Electronic Supplementary Material (ESI) for Faraday Discussions.

Engineering of spectator glycocalyx structures to evaluate molecular interactions at crowded cellular boundaries.

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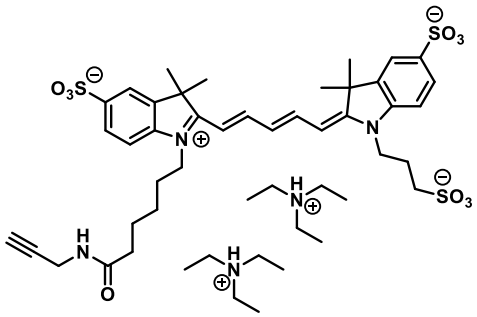
Supporting Information

Table of contents:

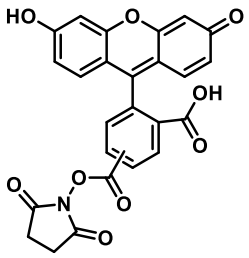
Instrumentation and general procedures.

Column chromatography was performed on a Biotage Isolera One automated flash chromatography system. Nuclear magnetic resonance (NMR) spectra were collected on a Bruker 300 MHz and a Jeol 500 MHz NMR spectrometers. Spectra were recorded in $CDCl_3$ or D_2O solutions at 293K and are reported in parts per million (ppm) on the δ scale relative to the residual solvent as an internal standard (for ^1H NMR: $CDCl_3$ = 7.26 ppm, D_2O = 4.79 ppm, for ^{13}C NMR: $CDCl_3$ = 77.0 ppm). HRMS (high-resolution mass spectrometry) analysis was performed on an Agilent 6230 ESI-TOFMS in positive ion mode. UV-Vis spectra were collected with a quartz cuvette using a Thermo Scientific Nanodrop2000c spectrophotometer. IR spectroscopy was performed on a Nicolet 6700 FT-IR spectrophotometer (Thermo Scientific). Size exclusion chromatography (SEC) was performed on a Hitachi Chromaster system equipped with an RI detector and two 5 µm, mixed bed, 7.8 mm I.D. x 30 cm TSKgel columns in series (Tosoh Bioscience). Organic soluble polymers were analyzed using an isocratic method with a flow rate of 0.7 mL/min in DMF (0.2% LiBr, 70 °C). For aqueous SEC, two 8 µm, mixed-M bed, 7.5 mm I.D. x 30 cm PL aquagel-OH columns in series (Agilent Technologies) were run in sequence using an isocratic method with a flow rate of 1.0mL/min in water (0.2M $NaNO_3$ in 0.01M Na_2HPO_4, pH = 7.0). AFM imaging of lactose glycopolymer **5** was performed on Nanoscope IV Scanning Probe Microscope (Veeco) in tapping mode using an 9991EVLR scanner and Tap150AL-G silicon AFM probes with aluminum reflex coating (Ted Pella). For imaging, polymers in aqueous solution were deposited on PELCO mica discs (Ted Pella). After 1 min, excess water was removed, and discs were allowed to dry at ambient pressure before imaging. Flow cytometry analysis was performed on live RBCs using a FACS Canto II cytometer (BD Biosciences). Red blood cells samples for bright field microscopy were prepared by diluting live cells in PBS 1:1 with Vectamount AQ aqueous mounting medium (Vector Labs H-5501) and imaged Keyence Fluorescent microscope. Scanning Electron Microscopy (SEM) was performed on an FEI Apreo Scanning Electron Microscope. RBCs containing 0nM, 2500nM, and 10000nM were prepared by fixing overnight in 2.5% glutaraldehyde solution at 4 °C, then were gradually dehydrated by washing with 0, 10, 20, 30, 50, 70, 80, 90, 100, 100% EtOH solutions. Prepared samples were then dried using Tousimis AutoSamdri 815A critical point dryer, and sputter coated with Iridium for 8 seconds using Emitech K575X Iridium Sputter coater. SEM imaging was done with ETD detector at HV 4.00KV, 0.1nA current.

Structure of Cy5-alkyne:



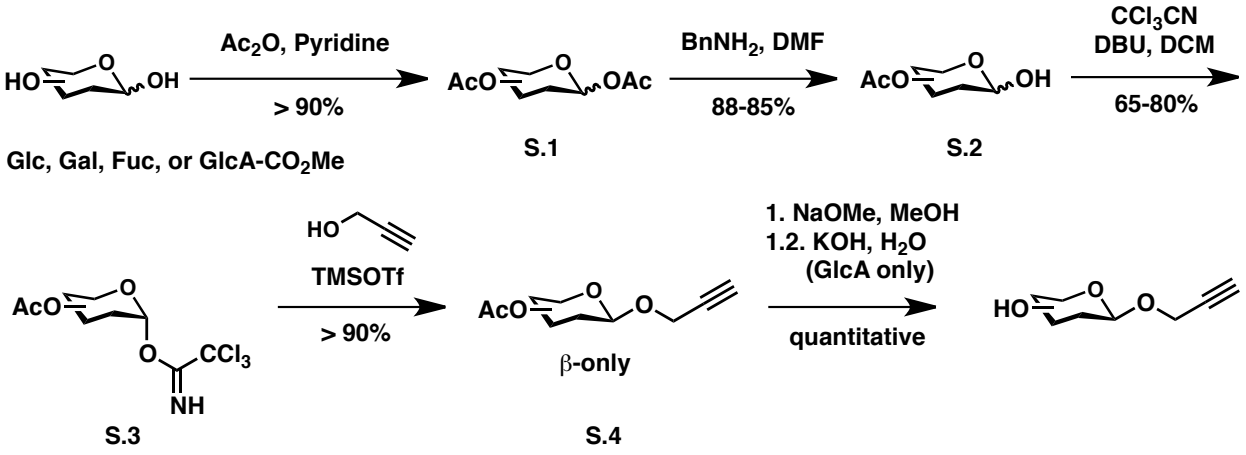
Structure of 5/6 Fluorescein-NHS:



Synthesis of propargyl glycosides.

β-propargyl glucoside (Glc),[31] galactoside (Gal),[31] fucoside (Fuc)[32] and glucuronopyranoside (GlcA)[33] were prepared according to published procedures. General procedures for the preparation of the glycosides according to Scheme S1 and ^{1}H NMR spectra for all intermediates and final glycosides are listed below.

Scheme S1. Preparation of propargyl glycosides via the Schmidt glycosylation.



General procedure for the preparation of peracetylated glycans (S.1). To glucose (Glc), galactose (Gal), fucose (Fuc), or glucopyranuronic acid methyl ester (GlcA-CO_2Me) (1.0 mmol) was added acetic anhydride (1.5 ml) and pyridine (1.5 ml). The reaction was cooled in an ice bath, and catalytic DMAP (50.0 mg, 0.1 mmol) was added. The mixture was brought to room temperature and stirred for 12 hrs. After this time, the reaction mixture was diluted with dichloromethane, washed with a saturated solutions of $NaHCO_3$ and NH_4Cl, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure to give peracetylated glycosides **S.1** in >90% yield. The crude products were used without further purification.

General procedure for anomeric hydroxyl deprotection in peracetylated glycans (S.2). To a solution of peracetylated glycans **S.1** (1.0 mmol) in dry DMF (4.0 mL) was added benzylamine (1.2 mmol). The mixture was stirred overnight. The reaction mixture was diluted with ethyl acetate and washed with a saturated solution of NH_4Cl, followed by brine. The organic phase was dried with anhydrous Na_2SO_4 and concentrated. The products were purified via column chromatography using 1:2 Hexanes/EtOAc to yield anomeric-OH deprotected glycans **S.2** as clear oils: S.2-Glc (95%) S.2-Gal (88%) S.2-Fuc (95%) S.2-GlcA (94%).

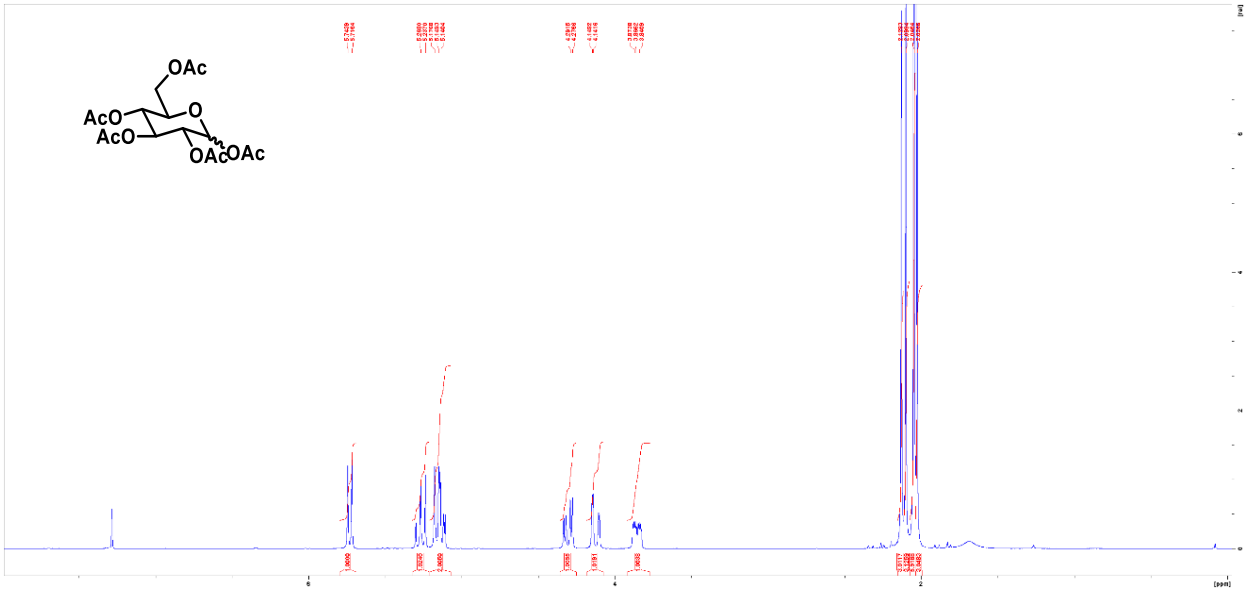
General procedure for synthesis of tricholoroacetimidate (TCA) glycosides (S.3). To a chilled (ice bath) solution of glycans **S.2** (1.0 mmol) in dry DCM (5.0 mL) were added trichloroacetonitrile (5.0 mmol) and DBU (0.1 mmol) under N2 atmosphere. After one hour, the reaction was concentrated under reduced pressure and the crude products were purified via column chromatography using 3:2 Hexanes/EtOAc to yield TCA glycosides **S.3** as light yellow oils: S.3-Glc (75%) S.3-Gal (80%) S.3-Fuc (65%) S.3-GlcA (70%).

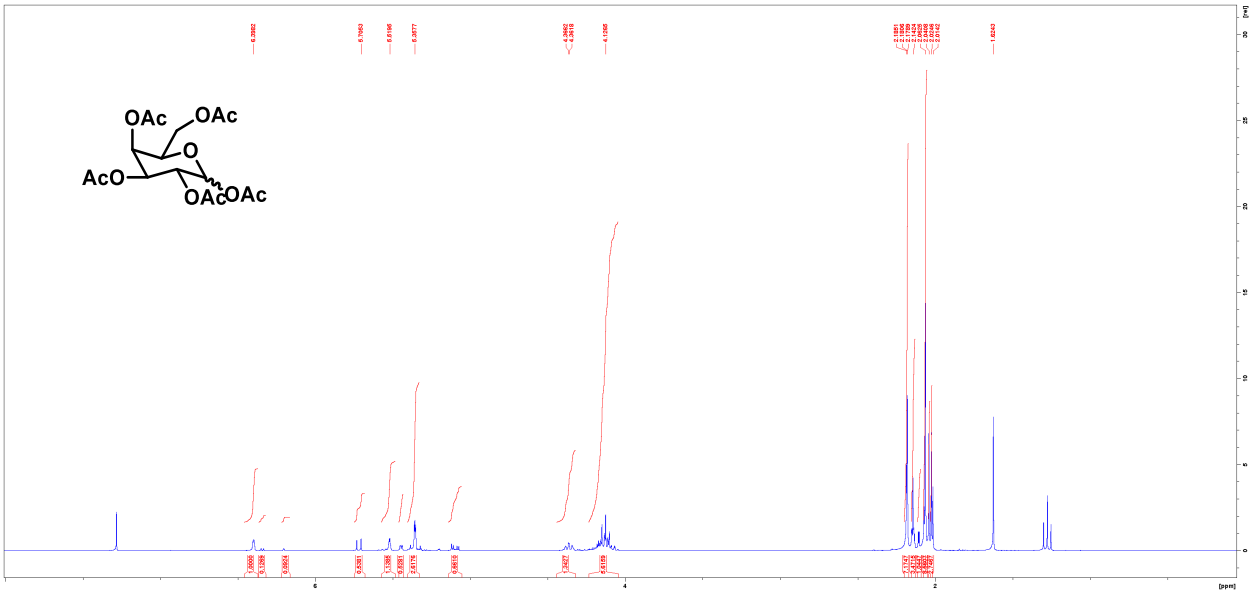
General procedure for the preparation of protected β-propargyl glycosides (S.4). To a solution of TCA glycan donors **S.3** (1.0 mmol) in dry DCM (5.0 mL) was added propargyl alcohol (5.0 mmol). The resulting solutions were cooled on ice, followed by the addition of TMSOTf catalyst (0.2 mmol). After 1h, the reaction was quenched with a saturated solution of $NaHCO_3$. The organic layer was separated, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude products were purified by column chromatography using 3:2 Hexanes/EtOAc on silica gel affording protected β-propargyl glycosides **S.4** as clear oils in >90% yield after purification.

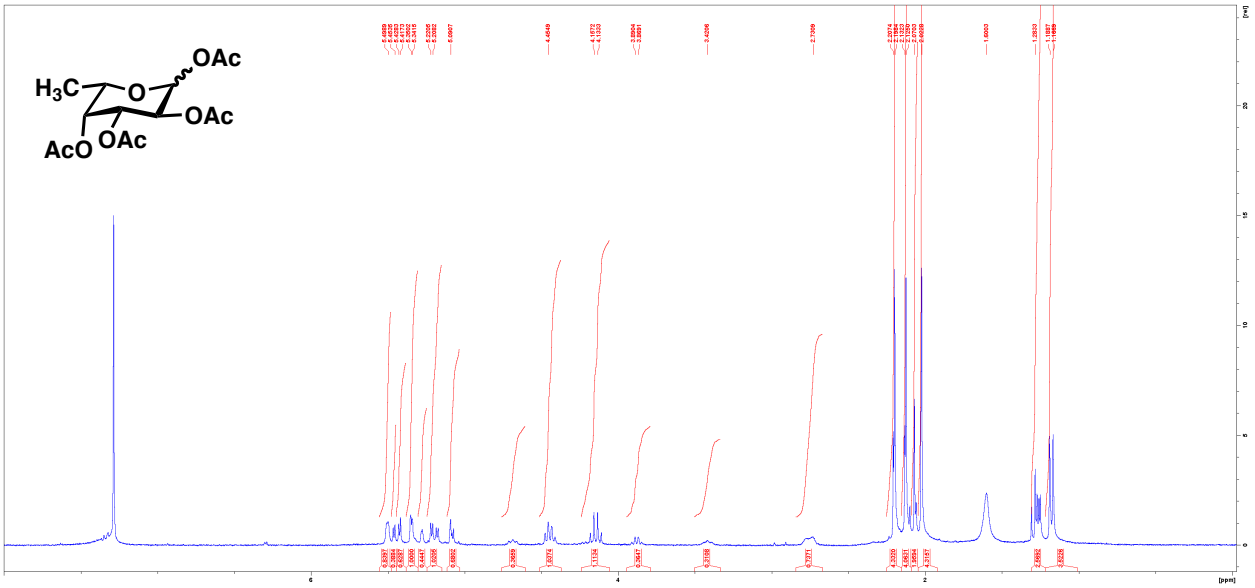
General procedure for the preparation of deprotected β-propargyl glycosides. To a solution of glycosides **S.4** (1.0 mmol) in dry methanol (5.0 mL) was added NaOMe (0.1 mmol) in methanol (0.1 mL). The reaction was stirred at room temperature for 2hrs, then was quenched by the addition of Dowex 50WX8-200 beads. The resin was removed by filtration and the filtrates were concentrated to yield pure deacetylated β-propargyl glycosides. The β-propargyl glucoronopyranoside methyl ester (1.0 mmol) was dissolved in MeOH and an aqueous solution of KOH (1.5 mmol, 10.0 mL) was added. After 1 hour, the reaction was quenched with the addition of Dowex 50WX8-200 ion-exchange resin. The resin was removed by filtration and the filtrate was concentrated to yield a pure fully deprotected β-propargyl glucoronopyranoside.

^{1}H NMR spectra of β-propargyl glycosides and synthetic intermediates.

Figure S1. ^{1}H NMR (300 MHz, $CDCl_3$) of peracetylated glycans **S.1**.






H3C
O
OAc
OAc
AcO
OAc

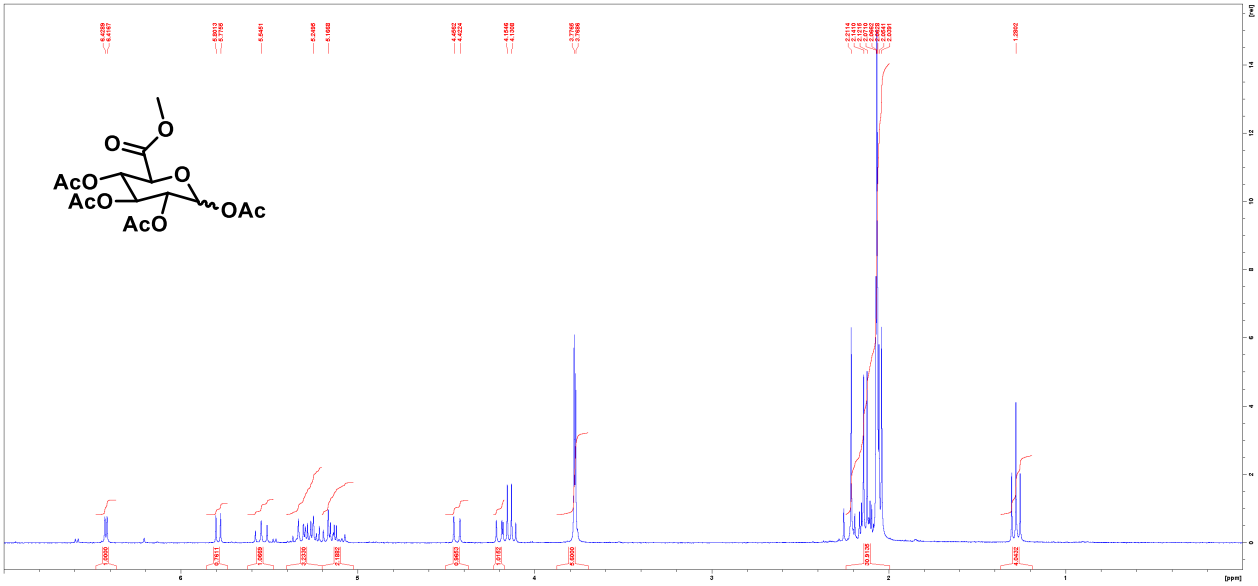
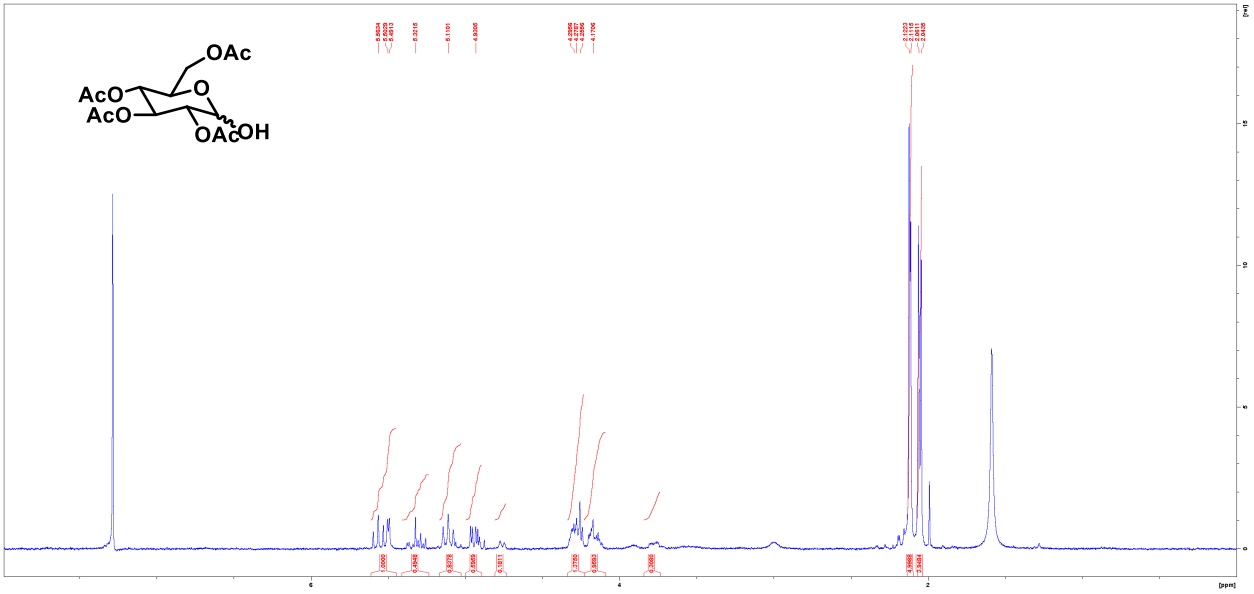
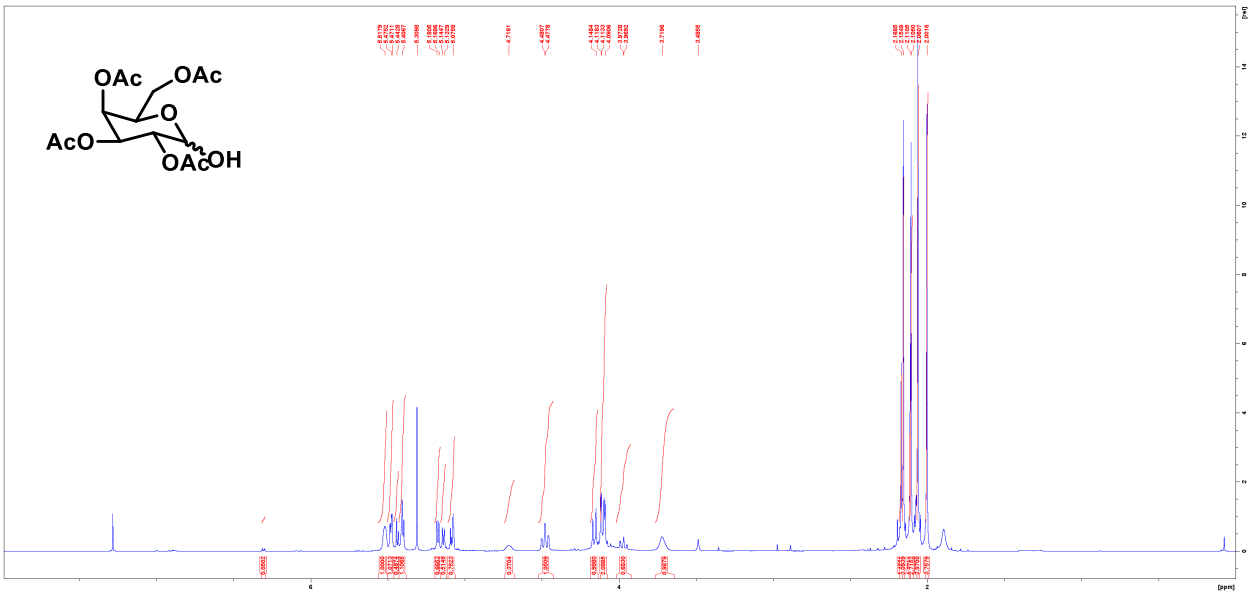
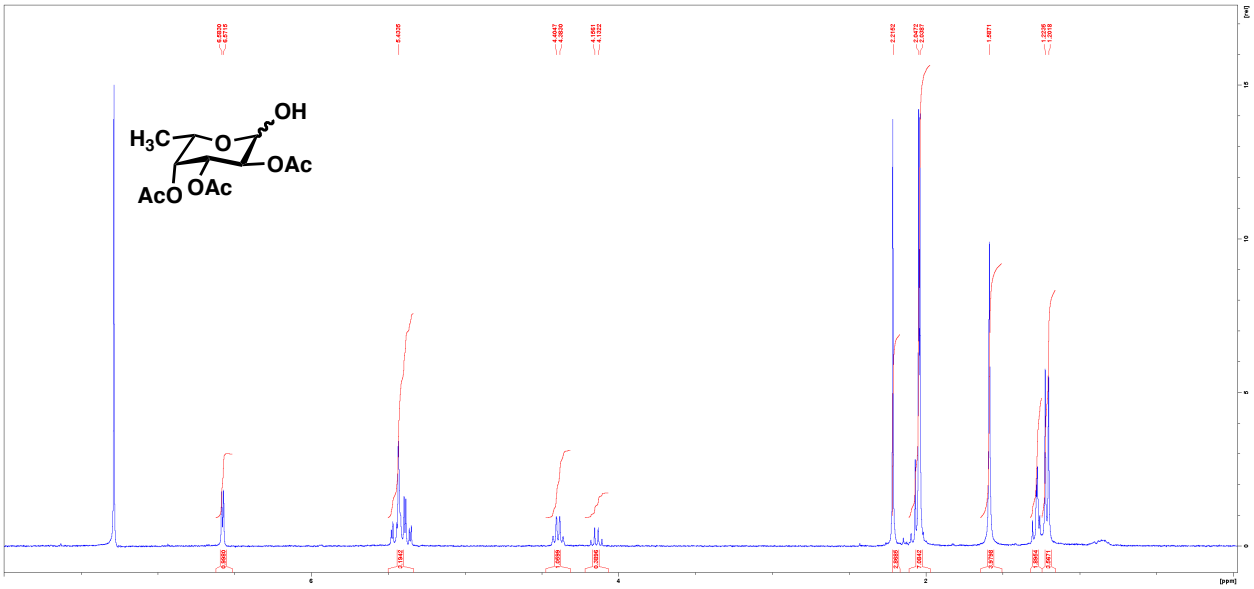

O
O
AcO
AcO
AcO
OAc

Figure S2. ^{1}H NMR (300 MHz, $CDCl_3$) of anomeric OH-deprotected peracetylated glycans **S.2**.






OH
H3C
O
OAc
AcO
OAc

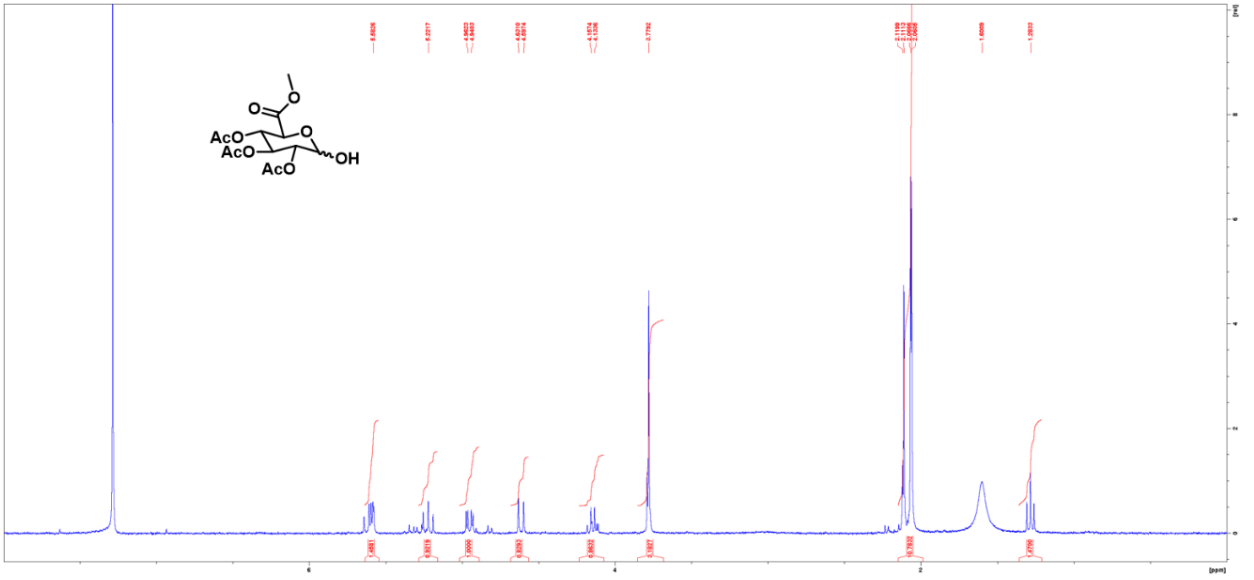
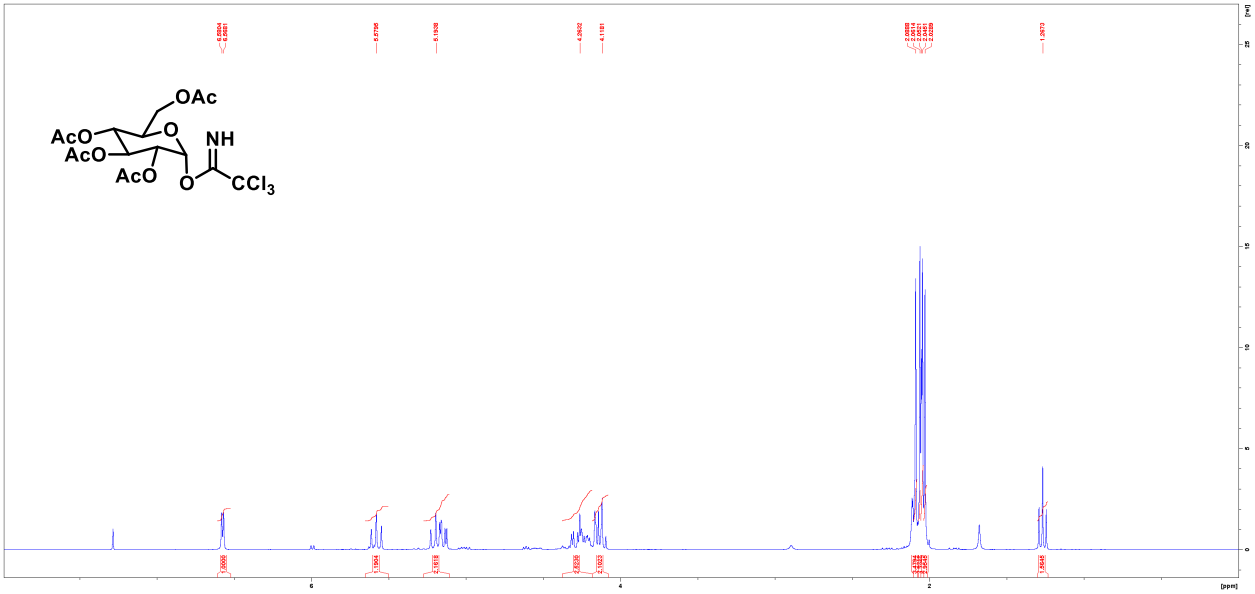
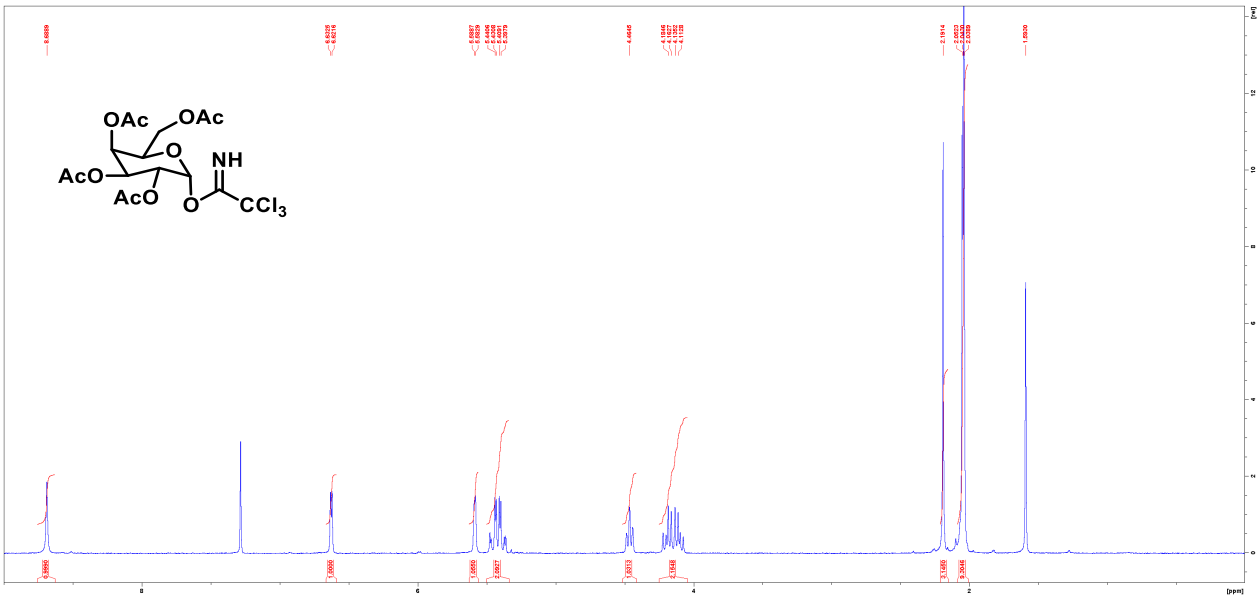
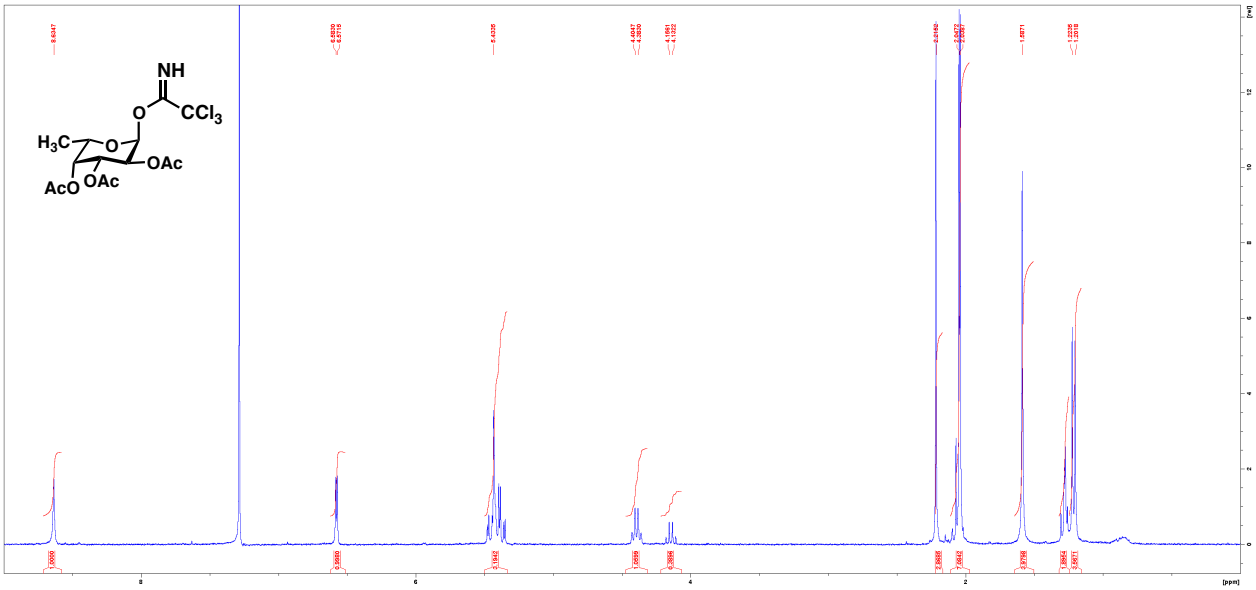

O
O
AcO
AcO
AcO
OH

Figure S3. ^{1}H NMR (300 MHz, $CDCl_3$) of anomeric TCA glycosides **S.3**.






NH
O
CCl3
H3C
O
OAc
AcO
OAc

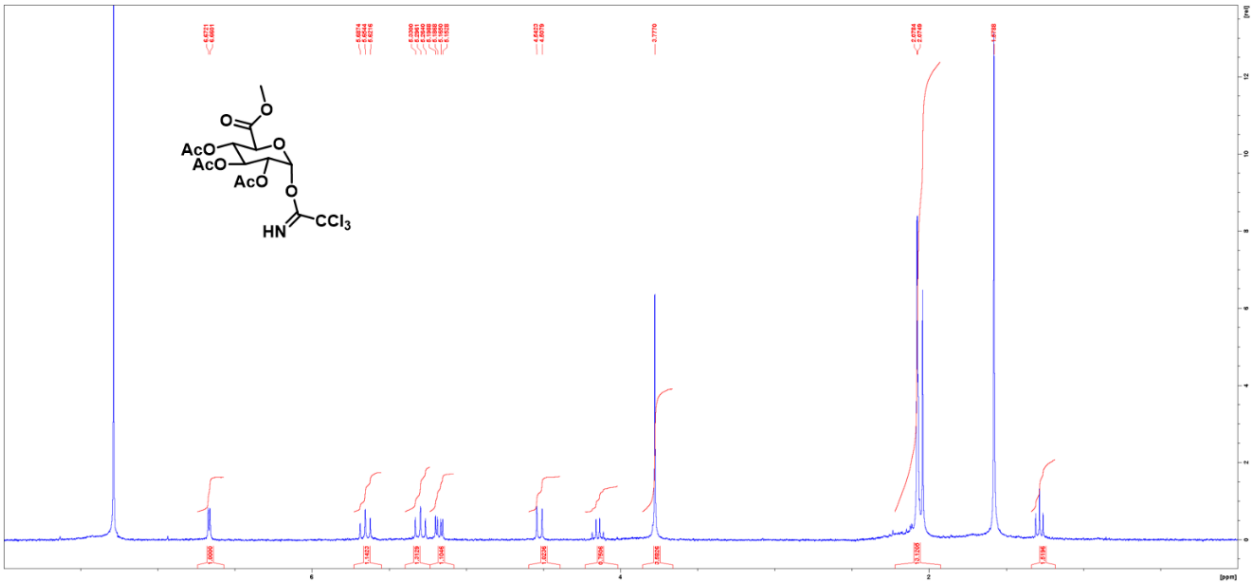
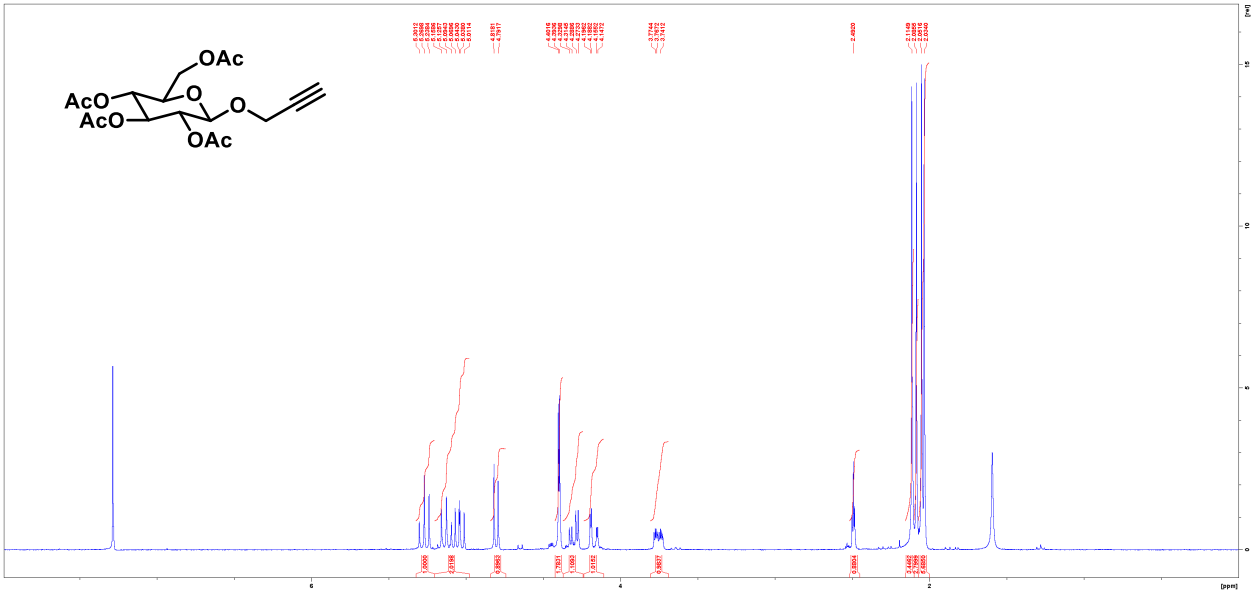
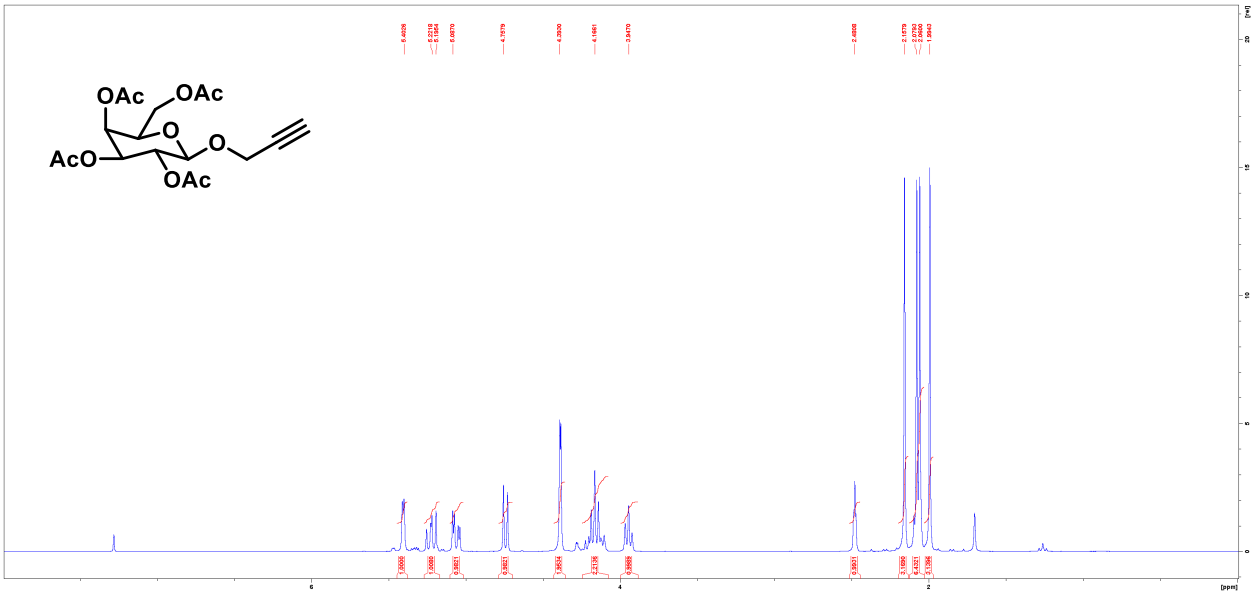
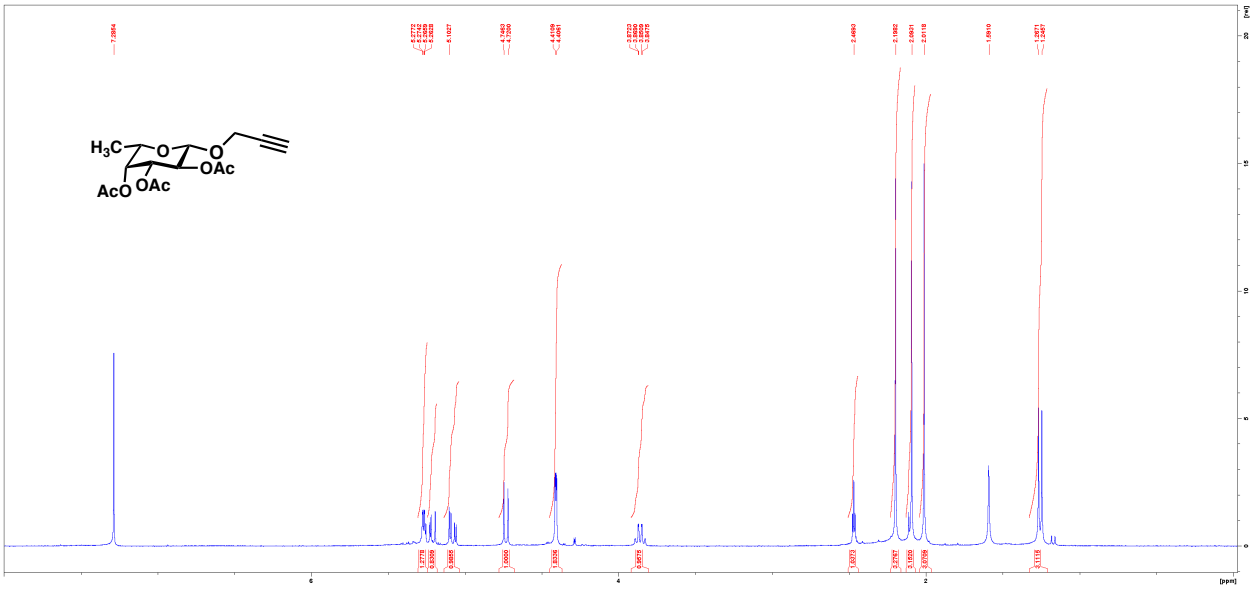

O
O
AcO
AcO
AcO
O
HN
CCl3

Figure S4. ^{1}H NMR (300 MHz, $CDCl_3$) of peracetylated β-propargyl glycosides **S.4**.






H3C
O
O
OAc
AcO
OAc

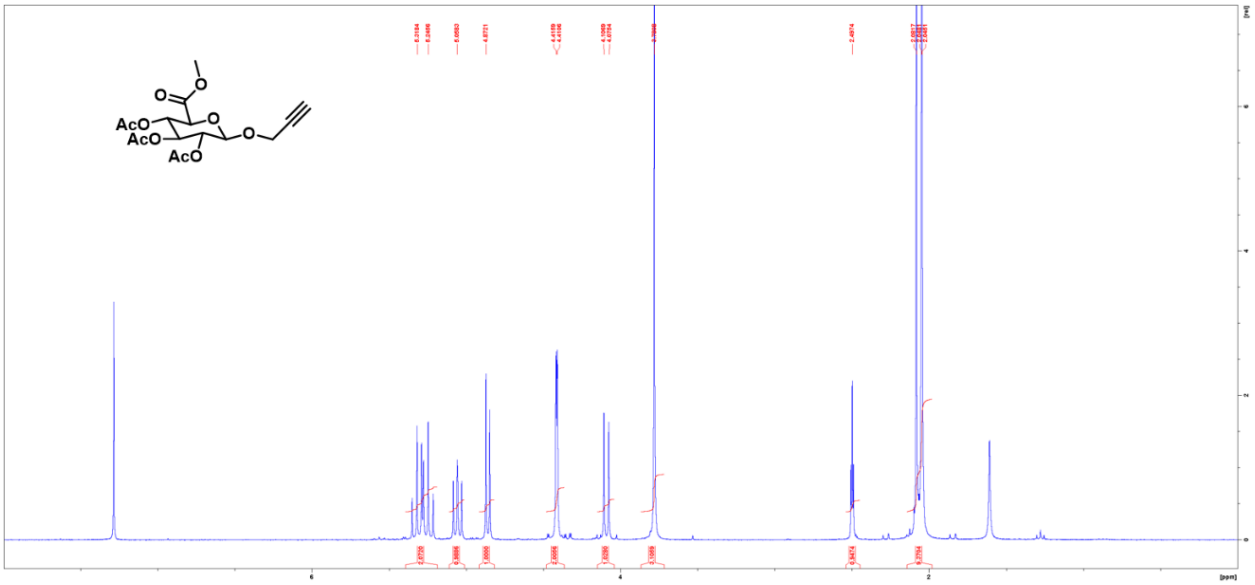
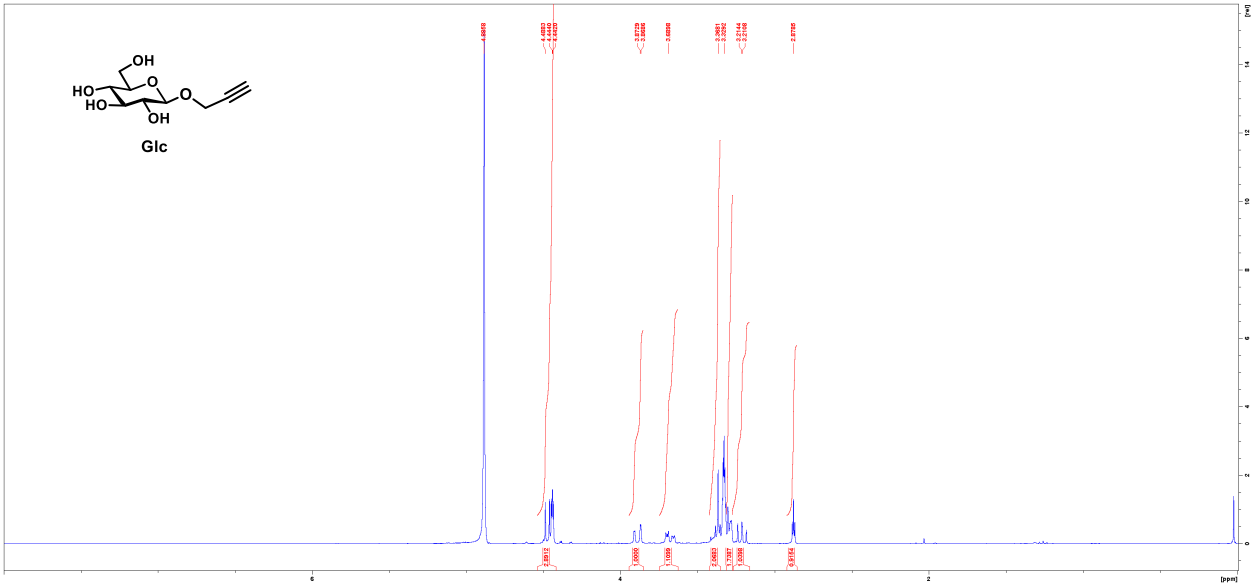
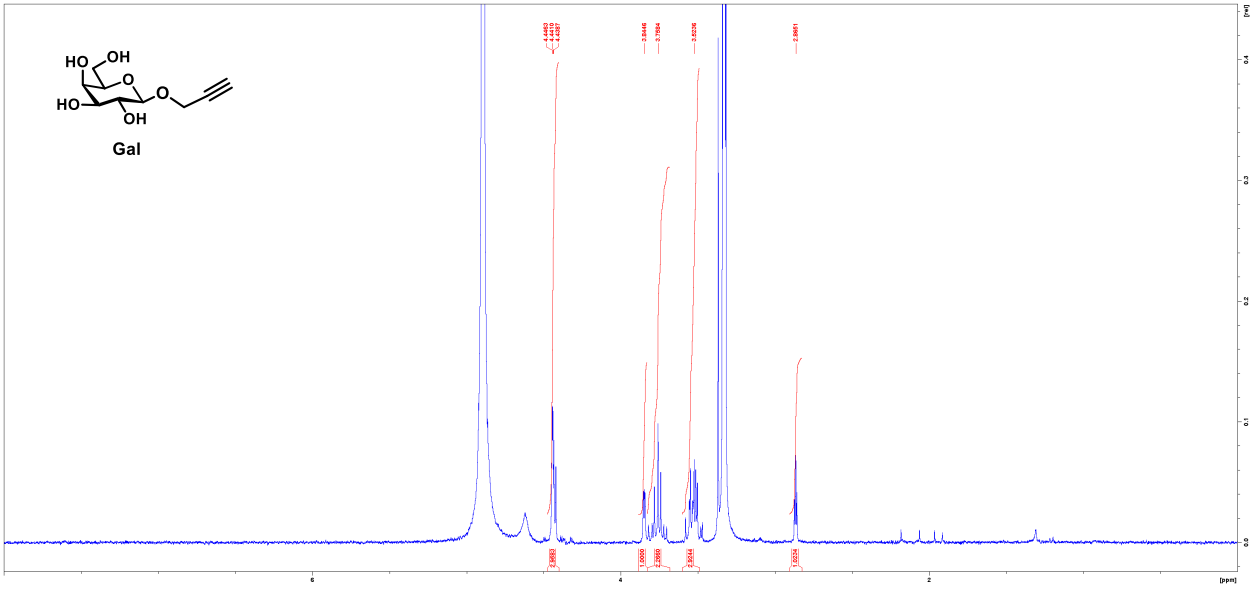
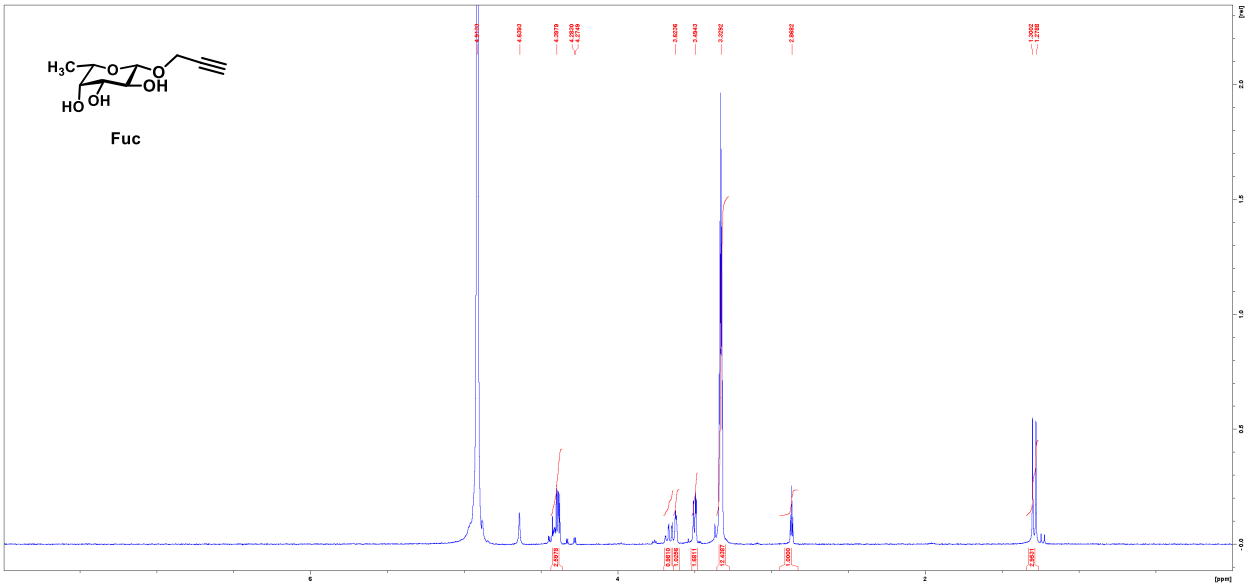
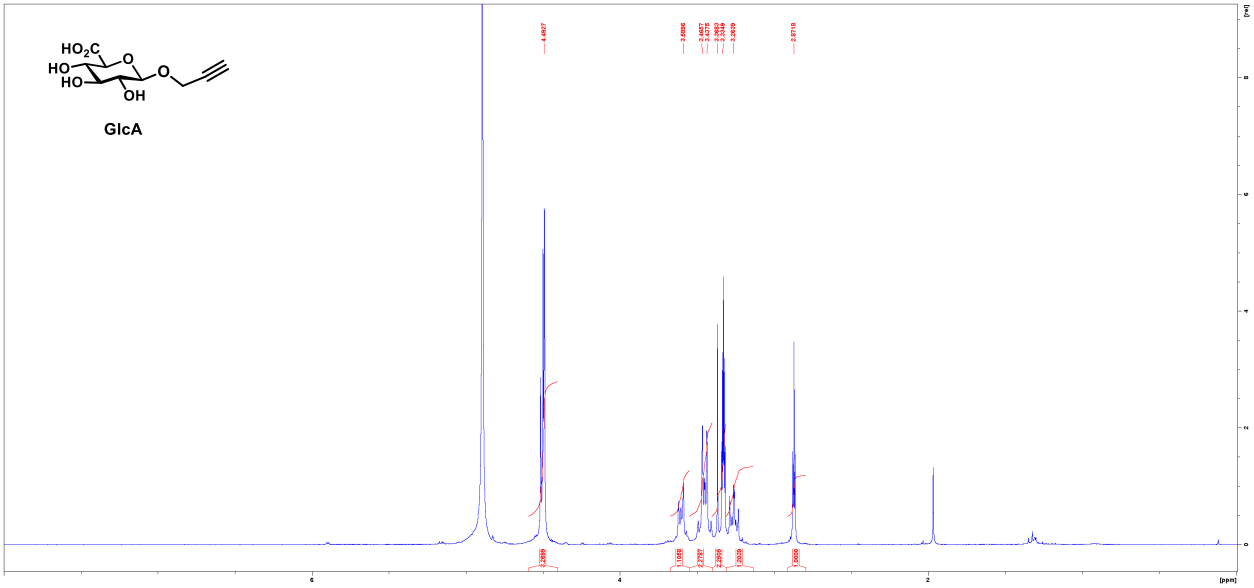

O
O
AcO
AcO
O
O
AcO

Figure S5. ^{1}H NMR (300 MHz, $CDCl_3$) of β-propargyl glycosides.






Fuc
[ppm]


GlcA
[ppm]

^{1}H NMR spectra of polymers 1, 3, 4, and 5.

Figure S6. ^{1}H NMR (300 MHz, D_2O) of pECH polymer **1**.

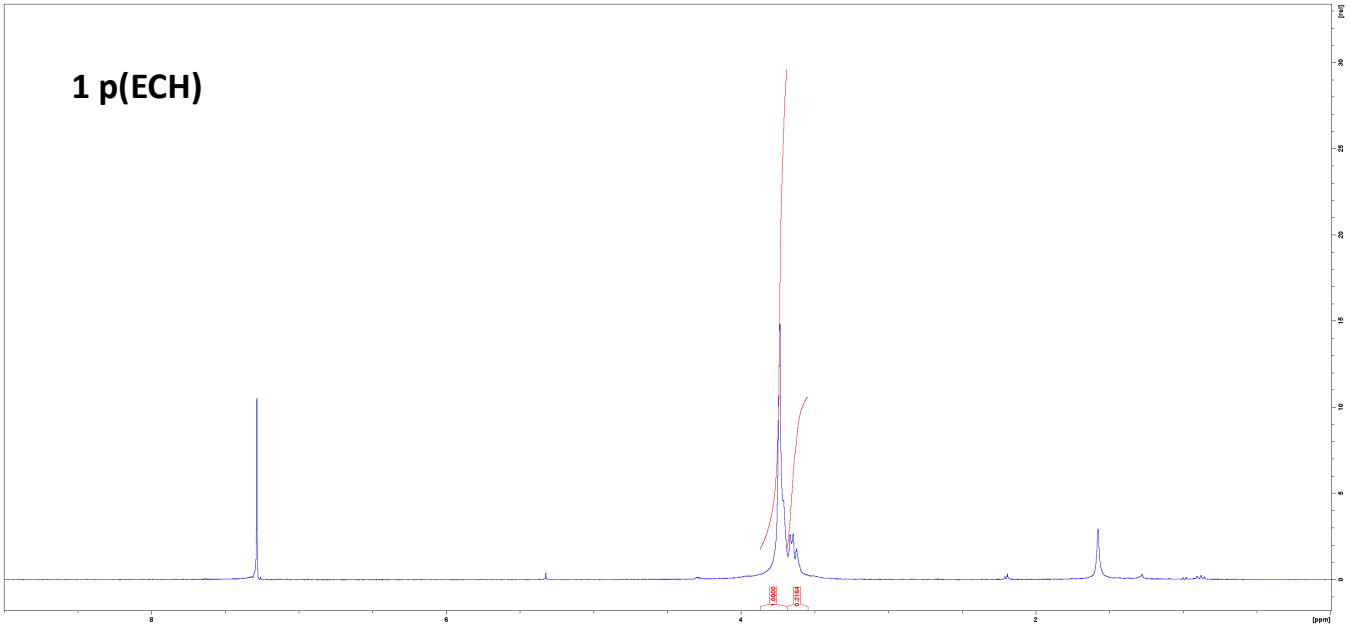


Figure S7. ^{1}H NMR (300 MHz, D_2O) of cholestanone-terminated pECH polymer **3**.

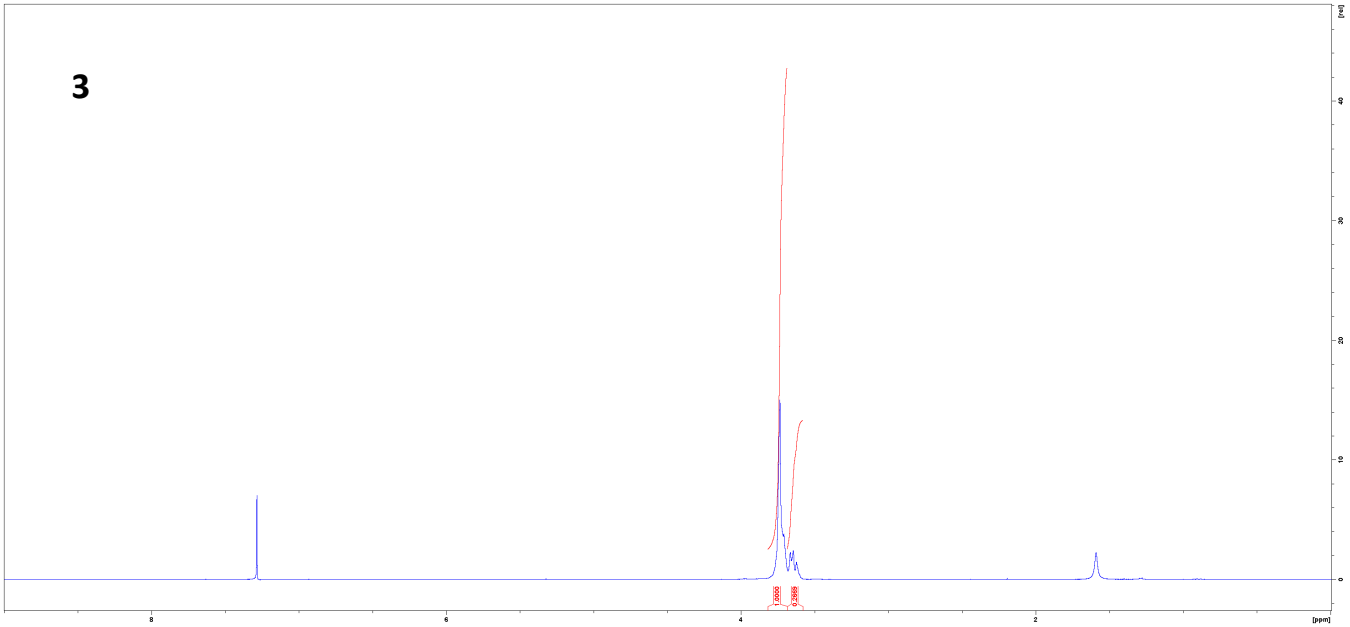


Figure S8. ^{1}H NMR (300 MHz, D_2O) of cholestanone-terminated pGA polymer **4**.

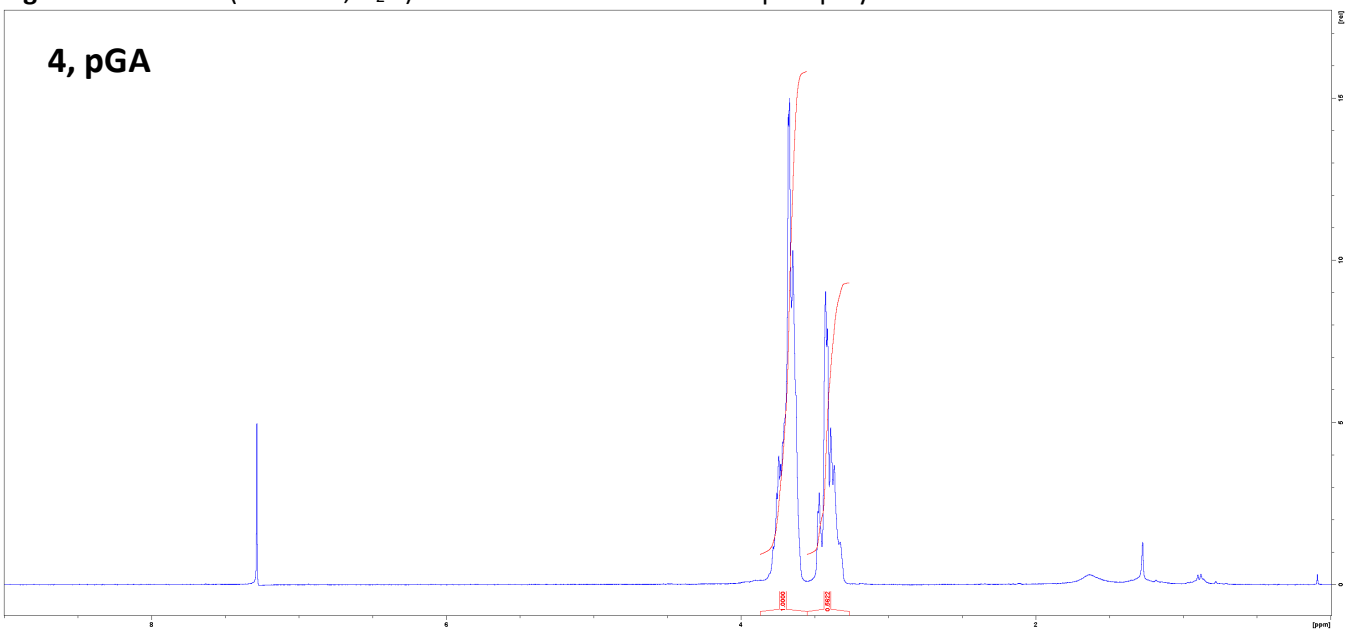


Figure S9. ^{1}H NMR (300 MHz, D_2O) of glucose glycopolymer **5-Glc**.

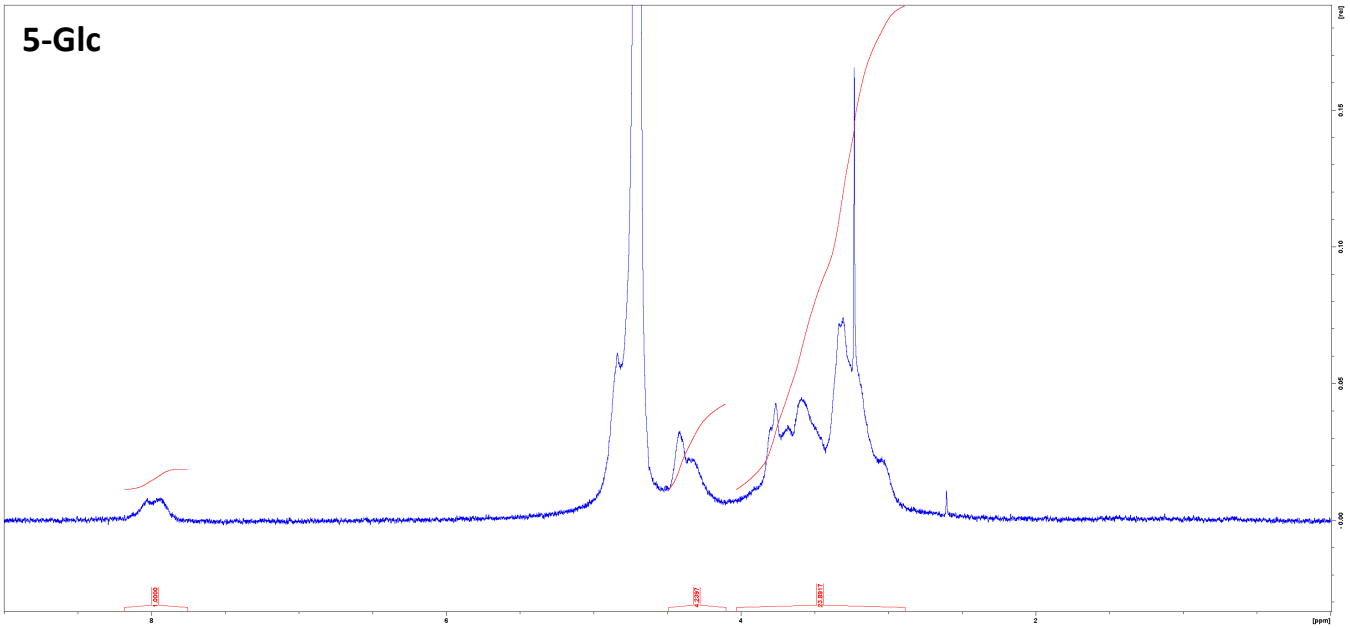


Figure S10. ^{1}H NMR (300 MHz, D_2O) of galactose glycopolymer **5-Gal**.

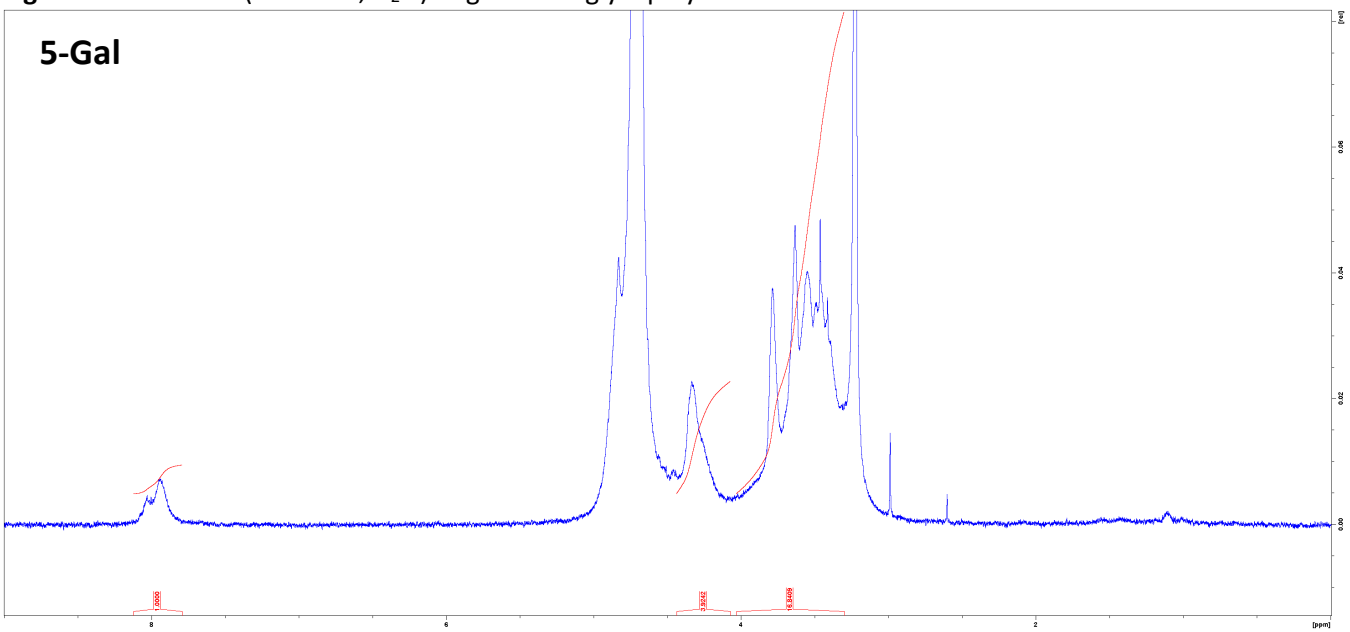


Figure S11. ^{1}H NMR (300 MHz, D_2O) of fucose glycopolymer **5-Fuc**.

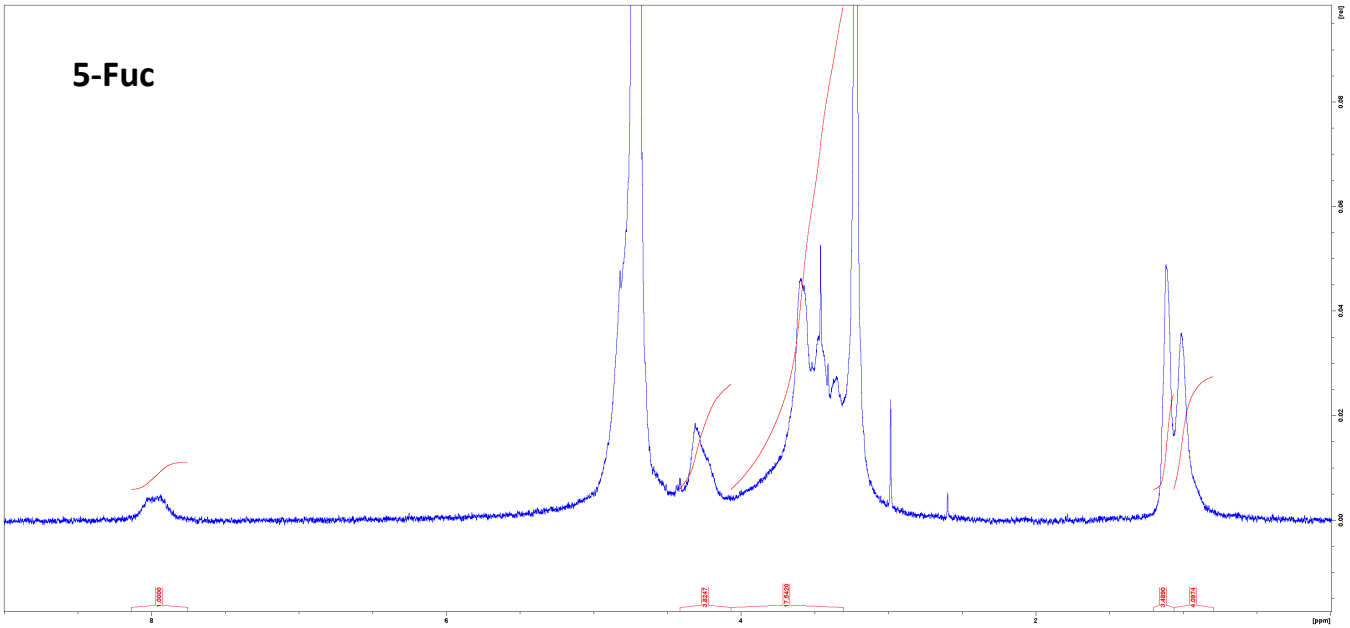


Figure S12. 1H NMR (300 MHz, D2O) of glucuronic acid glycopolymer **5-GlcA**.

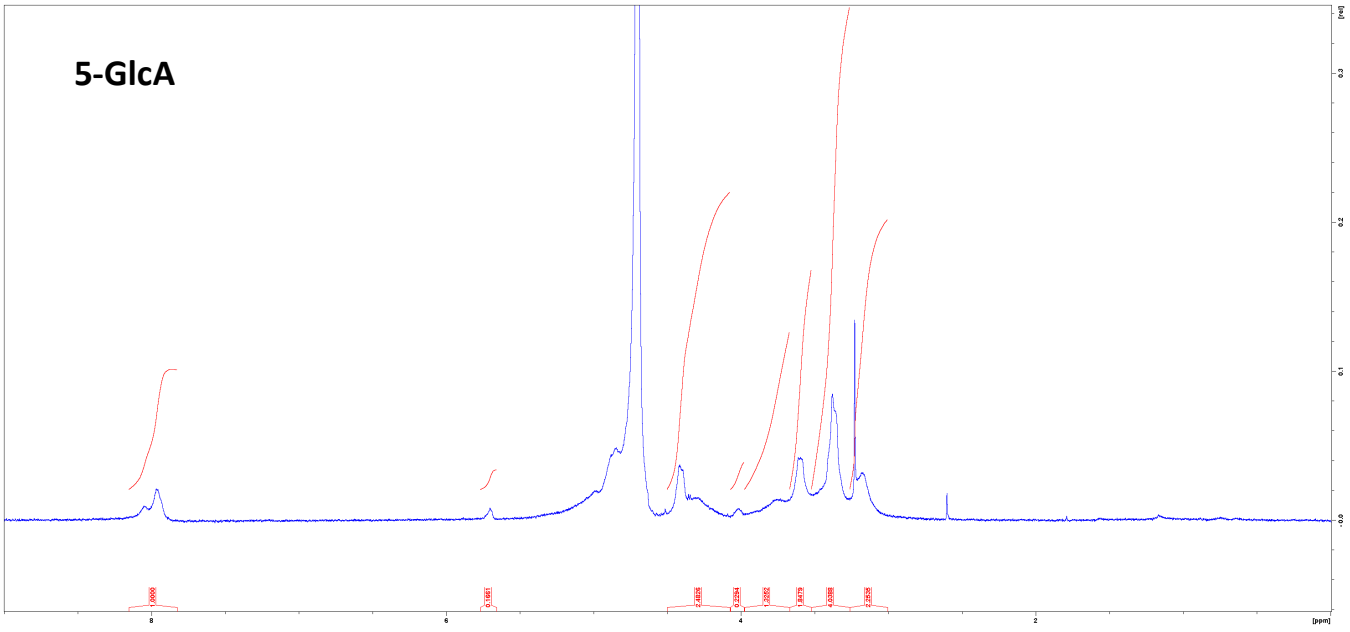
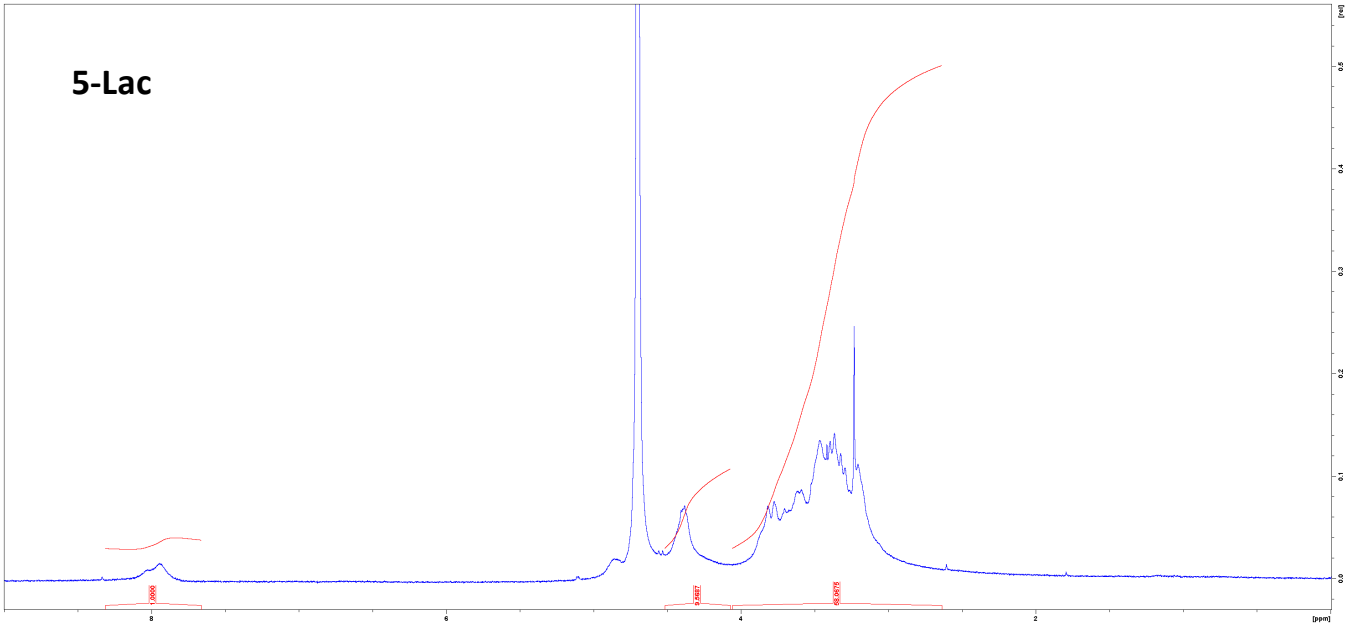


Figure S13. 1H NMR (300 MHz, D2O) of lactose glycopolymer **5-Lac**.



IR spectra of polymers 1, 3, 4, and 5.

Figure S15. IR spectra of pECH polymers **1** and **3**.

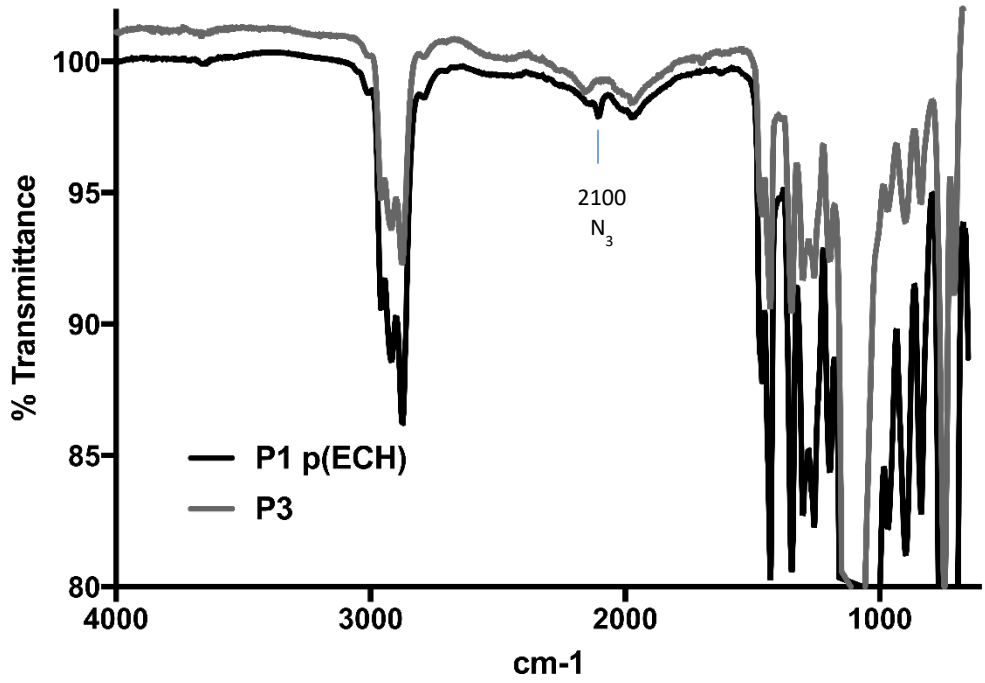


Figure S16. IR spectrum of pGA polymer **4**.

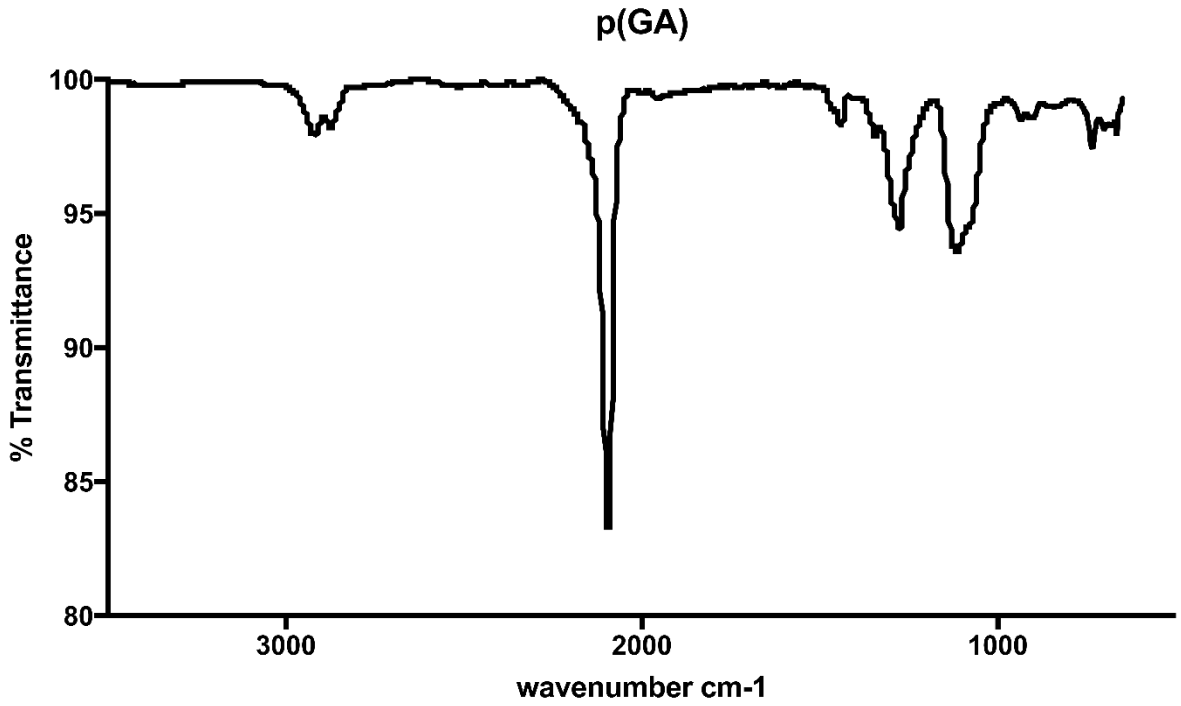
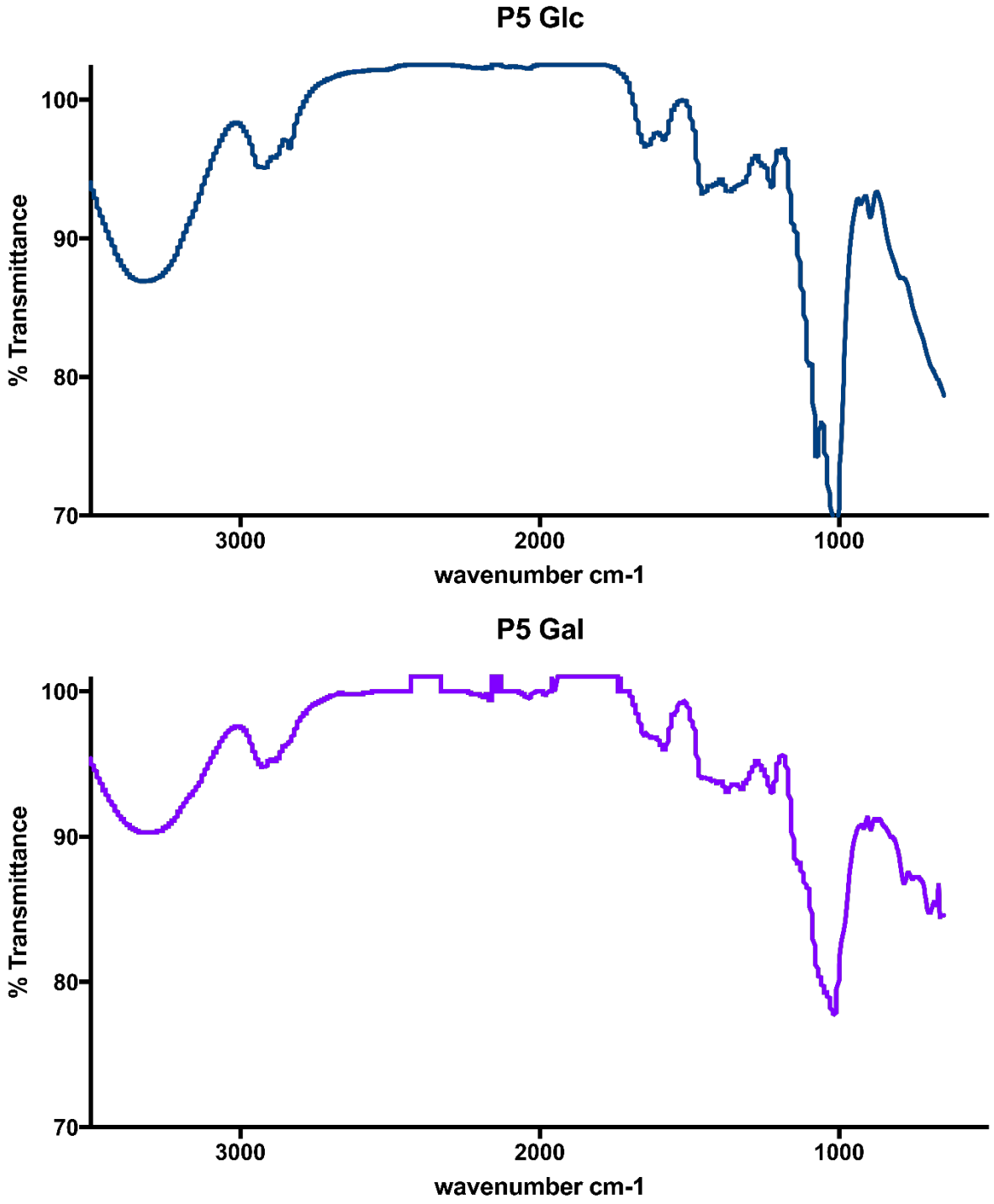
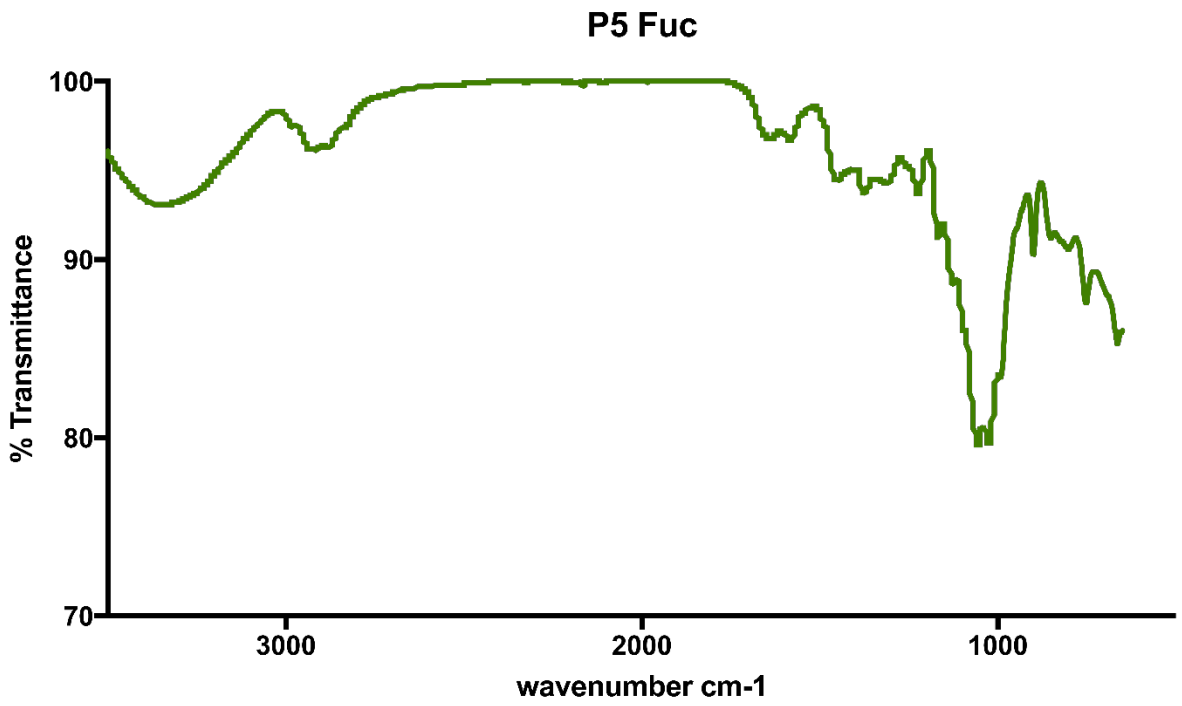
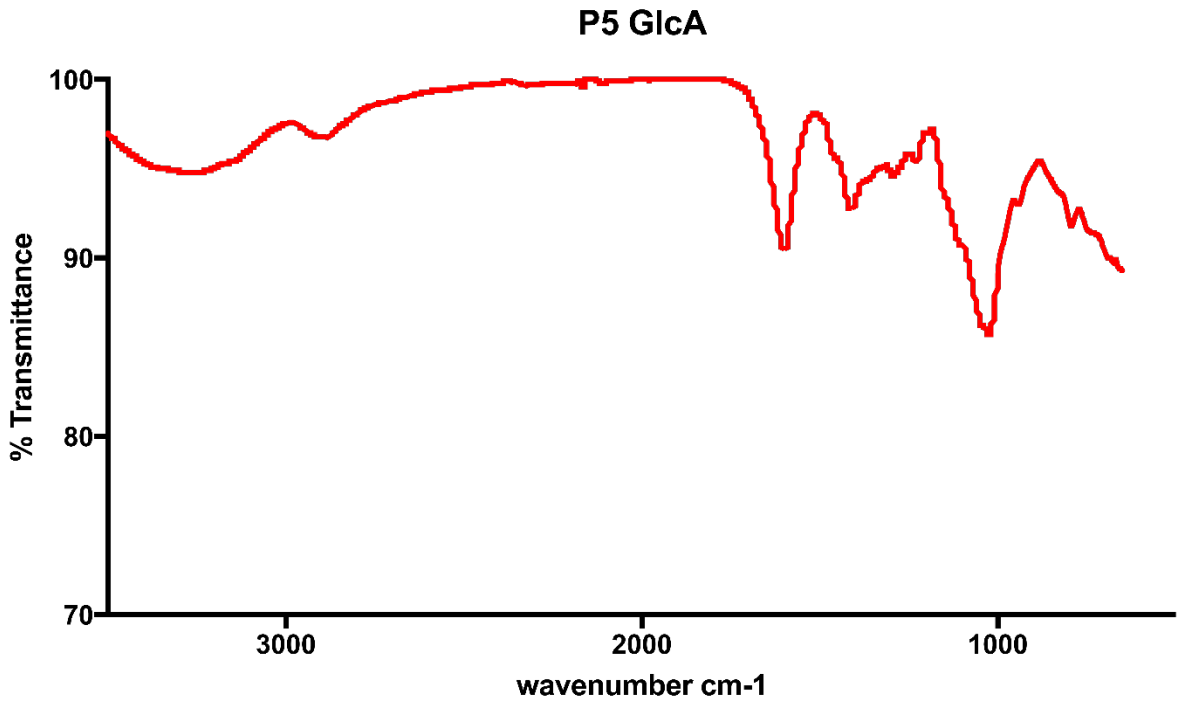


Figure S17. IR spectra of glycopolymers **5**.

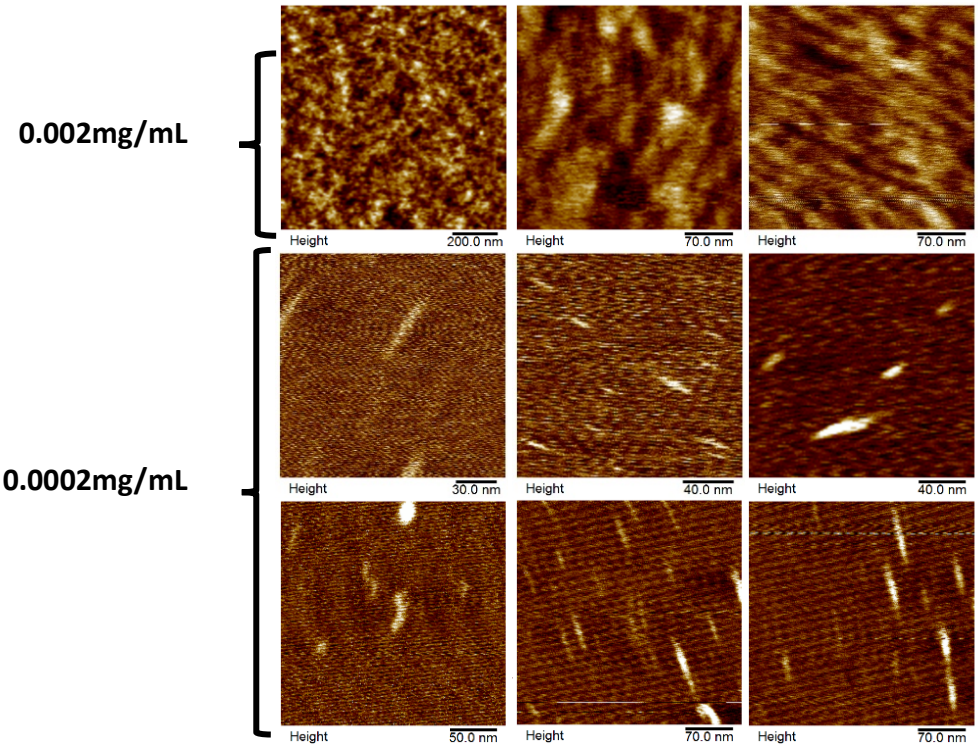



P5 Fuc
100
90
80
70
% Transmittance
3000
2000
1000
wavenumber cm-1


P5 GlcA
100
90
80
70
% Transmittance
3000
2000
1000
wavenumber cm-1

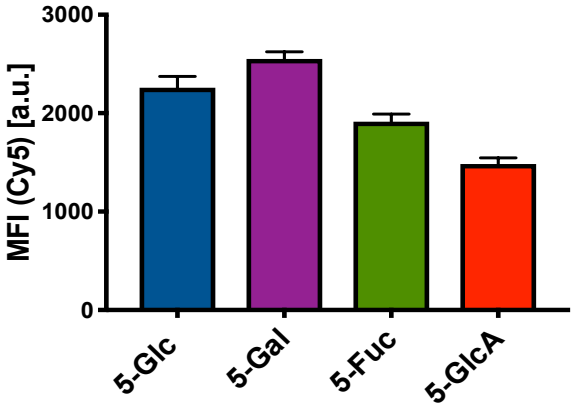
AFM analysis of lactose glycopolymer 5-Lac .

Figure S18. AFM images of **5-Lac** (expanded data for Figure 2D).



RBC remodeling with glycopolymers 5.

Figure S19. Relative levels of cell surface incorporation of glycopolymers **5** (2.5 μM).



polymer (2.5 μM)	
polymer	**relative surface incorporation (%)**
5-Glc	100
5-Gal	113
5-Fuc	85
5-GlcA	65

Figure S20. Relative incorporation of **P5** polymers vs equivalent polymer without cholestanone

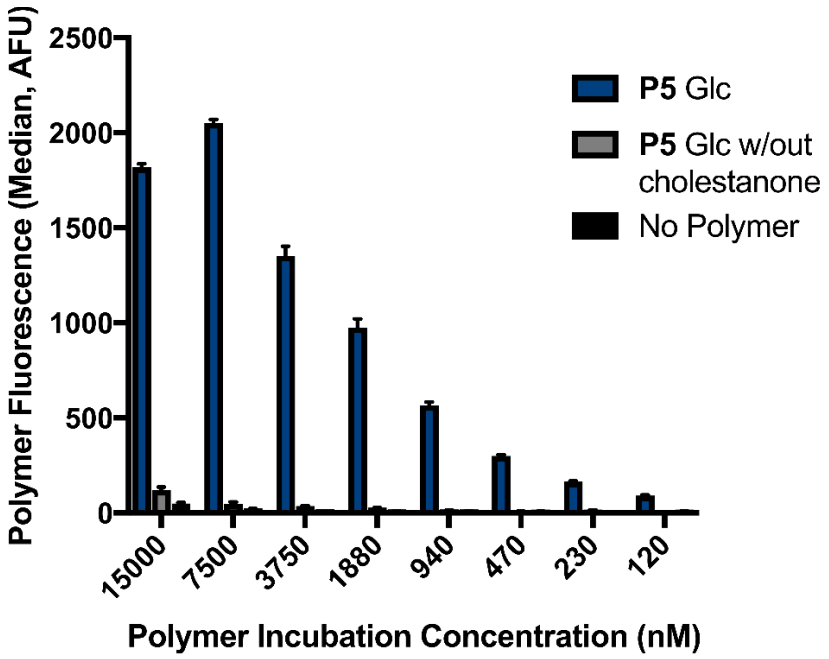


Figure S21. Bright field optical microscopy images of remodeled RBCs (associated with Figure 3C).

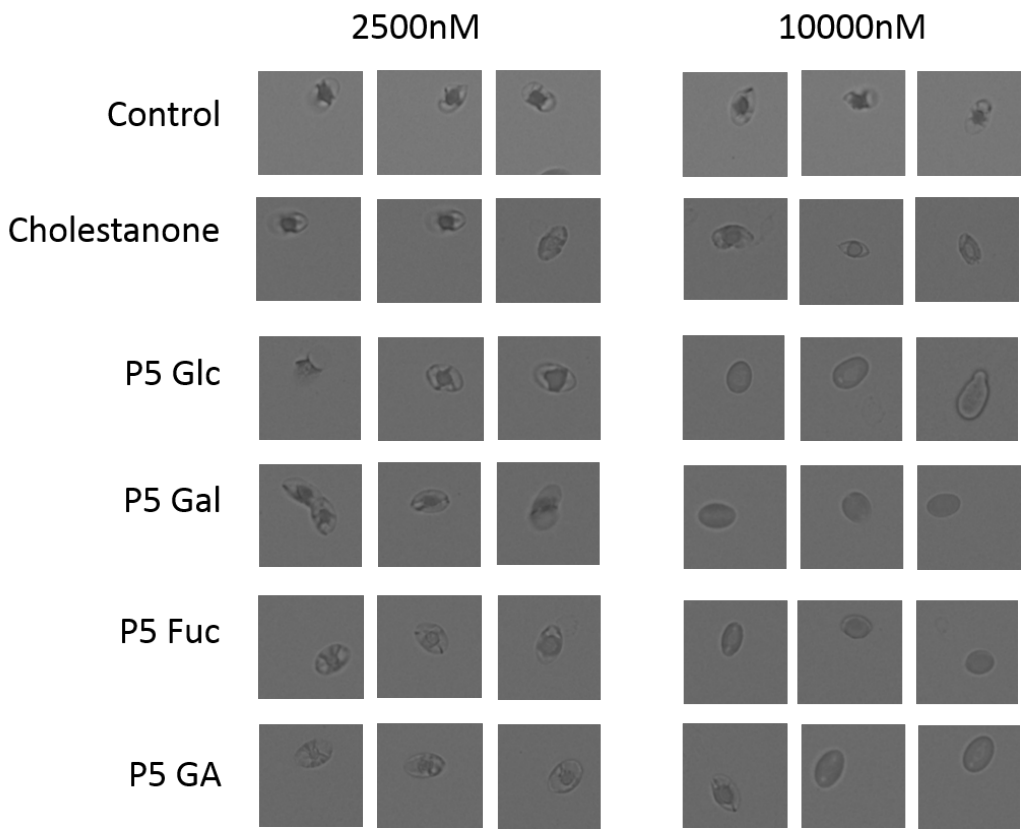
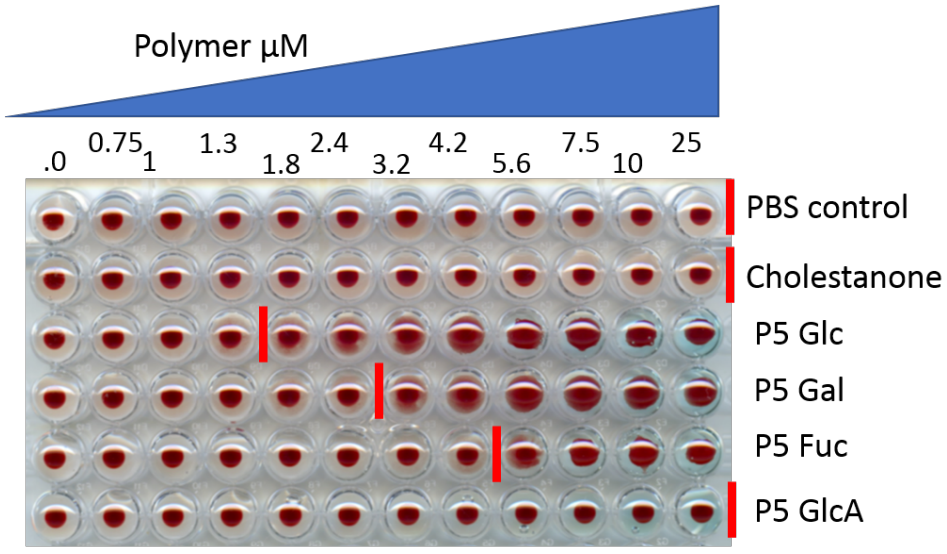


Figure S22. Sedimentation properties of RBCs remodeled with glycopolymers **5.**

*red line indicates point of settling inhibition.



Lectin agglutination of RBCs remodeled with glycopolymers 5.

Figure S23. ConA agglutination of RBCs treated with alkynyl cholestanone **2** or polymers **5** (2.5 μM).
*Red line indicates point of agglutination.

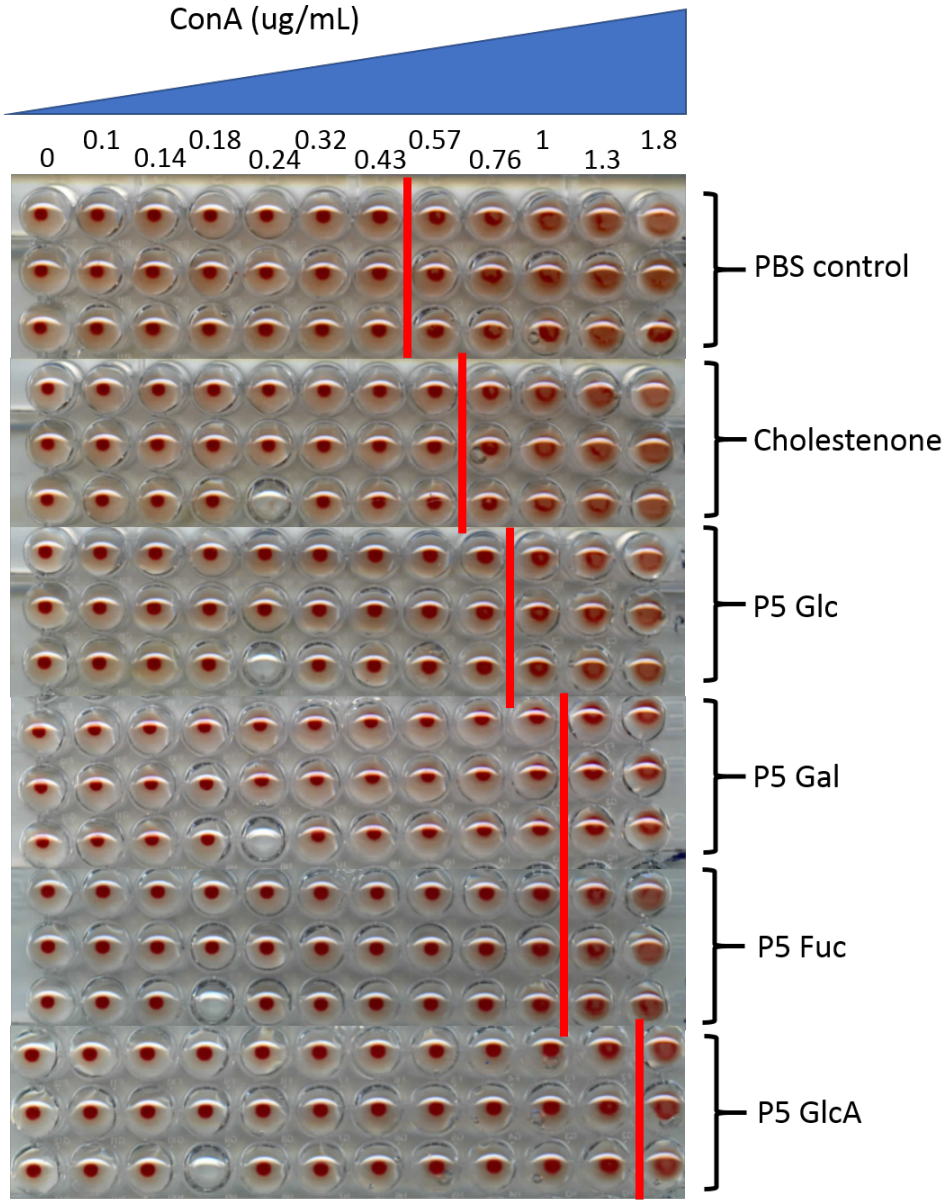
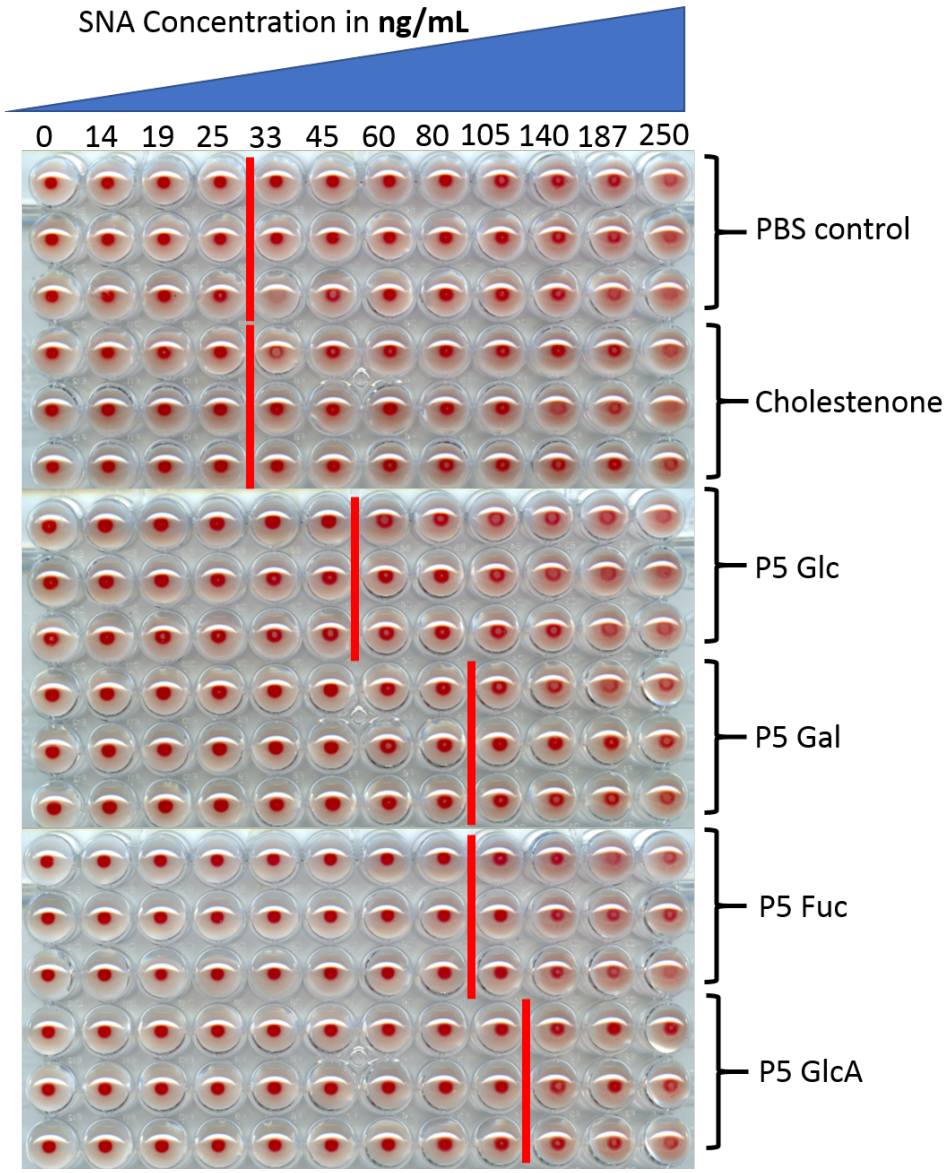


Figure S24. SNA agglutination of RBCs treated with alkynyl cholestanone **2** or polymers **5** (2.5 μM).
*Red line indicates point of agglutination.



Lectin binding to RBCs remodeled with glycopolymers 5 via flow cytometry.

Figure S25. Binding of ConA and SNA to RBCs with pre-treatment with alkynyl cholestanone **2** (associated with Figure 4).

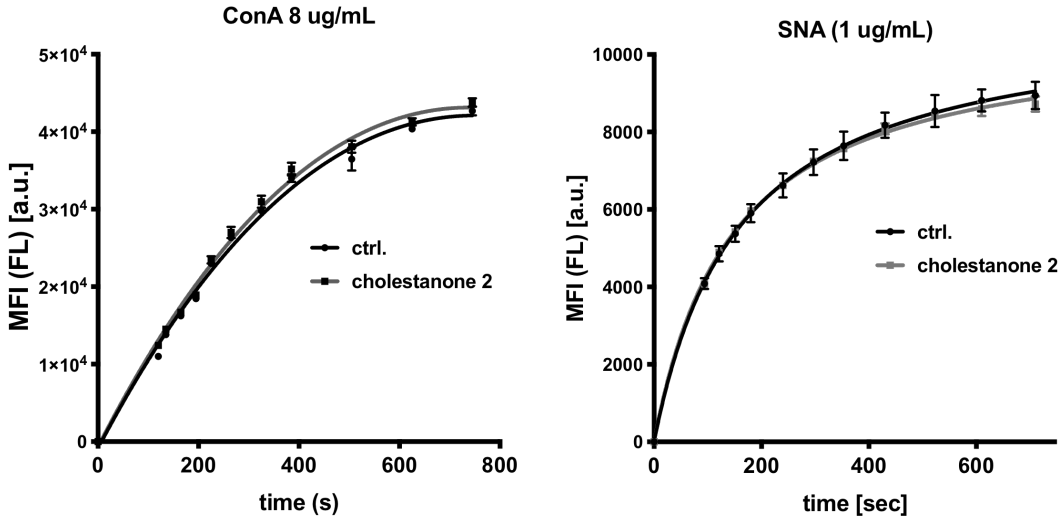
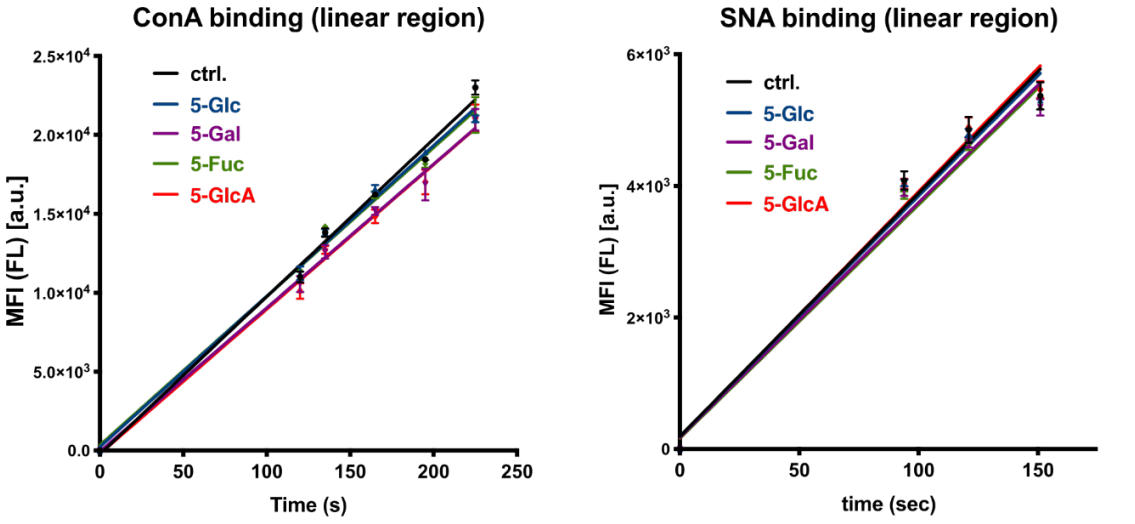


Figure S26. Initial rates of ConA and SNA binding to RBCs (associated with Figure 4).



ConA (8 μg/mL)			
polymer	**slope [s^{-1}]**	**R^2**	**P value (vs. ctrl)**
-/ctrl.	99.90 ± 2.781	0.9923	-
5-Glc	95.45 ± 2.395	0.9937	0.730 (ns)
5-Gal	91.07 ± 2.699	0.9913	0.0023 (**)
5-Fuc	94.29 ± 2.930	0.9904	0.0287 (*)
5-GlcA	91.65 ± 2.899	0.9901	0.0036 (**)

SNA (1 μg/mL)			
polymer	**slope [s^{-1}]**	**R^2**	**P value (vs. ctrl)**
-/ctrl.	36.98 ± 4.005	0.9771	-
5-Glc	36.56 ± 4.016	0.9764	0.9999 (ns)
5-Gal	35.69 ± 3.695	0.9790	0.9956 (ns)
5-Fuc	35.49 ± 3.448	0.9815	0.9916 (ns)
5-GlcA	37.49 ± 3.488	0.9830	0.9998 (ns)

References

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