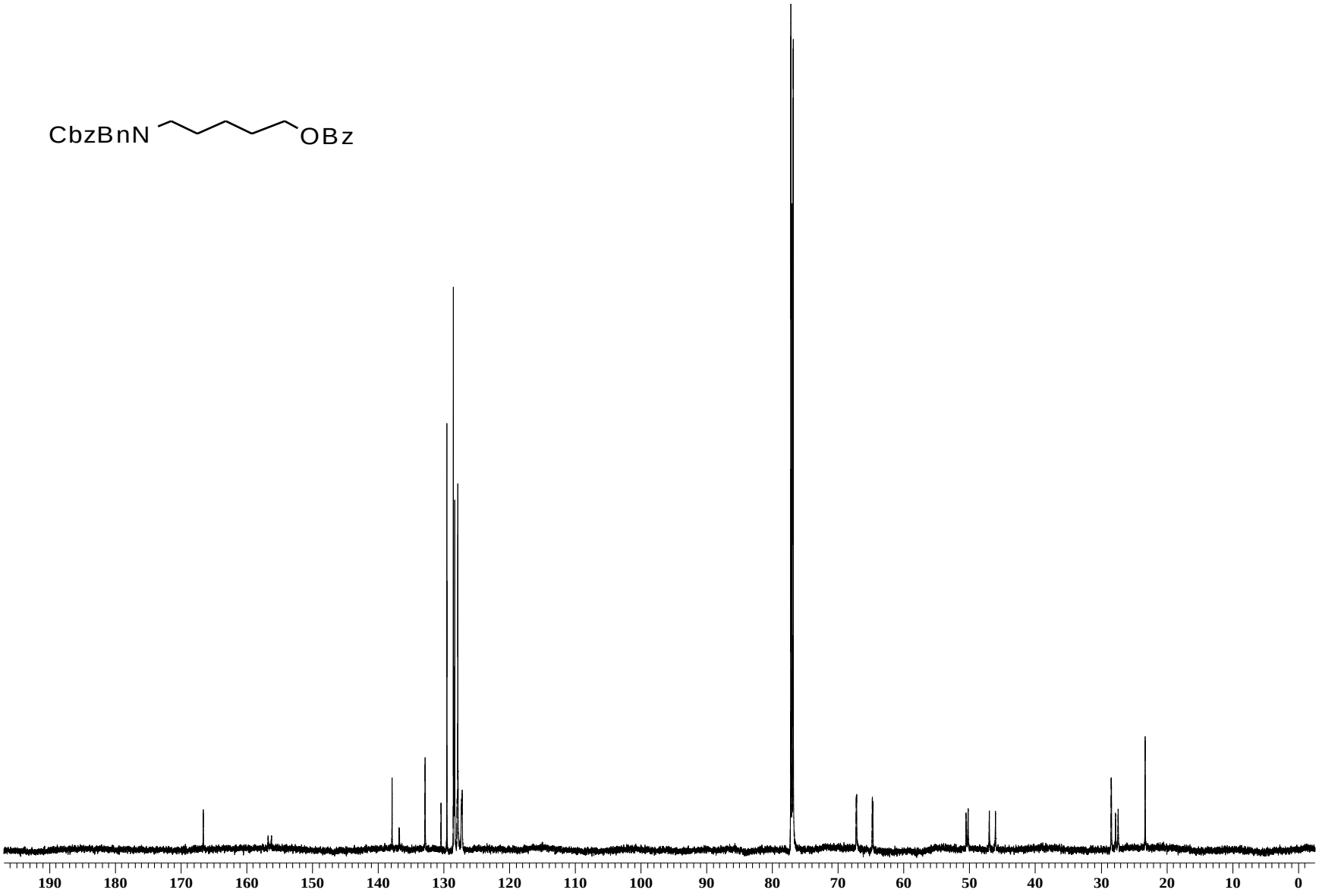


^{13}C NMR Spectrum of compound 11 ($CDCl_3$, 176 MHz).

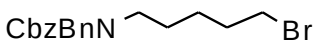


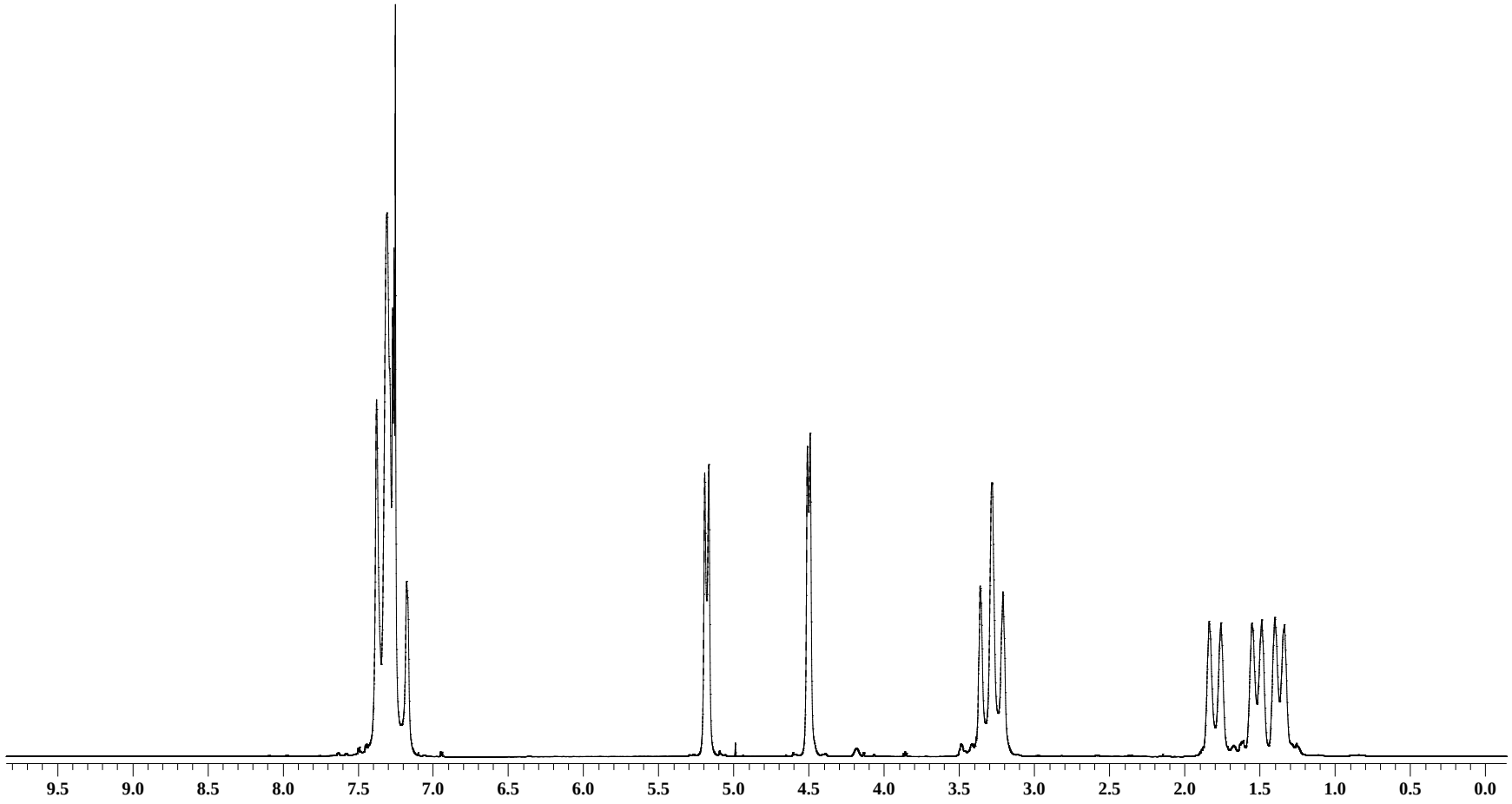


^{1}H NMR Spectrum of compound S13 ($CDCl_3$, 700 MHz).

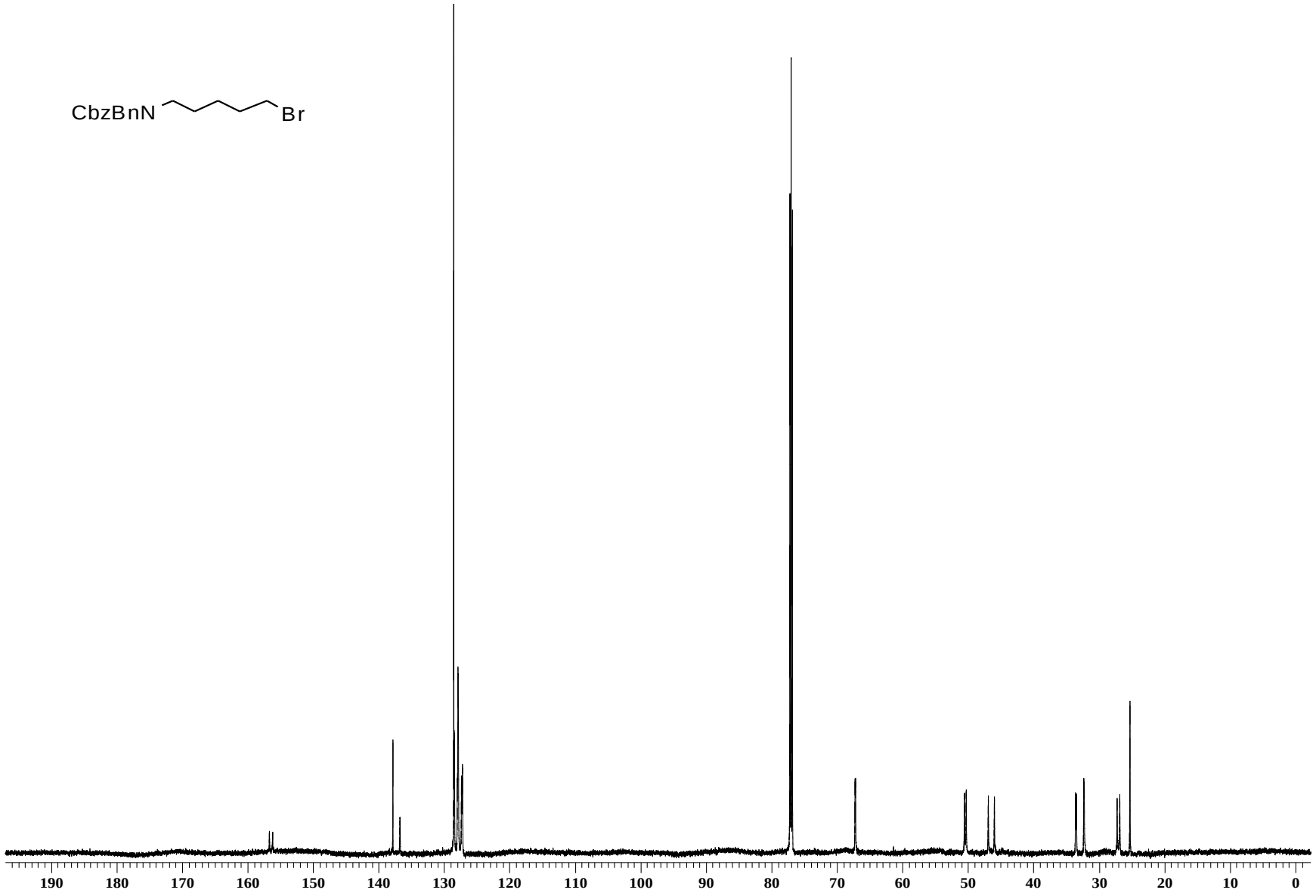


^{13}C NMR Spectrum of compound S13 ($CDCl_3$, 176 MHz).

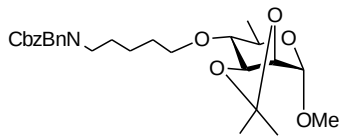


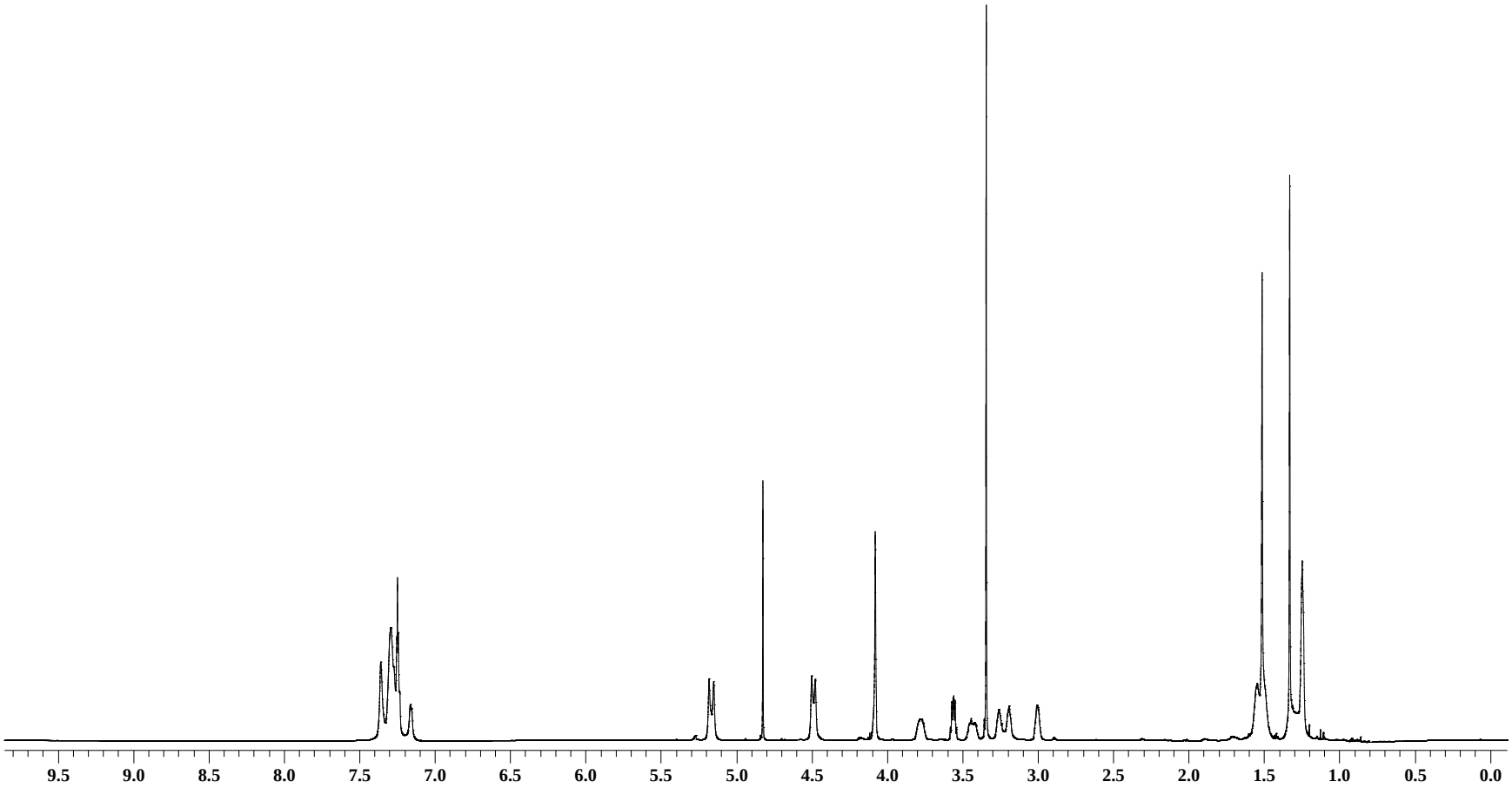


^{1}H NMR Spectrum of compound 12 ($CDCl_3$, 700 MHz).

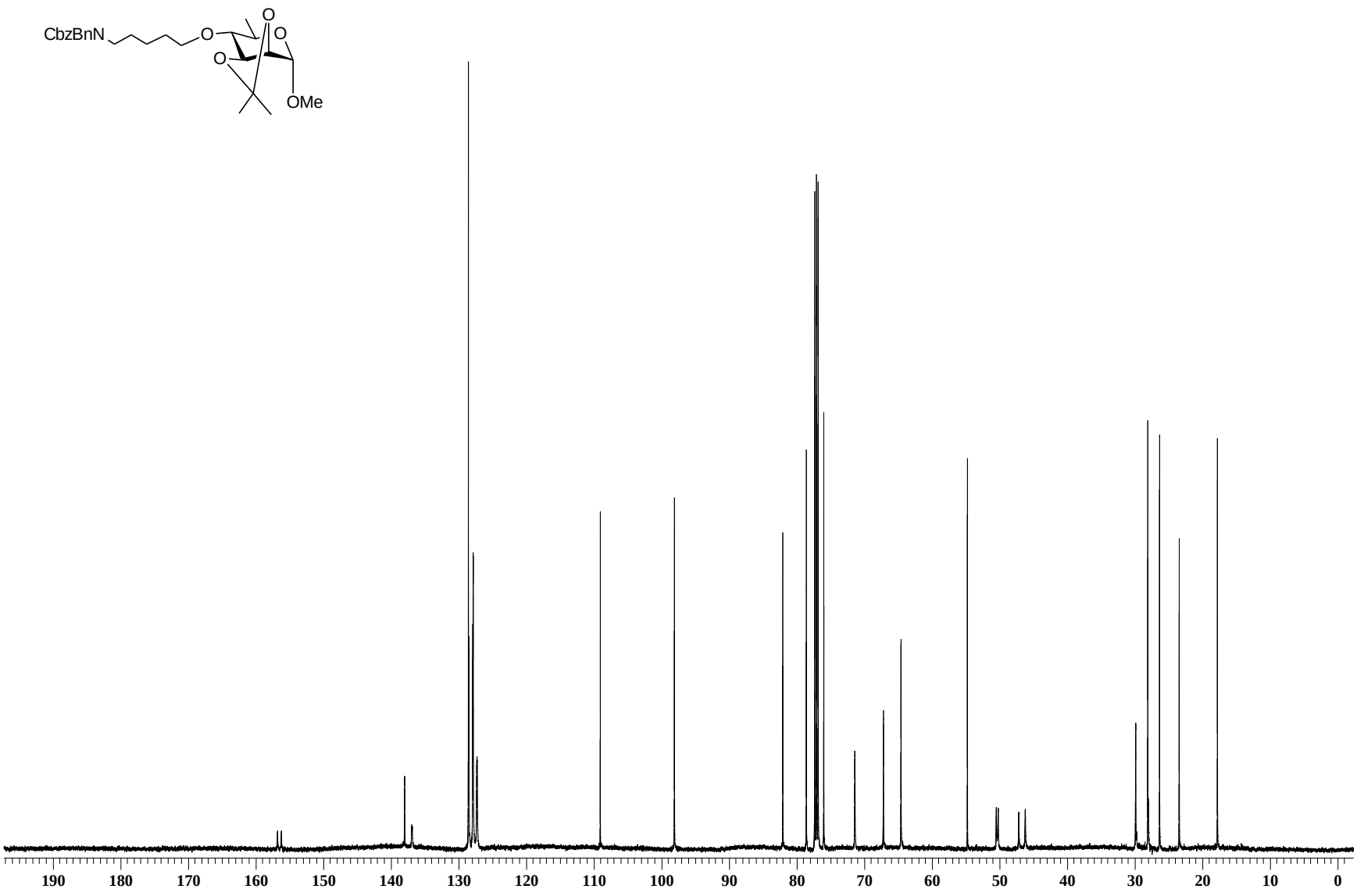


^{13}C NMR Spectrum of compound 12 ($CDCl_3$, 176 MHz).

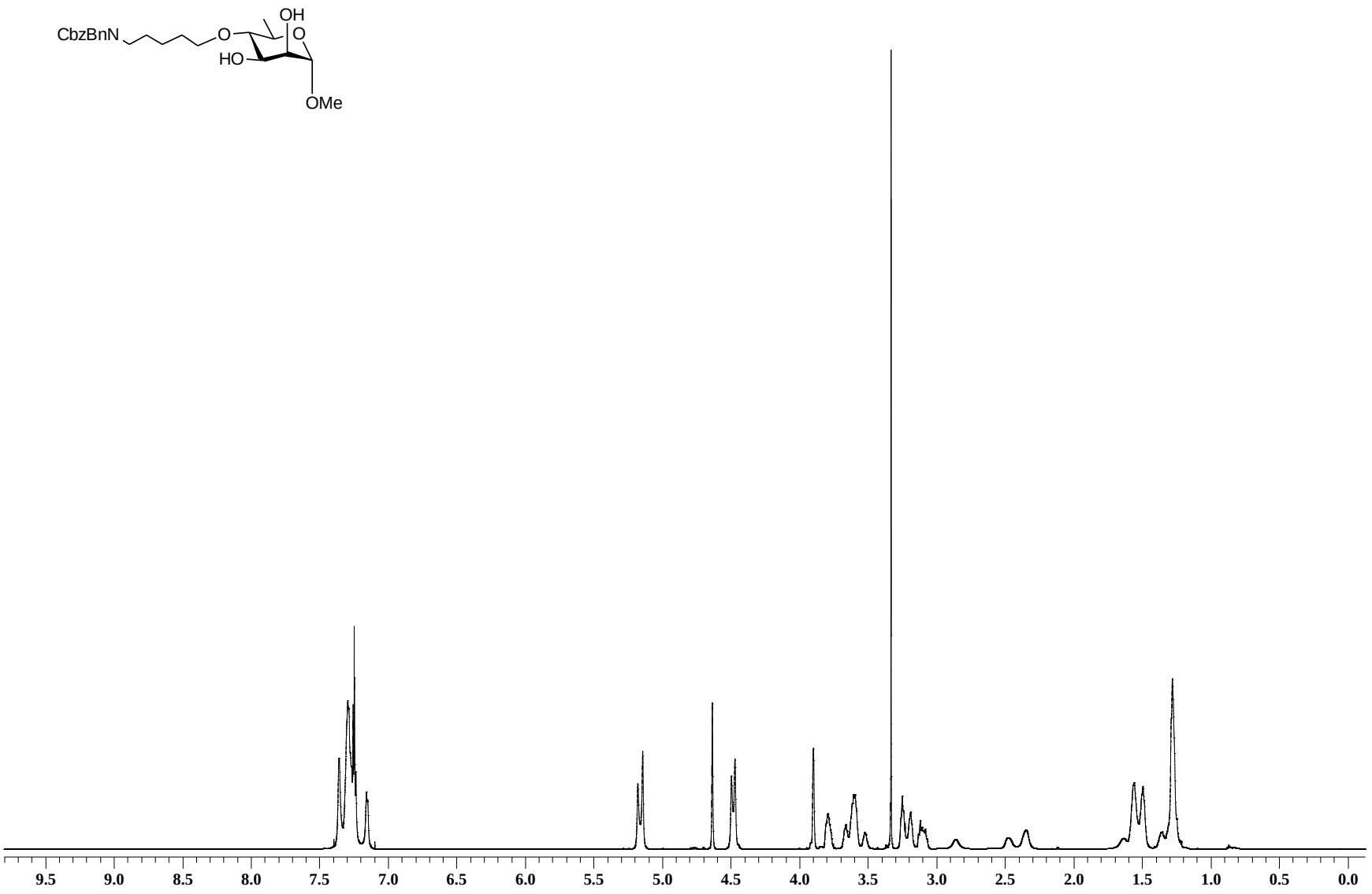




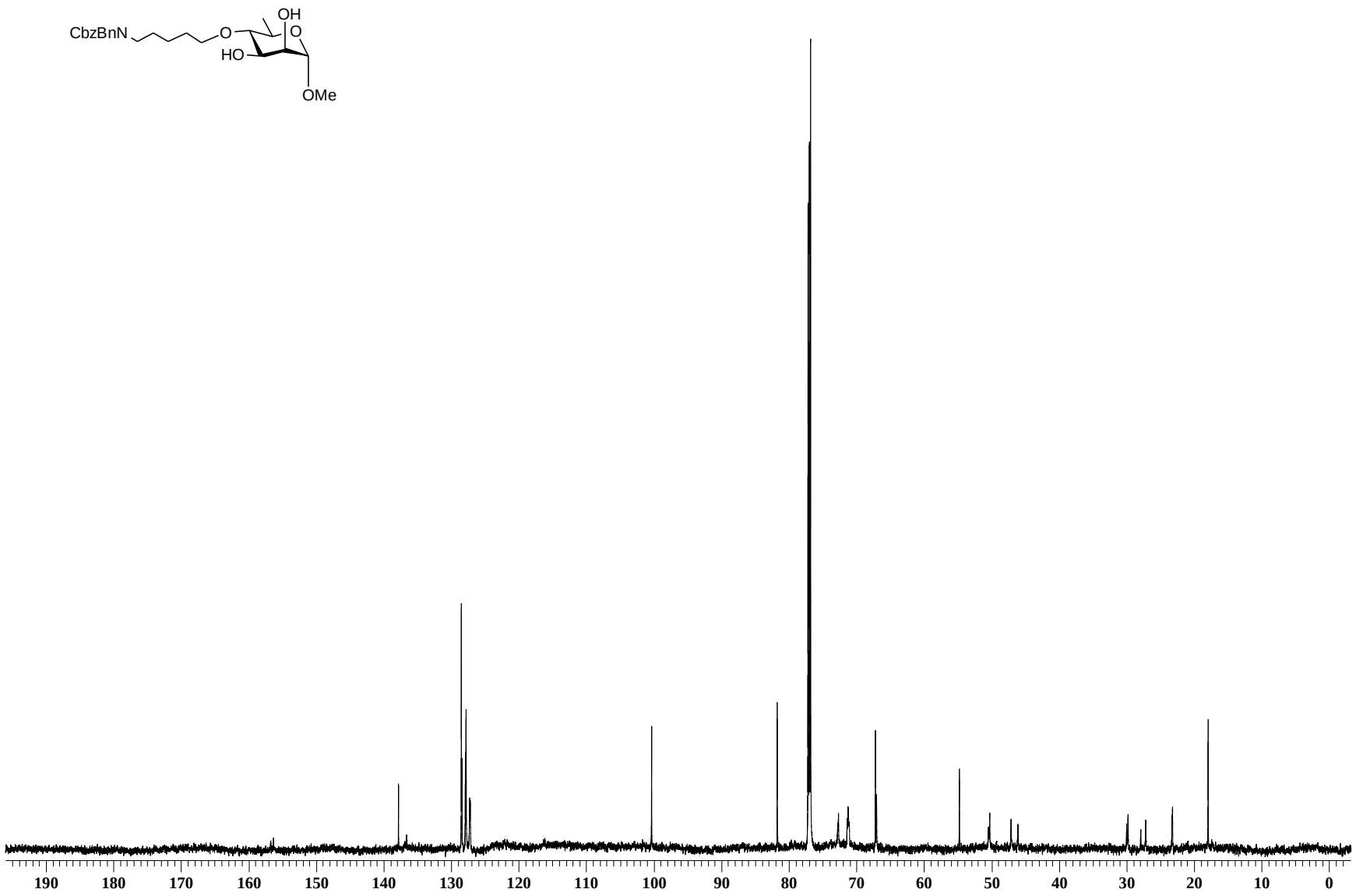
^{1}H NMR Spectrum of compound S14 ($CDCl_3$, 700 MHz).



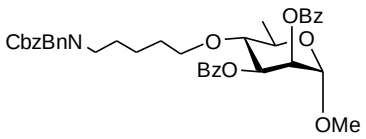
^{13}C NMR Spectrum of compound S14 ($CDCl_3$, 176 MHz).

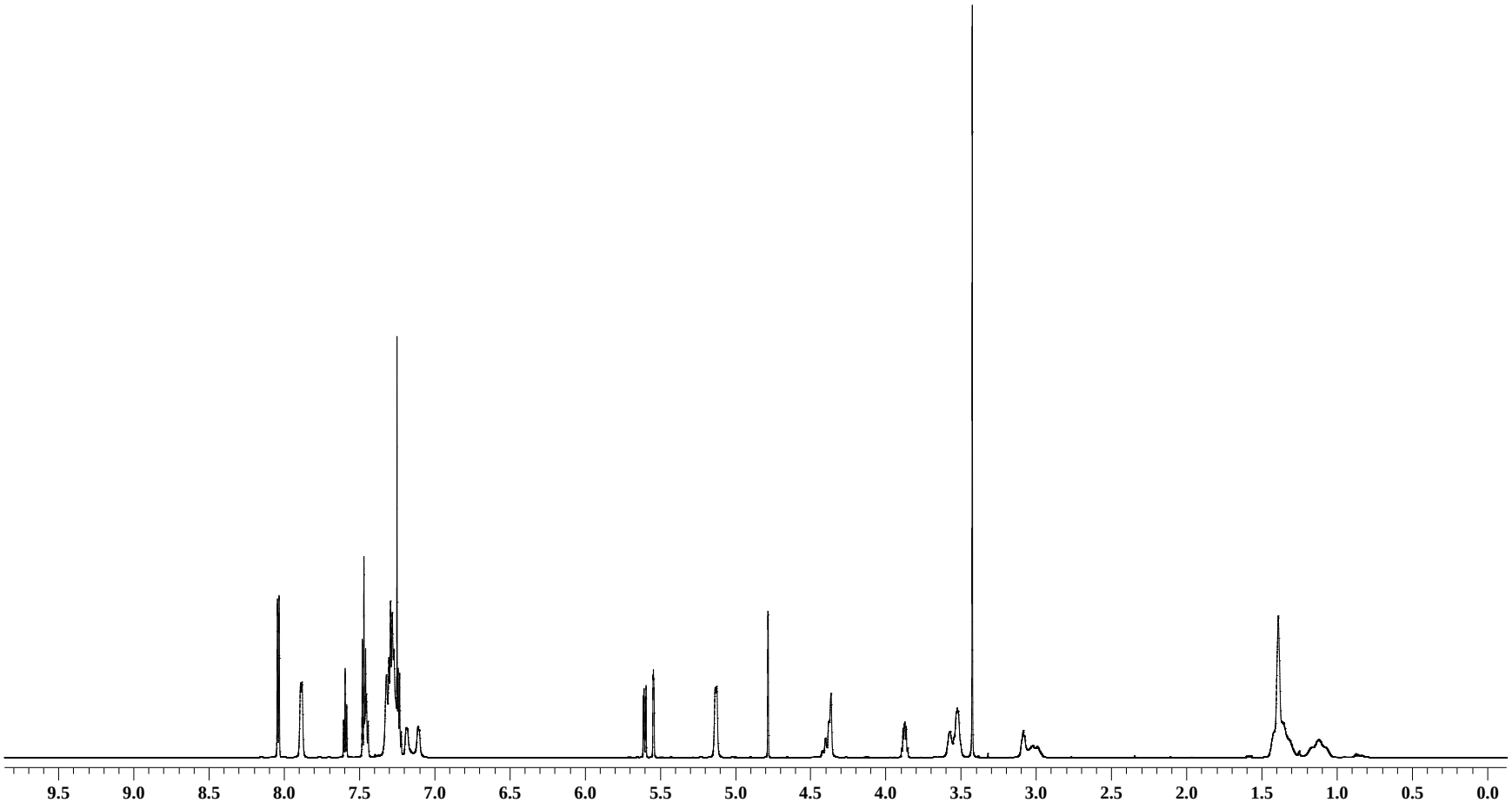


^{1}H NMR Spectrum of compound S15 ($CDCl_3$, 700 MHz).

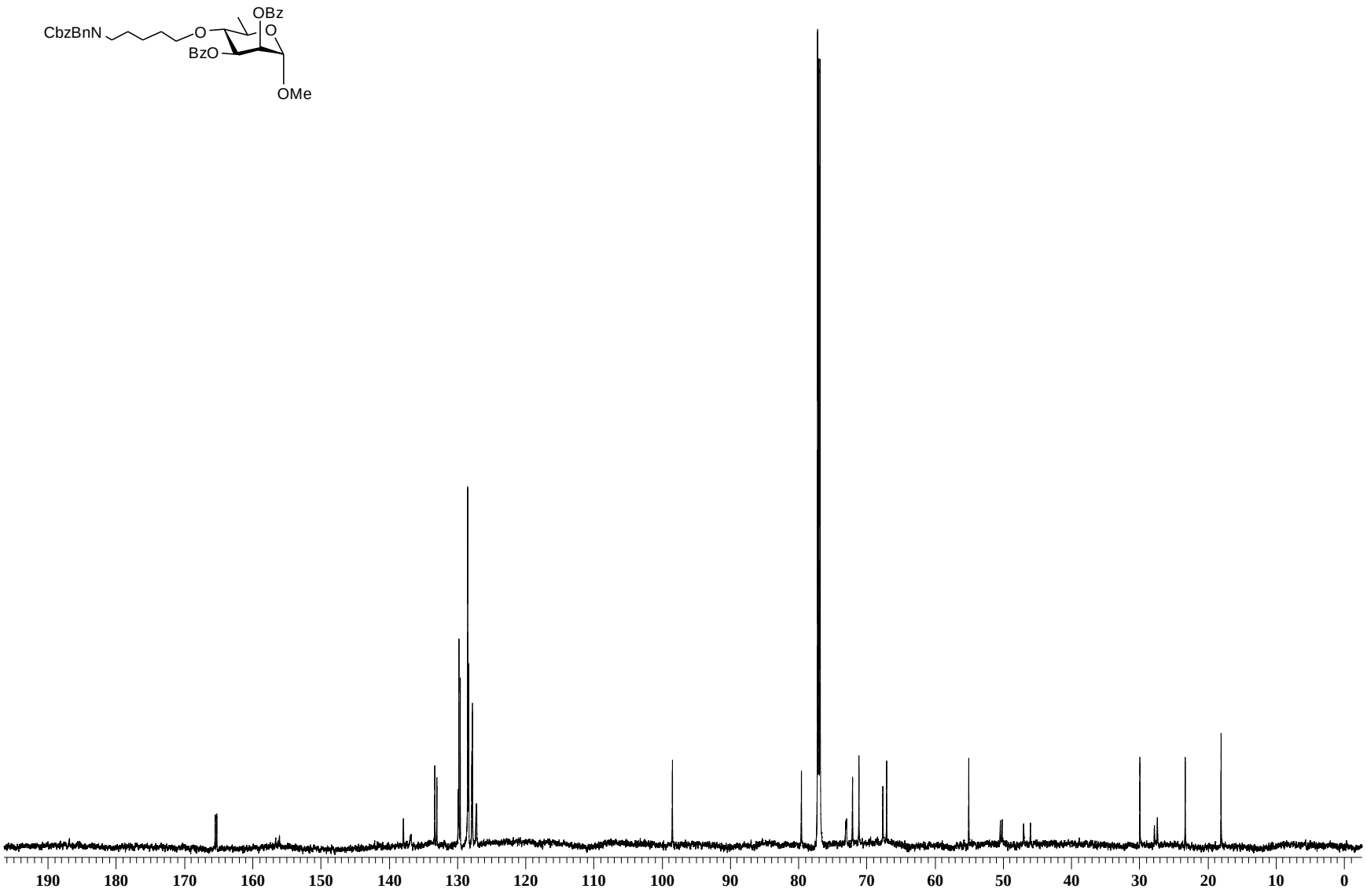


^{13}C NMR Spectrum of compound S15 ($CDCl_3$, 176 MHz).

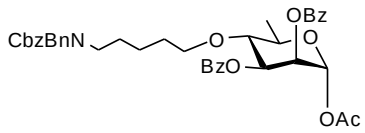


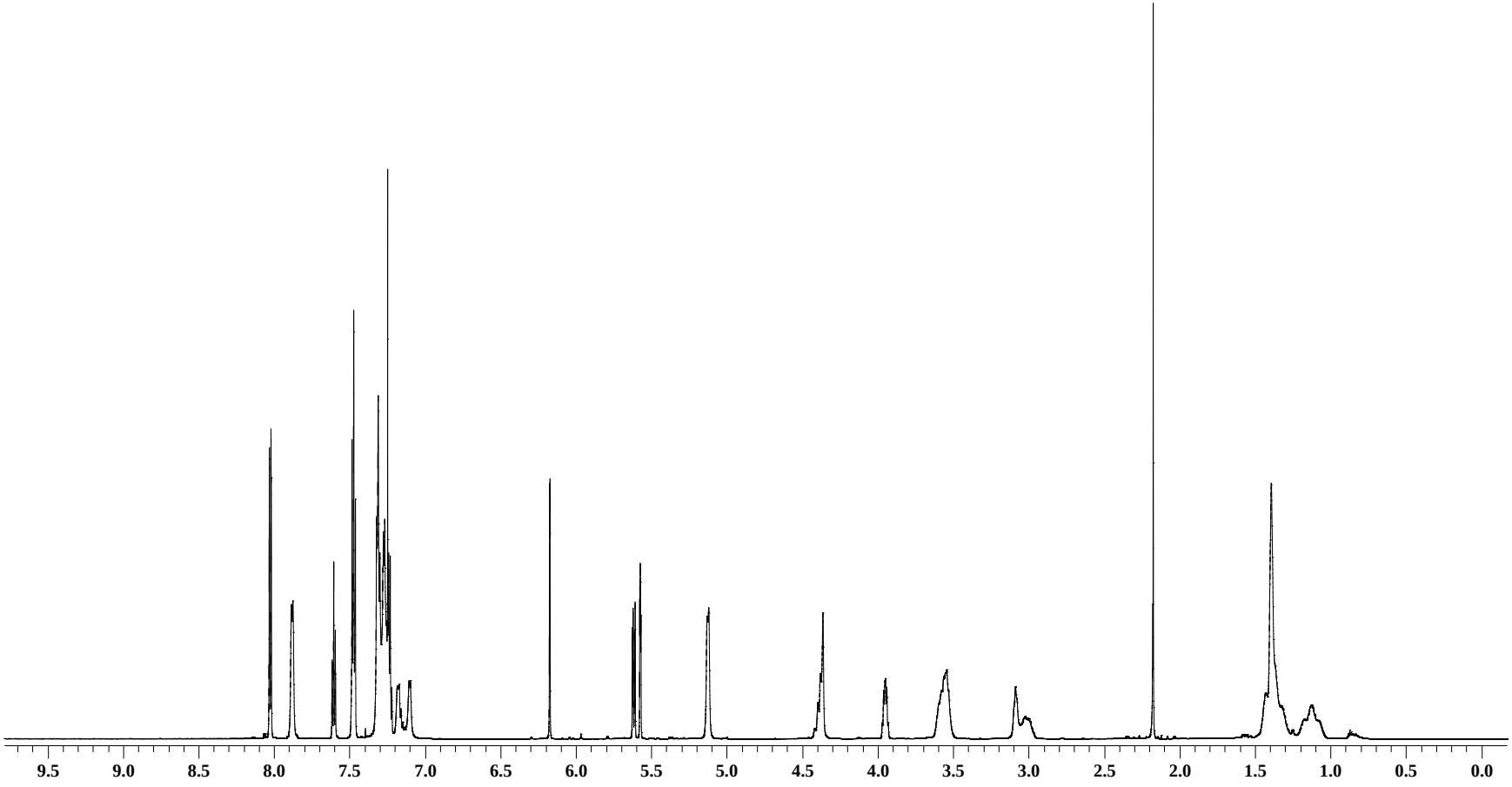


^{1}H NMR Spectrum of compound S16 ($CDCl_3$, 700 MHz).

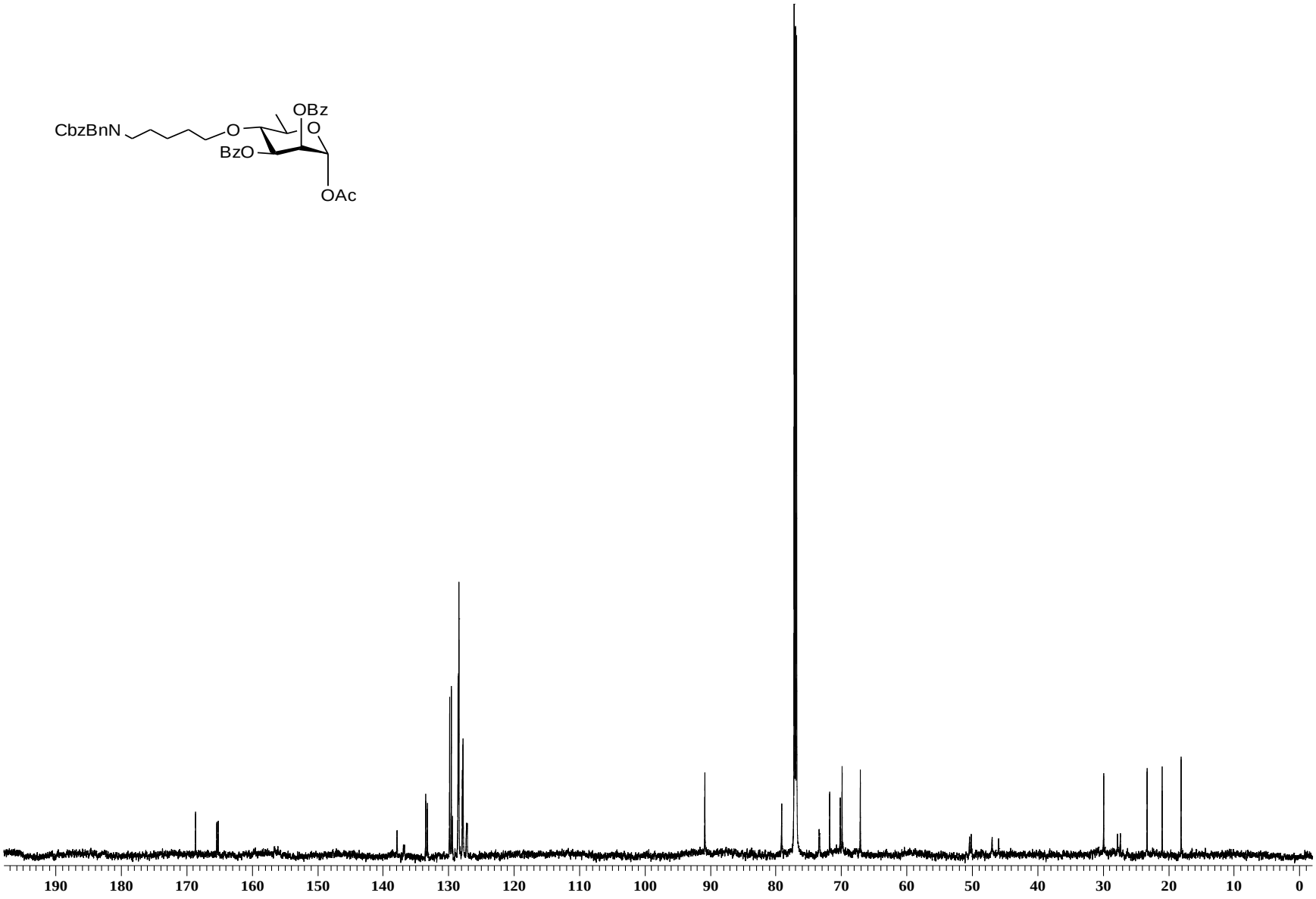


^{13}C NMR Spectrum of compound S16 ($CDCl_3$, 176 MHz).

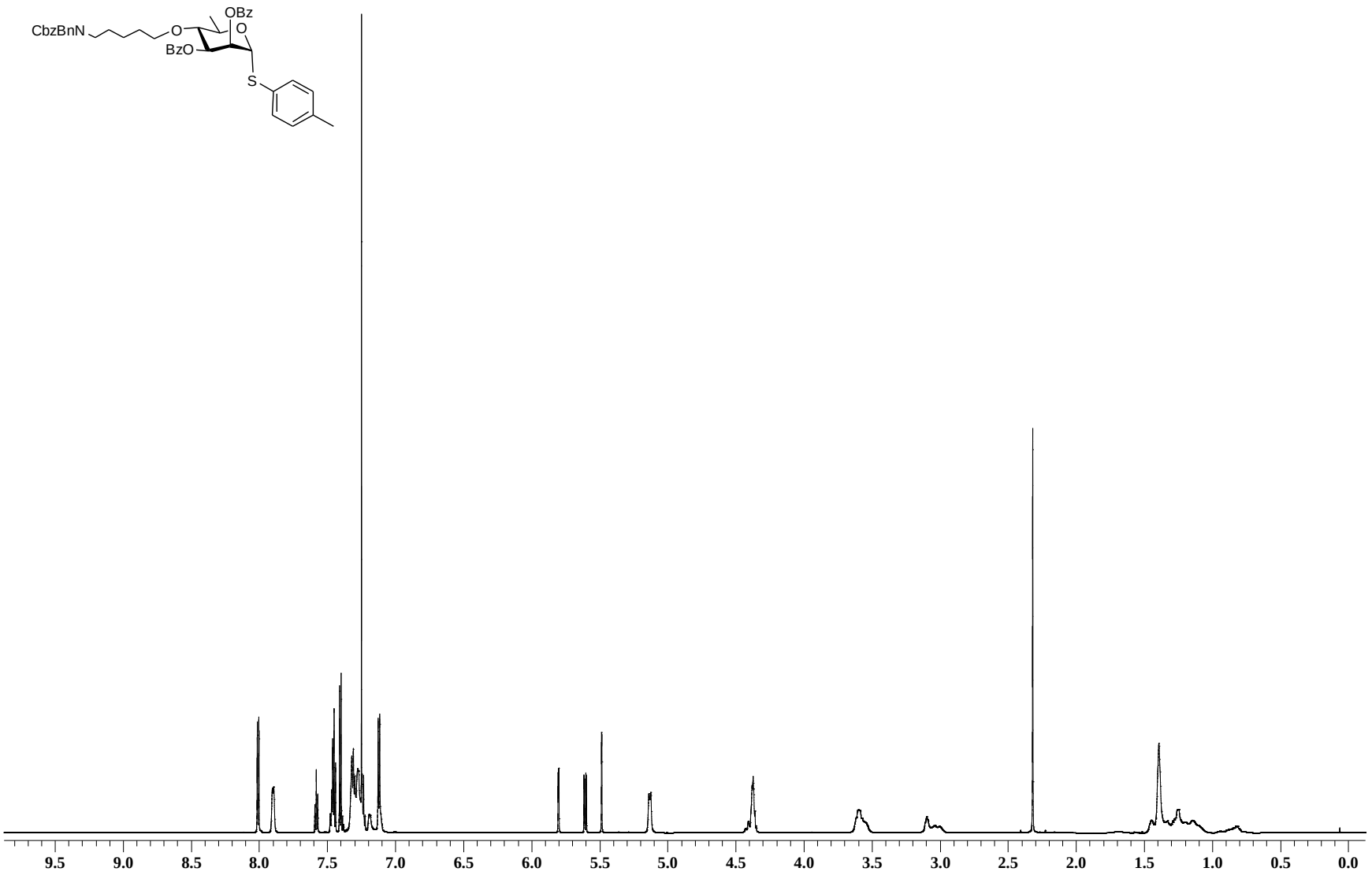




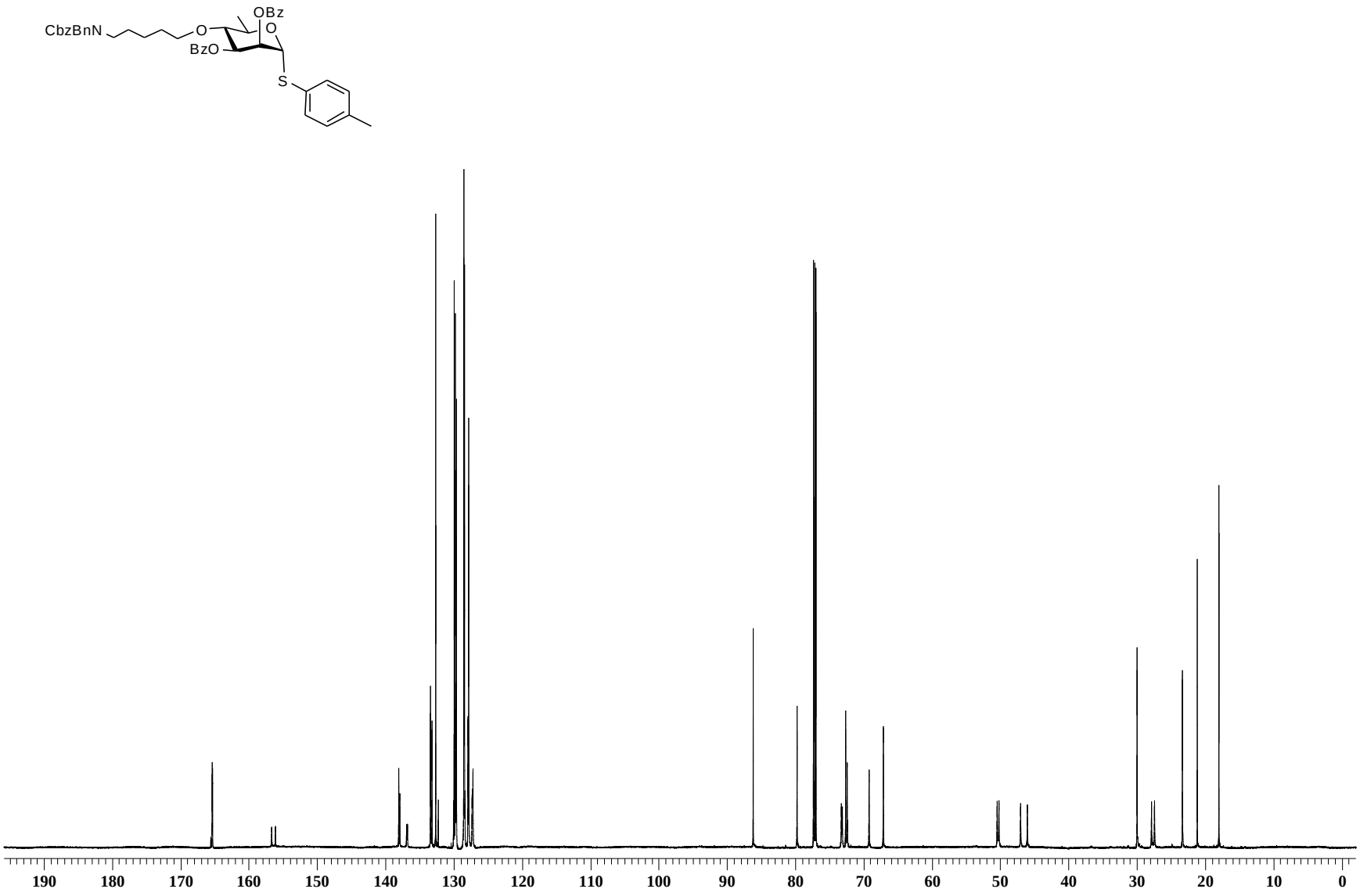
^{1}H NMR Spectrum of compound S17 ($CDCl_3$, 700 MHz).



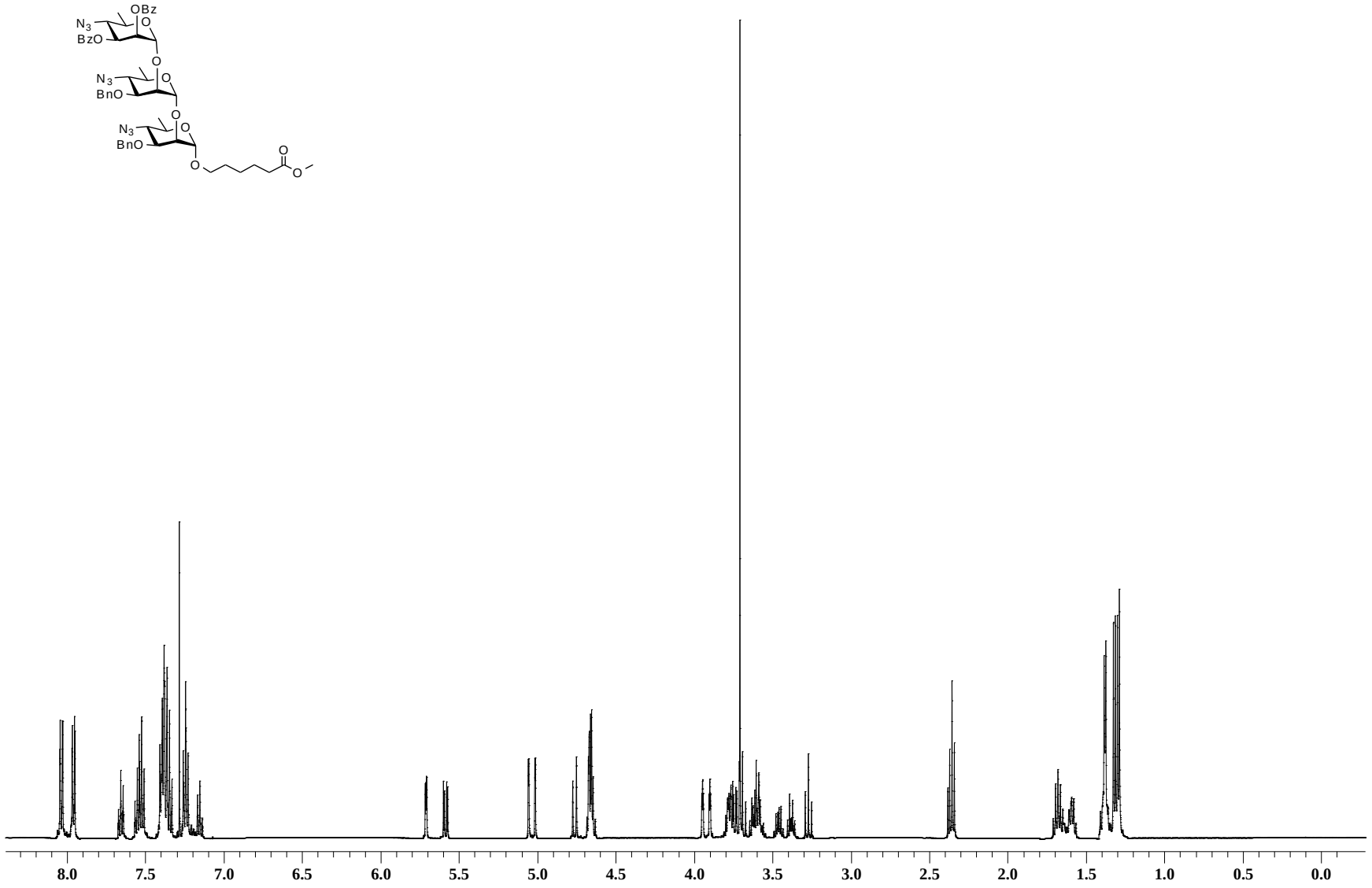
^{13}C NMR Spectrum of compound S17 ($CDCl_3$, 176 MHz).



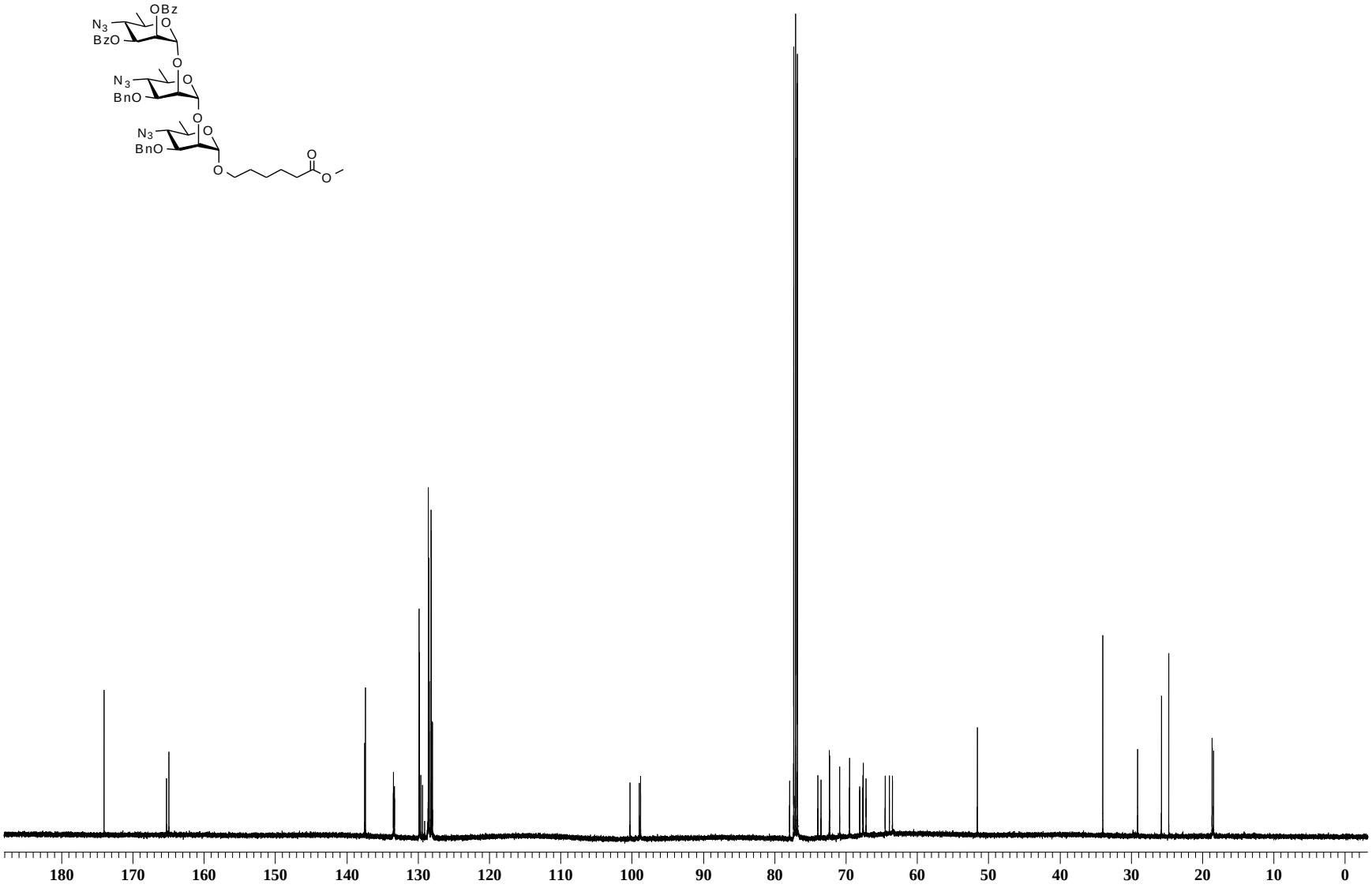
^{1}H NMR Spectrum of compound 13 ($CDCl_3$, 700 MHz).



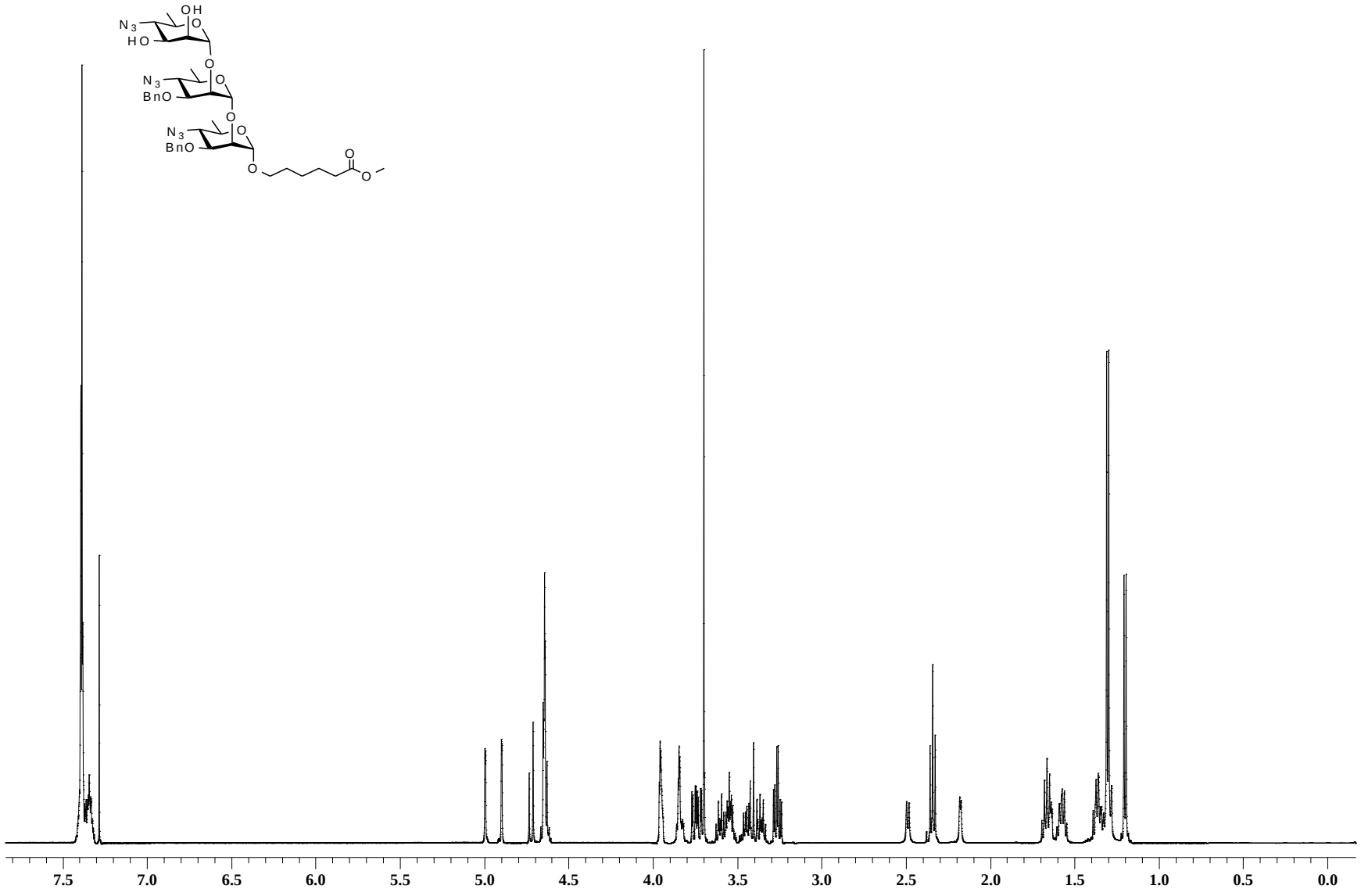
^{13}C NMR Spectrum of compound 13 ($CDCl_3$, 176 MHz).



^{1}H NMR Spectrum of compound S20 ($CDCl_3$, 500 MHz).



^{13}C NMR Spectrum of compound S20 ($CDCl_3$, 126 MHz).



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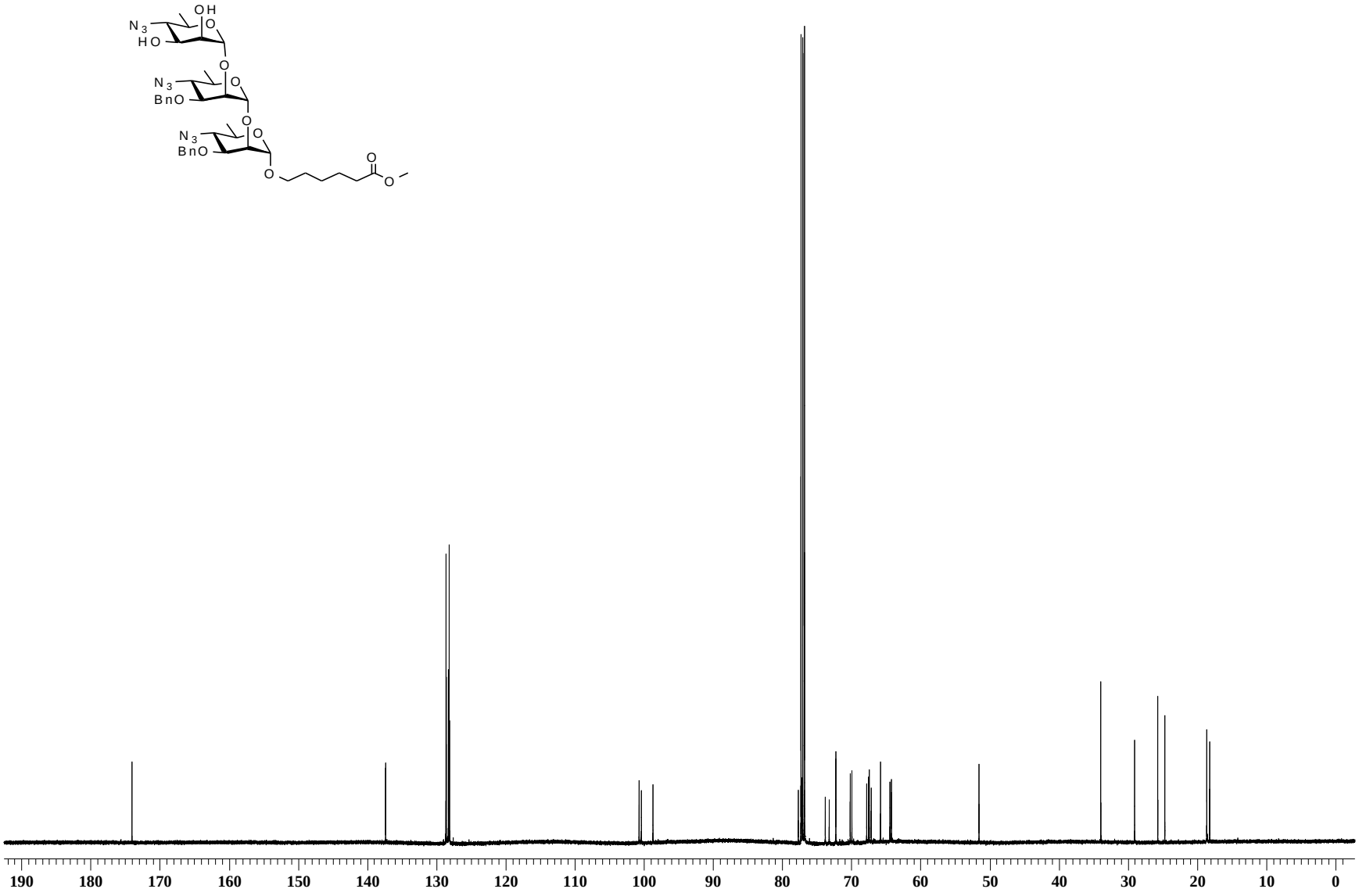
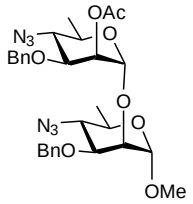
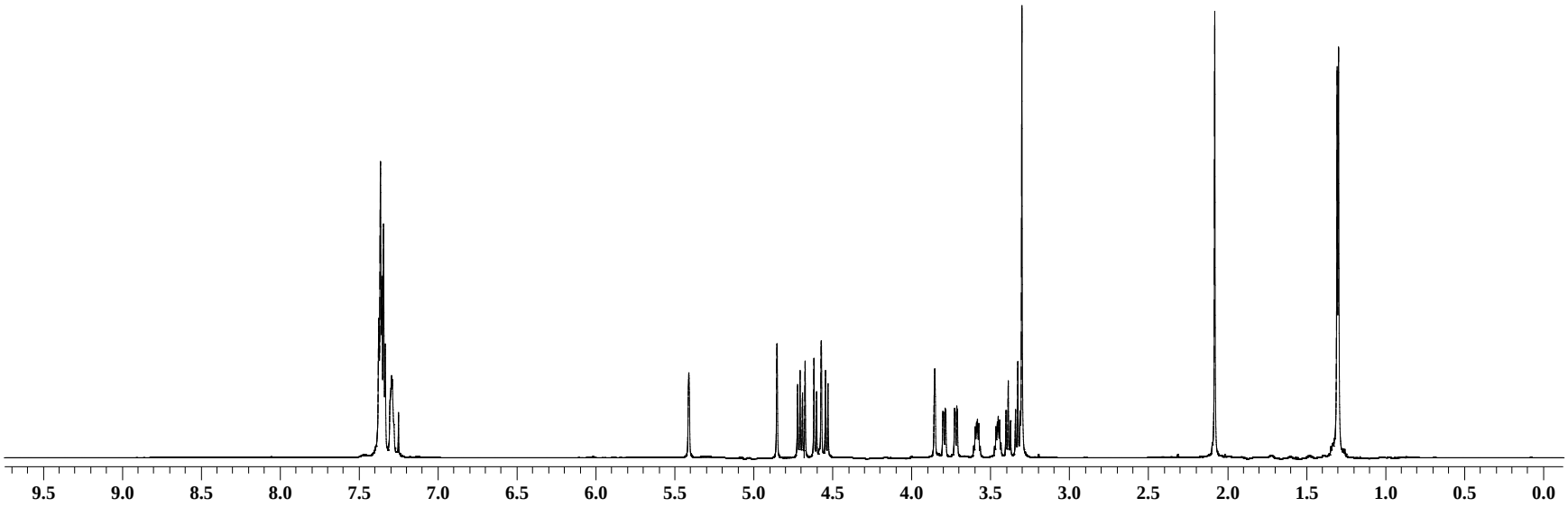
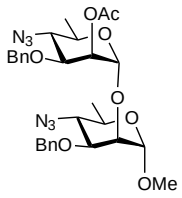


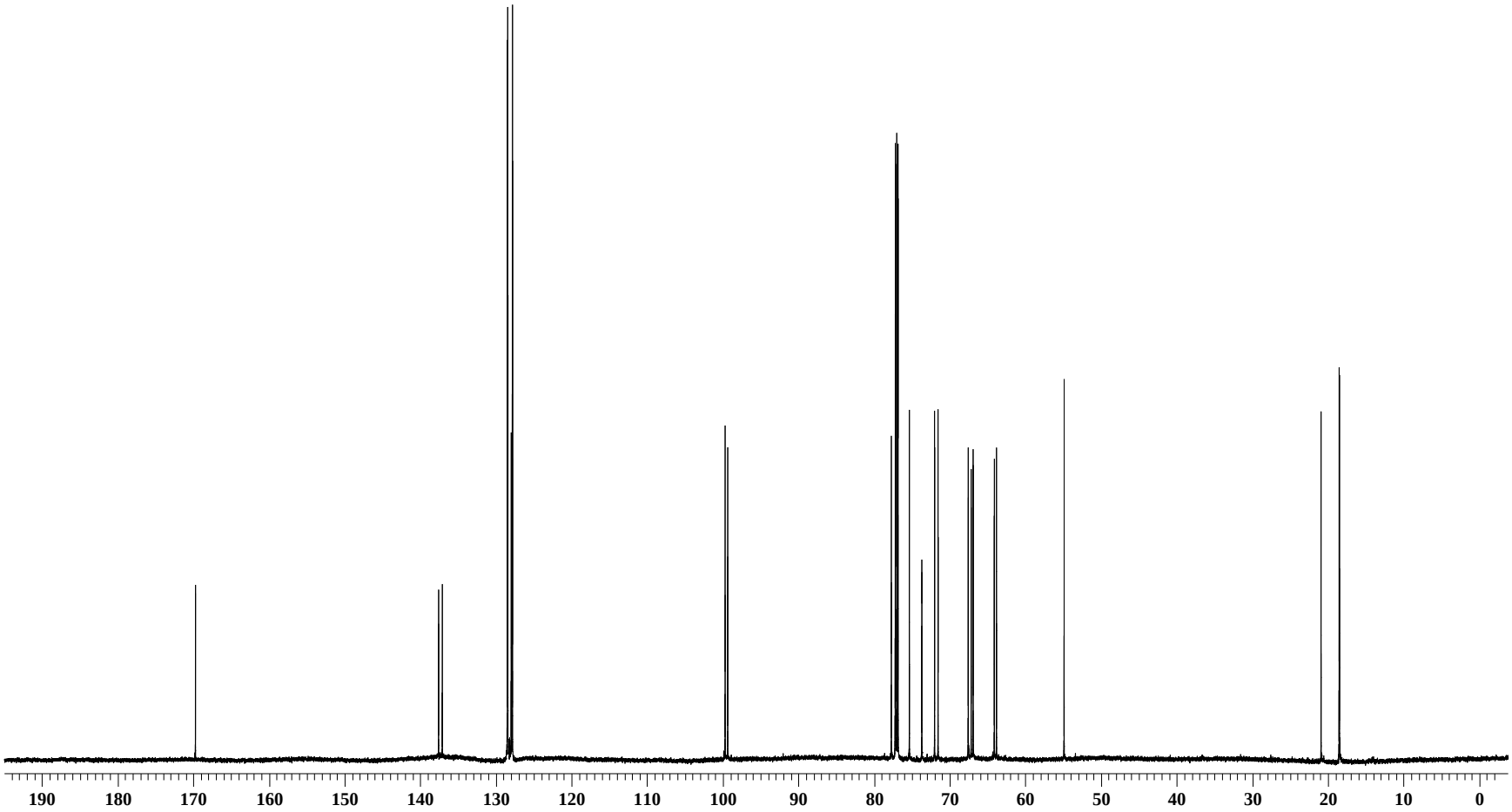
^{13}C NMR Spectrum of compound S21 ($CDCl_3$, 126 MHz).



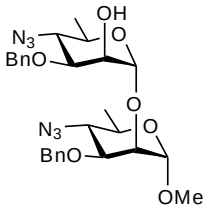


^{1}H NMR Spectrum of compound 14 ($CDCl_3$, 700 MHz).

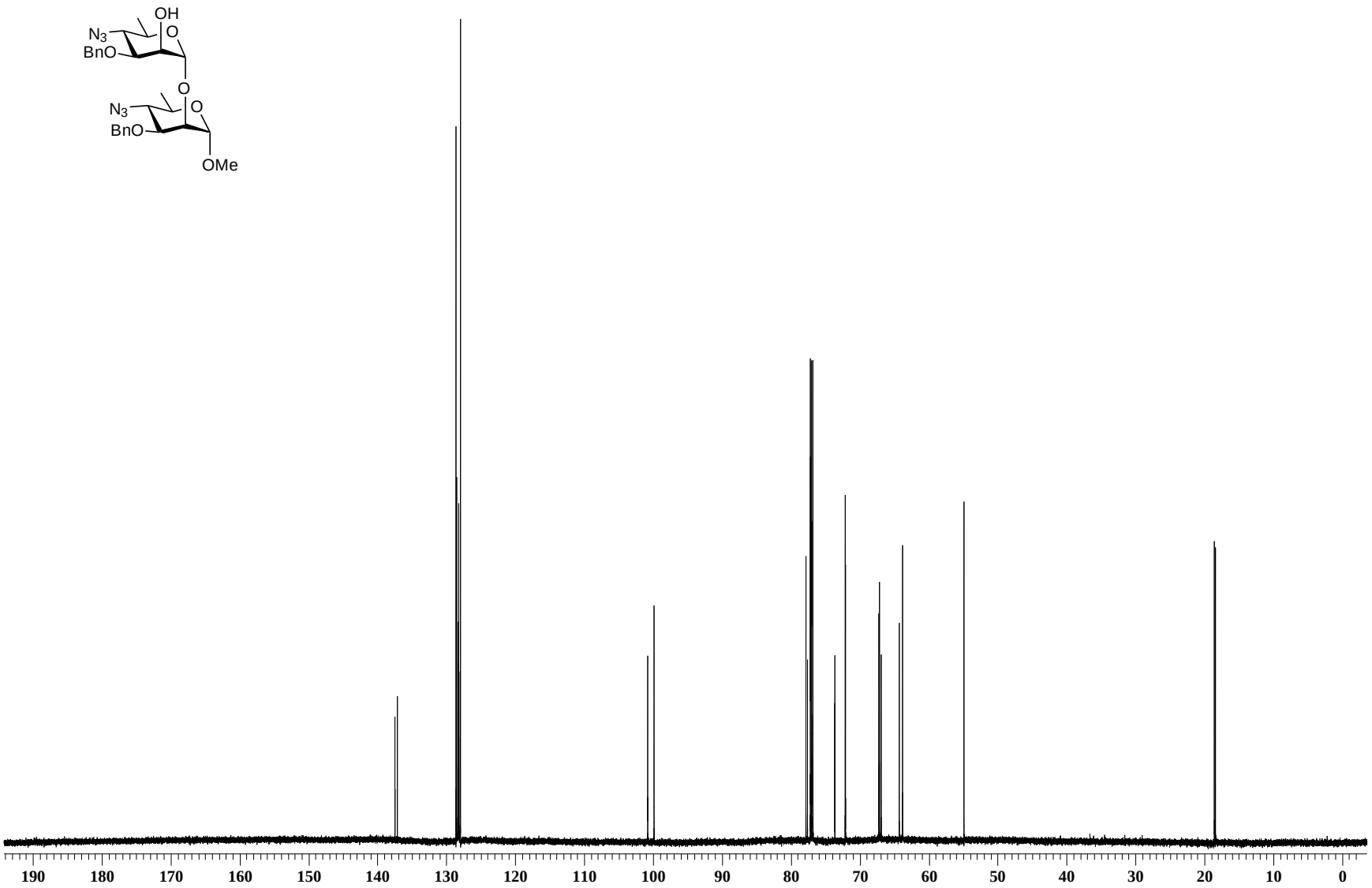




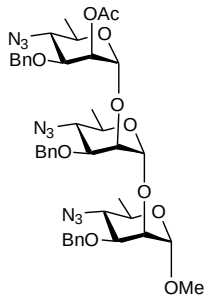
^{13}C NMR Spectrum of compound 14 ($CDCl_3$, 176 MHz).

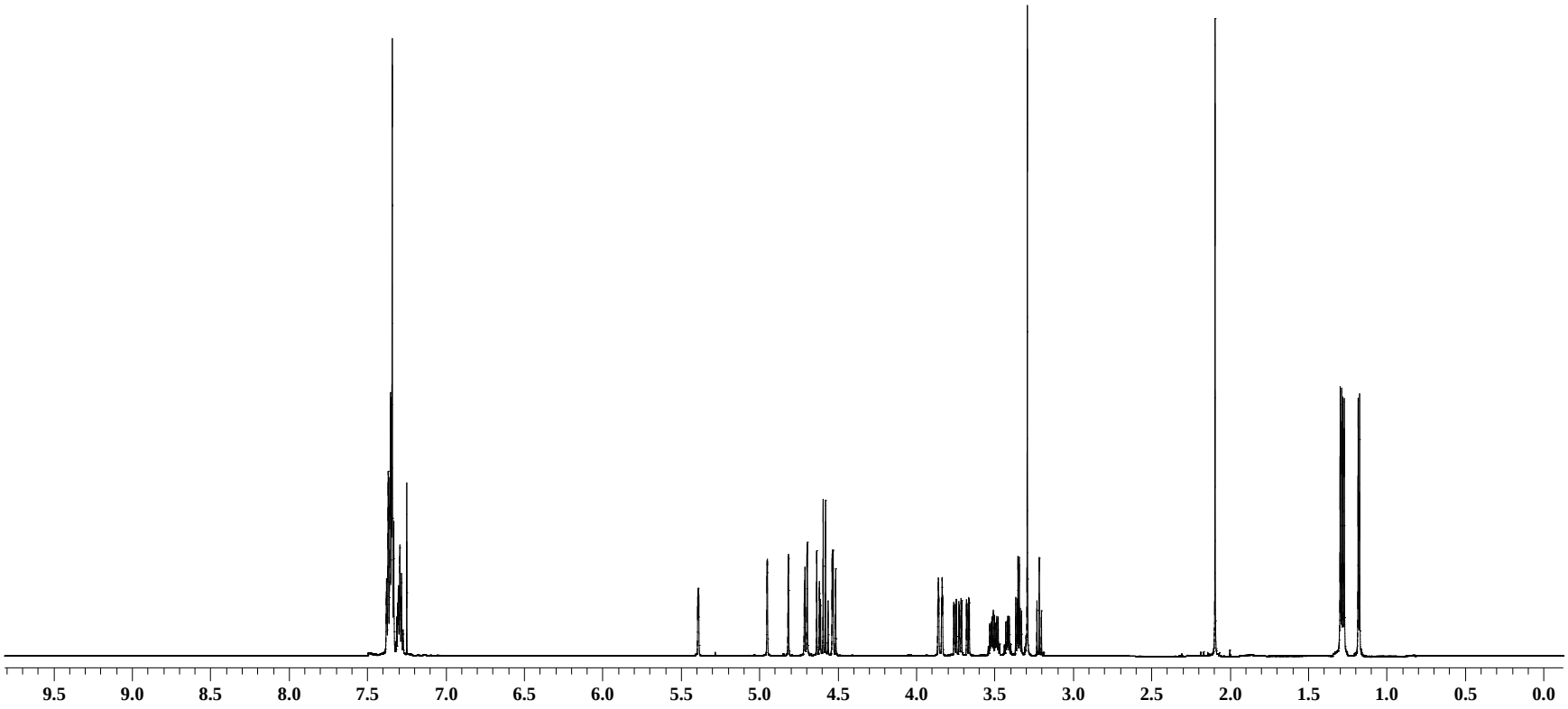


^{1}H NMR Spectrum of compound 15 ($CDCl_3$, 700 MHz).

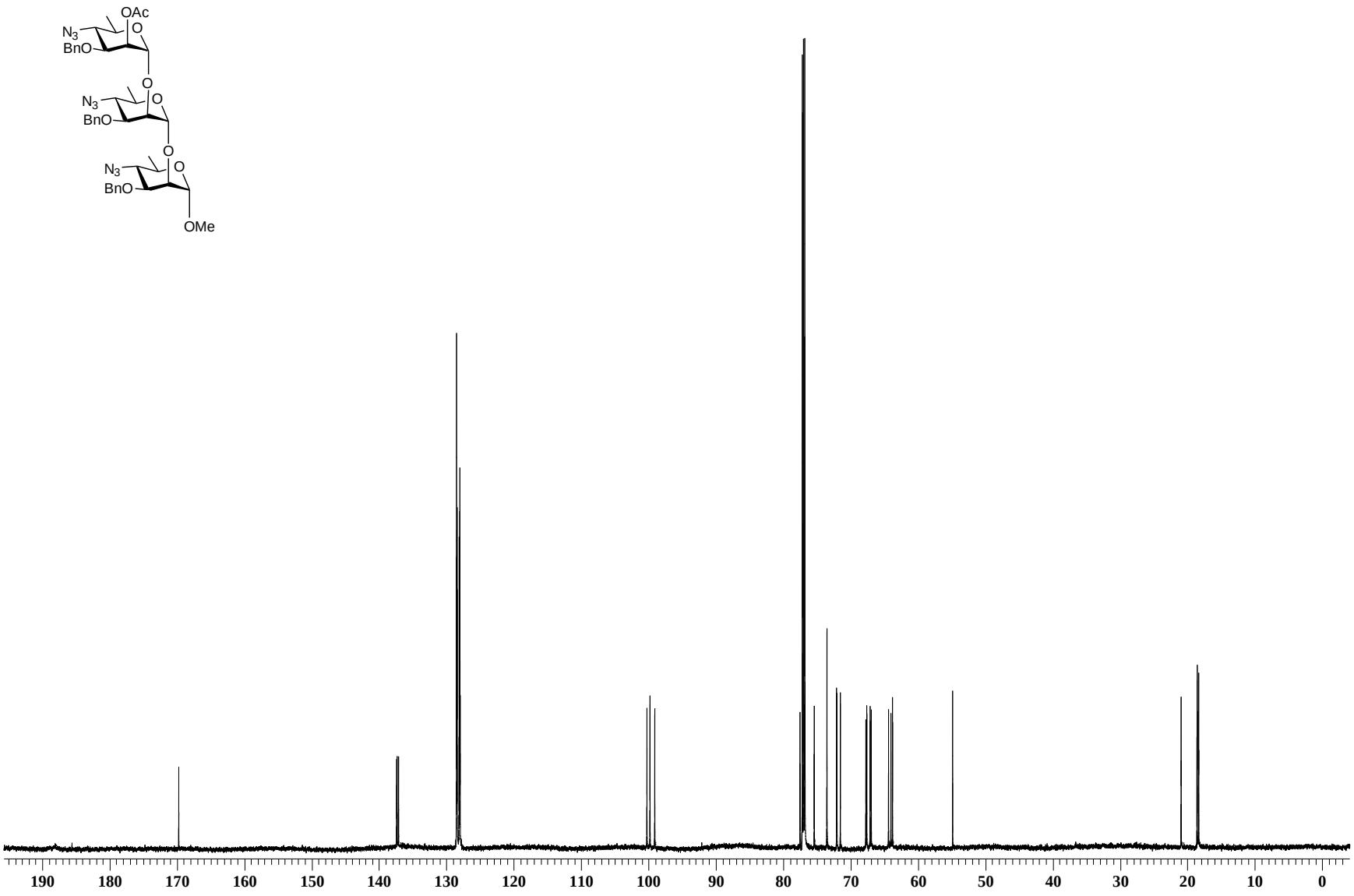


^{13}C NMR Spectrum of compound 15 ($CDCl_3$, 176 MHz).

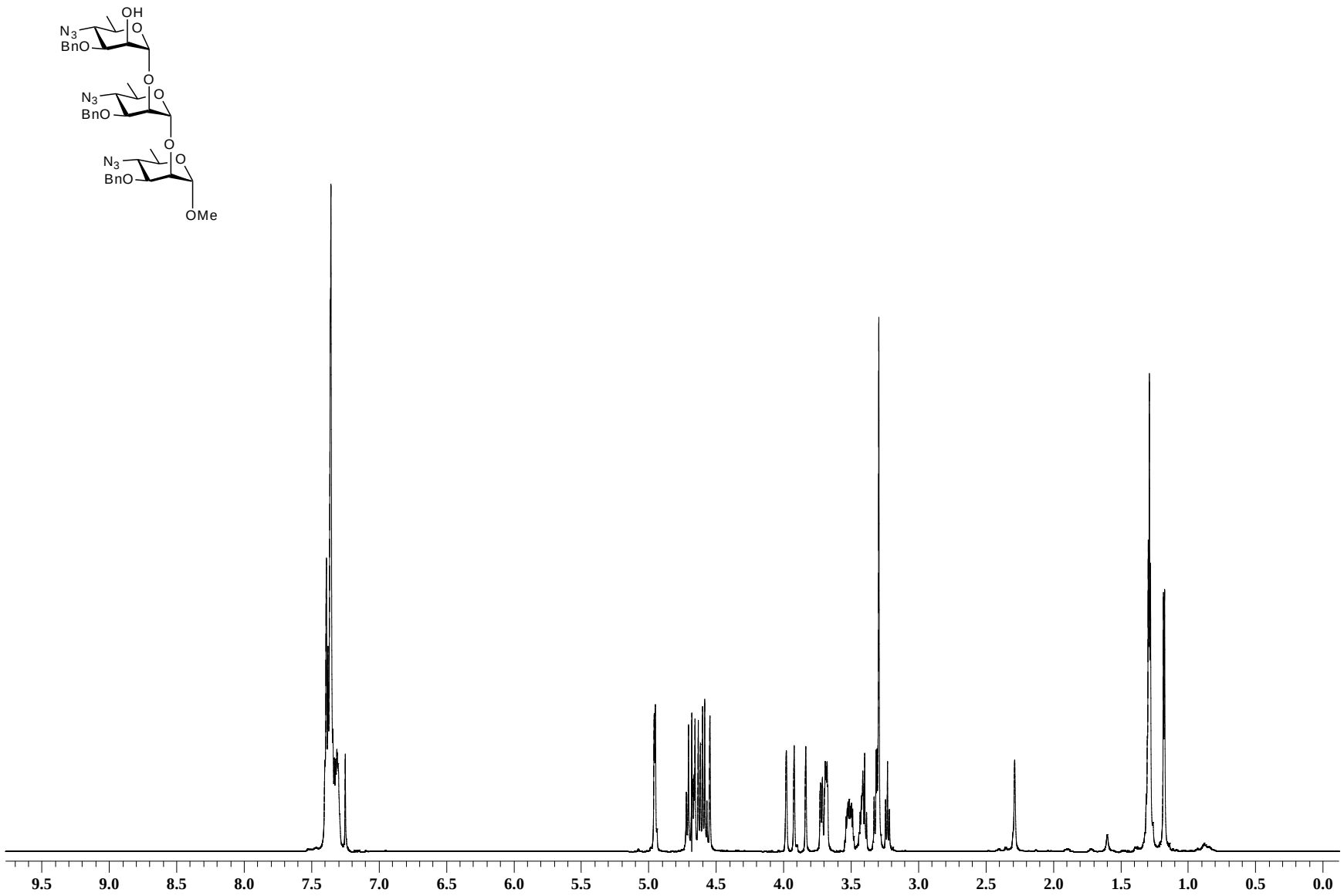




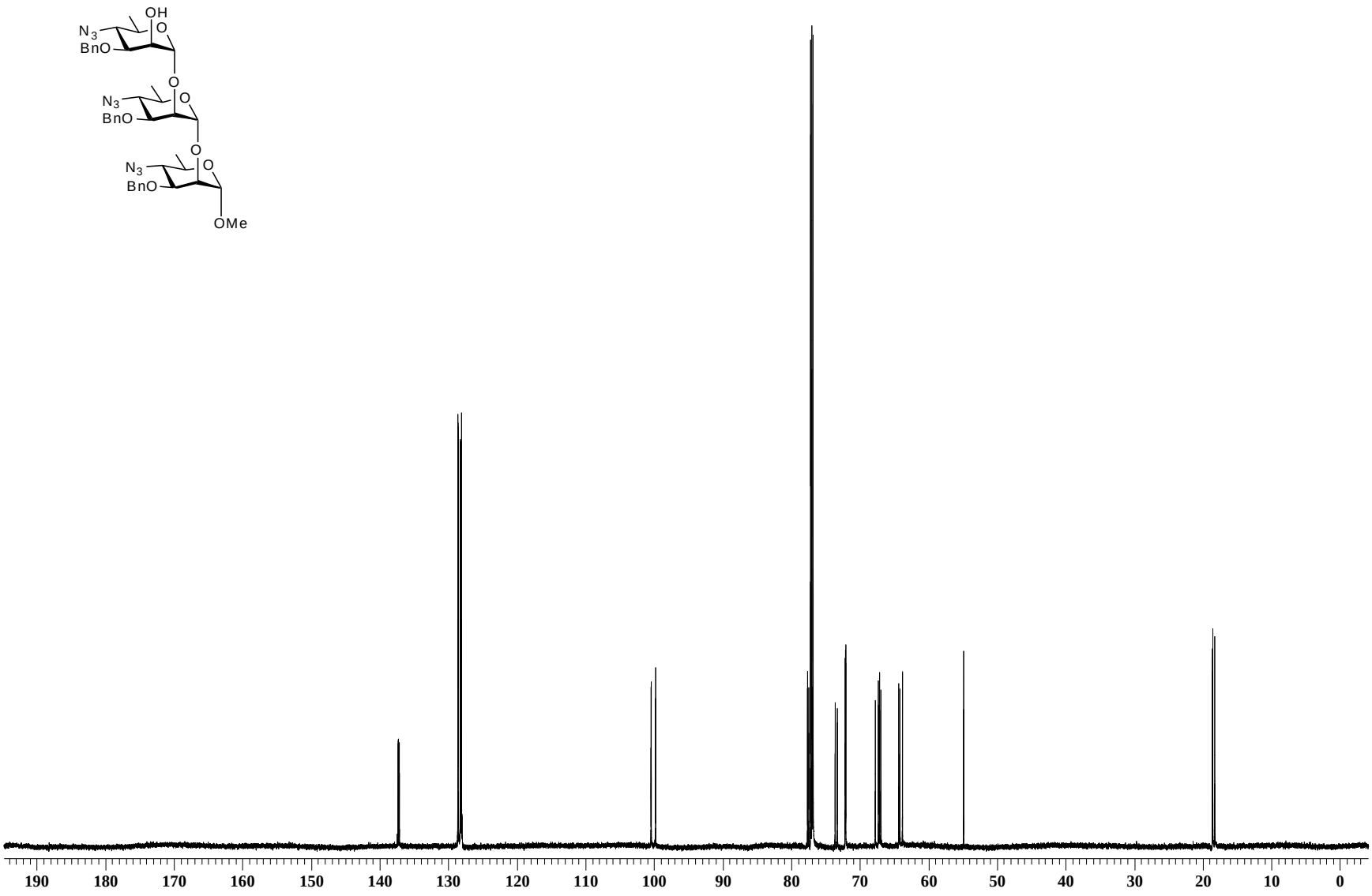
^{1}H NMR Spectrum of compound 16 ($CDCl_3$, 700 MHz).



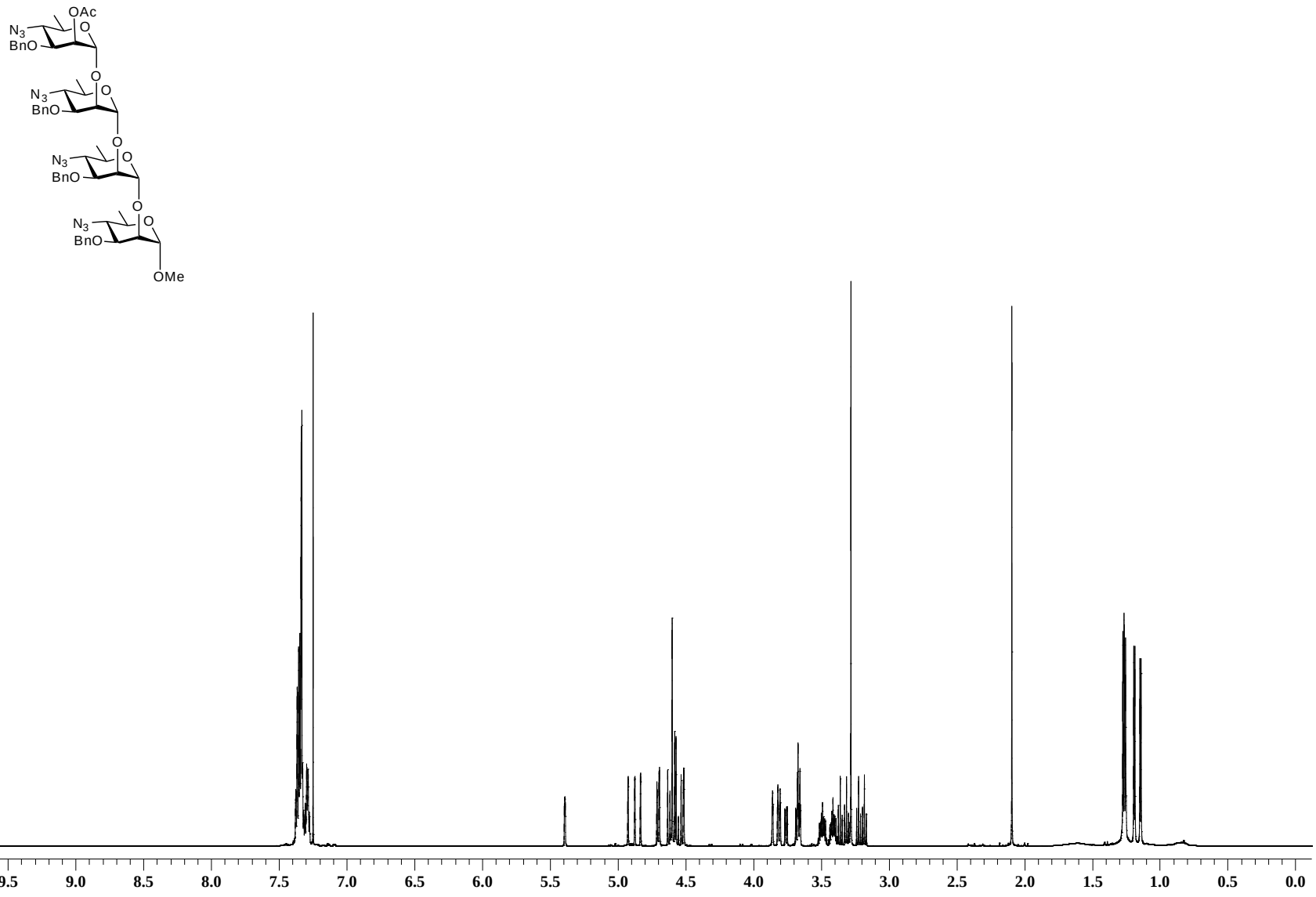
^{13}C NMR Spectrum of compound 16 ($CDCl_3$, 176 MHz).



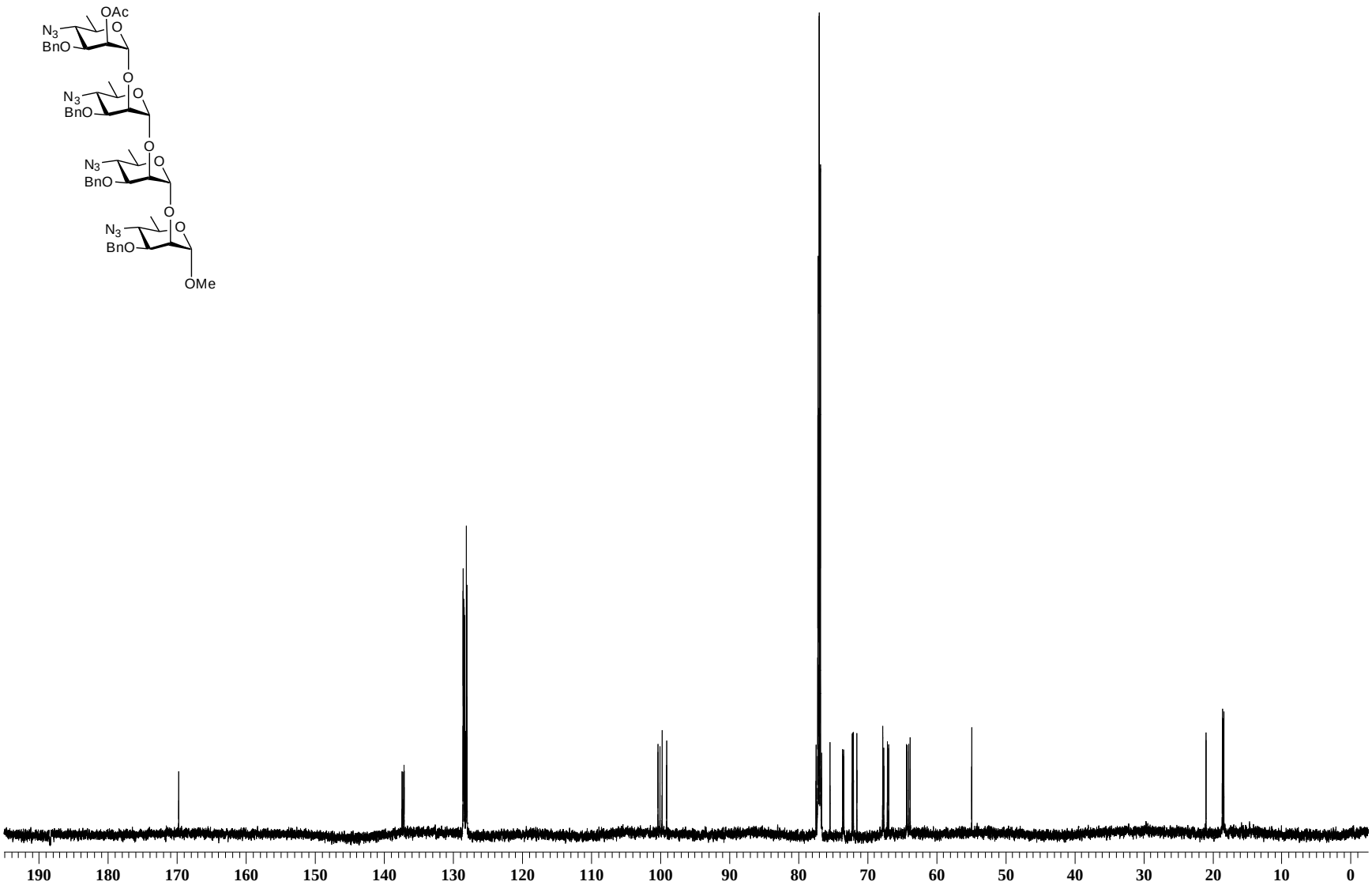
^{1}H NMR Spectrum of compound 17 ($CDCl_3$, 700 MHz).



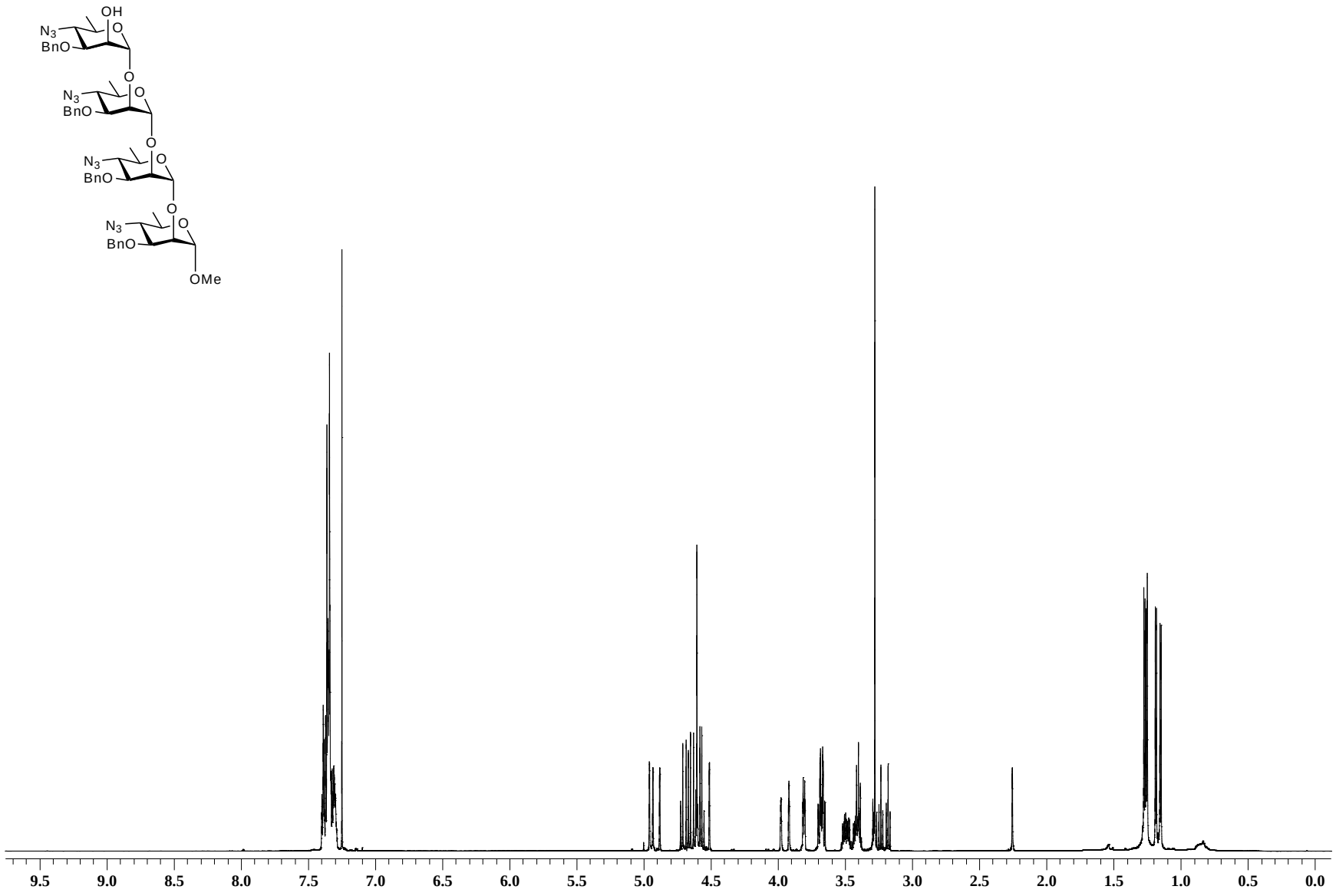
^{13}C NMR Spectrum of compound 17 ($CDCl_3$, 176 MHz).



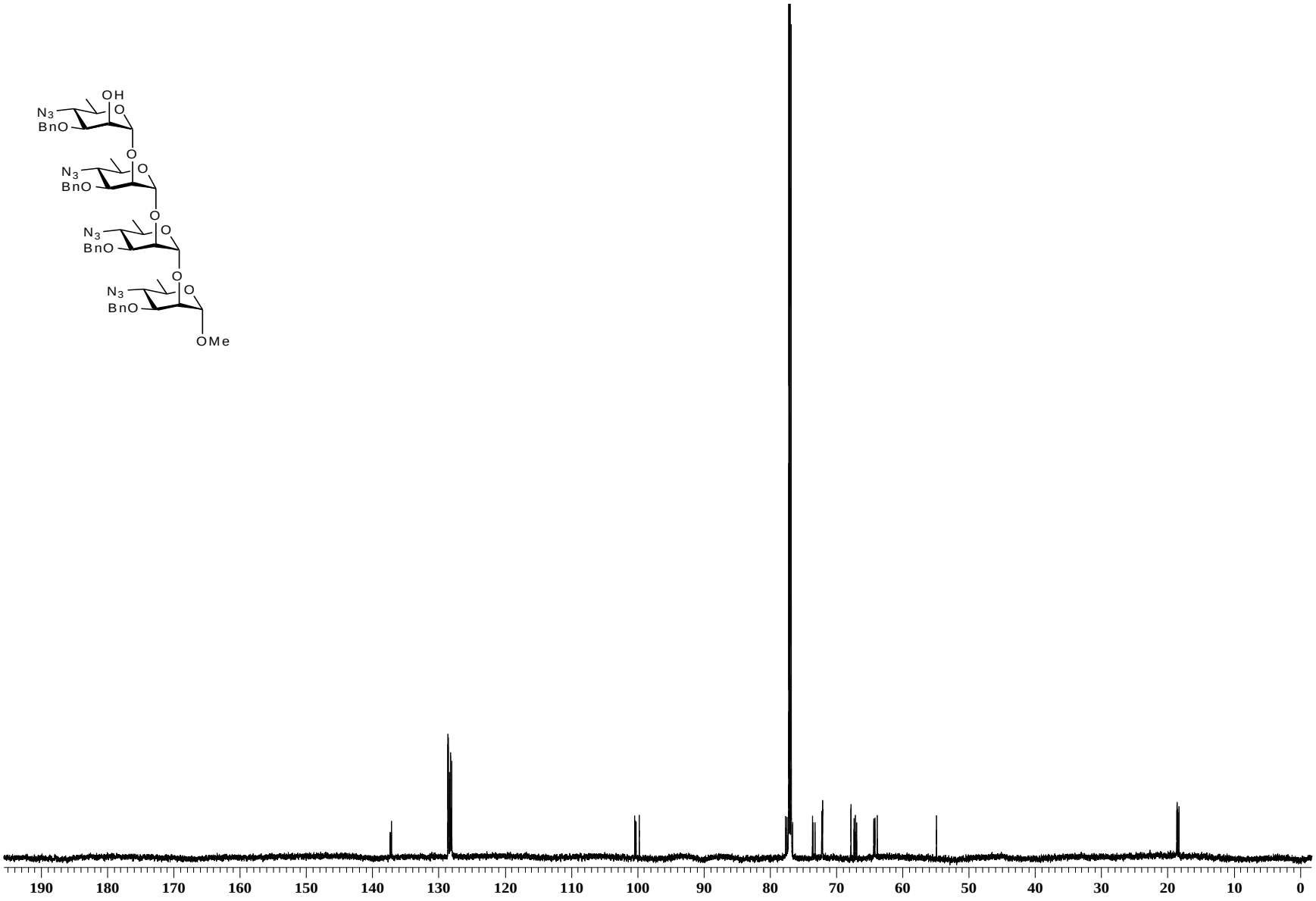
^{1}H NMR Spectrum of compound 18 ($CDCl_3$, 700 MHz).



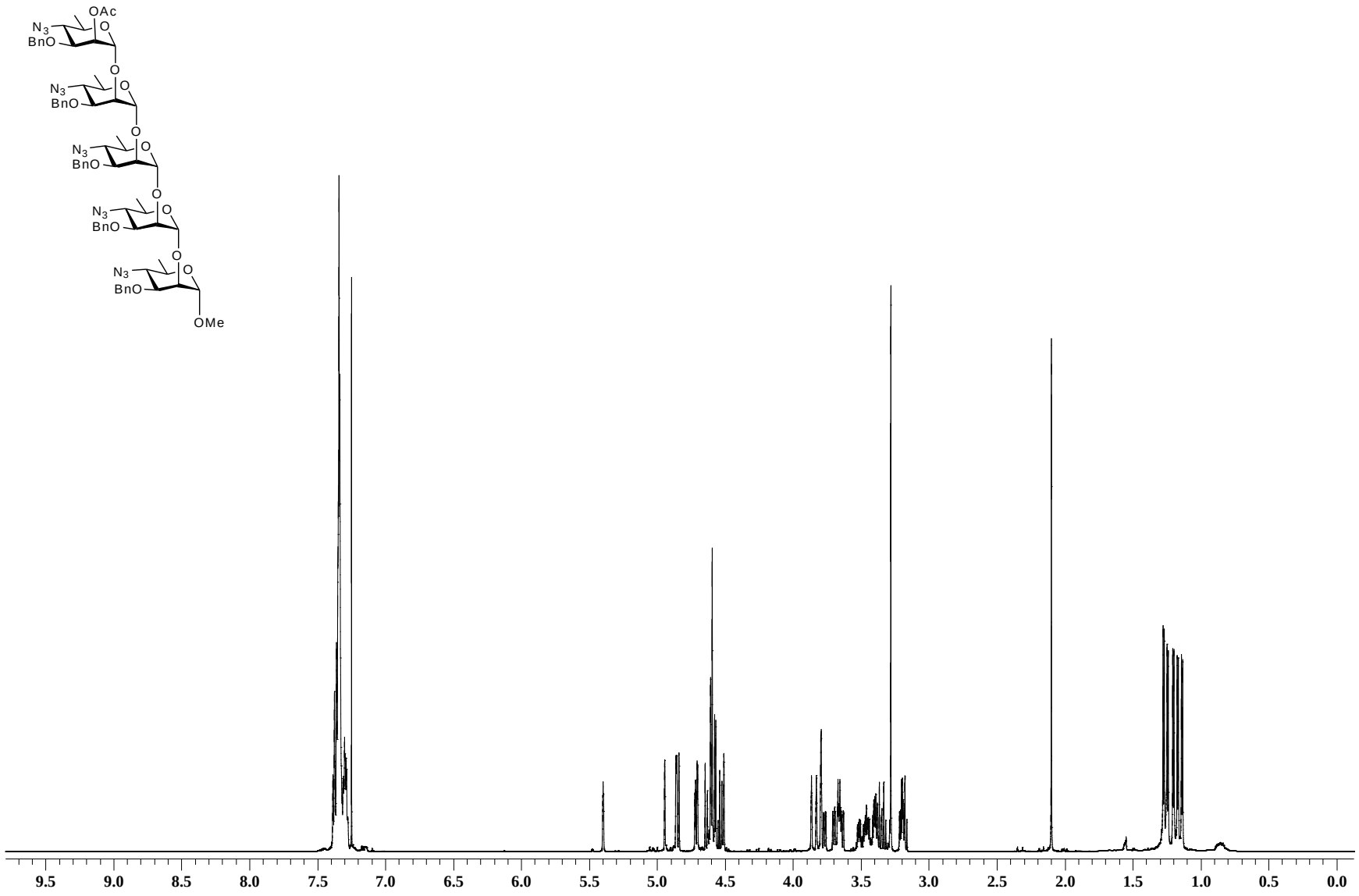
^{13}C NMR Spectrum of compound 18 ($CDCl_3$, 176 MHz).



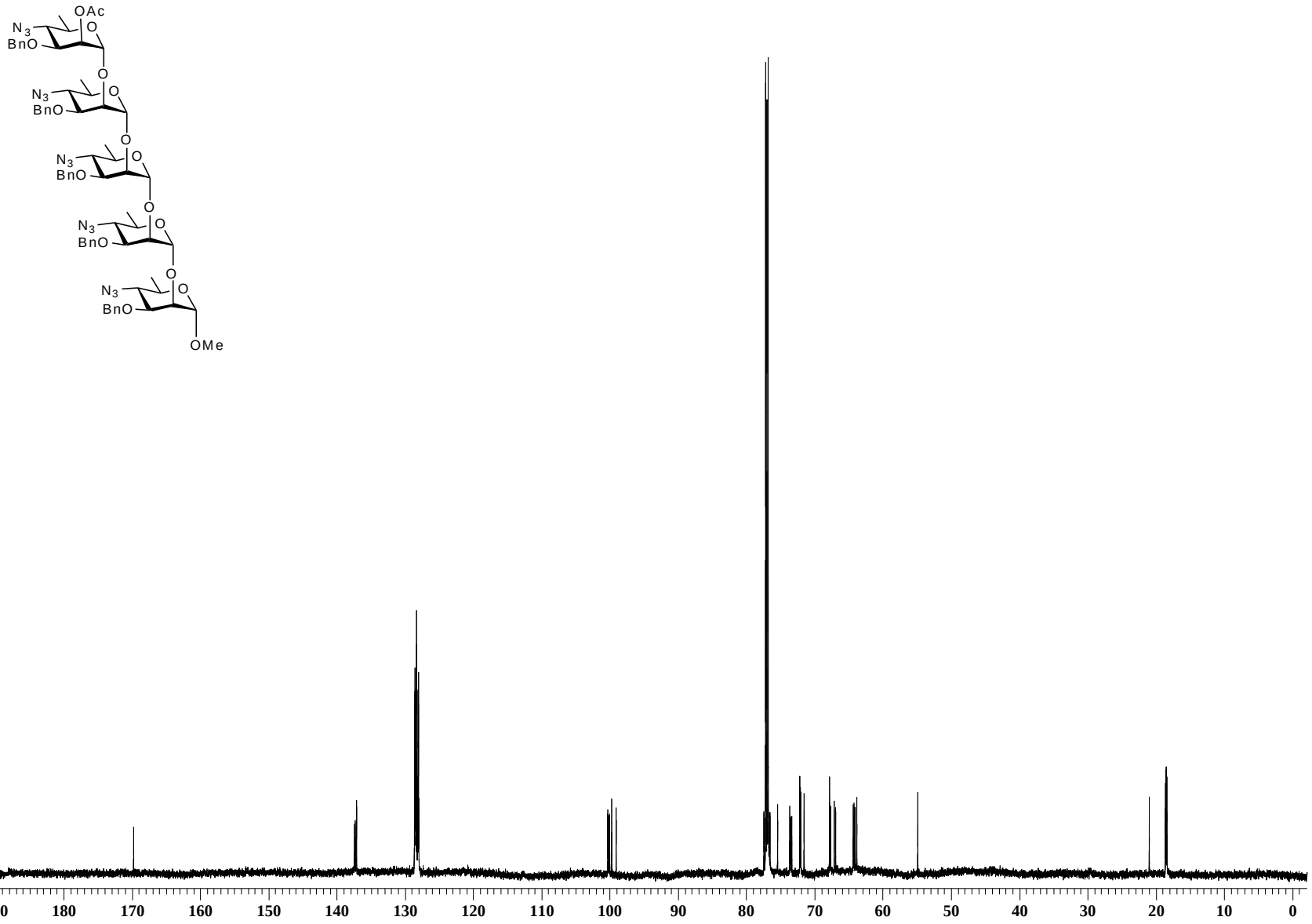
^{1}H NMR Spectrum of compound 19 ($CDCl_3$, 700 MHz).



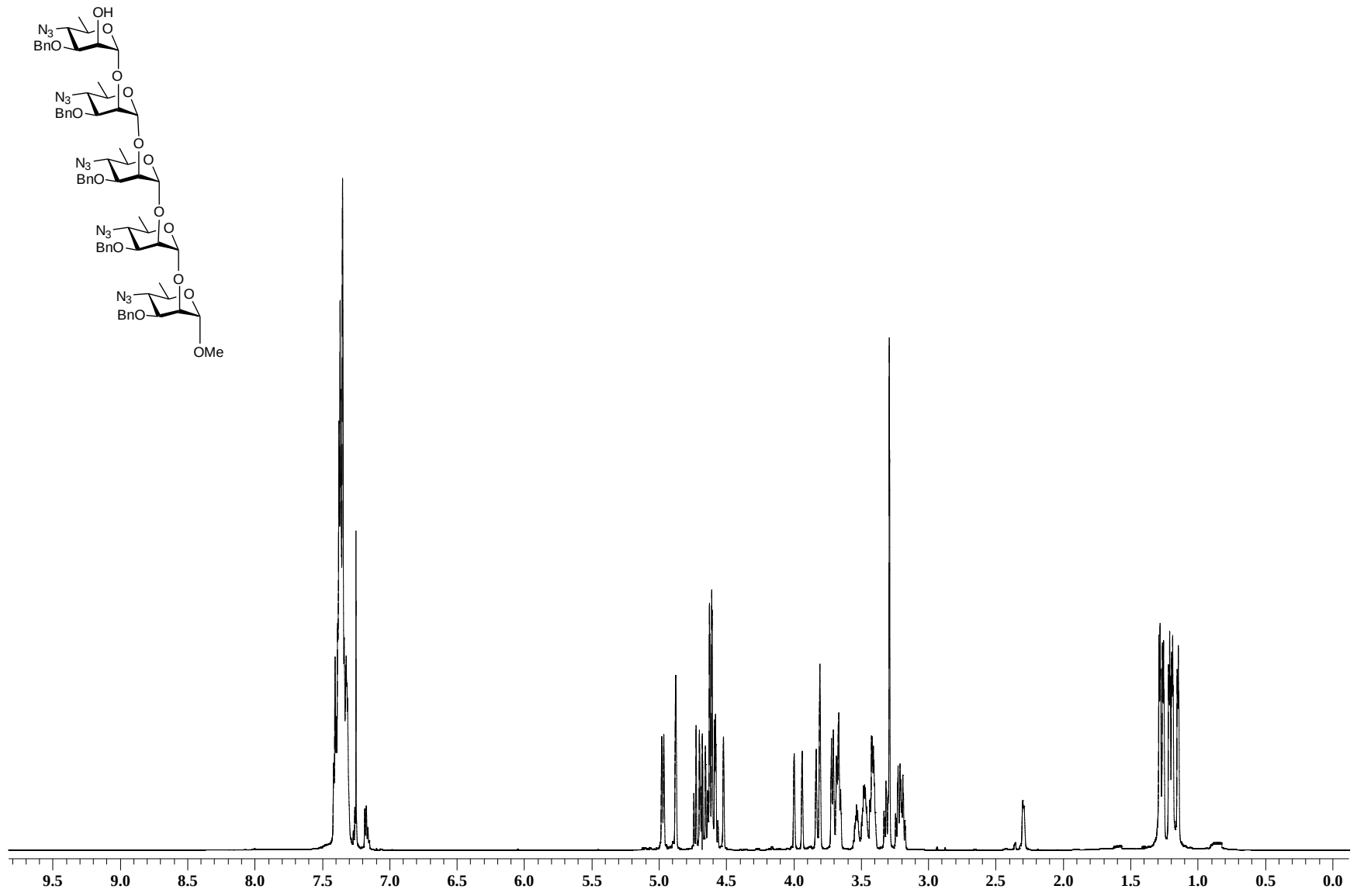
^{13}C NMR Spectrum of compound 19 ($CDCl_3$, 176 MHz).



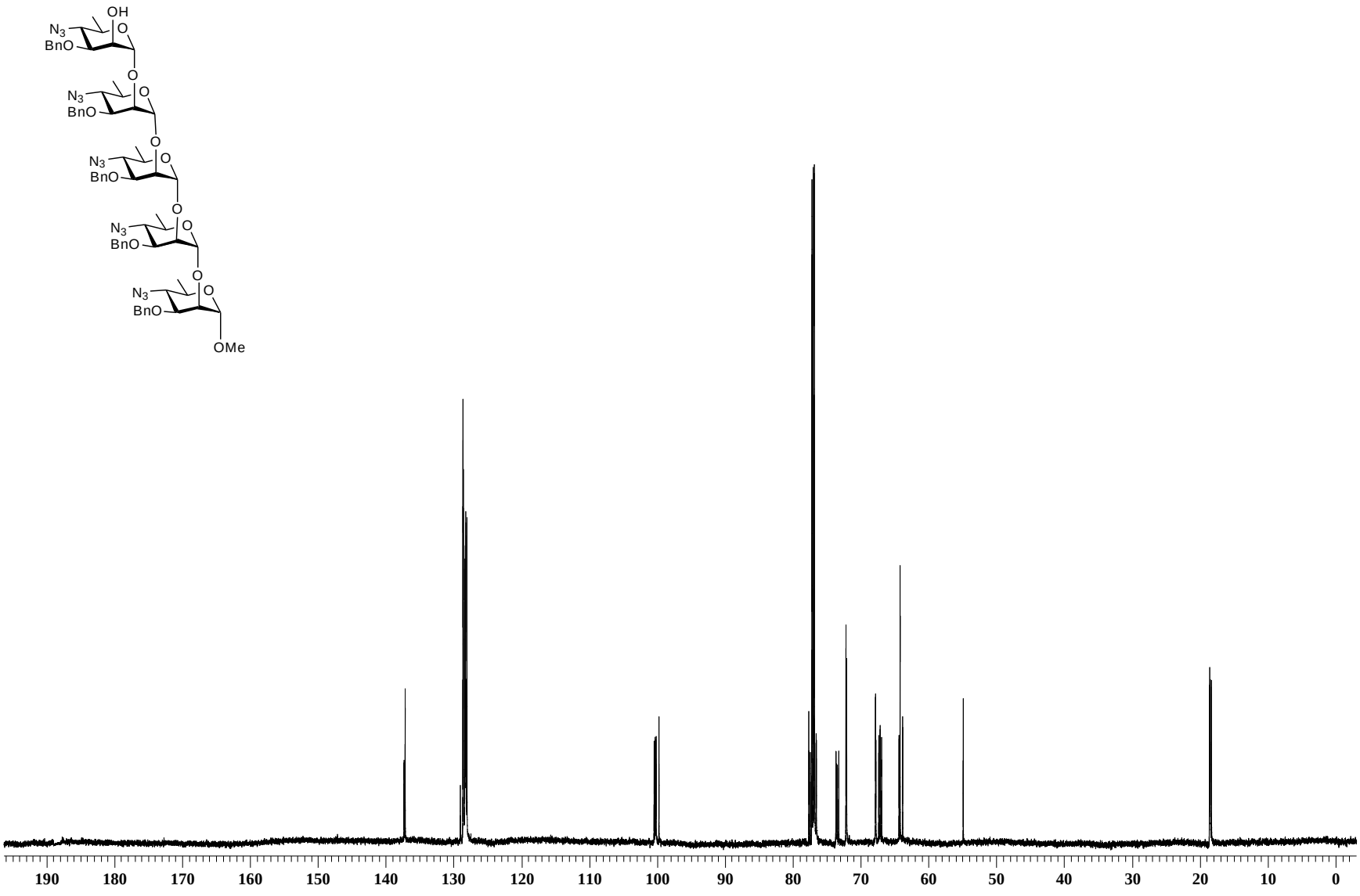
^{1}H NMR Spectrum of compound 20 ($CDCl_3$, 700 MHz).



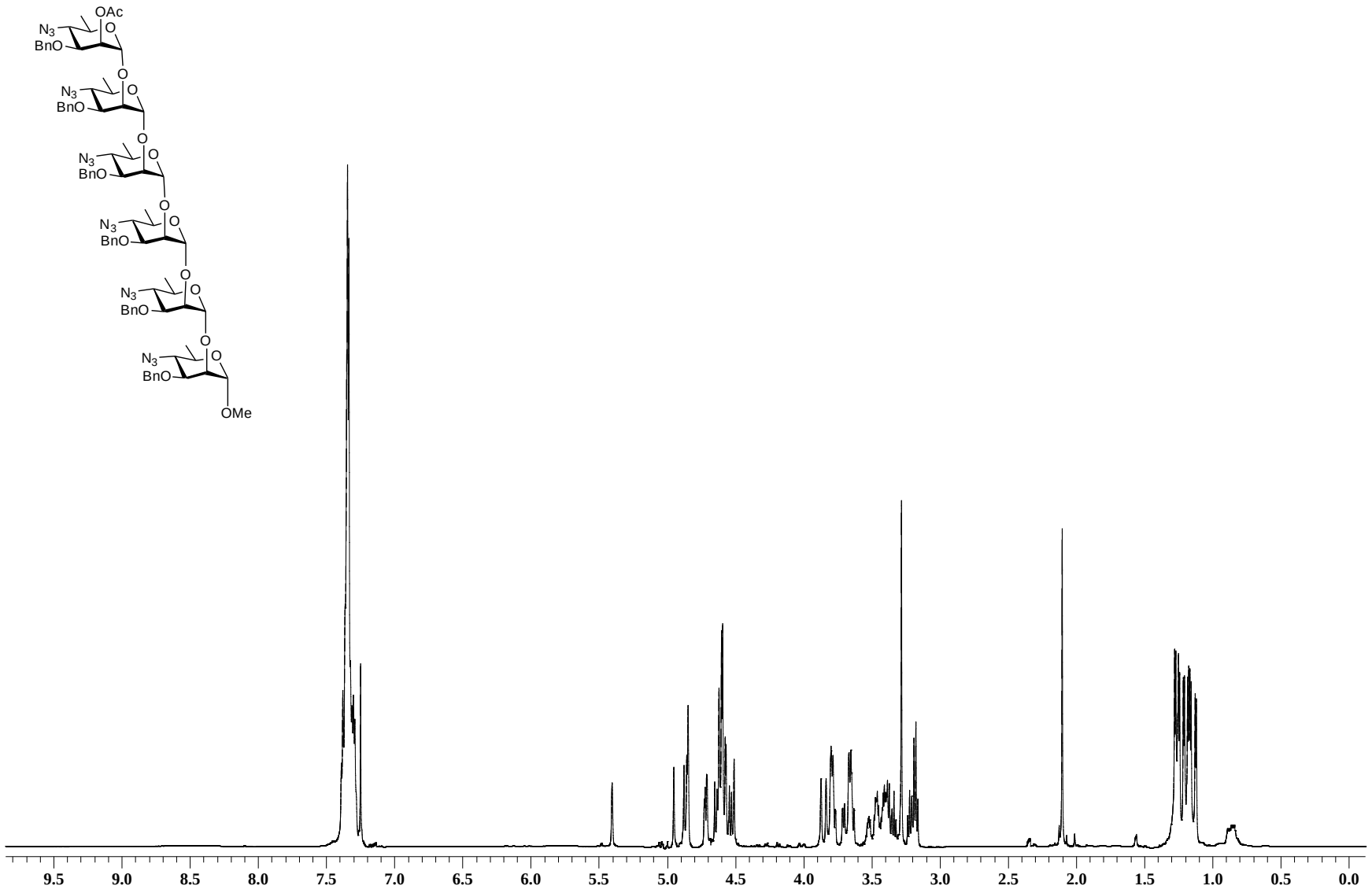
^{13}C NMR Spectrum of compound 20 ($CDCl_3$, 176 MHz).



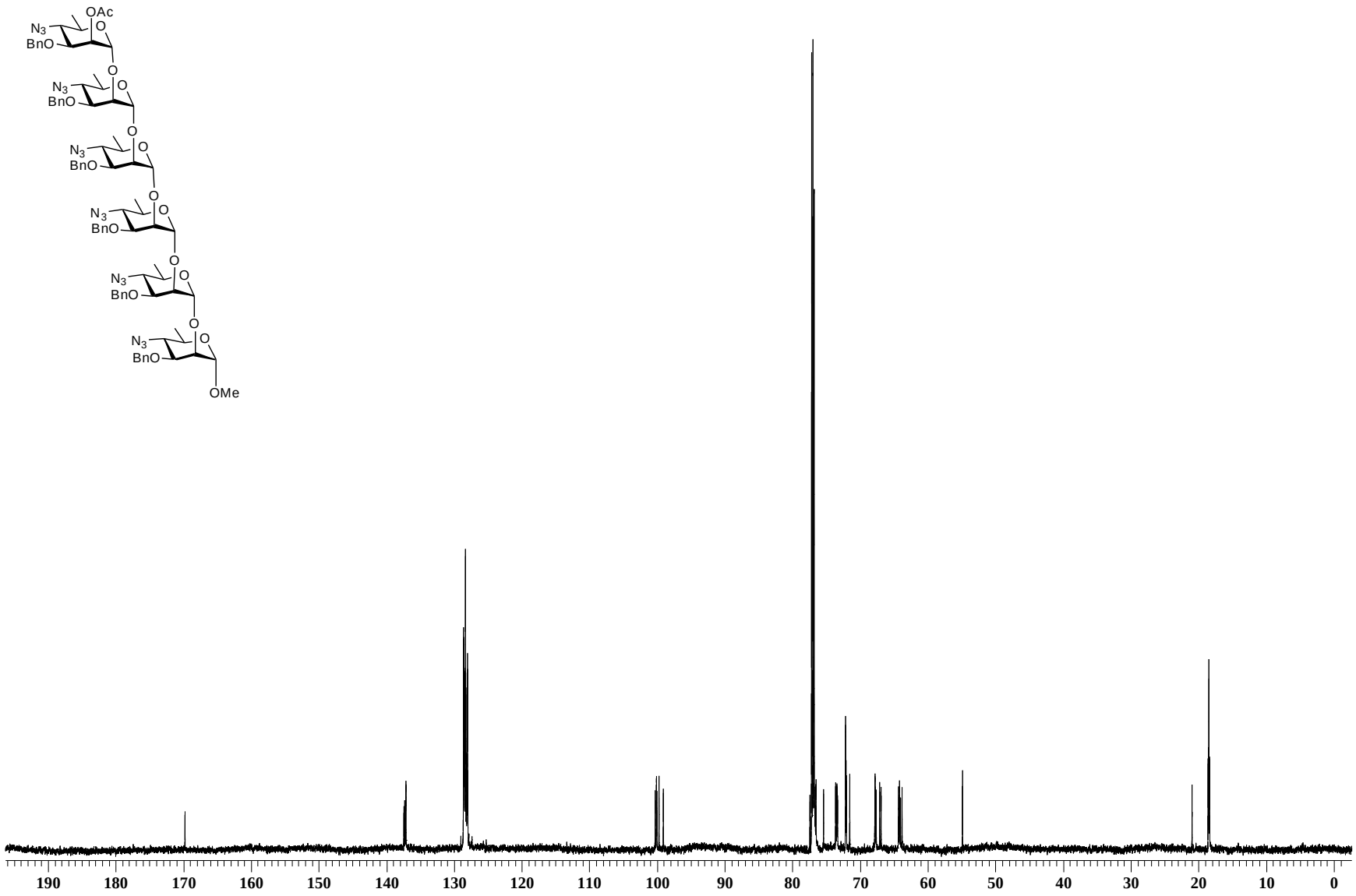
^{1}H NMR Spectrum of compound 21 ($CDCl_3$, 700 MHz).



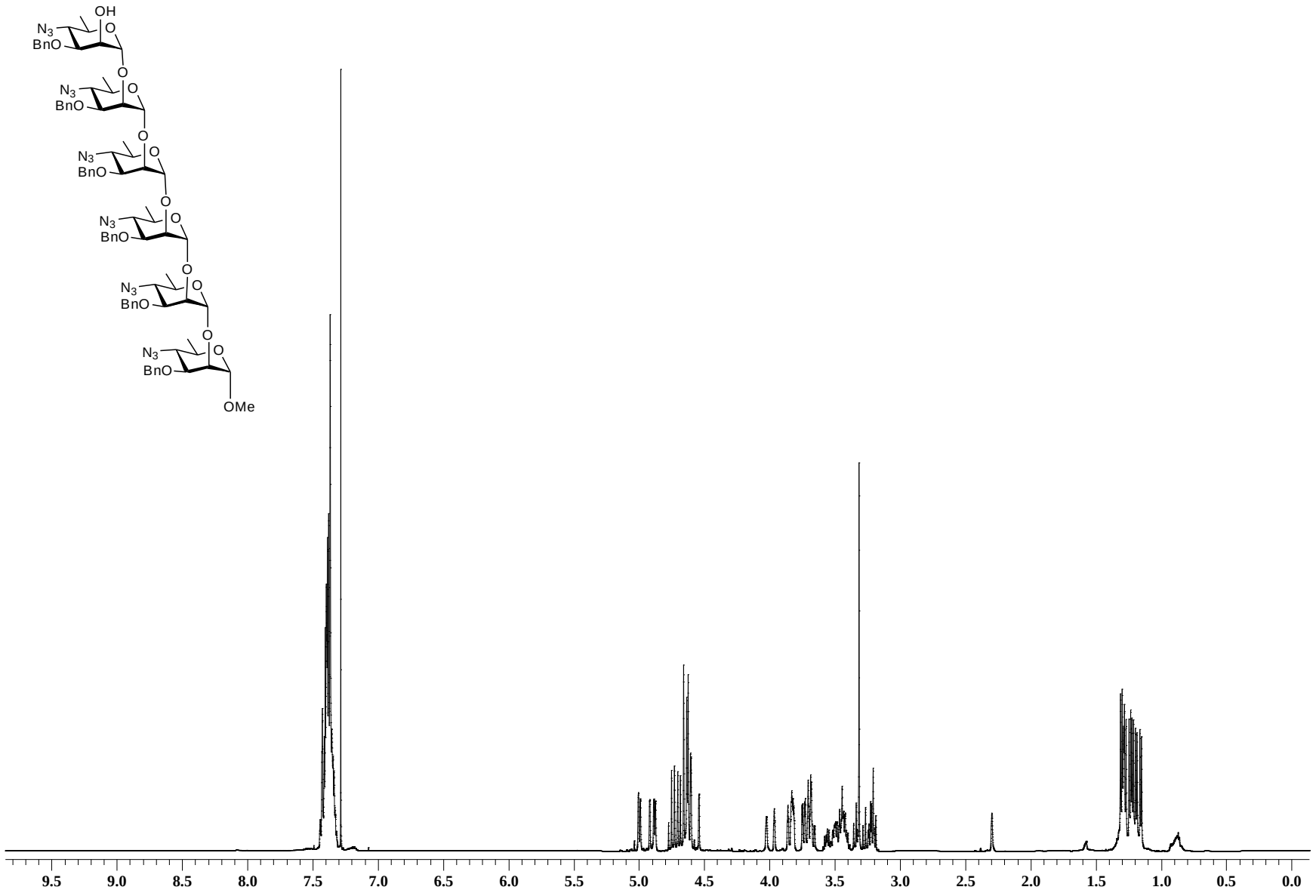
^{13}C NMR Spectrum of compound 21 ($CDCl_3$, 176 MHz).



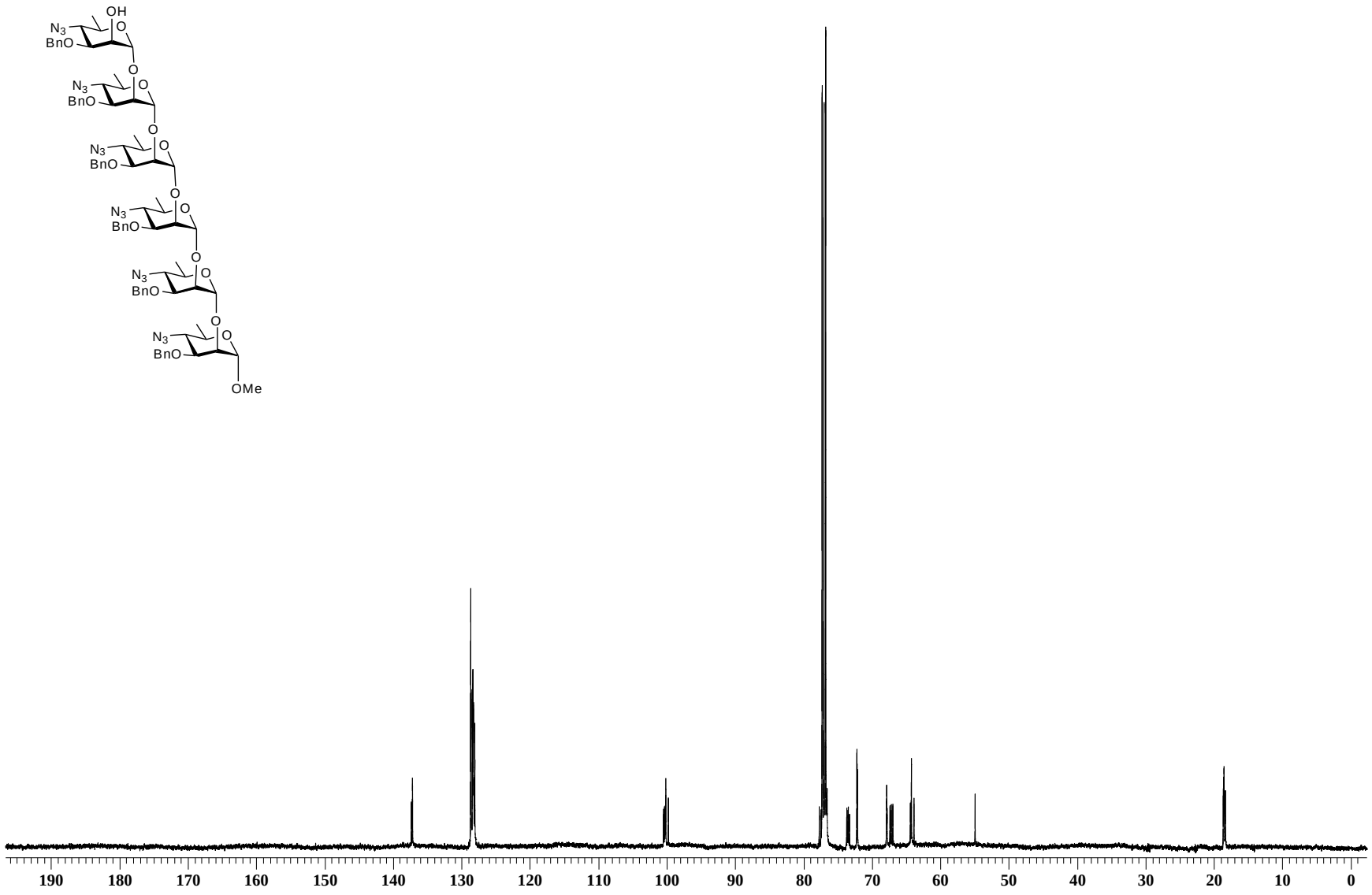
^{1}H NMR Spectrum of compound 22 ($CDCl_3$, 700 MHz).



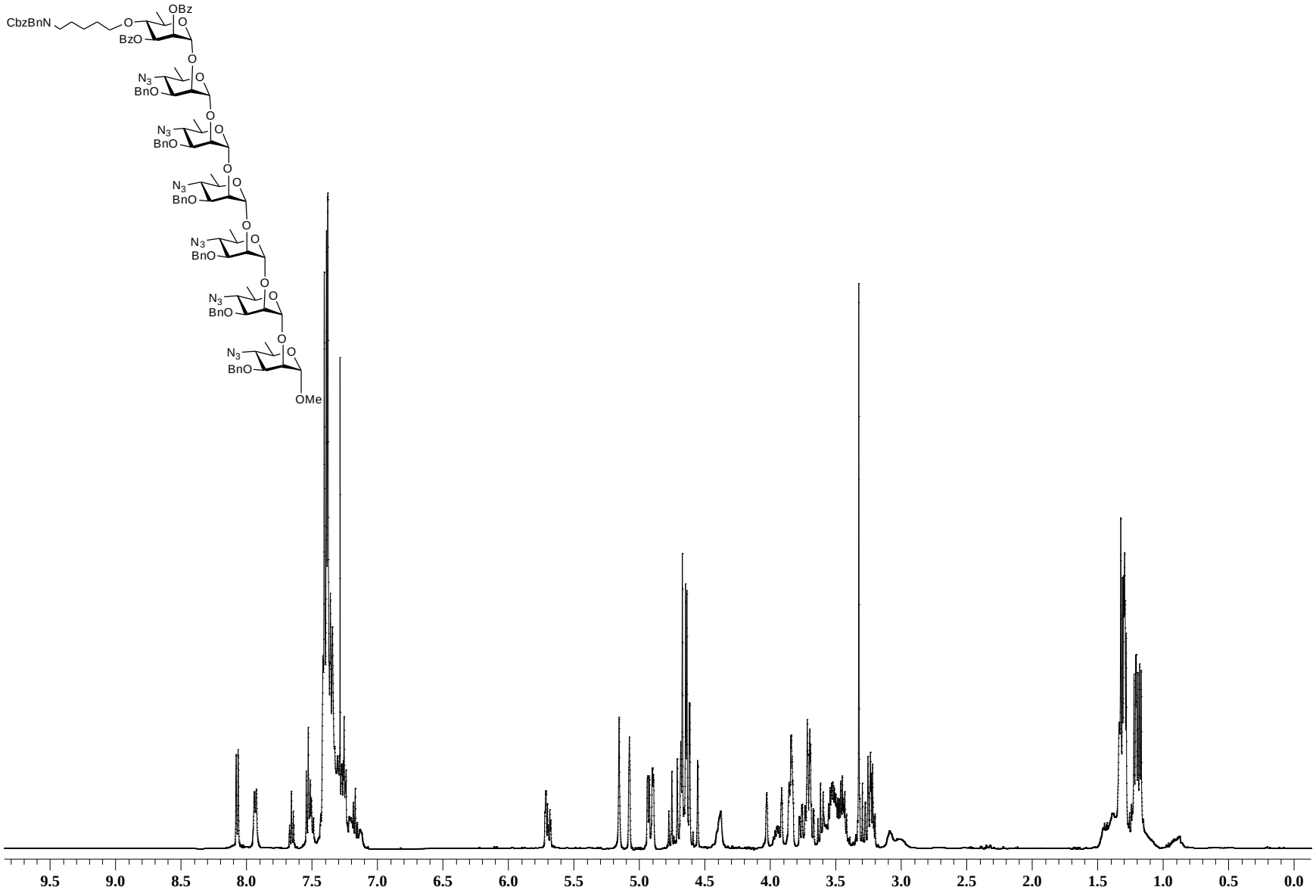
^{13}C NMR Spectrum of compound 22 ($CDCl_3$, 176 MHz).



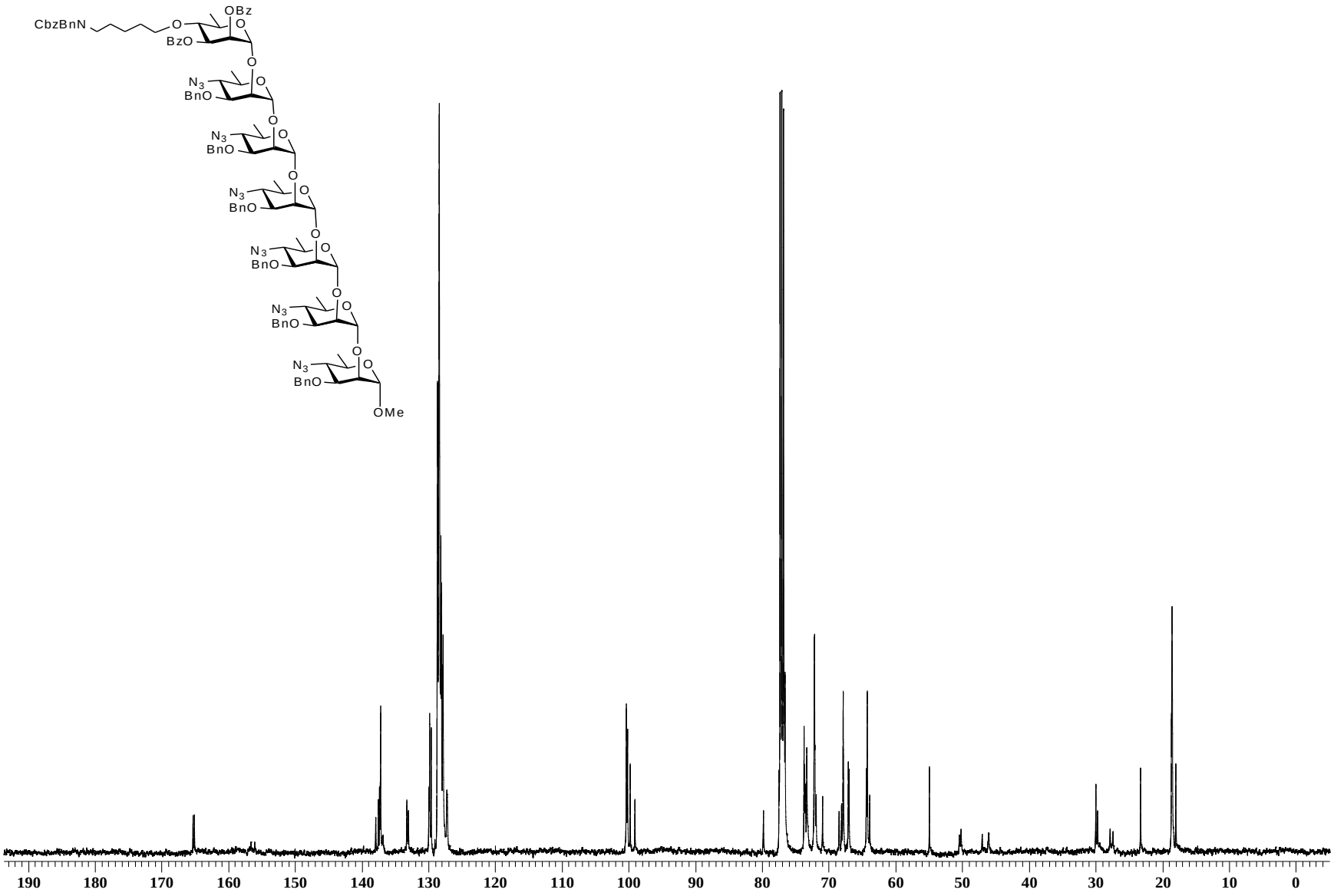
^{1}H NMR Spectrum of compound 23 ($CDCl_3$, 500 MHz).



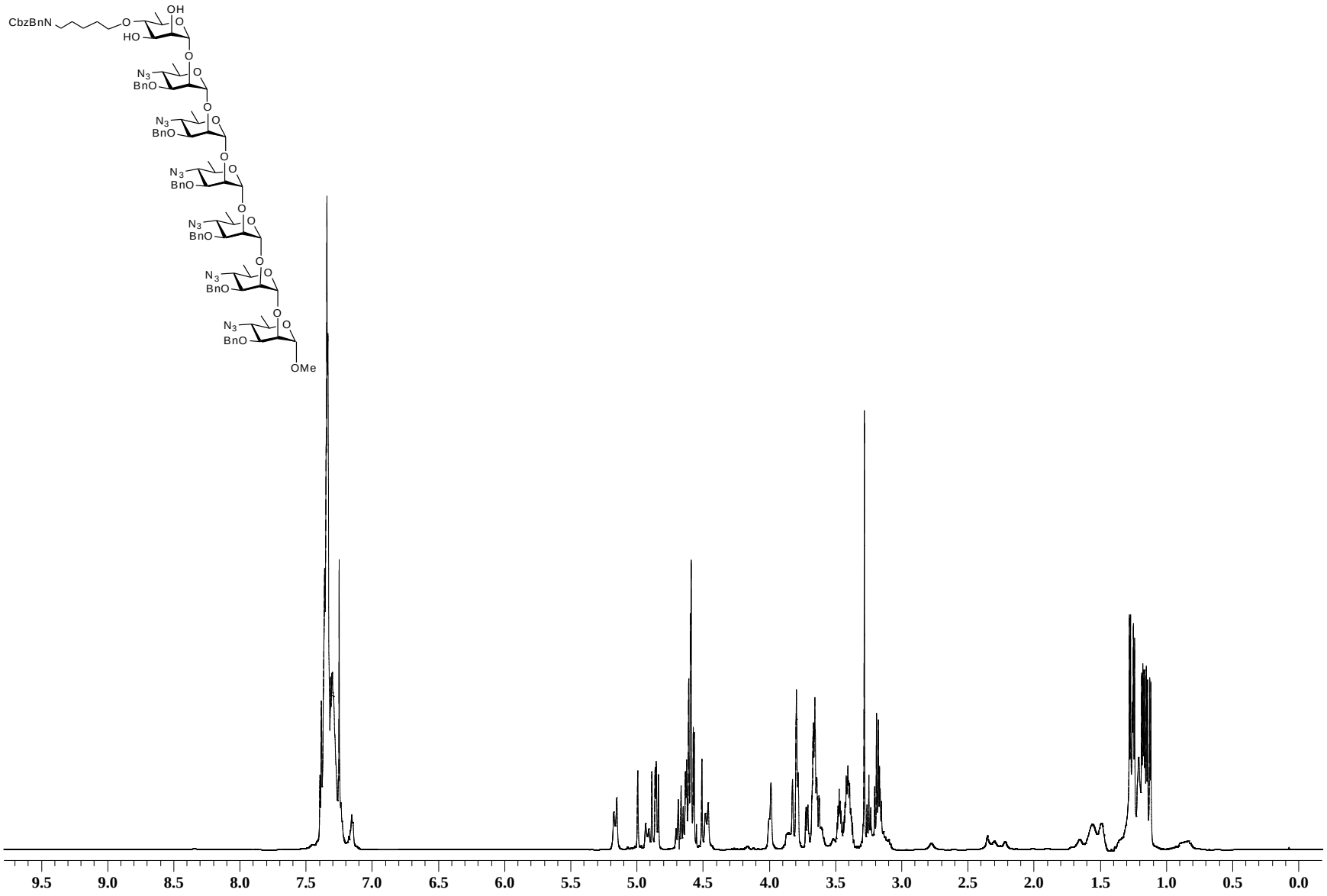
^{13}C NMR Spectrum of compound 23 ($CDCl_3$, 126 MHz).



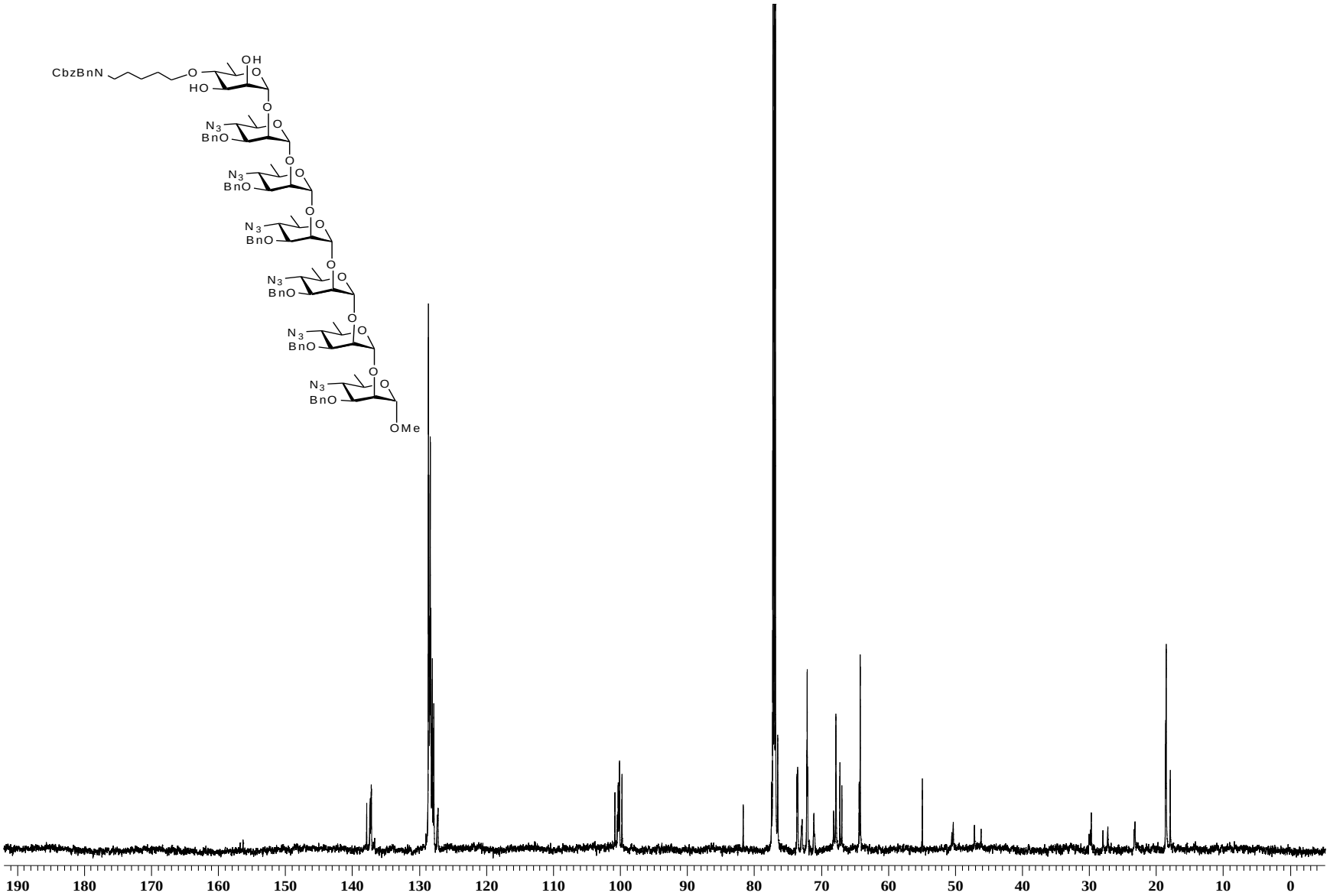
^{1}H NMR Spectrum of compound 24 ($CDCl_3$, 500 MHz).



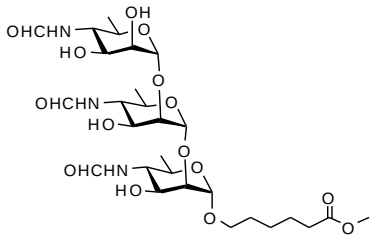
^{13}C NMR Spectrum of compound 24 ($CDCl_3$, 126 MHz).



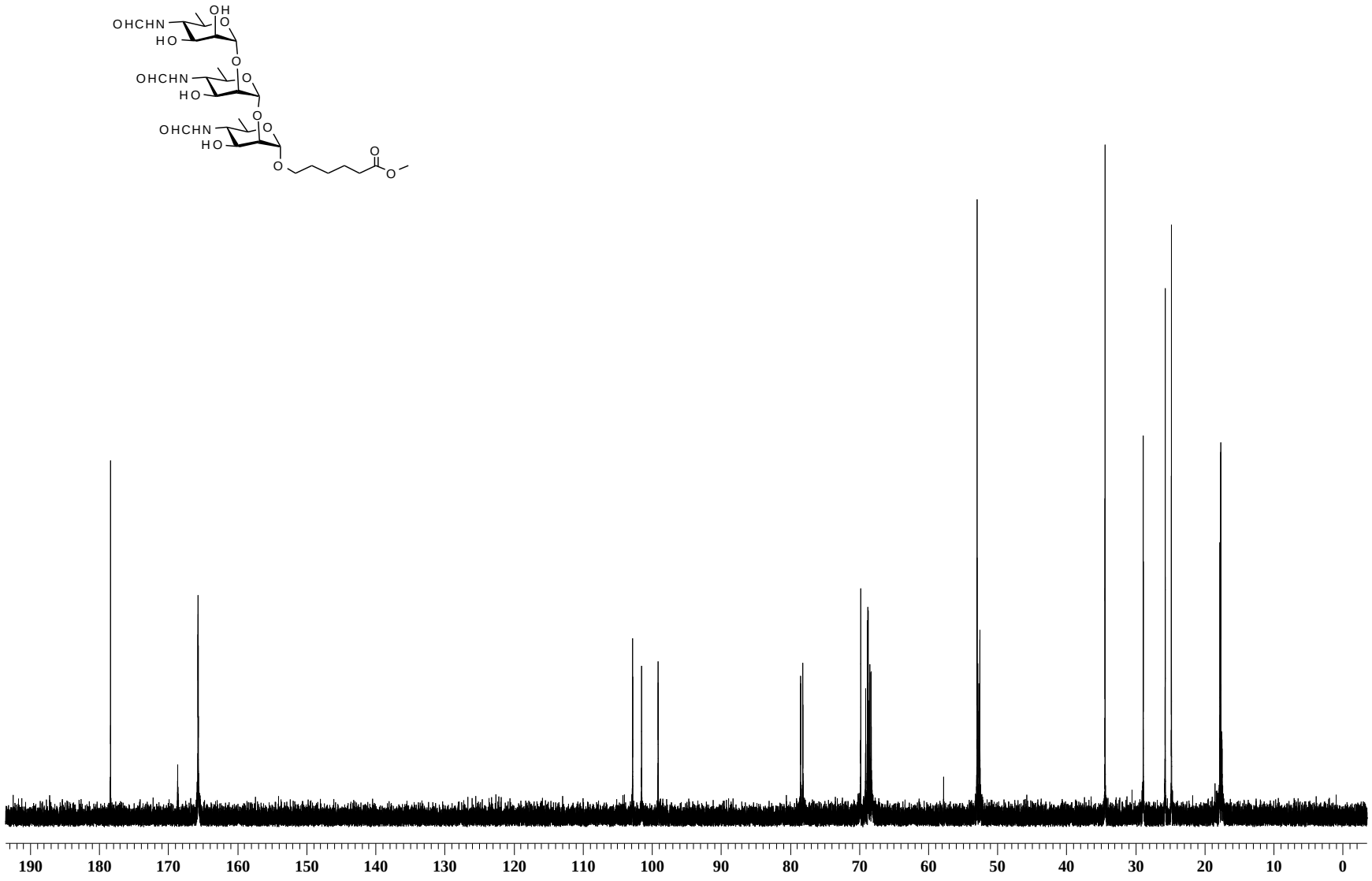
^{1}H NMR Spectrum of compound 25 ($CDCl_3$, 700 MHz).



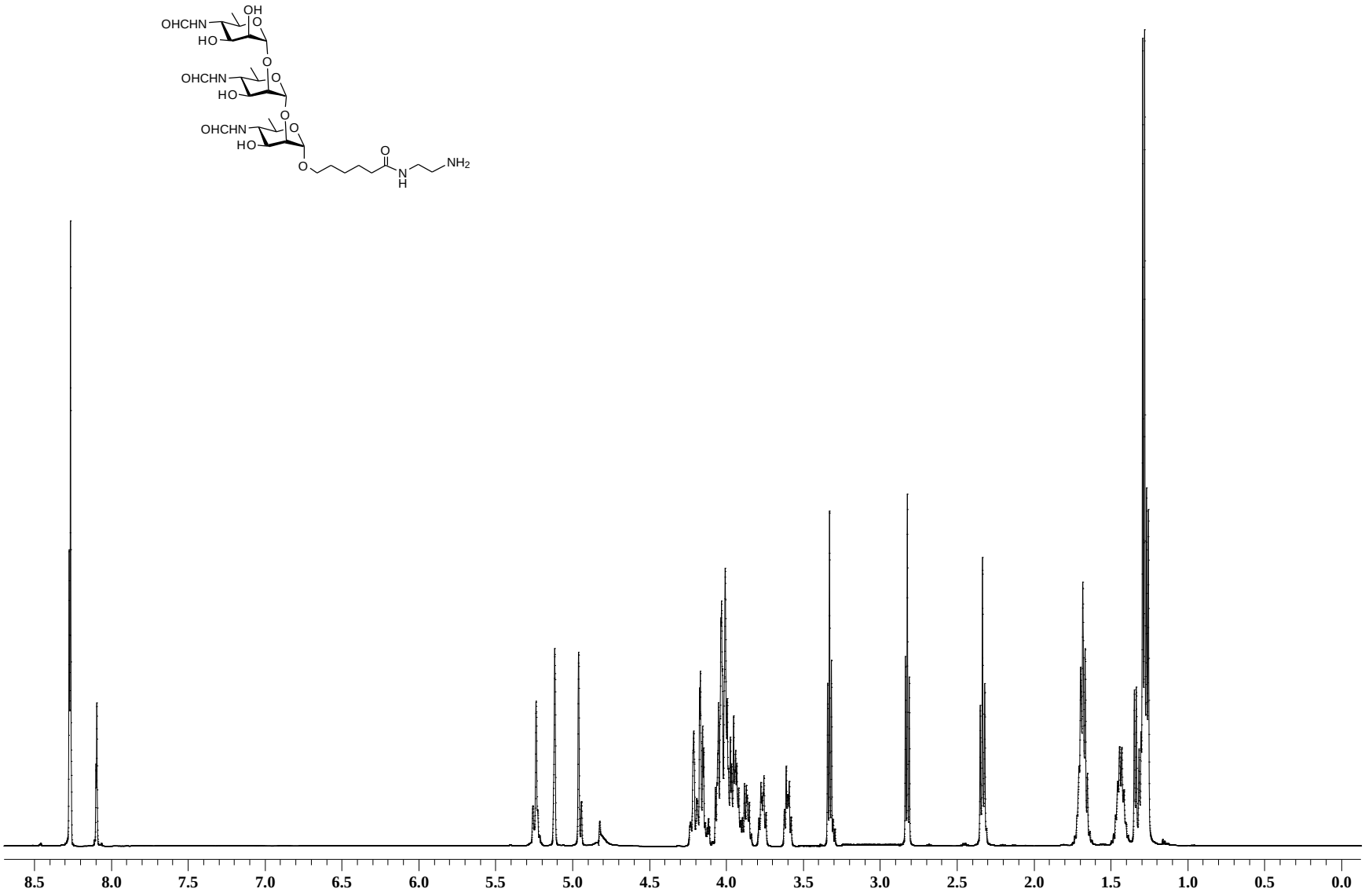
^{13}C NMR Spectrum of compound 25 ($CDCl_3$, 176 MHz).



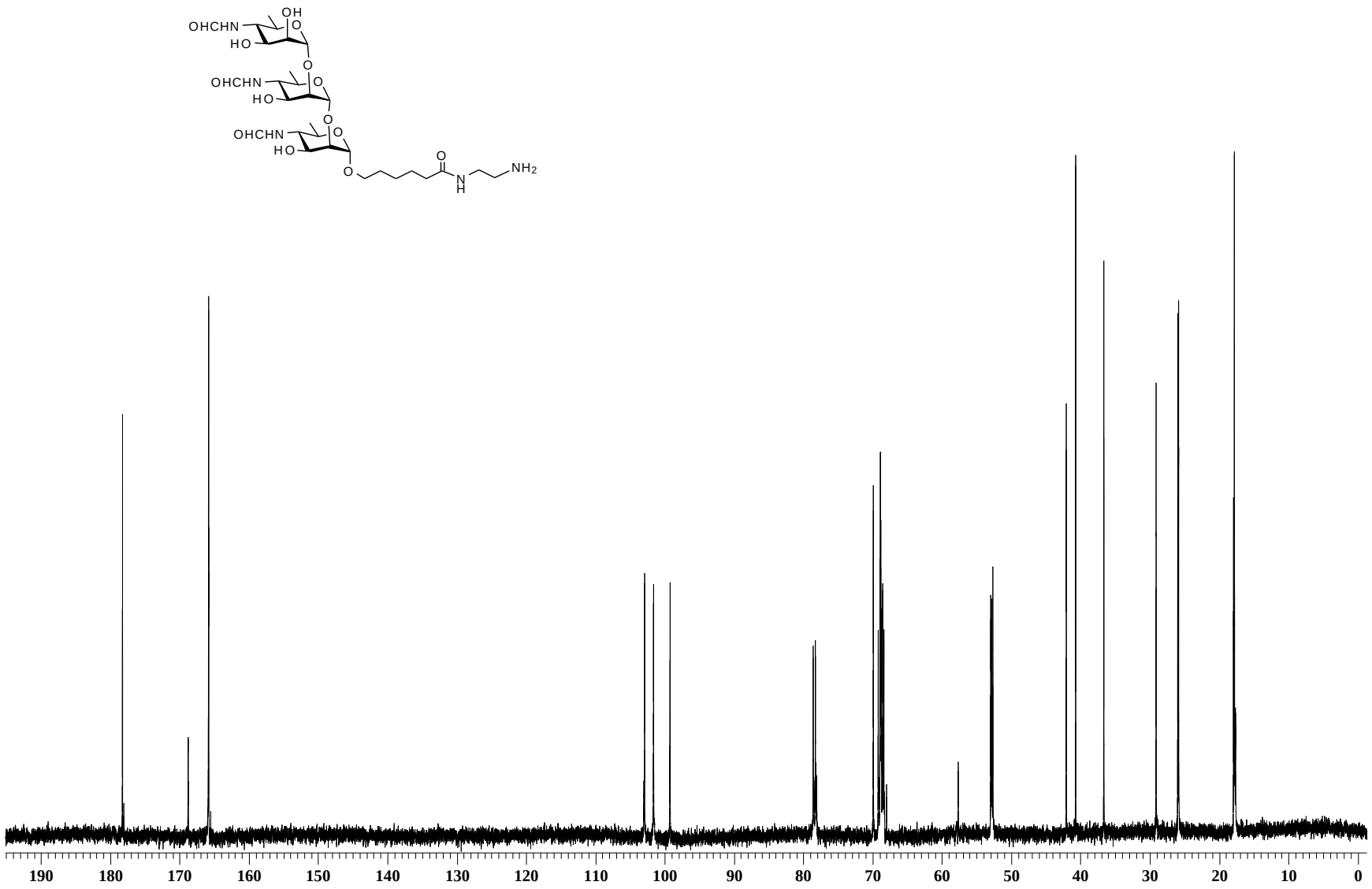
^{1}H NMR Spectrum of compound 4 (D_2O, 700 MHz).



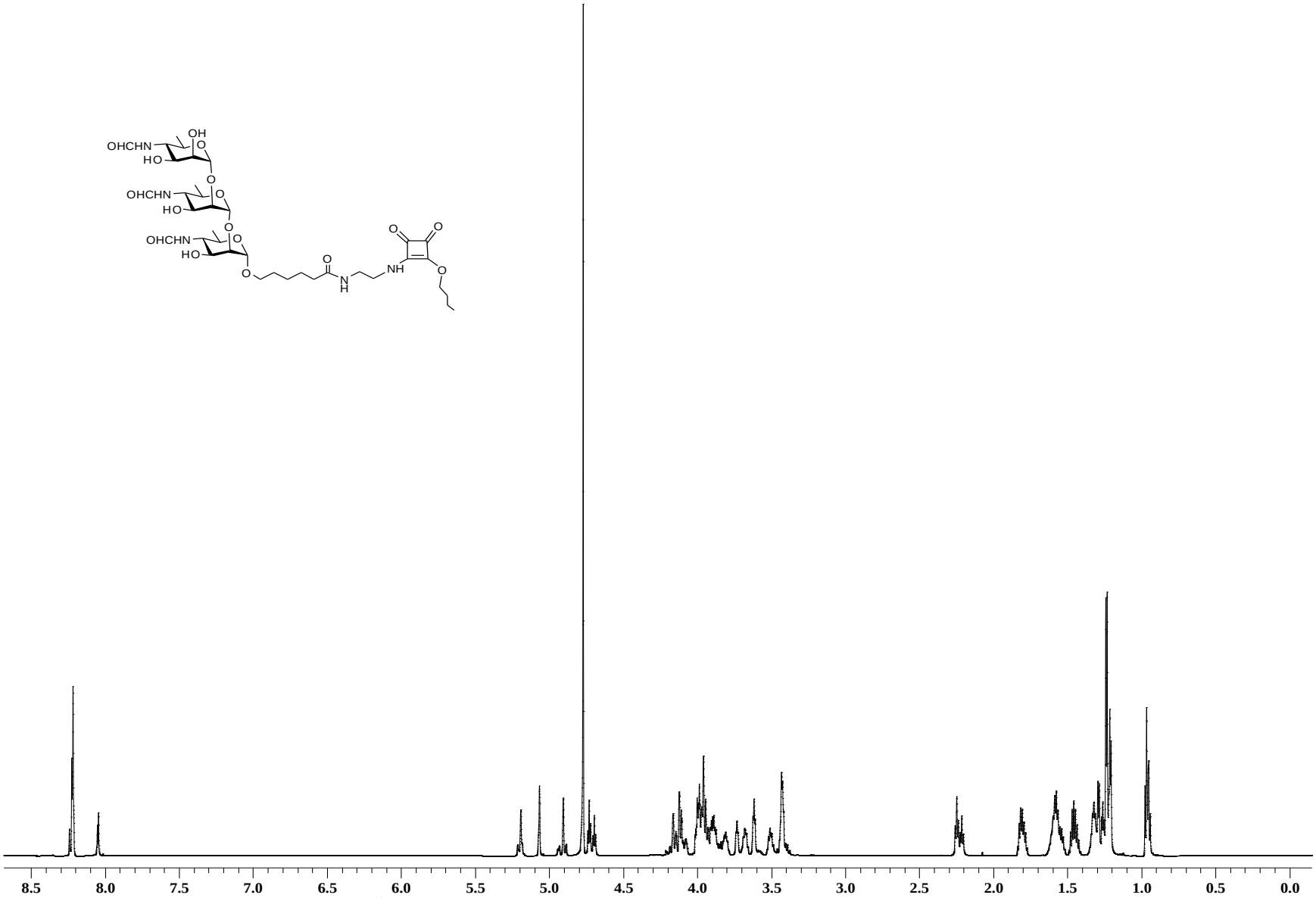
^{13}C NMR Spectrum of compound 4 (D_2O, 176 MHz).



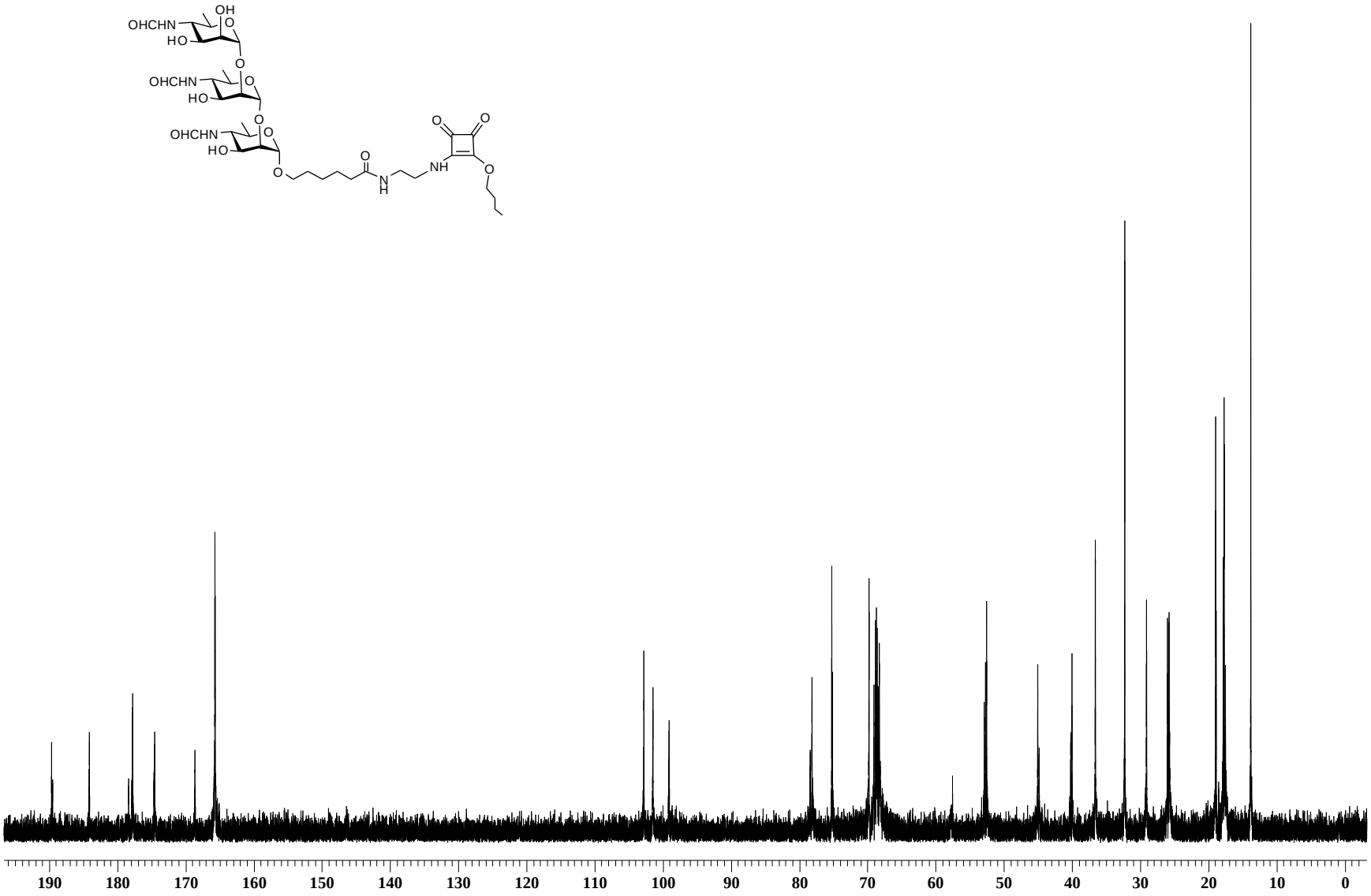
^{1}H NMR Spectrum of compound S24 (D_2O, 500 MHz).



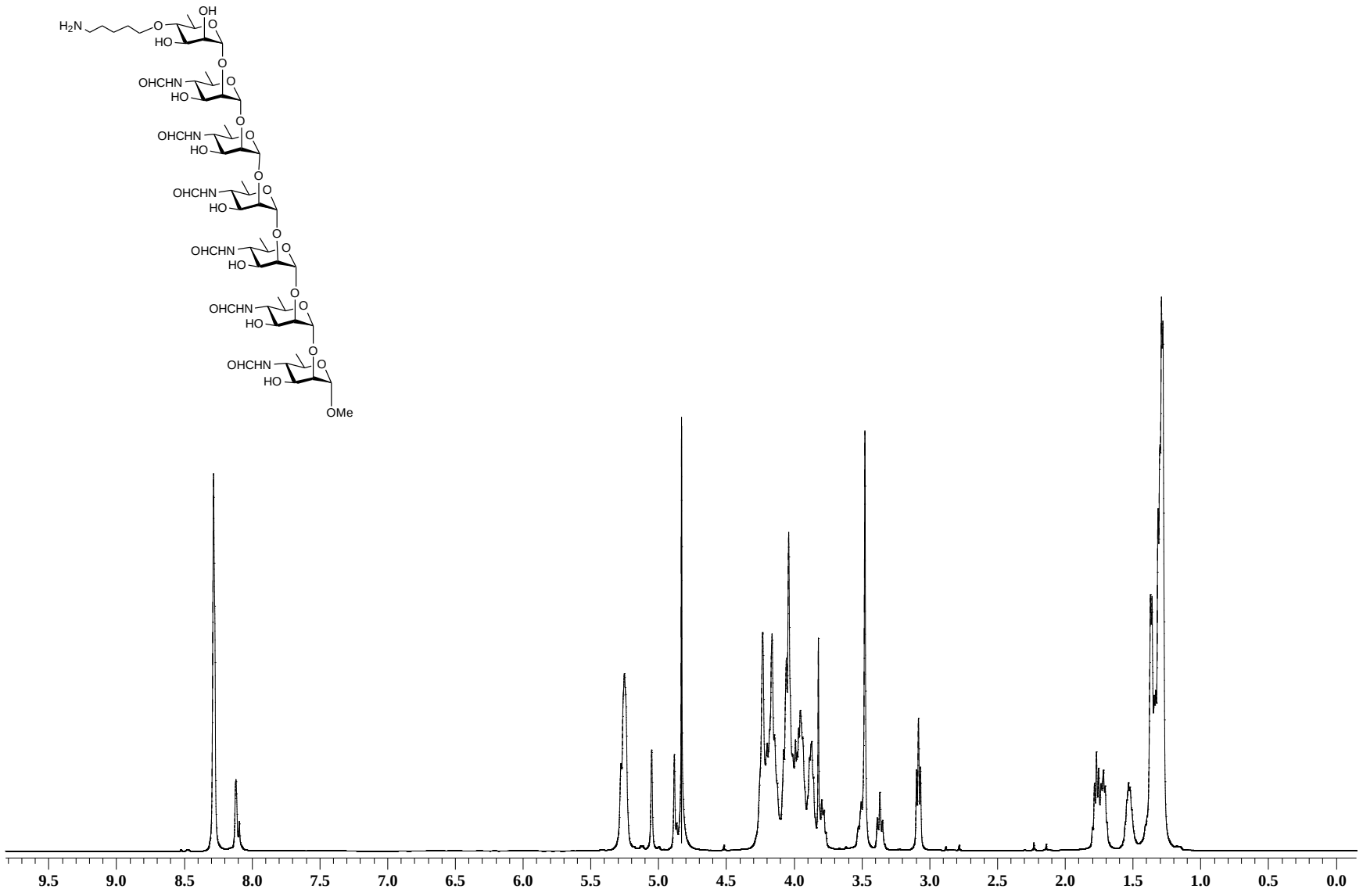
^{13}C NMR Spectrum of compound S24 (D_2O, 126 MHz).



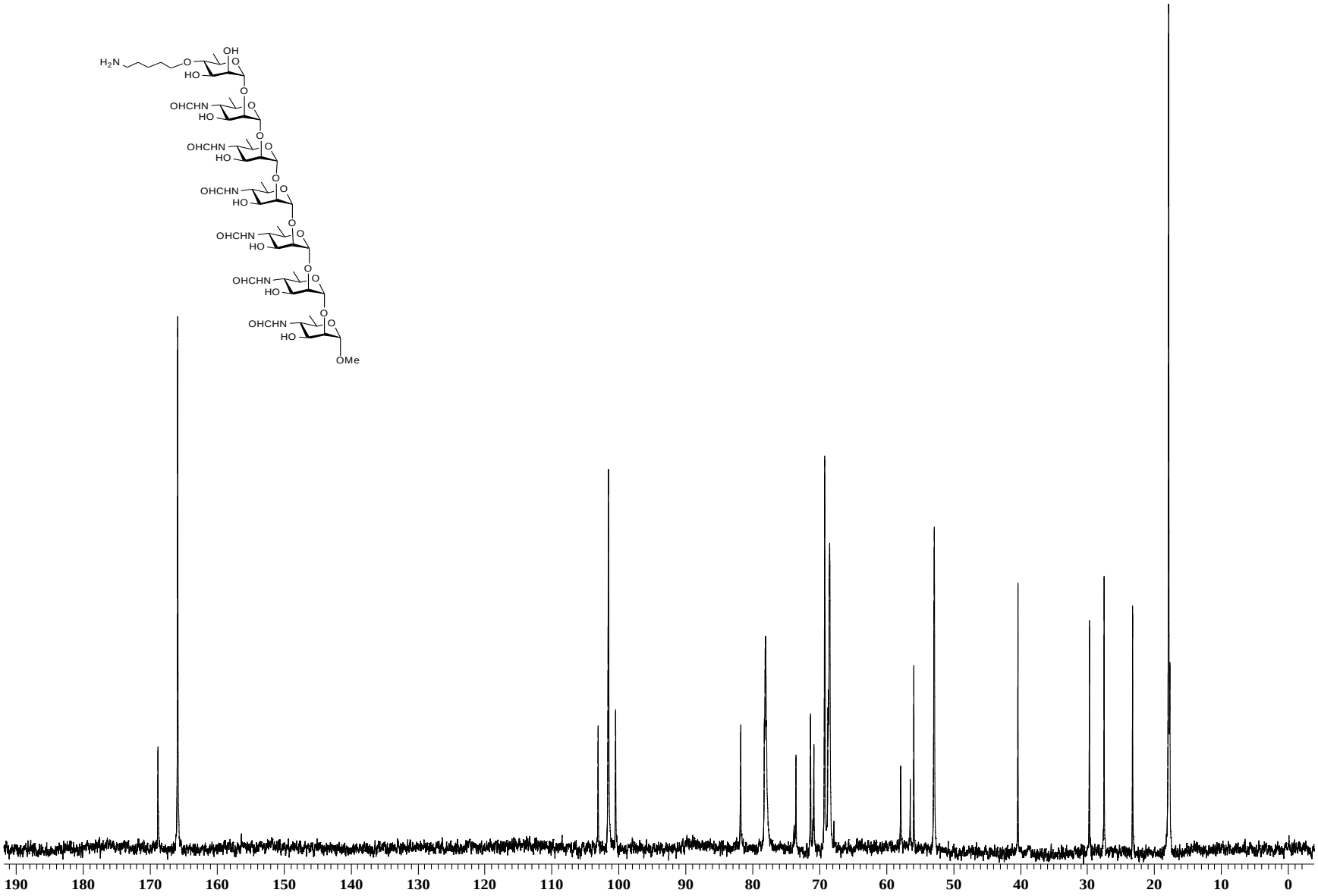
^{1}H NMR Spectrum of compound S25 (D_2O, 700 MHz).



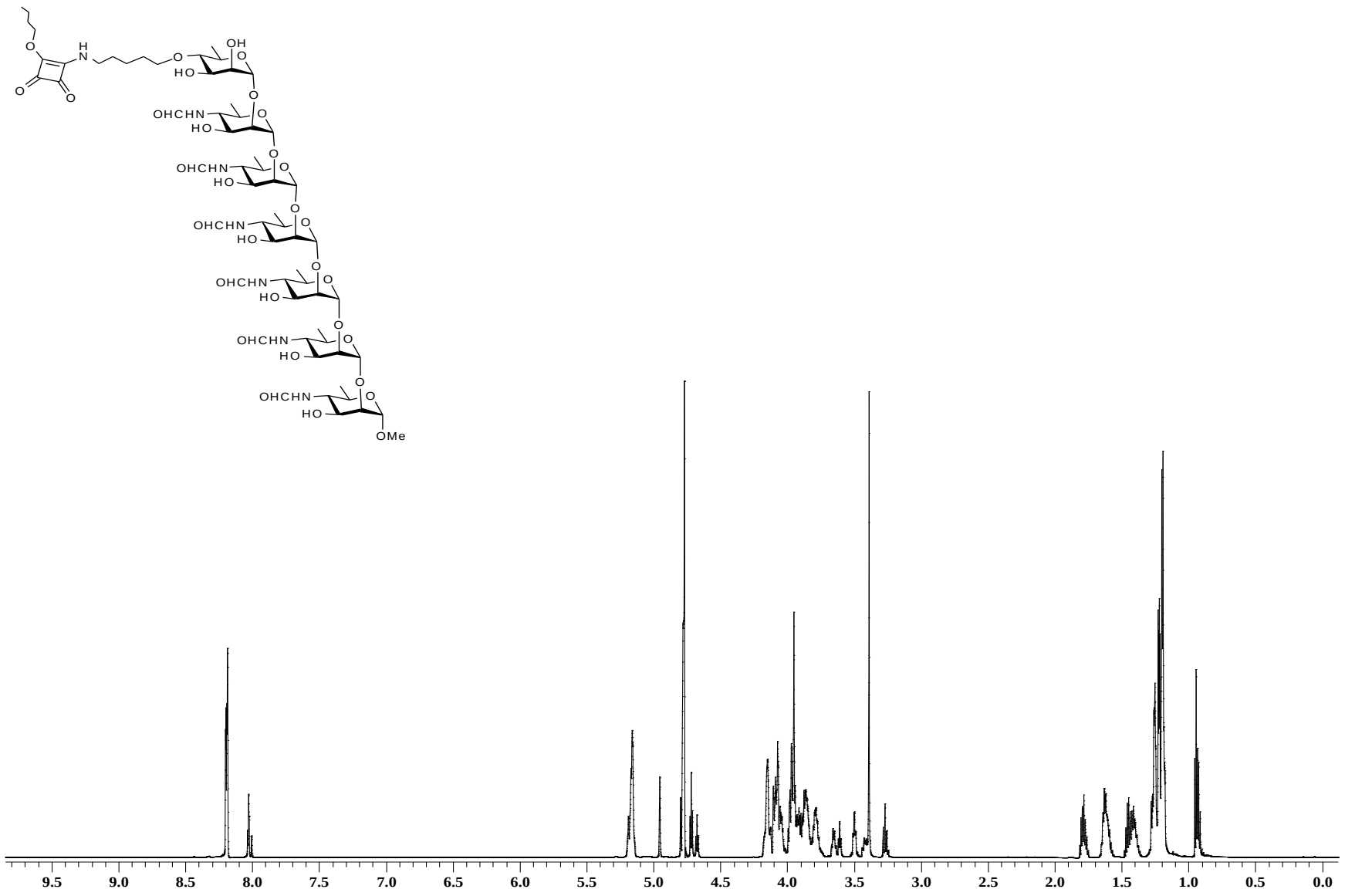
^{13}C Spectrum of compound S25 (D_2O, 176 MHz)



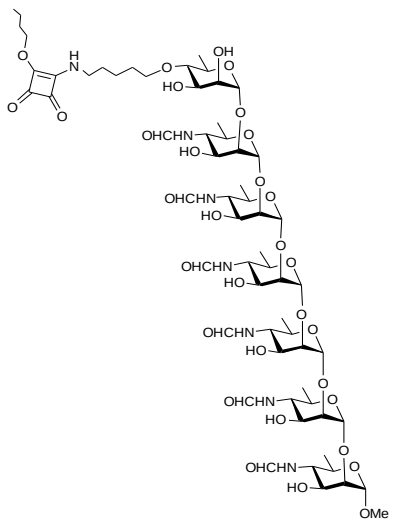
^{1}H NMR Spectrum of compound 8 (D_2O, 500 MHz).

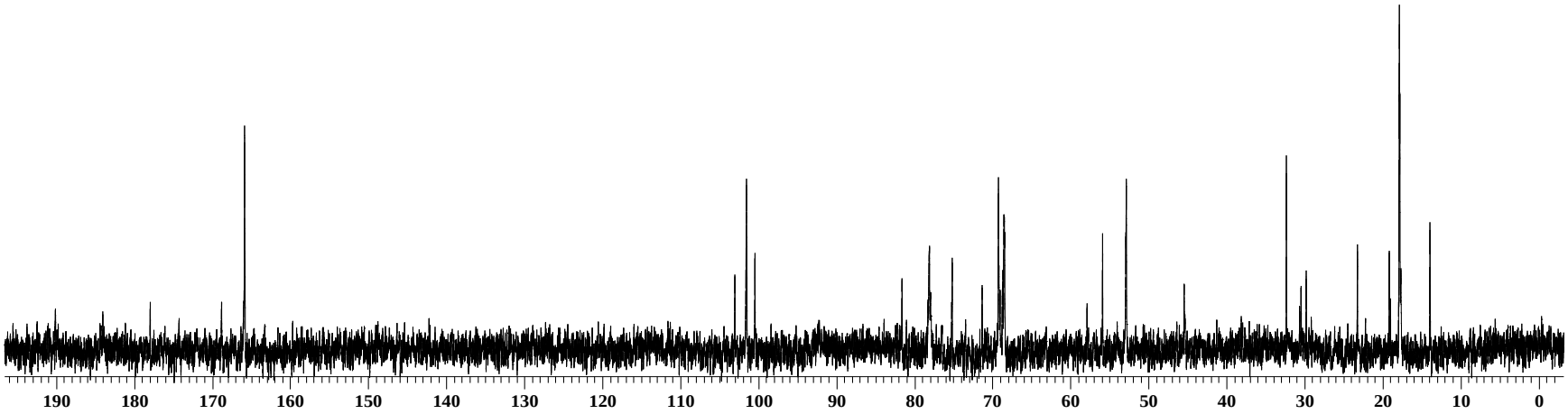


^{13}C NMR Spectrum of compound 8 (D_2O, 126 MHz).



^{1}H NMR Spectrum of compound S27 (D_2O, 500 MHz).





^{13}C NMR Spectrum of compound S27 (D_2O, 126 MHz).