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Supplementary Information

One-pot chemoenzymatic synthesis of glycopolymers from unprotected sugars via glycosidase-catalysed glycosylation using triazinyl glycosides

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1. Materials

D-Galactose (Gal), lactose (Lac) monohydrate and 2,6-lutidine were purchased from Nacalai Tesque, INC. (Kyoto, Japan). 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) and *N*-(2-hydroxyethyl)acrylamide (HEAAm) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). *N*-(2-Hydroxyethyl)methacrylamide (HEMAAm) was purchased from Combi-Blocks Inc. (San Diego, USA). HEAAm and HEMAAm were used after purification by activated alumina column. 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). β-Galactosidase from *Aspergillus oryzae*, cellulose from *Trichoderma reesei*, fluorescein isothiocyanate (FITC)-labelled peanut agglutinin from *Arachis hypogaea* (PNA) and FITC-labelled bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co. LLC. (St. Louis, USA). All other reagents were commercially available and used without further purification.

2. Measurements

The NMR spectra were recorded using Bruker BioSpin AV-300 and AV-600 spectrometers. The ESI-MS spectra were recorded using a Bruker Daltonics micrOTOF Q-III spectrometer. The HPLC and GPC measurements were conducted using a system consisting of a JASCO PU-2089 pump and a JASCO CO-2065 column oven. A JASCO UV-2075 ultraviolet detector and a JASCO RI-2031 refractive index detector were used for the HPLC and GPC analyses, respectively. A 5C_{18}-MS-II column (4.6 × 250 mm, Nacalai Tesque, INC.) was used for the HPLC analysis. 5% or 4% acetonitrile (MeCN)-containing water was used as the eluent at a flow rate of 1.0 mL/min at 30 °C to analyse the enzymatic reaction using HEAAm and HEMAAm, respectively. A Shodex OHpak SB-804 HQ column (8.0 × 300 mm, Showa Denko K.K.) was used for the GPC analysis using a phosphate buffer (20 mM, pH 7.0) as the eluent at a flow rate of 0.5 mL/min at 30 °C. Pullulan samples were used as standards. The fluorescence intensity was recorded using a JASCO FP-6500 spectrometer.

3. Synthesis of DMT-Gal

Gal (900 mg, 5.0 mmol) was dissolved in water (20 mL) and the solution was kept overnight at room temperature to achieve an αβ-configuration equilibrium. DMT-MM (2.80 g, 10.0 mmol) and 2,6-lutidine (0.6 mL, 5.0 mmol) were then added to the solution, and the resulting mixture was stirred at room temperature for 24 h. After concentration of the reaction mixture under reduced pressure, the product was purified by silica gel column chromatography (ethyl acetate/methanol = 5/1) and recrystallised from methanol to yield

4,6-dimethoxy-1,3,5-triazin-2-yl β-D-galactopyranoside (DMT-Gal, 639 mg, 2.0 mmol, 40.0%).

^{1}H NMR (300 MHz, D_2O): δ (ppm) 5.78 (1H, d, H1, J = 7.5 Hz), 3.97–3.94 (7H, m, OCH_3 and H4), 3.80 (1H, m, H5), 3.73 (1H, d, H2), 3.69 (1H, dd, H3), 3.66 (2H, d, H6). ^{13}C NMR (75 MHz, D_2O): δ (ppm) 173.3 and 172.0 (triazine), 97.6 (C1), 76.1 (C5), 72.4 (C3), 69.7 (C2), 68.4 (C4), 60.7 (C6), 55.9 (OCH_3). ESI-MS: Found: m/z 342.063, Calcd. for $C_{11}H_{17}N_3NaO_8$ ($[M+Na]^+$): m/z 342.091.

4. Enzymatic synthesis of glycomonomers using DMT-Gal

A general procedure for an enzymatic reaction is described. A mixture of DMT-Gal (3.2 mg, 10 μmol), HEAAm (30 or 50 μmol) and β-galactosidase (0.1 U) in 0.2 mL of buffer containing MeCN (0, 10 and 20 vol%) was incubated at 30 °C. An acetate (50 mM, pH 5.0) and a phosphate buffer (50 mM, pH 6.0) were used for the enzymatic reaction. The reaction mixtures were analysed by HPLC and detected by UV at 214 nm. The products were isolated by preparative HPLC using a combined system of a JASCO PU-2086 pump, a JASCO CO-2065 column oven, and a JASCO UV-2075 ultraviolet detector (214 nm). A 5C_{18}-MS-II column (20 × 250 mm, Nacalai Tesque, INC.) was used and the eluent was introduced at a flow rate of 12.0 mL/min at 30 °C.

1a. ^{1}H NMR (600 MHz, D_2O): δ (ppm) 6.22 (1H, dd, CH=C$\underline{H}_2$ (*cis*)), 6.12 (1H, d, C$\underline{H}$=CH_2), 5.70 (1H, d, CH=C$\underline{H}_2$ (*trans*)), 4.34 (1H, d, H1, J = 7.8 Hz), 3.94 (1H, m, O-C$\underline{H}_2$-CH_2-N), 3.85 (1H, d, H4), 3.75 (1H, m, O-C$\underline{H}_2$-CH_2-N), 3.69 (2H, m, H6), 3.62 (1H, m, H5), 3.57 (1H, dd, H3), 3.50–3.41 (3H, m, H2 and O-CH_2-C$\underline{H}_2$-N). ^{13}C NMR (150 MHz, D_2O): δ (ppm) 168.7 (C=O), 129.9 (-$\underline{C}$H=CH_2), 127.4 (-CH=$\underline{C}H_2$), 103.0 (C1), 75.2 (C5), 72.7 (C3), 70.8 (C2), 68.6 (C4), 68.4 (O-$\underline{C}H_2$-CH_2-N), 61.0 (C6), 39.4 (O-CH_2-$\underline{C}H_2$-N). ESI-MS: Found: m/z 300.117, Calcd. for $C_{11}H_{19}NNaO_7$ ($[M+Na]^+$): m/z 300.106.

1b. ^{1}H NMR (600 MHz, D_2O): δ (ppm) 5.64 (1H, s, -C(CH_3)=C$\underline{H}_2$), 5.39 (1H, s, -C(CH_3)=C$\underline{H}_2$), 4.33 (1H, d, H1, J = 7.8 Hz), 3.94 (1H, m, O-C$\underline{H}_2$-CH_2-N), 3.85 (1H, d, H4), 3.83 (1H, s, O-C$\underline{H}_2$-CH_2-N), 3.74 (2H, m, H6), 3.69 (1H, m, H5), 3.62 (1H, m, H3), 3.50–3.36 (3H, m, H2 and O-CH_2-C$\underline{H}_2$-N), 1.86 (3H, s, -C(C$\underline{H}_3$)=CH_2). ^{13}C NMR (150 MHz, D_2O): δ (ppm) 172.1 (C=O), 139.1 (-$\underline{C}$(CH_3)=CH_2), 121.1 (-C(CH_3)=$\underline{C}H_2$), 103.1 (C1), 75.2 (C5), 72.7 (C3), 70.7 (C2), 68.6 (C4), 68.4 (O-$\underline{C}H_2$-CH_2-N), 61.0 (C6), 39.5 (O-CH_2-$\underline{C}H_2$-N), 17.6 (-C($\underline{C}H_3$)=CH_2). ESI-MS: Found: m/z 314.158, Calcd. for $C_{12}H_{21}NNaO_7$ ($[M+Na]^+$): m/z 314.122.

5. One-pot synthesis of glycopolymers from Gal

A general procedure for the one-pot synthesis of glycopolymers is described. Gal (6.8 mg,

37.5 μmol) was dissolved in water (0.15 mL) and the solution was kept at room temperature overnight to achieve an αβ-configuration equilibrium. DMT-MM (20.7 mg, 75 μmol) and 2,6-lutidine (4.2 μL, 37.5 μmol) were added to the mixture and the resulting solution was stirred at room temperature for 24 h. To the resulting mixture consisting of DMT-Gal, a phosphate buffer (250 μL, 100 mM, pH 6.0) containing 10% MeCN, HEAAm (11.5 mg, 0.1 mmol) and β-galactosidase (0.2 U) were added, followed by incubation at 30 °C for 16 h. After heating the reaction mixture at 85 °C for 5 min, VA-044 (0.6 mg, 2 μmol) was added. The resulting mixture was kept under nitrogen bubbling for 15 min, sealed *in vacuo* and heated at 44 °C for 2 h. The product polymer was purified by dialysis (Spectra/Por 7, MWCO 3500) against deionised water and freeze-dried to yield the desired glycopolymer.

2a. ^{1}H NMR (300 MHz, D_2O): δ (ppm) 4.3–4.2 (H1 of Gal), 4.0–3.6 (H2–6 of Gal), 3.7–3.5 (O-C$\underline{H_2}$-CH_2-N), 3.4–3.1 (O-CH_2-C$\underline{H_2}$-N), 2.2–1.8 ((-CH_2-C$\underline{H}$-)$_n$), 1.8–1.3 ((-C$\underline{H_2}$-CH-)$_n$).

2b. ^{1}H NMR (300 MHz, D_2O): δ (ppm) 4.3–4.2 (H1 of Gal), 4.0–3.6 (H2–6 of Gal), 3.6–3.5 (O-C$\underline{H_2}$-CH_2-N), 3.3–3.1 (O-CH_2-C$\underline{H_2}$-N), 2.0–1.5 ((-C$\underline{H_2}$-C(CH_3)-)$_n$), 1.1–0.7 ((-CH_2- C(C$\underline{H_3}$)-)$_n$).

6. One-pot synthesis of a glycopolymer from Lac

Lac monohydrate (13.5 mg, 37.5 μmol) was dissolved in water (0.15 mL) and the solution was kept at room temperature overnight to achieve an αβ-configuration equilibrium. DMT-MM (20.7 mg, 75 μmol) and 2,6-lutidine (4.2 μL, 37.5 μmol) were added to the mixture and the resulting solution was stirred at room temperature for 24 h. To the resulting mixture consisting of 4,6-dimethoxy-1,3,5-triazin-2-yl β-lactoside (DMT-Lac), a phosphate buffer (250 μL, 100 mM, pH 6.0) containing 10% MeCN, HEAAm (11.5 mg, 0.1 mmol) and cellulase (0.2 U) were added, followed by incubation at 37 °C for 6 h. After heating the reaction mixture at 85 °C for 5 min, VA-044 (0.6 mg, 2 μmol) was added. The resulting mixture was kept under nitrogen bubbling for 15 min, sealed *in vacuo* and heated at 44 °C for 2 h. The product polymer was purified by dialysis (Spectra/Por 7, MWCO 3500) against deionised water and freeze-dried to yield the desired glycopolymer.

2c. ^{1}H NMR (600 MHz, D_2O): δ (ppm) 4.5–4.3 (H1 and H1' of Lac), 4.0–3.4 (H2–6 and H2'–6' of Lac), 3.7–3.5 (O-C$\underline{H_2}$-CH_2-N), 3.4–3.1 (O-CH_2-C$\underline{H_2}$-N), 2.2–1.8 ((-CH_2-C$\underline{H}$-)$_n$), 1.8–1.3 ((-C$\underline{H_2}$-CH-)$_n$).

7. Lectin binding test

FITC-labelled PNA or BSA with a final concentration of 0.8 μM and glycopolymer with a final concentration of 10 μg/mL were mixed in 40 μL of PBS(+) and the mixture was

incubated in the dark at 30 °C for 16 h. After centrifugation, the fluorescence intensity of the supernatant was measured by a fluorescence spectrophotometer (λ_{ex} = 495 nm, λ_{em} = 520 nm).

8. NMR spectra

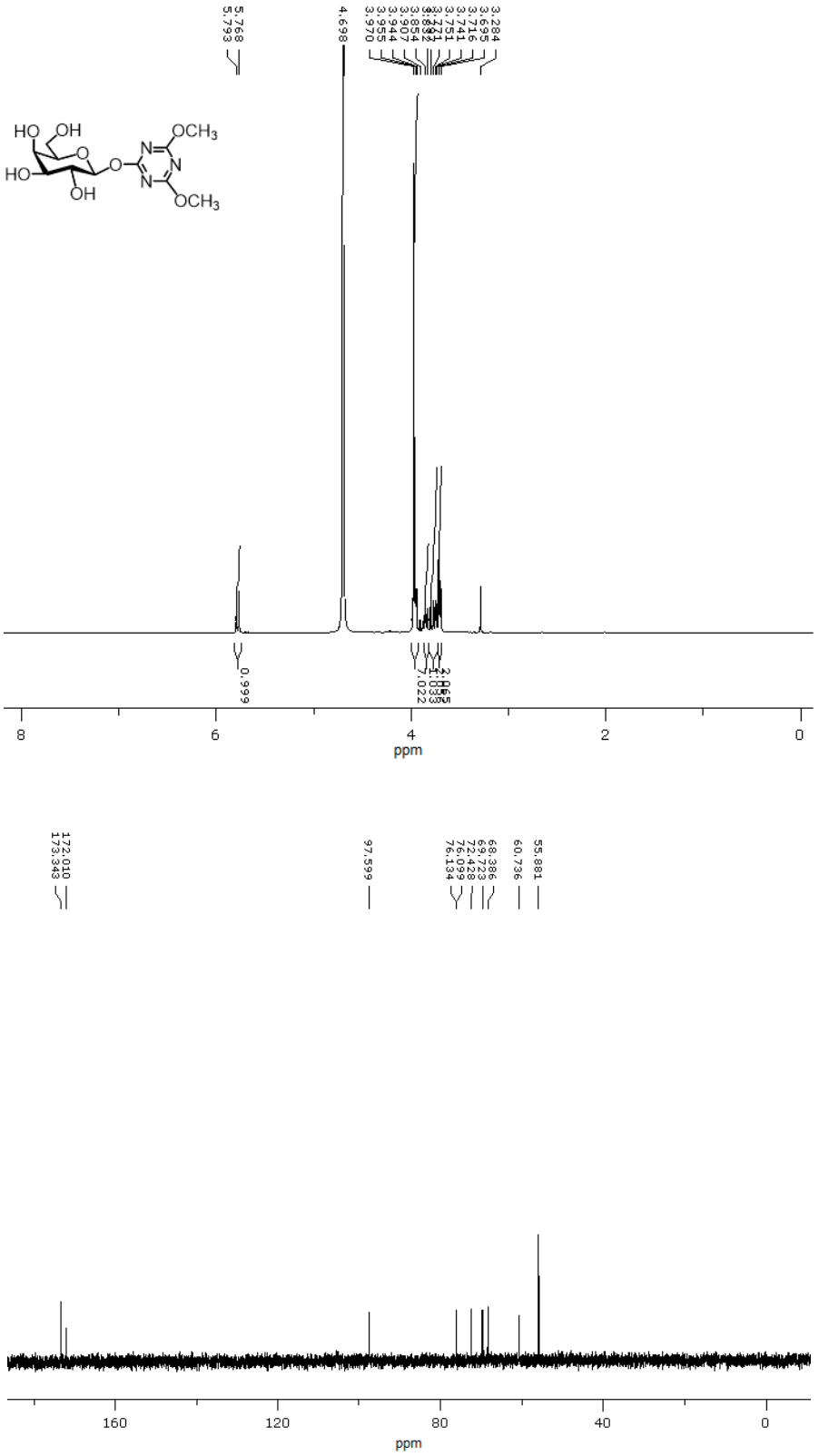

Fig. S1 ^{1}H and ^{13}C NMR spectra of DMT-Gal in D_2O.

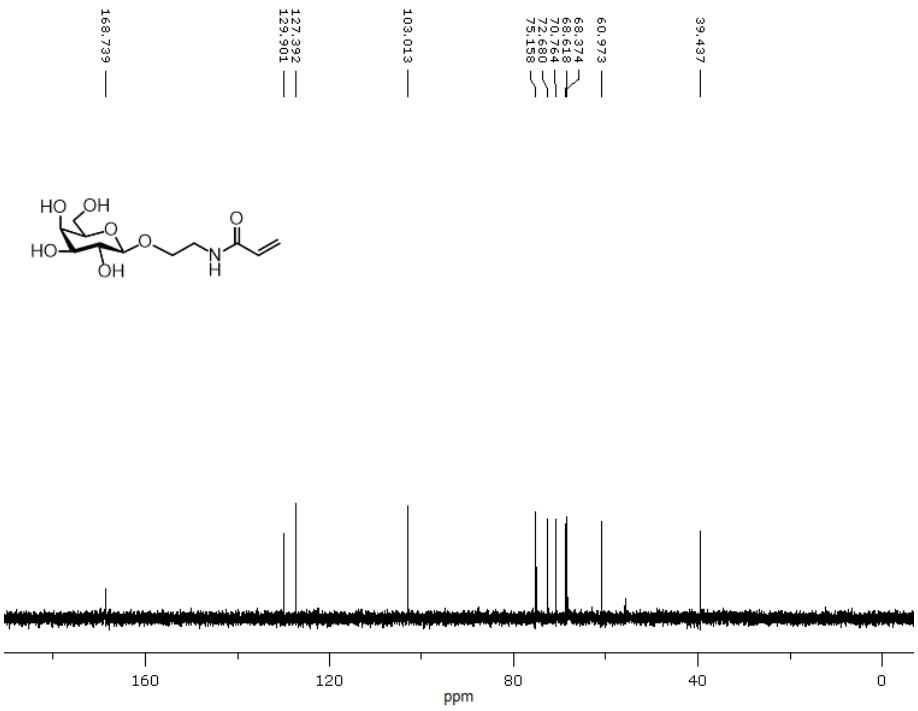

Fig. S2 ^{13}C NMR spectrum of **1a** in D_2O.

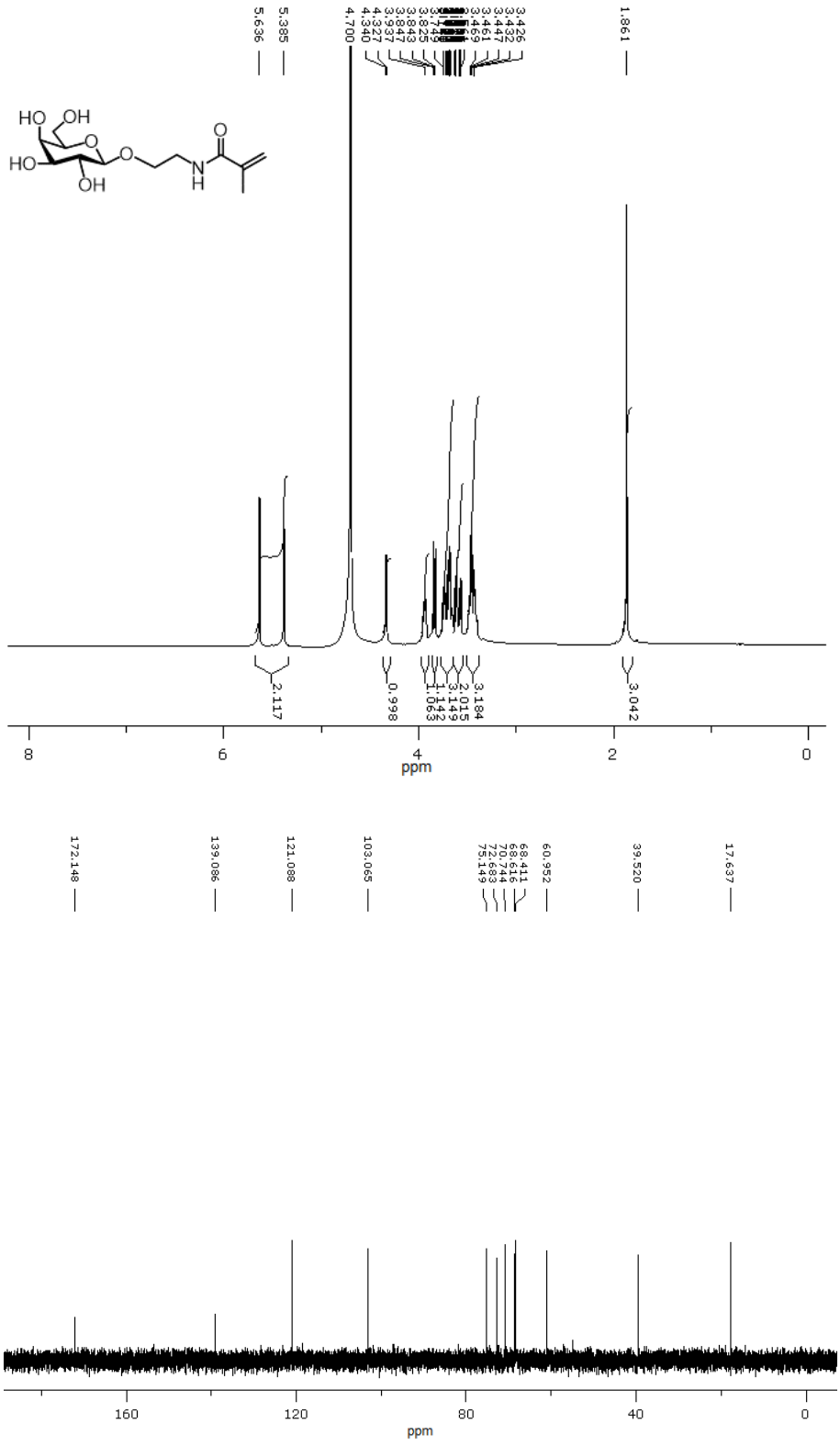

Fig. S3 ^{1}H and ^{13}C NMR spectra of **1b** in D_2O.

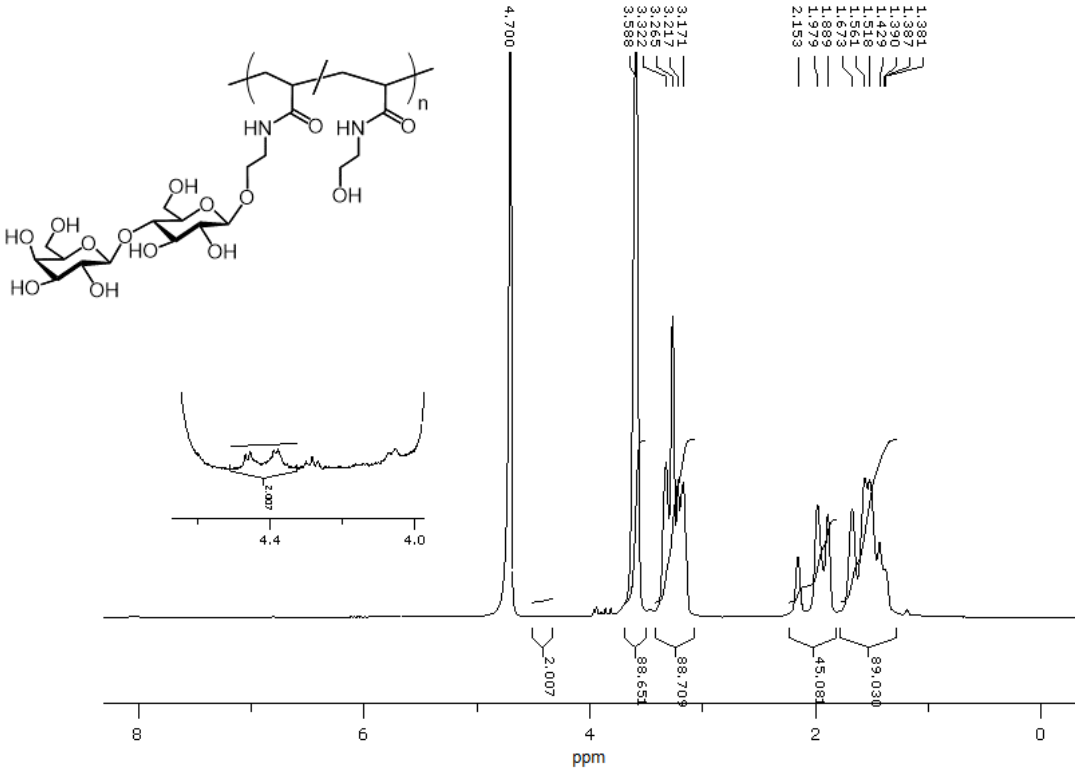

Fig. S4 ^{1}H NMR spectrum of Lac-bearing glycopolymer in D_2O.

9. HPLC chromatograms

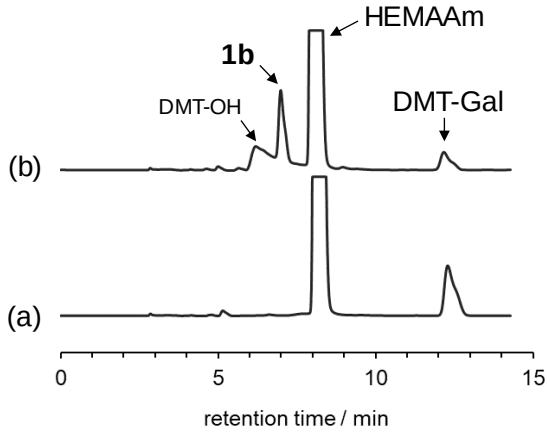

Fig. S5 HPLC chromatograms of the enzymatic reaction mixture for the synthesis of **1b** using DMT-Gal and five equivalents of HEMAAm in a buffer (pH 6) containing 10% MeCN. (a) 0 h and (b) 8 h.

10. GPC chromatograms

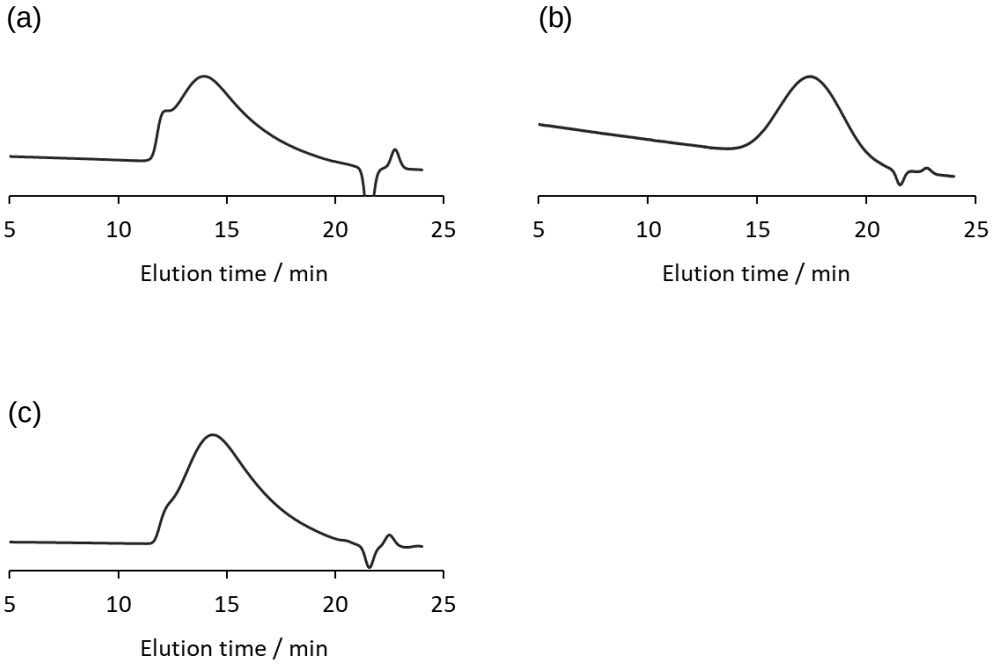

Fig. S6 GPC chromatograms of (a) **2a**, **2b** and (c) Lac-bearing glycopolymer.