Supporting Information for:

Glycopolymers with Secondary Binding Motifs Mimic Glycan Branching and Display Bacterial Lectin Selectivity in Addition to Affinity.

Mathew W. Jones, Lucienne Otten, Sarah-Jane Richards, Richard Lowery, Daniel J. Phillips, David M. Haddleton and Matthew I. Gibson*

Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

*Corresponding author

Phone: +44 247 652 4803

Fax: +44 247 652 4112

Email: m.i.gibson@warwick.ac.uk

Materials

Dichloromethane, diethyl ether, tetrahydrofuran, dichloromethane, triethylamine and dimethyl sulfoxide were all purchased from Fisher Scientific at laboratory reagent grade. Ethyl 2-bromoisobutyrate (99.0 %), glycidyl methacrylate (≥97.0 %), copper (I) bromide (98.0 %), anisole (≥99.0 %), benzoyl chloride (99.0 %), 1-naphthoyl chloride (97.0%), 2,4,6-trichlorobenzoyl chloride (97.0 %), decanoyl chloride (98.0 %), 4-chlorobenzoyl chloride (99.0 %), 4-bromobenzoyl chloride (98.0 %), 4-fluorobenzoyl chloride (98.0 %), α-bromoisobutyryl bromide (98.0 %), acetyl chloride (98.0 %), dichloroacetyl chloride (98.0 %), and 2,2'-bipyridyl (≥99.0 %) were all purchased from Sigma-Aldrich. *N*-ethyl-2-pyridylmethanimine[1] and β-D-1-propargyl galactose[2] were synthesised using previously reported methods. Fluorescein isothiocyanate (FITC) labelled peanut agglutinin (PNA) from *Arachis hypogaea* was purchased from Vector Laboratories. Galactocerebrosides (GCS), FITC labelled cholera toxin subunit B (CTxB), calcium chloride ($CaCl_2$), Sodium chloride (NaCl), manganese (II) chloride, HEPES powder, and Tris buffer were all purchased from Sigma-Aldrich, UK. 96-well high binding microtitre plates were purchased from Greiner Bio-one. Tris buffer containing 0.1 mmol $CaCl_2$ and 0.5 mmol NaCl (pH 8, TBS) was prepared in 250 mL of milliQ water (with a resistance >19 mOhms), and 10 mmol HEPES buffer containing 0.15M NaCl, 0.1mM $CaCl_2$ and 0.01mM Mn^{2+} (pH 7.5, HEPES) was prepared in 250 mL of milliQ water (with a resistance >19 mOhms).

Physical and analytical methods

NMR spectra were recorded on Bruker DPX-300 and DPX-400 spectrometers for ^{1}H NMR (400 MHz) and ^{13}C NMR (125 MHz). Chemical shifts are reported in ppm relative to the deuterated solvent resonances and spectra analysed with WIN-NMR software. GPC (DMF) was performed on a Varian 390-LC MDS system equipped with a PL-AS RT/MT

autosampler, a PL-gel 3 μm (50 × 7.5 mm) guard column, two PL-gel 5 μm (300 × 7.5 mm) mixed-D columns equipped with a differential refractive index, using DMF (with 1 $mg.mL^{-1}$ LiBr) as the eluent with a flow rate of 1.0 $mL.min^{-1}$ at 50 °C. Narrow molecular weight PMMA standards (200 - 1.0 × 10^6 $g.mol^{-1}$) were used for calibration using a second order polynomial fit. SEC (aqueous) was performed on a Varian 390-LC MDS system equipped with a PL-AS RT/MT autosampler, a PL-aquagel-OH 8 μm (50 × 7.5 mm) guard column, a PLaquagel-OH column set consisting of two 8 μm (300 × 7.5 mm) columns equipped with a differential refractive index detector using phosphate buffer (pH 8.2) as the eluent at a flow rate of 1.0 $mL.min^{-1}$. Narrow molecular weight PEO standards (100 - 1.26 × 10^6 $g.mol^{-1}$) were used for calibration using a second order polynomial fit. 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. Partition coeffecients (LogP) were estimated using ACD labs software.

Visualisation of protein structures

Image of one subunit of 2CHB (Merritt, E.A., Sarfaty, S., Jobling, M.G., Chang, T., Holmes, R.K., Hirst, T.R., Hol, W.G. (1997) Structural studies of receptor binding by cholera toxin mutants *Protein Science* **6**: 1516-1528) created with CCP4mg (McNicholas, S., Potterton, E., Wilson, K.S., Noble, M.E.M (2011) Presenting your structures: the CCP4mg molecular graphics software *Acta Cryst.* **67**: 386-394).

Fluorescence-linked sorbent assay for inhibitory activity

Peanut Agglutinin (PNA) 96-well microtitre plates were incubated for 16 h with 150 μL of 100 $\mu g.mL^{-1}$ Galactocerebroside (GCS) (in 95% ethanol, 5% water and heated to 45 °C). Unbound GCS was removed by washing extensively with water. Polymer solutions were made up as serial dilutions (up to 10 dilutions per sample from 0.1 $mg.mL^{-1}$ in water from a

stock solution of 1 $mg.mL^{-1}$ in DMSO). 10 μL of 100 $\mu g.mL^{-1}$ PNA-FITC in 10 mM HEPES with 0.15M NaCl, 0.1mM $CaCl_2$ and 0.01mM Mn^{2+}(pH 7.5) was added to 90 μL of polymer solution to a final concentration of 11 $\mu g.mL^{-1}$. 100 μL of the PNA/polymer solutions were then added to GCS coated wells and incubated at 37 °C for 30 mins. After this the wells were extensively washed with water and fluorescence was measured at excitation/emission wavelengths of 485/528 nm. All experiments were carried out in triplicate. Percentage inhibition was compared to relative to controls of pure PNA-FITC (with no polymer).

Glycan Notation and Microarray Analysis

Table S1. A table of the symbols representing sugar stereochemistries. All symbols as described by the consortium for functional glycomics.

Sugars	Hexoses	N-acetylhexosamines	Hexosamines
Galactose Stereochemistry			
Glucose Stereochemistry			
Mannose Stereochemistry			
Fucose Stereochemistry		N/A	N/A
Xylose Stereochemistry		N/A	N/A

Acidic sugars	Symbol
NeuAc	
NeuGc	
KDN	
GlcA	
IdoA	
GalA	
ManA	

To investigate the relative importance of glycan branching and secondary binding site interactions, microarray data for two galactose-binding proteins was extracted from the Consortium for Functional Glycomics database. Figure S1 shows the affinity of 5 sequentially modified carbohydrates to both peanut agglutinin (PNA) and cholera toxin (CTx). Upon changing from Gal (**1**) to GalNAc (**2**) there was no significant difference in lectin affinity for PNA or CTx. However, when Gal(β1-4)GalNAc (**3**) is used PNA's affinity is significantly increased compared to CTx, highlighting that connectivity and presentation of the sugars is crucial. Addition of a neuraminic acid branch (**4**) leads to a 200-fold increase in

CTx binding, but has little influence on PNA binding. This is despite the neuaminic acid (**5**) alone having negligible CTx affinity. The increased binding affinity of CTx to (**4**) is attributable to allosteric interactions of the neuraminic with a secondary binding pocket within CTx. While not intended as a complete bioinformatic study, this limited set of glycans demonstrate that controlled branching can improve specific and affinity of glycans by several orders of magnitude, which is more than observed by multivalency alone in many cases.

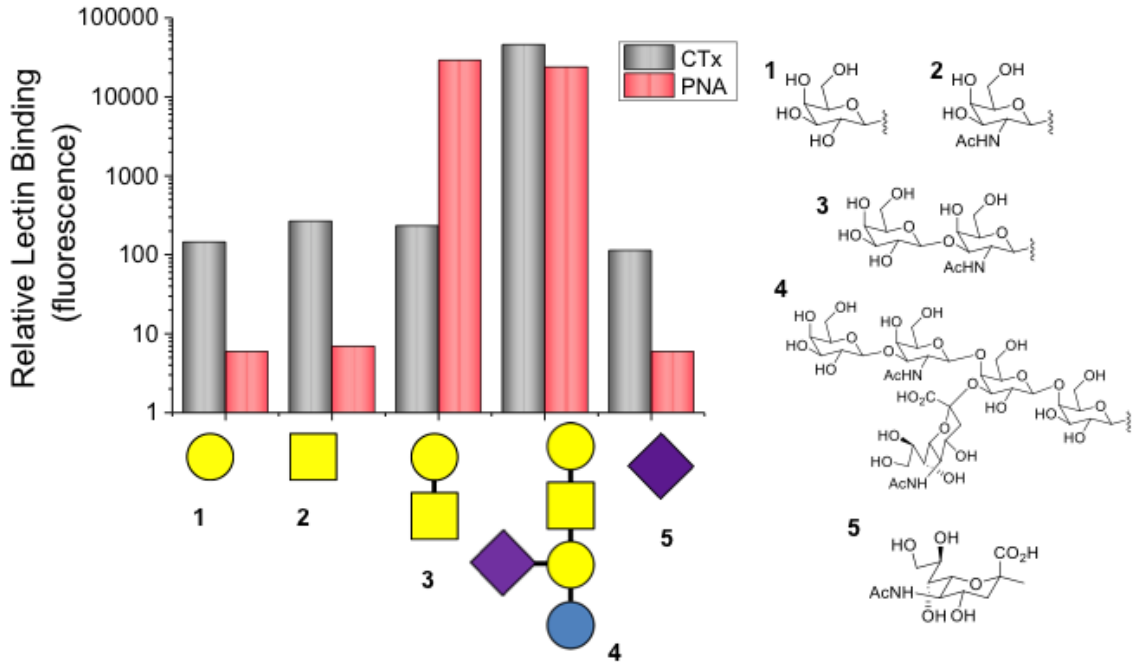
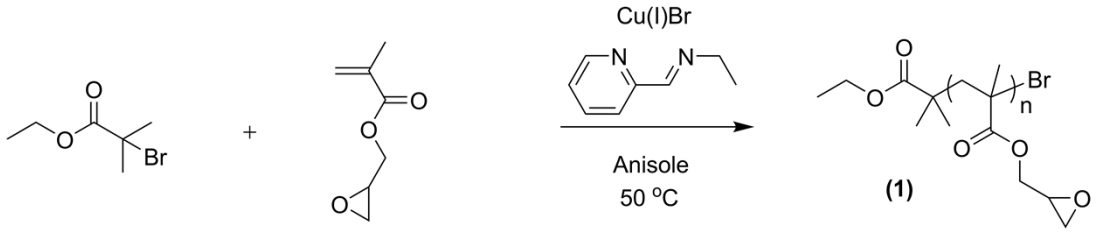


Figure S1. Glycan Microarray analysis of galactose-terminated glycans with PNA and CTx lectins taken from the CFG database (values were taken for the assay performed with 10 µg of each lectin).

Synthetic Procedures

Synthesis of poly(glycidyl methacrylate) (1)



To an oven dried Schlenk tube, ethyl 2-bromoisobutyrate (0.5489 g, 2.81 mmol), glycidyl methacrylate (40.00 g, 281 mmol), copper (I) bromide (0.4037 g, 2.81 mmol) and anisole (80 mL) were added. The tube was sealed and subjected to four freeze-pump-thaw cycles and left under a blanket of nitrogen. *N*-Ethyl-2-pyridylmethanimine (1.25 mL, 8.44 mmol) was added to the solution *via* a degassed syringe and the Schlenk tube immersed in an oil bath at 50 °C. Samples were taken hourly and analysed by ^{1}H NMR and GPC. After 6 hours, the reaction was quenched by immersing the flask in liquid nitrogen. The solution was then bubbled with air for 12 hours, passed through a short column of neutral alumina and the solvent removed under vacuum. The resulting crude mixture was redissolved in dichloromethane, precipitated three times into diethyl ether and dried under vacuum to yield the product as a white solid. Conversion (NMR): 84.2 %; M_n (theoretical): 12000 g.mol^{-1}; M_n (SEC): 22900 g.mol^{-1}; M_w/M_n (SEC): 1.20.

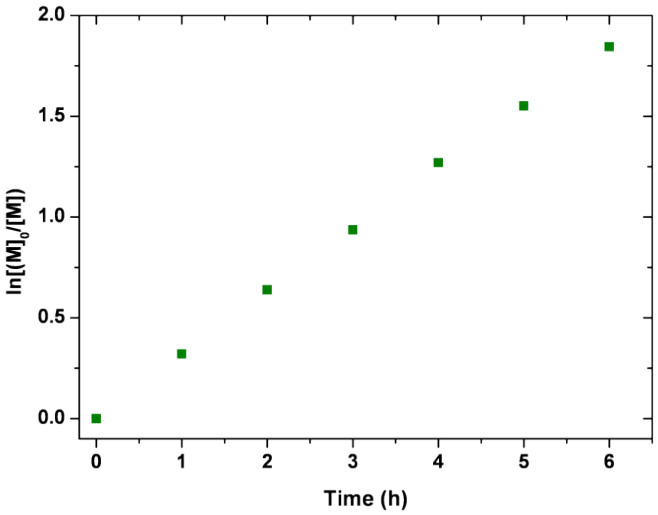


Figure S2. First order kinetic plot for the polymerisation of glycidyl methacrylate

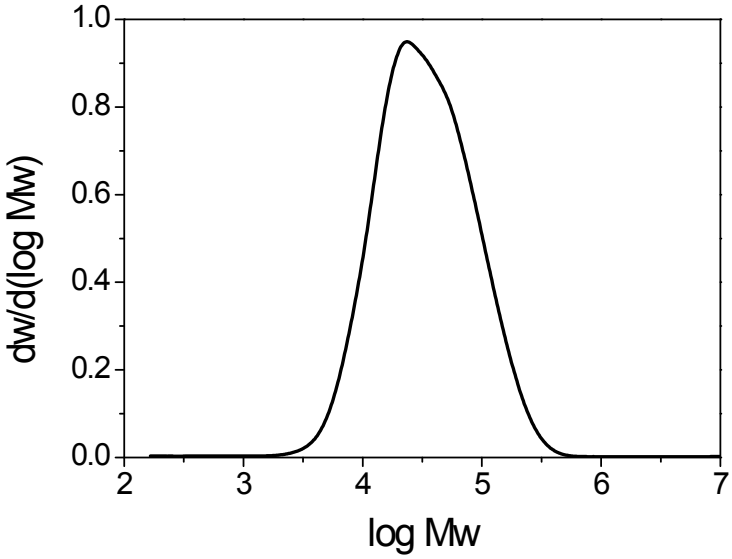
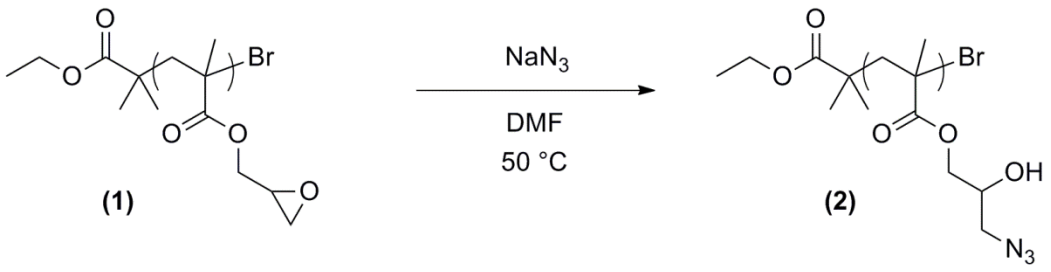


Figure S3. Molecular weight distribution curve of poly(glycidyl methacrylate) after 6 hour polymerisation time.

Synthesis of poly(2-hydroxy-3-azidopropyl methacrylate) (2)



Poly(glycidyl methacrylate) **(1)** (7.34 g, 0.051 mmol of polymer repeat unit) was dissolved in DMF (250 mL). Sodium azide (10.07 g, 0.155 mmol) and ammonium chloride (8.29 g, 0.155 mmol) were added to the solution and the mixture stirred for 24 hours. After this time, the mixture was poured into water and the solid collected by filtration. The polymer was washed several times with water and dried to leave an off-white solid. M_n (SEC) = 26000 g.mol^{-1}, M_w/M_n = 1.8.

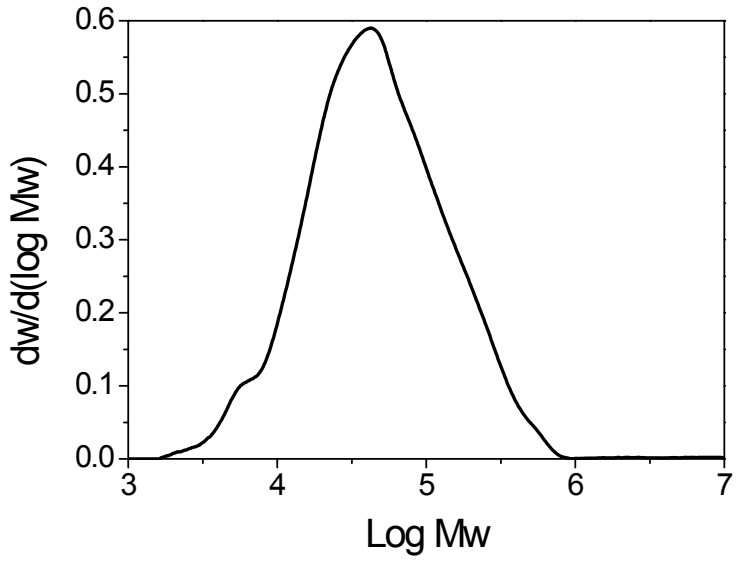


Figure S4. Molecular weight distribution curve of poly(2-hydroxy-3-azidopropyl methacrylate).

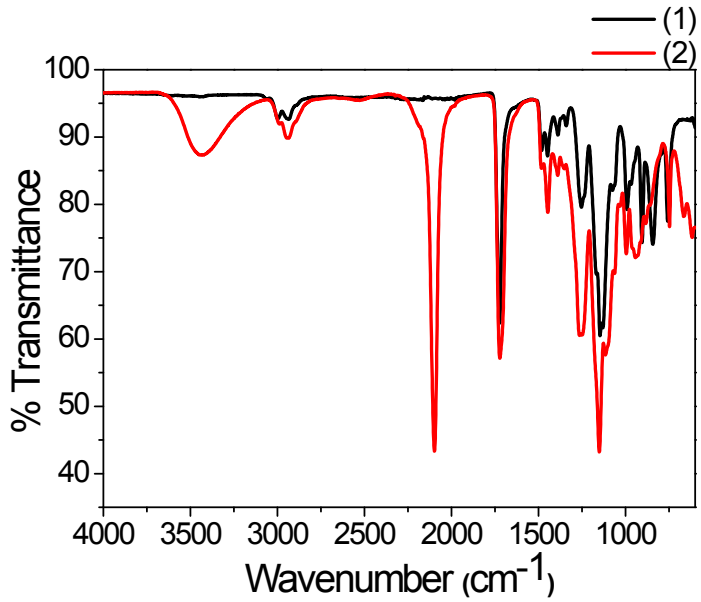
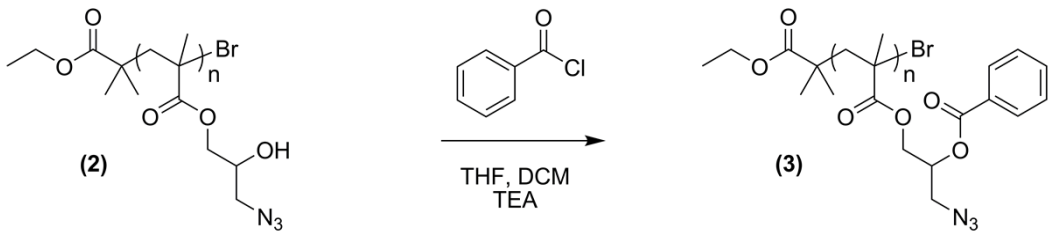


Figure S5. Infrared spectra of poly(glycidyl methacrylate), **(1)**, and poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**.

Modification of (2) with acid chlorides

Polymer **(2)** was modified with a series of commercially available acid chlorides and the reaction monitored by IR spectroscopy.

Modification of (2) with Benzoyl Chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3

eq. for each polymer repeat unit). Benzoyl chloride (0.46 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and benzoyl chloride (0.46 g, 3.24 mmol) were added to the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as an off-white powder.

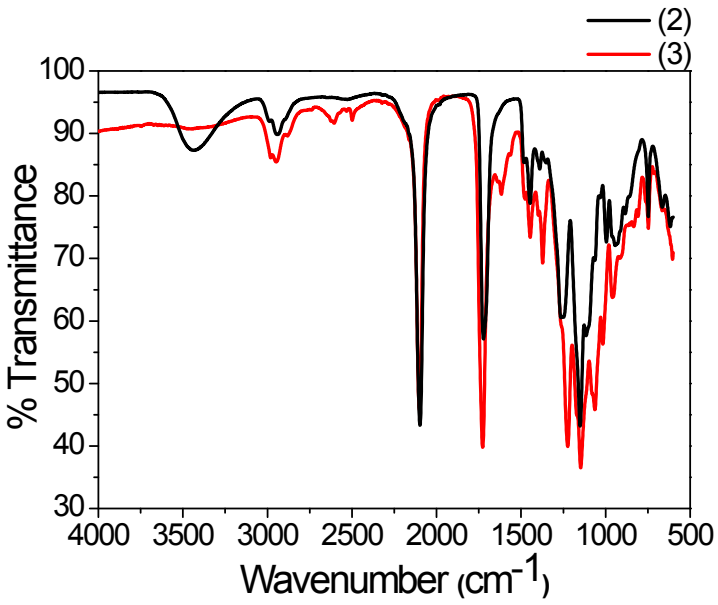
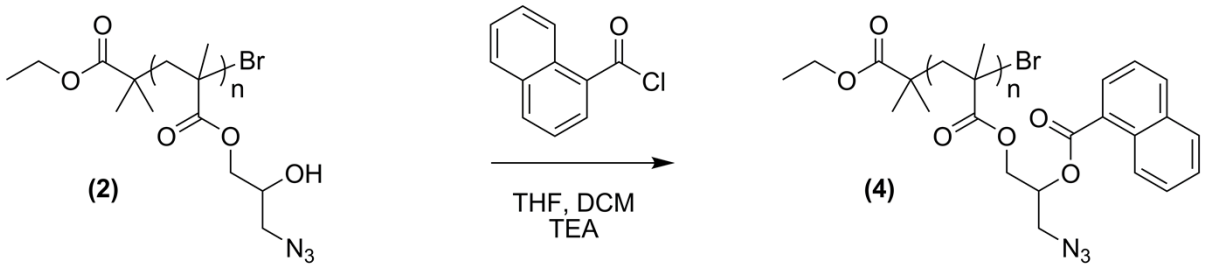


Figure S6. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with benzoyl chloride **(3)**.

Modification of (2) with 1-Naphthoyl Chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). 1-Naphthoyl chloride (0.62 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and 1-naphthoyl chloride (0.62 g, 3.24 mmol) were added to the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as a pale brown powder.

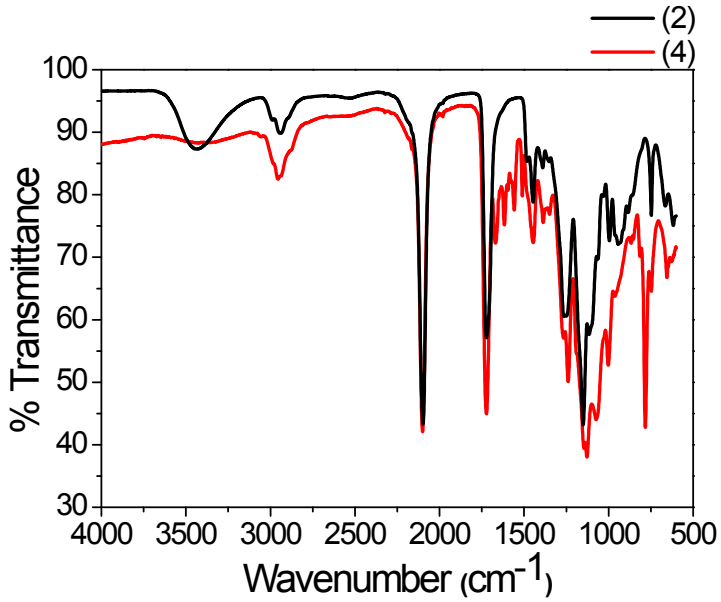
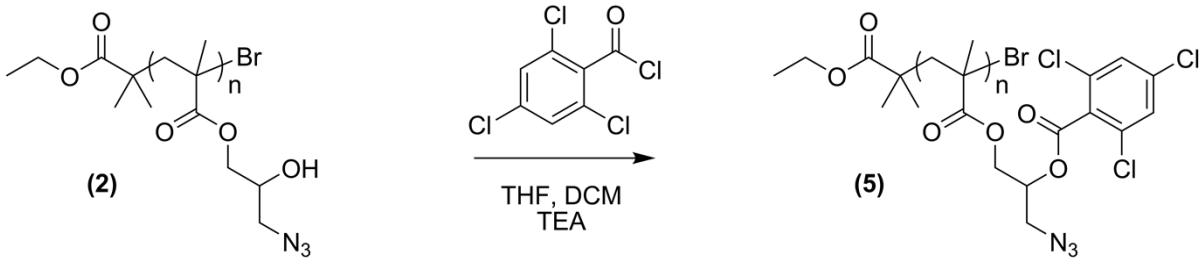


Figure S7. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with 1-naphthoyl chloride **(4)**.

Modification of (2) with 2,4,6-Trichlorobenzoyl Chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). 2,4,6-Trichlorobenzoyl chloride (0.79 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and 2,4,6-trichlorobenzoyl chloride (0.79 g, 3.24 mmol) were added to

the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as an off-white powder.

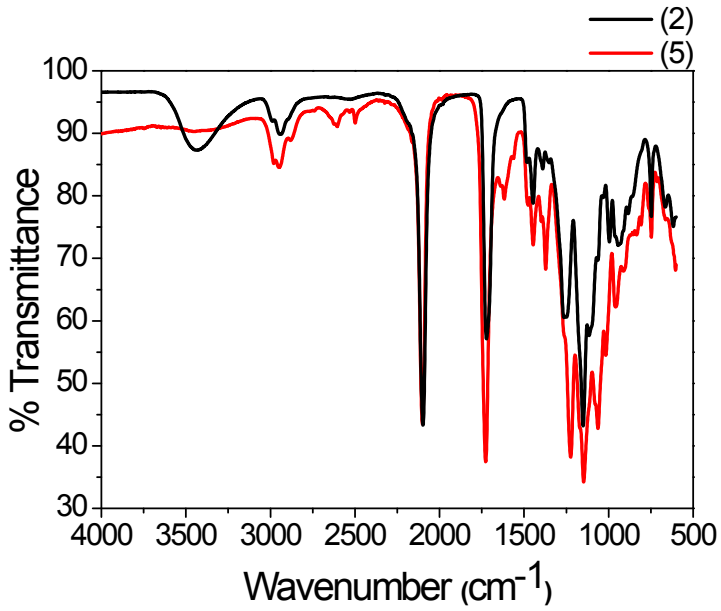
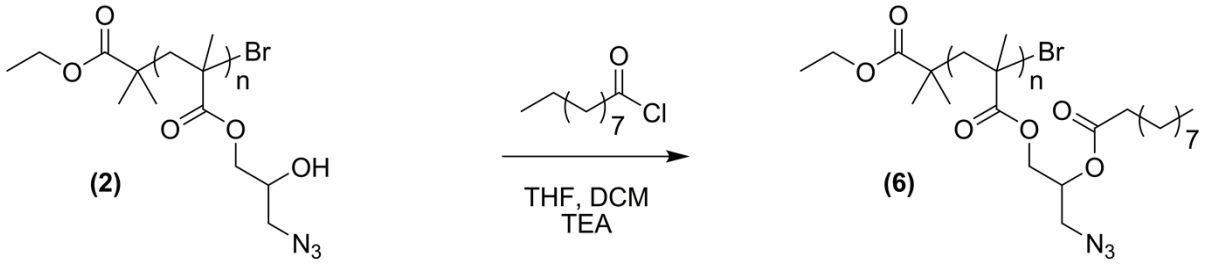


Figure S8. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with 2,4,6-trichlorobenzoyl chloride **(5)**.

Modification of (2) with Decanoyl chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). Decanoyl chloride (0.62 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and decanoyl chloride (0.62 g, 3.24 mmol) were added to the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as a white powder.

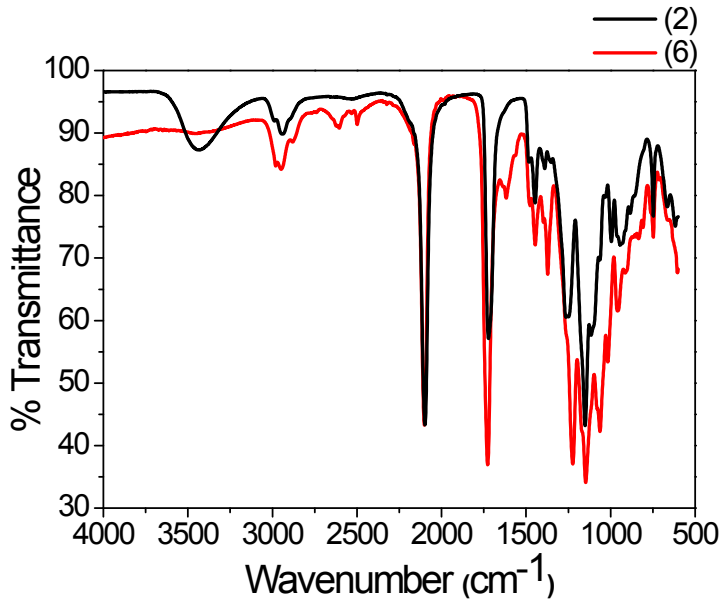
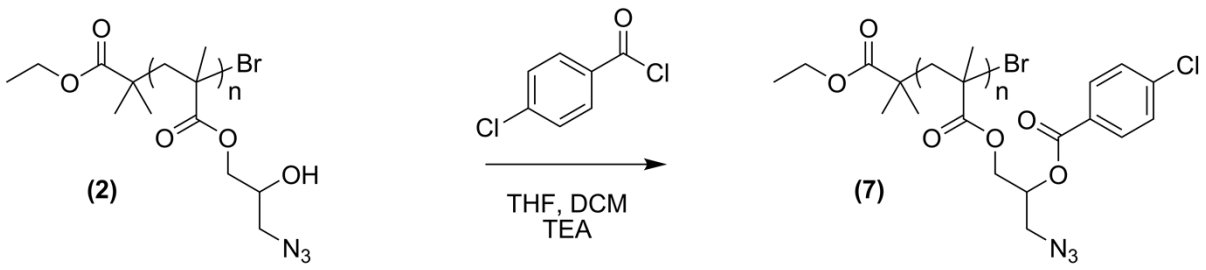


Figure S9. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with decanoyl chloride **(6)**.

Modification of (2) with 4-Chlorobenzoyl chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). 4-Chlorobenzoyl chloride (0.57 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and 4-chlorobenzoyl chloride (0.57 g, 3.24 mmol) were added to the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as an off-white powder.

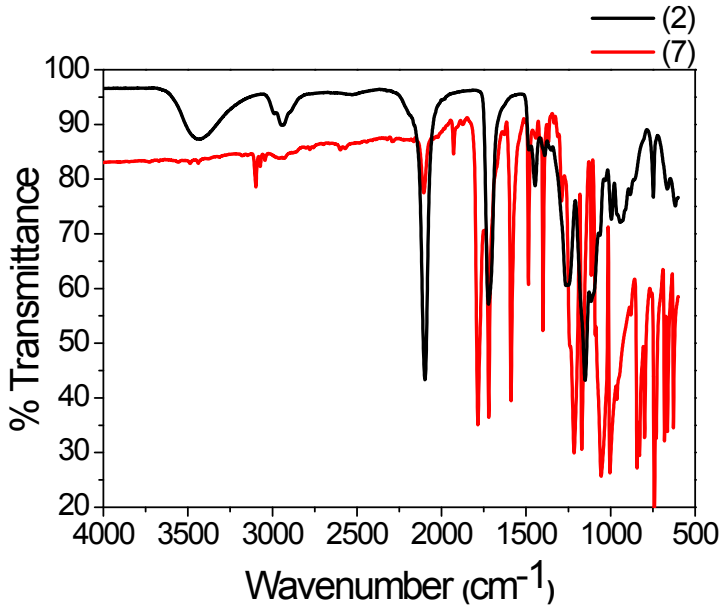
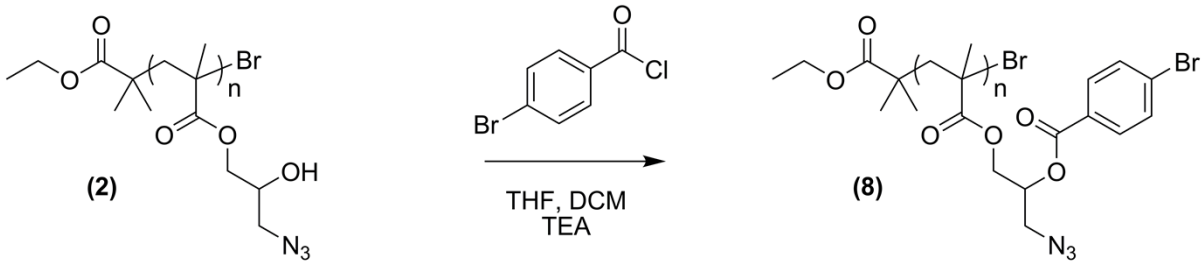


Figure S10. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with 4-chlorobenzoyl chloride **(7)**.

Modification of (2) with 4-Bromobenzoyl chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). 4-Bromobenzoyl chloride (0.71 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and 4-bromobenzoyl chloride (0.71 g, 3.24 mmol) were added to the solution and

allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as a white powder.

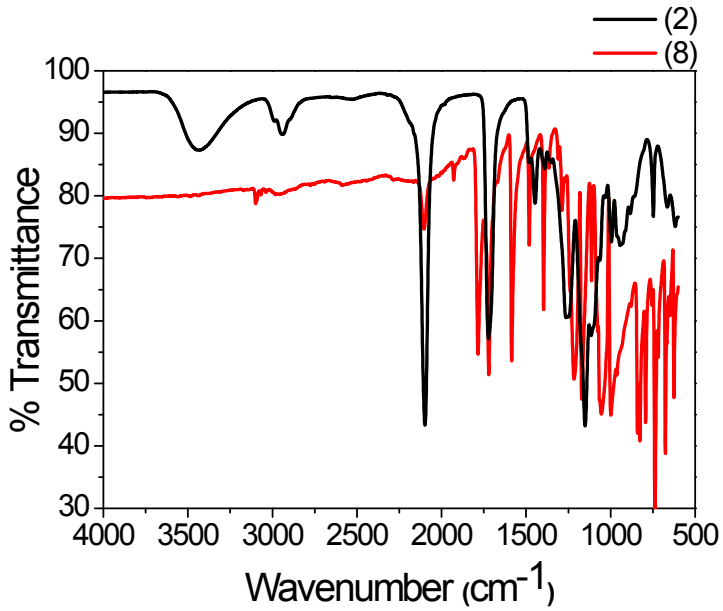
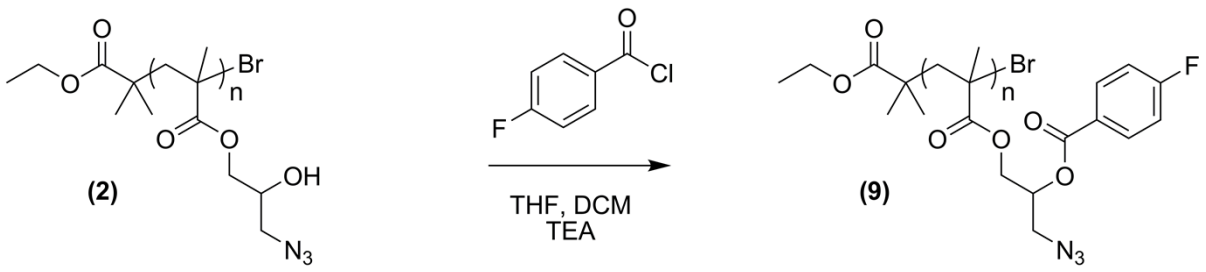


Figure S11. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with 4-bromobenzoyl chloride **(8)**.

Modification of (2) with 4-Fluorobenzoyl chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). 4-Fluorobenzoyl chloride (0.51 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and 4-fluorobenzoyl chloride (0.51 g, 3.24 mmol) were added to the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as an off-white powder.

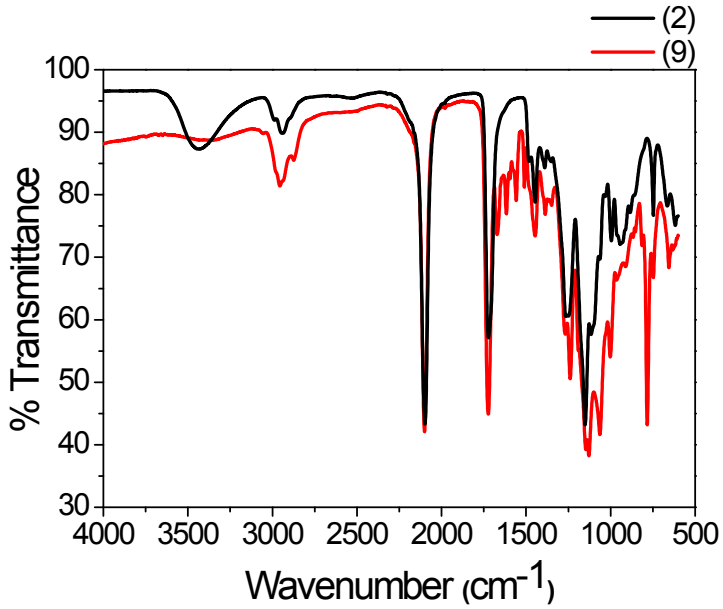
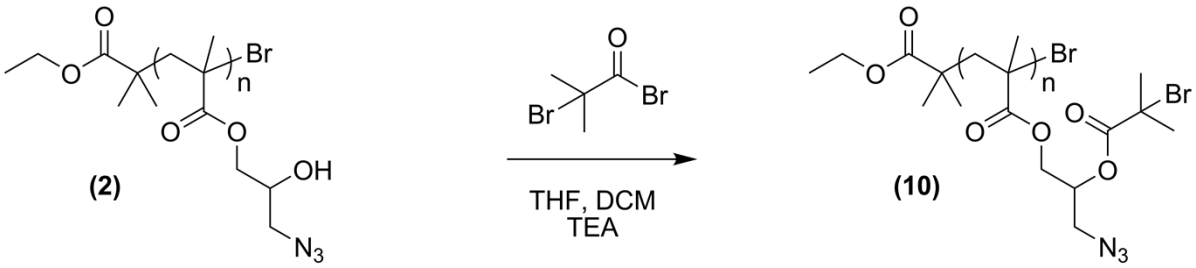


Figure S12. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with 4-fluorobenzoyl chloride **(9)**.

Modification of (2) with α-Bromoisobutyryl bromide



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). α-Bromoisobutyryl bromide (0.74 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24

mmol) and α-bromoisobutyryl bromide (0.74 g, 3.24 mmol) were added to the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as a pale brown powder.

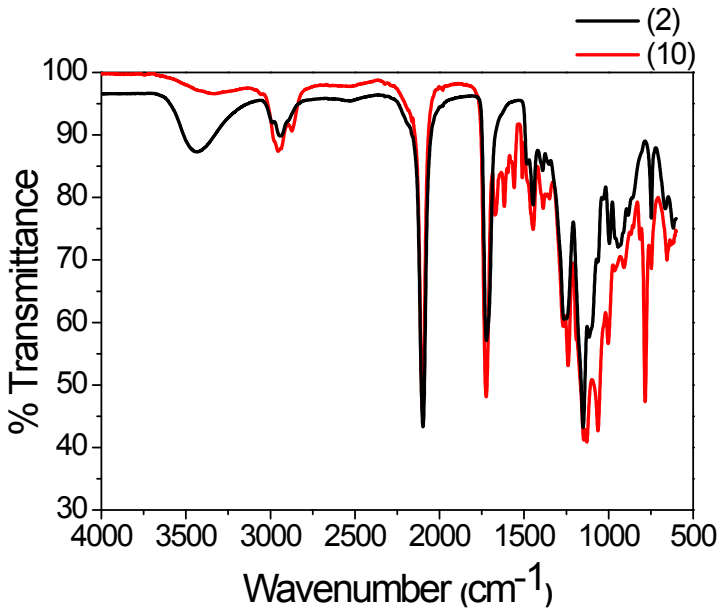
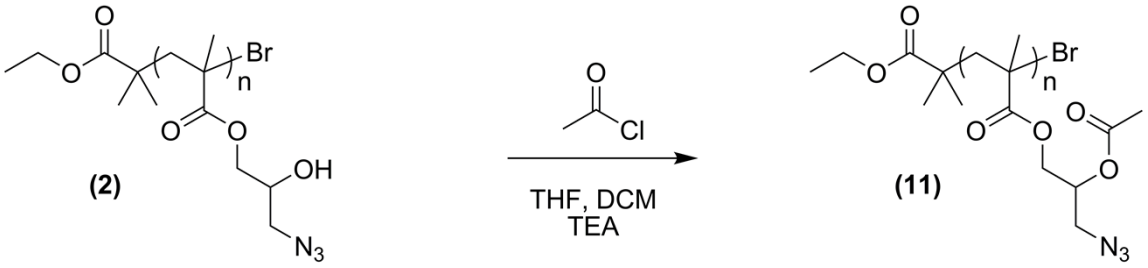


Figure S13. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with α-bromoisobutyryl bromide **(10)**.

Modification of (2) with Acetyl chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). Acetyl chloride (0.25 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and acetyl chloride (0.25 g, 3.24 mmol) were added to the solution and allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as an orange powder.

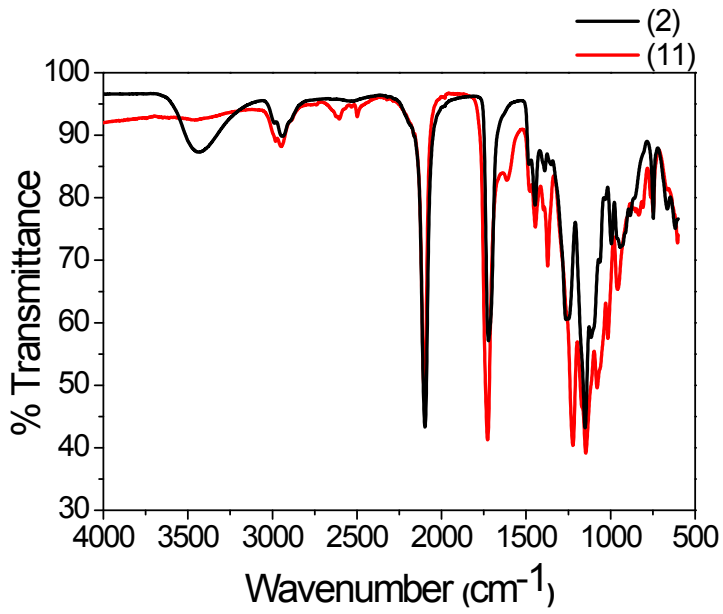
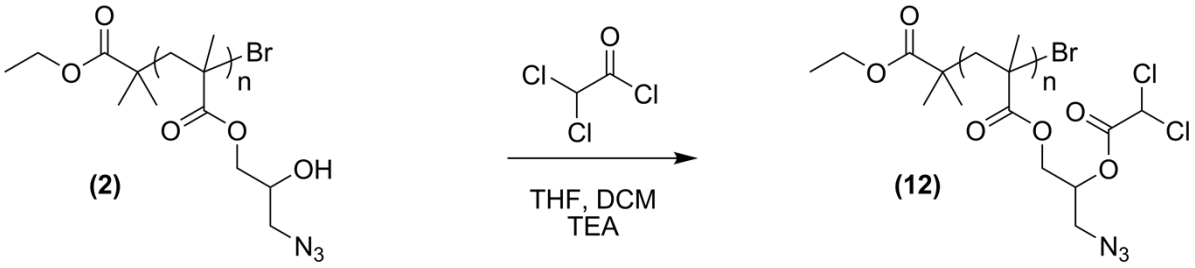


Figure S14. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with acetyl chloride **(11)**.

Modification of (2) with Dichloroacetyl chloride



Poly(2-hydroxy-3-azidopropyl methacrylate) (200 mg, 1.08 mmol of polymer repeat unit) was dissolved in anhydrous THF (50 mL), along with triethylamine (0.45 mL, 3.24 mmol – 3 eq. for each polymer repeat unit). Dichloroacetyl chloride (0.48 g, 3.24 mmol - 3 eq. for each polymer repeat unit) was dissolved in 50 mL of anhydrous DCM and added dropwise to the solution over a period of 30 minutes. Following complete addition, the solution was left to stir at room temperature for 24 hours. A further portion of triethylamine (0.45 mL, 3.24 mmol) and dichloroacetyl chloride (0.48 g, 3.24 mmol) were added to the solution and

allowed to stir for a further 24 hours. The solution was then diluted with 100 mL of DCM and quenched with 100 mL of water. The organic layer was washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed. The crude polymer solution was then redissolved in 50 mL of THF and twice precipitated into a 1:1 mixture of diethyl ether/petroleum ether. The solids were isolated by centrifugation and dried under vacuum to yield the product as a brown powder.

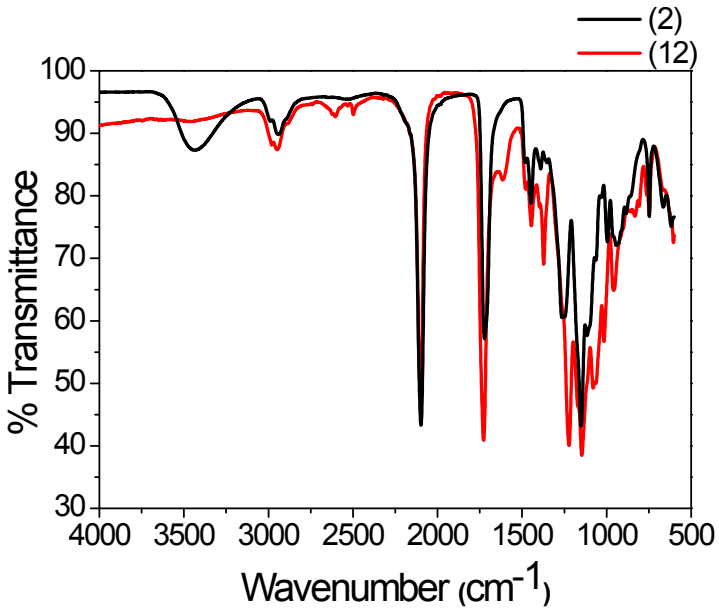
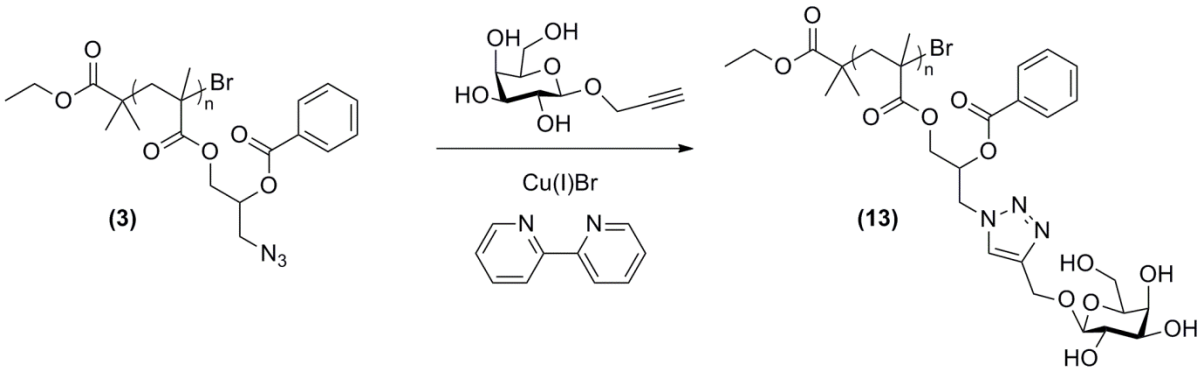


Figure S15. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following modification with dichloroacetyl chloride **(12)**.

1,3-Dipolar Cycloaddition of Polymers (2)-(12) with β-D-1-propargyl galactose

Reaction of (3) with β-D-1-propargyl galactose



Polymer (100 mg, 345.67 μmol), Cu(I)Br (4.9 mg, 34.16 μmol) and β-D-1-propargyl galactose (226 mg, 1.04 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (10.8 mg, 69.15 μmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

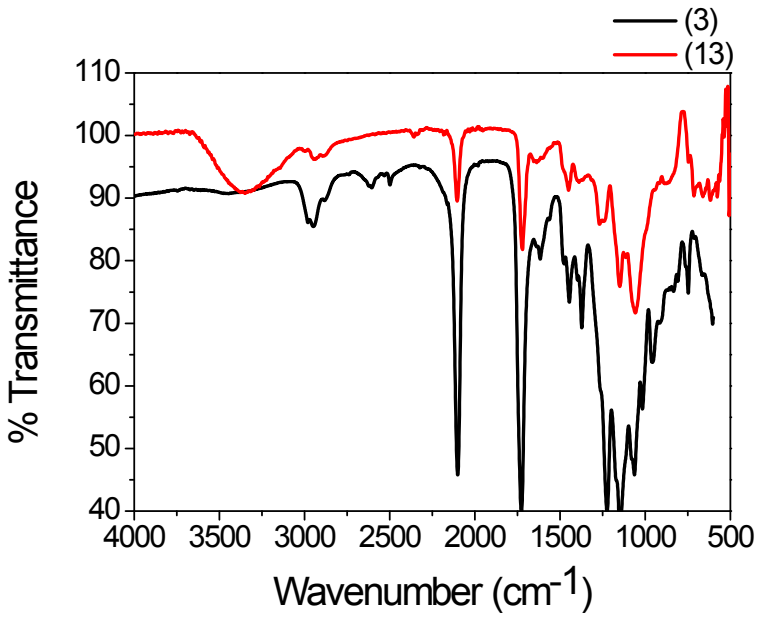
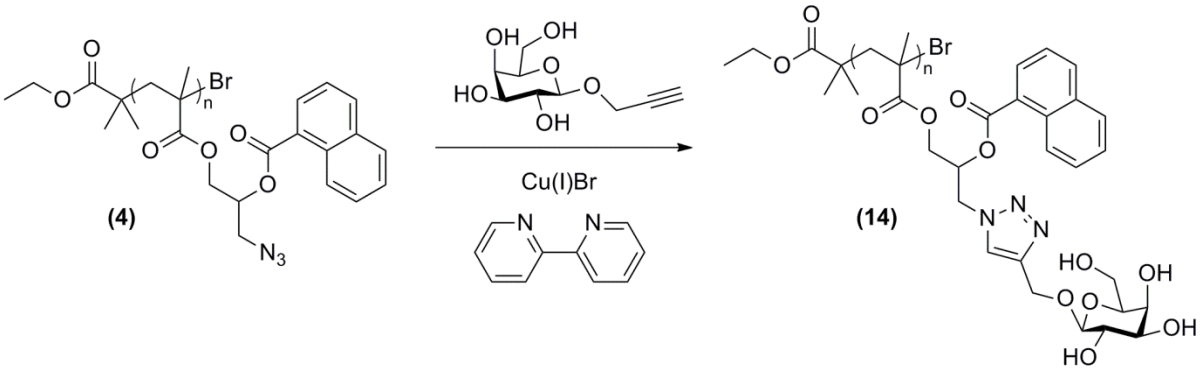


Figure S16. Infrared spectra of azide-functional polymer **(3)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(13)**.

Reaction of (4) with β-D-1-propargyl galactose



Polymer (160 mg, 471.49 µmol), Cu(I)Br (6.8 mg, 47.40 µmol) and β-D-1-propargyl galactose (308 mg, 1.41 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (14.8 mg, 94.76 µmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was

diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

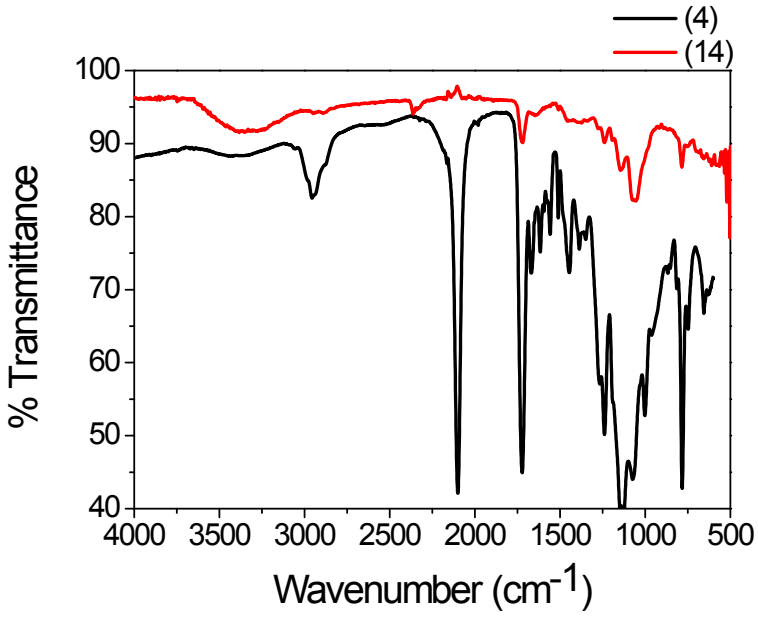
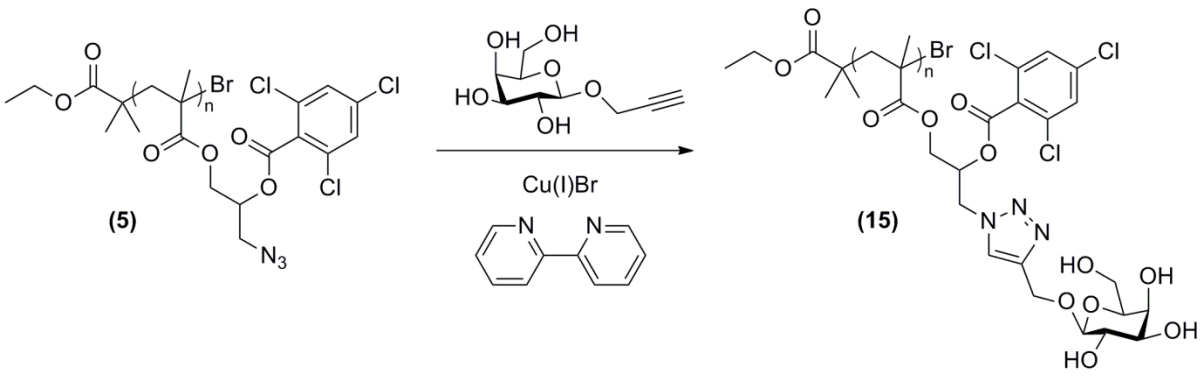


Figure S17. Infrared spectra of azide-functional polymer **(4)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(14)**.

Reaction of (5) with Galactose Alkyne



Polymer (177 mg, 450.82 µmol), Cu(I)Br (6.5 mg, 45.31 µmol) and β-D-1-propargyl galactose (295 mg, 1.35 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This

solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (14.1 mg, 90.28 μmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

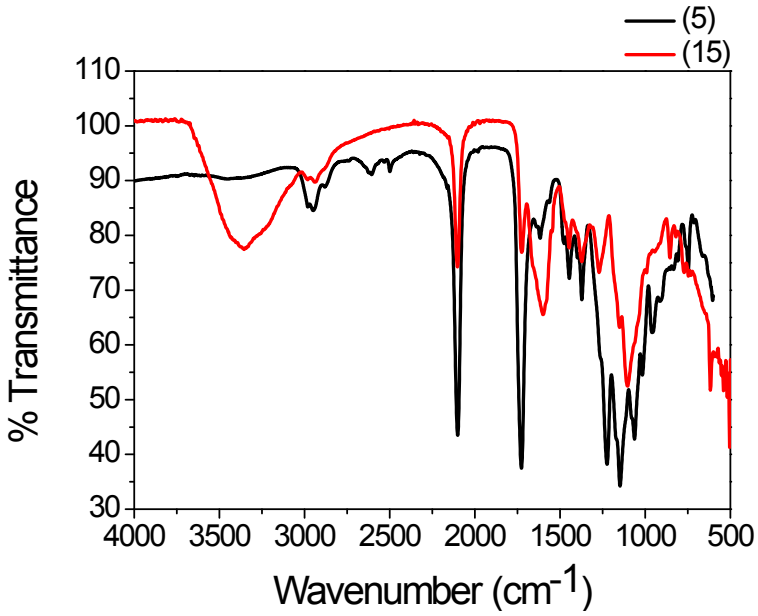
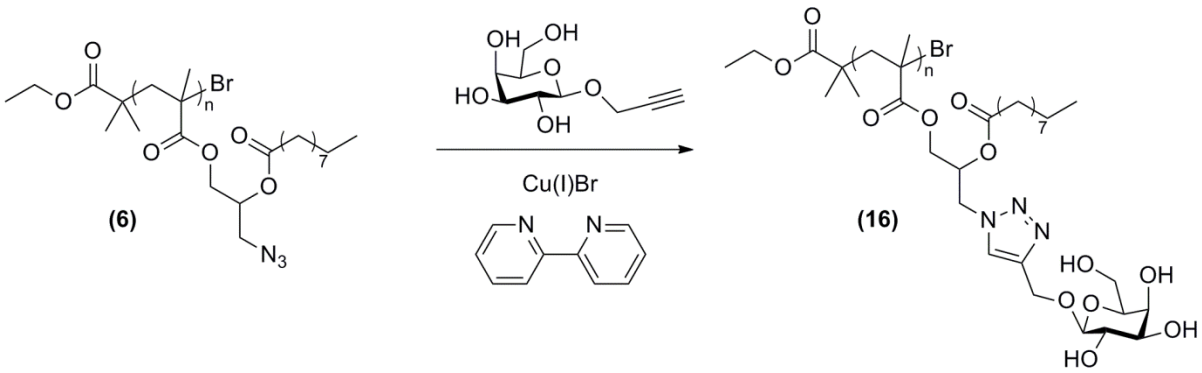


Figure S18. Infrared spectra of azide-functional polymer **(5)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(15)**.

Reaction of (6) with Galactose Alkyne



Polymer (200 mg, 589.22 µmol), Cu(I)Br (8.5 mg, 58.92 µmol) and β-D-1-propargyl galactose (385 mg, 1.76 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (18.4 mg, 117.81 µmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

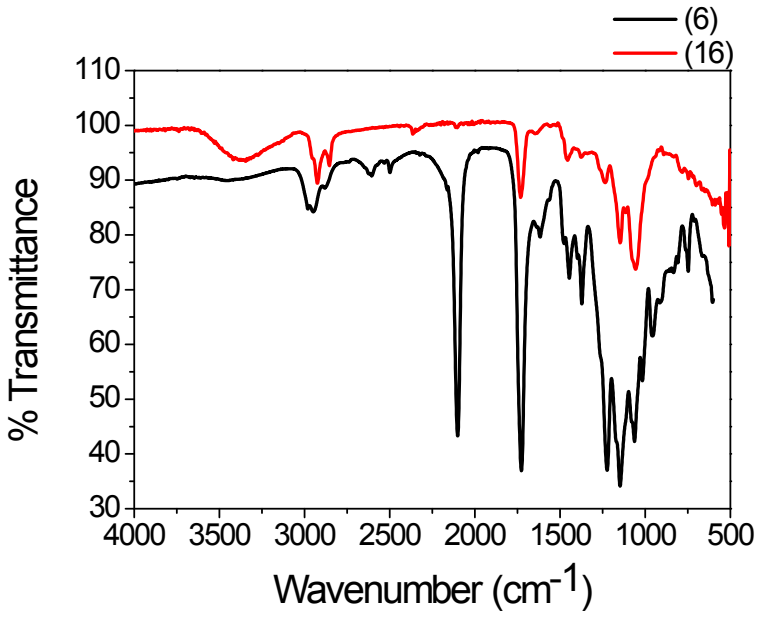
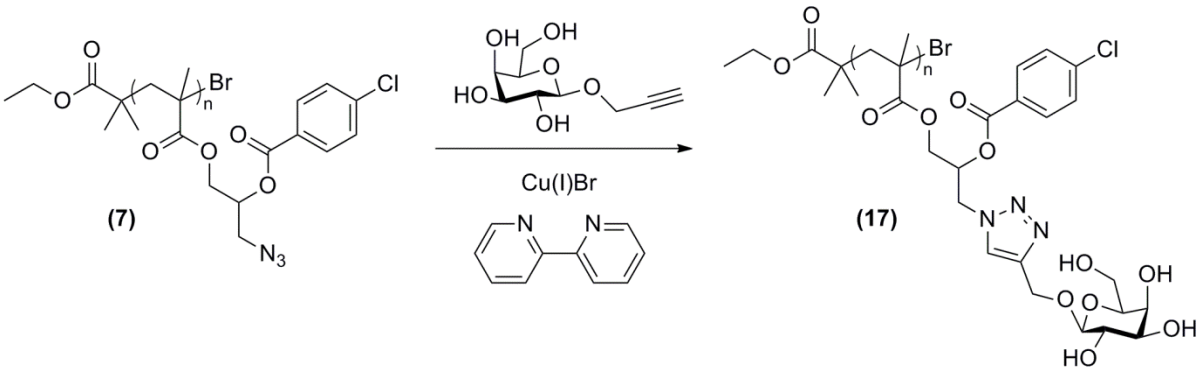


Figure S19. Infrared spectra of azide-functional polymer **(6)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(16)**.

Reaction of (7) with β-D-1-propargyl galactose



Polymer (280.0 mg, 865.92 μmol), Cu(I)Br (8.5 mg, 86.44 μmol) and β-D-1-propargyl galactose (566.0 mg, 2.59 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (27.0 mg, 172.88 μmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

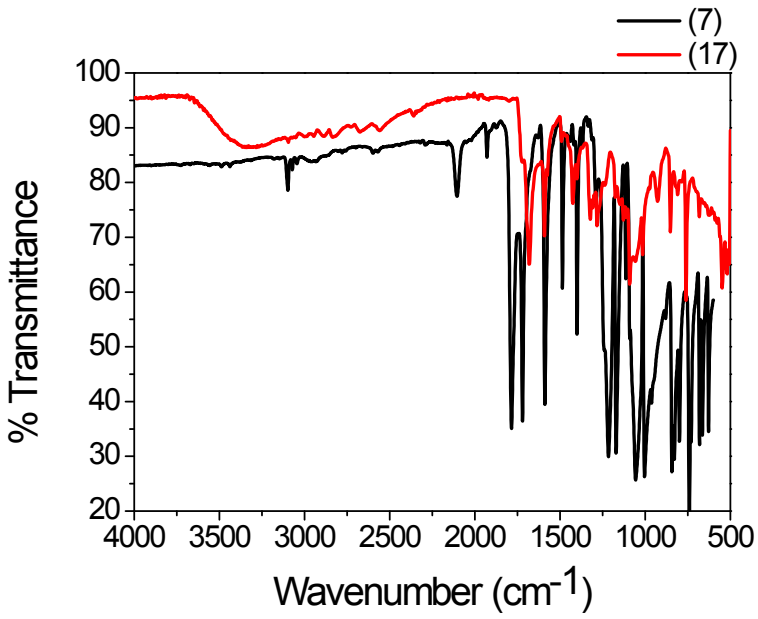
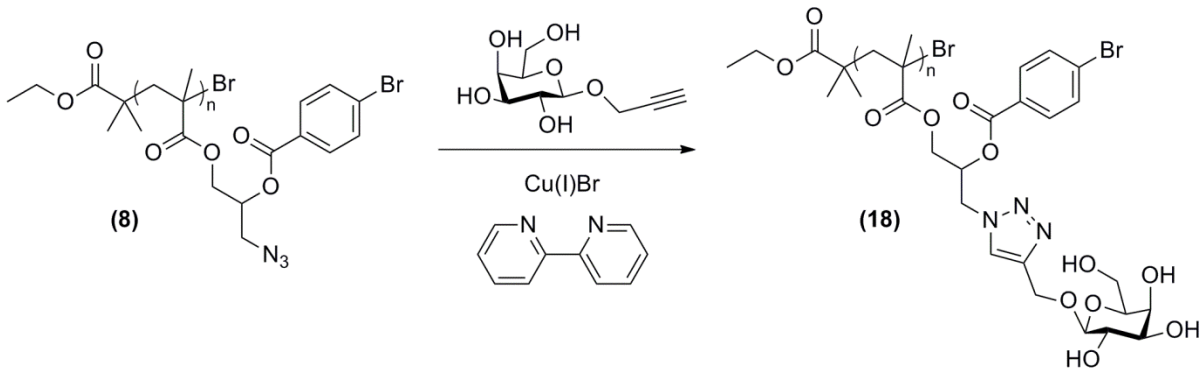


Figure S20. Infrared spectra of azide-functional polymer **(7)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(17)**.

Reaction of (8) with β-D-1-propargyl galactose



Polymer (180.0 mg, 488.89 µmol), Cu(I)Br (7.0 mg, 48.80 µmol) and β-D-1-propargyl galactose (320 mg, 1.47 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (15.3 mg, 97.96 µmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white power.

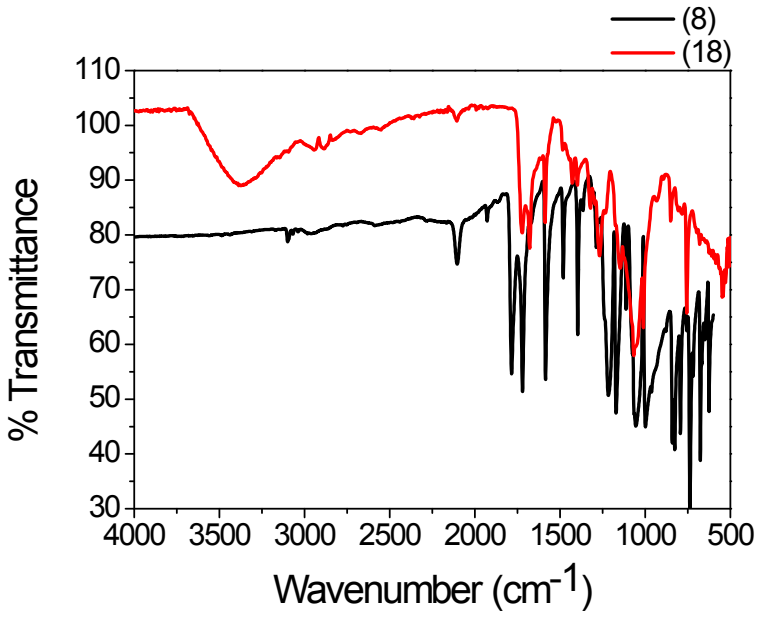
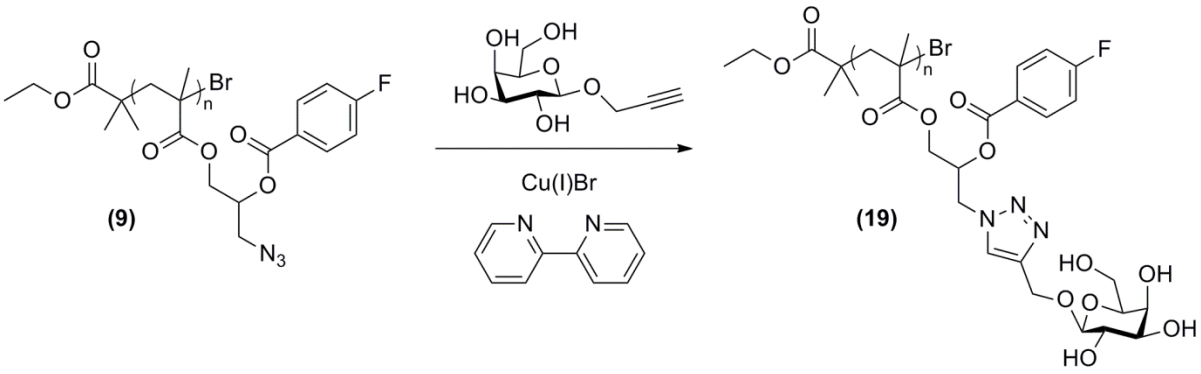


Figure S21. Infrared spectra of azide-functional polymer **(8)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(18)**.

Reaction of (9) with β-D-1-propargyl galactose



Polymer (180.0 mg, 585.80 μmol), Cu(I)Br (8.4 mg, 58.58 μmol) and β-D-1-propargyl galactose (383 mg, 1.76 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (18.3 mg, 117.17 μmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

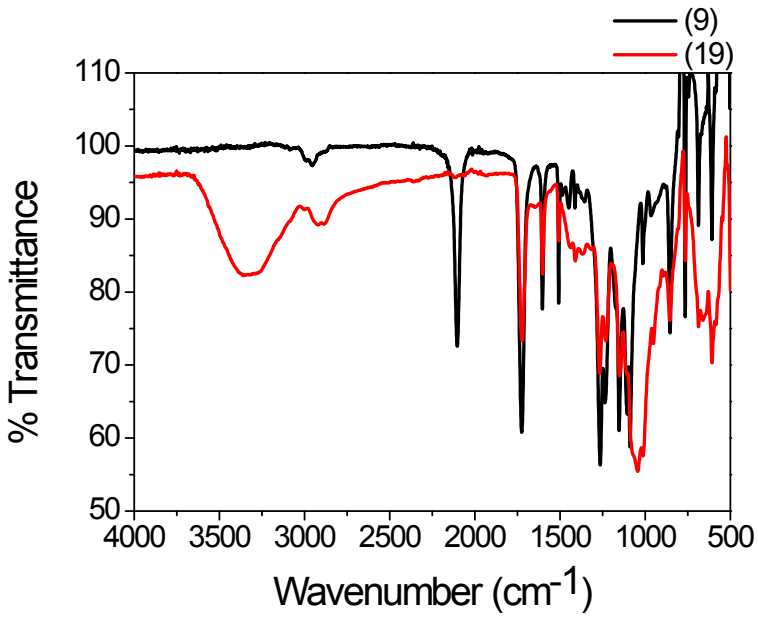
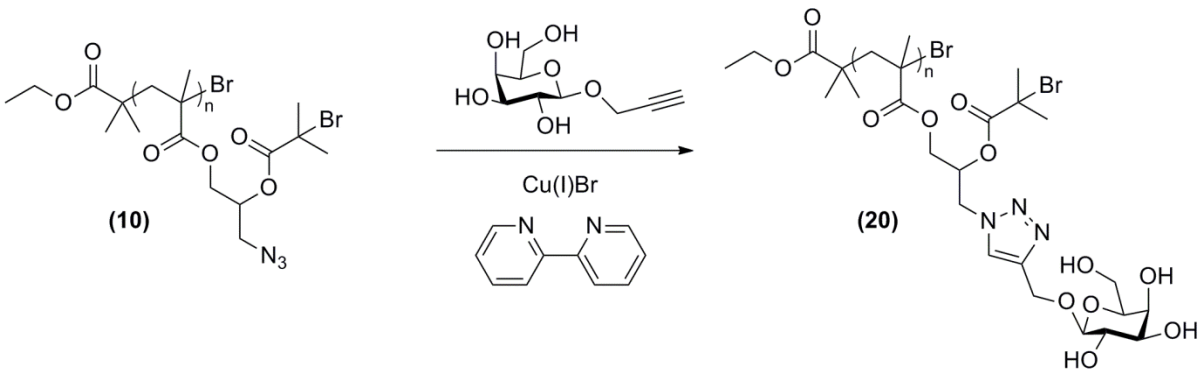


Figure S22. Infrared spectra of azide-functional polymer **(9)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(19)**.

Reaction of (10) with β-D-1-propargyl galactose



Polymer (54.0 mg, 161.59 µmol), Cu(I)Br (2.3 mg, 16.03 µmol) and β-D-1-propargyl galactose (105 mg, 481.21 µmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (5.0 mg, 32.27 µmol) was added

and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

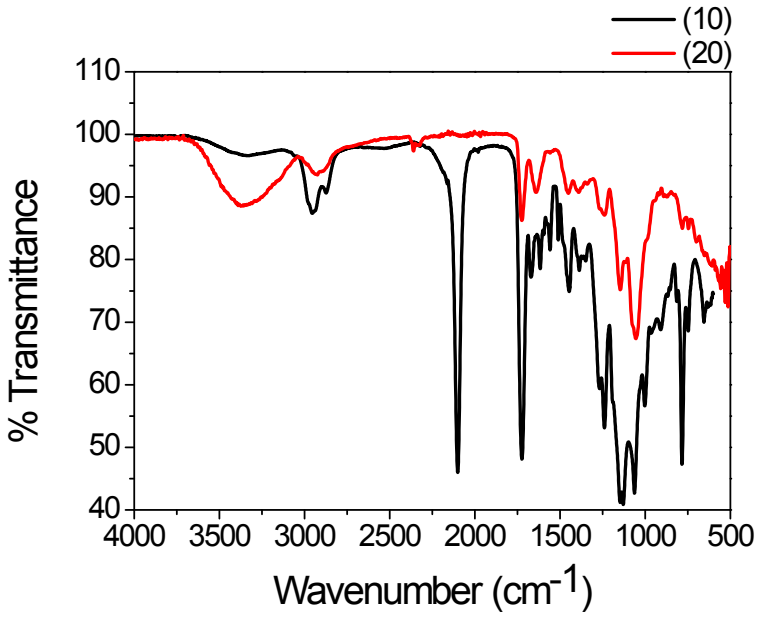
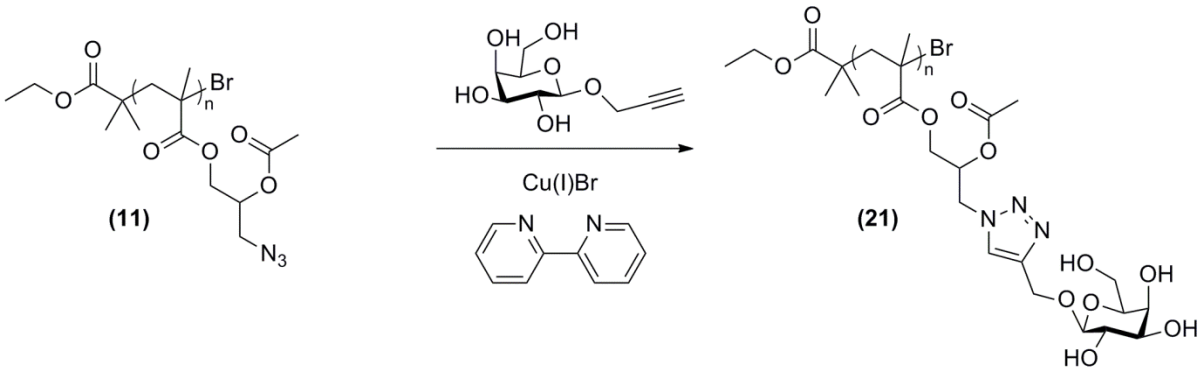


Figure S23. Infrared spectra of azide-functional polymer **(10)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(20)**.

Reaction of (11) with β-D-1-propargyl galactose



Polymer (140.0 mg, 616.17 µmol), Cu(I)Br (8.8 mg, 61.62 µmol) and β-D-1-propargyl galactose (403 mg, 1.85 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (19.2 mg, 122.94 µmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

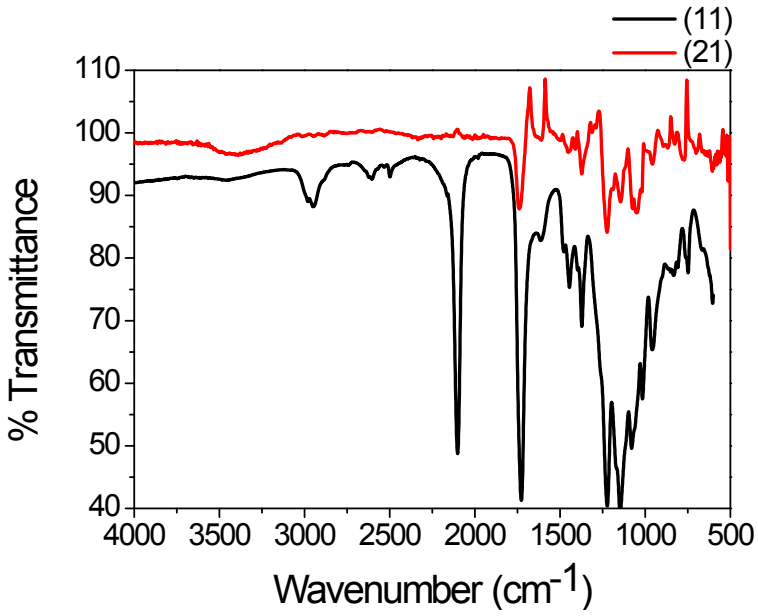
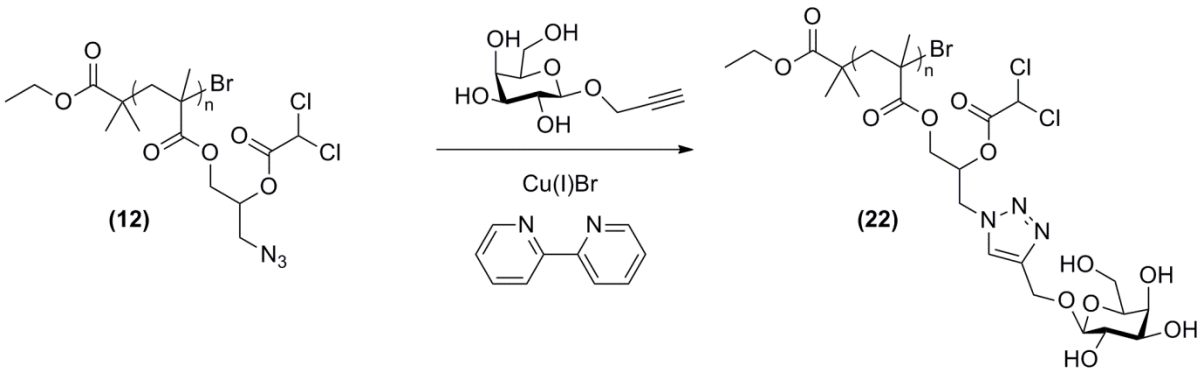


Figure S24. Infrared spectra of azide-functional polymer **(11)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(21)**.

Reaction of (12) Galactose Alkyne



Polymer (200.0 mg, 675.42 µmol), Cu(I)Br (9.7 mg, 67.54 µmol) and β-D-1-propargyl galactose (442 mg, 2.03 mmol) was dissolved in DMSO (8 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (21.1 mg, 135.10 µmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

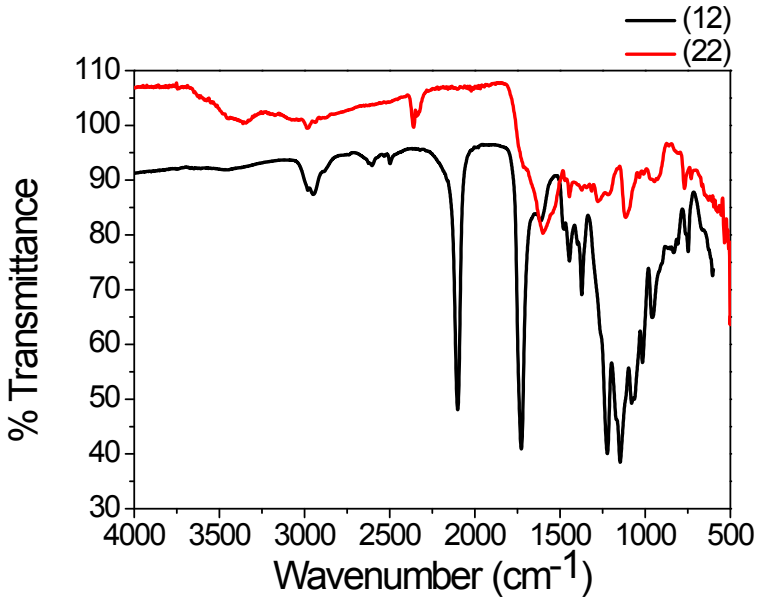
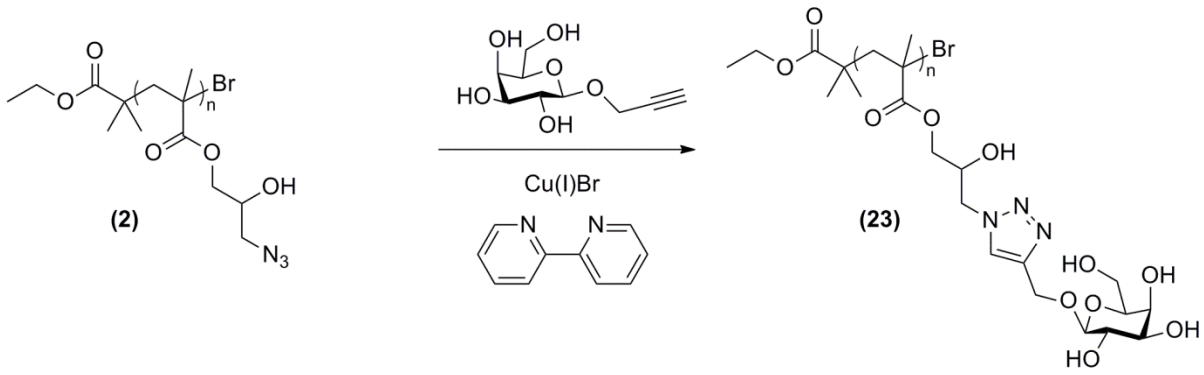


Figure S25. Infrared spectra of azide-functional polymer **(12)** and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(22)**.

Reaction of (2) with β-D-1-propargyl galactose



Polymer (200 mg, 1.08 mmol), Cu(I)Br (15.5 mg, 108.05 μmol) and β-D-1-propargyl galactose (710 mg, 3.25 mmol) was dissolved in DMSO (10 mL) in a Schlenk tube. This solution was degassed by a minimum of 3 freeze-pump-thaw cycles and frozen with liquid nitrogen. The Schlenk tube was then opened, 2,2'-bipyridyl (33.7 mg, 215.78 μmol) was added and the tube re-sealed. The frozen solution was evacuated three times, back-filled with dry nitrogen and left to defrost. After stirring at ambient conditions for 4 days, the solution was diluted with distilled water and dialysed against water for 3 days. The resulting suspension was centrifuged and the supernatant was lyophilised to leave an off-white powder.

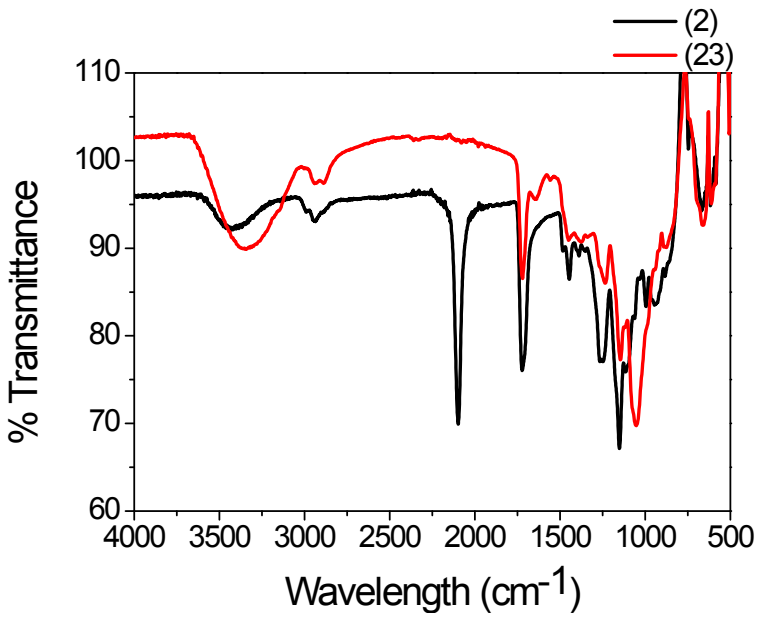


Figure S26. Infrared spectra of poly(2-hydroxy-3-azidopropyl methacrylate), **(2)**, and following 1,3-dipolar cycloaddition reaction with β-D-1-propargyl galactose **(23)**.

^{1}H NMR analysis of Post-Polymerization Modification

The synthetic route employed to obtain the library of polymers was designed to enable each reaction step to be monitored by IR-spectroscopy by the appearance/disappearance of diagnostic peaks, rather than NMR in which there are many overlapping peaks. Potential side reactions (such as the ester linkage being broken into carboxylic acid) would also be visible in IR. To demonstrate the successfully installation of the azide and carbohydrate groups, representative ^{1}H NMR spectra are shown below. The functional polymers obtained here generally displayed low solubility in most solvents (biochemical assays were undertaken < 1 mg.mL^{-1}). This complicated analysis as DMSO obscured several peaks of interest, and the intensity was rather low.

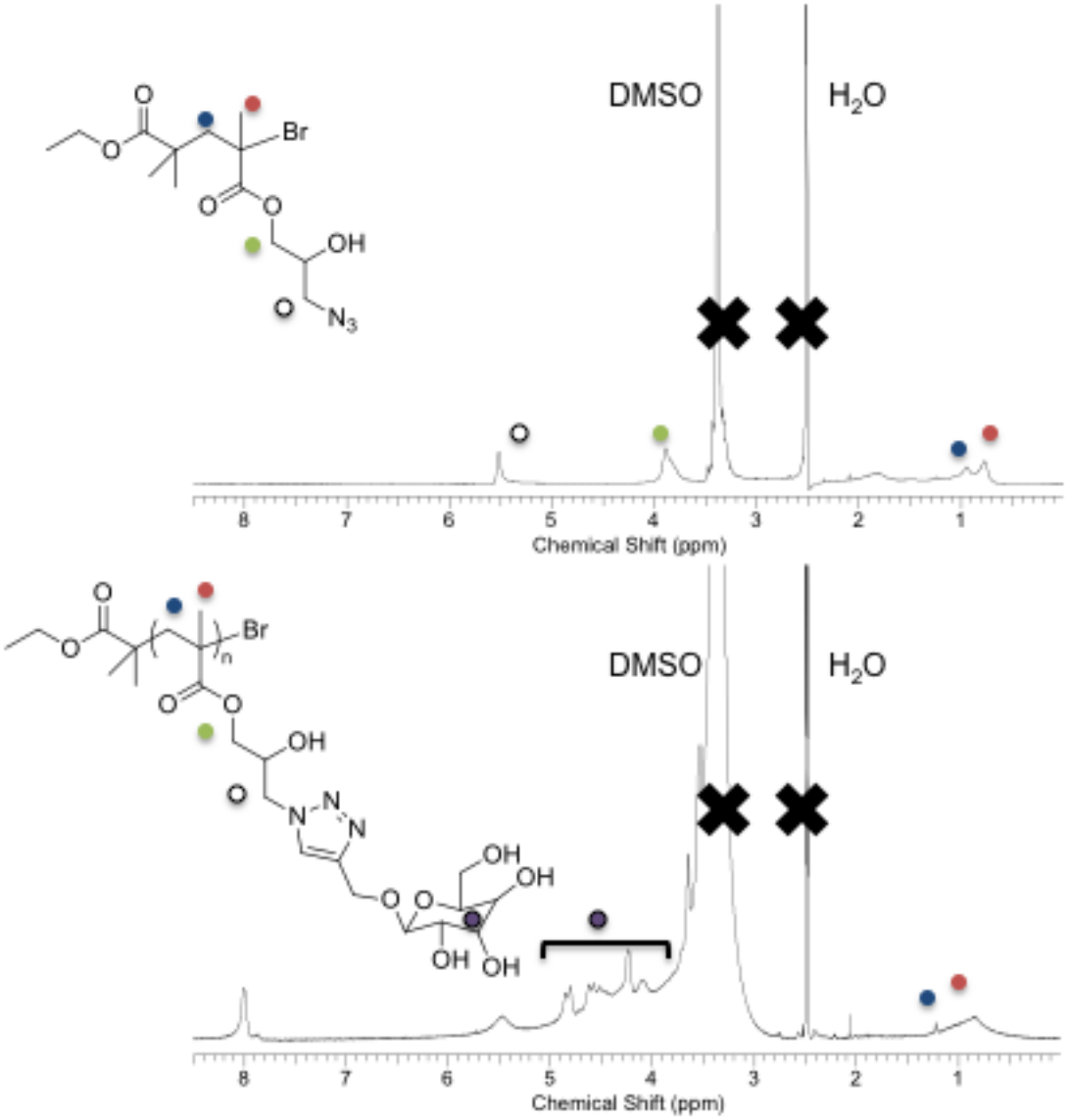


Figure S27. ^{1}H NMR analysis of representative polymers showing the successful installation of azide and carbohydrate groups.

Lectin Binding to Galactocerebroside Surface

Binding of each of the lectins to the galactocerebroside-functionalised surface was confirmed by measuring the fluorescence after incubation with a serial dilution of each lectin (Figure S28). As expected, greater binding (higher fluorescence) was observed with increasing lectin concentration.

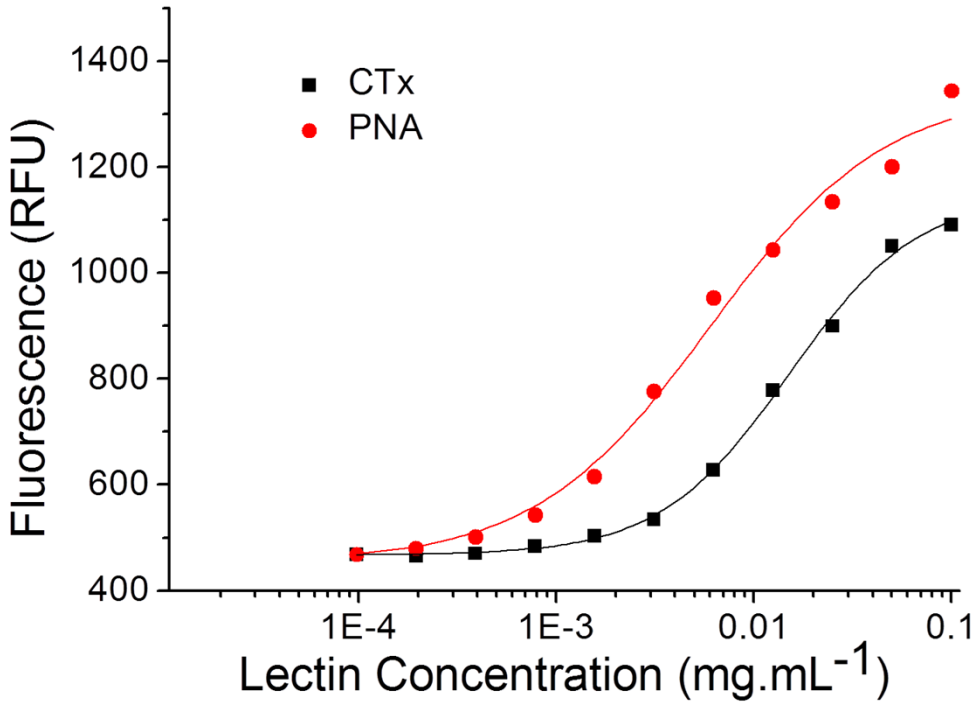


Figure S28. Binding curve of a serial dilution of PNA and CTx to a surface functionalised with 0.1 mg/ml of galactocerebroside.

Example glycopolymer inhibition curves

As anticipated, addition of the secondary binding motifs had a dramatic influence on the inhibitory potential of the glycopolymers. Figure S28 shows example inhibition curves for 3 glycopolymers **P1**, **P5** and **P9** against PNA. Addition of 4-chlorobenzyl (**P5**) lead to a dramatic reduction in binding activity with the MIC_{50} value increasing by a factor of 5. Conversely, introduction of a methyl group (**P9**) greatly enhanced inhibitory activity, with the MIC_{50} decreasing by 10-fold.

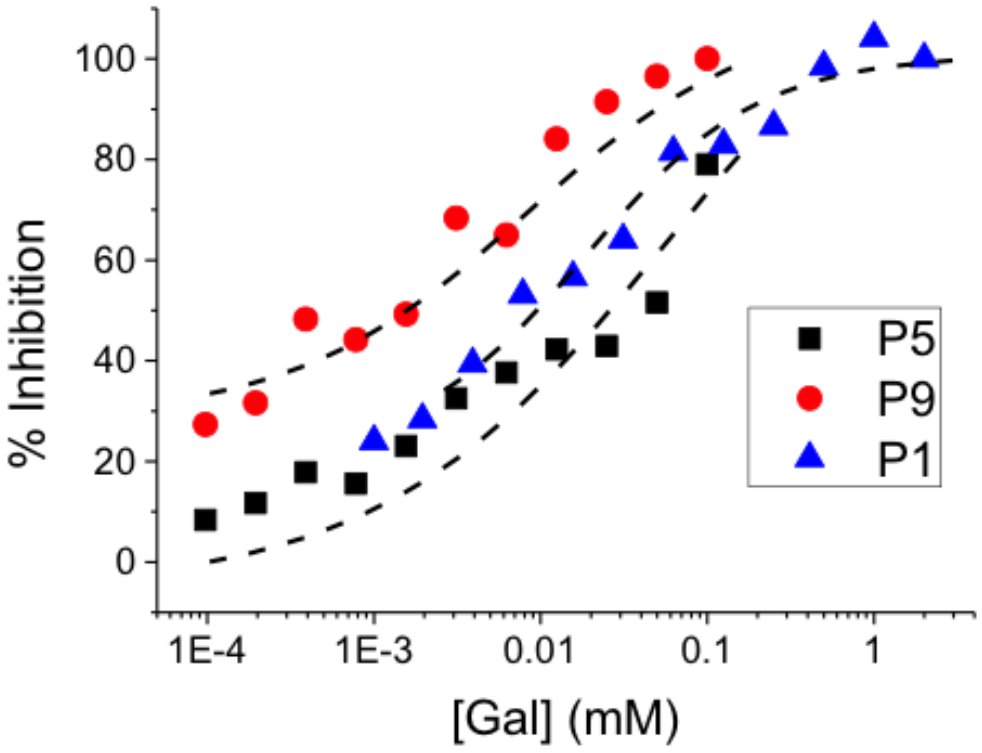


Figure S29. Example inhibition curves against PNA. Each point represents the average of 3 fluorescence measurements and the dotted line represents the line of best fit.

Correlation between Inhibition and Partition Coefficient

In small molecule drug design the partition coefficient, which is a measure of hydrophobicity is often used to assign hydrophobic interactions or to predict pharmacokinetics. The partition coefficient of a single repeat unit of each polymer was plotted against the observed MIC_{50} against both lectins, below. There was a peak of lower affinity (higher MIC_{50}) at intermediate values of LogP, but no clear trend emerged.

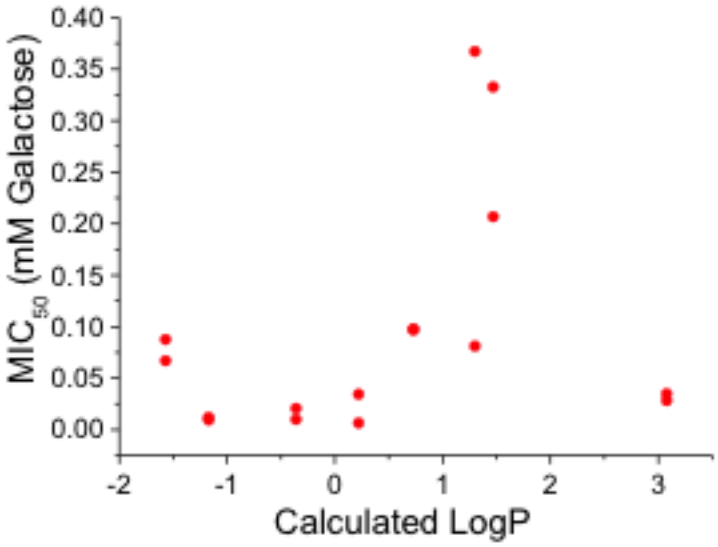


Figure S30. Plot showing relationship between (estimated) partition co-efficient of a single repeat unit of the polymers verses the observed MIC_{50} values for both lectins.

RCA_{120} Inhibitory Assay

Table S2. RCA_{120} inhibition compared to PNA

Polymer	**IC_{50} PNA (mM Gal)**	**IC_{50} RCA_{120} (mM Gal)**
P1	0.087	0.014
P5	0.36	0.017

Comparison of relative affinities of polymer to each lectin

Table S3 shows the relative affinities of each glycopolymer, relative to the control polymer (P1). P5 was selected as the most selective as it maintained its affinity towards CTx, but had significant decreased affinity towards PNA. P8 also demonstrates selectivity. However, this appear due to it have increase affinity towards both lectins.

Table S3. Relative affinity of glycopolymers compared to control polymer, P1

Polymer	**Px/P1 CTx**	**Px/P1 PNA**
P1	1	1
P4	0.52	0.32
P5	1.21	4.20
P6	4.98	2.36
P8	0.10	0.39
P9	0.18	0.11
P10	0.31	0.11
P11	1.45	1.12

Px is the polymer listed in the first column.

References:

[1] Haddleton, D. M.; Crossman, M. C.; Dana, B. H.; Duncalf, D. J.; Heming, A. M.; Kukulj, D.; Shooter, A. J. *Macromolecules* **1999,** *32,* 2110-2119.

[2] Schmid, S.; Mishra, A.; Bäuerle, P. *Chem. Commun.* **2011,** *47,* 1324-1326.