Supplementary Material (ESI) for Chemical Communications

Supporting Information

Glycopolymers via catalytic chain transfer polymerisation (CCTP), Huisgens cycloaddition and thiol-ene double click reactions

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1. Experimental

1.1 Materials

Copper(I) bromide (Aldrich, 98%) was purified according to the method of Keller and Wycoff.[1] α-Azido-D-mannose and β-azido-D-galactose were prepared as previously described[2] starting from peracetylated sugars prepared by slight modification of a known general protocol.[3] Trimethylsilyl-protected propargyl methacrylate was synthesised as previously described[4] starting from 3-(trimethylsilyl) propargyl alcohol (Alfa Aesar, 98%). Dimethylphenylphosphine (DMPP, Aldrich, 97%) was stored under nitrogen. Amberlite IR-120 (PLUS) ion-exchange resin was washed several times with methanol. All other reagents and solvents were obtained at the highest purity available from Sigma-Aldrich Chemical Company and used without further purification unless stated.

CAUTION! Although we have never experienced any adverse event when working with these products, organic azides are potentially explosive compounds which should be handled with utmost care!

1.2 Characterisation

All reactions were carried out using standard Schlenk techniques under an inert atmosphere of oxygen-free nitrogen, unless otherwise stated. NMR spectra were obtained on a Bruker DPX-400 and Bruker AV-400 spectrometer. All chemical shifts are reported in ppm (d) relative to tetramethylsilane, referenced to the chemical shifts of residual solvent resonances (^{1}H and ^{13}C). The following abbreviations were used to explain the multiplicities: d=doublet, m=multiplet, t=triplet. The degree of polymerisation of the oligomers $DP_{(NMR)}$ were calculated by comparing the integrals of the vinyl bond chain-end signals and appropriate peaks related to the polymer backbone. Molar mass distributions of oligomers **1a** and **1b** were measured using Size Exclusion Chromatography (SEC), on a system equipped with two PL gel 5 μm mixed D columns (300 x 7.5 mm) and one PL gel 5 μm guard column (50 x 7.5 mm; Polymer Laboratories, suitable for molecular weights between 200 and 400000 g/mol) with a differential refractive index detector calibrated with linear poly(methyl methacrylate) standards (200 – 3 x 10^5 g mol^{-1}). The system was eluted at 1.0 ml min^{-1} with a solution of chloroform/triethylamine 95:5 v/v. Analyte samples contained (0.5% volume) toluene as the flow rate marker.

Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell.

Mass spectra were recorded using a Micromass Autospec apparatus.

MALDI-TOF analysis was performed with a Bruker Ultraflex III equipped with a neodymium-doped yttrium aluminium garnet laser (Nd:YAG). The matrix solution was prepared by dissolving 0.1 g of 2,4-dihydroxybenzoic acid in a 10 mL mixture of equal volumes of ethanol and water. Trifluoroacetic acid was added was added in 0.1 % overall concentration and the polymer dissolved to a concentration of 1 mg cm^{-3}. The matrix solution (0.5µl) was applied to the stainless steel side and the solvent allowed to evaporate. The same volume of polymer sample solution was applied on top of the dried matrix, and the overall mixture allowed to dry. The sample was irradiated with 500 pulsed laser shots at a 37% laser power. Calibration was performed with various linear poly(ethylene glycol) standards.

RCA I recognition experiments were performed using an HPLC system equipped with a Shodex AFpak ARC-894 column, a Dionex P680 HPLC pump and a Gilson UV–Vis-55 detector. Turbidimetry was performed as described by Kiessling and coworkers[5] using a Varian Cary 50 Bio UV–Vis spectrometer, using 2 mL volume polycarbonate cuvettes (1 cm pathlength). Absorbance data was recorded at 420 nm for 10 min at 0.125 s. The steepest part of the initial aggregation was fit to determine the rate of aggregation.

1.3 Synthetic procedures

1.3.1 Azidation of cellobiose octaacetate: β-azido-D-cellobiose heptaacetate.

Trimethylsilyl azide (5.8 ml, 44 mmol) was added under nitrogen to a solution of D-cellobiose octaacetate (15 g, 22 mmol) in anhydrous CH_2Cl_2 (200 mL), followed by tin(IV) chloride (1.6 mL, 13 mmol). The mixture was stirred at ambient temperature for 12 h, then the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography (CC, SiO_2, ethyl acetate/petroleum ether 1:1

vol/vol). The relevant fractions were collected, combined and concentrated to dryness under reduced pressure. Obtained 13.8 g (94%) of β-azido-D-cellobiose heptaacetate as a white solid. ^{1}H NMR (400.03 MHz, DMSO-d_6, 298 K) δ = 1.91 (s, 3H, CH_3); 1.96 (6H, 2xCH_3); 1.98 (s, 3H, CH_3); 2.00 (s, 3H, CH_3); 2.02 (s, 3H, CH_3); 2.09 (s, 3H, CH_3); 3.79-3.88 (m, 1H); 3.90-4.10 (m, 4H); 4.20-4.28 (m, 1H); 4.33-4.40 (m, 1H); 4.61-4.68 (m, 1H); 4.69-4.75 (m, 1H); 4.79-4.85 (m, 1H); 4.85-4.92 (m, 1H); 5.02-5.07 (m, 1H); 5.17-5.30 (m, 2H). ^{13}C{^{1}H} NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 20.06 (1H, CH_3); 20.07 (1H, CH_3); 20.15 (1H, CH_3); 20.22 (1H, CH_3); 20.25 (1H, CH_3); 20.33 (1H, CH_3); 20.52 (1H, CH_3); 61,38 (1H, CH_2); 62.00 (1H, CH_2); 67,58 (1H, CH); 70.34 (1H, CH); 70.40 (1H, CH); 71.08 (1H, CH); 71.61 (1H, CH); 72.10 (1H, CH); 73.81 (1H, CH); 75.94 (1H, CH); 85.97 (1H, CHN_3); 99.45 (1H, CH); 168.94 (1C, CH_2OAc); 169.12 (1C, CH_2OAc); 169.15 (1C, CH_2OAc); 169.31 (1C, CH_2OAc); 169.52 (1C, CH_2OAc); 169.95 (1C, CH_2OAc); 170.20 (1C, CH_2OAc). FTIR (neat): $\tilde{\nu}$ = 2119, 1734, 1427, 1365, 1213, 1165, 1032, 950, 898 cm^{-1}.

1.3.2. Deprotection of cellobiose heptaacetate azide: β-azido-D-cellobiose.

Sodium methoxide (25% w/w solution in methanol, 24.2 mL, 106 mmol) was added to a dispersion of β-azido-D-cellobiose heptaacetate (10.0 g, 15.1 mmol) in methanol (550 mL). The mixture was stirred at ambient temperature for 3h. The mixture turned into a clear solution. Amberlite IR-120 (PLUS) ion-exchange resin was added and the reaction mixture was stirred for further 30 min. The resin was then removed by filteration and the resulting solution concentrated under reduced pressure. The crude product was purified by flash chromatography (CC, SiO_2, methanol/CH_2Cl_2 1:3, vol/vol). The relevant fractions were collected, combined and concentrated to dryness under reduced pressure. Obtained 3.02 g (54%, not optimized) of β-azido-D-cellobiose

as a white solid. ^{1}H NMR (400.03 MHz, DMSO-d_6, 298 K) δ = 2.95-3.01 (m, 1H); 3.01-3.09 (m, 2H); 3.11-3.22 (m, 2H); 3.30-3.45 (m, 4H); 3.58-3.66 (m, 1H); 3.66-3.77 (m, 2H); 4.25 (d, *J*=8.2Hz, 1H, CH); 4,56 (d, *J*=9.1Hz, 1H, CH). ^{13}C{^{1}H} NMR (100.59 MHz, DMSO, 298 K) δ = 59.93 (1H, CH_2); 60.97 (1H, CH_2); 69.97 (1H, CH); 73.04 (1H, CH); 73.23 (1H, CH); 74.79 (1H, CH); 76.40 (1H, CH); 76.74 (1H, CH); 76.96 (1H, CH); 79.54 (1H, CH); 89.65 (1H, CHN_3); 103.01 (1H, CH).

1.3.2 CCTP of TMS-protected propargyl methacrylate

A solution of TMS-protected propargyl methacrylate (2.00 g, 10.2 mmol), AIBN (10.0 mg 0.5 wt%) and mesitylene (100 μL, internal standard) in toluene (1.6 mL) was deoxygenated by 4 freeze-pump-thaw cycles. A deoxygenated solution of CoBF in toluene (392 μL, 1.30 mM) was subsequently added under nitrogen. The reaction was allowed to proceed at 60 °C for 19 h. After completion of the reaction, the crude polymerisation mixture was passed through a column of basic alumina to remove CoBF. The oligomer was isolated by precipitation into cold petroleum ether.

1.3.3 Deprotection of TMS-protected oligomer

Trimethylsilyl-protected oligomer (2.00 g, 10.2 mmol of trimethylsilyl groups) and acetic acid (875 μL, 1.5 equiv. mol/mol with respect to the alkyne-trimethylsilyl groups) were dissolved in THF (10 mL). Nitrogen was bubbled (ca. 10 min) and the solution was cooled to -20 °C. A 1.0 M solution of TBAF in THF (15.3 mL, 1.5 equiv. mol/mol with respect to the alkyne-trimethylsilyl groups) was added slowly via syringe. The resulting mixture was stirred at -20 °C for 30 min and then at ambient temperature overnight. The reaction solution was passed through a short silica pad in order to remove the excess of TBAF and the pad was subsequently washed with

additional THF. The resulting solution was then concentrated under reduced pressure and the **1** was recovered by precipitation in petroleum ether.

$DP_{(NMR)} = 23$, $M_{n(SEC)} = 2200$ g mol^{-1}, $M_w/M_n = 1.5$

1.3.4 Thiol-conjugation to propargyl methacrylate oligomer, general procedure

Propargyl methacrylate oligomer **1** (140 mg, 50 μmol of vinyl end groups) were dissolved into *d*-acetone (1.00 mL) in an NMR tube. Benzyl mercaptan was added (9.3 mg, 75 μmol). Finally, DMPP was added (6.9 mg, 50 μmol). The reaction was carried out overnight. The final benzyl-functionalised product **2** was precipitated from petroleum ether several times and dried under vacuum.

1.3.5 Reaction of end group functionalized poly(propargyl methacrylate) with sugar azide, general procedure

A solution of mannose azide, **5,** (58 mg, 0.28 mmol), benzyl-functionalised poly(propargyl methacrylate), **2**, (29 mg, 0.23 mmol of alkyne groups), bipyridine (7.0 mg, 47 μmol), triethylamine (5.0 mg, 47 μmol) and mesitylene (8.0 mg, internal standard) in DMSO (6.0 mL) was deoxygenated by three freeze-pump-thaw cycles. This solution was then transferred via cannula under nitrogen into a Schlenk tube, previously evacuated and filled with nitrogen, containing CuBr (3.0 mg, 23 μmol). The resulting solution was stirred overnight at 60 °C. When the reaction was completed, the reaction mixture was diluted with water and then dialysed against water for several days after which the product (**8**) could be recovered by freeze-drying.

2. Thio-Michael addition

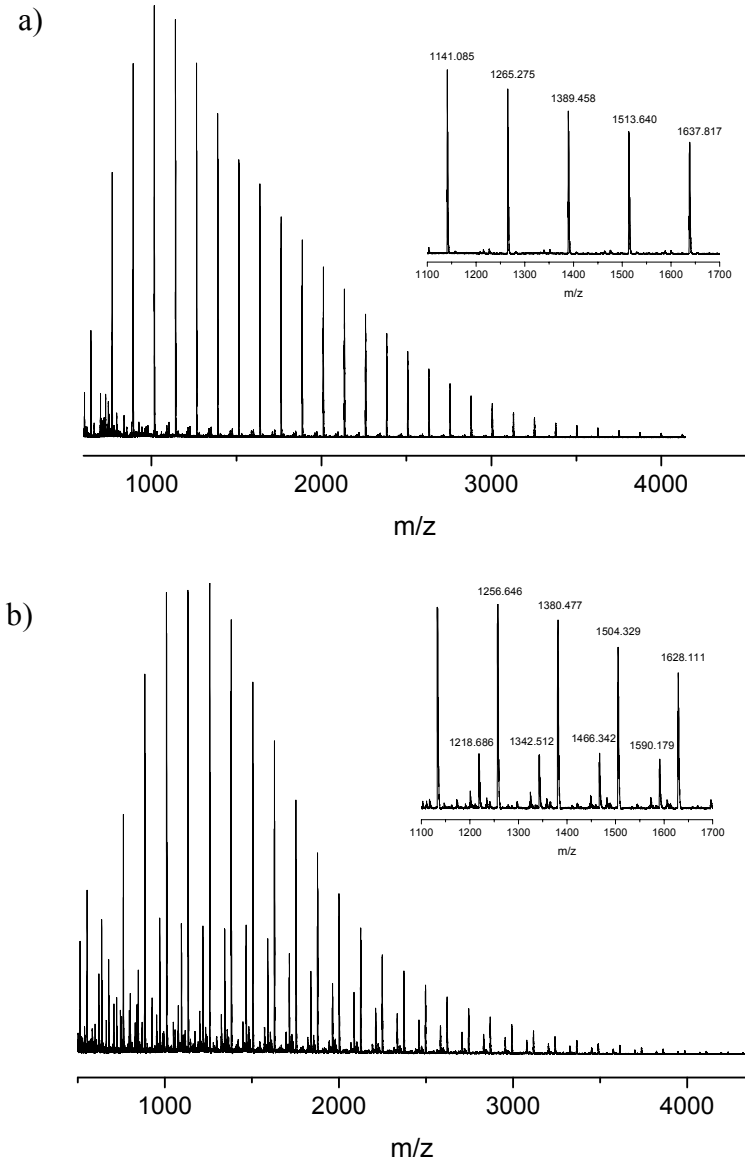

Fig. S1 MALDI-TOF traces for a) propargyl methacrylate oligomer **1**, and b) product after reaction with mercaptoethanol. The main distribution corresponds to the DMPP-conjugated oligomer, and the smaller distribution corresponds to the desired hydroxyethyl-functionalised product (**3**). The insets show an expansion of the same traces.

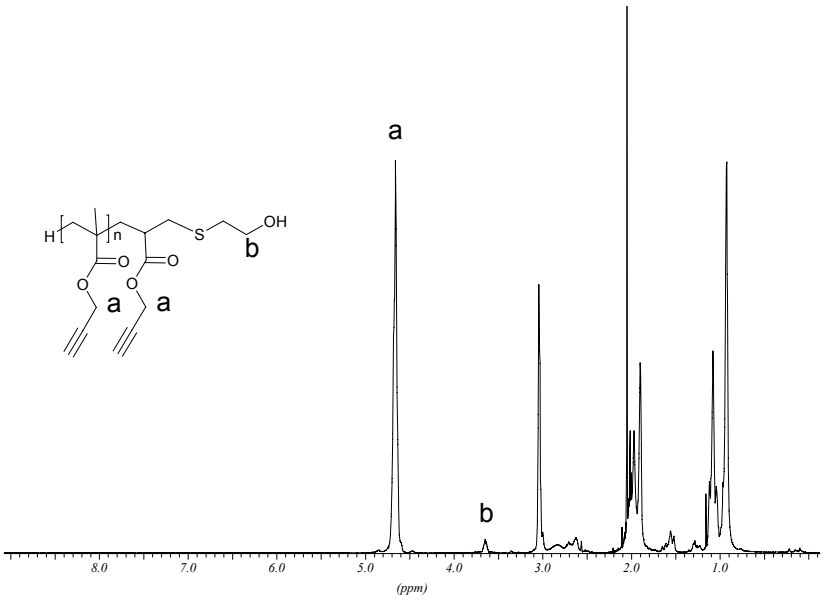

Fig. S2 ^{1}H NMR spectrum of hydroxyethyl-functionalised poly(propargyl methacrylate) **3.**

3. CuAAC reactions

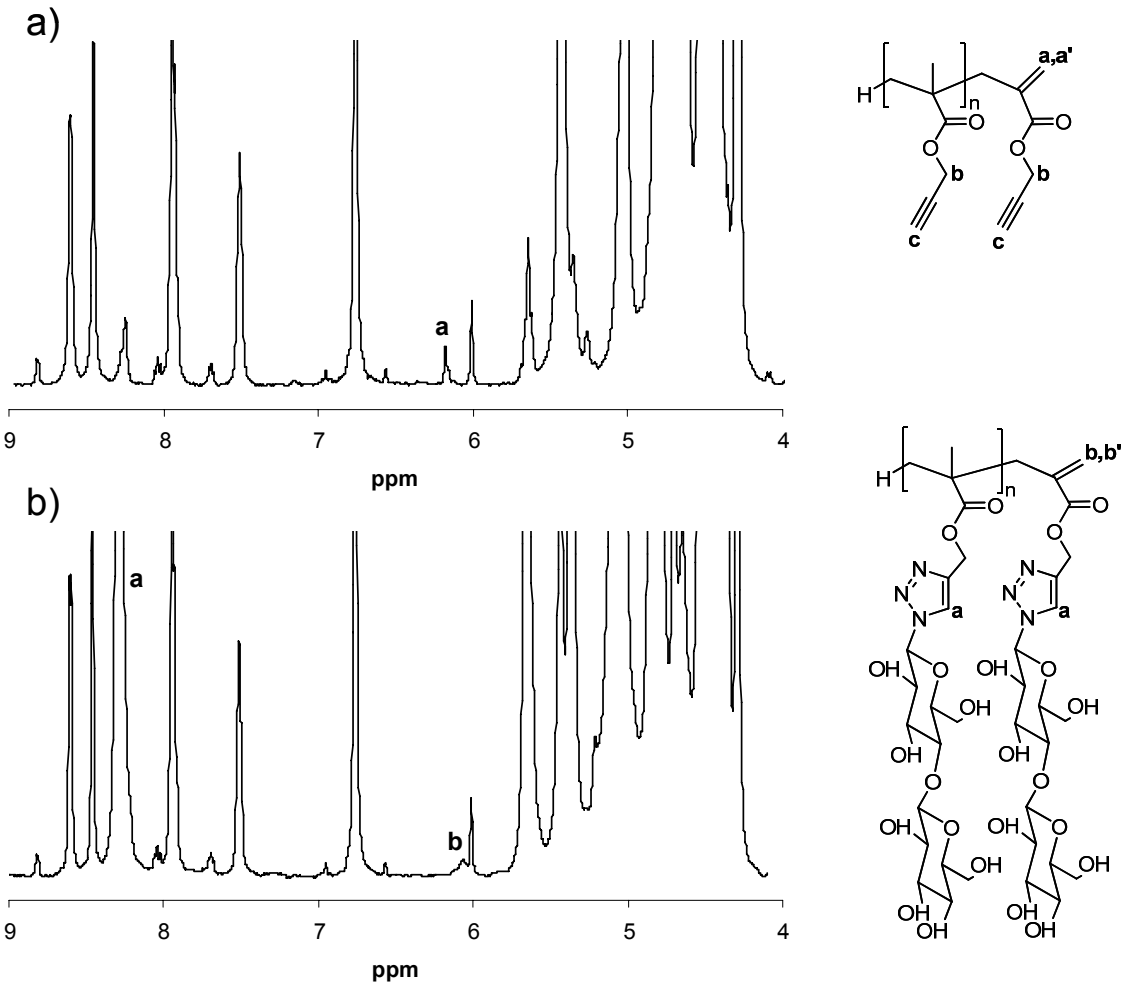

Fig. S3 The vinyl end group is retained in CuAAC. ^{1}H NMR spectra of the reaction mixtures for CuAAC side-chain functionalization a) before reaction b) after reaction. Poly(propargyl methacrylate) was reacted with cellobiose azide. The vinyl end group proton in poly(propargyl methacrylate) at 6.18 ppm shifts to 6.06 ppm as reaction proceeds.

4. Lectin recognition studies

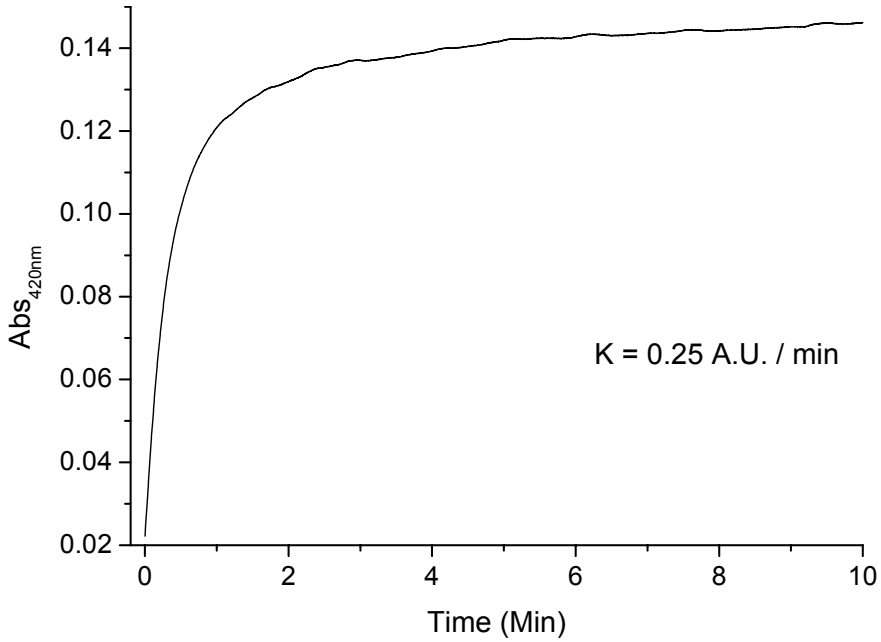

Fig. S4 Turbidimetry results for glycopolymer **8** in the presence of mannose-selective lectin Con A. The curve shown is an average of three separate runs. The aggregation rate constant K is calculated from the initial clustering rate (error +/- 5%).

5. References

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