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.

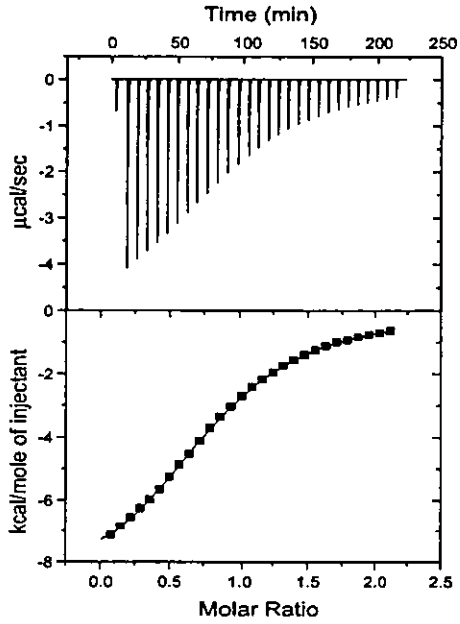


Figure 5. Calorimetric data for titration of concanavalin A, 0.21 mM, with trivalent ligand Fan(0)3-Man, 2.1 mM. Both protein and ligand were dissolved in buffer consisting of 50 mM 3,3-dimethylglutarate, 250 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$ adjusted pH 5.2. Top, raw (power vs time) data; bottom 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.[2Nd] 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, C*H*$_3$(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, C*H*$_3$(SAc)), 2.21, 2.14, 2.13, 2.12, 2.11, 2.06, 2.00 (21H, s, C*H*$_3$(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, CH_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, CH_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, CH_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–CH_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. $[\alpha]_D^{30}=+100.4°$ (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.

Acknowledgements

This work was supported by a Health and Labour Sciences Research Grant for Research on Advanced Medical Technology (14-N-015) from the Ministry of Health, Labour, and Welfare, Japan.

References and notes

1. Varki, A. *Glycobiology* **1993**, *3*, 97–130.
2. *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, 1999.
3. Kuo, C.-C.; Takahashi, N.; Swanson, A. F.; Dzeki, Y.; Hakomori, S.-I. *J. Clin. Invest.* **1996**, *98*, 2813–2818.
4. Helenius, A.; Trombetta, E. S.; Herbert, D. N.; Simons, J. F. *Trends Cell Biol.* **1997**, *7*, 193–200.
5. Wada, I.; Kai, M.; Imai, S.; Kanoh, H. *EMBO J.* **1997**, *16*, 5420–5432.
6. Ellgaad, L.; Helenius, A. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 181–191.
7. Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
8. (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720. (b) Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719–728.
9. (a) Lindhorst, Th. K. *Top. Curr. Chem.* **2002**, *218*, 201–235. (b) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696–703. (c) Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1997**, *3*, 1193–1199. (d) Roy, R. *Top. Curr. Chem.* **1997**, *187*, 241–274.
10. (a) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578. (b) Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* **2000**, *17*, 543–551. (c) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755–2794.
11. (a) Kawaguchi, K.; Lee, Y. C. *Proteins Nucleic Acids Enzymes* **1980**, *25*, 707–724. (b) Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225–281. (c) Connolly, D. T.; Townsend, R. R.; Kawaguchi, K.; Bell, W. R.; Lee, Y. C. *J. Biol. Chem.* **1982**, *257*, 939–945. (d) Lee, R. T.; Lin, P.; Lee, Y. C. *Biochemistry* **1984**, *23*, 4255–4261. (e) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321–327.
12. (a) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215–3237. (b) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742–748.
13. (a) Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *4*, 1949–1961. (b) Pagé, D.; Aravind, S.; Roy, R. *Chem. Commun.* **1996**, 1913–1914. (c) Pagé, D.; Roy, R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1765–1770. (d) Pagé, D.; Roy, R. *Bioconjugete Chem.* **1997**, *8*, 714–723. (e) Pagé, D.; Roy, R. *Glycoconjugete J.* **1997**, *14*, 345–356. (f) Roy, R.; Pagé, D.; Perez, S. F.; Bencomo, V. V. *Glycoconjugete J.* **1998**, *15*, 251–263. (g) Das, S. K.; Trono, M. C.; Roy, R. *Methods Enzymol.* **2003**, *362*, 3–17.
14. (a) Lindhorst, Th. K. *Nachr. Chem. Tech. Lab.* **1996**, *44*, 1073–1079. (b) Lindhorst, Th. K.; Kieburg, C.; Krallmann-Wenzel, U. *Glycoconjugate J.* **1998**, *15*, 605–613. (c) König, B.; Fricke, T.; Waßmann, A.; Krallmann-Wensel, U.; Lindhorst, Th. K. *Tetrahedron Lett.* **1998**, *39*, 2307–2310. (d) Kötter, S.; Krallmann-Wensel, U.; Ethlers, S.; Lindhorst, Th. K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2193–2200. (e) Dubber, M.; Lindhorst, Th. K. *J. Org. Chem.* **2000**, *65*, 5275–5281. (f) Lindhorst, Th. K.; Dubber, M.; Krallmann-Wenzel, U.; Ehlers, S. *Eur. J. Org. Chem.* **2000**, 2027–2034. (g) Horst, A. K.; Kötter, S.; Lindhorst, Th. K.; Ludwig, A.; Brandt, E.; Wagener, C. *Med. Microbiol. Immunol.* **2001**, *190*, 145–149. (h) Dubber, M.; Lindhorst, Th. K. *Synthesis* **2001**, 327–330. (i) Patel, A.; Lindhorst, Th. K. *J. Org. Chem.* **2001**, *66*, 2674–2680. (j) Röckendorf, N.; Sperling, O.; Lindhorst, Th. K. *Aust. J. Chem.* **2002**, *55*, 87–93. (k) Boysen, M. M. K.; Elsner, K.; Sperling, O.; Lindhorst, Th. K. *Eur. J. Org. Chem.* **2003**, 4376–4386. (l) Röchendorf, N.; Lindhorst, Th. K. *J. Org. Chem.* **2004**, *69*, 4441–4445. (m) Köhn, M.; Benito, J. M.; Ortiz Mellet, C.; Lindhorst, Th. K.; García Fernández, J. M. *ChemBioChem* **2004**, *5*, 771–777.
15. (a) Ashton, P. R.; Hounsell, E. F.; Jayaraman, N.; Nilsen,

T. M.; Spencer, N.; Fraser Stoddart, J.; Young, M. *J. Org. Chem.* **1998**, *63*, 3429–3437. (b) Nakata, E.; Nagase, T.; Shinkai, S.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 490–495.

16. Boysen, M. M. K.; Lindhorst, Th. K. *Tetrahedron* **2003**, *59*, 3895–3898.
17. (a) Matsuoka, K.; Terabatake, M.; Saito, Y.; Hagihara, C.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2709–2713. (b) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839–7842. (c) Matsuoka, K.; Saito, Y.; Terunuma, D.; Kuzuhara, H. *Kobunshi Ronbunshu* **2000**, *57*, 691–695. (d) Matsuoka, K.; Kurosawa, H.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Carbohydr. Res.* **2000**, *329*, 765–772. (e) Matsuoka, K.; Oka, H.; Koyama, T.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **2001**, *42*, 3327–3330. (f) Matsuoka, K.; Ohtawa, T.; Hinou, H.; Koyama, T.; Esumi, Y.; Nishimura, S.-I.; Hatano, K.; Terunuma, D. *Tetrahedron Lett.* **2003**, *44*, 3617–3620.
18. Nishikawa, K.; Matsuoka, K.; Kita, E.; Okabe, N.; Mizuguchi, M.; Hino, K.; Miyazawa, S.; Yamasaki, C.; Aoki, J.; Takashima, S.; Yamakawa, Y.; Nishijima, M.; Terunuma, D.; Kuzuhara, H.; Natori, Y. *Proc. Natl Acad. Sci. U.S.A.* **2002**, *99*, 7669–7674.
19. Takano, T.; Nakatsubo, F.; Murakami, K. *Carbohydr. Res.* **1990**, *203*, 341–342.
20. Houseman, B. T.; Gawalt, E. S.; Mrksich, M. *Langmuir* **2003**, *19*, 1522–1531.
21. Ravindranathan Karcha, K. P.; Jennings, H. J. *J. Carbohydr. Chem.* **1990**, *9*, 777–781.
22. Betaneli, V. I.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *107*, 285–291.
23. Zhang, J.; Kong, F. *Tetrahedron: Asymmetry* **2002**, *13*, 243–252.
24. (a) Pompipom, M. M. *Carbohydr. Res.* **1977**, *59*, 311–317. (b) Lee, E. E.; Wood, J. O. *Carbohydr. Res.* **1979**, *75*, 322–324. (c) Winnik, F. M.; Carver, J. P.; Krepinsky, J. J. *J. Org. Chem.* **1982**, *47*, 2701–2707. (d) Itoh, Y.; Tejima, S. *Chem. Pharm. Bull.* **1984**, *32*, 957–966. (e) Szurmai, Z.; Janossy, L.; Szlagyi, Z.; Vekey, K. *J. Carbohydr. Chem.* **1998**, *17*, 417–437.
25. (a) Terunuma, D.; Kato, T.; Nishio, R.; Matsuoka, K.; Kuzuhara, H.; Aoki, Y.; Nohira, H. *Chem. Lett.* **1998**, 59–60. (b) Terunuma, D.; Kato, T.; Nishio, R.; Aoki, Y.; Nohira, H.; Matsuoka, K.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2129–2134. (c) Tsuchida, T.; Shimazaki, C.; Hatano, K.; Matsuoka, K.; Aoki, Y.; Nohira, H.; Esumi, Y.; Terunuma, D. *Kobunshi Ronbunshu* **2003**, *60*, 561–568.
26. (a) van der Made, A. W.; van Leeuwen, P. W. N. M. *J. Chem. Soc., Chem. Commun.* **1992**, 1400–1401. (b) van der Made, A. W.; van Leeuwen, P. W. N. M.; de Wilde, J. C.; Brandes, R. A. C. *Adv. Mater.* **1993**, *5*, 466–468. (c) Muzafarov, A. M.; Gorbatsevich, O. B.; Rebrov, E. A.; Ignat'eva, G. M.; Chenskaya, T. B.; Myakushev, V. D.; Bulkin, A. F.; Papkov, V. S. *Vysokomol. Soedin., Ser. A Ser. B* **1993**, *35*, 1867–1872. (d) Seyferth, D.; Son, D. Y.; Rheingold, A. L.; Ostrander, R. L. *Organometallics* **1994**, *13*, 2682–2690.
27. (a) Winnik, F. M.; Brisson, J.-R.; Carver, J. P.; Krepinsky, J. J. *Carbohydr. Res.* **1982**, *103*, 15–28. (b) Madiyalakan, R.; Chowdhary, M. S.; Rana, S. S.; Matta, K. L. *Carbohydr. Res.* **1986**, *152*, 183–194.
28. (a) Williams, B. A.; Chervenak, M. C.; Toone, E. J. *J. Biol. Chem.* **1992**, *267*, 22907–22911. (b) Chervenak, M. C.; Toone, E. J. *J. Am. Chem. Soc.* **1994**, *116*, 10533–10539. (c) Chervenak, M. C.; Toone, E. J. *Bioorg. Med. Chem.* **1996**, *4*, 1963–1977. (d) Dimick, S. M.; Powell, S. C.; McMahon, S. A.; Moothoo, D. N.; Naismith, J. H.; Toone, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 10286–10296.
29. Edelhoch, H. *Biochemistry* **1967**, *6*, 1948–1954.
30. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Robers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.
31. Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L.-N. *Anal. Biochem.* **1989**, *179*, 131–137.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 57 (2004) 441–450
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

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

Atsushi Miyagawa[1], Hidehiro Kurosawa, Toshiyuki Watanabe, Tetsuo Koyama, Daiyo Terunuma, Koji Matsuoka*

Department of Functional Materials Science, Faculty of Engineering, Saitama University, 255 Shimo-okubo, Sakura, Saitama 338-8570, Japan

Received 26 February 2004; accepted 9 June 2004

Available online 8 July 2004

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

* Corresponding author. Tel./fax: +81-48-858-3099.
E-mail address: koji@fms.saitama-u.ac.jp (K. Matsuoka).
[1] Present address: Institute of Industrial Science, University of Tokyo, Meguro, Tokyo 153-8505, Japan.

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.06.001

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

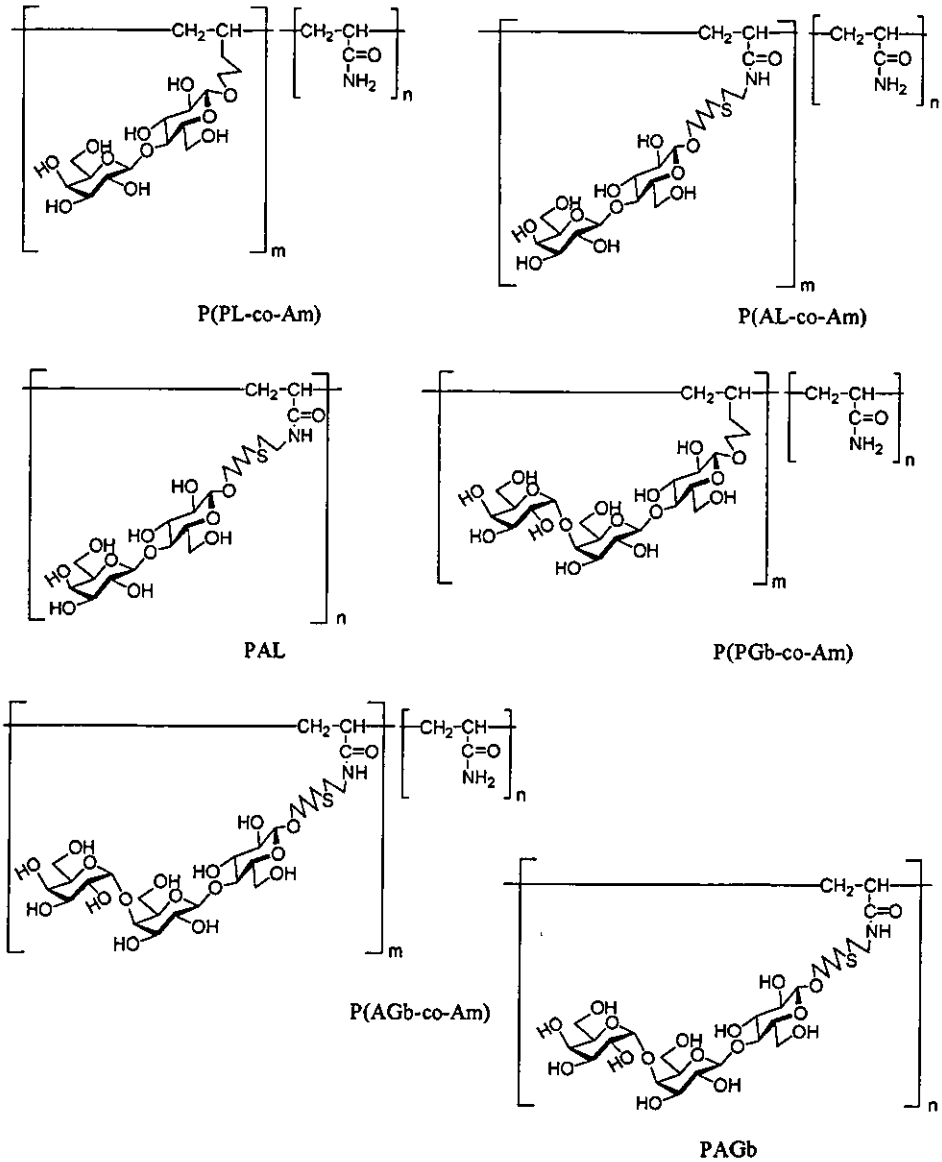
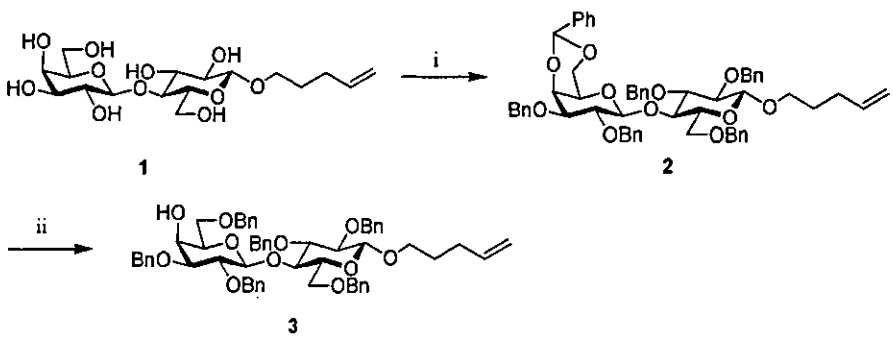
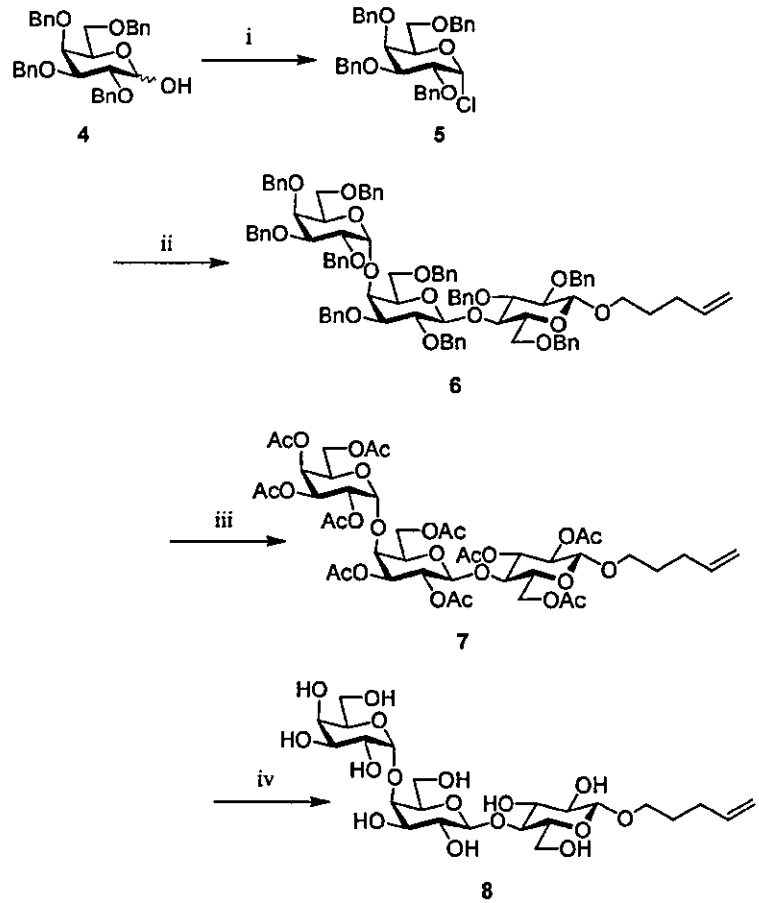


Fig. 1. Chemical structures of synthesized glycoconjugate polymers.

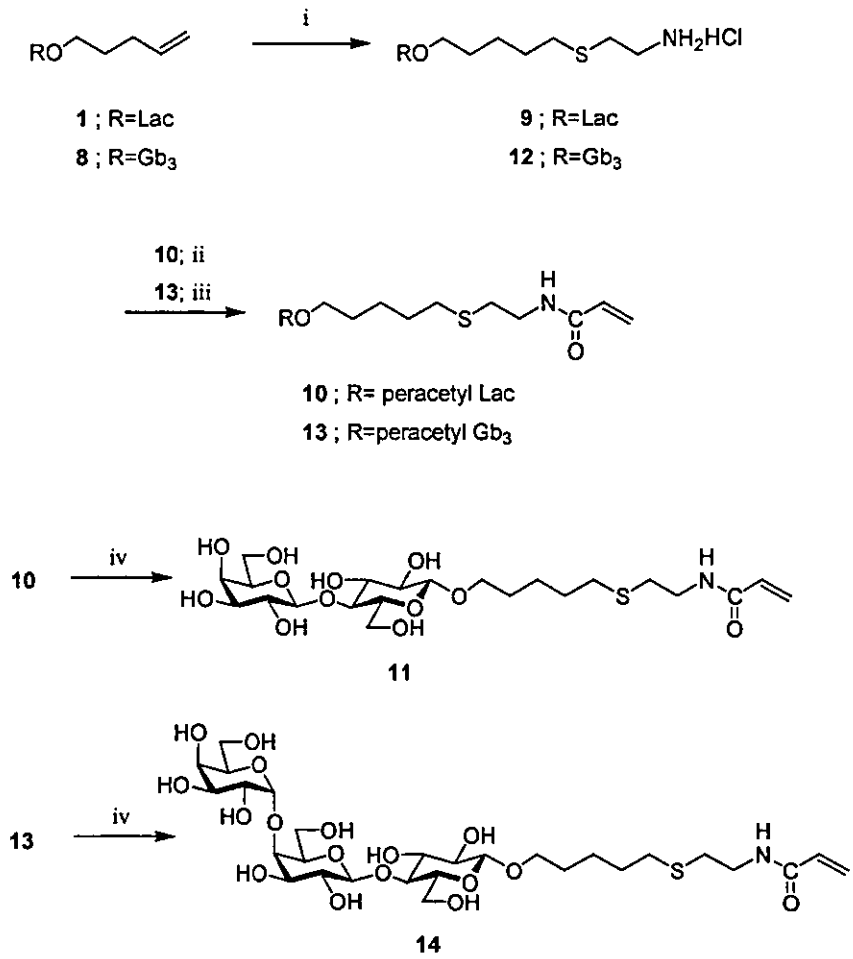


Scheme 1. Reagents and conditions: (i) $C_6H_5CH(OCH_3)_2$, CSA, 60°C, 2.5 h, under reduced pressure, then NaH, BnBr, DMF, rt, 1.5 h; (ii) $Me_3N{\cdot}BH_3$, $AlCl_3$, MS 4Å, THF, rt, 1 h.

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.

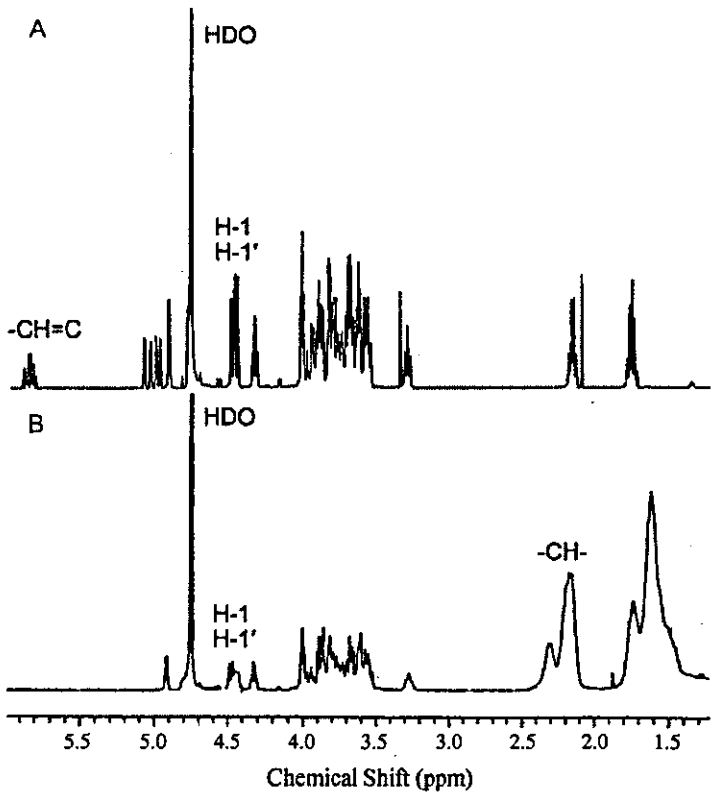


Fig. 2. ^1H NMR spectra of (A) glycosyl monomer **8**, (B) P(PGb-co-Am) in D_2O.

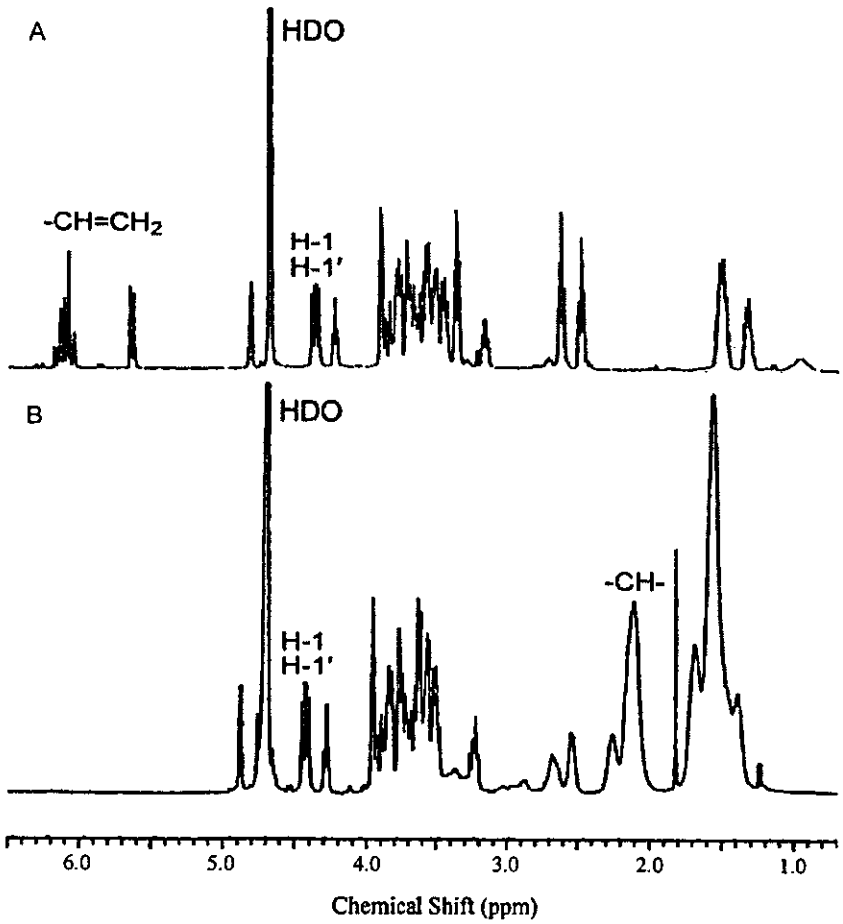


Fig. 3. ^{1}H NMR spectra of (A) glycosyl monomer **14**, (B) P(AGb-co-Am) in D_2O.

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.

Acknowledgements

We are grateful to Nishimura group of Hokkaido University for the SEC measurement of the polymer molecular weight, and to Natori and Nishikawa groups of International Medical Center of Japan for the polymer assay. We also thank Professor Hatanaka, K. and Dr Kasuya, M.K. of Institute of Industrial Science, University of Tokyo, for their critical reading of the manuscript and valuable discussions.

References

Arya, P., Kutterer, K. M. K., Qin, H., Roby, J., Barnes, M. L., Lin, S., Lingwood, C. A., & Peter, M. G. (1999). α Galactose based neoglycopeptides. Inhibition of verotoxin binding to globotriosyl ceramide. *Bioorganic and Medical Chemistry, 7*, 2823–2833.

Austin, P. W., Hardy, F. E., Buchanan, J. G., & Baddiley, J.2. (1965). 3,4, 6-Tetra-*O*-benzyl-D-galactosyl chloride and its use in the synthesis of a- and b-D-galactopyranosides. *Journal of the Chemical Society*, 1419–1424.

Bast, D. J., Banerjee, L., Clark, C., Read, R. J., & Brunton, J. L. (1999). The identification of three biologically relevant globotriaosyl ceramide receptor binding sites on the Verotoxin 1 B subunit. *Molecular Microbiology, 32*, 953–960.

Debenham, S. D., Cossrow, J., & Toone, E. J. (1999). Synthesis of α- and β-carbon-linked serine analogues of the P^k trisaccharide. *The Journal of Organic Chemistry, 64*, 9153–9163.

Dohi, H., Nishida, Y., Mizuno, M., Shinkai, M., Kobayashi, T., Takeda, T., Uzawa, H., & Kobayashi, K. (1999). Synthesis of an artificial glycoconjugate polymer carrying P^k-antigenic trisaccharide and its potent neutralization activity against Shiga-like toxin. *Bioorganic and Medical Chemistry, 7*, 2053–2062.

Gestwicki, J. E., Cairo, C. W., Strong, L. E., Oetjen, K. A., & Kiessling, L. L. (2002). Influencing receptor-ligand binding mechanisms with multivalent ligand architecture. *Journal of the American Chemical Society, 124*, 14922–14933.

Kitov, P. I., Sadowska, J. M., Mulvey, G., Armstrong, G. D., Ling, H., Pannu, N. S., Read, R. J., & Bundle, D. R. (2000). Shiga-like toxins are neutralized by tailored multivalent carbohydrate ligands. *Nature, 403*, 669–672.

Koto, S., Morishima, N., Miyata, Y., & Zen, S. (1976). Preparation of 2,3,4, 6-tetra-*O*-benzyl-D-mannose. *Bulletin of the Chemical Society of Japan, 49*, 2639–2640.

Lee, R. T., & Lee, Y. C. (1974). Synthesis of 3-(2-aminoethylthio)propyl glycosides. *Carbohydrate Research, 37*, 193–201.

Lee, Y. C., & Lee, R. T. (1995). Carbohydrate–protein interactions: basis of glycobiology. *Accounts of Chemical Research, 28*, 321–327.

Ling, H., Boodhoo, A., Hazes, B., Cummings, M. D., Armstrong, G. D., Brunton, J. L., & Read, R. J. (1998). Structure of the Shiga-like toxin I B-pentamer complexed with an analogue of its receptor Gb_3. *Biochemistry, 37*, 1777–1788.

Matsuoka, K., & Nishimura, S.-I. (1995). Synthetic glycoconjugates. 5. Polymeric sugar ligands available for determining the binding specificity of lectins. *Macromolecules, 28*, 2961–2968.

Matsuoka, K., Terabatake, M., Esumi, Y., Terunuma, D., & Kuzuhara, H. (1999). Synthetic assembly of trisaccharide moieties of globotriaosyl ceramide using carbosilane dendrimers as cores. A new type of functional glyco-material. *Tetrahedron Letters, 40*, 7839–7842.

Mylvaganam, M., & Lingwood, C. A. (1999). Adamantyl globotriaosyl ceramide: a monovalent soluble mimic which inhibits verotoxin binding to its glycolipid receptor. *Biochemical and Biophysical Research Communications, 257*, 391–394.

Nishikawa, K., Matsuoka, K., Kita, E., Okabe, N., Mizuguchi, M., Hino, K., Miyazawa, S., Yamasaki, C., Aoki, J., Takashima, S., Yamakawa, Y., Nishijima, M., Terunuma, D., Kuzuhara, H., & Natori, Y. (2002). A therapeutic agent with oriented carbohydrates for treatment of infections by Shiga toxin-producing *Escherichia coli* O157:H7. *Proceedings of the National Academy of Sciences, 88*, 7669–7674.

Nishimura, S.-I., Furuike, T., Matsuoka, K., Maruyama, K., Nagata, K., Kurita, K., Nishi, N., & Tokura, S. (1994). Synthetic glycoconjugates. 4. Use of ω-(acrylamido)alkyl glycosides for the preparation of cluster glycopolymers. *Macromolecules, 27*, 4876–4880.

Ogawa, T., Nakabayashi, S., & Kitajima, T. (1983). Synthesis of hexasaccharide unit of a complex type of glycan chain of a glycoprotein. *Carbohydrate Research, 114*, 225–236.

Roy, R. (1996). Syntheses and some applications of chemically defined multivalent glycoconjugates. *Current Opinion in Structural Biology, 6*, 692–702.

Roy, R., & Tropper, F. D. (1988). Synthesis of antigenic carbohydrate polymers recognized by lectins and antibodies. *Journal of the Chemical Society. Chemical Communications*, 1058–1060.

Soltyk, A. M., MacKenzie, C. R., Wolski, V. M., Hirama, T., Kitov, P. I., Bundle, D. R., & Brunton, J. L. (2002). A mutational analysis of the globotriaosyl ceramide-binding sites of verotoxin VT1. *The journal of Biological Chemistry, 277*, 5351–5359.

Takano, T., Nakatsubo, F., & Murakami, K. (1990). A facile allyl β-glycosylation in the presence of a benzyl protecting group, using boron trifuoride etherate. *Carbohydrate Research, 203*, 341–342.

Turnbull, W. B., & Stoddart, J. F. (2002). Design and synthesis of glycodendrimers. *Reviews in Molecular Biotechnology, 90*, 231–255.

Watanabe, M., Matsuoka, K., Kita, E., Igai, K., Higashi, N., Miyagawa, A., Watanabe, T., Yanoshita, R., Samejima, Y., Terunuma, D., Natori, Y., & Nishikawa, K. (2004). Oral therapeutic agents with highly clustered globotriose for treatment of shiga toxigenic *Escherichia coli* infections. *Journal of Infectious Diseases, 360*, 355–359.

van Seeventer, P. B., van Dorst, J. A. L. M., Siemerink, J. F., Kamerling, J. P., & Vliegenthart, J. F. G. (1997). Thiol addition to protected allyl glycosides: an improved method for the preparation of spacer-arm glycosides. *Carbohydrate Research, 300*, 369–373.

Prospects for Preventing Serious Systemic Toxemic Complications of Shiga Toxin–Producing *Escherichia coli* Infections Using Shiga Toxin Receptor Analogues

Mohamed A. Karmali

Laboratory for Foodborne Zoonoses, Health Canada, Guelph, and Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

(See the article by Watanabe et al., on pages 360–8.)

Shiga toxin (Stx)–producing *Escherichia coli* (STEC), also known as "verocytotoxin-producing *E. coli*" [1], are zoonotic pathogens that cause potentially fatal and often epidemic food- or waterborne illness [2–4] with a clinical spectrum that includes diarrhea, hemorrhagic colitis [5], and the hemolytic-uremic syndrome (HUS) [6, 7]. HUS, which is characterized by acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia, is the leading and a potentially fatal cause of acute renal failure in children [8–11]. There are no specific therapies for HUS or vaccines to prevent it. Histologically, HUS is characterized by widespread thrombotic microvascular lesions in the renal glomeruli, the gastrointestinal tract, and other organs, such as the brain, the pancreas, and the lungs [12–14]. These lesions probably result from primary Stx-mediated damage to microvascular endothelial cells [3, 4, 15–17]. The mechanism of this injury involves Stx binding to a specific receptor, globotriaosylceramide (Gb_3) [18], on the surface of endothelial cells [19] and toxin internalization by a receptor-mediated endocytic process [20], followed by toxin interaction with subcellular components that results in protein synthesis inhibition [19] or apoptosis [21].

STEC produce 2 major, serologically distinct Stx types, Stx1 and Stx2, which are AB_5 subunit toxins with 1 enzymatically active A subunit and 5 identical copies of the B subunit [22]. The B subunit pentamer is a doughnut-shaped structure composed of the individual subunits arranged symmetrically around a central α-helix–lined pore, over 1 face of which sits the A subunit [23]. The opposite face contains the receptor-binding domains of the B pentamer [24]. Stx2, which is found more often than Stx1 in the commonest STEC serotype, O157:H7 [25, 26], is reported to have a greater cytotoxic effect on human glomerular endothelial cells than does Stx1 in vitro [27] and thus may have a greater propensity than Stx1 to initiate HUS. Although the endothelial cell appears to be the main target of Stx action, there is evidence that these toxins also mediate biological effects on other cell types, such as renal tubular cells, mesangial cells, monocytes [16, 28], and platelets [16, 29]. Circulating proinflammatory cytokines, especially tumor necrosis factor (TNF)–α and interleukin (IL)–1β, stimulated by direct toxin action on monocytes [30], potentiate the action of Stx on endothelial cells by inducing expression of the receptor Gb_3 [31]. Binding of Stx to its target cells presumably initiates a complex chain of events, including coagulation and proinflammatory processes, that results in HUS [16, 28]. Blocking of Stx binding to endothelial cells to halt these events and prevent the development of HUS might be achieved by generation of specific Stx antibodies by active [32, 33] or passive [34–36] immunization or by the use of synthetic Gb_3 receptor analogues that competitively block toxin binding to the endothelial cell receptor, as discussed in the article by Watanabe et al. [37] in this issue of *The Journal of Infectious Diseases*.

Gb_3 (Pk blood group antigen) is composed of a sphingosine base to which are attached a fatty acid side chain and a terminal trisaccharide, Galα(1-4)-Galβ(1-4)-Glcβ1-, which binds to the Stx B pentamer. Although both Stx1 and Stx2 (including subtypes 2c and 2d) bind to Gb_3, their affinity for the molecule is variable, which probably reflects the fact that the amino acid sequence homology between the toxin-binding B subunits of the 2 toxins is only 57% [38]. Affinity is influenced

Received 16 October 2003; accepted 17 October 2003; electronically published 21 January 2004.

The views expressed in this article are solely those of the author and not necessarily those of Health Canada.

Reprints or correspondence: Dr. Mohamed Karmali, Laboratory for Foodborne Zoonoses, Health Canada, 110 Stone Rd. W, Guelph, Ontario N5G 1X8, Canada (mohamed_karmali@hc-sc.gc.ca).

The Journal of Infectious Diseases 2004; 189:355–9

0022-1899/2004/18903-0001$15.00

by difference in binding sites for Gb_3 in the toxins [39–41] and by the orientation of the terminal trisaccharide [42], which may be affected by a number of factors, including the length of the fatty acid side chain [41]. These considerations are important in designing synthetic receptor analogues, which, ideally, should bind both toxins with high affinity.

Theoretically, receptor analogues could be administered orally to block Stx produced in the gut from reaching the circulation or parenterally to block circulating Stx from binding to target endothelial cells. Although the precise timing of these events is not known, grounds for reasonable speculation may be based on clues from current knowledge of the natural history and pathophysiology of STEC-associated disease.

The initial symptoms of STEC infection, typically abdominal cramps and nonbloody diarrhea, manifest after an incubation period of ~3–5 days [2]. The mechanism of this initial diarrhea is not known, and there is no evidence that it is related to the action of Stxs. Approximately one-third of patients develop hemorrhagic colitis [2], which probably results from Stx interaction with endothelial cells lining the microvasculature of the gut lamina propria [43]. HUS occurs in approximately one-tenth to one-fourth of cases [2–4] and manifests ~1 week after the onset of diarrhea [2, 44]. Diagnostic studies of the detection of Stxs and STEC in stool samples from patients indicate that STEC bacterial counts and Stx titers in stool are probably already at their peak when the initial gastrointestinal symptoms appear, and the levels decrease thereafter [7, 44–46]. This is consistent with the occurrence of logarithmic growth, resulting in peak STEC bacterial counts and maximal toxin production, during the incubation period, a concept that is supported by observations of STEC growth in laboratory broth media that show maximal toxin titers during the STEC logarithmic growth phase and cessation of toxin production in the stationary phase [47]. Thus, Stx translocation from the gut to the circulation must occur at some point between the incubation period, when Stx is being actively produced, to ~1 week after the start of the illness, when HUS, the end result of toxin action, becomes clinically evident. Evidence based on elevated levels of plasma markers of thrombin generation, intravascular fibrin accretion, and fibrinolysis inhibition [48, 49] indicates that vascular activation has probably already occurred within 4 days after the onset of diarrhea [49], which suggests that toxin binding to its target cells must have occurred even earlier, perhaps by 2–3 days after the onset of symptoms. Thus, for optimal therapeutic effect, a receptor analogue would have to be administered at some point between the incubation period, before symptoms start, and a point within ~2–3 days after the onset of diarrhea. Presumably, the earlier the better. Unfortunately, experimental animal models of HUS have not been developed in which tests can be made to determine which therapies produce optimal effects. Consequently, investigators in Canada [50–52], Japan [53, 54], and Australia [55, 56] have actively pursued the development of potential therapeutic receptor analogue compounds.

The initial work was done in Canada, where Armstrong and colleagues [50, 52] found that Stx1 and Stx2 bound with high affinity to Pk trisaccharide covalently coupled to Chromosorb P (Synsorb; Johns-Manville Products), a calcinated, diatomaceous material used in gas-liquid chromatography. However, when Synsorb-Pk (R. Ippolito, Alberta Research Council, Edmonton, Alberta, Canada) was coincubated with Vero cells, it was much more efficient in neutralizing the Vero cell cytotoxicity of Stx 1 than that of Stx2 [50]. After a successful phase 1 clinical study of orally administered Synsorb-Pk [51], Armstrong et al. [52] implemented a multicenter, randomized, double-blind, placebo-controlled phase 2 clinical trial in children with diarrhea and confirmed STEC infection, close contact with an individual with HUS or STEC infection, or evidence of early signs of HUS. The results were inconclusive. A phase 3 trial that included individuals who had no evidence of HUS, who had experienced ≤4 days of diarrhea, and who had received a diagnosis of STEC infection confirmed by rapid tests was initiated in 1996, but this trial was prematurely terminated for reasons that were not publicized. Another clinical trial in Japan, which used historical controls, was inconclusive [57].

Trachtman et al. [58] postulated that the severity of diarrhea-associated HUS might be related to ongoing intestinal Stx production and systemic absorption and argued that administering Synsorb-Pk to patients after the onset of HUS might reduce the severity of disease. A multicenter, randomized, double-blind, placebo-controlled trial of Synsorb-Pk in 145 children with diarrhea-associated HUS that lasted from 1997 through 2001 failed to show that this compound could diminish the severity of HUS [58].

The terminal Gb_3 trisaccharide, Galα(1-4)-Galβ(1-4)-Glcβ1-, is one of the alternative structures for the outer core of the lipooligosaccharide surface coat of pathogenic *Neisseria* and *Haemophilus* species [59], an observation that prompted Paton et al. [55] to genetically engineer a nonpathogenic *E. coli* strain that expressed this structure on its surface as a potential "probiotic" agent against STEC infection. The recombinant bacterium CWG308:pJCP-Gb3 absorbed and neutralized Stx1, Stx2, Stx2c, and Stx2d very efficiently, and oral administration completely protected streptomycin-treated mice against an otherwise 100% fatal dose of B2F1, the most mouse-virulent strain used in this model [55]. Synsorb-Pk did not offer protection [60]. These protective effects in mice were confirmed using formaldehyde-killed CWG308:pJCP-Gb3 bacteria [55].

Although oral administration of a receptor blocker has the theoretic advantage of neutralizing toxin before the toxin translocates systemically, investigators in Japan [53] and Canada [61, 62] also investigated the feasibility of administering

soluble compounds parenterally. Nishikawa et al. [53] developed a series of dendritic (branched, treelike) polymers, referred to as "SUPER TWIGS," in which several Gb_3 trisaccharide molecules are present in different orientations. A SUPER TWIG (1)6 molecule with 6 trisaccharides had a high affinity for and in vitro neutralizing activity against both Stx1 and Stx2. The compound was tested in a mouse model in which mice were rendered highly susceptible to *E. coli* O157:H7 infection and to its systemic Stx toxemic complications by protein calorie malnutrition (PCM), which lasted for ~2 weeks before intragastric challenge with *E. coli* O157:H7. In this model, which was pioneered by Kurioka et al. [63], PCM mice (but not well-nourished mice) develop Stx-related neurologic symptoms 5 days after infection, and approximately three-fourths of the mice die by 10 days after infection. Kurioka et al. [63] found that inoculated *E. coli* O157:H7 multiplied in the intestines between day 2 after infection and day 4, when growth had reached its peak, an incubation period that is similar in duration to that in humans [2]. Stx was detectable in stool at day 2 after infection, and maximal titers were reached by day 4, paralleling the time of postulated peak toxin titers in humans. Stx was detected in blood only from day 3 to day 5 after infection. *E. coli* O157 strains producing Stx1, Stx2, or both were used, and 10-day mortality tended to be highest among mice challenged with strains that produced both toxins. When Nishikawa et al. [53] administered Stx2 intravenously with SUPER TWIG (1)6, the mice were completely protected from Stx2-induced fatality. When mice infected intragastrically with *E. coli* O157:H7 (producing Stx1 and Stx2) were given SUPER TWIG (1)6 intravenously twice a day from day 3 through day 6 after infection, a majority of treated mice survived, compared with untreated controls, none of which survived. Tailored multivalent soluble Gb_3 analogues constructed for intravenous use have also been developed in Canada. One of these compounds, "Starfish," which has an affinity for Stx1 and Stx2 that is several orders of magnitude higher than that of Synsorb-Pk [61], protected mice when it was injected subcutaneously in combination with a lethal dose of Stx1, but not when it was injected in combination with Stx2 [62]. On the other hand, a modified version of Starfish, called "Daisy" [62], protected mice against both Stx1 and Stx2 when toxin-inhibitor mixtures were administered parenterally. Furthermore, Daisy also protected streptomycin-treated mice from a fatal dose of the Stx2d-producing strain B2F1 [62].

The paper by Watanabe et al. [37] represents an investigation of the potential therapeutic efficacy of a new oral synthetic receptor analogue compound with a configuration different from that of SUPER TWIG, referred to as "Gb_3 polymer," which has an affinity for both Stx1 and Stx2 that is much higher than that exhibited by both parenterally administered SUPER TWIG (1)6 and orally administered Synsorb-Pk. This innovative compound was developed as a synthetic oral alternative to the recombinant CWG308: pJCP-Gb3 bacterium developed by Paton and colleagues [55, 56]. It is derived from linear polymers of acrylamide made with different densities of the Gb_3 trisaccharide Galα(1-4)-Galβ(1-4)-Glcβ1- molecules that are attached to the core acrylamide structure by a spacer and thus differs from the dendritic design of the SUPER TWIG compounds. The trisaccharide densities used were not critical for Stx1 binding, whereas high-affinity Stx2 binding was only observed in compounds with highly clustered trisaccharides.

Watanabe et al. [37] investigated the protective effect of intragastrically administered Gb_3 polymer against the systemic toxemic complications of *E. coli* O157:H7 using a protocol in PCM mice similar to that used earlier by Nishikawa et al. [53] to investigate the efficacy of intravenously administered SUPER TWIG molecules. Compared with saline control, Gb_3 polymer, administered intragastrically on days 3–5 after infection (corresponding to the time during the incubation period when gut toxin levels were in the exponential growth phase and Stx was detectable in the blood), exhibited a highly significant protective effect that was associated with a reduction of Stx2 titers (Stx1 was not tested, because Stx2 was considered more relevant clinically) in both blood and stool. The authors estimate that the Stx-binding capacity of Synsorb-Pk is at least 100,000 times lower than that of the Gb_3 polymer and suggest that this is probably because the density of trisaccharide displayed on the surface of Synsorb-Pk is 2000-fold lower than that on the Gb_3 polymer.

The abandonment of the Synsorb-Pk phase 3 clinical trial means we will not know for certain whether Synsorb-Pk would have succeeded clinically in preventing HUS despite its limited ability to neutralize the biological activity of Stx2 in vitro [50] and in the streptomycin-treated mouse model [60]. However, the Gb_3 polymer of Watanabe et al. [37] has clearly overcome the technical limitations associated with Synsorb-Pk. Gb_3 polymer has high binding affinity for both Stx1 and Stx2, is able to neutralize the biological activities of both toxins in cell culture, and is protective against otherwise fatal infection with *E. coli* O157 when the polymer is administered intragastrically in the PCM mouse model 3 days after infection has been initiated but while it is still in the incubation phase. Experimental evidence suggests that Gb_3 polymer has a substantially higher affinity for both Stx1 and Stx2 (Stx2c and Stx2d were not evaluated) than does Synsorb-Pk. Theoretically, therefore, Gb_3 polymer should have greater clinical efficacy than Synsorb-Pk. The challenge is to discover when in the course of infection an agent such as Gb_3 polymer should be administered to reap the desired benefit in reducing the risk that HUS will develop. This depends on whether toxin production in the gut and toxin translocation are prolonged processes that offer a wide window of opportunity for therapeutic inter-