Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Synthesis of Aryl Azide Chain-end Functionalized *N*-Linked Glycan Polymer and Its Photo-labelling of Specific Protein

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Experimental

Materials and methods

All solvents and reagents were purchased from commercial sources and were used as received. Glc, Gal, and Lac were purchased from Sigma (USA). Deionized water with a resistivity of 18 MΩ cm^{-1} was used as solvent in all polymerization reactions and dialysis experiments. Dialysis was performed using cellulose membrane with a molecular weight cutoff of 3 kDa with water as solvent. ^{1}H NMR spectra were measured at room temperature with a Bruker AV400 MHz spectrometer and D_2O was used as deuterated solvent. Aryl chloride chain-end functionalized *N*-lactosyl polymer synthesized in our previous report[1].

Glycomonomers (3a-3c) were synthesized in our previously reported methods[1].

Synthesis of glycosylamines (2a-2c) *via* Kochetkov method. A solution of saccharide(**1a-1c**) (5.0 mmol) and ammonium bicarbonate (389.0 mg, 5.0 mmol) in 20 mL of aqueous ammonia was kept in an oil bath at 40°C for 40 h and then was freeze-dried to afford glycosylamines (**2a-2c**), which were directly subjected to the next reaction without purification.

Acrylation of glycosylamines (2a-2c). The dry glycosylamines (**2a-2c**) obtained above were dissolved in 60 mL of CH_3OH-H_2O (1:1, v/v) and 3.0 g of Na_2CO_3 was added, respectively. The mixture was cooled in an ice bath for 30 min and then a solution of 1.28 mL of acryloyl chloride in 7 mL of THF was added dropwise. After that, the reaction mixture was stirred at 0°C for another 1 h. The organic solvents were evaporated under reduced pressure and the remaining aqueous layer was lyophilized to dryness, which were purified by silica gel column chromatography eluted with EtOAc and CH_3OH (5:3, v/v) afford glycomonomers (**3a-3c**).

Preparation of aryl azide chain-end functionalized *N*-linked glycan polymers (4a-4c)

Aryl azide chain-end functionalized *N*-glucosyl polymer (4a): 4-Azidoaniline (3.1 mg, 0.018 mmol) and 6.4 μL of HBF_4 solution (48 wt%, 0.054 mmol) were dissolved in 200 μL of degassed ddH_2O in a three-necked flask. The mixture solution was cooled in an ice bath for 30 min and a solution of sodium nitrite ($NaNO_2$, 2.8 mg, 0.034 mmol) in 200 μL of ddH_2O was added to react for 30 min in an atmosphere of N_2. Then, a degassed mixture solution of *N*-(prop-2-enoyl)-*β*-D-glucopyranosylamine (42.0 mg, 0.18 mmol), acrylamide (51.2 mg, 0.72 mmol) and NaOCN (1.2 mg, 0.018 mmol) dissolved in 1 mL of degassed ddH_2O was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60°C to react for 20 h. The resulting mixture was dialyzed against ddH_2O for 2 days to remove the impurity and then freeze-dried to yield the glycopolymer **4a** (72.0 mg, 58.0%). The molecular weight (*M*n) was about 33,300 with saccharide content at about 9,000 as determined by ^1H NMR spectrum.

Aryl azide chain-end functionalized *N*-galactosyl polymer (4b): 4-Azidoaniline (3.1 mg, 0.018 mmol) and 6.4 μL of HBF_4 solution (48 wt%, 0.054 mmol) were dissolved in 200 μL of degassed ddH_2O in a three-necked flask. The mixture solution was cooled in an ice bath for 30 min and a solution of sodium nitrite ($NaNO_2$, 2.8 mg, 0.034 mmol) in 200 μL of ddH_2O was added to react for 30 min in an atmosphere of N_2. Then a degassed mixture solution of *N*-(prop-2-enoyl)-*β*-D-galactopyranosylamine (42.0 mg, 0.18 mmol), acrylamide (51.2 mg, 0.72 mmol) and NaOCN (1.2 mg, 0.018 mmol) dissolved in 1 mL of degassed ddH_2O was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60°C to react for 20 h. The resulting mixture was dialyzed against ddH_2O for 2 days to remove the impurity and then freeze-dried to yield the glycopolymer **4b** (82.0 mg, 78.0%). The molecular weight (*M*n) was about 21,800 with saccharide content at about 9,000 as determined by ^1H NMR spectrum.

Aryl azide chain-end functionalized *N*-lactosyl polymer (4c). 4-Azidoaniline (12 mg, 0.07 mmol) and sodium nitrite ($NaNO_2$, 10 mg 0.14 mmol) were dissolved in 2.5 mL of H_2O-THF (1:1) in a three-necked flask. The mixture solution was cooled in an ice bath for 30 min and the 36 μL of HBF_4 solution (48 wt%, 0.276 mmol) was added to react for 1 h. Then, a degassed mixture of *N*-(prop-2-enoyl)-*β*-D-lactopyranosylamine (420 mg, 1.06 mmol) acrylamide (280 mg, 3.94 mmol) and NaOCN (10 mg, 0.153 mmol) dissolved in 2 mL of ddH_2O was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60°C to react for 20 h. The resulting mixture was dialyzed against ddH_2O for 2 days to remove the impurity and then freeze-dried to yield the glycopolymer **4c** (436 mg, 62.3%). The molecular weight (*M*n) was about 78,970 with saccharide content at about 19,500 as determined by ^1H NMR spectrum.

Preparation of aryl azide chain-end functionalized polyacrylamide. 4-Azidoaniline (7.2 mg, 0.042 mmol) and sodium nitrite ($NaNO_2$, 6 mg 0.084 mmol) were dissolved in 1.5 mL of H_2O-THF (1:1) in a three-necked flask. The mixture solution was cooled in an ice bath for 30 min and the 21.6 μL of HBF_4 solution (48 wt%, 0.166 mmol) was added to react for 1 h. Then a degassed solution of acrylamide (420 mg, 5.91 mmol) and NaOCN (6 mg, 0.0918 mmol) dissolved in 1.2 mL of ddH_2O was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60°C to react for 20 h. The resulting mixture was dialyzed against ddH_2O for 2 days to remove the impurity and then freeze-dried to yield the aryl azide chain-end functionalized polyacrylamide (350 mg, 83.4%). The molecular weight (*M*n) was about 44744 as determined by ^1H NMR spectrum.

UV-induced photo-crosslinking assay

Glycopolymers (2.8 mg, 3.57×10^{-8} mol) and lectin *Arachis hypogae* (0.6 mg, 5.45×10^{-9} mol, lyophilized powder, Sigma) were dissolved in 100 μL of PBS buffer (pH 7.4) solution containing 0.2% Tween 20 and allowed to react for 4 h in RT. Then, the mixture was degassed and exposed to a 366 nm UV source in the dark for 30 min while remaining on ice.

Characterization of photo-labeled lectin by SDS-PAGE

Laemmli Sample Buffer (Bio-Rad, #1610737) was prepared according to product guidelines. Sample taken directly from photo-crosslinking assay was added to Laemmli Sample Buffer to produce aliquots corresponding to 25 µg of protein (5 µL) for each sample and then boiled at 100°C for 20 min. Samples were then characterized in SDS-polyacrylamide gels containing 10% diH_2O, 12% acrylamide/Bis-acrylamide, 25% 1.5 M Tris-HCL pH 8.8, 0.1% SDS, 0.1% APS, 0.1% TEMED, and electrophoresis were performed at 120V for 90 min followed by silver staining (Pierce® Silver Stain Kit, Thermo Scientific).

^{1}H NMR spectra

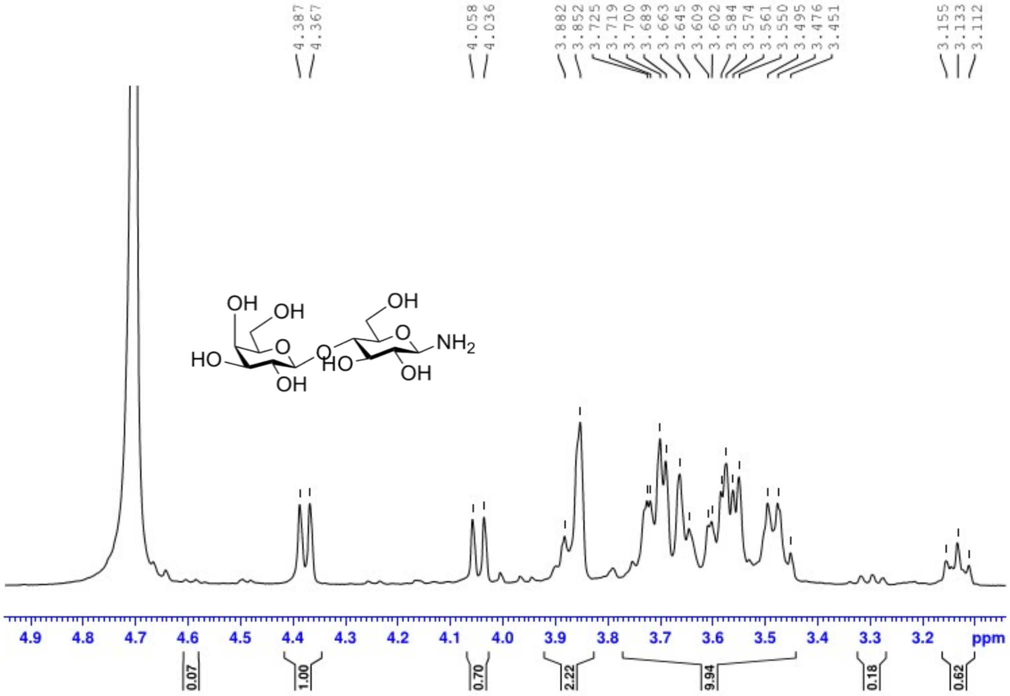

Fig. S1. ^{1}H NMR spectrum of *β*-D-lactopyranosylamine (**2c**) in D_2O.

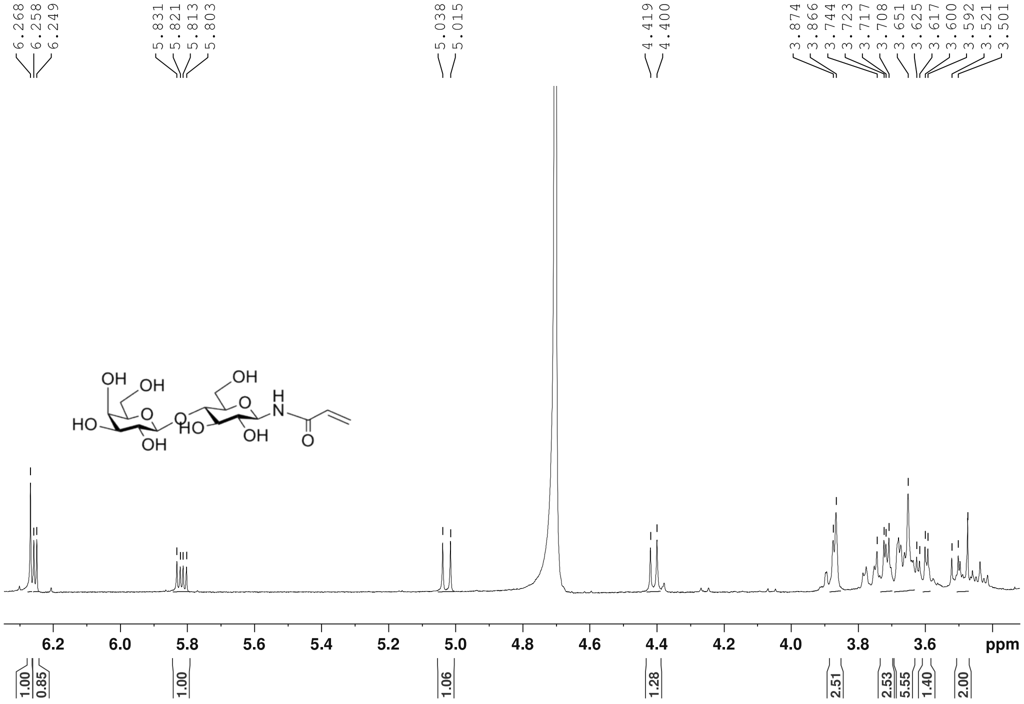

Fig. S2. ^{1}H NMR spectrum of *N*-(Prop-2-enoyl)-*β*-D-lactopyranosylamine (**3c**) in D_2O.

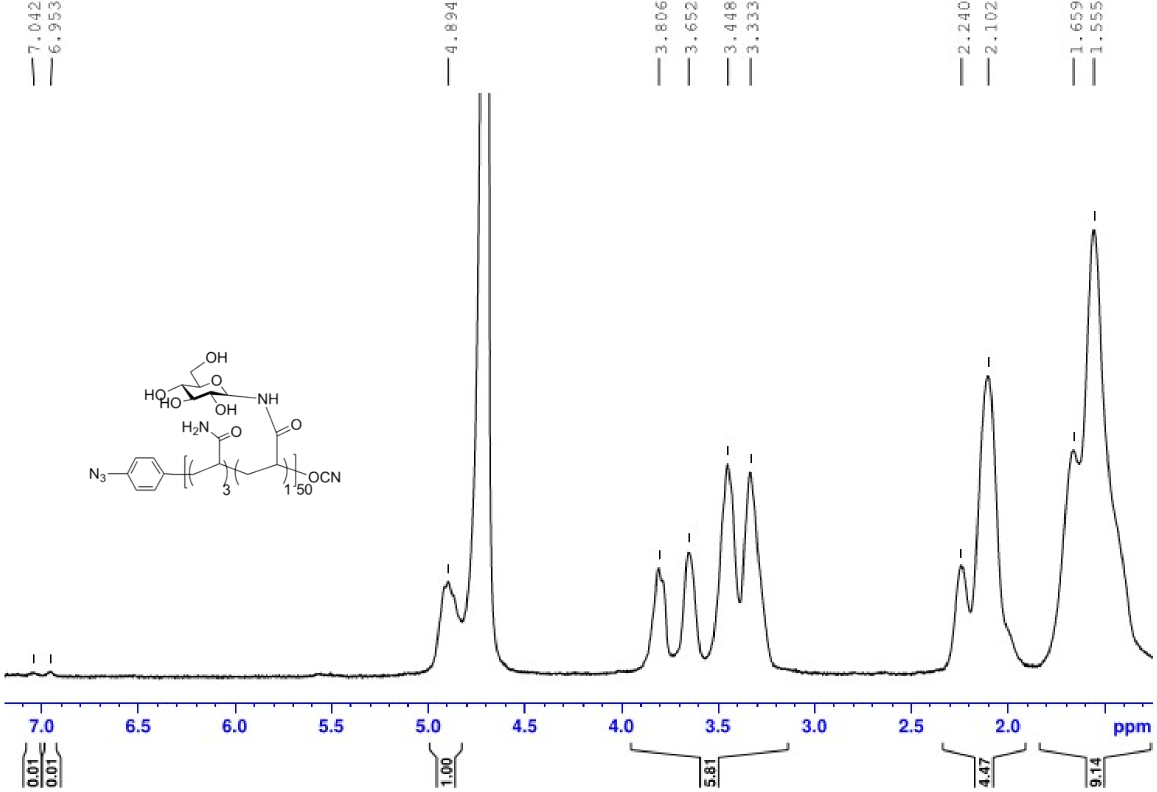

Fig. S3. ^{1}H NMR spectrum of glucosyl glycopolymer (**4a**) in D_2O.

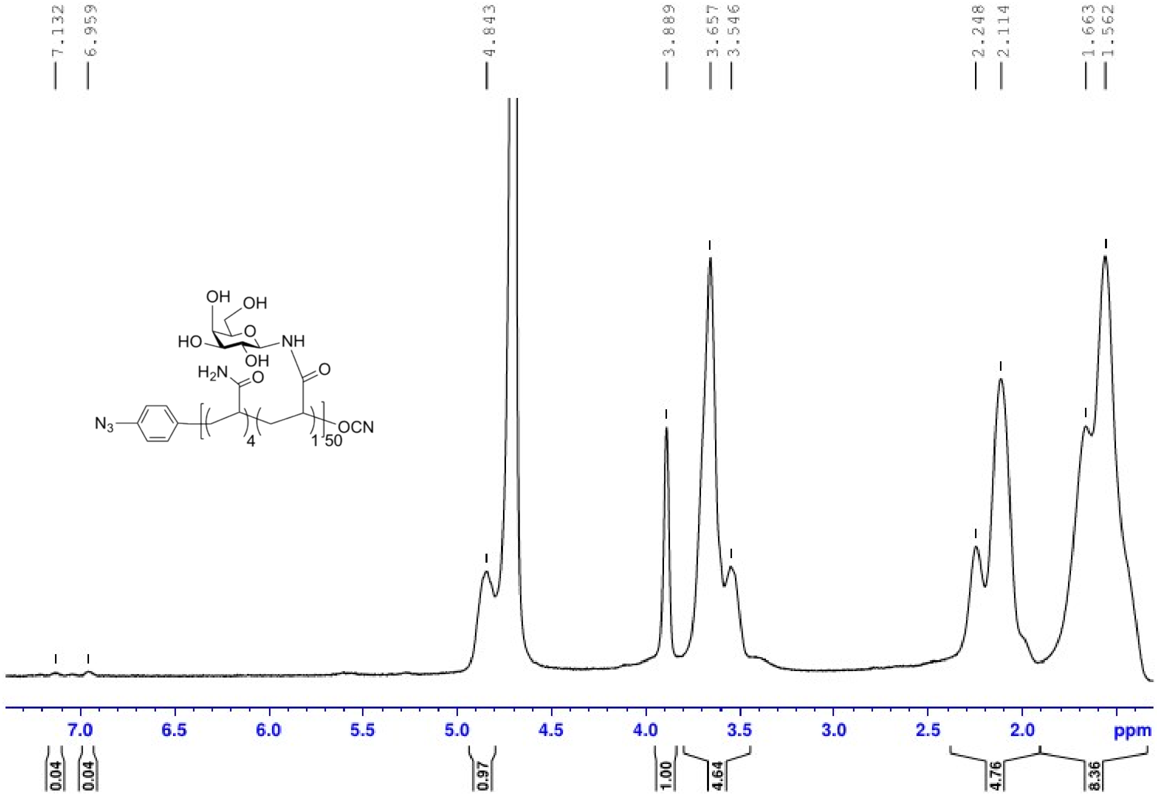

Fig. S4. ^{1}H NMR spectrum of galactosyl glycopolymer (**4b**) in D_2O.

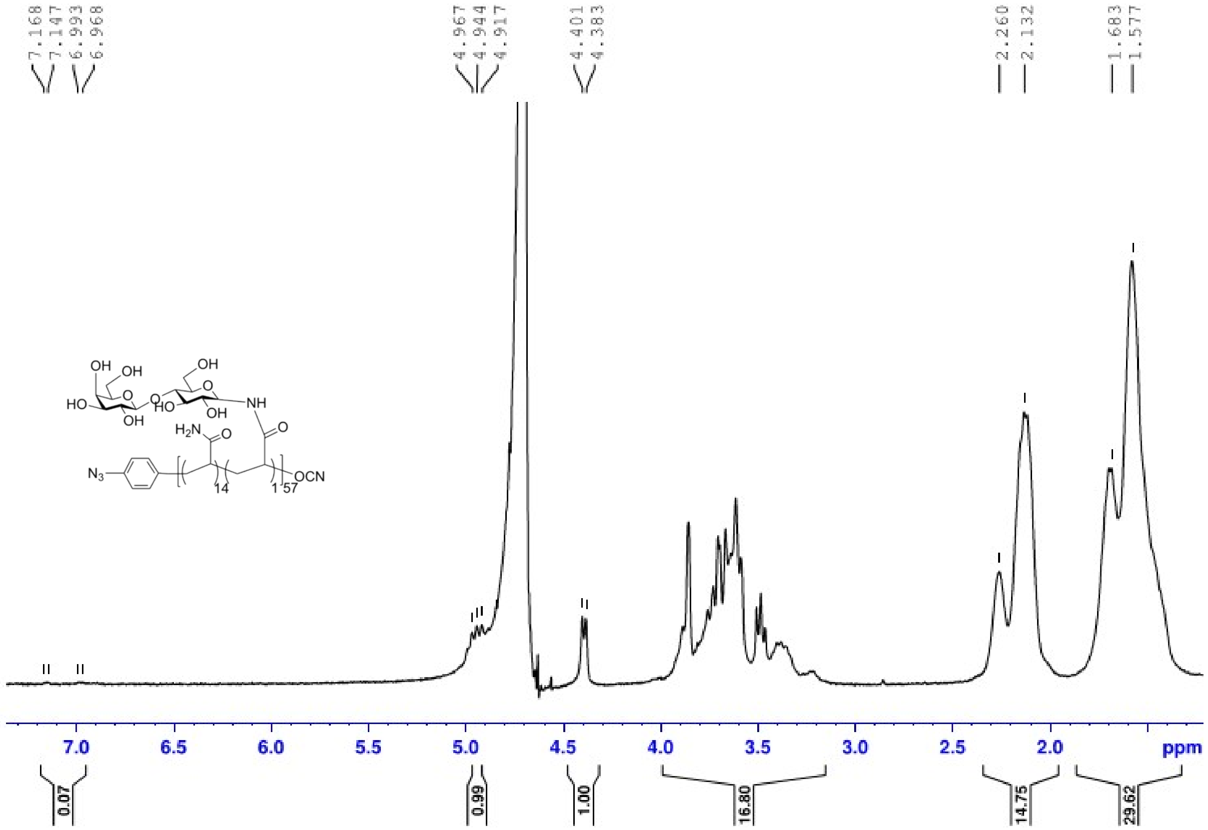

Fig. S5. ^{1}H NMR spectrum of lactosyl glycopolymer (**4c**) in D_2O.

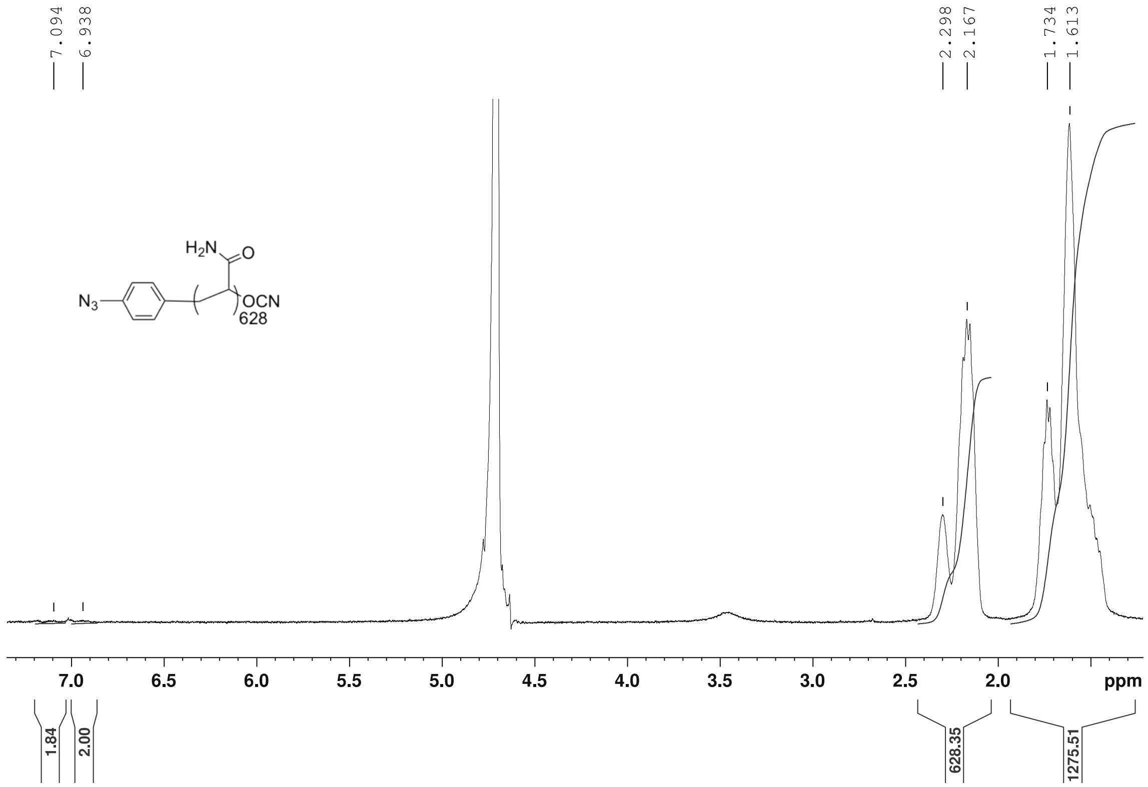

Fig. S6. ^{1}H NMR spectrum of polyacrylamide in D_2O.

Reference

1. Tang, J.; Ozhegov, E.; Liu, Y.; Wang, D.; Yao, X.; Sun, X. L. Straightforward Synthesis of N-Glycan Polymers from Free Glycans via Cyanoxyl Free Radical-Mediated Polymerization. *ACS Macro Lett.* **2017**, *6* (2), 107-111. https://doi.org/10.1021/acsmacrolett.6b00928.