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# Sialyl α(2 → 3) lactose clusters using carbosilane dendrimer core scaffolds as influenza hemagglutinin blockers ☆

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ABSTRACT

An efficient synthesis of a series of carbosilane dendrimers uniformly functionalized with sialyl α(2 → 3) lactose (Neu5Acα(2 → 3)Galβ(1 → 4)Glcβ1→) moieties was accomplished. The results of a preliminary study on biological responses against influenza virus hemagglutinin, using the sialyl lactose clusters showed unique biological activities on the basis of the structure–activity relationship according to the carbosilane scaffolds.



Carbosilanes are hybrid materials and show unique characteristics in view of the interdisciplinary region of organic chemistry and inorganic chemistry.[1] We have therefore selected carbosilanes as supporting materials for constructing glycodendrimers including bioactive carbohydrate moieties.[2] The chemical and biological stabilities of carbosilane dendrimers uniformly functionalized with carbohydrate moieties have been verified using Shiga toxin–carbohydrate interactions,[3] Dengue virus–carbohydrate interactions,[4] and influenza virus neuraminidase–thiosialoside cluster-type inhibitor interactions.[5]

Influenza viruses are unique because each virus has two glycoproteins with different roles on the surfaces of viral particles.[6] Hemagglutinin (HA) is one of the proteins and it shows lectin-like activity against sialyl oligosaccharides as specific receptors on host cells.[7] The other protein is a glycosidase, which is referred to as either sialidase or neuraminidase (NA), and it selectively creaves sialic acid residues from sialoglycoproteins as well as gangliosides on the surfaces of host cells.[8] Therefore, the virus is significantly attractive 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.[6] Many efforts have been made to prepare multivalent-type glycomaterials as HA blockers for inhibiting adhesion of the virus to a host cell.[9] 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 glycodendrimers as multivalent-type HA blockers. In this paper, we report a synthetic assembly of sialyl 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 glycodendrimers 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 Figure 1. A compound **2** having zero generation and three sugar moieties and **4** having first generation and six sugar moieties were prepared by the method previously reported.[10] A monomeric compound **1**[†] having a 4-pentenyl moiety as the aglycon was quantitatively prepared from a known compound **8** by a combination of transesterification and saponification to remove all protections

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

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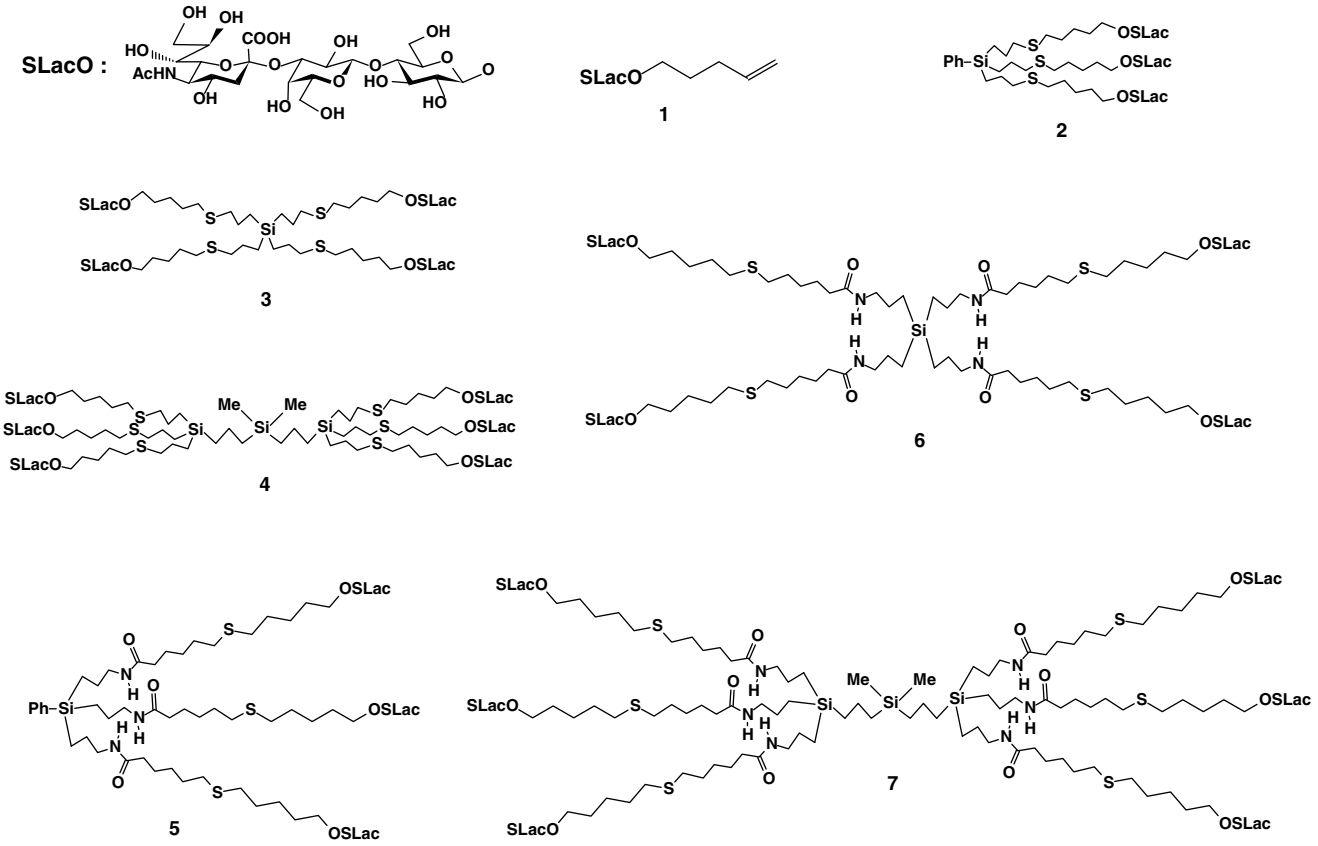
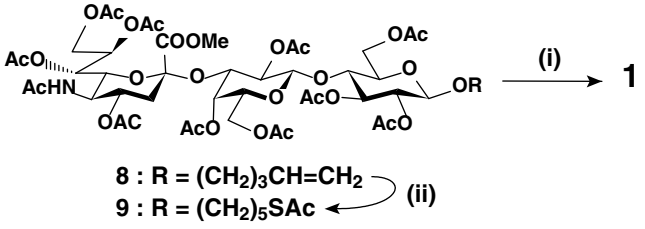



**Figure 1.** A series of multivalent-type synthetic substrates having sialyl α(2 → 3) lactose.

(Scheme 1), $[\alpha]_D^{18}$ − 3.4° (*c* 0.96, $H_2O$), $^1H$ NMR ($D_2O$) $\delta$ 4.40 (d, 1 H, $J_{1',2'}$ = 7.7 Hz, H-1′), 4.35 (d, 1 H, $J_{1,2}$ = 8.1 Hz, H-1), 2.63 (dd, 1 H, $J_{3''ax,3''eq}$ = 12.5 Hz, $J_{3''eq,4''}$ = 4.0 Hz, H-3″eq) 1.77 (t, 1 H, $J_{3''ax,4''}$ = 12.1 Hz, H-3″ax). The other transformation of **8** 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 **9** in high yield.

A series of alkyl halide-type carbosilane compounds as supporting materials for carbohydrates are shown in Figure 2. Dumbbell(1)6-type **15** was additionally synthesized by the method previously reported.[11] In brief, a known carbosilane having six bromine atoms at their terminal ends[3e] was converted into the azide, which was then transformed into the amine in the presence of $Ph_3P$ and water. The amine was further treated with 6-bromohexanoyl chloride to yield an amide **15** after chromatographic purification.

A synthetic scheme for construction of a series of glycodendrimers is illustrated in Scheme 2. Coupling reaction between the carbosilane dendrimer scaffolds **10**–**15** and sialyl lactose derivative **9** 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, Fan(0)3-type **16**,[10] Ball(0)4-type **17**, integral ratio of the H atoms by $^1H$ NMR: $SiCH_2$:H-1:H-1′ = 8:4:4, Dumbbell(1)6-type **18**,[10] Fan(0)3-amide-type **19**, integral ratio of the H atoms by $^1H$ NMR: $SiCH_2$:$CH_2SCH_2$:Ph:H-3″eq = 6:12:5:3, Ball(0)4-amide-type **20**, integral ratio of the H atoms by $^1H$ NMR: $SiCH_2$:$NCH_2$:H-1′ = 8:8:4, Dumbbell(1)6-amide-type **21**, integral ratio of the H atoms by $^1H$ NMR not determined due to intramolecular lactonization. Removal of all protective groups in the dendrimers was carried out by the same two-step procedure as that described for the preparation of **1** to give white powdery compounds after chromatographic purification, followed by lyophilization, Fan(0)3-type **2**,[10] Ball(0)4-type **3**, integral ratio of the H atoms by $^1H$ NMR: $SiCH_2$:H-1:H-1′:H-3″eq = 8:4:4:4, MALDI-TOF MS calcd for $[M+Na]^+$: 3158.4; found *m*/*z*: 3159.37, Dumbbell(1)6-type **4**,[10] Fan(0)3-amide-type **5**, integral ratio of the H atoms by $^1H$ NMR: $SiCH_2$:$CH_2SCH_2$:Ph:H-1:H-1′:H-3″eq = 6:12:5:3:3:3, MALDI-TOF MS calcd for $[M+Na]^+$: 2798.1; found *m*/*z*: 2799.26, Ball(0)4-amide-type **6**, integral ratio of the H atoms by $^1H$ NMR: $SiCH_2$:$NCH_2$:H-1:H-1′:H-3″eq = 8:8:4:4:4, MALDI-TOF MS calcd for $[M+Na]^+$: 3611.0; found *m*/*z*: 3610.44, Dumbbell(1)6-amide-type **7**, integral ratio of the H atoms by $^1H$ NMR: $CH_3Si$:H-1:H-1′:H-3″eq = 6:6:6:6, MALDI-TOF MS calcd for $[M+Na]^+$: 5561.4; found *m*/*z*: 5562.36.




**Scheme 1.** Reagents and conditions: (i) NaOMe, MeOH, rt, overnight, then 0.05 M aq. NaOH, rt, 2 h, 84%; (ii) AIBN, HSAc, 1,4-dioxane, 50 → 80 °C, 3 h, 99%.

Since the systematic synthesis of glycoclusters having sialyl α(2 → 3) lactose (Neu5Acα(2 → 3)Galβ(1 → 4)Glcβ1→) moieties **1**–**7** 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[12] was preliminarily per-

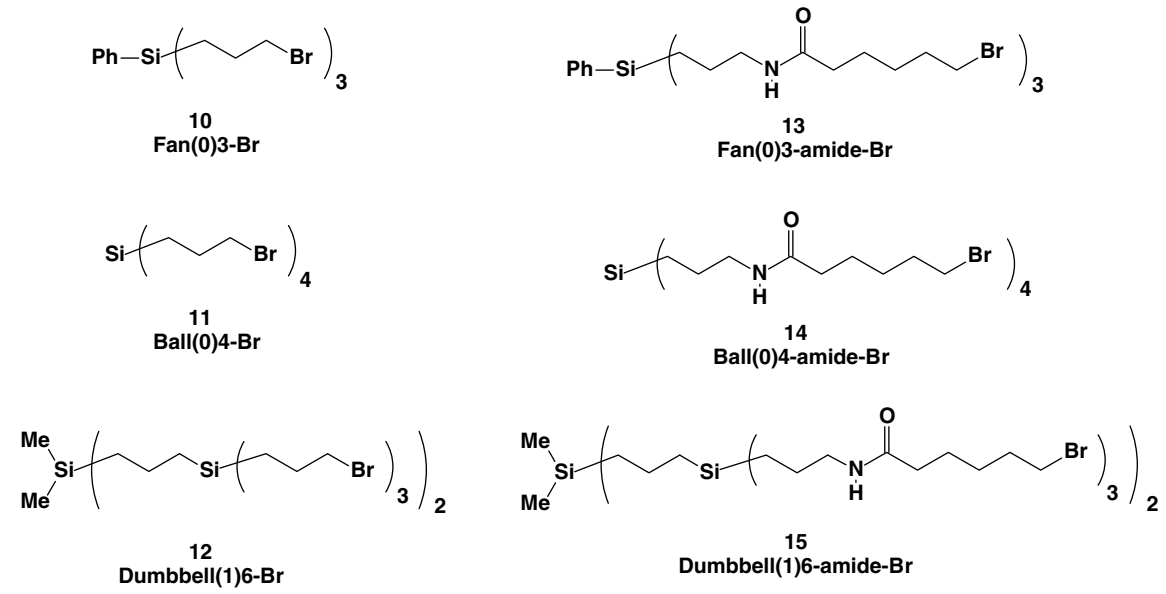
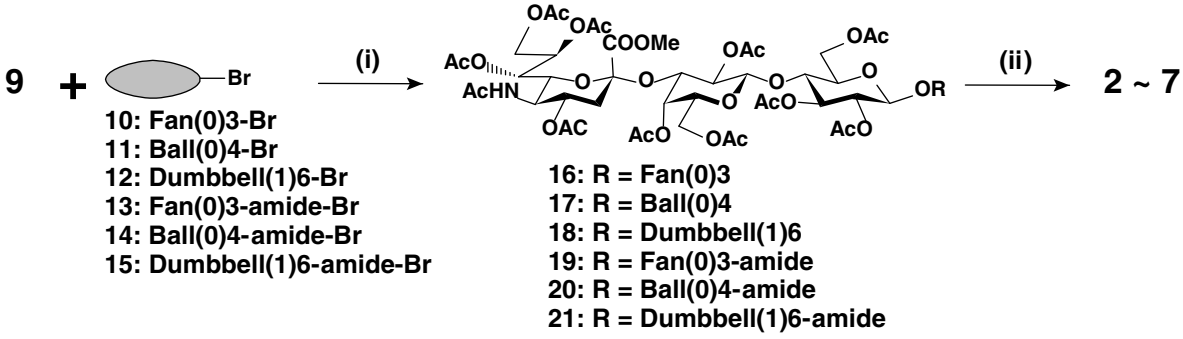



**Figure 2.** A series of alkyl halide-type carbosilane dendrimers uniformly functionalized with a bromo atom at each terminal end.




**Scheme 2.** Reagents and conditions: (i) NaOMe, MeOH–DMF, rt, then $Ac_2O$–Pyr, rt, then $CH_2N_2$, $Et_2O$, rt, for **16** (Ref. 10), for **17** (33%), for **18** (Ref. 10), for **19** (66%), for **20** (15%), for **21** (35%); (ii) NaOMe, MeOH, rt, overnight, then 0.05 M aqueous NaOH, rt, for **2** (Ref. 10), for **3** (94%), for **4** (Ref. 10), for **5** (quant.), for **6** (64%), for **7** (77%).

formed using various virus strains, and the results are summarized in Figure 3. The virus strains used in this study were human influenza viruses A/PR/8/34 (H1N1), A/Aich/2/68 (H3N2), and A/Memphis/1/71 (H3N2). The strain A/PR/8/34 recognizes Neu5Acα(2 → 3)Gal residues in glycoconjugates and binds to erythrocytes. A/Aich/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 glycodendrimers against hemagglutination of various human influenza viruses to erythrocytes was clearly observed when A/PR/8/34 and A/Aich/2/68 were used. Inhibitory potency of the glycodendrimers 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 virus surface. In addition, we found by using SARs methodology that Dumbbell(1)6-amide **7** showed the highest level of inhibitory activity in 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 degree of freedom of the sugar moieties depending on the spacer length and core shape, and different 3-D structures. This phenomenon is useful for consideration of synthetic construction of other glycodendrimers as well as expansion of the glycolibrary. Furthermore, these glycodendrimers having Neu5Acα(2 → 3)Gal residues at their terminal ends are promising agents for prevention of

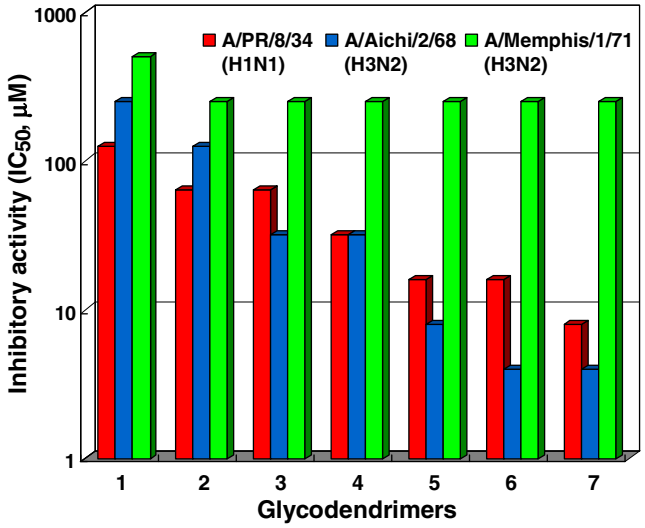



**Figure 3.** Inhibitory activity of a series of glycodendrimers against hemagglutination of various human influenza viruses. Concentrations of glycodendrimers were calculated on the basis of a sialyl α(2 → 3) lactose unit.

infection with avian influenza (H5N1), since avian influenza virus recognizes Neu5Acα(2 → 3)Gal residues on host cells.[13]

In conclusion, an efficient synthesis of a series of glycoclusters having sialyl α(2 → 3) lactose moieties **1** ∼ **7** was systematically accomplished using various carbosilane dendrimers as supporters for the oligosaccharides. Biological evaluations of these glycodendrimers against influenza virus were preliminarily carried out, and the results showed that a glycodendrimer having longer spacer-arms and most carbohydrate epitopes has the highest activity. The results suggested SARs in this biological response using the glycolibrary was observed. Experimental details for the syntheses of the series of glycodendrimers in this study and other biological activities will be reported elsewhere in the near future.

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## References and notes

1. For example, see Majoral, J.-P.; Caminade, A.-M. *Chem. Rev.* **1999**, *99*, 845. and references cited therein.
2. Yuasa, H. Ed. *Nanotechnology in carbohydrate chemistry*; Transworld Research Network: Kerala, 2006, Matsuoka, K.; Hatano, K.; Terunuma, D. pp. 89–102.
3. (a) 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; (b) Nishikawa, K.; Matsuoka, K.; Watanabe, M.; Igai, K.; Hino, K.; Hatano, K.; Yamada, A.; Abe, N.; Terunuma, D.; Kuzuhara, H.; Natori, Y. *J. Infect. Dis.* **2005**, *191*, 2097; (c) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839; (d) Matsuoka, K.; Terabatake, M.; Umino, A.; Esumi, Y.; Hatano, K.; Terunuma, D.; Kuzuhara, H. *Biomacromolecules* **2006**, *7*, 2274; (e) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Hatano, K.; Terunuma, D.; Kuzuhara, H. *Biomacromolecules* **2006**, *7*, 2284.
4. (a) Aoki, C.; Hidari, K. I. P. J.; Itonori, S.; Yamada, A.; Takahashi, N.; Kasama, T.; Hasebe, F.; Islam, M. A.; Hatano, K.; Matsuoka, K.; Taki, T.; Guo, C.-T.; Takahashi, T.; Sakano, Y.; Suzuki, T.; Miyamoto, D.; Sugita, M.; Terunuma, D.; Morita, K.; Suzuki, Y. *J. Biochem.* **2006**, *139*, 607; (b) Yamada, A.; Hatano, K.; Koyama, T.; Matsuoka, K.; Esumi, Y.; Terunuma, D. *Carbohydr. Res.* **2006**, *341*, 467.
5. Sakamoto, J.-I.; Koyama, T.; Miyamoto, D.; Yingsakmongkon, S.; Hidari, K. I. P. J.; Jampangern, W.; Suzuki, T.; Suzuki, Y.; Esumi, Y.; Hatano, K.; Terunuma, D.; Matsuoka, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 717.
6. Webster, R. G.; Bean, W. J.; Gorman, O. T.; Chambers, T. M.; Kawaoka, Y. *Microbiol. Rev.* **1992**, *56*, 152. and references cited therein.
7. For example, see Wiely, D. C.; Skehel, J. J. *Ann. Rev. Biochem.* **1987**, *56*, 365. and references cited therein.
8. For example, see Hayden, F. G.; Calfee, D. P. *Drugs* **1998**, *56*, 537. and references cited therein.
9. (a) Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686; (b) Tsuchida, A.; Kobayashi, K.; Matsubara, N.; Muramatsu, T.; Suzuki, T.; Suzuki, Y. *Glycoconjugate J.* **1998**, *15*, 1047; (c) Kamitakahara, H.; Suzuki, T.; Nishigori, N.; Suzuki, Y.; Kanie, O.; Wong, C.-H. *Angew. Chem. Int. Ed.* **1998**, *37*, 1524; (d) Fruike, T.; Aiba, S.; Suzuki, T.; Takahashi, T.; Suzuki, Y.; Yamada, K.; Nishimura, S.-I. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3000; (e) Makimura, Y.; Zhonghong, G.; Roy, R. *Int. Congress Series* **2001**, *1223*, 45; (f) Ohta, T.; Miura, N.; Fujitani, N.; Nakajima, F.; Niikura, K.; Sadamoto, R.; Guo, C.-T.; Suzuki, T.; Suzuki, Y.; Monde, K.; Nishimura, S.-I. *Angew. Chem. Int. Ed.* **2003**, *42*, 5186; (g) Ogata, M.; Murata, T.; Murakami, K.; Suzuki, T.; Hidari, K. I. P. J.; Suzuki, Y.; Usui, T. *Bioorg. Med. Chem.* **2007**, *15*, 1383.
10. Matsuoka, K.; Oka, H.; Koyama, T.; Esumi, Y.; Terunuma, D. *Tetrahedron Lett.* **2001**, *42*, 3327.
11. Matsuoka, K.; Terabatake, M.; Saito, Y.; Hagihara, C.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2709.
12. Suzuki, Y.; Suzuki, T.; Matsumoto, M. *J. Biochem. (Tokyo)* **1983**, *93*, 1621.
13. (a) Rogers, G. N.; Paulson, J. C. *Virology* **1983**, *127*, 361; (b) Shinya, K.; Kawaoka, Y. *Uirusu* **2006**, *56*, 85.