Supporting information

of

Glyco-stereoisomerism effect on hydrogelation of interacting polymers *via* dynamic covalent bond

Mingchang Lin, Pengfei Sun, Guosong Chen*, Ming Jiang

The Key Laboratory of Molecular Engineering of Polymers, Ministry of Education and Department of Macromolecular Science, Fudan University, Shanghai, 200433 China

Materials. Mannose and galactose were purchased from Shanghai Bangcheng chemical Co., Ltd. PEO 6K and tBMA was purchased from Sinopharm Chemical Reagent co. Ltd. 1,1,4,7,7-Pentamethyldiethylene triamine (PMDETA) (97%) and anisole were purchased from J&K Scientific Ltd. CuCl (99.99+%), CuBr (99.99+%) and glycidyl methacrylate (GMA, 98%) were purchased from Sigma-Aldrich. Anisole was distilled from calcium hydride (CaH₂) and stored under argon. N-Isopropylacrylamide (NIPAM) purchased from Tokyo Kasei Kagyo Co. and was recrystallized three times from benzene/hexane (65:35 v/v) prior to use. Azobisisobutyronitrile (AIBN, CP) supplied by Sinopharm Chemical before Reagent Co. was recrystallized from ethanol use. DMP (2dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid) was synthesized following procedures in literature.¹ GMA and tBMA were passed through a column of alumina to remove inhibitor and dried over CaH₂. It was then distilled under reduced pressure and stored under argon. THF and toluene were purified by distillation from sodium in the presence of benzophenone. DCM, DMF and THF 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 UV-light. HEPES buffer (20 mM, pH 7.4) was prepared by NaCl (50 mM), CaCl₂ (5 mM) and MnCl₂ (5 mM). All reactions were performed under nitrogen atmosphere. 1-(2'-propargyl)-α-D-mannose, 1-(2'-propargyl)-D-glucose ($\alpha:\beta = 10:4$), 1-(2'-propargyl) D-galactose ($\alpha:\beta = 10:3$), were synthesized according to the previous literatures² with their anomeric assignment reported in our previous study (¹H NMR in Figure S23)³. AABOB monomer was synthesized according to our previous reported procedures⁴.

Characterization. ¹H 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 columns, 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. The steady-state fluorescence measurements were recorded on a FLS 920 (EdinburgInstruments) spectro fluorophotometer. The Fourier transform infrared (FTIR) spectra were collected by a Nicolet 6700 spectrometer. The sample powder was mixed with KBr and tableting. QCM-D measurements were performed with a Q-Sense E4 system (Biolin Scientific AB). The rheological behavior of the samples was measured by a Bohlin GeminiHRnano Rheology equipment (Malvern, UK), fitted with a parallel plate (diameter of 40 mm) with the gap distance between the two parallel plates fixed at 0.15 mm and circulating environmental system for temperature control. Scanning electron microscopy (SEM) images were collected by a Field-Emission Scanning Electron Microscope (Ultra 55). The sample was prepared by coating the freeze-dried hydrogel with gold for 30 s.



Scheme S1 Synthetic procedures of the three glycopolymers PMan, PGal and PGlc.

Synthesis of glycopolymer precursor PGMA

PGMA was synthesized by ATRP according to the previous procedure⁵. Typically, a Schlenk tube was charged with 50 mg (0.25 mmol) initiator ethyl 2-bromoisobutyrate, 25 mg (0.174 mmol) CuBr, 10 mL (77.1 mmol) degassed GMA monomer and 9 mL anisole before the tube was sealed with a rubber septum. After three freeze-pump-thaw cycles, the glass reactor was immersed in an oil bath at 35 °C. After 5 min, 40 μ L (0.2 mmol) PMDETA was quickly injected into the tube to carry out polymerization. After 3 h, the

polymerization was quenched by liquid nitrogen. The sample was first passed through a column of neutral alumina to remove the catalyst. Then the eluent was concentrated and precipitated in diethyl ether for three times. The final product was obtained after drying under vacuum (4.8 g, 44%). ¹H NMR (CDCl₃, 400 MHz, δ): 4.30, 3.83 (d, -OCO-CH₂-), 3.23 (s, -CH-CH₂-O-), 2.84, 2.64 (d, -CH-CH₂-O-), 2.2-1.8 (br, -CH₂-C(CH₃)), 1.7-0.9 (br, -CH₂C(CH₃)).Based on GPC result, M_n of **PGMA** is calculated as 1.33×10^4 g/mol.



Figure S1. GPC of PGMA and PGMA-N₃.



Figure S2. ¹H NMR of PGMA in CDCl₃

Synthesis of PGMA-N₃

Ring-opening of PGMA with sodium azide was performed according to the previous procedure². Typically, 4.5 g PGMA (31.66 mmol of epoxide moieties) was dissolved in 80 mL *N*,*N*-dimethylformamide (DMF), 6.96 g (107.06 mmol) sodium azide and 5.64 g (107.06 mmol) ammonium chloride were added to the solution, and the mixture was stirred at 50 °C for 26 h. After removal of most DMF by rotavap, the residue was precipitated in water for three times. The final product was obtained after drying under vacuum (5.27 g, yield: 90 %). M_n of **PGMA-N₃** is measured as 2.88×10^4 g/mol by GPC. ¹H NMR (DMSO-*d*₆, 400 MHz, δ): 5.47 (s, -OCO-CH₂-CH(*OH*)-CH₂-N₃), 3.86 (d, -OCO-CH₂-CH(OH)-CH₂-N₃), 3.32 (s, -OCO-CH₂-CH(OH)-CH₂-N₃), 2.0-1.7 (br, -CH₂-C(CH₃)), 1.7 -0.6 (br, -CH₂C(CH₃)).



Figure S3. ¹H NMR of PGMA-N₃ in DMSO- d_6 .

Synthesis of Glycopolymer (PGlc/PGal/PMan)

The three glycopolymers are prepared from $PGMA-N_3$ via the same method. The preparation of PGal is described in detail as an example. A Schlenk tube was charged with 1 g (5.4 mmol of N₃-) PGMA-N₃, 1.7 g (1.4 equiv.) 1-(2'-propargyl)-D-galactose, 90 mg (7.8 mmol) CuBr and 30 mL DMF, before the tube was sealed with a rubber

septum. After three freeze-pump-thaw cycles, the glass reactor was immersed in an oil bath at 45 °C. After 10 min, 280 μ L (1.4 mmol) PMDETA was quickly injected into the tube to carry out polymerization. After 24 h, the polymerization was quenched by liquid nitrogen, and the mixture was dialyzed against water in a dialysis bag (MWCO 7000 Da) for 7 d. The final product was obtained after freeze-drying (1.76 g, yield: 65 %). ¹H NMR spectra of **PGal**, **PMan** and **PGIc** are shown in Figure S4-6.



Figure S4. ¹H NMR of PGlc in D₂O.



Figure S5. ¹H NMR of PGal in D₂O.



Figure S6. ¹H NMR of PMan in D₂O.



Figure S7 FT-IR Spectra of PGlc, PGal and PMan.

Synthesis of PNIPAm-co-PBOB (PBOB)

AABOB (0.203 g, 1 mmol), NIPAm (2.14 g, 19 mmol) and AIBN (20 mg, 0.1 mmol) were dissolved in dioxane (20 mL) in a sealed vial. The vial was deoxygenated with nitrogen for approximately 30 min and then placed in a preheated oil bath at 70 °C. The polymerization was quenched after 8 h, 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 (2.206 g, yield: 91%). From GPC result, the Mn was determined as 8600 g/mol (PDI = 3.17).



Figure S8. ¹H NMR of PBOB.



Figure S9. GPC of PBOB.

Hydrogel preparation. PBOB was mixed with the corresponding glycopolymer directly. To prepare the **PBOB** stock solution (100 mg/mL in H_2O , pH about 8.0), a tiny amount of base (0.1 M NaOH) was used first to dissolve the polymer. pH of the PBOB stock solution was test by pH paper.

The glycopolymer stock solution (100 mg/mL) was prepared by dissolving directly glycopolymer in PBS buffer (pH 7.4, salt concentration: 100 mM).

Then hydrogels are prepared simply by mixing 1 mL glycopolymer (100 mg/mL in PBS buffer (pH 7.4, salt concentration: 100 mM)) with 1 mL **PBOB** (100 mg/mL in H₂O, pH about 8.0) at room temperature. pH of the resulting gelation solution was always measured after mixing.

Rheology measurement. The rheological behavior of the samples was measured by a Bohlin GeminiHRnano Rheology equipment (Malvern, UK), fitted with a parallel plate (diameter of 40 mm) with the gap distance between the two parallel plates fixed at 0.15 mm and circulating environmental system for temperature control. The heating process was investigated by increasing temperature at a rate of 1 °C/min. Dynamic rheology measurements were performed by oscillatory frequency sweeps (strain-controlled) at the oscillation frequency of $0.1 \sim 1$ Hz and deformation 1%. The G', G'' and viscosity

variation data were collected in the same dynamic rheology experiment with the same temperature increase rate. Strain sweeps were performed prior to frequency sweeps to ensure that the % strain used was in the linear viscoelastic regime (Figure S22).



Figure S10. Picture of hydrogels Glc-5, Gal-5, Man-5.



Figure S11. Dynamic rheology measurement of viscosity of (a) Man-10, Gal-10, Glc-10 and (b) Man-5, Gal-5, Glc-5 as a function of temperature (constant shear frequency: 1 Hz).



Scheme S2. Chemical structure of N-PBOB and glycopolymers with RhB attachment.

Preparation of sample for FLS: To prepare the PNIPAm-co-PBOB-co-NBD stock solution (0.5 mg/mL in H_2O , pH about 8.0), a tiny amount of base (0.1 M NaOH) was used first to dissolve the polymer. pH of the PBOB stock solution was test by pH paper. The glycopolymer stock solution (0.5 mg/mL) was prepared by dissolving directly glycopolymer in deionized water.

The sample for FLS are prepared simply by mixing 1 mL glycopolymer (0.5 mg/mL in DIW) with 1 mL PNIPAm-co-PBOB-co-NBD (0.5 mg/mL in H_2O_7 pH about 8.0) at room temperature under vigorous stirring. Then 2 mL PBS buffer (pH 7.4, salt concentration: 100 mM) was added. pH of the resulting micelles solution was always measured after mixing.



Figure S12. Picture of sample Glc-1, Gal-1 and Man-1 after different mixing times (0 hr and 4 hr).



Figure S13. Pictures of hydrogels Glc-10 (1:3), Gal-10 (1:3), Man-10 (1:3). Weight ratio of hydrogel is 1:3 (glycopolymer :PBOB).





Figure S14. Pictures of hydrogel of Glc-10, Gal-10, Man-10 at different time.



Figure S15. SEM image of Man-10.



Figure S16. SEM image of Gal-10.



Figure S17. SEM image of Glc-10.



Figure S18. SEM image of Man-5.



Figure S19. SEM image of Man-1 4 hr



Figure S20. SEM image of Man-1 8 hr.

In vitro Cytotoxicity^{5b}

The *in vitro* cytotoxicity of our materials was evaluated by MTT assay with Hep G2 cells. The cells were seeded in a 96-well plates containing 100 μ L DMEM supplemented with 10% FBS at an initial density of 1 × 10⁴ cells per well. The cells were incubated at 37 °C under an atmosphere of 95% relative humidity and 5% CO₂ for 24 h. Then the cell culture media was replaced with fresh medium containing **PBOB/PGal** complex at different concentrations. After 24 h, 20 μ L of 5 mg mL⁻¹ MTT solution in PBS was added to each well and the plates were further incubated for another 4 h. Then the supernatant was removed by centrifugation for 10 min, and 200 μ L DMSO was added to each well. The optical absorbance was measured in a Multiskan MK3 microplate reader (Thermo Scientific) at 492 nm. Cells without nanoparticle treatment were used as control.



Figure S21. Cell viability of Hep G2 cells after incubation with **PBOB/PGal** complex (1:1) at different concentrations for 24 h. Black and white bar show different batches of the material. Standard derivation is obtained from three repeated experiments.



Figure S22. A strain sweep of (a) Man-10, Gal-10, Glc-10 and (b) Man-5, Gal-5, Glc-5 at 20 °C (shear frequency 1 Hz).



(a)



(b)



(c)

Figure S23. ¹H NMR of (a) 1-(2'-propargyl) D-galactopyranoside, (b) 1-(2'-propargyl)- α -D-mannopyranoside and (c) 1-(2'-propargyl) D-glucopyranoside, in MeOH- d_4 .

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