Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information

Synthetic glycopolymers as modulators of protein aggregation: influence of chemical composition, topology and concentration.

Joao do O,[a] Francesca Mastrotto,[b] Nora Francini,[a] Stephanie Allen,[a] Christopher F. van der Walle,[c] Snjezana Stolnik, [a] Giuseppe Mantovani[a]

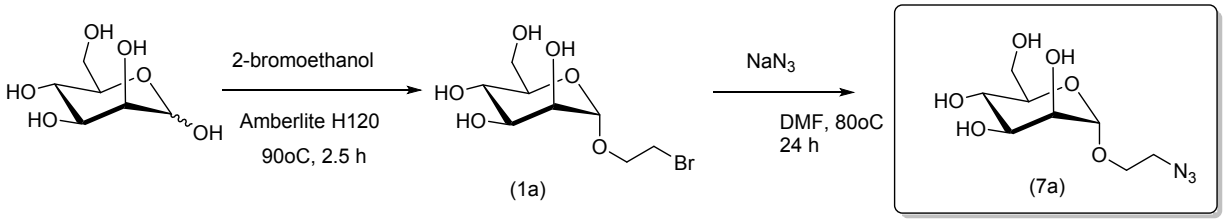
[a]Molecular Therapeutics and Formulation Division, School of Pharmacy, University of Nottingham, NG7 2RD, Nottingham, UK. [b]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via F. Marzolo 5, 35131 Padova, Italy Formulation Sciences, [c]Medimmune Ltd, Cambridge, CB21 6GH

Synthesis of sugar azides

Reagents. Benzyl 2-bromo-2-methylpropanoate initiator and (E)-*N*-ethyl-1-(pyridin-2-yl)methanimine ATRP ligand were prepared as described previously.[1] Trimethylsilyl propargyl methacrylate monomer, 2'-azidoethylsugars, and polymers were prepared as described in the supporting information. D-(+)- mannose, D-(+)- galactose, D-(+)-arabinose and *N*-acetyl-glucosamine were purchased from Alfa Aesar and used as received. Trehalose dihydrate was purchased from CalcioBiochem and used without any

further purification. Lactose and Cu(I)Br were purchased from Sigma Aldrich and used as received. All other reagents and solvents were obtained at the highest purity available from Aldrich Chemical Company or Fischer Scientific and used without further purification unless otherwise stated.

1. Synthesis of 2'-azidoethyl mannopyranoside (7a)



Scheme S1. Synthesis of 2'-azidoethyl mannopyranoside **(7a)**.

2'-bromoethyl mannopyranoside (1a)[2]. Amberlite H-120 (15 g) was suspended in 2-bromoethanol (60.0 mL, 847 mmol) and the mixture was heated at 90°C in a 250 mL round bottom flask equipped with a short pad of cotton wool to absorb the H_2O produced in the condensation reaction. After 30 min, solid D-mannose (15 g, 83 mmol) was added in a single portion. Samples were taken periodically for ^{13}C NMR analysis to assess reaction progression. After stirring at 90°C for further 2.5 h the suspension was filtered through a very short cotton wool pad to remove the solid catalyst, washing the filtered solid with additional 2-bromoethanol. An aliquot of the reaction was diluted with deuterium oxide and analysed by ^{13}C NMR. The spectrum revealed absence of starting material and the presence of glycoside **(1a)** with a diagnostic C1 anomeric carbon atom signal at 99.9 ppm. The excess of 2-bromoethanol was distilled off under reduced pressure, and the resulting sticky orange residue was dissolved in

MeOH and precipitated with Et_2O. The supernatant was decanted off and the residue was dissolved in water and washed five times with 100 mL of dichloromethane to remove residual unreacted 2-bromoethanol. The excess water was removed by freeze-drying the sample and the intermediate **(1a)** was subsequently purified by flash chromatography on SiO_2 (EtOAc/MeOH 9.5:0.5). Yield: 9.5 g (33 mmol), 40%.

*NOTE: care was take to make sure that **(1a)** was free of residual 2-bromoethanol, as this contaminant would have formed potentially explosive 2-azidoethanol in the subsequent step.*

^{1}H NMR (400.03 MHz, D_2O) δ (ppm) = 3.65 (t, *J* = 5.5 Hz, 2H, CH_2Br); 3.67-3.76 (m, 2H, H-4 + H-5); 3.76-3.86 (m, 2H, H-3 + H-6"); 3.86-3.95 (m, 2H, H-6' + OCH*H*CH_2Br); 3.99 (dd, *J* = 3.4, 1.7 Hz, 1H, H-2); 4.05 (dt, *J* = 6.4, 5.5 Hz, 1H, OC*H*HCH_2Br); 4.94 (d, *J* = 1.7 Hz, 1H, H-1).

^{13}C NMR (101 MHz, D_2O) δ (ppm) 31.64, 60.89, 66.59, 67.87, 69.75, 70.72, 73.20, 99.88 ppm.

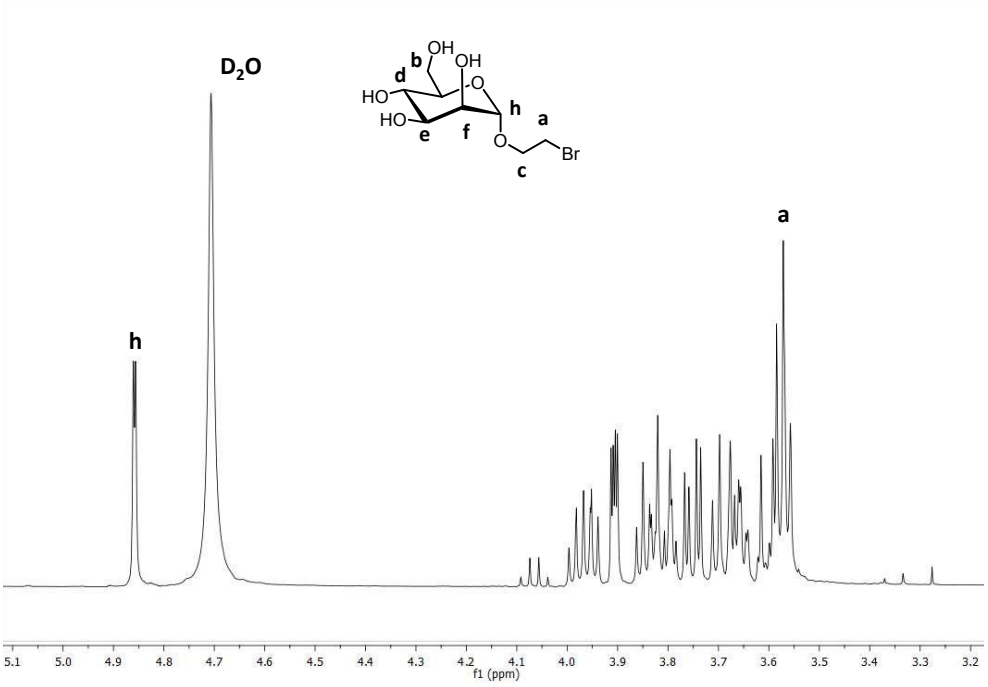


Figure S1. ^{1}H NMR spectrum of 2'-bromoethyl mannopyranoside **(1a)** in D_2O.

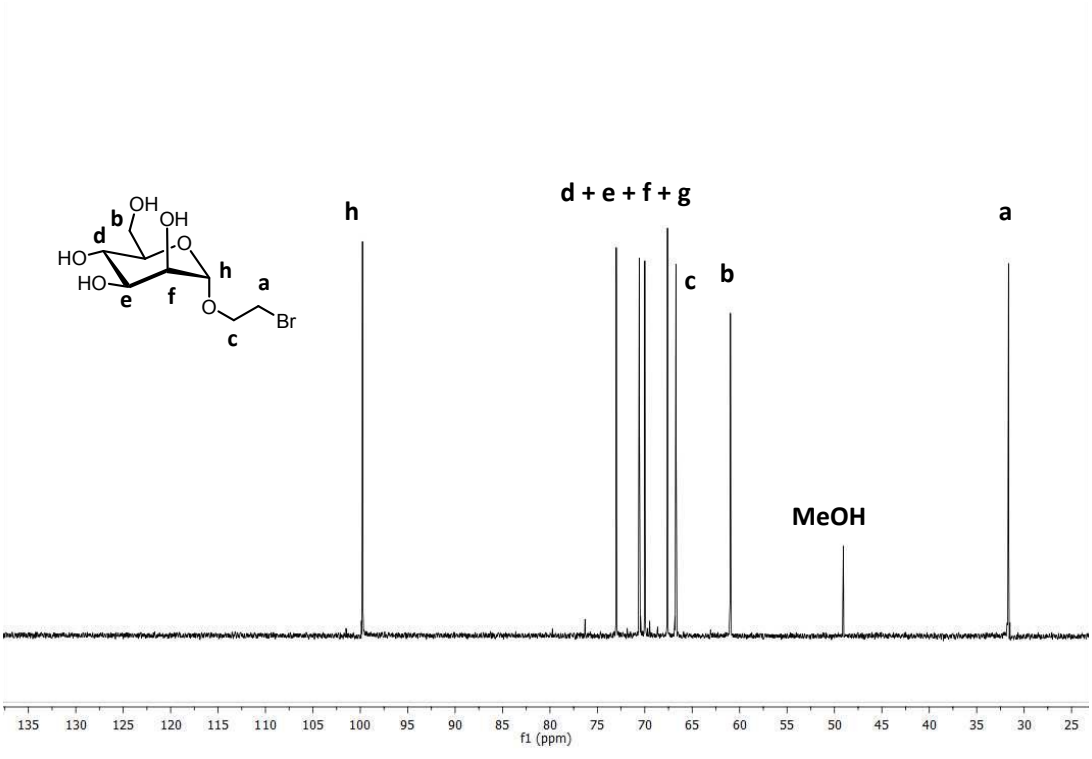


Figure S2. ^{13}C NMR spectrum of 2'-bromoethyl mannopyranoside **(1a)** in D_2O.

2'-azidoethyl mannopyranoside (7a)[3]. Sodium azide (3.46 g, 53.2 mmol) and 2-bromoethylmannopyranoside **(1a)** (9.5 g, 33 mmol) were dissolved in DMF (100 mL) and the resulting mixture was heated up at 50°C and stirred at this temperature overnight. A small aliquot was withdrawn and analysed by ^{13}C NMR in D_2O, revealing quantitative conversion of **(1a)** into the desired mannose azide **(7a)** (*C*H_2N_3 peak appearing at 50 ppm, with concomitant disappearance of *C*H_2Br at 31 ppm). Most of the DMF solvent was then removed under reduced pressure, and the resulting mixture was precipitated in Et_2O. The resulting oily residue was dissolved in methanol, and SiO_2 (30 g) was added. The solvent was removed under reduced pressure and the silica-supported reaction mixture was loaded onto a column previously filled with SiO_2 and pre-eluted with EtOAc/MeOH 19:1 vol/vol. After elution with the same solvent mixture, appropriate fractions were collected and the solvents were removed under pressure to give 2'-azidoethyl mannose **(7a)** as a white solid. Yield: 7.42 g (29.8 mmol), 90%.

^1H NMR (400 MHz, D_2O) δ (ppm) 3.52-3.67 (m, 2H, CH_2N_3); 3.71-3.78 (m, 2H, C*H*$_2$$CH_2N_3$); 3.78-3.88 (m, 2H, C*H*$_2$OH); 3.89-4.08 (m, 4H, 4 CH); 5.00 (d , *J* = 1. 7 Hz, 1H, CH).

^{13}C NMR (101 MHz, D_2O) δ (ppm) 50.37, 61.20, 66.30, 66.91, 70.06, 70.71, 73.21, 99.90 ppm.

FT-IR: v 3368, 3251, 2931, 2440, 2103, 1617, 1387, 1345, 1259, 1135, 1090, 1048, 967, 810, 677 cm^{-1}.

ESI-TOF Mass Spectrometry: expected *m/z* $[M-Na]^+$ 272.0853, found 272.0936.

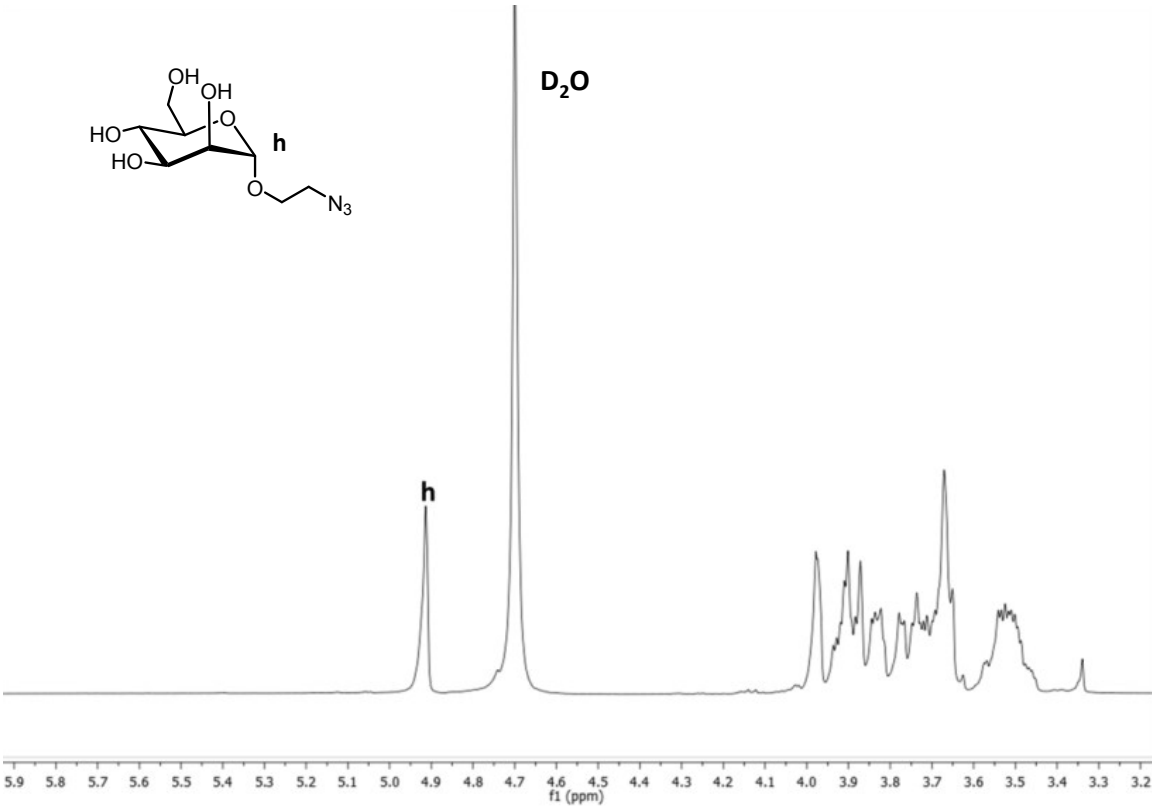


Figure S3. ^{1}H NMR spectrum of 2'-azidoethyl mannopyranoside **(7a)** in D_2O.

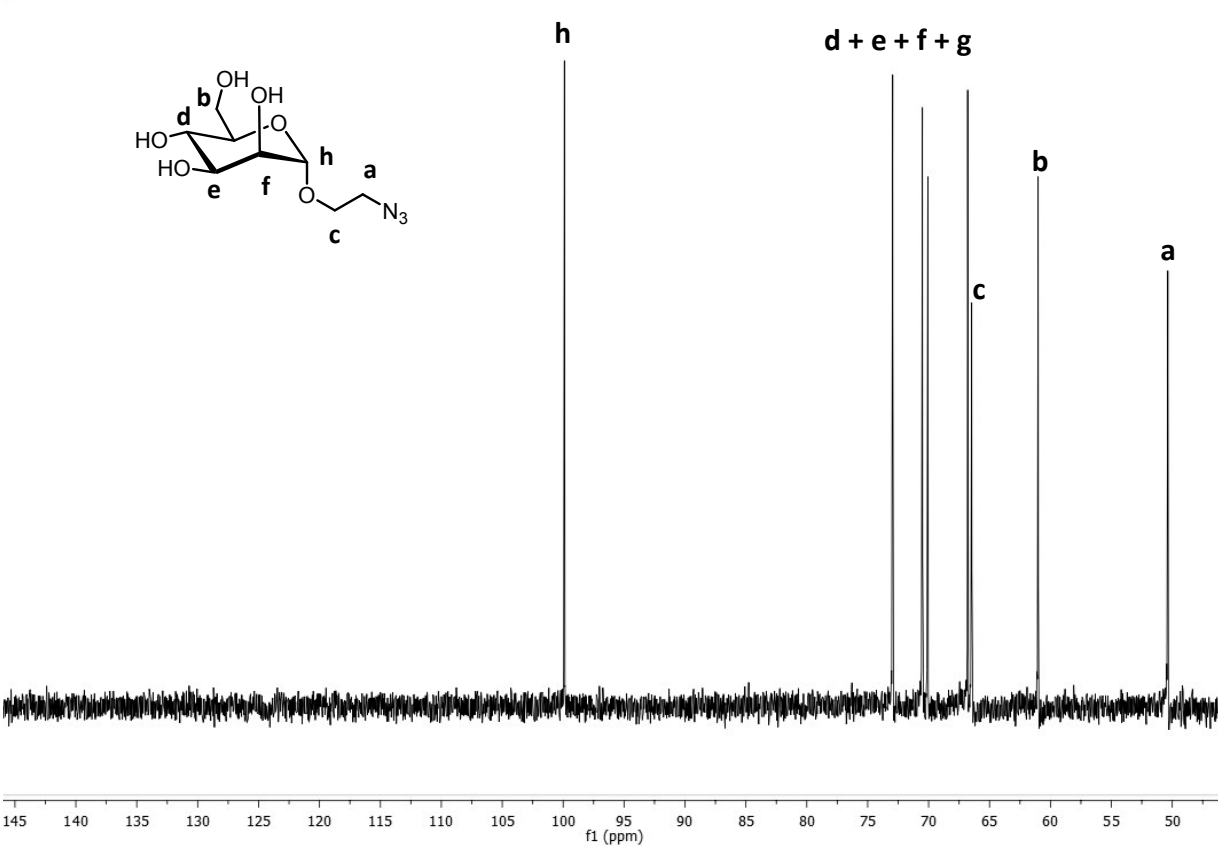
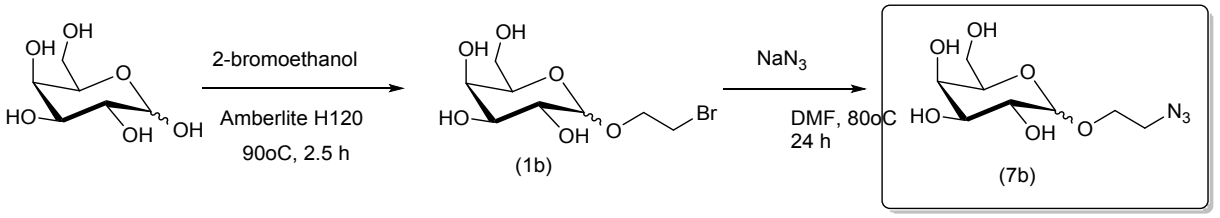


Figure S4. ^{13}C NMR spectrum of 2'-azidoethyl mannopyranoside **(7a)** in D_2O.

2 Synthesis of 2'-azidoethyl galactopyranoside (7b)



Scheme S2. Synthesis of 2'-azidoethyl galactopyranoside **(7b)**.

Conditions and protocol as reported above for 2'-bromoethyl mannopyranoside **(7b)**.

2'-bromoethyl galactopyranoside (1b). Conditions and protocol as reported for 2'-bromoethyl mannopyranoside **(1a)**.

Yield: 8.50 g (29.6 mmol), 53.3%.

*NOTE: care was take to make sure that **(1b)** was free of residual 2-bromoethanol, as this contaminant would have formed potentially explosive 2-azidoethanol in the subsequent step.*

^{1}H NMR (400 MHz, D_2O) δ (ppm)3.40-3.50 (m, 2H, CH_2Br); 3.60-3.7 (m, 2H, OCH_2CH_2Br); 3.53-3.63 (m, 2H, CH_2OH); 3.69-3.86 (m, 4H, 4 CH); 4.05 (m, 1H, CH); 4.95 (α, d , J = 3.7 Hz, 1H, CH); 4.42 (β, d , J = 7.8 Hz, 1H, CH).

^{13}C NMR (101 MHz, D_2O) δ (ppm) 31.1 (1C, CH_2Br); 60.9(1C, CH_2OH); 68.6 (1C, CH); 69.9 (1C, OCH_2CH_2Br); 70.8 (1C, CH); 72.7 (1C, CH); 75.2 (1C, CH); 102.9 (1C, CH anomeric). 31.4 (1C, CH_2Br); 61.1(1C, CH_2OH); 68.2 (1C, CH); 68.3 (1C, OCH_2CH_2Br); 69.2 (1C, CH); 69.4 (1C, CH); 71.2 (1C, CH); 98.47 (1C, CH anomeric).

Isolated galactose intermediate **(1b)** consisted of a mixture of 17% of beta and 83% of alpha anomers; hence the complex pattern of signals in both ^{1}H and ^{13}C NMR spectra (see Fig.S5 and S6).

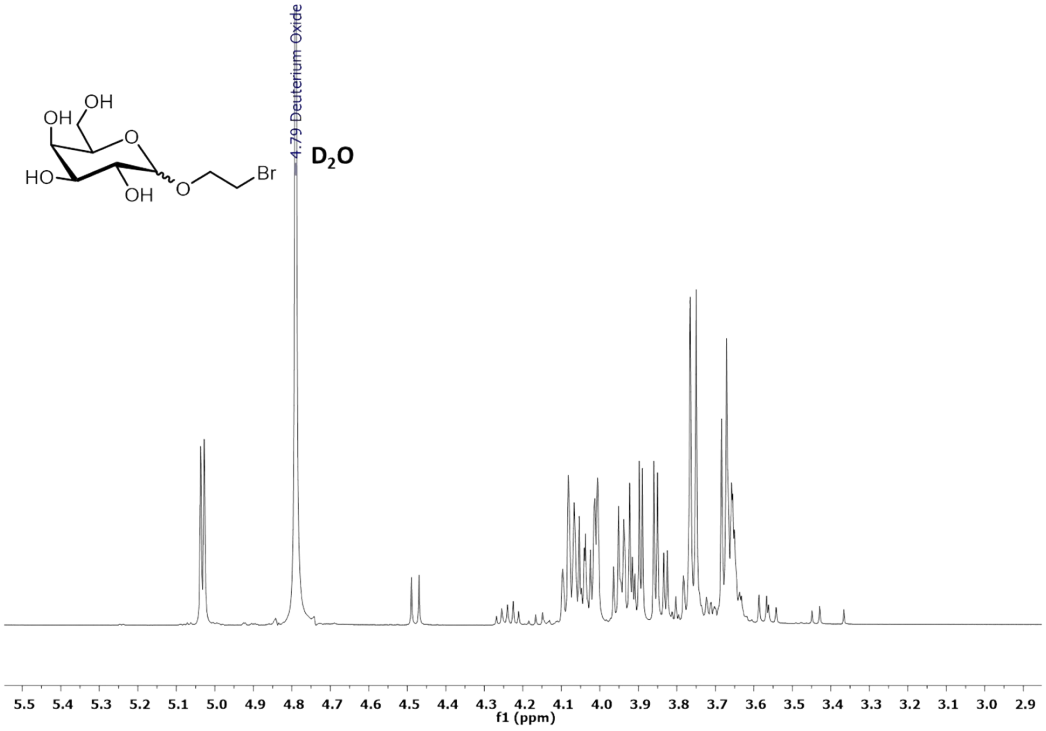


Figure S5. ^{1}H NMR spectrum of α/β 2'-bromoethyl galactopyranoside **(1b)** in D_2O.

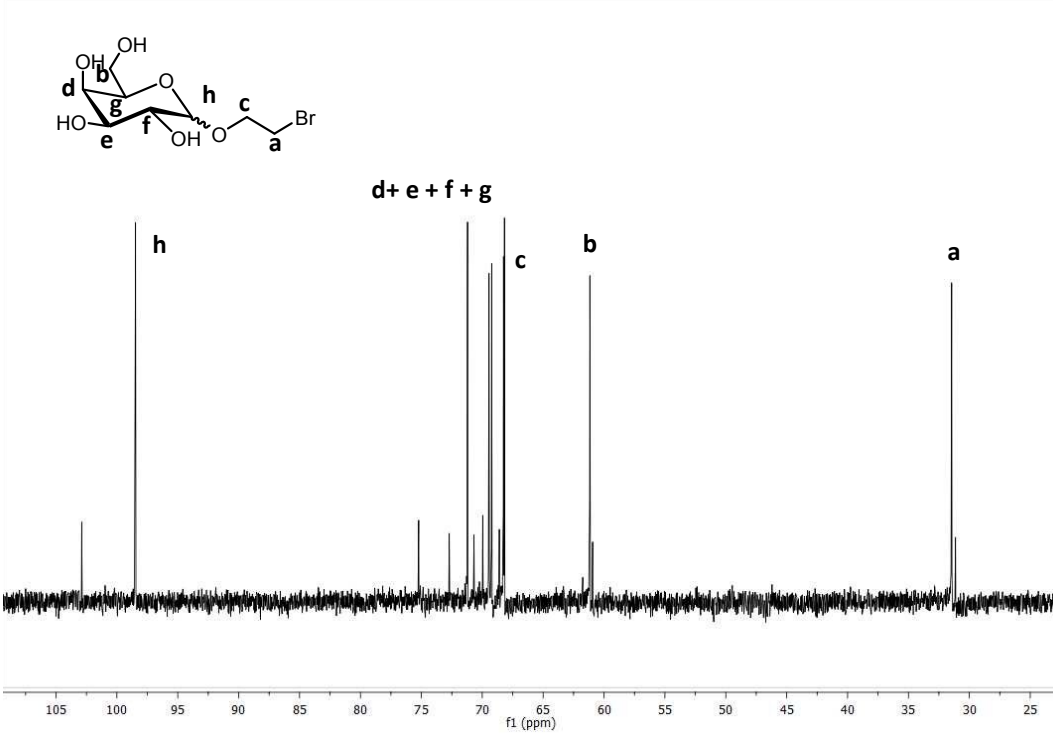


Figure S6. ^{13}C NMR spectrum of α/β 2'-bromoethyl galactopyranoside **(1b)** in D_2O.

2'-azidoethyl galactopyranoside (7b). Conditions and protocol as reported for 2'-azidoethyl mannopyranoside **(7a)**.

Yield: 6.3 g (25 mmol), 84%.

^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.40-3.55 (m, 2H, CH_2N_3); 3.55-3.75(m, 2H, C*H*$_2$$CH_2N_3$); 3.88-3.98 (m, 2H, C*H*$_2$OH); 4.10-4.32 (m, 4H, 4 CH); 4.36 (m, 1H, CH); 5.02 (α, d , J = 3.7 Hz, 1H, CH); 4.52 (β, d , J = 7.8 Hz, 1H, CH).

^{13}C NMR (101 MHz, D_2O): δ (ppm) α 50.5 (1C, CH_2N_3); 61.3 (1C, CH_2OH); 66.7 (1C, CH); 68.0 (1C, O*C*H_2CH_2Br); 69.2 (1C, CH); 69.3 (1C, CH); 71.2 (1C, CH); 98.4 (1C, CH anomeric). β= 50.6 (1C, CH_2N_3); 60.9(1C, CH_2OH); 61.8 (1C, CH); 64.3 (1C, O*C*$H_2CH_2N_3$); 68.4 (1C, CH); 70.7 (1C, CH); 72.7 (1C, CH); 102.9 (1C, CH anomeric).

FT-IR: v 3440, 2960, 2186, 1725, 1637, 1251, 1153, 1035, 943, 761 cm^{-1}.

ESI-TOF Mass Spectrometry: expected *m/z* $[M-Na]^+$ 272.0853, found 272.0858.

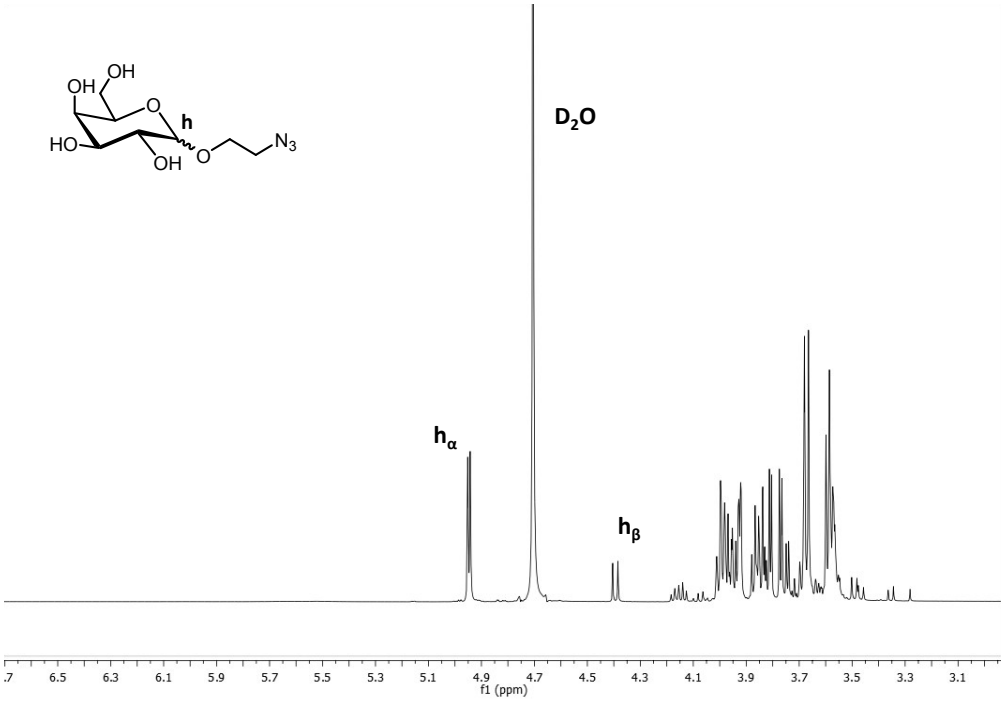


Figure S7. ^{1}H NMR spectrum of α/β 2'-azidoethyl galactopyranoside **(7b)** in D_2O.

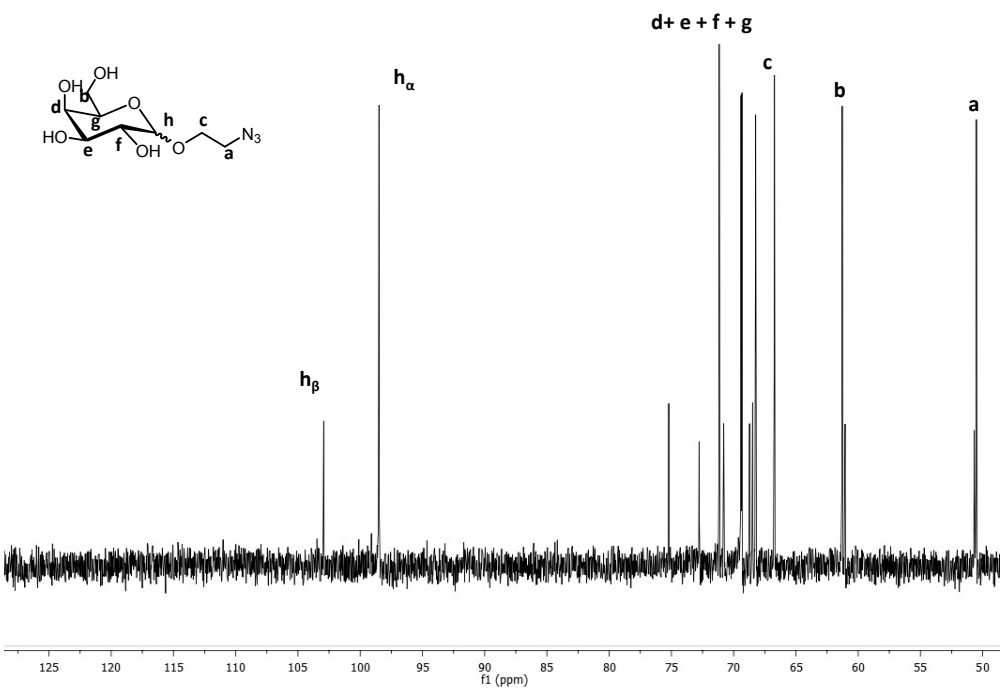
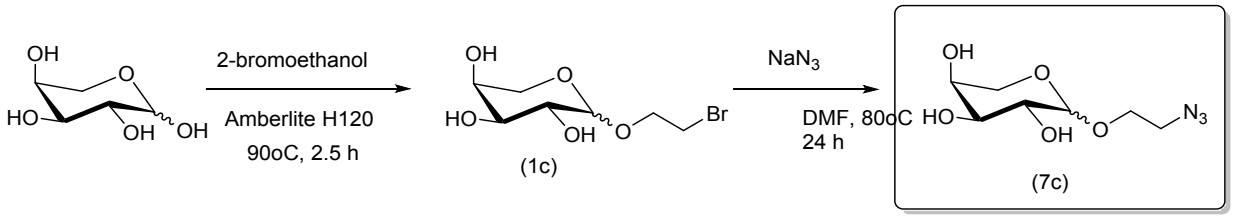


Figure S8. ^{13}C NMR spectrum of α/β 2'-azidoethyl galactopyranoside **(7b)** in D_2O.

3 Synthesis of 2'-azidoethyl arabinopyranoside (7c)



Scheme S3. Synthetic route to 2'-azidoethyl arabinofuranosyde **(7c)**.

2'-bromoethyl arabinopyranoside (1c). Conditions and protocol as reported for 2'-bromoethyl mannopyranoside **(1a)**.

Yield: 2.40 g (9.33 mmol), 28 %.

*NOTE: care was take to make sure that **(1c)** was free of residual 2-bromoethanol, as this contaminant would have formed potentially explosive 2-azidoethanol in the subsequent step.*

^{1}H NMR (400.03 MHz, D_2O) δ (ppm) 3.46 (dd, *J* = 6.0, 3.1 Hz, 2H, CH_2Br); 3.50-3.60 (m, 2H, H-4 + H-5); 3.72-3.74 (m, 2H, H-3 + H-6”); 3.78-3.80 (m, 2H, H-6’ + OCH*H*CH_2Br); 3.90 (dd, *J* = 12.8, 1.1 Hz, 1H, H-2); 3.92 (dt, *J* = 3.1, 1.6 Hz, 1H, OC*H*HCH_2Br); 4.90 (d, *J* = 3.6 Hz, 1H, H-1).

^{13}C NMR (101 MHz, D_2O) δ (ppm) 31.36, 63.0, 68.22, 68.24, 68.67, 68.94, 68.83 ppm.

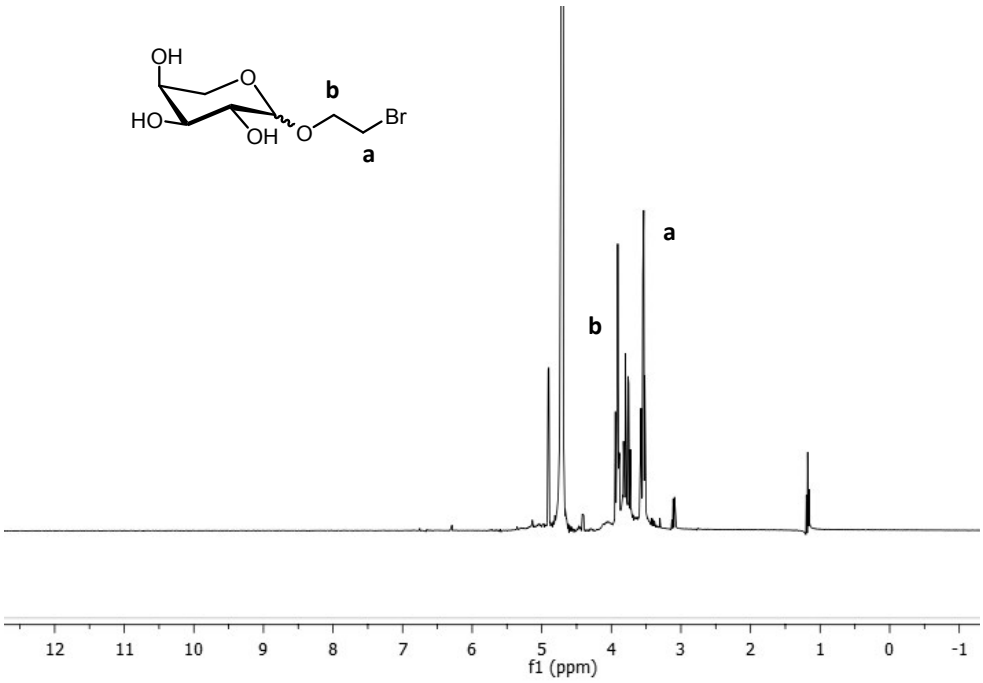


Figure S9. ^{1}H NMR spectrum of 2’-bromoethyl arabinopyranoside **(1c)** in D_2O.

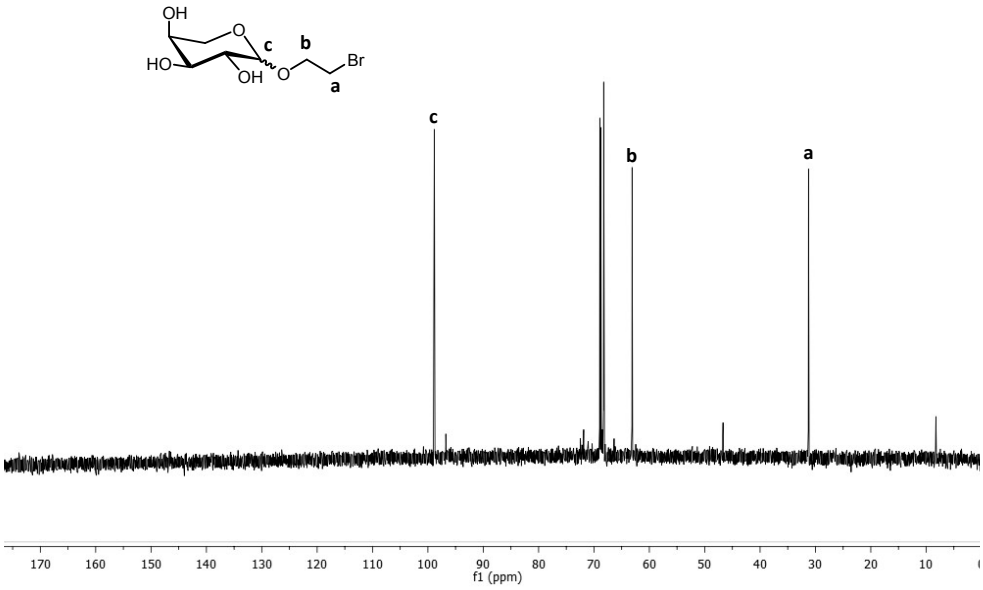


Figure S10. ^{13}C NMR spectrum of 2'-bromoethyl arabinopyranoside **(1c)** in D_2O.

2'-azidoethylarabinopyranoside (7c). Conditions and protocol as reported for 2'-azidoethyl mannose **(7a)**.

Yield: 1.96 g, (8.94 mmol), 94 %.

^1H NMR (400.03 MHz, D_2O) δ (ppm) 3.32 (dd, *J* = 6.0, 3.1 Hz, 2H, CH_2N_3); 3.50-3.60 (m, 2H, H-4 + H-5); 3.55-3.62 (m, 2H, H-3 + H-6"); 3.70-3.85 (m, 2H, H-6' + OCH*H*CH_2 N_3); 3.92 (dd, *J* = 12.8, 1.1 Hz, 1H, H-2); 4.15 (dt, *J* = 3.1, 1.6 Hz, 1H, OC*H*HCH_2 N_3); 4.85 (d, *J* = 3.6 Hz, 1H, H-1).

^{13}C NMR (101 MHz, D_2O) δ (ppm) 50.43, 62.87, 66.76, 68.11, 68.67, 69.02, 98.87.

FT-IR: v 3247, 2943, 2091, 1625, 1447, 1261, 1137, 1056, 1000, 954, 838, 773, 708 cm^{-1}.

ESI-TOF Mass Spectrometry: expected m/z $[M-Na]^+$ 242.0747, found 242.0759.

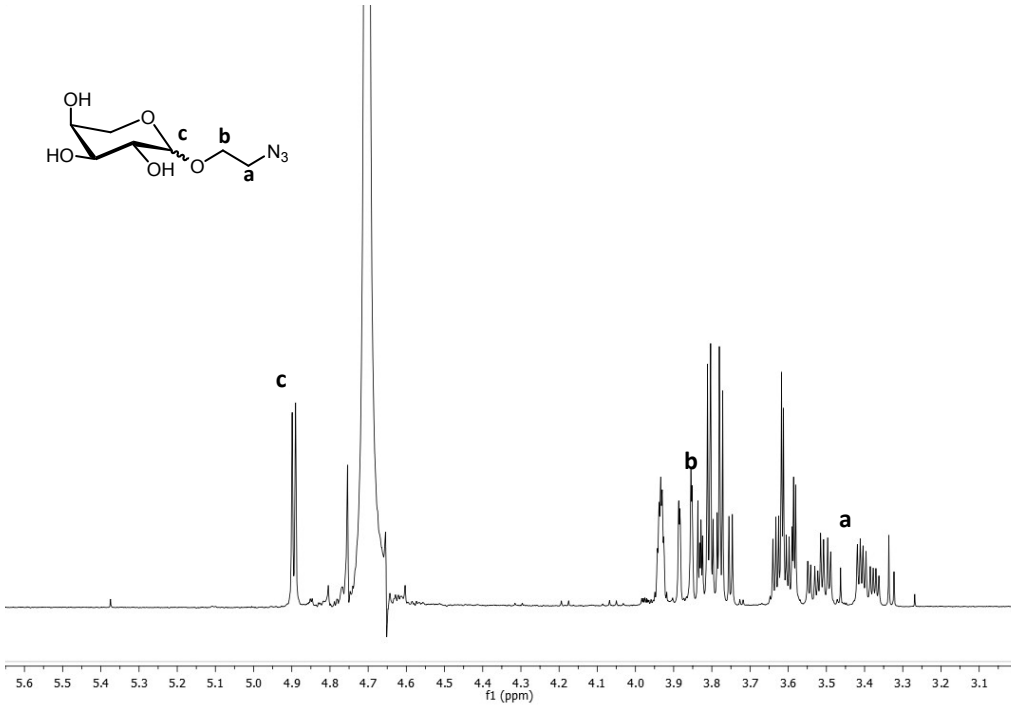


Figure S11. ^{1}H NMR spectrum of 2'-azidoethyl arabinopyranoside **(7c)** in D_2O.

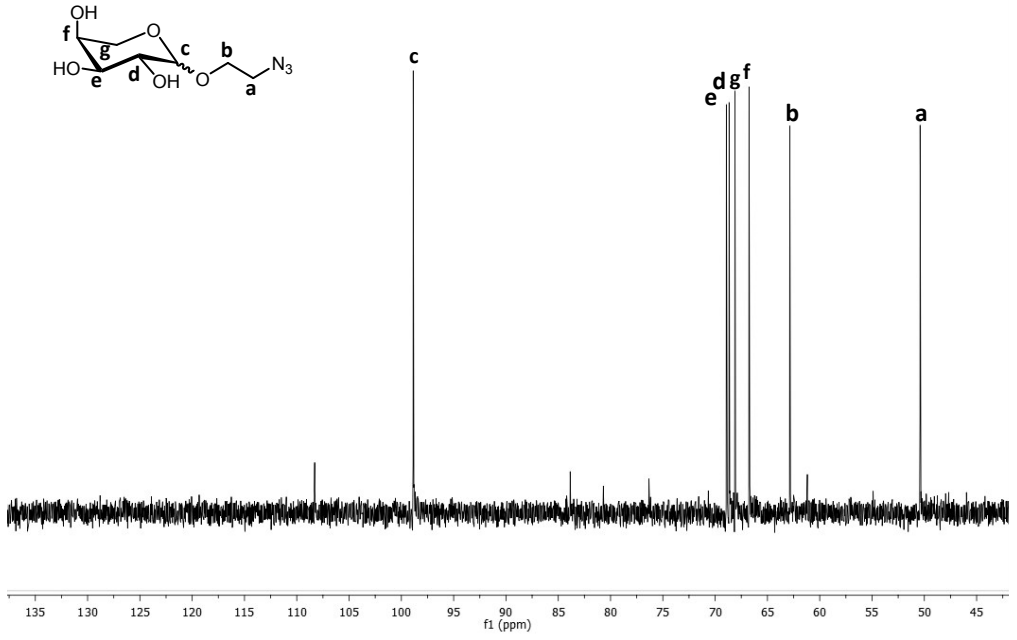
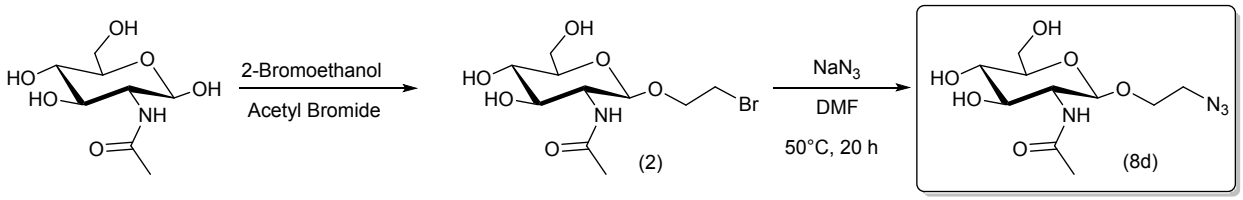


Figure S12. ^{13}C NMR spectrum of 2'-azidoethyl arabinopyranoside **(7c)** in D_2O.

4 Synthesis of 2'-azidoethyl-*N*-acetyl glucosamine (8d)



Scheme S4. Synthetic route to 2'-azidoethyl *N*-acetyl glucosamine **(8d)**.

Modified protocol from Roy, *et al*. [4]

The conditions utilised for the synthesis of mannose, galactose and arabinose azides **(7a)**, **(7b),** and **(7c)**, respectively, did not afford *N*-acetyl glucosamine azide **(8d)** in acceptable yields (i.e. > 10%). An alternative method, which utilised HBr generated in situ from acetyl bromide and 2-bromoethanol as the acid catalyst,[4] allowed to obtain the required 2'-bromoethyl *N*-acetyl glucosamine intermediate **(2)** in good yields.

2'-bromoethyl *N*-acetyl glucosamine (2). To a solution of *N*-acetyl-D-glucosamine (10.0 g, 45.2 mmol) in 2-bromoethanol (150 mL), acetyl bromide (4.26 g, 34.6 mmol) was added dropwise at 0°C. The reaction mixture was heated at 70°C for 4 hours and then stirred at room temperature for further 4 hours. Most of 2-bromoethanol was removed under reduced pressure, and the resulting brownish oily residue was dissolved in methylated spirit. The solution was decolorized by stirring it with activated charcoal and posterior filtration through a celite pad. The excess of 2-bromoethanol was remove under reduced pressure, and the resulting residue was purified by silica gel chromatography (EtOAc/MeOH 9.5:0.5) to yield 2'-bromoethyl *N*-acetyl glucosamine **(2)** as a viscous oil. Yield: 5.72 g (17.4 mmol), 39 %.

^{1}H NMR (400 MHz, D_2O) δ (ppm) 2.04 (s, 3H, NAc). 3.37– 3.43 (m, 1H), 3.44 – 3.98 (m, 10 H), 4.93 (dd, *J* = 7.4, 3.6 Hz, 1H).

^{13}C NMR (101 MHz, D_2O) δ (ppm) 21.98, 30.17, 61.57, 68.04, 69.60, 69.95, 70.89, 72.10, 96.84, 174.23.

*NOTE: care was take to make sure that **(2)** was free of residual 2-bromoethanol, as this contaminant would have formed potentially explosive 2-azidoethanol in the subsequent step.*

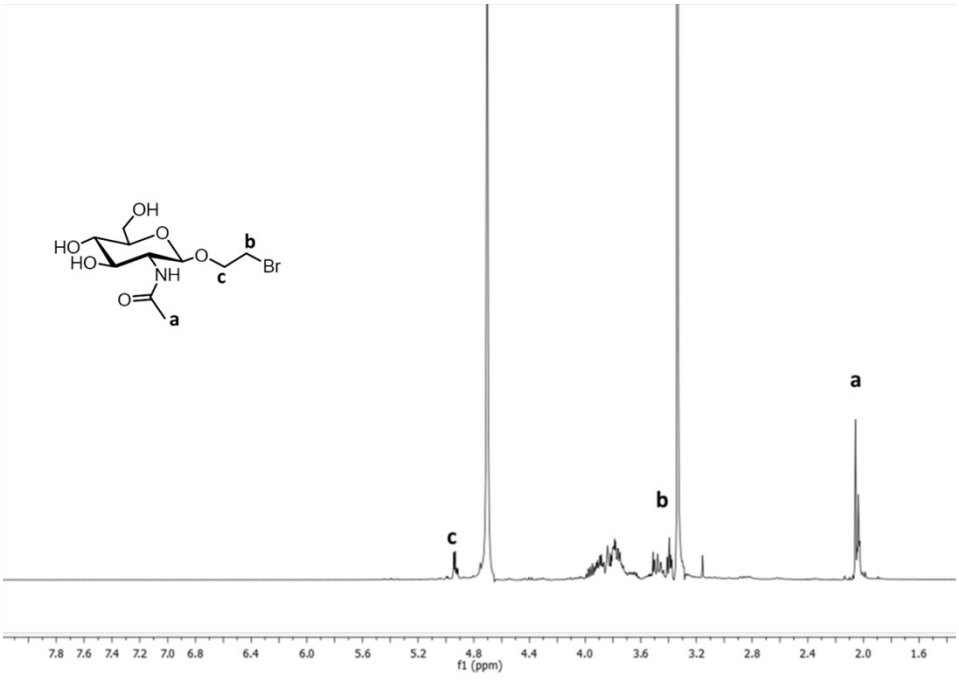


Figure S13. ^{1}H NMR spectrum of α/β-2'-bromoethyl *N*-acetyl glucosamine **(2)** in D_2O.

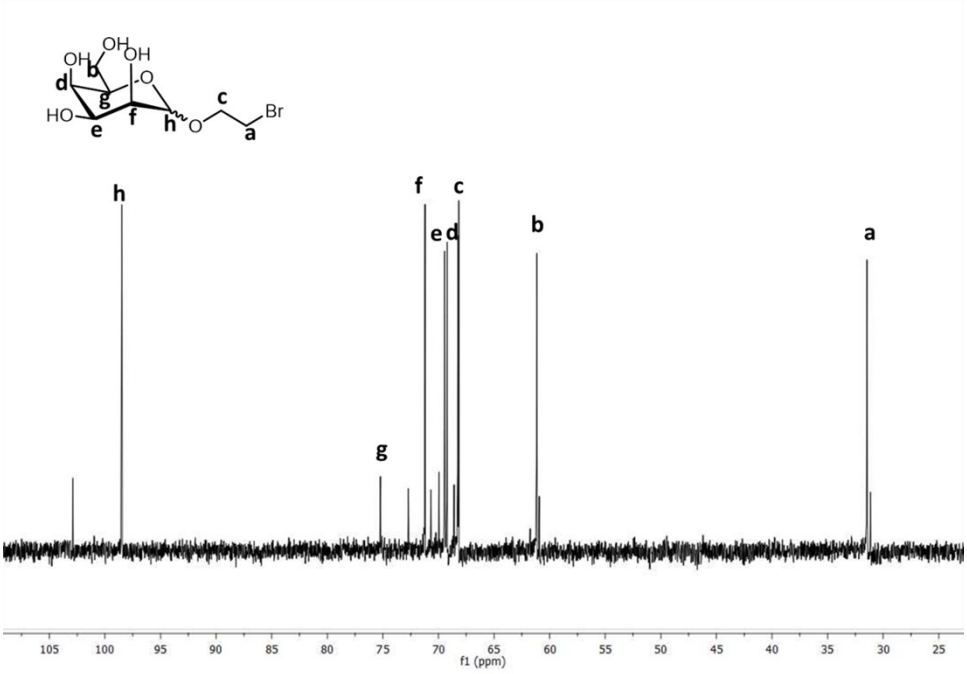


Figure S14. ^{13}C NMR spectrum of spectrum of α/β-2'-bromoethyl *N*-acetyl glucosamine **(2)** in D_2O.

2'-azidoethyl-*N*-acetyl glucosamine (8d). 2'-Bromo *N*-acetyl glucosamine **(2)** (5.72 g, 17.4 mmol) was dissolved in DMF (30 mL) and NaN_3 (1.0 eq, 1.15 g, 17.7 mmol) was added. The resulting solution was left to react overnight at 50°C under stirring, then the volume was reduced under reduced pressure, and the resulting residue dissolved in methanol and precipitated in Et_2O to remove residual traces of DMF. The oily residue was purified by flash chromatography (SiO_2) using 100% EtOAc and then EtOAc/MeOH 9:1 as the mobile phase. After removal of the solvent from the relevant fractions 2'-azidoethyl *N*-acetyl glucosamine **(8d)** was isolated as a pale yellow viscous oil (4.97 g, 17.1 mmol, 98% yield).

^1H NMR (400 MHz, D_2O) δ (ppm) 2.16 (s, 3H, NAc), 3.51 – 3.64 (m, 3H), 3.71– 4.01 (m, 7H), 4.99 (d, *J* = 3.6 Hz, 1H, H-1).

^{13}C NMR (101 MHz, D_2O) δ (ppm) 22.67, 49.17, 60.97, 61.64, 66.96, 70.11, 71.07, 72.17, 97.37, 174.82.

FT-IR: v 3344, 2932, 2321, 2104, 1654, 1531, 1438, 1387, 1254, 1099, 1018, 883 cm^{-1}.

ESI-TOF Mass Spectrometry: expected *m/z* $[M\text{-}Na]^+$ 313.1119, found 313.1132.

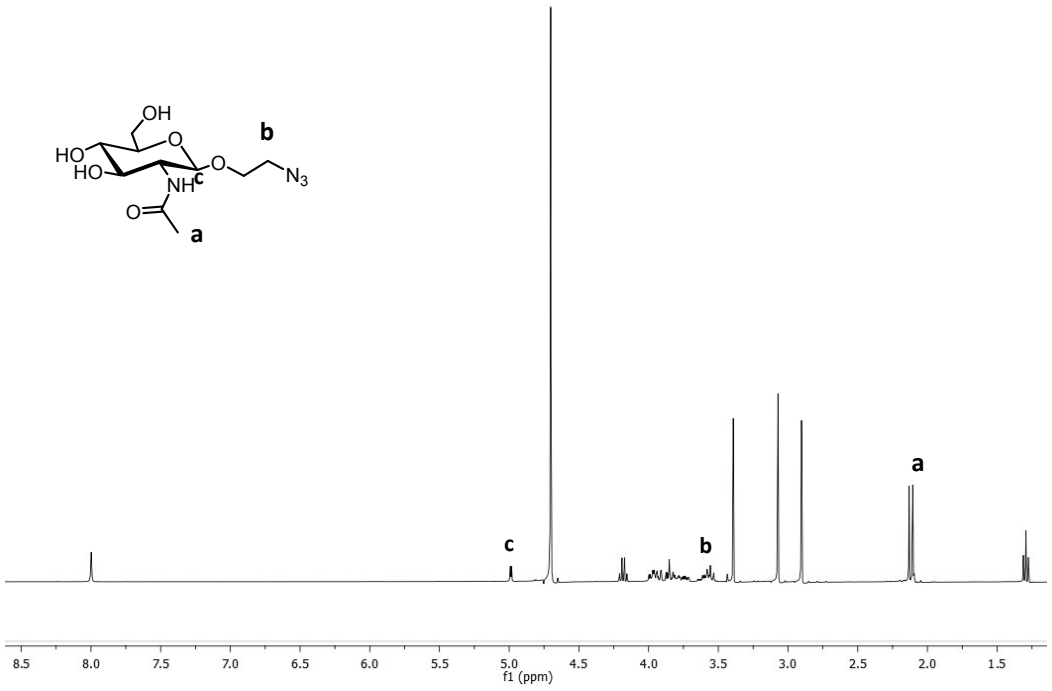


Figure S15. ^{1}H NMR spectrum of 2'-azidoethyl *N*-acetyl glucosamine **(8d)** in D_2O.

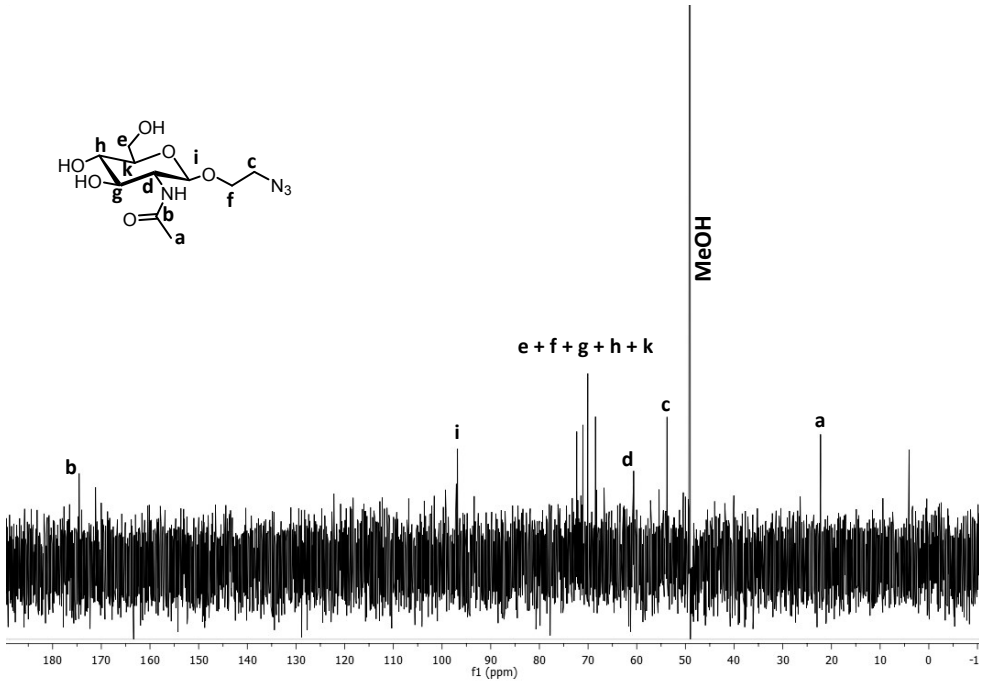


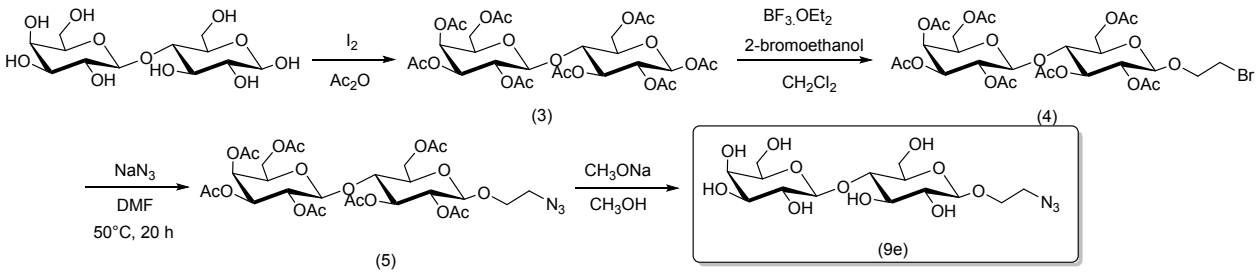
Figure S16. ^{13}C NMR spectrum of 2'-azidoethyl *N*-acetyl glucosamine **(8d)** in D_2O.

5 Synthesis of lactose azide (9e)

The synthesis of lactose azide **(9e)** was performed with a different procedure, as a first attempt using the same conditions as for mannose, galactose and arabinose led to the cleavage of the disaccharide β 1-4 linkage.

Lactose was peracetylated following the method reported by Mukhopadhyay *et al.*,[5] using I_2 as the catalyst, and a stoichiometric amount of acetic anhydride, to avoid longer work up and purification processes normally required when an excess of anhydride is used. After crystallization from MeOH, peracetylated lactose **(3)**, was reacted with 2-bromoethanol and $BF_3{\cdot}Et_2O$ in CH_2Cl_2 to give the corresponding 2'-bromoethyl *O*-glycoside **(4)**. Whilst the number of sugar signals in the ^1H NMR spectrum of **(4)** make its characterisation rather complicated, ^{13}C

NMR analysis showed the presence of only two anomeric signals at around 100 ppm, suggesting that the isolated glycoside **(4)** had a β anomeric configuration at both anomeric centres. Given the strong preference of peracetylated galactose residues - the part of lactose that is modified in this step is a Gal residue - to give β anomers in the presence of Lewis acids and *O*-nucleophiles, due to the anchimeric assistance of the acetate group at C_2,[1] these results may indicate preferential formation of the β, β lactose glycosyde. The reaction was monitored by ^{13}C NMR, by following the disappearance of the peak for the *O*-acetylated anomeric carbon at 92 ppm and mass spectroscopy. Due to the complexity of structure of 2-bromoethyl-peracetylated lactose ^{1}H and ^{13}C NMR spectra were of relatively difficult interpretation. Mass spectrometry analysis was in this case more diagnostic, with characteristic peak for $[M\text{-}Na]^+$ at 765 and 767 m/z. The following azidation step was performed with NaN_3 in DMF as described for the synthesis of 2'-azidoethyl mannose mannopyranoside, to give peracetylated 2'-azidoethyl lactose **(5)**, which was finally deprotected by treatment with sodium methoxide in MeOH, yielding the required 2-azidoethyl lactose **(9e)**.



Scheme S5. Synthesis of 2'-azidoethyl lactose **(9e)**.

Lactose octaacetate (3) (as reported by Mukhopadhyay et al. [6]). D-lactose (20.0 g, 58.4 mmol) was suspended in acetic anhydride (48.65 g, 476.5 mmol, 1.02 mol eq. per hydroxyl group) and

iodine was added as a solid (0.104 g, 0.410 mmol, 0.0009 mole eq. per hydroxyl group). *NOTE: once solid I_2 catalyst dissolves in the reaction mixture, this reaction can become rather exothermic. A condenser should be fitted to the reaction flask to avoid loss of product and spillage of reaction mixture.* The mixture was allowed to stir at room temperature overnight. The reaction was monitored by ^{1}H NMR and ^{13}C NMR in $CDCl_3$. After completion, the resulting dark yellow solution was diluted with CH_2Cl_2 (120 mL) and washed with 10% aq. $Na_2S_2O_3$ solution (120 mL). The organic layer was then washed three times with saturated aq. $NaHCO_3$ solution and finally with deionised water. The organic layer was dried over $MgSO_4$ and concentrated to an approximate volume of 50 mL. 100 mL of MeOH were then added and the resulting mixture was cooled to 4°C. Crystals rapidly formed, and these were filtered, washed with ice-cold methanol, and left overnight under reduced pressure to remove traces of residual solvent. Lactose octaacetate **(3)** crystals were isolated as white needles. Yield: 12.9 g (19.0 mmol), 32.6 %.

Note: although more **(3)** was still present in the mother liquor, no further crystals were recovered, and no further attempts to purify the mother liquor were taken. The reported yield refers to the crystals of analytically pure **(3)** isolated after the first crystallisation.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.22 – 1.96 (m, 24H, $OC(O)CH_3$), 3.77 – 3.99 (m, 2H), 4.09-4.12 (m, 2H), 4.47-4.96 (m, 2H), 5.09– 5.69 (m, 7H), 6.25 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 20.49, 20.60, 20.63, 20.67, 20.75, 20.82, 20.85, 20.94 60.84, 61.74, 66.60, 69.01, 70.52, 70.75, 70.96, 72.63, 73.50, 75.67, 91.53, 100.95, 168.82, 168.99, 169.53, 169.59, 170.3, 170.11, 170.29, 170.33.

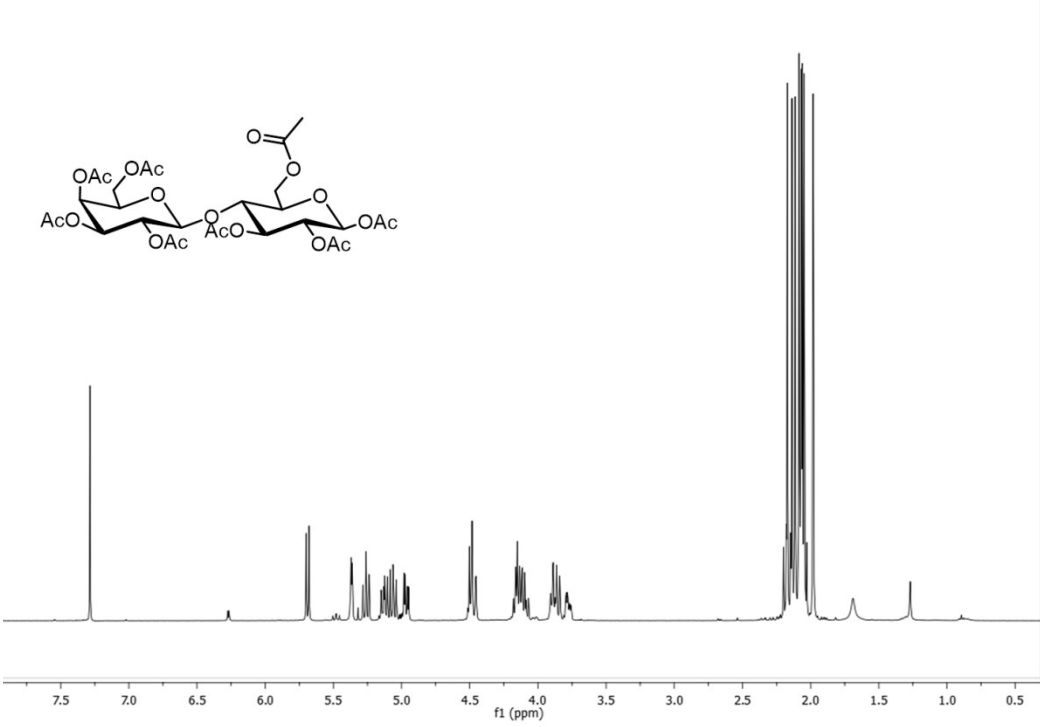


Figure S17. ^{1}H NMR spectrum of lactose octaacetate **(3)** in $CDCl_3$. For clarity only one of the OAc groups is shown in its expanded structure.

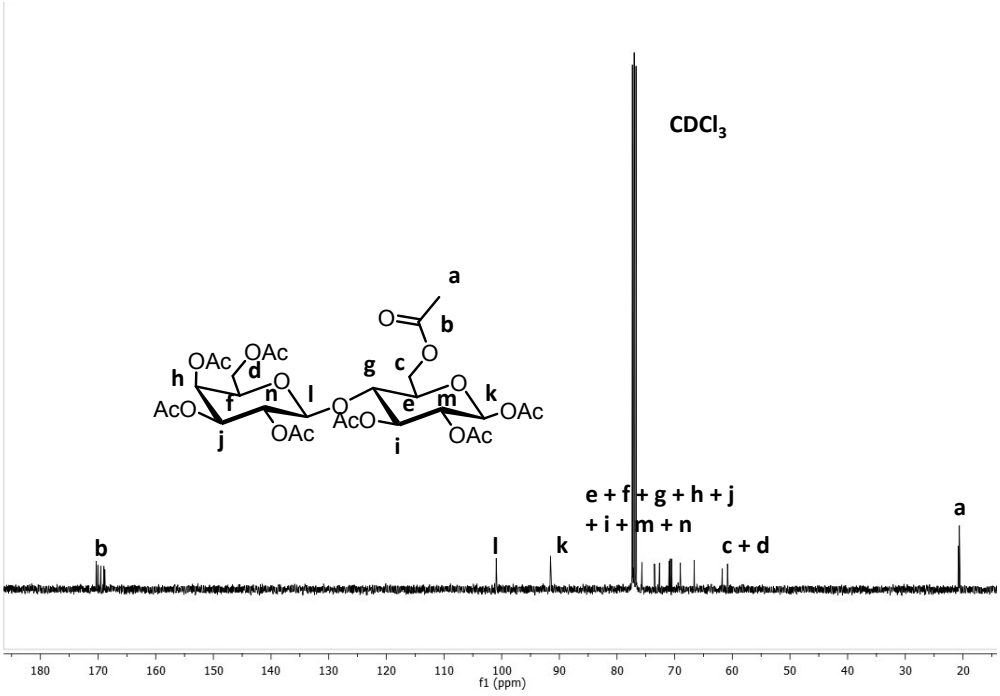


Figure S18. ^{13}C NMR spectrum of lactose octaacetate **(3)** in $CDCl_3$. For clarity only one of the OAc groups is shown in its expanded structure.

2'-bromoethyl peracetylated lactose (4). Peracetylated lactose **(3)** (12.9 g, 19.0 mmol) was dissolved in a solution of 2-bromoethanol (1.55 mL, 21.9 mmol) and CH_2Cl_2 (10 mL) under nitrogen. After complete dissolution of the protected sugar, $BF_3.OEt_3$ (2.6 mL, 20.8 mmol) was added drop wise via syringe over ca. 15 min. The yellow and highly viscous solution was subjected to ultrasonication for 2.5 hours, and the reaction was monitored by ^{13}C NMR, ^{1}H NMR and TLC (chloroform/MeOH 9.5:0.5). At completion, reaction mixture was diluted with ice-cold water (70 mL) and the resulting biphasic mixture was extracted with dichloromethane (70 mL). The aqueous layer was further washed three times with CH_2Cl_2. The organic layers, combined, were washed with a saturated aqueous solution of $NaHCO_3$ (70 mL), then again with water (70 mL), and dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure. The residue was solubilised in MeOH and the solvent was removed under reduced pressure. This procedure was repeated 4 times in order to eliminate traces of CH_2Cl_2, which could form explosive intermediates in the next step, by reaction with NaN_3. The residue was then analysed by ^{1}H NMR to confirm the absence of CH_2Cl_2 and 2-bromoethanol, and ^{19}F NMR to confirm the absence of $BF_3.OEt_3$. The product was purified by flash chromatography (EtOAc/Petroleum ether 1:3) to give 2'-bromoethyl peracetylated lactose **(4)** as a colourless oil. Yield: 14.72 g, 19.80 mmol, 95 %.

*NOTE: care was take to make sure that **(4)** was free of residual 2-bromoethanol, as this contaminant would have formed potentially explosive 2-azidoethanol in the subsequent step.*

^{13}C NMR (101 MHz, D_2O) δ (ppm) 17.95, 18.08, 18.21, 18.24, 18.29, 18.38, 18.49, 27.27, 57.83, 58.26, 59.31, 64.07, 66.57, 67.22, 68.16, 68.42, 68.87, 70.05, 70.21, 73.62, 98.21, 98.51, 166.50, 167.13, 167.16, 167.49, 167.57, 167.78, 168.58.

ESI-TOF Mass Spectrometry: expected m/z $[M-Na]^+$ 765.13 and 767.13, found 765.05 and 767.09.

Due to the relatively high molecular weight and complexity of this product the ^{1}H NMR spectrum was found to be of rather difficult interpretation (see Figure 7.20). However, ^{13}C NMR suggested that 2'-bromoethyl peracetylated lactose **(4)** isolated after flash chromatography was indeed pure. Mass spectrometry revealed two peaks at $[M+Na]^+$ 765 and 767, corresponding to the sodium adduct of **(4)** with its two ^{79}Br and ^{81}Br isotopes, confirming that the desired product **(4)** had indeed been obtained.

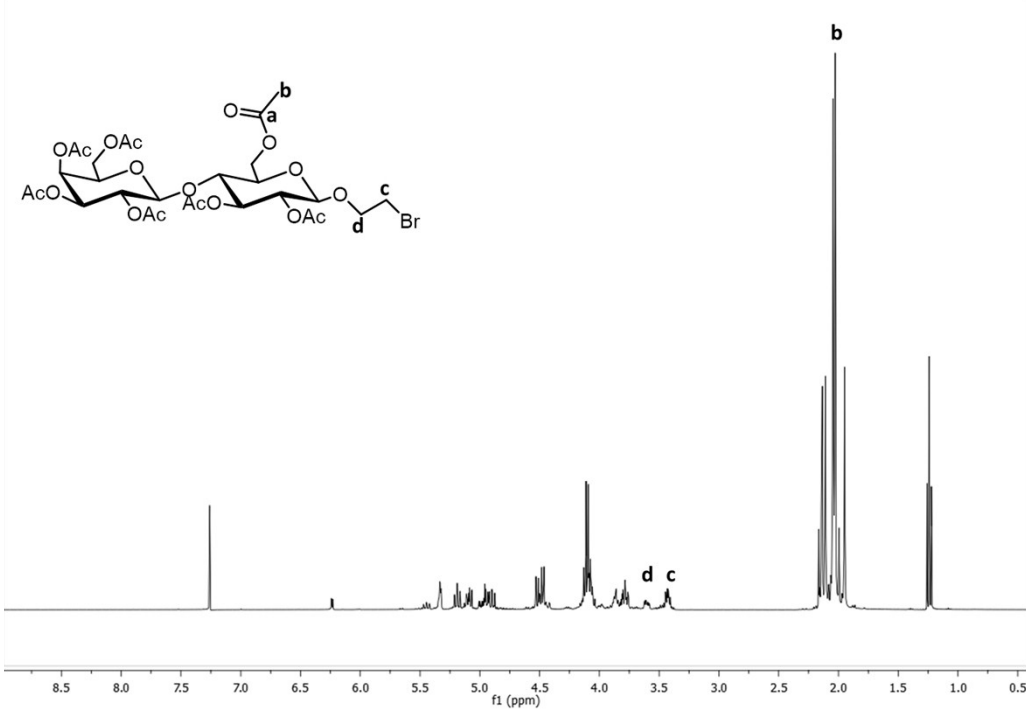


Figure S19. ^{1}H NMR spectrum of 2'-bromoethyl peracetylated lactose **(4)** in $CDCl_3$.

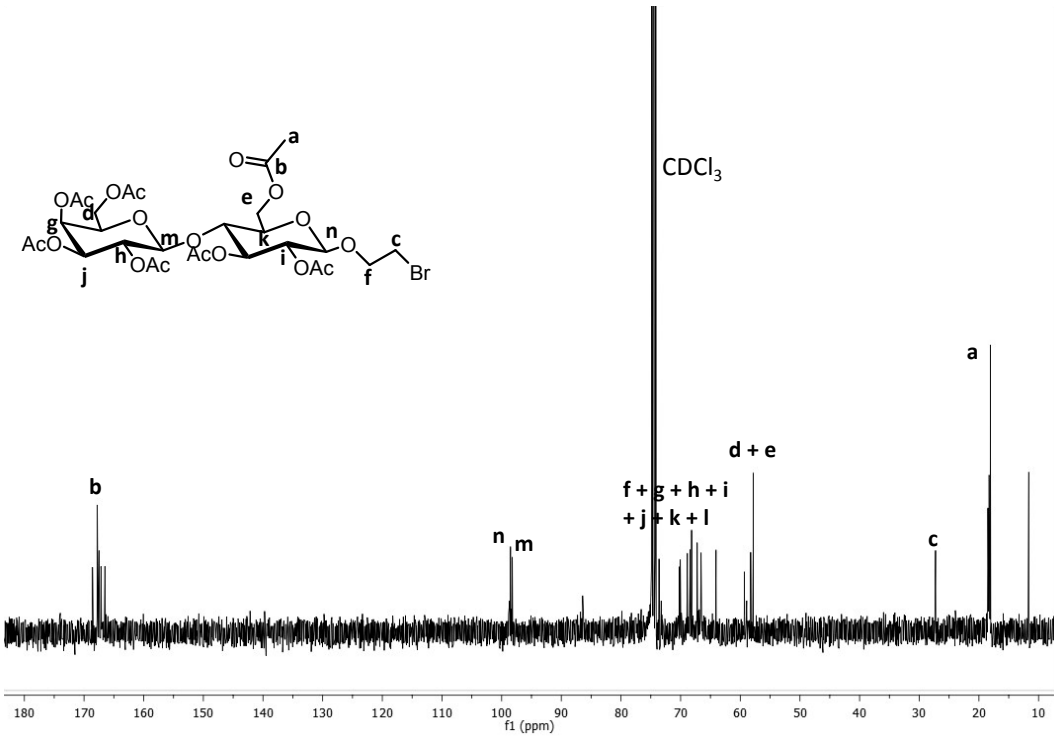


Figure S20. ^{13}C NMR spectrum of 2'-bromoethyl peracetylated lactose **(4)** in $CDCl_3$.

2'-azidoethyl peracetylated lactose (5). Compound **(4)** (14.7 g, 19.8 mmol) and NaN_3 (2.06 g, 31.7 mmol) were dissolved in DMF (80 mL) and the solution was left to react overnight under stirring at 50°C. The solvent was then removed under reduced pressure, and the residue was dissolved in acetone and then precipitated into water to remove residual traces of DMF. The residue was isolated by precipitation in H_2O, dissolved in diethyl ether and dried over $MgSO_4$. The solvent was removed under reduced pressure, and ^1H and ^{13}C NMR analysis confirmed quantitative conversion to the azide **(5)**, which was used for the following step without further purification. Yield: 8.0 g (11.3 mmol), 57 %.

^1H NMR spectrum is shown in Figure S21.

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 20.38, 20.51, 20.59, 20.66, 20.70, 20.72, 20.81, 50.41, 60.78, 61.78, 66.62, 68.56, 69.05, 70.55, 70.86, 71.41, 72.62, 72.73, 100.31, 100.90, 168.97, 169.53, 169.64, 169.89, 170.02, 170.21, 170.24 ppm.

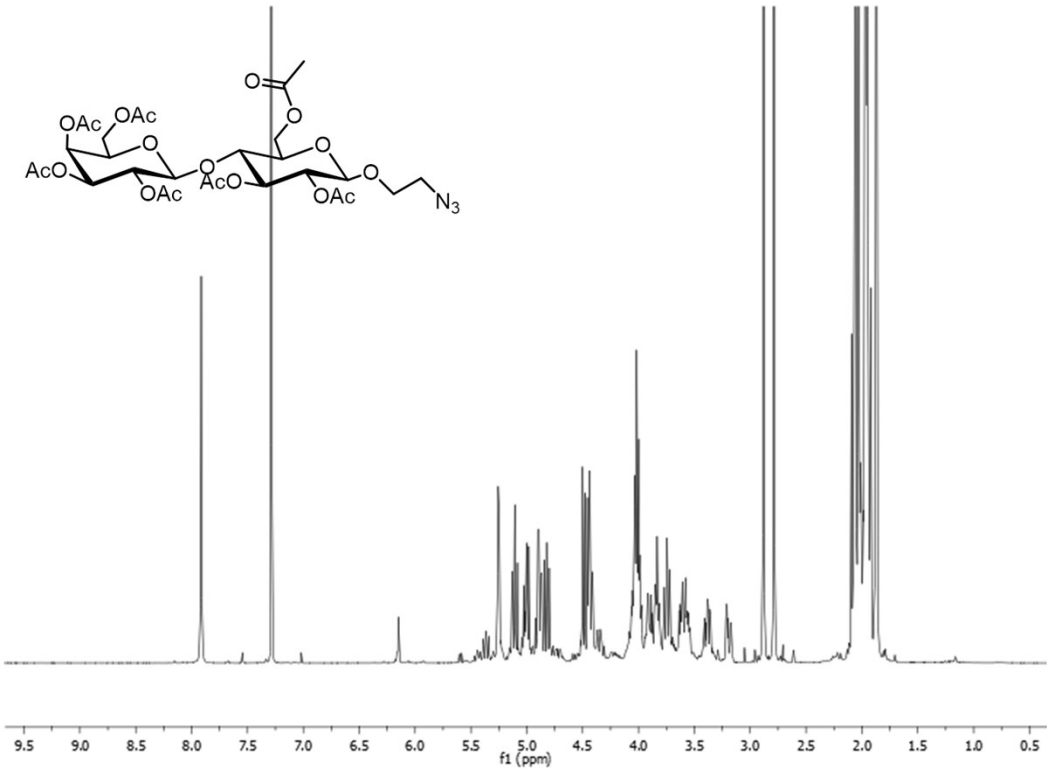


Figure S21. ^{1}H NMR spectrum of 2'-azidoethyl peracetylated lactose **(5)** in $CDCl_3$.

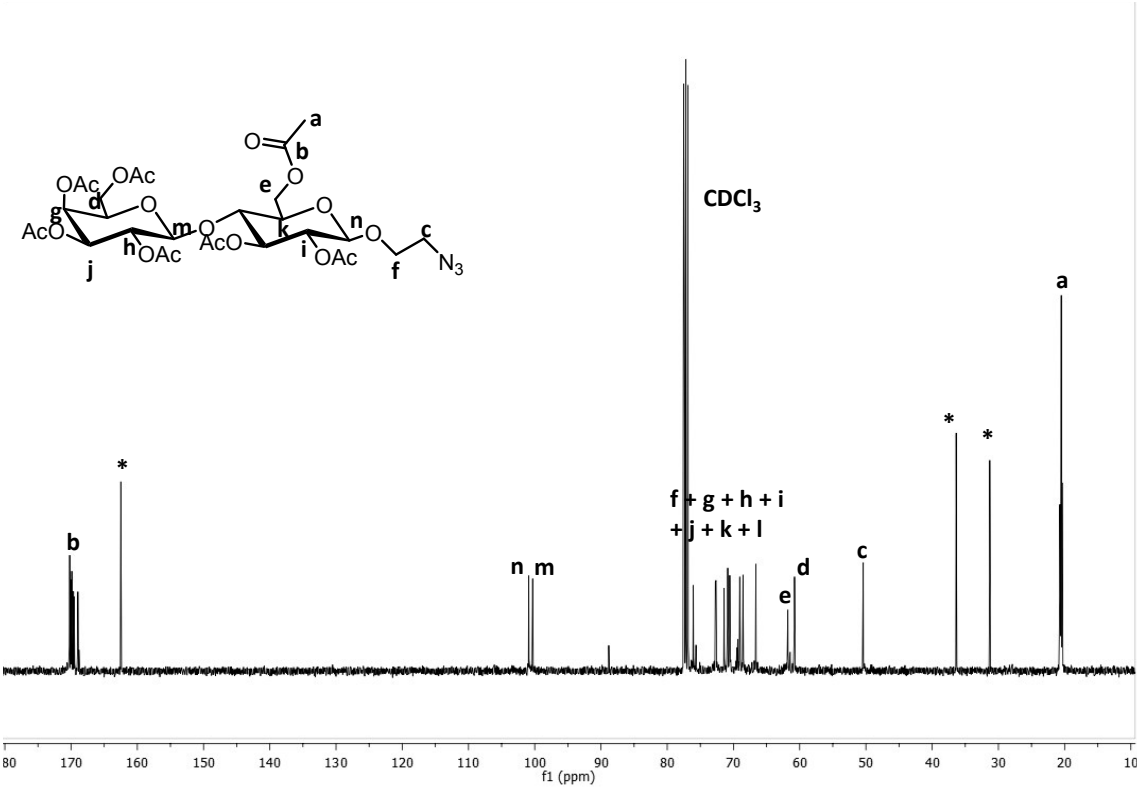


Figure S22. ^{13}C NMR spectrum of 2'-azidoethyl peracetylated lactose **(5)** in $CDCl_3$ (* DMF).

2'-azidoethyl lactose (9e). Peracetylated 2'-azidoethyl lactose **(5)** (7.90 g, 12.9 mmol) was dissolved in MeOH (300 mL). Sodium methoxide (0.67 g, 12mmol) was added, and the mixture was allowed to react for two hours. The volume was then reduced under vacuum and Amberlite IR 120H, 10 g, previously washed with water, was added. The reaction mixture was filtered and the solvent removed to give 2'-azidoethyl lactose **(9e)** as a white solid. Yield: 4.79 g (11.6 mmol), 89.9%.

^{1}H-NMR (400 MHz, MeOD) δ (ppm) ^{1}H NMR (400 MHz, D2O) δ 4.54 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.09 – 4.02 (m, 1H), 3.98 (dt, *J* = 8.3, 4.3 Hz, 1H), 3.93 (m, 2H), 3.89 – 3.85 (m, 1H), 3.85 – 3.79 (m, 2H), 3.78 – 3.75 (m, 2H), 3.74 – 3.72 (m, 2H), 3.67 (t, *J* = 3.8 Hz, 3H), 3.56 (dt, *J* = 7.3, 4.1 Hz, 2H).

^{13}C NMR (101 MHz, MeOD) δ (ppm) 50.57, 60.12, 61.05, 68.55, 68.59, 70.99, 72.57, 72.81, 74.38, 74.83, 75.37, 78.40, 102.19, 102.95.

FT-IR: v 3349, 2920, 2360, 2100, 1735, 1653, 1377, 1301, 1251, 1016, 888, 180, 663 cm^{-1}.

ESI-TOF Mass Spectrometry: expected *m/z* $[M\text{-}Na]^{+}$ 434.1381, found 434.1390.

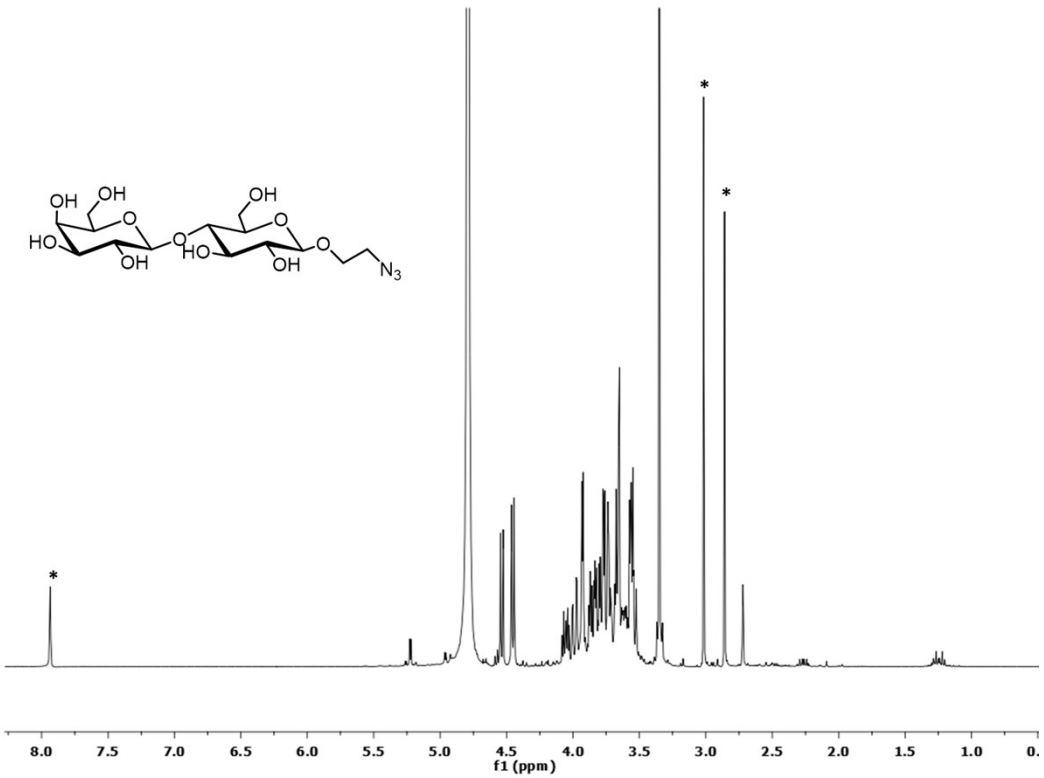


Figure S23. ^{1}H NMR spectrum of 2'-azidoethyl lactose **(9e)** in CD_3OD (* DMF).

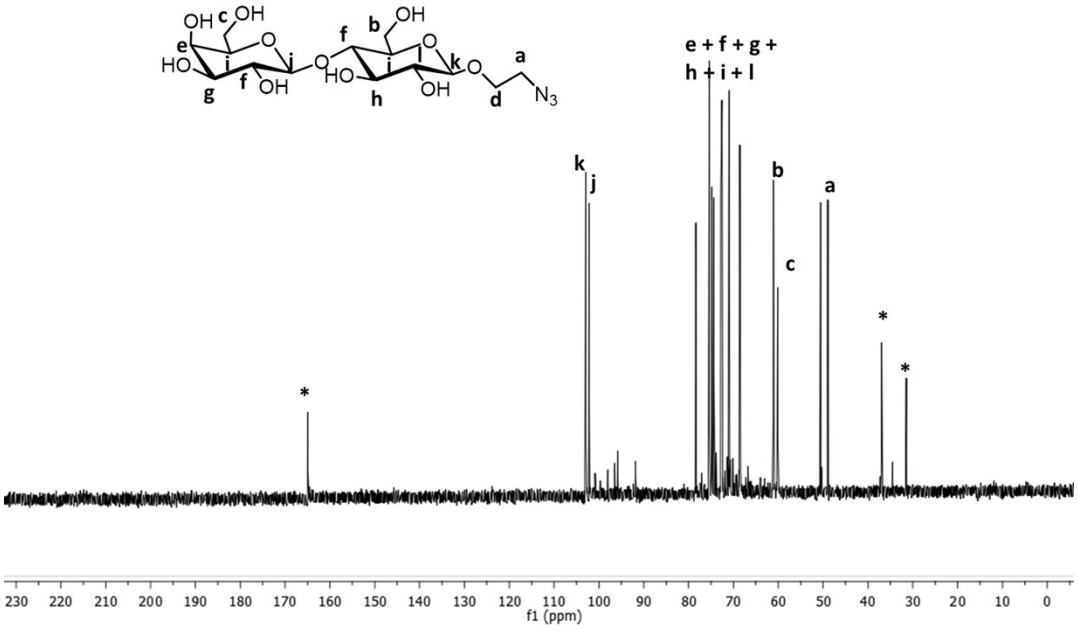
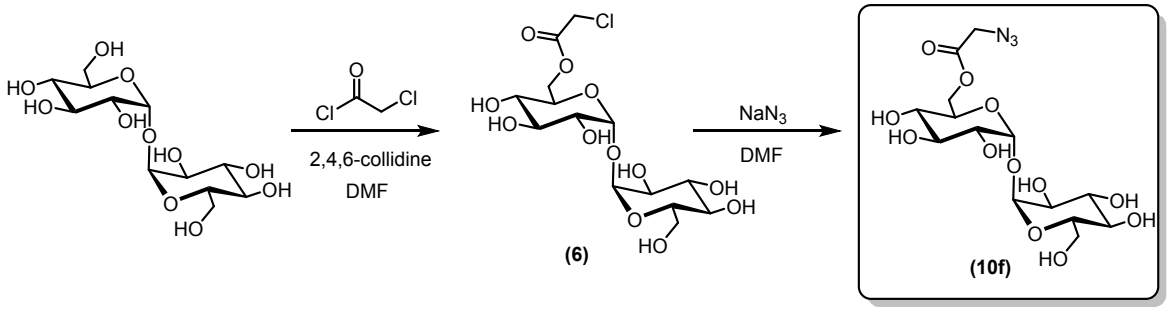


Figure S24. ^{13}C NMR spectrum of 2'-azidoethyl lactose **(9e)** in CD_3OD (* DMF).

6 Synthesis of trehalose azide (10f)

For the synthesis of trehalose **(10f)** a synthetic route adapted from Ishihara *et al.*, [7] was chosen. This involved reaction of D-(+)-trehalose dihydrate with chloroacetyl chloride in DMF using 2,4,6-collidine as a base, and at -50°C to favour reaction of the sugar primary hydroxyl groups over the more hindered secondary ones. Treatment of the reaction crude product – consisting of a statistical distribution of trehalose starting material, and mono and bis sugar alpha-chloroacetates - with sodium azide in DMF followed by flash chromatography on SiO_2 successfully afforded the desired trehalose monoazide **(10f)**.



Scheme S6. Synthesis of trehalose azide **(10f)**.

6- Chloroacetyl trehalose (6). Commercially available trehalose dihydrate (5.00 g, 13.2 mmol) was dissolved in DMF (20 mL) and 2,4,6-collidine (2.12 g, 17.53 mmol) was added. The solution was cooled down to -50°C, using a dry ice/acetonitrile cooling bath. Chloroacetyl chloride (1.81 g, 16.07 mmol) was added drop wise using a glass syringe and the reaction mixture was stirred at this temperature for 3 hours, then at room temperature for an additional hour. The progress of the reaction was monitored via mass spectroscopy. The reaction was then quenched with methanol, in order to react any potential residual chloroacetyl chloride, and precipitated in Et_2O. ^{13}C NMR analysis of the precipitate showed new signals at ca. 40 and 170 ppm, consistent with the presence of a chloroacetyl moiety in the trehalose structure (along with a pair of analogous signals deriving from unreacted chloroacetyl chloride). In comparison, ESI-TOF mass spectrometry was more diagnostic, with a main peak at 441.2 m/z for the $[M\text{-}Na]^+$ ion of the desired monoesterified product **(6)** (expected 441.1) and smaller ones for sodiated trehalose starting material and the 6,6'-diesterified trehalose byproduct, at 365.3 and 577.0, respectively, (Figure S25). The crude product was utilized for the following step without further purification.

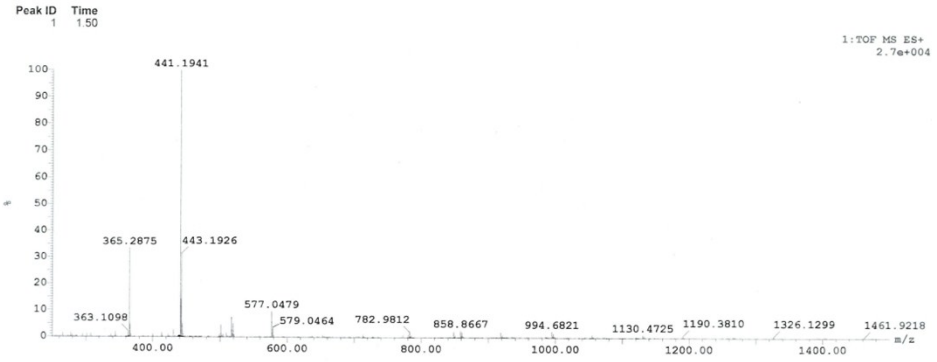


Figure S25. ESI-TOF mass spectroscopy of the crude mixture containing the desired 6-chloroacetyl trehalose **(6)**, along this unreacted trehalose, and 6'-diesterified trehalose byproduct. **(6)**: expected m/z $[M\text{-}Na]^+$ 441.0770, found 441.1941.

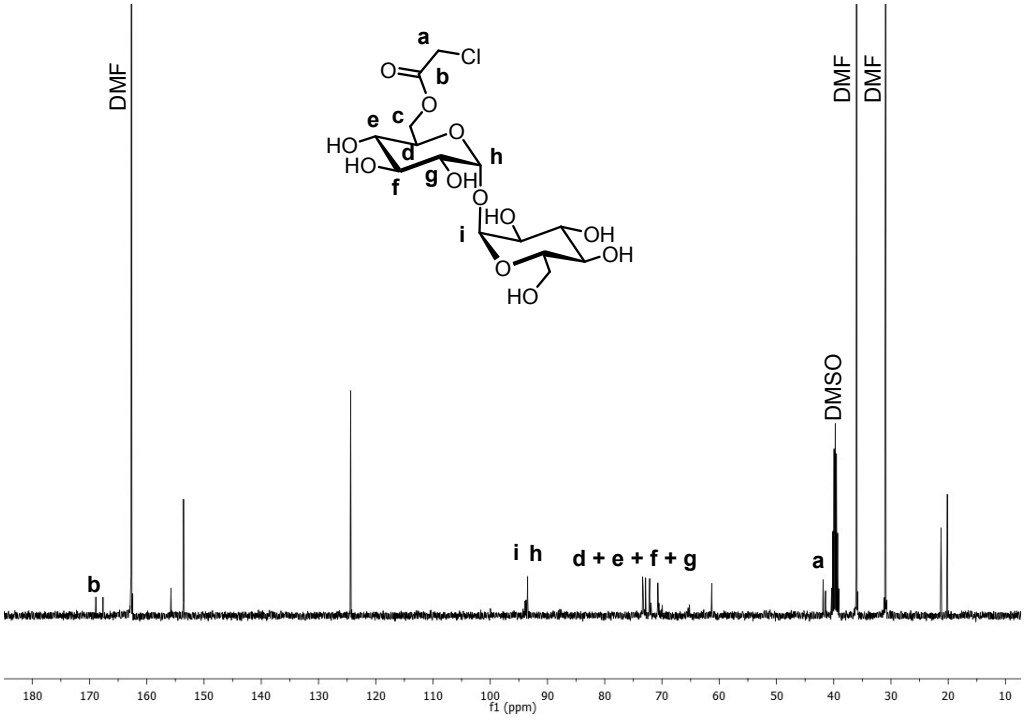


Figure S26. ^{13}C NMR spectrum of the crude reaction mixture containing the desired 6-chloroacetyl trehalose **(6)**, along this unreacted trehalose, and 6'-diesterified trehalose by-product.

Trehalose azide (10f). 6.0 g of the crude product from the previous step – consisting of a mixture of unreacted trehalose, mono- and bis-acetylated derivatives - was dissolved in DMF (10 mL) and NaN_3 (1.15 g, 17.7 mmol) was added to the solution. The mixture was left to react for 72 h at room temperature, under stirring. The reaction was monitored by ^{13}C NMR, by following the disappearance of the CH_2Cl signal at 40 ppm, and the appearance of a new peak for CH_2N_3 of the desired trehalose azide and its diazido byproduct at 49 ppm.

The reaction solution was then precipitated in Et_2O and the residue was purified by flash chromatography (SiO_2, EtOAc:MeOH 8:2) to give the pure product **(10f).** Yield: 1.17 g (2.75 mmol), 21% overall yield for the two steps.

^{13}C NMR (101 MHz, CD_3OD) δ (ppm) 49.67, 61.16, 64.13, 69.88, 70.35, 70.45, 71.67, 71.73, 72.49, 72.98, 73.14, 93.84, 93.97, 168.98.

FT-IR: v 3412, 2934, 2112, 1637, 1400, 1343, 1290, 1148, 1071, 976, 864, 781, 621 cm^{-1}.

ESI-TOF MS: expected m/z $[M-Na]^+$ 448.1174, found 448.1186.

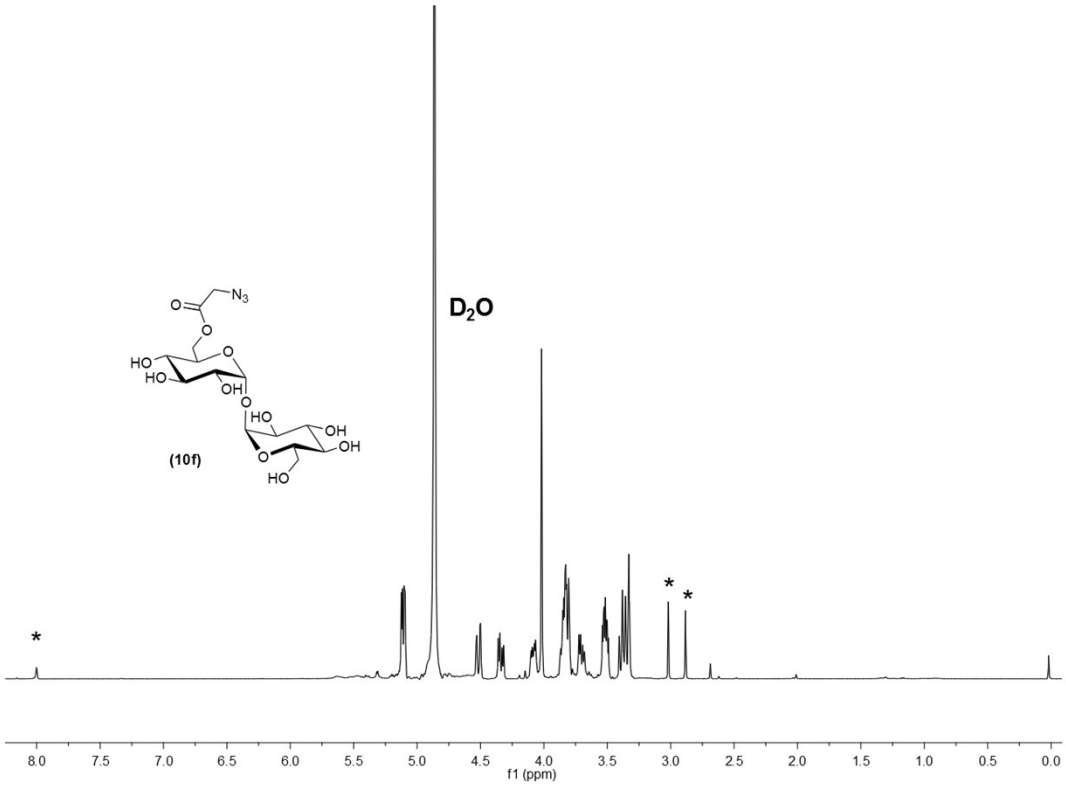


Figure S27. ^{1}H NMR spectrum of trehalose azide **(10f)** in D_2O.

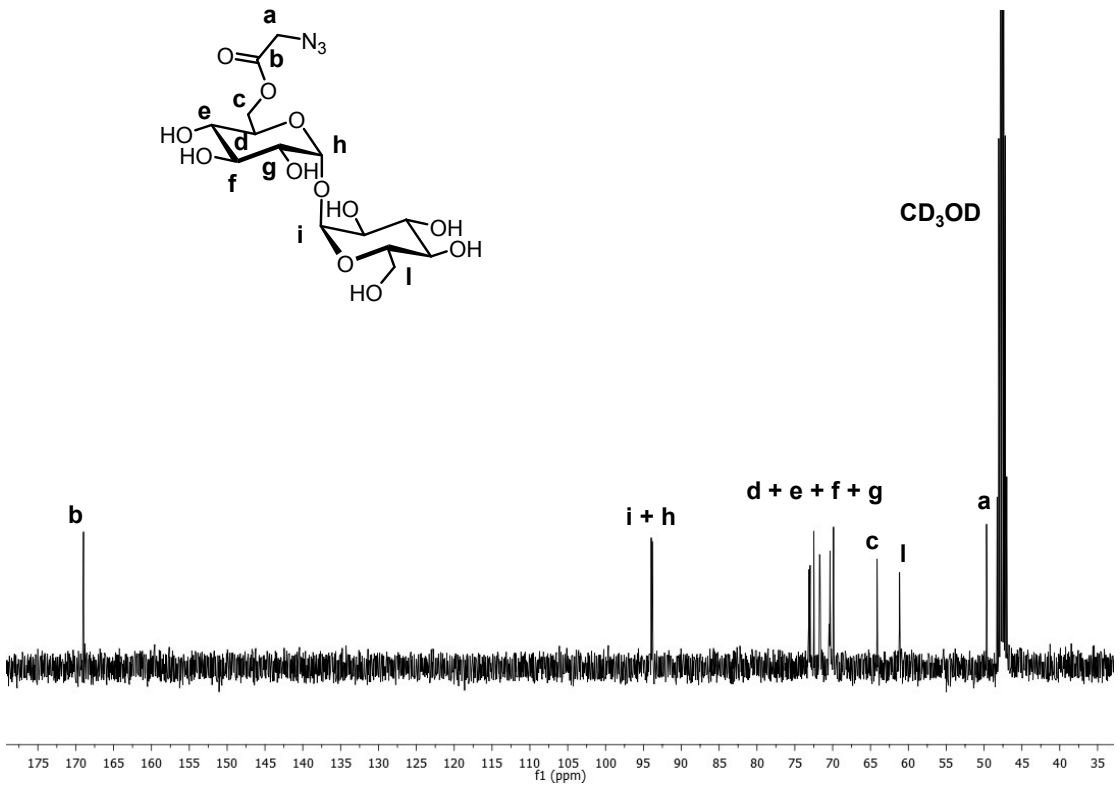
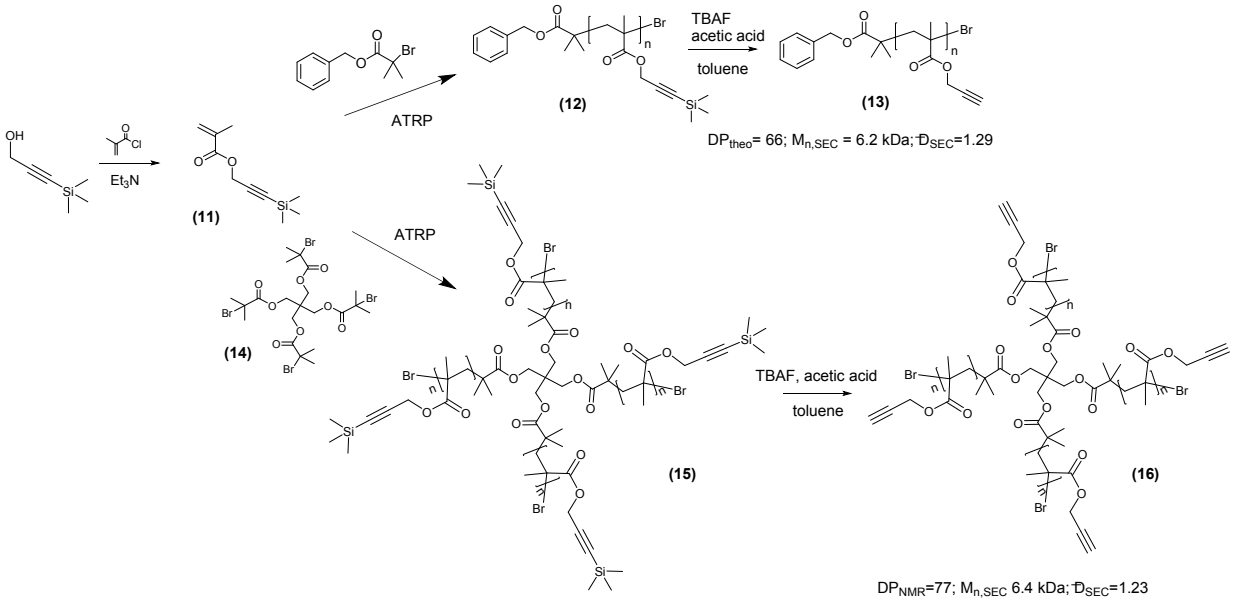


Figure S28. ^{13}C NMR spectrum of trehalose azide **(10f)** in CD_3OD.

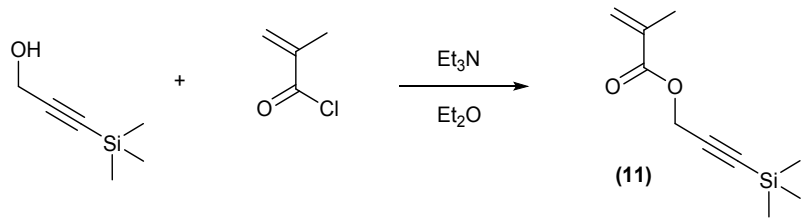
7 Synthesis of trimethylsilyl propargyl methacrylate polymers (13) and (16).

The first step for the preparation of the required "clickable" poly(propargyl methacrylate) scaffolds was the synthesis of trimethylsilyl-propargyl methacrylate monomer **(11)**. Commercially available trimethylsilyl propyn-1-ol was reacted with methacryloyl chloride in presence of trimethylamine as described by Ladmiral *et al*[1] and the crude product was purified by flash chromatography giving the desired monomer in 54% yield. Two clickable polymer, with linear **(13)** and star **(16)** architecture, were prepared by ATRP. Polymerization was carried out using the ATRP initiator of choice (I) , *N*-(ethyl)-2-pyridinmethanimide as ligand (L), Cu(I)Br, and the trimethylsilyl propargyl methacrylate monomer **(11)** (M), in a molar ratio of [M]:[I]:[L] [CuBr]= [100]:[1]:[2]: [1].



Scheme S7. Synthetic route to 3-trimethylsilyl-propargyl methacrylate monomer **(11)** and the corresponding linear **(13)** and star polymers **(16)**.

The synthesis of the trimethylsilyl propargyl methacrylate polymers and all intermediates was carried out as reported by Ladmiral *et al* and is described below. [1]



Scheme S8. Synthetic route to trimethylsilyl-propargyl methacrylate monomer **(11)**.

Trimethylsilyl methacrylate monomer (11). A solution of trimethylsilyl propyn-1-ol (10 g, 78 mmol) and EtN_3 (14.2 mL, 101 mmol) in Et_2O (100 mL) was cooled to 0°C and a solution of methacryloyl chloride (8.8 mL, 93 mmol) in Et_2O (50 mL) was added dropwise. The mixture was stirred at this temperature for 30 min, then at ambient temperature overnight. The precipitated

triethylammonium salt was removed by filtration, and the volatiles were removed under reduced pressure. The oily residue was subjected to flash chromatography (CC, SiO_2, petroleum ether 100% and when most of the product was already recovered, petroleum ether/Et_2O 50:1), yielding 8.17 g of monomer **(11)** (414 mmol, 53.4%).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.20 (s, 9H, $Si(CH_3)_3$), 1.95-1.98 (m, 3H, $CH_3C=CH_2$), 4.77 (s, 2H, OCH_2), 5.58- 5.59 (m, 1H, C=C*H*H), 6.16 (m, 1H, , C=CH*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 0.12, 18.30, 52.94, 92.07, 99.04, 126.23, 135.73, 166.57.

FT-IR: v 2960, 1723, 1638, 1452, 1366, 1314, 1292, 1251, 1147, 1035, 971, 942, 842, 813, 761 cm^{-1}.

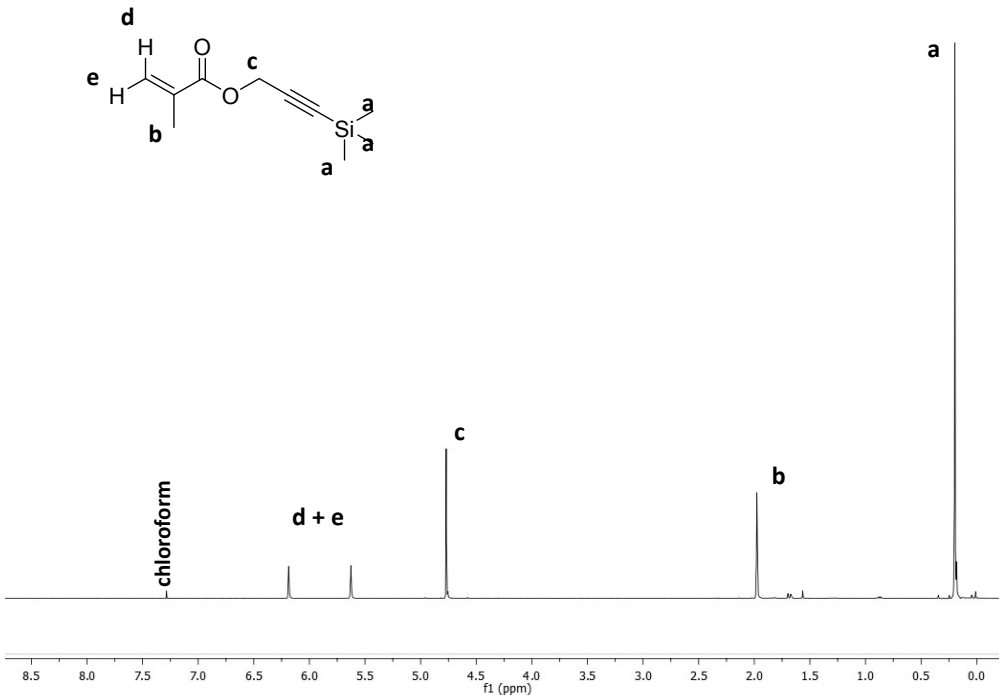


Figure S29. ^{1}H NMR spectrum of trimethylsilyl methacrylate monomer **(11)** in $CDCl_3$.

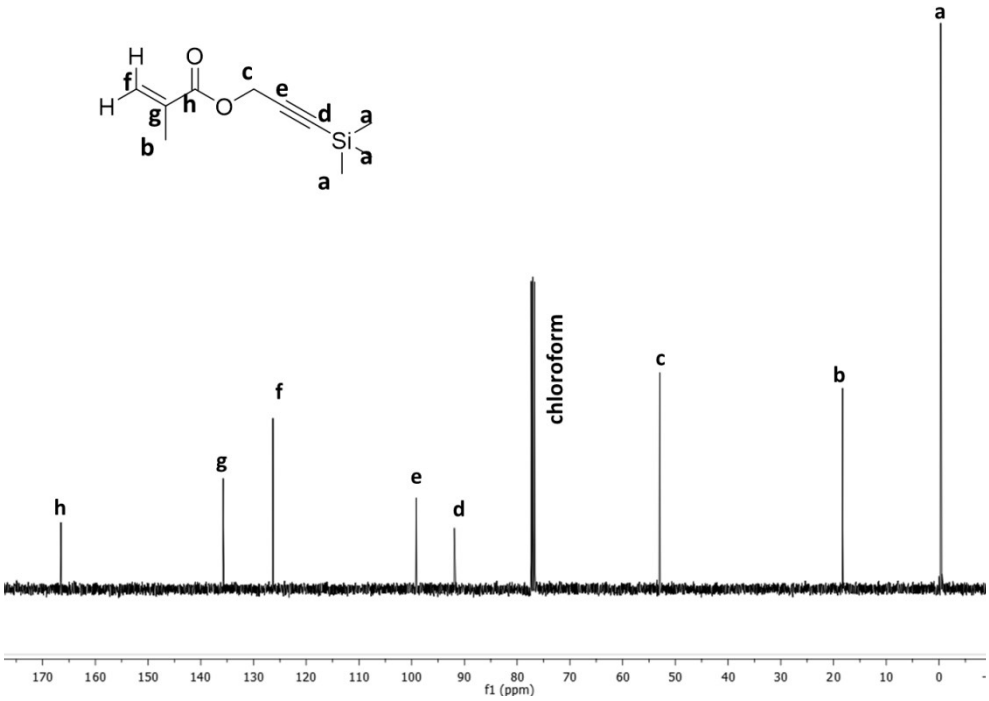
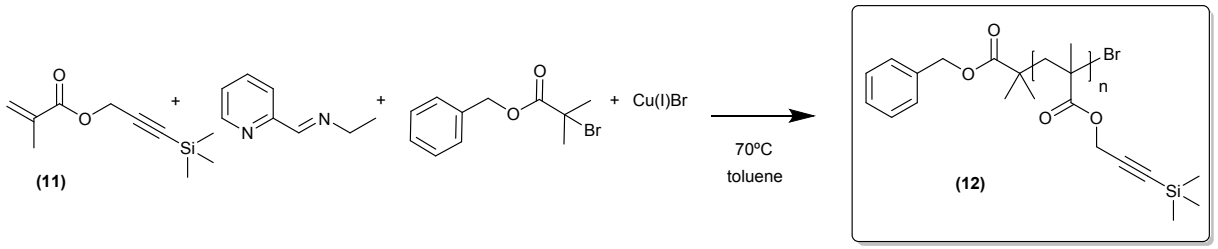


Figure S30. ^{13}C NMR spectrum of trimethylsilyl methacrylate monomer **(11)** in $CDCl_3$.

8 Synthesis of poly(propargyl methacrylate) linear polymer (13)

The first step involved the synthesis of linear TMS-protected poly(propargyl methacrylate) linear polymer **(12)** (Scheme S12). Polymerisation largely followed a linear pseudo first-order kinetic plot, indicating good control over the polymerisation process. The reaction was stopped at 88.6% of conversion, which was reached after 15 hours. The degree of polymerisation (DP, the number of polymer repeating units) was estimated by ^1H NMR, by comparing the signal of the methylene group in the initiator at 5.1 ppm with the methylene group in the polymer at 4.5 ppm, and was found to be 66.



Scheme S9. Synthesis of linear TMS-protected poly(trimethylsilyl-propargyl methacrylate) **(12)**.

N-(Ethyl)-2-pyridylmethanimide ligand (0.048 g, 0.36 mmol), trimethylsilyl-propargyl methacrylate monomer (3.50 g, 17.8 mmol), and benzyl 2-bromo-2-methylpropanoate initiator (0.064 g, 0.25 mmol) were charged into a dry Schlenk tube along with toluene (7.0 mL) as the solvent. The tube was sealed with a rubber septum and subjected to six freeze-pump-thaw cycles. This solution was then transferred via cannula under nitrogen into a second Schlenk tube, previously evacuated and filled with nitrogen, containing Cu(I)Br (0.025 g, 0.17 mmol) and a magnetic follower. The mixture became dark yellow immediately and progressively darker as time progressed. The temperature was adjusted at 70°C with constant stirring (t=0). Samples were removed periodically using a degassed syringe for molecular weight and conversion analysis. Once the desired conversion was reached, the reaction flask was cooled to room temperature and exposed to air. The reaction mixture was passed through a short neutral alumina column and subsequently washed with toluene, in order to remove traces of copper complexes. The conversion was monitored by analysing the decrease of the methylene group in the monomer (4.9 ppm) and the increase of the corresponding signal in the polymer (4.7 ppm). The polymerisation was stopped when conversion reached 90 % and the purified reaction mixture was used for the subsequent deprotection step without further purification.

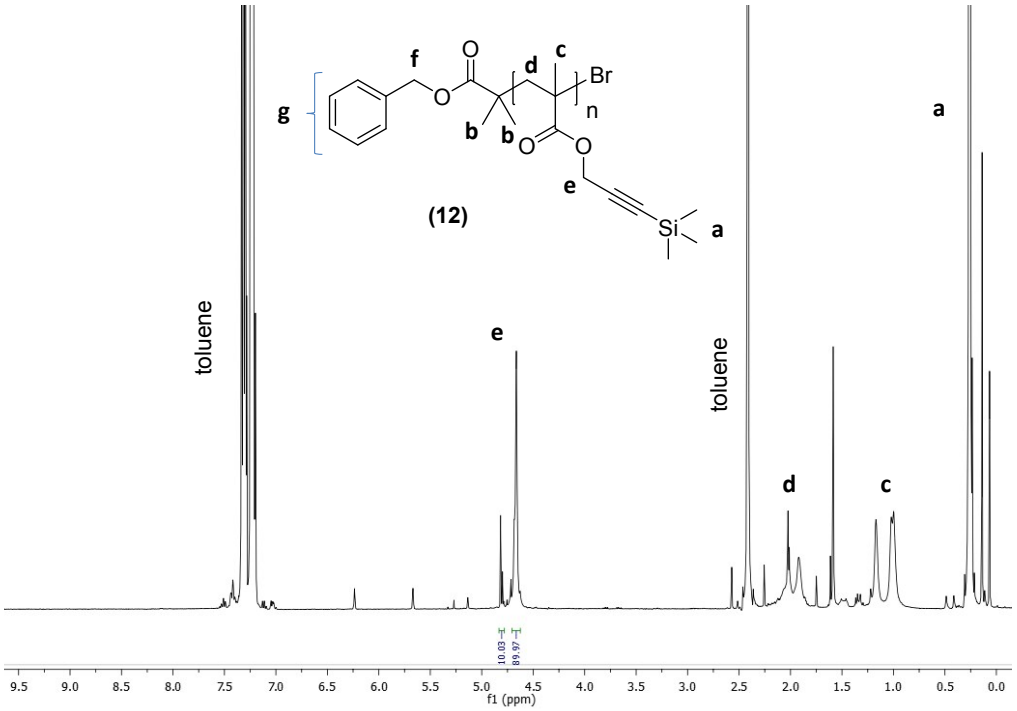
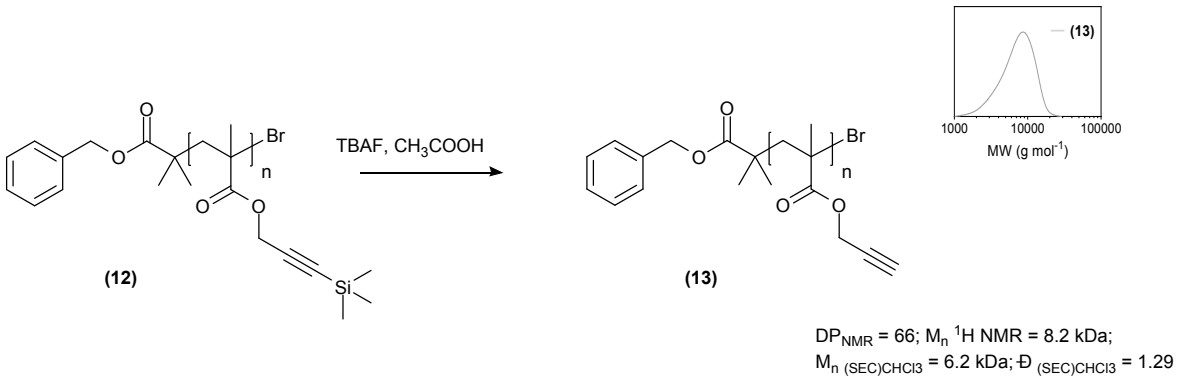


Figure S31. ^{1}H NMR spectrum of partially purified reaction mixture of TMS-protected linear poly(trimethylsilyl-propargyl methacrylate) **(12)**, in $CDCl_3$. This reaction mixture was used directly for the subsequent deprotection step.



Scheme S10. Deprotection of poly(trimethylsilyl-propargyl methacrylate) polymer **(12)** to give the required clickable linear polymer **(13)**. Inset: SEC_{CHCl3} trace of **(13)**.

Linear poly(propargyl methacrylate) (13). The partially purified reaction mixture from the previous step containing trimethylsilyl-protected polymer (3.50 g, 17.8 mmol of alkyne-trimethylsilyl groups) were dissolved in THF (40 mL) followed by addition of acetic acid (1.6 g, 27 mmol, 1.5 equiv. mol/mol with respect to the alkyne-trimethylsilyl groups). Nitrogen was bubbled and the brown solution was cooled to -20°C. A solution of 1 M TBAF in THF (26.8 mL, 26.8 mmol, 1.5 equiv. mol/mol with respect to the alkyne-trimethylsilyl groups) was added slowly *via* syringe. The resulting mixture was stirred at this temperature for 30 minutes and then left under stirring at room temperature overnight and the deprotection was monitored by ^{1}H NMR, following the disappearance of the alkyne-$SiMe_3$ peak at ca. 0.2 ppm. The deprotected polymer was precipitated twice in a mixture of water methanol 1:1 vol/vol to remove TBAF, and isolated by centrifugation. After confirming the absence of residual TBAF by ^{1}H NMR, the polymer was dissolved in chloroform, precipitated in petroleum ether. The final polymer **(13)** was collected by filtration and left under reduced pressure overnight to remove residual traces of solvent. Linear poly(propargyl methacrylate) (13) (0.48 g, 3.87 mmol of 1-alkyne clickable units) was isolated as an off-white solid. DP_{NMR} = 66; M_n ^{1}H NMR = 8.2 kDa; M_n $(SEC)_{CHCl_3}$ = 6.2 kDa; Đ $(SEC)_{CHCl_3}$ = 1.29.

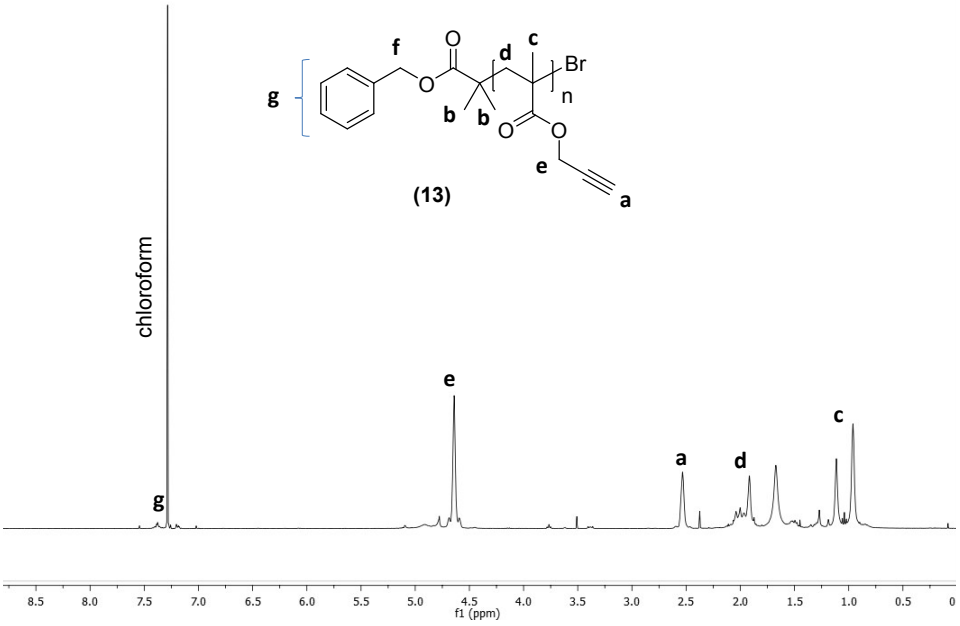
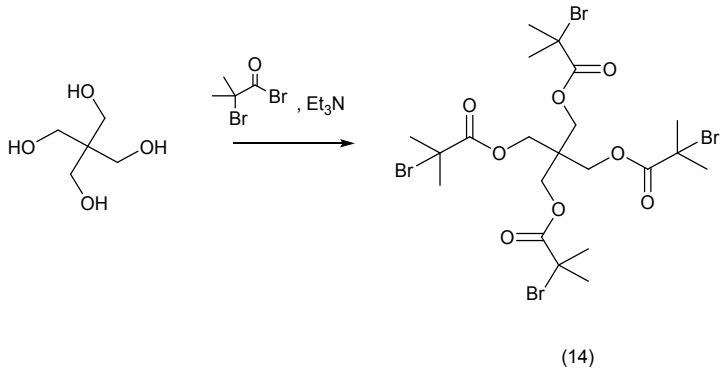


Figure S32. ^{1}H NMR spectrum of linear poly(propargyl methacrylate) **(13)** in $CDCl_3$.

9 Synthesis of four-arm initiator (14)

In order to obtain a four-arm star polymer, it was necessary to synthesise a suitable ATRP initiator. This was undertaken as described by Gao *et al*. [8] with some modifications.



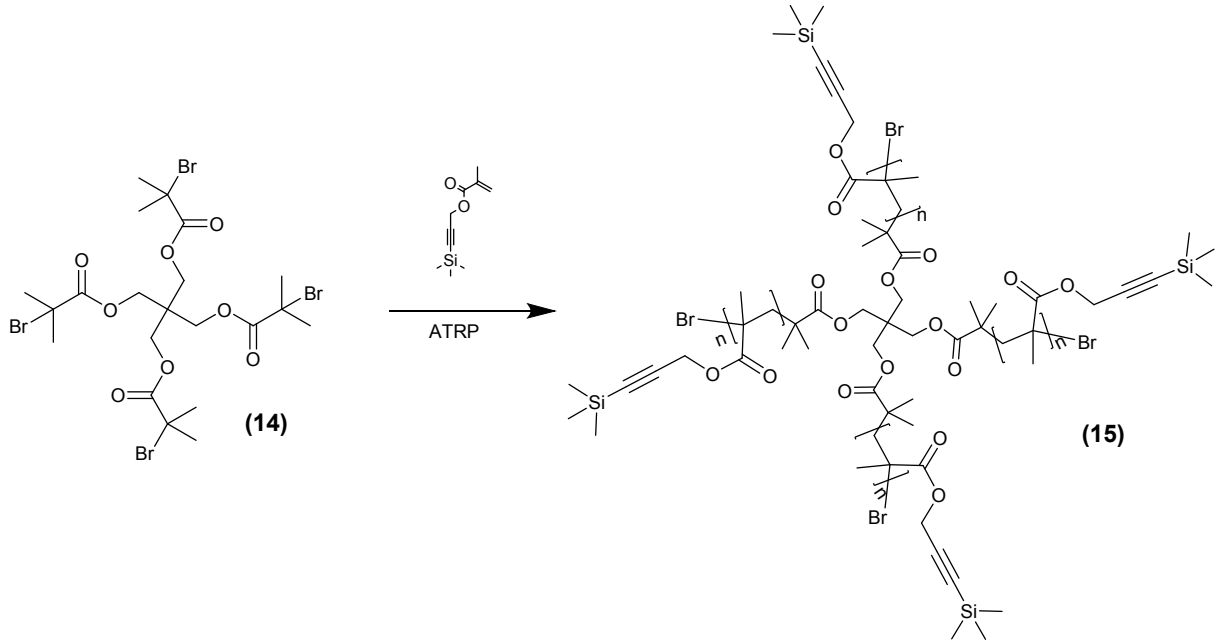
Scheme S11. Synthesis of four-arm ATRP initiator **(14)**.

A solution of pentaerythritol (2.37 g, 17.4 mmol) and Et_3N (12 mL, 87 mmol) in CH_2Cl_2 (10 mL) was cooled to 0°C and a 2-bromo-2-methylpropionyl bromide (10.8 mL, 87.4 mmol) was added drop wise over approximately 10 minutes. The mixture was stirred at this temperature for 30 min, then at ambient temperature overnight (18 hours). The volatiles were removed under reduced pressure. The crude product was purified by flash chromatography (CC, SiO_2, petroleum ether/ CH_2Cl_2 1:1; 100% CH_2Cl_2; 20% methanol) to give the initiator **(14)** (7.27 g, 9.93 mmol, 57.1%) as a colourless oil.

FT-IR: v 3465, 2103, 1948, 1596, 1489, 1326, 1153, 1008, 755, 694 cm^{-1}

10 Synthesis of 4-arm star poly (propargyl methacrylate) (16)

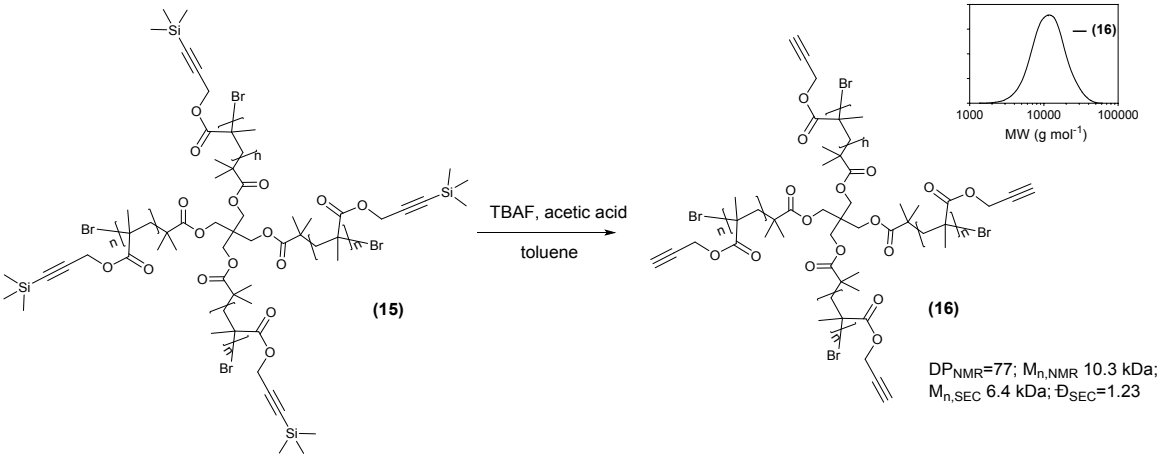
4-arm star poly (propargyl methacrylate) **(16)** polymer was synthesised as described for the analogous linear clickable polymer **(13)**.



Scheme S12. Synthesis of 4-arm star TMS-protected poly (propargyl methacrylate) **(15)**.

N-(Ethyl)-2-pyridylmethanimine ligand (0.072 mL, 0.51 mmol), trimethylsilyl-propargyl methacrylate monomer **(11)** (5.00 g, 25.5 mmol) and four-arm ATRP initiator **(14)** (0.186 g, 0.254 mmol) were charged into a dry Schlenk tube along with toluene (5.0 mL). The tube was sealed with a rubber septum and subjected to six freeze-pump-thaw cycles, and this solution was then transferred via cannula under nitrogen into a second Schlenk tube, previously evacuated and filled with nitrogen, containing Cu(I)Br (0.037 g, 0.26 mmol) and a magnetic follower. The mixture became dark yellow immediately and progressively darker. The temperature was adjusted at 70 °C with constant stirring (t=0). Samples were removed periodically (30', 60', 120', 270' and 360') using a degassed syringe for molecular weight and conversion analysis. At the end of the polymerization the mixture was diluted with 10 mL of toluene and air was bubbled through it for 4 h. The reaction mixture was passed through a short neutral alumina column eluting with

toluene to remove copper salts, and the volatiles were removed under reduced pressure. The molecular weight of the polymer was calculated by ^{1}H NMR by comparing the integral of the initiator benzylic CH_2 signal at 5.1 ppm to that of the C(O)OC*H*$_2$ groups of the propargyl pendant units, at 4.4 ppm. The same $M_{n,NMR}$ was estimated when the singlet of the $Si(CH_3)_3$ at 0.2 ppm was used instead of the signal at 4.4 ppm. The conversions were calculated via ^{1}H NMR by following the decreasing of the integrals of the monomer vinyl signals. Alternatively, the conversions were also calculated by comparison between the integrals relative to the C(O)OC*H*$_2$ protons of the monomer (bs, 4.6 ppm, decreasing with time) and the analogous C(O)OC*H*$_2$ protons in the polymer (broad signal, 4.4 ppm increasing with time). This mixture, polymer and unreacted monomer, was used directly for the next step without purification. $DP_{(NMR)}$= 75; $M_{n(NMR)}$ 15.5 kDa.



Scheme S13. Deprotection of TMS-protected star poly(propargyl methacrylate) **(15)** to give the star clickable scaffold **(16)** – inset: SEC_{CHCl3} trace of **(16)**.

The TMS-protected star polymer **(15)** was deprotected as described for the linear polymer **(12)**, to yield the required clickable poly(propargyl methacrylate) **(15)**. $M_{n\ SEC}$ 6.4 kDa, $Đ_{SEC}$ = 1.29, DP_{NMR} 77.

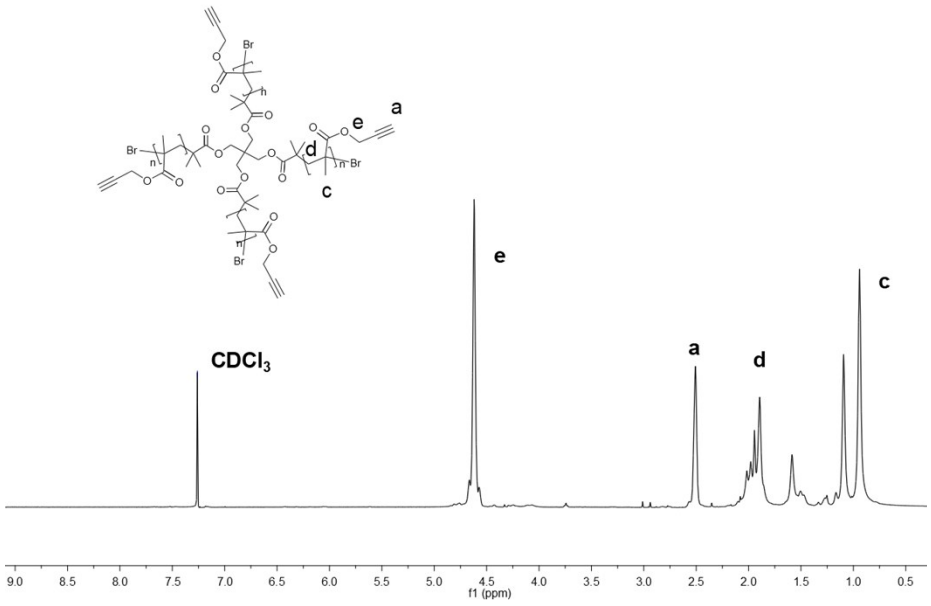
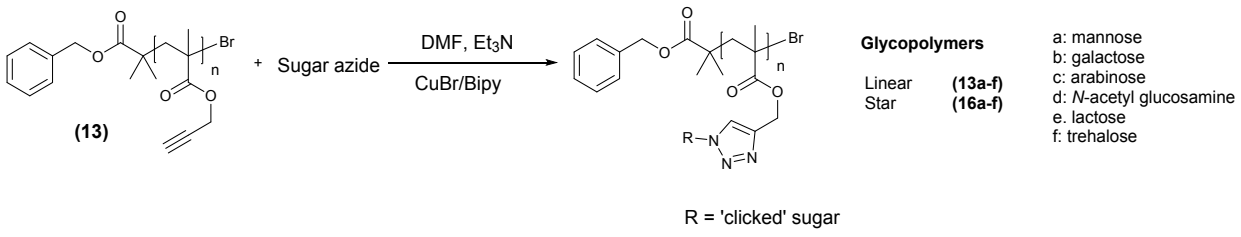


Figure S33. ^{1}H NMR spectrum of star poly(propargyl methacrylate) **(16)** in $CDCl_3$.

11 Synthesis of linear and star glycopolymers via CuAAC '*Click*' chemistry.



Scheme S14. General synthetic route to the glycopolymers utilized in this work.

The clickable linear or star polymer (60 mg, 0.48 mmol of alkyne repeating units), the azido-sugars (1.6 eq per alkyne clickable unit) and 2,2'-bipyridine ligand (9.8 mg, 0.063 mmol), were dissolved in DMF (2 mL) and the resulting solution was degassed by bubbling nitrogen for 15 min. Cu(I)Br (4.5 mg, 0.031 mmol), was then added and nitrogen was bubbled again into the resulting solution for further 10 min. The resulting solution was stirred at ambient temperature for 3 days and the reaction was monitored by ^{1}H NMR, following the formation of the broad signal at 5.1 ppm, corresponding to the C(O)OCH_2 moiety in the clicked polymer. The final clicked polymer was precipitated in Et_2O and the resulting residue was dissolved in deionised water and stirred with Cuprisorb® to remove traces of residual copper salts. After resin removal, the glycopolymer was subjected to dialysis (membrane of 1 kDa MWCO) against deionised water and freeze-dried.

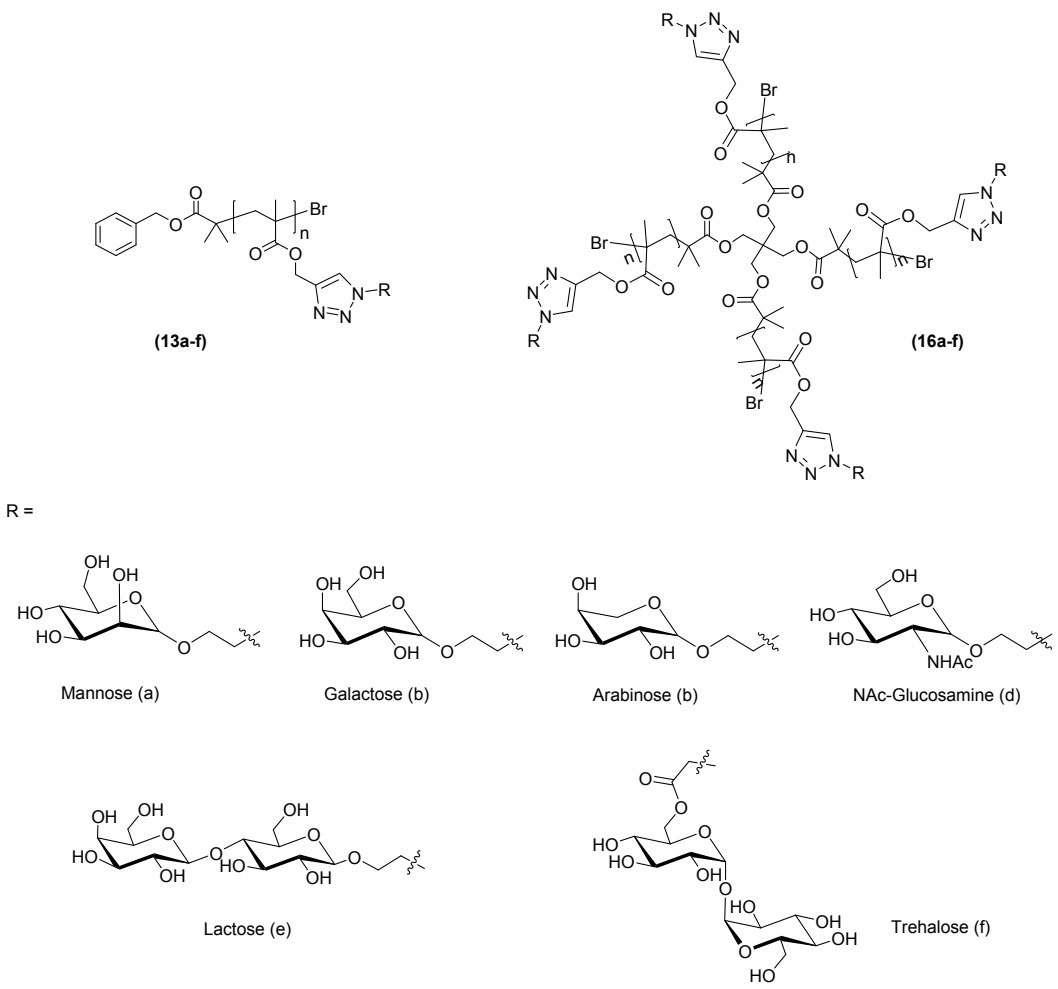


Chart S1. Linear **(13a-f)** and star **(16a-f)** glycopolymers synthetized in this work.

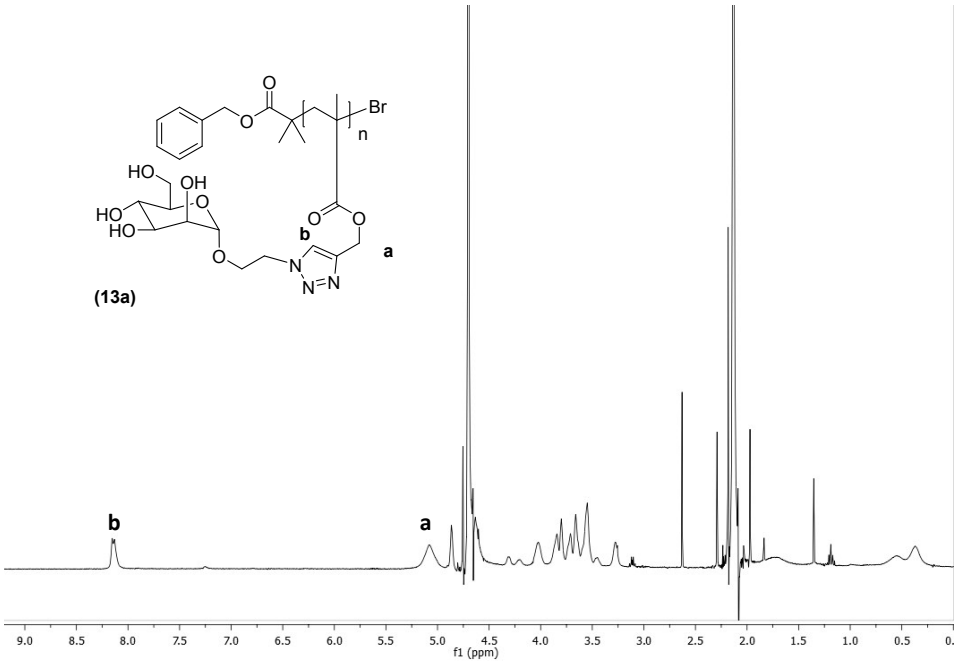


Figure S34. Representative spectrum of clicked glycopolymers: ^{1}H NMR spectrum of mannose linear homo-glycopolymer **(13a)** in D_2O.

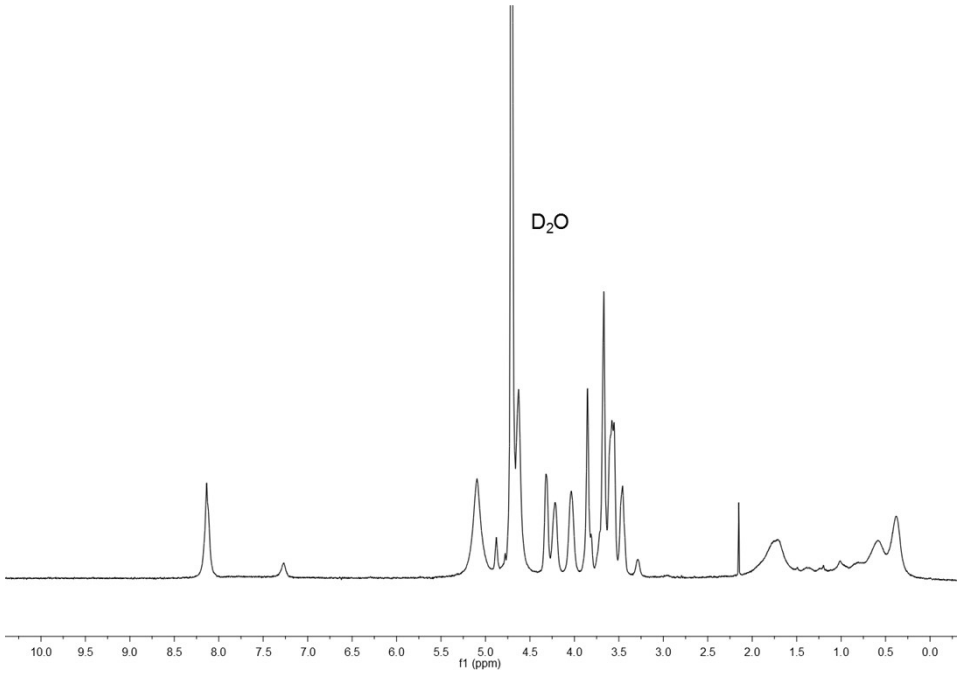


Figure S35. ^{1}H NMR spectrum of galactose linear homo-glycopolymer **(13b)** in D_2O.

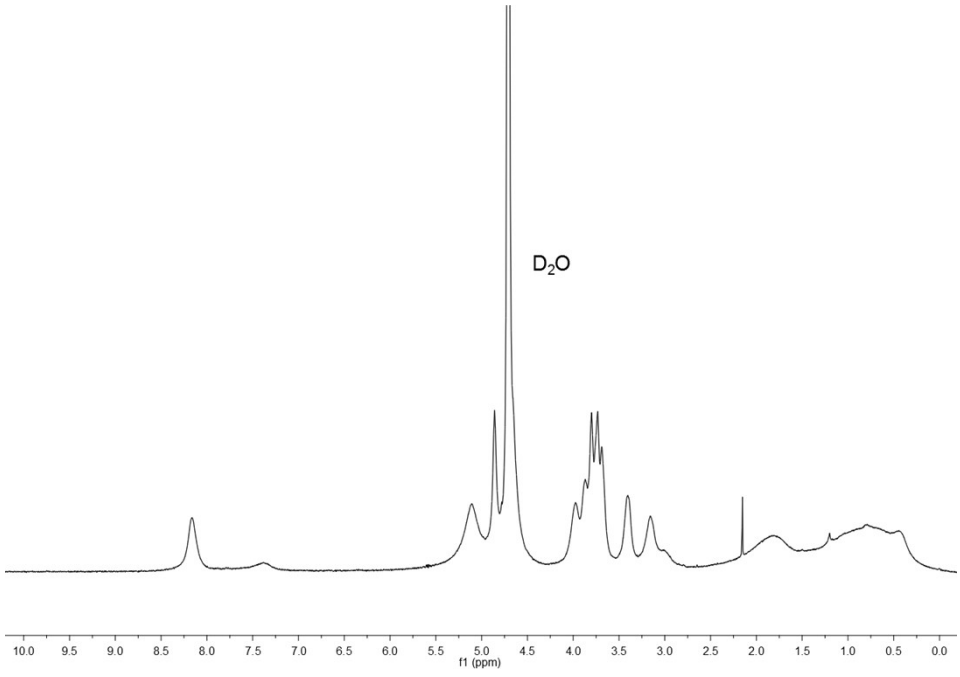


Figure S36. ^{1}H NMR spectrum of arabinose linear homo-glycopolymer **(13c)** in D_2O.

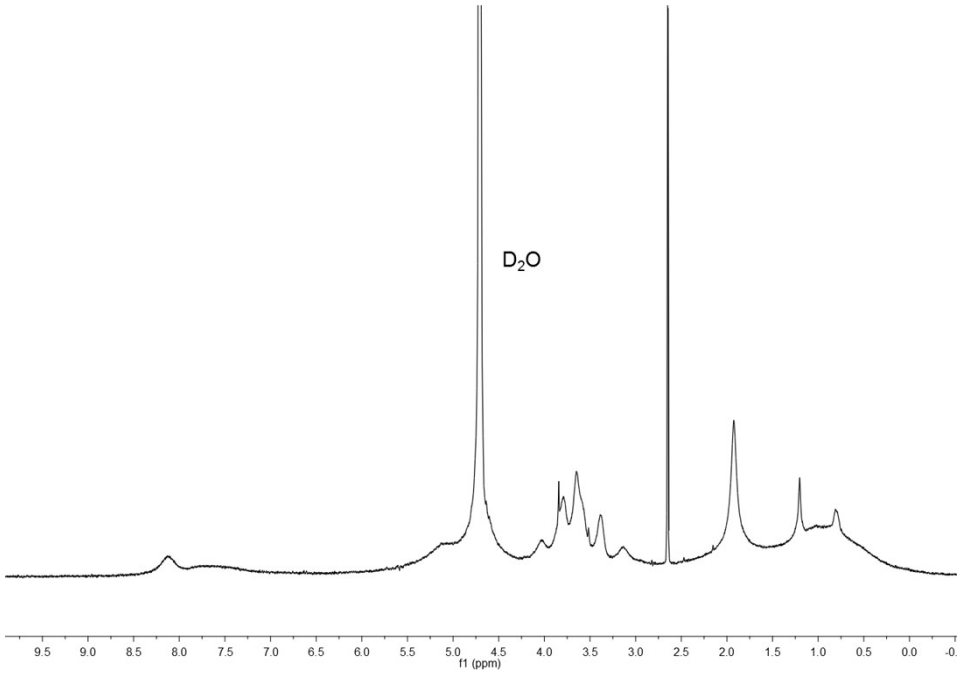


Figure S37. ^{1}H NMR spectrum of *N*-acetyl glucosamine linear homo-glycopolymer **(13d)** in D_2O.

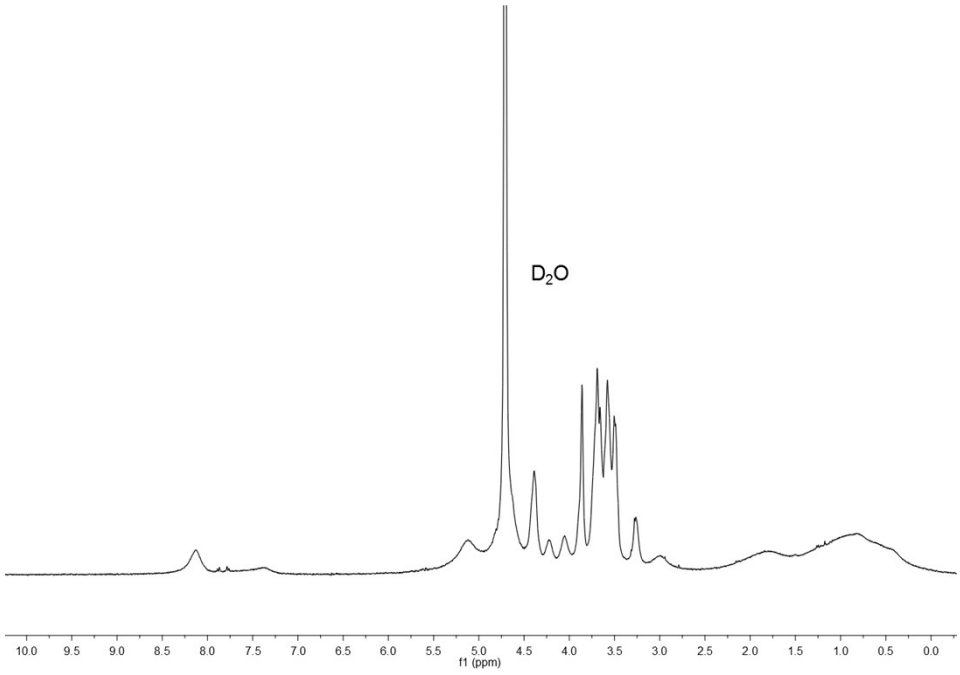


Figure S38. ^{1}H NMR spectrum of lactose linear homo-glycopolymer **(13e)** in D_2O.

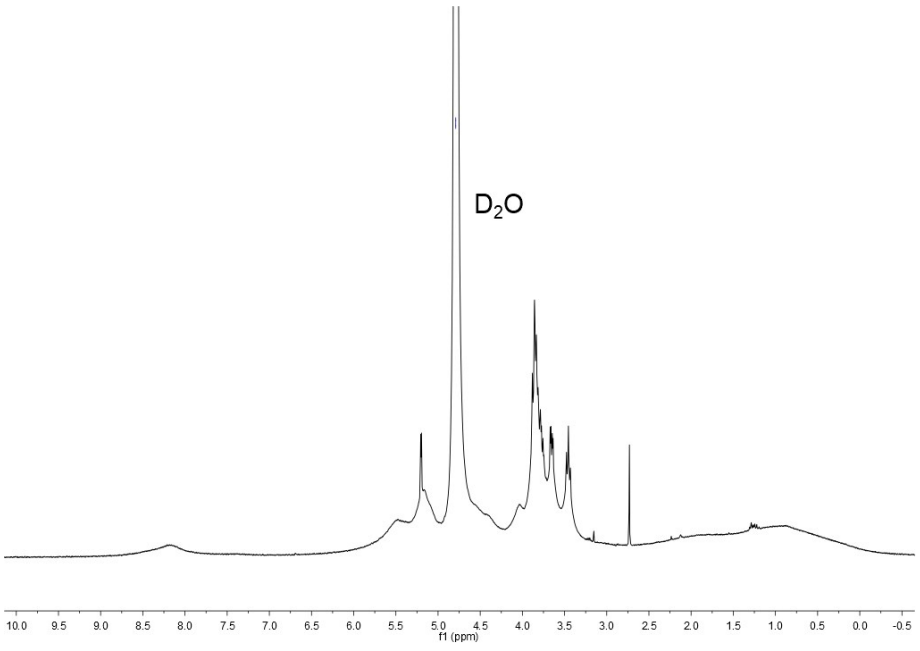


Figure S39. ^{1}H NMR spectrum of trehalose linear homo-glycopolymer **(13f)** in D_2O.

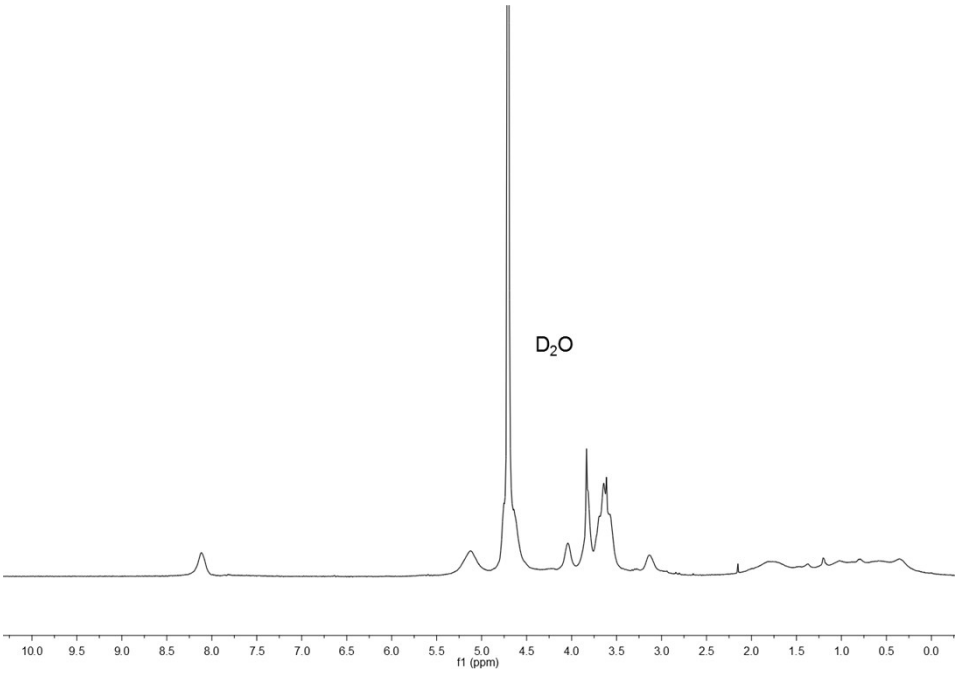


Figure S40. ^{1}H NMR spectrum of mannose star homo-glycopolymer **(16a)** in D_2O.

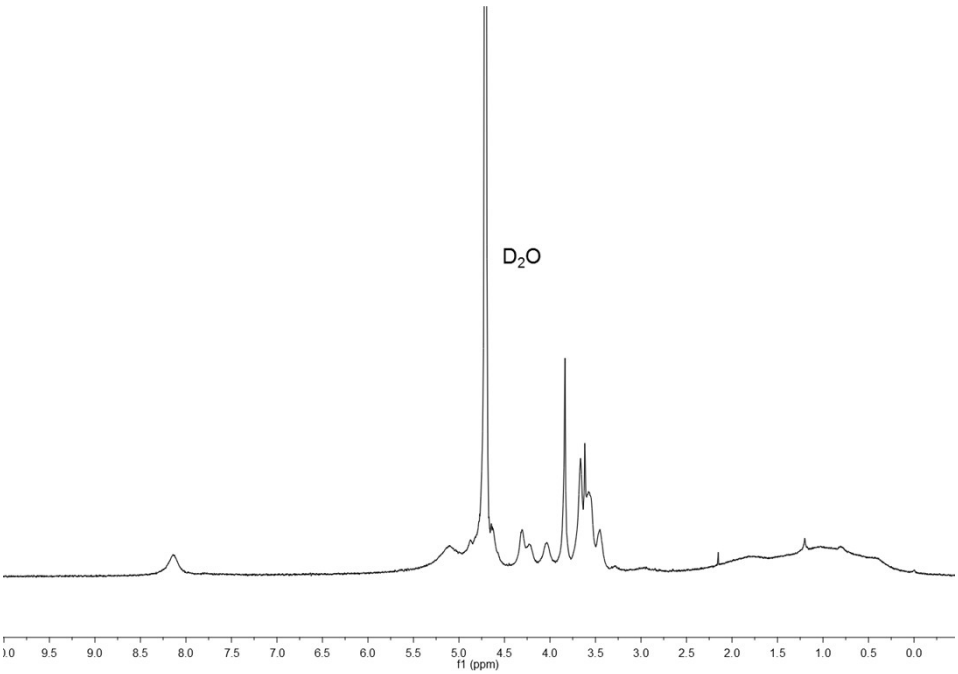


Figure S41. ^{1}H NMR spectrum of galactose star homo-glycopolymer **(16b)** in D_2O.

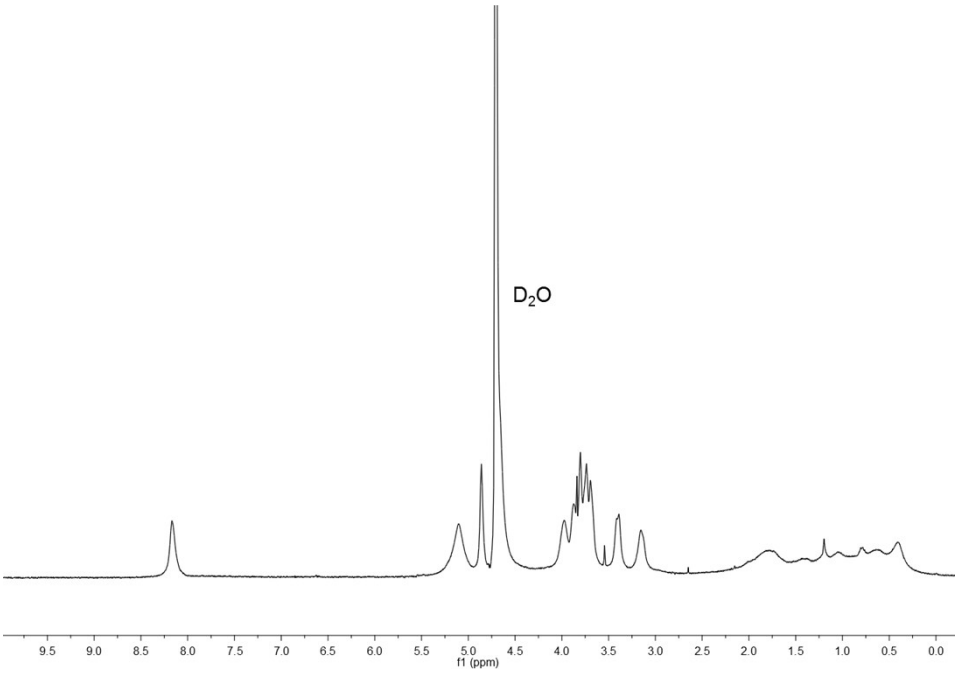


Figure S42. ^{1}H NMR spectrum of arabinose star homo-glycopolymer **(16c)** in D_2O.

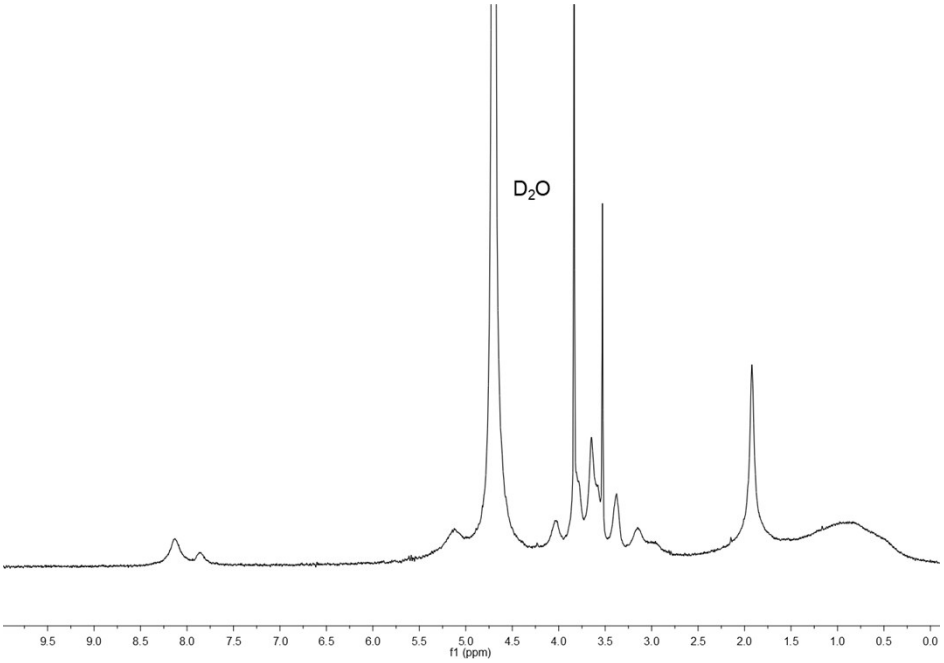


Figure S43. ^{1}H NMR spectrum of *N*-acetyl glucosamine star homo-glycopolymer **(16d)** in D_2O

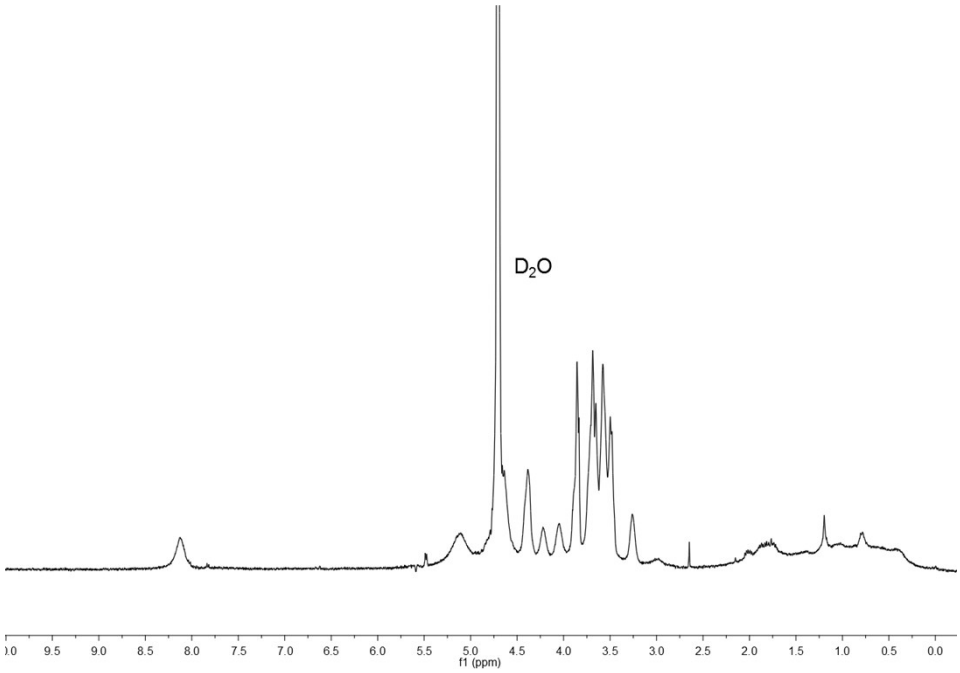


Figure S44. ^{1}H NMR spectrum of lactose star homo-glycopolymer **(16e)** in D_2O.

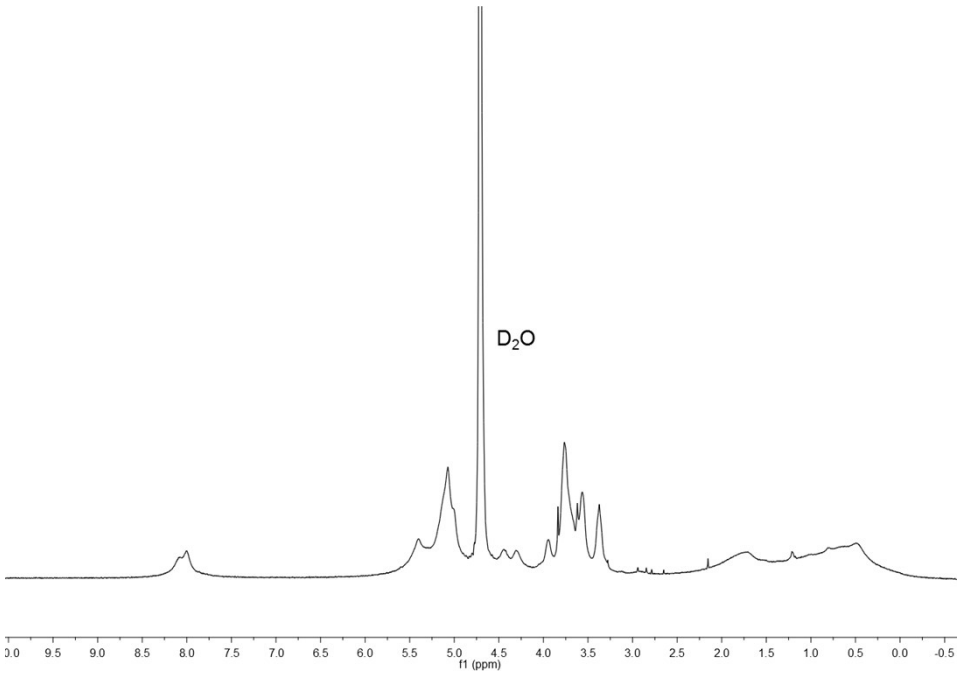


Figure S45. ^{1}H NMR spectrum of mannose star homo-glycopolymer **(16a)** in D_2O.

Determination of T_m by intrinsic fluorescence.

Tm1, Tm2, and Tm3 of mAb1 was determined by both intrinsic fluorescence method and DSC, which gave very similar values. In this initial experiment DSC was utilized as a method to further validate the intrinsic fluorescence method utilised in this study.

Table S1. T_m of native mAb1 as assessed by Intrinsic Fluorescence, and DSC.

Unfolding transition temperatures of protein alone (°C)	Intrinsic Fluorescence	DSC
T_m1 (T_m, $_{CH2}$)	69.46	69.04
T_m2 (T_m, $_{Fab}$)	78.70	78.85
T_m3 (T_m, $_{CH3}$)	85.05	86.51

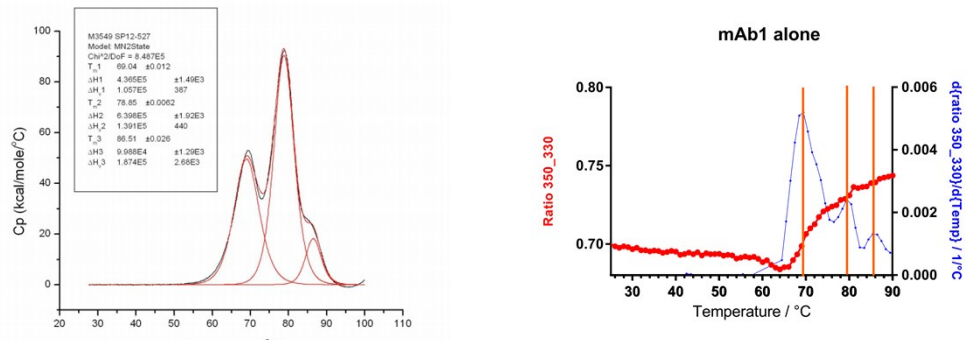


Figure S46. *Left:* DSC Profile of mAb1. Measurements were carried out as described in the *Materials and Methods* section of the main manuscript. *Right:* representative example (mAb1 alone) of intrinsic fluorescence profiles utilised in this study to estimate the unfolding transition temperatures (T_m) of CH2, Fab and CH3 domains of mAb1. Blue line represents the first derivative of the intrinsic fluorescence 350:330 nm ratio trace, used to estimate the T_m (orange vertical lines) of the three protein domains.

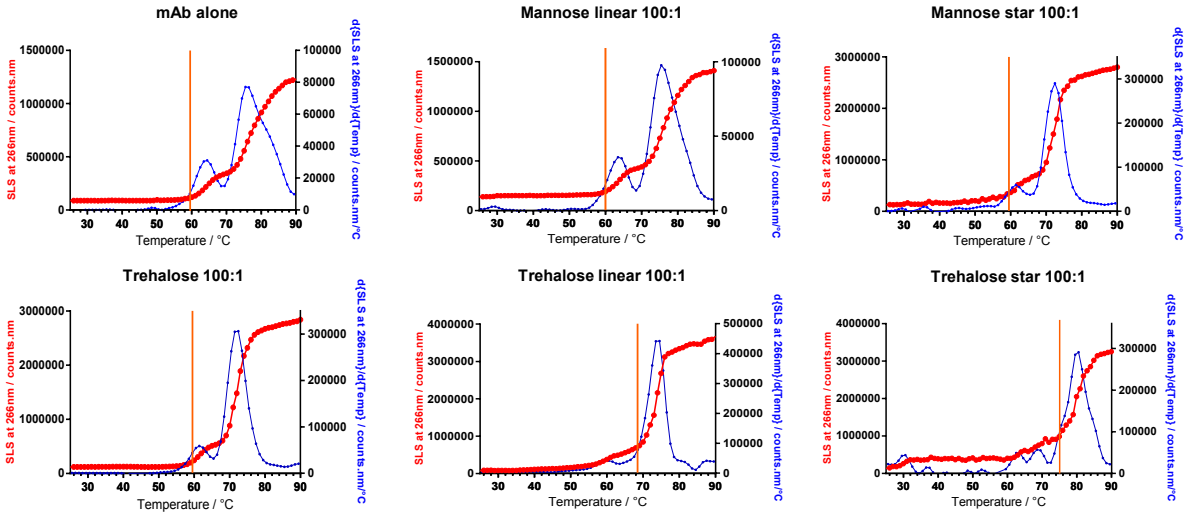


Figure S47. *Top:* typical colloidal stability profiles (red traces) obtained for mAb1 alone, and mAb1 + glycopolymers. Mannose linear **(13a)** and star **(16a)** glycopolymers are used here as representative examples. *Bottom:* trehalose-containing polymers, linear **(13f)** and star **(16f)**, had a distinct behaviour, with scattering profiles showing appreciably less pronounced initial aggregation, resulting in higher estimated T_{agg} values. All examples shown here are relative to 100:1 sugar:protein molar ratio. Blue lines represent the first derivative of the SLS traces used to estimate the T_{agg}. Orange vertical lines generated by the software define the calculated T_{agg} values. This figure shows plots of individual measurements; whilst averages of measurements ran in triplicate (N=3) with three measurements for each (n=3) are shown in Figure 2 in the manuscript. In all samples [mAb1] = 1.0 mg mL^{-1}, in 25 mM histidine buffer, pH 6.4. The presence of traces of residual copper species from the final CuAAC step of the synthesis of the glycopolymers was subsequently quantified by ICP for mannose **(16a)** and trehalose **(16f)** star polymers. The data showed a low copper concentration in the low micromolar range (0.60 and 1.8 µM of Cu, respectively, in the 100:1 samples tested), which is significantly lower that the concentration of free Cu which was shown to affect antibody stability in solution.[9]

References.

1. V. Ladmiral, G. Mantovani, G. J. Clarkson, S. Cauet, J. L. Irwin and D. M. Haddleton, *Journal of the American Chemical Society*, 2006, **128**, 4823-4830.
2. B. Davis, R. Lloyd and J. B. Jones, *J. Org. Chem*, 1998, **63**, 9614--9615.
3. A. Chernyak, G. Sharma, L. Kononov, P. Krishna, A. Levinsky, N. Kochetkov and A. Rao, *Carbohydrate research*, 1993, **223**, 303--309.
4. R. Roy and J. M. Kim, *Tetrahedron*, 2003, **59**, 3881--3893.

5. B. Mukhopadhyay, K. P. R. Kartha, D. A. Russell and R. A. Field, *The Journal of Organic Chemistry*, 2004, **69**, 7758-7760.
6. B. a. K. K. P. R. a. R. D. A. a. F. R. A. Mukhopadhyay, *The Journal of Organic Chemistry*, 2004, **69**, 7758--7760.
7. K. Ishihara, H. Kurihara and H. Yamamoto, *J. Org. Chem.* , 1993, **58**, 3791-3793

8. H. Gao and K. Matyjaszewski, *Macromolecules*, 2006, **39**, 4960–4965.
9. M. K. Joubert, Q. Luo, Y. Nashed-Samuel, J. Wypych and L. O. Narhi, *Journal of Biological Chemistry*, 2011, **286**, 25118-25133.