Synthetic Glycoconjugates. 5.[1] Polymeric Sugar Ligands Available for Determining the Binding Specificity of Lectins[†]

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ABSTRACT: Systematic syntheses of polymerizable *N*-acetyllactosamine and related disaccharide derivatives have been accomplished by introducing an *n*-pentenyl group at each reducing end as a simple and versatile polymerizable aglycon. Radical copolymerizations of these sugar monomers with acrylamide proceeded smoothly in water by means of ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine as initiators and to give water-soluble glycopolymers in good yields. In addition to chemical syntheses of glycopolymers, chemoenzymic galactosylation of the polymeric GlcNAc ligand was also completely performed by means of bovine galactosyl transferase activity in the presence of uridine 5′-diphosphogalactose as a sugar donor. These glycopolymers were demonstrated to exhibit enhanced binding capacity with lectins on the basis of polymeric sugar−cluster effect. It was suggested that the polymeric LacNAc ligand showed much higher affinity to *Erythrina corallodendron* lectin than other polymers having positional LacNAc isomers from the inhibitory assay of hemagglutination by *Erythrina corallodendron*. The order of the inhibitory effects of these polymers on hemagglutination by *Erythrina corallodendron* was Galβ(1−4)GlcNAc (LacNAc) ≫ Galβ(1−4)Glc (Lac) ≫ Galβ(1−3)GlcNAc > Galβ(1−6)GlcNAc ≅ Gal.

Introduction

Although sugar chains of the glycoconjugates could play important roles as biologically active structures on the cell surface, they usually bind to the peptide chains or the ceramides. This suggests that sugar chains usually act as partial structures of macromolecules or conjugated molecules. As it is known that the synthetic trisaccharide or small sugar derivatives exhibited only weak affinity to the hemagglutinin molecules,[2] it seems to be important to investigate how such weak carbohydrate−protein interactions are eventually amplified in the successful recognition processes, but with extreme carbohydrate specificity. In order to thoroughly understand these interactions on molecular level, our attention has been focused on glycoprotein models as the synthetic glycoconjugates which may be available for the investigation of the recognition processes related to sugar−protein interactions. Actually, synthetic polymers including well-defined sugar residues are used for biochemical purposes such as affinity chromatography of lectins, carbohydrate antigens, and cell culture systems.[3−5]

Recently we have reported a convenient preparation of water-soluble carbohydrate monomers which have *n*-pentenyl group (PentO; CH_2=$CHCH_2CH_2CH_2O$−) at the reducing end as a polymerizable functional group, and copolymers from the corresponding monomers with acrylamide.[6,7] This method has been applicable for the syntheses of a variety of sugar monomers containing *N*-acetyl-D-glucosamine (GlcNAc),[7] *N,N′*-diacetylchitobiose [GlcNAcβ(1−4)GlcNAc],[7] *N*-acetyllactosamine [Galβ-(1−4)GlcNAc],[6] and Le^x-type trisaccharide {Galβ(1−4)[Fucα(1−3)]GlcNAc} of tumor-associated carbohydrate antigens.[8]

N-Acetyllactosamine [*O*-(β-D-galactopyranosyl)-(1−4)-2-acetamido-2-deoxy-D-glucopyranose; Galβ(1−4)GlcNAc; LacNAc] is well known as a biologically important disaccharide core structure of lactosaminoglycans, tumor-associated antigenic carbohydrates, and many carbohydrate receptors in glycoproteins and glycolipids.[9−11] We have demonstrated a highly selective adhesion of rat hepatocyte on a polyacrylamide gel including LacNAc residues which clearly showed much higher affinity than another type of gel including *N,N′*-diacetylchitobiose,[6] since hepatic lectins have been reported to exhibit specific affinity to LacNAc containing sugar chains of glycoproteins.[12] It was also demonstrated for determining the sugar binding specificity of wheat germ agglutinin (WGA) that density of GlcNAc resides on the glycoprotein models significantly affected a successful combining to the subsites of WGA using high-density GlcNAc polymer prepared from ω-acryloyl amido type sugar monomer.[1]

We describe herein the syntheses and binding properties with lectins of new polymers having sequences related to LacNAc such as Gal, Lac, Galβ(1−3)GlcNAc (LacNAc-13), and Galβ(1−6)GlcNAc (LacNAc-16), respectively. The specific interaction of the glycopolymers with *Erythrina corallodendron* lectin will be discussed by means of inhibitory effects of glycopolymer ligands on hemagglutination induced by the lectin.

Results and Discussion

Figure 1 shows sugar monomers designated for investigating structural accessibility in the interaction between ECorL and lactosamine oligosaccharides. Compounds **1** and **3** were prepared according to the method reported previously through the sample reactions of oxazoline derivatives with *n*-pentenyl alcohol. Galactose derivative **2** and lactose derivative **6** were selected as sugar monomers to prepare glycopolymer ligands lacking the reducing GlcNAc residue. In order to evaluate the importance of the inter-glycosidic bond

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[†] Abbreviations: Gal, galactose; GlcNAc, *N*-acetyl-D-glucosamine; Lac, lactose; LacNAc, *N*-acetyllactosamine; LacNAc-13, Galβ(1−3)GlcNAc; LacNAc-16, Galβ(1−6)GlcNAc; Pent, CH_2=$CH(CH_2)_3$; GalT, galactosyl transferase; UDP-Gal, uridine 5′-diphosphogalactose; ECorL, *Erythrina corallodendron* lectin; TMSOTf, trimetylsilyltrifluoromethanesulfonate; CSA, camphorsulfonic acid; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; APS, ammonium persulfate; HPAEC-PAD, high-performance anion exchange chromatography with pulsed amperometric detection; PBS, phosphate-buffered saline.

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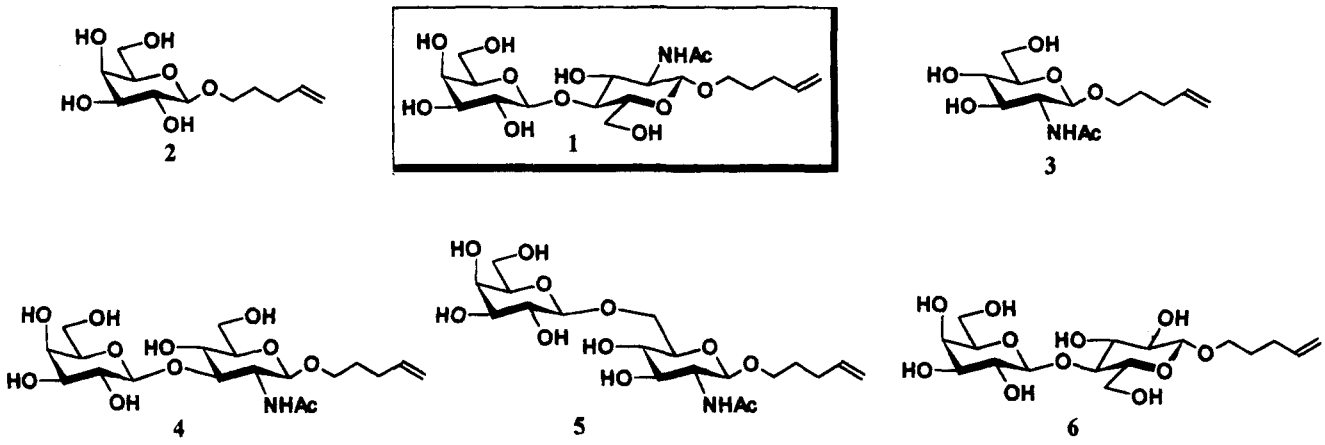
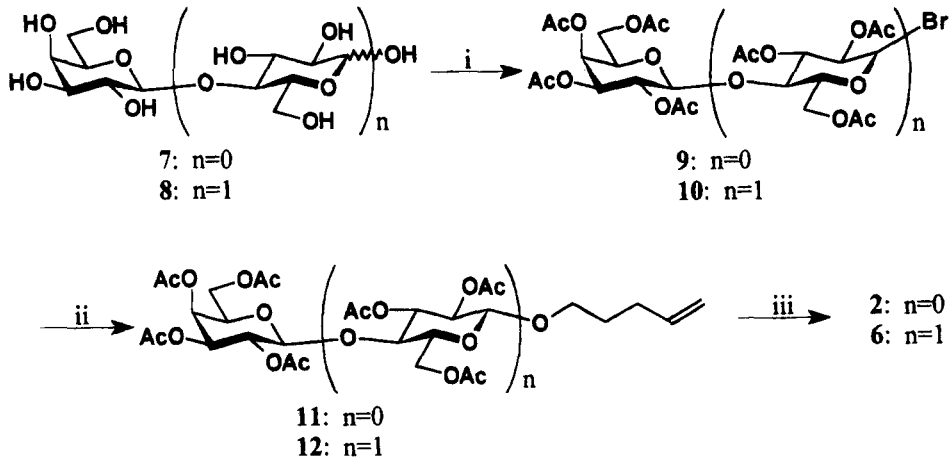


Figure 1. Glycosyl monomers related to LacNAc.

Scheme 1[a]



[a] Reagents and conditions: (1) Ac_2O, HBr/AcOH at room temperature, and then further HBr/AcOH at room temperature; (ii) PentOH, MS 4 Å, $Hg(CN)_2$, benzene–nitromethane, room temperature; (iii) NaOMe/MeOH, room temperature.

between Gal and GlcNAc residues, two positional isomers LacNAc-13 (**4**) and LacNAc-16 (**5**) were regarded as synthetic targets of this study. Since we have already demonstrated the versatility of *n*-pentenyl group for creating glycopolymers having branching oligosaccharide structure such as Le^x trisaccharide,[8] all sugar monomers used here were expected to show good polymerizability to give polymers with high molecular weights.

Preparation of *n*-Pentenyl Glycosides Derived from Galactose and Lactose. Our procedure for making *n*-pentenylated glycosides via an oxazoline derivative were generally applied for oligo- or monosaccharides which have a GlcNAc residue at the reducing end. Thus, Gal and Lac which are lacking the 2-acetamido group at reducing end were converted to *n*-pentenyl glycosides by employing the Helfrich method.[13] A galactosyl bromide **9** made by the one-pot synthesis of the Kartha's procedure[14] was allowed to react with 4-penten-1-ol in the presence of mercuric(II) cyanide [$Hg(CN)_2$] in benzene–nitromethane at room temperature (Scheme 1). The reaction mixture was purified by using chromatography on Sephadex LH-20 with 95% ethanol as the eluent, and subsequent silica gel chromatography gave the pure β-glycoside **11** in 89% yield. The per-*O*-acetate **11** was treated with sodium methoxide in methanol to give water-soluble **2** as an amorphous solid. ^{13}C NMR chemical shifts of compounds **2** and **6** are listed in Table 1.

Preparation of Galβ1-3GlcNAcβ1-OPent (LacNAc-13). Scheme 2 illustrates a synthetic route of LacNAc-13. Because of poor solubility of a benzylidene derivative of *n*-pentenyl GlcNAc **3** as a glycosyl acceptor, the key intermediate had to be changed to a benzylidene derivative of benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**13**).[15] Glycosyl acceptor **13** was coupled with galactosyl bromide **9** by means of $Hg(CN)_2$ as a catalyst to provide **14** in 68% yield. The disaccharide derivative **14** was converted to the per-*O*-acetate **15**, and then the trimethylsilyl trifluoromethanesulfonate (TMSOTf) was employed for the preparation of a reactive oxazoline derivative which was a convenient precursor for the glycoside formation. The oxazoline derivative was allowed to react with 4-penten-1-ol in the presence of a catalytic amount of camphorsulfonic acid (CSA) which is usually added until pH 3, and finally the *n*-pentenyl glycoside **16** was de-*O*-acetylated by the Zemplén method to give pure water-soluble **4** in good yield. The purity and structure of glycoside **4** was confirmed by ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum of compound **4** revealed the signal assignable to H-1 at δ 4.45 ppm as a doublet with $J_{1,2}$ 7.8 Hz, indicating that the glycosidic bond newly formed had the β configuration. Chemical shifts of the fully assigned ^{13}C NMR spectrum for **4** are listed in Table 1.

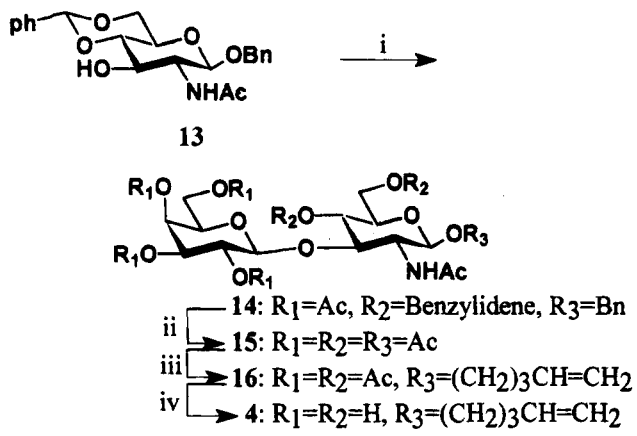
Preparation of Galβ(1–6)GlcNAcβ1-OPent (LacNAc-16). As shown in Scheme 3, we first prepared an intermediate **18** having an unprotected hydroxyl group at C-6 position as a glycosyl acceptor. Thus, a primary hydroxyl group at C-6 position of the *n*-pentenylated GlcNAc **3** was protected using triphenylmethyl (trityl) group and subsequently acetylated with acetic anhy-

Table 1. ^{13}C NMR Chemical Shifts[a] of Carbohydrate Monomers and Copolymers

	monomers				copolymers			
	2	**4**[c]	**5**	**6**	**2**	**4**[c]	**5**	**6**
C-1	102.85	103.5	101.11	102.13	102.77	103.5	101.24	102.06
C-2	70.87	57.3	55.66	72.96	70.88	57.0	55.71	72.97
C-3	72.95	85.2	73.77	74.54	72.97	85.2	73.86	74.57
C-4	68.73	73.1	70.01	78.84	68.77	73.2	70.06	78.83
C-5	75.04	75.2	74.95	74.79	75.08	77.9	74.98	74.80
C-6	60.94	63.5	68.59	60.38	61.02	63.6	68.77	60.38
C-1′		106.0	103.35	103.01		106.1	103.47	103.01
C-2′		73.5	70.81	71.06		73.4	70.88	71.05
C-3′		75.2	72.81	72.72		75.2	72.88	72.72
C-4′		71.2	68.69	68.66		71.2	68.77	68.66
C-5′		78.0	75.11	75.37		78.1	75.19	75.37
C-6′		64.5	60.97	61.02		63.6	65.05	61.02
C=O		177.0	174.30			177.0	174.24	
CH_3		24.9	22.26			25.1	22.42	
OCH_2	69.80	N.D.	69.75	69.93	70.36	N.D.	70.88	70.44
OCH_2CH_2	28.09	30.6	27.96	28.09				
CH_2CH=	29.30	31.8	29.21	29.30				
CH=CH_2	139.04	141.5	138.89	139.06				
CH=CH_2	114.75	117.4	114.77	114.78				
CH[b]					41.94	44.5	41.98	41.97
CH_2[b]					35.38	37.5	35.44	35.41
C=O[b]					179.40	182.1	179.42	179.42

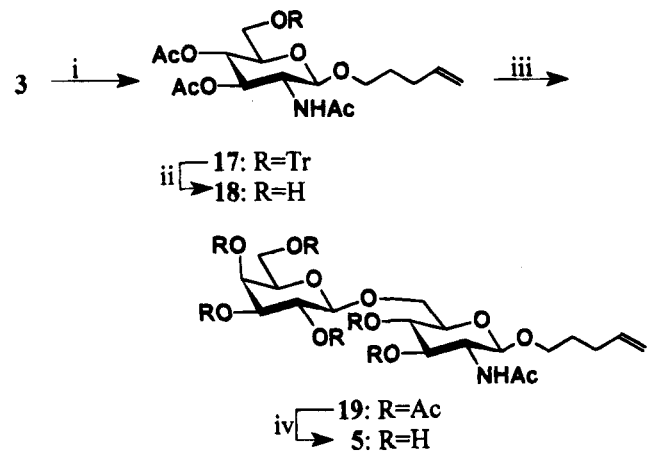
[a] Chemical shifts were determined from the signal of methanol (49.0 ppm) as the internal standard. [b] Signals were observed as multiplets owing to the polymer main chain structure. [c] Chemical shifts were determined from DSS instead of the methanol as the internal standard.

Scheme 2[a]



[a] Reagents and conditions: (i) **9**, $Hg(CN)_2$, benzene–nitromethane, 60 °C, 8 h; (ii) H_2/Pd-c, EtOH-AcOH-H_2O, 60 °C, 9 h, and then Ac_2O, pyridine; (iii) TMSOTf, $ClCH_2CH_2Cl$, 50 °C, 5 h, and then Et_3N, room temperature, and PentOH, CSA, $ClCH_2CH_2Cl$, 90 °C, 5 h; (iv) NaOMe/MeOH, room temperature, 3 h.

Scheme 3[a]



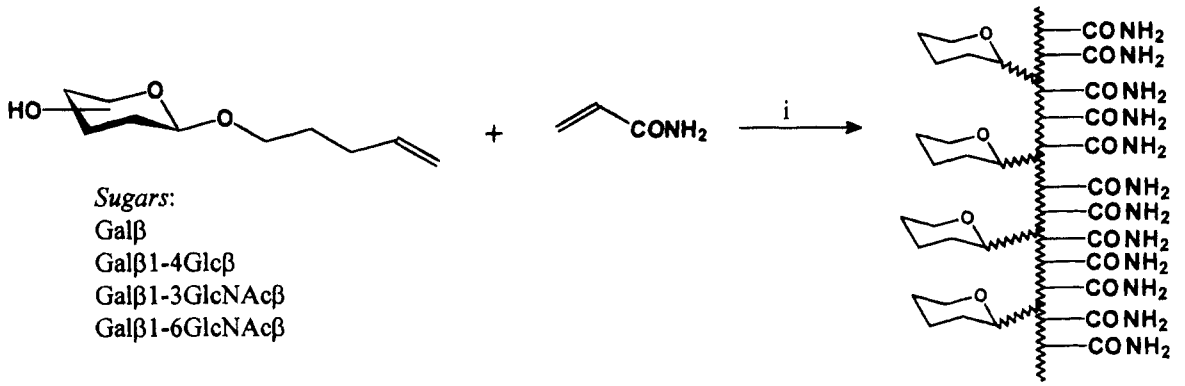
[a] Reagents and conditions: (i) TrCl, pyridine, 90 °C, 40 min, and then Ac_2O, room temperature, 3 h; (ii) 80% aqueous AcOH, 90 °C, 1 h; (iii) **9**, MS 4 Å, $Hg(CN)_2$, benzene–nitromethane; room temperature, 3 days; (iv) NaOMe/MeOH, room temperature, 4.5 h.

dride and pyridine to give derivative **17** in 80% yield. Next, compound **17** was treated with 80% aqueous acetic acid at 90 °C to remove the trityl protecting group to provide acceptor **18** in 76% yield without any acetyl migration to the adjacent hydroxyl group. Glycosidation of acetobromogalactose **9** as a donor and **18** as an acceptor was carried out in the presence of $Hg(CN)_2$ in 1:1 (v/v) of benzene–nitromethane at room temperature and gave disaccharide **19** in 21% yield. It is clearly suggested from ^1H NMR spectrum of disaccharide **19** that the formation of the glycoside between Gal and GlcNAc moiety was achieved as the desired β configuration (δ 4.56 ppm, $J_{1',2'}$ 8.0 Hz). Fully protected glycoside **19** was de-*O*-acetylated by Zemplén conditions to give water-soluble glycoside **5** in 95% yield. From the chemical shifts of the ^{13}C NMR spectrum of compound **5**, it was shown that only the C-6 signal of the GlcNAc residue distinctively deshielded and shifted to 68.6 ppm.

Radical Copolymerization of Water-Soluble Glycosides with Acrylamide. Carbohydrate monomers were copolymerized with acrylamide in deionized water for 2 h at room temperature using *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) as initiators[6] (Scheme 4), and the results of copolymerizations are summarized in Table 2. All new monomers exhibited good polymerizability in similar conditions as reported previously for the preparation of LacNAc polymers.[6] The unit ratio of the polymers abbreviated as a "polym comps" was determined by comparing the intensity of the integration of the protons for *N*-acetyl group (at 2.0 ppm) due to the GlcNAc residue and the methine group (2.2 ppm) due to the main chain of the polymer by ^1H NMR, or by the phenol–sulfuric acid assay[16] for carbohydrate analysis in the case of the polymers having pendant-type galactose and lactose. The molecular weights of the polymers were estimated to be high enough (more than 400 kDa) to achieve successful "polymeric sugar-cluster effects" in further binding study with lectins.

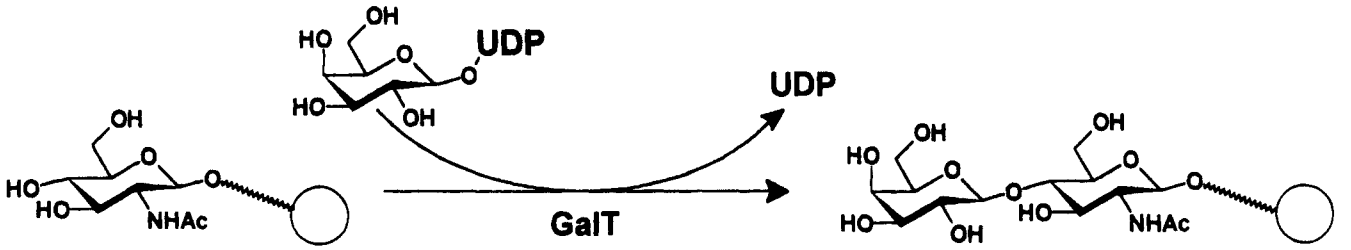
Enzymatic Preparation of Glycopolymer Having LacNAc Side Chains.[17] Chemoenzymatic syntheses

Scheme 4[a]



[a] Reagents and conditions: (i) TEMED, APS, H_2O, room temperature, 2 h.

Scheme 5[a]



[a] Reagents and conditions: α-Lactoalbumin, $MnCl_2$ (10 mM), 50 mM HEPES, pH 6.0, 37 °C, 24 h.

Table 2. Results of Copolymerization of Carbohydrate Monomers with Acrylamide

carbohydr monomer	monomer ratio[a]	total yield, %	polym compos[a]	sugar content, wt %	M_w,[b] kDa
Gal	1:4	72.2	1:5	40.7	>400
	1:10	93.9	1:10	25.2	>400
Lac	1:4	66.7	1:10	37.0	>400
	1:10	81.0	1:23	19.8	>400
LacNAc-13	1:10	78.7	1:17	27.2	>400
LacNAc-16	1:4	62.7	1:8	44.0	>400
	1:10	77.4	1:23	23.0	>400

[a] Ratio of carbohydrate monomer to acrylamide. [b] M_ws were estimated by the GPC method with asahipak G-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards].

of carbohydrates have been regarded as an important strategy for the preparation of structurally complex oligosaccharides owing to their stereo- and regioselectivity in the formation of glycoside linkages.[18] The galactosyl transferase (GalT) is a readily available and a well-evaluated enzyme.[19] Therefore, our interests were paid toward chemical and enzymatic syntheses of glycopolymers in order to establish more convenient and facile procedures for this purpose.

It has been known that galactosylation to a 4-OH position of the GlcNAc in oligosaccharides by β-1,4-GalT has succeeded to provide an *N*-acetyllactosamine-type oligosaccharide chains even in case of introduction of galactose to a sepharose−GlcNAc conjugate in approximately 20% yield.[20] Since Zehavi et al. also demonstrated the enzymatic elongation approach by using a water-soluble polymer substrate in which the polymer had pendant-type glucose residues,[21] galactosylation yield was found to be 36% owing to a poor accessibility of rigid polymer structures against enzymatic reactions. In order to enhance the transfer yield on the polymer chains, the *n*-pentenyl group was expected to provide the acceptor sugar side chains with an adequate spacer arm function. Actually, the distance between the main chain of the polymer and sugar moiety was estimated to be 7.48 Å. As anticipated, elongation of Gal into the GlcNAc polymer with GalT proceeded smoothly to give LacNAc-type polymer in quantitative yield (Scheme 5). The integration ratio of H-1 and H-1′ of the enzymatically synthesized polymer having LacNAc measured in ^1H NMR spectrum revealed this quantitative galactosylation (Figure 2B). And the spectrum was completely identical with that of the synthetic LacNAc polymer shown in Figure 2A. Moreover, high-performance anion exchange chromatography (HPAEC) analysis of acid hydrolysate of LacNAc polymer also supported this result as shown in Figure 2C. The calculated area of Gal and GlcNAc were found to be completely the same on the basis of elution profiles of standard samples.[22]

Inhibitory Effects of Glycopolymer Ligands on Hemagglutination Induced by *Erythrina corallodendron* lectin. As an example of biochemical availability of the glycopolymer ligands prepared here, inhibitory effects of these compounds on hemagglutination by *Erythrina corallodendron* lectin (ECorL) were examined. Although ECorL is a readily available plant lectin which mainly recognizes Lac and LacNAc,[23,24] a binding specificity assay had been done only for the simple sugar molecules. Therefore, glycopolymers having LacNAc-related disaccharides such as LacNAc-13 and LacNAc-16 were supposed to be useful ligands so as to discuss the significance of glycosidic linkage between Gal and GlcNAc residues. Although Tenenberg et al. have reported and discussed the binding specificity of glycolipid-type ligands with this lectin systematically,[25] there was no attempt to gain further information on the structural limitation of sugar ligands using positional isomers or their derivatives. The results of inhibitory effects of glycopolymers on the hemagglutination of human type B blood cells by ECorL are summarized in Table 3. The hemagglutination activity of the lectin was the most strongly inhibited by the

Table 3. Inhibitory Effects of Glycopolymers[a] on *Erythrina Corallodendron* Lectin Hemagglutination[b]

	ratio[c]							
	control	monomer LacNAc	1:10 Gal	1:10 GlcNAc	1:10 LacNAc-13	1:10 LacNAc-16	1:10 Lac	1:10 LacNAc
MC[d] (μM)	≫1000	>25	>1000	≫1000	>500	>1000	>25	>1

[a] Glycopolymers were derived from those *n*-pentenyl glycosides with acrylamide. [b] Fresh human type B red blood cells were treated with heparin and used as the concentration estimated to be 1% (v/v) in 0.05 M PBS (pH 7.3). The lectin concentration was estimated to be 2.0 μM by using the extinction coefficient A_{280}. [c] Monomer ratio of sugar to acrylamide. [d] Minimum concentration of inhibitory potency in the hemagglutination were estimated by the concentration of the sugar residue.

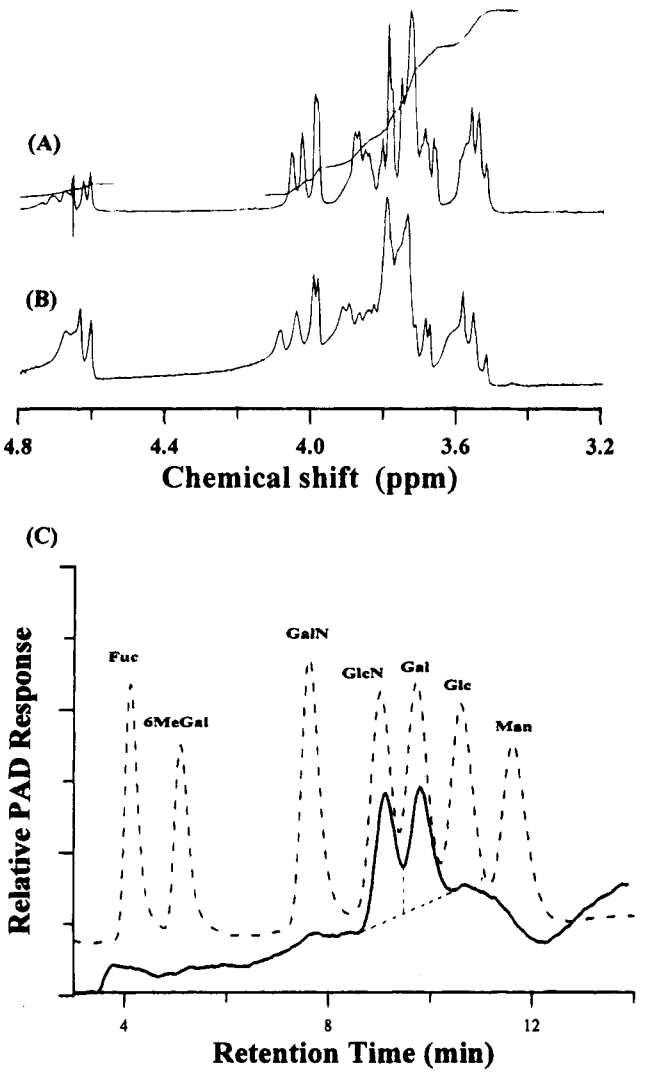


Figure 2. ^{H} NMR spectrum of (A) chemically (500 MHz) and (B) enzymatically (300 MHz) synthesized LacNAc polymer and (C) HPAEC patterns of 50 mM standard solution (broken line) and hydrolysate of LacNAc polymer (solid line).

polymeric ligand including LacNAc among the sugar ligands used in this experiment. The effect of the polymeric ligand including Lac was shown to be slightly weaker than that of polymeric LacNAc. On the other hand, glycopolymers of regioisomers of LacNAc such as LacNAc-13 and LacNAc-16 showed only a faint or weak inhibitory effect on the hemagglutination by lectin. It was apparently suggested that lctin recognizes specifically the interglycosidic linkage between Gal and GlcNAc residues. This suggests that the binding pocket of the lectin probably is tight and strictly designated for combining with the structures of Galβ(1−4)Glc or Galβ(1−4)GlcNAc. Use of the polymers having sugar residues is proved to be quite an efficient methodology for determining the binding specificity of a lectin. When the polymeric ligand is applied for such experiments, the affinity constant will be observed as exponentially amplified values rather than that of monomeric ligand at the same concentrations which is based on polymeric sugar-cluster effects. Usually, the binding constants of polymeric ligands are estimated to be 10^3 times as much as that of monomeric sugar itself, thus we could compare exactly the differences in the binding constants of sugar ligands with lectins. As shown in Table 3, it was clearly demonstrated that the order of the inhibitory effects of glycopolymers on hemagglutinination by lectin is LacNAc polymer ≫ Lac polymer, LacNAc monomer ≫ LacNAc-13 polymer > LacNAc-16 polymer, Gal polymer ≫ GlcNAc polymer.

In conclusion, we have systematically synthesized LacNAc-related oligosaccharides having an *n*-pentenyl group as a polymerizable aglycon, and these glycosides were smoothly converted to the water-soluble copolymers as the simple and convenient polymeric sugar ligands related to lactosaminoglycan models. The enzymatic elongation of the sugar chain in the GlcNAc-branching type polymer has been also demonstrated using GalT and UDP-Gal to give a quantitative yield of LacNAc-branching type polymer. It was suggested that these glycopolymer ligands are a new type of sugar-cluster ligands for lectins to discuss the binding specificity between sugar and sugar-binding proteins. This method will be applied for creating further complex glycoprotein mimetics and ligands such as sialic acid containing structures and sulfated oligosaccharides.

Experimental Section

General Procedures. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Benzene, chloroform ($CHCl_3$), dimethylformamide (DMF), nitromethane, and methanol (MeOH) were stored over molecular sieves (3 Å), and pyridine and triethylamine were stored with NaOH pallets before use. Powdered molecular sieves 4 Å (MS4Å) were dried over *in vacuo* at ca. 100 °C over night after pulverizing. Acrylamide was recrystallized from benzene. Melting points were determined with a Laboratory Devices melting point apparatus or Fisher-Johns melting point apparatus and were not corrected. ^{1}H NMR spectra were recorded at 270, 300, or 500 MHz with a JEOL JNM-GX270, Bruker AMX-300, or JEOL EX-500 spectrometer in chloroform-*d* or deuterium oxide. ^{13}C NMR spectra were recorded at 67.8 or 75 MHz with the same instruments. Tetramethylsilane (TMS), methanol (3.3 ppm for ^{1}H or 49.0 ppm for ^{13}C), or 3-(trimethylsilyl)propanesulfonic acid sodium salt (DSS) were used as internal standards. Ring-proton assignments in NMR were made by first-order analysis of the spectra and were supported by homonuclear decoupling experiments. Elemental analyses were performed with a Yanaco MT-3 CHNcorder or done by Galbraith Laboratories, Inc., on samples extensively dired ca. 24 h *in vacuo* (50 °C, 0.1 Torr) over phosphorus pentoxide. Average molecular weights of the polymers were estimated by the gel permeation chromatography (GPC) method with an Asahipak GS-510 column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 300 kDa, Shodex Standard P-82) were used as standards. 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). The solvent systems used are (A) 2:1, (B) 1:1, (C) 1:4 (v/v) toluene–ethyl acetate, (D) 5:4:1 (v/v/v) chloroform–ethyl acetate–methanol, and (E) 65:25:4 (v/v/v) chloroform–methanol–water. For detection of the intermediates, 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 carbohydrate) or (b) an aqueous solution of 5 wt % potassium permanganate and heated similarly (for double bond). Column chromatography was performed on silica gel (Wakogel c-200; 100–200 mesh, Wako Pure Chemical Industries, Osaka, Japan) or (Silica Gel 60; 0.015–0.040 mm, E. Merck). Dialyses were performed against deionized water using a dialysis tubing (molecular cut off, 12000–14000 kDa). All extractions were concentrated below 45 °C under diminished pressure. Galactosyl transferase from bovine milk (GalT; EC 2.4.1.90), uridine 5′-diphosphate galactose (UDP-Gal), and *Erythrina corallodendron* lectin (ECorL) were obtained from Sigma Co., St. Louis, MO.

***n*-Pentenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (11).** A mixture of powdered MS4Å (1 g), mercuric(II) cyanide [$Hg(CN)_2$] (2.48 g, 9.73 mmol), and 4-penten-1-ol (1.01 mL, 9.73 mmol) in 1:1 (v/v) of benzene–nitromethane (5 mL) was stirred for 40 min. To the mixture was added **9**[14] (2.0 g, 4.86 mmol), and the reaction mixture was stirred for 24 h at room temperature. The mixture was filtered, and the filtrate was evaporated under diminished pressure. The residue was dissolved in chloroform, and the suspension was filtered to remove precipitates of mercuric salts. The organic layer was washed with 1.5 M sodium chloride, dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo*. The residual syrup was subjected to the Sephadex LH-20 column (5 i.d. × 200 cm) with 95% ethanol as eluent. The fractions containing the glycoside **11** determined by TLC were combined and concentrated. The syrup was chromatographed on silica gel with 4:1 (v/v) toluene–ethyl acetate as the eluent to give pure **11** (1.80 g, 88.7%): R_f 0.57 (solvent A); ^{1}H NMR δ ($CDCl_3$) 1.70 (m, 2 H, OCH_2CH_2), 1.99, 2.05, 2.06, 2.15 (each s, 12 H, 4 $COCH_3$), 2.11 (m, 2 H, CH_2CH=), 3.71 (m, 2 H, OCH_2), 3.9 (m, 1 H, H-5), 4.12 (dd, 1 H, $J_{5,6a}$ 6.9 Hz and $J_{6a,6b}$ 11.2 Hz, H-6a), 4.19 (dd, 1 H, $J_{5,6b}$ 6.6 Hz, H-6b), 4.46 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.96–5.05 (m, 2 H, CH=CH_2), 5.02 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 5.21 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.39 (dd, 1 H, $J_{4,5}$ 0.8 Hz, H-4), and 5.72–5.86 (m, 1 H, CH=CH_2).

***n*-Pentenyl β-D-Galactopyranoside (2).** To a solution of **11** (1.80 g, 4.32 mmol) in methanol (20 mL) was added sodium methoxide (93.4 mg, 1.73 mmol), and the mixture was stirred for 15 h at room temperature. Dowex 50W X-8 (H^+) resin was added to neutralize the solution, and the suspension was filtered and evaporated to give a quantitative yield of carbohydrate monomer **2** (1.07 g): R_f 0.40 (solvent E): ^{1}H NMR δ (D_2O) 1.68 (m, 2 H, OCH_2CH_2), 2.10 (m, 2 H, CH_2CH=), 3.8 (m, 2 H, OCH_2), 3.45 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 3.59 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 3.87 (m, 1 H, H-4), 4.33 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.95–5.08 (m, 2 H, CH=CH_2), and 5.79–5.93 (m, 1 H, CH=CH_2); ^{13}C NMR δ (D_2O) are listed in Table 1.

Anal. Calcd for $C_{11}H_{20}O_6 \cdot 0.9H_2O$: C, 49.95; H, 8.30. Found: C, 50.18; H, 8.59.

***n*-Pentenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1–4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (12).** A mixture of MS4Å powder (3 g), $Hg(CN)_2$ (2.55 g, 10.0 mmol), and 4-penten-1-ol (1.56 mL, 15.0 mmol) in 1:1 (v/v) of benzene–nitromethane (10 mL) was stirred for 40 min. To the suspension was added **10**[14] (3.50 g, 5.00 mmol) at room temperature as described for the preparation of **11**. The reaction mixture was worked up in the same manner for **11**, and the residue was purified by chromatography on silica gel with 2:1 (v/v) toluene–ethyl acetate as the eluent to afford **12** (1.43 g, 40.3%): R_f 0.51 (solvent B); ^{1}H NMR δ ($CDCl_3$) 1.65 (m, 2 H, OCH_2CH_2), 1.97, 2.04, 2.05, 2.05, 2.07, 2.12, 2.16 (each s, 21 H, 7 $COCH_3$), 2.09 (m, 2 H, CH_2CH=), 3.60 (ddd, 1 H, $J_{5,6b}$ 5.0 Hz and $J_{5,6b}$ 2.0 Hz, H-5), 3.66 (m, 2 H, OCH_2), 3.80 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.88 (t, 1 H, $J_{5',6'}$ and $J_{5',6'b}$ 6 Hz, H-5′), 4.11 (m, 3 H, H-6a, 6′a, 6′b), 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.49 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1′), 4.47 (m, 1 H, H-6b), 4.89 (dd, $J_{2,3}$ 9.6 Hz, H-2), 4.95 (dd, 1 H, $J_{3'4'}$ 3.4 Hz, H-3′), 4.95–5.04 (m, 2 H, CH=CH_2), 5.11 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 5.20 (t, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 5.35 (d, $J_{4',5'}$ ~0 Hz, H-4′), and 5.78 (m, 1 H, CH=CH_2).

***n*-Pentenyl *O*-(β-D-Galactopyranosyl)-(1–4)-β-D-glucopyranoside (6).** Compound **6** was prepared from **12** (1.42 g, 2.02 mmol) by the procedure for the preparation of **2** (0.82 g, 98.9%): mp 162.5–165.5 °C (from ethanol); R_f 0.38 (solvent E); ^{1}H NMR δ (D_2O) 1.68 (m, 2 H, OCH_2CH_2), 2.09 (m, 2 H, CH_2CH=), 3.26 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 3.49 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-2′), 4.33 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.40 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.82–5.08 (m, 2 H, CH=CH_2), and 5.79–5.93 (m, 1 H, CH=CH_2); ^{13}C NMR δ (D_2O) are listed in Table 1.

Anal. Calcd for $C_{17}H_{30}O_{11} \cdot 0.8H_2O$: C, 48.06; H, 7.49. Found: C, 48.24; H, 7.71.

Benzyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1–3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (14). A solution of **13**[15] (3.0 g, 7.51 mmol) in 1:1 (v/v) of benzene–nitromethane (300 mL) was evaporated to ca. 50 mL. $Hg(CN)_2$ (1.71 g, 6.76 mmol) and a solution of **9** (3.24 g, 7.89 mmol) in benzene (30 mL) were added to the mixture, and the reaction mixture was stirred for 8 h at 60 °C. The solution was diluted by chloroform, washed with aqueous saturated sodium hydrogen bicarbonate and brine, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The syrup was chromatographed on silica gel eluted with 5:1 to 1:1 (v/v) toluene–ethyl acetate to give pure **14** (3.7 g, 67.5%): ^{1}H NMR δ ($CDCl_3$) 1.92–2.10 (5 s, 15 H, 5 $COCH_3$), 4.57 and 4.74 (each d, 2 H, CH_2-Ph), 4.66 (d, 1 H, $J_{1',2'}$ 9.3 Hz, H-1′), 5.24 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.52 (s, 1 H, benzylidene), 5.79 (d, 1 H, *J* 7.1 Hz, NH), and 7.26–7.47 (m, 10 H, aromatic).

Anal. Calcd for $C_{36}H_{43}O_{15}N_1 \cdot 1H_2O$: C, 57.82; H, 5.80; N, 1.87. Found: C, 58.08; H, 5.92; N, 2.01.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1–3)-2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (15).** A mixture of **14** (300 mg, 0.411 mmol), 10% palladium–carbon (300 mg) in 2:1:1 (v/v/v) of ethanol–acetic acid–water (20 mL) was continuously stirred under hydrogen atmosphere for 9 h at 60 °C. The mixture was neutralized with triethylamine until pH 7, filtered, and concentrated. The residual syrup was dissolved in pyridine (10 mL) and acetic anhydride (10 mL) at 0 °C. The mixture was stirred for 12 h at room temperature. The reaction mixture was evaporated, poured into ice–water, and extracted with chloroform. The chloroform layer was successively washed with 1 N sulfuric acid, saturated aqueous sodium hydrogen bicarbonate, and brine. The solution was dried over magnesium sulfate, filtered, and evaporated. The residue was purified by silica gel chromatography with 20:1 to 1:0 (v/v) chloroform–methanol to give pure **15** (200 mg, 71.8%): R_f 0.50 (solvent D); ^{1}H NMR δ ($CDCl_3$) 1.97–2.20 (8 s, 24 H, 8 $COCH_3$), 4.62 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 5.36 (d, 1 H, $J_{3',4'}$ 2.9 Hz, H-4′) 5.61 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), and 6.07 (d, 1 H, *J* 3.7 Hz, NH).

Anal. Calcd for $C_{28}H_{39}O_{18}N_1 \cdot 1H_2O$: C, 48.34; H, 5.94; N, 2.01. Found: C, 48.28; H, 5.73; N, 2.04.

***n*-Pentenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1–3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (16).** A solution of **15** (440 mg, 0.649 mmol) in dichloroethane (10 mL) was treated with trimethylsilyl trifluoromethanesulfonate (132 mL, 0.681 mmol) under a nitrogen atmosphere for 5 h at 50 °C. Triethylamine (272 mL, 1.95 mmol) was added to the mixture, and the solution was directly subjected to a column of silica gel with 100:200:1 (v/v/v) toluene–ethyl acetate–triethylamine as an eluent to give an oxazoline derivative (290 mg, 70.3%): R_f 0.56 (solvent D).

A solution of the freshly prepared oxazoline derivative (290 mg, 0.456 mmol) in dichloroethane (5 mL) was allowed to react with 4-penten-1-ol (141 μL, 1.37 mmol) in the presence of 10-camphorsulfonic acid-*d* (30 mg) under a nitrogen atmosphere for 5 h at 90 °C. The solution was cooled and diluted with chloroform, and the organic solution was washed with saturated aqueous hydrogen bicarbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with 100:1 to 1:0 (v/v) chloroform–methanol to give **16** (337 mg, 73.8%): ^{1}H NMR δ ($CDCl_3$) 1.67 (m, 2 H, OCH_2CH_2), 1.97–

2.15 (7 s, 21 H, 7 $COCH_3$), 4.52 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1'), 4.58 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1).

Anal. Calcd for $C_{31}H_{45}O_{17}N_1 \cdot 1H_2O$: C, 51.59; H, 6.56; N, 1.98. Found: C, 50.94; H, 6.22; N, 1.98.

***n*-Pentenyl *O*-(β-D-Galactopyranosyl)-(1−3)-2-acetamido-2-deoxy-β-D-glucopyranoside (4).** A white solid of **4** was prepared from **16** (240 mg, 0.341 mmol) by the procedure for the preparation of **2** (110 mg, 71.4%): mp 238 °C dec (from ethanol); ^{13}C NMR δ (D_2O) are listed in Table 1.

Anal. Calcd for $C_{19}H_{33}O_{11}N_1 \cdot 1H_2O$: C, 48.61; H, 7.51; N, 2.98. Found: C, 48.54; H, 7.36; N, 2.92.

***n*-Pentenyl 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(triphenylmethyl)-β-D-glucopyranoside (17).** A solution of **3** (4.9 g, 16.9 mmol) in pyridine (50 mL) was added with triphenylmethyl chloride (7.08 g, 25.4 mmol), and the mixture was stirred under nitrogen atmosphere for 40 min at 90 °C. The mixture was cooled to room temperature and added to acetic anhydride (16.5 mL, 0.17 mol). After 3 h, the reaction mixture was evaporated and poured into ice−water. The mixture was extracted with chloroform and worked up as the same manner described for **15**. The residue was applied to a silica gel column with 1:0 to 20:1 (v/v) chloroform−ethanol as eluent to give white powdery **17** (8.2 g, 80.4%) which was recrystallized from ethanol: mp 178−179 °C; ^1H NMR δ ($CDCl_3$) 1.72 (m, 2 H, OCH_2CH_2), 1.69, 1.94, 2.00 (each s, 9 H, 3 $COCH_3$), 2.16 (m, 2 H, CH_2CH=), 3.59 (m, 2 H, H-6a, 6b) 3.93 (m, 2 H, OCH_2), 4.64 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.97 (t, 1 H, $J_{4,5}$ 11 Hz, H-4), 5.14 (m, 2 H, CH=CH_2), 5.17 (t, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 5.84 (m, 2 H, NH and CH=CH_2), and 7.17−7.46 (m, 15 H, 3 Ph).

Anal. Calcd for $C_{35}H_{41}O_8N_1$: C, 69.63; H, 6.84; N, 2.32. Found: C, 69.48; H, 6.71; N, 2.24.

***n*-Pentenyl 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (18).** Compound **17** (1.7 g, 2.82 mmol) was treated with a solution of 80% aqueous acetic acid (25 mL) for 1 h at 90 °C. The solution was evaporated, and the residue was directly purified by silica gel chromatography with 1:2 (v/v) toluene−ethyl acetate as eluent to give **18** (0.8 g, 76.2%): mp 155−156 °C (from ethyl acetate−hexane); ^1H NMR δ ($CDCl_3$) 1.67 (m, 2 H, OCH_2CH_2), 1.96, 2.04, 2.05 (each s, 9 H, 3 $COCH_3$), 2.10 (m, 2 H, CH_2CH=), 2.38 (br s, 1 H, OH-6), 3.52 (m, 1 H, H-5), 3.60 (br dd, 1 H, $J_{5,6a}$ <1, H-6a), 3.70 (m, 2 H, OCH_2), 3.74 (br d, 1 H, $J_{5,6b}$ 13 Hz, H-6b), 3.88 (m, 1 H, H-2), 4.68 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.0 (m, 3 H, H-4, CH=CH_2), 5.33 (t, 1 H, $J_{2,3}$ 10.6 Hz and $J_{3,4}$ 9.4 Hz, H-3), 5.79 (m, 1 H, CH=CH_2), and 5.68 (d, 1 H, J 8.7 Hz, NH).

Anal. Calcd for $C_{17}H_{27}O_8N_1$: C, 54.68; H, 7.29; N, 3.75. Found: C, 54.46; H, 7.29; N, 3.71.

***n*-Pentenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1−6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (19).** A mixture of $Hg(CN)_2$ (1.89 g, 7.39 mmol), powdered MS4Å (3 g), and **18** (1.38 g, 3.70 mmol) in 10 mL of 1:1 (v/v) benzene−nitromethane was stirred for 40 min at room temperature. Glycosyl donor **9** (1.01 g, 2.46 mmol) was added to the mixture, and the mixture was stirred for 3 days at room temperature. The reaction mixture was filtered and evaporated, and the residue was dissolved in chloroform, and then filtered again to remove mercuric salts. The workup procedure was similar to that described for **11**. The product was purified by column chromatography on silica gel with 1:3 (v/v) toluene−ethyl acetate as eluent to give **19** (0.37 g, 21.4%): R_f 0.34 (solvent C); mp 122−123 °C (from ethyl acetate−hexane); ^1H NMR δ ($CDCl_3$) 1.69 (m, 2 H, OCH_2CH_2), 1.95, 1.98, 2.03, 2.03, 2.06, 2.06, 2.15 (each s, 21 H, 7 $COCH_3$), 2.13 (m, 2 H, CH_2CH=), 3.61 (dd, 1 H, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.65 (m, 2 H, OCH_2), 3.69 (m, 1 H, $J_{5,6b}$ 7.4 Hz and $J_{5,6b}$ 2 Hz, H-5), 3.79−3.92 (m, 2 H, H-6b, 5'), 3.82 (m, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 4.21 (m, 2 H, H-6'a, 6'b), 4.56 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.60 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.87 (t, $J_{4,5}$ 9.5 Hz, H-4), 4.99 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.95−5.05 (m, 2 H, CH=CH_2), 5.21 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.25 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 5.39 (d, 1 H, $J_{4',5'}$ 0.9 Hz, H-4'), 5.53 (d, 1 H, J 8.8 Hz, NH), and 5.80 (m, 1 H, CH=CH_2).

Anal. Calcd for $C_{31}H_{45}O_{17}N_1 \cdot 0.5H_2O$: C, 52.25; H, 6.50; N, 1.97. Found: C, 52.31; H, 6.54; N, 1.82.

***n*-Pentenyl *O*-(β-D-Galactopyranosyl)-(1−6)-2-acetamido-2-deoxy-β-D-glucopyranoside (5).** Compound **5**, recovered as a white solid, was prepared from **19** (133.3 mg, 0.189 mmol) by the method for preparing **2** (81.5 mg, 95.3%): R_f 0.17 (solvent E); mp 205−208 °C (from methanol); ^1H NMR δ (D_2O) 1.60 (m, 2 H, OCH_2CH_2), 1.99 (s, 3 H, $COCH_3$), 2.02 (m, 2 H, CH_2CH=), 4.18 (d, 1 H, J 11.4 Hz, H-6b), 4.40 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.47 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1'), 4.99 (m, 2 H, CH=CH_2), and 5.83 (m, 1 H, CH=CH_2); ^{13}C NMR δ (D_2O) are listed in Table 1.

Anal. Calcd for $C_{19}H_{33}O_{11}N_1 \cdot 1.5H_2O$: C, 47.70; H, 7.58; N, 2.93. Found: C, 47.89; H, 7.35; N, 2.88.

Copolymerization of Carbohydrate Monomers with Acrylamide. A solution of a carbohydrate monomer (100 mg) and 4 or 10 molar equiv of acrylamide in 1.0 mL of deionized water was degassed for a while using a water pump, and TEMED (0.1 molar equiv of the carbohydrate monomer) and APS (0.04 molar equiv of the carbohydrate monomer) was added. The reaction mixture was continuously stirred for 2 h at room temperature, diluted with 0.1 M acetic acid−pyridine buffer (pH 4.9), dialyzed against deionized water, and lyophilized to give the water-soluble copolymer as a white soft powder. The ^{13}C NMR data are shown in Table 1, and the physical data are shown in Table 2.

Enzymatic Galactosylation of a Polymer Having *N*-Acetyl-D-glucosamine.[15] Acceptor glycopolymer derived from GlcNAc **3** (10.0 mg, 12 μmol of GlcNAc), UDP-Gal (9.63 mg, 17 μmol), α-lactalbumine (100 μg), and GalT (1.0 unit) were incubated in 50 mM *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) (pH 6.0) for 24 h at 37 °C. The mixture was directly applied to a column of the Sephadex G-50 (2.5 i.d. × 100 cm) with 50 mM ammonium acetate as the eluent. The fractions containing glycopolymer were collected and freeze-dried to give a white powdery polymer having *N*-acetyllactosamine (11.8 mg) in quantitative yield: ^1H NMR δ (D_2O) 1.6 and 1.7 (br m, CH_2), 2.01 (s, 3 H, $COCH_3$), 2.2 and 2.3 (br m, CH), 3.52 (m, 1 H, H-2'), 3.55 (m, 1 H, H-5), 3.64 (d, 1H, H-3), 3.7 (m, 8 H, OCH_2, H-2, -3, -4, -5', -6'a, and -6'b), 3.83 (m, 1H, H-6a), 3.91 (d, 1 H, H-4'), 3.97 (br d, 1 H, H-6b), 4.43 (d, 1 H, H-1), 4.48 (d, 1 H, H-1').

High-Performance Anion Exchange Chromatography with Pulsed Amperometry Detection (HPAEC-PAD) Analysis. Acid hydrolysis of the polymer was carried out under 2 M trifluoroacetic acid at 100 °C for 2 h.[26] After the hydrolysis reaction, the solution was filtered to remove the main polymer chain, and the filtrate was successively subjected to the HPEAC-PAD analysis. HPAEC was carried out using Dionex BioLC (Sunnyvale, CA) equipped with a Carbopack PA-1 column and a pulsed amperometric detector (PAD-II). The quantitative analysis was carried out using a standard solution of monosaccharides of 50 mM solution respectively. The resulting pattern is shown in Figure 2C.

Effects of Glycopolymers on Hemagglutination by ECorL. The inhibitory activity of the synthetic polymers was tested in 0.05 M phosphate-buffered saline (PBS; pH 7.3) using a fresh suspension [ca. 4% (v/v)] of human type B red blood cells. The human blood cells were treated by heparin to avoid aggregation by themselves and used without further purification. The concentration was estimated to be 2.0 μM from absorbance at 280 nm, using the extinction coefficient $A^{0.1\%}_{1cm}$ 1.53. The procedure was as follows: Various concentrations of the test polymeric inhibitors (10 μL) were added to a suspension of the human type B red blood cells (10 μL), PBS (30 μL), and the ECorL solution (10 μL). The mixture was incubated for a few hours at room temperature before visual examination of hemagglutination activity. The minimum concentration of inhibitor required to inhibit the aggregation could be estimated by testing a dilution series of each test ligand.

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