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Synthetic construction of a Le^x determinant via gabriel amine synthesis and the glycopolymer involving highly clustered Le^x residues

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ABSTRACT

Synthetic construction of a Le^x determinant was accomplished via slightly modified Gabriel amine synthesis from three building blocks. Further transformations followed by polymerization of the Le^x derivative gave a glycopolymer having trisaccharidic units as pendant-type epitopes.

Lewis x determinant [Le^x; Galβ1 → 4(Fucα1 → 3)GlcNAc] is known as an extremely valuable core structure of glycoconjugates such as glycoproteins and glycolipids.[1] Synthetic assembly of the trisaccharide has been a very attractive challenge for synthetic chemists,[2] and the cluster-type trisaccharide is also very attractive from the point of view of biochemical as well as biomedical applications.[3] In our previous study,[4] synthesis of the Le^x trisaccharidic moiety and its polymer from a 1,6-anhydro-β-lactose as a key starting material was accomplished. In spite of the successful synthesis of a glycopolymer having trisaccharide moieties as pendant-type epitopes, density of the sugar moiety was low. Since the glycomonomer had an alkenyl moiety as polymerizable aglycon, polymerizable activity was moderate. Consequently a practical and versatile synthetic route for construction of a Le^x derivative as a glycomonomer having highly polymerizable potential is needed. In this Letter, we describe efficient preparation of trisaccharidic glycomonomer **1** via Gabriel amine synthesis[5] from three building blocks and the chemical conversion into a highly clustered glycopolymer.

Prior to construction of synthetic route for a Le^x determinant, 1,6-anhydro-β-mannnose[6] was selected as a key building block for GlcNAc residue as shown in Figure 1. Our synthetic plan, therefore, was convergent synthesis of the Le^x structure from D-galactose (Gal), L-fucose (Fuc), and D-mannose (Man) as key starting materials. Building blocks **A 2** and **B 3** were derived from Gal and Fuc, respectively, by means of Lewis acid-mediated glycosidation with 1-dodecanethiol (lauryl mercaptan).[7] Building block **C 4** was derived from 1,6-anhydro-β-Man via Gabriel amine synthesis[8] in order to shorten the typical synthetic route. Scheme 1 shows the preparation of each building block. A known β-acetogalactose **5** was allowed to react with lauryl mercaptan in the presence of BF_3–OEt_2 to furnish β-lauryl glycoside **2**[9] as building block **A** in 94.8% yield. Since building block **B 3** had to be a fully benzylated derivative in order to avoid neighboring participation by ester function in *O*-glycoside formation, protective groups of the fucosyl derivative were converted from acetate into benzylate. Thus, a known anomeric mixture **6** having an α:β ratio of 14:1 was treated with 4 M equiv of lauryl mercaptan by means of mediation of 1.5 M equiv of BF_3–OEt_2 to afford thioglycoside **7**[9] in 86.9% yield after chromatographic purification, which showed an anomeric mixture having α:β ratio of 20:1 according to the results of ^{1}H NMR. The acetate **7** underwent transesterification to provide corresponding alcohol **8**, which was further manipulated by Williamson ether synthesis to give benzyl ether **3** as building block **B** in 81.1% yield (two steps). For the preparation of building block **C**, Gabriel amine synthesis was preformed on a known alcohol **9**.[6] Thus **9** was converted into triflate **10** as an unstable intermediate, which was immediately treated with potassium phthalimide to provide phthaloyl derivative **11** as crystals in 84.7% yield (two steps),[†] $[\alpha]_D^{28}$ +34.7 (*c* 1.00, $CHCl_3$), mp 176.5–177.2 °C, ^{1}H NMR ($CDCl_3$) δ 5.47 (s, 1 H, H-1), 4.73 (d, 1 H, $J_{3,4}$ = 7.6 Hz, H-4), 4.30 (dd, 1 H, $J_{2,3}$ = 9.6 Hz, H-3), 4.15 (d, 1 H, H-2), and 2.16 (s, 3 H, Ac). The S_N2 reaction at C-2 by the anion from the phthalimide as the nucleophile involved Walden's inversion and gave a corresponding glucosaminyl

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† All new compounds with the specific rotation data gave satisfactory results of elemental analyses.

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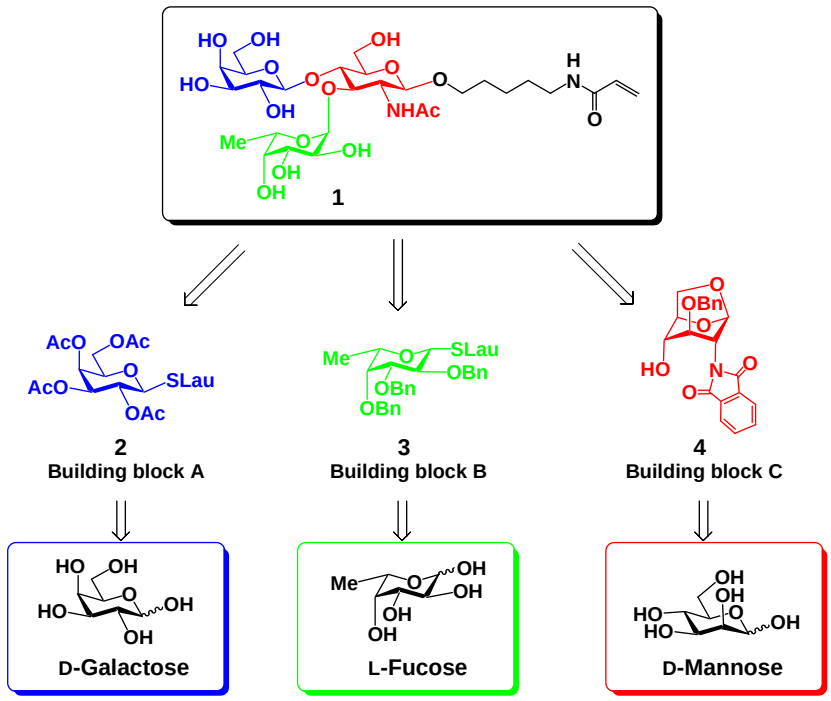
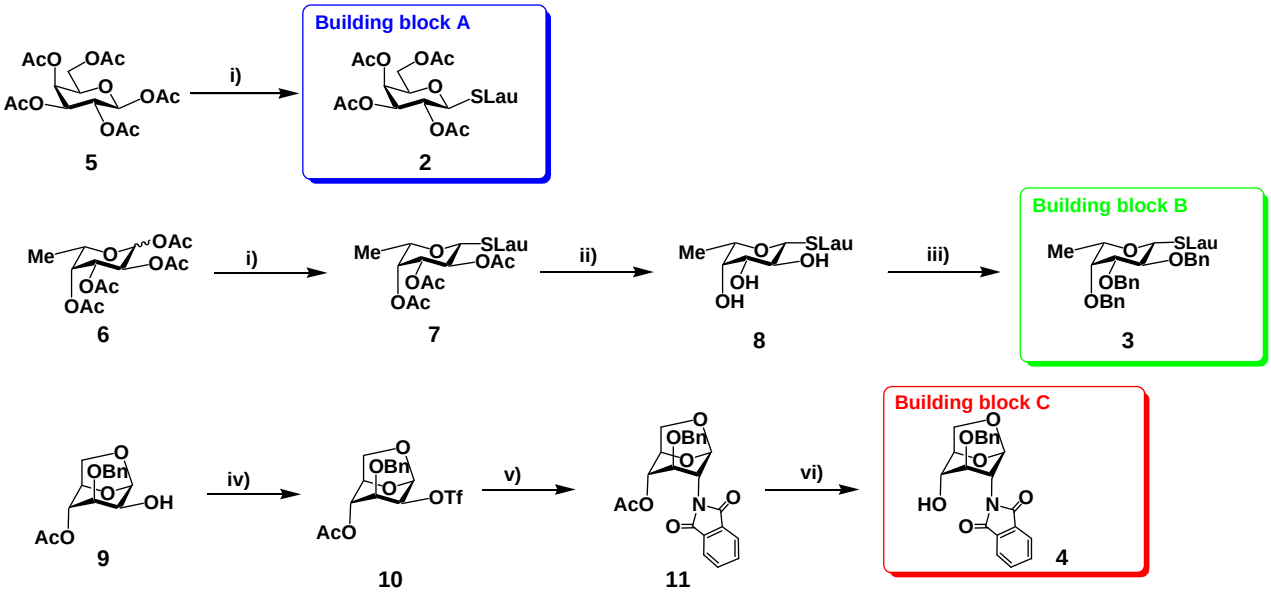


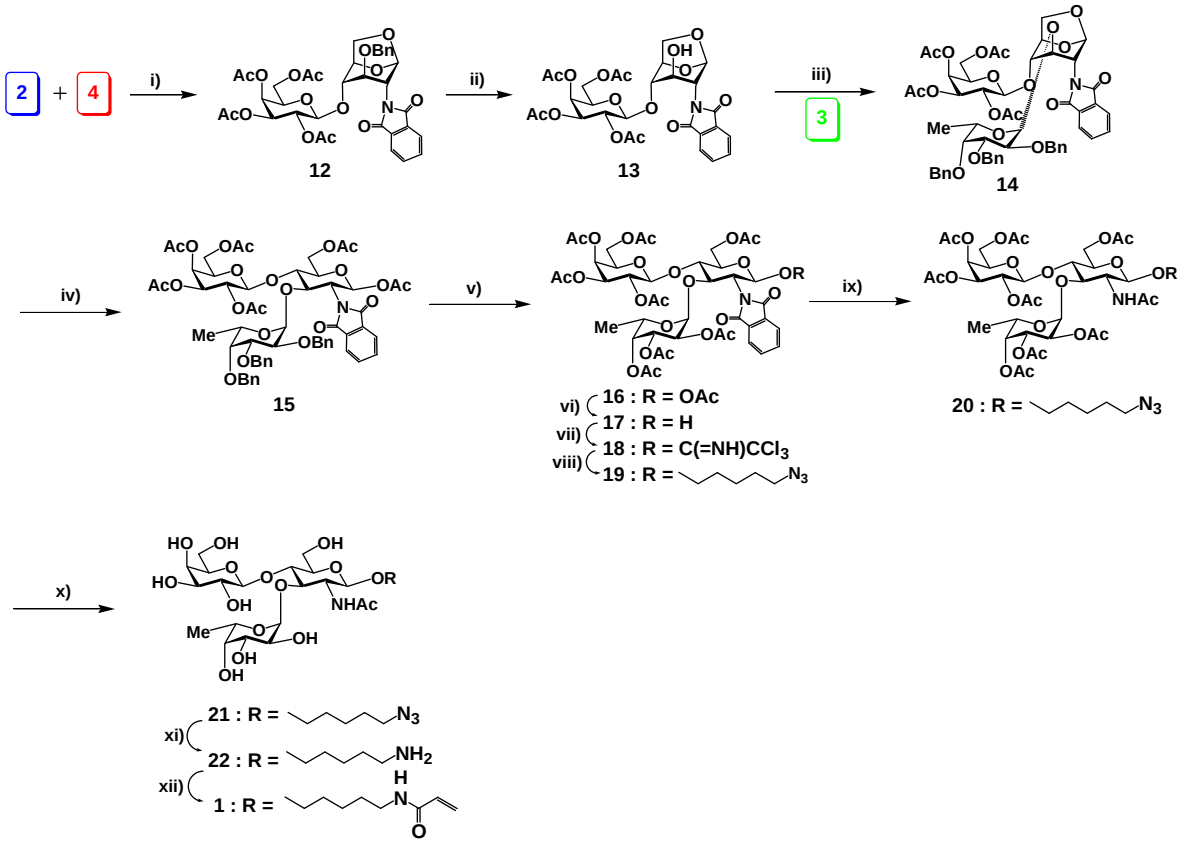
Figure 1. Synthetic plan for construction of Le^x derivative.



Scheme 1. Reagents and conditions: (i) $BF_3{\cdot}OEt_2$, $CH_3(CH_2)_{11}SH$, $ClCH_2CH_2Cl$, 0 °C → rt, 3.5–4 h, 94.8% for **2**, 86.9% for **7**, (ii) NaOMe, MeOH, rt, 4 h, quant., (iii) NaH, BnBr, DMF, 0 °C → rt, 5 h, 81.1%, (iv) Tf_2O, CH_2Cl_2–pyridine, 0 °C, 1 h, (v) potassium phthalimide, DMF, 45 °C, 10 h, 84.7% (two steps), (vi) K_2CO_3, DMF–MeOH, 0 °C, 3 h, then, toluene, reflux, 75.8%.

configuration. Transesterification[10] of acetate **11** gave alcohol **4** accompanied by a phthalimide ring-opened byproduct, which was refluxed in toluene in order to facilitate recyclization of the byproduct to afford building block **C** in 75.8% yield, $[\alpha]_D^{25}$ +59.6 (*c* 1.01, $CHCl_3$), mp 196.5–197.3 °C, ^{1}H NMR ($CDCl_3$) δ 5.52 (d, 1 H, $J_{4,OH}$ = 11.8 Hz, OH-4), 5.33 (s, 1 H, H-1), 4.46 (s, 1 H, H-2), 3.77 (s, 1 H, H-4), 3.76 (s, 1 H, H-3).

Since three building blocks were successfully constructed, our attention was turned to condensation of the building blocks. A schematic image of the assembly of these building blocks is shown in Scheme 2. Condensation of galactosyl donor **2** and glucosaminyl acceptor **4** was carried out in the presence of appropriate amounts of NIS and TMSOTf[11] in CH_2Cl_2 at −20 °C to give lactosaminyl disaccharide **12** involving newly formed β-glycosidic linkage in 83.8% yield, in which the benzyl moiety at C-3 was immediately deprotected by means of hydrogenolysis to furnish alcohol **13** as crystals in 85.8% yield, $[\alpha]_D^{26}$ −16.3 (*c* 1.00, $CHCl_3$), mp 302.4–303.9 °C, ^{1}H NMR ($CDCl_3$) δ 5.63 (s, 1 H, H-1), 4.62 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), 3.78 (d, 1 H, $J_{3,OH}$ = 2.8 Hz, OH-3), ^{13}C NMR ($CDCl_3$) δ 102.18 (C-1), 101.93 (C-1′). α-Stereoselective glycosida-



Scheme 2. Reagents and conditions: (i) NIS–TMSOTf, CH_2Cl_2, MS 4 Å, −20 °C, 1.5 h, 83.8%, (ii) Pd/C, H_2, EtOAc, rt, 5 h, 85.8%, (iii) NIS–TMSOTf, CH_2Cl_2, MS 4 Å, −15 °C, 1.5 h, 93.3%, (iv) CF_3COOH, Ac_2O, 0 °C, 1 h, 93.2%, (v) Pd/C, H_2, EtOAc, 45 °C, 4 h, then Ac_2O, Pyr, DMAP, 45 °C, 6 h, 99.4% (two steps), (vi) N_2H_4–AcOH, DMF, rt, 1 h, 96.9%, (vii) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 2 h, 97.8%, (viii) TMSOTf, 5-azidopentanol, CH_2Cl_2, −40 °C, 1 h, 70.7%, (ix) *n*-$BuNH_2$, MeOH, reflux, 2 days, then Ac_2O, pyridine, DMAP, rt, 20 h, 83.1% (two steps), (x) NaOMe, MeOH, rt, overnight, quant., (xi) Pd/C, H_2, MeOH, 0 °C → rt, 4 h, 83.7%, (xii) acryloyl chloride, Et_3N, MeOH, 0 °C → rt, 43.0%.

tion of Fuc for constructing trisaccharide **14** was performed using fucosyl donor **3** and acceptor **13** by means of same activation system as that for thioglycoside **2** to produce amorphous **14** having an α-fucosyl linkage in 93.3% yield, $[\alpha]_D^{31}$ −24.3 (*c* 1.00, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 5.60 (s, 1 H, H-1), 4.81 (d, 1 H, $J_{1'',2''}$ = 3.1 Hz, H-1″), 4.65 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), 1.10 (d, 3 H, $J_{5'',6''}$ = 6.4 Hz, H-6″), ^{13}C NMR ($CDCl_3$) δ 102.31 (C-1), 98.44 (C-1′), 97.92 (C-1″). In order to convert **14** into the corresponding glycosyl donor in one step, 1,6-anhydro ring opening of **14** by thiolysis[12] in the presence of Lewis acid was initially tried, but thioglycoside was not obtained. Because of acid lability of glycosidic bond between the Fuc moiety and the GlcN moiety, acetolysis in the presence of trifluoroacetic acid as a milder acid source[4] was applied for the fission of the 1,6-anhydro ring. Thus, **14** gave β-acetate **15** as a sole product in 93.2% yield, $[\alpha]_D^{23}$ +22.8 (*c* 1.00, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 6.27(d, 1 H, $J_{1,2}$ = 9.2 Hz, H-1), 4.80 (d, 1 H, $J_{1'',2''}$ = 3.2 Hz, H-1″), 4.59 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), ^{13}C NMR ($CDCl_3$) δ 99.72 (C-1′), 97.61 (C-1″), 89.99 (C-1). Benzyl protection in **15** was changed by the usual hydrogenolysis followed by acetylation to afford known **16**[13], which was further transformed into hemiacetal **17** in the presence of NH_2NH_2 in 96.9% yield, ^{1}H NMR ($CDCl_3$) δ 5.29 (br dd, 1 H, $J_{1,2}$ = 8.8 Hz, $J_{1,OH}$ = 7.2 Hz, H-1). Treatment of hemiacetal **17** with trichloroacetonitrile gave glycosyl imidate **18** in 97.8% yield, and the imidate was activated in the presence of TMSOTf,[14] coupled with azidoalcohol[15] to provide **19** having an azide function at the ω-position in the aglycon in 70.7% yield, $[\alpha]_D^{24}$ −65.1 (*c* 1.01, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 5.02 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 4.94 (d, 1 H, $J_{1'',2''}$ = 4.0 Hz, H-1″), 4.53 (d, 1 H, $J_{1',2'}$ = 8.4 Hz, H-1′), ^{13}C NMR ($CDCl_3$) δ 100.60 (C-1′), 98.05 (C-1″), 95.05 (C-1). Deprotection of phthaloyl protection in **19** followed by acetylation proceeded smoothly to afford an acetamide **20** in 83.1% yield, $[\alpha]_D^{25}$ −54.6 (*c* 0.19, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 5.50 (d, 1 H, $J_{NH,2}$ = 8.4 Hz, NH), 5.43 (d, 1 H, $J_{1'',2''}$ = 3.9 Hz, H-1″), 4.65(d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 4.48 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), ^{13}C NMR ($CDCl_3$) δ 100.40 (C-1′), 99.99 (C-1), 95.27 (C-1″). Quantitative de-O-acetylation by the Zemplén manner[10] for **20** was performed and gave azide alcohol **21**, which was further transformed into the corresponding amine **22** followed by acryloylation to give water-soluble glycomonomer **1** as a white powder after lyophilization in 36.0% yield (two steps), $[\alpha]_D^{25}$ −76.6 (*c* 0.96, $CHCl_3$), ^{13}C NMR (D_2O) δ 130.69 (CH=), 127.64 (CH_2=), 102.47 (C-1′), 101.54 (C-1), 99.27 (C-1″).

In our ongoing synthetic study of artificial glycoconjugates, synthetic assembly of carbohydrate moieties as pendant-type epitopes using linear polymers has been achieved using bioactive carbohydrates.[16] In order to obtain highly clustered Le^x determinants, the potential of radical polymerization as a monomer was examined by the previously reported method.[17] Thus, the homopolymerization of **1** proceeded efficiently in aqueous media to afford white powdery glycopolymer **23** in 83.5% yield after chromatographic purification by Sephadex G-50 followed by lyophilization, $\overline{Mn}$ 51 kDa, $\overline{Mw}$ 72 kDa, $\overline{Mw}/\overline{Mn}$ 1.4, ^{1}H NMR δ (D_2O) 5.11 (br s, 1 H, H-1″), 4.53 (br s, 1 H, H-1), 4.46 (br d, 1 H, $J_{1',2'}$ = 5.6 Hz, H-1′). ^{1}H NMR spectra of the glycomonomer **1** and the glycopolymer **23** are shown in Figure 2. Protons attached to C=C in the monomer **1** (a) at around 5–6 ppm completely vanished after radical polymerizations (b) and broadening of the peaks was observed. In addition, the weight-average molecular weight ($\overline{Mw}$) of the glycopolymer was estimated according to the results of size exclusion chromatography with 0.01 M aqueous NaCl solution as the eluent and the degree

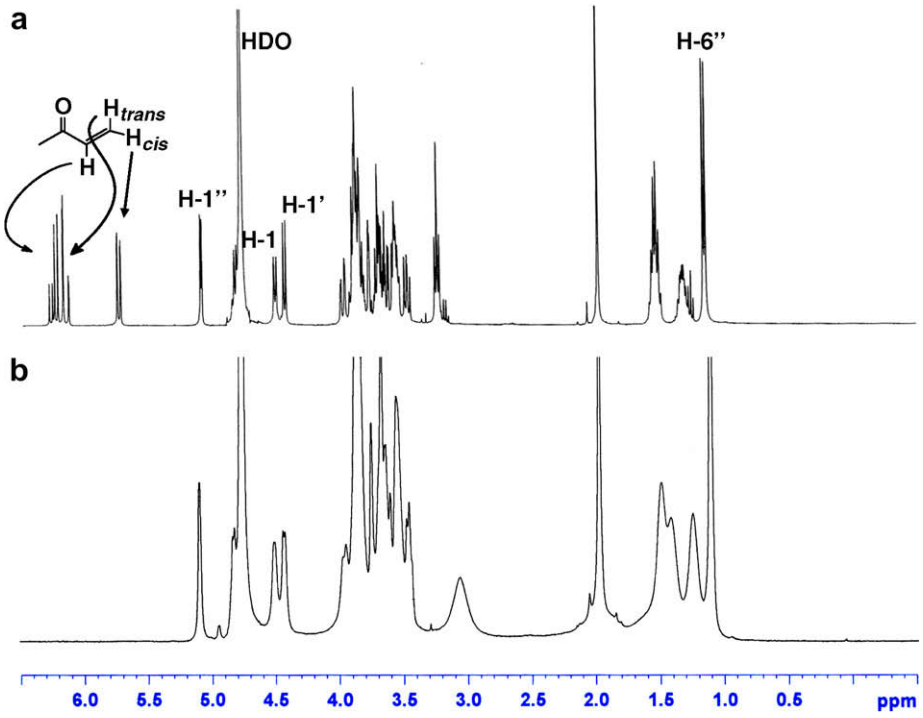
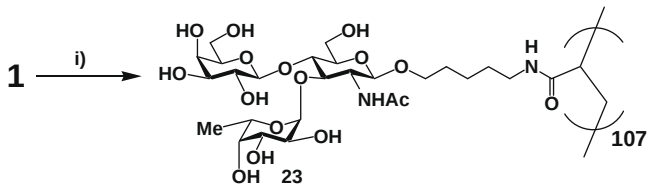


Figure 2. ^{1}H NMR spectra of (a) glycomonomer **1** and (b) homopolymer **23** in D_2O.



Scheme 3. Reagents and conditions: (i) APS, TEMED, H_2O, 50 °C, 3 h, 83.5%.

of polymerization of the glycopolymer **23** was estimated to be 107 on the basis of $\overline{Mw}$ (Scheme 3).

In summary, we have successfully described preparation of a Le^x derivative via Gabriel amine synthesis and its chemical modification to provide a water-soluble glycomonomer. Homopolymerization using the Le^x glycomonomer was performed to give a water-soluble glycopolymer having $\overline{Mw}$ 72 KDa in high yield, which displayed highly clustered glycoepitopes. Biological activity of the glycopolymer was preliminarily examined on the basis of fluorescence measurement using a plant lectin of *Lotus tetragonolobus*, which binds to an L-fucose residue.[18] Interestingly negative sugar clustering effect was observed since specific carbohydrate-protein interaction was interfered by highly clustered sugar residues. Further polymerization conditions including copolymerization conditions with acryl amide are now under investigation, and the biological activities of the glycopolymers will also be examined. The results of these experiments will be reported in the near future.

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