

Electronic Supplementary Information

Modular Synthesis and Immunological Evaluation of Suspected Allergenic Galactooligosaccharides

Hidde Elferink¹, Emiel Rossing¹, Chiung-Hui Huang^{2,3}, Bee Wah Lee^{2,3}, Linqiu Cao⁴, Dianne J.
Delsing⁴, Andre Groeneveld⁴, Thomas J. Boltje^{1*}

¹Cluster for Molecular Chemistry, Institute for Molecules and Materials, Radboud University
Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

²Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore

³Department of Paediatrics, National University Hospital, Singapore

⁴FrieslandCampina, Amersfoort, The Netherlands

***Corresponding Author:** Thomas J. Boltje, Cluster for Molecular Chemistry, Institute for Molecules
and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The
Netherlands, Tel: +31 (0)2436 52331, E-mail: t.boltje@ru.nl

Table of Contents

General Methods Allergenicity of 4PY and 4PX	2
Subjects	2
Basophil activation test	2
General Synthetic Methods	2
Experimental Procedures	3
NMR Spectra	23

General Methods Allergenicity of 4PY and 4PX

Subjects

Subjects with confirmed GOS-related allergy were selected from the cohort previously studied for the prevalence of GOS-related allergy in a Singapore atopic population, as described in the paper by Soh et al., 2015¹. Five adult subjects with confirmed GOS-related allergy were recalled to the clinic for a blood sample drawing to test allergenicity of the GOS-structure 4PX, for testing of 4PY blood was drawn from three subjects.

This study was approved by the National University Hospital of Singapore institutional ethical review board (DSRB). Written consent of all subjects was obtained prior to the start of the study.

Basophil activation test

A Basophil Activation Test was performed on blood samples from GOS allergic subjects. Heparinized peripheral blood aliquots (100 μ L) were pre-incubated at 37°C for 5 minutes and then incubated with 100 μ L of PBS (negative control), anti-IgE antibody (positive control, G7-18; BD Biosciences, USA) or diluted GOS samples for 15 minutes (37°C). After incubation with GOS, cells were washed in PBS-EDTA (20 mmol/L) and then incubated with phycoerythrin-labeled anti-human IgE (Ige21; eBioscience, USA), allophycocyanin-labeled anti-human CD203c (NP4D6; BioLegend, USA), and fluorescein isothiocyanate-labeled anti-human CD63 (MEM-259, BioLegend) mAbs for 20 minutes at 4°C. Expression of CD203c and CD63 are both markers for basophil activation. Thereafter, samples were subjected to erythrocyte lysis with 2 mL of FACS Lysing Solution (BD Biosciences). Cells were then washed, resuspended in 1% BSA/PBS, and analyzed by means of FACSCalibur (BD Biosciences). Basophils were detected on the basis of side-scatter characteristics and expression of IgE (IgE^{high}).

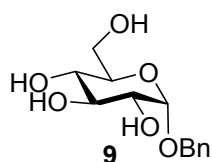
General Synthetic Methods

¹H and ¹³C NMR spectra were recorded on a Bruker 400 MHz or 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), or residual solvents as the internal standard. NMR data is presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet and/or multiple resonances), coupling constant in hertz (Hz), integration. All NMR signals were assigned on the basis of ¹H NMR, ¹³C NMR, COSY, HSQC and TOCSY experiments. Mass spectra were recorded on an JEOL AccuTOF CS JMST100CS mass spectrometer. Automatic flash column chromatography was performed using Biotage Isolera Spektra One, using SNAP cartridges (Biotage, 30-100 μ m, 60 Å), 10-50 g. TLC-analysis was conducted on Silicagel F254 (Merck KGaA) with detection by UV-absorption (254nm) where applicable, and by spraying with 10% sulphuric acid in methanol followed by charring at \approx 300°C. DCM, THF and toluene were freshly distilled. Molecular sieves (4Å) were flame activated under

vacuum prior to use. All inert reactions were carried out under argon atmosphere using flame-dried flasks.

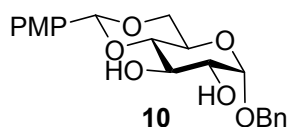
Experimental Procedures

Benzyl α -D-glucopyranoside (**9**)



To a solution of D-glucose (9.00 g, 50.0 mmol) in benzylalcohol (45.0 mL, 433 mmol) acetyl chloride (1.00 mL, 14.0 mmol) was added. The mixture was heated to 80 °C and left under stirring for 16 hrs. The mixture was decanted trice by addition of 250 mL diethyl ether in heptane (1:6, 1:1) and 150 mL pure diethyl ether respectively to remove most of the benzyl alcohol. The resulting emulsion was purified by silica column chromatography (10% MeOH in DCM) to afford **9** (8.1 g, 60 %) as a pale oil. **TLC** (10/90, MeOH/DCM, v/v) R_f =0.32; **¹H NMR** (500 MHz, CD₃OD) *major anomer* δ 7.45 – 7.42 (m, 2H, *o*-CH, benzyl), 7.35 (td, J = 7.8, 7.3, 1.6 Hz, 2H, *m*-CH, benzyl), 7.31 – 7.27 (m, 1H, *p*-CH, benzyl), 4.95 (d, J = 11.6 Hz, 1H, *CHH*, benzyl), 4.69 (d, J = 11.8 Hz, 1H, *CHH*, benzyl), 4.38 (d, J = 7.7 Hz, 1H, H-1_{ax}), 3.92 (dd, J = 11.9, 2.2 Hz, 1H, H-6_a), 3.76 – 3.69 (m, 1H, H-6_b), 3.42 – 3.24 (m, 4H, H-2; H-3; H-4; H-5) **¹³C NMR** (126 MHz, CD₃OD) δ 137.66 (C, benzyl), 127.88 (*m*-CH, benzyl), 127.80 (*o*-CH, benzyl), 127.30 (*p*-CH, benzyl), 101.88 (C-1), 76.69 (C-4), 76.61 (C-5), 73.73 (C-2), 70.35 (CH₂, benzyl), 70.29 (C-3), 61.42 (C-6).

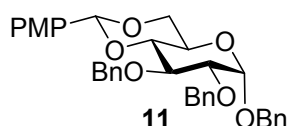
Benzyl 4,6-*p*-methoxy-benzylidene- α -D-glucopyranoside (**10**)



To a solution of **9** (7.00 g, 25.9 mmol) and anisaldehyde dimethylacetal (6.79 mL, 38.8 mmol) in dry DMF (129 mL) *p*-toluene sulfonic acid monohydrate (0.985 g, 5.18 mmol) was added. The mixture was stirred at 50 °C under N₂ atmosphere. After 4 hrs the reaction was quenched by addition of triethylamine (10 mL). The mixture was concentrated *in vacuo* and crystallized from EtOAc / heptane to afford **10** (7.0 g, 70%) as a white solid. **TLC** (60/40 EtOAc/n-heptane, v/v) R_f =0.21; **¹H NMR** (500 MHz, Chloroform-*d*) δ 7.45 – 7.30 (m, 7H, CH Ar), 6.93 – 6.85 (m, 2H, CH Ar), 5.49 (s, 1H, CH acetal PMP), 5.01 (d, J = 4.0 Hz, 1H, H-1), 4.78 (d, J = 11.7 Hz, 1H, *CHH*Ph), 4.57 (d, J = 11.7 Hz, 1H, *CHH*Ph),

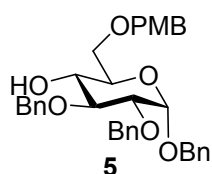
4.22 (dd, $J = 10.2, 4.9$ Hz, 1H, H-6a), 3.95 (t, $J = 9.2$ Hz, 1H, H-3), 3.85 (td, $J = 9.9, 4.9$ Hz, 1H, H-5), 3.80 (s, 3H, OCH_3), 3.71 (t, $J = 10.3$ Hz, 1H, H-6b), 3.64 (td, $J = 9.5, 4.1$ Hz, 1H, H-2), 3.49 (t, $J = 9.4$ Hz, 1H, H-4), 2.74 (s, 1H, 3-OH), 2.25 (d, $J = 10.0$ Hz, 1H, 2-OH); ^{13}C NMR (126 MHz, CDCl_3) δ 160.25, 136.65, 129.50, 128.65, 128.27, 128.19, 127.61, 113.70, 101.84, 98.11(C-1), 80.86(C-4), 72.91(C-2), 71.90(C-3), 70.20, 68.84(C-6), 62.73(C-5), 55.32; **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{21}\text{H}_{24}\text{NaO}_7$, 411.1420; found, 411.1415.

Benzyl 2,3-di-*O*-benzyl-4,6-*p*-methoxy-benzylidene- α -D-glucopyranoside (**11**)



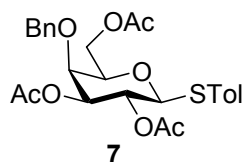
To a cooled 0°C solution of **10** (5.8 g, 14.9 mmol) in DMF (75 mL) 60% w/w NaH (2.99 g, 74.7 mmol) was added portion wise under stirring. After 20 minutes (bromomethyl)benzene (5.33 mL, 44.8 mmol) was added and the mixture was left under stirring for 16 hrs slowly allowing to heat up to room temperature. The suspension was quenched by addition of methanol (20 mL) and diluted with DCM (150 mL). The resulting suspension was washed with saturated NaHCO_3 (100 mL) and brine (100 mL) and the organic layer was dried over Na_2SO_4 . After filtration, the resulting solution was concentrated *in vacuo*. Silica column chromatography (0% – 20% EtOAc in n-heptane) of the residue afforded **11** (7.1 gram, 83% yield) as white solid. **TLC** (20/80 EtOAc/n-heptane, v/v) $R_f=0.34$; ^1H NMR (500 MHz, CDCl_3) δ 7.49 – 7.21 (m, 17H, CH Ar), 6.98 – 6.85 (m, 2H, CH Ar), 5.50 (s, 1H, CH acetal PMP), 4.91 (d, $J = 11.2$ Hz, 1H, CHHPh), 4.86 – 4.81 (m, 2H, H-1, CHHPh), 4.74 (d, $J = 12.0$ Hz, 1H, CHHPh), 4.73 (d, $J = 12.3$ Hz, 1H, CHHPh), 4.61 – 4.55 (m, 2H, CHHPh), 4.18 (dd, $J = 10.2, 4.9$ Hz, 1H, H-6a), 4.10 (t, $J = 9.3$ Hz, 1H, H-3), 3.89 (td, $J = 10.0, 4.9$ Hz, 1H, H-5), 3.81 (s, 3H, OCH_3), 3.67 (t, $J = 10.3$ Hz, 1H, H-6b), 3.59 (t, $J = 9.4$ Hz, 1H, H-4), 3.55 (dd, $J = 9.3, 3.8$ Hz, 1H, H-2); ^{13}C NMR (126 MHz, CDCl_3) δ 160.00, 138.85, 138.19, 136.99, 129.93, 128.46, 128.41, 128.37, 128.30, 127.99, 127.97, 127.92, 127.80, 127.76, 127.65, 127.55, 127.34, 113.59, 101.23, 96.57(C-1), 82.19(C-4), 79.28(C-2), 78.72(C-3), 75.34, 73.47, 72.13, 69.34, 68.99(C-6), 62.67(C-5), 55.32; **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{35}\text{H}_{36}\text{NaO}_7$, 591.2359; found, 591.2354.

Benzyl 2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl- α -D-glucopyranoside (**5**)



To a solution of **11** (380 mg, 0.688 mmol) was dissolved in dry THF under slenk conditions. The mixture was cooled down to -78°C . 1.0 M solution of BH_3 in THF (3.01 ml, 3.01 mmol) and a 1.0 M solution of dibutyl(trifluoromethylsulfonyloxy)borane in toluene (2.01 ml, 2.01 mmol) were added dropwise. The mixture was stirred at -78 for 1h. The reaction was allowed to warm up to rt in 20 min and quenched with triethylamine and methanol. The reaction was diluted with EtOAc (100 mL) washed with saturated NaHCO_3 (50 mL) brine (50mL) dried over Na_2SO_4 and concentrated *in vacuo*. Silica column chromatography (0% – 40% EtOAc in n-heptane) of the residu afforded **5** (370mg, 97% yield). **TLC** (40/60 EtOAc/toluene v/v%) $R_f=0.75$; **^1H NMR** (500 MHz, Chloroform-*d*) δ 7.44 – 7.21 (m, 17H, CH Ar), 6.90 – 6.82 (m, 2H, CH Ar), 5.01 (d, $J = 11.4$ Hz, 1H, CHHpMP), 4.85 (d, $J = 3.6$ Hz, 1H, H-1), 4.75 (d, $J = 11.4$ Hz, 1H, CHHpMP), 4.71 (d, $J = 12.3$ Hz, 1H, CHHPh), 4.63 (d, $J = 11.9$ Hz, 1H, CHHPh), 4.56 – 4.49 (m, 3H, 3 x CHHPh), 4.46 (d, $J = 11.8$ Hz, 1H, CHHPh), 3.85 (t, $J = 9.2$ Hz, 1H, H-3), 3.79 – 3.74 (m, 4H, OCH_3 , H-5), 3.65 – 3.57 (m, 3H, H-6a, H-6b, H-4), 3.52 (dd, $J = 9.6, 3.6$ Hz, 1H, H-2), 2.44 (s, 1H, 4-OH); **^{13}C NMR** (126 MHz, CDCl_3) δ 159.23, 138.91, 138.09, 137.20, 130.11, 129.31, 128.55, 128.42, 128.41, 127.96, 127.94, 127.88, 127.80, 127.79, 113.79, 95.52(C-1), 81.55(C-3), 79.56(C-2), 75.42, 73.25, 72.76, 70.97(C-4), 70.17(C-5), 69.17, 69.07(C-6), 55.29; **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{35}\text{H}_{38}\text{NaO}_7$, 593.2515; found, 593.2511.

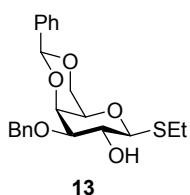
Tolyl 2,3,6-tri-*O*-acetyl-4-*O*-benzyl-1-thio- β -D-galactopyranoside (**7**)



Tolyl 4,6-benzylidene-1-thio- β -D-galactopyranose **16²** (4.00 gr, 8.72 mmol) was dissolved in 10 ml dry THF. The solution was cooled down to 0°C and 1.0M BH_3 in THF (44 mL) was added under stirring and N_2 atmosphere. After addition of 1.0 M dibutyl(trifluoromethylsulfonyloxy)borane in DCM (11 mL) the reaction stirred for 70 minutes at 0°C . The reaction was quenched by addition of triethylamine (8.0 mL) and methanol (40 mL). The mixture was concentrated *in vacuo* and the pale yellow residue was purified by silica column (0 – 40% EtOAc in n-heptane) to afford **17** (3.0 g, 73%) as colourless oil. **TLC** (50/50 EtOAc/heptane v/v) $R_f=0.33$. This was subsequently acetylated by addition of pyridine (20 mL) and acetic anhydride (6.15 mL, 65.1 mmol). After completion was observed by TLC after 16 hours, the solution was concentrated *in vacuo* by co-evaporation with toluene to afford **7** as a white solid (3.01g, 92%). **TLC** (30/70 EtOAc/n-heptane, v/v) $R_f=0.29$; **^1H NMR** (500 MHz, CDCl_3) δ 7.43 – 7.38 (m, 2H, CH Ar), 7.37 – 7.23 (m, 5H, CH Ar), 7.07 (d, $J = 7.9$ Hz, 2H, CH Ar), 5.38 (t, $J = 9.9$ Hz, 1H, H-2), 4.99 (dd, $J = 9.9, 3.0$ Hz, 1H, H-3), 4.74 (d, $J = 11.6$ Hz, 1H, CHH Benzyl), 4.61 (d, $J = 9.9$ Hz, 1H, H-1), 4.52 (d, $J = 11.6$ Hz, 1H, CHH Benzyl), 4.28 (dd, $J = 11.2, 6.7$ Hz, 1H, H-6A), 4.07 (dd, $J =$

11.2, 6.2 Hz, 1H, H-6B), 3.97 – 3.88 (m, 1H, H-4), 3.78 – 3.66 (m, 1H, H-5), 2.32 (s, 3H, CH₃ Tol), 2.08 (s, 1H, CH₃ Ac), 2.01 (s, 1H, CH₃ Ac), 2.00 (s, 1H, CH₃ Ac). **¹³C NMR** (126 MHz, CDCl₃) δ 170.40, 170.34, 169.40, 138.09, 137.46, 132.95, 129.57, 128.94, 128.41, 128.05, 127.93, 86.73 (C-1), 75.88 (C-5), 75.03 (C-3), 74.85, 73.70 (C-4), 68.00 (C-2), 62.55 (C-6), 21.18, 20.89, 20.83, 20.78; **HR-ESI-TOF/MS** (*m/z*): [M+Na]⁺ calcd. for C₂₆H₃₀NaO₈S, 525.1559; found, 525.1554.

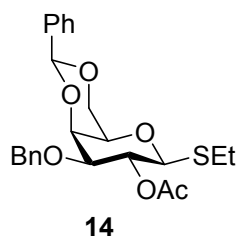
Ethyl-3-*O*-benzyl-4,6-benzylidene-1-thio-β-D-galactopyranoside (13**)³**



To a solution of **12**³ (6.00 g, 19.2 mmol, 1 eq.) in toluene (40 ml), dibutylstannanone (4.78 g, 19.1 mmol, 1 eq.) was added. The mixture was refluxed for 4 hrs. The solvent was concentrated *in vacuo* and the resulting residue was dissolved in DMF (100 ml). To the solution, cesium fluoride (5.84 g, 38.4 mmol, 2 eq.) and benzyl bromide (2.74 ml, 23.1 mmol, 1.2 eq.) were added and the mixture was allowed to stir overnight at rt. The reaction mixture was then concentrated *in vacuo* and purified over by silica column chromatography (20-80% EtOAc/n-heptane) to yield the product (**13**) (4.63 g, 11.5 mmol, 60%). **TLC** (80/20 EtOAc/n-heptane, v/v); R_f=0.75 **¹H NMR** (500 MHz, CDCl₃) δ 7.59 – 7.43 (m, 2H, *ortho*-H benzylidene), 7.43 – 7.22 (m, 8H, Ar), 5.45 (s, 1H, PhCHO₂), 4.77 (d, *J* = 2.9 Hz, 2H, CH₂, bn), 4.35 (d, *J* = 9.6 Hz, 1H, H-1), 4.32 (dd, *J* = 12.4, 1.7 Hz, 1H, H-6a), 4.18 (dd, *J* = 3.5, 1.2 Hz, 1H, H-4), 4.07 (td, *J* = 9.4, 1.6 Hz, 1H, H-2), 3.97 (dd, *J* = 12.4, 1.8 Hz, 1H, H-6b), 3.50 (dd, *J* = 9.2, 3.4 Hz, 1H, H-3), 3.42 (q, *J* = 1.6 Hz, 1H, H-5), 2.84 (dq, *J* = 12.5, 7.5 Hz, 1H, CHH, SEt), 2.75 (dq, *J* = 12.6, 7.5 Hz, 1H, CHH, SEt), 2.55 (d, *J* = 1.6 Hz, 1H, OH), 1.33 (t, *J* = 7.5 Hz, 3H, CH₃, SEt); **¹³C NMR** (126 MHz, CDCl₃) δ 138.06 (C, bn), 137.83 (C, benzylidene), 129.01(*para*-CH, benzylidene), 128.48 (*meta*-CH, Bn), 128.18 (*meta*-CH, benzylidene), 127.90 (*para*-CH, Bn), 127.88 (*ortho*-CH, Bn), 126.40 (*Ortho*-CH, benzylidene), 101.29 (CH₂, benzylidene), 85.32 (C-1), 80.32 (C-3), 73.55 (C-4), 71.57 (CH₂, Bn), 70.16 (C-5), 69.46 (C-6), 68.03 (C-2), 22.96 (CH₂, SEt), 15.30 (CH₃, SEt). **HR-ESI-TOF/MS** (*m/z*): [M+Na]⁺ calcd. for C₂₂H₂₆NaO₅S, 425.13986; found, 425.13950.

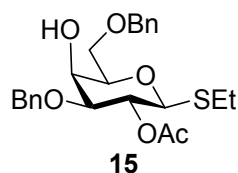
Comment [HE]: Reference added

Ethyl-2-*O*-acetyl-3-*O*-benzyl-4,6-benzylidene-1-thio- β -D-galactopyranoside (**14**)⁵



Galactose derivative **13**³ (2.81 g, 6.98 mmol, 1 eq.) was dissolved pyridine (30 ml). To the solution, acetic anhydride (3.30 ml, 34.9 mmol, 5 eq.) was added and the reaction was allowed to stir overnight at rt. The mixture was concentrated *in vacuo* and dissolved in EtOAc (50 ml) and washed with 1M HCl (2x 25 ml), saturated aqueous NaHCO₃ (2x 25 ml) and brine (25 ml). The solvent of the organic layer was then evacuated *in vacuo*. Silica column chromatography of the residue (20-50% EtOAc/heptane) yielded the product (**14**) (1.58 g, 3.55 mmol, 51%). **TLC** (35/65, EtOAc/n-heptane, v/v); R_f =0.23 **¹H NMR** (500 MHz, Chloroform-*d*) δ 7.70 – 7.46 (m, 2H, *ortho*-H benzylidene), 7.44 – 7.18 (m, 8H, CH, Ar), 5.67 – 5.35 (m, 2H, H-2; CH, benzylidene), 4.72 – 4.61 (m, 2H, CH₂, bn), 4.36 (d, J = 9.8 Hz, 1H, H-1), 4.31 (dd, J = 12.5, 1.6 Hz, 1H, H-6a), 4.21 (dd, J = 3.4, 1.1 Hz, 1H, H-4), 3.97 (dd, J = 12.4, 1.8 Hz, 1H, H-6b), 3.60 (dd, J = 9.6, 3.4 Hz, 1H, H-3), 3.40 (q, J = 1.5 Hz, 1H, H-5), 2.88 (dq, J = 12.2, 7.4 Hz, 1H, CHH, SEt), 2.72 (dq, J = 12.2, 7.5 Hz, 1H, CHH, SEt), 2.07 (s, 3H, CH₃, Ac), 1.28 (t, J = 7.5 Hz, 3H, CH₃, SEt); **¹³C NMR** (126 MHz, CDCl₃) δ 169.57 (CO, Ac), 138.05 (C, bn), 137.72 (C, benzylidene), 129.07 (*para*-CH, benzylidene), 128.40 (*meta*-CH, bn), 128.22 (*meta*-CH, benzylidene), 127.80 (*para*-CH, bn), 127.58 (*ortho*-CH, bn), 126.47 (*ortho*-CH, benzylidene), 101.38 (CH, benzylidene), 82.85 (C-1), 78.44 (C-3), 73.52 (C-4), 71.20 (CH₂, bn), 70.07 (C-5), 69.36 (C-6), 68.12 (C-2), 22.64 (CH₂, SEt), 21.07 (CH₃, Ac), 14.82 (CH₃, SEt). **HR-ESI-TOF/MS** (m/z): [M+Na]⁺ calcd. for C₂₄H₂₈NaO₆S, 467.1504; found, 467.1501.

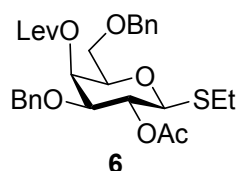
Ethyl 2-*O*-Acetyl-3,6-di-*O*-benzyl -1-thio- β -D-galactopyranoside (**15**)



Ethyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-benzylidene-1-thio- β -D-galactopyranoside⁵ **14** (4.00g, 9.00 mmol) was dissolved in a mixture of diethyl ether/DCM 1:2 (90 mL). To this solution sodium cyanoborohydride (5.65 g, 90 mmol) was added. Next, TFA (13.86 mL, 180 mmol) was added dropwise and the reaction stirred for 48 hrs until completion at rt under N₂. The mixture was diluted with EtOAc (200 mL) and washed twice with NaHCO₃ (50 mL) and once with brine (50 mL). The

organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*. Silica column chromatography (20% – 40% EtOAc in n-heptane) of the residue afforded **15** (4.01 gr, quant.) as pale oil. **TLC** (50/50 EtOAc/n-heptane, v/v) $R_f=0.57$; **^1H NMR** (500 MHz, CDCl_3) δ 7.41 – 7.23 (m, 10H, CH Ar), 5.26 (t, $J = 9.7$ Hz, 1H, H-2), 4.70 (d, $J = 12.2$ Hz, 1H, CHH Benzyl), 4.58 (s, 2H, CHH Benzyl), 4.55 (d, $J = 12.1$ Hz, 1H, CHH Benzyl), 4.32 (d, $J = 10.0$ Hz, 1H, H-1), 4.15 – 4.12 (m, 1H, H-4), 3.79 (dd, $J = 9.8, 6.3$ Hz, 1H, H-6A), 3.73 (dd, $J = 9.8, 5.7$ Hz, 1H, H-6B), 3.61 (t, $J = 6.0$ Hz, 1H, H-5), 3.51 (dd, $J = 9.3, 3.3$ Hz, 1H, H-3), 2.80 – 2.62 (m, 2H, CHH SEt), 2.56 (s, 1H, 4-OH), 2.04 (s, 3H, CH_3 Acetyl), 1.24 (t, $J = 7.5$ Hz, 3H, CH_3 SEt). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 171.06, 137.17, 136.53, 128.85, 128.76, 128.75, 128.35, 128.31, 128.15, 83.76 (C-1), 79.87 (C-3), 76.51 (C-5), 74.09, 72.57, 69.59 (C-6), 69.34 (C-2), 67.79 (C-4), 24.52, 21.16, 14.92; **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{24}\text{H}_{30}\text{NaO}_6\text{S}$, 469.1661; found, 469.1654.

Ethyl 2-*O*-Acetyl-3,6-di-*O*-benzyl-4-*O*-levuloyl-1-thio- β -D-galactopyranoside (**6**)



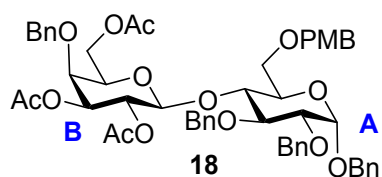
To a solution of **15** (1.8 g, 4.03 mmol) in DCM (40 mL) levulinic acid (0.702g, 6.05mmol) was added. The acid was activated by addition of DCC(1.248g, 6.05mmol) and DMAP(0.098g, 0.806mmol). The reaction was left under stirring and N_2 for 3 hrs at rt. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0% -> 30 % EtOAc in n-heptane). **TLC** (40/60, EtOAc/n-heptane, v/v) $R_f=0.42$; **^1H NMR** (500 MHz, CDCl_3) δ 7.44 – 7.17 (m, 10H, CH Ar), 5.66 (dd, $J = 3.3, 0.7$ Hz, 1H, H-4), 5.12 (t, $J = 9.8$ Hz, 1H, H-2), 4.67 (d, $J = 12.3$ Hz, 1H, CHH Benzyl), 4.52 (s, 2H, CHH Benzyl), 4.38 (t, $J = 10.9$ Hz, 2H, H-1, CHH Benzyl), 3.74 (ddd, $J = 6.9, 5.7, 0.9$ Hz, 1H, H-5), 3.63 (dd, $J = 9.5, 5.7$ Hz, 1H, H-6A), 3.57 – 3.47 (m, 2H, H-3, H-6B), 2.90 – 2.56 (m, 2H, CHH SEt), 2.15 (s, 3H, CH_3 Lev), 2.03 (s, 1H, CH_3 Acetyl), 1.59 (s, 1H), 1.25 (t, $J = 7.5$ Hz, 1H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 206.46, 172.13, 169.73, 137.86, 137.76, 128.57, 128.46, 128.27, 127.98, 127.96, 127.92, 83.93 (C-1), 77.85 (C-3), 76.31 (C-5), 73.88, 71.13, 69.11 (C-2), 68.14, 66.72 (C-4), 38.23, 29.93, 28.21, 24.15, 21.09, 15.00; **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{29}\text{H}_{36}\text{NaO}_8\text{S}$, 567.2029; found, 567.2024.

General glycosylation procedure A

To a solution of the acceptor and the the thioglycosyl donor (1.1 equivalents) in DCM (0.1 M), mol sieves (4\AA) were added under a nitrogen atmosphere. The reaction stirred for 15 minutes and was

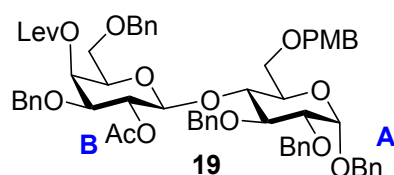
cooled to 0°C. The thioglycoside was activated by addition of NIS (1.3 equivalents) and triflic acid (0.2 eq.) The reaction was stirred to completion and quenched by addition of triethylamine. The mixture was diluted by addition of DCM, filtered and washed with 10% aqueous thiosulfate solution, sat. aqueous NaHCO₃ and brine. The organic layer was dried (MgSO₄), filtered and purified by silica column chromatography (x% ethyl acetate in n-heptane).

Benzyl 2,3,6-tri-O-acetyl-4-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-O-benzyl-6-O-para-methoxybenzyl-α-D-glucopyranoside (18)



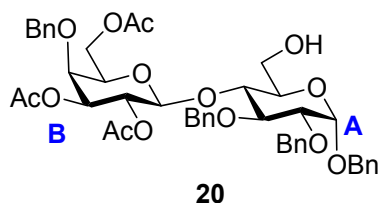
According to the general glycosylation procedure. **5** (0.400 g, 0.701 mmol), **7** (0.423 g, 0.841 mmol). Purified by silica column chromatography (5% – 40% EtOAc in n-heptane) of the residue afforded **18** (470mg, 71%) as a clear oil. **TLC** (40/60 EtOAc/n-heptane, v/v) R_f =0.33; **¹H NMR** (500 MHz, CDCl₃) δ 7.45 – 7.15 (m, 22H, CH Ar), 6.89 (d, J = 8.7 Hz, 2H, CH Ar), 5.28 (dd, J = 10.4, 7.9 Hz, 1H, H(B)-2), 4.95 (d, J = 10.8 Hz, 1H, CHH Benzyl), 4.79 (d, J = 3.7 Hz, 1H, H(A)-1), 4.78 – 4.62 (m, 7H, H(B)-3, CHH Benzyl), 4.56 (d, J = 12.5 Hz, 1H, CHH Benzyl), 4.52 (d, J = 12.1 Hz, 1H, CHH Benzyl), 4.49 (dd, J = 9.7, 1.7 Hz, 2H, H(B)-1, CHH PMB), 4.35 (d, J = 11.8 Hz, 1H, CHH PMB), 3.99 (dd, J = 10.9, 5.5 Hz, 1H, H(B)-6A), 3.91 (m, 3H, H(B)-6B, H(A)-3, H(A)-4), 3.82 – 3.79 (m, 4H, H(B)-4, CH₃ PMB), 3.69 – 3.63 (m, 2H, H(A)-6A, H(A)-5), 3.51 – 3.44 (m, 2H, H(A)-6B, H(A)-2), 3.34 (dd, J = 7.4, 6.1 Hz, 1H, H(B)-5), 2.01 (s, 3H, CH₃ Acetyl), 1.94 (s, 3H, CH₃ Acetyl), 1.94 (s, 2H, CH₃ Acetyl). **¹³C NMR** (126 MHz, Chloroform-*d*) δ 170.42, 170.09, 169.21, 159.39, 139.33, 138.40, 137.72, 137.33, 130.02, 129.57, 128.45, 128.39, 128.32, 128.12, 128.08, 128.04, 127.98, 127.94, 127.92, 127.84, 127.74, 127.66, 127.11, 113.93, 100.23 (C-1(B)), 95.96 (C-1(A)), 79.96 (C-3(A)), 79.15 (C-2(A)), 76.65 (C-4(A)), 75.28, 75.07, 74.24 (C-3(B)), 73.59 (C-4(B)), 73.27, 73.18, 71.79 (C-5(B)), 70.47 (C-2(B)), 70.22 (C-5(A)), 69.36, 67.43 (C-6(A)), 61.70 (C-6(B)), 55.27, 20.86, 20.75; **HR-ESI-TOF/MS** (m/z): [M+Na]⁺ calcd. for C₅₄H₆₀NaO₁₅, 971.38299; found, 971.38028.

Benzyl 2-O-acetyl-3,6-di-O-benzyl-4-O-levuloyl-β-D-galactopyranosyl-(1→4)-2,3-di-O-benzyl-6-O-para-methoxybenzyl-α-D-glucopyranoside (19)



According to the general glycosylation procedure. **5** (0.300 g, 0.526 mmol), **6** (0.315 g, 0.578 mmol). Purified by silica column chromatography (0% – 50% EtOAc in n-heptane) of the residue afforded **19** (240 mg, 43%) as a clear oil. **TLC** (60/40, EtOAc/n-heptane, v/v%): R_f =0.33; **¹H NMR** (500 MHz, Chloroform-*d*) δ 7.47 – 7.41 (m, 4H, *CH* Ar), 7.41 – 7.21 (m, 23H, *CH* Ar), 6.88 – 6.80 (m, 2H, *CH* Ar), 5.60 (d, J = 3.3 Hz, 1H, H-4(B)), 5.07 (dd, J = 10.0, 8.1 Hz, 1H, H-2(B)), 5.02 (d, J = 10.6 Hz, 1H), 4.87 – 4.81 (m, 2H, H-1(A)), 4.76 – 4.52 (m, 3H), 4.45 (d, J = 11.8 Hz, 1H), 4.41 – 4.29 (m, 4H, H-1(B),), 3.99 – 3.85 (m, 2H, H-3(A), H-4(A)), 3.75 (s, 3H), 3.71 (td, J = 11.3, 10.7, 2.5 Hz, 2H, H-5(A), H-6A(A)), 3.54 – 3.48 (m, 2H, H-2(A), H-6B(A)), 3.47 – 3.37 (m, 3H, H-5(B), H-6A(B), H-6B(B)), 3.30 (dd, J = 9.9, 3.4 Hz, 1H, H-3(B)), 2.86 – 2.65 (m, 2H), 2.64 – 2.54 (m, 2H), 2.12 (s, 3H), 1.99 (s, 3H); **¹³C NMR** (126 MHz, Chloroform-*d*) δ 206.10, 171.94, 169.08, 159.26, 139.33, 138.31, 137.96, 137.84, 137.23, 129.96, 129.62, 128.32, 128.25, 128.03, 127.99, 127.83, 127.78, 127.65, 127.61, 127.60, 127.51, 127.21, 113.79, 100.41 (C-1(B)), 95.87 (C-1(A)), 79.81 (C-3(A)), 79.08 (C-2(A)), 77.37 (C-3(B)), 76.74 (C-4(A)), 75.27, 73.53, 73.20, 73.04, 71.96 (C-5(B)), 71.30 (C-2(B)), 71.03, 70.23 (C-5(A)), 69.29, 67.24 (C-6(A), C-6(B), 66.06 (C-4(B)), 55.17, 37.98, 29.65, 28.01, 20.95; **HR-ESI-TOF/MS** (m/z): $[M+Na]^+$ calcd. for C₆₂H₆₈NaO₁₅, 1075.4456; found, 1075.4437.

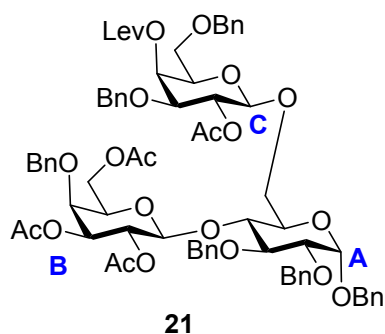
Benzyl **2,3,6-tri-O-acetyl-4-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2,3-di-O-benzyl- α -D-glucopyranoside (20)**



To a well stirred emulsion of **18** (840 mg, 0.885 mmol) in DCM (9 mL) and water (1 mL) DDQ (0.201 g, 0.885 mmol) was added. The resulting solution was stirred for 2 hrs at rt in the dark. The mixture was diluted with CH₂Cl₂ (50 mL) and washed with an aqueous mixture of ascorbic acid (1.5 %), citric acid (0.7%) and NaOH (0.9%) twice, once with 10 ml and once with 5 ml. The resulting organic layer was slightly pale yellow. The organic layer was dried (MgSO₄), filtered and the filtrate was concentrated *in vacuo*. Silica column chromatography (20% \rightarrow 70% - EtOAc in heptane) of the residue afforded **20** (520 mg, 71%) as colourless oil. Part of the starting material (170 mg) was reclaimed. **TLC** (60/40 EtOAc/n-heptane, v/v) R_f =0.50; **¹H NMR** (500 MHz, CDCl₃) δ 7.42 – 7.17 (m, 20H, *CH* Ar), 5.35 (dd, J = 10.4, 7.9 Hz, 1H, H(B)-2), 4.94 (s, 1H, *CHH* Benzyl), 4.90 (dd, J = 10.4, 3.0 Hz, 1H, H(B)-3), 4.82 (d, J = 7.9 Hz, 1H, H(B)-1), 4.76 (d, J = 3.7 Hz, 1H, H(A)-1), 4.74 (d, J = 11.6 Hz, 1H, *CHH* Benzyl), 4.65 (dd, J =

12.2, 7.5 Hz, 2H, *CHH* Benzyl), 4.54 (d, $J = 12.3$ Hz, 2H, *CHH* Benzyl), 4.50 (dd, $J = 11.8, 4.2$ Hz, 2H, *CHH* Benzyl), 4.00 (t, $J = 9.2$ Hz, 1H, H(A)-3), 3.96 – 3.92 (m, 2H, H(B)-6AB), 3.81 (dt, $J = 11.3, 6.6$ Hz, 2H, H(A)-4, H(B)-4), 3.70 – 3.59 (m, 1H, H(A)-6AB, H(A)-5), 3.47 – 3.41 (m, 2H, H(B)-5, H(A)-2), 2.06 (s, 3H, CH_3 Ac), 2.01 (s, 3H, CH_3 Ac), 1.87 (s, 3H, CH_3 Ac), 1.64 (broad s, 1H, 4(A)-OH); ^{13}C NMR (126 MHz, Chloroform-*d*) δ 170.57, 170.10, 169.74, 139.42, 138.22, 137.68, 137.20, 128.56, 128.53, 128.48, 128.46, 128.36, 128.26, 128.08, 128.06, 127.88, 127.17, 127.15, 101.15 (C-1(B)), 95.84 (C-1(A)), 80.18 (C-3(A)), 79.78 (C-2(A)), 76.87 (C-4(A)), 75.09, 74.96, 74.29 (C-3(B)), 73.41, 73.26 (C-4(B)), 72.11 (C-5(B)), 70.79 (C-2(B)), 70.65 (C-5(A)), 69.61, 61.84 (C-6(B)), 60.89 (C-6(A)), 20.97, 20.96, 20.79; **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{46}\text{H}_{52}\text{NaO}_{14}$, 851.3255; found, 851.3201.

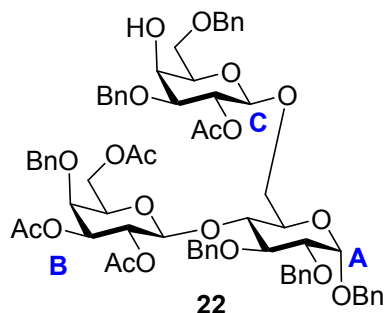
Benzyl 2,3,6-tri-*O*-acetyl-4-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-[2-*O*-Acetyl-3,6-di-*O*-benzyl-4-*O*-levuloyl- β -D-galactopyranosyl-(1 \rightarrow 6)]-2,3-di-*O*-benzyl- α -D-glucopyranoside (21)



According to the general glycosylation procedure **20** (510 mg, 0.615 mmol) and **6** (503 mg, 0.923 mmol). Silica gel column chromatography (10% \rightarrow 50% - EtOAc in *n*-Heptane) afforded **21** (550 mg, 68%) as a colourless oil with minor impurities of trehalose-like structures. **TLC** (40/60 EtOAc/*n*-heptane, v/v) R_f =0.36; ^1H NMR (500 MHz, Chloroform-*d*) δ 7.52 – 7.09 (m, 28H, *CH* Ar), 5.66 – 5.56 (m, 1H, H-4(C)), 5.32 (dd, $J = 10.4, 7.9$ Hz, 1H, H-2(B)), 5.13 (dd, $J = 10.0, 8.1$ Hz, 1H, H-2(C)), 4.94 – 4.82 (m, 2H, H-3(B)), 4.78 – 4.70 (m, 2H, H-1(Aa)), 4.70 – 4.63 (m, 2H), 4.60 (dd, $J = 7.8, 4.7$ Hz, 2H, H-1(B)), 4.53 – 4.43 (m, 5H, H-1(C)), 4.37 (d, $J = 12.3$ Hz, 1H), 3.97 (dd, $J = 11.5, 6.9$ Hz, 2H, H-3(A), H-6A(A)), 3.92 – 3.87 (m, 1H, H-6A(B), H-6B(B)), 3.82 – 3.71 (m, 3H, H-4(B), H-5(C), H-5(A)), 3.68 – 3.60 (m, 2H, H-6B(A), H-6A(C)), 3.60 – 3.53 (m, 2H, H-4(A), H-6B(C)), 3.53 – 3.49 (m, 1H, H-4(C)), 3.48 – 3.40 (m, 2H, H-5(B), H-2(A)), 2.87 – 2.44 (m, 4H, *CHH* Lev), 2.16 (s, 3H, Ac), 2.14 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.89 (s, 3H, Ac), 0.86 (s, 3H, CH_3 Lev). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 206.46, 172.15, 170.49, 170.12, 169.49, 169.43, 139.40, 138.25, 137.90, 137.74, 137.66, 137.22, 128.51, 128.45, 128.43, 128.37, 128.32, 128.18, 127.92, 127.91, 127.88, 127.86, 127.75, 101.66 (C-1(C)), 100.74 (C-1(B)), 94.84 (C-1(A)), 79.62 (C-2(A)), 79.39 (C-3(A)), 78.09 (C-4(A)), 76.84 (C-3(C)), 75.09, 74.88, 74.09 (C-3(A)), 73.80 (C-3(A)), 73.47 (C-4(A)), 73.10, 72.54 (C-5(C)),

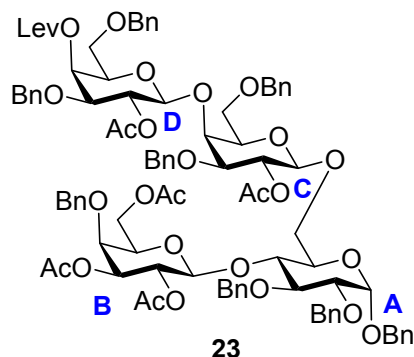
71.93(C-5(B)), 71.12, 70.71(C-2(B)), 70.51 (C-2(C)), 70.22 (C-5(A)), 68.64, 67.96 (C-6(C)), 67.82 (C-6(A)), 66.42 (C-4(C)), 61.77 (C-6(B)), 38.17, 35.53, 31.98, 29.87, 28.16, 22.80, 21.00, 20.93, 20.89, 20.77; **HR-ESI-TOF/MS** (m/z): $[M+Na]^+$ calcd. for $C_{73}H_{82}NaO_{22}$, 1333.5195; found, 1333.5194.

Benzyl 2,3,6-tri-O-acetyl-4-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-[2-O-Acetyl-3,6-di-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 6)]-2,3-di-O-benzyl- α -D-glucopyranoside (22)



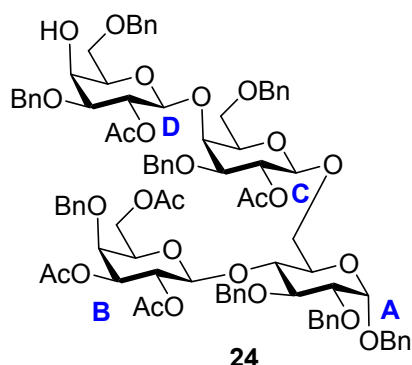
To a solution of **21** (510 mg, 0.389 mmol) in a mixture of toluene/ethanol (7.6 mL, 2/1, v/v), hydrazine acetate (54 mg, 0.538 mmol) was added. The reaction was stirred at rt until completion and quenched with acetone. The resulting mixture was diluted with ethyl acetate (50 mL) and washed with sat. aqueous $NaHCO_3$ (30 mL), brine (30 mL), dried (Na_2SO_4) and filtered. Silica column chromatography (0% – 50% EtOAc in n-heptane) afforded **22** (380 mg, 81%) as an amorphous solid. **TLC** (50/50 EtOAc/n-heptane, v/v) R_f =0.75; **1H NMR** (500 MHz, Chloroform- d) δ 7.44 – 7.15 (m, 34H, CH Ar), 5.32 (dd, J = 10.4, 7.9 Hz, 1H, H-2(B)), 5.22 (dd, J = 9.6, 7.9 Hz, 1H, H-2(C)), 4.92 (d, J = 11.2 Hz, 1H), 4.89 (dd, J = 10.4, 3.0 Hz, 1H, H-3(B)), 4.83 (d, J = 11.1 Hz, 1H), 4.76 – 4.65 (m, 4H, H-1(A)), 4.64 – 4.55 (m, 4H, H-1(B)), 4.54 – 4.43 (m, 5H, H-1(C)), 4.09 (d, J = 2.7 Hz, 1H, H-4(C)), 4.00 – 3.86 (m, 4H, H-3(A), H-6A(A), H6-A(B), H-6B(B)), 3.86 – 3.70 (m, 4H, H-6A(C), H-6B(C), H-5(A), H-4(B)), 3.70 – 3.59 (m, 3H, H-6B(A), H-5(C), H-4(A)), 3.52 – 3.46 (m, 2H, H-3(C), H-5(B)), 3.44 (dd, J = 9.6, 3.7 Hz, 1H, H-2(A)), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.89 (s, 3H); **^{13}C NMR** (126 MHz, Chloroform- d) δ 170.51, 170.19, 169.64, 169.61, 139.48, 138.36, 138.14, 137.74, 137.55, 137.29, 128.64, 128.56, 128.54, 128.48, 128.42, 128.40, 128.28, 128.15, 128.04, 127.98, 127.91, 127.88, 127.74, 127.40, 127.14, 101.36 (C-1(C)), 100.75(C-1(B)), 95.00 (C-1(A)), 79.56 (C-3(A)), 79.53 (C-2(A)), 78.81 (C-3(C)), 78.09 (C-4(A)), 75.10, 75.00, 74.17, 73.77 (C-3(B)), 73.73 (C-5(C)), 73.61 (C-4(B)), 73.17 (C-5(B)), 71.90, 71.51, 70.69(C-2(B)), 70.58 (C-2(C)), 70.22 (C-5(A)), 68.92, 68.71(C-6(C)), 67.20 (C-6(A)), 66.08 (C-4(C)), 61.88 (C-6(B)), 21.09, 20.97, 20.96, 20.82; **HR-ESI-TOF/MS** (m/z): $[M+Na]^+$ calcd. for $C_{68}H_{76}NaO_{20}$, 1235.4828; found, 1235.4790.

Benzyl 2-O-Acetyl-3,6-di-O-benzyl-4-O-levuloyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-O-Acetyl-3,6-di-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 6)-[2,3,6-tri-O-acetyl-4-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)]-2,3-di-O-benzyl- α -D-glucopyranoside (23**)**



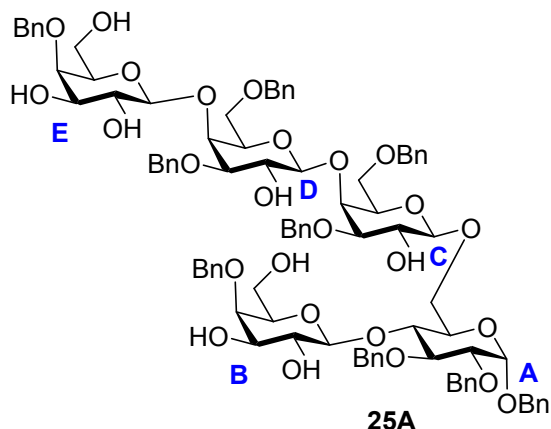
According to the general glycosylation procedure **22** (398 mg, 0.303 mmol) and **6** (247 mg, 0.454 mmol). Silica gel column chromatography (10% \rightarrow 50% - EtOAc in n-heptane) afforded **23** (433 mg, 84%) as a colourless oil with some minor trehalose-like impurities. **TLC** (60/40, EtOAc/n-heptane, v/v%): R_f =0.60; **$^1\text{H NMR}$** (500 MHz, Chloroform-*d*) δ 7.43 – 7.16 (m, 40H, *CH* Ar), 5.55 (d, J = 3.2 Hz, 1H, H-4(D)), 5.32 (dd, J = 10.3, 7.9 Hz, 1H, H-2(B)), 5.17 (dd, J = 10.0, 8.0 Hz, 1H (H-2(C)), 5.03 (dd, J = 10.0, 8.1 Hz, 1H, H-2(D)), 4.94 (d, J = 11.1 Hz, 1H), 4.86 (dd, J = 10.4, 3.0 Hz, 1H, H-3(B)), 4.81 (d, J = 11.1 Hz, 1H), 4.75 – 4.34 (m, 18H, H-1(A), H-1(B), H-1(C), H-1(D)), 4.15 (d, J = 2.2 Hz, 1H, H-4(C)), 3.98 – 3.86 (m, 4H, H-6A(A), H-6A(B), H-6B(B), H-3(A)), 3.80 – 3.73 (m, 3H, H-4(B), H-5(A), H-6A(C)), 3.72 – 3.51 (m, 5H, H-6B(C), H-6B(A), H-4(A), H-5(C), H-5(D)), 3.50 – 3.40 (m, 6H, H-6A(D), H-6B(D), H-5(B), H-3(D), H-2(A), H-3(C)), 2.83 – 2.50 (m, 4H, Lev), 2.15 (s, 1H), 2.08 (s, 1H), 2.06 (s, 1H), 2.01 (s, 1H), 1.96 (s, 1H), 1.88 (s, 1H); **$^{13}\text{C NMR}$** (126 MHz, Chloroform-*d*) δ 206.51, 172.09, 170.40, 170.10, 170.00, 169.91, 169.20, 139.48, 138.55, 138.39, 137.83, 137.81, 137.75, 137.23, 128.50, 128.47, 128.43, 128.37, 128.32, 128.19, 128.07, 128.06, 127.94, 127.91, 127.90, 127.82, 127.79, 127.76, 127.69, 127.43, 127.32, 101.27 (C-1(C)), 101.00 (C-1(D)), 100.98 (C-1(B)), 94.75 (C-1(A)), 80.13 (C-3(C)), 79.73 (C-3(A)), 79.38 (C-2(A)), 78.15 (C-4(A)), 76.77 (C-3(D)), 75.07, 75.03, 74.16 (C-3(B)), 73.97 (C-5(C)), 73.73 (C-4(B)), 73.54, 73.50, 73.11, 72.21 (C-5(D)), 71.78 (C-5(B)), 71.36 (C-4(C)), 71.18, 70.70 (C-2(D)), 70.54 (C-2(C)), 70.35 (C-2(B)), 70.30 (C-5(A)), 69.35 (C-6(C)), 68.44, 68.01 (C-6(D)), 66.65 (C-6(A)), 66.54 (C-4(D)), 61.77 (C-6(B)), 38.10, 29.91, 28.14, 21.02, 20.96, 20.92, 20.85, 20.76. **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{95}\text{H}_{106}\text{NaO}_{28}$, 1717.67683; found, 1717.67570.

Benzyl 2-O-Acetyl-3,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-2-O-Acetyl-3,6-di-O-benzyl-β-D-galactopyranosyl-(1→6)-[2,3,6-tri-O-acetyl-4-O-benzyl-β-D-galactopyranosyl-(1→4)]-2,3-di-O-benzyl-α-D-glucopyranoside (24)



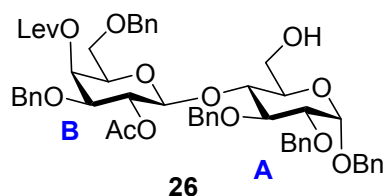
To a solution of **22** (421 mg, 0.249 mmol) in a mixture of toluene/ethanol (5.0 mL, 2/1, v/v), hydrazine acetate (46 mg, 0.497 mmol) was added. The reaction was stirred at rt until completion and quenched with acetone. The resulting mixture was diluted with ethyl acetate (50 mL) and washed with sat. aqueous NaHCO₃ (30 mL), brine (30 mL), dried (Na₂SO₄) and filtered. Silica column chromatography (0% – 60% EtOAc in n-heptane) afforded **24** (310 mg, 78%) as an amorphous solid. **TLC** (60/40, EtOAc/n-heptane, v/v): R_f=0.57; **¹H NMR** (500 MHz, Chloroform-*d*) δ 7.57 – 7.07 (m, 40H), 5.33 (dd, *J* = 10.4, 7.9 Hz, 1H, H-2(B)), 5.19 (dd, *J* = 10.0, 8.0 Hz, 1H, H-2(C)), 5.10 (dd, *J* = 9.8, 8.0 Hz, 1H, H-2(D)), 4.95 (d, *J* = 11.0 Hz, 1H), 4.86 (dd, *J* = 10.5, 3.1 Hz, 1H, H-3(B)), 4.81 – 4.40 (m, 18H, H-1(A), H-1(D), H-1(B), H-1(C)), 4.16 (d, *J* = 2.3 Hz, 1H, H-4(C)), 4.01 (d, *J* = 3.3 Hz, 1H, H-4(D)), 3.98 – 3.84 (m, 4H, H-6A(A), H-6A(B), H-6(B), H-3(B)), 3.82 – 3.61 (m, 7H, H-4(B), H-6A(D), H-6A(C), H-6B(D), H-5(A), H-6A(B), H-4(A)), 3.61 – 3.51 (m, 2H, H-6B(C), H-5(C)), 3.50 – 3.38 (m, 5H, H-5(D), H-5(B), H-3(D), H-3(C), H-2(A)), 2.73 (d, *J* = 2.9 Hz, 1H, 4-OH(D)), 2.09 (s, 3H), 2.07 (s, 2H), 2.03 (s, 3H), 1.96 (s, 2H), 1.88 (s, 3H). **¹³C NMR** (126 MHz, Chloroform-*d*) δ 170.64, 170.17, 170.04, 170.02, 169.23, 139.41, 138.53, 138.41, 137.97, 137.81, 137.77, 137.58, 137.17, 100.88 (C-1(C)), 100.84 (C-1(D)), 100.78 (C-1(B)), 94.83 (C-1(A)), 80.14 (C-3(C)), 79.70 (C-3(A)), 79.13 (C-2(A)), 78.52 (C-3(D)), 78.06 (C-4(A)), 75.19, 74.99, 74.17 (C-3(B)), 74.03 (C-5(C)), 73.65 (C-4(B)), 73.63, 73.40, 73.25 (C-5(B)), 73.10, 72.05, 71.65 (C-5(D)), 71.48, 70.94 (C-4(C)), 70.76 (C-2(D)), 70.25 (2C, C-5(A), C-2(C)), 70.13 (C-2(B)), 69.42 (C-6(D)), 68.79 (C-6(C)), 68.45, 66.15 (C-4(D)), 66.03 (C-6(A)), 61.75 (C-6(B)), 21.00, 20.93, 20.89, 20.84, 20.72; **HR-ESI-TOF/MS** (*m/z*): [M+Na]⁺ calcd. for C₉₀H₁₀₀NaO₂₆, 1619.6401; found, 1619.6366.

Benzyl 4-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-3,6-di-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-3,6-di-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 6)-[4-O-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)]-2,3-di-O-benzyl- α -D-glucopyranoside (25A**)**



According to the general glycosylation procedure **24** (155 mg, 0.100 mmol) and **7** (75 mg, 0.150 mmol). Silica gel column chromatography (0% \rightarrow 50% - EtOAc in n-Heptane) afforded **25** as a mixture with minor inseparable impurities (trehalose and tetrasaccharide). **TLC** (60/40, EtOAc/n-heptane, v/v) R_f =0.75; **HR-ESI-TOF/MS** (m/z): $[M+Na]^+$ calcd. for $C_{109}H_{122}NaO_{34}$, 1997.7715; found, 1997.7699. The mixture was deacetylated after isolation by dissolving the compound in THF/MeOH (1 mL, 1/1, v/v) and subsequent addition of KOtBu (pH 10). The mixture was stirred for 16 hours and neutralized by addition of Dowex H⁺. The suspension was filtered and concentrated *in vacuo*. Silica gel column chromatography (0% \rightarrow 10% - MeOH in DCM) gave **25A** amorphous solid (22 mg, 14% over two steps). **TLC** (10/90 MeOH/DCM v/v%) R_f =0.70; **¹H NMR** (500 MHz, Methanol-*d*₄) δ 7.51 – 7.03 (m, 45H), 5.04 (d, J = 9.9 Hz, 1H), 4.99 (d, J = 11.2 Hz, 1H), 4.94 (d, J = 11.3 Hz, 1H), 4.88– 4.80 (m, 1H), 4.80 – 4.67 (m, 5H), 4.67 – 4.50 (m, 12H), 4.49 – 4.32 (m, 6H), 4.22 (d, J = 2.9 Hz, 1H), 4.15 – 4.05 (m, 2H), 3.99 – 3.95 (m, 1H), 3.94 – 3.80 (m, 4H), 3.79 – 3.34 (m, 22H); **¹³C NMR** (126 MHz, Methanol-*d*₄) δ 139.06, 138.88, 138.31, 138.25, 138.16, 138.13, 138.11, 138.08, 137.29, 104.28, 103.74, 103.51, 102.59, 95.42, 82.09, 81.27, 80.24, 78.91, 77.33, 76.66, 75.88, 75.82, 75.50, 75.35, 75.08, 75.02, 74.47, 74.37, 74.12, 73.51, 73.42, 73.02, 72.94, 72.75, 72.64, 72.52, 72.30, 72.13, 71.82, 70.45, 70.35, 69.66, 69.61, 68.97, 61.43, 60.82; **LR-ESI/MS** (m/z): $[M+Na]^+$ calcd. for $C_{93}H_{106}O_{26}$, 1661.7; found, 1661.9

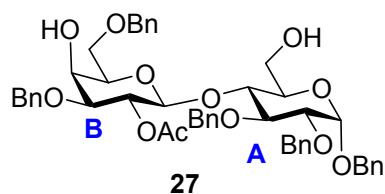
Benzyl 2-O-acetyl-3,6-di-O-benzyl-4-O-levuloyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2,3-di-O-benzyl- α -D-glucopyranoside (26)



To a well stirred emulsion of **19** (240mg, 0.495mmol) in a mixture of CH_2Cl_2 and water (9/1=v:v, 4.5 mL) DDQ (0.169g, 0.743mmol) was added. The resulting solution was stirred for 3 hrs at rt in the dark. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with an aqueous mixture of Ascorbic Acid (1.5 %), citric acid (0.7%) and NaOH (0.9%) twice, once with 10 ml and once with 5 ml. This reduced the left over DDQ. The resulting organic layer was slightly pale yellow. The organic layer was dried (MgSO_4), filtered and the filtrate was concentrated *in vacuo*. The residue was dry loaded on Silica gel column chromatography (20% \rightarrow 50% - EtOAc in heptane) of the residue afforded the product (130mg, 61%) as colourless oil. **TLC** (50/50 EtOAc/n-heptane, v/v) R_f =0.50; **^1H NMR** (500 MHz, CDCl_3) δ 7.42 – 7.16 (m, 25H, CH Ar), 5.57 (d, J = 3.2 Hz, 1H, H(B)-4), 5.08 (dd, J = 9.9, 8.1 Hz, 1H, H(B)-2), 4.96 (d, J = 11.1 Hz, 1H, CHH Benzyl), 4.91 (d, J = 11.1 Hz, 1H, CHH Benzyl), 4.75 (d, J = 3.7 Hz, 1H, H(A)-1), 4.67 (d, J = 8.2 Hz, 1H, H(B)-1), 4.64 (dd, J = 10.2, 3.2 Hz, 3H CHH Benzyl), 4.53 (d, J = 12.4 Hz, 1H, CHH Benzyl), 4.50 (d, J = 12.0 Hz, 1H, CHH Benzyl), 4.37 – 4.26 (m, 2H, CHH Benzyl), 3.98 (t, J = 9.2 Hz, 1H, H(A)-3), 3.76 (t, J = 9.3 Hz, 1H, H(A)-4), 3.67 – 3.58 (m, 5H, H(A)-6AB, H(A)-5), 3.53 (dd, J = 7.6, 5.9 Hz, 1H, H(B)-5), 3.45 – 3.36 (m, 3H, H(B)-3; H(A)-2; H(B)-6A), 3.32 (dd, J = 9.2, 5.4 Hz, 1H, H(B)-6B), 2.83 – 2.75 (m, 1H, CHH Lev), 2.74 – 2.65 (m, 1H, CHH Lev), 2.64 – 2.54 (m, 2H, CHH Lev), 2.12 (s, 3H, CH_3 Lev), 2.01 (s, 3H, CH_3 Acetyl); **^{13}C NMR** (126 MHz, Chloroform-d) δ 206.34, 172.11, 169.70, 139.50, 138.22, 137.94, 137.78, 137.18, 128.54, 128.46, 128.45, 128.43, 128.27, 128.19, 128.06, 128.01, 127.91, 127.85, 127.83, 127.30, 127.28, 101.30 (C-1(B)), 95.79 (C-1(A)), 80.01 (C-3(A)), 79.72 (C-2(A)), 77.12 (C-3(B)), 76.97 (C-4(A)), 75.03, 73.67, 73.29, 72.16 (C-5(A)), 71.63 (C-2(B)), 71.04, 70.74 (C-5(B)), 69.54, 67.44 (C-6(B)), 66.10 (C-4(B)), 60.82 (C-6(A)), 38.19, 29.86, 28.17, 21.08. **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{54}\text{H}_{60}\text{NaO}_{14}$, 955.3881; found, 955.3850.

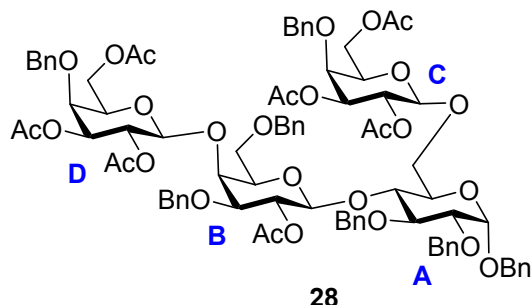
Benzyl

2-*O*-acetyl-3,6-di-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzyl- α -D-glucopyranoside (27)



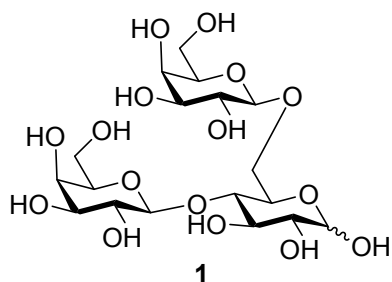
To a solution of **26** (500 mg, 0.536 mmol) in a mixture of toluene/ethanol (10.0 mL, 2/1, v/v), hydrazine acetate (99 mg, 1.07 mmol) was added. The reaction was stirred at rt until completion and quenched with acetone. The resulting mixture was diluted with ethyl acetate (50 mL) and washed with sat. aqueous NaHCO₃ (30 mL), brine (30 mL), dried (Na₂SO₄) and filtered. Silica column chromatography (0% – 60% EtOAc in n-heptane) afforded **24** (400 mg, 89%) as an amorphous solid. **TLC** (60/40 EtOAc/n-heptane, v/v) *R*_f=0.21; **¹H NMR** (500 MHz, Chloroform-*d*) δ 7.44 – 7.10 (m, 25H, CH Ar), 5.17 (dd, *J* = 9.8, 8.0 Hz, 1H, H-2(B)), 4.97 (d, *J* = 11.1 Hz, 1H), 4.88 (d, *J* = 11.1 Hz, 1H), 4.75 (d, *J* = 3.6 Hz, 1H, (H-1(A))), 4.70 – 4.60 (m, 4H, H-1(B))), 4.56 – 4.44 (m, 3H), 4.39 (d, *J* = 11.9 Hz, 1H), 4.34 (d, *J* = 11.9 Hz, 1H), 4.04 (d, *J* = 3.2 Hz, 1H, H-4(B)), 3.98 (t, *J* = 9.2 Hz, 1H, (H-3(B))), 3.75 (dd, *J* = 9.9, 8.8 Hz, 1H, H-4(A)), 3.71 – 3.55 (m, 4H, H-6A(B), H-6A(A), H-6B(A), H-5(B)), 3.48 – 3.28 (m, 4H, H-6B(B), H-2(A), H-3(B), H-5(A)), 2.44 (s, 1H, 4-OH(B)), 2.01 (s, 3H), 1.63 (broad doublet, 1H, 6-OH(A)); **¹³C NMR** (126 MHz, Chloroform-*d*) δ 169.83, 139.63, 138.30, 138.24, 137.56, 137.24, 128.67, 128.55, 128.49, 128.46, 128.23, 128.16, 128.06, 128.04, 127.86, 127.84, 127.77, 127.49, 127.20, 101.20 (C-1(B)), 95.85 (C-1(A)), 80.06 (C-3(A)), 79.76 (C-2(A)), 79.12 (C-3(B)), 75.09, 73.69, 73.32 (C-5(A)), 73.25, 71.64 (C-2(B)), 71.37, 70.85 (C-5(B)), 69.55, 68.41 (C-6(B)), 65.55 (C-4(B)), 60.95 (C-6(A)), 21.15; **HR-ESI-TOF/MS** (*m/z*): [M+Na]⁺ calcd. for C₄₉H₅₄NaO₁₂, 857.3513; found, 857.3493.

Benzyl 2-*O*-Acetyl-3,6-di-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4) -2,3,6-tri-*O*-acetyl-4-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-[2-*O*-Acetyl-3,6-di-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 6)]-2,3-di-*O*-benzyl- α -D-glucopyranoside (28)



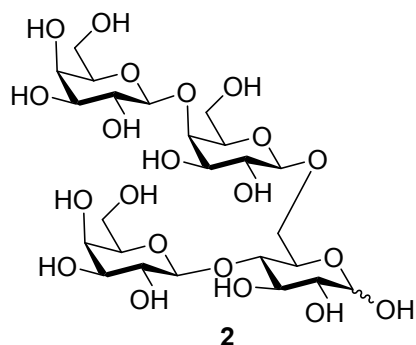
According to the general glycosylation procedure **27** (350 mg, 0.419 mmol) and **6** (526 mg, 1.05 mmol). Silica gel column chromatography (10% \rightarrow 50% - EtOAc in n-Heptane) afforded **28** (550 mg, 82%) as a colourless oil. **TLC** (40/60 EtOAc/n-heptane, v/v) R_f =0.38; **$^1\text{H NMR}$** (500 MHz, Chloroform-*d*) δ 7.51 – 7.45 (m, 2H), 7.44 – 7.41 (m, 2H), 7.39 – 7.14 (m, 30H), 6.95 (tt, J = 7.6, 1.3 Hz, 1H), 5.42 (ddd, J = 14.9, 10.4, 7.9 Hz, 2H, H-2(D), H-2(C)), 5.11 (dd, J = 10.0, 7.8 Hz, 1H, H-2(B)), 5.01 – 4.91 (m, 3H, H-3(C), H-3(D)), 4.86 – 4.45 (m, 14H, H-1(A), H-1(C), H-1(D), 11 \times CHHPh), 4.33 (d, J = 7.8 Hz, 1H, H-1(B)), 4.29 – 4.01 (m, 7H, H-6A(C), H-6B(C), H-6A(D), H-6B(D), H-4(B), 2 \times CHHPh), 3.99 – 3.87 (m, 4H, H-6A(A), H-4(C), H-4(D), H-3(A)), 3.77 (ddd, J = 10.3, 5.7, 1.9 Hz, 1H, H-5(A)), 3.74 – 3.67 (m, 1H, H-5(D)), 3.66 – 3.57 (m, 2H, H-5(C), H-6B(A)), 3.56 – 3.31 (m, 5H, H-6A(B), H-4(A), H-3(B), H-2(A), H-5(B)), 3.18 (dd, J = 10.1, 5.4 Hz, 1H, H-6B(B)), 2.14 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.95 (s, 2H), 1.92 (s, 3H); **$^{13}\text{C NMR}$** (126 MHz, Chloroform-*d*) δ 170.58, 170.45, 170.35, 170.34, 169.85, 169.40, 169.32, 139.17, 138.60, 138.49, 137.84, 137.63, 137.42, 137.19, 128.63, 128.57, 128.54, 128.51, 128.49, 128.45, 128.40, 128.36, 128.33, 128.31, 128.26, 128.21, 128.14, 128.07, 128.04, 127.98, 127.93, 127.86, 127.81, 127.75, 127.71, 127.59, 127.53, 127.39, 127.32, 127.02, 101.11 (C-1(D)) , 100.86 (2C, C-1(B), C-1(C)), 94.95 (C-1(A)), 80.13 (C-3(D)), 79.20 (C-3(A)), 78.97(C-2(A)), 77.99 (C-4(A)), 75.68, 75.36, 75.06, 74.97, 74.93-73.67 (5C, C-3(C), C-3(D), C-4(D), C-4(C), C-5(B)), 73.35, 73.33, 72.19, 72.16 (C-5(D)), 71.77(2C, C-2(B), C-5(C)), 71.30 (C-4(B)), 70.48 (C-5(A)), 69.38 (C-2(D)), 69.08 (C-2(C)), 68.71 (C-6(B)), 68.37, 66.40 (C-6(A)), 62.19-62.13 (2C, C-6(D), C-6(C)), 29.57(Acetone), 20.88, 20.82, 20.79, 20.77, 20.75; **HR-ESI-TOF/MS** (m/z): $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{97}\text{H}_{106}\text{NaO}_{26}$, 1709.6870; found, 1709.6936.

β -D-galactopyranosyl-(1 \rightarrow 4)-[β -D-galactopyranosyl-(1 \rightarrow 6)]-D-glucopyranose (1)



22 (80 mg, 0.066 mmol) was deacetylated by dissolving the compound in THF/MeOH (1 mL, 1/1, v/v) and subsequent addition of KOtBu (pH 10). The mixture was stirred for 16 hours and neutralized by addition of Dowex H⁺. The suspension was filtered and concentrated *in vacuo* and hydrogenated with H₂ over Pd/C in H₂O/tBuOH/MeOH (2.5 mL, 1/1/0.5, v/v/v) for 48 hours. The mixture was filtered over Celite and rinsed with water. The resulting solution was freeze dried to obtain **1** (24 mg, 61% over two steps); **¹H NMR** (500 MHz, Deuterium Oxide) δ 5.23 (dd, *J* = 3.9, 1.5 Hz, 1H), 4.69 (dd, *J* = 8.0, 1.5 Hz, 1H), 4.52 (dd, *J* = 7.9, 1.5 Hz, 2H), 4.45 (ddd, *J* = 7.3, 5.5, 1.5 Hz, 2H), 4.30 (dt, *J* = 11.2, 1.6 Hz, 1H), 4.23 (dt, *J* = 11.3, 1.8 Hz, 1H), 4.12 – 4.03 (m, 1H), 4.02 – 3.88 (m, 7H), 3.88 – 3.49 (m, 27H), 3.31 (ddd, *J* = 9.5, 8.0, 1.5 Hz, 1H). **¹³C NMR** (126 MHz, Deuterium Oxide) δ 103.12, 103.10, 102.81, 102.77, 95.90, 91.88, 77.79, 77.77, 75.23, 75.10, 75.07, 74.27, 73.70, 73.57, 72.65, 72.47, 71.34, 71.11, 70.92, 70.61, 68.96, 68.65, 68.59, 68.56, 67.48, 67.42, 61.07, 61.05, 60.98; **HR-ESI-TOF/MS** (*m/z*): [M+Na]⁺ calcd. for C₁₈H₃₂NaO₁₆, 527.15880; found, 527.15776.

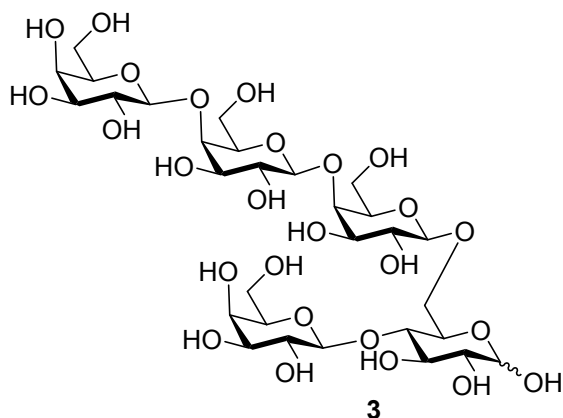
β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 6)-[β -D-galactopyranosyl-(1 \rightarrow 4)]-D-glucopyranose (2)



24 (50 mg, 0.031 mmol) was deacetylated by dissolving the compound in THF/MeOH (1 mL, 1/1, v/v) and subsequent addition of KOtBu (pH 10). The mixture was stirred for 16 hours and neutralized by addition of Dowex H⁺. The suspension was filtered and concentrated *in vacuo* and hydrogenated with H₂ over Pd/C in H₂O/tBuOH/MeOH (2.5 mL, 1/1/0.5, v/v/v) for 48 hours. The mixture was filtered

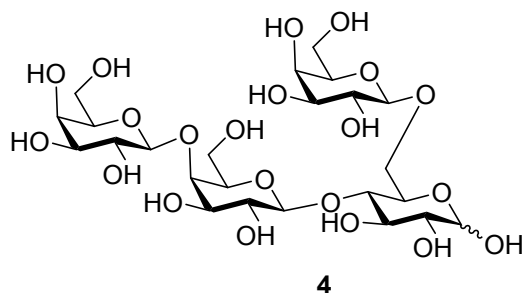
over Celite and rinsed with water. The resulting solution was freeze dried to obtain **2** (10 mg, 48% over two steps). **¹H NMR** (500 MHz, Deuterium Oxide) δ 5.23 (d, J = 3.5 Hz, 1H), 4.69 (d, J = 8.0 Hz, 2H), 4.60 (d, J = 7.8 Hz, 3H), 4.55 – 4.45 (m, 5H), 4.28 (d, J = 11.2 Hz, 2H), 4.21 (d, J = 17.3 Hz, 4H), 4.09 (d, J = 8.4 Hz, 1H), 4.01 – 3.50 (m, 59H), 3.31 (t, J = 8.7 Hz, 2H); **¹³C NMR** (126 MHz, Deuterium Oxide) δ 104.75, 103.64, 103.56, 103.35, 103.32, 96.41, 92.40, 78.44, 78.41, 77.65, 77.60, 75.77, 75.62, 74.78, 74.76, 74.74, 74.23, 74.11, 73.60, 73.26, 73.01, 71.90, 71.62, 71.44, 69.51, 69.13, 69.09, 68.14, 61.60, 61.57, 61.51, 61.04; **HR-ESI-TOF/MS** (m/z): $[M+Na]^+$ calcd. for $C_{24}H_{42}NaO_{21}$, 689.2116; found, 689.2096.

β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 6)-[β -D-galactopyranosyl-(1 \rightarrow 4)]-D-glucopyranose (3**)**



25A (22 mg, 0.031 mmol) hydrogenated with H_2 over Pd/C in $H_2O/tBuOH/MeOH$ (2.5 mL, 1/1/0.5, v/v/v) for 48 hours. The mixture was filtered over Celite and rinsed with water. The resulting solution was freeze dried to obtain **3** (10 mg, 90%). **¹H NMR** (500 MHz, Deuterium Oxide) δ 5.08 (d, J = 3.6 Hz, 1H), 4.55 – 4.52 (m, 3H), 4.50 (d, J = 7.9 Hz, 4H), 4.47 – 4.42 (m, 4H), 4.40 – 4.31 (m, 8H), 4.13 (d, J = 11.2 Hz, 3H), 4.04 (d, J = 9.7 Hz, 9H), 3.97 – 3.37 (m, 114H), 3.20 – 3.12 (m, 2H). **¹³C NMR** (126 MHz, Deuterium Oxide) δ 104.65, 104.63, 104.58, 103.38, 103.33, 103.11, 103.08, 96.19, 92.18, 78.16, 78.14, 77.97, 77.89, 77.40, 75.56, 75.45, 74.73, 74.64, 74.61, 74.56, 74.01, 73.88, 73.52, 73.46, 73.03, 72.77, 72.10, 71.65, 71.40, 71.21, 69.27, 67.93, 61.38, 61.36, 61.28, 61.01, 60.85; **HR-ESI-TOF/MS** (m/z): $[M+Na]^+$ calcd. for $C_{30}H_{52}NaO_{26}$, 851.2644; found, 851.2647.

β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)-[- β -D-galactopyranosyl-(1 \rightarrow 6)]-D-glucopyranose (4**)**

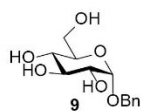


28 (100 mg, 0.031 mmol) was deacetylated by dissolving the compound in THF/MeOH (1 mL, 1/1, v/v) and subsequent addition of KOtBu (pH 10). The mixture was stirred for 16 hours and neutralized by addition of Dowex H⁺. The suspension was filtered and concentrated *in vacuo* and hydrogenated with H₂ over Pd/C in H₂O/tBuOH/MeOH (2.5 mL, 1/1/0.5, v/v/v) for 48 hours. The mixture was filtered over Celite and rinsed with water. The resulting solution was freeze dried. As incomplete conversion was observed by ¹H NMR, multiple hydrogenation cycles were performed without success, showing that the aromatic impurity was not attached to the glycan. Finally, the product was purified by C18 silica column purification to remove the aromatic impurities obtaining **4** (11 mg, 26% over two steps, see NMR-spectrum for purity). **¹H NMR** (500 MHz, Deuterium Oxide) δ 5.24 (d, J = 3.8 Hz, 1H), 4.69 (d, J = 8.0 Hz, 1H), 4.65 (d, J = 7.9 Hz, 4H), 4.64 – 4.59 (m, 9H), 4.56 (d, J = 7.9 Hz, 2H), 4.46 (dd, J = 7.8, 2.7 Hz, 8H), 4.31 (dd, J = 11.3, 1.8 Hz, 1H), 4.28 – 4.03 (m, 34H), 4.02 – 3.53 (m, 126H), 3.31 (dd, J = 9.5, 8.0 Hz, 1H); **¹³C NMR** (126 MHz, Deuterium Oxide, before C18 purification) δ 104.21, 104.17, 103.42, 103.31, 103.07, 103.04, 95.89, 91.86, 80.96, 78.63, 77.74, 77.15, 75.15, 75.10, 75.08, 74.50, 74.36, 74.25, 74.19, 73.66, 73.55, 72.98, 72.90, 72.78, 72.65, 72.60, 72.32, 72.16, 71.67, 71.50, 71.41, 71.35, 71.32, 71.06, 70.78, 70.59, 70.39, 69.51, 69.24, 68.93, 68.64, 68.58, 61.01, 60.96, 60.76, 60.71, 60.53. **HR-ESI-TOF/MS** (*m/z*): [M+Na]⁺ calcd. for C₂₄H₄₂NaO₂₁, 689.21163; found, 689.21066.

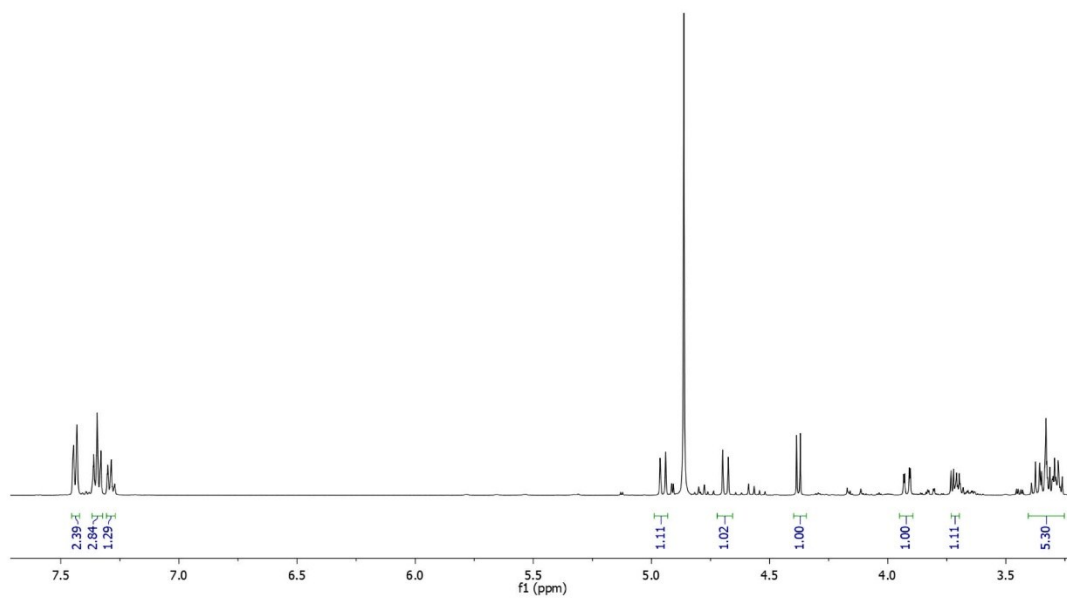
Detailed Characterization of deprotected oligosaccharides **1-3**

Compound 1			Compound 2			Compound 3		
	¹ H	¹³ C		¹ H	¹³ C		¹ H	¹³ C
Aα-1	5.22	91.9		5.07	92.4		5.07	92.2
Aα-2	3.58	71.1		3.44	73.3		3.44	71.4
Aα-3	3.82	71.3		3.63	71.9		3.69	71.7
Aα-4	3.8	77.8		3.67	78.4		3.65	78.2
Aα-5	4.08	69.0		3.93	69.5		3.92	69.3
Aα-6a	4.21	67.5		4.05	68.1		4.06	67.9
Aα-6b	3.96	67.5		3.82			3.81	67.9
Aβ-1	4.68	95.9		4.51	96.4		4.53	96.2
Aβ-2	3.31	73.7		3.14	74.2		3.16	74.0
Aβ-3	3.63	74.3		3.47	74.7		3.48	74.6
Aβ-4	3.77	77.8		3.6	78.4		3.61	77.9
Aβ-5	3.74	73.5		3.6	74.1		3.88	77.4
Aβ-6a	4.28	67.4		4.1	68.1		4.12	67.9
Aβ-6b	3.91	67.4		3.75			3.75	67.9
B-1	4.51	102.8		4.34	103.3		4.36	103.1
B-2	3.54	70.9		3.39	71.4		3.38	71.2
B-3	3.66	72.5		3.5	73.3		3.52	72.8
B-4	3.92	68.6		3.76	69.1		3.78	68.9
B-5	3.69	75.1		3.53	75.6		3.54	74.7(d)
B-6a	3.8	61.1		3.68	61.0		3.66	61.4
B-6b	3.75	61.1		3.62			3.62	61.4
C-1	4.45/44	103.1		4.31	103.6		4.33	103.4
C-2	3.54	70.6		3.49	71.6		3.48	71.4
C-3	3.66	72.7		3.59	73.6		3.62	73.4
C-4	3.92	68.7		4.01	77.6(d)		4.03	77.9
C-5	3.69	75.2		3.57	74.8(d)		3.54	74.7
C-6a	3.8	61.0		3.6	61.6 (d)		3.66	61.3
C-6b	3.75	61.0		3.51			3.62	61.3
D-1				4.43	104.8		4.5	104.6
D-2				3.42	71.9		3.52	72.1
D-3				3.74	73.0		3.63	73.5
D-4				3.5	69.1		4.03	77.4
D-5				3.53	75.8		3.54	75.4
D-6a				3.6	61.5		3.66	60.8
D-6b				3.6			3.62	60.8
E-1							4.45	104.6
E-2							3.43	71.4
E-3							3.51	73.0
E-4							3.76	68.9
E-5							3.54	75.6
E-6a							3.66	61.0
E-6b							3.62	61.0

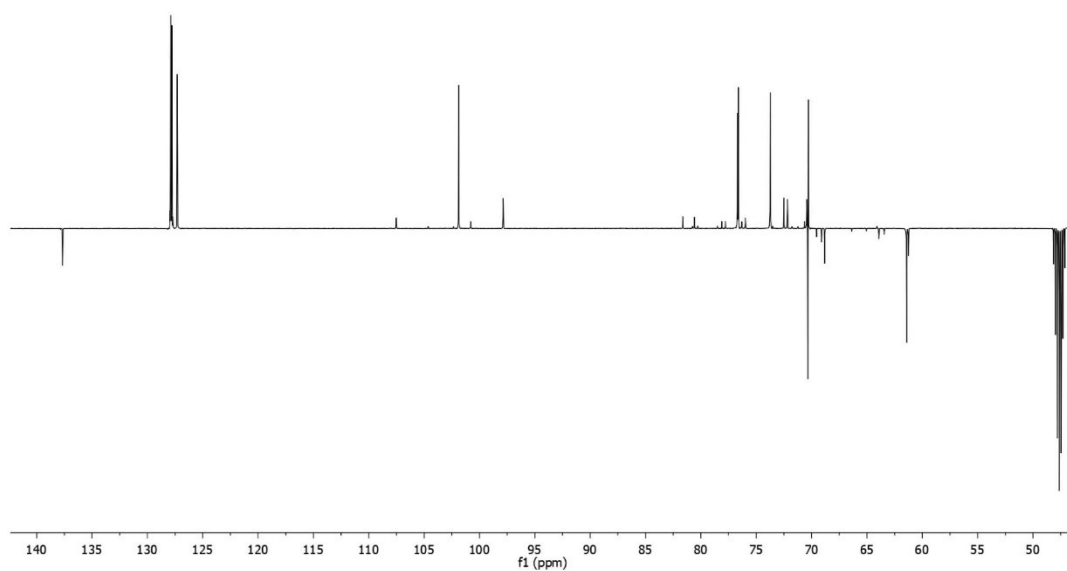
NMR Spectra

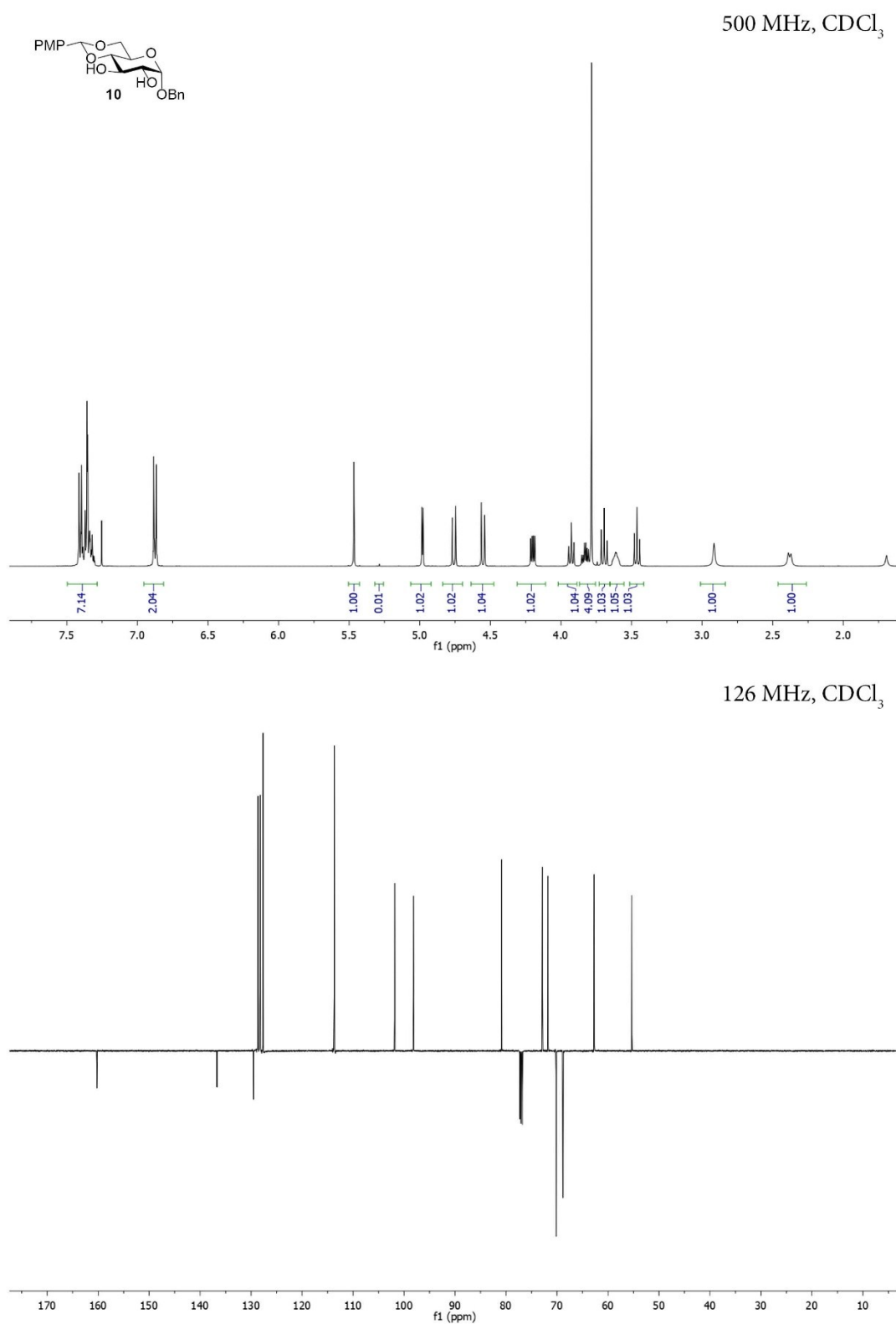


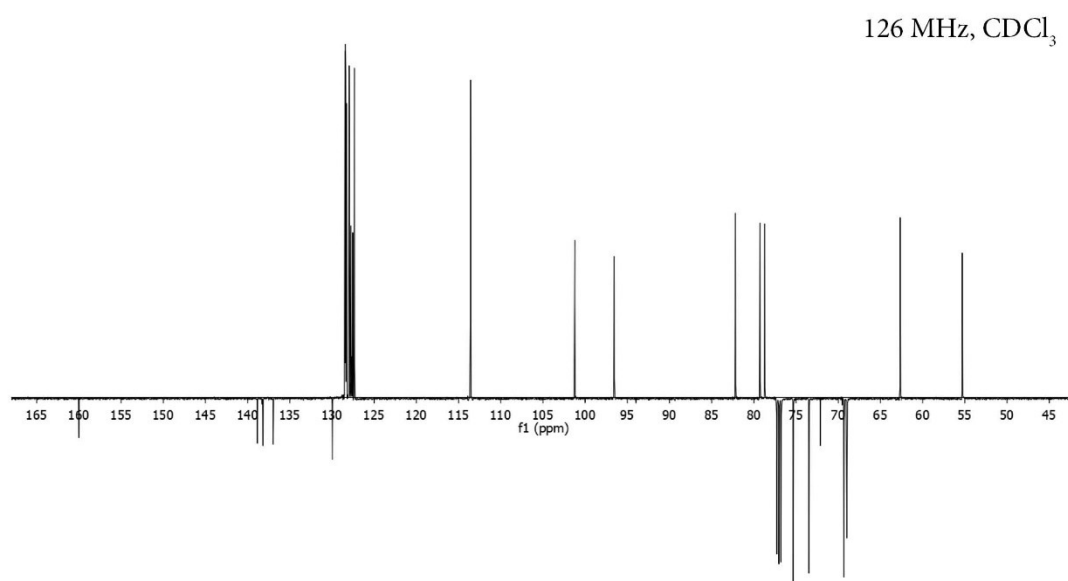
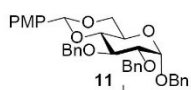
500 MHz, CDCl₃

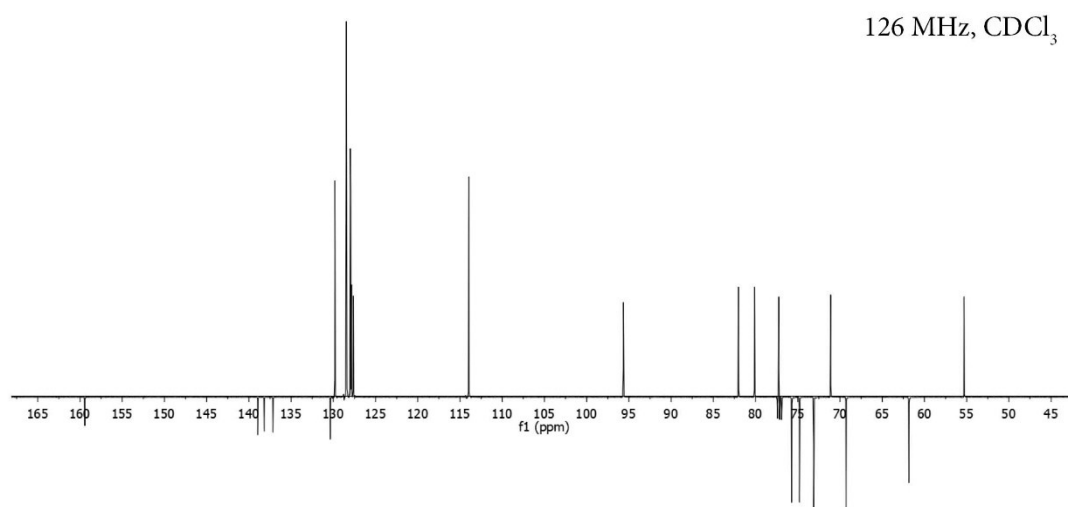
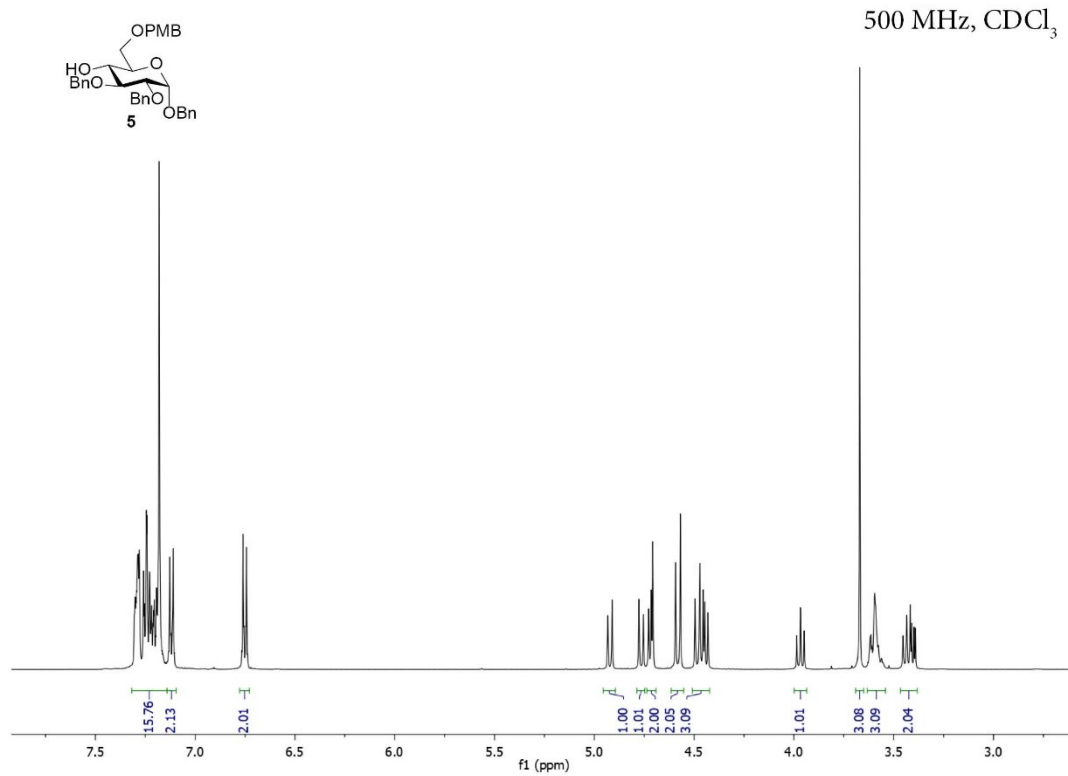


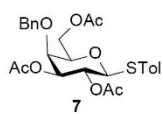
126 MHz, CDCl₃



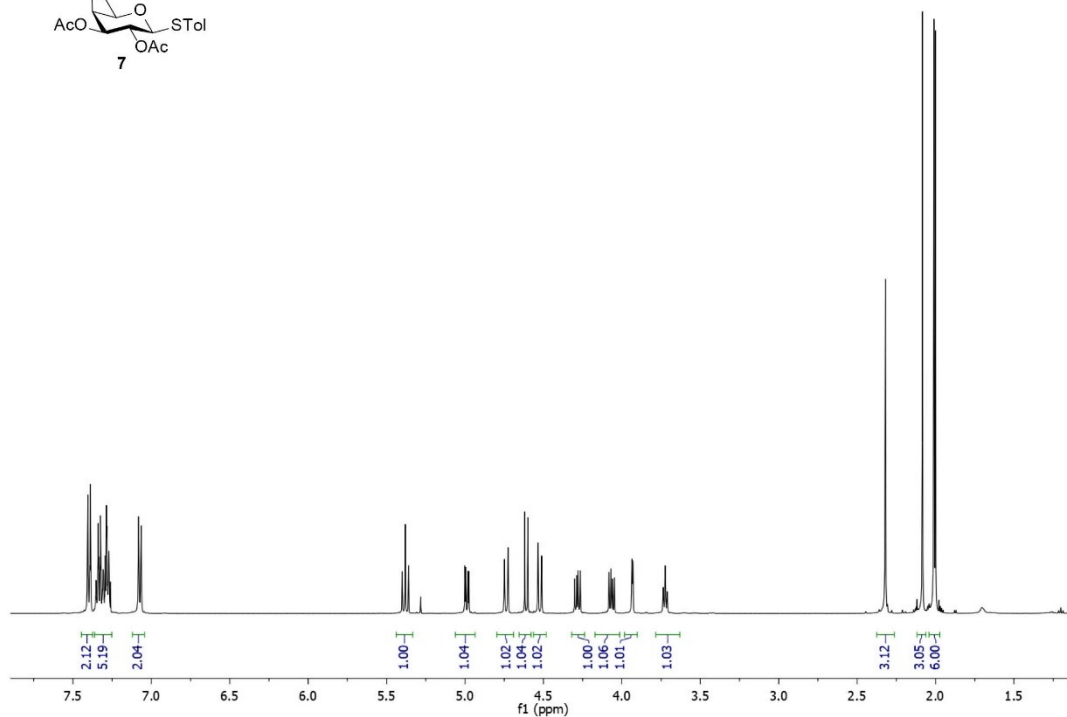




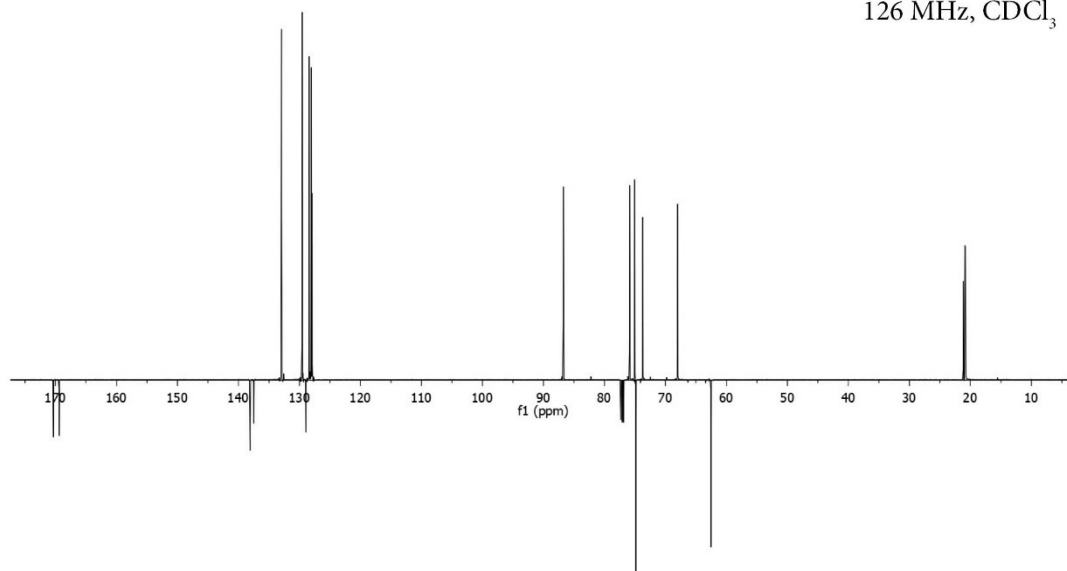


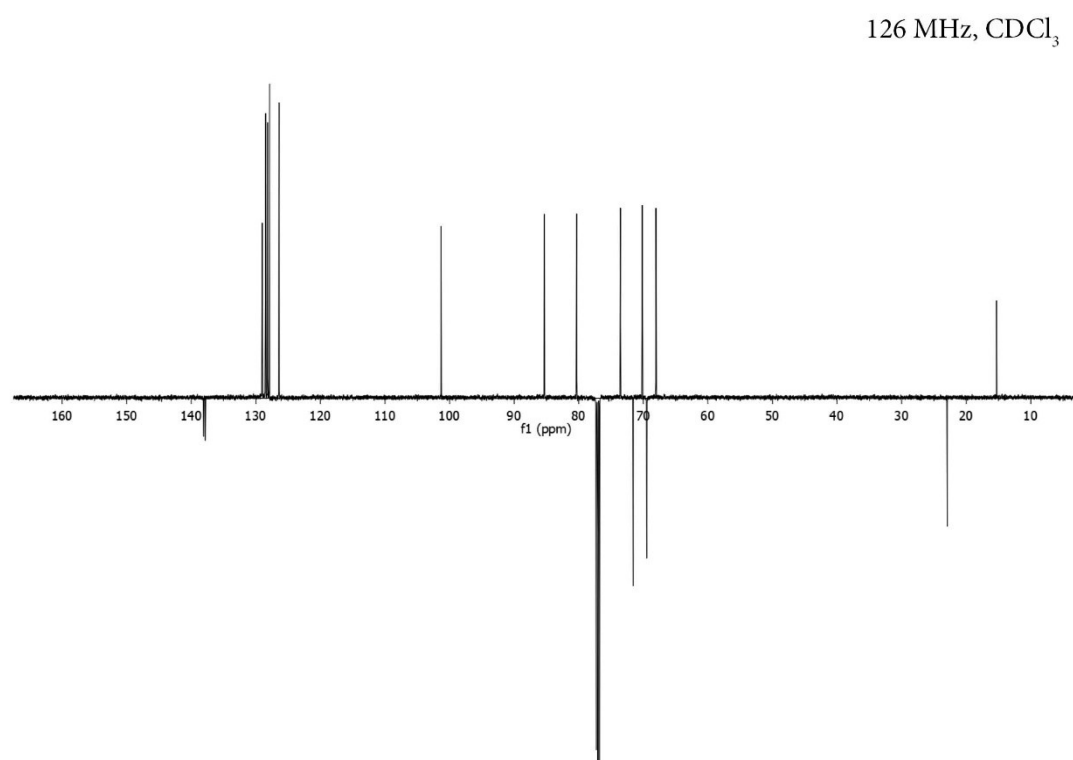
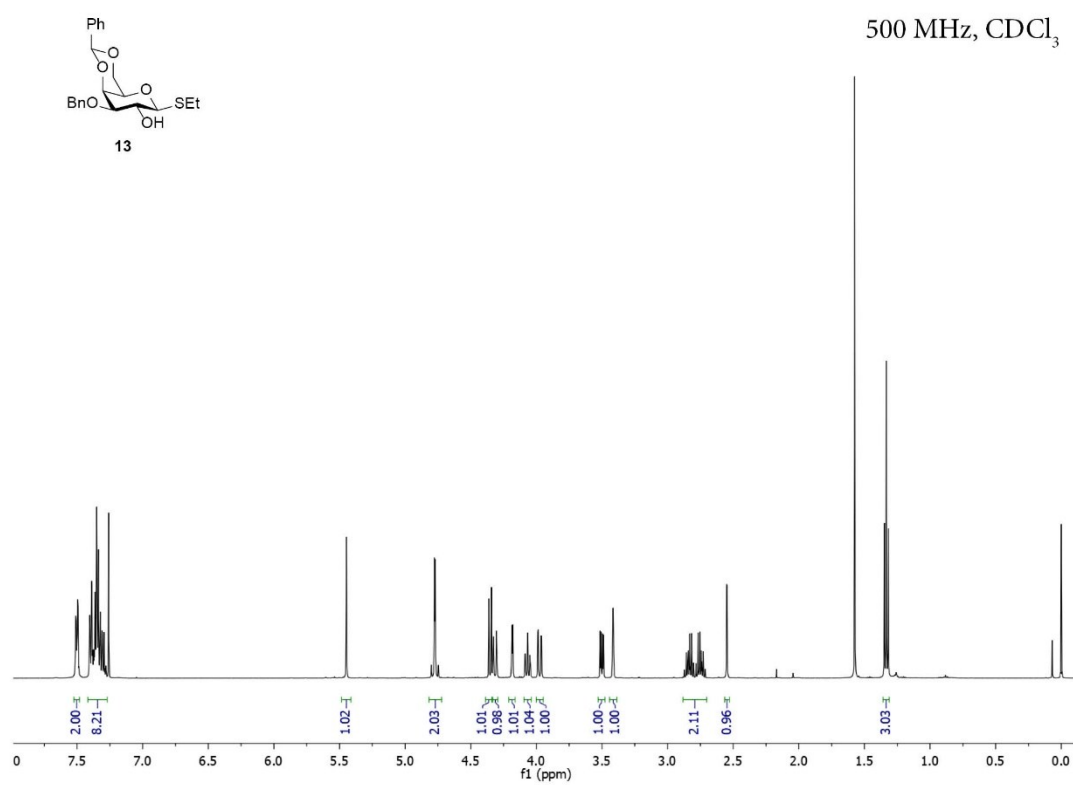


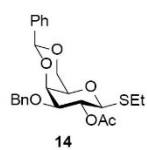
500 MHz, CDCl₃



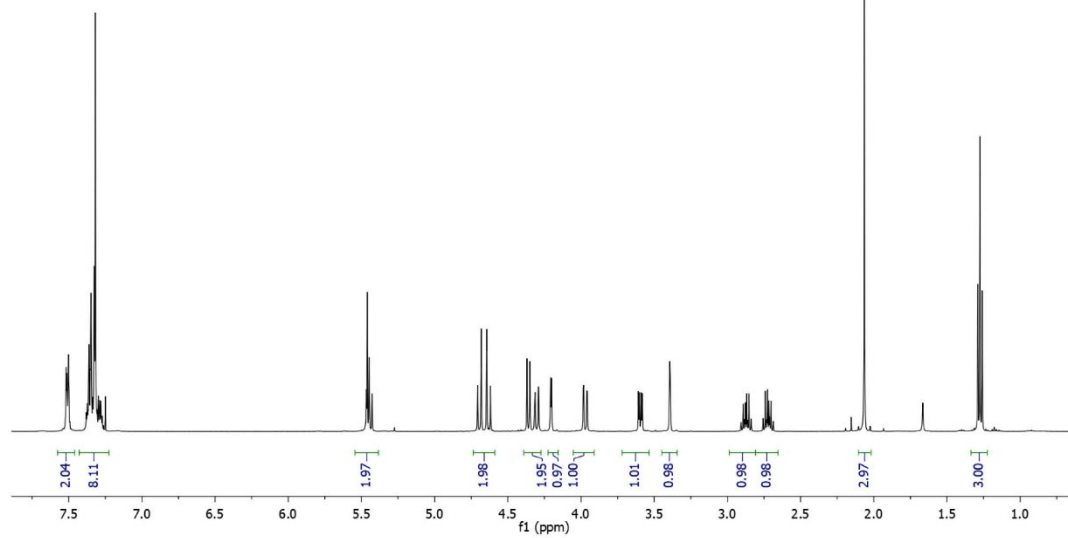
126 MHz, CDCl₃



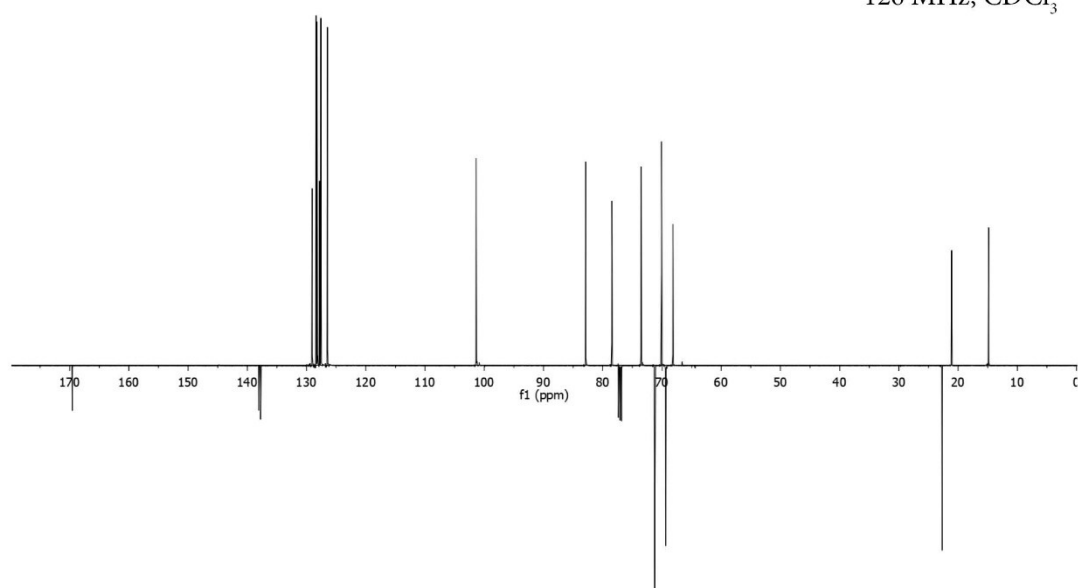


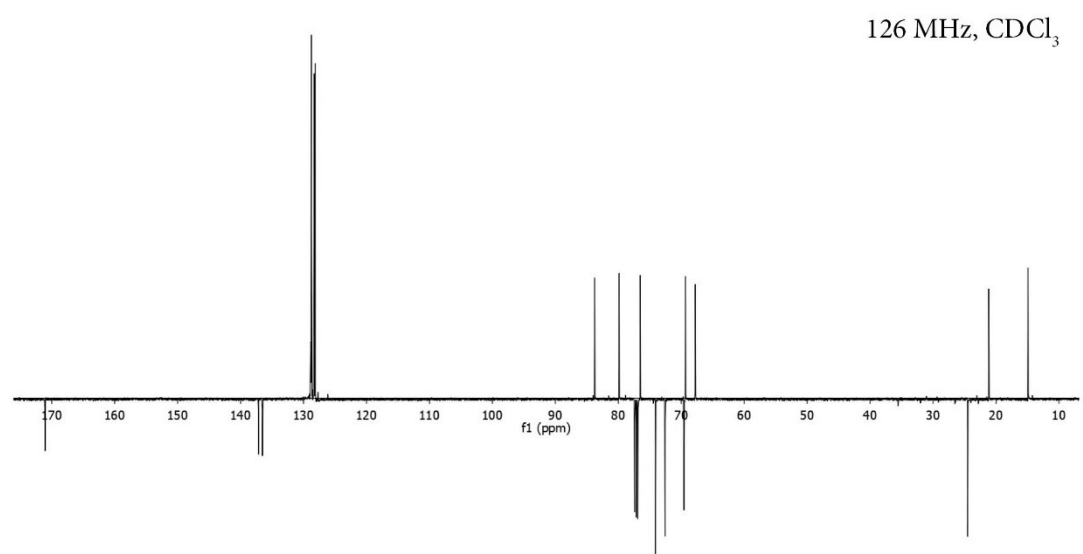
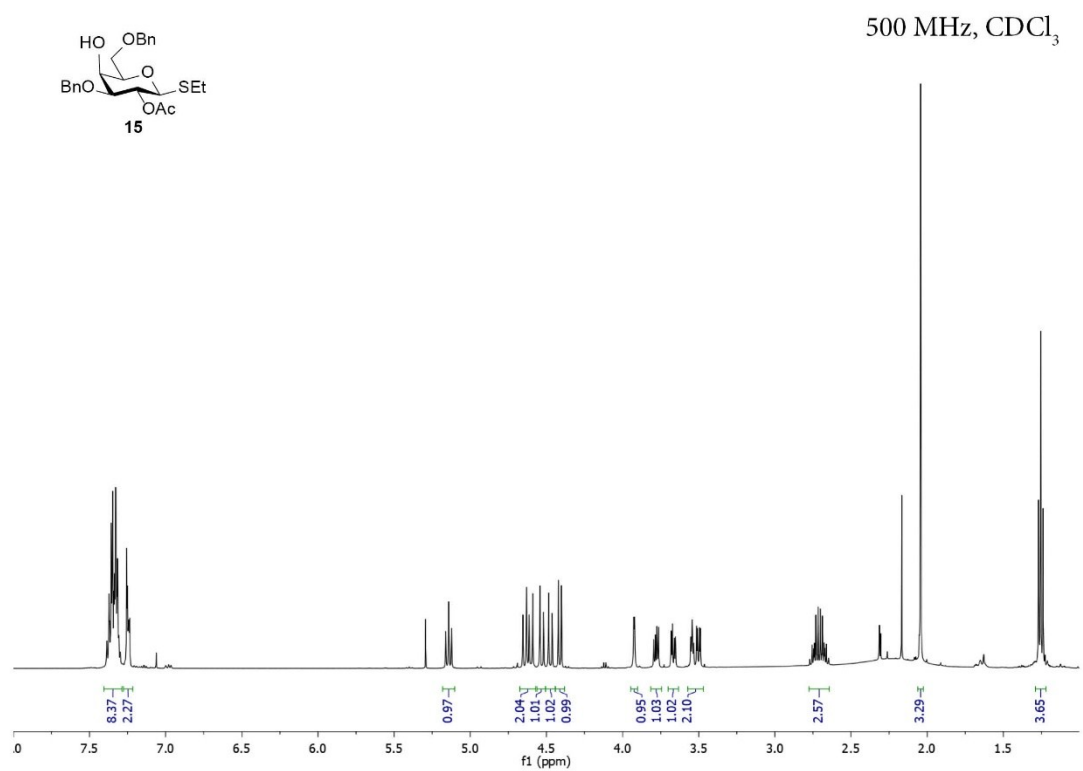


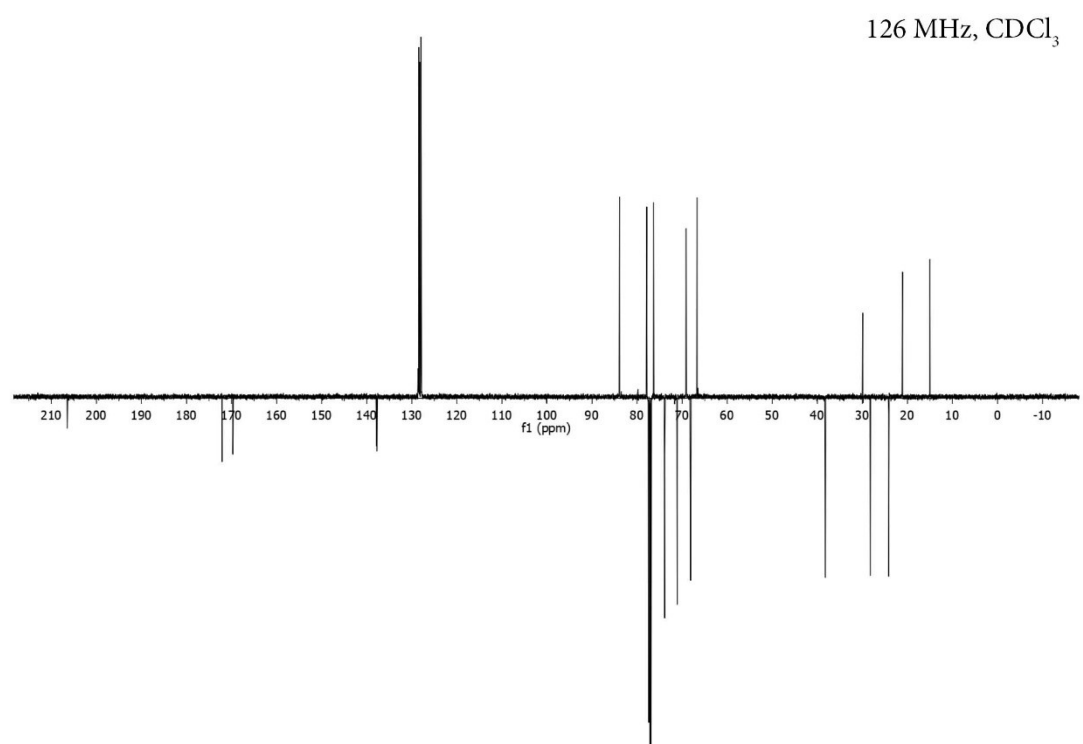
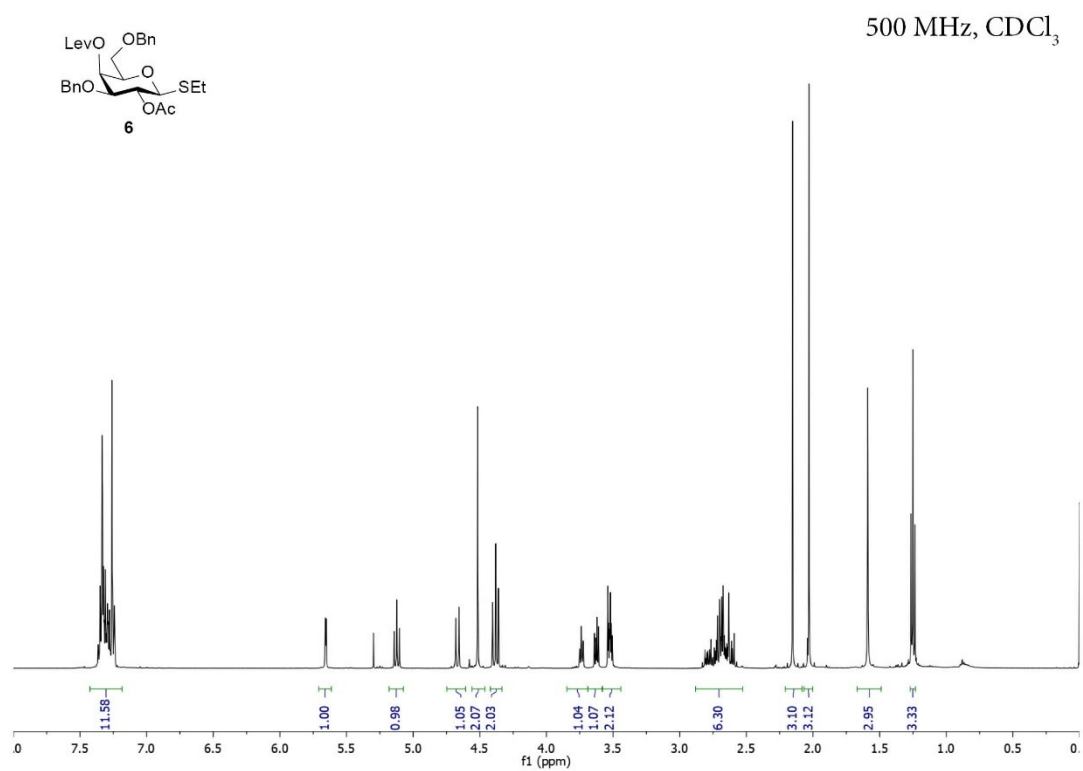
500 MHz, CDCl₃

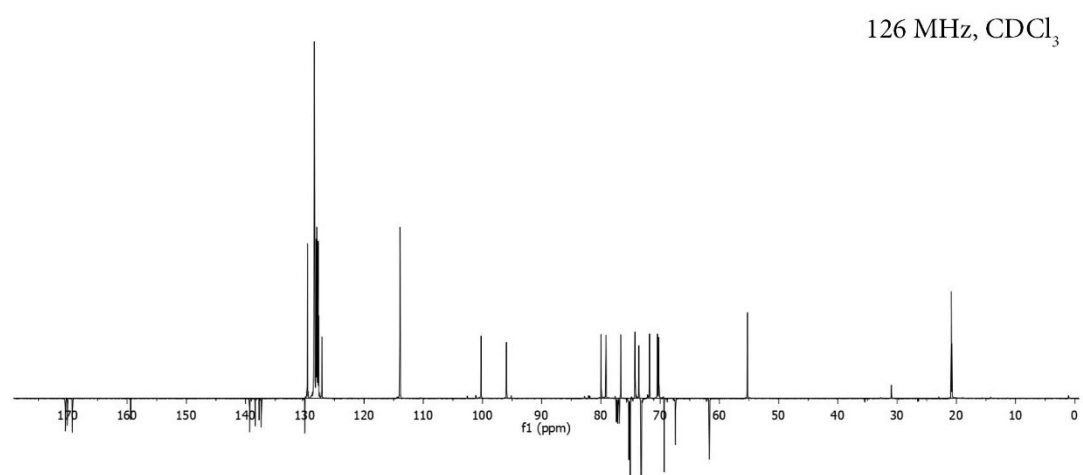
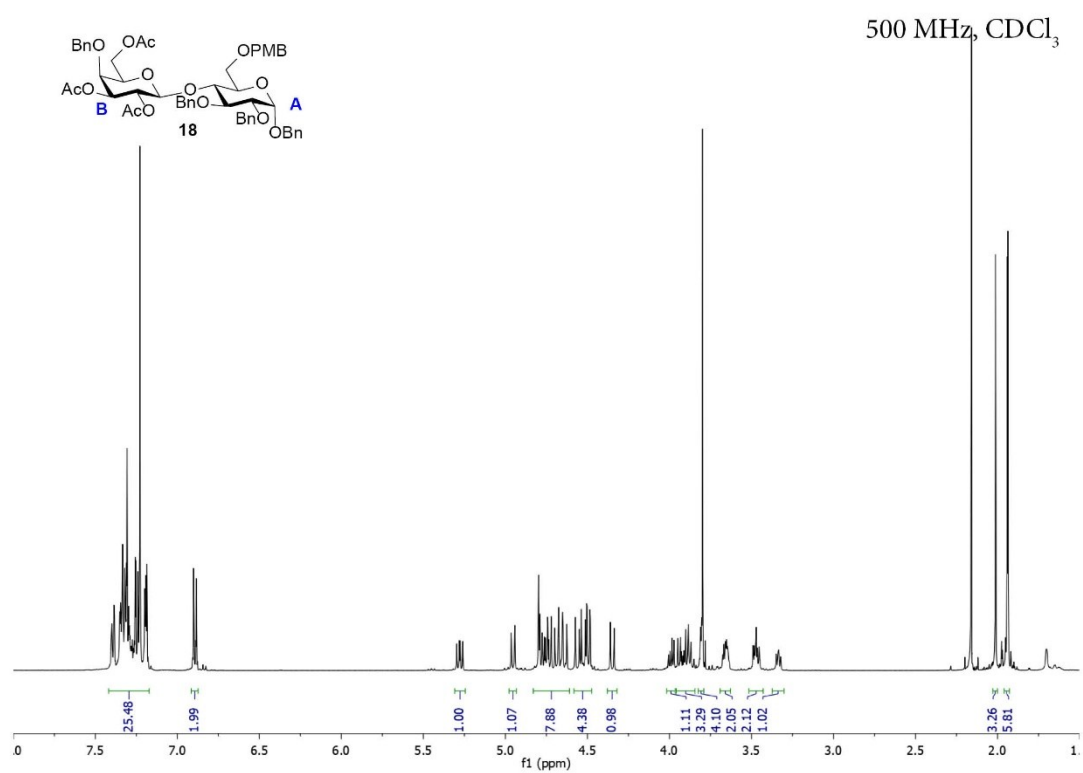


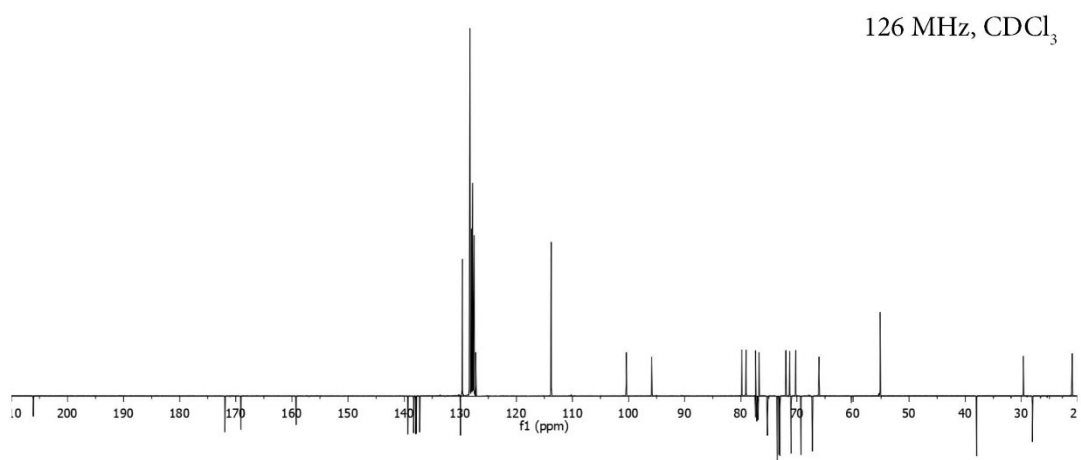
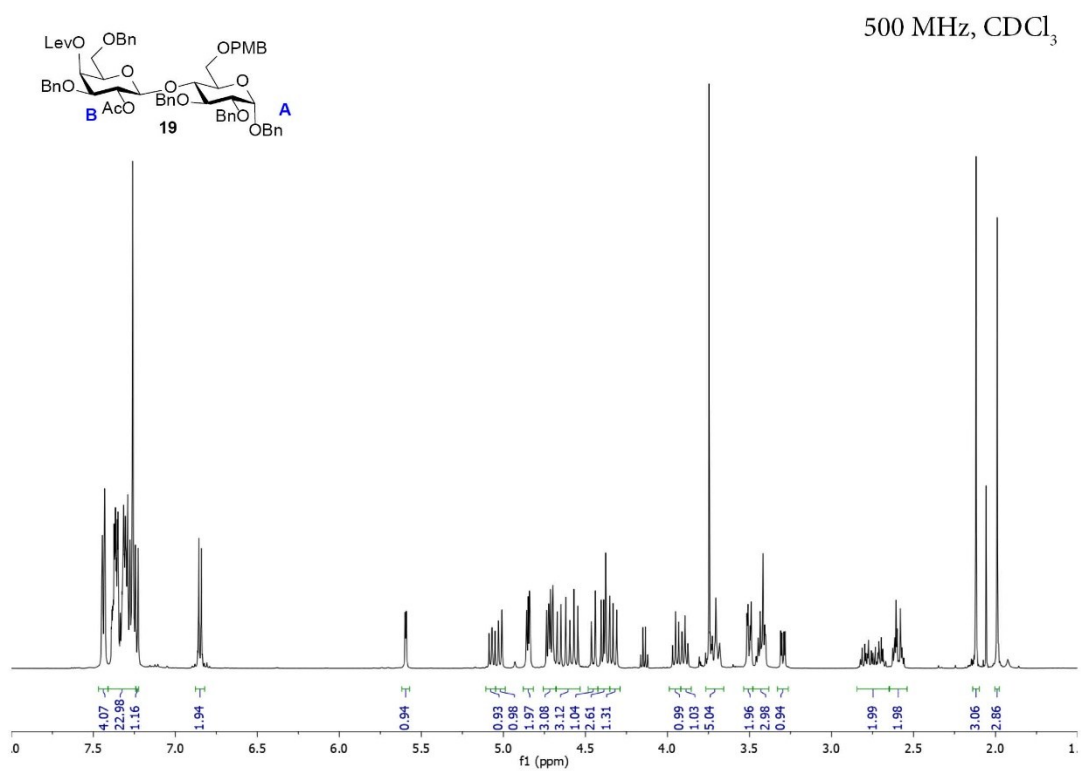
126 MHz, CDCl₃

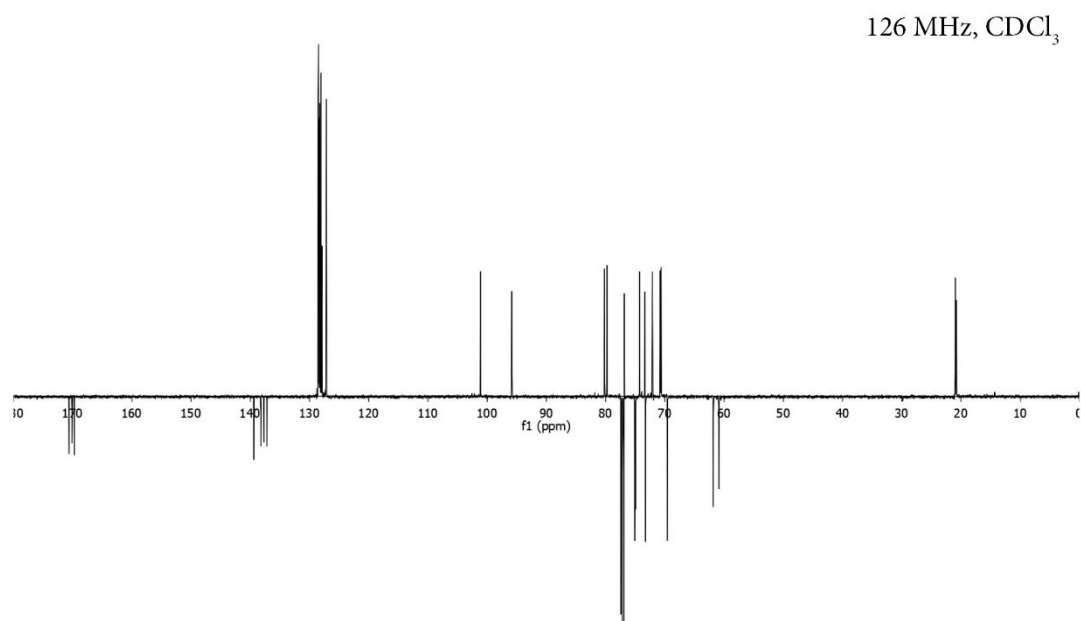
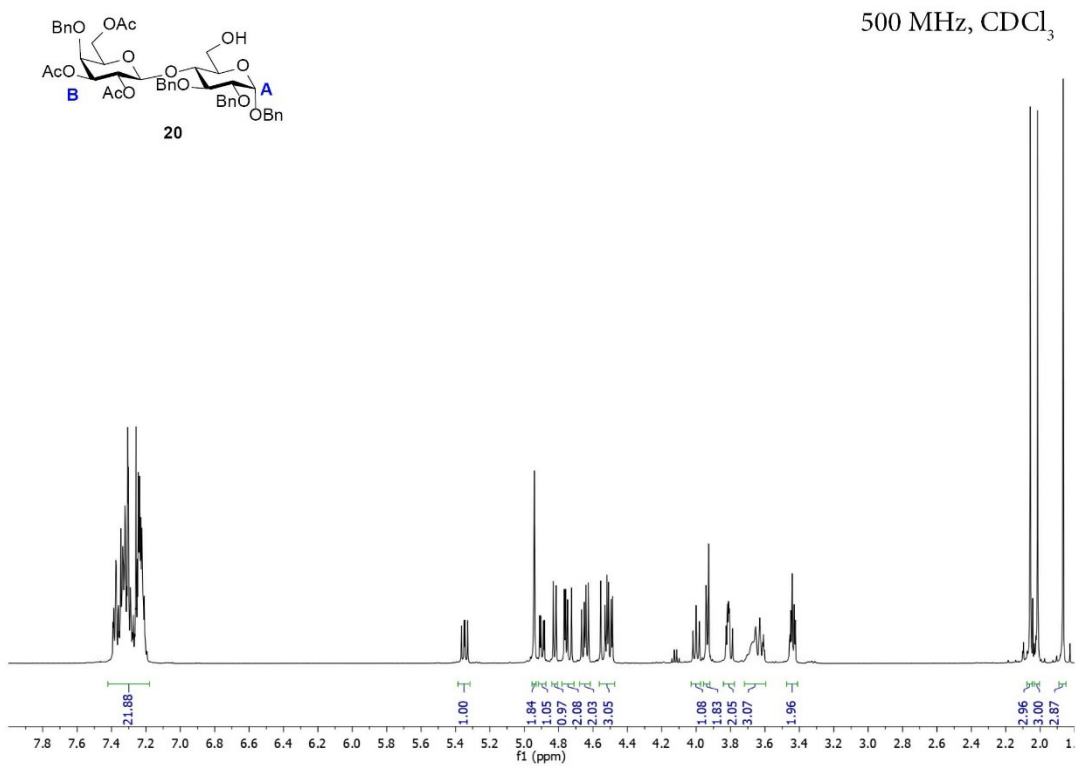


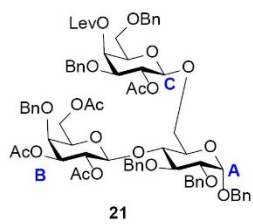




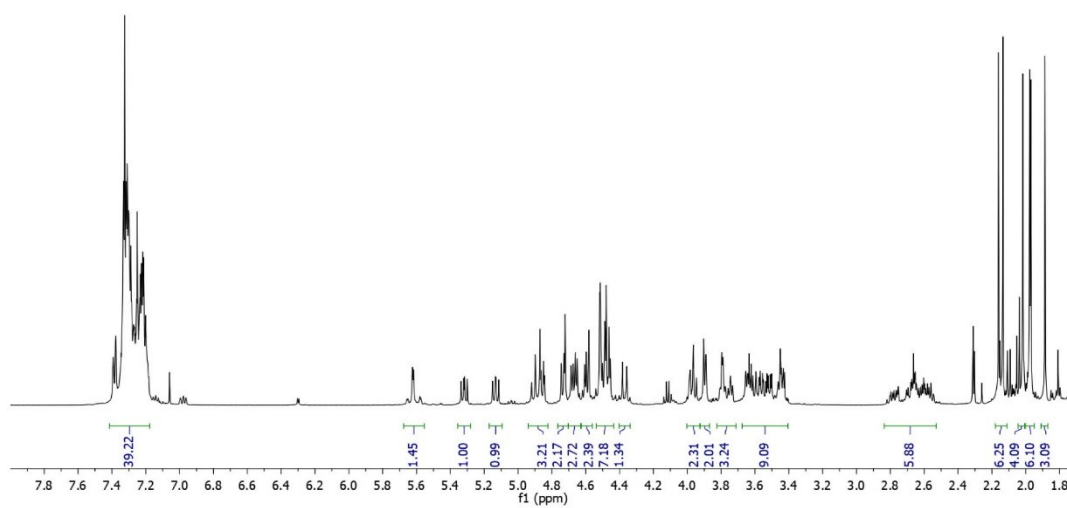




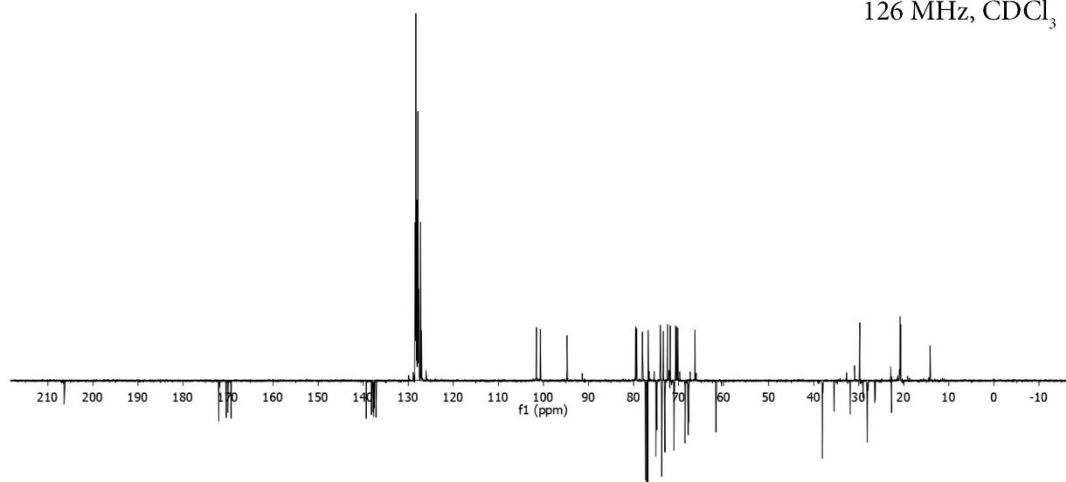


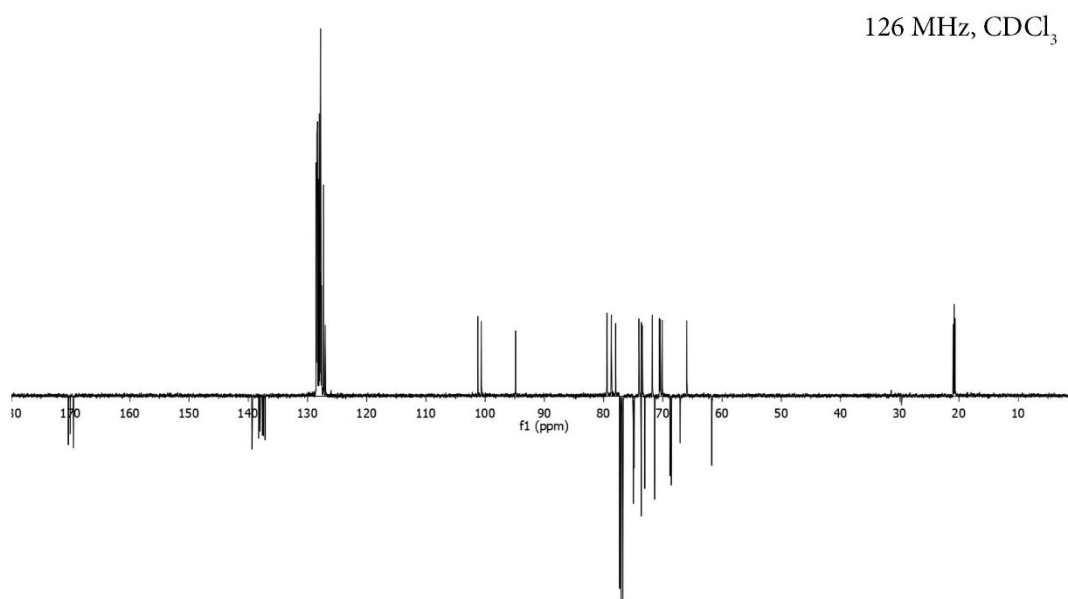
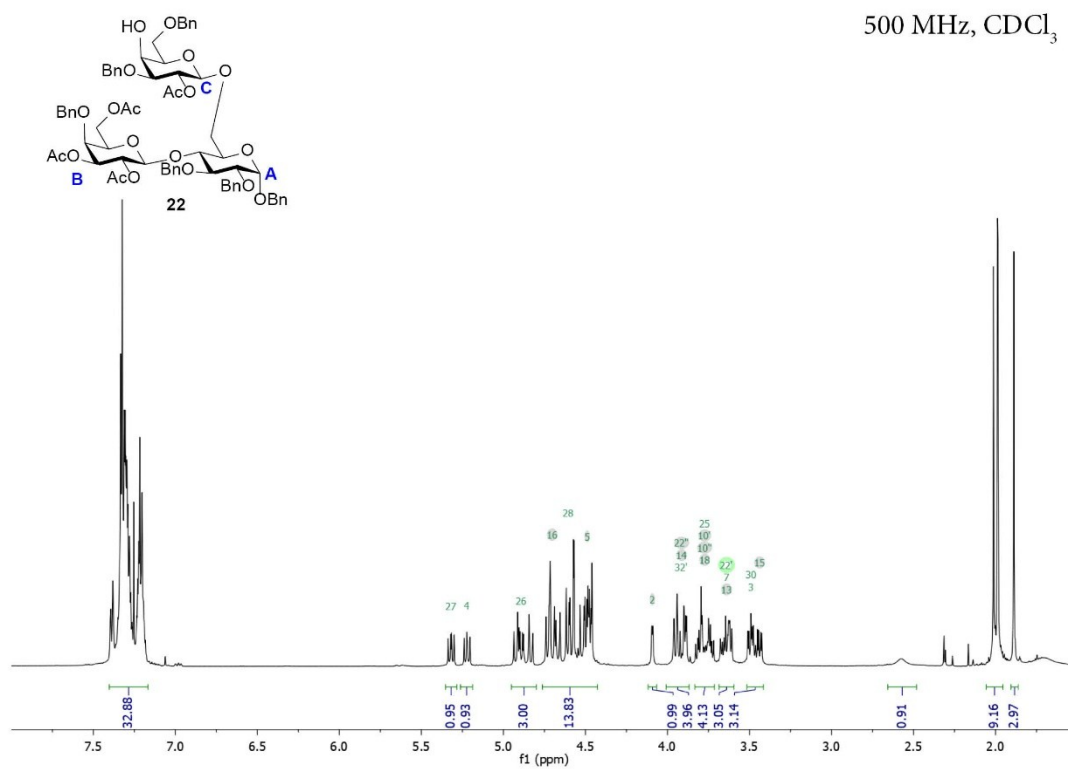


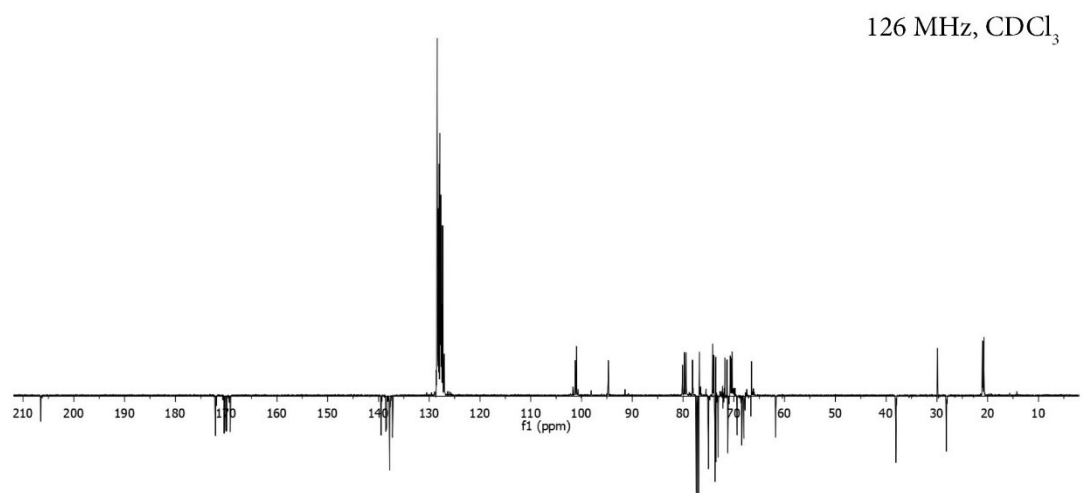
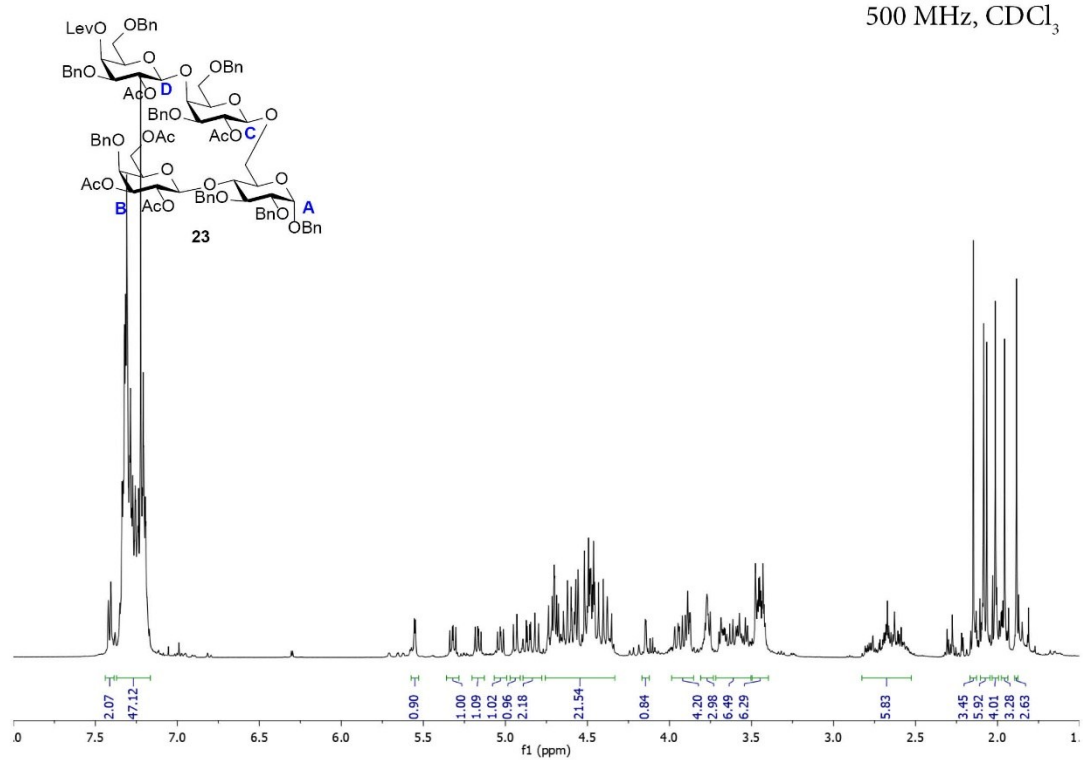
500 MHz, CDCl₃

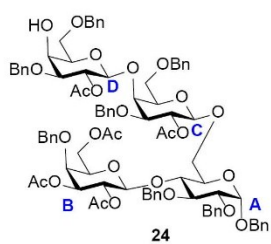


126 MHz, CDCl₃

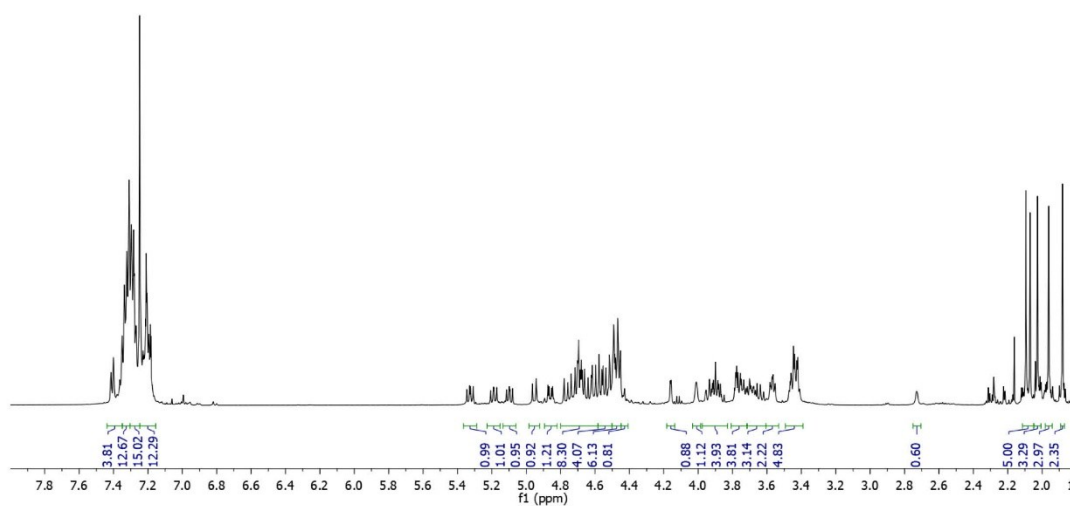




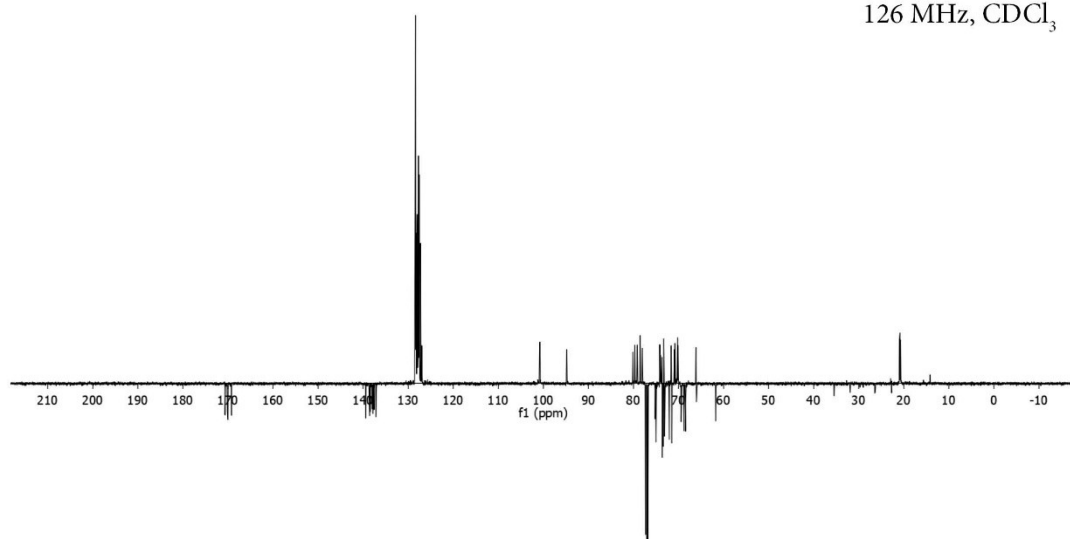


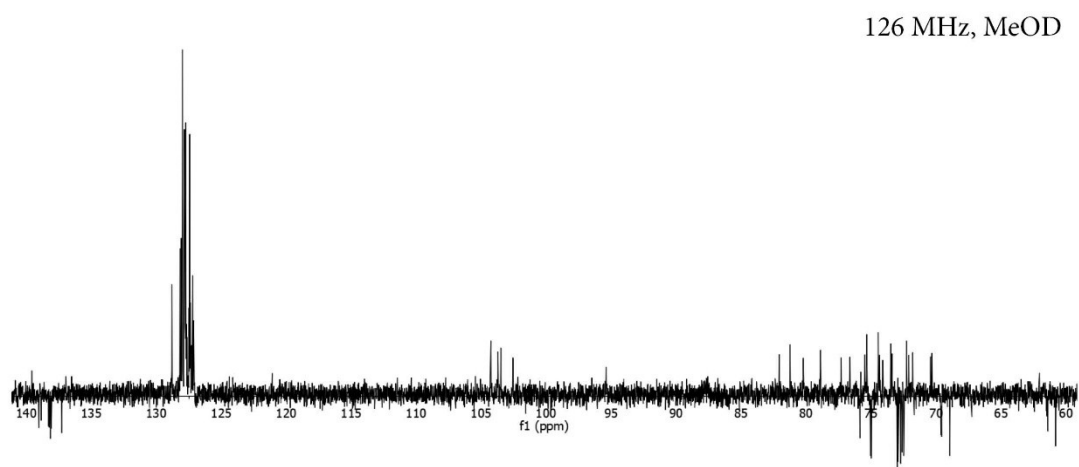
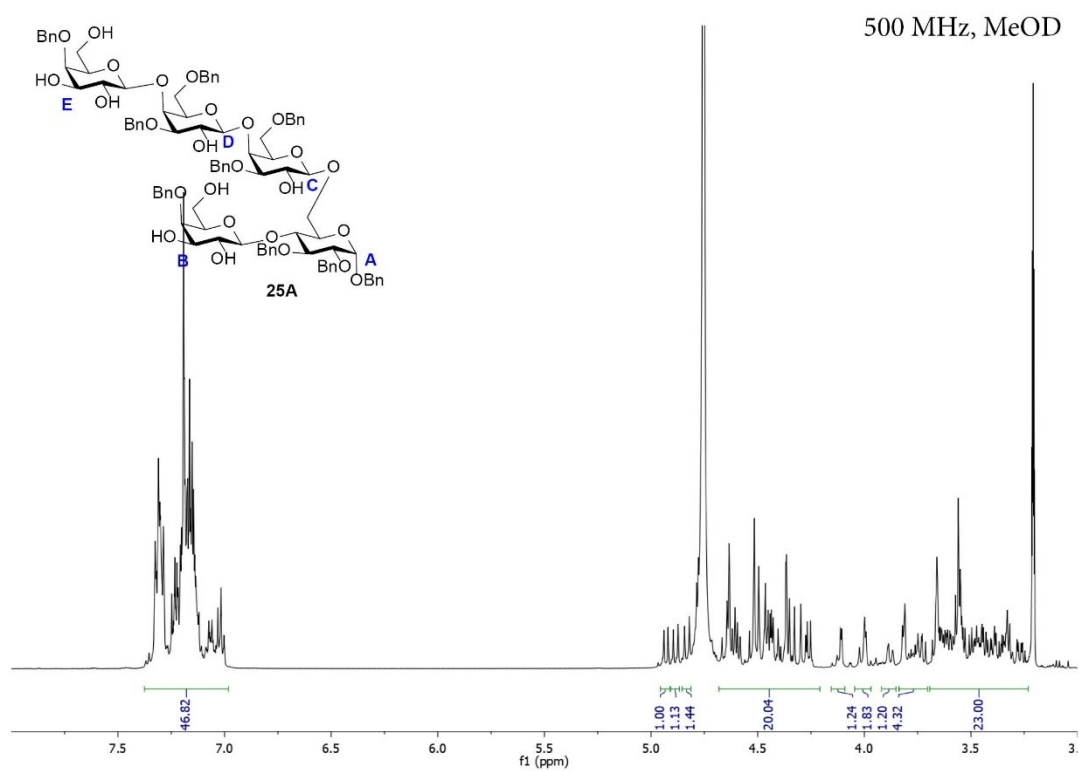


500 MHz, CDCl₃

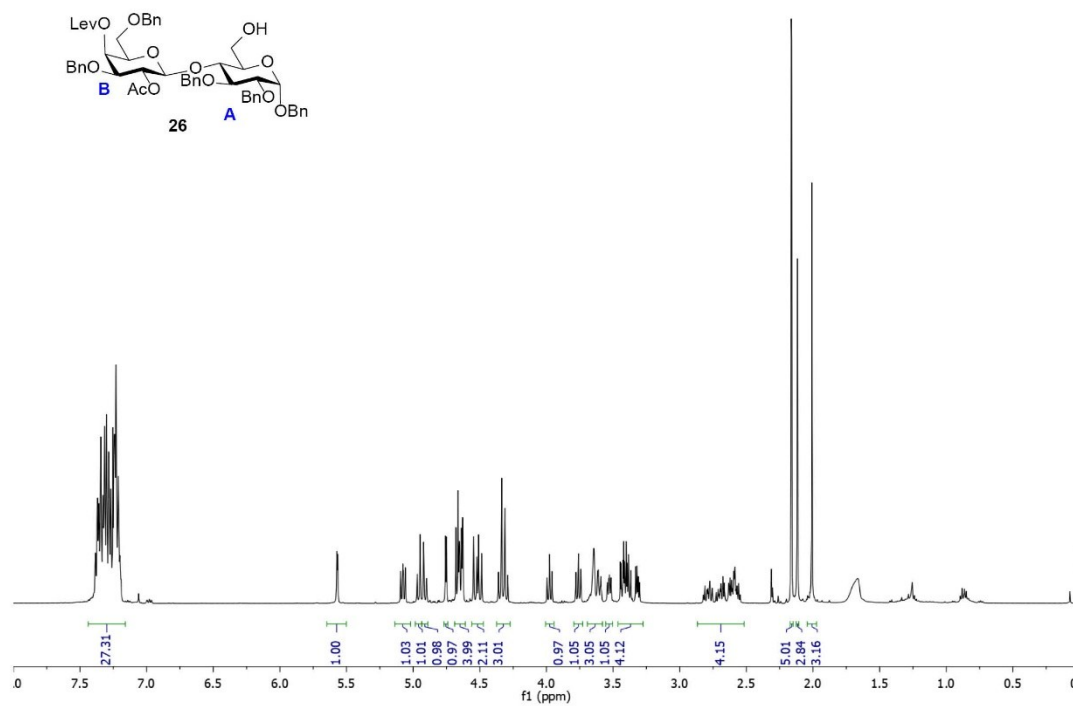


126 MHz, CDCl₃

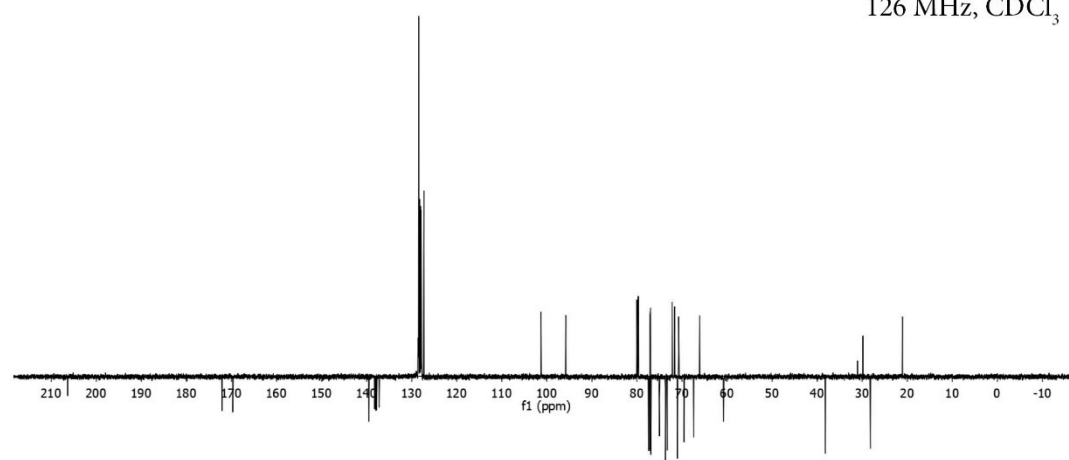




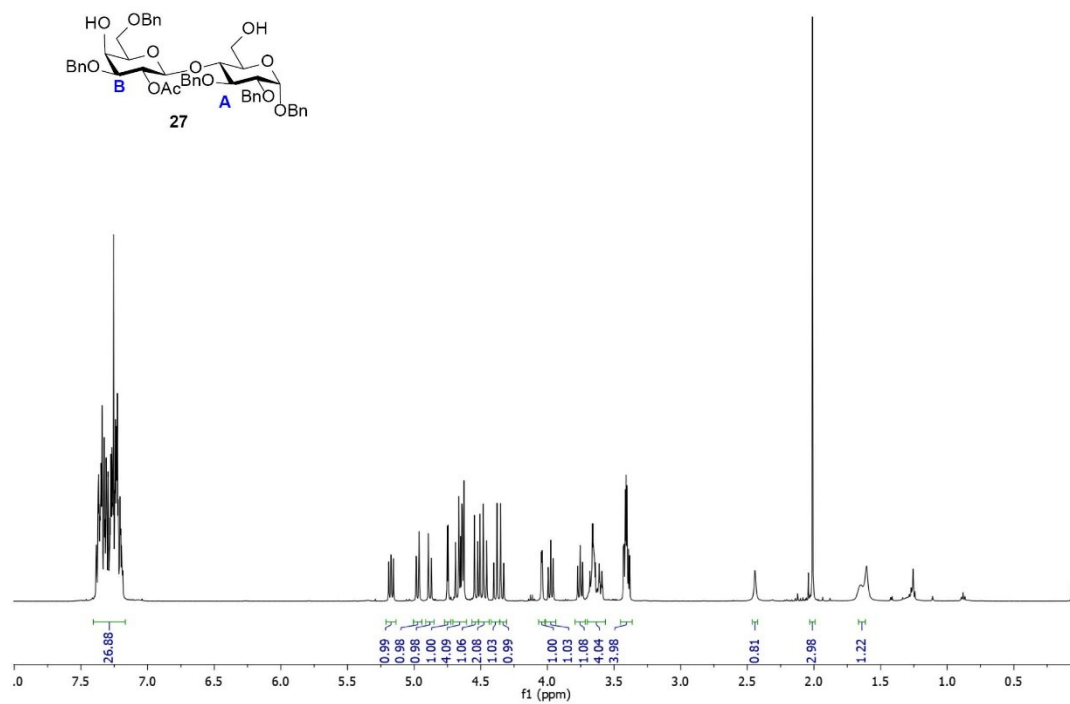
500 MHz, CDCl₃



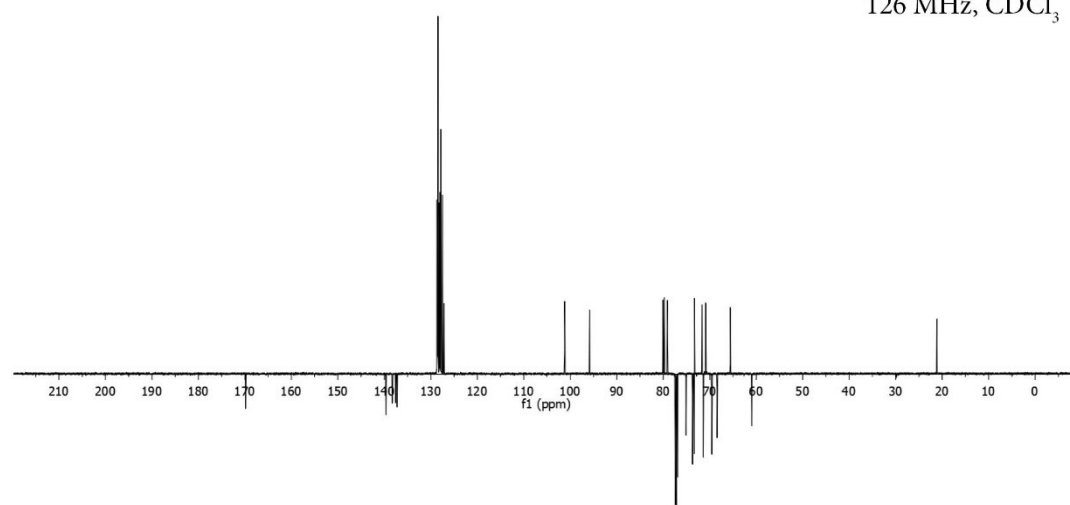
126 MHz, CDCl₃

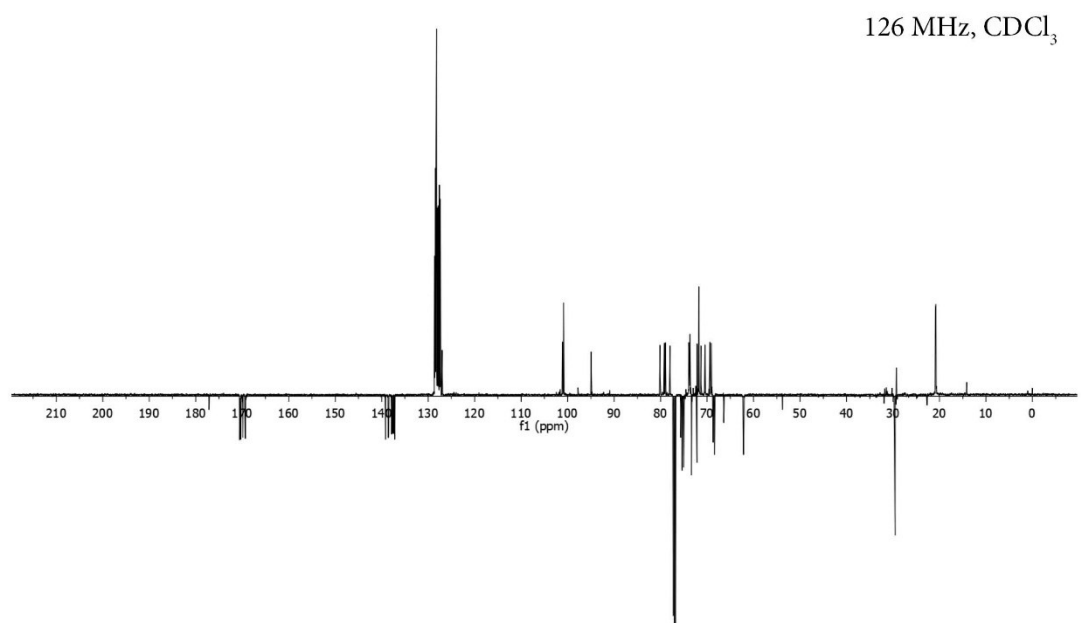
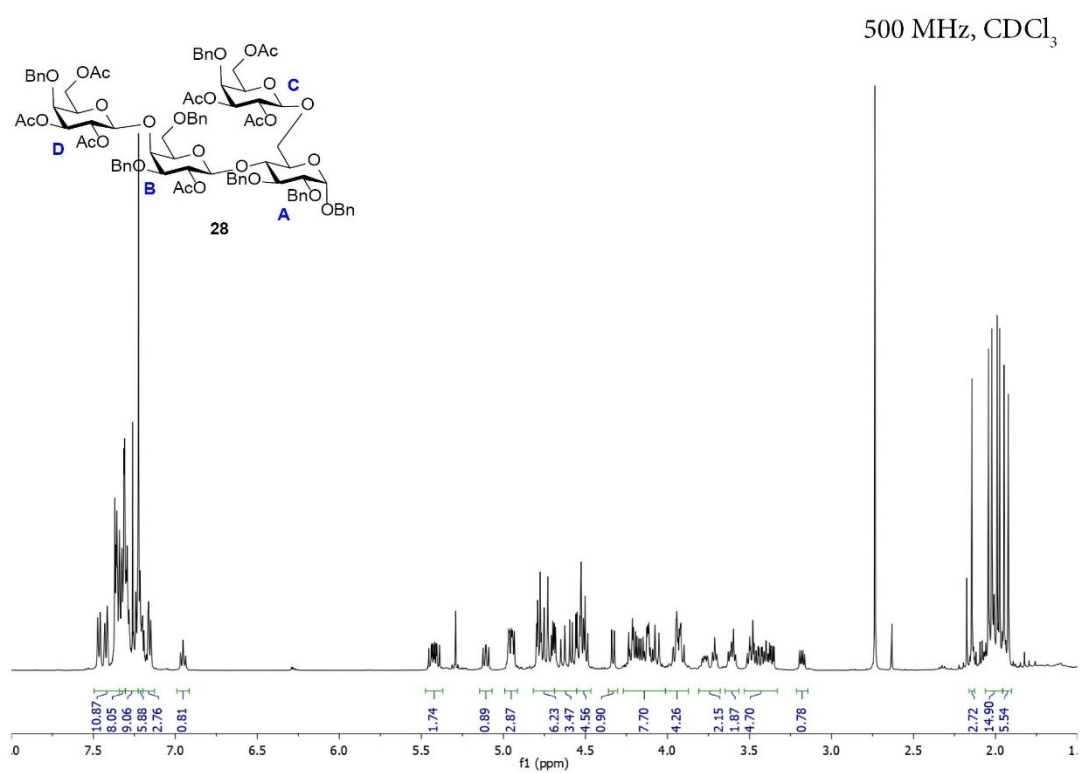


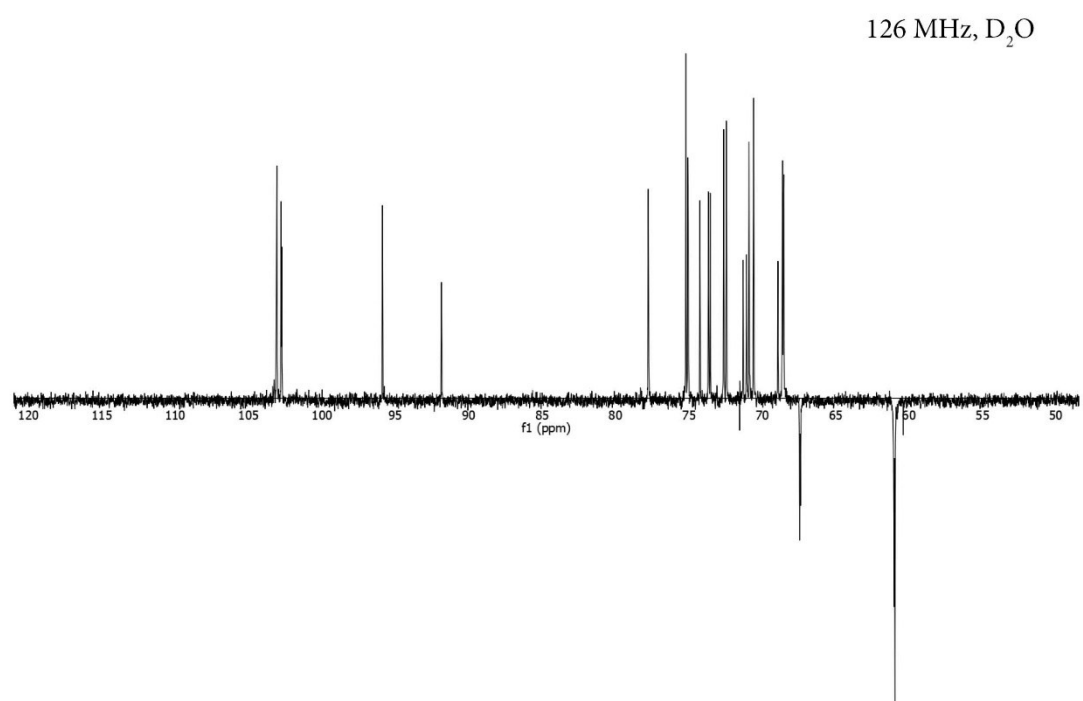
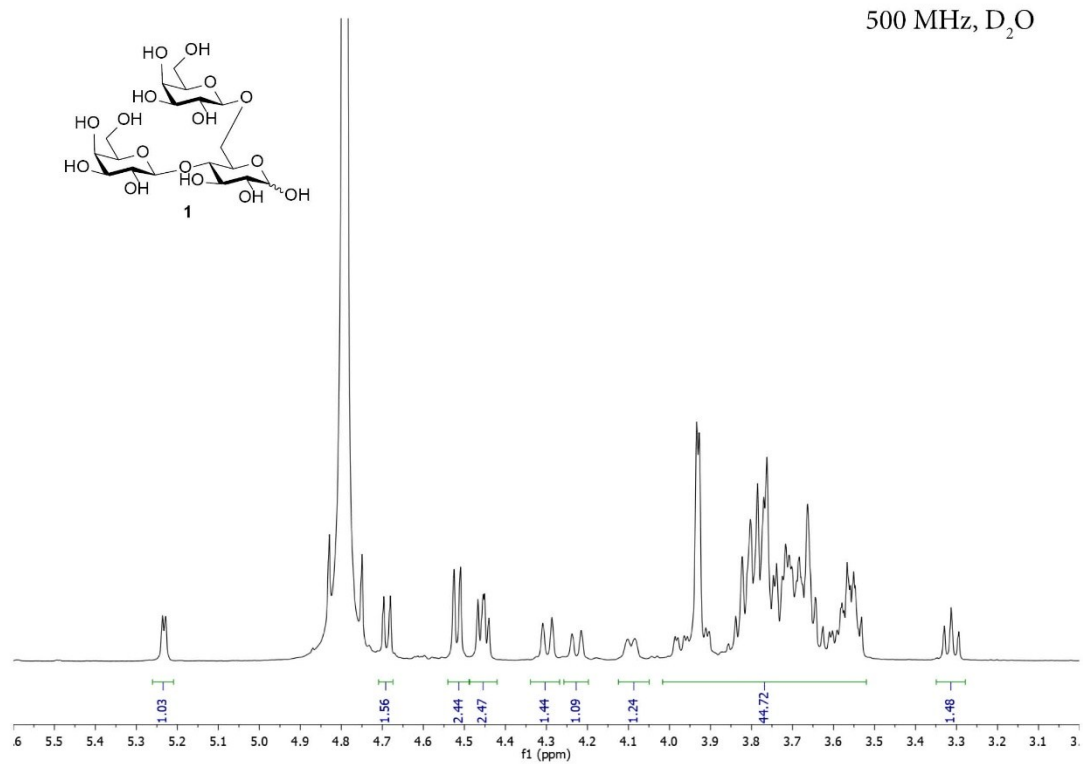
500 MHz, CDCl₃

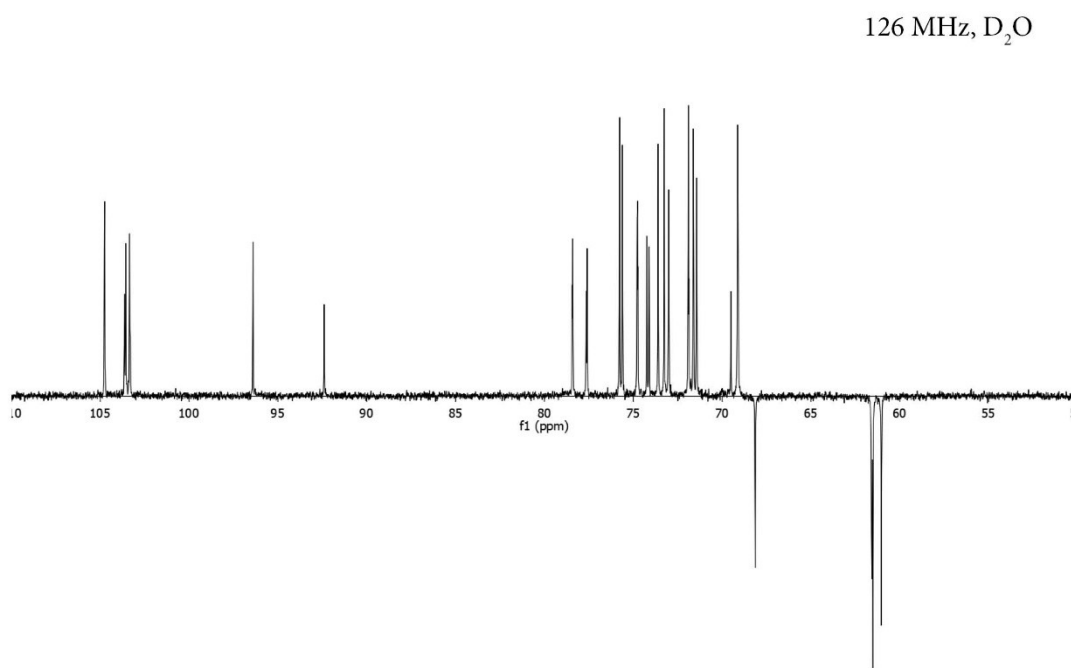
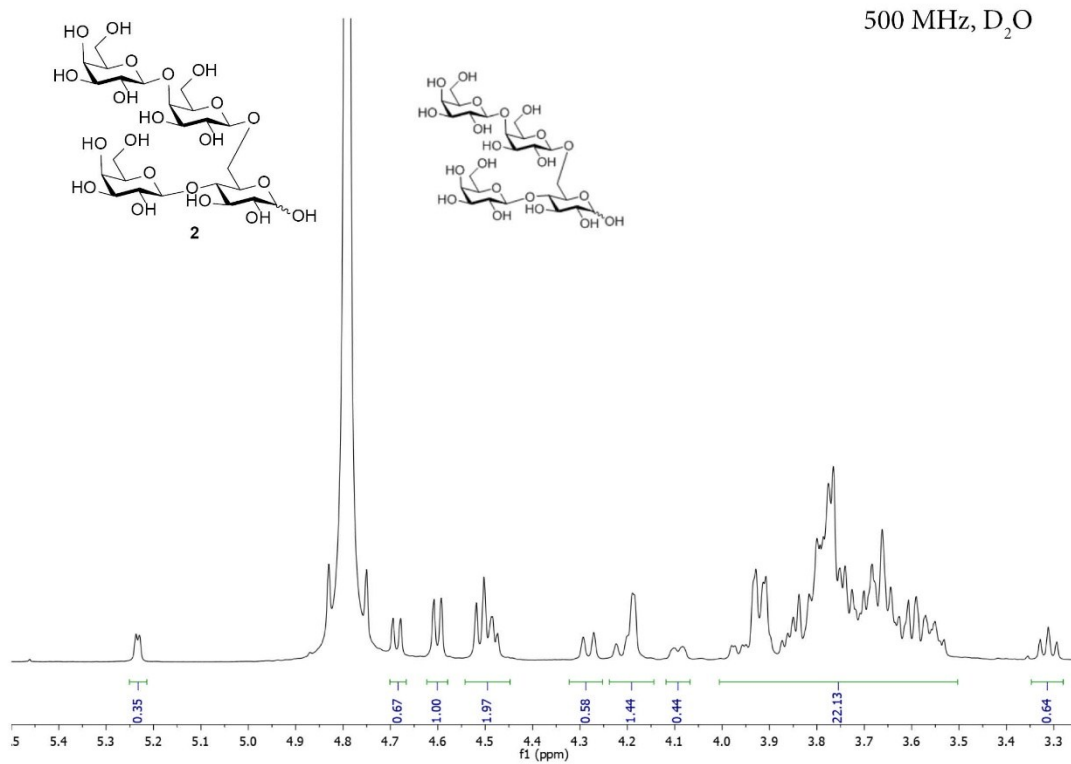


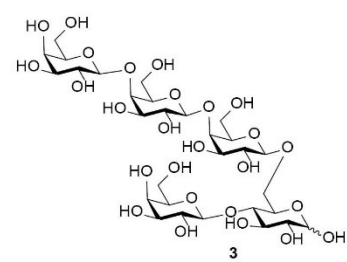
126 MHz, CDCl₃



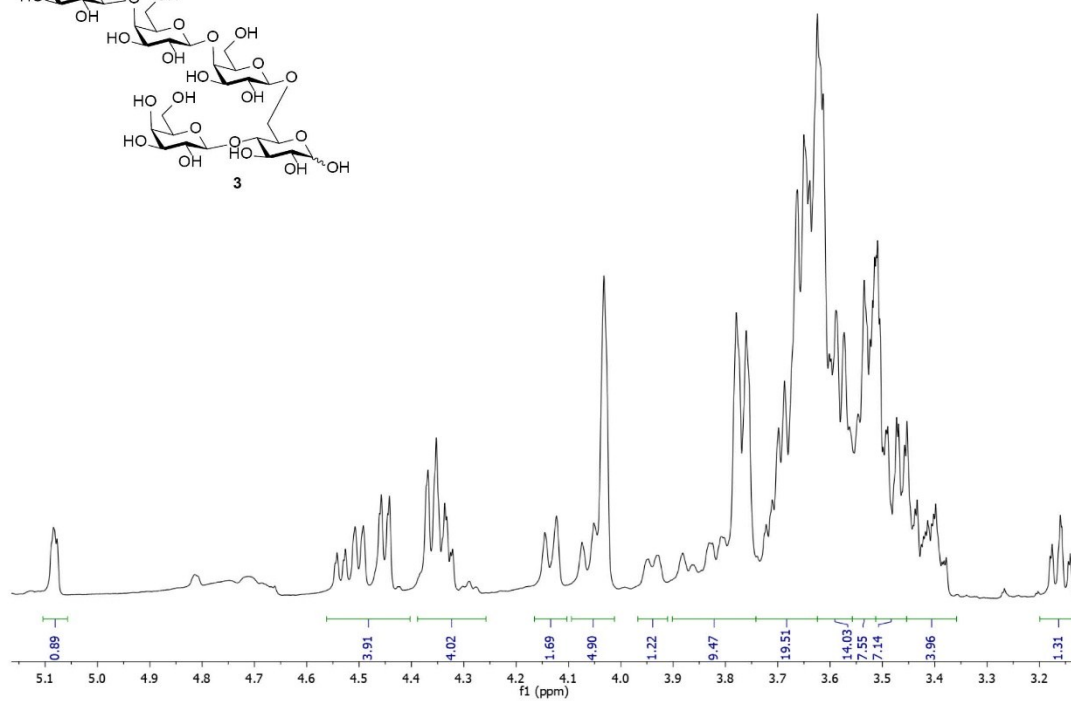




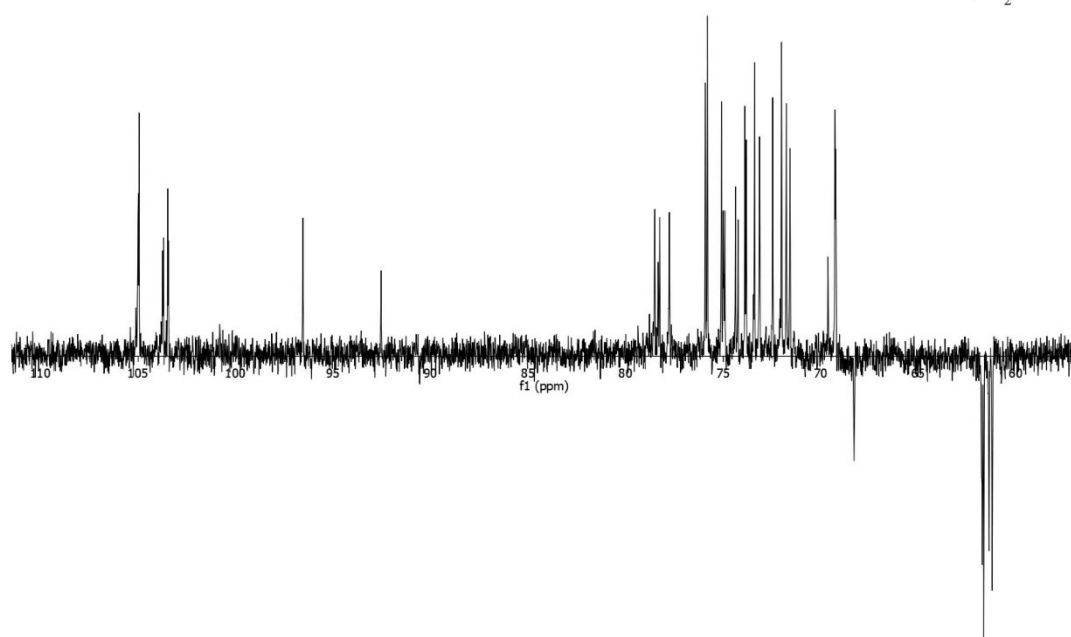


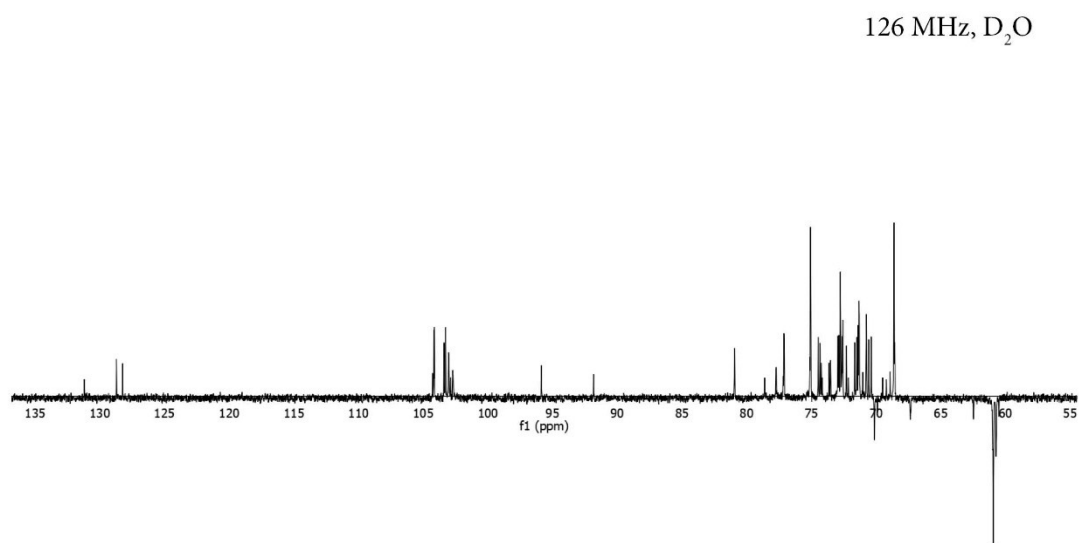
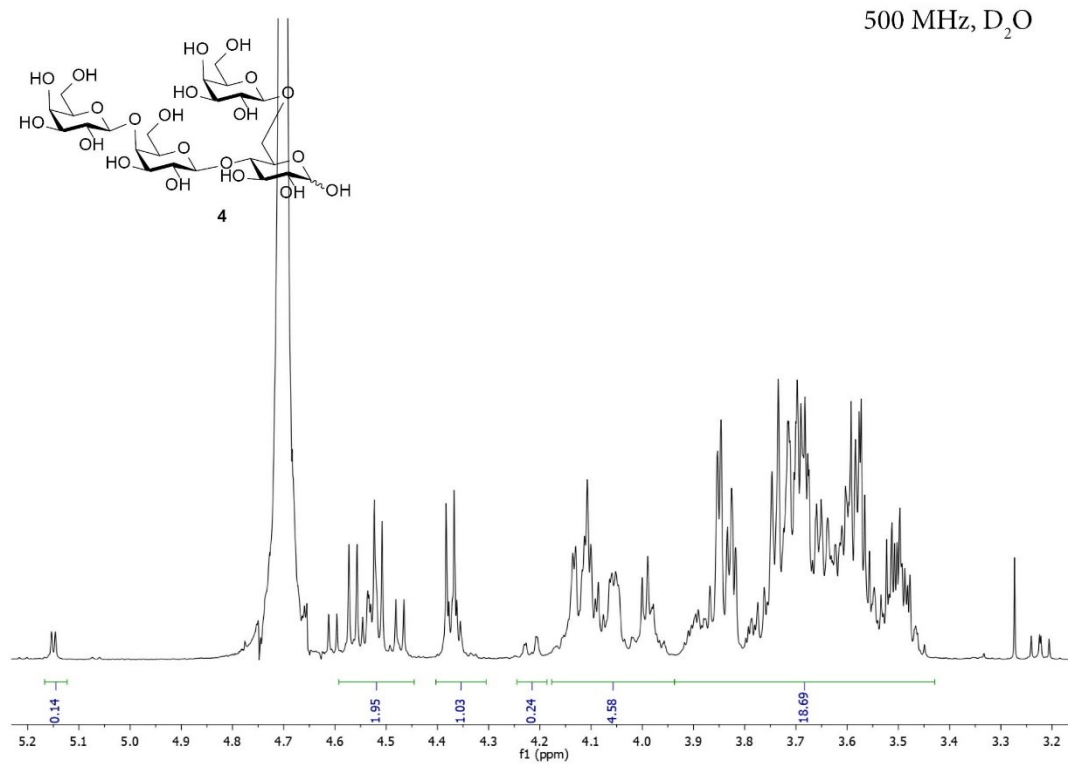


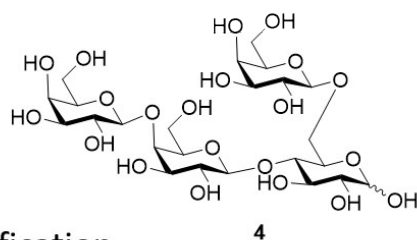
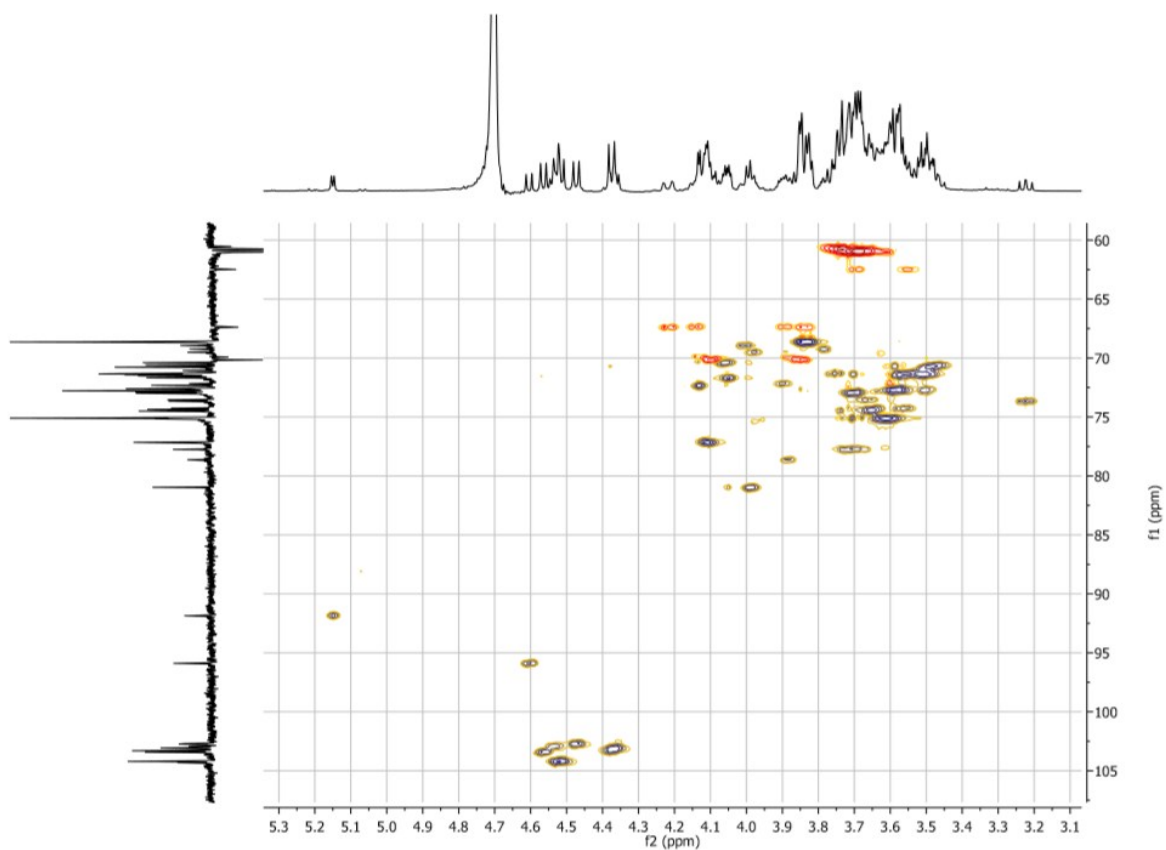
500 MHz, D₂O



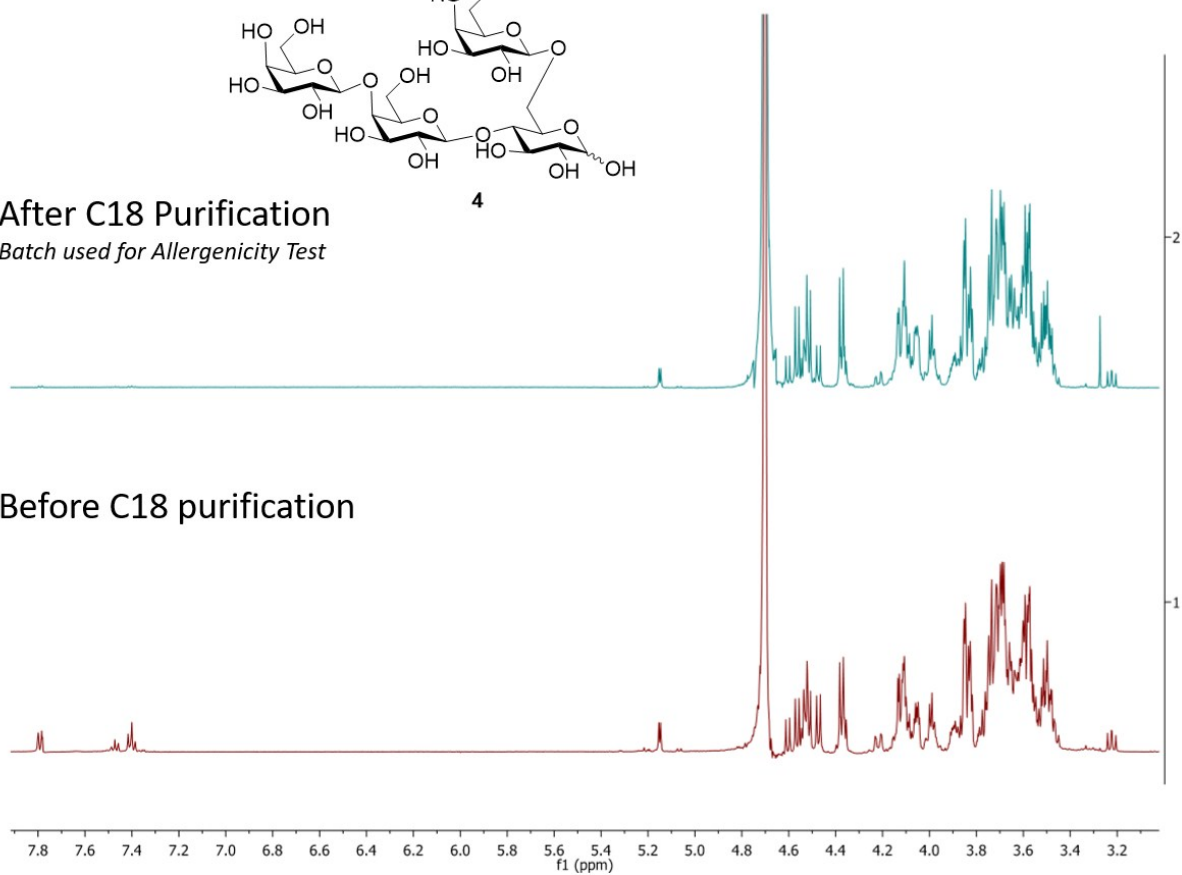
126 MHz, D₂O







After C18 Purification
Batch used for Allergenicity Test



1. J. Y. Soh, C.-H. Huang, W. C. Chiang, G. V. Llanora, A. J. Lee, W. Loh, Y. L. C. Chin, V. Y. J. Tay, Y. H. Chan, D. Dianne and B. W. Lee, *Allergy*, 2015, **70**, 1020-1023.
2. K.-K. T. Mong, C.-S. Chao, M.-C. Chen and C.-W. Lin, *Synlett*, 2009, **2009**, 603-606.
3. S. S. Mandal, G. Liao and Z. Guo, *RSC Advances*, 2015, **5**, 23311-23319.
4. J. Lindberg, S. C. T. Svensson, P. Pålsson and P. Konradsson, *Tetrahedron*, 2002, **58**, 5109-5117.
5. A. Santra, G. Guchhait and A. K. Misra, *Green Chemistry*, 2011, **13**, 1345-1351.