Supporting Information

Evaluation of Anti α-D-Glc*p*-(1→4)-α-D-Glc*p* (GAGA4) IgM Antibodies as a Biomarker for Multiple Sclerosis

Chriselle D. Braganza,[a,b] Kristiana T. Santoso,[a,b] Emma M. Dangerfield,[a,b] Anne C. La Flamme,[b,c] Mattie S. M. Timmer,[a,b]* Bridget L. Stocker[a,b]*

[a]School of Chemical and Physical Sciences, Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand.

[b]Centre for Biodiscovery, Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand.

[c]Malaghan Institute of Medical Research, Victoria University of Wellington, P.O. Box 7060, Wellington 6242, New Zealand.

Table of Contents

Biology Experimental

Patient Recruitment and Blood Sample Collection

Serum sample collection: Subjects (n = 41; 80.5% female) with RRMS (age = 40 +/- 11; range 17-74) were recruited from the Neurology Department at Wellington Hospital where most (37/41; 90%) were receiving monthly infusions of natalizumab, and blood was collected prior to infusion. RRMS patients were excluded if they had malignancy, renal impairment, or other intercurrent illness. Healthy volunteers (n = 10; 50% female) were recruited from Victoria University of Wellington (age = 47 +/- 8; range 35-59). Blood was collected into an SST tube (Beckton Dickinson, Franklin Lakes, NJ USA), and the serum stored at -80˚C. All procedures were approved by the New Zealand Northern A Health and Disability Committee (approval 17/NTA/46).

Plasma sample collection: The blood was collected from 6 healthy volunteers aged 22-51 years (4 females, 2 males) and 8 MS patients (4 RRMS, 3 secondary progressive and 1 progressive relapsing), aged 26-58 years (7 females, 1 male), and receiving no disease modifying therapies. Samples were collected into CPT™ tubes (Beckton Dickinson, Franklin Lakes, NJ USA) and centrifuged at 1600 x g for 25 minutes after blood collection. The upper plasma layer was then collected in a separate Eppendorf tube and stored at -80˚C for testing in an ELISA. All procedures were approved by the Victoria University of Wellington Human Ethics Committee (RM20738).

ELISA for Measuring Anti-glycan IgM Levels

The IgM levels against each antigen were measured for every serum or plasma sample individually using an ELISA. Each antigen (10 µg/mL in PBS), including the linker negative control, was adsorbed onto separate rows of flat-bottomed Immuno-Maxisorp 96-well plates (Nunc, Roskilde, Denmark). The plates were incubated overnight at 4 °C, washed with 0.2% Tween20 in PBS (wash buffer) three times, and blocked (1% non-fat milk in PBS) for 2 hours to prevent non-specific IgM antibody binding.[1] After washing with wash buffer, the serum samples (1:500 in block) were added to the plates in triplicate, and the plates incubated at room temperature for 2 hours. The plates were washed with wash buffer, then incubated with horseradish peroxidase (HRP)-conjugated goat anti-human IgM specific secondary antibody (Thermo Scientific, Illinois, USA) (1:2000 in block) for 2 hours at room temperature in order

to detect any bound anti-glycan IgM antibodies. Following washing with wash buffer, 3, 3′, 5, 5′-tetramethylbenzidine (BD Biosciences, California, USA) was added to the plates, left for 8 min, and then the enzymatic reaction quenched via the addition of 5% sulphuric acid. The optical density (OD) was then measured at 460 nm using a Perkin Elmer Enspire™ 2300 Multilabel plate reader. The results reported are those obtained from direct OD measurements.

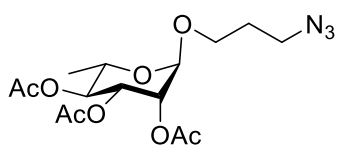
Statistical Analysis

Statistical significance of differences was assessed using the non-parametric Mann-Whitney U Test, using Prism v7 software (GraphPad, CA, USA), where $p<0.05$ was considered statistically significant.

Chemistry Experimental

General Experimental

All reactions were performed under an atmosphere of argon. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm with fluorescent indicator UV_{254}) via detection by UV absorption (254 nm) where relevant and dipping in 10% H_2SO_4 in EtOH followed by charring or dipping in a solution of $KMnO_4$ (0.05 M), K_2CO_3 (0.4 M), and NaOH (0.06%) in water. Column chromatography was performed using Pure Science silica gel (40–63 μm). All solvents were removed by evaporation under reduced pressure. High resolution mass spectra (HRMS) were recorded on an Agilent 6530 Q-TOF mass spectrometer utilising a JetStream™ electro-spray ionisation (ESI) source in positive or negative mode. Optical rotations were recorded on a Autopol II (Rudolph Research Analytical) at 589 nm (sodium D line). Infrared (IR) spectra were recorded as thin films using either a Bruker Platinum-ATR spectrometer. Nuclear magnetic resonance spectra (NMR) were obtained at 20 °C in $CDCl_3$ or D_2O using a Varian INOVA operating at 500 MHz. Chemical shifts are given in ppm (δ) relative to the solvent residual peak. NMR peak assignments were made using COSY, HSQC, and HMBC 2D experiments.



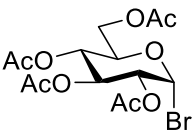
Formation of 3-azidopropyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranoside (**14**)

Peracetylated L-rha (**13**, 90 mg, 0.26 mmol) and 3-azidopropanol (**12**, 39 mg, 0.39 mmol) were coevaporated with anhydrous toluene (2 mL) three times to

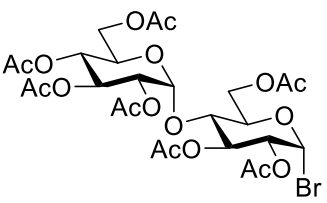
remove any traces of water. The mixture was then dissolved in freshly distilled CH_2Cl_2 (5 mL) and cooled to 0 °C under argon, followed by the slow addition of $BF_3.Et_2O$ (98 µL, 0.78 mmol). The mixture was brought to room temperature, and then stirred for 16 hours, after which all the starting material was consumed. The reaction was quenched with sat. $NaHCO_3$ (aq.) and extracted with CH_2Cl_2. The organic phase was washed with brine and H_2O, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The oil was purified by flash silica gel column chromatography (20:1 → 1:1 PE : EtOAc) to give **14** (78 mg, 0.21 mmol, 79%) as a colourless oil. $[\alpha]_D^{23}$ = -65° (*c* = 1.0, CH_2Cl_2); IR (film) 2096, 1743, 1369, 1217, 1046 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.26 (dd, *J* = 9.9, 3.5 Hz, 1H), 5.23-5.20 (m, 1H), 5.06 (t, *J* = 9.9 Hz, 1H), 4.72 (s, 1H), 3.87-3.80 (m, 1H), 3.82-3.75 (m, 1H), 3.52-3.45 (m, 1H), 3.42 (t, *J* = 6.6 Hz, 2H), 2.14 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.93-1.81 (m, 2H), 1.22 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.2, 170.1, 97.6, 71.1, 69.9, 69.2, 66.6, 64.7, 48.3, 28.9, 21.0, 20.9, 20.8, 17.5; HRMS calcd. for $C_{15}H_{27}N_4O_8$ ($[M+NH_4]^+$) 391.1823; found 391.1892. Experimental data matched those reported in literature.[2]

General procedure for the formation of peracetylated glycosyl bromides

To a solution of the peracetylated sugar in freshly distilled CH_2Cl_2 (20 mL/mmol), HBr-HOAc (2 mL/mmol) was added and the solution was stirred for 4 hours at room temperature. The reaction was quenched by the addition of sat. $NaHCO_3$ (aq.) and the product was then extracted using CH_2Cl_2, washed with brine and H_2O, before being dried over $MgSO_4$, filtered and concentrated. The product was then purified using silica gel flash column chromatography (10:1 → 0:1, PE : EtOAc).

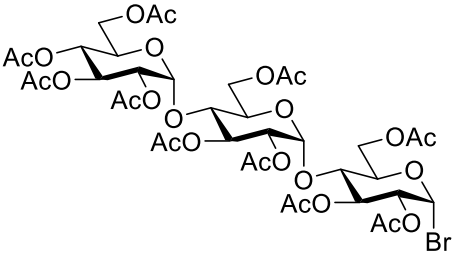


2,3,4,6-Tetra-*O*-acetyl-α-D-glucosyl bromide. The general procedure for the formation of peracetylated glycosyl bromides was carried out on peracetylated D-glucose (**17a**, 100 mg, 0.26 mmol) to yield 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (85 mg, 0.21 mmol, 81%) as a white solid. Experimental data matched those reported in literature.[3]



2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-α-D-maltosyl bromide. The general procedure for the formation of peracetylated glycosyl bromides was carried out on peracetylated D-maltose (**17b**, 100 mg, 0.15 mmol) to yield 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-maltosyl

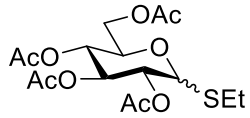
bromide (74 mg, 0.11 mmol, 72%) as a white solid. Experimental data matched those reported in literature.[4]



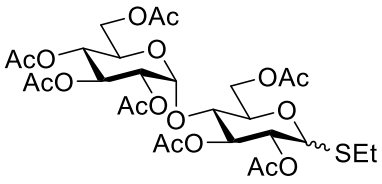
2,3,6,2′,3′,6′,2″,3″,4″,6″-Deca-*O*-acetyl-α-D-maltotriosyl bromide. The general procedure for the formation of peracetylated glycosyl bromides was carried out on peracetylated D-maltotriose (**17c**, 100 mg, 0.10 mmol) to yield 2,3,6,2′,3′,6′,2″,3″,4″,6″-deca-*O*-acetyl-α-D-maltotriosyl bromide (67 mg, 0.068 mmol, 66%) as a colourless oil. Experimental data matched those reported in literature.[5]

General procedure for the formation of thioglycosides

The peracetylated sugar was coevaporated with toluene three times to remove traces of water before being dissolved in freshly distilled CH_2Cl_2 (10 mL/mmol). Activated 4 Å molecular sieves were then added, followed by EtSH (2 equiv.). The reaction was then left to stir under an argon atmosphere for 45 min at 0 °C, followed by the slow addition of $SnCl_4$ (1.5 equiv.). The mixture was then warmed to room temperature and stirred for 18 hours. The reaction was quenched by the addition of sat. $NaHCO_3$ (aq.) and the product extracted with CH_2Cl_2. The combined organic layers were washed with brine and H_2O before being dried with anhydrous $MgSO_4$, which was filtered off and the filtrate concentrated *in vacuo*. The products were then subjected to flash column chromatography (10:1 → 1:1, PE : EtOAc) to yield the desired thioglycosides.



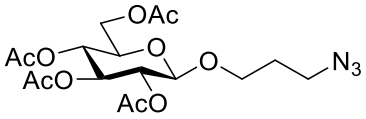
Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-D-glucopyranoside. The general procedure for the formation of thioglycosides was carried out on **17a** (1 g, 2.6 mmol) to yield ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-D-glucopyranoside (806 mg, 2.1 mmol, 79%) as a white solid. Experimental data matched those reported in literature.[6]



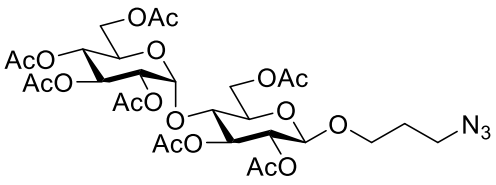
Ethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-thio-D-maltoside. The general procedure for the formation of thioglycosides was carried out on **17b** (1 g, 1.5 mmol) to yield ethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-thio-D-maltoside (622 mg, 0.91 mmol, 62%) as a white solid. Experimental data matched those reported in literature.[7]

General procedure for the formation of β-glycosides

The glycosyl bromide and 3-azidopropanol (3 equiv.) were coevaporated with toluene three times. The mixture was then dissolved in freshly distilled CH_2Cl_2 (10 mL/mmol) and AgOTf (1.2 equiv.) was added and the solution was stirred under argon overnight at room temperature. The reaction was quenched by the addition of sat. $NaHCO_3$ (aq.) and the product was then extracted using CH_2Cl_2, washed with brine and H_2O, before being dried over $MgSO_4$, filtered and concentrated. The product was purified using silica gel flash column chromatography (10:1 → 0:1, PE : EtOAc).

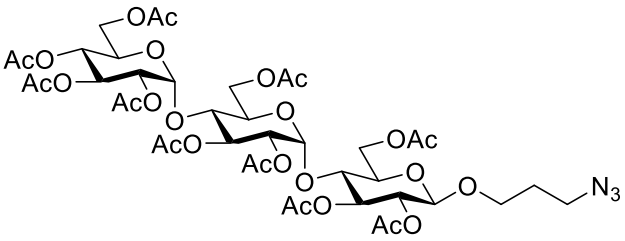


3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside. The general procedure for the formation of β-glycosides above was carried out on 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (80 mg, 0.19 mmol) to yield 3-azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (60 mg, 0.14 mmol, 71%) as a colourless oil. $[\alpha]_D^{23}$ = -10° (*c* = 1.0, CH_2Cl_2); IR (film) 2096, 1745, 1367, 1214, 1034 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.20 (t, *J* = 9.7 Hz, 1H), 5.07 (t, *J* = 9.7 Hz, 1H), 4.98 (t, *J* = 8.8 Hz, 1H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.25 (dd, *J* = 12.3, 4.4 Hz, 1H), 4.13 (d, *J* = 12.3 Hz, 1H), 3.98-3.90 (m, 1H), 3.72-3.66 (m, 1H), 3.64-3.56 (m, 1H), 3.40-3.31 (m, 2H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.91-1.76 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.4, 169.5, 169.4, 100.9, 72.9, 72.0, 71.4, 68.5, 66.6, 62.0, 48.1, 29.1, 20.9, 20.8, 20.7(4), 20.7(3); HRMS calcd. for $C_{17}H_{25}N_3O_{10}Na$ ($[M+Na]^+$) 454.1432; found 454.1448. Experimental data matched those reported in literature.[8]



3-Azidopropyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-maltoside. The general procedure for the formation of β-glycosides was carried out on 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-maltosyl bromide (60 mg, 0.086 mmol) to yield 3-azidopropyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-maltoside (40 mg, 0.056

mmol, 64%) as a colourless oil. $[\alpha]_D^{23}$ = +50° (*c* = 1.0, CH_2Cl_2); IR (film) 2099, 1744, 1368, 1223, 1036 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.40 (d, *J* = 4.0 Hz, 1H), 5.35 (t, *J* = 10.2 Hz, 1H), 5.24 (t, *J* = 10.2 Hz, 1H), 5.09 (t, *J* = 10.2 Hz, 1H), 4.84 (dd, *J* = 10.6, 4.0 Hz, 1H), 4.81 (t, *J* = 8.5 Hz, 1H), 4.51 (d, *J* = 7.9 Hz, 1H), 4.51-4.43 (m, 1H), 4.22 (dt, *J* = 12.3, 3.6 Hz, 2H), 4.07-3.86 (m, 4H), 3.70-3.64 (m, 1H), 3.63-3.55 (m, 1H), 3.38-3.29 (m, 2H), 2.13 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 2.01 (s, 6H), 1.99 (s, 6H), 1.89-1.75 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.6, 170.3, 170.1, 169.8, 169.6, 100.4, 95.7, 75.5, 72.8, 72.3, 72.2, 70.1, 69.5, 68.6, 68.1, 66.6, 62.9, 61.6, 48.1, 29.1, 21.0, 20.9, 20.8, 20.7(6), 20.7(3), 20.7(1), 20.7(0); HRMS calcd. for $C_{29}H_{41}N_3O_{18}Na$ ($[M+Na]^+$) 742.2277; found 742.2293. Experimental data matched those reported in literature.[9]

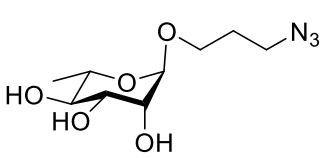


3-Azidopropyl 2,3,6,2′,3′,6′,2″,3″,4″,6″-deca-*O*-acetyl-β-D-maltotrioside. The general procedure for the formation of β-glycosides was carried out on 2,3,6,2′,3′,6′,2″,3″,4″,6″-deca-*O*-acetyl-α-D-maltotriosyl bromide (50 mg, 0.051 mmol) to yield 3-azidopropyl 2,3,6,2′,3′,6′,2″,3″,4″,6″-deca-*O*-acetyl-β-D-maltotrioside (30 mg, 0.030 mmol, 58%) as a colourless oil. $[\alpha]_D^{23}$ = +53° (*c* = 1.0, CH_2Cl_2); IR (film) 2098, 1740, 1367, 1211, 1024 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.44-5.20 (m, 5H), 5.07 (t, *J* = 10.6 Hz, 1H), 4.85 (dd, *J* = 10.7, 4.0 Hz, 1H), 4.81 (t, *J* = 9.0 Hz, 1H), 4.73 (dd, *J* = 10.4, 4.0 Hz, 1H), 4.52 (d, *J* = 8.0 Hz, 1H), 4.47 (t, *J* = 10.7 Hz, 2H), 4.31 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.24 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.18 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.05 (d, *J* = 12.0 Hz, 1H), 4.01-3.88 (m, 4H), 3.74-3.68 (m, 1H), 3.64-3.57 (m, 1H), 3.55-3.46 (m, 1H), 3.39-3.30 (m, 2H), 2.17 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 2.00 (s, 6H), 1.98 (s, 3H), 1.91-1.77 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.7, 170.6(7), 170.6(2), 170.5, 170.2, 170.0, 169.8(5), 169.8(3), 169.6, 100.4, 95.9, 95.8, 75.4, 73.9, 72.6, 72.3, 72.2, 71.9, 70.6, 70.2, 69.5, 69.1, 68.7, 68.0, 66.6, 63.0, 62.5, 61.5, 48.1, 29.1, 21.1, 21.0, 20.9, 20.8, 20.7(9), 20.7(4), 20.7(0); HRMS calcd. for $C_{41}H_{57}N_3O_{26}Na$ ($[M+Na]^+$) 1030.3123; found 1030.3140.

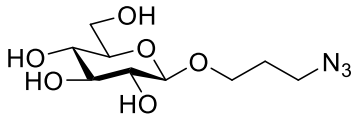
General procedure for deacetylation

The peracetylated compound was dissolved in MeOH (50 mL/mmol) before NaOMe (0.1 eq) was added to the solution. The reaction mixture was allowed to stir overnight at room

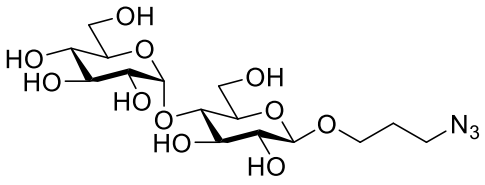
temperature, after which complete conversion of starting material was achieved. The solution was neutralised using Dowex H^+, followed by filtration of the beads and concentration of the filtrate *in vacuo* to afford the product. Products were used directly without further purification.



3-Azidopropyl α-L-rhamnopyranoside (15). The general procedure for deacetylation was carried out on **14** (50 mg, 0.13 mmol) to yield **15** quantitatively as a colourless oil. $[\alpha]_D^{23}$ = -41° (*c* = 1.0, CH_3OH); IR (film) 3392, 2924, 2098, 1052 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.76 (s, 1H), 3.95-3.89 (m, 1H), 3.81-3.71 (m, 2H), 3.66-3.57 (m, 1H), 3.52-3.44 (m, 2H), 3.43-3.33 (m, 2H), 1.93-1.79 (m, 2H), 1.31 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 99.9, 73.1, 71.8, 71.1, 68.3. 64.4, 48.5, 28.8, 17.6; HRMS calcd. for $C_9H_{17}N_3O_5Na$ ($[M+Na]^+$) 270.1060; found 270.1071. Experimental data matched those reported in literature.[2]

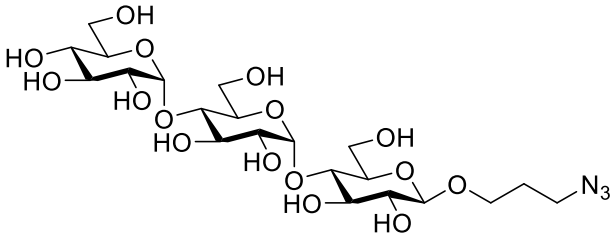


3-Azidopropyl β-D-glucopyranoside (20a). The general procedure for deacetylation was carried out on 3-azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (50 mg, 0.12 mmol) to yield **20a** quantitatively as a colourless oil. $[\alpha]_D^{23}$ = -19° (*c* = 1.0, CH_3OH); IR (film) 3357, 2923, 2096, 1032 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.44 (d, *J* = 7.9 Hz, 1H), 4.02-3.95 (m, 1H), 3.90 (d, *J* = 12.1 Hz, 1H), 3.78-3.70 (m, 1H), 3.70 (dd, *J* = 12.1, 5.7 Hz, 1H), 3.51-3.40 (m, 4H), 3.36 (t, *J* = 9.7 Hz, 1H), 3.25 (t, *J* = 9.7 Hz, 1H), 1.90 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 102.2, 75.8, 75.6, 73.0, 69.9, 67.2, 60.7, 47.8, 28.2; HRMS calcd. for $C_9H_{17}N_3O_6Na$ ($[M+Na]^+$) 286.1010; found 286.1016. Experimental data matched those reported in literature.[10]

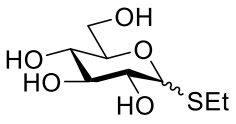


3-Azidopropyl β-D-maltoside (20b). The general procedure for deacetylation was carried out on 3-azidopropyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-maltoside (50 mg, 0.069 mmol) to yield **20b** quantitatively as a colourless oil. $[\alpha]_D^{23}$ = +61° (*c* = 1.0, CH_3OH); IR (film) 3334, 2923, 2096, 1023 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 5.40 (d, *J* = 3.5 Hz, 1H), 4.47 (d, *J* = 8.3 Hz, 1H), 4.00 (dt, *J* = 10.4, 6.5 Hz, 1H), 3.94 (dd, *J* = 12.4, 1.8 Hz, 1H), 3.85 (dd, *J* = 12.1, 1.8 Hz, 1H), 3.81-3.54 (m, 9H), 3.46 (t, *J* = 6.4 Hz, 2H), 3.41 (t, *J* = 9.4 Hz, 1H), 3.30 (dd, *J* = 9.4, 7.5 Hz, 1H), 1.91 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 102.0, 99.4, 76.2, 76.0, 74.4, 73.0, 72.9,

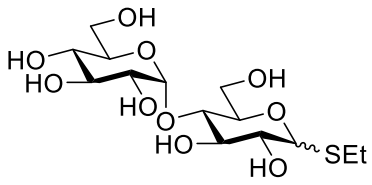
72.8, 71.6, 69.2, 67.2, 60.4, 47.8, 28.1; HRMS calcd. for $C_{15}H_{27}N_3O_{11}Na$ ([M+Na]$^+$) 448.1538; found 448.1594.



3-Azidopropyl β-D-maltotrioside (20c). The general procedure for deacetylation was carried out on 3-azidopropyl 2,3,6,2′,3′,6′,2″,3″,4″,6″-deca-*O*-acetyl-β-D-maltotrioside (30 mg, 0.029 mmol) to yield **20c** quantitatively as a colourless oil. $[\alpha]_D^{23}$ = +73° (*c* = 1.0, CH_3OH); IR (film) 3329, 2927, 2100, 1024 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 5.39 (d, *J* = 3.4 Hz, 2H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.03-3.90 (m, 3H), 3.89-3.54 (m, 15H), 3.46 (t, *J* = 7.0 Hz, 2H), 3.41 (t, *J* = 9.3 Hz, 1H), 3.30 (t, *J* = 9.3 Hz, 1H), 1.91 (p, *J* = 6.9 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 102.0, 99.6, 99.4, 76.9, 76.6, 76.1, 74.4, 73.2, 72.9, 72.8, 72.6, 71.6, 71.4, 71.1, 69.2, 67.2, 60.6, 60.4, 47.8, 28.1; HRMS calcd. for $C_{21}H_{37}N_3O_{16}Na$ ([M+Na]$^+$) 610.2066; found 610.2033.



Ethyl 1-thio-D-glucopyranoside. The general procedure for deacetylation was carried out on ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-D-glucopyranoside (200 mg, 0.51 mmol) to yield ethyl 1-thio-D-glucopyranoside quantitatively as a white solid. Experimental data matched those reported in literature.[11]

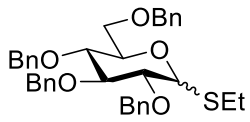


Ethyl 1-thio-D-maltopyranoside. The general procedure for deacetylation was carried out on ethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-thio-D-maltoside (100 mg, 0.29 mmol) to yield ethyl 1-thio-D-maltopyranoside quantitatively as a white solid. Experimental data matched those reported in literature.[7]

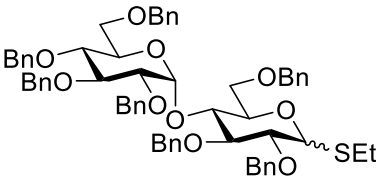
General procedure for benzylation

The deacetylated thioglycoside was coevaporated with DMF three times, leaving DMF (10 mL/mmol) on the final coevaporation. Under an argon atmosphere, BnBr (15 equiv.) and TBAI (0.15 equiv.) were added. The solution was then cooled to 0 °C and NaH (14 equiv.) was added slowly in portions. The reaction was allowed to warm to room temperature and then heated to 80 °C and was left to stir under an argon atmosphere for 16 h. MeOH (5 mL) was then added

to quench the excess BnBr before sat. $NaHCO_3$ (aq.) was added. The product was extracted with EtOAc, and the combined organic layers washed with H_2O and brine. The organic layer was then dried using anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The product was then isolated using silica gel flash column chromatography (20:1 → 2:1, PE : EtOAc).



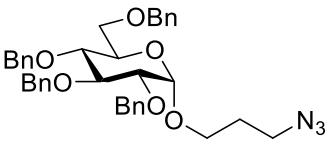
Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-D-glucopyranoside. The general procedure for benzylation was carried out on ethyl 1-thio-α-D-glucopyranoside (55 mg, 0.25 mmol) to yield ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-D-glucopyranoside (103 mg, 0.18 mmol, 72%) as a colourless oil. Experimental data matched those reported in literature.[12]



Ethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-1-thio-D-maltoside. The general procedure for benzylation was carried out on ethyl 1-thio-D-maltopyranoside (36 mg, 0.093 mmol) to yield ethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-1-thio-D-maltoside (59 mg, 0.058 mmol, 65%). Experimental data matched those reported in literature.[13]

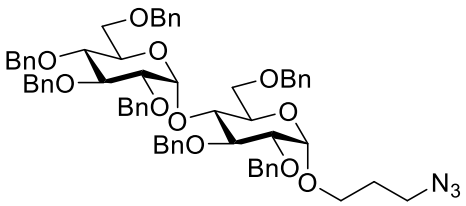
<u>General procedure for the formation of α-glycosides</u>

The perbenzylated donor and 3-azidopropanol (6 equiv.) were coevaporated with DMF three times, leaving DMF (10 mL/mmol) on the final coevaporation. Activated 4 Å molecular sieves were then added, which was then followed by the addition of 20 mL/mmol of freshly distilled CH_2Cl_2. Activators $CuBr_2$ (3 equiv.) and TPABr (3 equiv.) were added to the solution, which turned dark green. The mixture was refluxed at 55 °C for 24 hours before all starting material was consumed. The reaction was quenched by the addition of sat. $NaHCO_3$ (aq.) and the product was then extracted using CH_2Cl_2. The combined organic layers were washed with $Na_2S_2O_3$ (aq.) and brine, before being dried over $MgSO_4$, filtered and concentrated. The resulting pale yellow oil was purified by flash chromatography (0:1 → 1:50, EtOAc : toluene) to yield the desired α-glycoside.



3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (19a). The general procedure the formation of α-glycosides was carried out on **18a** (50 mg, 0.086 mmol) to yield **19a** (38 mg, 0.061 mmol, 68%) as a colourless oil. $[\alpha]_D^{23}$ = -49° (*c* = 1.0, CH_2Cl_2); IR (film) 2920, 2094, 1453,

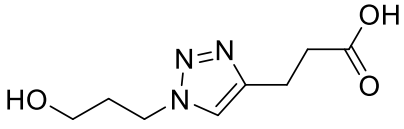
1359, 1066, 1027 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35-7.14 (m, 20 H), 4.99 (d, *J* = 11.1 Hz, 1H), 4.88-4.70 (m, 4H), 4.62 (dd, *J* = 12.1, 7.5 Hz, 2H), 4.47 (d, *J* = 12.1 Hz, 2H), 3.97 (t, *J* = 9.0 Hz, 1H), 3.79-3.68 (m, 3H), 3.68-3.52 (m, 3H), 3.51-3.33 (m, 3H), 1.97-1.81 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0, 138.4, 138.3, 138.1, 128.5-127.7, 97.4, 82.2, 80.2, 77.8, 75.8, 75.3, 73.6, 73.4, 70.5, 68.6, 64.9, 48.5, 29.0; HRMS calcd. for $C_{37}H_{45}N_4O_6$ ($[M+NH_4]^+$) 641.3334; found 641.3311.



3-Azidopropyl 2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-D-maltoside (19b). The general procedure the formation of α-glycosides was carried out on **18b** (50 mg, 0.049 mmol) to yield **19b** (34 mg, 0.032 mmol, 65%) as a colourless oil. $[\alpha]_D^{23}$ = +51° (*c* = 1.0, CH_2Cl_2); IR (film) 2924, 2108, 1445, 1153, 1001 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.29-7.10 (m, 35H), 5.70 (d, *J* = 3.3 Hz, 1H), 5.05 (d, *J* = 11.5 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 4.84-4.78 (m, 3H), 4.75 (d, *J* = 3.3 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.60-4.50 (m, 6H), 4.43 (d, *J* = 10.8 Hz, 1H), 4.29 (d, *J* = 12.3 Hz, 1H), 4.09-4.04 (m, 2H), 3.92 (t, *J* = 9.3 Hz, 1H), 3.87-3.82 (m, 2H), 3.77-3.72 (m, 2H), 3.68-3.61 (m, 3H), 3.52-3.39 (m, 6H), 1.92 (p, *J* = 6.1 Hz, 2H); ^{13}C NMR (125 MHz, CDCl3) δ 139.1, 138.9, 138.6, 138.3, 138.2, 138.1, 128.6, 128.5-128.4, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 126.9, 97.0, 96.9, 82.1, 82.0, 80.5, 79.5, 77.8, 75.7, 75.1, 74.4, 73.6, 73.4, 73.3, 72.6, 71.1, 69.9, 69.1, 68.3, 64.9, 48.5, 29.0; HRMS calcd. for $C_{64}H_{73}N_4O_{11}$ ($[M+NH_4]^+$) 1073.5270; found 1073.5273.

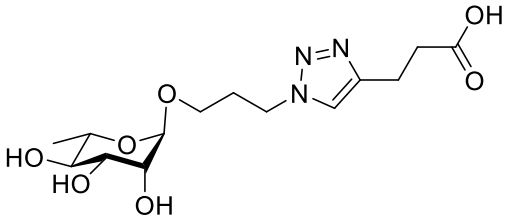
General CuAAC procedure

To a solution of azide in 1:1 acetone : H_2O, 4-pentynoic acid (2 equiv.), (+)-sodium L-ascorbate (2.5 eq) and $CuSO_4$-$5H_2O$ (1 eq) were added and the resulting mixture was stirred at room temperature overnight. The resulting aq.ueous solution was concentrated and purified by flash silica gel and C-18 reversed-phase chromatography.

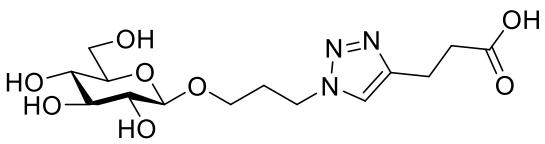


3-(1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (16a). The general CuAAC procedure described above was carried out on **12** (50 mg, 0.49 mmol) to yield **16a (**81 mg, 0.41 mmol, 82%) as a colourless oil. $[\alpha]_D^{23}$ = -2° (*c* = 1.0, CH_3OH); IR (film) 3350, 2936, 1721, 1217, 1059 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 7.78 (s, 1H), 4.44 (t, *J* = 6.9 Hz, 2H), 3.52

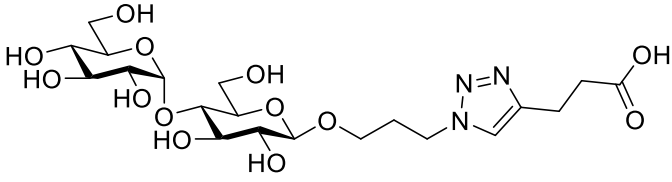
(t, *J* = 6.2 Hz, 2H), 2.96-2.89 (m, 2H), 2.63-2.55 (m, 2H), 2.08 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 179.9, 123.5, 58.0, 47.0, 31.7, 21.1; HRMS calcd. for $C_8H_{13}N_3O_3Na$ ($[M+Na]^+$) 222.0849; found 222.0856.



3-(1-(3-(α-L-Rhamopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (16b). The general CuAAC procedure described above was carried out on **15** (50 mg, 0.20 mmol) to yield **16b** (54 mg, 0.16 mmol, 77%) as a colourless oil. $[\alpha]_D^{23}$ = -36° (*c* = 1.0, CH_3OH); IR (film) 3349, 2930, 1716, 1221, 1051 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 7.81 (s, 1H), 4.63 (s, 1H), 4.48 (t, *J* = 6.8 Hz, 2H), 3.80-3.76 (m, 1H), 3.66-3.60 (m, 1H), 3.60 (dd, *J* = 9.7 Hz, 3.7 Hz, 1H), 3.56-3.50 (m, 1H), 3.44-3.38 (m, 1H), 3.40-3.34 (m, 1H), 2.97-2.90 (m, 2H), 2.63-2.54 (m, 2H), 2.19 (p, *J* = 6.4 Hz, 2H), 1.21 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (125 MHz, D_2O) δ 175.1, 123.5, 99.7, 71.9, 70.1, 69.9, 68.4, 64.3, 47.6, 28.8, 21.3, 16.4; HRMS calcd. for $C_{14}H_{24}N_3O_7$ ($[M+H]^+$) 346.1609; found 346.1634.

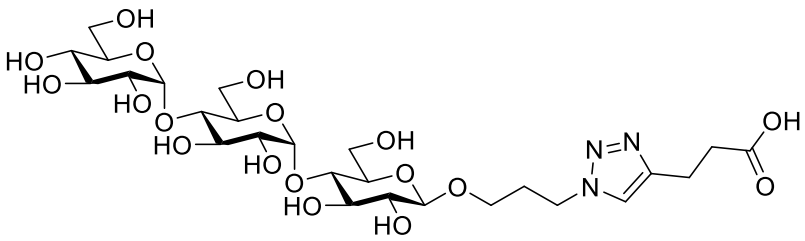


3-(1-(3-(β-D-Glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid. The general procedure CuAAC described above was carried out on **20a** (20 mg, 0.076 mmol) to yield 3-(1-(3-(β-D-glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (22 mg, 0.061 mmol, 79%) as a colourless oil. $[\alpha]_D^{23}$ = -7° (*c* = 0.2, CH_3OH); IR (film) 3331, 2923, 2098, 1262, 1033 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 7.82 (s, 1H), 4.50 (t, *J* = 7.0 Hz, 2H), 4.38 (d, *J* = 8.1 Hz, 1H), 3.91-3.81 (m, 2H), 3.68 (dd, *J* = 12.3, 5.8 Hz, 1H), 3.59-3.50 (m, 1H), 3.46 (t, *J* = 8.8 Hz, 1H), 3.43-3.30 (m, 2H), 3.24 (t, *J* = 8.0 Hz, 1H), 2.98 (t, *J* = 7.3 Hz, 2H), 2.73 (t, *J* = 7.3 Hz, 2H), 2.22-2.13 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 177.5, 146.5, 123.5, 102.1, 75.8, 75.6, 73.0, 69.5, 66.3, 60.6, 46.9, 33.3, 29.4, 20.1; HRMS calcd. for $C_{14}H_{24}N_3O_8$ ($[M+H]^+$) 362.1558; found 362.1579.

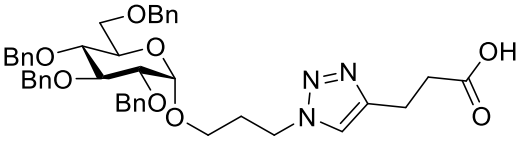


3-(1-(3-(β-D-Maltosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid. The general CuAAC procedure described above was carried out on **20b** (20 mg, 0.047 mmol) to yield 3-(1-(3-(β-D-maltosyloxy)propyl)-1*H*-1,2,3-triazol-4-

yl)propanoic acid (18 mg, 0.034 mmol, 73%) as a colourless oil. $[\alpha]_D^{23}$ = +4° (*c* = 0.2, CH_3OH); IR (film) 3334, 2929, 2098, 1262, 1036 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 7.83 (s, 1H), 5.40 (d, *J* = 3.8 Hz, 1H), 4.51 (t, *J* = 6.6 Hz, 2H), 4.41 (d, *J* = 7.3 Hz, 1H), 3.94-3.81 (m, 3H), 3.80-3.52 (m, 9H), 3.41 (t, *J* = 9.0 Hz, 1H), 3.29 (t, *J* = 9.0 Hz, 1H), 3.00 (t, *J* = 8.0 Hz, 2H), 2.73 (t, *J* = 8.0 Hz, 2H), 2.24-2.14 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 177.7, 146.8, 123.5, 102.0, 99.5, 76.6, 76.1, 74.4, 72.9, 72.7, 72.6, 71.6, 69.2, 66.4, 60.6, 60.4, 47.0. 33.5, 29.4, 20.3; HRMS calcd. for $C_{20}H_{34}N_3O_{13}$ ($[M+H]^+$) 524.2086; found 524.2082.

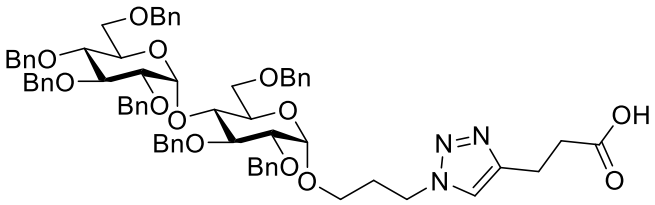


3-(1-(3-(β-D-Maltotriosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid. The general CuAAC procedure described above was carried out on **20c** (20 mg, 0.034 mmol) to yield 3-(1-(3-(β-D-maltotriosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (17 mg, 0.025 mmol, 74%) as a colourless oil. $[\alpha]_D^{23}$ = +23° (*c* = 0.5, CH_3OH); IR (film) 3341, 2946, 1730, 1223, 1034 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 7.84 (s, 1H), 5.39 (d, *J* = 3.4 Hz, 2H), 4.52 (d, *J* = 6.9 Hz, 2H), 4.42 (d, *J* = 7.7 Hz, 1H), 4.00-3.52 (m, 18H), 3.42 (t, *J* = 9.4 Hz, 1H), 3.30 (t, *J* = 9.3 Hz, 1H), 3.00 (t, *J* = 7.4 Hz, 2H), 2.73 (t, *J* = 6.5 Hz, 2H), 2.25-2.15 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 178.0, 123.5, 102.0, 99.6, 99.3, 76.8, 76.6, 76.0, 74.4, 73.2, 72.9, 72.7, 72.6, 71.6, 71.4, 71.2, 69.2, 66.4, 60.5, 60.3, 46.9, 33.8, 29.4, 20.4; HRMS calcd. for $C_{26}H_{44}N_3O_{18}$ ($[M+H]^+$) 686.2614; found 686.2629.



3-(1-(3-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid. The general CuAAC procedure described above was carried out on **19a** (30 mg, 0.051 mmol) to yield 3-(1-(3-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (24 mg, 0.033 mmol, 68%) as a colourless oil. $[\alpha]_D^{23}$ = -30° (*c* = 1.0, CH_2Cl_2); IR (film) 3336, 2922, 1732, 1453, 1070 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41-7.09 (m, 21H), 4.99 (d, *J* = 11.0 Hz, 1H), 4.90-4.78 (m, 3H), 4.73 (d, *J* = 3.5 Hz, 1H), 4.66 (d, *J* = 12.2 Hz, 1H), 4.58 (d, *J* = 12.6 Hz, 1H), 4.51-4.37 (m, 4H), 3.99 (t, *J* = 9.4 Hz, 1H), 3.78-3.53 (m, 6H), 3.37-3.27 (m, 1H), 2.96 (t, *J* = 7.1 Hz, 2H), 2.71 (t, *J* = 7.1 Hz, 2H),

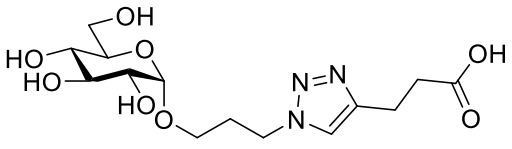
2.23-2.08 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8, 138.3, 138.0, 137.9, 128.8-127.7, 122.3, 97.5, 82.1, 80.2, 77.9, 75.8, 75.4, 73.6, 70.6, 68.6, 64.3, 47.1, 33.6, 31.3, 31.1, 20.8; HRMS calcd. for $C_{42}H_{48}N_3O_8$ ([M+H]$^+$) 722.3436; found 722.3482.



3-(1-(3-(2,3,6,2′,3′,4′,6′-Hepta-*O*-benzyl-α-D-maltopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid. The general CuAAC procedure described above was carried out on **19b** (30 mg, 0.029 mmol) to yield 3-(1-(3-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-D-maltopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (21 mg, 0.018 mmol, 65%) as acolourless oil. $[\alpha]_D^{23}$ = +54° (*c* = 1.0, CH_2Cl_2); IR (film) 3334, 2921, 1724, 1453, 1025 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49-7.03 (m, 36H), 5.70 (d, *J* = 3.6 Hz, 1H) , 5.06 (d, *J* = 12.0 Hz, 1H), 4.88 (t, *J* = 11.0 Hz, 2H), 4.84-4.67 (m, 4H), 4.64-4.55 (m, 3H), 4.56-4.37 (m, 6H), 4.29 (d, *J* = 12.0 Hz, 1H), 4.15-4.00 (m, 2H), 3.93 (t, *J* = 10.1 Hz, 1H), 3.86-3.77 (m, 2H), 3.73 (d, *J* = 10.0 Hz, 1H), 3.70-3.55 (m, 4H), 3.55-3.46 (m, 2H), 3.41 (d, *J* = 10.6 Hz, 1H), 3.37-3.28 (m, 1H), 3.03-2.94 (m, 2H), 2.73-2.67 (m, 2H), 2.23-2.12 (m, 2H); ^{13}C NMR: (125 MHz, $CDCl_3$) δ 139.0, 138.8, 138.4, 138.2, 138.1, 138.0, 137.8, 129.0-126.0, 97.0, 96.8, 82.0, 80.5, 79.3, 77.4, 75.6, 75.1, 74.3, 73.6, 73.5, 73.4, 73.3, 72.5, 71.1, 70.1, 69.1, 68.2, 64.2, 47.0, 33.6, 30.0, 20.8; HRMS calcd. for $C_{69}H_{76}N_3O_{13}$ ([M+H]$^+$) 1154.5373; found 1154.5388.

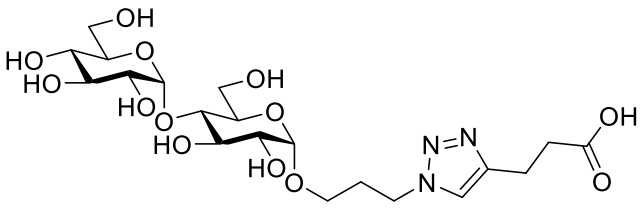
<u>General procedure for hydrogenation</u>

The benzylated glycoside was dissolved in MeOH $Pd(OH)_2/C$ (0.2 equiv.) was added. H_2 gas was then bubbled through the solution, and the reaction was left to stir for 16 h at room temperature. The reaction mixture was then filtered through celite to remove $Pd(OH)_2/C$ and concentrated *in vacuo* before it was purified using silica gel flash column chromatography (4:1 → 0:1, PE : EtOAc).



3-(1-(3-(α-D-Glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid. The general procedure for hydrogenation was carried out on 3-(1-(3-(2,3,4,6-tetra-*O*-benzyl-α-D-

glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (20 mg, 0.028 mmol) to yield 3-(1-(3-(α-D-glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (9 mg, 0.024 mmol, 88%) as a colourless oil. $[\alpha]_D^{23}$ = +19 (*c* = 0.2, CH_3OH); IR (film) 3333, 2923, 1569, 1032 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 7.82 (s, 1H), 4.83 (d, *J* = 3.7 Hz, 1H), 4.51 (t, *J* = 6.4 Hz, 2H), 3.79-3.69 (m, 2H), 3.68-3.60 (m, 2H), 3.58-3.49 (m, 2H), 3.44-3.34 (m, 2H), 2.97 (t, *J* = 7.2 Hz, 2H), 2.68 (t, *J* = 7.2 Hz, 2H), 2.29-2.14 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 178.5, 147.1, 123.3, 98.2, 72.9, 71.6, 71.2, 69.4, 64.2, 60.3, 47.2, 34.1, 29.0, 20.6; HRMS calcd. for $C_{14}H_{24}N_3O_8$ ($[M+H]^+$) 362.1558; found 362.1622.



3-(1-(3-(α-D-Maltosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid. The general procedure for hydrogenation was carried out on 3-(1-(3-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-D-maltopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (20 mg, 0.017 mmol) to yield 3-(1-(3-(α-D-maltosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (6 mg, 0.012 mmol, 71%) as a colourless oil. $[\alpha]_D^{23}$ = +12° (*c* = 0.2, CH_3OH); IR (film) 3332, 2925, 1567, 1030 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 7.84 (s, 1H), 5.37 (d, *J* = 4.1 Hz, 1H), 4.82 (d, *J* = 4.1 Hz, 1H), 4.59-4.43 (m, 2H), 3.90-3.81 (m, 2H), 3.80-3.68 (m, 5H), 3.67-3.49 (m, 5H), 3.47-3.35 (m, 2H), 2.99 (t, *J* = 7.1 Hz, 2H), 2.73 (t, *J* = 7.1 Hz, 2H), 2.32-2.15 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 177.7, 146.7, 123.2, 99.7, 98.0, 76.8, 73.4, 72.8, 72.6, 71.7, 71.0, 70.1, 69.2, 64.4, 60.4, 60.2, 47.3, 33.6, 28.8, 20.3; HRMS calcd. for $C_{20}H_{33}N_3O_{13}Na$ ($[M+Na]^+$) 546.1906; found 546.1923.

General procedure for NHS activation of acids

TSTU (1 equiv.) and Et_3N (1 equiv.) were added to a solution of the free acid in anhydrous DMF (5 mL/mmol), and the resulting solution was stirred for 1 hour at room temperature. The reaction was monitored using HRMS, which indicated complete consumption of the free acid. The reaction mixture was then concentrated to give the activated NHS linked ester for direct use in the next step.

Conjugation with bovine serum albumin (BSA)

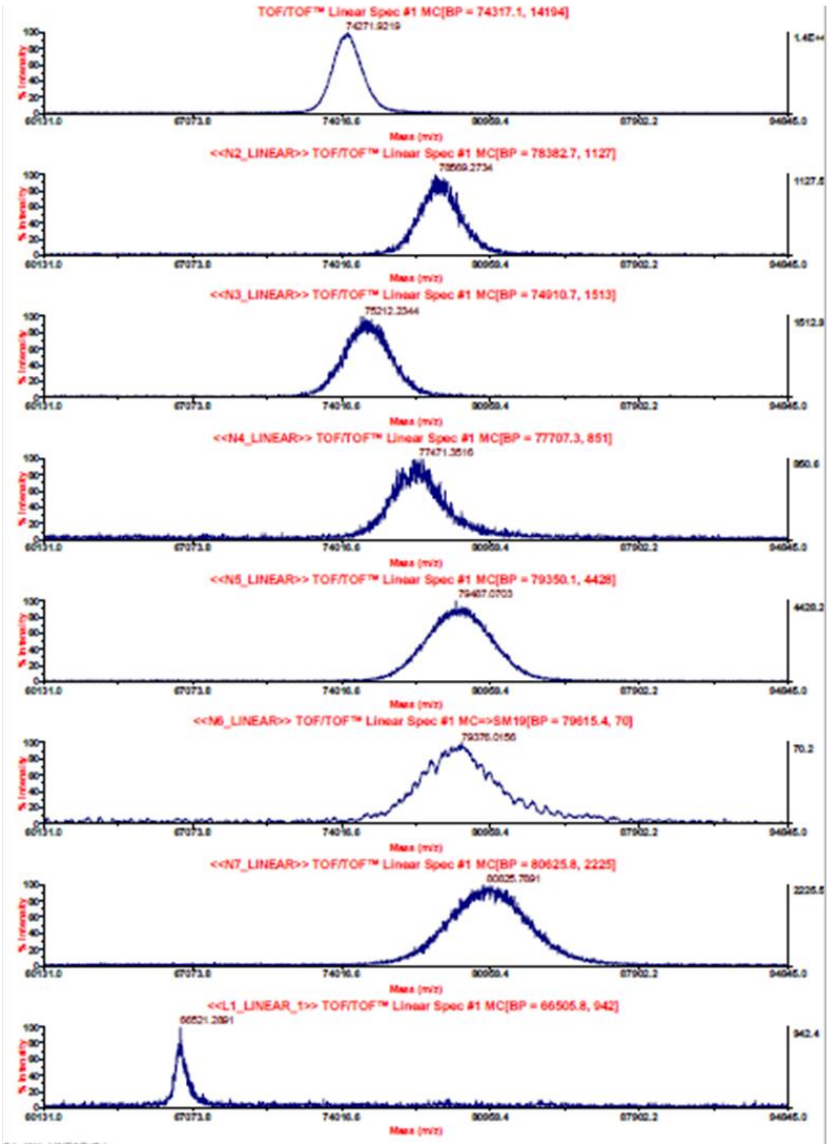
The NHS esters in solution (10 mg/mL in 3x PBS, pH 7.4) were added to the same volume of BSA solution (10 mg/mL in 3x PBS pH 7.4) and was stirred at room temperature for 1 h. The reaction was monitored using MALDI-TOF analysis whereby consumption of BSA indicated successful conjugation (Supplementary Figure S1).

MALDI-TOF Analysis

To a solution of cinnapinic acid (15 mg) in MeCN:H_2O (1 mL, 1/1, v/v), TFA (1 µL) was added and the mixture was vortexed for 3 min and centrifuged for 10 min, to obtain the matrix solution. A 1–2 µL aliquot of the glycoprotein (~1.0 mg/mL) was added to 20 µL of the matrix solution and a pipette was used to mix the sample. Next, 2 µL of the sample/matrix was spotted on a MALDI-plate, dried for 1 h, and measured using MALDI-TOF.

References

1. K. Vedhagiri, S. Velineni, J. F. Timoney, S. Shanmughapriya, P. Vijayachari, R. Narayanan and K. Natarajaseenivasan, *Pathog. Glob. Health*, 2013, **107**, 130-135.
2. C. Stefano, Z. Huili, Z. Jing, A. Martin, P. Daniela, P. Werner, H. Qi-Ying, B. Francesco and A. Roberto, *ChemBioChem*, 2014, **15**, 836-843.
3. Y. A. Lin, J. M. Chalker and B. G. Davis, *J. Am. Chem. Soc.*, 2010, **132**, 16805-16811.
4. B. Henri and K. Christian, *Z. Anorg. Allg. Chem.*, 2005, **631**, 2555-2562.
5. D. P. Gamblin, P. Garnier, S. J. Ward, N. J. Oldham, A. J. Fairbanks and B. G. Davis, *Org. Biomol. Chem.*, 2003, **1**, 3642-3644.
6. C. S. Barry, E. J. Cocinero, P. Çarçabal, D. P. Gamblin, E. C. Stanca-Kaposta, S. M. Remmert, M. C. Fernández-Alonso, S. Rudić, J. P. Simons and B. G. Davis, *J. Am. Chem. Soc.*, 2013, **135**, 16895-16903.
7. S. Singh, M. Scigelova, P. Critchley and D. H. G. Crout, *Carbohydr. Res.*, 1997, **305**, 363-370.
8. Y. Wang, K. El-Boubbou, H. Kouyoumdjian, B. Sun, X. Huang and X. Zeng, *Langmuir*, 2010, **26**, 4119-4125.
9. S. André, M. Lahmann, H.-J. Gabius and S. Oscarson, *Mol. Pharm.*, 2010, **7**, 2270-2279.
10. W.-Y. Lu, X.-W. Sun, C. Zhu, J.-H. Xu and G.-Q. Lin, *Tetrahedron*, 2010, **66**, 750-757.
11. T. Angles d'Ortoli and G. Widmalm, *Tetrahedron*, 2016, **72**, 912-927.
12. A. Kitowski, E. Jiménez-Moreno, M. Salvadó, J. Mestre, S. Castillón, G. Jiménez-Osés, O. Boutureira and G. J. L. Bernardes, *Org. Lett.*, 2017, **19**, 5490-5493.
13. G. H. Veeneman and J. H. van Boom, *Tetrahedron Lett.*, 1990, **31**, 275-278.

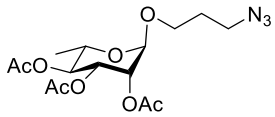


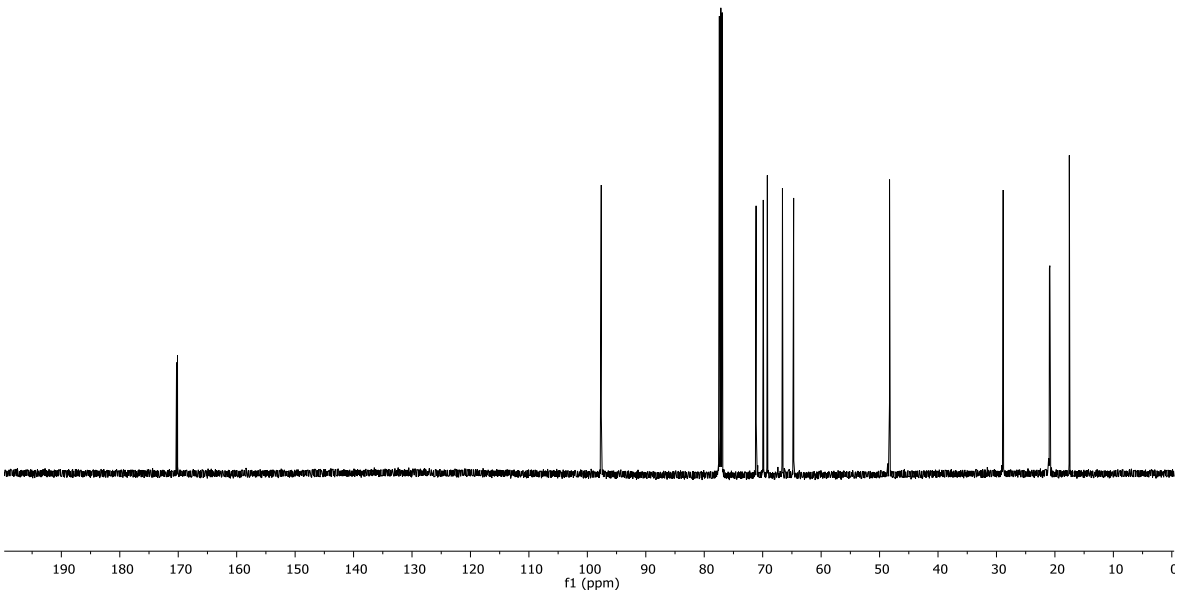
Antigen	# of Antigens per BSA Molecule
Linker	42
Rha	35
β-Glc	25
α-Glc	31
GAGB4	25
GAGA4	25
GAGAGB4	21
BSA	

Supplementary Figure S1. MALDI-TOF spectra of antigen-conjugated BSA, where the depletion of unconjugated BSA indicated successful conjugation. From top to bottom: Linker-BSA, Rha-BSA, β-Glc-BSA, α-Glc-BSA, GAGB4-BSA, GAGA4-BSA, GAGAGB4-BSA, BSA only.

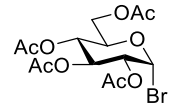
NMR Spectra

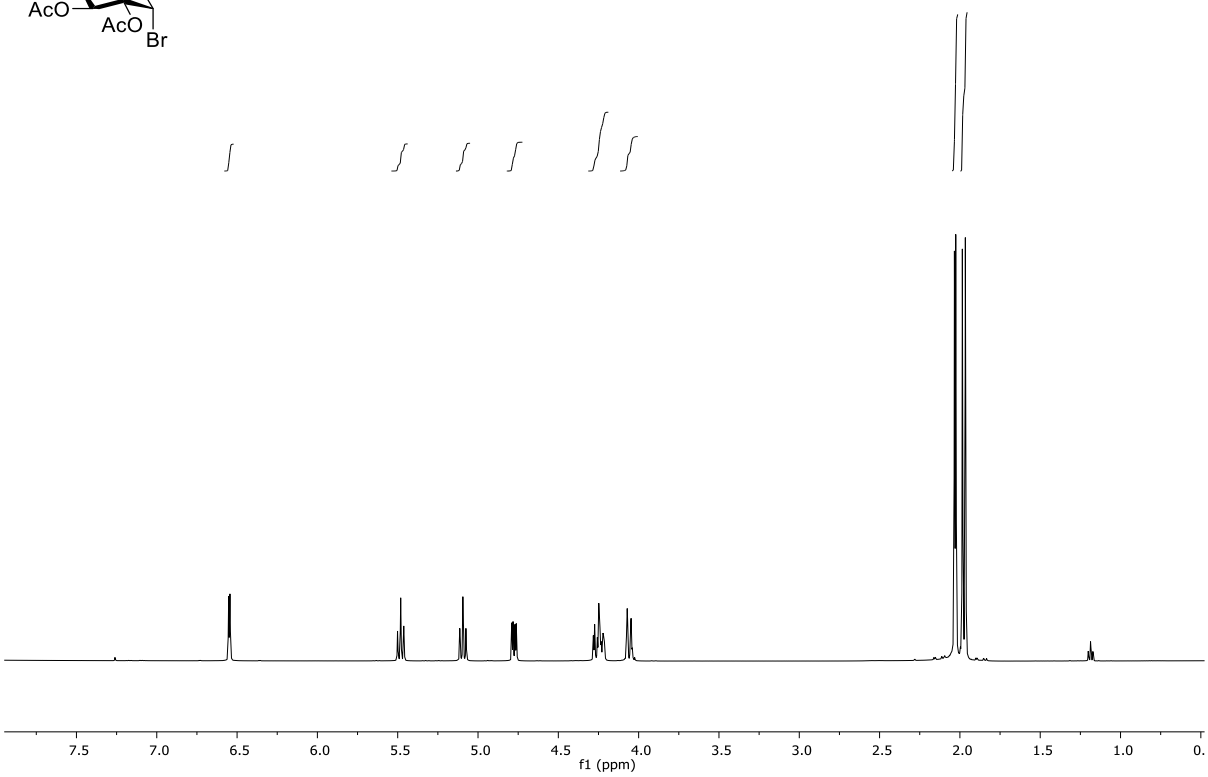
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-Azidopropyl 2,3,4,-tri-*O*-acetyl-α-L-rhamnopyranoside (14)**

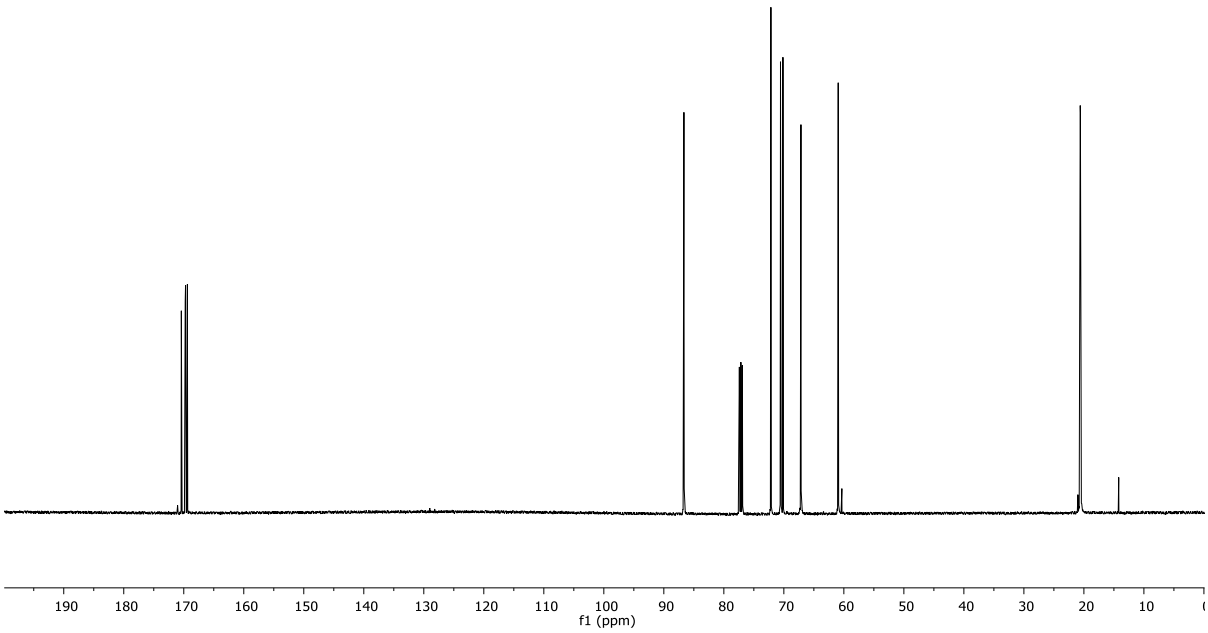




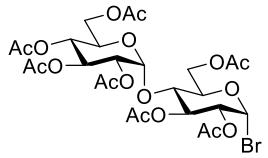
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **2,3,4,6-Tetra-*O*-acetyl-α-D-glucosyl bromide**

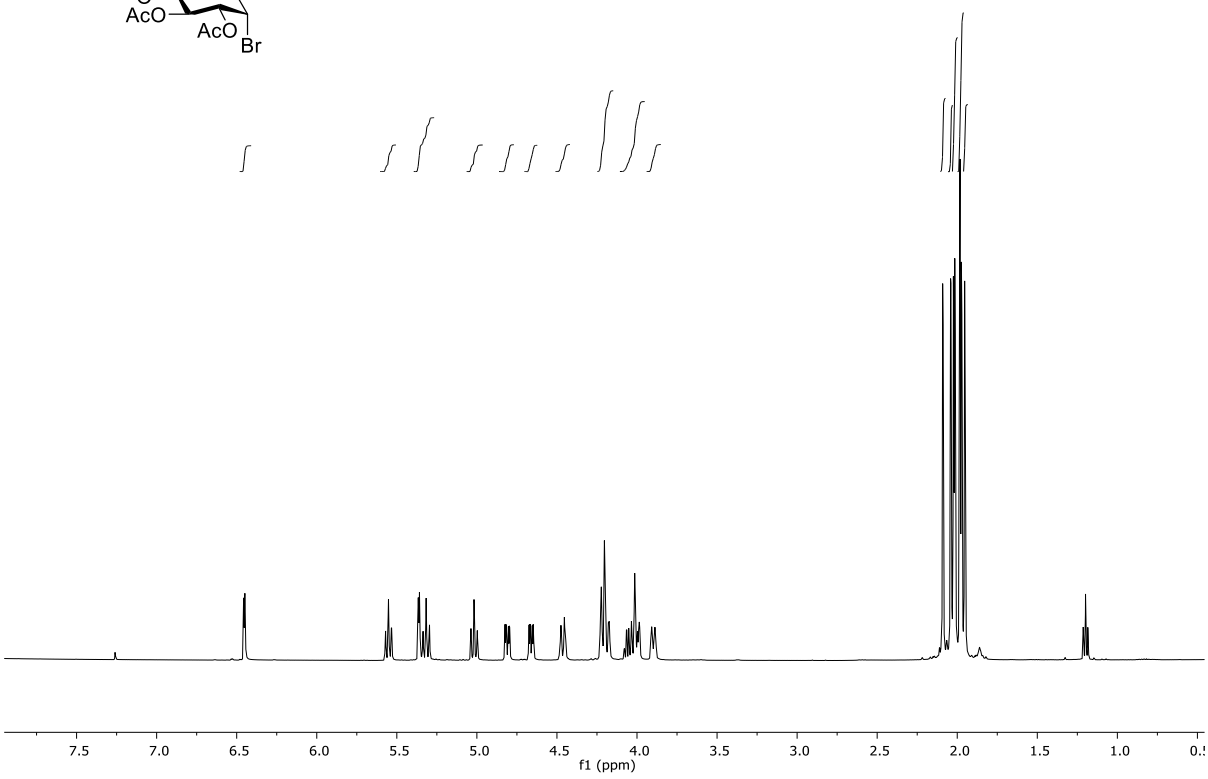


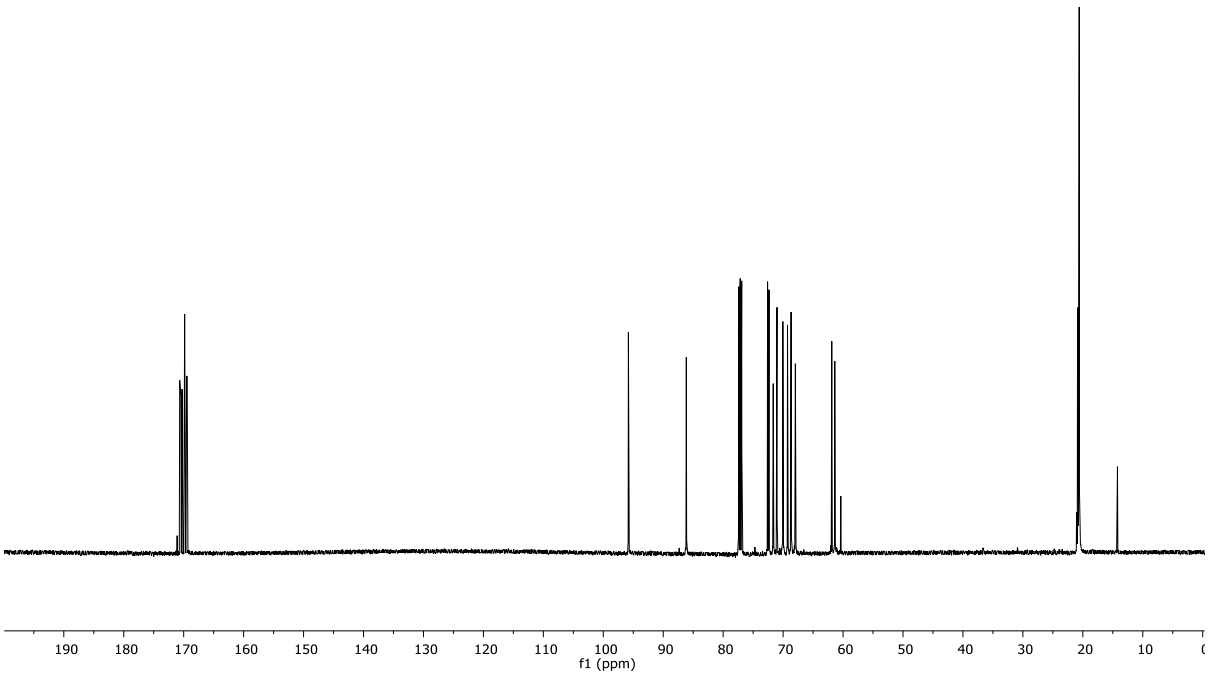




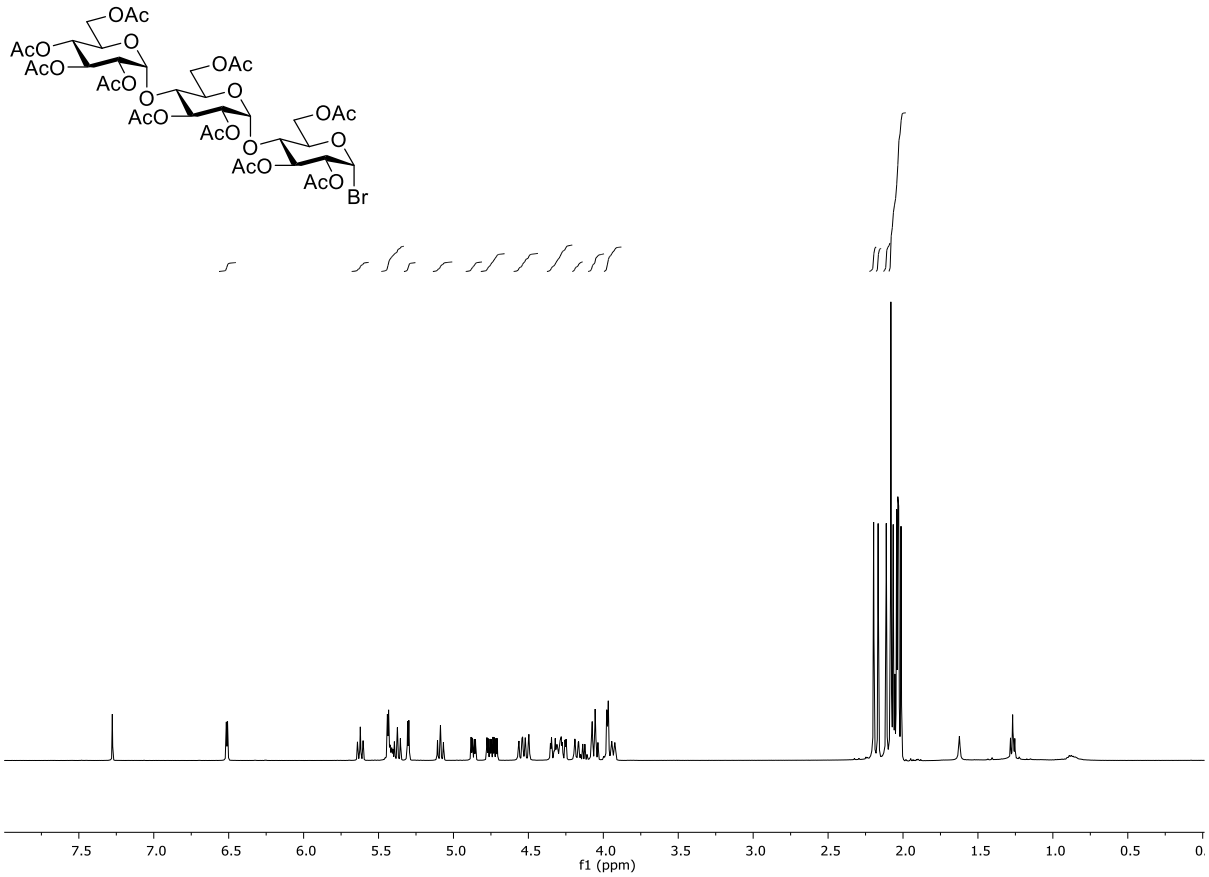
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-α-D-maltosyl bromide**

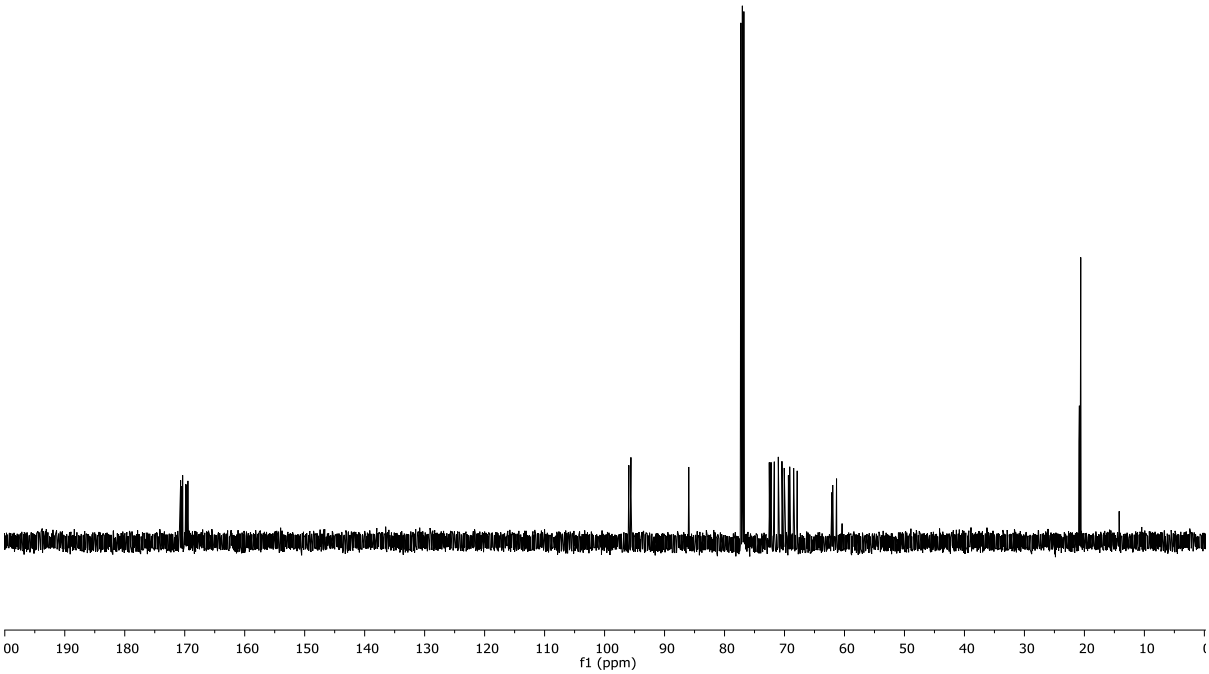




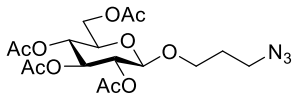


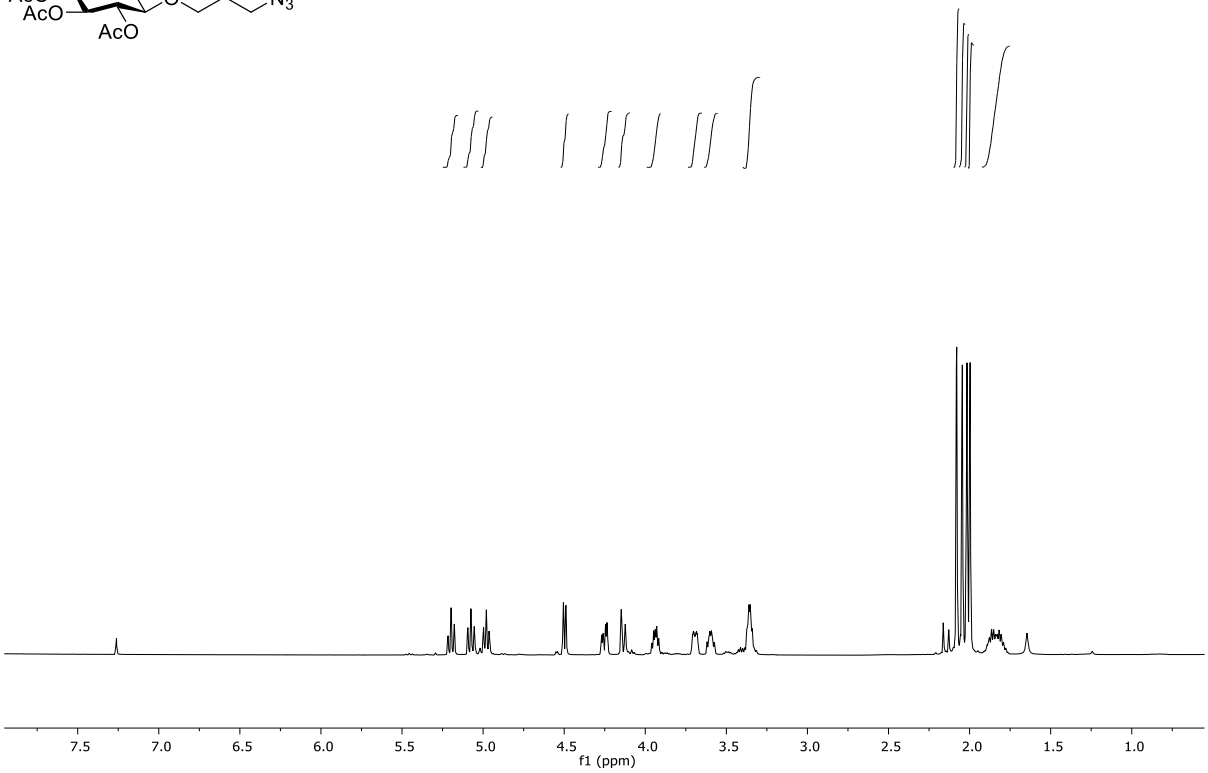
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **2,3,6,2′,3′,6′,2″,3″,4″,6″-Deca-*O*-acetyl-α-D-maltotriosyl bromide**

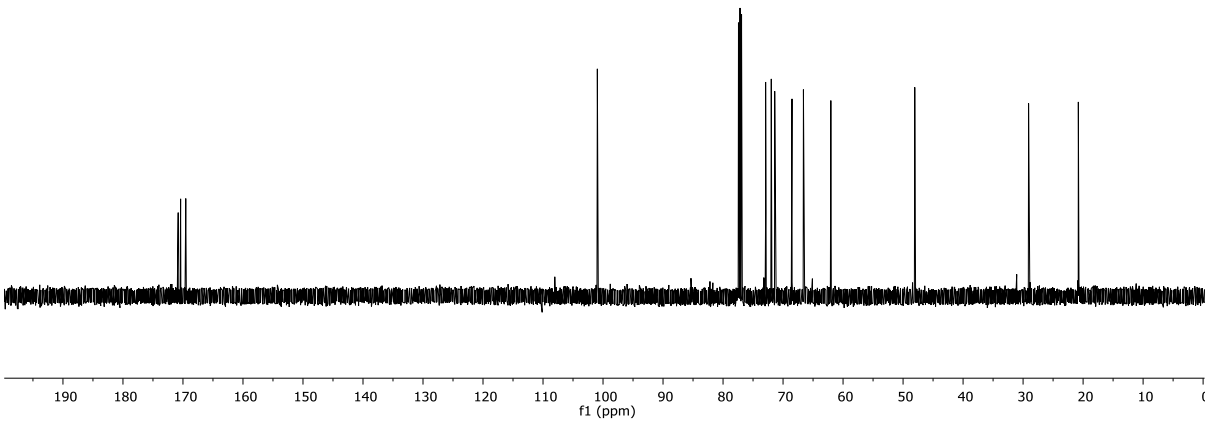




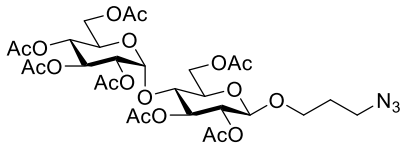
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside**

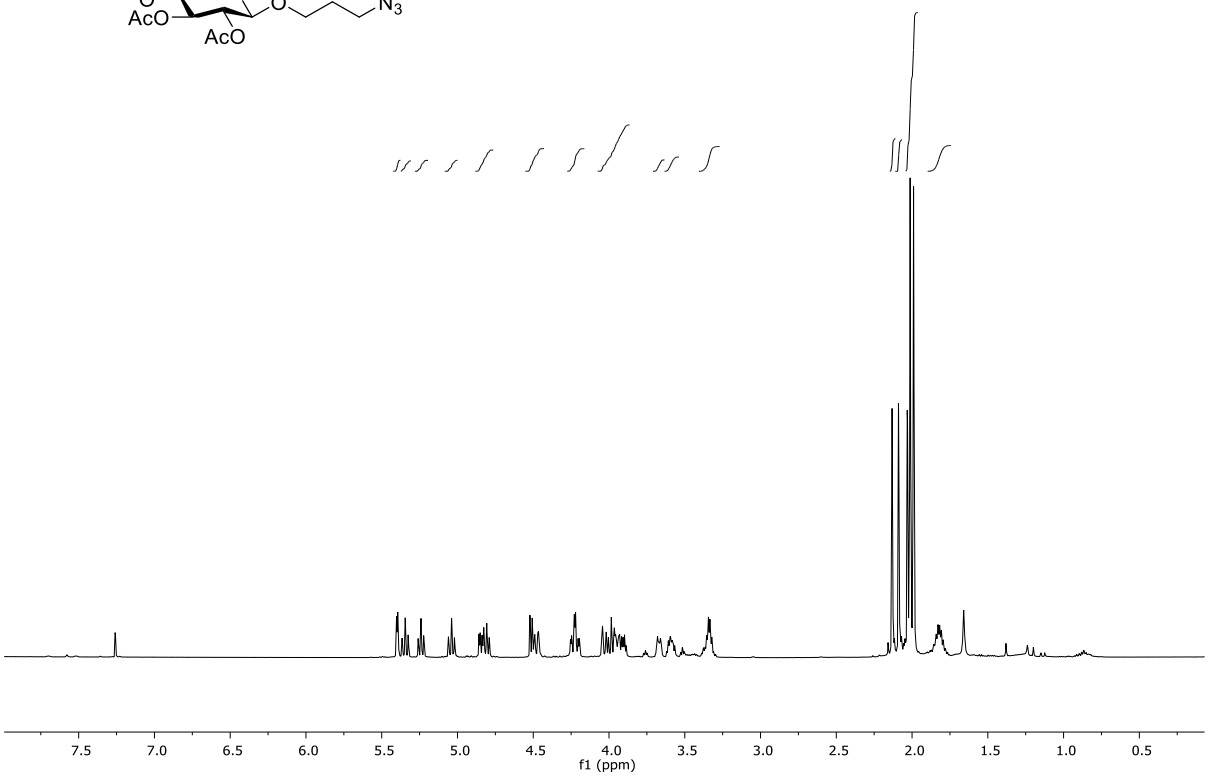


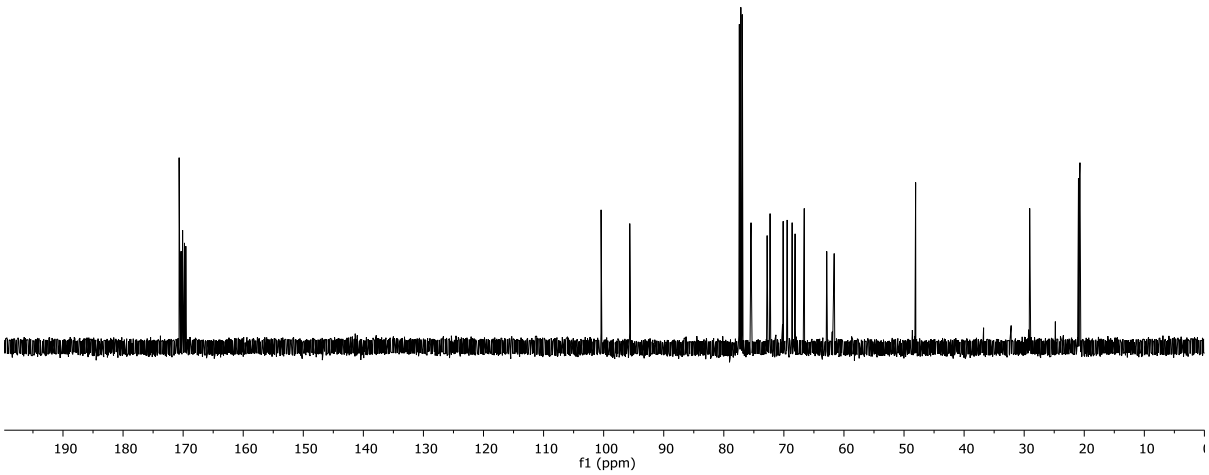




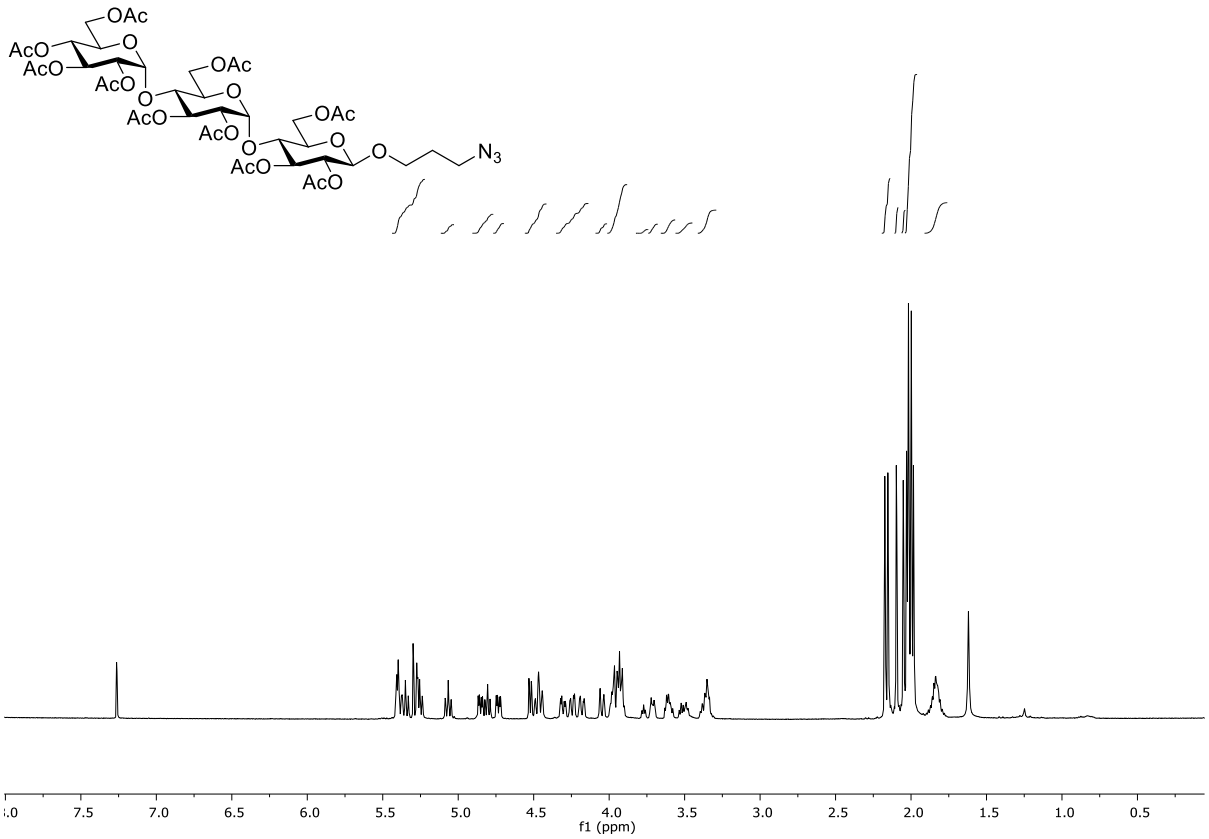
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-Azidopropyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-maltoside**

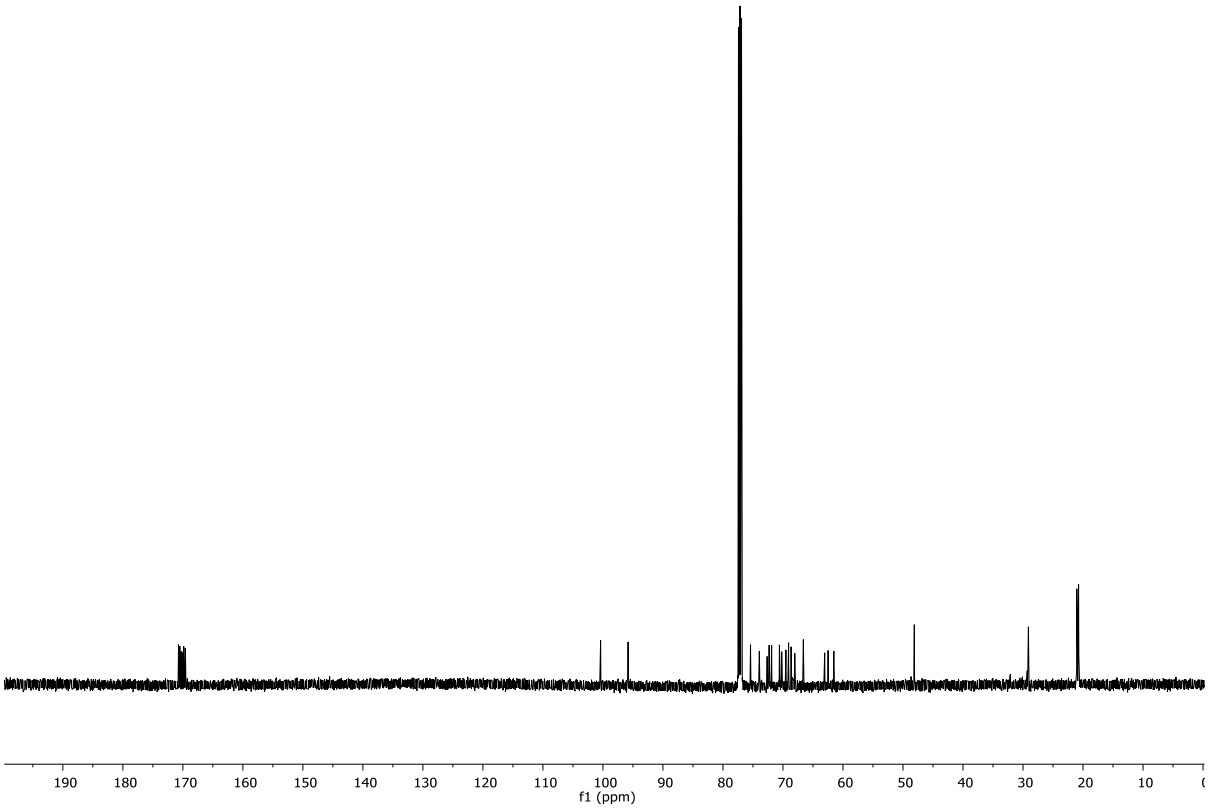




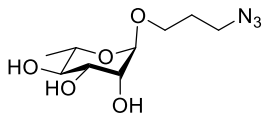


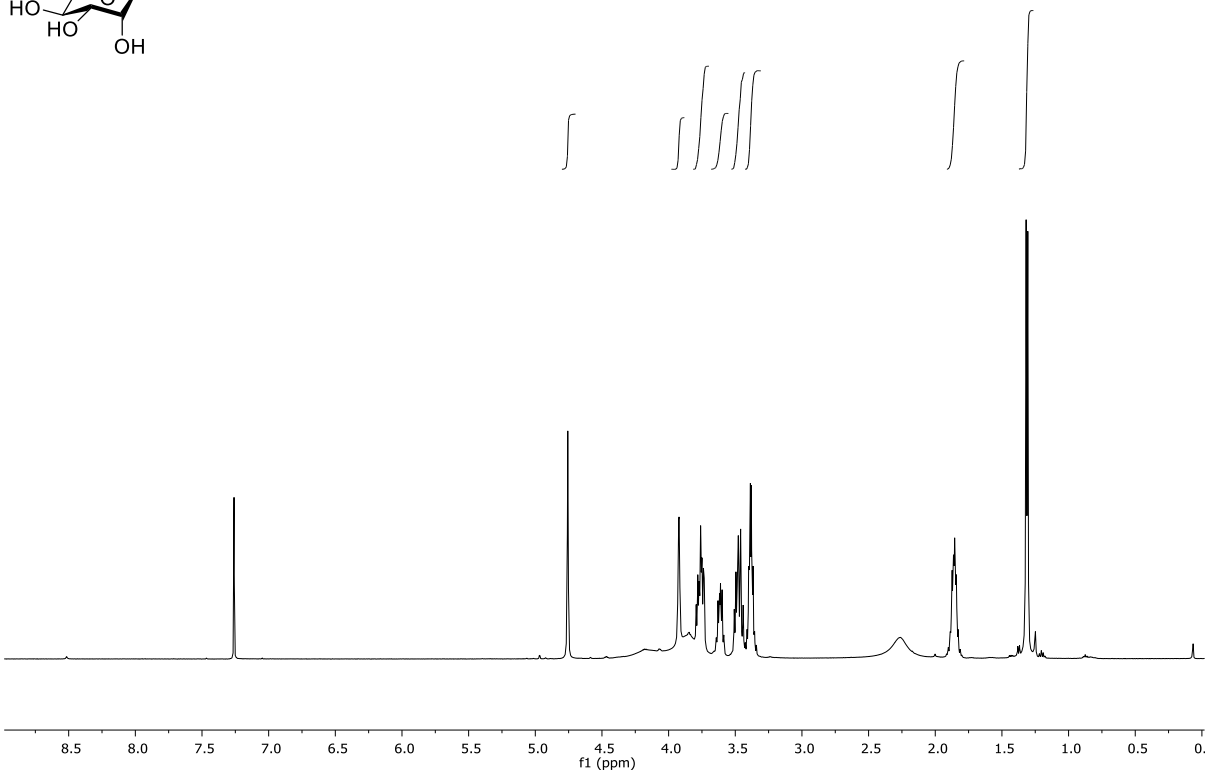
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-Azidopropyl 2,3,6,2′,3′,6′,2″,3″,4″,6″-deca-*O*-acetyl-β-D-maltotriopyranoside**

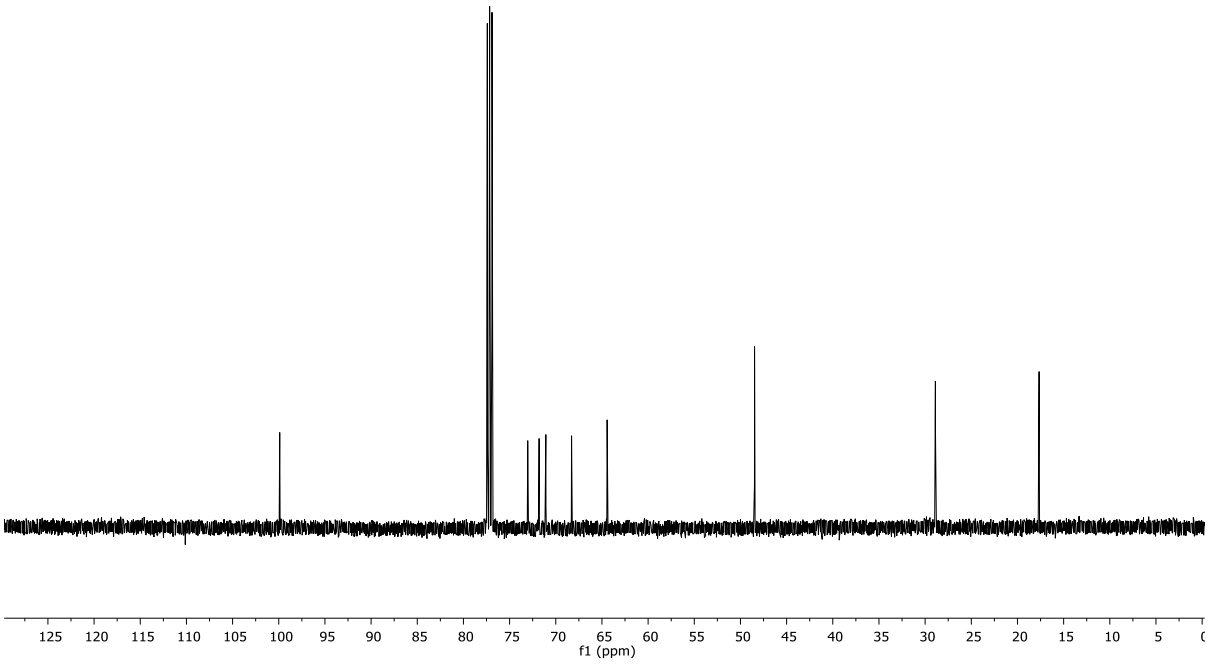




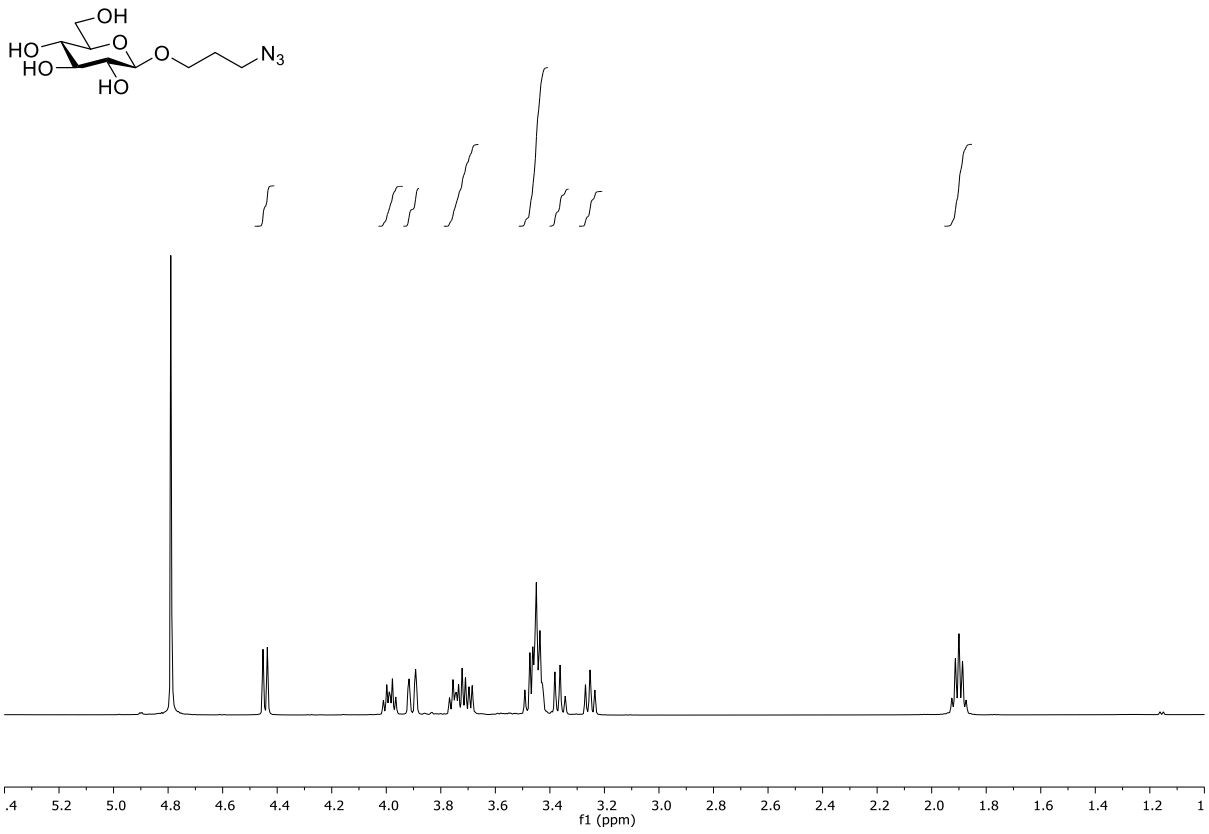
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-Azidopropyl α-L-rhamnopyranoside (15)**

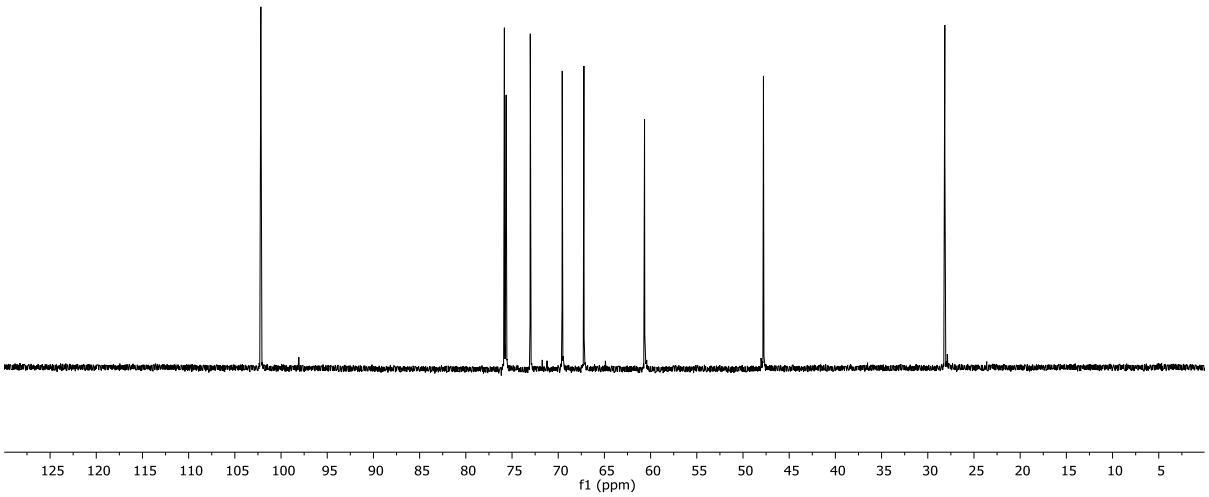




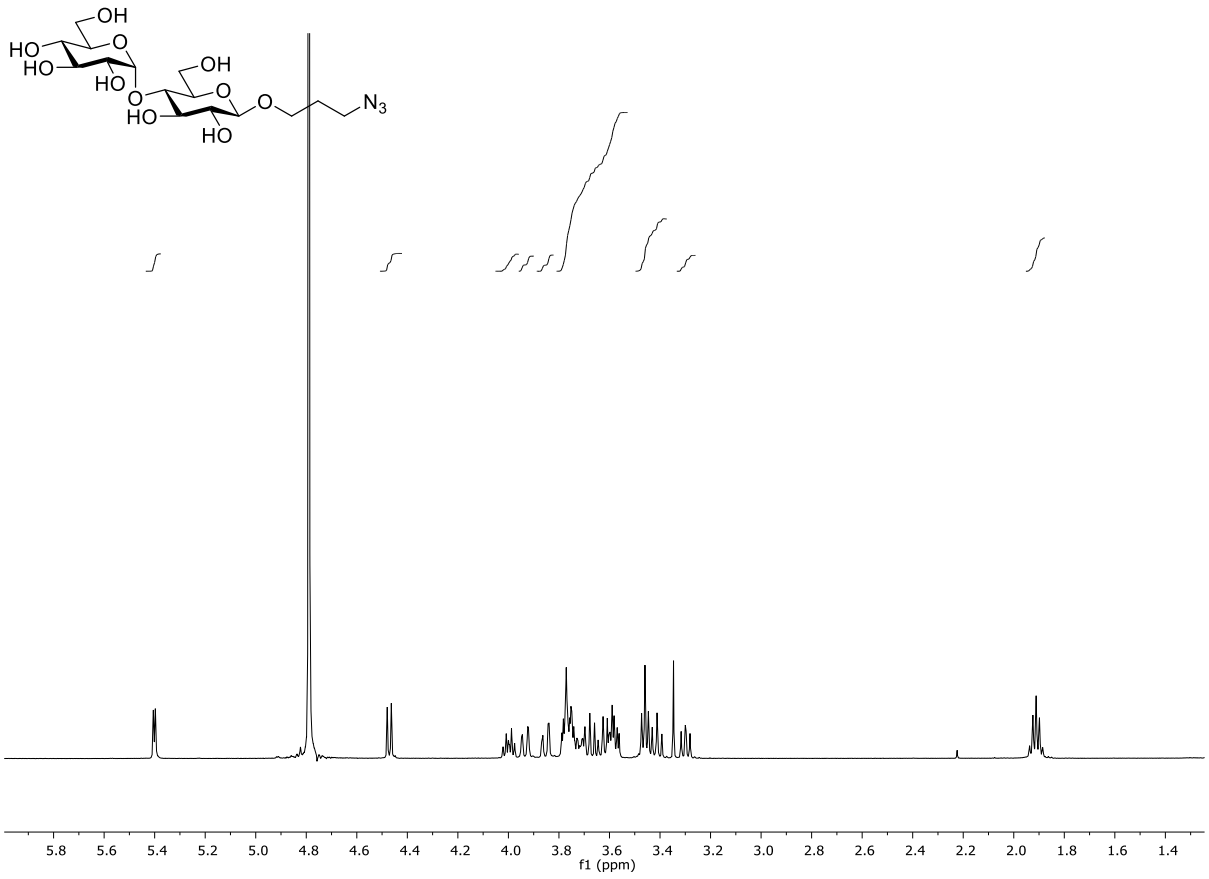


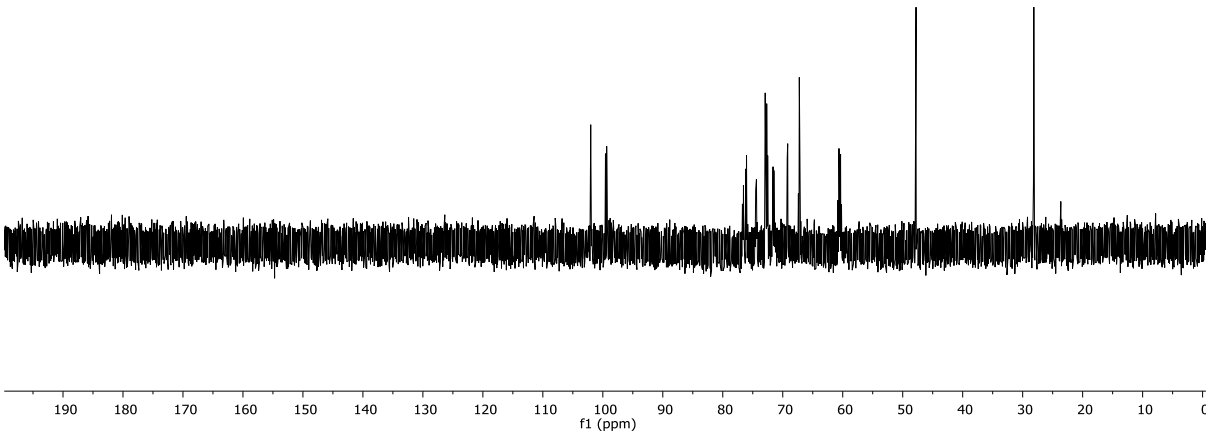
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-Azidopropyl β-D-glucopyranoside (20a)**



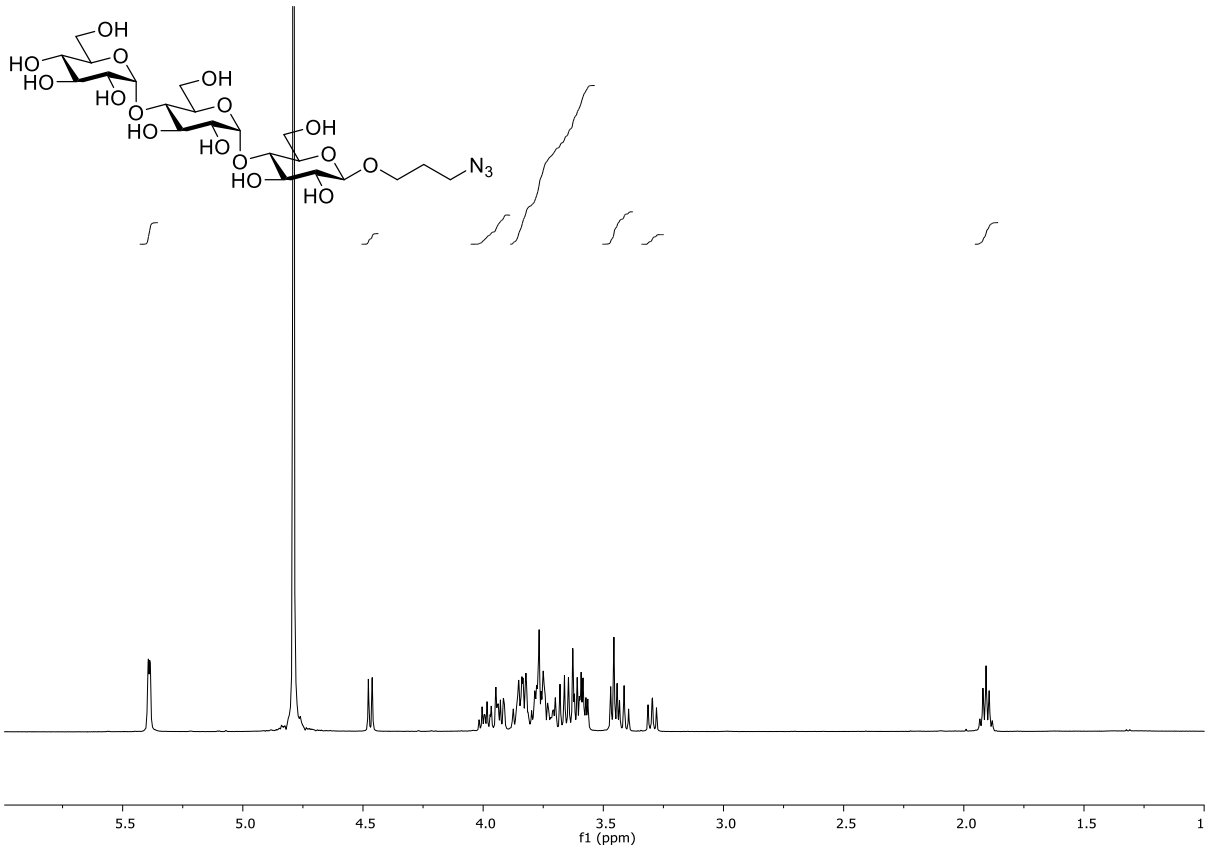


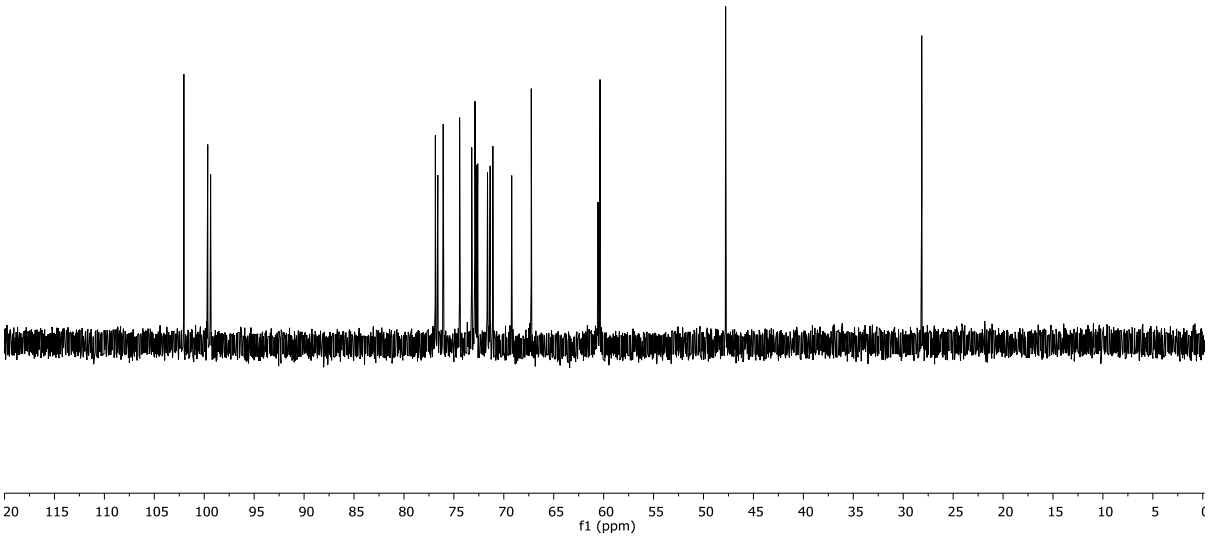
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-Azidopropyl β-D-maltopyranoside (20b)**



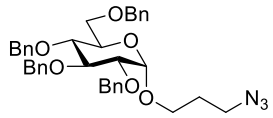


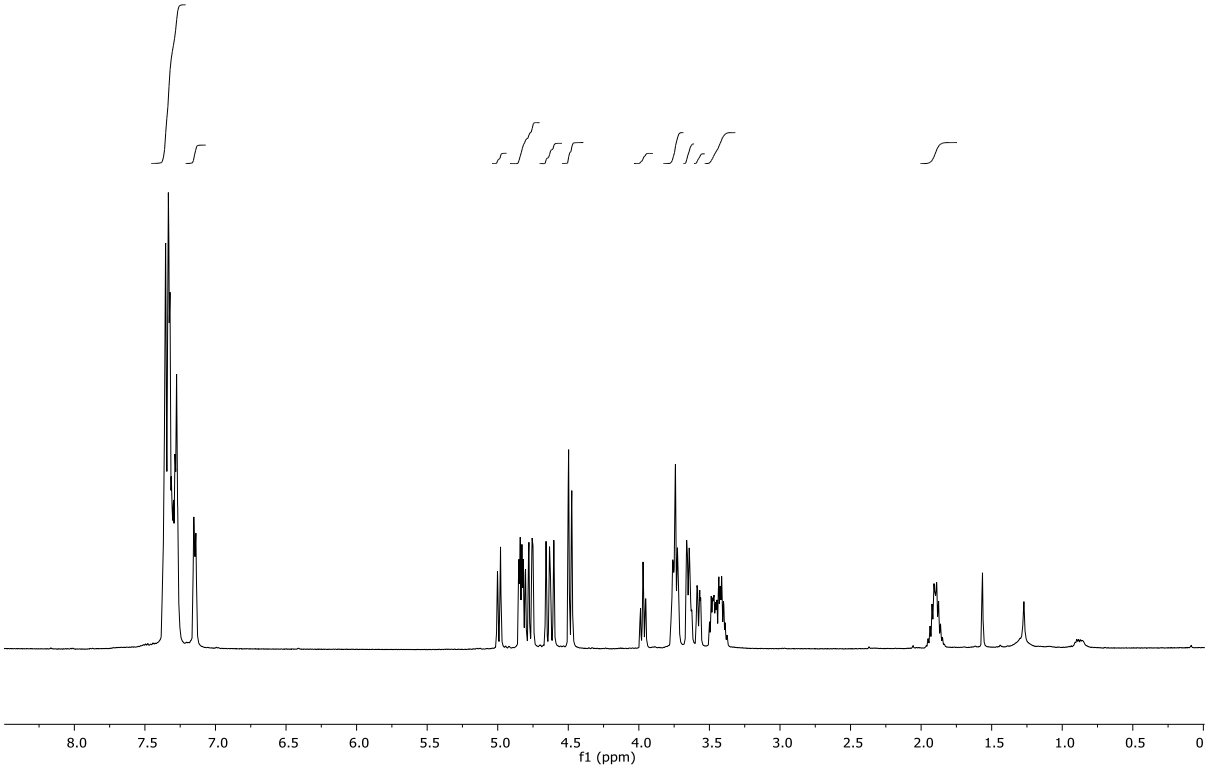
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-Azidopropyl β-D-maltotrioside (20c)**

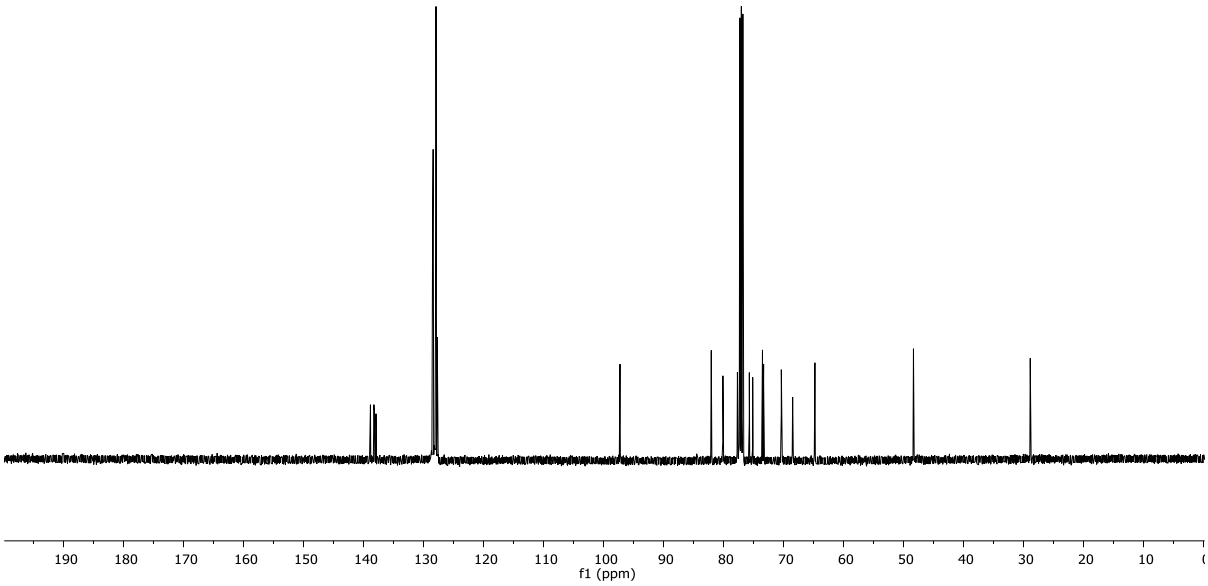




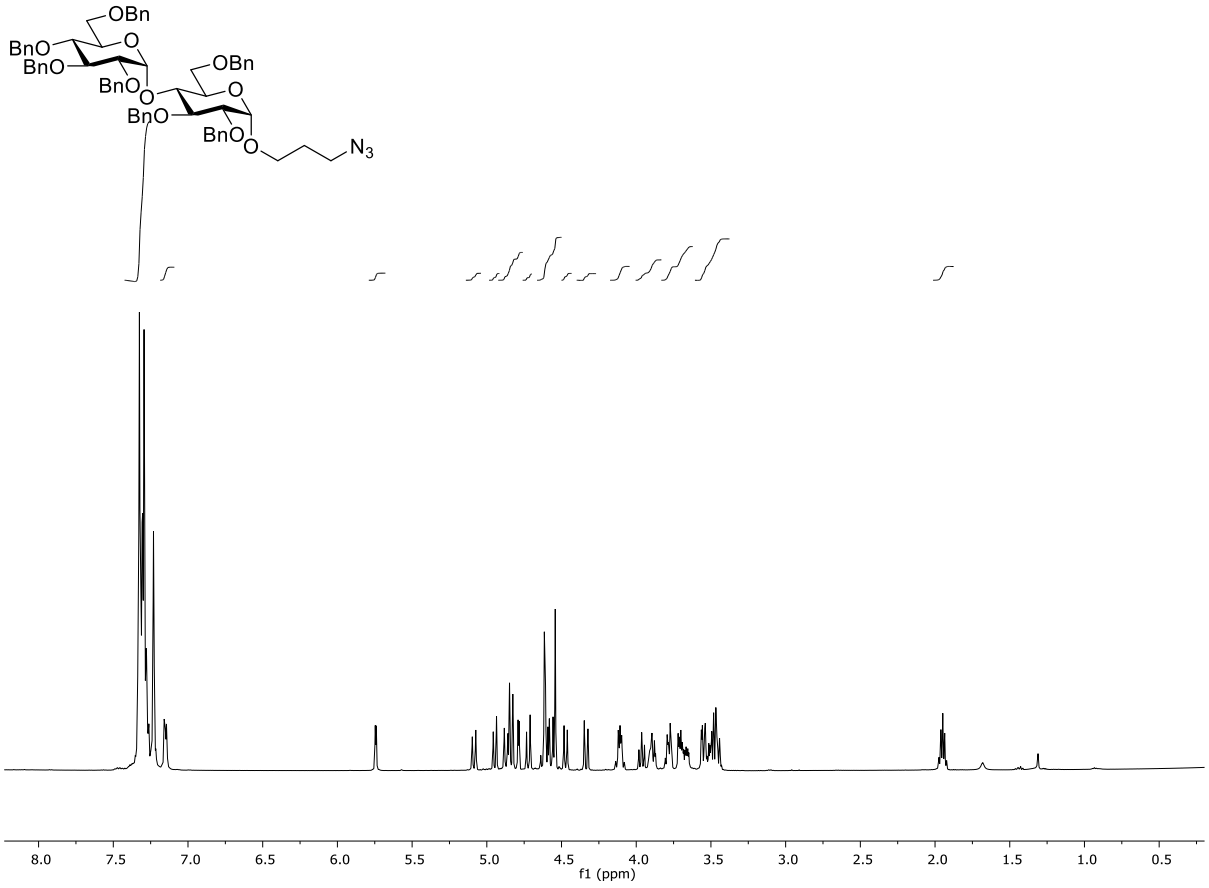
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (19a)**

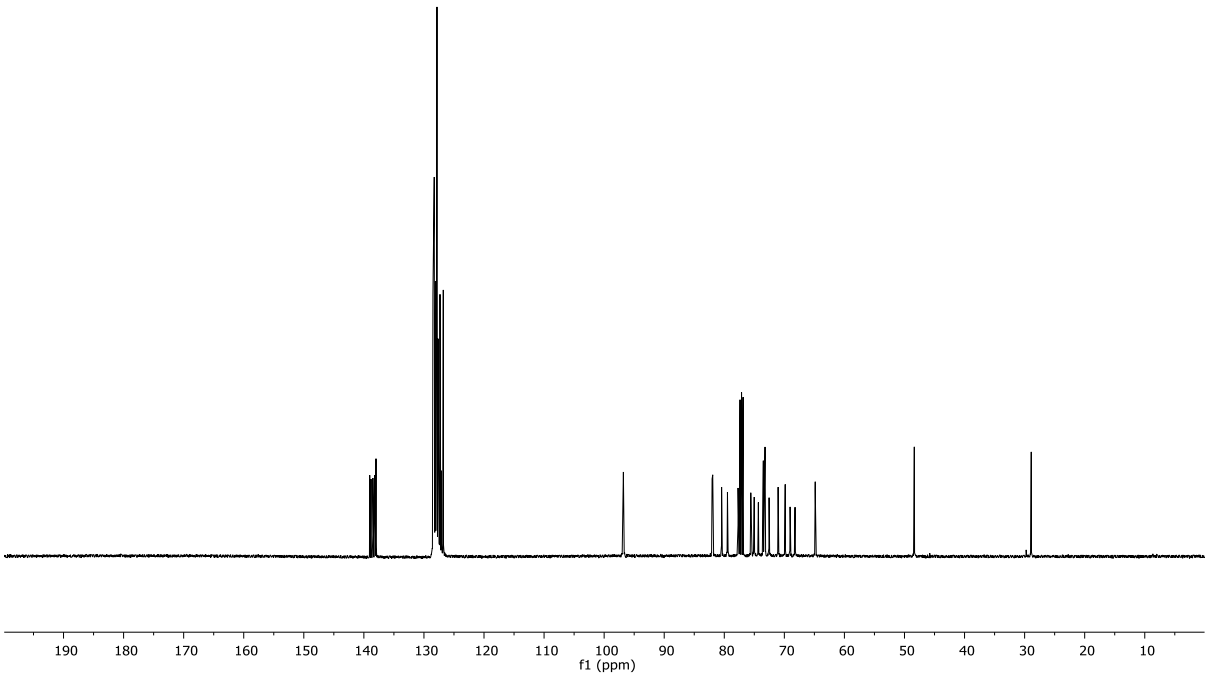




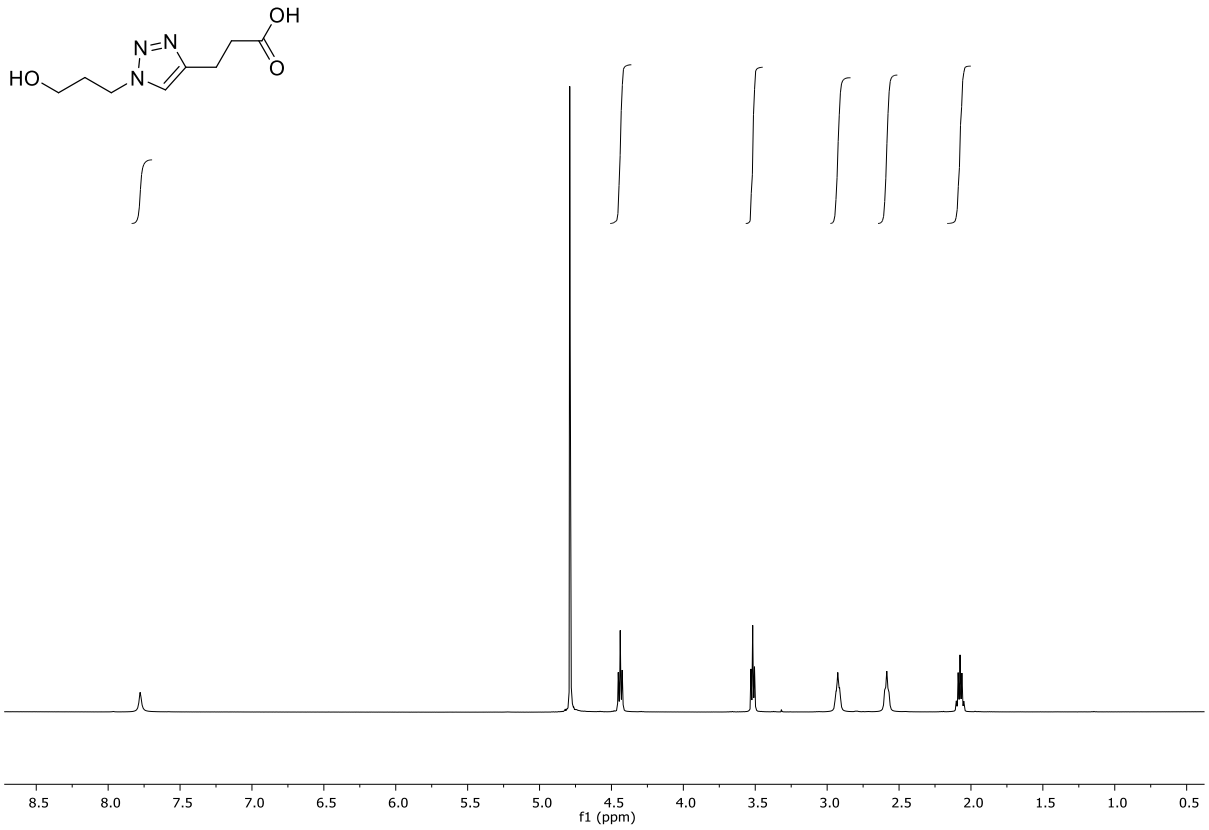


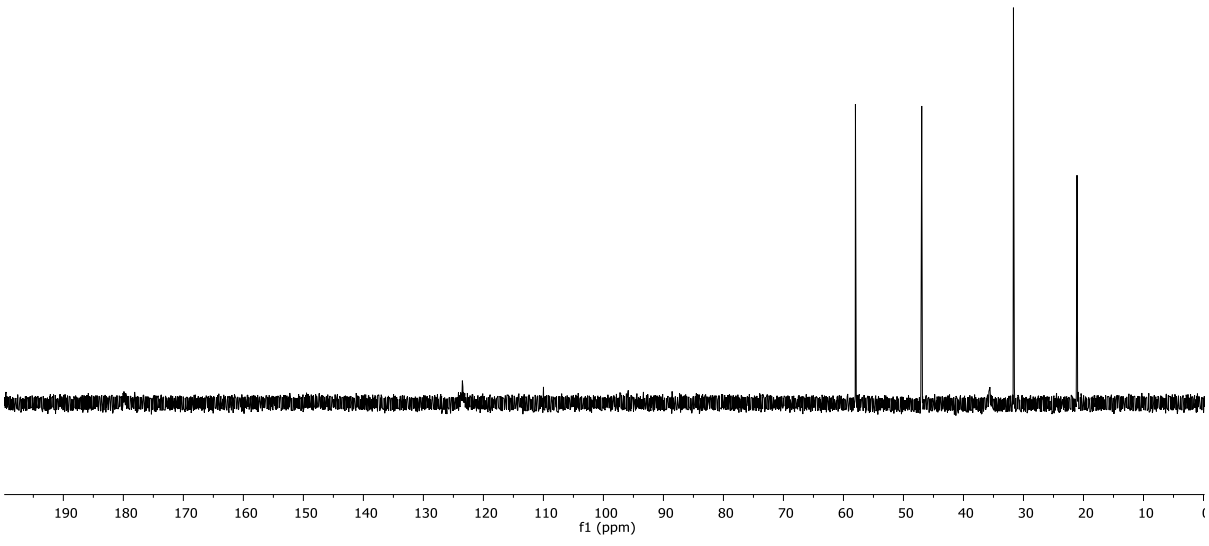
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-Azidopropyl 2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-D-maltoside (19b)**



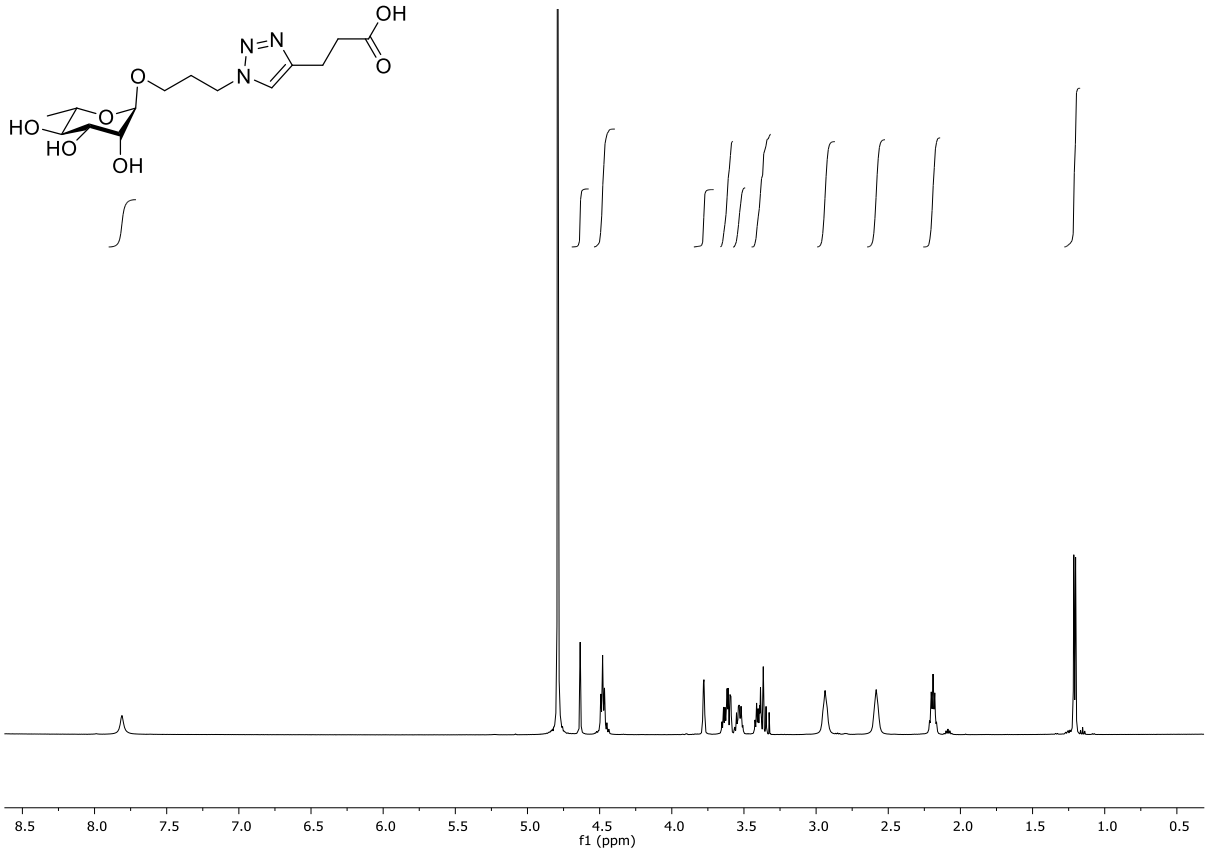


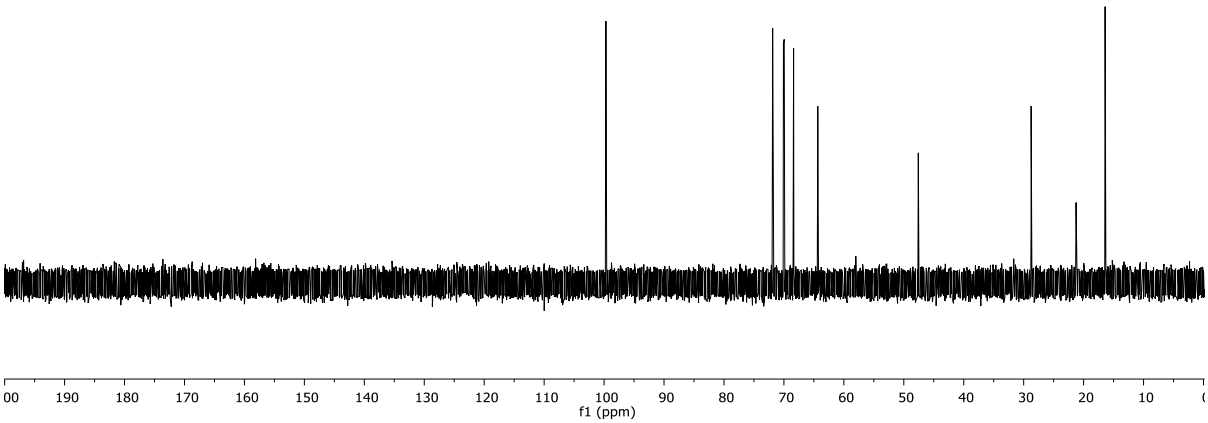
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-(1-(3-Hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (16a)**



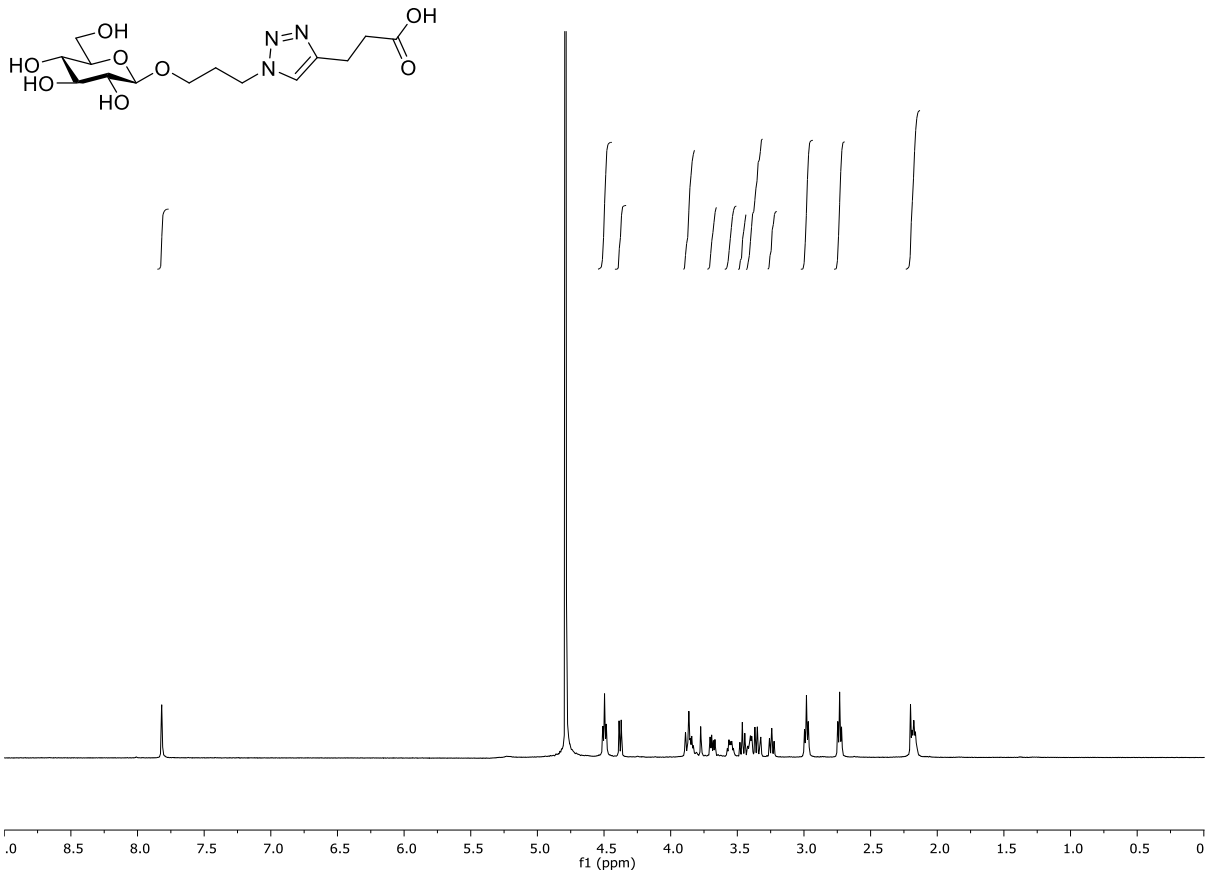


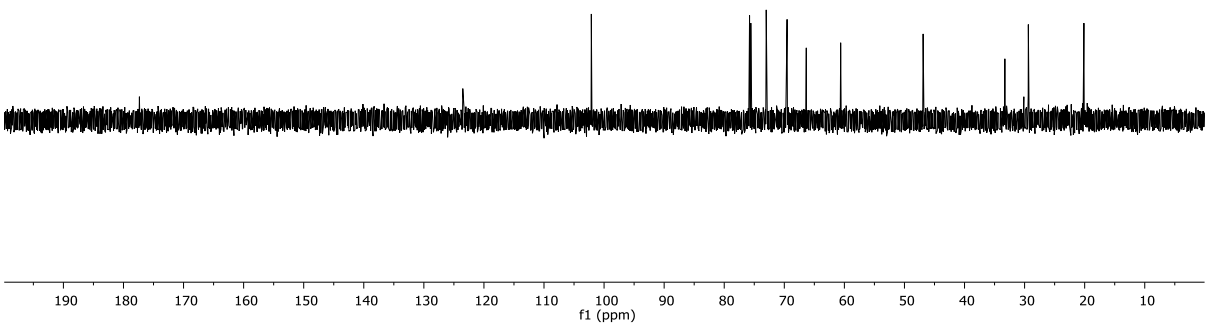
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-(1-(3-(α-L-Rhamopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (16b)**



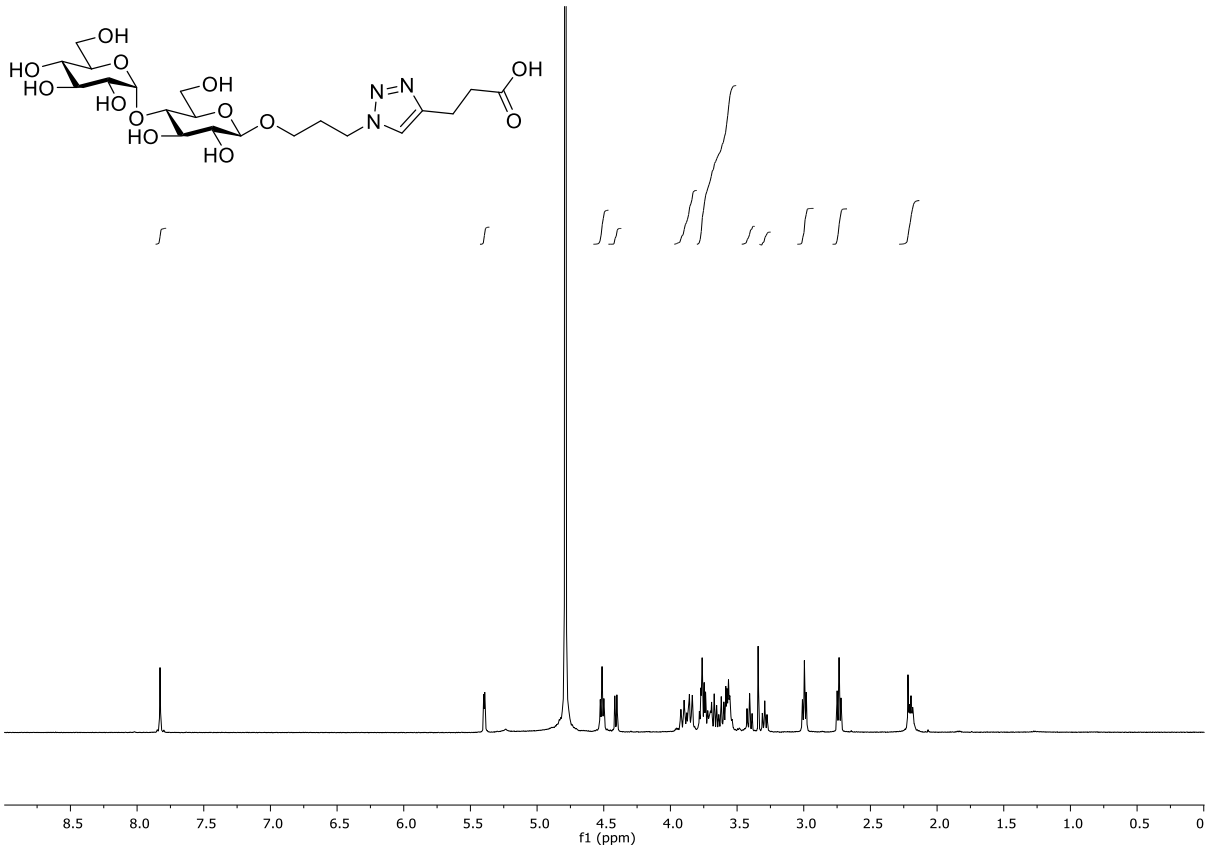


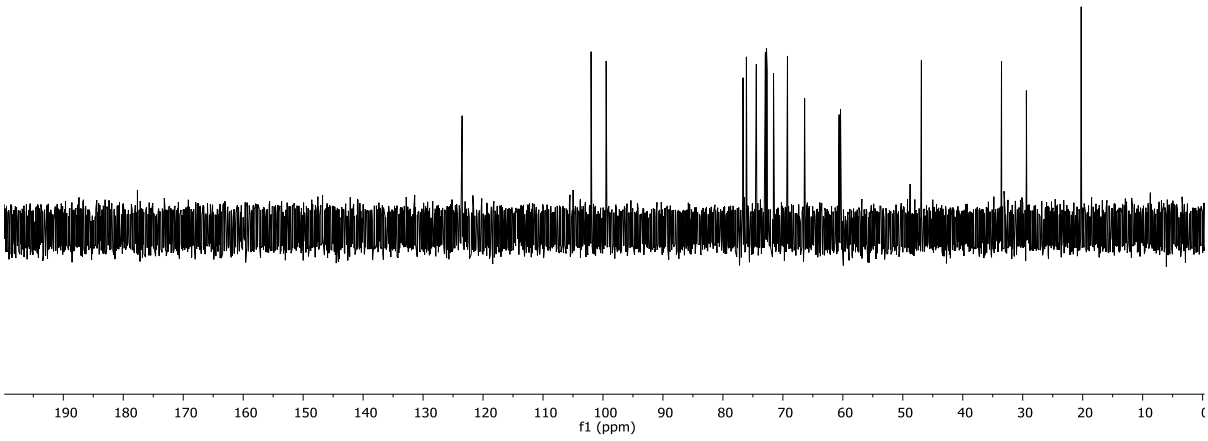
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-(1-(3-(β-D-Glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid**



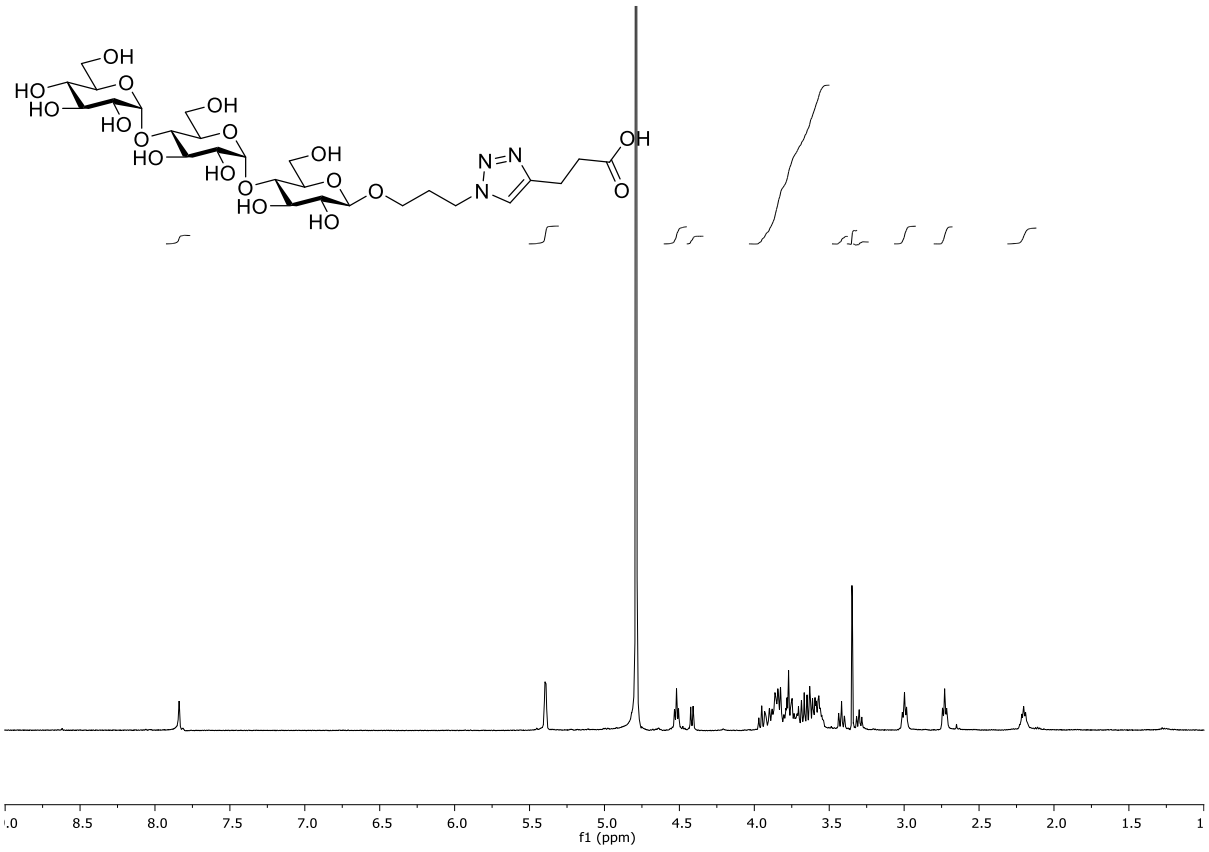


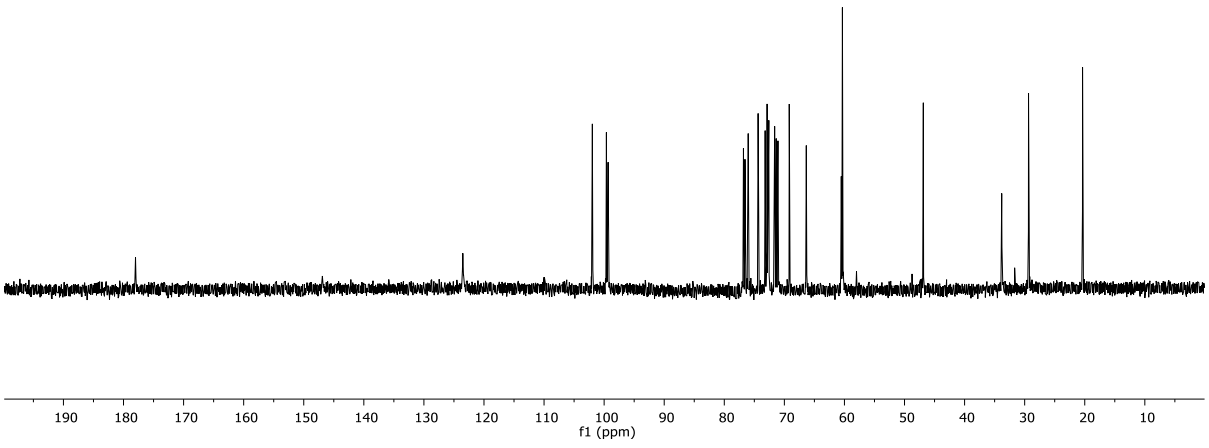
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-(1-(3-(β-D-Maltosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid**



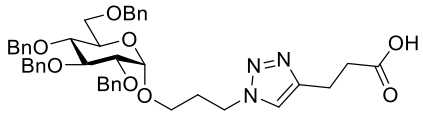


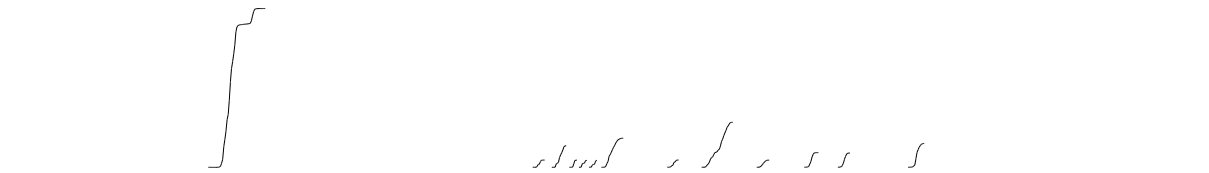
^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-(1-(3-(β-D-Maltotriosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid**

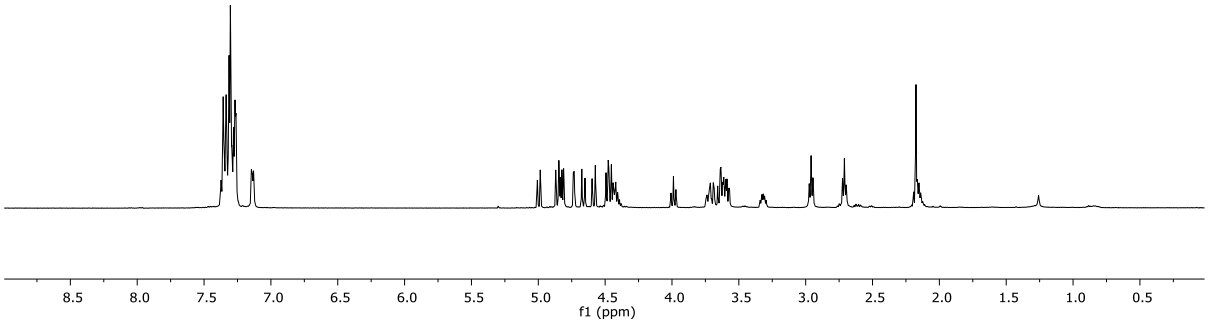


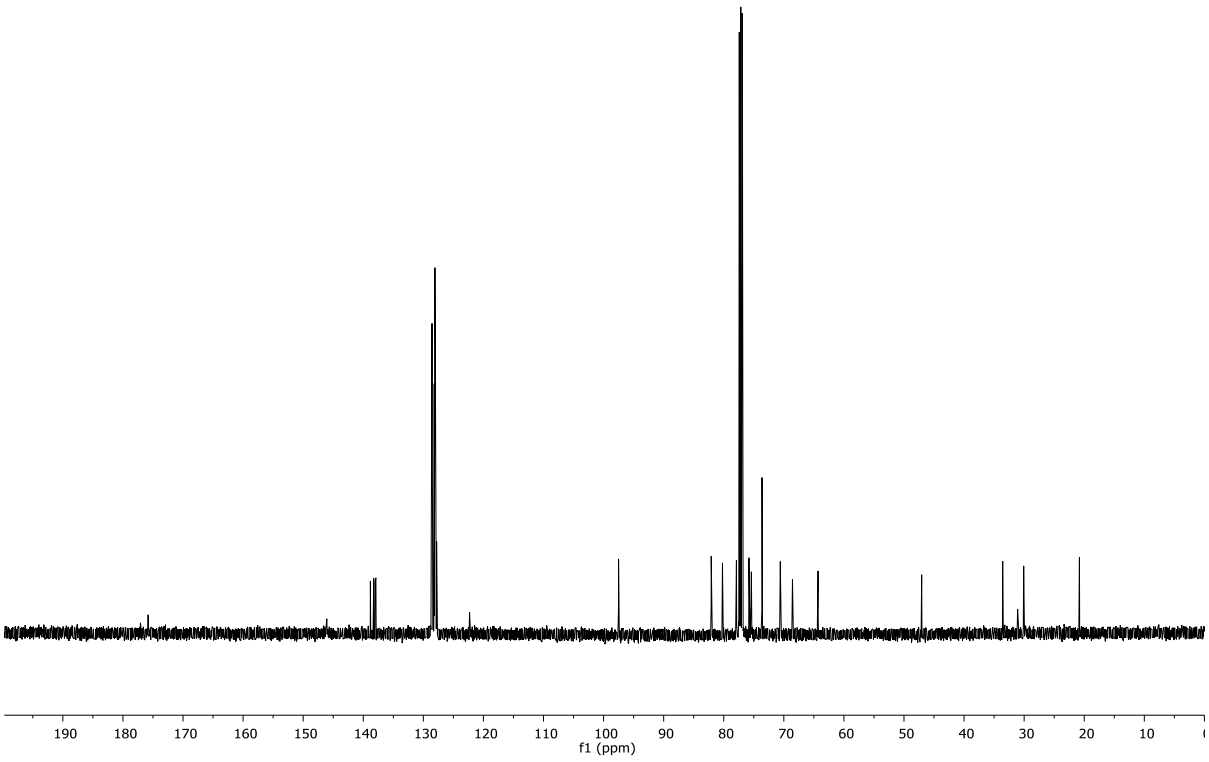


^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-(1-(3-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid**

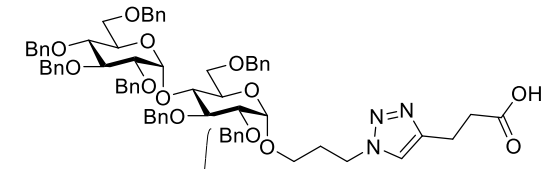


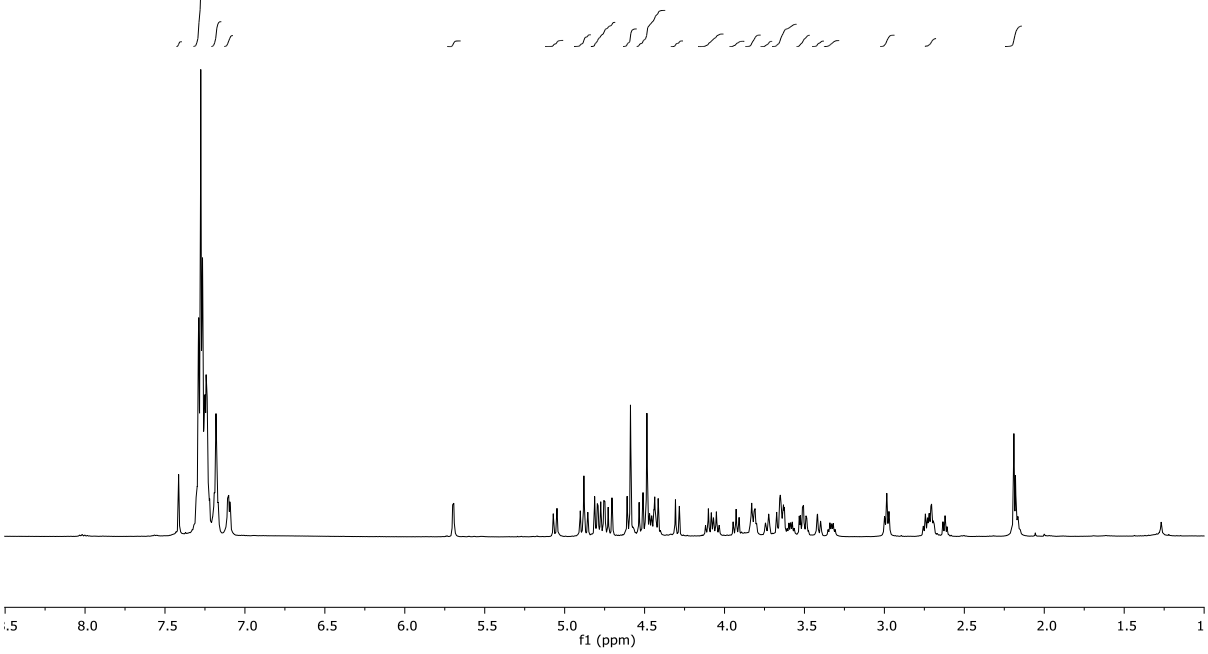


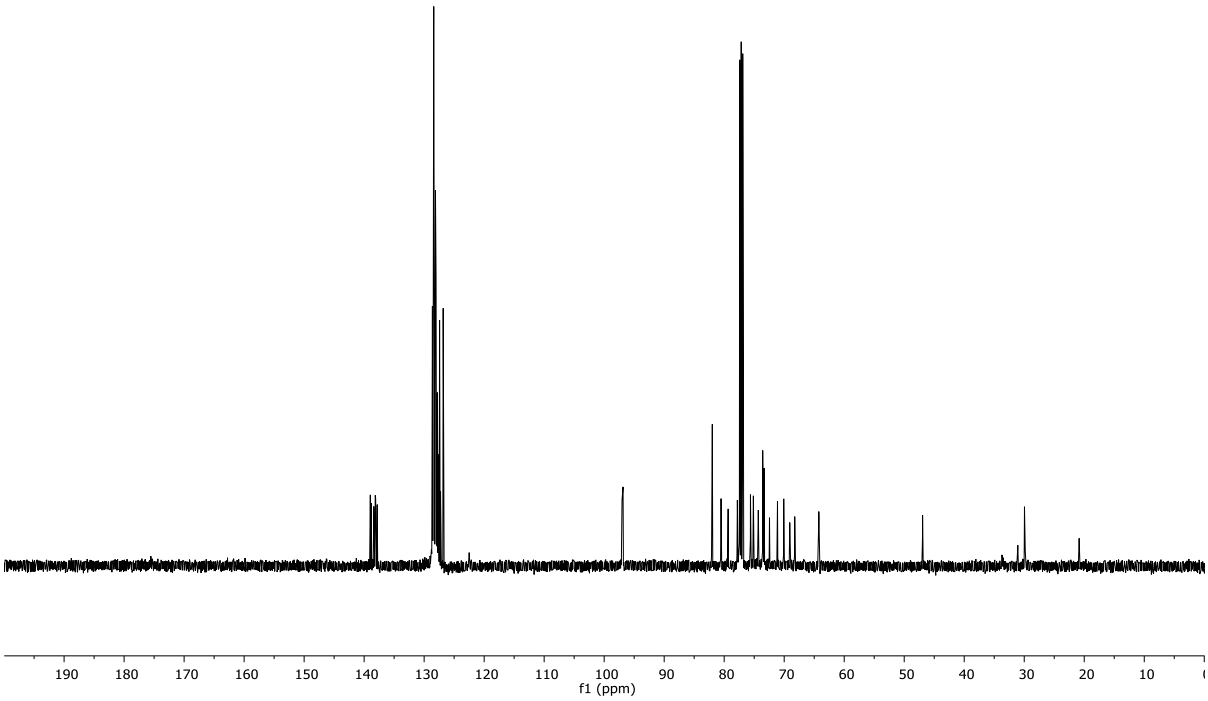




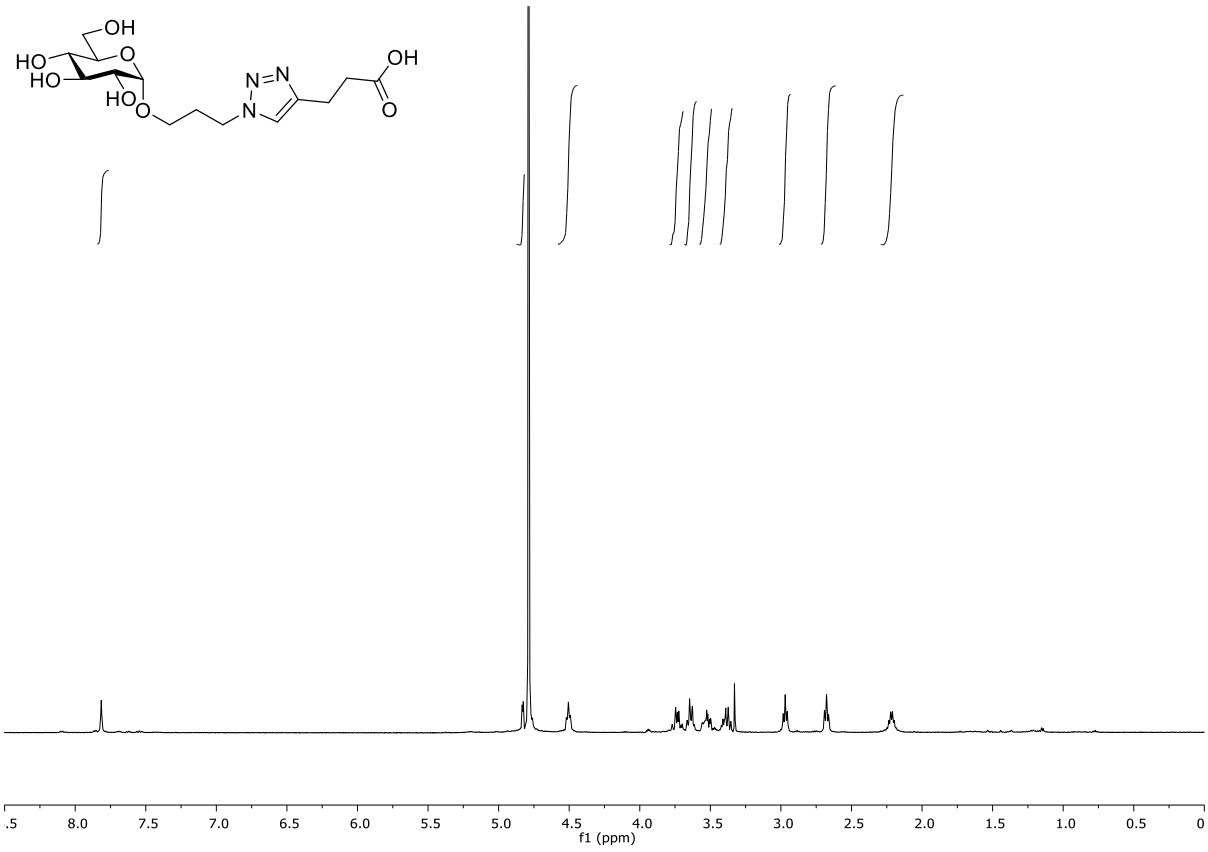
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **3-(1-(3-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-*α*-D-maltopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid**

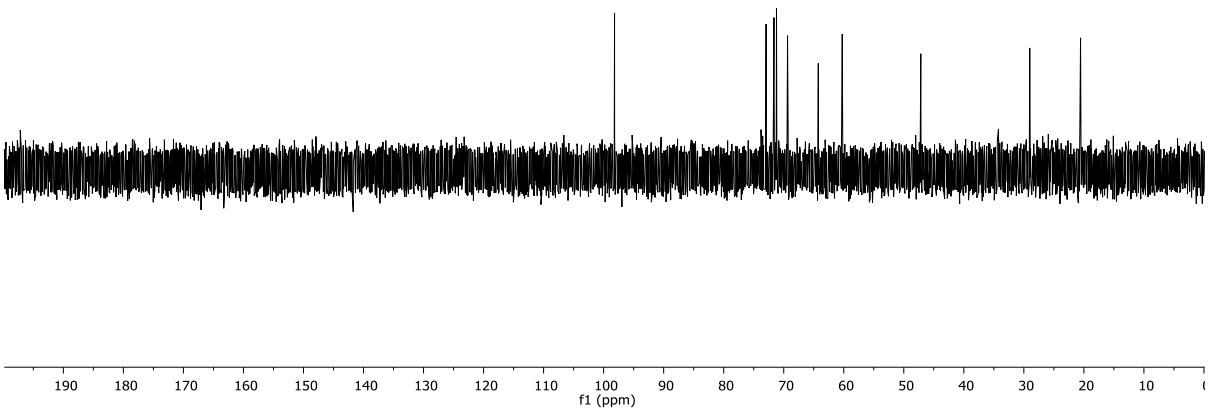






^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-(1-(3-(α-D-Glucopyranosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid**





^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) of **3-(1-(3-(α-D-Maltosyloxy)propyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid**