Synthetic Glycoconjugates. 4.[1] Use of ω-(Acrylamido)alkyl Glycosides for the Preparation of Cluster Glycopolymers

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ABSTRACT: A simple and efficient method for the syntheses of clustering-sugar homopolymers from ω-(acrylamido)alkyl glycosides of *N*-acetyl-β-D-glucosamine (Glc*p*NAc) is described. Radical polymerization of the new glycosides proceeded smoothly in an aqueous solution in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine and gave water-soluble homopolymers having high-density sugar branches as a novel class of cluster glycosides. The apparent association constant of wheat germ agglutinin (WGA) with poly[3-(*N*-acryloylamino)propyl 2-acetamido-2-deoxy-β-D-glucopyranoside] was determined by measuring the change in fluorescence intensity produced by various concentrations of polymeric ligand and found to be approximately 10^8 M^{-1}. Addition of the cluster type Glc*p*NAc polymer to WGA induced much greater enhancement of the fluorescence intensity and a significant blue shift of the fluorescence emission maximum of WGA than did addition of the low-density Glc*p*NAc polymer derived from *n*-pentenyl glycoside.

Introduction

The significance of sugar density on glycoproteins in the specific interaction between oligosaccharide chains and receptors was first proposed and reported by Lee et al. on the basis of chemically designed "cluster glycosides"[2] or "neoglycoproteins".[3] They have elegantly demonstrated for the galactose/*N*-acetylgalactosamine–hepatocyte binding systems that a "cluster" or "multipoint" sugar–ligand interaction may be involved in the successful binding process.[4] Actually, this cluster theory has recently been shown to be applicable to a number of carbohydrate–receptor bindings in relation to cell–cell interactions. For example, it was reported that some inhibitors containing multivalent-type *N*-acetylneuraminic acid showed markedly amplified inhibitory effects on hemagglutination by influenza virus.[5] Furthermore, it was also found that bivalent-type sialyl Lewis *x* (SLe^x) exhibited much a higher affinity for the specific receptor proteins, selectin families.[6]

In the preceding papers, we have reported an efficient method for the syntheses of biochemically useful glycoprotein models having pendant oligosaccharides based on the radical copolymerization of *n*-pentenyl glycosides with acrylamide.[7,8] Using this method, successful synthesis of the polymer carrying tumor-associated Le^x-type trisaccharide branches has recently been reported.[1] Chemoenzymatic preparation of a glycoconjugate polymer having a sialylα(2→3)Galpβ(1→4)Glc*p*NAc structure was also performed on the basis of bovine galactosyl transferase and *Trypanosoma cruzi trans*-sialidase.[9]

Molecular design of water-soluble polymers containing sugar side chains is gaining interest as one of the most effective methods to increase the density of carbohydrate moieties. High-density sugar ligands based on the polymerizable glycosides seem to be effective and potential reagents not only for biomimetic models of glycoconjugates but also for therapeutic or diagnostic purposes in biomedical fields. Thus, our interest is now focused on the preparation of homopolymers from sugar monomers that might serve as high-density and clustering carbohydrate ligands. Although the *n*-pentenyl glycosides have proved to be versatile polymerizable glycosides for the design of a variety of glycoconjugate models and the sugar contents in the copolymers could be adjusted by the feed ratio of polymerizable glycosides and acrylamide as needed, homopolymers from these ω-alkenyl-type glycosides could not be obtained under this reaction condition owing to the innate low chemical reactivity of simple olefin-type aglycons.[8] The present paper describes a facile and efficient method for the preparation of a new type of sugar homopolymers, "cluster glycopolymers", from highly reactive glycosides having ω-(acrylamido)alkyl-type aglycons. The specific interaction of this cluster glycopolymer with wheat germ agglutinin (WGA), a plant lectin which agglutinates erythrocytes and other types of cells through binding with oligosaccharides containing *N*-acetyl-β-D-glucosamine (Glc*p*NAc),[10] is also preliminarily discussed on the basis of a spectrofluorometric investigation.

Results and Discussion

Synthesis of ω-(Acrylamido)alkyl Glycosides and Their Polymers. Scheme 1 indicates the synthetic route of Glc*p*NAc derivatives **1** and **2**[11] as tentative candidates of the reactive model monomers with appropriate spacer-arm structure for the polymerization of a variety of oligosaccharides containing GLc*p*NAc residue at the reducing end. Initially, precursors of polymerizable aglycons, *N*-protected simple aminoalcohols, 3-[[*N*-[(benzyloxy)carbonyl]amino]propan-1-ol (**3**), and 6-[[*N*-[(benzyloxy)carbonyl]amino]hexan-1-ol (**4**),[12] were prepared. Coupling reactions of oxazoline derivative **5** with *N*-protected aminoalcohols **3** and **4** proceeded smoothly in the presence of 10-camphorsulfonic acid (CSA) as the promoter and gave the corresponding intermediates **6** and **7**. General *N*-deprotection by hydrogenation and the following *N*-acryloylation afforded derivatives **8** and **9** as peracetates. Finally, *O*-deacetylation by the usual Zem-

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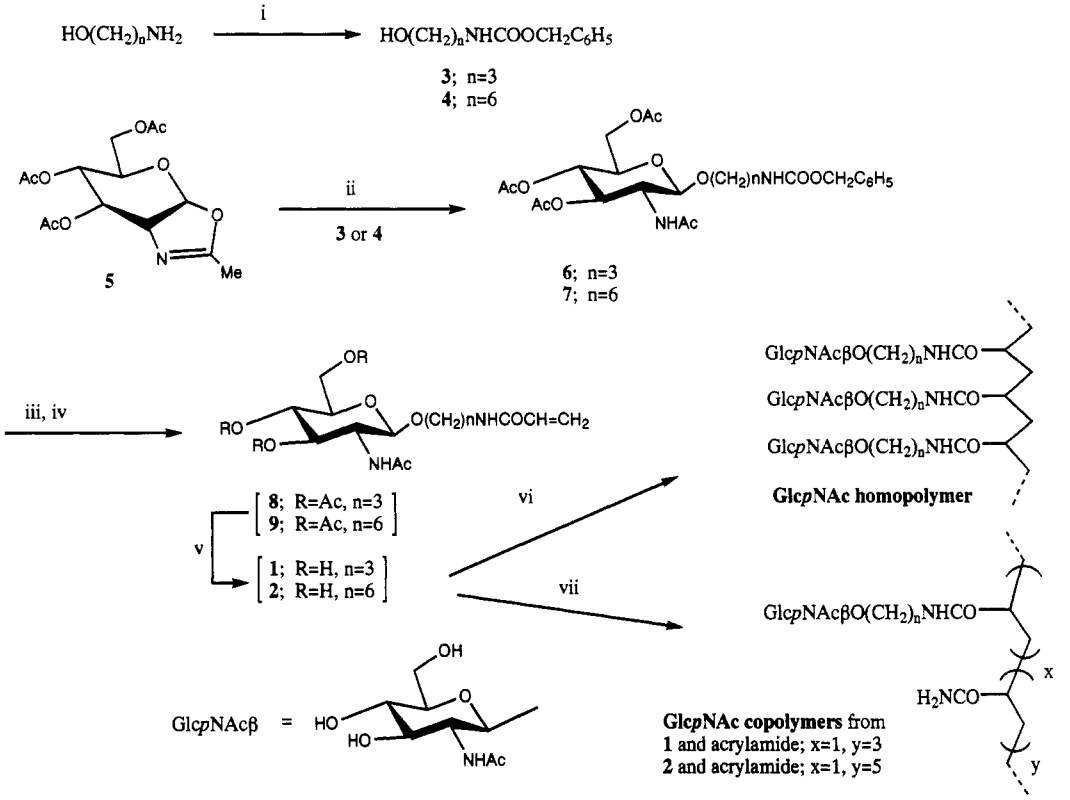
Scheme 1[a]



[a] Reagents and conditions: (i) $C_6H_5CH_2OCOCl$, $NaHCO_3$ (aq), rt, 3 h; (ii) CSA, $ClCH_2CH_2Cl$, 90 °C, 2 h; (iii) H_2/Pd-c, MeOH, rt, 2 h; (iv) CH_2=CHCOCl, Et_3N, THF, 0 → 25 °C, 24 h; (v) NaOMe/MeOH, rt, 3 h; (vi) TEMED, APS, H_2O, 25 °C, 2 h; (vii) CH_2=$CHCONH_2$, TEMED, APS, H_2O, 25 °C, 2 h.

Table 1. Polymerizations of ω-(Acrylamido)alkyl glycosides of *N*-Acetyl-D-glucosamine

sugar monomer	monomer ratio[a]	total yield (%)	polymer compos[a]	sugar (wt %)	$[\alpha]_D$ (deg)	η_{inh}[b] (dL/g)	M_w[c] × 10^3
1	1:0[d]	100	1:0	100	−31.0	0.41	220
1	1:4	91.8	1:3	61.1	−21.9	1.97	>300
2	1:0[d]	35.0[e]	1:0	100	−18.6	0.12	9
2	1:4	90.0[e]	1:5	51.0	−7.1	0.35	70

[a] Ratio of carbohydrate monomer to acrylamide. [b] In water at 25 °C. [c] M_w's were determined by the GPC method with an Asahipack GS-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 (×10^3); Shodex Standard P-82) were used as standards]. [d] Homopolymerization of glycoside (polymerization without acrylamide). [e] 1:1 (v/v) EtOH–H_2O was used for the polymerization solvent, owing to poor solubility in water.

plen procedure gave monomers **1** and **2**. All new compounds prepared here gave satisfactory analytical and spectroscopic data.

Radical polymerizations of ω-acrylamido-type monomers **1** and **2** were carried out in deionized water in the presence of *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS), and the products were purified according to the method reported previously.[7] As anticipated, these new compounds showed an excellent polymerizability compared with those of the glycosides having simple ω-alkenyl-type aglycons.[8] As seen in Table 1, the polymerization reaction proceeded efficiently at room temperature even in the absence of acrylamide and afforded desired homopolymers from derivatives **1** and **2** in 100 and 35% yield, respectively. Moreover, polymerization of glycosides with acrylamide yielded the corresponding copolymers containing desirable amounts of Glc*p*NAc residues in high yields. Since polymers from **1** exhibited much higher molecular weight than those of polymers from **2**, the ω-(acryloylamino)-propyl group seems to be a practically available "polymerizable aglycon" having high reactivity, a moderate spacer-arm structure (8.822 Å), and a good solubility in water.[13] Fully assigned ^1H- and ^{13}C-NMR spectra of polymers from derivative **2** are shown in Figure 1, and all data of chemical shifts of the polymers prepared here are summarized in Table 2 together with those of the monomer glycosides.

Interaction of Cluster Glycopolymer with WGA. It has long been suggested that the value of the binding constants of WGA with chitin oligomers [(Glc*p*Nac)$_n$] increases with the length of these oligosaccharides. Results of equilibrium dialysis showed that association constants for the binding to WGA of Glc*p*NAc, (Glc*p*NAc)$_2$, and (Glc*p*NAc)$_3$ were found to be 1.3×10^3, 2.0×10^4, and 8.3×10^4 M^{-1}, respectively.[14] Fluorescence methods have been also used extensively to study the specific interaction of WGA with sugars and to evaluate the binding site structure of WGA.[15-17] For example, Privat et al.[16] reported that changes of tryptophan fluorescence by addition of (Glc*p*NAc)$_n$ depend on the ligand size and all ligands but monomeric Glc*p*NAc produce a 10-nm shift toward shorter wavelength and 46% enhancement of fluorescence intensity. The binding constants of chitin oligomers to WGA were determined to be 6.9×10^2 (Glc*p*NAc), 4.5×10^3 [(Glc*p*NAc)$_2$], 2.0×10^4 [(Glc*p*NAc)$_3$], and 2.3×10^4 M^{-1} [(Glc*p*NAc)$_4$].[16] Sharon et al. clearly demonstrated by further systematic investigation on the interaction of WGA and 4-methylumbelliferyl chitooligosaccharides that each binding site of WGA consists of three adjacent subsites.[17]

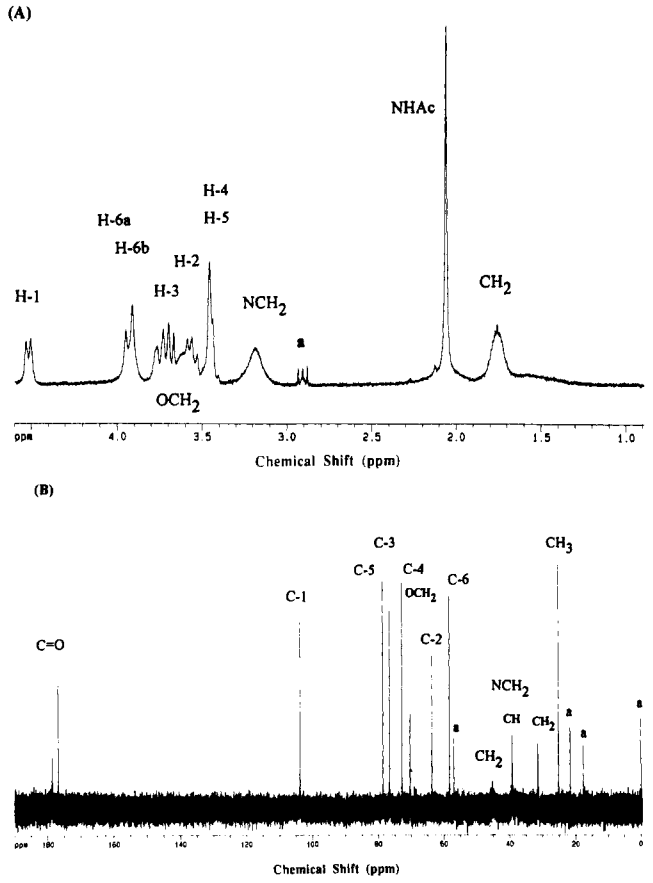


Figure 1. ^{1}H- and ^{13}C-NMR spectra of cluster glycopolymer from glycoside 1 in D_2O. a indicates DSS. (A) ^{1}H-NMR spectrum at 25 °C; (B) ^{13}C-NMR spectrum at 50 °C.

Table 2. ^{13}C Chemical Shifts of Glycosides and Polymers (in ppm from DSS[a])

	carbohydr monomer		homopolymer		copolymer	
compd	1	2	1	2	1	2
C-1	104.0	103.7	103.8	103.7	103.8	103.7
C-2	58.4	58.3	58.3	58.3	58.3	58.3
C-3	76.6	76.5	76.6	76.6	76.5	76.5
C-4	72.8	72.9	72.8	72.8	72.8	72.9
C-5	78.7	78.5	78.6	78.5	78.5	78.5
CH_2=CH	132.5	132.9				
CH_2=CH	130.0	129.3				
CH_2	31.4	31.1	31.5	31.3	31.2	31.2
		30.8		31.2		30.9
		28.3		28.7		28.5
		27.3		27.5		27.3
OCH_2	70.9	72.7	70.4	72.7	70.4	72.7
NCH_2	39.4	41.9	39.1	42.1	39.1	42.0
CH			38.0	38.0	38.3	38.0
CH_2[b]			45.0	45.0	44.5	44.5
C=O[c]	177.4	176.9	176.8	176.6	177.1	176.9
C=O[d]	171.3	171.0	178.7	178.7	179.0	178.8
$CONH_2$					182.0	182.0
CH_3	25.0	24.8	25.1	25.0	24.9	24.9

[a] 3-(Trimethylsilyl)propanesulfonic acid sodium salt. [b] Methylene carbons due to the main chain. [c] Signals due to the carbonyl group of the Glc*p*NAc residue. [d] Signals due to the carbonyl groups of aglycons.

If clustering Glc*p*NAc residues on the glycopolymer possesses the appropriate flexibility to bind all subsites in the binding site, or bind at least two of them, the binding of cluster Glc*p*NAc polymer (cluster-Glc*p*NAc) to WGA might induce significant enhancement of the fluorescence intensity and a shift of the fluorescence emission maximum to shorter wavelength observed in the cases for the bindings of chitooligosaccharides with WGA. Figure 2 shows the emission spectra of WGA and of its complexes with cluster-

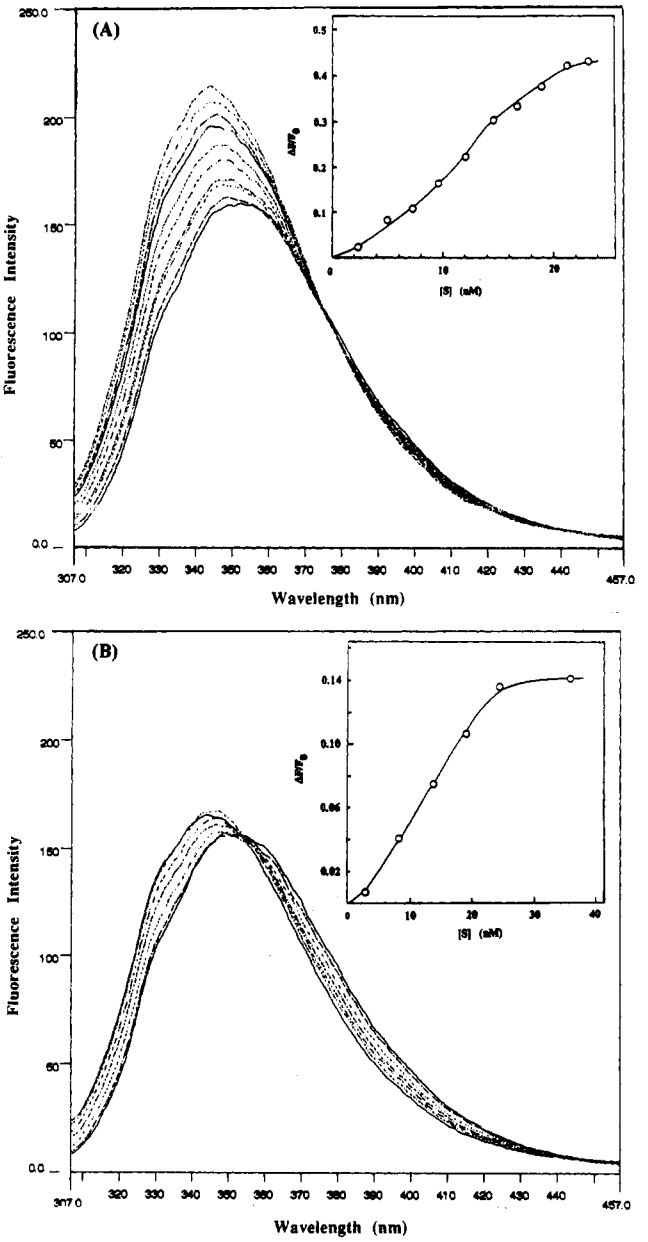


Figure 2. Changes in fluorescence emission spectra of WGA (0.65 μM, 3.0 mL of 25 mM Tris-HCl buffer containing 1.25 M NaCl and 25 mM $CaCl_2$, pH 7.8, 5.0 °C) upon addition of (A) 10-μL aliquots of cluster Glc*p*NAc polymer (0.76 μM) and (B) 20-μL aliquots of low-density Glc*p*NAc polymer (0.88 μM). ΔF is a change of the fluorescence intensity at the fluorescence maximum wavelength of a solution containing the protein with a total ligand concentration [S], and F_0 is the fluorescence intensity of protein alone.

Glc*p*NAc (Figure 2A) and with the low-density Glc*p*NAc polymer (LD-Glc*p*NAc; the ratio of Glc*p*NAc residue/acrylamide residue of the polymer = 1:8) derived from the known *n*-pentenyl glycoside[8] (Figure 2B) at pH 7.8. When protein was saturated with cluster-type polymer, the maximum fluorescence intensity was enhanced by 43% and the emission maximum was shifted from 349 to 343 nm (Figure 2A). In contrast, LD-Glc*p*NAc induced only 14% enhancement of the fluorescence intensity and a 3-nm shift toward shorter wavelengths (Figure 2B).

Thus, although the results suggested that the environment of tryptophan residues located at or near the binding sites of WGA is altered from hydrophilic to relatively more hydrophobic upon interaction with both glycopolymers, the combining of cluster Glc*p*NAc to the subsites seems to be more effective than that of LD-Glc*p*NAc and provides a very similar profile with those of chitooligosaccharides

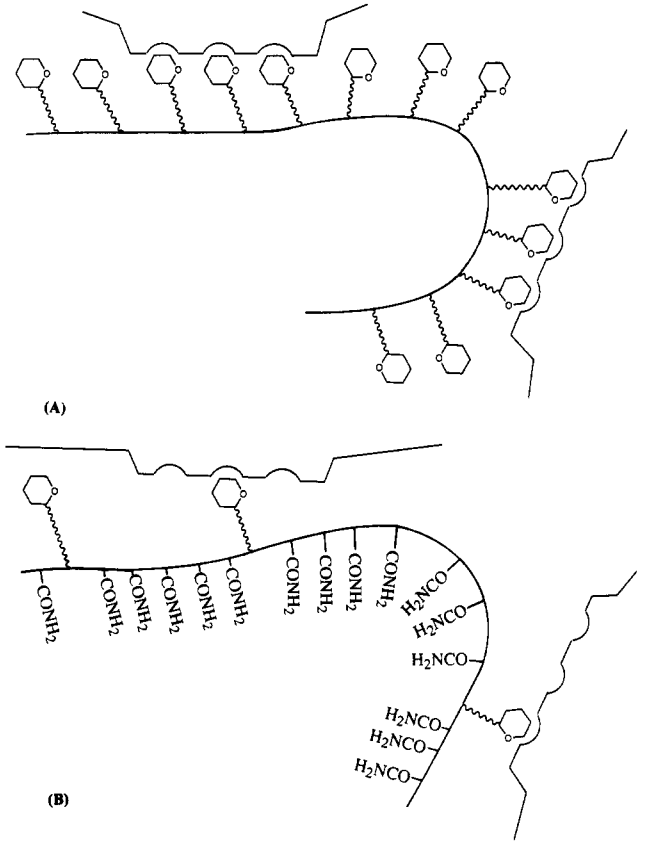


Figure 3. Models of multivalent binding of polymeric Glc*p*NAc ligands to the subsites of WGA. (A) Cluster Glc*p*NAc polymer; (B) low-density Glc*p*NAc polymer.

which tightly bind to the subsites. In a plot of $\Delta F/F_0$ versus [S], apparent association constants (K_{app}) of these polymers with WGA based on the concentration required for 50% enhancement of the fluorescence intensity are preliminarily estimated to be approximately 9×10^7 and 7×10^7 M^{-1}, respectively. These results clearly indicated that the lectin showed a much higher affinity for polymeric Glc*p*NAc ligands than chitooligosaccharides. Interestingly, the combining curve of the cluster Glc*p*NAc has a somewhat sigmoidal shape characteristic of positive cooperativity in the binding of the two macromolecules. The plausible models we propose regarding the interaction between glycopolymers and WGA are shown in Figure 3; it illustrates the binding profile of each glycopolymer ligand with the subsites of WGA. Further evaluation including the stoichiometric analyses of the binding is currently under investigation, and the results will be discussed elsewhere.

In conclusion, a facile and an efficient synthetic method for the preparation of cluster glycopolymers from ω-(acryloylamino)alkyl glycosides is described above. It was suggested that these glycopolymers are specific and effective sugar ligands for lectins. This method will enhance considerably our ability to design and synthesize a variety of glycoconjugate mimetics for investigating the nature of cell surface receptors which specifically interact with sugar molecules. Work is in progress to extend this method to the syntheses of cluster glycoconjugates having more complex oligosaccharides including *N*-acetylneuraminic acid and to evaluate the biological activities of these cluster glycopolymers.

Experimental Section

General Procedure. Melting points were determined with a Laboratory Devices melting point apparatus and are uncorrected. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. 1,2-Dichloroethane, ethyl acetate, and pyridine were stored over molecular sieves (3 Å) for several days before use. Acrylamide was recrystallized from benzene before use. Wheat germ agglutinin (WGA, activity < 20 μg/mL) was purchased from Sigma. Optical rotations were determined with a Jasco DIP-370 digital polarimeter at 23 °C. ^1H and proton-decoupled carbon NMR spectra were recorded at 270 and 67.8 MHz, respectively, with a JEOL JNM-GX270 spectrometer in chloroform-*d* or deuterium oxide, using tetramethylsilane (TMS), methanol, or 3-(trimethylsilyl)propanesulfonic acid sodium salt (DSS) as internal standards. Average molecular weights were estimated by gel permeation chromatography (GPC) with an Asahipak GS-510 column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 K; Shodex Standard P-82) were used as standards. Elemental analyses were performed with a Yanaco MT-3 CHNcorder on samples extensively (ca. 24 h) dried in vacuo (50 °C, 0.1 Torr) over phosphorus pentoxide. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For detection of the components, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) methanol–concentrated sulfuric acid–*p*-anisaldehyde and heated for a few minutes (for carbohydrates), (b) a solution of 5 wt % ninhydrin in ethanol and heated for a few minutes (for amino groups), or (c) an aqueous solution of 5 wt % potassium permanganate and heated similarly (for C–C double bonds). Column chromatography was performed on silica gel (Wakogel C-200; 100–200 mesh, Wako Pure Chemical Industries Co., Ltd., Japan). All extracted solutions were concentrated below 44 °C under diminished pressure.

3-[*N*-[(Benzyloxy)carbonyl]amino]propan-1-ol (3). To a solution of 3-amino-1-propanol (7.6 mL, 0.1 mol) and sodium hydrogen carbonate (21.8 g, 0.26 mol) in water were added dropwise a mixture of (benzyloxy)carbonyl chloride (20.8 mL, 0.13 mol) and ether (20 mL), and the mixture was stirred for 3 h at room temperature. The mixture was filtered with Celite, extracted with ether (200 mL), washed with water, dried over magnesium sulfate, and evaporated. The residual syrup was chromatographed on silica gel eluted first with 20:1 and then with 2:1 (v/v) toluene–ethyl acetate as an eluant to give compound **3** (15.7 g): mp 43 °C.

Anal. Calcd for $C_{11}H_{15}O_3N$: C, 63.07; H, 7.16; N, 6.69. Found: C, 63.27; H, 7.25; N, 6.80.

3-[[(Benzyloxy)carbonyl]amino]propyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (6). A solution of oxazoline derivative **5**[7] (3.9 g, 11.8 mmol) and **3** (5.0 g, 23.7 mmol) in 1,2-dichloroethane (15 mL) was stirred under a nitrogen atmosphere for 2 h at 90 °C in the presence of CSA (50 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice–water. The extract with chloroform was washed successively with aqueous sodium hydrogen carbonate and water, dried over magnesium sulfate, filtered, and evaporated. The residue was purified by chromatography on silica gel with 20:1 (v/v) toluene–ethyl acetate to afford compound **6** (5.7 g, 89%): mp 145–146 °C; $[\alpha]_D$ −7.4° (*c* 0.229, chloroform); ^1H-NMR ($CDCl_3$) δ 1.77 and 1.84 (m, 2 H, CH_2), 1.94, 2.03, and 2.07 (all s, 12 H, 4 $COCH_3$), 3.55 and 3.94 (m, 2 H, OCH_2), 3.56 (m, 1 H, H-5), 3.96 (ddd, 1 H, J = 8.9 Hz, H-2), 4.12 (dd, 1 H, J = 2.3 and 12.2 Hz, H-6a), 4.23 (dd, 1 H, J = 4.5 and 12.2 Hz, H-6b), 4.31 (d, 1 H, J = 8.3 Hz, H-1), 4.95 (m, 1 H, NHCOO), 5.0–5.16 (m, 4 H, H-3, H-4, and $PhCH_2$), 6.38 (d, 1 H, J = 8.6 Hz, NH), and 7.31–7.40 (m, 5 H, aromatic).

Anal. Calcd for $C_{25}H_{34}O_{11}N_2$: C, 55.73; H, 6.31; N, 5.20. Found: C, 55.51; H, 6.32; N, 5.26.

6-[[(Benzyloxy)carbonyl]amino]hexyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (7). A solution of oxazoline derivative **5** (8.0 g, 24.3 mmol) and 6-[*N*-[(benzyloxy)-carbonyl]amino]hexanol (**4**)[12] (12.2 g, 48.6 mmol) in 1,2-dichloroethane (50 mL) was stirred under a nitrogen atmosphere for 2 h at 90 °C in the presence of CSA (50 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice–water. The chloroform extract was washed successively with aqueous sodium hydrogen carbonate and water, dried over magnesium sulfate, filtered, and evaporated. The residue was purified by chromatography on silica gel with 1:1 (v/v) toluene–

ethyl acetate to afford compound 7 (8.8 g, 62%): mp 111–113 °C (lit.[18] mp 112–114 °C, lit.[19] mp 96–98 °C); ^{1}H-NMR ($CDCl_3$) δ 1.35 and 1.50 (m, 8 H, CH_2), 1.93, 2.02, 2.03, and 2.08 (all s, 12 H, $COCH_3$), 3.19 (m, 2 H, NCH_2), 3.46 and 3.80 (m, 2 H, OCH_2), 3.63 (br d, 1 H, H-5), 3.85 (ddd, 1 H, H-2), 4.09 (dd, 1 H, J = 2.3 and 12.2 Hz, H-6a), 4.25 (dd, 1 H, J = 4.5 and 12.2 Hz, H-6b), 4.63 (d, 1 H, J = 8.2 Hz, H-1), 4.87 (br s, 1 H, NHCOO), 5.06 (t, 1 H, J = 9.6 Hz, H-4), 5.12 (dd, 2 H, J = 16.2 Hz, $PhCH_2$), 5.29 (t, 1 H, J = 9.6 Hz, H-3), 5.93 (d, 1 H, J = 8.6 Hz, NH), and 7.30–7.37 (m, 5 H, aromatic).

Anal. Calcd for $C_{28}H_{40}O_{11}N_2$: C, 57.90; H, 6.89; N, 4.82. Found: C, 58.08; H, 6.96; N, 4.91.

3-(*N*-Acryloylamino)propyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (8). Compound 6 (4.7 g, 8.72 mmol) was hydrogenated in the presence of 10% palladium on carbon (0.4 g) in methanol (100 mL) for 2 h at room temperature. The reaction was monitored by TLC in 65:25:4 (v/v/v) chloroform–ethyl acetate–methanol. The mixture was filtered and evaporated to give the crude 3-aminopropyl glycoside.

To a solution of the crude 3-aminopropyl glycoside in THF (40 mL) were added triethylamine (1.57 mL) and freshly distilled acryloyl chloride (0.90 mL) at 0 °C, and the mixture was stirred for 24 h at room temperature. The mixture was poured into ice–water and extracted with chloroform. The organic layer was washed with brine, dried, and evaporated. The residue was subjected to silica gel chromatography with 1:2 (v/v) toluene–ethyl acetate as eluant to yield 8 (3.2 g, 80%): mp 149–150 °C; $[\alpha]_D$ −51.4° (c 0.23, chloroform); ^{1}H-NMR ($CDCl_3$) δ 1.37 and 1.53 (m, 2 H, CH_2), 1.95, 2.03, 2.04, and 2.08 (all s, 12 H, $COCH_3$), 3.19 and 3.46 (m, 2 H, NCH_2), 3.75 and 4.05 (m, 2 H, OCH_2), 3.67 (m, 1 H, H-5), 4.08 (dd, 1 H, H-2), 4.12 (dd, 1 H, J = 2.4 and 12.0 Hz, H-6a), 4.27 (dd, 1 H, J = 4.5 and 12.2 Hz, H-6b), 4.47 (d, 1 H, J = 8.5 Hz, H-1), 5.12 (t, 1 H, J = 9.3 Hz, H-4), 5.14 (t, 1 H, J = 9.7 Hz, H-3), 5.65 [dd, 1 H, J = 3.7 and 8.3 Hz, CH=CH_2 (cis)], 6.27–6.30 (m, 2 H, CH=CH_2 (trans)], 6.31 (m, 1 H, CH_2NHCO), and 6.53 (d, 1 H, J = 9.0 Hz, NH).

Anal. Calcd for $C_{20}H_{30}O_{10}N_2$: C, 51.79; H, 6.65; N, 6.04. Found: C, 51.79; H, 6.76; N, 6.14.

3-(*N*-Acryloylamino)propyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (1). To a solution of compound 8 (0.8 g, 1.75 mmol) in dry methanol (30 mL) was added sodium methoxide (28 mg), and the mixture was stirred for 1 h at room temperature. It was made neutral with Dowex 50W-X8 (H^+) resin, filtered, and evaporated in vacuo to give a quantitative yield of 1 (0.5 g): mp 168–169 °C; $[\alpha]_D$ −29.3° (c 0.23, water); ^{1}H-NMR (D_2O) δ 1.80 (m, 2 H, CH_2), 2.03 (s, 3 H, $NHCOCH_3$), 3.30 (m, 2 H, NCH_2), 3.44 (m, 2 H, H-4 and H-5), 3.53 (t, 1 H, J = 8.8 Hz, H-3), 3.62 and 3.94 (each dd, 2 H, OCH_2), 3.70 (ddd, 1 H, J = 10.2 Hz, H-2), 3.74 (dd, 1 H, J = 12.5 Hz, H-6a), 3.93 (dd, 1 H, J = 4.5 and 12.1 Hz, H-6b), 4.49 (d, 1 H, J = 8.4 Hz, H-1), 5.74 [dd, 1 H, J = 1.9 and 10.0 Hz, CH=CH_2 (cis)], and 6.13–6.30 [m, 2 H, CH=CH_2 (trans)].

Anal. Calcd for $C_{14}H_{24}O_7N_2$: C, 49.79; H, 7.34; N, 8.29. Found: C, 49.69; H, 7.31; N, 8.32.

6-(*N*-Acryloylamino)hexyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (2).[11] Compound 7 (2.2 g, 3.79 mmol) was hydrogenated and subsequently *N*-acryloylated according to the method described for the preparation of 8 to afford compound 9 (1.0 g, 53%): mp 159 °C; $[\alpha]_D$ −15.6° (c 0.205, chloroform); ^{1}H-NMR ($CDCl_3$) δ 1.37 and 1.54 (m, 8 H, CH_2), 1.95, 2.03, 2.04, and 2.08 (all s, 12 H, $COCH_3$), 3.35 (m, 2 H, NCH_2), 3.48 and 3.88 (m, 2 H, OCH_2), 3.70 (br d, 1 H, H-5), 3.86 (ddd, 1 H, J = 8.3 Hz, H-2), 4.15 (dd, 1 H, J = 12.0 Hz, H-6a), 4.25 (dd, 1 H, J = 4.5 and 12.0 Hz, H-6b), 4.67 (d, 1 H, J = 8.5 Hz, H-1), 5.07 (t, 1 H, J = 10.0 Hz, H-4), 5.32 (t, 1 H, J = 9.6 Hz, H-3), 5.65 [dd, 1 H, J = 10.0 Hz, CH=CH_2 (cis)], 6.03 (br s, 1 H, CH_2NH), 6.15–6.35 (m, 2 H, CH=CH_2 (trans)], and 6.29 (d, 1 H, NH).

Treatment of acetate 9 (0.9 g, 1.8 mmol) with sodium methoxide (29 mg) in dry methanol, as described for the preparation of 1, gave a quantitative yield of 2 (0.7 g): mp 171 °C.

Anal. Calcd for $C_{17}H_{30}O_7N_2$: C, 53.44; H, 8.14; N, 7.33. Found: C, 53.69; H, 8.44; N, 7.33.

Polymerization. A typical polymerization procedure is as follows: A solution of monomer 1 (99.5 mg, 0.30 mmol) in deionized water (1.0 mL) was deaerated by a water aspirator for 20 min, to which are added TEMED (4.5 μL, 30 μmol) and APS (2.44 mg, 10.7 μmol). The mixture was stirred for 17 h at room temperature, diluted with 1.9 mL of a 0.1 M pyridine–acetic acid buffer (pH 5.1), dialyzed against deionized water for 24 h, and freeze-dried to give a white powdery polymer in a quantitative yield. Copolymerization of glycosides with acrylamide was performed as described previously.[7]

Fluorescence Measurements. Emission spectra of WGA induced by excitation at 295 nm were uncorrected and were recorded with a Hitachi 650-60 fluorescence spectrophotometer or a Perkin-Elmer LS-50B luminescence spectrometer. The solutions were contained in 1-cm quartz cuvettes, mounted in thermostated holders, and the measurements were carried out at 5 °C in order to remove the effect of nonspecific binding on the spectra. The concentration of WGA was estimated to be 0.65 μM by using the absorption coefficient at 280 nm (1.27 mg^{-1} cm^2 in 0.1 M CH_3COONa/CH_3COOH, 0.5 M NaCl, pH 4.7).[20]

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References and Notes

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