First synthesis of *C. difficile* PS-II cell wall polysaccharide repeating unit

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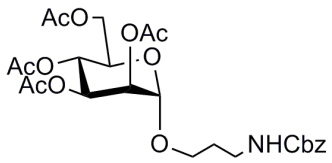
General Methods

All chemicals were of reagent grade, and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} (Sigma Aldrich); after exam under UV light, compounds were visualized by heating with 10% (v/v) ethanolic H_2SO_4. In the work up procedures, organic solutions were washed with the amounts of the indicated aqueous solutions, then dried with anhydrous Na_2SO_4, and concentrated under reduced pressure at 30–50ºC on a water bath. Column chromatography was performed on Silica Gel 60 (Sigma Aldrich, 0.040–0.063 nm) or using pre-packed silica cartridges RediSep (Teledyne-Isco, 0.040–0.063 nm) SiliaSep HP (Silicycle, 0.015–0.040 nm) or Supelco (Sigma Aldrich, spherical silica 0.040–0.075 nm). Unless otherwise specified, a gradient 0 $\rightarrow$ 100% of the elution mixture was applied in a Combiflash R_f (Teledyne-Isco) or Spot II (Armen) instrument. Solvent mixtures less polar than those used for TLC were used at the onset of separation. ^{1}H NMR spectra were measured at 400 MHz and 298 K with a Bruker Avance^{III} 400 spectrometer; δ_H values are reported in ppm, relative to internal Me_4Si (δ_H = 0.00, $CDCl_3$); solvent peak for D_2O was calibrated at 4.79 ppm. ^{13}C NMR spectra were measured at 100 MHz and 298 K with a Bruker Avance^{III} 400 spectrometer, except for compound **2**, that was recorded at 150 MHz and 298 K with a Bruker DRX 600 spectrometer; δ_C values are reported in ppm relative to the signal of $CDCl_3$ (δ_C = 77.0, $CDCl_3$) when possible or internal acetone (δ_C = 30.9, D_2O). Assignments of NMR signals were made by homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometer. Assignment of ^{13}C NMR spectra of some compounds was aided by comparison with spectra of related substances reported previously from this laboratory or elsewhere. When reporting assignments of NMR signals, sugar residues in oligosaccharides are indicated with capital letters, uncertain attributions are denoted "/". Nuclei associated with the linker are denoted with a prime. Exact masses were measured by electron spray ionization cut-off spectroscopy, using a Q-Tof *micro* Macromass (Waters) instrument. Structures of these compounds follow unequivocally from the mode of synthesis, NMR data and *m/z* values found in their mass spectra. Optical rotation was measured with a P-2000 Jasco polarimeter. Hydrogenation reactions were performed in a continuous flow reactor H-Cube (Thalesnano) instrument, using packed catalyst

cartridges CatCart.

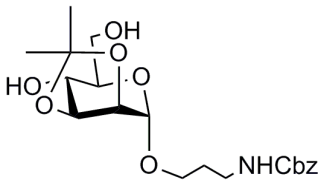
Experimental procedures and characterization of compounds



11

3-(Benzyloxycarbonyl)aminopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 11. Trichloroacedimidate donor **10** (31.00 g, 61 mmol) and 3-(benzyloxycarbonyl)amino propanol (16.00 g, 73 mmol) were dissolved in dry dichloromethane (150 ml), under nitrogen atmosphere, then the mixture was cooled to -10°C and TMSOTf (105 μl, 0.6 mmol) was slowly added. The mixture was stirred overnight allowing it to warm to room temperature, when TLC showed the reaction was complete (1:1 cyclohexane-EtOAc). The mixture was neutralized with triethylamine and concentrated. Chromatography of the crude mixture (9:1 → 1:1 toluene-EtOAc) gave 14.50 g of foamy product **11** (43%). $[\alpha]_D^{24}$= +17.6 (c 0.55, $CHCl_3$).

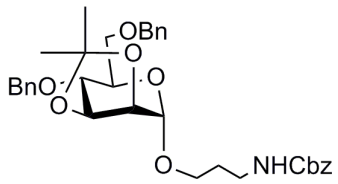
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39–7.27 (m, 5 H, Ph), 5.32 (dd, $J_{2,3}$ = 3.5, $J_{3,4}$ = 9.9 Hz, 1 H, H-3), 5.30–5.20 (m, 2 H, H-2,4), 5.11 (br t, J = 5.5 Hz, 1 H, NH), 5.09 (s, 2 H, CH_2^{Cbz}), 4.76 (s, 1 H, H-1), 4.28 (dd, $J_{5,6a}$ = 5.3, $J_{6a,6b}$ = 12.2 Hz, 1 H, H-6a), 4.11 (dd, $J_{5,6b}$ = 2.7 Hz, 1 H, H-6b), 4.00 (m, 1 H, H-5), 3.80 (m, 1 H, H-1′a), 3.47 (m, 1 H, H-1′b), 3.26 (m, 2 H, H-3′), 2.5, 2.08, 2.04, 1.99 (4 s, 12 H, 4 × CH_3CO), 1.80 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.65, 169.83, 169.76, 169.69 (4 × CO), 156.42 (CONH), 136.51–127.71 (Ar), 97.60 (C-1), 69.46 (C-2), 69.01 (C-3), 68.46 (C-5), 66.55 (CH_2^{Cbz}), 66.09 (C-4), 65.86 (C-1′), 62.52 (C-6), 38.13 (C-3′), 29.55 (C-2′), 20.97, 20.81, 20.72, 20.65 (4 × CH_3CO). ESI HR-MS ($C_{25}H_{33}NO_{12}$): *m/z* = ($[M+H]^+$ found 540.2088; calcd 540.2081).



12

3-(Benzyloxycarbonyl)aminopropyl 2,3-*O*-isopropylidene-α-D-mannopyranoside 12. A solution of the linker equipped Man **11** (8.50 g, 2 mmol) was dissolved in MeOH (200 ml), when 1 M methanolic solution of NaOMe was added until pH was strongly alkaline. The mixture was stirred overnight (TLC, 7:3 cyclohexane-EtOAc), then it was neutralized with Dowex H^+. After filtration, the filtrate was concentrated and re-dissolved in 1:1 acetone-acetone dimethyl acetale mixture (150 ml). The mixture was stirred for 1 h in the presence of catalytic *p*-TsOH (0.75 g). After the starting material disappeared (TLC, 7:3 cyclohexane-EtOAc), 75 ml of water were added and stirring was continued for further 6 h. The mixture was concentrated and purified on silica gel (4:1 → 0:10 toluene-EtOAc) to afford 6.50 g of 2,3-*O*-isopropylidene product **12** (70%). $[\alpha]_D^{24}$= +16.5 (c 0.23, $CHCl_3$).

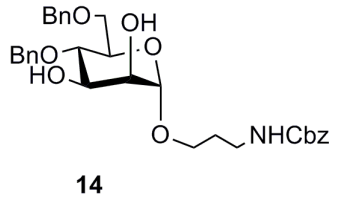
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.41–7.17 (m, 5 H, Ph), 5.15–5.05 (m, 3 H, CH_2^{Cbz}, NH), 4.99 (s, 1 H, H-1), 4.16–4.10 (m, 2 H, H-3,4), 3.89–3.76 (m, 3 H, H-2,5,1′a), 3.74 (m, 1 H, H-6a), 3.62 (m, 1 H, H-6b), 3.59 (m, 1 H, , H-1′b), 3.37 (m, 2 H, H-3′), 2.85 (br s, 2 H, OH-4,6), 1.82-1.77 (m, 2 H, H-2′), 1.52, 1.35 (2 s, 6 H, 2 × CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.51 (CONH), 136.46–125.26 (Ar), 109.61 (*C*(CH_3)$_2$), 97.33 (C-1), 78.32, 75.55 (C-3,4), 69.99 (C-6), 69.75 (C-2/5), 65.69 (CH_2^{Cbz}), 65.22 (*C*-1’), 62.64 (C-2/5), 38.32 (C-3′), 29.59 (C-2′), 27.93, 26.12 (2 × CH_3). ESI HR-MS ($C_{20}H_{29}NO_8$): *m/z* = ([*M*+Na]$^+$ found 434.1797; calcd 434.1791).



13

3-(Benzyloxycarbonyl)aminopropyl 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-α-D-mannopyranoside 13. To a solution of mannopyranoside **12** (6.50 g, 15.8 mmol) in THF (150 ml) containing 5% of water, powdered NaOH (3.16 g, 79 mmol), BnBr (12.3 ml, 105 mmol) and 18-crown-6 (0.50 g) were added, and the mixture was stirred at room temperature. After 72 h TLC (7:3 cyclohexane-EtOAc) showed the presence of one major spot, so the mixture was concentrated and purified on silica gel (10:0 → 9:1 → 0:10 cyclohexane-EtOAc) to give 6.60 g of compound **13** (71%). $[\alpha]_D^{24}$= +53.0 (c 0.28, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.40–7.23 (m, 15 H, 3 × Ph), 5.55 (br t, 1 H, J = 5.6 Hz, NH), 5.09, 5.03 (2 d, ^2J = 12.2 Hz, 2 H, CH_2^{Cbz}), 5.00 (s, 1 H, H-1), 4.82, 4.50 (2 d, ^2J = 11.5 Hz, 2 H, CH_2Ph), 4.58 (s, 2 H, CH_2Ph), 4.29 (t, J = 6.6. Hz, 1 H, H-4), 4.12 (d, $J_{3,4}$ = 6.0 Hz, 1 H, H-3), 3.87 (m, 1 H, H-1′a), 3.76–3.70 (m, 2 H, H-6), 3.52–3.48 (m, 2 H, H-2,1′b), 3.44–3.31 (m, 2 H, H-5,3′a), 3.17 (m, 1 H, H-3′b), 1.80 (m, 2 H, H-2′), 1.49, 1.35 (2 s, 6 H, 3 × CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.44 (CONH), 137.98–127.54 (Ar), 109.28 ($C(CH_3)_2$), 97.04 (C-1), 78.80 (C-3), 75.87 (C-4,5), 73.21, 72.77 (2 × CH_2Ph), 69.14 (C-6), 68.64 (C-2), 66.51 (CH_2^{Cbz}), 64.53 (C-1′), 37.84 (C-3′), 29.32 (C-2′), 27.90, 26.21 (2 × CH_3). ESI HR-MS ($C_{34}H_{41}NO_8$): *m/z* = ([*M*+H]$^+$ found 592.2899; calcd 592.2910).

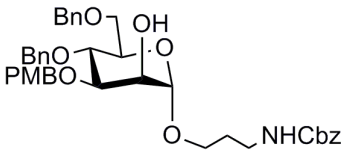


14

3-(Benzyloxycarbonyl)aminopropyl 4,6-di-*O*-benzyl-α-D-mannopyranoside 14. The compound **13** was dissolved in 90% AcOH-H_2O (100 ml) and stirred overnight at 50°C. When the reaction was complete (TLC, 1:1 cyclohexane-EtOAc) the mixture was

concentrated and purified on silica gel (4:1 → 1:9 toluene-EtOAc) to afford 5.68 g of product **14** (89%).$[\alpha]_D^{24}$= +54.8 (c 0.6, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39–7.13 (m, 15 H, 3 × Ph), 5.18 (br t, 1 H, J = 5.2 Hz, NH), 5.09, 5.05 (2 d, ^2J = 12.0 Hz, 2 H, CH_2^{Cbz}), 4.82 (s, 1 H, H-1), 4.70, 4.55 (2 d, ^2J = 11.4 Hz, 2 H, CH_2Ph), 4.63, 4.54 (2 d, ^2J = 12.0 Hz, 2 H, CH_2Ph), 3.92–3.85 (m, 2 H, H-2,3), 3.78 (m, 1 H, H-1′a), 3.72–3.63 (m, 4 H, H-5,6, incl. t, 3.65 J = 9.0 Hz, H-4), 3.47 (m, 1 H, H-1′b), 3.33 (m, 1 H, H-3′a), 3.21 (m, 1 H, H-3′b), 2.52 (m, 2 H, OH-2,3), 1.77 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.43 (CONH), 138.09–127.73 (Ar), 99.51 (C-1), 75.80 (C-4), 74.74, 73.47 (2 × CH$_2$Ph), 71.84 (C-3), 71.06 (C-5), 71.00 (C-2), 68.74 (C-6), 66.60 (CH$_2^{Cbz}$), 65.20 (C-1′), 38.27 (C-3′), 29.44 (C-2′). ESI HR-MS ($C_{31}H_{37}NO_8$): m/z = ([M+H]$^+$ found 552.2595; calcd 552.2597).

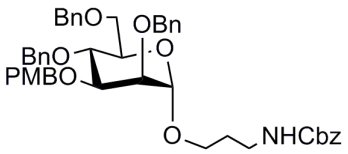


15

3-(Benzyloxycarbonyl)aminopropyl 4,6-di-*O*-benzyl-3-*p*-methoxybenzyl-α-D-mannopyranoside 15. A suspension of diol **14** (5.30 g, 10.3 mmol) and Bu_2SnO (3.57 g, 14.4 mmol) in toluene (100 ml) containing pre activated 4 Å MS was stirred under reflux for 1 h. Then temperature was decreased to 60°C and PMBBr (2.1 ml, 14.4 mmol) was added followed by TBAI (5.3 g, 14.4 mmol). After stirring overnight the reaction was complete (TLC, 7:3 cyclohexane-EtOAc). The mixture was filtered and concentrated. The residue was chromatographed (10:0 → 9:1 toluene-EtOAc) to give 4.55 g of product **15** (69%). $[\alpha]_D^{24}$= +39.5 (c 0.13, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39–7.22 (m, 15 H, 3 × Ph), 7.21–6.83 (m, 4 H, *p*-OMe-Ph), 5.31 (br t, 1 H, J = 5.6 Hz, NH), 5.10, 5.05 (2 d, ^2J = 11.9 Hz, 2 H, CH_2^{Cbz}), 4.86 (s, 1 H, H-1), 4.79, 4.46 (2 d, ^2J = 11.0 Hz, 2 H, CH_2Ph), 4.63–4.54 (m, 4 H, 2 × CH_2Ph), 3.98 (s, 1 H, H-2), 3.82–3.61 (m, 9 H, H-3,4,5,6, H-1′a, incl. s, 3.79, OMe), 3.50 (m, 1 H, H-1′b), 3.36 (m, 1 H, H-3′a), 3.23 (m, 1 H, H-3′b), 2.49 (s, 1 H, OH-2), 1.78 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.39 (CONH), 138.10–127.56 (Ar),

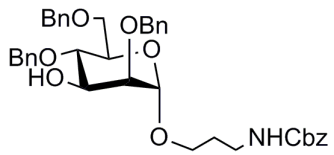
113.91 (C_q^{PMB}), 99.15 (C-1), 80.02 (C-3), 75.11 (CH_2Ph), 74.20 (C-4), 73.30, 71.54 (2 × CH_2Ph), 71.33 (C-5), 68.82 (C-6), 68.35 (C-2), 66.58 (CH_2^{Cbz}), 65.21 (C-1′), 55.24 (OMe), 38.33 (C-3′), 29.34 (C-2′). ESI HR-MS ($C_{39}H_{45}NO_9$): *m/z* = ([*M*+H]$^+$ found 672.3155; calcd 672.3173).



16

3-(Benzyloxycarbonyl)aminopropyl 2,4,6-tri-*O*-benzyl-3-*p*-methoxybenzyl-α-D-mannopyranoside 16. To a solution of the 2-hydroxy mannopyranoside **15** (3.60 g, 5.3 mmol) in THF (50 ml) containing 5% of water, powdered NaOH (900 mg, 21.4 mmol), BnBr (2.54 ml, 21.4 mmol) and 18-crown-6 (0.250 mg) were added and the mixture was stirred for 5 d, monitoring by TLC (7:3 cyclohexane-EtOAc). Then the mixture was concentrated and purified on silica gel to give 3.47 g of product **16** (85%). $[\alpha]_D^{24}$= +49.3 (c 0.48, $CHCl_3$).

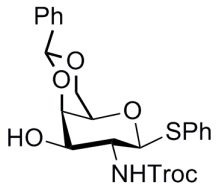
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.37–6.83 (m, 24 H, 5 × Ar), 5.29 (br t, 1 H, *J* = 5.6 Hz, NH), 5.11, 5.03 (2 d, ^2J = 12.2 Hz, 2 H, CH_2^{Cbz}), 4.85, 4.44 (2 d, ^2J = 10.8 Hz, 2 H, CH_2Ph), 4.81 (d, *J* = 1.7 Hz, 1 H, H-1), 4.79, 4,67 (2 d, ^2J = 12.4 Hz, 2 H, CH_2Ph), 4.59, 4.56 (2 d, ^2J = 12.5 Hz, 2 H, CH_2Ph), 4.51 (s, 2 H, CH_2Ph), 3.87–3.61 (m, 10 H, H-2,3,4,5,6, H-1′a, incl. s, 3.79, OMe), 3.42 (m, 1 H, H-1′b), 3.32 (m, 1 H, H-3′a), 3.17 (m, 1 H, H-3′b), 1.73 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.40 (CONH), 138.29–127.48 (Ar), 113.71 (C_q^{PMB}), 98.07 (C-1), 79.95 (C-3), 75.05, 74.88, 73.23, 72.70, 72.11, 71.92 (3 × CH_2Ph, C-4,5,6), 69.14 (C-2), 66.55 (CH_2^{Cbz}), 65.07 (C-1′), 55.24 (OMe), 38.25 (C-3′), 29.34 (C-2′). ESI HR-MS ($C_{46}H_{51}NO_9$): *m/z* = ([*M*+H]$^+$ found 779.3902; calcd 779.3908).



9

3-(Benzyloxycarbonyl)aminopropyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 9. To a solution of the 3-*O*-PMB protected sugar **16** (2.00 g, 2.63 mmol) in CH_2Cl_2 (27 ml) moistened with water (3 ml), DDQ (750 mg, 3.31 mmol) was added and the mixture was stirred for 1 h. After 1 h TLC (1:1 cyclohexane-EtOAc) showed the reaction was complete. The mixture was partitioned with 10% sodium thiosulfate, then combined organic layers were concentrated and purified on silica gel (cyclohexane-EtOAc) to afford 1.35 g of **9** as solid product (80%). White crystals from EtOAc: m.p. 81–82°C. $[\alpha]_D^{24}$= +32.7 (c 0.22, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.40–7.17 (m, 20 H, 4 × Ph), 5.23 (br t, 1 H, J = 6.3 Hz, NH), 5.09, 5.04 (2 d, ^2J = 12.0 Hz, 2 H, CH_2^{Cbz}), 4.88 (s, 1 H, H-1), 4.83, 4.50 (2 d, ^2J = 11.0 Hz, 2 H, CH_2Ph), 4.73, 4.57 (2 d, ^2J = 11.3 Hz, 2 H, CH_2Ph), 4.60, 4.54 (2 d, ^2J = 12.4 Hz, 2 H, CH_2Ph), 3.94 (ddd, $J_{2,3}$ = 3.8, $J_{3,4}$ = $J_{3,OH}$= 9.2 Hz, 1 H, H-3), 3.76 (m, 1 H, H-1′a), 3.74–3.57 (m, 5 H, H-2,4,5,6), 3.45 (m, 1 H, H-1′b), 3.34 (m, 1 H, H-3′a), 3.18 (m, 1 H, H-3′b), 2.32 (d, 1 H, OH-3), 1.75 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.40 (CONH), 138.21–127.55 (Ar), 96.95 (C-1), 78.37 (C-2), 77.20 (C-4), 76.99, 74.82, 73.29 (3 × CH$_2$Ph), 71.83 (C-3), 71.15 (C-5), 69.03 (C-6), 66.54 (CH$_2^{Cbz}$), 64.99 (C-1′), 38.13 (C-3′), 29.42 (C-2′). ESI HR-MS ($C_{38}H_{43}NO_8$): *m/z* = ([M+H]$^+$ found 642.3033; calcd 642.3067).

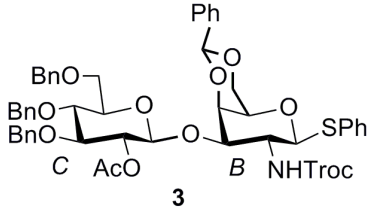


7

Phenylthio 4,6-*O*-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside 7. Phenylthio 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxy carbonylamino)-β-D-galactopyranoside **17**[1] (25.00 g, 45 mmol) was dissolved in MeOH

(100 ml), to which 0.25 M methanolic solution of NaOMe was added dropwise at 0°C until pH = 9. After stirring for 3 h at 0°C, the reaction was complete (TLC, cyclohexane-EtOAc 7:3). The mixture was neutralized with Dowex H^+ and filtrated. The filtrate (45 mmol) was re-dissolved in AcCN (100 ml), and $PhCH(OMe)_2$ (20.5 ml, 134 mmol) followed by *p*-TsOH (1.28 g, 0.68 mmol) were added at 0°C. The mixture was stirred for 2 h at ambient temperature, when TLC (cyclohexane-EtOAc 1:1) showed the product was formed. After evaporation of solvent, the residue was chromatographed (cyclohexane-EtOAc) to afford 17.00 g of **7** as solid product (72% over two steps). White crystals from EtOAc: m.p. 171–172°C. $[\alpha]_D^{24}$= -22.7 (c 0.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64–7.27 (m, 10 H, 2 × Ph), 5.69 (m, 1 H, NH), 5.54 (s, 1 H, PhC*H*), 4.90 (d, $J_{1,2}$ = 10.0 Hz, 1 H, H-1), 4.75, 4.69 (2 d, ^2J = 12.0 Hz, 2 H, CH_2^{Troc}), 4.37 (d, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6a), 4.22 (d, $J_{3,4}$ = 3.2 Hz, 1 H, H-4), 4.04 (d, 1 H, H-6b), 3.96 (dd, $J_{3,2}$ = 9.0, 1 H, H-3), 3.69 (q, $J_{2,NH}$ = 9.9 Hz, 1 H, H-2), 3.56 (s, 1 H, H-5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 154.42 (CONH), 137.46–126.47 (Ar), 101.28 (Ph*C*H), 95.55 (CCl_3), 84.98 (C-1), 75.00 (C-4), 74.59 (CH_2^{Troc}), 71.32 (C-3), 69.91 (C-5), 69.20 (C-6), 53.38 (C-2). ESI HR-MS ($C_{22}H_{22}Cl_3NO_5S$): *m/z* = ([*M*+H]$^+$ found 534.0302; calcd 534.0312).

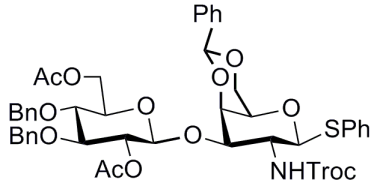


3

Phenylthio 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside 3. To a mixture of acceptor **7** (880 mg, 1.38 mmol) and donor **6** (575 mg, 1.1 mmol) in 2:1 CH_2Cl_2-hexane (12 ml), TMSOTf (7.5 μl, 0.042 mmol) was added at -10 °C under nitrogen atmosphere. After 15 min TLC (7:3 cyclohexane-EtOAc) showed formation of the product. The mixture was neutralized with a few drops of triethylamine and concentrated. Chromatography of the residue (toluene-EtOAc) gave the desired disaccharide **3** as a white solid (900 mg, 82%). White crystals from EtOAc: m.p.

149–150°C. $[\alpha]_D^{24}$= +5.8 (c 0.2, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.59–7.17 (m, 25 H, 5 × Ph), 5.44 (d, $J_{NH,2}$ = 6.4 Hz, 1 H, NH), 5.43 (s, 1 H, PhC*H*), 5.21 (d, $J_{1,2}$ = 10.1 Hz, 1 H, H-1^B), 4.95 (t, J = 8.2 Hz, 1 H, H-2^C), 4.81, 4.50 (2 d, ^2J = 12.0 Hz, 2 H, C*H*$_2$Ph), 4.76, 4.62 (2 d × 2, ^2J = 11.3 Hz, 4 H, C*H*$_2$Ph, CH_2^{Troc}), 4.63, 4.50 (2 d, ^2J = 11.3 Hz, 2 H, C*H*$_2$Ph), 4.50 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-1^C), 4.39–4.35 (m, 2 H, H-3^B,4^B), 4.27 (d, $J_{6a,6b}$ = 12.0 Hz, 1 H, H-6a^B), 3.82 (d, 1 H, H-6b^B), 3.70 (dd, $J_{5,6a}$ = 1.7, $J_{6a,6b}$ = 10.3 Hz, 1 H, H-6a^C), 3.71–3.52 (m, 4 H, H-2^B,3^C,4^C,6b^C), 3.43–3.39 (m, 2 H, H-5^{B,C}), 1.89 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.48 (CO), 153.68 (CONH), 137.96–125.28 (Ar), 100.68 (*C*HPh), 100.59 (C-1^C), 95.54 (CCl_3), 84.15 (C-1^B), 83.01, 77.90 (C-3^C,4^C), 75.86, 75.82 (C-3^B,4^B), 75.03 (2 × CH_2), 74.47 (C-5^C), 74.27, 73.55 (2 × CH_2), 72.82 (C-2^C), 69.96 (C-5^B), 69.20 (C-6^{B,C}), 51.22 (C-2^B), 20.84 (*C*H_3CO). ESI HR-MS ($C_{51}H_{52}Cl_3NO_{12}S$): *m/z* = ([*M*+Na]$^+$ found 1030.2217; calcd 1030.2247); ([*M*+K]$^+$ found 1046.1865; calcd 1046.1913).

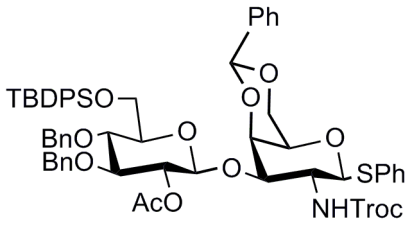


19

Phenylthio 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside 19. To a solution of acceptor **7** (571 mg, 1.1 mmol) and donor **18** (880 mg, 1.38 mmol) in 1:1 CH_2Cl_2-hexane (30 ml), TMSOTf (2.5 μl, 0.014 mmol) was added at -30°C under nitrogen atmosphere. After 15 min the mixture became cloudy and the flask was brought to ambient temperature. TLC (3:2 cyclohexane-EtOAc) showed the reaction had taken place. The reaction mixture was neutralized with few drops of triethylamine, and concentrated. The residue was chromatographed on silica gel to afford 530 mg of disaccharide **19** (52%). $[\alpha]_D^{24}$= +23.94 (c 0.23, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.65–7.17 (m, 20 H, 4 × Ph), 5.53 (s, 1 H, PhC*H*), 5.37 (d, $J_{NH,2}$ = 6.9 Hz, 1 H, NH), 5.28 (d, $J_{1,2}$ = 10.0 Hz, 1 H, H-1^B), 4.94 (t, J = 8.8 Hz, 1 H, H-2^C), 4.82–4.75 (m, 3 H, 3 × *H*CH), 4.65-4.55 (m, 5 H, H-6a^C), 4.40–4.33 (m, 3 H, H-

3^B,4^B,$6a^B$), 4.06–4.01 (m, 2 H, H-$6b^B$,$6b^C$), 3.61–3.51 (m, 4 H, H-2^B,3^C,4^C,5^B), 3.44 (m, 1 H, H-5^C), 2.00, 1.91 (2 × s, 6 H, 2 × CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.57, 169.48 (2 × CO), 153.63 (CONH), 138.24–126.08 (Ar), 101.23 (*C*HPh), 100.51 (C-1^C), 95.48 (CCl_3), 84.06 (C-1^B), 82.75, 77.20 (C-3^C,4^C), 75.64, 75.24 (C-3^B,4^B), 75.09, 75.00, 74.15 (3 × CH_2), 73.09 (C-5^C), 72.76 (C-2^C), 70.02 (C-5^B), 69.23 (C-6^B), 62.20 (C-6^C), 51.13 (C-2^B), 20.84, 20.80 (2 × *C*H_3CO). ESI HR-MS ($C_{46}H_{48}Cl_3NO_{13}S$): *m/z* = ([*M*+H]$^+$ found 960.1965; calcd 960.1990).



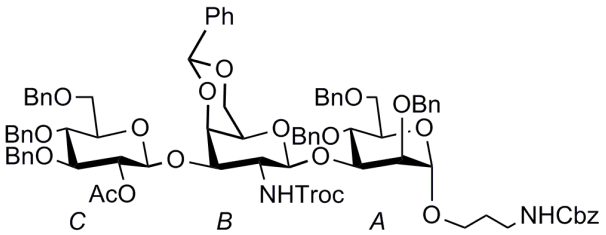
4

Phenylthio 2-*O*-acetyl-3,4-di-*O*-benzyl6-O-*tert*-butyldiphenylsilyl--β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxy carbonylamino)-β-D-galactopyranoside 4. A solution of disaccharide **19** (830 mg, 0.87 mmol) in MeOH (50 ml) was made alkaline (pH = 9) by dropwise addition of 0.25 M methanolic solution of NaOMe. The mixture was stirred overnight at 0°C, when TLC (3:2 cyclohexane-EtOAc) showed the formation of a lower moving spot. The mixture was neutralized with Dowex H^+ and filtrated. The filtrate was concentrated and purified on silica gel to afford 575 mg of 6-de-*O*-acetylated product **20** (73%).

Phenylthio 2-O-*acetyl-3,4-di*-O-*benzyl-β*-D-*glucopyranosyl-(1→3)-4,6*-O-*benzylidene-2 Deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β*-D-*galactopyranoside* **20**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.65–7.21 (m, 20 H, 4 × Ph), 5.50 (s, 1 H, PhC*H*), 5.33 (d, $J_{NH,2}$ = 7.3 Hz, 1 H, NH), 5.26 (d, $J_{1,2}$ = 9.6 Hz, 1 H, H-1^B), 4.94 (t, *J* = 8.5 Hz, 1 H, H-2^C), 4.87–4.55 (m, 7 H, 2 × C*H*$_2$Ph, CH_2^{Troc}, H-1^C), 4.51 (br d, $J_{2,3}$ = 10.6 Hz, 1 H, H-3^B), 4.37–4.33 (m, 2 H, H-4^B,$6a^B$), 4.02 (d, $J_{6a,6b}$ = 12.1 Hz, 1 H,$6b^B$), 3.74–3.62 (m, 3 H, H-2^B,6^B), 3.59–3.47 (m, 3 H, H-3^C,4^C,5^B), 3.30 (m, 1 H, H-5^C), 1.87 (s, 3 H, CH_3CO). ESI HR-MS ($C_{44}H_{46}Cl_3NO_{12}S$): *m/z* = ([*M*+H]$^+$ found 918.1892; calcd 918.1885).

To a solution of the 6-OH disaccharide **20** (550 mg, 0.59 mmol) in DMF (4 ml), *t*-butyldiphenylsylilchloride (0.31 ml, 1.2 mmol) and imidazole (82 mg, 1.2 mmol) were added. After stirring for 24 h TLC (4:1 cyclohexane-EtOAc) showed the reaction was complete The mixture was concentrated, and the residue was purified on silica gel (cyclohexane-EtOAc) to give 630 mg of foamy product **4** (92%). $[\alpha]_D^{24}$= -12.90 (c 0.11, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.71–7.03 (m, 30 H, 6 × Ph), 5.43 (s, 1 H, PhC*H*), 5.18 (d, $J_{1,2}$ = 10.0 Hz, 1 H, H-1^B), 5.17 (d, $J_{NH,2}$ = 7.2 Hz, 1 H, NH), 4.99 (t, J = 8.1 Hz, 1 H, H-2^C), 4.80, 4.64 (2 d, ^2J = 11.2 Hz, 2 H, CH_2Ph), 4.80, 4.50 (2 d, ^2J = 10.5 Hz, 2 H, CH_2Ph), 4.76, 4.62 (2 d, ^2J = 11.2 Hz, 2 H, CH_2^{Troc}), 4.61 (d, $J_{1,2}$ = 7.4, 1 H, H-1^C), 4.40 (br s, 1 H, H-4^B), 4.38 (br d, $J_{2,3}$ = 10.6 Hz, 1 H, H-3^B), 4.25 (d, $J_{6a,6b}$ = 12.1 Hz, 1 H, 6a^B), 4.03 (d, $J_{6a,6b}$ = 10.5 Hz, 1 H, 6a^C), 3.89 (dd, $J_{5,6}$ = 5.4 Hz, 1 H, 6b^C), 3.77 (d, 1 H, 6b^B), 3.70–3.57 (m, 3 H, H-2^B,3^C,4^C), 3.49 (m, 1 H, H-5^C), 3.34 (s, 1 H, H-5^B), 1.94 (s, 3 H, CH_3CO), 1.10 (s, 9 H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.66 (CO), 153.69 (CONH), 137.89–126.36 (Ar, *C*$(CH_3)_3$), 101.80 (C-1^C), 100.49 (*C*HPh), 95.47 (CCl_3), 84.29(C-1^B), 82.96, 77.62 (C-3^C,4^C), 76.27, (C-5^C), 76.12, 75.85 (C-3^B,4^B), 75.09, 74.91, 74.17 (3 × CH_2), 72.94 (C-2^C), 70.00 (C-5^B), 69.90 (C-6^B), 63.01 (C-6^C), 51.49 (C-2^B), 26.88 (*t*-Bu), 20.87 (*C*H_3CO). ESI HR-MS ($C_{60}H_{64}Cl_3NO_{12}SSi$): *m/z* = ([*M*+H]$^+$ found 1178.2897; calcd 1178.2882).

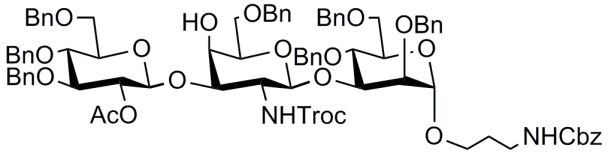


21

3-(Benzyloxycarbonyl)aminopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxy carbonylamino)-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 21. A solution of acceptor **9** (1.18 g, 1.84 mmol) and donor **3** (2.26 g, 2.27 mmol) in CH_2Cl_2 (25 ml) was stirred at -40°C in presence of 4 Å MS, under

nitrogen atmosphere. After addition of NIS (0.53 g, 2.33 mmol) and TfOH (38.6 μl, 0.44 mmol) the mixture turned immediately red and TLC (7:3 cyclohexane-EtOAc) showed that a new spot was formed. The reaction mixture was washed with 10% NaS_2O_3-*aq* $NaHCO_3$. Combined organic layers were dried on Na_2SO_4, filtered and purified on silica gel (cyclohexane-EtOAc) to yield trisaccharide **21** (2.2 g, 77%). $[\alpha]_D^{24}$= +46.5 (c=0.05, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.53–7.13 (m, 40 H, 8 × Ph), 5.59 (d, $J_{NH,2}$ = 7.0 Hz, 1 H, NH^B), 5.43 (s, 1 H, PhC*H*), 5.37 (br t, *J* = 5.2 Hz,1 H, NH^{Cbz}), 5.05 (br s, 2 H, CH_2^{Cbz}), 5.03 (d, $J_{1,2}$ = 8.7 Hz, 1 H, H-1^B), 5.02–4.95 (m, 2 H, H-2^C, *H*CH), 4.78–4.74 (m, 3 H, 2 × *H*CH, incl. s, 4.75, H-1^A), 4.63–4.46 (m, 11 H, 10 × *H*CH, H-1^C), 4.42–4.34 (m, 2 H, *H*CH, H-3^B), 4.28 (d, $J_{3,4}$ = 2.6 Hz, 1 H, H-4^B), 4.11 (m, 1 H, H-3^A), 3.99 (d, $J_{6a,6b}$ = 12.1 Hz, 1 H, H-$6a^B$), 3.89–3.48 (m, 12 H, H-$2^{A,B}$,3^C,$4^{A,C}$,5^A,$6a^{A,C}$,$6b^{A,B,C}$,1′a), 3.43 (m, 1 H, H-1′b), 3.36–3.27 (m, 2 H, H-5^C,3′a), 3.22 (s, 1 H, H-5^B), 3.17 (m, 1 H, H-3b′), 1.89 (s, 3 H, CH_3CO), 1.72 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.55 (CO), 156.45, 153.80 (2 × CONH), 138.44–126.32 (Ar), 101.54 (C-1^C), 100.64 (*C*HPh), 99.91 (C-1^B), 97.64 (C-1^A), 95.40 (CCl_3), 82.91 (C-$3/4^C$), 78.69 (C-3^A), 77.91 (C-$3/4^C$), 75.71, 75.82 (C-2^A,4^B), 74.96, 74.92, 74.61, 74.43, 74.31, 74.10 (4 × CH_2, C-4^A, C-3^B), 73.44 (2 × CH_2), 73.10 (C-2^C), 72.72, 72.46 (2 × CH_2), 72.00 (C-5^A), 69.17 (C-6^A), 68.76 (C-5^C), 68.17, 67.96 (C-$6^{B,C}$), 66.48 (C-5^B), 64.74 (C-1′), 53.68 (C-2^B), 37.87 (C-3′), 29.40 (C-2′), 20.83 (*C*H_3CO). ESI HR-MS ($C_{83}H_{89}Cl_3N_2O_{20}$): *m/z* = ([*M*+Na]$^+$ found 1561.4944 calcd 1561.4972); ([*M*+K]$^+$ found 1577.4655 calcd 1577.4711).

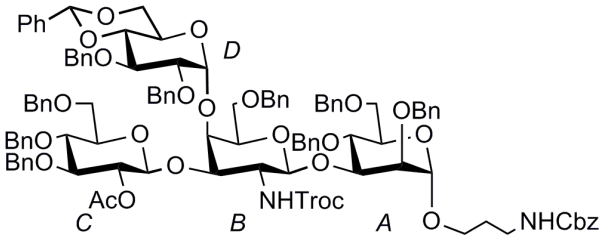


22

3-(Benzyloxycarbonyl)aminopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-6-*O*-benzyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonyl amino)-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 22. The starting trisaccharide **21** (330 mg, 0.2 mmol) was dissolved in dry acetonitrile (30

ml) under nitrogen atmosphere and treated with trimethylamineborane (83 mg, 1.08 mmol) and $BF_3{\cdot}Et_2O$ (0.176 ml, 1.08 mmol) at 0°C. After stirring for 1 h at 0°C, the mixture was quenched with triethylamine and MeOH and concentrated. Chromatography of the residue (cyclohexane-EtOAc) afforded 265 mg of syrupy product **22** (80%). $[\alpha]_D^{24}$= +50.06 (c=0.36, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38–7.17 (m, 40 H, 8 × Ph), 5.40 (br t, J = 5.2 Hz, 1 H, NH^{Cbz}), 5.04 (br s, 2 H, CH_2^{Cbz}), 4.97 (t, J=8.4 Hz, 1 H, H-2^C), 4.91 (d, $J_{NH,2}$ = 6.6 Hz, 1 H, NH^B), 4.80 (d, $J_{1,2}$ = 7.5 Hz, 1 H, H-1^B), 4.79 (s, 1 H, H-1^A), 4.77–4.74 (m, 4 H, *H*CH), 4.68–4.62 (m, 2 H, *H*CH), 4.55–4.33 (m, 11 H, 10 × *H*CH, H-1^C), 4.11–4.08 (m, 3 H, H-3^B, H-4^B, H-3^A), 3.89–3.42 (m, 16 H, H-$2^{A,B}$,3^C,$4^{A,C}$,$5^{A,B,C}$,$6a^{A,B,C}$,$6b^{A,B,C}$,H-1′), 3.30 (m, 1 H, H-3′a), 3.14 (m, 1 H, H-3′b), 2.73 (br s, 1H, OH-4^B), 1.91 (s, 3 H, CH_3CO), 1.70 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.44 (CO), 156.46, 153.64 (2 × CONH), 138.78–127.47 (Ar), 101.49 (C-1^C), 99.34 (C-1^B), 98.17 (C-1^A), 95.34 (CCl_3), 82.61 (C-3^C), 78.32 (C-3^B), 77.65 (C-4^B), 77.65 (C-4^C), 77.18 (C-2^A), 75.04 (2 × CH_2), 75.33 (C-5^B), 75.00 (C-$5^{A/C}$), 74.86, 74.21, 73.81, (3 × CH_2), 73.41 (C-4^A), 73.11 (CH_2), 72.74 (C-2^C), 72.60 (2 × CH_2), 71.87 (C-$5^{A/C}$), 69.39, 69.17, 68.57 (3 × C-$6^{A/B/C}$), 67.75 (C-3^A), 66.42 (CH_2^{Cbz}), 64.64 (C-1′), 54.36 (C-2^B), 37.86 (C-3′), 29.35 (C-2′), 20.81 (C*H*$_3$CO). ESI HR-MS ($C_{83}H_{91}Cl_3N_2O_{20}$): *m/z* = ([*M*+Na]$^+$ found 1563.5134; calcd 1563.5128); ([*M*+K]$^+$ found 1579.4945; calcd 1579.4868).



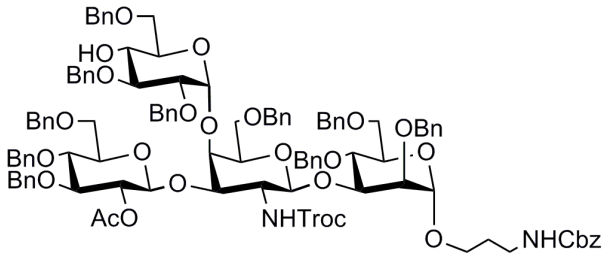
23

3-(Benzyloxycarbonyl)aminopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-[6-*O*-benzyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonyl amino)-β-D-galactopyranosyl-(1→3)-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)]-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 23. A solution of acceptor **22** (600 mg, 0.389 mmol) and donor **8** (287 mg, 0.583 mmol) in 4:1 toluene-

dioxane (10 ml) containing 4 Å MS was stirred at 0°C, under nitrogen atmosphere. After addiction of NIS (131 mg, 0.583 mmol) and TfOH (13.8 µl, 0.156 mmol) the mixture turned immediately red and the reaction mixture was stirred at room temperature. After 5 h further portions of NIS (20 mg, 0.089 mmol) and TfOH (3.4 µl, 0.039) were added, and stirring was continued for 3 h, when TLC (7:3 toluene-EtOAc) showed that the reaction was complete. Triethylamine was added to neutralize the reaction and the mixture was concentrated. The residue was purified on silica gel (95:5→1:1 Toluene-EtOAc) to yield 680 mg of tetrasaccaride **23** (89%). $[\alpha]_D^{24}$= +33.8 (c 0.80, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.50–7.11 (m, 55 H, 11 × Ph), 5.53 (s, 1 H, PhC*H*), 5.42 (m, 1 H, NH^{Cbz}), 5.11 (d, $J_{1,2}$ = 2.3 Hz, 1H, H-1^D), 5.04–5.00 (m, 4 H, NH^B, $CH_2{}^{Cbz}$, H-2^C), 4.84 (d, $J_{1,2}$ = 7.84 Hz, 1 H, H-1^B), 4.83 (d, ^2J = 11.7 Hz, 1H, *H*CH), 4.78 (s, 1 H, H-1^A), 4.75–4.71 (m, 2 H, *H*CH), 4.67–4.39 (m, 16 H, 14 × *H*CH, H-3^A, H-1^C), 4.32 (d, ^2J = 12.04 Hz, 1 H, *H*CH), 4.25–4.03 (m, 8 H, H-$3^{B,D}$,$4^{A,B}$,$6^{D/B}$, $CH_2{}^{Troc}$), 3.76–3.27 (m, 18 H, H-$2^{A,B,D}$,3^C,$4^{C,D}$,$5^{A,B,C,D}$,$6^{A,B/D,C}$, 1'), 3.28 (m, 1 H, H-3′a), 3.11 (m, 1 H, H-3′b), 1.92 (s, 3 H, CH_3CO), 1.71 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.04 (CO), 156.34, 153.65 (2 × CONH), 138.81–123.48 (Ar), 101.81 (C-1^C), 101.49 (*C*HPh), 100.18 (C-1^B), 99.12 (C-1^D), 98.12 (C-1^A), 95.40 (CCl_3), 82.82 (C-3^C), 82.52, 80.11 (C-2^D), 78.54 (C-3^D), 78.14 (C-4^B), 77.53, 77.32, 77.02 (C-3^B), 76.52, 76.19, 74.87, 74.81, 74.78 (3 × CH_2), 74.54, 73.93 (CH_2), 73.89, 73.55, 73.21, 73.02, 72.90, 72.81 (5 × CH_2), 72.52 (C-2^C), 72.36 ($CH_2{}^{Troc}$), 71.70 (C-3^A), 69.33, 69.09, 68.52 (C-$6^{A/B/C/D}$), 66.28 ($CH_2{}^{Cbz}$), 64.32 (C-1′), 62.85 (C-4^A), 54.78 (C-2^B), 37.52 (C-3′), 29.18 (C-2′), 20.84 (C*H*$_3$CO).

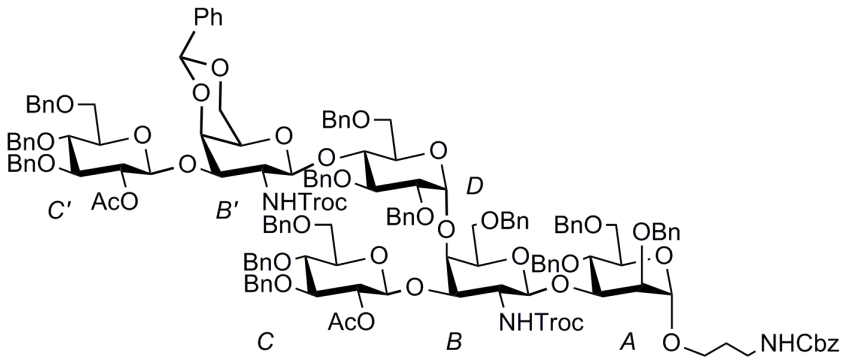
ESI HR-MS ($C_{110}H_{117}Cl_3N_2O_{25}$): *m/z* = ([*M*+Na]$^+$ found 1993.6755; calcd 1993.6909); ([*M*+K]$^+$ found 2009.6423; calcd 2009.6648).



5

3-(Benzyloxycarbonyl)aminopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-[6-*O*-benzyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonyl amino)-β-D-galactopyranosyl-(1→3)-2,3-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4))]-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 5. The starting tetrasaccharide **23** (230 mg, 0.117 mmol) was dissolved in dry acetonitrile (26 ml) under nitrogen atmosphere and treated with trimethylamineborane (43 mg, 0.584 mmol) and $BF_3{\cdot}Et_2O$ (0.072 ml, 0.584 mmol) at 0°C. After 1 h at 0°C, the mixture was quenched with triethylamine and MeOH and concentrated. Chromatography of the residue (cyclohexane-EtOAc) afforded 220 mg of product **5** (95%). $[\alpha]_D^{25}$= +58.08 (c=0.13, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.29–7.08 (m, 55 H, 11 × Ph), 5.36 (m, 1 H, NH^{Cbz}), 5.02 (d, $J_{1,2}$=2.5 Hz, 1 H, H-1^D), 4.96-4.92 (m, 3 H, NH^B, CH_2^{Cbz}), 4.74–4.69 (m, 9 H, 6 × *H*CH, H-2^C, incl. d, 4.72, $J_{1,2}$=2.7 Hz, H-1^A; d, 4.70 $J_{1,2}$ = 8.5 Hz, H-1^B), 4.55–4.25 (m, 18 H, 16 × *H*CH, H-5^D, incl. d, 4.34, $J_{1,2}$ = 7.7 Hz, H-1^C), 4.18 (br s, 1 H, H-4^B), 4.16–4.03 (m, 4 H, 2 × *H*CH, H-3^B, H-3^A), 3.85–3.37 (m, 19 H, H-$2^{A,B,D}$,$3^{C,D}$,$4^{A,C,D}$,$5^{A,B,C}$,$6^{A,B,C}$,1′) 3.23 (m, 1 H, H-3′a), 3.07 (m, 1 H, H-3′b), 2.86 (br s, 1 H, OH-4^D), 1.85 (s, 3 H, CH_3CO), 1.64 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.76 (CO), 156.46, 153.80 (2 × CONH), 139.14-127.29 (Ar), 101.60 (C-1^C), 99.71 (C-1^B), 98.11 (C-1^A), 97.47 (C-1^D), 95.51 (CCl_3), 82.63 (C-3^C), 81.78 (C-4^D), 79.63, 77.83, 77.50 (C-3^A), 76.80, 76.01 (C-3^B), 75.33 (C-2^D), 75.04, 75.03 (3 × CH_2), 74.97, 73.92, 73.95 (C-4^B,5^B), 73.58, 73.55, 73.26, 73.18, 73.05 (6 × CH_2), 72.92 (C-2^C), 72.83 ,72.40 (2 × CH_2), 71.82 (C-4^D), 71.70, 70.55 (C-5^D), 69.57, 69.49, 68.60, 68.36 (C-$6^{A,B,C,D}$), 66.46 (CH_2^{Cbz}), 64.39 (C-1′), 54.95 (C-2^B), 37.63 (C-3′), 29.31 (C-2′), 20.95 (*C*H_3CO). ESI HR-MS ($C_{110}H_{119}Cl_3N_2O_{25}$): *m/z* = ([*M*+Na]$^+$ found 1995.7106; calcd 1995.7065); ([*M*+K]$^+$ found 2011.6824; calcd 2011.6805).

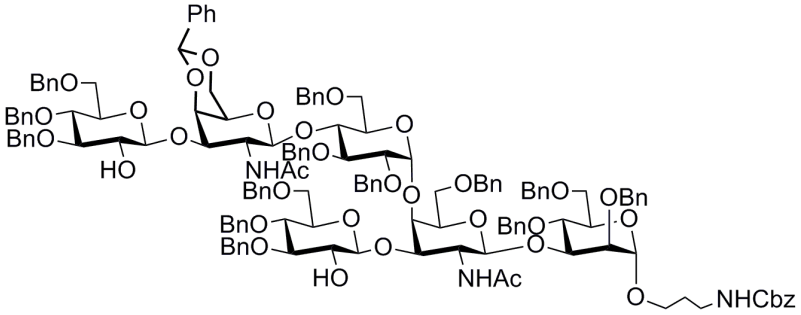


24

3-(Benzyloxycarbonyl)aminopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-O-benzilidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-6-O-benzyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 24. A solution of acceptor **5** (100 mg, 0.051 mmol) and donor **3** (83 mg, 0.083 mmol) in CH_2Cl_2 (3 ml) containing 4 Å MS was stirred at 0°C, under nitrogen atmosphere. After addiction of NIS (18 mg, 0.082 mmol) and TfOH (18 ul, 0.02 mmol) the mixture turned immediately red and the reaction mixture was stirred at room temperature for 8 h. When TLC (Toluene-EtOH 9:1) showed the reaction was complete, it was neutralized with a drop of triethylamine and concentrated. The residue was purified on silica gel (95:5→1:1 toluene-AcOEt) to yield 75 mg of hexasaccaride (50%) **24**. $[\alpha]_D^{24}$= +23.5 (c 0.25, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.44–7.14 (m, 75 H, 15 × Ph), 5.68, 5.57 (2 m, 2 H, 2 × $NH^{B,B'}$), 5.40 (m, 1 H, NH^{Cbz}), 5.37 (s, 1 H, Ph*C*H), 5.16 (d, $J_{1,2}$=1.2 Hz, 1 H, H-1^D), 5.10–4.87 (m, 9 H, CH_2^{Cbz}, 2 × *H*CH, H-$2^{C,C'}$, H-$1^{A,B,B'}$), 4.82–4.70 (m, 8 H, 8 × *H*CH), 4.64–4.37 (m, 20 H, 18 × *H*CH, H-$1^{C,C'}$), 4.31–3.43 (m, 37 H, 2 × *H*CH, H-$2^{A,B,B',D}$,$3^{A,B,B',C,C',D}$,$4^{A,B,B',C,C',D}$,$5^{A,B/B',C,C',D}$,$6^{A,B,B',C,C',D}$,1′), 3.23 (m, 1 H, H-3′a), 3.14 (m, 1 H, H-3′b), 2.96 (s, 1 H, H-$5^{B/B'}$), 1.91, 1.87 (2 × s, 6 H, 2 × C*H*$_3$CO), 1.72 (m, 2 H, H-2′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.82, 169.45 (2 × CO), 156.42, 153.76 (3 × CONH), 139.14–125.24 (Ar), 102.09, 101.96 (C-$1^{C,C'}$), 100.28 (*C*HPh), 99.62, 98.84 (C-$1^{B,B'}$), 97.97 (C-$1^{A,D}$), 97.97 (C-1^D), 97.78, 95.50 (2 × CCl_3), 83.51, 82.94, 82.62, 81.01,

80.60, 80.47, 80.27, 79.99, 79.79, 78.86, 77.91, 77.89, 77.32, 77.09, 76.68, 75.70, 74.93, 74.64, 73.94, 73.45, 73.30, 73.16, 73.08, 72.97, 72.42 (C-$2^{C/C'}$), 71.83 (C-$2^{C/C'}$), 70.83, 69.50, 69.20, 68.74, 68.62, 68.00, 67.12, 66.42 (CH_2^{Cbz}), 66.10, 64.13 (C-1′), 54.83 (C-$2^{B/B'}$), 54.50 (C-$2^{B/B'}$), 37.47 (C-3′), 29.19 (C-2′), 21.40, 20.79 (2 × *C*H_3CO). ESI HR-MS ($C_{155}H_{165}Cl_6N_3O_{37}$): *m/z* = ([*M*+Na]$^+$ found 2892.9521; calcd 2892.9151).

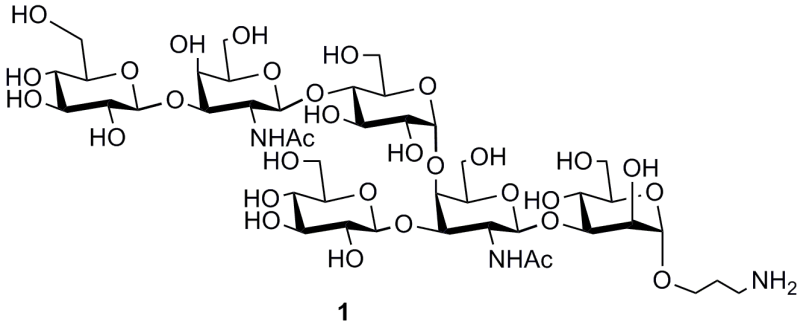


25

3-(Benzyloxycarbonyl)aminopropyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-O-benzilidene-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 25. The hexasaccharide **24** (87 mg, 0.032 mmol) was dissolved in THF (5 ml) to which 3 M NaOH (0.5 ml) was added. After refluxing for 2 d (TLC, 7:3 cyclohexane-EtOAc), the mixture was neutralized with 0.1% HCl and concentrated. The residue was re-dissolved in 2:3 Ac_2O-MeOH (5 ml) and stirred overnight, when TLC (17:1 toluene-EtOH) showed disappearance of the starting material. After concentration, the residue was purified on silica gel (97:3 toluene-EtOH) to afford 68 mg of product **25** (84%). $[\alpha]_D^{24}$= +34.06 (c 0.29, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.45–7.06 (m, 75 H, 15 × Ph), 5.81 (d, $J_{NH,2}$ =6.12 Hz, 1 H, $NH^{B/B'}$), 5.66 (d, $J_{NH,2}$ =5.8 Hz, 1 H, $NH^{B/B'}$), 5.36 (m, 2 H, NH^{Cbz}, PhC*H*), 5.08 (d, $J_{1,2}$=2.4 Hz, 1 H, H-1^D), 5.04–4.87 (m, 5 H, CH_2^{Cbz}, 2 × *H*CH), 4.83–4.57 (m, 10 H, 8 × *H*CH), 4.54–4.29 (m, 16 H, 16 × *H*CH), 4.27–3.35 (m, 39 H, H-$1^{C,C'}$, $2^{A,B,B',C,C',D}$, $3^{A,B,B',C,C',D}$,$4^{A,B,B',C,C',D}$,$5^{A,B/B',C,C',D}$,$6^{A,B,B',C,C',D}$,1′), 3,17 (m, 1 H, H-3′a), 3.08 (m, 1 H, H-3′b), 2.96 (s, 1 H, H-$5^{B/B'}$), 1.70 (s, 3 H, C*H*$_3$CO), 1.69–1.59 (m, 2 H, H-2′), 1.59 (s, 3 H,

C*H*$_3$CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.39, 172.05, 156.60 (3 × CONH), 139.40–126.39 (Ar), 104.50 and 104.06 (C-$1^{C,C'}$), 100.73 (C*H*Ph), 99.93 and 98.96 (C-$1^{B,B'}$), 98.18 (C-1^{A}), 97.97 (C-1^{D}), 84.46, 80.32, 79.78, 79.46, 77.97, 77.81, 77.30, 77.20, 76.98, 76.49, 76.31, 75.70, 75.59, 75.12, 75.02, 74.95, 74.90, 74.67, 74.34, 74.04, 73.78, 73.39, 73.25, 73.04, 72.74, 71.77, 70.54, 69.37, 68.90, 68.18, 67.92, 66.45 (CH_2^{Cbz}), 64.42 (C-1′), 60.37, 53.99 (C-$2^{B,B'}$), 37.72 (C-3′), 29.68 (C-2′), 23.52, 20.46 (2 × *C*H$_3$CO). ESI HR-MS ($C_{149}H_{163}N_3O_{33}$): *m/z* = ([*M*+H]$^+$ found 2523.1301 calcd 2523.1247).



1

Aminopropyl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-mannopyranoside 1. Compound **25** was deprotected by flow chemistry, using a H-Cube Thales-Nano system.

The protected hexasaccaride (35 mg, 0.014 mmol) was dissolved in 9:1 EtOH-CH_3COOH (30 ml) and hydrogenated over a 10% Pd/C cartridge at 40°C and pressure = 10 bar. The mixture was flown for 1 d, then the solvent was evaporated and the recovered crude material was purified on a C-18 Isolute SPE cartridge, giving 14 mg of the final hexasaccharide **1** (90%). $[\alpha]_D^{24}$= +26.09 (c 0.43, H_2O).

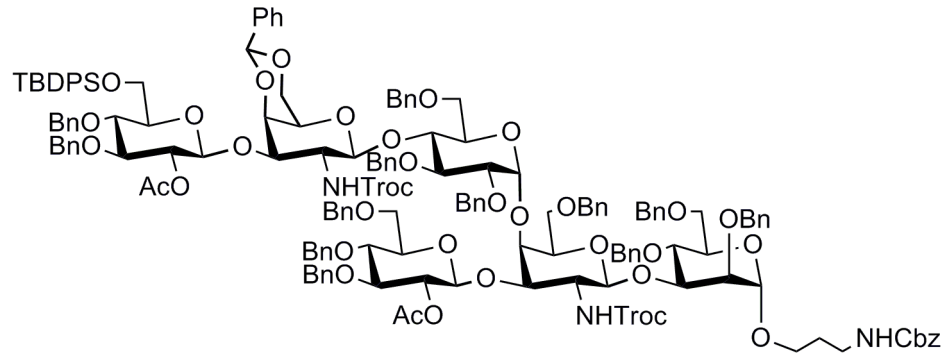
^{1}H and ^{13}C NMR data are reported in Table 1.

ESI HR-MS ($C_{43}H_{75}N_3O_{31}$): *m/z* = ([*M*+H]$^+$ found 1130.4412 calc 1130.4463); ([*M*+Na]$^+$ found 1152.4125 calcd 1152.4282).

Table 1. ^{1}H and ^{13}C NMR[a] δ (ppm), recorded at 400 MHz, D_2O, 298 K, of hexasaccharide **1**

	α-Man ***(A)***	***β-GalNAc*** ***(B)***	***β-Glc*** ***(C)***	***β-GalNAc*** ***(B′)***	***β-Glc*** ***(C′)***	***α-Glc*** ***(D)***	***Linker***
H-1	4.86	4.76 $J_{1,2}$=8.6 Hz	4.49 $J_{1,2}$=7.8 Hz	4.60 $J_{1,2}$=8.6 Hz	4.41 $J_{1,2}$=7.8 Hz	4.95 $J_{1,2}$=3.4 Hz	
C-1	100.5	100.3	105.3	102.3	106.0	99.6	
H-2	4.00	4.00	3.29	4.00	3.07	3.52	
C-2	68.6	52.8	73.6	52.3	74.2	72.1	
H-3	4.00	3.90	3.45	3.90	3.46	3.97	
C-3	79.1	79.4	76.3	80.7	76.4	72.3	
H-4	3.73	4.26	3.41	4.18	3.37	3.66	
C-4	65.9	75.5	70.3	68.6	70.6	79.8	
H-5	3.60	3.76	3.36	3.76	3.39	4.29	
C-5	73.7	76.0	76.4	76.0	76.2	73.6	
H-6	3.76, 3.89	3.71, 3.89	3.90, 4.18	3.75, 3.90	3.71, 3.88	3.66, 3.82	
C-6	61.1	61.3	65.7	61.6	61.8	60.3	
H-1'							3.61, 3.81
C-1'							65.7
H-2'							1.98
C-2'							27.6
H-3'							3.10
C-3'							38.3

[a]^{1}H and ^{13}C NMR resonances were assigned based on HMQC experiment

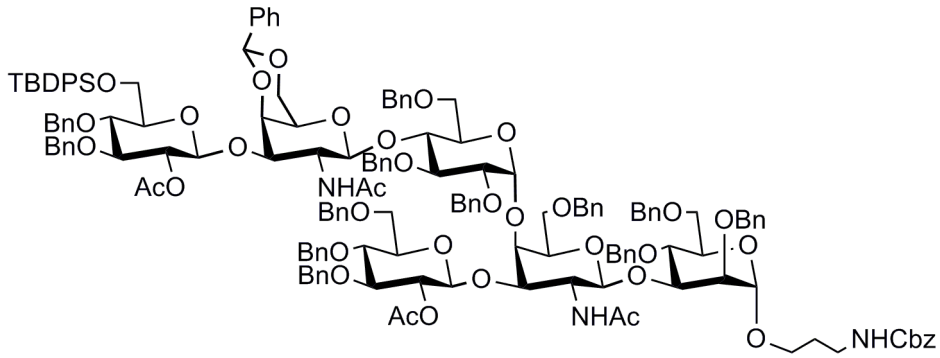


26

3-(Benzyloxycarbonyl)aminopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl--β-D-glucopyranosyl-(1→3)-[6-*O*-*tert*butyldiphenylsilyl-2-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxy carbonylamino)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-6-*O*-benzyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonyl amino)-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 26. A solution of acceptor **5** (203 mg, 0.11 mmol) and donor **4** (180 mg, 0.16 mmol) in CH_2Cl_2 (3 ml) was stirred at 0°C in presence of 4 Å MS, under nitrogen atmosphere. After addition of NIS (39.6 mg, 0.018 mmol) and TfOH (4 µl, 0.05 mmol) the mixture turned immediately red and the reaction mixture was stirred for 6 h at 0°C. When TLC (toluene-EtOH 17:1) showed the reaction was complete, it was quenched with a drop of triethylamine and concentrated. The residue was purified on silica gel (95:5→1:1 cyclohexane-EtOAc) to yield 184 mg of hexasaccharide **26** (62%). $[\alpha]_D^{24}$= +18.4 (c 0.5, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.66–7.03 (m, 80 H, 16 × Ph), 5.89 (d, $J_{NH,2}$ = 6.5 Hz, 1 H, NH), 5.78 (d, $J_{NH,2}$ = 6.0 Hz, 1 H, NH), 5.39 (m, 1 H, NH^{Cbz}), 5.31 (s, 1 H, PhC*H*), 5.16 (d, $J_{1,2}$ = 2.1 Hz, 1 H, H-1^D), 5.05–4.96 (m, 5 H, H-$2^{C/C'}$, CH_2^{Cbz}, incl. 5.03 and 5.00, H-1^B,1^B), 4.89 (t, J = 8.6 Hz, 1 H, H-$2^{C/C'}$), 4.87–4.38 (m, 25 H, 12 × CH_2, incl. s, 4.76, H-1^A), 4.37–3.62 (m, 40 H, 2 × CH_2, H-$2^{A,B/B',D}$,$3^{A,B,B',C,C',D}$,$4^{A,B,B',C,C',D}$,$5^{A,B/B',C,C',D}$, $6^{A,B,B',C,C',D}$,1′, H-$1^{C,C'}$), 3.30–3.20 (m, 2 H, H-$2^{B/B'}$,3′a), 3.15 (m, 1 H, H-3′b), 2.95 (s, 1 H, H-$5^{B/B'}$), 1.87, 1.92 (2 × s, 6 H, 2 × CH_3CO), 1.73 (m, 2 H, H-2′), 1.05 (s, 9 H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.11, 169.70 (2 × CO), 156.51, 153.69, 153.87 (3 ×

CONH), 139.73–126.16 (Ar, *C*(CH_3)$_3$), 102.38, 102.20 (C-$1^{C,C'}$), 100.13 (*C*HPh), 99.80, 98.83 (C-$1^{B,B'}$), 97.97 (C-1^{A}), 97.83 (C-1^{D}), 95.87, 95.54 (2 × CCl_3), 83.05, 82.60 (C-$3^{B,B'}$), 80.76, 80.56, 78.96, 78.14 (C-$3^{C,C'}$,$4^{C,C'}$), 77.24, 76.72, 76.35, 75.96, 75.82, 75.10 (C-$3^{A,D}$,$4^{A,B,B',D}$,$5^{C,C'}$), 74.99, 74.49, 73.97, 73.69, 73.38, 73.18, 73.02, 72.74, 72.42, 71.89, 70.86, 69.55, 69.08, 68.78, 68.57, 68.20, 67.98, 67.95, 66.50, 66.22, 64.25 (C-1′), 63.03, 55.07 (C-$2^{B/B'}$), 54.56 (C-$2^{B/B'}$), 37.55 (C-3′), 29.37 (C-2′), 26.81 (*t*-Bu), 21.10, 20.88 (2 × *C*H_3CO). ESI HR-MS ($C_{164}H_{177}Cl_6N_3O_{37}Si$): *m/z* = ([*M*+Na]$^+$ found 3040.9955; calcd 3040.9859).

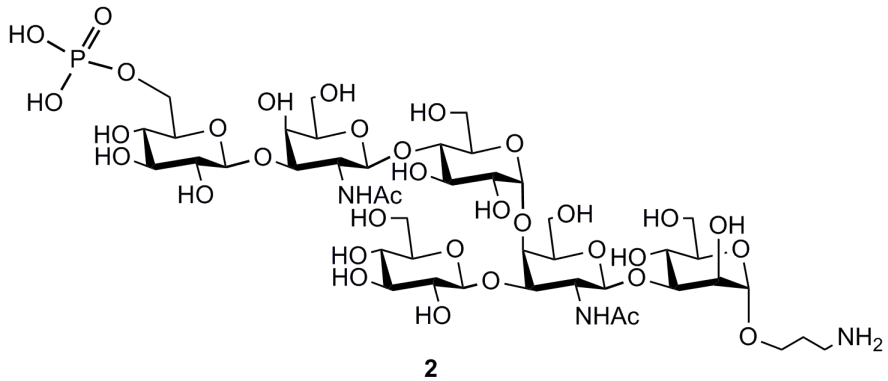


27

3-(Benzyloxycarbonyl)aminopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-[6-*O*-*tert*butyldiphenylsilyl-2-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 27. The hexasaccharide **26** (280 mg, 0.09 mmol) was dissolved in THF (5 ml) and 3 M NaOH (0.5 ml) was added. After refluxing for 48 h (TLC, 15:1 toluene-EtOH), the mixture was neutralized with 0.1 % HCl and concentrated. The residue was re-dissolved in 2:3 pyridine-Ac_2O (5 ml) and stirred overnight, when TLC (17:1 toluene-EtOH) showed disappearance of the starting material. After concentration, the residue was purified on silica gel (20:1 toluene-EtOH) to afford 180 mg of product **27** (76%). $[\alpha]_D^{25}$= +47.42 (c 0.6, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.72–7.01 (m, 80 H, 16 × Ph), 5.48–5.35 (m, 3 H, PhC*H*, 2 NH), 5.23–4.88 (m, 7 H, NH^{Cbz}, $2^{C,C'}$, H-$1^{B,B'}$, *H*CH, incl. d, 5.14, $J_{1,2}$ = 3.1 Hz,

H-1^{D}), 4.88–4.40 (m, 28 H, 25 × *H*CH, incl. s, 4.82 H-1^{A}, H-1$^{C,C'}$) 4.38–3.38 (m, 33 H, H-2^{A,D},3$^{A,B,B',C,C',D}$,4$^{A,B,B',C,C',D}$,5$^{A,B/B',C,C',D}$,6$^{A,B,B',C,C',D}$,1′), 3.29–3.18 (m, 5 H, 2$^{B,B'}$,5$^{B/B'}$,3′), 1.89–1.71 (m, 14 H, H-2′, incl. 4 × s, 1.88, 1.83, 1.78, 1.72, 4 × CH_3CO), 1.05 (s, 9 H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.70, 171.37, 170.20, 169.32, 156.38 (5 × CO), 139.85–125.20 (Ar, *C*(CH_3)$_3$), 101.80, 101.78 (C-1$^{C,C'}$), 99.72 (*C*HPh), 99.81 (C-1$^{B,B'}$), 97.41 (C-1^{A,D}), 83.07, 82.50 (C-3$^{B,B'}$), 81.00, 80.76, 79.76, 79.51, 77.82, 75.96, 75.70, 75.23, 75.00, 74.97, 74.73, 74.35, 73.65, 73.44, 72.99, 72.87, 72.46, 72.11, 71.82, 71.60, 70.45, 69.44, 68.57, 67.89, 66.31, 65.77, 64.47 (C-1′), 63.01, 55.43 (C-2$^{B/B'}$), 53.71 (C-2$^{B/B'}$), 36.94 (C-3′), 29.25 (C-2′), 26.77 (*t*-Bu), 24.06, 23.38, 21.10, 20.88 (4 × *C*H_3CO). ESI HR-MS ($C_{162}H_{179}N_3O_{35}Si$): *m/z* = ([*M*+Na]$^+$ found 2777.2048; calcd 2777.1986).



2

3-Aminopropyl β-D-glucopyranosyl-(1→3)-[6-*O*-phospho-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-)-β-D-galactopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-mannopyranoside 2. To a solution of the silylated hexasaccharide **27** (95 mg, 0.034 mmol) in THF (3 ml) 0.1 M TBAF in THF (1 ml, 0.1 mmol) was added at 0°C. After stirring for 2 h at room temperature TLC (17:1 toluene-EtOH) showed complete deprotection. The solvent was evaporated and the residue was purified on silica gel (20:1 toluene-EtOH) to afford 85 mg of product **28** (94%). The product showed disappearance of the *t*-Bu signal at ^{1}H NMR. ESI HR-MS ($C_{146}H_{161}N_3O_{35}$): *m/z* = ([*M*+Na]$^+$ found 2539.0872; calc 2539.0808).

1H-Tetrazole 0.45 M in acetonitrile (1.8 ml, 0.8 mmol) was added to a solution of the foregoing hexasaccharide **28** (65 mg, 0.026 mmol) and N,N-diethyl-1,5-dihydro-3H-2,4,3-benzodioxaphosphepin-3-amine (19 mg, 0.08 mmol) in CH_2Cl_2 (8 mL). After the reaction mixture was stirred at room temperature for 40 min, TLC (17:1 toluene-EtOH) showed formation of a new product. The mixture was cooled to -20°C, then 3-chloroperoxybenzoic acid (*m*-CPBA) (50 mg, 50–55% wt, 0.11 mmol) was added. The reaction mixture was stirred at -20°C for 20 min (TLC, 17:1 toluene-EtOH), and then quenched by addition of aq $NaHCO_3$ (3 ml) and diluted with CH_2Cl_2 (10 mL). The solution was washed with aq $NaHCO_3$ and brine. After work up the organic phase was concentrated, and the residue was purified on silica gel to give 58 mg of phosphorylated product **29** (81%). Introduction of phosphate group was confirmed by ^{31}P-NMR and ESI-MS analysis. ^{31}P NMR ($CDCl_3$, 162 MHz): δ = -0.36 ppm. ESI HR-MS ($C_{154}H_{168}N_3O_{38}P$): *m/z* = ([*M*+Na]$^+$ found 2721.0991; calcd 2721.0941).

The phosphorylated hexasaccharide **29** was then deprotected in flow chemistry, using a H-Cube Thales-Nano system. Compound **29** (38 mg, 0.014 mmol) was dissolved in MeOH-H_2O 9:1 (10 ml) and hydrogenated for 6 h, over a Pd/C 10% cartridge at room temperature and atmospheric pressure. The solvent was then evaporated and the crude material was dissolved in 1:1 MeOH-H_2O (2 ml). A 0.5 M methanolic solution of NaOMe was added until pH = 9 and the mixture was left to react at room temperature over night. The mixture was then neutralized with aq 0.1% HCl and evaporated. The obtained crude was desalted using a G10 PD MiniTrap^{TM} GE Healthcare cartridge, giving 19 mg of the final hexasaccharide **2** (99%). ^{1}H and ^{13}C NMR data are reported in Table 2. $[\alpha]_D^{24}$= +18.68 (c 0.23, H_2O).

^{31}P NMR (D_2O, 162 MHz): δ = 4.89. ESI HR-MS ($C_{43}H_{76}N_3O_{34}P$): *m/z* = ([*M*+H]$^+$ found 1210.4080 calcd 1210.4126); ([*M*+Na]$^+$ found 1232.3951 calc 1232.3946).

Table 2. ^{1}H and ^{13}C-NMR[a] δ (ppm), recorded at 400 MHz, D_2O, 298 K, of hexasaccharide **2**

	α-Man ***(A)***	***β-GalNAc*** ***(B)***	***β-Glc*** ***(C)***	***β-GalNAc*** ***(B')***	***β-Glc*** ***(C')***	***α-Glc*** ***(D)***	***Linker***
H-1	4.86	4.76	4.49	4.60	4.41	4.95	
		$J_{1,2}$=8.6 Hz	$J_{1,2}$=7.8 Hz	$J_{1,2}$=8.6 Hz	$J_{1,2}$=7.8 Hz	$J_{1,2}$=3.4 Hz	
C-1	100.6	100.5	105.6	102.5	106.0	99.6	
H-2	4.02	4.01	3.32	4.02	3.07	3.53	
C-2	68.9	53.0	74.1	53.0	74.2	72.4	
H-3	4.02	4.00	3.49	3.90	3.45	3.97	
C-3	79.5	79.4	76.4	80.4	76.4	72.3	
H-4	3.74	4.26	3.48	4.22	3.31	3.66	
C-4	66.2	75.6	70.6	68.4	70.6	79.8	
H-5	3.60	3.81	3.58	3.76	3.36	4.30	
C-5	65.6	76.3	76.2	76.3	76.2	71.0	
H-6	3.69, 3.80	3.67, 3.90	3.90, 4.21	3.76, 3.90	3.70, 3.91	3.66, 3.82	
C-6	61.2	61.9	65.7	61.7	63.7	60.7	
H-1'							3.61, 3.81
C-1'							65.7
H-2'							1.98
C-2'							27.6
H-3'							3.10
C-3'							38.3

[a]^{1}H and ^{13}C NMR resonances were assigned based on HMQC experiment

Table 1. Comparison of NMR[a] δ (ppm) between hexasaccharide **2** and PS-II repeating unit[b]

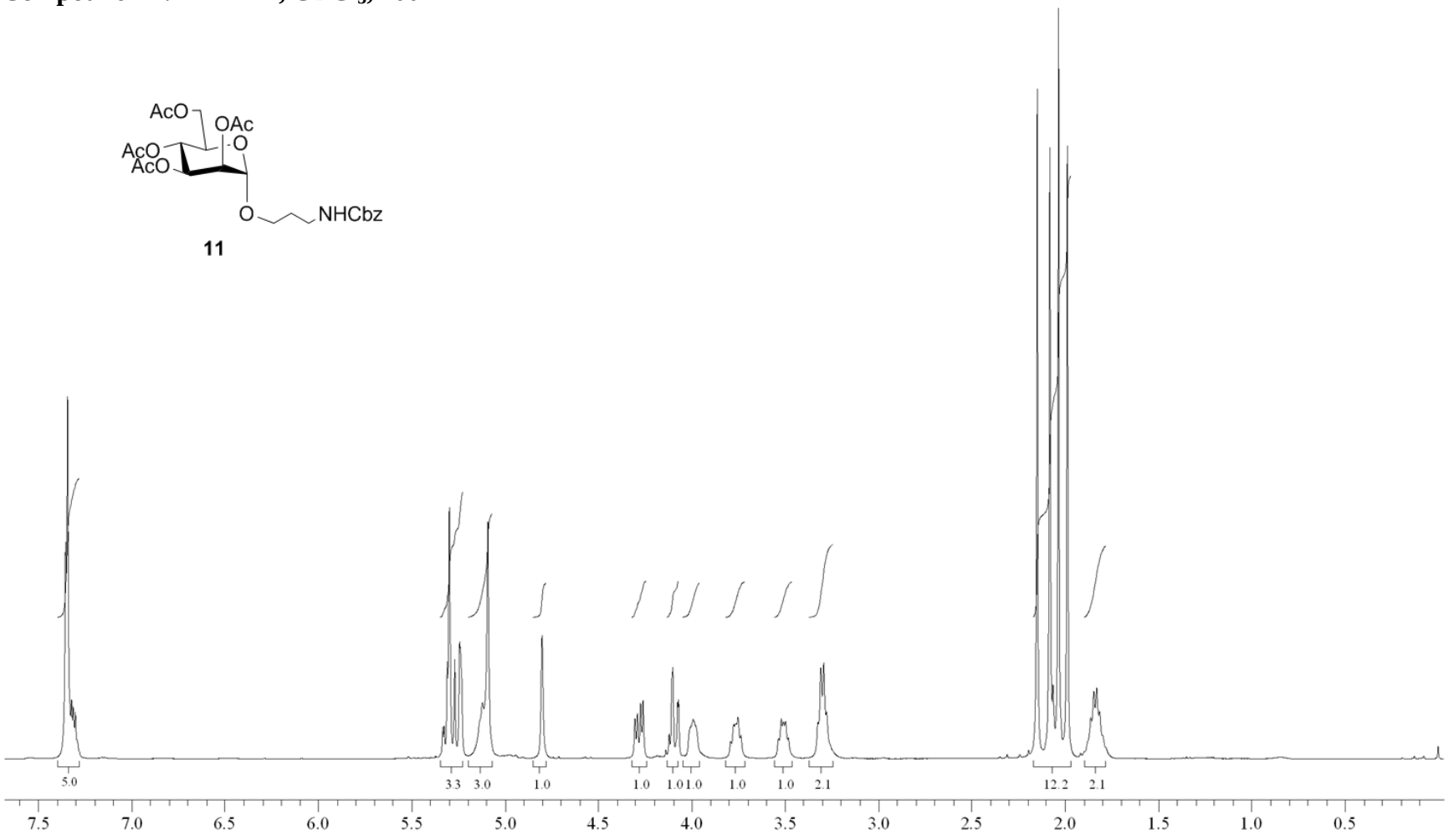
	α-Man (A)	β-GalNAc (B)	β-Glc (C)	β-GalNAc (B′)	β-Glc (C′)	α-Glc (D)
H-1	4.86	4.76	4.49	4.60	4.41	4.95
	5.44	*4.76*	*4.53*	*4.64*	*4.45*	*4.99*
C-1	100.6	100.5	105.6	102.5	106.0	99.6
	97.0	*100.7*	*105.5*	*102.3*	*106.0*	*99.6*
H-2	4.02	4.01	3.32	4.02	3.07	3.53
	4.07	*4.09*	*3.34*	*4.05*	*3.09*	*3.56*
C-2	68.9	53.0	74.1	53.0	74.2	72.4
	69.2	*53.1*	*73.8*	*52.5*	*74.1*	*72.3*
H-3	4.02	4.00	3.49	3.90	3.45	3.97
	4.07	*4.00*	*3.49*	*3.93*	*3.49*	*4.01*
C-3	79.5	79.4	76.4	80.4	76.4	72.3
	79.1	*79.6*	*76.4*	*80.9*	*76.4*	*72.3*
H-4	3.74	4.26	3.48	4.22	3.31	3.66
	3.89	*4.30*	*3.48*	*4.22*	*3.38*	*3.70*
C-4	66.2	75.6	70.6	68.4	70.6	79.8
	65.6	*75.5*	*70.2*	*68.7*	*70.7*	*79.6*
H-5	3.60	3.81	3.58	3.76	3.36	4.30
	3.83	*3.81*	*3.57*	*3.78*	*3.41*	*4.33*
C-5	65.6	76.3	76.2	76.3	76.2	71.0
	74.8	*76.2*	*75.4*	*76.2*	*76.7*	*70.8*
H-6	3.69, 3.80	3.67, 3.90	3.90, 4.21	3.76, 3.90	3.95, 4.00	3.66, 3.82
	n.d.	*n.d.*	*4.07, 4.19*	*n.d.*	*3.74, 3.93*	*3.68, 3.84*
C-6	61.2	61.9	65.7	61.7	63.7	60.7
	n.d.	*n.d.*	*65.7*	*n.d.*	*61.7*	*60.4*

[a] NMR were measured at 400 MHz, 298 K. [b] Data of PS-II are reported in italic

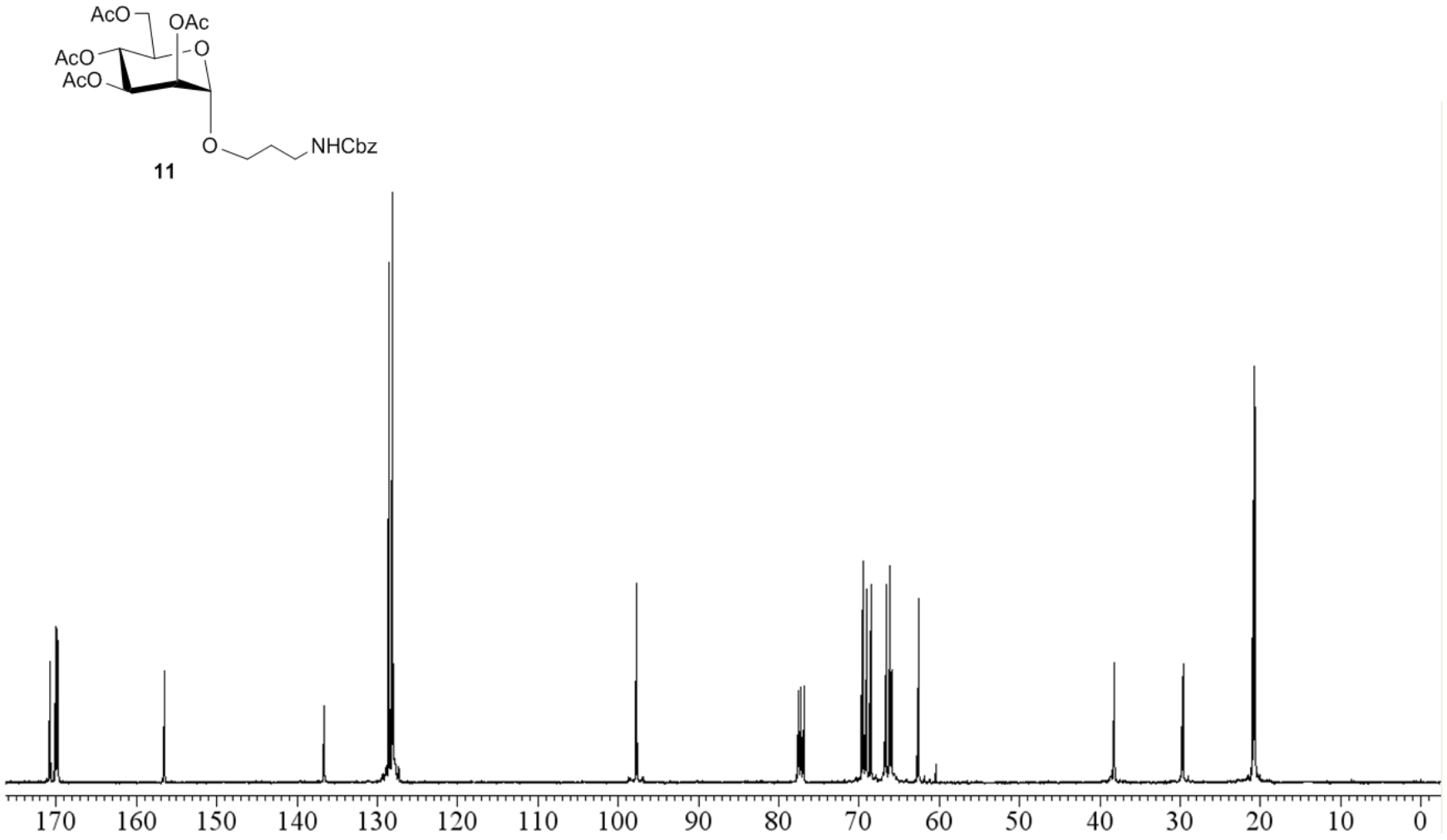
[1]M. Nitz, D. R. B. *J. Org. Chem.* **2000**, *65*, 3064.
[2] Haag, Eur. JOC, 2007

Spectra of compounds

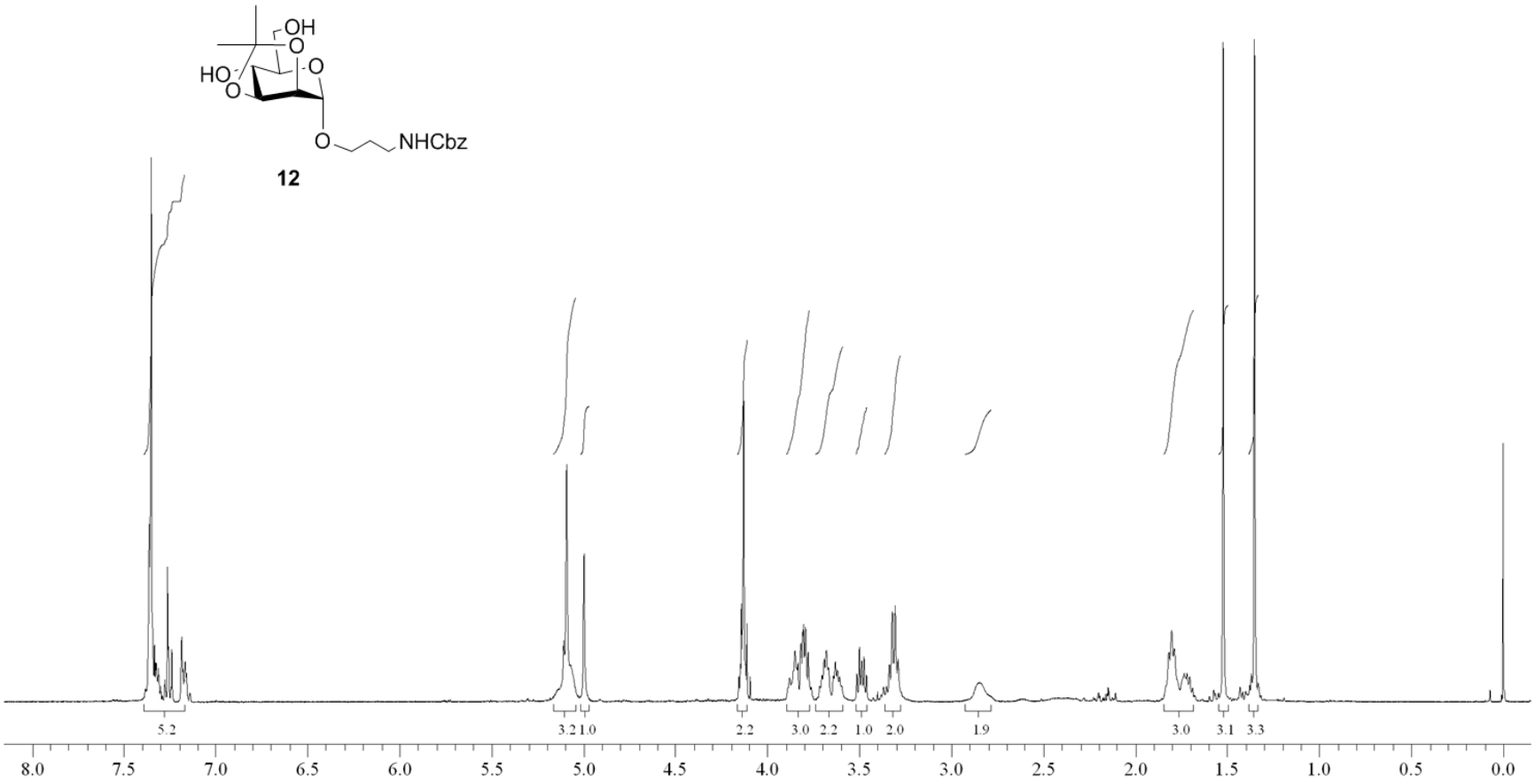
Compound 11: ^{1}H NMR, $CDCl_3$, 400 MHz



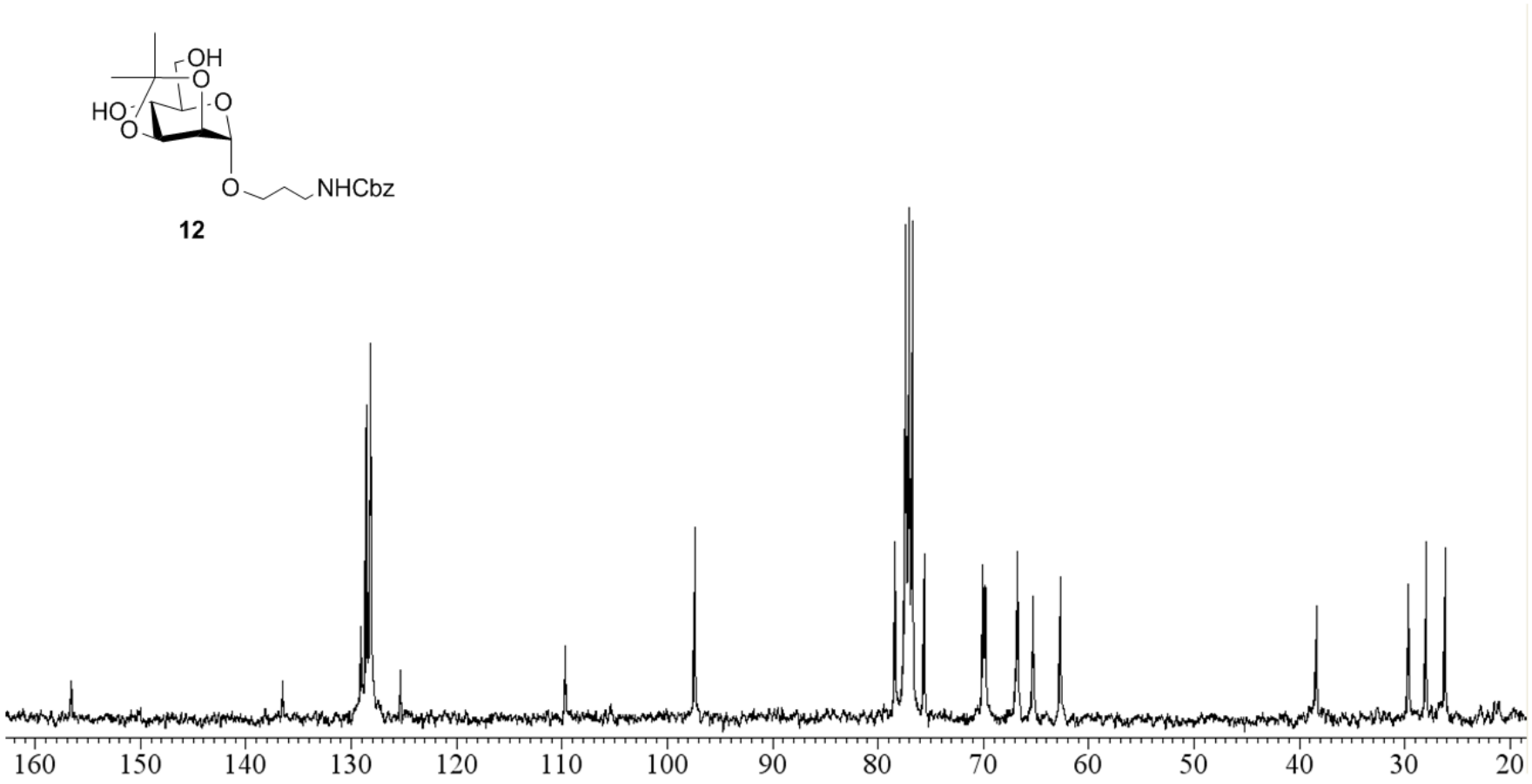
Compound 11: ^{13}C NMR, $CDCl_3$, 100 MHz



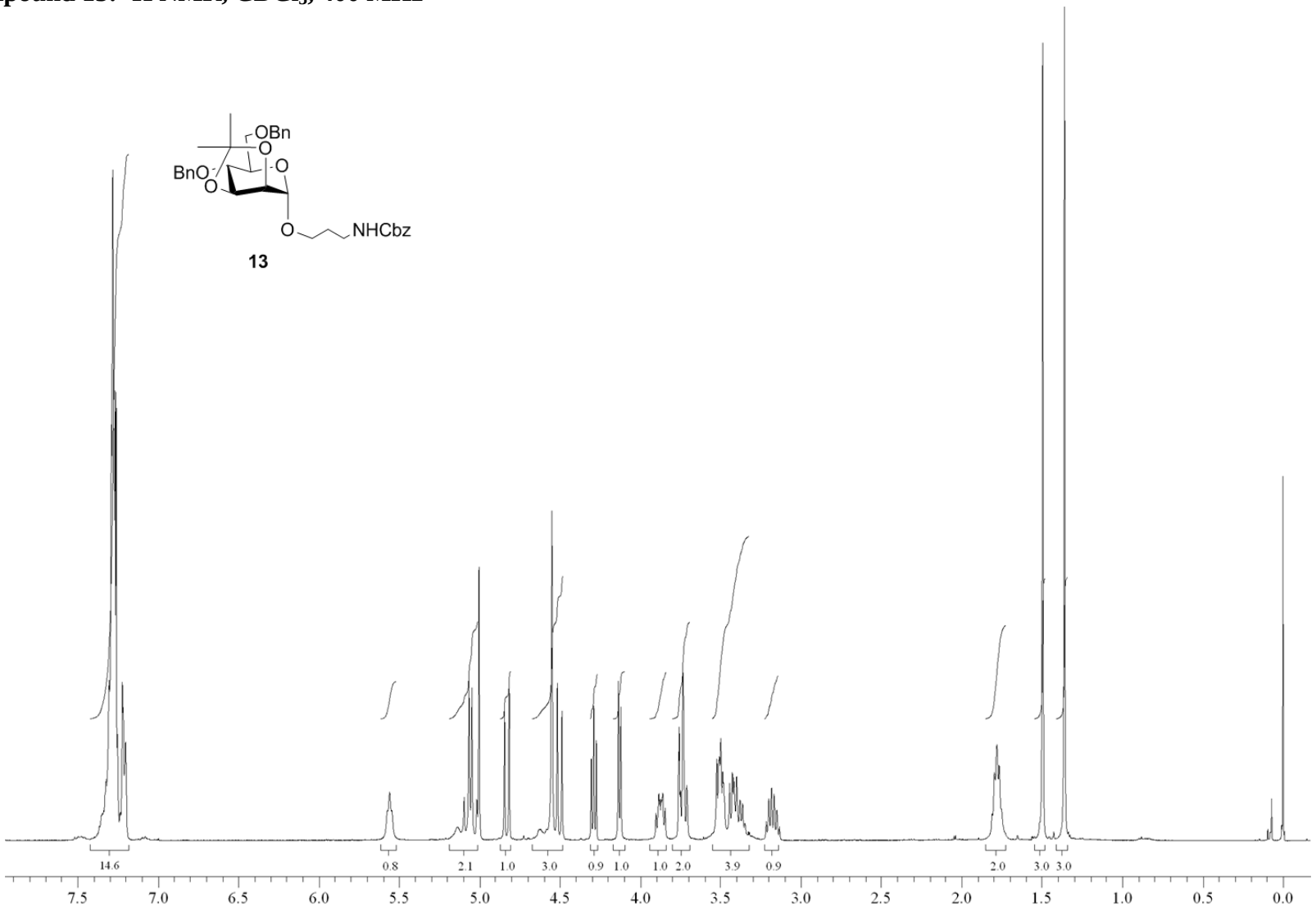
Compound 12: ^{1}H NMR, $CDCl_3$, 400 MHz



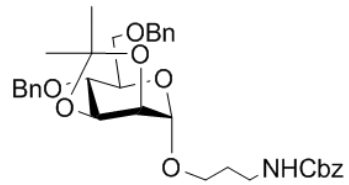
Compound 12: ^{13}C NMR, $CDCl_3$, 100 MHz



Compound 13: ^{1}H NMR, $CDCl_3$, 400 MHz

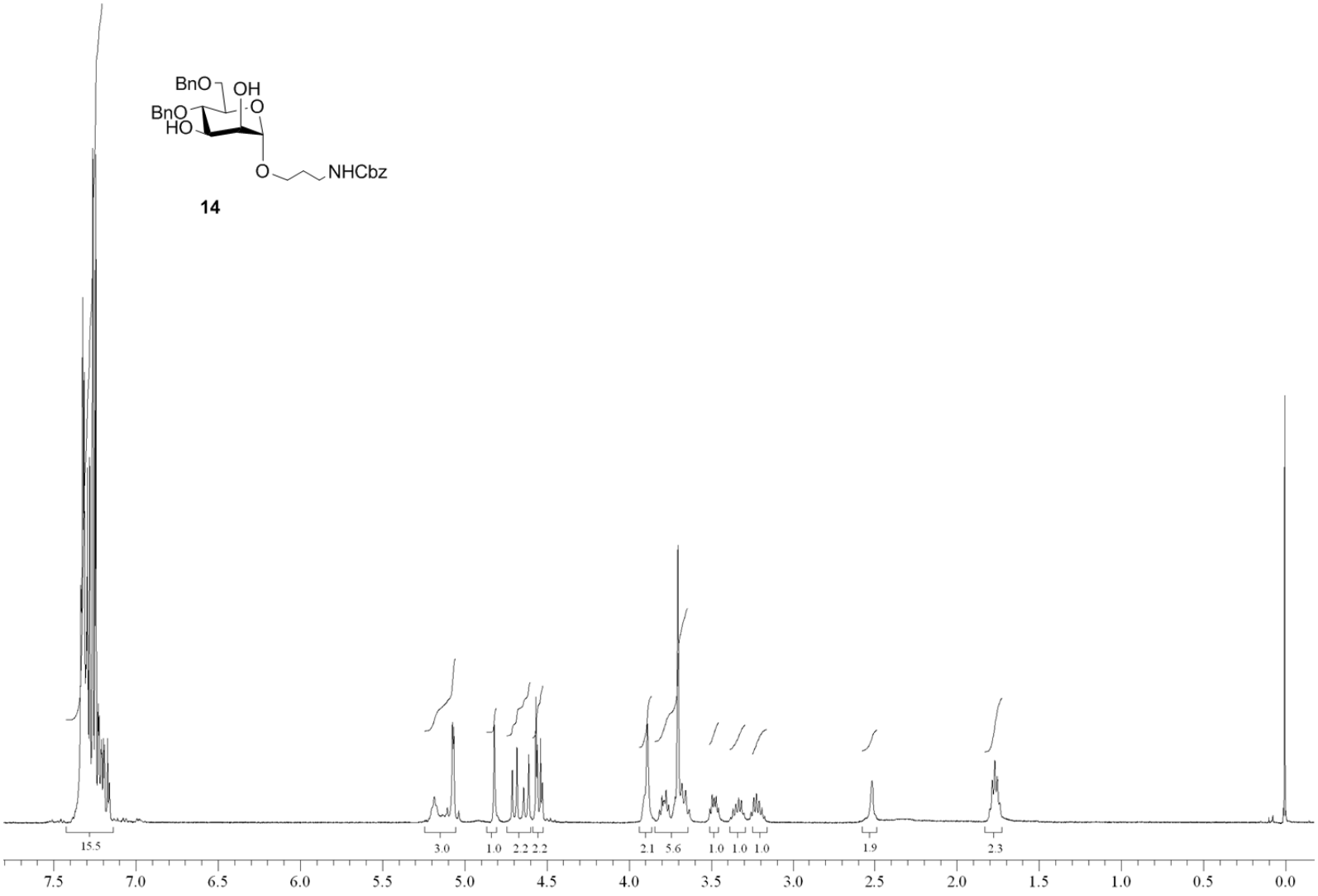


Compound 13: ^{13}C NMR, $CDCl_3$, 100 MHz

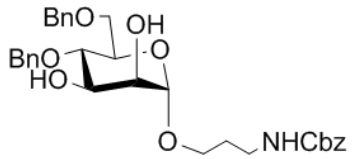


13

Compound 14: ^{1}H NMR, $CDCl_3$, 400 MHz



Compound 14: ^{13}C NMR, $CDCl_3$, 100 MHz



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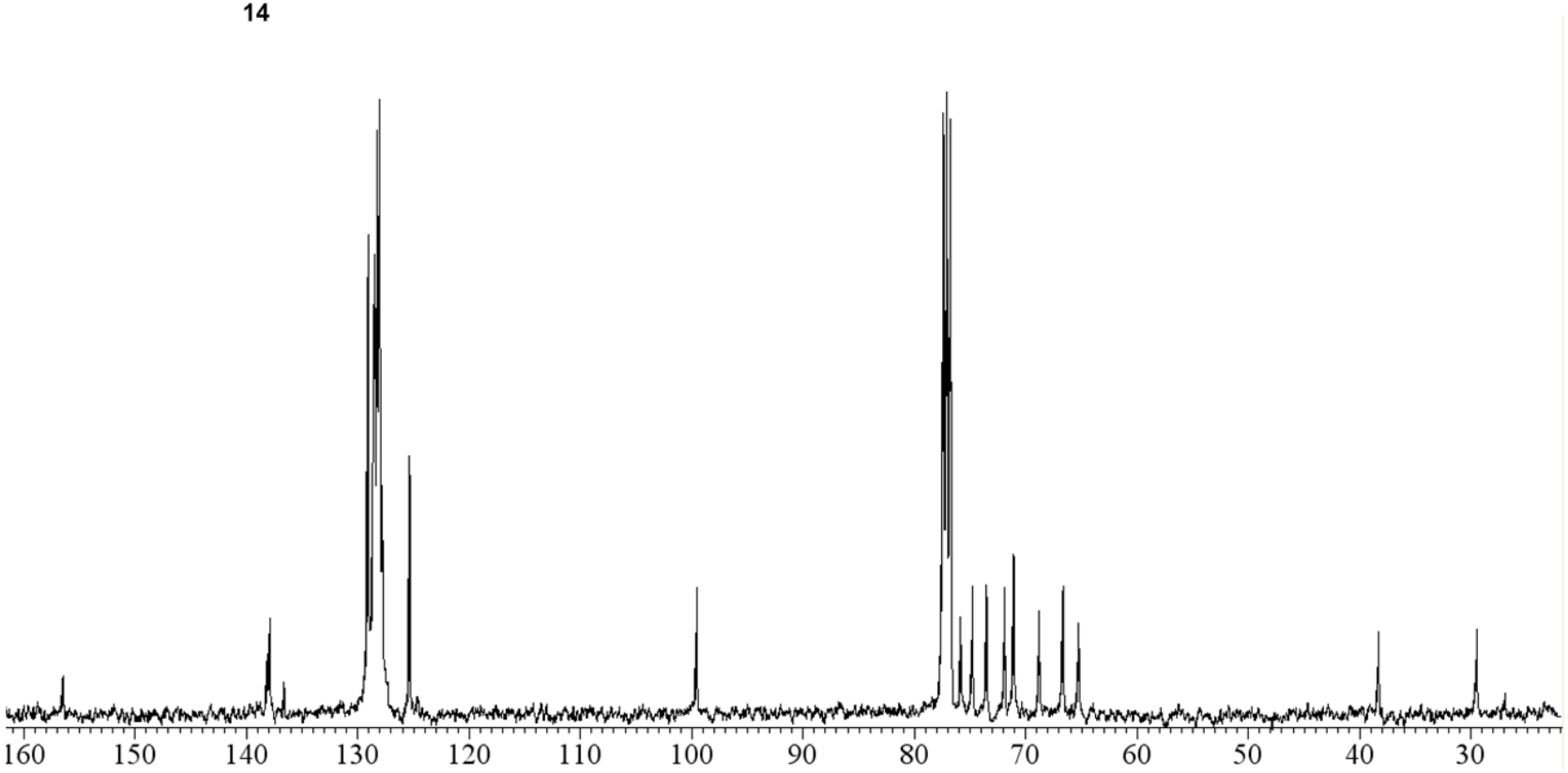
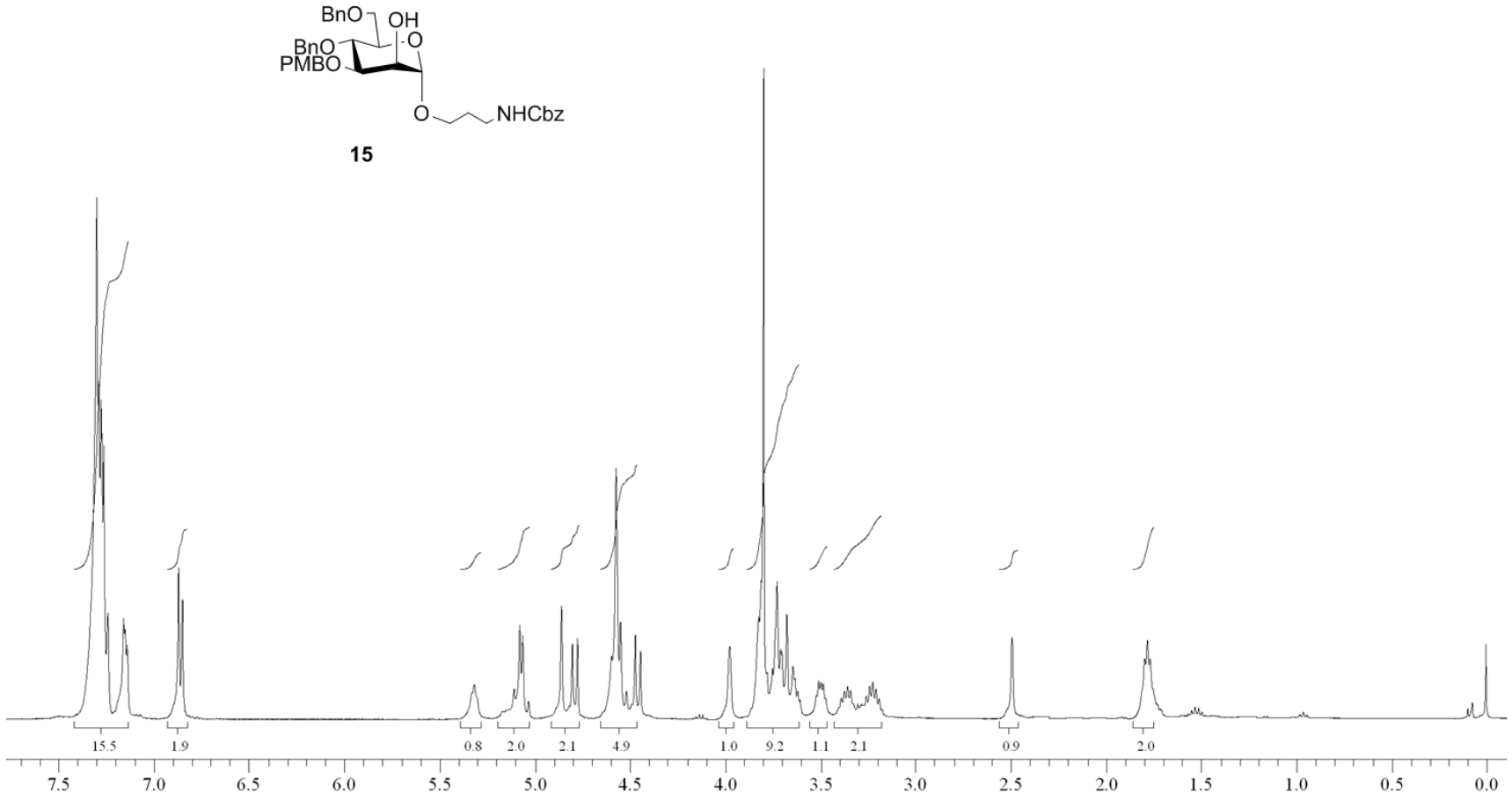
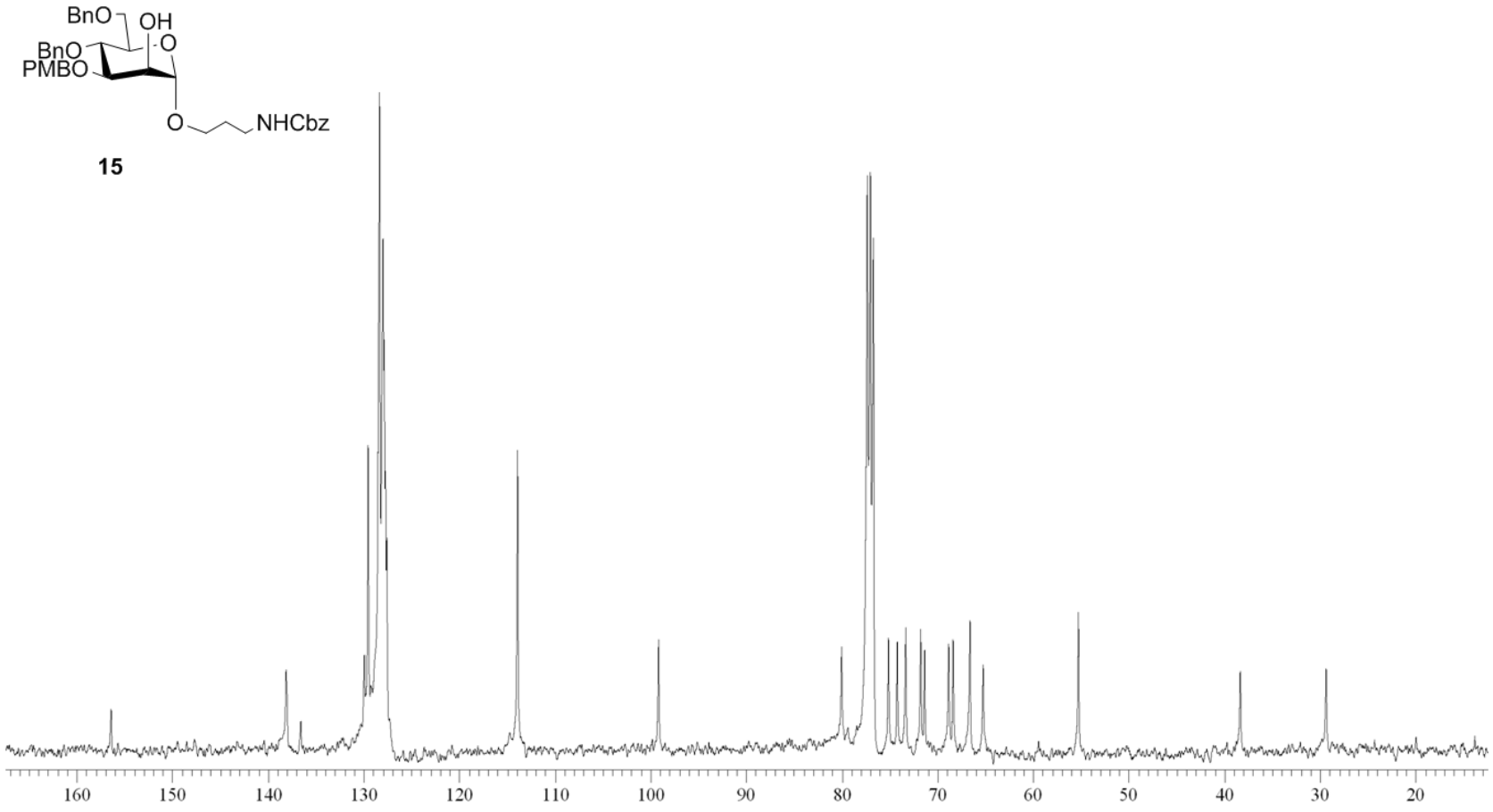


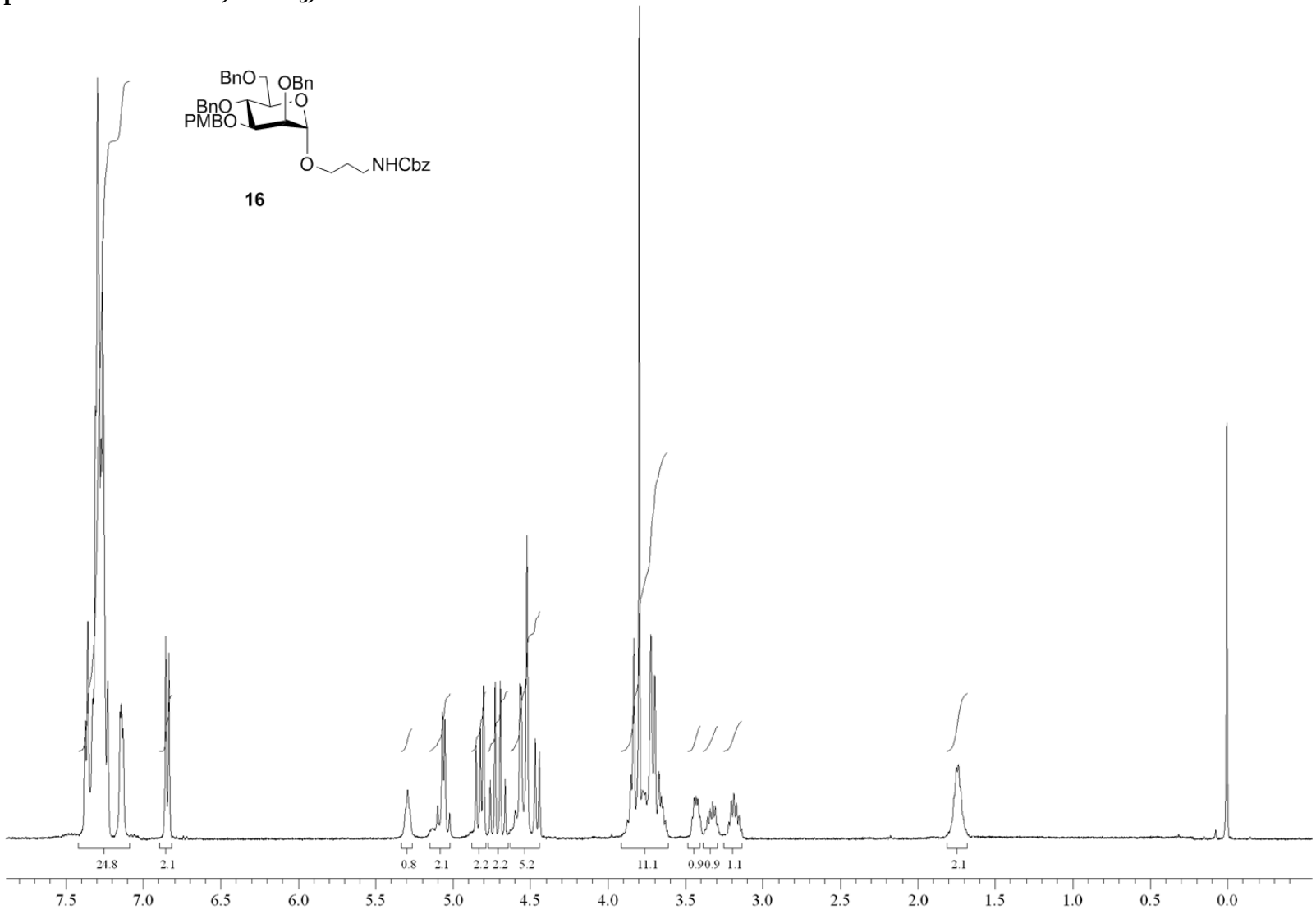
Compound 15: ^{1}H NMR, $CDCl_3$, 400 MHz



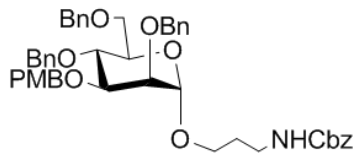
Compound 15: ^{13}C NMR, $CDCl_3$, 100 MHz



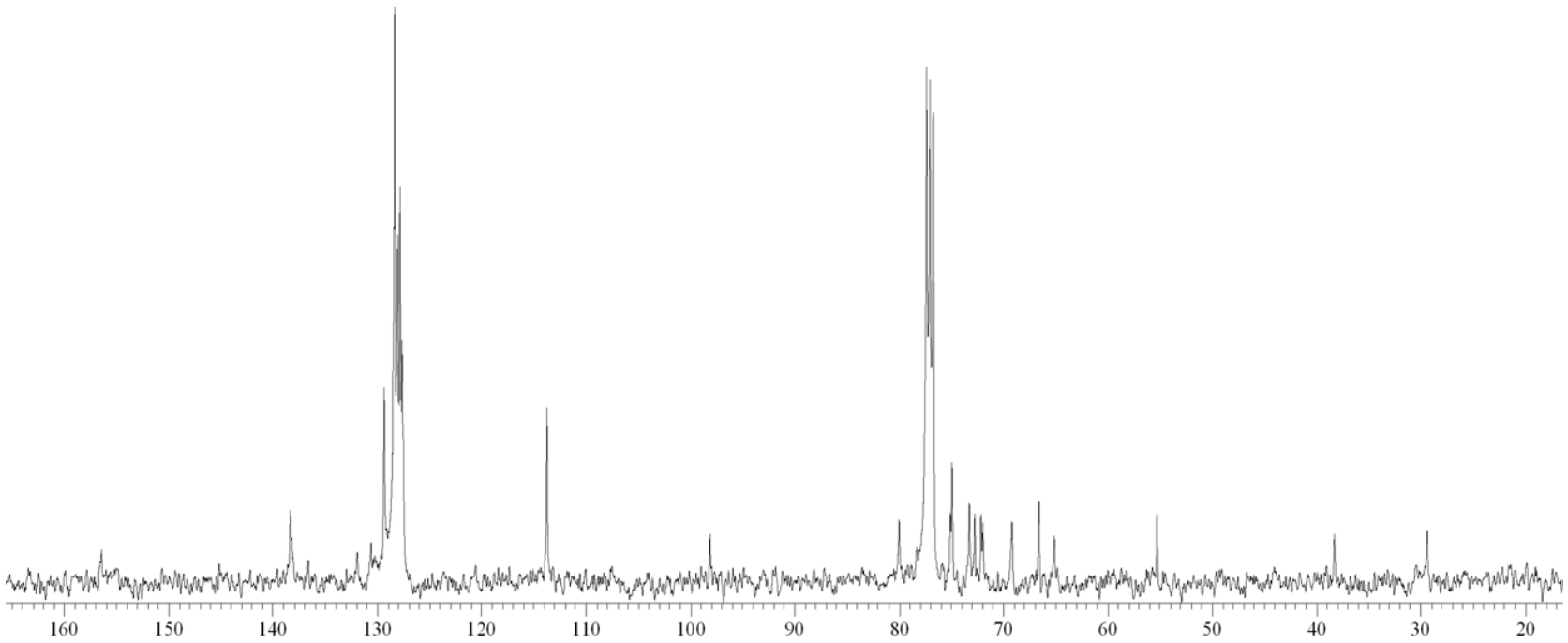
Compound 16: ^{1}H NMR, $CDCl_3$, 400 MHz



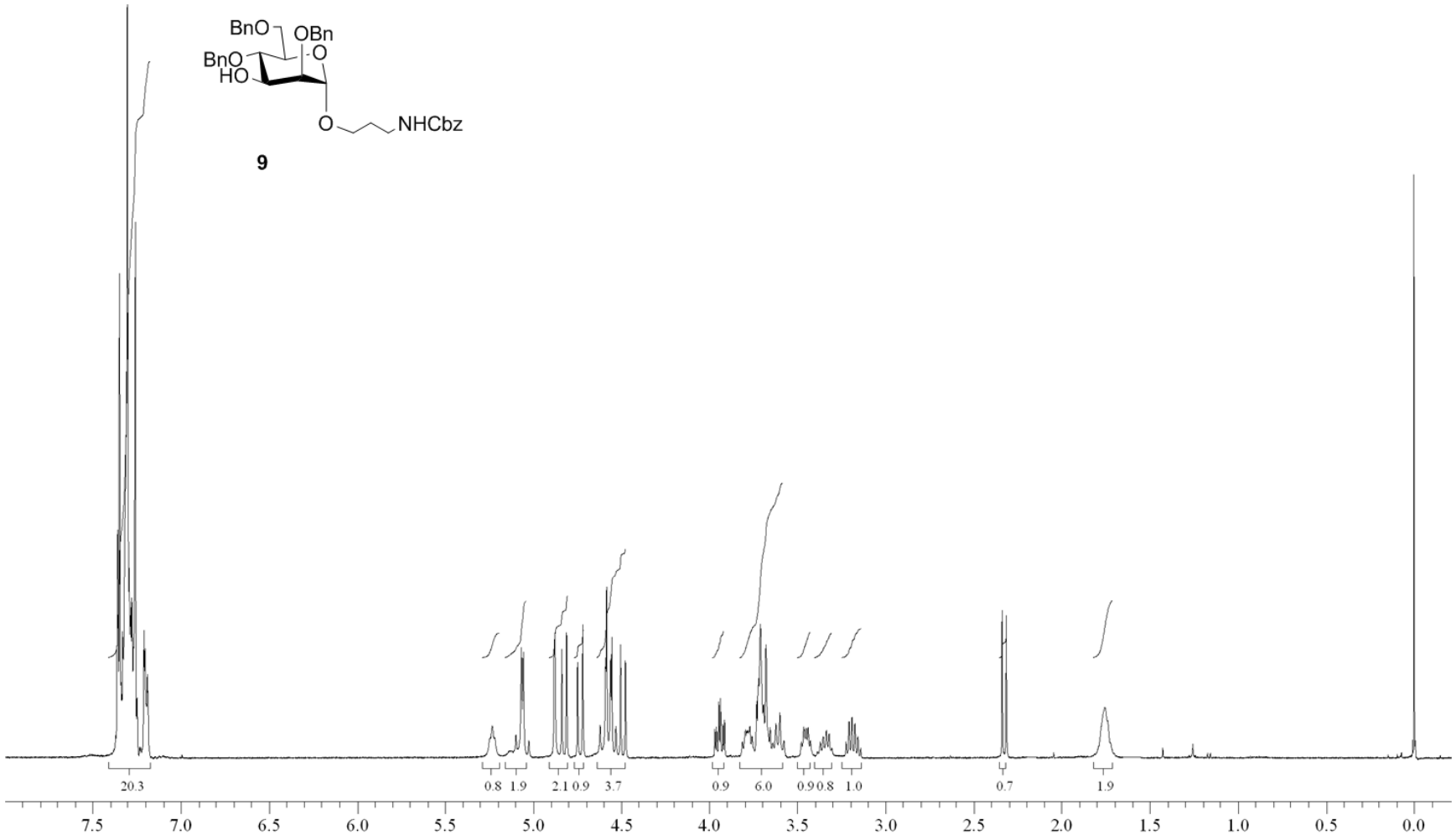
Compound 16: ^{13}C NMR, $CDCl_3$, 100 MHz



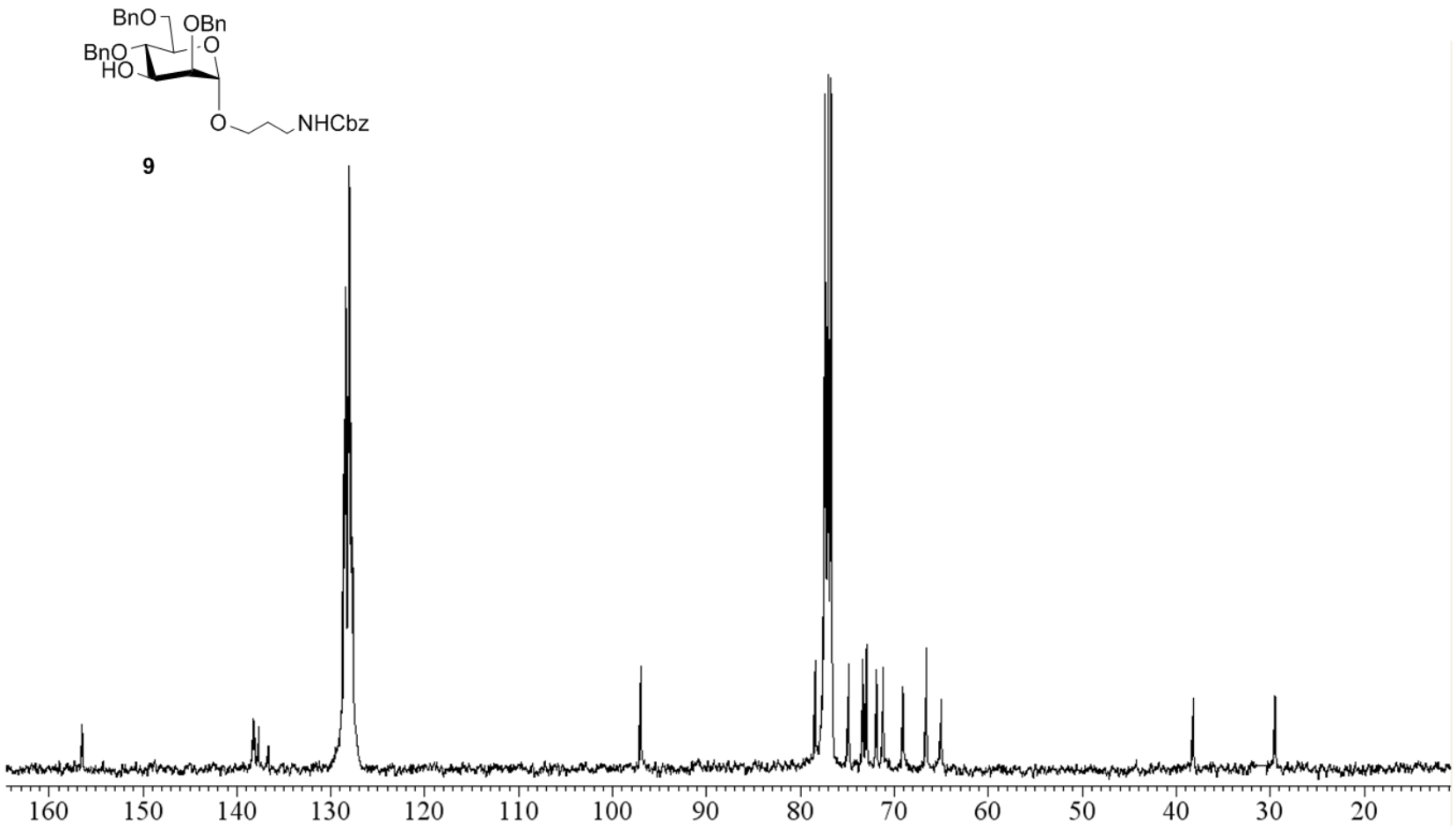
16



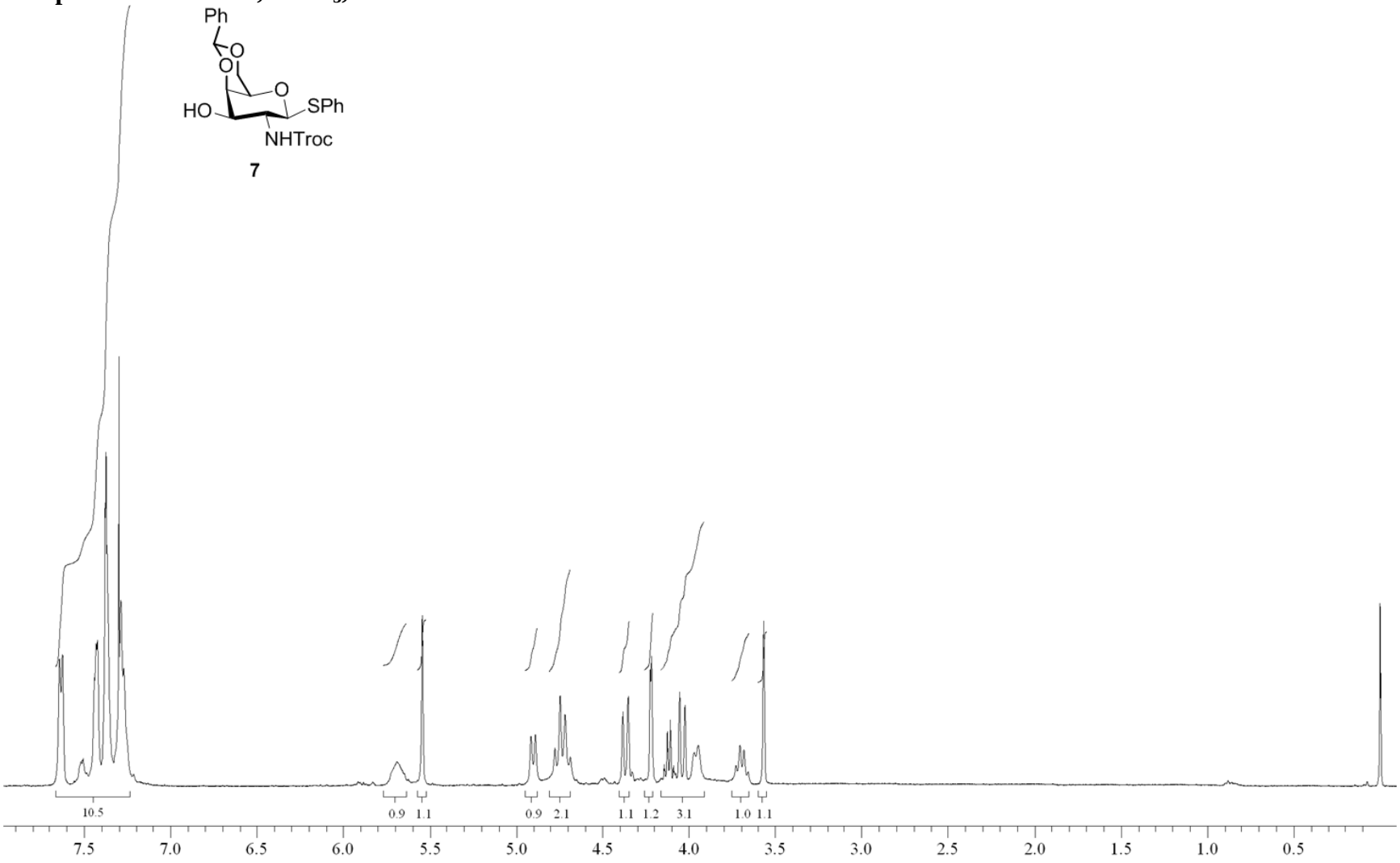
Compound 9: ^{1}H NMR, $CDCl_3$, 400 MHz



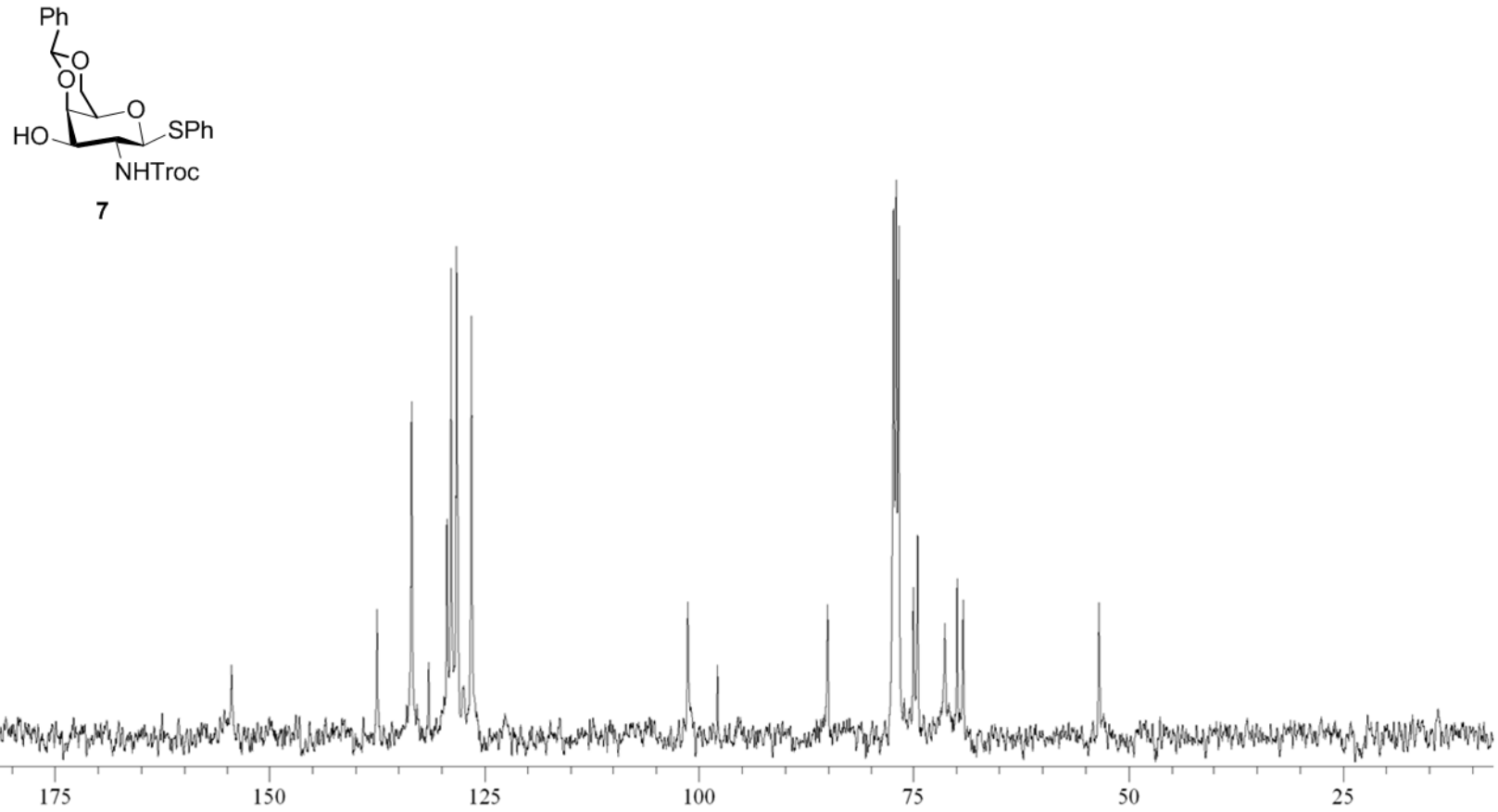
Compound 9: ^{13}C NMR, $CDCl_3$, 100 MHz



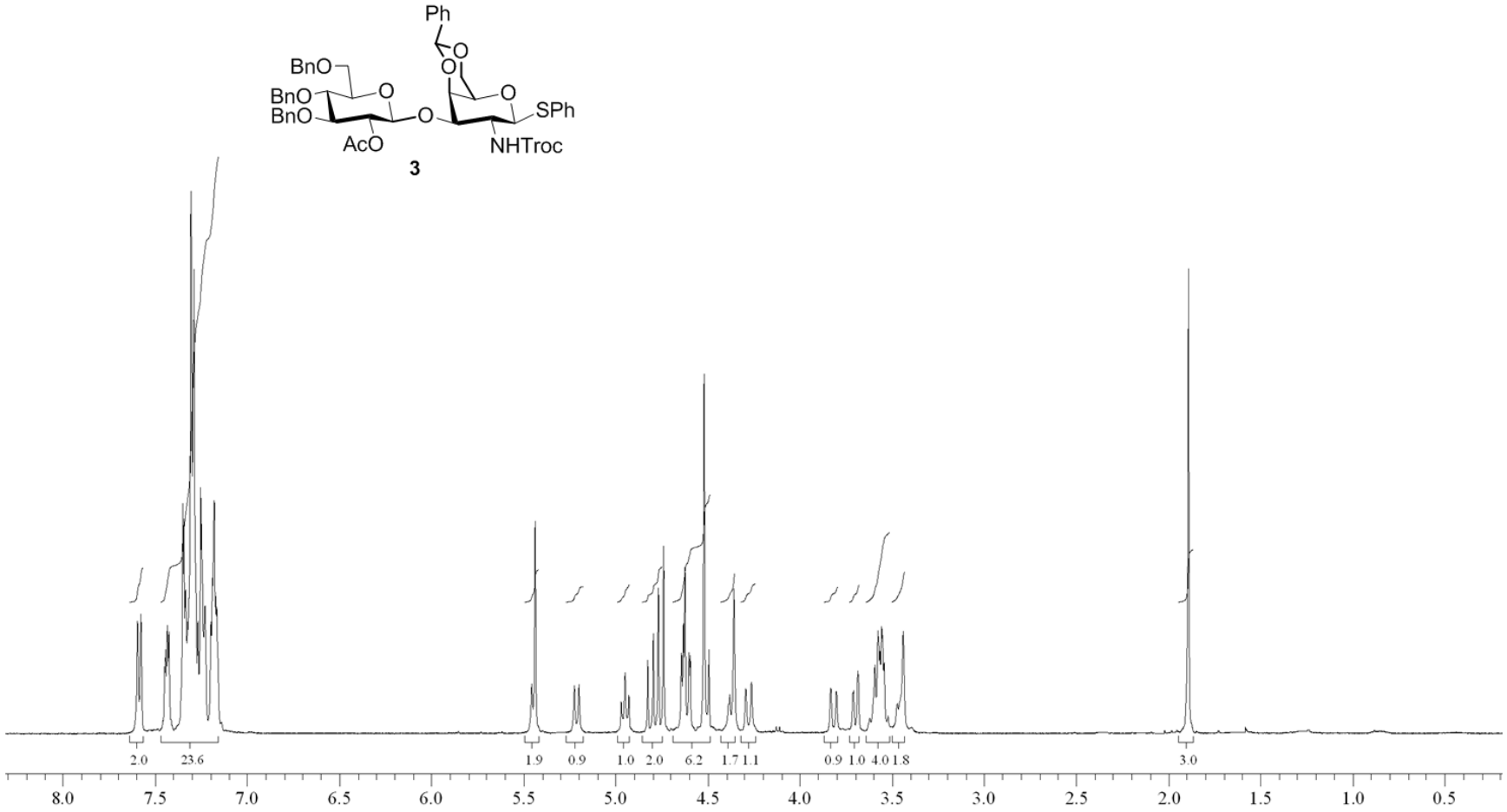
Compound 7: ^{1}H NMR, $CDCl_3$, 400 MHz



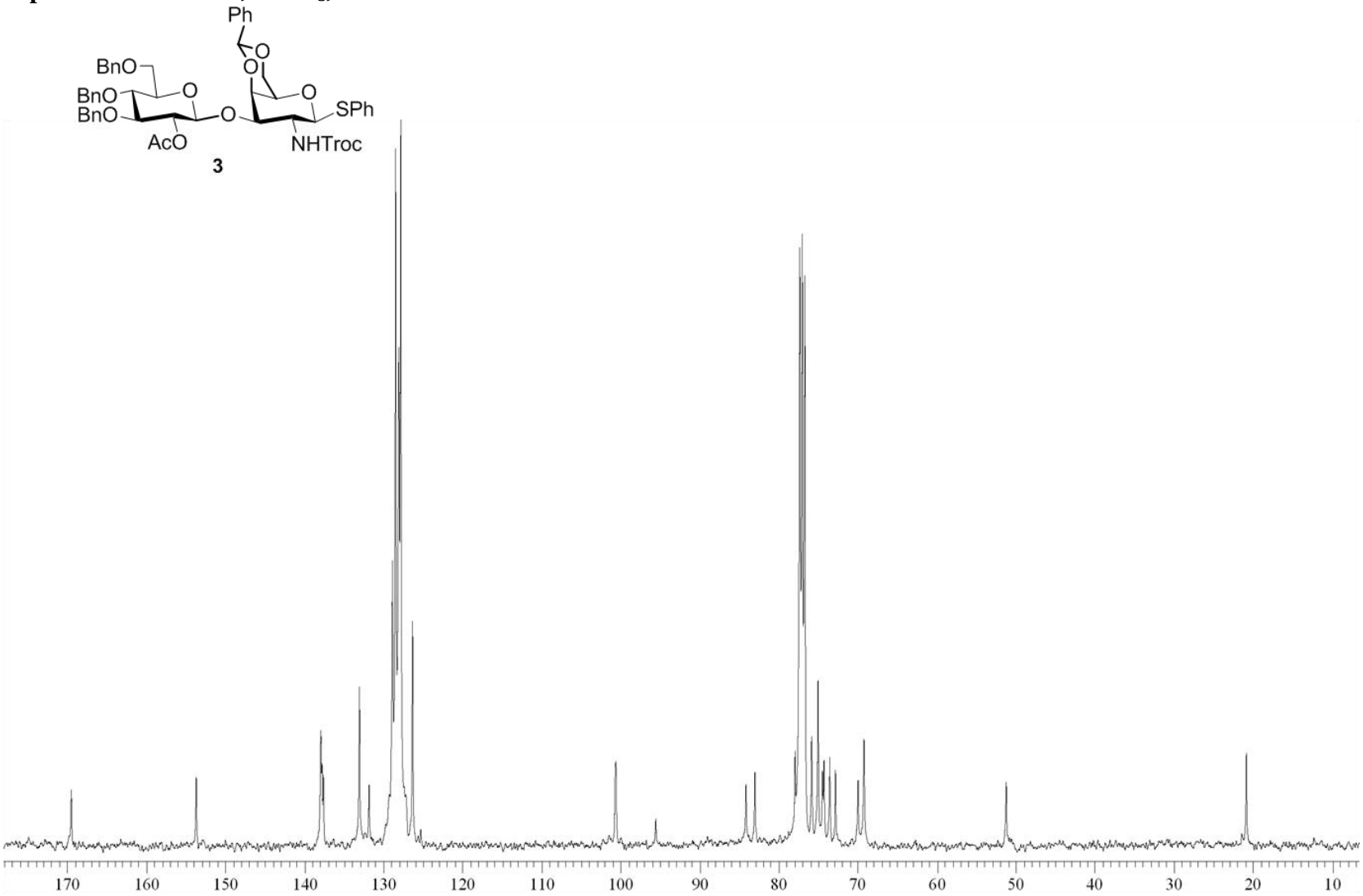
Compound 7: ^{13}C NMR, $CDCl_3$, 100 MHz



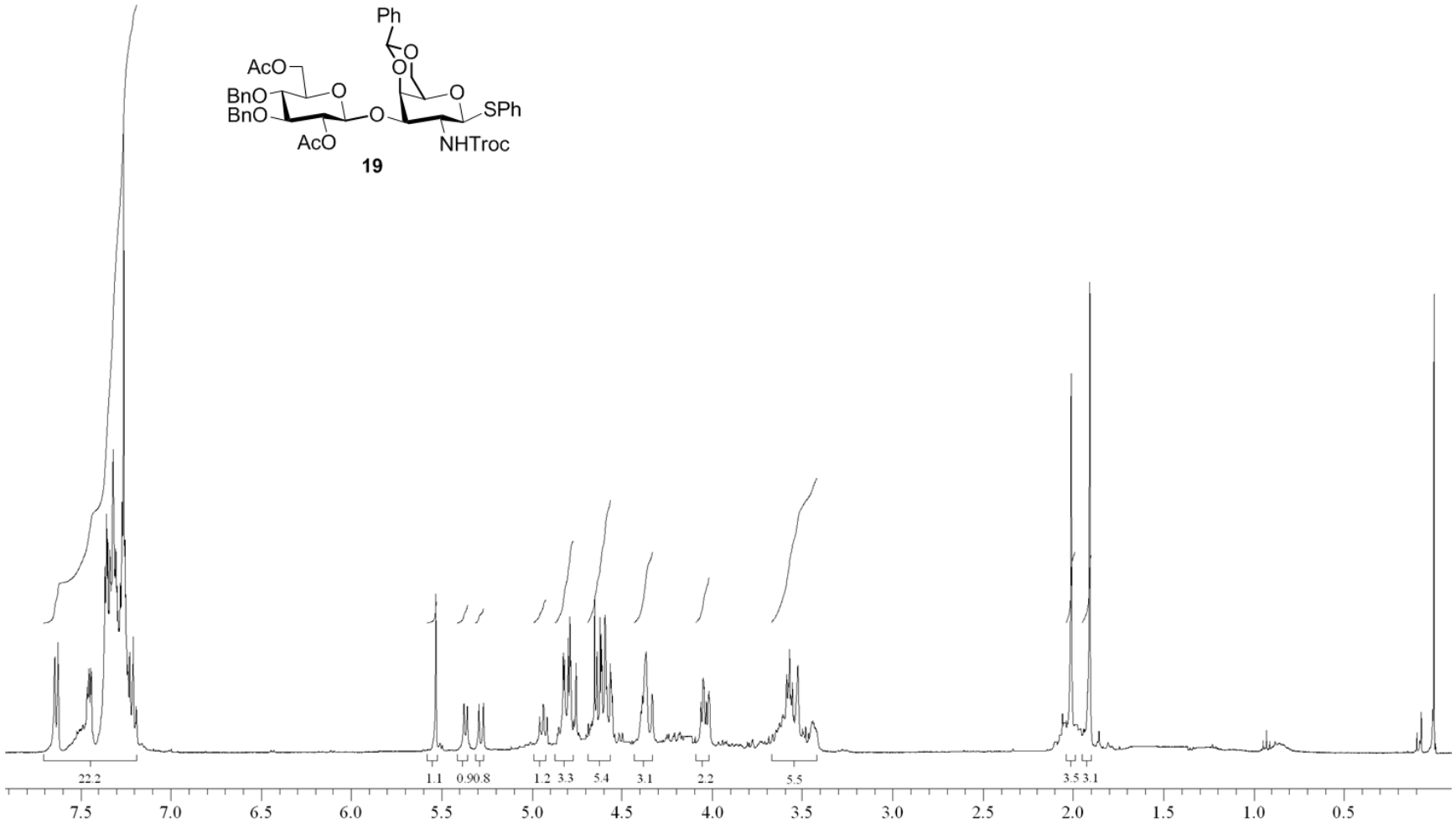
Compound 3: ^{1}H NMR, $CDCl_3$, 400 MHz



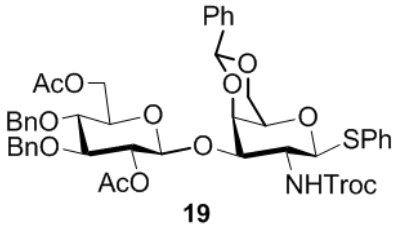
Compound 3: ^{13}C NMR, $CDCl_3$, 100 MHz



Compound 19: ^{1}H NMR, $CDCl_3$, 400 MHz



Compound 19: ^{13}C NMR, $CDCl_3$, 100 MHz



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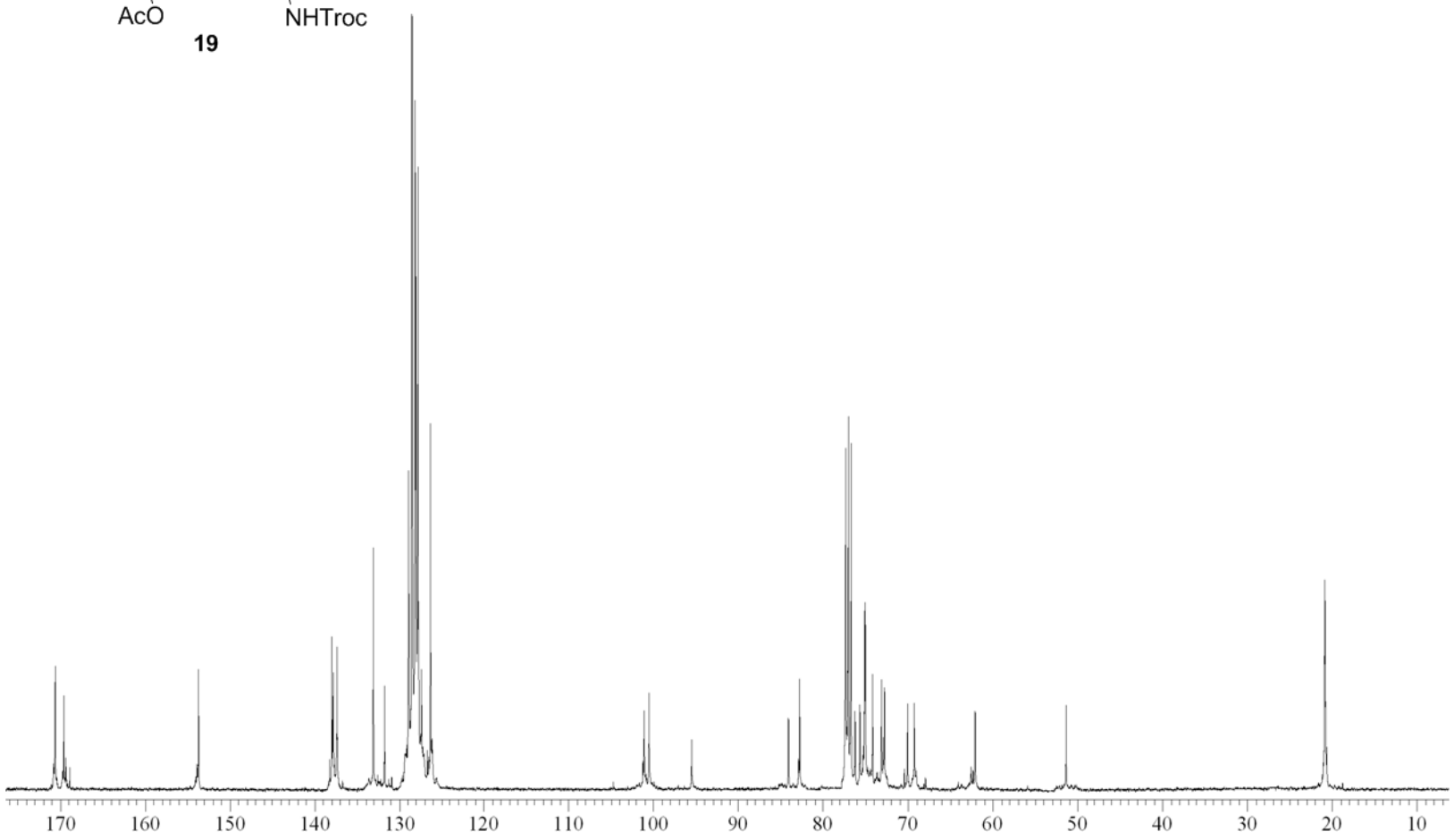
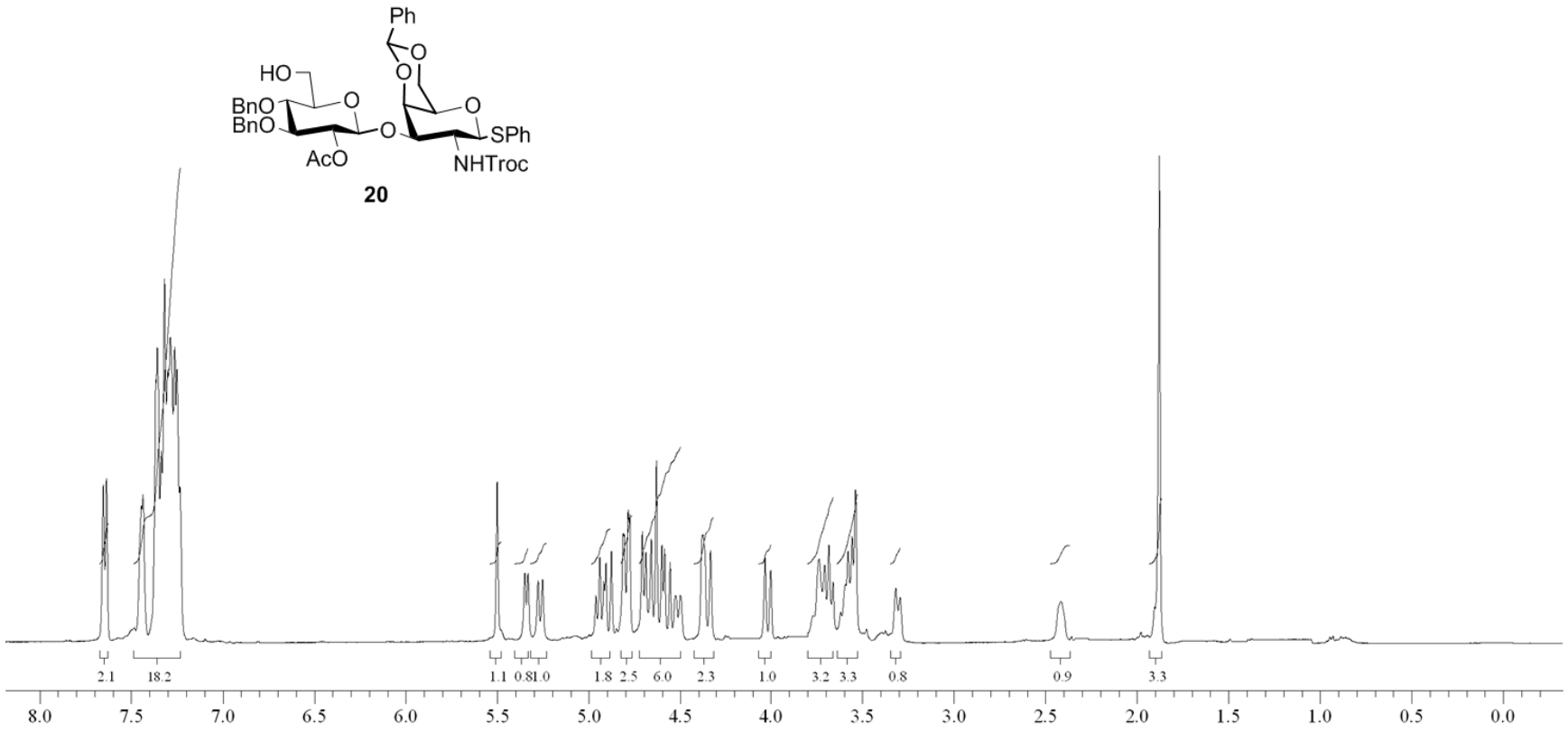
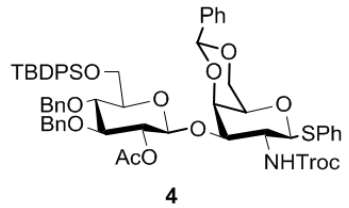


Compound 20: ^{1}H NMR, $CDCl_3$, 400 MHz

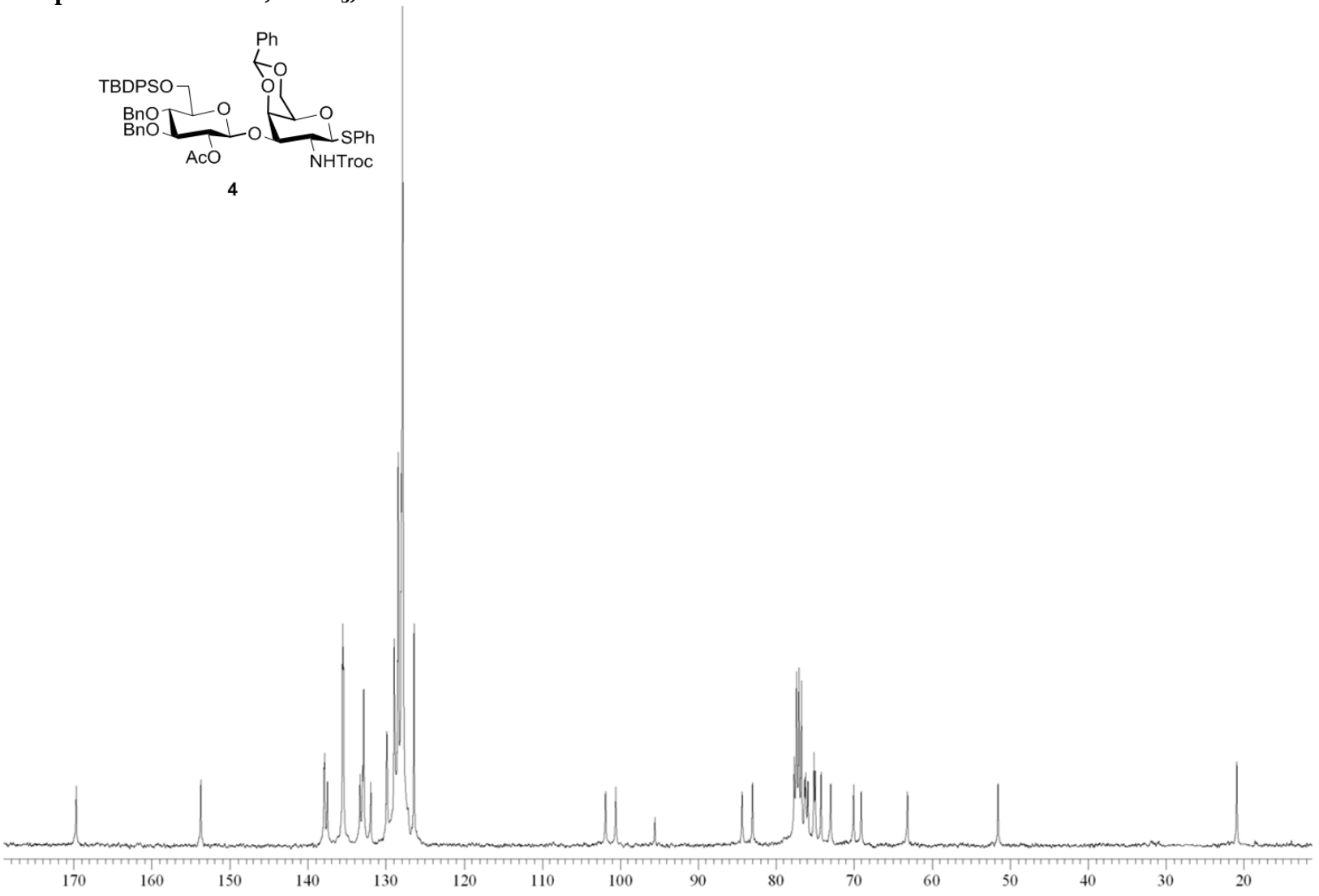


Compound 4: ^{1}H NMR, $CDCl_3$, 400 MHz

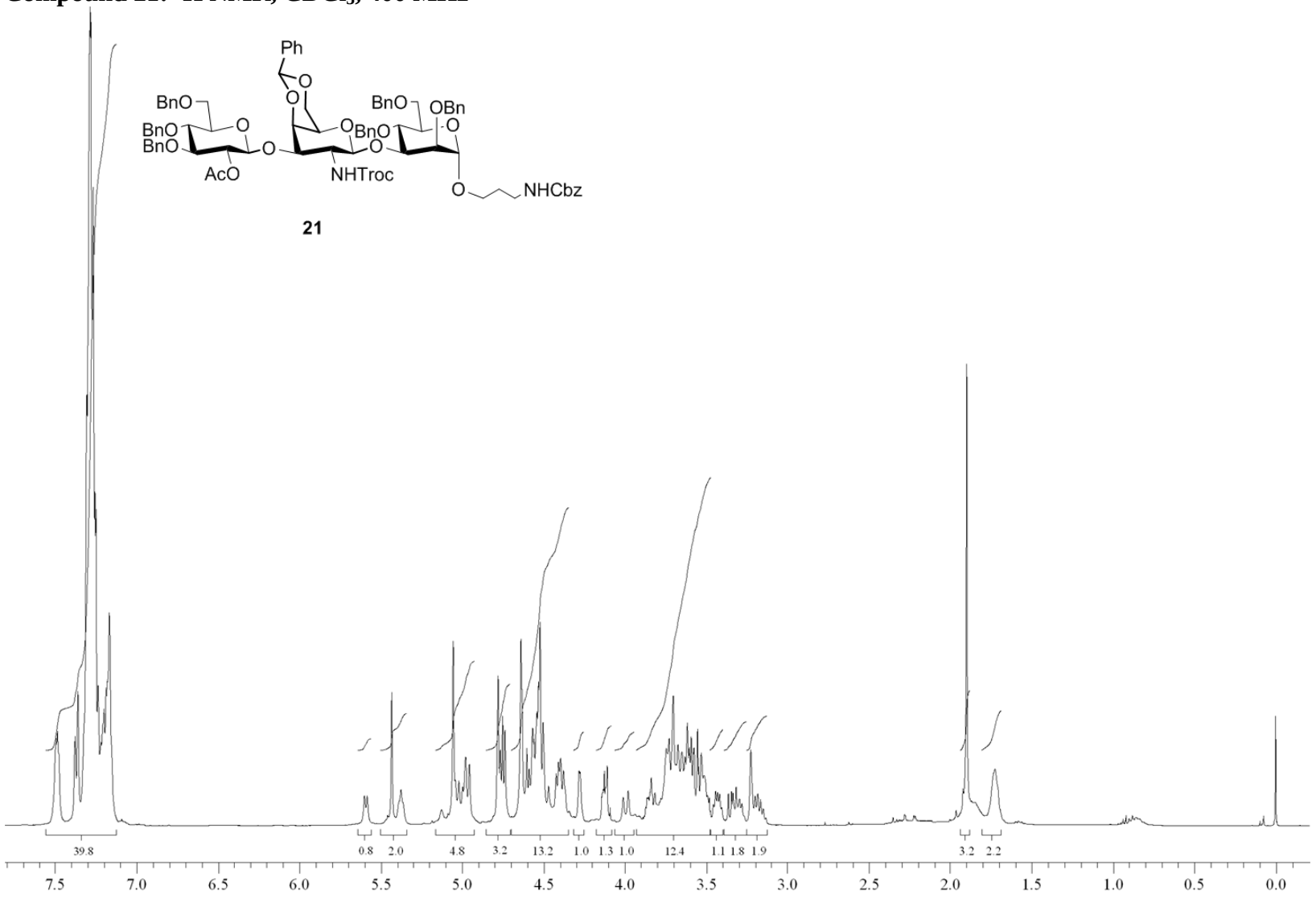


4

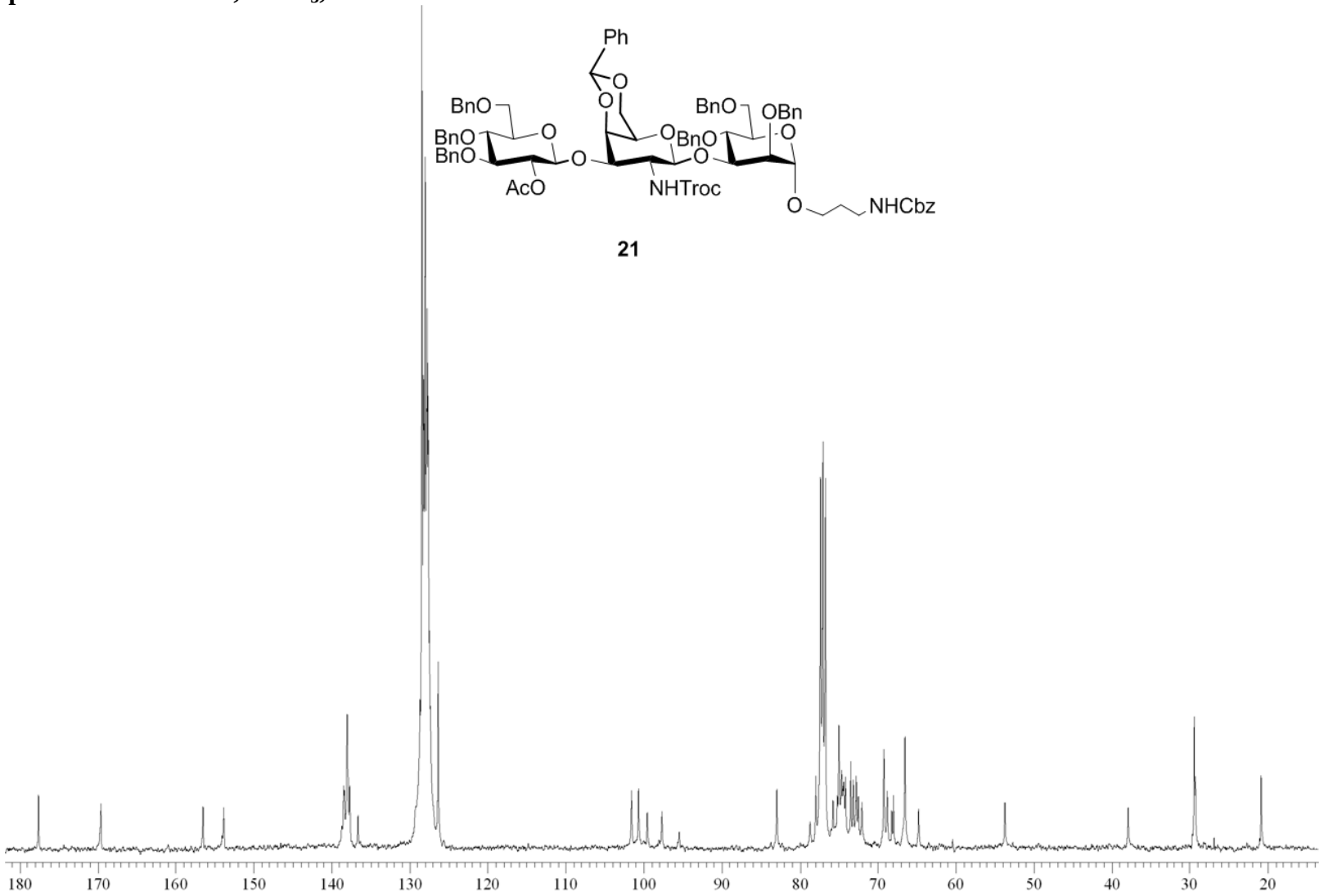
Compound 4: ^{13}C NMR, $CDCl_3$, 400 MHz



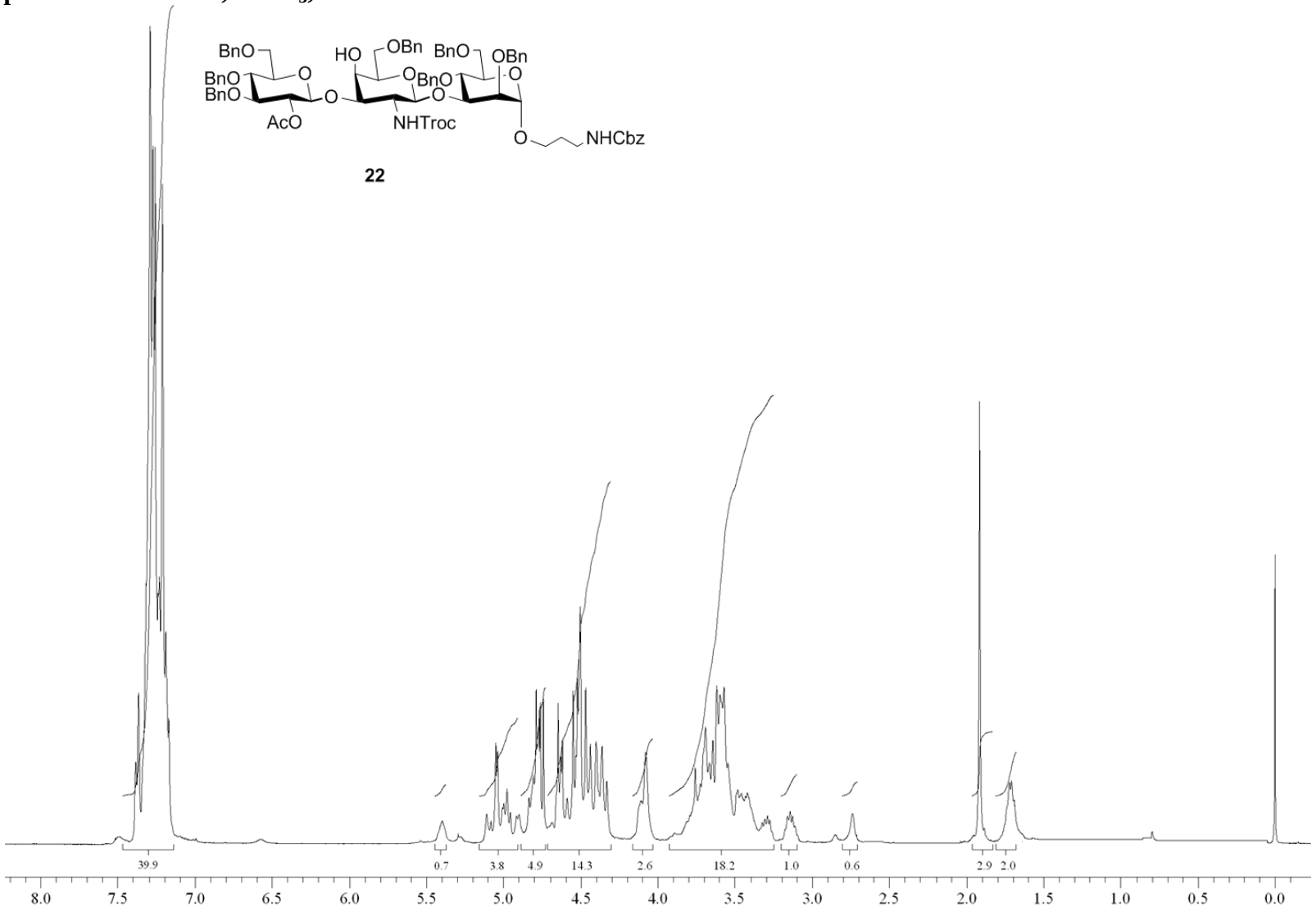
Compound 21: ^{1}H NMR, $CDCl_3$, 400 MHz



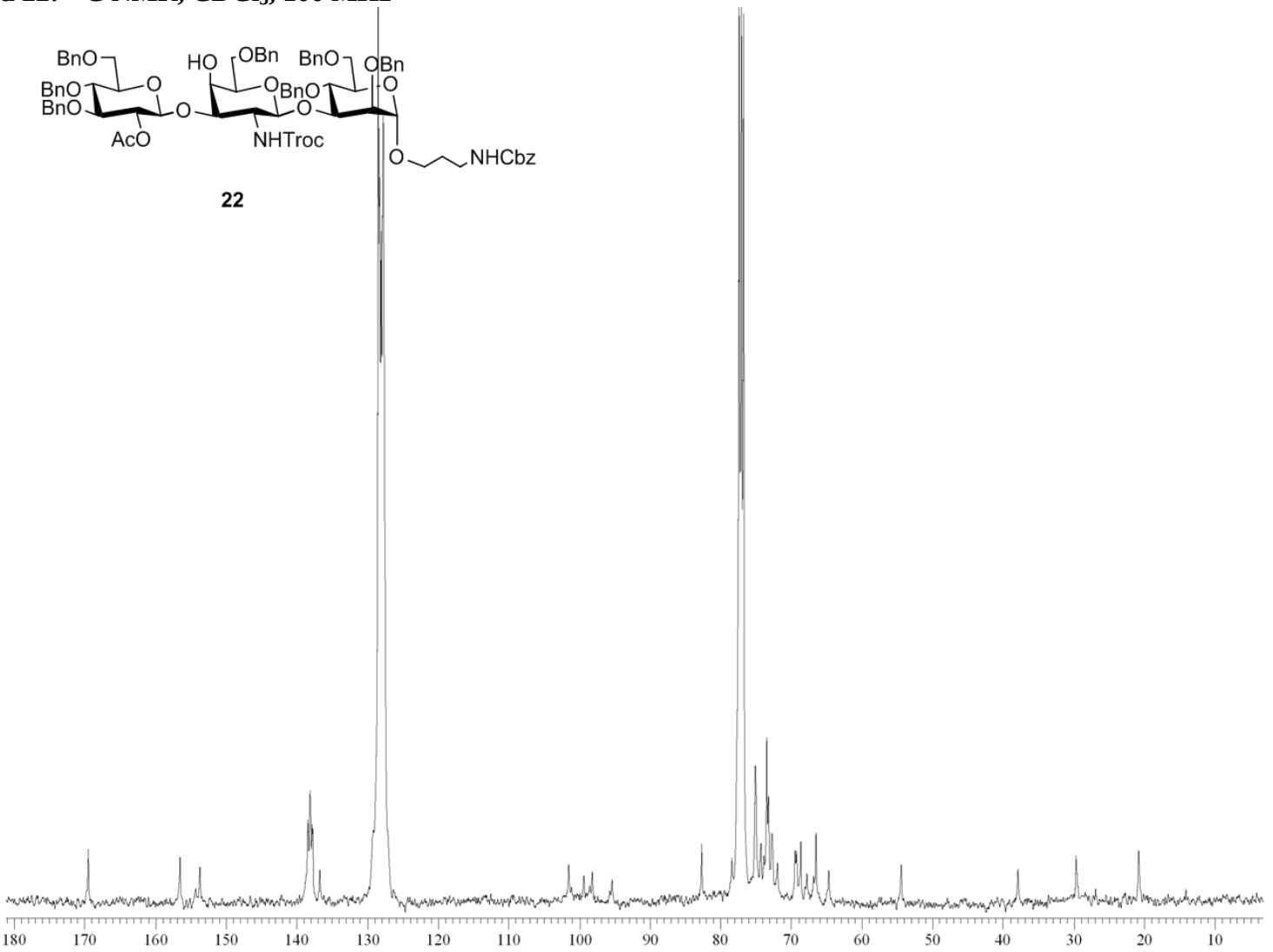
Compound 21: ^{13}C NMR, $CDCl_3$, 100 MHz



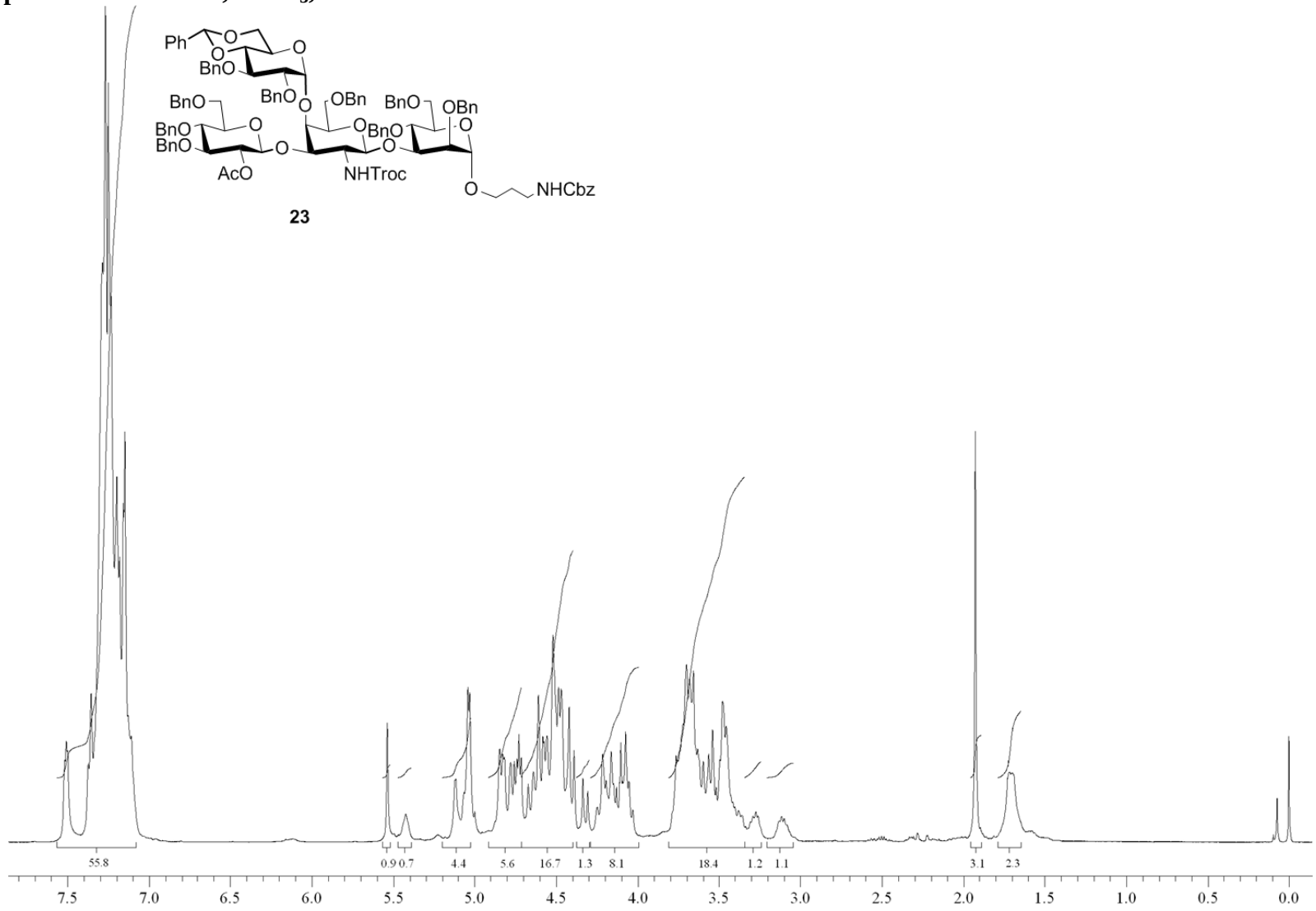
Compound 22: ^{1}H NMR, $CDCl_3$, 400 MHz



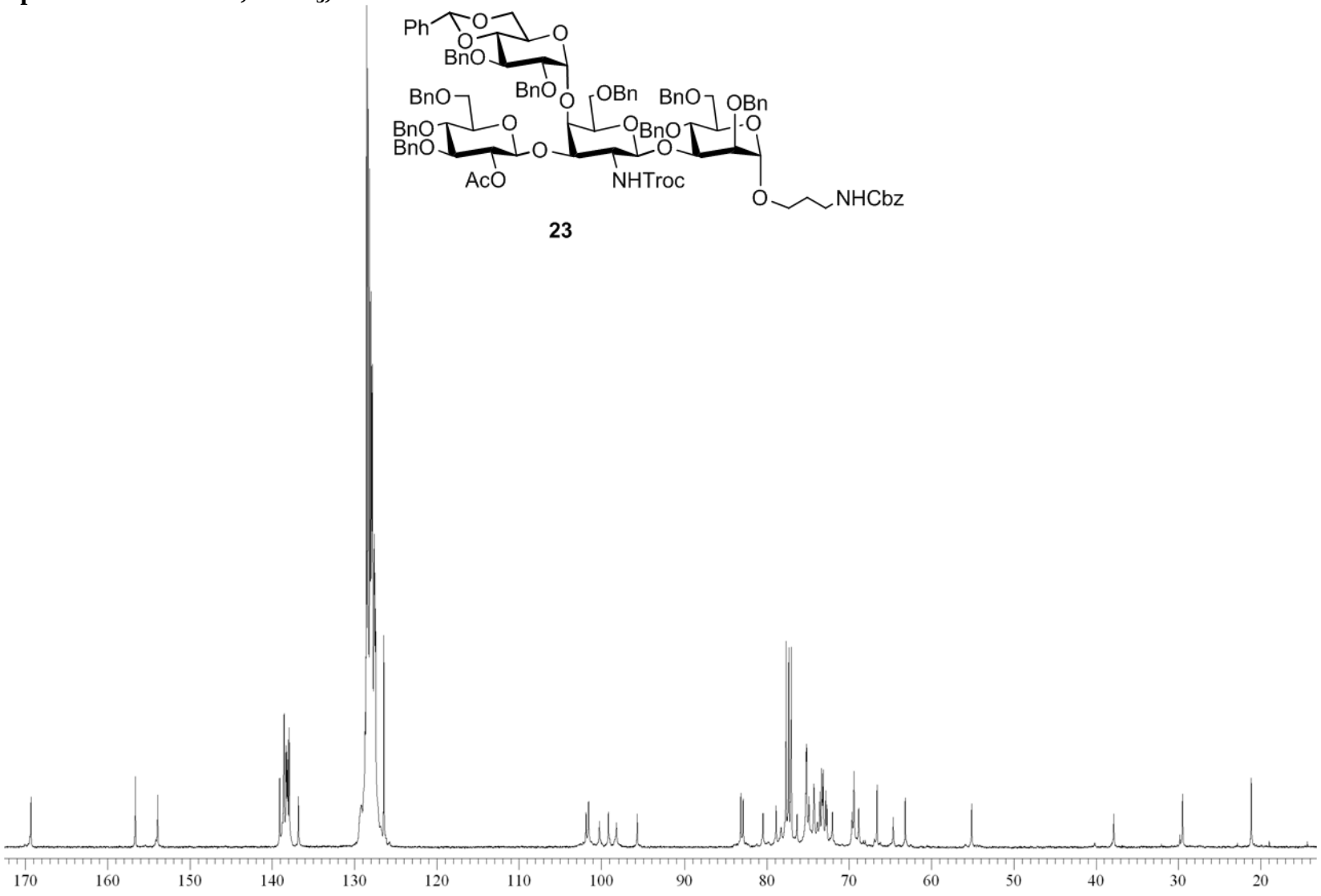
Compound 22: ^{13}C NMR, $CDCl_3$, 100 MHz



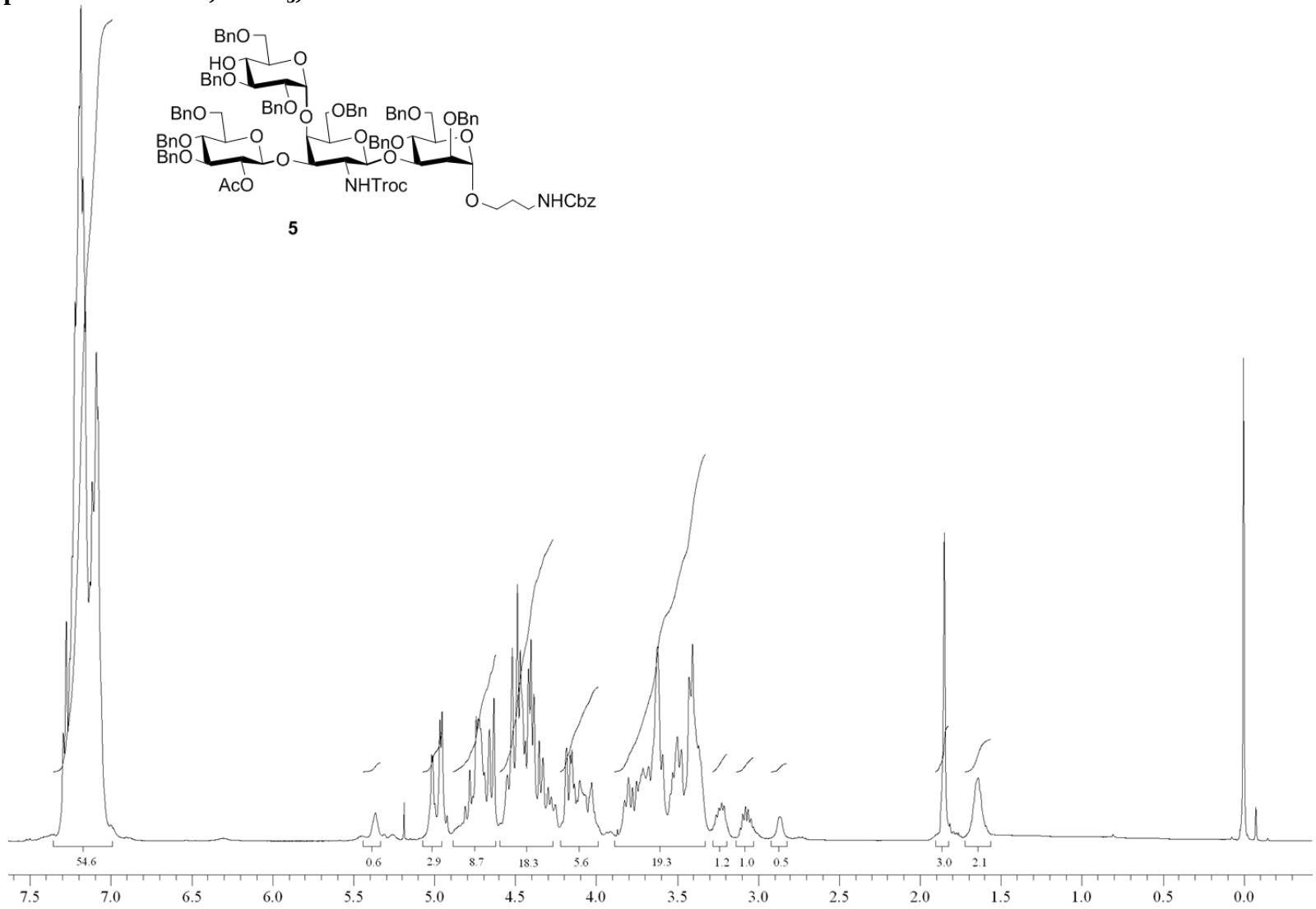
Compound 23: ^{1}H NMR, $CDCl_3$, 400 MHz



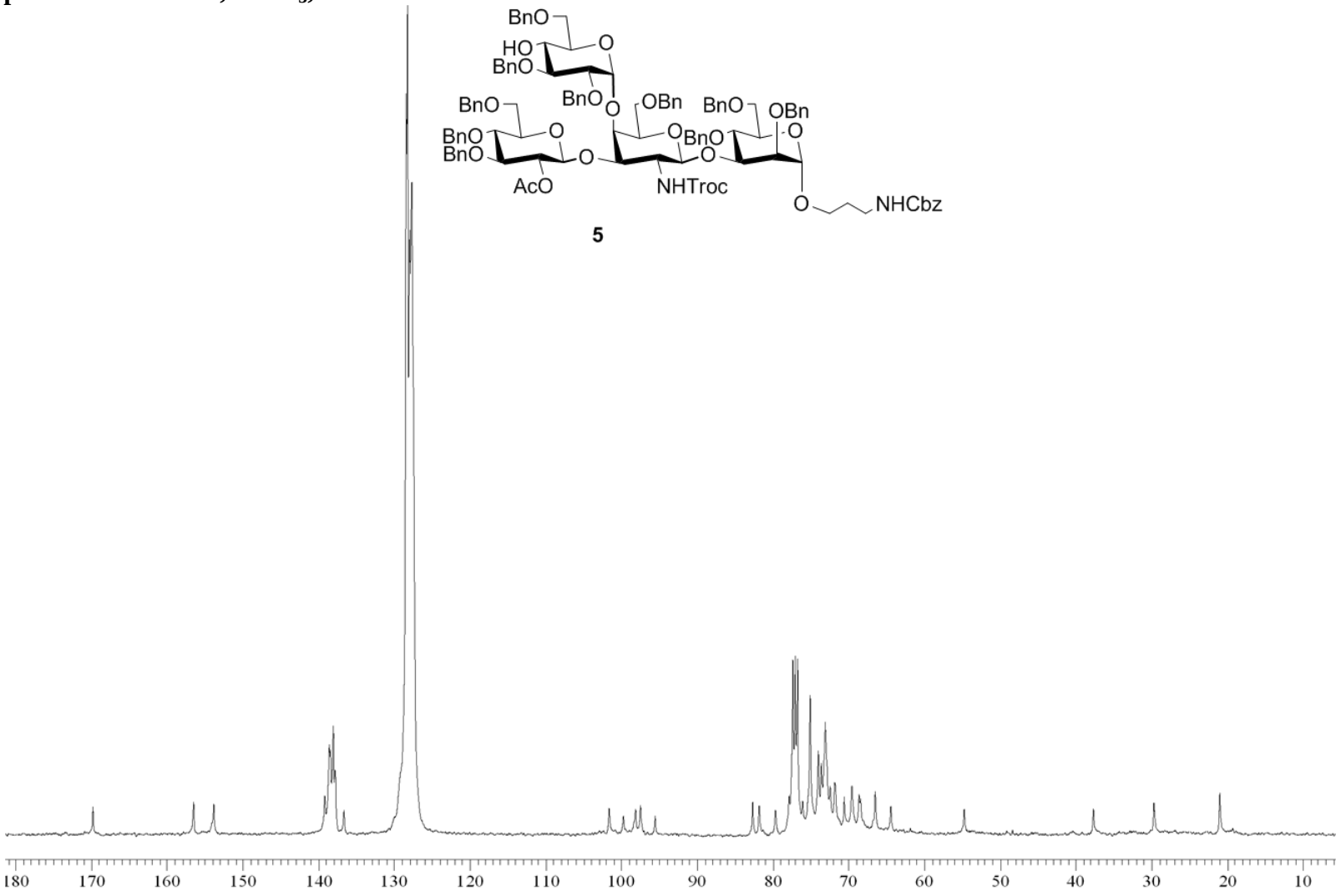
Compound 23: ^{13}C NMR, $CDCl_3$, 100 MHz



Compound 5: ^{1}H NMR, $CDCl_3$, 400 MHz

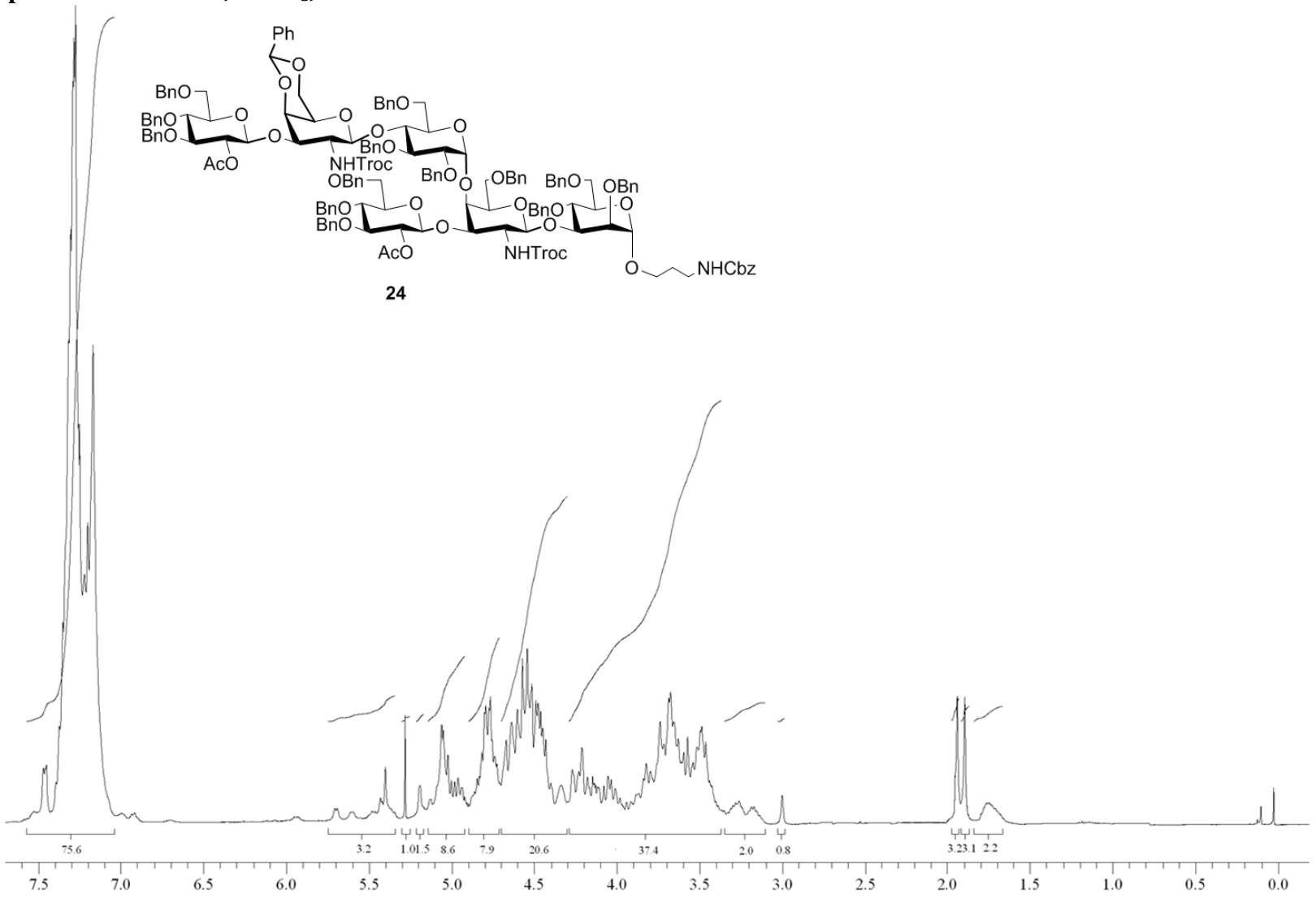


Compound 5: ^{13}C NMR, $CDCl_3$, 100 MHz

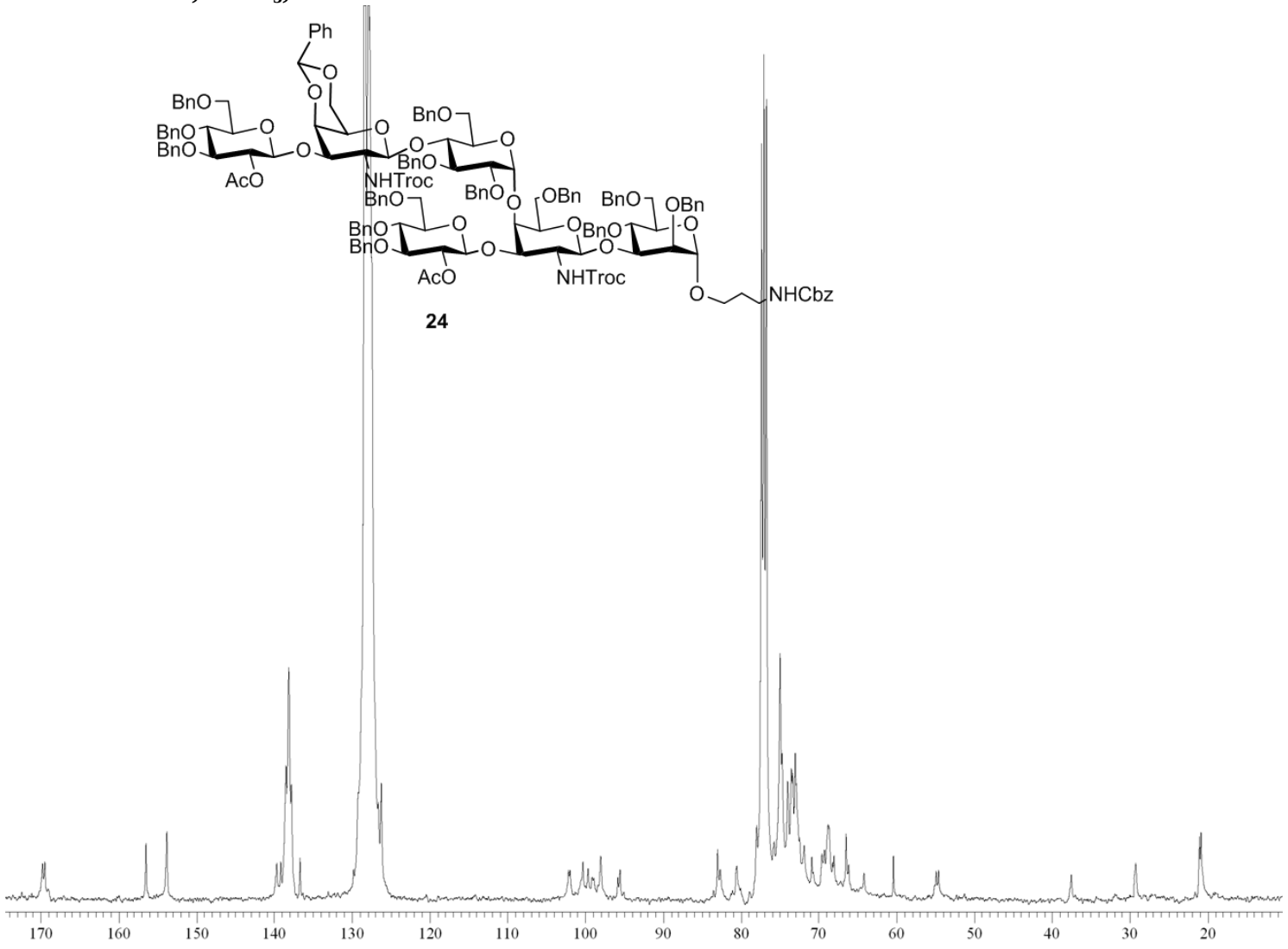


5

Compound 24: ^{1}H NMR, $CDCl_3$, 400 MHz

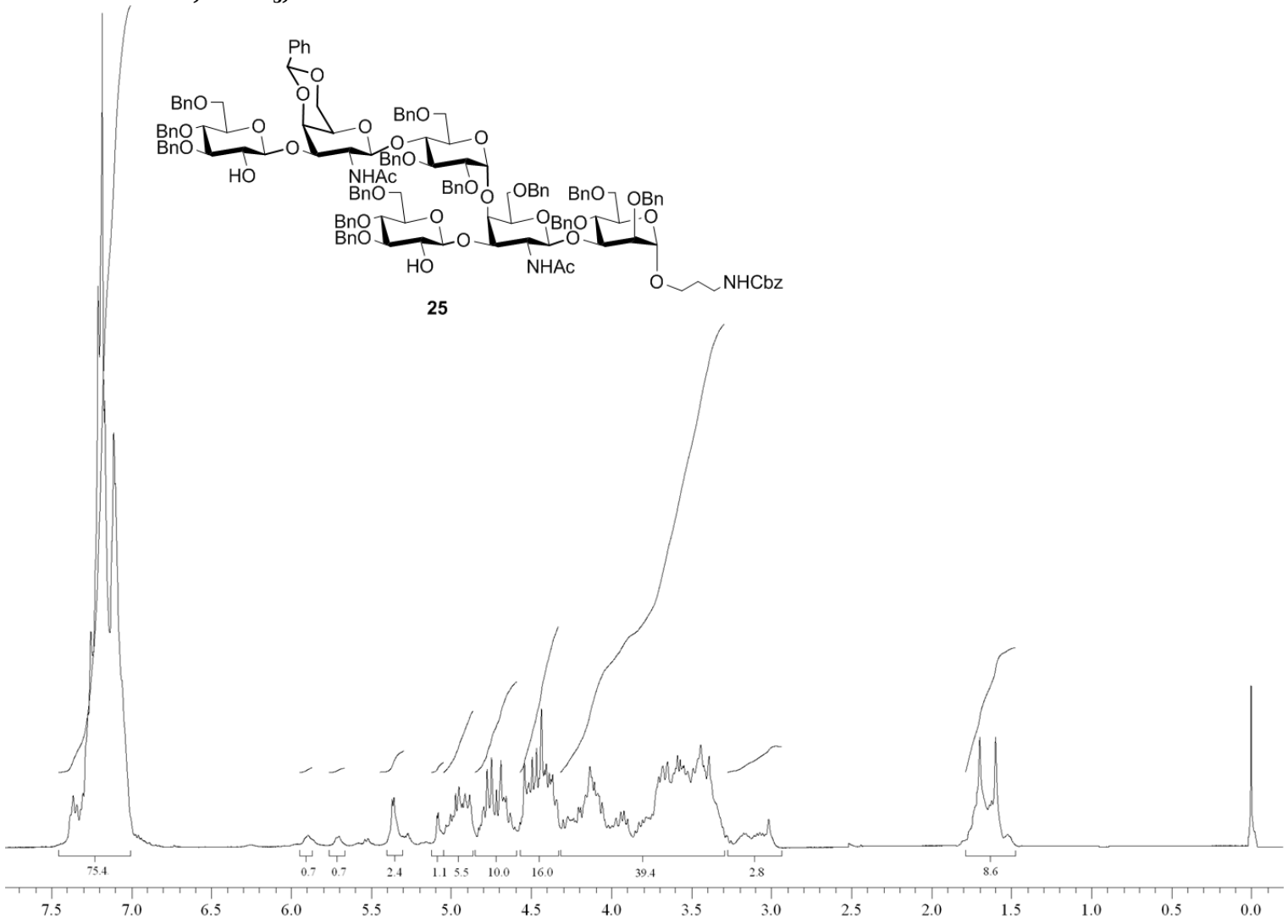


Compound 24: ^{13}C NMR, $CDCl_3$, 100 MHz

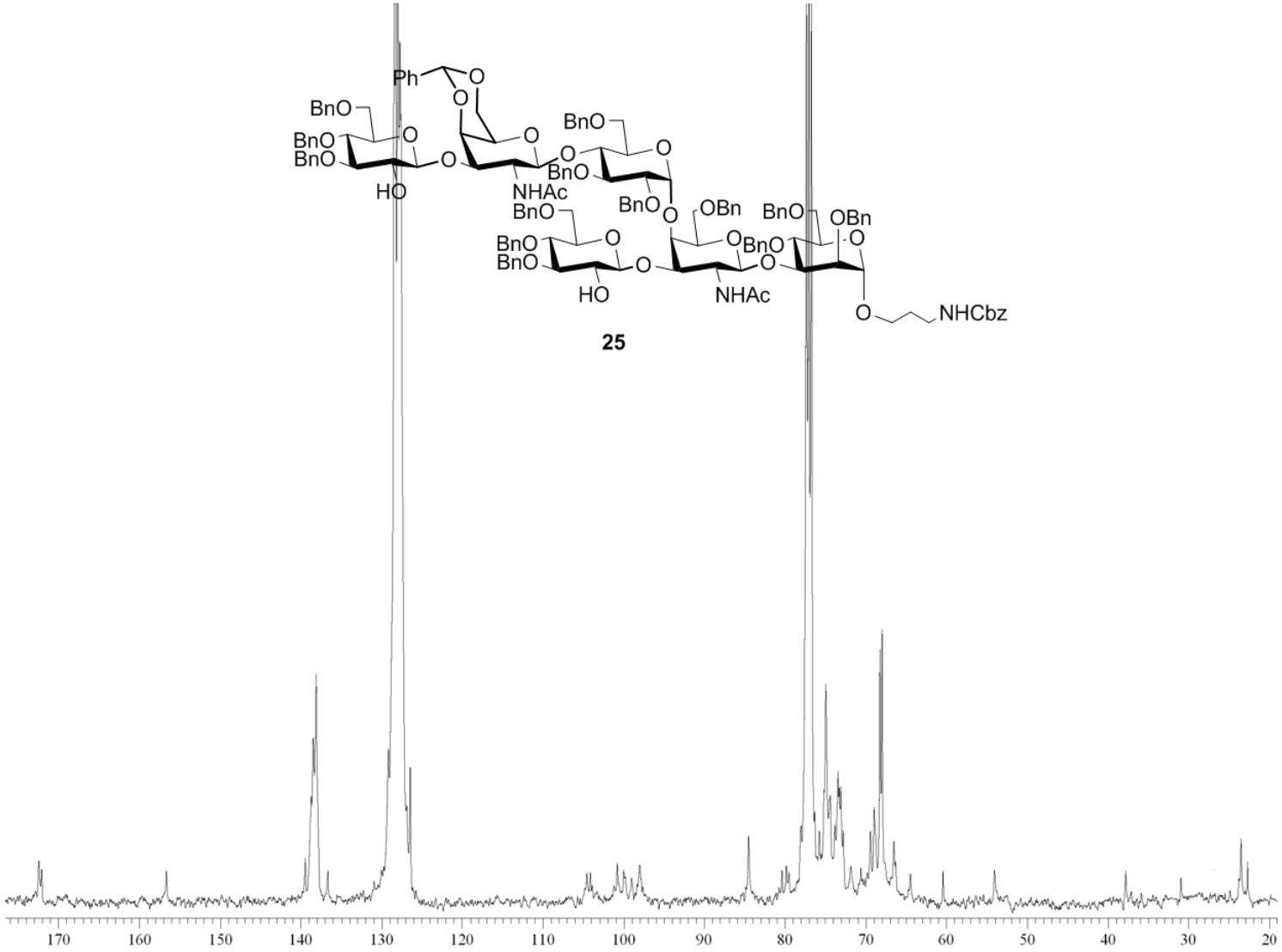


24

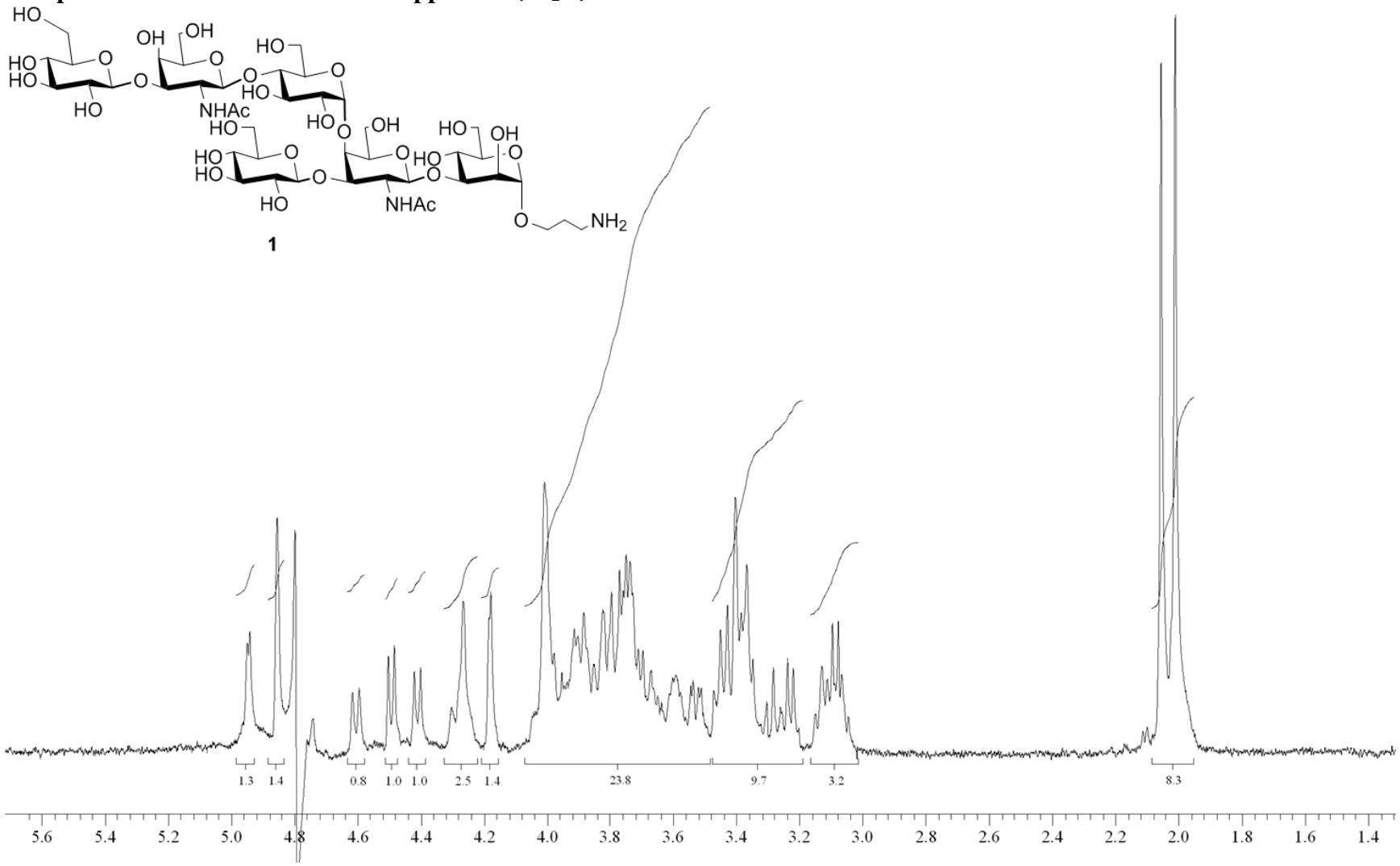
Compound 25: ^{1}H NMR, $CDCl_3$, 400 MHz



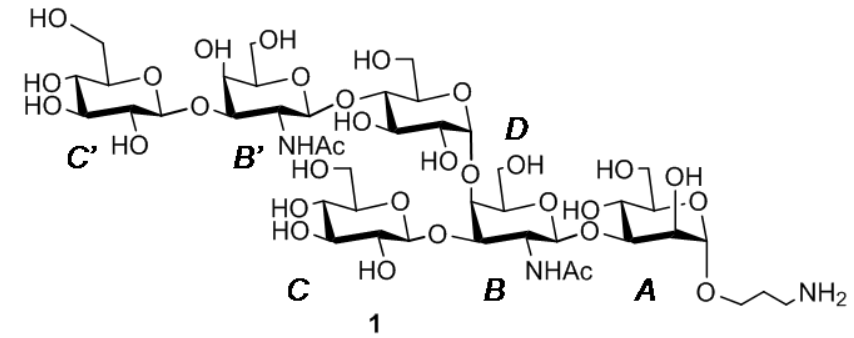
Compound 25: ^{13}C NMR, $CDCl_3$, 100 MHz

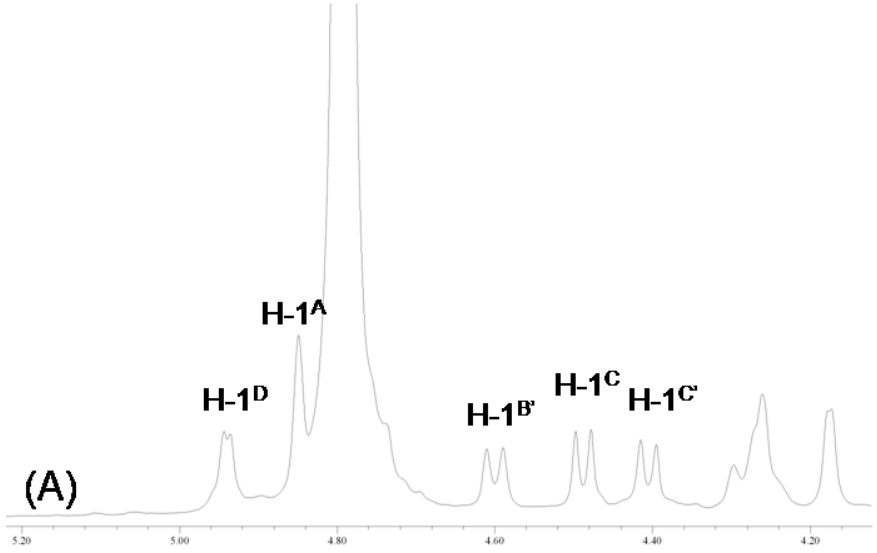


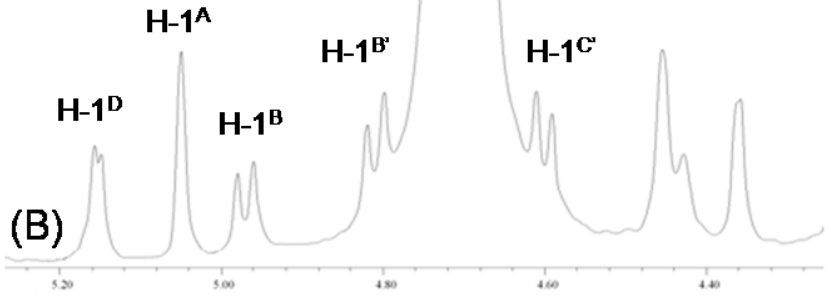
Compound 1: ^{1}H NMR with water suppression, D_2O, 400 MHz



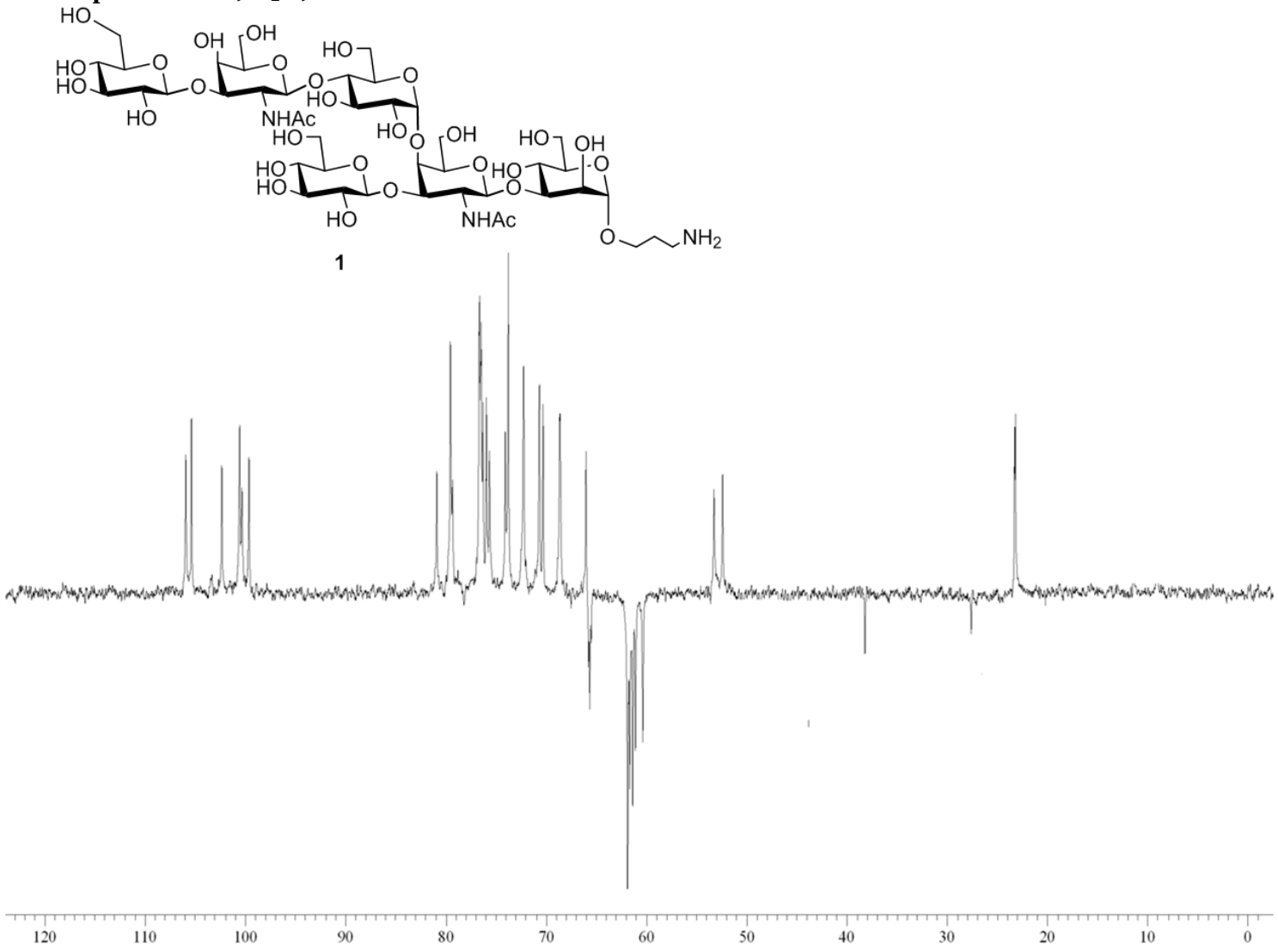
Compound 1: ^{1}H NMR, D_2O, 400 MHz, zoom of anomeric region at 298 (A) and 323 K (B)



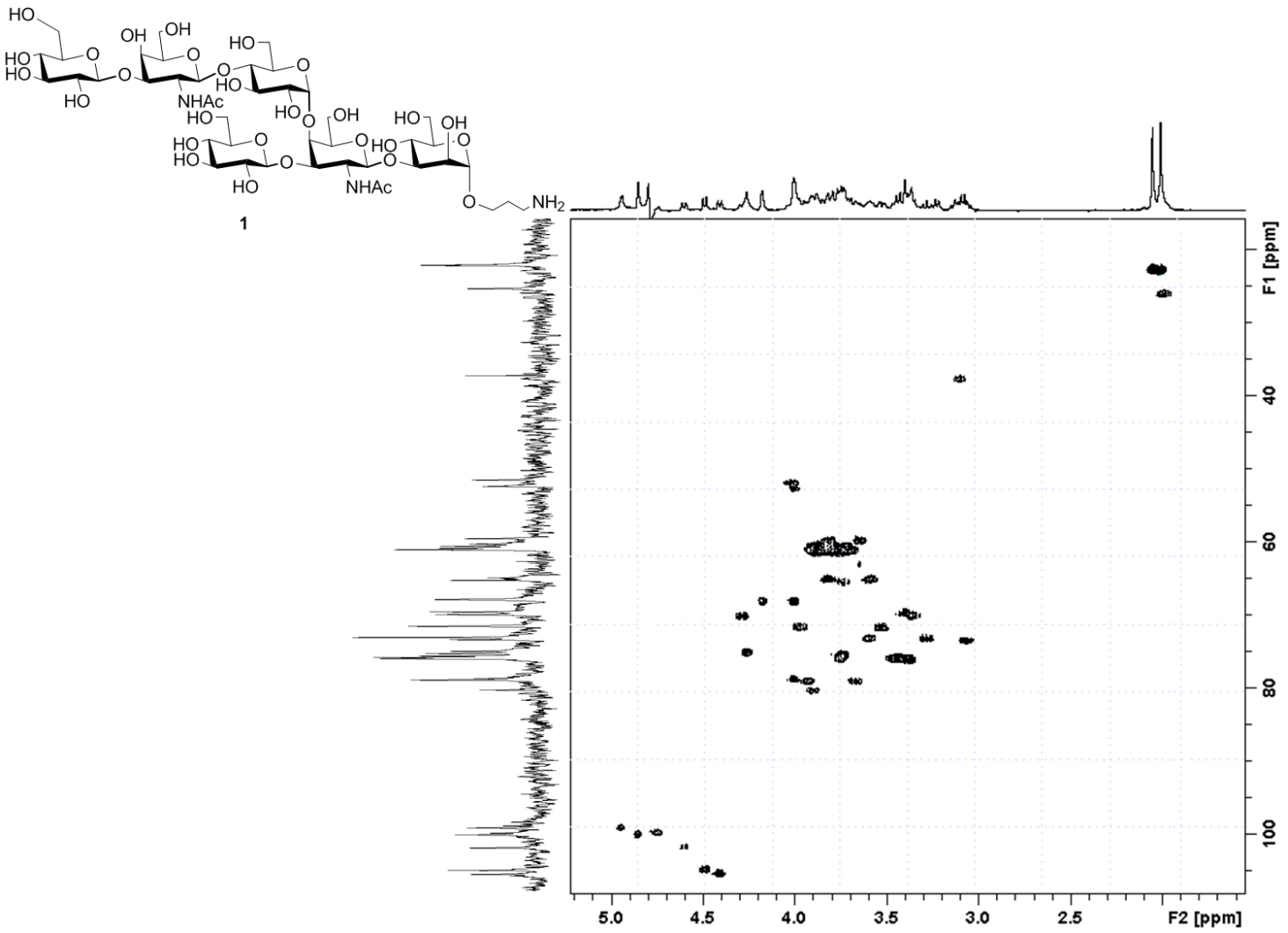




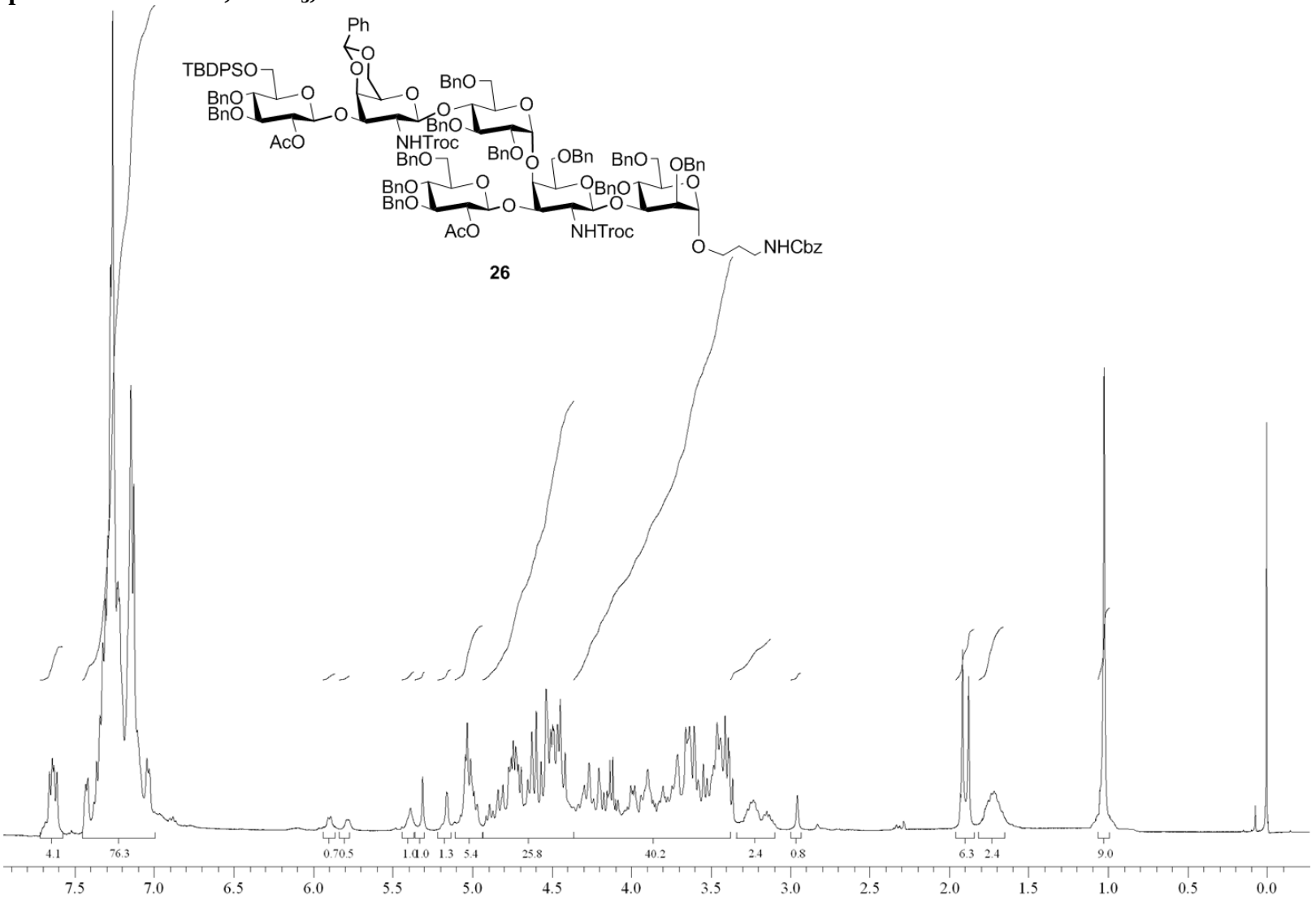
Compound 1: dept 135 NMR, D_2O, 100 MHz



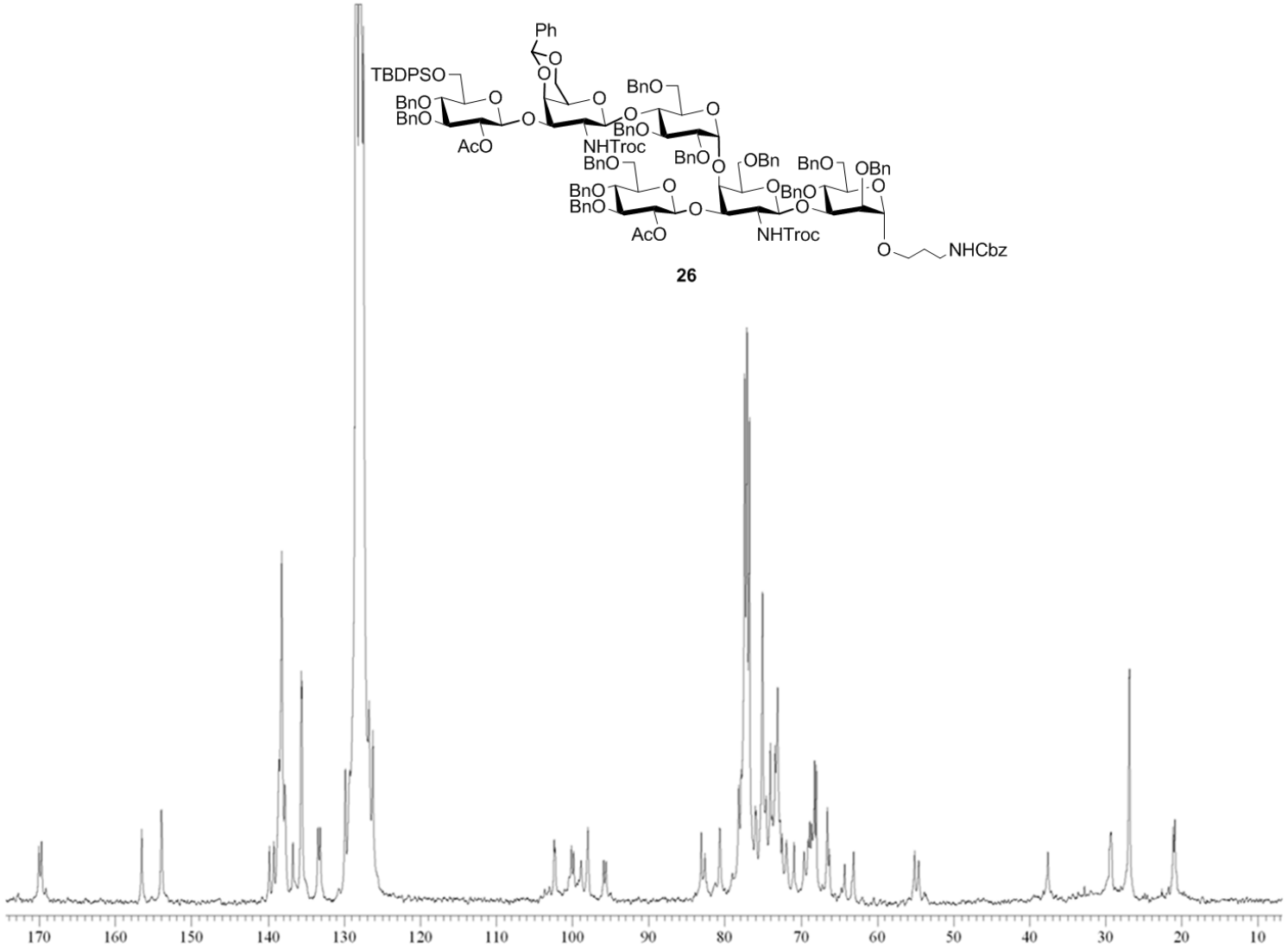
Compound 1: $^{1}H^{13}C$ HMQC NMR, D_2O, 400 MHz



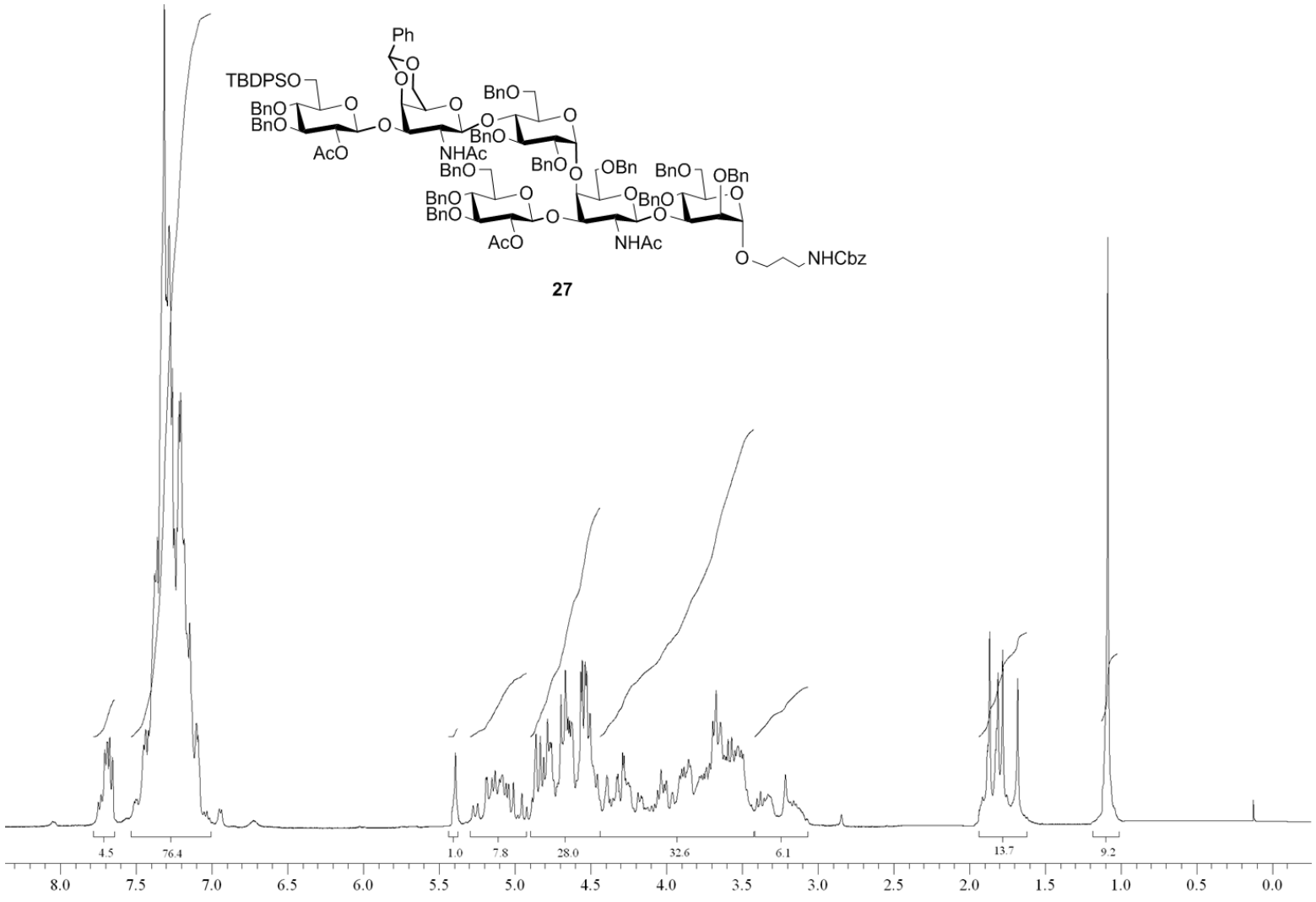
Compound 26: ^{1}H NMR, $CDCl_3$, 400 MHz



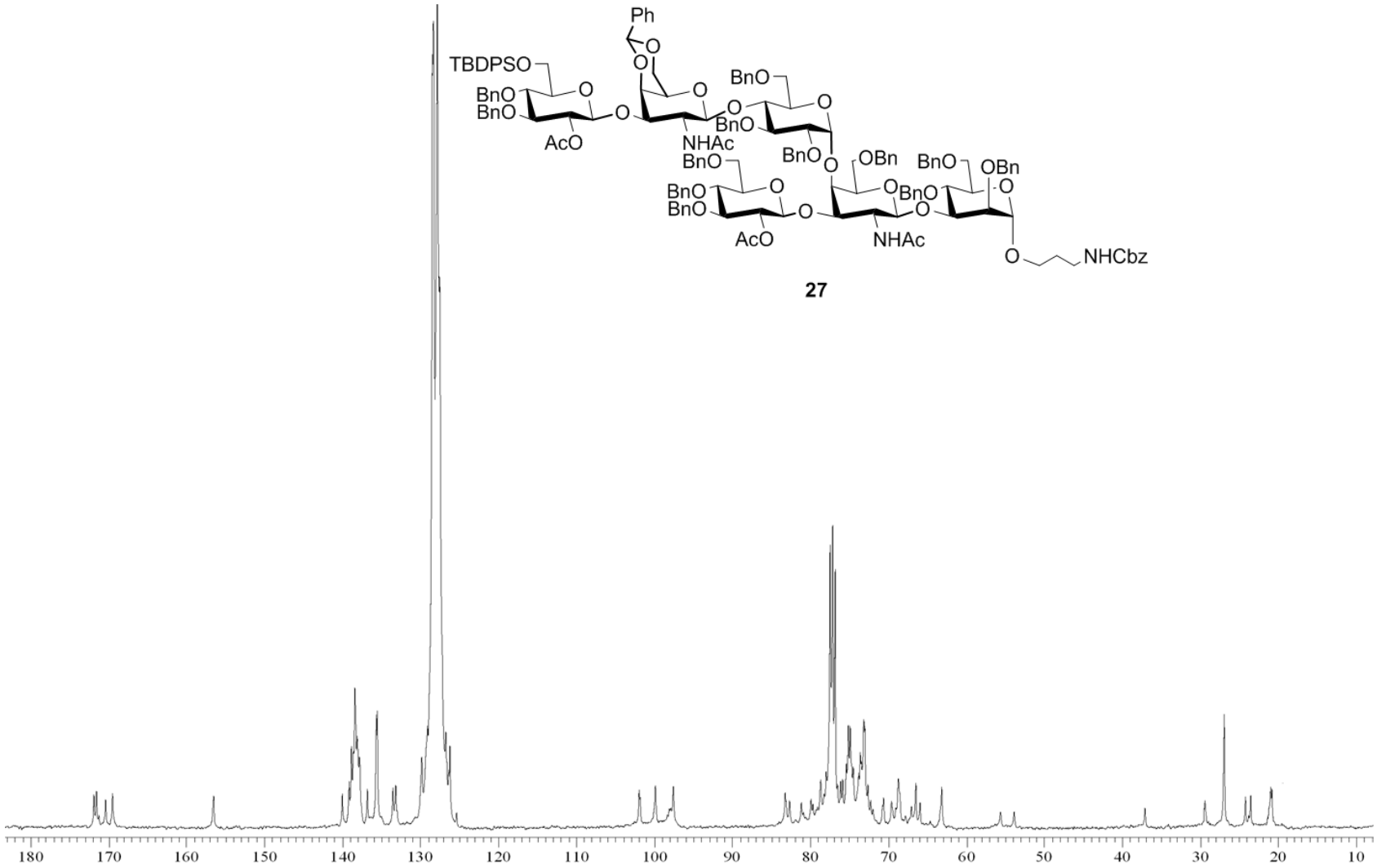
Compound 26: ^{13}C NMR, $CDCl_3$, 100 MHz



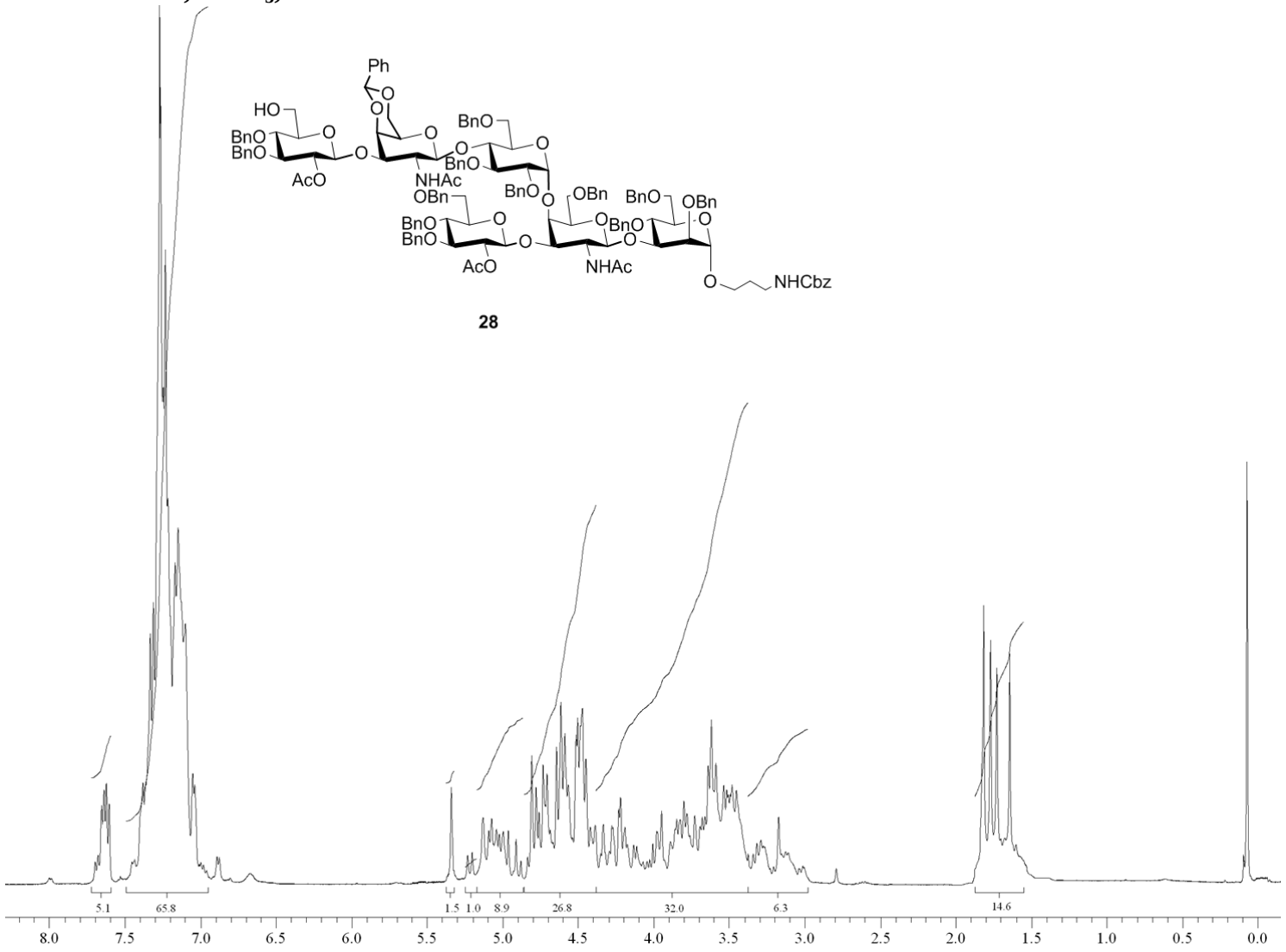
Compound 27: ^{1}H NMR, $CDCl_3$, 400 MHz



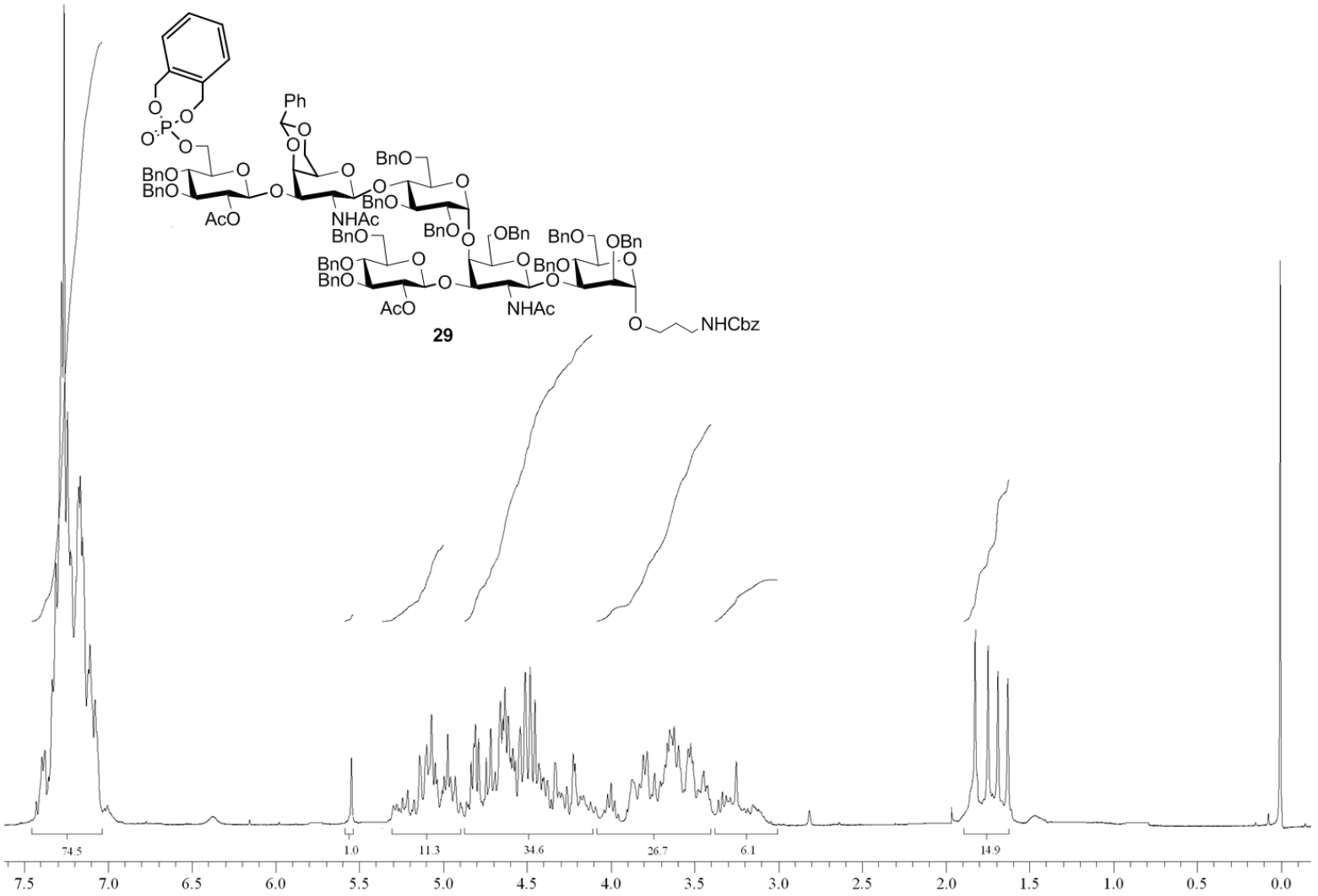
Compound 27: ^{13}C NMR, $CDCl_3$, 100 MHz



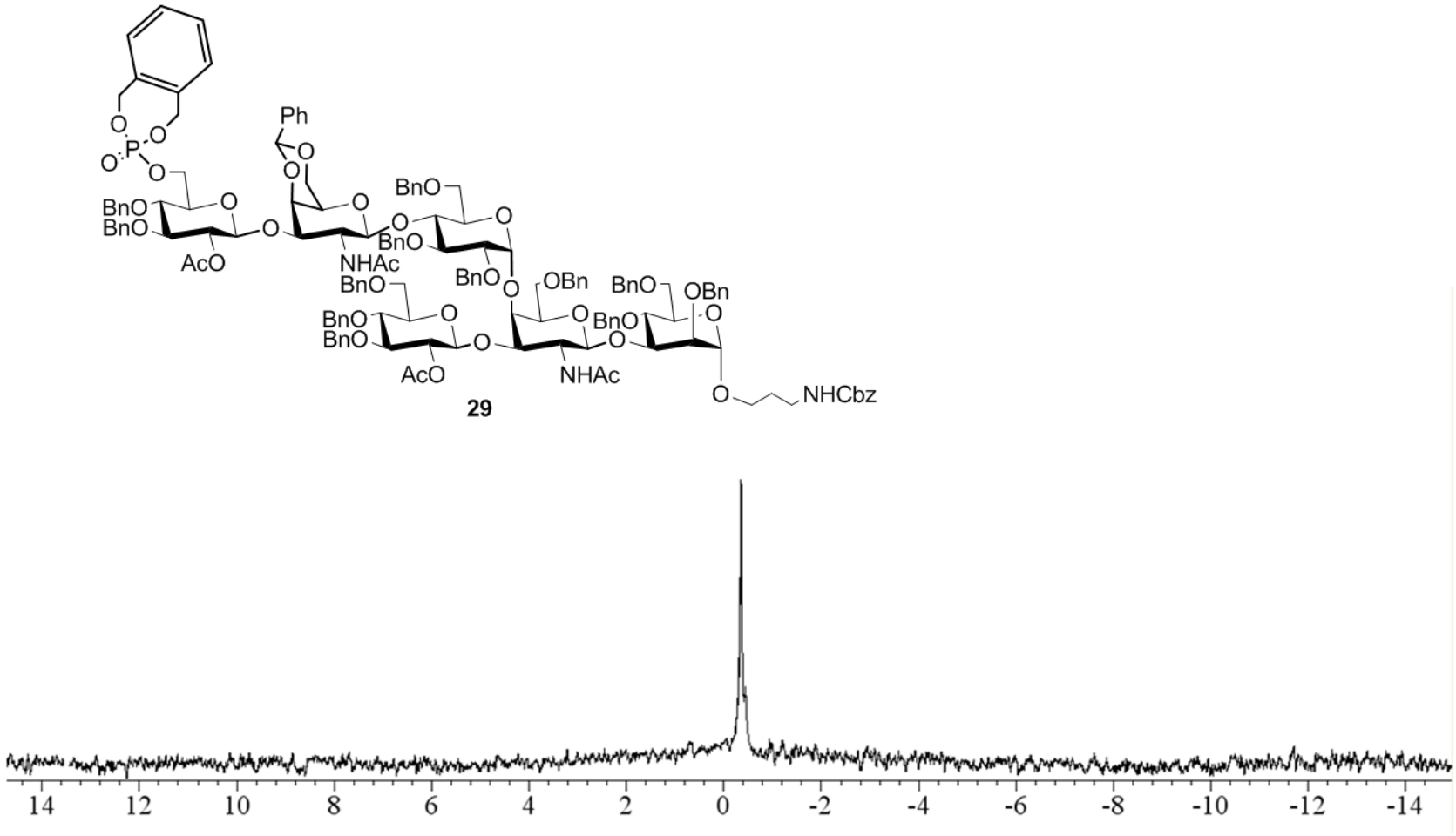
Compound 28: ^{1}H NMR, $CDCl_3$, 400 MHz



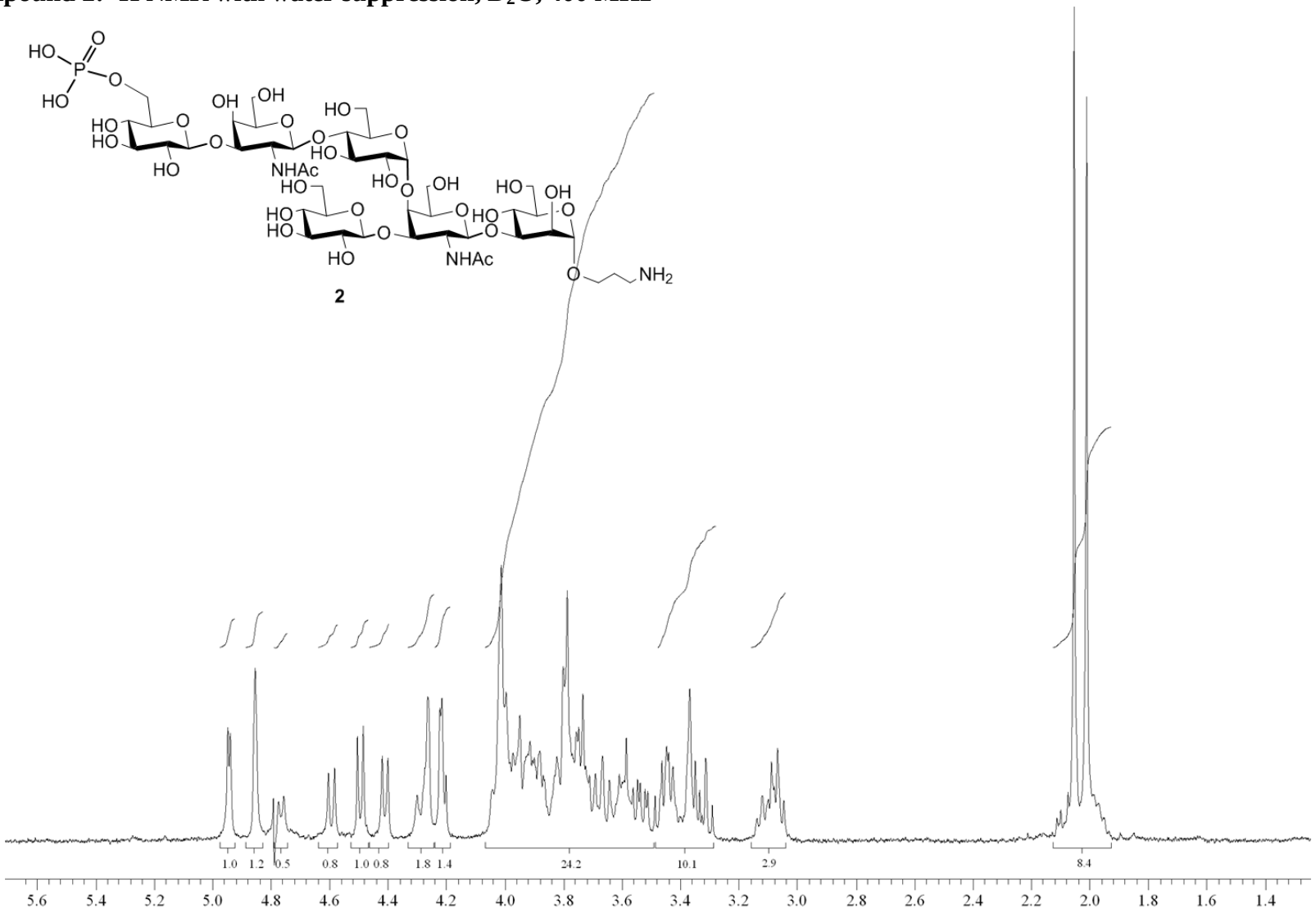
Compound 29: ^{1}H NMR, $CDCl_3$, 400 MHz



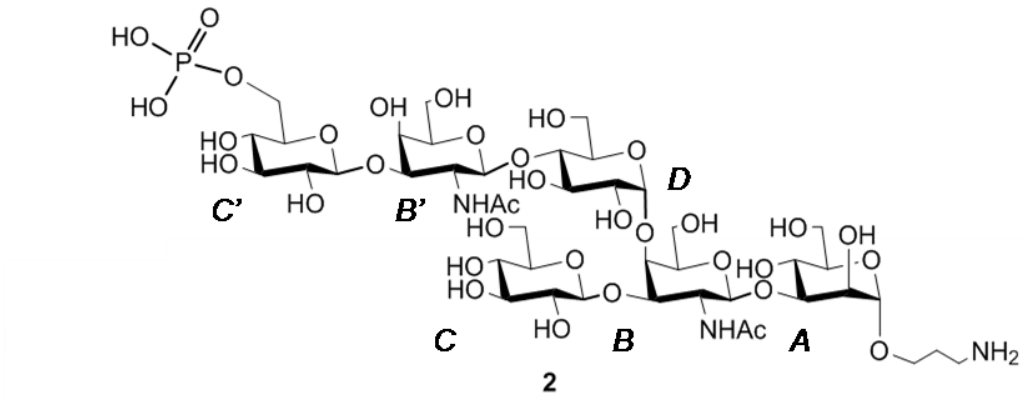
Compound 29: ^{31}P NMR, $CDCl_3$, 162 MHz

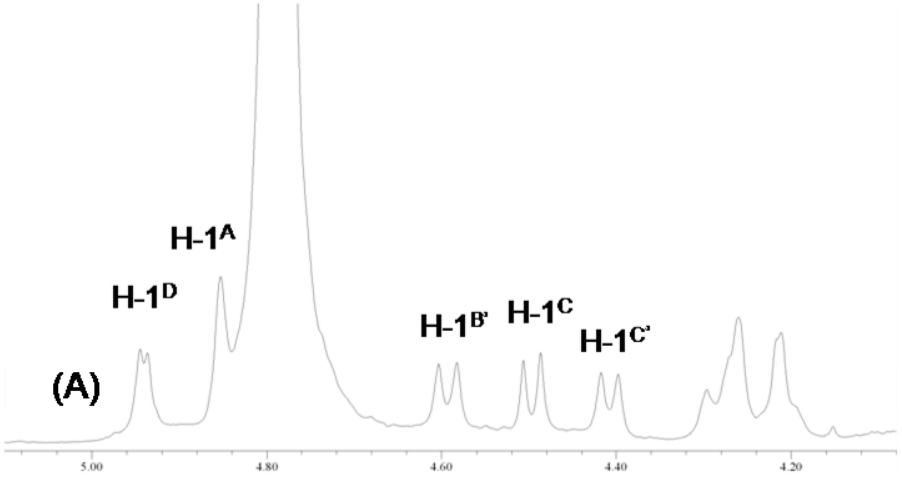


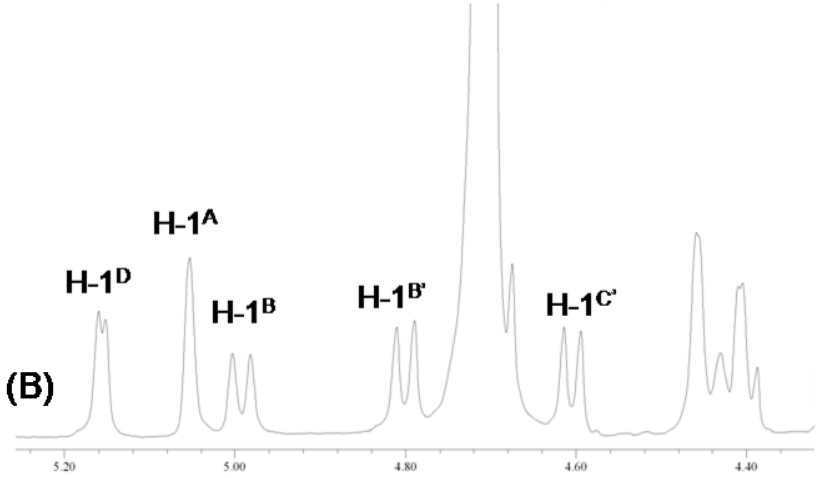
Compound 2: ^{1}H NMR with water suppression, D_2O, 400 MHz



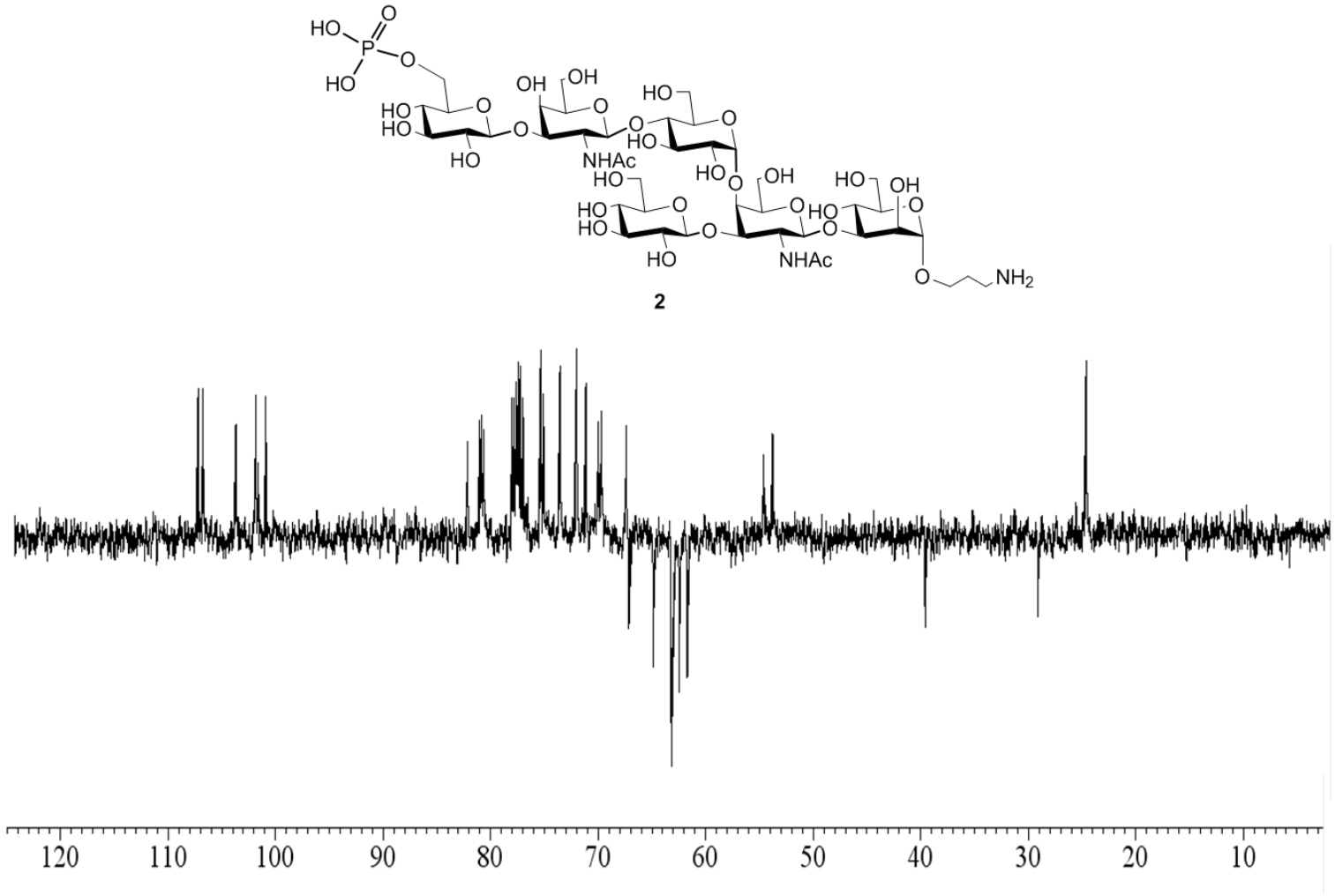
Compound 1: ^{1}H NMR, , D_2O, 400 MHz, zoom of anomeric region at 298 (A) and 323 K (B)







Compound 2: dept 135 NMR, D_2O, 150 MHz



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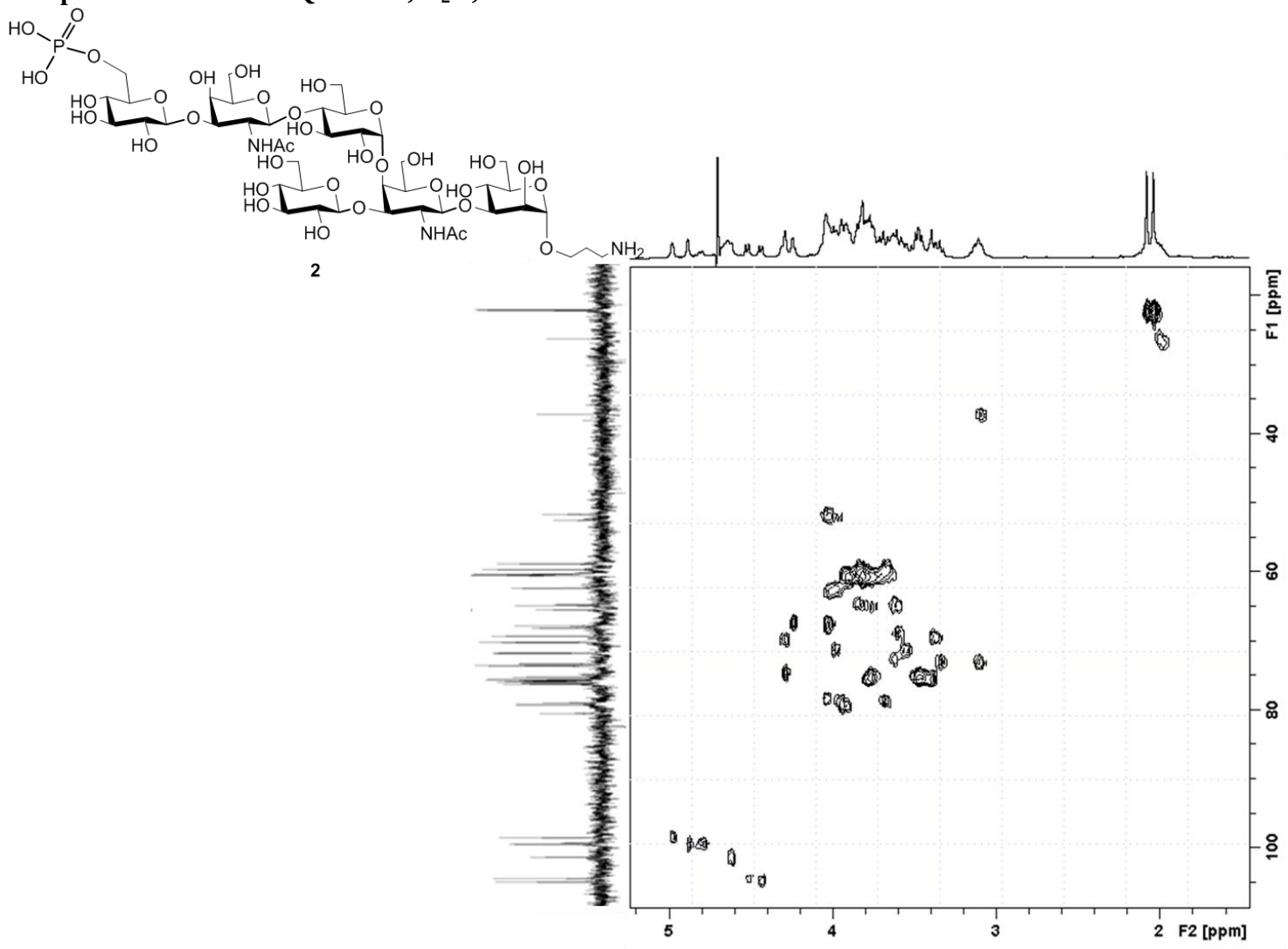
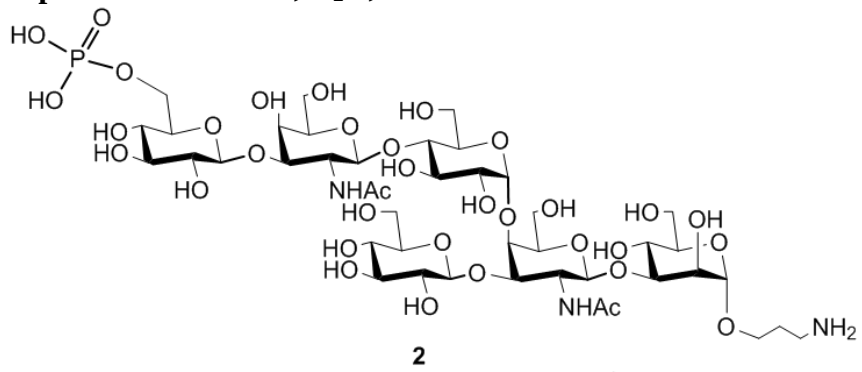
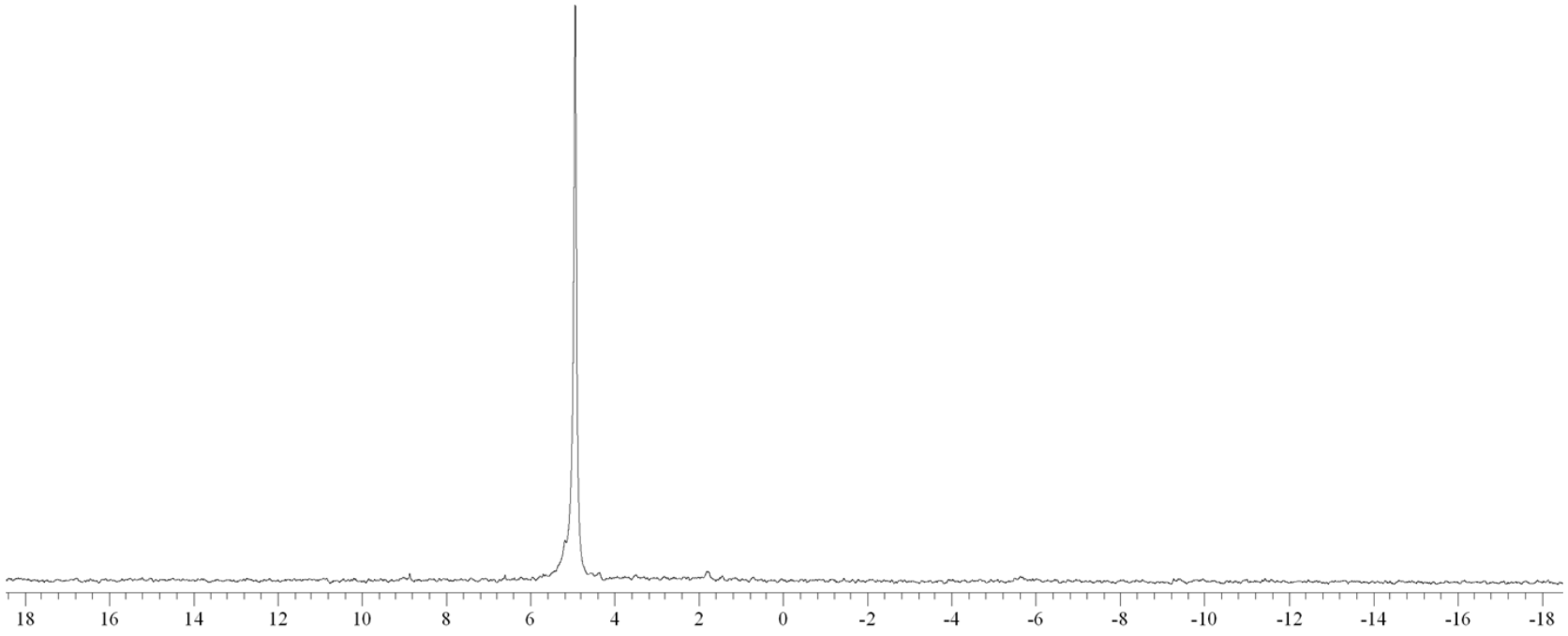
Compound 2: $^{1}H^{13}C$ HMQC NMR, D_2O, 400 MHz



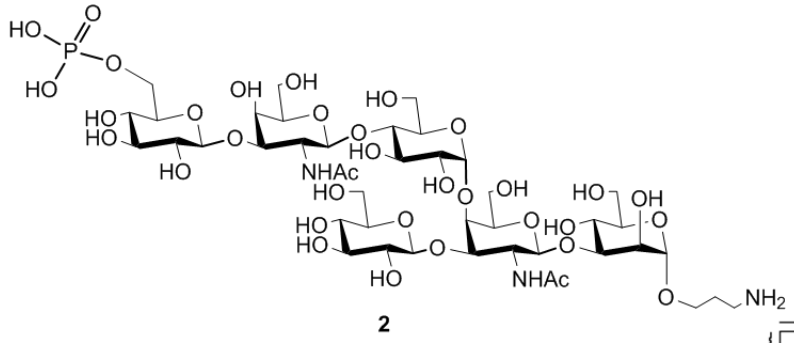
Compound 2: ^{31}P NMR, D_2O, 162 MHz



2



Compound 2: $^{1}H^{31}P$ HMQC NMR, D_2O, 400 MHz



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