Synthesis of branched and linear 1,4-linked galactan oligosaccharides

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Contents

1. General considerations

Starting materials, reagents and solvents were purchased from commercial suppliers and have been used without further purification. All solvents are HPLC-grade. The dry solvents were taken from stills where THF was distilled from sodium/benzophenone whereas Et_2O, CH_2Cl_2, and Et_3N were distilled from CaH_2. DMF and MeCN were dried over 4Å molecular sieves. TLC was performed on Merck Aluminium Sheets precoated with silica, C-60 F_{254} plates. Compounds were visualized by charring after dipping in CAM stain (cerium(IV) sulphate (1.6 g) and ammonium molybdate (40 g) in 10 % sulphuric acid (800 mL). Eluent systems are specified for each R_f-value, and ratios are given as volume ratios. Evaporation of solvents was performed with a VWR International Laborota 400 under reduced pressure (*in vacuo)* at temperatures ranging between 35-45°C. Traces of solvent were removed under reduced pressure by means of an oil pump.

H_2-, and N_2-atmosphere were achieved by applying a rubber septum and a balloon filled with the relevant gas. Flash chromatography was performed using Matrex 60 Å silica gel (35-70 µm) as the stationary phase by the general procedure developed by Still *et al*.[179] The eluent system is specified under the protocol for each synthesis. Eluent ratios are given as volume ratios.

IR analysis was done on a Bruker Alpha-P FT-IR instrument where solid compound is applied directly onto the instrument.

300 MHz ^{1}H-NMR- and 75 MHz ^{13}C-NMR-spectras were recorded on a Varian Mercury 300 B spectrometer, while 400 MHz ^{1}H-NMR- and 101 MHz ^{13}C-NMR-spectras were obtained from a Bruker Ascend 400 spectrometer. Finally 800 MHz ^{1}H-NMR- and 200 MHz ^{13}C-NMR-spectras were recorded from a Bruker Avance 800 spectrometer. Chemical shifts were measured in parts per million (ppm) and coupling constants in hertz (Hz). Solvents used were either $CDCl_3$, CD_3OD, D_2O or d_6-DMSO.

Optical rotation was measured on a Perkin Elmer Model 341 Polarimeter. Solvents used were either $CDCl_3$, CD_3OD, D_2O or DMSO-d_6.

Melting points were measured on a Stuart melting point SMP30 and given in degrees Celsius (°C) uncorrected.

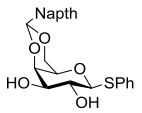
Analysis was run on a Waters AQUITY UPLC system equipped with PDA and SQD MS detector. Column: AQUITY UPLC BEH C18 1.7µm, 2.1 x 50mm. Column temp: 65 º C.

Flowrate: 0.6 ml/min. Solvent A: 0.1% formic acid in water, Solvent B: 0.1% formic in MeCN. Gradient: 5% B to 100% B in 2.4 min, hold 0.1 min, total run time – 2.6 min.

High-resolution LC-DAD-MS was performed in an Agilent 1100 system equipped with a photodiode array detector (DAD) and coupled to a LCT orthogonal time-of-flight mass spectrometer (Waters-Micromass, Manchester, UK) with Z-spray electrospray ionization (ESI) source and a LockSpray probe and controlled MassLynx 4.0 software. LC-MS calibration from *m/z* 100-900 was done with a PEG mixture. Standard separation involved a LUNA 2 column with a MeCN (50 ppm TFA) in water gradient starting from 15% to 100% over 25 minutes with a flow rate of 0.3 mL/min.

2. Experimental Procedures

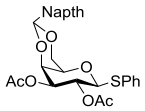
Phenyl 4,6-*O*-(2-naphthyl)methylene-1-thio-β-D-galactopyranoside (S1)



To a solution of 2-naphthaldehyde (7.7 g, 49.3 mmol) and camphorsulphonic acid (255 mg, 1.1 mmol) in MeCN (200 mL) was added thiophenylgalactoside (10 g, 36.5 mmol). The flask was equipped with a distillation unit and the mixture was heated to 95°C for 1.5 h. During that period app. 100 mL of MeCN-MeOH was distilled off. The distillation unit was replaced with a condenser and the reaction mixture was heated to reflux until TLC showed completion of the reaction (1 h). The reaction mixture was concentrated and purified by flash chromatography (19:1 CH_2Cl_2/MeOH) to afford **S1** as a white crystalline powder. R_f 0.14 (1:1 EtOAc/toluene)
Yield: 14.7 g (98%)

mp: 129.5 - 132°C
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$= -21.3° (c 1.0, $CDCl_3$)
IR(neat, cm^{-1}): 3600-3000, 3057.04, 2976.16, 2904.45, 2857.50, 1402.74, 1354.27, 1094.07, 1068.42, 1041.28
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 – 7.73 (m, 4H, Ar-*H*), 7.69 – 7.58 (m, 2H, Ar-*H*), 7.50 – 7.39 (m, 3H, Ar-*H*), 7.30 – 7.17 (m, 3H, Ar-*H*), 5.60 (s, 1H, ArC*H*$(OR)_2$), 4.45 (d, 1H, *J* = 9.2 Hz, H-1), 4.36 (dd, 1H, *J* = 12.5, 1.2 Hz, H-6b), 4.19 (d, 1H, *J* = 1.7 Hz, H-4), 4.01 (dd, 1H, *J* = 12.5, 1.2 Hz, H-6a), 3.71 – 3.61 (m, 2H, H-2, H-3), 3.51 (dd, 1H, *J* = 1.2, 1.5 Hz, H-5)
^{13}C NMR (75 MHz, $CDCl_3$) δ 135.20 (1C), 134.01 (1C), 133.82 (2C), 133.02 (1C), 131.12 (1C), 129.16 (2C), 128.54 (1C), 128.36 (1C), 127.97 (1C), 126.79 (1C), 126.50 (1C), 126.16 (1C), 124.28 (1C), 101.66, 87.15, 75.69, 73.87, 70.19, 69.52, 68.84.
HR-MS: calc. $[M+H]^+$: 411.1266 found $[M+ H]^+$: 411.1249

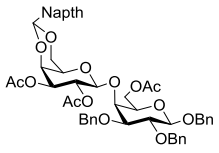
Phenyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-1-thio-β-D-galactopyranoside (6)



S1 (12 g ; 29.23 mmol) was dissolved in CH_2Cl_2 (250 mL). Et_3N (12.2 mL; 87.7 mmol), DMAP (71 mg; 0.58 mmol) and acetic anhydride (6.9 mL; 73.1 mmol) were added to the solution and the reaction mixture was stirred until TLC revealed full conversion of the starting material (2 h). The reaction was quenched with MeOH (5 mL), washed with water (2x200 mL), dried over $MgSO_4$ and concentrated. The product was purified by flash chromatography (CH_2Cl_2/MeOH 19:1) to afford **6** as a white crystalline powder. R_f 0.71 (1:1 EtOAc/toluene)
Yield: 14.02 g (98%)

mp: 165.4 - 167°C
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$= +36.0° (c 1.0, $CDCl_3$)
IR(neat, cm^{-1}): 3059.39, 2974.36, 2906.11, 2863.98, 2803.27, 1751.73, 1243.25, 1216.57, 1097.35, 1031.81, 993.68
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H, Ar-*H*), 7.60 – 7.52 (m, 2H, Ar-*H*), 7.50 – 7.38 (m, 3H, Ar-*H*), 7.28 – 7.17 (m, 3H, Ar-*H*), 5.56 (s, 1H, ArC*H*$(O\text{-}R)_2$), 5.31 (t, 1H, *J* = 9.9 Hz, H-2), 4.96 (dd, 1H, *J* = 9.9, 3.5 Hz, H-3), 4.66 (d, 1H, *J* = 9.9 Hz, H-1), 4.36 (d, 1H, *J* = 3.5 Hz, H-4), 4.36 (dd, 1H, *J* = 12.5, 1.7 Hz, H-6b), 4.02 (dd, 1H, *J* = 12.5, 1.7 Hz, H-6a), 3.56 (t, 1H, *J* = 1.7 Hz, H-5), 2.03 (s, 3H, CH_3), 1.96 (s, 3H, CH_3).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.96 (1C), 169.32 (1C), 135.05 (1C), 134.01 (1C), 133.83 (2C), 133.02 (1C), 131.52 (1C), 129.05 (2C), 128.62 (1C), 128.38 (1C), 128.30 (1C), 127.96 (1C), 126.63 (1C), 126.30 (1C), 126.11 (1C), 124.38 (1C), 101.62 (1C), 85.45 (1C), 73.80 (1C), 73.41 (1C), 69.99 (1C), 69.41 (1C), 67.03 (1C), 21.16 (2C, CO*C*H_3).
HR-MS: calc. $[M+NH_4]^+$: 512.1743 found $[M+ NH_4]^+$: 512.1755

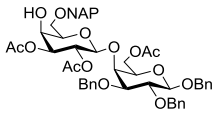
Benzyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (8)



A mixture of **6** (2.0 g; 4.01 mmol) and **7** (2.58 g; 5.21 mmol) was dried azeotropically with benzene (2x30 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (25 mL) and dry MeCN (25 mL), cooled to -20 °C, followed by addition of NIS (1.20 g; 5.33 mmol) and TESOTf (212 mg; 0.80 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (2 h). The solution was diluted with CH_2Cl_2 (150 mL) and washed with sat. aq. NaS_2O_3 (100 mL) and sat. aq. $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography (5:1 toluene/EtOAc) to afford **8** as a white crystalline material. R_f 0.58 (1:2 EtOAc/toluene)
Yield: 2.70 g (77%)

mp: 152.3-154.1°C
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$= +47.1° (c 1.0, $CDCl_3$)
IR(neat, cm^{-1}): 3062.68, 3030.40, 2868.79, 1737.93, 1222.87, 1057.46
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 – 7.72 (m, 4H, Ar-*H*), 7.56 (dd, 1H, *J* = 8.5, 1.6 Hz, Ar-*H*), 7.44 – 7.35 (m, 2H, Ar-*H*), 7.33 – 7.17 (m, 15H, Ar-*H*), 5.53 (s, 1H, ArC*H*(OR)$_2$), 5.33 (dd, 1H, *J* = 10.4, 7.8 Hz, H-2'), 4.90 (d, 1H, *J* = 2.0 Hz, PhC*H*$_2$O), 4.87 (d, 1H, *J* = 1.5 Hz, PhC*H*$_2$O), 4.85 (d, 1H, *J* = 9.0 Hz, H-3'), 4.74 – 4.54 (m, 5H, PhC*H*$_2$O, H-1'), 4.50 (dd, 1H, *J* = 11.9, 4.5 Hz, H-6b), 4.33 (d, 1H, *J* = 7.6 Hz, H-1), 4.30 – 4.13 (m, 2H, H-4', H-6b'), 4.03 – 3.91 (m, 2H, H-6a, H6a'), 3.70 – 3.62 (m, 1H, H-5'), 3.58 (dd, 1H, *J* = 9.7, 7.6 Hz, H-2), 3.47 (dd, 1H, *J* = 7.0, 4.5 Hz, H-5), 3.39 (dd, 1H, *J* = 9.7, 2.9 Hz, H-3), 3.29 (s, 1H, H-4), 1.98 (s, 6H, C*H*$_3$), 1.82 (s, 3H, C*H*$_3$).
^{13}C NMR (75 MHz, $CDCl_3$) δ 171.23 (1C), 171.18 (1C), 169.64 (1C), 138.82 (1C), 138.52 (1C), 137.71 (1C), 135.16 (1C), 134.08 (1C), 133.10 (1C), 128.72-127.81 (15C), 126.58 (1C), 126.26 (1C), 126.19 (1C), 124.40 (1C), 102.47 (1C), 101.93 (1C), 101.73 (1C), 81.55 (1C), 79.49 (1C), 75.53 (1C), 74.64 (1C), 73.73 (1C), 73.53 (1C), 72.39 (1C), 72.16 (1C) , 70.88 (1C), 69.18 (1C), 68.22 (1C), 66.38 (1C), 64.42 (1C), 21.26 (1C), 21.23 (1C), 21.11 (1C).
HR-MS: calc. $[M+NH_4]^+$: 894.3701 found $[M+ NH_4]^+$: 894.3695

Benzyl 2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (9)

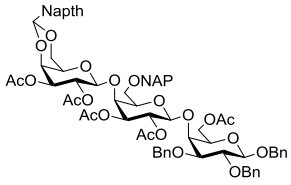


To a solution of **8** (2.6 g; 2.96 mmol) in dry THF (60 mL), $NaCNBH_3$ (3.73 g; 59.30 mmol) was added. The mixture was stirred at 22 °C and a 2M solution of HCl in dry ether was added dropwise until gas development ceased and the mixture remained acidic (pH 3-4). The reaction mixture was concentrated to 4 ml, diluted with CH_2Cl_2 (150 mL) and neutralized with sat. aq. $NaHCO_3$ (80 mL). The aqueous phase was extracted with CH_2Cl_2 (3x100 mL). The combined organic phases were washed with water (500 mL), dried over $MgSO_4$, filtered and evaporated. The product was purified by flash chromatography (3:1 toluene/EtOAc) to afford **9** as a colorless crystalline powder. R_f 0.49 (1:2 EtOAc/toluene)
Yield: 2.16 g (83%)

mp: 133.5-134.8°C
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$= +46.4° (c 1.0, $CHCl_3$)
IR(neat, cm^{-1}): 3600-3400, 3030.61, 2916.50, 2868.86, 1738.02, 1366.16, 1226.88, 1063.52
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 – 7.71 (m, 3H, Ar-*H*), 7.69 (s, 1H, Ar-*H*), 7.43 – 7.33 (m, 3H, Ar-*H*), 7.33 – 7.15 (m, 15H, Ar-*H*), 5.20 (dd, 1H, *J* = 10.3, 7.8 Hz, H-2'), 4.90 – 4.78 (m, 3H, H-1', ArC*H*$_2$O), 4.67 – 4.51 (m, 7H, H-6b, ArC*H*$_2$O), 4.37 – 4.29 (m, 2H, H-1, H-6b'), 4.19 (dd, 1H, *J* = 11.8, 7.2 Hz, H-3'), 4.05 (d, 1H, *J* = 2.7 Hz, H-6a), 3.87 (d, 1H, *J* = 2.4 Hz, H-6a'), 3.68 (m, 2H, H-4', H-5'), 3.59 – 3.49 (m, 2H, H-2, H-4), 3.43 (dd, 1H, *J* = 6.7, 5.0 Hz, H-5), 3.34 (dd, 1H, *J* = 9.7, 2.9 Hz, H-3), 2.01 (s, 3H, C*H*$_3$), 1.91 (s, 3H, C*H*$_3$), 1.78 (s, 3H, C*H*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.04 (1C), 170.64 (1C), 169.89 (1C), 138.76 (1C), 138.33 (1C), 137.66 (1C), 135.46 (1C), 133.45 (1C), 133.26 (1C), 128.66 (2C), 128.57 (2C), 128.52 (3C), 128.28 (2C), 128.23 (2C), 128.12 (1C), 128.04 (1C), 127.93 (2C), 127.85 (2C), 126.85 (1C), 126.42 (1C), 126.23 (1C), 125.91 (1C), 102.48(1C), 102.15 (1C), 81.39 (1C), 79.45 (1C), 75.53 (1C), 74.56 (1C), 74.10 (1C), 73.61 (1C), 73.57 (1C), 73.33 (1C), 72.17 (1C), 70.88 (1C), 69.70 (1C), 69.46 (1C), 68.03 (1C), 64.36 (1C), 21.19 (1C), 21.12 (1C), 21.00 (1C).
HR-MS: calc. $[M+NH_4]^+$: 896.3858 found $[M+ NH_4]^+$: 896.3824

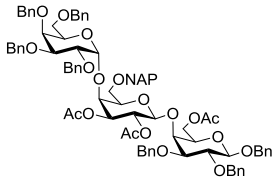
Benzyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (11)



A mixture of **9** (1.9 g; 2.16 mmol) and **6** (1.50 g; 3.02 mmol) was dried azeotropically with benzene (2x30 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (20 mL) and dry MeCN (20 mL), cooled to -20°C, followed by addition of NIS (0.69 g; 3.09 mmol) and TESOTf (114 mg; 0.43 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (3 h). The solution was diluted with CH_2Cl_2 (100 mL) and washed with sat. aq. NaS_2O_3 (100 mL) and sat. aq. $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography (3:1 toluene/EtOAc) to afford **11** as a white crystalline material
Yield: 2.21 g (81%) R_f 0.41 (2:1 toluene/EtOAc)

mp: 87.4 – 88.3°C
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$= -18.7° (c 1.0, $CDCl_3$)
IR(neat, cm^{-1}): 3062.10, 3030.30, 2919.45, 2865.48, 1744.70, 1368.26, 1241.72, 1222.54, 1060.61
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (s, 1H, Ar-*H*), 7.88 – 7.78 (m, 3H, Ar-*H*), 7.71 – 7.57 (m, 4H, Ar-*H*), 7.51 (d, *J* = 6.7 Hz, 1H, Ar-*H*), 7.47 – 7.41 (m, 2H, Ar-*H*), 7.35 – 7.17 (m, 18H, Ar-*H*), 5.60 (s, 1H, ArC*H*$(OR)_2$), 5.33 (dd, 1H, *J* = 10.5, 7.9 Hz, H-2''), 4.92 (m, 5H, H-1'', H-2', H-3'', ArCH_2O), 4.75 – 4.51 (m, 8H, H-1', H-6b, ArCH_2O), 4.38 – 4.15 (m, 5H, H-1, H-3', H-4'', H-6b', H-6b''), 4.06 (d, 1H, *J* = 12.2 Hz, H-6a), 3.97 – 3.76 (m, 3H, H-4', H-6a', H6a''), 3.62 – 3.45 (m, 3H, H-2, H-5', H-5''), 3.46 – 3.38 (m, 1H, H-5), 3.36 (s, 1H, H-4), 3.33 (dd, 1H, *J* = 9.8, 2.9 Hz, H-3), 2.14 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.84 (s, 3H, CH_3), 1.81 (s, 3H, CH_3).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.89, 170.762, 170.16, 169.52, 169.33, 138.49, 138.14, 137.42, 135.80, 135.07, 133.73, 133.13, 132.78, 128.93, 128.41-125.62 (m, 29C), 102.13, 101.13, 100.98, 81.27, 79.13, 75.09, 73.47, 73.29, 73.11, 72.47, 71.92, 71.77, 70.50, 69.81, 68.95, 68.45, 66.26, 63.80

Benzyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (13)

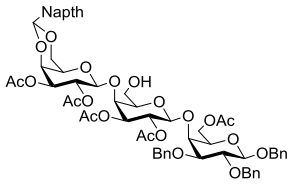


A mixture of **9** (1.2 g; 1.37 mmol) and **10** (1.21 g; 1.91 mmol) was dried azeotropically with benzene (2x30 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (10 mL) and dry Et_2O (10 mL), cooled to -20 °C, followed by addition of NIS (0.44 g; 1.95 mmol) and TESOTf (72 mg; 0.27 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (4 h). The solution was diluted with CH_2Cl_2 (100 mL) and washed with sat. aq. NaS_2O_3 (80 mL) and sat. aq. $NaHCO_3$ (80 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography (4:1 toluene/EtOAc) to afford **13** as a white crystalline material
Yield: 1.61 g (84%) R_f 0.62 (2:1 toluene/EtOAc)

mp: 54.2 – 55.1°C

$[\alpha]_D^{20}$= -27.2° (c 1.0, $CHCl_3$)
IR(neat, cm^{-1}): 3062.57, 3030.41, 2925.39, 2866.92, 1742.01, 1365.91, 1236.91, 1065.61
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 – 7.64 (m, 3H, Ar-*H*), 7.47 (s, 1H, Ar-*H*), 7.37 (m, 4H, Ar-*H*), 7.32 – 7.09 (m, 34H, Ar-*H*), 5.21 (dd, 1H, *J* = 10.6, 7.7 Hz, H-2'), 4.95 – 4.72 (m, 8H, ArCH_2O), 4.65 – 4.45 (m, 7H, H-1', H-6b, ArCH_2O), 4.39 – 4.17 (m, 8H, H-1, H-1'', H-3', H-6b', H-6b'', ArCH_2O), 4.08 (m, 3H, H-4'', H-6a, H-6a''), 3.97 (dd, 1H, *J* = 9.5, 3.3 Hz, H-6a'), 3.81 (m, 2H, H-3'', H-4'), 3.56 (m, 3H, H-2, H-5', H-5''), 3.47 – 3.28 (m, 4H, H-2'', H-3, H-4, H-5), 1.85 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 1.74 (s, 3H, CH_3).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.78 (2C), 169.51, 139.10, 138.94, 138.80, 138.25, 138.10, 137.60, 135.56, 133.32, 133.10, 128.46-127.84 (m, 38C), 127.55, 126.54, 126.22, 126.01, 125.79, 102.52 (2C), 100.35(1C), 81.26, 79.51, 79.03, 75.47, 75.12, 74.43, 74.19, 73.73, 73.57, 73.40, 73.12, 72.70, 72.05, 70.94, 69.88, 69.59, 68.22, 67.63, 63.87, 20.89 (2C), 20.74 (1C).

Benzyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (12)

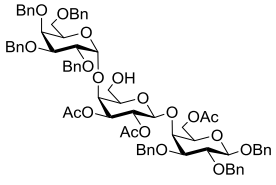


To a solution of **11** (1.1 g; 0.87 mmol) in CH_2Cl_2 (8 mL) and methanol (2 mL), DDQ (0.27 g; 1.18 mmol) and three drops of water were added and the reaction was stirred at 22 °C. After complete conversion (2 h) the mixture was concentrated and the residue was purified by flash chromatography (19:1 CH_2Cl_2/MeOH followed by another column with the eluent system 3:1 toluene/EtOAc). R_f 0.31 (2:1 toluene/EtOAc).

Yield: 0.77 g (79%)

mp: 114.3 – 115.4°C
$[\alpha]_D^{20}$= 31.5° (c 1.0, $CHCl_3$)
IR(neat, cm^{-1}): 3600-3400, 3063.06, 3030.43, 2869.65, 1742.52, 1367.26, 1220.37, 1055.46
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.74 (m, 4H, Ar-*H*), 7.56 (dd, 1H, *J* = 8.5, 1.6 Hz, Ar-*H*), 7.46 – 7.39 (m, 2H, Ar-*H*), 7.34 – 7.16 (m, 15H, Ar-*H*), 5.57 (s, 1H, ArC*H*$(OR)_2$), 5.31 (dd, 1H, *J* = 10.5, 7.9 Hz, H-2''), 5.08 – 4.77 (m, 5H, H-1'', H-3'', ArCH_2O), 4.69 (d, 1H, *J* = 11.8 Hz, H-2'), 4.63 – 4.49 (m, 5H, H-1', H-6b, ArCH_2O), 4.40 – 4.26 (m, 3H, H-1, H-4'', H-6b''), 4.21 – 4.11 (m, 2H, H-3', H-6b'), 4.03 (d, 1H, *J* = 11.3 Hz, H-6a), 3.86 (d, 1H, *J* = 2.6 Hz, H-6a'), 3.82 – 3.67 (m, 2H, H-4', H-6a''), 3.58 – 3.46 (m, 3H, H-2, H-5, H-5''), 3.42 (t, 1H, *J* = 5.8 Hz, H-5'), 3.36 (dd, 1H, *J* = 9.7, 2.9 Hz, H-3), 3.18 (t, 1H, *J* = 7.3 Hz, H-4), 2.12 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.80 (s, 3H, CH_3).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.97, 170.83, 170.24, 169.351 (2C), 138.47, 138.13, 137.37, 134.61, 133.75, 132.74, 128.34-127.47 (m, 18C), 126.30, 125.96, 125.77, 123.82, 102.22, 101.56 (2C), 101.36, 81.09, 79.15, 75.16, 74.51, 73.81 (2C), 73.26, 73.14, 72.56, 71.74, 71.48, 70.64, 69.46, 68.63 (2C), 66.50, 63.81, 60.36, 20.83-20.59 (5C).

Benzyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (14)

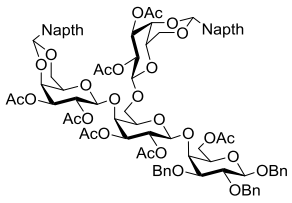


To a solution of **13** (0.92 g; 0.66 mmol) in CH_2Cl_2 (8 mL) and methanol (2 mL), DDQ (0.18 g; 0.79 mmol) and three drops of water were added and the reaction was stirred at 22 °C. After complete conversion (2 h) the mixture was concentrated and the residue was purified by flash chromatography (19:1 CH_2Cl_2/MeOH followed by another column with the eluent system 4:1 toluene/EtOAc). R_f 0.47 (2:1 toluene/EtOAc)
Yield: 0.72 g (87%)

mp: 61.2 – 63.5°C
$[\alpha]_D^{20}$= -15.7° (c 1.0, $CHCl_3$)

IR(neat, cm^{-1}): 3600-3400, 3063.13, 3030.31, 2917.56, 2868.89, 1746.19, 1365.82, 1221.71,1052.50
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.12 (m, 35H, Ar-*H*), 5.16 (dd, 1H, *J* = 10.7, 7.7 Hz, H-2'), 4.93 – 4.70 (m, 7H, PhC*H*$_2$O), 4.70 – 4.46 (m, 7H, PhC*H*$_2$O), 4.43 (d, 1H, *J* = 6.4 Hz, H-1'), 4.39 – 4.30 (m, 2H, H-1, H-1''), 4.27 (dd, 2H, *J* = 6.1, 3.6 Hz, H-4', H-6b), 4.21 (dd, 1H, *J* = 9.3, 5.9 Hz, H-3'), 4.08 (s, 1H, H-6b''), 4.02 (s, 1H, H-6a), 3.98 (dd, 2H, *J* = 6.2, 2.6 Hz, H-4'', H-5''), 3.77 (d, 1H, *J* = 2.5 Hz, H-6a''), 3.70 – 3.29 (m, 9H, H-2, H-2'', H-3, H-3'', H-4, H-5, H-5', H-6a', H-6b'), 1.99 (s, 3H, C*H*$_3$), 1.85 (s, 3H, C*H*$_3$), 1.76 (s, 3H, C*H*$_3$).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.78, 170.61, 169.15, 138.75, 138.52, 138.42, 138.21, 138.03, 137.48, 137.36, 128.21-127.42 (m, 45), 102.44 (2C), 101.26, 81.01, 79.22, 78.76, 75.65, 75.29, 74.96, 74.68, 74.56, 74.40, 73.32, 72.78, 72.20, 71.56, 70.83, 69.61, 69.28, 67.93, 63.53, 60.13, 20.85-20.69 (3C).

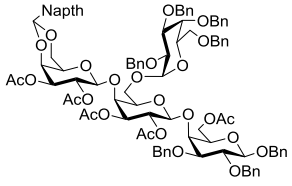
Benzyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl-(1→4)-[2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl-(1→6)]-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (15)



A mixture of **12** (450 mg; 0.40 mmol) and **6** (277 mg; 0.56 mmol) was dried azeotropically with benzene (2 x 5 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (2.5 mL) and dry MeCN (2.5 mL), cooled to -20°C, followed by addition of NIS (130 mg; 0.58 mmol) and TESOTf (21 mg; 0.08 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (2 h). The solution was diluted with CH_2Cl_2 (40 mL) and washed with sat. aq. NaS_2O_3 (40 mL) and sat. aq. $NaHCO_3$ (40 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography (3:1 toluene/EtOAc) to afford **15** as a white crystalline material. R_f 0.25 (2:1 toluene/EtOAc)
Yield: 452 mg (75%)

mp: 143.4 – 144.2°C
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$= -42.5° (c 1.0, $CHCl_3$)
IR(neat, cm^{-1}): 3062.41, 3030.38, 2867.75, 1742.18, 1367.70, 1242.11, 1220.49, 1061.20
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 – 7.67 (m, 9H, Ar-*H*), 7.59 (dd, 1H, *J* = 8.4, 1.5 Hz, Ar-*H*), 7.51 – 7.14 (m, 19H, Ar-*H*), 5.51 (s, 1H, ArC*H*(OR)$_2$), 5.40 (s, 1H, ArC*H*(OR)$_2$), 5.28 (m, 2H, H-2'', Gal-1,6 H-2), 5.05 – 4.52 (m, 11H, H-1'', Gal-1,6 H-1, H-2', H-3'', Gal-1,6 H-3, ArC*H*$_2$O), 4.44 (d, 1H, *J* = 7.8 Hz, H-1), 4.40 – 3.90 (m, 11H, H-3', H-4'', Gal-1,6 H-4, H-6a, H-6a', H-6a'', Gal-1,6 H-6a, H-6b, H-6b', H-6b'', Gal-1,6 H-6b), 3.84 (dd, 1H, *J* = 12.0, 7.9 Hz, H-4'), 3.51 (m, 6H, H-2, H-4, H-5, H-5', H-5'', Gal-1,6 H-5), 3.36 (dd, 1H, *J* = 9.7, 2.8 Hz, H-3), 3.12 (s, 3H, H-4), 2.12 (s, 3H, C*H*$_3$), 2.09 (s, 3H, C*H*$_3$), 2.02 (s, 3H, C*H*$_3$), 2.00 (s, 3H, C*H*$_3$), 1.95 (s, 3H, C*H*$_3$), 1.87 (s, 3H, C*H*$_3$), 1.82 (s, 3H, C*H*$_3$).
^{13}C NMR (75 MHz, $CDCl_3$) δ 171.09 (2C), 170.66, 170.44, 169.67, 169.54, 169.23, 138.74, 138.39, 137.65, 135.49, 135.19, 133.93, 133.76, 132.98, 128.53-127.633 (m, 14C), 126.60, 126.33, 126.05, 125.80, 124.35, 124.17, 102.22, 101.49, 101.30 (2C), 101.04 (2C), 81.48, 79.44, 75.25, 73.78, 73.39, 72.44, 72.06, 71.87, 70.66, 69.77, 69.57, 69.11, 68.84, 66.36, 66.24, 65.28, 21.17-20.85 (m, 7C).

Benzyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl)-(1→4)-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→6)]-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (16)

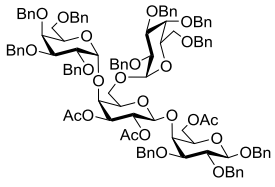


A mixture of **12** (450 mg; 0.40 mmol) and **10** (355 mg; 0.56 mmol) was dried azeotropically with benzene (2 x 5 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (2.5 mL) and dry Et_2O (2.5 mL), cooled to -20°C, followed by addition of NIS (130 mg; 0.58 mmol) and TESOTf (21 mg; 0.08 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (4 h). The solution was diluted with CH_2Cl_2 (40 mL) and washed with sat. aq. NaS_2O_3 (40 mL) and sat. aq. $NaHCO_3$ (40 mL). The organic phase was dried over $MgSO_4$,

filtered and concentrated. The product was purified by flash chromatography (4:1 toluene/EtOAc) to afford a white crystalline material. R_f 0.44 (2:1 toluene/EtOAc)
Yield: 514 mg (78%)

mp: 85.8 – 86.4°C
$[\alpha]_D^{20}$ = 31.2° (c 1.0, $CHCl_3$)
IR(neat, cm^{-1}): 3062.96, 3030.63, 2868.58, 1746.69, 1367.42, 1220.78, 1058.26
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 – 7.65 (m, 4H, Ar-*H*), 7.54 (dd, 1H, *J* = 8.5, 1.6 Hz, Ar-*H*), 7.42 – 7.05 (m, 37H, Ar-*H*), 5.48 (s, 1H, ArC*H*$(OR)_2$), 5.27 (dd, 1H, *J* = 10.5, 7.9 Hz, H-2''), 5.03 (dd, 1H, *J* = 10.3, 7.7 Hz, H-2'), 4.93 – 4.74 (m, 7H, H-1'', ArCH_2O), 4.70 (d, 1H, *J* = 7.7 Hz, H-3''), 4.63 – 4.39 (m, 9H, H-1, H-1', H-6b, ArCH_2O), 4.37 – 4.03 (m, 7H, Gal-1,6 H-1, H-3', H-4'', H-6a, H-6b'', Gal-1,6 H-6b, ArCH_2O), 4.00 – 3.65 (m, 7H, H-2, Gal-1,6 H-2, Gal-1,6 H-3, H-4', Gal-1,6 H-4, Gal-1,6 H-5, H-6a', H-6a''), 3.61 (t, 1H, *J* = 5.4 Hz, H-5''), 3.55 – 3.33 (m, 4H, H-3, H-5, H-5', Gal-1,6 H-6a), 3.28 (dd, H1, *J* = 9.8, 2.8 Hz, H-4), 2.12 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.90 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 1.60 (s, 3H, CH_3).
^{13}C NMR (75 MHz, $CDCl_3$) δ 171.078 (2C), 170.38, 169.56 (2C), 139.24, 138.96, 138.79, 138.44, 138.22, 137.69, 135.15, 133.90, 132.97, 128.41-127.53 (m, 39C), 126.35, 126.04, 125.91, 124.28, 102.22, 101.40 (3C), 98.58, 81.45, 79.40, 75.29, 74.90, 73.84, 73.49, 73.24, 72.58, 72.28, 72.02, 70.62, 69.97, 69.39, 69.00, 68.74, 67.43, 66.49, 64.36, 21.06-20.86 (m, 5C).

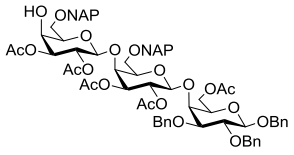
Benzyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→6)]-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (17)



A mixture of **14** (310 mg; 0.25 mmol) and **10** (218 mg; 0.34 mmol) was dried azeotropically with benzene (2 x 5 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (2.5 mL) and dry Et_2O (2.5 mL), cooled to -20°C, followed by addition of NIS (79 mg; 0.35 mmol) and TESOTf (13 mg; 0.05 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (2 h). The solution was diluted with CH_2Cl_2 (40 mL) and washed with sat. aq. NaS_2O_3 (40 mL) and sat. aq. $NaHCO_3$ (40 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography (4:1 toluene/EtOAc) to afford a white crystalline material. R_f 0.55 (2:1 toluene/EtOAc)
Based on ^{1}H NMR and ^{13}C NMR the product is a mixture of the α- and β-product and is therefore deprotected prior to characterization. Separated product: 355 mg (81%)

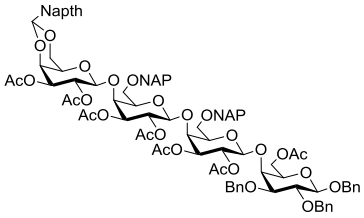
mp: 44.7-45.9°C
IR (neat, cm^{-1}): 3062.83, 3029.99, 2919.27, 2868.25, 1750.43, 1453.96, 1365.76, 1237.76, 1094.02, 1054.77
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 6.90 (m, 55H, Ar-*H*), 5.19 (dd, 1H, *J* = 10.7, 7.7 Hz, H-2'), 5.00 – 3.21 (m, 49H (mangler 14H se appendix), H-1, H-1', H-1'', Gal-1,6 H-1, H-2, H-2'', Gal-1,6 H-2, H-3, H-3', H-3'', Gal-1,6 H-3, H-4, H-4', H-4'', Gal-1,6 H-4, H-5, H-5', H-5'', Gal-1,6 H-5, H-6a, H-6a', H-6a'', Gal-1,6 H-6a, H-6b, H-6b', H-6b'', Gal-1,6 H-6b), 1.90 (s, 3H, CH_3), 1.86 (s, 3H, CH_3), 1.72 (s, 3H, CH_3).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.67 (2C), 169.53, 139.27, 139.03, 138.86, 138.68, 138.25, 138.14, 137.61, 128.44-127.21 (m, 59C), 102.60, 102.43, 99.97, 98.73, 81.22, 79.53, 78.86, 76.20, 75.47, 75.12, 74.85, 73.62, 73.52, 73.33, 72.94, 72.73, 72.41, 72.03, 70.89, 69.92, 69.54, 69.12, 68.28, 63.88, 21.07, 20.77, 20.67.

Benzyl 2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (S2)



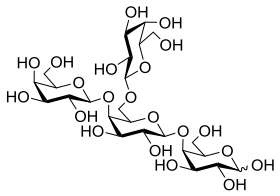
To a solution of **11** (1.0 g; 0.79 mmol) in dry THF (20 mL), $NaCNBH_3$ (1.0 g; 15.81 mmol) was added. The mixture was stirred at room temperature and a 2M solution of HCl in dry ether was added drop wise until gas development ceased and the mixture remained acidic (pH 3-4). The mixture was concentrated to 2 ml, diluted with CH_2Cl_2 (50 ml) and the mixture was neutralized with sat. aq. $NaHCO_3$ (50 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 50 ml). The combined organic phases were washed with water (200 mL), dried over $MgSO_4$, filtered and evaporated. The product was purified by flash chromatography (4:1 toluene/EtOAc) to afford a colorless crystalline powder. **S2** was used for the following reaction without characterization. R_f 0.35 (2:1 toluene/EtOAc)
Yield: 290 mg (74%)

Benzyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (18)



A mixture of **S2** (760 mg; 0.60 mmol) and **6** (416 mg; 0.84 mmol) was dried azeotropically with benzene (2 x 5 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (5 mL) and dry MeCN (5 mL), cooled to -20°C, followed by addition of NIS (196 mg; 0.87 mmol) and TESOTf (32 mg; 0.12 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (2 h). The solution was diluted with CH_2Cl_2 (40 mL) and washed with sat. aq. NaS_2O_3 (40 mL) and sat. aq. $NaHCO_3$ (40 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography (4:1 toluene/EtOAc) to afford a white crystalline material. **18** was deprotected prior to characterization. R_f 0.44 (1:1 toluene/EtOAc)
Yield: 749 mg (76%)

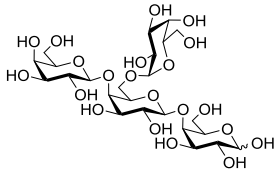
β-D-Galactopyranosyl-(1→4)-[α-D-galactopyranosyl-(1→6)]-β-D-galactopyranosyl-(1→4)-D-galactopyranose (3)



15 (323 mg; 0.21 mmol) was dissolved in MeOH (6 mL) and THF (2 mL) and 5 drops of freshly prepared 1 M NaOMe in MeOH was added. The reaction mixture was stirred at room temperature until full conversion was observed by TLC (24 hours). The reaction was quenched with Amberlite IR-120 (H^+), filtered and concentrated. The product was purified by flash chromatography (9:1 EtOAc/MeOH) to yield colorless crystals.
The product was next dissolved in MeOH (6 mL), THF (2 mL) and AcOH (0.5 mL). 10% Pd/C (132 mg; 0.18 mmol) was added and an atmosphere of H_2 (1 atm.) was installed. The reaction was stirred for 2 h, then water (2 mL) was added. The reaction was stirred until TLC indicated full conversion (72 h), filtered through celite, and concentrated to give a white solid. R_f 0.68 (3:1 MeOH/H_2O)
Yield: 67 mg (81%)

^{1}H NMR (400 MHz, D_2O) δ 4.69 – 4.57, 4.46, 4.22 – 4.10, 4.04 – 3.48.
^{13}C NMR (101 MHz, D_2O) δ 106.85, 106.62, 106.06, 98.88, 81.24, 80.26, 79.94, 77.61, 76.67, 76.27, 75.76, 75.26, 74.77, 74.22, 73.97, 73.24, 72.64, 72.21, 71.34, 71.14, 63.47, 63.16.
HR-MS: calc. $[M+H]^+$: 667.2297 found $[M+H]^+$: 667.2275

β-D-Galactopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→6)]-β-D-galactopyranosyl-(1→4)-D-galactopyranose (4)

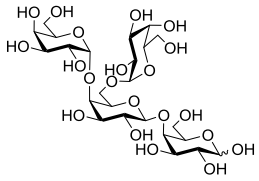


16 (465 mg; 0.28 mmol) was dissolved in MeOH (6 mL) and THF (2 mL) and 6 drops of freshly prepared 1 M NaOMe in MeOH was added. The reaction mixture was stirred at room temperature until full conversion was observed by TLC (24 h). The reaction was quenched with Amberlite IR-120 (H^+), filtered and concentrated. The product was purified by flash chromatography (9:1 CH_2Cl_2/MeOH) to yield colorless crystals.

The product was next dissolved in MeOH (6 mL), THF (2 mL) and AcOH (0.5 mL). 10% Pd/C (223 mg; 0.18 mmol) was added and an atmosphere of H_2 (1 atm.) was installed. The reaction was stirred for 2 h, and then water (2 mL) was added. The reaction was stirred until TLC indicated full conversion (72 h), filtered through celite, and concentrated to give a white solid. R_f 0.72 (3:1 MeOH/H_2O)
Yield: 92 mg (79%)

^{1}H NMR (800 MHz, D_2O) δ 5.02, 4.70 – 4.59, 4.30 – 4.11, 4.03 – 3.50.
^{13}C NMR (200 MHz, D_2O) δ 107.46, 106.99, 106.28, 101.11, 99.11, 95.06, 82.47, 81.55, 80.41, 80.20, 77.99, 77.84, 77.26, 76.51, 76.01, 75.75, 75.49, 75.09, 74.72, 74.43, 74.21, 73.62, 72.83, 72.44, 72.23, 71.92, 71.66, 71.41, 70.96, 69.83, 63.87.
HR-MS: calc. $[M+H]^+$: 667.2297 found $[M+ H]^+$: 667.2267

α-D-Galactopyranosyl-(1→4)-[α-D-galactopyranosyl)-(1→6)]-β-D-galactopyranosyl-(1→4)-D-galactopyranose (5)

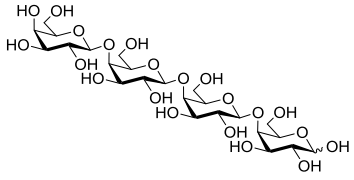


17 (200 mg; 0.11 mmol) was dissolved in MeOH (6 mL), THF (2 mL) and AcOH (0.5 mL). 10% Pd/C (223 mg; 0.18 mmol) was added and an atmosphere of H_2 (1 atm.) was installed. The reaction was stirred for 2 h, then water (2 mL) was added. The reaction was stirred until TLC indicated full conversion (48 h), filtered through celite, and concentrated to give a white solid.

The product was next dissolved in MeOH (6 mL) and THF (2 mL) and 6 drops of freshly prepared 1 M NaOMe in MeOH was added. The reaction mixture was stirred at room temperature until full conversion was observed by TLC (24 hours). The reaction was quenched with Amberlite IR-120 (H^+), filtered and concentrated. The product was purified by flash chromatography (2:1 toluene/EtOAc) to yield colorless crystals. R_f 0.73 (3:1 MeOH/H_2O)
Yield 269 mg (90%)

^{1}H NMR (400 MHz, D_2O) δ 5.05 – 4.95, 4.73 – 4.66, 4.59, 4.42, 4.27 – 3.49.
^{13}C NMR (101 MHz, D_2O) δ 107.69, 107.43, 106.58, 105.95, 102.89, 101.40, 99.08, 95.03, 84.49, 82.71, 81.83, 81.30, 79.59, 79.42, 77.86, 77.33, 76.76, 76.33, 76.04, 75.41, 75.06, 74.80, 74.31, 73.76, 73.37, 72.91, 72.248, 72.10, 71.87, 71.72, 71.46, 70.89, 68.66, 66.06, 64.09, 63.81, 63.22.
HR-MS: calc. $[M+Na]^+$: 689.2117 found $[M+ Na]^+$: 689.2095

β-D-Galactopyranosyl-(1→4)-(β-D-galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-D-galactopyranose (1)



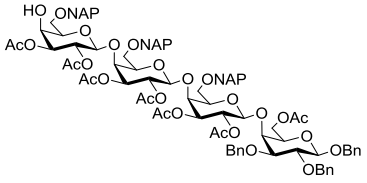
18 (260 mg; 0.16 mmol) was dissolved in MeOH (6 mL) and THF (2 mL) and 5 drops of freshly prepared 1 M NaOMe in MeOH was added. The reaction mixture was stirred at room temperature until full conversion was observed by TLC (24 hours). The reaction was quenched with Amberlite IR-120 (H^+), filtered and concentrated. The product was purified by flash chromatography (4:1 EtOAc/MeOH) to yield colorless crystals.

The product was next dissolved in MeOH (6 mL), THF (2 mL) and AcOH (0.5 mL). 10% Pd/C (223 mg; 0.18 mmol) was added and an atmosphere of H_2 (1 atm.) was installed. The reaction was stirred for 2 h, then water (2 mL) was added. The reaction was stirred until TLC indicated full conversion (48 h), filtered through celite, and concentrated to give a white solid. R_f 0.74 (3:1 MeOH/H_2O)

Yield: 72 mg (83%)

^{1}H NMR (800 MHz, D_2O) δ 4.68 – 4.59, 4.19, 4.14, 3.96, 3.93 – 3.65, 3.62, 3.58.
^{13}C NMR (200 MHz, D_2O) δ 107.06 (3C), 99.12, 95.04, 82.43, 81.54, 80.59, 80.30, 79.87, 79.40, 77.87, 77.20, 75.98, 75.48, 74.97, 74.54, 74.10, 72.58, 72.42, 72.04, 71.36, 63.69, 63.45, 63.29
HR-MS: calc. $[M+Na]^+$: 689.2117 found $[M+ Na]^+$: 689.2089

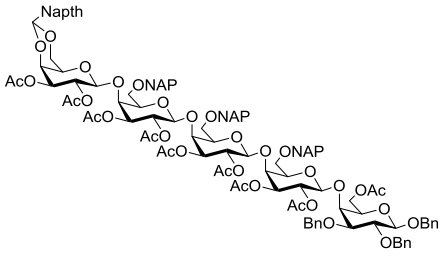
Benzyl 2,3-di-O-acetyl-6-O-(2-naphthyl)methyl-β-D-galactopyranosyl-(1→4)-(2,3-di-O-acetyl-6-O-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-(2,3-di-O-acetyl-6-O-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-6-O-acetyl-2,3-di-O-benzyl-β-Dgalactopyranoside (S3)



To a solution of **18** (390 mg; 0.24 mmol) in dry THF (6 mL), $NaCNBH_3$ (297 mg; 4.73 mmol) was added. The mixture was stirred at room temperature and a 2M solution of HCl in dry ether was added dropwise until gas development ceased and the mixture remained acidic (pH 3-4). The mixture was concentrated to 2 ml, diluted with CH2Cl2 (50 ml) and the mixture was neutralized with sat. aq. NaHCO3 (50 mL). The aqueous phase was extracted with CH2Cl2 (3 x 50 ml). The combined organic phase were washed with water (200 mL), dried over MgSO4, filtered and evaporated.. R_f 0.36 (1:1 toluene/EtOAc)
Yield: 290 mg (74%)
mp: 81.5 – 82.1°C

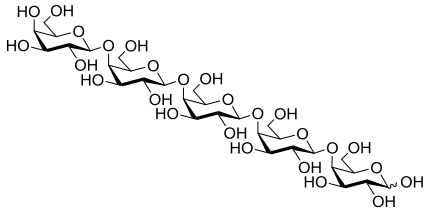
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 – 6.89 (m, 36H), 5.35 (dd, *J* = 10.2, 7.8 Hz, 1H), 5.07 – 3.09 (m, 39H), 2.19 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.77 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.99-169.63 (7C), 138.74, 138.33, 137.65, 136.30, 135.85, 135.01, 133.51, 133.32, 133.10, 128.47-127.63 (m, 33C), 126.78, 126.60, 126.31, 126.17, 125.96, 125.81, 125.57, 103.37, 101.52, 101.34, 100.93, 81.44, 79.40, 75.35, 73.79, 73.62, 73.25, 72.68, 72.24, 70.76, 69.90, 69.57, 69.27, 69.11, 67.87, 64.09, 21.03-20.84 (7C).

Benzyl 2,3-di-*O*-acetyl-4,6-*O*-(2-naphthyl)methylene-β-D-galactopyranosyl-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-6-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (19)



A mixture of **S3** (240 mg; 0.15 mmol) and **6** (108 mg; 0.22 mmol) was dried azeotropically with benzene (2 x 5 mL) and subjected to vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (5 mL) and dry MeCN (5 mL), cooled to -20°C, followed by addition of NIS (50 mg; 0.23 mmol) and TESOTf (8 mg; 0.03 mmol). The reaction mixture was stirred at -20°C until TLC revealed full conversion of the donor (2 h). The solution was diluted with CH_2Cl_2 (40 mL) and washed with sat. aq. NaS_2O_3 (40 mL) and sat. aq. $NaHCO_3$ (40 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography (3:1 toluene/EtOAc) to afford a white crystalline material. **19** was deprotected prior to characterization. R_f 0.26 (1:1 toluene/EtOAc)
Yield: 206 mg (71%)

Benzyl-β-D-galactopyranosyl-(1→4)-(β-D-galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-D-galactopyranose (2)



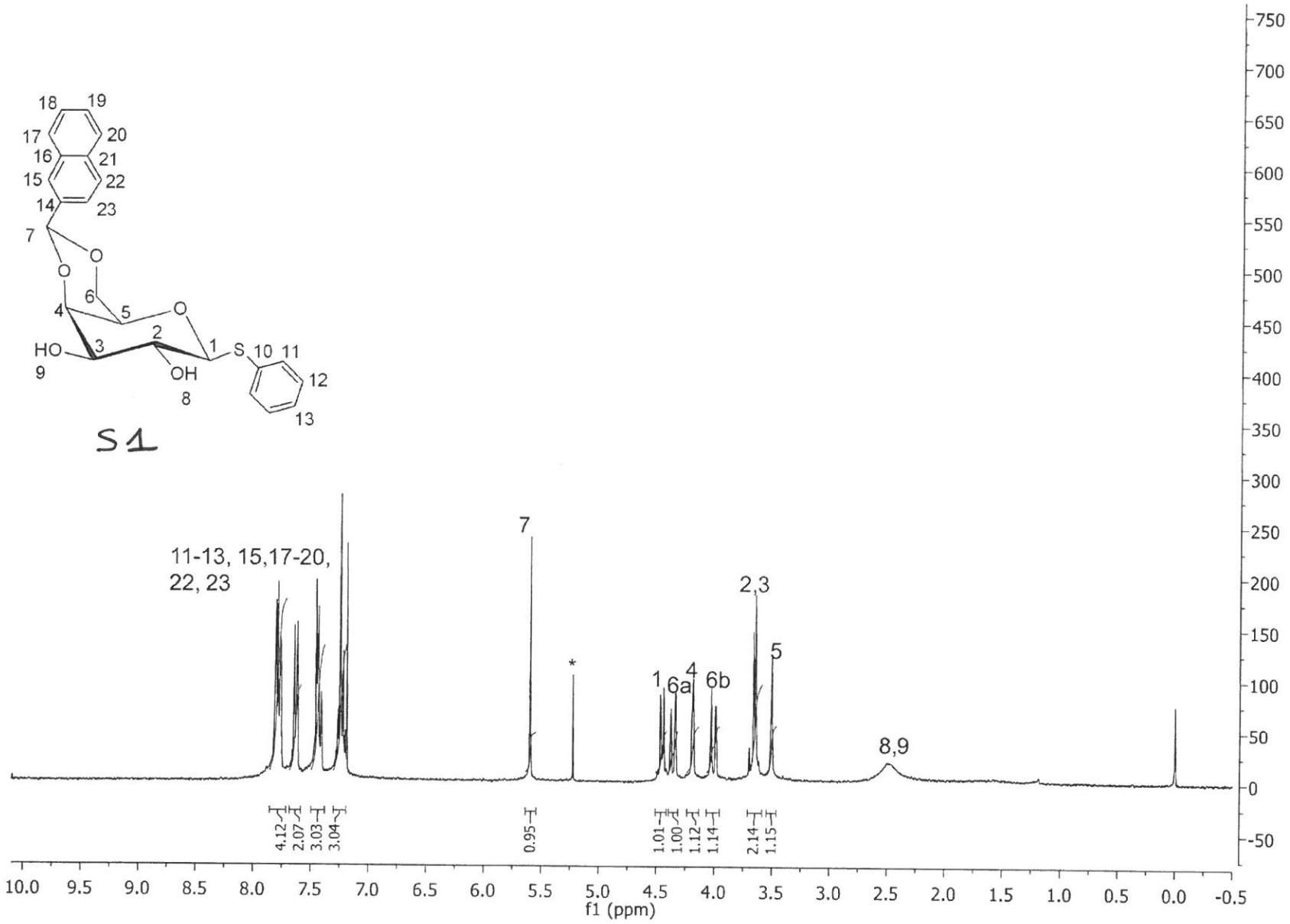
19 (200 mg; 0.10 mmol) was dissolved in MeOH (6 mL) and THF (2 mL) and 5 drops of freshly prepared 1 M NaOMe in MeOH was added. The reaction mixture was stirred at room temperature until full conversion was observed by TLC (72 hours). The reaction was quenched with Amberlite IR-120 (H+), filtered and concentrated. The product was purified by flash chromatography (4:1 EtOAc/MeOH) to yield colorless crystals. R_f 0.21 (9:1 EtOAc/MeOH).

The product (130 mg; 0.078 mmol) was dissolved in MeOH (6 mL), THF (2 mL) and AcOH (0.5 mL). 10% Pd/C (83 mg; 0.078 mmol) was added and an atmosphere of H_2 (1 atm.) was installed. The reaction was stirred for 2 h, then water (2 mL) was added. The reaction was stirred until TLC indicated full conversion (48 h), filtered through celite, and concentrated to give a white solid. R_f 0.68 (3:1 MeOH/H_2O)
Yield: 54 mg (84%)

^{1}H NMR (800 MHz, D_2O) δ 5.23 , 4.55 – 4.48, 4.23 – 3.96, 3.95 – 3.49.

^{13}C NMR (200 MHz, D_2O) δ 107.04 (4C), 99.12 , 95.02, 80.58, 80.21, 79.86, 77.86, 77.20, 76.00, 75.48, 74.97, 74.53, 74.09, 72.41, 71.35, 63.69, 63.44

HR-MS: calc. [M+Na]+: 851.2645 found [M+ Na]+: 851.2625.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16
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15
22
14
23
7
O
O
6
4
5
O
2
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S
10
11
HO
3
OH
9
8
12
13
S1
11-13, 15,17-20,
22, 23
7
*
1
6a
4
6b
2,3
5
8,9
4.12
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3.03
3.04
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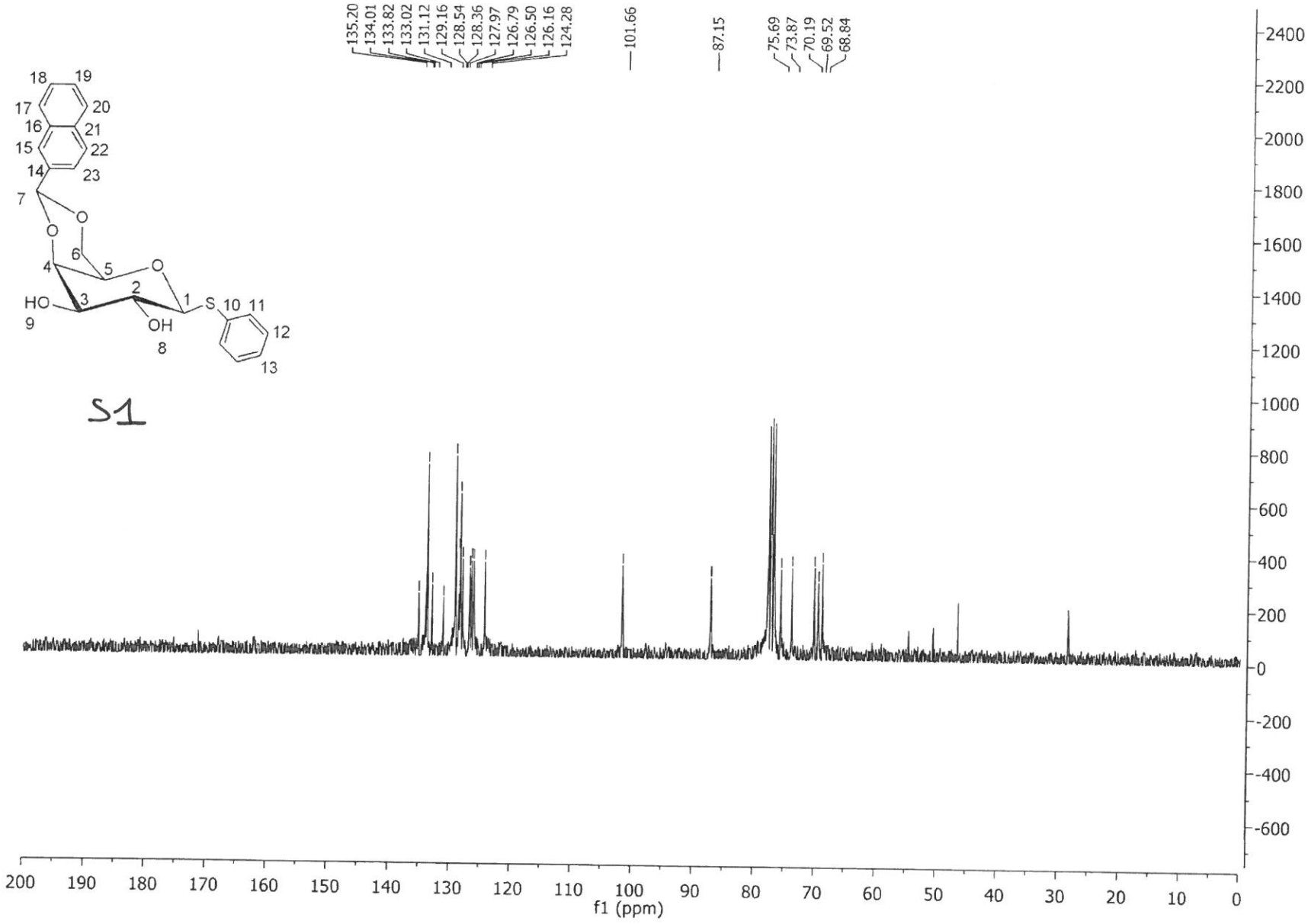
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101.66
87.15
75.69
73.87
70.19
69.52
68.84
18
19
17
20
16
21
15
22
14
23
7
O
O
6
5
O
4
2
1
S
10
11
12
13
HO
9
3
OH
8
S1
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
-400
-600
200
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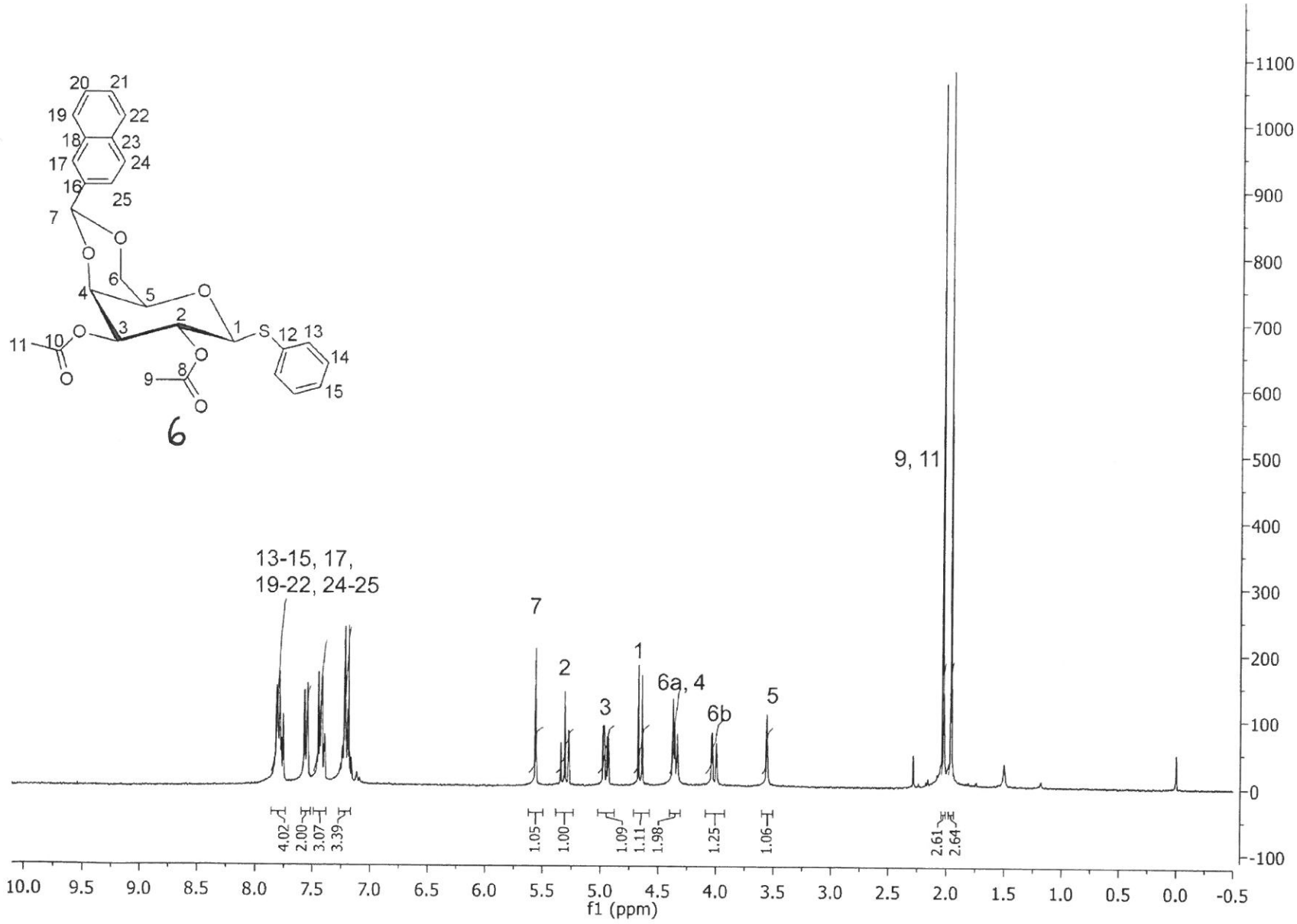
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13-15, 17,
19-22, 24-25
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2
3
1
6a, 4
6b
5
9, 11
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1.00
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1.11
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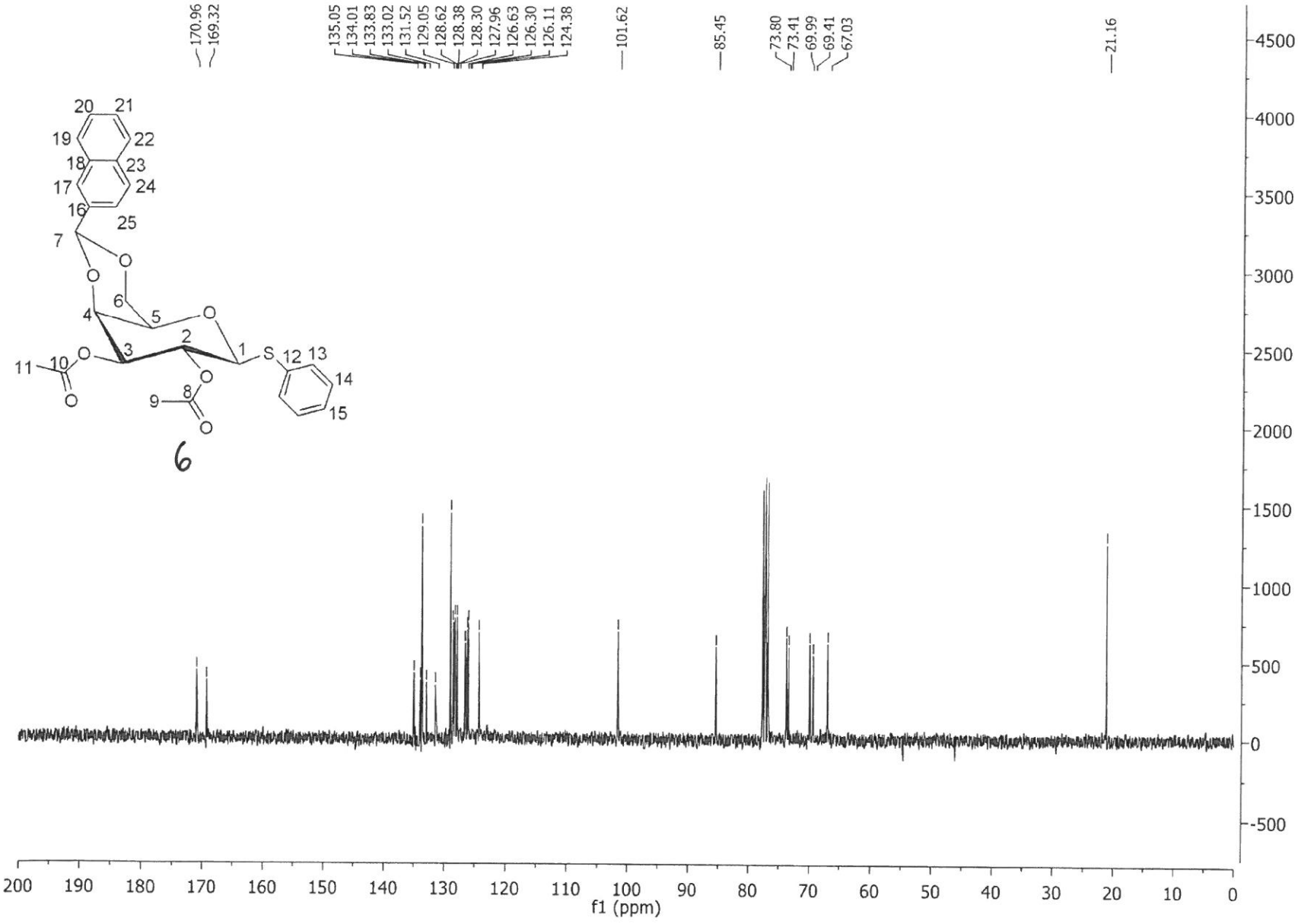
f1 (ppm)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129.05
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128.30
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126.63
126.30
126.11
124.38
101.62
85.45
73.80
73.41
69.99
69.41
67.03
21.16
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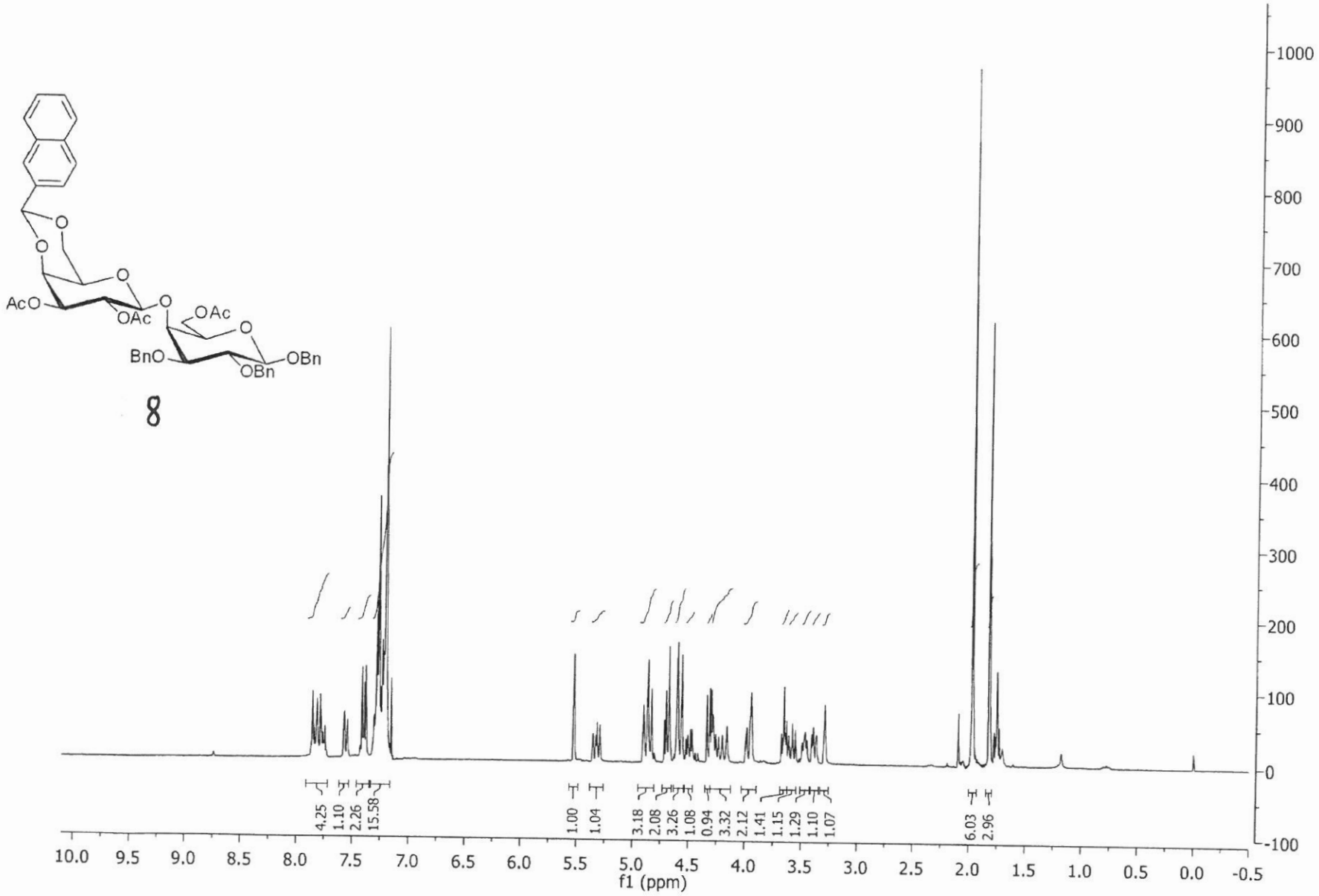
f1 (ppm)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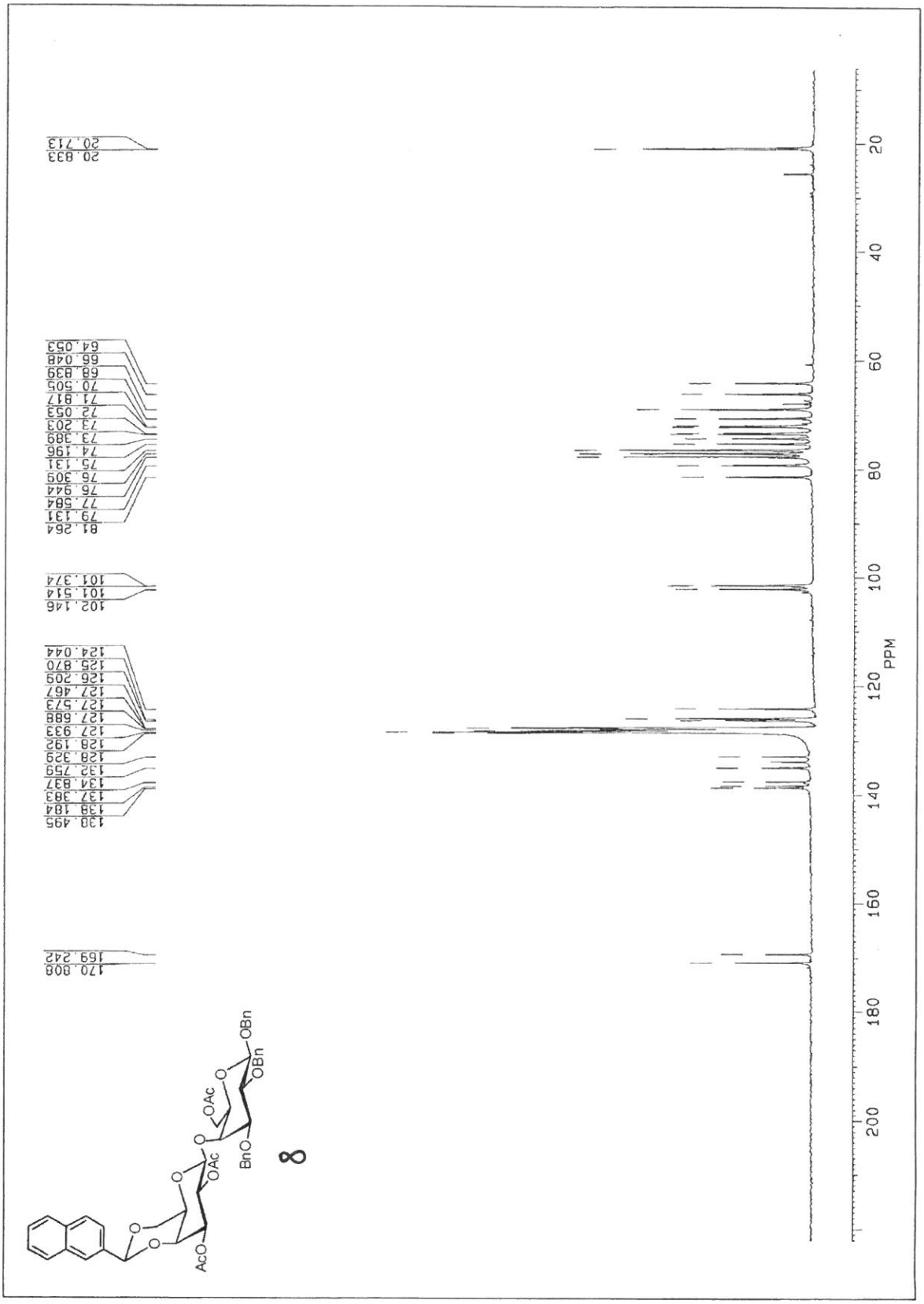
f1 (ppm)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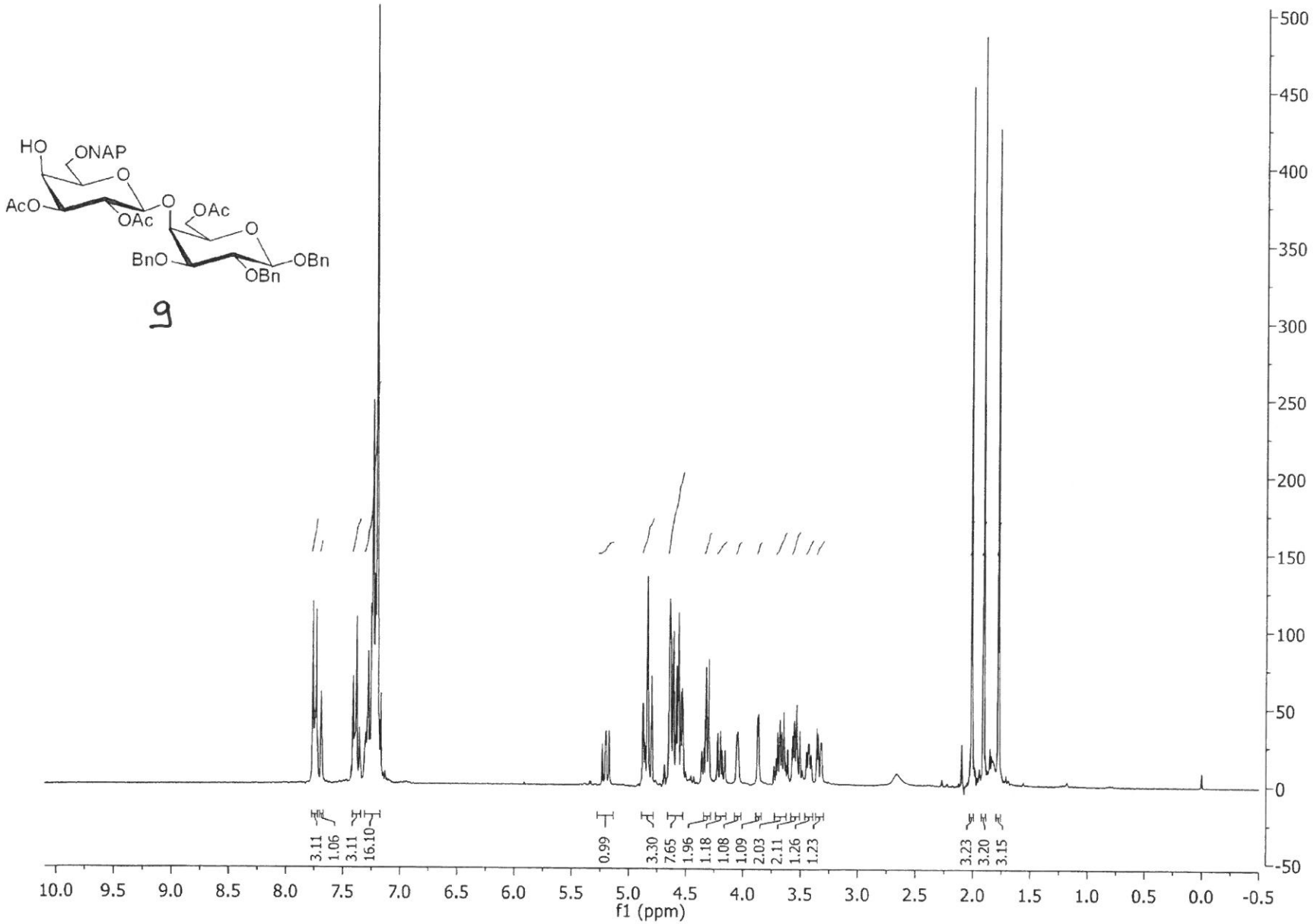
OBn
8
PPM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OBn
OBn
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7.65
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1.09
2.03
2.11
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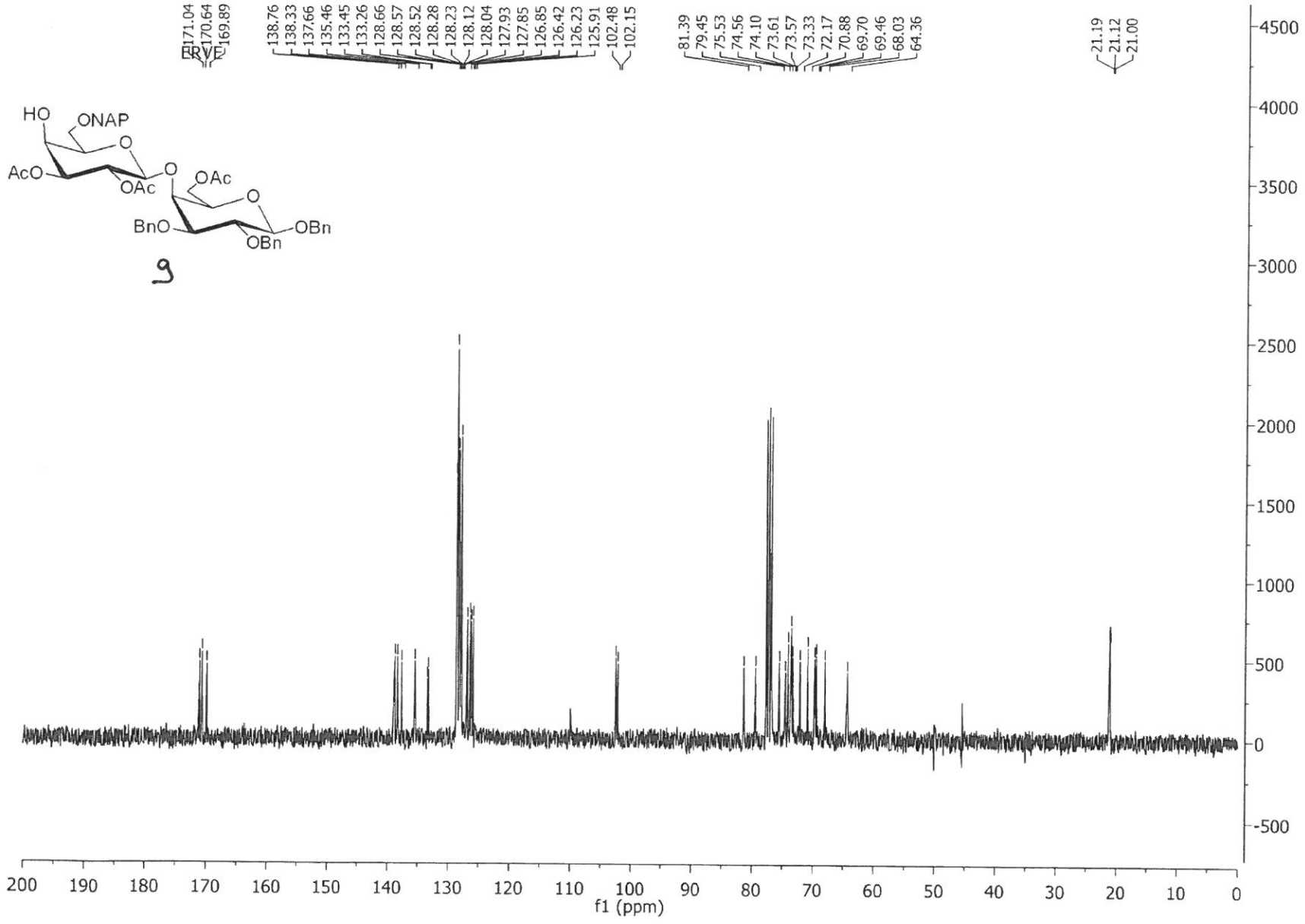
3.23
3.20
3.15
f1 (ppm)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128.23
128.12
128.04
127.93
127.85
126.85
126.42
126.23
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102.15
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79.45
75.53
74.56
74.10
73.61
73.57
73.33
72.17
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69.70
69.46
68.03
64.36
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OAc
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OAc
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BnO
OBn
OBn
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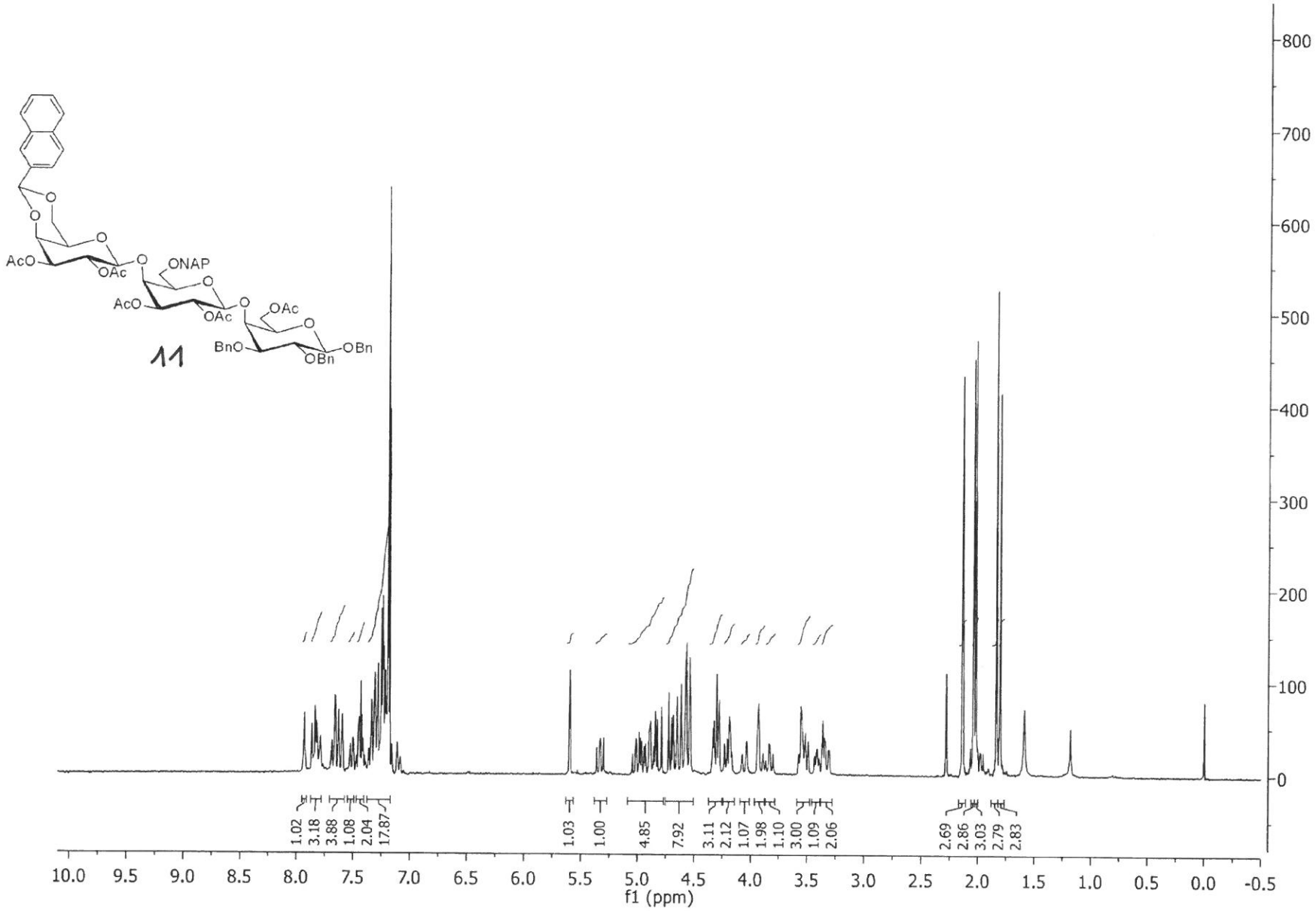
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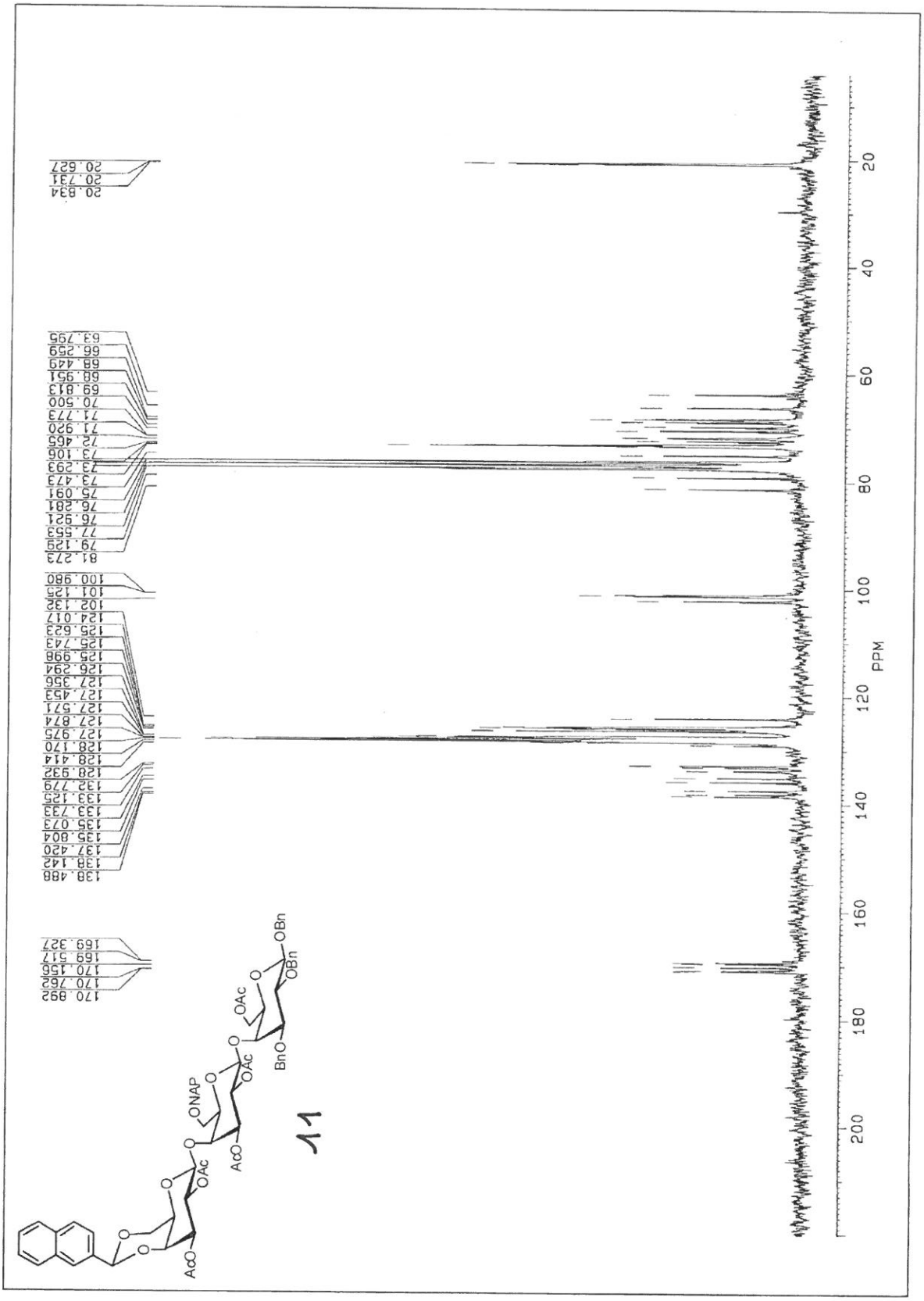
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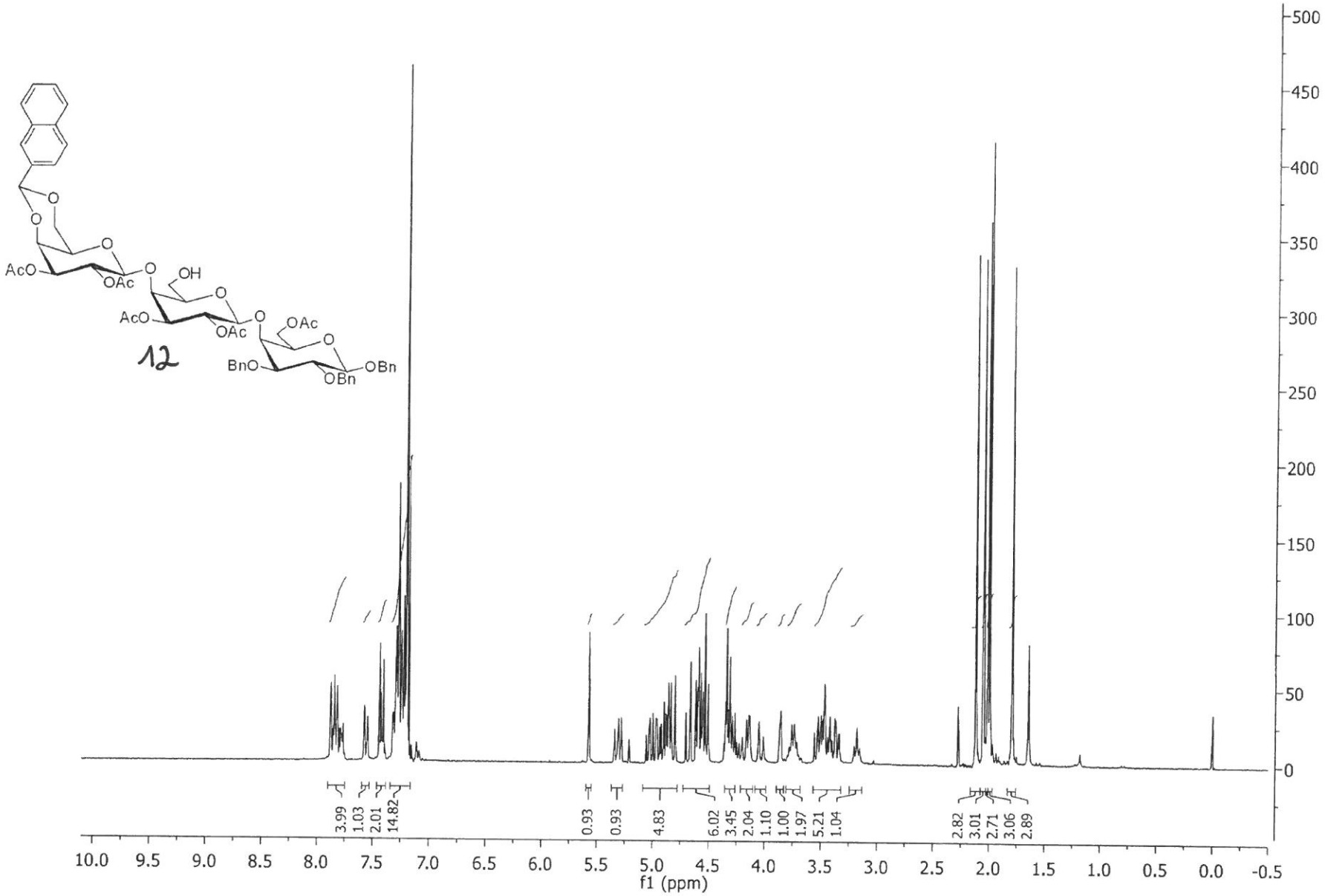
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0.93
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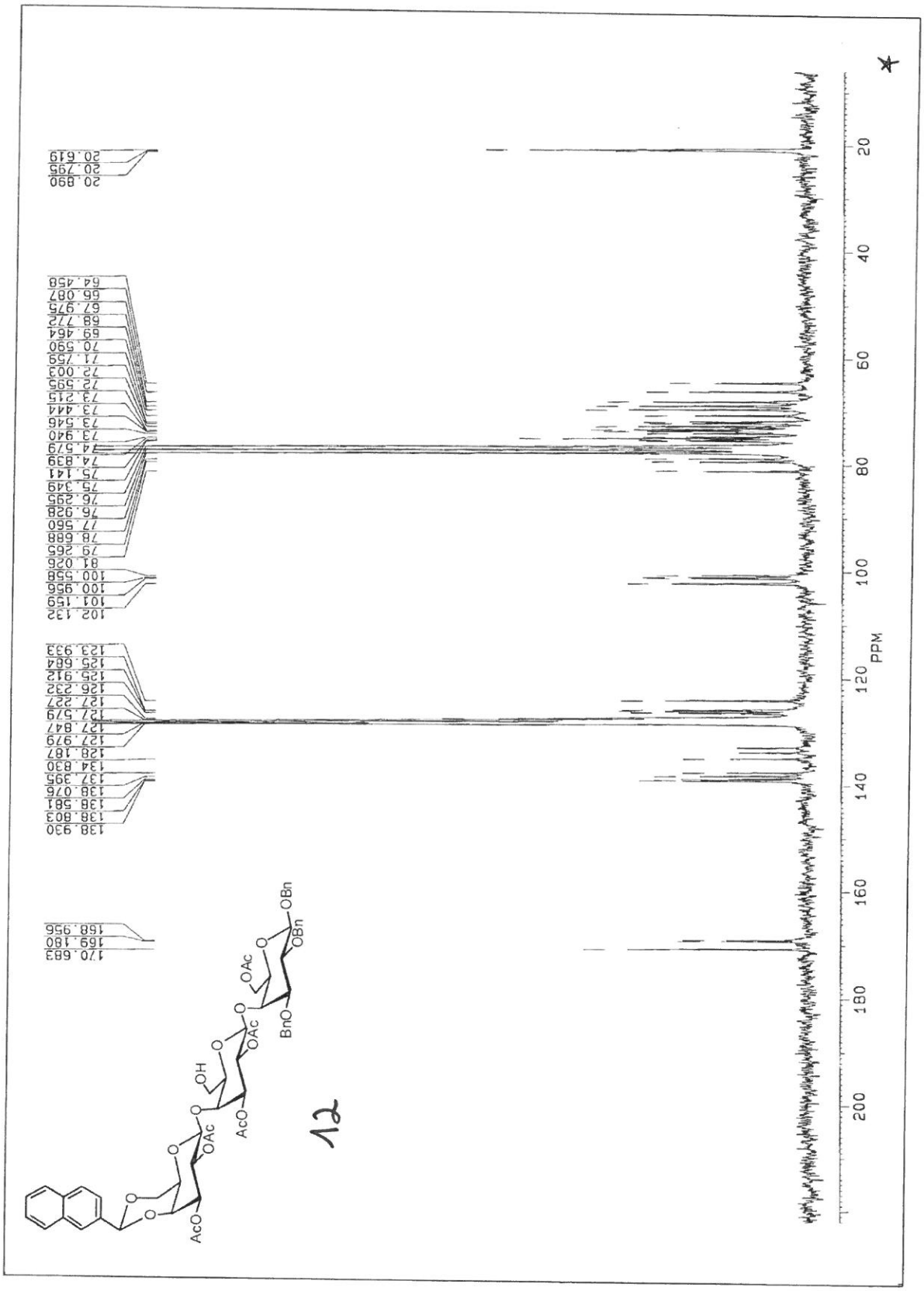
f1 (ppm)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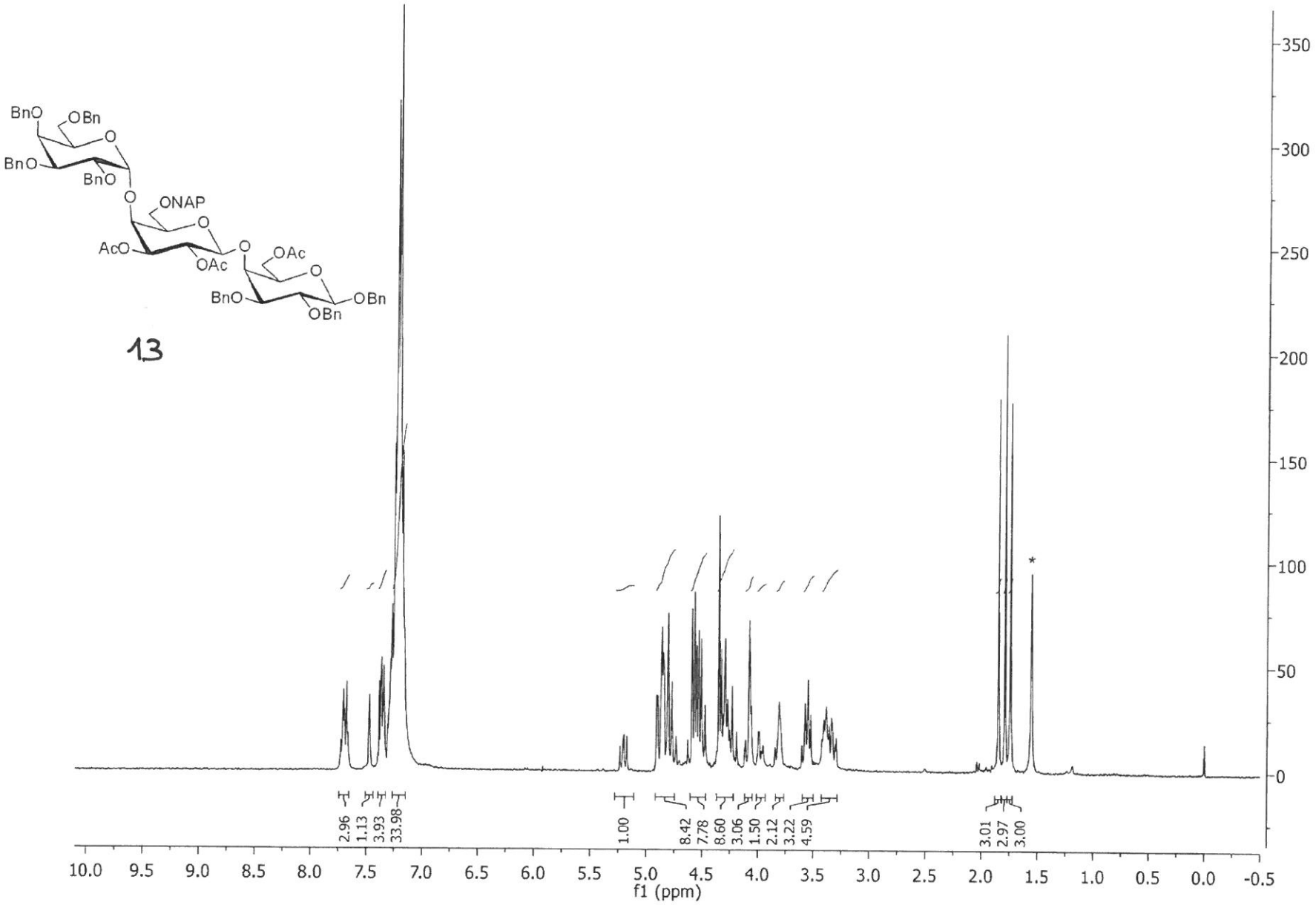
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3.01
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3.00
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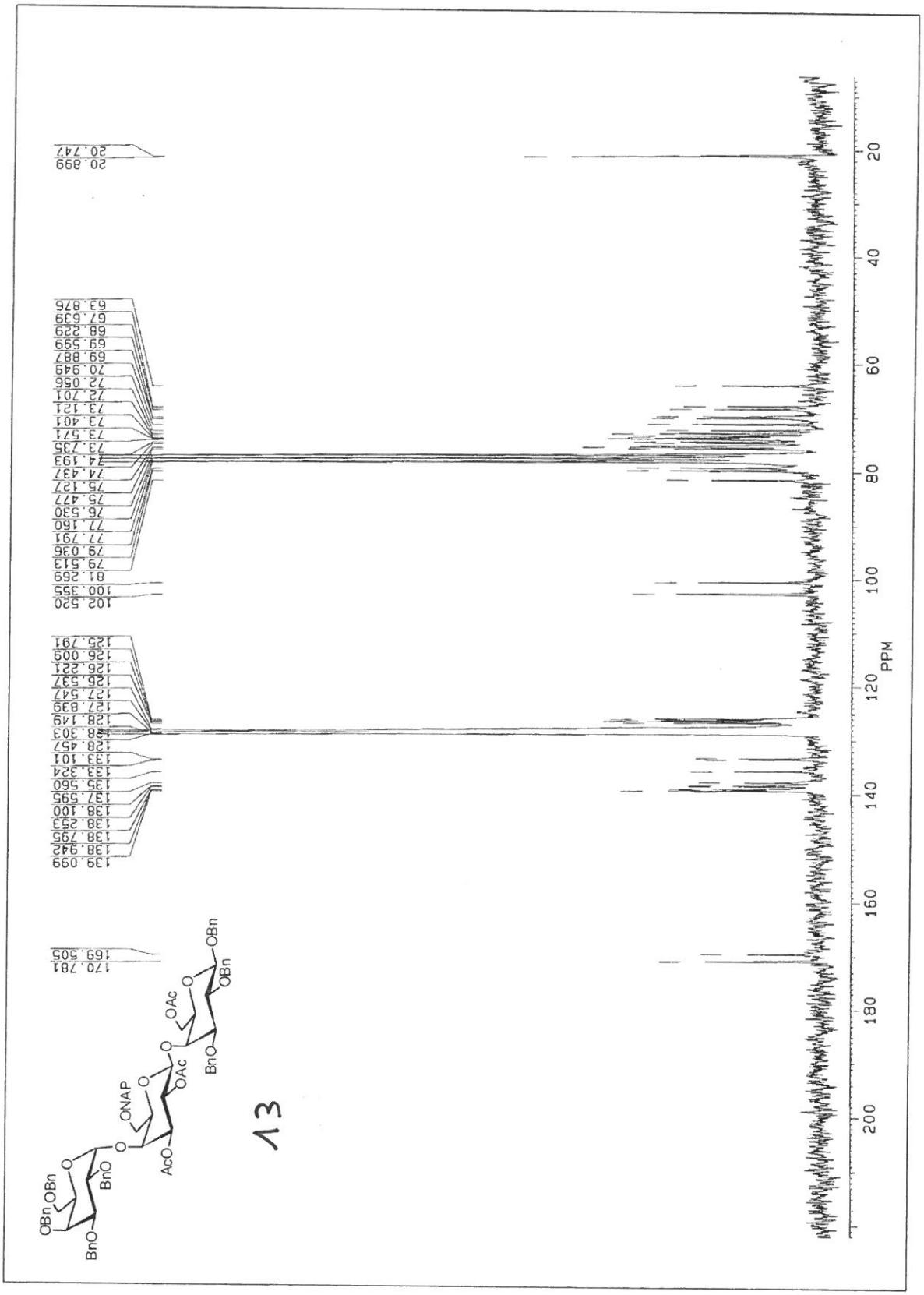
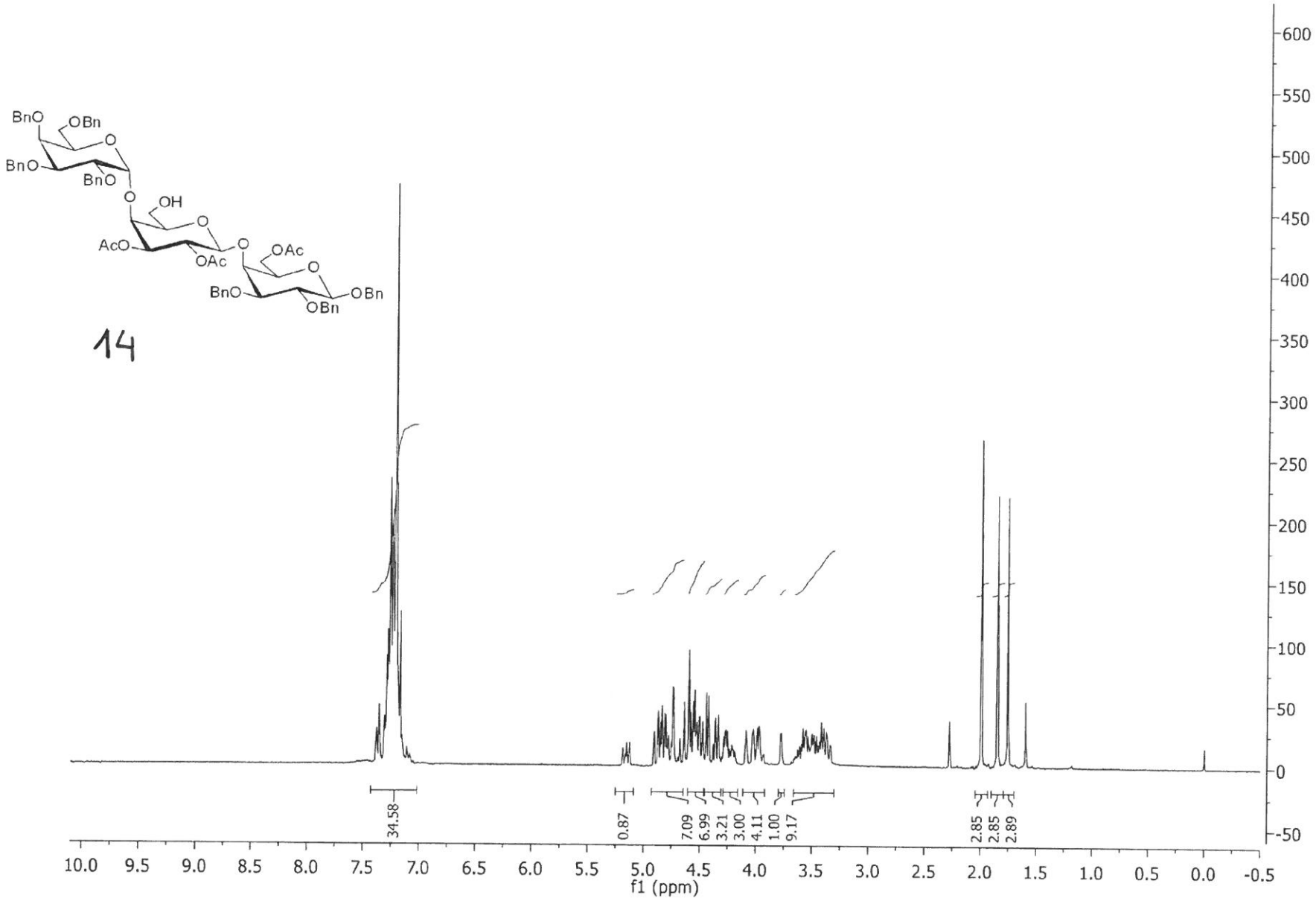
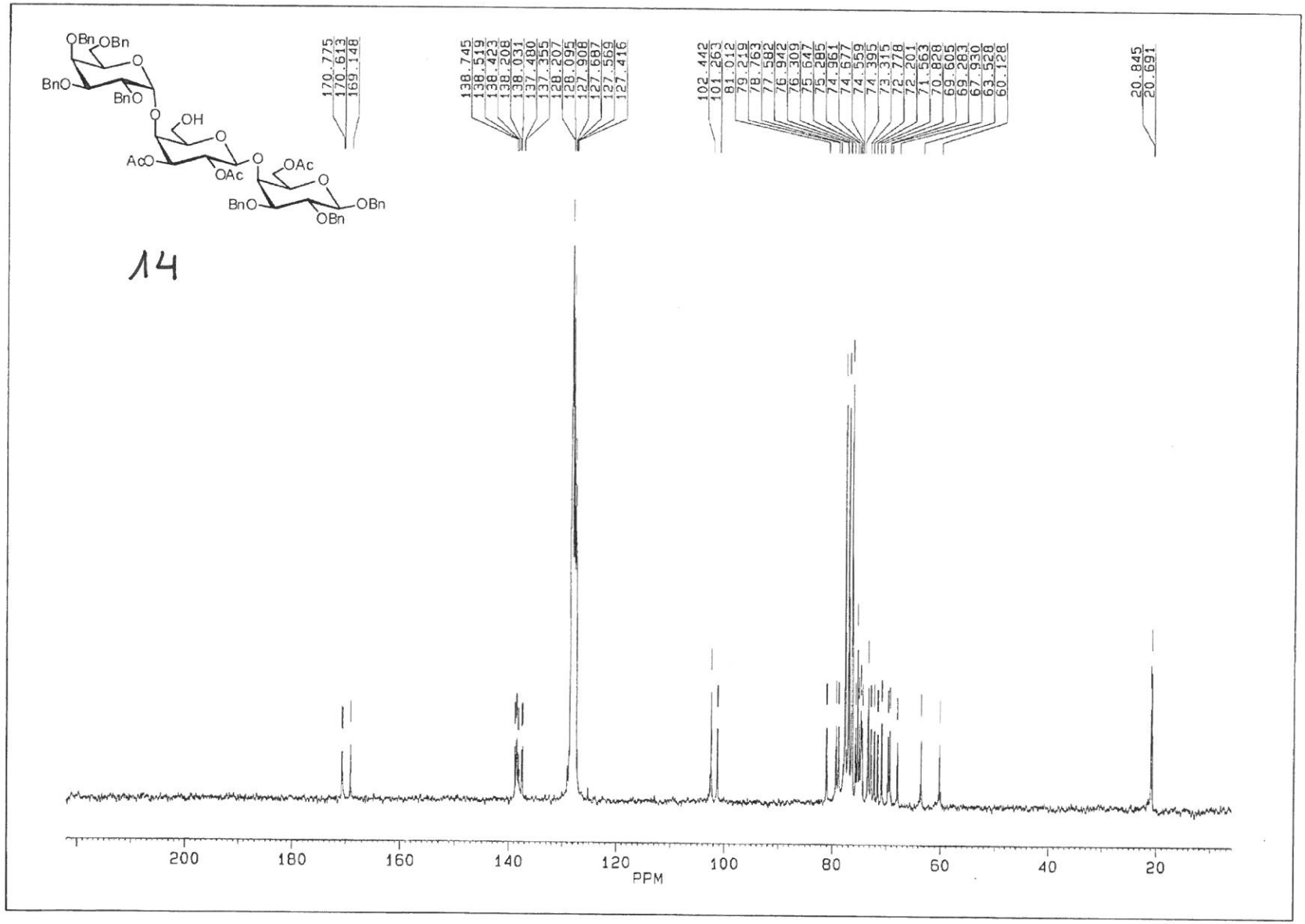
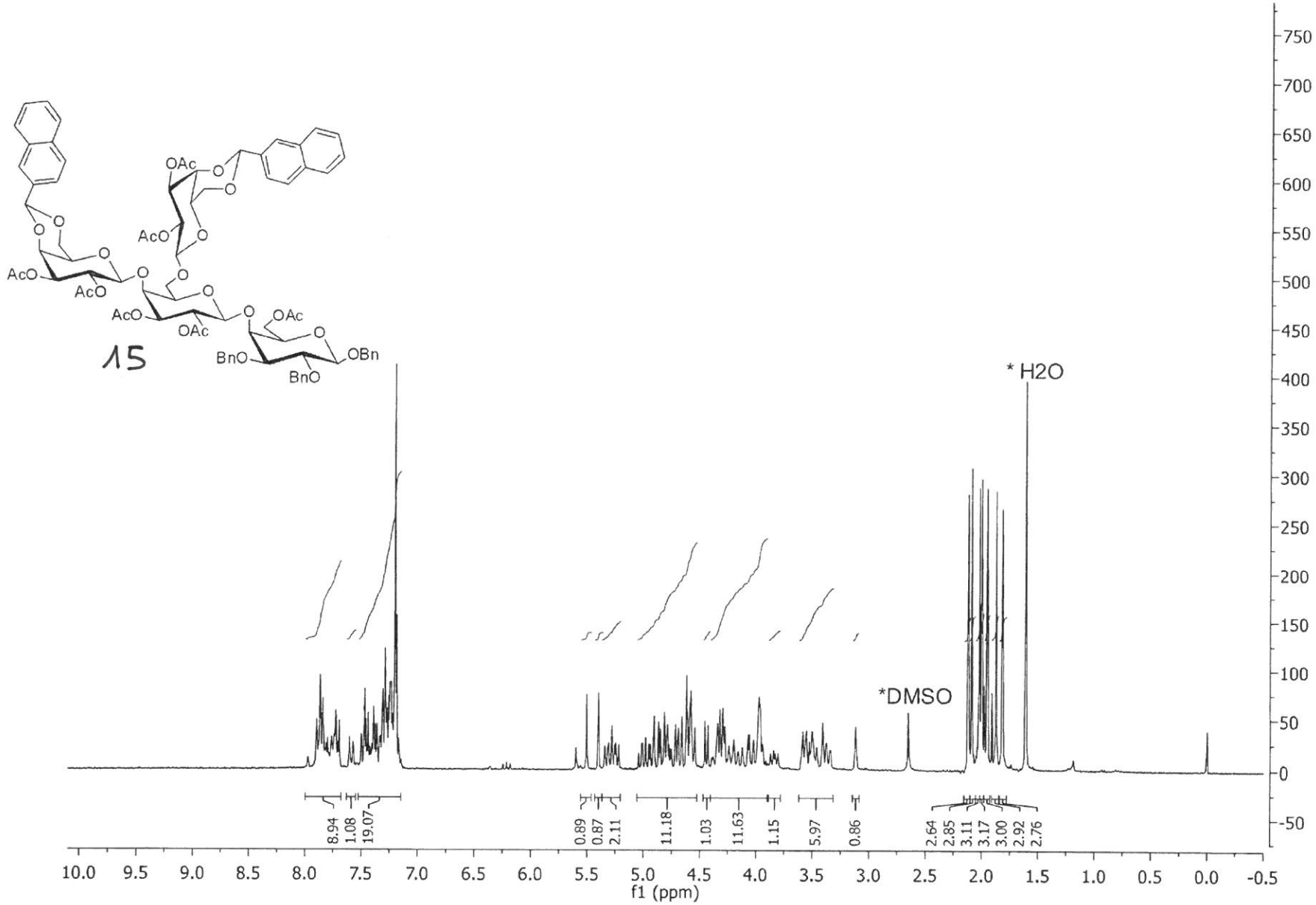
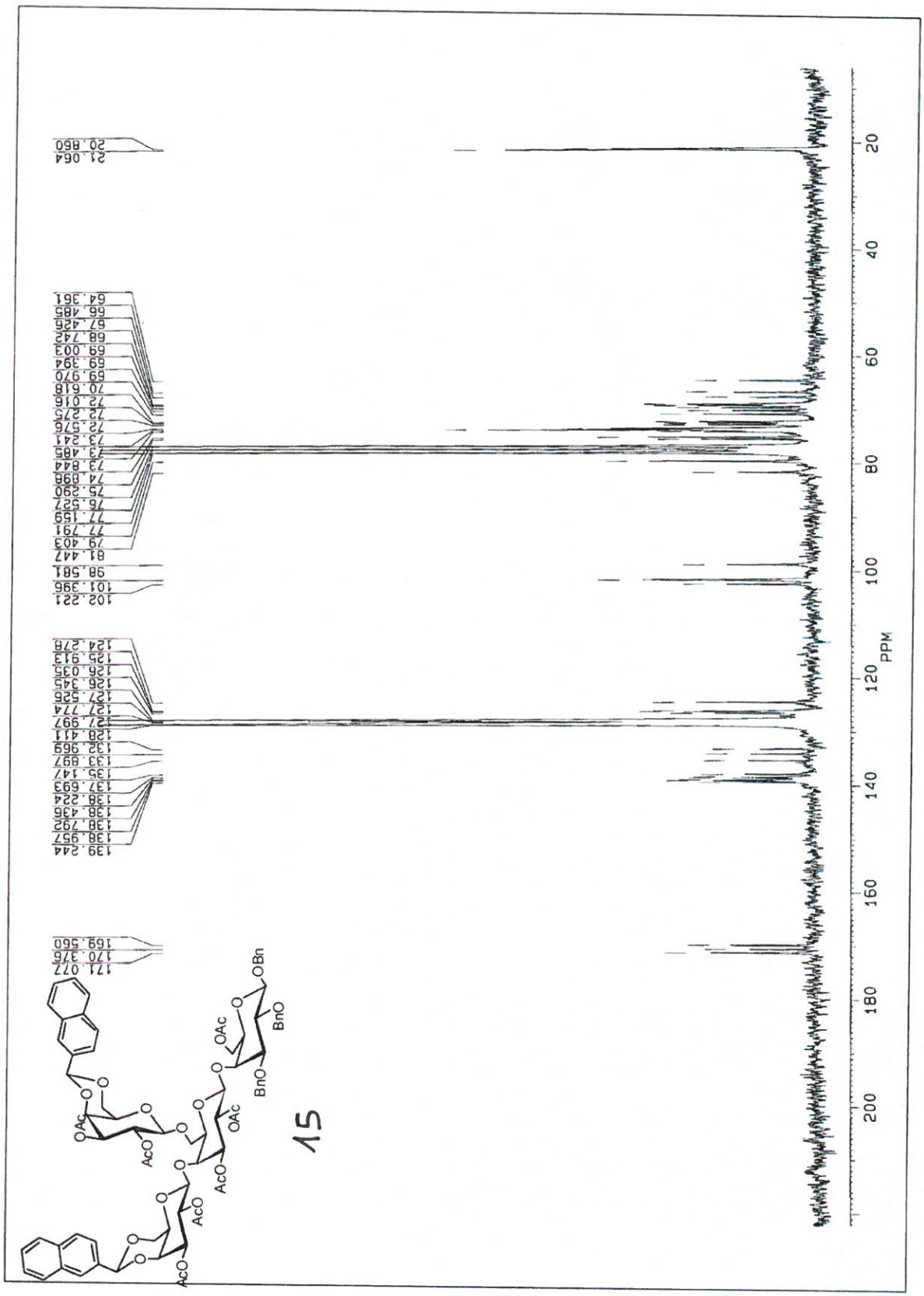
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

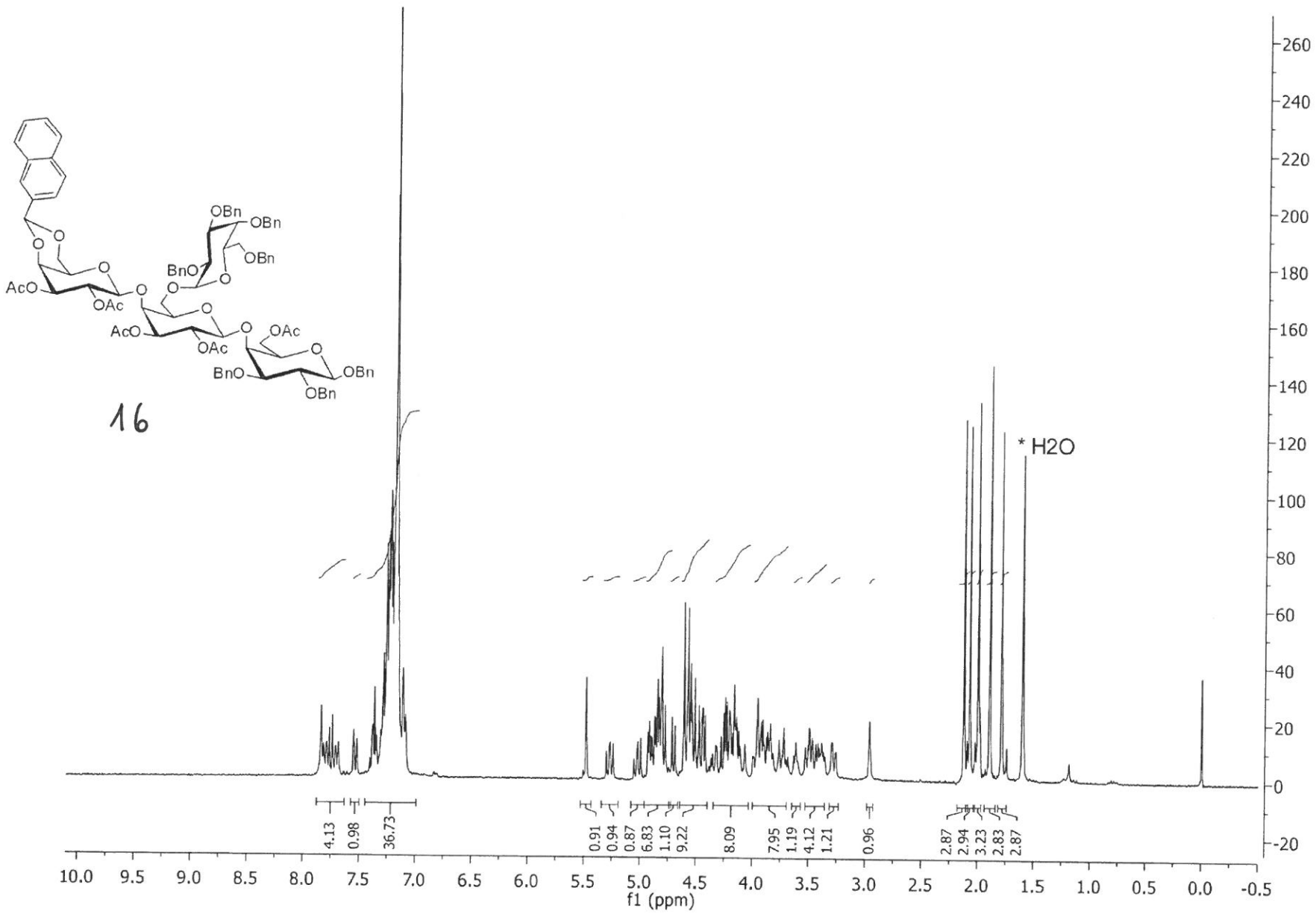

13
PPM

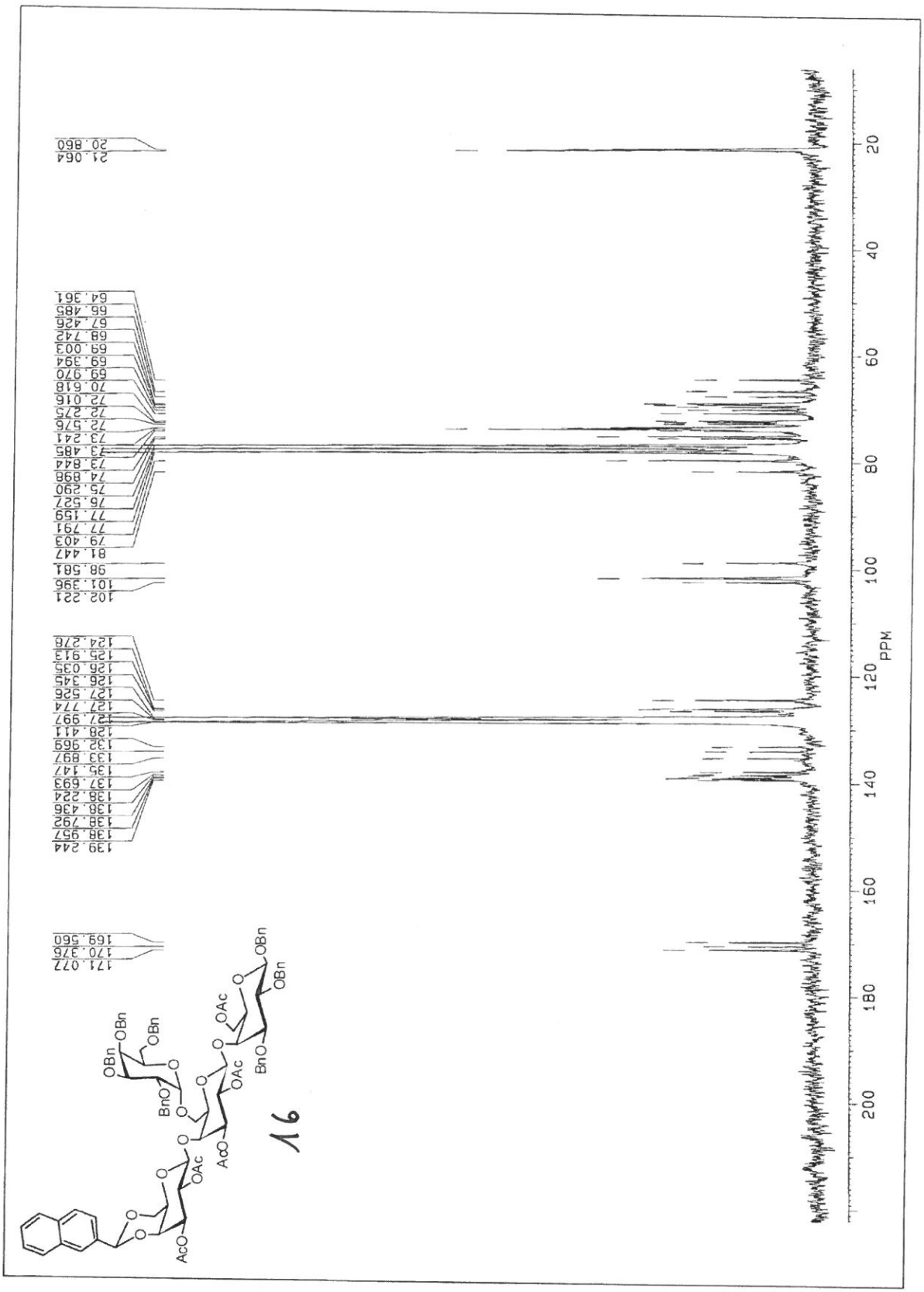

BnO
OBn
BnO
BnO
O
OH
O
AcO
OAc
O
OAc
O
BnO
OBn
OBn
14
34.58
0.87
7.09
6.99
3.21
3.00
4.11
1.00
9.17
2.85
2.85
2.89
f1 (ppm)

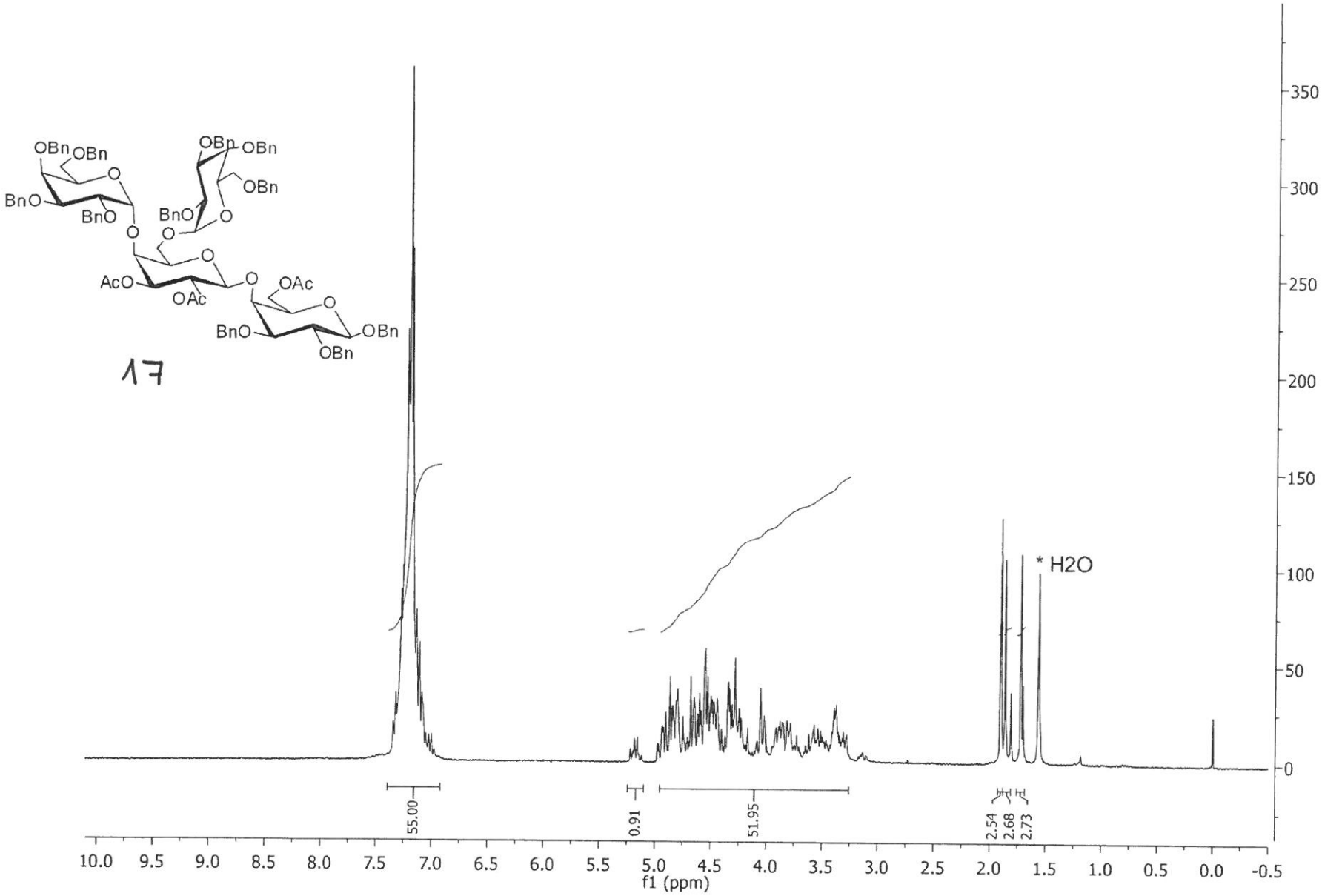

OBn
OBn
BnO
BnO
OH
AcO
OAc
OAc
BnO
OBn
OBn
14
170.775
170.613
169.148
138.745
138.519
138.423
138.208
138.031
137.480
137.355
128.207
128.095
127.908
127.687
127.560
127.416
102.442
101.263
81.012
79.219
78.763
77.582
76.942
76.309
75.647
75.285
74.961
74.677
74.559
74.395
73.315
72.778
72.201
71.563
70.828
69.605
69.283
67.930
63.528
60.128
20.845
20.691
200
180
160
140
120
PPM
100
80
60
40
20

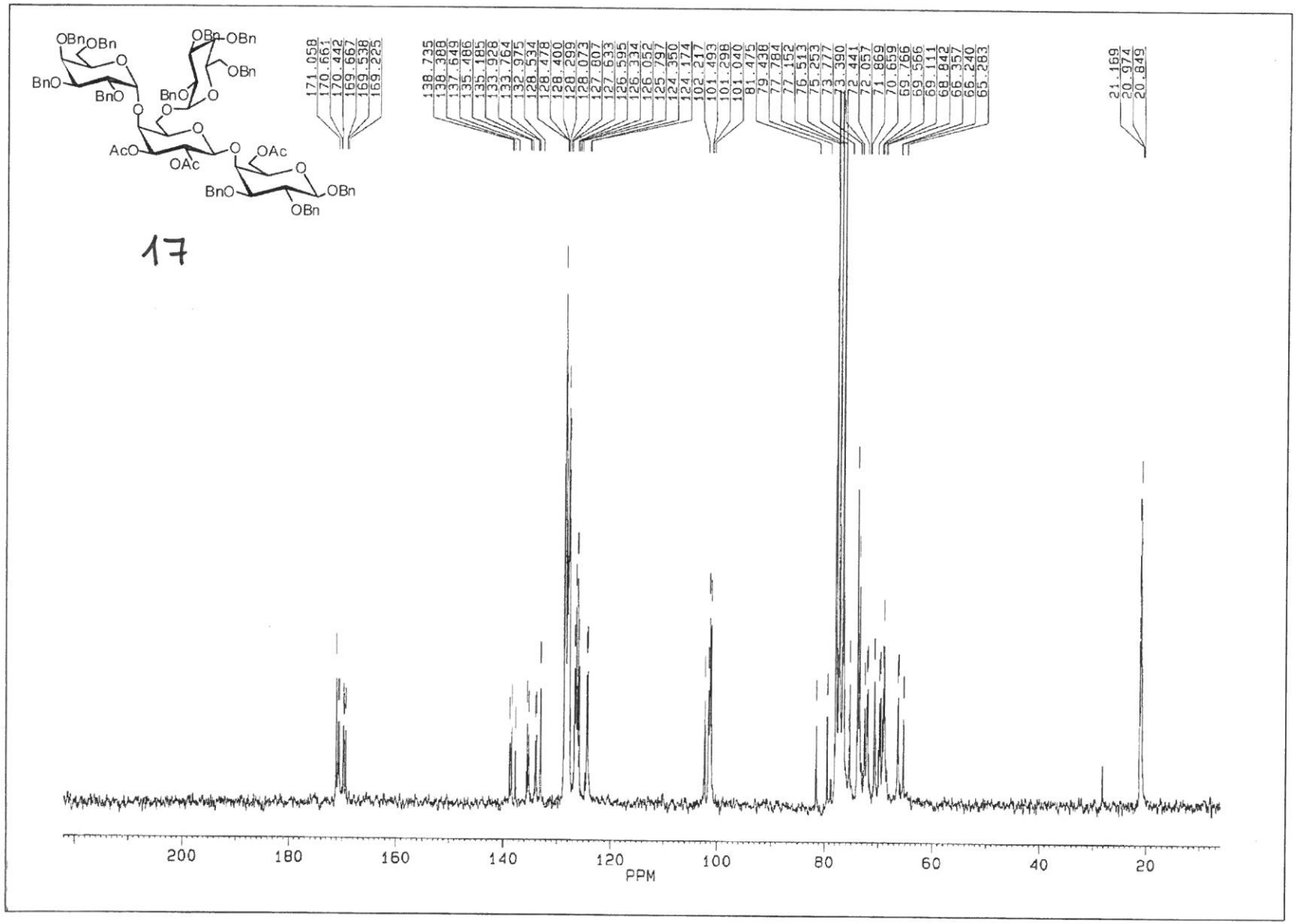

15
OAc
AcO
AcO
AcO
OAc
AcO
AcO
OAc
BnO
BnO
OBn
* H2O
*DMSO
8.94
1.08
19.07
0.89
0.87
2.11
11.18
1.03
11.63
1.15
5.97
0.86
2.64
2.85
3.11
3.17
3.00
2.92
2.76
f1 (ppm)

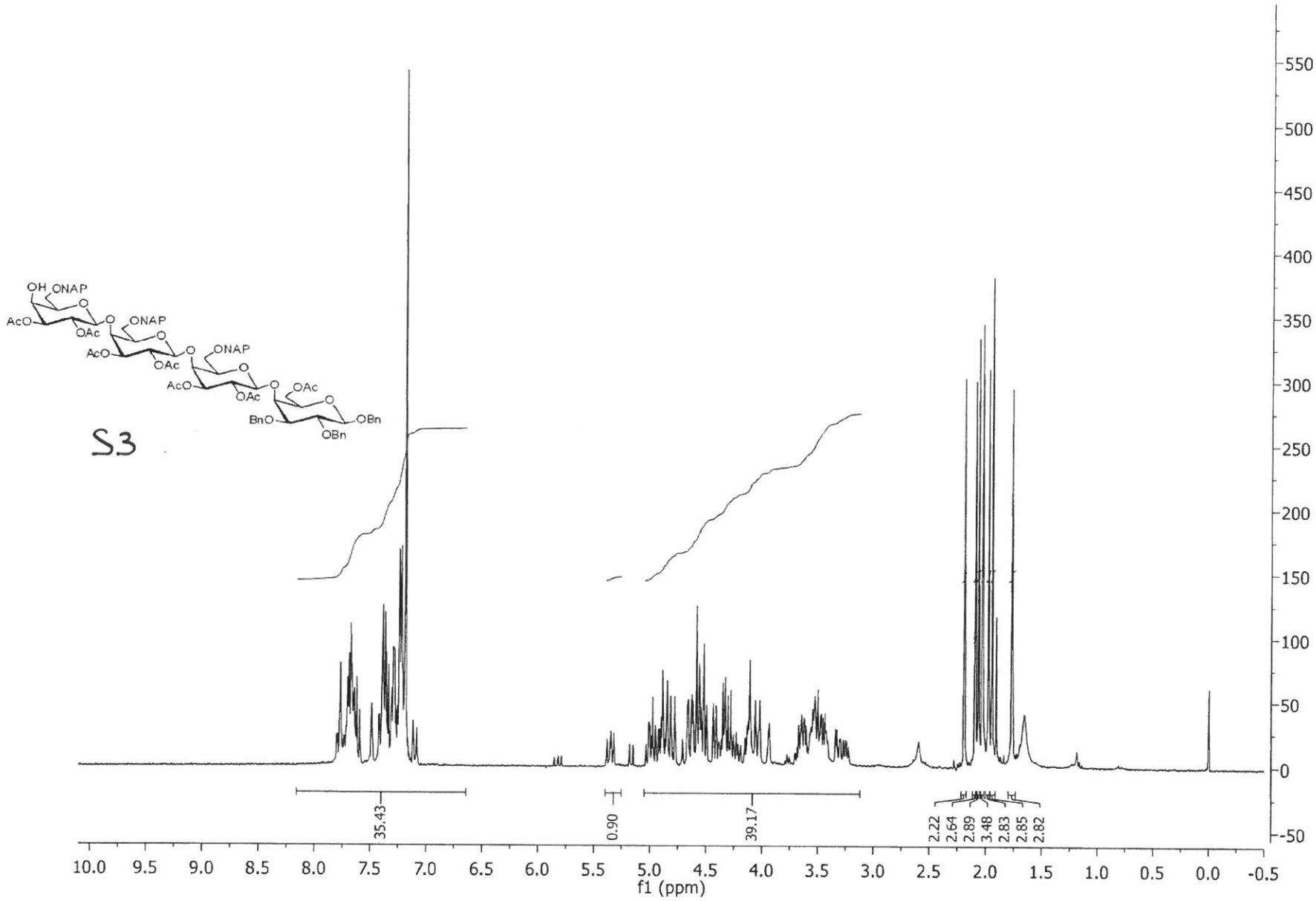

15
PPM

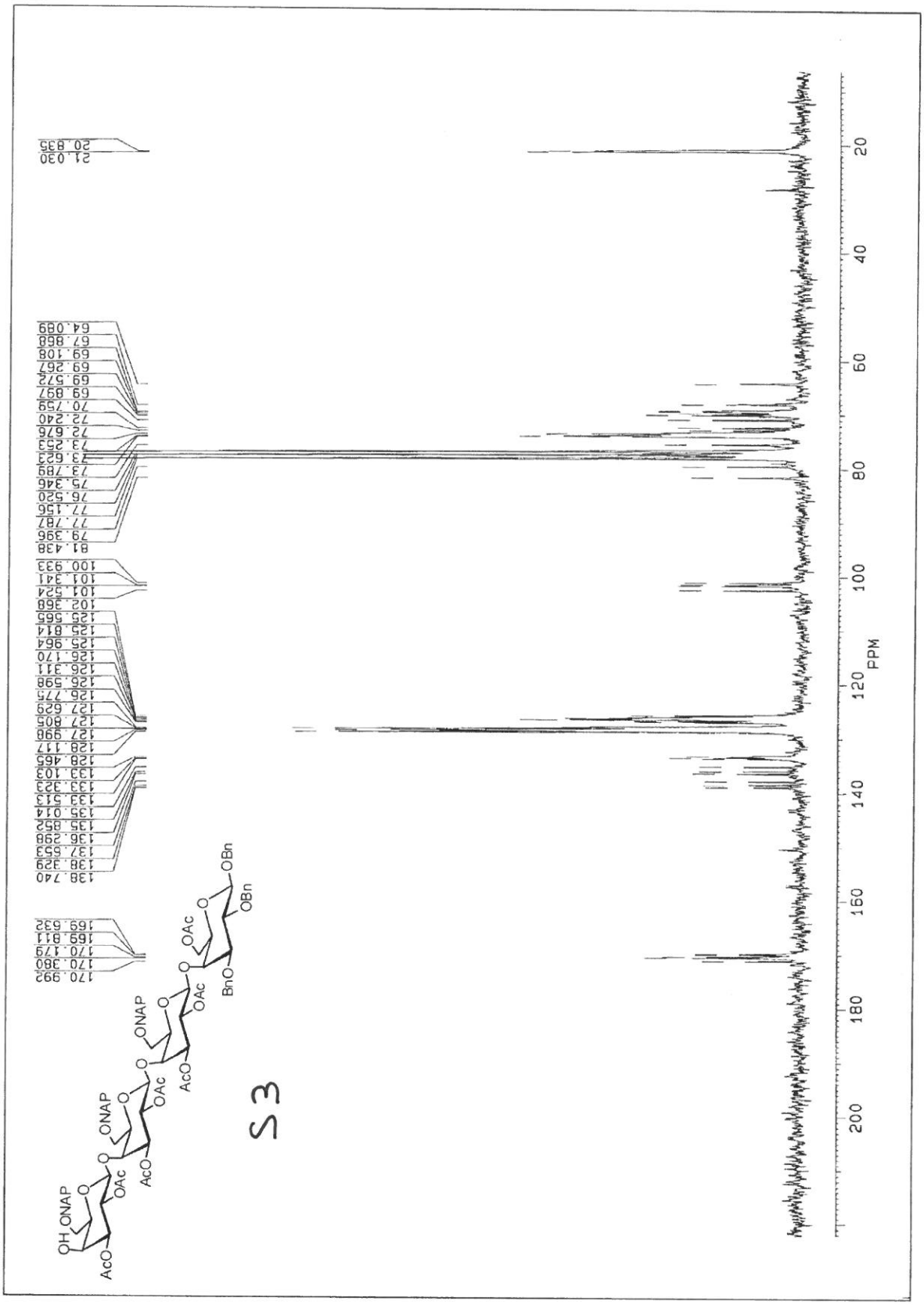

OBn
OBn
OBn
BnO
AcO
OAc
AcO
OAc
OAc
BnO
OBn
OBn
16
* H2O
4.13
0.98
36.73
0.91
0.94
0.87
6.83
1.10
9.22
8.09
7.95
1.19
4.12
1.21
0.96
2.87
2.94
3.23
2.83
2.87
f1 (ppm)

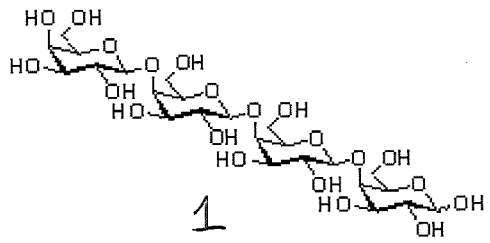

16
PPM


OBn
OBn
BnO
BnO
O
AcO
OAc
OAc
BnO
OBn
OBn
17
* H2O
55.00
0.91
51.95
2.54
2.68
2.73
f1 (ppm)


17
171.058
170.661
170.442
169.667
169.536
169.225
138.735
138.388
137.649
135.486
135.185
133.928
133.764
132.975
128.534
128.478
128.400
128.299
128.073
127.807
127.633
126.595
126.334
126.052
125.797
124.350
124.174
102.217
101.493
101.298
101.040
81.475
79.438
77.784
77.152
76.513
75.253
73.777
73.390
72.441
72.057
71.869
70.659
69.766
69.566
69.111
68.842
66.357
66.240
65.283
21.169
20.974
20.849
200
180
160
140
120
100
80
60
40
20
PPM

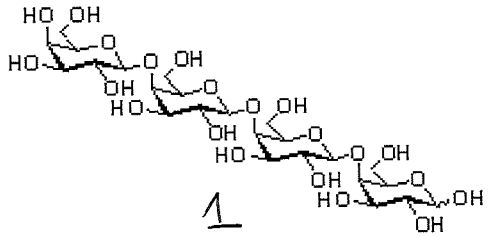

S3
35.43
0.90
39.17
2.22
2.64
2.89
3.48
2.83
2.85
2.82
f1 (ppm)


S3
OH
ONAP
AcO
OAc
AcO
ONAP
OAc
AcO
ONAP
OAc
BnO
OBn
OBn
PPM

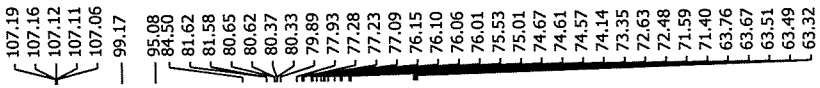


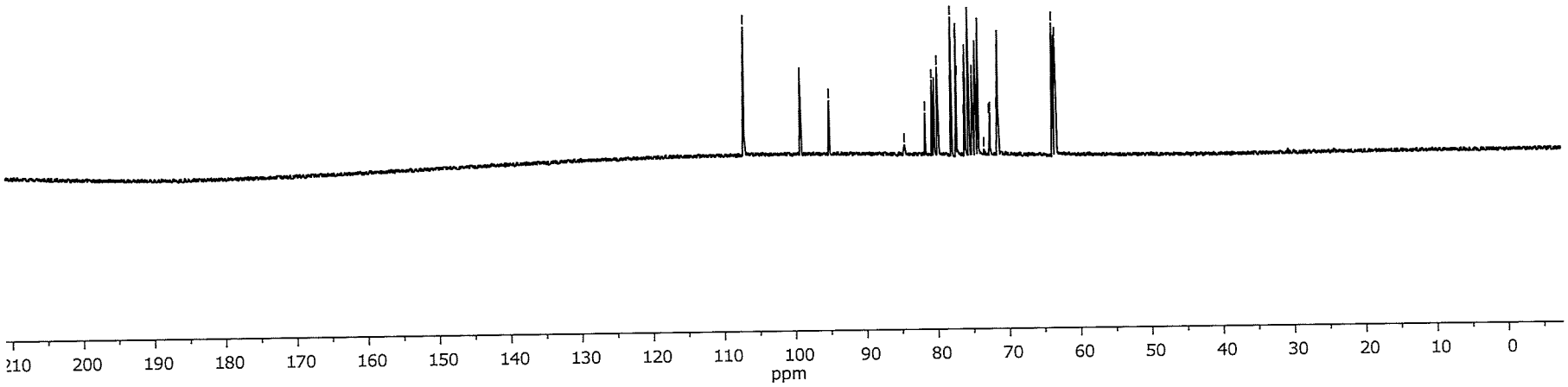
1

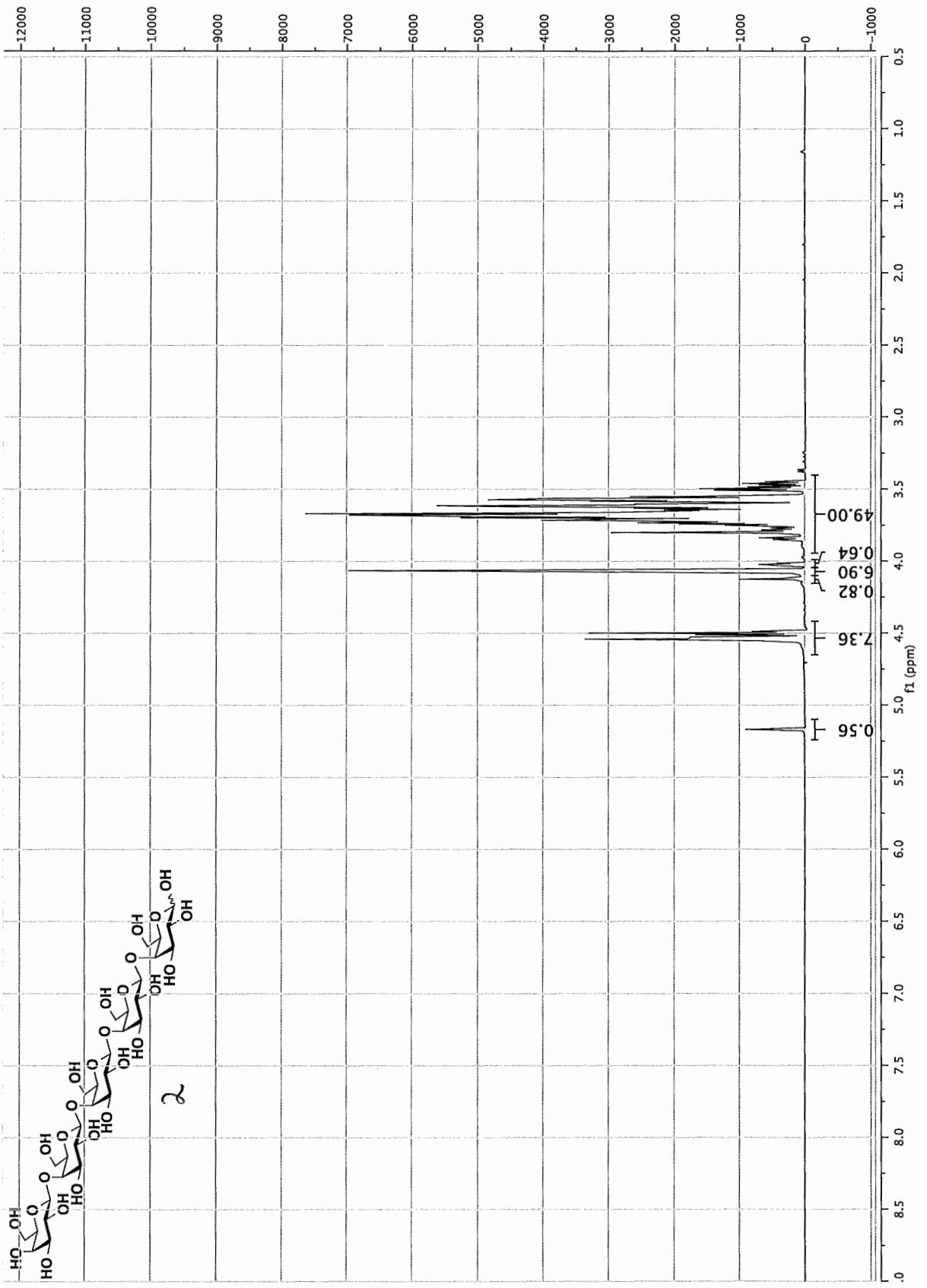
0.47
4.77
0.49
3.35
0.54
0.52
24.50
1.42
1.00
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm

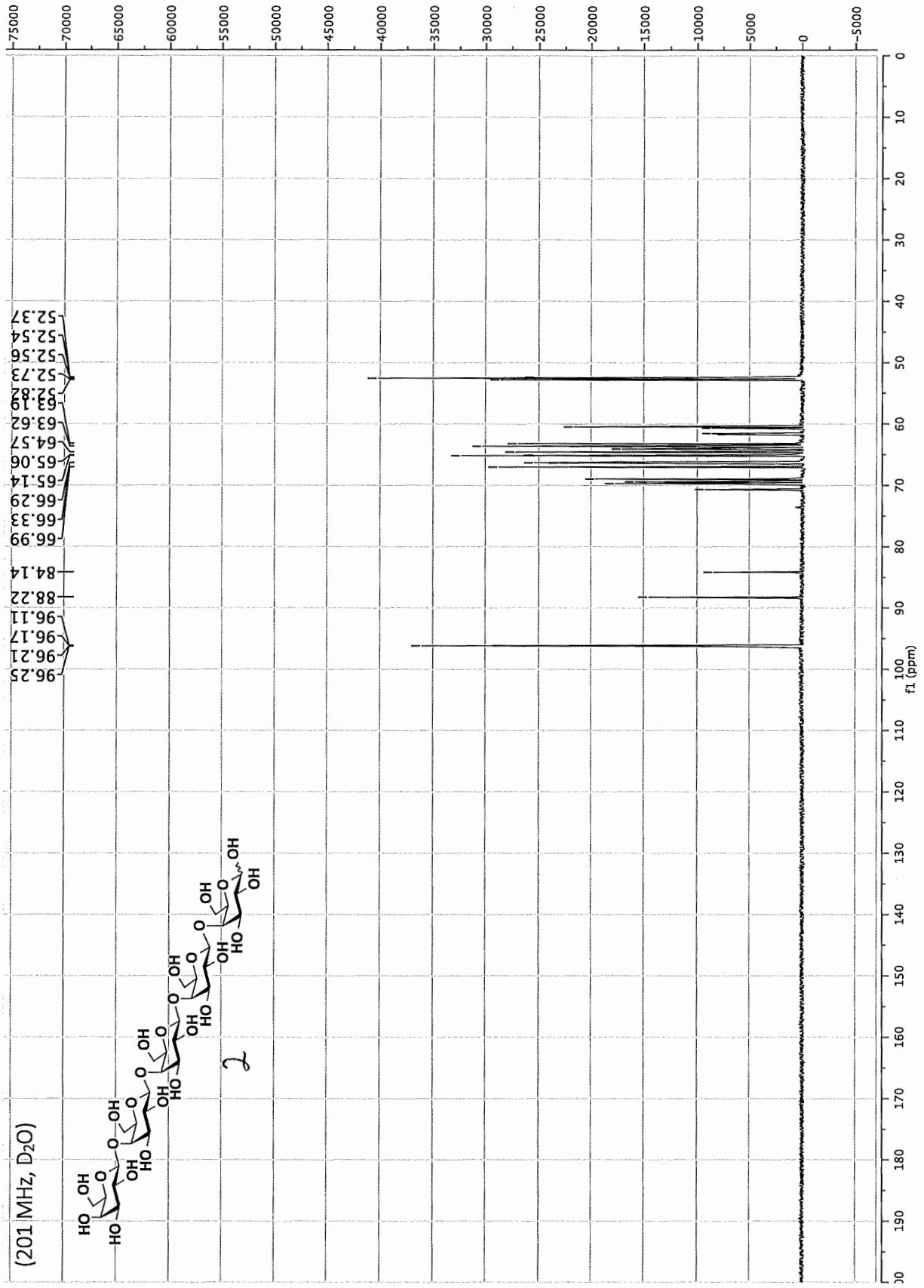


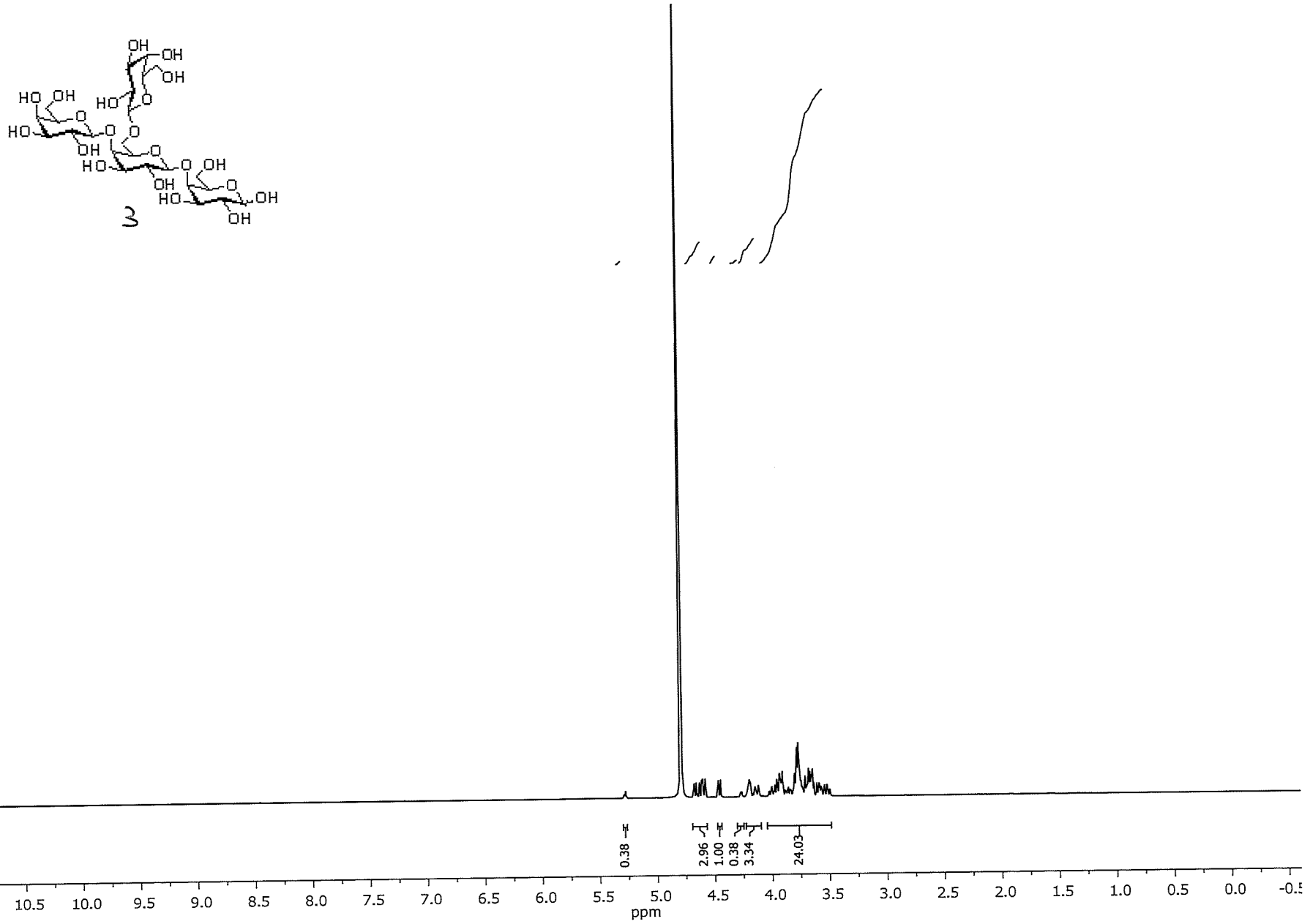
1

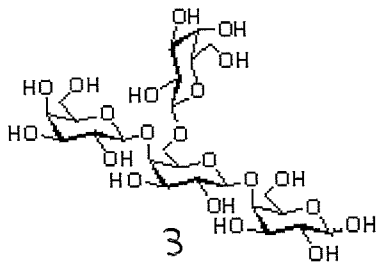

107.19
107.16
107.12
107.11
107.06
99.17
95.08
84.50
81.62
81.58
80.65
80.62
80.37
80.33
79.89
77.93
77.28
77.23
77.09
76.15
76.10
76.06
76.01
75.53
75.01
74.67
74.61
74.57
74.14
73.35
72.63
72.48
71.59
71.40
63.76
63.67
63.51
63.49
63.32

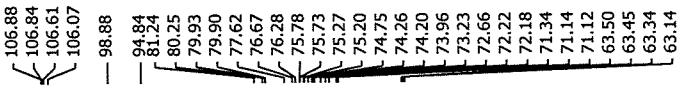

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

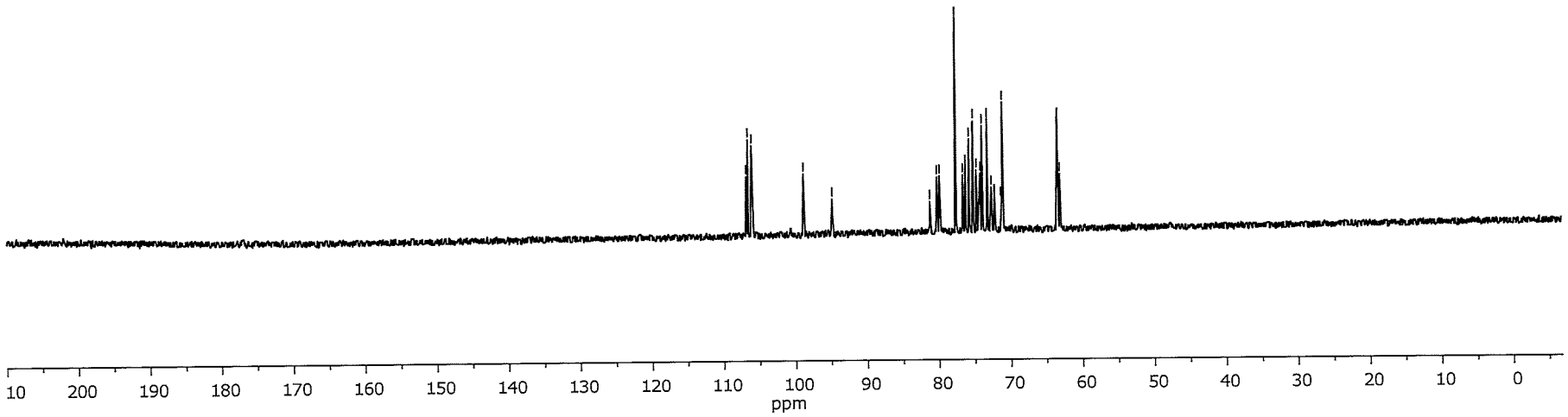

2
49.00
0.64
6.90
0.82
7.36
0.56
f1 (ppm)

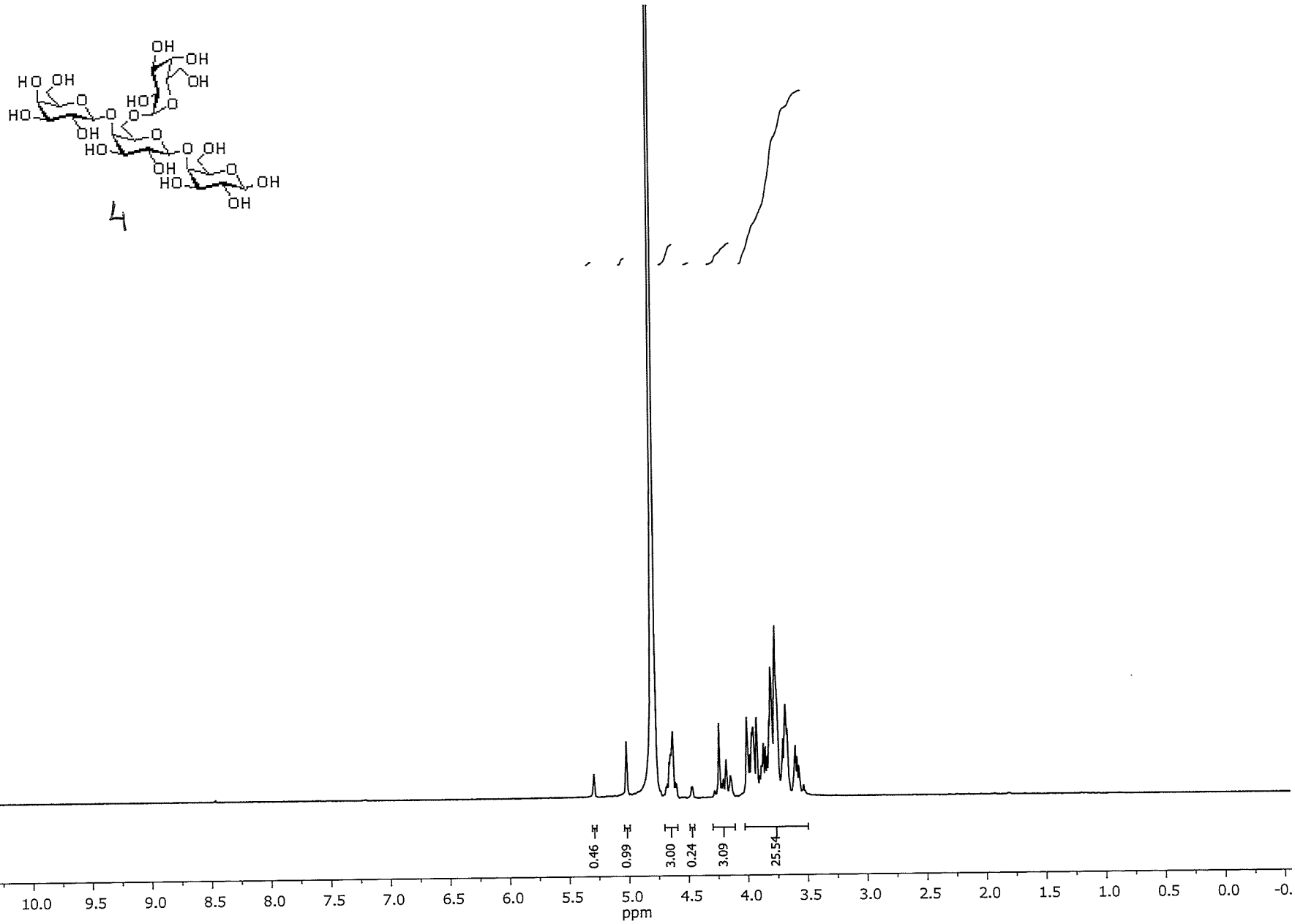

(201 MHz, D2O)
96.25
96.21
96.17
96.11
88.22
84.14
66.99
66.33
66.29
65.14
65.06
64.57
63.62
63.19
52.82
52.73
52.56
52.54
52.37
f1 (ppm)

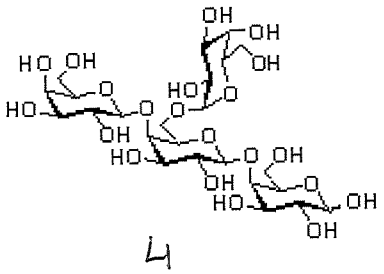

3
0.38
2.96
1.00
0.38
3.34
24.03
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm


OH
OH
OH
HO
OH
HO
O
HO
O
O
O
OH
O
HO
O
OH
OH
O
HO
OH
OH
3


106.88
106.84
106.61
106.07
98.88
94.84
81.24
80.25
79.93
79.90
77.62
76.67
76.28
75.78
75.73
75.27
75.20
74.75
74.26
74.20
73.96
73.23
72.66
72.22
72.18
71.34
71.14
71.12
63.50
63.45
63.34
63.14

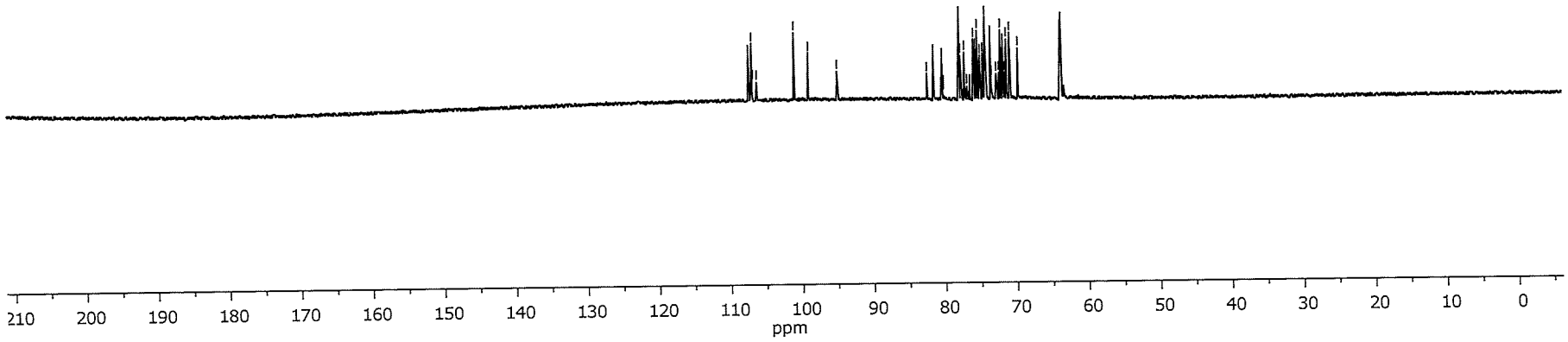

10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

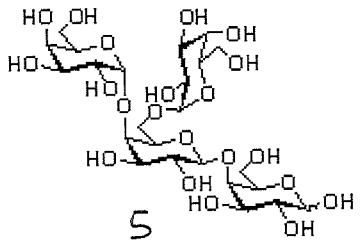

4
0.46
0.99
3.00
0.24
3.09
25.54
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.
ppm



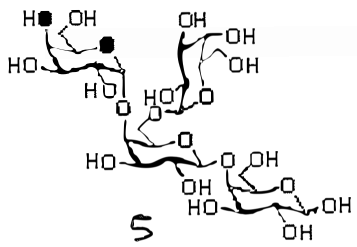
4

107.51
107.48
107.01
107.00
106.85
106.31
101.11
99.11
95.08
95.07
82.53
81.59
80.43
80.39
78.02
77.86
77.27
76.07
76.04
76.01
75.97
75.78
75.77
75.52
75.48
75.44
75.09
74.77
74.71
74.43
74.20
73.64
73.48
72.83
72.45
72.23
71.91
71.67
71.43
71.39
71.36
70.96
69.81
64.03
63.91
63.86
63.79
63.74

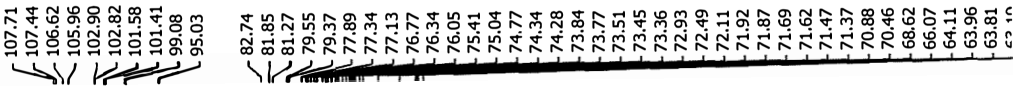



HO
OH
OH
OH
HO
HO
HO
HO
O
O
O
O
O
O
OH
OH
HO
OH
OH
5

0.30
2.00
0.91
0.87
1.49
26.03
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm



5


107.71
107.44
106.62
105.96
102.90
102.82
101.58
101.41
99.08
95.03
82.74
81.85
81.27
79.55
79.37
77.89
77.34
77.13
76.77
76.34
76.05
75.41
75.04
74.77
74.34
74.28
73.84
73.77
73.51
73.45
73.36
72.93
72.49
72.11
71.92
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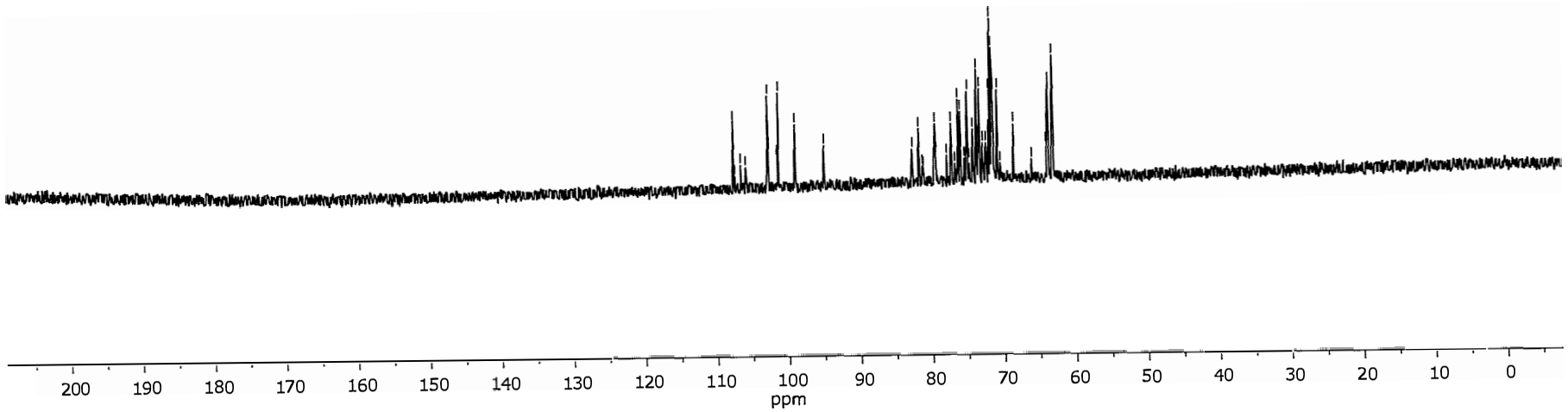
70.46
68.62
66.07
64.11
63.96
63.81


200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

4. Crystallization and structure determination

19 mg/ml galectin-3C in buffer (10 mM Na phosphate buffer pH 7.5, 100 mM NaCl, 10 mM β-mercaptoethanol and 0.02 % NaN_3) was produced as described previously.[1] 100 mM solution of **2** was made by dissolving 1 mg of **2** in 12 µl milliQ water. 3.5 µl galectin-3C (19 mg/ml in buffer as above) were mixed with 3.5 µl **2** and incubated on ice for one hour to obtain a final concentration of 50 mM **2**. The sample was then centrifuged at 10 000 rpm for 5 min at 4 °C. Crystallisation trials were setup in NeXtal plates as follows: 2 µl Gal3C:GalP complex were mixed with 2 µl reservoir solution and equilibrated against 1 ml of reservoir solution containing 30% PEG 4000, 0.1 M Tris/HCl pH 75m 0.1 M MgCl2, 0.4 M NaSCN, 8 mM β-mercaptoethanol. A crystal measuring approximately 0.05 x 0.1 mm was briefly soaked in a cryoprotectant solution consisting of 19% PEG 4000, 11% glycerol, 65 mM Tris/HCl pH 7.5, 65 mM $MgCl_2$, 190 mM NaSCN, 6 mM β-mercaptoethanol, 25 mM **2**. After soaking, the crystal was flash-cooled in liquid N_2.

Data to 1.4 Å resolution were collected at 100 K at station I911-3 of the MAX IV Laboratory, Lund, Sweden (λ = 1.0000 Å), equipped with a 225 mm marMosaic detector. Three hundred and thirteen images with 0.5° rotation were collected. All data were integrated using XDS and scaled using XSCALE.[2] The structure was solved by difference Fourier using PDB entry 3ZSK, the structure of Gal3C in complex with glycerol at 0.9 Å resolution,[3] with glycerol removed. Double conformations present in the higher resolution structure 3ZSK were deleted depending on their presence or absence in the electron density maps. Alternate conformations only seen in the GalP complex were added based on the same criteria. After initial refinement of the protein coordinates in Refmac5,[4] two of the galactose rings of GalP were fitted to the electron density using Coot.[5] The structures were refined until convergence. Individual anisotropic B-factors for each atom were refined. Hydrogen atoms were added in the riding positions. Water molecules were added to positive difference density peaks more than 5 standard deviations above the mean and present in 2m|Fo|-d|Fc| maps at the 1σ level.

Resolution range (Å)	27.7 - 1.41 (1.49 - 1.41)
Space group	$P2_12_12_1$
Unit cell a ,b, c (Å)	36.1, 58.2, 63.0
Total observations	151483
Unique reflections	25876
Multiplicity	5.9 (4.2)
Completeness (%)	98.2 (90.2)
Mean I/σ(I)	19.8 (3.7)
Wilson B-factor (Å^2)	19.2
R_{sym} (I)	0.053 (0.351)
CC(1/2) (I)	0.999 (0.885)
R_{model} (F)	0.116 (0.186)
R_{free} (F)	0.153 (0.211)
number of atoms	2800
protein	1236
compound 2*	23
chloride ions	2
water molecules	217
rms deviations from ideal geometry	
bonds, Å	0.005
angles, °	1.23
Ramachandran outliers (%)	0
average B-factor (Å^2)	12.7
macromolecules	14.2
compound 2*	35.0
chloride ions	31.8
solvent	32.2

Table S1: Data collection and refinement statistics. Figures in parentheses are for the highest resolution shell. Other relevant quality indicators can be easily extracted from the PDB file header. *Includes two galactose rings out of five present in ligand **2**. The structure has been analysed using the Molprobity server[6] and belongs to the 98th percentile comparing structures with similar resolution.

(1) Diehl, C.; Engström, O.; Delaine, T.; Håkansson, M.; Genheden, S.; Modig, K.; Leffler, H.; Ryde, U.; Nilsson, U. J.; Akke, M. Protein Flexibility and Conformational Entropy in Ligand Design Targeting the Carbohydrate Recognition Domain of Galectin-3. *J. Am. Chem. Soc.* **2010**, *132*, 14577–14589.

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