Glyco-silicon Functional Materials as Anti-influenza Virus Agents

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Abstract: This review shows introduction of glycoclusters using carbosilanes as core scaffolds, preparations of glycoclusters and their excellent properties as well as functions. Since a dendrimer has unique advantages such as single molecular weight, regularity of structure and easy control of shape and size, dendrimers are utilized in various research areas. Results of syntheses and biological evaluations of the carbosilane dendrimers having carbohydrate moieties for influenza viruses are presented.

Keywords: Dendrimers, Carbosilanes, Carbohydrates, Glycodendrimers, Influenza viruses.

INTRODUCTION

Carbosilanes are hybrid materials and show unique characteristics from the viewpoint of interdisciplinary region of organic chemistry and inorganic chemistry [1]. We have therefore selected carbosilanes as supporting materials for constructing glycoclusters including bioactive carbohydrate moieties [2]. The chemical and biological stabilities of carbosilane dendrimers uniformly functionalized with carbohydrate moieties have been verified using Shiga toxincarbohydrate interactions [3-7], Dengue virus-carbohydrate interactions [8, 9], and lectin-carbohydrate interactions [10-12]. We call a series of carbosilanes having carbohydrate moieties "Glyco-silicon Functional Materials". In this review, we describe results of synthetic studies on carbosilane compounds uniformly functionalized with sialyl carbohydrate moieties through covalent bonds and biological evaluations as inhibitors for influenza viruses [13-17].

ATTRACTIVE PURIFICATION METHOD PRIOR TO ASSEMBLY OF CARBOHYDRATE MOIETIES USING CARBOSILANE SCAFFOLDS

Because of the importance of sialyl oligosaccharides in biological systems, much interest has been shown in the synthesis of sialyl oligosaccharides [18]. Therefore, many efforts have been devoted to the development of sialic acid chemistry [19-20]. We have also been synthesizing sialyl oligosaccharides and their glycoclusters by chemical and enzymatic methods, and the glycoclusters have been used in biochemical and biomedical fields [21-24]. In our ongoing study on the synthesis of sialyl oligosaccharides having thioglycosidic linkages, we have encountered the problem of an inseparable mixture of a desired sialyl derivative and the 2,3-didehydro sialic acid byproduct being obtained in some cases. A similar phenomenon has been reported by von Itzstein's group, and the separation of products was achieved by using HPLC purification methodology with a reverse-phase C_{18} column after deprotection of the product mixture [25]. An alternative and a highly convenient purification method for isolation of the sialyl oligosaccharides without the 2,3-didehydro sialic acid byproduct is required. Differences in the molecular sizes of the products led us to consider size exclusion chromatography (SEC), which filters molecules based on differences in molecular weights. Consequently, SEC was used for separation of the products. This section deals with a convenient method for removal of the 2,3-didehydro sialic acid byproduct in the reaction mixture by the SEC method [26].

Scheme (**1**) summarizes production of the 2,3-didehydro sialic acid byproduct **3** [27] accompanied by thioacetate **2** as the major product in the simple S_N2 reaction of known anomeric chloride **1** and KSAc [28]. In the reaction, simultaneous elimination of HCl from **2** giving a side product **3** was often observed. We have tried a variety of purification methods, but direct separation of **2** and **3** unfortunately failed. Therefore, we next turned our attention to purification of sialooligosaccharide after forming corresponding thioglycosides. Condensation of 6-bromo-6-deoxy-D-glucoside **4** [29] with thioacetate **2**, after producing a thiolate anion by treatment of **2** with diethylamine [30, 31], followed by chromatographic purification using silica gel gave a mixture of disaccharide **5** having an *endo*-thioglycosidic linkage and glycal **3**. Fig. (**1A**) shows the ^{1}H NMR spectrum of the mixture of **5** and **3**. The inseparable mixture was subjected to a recycle-type SEC system using chloroform as the eluent. The chromatographic profile of the separation of **5** and **3** is shown in Fig. (**2**). The peak of the inseparable mixture by silica gel chromatography was initially one peak (Peak I), which was gradually split into two peaks (Peak II and Peak III) after several recyclings. An impurity was eluted later than Peak I and was cut off at the first stage. When the complete separation was monitored (4th cycle), Peak II and Peak III were independently fractionated. Structural elucidation and a purity check of thioacetate **2**, glycal **3**, and disaccharide **5** were carried out by means of ^{1}H

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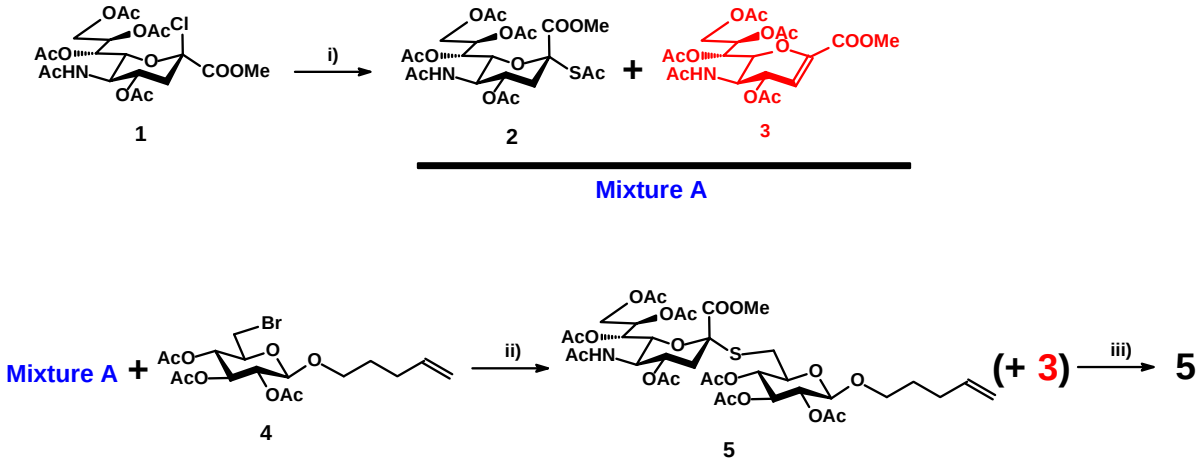
NMR spectroscopic analyses, the results of which are shown in Fig. (**1B-D**). Since Fig. (**1D**) clearly shows disaccharide **5** without impurities, attempts to purify the inseparable mixture by using the SEC method were deemed likely to succeed. The final yield of the isolated product **5** was 78.8%.

In this section, the purification of thiosialosides is introduced by means of the SEC method as a convenient and a useful tool. This methodology is applicable for the separation of other thiosialoside mixtures, usual sialosides, synthetic oligosaccharides, and dendritic compounds.

USE OF SIALYL LACTOSE AS A RECEPTOR FOR HEMAGGLUTININ OF INFLUENZA VIRUSES

Influenza viruses are unique because each virus has two glycoproteins with different roles on the surfaces of viral particles [32]. Hemagglutinin (HA) is one of the proteins and it shows lectin-like activity against sialyl oligosaccharides as specific receptors on host cells [33]. The other protein is a glycosidase, which is referred to as either sialidase or neuraminidase (NA), and it selectively cleaves sialic acid residues from sialoglycoproteins as well as gangliosides on the surfaces of host cells [34]. 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 [32]. Many efforts have been made to prepare multivalent-type glycomaterials as HA blockers for inhibiting adhesion of the virus to a host cell [35-41]. We have also established a procedure for coupling between the carbosilane dendrimers and sialic acid derivatives including a sugar moiety of GM3 to afford corresponding glycoclusters as multivalent-type HA blockers. In this section, we report a synthetic assembly of sialyl



Scheme (1). *Reagents and conditions*: (i) KSAc (5 molar excess), CH_2Cl_2, 0 °C→rt, 2 d; (ii) diethylamine (10 molar excess to Br residues), DMF, 0 °C→rt, overnight; (iii) purified by SEC.

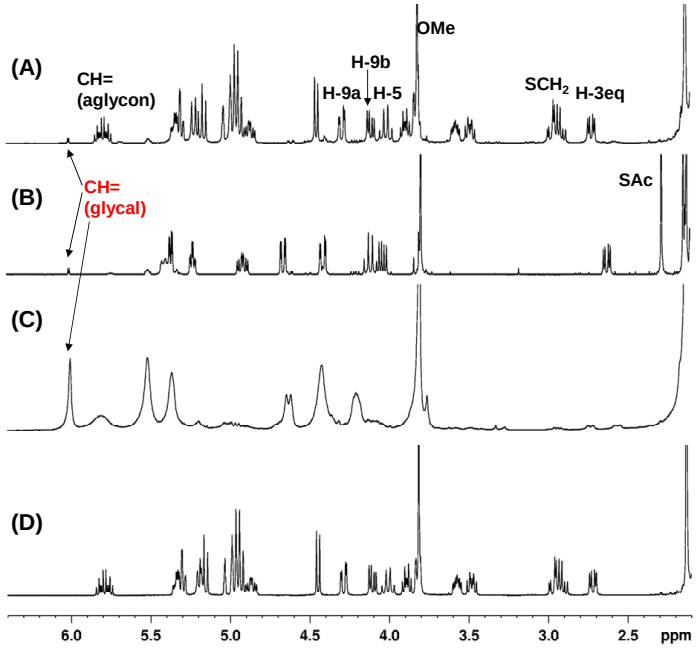


Fig. (1). ^1H NMR spectra at 400 MHz in $CDCl_3$ of (**A**) mixture of disaccharide **5** and glycal **3**, (**B**) thioacetate **2**, (**C**) glycal **3**, and (**D**) pure disaccharide **5**.

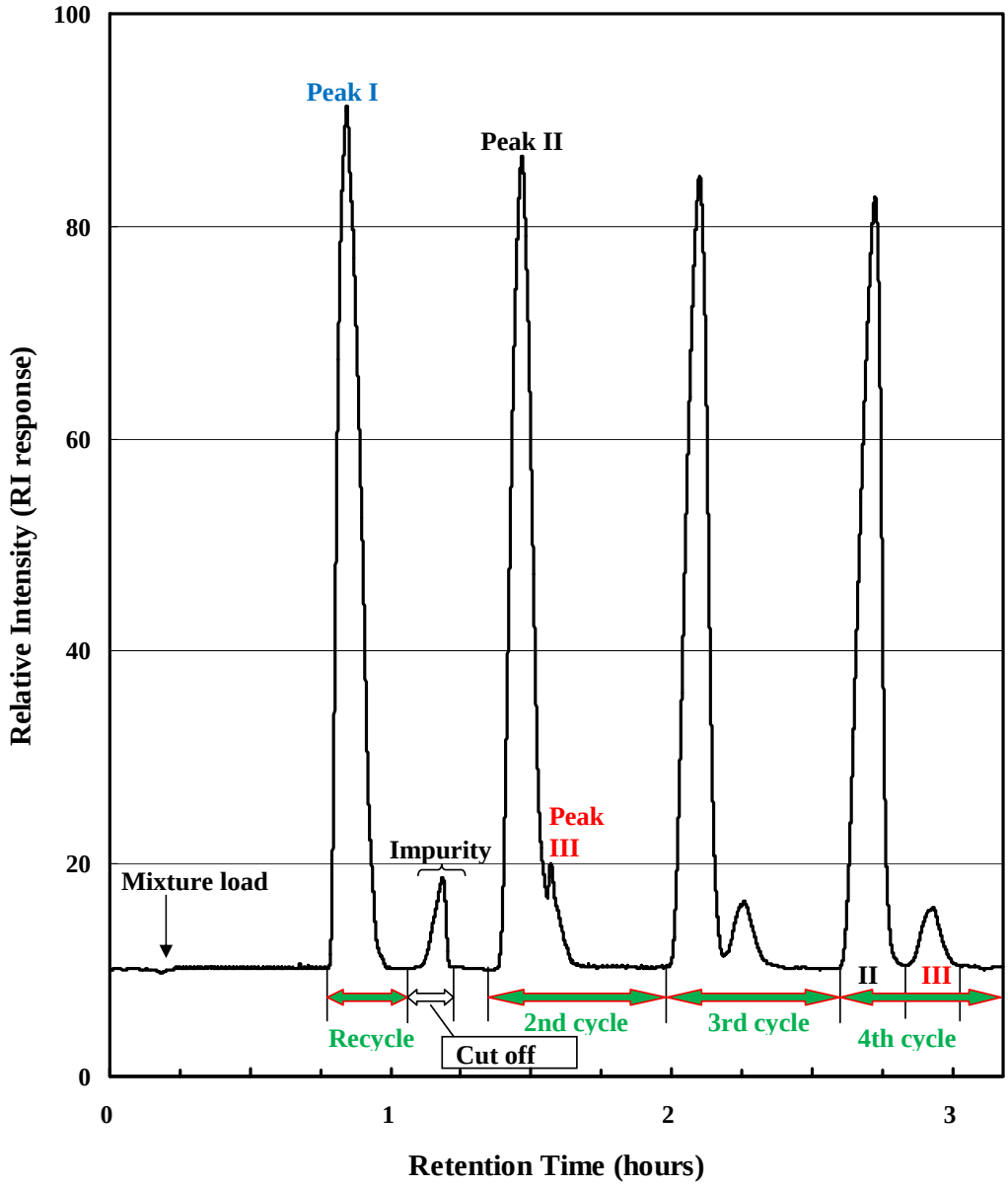


Fig. (2). SEC profile of the separation of disaccharide **5** and glycal **3**. Preparative SEC was performed by an SEC recycling apparatus [HLC-50G system (Shimamura Instruments Works, Co., Tokyo, Japan)] using tandem-bonded Shodex H-2001L (I.D., 20.0 mm x 600 mm) and H-2002 (I.D., 20.0 mm x 500 mm) columns. The columns were equilibrated with $CHCl_3$ at ambient temperature and the flow rate of the apparatus was 3.8 mL/min. A refractive index (RI) detector was used for monitoring the chromatograms.

α(2→3) lactosyl (Neu5Acα(2→3)Galβ(1→4)Glcβ1→) moieties using a series of carbosilane dendrimer scaffolds and the results of biological evaluations of the glycoclusters as potential candidates for influenza virus hemagglutinin blockers.

Our synthetic target compounds having unique shapes, generations, and different numbers of saccharide residues are shown in Fig. (**3**) [15, 17]. A monomeric compound **6** having a 4-pentenyl moiety as the aglycon was quantitatively prepared from a known compound **13** [13] by a combination of transesterification and saponification to remove all protections (Scheme **2**). Another transformation of **13** was also performed at the terminal C=C double bond by a radical addition of thioacetic acid in the presence of AIBN as the radical initiator to furnish thioacetate **14**. A series of alkyl halide-type carbosilane compounds as supporting materials for carbohydrates is shown in Fig. (**4**). A schematic diagram for construction of a series of glycoclusters is shown in Scheme **2**. Coupling reaction between the carbosilane dendrimer scaffolds **15**–**20** and sialyl lactose derivative **14** was performed in a one-pot reaction in the presence of sodium methoxide in methanol–DMF solvent systems, followed by the usual reacetylation to provide fully protected carbosilane dendrimers having sialyl lactose moieties at each terminal and disulfide **27**. Removal of all protective groups in the dendrimers was carried out by the same two-step procedure as that described for the preparation of **6** to give white powdery compounds after chromatographic purification, followed by lyophilization.

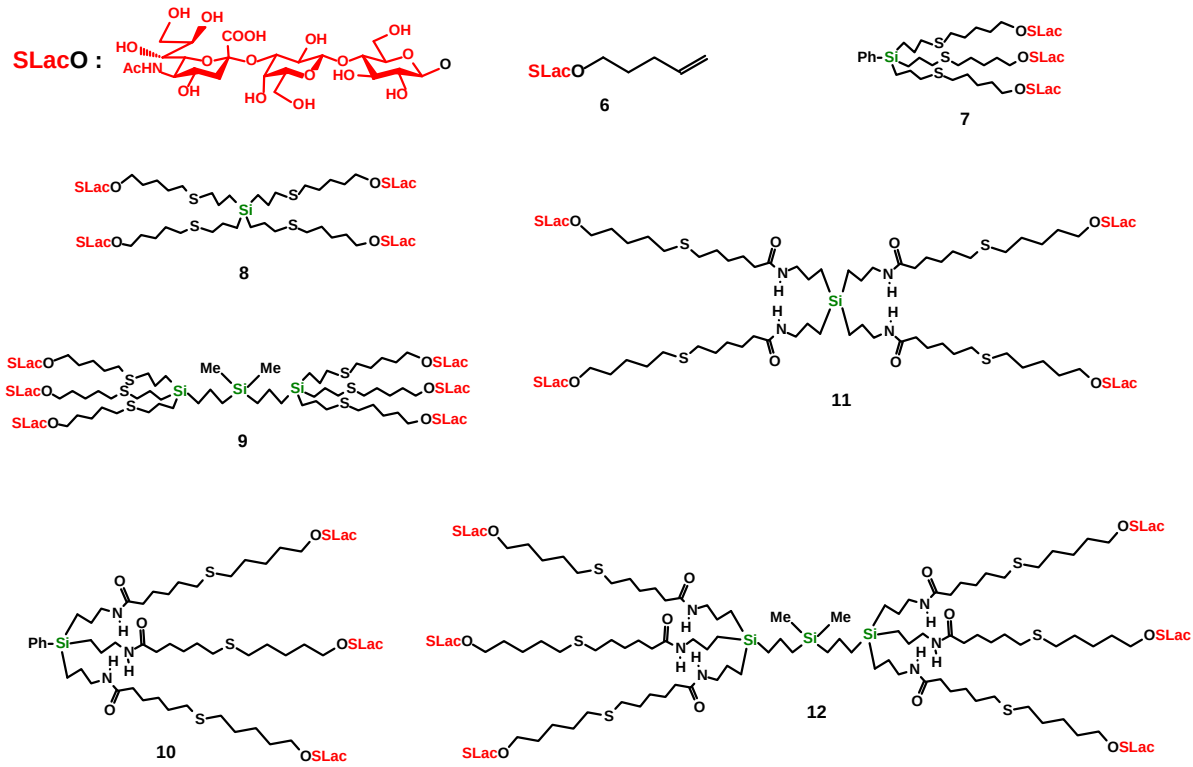


Fig. (3). A series of Glyco-silicon functional materials as multivalent-type synthetic substrates having sialyl α(2→3) lactose moieties.

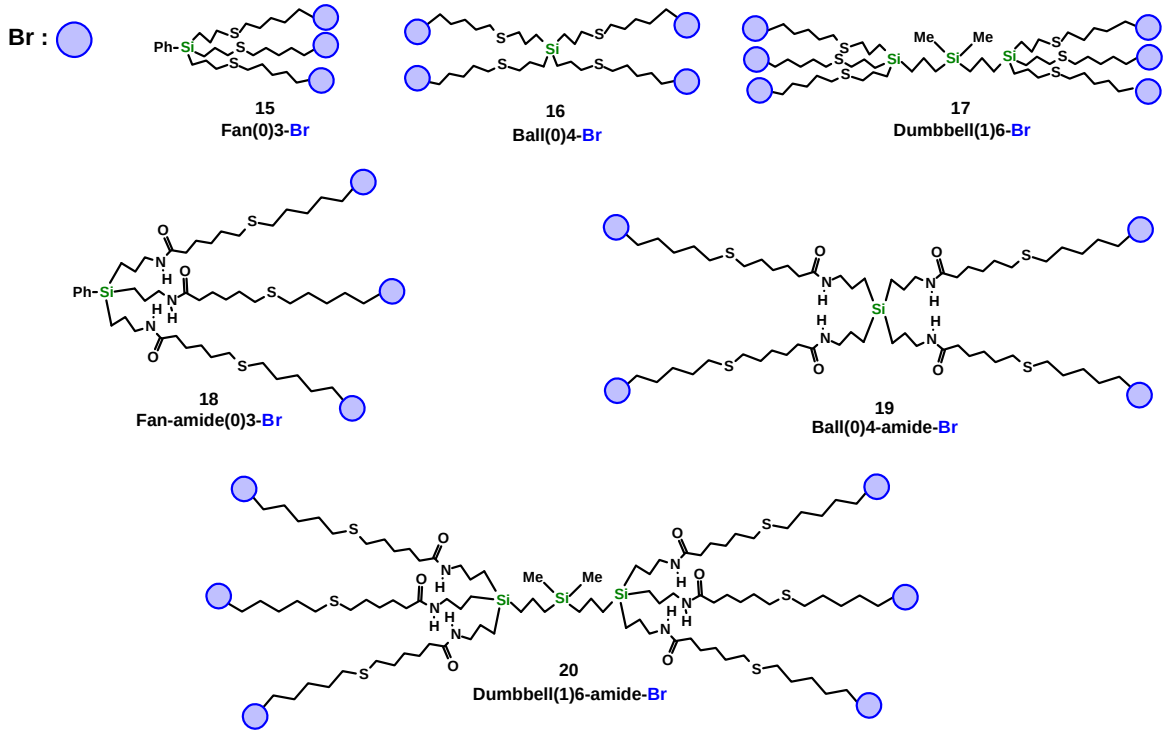


Fig. (4). A series of Glyco-silicon functional materials as multivalent-type synthetic core scaffolds having bromine atoms at each ω-terminal.

Since the systematic synthesis of glycoclusters having sialyl α(2→3) lactose (Neu5Acα(2→3)Galβ(1→4)Glcβ1→) moieties **6**–**12** was efficiently accomplished, our attention was directed toward the structure–activity relationship (SAR) of the sialyl compounds as HA blockers against influenza virus. Thus, the hemagglutinin inhibition (HAI) assay [42] was preliminarily performed using various virus strains, and the results are summarized in Table **1**. The virus strains used in this study were human influenza viruses A/PR/8/34 (H1N1), A/Aichi/2/68 (H3N2), and A/Memphis/1/71 (H3N2). The strain A/PR/8/34 recognizes Neu5 Acα(2→3)Gal residues in glycoconjugates and binds to

erythrocytes. A/Aichi/2/68-type virus recognizes both Neu5Acα(2→3)Gal and Neu5Acα(2→6)Gal residues, and A/Memphis/1/71-type virus recognizes Neu5Acα(2→6)Gal residues. Inhibitory activity of the series of glycoclusters against hemagglutination of various human influenza viruses to erythrocytes was clearly observed when A/PR/8/34 and A/Aichi/2/68 were used. Inhibitory potency of the glycoclusters against A/Memphis/1/71 was weak because of an unsuitable carbohydrate structure for the HA on the virus strain. These results strongly suggested that oligosaccharide chains of the glycoclusters were closely recognized by the corresponding HA on the viral surface. In addition, we found that Dumbbell(1)6-amide **12** showed the highest level of inhibitory activity in the glycolibrary. These carbosilane dendrimers uniformly functionalized with sialyl α(2→3) lactose moieties have unique characteristics, such as different numbers of sugar moieties depending on the core structure, different degrees of freedom of the sugar moieties depending on the spacer length and core shape, and different 3-D structures. These unique characteristics are useful for consideration of synthetic construction of other glycoclusters as well as expansion of the glycolibrary.

In order to further elucidate the SARs of the glycoclusters against influenza virus, hemolysis inhibition assay and infection inhibition assay were carried out using human influenza virus (A/PR/8/34) strain. The strain A/PR/8/34 was chosen for both hemolysis inhibition assay and infection inhibition assay because the virus strain of A/PR/8/34 strongly recognizes the Neu5Acα(2→3)Gal structure, which was appropriate for our synthesized glycoclusters. The inhibitory activities of glycoclusters in the hemolysis inhibition assay of influenza virus to erythrocytes are shown in Fig. (**5**), where concentrations of glycoclusters are represented by [SLac], which was corrected on the basis of a Neu5Acα(2→3)Galβ(1→4)Glc unit. According to Fig. (**5**), both Dumbbell(1)6-SLac **9** and Dumbbell(1)6-amide-SLac **12** showed strong inhibitory activity. The results suggested that the shape of the core dendrimer has a remarkable influence on the inhibitory activities in the HAI assay. The half maximal inhibitory concentration (IC_{50}) values of the inhibitors from Fig. (**5**) were estimated and are shown in Table **2**. Other glycoclusters also have stronger inhibitory activity than that of Pent-SLac **6**, and the order of the inhibitory effects of the glycolibrary in the hemolysis inhibition assay was **9** [Dumbbell(1)6-SLac] = **12** [Dumbbell(1)6-amide-SLac] > **10** [Fan(0)3-amide-SLac] = **11** [Ball

Table 1. Inhibitory Activities of a Series of Glycoclusters Against Hemagglutination of Human Influenza Viruses (Type-A) to Erythrocytes

Compounds	Inhibition activities (μM)		
	A/PR/8/34	A/Aichi/2/68	A/Memphis/1/71
	(H1N1)	(H3N2)	(H3N2)
6 [Pent-SLac]	125	250	500
7 [Fan(0)3-SLac]	64	125	250
8 [Ball(0)4-SLac]	64	32	250
9 [Dumbbell(1)6-SLac]	32	32	250
10 [Fan(0)3-amide-SLac]	16	8	250
11 [Ball(0)4-amide-SLac]	16	4	250
12 [Dumbbell(1)6-amide-SLac]	8	4	250

Values are IC_{50} values based on a sialyl lactose unit.

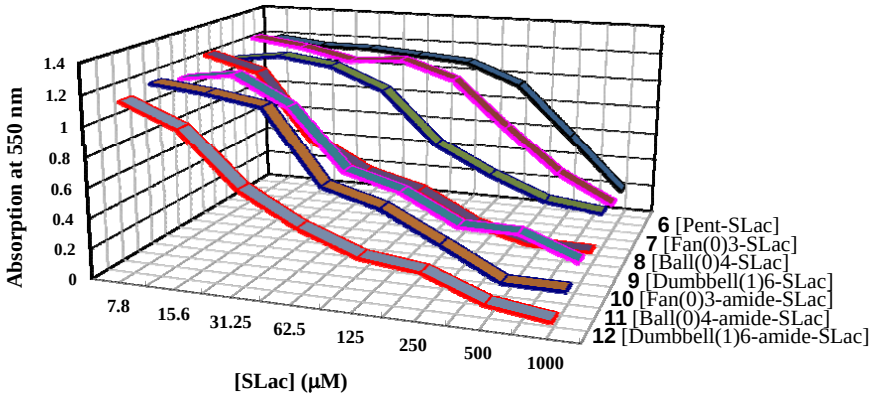


Fig. (5). Inhibitory activities of glycoclusters having sialyl α(2→3) lactose moieties (**6** – **12**) against hemolysis of human influenza virus (A/PR/8/34 (H1N1)) to erythrocytes. Concentrations of glycoclusters are represented by [SLac], which was calculated on the basis of a sialyl α(2→3) lactose unit.

(0)4-amide-SLac] > **8** [Ball(0)4-SLac] > **7** [Fan(0)3-SLac] > **6** [Pent-SLac].

Neutralization assays of glycoclusters to the infection of influenza virus (A/PR/8/34) using Madin-Darby canine kidney (MDCK) cells were also performed and the results are shown in Fig. (**6**). As was found in hemolysis inhibition assays, both Dumbbell(1)6-SLac **9** and Dumbbell(1) 6-amide-SLac **12** showed strong inhibitory activity compared to the activities of others. IC^{50} of the glycolibrary in the neutralization assay was estimated from the results shown in Fig. (**6**) and the values are summarized in Table **2**. All glycoclusters having multivalent sialyl lactose moieties showed higher levels of inhibitory activities against human influenza virus than that of monomeric-type Pent-SLac **6**. Dumbbell(1)6-amide-SLac **12** showed 22-times stronger inhibitory activity than that of **6**. The order of the inhibitory effects of the glycolibrary in the infection inhibition assay was **12** [Dumbbell(1)6-amide-SLac] > **9** [Dumbbell(1)6-SLac] > **11** [Ball(0)4-amide-SLac] > **8** [Ball(0)4-SLac] > **10** [Fan(0)3-amide-SLac] > **7** [Fan(0)3-SLac] > **6** [Pent-SLac]. Furthermore, these glycoclusters having a Neu5Acα(2→3)Gal residue at each terminal end are promising agents for prevention of infection with avian influenza virus (H5N1), since avian influenza virus has been shown to recognize Neu5Acα(2→3)Gal residues on host cells [43, 44].

In conclusion of this section, an efficient synthesis of a series of glycoclusters having sialyl α(2→3) lactose moieties **6**–**12** was systematically accomplished using various carbosilane dendrimers as supporters for the oligosaccharides. Biological evaluations of these glycoclusters against influenza virus were carried out, and the results showed that a glycocluster having longer spacer-arms and most carbohydrate epitopes has the highest activity.

USE OF THIOSIALOSIDES AS INHIBITORS FOR NEURAMINIDASE OF INFLUENZA VIRUSES

Influenza viruses have different types of carbohydrate-related proteins on their surfaces as described above [32]. Therefore, 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 [33]. Neuraminidase inhibitors, such as zanamivir [45] and oseltamivir [46], have been synthesized and widely used as therapeutic agents in the clinical treatment of influenza A and B viruses [47]. These drugs have extremely high inhibitory potencies for the release of influenza virions from infected cells; however, NA inhibitor-resistant viruses have already been generated [48, 49]. 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

Table 2. Inhibitory Activities of a Series of Glycoclusters Against Human Influenza Virus (A/PR/8/34 (H1N1) Strain).

Compounds	Inhibition activities (μM)		
	Hemagglutination*	Hemolysis (IC_{50})	Infection (IC_{50})
6 [Pent-SLac]	125	500	125
7 [Fan(0)3-SLac]	64	250	64
8 [Ball(0)4-SLac]	64	125	32
9 [Dumbbell(1)6-SLac]	32	32	7.8
10 [Fan(0)3-amide-SLac]	16	62.5	25
11 [Ball(0)4-amide-SLac]	16	62.5	16
12 [Dumbbell(1)6-amide-SLac]	8	32	5.6

Concentrations of glycodendrimers were calculated on the basis of a sialyl lactose unit.
*The data are also shown in Table **1**

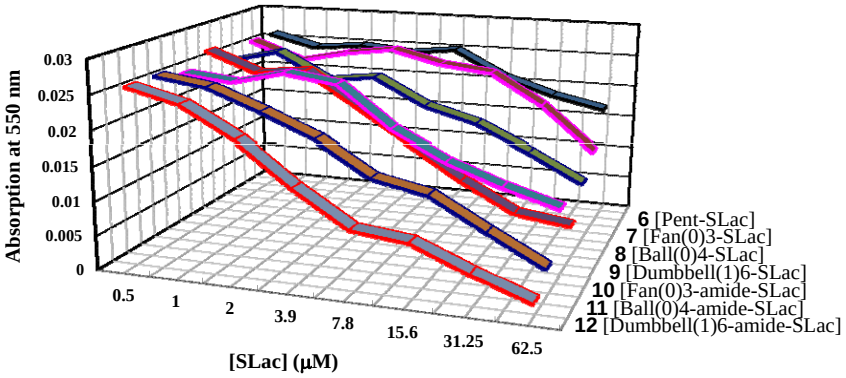


Fig. (6). Inhibitory activities of glycoclusters having sialyl α(2→3) lactose moieties (**6** – **12**) against infection of human influenza virus (A/PR/8/34 (H1N1)) to MDCK cells. Concentrations of glycoclusters are represented by [SLac], which was calculated on the basis of a sialyl α(2→3) lactose unit.

acid residues by a sialidase [45, 46]. 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 [20]. However, since monomeric sialoside does not have inhibitory potency for NA, we planned to use the sugar-clustering effect [50, 51]. In addition, NAs on the virus surface display tetrameric structures [52, 53] and a glycocluster is expected to be a promising multivalent-type therapeutic agent [3, 4].

In this section, we describe the synthetic assembly of thiosialoside moieties using a series of carbosilane dendrimers as the core frames and the results of preliminary biological evaluations using influenza viruses.

Schematic structures of target dendrimers are shown in Fig. (**7**). For investigation of the SAR for human influenza NAs, we selected a series of carbosilane dendrimers that consist of Fan(0)3, Ball(0)4, Dumbbell(1)6 and Ball(1)12, named by their shapes, generation number and number of

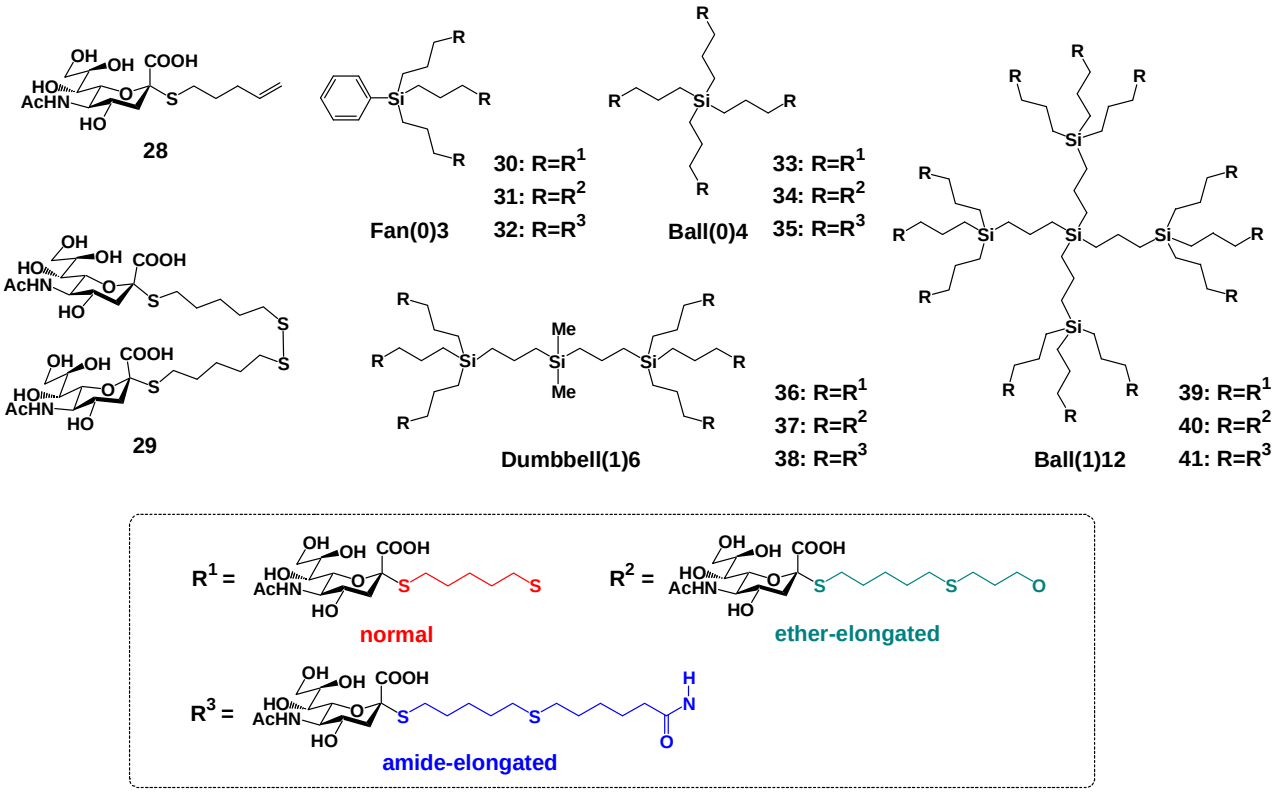
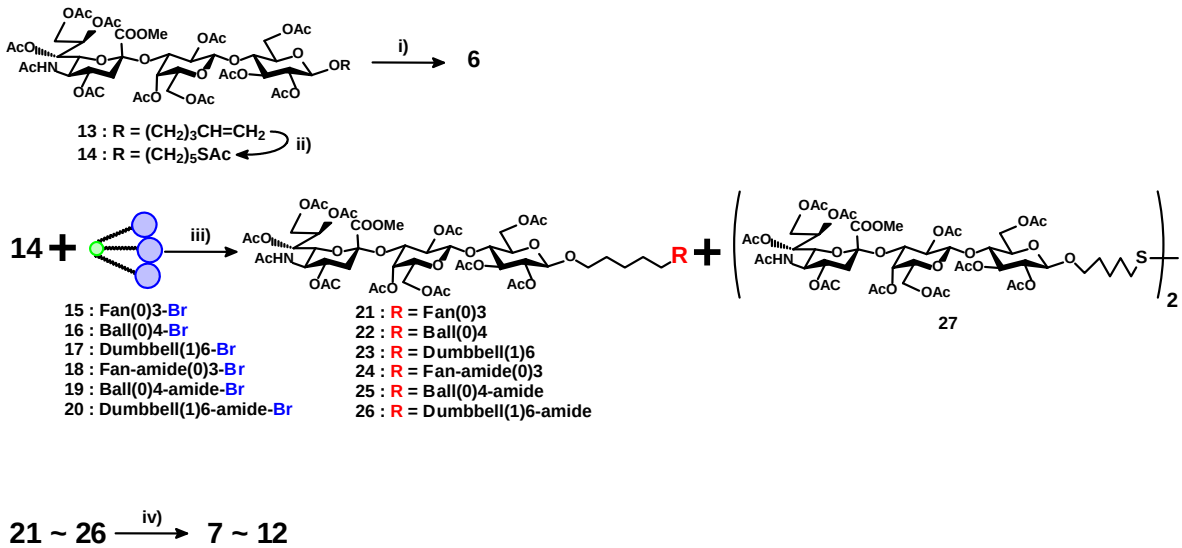


Fig. (7). A series of Glyco-silicon functional materials as multivalent-type synthetic substrates having thioglycoside-type sialic acid residues on each terminal end.



Scheme (2). *Reagents and conditions*: (i) NaOMe, MeOH, rt, overnight, then 0.05 M aq NaOH, rt, 2 h; (ii) AIBN, HSAc, 1,4-dioxane, 50→80 °C, 3 h; (iii) NaOMe, MeOH–DMF, rt, then Ac_2O–Pyr, rt, then CH_2N_2, Et_2O, rt; (iv) NaOMe, MeOH, rt, then 0.05 M aq NaOH, rt.

Table 3. Preliminary Results of Inhibition Assays of a Series of Glycoclusters Against Human Influenza Virus Sialidases

Compounds	Inhibitory potency (mM)[a]	
	A/Memphis/1/71 (H3N2)	A/PR/8/34 (H1N1)
28 [*S*-Neu5Ac]	N.D.[b]	N.D.[b]
29 [*S*-Neu5Ac$_2$]	N.D.[b]	N.D.[b]
30 [Fan(0)3-*S*-Neu5Ac]	N.D.[b]	N.D.[b]
31 [Fan(0)3-ether-*S*-Neu5Ac]	5.00	5.00
32 [Fan(0)3-amide-*S*-Neu5Ac]	7.1	2.7
33 [Ball(0)4-*S*-Neu5Ac]	N.D.[b]	N.D.[b]
34 [Ball(0)4-ether-*S*-Neu5Ac]	5.00	5.00
35 [Ball(0)4-amide-*S*-Neu5Ac]	8.8	4.8
36 [Dumbbell(1)6-*S*-Neu5Ac]	N.D.[b]	N.D.[b]
37 [Dumbbell(1)6-ether-*S*-Neu5Ac]	1.25	5.00
38 [Dumbbell(1)6-amide-*S*-Neu5Ac]	8.1	2.8
39 [Ball(1)12-*S*-Neu5Ac]	7.0	5.0
40 [Ball(1)12-ether-*S*-Neu5Ac]	6.5	6.4
41 [Ball(1)12-amide-*S*-Neu5Ac]	10.0 <	7.2

[a]IC_{50} values are indicated in milimolar concentration (mM) based on a monomeric sugar unit concentration. [b]N.D. means not determined due to weak inhibition.

terminal ends. In addition, on each framework, novel ether-linkage dendrimers [5-7] as well as known normal aliphatic dendrimers [14, 16] and amide-linkage dendrimers [54, 55] were used for clustering of thiosialosides.

Since a series of thiosialoside clusters **30**-**41** as well as thioglycosidic monomer **28** and dimer **29** had been prepared, biological evaluation of the thiosialosides for inhibitory activity against neuraminidases of two subtypes of human influenza virus (H1N1 and H3N2) was performed. The IC_{50} values measured by a previously reported method [56, 57] are summarized in Table **3**. Interestingly, all of the ether- and amide-elongated sialodendrimers and Ball(1)12-*S*-Neu5Ac (**39**) showed inhibitory potencies not only for H3N2-type sialidase but also for H1N1-type sialidase in the mM range, although monomeric sialoside **28** and dimeric sialoside **29** as well as normal aliphatic-type dendrimers **30**, **33**, **36** did not show any inhibitory activities. The most potent inhibitory activities were observed when Dumbbell(1)6-ether-*S*-Neu5Ac (**37**) for H3N2-type sialidase and Fan(0)3-amide-*S*-Neu (**32**) as well as Dumbbell(1)6-amide-*S*-Neu (**38**) for H1N1-type sialidase were used as substrates. Among the normal aliphatic-type dendrimers (**30**, **33**, **36**, **39**), only Ball(1)12-type dendrimer **39** showed inhibitory activity, indicating that the glycocluster effect effectively functioned with enhancing the binding affinity. However, among ether- and amide-elongated dendrimers, Ball(1)12-ether-Neu5Ac (**40**) and Ball(1)12-amide-Neu5Ac (**41**) interestingly showed the weakest inhibition potencies, respectively, indicating that the cluster effect functioned in an adverse direction. According to these results, elongation of the dendrimer may have a more favorable influence than increase in the number of sialic acid moieties for inhibitory activities against sialidases. Therefore, it is thought that the distance between the sialic acid moieties as well as the degree of freedom of the sialic acid moieties are important for effective binding to active sites on the tetrameric sialidases on virus surfaces.

CONCLUDING REMARKS

In this paper, we introduced some glycoclusters having sialic acid residues and showed that these dendrimers have excellent properties as well as functions. Since a dendrimer has unique advantages such as single molecular weight, regularity of structure, and easy control of shape and size, dendrimers are utilized in various research areas. Carbosilane dendrimers uniformly functionalized with sialic acid as an epitope for influenza viruses are of great interest for therapeutic use against viruses. We hope that the information presented in this review will be useful for scientists in various research fields.

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CONFLICT OF INTEREST

None.

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