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Novel linear polymers bearing thiosialosides as pendant-type epitopes for influenza neuraminidase inhibitors

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Abstract—A conventional synthesis of α-thioglycoside of sialic acid as a glycomonomer was accomplished. Radical copolymerization of the glycomonomer with vinyl acetate proceeded smoothly to afford a new class of glycopolymers having thiosialoside residues, in which all protection was removed by a combination of transesterification and saponification to provide a water-soluble thiosialoside cluster. The results of a preliminary study on biological responses against influenza virus neuraminidases using the thiosialoside polymer as a candidate for a neuraminidase inhibitor showed that the glycopolymer has potent inhibitory activity against the neuraminidases.

Synthetic glycoclusters are of great interest in biochemical as well as biomedical fields because the multivalent compounds have various advantages such as (1) simplicity of the synthetic process, (2) obvious epitope structures in the molecule, and (3) chemical and biological stabilities.[1] Among the various synthetic glycoclusters, glycopolymers are attractive candidate for manufacturing therapeutic reagents.[2] We have previously reported glycopolymers having trisaccharidic epitopes of globotriaosyl ceramide (Gb3; Galα1 → 4Galβ1 → 4Glcβ1 → Cer)[3] and their biological evaluations.[4] The results of a study on biological evaluations showed that the activity of the glycopolymers was highly enhanced by the sugar-cluster effect, and the therapeutic treatment for mice was also demonstrated.[4] A sialic acid (Neu5Ac) is a unique monosaccharide and is specifically recognized by various carbohydrate-related proteins.[5] Several proteins in influenza virus are the carbohydrate-related proteins. In general, influenza viruses have different types of carbohydrate-related proteins on their viral particle surfaces,[6] such as hemagglutinin (HA), which is a kind of lectin and specifically binds to sialyl oligosaccharides on host cells,[7] and 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.[8] Neuraminidase inhibitors, such as zanamivir[9] and oseltamivir,[10] have been synthesized and widely used as therapeutic agents in the clinical treatment of influenza A and B viruses.[11] Although these drugs have extremely high inhibitory potencies to the release of influenza virions from infected cells, NA inhibitor-resistant viruses have already been generated.[12] Oseltamivir-resistant avian influenza virus has been isolated, and the death of a human from influenza A virus (H5N1) infection has recently been reported.[13] Since the inhibitors are not naturally obtained and are designed as transition-state analogues after cleavage of sialic acid residues by an

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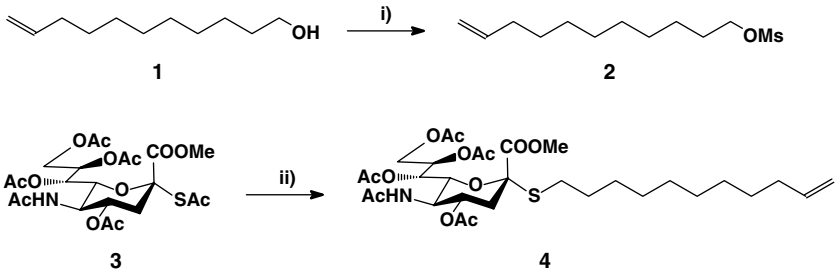
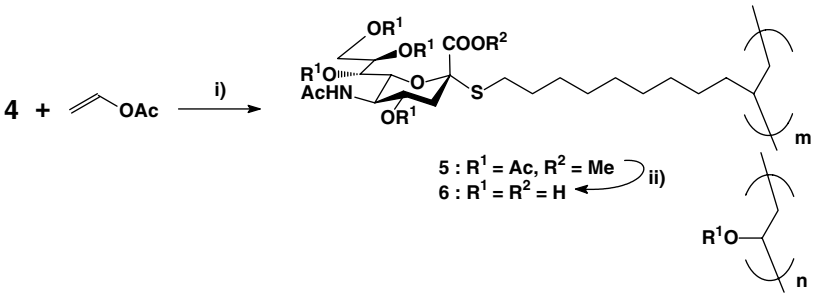


Scheme 1. Reagents and conditions: (i) MsCl, Pyr, 0 °C, 3 h; (ii) **2**, K_2CO_3, MeOH, 0 °C → rt, overnight, then Ac_2O–Pyr, rt, overnight.



Scheme 2. Reagents and conditions: (i) AIBN, reflux temp; (ii) NaOMe, MeOH, rt, 3 h, then 0.5 M aqueous NaOH, rt, 2 days.

NA, a therapeutic treatment of influenza using oseltamivir would result in the oseltamivir-resistant virus.[12a] It seems that an epitope of the neuraminidase of influenza virus is a natural sialic acid, and the resistant virus therefore appeared during treatment of the infection. Therefore, we planned to synthesize a new polymeric thiosialoside cluster as an additional antiviral agent because thioglycosidic linkage is usually not hydrolyzed by glycosidases such as NA.[14] However, since monomeric sialoside does not have any potent inhibitory activity for NA, we utilized the sugar-cluster effect for enhancement of the binding activities.[15] In addition, a thiosialoside polymer is expected to show enhancement of the binding activity, since NAs display tetrameric structures on the virion surface.[16] In this paper, we describe syntheses of thiosialoside polymers and preliminary biological evaluations of the activity of the polymers against influenza virus NAs.

The synthetic scheme of a convenient pathway for preparation of carbohydrate monomer **4** is shown in Scheme 1. Commercially available ω-undecylenyl alcohol (10-undecen-1-ol) **1** was selected as a precursor of aglycon because the alkenyl groups have a C=C double bond at an end terminal, which has polymerizable potency with other monomers.[17] In addition, the alcohol has a long aliphatic chain, which can increase the degree of freedom of a carbohydrate moiety bearing from linear backbone after polymerization and has an adequate hydrophobicity as a lipid-like component. Thus, *O*-mesylate **2** having a leaving group was prepared from **1** in quantitative yield. Known thioacetate of sialic acid **3**[18] was treated with mesylate **2** in the presence of potassium carbonate to give α-thioglycoside **4**[†] in 92% yield after re-acetylation, $[\alpha]_D^{30}$ +28.7° (*c* 1.0, $CHCl_3$), $J_{7,8}$ = 8.2 Hz, $\Delta\delta$ |H-9a-H9b| = 0.20 ppm.[19]

Since the preparation of glycomonomer **4** was accomplished, we directed our attention to utilization of the monomer for radical polymerization. We first investigated a radical polymerization of deprotected **4** with an acrylamide in water by a previously reported method.[3] However, a glycopolymer having sialic acid residues was not obtained in the present study. Therefore, a direct copolymerization of glycomonomer **4** having protecting groups with vinyl acetate (VA) was examined. The synthetic scheme of the reaction is shown in Scheme 2. A polymerization of **4** with VA was initially carried out in the presence of AIBN as a radical initiator in MeOH as a solvent, but a polymeric product was unfortunately not obtained. Consequently our synthetic plan had to be changed. Bulk polymerization was one of the suitable procedures for preparation of a polymer. Thus, the copolymerization of **4** with VA as both a comonomer and a solvent was investigated. The results of the radical polymerization, in which the monomer ratio of the polymerization reaction was fixed at 1:10 and the reaction temperature as well as reaction time were changed, are summarized in Table 1. The results of polymerization showed that the reaction temperature of 90 °C and the reaction time of 3 h gave the best result. The effects of different amounts of AIBN as an initiator were then examined, and the results are summarized in Table 2. The yields of the polymerization were gradually increased until around 40% according to addition of AIBN. The weight–average molecular weights ($\overline{Mw}$) of the glycopolymers were around 20 kDa as estimated by the results of size exclusion chromatography (SEC), and SEC experiments also gave information on the degree of polydispersion ($\overline{Mw}/\overline{Mn}$) of the polymers, which was estimated to be around 1.7. ^{1}H NMR spectra

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

Table 1. Results of radical polymerization of **4** with vinyl acetate (VA)

Entry	Monomer ratio sugar:VA	Temp (°C)	Time (h)	Polym. comp.[a]	Sugar content (wt%)	Total yield (%)
1	1:10	60	12	—	—	—[c]
2	1:10	80	6	1:11	41.1	14.7
3	1:10	rt ~ 80	3	N.D.[b]	N.D.[b]	6.1
4	1:10	rt ~ 90	6	N.D.[b]	N.D.[b]	6.1
5	1:10	90	3	1:7	52.3	31.2
6	1:10	90	6	1:6	56.1	17.4
7	1:20	90	6	1:23	25.0	57.1

[a] Polymer compositions of sugar unit:vinyl acetate unit were estimated on the basis of the results of ^{1}H NMR spectra.
[b] N.D. means 'not determined' due to small amount of the polymer.
[c] Polymerization reaction did not proceed.

Table 2. Effects of the initiator (AIBN) for the radical polymerization in 1:10 of sugar/vinyl acetate (monomer ratio) at 90 °C for 3 h

Entry	AIBN (mg)	Total yield (%)	Polym. comp.[a]	Sugar content (wt%)	$\overline{Mw}$ (kDa)[b]	$\overline{Mw}/\overline{Mn}$
1	0.69	6.9	1:9	46.0	17.4	1.6
2	1.10	26.0	1:9	46.0	16.3	1.8
3	1.57	30.4	1:9	46.0	18.3	1.7
4	2.02	40.0	1:9	46.0	24.3	1.9
5	2.47	37.3	1:10	43.4	17.5	1.8
6	3.02	39.0	1:9	46.0	16.4	1.6

[a] Polymer compositions of sugar unit:acrylamide unit were estimated on the basis of the results of ^{1}H NMR spectra.
[b] The weight–average molecular weights were estimated by size-exclusion chromatography in THF using tandem-bonded Tosoh TSKgel SuperHZM-M × 2 columns. Calibration curves were obtained using polystyrene standards (0.5, 1.0, 2.5, 5.87, 9.49, 17.1, 37.2, 98.9, 189, 397, 707, and 1110 kDa; TSK standard, PS-oligomer kit).

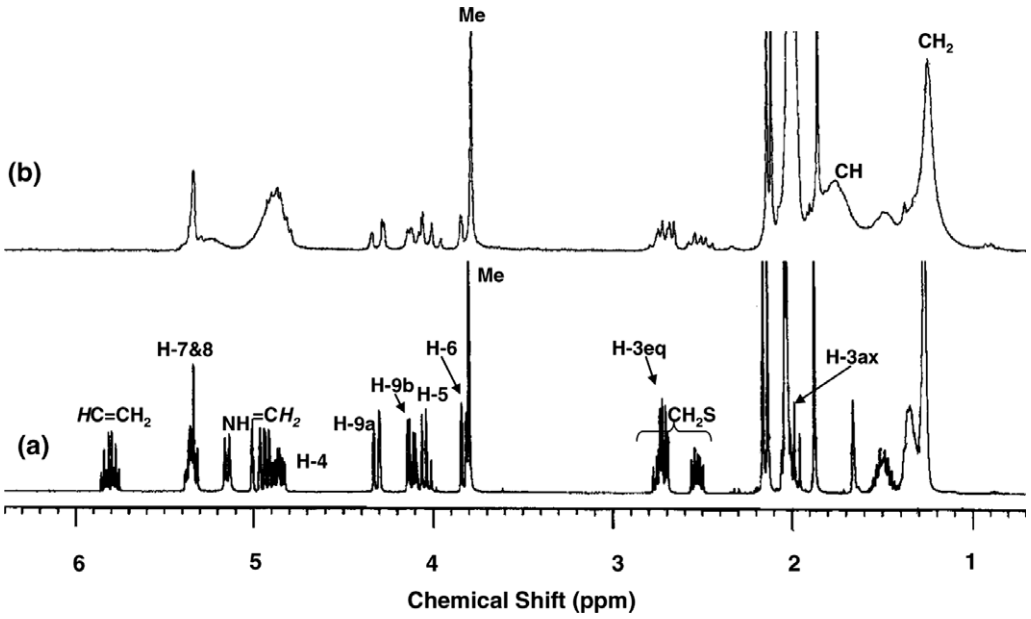


Figure 1. ^{1}H NMR spectra of (a) a glycomonomer **4** and (b) a glycopolymer **5**.

of the glycomonomer **4** and the glycopolymer **5** in $CDCl_3$ are shown in Figure 1. Signals due to proton binding to a C=C double bond of aglycon on **4** appeared at around 6–5 ppm (Fig. 1a), and the signals disappeared after polymerization (Fig. 1b).

Given the success of preparation of glycopolymers having thiosialoside residues by radical polymerization, complete removal of the protection in the polymer was our next objective. Transesterification and subsequent saponification of **5** gave water-soluble glycopolymer **6** after gel filtration to remove low molecular-weight byproducts. Structural elucidation of **6** was performed by a combination of spectroscopic analyses, including NMR and IR. IR spectra of the fully protected glycomonomer **5** and the deprotected glycopolymer **6** are shown in Figure 2. Disappearance of a characteristic infrared absorption band of C=O functional group as stretching vibration at around 1740 cm^{-1} and appearance of a characteristic infrared absorption band of O–H functional group as a stretching vibration at around 3400 cm^{-1} in **6** (Fig. 2b) provided evidence of a completely deprotected structure.

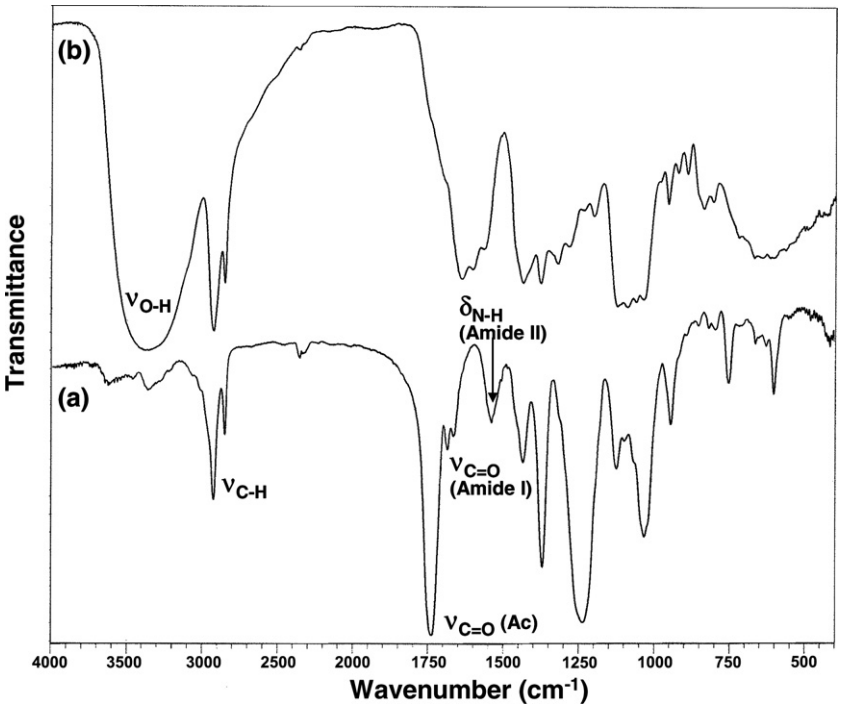


Figure 2. IR spectra of (a) a fully protected glycopolymer **5** and (b) a deprotected glycopolymer **6**.

Table 3. Preliminary results of inhibition assays for influenza virus sialidases

Compound[a]	Influenza virus subtype	
	A/Memphis/1/71 (H3N2)	A/PR/8/34 (H1N1)
6	2.5	10

IC_{50} values are indicated in millimolar concentration based on a monomeric sugar unit concentration.
[a] The structure is shown in Scheme 2.

Preliminary results of inhibitory activity of a glycopolymer **6** (*m*:*n* = 1:7) against influenza virus NAs[20] are summarized in Table 3. Interestingly, the glycopolymer showed inhibitory activity potencies not only against H3N2-type NA but also against H1N1-type NA, while a similar glycopolymer having thiosialoside with a shorter spacer-arm length than that of **6** did not show any activity.[21] The results suggested that the degree of freedom of the sialoside moiety in the glycopolymer is an important factor for effective inhibition of tetrameric NAs expressed on viral particles.

In summary, an efficient synthesis of a new class of glycopolymers **6** having thioglycosidic linkage of sialic acid was accomplished by a convenient radical polymerization protocol. Biological responses of **6** against influenza virus NAs were preliminarily evaluated, and the results showed that **6** has inhibitory activity against viral NAs. Further manipulations of a series of glycopolymers having thiosialoside moieties are now in progress. In addition, we have recently reported glycodendrimers as sialidase inhibitors and the multivalency effect was also observed to enhance weak binding efficiency.[22] The details of the results presented here and the further synthetic procedures as well as biological activities will be reported elsewhere.

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