ELSEVIER

Carbohydrate Research 305 (1998) 443–461

CARBOHYDRATE RESEARCH

High performance polymer supports for enzyme-assisted synthesis of glycoconjugates [1]

Kuriko Yamada, Eriko Fujita, Shin-Ichiro Nishimura *

Laboratory for Bio-Macromolecular Chemistry, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan

Received 17 June 1997; accepted 14 October 1997

Abstract

Efficient and practical methodology for the construction of carbohydrates, including oligosaccharide derivatives and sphingoglycolipids, was established on the basis of a water-soluble polymer supports having unique linkers that can be cleaved by specific conditions. Novel glycomonomers for the construction of polymer supports were synthesized and copolymerized with acrylamide to give three types of water-soluble glycopolymers having primer sugars through the specific linkers containing (i) *p*-substituted benzyl group, (ii) L-phenylalanine residue, and (iii) ceramide-mimetic L-serine derivative, respectively. These glycopolymers were employed for sugar elongation reactions with glycosyl transferases such as GlcNAc β1,4-galactosyl transferase, βGal1 $\rightarrow$ 3/4GlcNAc α-2,6-sialyl transferase, and βGal1 $\rightarrow$ 3/4GlcNAc α-2,3-sialyl transferase in the presence of each sugar nucleotide as glycosyl donor to afford polymers having *N*-acetyllactosamine, sialyl α-(2 $\rightarrow$ 6) *N*-acetyllactosamine, and sialyl α-(2 $\rightarrow$ 3) lactose residues in excellent yield. Subsequent hydrogenolysis, hydrolysis with α-chymotrypsin, or transglycosylation to ceramide with ceramide glycanase proceeds smoothly to give *N*-acetyllactosamine, a versatile sialyl α-(2 $\rightarrow$ 6) *N*-acetyllactosamine derivative having a terminal amino group, and ganglioside GM3 in high yield.

Keywords: Enzyme-assisted synthesis; Glycosyl transferases; Polymer supports; Water-soluble glycopolymers; Sugar primers; Specific linkers

1. Introduction

Easy, versatile, and practical methodology for the construction of glycoconjugates of higher structural complexity and in a combinatorial fashion is of particular interest to carbohydrate chemistry and glycotechnology. Although several methods for polymer-supported chemical synthesis of oligosaccharides

* Corresponding author. Laboratory for Bio-Macromolecular Chemistry, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Nishi-8, Kita-10, Kita-ku, Sapporo 060, Japan. Tel.: +81-11-706-3807; fax: +81-11-706-3435; e-mail: nishimura@indy.polymer.sci.hokudai.ac.jp.

[1] For preliminary accounts of this work, see Refs. [1–3].

PII S0008-6215(97)10051-9

have been reported [4–17], new technologies that advance this field are still very much in demand. On the other hand, enzyme-assisted strategy for the synthesis of oligosaccharides is recognized as one of the promising practical alternatives to chemical synthesis because of highly stereo- and regioselective reactions with no tedious protection/deprotection steps [18–20]. Glycosyltransferases have also become valuable reagents for the synthesis of carbohydrates due to increasing availability via recombinant DNA technology [21–23], however, no viable methodology with both satisfactory yield and ease of procedures has been reported for polymer-supported enzymatic synthesis of carbohydrates [24–28]. Therefore, the advent of new technologies that amplifies efficiency and versatility of enzymatic strategies is strongly required recently.

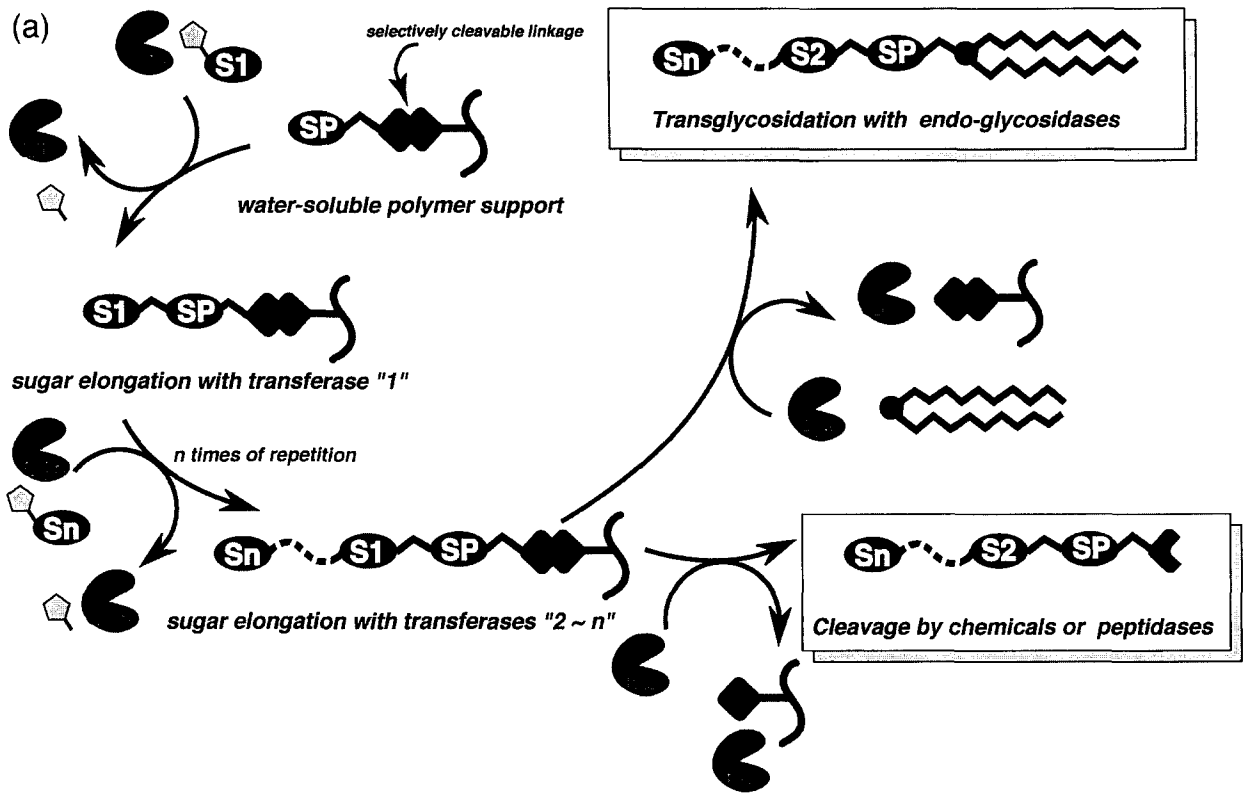
As part of an ongoing project on the synthesis of glycopolymers as convenient tools in glycobiology and glycotechnology [29], our attention has been focused on specific 'polymeric sugar-cluster effects' by these macromolecules on the interaction with proteins. It was clearly demonstrated that some plant lectins recognized specifically synthetic multivalent glycoconjugates with remarkably enhanced affinities ($K_a = 10^5 \sim 10^8$ M^{-1}). It was suggested that exponentially amplified affinities rather than those of the corresponding mono- or oligomeric sugar ligands at the same concentrations were observed in many cases. For example, it is now well accepted that some synthetic glycoconjugates having multivalent *N*-acetyl neuraminic acid (Neu5Ac) residues are novel class of potent inhibitors against infections of influenza viruses through the specific interactions with hemagglutinins (HA) [30–34]. The inhibition constants of glycopolymers having a variety of sialosides against viral infections are reported to be $10^{-6} \sim 10^{-10}$ M. Moreover, we also demonstrated that GlcNAc residues attached to a water-soluble polyacrylamide by a suitable spacer-arm allowed quantitative galactosylation with bovine galactosyl transferase, and partial sialylation of the Gal residue by *Trypanosoma cruzi trans*-sialidase [35]. The success of this enzymatic assembly of oligosaccharides on flexible water-soluble polymer supports is also critically dependent on the 'polymeric sugar-cluster effects' which promote the successful binding of the sugar residues with enzymes. In this paper, we describe a facile and versatile strategy for enzymatic synthesis of glycoconjugates using specially designed water-soluble polymer supports having specific linkers that can manifest controlled release of carbohydrates.

2. Results and discussion

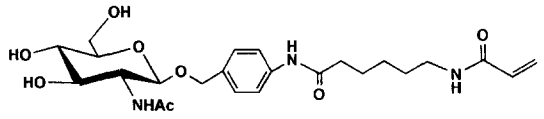
Fig. 1 illustrates the general concept of polymer-supported enzymatic synthesis (Fig. 1A) and chemical structures of three glycomonomers **1–3** designed in the present study (Fig. 1B). Water-soluble polyacrylamide copolymers, prepared by radical copolymerization of these glycomonomers with acrylamide as backbone structure were employed for sugar elongation reactions by some glycosyltransferases. Enzymatic extension of the carbohydrate chains on water-soluble polymer with flexible anchors has advantages of facilitating efficient separation of the product from the reactants, and permitting high reactivity expected of simple soluble molecules and high efficiency based on 'polymeric sugar-cluster effects'. It should also be noted that easy monitoring and characterization of each reaction step can be carried out by NMR measurements due to excellent solubility and homogeneity of glycopolymers. Since these glycomonomers contain unique linkers that can be selectively cleaved by some mild conditions such as hydrogenolysis, hydrolysis with proteases, and transglycosylations with carbohydrases, the present strategy will permit feasible and efficient procedures for the synthesis of a variety of carbohydrates.

Hydrogen-sensitive primer.—The synthetic route for the glycomonomers containing a *p*-substituted benzyl group as a hydrogen-sensitive linker is shown in Scheme 1. Firstly, *p*-nitrobenzyl glycoside **5** was prepared by simple coupling of an oxazoline derivative **4** with *p*-nitrobenzyl alcohol. Selective hydrogen transfer reaction and subsequent condensation with 6-acrylamido hexanoic acid **8** afforded the intermediate **9**. Finally, de-*O*-acetylation under the Zemplen condition gave the polymerizable glycoside **1**. Radical copolymerization of **1** with acrylamide proceeded smoothly in the presence of ammonium persulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) as promoters and yielded glycopolymers having GlcNAc branches **10** in high yield. To evaluate the importance of the flexible anchor between primer sugar and the polyacrylamide main chain, the water-soluble polymer **7** was also prepared from **6** in a similar manner.

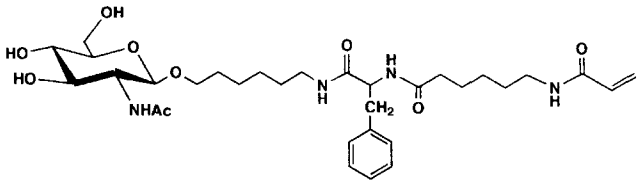
Enzymatic sugar elongation of primers **7** and **10** was examined with bovine milk galactosyl transferase according to the method reported previously [36–38]. As expected, the effect of the length of the spacer-arm moiety on the efficiency of the glycosylation was evident, and quantitative galactosylation of the primer **10** was achieved in the presence of only a



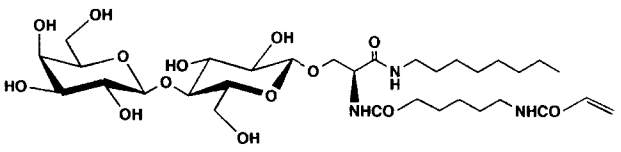
(b)



Monomer (**1**)



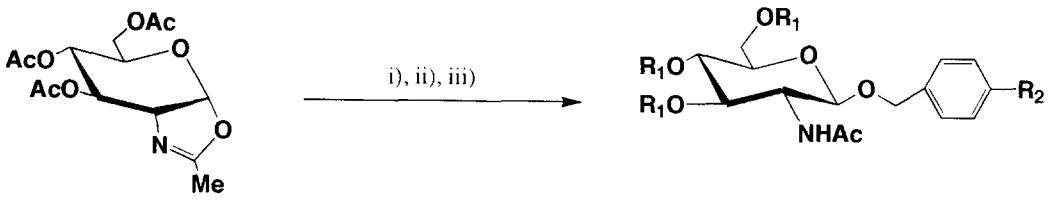
Monomer (**2**)



Monomer (**3**)

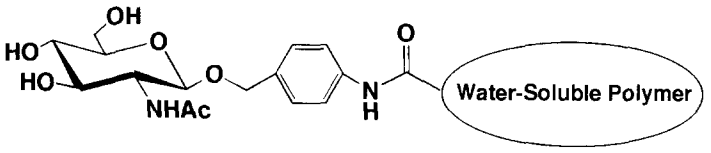
Fig. 1. (a) Concept of polymer-assisted enzymatic synthesis and (b) structures of glycomonomers used in this study.

small excess of uridine 5′-diphospho galactose (UDP-Gal) (1.2 mol/equiv. to acceptor GlcNAc residues). On the other hand, only 30% galactosylation was observed in the case of the short-armed primer **7** (Scheme 2). The ^{1}H NMR spectrum of **11** showed clearly that two equivalent integration of anomeric

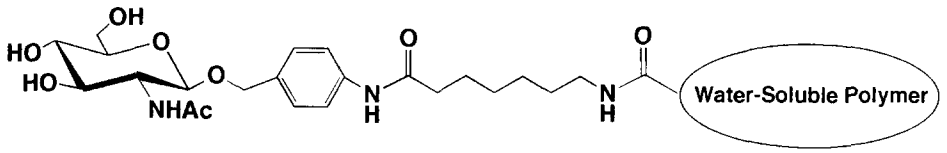


4

	R_1	R_2
5	Ac	NO_2
6	Ac	$NHCOCH{=}CH_2$
9	Ac	$CO(CH_2)_5NHCOCH{=}CH_2$
1	H	$CO(CH_2)_5NHCOCH{=}CH_2$



7



10

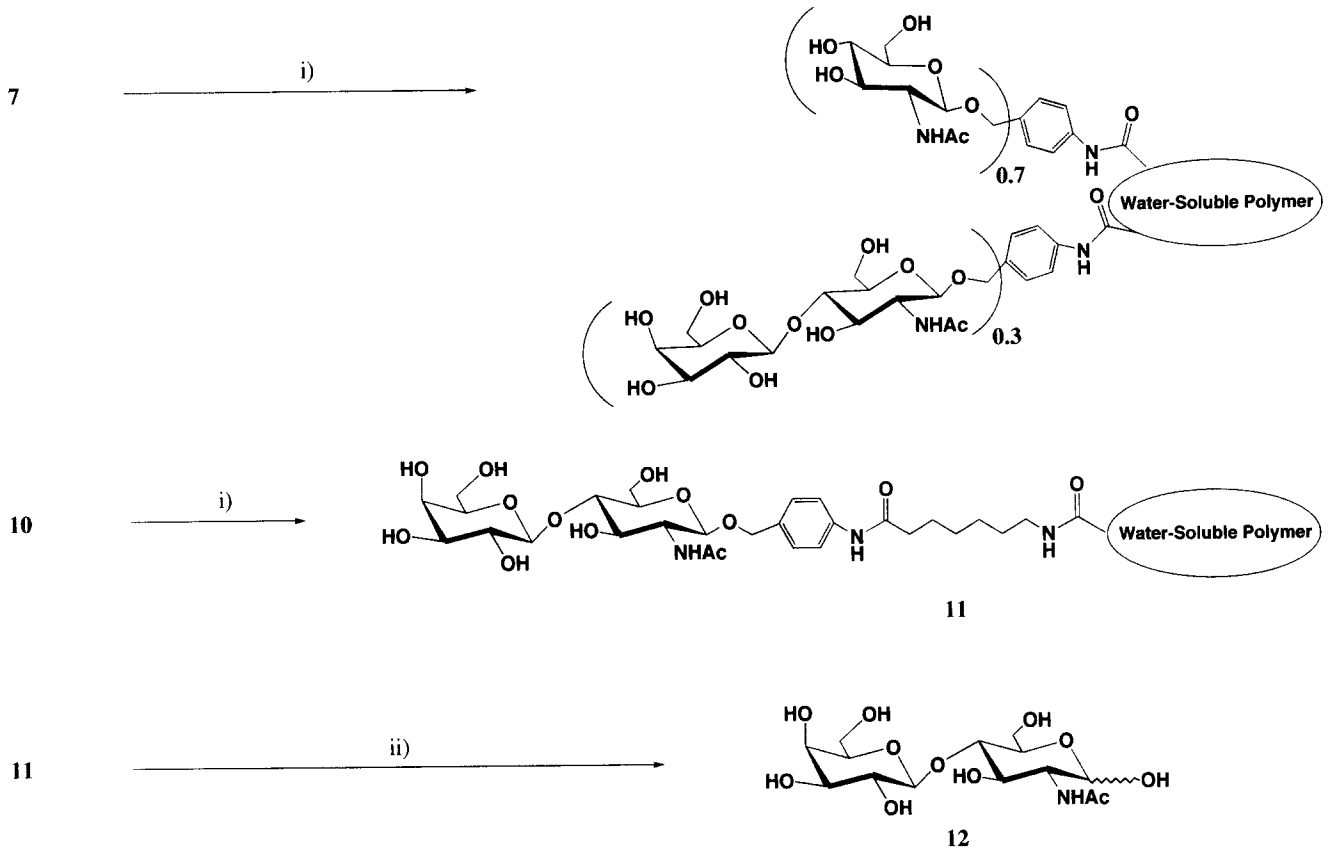
Scheme 1. (i) $HOCH_2C_6H_4NO_2$, CSA/dichloroethane, (ii) $HCOONH_4$, Pd/C, MeOH, then, (iii) $CH_2{=}CHCOCl$, Et_3N, MeOH for compound **6**, or $CH_2{=}CHCONH(CH_2)_5COOH$ (**8**), Et_3N, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl, CH_2Cl_2–DMF for compound **9**, (iv) NaOMe, MeOH.

protons at 4.45 and 4.50 ppm were observed concomitant with disappearance of a signal at 3.42 ppm attributable to the H-4 of an unsubstituted GlcNAc residue, suggesting a complete substitution of the GlcNAc residues with Gal residues. Hydrogenolysis in the presence of palladium on carbon generated a disaccharide product, *N*-acetyllactosamine, which was purified on Sephadex LH-20 with 95:5 ethanol–water as eluant with a high recovery (> 95%).

α-Chymotrypsin-sensitive primer.—As an extension of a novel strategy using water-soluble polymer supports, our interest was directed to the design of primers bearing linkers specifically cleaved by some peptidases. We selected and synthesized a polymer **20** containing a L-phenylalanine residue in the spacer-arm moiety as α-chymotrypsin-sensitive structure.

The synthetic route of the new primer GlcNAc-polymer having a peptidase-sensitive spacer moiety is shown in Scheme 3. The oxazoline derivative **4** was coupled with a readily preparable 1-amino-6-(*N*-benzyloxycarbonyl-L-phenylalanyl)-hexanol **13** to give **14** in moderate yield. Next, *N*-deprotection of the phenylalanine segment and subsequent condensation with 6-acrylamido hexanoic acid **8** afforded a polymerizable GlcNAc derivative **19**. Finally, usual de-*O*-acetylation of **19** (83%) and copolymerization of the sugar monomer **2** (93%) gave the primer polymer **20**. The primer **17** was also prepared to discuss the effect of anchor moiety between phenylalanine and polymer main chain on the accessibility of α-chymotrypsin.

Galactosylation with bovine milk galactosyltransferase and subsequent sialylation with rat liver α-2 → 6 sialyl transferase were carried out according to the conditions shown in Scheme 4. A typical elution pattern of gel filtration chromatography on Sephadex



Scheme 2. (i) UDP-Gal, Gal-T, HEPES buffer (pH 6.0), 37 °C, 24 h, (ii) H_2, Pd/C, water–MeOH.

G-25 after glycosylation reactions with galactosyl and sialyltransferases is shown in Fig. 2. As expected, high efficiency of the sugar-transfer reactions was achieved using only a small excess of UDP-Gal or cytidine 5′-monophospho-*N*-acetyl neuraminic acid (CMP–Neu5Ac) yielding the polymer **22** bearing sialyl α-(2 → 6)-LacNAc branches. As shown in the ^{1}H NMR spectrum of **22**, all protons were clearly assigned and quantitative transfers of Gal and Neu5Ac residues can also be estimated from the integration data of ring protons. Consequently, a useful sialyl α-(2 → 6)-LacNAc derivative **23** having terminal amino group at the reducing end was successfully released from the polymer chains by treating with α-chymotrypsin at 40 °C for 24 h in 72% overall yield from the primer polymer **20** (Fig. 3). It was preliminarily demonstrated that 6-aminohexyl glycoside of sialooligosaccharide can be utilized for conjugation with bovine serum albumin to give a novel type of neoglycoprotein [2]. On the contrary, although quantitative galactosylation was also observed in the case of the reaction of the primer **17**, this disaccharide product could not be released from the polymer support by hydrolytic action of α-chymotrypsin. This suggests the importance of a flexible spacer-arm between phenylalanine and the polymer backbone for rendering the releasing point accessible to the peptidase.

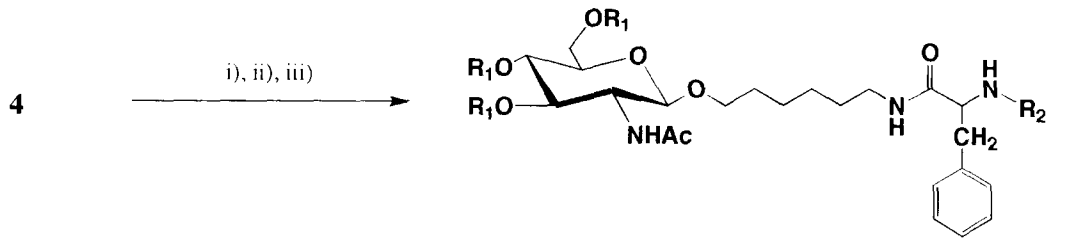
Relationship between sugar-distribution and transfer efficiency.—Efficiency of sugar elongation reaction by glycosyl transferases seems to be significantly dependent on the sugar density and its distribution on the primers. To understand and regulate the distribution and the density of primer sugars on polymer support, monomer reactivity ratio in copolymerization was determined according to the method by Fineman and Ross [39]. The copolymerization of two monomers M_1 (acrylamide) and M_2 (glycomonomer) is described schematically as follows:

$$M_1\cdot + M_1 \xrightarrow{k_{11}} M_1\cdot$$

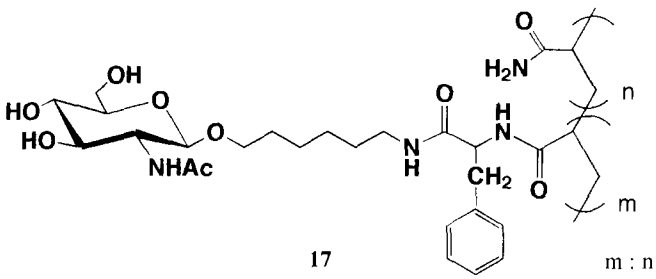
$$M_1\cdot + M_2 \xrightarrow{k_{12}} M_2\cdot$$

$$M_2\cdot + M_2 \xrightarrow{k_{22}} M_2\cdot$$

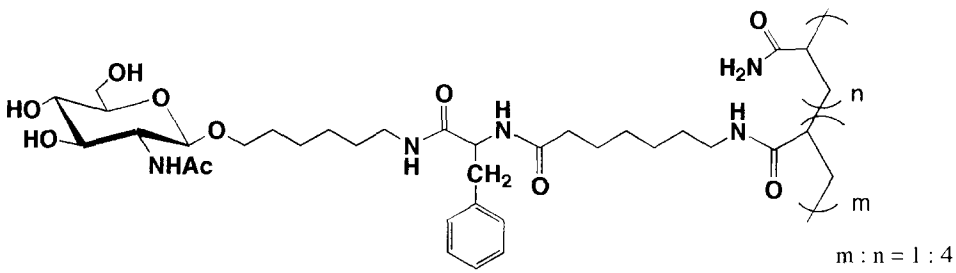
$$M_2\cdot + M_1 \xrightarrow{k_{21}} M_1\cdot$$



	R_1	R_2
14	Ac	$COOCH_2Ph$
15	Ac	$COCH{=}CH_2$
16	H	$COCH{=}CH_2$
19	Ac	$CO(CH_2)_5NHCOCH{=}CH_2$
2	H	$CO(CH_2)_5NHCOCH{=}CH_2$



17 m : n = 1 : 4



20 m : n = 1 : 4

Scheme 3. (i) Compound **13**, CSA/CH_2Cl_2, (ii) H_2, Pd/C, MeOH, then, $CH_2{=}CHCOCl$, Et_3N, THF for **15**, or compound **8**, EEDQ, benzene–EtOH for **19**, (iii) NaOMe/MeOH.

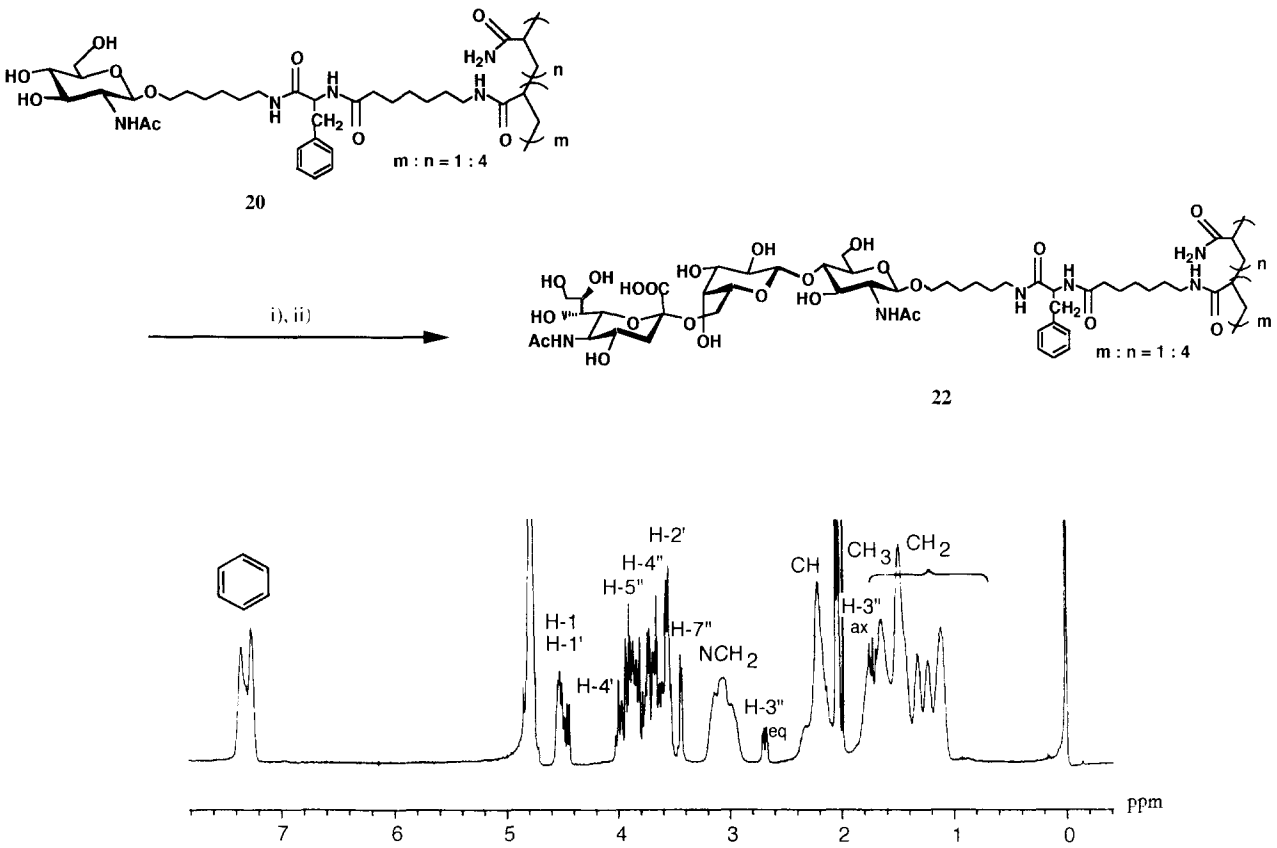
The monomer reactivity ratios are given by $r_1 = k_{11}/k_{12}$ and $r_2 = k_{22}/k_{21}$, and the copolymer composition equation which relates the polymer composition to the monomer composition is given by

$$d[M_1]/d[M_2] = [M_1]/[M_2]\{(r_1[M_1] + [M_2]) /([M_1] + r_2[M_2])\}, \quad (1)$$

where $[M_1]$ and $[M_2]$ refer to the monomer composition of M_1 and M_2 when the copolymerization started, and $d[M_1]$ and $d[M_2]$ to the compositions of M_1 and M_2 in the generated polymers at the initial stage ($< 5\%$ dp [2]) of chain propagation reaction, respectively. If $d[M_1]/d[M_2] = y$ and $[M_1]/[M_2] = x$, then Eq. (1) can be rewritten as

$$x/y(y-1) = r_1(x_2/y) - r_2 \quad (2)$$

By using y values estimated from the integration data of ^1H NMR spectra, a plot of x^2/y versus $x/y(y-1)$ is a straight line whose slope is r_1 and whose intercept is minus r_2 (Fig. 4). Consequently, monomer reactivity ratios were determined to be $r_1 = 0.62$ and $r_2 = 0.85$, suggesting that the glycomonomer shows similar reactivity to that of acryl-



Scheme 4. (i) UDP-Gal, Gal-T, HEPES buffer, (ii) CMP–Neu5Ac, α-2,6-sialyl-T, sodium cacodylate buffer (pH 7.40), 37 °C, 48 h.

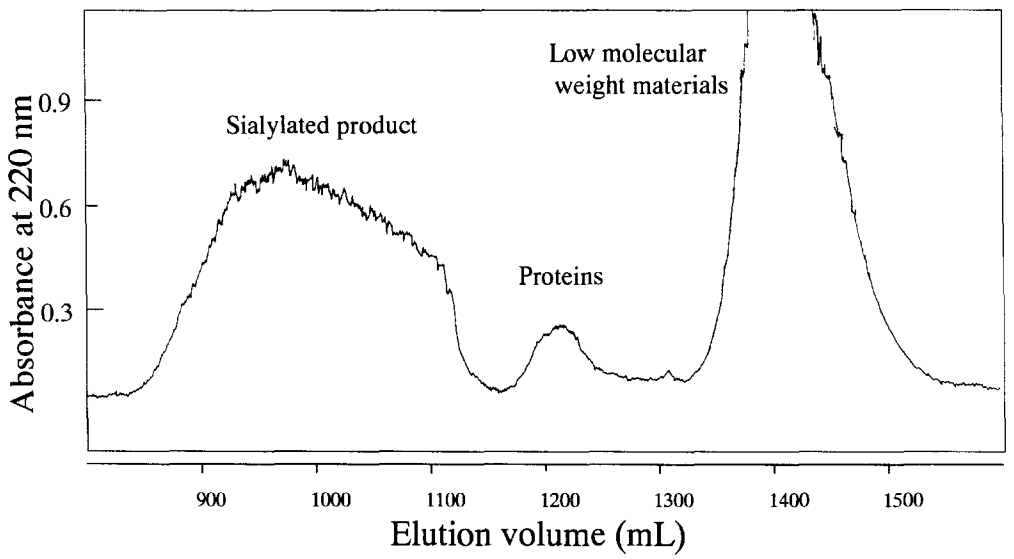


Fig. 2. A typical elution pattern of gel filtration chromatography on Sephadex G-25 column of enzymatically sialylated product **22**.

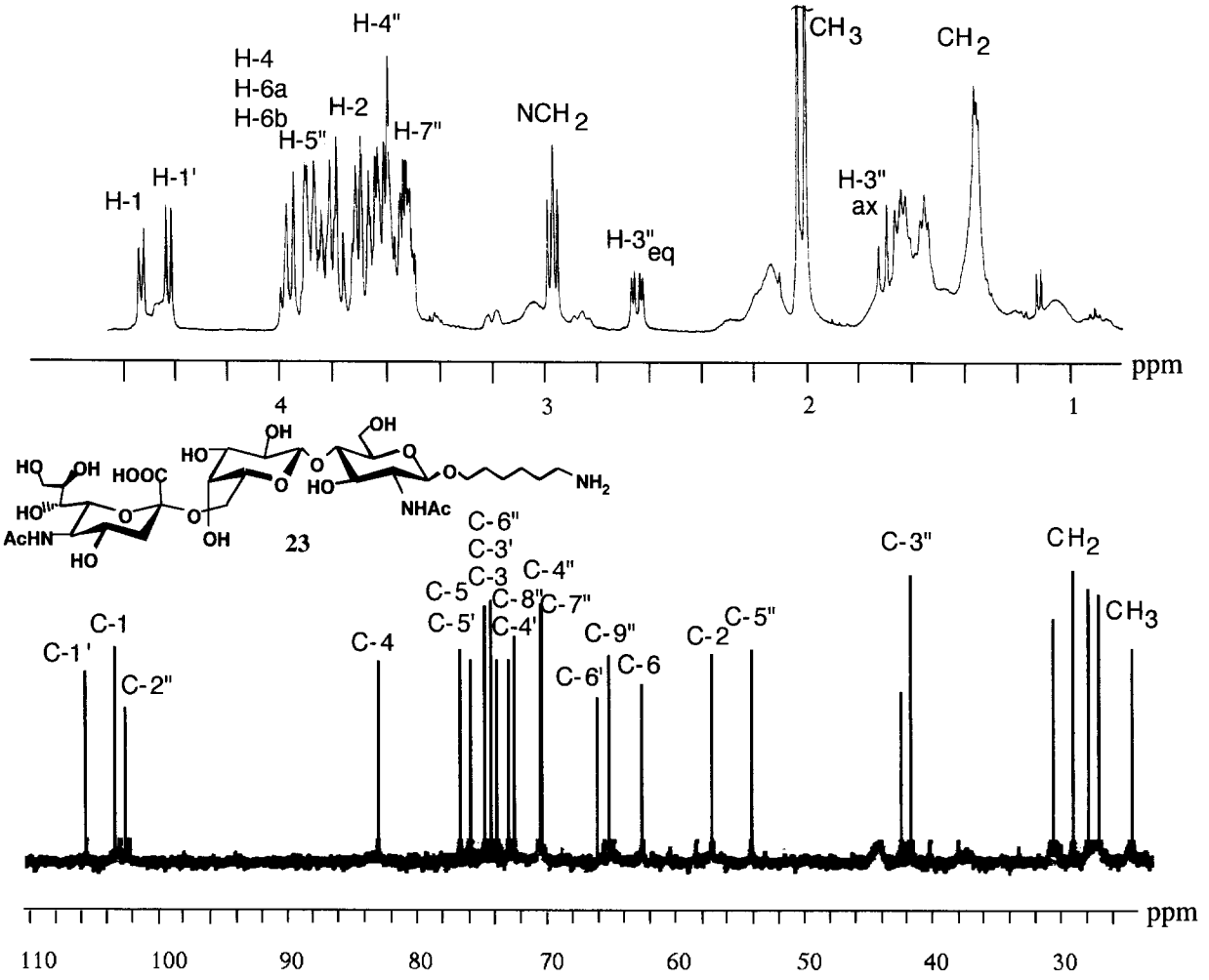


Fig. 3. ^{1}H and ^{13}C NMR spectra of compound **23** released from polymer support in deuterium oxide at room temperature.

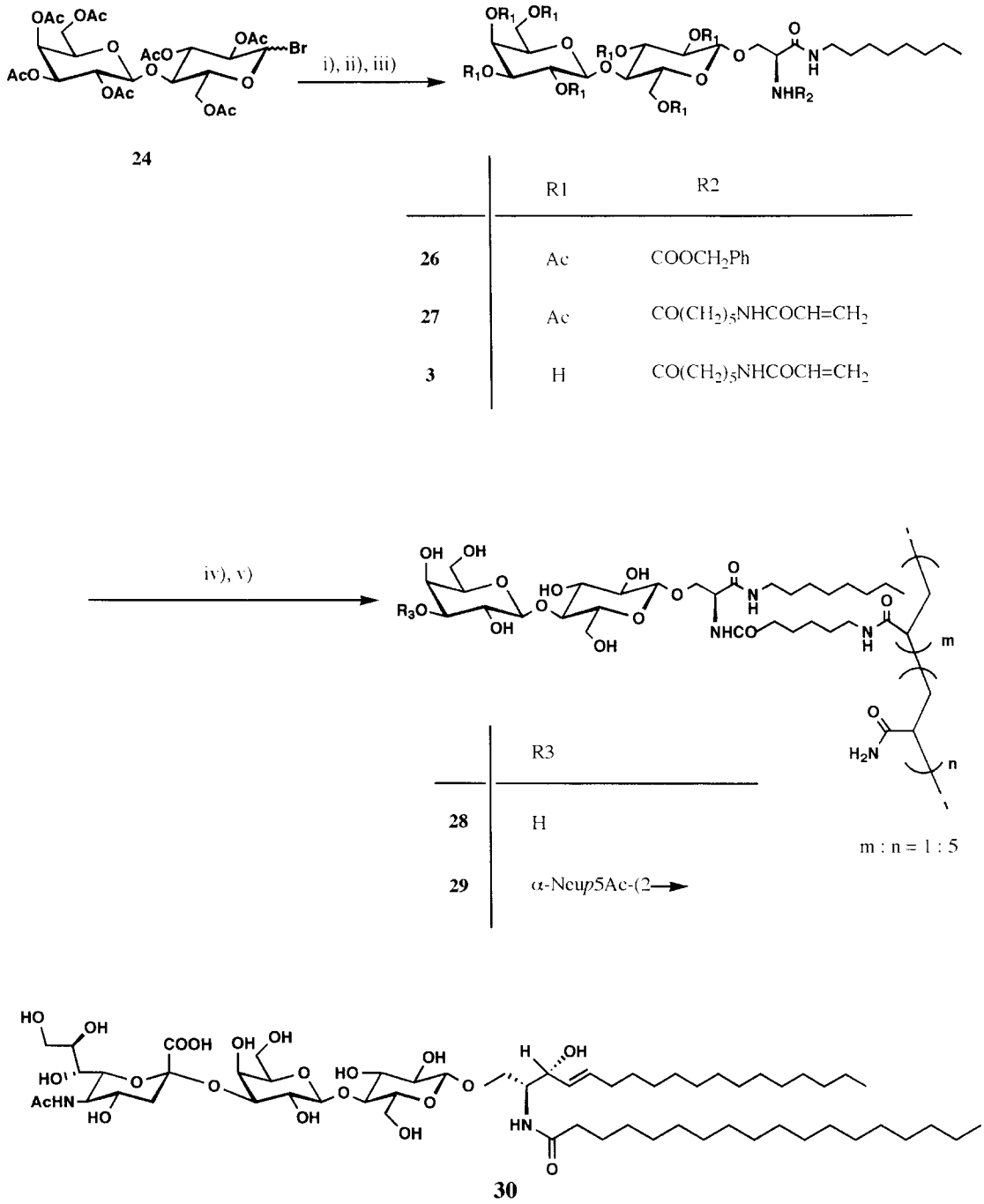
amide and primer sugar residues distributed quite randomly in the polymer supports [40]. In addition, it was also suggested that the polymer composition (y) can be predicted and controlled by the composition of monomers (x) as shown in Fig. 5 and

$$y = 0.728x \log_{10} x + 4.798 \quad (3)$$

was obtained by curve fitting with the least-squares method, where x refers to the ratio of the composition of acrylamide and glycomonomer (acrylamide:glycomonomer) and y refers to the ratio of the composition of acrylamide unit and sugar unit in the polyacrylamide copolymers.

Next, several polymer supports having different sugar densities (1:4 ~ 1:46 sugar–acrylamide) were prepared on the basis of Eq. (3) and used for the enzymatic galactosylation reaction to optimize 'polymeric sugar-cluster effect' on the interaction of glycosyl transferases with water-soluble glycopolymers as acceptor substrates. The results, obtained from ^{1}H NMR spectra, were in good agreement with those estimated from the sugar analysis of the products by DIONEX-PAD method [41] as shown in Fig. 6. It was clearly suggested that primers with a sugar ratio (sugar acrylamide) from 1:4 to 1:10 exhibited excellent property as substrates of galactosyl transferase and their yields were almost quantitative. However, the efficiency of enzymatic actions were apparently reduced by decrease of the ratio of sugar composition on the polymer supports. Interestingly, the polymer having 1:46 of low sugar density showed only 40% of galactosylation reaction. Therefore, it was concluded that primer sugars on the polymer supports should be distributed randomly and clustered with

[2] The degrees of polymerization at 0, 5, 10, 20, 30, and 60 min were estimated by the integration of the signals due to C=C double bond on ^{1}H NMR spectra. Since 3 ~ 5% of polymerization was observed from 10 to 20 min after the initiation reaction, we employed the data at 10 min to determine the values of $d[M_1]$ and $d[M_2]$ at the initial stage.



Scheme 5. (i) Compound **25**, AgOTf, CH_2Cl_2, −20 °C, (ii) H_2, Pd–C, MeOH, r.t., 24 h, then, compound **8**, EEDQ, EtOH–benzene, (iii) NaOMe, MeOH, (iv) acrylamide, TEMED, APS, Me_2SO–water, (v) CMP–NeuAc, α-2 → 3-sialyl-T, sodium cacodylate buffer (pH 7.49), 37 °C, 3 days, then, ceramide, ceramide glycanase, Triton CF-54, 50 mM sodium citrate buffer (pH 6.0), 37 °C, 17 h.

relatively high density (1:4 ~ 1:10 sugar–acrylamide) to achieve satisfactory yields. It should also be noted that the polymers having different 'coiled' conformation may influence the efficiency of enzymatic glycosylations.

(C) Lactosyl ceramide mimetic primer.—In order to extend the polymer-supported enzymatic strategy to the synthesis of sphingoglycolipids, the amphiphilic primer **28** was also designed and prepared as indicated in Scheme 5. The described strategy combines: (i) synthesis and utilization of a new lactosyl ceramide-mimetic monomer **3** for polymer-supported synthesis, (ii) high efficiency in glycosylation based on 'polymeric glycoside-cluster effects' and

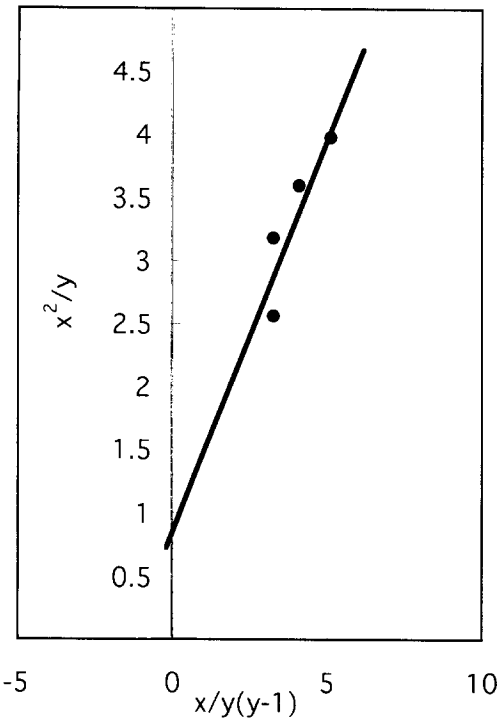


Fig. 4. Plot of x^2/y vs. $x/y(y-1)$ for acrylamide (M_1) glycomonomer (M_2) copolymerization with least squares straight line.

easy purification of the products as suggested in the cases of both hydrogen-sensitive and α-chymotrypsin-sensitive primers, and (iii) versatile and practical procedure for the transfer of carbohydrates from polymer to ceramides by unique transglycosylation activity of ceramide glycanase found by Zhou et al. [42], Li et al. [43] and Li and Li [44].

Ganglioside GM3 (**30**) was selected as a target for evaluation of the present synthetic methodology in comparison with chemical syntheses of this compound [45–49]. Although this trisaccharide sequence

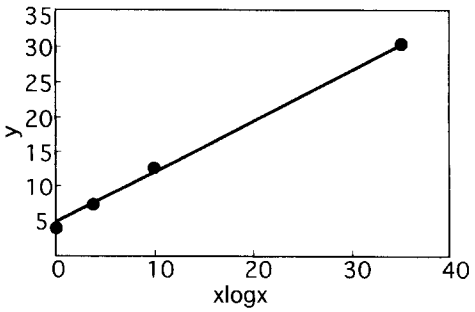


Fig. 5. Plot of y vs. $x\log_{10}x$; x and y refer to the ratio of the composition of acrylamide and glycomonomer [acrylamide (mol)/glycomonomer (mol)] and polymer composition [acrylamide unit (mol)/carbohydrate unit (mol)].

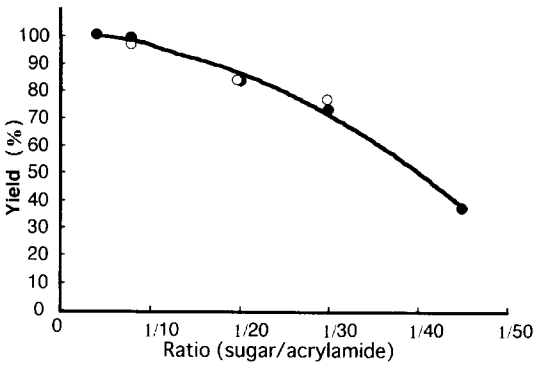


Fig. 6. Relationship between enzymatic galactosylation and the ratio of polymer composition (sugar unit:acrylamide unit). ○; NMR method, ●; DIONEX-PAD method.

[α-NeuAc-(2 → 3)-β-D-Gal (1 → 4)-β-D-Glc] was synthesized previously from methyl lactoside and CMP–NeuAc [50] by use of rat liver βGal1 → 3/4GlcNAc α-2 → 3-sialyltransferase [51] in modest yield (39%), the advent of new technologies that amplifies efficiency of enzymatic strategies is still very much in demand.

For the purpose of the present technology, the polymerizable lactose derivative **3** was designed to allow simple preparation of water-soluble polyacrylamide having lactose residues through a ceramide mimetic linker derived from a readily available L-serine derivative **25** (Scheme 5). The lactosyl ceramide (LacCer) mimetic glycopolymer **28** was obtained in 92% yield by radical copolymerization of the monomeric precursor **3** with acrylamide in deoxygenated water according to the procedure as described above. This primer support was employed for sialylation reaction using porcine liver βGal1 → 3/4GlcNAc α-2 → 3-sialyltransferase in the presence of CMP–NeuAc according to the previous report [51]. The product **29**, obtained in quantitative yield, was conveniently isolated by a simple column of Sephadex G-25 gel, which can be monitored by NMR measurements. Finally, treatment of **29** with leech ceramide glycanase in the presence of an excess of ceramide as an acceptor and subsequent chromatography by a column of Sephadex LH-20 gel gave ganglioside GM3 (**30**) in 63% yield.

The polymer-assisted enzymatic process described here afforded GM3 in three steps with 58% overall yield from a readily available precursor **3**, a remarkable improvement in both ease of synthesis and overall yield compared to those of chemical and/or enzymatic synthesis reported previously. The versatility of this synthetic method in the synthesis of longer and

more complicated sphingoglycolipids are under investigation and the results will be reported in elsewhere shortly.

3. Conclusion

A versatile and efficient technology for the synthesis of carbohydrates was established on the basis of newly prepared polymer-supports having specific linkers. It was clearly demonstrated that these high performance polymers facilitate enzymatic synthesis of oligosaccharides and sphingoglycolipids. The success of the present strategy is significantly dependent on the excellent water-solubility and the multivalency of clustered glycopolymers that provide enzymatic synthesis with high efficiency of the glycosylations and easy separations of the products. It should also be noted that each step for enzymatic sugar-elongations of water-soluble polymers can be clearly characterized by NMR spectroscopy. Since large-scale preparation of both recombinant glycosyltransferases and sugar nucleotides are now possible [21–23], the present methodology using water-soluble polymer-supports should be widely applicable both for the synthesis of various naturally occurring glycoconjugates and combinatorial synthesis of carbohydrate libraries.

4. Experimental

General methods.—Unless otherwise stated, all commercially available solvents and reagents were used without further purification. $CHCl_3$, CH_2Cl_2, dichloroethane, Me_2SO, tetrahydrofuran, and MeOH were stored over molecular sieves 3 Å before use. Et_3N and pyridine were stored over NaOH pellets. MS 4 Å was dried under reduced pressure at ca. 100 °C overnight before use. Melting points (uncorrected) were determined with a Fisher-Johns apparatus. Optical rotations were determined with a Perkin Elmer 343 digital polarimeter at 23 °C. ^1H and ^{13}C NMR spectra were recorded with JEOL EX-400 or α-400 spectrometer at 400 MHz (^1H NMR) and 100 MHz (^{13}C NMR) in $CHCl_3$-*d*, MeOH-d_3, Me_2SO-d_6 or deuterium oxide using Me_4Si or TSP-d_4 as internal standard. Assignment of the ring-protons was made by first-order analysis of the spectra, and was confirmed by H–H COSY spectra. Samples were dried (ca. 24 h) in vacuo (50 °C, 0.1 Torr) over P_5O_2 powder before elemental analyses. Chemical reactions were monitored by thin-layer chromatography (TLC) on precoated plates of Silica Gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt). Column chromatography was performed on silica gel (Silica gel 60; 0.015–0.040 mm, E. Merck). 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. Enzymatic reactions were also performed using commercially available materials. Galactosyl transferase from bovine milk (Sigma), sialyl transferase from rat liver (Sigma), sialyl transferase from porcine liver (Sigma), [3] and α-chymotrypsin from bovine pancreas (Wako pure chemicals) were used without further purification. Sugar compositions of glycopolymers were determined from both the integration data of ^1H NMR spectra and high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) analysis according to the method described previously [41]. Acid hydrolysis of glycopolymers (1 mg of each polymer) was carried out under 2 M CF_3COOH at 100 °C for 2 h. After the hydrolysis reaction, the solution was filtered to remove the main polymer, and the filtrate was successively subjected to the HPAEC-PAD analysis. HPAEC was carried out using Dionex Bio LC (Sunnyvale, CA) equipped with a carbopack PA-1 column and a pulsed amperometric detector (PAD-II). The quantitative analysis was carried out using a standard solution of monosaccharides of 50 mM solution, respectively.

p-*Nitrobenzyl 3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside* (**2**).—To a soln of **4** (1.4 g, 4.25 mmol) in dichloroethane (20 mL) was added *p*-nitrobenzyl alcohol (5.2 g, 21.0 mmol) and D-camphor-10-sulfonic acid (110 mg, 0.47 mmol). The mixture was stirred at 80 °C for 2 h, then Et_3N (2.0 mL, 14.33 mmol) was added. The soln was concentrated under reduced pressure to give a syrupy crude product. The residual syrup was directly chromatographed on a silica gel column and eluted with 200:40:5 $CHCl_3$–EtOAc–MeOH to give the glycoside **5** (1.82 g, 89%): $[\alpha]_D^{23} \sim 0°$ (*c* 0.10, $CHCl_3$); ^1H NMR ($CHCl_3$) δ 8.19 (d, 2 H, –Ph–NO_2 *m*), 7.47 (d, 2 H, –Ph–NO_2 *o*), 5.57 (d, 1 H, NH, Glc), 5.26 (t, 1 H, H-3), 5.11 (t, 1 H, H-4), 5.01 (d, 1 H, –CH_2–Ph),

[3] Although some of the sugar nucleotides are still not available, recombinant glycosyl transferases including sialyl transferases and glycosyl donors are now obtainable from Calbiochem®.

4.77 (d, 1 H, H-1), 4.70 (d, 1 H, $-CH_2-$Ph), 4.27 (dd, 1 H, H-6_a), 4.17 (dd, 1 H, H-6_b), 4.02 (q, 1 H, H-2), 3.73 (ddd, 1 H, H-5), and 2.10, 2.05, 2.04, 1.96 (each s, 12 H, 4 Ac). Anal. Calcd. for $C_{21}H_{26}O_{11}N_2$: C, 52.28; H, 5.43; N, 5.81. Found: C, 52.26; H, 5.48; N, 5.81.

p-*Acrylamidobenzyl 3,4,6-tri*-O-*acetyl-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**6**).—To a soln of **2** (655.3 mg, 1.36 mmol) in MeOH (28 mL) was added ammonium formate (1.71 g, 27.2 mmol) and 10% Pd–C (142 mg). The mixture was stirred at room temperature for 5 min, the mixture was filtered with celite and the filtrate was evaporated under reduced pressure. The residual syrup was dissolved in $CHCl_3$, washed with water and dried over Na_2SO_4. The soln was filtered and evaporated to give *p*-aminobenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside. This intermediate was subsequently used for the next step without further purification.

To the soln of amino component (615 mg, 1.36 mmol) in MeOH (20 mL) was added Et_3N (0.19 mL, 1.36 mmol) and a soln of acryloyl chloride (137.9 μL, 1.63 mmol) in THF (5 mL) at 0 °C, dropwisely. The mixture was stirred for 2 h. The soln was poured into ice-water and extracted with $CHCl_3$. The organic layer was washed with brine, dried, and evaporated to give a quantitative yield of *p*-acrylamidobenzyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside **6** (688 mg, ~ 100%): ^{1}H NMR ($CDCl_3$) δ 9.54 (s, 1 H, NH_{Ph}), 7.71 (d, 2 H, –Ph-*m*), 7.19 (d, 2 H, –Ph-*o*), 6.76 (d, 1 H, NH, Glc), 6.64 (dd, 1 H, C*H*=CH_2), 6.41 (dd, 1 H, CH=CH_{2trans}), 5.70 (dd, 1 H, CH=CH_{2cis}), 5.28 (t, 1 H, H-3), 5.05 (t, 1 H, H-4), 4.80 (d, 1 H, $-CH_2-$Ph), 4.74 (d, 1 H, H-1), 4.52 (d, 1 H, $-CH_2-$Ph), 4.27 (dd, 1 H, H-6_a), 4.13 (dd, 1 H, H-6_b), 3.98 (q, 1 H, H-2), 3.65 (ddd, 1 H, H-5), 2.10, 2.02, 2.01, 1.90 (each s, 12 H, 4 Ac).

Polyacrylamide copolymer **7** *from glycomonomer* **6**.—To a soln of **6** (23.2 mg, 45.8 μmol) in MeOH (5 mL) was added NaOMe (2.2 mg, 40.72 μmol). The mixture was stirred at room temperature for 5 h. It was made neutral with Dowex 50W-X8 (H+) resin, filtered, and evaporated to give a quantitative yield of *p*-acrylamidobenzyl 2-acetamido-2-deoxy-β-D-glucopyranoside. This deprotected glycomonomer was subsequently used for the next step without further purification.

A soln of glycomonomer (17.42 mg, 45.8 μmol) and acrylamide monomer (32.55 mg, 458 μmol) in 3:1 Me_2SO–water (1 mL) was deaerated by a water aspirator for 20 min, to which were added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) (1.38 μL, 9.06 μmol) and ammonium peroxodisulfate (APS) (0.84 mg, 3.66 μmol). The mixture was stirred at room temperature for 24 h, diluted with deionized water (1 mL), directly subjected to gel filtration chromatography on Sephadex G-50 column and eluted with 50 mM aq CH_3COONH_4. The void fractions were collected and concentrated to 10 mL, and the syrupy soln was lyophilized to afford polymer **7** as an amorphous powder (30 mg, 61%): ^{1}H NMR (deuterium oxide) δ 7.37 (br d, 4 H, –Ph–), 4.70 (br d, 2 H, OCH_2Ph), 4.48 (d, 1 H, *J* 8 Hz, H-1), 3.89 (d, 1 H, *J* 12 Hz, H-6_b), 3.70 (br d, 1 H, *J* 14 Hz, H-6_a), 3.64 (t, 1 H, *J* 10 Hz, H-3), 3.53 (m, 1 H, H-2), 3.43 (br d, 2 H, H-4 and H-5), 2.3–2.1 (m, 10.7 H, –CH–), 1.90 (s, 3 H, $COCH_3$), and 1.7–1.4 (m, 26.9 H, $-CH_2-$).

6-N-*Acrylamido hexanoic acid* (**8**).—To a soln of 6-amino-hexanoic acid (5.0 g, 38.1 mmol) in 1.27 M aq NaOH (30 mL) was added a soln of acryloyl chloride (3.87 mL, 45.7 mmol) in THF (10 mL) at 0 °C for 2 h. The reaction mixture was adjusted to be pH 3 by addition of 1 N aq HCl. The mixture was poured into ice-water and extracted with EtOAc. The organic layer was washed with water, dried, and evaporated. The crude material was then recrystallized from EtOAc–hexane to afford pure **8** (4.8 g, 68%): mp 65 ~ 66 °C; ^{1}H NMR (CD_3OD) δ 6.13 (dd, 1 H, C*H*=CH_2), 6.11 (dd, 1 H, CH=CH_{2trans}), 5.55 (dd, 1 H, CH=CH_{2cis}), 3.16 (t, 2 H, CH_2), 2.21 (t, 2 H, CH_2), 1.55, 1.47, 1.31 (each q, 6 H, 3 CH_2).

p-N-*[(N-Acrylamido)-pentylcarbonyl]-benzyl 3,4,6-tri*-O-*acetyl-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**9**).—Compound **2** (800 mg, 1.66 mmol) was hydrogenated according to the method described for the preparation of **6** to afford the intermediate. To the mixture of crude amino component (751 mg, 2.06 mmol) and **8** (307 mg, 1.66 mmol) in 10:1 dichloroethane–DMF (22.0 mL) was added Et_3N (0.231 mL, 1.66 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (325 mg, 1.66 mmol) at 0 °C. The mixture was stirred at room temperature for 22 h. The soln was diluted with $CHCl_3$ (30 mL), washed with 1 N aq H_2SO_4, saturated aq $NaHCO_3$, and brine. The soln was dried over Na_2SO_4, filtered, and the filtrate was evaporated. The residual syrup was directly chromatographed on a silica gel column and eluted with 30:1 $CHCl_3$–EtOH to give the glycoside **9** (520 mg, 51%): $[\alpha]_D^{23}$ −36.9°; ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 1 H, NH), 7.52 (d, 2 H, –Ph-*m*), 7.24 (d, 2 H, –Ph-*o*), 6.27 (dd, 1 H, C*H*=CH_2), 6.10 (dd, 1 H, CH=CH_{2trans}), 5.63 (dd, 1 H, CH=CH_{2cis}), 5.19 (t, 1 H, H-3), 5.08 (t, 1 H,

H-4), 4.84 (d, 1 H, $-CH_2-Ph$), 4.59 (d, 1 H, H-1), 4.56 (d, 1 H, $-CH_2-Ph$), 4.27 (dd, 1 H, H-6_a), 4.16 (dd, 1 H, H-6_b) 3.96 (q, 1 H, H-2), 3.64 (ddd, 1 H, H-5), 3.35 (q, 2 H, CH_2), 2.38 (t, 2 H, CH_2), 2.11, 2.05, 2.02, 1.91 (each s, 12 H, 4 Ac), 1.77, 1.58, 1.42 (each t, 6 H, 3 CH_2). Anal. Calcd. for $C_{30}H_{41}O_{11}N_3$: C, 58.15; H, 6.67; N, 6.78. Found: C, 57.80; H, 6.69; N, 6.50.

p-N-*[(N-Acrylamido)-pentylcarbonyl]-benzyl 2-acetamido-2-deoxy-β-*D-*glucopyranoside* (**1**).—To a soln of **9** (132 mg, 0.213 mmol) in MeOH (15 mL) was added NaOMe (10.0 mg, 0.181 mmol). The soln was stirred at room temperature for 15 h. The mixture was neutralized with Dowex 50W-X8 (H+). The filtrate was evaporated to afford the pure glycomonomer **1** as an amorphous powder (104 mg, 99%): $[\alpha]_D^{23}$ −3.3°; ^{1}H NMR (Me_2SO-d_6) δ 9.88 (s, 1 H, NH_{Ph}), 8.07 (t, 1 H, NH), 7.70 (d, 1 H, NH, Glc), 7.53 (d, 2 H, -Ph-*m*), 7.18 (d, 2 H, -Ph-*o*), 6.19 (dd, 1 H, C*H*=CH_2), 6.04 (dd, 1 H, CH=CH_{2trans}), 5.54 (dd, 1 H, CH=CH_{2cis}), 5.07 (br s, 1 H, OH), 4.99 (br s, 1 H, OH), 4.69 (d, 1 H, $-CH_2-Ph$), 4.58 (br s, 1 H, OH), 4.44 (d, 1 H, $-CH_2-Ph$), 4.31 (d, 1 H, H-1), 3.70 (ddd, 1 H, H-5), 3.45 (dd, 2 H, H-3 and H-4), 3.39 (s, 6.6 H, 2.2 × CH_3OH), 3.33 (m, 1 H, H-6_a), 3.13–3.06 (m, 4 H, CH_2, H-6_b and H-2), 2.28 (t, 2 H, CH_2), 1.79 (s, 3 H, NAc), 1.57, 1.43, 1.30 (each dt, 6 H, 3 CH_2). Anal. Calcd. for $C_{24}H_{35}O_8N_3 \cdot 2.2MeOH$: C, 55.79; H, 7.83; N, 7.45. Found: C, 55.54; H, 7.25; N, 7.61.

Polyacrylamide copolymer **10** from the glycomonomer **1**.—A soln of **1** (62.0 mg, 125.6 μmol) and acrylamide monomer (44.6 mg, 628 μmol) in 3:1 Me_2SO–water (1 mL) was deaerated by a water aspirator for 20 min, to which were added TEMED (7.52 μL, 50.2 μmol) and APS (4.6 mg, 20.1 μmol). The mixture was stirred at room temperature for 24 h, diluted with deionized water (2 mL), directly subjected to gel filtration chromatography on Sephadex G-50 column and eluted with 50 mM aq CH_3COONH_4. The void fractions were collected and concentrated to 10 mL, and the syrupy soln was lyophilized, and substituted with deionized water twice, to afford polymer **10** as an amorphous powder (89 mg, 83%): ^{1}H NMR (D_2O) δ 7.36 (br d, 4 H, –Ph–), 4.60 (m, 2 H, OCH_2Ph), 4.49 (br d, 1 H, H-1), 3.93 (br d, 1 H, *J* 12 Hz, H-6_b), 3.74 (br d, 1 H, *J* 12 Hz, H-6_a), 3.68 (t, 1 H, *J* 10 Hz, H-3), 3.48 (m, 1 H, H-2), 3.42 (m, 2 H, H-4 and H-5), 3.17 (br s, 2 H, NCH_2), 2.4–2.1 (br m, 10.9 H, $COCH_2$ and –CH–), 1.92 (s, 3 H, Ac), 1.8–1.4 (br m, 20.5 H, CH_2).

Galactosylation of the primer polymer **10**.—The primer polymer **10** (30.0 mg, ~ 33 μmol of GlcNAc residue), uridine-5′-di-phospho-galactose (UDP-Gal) (22 mg, 40 μmol), α-lactalbumin (200 μg) and galactosyl transferase from bovine milk (0.5 unit) were incubated in 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 6.0, 1.0 mL) at 37 °C for 24 h. The reaction mixture was directly purified by chromatography on Sephadex G-50 column (φ 25 mm × 1000 mm) and eluted with 50 mM aq CH_3COONH_4. The polymer fractions were collected and concentrated to 10 mL, and the syrupy soln was lyophilized to give a glycopolymer having LacNAc branches, **11** (32.0 mg, quantitative yield): ^{1}H NMR (D_2O) δ 7.38 (br d, 4 H, –Ph–), 4.60 (m, 2 H, OCH_2Ph), 4.50 (br d, 1 H, H-1), 4.47 (d, 1 H, *J* 8 Hz, H-1′), 4.0 (br d, 1 H, H-6b), 3.91 (d, 1 H, H-4′), 3.75–3.65 (m, 2 H, H-3 and H-4), 3.55–3.50 (m, 2 H, H-2 and H-3′), 3.42 (br s, 1 H, H-5), 3.17 (br s, 2 H, NCH_2), 2.4–2.1 (br b, 12.8 H, $COCH_2$ and –CH–), 1.91 (s, 3 H, Ac), 1.8–1.35 (br m, 18.0 H, CH_2).

Release of N-*acetyllactosamine from the glycopolymer* **12**.—To a soln of polymer **11** (28.0 mg, 25.9 μmol) in 3:1 water–EtOH (10 mL) was added 10% Pd/C (50 mg). The mixture was stirred under H_2 atmosphere at room temperature for 24 h. The mixture was filtered with celite, and the filtrate was evaporated under reduced pressure. The residual syrup was subjected to gel filtration chromatography on Sephadex LH-20 column and eluted with 95:5 EtOH–water to afford disaccharide **12** (9.4 mg, 95%): ^{1}H NMR (deuterium oxide) δ 5.20 (d, 1 H, *J* 2.3 Hz, H-1_α), 4.46 (d, 2 H, *J* 7.7 Hz, H-1_β and H-1′), 2.04 (s, 3 H, $COCH_3$); ^{13}C NMR (deuterium oxide) δ 177.1 (C=O), 105.6 (C-1), 97.5 (C-1_β), 93.2 (C-1_α), 81.4 (C-4_α), 81.0 (C-4β), 78.0 (C-5′), 77.5 (C-5_β), 75.1 (C-3_β), 73.6 (C-2′), 72.9 (C-5_α), 71.9 (C-3_α), 71.2 (C-4′), 63.6 (C-6′), 62.6 (C-6), 58.8 (C-2_β), 56.3 (C-2_α), 24.8 (CH_3).

N-*(Benzyloxycarbonyl-*L-*phenylalanyl)-6-amino-1-hexanol* (**13**).—To a soln of *N*-benzyloxycarbonylphenylalanine (2.99 g, 10.0 mmol) and 6-amino-1-hexanol (1.29 g, 11.0 mmol) in 1:1 benzene–EtOH (20 mL) was added *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (2.47 g, 10.0 mmol). The mixture was stirred at room temperature for 24 h. The clear soln was concentrated to give a residual syrupy material. The crude product was then recrystallized from benzene to afford the pure compound **13** (3.39 g, 85%): mp 109–110 °C; $[\alpha]_D^{23}$ +7.6° (*c* 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 10 H, –Ph),

5.72 (br s, 1 H, NH), 5.44 (br s, 1 H, NH), 5.09 (s, 2 H, OCH_2–Ph), 4.34 (q, 1 H, $CH_{\alpha Phe}$), 3.62 (t, 2 H, *J* 6.6 Hz, CH_2OH), 3.08 (m, 4 H, CH_2N, CHCH_2–Ph), 1.40 (m, 8 H, 4 CH_2). Anal. Calcd. for $C_{23}H_{30}O_4N_2 \cdot 0.2H_2O$: C, 68.70; H, 7.62; N, 6.97. Found: C, 68.80; H, 7.76; N, 6.99.

N-*(Benzyloxycarbonyl-*L*-phenylalanyl)-6-aminohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**14**).—To a soln of the oxazoline derivative **4** (1.56 g, 4.56 mmol) and **13** (3.63 g, 9.12 mmol) in dichloroethane (20 mL) was added DL-camphor-10-sulfonic acid (CSA) to be pH 2–3 at 70 °C. After 30 min, the mixture was cooled down to room temperature, and diluted with $CHCl_3$, and the organic layer was washed with saturated $NaHCO_3$ solution (×2). The soln was dried over $MgSO_4$, filtered and evaporated. The residual syrup was chromatographed on a silica gel column and eluted with $CHCl_3$ to give the glycoside **14** (2.0 g, 60%): $[\alpha]23_D$ −0.3° (*c* 0.59, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.43–7.32 (m, 10 H, 2-Ph), 6.28 (br d, 1 H, NH_{Glc}), 6.03 (br s, 1 H, NH_{hex}), 5.61 (br d, 1 H, NH_{Phe}), 5.36 (t, 1 H, H-3), 5.20–5.03 (m, 3 H, H-4 and OCH_2–Ph), 4.71 (d, 1 H, H-1), 4.42 (dd, 1 H, $H_{\alpha Phe}$), 4.30 (dd, 1 H, H-6_a), 4.17 (d, 1 H, H-6_b), 3.72 (m, 1 H, H-5), 3.32–3.07 (m, 4 H, $H_{\beta Phe}$ and NCH_2), 2.09–1.94 (m, 9 H, 3 OAc), 1.74 (s, 3 H, NAc), 1.36–1.14 (m, 8 H, 4 CH_2). Anal. Calcd. for $C_{37}H_{49}O_{12}N_3$: C, 61.06; H, 6.79; N, 5.77. Found: C, 60.87; H, 6.83; N, 5.73.

N-*(Acrylamido-*L*-phenylalanyl)-6-aminohexyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranoside* (**15**).—Palladium on carbon—10% (100 mg) was added to a solution of **14** (1.04 g, 1.42 mmol) in MeOH (30 mL) and the mixture was stirred under hydrogen atmosphere at room temperature for 24 h. The mixture was filtered with celite and the filtrate was evaporated to give crude 6-*N*-(L-phenylalanyl)-aminohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside. This intermediate was used for the next step without further purification.

To a mixture of the crude amino component (843 mg, ~ 1.42 mmol) in THF (20 mL) was added Et_3N (0.20 mL, 1.44 mmol) and a soln of acryloyl chloride (144.7 μL, 1.71 mmol) in THF (5 mL) at 0 °C, dropwise. To maintain the pH of the reaction mixture at pH 8.5, Et_3N was added appropriately and the mixture was stirred for 2 h. The soln was then evaporated under reduced pressure and extracted with $CHCl_3$ and it was washed with saturated aq $NaHCO_3$ and brine. The soln was dried over $MgSO_4$, filtered, and evaporated. The residual syrup was chromatographed on a silica gel column and eluted with 50:1 $CHCl_3$–MeOH to give compound **15** (428 mg, 46%): $[\alpha]_D^{23}$ −16.6° (*c* 0.28, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.31–7.20 (m, 5 H, –Ph), 6.26 (dd, 1 H, COC*H*=CH_2), 6.17 (dd, 1 H, COCH=CH_{2trans}), 5.66 (dd, 1 H, COCH=CH_{2cis}), 5.32 (t, 1 H, H-3), 5,09 (t, 1 H, H-4), 4.75 (m, 1 H, $H_{\alpha Phe}$), 4.64 (d, 1 H, H-1), 4.27 (dd, 1 H, H-6_a), 4.13 (dd, 1 H, H-6_b), 3.85–3.80 (m, 1 H, H-5), 3.71–3.67 (m, 1 H, H-2), 3.27–3.01 (m, 4 H, NCH_2 and $H_{\beta Phe}$), 2.17, 2.08, 2.03 (each s, 9 H, 3 OAc), 1.91 (s, 3 H, NAc), 1.54–1.17 (m, 8 H, 4 CH_2). Anal. Calcd. for $C_{32}H_{45}O_{11}N_3 \cdot 0.2H_2O$: C, 59.01; H, 6.99; N, 6.45. Found: C, 58.92; H, 6.98; N, 6.45.

N-*(Acrylamido-*L*-phenylalanyl)-6-aminohexyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**16**).—To a soln of **15** (416 mg, 0.64 mmol) in 1:1 THF–MeOH (10 mL) was added NaOMe (12.9 mg, 0.23 mmol). The soln was stirred at room temperature for 1 h. The mixture was neutralized with Dowex 50W-X8 (H +). The filtrate was evaporated to afford the pure glycomonomer **16** as an amorphous powder (335 mg, > 99%): $[\alpha]23_D$ −9.0° (*c* 0.69, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 8.36 (br d, 1 H, NH_{Phe}), 8.02 (br s, 1 H, NH_{hex}), 7.74 (br d, 1 H, NH_{Glc}), 7.24–7.19 (m, 5 H, –Ph), 6.28 (dd, 1 H, *J* 6.6 and 10.3 Hz, COC*H*=CH_2), 6.03 (dd, 1 H, *J* 16.5 Hz, COCH=CH_{2trans}), 5.57 (dd, 1 H, *J* 9.9 Hz, COCH=CH_{2cis}), 4.53 (br s, 1 H, $H_{\alpha Phe}$), 4.27 (d, 1 H, *J* 8.1 Hz, H-1), 3.70–3.26 (m, 6 H, *J* 7.7, 8.8, 9.5, and 11.7 Hz, H-6_a, H-6_b, H-5, H-4, H-3 and H-2), 3.08–2.77 (m, 6 H, NCH_2, OCH_2 and $H_{\beta Phe}$), 1.81 (s, 3 H, NAc), 1.41–0.86 (m, 8 H, 4 CH_2). Anal. Calcd. for $C_{26}H_{39}O_8N_3 \cdot 0.9H_2O$: C, 58.07; H, 7.83; N, 7.81. Found: C, 58.04; H, 7.36; N, 7.81.

Polyacrylamide copolymer **17** from the glycomonomer **16**.—To a soln of **16** (100 mg, 0.192 mmol) in Me_2SO (1.5 mL) was added a soln of acrylamide (54.30 mg, 0.764 mmol) in water (0.6 mL). This clear solution was deaerated for a while using water pump, to which was added TEMED (11.52 μL, 76.4 μmol) and APS (6.96 mg, 30.56 μmol). The solution was stirred at 50 °C for 24 h. The reaction mixture was directly subjected to gel filtration chromatography on a Sephadex G-25 column (φ 30 mm × 400 mm) and eluted with 10 mM aq CH_3COONH_4. The polymer containing fractions were collected and evaporated to small volume (~ 10 mL), and the syrupy soln was lyophilized to afford polymer **17** as an amorphous powder (131 mg, 85%): Molecular weight ~ 380,000 (GPC method); ^{1}H NMR (deuterium oxide) δ 7.30 (br d, 5 H, –Ph), 4.49 (br s, 1 H, H-1), 3.44 (br s, 1 H, H-4), 2.03 (s, 3 H, NAc).

Galactosylation of the primer polymer **17**.—Primer polymer **17** (10.0 mg, 13.58 μmol of GlcNAc residue), uridine-5′-diphospho-galactose (UDP-Gal) (10.38 mg, 17.65 μmol), α-lactalbumin (80.4 μg) and galactosyl transferase from bovine milk (1 unit) were incubated in 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 6.0, 1.0 mL) at 37 °C for 24 h. The reaction mixture was directly purified by chromatography on Sephadex G-25 column [4] (ϕ 30 mm × 400 mm) eluted with 10 mM aqueous CH_3COONH_3. The polymer fractions were pooled and lyophilized to give a glycopolymer having LacNAc branches (12 mg): ^{1}H NMR (deuterium oxide) δ 7.35 (br d, 5 H, –Ph), 4.49 (m, 2 H, H-1 and H-1′), 3.94 (d, 1 H, H-4′), 3.85 (br s, 1 H, H-3′), 3.51 (t, 1 H, H-2′), 2.03 (s, 3 H, NAc).

N-(N-*Acrylamido-hexanoyl*)-L-*phenylalanyl*-6-*aminohexyl 2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy*-β-D-*glucopyranoside* (**19**).—Compound **14** (1.50 g, 2.06 mmol) was hydrogenated according to the procedure described for the preparation of **15** to afford an intermediate. To the mixture of crude amino component (1.22 g, ~ 2.06 mmol) and **8** (420 mg, 2.23 mmol) in 1:1 EtOH–benzene (40 mL) was added EEDQ (550 mg, 2.23 mmol). The mixture was stirred at room temperature for 24 h. The clear soln was evaporated. The crude material was then recrystallized from EtOH to afford the pure compound **19** (1.2 g, 77%): $[\alpha]_D^{23}$ −4.5° (*c* 0.83, Me_2SO); ^{1}H NMR ($CDCl_3$) δ 8.00–7.84 (m, 4 H, 4 NH), 7.25–7.14 (m, 5 H, –Ph), 6.19 (dd, 1 H, COC*H*=CH_2), 6.06 (dd, 1 H, COCH=CH_{2trans}), 5.54 (dd, 1 H, COCH=CH_{2cis}), 5.07 (t, 1 H, H-3), 4.80 (t, 1 H, H-4), 4.58 (d, 1 H, H-1), 4.47–4.41 (m, 1 H, $H_{\alpha Phe}$), 4.17 (dd, 1 H, H-6_a), 3.99 (dd, 1 H, H-6_b), 3.82–3.77 (m, 1 H, H-5), 3.72–3.64 (m, 1 H, H-2), 3.07–2.89 (m, 5 H, 2 NCH_2 and $H_{\beta Phe}$), 1.20, 1.95, 1.90 (each s, 9 H, 3 OAc), 1.74 (s, 3 H, NAc), 1.45–1.07 (m, 14 H, 7 CH_2). Anal. Calcd. for $C_{38}H_{56}O_{12}N_4 \cdot 0.5H_2O$: C, 59.28; H, 7.46; N, 7.28. Found: C, 59.39; H, 7.30; N, 7.22.

N-(N-*Acrylamido-hexanoyl*)-L-*phenylalanyl*-6-*aminohexyl 2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**2**).—Treatment of the peracetylated derivative **19** (590 mg, 0.78 mmol) with NaOMe (16.9 mg, 0.31 mmol) in dry MeOH, as described for the preparation of **1**, gave the glycomonomer **2** (413 mg, 83%): $[\alpha]_D^{23}$ −1.5° (*c* 0.29, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 7.95 (m, 2 H, N*H*COCH=CH_2, NH_{Phe}), 7.85 (s, 1 H, *J* 8.4 Hz, NH_{Glc}), 7.18 (m, 5 H, –Ph), 6.18 (dd, 1 H, COC*H*=CH_2), 6.03 (d, 1 H, *J* 16.8 Hz, COCH=CH_{2trans}), 5.52 (d, 1 H, *J* 9.6 Hz, COCH=CH_{2cis}), 4.87 (d, 2 H, OCH_2), 4.46 (m, 2 H, H-4 and $H_{\alpha Phe}$), 4.23 (d, 1 H, *J* 7.5 Hz, H-1), 3.67 (s, 1 H, H-3), 3.40 (m, 2 H, H-2 and H-5), 2.96–2.71 (m, 8 H, H-$6_{a,b}$, 2 × CH_2, and CH_2–Ph), 2.00 (s, 2 H, $COCH_2$), 1.80 (s, 3 H, NAc), and 1.34–1.10 (m, 16 H, 8 × CH_2). Anal. Calcd. for $C_{32}H_{50}O_9N_4$: C, 60.55; H, 7.94; N, 8.83. Found: C, 60.23; H, 7.89; N, 8.71.

Polyacrylamide copolymer from the glycomonomer **2**.—To a soln of the glycomonomer **2** (150 mg, 0.236 mmol) in Me_2SO (2.0 mL) was added an aq soln of acrylamide (67.19 mg, 0.945 mmol) in water (1.0 mL). This clear soln was deaerated for a while using water pump, to which was added TEMED (14.25 μL, 94.4 μmol) and APS (8.62 mg, 37.76 μmol). The soln was stirred at 50 °C for 24 h. The reaction mixture was directly subjected to gel filtration chromatography on Sephadex G-25 column (ϕ 30 mm × 400 mm) and eluted with 10 mM aq CH_3COONH_4. Polymer containing fractions were collected and evaporated to a small volume (~ 10 mL), and the syrupy soln was lyophilized to afford polymer **20** as an amorphous powder (202 mg, 93%): Mw ~ 380,000 (GPC); ^{1}H NMR (D_2O) δ 7.29 (m, 5 H, –Ph), 4.50 (br t, 2 H, H-1 and Hα_{Phe}), 3.89 (m, 1 H, H-4), 3.71 (m, 1 H, H-2), 3.44 (br d, 3 H, H-3, and OCH_2), 3.12–2.86 (m, 4 H, CH_2N, CHCH_2–Ph), 2.20 (br s, 5 H, 5 CH), 1.80–1.15 (m, 26 H, 13 × CH_2).

Galactosylation and sialylation of the primer polymer **20**.—Primer polymer **20** (15.0 mg, ~ 16.3 μmol of GlcNAc), uridine-5′-diphospho-galactose (UDP-Gal) (12.48 mg, 21.22 μmol), α-lactalbumin (130 μg), galactosyltransferase (1 unit) were incubated in 50 mM HEPES buffer (1.0 mL) at 37 °C. After reaction for 24 h, the mixture was directly purified by chromatography on Sephadex G-25 column (ϕ 30 mm × 400 mm) and eluted with 10 mM aq CH_3COONH_4. The polymer fractions were collected and lyophilized to give a glycopolymer having LacNAc branches (17.2 mg). The efficiency of the galactosylation reaction was estimated by the integration data of anomeric protons of ^{1}H NMR spectrum: ^{1}H NMR (deuterium oxide) δ 7.26 (br d, 5 H, –Ph), 4.48 (m, 2 H, H-1 and H-1′), 3.94 (d, 1 H, H-4′), 3.56 (t, 1 H, H-2′), 2.02 (s, 3 H, NAc). This material was

[4] Recently, the reaction mixtures are heated at 100 °C for 30 min before chromatography to remove denatured protein contamination by simple filtration.

used for subsequent sialylation without further characterization as follows.

A soln of bovine serum albumin (BSA) (10.0 mg), NaN_3 (4.67 mg, 71.9 μmol) and manganese(II) chloride tetrahydrate (1.56 mg, 7.9 μmol) dissolved in 50 mM sodium cacodylate buffer (5.0 mL) was prepared and the pH of the soln was adjusted to 7.40 with aq HCl. To the above described soln (1 mL) was added the glycopolymer carrying LacNAc residues (35.3 mg, ~ 32.6 μmol of LacNAc residues), cytidine-5′-monophospho-*N*-acetyl neuraminic acid (CMP–Neu5Ac) (32.5 mg, 52.9 μmol), carf intestinal alkaline phosphate (CIAP) (20 unit) and rat liver α-2 → 6-sialyltransferase (0.1 unit). The mixture was incubated at 37 °C for 48 h. The reaction mixture was directly purified by chromatography on Sephadex G-25 column (ϕ 30 mm × 400 mm) eluted with 10 mM aq CH_3COONH_4. The polymer fractions were collected and lyophilized to give a glycopolymer having sialyl-LacNAc branches, **22** (33.0 mg, quantitative yield from the integration data of ^{1}H NMR spectrum); ^{1}H NMR (deuterium oxide) δ 7.26 (br d, 5 H, –Ph), 4.48 (m, 2 H, H-1 and H-1′), 3.94 (d, 1 H, H-4′), 3.56 (t, 1 H, H-2′), 2.68 (dd, 1 H, H-3″$_{eq}$), 2.02 (s, 3 H, NAc), 1.74 (d, 1 H, H-3″$_{ax}$).

Treatment of the LacNAc carrying polymer **17** and sialyl - LacNAc carrying polymer **22** with α - chymotrypsin.—Polymer **17** (20.0 mg, 22.3 μmol) and α-chymotrypsin (1.0 mg, 1000 unit) were incubated in 80 mM Tris–HCl buffer (pH 7.8, 1.0 mL) containing 0.1 M $CaCl_2$ at 40 °C for 48 h. Similarly, polymer **22** (33.0 mg, 24.0 μmol) and α-chymotrypsin (1.0 mg, 1000 unit) were incubated in 80 mM Tris–HCl buffer (0.1 M $CaCl_2$) (1.0 mL) at 40 °C for 24 h. The reaction mixture was directly purified by chromatography on Sephadex G-15 column (ϕ 25 mm × 350 mm) and eluted with deionized water. The sugar fractions were collected and lyophilized. Compound **23** (15.0 mg, 87%): ^{1}H NMR (deuterium oxide) δ 4.56 (d, 1 H, *J* 7.3 Hz, H-1), 4.45 (d, 1 H, *J* 8.0 Hz, H-1′), 4.00 (br d, 3 H, H-6$_b$, H-4, and H-6′$_b$), 3.97 (m, 1 H, H-4′), 3.92 (br d, 1 H, *J* 3.81 Hz, H-5″), 3.72 (dd, 1 H, *J* 10 Hz, H-2), 3.67–3.62 (m, 3 H, H-3, H-3′, H-4″), 3.59–3.52 (m, 5 H, H-5, H-2′, H-3, H-6′$_a$, and H-7″), 3.00 (t, 2 H, *J* 7.5 Hz, NCH_2), 2.67 (dd, 1 H, *J* 4.7 and 12.5 Hz, H-3″$_{eq}$), 2.06–2.03 (each s, 2 H, 2 $COCH_3$), 1.74 (d, 1 H, *J* 12.4 Hz, H-3″$_{ax}$), 1.68–1.37 (m, 8 H, 4 CH_2); ^{13}C NMR (D_2O) δ 177.8, 177.3, and 176.4 (C=O), 106.4 (C-1′), 103.8 (C-1), 103.0 (C-2″), 83.7 (C-4), 77.4 (C-5), 76.6 (C-5), 75.4 (C-6″), 75.3 (C-3′), 74.6 (C-3), 73.7 (C-8″), 73.2 (C-2″), 71.3 (C-4, C-4″), 71.1 (C-7″), 66.3 (C-6″), 65.5 (C-9″), 63.3 (C-6), 57.8 (C-2), 54.7 (C-5″), 43.0 (OCH_2), 42.3 (C-3″), 31.2, 29.5, 28.1, and 27.5 (CH_2), 25.2 and 24.9 (CH_3).

N-*Benzyloxycarbonyl*-L-*serinoctylamide* (**25**).—To a soln of *N*-benzyloxycarbonyl-L-serine (3.0 g, 12.5 mmol) and *n*-octylamine (2.28 mL, 13.8 mmol) in 1:1 EtOH–benzene (30 mL) was added EEDQ (3.4 g, 13.8 mmol). The mixture was stirred at room temperature for 24 h. The clear soln was concentrated to give a syrupy crude product. The crude material was then recrystallized from benzene to afford the pure compound **25** (3.4 g, 78%): mp 95 °C; $[\alpha]_D$ −14.1° (*c* 0.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 5 H, –Ph), 6.59 (br s, 1 H, NH_{Ser}), 5.87 (br d, 1 H, NH), 4.17 (ddd, 1 H, $H_{\alpha Ser}$), 4.09 (dd, 1 H, $H_{\beta Ser}$), 3.62–3.65 (dd, $H_{\beta Ser}$), 3.22 (dd, 2 H, $NHCH_2$), 1.48–1.26 (m, 14 H, 7 × CH_2), 0.88 (t, 3 H, CH_3). Anal. Calcd. for $C_{19}H_{30}O_4N_2 \cdot 0.2H_2O$: C, 64.45; H, 8.65; N, 7.91. Found: C, 64.39; H, 8.69; N, 7.98.

N-*(Benzyloxycarbonyl)*-O-*(2′,3′,4′,6′-tetra*-O-*acetyl*-β-D-*galactopyranosyl)*-*(1 → 4)*-*(2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosyl)*-L-*serinoctylamide* (**26**).—Powdered molecular sieves 4 Å was added to a soln of the readily available lactosyl bromide **24** (3.1 g, 4.43 mmol) and **25** (1.03 g, 2.95 mmol) in CH_2Cl_2 (20 mL). Silver triflate (1.14 g, 4.43 mmol) was then added to the mixture at −20 °C. The reaction mixture was stirred under nitrogen atmosphere at −20 °C for 24 h. The mixture was filtered with celite, and the filtrate was washed with brine. The soln was dried over $MgSO_4$, filtered, and evaporated. The residual syrup was chromatographed on a silica gel column and eluted with 2:1 toluene–EtOAc to give the glycoside **26** (1.49 g, 48%): $[\alpha]_D^{23}$ +6.8° (*c* 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ph), 6.33 (br s, 1 H, NH), 5.62 (br s, 1 H, NH), 5.35 (dd, 1 H, H-4′), 5.17–5.07 (m, 2 H, H-3 and H-2′), 4.96 (dd, 1 H, H-3′), 4.86 (dd, 1 H, H-2), 4.54–4.47 (m, 4 H, H-1, H-1′, H-6$_b$ and H-6′$_b$), 4.15–3.66 (m, 8 H, Hα_{Ser}, H-6$_a$, H-6′$_a$, H-5′, H-5, H-4 and $COCH_2$), 3.21 (br t, 4 H, 2 × $NHCH_2$), 2.78 (t, 2 H, 2 × $COCH_2$), 2.20–1.93 (m, 21 H, 7 × OAc), 1.62–1.28 (m, 10 H, 5 × CH_2), 0.88 (t, 3 H, CH_3). Anal. Calcd. for $C_{45}H_{64}O_{21}N_2 \cdot 0.8H_2O$: C, 54.96; H, 6.72; N, 2.85. Found: C, 54.89; H, 6.63; N, 2.72.

N-*[(*N-*Acrylamido)-pentanoyl]*-O-*(2′,3′,4′,6′-tetra*-O-*acetyl*-β-D-*galactopyranosyl)*-*(1 → 4)*-*(2,3,6-tri*-O-*acetyl* - β - D - *glucopyranosyl)* - L - *serinoctylamide* (**27**).—10% Palladium on carbon (300 mg) was added to a solution of **26** (1.5 g, 1.55 mmol) in MeOH (40 mL) and the mixture was stirred under hydrogen

atmosphere at room temperature for 2 h. The mixture was filtered with celite and the filtrate was evaporated to give crude *O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serinoctylamide. This intermediate was used for the next step without further purification.

A mixture of crude amino component (1.29 g, ~ 1.55 mmol), **8** (315 mg, 1.70 mmol), and EEDQ (421 mg, 1.70 mmol) in 1:1 EtOH–benzene (40 mL) was stirred at room temperature for 24 h. The clear solution was concentrated under reduced pressure. The residue was purified by silica gel chromatography with 50:1 $CHCl_3$–MeOH as eluant to give compound **27** (1.21 g, 78% from compound **26**): $[\alpha]_D^{23}$ +3.8° (*c* 0.37, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.43 (t, 1 H, NH), 6.39 (d, 1 H, NH_{Ser}), 6.28 (dd, 1 H, C*H*=CH_2), 6.10 (dd, 1 H, CH=CH_{2trans}), 5.62 (dd, 1 H, CH=CH_{2cis}), 5.35 (dd, 1 H, H-4′), 5.20–5.09 (m, 2 H, H-3 and H-2′), 4.97 (dd, 1 H, H-3′), 4.86 (dd, 1 H, H-2), 4.62–4.49 (m, 4 H, H-1, H-1′, H-6_b and H-$6'_b$), 4.16–4.05 (m, 3 H, Hα_{Ser}, H-6_a and H-$6'_a$), 3.95 (dd, 1 H, H-5′), 3.88 (dt, 1 H, H-4), 3.81–3.66 (m, 3 H, H-5 and $COCH_2$), 3.36–3.15 (m, 4 H, 2 × $NHCH_2$), 2.23 (t, 2 H, 2 × $COCH_2$), 2.16–1.97 (m, 21 H, 7 × OAc), 1.73–1.29 (m, 18 H, 9 × CH_2), 0.88 (t, 3 H, CH_3). Anal. Calcd. for $C_{46}H_{71}O_{21}N_3 \cdot 0.33H_2O$: C, 54.81; H, 7.16; 4.17. Found: C, 54.82; H, 7.16; N, 4.12.

N-*[(*N*-Acrylamido)-pentanoyl]*-O-β-D-*galacto-pyranosyl)-(1 → 4)-(*β-D-*glucopyranosyl)*-L-*serinocty-lamide* (**3**).—To a soln of **5** (394 mg, 0.39 mmol) in 1:1 THF–MeOH (10 mL) was added NaOMe (8.49 mg, 0.157 mmol). The mixture was stirred at room temperature for 1 h. The soln was neutralized with Dowex 50W-X8 (H +) and filtered with celite. The filtrate was evaporated to afford the pure glycomonomer **3** as an amorphous powder (278 mg, > 99%): $[\alpha]_D^{23}$ +8.2° (*c* 0.25, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 8.04 (t, 1 H, NH), 7.91 (d, 1 H, NH_{Ser}), 7.74 (t, 1 H, NH), 6.21 (dd, 1 H, *J* 7.0 and 10.1 Hz, C*H*=CH_2), 6.06 (dd, 1 H, *J* 2.3 and 14.8 Hz, CH=CH_{2trans}), 5.55 (dd, 1 H, *J* 2.3 and 7.8 Hz, CH=CH_{2cis}), 5.19 (br s, 2 H, CH_2), 5.10 (br s, 2 H, CH_2), 4.61 (br t, 2 H, H-1 and H-1′), 4.52 (d, 1 H, H-4), 4.43 (ddd, 1 H, *J* 5.3 Hz, Hα_{Ser}), 4.18 (m, 2 H, CH_2), 3.91 (dd, 1 H, Hβ_{Ser}), 3.77 (m, 1 H, *J* 11.5 Hz, H-2), 3.62–3.29 (m, 7 H, H-3, H-2′, Hβ_{Ser} and 2 × CH_2), 3.10 (dd, 2 H, NHCH_2), 3.04 (dd, 2 H, NHCH_2), 2.15 (t, 2 H, $COCH_2$), 1.53–1.24 (m, 8 H, 4 × CH_2), 0.86 (t, 3 H, CH_3); ^{13}C NMR (Me_2SO-d_6) δ 172.2, 169.3, and 164.4 (C=O), 131.9 (CH=CH_2), 124.7 (CH=CH_2), 103.9 (C-1), 103.2 (C-1′), 80.6 (C-4), 75.5 (C-4′), 74.8 (C-5′), 74.6 (C-5), 73.2 (C-3′), 73.0 (C-3), 70.5 (C-2′), 69.8 (C-2), 68.1 (C-6′), 67.4 (C-6), 60.4 ($C_{\beta Ser}$), 52.5 ($C_{\alpha Ser}$), 38.6, 38.4, 35.1, 31.2, 28.9, 28.8, 28.7, 28.6, 26.3, 26.1, 24.8, and 22.1 (CH_2), 13.9 (CH_3). Anal. Calcd. for $C_{32}H_{57}O_{14}N_3$: C, 54.30; H, 8.12; N, 5.94. Found: C, 54.88; H, 8.20; N, 5.06.

Polyacrylamide copolymer **28** from the glycomonomer **3**.—To a soln of **3** (150 mg, 0.21 mmol) in Me_2SO (2.0 mL) was added an aqueous soln of acrylamide (60.3 mg, 0.84 mmol) in water (2.0 mL). This clear soln was deaerated for a while using water pump, to which was added TEMED (12.7 μL, 84.0 μmol) and APS (7.67 mg, 33.6 μmol). The soln was stirred under nitrogen atmosphere at 50 °C for 24 h. The reaction mixture was directly subjected to gel filtration chromatography on Sephadex G-25 column (φ 30 mm × 400 mm) and eluted with deionized water. Polymer containing fractions were collected and concentrated to small volume, and the syrupy soln was lyophilized to afford polymer **28** as an amorphous powder (193 mg, 92%): Mw > 380,000 (GPC method). ^{1}H NMR (deuterium oxide) δ 4.34–4.32 (m, 4 H, H-1, H-1′, H-4 and Hα_{Ser}), 4.02 (br d, 2 H, H-3 and H-3′), 3.84–3.78 (m, 4 H, H-2, H-2′, H-6 and H-6′), 3.61 (m, 2 H, H-5 and H-5′), 3.50–3.39 (m, 2 H, H-4′ and Hβ_{Ser}), 3.19 (d, 1 H, Hβ_{Ser}), 3.04 (m, 2 H, CH_2), 2.19 (m, 4 H, 2 × $NHCH_2$), 2.06 (m, 2 H, $COCH_2$), 1.61–1.35 (m, 13 H, 3 × CH_2 and 7 × CH), 1.12 (s, 12 H, 6 × CH_2), 0.71 (s, 3 H, CH_3).

Polyacrylamide having GM3 trisaccharides (**29**).—Primer polymer **28** (22 mg, ~ 20 μmol of lactose residue), cytidine-5′-monophospho–*N*-acetyl neuraminic acid (CMP–NeuAc) (15.0 mg, 24.4 μmol), α-(2 → 3)-sialyltransferase (0.3 unit), bovine serum albumin (BSA) (4.0 mg), and carf intestinal alkaline phosphatase (CIAP) (20 unit) were incubated in 50 mM sodium cacodylate buffer (pH 7.4, 2.0 mL) containing manganese(II) chloride (0.62 mg) and Triton CF-54 (10 μL) at 37 °C for 72 h. The reaction mixture was directly purified by chromatography on Sephadex G-25 column (φ 30 mm × 400 mm) eluted with deionized water. The polymer fractions were collected and lyophilized to give a glycopolymer having GM3 trisaccharide, **29** (22.0 mg, quantitative sialylation was estimated from integration data of ^{1}H NMR spectrum): ^{1}H NMR (D_2O) δ 4.16–3.57 (m, 11 H, H-1, H-1′, H-2, H-3, H-3′, H-4, H-4″, H-5, H-5″, H-6 and H-7″), 3.35 (d, 1 H, Hβ_{Ser}), 3.23–3.18 (m, 2 H, CH_2), 2.77 (dd, 1 H, H-$3''_{eq}$), 2.35–2.14 (m,

$2 \times NHCH_2$ and $COCH_2$), 2.05 (s, 3 H, NHAc), 1.81 (t, 1 H, H-$3''_{ax}$), 1.66–1.13 (m, 34 H, $14 \times CH_2$ and $6 \times CH$), 0.79 (t, 3 H, CH_3); ^{13}C NMR (D_2O) δ 182.2, 177.9, and 176.6 ($4 \times C{=}O$), 105.7 (C-1), 105.1 (C-1′), 104.9 (C-2″), 81.5 (C-4), 65.5 (C-6′), 63.8 (C-6), 63.1 ($C_{\beta Ser}$), 54.6 ($C_{\alpha Ser}$), 46.4 (C-3″), 44.7, 42.3, 38.1, 37.5, 34.0, 31.3, 28.8, 27.6, and 24.9 (CH_2), 16.6 and 16.4 (CH_3).

Transglycosylation reaction by ceramide glycanase.—To a mixture of glycopolymer **29** (22.0 mg, ~ 16.2 μmol of sialyllactose residue) and ceramide (Funakoshi, Japan) (50 mg, 78.7 μmol) in 50 mM sodium citrate buffer (pH 6.0, 1 mL) was added Toriton CF-54 (1 drop), and the mixture was sonicated for 1 min in an ultrasonic water bath. The reaction was initiated by the addition of ceramide glycanase from leech (0.01 unit) and incubated at 37 °C for 17 h. This mixture was directly chromatographed on Sephadex LH-20 column and eluted with 60:30:4.4 $CHCl_3$–MeOH–water to give GM3 (**1**) (12 mg, 63% calculated from glycopolymer **29**). A faint amount of hydrolytic product such as sialyllactose was obtained by the chromatographic purification: ^1H NMR (49:1) Me_2SO-d_6–deuterium oxide δ 5.55 (dt, 1 H, *J* 6.6 Hz, H-5cer), 5.37 (dd, 1 H, *J* 6.8 Hz, H-4cer), 4.20 (d, 1 H, *J* 7.8 Hz, H-1′), 4.14 (d, 1 H, *J* 7.8 Hz, H-1), 2.75 (dd, 1 H, *J* 5.0 and 12.0 Hz, H-$3''_{eq}$), 1.89 (s, 3 H, NAc), 1.38 (t, 1 H, *J* 12.0 Hz, H-3″ax), 1.25 (br s, CH_2 of ceramide), and 0.85 (t, 6 H, *J* 6.8 Hz, $CH_3 \times 2$). Anal. Calcd. for $C_{63}H_{116}O_{21}N_2$: C, 61.14; H, 9.45; N, 2.26. Found: C, 61.36; H, 9.49; N, 2.22.

Acknowledgements

The authors are grateful to Professor Yuan C. Lee (Johns Hopkins University) and Dr. Koji Matsuoka (Saitama University) for their valuable discussion and suggestions. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture (09240101) and by a grant for the Original Industrial Technology R&D Promotion Program of the New Energy and Industrial Technology Development Organization (NEDO). Financial supports from the Sumitomo Foundation, Izumi Foundation, and Toray Science Foundation are also gratefully acknowledged.

References

[1] S.-I. Nishimura, K. Matsuoka, and Y.C. Lee, *Tetrahedron Lett.*, 35 (1994) 5657–5660.
[2] K. Yamada and S.-I. Nishimura, *Tetrahedron Lett.*, 36 (1995) 9493–9496.
[3] S.-I. Nishimura and K. Yamada, *J. Am. Chem. Soc.*, 119 (1997) 10555–10556.
[4] J.M.J. Frechet, *Polymer-Supported Reactions in Organic Synthesis*, Wiley, New York, 1980, 407 pp.
[5] G.H. Veeneman, S. Notermans, R.M.J. Liskamp, G.A. van der Marel, and J.H. van Boom, *Tetrahedron Lett.*, 28 (1987) 6695–6698.
[6] S.J. Danishefsky, K.F. McClure, J.T. Randolph, and R.B. Ruggeri, *Science*, 260 (1993) 1307–1309.
[7] J.T. Randolph and S.J. Danishefsky, *Angew. Chem. Int. Ed. Engl.*, 33 (1994) 1470–1473.
[8] L. Yan, C.M. Taylor, R. Goodnow Jr., and D. Kahne, *J. Am. Chem. Soc.*, 116 (1994) 6953–6954.
[9] J.T. Randolph, K.F. McClure, and S.J. Danishefsky, *J. Am. Chem. Soc.*, 117 (1995) 5712–5719.
[10] J. Rademann and R.R. Schmidt, *Tetrahedron Lett.*, 37 (1996) 3989–3990.
[11] M. Adinolfi, G. Barone, L. de Napoli, A. Iadonisi, and G. Piccialli, *Tetrahedron Lett.*, 37 (1996) 5007–5010.
[12] S.P. Douglas, D.M. Whitfield, and J.J. Krepinsky, *J. Am. Chem. Soc.*, 113 (1991) 5095–5097.
[13] Y. Ito, O. Kanie, and T. Ogawa, *Angew. Chem. Int. Ed. Engl.*, 35 (1996) 2510–2512.
[14] D.M. Whitfield, S.P. Douglas, and J.J. Krepinsky, *Tetrahedron Lett.*, 33 (1992) 6795–6798.
[15] R. Verduyn, P.A.M. van der Klein, M. Douwes, G.A. van der Marel, and J.H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 112 (1993) 464.
[16] S.P. Douglas, D.M. Whitfield, and J.J. Krepinsky, *J. Am. Chem. Soc.*, 117 (1995) 2116–2117.
[17] K.C. Nicolaou, N. Winssinger, J. Pastor, and F. DeRoose, *J. Am. Chem. Soc.*, 119 (1997) 449–450.
[18] E.J. Toone, E.S. Simon, M.D. Bednarski, and G.M. Whitesides, *Tetrahedron*, 45 (1989) 5365–5422.
[19] S. David, C. Auge, and C. Gautheron, *Adv. Carbohydr. Chem. Biochem.*, 49 (1991) 175–237.
[20] Y. Ichikawa, G.C. Look, and C.-H. Wong, *Anal. Biochem.*, 202 (1992) 215–238.
[21] K.J. Colley, E.U. Lee, B. Alder, J.K. Broune, and J.C. Paulson, *J. Biol. Chem.*, 264 (1989) 17,619–17,622.
[22] W. Gillespie, S. Kelm, and J.C. Paulson, *J. Biol. Chem.*, 267 (1992) 21,004–21,010.
[23] D.X. Wen, B.D. Livingston, K.F. Medrihdszky, S. Kelm, A.L. Burlingame, and J.C. Paulson, *J. Biol. Chem.*, 267 (1992) 21,011–21,019.
[24] U. Zehavi, S. Sadeh, M. Herchman, and S. Kopper, *Carbohydr. Res.*, 124 (1983) 23–34.
[25] U. Zehavi, M. Harchman, R.R. Schmidt, and T. Bar, *Glycoconj. J.*, 7 (1990) 229–234.
[26] U. Zehavi, M. Harchman, and S. Kopper, *Carbohydr. Res.*, 228 (1992) 255–263.
[27] M. Shulster, P. Wang, J.C. Paulson, and C.-H. Wong, *J. Am. Chem. Soc.*, 116 (1994) 1135–1136.
[28] M. Meldal, F.-I. Auzanneau, O. Hindsgaul, and M. Palcic, *J. Chem. Soc. Chem. Commun.*, (1994) 1849–1850.
[29] For example, T. Furuike, N. Nishi, S. Tokura, and S.-I. Nishimura, *Macromolecules*, 28 (1995) 7241–

7247 and S.-I. Nishimura and Y.C. Lee, in S. Dumitriu (Ed.), *In Structural Diversity and Functional Versatility of Polysaccharides*, Marcel Dekker, 1997, in press.
[30] R. Roy, C.A. Rafgerriere, R.A. Pon, and A. Gamian, *Methods Enzymol.*, 247 (1994) 351–361.
[31] R. Roy, F.O. Andersson, G. Harms, S. Kelm, and R. Shauer, *Angew. Chem. Int. Ed. Engl.*, 31 (1992) 1478–1481.
[32] L.V. Mochalova, A.B. Tuzikov, V.P. Marinina, A.S. Gambaryan, N.E. Byramova, N.V. Bovin, and M.N. Matrosovich, *Antivir. Res.*, 23 (1994) 179–190.
[33] S.-K. Choi, M. Mammem, and G.M. Whitesides, *Chem. Biol.*, 3 (1996) 97–104.
[34] T. Suzuki, G. Horiike, Y. Yamazaki, K. Kawabe, H. Masuda, D. Miyamoto, M. Matsuda, S.-I. Nishimura, T. Yamagata, T. Ito, H. Kida, Y. Kawaoka, and Y. Suzuki, *FEBS Lett.*, 404 (1997) 192–196.
[35] S.-I. Nishimura, K.B. Lee, K. Matsuoka, and Y.C. Lee, *Biochem. Biophys. Res. Commun.*, 199 (1994) 249–254.
[36] C.-H. Wong, S.L. Haynie, and G.M. Whitesides, *J. Org. Chem.*, 47 (1982) 5416–5418.
[37] P.R. Rosevear, H.A. Nunez, and R. Barker, *Biochemistry*, 21 (1982) 1421–1431.
[38] M.M. Palcic, O.P. Srivastava, and O. Hindsgaul, *Carbohydr. Res.*, 159 (1987) 315–324.
[39] M. Fineman and S.D. Ross, *J. Polymer Sci.*, 5 (1950) 259–265.
[40] F.R. Mayo and F.M. Lewis, *J. Am. Chem. Soc.*, 66 (1944) 1594–1601.
[41] J.Q. Fan, Y. Namiki, K. Matsuoka, and Y.C. Lee, *Anal. Biochem.*, 219 (1994) 375–378.
[42] B. Zhou, S.-C. Li, R.A. Laine, R.T.C. Huang, and Y.-T. Li, *J. Biol. Chem.*, 264 (1989) 12,272–12,277.
[43] Y.-T. Li, B.Z. Carter, B.N.N. Rao, H. Schweingruber and S.-C. Li, *J. Biol. Chem.*, 266 (1989) 10,723–10,726.
[44] Y.-T. Li and S.-C. Li, in Y.C. Lee and R.T. Lee (Eds.), *Neoglycoconjugates, Preparation and Applications*, Academic Press, San Diego, 1994, 251 pp.
[45] M. Sugimoto and T. Ogawa, *Glycoconj. J.*, 2 (1985) 5–9.
[46] M. Numata, M. Sugimoto, S. Shibayama, and T. Ogawa, *Carbohydr. Res.*, 174 (1988) 73–85.
[47] T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 188 (1989) 71–80.
[48] M. Numata, M. Sugimoto, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 203 (1990) 205–217.
[49] Y. Ito and J.C. Paulson, *J. Am. Chem. Soc.*, 115 (1993) 1603–1605.
[50] S. Sabesan and J.C. Paulson, *J. Am. Chem. Soc.*, 108 (1986) 2068–2080.
[51] J. Weinstein, U. de Souza-e-Silva, and J.C. Paulson, *J. Biol. Chem.*, 257 (1982) 13,845–13,853.