A New Type of Artificial Glycoconjugate Polymer: A Convenient Synthesis and Its Interaction with Lectins

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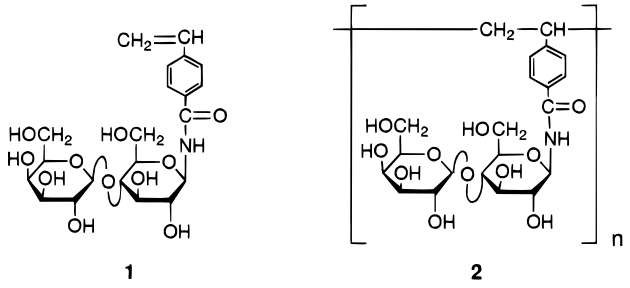
ABSTRACT: A convenient synthetic route to a new type of artificial glycoconjugate polymer has been designed to develop biomedical materials using oligosaccharide moieties as recognition signals. An amino function was introduced to the reducing end of lactose and *N*,*N*′-diacetylchitobiose with ammonium hydrogen carbonate and then was allowed to react with *p*-vinylbenzoyl chloride. The *N*-glycosidation proceeded stereospecifically in one flask to give only the *β*-glycoside without any protection and deprotection steps. The resulting *p*-vinylbenzamide glycoside derivatives were homo- and copolymerized with acrylamide using 2,2′-azobisisobutyronitrile as initiator in dimethyl sulfoxide at 60 °C. The interaction of the glycopolymers with lectins was investigated by means of a two-dimensional immunodiffusion test in agar and inhibition of the hemagglutinating activity. The specificity of lectins with these glycopolymers was similar to that reported for naturally-occurring glycoconjugates. Binding between wheat germ agglutinin lectin (WGA) and poly((*p*-vinylbenzamido)-*β*-diacetylchitobiose) was increased by 10^3 times compared with that of the oligosaccharide itself. The enhancement was attributed to the presence of the hydrophobic phenyl aglycon as well as the high density, multiantennary disaccharide ligands along the polymer chain. The present synthetic method is useful to introduce biologically important, complex oligosaccharides into glycopolymers.

Introduction

Increasing attention has been paid to synthetic polymers substituted with pendant oligosaccharide moieties as biological recognition signals.[1−3] Cell-specific culture substrata, artificial antigens, and targeted drug delivery systems are important targets of these glycopolymers. These applications are based on biological recogntion phenomena, that is, specific interactions of carbohydrates along polymer materials with proteins on cell surfaces. Our strategy of molecular designing is to construct amphiphilic structures by arranging hydrophobic main chains and hydrophilic pendant oligosaccharides via convenient synthetic routes.[4,5] Highly concentrated glyco signals along the hydrophobic main chains can interact with various types of carbohydrate-binding proteins. Among them, a lactose-carrying styrene polymer, poly(*N*-(*p*-vinylbenzyl)-4-*O*-*β*-D-galactopyranosyl-D-gluconamide), was found to be a useful substratum for culture of hepatocytes.[6−8] Dynamic interaction functioned between clustered galactose ligands along the homopolymer chains on the surface of the dish and asialoglycoprotein receptors on the surface of the cells. We also synthesized another type of amphiphilic glycopolymer consisting of a poly(acryl-(aminophenyl)) backbone and chemoenzymatically synthesized oligosaccharides.[9]

The present paper proposes a simpler synthesis of a new type of artificial glycoconjugate polymer starting from lactose and *N*,*N*′-diacetylchitobiose. Scheme 1



1 **2**

represents the path way from *N*,*N*′-diacetylchitobiose via the following three steps: (1) introduction of an amino function to the reducing end of oligosaccharide,[10−12] (2) amidation of the amino function with *p*-vinylbenzoyl chloride to give the (*p*-vinylbenzamido)-glycoside derivative, and (3) its radical homo- and copolymerization with acrylamide. The following abbreviations are used for these monomeric and polymeric compounds in this paper: 4-*O*-(*β*-D-galactopyranosyl)-*N*-(4-vinylbenzoyl)-*β*-D-glucopyranosylamine ((*p*-vinylbenzamido)-*β*-lactose, **1**), poly((*p*-vinylbenzamido)-*β*-lactose) (**2**), 2-acetamide-4-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-2-deoxy-*N*-(4-vinylbenzoyl)-*β*-D-glucopyranosylamine ((*p*-vinylbenzamido)-*β*-diacetylchitobiose, **3**), and poly((*p*-vinylbenzamido)-*β*-diacetylchitobiose) (**4**). The interaction with lectins was investigated by means of inhibition of hemagglutination and double diffusion in agar.

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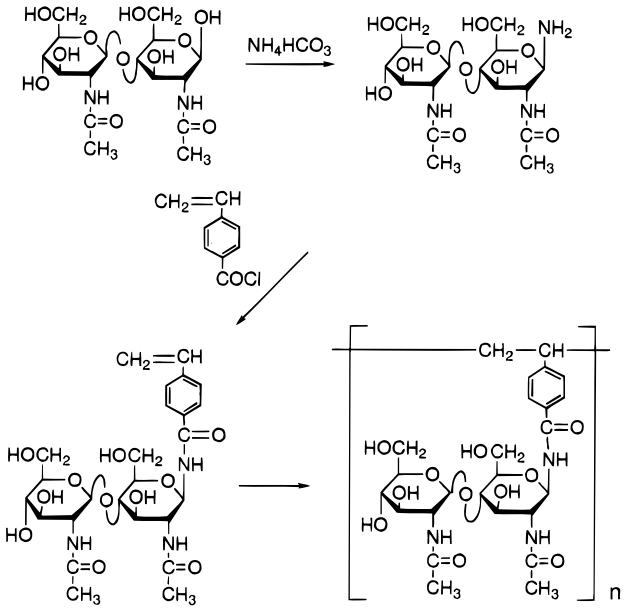
Scheme 1. Convenient Synthetic Route to a New Type of Artificial Glycoconjugate Polymer



3 **4**

Table 1. ^{1}H- and ^{13}C-NMR Spectral Evidence of a N-β-Anomeric Linkage in (*p*-Vinylbenzamido)-β-lactose (1) and (*p*-Vinylbenzamido)-β-diacetylchitobiose (3)

	^{1}H-NMR δ, ppm (J,Hz)			13C-NMR δ, ppm (J,Hz)		
	H-1′ ($J_{1',2'}$)	H-1 ($J_{1,2}$)	CONH ($J_{1,NH}$)	C′-1 ($J_{C-1',H-1'}$)	C-1 ($J_{C-1,H-1}$)	CONH
1	4.25 (7.4)	5.01 (9.2)	8.90 (8.6)	103.7 (159.7)	80.0 (152.8)	166.3
3	4.40 (8.1)	5.01 (9.6)	8.66 (8.1)	103.5 (158.2)	80.8 (154.1)	170.2

Results and Discussion

A Convenient Synthetic Route to a New Type of Oligosaccharide-Carrying Styrene Derivative. An amino function was derived from the reducing terminals of lactose and *N*,*N*′-diacetylchitobiose with ammonium hydrogen carbonate [10−12] and then allowed to react with *p*-vinylbenzoyl chloride without isolation of the intermediate glycosylamine. The two-step procedure was carried out practically in one flask. The yield of (*p*-vinylbenzamido)-β-lactose (**1**) and (*p*-vinylbenzamido)-β-*N*,*N*′-diacetylchitobiose (**3**) was 74 and 83% respectively. These compounds were soluble in water, dimethyl sulfoxide, *N*,*N*-dimethylformamide, and pyridine.

Unambiguous assignments of ^{1}H- and ^{13}C-NMR absorptions were made on the basis of two-dimensional H−H COSY and C−H COSY techniques and summarized in Experimental Section. Table 1 lists the ^{1}H- and ^{13}C-NMR data which demonstrate the N-linked β-anomeric bond between glycosyl and vinylbenzamide residues. The N-β-linked anomeric proton signals (H-1) appeared at δ 5.01 ppm ($J_{1,2}$ = 9.2 Hz) for **1** and δ 5.01 ppm ($J_{1,2}$ = 9.6 Hz) for **3**. The proton signals at δ 4.25 ppm ($J_{1',2'}$ = 7.4 Hz) and δ 4.40 ppm ($J_{1',2'}$ = 8.1 Hz) were assignable respectively to the non-reducing terminal H-1′ of O-linked β-anomeric galactopyranose (Galβ1-4Glc) and 2-acetamido-2-deoxyglucopyranose (GlcNAcβ1-4GlcNAc). The N-β-linked protons showed the lower chemical shifts and the larger coupling constants than those of O-β-linked protons. In the ^{13}C-NMR spectra, N-β-linked C-1β signals were distinct in the higher chemical shifts and the smaller heteronuclear coupling constants (INEPT), compared to those of O-β-linked C-1′ signals.[13] The benzamide protons at δ 8.90 ppm ($J_{1,NH}$ = 8.6 Hz) and δ 8.66 ppm ($J_{1,NH}$ = 8.1 Hz) were due to the N-linked β-anomeric carbon.[14] No other minor products, such as N-α-linked glycosyl compounds and vinylbenzoate ester compounds, were detected.

The *N*-glycosidation proceeded stereospecifically in one flask to give only the β-glycoside without any protection and deprotection steps. Such β-anomeric configuration is assumed to be due to reversed anomeric effect which is related to nonbonding electron pairs of ring-oxygen and electron-poor nitrogen atoms of the amide group. The β-anomeric N-linked structure is distinct from the open chain structure of the previously reported oligosaccharide-carrying styrene monomers[4,5] and also from the α- or β-anomeric O-linked sturcture of oligosaccharide-carrying acrylaminophenyl monomers.[9] This method will be useful to introduce more biologically important, complex oligosaccharides.

Synthesis of Artificial Glycoconjugate Polymers. Table 2 summarizes homo- and copolymerizations with acrylamide using 2,2′-azobisisobutyronitrile as initiator in dimethyl sulfoxide at 60 °C. Copolymer composition was estimated by the ^{1}H-NMR area ratio of amide, phenyl, anomer H-1, and main chain methylene proton signals. The compositions of the vinylbenzamide derivatives in copolymers were higher than those in the feed. The monomer reactivity of the vinylbenzamide derivatives was higher than that of acrylamide. The lactose homopolymer **2** was soluble in water and dimethyl sulfoxide and insoluble in *N*,*N*-dimethylformamide, pyridine, and methanol. The chitobiose homopolymer **4** showed similar solubility but partially soluble in *N*,*N*-dimethylformamide.

The chemical shifts and assignments of ^{13}C-NMR spectra of the homopolymers **2** and **4** in D_2O are summarized in the Experimental Section. The main chain methylene, methyne, and carbonyl signals were too broad to be detected, and some signals of the benzamide moiety became broad. The signals of the N-β-linked glucopyranose residue were also smaller than those of the non-reducing terminal galactopyranosyl residue. The signal broadening of the N-β-linked glucopyranose residue seems to be caused by restricted mobility of the polymeric main chain as well as restricted mobility due to the amphiphilic structural conformations in water. This tendency was observed in the ^{13}C-NMR spectra of previously reported glycopolymers.[4,6] The signal broadening of the present polymers was more prominent than those of the corresponding polyacrylamide derivatives.[9]

Limiting viscosity numbers [η] of some polymers were determined in Me_2SO and in water as follows. Polymer **2** (no. 2): 0.64 in Me_2SO and 0.12 in water. Polymer **4** (no. 8): 0.31 in Me_2SO and 0.07 in water. The small intrinsic viscosity in water was a reflection of the assumed tightly-coiled conformations of these polymers in water.

The molecular weights of some polymers were determined with a multi-angle laser light scattering (MALLS) photometer to be 1.6×10^5 to 4.3×10^5. The distribution was rather wide (M_w/M_n = 1.5−2.4). The molecular weight estimated by size exclution chromatography was also listed in Table 1, although the determination was less reliable.

Interaction of Artificial Glycopolymers with Lectins. In order to estimate the molecular recognition ability of lactose- and *N*,*N*′-diacetylchitobiose-carrying polymers, the interactions between lectins and glyco-

Table 2. Polymerization of (*p*-Vinylbenzamido)-*β*-lactose (1) and (*p*-Vinylbenzamido)-*β*-diacetylchitobiose (3)

expt. no.	monomer	amount, g	g of acrylamide	mol fr in feed[b]	mol % of AIBN	mL of Me_2SO	mL of H_2O	yield, %	mol fr in copolymer	M_n[c] $\times 10^{-5}$	M_w[c] $\times 10^{-5}$	M_n[d] $\times 10^{-5}$	$[\alpha]_D^{25}$
1	**1**	0.23	0	1.0	1.0	1.0	0	86	1.0				
2		1.20	0	1.0	1.0	5.0	0	95	1.0			1.3[e]	+33.1[f]
16		1.00	0	1.0	1.0	5.0	3.0	68	1.0	4.3	27.5	1.7	
4		0.20	0.20	0.11	0.5	10.0	0	45	0.39			3.4	+33.0[f]
17		0.40	0.40	0.11	0.5	3.9	2.5	63	0.18	1.6	2.4	1.5	
18		0.10	0.40	0.04	0.5	2.1	0.9	66	0.04	2.4	4.1	1.9	
7	**3**	0.32	0	1.0	1.0	1.3	0	10	1.0				
8		0.32	0	1.0	2.0	1.4	0	63	1.0			0.51	
3		0.15	0	1.0	1.0	1.0	0	100	1.0			8.0	−22.8[f]
6		0.10	0.10	0.11	0.5	2.0	0	23	0.36			2.2	−4.0[g]
10		0.40	0.40	0.03	0.5	2.0	0	40	0.02			2.5	

[a] At 60 °C, 15 h. [b] Mole fraction of (*p*-vinylbenzamido)oligosaccharides. [c] By multiangle laser light scattering using 100 mM aqueous urea solution. [d] By SEC using H_2O eluent and pullulan standard. [e] Using Me_2SO eluent. [f] *c*, 0.2 g/dL. [g] *c*, 0.1 g/dL.

Table 3. Inhibition of Hemagglutinating Activity of Lectins by Glycopolymers[a]

inhibitor	minimum inhibitory concentration, M[b] (mg/mL) ECA[c]	PNA[e]	RCA[d]	WGA[f]	DSA[g]
		Gal*β*1-4Glc			
homopolymer **2**	3×10^{-4} (0.13)	3×10^{-4} (0.16)	5×10^{-4} (0.25)	N.I.[h]	N.I.
monomer **1**	1×10^{-4} (0.05)	7×10^{-4} (0.31)	1×10^{-2} (5.0)	N.I.	N.I.
sugar	5×10^{-4} (0.16)	5×10^{-4} (0.16)	3×10^{-2} (10)	N.I.	N.I.
		GlcNAc*β*1-4GlcNAc			
homopolymer **4**	N.I.	N.I.	N.I.	1×10^{-6} (0.001)	N.I.
monomer **3**	N.I.	N.I.	N.I.	2×10^{-5} (0.01)	N.I.
sugar	N.I.	N.I.	N.I.	3×10^{-3} (1.3)	N.I.

[a] [Lectin] = 4[minimum concentration required for hemagglutination]. [b] Molarity (mol L^{-1}) of oligosaccharide unit. [c] Erithrina cristagalli. [d] Ricinus communis. [e] Arachis hypogaea. [f] Triticum vulgaris. [g] Datura stramonium. [h] N.I., Not inhibited by 20 mg/mL (0.1−0.04 mM).

polymers were investigated by means of a two-dimensional immunodiffusion test in agar and inhibition of the hemagglutinating activity of lectin by glycopolymers. The lectins were obtained from Sigma, and their abbreviations, taxonomic names, and binding oligosaccharides were as follows: ECA (*Erythrina cristagalli*, Gal*β*1-4GlcNAc),[15] PNA (peanut, *Arachis hypogaea*, Gal*β*1-3GalNAc),[16] RCA_{120} (*Ricinus communis*, Gal*β*),[17] WGA (wheat germ, *Triticum vulgalis*, GlcNAc*β*1-4GlcNAc),[18−20] and DSA (Jimson weed, *Datura stramonium*, Gal*β*1-4GlcNAc).[21]

Two-dimensional immunodiffusion tests in agar were carried out using PNA and WGA. Sharp precipitation bands appeared on the gel between the wells of PNA and lactose-carrying polymer **2** and between the wells of WGA and chitobiose-carrying polymer **4**. Control experiments using other combinations between PNA and **4** as well as between WGA and **2** yielded no precipitation bands. Lectins were insolubilized through specific cross-linking with carbohydrate ligands along the polymer chains. Each carbohydrate ligand was recognized specifically and strongly by the corresponding lectin. This method was useful to detect the specificity between lectins and glycopolymers rapidly and easily. However, it is merely a qualitative test and hence the following hemagglutination test was carried out as a measure of quantification.

Table 3 summarizes inhibitory effects of glycopolymers on the hemagglutination of human A-type blood cells by lectins. The minimum concentrations of glycopolymers are listed as molarity (mol/L) of oligosaccharide unit and as weight concentration (mg/mL) of the polymer. If the concentration of polymeric molecules was adapted, the minimum concentration becomes much lower than the values in Table 3.

Inhibition was observed between **2** and the galactose-binding lectins (ECA, PNA, and RCA) and between **4** and chiobiose-binding lectin (WGA). No inhibition was observed between the combinations of **2** with WGA and DSA and of **4** with ECA, PNA, RCA, and DSA. The qualitative results on double diffusion and hemmagglutination were the same as the specificities of these lectins reported for glycoproteins, glycolipids, and oligosaccharides.

The following comparison was made on the glycopolymers with the corresponding monomeric substances and oligosaccharide themselves. The minimum inhibition concentration of lactose-carrying glycopolymer **2** was almost the same as those of the corresponding sugars and monomer **1** for ECA and PNA. For RCA lectin, the inhibition activity of the monomeric substance was also the same as that of the corresponding sugar, but glycopolymer **2** increased the ability by 10^2. This tendency was enhanced for the combination between WGA and chitobiose-related substances. The inhibition ability of the monomeric compound **3** was 10^2 times of that of the corresponding oligosaccharide. The polymer **4** increased by 10^3 the inhibition of the oligosaccharide.

The strong interaction between **4** and WGA can be interpreted as follows. Interaction with WGA is reported to be enhanced by the presence of hydrophobic aglycon and by the multiantennary oligosaccaharide chains.[22−24] The polymer **4** has both of these features: the hydrophobic effect of the styrene residue and the numerous multiantennary or clustered oligosaccharide terminals. As pointed out previously,[6,25] the numerous multiantennary or clustered oligosaccharides are effected by the two factors. First, oligosaccharides are attached to every repeating unit along the polymer chain. Second, the hydrophobic polystyrene main chain is buried inside the molecule in water to form a hydrophobic core that is sheltered from water, and hence the oligosaccharide chains tend to gather on the outside of the polymer.

The strong binding of these glycopolymers with specific lectins is of promise in applying the polymers

as a tool to elucidate lectin-mediated biosignaling on the level of cells or organisms and to assess lectin-dependent cell adhesion as well as relevant techniques of molecular biology.

Experimental Section

NMR spectra were recorded with a JEOL JNM-FX-270 Fourier transform NMR spectrometer. IR spectra were taken with a Japan Spectroscopic Co. (JASCO) A-3 grating infrared spectrophotometer. Optical rotations were determined with a JASCO DIP-181 digital polarimeter using a water-jacketed 1 dm cell at 25 °C. Absolute molecular weights of polymers were determined with a DAWN DSP-F multiangle laser light scattering (MALLS) photometer (Wyatt Technology) equipped with a Shodex SB-806MHQ column and a Shodex RI-71 detector. Eluent was 100 mM aqueous urea solution (pH 7.0). Size exclusion chromatography (SEC) was conducted with a JASCO BIP-1 high performance liquid chromatograph using Shodex KF-803 and KF-804 columns and dimethyl sulfoxide (Me_2SO) as the eluent, and with a JASCO 800 high-performance liquid chromatograph on Shodex B804 + B805 columns using water as eluent. Thin layer chromatography (TLC) was carried out with Merck TLC plate precoated with silica gel 60. Preparative chromatography was carried out using a Yamazen preparative liquid chromatograph.

p-Vinylbenzoyl chloride was prepared from *p*-vinylbeozoic acid (Hokko Chemical Industry Co. Ltd., Tokyo, Japan) according to the published procedure. *N*,*N*′-Diacetylchitobiose was supplied by Yaizu Suisan Kagaku Co. Ltd. (Yaizu, Shizuoka, Japan). The lectins used were purchased from Sigma.

4-*O*-(*β*-D-Galactopyranosyl)-*N*-(4-vinylbenzoyl)-*β*-D-glucopyranosylamine ((*p*-vinylbenzamido)-*β*-lactose, 1). Lactose (1.0 g, 3.0 mmol) was dissolved in water (50 mL) and ammonium hydrogen carbonate was added until a portion of solid salt remained undissolved. The mixture was stirred in an open vessel at 37 °C for 4 days. Ammonium hydrogen carbonate (total 56.5 g) was added at intervals to ensure saturation. TLC (ethyl acetate:acetic acid:methanol:water = 4:3:3:2 in volume): R_f of lactose = 0.58 and of β-lactosylamine = 0.48. When TLC indicated no more conversion, the mixture was diluted with water (100 mL) and concentrated to 10 mL. The residue was diluted to 150 mL with water and concentrated to 5 mL. This procedure was repeated twice.

Sodium carbonate (2.0 g) and methanol (20 mL) were added to the crude solution of the lactosylamine in water (20 mL). The mixture was stirred magnetically at 0 °C for 2 h, and *p*-vinylbenzoyl chloride (2.0 mL, 14.4 mmol) in tetrahydrofuran (5.0 mL) was added. TLC (ethyl acetate:acetic acid:methanol: water = 4:3:1:1 in volume): R_f of β-lactosylamine = 0.36 and of the product = 0.60. After 5 h, the mixture was washed with chloroform (50 mL × 3) to remove unreacted *p*-vinylbenzoyl chloride. The solution was concentrated to 5−10 mL, and the crystallized product was chromatographed on a TSKgel HW-40S column (eluent, methanol-water 4:1 in volume). Unreactive oligosaccharide and a byproduct, *p*-vinylbenzamide, were removed by the procedure. The byproduct was formed by the reaction between remaining ammonima and an excess amount of *p*-vinylbenzoyl chloride

Fractions containing the aimed product were collected and lyophilized. Yield was 1.04 g (74%).

$[\alpha]_D^{25}$: +36.1 (*c* 0.2 in Me_2SO); ^{1}H-NMR (Me_2SO-d_6): δ 3.41−3.74 (m, 12H, from sugar), 4.25 (d, 1H, $J_{1',2'}$ = 7.3 Hz, H′-1), 5.01 (m, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 5.38 {d, 1H, J = 10.8 Hz, CH_2=CH (cis)}, 5.97 {d, 1H, J = 17.6 Hz, CH_2=CH (trans)}, 6.80 (dd, 1H, J = 10.8 and 17.6 Hz, CH_2=CH−), 7.58 and 7.90 (d, 4H, J = 8.1 Hz, C_6H_4), 8.90 (d, 1H, J = 8.6 Hz, CONH).

^{13}C-NMR (Me_2SO-d_6): δ 60.3 (C-6,C′-6), 68.1 (C′-4), 70.5 (C′-2), 71.5 (C′-3), 73.1 (C-2), 75.4 (C-3), 75.7 (C′-5), 76.5 (C-5), 80.0 (C-1), 80.6 (C-4), 103.7 (C′-1), 116.3 (*C*H_2=CH−), 133.1 and 139.9 {phenyl (*ipso*)}, 125.8 and 127.9 {phenyl (*meta* and *ortho*)}, 135.8 ((CH$_2$=*C*H−), 166.9 (C=O).

IR (cm^{-1}): 3350, ν_{O-H}; 2930, ν_{C-H}; 1660, $\nu_{C=O}$(amide); 1540, δ_{N-H}(amide); 780, δ_{C-H}.

2-Acetamido-4-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-2-deoxy-*N*-(4-vinylbenzoyl)-*β*-D-glucopyranosylamine ((*p*-vinylbenzamido)-*β*-diacetylchitobiose, 3). *N*,*N*′-Diacetylchitobiose (0.2 g, 0.48 mmol) was dissolved in water (50 mL), and ammonium hydrogen carbonate was added until a portion of the solid salt remained undissolved. The mixture was stirred in an open vessel at 37 °C for 3 days. Ammonium hydrogen carbonate (total 17.7 g) was added at intervals to ensure saturation. TLC (ethyl acetate:acetic acid:methanol: water = 4:3:3:2 in volume): R_f of lactose = 0.49 and of β-lactosylamine = 0.38. When TLC indicated no more conversion, the mixture was diluted with water (50 mL) and concentrated to 5 mL. The residue was diluted to 100 mL with water and concentrated to 5 mL. This procedure was repeated twice.

Sodium carbonate (0.2 g) and methanol (4.0 mL) were added to the crude solution of the lactosylamine in water (4.0 mL). The mixture was stirred magnetically at 0 °C for 2 h, and *p*-vinylbenzoyl chloride (0.50 mL, 3.6 mmol) in tetrahydrofuran (2.0 mL) was added. TLC (ethyl acetate:acetic acid:methanol: water = 4:3:1:1 in volume): R_f of β-*N*,*N*′-diacetylchitobiosylamine = 0.26 and of the product = 0.65. After 5 h, the mixture was washed with chloroform (50 mL × 3) to remove unreacted *p*-vinylbenzoyl chloride. The solution was concentrated to 5−10 mL and the crystallized product was chromatographed on a TSKgel HW-40S column (eluent, methanol−water 4:1 in volume). Fractions containing the aimed product were collected and lyophilized. Yield was 0.22 g (83%).

$[\alpha]_D^{25}$: −26.8 (*c* 0.2 in Me_2SO); ^{1}H-NMR (Me_2SO-d_6): δ 1.77 (s, 3H, NHCOCH_3′), 1.84 (s, 3H, NHCOCH_3), 3.01−3.84 (m, 12H, from sugar), 4.40 (d, 1H, $J_{1',2'}$ = 7.3 Hz, H′-1), 5.01 (m, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 5.38 {d, 1H, J = 10.8 Hz, CH_2=CH (cis)}, 5.97 {d, 1H, J = 17.6 Hz, CH_2=CH (trans)}, 6.79 (dd, 1H, J = 10.8 and 17.6 Hz, CH_2=CH−), 7.57, 7.77 (d, 4H, J = 8.1 Hz, C_6H_4), 8.11 (d, 1H, J = 7.6 Hz, NHCOCH$_3$), 8.66 (d, 1H, J = 8.1 Hz, CONH).

^{13}C-NMR (Me_2SO-d_6): δ 22.6 (CH_3), 22.9 (C′H$_3$), 54.4 (C-2), 55.7 (C′-2), 59.7(C-6), 61.2 (C′-6), 71.0 (C′-4), 72.2 (C-3), 74.3 (C′-3), 76.8 (C-5), 77.0 (C′-5), 80.2 (C-1), 80.8 (C-4), 103.5 (C′-1), 116.3 (*C*H$_2$=CH−), 133.1 and 140.3 {phenyl (*ipso*)}, 126.0 and 127.6 {phenyl (*meta* and *ortho*)}, 135.9((CH$_2$=*C*H−), 166.1 (C=O), 169.4 (C=O from sugar), 170.2 (C′=O from sugar).

IR (cm^{-1}): 3350, ν_{O-H}; 2900, ν_{C-H}; 1655, $\nu_{C=O}$(amide); 1550, δ_{N-H}(amide); 780, δ_{C-H}.

Polymerization. Prescribed amounts of the monomer and azobisisobutyronitrile were charged in a glass ampule and dissolved in dimethyl sulfoxide. The ampule was placed in a carbon dioxide−methanol bath, and the solution was frozen and degassed. The procedure was repeated three times. The ampule was sealed under a reduced pressure and maintained in a thermostat at 60 ± 0.05 °C. The ampule was chilled, and the solution was poured into an excess amount of cold methanol. The product was reprecipitated from its aqueous solution into methanol. The precipitate was dissolved in water, dialyzed in a cellulose tube (cutoff molecular weight, 3500; diameter, 11 mm; thickness, 0.03 mm; Nacalai Tesque, Kyoto, Japan) against water for 3 days, and freeze-dried to give a white powdery polymer.

Poly((*p*-vinylbenzamido)-*β*-lactose) (2). ^{1}H-NMR (Me_2SO-d_6): δ 1.54 (main chain CH_2−CH−), 5.07 (H-1), 3.27−4.65 (other protons from sugar), 6.54 and 7.52 (C_6H_4), 8.69 (CONH). ^{13}C-NMR (Me_2SO-d_6): δ ∼40.3 (broad, main chain CH_2−CH−), 60.6 (C-6 and C′-6), 68.1 (C′-4), 70.8 (C′-2), 71.8 (C′-3), 73.1 (C-2), 75.2 (C-3 and C′-5), 76.7 (C-5), 80.0 (C-1), 80.7 (C-4), 103.7 (C′-1), 127.3 (C_6H_4 meta and ortho), 131.6, 148.0 (C_6H_4 ipso and para), 167.0 (CO).

Poly((*p*-vinylbenzamido)-*β*-diacetylchitobiose) (4). ^{1}H-NMR (Me_2SO-d_6): δ 1.68 (main chain CH_2−CH−), 1.89 (NHCOCH_3), 5.06 (H-1), 3.13−4.84 (other protons from sugar), 6.40 and 7.42 (C_6H_4), 7.65 (N*H*COCH′$_3$), 7.88 (N*H*COCH$_3$), 8.38 (CONH). ^{13}C-NMR (Me_2SO-d_6): δ 23.0 (NHCO*C*H$_3$ and NHCO*C*′H$_3$), ∼40.4 (broad, main chain CH_2−CH−), 50.4 (C-2), 55.6 (C′-2), 60.7 (C-6 and C′-6), 70.4 (C′-4), 74.2 (C-3 and C′-3), 77.0 (C-5 and C′-5), 79.7 (C-1), 81.2 (C-4), 101.8 (C′-1), 127.1 (C_6H_4 meta and ortho), 131.5 and 148.0 (C_6H_4 ipso and para), 166.7−170.3 (CO, NH*C*OCH$_3$, and NH*C*OCH′$_3$).

Two-Dimensional Immunodiffusion Test in Agar.[26] Agarose (100 mg), poly(ethylene glycol) (300 mg), and sodium azide (100 mg) were dissolved in the buffer solution (9.5 g) at 80 °C. The solution was poured into Petri dishes (60 mm o.d.) to form a thin gel layer of 3 mm thick. Wells (diameter, 4 mm o.d.; distance between well centers, 10 mm) were formed with a glass capillary. Aqueous lectin solution (20 μL) and aqueous polymer solutions (20 μL) were added respectively to the central and peripheral wells with micro syringes, and the plate was stored at 30 °C for 24−48 h.

Inhibition Assay of Hemagglutination of Red Blood Cells by Glycopolymers.[27] Human blood (Type A, 4 mL) was collected, immediately mixed with a small amount of 3.8% aqueous sodium citrate solution, and then centrifuged (1000*g*) to remove leukocytes and plasma. The precipitate was mixed with PBS (10 mM phosphate buffer saline solution, pH 7.4) and centrifuged three times. The resulting erythrocytes (0.5 mL) were suspended into 4.5 mL of PBS containing actinase E (Kaken Seiyaku Co.), shaken at 45 °C for 30 min, centrifuged (1000*g*), and washed with PBS three times. The erythrocytes (0.3 mL) were diluted with PBS containing 0.9% NaCl to 10 mL to make actinase E-treated 3%-erythrocytes in PBS solution.

The 2-fold dilution series (20 μL) of lectins were prepared in 96-hole microtiter U-plates. After 1 h, the erythrocyte suspension (20 μL) was added and incubated at 30 °C for 1 h. Agglutination of erythrocytes was carefully observed, the minimum concentration of lectin required for agglutination of erythrocytes was determined, and its 4-fold concentration was used for the following inhibition assay.

The 2-fold dilution series (20 μL) of oligosaccharides, oligosaccharide-substituted styrene monomers, and glycopolymers were prepared in 96-hole microtiter U-plates. An aliquote (20 μL) of the lectin solution was added to each hole and incubated for 1 h. The erythrocyte suspension (40 μL) was added and incubated for 1 h. The minimum concentration of oligosaccharides and glycopolymers required to inhibit erythrocyte agglutination was determined.

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