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Synthesis of carbosilane dendrimers having peripheral mannose and mannobiose

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Abstract—The mannose monosaccharide derivative, acetylthiopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (Man), and the mannobiose derivative, acetylthiopropyl 2,4,6-tri-*O*-acetyl-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (α-1,3-Man), were synthesized respectively. These mannose derivatives were introduced into carbosilane dendrimer scaffolds of the zero and first generations. As a result, six carbosilane dendrimers were functionalized by Man and α-1,3-Man. Isothermal titration microcalorimetry was done to determine binding assay between mannose moieties of carbosilane dendrimer and concanavalin A. It was found that carbosilane dendrimers bound more efficiently to concanavalin A than free mannose (Me-α-Man) and mannobiose (Me-α-1,3-Man).

1. Introduction

Oligosaccharide chains in natural glycoconjugates which contain glycoproteins, glycolipids, and proteoglycans components of extracellular matrixes and cell surfaces play crucial roles in a variety of biological systems.[1,2] Mannose is one of the important and characteristic monosaccharides in *N*-glycans (asparagine-linked oligosaccharides).[3] A group of *N*-glycans, which contain high levels of mannose residues, is called a high-mannose type. The majority of nascent peptides in the endoplasmic reticulum (ER) are *N*-glycosylated with high-mannose type oligosaccharides.[4,5] The functions of high-mannose type oligosaccharides in the ER glycoprotein quality control have attracted recent attention.[6]

The interactions between lectins (carbohydrate-binding proteins),[7] and carbohydrates in glycoconjugates play principal roles in many cellular recognition processes.[8] At the monosaccharide level these interactions typically have weak affinities (K_D in mM). However, multivalent carbohydrates are known to greatly enhance interaction between the binding proteins (lectins) and the ligands involving carbohydrates.[9] This phenomenon, called 'the cluster effect',[10] enticed to generate a large number of functional neoglyconconjugates to achieve superior binding affinity.[11] Dendrimers, one of the typical forms to manifest the cluster effect, are targets of intensive investigation of the cluster effect, because their structures are easy to control and prepare.[12] Glycodendrimers with peripheral mannosyl group as one of the neoglycoconjugates have been shown to mimic the structure and functions of the high mannose type *N*-glycans.[13–15]

Large number of dendrimers with mannose moieties have been synthesized.[13–15] However, only one case of glycocoating carbosilane dendrimer with mannose moiety has been synthesized by Lindhorst et al.[16] as far as we know. They described the pathway to introduce mannose derivatives into carbosilane dendrimer scaffold via a hydrosilylation reaction of a protected allyl mannoside with a carbosilane containing Si–H end groups in the presence of a platinum catalyst, thus leading to an Si–C linked structure.

The carbosilane dendrimer scaffold is easy to control the number of branches at each generation and the chain length between the terminal silicon. We have been preparing

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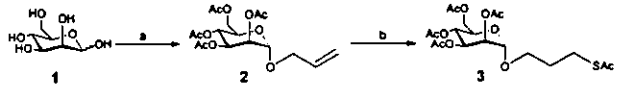
carbosilane dendrimers with peripheral functional carbohydrate moieties by a different route.[17] Our approach to vary the molecular design of carbohydrates containing carbosilane dendrimers is to control the methylene chain length of dendrimer scaffolds and the aglycon moiety length of carbohydrates. Peripheral globotriose clustered on carbosilane dendrimers were synthesized for the purpose of neutralizing Shiga-toxin producing *Escherichia coli* O157:H7.[18]

In this article, we describe syntheses of carbosilane dendrimers with peripheral mannose, and their characterization by spectrometric methods. We also determined the binding assay of concanavalin A (Con A), by the means of isothermal titration microcalorimetry (ITC).

2. Results and discussion

2.1. Preparation of mannose monosaccharide derivative (Man)

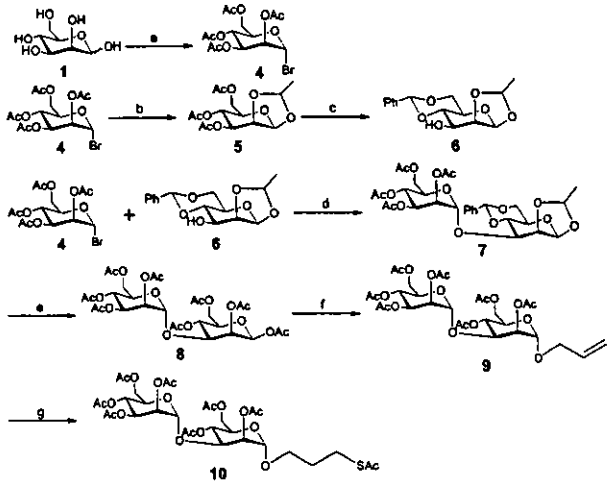
Scheme 1 summarizes the synthetic steps of mannose monosaccharide derivative, acetylthiopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (Man; **3**). The treatment of penta-*O*-acetylmannopyranose with allyl alcohol in the presence of borone trifluoride diethyl ether complex as Lewis acid produces allyl tetraacetylmannose (**2**).[17b,19] Compound **3** was synthesized by the anti-Markovnikov addition of the thio group to the allyl moiety of **2** although **3**, which is synthesized by another synthetic method of activating 2,2′-azobisisobutyronitrile (AIBN) irradiated in a photochemical reactor.[20] In this reaction, AIBN was activated by heat at 80 °C.[17d,e] Each NMR signal of **3** was assigned by following measurements: ^{1}H, ^{13}C, DEPT, HH, and HC COSY. Chemical shifts of **3** are described in Section 4.



Scheme 1. (a) $AcONa/Ac_2O$, then allyl alcohol, BF_3-OEt_2/CH_2Cl_2, 70% (2 steps); (b) AcSH, AIBN/1,4-dioxane, 73%.

2.2. Preparation of mannose disaccharide derivative (α-1,3-Man)

Mannose disaccharide derivative, 1-*O*-(3′-acetylthiopropyl)-2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-D-mannopyranosyl) D-mannopyranose (α-1,3-Man; **10**), was synthesized starting from D-mannose (Scheme 2). D-Mannose was converted to 1-bromo 2,3,4,6-tetra-*O*-acetyl mannose (**4**)[21] which will be used as a glycosyl acceptor and will also lead to a donor. Compounds **5** and **6** were synthesized by the method described in the literature:[22,23] 1,2-*O*-ethylidene protection of **4** was prepared by using $NaBH_4$ in acetonitrile at room temperature,[22] then 4,6-*O*-benzylidene protection of **5** using benzaldehyde dimethyl acetal and 1,10-camphorsulfonic acid.[23] Glycosylation of **6** with **4** in the presence in AgOTf, the reagent which was used for the formation of α-glycoside, in dichloromethane at −20 °C proceeded stereoselectively to give **7**. Compound **7**



Scheme 2. (a) HBr-AcOH, Ac_2O, quant; (b) $NaBH_4$/MeCN, 64%; (c) NaOMe/MeOH, then $PhCH(OMe)_2$, CSA/DMF, quant. (2 steps); (d) AgOTf, MS4A/CH_2Cl_2, 66%; (e) 90% CF_3COOH aq, then $AcONa/Ac_2O$, 64% (2 steps); (f) allyl alcohol, BF_3-OEt_2/CH_2Cl_2, 43%; (g) AcSH, AIBN/1,4-dioxane, 97%.

was assigned by measurements of the high resolution mass and NMR spectra to form the α-1,3-glycoside bond with both mannose moieties. Next **7** was treated with aqueous trifluoroacetic acid (90% v/v) to remove both 1,2-*O*-ethylidene and 4,6-*O*-benzylidene groups, followed by acetylation with acetic anhydride and sodium acetate to provide **8**.[24] 1-Allylation[17b,19] and thioacetylation[17d,e] of the allyl moiety were synthesized by the same method to mannose monosaccharide to give **10**.

2.3. Preparation of carbosilane dendrimders having peripheral mannose

For the introduction of mannose derivatives, we used three carbosilane dendrimer scaffolds: three-branched (Fan(0)3-Br), four-branched (Ball(0)4-Br), and six-branched (Dumbbell(1)6-Br), as described in Figure 1. Fan(0)3-Br and Ball(0)4-Br are the zero generation scaffolds which were prepared with triallylphenylsilane and tetraallylsilane by following three reaction steps: hydroxylation, mesylation, and bromination.[17b,25] On the other hand, Dumbbell(1)6-Br is the first generation carbosilane dendrimer scaffold,

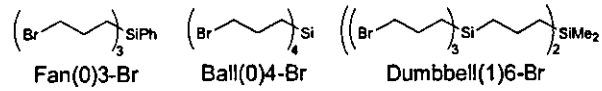
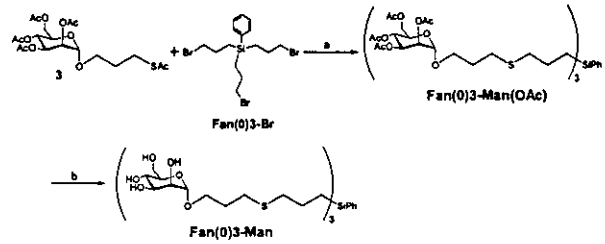


Figure 1. Carbosilane dendrimer scaffolds.



Scheme 3. (a) NaOMe/MeOH, DMF, then Ac_2O/pyridine, 66% (2 steps) and (b) NaOMe/MeOH, then 0.1 mol/l NaOH aq, 61%.

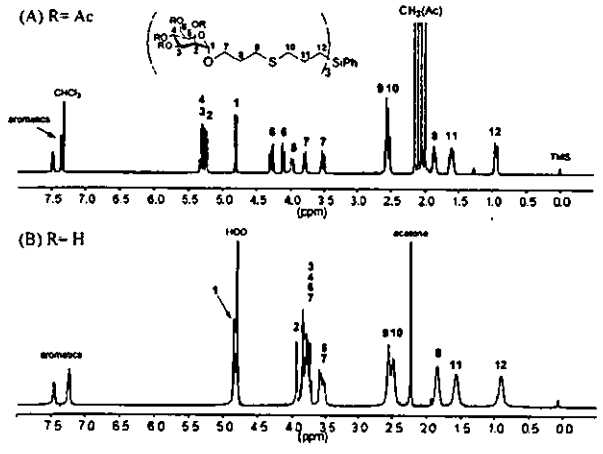


Figure 2. ^{1}H NMR spectra (400 MHz, $CDCl_3$ or D_2O): (A) Fan(0)3-Man(OAc) and (B) Fan(0)3-Man.

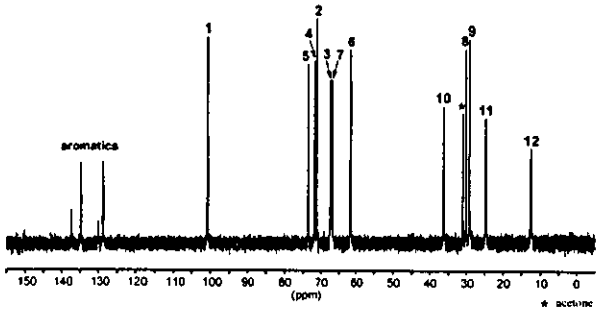


Figure 3. ^{13}C NMR spectrum (100 MHz, D_2O) of Fan(0)3-Man.

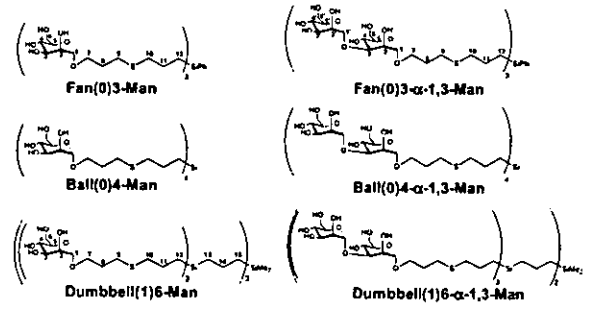


Figure 4. Carbosilane dendrimers having peripheral mannose and mannobiose moieties.

prepared with allylation of dichlorodimethylsilane followed by hydrosilylation[17d,25,26] with the first generation skeleton. The resulting reactions were the same as for the zero generation carbosilane dendrimer scaffolds.

Introduction of Man and Man-α-1,3-Man to carbosilane dendrimer scaffolds was done concurrently with deacetylation, that is, using sodium methoxide/methanol and *N,N*-dimethylformamide (Scheme 3). This reaction includes de-*O*- and -*S*-acetylation, followed by S_N2 replacement reaction, and then acetylation for purification by silica gel and gel permeation chromatography. After purification by means of recycling GPC, products of mannose-coated carbosilane dendrimers were obtained, and disulfide byproducts (Man-SS-Man or α-1,3-Man-SS-α-1,3-Man) was removed.

In summary, six carbosilane dendrimers were synthesized, and functionalized with acetyl-protected derivatives of mannose or mannose disaccharide (α-1,3-Man). The yields of addition of mannose monosaccharide were 62–76%, and those of α-1,3-Man were 30–35%. The difference in the yields between the mannose and mannobiose derivatives may be due to the bulkier structure of mannobioside.

All synthesized dendrimers were characterized by ^{1}H and ^{13}C NMR and high resolution mass spectrometry. From the results of high resolution mass spectrometry, the proton or sodium ion adduct peaks, $[M+H]^+$ or $[M+Na]^+$, were determined and these showed good agreement with the calculated values, within the ± 5 ppm error margins. Moreover, from the measurement of ^{1}H NMR measurements, we found the new signal at ca. 2.5 ppm (Fig. 2A) which showed that a bond was formed between the sulfur atom of saccharide moiety and the methylene carbon of the corresponding carbosilane dendrimer scaffold. Thus, these spectrometric results confirmed the structures of a carbosilane dendrimer with peripheral mannose and mannobiose.

The dendrimers with acetylated mannose moieties were deacetylated by sodium methoxide/methanol, deacetylation is saponification to yield the corresponding carbosilane dendrimers with peripheral mannose and mannose disaccharide, and then purified by gel filtration. All six types of carbosilane dendrimers functionalized by peripheral mannose moieties were synthesized and characterized by the measurements of ^{1}H and ^{13}C NMR, and high resolution mass spectrometry. Figures 2B and 3 show ^{1}H and ^{13}C NMR spectra of Fan(0)3-Man, respectively. Signals of methyl proton from the acetyl groups in mannose moiety

Table 1. ^{13}C NMR spectroscopic data (δ values) of carbosilane dendrimers functionalized peripheral mannose moieties (I)

Mannose moieties	C-1 C-1′	C-2 C-2′	C-3 C-3′	C-4 C-4′	C-5 C-5′	C-6 C-6′
Fan(0)3-Man	100.7	71.1	67.2	71.7	73.5	61.5
Ball(0)4-Man	100.6	71.0	67.2	71.6	73.4	61.5
Dumbbell(1)6-Man	100.1	70.7	66.8	71.2	72.8	60.9
Fan(0)3-α-1,3-Man	100.5	70.5	79.3	66.7	73.8	61.1
	102.9	70.8	71.0	67.1	73.4	61.4
Ball(0)4-α-1,3-Man	100.6	70.6	79.4	66.9	73.8	61.3
	103.0	70.9	71.1	67.3	73.5	61.5
Dumbbell(1)6-α-1,3-Man	100.7	70.7	79.6	67.0	74.0	61.5
	103.1	71.0	71.3	67.5	73.6	61.8

Table 2. ^{13}C NMR spectroscopic data (δ values) of carbosilane dendrimers functionalized peripheral mannose moieties (II)

Dendrimer scaffolds	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15
Fan(0)3-Man	66.9	30.1	29.1	36.2	24.6	12.4			
Ball(0)4-Man	66.9	30.1	29.1	36.3	24.8	12.5			
Dumbbell(1)6-Man	66.3	29.5	28.6	35.8	24.4	12.0	19.1	20.3	21.0
Fan(0)3-α-1,3-Man	66.1	29.8	28.9	35.9	24.1	12.1			
Ball(0)4-α-1,3-Man	66.3	29.8	29.0	36.2	24.8	12.4			
Dumbbell(1)6-α-1,3-Man	66.4	30.0	29.2	36.5	25.0	12.6	18.1	19.4	21.0

disappeared in Figure 2B, which is distinct from Figure 2A. This is in agreement with the results of ^{13}C NMR measurements. Figure 4 shows all six carbosilane dendrimers. Signals of ^{13}C NMR spectra are all assigned and partly listed in Table 1 (mannose moieties) and Table 2 (carbosilane dendrimer scaffolds). Signals from mannose moieties were assigned according to the published results.[24e,27] In Table 2, each signal displays good agreement in spite of the differences between dendrimer scaffolds and saccharides. Characteristic signals are observed on Fan(0)3- and Dumbbell(1)6-scaffolds: these were four signals of phenyl carbons at about 130 ppm on Fan(0)3 types, and the signal of methyl carbon binding core silicon atom at about −2 ppm on Dumbbell(1)6 types.

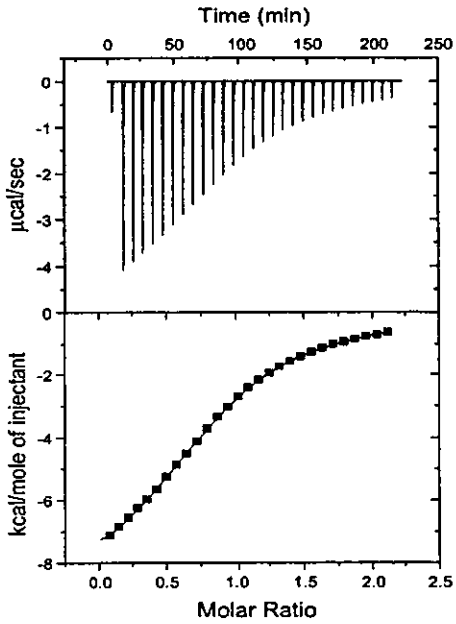


Figure 5. Calorimetric data for titration of concanavalin A, 0.21 mM, with trivalent ligand Fan(0)3-Man, 2.1 mM. Both protein and ligand were dissolved in buffer consisting of 50 mM 3,3-dimethylglutarate, 250 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$ adjusted pH 5.2. Top, raw (power vs time) data; bottom integrated heat vs molar ratio of ligand. Solid line shows best fit of data using a one-site model: $n=0.85$; $K=2.09\times10^4$; $\Delta H=-9.2$ kcal mol^{-1}. A fit to a two-site model does not provide a statistically superior fit.

2.4. Binding affinity between carbosilane dendrimers and concanavalin A

Con A is one of the most widely used lectins in biological studies. Mannose residue is recognized specifically by Con A. Recently, isothermal titration microcalorimetry (ITC) has been utilized to study protein–carbohydrate interaction.[10a] A soluble protein is titrated with aliquots of a soluble ligand in this measurement. The heat produced during ligand addition serves as a reporter signal for binding, which is yielded a binding constant, which, in turn can be related to the free energy of binding. Since this technique also directly measures binding enthalpies, an entropy of binding can be readly calculated.

The bindings of tri-, tetra-, and hexavalent ligands Fan(0)3-Man, Ball(0)4-Man, and Dumbbell(1)6-Man to dimeric Con A (pH 5.2) were evaluated by titration microcalorimetry. The titration microcalorimetry of all multivalent ligands yielded curves indicative of simple reversible binding. Figure 5 shows one of the titration curves for the carbosilane dendrimer with peripheral mannose moieties (Fan(0)3-Man) when bound to Con A in glutarate buffer (top) and the resulting one-site fit of the integrated differential power signal with respect to time (bottom). Table 3 lists the calculated binding constant and other parameters of binding between the carbosilane dendrimers and Con A. All of the carbosilane dendrimers have higher binding constant values with Con A, K, than the non-dendric mannose derivatives, Me-α-Man and Me-α-1,3-Man,[28] demonstrating cluster glycoside effect. As for monosaccharide types of the

Table 3. Binding of carbosilane dendrimers to concanavalin A

	K/M^{-1}	ΔG	ΔH	$T\Delta S^a$
		kcal mol^{-1}		
Man	4.2×10^3	−4.9	−2.8	2.2
Me-α-Man[b]	7.6×10^3	−5.3	−6.8	−1.5
Fan(0)3-Man	2.1×10^4	−5.9	−9.2	−3.3
Ball(0)4-Man	2.2×10^4	−5.9	−5.6	0.3
Dumbbell(1)6-Man	6.0×10^4	−6.5	−3.6	3.0
Me-α-1,3-Man[b]	3.0×10^4	−6.0	−7.4	−1.4
Fan(0)3-α-1,3-Man	7.9×10^4	−6.7	−14.1	−7.4
Ball(0)4-α-1,3-Man	9.1×10^4	−6.8	−9.8	−3.0
Dumbbell(1)6-α-1,3-Man	6.1×10^4	−6.5	−4.2	2.3

[a] 298 K.
[b] See Ref. 28c.

synthesized carbosilane dendrimers, the magnitude of the effects depend on the amount of mannose in a dendrimer. In the case of three-branched dendrimer scaffolds having peripheral mannose, K value of the carbosilane dendrimer was higher than that of the non-carbosilane dendrimer, and other thermodynamic parameters were similar values.[28d] However, the multivalency effect was not clearly measured in the mannobiose-type of carbosilane dendrimers, because these dendrimers became highly aggregated during titration and the orientation of the saccharides could not match tightly to the binding pockets of Con A.

3. Conclusion

We synthesized six carbosilane dendrimers with peripheral mannose and mannobiose. The structures of these dendrimers were characterized by measurements of NMR and mass spectrometry. Isothermal titration microcalorimetry (ITC) was done for determining the binding assay between the carbosilane dendrimer and concanavalin A (Con A). It was found that the carbosilane dendrimers bound to Con A more frequently than to free mannose (Me-α-Man) and mannobiose (Me-α-1,3-Man), thus showing the cluster effect.

4. Experimental

4.1. Analyses and GPC

NMR spectra were recorded with a Bruker DRX-400, AM-400, and a Valian Gemini-2000 spectrometer. Fast atom bombardment (FAB) and electron spray ionization (ESI) mass spectra were obtained with a JEOL JMS-HX110A spectrometer and a JEOL JMS-T100LC spectrometer, respectively. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Isothermal titration microcalorimetry was performed using the MicroCal Omega titration microcalorimeter. High resolution mass spectrometry (HRMS) measurements were valid to ±5 ppm. Recycling preparative GPC was performed with a LC-908W (Japan Analytical Industry Co., Ltd) connected to an RI detector RI-5 (column, JAIGEL-1H-A and JAIGEL-2H-A; solvent, chloroform).

4.2. Materials

Concanavalin A (Type IV, lot No. 102K7044) was purchased from Sigma Chemical Company and dialyzed with a glutarate buffer. Protein concentration was determined by the method of Edelhoch.[29] Carbohydrate concentrations were determined by phenol-sulfuric acid method.[30] For calorimetric measurements, water was purified with a Millpore purification system that involved passage through reverse osmosis, charcoal, and two ion exchange filters to attain resistance of $>10\ M\Omega\ cm^{-1}$.

4.3. Reactions

4.3.1. Acetylthiopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (3). D-Mannose (5.00 g, 27.8 mmol) was acetylated to yield penta-*O*-acetyl-α-D-mannose by using a mixture of sodium acetate (2.51 g, 30.62 mmol) and acetic anhydride (25.0 mL, 263 mmol). Under an argon atmosphere, penta-*O*-acetyl-α-D-mannopyranose was dissolved in dry-dichloromethane (123 mL) and allyl alcohol (9.50 mL, 139 mmol) was added, then the mixture was cooled to −5 °C. Boron trifluoride diethyl ether complex (94 mL, 742 mmol) was dropped into the solution. The reaction solution was stirred for 30 min at 0 °C, then stirred for 54 h at room temperature. After the reaction, the solution was poured into ice-water, washed with water, saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with toluene–ethyl acetate (5:1 (v/v)) as eluent to yield pure **2** (7.53 g, 70% (2 steps)).

To a stirred solution of **2** (3.65 g, 9.40 mmol) and thioacetic acid (13.4 mL, 188 mmol) in 1,4-dioxane (2.0 mL), 2,2′-azobisisobutyronitrile (AIBN; 7.72 g, 47.0 mmol) was added at 50 °C under an argon atmosphere. The mixture was stirred for 2.5 h at 80 °C, then cooled to room temperature. Cyclohexene (5.0 mL, 49.3 mmol) was added, and the mixture was stirred at room temperature for 30 min. After evaporation, silica gel chromatography of the residual syrup (toluene–ethyl acetate 10:1–5:1–3:1) yielded sulfide **3** (3.16 g, 73%): ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.33 (1H, m, H-3), 5.28 (1H, m, H-4), 5.24 (1H, dd, H-2, $J_{1,2}=1.61$ Hz, $J_{2,3}=3.21$ Hz), 4.81 (1H, H-1), 4.28 (1H, dd, H-6a, $J_{5,6a}=5.35$ Hz, $J_{6a,6b}=12.31$ Hz), 4.11 (1H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.31$ Hz), 3.98 (1H, m, H-5), 3.77 (1H, m, $OCH_2CH_2CH_2S$), 3.52 (1H, m, $OCH_2CH_2CH_2S$), 2.97 (2H, t, $OCH_2CH_2CH_2S$, $J=6.96$ Hz), 2.34 (3H, s, CH_3(SAc)), 2.16, 2.12, 2.06, 2.00 (12H, s, CH_3(OAc)), 1.91 (2H, m, $OCH_2CH_2CH_2S$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 195.2 (C, C=O(SAc)), 170.3, 169.7, 169.5, 169.4 (C, C=O(Ac)), 97.4 (CH, C-1), 69.2 (CH, C-2), 68.8 (CH, C-3), 68.3 (CH, C-5), 66.5 (CH_2, $OCH_2CH_2CH_2S$), 65.8 (CH, C-4), 62.2 (CH_2, C-6), 30.3 (CH_3, CH_3(SAc)), 28.9 (CH_2, $OCH_2CH_2CH_2S$), 25.5 (CH_2, $OCH_2CH_2CH_2S$), 20.6, 20.5, 20.41, 20.39 (CH_3, CH_3(OAc)).

4.3.2. 4,6-*O*-Benzylidene-1,2-ethylidene-β-D-manno-pyranoside (6).[23] Under an argon atmosphere, 1-bromo 2,3,4,6-tetra-*O*-acetyl mannose ((**4**)[21]; 22.9 g, 55.6 mmol) was dissolved in acetonitrile (130 mL), and sodium borohydrate (10.5 g, 278 mmol) was added, then the mixture was stirred for 22 h at room temperature. After the reaction, the solution was diluted with ethyl acetate, washed with water and brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with *n*-hexane–ethyl acetate (5:1–3:1–2:1) yielded pure **5**[22] (11.8 g, 64%).

Under an argon atmosphere, **5** (5.73 g, 17.3 mmol) was dissolved in methanol (5.0 mL), and sodium methoxide (0.14 g, 2.60 mmol) was added, then the mixture was stirred for 1 h at room temperature. After the reaction, IR120B (H^+) resin was added to neutralize the reaction solution, and the suspension was filtered and evaporated. The residue was dissolved in *N*,*N*-dimethylformamide (15.0 mL).

Benzaldehyde dimethylacetal (3.70 mL, 24.6 mmol) and (+)-10-camphorsulfonic acid (379 mg, 1.63 mmol) was added, and the mixture was stirred over evaporation for 6 h at 30 °C. The solution was cooled to room temperature, and triethylamine (0.45 mL, 3.34 mmol) added to neutralize. The solution was evaporated, and purified by silica gel column chromatography with *n*-hexane–ethyl acetate (10:1–5:1–3:1–1:1) as eluent to yield pure **6** (5.08 g, quant. (2 steps)).

4.3.3. 4,6-*O*-Benzylidene-1,2-ethylidene-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (7). 4,6-*O*-Benzylidene-1,2-ethylidene-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**7**). A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**4**) (298 mg, 0.72 mmol) and 4,6-*O*-benzylidene-1,2-ethylidene-β-D-mannopyranoside (**6**) (100 mg, 0.34 mmol) in anhydrous dichloromethane (8.0 mL) was stirred in the presence of activated MS4A (1.0 g) and silver trifluoromethanesulfonate (228 mg, 0.89 mmol) was added under an argon atmosphere. The reaction mixture was stirred for 2 h at −20 °C. Further, silver trifluoromethanesulfonate (113 mg, 0.44 mmol) was added to the mixture under an argon atmosphere, and the mixture was stirred for 40 min at −20 °C. Sodium carbonate (302 mg, 2.85 mmol) was added to the reaction solution, then the solution was filtered through a celite bed, diluted with chloroform, washed with saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. Then the solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with toluene–ethyl acetate (5:1) as eluent to yield pure **7** (139 mg, 66%): HRMS (ESI); calcd for $C_{29}H_{36}O_{15}Na$ $[M+Na]^+$ 647.1952, found 647.1936. $[\alpha]_D^{33}=-16.2°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 7.45–7.33 (5H, m, Ph), 5.59 (1H, s, C*H*(4,6-bndn)), 5.45 (1H, m, H-3′), 5.40 (1H, m, H-4′), 5.34 (1H, q, $J=5.35$ Hz, C*H*-(1,2-etdn)), 5.30–5.25 (2H, m, H-1, 2′), 5.19 (1H, d, $J_{1',2'}=1.61$ Hz, H-1′), 4.33–4.22 (4H, m, H-2, 3, 6a, 6′a), 4.11–4.04 (3H, m, H-4, 5′, 6′b), 3.78 (1H, m, H-6b), 3.39 (1H, m, H-5), 2.11, 2.09, 2.05, 1.99 (12H, s, Ac), 1.54 (3H, d, $J=5.35$ Hz, C*H*$_3$-(1,2-etdn)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.5, 169.8, 169.7, 169.6 (C, *C*=O (Ac)), 136.9 (C, *C* (Ph)), 128.9, 128.2, 125.9 (CH, *C*H(Ph)), 104.7 (CH, *C*H (1,2-etdn)), 101.1 (CH, *C*H (4,6-bndn)), 99.5 (CH, C-1′), 96.8 (CH, C-1), 79.6 (CH, C-3), 76.9 (CH, C-4), 75.9 (CH, C-5′), 69.3 (CH, C-4′), 68.80 (CH, C-3′), 68.77 (CH, C-2), 68.4 (CH_2, C-6), 66.4 (CH, C-2′), 65.7 (CH, C-5), 62.6 (CH_2, C-6′), 21.8 (CH_3, *C*H$_3$-(etdn)), 20.77, 20.75, 20.70, 20.66 (CH_3, *C*H$_3$-(Ac)).

4.3.4. Allyl 2,4,6-tri-*O*-acetyl-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (9). A solution of **7** (860 mg, 1.38 mmol) in 90% (v/v) aqueous trifluoroacetic acid (10 mL) was stirred for 22 h at room temperature. The solution was cooled in an ice-water bath and neutralized with sodium carbonate. Then the solution was evaporated and dried with a vacuum pump. Sodium acetate (229 mg, 2.79 mmol) and acetic anhydride (15 mL, 158 mmol) were added to the residue, and the reaction mixture was stirred for 1 h at 110 °C. To the reaction mixture was added ice-water, and the mixture was extracted with chloroform. The extract was washed with saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with *n*-hexane–ethyl acetate (1:1–1:2) as eluent to yield pure **8** (598 mg, 64% (2 steps)).

Under an argon atmosphere, **8** (4.08 g, 6.01 mmol) was dissolved in dry-dichloromethane (27 mL) and allyl alcohol (2.1 mL, 30.7 mmol) was added, then cooled to −5 °C. Boron trifluoride diethyl ether complex (8.0 mL, 63.1 mmol) was dropped into the solution. The reaction solution was stirred for 30 min at 0 °C, then stirred for 71 h at room temperature. After the reaction, the solution was poured onto ice-water, washed with water, saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with toluene–ethyl acetate (5:1–3:1–2:1–1:1–0:1) as eluent to yield pure **9** (1.73 g, 43%): ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.87 (1H, m, $OCH_2CH{=}CH_2$), 5.34–5.18 (6H, m, H-2, 3, 4, 3′, $OCH_2CH{=}CH_2$), 5.01 (1H, m, H-2′), 5.00 (1H, d, H-1′ $J_{1',2'}=1.61$ Hz), 4.88 (1H, H-1), 4.30–3.98 (8H, m, H-6, 4′, 5′, 6′, $OCH_2CH{=}CH_2$), 3.90 (1H, ddd, H-5, $J_{4,5}=10.17$ Hz, $J_{5,6a}=5.35$ Hz, $J_{5,6b}=2.68$ Hz), 2.21, 2.14, 2.13, 2.113, 2.106, 2.06, 1.99 (21H, s, CH_3(OAc)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.65, 170.62, 170.4, 170.0, 169.9, 169.8, 169.5 (C, *C*=O(Ac)), 132.8 (CH, $OCH_2CH{=}CH_2$), 118.5 (CH_2, $OCH_2CH{=}CH_2$), 98.8 (CH, C-1′), 96.5 (CH, C-1), 74.6 (CH, C-3), 70.9 (CH, C-2), 69.9 (CH, C-2′), 69.3 (CH, C-5′), 68.7 (CH, C-5), 68.5 (CH_2, $OCH_2CH{=}CH_2$), 68.2 (CH, C-3′), 67.7 (CH, C-4′), 65.9 (CH, C-4), 62.5, 62.4 (CH_2, C-6, 6′), 20.9, 20.8, 20.73, 20.71, 20.63, 20.60, 20.59 (CH_3, *C*H$_3$(Ac)).

4.3.5. Acetylthiopropyl 2,4,6-tri-*O*-acetyl-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (10). AIBN (2.11 g, 12.8 mmol) was added to a stirred solution of **9** (1.73 g, 2.56 mmol) and thioacetic acid (3.7 mL, 52.0 mmol) in 1,4-dioxane (1.5 mL) at 50 °C under an argon atmosphere. The mixture was stirred for 3 h at 80 °C, then cooled to room temperature. Cyclohexene (1.5 mL, 14.8 mmol) was added, and the mixture was stirred for 30 min at room temperature. After evaporation, the residue was purified by silica gel column chromatography with toluene–ethyl acetate (10:1–5:1–3:1–2:1) and size exclusion chromatography (Sephadex LH-20; eluent: methanol) as eluent to yield pure **10** (1.87 g, 97%): HRMS (ESI); calcd for $C_{31}H_{44}O_{19}SNa$ $[M+Na]^+$ 775.2095, found 775.2065. $[\alpha]_D^{33}=+33.8°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.29, 5.26 (2H, m, H-3, 4), 5.23–5.19 (2H, m, H-2, 3′), 5.02 (1H, dd, H-2′, $J_{1',2'}=1.61$ Hz, $J_{2',3'}=2.14$ Hz), 5.01 (1H, d, H-1′, $J_{1',2'}=1.61$ Hz), 4.82 (1H, d, H-1, $J_{1,2}=1.60$ Hz), 4.31–4.21 (2H, m, H-6a, 6′a), 4.16 (1H, dd, H4′, $J_{3',4'}=3.75$ Hz, $J_{4',5'}=10.17$ Hz), 4.13–4.04 (3H, m, H-6b, 5′, 6′b), 3.86 (3H, m, H-5), 3.73 (1H, m, $OCH_2CH_2CH_2S$), 3.50 (1H, m, $OCH_2CH_2CH_2S$), 2.94 (2H, t, $OCH_2CH_2CH_2S$, $J=6.96$ Hz), 2.34 (3H, s, CH_3(SAc)), 2.21, 2.14, 2.13, 2.12, 2.11, 2.06, 2.00 (21H, s, CH_3(OAc)), 1.88 (2H, m, $OCH_2CH_2CH_2S$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 195.2 (C, *C*=O(SAc)), 170.5, 170.4, 170.2, 169.8, 169.7,

169.6, 169.4 (C, *C*=O(OAc)), 98.8 (CH, C-1′), 97.3 (CH, C-1), 74.8 (CH, C-3), 70.7 (CH, C-2), 69.8 (CH, C-2′), 69.2 (CH, C-5′), 68.7 (CH, C-5), 68.1 (CH, C-3′), 67.5 (CH, C-4′), 66.4 (CH_2, $OCH_2CH_2CH_2S$), 65.7 (CH, C-4), 62.4, 62.2 (CH_2, C-6, 6′), 30.4 (CH_3, CH_3(SAc)), 29.1 (CH_2, $OCH_2CH_2CH_2S$), 25.6 (CH_2, $OCH_2CH_2CH_2S$), 20.8, 20.7, 20.60, 20.56, 20.49, 20.45 (CH_3, CH_3(OAc)).

4.3.6. Introduction of mannose and mannobiose into carbosilane dendrimer scaffolds: Fan(0)3-Man(OAc). Under an argon atmosphere, a mixture of **3** (348 mg, 0.75 mmol) and a dendrimer scaffold (for example, Fan(0)-Br: 56.4 mg, 0.12 mmol) was dissolved in *N*,*N*-dimethylformamide (0.5 mL) and methanol (0.5 mL), and stirred at room temperature for 20 min. Sodium methoxide in methanol solution (1.0 M, 0.75 mL, 0.75 mmol) was added to the reaction solution and stirred at room temperature over night. Acetic acid (0.1 mL) was added to the reaction solution, and stirred at room temperature for 10 min, then evaporated in vacuo. The residue was suspended in a mixture of pyridine (0.5 mL) and acetic anhydride (1.0 mL, 10.5 mmol), and stirred at room temperature over night. The reaction mixture was evaporated in vacuo, added to ice-water and chloroform, then washed with 1 M hydrochloric acid, saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with hexane–ethyl acetate (1:1–1:2–0:1) as the eluent to produce pure Fan(0)3-Man(OAc): Yield 136 mg (76% (2 steps)). HRMS (ESI): Calcd for $C_{66}H_{98}O_{30}S_3SiNa$ $[M+Na]^+$ 1517.4972, found 1517.4990. $[\alpha]_D^{32}=+42.3°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 7.50–7.34 (5H, Ph), 5.34–5.24 (6H, m, H-3, 4), 5.23 (3H, dd, H-2, $J_{1,2}=1.60$ Hz, $J_{2,3}=2.14$ Hz), 4.81 (3H, d, H-1, $J_{1,2}=1.60$ Hz), 4.28 (3H, dd, H-6a, $J_{5,6a}=5.35$ Hz, $J_{6a,6b}=12.32$ Hz), 4.11 (3H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.32$ Hz), 3.98 (3H, m, H-5), 3.80 (3H, m, H-7a), 3.52 (3H, m, H-7b), 2.55 (6H, t, H-9, $J_{8,9}=6.96$ Hz), 2.53 (6H, t, H-10, $J_{10,11}=6.96$ Hz), 2.16 (9H, s, CH_3(Ac)), 2.10 (9H, s, CH_3(Ac)), 2.04 (9H, s, CH_3(Ac)), 1.99 (9H, s, CH_3(Ac)), 1.86 (6H, m, H-8), 1.60 (6H, m, H-11), 0.94 (6H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 169.8, 169.7, 169.5 (C, *C*=O(Ac)), 136.0 (C, Ph), 133.8, 129.0, 127.8 (CH, Ph), 97.4 (CH, C-1), 69.4 (CH, C-2), 68.9 (CH, C-3), 68.3 (CH, C-5), 66.4 (CH_2, C-7), 66.0 (CH, C-4), 62.2 (CH_2, C-6), 35.6 (CH_2, C-10), 28.9 (CH_2, C-8), 28.4 (CH_2, C-9), 23.8 (CH_2, C-11), 20.7, 20.6, 20.53, 20.51 (CH_3, CH_3(Ac)), 11.7 (CH_2, C-12).

Another carbosilane dendrimer with peripheral mannose or mannobiose acetate was prepared by the same method as Fan(0)3-Man. The mannobiose-bearing carbosilane dendrimers were synthesized using compound **10**.

Ball(0)4-*Man*(*OAc*). Yield 120.3 mg (66% (2 steps)). HRMS (FAB): Calcd for $C_{80}H_{125}O_{40}S_4Si$ $[M+H]^+$ 1881.6399, found 1881.6445. $[\alpha]_D^{29}=+45.1°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.32 (1H, m, H-3), 5.28 (1H, m, H-4), 5.23 (1H, dd, H-2, $J_{1,2}=1.60$ Hz, $J_{2,3}=2.14$ Hz), 4.82 (1H, d, $J_{1,2}=1.60$ Hz, H-1), 4.29 (1H, dd, H-6a, $J_{5,6a}=5.35$ Hz, $J_{6a,6b}=12.32$ Hz), 4.12 (1H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.32$ Hz), 3.99 (1H, ddd, H-5, $J_{4,5}=9.63$ Hz, $J_{5,6a}=5.35$ Hz, $J_{5,6b}=2.14$ Hz), 3.83 (1H, m, H-7a), 3.56 (1H, m, H-7b), 2.60 (2H, t, H-9, $J_{8,9}=6.96$ Hz), 2.53 (2H, t, H-10, $J_{10,11}=6.96$ Hz), 2.16 (3H, s, CH_3(Ac)), 2.11 (3H, s, CH_3(Ac)), 2.05 (3H, s, CH_3(Ac)), 2.00 (3H, s, CH_3(Ac)), 1.90 (2H, m, H-8), 1.58 (2H, m, H-11), 0.67 (2H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 169.8, 169.6, 169.5 (C, *C*=O(Ac)), 97.4 (CH, C-1), 69.3 (CH, C-2), 68.9 (CH, C-3), 68.3 (CH, C-5), 66.4 (CH_2, C-7), 65.9 (CH, C-4), 62.2 (CH_2, C-6), 35.7 (CH_2, C-10), 28.9 (CH_2, C-8), 28.4 (CH_2, C-9), 23.9 (CH_2, C-11), 20.7, 20.54, 20.48, 20.45 (CH_3, CH_3(Ac)), 11.7 (CH_2, C-12).

Dumbbell(1)6-*Man*(*OAc*). Yield 141.6 mg (62% (2 steps)). HRMS (FAB): Calcd for $C_{128}H_{205}O_{60}S_6Si_3$ $[M+H]^+$ 2978.0622, found 2978.0669. $[\alpha]_D^{29}=+40.1°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.32 (3H, m, H-3), 5.28 (3H, m, H-4), 5.23 (3H, dd, H-2, $J_{1,2}=1.61$ Hz, $J_{2,3}=2.14$ Hz), 4.82 (3H, d, $J_{1,2}=1.61$ Hz, H-1), 4.29 (3H, dd, H-6a, $J_{5,6a}=4.82$ Hz, $J_{6a,6b}=12.32$ Hz), 4.12 (3H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.32$ Hz), 3.99 (3H, ddd, H-5, $J_{4,5}=9.63$ Hz, $J_{5,6a}=4.82$ Hz, $J_{5,6b}=2.14$ Hz), 3.83 (3H, m, H-7a), 3.55 (3H, m, H-7b), 2.60 (6H, t, H-9, $J_{8,9}=6.96$ Hz), 2.53 (6H, t, H-10, $J_{10,11}=6.96$ Hz), 2.16 (9H, s, CH_3(Ac)), 2.11 (9H, s, CH_3(Ac)), 2.05 (9H, s, CH_3(Ac)), 2.00 (9H, s, CH_3(Ac)), 1.90 (6H, m, H-8), 1.57 (6H, m, H-11), 1.31 (2H, m, H-14), 0.67–0.62 (8H, m, H-12, 13), 0.56 (2H, m, H-15), −0.04 (3H, s, CH_3(Si–Me)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 169.8, 169.6, 169.5 (C, *C*=O(Ac)), 97.4 (CH, C-1), 69.3 (CH, C-2), 68.9 (CH, C-3), 68.3 (CH, C-5), 66.4 (CH_2, C-7), 65.9 (CH, C-4), 62.2 (CH_2, C-6), 35.8 (CH_2, C-10), 28.9 (CH_2, C-8), 28.4 (CH_2, C-9), 24.0 (CH_2, C-11), 20.7, 20.55, 20.49, 20.46 (CH_3, CH_3(Ac)), 20.2 (CH_2, C-15), 18.1 (CH_2, C-14), 16.9 (CH_2, C-13), 11.9 (CH_2, C-12), −3.4 (CH_3, CH_3(Si–Me)).

Fan(0)3-α-1,3-*Man*(*OAc*). Yield 61.2 mg (30% (2 steps)). HRMS (ESI): Calcd for $C_{102}H_{146}O_{54}S_3SiNa$ $[M+Na]^+$ 2381.7508, found 2381.7485. $[\alpha]_D^{32}=+32.3°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 7.47–7.34 (5H, Ph), 5.29, 5.26 (6H, m, H-3, 4), 5.23–5.19 (6H, m, H-2, 3′), 5.02 (3H, m, H-2′), 4.99 (3H, H-1′), 4.82 (3H, H-1), 4.29–4.21 (6H, m, H-6a, 6′a), 4.13 (3H, dd, H-4′, $J_{3',4'}=3.75$ Hz, $J_{4',5'}=10.17$ Hz), 4.12–4.03 (9H, m, H-6b, 5′, 6′b), 3.86 (3H, ddd, H-5, $J_{4,5}=10.17$ Hz, $J_{5,6a}=5.36$ Hz, $J_{5,6b}=2.14$ Hz), 3.75 (3H, m, H-7a), 3.51 (3H, m, H-7b), 2.51 (12H, t, H-9, 10, $J_{8,9}=J_{10,11}=6.96$ Hz), 2.21, 2.14, 2.13, 2.102, 2.099, 2.05, 1.99 (63H, s, CH_3(Ac)), 1.84 (6H, m, H-8), 1.58 (6H, m, H-11), 0.92 (6H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 170.3, 170.2, 169.8, 169.65, 169.59, 169.4 (C, *C*=O(Ac)), 135.9 (C, Ph), 133.8, 129.1, 127.8 (CH, Ph), 98.8 (CH, C-1′), 97.2 (CH, C-1), 74.9 (CH, C-3), 70.7 (CH, C-2), 69.7 (CH, C-2′), 69.2 (CH, C-5′), 68.5 (CH, C-5), 68.0 (CH, C-3′), 67.4 (CH, C-4′), 66.3 (CH_2, C-7), 65.7 (CH, C-4), 62.3, 62.1 (CH_2, C-6, 6′), 35.6 (CH_2, C-10), 28.8 (CH_2, C-8), 28.4 (CH_2, C-9), 23.7 (CH_2, C-11), 20.7, 20.61, 20.58, 20.52, 20.46, 20.42 (CH_3, CH_3(Ac)), 11.7 (CH_2, C-12).

Ball(0)4-α-1,3-*Man*(*OAc*). Yield 81.1 mg (35% (2 steps)). HRMS (FAB): Calcd for $C_{128}H_{189}O_{72}S_4Si$ $[M+H]^+$ 3033.9780, found 3033.9751. $[\alpha]_D^{32}=+33.9°$ ($c=1.0$ in

$CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.30, 5.26 (2H, m, H-3, 4), 5.23–5.19 (2H, m, H-2, 3′), 5.02 (1H, m, H-2′), 5.00 (1H, H-1′), 4.83 (1H, H-1), 4.29–4.21 (2H, m, H-6a, 6′a), 4.14 (1H, m, H4′), 4.13–4.03 (3H, m, H-6b, 5′, 6′b), 3.88 (1H, m, H-5), 3.79 (1H, m, H-7a), 3.54 (1H, m, H-7b), 2.56 (2H, t, H-9, $J_{8,9}$=6.96 Hz), 2.51 (2H, t, H-10, $J_{10,11}$=6.96 Hz), 2.21, 2.14, 2.13, 2.11, 2.05, 1.99 (21H, s, CH_3(Ac)), 1.87 (2H, m, H-8), 1.57 (2H, m, H-11), 0.65 (2H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.5, 170.4, 170.3, 169.9, 169.71, 169.65, 169.4 (C, *C*=O(Ac)), 98.8 (CH, C-1′), 97.3 (CH, C-1), 75.0 (CH, C-3), 70.8 (CH, C-2), 69.8 (CH, C-2′), 69.2 (CH, C-5′), 68.6 (CH, C-5), 68.1 (CH, C-3′), 67.4 (CH, C-4′), 66.4 (CH_2, C-7), 65.7 (CH, C-4), 62.4, 62.2 (CH_2, C-6, 6′), 35.9 (CH_2, C-10), 28.9 (CH_2, C-8), 28.6 (CH_2, C-9), 24.0 (CH_2, C-11), 20.8, 20.68, 20.65, 20.58, 20.53, 20.47 (CH_3, *C*H_3(Ac)), 11.9 (CH_2, C-12).

Dumbbell(*1*)6-α-1,3-*Man*(*OAc*). Yield 61.3 mg (31% (2 steps)). HRMS (FAB): Calcd for $C_{200}H_{301}O_{108}S_6Si_3$ $[M+H]^+$ 4706.5693, found 4706.5679. $[\alpha]_D^{33}=+33.0°$ (c=1.0 in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.30, 5.26 (6H, m, H-3, 4), 5.23–5.19 (6H, m, H-2, 3′), 5.02 (3H, m, H-2′), 4.99 (3H, d, H-1′, $J_{1',2'}$=1.61 Hz), 4.83 (3H, H-1), 4.29–4.21 (6H, m, H-6a, 6′a), 4.14 (3H, dd, H-4′, $J_{3',4'}$=3.75 Hz, $J_{4',5'}$=10.17 Hz), 4.13–4.03 (9H, m, H-6b, 5′, 6′b), 3.87 (3H, m, H-5), 3.79 (3H, m, H-7a), 3.54 (3H, m, H-7b), 2.56 (6H, t, H-9, $J_{8,9}$=6.96 Hz), 2.51 (6H, t, H-10, $J_{10,11}$=6.96 Hz), 2.21, 2.14, 2.13, 2.11, 2.05, 1.99 (63H, s, CH_3(Ac)), 1.87 (6H, m, H-8), 1.56 (6H, m, H-11), 1.29 (2H, m, H-14), 0.65–0.60 (8H, m, H-12, 13), 0.54 (2H, m, H-15), −0.05 (3H, s, CH_3(Si–Me)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.5, 170.4, 170.3, 169.9, 169.75, 169.70, 169.5 (C, *C*=O(Ac)), 98.9 (CH, C-1′), 97.3 (CH, C-1), 75.1 (CH, C-3), 70.8 (CH, C-2), 69.8 (CH, C-2′), 69.3 (CH, C-5′), 68.6 (CH, C-5), 68.1 (CH, C-3′), 67.5 (CH, C-4′), 66.5 (CH_2, C-7), 65.8 (CH, C-4), 62.4, 62.2 (CH_2, C-6, 6′), 35.9 (CH_2, C-10), 29.0 (CH_2, C-8), 28.6 (CH_2, C-9), 24.1 (CH_2, C-11), 20.8, 20.72, 20.70, 20.62, 20.57, 20.52 (CH_3, *C*H_3(Ac)), 20.4 (CH_2, C-15), 18.2 (CH_2, C-14), 17.0 (CH_2, C-13), 12.0 (CH_2, C-12), −3.4 (CH_3, *C*H_3(Si–Me)).

4.3.7. Deprotection of carbosilane dendrimers with mannose and mannobiose: Fan(0)3-Man. A solution of sodium methoxide in methanol (12.7 mg, 235 μmol) was added to a solution of Fan(0)3-Man(OAc) (135.8 mg, 90.8 μmol) in methanol (1.5 mL) at room temperature under an argon atmosphere. The solution was stirred for 1 h, then the aqueous solution of sodium hydroxide (0.1 M) was added and was stirred at room temperature over night. After neutralizing with acetic acid, the solution was evaporated in vacuo. The residue was subjected to Sephadex G-25 size exclusion chromatography eluting with 5% (v/v) aqueous solution of acetic acid. The fractions containing carbosilane dendrimer were combined and lyophilized to yield Fan(0)3-Man as a white solid (54.8 mg (61%)): HRMS (ESI): Calcd for $C_{42}H_{74}O_{18}S_3SiNa$ $[M+Na]^+$ 1013.3704, found 1013.3696. $[\alpha]_D^{27}=+49.0°$ (c=1.0 in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 7.52–7.19 (5H, m, Ph), 4.84 (3H, H-1), 3.93 (3H, m, H-2), 3.89–3.64 (15H, m, H-3, 4, 6, 7a), 3.64–3.45 (6H, m, H-5, 7b), 2.55 (6H, m, H-9), 2.48 (6H, m, H-10), 1.84 (6H, m, H-8), 1.56 (6H, m, H-11), 0.89 (6H, m, H-12); ^{13}C NMR (100 MHz, D_2O) δ (ppm); 137.3 (C, Ph), 134.8, 129.8, 128.8 (CH, Ph), 100.7 (CH, C-1), 73.5 (CH, C-5), 71.7 (CH, C-4), 71.1 (CH, C-2), 67.2 (CH, C-3), 66.9 (CH_2, C-7), 61.5 (CH_2, C-6), 36.2 (CH_2, C-10), 30.1 (CH_2, C-8), 29.1 (CH_2, C-9), 24.6 (CH_2, C-11), 12.4 (CH_2, C-12).

Another carbosilane dendrimer with peripheral mannose or mannobiose acetate was deacetylated by the same method as Fan(0)3-Man. Carbosilane dendrimers with peripheral mannose or mannobiose which have no protective group of saccharide moieties were synthesized.

Ball(0)4-*Man*. Yield 64.8 mg (82%). HRMS (ESI): Calcd for $C_{48}H_{92}O_{24}S_4SiNa$ $[M+Na]^+$ 1231.4528, found 1231.4581. $[\alpha]_D^{24}=+52.7°$ (c=1.0 in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 4.87 (1H, H-1), 3.95 (1H, m, H-2), 3.89–3.78 (4H, m, H-3, 6a, 7a), 3.74 (1H, m, H-6b), 3.61 (2H, m, H-5, 7b), 2.65 (4H, m, H-9, 10), 1.93 (2H, m, H-8), 1.66 (2H, m, H-11), 0.76 (2H, m, H-12); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 100.6 (CH, C-1), 73.4 (CH, C-5), 71.6 (CH, C-4), 71.0 (CH, C-2), 67.2 (CH, C-3), 66.9 (CH_2, C-7), 61.5 (CH_2, C-6), 36.3 (CH_2, C-10), 30.1 (CH_2, C-8), 29.1 (CH_2, C-9), 24.8 (CH_2, C-11), 12.5 (CH_2, C-12).

Dumbbell(1)6-*Man*. Yield 33.6 mg (81%). HRMS (FAB): Calcd for $C_{80}H_{156}O_{36}S_6Si_3Na$ $[M+Na]^+$ 1991.7906, found 1991.7937. $[\alpha]_D^{30}=+46.3°$ (c=1.0 in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 4.90 (3H, d, $J_{1,2}$=1.0 Hz, H-1), 3.99 (3H, m, H-2), 3.92–3.75 (15H, m, H-3, 4, 6, 7a), 3.68–3.55 (6H, m, H-5, 7b), 2.66 (6H, m, H-9), 2.62 (6H, m, H-10), 1.93 (6H, m, H-8), 1.66 (6H, m, H-11), 1.48 (2H, m, H-14), 0.82–0.65 (10H, m, H-12, 13, 15), 0.06 (3H, s, Si–CH_3); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 100.1 (CH, C-1), 72.8 (CH, C-5), 71.2 (CH, C-4), 70.7 (CH, C-2), 66.8 (CH, C-3), 66.3 (CH_2, C-7), 60.9 (CH_2, C-6), 35.8 (CH_2, C-10), 29.5 (CH_2, C-8), 28.6 (CH_2, C-9), 24.4 (CH_2, C-11), 21.0 (CH_2, C-15), 20.3 (CH_2, C-14), 19.1 (CH_2, C-13), 12.0 (CH_2, C-12), −2.7 (CH_3, Si–*C*H_3).

Fan(0)3-α-1,3-*Man*. Yield 44.6 mg (quant.). HRMS (FAB): Calcd for $C_{60}H_{104}O_{33}S_3SiNa$ $[M+Na]^+$ 1499.5289, found 1499.5278. $[\alpha]_D^{22}=+78.7°$ (c=0.87 in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 7.52–7.25 (5H, m, Ph), 5.14 (3H, H-1′), 4.82 (3H, H-1), 4.08 (6H, m, H-2, 2′), 3.92–3.50 (36H, m, H-3, 4, 5, 6, 3′, 4′, 5′, 6′, 7), 2.52 (12H, m, H-9, 10), 1.85 (6H, m, H-8), 1.57 (6H, m, H-11), 0.91 (6H, m, H-12); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 137.1 (C, Ph), 134.6, 129.8, 128.6 (CH, Ph), 102.9 (CH, C-1′), 100.5 (CH, C-1), 79.3 (CH, C-3), 73.8 (CH, C-5), 73.4 (CH, C-5′), 71.0 (CH, C-3′), 70.8 (CH, C-2′), 70.5 (CH, C-2), 67.1 (CH, C-4′), 66.7 (CH, C-4), 66.1 (CH_2, C-7), 61.4 (CH_2, C-6′), 61.1 (CH_2, C-6), 35.9 (CH_2, C-10), 29.8 (CH_2, C-8), 28.9 (CH_2, C-9), 24.1 (CH_2, C-11), 12.1 (CH_2, C-12).

Ball(0)4-α-1,3-*Man*. Yield 75.5 mg (quant.). HRMS (ESI): Calcd for $C_{72}H_{132}O_{44}S_4SiNa$ $[M+Na]^+$ 1879.6641, found 1879.6622. $[\alpha]_D^{20}=+100.4°$ (c=1.0 in H_2O). ^1H NMR (200 MHz, D_2O) δ (ppm); 5.14 (H-1′), 4.92 (1H, H-1), 4.15 (2H, m, H-2, 2′), 4.08–3.60 (12H, m, H-3, 4, 5, 6, 3′, 4′, 5′, 6′, 7), 2.71 (4H, m, H-9, 10), 2.02 (2H, m, H-8), 1.73 (2H, m, H-11), 0.82 (2H, m, H-12); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 103.0 (CH, C-1′), 100.6 (CH, C-1), 79.4 (CH, C-3), 73.8 (CH, C-5), 73.5 (CH, C-5′), 71.1 (CH, C-3′), 70.9 (CH,

C-2′), 70.6 (CH, C-2), 67.3 (CH, C-4′), 66.9 (CH, C-4), 66.3 (CH_2, C-7), 61.5 (CH_2, C-6′), 61.3 (CH_2, C-6), 36.2 (CH_2, C-10), 29.8 (CH_2, C-8), 29.0 (CH_2, C-9), 24.8 (CH_2, C-11), 12.4 (CH_2, C-12).

Dumbbell(1)6-α-1,3-*Man*. Yield: 33.8 mg (90%). HRMS (ESI): Calcd for $C_{116}H_{216}O_{66}S_6Si_3Na_2/2$ $[M+2Na]^{2+}/2$ 1493.5487, found 1493.5482. $[\alpha]_D^{29}=+48.3°$ ($c=1.0$ in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 5.15 (3H, H-1′), 4.86 (3H, H-1), 4.09 (6H, m, H-2, 2′), 4.00–3.65 (33H, m, H-3, 4, 5, 6, 3′, 4′, 5′, 6′, 7a), 3.63 (3H, m, H-7b), 2.65 (12H, m, H-9, 10), 1.94 (6H, m, H-8), 1.65 (6H, m, H-11), 1.46 (2H, m, H-14), 0.75 (8H, m, H-12, 13), 0.69 (2H, m, H-15), 0.05 (3H, s, Si–CH_3); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 103.1 (CH, C-1′), 100.7 (CH, C-1), 79.6 (CH, C-3), 74.0 (CH, C-5), 73.6 (CH, C-5′), 71.3 (CH, C-3′), 71.0 (CH, C-2′), 70.7 (CH, C-2), 67.5 (CH, C-4′), 67.0 (CH, C-4), 66.4 (CH_2, C-7), 61.8 (CH_2, C-6′), 61.5 (CH_2, C-6), 36.5 (CH_2, C-10), 30.0 (CH_2, C-8), 29.2 (CH_2, C-9), 25.0 (CH_2, C-11), 21.0 (CH_2, C-15), 19.4 (CH_2, C-14), 18.1 (CH_2, C-13), 12.6 (CH_2, C-12), −1.6 (CH_3, Si–CH_3).

4.4. Calorimetry

Isothermal titration microcalorimetry was performed using the MicroCal Omega titration microcalorimeter. Details of instrument design and data analysis are described by Wiseman et al.[31] A solution of concanavalin A (0.21 mM) in a buffer of 50 mM 3,3-dimethylglutarate, 250 mM NaCl, and 1 mM each of $CaCl_2$ and $MnCl_2$ at pH 5.2 were placed in the sample cell. Carbosilane dendrimer solutions ([mannose] = 2.1 mM) in a buffer identical to that used for protein solutions were added in 10 μL increments during 30 s, with 3 min intervals between injections. Each calorimetric titration was performed at a sample cell temperature of 298 K. Protein concentrations were determined spectrometrically using an extinction coefficient of $\varepsilon_{280}=1.24$ for a 1 mg/mL of solution.

The heat evolved upon each injection was digitally recorded, and the data were integrated to generate a titration curve upon completion of the experiment. The stoichiometry of the association, n, binding constant, K, and the change in enthalpy, ΔH, were obtained from a nonlinear least-squares fit using the Origin software program. All data are presented on a valency-corrected basis.

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Synthesis of glycoconjugate polymer carrying globotriaose as artificial multivalent ligand for Shiga toxin-producing *Escherichia coli* O157: H7

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Abstract

As an artificial ligand, a glycoconjugate polymer carrying carbohydrate moiety of lactosyl ceramide or globotriaosyl ceramide (Gb_3) was synthesized. Gb_3 is known as the receptor of Shiga toxin-producing *Escherichia coli* O157: H7. The preparation of the glycoconjugate polymer initially involves the construction of the carbohydrate moiety of Gb_3 derivative which has *n*-pentenyl group as polymerizable group. In addition, the *n*-pentenyl group of the Gb_3 derivative was modified and different polymerizable groups such as acrylamide group were introduced at ω-position of the aglycon. Radical polymerization of the synthesized glycosyl monomers with or without acrylamide proceeded smoothly in water using ammonium persulfate and *N*, *N*, *N'*, *N'*-tetramethylethylenediamine as usual initiator system and gave water-soluble glycoconjugate polymers having various polymer compositions. These polymers have the potential to neutralize Shiga toxin by reason of cluster effect and multivalency.

Keywords: *Escherichia coli*; Shiga toxins; Glycoconjugate polymer; Radical polymerization; Carbohydrates; Globotriaose

1. Introduction

The importance of cell-surface carbohydrates in initiating a wide variety of biological and pathological processes is now well recognized (Arya et al., 1999). Glycoconjugate polymers carrying biologically active carbohydrates as pendant groups constitute a new class of biomimetic and biomedical materials. They have provided access to many new methodologies in cell cultivation, tumor detection and diagnosis, and trapping of viruses and toxins. Their wide range of utility can be ascribed primarily to the widely occurring carbohydrate-binding proteins on the surfaces of cells, bacteria, and viruses (Debenham, Cossrow, & Toone, 1999; Dohi et al., 1999; Mylvaganam & Lingwood, 1999). Moreover, multivalency or cluster effects of carbohydrate integrate the binding affinity of glycoconjugate polymers to carbohydrate-binding proteins and contribute much to extend their potential utility (Gestwicki, Cairo, Strong, Oetjen, & Kiessling, 2002; Lee & Lee, 1995; Roy, 1996; Turnbull & Stoddart, 2002).

Shiga toxins (Stxs; Stx1 and Stx2) produced by pathogenic *Escherichia coli* O157: H7 have been associated with diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. The Stxs are a family of AB_5 subunit toxins. The enzymatic A subunit (32 kDa) is non-covalently associated with the pentamer of receptor-binding B subunits (7.5 kDa). The B-pentamer specifically binds to the globotriaosyl ceramide [Gb_3, Galα(1-4)Galβ(1-4)Glcβ-ceramide], a cell surface glycolipid (Kitov et al., 2000; Ling et al., 1998; Nishikawa et al., 2002; Soltyk et al., 2002). The importance of the B-pentamer–Gb_3 interaction is clearly illustrated by the fact that all cells susceptible to Stxs express Gb_3 on their cell surface, whereas cells that do not express Gb_3 are resistant to the toxins. Therefore, binding to the cell surface is a crucial initial step in cytotoxicity of Stxs (Bast, Banerjee, Clark, Read, & Brunton, 1999).

We describe herein the syntheses of a couple of new glycoconjugate polymers carrying the trisaccharide (globotriaose) moieties of Gb_3 as an artificial receptor for Stxs. These polymerizable saccharide derivatives having *n*-pentenyl group or acrylamide group at the ω-position of

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the aglycon are polymerized or co-polymerized with acrylamide. These artificial glycoconjugate polymers have the therapeutic potential for neutralization of Stxs because the polymers are water-soluble and the binding affinity is enhanced by cluster effects.

2. Results and discussion

2.1. Synthesis of polymerizable globotriose derivative

Our strategy for preparing the polymerizable globotriaose derivative involves the introduction of an olefin, *n*-pentenyl group, at the aglycon unit of the glycosyl acceptor and subsequent glycosidation with glycosyl donor (Matsuoka, Terabatake, Esumi, Terunuma, & Kuzuhara, 1999). After assembling the globotriaose structure, *n*-pentenyl group was modified, and to the ω-position of the aglycon was introduced an acrylamide group (Fig. 1). Consequently, glycosyl monomers having different polymerizable groups (*n*-pentenyl and acrylamide group) were prepared, respectively.

Scheme 1 describes the synthesis of glycosyl acceptor **3**. *n*-Pentenyl β-lactoside **1** which was prepared according to Matsuoka and Nishimura (1995), and Takano, Nakatsubo, and Murakami (1990) was selectively protected by formation of a benzylidene acetal intermediate. Subsequent benzylation of remaining OH groups afforded **2**. Selective reductive cleavage by treatment of **2** with $AlCl_3$ in the presence of $BH_3{\cdot}NMe_3$ in THF gave glycosyl acceptor **3** with 4′-OH in 76.3% yield.

Compound **4** (Koto, Morishima, Miyata, & Zen, 1976) gave the glycosyl donor **5** (Austin, Hardy, Buchanan, & Baddiley, 1965) quantitatively by treatment with $SOCl_2$ in

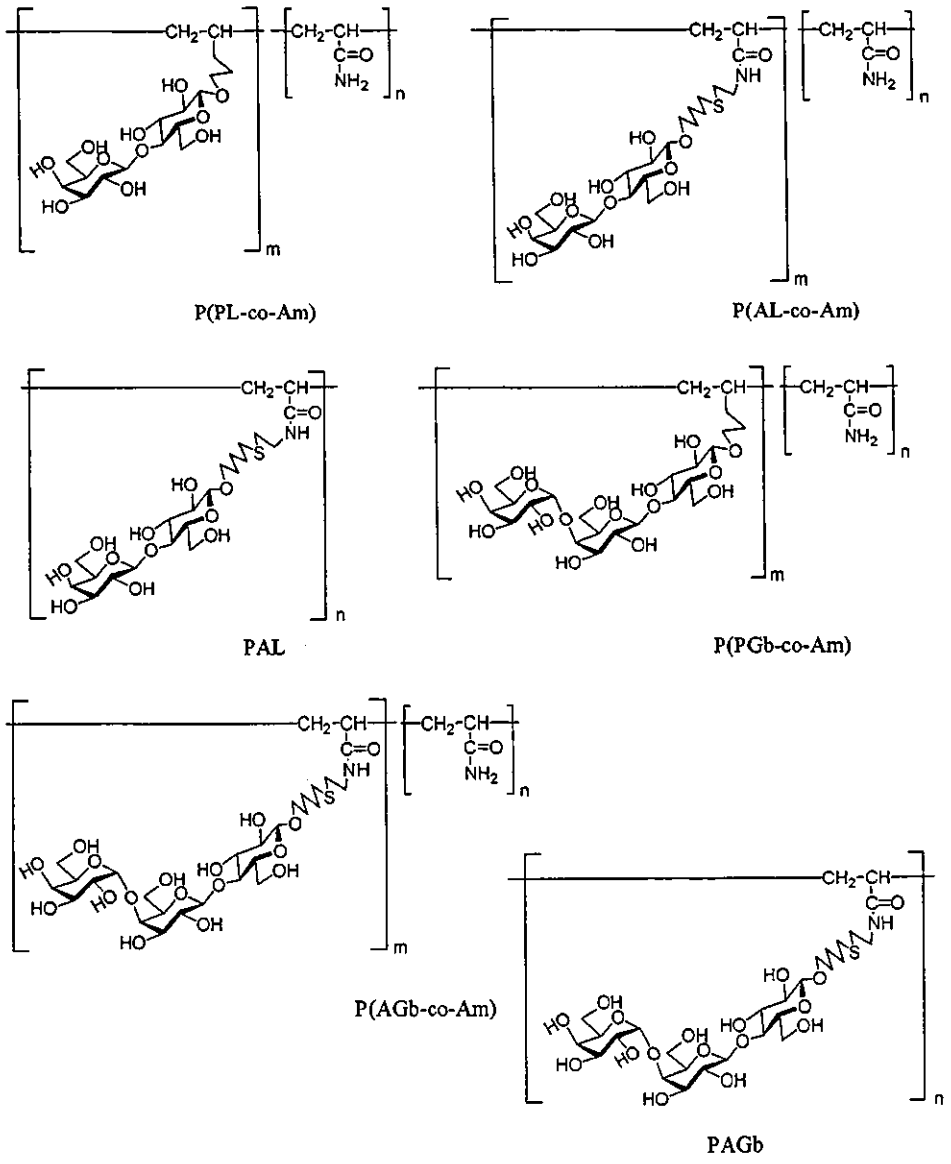
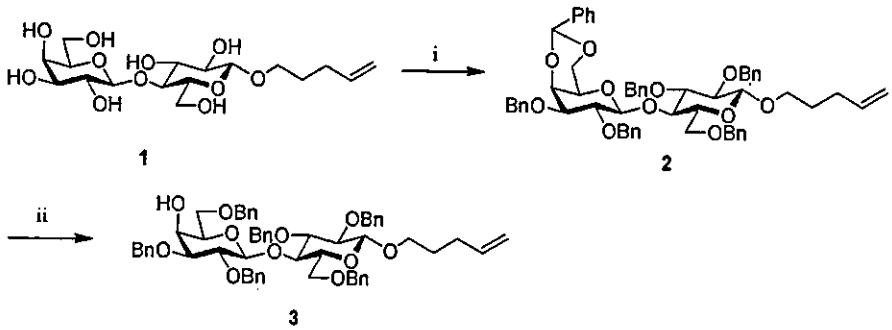
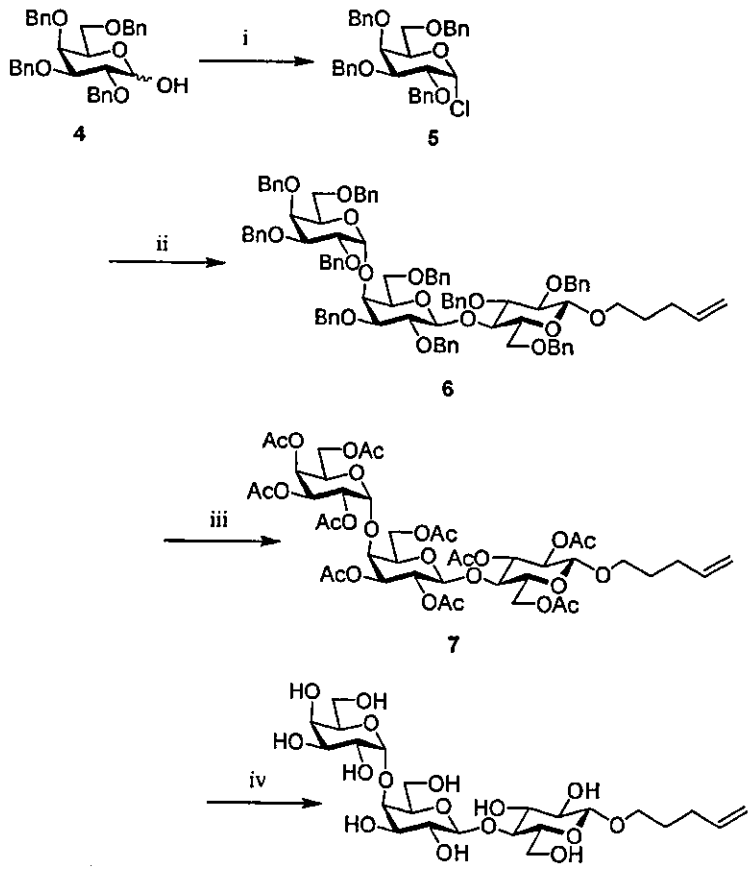


Fig. 1. Chemical structures of synthesized glycoconjugate polymers.

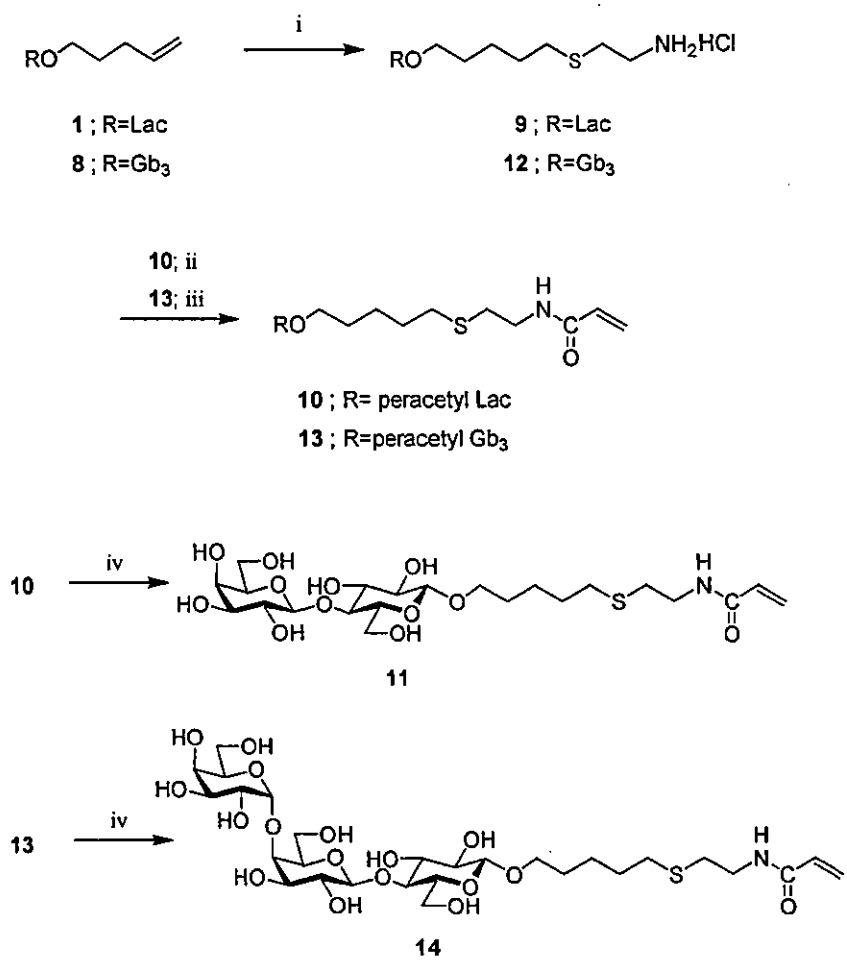


Scheme 1. Reagents and conditions: (i) $C_6H_5CH(OCH_3)_2$, CSA, 60°C, 2.5 h, under reduced pressure, then NaH, BnBr, DMF, rt, 1.5 h; (ii) $Me_3N{\cdot}BH_3$, $AlCl_3$, MS 4Å, THF, rt, 1 h.

the presence of DMF (Ogawa, Nakabayashi, & Kitajima, 1983). Stereoselective glycosidation (shown in Scheme 2) of glycosyl acceptor **3** with glycosyl donor **5** promoted by silver trifluoromethansulfonate in ether at −20 °C gave globotriaose derivative **6** in 71.3% yield. The NMR spectrum of the product confirms the α-linkage of the newly formed glycosidic bond [^{13}C NMR ($CDCl_3$) δ: 103.51 (β; C-1′), 102.80 (β; C-1), 100.63 (α; C-1″)]. Debenzylation of **6** without affecting the terminal double bond of the *n*-pentenyl aglycon was accomplished by Birch reduction. However, in the case of globotriaose derivatives having butenyl or allyl aglycon unit, these aglycons were slightly cleaved by Birch reduction. Compound **6** was treated with Na in liquid NH_3 at −78 °C, followed by acetylation to afford fully acetylated *n*-pentenyl globotriaose derivative **7**. After purification of **7**, subsequent deacetylation gave water-soluble *n*-pentenyl β-globotriaoside **8**, a glycosyl monomer with free hydroxyl groups and the *n*-pentenyl (olefin moiety) as polymerizable group.



Scheme 2. Reagents and conditions: (i) $SOCl_2$, DMF, $ClCH_2CH_2Cl$, 0 °C → rt, 20 h. (ii) AgOTf, MS 4Å, Et_2O, −20 °C, 3.5 h; (iii) Na, liq. NH_3, −78 °C, 20 min, then Ac_2O, pyridine, rt, 21 h; (iv) NaOMe, MeOH, rt, 16 h.



Scheme 3. Reagents and conditions: (i) $HSCH_2CH_2NH_2{\cdot}HCl$, MeOH, $h\nu$ (254 nm), 0 °C, 2.5 h; (ii) $CH_2{=}CHCOCl$, Et_3N, MeOH, 0 °C, then Ac_2O, pyridine, rt, 15 h; (iii) $CH_2{=}CHCOCl$, $NaHCO_3$, MeOH, 0 °C, then Ac_2O, pyridine, rt, 13 h; (iv) NaOMe, MeOH, rt, 3.0 h.

Scheme 3 describes the syntheses of glycosyl monomers having acrylamide group at the terminal. Initially, to *n*-pentenyl β-lactoside **1** was introduced an amino group at ω-position of the aglycon. *n*-Pentenyl β-lactoside **1** and cysteamine hydrochloride were irradiated (254 nm), yielding the amino terminated thioether **9** (Lee & Lee, 1974; Roy & Tropper, 1988; van Seeventer, van Dorst, Siemerink, Kamerling, & Vliegenthart, 1997). Then the amino group of **9** was *N*-acryloylated and then acetylated to give fully protected derivative. After usual purification, deacetylated **10** gave lactose monomer **11** having an acrylamide group at ω-position of the aglycon. By the same procedure, globotriaose monomer **14** was obtained. Radical addition of *n*-pentenyl β-globotriaoside **8** proceeded, followed by *N*-acryloylation and acetylation to afford **13** which was deacetylated to give globotriaose monomer **14** having an acrylamide group at ω-position of the aglycon.

2.2. Radical polymerization of glycoconjugate polymers

Glycosyl monomers were polymerized or copolymerized with acrylamide in distilled water at room temperature using *N*, *N*, *N'*, *N'*-tetramethylenediamine (TEMED) and ammonium persulfate (APS) as initiators (Matsuoka & Nishimura, 1995; Nishimura et al., 1994), and the products were purified by gel filtration.

The results of polymerization and copolymerization are summarized in Table 1. The unit ratio of the polymers abbreviated as 'polymer comps' was determined from the ^1H NMR results by comparing the intensity of the integration of the protons for 1, $1'$-positions of lactose or globotriaose (at 4.4 ppm) due to glycosyl residue, and methine group (at 2.2 ppm) due to the main chain of the polymer (Figs. 2 and 3).

The sugar content of the polymer was determined as percent by weight of the glycosyl monomer in the polymer. As shown in Table 1, polymer composition was affected by the glycosyl monomer. The factors affecting polymerization involves the difference of the polymerizability of *n*-pentenyl and acrylamide groups that glycosyl monomers have and steric hindrance of bulky glycosides. Gb_3 was bulkier than lactose due to the additional α-galactose residue. However, the polymer molecular weight and sugar content seemed enough to inhibit

Table 1
Results of polymerization of glycosyl monomers with acrylamide

Polymer	Glycosyl monomer	Monomer ratio[a]	Total yield (%)	Polymer compsa	Sugar content (wt %)	Mw[b] (kDa)
P(PL-co-Am)	**1**	1:10	86.5	2:27	30	81.2
P(AL-co-Am)	**11**	1:10	92.2	1:6	55.9	<10
PAL	**11**	1:0	92.0	1:0	100	46.5
P(PGb-co-Am)	**13**	1:10	83.9	1:25	24.4	147
P(AGb-co-Am)	**14**	1:10	48.0	1:12	45.2	73.1
PAGb	**14**	1:0	80.0	1:0	100	36

[a] Ratio of glycosyl monomer to acrylamide.
[b] Mws were estimated by SEC method with Asahipack G-510 column (pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards).

cytotoxicity of Shiga toxins. These glycoconjugate polymers were assayed in vitro and glycoconjugate polymers carrying Gb_3 were found effective for neutralization of Shiga toxins, not only for Stx1 but also the clinically more relevant Stx2. Moreover, glycoconjugate polymers carrying Gb_3 were also found effective in vivo. Details of these results are discussed elsewhere (Watanabe et al., 2004).

In conclusion, we synthesized glycoconjugate polymers having lactose and globotriaose residues as biologically active pendants. These monomers of glyco-polymers were systematically synthesized. The construction of the trisaccharide moiety was accomplished from D-galactose and D-lactose by several chemical steps. The carbohydrate derivatives having *n*-pentenyl group at the aglycon were efficiently synthesized, and the elongation of the aglycon was performed to afforded corresponding glycosyl monomers having an acrylamide group. Polymerization of those monomers was accomplished and the results suggested that the acrylamide-type aglycon was found to be a better polymerizable group. This phenomenon gave us the glycosyl monomers having acrylamide group had appropriate length of flexible spacer arm and showed grater polymerizability. The glycoconjugate polymers having acrylamide-type aglycon had stronger neutralization potency against both Stxs.

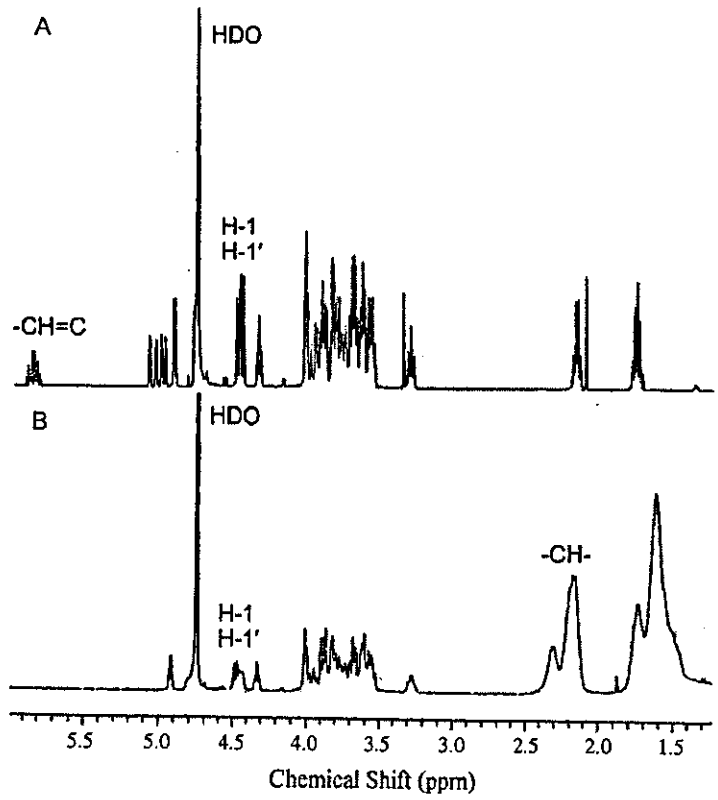


Fig. 2. ^1H NMR spectra of (A) glycosyl monomer **8**, (B) P(PGb-co-Am) in D_2O.

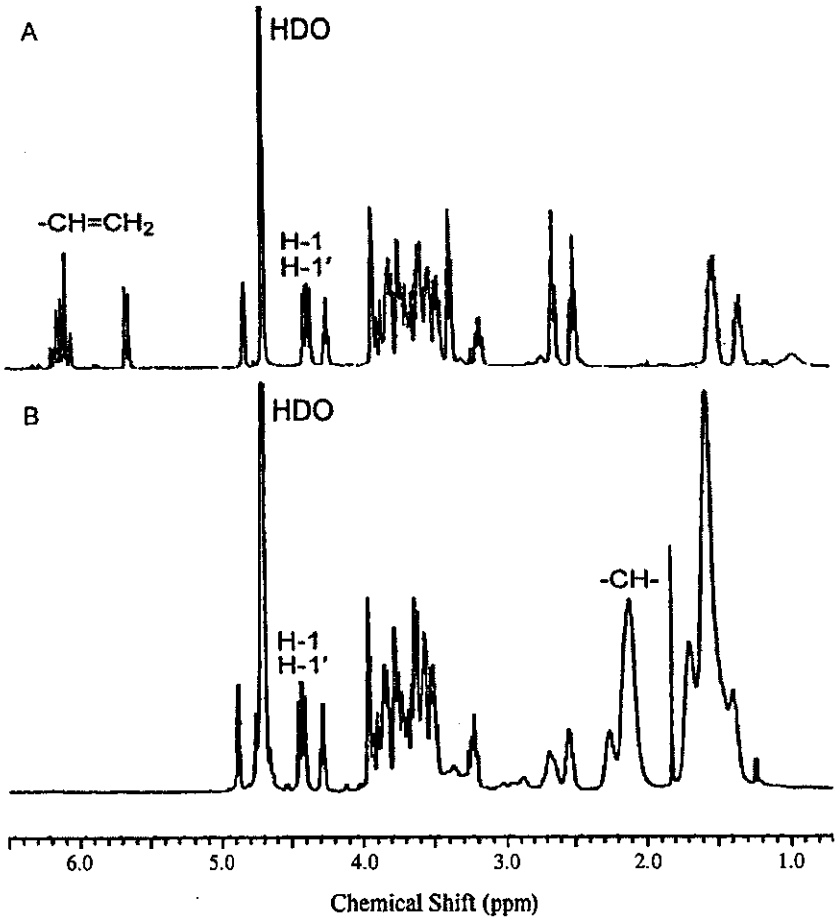


Fig. 3. ^{1}H NMR spectra of (A) glycosyl monomer **14**, (B) P(AGb-co-Am) in D_2O.

3. Experimental section

3.1. General procedures

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. *N,N*-Dimethylformamide (DMF), tetrahydrofuran (THF), 1,2-dichloroethane, dichloromethane, and pyridine were stored over molecular sieves 4 Å. Methanol was stored over molecular sieves 3 Å. Powdered molecular sieves were dried in vacuo at ca. 180 °C in 2 h. Acrylamide was recrystallized from benzene. The optical rotations were determined with a JASCO DIP-1000 digital polarimeter. IR spectra were measured in KBr disc for solid samples, or film on KBr for liquid samples with JASCO FT/IR-300E. ^{1}H NMR spectra were recorded at 200 or 400 MHz with Varian Gemini-200 or Bruker AM-400 spectrometer in chloroform-*d* or deuterium oxide. ^{13}C NMR spectra were recorded at 50.3 or 100.6 MHz with the same instruments. Tetramethylsilane (TMS), HDO (4.78 ppm) were used as internal standards. Proton assignments in NMR were made by first-order analysis of spectra, and supported by homonuclear decoupling experiments. Elemental analyses were performed with a Fisons EA1108 on samples extensively dried ca. 24 h in vacuo over phosphorus pentoxide. Average molecular weights of the polymers were estimated by size exclusion chromatography (SEC) method with a Shodex Asahipak GS-510 7E column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, 300 kDa, Shodex Standard P-82) were used as standards. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (layer thickness, 0.25 mm; E. Merk, Darmstadt, Germany). For detection of intermediates, TLC sheets were dipped with (a) a solution of 85:10:5 (v/v/v) methanol–*p*-anisaldehyde–concentrated sulfuric acid and heated for a few minutes (for carbohydrates); (b) an aqueous solution of 5 wt % potassium permanganate and heated similarly (for double bond). Column chromatography was performed on silica gel (Silica Gel 60; 40–63 μm, E. Merck), or (Silica Gel 60, spherical neutral; 40–100 μm, E. Merck).

3.2. n-Pentenyl 4-O-(2,3-di-O-benzyl-4,6-O-benzilidene-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (2)

To a solution of **1** (500 mg, 1.22 mmol) in DMF (2.5 ml) was added benzaldehyde dimethylacetal (275 μl, 1.83 mmol)

and (±)-camphor-10-sulfonic acid (28.3 mg, 122 μmol), and the mixture was stirred over evaporation at 60 °C for 2.5 h. The solution was cooled to room temperature, and triethylamine (34 μl, 244 μmol) added to neutralize. The solution was evaporated to give an intermediate mixture. A part of mixture was crystallized from 2-propanol to give a white crystals having m.p. 179–180 °C. The whole mixture was dissolved in DMF (15 ml), and the solution was added dropwise to NaH (420 mg, 17.6 mmol) in DMF (15 ml). Then benzyl bromide (1.39 ml, 11.7 mmol) was added dropwise to the reaction mixture, and the mixture was stirred at room temperature. After 40 min, the reaction was quenched with methanol and the mixture was evaporated. The residue was extracted with diethyl ether and washed with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 10:1 (v/v) toluene–ethyl acetate to give **2** (359 mg, 61.8%) as a syrup: $[\alpha]_D^{27.5} = +12.3°$ ($c = 1.60$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.20 (m, 30 H, Ph × 6), 5.81 (m, 1 H, –CH=C), 5.45 (s, 1 H, Ph–CH–O_2–), 4.46 (d, 1 H, $J_{1',2'} = 7.5$ Hz, H-1′), 4.37 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.02 (br-d, 1 H, $J_{3',4'} = 3.2$ Hz, H-4′), 3.93 (t, 1 H, $J_{4,5} = 6.6$ Hz, H-4), 3.86 (dd, 1 H, $J_{5,6b} = 4.6$ Hz, H-6b), 3.85 (dd, 1 H, $J_{6a,6b} = 10.7$ Hz, H-6a), 3.74 (m, 2 H, –OCH_2–), 3.62 (t, 1 H, $J_{2,3} = 8.8$ Hz, H-3), 3.53 (ddd, 1 H, $J_{5,6a} = 2.9$ Hz, H-5), 2.15 (m, 2 H, –CH_2–C=C), 1.75 (m, 2 H, –OC–CH_2–); Anal. $C_{59}H_{64}O_{11}$. Calcd: C, 74.66; H, 6.80. Found: C, 74.52; H, 6.80.

3.3. n-Pentenyl 4-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (3)

To a solution of **2** (119 mg, 125 μmol) in THF (1.95 ml) was added molecular seives 4 Å powder (119 mg) and stirred at 0 °C for 30 min. To subsequent solution was added trimethylamine–borane (63.8 mg, 875 μmol) and then aluminum chloride (117 mg, 875 μmol) added in numbers. The solution was stirred at room temperature for 1 h. The solution was filtered through Celite pad and the filtrate was extracted with chloroform and washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 3:1 (v/v) hexane–ethyl acetate to give **3** (91 mg, 76.3%) as an amorphous powder: $[\alpha]_D^{25.9} = +18.9°$ (c 1.91, $CHCl_3$); IR (KBr) ν 3506 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (m, 30 H, Ph × 6), 5.81 (m, 1 H, –CH=C), 4.53 (br-s, 1 H, H-1′), 4.44 (br-s, 1 H, H-1), 4.01 (m, 1 H, H-4′), 3.94 (br-d, 1 H, $J = 8.6$ Hz, H-4), 2.12 (m, 2 H, –CH_2–C=C), 1.65 (m, 2 H, –OC–CH_2–); Anal. $C_{59}H_{66}O_{11}$. Calcd: C, 74.50; H, 6.99. Found: C, 74.48; H, 7.05.

3.4. 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl chloride (5)

To a solution of **4** (4.00 g, 7.40 mmol) in 1,2-dichloroethane (30 ml) was added DMF (290 μl, 3.70 mmol) and cooled at 0 °C. To the solution was added thionyl chloride (3.22 ml, 444 mmol) and stirred at 0 °C for 20 h. The solution was filtered through silica gel and concentrated to give **5** (4.14 g, 100%): ^1H NMR ($CDCl_3$) δ 7.28 (m, 20 H, Ph × 4), 6.14 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-1), 4.45 (dd, 1 H, $J_{3,4} = 18.8$ Hz, H-3), 4.22 (dd, 1 H, $J_{2,3} = 11.9$ Hz, H-2).

3.5. n-Pentenyl 4-O-[4-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-β-D-glucopyranoside (6)

To a solution of **3** (4.35 g, 4.57 mmol) and **5** (6.10 g, 10.9 mmol) in distilled diethyl ether (200 ml) was added molecular seives 4 Å powder (4.14 g) and stirred for 30 min. To the mixture was added silver trifluoromethansulfonate (3.52 g, 13.7 mmol) and stirred at −20 °C for 3.5 h. The solution was diluted with chloroform and filtered through a pad of Celite, and the filtrate was extracted with chloroform and washed successively with aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 8:1 (v/v) hexane–ethyl acetate to give **6** (4.80 mg, 71.3%) as a syrup: $[\alpha]_D^{25.4} = +33.5°$ (c 0.51, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.22 (m, 50 H, Ph × 10), 5.80 (m, 1 H, –CH=C), 2.13 (m, 2 H, –CH_2–C=C), 1.73 (m, 2 H, –OC–CH_2–); ^{13}C NMR ($CDCl_3$) δ 103.51 (C-1′), 102.80 (C-1), 100.63 (C-1″), 114.79 (–C=CH_2); Anal. $C_{93}H_{100}O_{16}$. Calcd: C, 75.79; H, 6.84. Found: C, 75.83; H, 6.86.

3.6. n-Pentenyl 4-O-[4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-galactopyranosyl]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (7)

Na (1.97 g, 85.8 mmol) was added to liquid NH_3 (90 ml) at −78 °C and a solution of **6** (3.16 g, 2.15 mmol) in 1,2-dimethoxyethane (20 ml) was added dropwise to the mixture. After the mixture was stirred at −78 °C for 20 min, ammonium chloride (4.59 g, 85.8 mmol) was added to the reaction mixture and the mixture was stirred for 3 h. The mixture was evaporated and the residue was stirred with pyridine (45 ml) and acetic anhydride (30 ml) at room temperature for 21 h. The mixture was poured into ice–water. The extract with chloroform was washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 1:1 (v/v) hexane–ethyl acetate to give syrupy **7**

(1.53 g, 71.7%): $[\alpha]_D^{24.0} = +40.5°$ (*c* 1.11, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.78 (m, 1 H, –CH=C), 5.39 (dd, 1 H, $J_{3'',4''} = 3.3$ Hz, $J_{2''3''} = 11.0$ Hz, H-3″), 5.20 (t, 1 H, $J_{2,3} = 9.2$ Hz, $J_{3,4} = 9.4$ Hz, H-3), 5.18 (dd, 1 H, $J_{1'',2''} = 3.8$ Hz, H-2″), 5.10 (dd, 1 H, $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 10.8$ Hz, H-2′), 5.00 (m, 1 H, –C=CH_2), 4.99 (d, 1 H, $J_{1'',2''} = 2.9$ Hz, H-1″), 4.89 (br-t, 1 H, H-2), 4.73 (dd, 1 H, $J_{2',3'} = 10.9$ Hz, $J_{3',4'} = 2.4$ Hz, H-3′), 4.52 (d, 1 H, $J_{1',2'} = 7.6$ Hz, H-1′), 4.46 (d, 1 H, $J_{1,2} = 7.7$ Hz, H-1), 4.45 (m, 3 H, $J_{6a,6b} = 11.1$ Hz, $J_{5,6b} = 6.3$ Hz, H-6b, 6a″ and 6b″), 4.13 (m, 4 H, H-6a, 6a′, 6b′and 5″), 4.01 (br-d, 1 H, H-4′), 3.84 (t, 1 H, $J_{4,5} = 9.0$ Hz, H-4), 3.81 (m, 2 H, –OCH_2–), 3.79 (t, 1H, $J_{5',6b'} = 9.4$ Hz, H-5′), 3.48 (ddd, 1 H, $J_{5,6a} = 3.5$ Hz, $J_{5,6b} = 4.8$ Hz, $J_{4,5} = 9.6$ Hz, H-5), 2.08 (m, 32 H, –OAc × 10, –CH_2–C=C), 1.75 (m, 2 H, –OC–CH_2–); ^{13}C NMR ($CDCl_3$) δ 101.02 (C-1′), 100.49 (C-1), 99.54 (C-1″), 114.99 (–C=CH_2); Anal. $C_{43}H_{60}O_{26}$. Calcd: C, 52.01; H, 6.09. Found: C, 52.38; H, 6.15.

3.7. n-Pentenyl 4-O-[4-O-(α-D-galactopyranosyl)- β-D-galactopyranosyl]-β-D-glucopyranoside (8)

To a solution of **7** (1.10 g, 11.0 mmol) in methanol (11 ml) was added sodium methoxide (59.9 mg, 1.10 mmol), and the mixture was stirred for 16 h at room temperature. IR-120B (H^+) resin (875 μl) was added to neutralize the solution, and the suspension was filtered and evaporated to give **8** (632 mg, 99.9%): ^1H NMR (D_2O) δ 5.87 (m, 1 H, –CH=C), 5.05 (dd, 1 H, $J_{trans} = 17.1$ Hz, $J_{gem} = 1.35$ Hz, –C–C=CH), 4.98 (dd, 1 H, $J_{cis} = 10.2$ Hz, –C–C=CH), 4.90 (d, 1 H, $J_{1'',2''} = 3.8$ Hz, H-1″), 4.46 (d, 1 H, $J_{1',2'} = 8.0$ Hz, H-1′), 4.43 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1), 3.25 (t, 1 H, H-2), 2.10 (m, 2 H, –CH_2–C=C), 1.68 (m, 2 H, –OC–CH_2–).

3.8. 5-(2-aminoethylthio) pentyl 4-O-(β-D-galactopyranosyl)- β-D-glucopyranoside hydrochloride (9)

To a solution of **1** (200 mg, 487 μmol) in MeOH (3.0 ml) was added 2-aminoethanthiol hydrochloride (277 mg, 2.44 mmol) and irradiated with ultraviolet light (254 nm) at 0 °C for 3 h. The mixture was concentrated and then the residue was purified by gel filtration with aqueous 5% acetic acid to give crude **9** (294 mg) containing acetic acid as an impurity, which was used for next step without further purification: ^1H NMR (D_2O) δ 3.09 (dd, 2 H, –CH_2–N–C–), 2.74 (dd, 2 H, –S–CH_2–), 2.49 (dd, 2 H, –CH_2–S–).

3.9. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyrosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (10)

Compound **9** (100 mg, 191 μmol) was dissolved in methanol (1.0 ml) and cooled at 0 °C. To the solution was simultaneously added triethylamine (41.4 μl, 573 μmol) and acryloyl chloride (19.5 μl, 229 μmol) dropwise five times. After removal of the solvent, acetic anhydride (3.0 ml) and pyridine (3.0 ml) was added to the mixture at room temperature and the mixture was stirring for 15 h, and concentrated. The residue was extracted with chloroform and washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 1:3 (v/v) toluene–ethyl acetate to give corresponding **10** (132 mg, 82.6%): $[\alpha]_D^{14.9} = -14.4°$ (*c* 1.36, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.30 (dd, 1H, $J_{trans} = 17.0$ Hz, $J_{gem} = 1.42$ Hz, –C–C=CH), 6.13 (m, 1 H, –NH–), 6.13 (dd, 1 H, $J_{cis} = 10.2$ Hz, –C–CH=C), 5.66 (dd, 1 H, –C–C=CH), 5.35 (br-d, 1 H, H-4″), 3.52 (m, 2 H, –CH_2–N–), 2.69 (t, 2 H, $J = 6.4$, –S–CH_2–C–N–), 2.52 (t, 2 H, $J = 7.2$, –C–CH_2–S–), 2.00 (m, 30 H, –OAc × 10), 1.58 (m, 4 H, –CH_2– × 2), 1.43 (m, 2 H, –CH_2–); Anal. $C_{36}H_{53}N_1O_{19}S_1$. Calcd: C, 51.73; H, 6.39; N, 1.68. Found: C, 51.79; H, 6.41; N, 1.51.

3.10. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-(β-D-galactopyranosyl)- β-D-glucopyranoside (11)

To a solution of **10** (950 mg, 1.14 mmol) in methanol (12 ml) was added sodium methoxide (43.0 mg, 795 μmol), and the mixture was stirred for 2.5 h at room temperature. IR-120B (H^+) resin (628 μl) was added to neutralize the solution, and the suspension was filtered and evaporated to give **11** (612 mg, 99.4%): m.p.: 159 °C; IR (KBr) ν 3420 (N–H), 2920 (O–H), 1653 (C=O), 1627 (N–H) cm^{-1}; ^1H NMR (D_2O) δ 6.10 (m, 1 H, –CH=C), 5.66 (br-d, 1 H, –C=CH), 4.35 (m, 2 H, H-1′ and 1), 3.82 (m, 1 H, H-4′), 3.68 (m, 1 H, H-3′), 3.56 (m, 2 H, H-3, 5), 3.45 (m, 2 H, H-2, 4), 3.38 (m, 2 H, –CH_2–N–), 3.20 (m, 3 H, H-2, –O–CH–), 2.64 (m, 2 H, –CH_2–S–), 2.49 (m, 2 H, –S–CH_2–), 1.51 (m, 4 H, –CH_2– × 2), 1.28 (m, 2 H, –CH_2–); Anal. $C_{36}H_{53}N_1O_{19}S_1 \cdot 0.25\ H_2O$. Calcd: C, 48.38; H, 7.29; N, 2.56. Found: C, 48.35; H, 7.05; N, 2.44.

3.11. 5-(2-aminoethylthio) pentyl 4-O-[4-O-(α-D-galactopyranosyl)- β-D-galactopyranosyl]-β-D-glucopyranoside hydrochloride (12)

To a solution of **8** (200 mg, 349 μmol) in MeOH (2.0 ml) was added 2-aminoethanthiol hydrochloride (198 mg, 1.75 mmol) and irradiated with ultraviolet light (254 nm) at 0 °C for 3 h. The mixture was concentrated and then the residue was purified by gel filtration with aqueous 5% acetic acid to give **12** (241 mg, 100%): ^1H NMR (D_2O) δ 3.09 (dd, 2 H, –CH_2–N–C–), 2.73 (dd, 2 H, –S–CH_2–), 2.47 (dd, 2 H, –CH_2–S–).

3.12. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-[4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-galactopyranosyl]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (13)

Compound **12** (225 mg, 328 μmol) was dissolved in methanol (3.0 ml) and cooled at 0 °C. Then to the solution was added sodium hydrogen carbonate (165 mg, 1.97 mmol) and acryloyl chloride (80.0 μl, 984 μmol) dropwise four times. To the mixture was added acetic anhydride (7.0 ml) and pyridine (10.0 ml) at room temperature for 13 h and concentrated. The residue was diluted with chloroform and washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 1:3 (v/v) toluene–ethyl acetate to give **13** (163 mg, 44.2%): $[\alpha]_D^{14.6} = +34.3°$ (*c* 1.09, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.24 (dd, 1 H, $J_{trans} = 17.1$ Hz, $J_{gem} = 1.1$ Hz, –C–C=CH), 6.22 (m, 1 H, –NH–), 6.09 (dd, 1 H, $J_{cis} = 10.2$ Hz, –C–C=CH), 5.60 (dd, 1 H, –C–C=CH), 5.53 (br-d, 1 H, H-4″), 5.33 (dd, 1 H, $J_{2'',3''} = 11.2$ Hz, $J_{3'',4''} = 3.2$ Hz H-3″), 5.14 (t, 1 H, $J_{2,3} = 9.1$ Hz, H-3), 5.13 (dd, 1 H, $J_{1'',2''} = 3.5$ Hz, H-2″), 5.05 (dd, 1 H, $J_{1',2'} = 7.5$ Hz, $J_{2',3'} = 10.7$ Hz, H-2′), 4.94 (d, 1 H, H-1″), 4.83 (dd, 1 H, $J_{1,2} = 8.0$ Hz, H-2), 4.69 (dd, 1 H, $J_{3',4'} = 2.7$ Hz, H-3′), 4.47 (d, 1 H, H-1′), 4.44 (m, 2 H, H-6a″, 6b″), 4.42 (d, 1 H, H-1), 4.38 (dd, 1 H, $J_{5,6b} = 6.7$ Hz, $J_{6a,6b} = 11.5$ Hz, H-6b), 4.07 (m, 4 H, H-6a, 6a′, 6b′ and 5″), 3.97 (br-d, 1 H, H-4′), 3.77 (m, 3 H, H-4, $-OCH_2$), 3.74 (t, 1 H, $J_{5',6a'} = J_{5',6b'} = 3.7$ Hz, H-5′), 3.43 (m, 2 H, $-CH_2-N-$), 2.63 (t, 2 H, $J = 2.6$ Hz, $-S-CH_2-C-N-$), 2.47 (t, 2 H, $J = 2.5$, $-C-CH_2-S-$), 1.99 (m, 30 H, –OAc × 10), 1.52 (m, 4 H, $-CH_2-$ × 2), 1.37 (m, 2 H, $-CH_2-$); Anal. $C_{48}H_{69}N_1O_{27}S_1$. Calcd: C, 51.29; H, 6.19; N, 1.25. Found: C, 51.27; H, 6.18; N, 1.22.

3.13. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-[4-O-(α-D-galactopyranosyl)- β-D-galactopyranosyl]-β-D-glucopyranoside (14)

To a solution of **13** (225 mg, 200 μmol) in methanol (2.85 ml) was added sodium methoxide (10.8 mg, 200 μmol), and the mixture was stirred for 8 h at room temperature. IR-120B (H^+) resin (158 μl) was added to neutralize the solution, and the suspension was filtered and evaporated to give **14** (141 mg, 100%): IR (KBr) ν 3445 (N–H), 2922 (O–H), 1654 (C=O), 1623 (N–H) cm^{-1}; ^1H NMR (D_2O) δ 6.10 (m, 2 H, –CH=C, –C=CH), 5.66 (d, 1 H, –C=CH), 4.83 (d, 1 H, H-1″), 4.39 (d, 1 H, H-1′), 4.34 (d, 1 H, H-1), 4.23 (br-t, 1 H, H-5″), 3.92 (br-d, 1 H, H-4′), 3.80 (m, 1 H, H-5′), 3.73 (m, 1 H, H-2″), 3.62 (m, 2 H, H-3′ and 4″), 3.52 (m, 2 H, H-3 and 5), 3.46 (m, 2 H, H-3″, 2′ and 4), 3.36 (m, 2 H, $-CH_2-N-$), 3.24 (s, 2 H, $-OCH_2-$), 3.18 (t, 1 H, H-2), 2.65 (m, 2 H, $-S-CH_2-$), 2.51 (m, 2 H, $-CH_2-S-$), 1.52 (m, 4 H, $-CH_2-$ × 2), 1.30 (m, 2 H, $-CH_2-$); ^{13}C NMR (D_2O) δ 168 (–C=O), 130 (–*C*=C), 128 (–C=*C*), 103 (C-1′), 102 (C-1), 100 (C-1″).

3.14. Copolymerization of glycosyl monomer with acrylamide

A solution of the glycosyl monomer **1** (100 mg, 185 μmol) and 10 molar equiv of acrylamide (131 mg, 1.85 mmol) in distilled water (2.0 ml) was degassed using a diaphragm pump, and TEMED (2.74 μl, 18.5 μmol) and APS (1.67 mg, 7.3 μmol) were added. The reaction mixture was continuously stirred for 2 h at room temperature, diluted with 0.1 M acetic acid–pyridine buffer (pH 5.00), purified by using gel filtration (Sephadex G-50), and lyophilized to give the water-soluble copolymer as a white powder. The same procedure was carried out for each of the monomers **8**, **11** and **14**.

3.15. Polymerization of glycosyl monomer

A solution of a glycosyl monomer **11** or **14** (100 mg, 185 μmol) in distilled water (2.0 ml) was degassed using a diaphragm pump, and TEMED (2.74 μl, 18.5 μmol) and APS (1.67 mg, 7.3 μmol) were added. The reaction mixture was continuously stirred for 2 h at room temperature, diluted with 0.1 M acetic acid–pyridine buffer (pH 5.00), purified using gel filtration (Sephadex G-50), and lyophilized to give the water-soluble copolymer as a white powder.

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