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Synthesis of sialic acid derivatives having a C═C double bond substituted at the C-5 position and their glycopolymers

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ABSTRACT

Glycomonomers of sialic acid in which the acetamide group at C-5 was converted into two kinds of C═C double bond substituents were prepared and the fully protected glycomonomers were directly polymerized before deprotection steps. Radical polymerization with acrylamide in DMF in the presence of ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine proceeded smoothly and gave corresponding sialopolymers. Interestingly glycomonomers had hemagglutination inhibitory activities not only for H1N1 but also for H3N2 of human influenza virus strains.

The cell surface of a eukaryotic organism is covered with various glycoconjugates, including glycolipids and glycoproteins. Carbohydrates are closely related to a wide range of biological processes, such as growth and differentiation of cells, cell–cell adhesions, immune responses, malignant alterations and oncogenesis.[1] Sialic acids, which are usually located at the terminal positions of glycoconjugates, play a significant role in biological systems. Influenza viruses are classified into types A, B and C on the basis of the major internal proteins, such as nucleoprotein, and each type of virus interacts with host cells through sialylated oligosaccharides[2] on the host cells. Hemagglutinin (HA) on the surface of an influenza virus particle attaches to the terminal sialic acid residues of glycoconjugates, allowing viruses to invade the host cell. After budding, multiplied viruses interact with sialic acid residues on the surface of the infected host cell. Then neuraminidase (NA), which is an alternative enzyme on the viral surface, divides the interaction between sialic acid and HA, enabling the multiplied viruses to spread to uninfected cells.

Oseltamivir,[3] one of the NA inhibitors, is a transition-state analog of sialic acid in the course of hydrolysis of the sialyl glycoside by NA, and it is known as an effective therapeutic agent for influenza infection.[4] However, its structure differs from that of natural sialic acid. Due to its structure, influenza viruses have become oseltamivir-resistant, and infection in humans with drug-resistant mutant viruses has been reported. Several patients have died because of the ineffectiveness of the drug.[5] There have been many efforts to develop an anti-influenza drug based on the sugar-clustering effect of sialic acid.[6] We have also explored potential agents against HA and NA of influenza virus based on the naturally obtainable sialic acid moiety. A C-5-modified sialic acid derivative has been reported by several groups,[7] however no polymeric compound of the C-5-modified sialic acid has been reported. Only a C-4-modified sialic acid derivative and the glycopolymer have been reported.[8] In this letter, the synthesis of novel sialic acid derivatives modified at C-5 and polymerization of the sialic acid derivatives are described. Since a monomeric carbohydrate does not have strong inhibitory potency against carbohydrate-binding proteins, glycoside clustering effect[9] is used for enhancement of the weak binding activity.[10] One of the most effective and facile methods to obtain this effect is polymerization with acrylamide[11] in order to construct a glycopolymer having a large number of glycoepitopes. Accordingly, we planned to prepare cluster compounds by simple polymerization and we describe herein the synthesis of novel sialic acid derivatives and the polymers.

Anomeric mixtures **2** were derivatized from neuraminic acid **1** by Sinaÿ's protocol[12] (Scheme 1). The acetate **2** gave anomeric mixture of lauryl thioglycoside **3** by treatment with 1-dodecanthiol in the presence of Lewis acid in dichloromethane, which was separated into **3α** and **3β** by means of chromatographic purification.[13]

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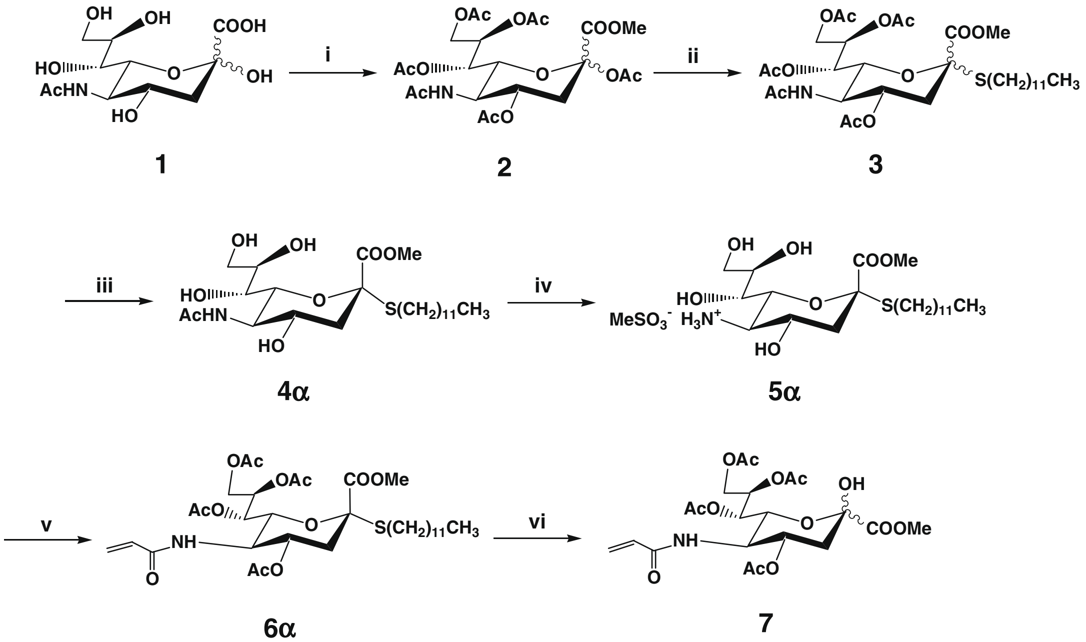

Scheme 1. Reagents and conditions: (i) Ac_2O, Pyr., rt, overnight, then CH_2N_2, 0 °C (88%); (ii) $CH_3(CH_2)_{11}SH$, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 3 h (81%); (iii) MeONa, MeOH, rt, overnight; (iv) $MeSO_3H$, MeOH, 60 °C, overnight (88% in two steps); (v) acryloyl chloride, Et_3N, MeOH, 0 °C, then DMAP, Ac_2O, Pyr., rt, overnight (61%); (vi) NIS, H_2O–$(CH_3)_2CO$ (1:9), rt, 1 h (quant.).

After deacetylation of **3α** under Zemplén conditions, the acetamide **4α** was treated with methanesulfonic acid[14] to provide ammonium salt **5α** in 88% yield (two steps). The amino group of **5α** was N-acryloylated under Schotten–Baumann conditions and then acetylated to give the fully protected derivative **6α** in 61% yield having a terminal C=C double bond,[†] $[\alpha]_D^{31}$ -25 (c 1.27, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 5.31 (dd, 1H, $J_{6,7} = 2.1$ Hz and $J_{7,8} = 6.9$ Hz, H-7), 4.95 (ddd, 1H, $J_{3ax,4} = 11.0$ Hz, $J_{3eq,4} = 4.3$ Hz and $J_{4,5} = 11.0$ Hz, H-4), 4.34 (dd, 1H, $J_{8,9a} = 2.6$ Hz and $J_{9a,9b} = 12.5$ Hz, H-9a), 4.12 (m, 1H, H-9b), 2.75 (dd, 1H, $J_{3ax,3eq} = 12.8$ Hz, H-3eq).[15] To examine the effect of the aglycon moiety, a hydroxyl group was introduced at an anomeric position. The reaction of thioglycoside **6α** with NIS in H_2O–acetone (1:9)[16] quantitatively produced the corresponding hemiketal **7**, $[\alpha]_D^{30}$ -2.1 (c 1.13, $CHCl_3$).

As shown in Scheme 2, each anomer of the ammonium salts **5** was further treated with 6-bromohexanoyl chloride followed by acetylation to give the alkyl halide **8α** in 40% yield and **8β** 51% yield, respectively. A bromine atom of **8** was quantitatively displaced by an azido group by the usual S_N2 to yield the compound **9**. After deacetylation of the azide **9**, hydrogenation of **10** was carried out in the presence of $Pd(OH)_2/C$ under H_2 atomosphere followed by Schotten–Baumann reaction and reacetylation to afford the compound **12** having a longer aliphatic spacer-arm than that of **6**, **12α** in 34% yield (two steps) from **10α**; $[\alpha]_D^{32}$ $+20$ (c 1.27, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 5.29 (dd, 1H, $J_{6,7} = 1.8$ Hz and $J_{7,8} = 7.8$ Hz, H-7), 4.88 (ddd, 1H, $J_{3ax,4} = 11.0$ Hz, $J_{3eq,4} = 4.5$ Hz and $J_{4,5} = 11.0$ Hz, H-4), 4.34 (dd, 1H, $J_{8,9a} = 2.5$ Hz and $J_{9a,9b} = 12.4$ Hz, H-9a), 4.11 (dd, 1H, $J_{8,9b} = 5.6$ Hz, H-9b), 2.72 (dd, 1H, $J_{3ax,3eq} = 12.5$ Hz, H-3eq), **12β** in 76% yield (two steps) from **10β**; $[\alpha]_D^{32}$ -42 (c 1.27, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 5.42 (t, 1H, $J_{6,7} = J_{7,8} = 2.3$ Hz, H-7), 5.29 (ddd, 1H, $J_{3ax,4} = 11.0$ Hz, $J_{3eq,4} = 4.7$ Hz and $J_{4,5} = 10.4$ Hz, H-4), 4.83 (dd, 1H, $J_{8,9a} = 2.2$ Hz and $J_{9a,9b} = 12.4$ Hz, H-9a), 4.19 (dd, 1H, $J_{8,9b} = 8.3$ Hz, H-9b), 2.52 (m, 1H, H-3eq). The same reaction as that described for the preparation of **7** was performed for the acetate **12β**, giving the hemiketal **13** in 80% yield, $[\alpha]_D^{31}$ $+1.9$ (c 1.07, $CHCl_3$).

After removal of protective groups of the glycomonomers, polymerization of the monomers was carried out in aqueous media.[17] The reaction proceeded, but all trials gave unsoluble gel-state products. Consequently, we needed to change our methodology of the polymerization in order to accomplish our research objective. Thus, the step of deprotection of the products was changed after polymerization. Radical polymerizarion of glycomonomers **6α**, **7**, **12α**, **12β** and **13** with varous ratios of acrylamide in DMF in the presence of APS—TEMED as a radical initiator system proceeded smoothly and produced the corresponding copolymers **14α**, **15**, **16α**, **16β** and **17**, respectively (Scheme 3). When solubility of the polymers in organic solvents was examined, the polymers showed unique solubility dependent on the polymer compositions based on the sugar–acrylamide unit ratio. Therefore, polymers that are soluble in ethyl acetate were purified by gel filtration (Sephadex LH-20) with ethyl acetate as the eluent, and transesterification of the purified polymers, followed by saponification yielded corresponding water-soluble glycopolymers. On the other hand, polymers that are insoluble in ethyl acetate were directly treated with 1 M NaOMe in MeOH without chromatographic purification. After 3 h, 0.2 M NaOH aq was added to induce saponification, and the mixture was further stirred overnight at room temperature. The solution containing sodium salts of carboxylic acid in sialic acid residues was purified by dialysis against water and lyophilized to furnish white powdery copolymer **18α**, **19**, **20α**, **20β** or **21** as the sodium salt, **18α** in 91% yield; x:y = 11:1, $\overline{Mw}$ 49 kDa, $\overline{Mw}/\overline{Mn} = 1.3$, **19** in 80% yield; x:y = 13:1, $\overline{Mw}$ 300 kDa, $\overline{Mw}/\overline{Mn} = 2.0$, **20α** in 87% yield; x:y = 10:1, $\overline{Mw}$ 100 kDa, $\overline{Mw}/\overline{Mn} = 2.4$, **20β** in 35% yield; x:y = 10:1, $\overline{Mw}$ 50 kDa, $\overline{Mw}/\overline{Mn} = 1.5$, or **21** in 14% yield; x:y = 2:1, $\overline{Mw}$ 66 kDa, $\overline{Mw}/\overline{Mn} = 1.2$.

Since the synthesis of sialic acid derivatives having a C=C double bond substituent at C-5 position and their glycopolymers was accomplished, our attention was directed to biological activities of a series of novel sialic polymers and monomers for influenza virus HA. In addition to the polymers, monomeric candidates for biological evaluations were prepared from **6α**, **7** and **12α** by means

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[†] All new compounds with the specific rotation data gave satisfactory results of elemental analyses.