Supporting Information

Facile Preparation of Hyperbranched Glycopolymer via AB_3* Inimer Promoted by a Hydroxy/Cerium (IV) Redox Process

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Experimental section

Materials.

Methyl α-D-glucoside, vinyl methacrylate and ceric ammonium nitrate were purchased from J&K Chemicals. Novozym 435 was purchased from Novozymes Biotech. 2,2-Azobis-(isobutyronitrile) (AIBN, Wako Chemicals) was recrystallized from ethanol before used. The solvent was dried and distilled by a standard process before used in synthesis or polymerization. Distilled water was used throughout the study. High-purity argon was used for degassing. The monomer 6-*O*-MMAGlc was synthesized according to the reference [1].

Syntheses

Synthesis of linear poly(6-O-MMAGlc)(P1).

6-*O*-MMAGlc (262 mg, 1.0 mmol) were dissolved in 5 mL dimethylsulfoxide. The solution was degassed under room temperature and then AIBN (8 mg, 0.05 mmol) was added under argon atmosphere. The polymerization was heated at 70 ºC for 5 h and then precipitated in acetone to give **P1** as a white solid. ^{1}H NMR (600 MHz, CD_3SOCD_3, δ): 5.16 (H-12), 4.96 (H-13), 4.82 (H-14), 4.59 (H-1), 4.18 and 3.81 (H6), 3.57 (H-5), 3.33 (H-7), 3.27 (H-2), 3.04 (H-4), 1.75 (H-10'), 0.95 and 0.81 (H-11).

Synthesis of hyperbranched poly(6-O-MMAGlc)(P2-P6).

A typical procedure is described below. 6-*O*-MMAGlc (131 mg, 0.5 mmol) were dissolved in 2.5 mL DMSO. The solution was degassed under room temperature and then ceric ammonium nitrate (274 mg, 0.5 mmol) was added under argon atmosphere.

The system was heated at 35 ºC for 4 h and then precipitated into excess acetone, centrifuged, and washed thoroughly by acetone to give P6 as a white solid. **P2**: ^{1}H NMR (600 MHz, CD_3SOCD_3, δ): 5.16 (H-12), 4.95 (H-13), 4.81 (H-14), 4.59 (H-1), 4.18 and 3.79 (H6), 3.91 (H-15), 3.55 (H-5), 3.32 (H-7), 3.26 (H-2), 3.03 (H-4), 1.76 (H-10'), 0.96 and 0.82 (H-11); **P3**: ^{1}H NMR (600 MHz, CD_3SOCD_3, δ): 5.14 (H-12), 4.93 (H-13), 4.79 (H-14), 4.59 (H-1), 4.18 and 3.80 (H6), 3.91 (H-15), 3.57 (H-5), 3.33 (H-7), 3.26 (H-2), 3.03 (H-4), 1.74 (H-10'), 0.97 and 0.81 (H-11); **P4**: ^{1}H NMR (600 MHz, CD_3SOCD_3, δ): 5.18 (H-12), 4.97 (H-13), 4.84 (H-14), 4.59 (H-1), 4.17 and 3.78 (H6), 3.92 (H-15), 3.55 (H-5), 3.32 (H-7), 3.26 (H-2), 3.04 (H-4), 1.77 (H-10'), 0.95 and 0.81 (H-11); **P5**: ^{1}H NMR (600 MHz, CD_3SOCD_3, δ): 5.16 (H-12), 4.97 (H-13), 4.82 (H-14), 4.59 (H-1), 4.16 and 3.78 (H6), 3.91 (H-15), 3.56 (H-5), 3.32 (H-7), 3.26 (H-2), 3.03 (H-4), 1.78 (H-10'), 0.98 and 0.81 (H-11); **P6**: ^{1}H NMR (600 MHz, CD_3SOCD_3, δ): 5.17 (H-12), 4.98 (H-13), 4.83 (H-14), 4.59 (H-1), 4.16 and 3.79 (H6), 3.93 (H-15), 3.56 (H-5), 3.32 (H-7), 3.26 (H-2), 3.04 (H-4), 1.75 (H-10'), 0.96 and 0.80 (H-11).

Dialysis

The dialysis bags (Green Bird Biotech, CE) with the molecular weight cut off MWCO of 1000 Da were used to purify the obtained glycopolymers. The isolated products were obtained by dialysis for 5 days. 500 mL deionized water was used and changed per 12 h. The solution in bags was collected and freeze-dried to give the product. **P6***: ^{1}H NMR (600 MHz, CD_3SOCD_3, δ): 5.15 (H-12), 4.94 (H-13), 4.82 (H-14), 4.59 (H-1), 4.17 and 3.83 (H6), 3.92 (H-15), 3.56 (H-5), 3.40 (H-7), 3.26 (H-2), 3.03 (H-4), 1.73 (H-10'), 0.98 and 0.81 (H-11);

Characterizations

^{1}H (600 MHz) NMR and ^{13}C (150 MHz) NMR spectra were performed on a Bruker AV600 spectrometer using tetramethylsilane (TMS) as internal standard.

The molecular weight, polydispersity and intrinsic viscosity [η] were determined by Viscotek 270-doul detector-Size Exclusion Chromatography which equipped with differential refractive index (RI), viscometer, a Waters Wat011545 gel permeation chromatographic column and two-angle light scattering (LS) triplet detectors. For LS, the laser wavelength was 530 nm. 0.1 mol/L $NaNO_3$ aqueous solution was used as eluent at a flow rate 1.0 mL/min, and the Polyethylene oxide std-PEO was used for calibration at 30 ^{o}C. The product dissolved in 0.1 mol/L $NaNO_3$ solution, and passed through 0.2 μm filter before injection. The OmniSEC software was used for data processing. The $M_{w,\ MALLS}$ were given by inputting the specific refractive index increments (dn/dC) which were collected from Brookhaven BI-DNDC Differential Refractometer. The dn/dC value of the prepared polymers in aqueous solution were determined as 0.132-0.137 mL/g.

The dynamic light scattering (DLS) measurement was performed by using a

commercialized spectrometer from Brookhaven Instrument Corporation (BI-200SM Goniometer, Holtsville, NY). A 17 mW He−Ne laser (Research Electro-Optics, Inc.) operating at 633 nm was used as the light source. The intensity−intensity time correlation function $G^{(2)}(\tau)$ in the self-beating mode was measured. A Laplace inversion program, CONTIN, was used to process the data to obtain the line width distribution ($G(\Gamma)$) and diffusion coefficient (D). The diffusion coefficient D and $G(\Gamma)$ can be further converted into the hydrodynamic radius R_h and $f(R_h)$ by using the Stokes−Einstein equation, respectively:

$$D = \frac{k_B T}{6\pi\eta R_h} \tag{S1}$$

where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the viscosity of the solvent, respectively.

Lysozyme sample preparation

Lysozyme sample was prepared by dissolving lysozyme in hydrochloric acid (pH=2.0) containing 140 mM NaCl and 0.01% (w/v) NaN_3 with or without poly(6-*O*-MMAGlc). The prepared solution were incubated at 55 °C for 168 h without stirring. The formation of the fibril was monitored by ThT fluorescence assay.

ThT fluorescence measurment:

Thioflavin T (ThT) stock solution of 10 μM was prepared by dissolving ThT in phosphate buffer solution (pH=7.4). Incubated lysozyme sample of 60 μL was diluted with 2 mL ThT stock solution and the ThT fluorescence measurement was conducted by exciting samples at 440 nm and recording the emission intensity at 486 nm on an F-7000 FL spectrophotometer. Fluorescence measurements were performed in a quartz cuvette with 1 cm excitation light path. Excitation slits and emission slits were set to 20 nm and 10 nm, respectively. If I_0 was the ThT fluorescence intensity in absence of polymer and I was the ThT fluorescence intensity in presence of polymer, the inhibition ratio (C) could be calculated according to following equation:

$$C = \frac{I_0 - I}{I_0} \tag{S2}$$

NMR and GPC Data

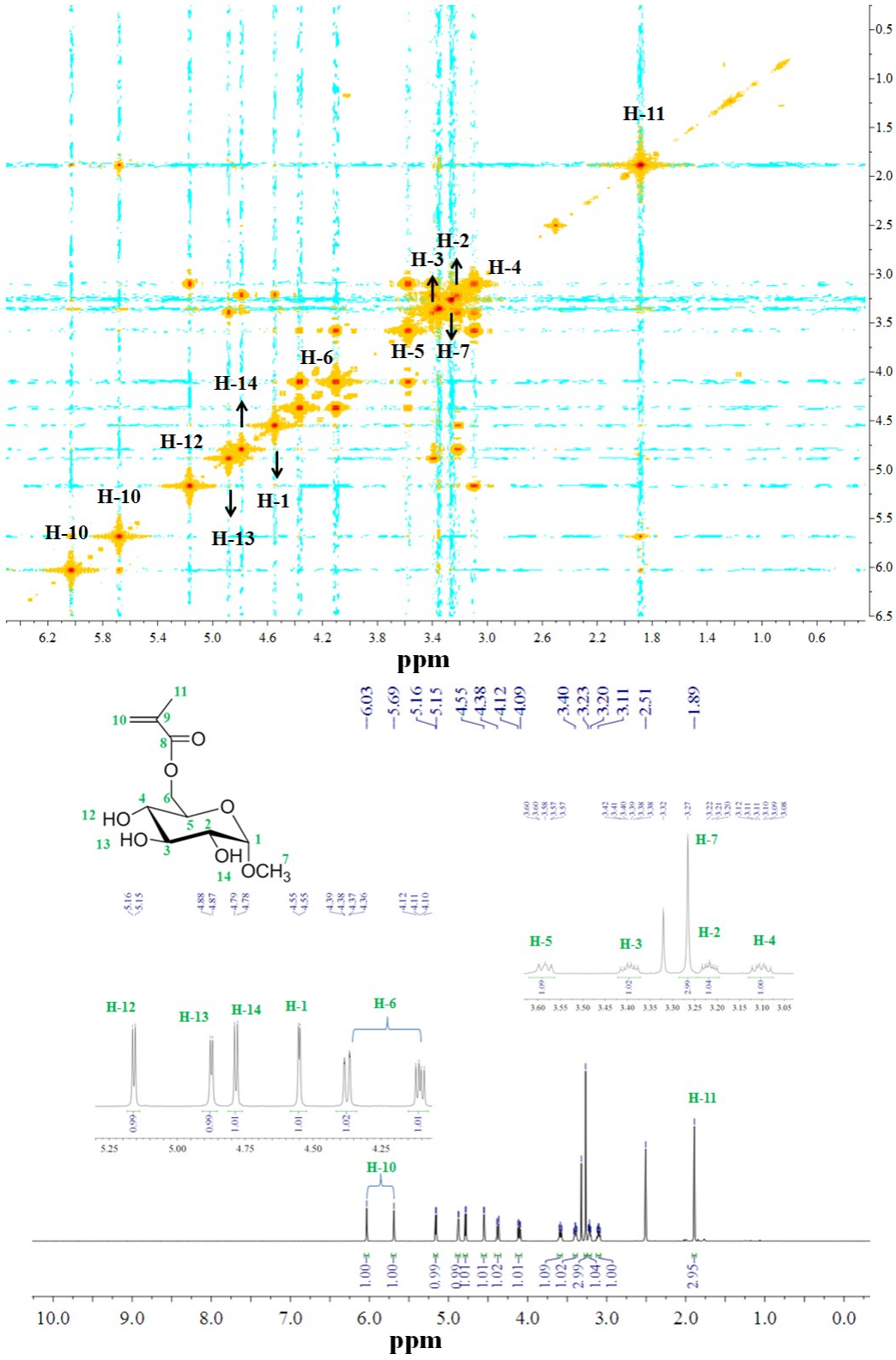

Fig.S1 The ^{1}H-^{1}H cosy and ^{1}H NMR spectrum of 6-*O*-MMAGlc measured in CD_3SOCD_3.

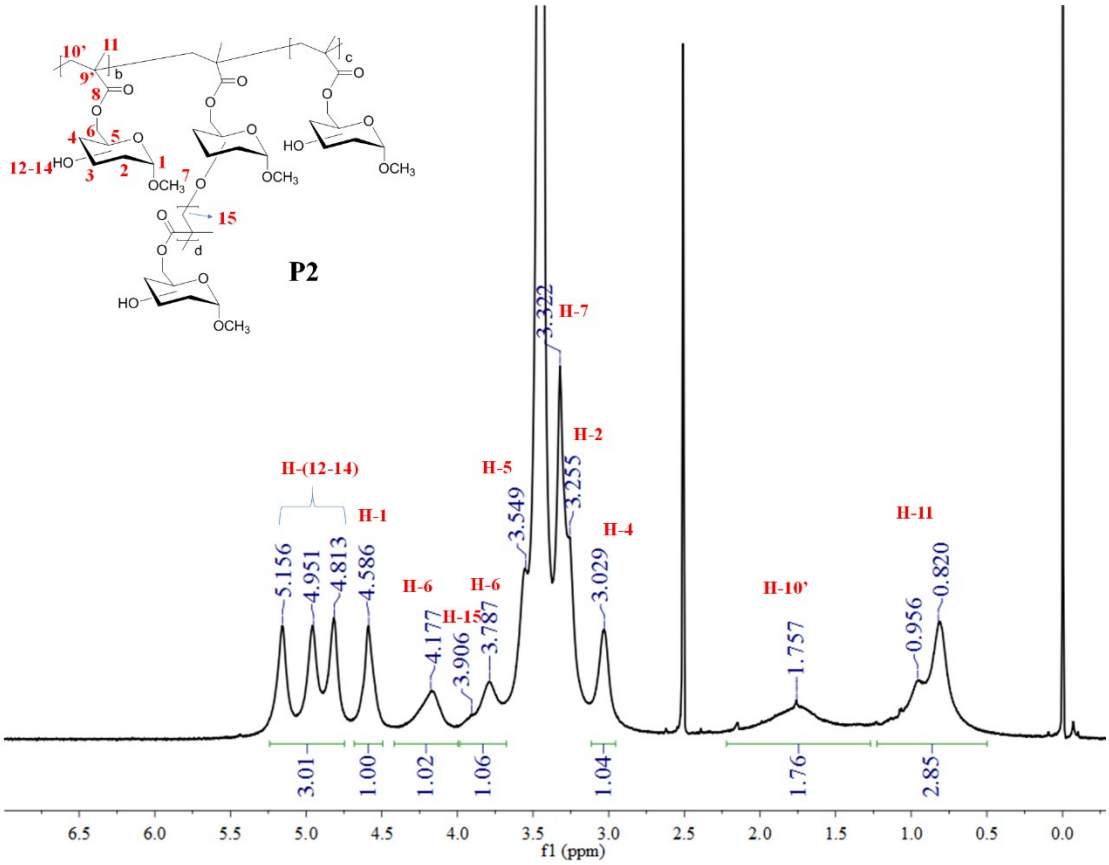

Fig.S2 The ^{1}H NMR spectrum of P2 measured in CD_3SOCD_3.

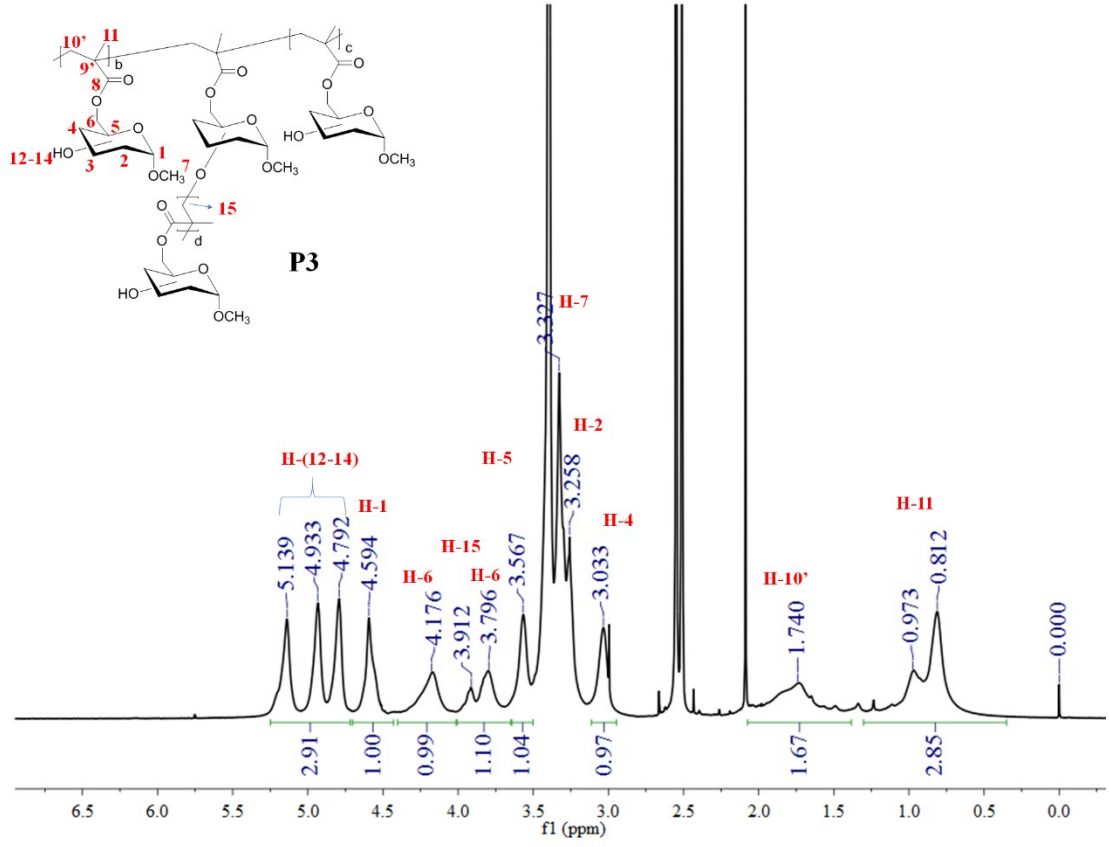

Fig.S3 The ^{1}H NMR spectrum of P3 measured in CD_3SOCD_3.

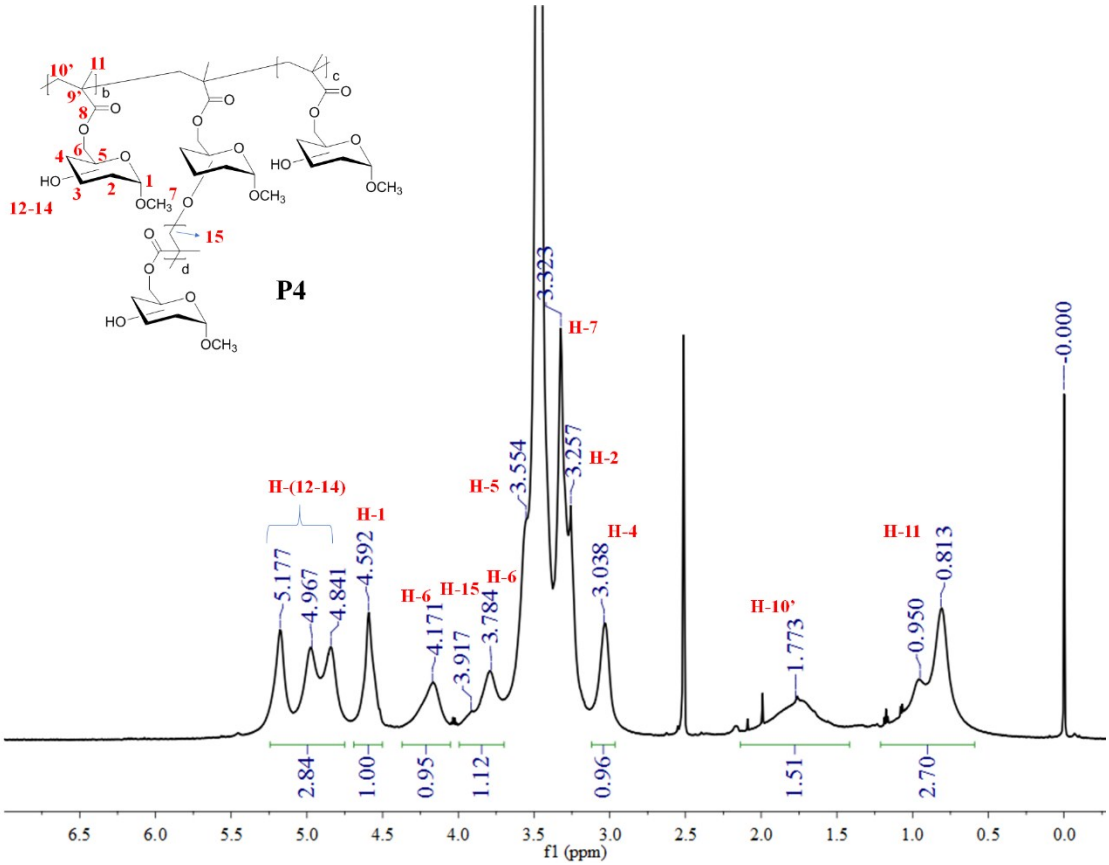

Fig.S4 The ^{1}H NMR spectrum of P4 measured in CD_3SOCD_3.

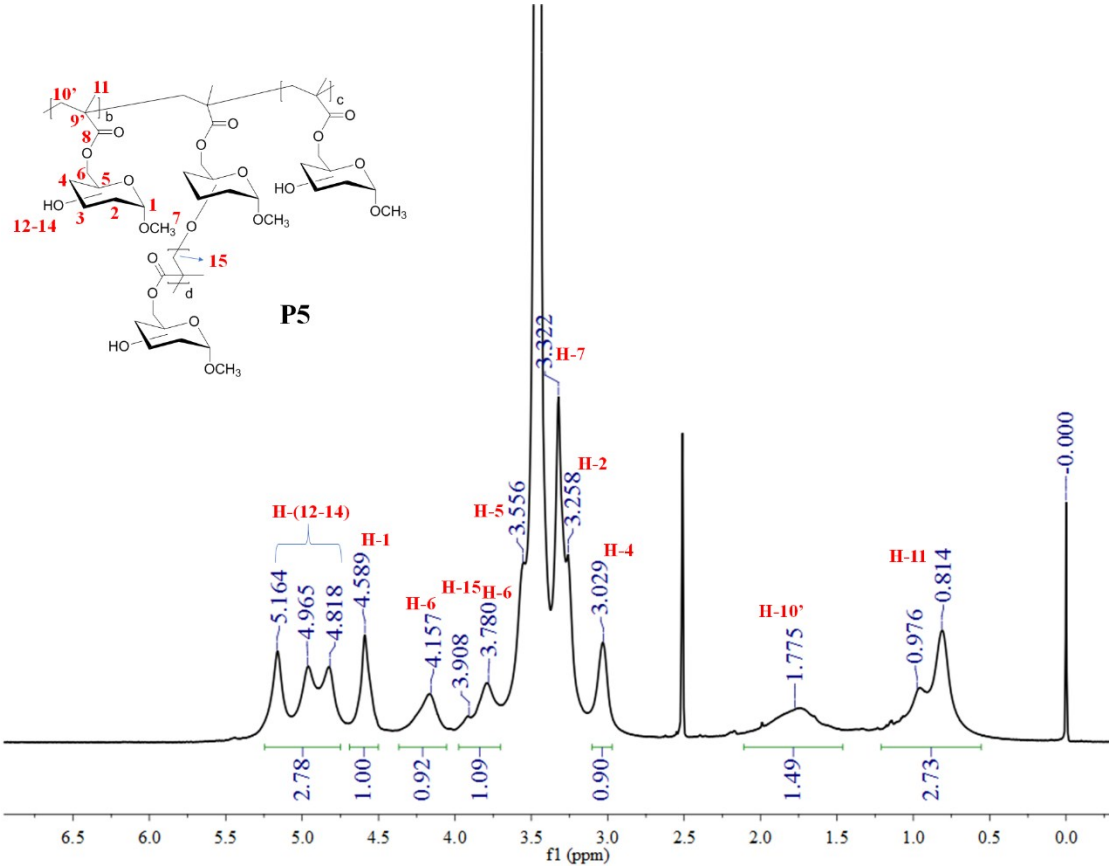

Fig.S5 The ^{1}H NMR spectrum of P5 measured in CD_3SOCD_3.

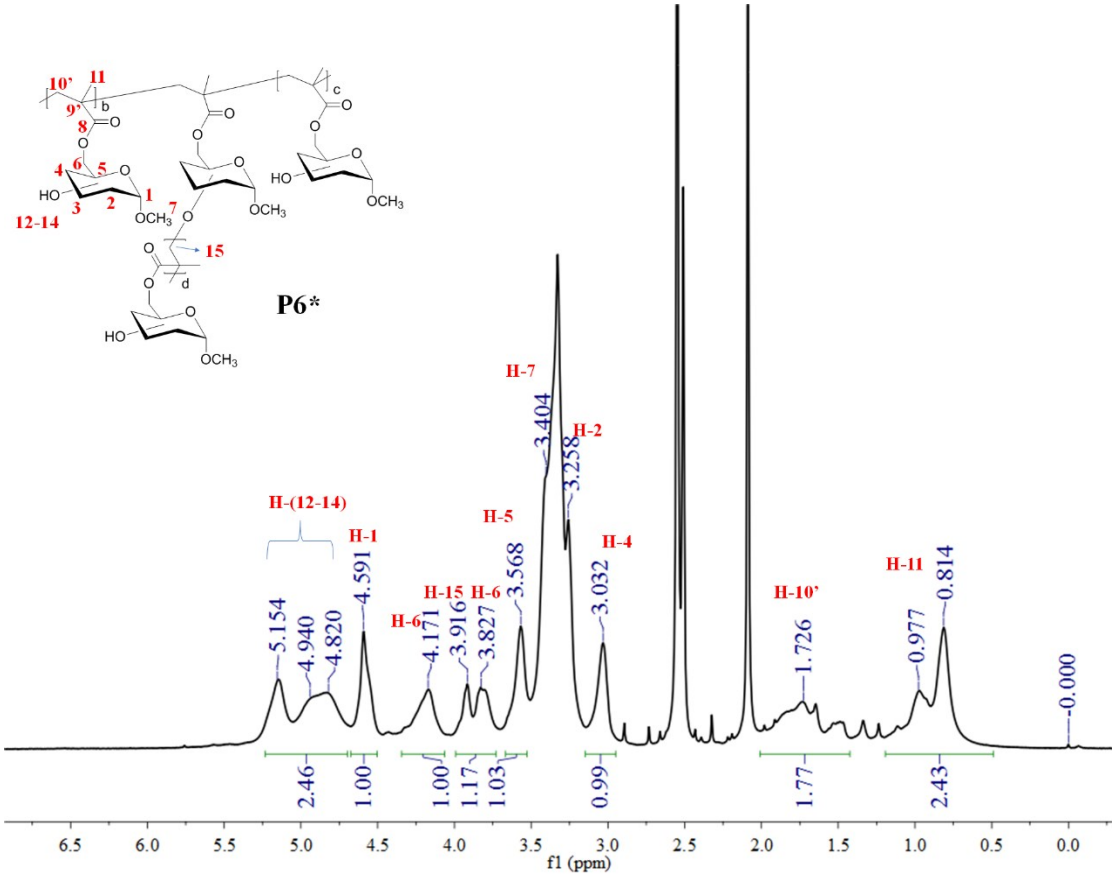

Fig.S6 The ^{1}H NMR spectrum of P6* measured in CD_3SOCD_3.

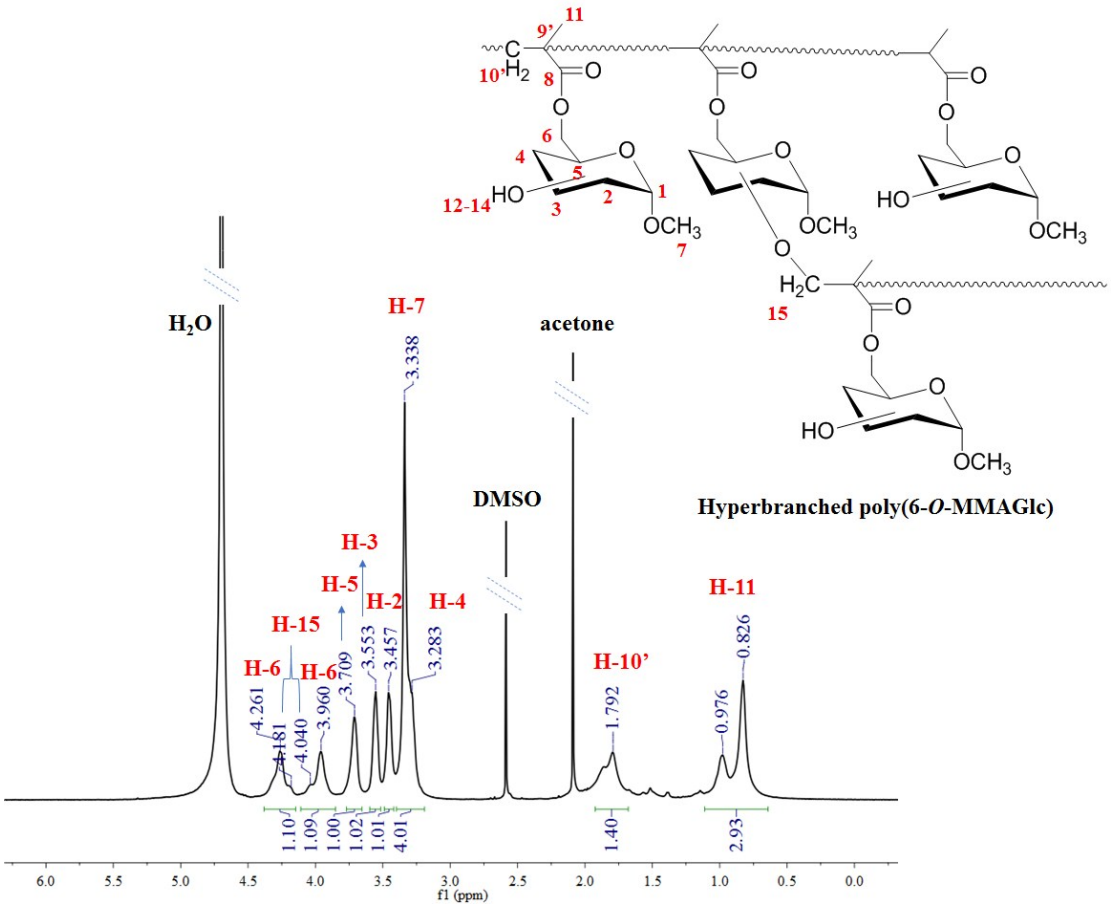

Fig.S7. ^{1}H NMR spectrum of P6 performed in D_2O

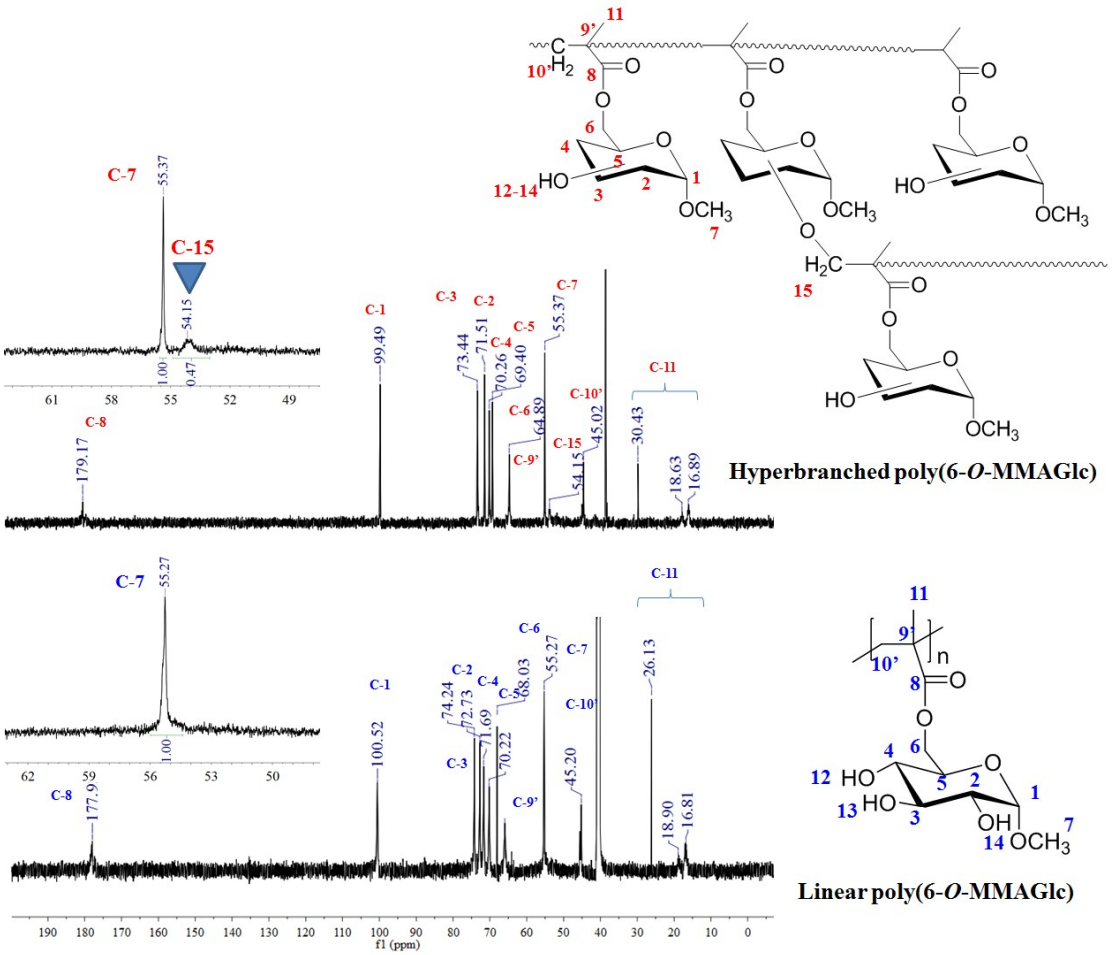

Fig.S8. ^{13}C NMR spectra of P1 and P6 performed in DMSO-d_6 with decoupling.

Calculation of the *DB* value based on the ^{13}C NMR spectra with decoupling

The resonances in the ^{13}C NMR spectrum of P1 with decoupling performed in DMSO-d_6 have been completely assigned, while the newly emerged peak at 54.15 ppm in the ^{13}C NMR spectrum of P6 can be attributed to the Carbon-15 (H. Bazin, A. Bouchu and G. Descotes, *J. Carbohydr. Chem.*, 1995, **14**, 1187-1207).

As shown in manuscript, the *DB* value can be given as:

$$DB = \frac{BP}{3DP_{mol}} \tag{S3}$$

where *BP* was denoted as a number of branching points, DP_{mol} was denoted as overall number of incorporated monomer units.

As a result, the $3DP_{mol}$ in eq.(2) is equivalent to $3A_{C7}$. The *BP* in eq.(2) is equal to the amount of Carbon-15 groups, which can be calculated by A_{C15}. So the *DB* in eq.(2) can be rewritten as follows for our ^{13}C NMR measurement:

$$DB = \frac{A_{C15}}{3A_{C7}} \quad \text{(S4)}$$

According to the eq.(4), *DB* value of P1 is calculated as 0, which is in accordance with result calculated by ^{1}H NMR measurement. As for P6, the *DB* value is calculated as 0.16 based on eq.(4), which is closed to that calculated by ^{1}H NMR measurement (0.18).

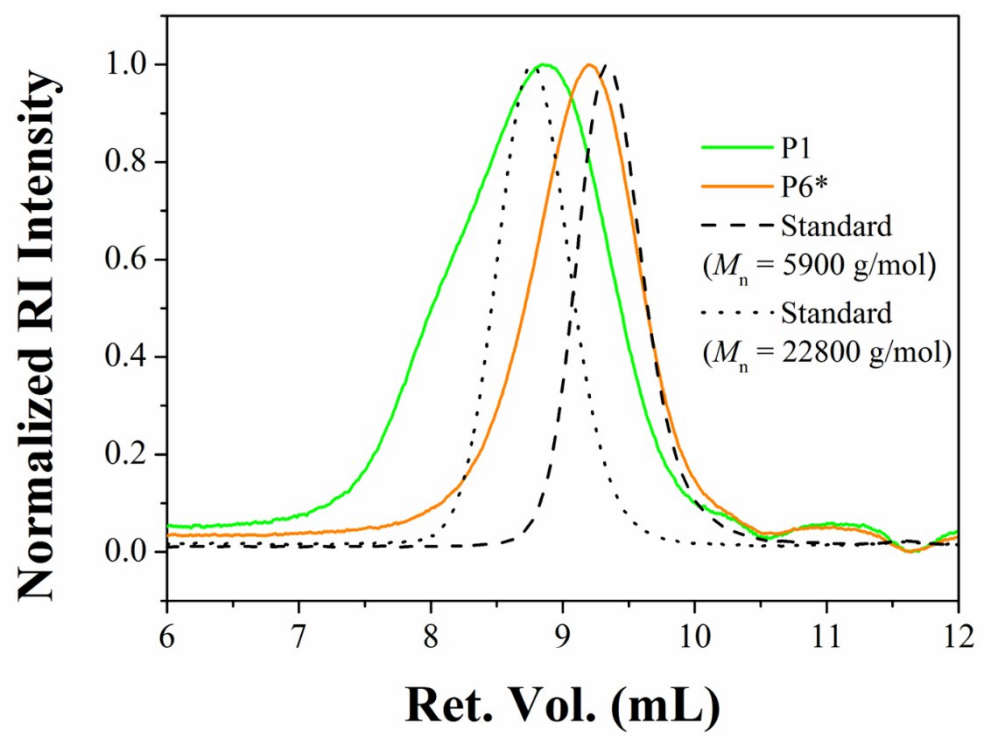

Fig.S9 Evolution of the GPC traces (RI signal) for the linear poly(6-*O*-MMAGlc) (P1), hyperbranched poly(6-*O*-MMAGlc) (P6*) and standard samples.

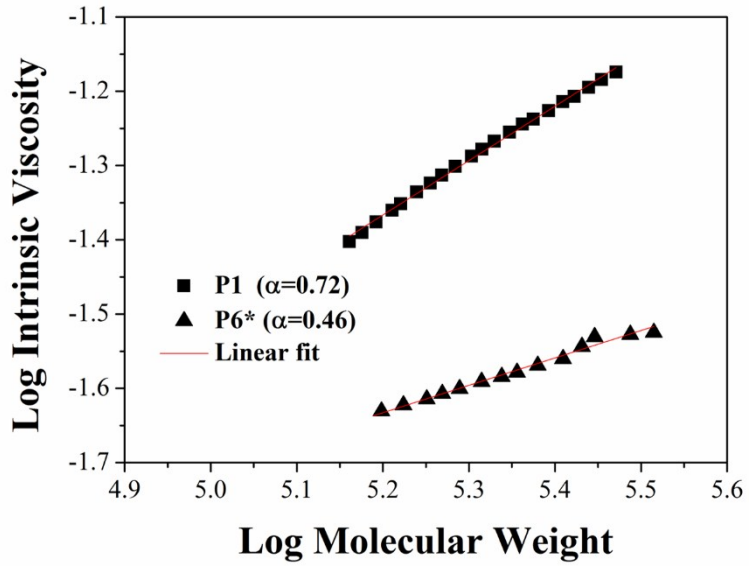

Fig.S10 The calculation of Mark-Houwink exponents (α) of P1 and P6* by plotting Log Intrinsic Viscosity to Log Molecular Weight.

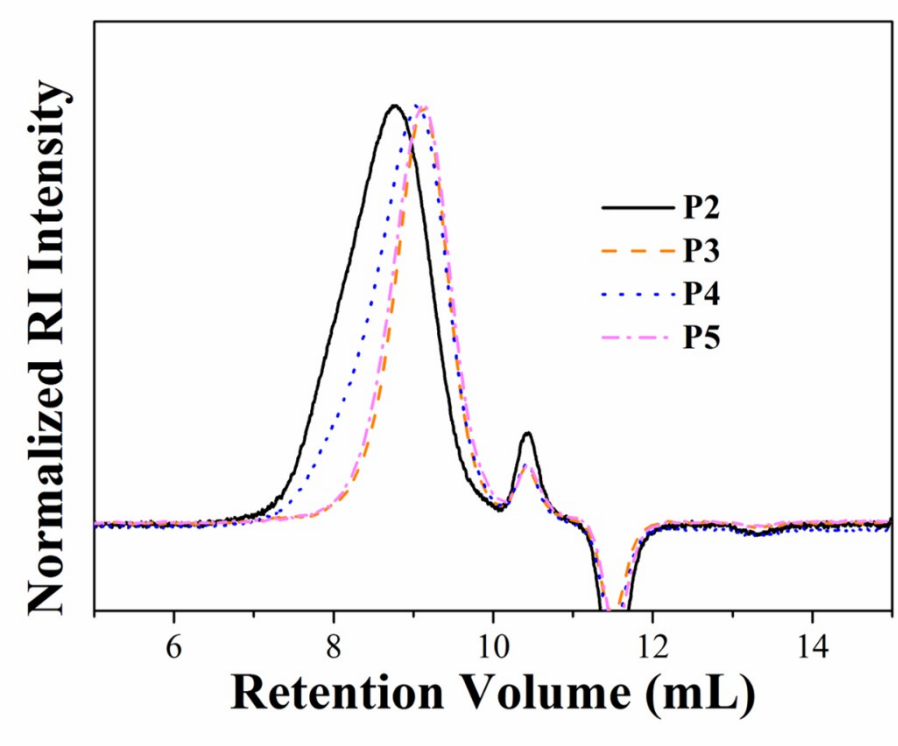

Fig.S11. Evolution of the GPC traces (RI signal) for P2-P5.

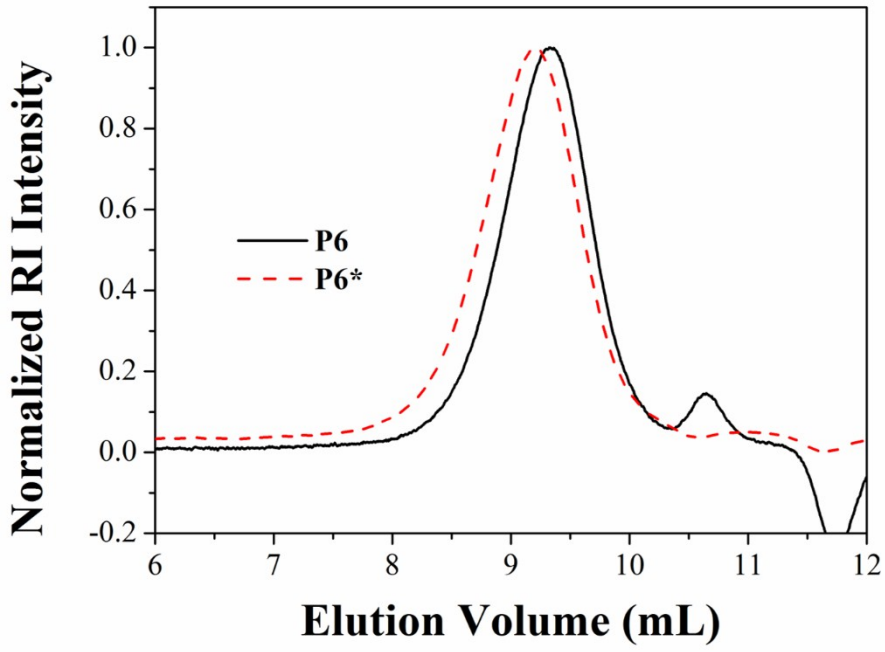

Fig.S12. Evolution of the GPC traces before (P6) and after dialysis (P6*)

Real-Time ^{1}H NMR measurement

In order to monitor the consumption of the vinyl groups over the reaction and quantify the amount of unreacted vinyl groups, we performed a series of ^{1}H NMR measurement in real-time. The reaction was conducted in CD_3SOCD_3 in which the feed ratio of Ce(IV) to 6-*O*-MMAGlc is 1:1.

The sample solution was withdrawn at 5 min, 10 min, 20 min, 30 min, 45 min, 60 min, 80 min, 100 min and 120 min and subjected into NMR for analysis immediately. The ^{1}H NMR spectra are shown in Fig.S11 without any purification or separation procedures. During the reaction, the peaks of the protons in vinyl groups (H-10, 6.03 and 5.69 ppm) shift to a higher magnetic field (1.86 ppm), implying the transformation of vinyl groups into alkyl chain. At the same time, the peak of protons in methyl groups (H11, 1.89 ppm) also shift to a higher magnetic field at 0.80 ppm. The consumption (%) of the vinyl groups, equal to the conversion rate (%) of the vinyl units into polymer chains, was calculated by defining the integration of H5 as 1.0 (3.54 ppm). The consumption increases with the reaction proceeds and exceeds 90 % after 100 min (Fig.S14).

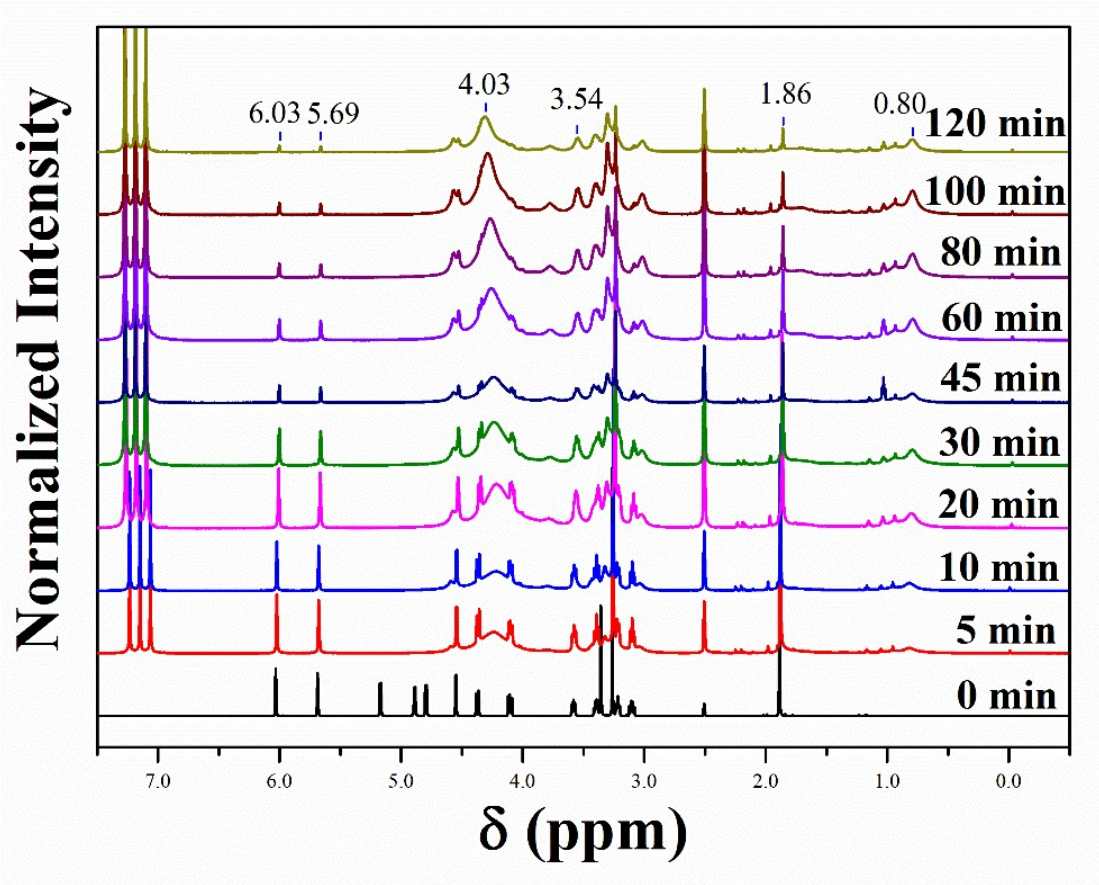

Fig.S13. ^{1}H NMR spectra of the reaction solution withdrawn at given times

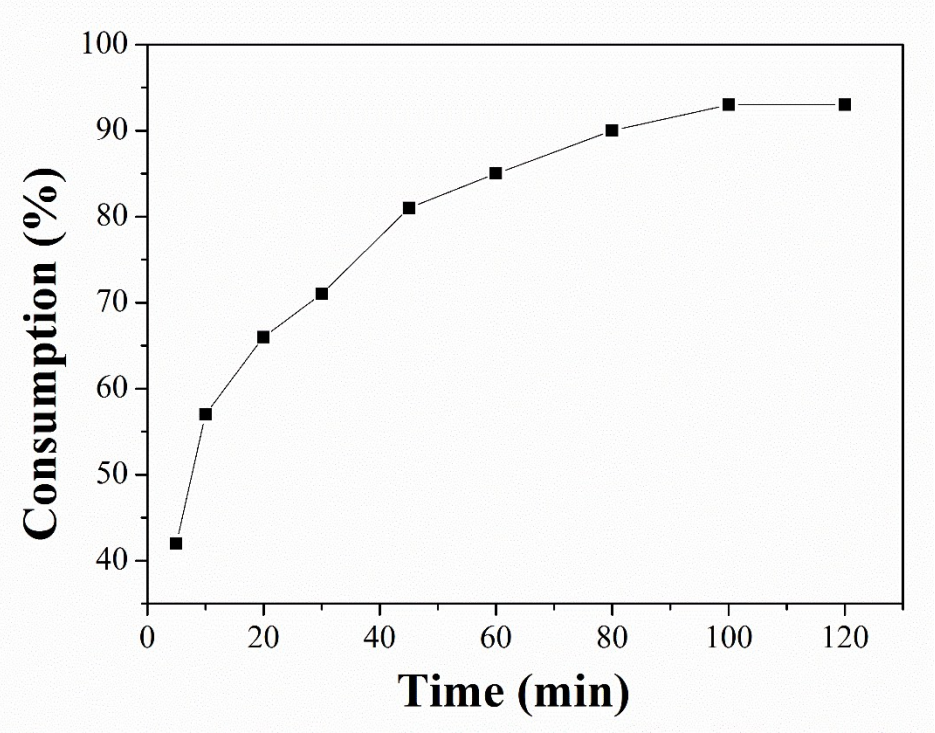

Fig.S14. Plots of the Consumption (%) of the vinyl groups *vs* the reaction time

Reference

1. Albertin, L.; Stenzel, M.; Barner-Kowollik, C.; Foster, L.; Davis, T., Well-Defined Glycopolymers from RAFT Polymerization: Poly(Methyl 6-*O*-methacryloyl-α-D-glucoside) and Its Block Copolymer with 2-Hydroxyethyl Methacrylate. *Macromolecules* **2004,** *37* (20), 7530-7537.