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Thiosialoside clusters using carbosilane dendrimer core scaffolds as a new class of influenza neuraminidase inhibitors☆

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Abstract—An efficient synthesis of a series of carbosilane dendrimers uniformly functionalized with α-thioglycoside of sialic acid was accomplished. The results of a preliminary study on biological responses against influenza virus sialidases using thiosialoside clusters showed that some of the glycodendrimers have inhibitory potencies against the sialidases.

Influenza viruses have different types of carbohydrate-related proteins on their surfaces.[1] One of the proteins is hemagglutinin (HA), which is a kind of lectin and binds to sialyl oligosaccharides as specific receptors on host cells.[2] The other one is glycosidase, which is referred to as either sialidase or neuraminidase (NA) and cleaves sialic acid residues from sialoglycoproteins as well as gangliosides on the surfaces of the host cells.[3] Therefore, the virus is significantly unique and the proteins on the surfaces of influenza viruses have completely different roles, such as adhesion to the host cell and secession from the host cell.[1] Neuraminidase inhibitors, such as zanamivir[4] and oseltamivir,[5] have been synthesized and widely used as therapeutic agents in the clinical treatment of influenza A and B viruses.[6] These drugs have extremely high inhibitory potencies to the release of influenza virions from infected cells; however, NA inhibitor-resistant viruses have already been generated.[7] Although the specific ligand of NA of influenza viruses A and B is natural sialic acid, the inhibitors are designed as transition-state analogues after cleavage of sialic acid residues by a sialidase.[4,5] Therefore, we examined the use of thioglycoside of sialic acid as a natural epitope for an NA inhibitor because thioglycosidic linkage is usually not hydrolyzed by glycosidases, such as NA.[8] However, since monomeric sialoside does not have inhibitory potency for NA, we planned to use the sugar-clustering effect.[9] In addition, NAs on the virus surface display tetrameric structures[10] and a glycocluster is expected to be a promising multivalent-type therapeutic agent.[11]

As to glycoclusters, we have reported syntheses of various carbosilane dendrimers having bioactive carbohydrate determinants on the terminal ends[12] and biological activities of the compounds.[11] In this paper, we describe the synthetic assembly of thiosialoside moieties using a series of carbosilane dendrimers as the core

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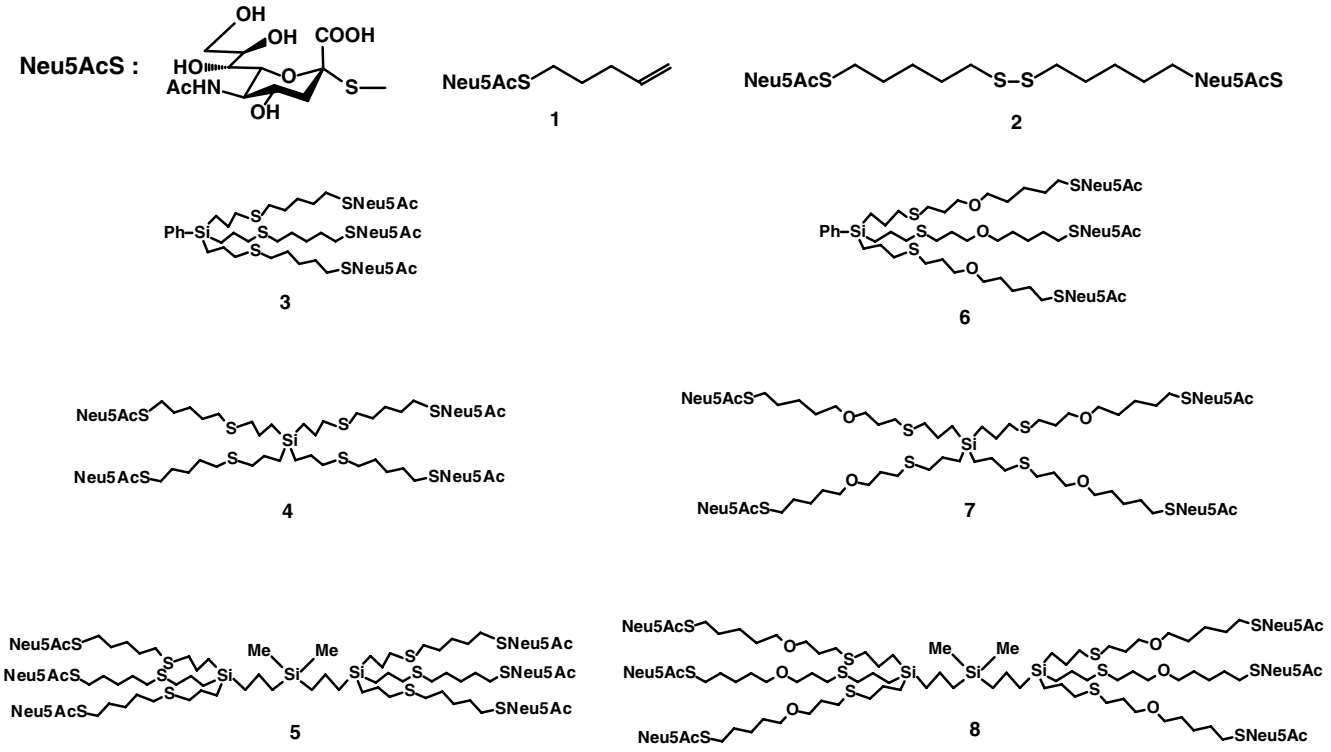


Figure 1. A series of multivalent-type synthetic substrates having thioglycosidic linkages of sialic acids.

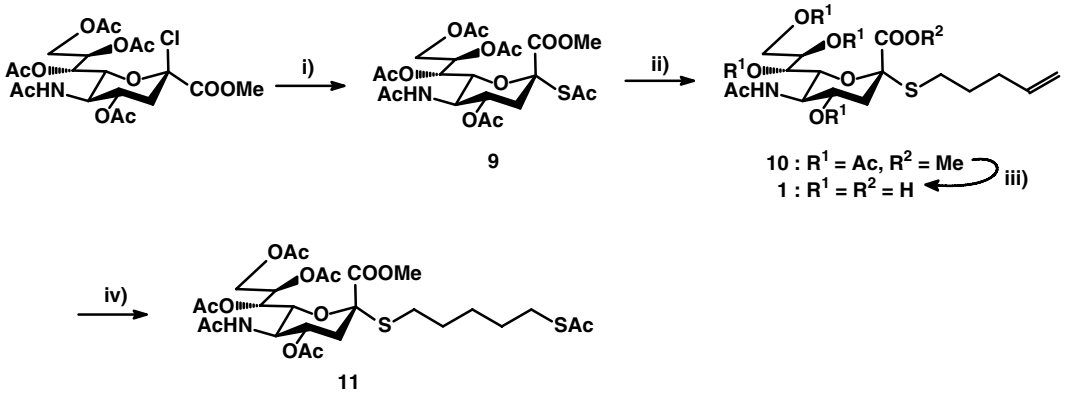
frames and the preliminary biological evaluations using influenza viruses.

Schematic structures of target dendrimers are shown in Figure 1, where monomeric substrate **1**, dimeric substrate **2**, trimeric substrates **3** and **6**, which have fan shapes, tetrameric substrates **4** and **7**, which have ball shapes, and hexameric substrates **5** and **8**, which have dumbbell shapes, are illustrated.

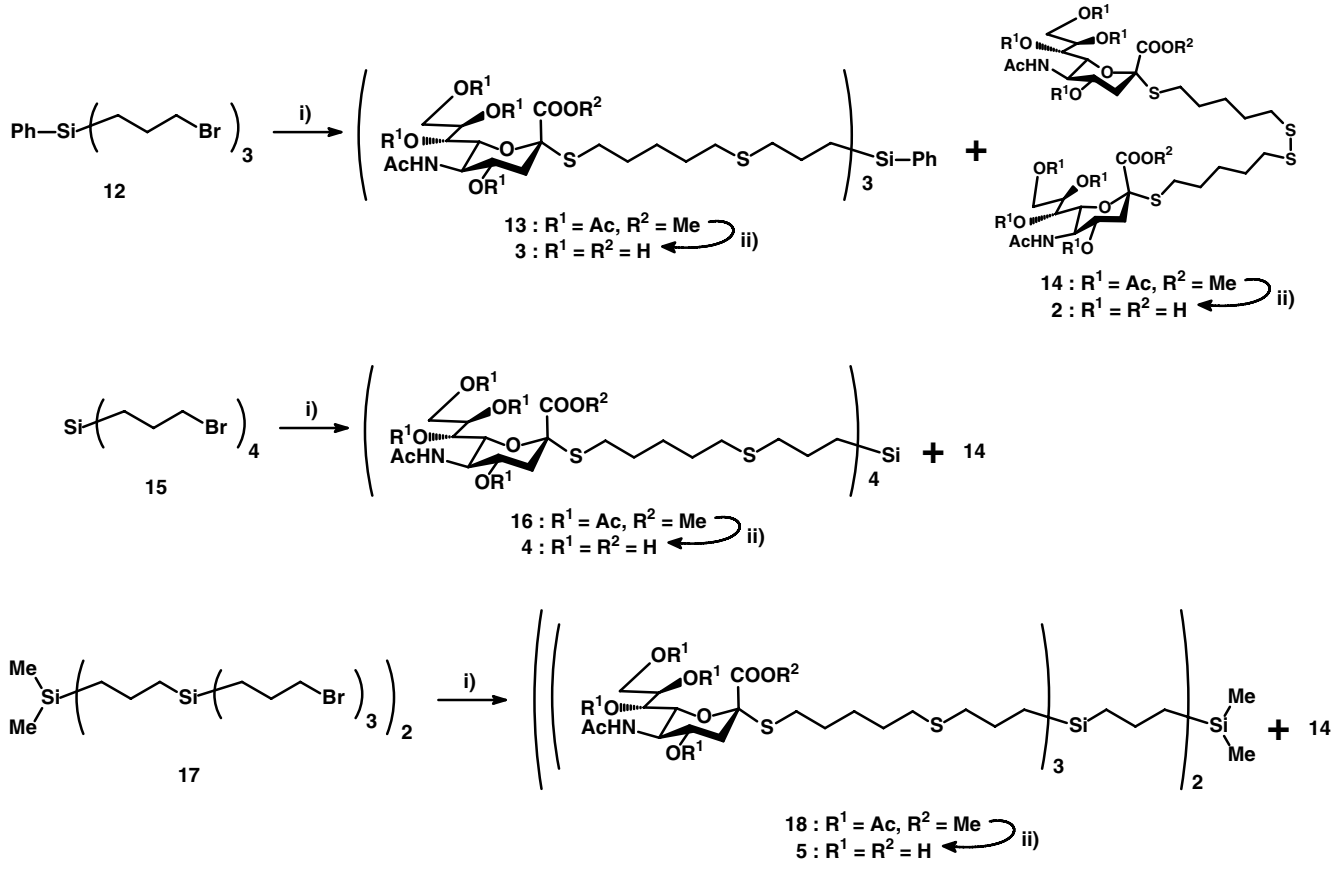
The synthetic scheme for preparation of a common thioacetate **11** of sialic acid having thioglycosidic linkage is shown in Scheme 1. Known chloride was converted into α-thioacetate **9** by the previously reported procedures,[13] and **9** was allowed to react with 5-bromo-pentene in the presence of potassium carbonate in methanol to give thioglycoside **10**[†] in 84.2% yield after re-acetylation followed by chromatographic purification, $[\alpha]_D^{27}$ +29° (*c* 1.13, $CHCl_3$), $J_{7,8}$ = 8.2 Hz, $\Delta\delta$ |H-9a-H9b| = 0.20 ppm.[14] Transesterification and subsequent saponification to **10** gave water-soluble sialoside **1** as a monomeric substrate for the biological assay in quantitative yield after lyophilization. Quantitative transformation of the terminal double bond into corresponding primary thioacetate was accomplished by a radical addition of thioacetic acid in the presence of AIBN[15] to afford **11**, $[\alpha]_D^{28}$ +25° (*c* 1.11, $CHCl_3$).

Construction of carbosilane dendrimers **3**–**5** functionalized with thioglycosides of sialic acid is summarized in Scheme 2. Known tribromide **12**[12b] was condensed with 6 equivalent molar of thioacetate **11**, which means the ratio of Br/SAc is 1:2, in the presence of sodium methoxide in methanol—DMF followed by re-acetylation to afford fully protected **13** in 80.2% yield accompanied by a disulfide **14**, **13**, $[\alpha]_D^{33}$ +26° (*c* 1.10, $CHCl_3$), integral ratio of the H atoms by ^{1}H NMR/$SiCH_2$/CH_2SCH_2/Ph/H-8 = 6:12:5:3, HRMS (ESI^+) calcd for $[M+H]^+$: 2056.71535; found *m*/*z*: 2056.72008, and **14**, $[\alpha]_D^{28}$ +28° (*c* 1.13, $CHCl_3$), FABMS calcd for $[M+H]^+$: 1217.4; found *m*/*z*: 1218.0. Deprotection of **13** was accomplished by a combination of Zemplén's manner and alkali hydrolysis to provide water-soluble **3** in 87.4% yield after chromatographic purification by using Sephadex G-25, $[\alpha]_D^{29}$ +28° (*c* 1.03, H_2O), HRMS (ESI^+) calcd for $[M+H]^+$: 1510.54162; found *m*/*z*: 1510.53494. The same reaction was applied for known tetrabromide **15**[12c] and the coupling reaction proceeded smoothly to yield **16** (75.2%), in which ester functions were removed by the method described for **3** to give white powdery **4** in 99.5% yield, **16**, $[\alpha]_D^{24}$ +27° (*c* 1.19, $CHCl_3$), integral ratio of the H atoms by ^{1}H NMR/$SiCH_2$/CH_2SCH_2/H-8 = 8:16:4, HRMS (ESI^+) calcd for $[M+Na]^+$: 2651.88866; found *m*/*z*: 2651.87993, and **4**, $[\alpha]_D^{31}$ +23° (*c* 1.06, H_2O), HRMS (ESI^+) calcd for $[M+H]^+$: 1901.67508; found *m*/*z*: 1901.66668. The same reaction was also carried out for **17**[12c] and the condensed product **18** having hexa-sialic acid moieties in the molecule was obtained in 53.5% yield, which was then treated in same manner as that described for **3** to give quantitatively **5** as a white powder after lyophilization, **18**, $[\alpha]_D^{24}$ +24° (*c* 0.664, $CHCl_3$), integral ratio of the H atoms by ^{1}H NMR/$SiCH_3$/$SiCH_2$/CH_2SCH_2/H-8 = 6:20:24:6, HRMS (ESI^+) calcd for $[M+2H]^{2+}$/2: 2072.71705; found *m*/*z*:

[†] All new compounds with specific rotation data gave satisfactory results of elemental analyses or high-resolution mass spectra.



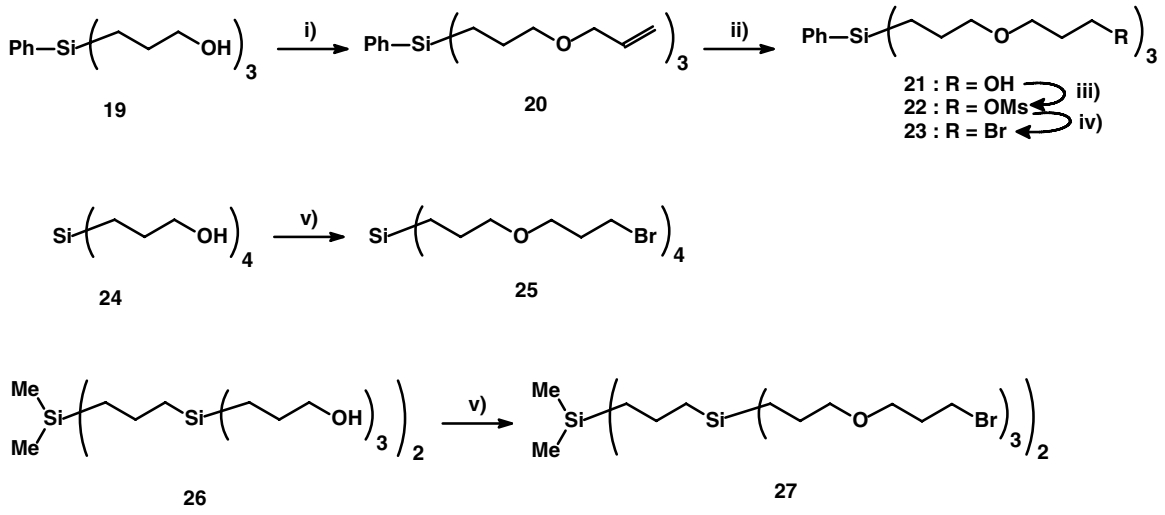
Scheme 1. Reagents and conditions: (i) Ref. 13; (ii) 5-Bromo-1-pentene, K_2CO_3, MeOH—DMF, 0°C → rt, overnight, then Ac_2O—Pyr, rt, overnight; (iii) NaOMe, MeOH, rt, 0.5 h, then 0.5 M aqueous NaOH, rt, overnight; (iv) AIBN, HSAc, 1,4-dioxane, 50 °C → 80°C, 3 h.



Scheme 2. Reagents and conditions: (i) **11** (2 molar excess vs SAc), NaOMe, MeOH—DMF, 0°C → rt, overnight, then Ac_2O—Pyr, rt, overnight; (ii) NaOMe, MeOH, rt, 3 h, then 0.5 M aqueous NaOH, rt, overnight.

2072.71684, and **5**, $[\alpha]_D^{27}$ +23° (*c* 0.99, H_2O), HRMS (ESI^+) calcd for $[M+2H]^{2+}/2$: 1504.5614; found *m*/*z*: 1504.5558. Removal of protective groups in dimeric sialoside **14** was also carried out in a similar manner as that described for **3** to give quantitatively **2** as a white powder after lyophilization, $[\alpha]_D^{30}$ +25° (*c* 1.03, H_2O), FABMS calcd for $[M+H]^+$: 853.3; found *m*/*z*: 853.0.

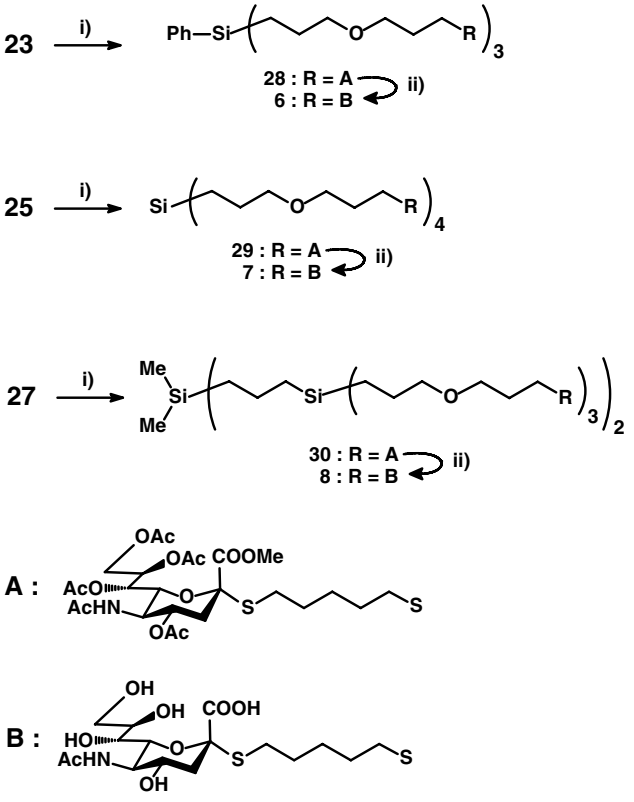
Given the success of synthetic assembly of thiosialoside using various carbosilane scaffolds, our attention was directed toward an elongation of spacer length between the dendritic core and sugars, because the binding sites of sialic acid residue in tetrameric sialidases in influenza virus are located around in 40–50 distances.[8] Therefore, an elongation of each spacer-arm in the known dendrimer scaffolds was first needed, and the synthetic route for the preparation of dendrimer scaffolds and the construction of glycodendrimers using elongated core frames are shown in Scheme 3. The starting trihydroxy compound **19**[16] was subjected to Williamson's ether synthesis with allyl bromide giving triallyl derivative **20**, which underwent hydroboration to give **21**. The



Scheme 3. Reagents and conditions: (i) Allyl bromide, NaH, THF, 0 → 70 °C, 2 h; (ii) 1 M BH_3—THF, cyclohexene, THF, 0 °C → rt, 3 h, then 3 M aqueous NaOH, H_2O_2, 60°C, overnight; (iii) MsCl, Pyr, 0°C, 3 h; (iv) NaBr, DMF, 60 °C, overnight; (v) (i)–(iv).

alcohols were all *O*-mesylated to afford **20** and replaced with bromo anions to give tribromo compound **23** in 55.4% yield (4 steps), FABMS calcd for $[M+H]^+$: 643.1; found *m*/*z*: 642.7. The same reaction sequences were performed for known alcohols **24**[16] and **26**,[12c] and all of the reactions proceeded smoothly to provide tetrabromo compound **25** in 54.4% yield (4 steps) and hexabromo compound **27** in 39.4% yield (4 steps), respectively, **25**, FABMS calcd for $[M+H]^+$: 745.0; found *m*/*z*: 748.5. Although the molecular ion peak of **27** was unfortunately not observed at this stage, a combination of the results of elemental analysis, IR, and ^1H and ^{13}C NMR for **27** supported its structure.

Coupling of **11** with **23**, **25**, and **27** was performed in the same way as that for the preparation of **13**, giving fully protected dendrimers **28** (55.9% yield), **29** (59.2% yield), and **30** (36.1% yield), which carry three, four, and six thiosialoside moieties, respectively, **28**, HRMS (ESI^+) calcd for $[M+Na]^+$: 2252.82289; found *m*/*z*: 2252.82345, **29**, HRMS (ESI^+) calcd for $[M+2Na]^{2+}/2$: 1453.52295; found *m*/*z*: 1453.51809, and **30**, HRMS (ESI^+) calcd for $[M+3Na]^{3+}/3$: 1505.55837; found *m*/*z*: 1505.55195. Finally, transesterification and subsequent saponification of **28**, **29**, and **30** gave corresponding water-soluble **6**, **7**, and **8** in quantitative yields, respectively, **6**, $[\alpha]_D^{24}$ +25° (*c* 1.07, H_2O), HRMS (ESI^+) calcd for $[M+Na]^+$: 1706.64916; found *m*/*z*: 1706.65283, **7**, $[\alpha]_D^{25}$ +29° (*c* 1.12, H_2O), HRMS (ESI^+) calcd for $[M+Na]^+$: 2155.82448; found *m*/*z*: 2155.81847, and **8**, $[\alpha]_D^{25}$ +20° (*c* 1.05, H_2O), HRMS (ESI^-) calcd for $[M-2H]^{2-}/2$: 1676.67135; found *m*/*z*: 1676.67301 (see Scheme 4).



Scheme 4. Reagents and conditions: (i) **11** (2 molar excess vs SAc), NaOMe, MeOH—DMF, 0 °C → rt, overnight, then Ac_2O—Pyr, rt, overnight; (ii) NaOMe, MeOH, rt, 3 h, then 0.5 M aqueous NaOH, rt, overnight.

Preliminary results of inhibitory activity[17] of a series of thiosialoside clusters against influenza virus sialidases are summarized in Table 1. Interestingly, dendrimers **6**, **7**, and **8** showed inhibitory potencies not only for H3N2-type sialidase but also for H1N1-type sialidase, while monomeric substrate **1** and dimeric one **2** as well as dendrimers **3**, **4**, and **5** did not show any activities. The results suggested that topological orientation of each sialoside and distances between the sugar moieties are important for effective binding to the binding sites in the tetrameric sialidases on virus surfaces. Indeed, dendrimers **6**, **7**, and **8** having longer spacer-arm moieties than those of dendrimers **3**, **4**, and **5** show the inhibitory activities.

Table 1. Preliminary results of inhibition assays for influenza virus sialidases

Compound[a]	Influenza virus subtype	
	A/Menphis/1/71 (H3N2)	A/PR/8/34 (H1N1)
1	ND[b]	ND[b]
2	ND[b]	ND[b]
3	ND[b]	ND[b]
4	ND[b]	ND[b]
5	ND[b]	ND[b]
6	5.00	5.00
7	5.00	5.00
8	1.25	5.00

IC_{50} values are indicated in mM concentration.
[a] The structures are shown in Figure 1.
[b] ND means not determined due to weak binding potency.

In conclusion, an efficient synthesis of a series of dendrimers having thioglycosidic linkage of sialic acid **3**–**8** was accomplished using functionalized thioglycoside of sialic acid and brominated dendrimers. Biological responses of these dendrimers against influenza virus sialidases were preliminary evaluated, and the results showed that some of the dendrimers have inhibitory potency for the sialidase. Further manipulations of dendritic core structures and the introduction of sialoside into the scaffolds to broaden the library are now in progress. The details of the results presented here and further synthetic procedures including other homologues and biological activities will be reported elsewhere in the near future.

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