Polymer Journal, Vol. 30, No. 8, pp 653—658 (1998)

Synthesis and Functions of a Glycopolymer Carrying Galβ1→4(GlcNAc)$_3$ Tetrasaccharide

Kazukiyo KOBAYASHI,† Shoko KAMIYA, Minoru MATSUYAMA,*
Takeomi MURATA,* and Taichi USUI*

Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464-8603, Japan
**Faculty of Agriculture, Shizuoka University, Ohya, Shizuoka 422-8529, Japan*

(Received February 16, 1998)

ABSTRACT: Tetrasaccharide Galβ1→4(GlcNAc)$_3$ was synthesized from *N,N',N''*-triacetylchitotriose (GlcNAc)$_3$ and lactose using transglycosylation with a β-D-galactosidase from *Bacillus circulans*. The reducing terminal of Galβ1→4(GlcNAc)$_3$ was oxidized and connected to *p*-vinylbenzylamine *via* amide linkage, and the resulting oligosaccharide-substituted styrene monomer was polymerized with the radical initiator, 2,2'-azobis(2-amidinopropane) dihydrochloride at 60°C. Glycopolystyrene was found to bind strongly with wheat germ agglutinin (WGA) and tomato (*Lycopersicon esculentum*) agglutinin (LEA) by inhibition of hemagglutination and double diffusion.

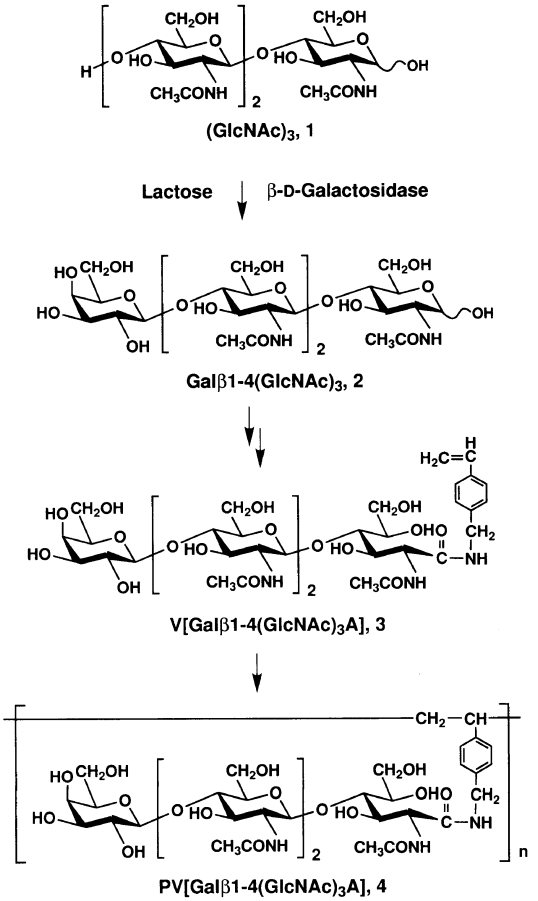
KEY WORDS Oligosaccharides / Glycopolymers / Lectins / Enzymatic Synthesis / Recognition /

Cell surface carbohydrates from glycoproteins, glycolipids, proteoglycans, and capsular polysaccharides play important roles in biological events.[1,2] Carbohydrate-protein interaction is usually weak and multivalent oligosaccharide chains are required to target cell surface carbohydrate receptors and inhibit host infection by pathogens.[3] Glycopolymers carrying pendant oligosaccharide chains can be regarded as multivalent ligands known to interact strongly with lectins and antibodies.[4,5] Glycopolymers have been synthesized and applied as biomedical materials having biological recognition signals.[6]

The *N*-acetyllactosamine (Galβ1→4GlcNAc) unit is a major component of oligosaccharide chains in glycoproteins and glycolipids, and behaves as differentiation antigens, tumor-associated antigens, and components of receptor systems.[7,8] Usui *et al.*[9-11] reported the gram-scale synthesis of *N*-acetyllactosamine and its *p*-nitrophenyl derivative using an industrially important enzyme, β-D-galactosidase from *Bacillus circulans*. *p*-Nitrophenyl *N*-acetyllactosaminide was converted to the acrylaminophenyl derivative and then the corresponding glycopolymer PAP(Galβ1→4GlcNAcβ) (**5**).[12] Analogous glycopolymers having related oligosaccharides were also reported by several groups.[13-18]

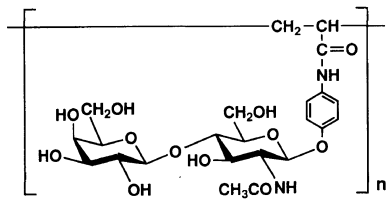
This paper describes the chemo-enzymatic synthesis of an *N*-acetyllactosamine-containing glycopolymer according to Scheme 1 *via* the following three step procedures.[18,19] (1) A β1→4-linked tetrasaccharide Galβ1→4(GlcNAc)$_3$ was synthesized from *N,N',N''*-triacetylchitotriose in the presence of a β-D-galactosidase of *Bacillus circulans*. (2) Oxidation of the oligosaccharide and subsequent amidation with *p*-vinylbenzylamine gave a vinylbenzyl-oligosaccharide-amide abbreviated as V[Galβ1→4(GlcNAc)$_3$A] in this paper. (3) Radical homopolymerization yielded a glycopolymer PV[Galβ1→4(GlcNAc)$_3$A] (**4**).

Interaction of the glycopolymer **4** with wheat germ agglutinin (WGA) and tomato (*Lycopersicon esculentum*) agglutinin (LEA) lectins was investigated by two-dimensional immunodiffusion in agar and inhibition of lectin-induced hemagglutination. Comparison was made using analogous homopolymers carrying *N*-acetyllactosamine and chitooligosaccharides. Chart 1 illustrates the chemical structures and abbreviations of these glycopolymers.

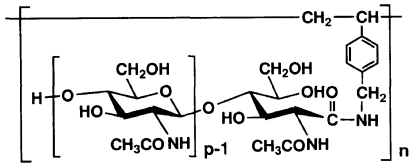


Scheme 1. Synthesis of a glycopolymer PV[Galβ1→4(GlcNAc)$_3$A] from *N,N',N''*-triacetylchitotriose.

† To whom correspondence should be addressed (Tel: +81-52-789-2488, Fax: +81-52-789-2528, e-mail: kobayash@mol.nagoya-u.ac.jp).

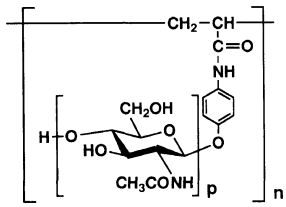


PAP(Galβ1-4GlcNAcβ), 5



p = 4 : PV(GlcNAc)$_4$A, 6

3 : PV(GlcNAc)$_3$A, 7



p = 2 : PAP[(GlcNAc)$_2$β], 8

1 : PAP(GlcNAcβ), 9

Chart 1.

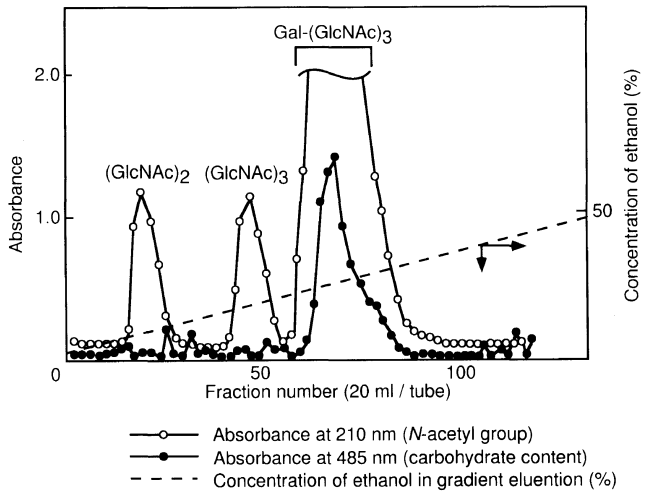


Figure 1. Charcoal-Celite column chromatograms of transglycosylation products after treatment of the crude product with *N*-acetylhexosaminidase.

RESULTS AND DISCUSSION

Enzymatic Synthesis of Galβ1→4(GlcNAc)$_3$ (2)

N,N′,N″-Triacetylchitotriose (GlcNAc)$_3$ was treated with an equimolar amount of lactose in the presence of β-D-galactosidase of *Bacillus circulans* origin. The product was purified by charcoal-Celite column chromatography with a linear water–ethanol gradient. The fractions were monitored by absorbance at 485 nm (total carbohydrate) after phenol–sulfuric acid treatment and at 210 nm (*N*-acetyl group). The main fraction was treated with *N*-acetylhexosaminidase to digest selectively the contaminated chitotriose and chromatographed again. As shown in Figure 1, galactosylated tetrasaccharide Galβ1→4(GlcNAc)$_3$ (**2**) was eluted as the main peak and separated from (GlcNAc)$_3$ and (GlcNAc)$_2$. The main fractions were collected, concentrated, and freeze-dried to give **2** as a colorless powder in a yield of 0.90 g (14% from (GlcNAc)$_3$; purity, 95%).

The presence of the non-reducing terminal galactose residue in **2** was confirmed by ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR signal at δ 4.47 ppm (d, $J_{1,2}=7.7$ Hz) was assignable to H-1 of β-anomeric galactopyranose introduced into the non-reducing terminal by analogy with *N*-acetyllactosamine.[12] ^{13}C NMR signals due to the galactopyranose unit appeared at δ 105.7 (C-1); 78.2 (C-5); 75.3 (C-3); 73.8 (C-2); 71.4 (C-4); 63.8 ppm (C-6) in addition to the signals of (GlcNAc)$_3$ unit.

Transglycosylation on *N,N′,N″*-triacetylchitotriose (GlcNAc)$_3$ proceeded regioselectively to give Galβ1→4 linked (GlcNAc)$_3$, in contrast to the finding that transglycosylation on *N,N′*-diacetylchitobiose (GlcNAc)$_2$ yields not only the main product Galβ1→4(GlcNAc)$_2$ but also a regioisomer Galβ1→6(GlcNAc)$_2$.[20] Regioselectivity on *N*-acetylglucosamine (GlcNAc) and *p*-nitrophenyl *N*-acetylglucosamine (*p*NP-GlcNAcβ) was further decreased. Similar lowered regioselectivity was observed for the transglycosilation using the same β-D-galactosidase on maltose, in contrast to the high regioselective Galβ1→4 glycosylation onto maltotriose and maltotetraose.[20]

Synthesis of a Glycopolymer Carrying Galβ1→4(GlcNAc)$_3$ Units

The reducing terminal of **2** was oxidized and the resulting oligosaccharide lactone was allowed to react with *p*-vinylbenzylamine. Figure 2 shows the ^{13}C NMR spectrum of product **3**. There appeared the vinylbenzyl (c—h and i), Galβ1→4(GlcNAc)$_3$ (a and Arabic numerals), and gluconamide C-1 carbonyl moieties (b).

The radical homopolymerization of **3** was carried out using 2,2′-azobis(2-amidinopropane)dihydrochloride as initiator in water at 60°C. White powdery homopolymer **4** was obtained. Homopolymers (**6** and **7**) carrying (GlcNAc)$_4$ and (GlcNAc)$_3$ were prepared similarly. These monomers and polymers were soluble in water.

Interaction of the Glycopolymers with Lectins

Two-dimensional immunodiffusion profiles are shown in Figure 3 and inhibition of hemagglutination is summarized in Table I. Minimum concentrations of the glycopolymers are listed as molarity (mol l^{-1}) of the oligosaccharide unit. WGA, a dimeric lectin with four potential binding sites per monomeric unit, exhibits specificity toward GlcNAc and NeuAc residues.[21–25] Multiple independent binding sites give rise to enormous increase in affinity when the lectin is presented with multiple ligands. It is also reported[26] to bind poly(*N*-acetyllactosamine) (3Galβ1→4GlcNAcβ1→)$_n$ chains. LEA (molecular weight of 7.1×10^4) is reported[27–30] to recognize tetrasaccharide (GlcNAc)$_4$ more strongly than trisaccharide (GlcNAc)$_3$ and to interact with high affinity with three or more linear repeating disaccharide units (3Galβ1→4GlcNAcβ1→)$_n$ ($n\geq3$).

Table I confirms that these two lectins exhibit similar

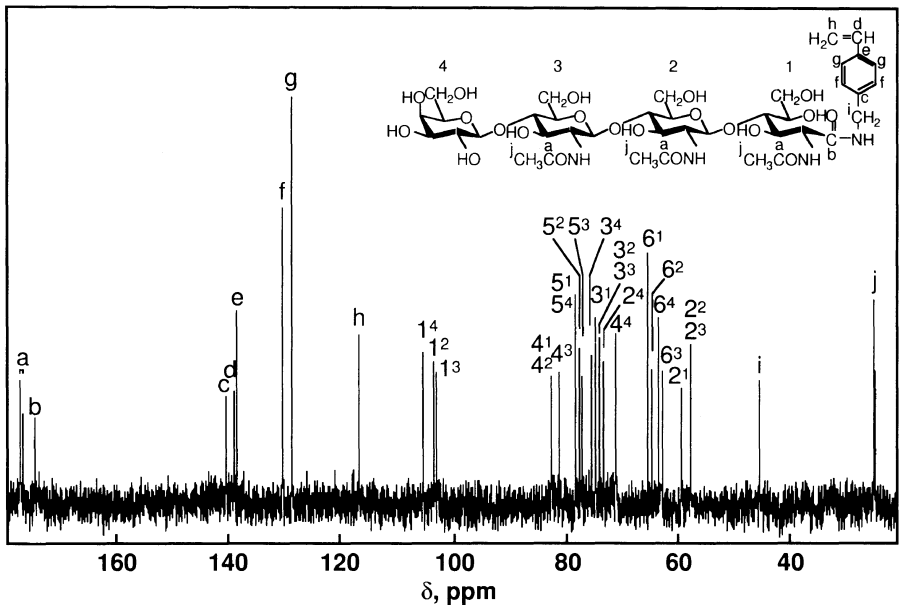


Figure 2. ^{13}C NMR spectrum of **2**. Concn, 5%; in D_2O; temp, 70°C; reference, TPS; 67.8 MHz. Sugar units of Gal-(GlcNAc)$_3$ are numbered from the reducing terminal in series and designated as the superscript. 4^1 represents C-4 of the reducing terminal GlcNAc residue and 1^4, C-1 of the nonreducing terminal Gal residue.

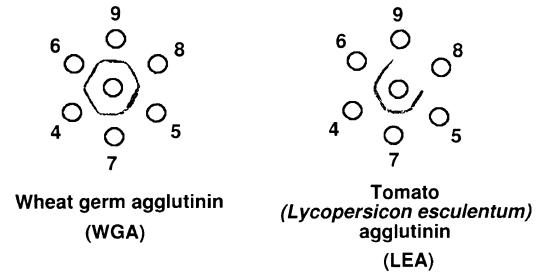


Figure 3. Two-dimensional immunodiffusion profiles. Photographs of the agar gels were obtained with a scanner.

specificity toward the oligosaccharides as reported in the literature.[27-30] WGA- and LEA-induced hemagglutinations were inhibited by (GlcNAc)$_4$, (GlcNAc)$_3$, and (GlcNAc)$_2$ in 10^{-5} to 10^{-3} M but not by Galβ1→4(GlcNAc)$_3$, Galβ1→4GlcNAc, and GlcNAc in the concentration listed.

The glycopolymers showed different specificity to these lectins. Sharp precipitation bands appeared on the gel between the central well of WGA and peripheral wells of all glycopolymers. These glycopolymers at 10^{-6} to 10^{-7} M inhibited WGA-induced hemagglutination strongly. Inhibition ability was much stronger than that of the corresponding oligosaccharides. Diffusion using LEA lectin showed sharp precipitation bands with the glycopolymers **4**, **5**, **6**, and **7**, but no bands with **8** and **9**. Similar results were obtained by inhibition tests of LEA-induced hemagglutination. Inhibition by **4**, **5**, **6**, and **7** was much stronger than by the corresponding oligosaccharides, but no inhibition was observed by **8** and **9** at the concentrations listed. Glycopolymers **4**, **5**, and **6** inhibited LEA-induced hemagglutination slightly stronger than WGA-induced hemagglutination and that glycopolymers **8** and **9** inhibited WGA-induced hemagglutination but not LEA-induced hemagglutination. Little difference was observed between glycopolymers **4** and **5** in binding with WGA and LEA. Against our expectation, the effect of the tetrasaccharide superior to the disaccharide was not detected.

Table I. Inhibition of lectin-induced hemagglutination[a,b]

Inhibitor	Minimum inhibitory concentration (M[c])	
	Wheat germ agglutinin (WGA)	Tomato (*Lycopersicon esculentum*) agglutinin (LEA)
Glycopolymers		
PV[Galβ1→4(GlcNAc)$_3$A], **4**	1×10^{-6}	2×10^{-7}
PAP(Galβ1→4GlcNAcβ), **5**	4×10^{-6}	3×10^{-7}
PV(GlcNAc)$_4$A, **6**	1×10^{-7}	8×10^{-8}
PV(GlcNAc)$_3$A, **7**	1×10^{-6}	4×10^{-6}
PAP[(GlcNAc)$_2\beta$], **8**	1×10^{-7}	N.I. (4×10^{-4})
PAP(GlcNAcβ), **9**	1×10^{-6}	N.I. (2×10^{-3})
Oligosaccharides		
Galβ1→4(GlcNAc)$_3$	N.I. (2×10^{-3})	N.I. (2×10^{-3})
Galβ1→4GlcNAc	N.I. (5×10^{-2})	N.I. (5×10^{-2})
(GlcNAc)$_4$	1×10^{-4}	6×10^{-5}
(GlcNAc)$_3$	5×10^{-4}	5×10^{-4}
(GlcNAc)$_2$	3×10^{-3}	3×10^{-3}
GlcNAc	N.I. (5×10^{-2})	N.I. (5×10^{-2})

[a] [Lectin] = 4 × [minimum concentration required for hemagglutination]. WGA = 0.1 μg ml^{-1} and LEA = 1 μg ml^{-1}. [b] N.I. means hemagglutination was not inhibited by the concentration indicated in parentheses. [c] Molarity (mol l^{-1}) of oligosaccharide unit.

These two lectins have similar recognition ability toward linear repeating chains of (3Galβ1→4GlcNAcβ1→)$_n$ and (GlcNAc)$_n$.[27-30] The present experiments using the glycopolymers can compare the binding ability of the lectins toward branched or antennary Galβ1→4(GlcNAcβ)$_n$ and (GlcNAc)$_n$ units. WGA and LEA lectins exhibit strong binding toward antennary Galβ1→4(GlcNAcβ)$_3$, Galβ1→4GlcNAc, (GlcNAc)$_4$, and (GlcNAc)$_3$ units. Antennary (GlcNAc)$_2$ and GlcNAc units were recognized by

WGA but not by LEA. The present results suggest that LEA binds to the outer disaccharide Galβ1→4GlcNAcβ units. This can be regarded as exo-type recognition, in contrast to endo-type recognition of WGA which binds to the inner GlcNAc units.[26]

Much higher inhibition activity of the glycopolymers than the oligosaccharides is attributable to the high density or cluster effect of the ligands along the glycopolymer main chain.[3-5,24,25]

EXPERIMENTAL

General

NMR spectra were recorded on a JEOL JNM-FX-270 spectroscope operating at 270 MHz (pulse width 5.0 μs, scan times 16, and relaxation delay 137.9 μs) for ^{1}H NMR and at 67.80 MHz (pulse width 3.9 μs, scan times 1.2×10^4, and relaxation delay 24.0 μs) for ^{13}C NMR. In the following description of NMR data, sugar units of Galβ1→4(GlcNAc)$_3$ are numbered from the reducing terminal in series and designated as the superscript. Optical rotations were recorded with a JASCO DIP-181 digital polarimeter using a water-jacketed 1 dm cell at 25°C. High performance liquid chromatography (HPLC) was recorded with a Shimadzu liquid chromatography LC-6A on Asahipack NH_2-50 column ($CH_3CN : H_2O = 7 : 3$) and UV detector (wavelength, 210 nm).

Materials

β-D-Galactosidase (EC 3.2.1.23; from *Bacillus circulans*) was purchased from Daiwa Kasei Ltd. β-*N*-Acetylhexosaminidase (EC 3.2.1.52) was a gift from Kyowa Hakko Ltd. WGA was purchased from Seikagaku Kogyo Ltd. and LEA from Sigma. *p*-Vinylbenzylamine was prepared by the previously reported method.[18] 2,2′-Azobis(2-amidinopropane) dihydrochloride (Wako Pure Chemicals) was recrystallized from water.

Enzymatic Synthesis of O-β-D-Galactopyranosyl-[O-β-2-acetamide-2-deoxy-D-glucopyranosyl-(1→4)-]$_2$2-acetamide-2-deoxy-D-glucose (**2**; *Galβ1→4(GlcNAc)$_3$*)

N,*N*′,*N*″-Triacetylchitotriose (5.08 g, 8.10 mmol) and lactose (2.92 g, 8.10 mmol) were dissolved in a mixture of 20 mM acetic acid buffer (pH = 5.0, 92 ml). β-D-Galactosidase (7.6 units) was added and the solution was incubated at 30°C for 24 h. The solution was heated at 95°C for 10 min to deactivate the enzyme, and centrifuged ($20700 \times g$, 15 min). The supernatant was applied to a charcoal-Celite column (ϕ 5.5 × 60 cm) equilibrated with water. The product was eluted with a linear gradient of 5 L of water to 5 L of 50% ethanol aqueous solution and fractions (60 ml/tube) were collected. The sugar residue was detected by an absorbance at 485 nm after the treatment with phenol–sulfuric acid and the *N*-acetyl group was detected by an absorbance at 210 nm. Fractions 86—94 containing both the sugar residue and *N*-acetyl group were collected, concentrated, and freeze-dried. The product was a mixture of **2** and unreactive (GlcNAc)$_3$ (8 : 1) according to HPLC.

The crude product (1.24 g) was dissolved in a 20 mM acetic acid buffer (pH = 5.0, 309 ml). *N*-Acetylhexosaminidase (0.62 units) was added and the solution was incubated at 40°C for 5 h. The solution was heated at 95°C for 10 min to deactivate the enzyme, and centrifuged ($20700 \times g$, 15 min). The supernatant was applied to a charcoal-Celite column (ϕ 2.2 × 95 cm) equilibrated with water. The product was eluted with a linear gradient of 1.5 L of water to 1.5 L of 50% ethanol aqueous solution. Fractions (20 ml/tube) were collected, and the sugar component and *N*-acetyl group were assayed. Fractions 66—84 containing both the sugar residues and *N*-acetyl group were collected, concentrated, and freeze-dried. The yield was 0.90 g (1.14 mmol, 14%, purity: 95%).

^{1}H NMR (D_2O; 5.0%; TPS; 70°C): δ 5.19 (d, J = 2.2 Hz, H-1α); 4.70 (d, J = 7.3 Hz, H-1$^1\beta$); 4.61 (d, J = 7.7 Hz, H-1^3); 4.59 (d, J = 7.7 Hz, H-1^2); 4.47 (d, J = 7.7 Hz, H-1^4); 4.49—3.35 (m, sugar residue); 2.04 ppm (s, NHCOCH_3). ^{13}C NMR (D_2O; 5.0%; TPS; 70°C): δ 177.4 (NH*C*OCH$_3$$^1\beta$, NH*C*OCH$_3$^{2}, and NH*C*OCH$_3$^{3}); 177.3 (NH*C*OCH$_3$$^1\alpha$); 105.7 (C-1^4); 104.1 (C-1^2 and C-1^3); 97.6 (C-1$^1\beta$); 93.3 (C-1$^1\alpha$); 82.5 (C-4$^1\alpha$); 82.0 (C-4$^1\beta$); 81.9 (C-4^2); 81.1 (C-4^3); 78.2 (C-5^4); 77.6 (C-5^2); 77.4 (C-5$^1\beta$); 77.3 (C-5^3); 75.3 (C-3^4); 74.9 (C-3$^1\beta$, C-3^2 and C-3^3); 73.8 (C-2^4); 72.8 (C-5$^1\alpha$); 72.1 (C-3$^1\alpha$); 71.4 (C-4^4); 63.8 (C-6^4); 62.9 (C-6^2); 62.8 (C-6^1 and C-6^3); 58.9 (C-2$^1\beta$); 57.9 (C-2^2 and C-2^3); 56.4 (C-2$^1\alpha$); 24.9 (NHCO*C*H$_3$$^1\beta$, NHCO*C*H$_3$^{2}, and NHCO*C*H$_3$^{3}); 24.7 ppm (NHCO*C*H$_3$$^1\alpha$).

N-p-Vinylbenzyl-O-β-D-galactopyranosyl-[O-β-2-acetamide-2-deoxy-D-glucopyranosyl-(1→4)-]$_2$2-acetamide-2-deoxy-D-gluconamide (*V[Galβ1→4(GlcNAc)$_3$A]*, **3**)

The enzymatically synthesized oligosaccharide (**2**; 1.50 g; 1.90 mmol) was dissolved in water (20 ml). Iodine (1.3 g) solution in methanol (25 ml) was added to the solution and then 4% potassium hydroxide in methanol solution (40 ml) was added dropwise at 40°C for 3.5 h. The mixture was cooled and poured into cold diethyl ether (100 ml). The precipitated product was filtered, washed with cold methanol, and centrifuged ($20700 \times g$, 15 min). The precipitate was dissolved into water (12 ml) and silver carbonate (96 mg) was added. The suspension was stirred overnight at room temperature and centrifuged. The supernatant was treated with Amberlite IR-120 (H^+). The acidic eluate was collected, concentrated in a rotary evaporator, and dried. The product was dissolved in methanol–ethanol (6 : 1) solution and concentrated under reduced pressure. This procedure was repeated several times and the resulting white powder was dried *in vacuo*. Yield of Gal-(GlcNAc)$_3$ lactone was 1.42 g (95%).

The lactone (1.42 g; 1.80 mmol) was dissolved in ethylene glycol (10 ml). *p*-Vinylbenzylamine (0.48 g; 3.6 mmol) solution in ethylene glycol (3 ml) was added to the solution and heated at 70°C for 13 h. The solution was dissolved in water (100 ml) and washed with chloroform (50 ml × 2). The aqueous layer was concentrated and centrifuged. To remove unreactive oligosaccharide derivatives, the crude filtrate was purified with a Toyopearl HW-40S chromatography equipped with a ultra violet (UV) detector using a mixture of water and methanol (3 : 1) as eluent. Fractions (20 ml/tube) were collected, and the styrene moiety and sugar residues were detected by absorbance at 250 nm and 485 nm by the phenol–sulfuric acid method. Fractions 28—34 contain-

ing the styrene moiety and sugar residue were collected and concentrated. The solution was poured into cold acetone and centrifuged. The precipitate was dried *in vacuo*. Yield was 0.35 g (0.38 mmol, 21%). $[\alpha]_D^{25} = +4.9°$ (*c* 1.0 in water).

^{1}H NMR (D_2O; 5.0%; TPS; 70°C): δ 7.46 (d, J= 7.8 Hz, *o*-ph); 7.27 (d, J=7.8 Hz, *m*-ph); 6.78 (dd, J=11.0 and 17.7 Hz, $CH=CH_2$); 5.82 (d, J=17.7 Hz, $CH=CH_2$ (*cis*)); 5.31 (d, J=11.0 Hz, $CH=CH_2$ (*trans*)); 4.65—4.53 (m, H-1^2, H-1^3, and H-1^4); 4.49—3.35 (m, sugar residue); 2.09 (s, $NHCOCH_3$^{1}); 2.05 ppm (s, $NHCOCH_3$^{2} and $NHCOCH_3$^{3}). ^{13}C NMR (D_2O; 5.0%; TPS; 70°C): δ 177.2 and 176.9 ($NHCOCH_3$); 164.6 (CONH); 140.5 (*p*-ph); 139.5 ($CH=CH_2$); 139.2 (*ipso*-ph); 130.5 (*m*-ph); 129.2 (*o*-ph); 117.1 ($CH=CH_2$); 105.8 (C-1^4); 104.1 (C-1^2); 103.8 (C-1^3); 82.5 (C-4^1 and C-4^2); 81.6 (C-4^3); 78.2 (C-5^1 and C-5^4); 77.7 (C-5^2); 77.4 (C-5^3); 75.5 (C-3^4); 75.0 (C-3^1); 74.5 (C-3^2 and C-3^3); 73.9 (C-2^4); 71.4 (C-4^4); 65.5 (C-6^1); 64.8 (C-6^2); 63.8 (C-6^4); 63.0 (C-6^3); 59.2 (C-2^1); 58.0 (C-2^2 and C-2^3); 45.5 ($NHCH_2$); 25.1 and 24.8 ppm ($NHCOCH_3$).

*N-p-Vinylbenzyl-[O-β-2-acetamide-2-deoxy-*D*-glucopyranosyl-(1→4)-]$_2$2-acetamide-2-deoxy-*D*-gluconamide [V(GlcNAc)$_3$A]*

The preparative method was similar to that of **3**. Yield was 25%. $[\alpha]_D^{25} = +9.3°$ (*c* 1.0 in water). ^{1}H NMR (D_2O; 5.0%; TPS; 70°C): δ 7.48 (d, J=8.0 Hz, *o*-ph); 7.26 (d, J=8.0 Hz, *m*-ph); 6.78 (dd, J=11.0 and 17.0 Hz, $CH=CH_2$); 5.84 (d, J=17.6 Hz, $CH=CH_2$ (*cis*)); 5.30 (d, J=11.5 Hz, $CH=CH_2$ (*trans*)); 4.53 (d, J=7.0 Hz, H-1^3); 4.46 (broad-s, H-1^2); 4.28—3.39 (m, sugar residue); 2.05 ppm (s, $NHCOCH_3$). ^{13}C NMR (D_2O; 5.0%; TPS; 70°C): δ 177.6 and 177.3 ($NHCOCH_3$); 174.9 (CONH); 140.3 (*p*-ph); 139.5 ($CH=CH_2$); 139.1 (*ipso*-ph); 130.5 (*m*-ph); 129.3 (*o*-ph); 117.2 ($CH=CH_2$); 104.3 (C-1^2); 103.8 (C-1^3); 82.4 (C-4^1); 82.3 (C-4^2); 78.7 (C-5^1); 77.3 (C-5^2); 76.3 (C-5^3); 74.9 (C-3^1); 74.0 (C-3^2); 72.5 (C-3^3); 71.6 (C-4^3); 64.7 (C-6^1); 63.4 (C-6^2); 63.1 (C-6^3); 59.5 (C-2^1); 58.4 (C-2^2); 57.4 (C-2^3); 45.5 ($NHCH_2$); 25.0 and 24.8 ppm ($NHCOCH_3$).

Homopolymerization of **3**

A solution of 2,2′-azobis(2-amidinopropane)dihydrochloride (12 mg/5 ml) in water was prepared. V[Gal-(GlcNAc)$_3$A] monomer (80 mg; 0.087 mmol) was dissolved in a mixture of the initiator solution (0.1 ml) and water (0.3 ml) in an ampoule. The solution was cooled to −78°C, and deaerated *in vacuo*. The ampoule was sealed *in vacuo* and kept at 60°C in a thermostated water bath for 15 h. The solution was poured into methanol (50 ml), and the supernatant was removed by decantation. The precipitated product was dissolved in water (1 ml), dialyzed, and freeze-dried. Yield was 94%. $[\alpha]_D^{25} = +9.2°$ (*c* 0.14 in water).

^{1}H NMR (D_2O; 5.0%; TPS; 70°C): δ 6.96 and 6.47 (phenyl); 4.62 (H-1^2, H-1^3, and H-1^4); 4.49—3.44 (sugar residue); 2.01 ($NHCOCH_3$); 1.60 ppm (main chain, CH_2CH). ^{13}C NMR (D_2O; 5.0%; TPS; 70°C): δ 177.0 and 176.7 ($NHCOCH_3$); 174.5 (CONH); 130.4 (phenyl); 105.8 (C-1^4); 104.2 (C-1^2); 103.8 (C-1^3); 83.3 (C-4^1); 82.6 (C-4^2); 81.7 (C-4^3); 78.1 (C-5^1 and C-5^4); 77.7 (C-5^2 and C-5^3); 75.5 (C-3^4); 75.0 (C-3^1); 74.6 (C-3^2 and C-3^3); 73.9 (C-2^4); 71.4 (C-4^4); 64.8 (C-6^1 and C-6^2); 63.8 (C-6^4); 63.1 (C-6^3); 59.3 (C-2^1); 58.1 (C-2^2 and C-2^3); 45.5 ($NHCH_2$); 25.2 ppm ($NHCOCH_3$).

Homopolymerization of V(GlcNAc)$_3$A

The preparative method was similar to that of **4**. Yield was 87%. $[\alpha]_D^{25} = +11.7°$ (*c* 0.20 in water). ^{1}H NMR (D_2O; 5.0%; TPS; 70°C): δ 7.00 and 6.53 (phenyl); 4.68 (H-1^2 and H-1^3); 4.38—3.50 (sugar residue); 2.11 ($NHCOCH_3$); 1.44 ppm (main chain, CH_2CH). ^{13}C NMR (D_2O; 5.0%; TPS; 70°C): δ 176.8, 176.5, and 176.5 ($NHCOCH_3$); 174.7 (CONH); 146.9 (*p*-ph); 138.3 (*ipso*-ph); 129.8 (*m*-ph and *o*-ph); 104.4 (C-1^2); 103.9 (C-1^3); 83.3 (C-4^1); 82.8 (C-4^2); 79.0 (C-5^1); 77.6 (C-5^2); 76.7 (C-5^3); 75.3 (C-3^1); 74.8 (C-3^2); 73.1 (C-3^3); 73.0 (C-4^3); 64.9 (C-6^1); 63.8 (C-6^2); 63.2 (C-6^3); 59.4 (C-2^1); 58.6 (C-2^2); 58.2 (C-2^3); 45.5 ($NHCH_2$); 25.3 ppm ($NHCOCH_3$).

Two-Dimensional Immunodiffusion Test in Agar[31]

A solution of agar gel (4% w/w) in 0.1 M phosphoric acid buffer solution (pH=7.2) containing several drops of sodium azide aqueous solution (1%) was poured into a Petri dish to form a thin gel layer. Wells (4 mm o.d.; distance between well centers, 10 mm) were formed. Aqueous lectin solution (WGA: 2 mg ml^{-1}, LEA: 5 mg ml^{-1}) and aqueous polymer solution (0.5—6 mg ml^{-1}) were added respectively to the central and peripheral wells, and the plate was stored at 30°C for 48 h.

Inhibition Assay of Hemagglutination of Red Blood Cells by Glycopolymer

Two-fold dilution series (20 μl) of oligosaccharides and glycopolymers were prepared in 96-holes microtiter U-plates. An aliquot (20 μl) of the lectin solution was added to each hole and incubated at 30°C for 1 h. An erythrocyte suspension (40 μl) was added to the hole and incubated at 30°C for 1 h. Agglutination of erythrocytes was carefully observed and minimum concentrations of oligosaccharides and glycopolymers required to inhibit erythrocyte agglutination were determined.

Acknowledgments. The financial aid given by the Regional Leading Research Project of Shizuoka Prefecture from the Ministry of Science and Technology, Japan, is greatly appreciated.

REFERENCES

1. J. Montreuil, J. F. G. Vliegenthart, and H. Schachter, Ed., "Glycoproteins," Elsevier, Amsterdam, 1995.
2. R. A. Dwek, *Chem. Rev.*, **96**, 683 (1996).
3. Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, **28**, 321 (1995).
4. R. Roy, *Trends Glycosci. Glycotech.*, **8**, 79 (1996).
5. L. L. Kiessling and N. L. Pohl, *Chem. Biol.*, **3**, 71 (1996).
6. Y. C. Lee, in "Neoglycoconjugates: Preparation and Applications," Y. C. Lee and R. T. Lee, Ed., Academic Press, San Diego, 1994, pp 3—21.
7. J. C. Paulson, *Trends Biochem. Sci.*, **14**, 272 (1983).
8. T. Feizi, *Trends Biochem. Sci.*, **16**, 84 (1991).
9. T. Murata and T. Usui, *Biosci. Biotech. Biochem.*, **61**, 1059 (1997).
10. K. Sakai, R. Katsumi, H. Ohi, T. Usui, and Y. Ishido, *J. Carbohydr. Chem.*, **11**, 553 (1992).
11. T. Usui, S. Kubota, and H. Ohi, *Carbohydr. Res.*, **244**, 315 (1993).
12. K. Kobayashi, N. Kakishita, M. Okada, T. Akaike, and T. Usui, *J. Carbohydr. Chem.*, **13**, 753 (1994).

13. R. Roy and F. D. Tropper, *Glycoconj. J.*, **5**, 203 (1988).
14. K. Hatanaka, Y. Ito, A. Maruyama, Y. Watanabe, T. Akaike, K. Ishio, and T. Uryu, *Macromolecules*, **26**, 1483 (1993).
15. K. Matsuoka and S.-I. Nishimura, *Macromolecules*, **28**, 2961 (1995).
16. T. Furuike, N. Nishi, S. Tokura, and S.-I. Nishimura, *Macromolecules*, **28**, 7241 (1995).
17. K. Kobayashi and A. Tsuchida, *Macromolecules*, **30**, 2016 (1997).
18. K. Kobayashi, K. Aoki, H. Sumitomo, and T. Akaike, *Makromol. Chem., Rapid Commun.*, **11**, 477 (1990).
19. K. Kobayashi, H. Sumitomo, and Y. Ina, *Polym. J.*, **17**, 567 (1985).
20. M. Takada, K. Ogawa, S. Saito, T. Murata, and T. Usui, *J. Biochem.*, **123**, 508 (1998).
21. C. S. Wright, *J. Mol. Biol.*, **141**, 267 (1980).
22. C. S. Wright, *J. Mol. Biol.*, **209**, 475 (1989).
23. C. S. Wright, *J. Biol. Chem.*, **267**, 14345 (1992).
24. J. M. Rini, *Annu. Rev. Biophys. Biomol. Struct.*, **24**, 551 (1995).
25. K. Drickamer, *Nature Struc. Biol.*, **2**, 437 (1995).
26. J. T. Gallagher, A. Morris, and T. M. Dexter, *Biochem. J.*, **231**, 115 (1985).
27. M. S. Nachbar, J. D. Oppenheim, and J. O. Thomas, *J. Biol. Chem.*, **255**, 2056 (1980).
28. R. K. Merkle and R. D. Cummings, *J. Biol. Chem.*, **262**, 8179 (1987).
29. H. Kawashima, S. Sueyoshi, H. Li, K. Yamamoto, and T. Osawa, *Glycoconj. J.*, **7**, 323 (1990).
30. J. M. Callaghan, B. H. Toh, J. M. Pettitt, D. C. Humphris, and P. A. Gleeson, *J. Cell Sci.*, **95**, 563 (1990).
31. I. J. Goldstein, "Methods in Carbohydrate Chemistry," Vol. 6, J. N. BeMiller and R. L. Whistler, Ed., Academic Press, New York, N.Y., 1972, pp 106.