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Syntheses and Vero toxin-binding activities of carbosilane dendrimers periphery-functionalized with galabiose

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Abstract—Carbosilane dendrimers bearing galabiose (Galα1–4Gal) with three, four, and six galabiose units at the periphery of the dendrimers were synthesized for use as artificial inhibitors against Shiga toxins (Stxs) produced by *Escherichia coli* O157:H7. The galabiose unit, prepared from penta-*O*-acetyl-β-D-galactopyranose, was linked with carbosilane dendrimers of three shapes to afford acetyl-protected glycodendrimers in good yields. De-O-acetylation of the clusters was carried out in the presence of NaOMe and then aq NaOH to give the desired three shapes of galabiose-coated carbosilane dendrimers. Their biological activities toward Stxs were evaluated by kinetic analysis, binding assays, and cytotoxic assays.

1. Introduction

Protein–carbohydrate interactions play important roles in many biological phenomena such as processes of cell adhesion with proteins of pathogens.[1] Although the interactions between single carbohydrate ligands and protein receptors are usually weak, it is known that clustering of carbohydrate ligands induces enhancement of the interaction due to the glycoside cluster effect.[2] Artificial clustering carbohydrate models,[2,3] i.e., glycopolymers and glycodendrimers, have been utilized extensively for the enrichment of protein–carbohydrate interaction. Recently, a large number of neoglycoconjugates carrying a globotriaose cluster have been synthesized by several groups,[4] and their activities have been evaluated.[5]

Shiga toxins (Stxs: Stx1 and Stx2) produced by *Escherichia coli* O157:H7, known as Vero toxins, cause diarrhea and hemolytic uremic syndrome (HUS). Stxs are multimeric toxins consisting a single catalytically active A subunit and a pentamer of B subunits.[6] The B subunit is responsible for binding to globotriaose (Galα1–4Galβ1–4Glc).[7] Successful preparation of carbosilane dendrimers periphery-functionalized with globotriaose for the purpose of neutralizing Stxs has been reported.[4a] It was found that the dendrimers carrying globotriaose, named Dumbbell(1)6 and Dumbbell(2)18 (Fig. 1), completely inhibited Stxs in vivo.[5a]

On the other hand, galabiose (Galα1–4Gal), the constitutive part of globotriaose, is also known to have the ability to bind to Stxs.[7] Enhancement of the binding ability by clustering of galabiose would be advantageous compared to clustering of globotriaose from the viewpoint of practical use for a Stxs neutralizer, because preparation of the former disaccharide cluster is easier than that of the latter trisaccharide one.

Magnusson[8a] and Pieters[8b] independently reported the preparation of galabiose clusters using dendrimers and the results of evaluation of their potency for inhibiting *Streptococcus suis*. We describe herein the syntheses and Stxs binding activities of dendritic galabiose clusters using a carbosilane dendrimer as a glycocluster backbone.

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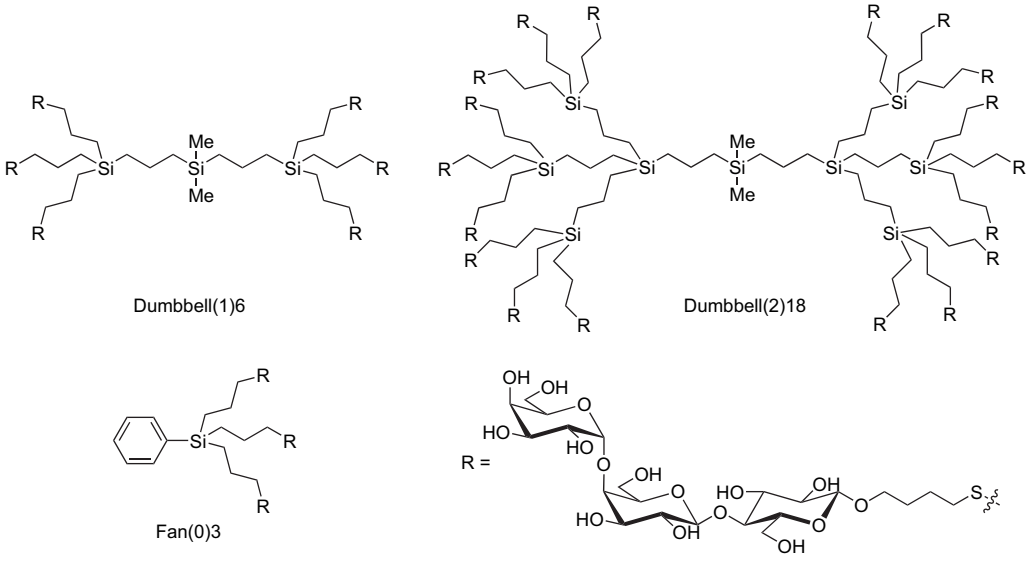


Figure 1. Structure of globotriaose cluster compounds.

2. Results and discussion

2.1. Syntheses of glycoclusters

In the course of our studies on the synthesis of an Stxs inhibitor,[4a] the preparation and characterization of new carbosilane dendrimers of three shapes having galabiose were carried out. One essential point for the molecular design of a carbosilane dendrimer bearing peripheral galabiose is adjustment of the molecular size to that of Stxs adhesion-point distance, ca. 46 Å (The information is obtained from crystallographic analysis.), because the size of galabiose is smaller than that of the already reported Dumbbell(1)6 for globotriaose (Fig. 1). Thus, a carbosilane dendrimer larger than that of Dumbbell(1)6 was designed. Another essential point is that the carbosilane dendrimer derivative must have sufficient water solubility. The water solubility of the glycocluster is influenced by the balance between hydrophobicity of the carbosilane dendrimer and hydrophilicity of the saccharide used. Either an increase of hydrophobicity by using a longer-armed carbosilane dendrimer or a decrease of hydrophilicity by using a disaccharide results in low water solubility of the galabiose clusters.

At first, new carbosilane dendrimers shown in Figure 2 were designed. The carbosilane dendrimers were prepared in a manner similar to that described in the literature (Scheme 1).[9] The hydrosilylation reaction of commercially available triallylmethylsilane (**1**) with chlorodimethylsilane catalyzed by H_2PtCl_6 followed by alkylation with allyl grignard reagent gave tri-branched dendrimer **2** in 31% yield. In the reaction mixture, a small amount of probably olefin-rearranged by-products was detected by ^{1}H NMR spectra, 5.93–6.08 (m), 5.58–5.63 (m), 1.82 (d, J=1.6, 6.2 Hz) ppm. The by-products were carefully removed by distillation. Both tetra-branched dendrimer (**4**) and hexa-branched dendrimer (**5**) were also synthesized from bis(allyldimethylsilylpropyl)dimethylsilane (**3**)[10] using the corresponding chlorosilanes. The ω-brominated carbosilane dendrimers **6–8** were prepared by: (i) hydroxylation of terminal olefin, (ii) mesylation, and then (iii) bromination using excess sodium bromide. The carbosilane dendrimers **6**, **7**, and **8** were obtained in 18%, 41%, and 15% yields, respectively. The structures of these bromide dendrimers were confirmed by ^{1}H, ^{13}C NMR, and FABMS spectra.

On the other hand, galabiose derivatives were synthesized by a procedure closely analogous to that used for the synthesis of a globotriaose derivative (Scheme 2).[4a] Reductive opening of the 4,6-*O*-benzylidene ring of 4-pentenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopylanoside (**9**)[11] gave 4-pentenyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**10**) in 90% yield. Stereoselective glycosylation of **10** and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[12] was carried out in the presence of AgOTf as a promoter in ether at −20 °C to afford galabioside (**11**) in 71% yield. The α-galactose linkage was confirmed by the ^{1}H NMR signal at δ 5.02 ppm ($J_{1',2'}$=1.2 Hz, H-1′) and the ^{13}C NMR

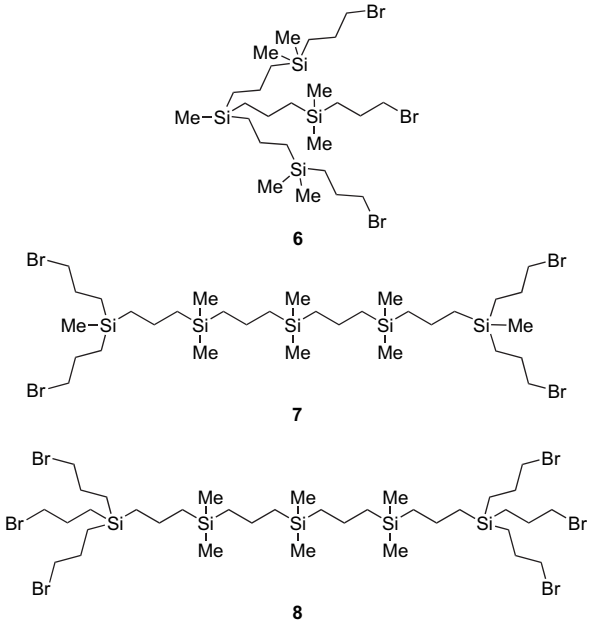
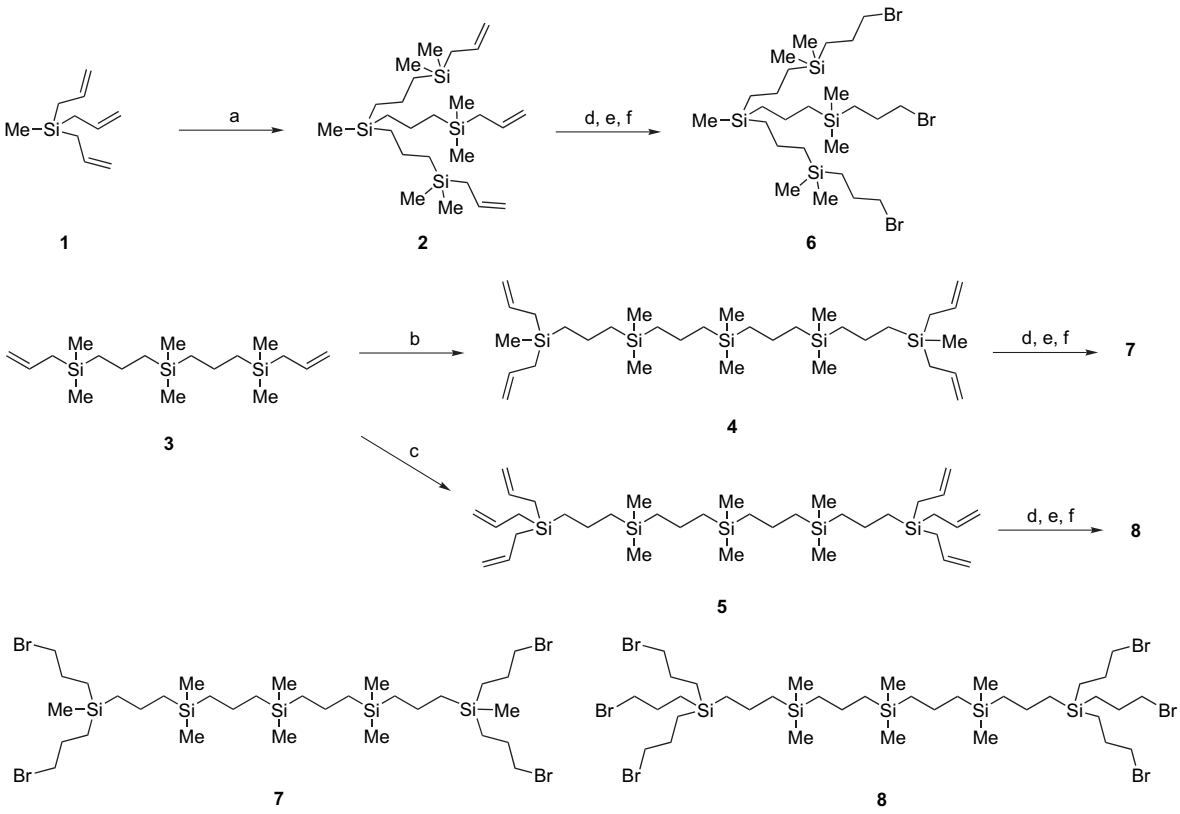


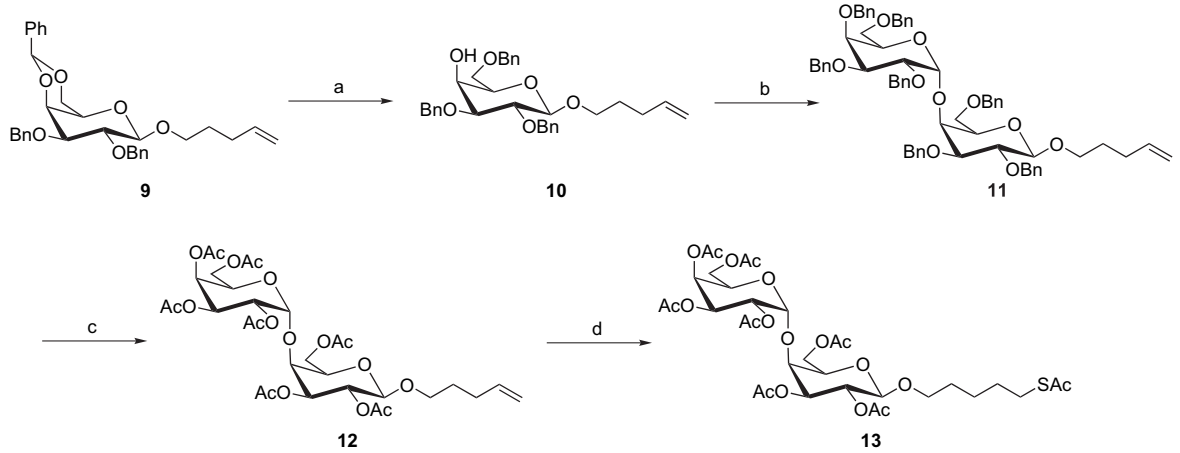
Figure 2. Structure of new carbosilane dendrimers.



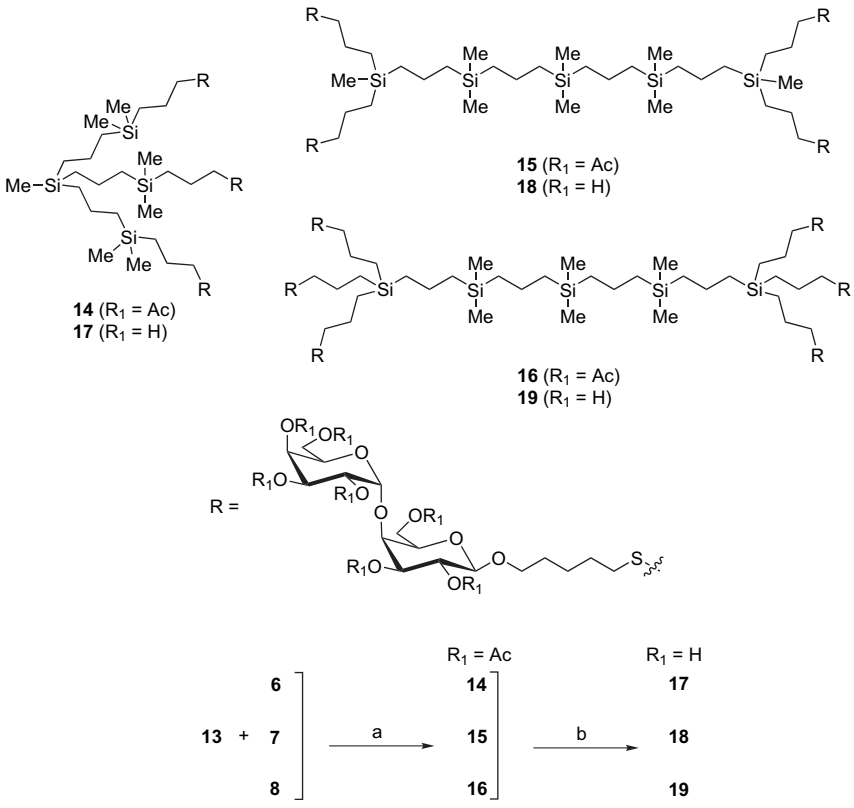
Scheme 1. Reagents and conditions: (a) $HSiMe_2Cl$, Speier cat., THF, 50 °C, then allyl grignard, reflux (31%); (b) $HSiMeCl_2$, Speier cat., THF, 40 °C, then allyl grignard, reflux (81%); (c) $HSiCl_3$, Speier cat., THF, rt-reflux, then allyl grignard, reflux (37%); (d) cyclohexylborane, THF, then 3 M NaOH aq, 30% H_2O_2 aq, 60 °C; (e) MsCl, Py, −20 °C; (f) NaBr, DMF, 80 °C.

signals at δ 103.87 and 100.37 ppm (C-1 and C-1′, respectively). Elemental analysis and FABMS spectrum ($[M-H]^+$ 1039.0) also support the disaccharide structure. Debenzylation of **11** without affecting the terminal double bond of the aglycon was conducted through Birch reduction. Removal of the benzyl groups from **11** followed by acetylation afforded acetyl-protected galabioside (**12**) in 38% yield (Scheme 2), and about 60% of the starting compound **11** was recovered. Acetylthio function was introduced into **12** carrying an olefin terminal by treatment with AcSH and AIBN to give **13** in 93% yield after purification by silica gel column chromatography. The structure of **13** was confirmed by ^1H and ^{13}C NMR spectra, FABMS spectrum, and elemental analysis. The signals at 2.86 (t, 2H, J=7.2 Hz, CH_2SAc) and 2.32 (s, 3H, CH_2SAc) ppm in the ^1H NMR spectrum of **13** prove the formation of acetylthio function.

Finally, acetylated glycocluster Fan(1)3-Ga2OAc (**14**) was synthesized by coupling of the dendrimer **6** with the acetylthio-terminated galabiose **13** (2.1 equiv for each terminal branch) in the presence of NaOMe (1.1 equiv for sugar



Scheme 2. Reagents and conditions: (a) $AlCl_3$, Me_3N–BH_3, MS4A, THF, rt (90%); (b) 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl chloride, AgOTf, MS4A, Et_2O, −20 °C (71%); (c) Na, liq. NH_3, dimethoxyethane, −72 °C, then Ac_2O, Py, rt (38%); (d) AcSH, AIBN, 1,4-dioxane, 80 °C (93%).



Scheme 3. (a) NaOMe, DMF, MeOH, rt, then Ac_2O, Py, rt (86% for **14**, 72% for **15**, and 75% for **16**); (b) NaOMe, MeOH, rt, then dil NaOH aq, rt (91% for **17**, 99% for **18**, 98% for **19**).

Table 1. HRMS (ESI) of galabiose clusters

Compounds	Formula	*m/z* (Calculated)	*m/z* (Found)
Fan(1)3-Ga2OAc (**14**)	$C_{118}H_{192}O_{54}S_3Si_4+Na^+$	2704.04148	2704.04324
Dumbbell(2)4-Ga2OAc (**15**)	$C_{156}H_{252}O_{72}S_4Si_5+2Na^+$	1795.67912	1795.68283
Dumbbell(2)6-Ga2OAc (**16**)	$C_{222}H_{348}O_{108}S_6Si_5+2Na^+$	2559.93525	2559.92997
Fan(1)3-Ga2 (**17**)	$C_{76}H_{150}O_{33}S_3Si_4+Na^+$	1821.81962	1821.81874
Dumbbell(2)4-Ga2 (**18**)	$C_{100}H_{196}O_{44}S_4Si_5+Na^+$	2392.07263	2392.07023
Dumbbell(2)6-Ga2 (**19**)	$C_{138}H_{264}O_{66}S_6Si_5+2Na^+$	1677.71339	1677.71763

moiety) (Scheme 3).[13] Purification by recycling preparative HPLC afforded pure **14** in 86% yield. Dumbbell(2)4-Ga2OAc (**15**) and Dumbbell(2)6-Ga2OAc (**16**) were prepared from the corresponding dendrimers in a manner similar to that described above in 72% and 75% yields, respectively. De-O-acetylation by NaOMe in MeOH and successive saponification of **14**, **15**, and **16** gave target compounds Fan(1)3-Ga2 (**17**), Dumbbell(2)4-Ga2 (**18**), and Dumbbell(2)6-Ga2 (**19**) in 91%, 99%, and 98% yields, respectively. The galabiose clusters **18** and **19** were soluble in an aqueous solution. Fortunately, the level of water solubility was adequate for biological assay systems, although that of **17** was low. All the synthesized acetyl- and de-acetylated clusters were fully characterized by high-resolution mass spectrometry (Table 1). The results showed good agreement with the calculated values for the expected structures.

2.2. Inhibition studies of the glycoclusters

The synthesized galabiose cluster compounds were assessed by using a BIAcore instrument. The dissociation constants (K_D) of these clusters to the Stx1 and Stx2 B subunits were determined by Scatchard plot analysis. Compound **19** showed K_D values of 1.3 and 1.6 μM for Stx1 and Stx2, respectively (Table 2). Previously reported K_D values of hexavalent globotriaose cluster Dumbbell(1)6 were 0.11 and 0.21 μM for Stx1 B subunit and Stx2 B subunit, respectively.[5a,14] Therefore, the potency of **19** is one-tenth of that of Dumbbell(1)6. However, the K_D values of **17** are similar to those of trivalent globotriaose cluster Fan(0)3 (structure of Fan(0)3 is shown in Fig. 1). The potency of the clusters **17**,

Table 2. Kinetic analysis of dendrimers having oligosaccharide toward Stxs

Dendrimers	Stx1 B subunit	Stx2 B subunit
	K_D, μM	K_D, μM
Fan(1)3-Ga2 (**17**)	61.1	53.9
Dumbbell(2)4-Ga2 (**18**)	10.5	10.1
Dumbbell(2)6-Ga2 (**19**)	1.3	1.6
Fan(0)3[a]	64.8	124
Dumbbell(1)6[a]	0.11	0.21

[a] See Refs. 5a and 14.

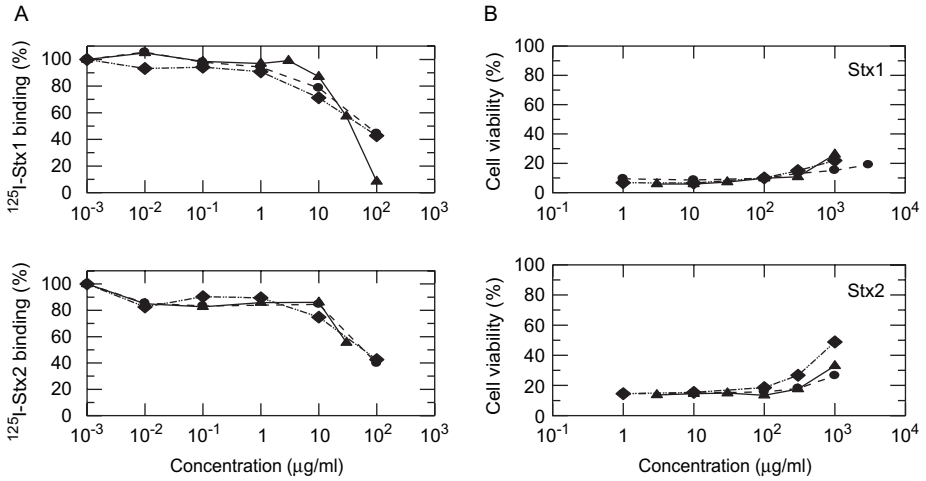


Figure 3. Inhibitory effects of carbosilane dendrimers having galabiose on the biological activities of Stxs in Vero cells. (A) Results of ^{125}I-Stxs binding assay. (B) Results of cytotoxicity assay using Vero cells. The symbols ▲, ●, and ◆ indicate Fan(1)3-Ga2 (**17**), Dumbbell(2)4-Ga2 (**18**), and Dumbbell(2)6-Ga2 (**19**), respectively.

18, and **19** was further evaluated by ^{125}I-labeled Stxs (^{125}I-Stxs) binding assay and cytotoxic assay. It was found that the clusters **17**, **18**, and **19** inhibited the binding of ^{125}I-Stx1 and ^{125}I-Stx2 to Vero cells (Fig. 3A). The IC_{50} values in the ^{125}I-Stxs binding assay are shown in Table 3. The minimum IC_{50} value was 13.6 μM, which is larger than that of Dumbbell(1)6.[5a] On the other hand, **17**, **18**, and **19** showed very weak inhibitory effects (Fig. 3B) in the cytotoxic assay. Only the IC_{50} value of **19** for Stx2 was determined (362 μM). Interestingly, the inhibitory potency in these analyses of **19** is lower than that of Dumbbell(1)6, although the potency of **17** is similar to that of Fan(0)3. The intra-sugar distances between each branches of **17**, about 29 Å, are similar to those of globotriaose clusters, about 27 Å. In contrast, the intra-sugar distances of **19**, about 15 Å, are shorter than those of globotriaose clusters. These results suggest that a long intra-sugar distance in dendritic galabiose is preferable for high binding activity of Stxs.

Table 3. Determination of the IC_{50} values in ^{125}I-Stxs binding assay

Dendrimers	Stx1 B subunit	Stx2 B subunit
	IC_{50}, μM	IC_{50}, μM
Fan(1)3-Ga2 (**17**)	18.9	17.8
Dumbbell(2)4-Ga2 (**18**)	23.6	23.6
Dumbbell(2)6-Ga2 (**19**)	14.2	13.6
Fan(0)3[a]	21.4	>50
Dumbbell(1)6[a]	0.08	0.50

[a] See Ref. 5a.

3. Conclusion

A series of new carbosilane dendrimers periphery-functionalized with galabiose was synthesized. The synthesized dendrimers having galabiose were evaluated by biological potency of Stx1 and Stx2 using kinetic analysis, binding assays, and cytotoxic assays. The results show that about 29 Å intra-sugar distance between each branches is required for increasing binding activity toward Stxs.

4. Experimental

4.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 and DRX 400, at 400 and 400 MHz for proton and at 100 and 100 MHz for carbons, respectively. Proton chemical shifts are given in parts per million with the use of tetramethylsilane (0 ppm) or residual solvent peaks as internal standard. NMR signals were assigned by ^{1}H, ^{13}C, HH, and HC COSY measurements. FAB and ESI mass spectra were obtained with a JEOL JMS-HX110A spectrometer and a JEOL JMS-T100LC spectrometer, respectively. Optical rotations were recorded on a JASCO DIP-1000 digital polarimeter at ambient temperature, using a 10-cm micro cell. Recycling preparative HPLC was performed with a LC-908 or LC-918W (Japan Analytical Industry Co., Ltd) connected to an RI detector RI-5. The computer generated low energy conformations were calculated using the CaChe software (Fujitsu Inc.).

4.1.1. Tris[3-(allyldimethylsilyl)propyl]methylsilane (2). A solution of chlorodimethylsilane (16.8 g, 177.6 mmol) in THF (3 mL) was added dropwise to a mixture of **1** (1.97 g, 11.8 mmol) and Speier catalyst (six drops) in THF (3 mL) under Ar, and the reaction mixture was stirred at 50 °C for 24 h. Chlorodimethylsilane and THF were eliminated by heating the mixture. The residue was dissolved in THF (25 mL) and a solution of allyl grignard reagent (88.8 mmol) in diethyl ether (45 mL) was added to the reaction solution at 0 °C. The mixture was refluxed overnight and then 1 M HCl aq was added dropwise to the resulting mixture. The mixture was extracted with diethyl ether and washed with water and brine. The organic layer was dried (Na_2SO_4) and the filtrate concentrated. Distillation of the residue gave compound **2** (1.73 g, 31%). Bp 220 °C/4 torr. ^{1}H NMR ($CDCl_3$) δ 5.75–5.81 (m, 3H, –C*H*=CH_2), 4.80–4.86 (m, 6H, –CH=CH_2), 1.52 (d, 6H, J=16.8 Hz, SiCH_2CH=CH_2), 1.32–1.34 (m, 6H, $SiCH_2CH_2CH_2Si$), 0.53–0.61 (m, 12H, $SiCH_2$), 0.01 (s, 21H, Si–CH_3); ^{13}C NMR ($CDCl_3$) δ 135.28 (–*C*H=CH_2), 112.54 (–CH=*C*H_2), 23.53, 19.72, 19.01, 18.38, −3.88, −4.97.

4.1.2. Bis(diallylmethylsilylpropyldimethylsilylpropyl)-dimethylsilane (4). A solution of dichloromethylsilane (3.38 g, 29.3 mmol) in THF (10 mL) was added dropwise to a mixture of **3** (2.00 g, 5.87 mmol) and Speier catalyst (five drops) in THF (10 mL) under Ar, and the reaction mixture was stirred at 40 °C for 39 h. The reaction mixture was then processed in the same way described for compound **2**. The residue was chromatographed on silica gel with hexane as eluent to give **4** (2.82 g, 81%). ^{1}H NMR ($CDCl_3$) δ 5.73–5.84 (m, 4H, –C*H*=CH_2), 4.81–4.90 (m, 8H, –CH=C*H*$_2$), 1.55 (d, 8H, J=8.2 Hz, SiC*H*$_2$CH=CH_2), 1.26–1.41 (m, 8H, $SiCH_2CH_2CH_2Si$), 0.49–0.64 (m, 16H, $SiCH_2$), −0.01 (s, 6H, $SiCH_3$), −0.05 (s, 18H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 134.86 (–*C*H=CH_2), 112.99 (–CH=*C*H$_2$), 21.48, 20.13, 20.09, 18.43, 18.19, 17.80, −3.21, −5.74.

4.1.3. Bis(triallylsilylpropyldimethylsilylpropyl)dimethylsilane (5). A solution of trichlorosilane (2.38 g, 17.6 mmol) in THF (3 mL) was added dropwise to a mixture of **3** (2.00 g, 5.87 mmol) and Speier catalyst (six drops) in THF (4 mL) under Ar, and the reaction mixture was stirred at room temperature overnight and then refluxed for 2 h. The reaction mixture was then processed in the same way described for compound **4** to give **5** (1.40 g, 37%). ^{1}H NMR ($CDCl_3$) δ 5.73–5.82 (m, 6H, –C*H*=CH_2), 4.85–4.91 (m, 12H, –CH=C*H*$_2$), 1.58 (d, 12H, J=8.1 Hz, SiC*H*$_2$CH=CH_2), 1.25–1.40 (m, 8H, $SiCH_2CH_2CH_2Si$), 0.63–0.67 (m, 4H, $CH_2CH_2SiCH_2CH$=CH_2), 0.52–0.57 (m, 12H, $SiCH_2$), −0.05 (s, 18H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 134.50 (–*C*H=CH_2), 113.44 (–CH=*C*H$_2$), 20.23, 20.14, 20.07, 19.72, 18.42, 18.10, 16.28, −3.20, −3.23.

4.1.4. Tris(3-bromopropyldimethylsilylpropyl)methylsilane (6).

4.1.4.1. Synthesis of tris(3-hydroxypropyldimethylsilylpropyl)methylsilane. A solution of **2** (0.98 g, 2.1 mmol) in THF (4 mL) was added to a solution of cyclohexylborane, which was prepared from borane/THF complex (4.7 mL of 1 M solution) and cyclohexene (0.39 g, 4.7 mmol) in THF at 0 °C under Ar. After stirring at room temperature for 2 h, MeOH (3 mL) was added to the reaction mixture. Aqueous NaOH (5 mL of 3 M solution) and H_2O_2 (2 mL of 30% solution) were added to the resulting solution at 0 °C, subsequently. The reaction mixture was stirred at 60 °C for 1 h. The resulting mixture was extracted with THF; the solution was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by reprecipitation from cold hexane (−30 °C) to yield the title compound (0.79 g, 73%). ^{1}H NMR ($CDCl_3$) δ 3.58 (t, 6H, J=6.8 Hz, C*H*$_2$OH), 1.64 (br s, 3H, OH), 1.50–1.58 (m, 6H, C*H*$_2$CH$_2$OH), 1.27–1.35 (m, 6H, $SiCH_2CH_2CH_2Si$), 0.52–0.60 (m, 12H, SiC*H*$_2$CH$_2$C*H*$_2$Si), 0.45–0.50 (m, 6H, SiC*H*$_2$CH$_2$CH$_2$Br), −0.04 (s, 18H, $CH_2Si(CH_3)_2CH_2$), −0.09 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 65.83, 27.17, 19.99, 18.78, 18.43, 10.89, −3.37, −4.93.

4.1.4.2. Synthesis of tris(3-methylsulfonyloxypropyldimethylsilylpropyl)methylsilane. Methanesulfonyl chloride (1.8 mL, 32.2 mmol) was added dropwise to a solution of the above hydroxylated compound (1.29 g, 1.94 mmol) in pyridine (7 mL) at −20 °C under Ar, and the reaction mixture was stirred at same temperature for 3 h. The solution was diluted with $CHCl_3$ (10 mL), and water (10 mL) was added dropwise to the solution. The mixture was extracted with $CHCl_3$, and washed with 5% H_2SO_4 aq (v/v), 5% $NaHCO_3$ aq (w/w) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (1:100 MeOH/$CHCl_3$) to yield the title compound (1.86 g, 98%). ^{1}H NMR ($CDCl_3$) δ 4.15 (t, 6H, J=6.9 Hz, CH_2O), 2.98 (s, 9H, SO_2CH_3), 1.67–1.75 (m, 6H, C*H*$_2$CH$_2$O), 1.27–1.31 (m, 6H, $SiCH_2CH_2CH_2Si$), 0.48–0.56 (m, 18H, $SiCH_2$), −0.04 (s, 18H, $CH_2Si(CH_3)_2CH_2$), −0.09 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 72.54, 37.28, 23.89, 19.73, 18.66, 18.21, 10.62, −3.54, −5.05.

4.1.4.3. Synthesis of 6. NaBr (4.89 g, 45.5 mmol) was added to a solution of above mesylated compound (2.39 g, 3.17 mmol) in DMF (17 mL) under Ar. The mixture was heated at 80 °C for 3 h. The resulting mixture was diluted with water and toluene. The solution was extracted with toluene, washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (1:99 EtOAc/hexane) to yield compound **6** (1.78 g, 79%). ^{1}H NMR ($CDCl_3$) δ 3.38 (t, 6H, J=7.1 Hz, CH_2Br), 1.81–1.85 (m, 6H, C*H*$_2$CH$_2$Br), 1.29–1.31 (m, 6H, $SiCH_2CH_2CH_2Si$), 0.52–0.62 (m, 18H, SiC*H*$_2$), −0.02 (s, 18H, $CH_2Si(CH_3)_2CH_2$), −0.08 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 37.18 (CH_2Br), 28.01 (*C*H$_2$CH$_2$Br), 19.90, 18.97, 13.39, 14.64, −3.37, −4.96. FABMS (negative): $[M+Br]^-$ 786.8.

4.1.5. Bis(bis(3-bromopropyl)methylsilylpropyldimethylsilylpropyl)dimethylsilane (7).

4.1.5.1. Synthesis of bis(bis(3-hydroxypropyl)methylsilylpropyldimethylsilylpropyl)dimethylsilane. Reaction conditions and workup were as described in Section 4.1.4.1, with compound **4** (2.00 g, 3.37 mmol). The residue was purified by reprecipitation from hexane then a mixture of 1:5 EtOAc/hexane again to yield title compound (1.34 g, 60%). ^{1}H NMR (DMSO-d_6) δ 4.40 (t, 4H, J=5.2 Hz, –OH), 3.28–3.33 (m, 8H, C*H*$_2$OH), 1.26–1.41 (m, 16H, $SiCH_2CH_2$), 0.47–0.53 (m, 16H, $SiCH_2$), 0.39–0.43 (m, 8H, $SiCH_2$), −0.09 and −0.10 (each s, 24H, $SiCH_3$); ^{13}C NMR (DMSO-d_6) δ 64.13, 27.19, 19.82, 19.70, 19.68, 18.27, 18.209.53, −2.91, −4.90.

4.1.5.2. Synthesis of bis(bis(3-methylsulfonyloxypropyl)methylsilylpropyldimethylsilylpropyl)dimethylsilane. Reaction conditions and workup were as described in Section 4.1.4.2, with above hydoxylated compound (1.29 g, 1.94 mmol). Title compound (1.86 g, 98%) was obtained. ^{1}H NMR δ 4.15 (t, 8H, J=6.8 Hz, CH_2O), 2.99 (s, 12H, SO_2CH_3), 1.69–1.73 (m, 8H, $SiCH_2CH_2CH_2O$), 1.27–1.31 (m, 8H, $SiCH_2CH_2CH_2Si$), 0.50–0.61 (m, 24H, $SiCH_2$), −0.02 and −0.07 (each s, 24H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 72.29, 37.31, 23.81, 20.14, 20.03, 19.99, 18.32, 18.21, 18.01, 9.10, −3.27, −5.52.

4.1.5.3. Synthesis of 7. Reaction conditions and workup were as described in Section 4.1.4.3, with above mesylated compound (1.81 g, 1.85 mmol). The compound **7** (1.40 g, 83%) was obtained. ^{1}H NMR δ 3.38 (t, 8H, J=7.0 Hz, CH_2Br), 1.79–1.86 (m, 8H, C*H*$_2$CH$_2$Br), 1.29–1.33 (m, 8H, $SiCH_2CH_2CH_2Si$), 0.52–0.65 (m, 24H, $SiCH_2$), −0.02 and −0.05 (each s, 24H, $SiCH_3$); ^{13}C NMR ($CDCl_3$)

δ 37.05, 27.83, 20.18, 20.11, 20.06, 18.40, 18.28, 18.23, 12.99, −3.19, −5.26. FABMS (negative): $[M+Br]^-$ 995.0.

4.1.6. Bis(tris(3-bromopropyl)silylpropyldimethylsilylpropyl)dimethylsilane (8).

4.1.6.1. Synthesis of bis(tris(3-hydroxypropyl)silylpropyldimethylsilylpropyl)dimethylsilane. Reaction conditions and workup were as described in Section 4.1.4.1, with compound **5** (1.38 g, 2.14 mmol). The residue was purified by reprecipitation from a mixture of 1:5 EtOAc/hexane to yield the title compound (1.35 g, 84%). ^{1}H NMR (DMSO-d_6) δ 4.40 (t, 6H, J=5.2 Hz, –OH), 3.28–3.37 (q, 12H, J=6.6 Hz, CH_2OH), 1.26–1.60 (m, 20H, SiCH$_2$CH_2CH$_2$), 0.50–0.56 (m, 16H, CH_2SiCH_2CH$_2$CH$_2$OH), 0.40–0.44 (m, 12H, SiCH$_2$), −0.08 (s, 18H, SiCH$_3$); ^{13}C NMR (DMSO-d_6) δ 64.20, 27.21, 19.94, 19.69, 19.65, 18.24, 18.21, 17.00, 8.15, −2.89.

4.1.6.2. Synthesis of bis(tris(3-methylsulfonyloxypropyl)silylpropyldimethylsilylpropyl)dimethylsilane. Reaction conditions and workup were as described in Section 4.1.4.2, with the above hydoxylated compound (1.03 g, 1.37 mmol). Purification by chromatography on silica gel (1:50 MeOH/$CHCl_3$) yielded the title compound (1.62 g, 96%). ^{1}H NMR δ 4.17 (t, 12H, J=6.4 Hz, CH$_2$O), 3.02 (s, 18H, SO$_2$CH$_3$), 1.70–1.76 (m, 12H, CH_2CH$_2$O), 1.28–1.31 (m, 8H, SiCH$_2$CH_2CH$_2$Si), 0.52–0.67 (m, 28H, SiCH$_2$), −0.05 (s, 18H, SiCH$_3$); ^{13}C NMR ($CDCl_3$) δ 72.11, 37.34, 23.75, 20.31, 20.02, 19.98, 18.31, 18.23, 16.56, 7.57, −3.26, −3.29.

4.1.6.3. Synthesis of 8. Reaction conditions and workup were as described in Section 4.1.4.3, with above mesylated compound (2.08 g, 1.70 mmol). Purification was effected by column chromatography on silica gel (5:95 EtOAc/hexane) to yield compound **8** (0.98 g, 51%). ^{1}H NMR ($CDCl_3$) δ 3.39 (t, 12H, J=6.8 Hz, CH$_2$Br), 1.79–1.85 (m, 12H, CH_2CH$_2$Br), 1.30–1.32 (m, 8H, SiCH$_2$CH_2CH$_2$Si), 0.52–0.69 (m, 28H, SiCH_2), −0.05 (s, 18H, SiCH$_3$); ^{13}C NMR ($CDCl_3$) δ 30.99, 27.67, 20.32, 20.12, 20.05, 18.40, 18.28, 16.79, 11.44, −3.19. FABMS (negative): $[M+Br]^-$ 1208.8.

4.1.7. 4-Pentenyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (10) one half hydrate. A mixture of compound **9** (5.03 g, 9.75 mmol) and molecular sieves (4 Å powder, 5.0 g) in THF (80 mL) was stirred at room temperature for 2 h under Ar, trimethylamine boron complex (4.98 g, 68.2 mmol) was added. Aluminum chloride (9.10 g, 68.2 mmol) was added to the reaction mixture in some portion at 0 °C. After stirring at room temperature for 4 h, the resulting mixture was filtered through Celite and filtrate was diluted with $CHCl_3$. The solution was poured into ice-water and extracted with $CHCl_3$. The organic solution was washed with water, saturated $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel (1:4 EtOAc/hexane) to give compound **10** in hydrated form (4.54 g, 90%). $[\alpha]_D^{31}$ +7.6 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.28–7.37 (m, 15H, aromatics), 5.76–5.87 (m, 1H, –CH=CH$_2$), 4.94–5.03 (m, 2H, –CH=CH_2), 4.91 (d, 1H, J=11.2 Hz, CH_2Ph), 4.73 (d, 1H, J=11.6 Hz, CH_2Ph), 4.72 (s, 2H, CH_2Ph), 4.59 (s, 2H, CH_2Ph), 4.35 (d, 1H, $J_{1,2}$=7.7 Hz, H-1), 4.02 (d, 1H, $J_{3,4}$=3.2 Hz, H-4), 3.93–3.98 (m, 1H, one of OCH_2CH$_2$CH$_2$CH=CH$_2$), 3.80 (dd, 1H, J=6.0 Hz, J=9.9 Hz, H-6a), 3.72 (dd, 1H, J=6.0 Hz, J=9.9 Hz, H-6b), 3.64 (dd, 1H, $J_{1,2}$=7.8 Hz, $J_{2,3}$=9.2 Hz, H-2), 3.53–3.57 (m, 2H, H-5 and one of OCH_2CH$_2$CH$_2$CH=CH$_2$), 3.49 (dd, 1H, $J_{2,3}$=9.4 Hz, $J_{3,4}$=3.4 Hz, H-3), 2.13–2.19 (m, 2H, OCH$_2$CH$_2$CH_2CH=CH$_2$), 1.69–1.80 (m, 2H, OCH$_2$CH_2CH$_2$CH=CH$_2$), 1.60 (br s, 2H, –OH and H_2O); ^{13}C NMR ($CDCl_3$) δ 138.55, 138.05, 137.96, 137.85, 128.39, 128.37, 128.25, 128.05, 127.81, 127.77, 127.72, 127.69, 127.56, 114.80 (–C=CH_2), 103.63 (C-1), 80.55, 78.91, 75.14, 73.65, 73.10, 72.34, 69.17, 66.83 (C-4), 30.17, 28.91; Anal. Calcd for $C_{32}H_{38}O_6$+0.5H_2O: C, 72.84; H, 7.45. Found: C, 73.08; H, 7.35. FABMS (positive): $[M+Na]^+$ 541.3.

4.1.8. 4-Pentenyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (11). A mixture of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (17.4 g, 31.0 mmol), compound **10** (7.58 g, 14.6 mmol), and molecular sieves (4 Å powder, 7.6 g) in diethyl ether (300 mL) was stirred at room temperature for 1 h under Ar. Silver triflate (12.0 g, 47.0 mmol) was added to the mixture at −20 °C under darkness and reaction mixture was stirred at same temperature for 2 h. The insoluble solids were separated by filtration through a Cerite bed, the filtrate was diluted with $CHCl_3$ and washed with ice-water, saturated $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel (3:97 then 5:95 EtOAc/toluene) to yield compound **11** (10.8 g, 71%). $[\alpha]_D^{31}$ +41.0 (c 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.16–7.38 (m, 35H, aromatics), 5.76–5.86 (m, 1H, –CH=CH$_2$), 5.02 (d, 1H, $J_{1',2'}$=1.3 Hz, H-1′), 4.87–4.99 (m, 5H, –CH=CH2, CH_2Ph), 4.77–4.80 (m, 4H, CH_2Ph), 4.68 (d, 1H, J=11.9 Hz, CH_2Ph), 4.55 (d, 1H, J=3.1 Hz, CH_2Ph), 4.52 (d, 1H, J=4.6 Hz, CH_2Ph), 4.12–4.45 (m, 1H, H-5′), 4.31 (d, 1H, $J_{1,2}$=7.6 Hz, H-1), 4.26 (d, 1H, J=11.8 Hz, CH_2Ph), 4.21 (d, 1H, J=11.8 Hz, CH_2Ph), 4.10–4.17 (m, 5H, H-2′, H-3′, H-4′ and CH_2Ph), 4.02 (d, 1H, $J_{3,4}$=2.4 Hz, H-4), 3.91–3.98 (m, 2H, H-6a and one of OCH_2CH$_2$CH$_2$CH=CH$_2$), 3.67 (dd, 1H, $J_{1,2}$=7.6 Hz, $J_{2,3}$=9.9 Hz, H-2), 3.51–3.57 (m, 4H, H-5, H-6b, H-6′a and one of OCH_2CH$_2$CH$_2$CH=CH$_2$), 3.38 (dd, 1H, $J_{2,3}$=9.9 Hz, $J_{3,4}$=2.8 Hz, H-3), 3.24 (dd, 1H, J=4.7 Hz, J=8.3 Hz, H-6′b), 2.15–2.19 (m, 2H, OCH$_2$CH$_2$CH_2CH=CH$_2$), 1.76–1.78 (m, 2H, OCH$_2$CH_2CH$_2$CH=CH$_2$); ^{13}C NMR ($CDCl_3$) δ 138.90, 138.74, 138.67, 138.60, 138.06, 137.99, 128.25, 128.19, 128.16, 128.14, 128.10, 128.02, 127.96, 127.86, 127.72, 127.50, 127.47, 127.35, 127.24, 114.79, 103.87 (C-1), 100.37 (C-1′), 80.86, 78.91, 78.86, 76.51, 75.02, 74.80, 74.76, 74.64, 73.58, 73.51, 73.07, 72.95, 72.27, 72.17, 69.57, 69.26, 69.19, 67.97, 67.87, 30.12, 28.89; Anal. Calcd for $C_{66}H_{72}O_{11}$: C, 76.13; H, 6.97. Found: C, 75.99; H, 7.01. FABMS (positive): $[M-H]^+$ 1039.0. $[M+Na]^+$ 1062.9.

4.1.9. 4-Pentenyl (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (12). A solution of compound **11** (5.16 g, 4.92 mmol) in dimethoxyethane (30 mL) was added dropwise to sodium (4.56 g, 198 mmol) in liq. NH_3 (150 mL) at −72 °C. After stirring at same temperature for 45 min, NH_4Cl (10.6 g, 198 mmol) in limited amounts was added to the reaction

mixture. The mixture was allowed to warm at room temperature and stirred overnight, and then evaporated to dryness. The residue was treated with pyridine (33.5 mL, 420 mmol) and Ac_2O (19.7 mL, 210 mmol) at room temperature for 19 h. The mixture was poured into ice-water and extracted with $CHCl_3$. The solution was washed with 1 M HCl aq, saturated $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel (1:1 EtOAc/hexane) to give compound **12** (1.32 g, 38%) and 3.26 g of the starting compound. $[\alpha]_D^{29}$ +73.5 (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.75–5.85 (m, 1H, –C*H*=CH_2), 5.57 (d, 1H, $J_{3',4'}$=3.1 Hz, H-4′), 5.39 (dd, 1H, $J_{2',3'}$=10.9 Hz, $J_{3',4'}$=3.1 Hz, H-3′), 5.16–5.21 (m, 2H, H-2 and H-2′), 4.96–5.04 (m, 3H, H-1′, –CH=C*H*$_2$), 4.82 (dd, 1H, $J_{2,3}$=10.7 Hz, $J_{3,4}$=2.4 Hz, H-3), 4.54 (t, 1H, *J*=6.9 Hz, H-5′), 4.44–4.48 (m, 2H, H-1 and H-6b), 4.06–4.25 (m, 4H, H-4, H-6a and H-6′ab), 3.87–3.93 (m, 1H, one of OC*H*$_2$CH$_2$CH$_2$CH=CH$_2$), 3.78 (t, 1H, *J*=6.6 Hz, H-5), 3.47–3.53 (m, 1H, one of OC*H*$_2$CH$_2$CH$_2$CH=CH$_2$), 1.99–2.13 (m, 23H, –OAc, OCH$_2$CH$_2$C*H*$_2$CH=CH$_2$), 1.64–1.76 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CH=CH$_2$); ^{13}C NMR ($CDCl_3$) δ 170.70, 170.55, 170.45, 170.41, 170.10, 169.72, 169.06, 137.90 (–*C*H=CH$_2$), 114.95 (–CH=*C*H$_2$), 101.17 (C-1), 99.30 (C-1′), 72.75 (C-3), 71.79 (C-5), 69.12, 68.76, 68.56, 67.81 (C-4′), 67.35 (C-3′), 66.99 (C-5′), 61.94 (C-6), 60.46, 29.84, 28.55, 20.92, 20.73, 20.66, 20.64, 20.59; Anal. Calcd for $C_{31}H_{44}O_{18}$: C, 52.84; H, 6.29. Found: C, 52.66; H, 6.23. FABMS (positive): $[M+Na]^+$ 726.9.

4.1.10. 5-Acetylthiopentyl (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (13). A solution of compound **12** (1.41 g, 2.00 mmol) in 1,4-dioxane (1 mL) was treated with thioacetic acid (3.0 ml, 42.6 mmol) and AIBN (0.679 g, 4.26 mmol) at 80 °C for 2 h under Ar. Cyclohexene (432 μL, 4.26 mmol) was added with stirring for 10 min. The resulting solution was purified by column chromatography on silica gel (1:1 EtOAc/hexane) to yield compound **13** (1.46 g, 93%). $[\alpha]_D^{32}$ +66.1 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.57 (d, 1H, $J_{3',4'}$= 2.6 Hz, H-4′), 5.39 (dd, 1H, $J_{2',3'}$=11.0 Hz, $J_{3',4'}$=3.3 Hz, H-3′), 5.15–5.21 (m, 2H, H-2 and H-2′), 5.00 (d, 1H, $J_{1',2'}$= 3.6 Hz, H-1′), 4.81 (dd, 1H, $J_{2,3}$=10.8 Hz, $J_{3,4}$=2.7 Hz, H-3), 4.53 (t, 1H, *J*=6.6 Hz, H-5′), 4.43–4.47 (m, 2H, H-1 and H-6b), 4.06–4.19 (m, 4H, H-4, H-6a and H-6′ab), 3.85–3.91 (m, 1H, one of OC*H*$_2$CH$_2$), 3.78 (t, 1H, *J*= 6.6 Hz, H-5), 3.45–3.51 (m, 1H, one of OC*H*$_2$CH$_2$–), 2.86 (t, 2H, *J*=7.2 Hz, C*H*$_2$SAc), 2.32 (s, 3H, SAc), 2.13 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.55–1.66 (m, 4H, OCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$S), 1.39–1.48 (m, 2H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$S); ^{13}C NMR ($CDCl_3$) δ 195.83 (SC=O), 170.68, 170.53, 170.43, 170.39, 170.08, 169.71, 169.05, 101.09 (C-1), 99.31 (C-1′), 72.72 (C-3), 71.80 (C-5), 69.51 (O*C*H$_2$CH$_2$), 68.69, 68.54, 67.80, 67.33, 66.98, 61.93, 60.44, 30.57, 29.16, 28.89, 28.83, 25.03, 20.90, 20.72, 20.63, 20.58. Anal. Calcd for $C_{33}H_{48}O_{19}S$: C, 50.76; H, 6.20. Found: C, 50.47; H, 6.10. FABMS (positive): $[M+H]^+$ 781.2. $[M+Na]^+$ 803.2.

4.1.11. Fan(1)3-Ga2OAc (14). A mixture of **6** (22.1 mg, 31.0 μmol) and **13** (152.6 mg, 195.0 μmol) was dissolved in a mixture of DMF (0.25 mL) and MeOH (0.25 mL), the solution was treated with NaOMe (11.6 mg, 215 μmol) at room temperature for 21 h. Acetic acid (0.25 mL) was then added to the mixture, the resulting solution was evaporated under reduced pressure. The residue was suspended in a mixture of pyridine (6 mL) and acetic acid (3 mL). The suspension was stirred for 5 h at room temperature, after resulting solution was poured into ice-water and extracted with EtOAc. The organic solution was washed with 1 M HCl aq, saturated $NaHCO_3$ aq, and brine. Organic phase was dried ($MgSO_4$) and concentrated. Purification by recycling preparative HPLC (column, JAIGEL-1H and 2H; solvent, chloroform) afforded Fan(1)3-Ga2OAc **14** (75.6 mg, 86%). $[\alpha]_D^{33}$ +59.0 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.57 (d, 3H, $J_{3',4'}$=2.8 Hz, H-4′), 5.39 (dd, 3H, $J_{2',3'}$=11.0 Hz, $J_{3',4'}$=3.3 Hz, H-3′), 5.15–5.21 (m, 6H, H-2 and H-2′), 5.00 (d, 3H, $J_{1',2'}$=3.6 Hz, H-1′), 4.81 (dd, 3H, $J_{2,3}$=10.8 Hz, $J_{3,4}$=2.7 Hz, H-3), 4.53 (t, 3H, *J*=6.7 Hz, H-5′), 4.43–4.48 (m, 6H, H-1 and H-6b), 4.06–4.20 (m, 12H, H-4, H-6a and H-6′ab), 3.88–3.90 (m, 3H, one of OC*H*$_2$CH$_2$), 3.77 (t, 3H, *J*=6.6 Hz, H-5), 3.46–3.48 (m, 3H, one of OC*H*$_2$CH$_2$–), 2.50 (t, 12H, *J*=7.5 Hz, CH$_2$S), 1.99–2.13 (m, 63H, OAc), 1.53–1.61 (m, 18H, OCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$SCH$_2$C*H*$_2$CH$_2$Si), 1.43–1.47 (m, 6H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$S), 1.26–1.32 (m, 6H, SiCH$_2$C*H*$_2$CH$_2$Si), 0.51–0.60 (m, 18H, SiCH$_2$), −0.04 (s, 18H, SiCH$_3$), −0.09 (s, 3H, SiCH$_3$); ^{13}C NMR ($CDCl_3$) δ 170.68, 170.53, 170.43, 170.39, 170.08, 169.71, 169.06, 101.13 (C-1), 99.31 (C-1′), 72.71, 71.79, 69.71, 68.71, 68.54, 67.80, 67.33, 66.98, 61.92, 60.45, 35.94, 31.98, 29.41, 29.03, 25.16, 24.38, 20.90, 20.72, 20.66, 20.62, 20.58, 19.98, 18.74, 18.37, 15.13, −3.39, −5.01; HRMS (ESI) Anal. Calcd for $C_{118}H_{192}O_{54}S_3Si_4$ $[M+Na]^+$: 2704.04148. Found: 2704.04324.

4.1.12. Dumbbell(2)4-Ga2OAc (15). A coupling reaction between **7** (60.8 mg, 66.3 μmol) and **13** (312.5 mg, 400.0 μmol) was carried out in the same manner as described in synthesis of **14** to give Dumbbell(2)4-Ga2OAc **15** (169.6 mg, 72%) after purification by recycling preparative HPLC (column, JAIGEL-2H and 2.5H; solvent, chloroform). $[\alpha]_D^{34}$ +61.1 (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.57 (d, 4H, $J_{3',4'}$=2.4 Hz, H-4′), 5.39 (dd, 4H, $J_{2',3'}$=11.0 Hz, $J_{3',4'}$=3.2 Hz, H-3′), 5.15–5.21 (m, 8H, H-2 and H-2′), 5.00 (d, 4H, $J_{1',2'}$=3.6 Hz, H-1′), 4.81 (dd, 4H, $J_{2,3}$= 10.8 Hz, $J_{3,4}$=2.7 Hz, H-3), 4.52 (t, 4H, *J*=7.3 Hz, H-5′), 4.43–4.48 (m, 8H, H-1 and H-6b), 4.06–4.20 (m, 16H, H-4, H-6a and H-6′ab), 3.88–3.90 (m, 4H, one of OC*H*$_2$CH$_2$), 3.78 (t, 4H, *J*=6.6 Hz, H-5), 3.46–3.48 (m, 4H, one of OC*H*$_2$CH$_2$–), 2.50 (t, 16H, *J*=6.4 Hz, CH$_2$S), 1.98–2.22 (m, 84H, OAc), 1.53–1.61 (m, 24H, OCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$ SCH$_2$C*H*$_2$CH$_2$Si), 1.43–1.46 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$ CH$_2$S), 1.27–1.33 (m, 8H, SiCH$_2$C*H*$_2$CH$_2$Si), 0.52–0.61 (m, 28H, SiCH$_2$), −0.05 and −0.06 (s, 24H, SiCH$_3$); ^{13}C NMR ($CDCl_3$) δ 170.70, 170.54, 170.44, 170.40, 170.10, 169.72, 169.06, 101.15 (C-1), 99.34 (C-1′), 72.73, 71.80, 69.72, 68.72, 68.55, 67.80, 67.34, 66.99, 61.92, 60.46, 36.00, 32.04, 29.41, 29.05, 25.17, 24.33, 20.93, 20.75, 20.68, 20.65, 20.60, 20.21, 20.10, 18.36, 13.58, −3.22, −5.28; HRMS (ESI) Anal. Calcd for $C_{156}H_{252}O_{72}S_4Si_5$ $[M+2Na]^{2+}/2$: 1795.67912. Found: 1795.68283.

4.1.13. Dumbbell(2)6-Ga2OAc (16). A coupling reaction between **8** (49.3 mg, 43.6 μmol) and **13** (316.5 mg, 405.0 μmol) was carried out in the same manner as

described in synthesis of **14** to give Dumbbell(2)6-Ga2OAc **16** (166.2 mg, 75%) after purification by recycling preparative HPLC (column, JAIGEL-2.5H and 3H; solvent, chloroform). $[\alpha]_D^{35}$ +61.1 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.57 (d, 6H, $J_{3',4'}$=2.4 Hz, H-4′), 5.38 (dd, 6H, $J_{2',3'}$=11.0 Hz, $J_{3',4'}$=3.3 Hz, H-3′), 5.15–5.21 (m, 12H, H-2 and H-2′), 5.00 (d, 6H, $J_{1',2'}$=3.6 Hz, H-1′), 4.81 (dd, 6H, $J_{2,3}$= 10.7 Hz, $J_{3,4}$=2.6 Hz, H-3), 4.53 (t, 6H, *J*=6.7 Hz, H-5′), 4.43–4.48 (m, 12H, H-1 and H-6b), 4.07–4.20 (m, 24H, H-4, H-6a and H-6′ab), 3.88–3.91 (m, 6H, one of OCH_2CH_2), 3.78 (t, 6H, *J*=6.6 Hz, H-5), 3.46–3.50 (m, 6H, one of OCH_2CH_2–), 2.50 (t, 24H, *J*=6.8 Hz, CH_2S), 1.99–2.23 (m, 126H, OAc), 1.58–1.62 (m, 36H, $OCH_2CH_2CH_2CH_2CH_2SCH_2CH_2CH_2Si$), 1.42–1.46 (m, 12H, $OCH_2CH_2CH_2CH_2CH_2S$), 1.25–1.31 (m, 8H, $SiCH_2CH_2CH_2Si$), 0.54–0.61 (m, 28H, $SiCH_2$), −0.05 (s, 18H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.60, 170.46, 170.35, 170.30, 170.02, 169.64, 168.99, 101.05 (C-1), 99.24 (C-1′), 72.62, 71.70, 69.64, 68.63, 68.45, 67.71, 67.25, 66.89, 61.83, 60.38, 35.97, 34.04, 32.00, 29.96, 29.30, 29.10, 28.96, 27.75, 25.24, 25.06, 24.18, 23.13, 20.84, 20.66, 20.59, 20.56, 20.51, 20.27, 20.02, 18.39, 18.25, 16.96, 12.01, 11.40, −3.32; HRMS (ESI) Anal. Calcd for $C_{222}H_{348}O_{108}S_6Si_5$ $[M+2Na]^{2+}/2$: 2559.93525. Found: 2559.92997.

4.1.14. Fan(1)3-Ga2 (17). A solution of Fan(1)3-Ga2OAc **14** (103.6 mg, 29.2 μmol) in MeOH (1 mL) was treated with 28% NaOMe methanolic solution (20 μL) at room temperature for 1 h, DMF (7 mL) and 0.1 M NaOH aq (6 mL) were then added to the reaction mixture. After overnight, the solution was neutralized by Amberlite IR120B (H^+) resin. The resin was filtered off and filtrate was concentrated to dryness. Purification of the crude product was carried out by gel permeation chromatography (Sephadex G50, 5% HOAc aq eluent) to give Fan(1)3-Ga2 **17** (47.8 mg, 91%) as white powder after lyophilization. $[\alpha]_D^{31}$ +52.8 (*c* 1.1, DMSO); ^1H NMR (CD_3OD) δ 4.97 (s, 3H), 4.27–4.32 (m, 6H), 44.06–4.12 (m, 3H), 4.00 (d, 3H, *J*=2.5 Hz), 3.78–3.92 (m), 3.68–3.76 (m), 3.47–3.63 (m), 2.51 (t, 12H, *J*=7.2 Hz, CH_2S), 1.49–1.67 (m, 18H, $OCH_2CH_2CH_2CH_2CH_2SCH_2CH_2CH_2Si$), 1.43–1.47 (m, 6H, $OCH_2CH_2CH_2CH_2CH_2S$), 1.37–1.40 (m, 6H, $SiCH_2CH_2CH_2Si$), 0.57–0.65 (m, 18H, $SiCH_2$), −0.03 (s, 18H, $SiCH_3$), −0.06 (s, 3H, $SiCH_3$); ^{13}C NMR (CD_3OD) δ 105.16, 102.51, 78.96, 76.08, 74.70, 72.82, 72.60, 71.38, 71.16, 71.05, 70.80, 62.63, 60.89, 36.80, 32.85, 30.76, 30.49, 26.38, 25.58, 21.04, 19.83, 19.79, 15.86, 14.46, −2.97, −4.67; HRMS (ESI) Anal. Calcd for $C_{76}H_{150}O_{33}S_3Si_4$ $[M+Na]^+$: 1821.81962. Found: 1821.81874.

4.1.15. Dumbbell(2)4-Ga2 (18). A mixture of Dumbbell(2)4-Ga2OAc **15** (50.1 mg, 14.0 μmol) and 1 M NaOMe methanolic solution (1 mL) was stirred at room temperature for 4 h, 0.25 M NaOH aq (3 mL) was then added to the reaction mixture. The solution was stirred for 14 h at room temperature. Workup and purification were as described in synthesis of Fan(1)3-Ga2 **17**. Dumbbell(2)4-Ga2 **18** (31.6 mg, 99%) was obtained as white powder after lyophilization. $[\alpha]_D^{28}$ +47.6 (*c* 1.1, H_2O); ^1H NMR (D_2O) δ 5.02 (br s, 4H), 4.36 (br s, 4H), 4.04 (br s), 3.55–3.90 (m), 2.55 (br s, 16H, CH_2S), 1.26–1.62 (m, 40H), 0.60 (br s, 24H, $SiCH_2$), −0.01 (br s, 24H, $SiCH_3$); ^{13}C NMR (D_2O) δ 103.47, 110.49, 76.97, 74.82, 72.80, 71.04, 70.32, 69.47, 69.21, 69.00, 60.82, 59.70, 35.93, 32.00, 29.61, 29.22, 25.25, 24.44, 20.36, 18.64, 13.51, −2.55, −4.55; HRMS (ESI) Anal. Calcd for $C_{100}H_{196}O_{44}S_4Si_5$ $[M+Na]^+$: 2392.07263. Found: 2392.07023.

4.1.16. Dumbbell(2)6-Ga2 (19). A mixture of Dumbbell(2)6-Ga2OAc **16** (35.2 mg, 7.0 μmol) and 1 M NaOMe methanolic solution (1 mL) was stirred at room temperature for 3 h, 0.25 M NaOH aq (3 mL) was then added to the reaction mixture. The solution was stirred overnight at room temperature. Workup and purification were as described in synthesis of Fan(1)3-Ga2 **17**. Dumbbell(2)6-Ga2 **19** (22.4 mg, 98%) was obtained as white powder after lyophilization. $[\alpha]_D^{31}$ +56.7 (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 4.97 (s, 12H), 4.32 (br s, 12H), 4.00 (br s, 12H), 3.51–3.86 (m), 2.53 (br s, 24H, CH_2S), 1.19–1.61 (m, 98H), 0.57 (br s, 28H, $SiCH_2$), −0.03 (br s, 18H, $SiCH_3$); ^{13}C NMR (D_2O) δ 103.29, 100.34, 76.84, 74.70, 72.64, 70.92, 70.25, 69.31, 69.06, 68.81, 60.67, 59.61, 35.81, 31.88, 29.49, 29.09, 25.11, 24.30, 20.30, 20.13, 18.59, 11.90, −2.60; HRMS (ESI) Anal. Calcd for $C_{138}H_{264}O_{66}S_6Si_5$ $[M+2Na]^{2+}/2$: 1677.71339. Found: 1677.71763.

4.2. Kinetic analysis of dendrimers carrying galabiose binding to immobilized B subunits

The dendrimer binding to immobilized recombinant histidine-tagged Stx1 B (1B-His) and Stx2 subunits (2B-His) was quantified using a BIAcore instrument (BIAcore, Uppsala, Sweden) as described previously.[5b] Ni^{2+} was fixed on a nitrilotriacetic acid sensor chip (BIAcore), and recombinant 1B-His or 2B-His (10 μg/mL) was injected into the system, where it was immobilized on the chip. Various concentrations of the dendrimers were injected (time 0) over the immobilized 1B-His or 2B-His at flow rate of 20 μL/min to reach plateau at 25 °C. The binding kinetics were analyzed by Scatchard plot using the software BIAEVALUATION 3.0 (BIAcore).

4.3. ^{125}I-Stx binding assay

^{125}I-Stx binding assay was performed as described previously.[5a] Vero cells were treated with ^{125}I-Stx1 or ^{125}I-Stx2 (1 μg/mL) in the absence or presence of the desired amount of a given compound for 30 min at 4 °C. After extensive washing, the cells were dissolved in lysis solution (0.1 M NaOH, 0.5% SDS). Recovered radioactivity was measured by a γ-counter (Packard).

4.4. Cytotoxicity assay

Subconfluent Vero cells in a 96-well plate were treated with Stx1 or Stx2 (10 pg/mL) in the absence or presence of the desired amount of a given compound for 72 h. The relative number of living cells was determined by using a WST-1 Cell Counting Kit (Wako Pure Industries).

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