Synthetic Glycoconjugates. 3.[1] An Efficient Synthesis of a Glycoprotein Model Having a Le^x-Type Trisaccharide Sequence of Tumor-Associated Carbohydrate Antigen[2]

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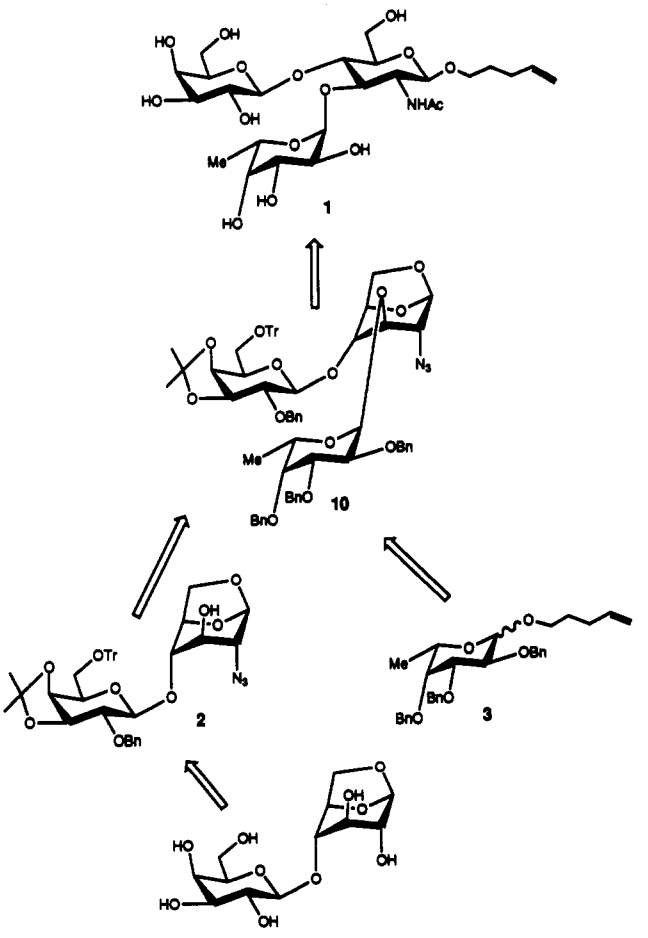
ABSTRACT: A unique and efficient synthetic strategy for the macromolecule containing the tumor-associated antigenic oligosaccharide, Lewis x (Le^x), is described. A novel standardized intermediate derived from an available 1,6-anhydro-β-lactose remarkably facilitated the conventional synthetic route of a peracetate of Galpβ(1→4)[Fucpα(1→3)]GlcpNAc as an important precursor for the preparation of the targeted polymerizable glycoside. Introduction of an *n*-pentenyl group at the anomeric position into the peracetate of Le^x through a reactive oxazoline derivative successfully afforded a new type of carbohydrate monomer. Copolymerization of the glycoside with acrylamide proceeded smoothly and gave a biochemically interesting glycoprotein model having a pendant Le^x structure.

Introduction

As a result of their structural and biochemical significance, a variety of oligosaccharide sequences containing *N*-acetyllactosamine [Galpβ(1→4)GlcpNAc] as an invariable "core" structure are of growing importance. Especially, recent discoveries in glycobiology identifying Lewis x (Le^x) and sialyl Le^x (SLe^x) as the binding ligands in cell–cell interactions generated considerable excitement not only in biological science but also in chemical and medical circles.[3] For example, glycosphingolipids and glycoproteins carrying the Le^x determinant are known to accumulate in a wide variety of tumor cells and identified as embryonic antigens specifically expressed at the morula stage.[4–6] In relation to the biological functions of the Le^x determinant in F9 embryonal carcinoma cell adhesion, Hakomori and his co-workers reported evidence suggesting a crucial role for Le^x in the specific carbohydrate–carbohydrate interactions.[7,8] Since the Le^x type substances are conspicuously absent in normal hepatocytes, colonic mucosa, and granulocytes, Le^x and related structural families have been regarded as one of the specific markers of tumor-associated antigens coupled with their potential usefulness in diagnostics and immunotherapy. Moreover, some classes of gangliosides or glycoproteins carrying SLe^x structures, sialylated species of the Le^x family, have been found to exhibit a significant role as the binding ligands of the selectin family such as E-selectin [endothelial-leukocyte adhesion molecule-1 (ELAM-1)], P-selectin [granule membrane protein-140 (GMP-140)], and L-selectin [leukocyte adhesion molecule-1 (LAM-10)].[9–12]

Increasing interest in Le^x-type oligosaccharides necessitates the development of practical methodology for the design and synthesis of a variety of model ligands having this class of carbohydrate determinants. In connection with the role of selectins in these ectobiological processes, artificially designed selectin-binding molecules are emerging as important biochemical tools and potential agents

Scheme 1. Retrosynthetic Analysis of a Polymerizable Le^x Derivative 1



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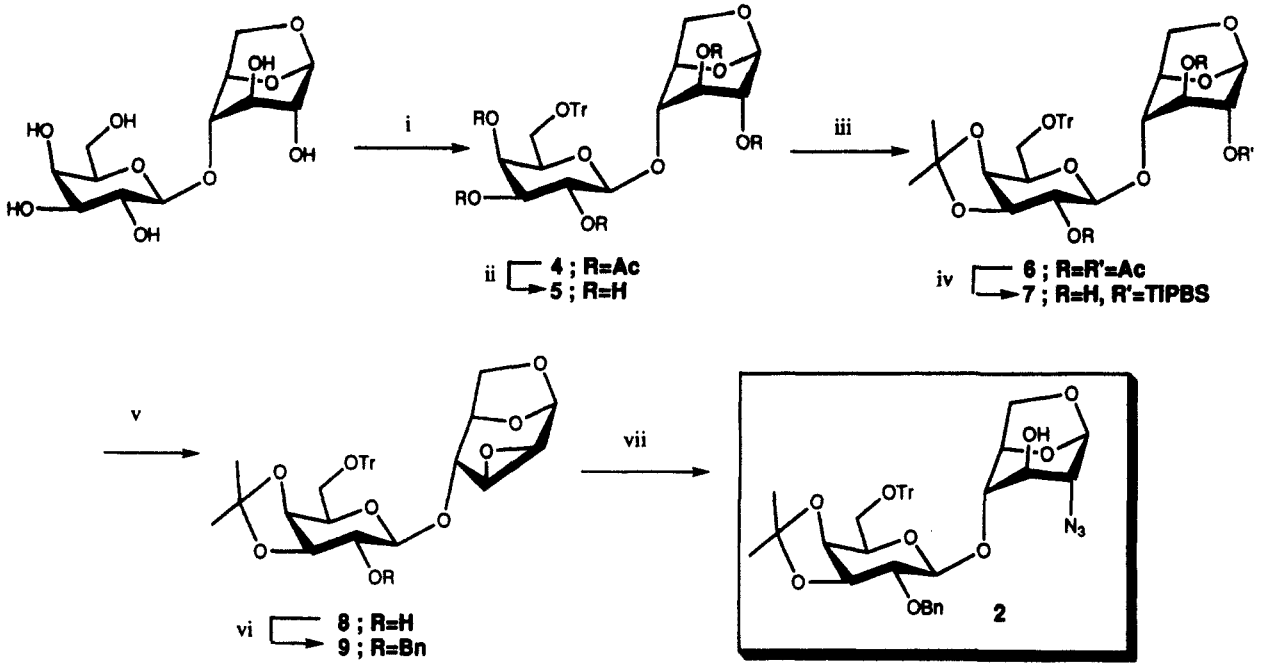
Scheme 2[a]



[a] Reagents and conditions: (i) triphenylmethyl chloride, (dimethylamino)pyridine, pyridine, 90 °C, 24 h; (ii) NaOMe/MeOH–THF, 25 °C, 3 h; (iii) $Me_2C(OMe)_2$, 10-camphorsulfonic acid, DMF, 60 °C, 20 mmHg, 6 h; then Ac_2O, pyr, 25 °C, 16 h; (iv) NaOMe/MeOH–THF, triisopropylbenzenesulfonyl chloride, (dimethylamino)pyridine, pyridine, 25 °C, 16 h; (v) NaOH(aq)/MeOH–THF, 25 °C, 16 h; (vi) BnBr, BaO, $Ba(OH)_2 \cdot 8H_2O$, DMF, 25 °C, 24 h; (vii) NaN_3, NH_4Cl, DMF–H_2O, 120 °C, 24 h.

for inflammation or related diseases. Indeed, much attention and effort have been paid toward the synthetic studies on the Le^x family as examples of the most complex oligosaccharides ever to be targeted for synthesis.[13-15]

In the preceding papers,[1,16] we demonstrated the efficacy of the *n*-pentenyloxy group as an anchoring device with adequate polymerizability and wide applicability for the preparation of novel types of glycoconjugate models containing *N*-acetyl-D-glucosamine, *N,N'*-diacetylchitobiose, and *N*-acetyllactosamine. Thus, our attention is now focused on the expansion of this methodology into the first synthesis of a macromolecule having pendant Le^x structure. We describe herein a unique and efficient approach for the synthesis of this polymer using new 1,6-anhydro-β-lactose derivatives as key intermediates.

Results and Discussion

Oligosaccharide Synthesis. Although the stepwise glycosidation procedures of each monosaccharide synthon have been widely utilized and have succeeded in the chemical syntheses of Le^x and its related derivatives including the sialyl Le^x structure,[13-15] these methods seem to contain rather complicated and long procedures and this sometimes has made oligosaccharide syntheses difficult. The growing interests and needs for the rapid and sophisticated syntheses of biologically important oligosaccharides have prompted us to develop a new synthetic approach to them based on chemospecific manipulations of key disaccharide materials.[2,17-19]

In the present study, we selected a conformationally restricted 1,6-anhydro-β-lactose[20] as a key starting material in order to facilitate the synthetic procedure of Le^x–trisaccharide monomer **1**. As illustrated in Scheme 1, a finely designed glycosyl acceptor **2** derived from 1,6-anhydro-β-lactose is regarded as an effective intermediate for facile glycoside formation with a known fucose derivative **3**.

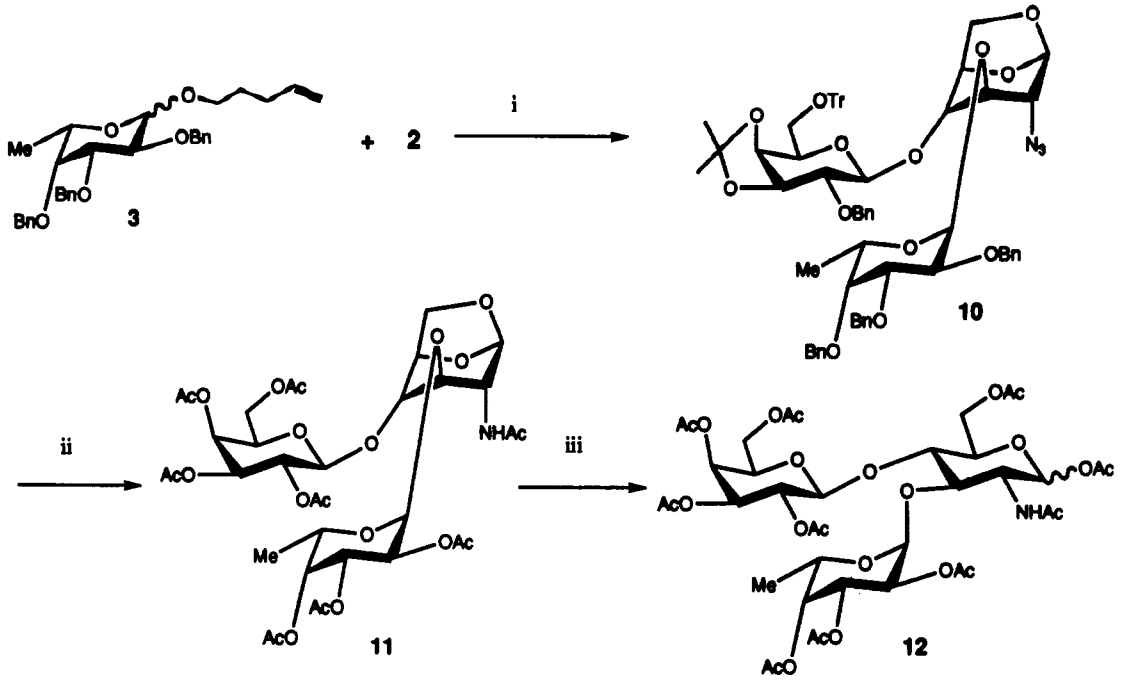
(A) Key Glycosyl Acceptor. Scheme 2 summarizes a synthetic strategy for a key glycosyl acceptor **2** having an unprotected hydroxy group at the C-3 position of the reducing Glc*p*NAc residue from 1,6-anhydro-β-lactose.

The scheme planned here contains two important steps for the regioselective modifications of 1,6-anhydro-β-lactose derivatives: (a) protection of the highly reactive 3'-OH group of the Gal*p* group prior to the introduction of a suitable leaving group at the C-2 position and (b) selective introduction of a sterically hindered leaving group, 2,4,6-triisopropylbenzenesulfonyl. With respect to the order of reactivites of secondary hydroxyl groups of 1,6-anhydro-4',6'-*O*-benzylidene-β-lactose, Tejima and his co-workers had already examined the introduction of protective groups or leaving groups by using benzoylation (3' > 2 > 3 > 2') and tosylation reactions (2 > 3' > 3 > 2') in the previous papers.[21-23] Moreover, Kuzuhara and Morikawa revealed that the much higher reactivity of the 3'-OH group often made further regioselective modifications of this disaccharide difficult.[20]

Initially, a readily preparable peracetate of 1,6-anhydro-6'-*O*-trityl-β-lactose, **4**,[2] was de-*O*-acetylated to give derivative **5** having five hydroxyl groups at C-2, C-3, C-2', C-3', and C-4' positions. As anticipated, protection of the 3'-OH group of **5** facilitated the further regioselective modifications and conversions of this disaccharide. Indeed, ketalization of compound **5** proceeded smoothly and gave the 3',4'-*O*-isopropylidene derivative as peracetate **6**. Next, the intermediate **6** was converted into a sterically hindered triisopropylbenzenesulfonyl (TIPBS) derivative **7** with position specificity.[24] Treatment of diol **7** with aqueous sodium hydroxide solution gave epoxide **8** in high yield and subsequent benzylation of the epoxide afforded 2'-*O*-benzyl derivative **9**. Finally, nucleophilic attack by the azide ion of the epoxide **9** occurred stereospecifically to yield the key synthon **2** having an unprotected hydroxyl group at the C-3 position.

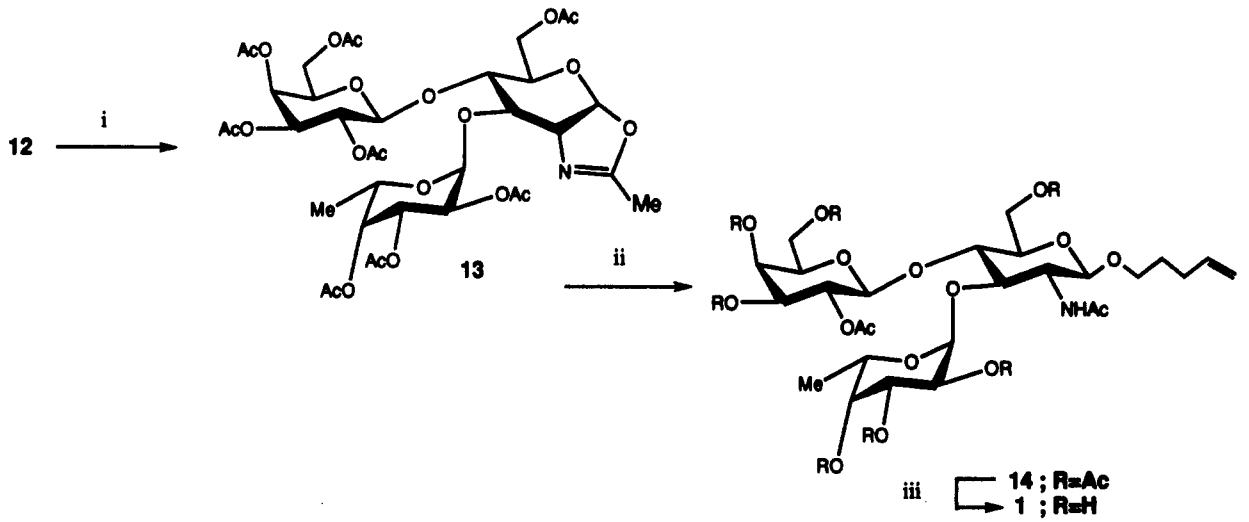
(B) Trisaccharide Synthon. The synthetic strategy of the Le^x structure using 1,6-anhydro-β-lactose derivatives described herein is considerably shorter and simpler than those previously reported and has the potential to provide

Scheme 3[a]



[a] Reagents and conditions: (i) $I(collidine)_2ClO_4$, 4 Å MS, CH_2Cl_2–Et_2O, 25 °C, 24 h; (ii) Pd/C, H_2, 25 °C, 48 h; AcOH–H_2O, 90 °C, 5 h; Ac_2O, pyridine, 25 °C, 16 h; (iii) trifluoroacetic acid/Ac_2O, 25 °C, 16 h.

Scheme 4[a]

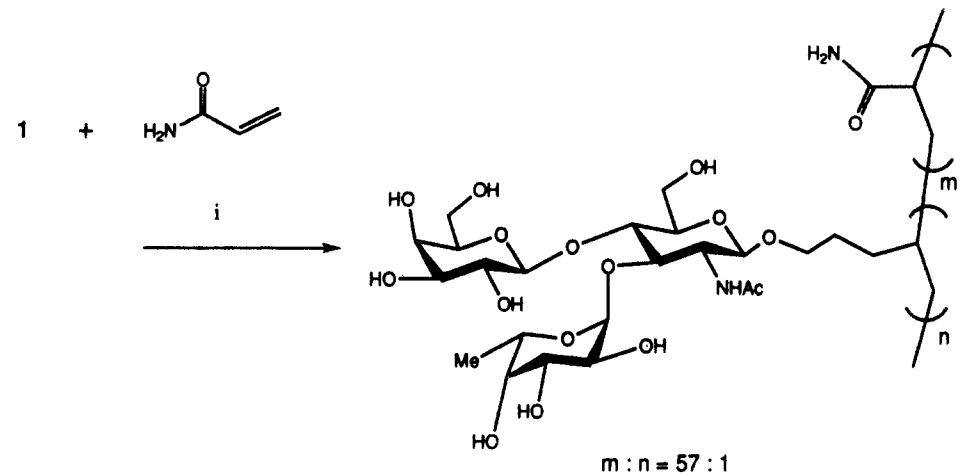


[a] Reagents and conditions: (i) trimethylsilyl trifluoromethanesulfonate, $ClCH_2CH_2Cl$, 50 °C, 3 h; (ii) $HO(CH_2)_3CH{=}CH_2$, 10-camphorsulfonic acid, 90 °C, 1 h; (iii) NaOMe/MeOH, 25 °C, 24 h.

large-scale preparation of functional oligosaccharides for further biochemical research. Actually, the coupling reaction of the key acceptor **2** with a known fucosyl donor **3** under Fraser-Reid's condition[25] gave rise to the corresponding trisaccharide intermediate **10** in 55% yield. Stereochemistry in the anomeric position of the L-fucose residue was apparently determined to be the α configuration by the lower vicinal coupling constant ($J_{1,2} < 1$ Hz) of H-1″ at 4.84 ppm in the proton NMR spectrum. Deprotection by hydrogenation and complete acetylation of compound **10** afforded the Le^x type precursor **11** containing a 1,6-anhydro-β-Glc*p*NAc residue. Conformational conversion of derivative **11** (1C→C1 conformation in the reducing Glc*p*NAc residue) by treatment with trifluoroacetic acid–acetic anhydride successfully provided the important Le^x trisaccharide sequence (**12**) as a peracetate (Scheme 3).

Preparation and Copolymerization of a Polymerizable Oligosaccharide. Since we have already demonstrated that a simple *n*-pentenyl group exhibited excellent properties as a convenient polymerizable aglycone having a spacer-arm function for the incorporation of bulky sugar moieties,[16] we examined the versatility of this procedure in the case for the preparation of a monomer containing further complicated Le^x–trisaccharide structure. According to the method described before,[1] peracetate **12** was converted into *n*-pentenyl glycoside **14** via an oxazoline derivative **13**. (Trimethylsilyl)trifluoromethanesulfonate (TMSOTf)[26] was allowed to promote this oxazoline formation and the following glycosylation with 4-penten-1-ol in the presence of 10-camphorsulfonic acid gave stereoselectively β-glycoside **14**. Finally, general de-*O*-acetylation of **14** by the Zemplen method afforded a polymerizable and water-soluble Le^x monomer **1** in quantitative yield (Scheme 4). The chemical structure of oligosaccharide monomer **1** was clearly elucidated by the ^{13}C NMR spectrum in D_2O, as shown in Figure 1.

Scheme 5[a]



[a] Reagents and conditions: *N,N,N′,N′*-tetramethylethylenediamine, $(NH_4)_2S_2O_8$, H_2O, 25 °C, 48 h.

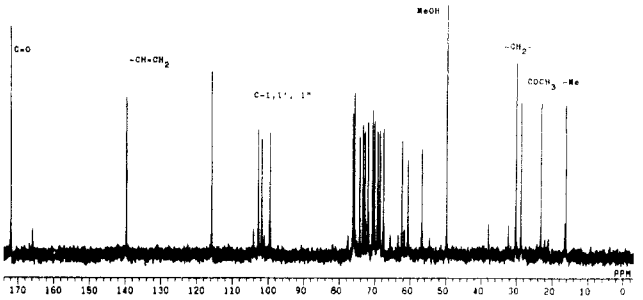


Figure 1. ^{13}C-NMR spectrum of the Le^x monomer 1 measured in D_2O at 27 °C.

Copolymerization of carbohydrate monomer 1 with acrylamide in water was carried out in the same manner as in the case for polymers containing Glc*p*NAc, Glc*p*NAc β(1→4)Glc*p*NAc, and Gal*p*β(1→4)Glc*p*NAc.[1] Actually, radical copolymerization in the presence of ammonium peroxodisulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) proceeded smoothly and gave the water-soluble carbohydrate polymer showing high viscosity in 75% yield (Scheme 5). The resulting polymer was calculated to contain 12.9 wt % carbohydrate moiety by integration of the signals due to methine (2.2 ppm) of the main chain and *N*-acetyl protons of Glc*p*NAc residues in the ^{1}H NMR spectrum measured in D_2O at 25 °C (Figure 2A). This suggested that the unit ratio of carbohydrate to acrylamide in the polymer was approximately 1:57. From the spectrum measured at 50 °C and its homonuclear decoupling experiment (Figure 2B,C), a broad doublet signal at 4.7 ppm overlapped with the signal due to HDO in Figure 2A was clearly assigned to be H-5″ of Fuc*p* residues which coupled with H-6″ at 1.1 ppm in addition to three signifiicant anomeric protons. Moreover, the ^{13}C NMR spectrum of the copolymer from 1 and acrylamide in D_2O showed appropriate signals attributed to the ring carbons of sugar domains besides the carbons of polyacrylamide main chains (Figure 3). Owing to broadening of the C-1 signal, only two anomeric carbon signals due to C-1′ and C-1″ were observed in this spectrum. The resulting water-soluble polymer exhibited high viscosity, and the number-average molecular weight determined by aqueous phase GPC was 280K.

In conclusion, we succeeded in the novel and efficient synthesis of the artificial glycoconjugate containing tumour-associated Le^x branches. A fully functionalized *O*-(2-*O*-benzyl-3,4-*O*-isopropylidene-6-*O*-trityl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (2) was shown to be an excellent lactosaminyl acceptor for the preparation of the Le^x trisaccharide sequence. Introduction of the *n*-pentenyl group as a convenient polymeriable aglycone into the trisaccharide synthon and subsequent copolymerization of this reactive glycoside with acrylamide gave a new glycoprotein model having the pendant Le^x structure. We are currently studying enzymatic introduction of *N*-acetylneuraminic acid and its analogs to this unique and versatile polymeric substrate, and the results will be reported in elsewhere.

Experimental Section

General Procedures. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. 1,2-Dichloroethane, ethyl acetate, and pyridine were stored over molecular sieves (3 Å) for several days before use. Acrylamide was recrystallized from benzene before use. Melting points were determined with a Laboratory Devices melting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-370 digital polarimeter at 23 °C. IR spectra were recorded with a JASCO IR-700. ^{1}H NMR and proton decoupled carbon NMR spectra were recorded at 270 and 67.8 MHz, respectively, with a JEOL JNM-GX270 spectrometer in chloroform-*d* or deuterium oxide, using tetramethylsilane (TMS), methanol, or 3-(trimethylsilyl)propanesulfonic acid sodium salt (DSS) as internal standards. Ring proton assignments in NMR were made by first-order analysis of the spectra and were supported by homonuclear decoupling experiments. The average molecular weight was estimated by gel permeation chromatography (GPC) with an Asahipak GS-510 column, and pullulans (5.8, 12.2-, 23.7-, 48.0-, 100-, 186-, and 380K, Shodex Standard P-82) were used as standards. Elemental analyses were performed with a Yanaco MT-3 CHNcorder on the samples extensively (ca. 24 h) dried in vacuo (50 °C, 0.1 Torr) over phosphorus pentoxide. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60F_{254} (layer thickness, 0.25 mm; E. Merck, Darmmstadt, Germany). For detection of the components, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) methanol–concentrated sulfuric acid–*p*-anisaldehyde and heated for a few minutes (for carbohydrates) or (b) an aqueous solution of 5 wt % potassium permanganate and heated similarly (for C–C double bonds). Column chromatography was performed on silica gel (Wakogel C-200; 100–200 mesh, Wako Pure Chemical Industries Co. Ltd., Japan). All extractions were concentrated below 45 °C under diminished pressure.

***O*-(2,3,4-Tri-*O*-acetyl-6-*O*-(triphenylmethyl)-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (4).** To a solution of 1,6-anhydro-β-lactose[20] (13.0 g, 22.9 mmol) in pyridine (268 mL) were added triphenylmethyl chloride (26.2 g, 92 mmol) and 4-(dimethylamino)pyridine (DMAP) (1.4 g, 11.5 mmol), and the mixture was stirred under a nitrogen atmosphere for 24 h at 90 °C. The solution was cooled to 0 °C and added with acetic anhydride (67 mL). The mixture

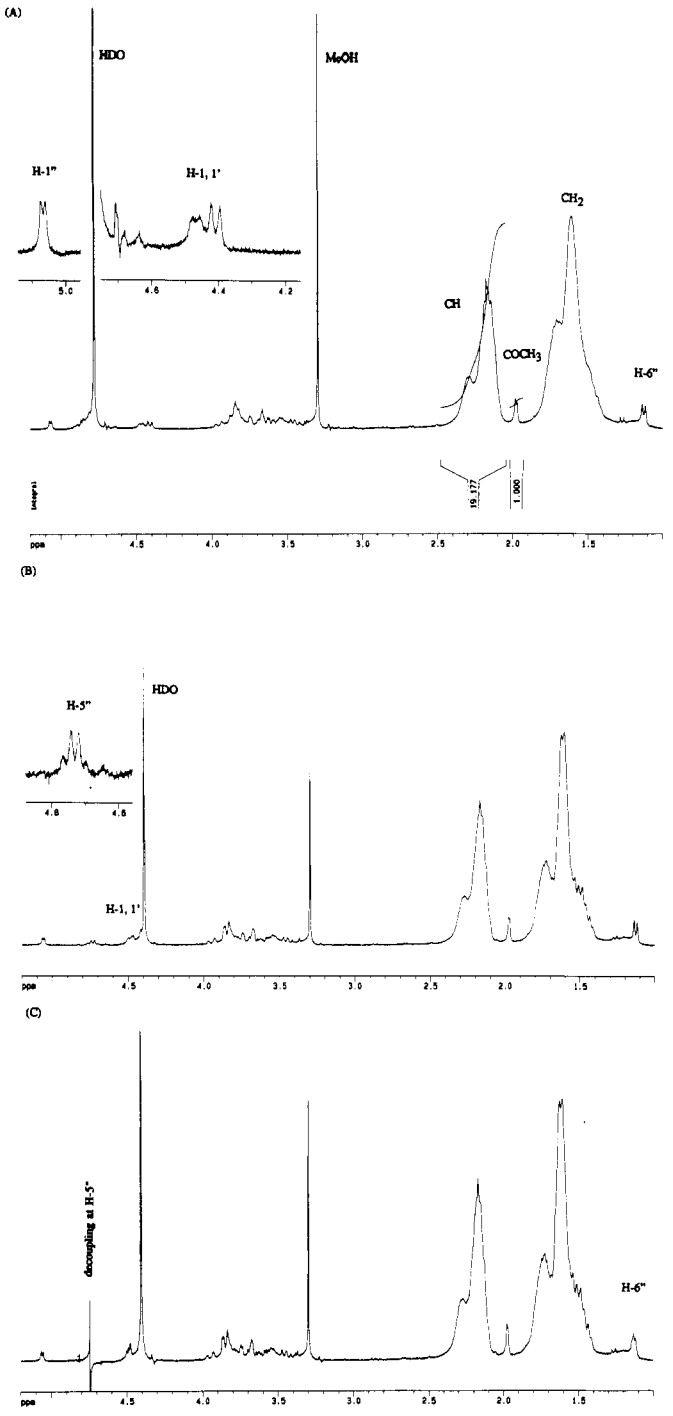


Figure 2. ^{1}H-NMR spectra of copolymer from 1 and acrylamide in D_2O at (A) 25 °C, (B) 50 °C, and (C) homonuclear decoupling experiment at 50 °C.

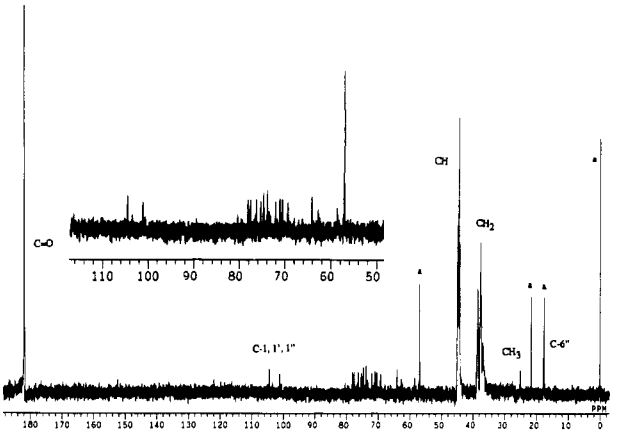


Figure 3. ^{13}C-NMR spectrum of copolymer from 1 and acrylamide in D_2O at 50 °C. a indicates DSS.

was stirred for 12 h at room temperature and then evaporated. After the usual workup, silica gel column chromatography (20:1, v/v, toluene–ethyl acetate) of the residue gave **4** which crystallized from ethanol–chloroform (1:1, v/v) (15.2 g, 85%): mp 187–189 °C; $[\alpha]_D$ −63.4° (*c* 0.10, chloroform); ^{1}H NMR δ ($CDCl_3$) 1.89, 1.98, 2.00, 2.03, and 2.04 (all s, 15 H, 5 OCOC*H*$_3$), 3.05 (t, 1 H, *J* 8.6 Hz, H-5′), 3.35 (dd, 1 H, *J* 5.6 and 8.8 Hz, H-6′a), 3.59 (s, 1 H, H-4), 3.77 (t, 1 H, *J* 7.5 Hz, H-6 a), 3.85 (br t, 1 H, *J* 5.9 Hz, H-6′b), 3.96 (d, 1 H, *J* 7.6 Hz, H-6b), 4.48 (s, 1 H, H-2), 4.61 (d, 1 H, *J* 5.1 Hz, H-5), 4.74 (d, 1 H, *J* 7.8 Hz, H-1′), 4.98 (s, 1 H, H-3), 5.07 (dd, 1 H, *J* 3.2 and 10.3 Hz, H-3′), 5.20 (dd, 1 H, *J* 7.8 and 10.5 Hz, H-2′), 5.41 (s, 1 H, H-1), 5.57 (d, 1 H, *J* 3.2 Hz, H-4′), and 7.19–7.37 (m, 15 H, aromatic). Anal. Calcd for $C_{41}H_{44}O_{15}$: C, 63.44; H, 5.64. Found: C, 63.40; H, 5.71.

***O*-(6-*O*-(Triphenylmethyl)-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-β-D-glucopyranose (5).** A solution of compound **4** (15.2 g, 19.4 mmol) and sodium methoxide (140 mg, 2.59 mmol) in 1:1 (v/v) methanol–tetrahydrofuran (400 mL) was stirred for 3 h at room temperature. The mixture was made neutral with the Dowex 50W X8 (H^+) resin, and the suspension was filtered. The filtrate was evaporated to give a quantitative yield of **5** (10.9 g) as amorphous powder: $[\alpha]_D$ −48.1° (*c* 0.262, chloroform). Anal. Calcd for $C_{31}H_{34}O_{10}\cdot 0.5H_2O$: C, 64.69; H, 6.13. Found: C, 64.35; H, 6.01.

***O*-(2-*O*-Acetyl-3,4-*O*-isopropylidene-6-*O*-(triphenylmethyl)-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (6).** A solution of **5** (10.9 g, 19.2 mmol), 2,2-dimethoxypropane (3.0 g, 28.8 mmol), and 10-camphorsulfonic acid-*d* (CSA) (68 mg) in dimethyl formamide (67 mL) was stirred under reduced pressure (20 mmHg) for 6 h at 60 °C, and then diluted with pyridine (68 mL). To the reaction mixture was added acetic anhydfide (55 mL), and the mixture was stirred for 16 h at room temperature. The mixture was poured into ice–water and extracted with chloroform. The organic layer was successively washed with 1 N sulfuric acid, saturated sodium hydrogen bicarbonate, and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with 30:1 (v/v) toluene–ethyl acetate as the eluant to afford compound **6** (8.8 g, 57 %): $[\alpha]_D$ −51.7° (*c* 0.23, chloroform); ^{1}H NMR δ ($CDCl_3$) 1.33 and 1.52 [each s, 6 H, $(CH_3)_2C$], 1.96, 1.98, and 2.08 (all s, 9 H, 3 OCOC*H*$_3$), 3.39–3.51 (m, 2 H, H-6′a,b), 3.63 (s, 1 H, H-4), 3.74 (dd, 1 H, *J* 5.1 and 7.4 Hz, H-6a), 3.89 (dt, 1 H, *J* 1.6 and 7.0 Hz, H-5′), 3.94 (d, 1 H, *J* 7.6 H z, H-6b), 4.15 (dd, 1 H, *J* <1 and 7.3 Hz, H-3′), 4.24 (dd, 1 H, *J* 1.6 and 4.9 Hz, H-4′), 4.52 (s, 1 H, H-2), 4.64 (s, 1 H, H-5), 4.66 (d, 1 H, *J* 8.3 Hz, H-1′), 4.98 (s, 1 H, H-3), 5.02 (t, 1 H, *J* 8.3 Hz, H-2′), 5.41 (s, 1 H, H-1), and 7.13–7.45 (m, 15 H, aromatic). Anal. Calcd for $C_{40}H_{44}O_{13}\cdot 0.8H_2O$: C, 64.14; H, 6.15. Found: C, 64.60; H, 6.57.

***O*-(3,4-Isopropylidene-6-*O*-(triphenylmethyl)-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-*O*-((triisopropylbenzyl)sulfonyl)-β-D-glucopyranose (7).** To a solution of **6** (8.8 g, 12 mmol) in 1:1 (V/V) methanol–tetrahydrofuran (150 mL) was added sodium methoxide (100 mg), and the mixture was stirred for 4 h at room temperature. It was made neutral with Dowex 50W-X8 (H^+) resin, filtered, and evaporated in vacuo. The residual syrup was dissolved in pyridine (80 mL), and to the solution was dropwise added a solution of triisopropylbenzenesulfonyl chloride (TIPBS-Cl) (16.3 g, 54 mmol) and (dimethylamino)pyridine (DMAP) (4.4 g, 36 mmol) in 1:1 (v/v) chloroform–pyridine (30 mL). The mixture was stirred under a ntirogen atmosphere for 16 h at room temperature. The solution was poured into ice–water and extracted with chloroform. The organic layer was successively washed with 1 N sulfuric acid, water, saturated aqueous hydrogen bicarbonate, and brine, dried, and evaporated. The residue was purified by silica gel chromatography with 100:4:1 (v/v/v) chloforom–methanol–triethylamine as an eluant to give amorphous powdery **6** (8.5 g, 81%): $[\alpha]_D$ 2.6° (*c* 0.10, chloroform); ^{1}H NMR δ ($CDCl_3$) 1.22–1.27 [m, 18 H, 3 $(CH_3)_2CH$, TIPBS], 1.32 and 1.49 [each s, 6 H, $(CH_3)_2C$, isopropylidene], 2.90 (m, 1 H, H-5′), 2.95 and 3.29 (each br s, 2 H, 3- and 2′-OH), 3.55 (t, 1 H, *J* 10 Hz, H-2′), 3.61–4.04 [m, 8 H, H-4 , H-6a, H-6b, H-6′a, H-6′b, and 3 $(CH_3)_2CH$], 4.10–4.19 (m, 3 H, H-2, H-3, and H-3′), 4.26 (d, 1 H, *J* 8.3 Hz, H-1′), 4.37 (d, 1 H, *J* 4.4 Hz, H-4′), 4.61 (d, 1 H, *J* 5.1 Hz, H-5), 5.45 (s, 1 H,

H-1), and 7.17–7.60 (m, 17 H, aromatic). Anal. Calcd for $C_{49}H_{60}O_{12}S_1$: C, 66.05; H, 7.01. Found: C, 66.01; H. 6.80.

***O*-(3,4-*O*-Isopropylidene-6-*O*-(triphenylmethyl)-β-D-galactopyranosyl)-(1→4)-1,6:2,3-dianhydro-β-D-mannopyranose (8).** To a solution of 7 (7.5 g, 8.6 mmol) in 1:1 (v/v) methanol–tetrahydrofuran (86 mL) was added an aqueous solution of 1 N sodium hydroxide (25.8 mL), and the solution was stirred for 16 h at room temperature overnight. The reaction mixture was concentrated and diluted with chloroform. The organic layer was washed with water, dried, and evaporated. The residue was purified on a silica gel column with 50:40:2 (v/v/v) chloroform–ethyl acetate–methanol as the eluant to give a crystalline mass of 8 (4.4 g, 87%): mp 200–202 °C; $[\alpha]_D$ −26.7° (*c* 0.21, chloroform); ^{1}H NMR δ ($CDCl_3$) 1.35 and 1.50 [each s, 6 H, $(CH_3)_2C$], 2.71 (d, 1 H, *J* 2.7 Hz, 2′-OH), 3.42 (d, 1 H, *J* 2.9 Hz, H-2), 3.45–3.50 (m, 3 H, H-3, H-6′a, and H-6′b), 3.62 (dt, 1 H, *J* 2.0 and 8.3 Hz, H-2′), 3.73 (d, 2 H, *J* 4.6 Hz, H-6a and H-6b), 3.83 (dt, 1 H, *J* 2.0 and 5.4 Hz, H-5′), 3.95 (s, 1 H, H-4), 4.07 (dd, *J* 2.0 and 7.1 Hz, H-3′), 4.22 (dd, 1 H, *J* 2.0 and 5.4 Hz, H-4′), 4.35 (d, 1 H, *J* 8.3 Hz, H-1′), 4.52 (br t, 1 H, *J* 4.6 Hz, H-5), 5.70 (d, 1 H, *J* 2.9 Hz, H-1), and 7.25–7.48 (m, 15 H, aromatic). Anal. Calcd for $C_{34}H_{36}O_9$: C, 69.37; H, 6.16. Found: C, 69.65; H, 6.44.

***O*-(2-*O*-Benzyl-3,4-*O*-isopropylidene-6-*O*-(triphenylmethyl)-β-D-galactopyranosyl)-(1→4)-1,6:2,3-dianhydro-β-D-mannopyranose (9).** To a solution of 8 (3.5 g, 5.95 mmol) in dimethylformamide (30 mL) were added barium hydroxide octahydrate (2.81 g, 8.9 mmol), barium oxide (2.74 g, 17.8 mmol), and benzyl bromide (7.5 g, 44.6 mmol) at 0 °C. The reaction mixture was stirred for 24 h at room temperature and diluted with ethyl acetate (40 mL). The solution was then filtered with Celite, and the filtrate was washed with brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with 200:5:1 (v/v/v) chloroform–ethyl acetate–triethylamine to give 9 as a white crystalline powder (3.6 g, 89%): mp 158–160 °C; $[\alpha]_D$ 2.4° (*c* 0.515, chloroform); ^{1}H NMR δ ($CDCl_3$) 1.34 and 1.37 [each s, 6 H, $(CH_3)_2C$], 3.41–3.52 (m, 5 H, H-2, H-3, H-2′, H-6′a, and H-6′b), 3.70 (d, 2 H, *J* 4.4 Hz, H-6a and H-6b), 3.79 (m, 1 H, H-5′), 3.90 (s, 1 H, H-4), 4.17 (m, 2 H, H-3′ and H -4′), 4.45–4.49 (m, 2 H, *J* 7.9 Hz, H-5 and H-1′), 4.83 (s, 2 H, CH_2–Ph), 5.72 (d, 1 H, *J* 2.7 Hz, H-1), and 7.23–7.43 (m, 20 H, aromatic). Anal. Calcd for $C_{41}H_{42}O_9$: C, 72.44; H, 6.38. Found: C, 72.31; H, 6.34.

***O*-(2-*O*-Benzyl-3,4-*O*-isopropylidene-6-*O*-trityl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (2).** A mixture of 9 (3.1 g, 4.56 mmol), sodium azide (1.95 g, 30 mmol), and ammonium chloride (3.74 g, 70 mmol) in 10:1 (v/v) dimethylformamide–water (33 mL) was stirred for 24 h at 120 °C. The mixture was poured into ice–water and extracted with ethyl acetate (200 mL). The organic layer was washed with water, dried, filtered, and evaporated. The residual syrup was purified on a silica gel column with 40:1 (v/v) chloroform–ethyl acetate as the eluant, to give amorphous 2 (2.1 g, 65%): $[\alpha]_D$ −6.1° (*c* 0.215, chloroform); ^{1}H NMR δ ($CDCl_3$) 1.28 and 1.34 [each s, 6 H, $(CH_3)_2C$], 3.20 (d, 2 H, *J* 6.4 Hz, H-2 and 3-OH), 3.43–3.65 (m, 6 H, H-4, H-5, H-6a, H-6b, H-2′, and H-6′a), 3.76 (d, 1 H, *J* 7.1 Hz, H-6′b), 3.85 (m, 1 H, H-3), 4.01 (d, 1 H, *J* 6.1 Hz, H-4′), 4.10 (t, 1 H , *J* 6.1 Hz, H-3′), 4.32 (d, 1 H, J 7.6 Hz, H-1′), 4.50 (d, 1 H, *J* 5.1 Hz, H-5), 4.80 (d, 2 H, *J* 3.9 Hz, CH_2Ph), 5.32 (s, 1 H, H-1), and 7.23–7.46 (m, 20 H, aromatic). Anal. Calcd for $C_{41}H_{43}O_9N_3$: C, 68.22; H, 6.00; N, 5.82. Found: C, 68.21; H, 6.08; N, 5.86.

***O*-(2-*O*-Benzyl-3,4-*O*-isopropylidene-6-*O*-(triphenylmethyl)-β-D-galactopyranosyl)-(1→4)-[*O*-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (10).** A solution of 2 (900 mg, 1.25 mmol) and an anomeric mixture of *n*-pentenyl 2,3,4-tri-*O*-benzyl-L-fucopyranoside 3 (319 mg, 1.37 mmol) prepared by the conventional Fischer procedure[27] in dichloroethane–ether (20 mL, 1:4) was stirred with powdered molecular sieves 4 Å (2.0 g) and iodonium di-*s*-collidine perchlorate (644 mg, 1.37 mmol) for 24 h under a nitrogen atmosphere at room temperature. The reaction was monitored by tlc with 6:1 (v/v) benzene–ethyl acetate as a solvent system. The mixture was diluted with dichloroethane (30 mL) and filtered with Celite. The filtrate was successively washed with 10 wt % aqueous sodium thiosulfate, saturated sodium hydrogen bicarbonate, and brine, dried, filtered, and evaporated. The residual syrup was chromatographed on silica gel with 30:1 (v/v) toluene–ethyl acetate as eluant to give trisaccharide 10 (698 mg, 50% based on 2). Glycosyl acceptor 2 (681 mg) was also recovered. Compound 10: $[\alpha]_D$ −16.7° (*c* 0.205, chloroform); ^{1}H NMR δ ($CDCl_3$) 1.02 (d, 1 H, *J* 6.35 Hz, H-6″), 1.34 and 1.36 [each s, 6 H, $(CH_3)_2C$], 3.35 (m, 1 H, H-5′), 3.45 (dd, 1 H, *J* 7.3 Hz, H-2′), 3.67–3.75 (m, 2 H, H-5′ and H-3″), 3.85 (dd, 1 H, *J* 3.9 Hz, H-5″), 3.94 (d, 1 H, *J* 3.9 Hz, H-4″), 3.97 (t, 1 H, *J* 8.1 Hz, H-2″), 4.13 (t, 1 H, *J* 6.7 Hz, H-3′), 4.27 (dd, 1 H, *J* <1 and 7.9 Hz, H-4′), 4.39 (d, 1 H, *J* 7.8 Hz, H-1′), 4.84 (m, 3 H, *J* <1 Hz, H-1″ and $PhCH_2$), 5.50 (s, 1 H, H-1), and 7.15–7.50 (m, 35 H, aromatic). Anal. Calcd for $C_{68}H_{71}O_{13}N_3$: C, 71.75; H, 6.29; N, 3.39. Found: C, 72.02; H, 6.38; N, 3.77.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose (11).** A solution of trisaccharide 10 (1.7 g, 1.49 mmol) in 1:1 (v/v) methanol–ethyl aetate (30 mL) was hydrogenated in the presence of 10% palladium–carbon (200 mg) at room temperature for 48 h and filtered with Celite. The filtrate was concetrated, and the residue was treated with 4:1 (v/v) acetic acid–water (20 mL) at 90 °C for 5 h. The mixture was evaporated, and the residual syrup was treated with acetic anhydride (5 mL), pyridine (20 mL), and triethylamine (0.5 mL) for 16 h at room temperature. The extraction with chloroform was washed in the usual manner, dried, and chromatographed on silica gel with 50:48:1 (v/v/v) chloroform–ethyl acetate–methanol as eluant to give peracetate 11 (608 mg, 51% from 10): ^{1}H-NMR δ ($CDCl_3$) 1.14 (d, 1 H, *J* 6.4 Hz, H-6″), 2.00, 2.01, 2.05, 2.07, 2.08, 2.13, 2.16, and 2.19 (each s, 24 h, 8 $COCH_3$), 4.02 (d, 1 H, *J* 7.9 Hz, H-2), 4.47 (d, 1 H, *J* 4.9 Hz, H-5″), 4.54 (d, 1 H, *J* 7.3 Hz, H-1 ′), 5.07 (d, 1 H, *J* 3.7 Hz, H-4′), 5.22 (m, 1 H, H-2′), 5.26 (s, 1 H, H-1), 5.37 (d, 1 H, *J* 3.4 Hz, H-3′), and 6.20 (d, 1 H, *J* 8.8 Hz, NH).

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-1,6-di-*O*-2-deoxy-D-glucopyranose (12).** Compound 11 (222 mg, 0.276 mmol) was dissolved in acetic anhydride (4 mL) and trifluoroacetic acid (0.54 mL, 8.0 mmol). After being kept overnight at room temperature, the solution was concentrated, and the residue was coevaporated with toluene. The syrupy product was chromatographed on silica gel with 50:48:1 (v/v/v) chloroform–ethyl acetate–methanol as eluant to yield an anomeric mixture of 12 (260 mg, 99%): ^{1}H NMR δ ($CDCl_3$) 1.24 (br d, 3 H, H-6″), 1.96–2.22 (each s, 30 H, 10 $COCH_3$), 4.47 (d, 1 H, *J* 8.3 Hz, H-1′), 4.49 (s, 1 H, H-1″), 4.97 (d, 1 H, *J* 2.2 Hz, H-5′), 5.07 (m, 1 H, H-2′), 5.22 (dd, 1 H, *J* 3.2 and 11.0 Hz, H-3′), 5.42 (m, 1 H, H-4′), and 5.92 (br d, 1 H, *J* 3.7 Hz, H-1). Anal. Calcd for $C_{39}H_{53}O_{24}N{\cdot}2H_2O$: C, 48.36; H, 6.09; N, 1.48. Found: C, 47.99; H, 6.00; N, 1.75.

***n*-Pentenyl *O*-(β-D-Galactopyranosyl)-(1→4)-[*O*-(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1).** A solution of 12 (261.2 mg, 0.288 mmol) in 1,2-dichloroethane (5.0 mL) was treated with (trimethylsilyl)trifluoromethanesulfonate (58.4 μL, 0.303 mmol) under a nitrogen atmosphere, and the mixture was stirred for 3 h at 50 °C. Triethylamine (0.20 mL, 1.44 mmol) was added, and the solution was applied directly to a column of silica gel and eluted with 100:200:1 (v/v/v) toluene–ethyl acetate–triethylamine to give the syrupy oxazoline derivative 13 (147 mg, 60%): R_f 0.51 [5:4:1 (v/v/v) chloroform–ethyl acetate–methanol].

Freshly prepared 13 (147 mg, 0.173 mmol) in dry 1,2-dichloroethane (10 mL) was allowed to react with 4-penten-1-ol (100 μL, 0.968 mmol) in the presence of CSA (40.3 mg, 17.3 μmol), and the solution was stirred under a nitrogen atmosphere for 1 h at 90 °C, cooled, and poured into ice–water. The organic layer was washed successively with aqueous sodium hydrogen carbonate and water, dried, and evaporated. The residue was chromatographed on silica gel with 1:1 (v/v) toluene–ethyl acetate as eluant to give amorphous powdery 14 (61 mg, 38%), and the glycoside 14 was directly used for the next deacetylation step: R_f 0.52 [5:4:1 (v/v/v) chloroform–ethyl acetate–methanol]; ^{1}H NMR δ ($CDCl_3$) 1.21 (d, 3 H, *J* 6.3 Hz, H-6″), 1.64 (m, 2 H, OCH_2CH_2), 1.97, 1.98, 1.99, 2.07, 2.09, 2.12, 2.14, 2.16, and 2.20 (each s, 27 H, 9 $COCH_3$), 2.17 (m, 2 H, $CH_2CH{=}CH_2$), 3.45 (m, 1 H, H-5), 3.60 (m, 2 H, OCH_2), 3.80–4.17 (m, 4 H, H-2, H-4, H-6′a, and H-6′b), 4.30 (dd, 1 H, *J* 7.5 and 11.5 Hz, H-6a), 4.48 (d, 1 H, *J* 8.1 Hz,

H-1), 4.53 (dd, 1 H, H-6b), 4.60 (d, 1 H, *J* 8.6 Hz, H-1′), 4.87 (dd, 1 H, *J* 7.1 Hz, H-5″), 4.94–5.09 (m, 5 H, H-2′, H-3′, H-1″, and CH=CH_2), 5.20 (dd, 1 H, *J* 2.2 and 7.6 Hz, H-3″), 5.37–5.43 (m, 2 H, *J* 3.2 Hz, H-4′ and H-4″), 5.58 (d, 1 H, *J* 8.8 Hz, NH), 5.78 (m, 1 H, C*H*=CH_2).

To a solution of **14** (60.4 mg, 65 μmol) in dry methanol (10 mL) was added sodium methoxide (1.0 mg, 19 μmol), and the mixture was stirred for 24 h at room temperature. It was made neutral with Dowex 50W X-8 (H^+) resin, and the suspension was filtered. The filtrate was evaporated to give a quantitative yield of carbohydrate monomer **1** (38.8 mg): R_f 0.6 [3:2 (v/v) *n*-propanol–water]; $[\alpha]_D$ −75.8° (*c* 0.387, methanol); ^{1}H NMR δ (D_2O) 1.12 (d, 3 H, *J* 6.6 Hz, H-6″), 1.59 (m, 2 H, OCH_2CH_2), 1.98 (s, 3 H, $COCH_3$), 2.01 (m, 2 H, CH_2CH=CH_2), 4.40 and 4.47 (each d, 2 H, *J* 7.8 Hz, H-1 and H-1′), 4.95–5.05 (m, 3 H, H-1″ and CH=CH_2), and 5.81 (m, 1 H, C*H*=CH_2); ^{13}C NMR δ (D_2O) 16.2 (C-6″), 23.2 (CH_3), 28.8 (CH_2CH=CH_2), 30.2 (OCH_2CH_2), 56.8 (C-2), 60.7 (C-6), 61.7 (C-6′), 67.6, 68.6, 69.3, 70.1, 70.7, 72.0, 72.8, 73.4, 74.3, 75.8, 76.2, and 77.6 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″, and OCH_2), 99.5 (C-1″), 101.8 (C-1), 102.7 (C-1′), 115.8 and 140.0 (CH=CH_2), and 171.9 (C=O).

Copolymer from Oligosaccharide Monomer and Acrylamide. A mixture of monomer **15** (38.8 mg, 65 μmol) and acrylamide (91.9 mg, 1.29 mmol) in deionized water (1 mL) was deaerated by using water pump for 20 min, to which were added TEMED (3.95 μL, 26.1 μmol) and APS (2.1 mg, 9.20 μmol). The mixture was stirred for 48 h at room temperature, diluted with 0.1 M pyridine–acetic acid buffer (pH 5.1), dialyzed against deionized water for 3 days, and freeze-dried to give a water-soluble polymer as an amorphous white powder (98.3 mg, 75.3%): $[\alpha]_D$ −8.6° (*c* 0.305, water); η_{inh} 0.87 (*c* 0.305, 25 °C, water); MW 280K (GPC method); ^{1}H NMR δ (D_2O) 1.1 (br d, H-6″), 1.5–1.8 (m, $-CH_2-$), 2.0 (s, $COCH_3$), 2.2 and 2.3 (–CH–), 3.3–4.0 (ring protons of sugar moieties), 4.4–4.6 (br d, H-1 and H-1′), 4.7 (br d, H-5″), and 5.0 (br s, H-1″).

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