Electronic Supplementary Material (ESI) for Polymer Chemistry

Supporting Information

of

Polymeric vesicle mimicking *glycocalyx* (PV-*Gx*) for studying carbohydrate-protein interactions in solution

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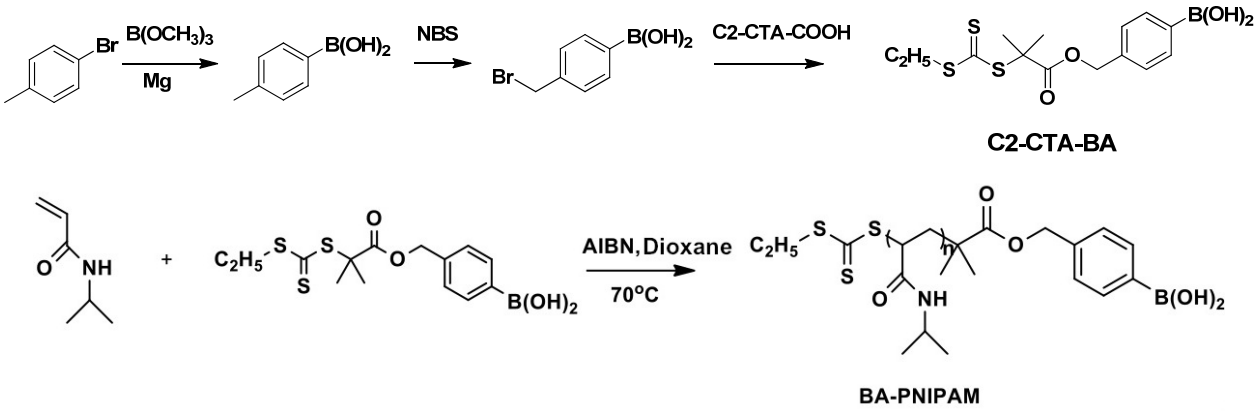
Materials. *N*-Isopropylacrylamide (NIPAM) purchased from Tokyo Kasei Kagyo Co. was recrystallized three times from benzene/hexane (65:35 v/v) prior to use. Azobisisobutyronitrile (AIBN, CP) supplied by Sinopharm Chemical Reagent Co., was recrystallized from ethanol before use. Concanavalin A (Con A), peanut agglutinin (PNA), Erythrina cristagalli (ECA) were purchased from Sigma-Aldrich or Shanghai Shrek Biotechnology Co., Ltd. DCM and DMSO were distilled before use. Unless specially mentioned, all other chemicals were used as received. The reactions were monitored and the R_f values were determined using analytical thin layer chromatography (TLC). The TLC plates were visualized by immersion into 5% sulfuric acid solution in ethanol followed by heating on a hot plate.

Characterization. ^{1}H NMR spectra were recorded with a JEOL ECA-400 spectrometer. Gel permeation chromatography (GPC) analysis was carried out with a Waters Breeze 1515 GPC analysis system with two PL mix-D column, using DMF with 0.5 M LiBr as eluents at the flow rate of 1 mL/min at 80°C and PEO calibration kit (purchased from TOSOH) as the calibration standard. GPC analysis using water with 0.02 M $NaNO_3$ as eluents was carried out with a Waters Breeze 1525 GPC analysis system with three TOSCH column, at the flow rate of 1.0 mL/min at 25°C and PEO calibration kit (purchased from TOSOH) as the calibration standard. Molecular weight of glycopolymer was determined by Wyatt MALLS detector with pre-measured dn/dC value. UV-vis spectroscopy was recorded in a conventional quartz cell (light path 10 mm) on a Perkin-Elmer Lambda 35 spectrophotometer. Dynamic and Static light scattering studies were conducted using ALV/5000E laser light scattering (LLS) spectrometers at scattering angles from 18° - 90°, CONTIN analysis was used for the extraction of R_h data. In this paper, we use the R_h result collected at 90°, because no obvious scattering angle dependence of R_h has been observed. Aqueous solution (pH 9) was used for characterizations, including fluorescence spectroscopy, dynamic light scattering and UV-vis spectroscopy, to ensure the dynamic covalent bond between boron and sugars. This pH value was obtained by adding aqueous solution of NaOH (1 M) dropwise. Samples were first dissolved in water (pH 9) at different concentrations, then were heated at a certain temperature until stable assembles were observed. Concentrated lectin solutions were first prepared and filtered through Millipore membrane (pore size 450 μm), than directly added into the vesicle solutions by syringe. The refractive index increments (dn/dC) of the protected glycopolymers (**PGlc**-P and **PGal**-P) were determined to be in DMF by a precise differential refractometer at 632.8 nm[1]. The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) measurement was performed using a Perspective Biosystem Voyager DE-STR MALDI-TOF MS (Applied Biosystems, Framingham, MA), using DHB as matrix in reflection mode. IR data were acquired from the NEXUS-470 by transmission mode and FLS data were obtained by FLS 920. TEM Transmission electron microscopy (TEM) images were taken with a JEOL 2011 microscope (Japan) operating at 200 kV. For the TEM

measurements, the aqueous solution of sample was dropped onto carbon film Cu grid and then stained with OsO_4 vapor after drying.



Scheme S1. Synthesis of RAFT CTA and polymerization for BA-PNIPAM.

Synthesis of chain transfer agents (CTA). C_2-CTA-CA[2] was synthesized according to procedures described in the literature. 4-bromomethylphenyboronic acid was synthesized via two steps according to the literature[3], and then reacted with C_2-CTA-CA to obtain the desired CTA (C_2-CTA-BA); their synthesis procedure can be found in our previous work[4].

^{1}H NMR of C_2-CTA-BA: (400 MHz, $CDCl_3$, ppm): δ 8.18 (d, 2H, C2***H***-Ar and C6***H***-Ar); 7.46 (d, 2H, C3***H***-Ar and C5***H***-Ar); 5.19 (s, 2H, Ar-C***H***$_2$OCO), 3.30 (q, 2H, CH_3-C***H***$_2$-S-C=S), 1.74 (s, 6H, -S-C(C***H***$_3$)$_2$-CO),1.34 (t, 3H, C***H***$_3$-CH_2-S-C=S).

RAFT polymerization for BA-PNIPAM.

NIPAM (1 g, 8.85 mmol, 100 equiv.), C_2-CTA-BA (0.089 mmol, 1.0 equiv.), AIBN (0.018 mmol, 0.2 equiv.) and 3 mL 1,4-dioxane were sealed in a flask equipped with a magnetic stir bar, followed by three freeze-thaw cycles. The reaction flask filled with argon was placed in a preheated oil bath at 70°C. The polymerization was online monitored by GPC-MALLS and quenched by removing the reaction flask from heat followed by cooling in liquid nitrogen immediately. The polymer was precipitated into cold ethyl ether, filtrated and then dissolved in THF and precipitated again. The procedure was repeated for three times and the polymer was obtained as yellow powder after drying under vacuum at room temperature for 12 h. GPC exhibited satisfactory PDI (=1.10). The polymer was also characterized by ^{1}H NMR.

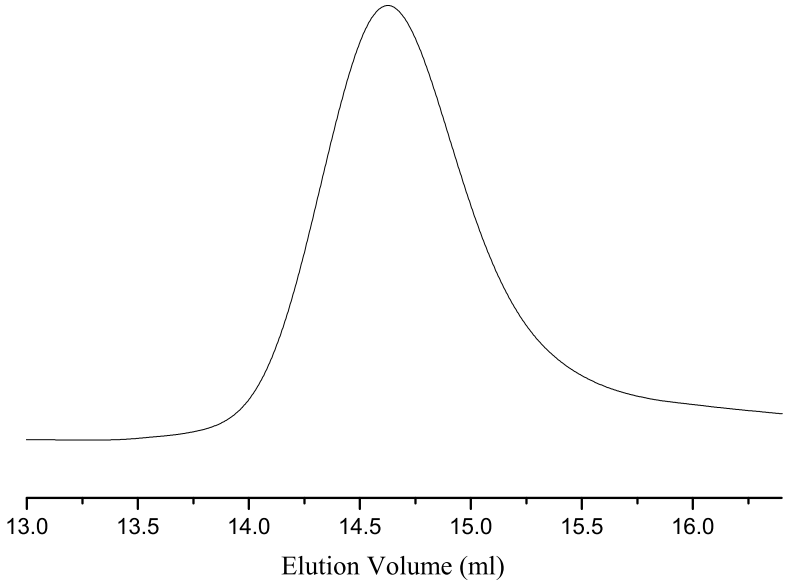
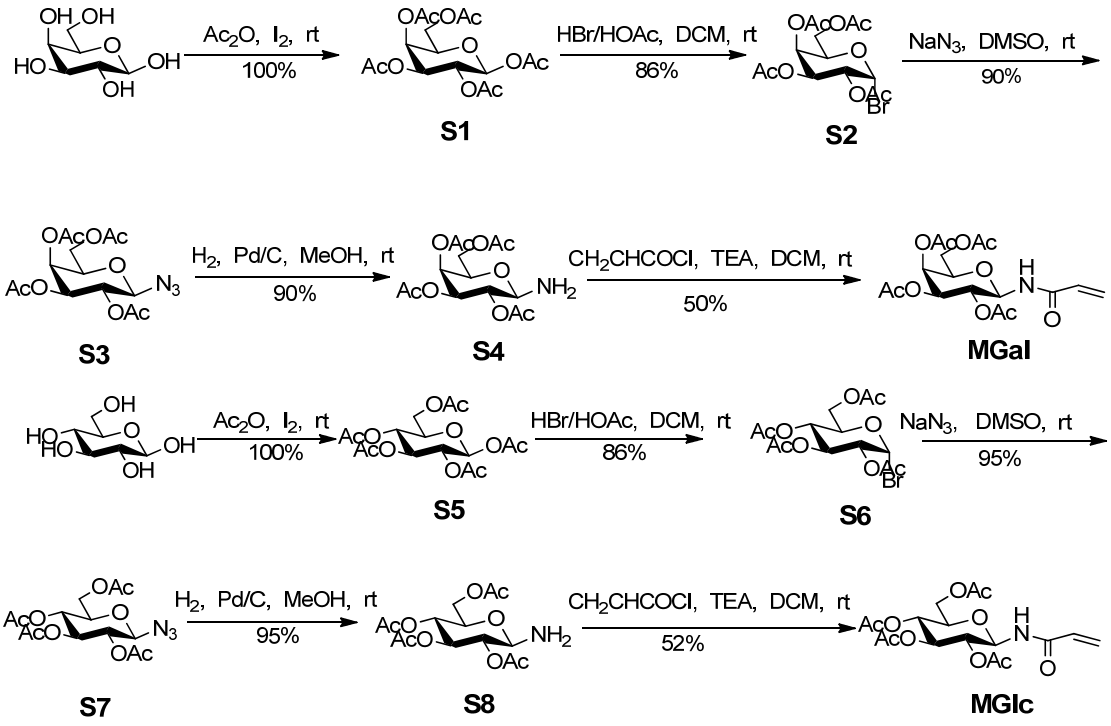


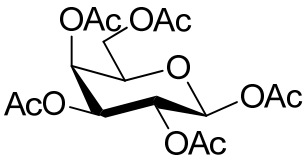
Figure S1. GPC of **BA-PNIPAM** using DMF as eluent ($M_w = 7\times10^3$ (calculated from ^{1}H NMR), PDI =1.10).

Synthesis of the monomers for glycopolymers



Scheme S2. Synthetic routes of sugar monomer MGal and MGlc.

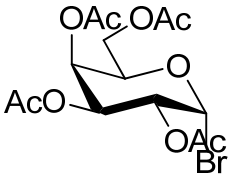
Pentaacetyl-β-D-galactopyranose (S1)



S1

The compound was synthesized following procedures in literature[5]. Briefly, galactose (10.00 g, 55.6 mmol) and iodine (0.22 g, 0.87 mmol) were added to acetic anhydride (100 mL, 1.06 mol). The reaction mixture was stirred at room temperature until the solid was totally dissolved and then extracted with CH_2Cl_2 three times. The CH_2Cl_2 solution was washed with aqueous saturated Na_2SO_3 followed by saturated $NaHCO_3$. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated.

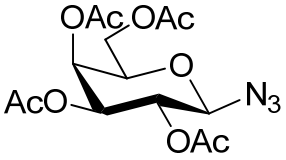
2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl bromide (S2)



S2

The compound was synthesized following procedures in literature[6]. HBr (33 % in HOAc, 25 mL) was added into the CH_2Cl_2 solution (60 mL) of pentaacetyl-β-D-galactopyranose **S1** (9.00 g, 21.2 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 2 h, TLC (ethyl acetate/hexane 1:2) indicated the formation of a product with complete consumption of the starting material. The reaction mixture was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), and the aqueous layer was re-extracted with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was evaporated, and then the obtained solid was purified by chromatography (ethyl acetate/hexanes; 1:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.69 (d, 1H, C**H**•C-1), 5.52 (d, 1H, C**H**•C-3), 5.40 (dd, 1H, C**H**•C-5), 5.04 (dd, 1H, C**H**•C2), 4.48 (t, 1H, C**H**•C-4), 4.08-4.21 (m, 2H, C**H**$_2$•C-6), 2.04-2.14 (s, 12H, C**H**$_3$CO)

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide (S3)

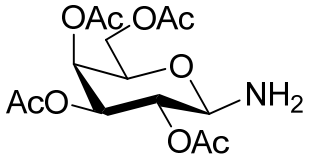


(S3)

The compound was synthesized following procedures reported in literature[7]. Sodium azide (1.94 g, 29.3 mmol) was added to the solution of 2,3,4,6-Tetra-*O*-acetyl-β-glactopyranosyl bromide **S2** (8.40 g, 19.9

mmol) in dry DMSO (50 mL). The reaction mixture was stirred at room temperature for 10 min. The solution was diluted with water (100 mL) and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.37 (d, 1H, C**H**•C-3), 5.10 (t, 1H, C**H**•C-2), 4.99 (dd, 1H, C**H**•C-4), 4.55-4.58 (d, 1H, C**H**-N_3•C-1), 4.11-4.13 (dd, 2H, C$\mathbf{H_2}$•C-6), 3.97-4.00 (m, 1H, C**H**•C-5), 1.94-2.12 (s, 12H, C$\mathbf{H_3}$CO).

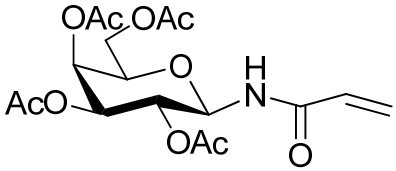
2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl amine (S4)



S4

The compound was synthesized following procedures reported in literature[6]. Palladium on carbon hydrogenation reagent (25%, 2 g) was added to the solution of 2,3,4,6-Tetra-*O*-acetyl-β-galactopyranosyl azide **S3** (6.30 g, 16.4 mmol) in dry CH_3OH (100 mL). The reaction mixture was stirred at room temperature under H_2 atmosphere for several hours until TLC indicated the complete consumption of the starting material. The solid was filtered over celite, and the solvent was evaporated. The crude product was purified by chromatography (ethyl acetate/hexanes; 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.35 (d, 1H, C**H**•C-1), 4.97-5.00 (m, 2H, C**H**•C-2, C**H**· C3), 4.04-4.10 (m, 2H, C**H**•C-5, C**H**•C-6), 3.(d, 1H, C**H**•C-4), 1.94-2.11 (s, 12H, C$\mathbf{H_3}$CO)

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl Acrylamide (MGal)

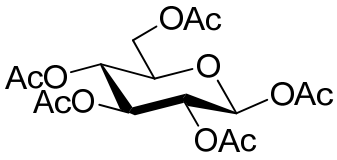


To the solution of 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl amine **S4** (5.00 g, 13.9 mmol, 1 equiv.) in CH_2Cl_2 (100 mL) with Et_3N (5.8 mL, 41.7 mmol, 3.0 equiv.) at 0 °C, acryloyl chloride (2.1 mL, 27.8 mmol, 2.0 equiv.) was added dropwise. The mixture was stirred at room temperature for 2 h, when TLC indicated the complete conversion of starting material. The reaction mixture was quenched with saturated $NaHCO_3$, and the layers were separated. The organic layer was dried over $MgSO_4$, filtered and evaporated. The product was purified by chromatography (ethyl acetate/hexanes; 1:1).

^{1}H NMR (400MHz, $CDCl_3$): δ 6.50-6.53 (d, 1H, C$\mathbf{H_2}$CHCO), 6.26-6.30 (d, 1H, C$\mathbf{H_2}$CHCO), 6.04 (dd, 1H, CH_2C**H**CO), 5.70 (d, 1H, C**H**·C-1), 5.43 (d, 1H, C**H**·C-3), 5.29 (dd, 1H, CH•C-4), 5.13 (d, 2H, C$\mathbf{H_2}$•C-6), 4.05-4.13 (m, 3H, C**H**•C-2, C**H**·C-5, CHN**H**), 2.0 (s, 12H, C$\mathbf{H_3}$CO); ^{13}C NMR (400Hz, $CDCl_3$): δ 170.3-171.7 (4C, CH_3**C**O), 165.6 (1C, **C**O**C**HCH_2), 130.3 (1C, CO**C**HCH_2), 128.7 (1C,

COCH**C**H_2), 78.9(1C, C-1), 67.4-72.6 (4C, C-2~C-5), 61.4 (1C, C-6), 20.8 (4C, **C**H_3CO). Maldi-TOF MS (M+Na^+): 424.0778 (Found), 424.1214 (Calculated).

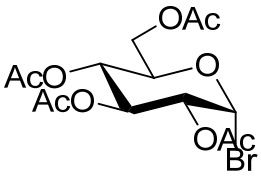
Pentaacetyl-β-D-glucopyranose (S5)



S5

Similar procedure as S1 was employed.

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl bromide (S6)

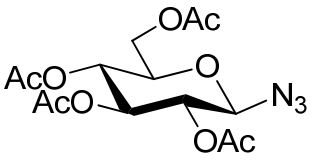


S6

Similar procedure as S2 was employed.

^{1}H NMR (400MHz, $CDCl_3$): δ 6.62 (d, 1H, C**H**•C-1), 5.56 (t, 1H, C**H**•C-3), 5.17 (t, 1H, C**H**•C-2), 4.85 (dd, 1H, C**H**-C5), 4.32 (m, 2H, C$\mathbf{H}_2$•C-6), 4.14 (d, 1H, C**H**•C-4), 2.04-2.11 (s, 12H, C$\mathbf{H}_3$CO)

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (S7)

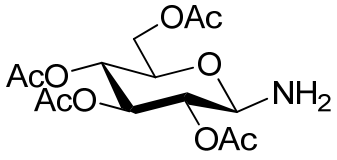


S7

Similar procedure as S3 was employed.

^{1}H NMR (400MHz, $CDCl_3$): δ 5.219 (t, 1H, C**H**•C-3), 5.11 (t, 1H, C**H**•C-2), 4.96 (t, 1H, C**H**•C-4), 4.64-4.66 (d, 1H, C**H**-N_3•C-1), 4.15-4.18, 4.25-4.30 (dd, 2H, C$\mathbf{H}_2$•C-6), 3.79-3.80 (m, 1H, C**H**•C-5), 2.01-2.10 (s, 12H, C$\mathbf{H}_3$CO)

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl amine (S8)

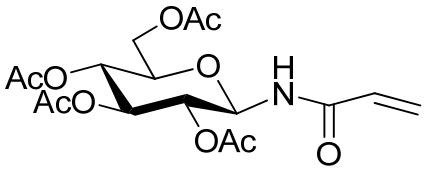


S8

Similar procedure as S4 was employed.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (t, 1H, C**H**·C-3), 5.03 (t, 1H, C**H**·C-2), 4.82 (t, 1H, C**H**·C-4), 4.18-4.24 (m, 2H, C**H$_2$**·C-6), 4.08-4.11 (dd, 1H, C**H**NH$_2$·C-1), 3.66-3.70 (m, C**H**·C-5), 2.00-2.09 (s, 12H, C**H$_3$**CO)

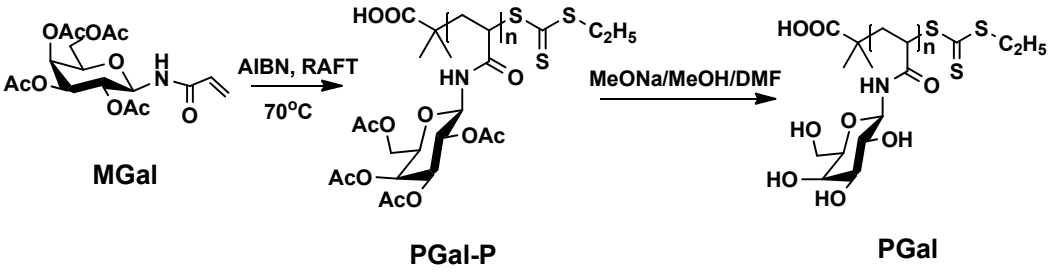
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Acrylamide (MGlc)



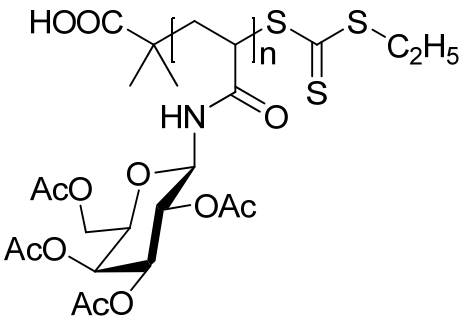
Similar procedure as **MGal** was employed.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.64-6.66 (d, 1H, C**H**NH·C-1), 6.24,6.29 (d, 1H, C**H$_2$**CHCO), 6.04 (dd, 1H, C**H**·C-2), 5.70 (d, 1H, **CH**·C1), 5.29 (dd, 2H, C**H$_2$**·C-6), 5.03 (t, 1H, C**H**·C-3), 4.92 (t, 1H, C**H**·C-4), 4.27 (dd, 1H, CHN**H**), 4.05 (m, 1H, C**H**·C2), 3.82 (m, 1H, C**H**·C-5), 2.0 (s, 12H, C**H$_3$**CO); ^{13}C NMR (400Hz, $CDCl_3$): δ 170.3-171.7 (4C, CH_3**C**O), 165.6 (1C, **C**OCHCH$_2$), 130.3(1C, CO**C**HCH$_2$), 128.7 (1C, COCH**C**H$_2$), 78.9 (1C, C-1), 67.4-72.6 (4C, C-2—C-5), 61.4 (1C, C-6), 20.8 (4C, **C**H$_3$CO).

Maldi-TOF MS (M+Na$^+$): 424.2233 (Found), 424.1214 (Calculated)



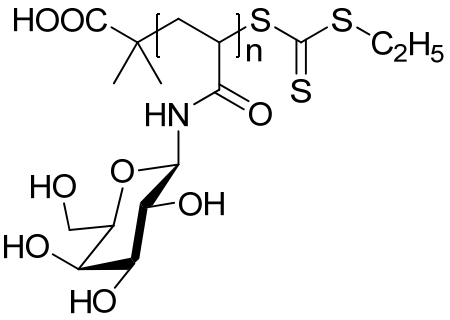
Poly(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl acrylamide) (PGal-P)



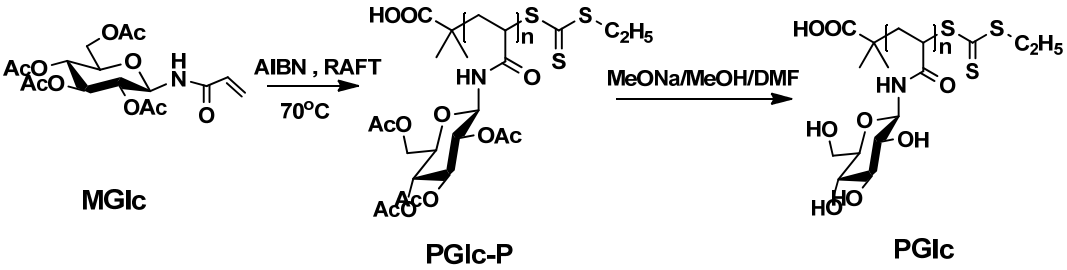
To a solution of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl acrylamide (2 g, 5 mmol, 2.0 equiv.) in 1,4-dioxane water was added S-ethyl-S'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (CTA, 18.2 mg, 0.05 mmol, 1.0 eqv.) and AIBN (4.1 g, 0.025 mmol, 0.2 equiv.). The solution was degassed by three freeze-pump-thaw cycles and purged with Ar before sealing. The solution was stirred at 65°C for several hours and online monitored by GPC-MALLS until the conversion rate was over 70%.The polymerization was quenched by removing the reaction flask from heat followed by cooling in liquid nitrogen immediately.

The reaction mixture was concentrated in vacuum and precipitated into cold diethyl ether, filtrated and then dissolved in THF and precipitated again. The procedure was repeated for three times and the polymer was obtained as lemon yellow powder after drying under vacuum at room temperature for 12 h.

Poly(β-D-galactopyranosyl acrylamide) (PGal)

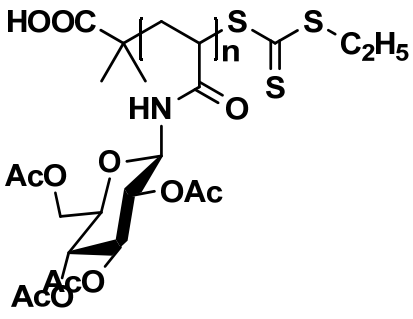


Sodium methoxide (30% in methanol, 30 mL) was added to a solution of poly(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl) (1.4 g) in DMF (100 mL). The reaction mixture was stirred at room temperature for 2 h, and then the mixture was concentrated in vacuum. The product was dissolved in water and precipitated into methanol. The procedure was repeated for three times and the polymer was obtained as lemon yellow powder after drying under vacuum at room temperature for 12 h (Yield: 70%). GPC exhibited satisfactory PDI =1.20. The polymer was also characterized by ^{1}H NMR.



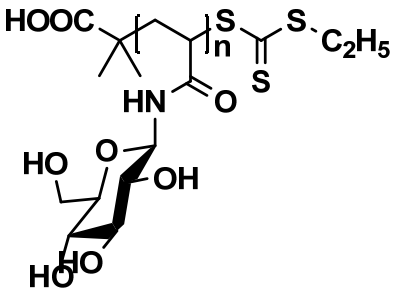
Poly(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Acrylamide) (PGlc-P)

The procedure is similar to **PGal**-P



Poly(β-D-glucopyranosyl Acrylamide) (PGlc)

The procedure is similar to **PGal**.



GPC exhibited satisfactory PDI (=1.18) for the final polymer and was also characterized by ^{1}H NMR.

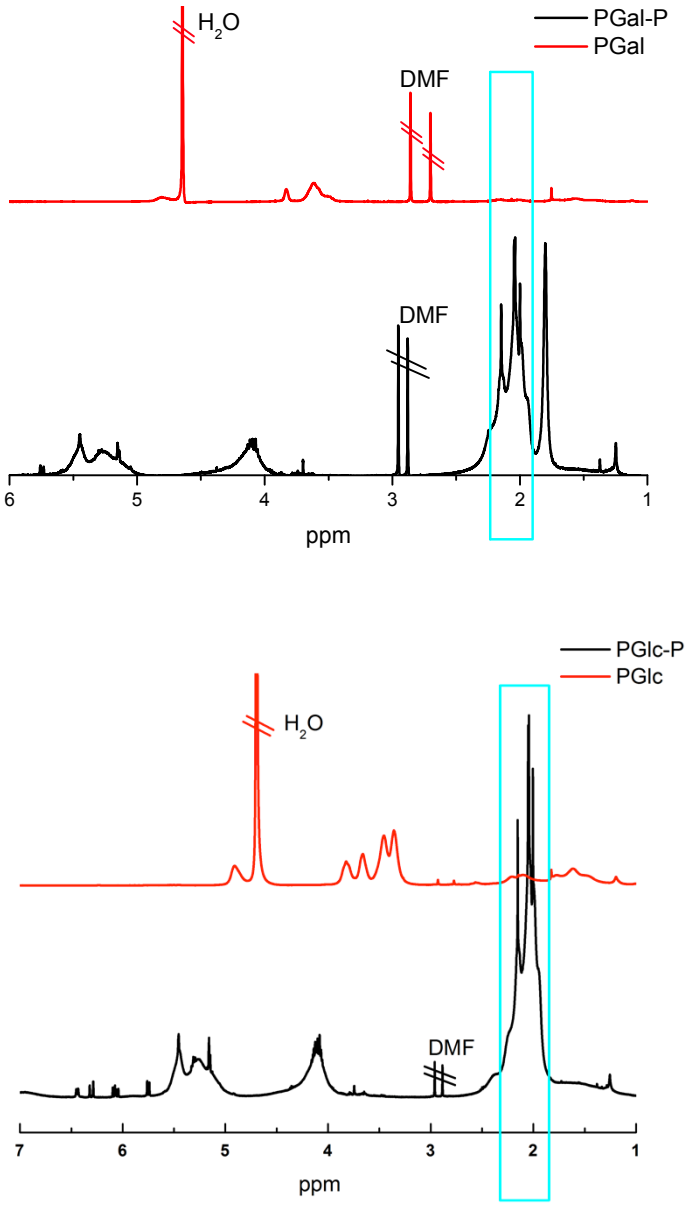


Figure S2. ^{1}H NMR of **PGal** and **PGlc** before and after acetate removal.

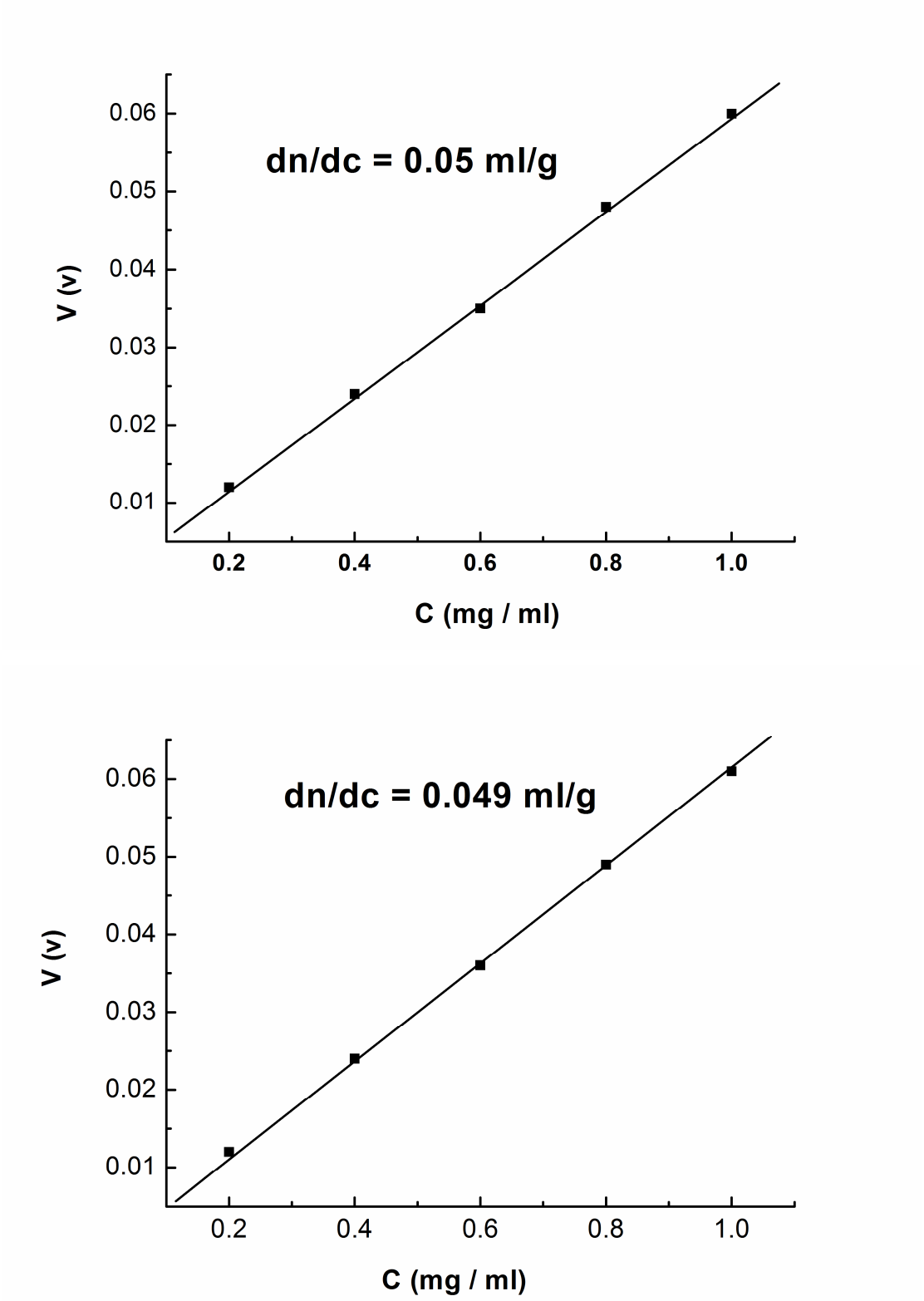


Figure S3. dn/dC results for M_w calculation in DMF by MALLS (up: **PGlc**-P, down: **PGal**-P).

$dn/dV=9.02\times10^{-4}\ v^{-1}$

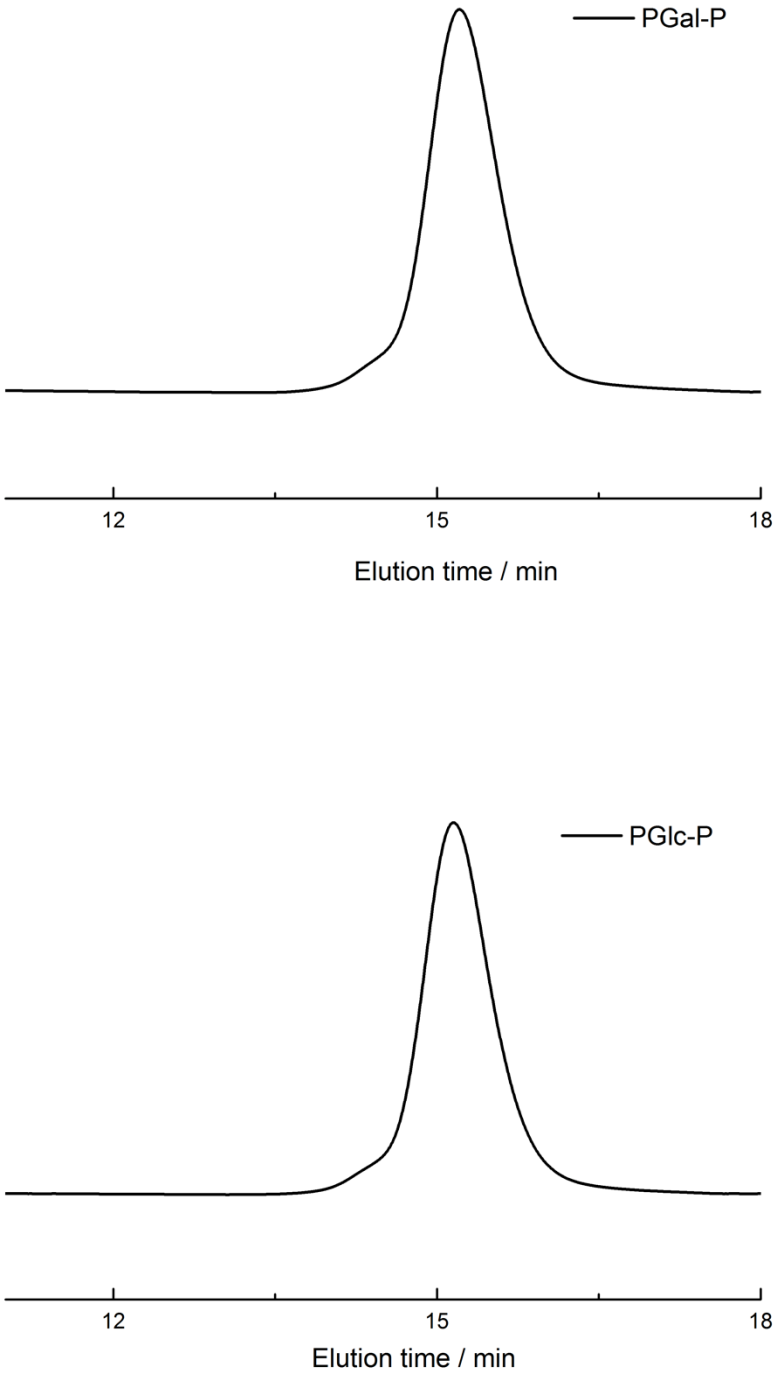


Figure S4. GPC of **PGal**-P ($M_w = 2.4\times10^4$, PDI =1.18) and **PGlc**-P ($M_w = 2.5\times10^4$, PDI =1.19) using DMF as eluent.

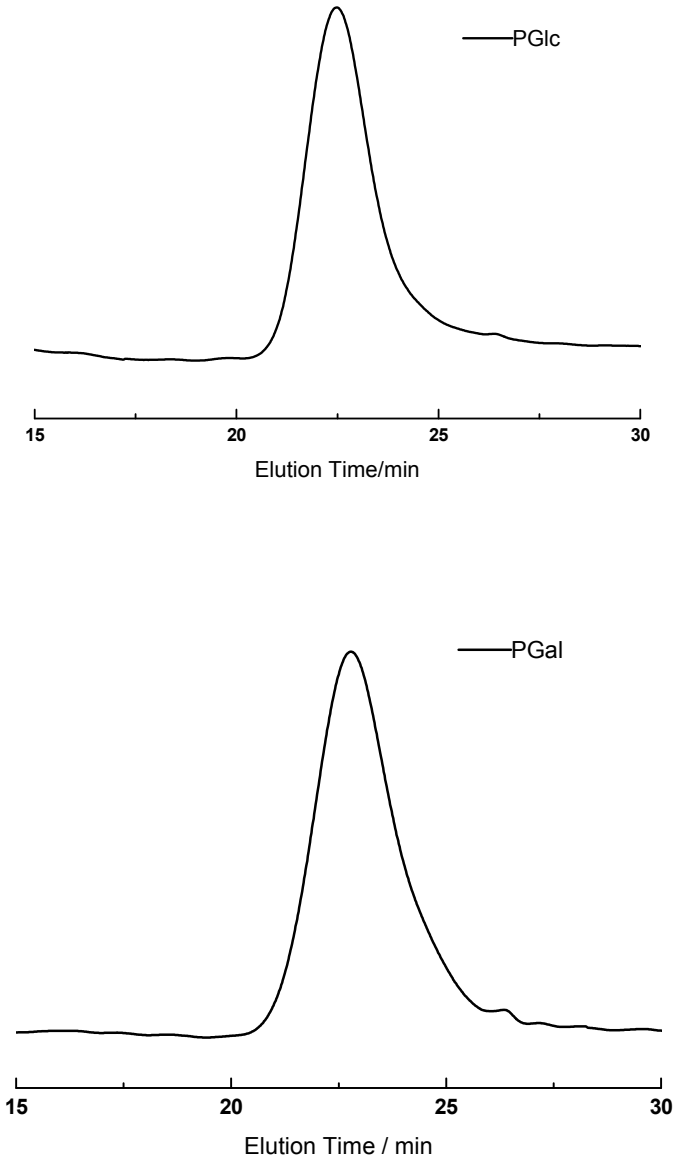


Figure S5. GPC of **PGal** ($M_w = 1.4\times10^4$, PDI =1.18) and **PGlc** ($M_w = 1.4\times10^4$, PDI =1.20) using water as eluent.

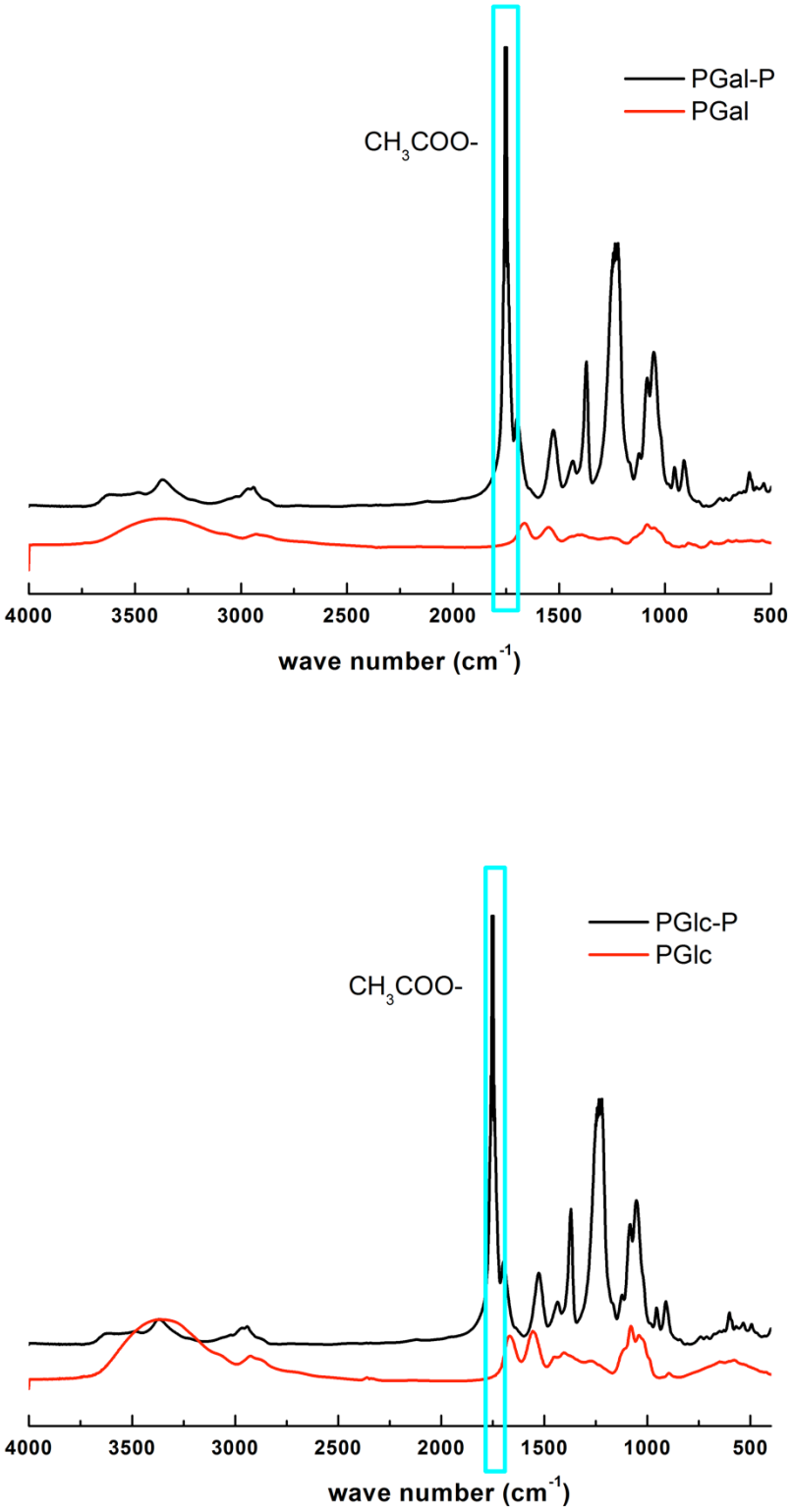


Figure S6. FT-IR of **PGal** and **PGlc** before and after acetate removal.

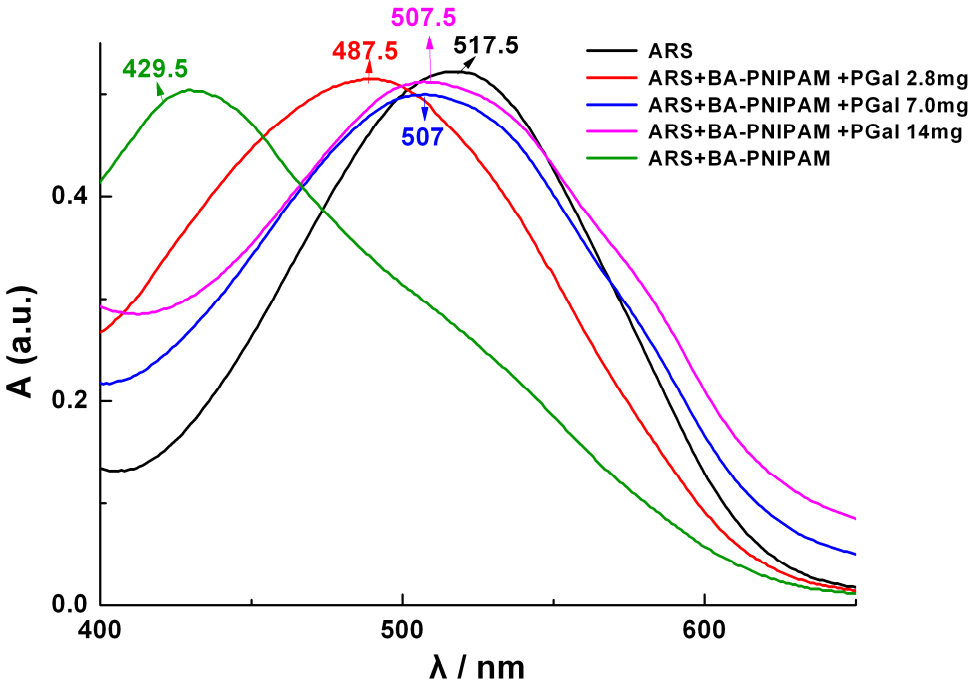


Figure S7. UV-vis spectroscopy of ARS (10^{-4}M) with BA-PNIPAM (10^{-3}M) in the absence and presence of **PGal** in 2 mL aqueous solution (pH 9.0).

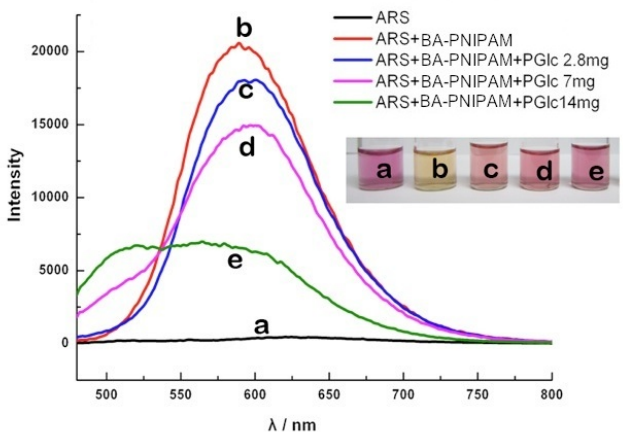


Figure S8. Fluorescence intensity of ARS (10^{-4}M) with BA-PNIPAM (10^{-3}M) in the absence and presence of **PGlc** in 2 mL aqueous solution (pH 9.0). Inset: Photo of the corresponding samples.

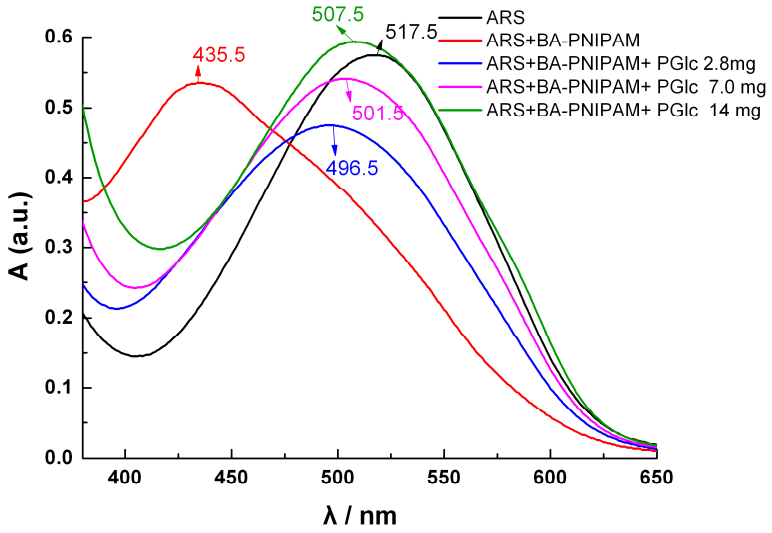


Figure S9. UV-vis spectroscopy of ARS (10^{-4}M) with BA-PNIPAM (10^{-3}M) in the absence and presence of **PGlc** in 2 mL aqueous solution (pH 9.0).

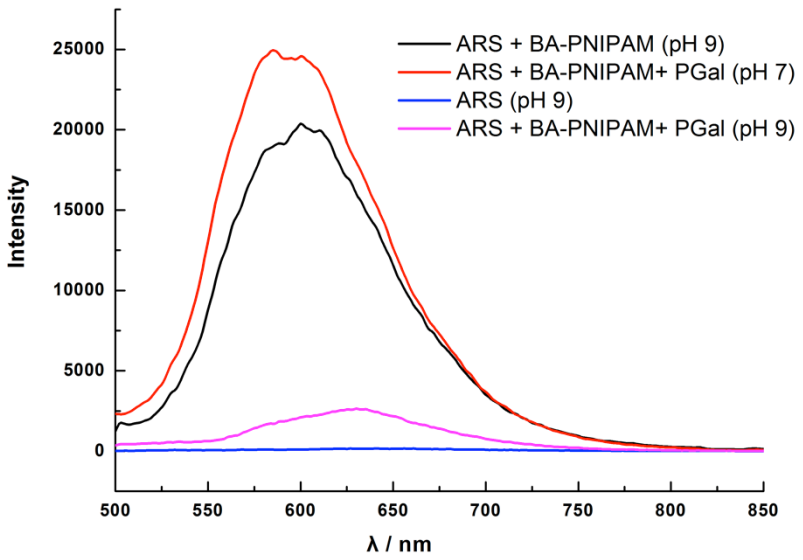


Figure S10. Demonstration of the reversibility of dynamic covalent bond by adding acid into the mixture of BA-PNIPAM, ARS and **PGlc** in aqueous solution (pH 9.0).[8]

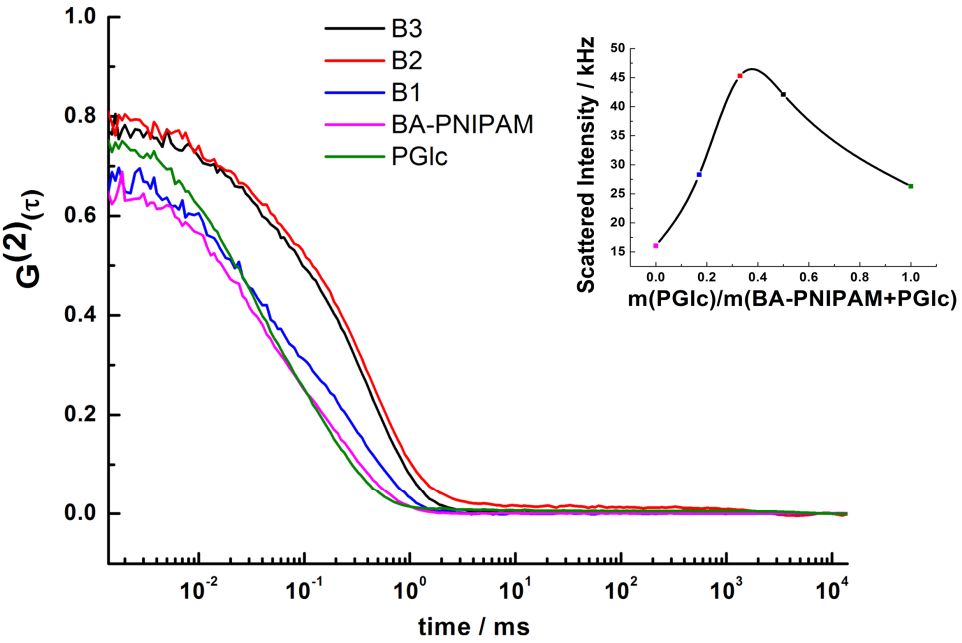
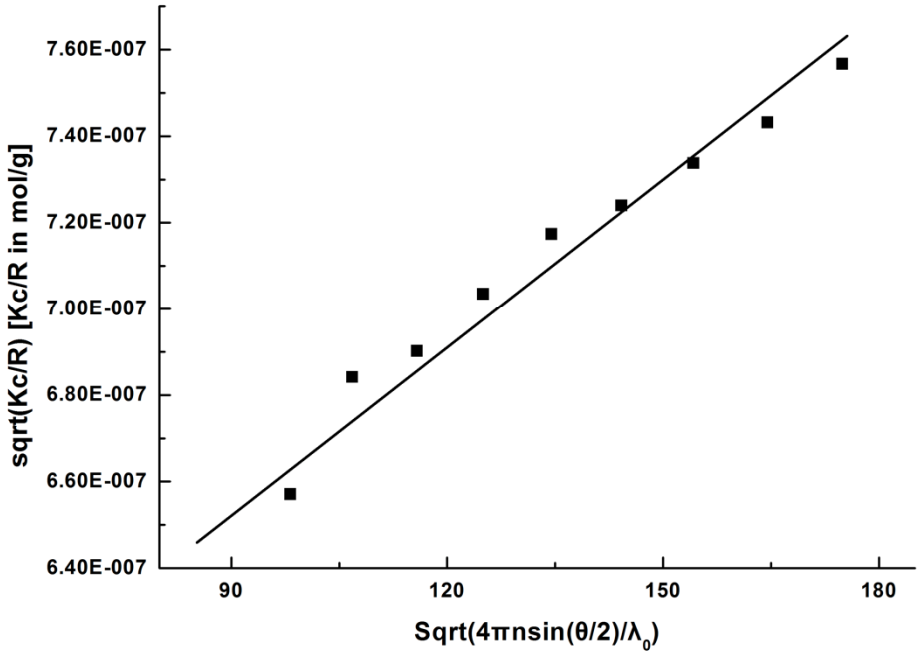
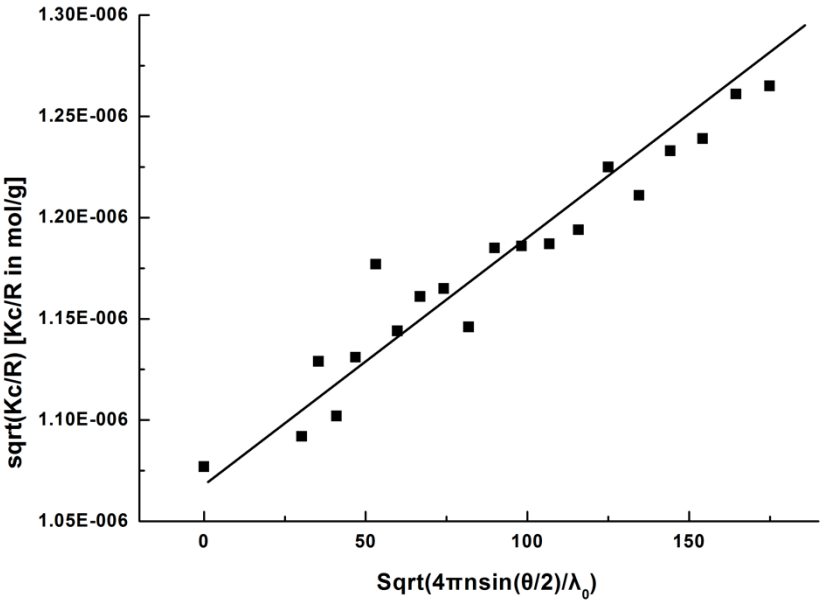


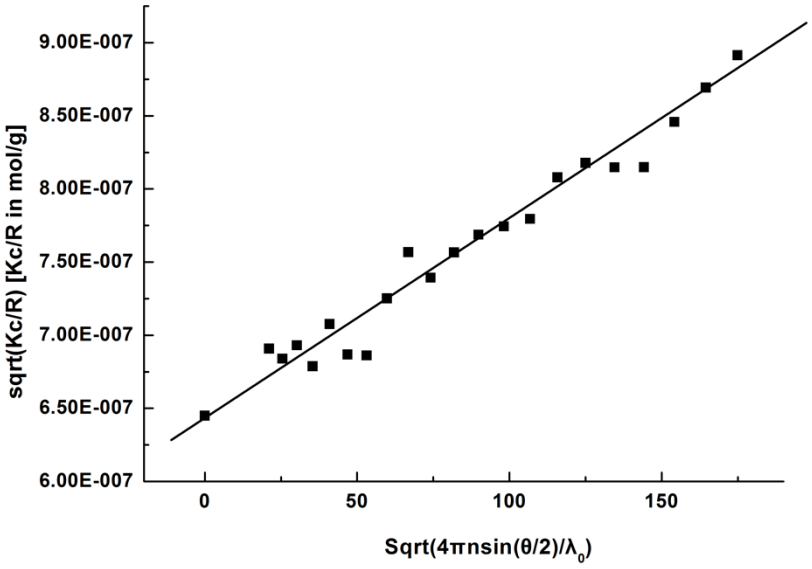
Figure S11. Auto correlation function obtained by DLS analysis (θ = 90º) of graft-like complex formed in different ratio between **PGlc** and BA-PNIPAM (BA-PNIPAM: 1 mg/mL; B1: BA-PNIPAM 0.17 mg/mL and **PGlc** 0.83 mg/mL; B2: BA-PNIPAM 0.33 mg/mL and **PGlc** 0.66 mg/mL; B3: BA-PNIPAM 0.5 mg/mL and **PGlc** 0.5 mg/mL; **PGlc**: 1 mg/mL). Inset: Dependence of the scattered intensity on the mixture ratio (m(PGlc)/m(PGlc+BA-PNIPAM)), where the total weight concentration of polymers was fixed to 1 mg/mL



(a)

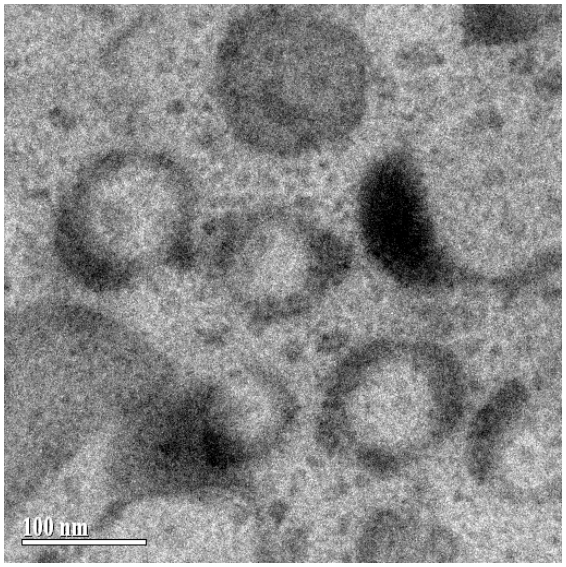


(b)



(c)

Figure S12. R_g (Zimm plot) of vesicles formed by BA-PNIPAM (a), **V-PGal** (b) and **V-PGlc** (c), respectively.



(d)

Figure S13. (d) TEM image of **V-PGal** after OsO_4 staining.

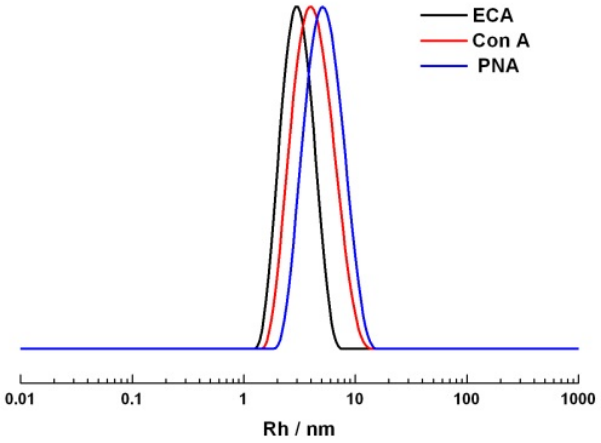


Figure S14. DLS profiles of PNA, ECA and ConA.

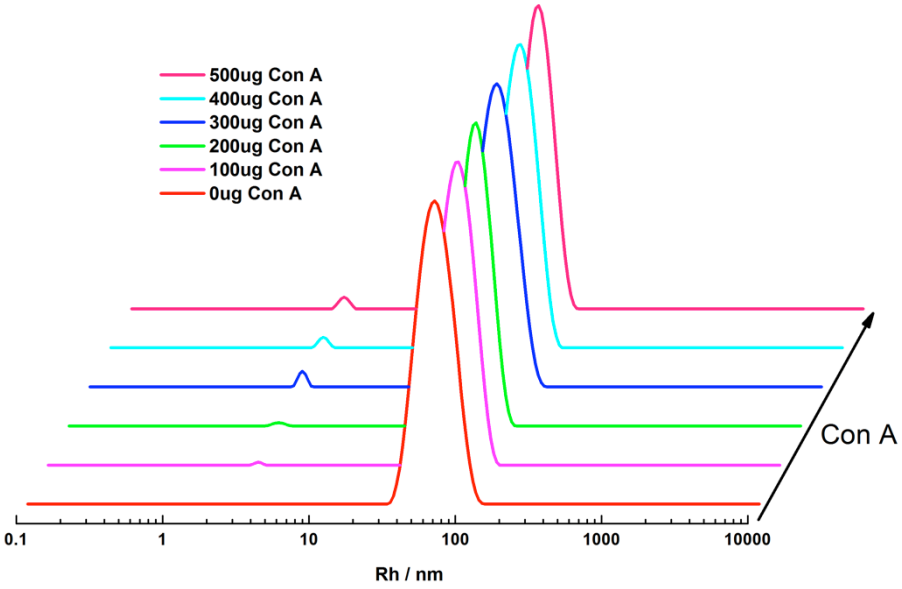


Figure S15. DLS profiles of ConA titration to **V-PGlc**.

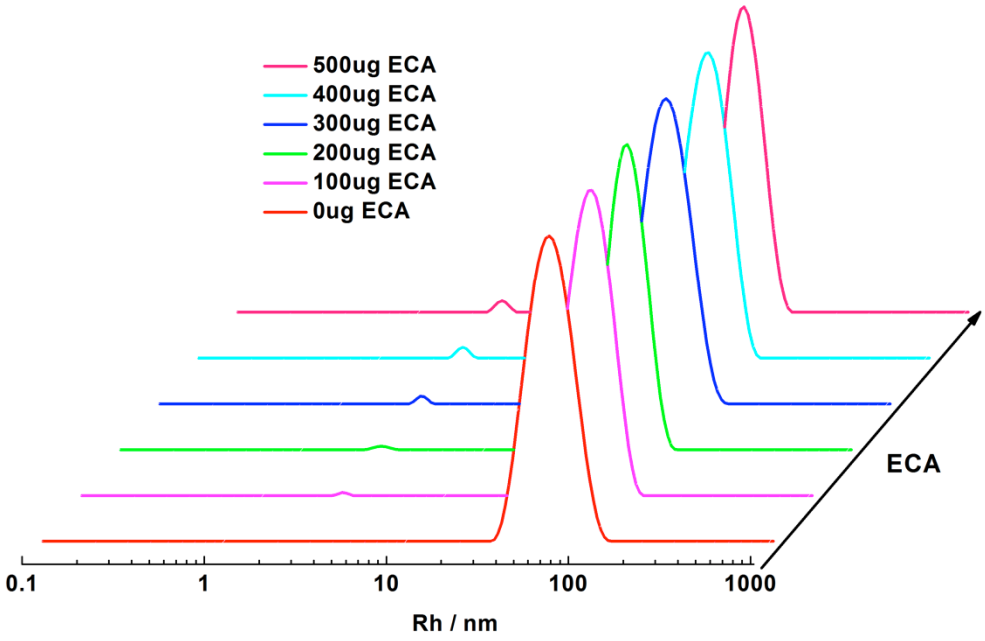


Figure S16. DLS profiles of ECA titration to **V-PGlc**.

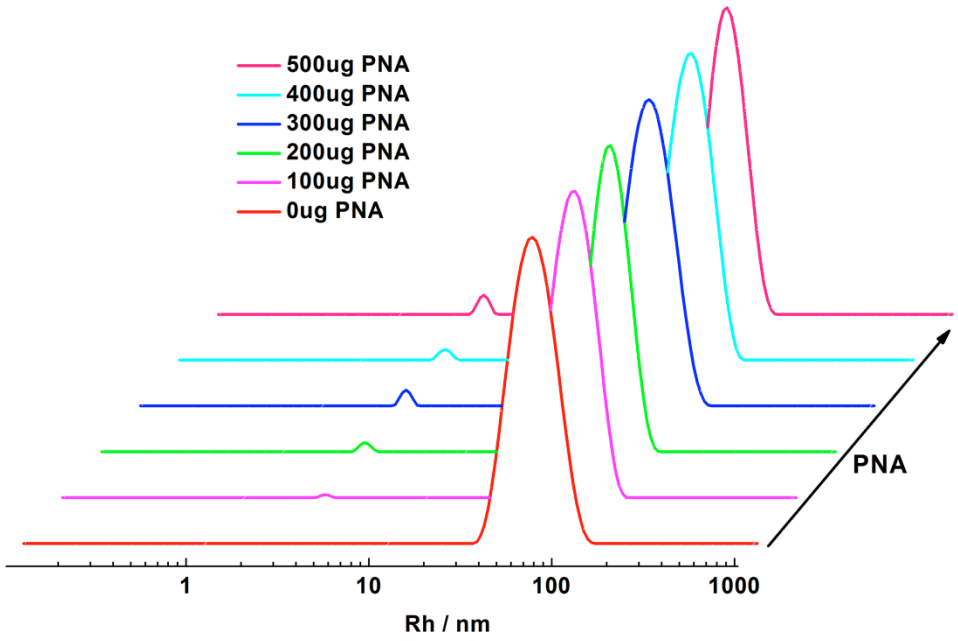


Figure S17. DLS profiles of PNA titration to **V-PGlc**.

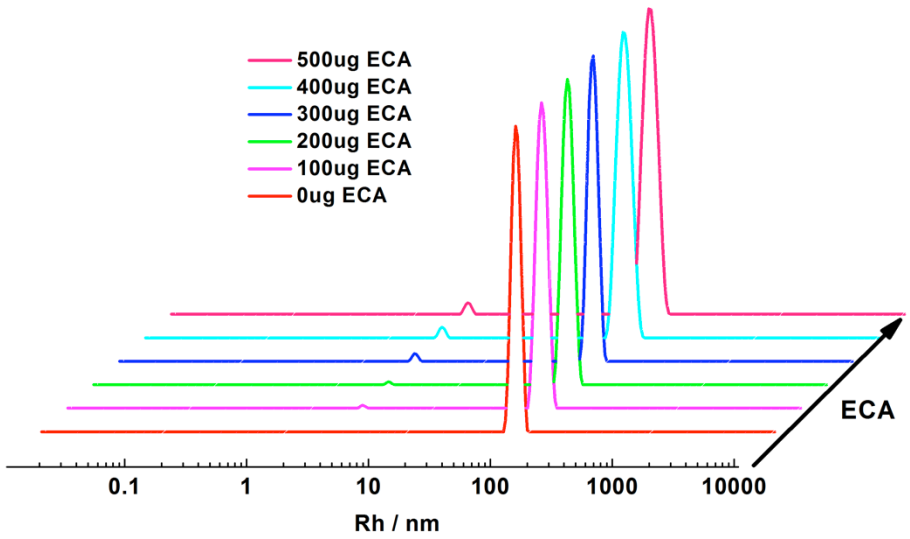


Figure S18. DLS profiles of ECA titration to V-PNIPAM as negative control.

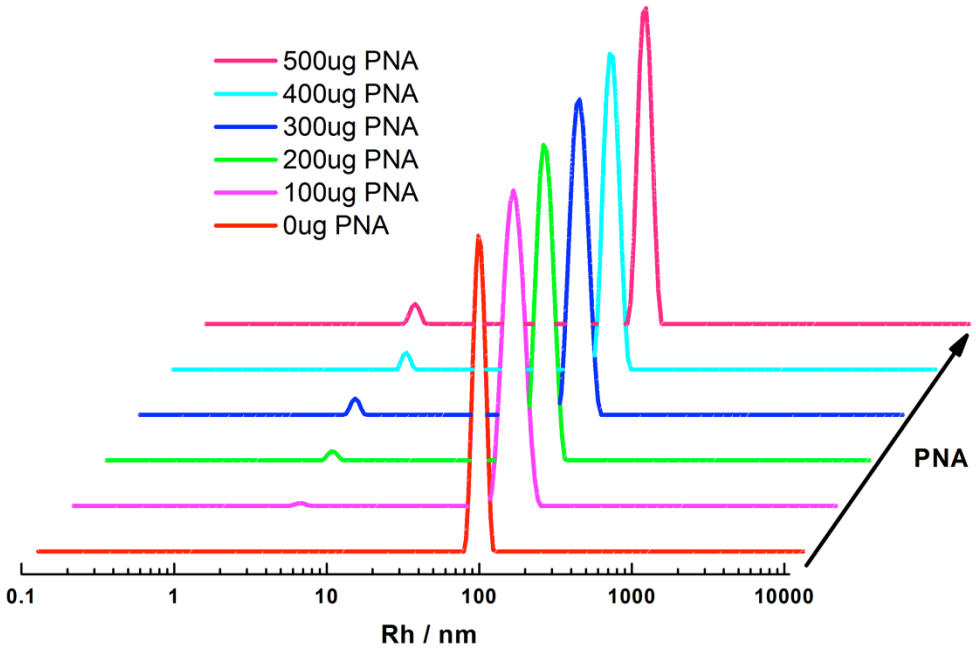


Figure S19. DLS profiles of PNA titration to V-PNIPAM as negative control.

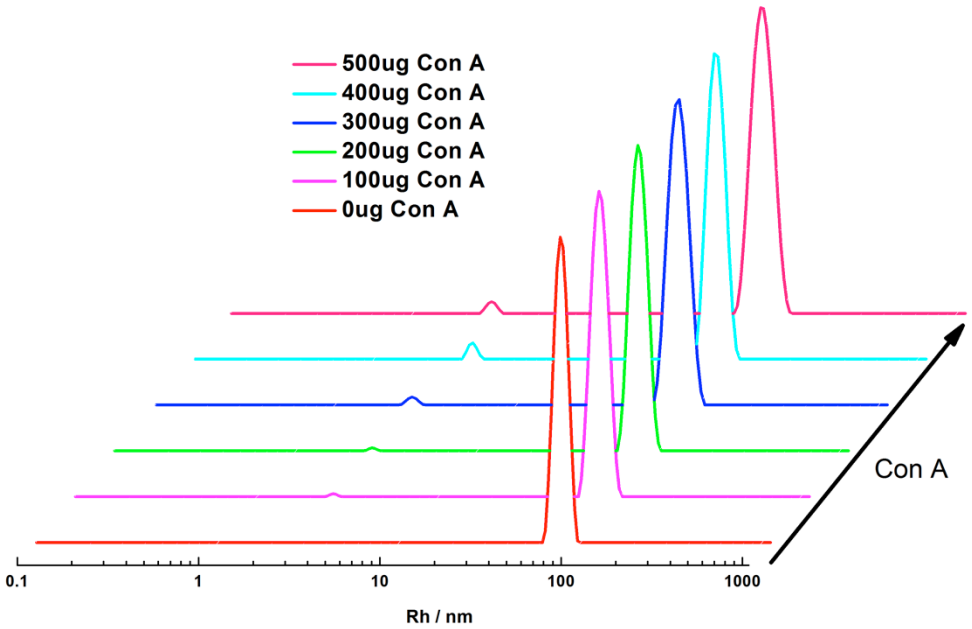
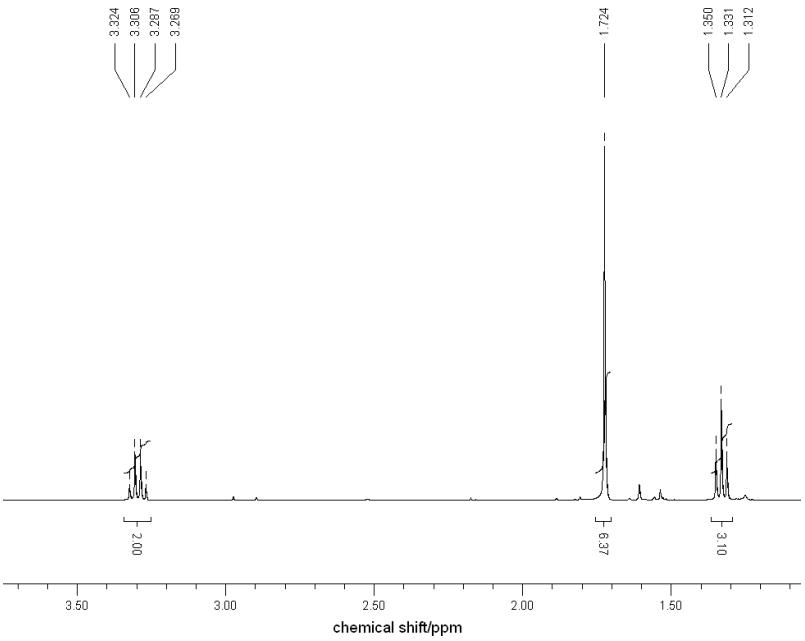
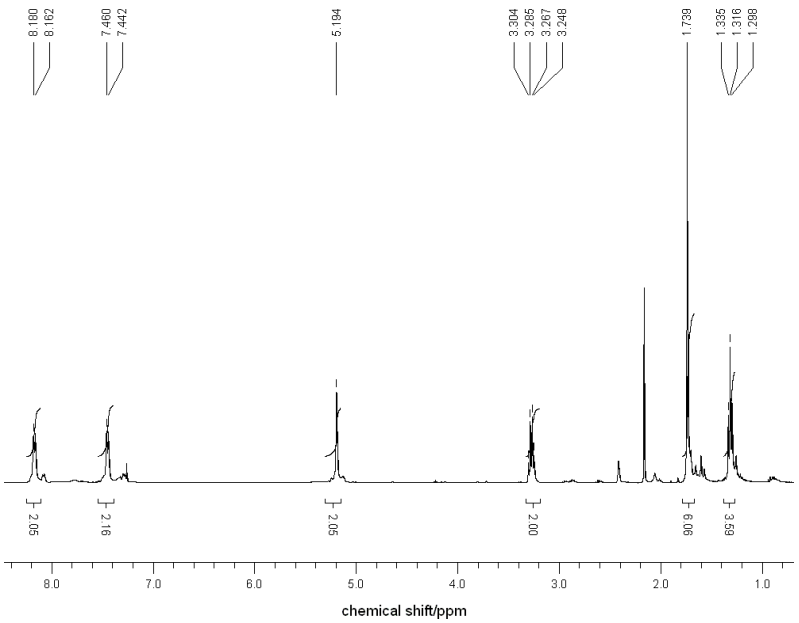


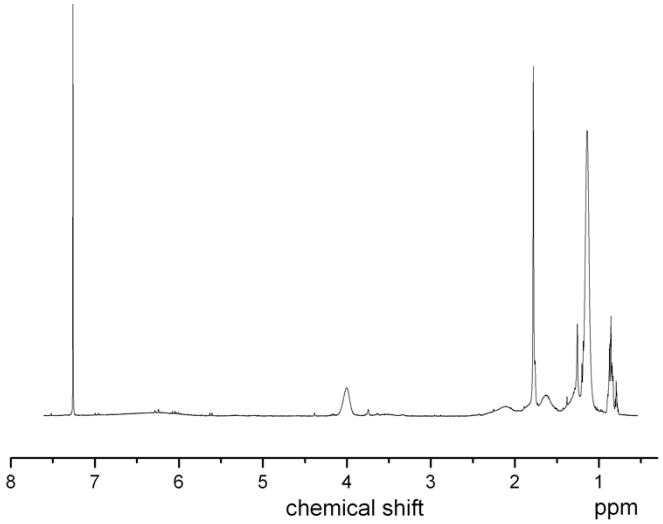
Figure S20. DLS profiles of ConA titration to V-PNIPAM as negative control.



^{1}H NMR spectrum of C_2-CTA-CA in $CDCl_3$

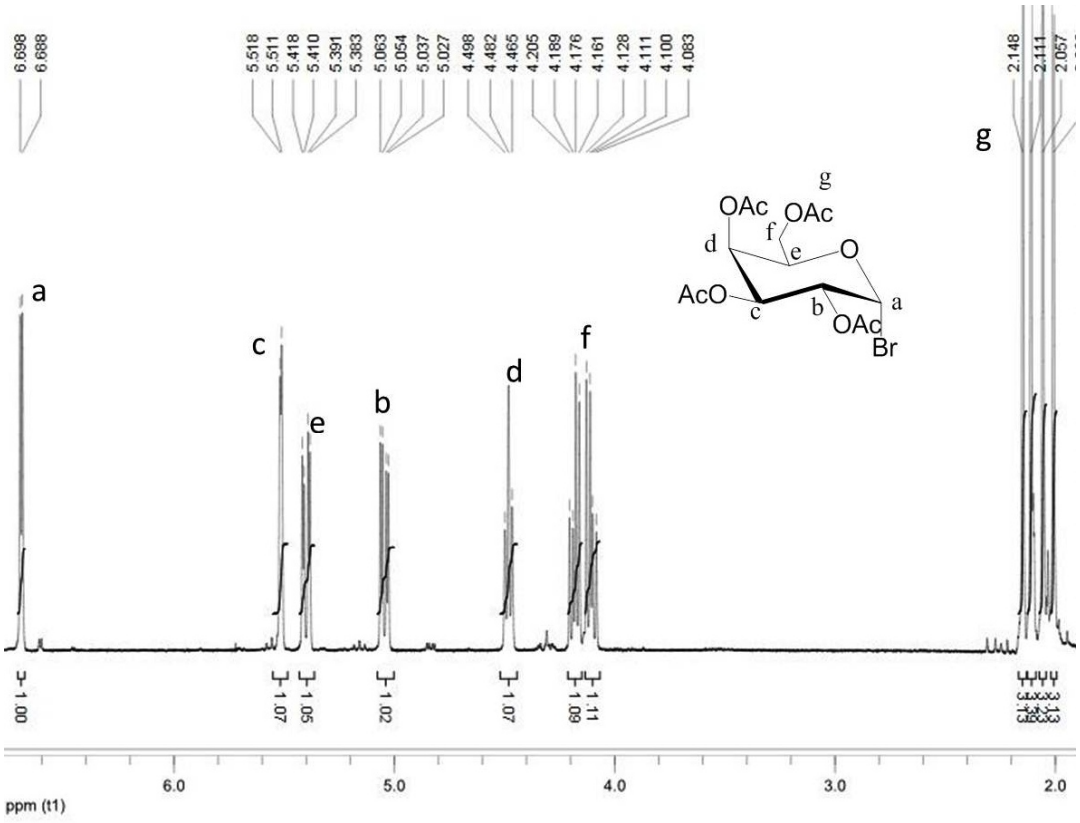


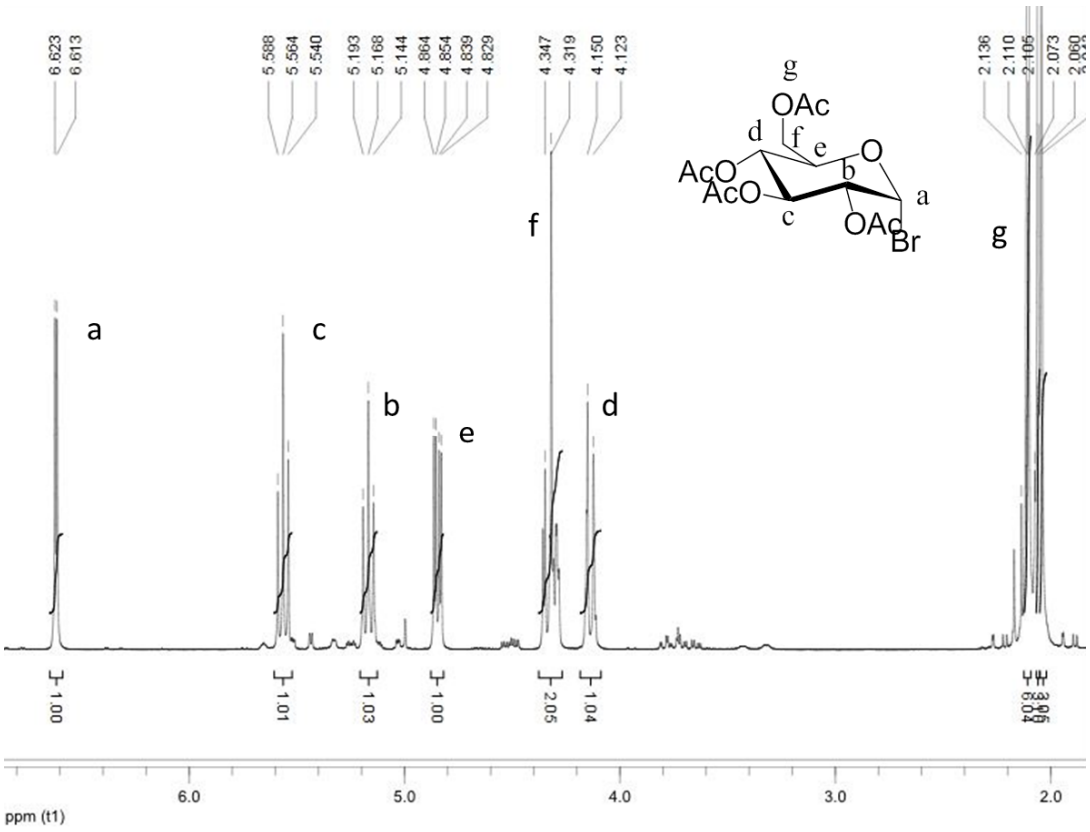
^{1}H NMR spectrum of C_2-CTA-BA in $CDCl_3$

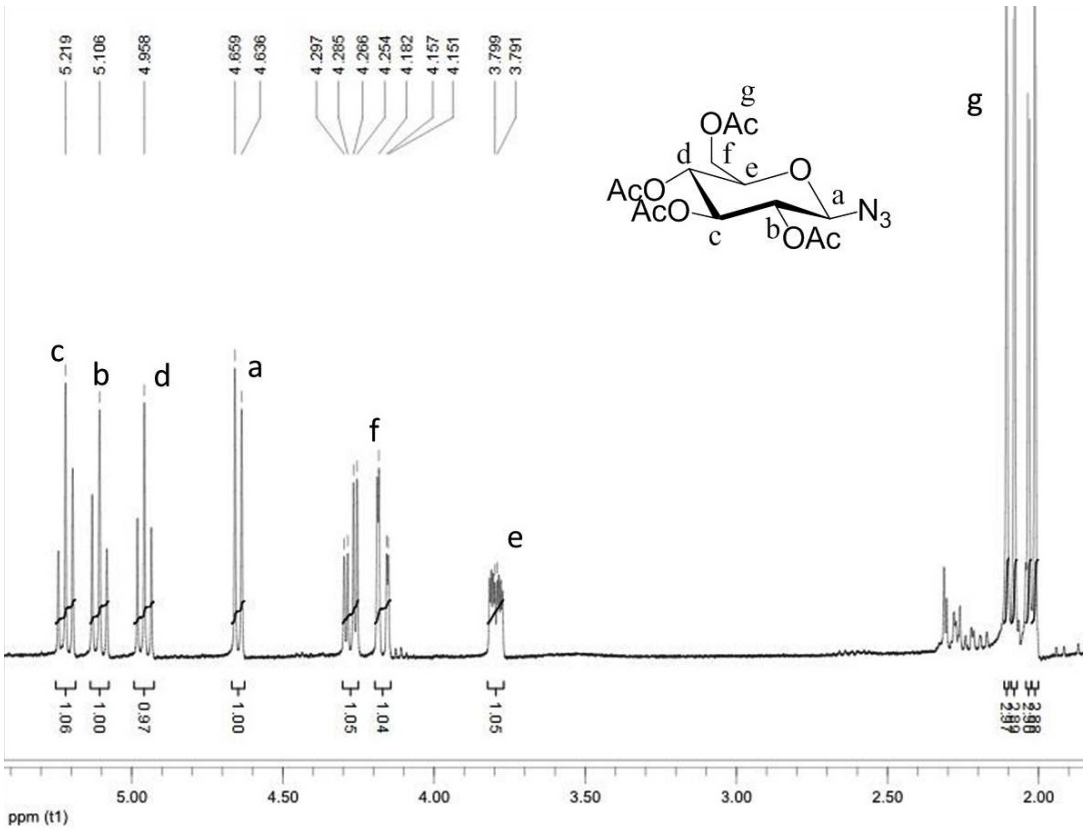


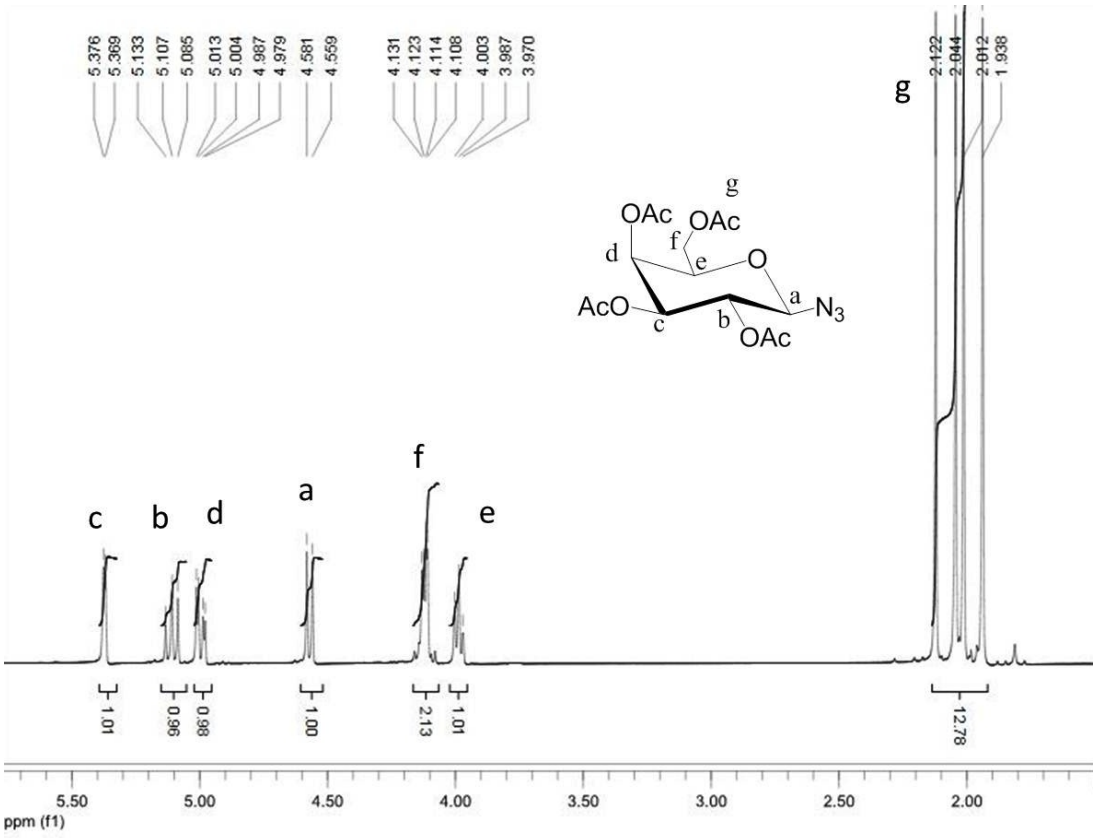
^{1}H NMR spectrum of BA-PNIPAM in $CDCl_3$

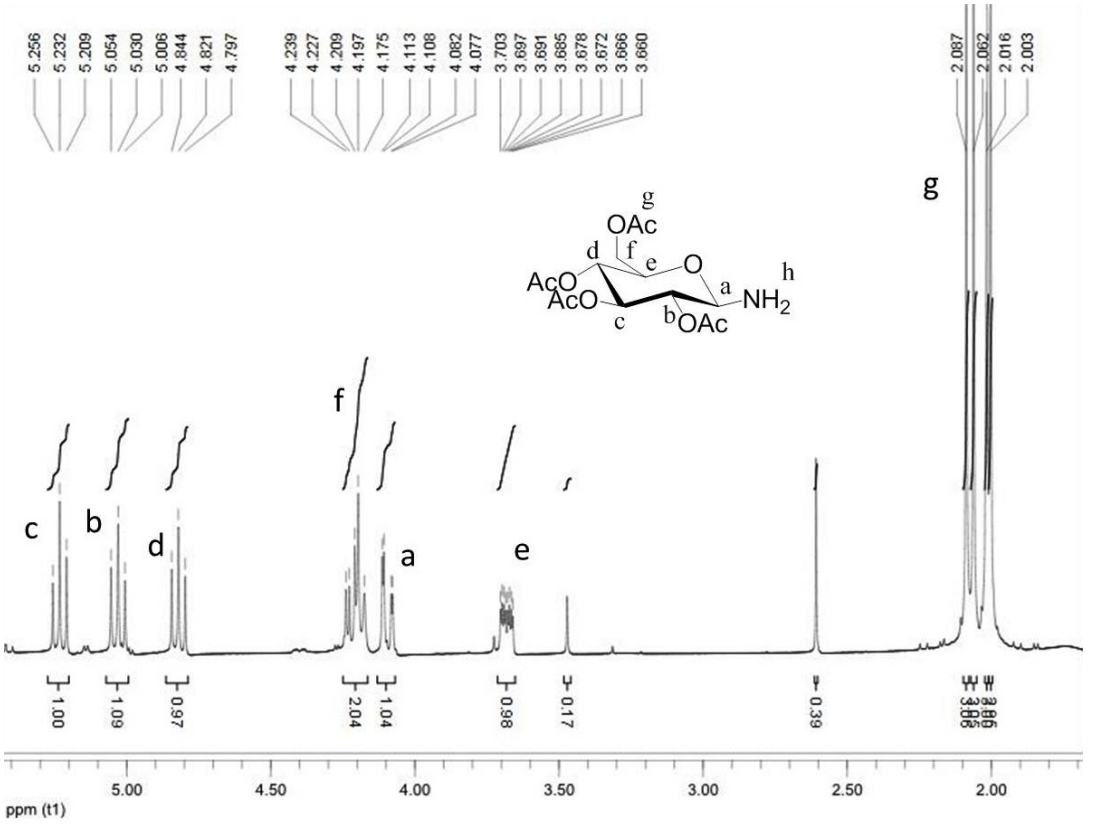
^{1}H and ^{13}C NMR and Maldi-TOF characterizations of sugar monomers and corresponding precusors.

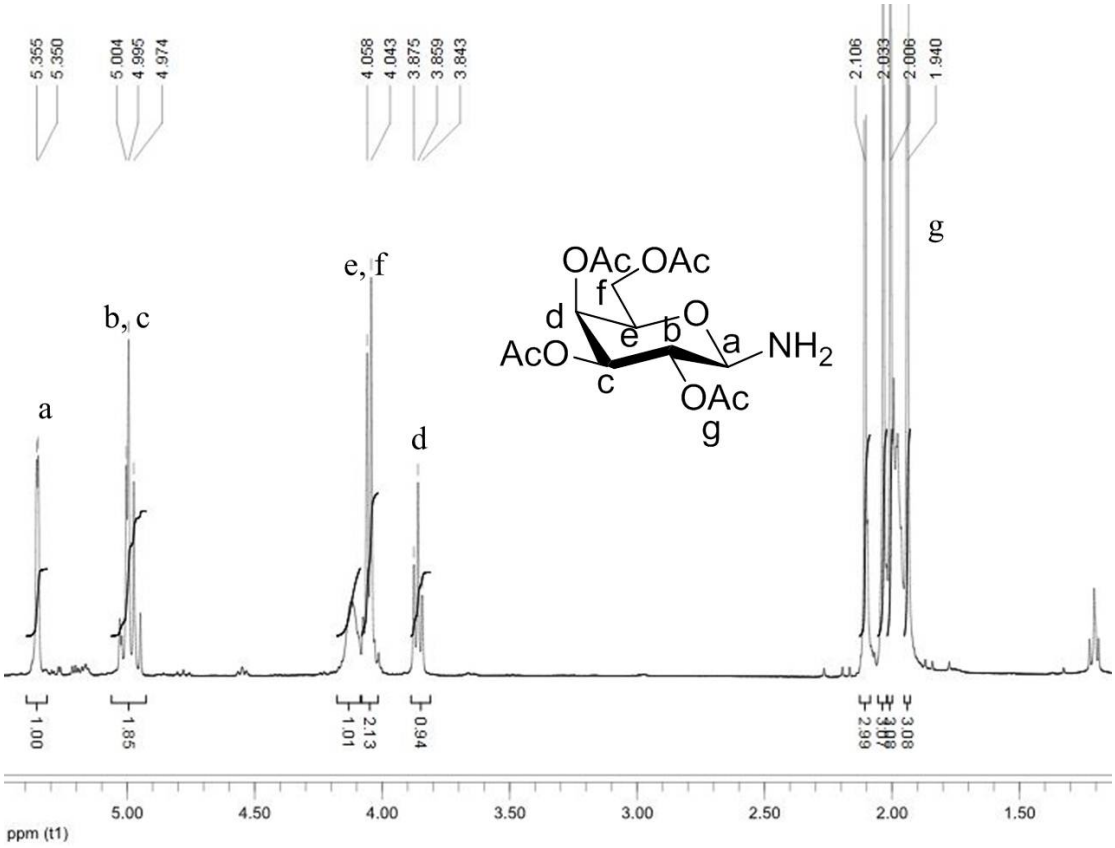


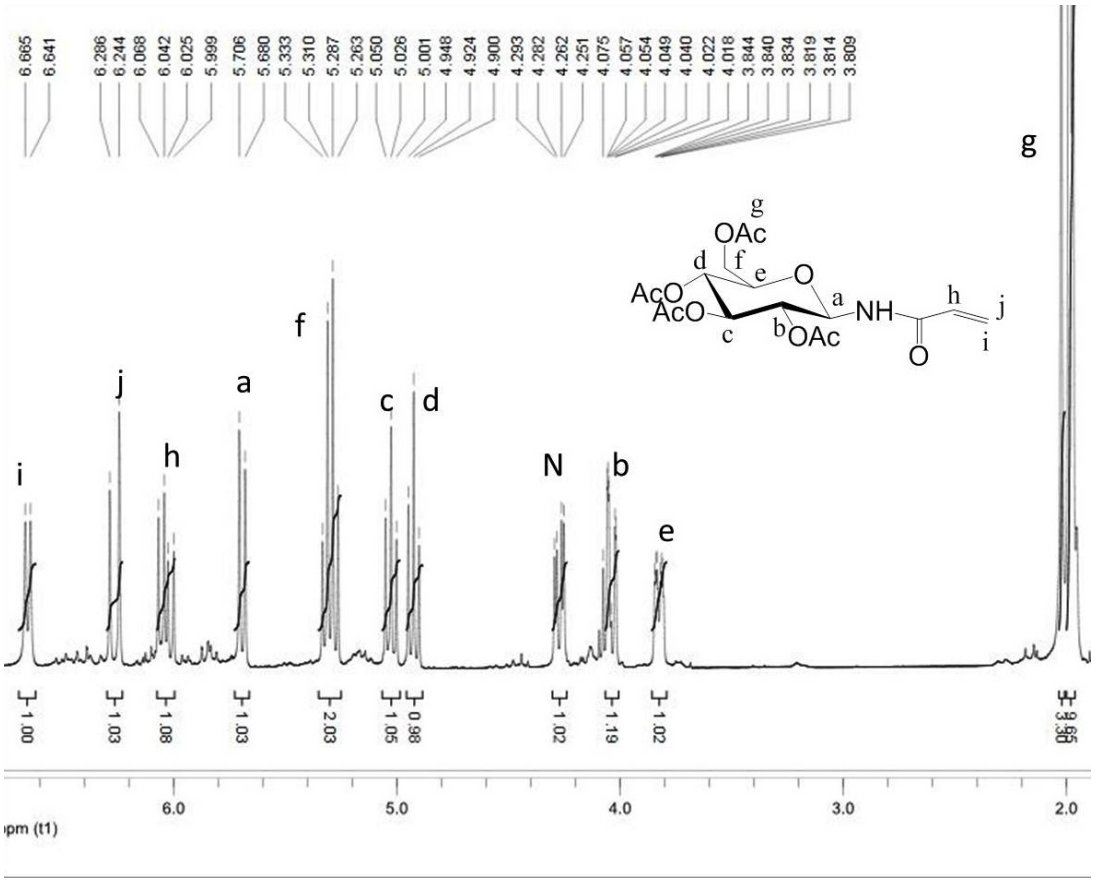


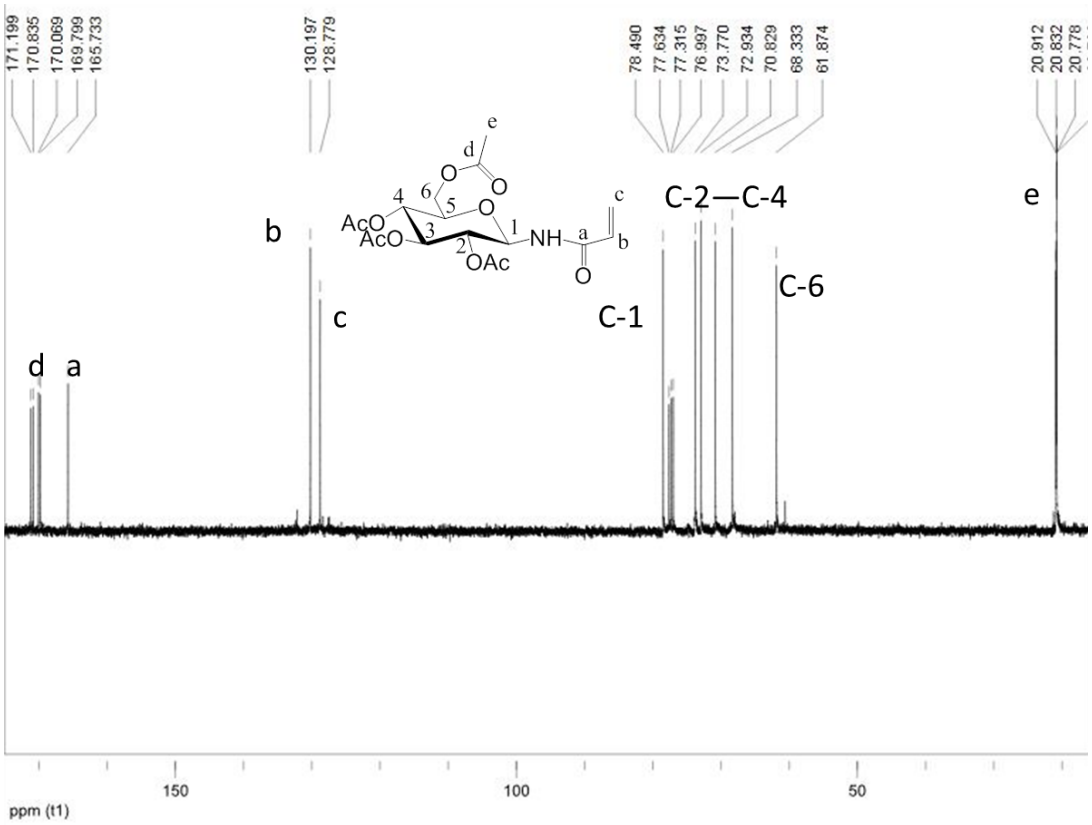


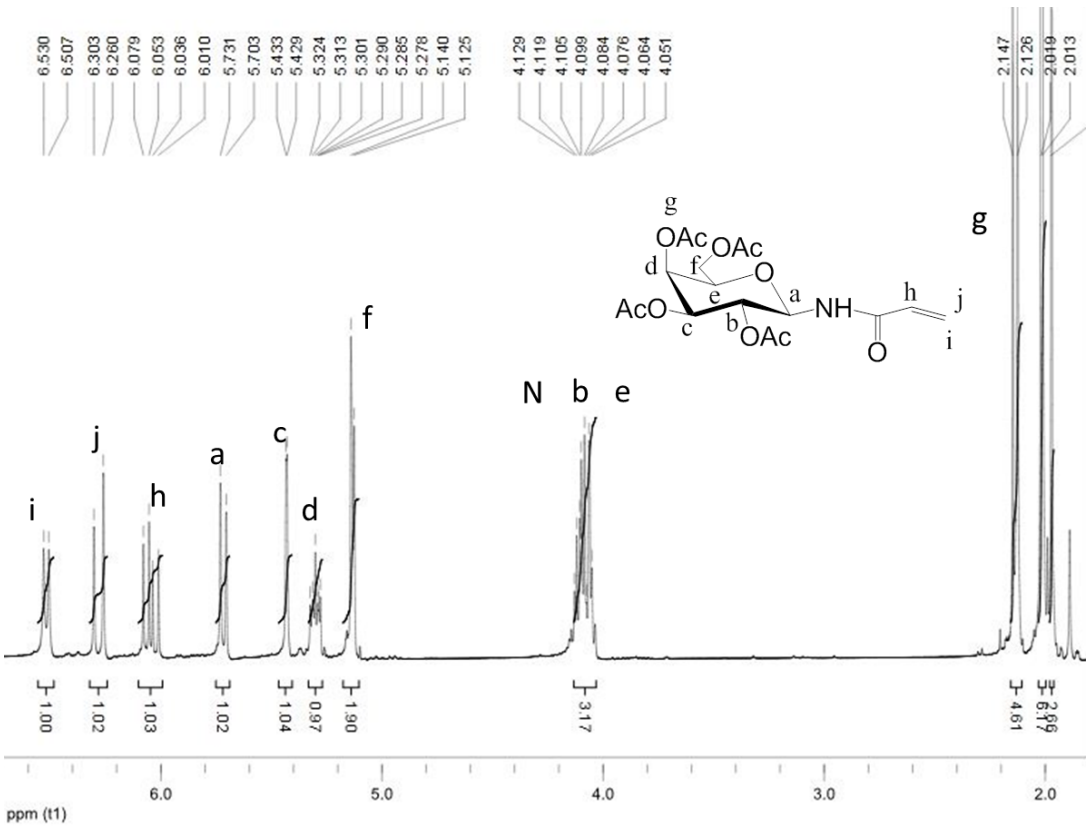


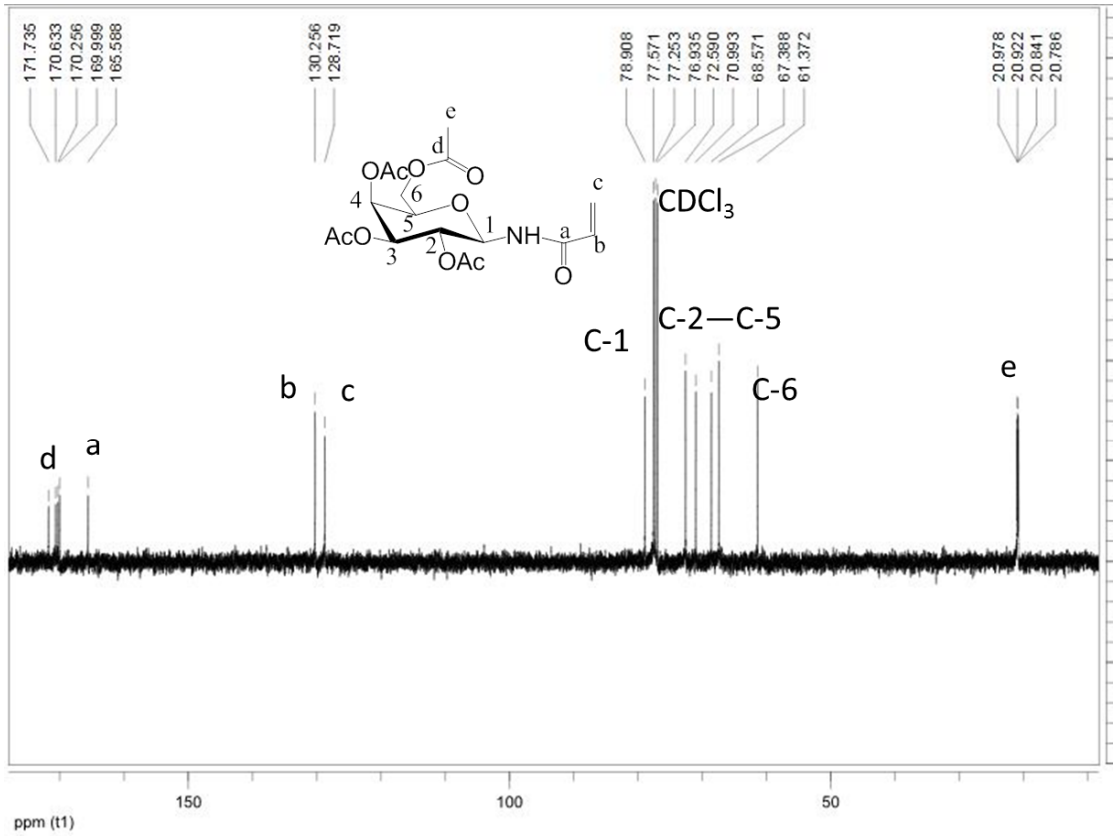


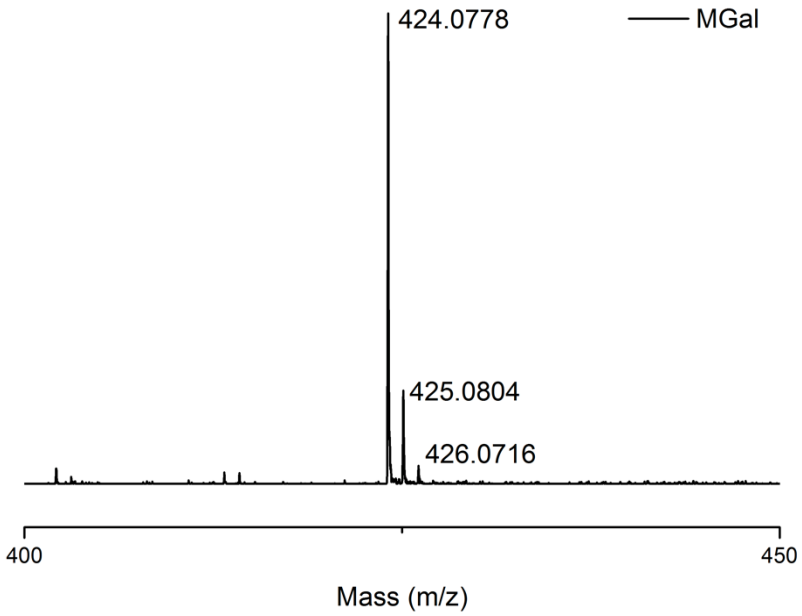


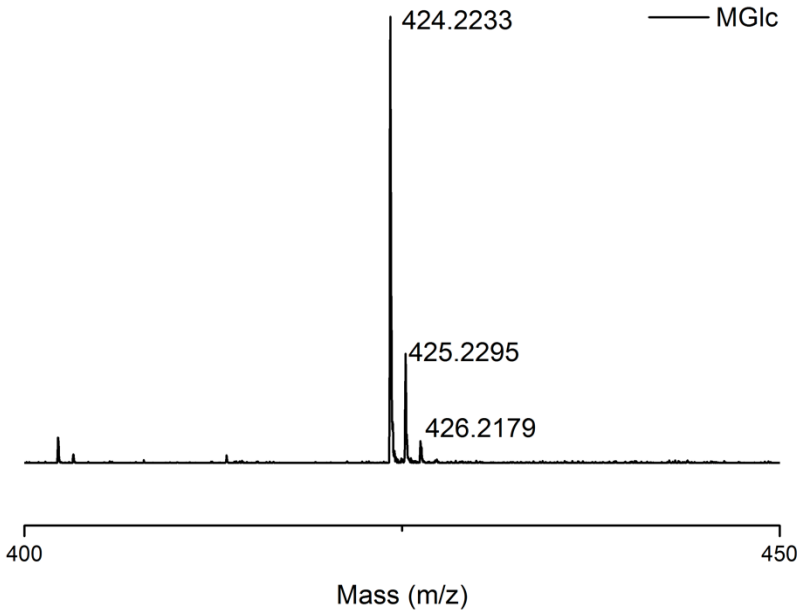












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