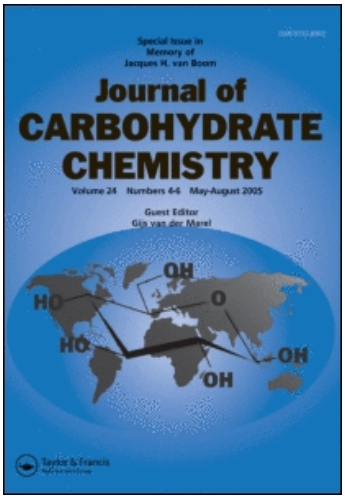

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CHEMO-ENZYMATIC SYNTHESIS OF A GLYCOPOLYMER CARRYING CLUSTERED-*N*-ACETYL-β-LACTOSAMINE MOIETIES

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ABSTRACT

A polyacrylamide derivative having a β-linked *N*-acetyllactosamine moiety, a major component of oligosaccharide chains of glycoproteins, on each repeating unit was synthesized via a chemo-enzymatic process. *p*-Nitrophenyl *N*-acetyl-β-lactosaminide was prepared by an one-step enzymatic synthesis, using a β-galactosidase as catalyst, from *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide as the glycosyl acceptor and lactose as the glycosyl donor. The nitro group was reduced to an amino function, which was then allowed to react with either acryloyl chloride or acrylic acid. The resulting *p*-acryloylaminophenyl *N*-acetyl-β-lactosaminide was polymerized with azobisisobutyronitrile as initiator in dimethyl sulfoxide to give a homopolymer with a number-average molecular weight ($\overline{M}_n$) of 3.2 x 10^5. High molecular-weight polymers carrying α-lactose, *N*-acetyl-β-D-glucosamine, and α-D-glucopyranose were also synthesized as reference polymers in similar manners. Solution properties of these poly-*p*-acryloylaminophenyl derivatives are discussed on the basis of the hydrophilic-hydrophobic unit structures.

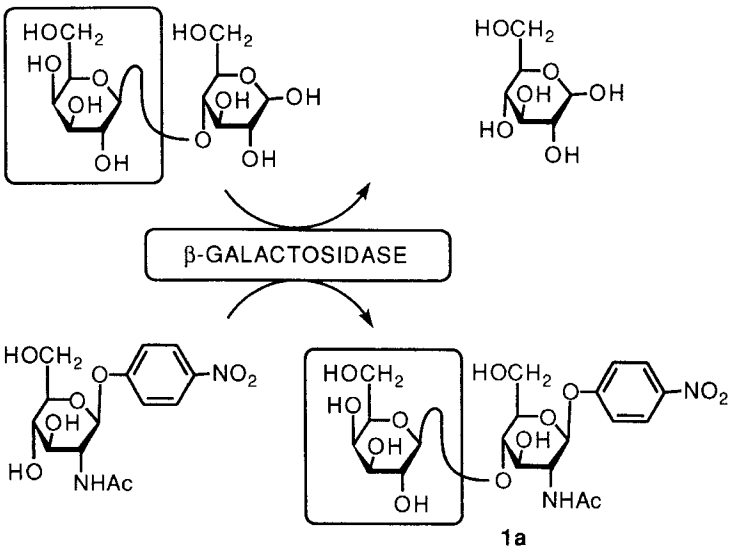
INTRODUCTION

Increasing attention has been paid recently to synthetic polymers substituted with pendant carbohydrate moieties. Several different types of these polymers, termed glycopolymers,[1] have been used in biomedical applications such as cell-specific culture substrata,[2-6] artificial antigens,[7,8] and targeted drug delivery systems.[9] These polymers are also useful as tools for investigating biological recognition phenomena using lectins and anti-carbohydrate monoclonal antibodies.[10-16] Attempts have been made to introduce more biologically important, complex oligosaccharides as well as to design simpler synthetic routes. Synthetic strategies using enzymes as catalyst have been noted recently,[17-21] in order to overcome the difficulties of chemical synthesis of biologically important oligosaccharides. However, little has been reported on chemo-enzymatic synthesis of glycopolymers.[22, 23]

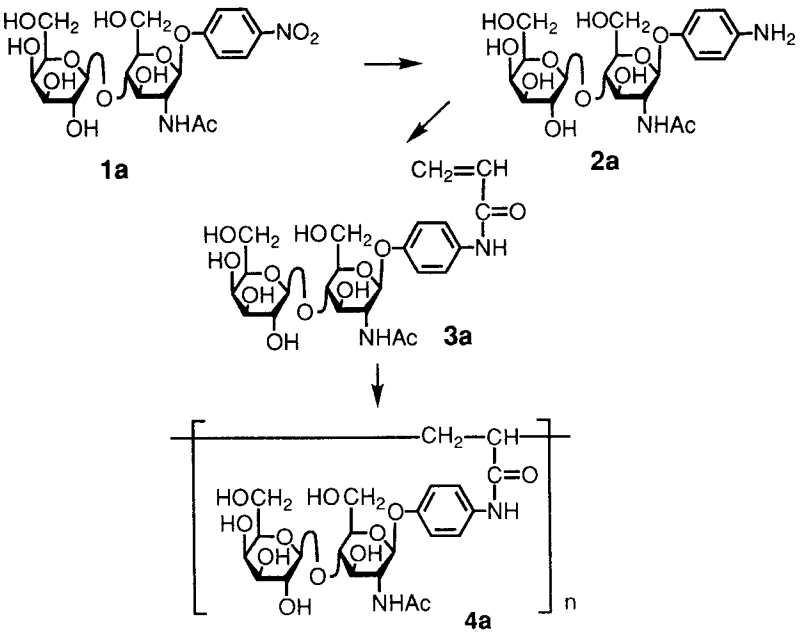
Usui *et al.*[21,24,25] reported several preparative-scale syntheses of oligosaccharides via kinetic approaches employing transglycosylation activity of glycosidases. For instance,[25] *p*-nitrophenyl *N*-acetyl-β-lactosaminide was synthesized employing *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide as a glycosyl acceptor and lactose as a glycosyl donor in one-step procedure using a β-galactosidase from *Bacillus circulans* (Scheme 1). The *N*-acetyllactosamine residue is a major component of oligosaccharide chains of glycoproteins and glycolipids, and can behave as differentiation antigens, tumor-associated antigens, and components of receptor systems.[26,27] Glycopolymers carrying pendant *N*-acetyllactosamine signals are expected to exhibit interesting functions in biological recognition events. The enzymatically synthesized *N*-acetyllactosamine derivative (*p*-nitrophenyl *N*-acetyl-β-lactosaminide) is an attractive starting substance for glycopolymers.

This paper describes the synthesis of the glycopolymer according to Scheme 2 via (1) reduction of the nitro function to the *p*-aminophenyl glycoside (**2a**), (2) introduction of acryloyl function to give the *p*-acryloylaminophenyl glycoside monomer (**3a**), and (3) radical polymerization to yield the homopolymer (**4a**). Other three corresponding types of homopolymers also have been synthesized according to the convenient synthetic route. Figure 1 depicts the polymers carrying α-lactose (**4b**), *N*-acetyl-β-D-glucosamine (**4c**), and α-D-glucopyranose (**4d**) moieties, as reference samples of **4a**.

The following numberings and abbreviations as represented in Scheme 2 and Figure 1 are used in this paper: *p*-nitrophenyl- (*p*NP-, **1**); *p*-aminophenyl-



Scheme 1. Enzymatic synthesis of *p*-nitrophenyl *N*-acetyl-β-lactosaminide (1a)



Scheme 2. Synthesis of *N*-acetyl-β-lactosamine-carrying glycopolymer

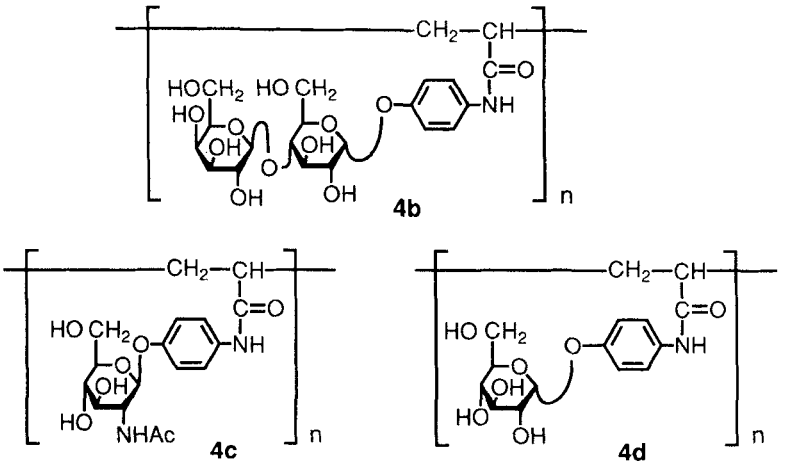


Fig. 1. Glycopolymers prepared as the reference samples of 4a

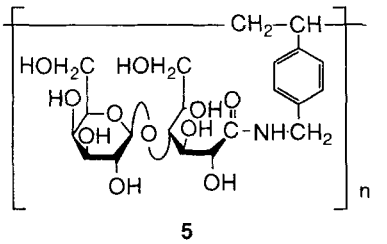


Fig. 2. Lactose-carrying polystyrene useful as hepatocyte-specific culture substratum

(**2**); *p*-acryloylaminophenyl- (**3**); poly-*p*-acryloylaminophenyl- (**4**) derivatives; 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (β-LacNAc, **a**); 4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (α-Lac, **b**); 2-acetamido-2-deoxy-β-D-glucopyranoside (β-GlcNAc, **c**); α-D-glucopyranoside (α-Glc, **d**).

The resulting *p*-acryloylaminophenyl glycopolymers have been found to show characteristic solution properties which are assumed to result from the amphiphilic structures consisting of a hydrophobic main chain and hydrophilic pendant glycosides. These characteristics of the homopolymers are distinct from those of the copolymers with acrylamide,[1,12] while copolymerizations

with various kinds of vinyl compounds provide an interesting strategy for designing biomaterials. The solution properties of glycopolymers (**4a-4d)** are comparable to those of the corresponding glycopolymers of styrene derivatives previously reported.[28,29] In Figure 2 is represented the lactose-carrying polystyrene, poly[*N*-*p*-vinylbenzyl-4-*O*-β-D-galactopyranosyl-D-gluconamide] (**5**), the structure of which is similar to **4b** in some respects. It has been reported [3-6] that the glycopolymer **5** is a useful substratum for culture of hepatocytes because the dynamic interactions function between asialoglycoprotein receptors on the surface of the cells and clustered-galactose ligands along the polymer chains on the surface of the dish. We expect that the glycopolymers **4a-4d** synthesized in this paper will exhibit effective biological recognitions due to high density or clustered glycosignals.[30,31]

RESULTS AND DISCUSSION

Hydrogenation of the nitorophenyl group of **1** was carried out with palladium black as catalyst in a mixture of water and methanol (1 : 1 in volume), and *p*-aminophenyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**2a**) was isolated by crystallization from methanol. The amino function was unstable and the other aminophenyl derivatives (**2b**, **2c**, and **2d**) were used immediately for the next step without isolation. *p*-Acryloylaminophenyl glycosides (**3a**-**3d**) were prepared with either acryloyl chloride or acrylic acid. Amidation using acrylic acid and water-soluble carbodiimide in the presence of a radical inhibitor in water/dioxane or in water was preferred because less amounts of ester by-products were formed. An excess amount of acrylic acid brought about polymerization of the resulting monomer during purification procedures.[1] Production of polymerizates was depressed by using an equimolar amount of acrylic acid and by concentrating the monomeric compound through freeze-drying. The overall yields of **3** from **1** were in the range of 69 -78 %.

The linkage between lactose and *p*-phenyl in **3b** is α-anomeric, as judged by the ^{1}H- (H-1, δ 5.48, J = 3.9 Hz) and ^{13}C NMR (C-1', δ 103.8; C-1, δ 97.6) spectral data and the optical rotation ($[\alpha]_D^{25}$ + 40.6 °), all of which were distinct from the data of the β-linked compound, *p*-acryloylaminophenyl 4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (H-1, δ 5.14, J = 7.8 Hz; C-1', δ 103.7; C-1, δ 100.2; $[\alpha]_D^{25}$ - 26.7 °) reported by Roy *et al.* [1]

TABLE 1. Polymerization of *p*-acryloylaminophenyl glycosides [a]

Monomer,			Me_2SO,	Time,	Yield,	[η], dl/g [b]		$\overline{M}_w$ [c]	$[\alpha]_D^{25}$ [d]
	Glycoside	g	ml	h	%	Me_2SO	H_2O	x 10^{-5}	deg.
3a	β-LacNAc	1.1	2.5	12	91	-	0.19	3.2	-5.2
3b	α-Lac	1.5	3.0	9	50	0.38	0.20	3.0	+42.3
"	"	3.0	8.0	12	45	0.20	0.10	1.4	+41.3
3c	β-GlcNAc	1.8	5.0	8	85	0.66	0.21	4.4	-11.2
3d	α-Glc	2.3	5.0	5.5	80	0.92	0.62	1.4	+136.6

a. Azobisisobutyronitrile, 1 mol%; 60°C.

b. At 25 °C.

c. Determined by light scattering method (in water at 25 °C).

d. c 1.0 in water.

Compounds **3a**, **3b**, and **3d** were solubule in water, dimethyl sulfoxide (Me_2SO), dimethylformamide, pyridine, ethanol, and methanol. Compound **3c** was soluble in these solvents but not in water. Since their solubilities in Me_2SO were higher than those in water, their polymerizations were carried out at rather high monomer concentrations in Me_2SO with azobisisobutyronitrile (1 mol%) as initiator at 60 °C. The polymerizations proceeded homogeneously to give viscous solutions. Table 1 summarizes the representative data of the polymerizations using 1 to 3 g of these monomers.

All of the polymers, obtained as white powders, were soluble in Me_2SO, but their solubilities in water and dimethylformamide were slightly different as follows. Poly(*p*-acryloylaminophenyl=α-Lac) (**4b**) was soluble in water, but insoluble in dimethylformamide; poly(*p*-acryloylaminophenyl=β-LacNAc) (**4a**) was soluble in water, but swollen in dimethylformamide; poly(*p*-acryloylaminophenyl=α-Glc) (**4d**) was soluble in both solvents; poly(*p*-acryloylaminophenyl=β-GlcNAc) (**4c**) was slightly soluble in water and soluble in dimethylformamide. The solution properties of these polymers changed from

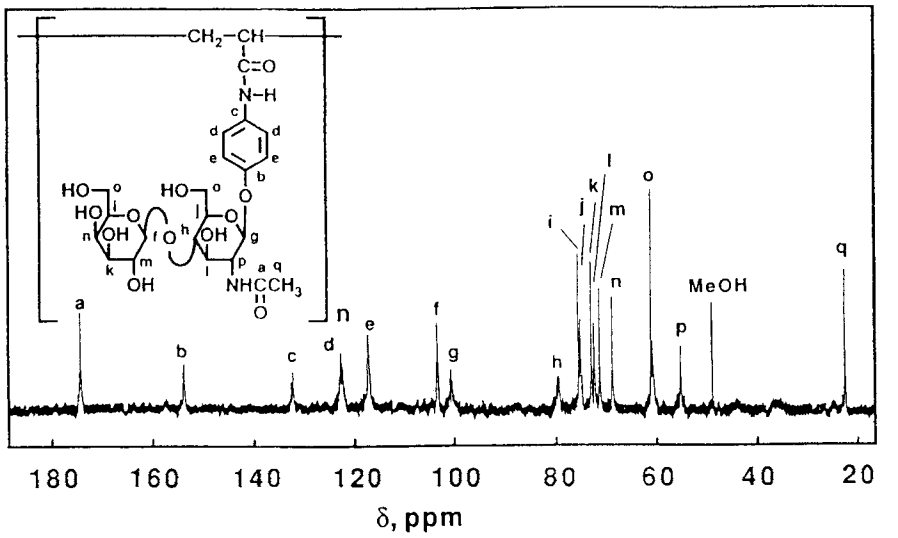


Fig. 3. ^{13}CNMR spectrum of poly(*p*-acryloylaminophenyl N-acetyl-β-lactosaminide) (4a)
(10 % in D_2O, 80 °C, methanol standard, 67.8 MHz)

hydrophilic to hydrophobic in the order of **4b** > **4a** > **4d** > **4c** according to the chemical structures of the glycosides.

It is worth to note that, in spite of insolubility of monomeric compound **3c** in water, the corresponding polymer **4c** was soluble in water, although the solubility is not high. We assume that the solubility of **4c** is increased owing to the conformation of the polymer chain: the hydrophobic moieties along the polymer chain are occluded in the inside of the molecule and the hydrophilic carbohydrate moieties are protruded outside, in contrast to the exposed hydrophobic moiety of **3c**. Such an increase of solubility in water through polymerization was previously observed for *N*-*p*-vinylbenzyl-D-gluconamide and its homopolymer.[28] The characteristic conformations may apply to other polymers **4a**, **4b**, and **4d**, as suggested in the following findings.

The ^{13}CNMR spectrum of **4a** in D_2O is depicted in Figure 3. The chemical shifts were similar to those of **3a**, except for the absence of vinyl signals. However, some signals of the phenyl moiety (peak b - e) became broad, and the main chain methylene, methine, and carbonyl signals were too broad to be detected. The signal broadening 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 mentioned above, although the signal broadening of the present polymers was not so prominent as that of the corresponding polystyrene derivatives.[29]

Formation of high molecular-weight polymers was suggested by rather high limiting viscosity numbers [η] of the polymers determined in Me_2SO. The limiting viscosity numbers of the polymers determined in water was lower than the respective one determined in Me_2SO. This is also a reflection of the assumed conformations.[29]

Estimation of molecular weights of these polymers was attempted by gel-permeation chromatography using the following two different column/eluent combinations: (1) Shodex OHpak KB-802 and KB-803 columns with water eluent and (2) Shodex KF-803 and KF-804 columns with Me_2SO eluent. Several standard pullulan samples ($\bar{M}_n$ or $\bar{M}_w$ = 5.3 x 10^3 - 2.36 x 10^6) were used as references for both combinations. The average molecular weights estimated from the two chromatograms of one polymer sample were quite different with each other. For an example, the polymer **4a** had $\bar{M}_n$ = 1.9 x 10^6 ($\bar{M}_w/\bar{M}_n$ = 3.0) (water eluent) and $\bar{M}_n$ = 2.4 x 10^4 ($\bar{M}_w/\bar{M}_n$ = 24) (Me_2SO eluent). There was a similar tendency for the other polymers. Molecular size of these polymers could not be estimated by gel-permeation chromatography.

More precise weight-average molecular weights ($\bar{M}_w$) determined by light scattering were in the range of 1.4 x 10^5 to 4.4 x 10^5 as shown in Table 1. Even in these measurements, Zimm plots gave distorted rectilinear grids, suggestive of the possibility of molecular association of these polymers.

In summary, the poly-*p*-acryloylaminophenyl mono- and disaccharide derivatives showed unique solution properties in connection with solubility, NMR spectra, gel-permeation chromatograms, solution viscosities, and light scattering measurements. It is assumed that the properties of the polymers originate from their unique conformations owing to the hydrophilic-hydrophobic unit structures. The hydrophobicity of the *p*-acryloylaminophenyl portion is not so strong as that of the *p*-vinylbenzyl portion of polystyrene derivatives because the amide group is rather hydrophilic. These polymers may have tendencies to be adsorbed to solid surface and to form molecular aggregates (supramolecular conformations). These characteristics should be useful for applications using the polymers as cell-specifc culture substrata and other biomedical materials.

EXPERIMENTAL

General Procedures. IR spectra were recorded with a Japan Spectroscopic Co. (JASCO) A-3 grating spectrophotometer. NMR spectra were recorded with a Japan Electro Optics Co. JNM-FX-270 Fourier transform NMR spectrometer. Optical rotations were determined at 25 °C on a JASCO DIP-181 digital polarimeter using a water-jacketed 1 dm cell. Gel-permeation chromatography 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 880 high performance liquid chromatograph using Shodex OHpak KB-802 and KB-803 columns and water as the eluent. Weight-average molecular weights were determined with an Otsuka Electronics SLS-600 light scattering photometer using water as solvent at 25 °C.

Materials. *p*-Nitrophenyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (*p*NP=β-LacNAc, **1a**) was prepared according to the method reported.[25] *p*-Nitrophenyl *N*-acetyl-β-D-glucosaminide (**1c**) and *p*-nitrophenyl α-D-glucopyranoside (**1d**) were kindly supplied respectively by Yaizu Suisankagaku Industry (Yaizu, Japan) and Nihon Shokuhin Kako (Fuji, Japan).

***p*-Aminophenyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (*p*-aminophenyl=β-LacNAc, 2a).** *p*NP=β-LacNAc (**1a**, 150 mg, 0.3 mmol) was dissolved in a mixture of methanol (7.5 mL) and water (7.5 mL). Hydrogenation was carried out in the presence of palladium on carbon (7.5 mg) in a hydrogen atmosphere. A TLC spot (ethanol) of R_f = 0.51 (**1a**) was converted to R_f = 0.40 (**2a**). After 2 h, the palladium on carbon catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give a white powdery product. It was recrystallized from methanol: yield 45 mg (32 %); mp 239-241 °C; $[\alpha]_D^{25}$ -1.1 ° (*c* 0.30, water).

^1H NMR (D_2O, ref. TPS) δ 6.94 (d, 2H, *J* = 8.8 Hz, *m*-Ph), 6.81 (d, 2H, *J* = 8.4 Hz, *o*-Ph), 5.01 (d, 1H, *J* = 8.4 Hz, H-1), 4.51 (d, 1H, *J* = 7.7 Hz, H-1'), 4.8 (s, HO-), 4.1-3.5 (m, ring-H), and 2.05 (3H, s, CH_3). ^{13}C NMR (D_2O, ref. TPS) δ 177.6 (CH_3-C=O), 153.1 (*ipso*-Ph attached to the phenolic oxygen), 144.8 (*p*-Ph), 121.6 (*m*-Ph), 120.3 (*o*-Ph), 105.7 (C-1'), 103.6 (C-1), 81.1 (C-4), 78.2 (C-5'), 77.8 (C-5), 75.4 (C-3'), 75.1 (C-3), 73.8 (C-2'), 71.4 (C-4'), 63.9 (C-6'), 62.8 (C-6), 57.9 (C-2), and 25.0 (CH_3).

Anal. Calcd for $C_{20}H_{30}O_{11}N_2$: C, 50.63; H, 6.37; N, 5.90. Found C, 50.60; H, 6.28; N, 5.78.

***p*-Acryloylaminophenyl 2-acetamido-2-deoxy-4-*O*-β-D-galacto-pyranosyl-β-D-glucopyranoside (*p*-acryloylaminophenyl=β-LacNAc, 3a).** Hydrogenation of *p*NP=β-LacNAc (**1a**, 1.5 g, 3 mmol) in a mixture of methanol (75 mL) and water (75 mL) was carried out in the presence of palladium on carbon (270 mg) in a hydrogen atmosphere for 8.5 h. The palladium on carbon was removed by filtration and the filtrate was concentrated under reduced pressure. The residue (**2a**) was dissolved in 50 mL of water and a trace of 2,6-di-*tert*-butyl-4-methylphenol was added as radical inhibitor. The flask was cooled at 5 °C; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.58 g, 3.0 mmol) and then acrylic acid (0.22 mL, 3.2 mmol) were added dropwise. The solution was stirred in a cooling room at 5 °C for 24 h. TLC (ethanol) R_f = 0.48. Water was removed by freeze-drying and the product was purified by silica gel chromatography (eluent, ethanol): yield 1.1 g (69 %); mp 227-228 °C; $[\alpha]_D^{25}$ 7.0 ° (*c*, 0.10 water/ ethanol 1 : 1 in volume).

^{1}H NMR (D_2O, 50 °C, ref. CH_3OH δ 3.34) δ 7.44 (d, 2H, *J* = 9.2 Hz, *m*-Ph), 7.08 (d, 2H, *J* = 9.2 Hz, *o*-Ph), 6.41 (d x d, 1H, *J* = 17.2 and 9.9 Hz, =CH-), 6.29 (d x d, 1H, *J* = 17.2 and 1.8 Hz, (*Z*)-H-C=C-), 5.85 (d x d, 1H, *J* = 9.9 and 1.8 Hz, (*E*)-H-C=C-), 5.15 (d, 1H, *J* = 8.1 Hz, H-1), 4.50 (d, 1H, *J* = 7.4 Hz, H-1'), 4.22 (s, HO-), 4.0-3.5 (m, ring-H), and 2.01 (3H, s, CH_3). ^{13}C NMR (D_2O, 50 °C, ref. CH_3OH δ 49.0) δ 174.6 (CH_3-C=O), 166.6 (=CHC=O), 154.0 (*ipso*-Ph attached to the phenolic oxygen), 132.2 (=CH-), 130.4 (CH_2=), 128.1 (*p*-Ph), 123.3 (*m*-Ph), 117.3 (*o*-Ph), 102.9 (C-1'), 99.7 (C-1), 78.5 (C-4), 75.3 (C-5'), 74.9 (C-5), 72.6 (C-3'), 72.1 (C-3), 71.0 (C-2'), 68.6 (C-4'), 61.0 (C-6'), 60.1 (C-6), 55.0 (C-2), and 22.2 (CH_3).

***p*-Nitrophenyl 4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (*p*-NP=α-Lac, 1b).** Octa-*O*-actyllactoside (162 g, 240 mmol), *p*-nitrophenol (130 g, 930 mmol), and zinc chloride (1.5 g, 11 mmol) were heated in an oil bath at 140 °C under reduced pressure with magnetic stirring for 1 h. The mixture was dissolved in 500 mL of benzene and washed with 330 mL of 1M sodium hydroxide several times until the yellow color disappeared. The solution was washed with 30 mL of water twice, dried with calcium chloride, and concentrated on a rotary evaporator to give a white powdery product (110 g, 60 %); TLC (ethyl acetate/hexane 2/1) R_f = 0.58. The powder was dissolved in 970 mL of methanol and the solution was treated with 0.2 N sodium methoxide (30 mL) at 60 °C for 5 min. The solution was concentrated on a rotary

evaporator, and the product was purified by crystallization from methanol; yield 29 g (45 %).

***p*-Acryloylaminophenyl 4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (*p*-acryloylaminophenyl=α-Lac, 3b).** Compound **1b** (5.0 g, 11 mmol) was dissolved in a mixture of water and methanol (1:1 in volume) (300 mL), to which was added palladium on carbon (0.5 g). The mixture was stirred in a hydrogen atmosphere at room temperature for 6.5 h. The catalyst was removed by filtration, and the filtrate was concentrated. The product was dissolved in water/dioxane (1 : 2 in volume, 120 mL) and cooled in an ice bath. A small amount of 2,6-di-*tert*-butyl-4-methylphenol, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.3 g, 12 mmol), and then acrylic acid (0.81 mL, 12 mmol) were added, and the solution was stirred for 24 h and concentrated in a rotary evaporator; TLC (ethanol) R_f = 0.54 (**1b**), 0.44 (**2b**), and 0.60 (**3b**). The product was purified by silica gel chromatography; yield 4.0 g (74 %); mp 170-172 °C; $[\alpha]_D^{25}$ 40.6 ° (*c*, 1.0 Me_2SO).

^1H NMR (D_2O, 50 °C, ref. acetone δ 2.10) δ 7.33 (d, 2H, *J* = 8.8 Hz, *m*-Ph), 7.06 (d, 2H, *J* = 8.8 Hz, *o*-Ph), 6.28 (d x d, 1H, *J* = 17.0 and 9.6 Hz, =CH-), 6.20 (d x d, 1H, *J* = 17.0 and 1.8 Hz, (*Z*)-H-C=C-), 5.74 (d x d, 1H, *J* = 9.6 and 1.8 Hz, (*E*)-H-C=C-), 5.48 (d, 1H, *J* = 3.9 Hz, H-1), 4.40 (s, HO-), and 4.4-3.5 (m, ring-H). ^{13}C NMR (Me_2SO-d_6, ref. TMS) δ 162.8 (=CH<u>C</u>=O), 152.8 (*ipso*-Ph), 133.5 (=<u>C</u>H-), 132.0 (<u>C</u>H_2=), 126.4 (*p*-Ph), 120.6 (*m*-Ph), 117.1 (*o*-Ph), 103.8 (C-1'), 97.6 (C-1), 80.2 (C-4), 75.5, 73.3 71.4, 70.6, 68.1 (C-4'), and 60.4 and 59.9 (C-6 and C-6').

***p*-Acryloylaminophenyl 2-acetamido-2-deoxy-β-D-gluco-pyranoside (*p*-acryloylaminophenyl=β-GlcNAc, 3c).** Compound **1c** (2.0 g, 5.8 mmol) was dissolved in water / methanol (1:1 in volume) (200 mL), to which was added palladium on carbon (360 mg). The mixture was stirred in a hydrogen atomosphere at room temperature for 8 h. The catalyst was removed by filtration, and the filtrate was concentrated. The product was dissolved in water (50 mL) and cooled in an ice bath. A small amount of 2,6-di-*tert*-butyl-4-methylphenol, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.1 g, 5.8 mmol), and then acrylic acid (0.40 mL, 5.8 mmol) were added, and the solution was stirred for 24 h and concentrated on a rotary evaporator. TLC (ethanol) R_f = 0.66 (**1c**), 0.47 (**2c**), and 0.65 (**3c**). The product was purified by silica gel chromatography; yield 1.5 g (72 %); mp 243-245 °C; $[\alpha]_D^{25}$ 9.0 ° (*c*, 1.0 Me_2SO).

^{1}H NMR (Me_2SO-d_6, ref. TMS) δ 7.82 (d, 1H, J = 8.9 Hz, CH_3CONH-), 7.58 (d, 2H, J = 8.9 Hz, *m*-Ph), 6.94 (d, 2H, J = 8.9 Hz, *o*-Ph), 6.42 (d x d, 1H, J = 17.0 and 9.9 Hz, =CH-), 6.23 (d x d, 1H, J = 17.0 and 2.1 Hz, (*Z*)-H-C=C-), 5.72 (d x d, 1H, J = 9.9 and 2.1 Hz, (*E*)-H-C=C-), 5.08 (2d, 2H, OH-3 and OH-4), 4.91 (d, 1H, J = 8.5 Hz, H-1), 4.62 (t, 1H, J = 5.7 Hz, OH-6), 3.37 (s, HOD), 3.8-3.2 (m, ring-H), 1.82 (s, 3H, CH_3), and 1.34 (s, 1H, CONHPh). ^{13}C NMR (Me_2SO-d_6) δ 169.3 (CH_3-C=O), 162.8 (=CHC=O), 153.6 (*ipso*-Ph), 133.5 (=CH-), 131.9 (CH_2=), 126.5 (*p*-Ph), 120.6 (*m*-Ph), 116.7 (*o*-Ph), 99.6 (C-1), 77.2 (C-5), 74.1 (C-3), 70.3 (C-4), 60.7 (C-6), 55.5 (C-2), and 23.1 (CH_3).

***p*-Acryloylaminophenyl α-D-glucopyranoside (*p*-acryloylaminophenyl=α-Glc, 3d).** A solution of **1d** (1.0 g, 3.3 mmol) in water/methanol (1:1 in volume) (100 mL) was stirred with palladium on carbon (180 mg) in a hydrogen atomosphere at room temperature for 7 h, the catalyst was removed by filtration, and the filtrate was concentrated. The product was dissolved in water/dioxane (1 : 2 in volume, 20 mL), a small amount of 2,6-di-*tert*-butyl-4-methylphenol was added and the solution was cooled in an ice bath; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.77 g, 4.0 mmol) and then acrylic acid (0.46 mL, 6.7 mmol) were added; the solution was stirred for 48 h and concentrated on a rotary evaporator; the product was purified by silica gel chromatography; yield 0.83 g (78 %); mp 170-172 °C; $[\alpha]_D^{25}$ +149.9 ° (*c* 1.0, water).

^{1}H NMR (D_2O, 80 °C, ref. acetone δ 2.10) δ 7.34 (d, 2H, J = 8.8 Hz, *m*-Ph), 7.07 (d, 2H, J = 8.8 Hz, *o*-Ph), 6.30 (d x d, 1H, J = 17.2 and 9.9 Hz, =CH-), 6.19 (d x d, 1H, J = 17.2 and 1.8 Hz, (*Z*)-H-C=C-), 5.74 (d x d, 1H, J = 9.9 and 1.8 Hz, (*E*)-H-C=C-), 5.48 (d, 1H, J = 3.5 Hz, H-1), 4.13 (s, HO-), and 3.9-3.4 (m, ring-H). ^{13}C NMR (D_2O, 60 °C, ref. CH_3CH_2OH δ 57.0) δ 166.3 (CH_3-C=O), 153.1 (*ipso*-Ph), 131.3 (=CH-), 129.8 (CH_2=), 127.8 (*p*-Ph), 123.0 (*m*-Ph), 117.3 (*o*-Ph), 96.9 (C-1), 72.6 (C-3), 72.1 (C-2), 70.7 (C-5), 68.9 (C-4), and 59.8 (C-6).

Polymerization. Azobisisobutyronitrile (AIBN) was purified by recrystallization from ethanol. Me_2SO was distilled under reduced pressure. Prescribed amounts of monomer and initiator were dissolved in Me_2SO in a polymerization ampule. The solution was frozen and degassed three times under reduced pressure by placing the ampule in a solid carbon dioxide-methanol bath. The ampule was sealed under reduced pressure and kept in a thermostat at 60 °C. The polymerization was terminated by pouring the solution

into an excess amount of cold methanol. After centrifugation, polymeric product was reprecipitated from its Me_2SO solution into methanol three times and dialyzed in a cellulose tube against water. A white powdery polymer was isolated by freeze-drying from an aqueous solution.

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