Bioorganic & Medicinal Chemistry 20 (2012) 446–454

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of sialic acid derivatives containing a long hydrophobic chain at the anomeric position and their C-5 linked polymers as potent influenza virus inhibitors

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ARTICLE INFO

Article history:
Received 29 August 2011
Revised 21 October 2011
Accepted 21 October 2011
Available online 26 October 2011

Keywords:
Sialic acid
Hemagglutinin
Neuraminidase
Glycoclusters
Influenza viruses
Glycopolymers
Inhibitors
Thioglycosides

ABSTRACT

Conversions of the C-5 acetamide group in sialic acid into two kinds of C=C double bond substituents were accomplished under Shotten–Baumann conditions. The polymerizable glycomonomers also contain a hydrophobic chain or hydroxyl group at the anomeric position. Radical polymerizations of the fully protected glycomonomers were carried out with acryl amide in the presence of ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), followed by deprotection to furnish water-soluble glycopolymers. The activities of the deprotected glycopolymers and glycomonomers against human influenza viruses (H1N1 and H3N2) and avian influenza virus (H5N3) were evaluated. Biological evaluations showed that the glycomonomers containing a long hydrophobic chain at the anomeric position had both hemagglutination and neuraminidase inhibitory activities.

1. Introduction

Carbohydrates on the cell surface of eukaryotic organisms have various biological roles including roles in growth and differentiation of cells, cell–cell adhesion, immune response, and oncogenesis.[1] Sialic acids, nine-carbon carbohydrates, are usually located at the terminal position of glycoconjugates. Due to the location of sialic acid residues, sialic acids play major roles in various biological processes.

Influenza infection, one of the biological processes, is caused by sialic acid—binding lectins.[2] Influenza viruses have two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), on the viral surfaces.[3] HA specifically attaches to the sialic acid residue of the terminal of oligosaccharide chains on the host cell surface, and it enables the virus to multiple in the host cell.[4] Although multiplied viruses are also bound to the sialic acid residue on the host cell surface, NA cuts the sialosidic linkage between HAs and glycoconjugates, and it enables viruses to expand to uninfected host cells.[5]

Chemical modifications of sialic acid at the anomeric position and results of biological investigations for potent influenza activities have been reported by many researchers.[6] In addition, a C-4 linked sialic acid polymer was synthesized and investigated by Wu et al., and it was shown to have potent inhibitory activity for influenza HA.[7] However, syntheses and evaluations of C-5-modified sialic acid derivatives have rarely been reported. Matrosovich et al. reported that conversion of the acetamide group at the C-5 position into a thioacetamido group increased binging affinity to influenza viruses.[8] Additionally, Whiteside et al. showed that the hydrophobic chain length at the C-5 position of a sialic acid derivative is related to inhibitory activity and that a derivative having a long hydrophobic chain at the C-5 position caused strong hemagglutination inhibition.[9] Therefore, we planned to develop C-5-modified sialic acid derivatives as novel inhibitors. Since a glycomonomer usually shows weak inhibitory potency for lectins, the glycoside cluster effect is required to enhance the affinity.[10] Thus, the prepared compounds were polymerized at the C-5 position to lead to a glycoside cluster effect.[11] We describe here the synthesis and results of biological evaluations of novel sialic acid derivatives and their glycopolymers.

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doi:10.1016/j.bmc.2011.10.064

2. Results and discussion

Lauryl thioglycoside **1α** was selected as a starting material for construction of C-5-modified sialic acid derivatives because of its availability and convenient handling. Thus, lauryl thioglycoside **1α** was easily prepared from neuraminic acid by using Sinaÿ's protocol[12] and glycosylation with 1-dodecanthiol.[13] Schotten–Baumann reactions were key steps to construct sialic acid derivatives having C═C double bond substituents at the C-5 position.

2.1. Synthesis of C-5-modified sialic acid derivatives

After deacetylation of lauryl thioglycoside **1α** under Zemplén conditions, acetamide was converted into ammonium salt by treatment with methanesulfonic acid in methanol at 60 °C overnight (Scheme 1).[14] Schotten–Baumann reaction of ammonium salt with acryloyl chloride followed by acetylation proceeded smoothly and gave the fully protected derivative **2α** having a C═C double bond at the C-5 position in 54% yield. In order to evaluate the effect of the long hydrophobic aglycon moiety, the thiolauryl substituent was converted into a hydroxyl group. The conversion was accomplished by reaction of thioglycoside **2α** with NIS in H_2O–acetone (1:9) to give hemiketal **3** quantitatively.[15] Quantitative conversions of acetates **2α** and **3** into **4α** and **5** were accomplished in the presence of NaOMe in MeOH followed by treatment with NaOH aq.

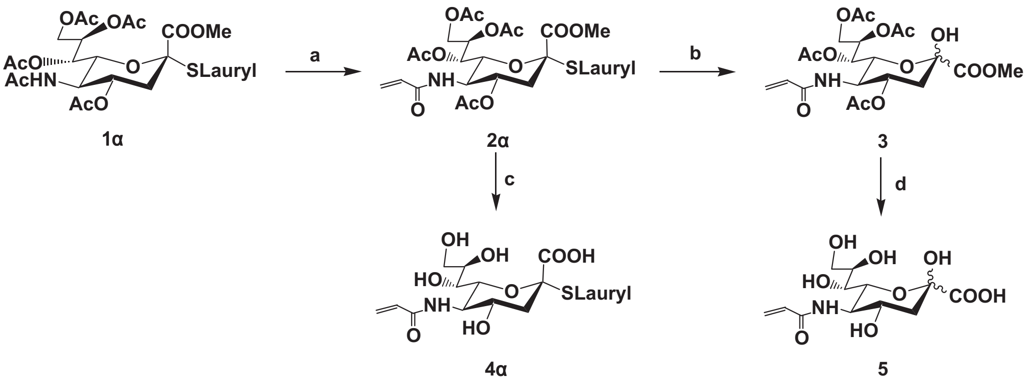
2.2. Synthesis of C-5-modified sialic acid derivatives having a longer aliphatic spacer-arm

We also aimed to construct sialic acid derivatives having a longer aliphatic spacer-arm at the C-5 position. The synthetic plan is summarized in Scheme 2. Deprotected lauryl thioglycoside **6** was treated with methanesulfonic acid in methanol at 60 °C overnight to give the ammonium salt. The ammonium salt was treated with 6-bromohexanoyl chloride in the presence of triethylamine in MeOH. This reaction needed to be carried out at a low temperature because a bromine atom would be replaced by a chlorine atom at room temperature. Therefore, Schotten–Baumann reaction was carried out at −15 °C, and the conversion of β-ammonium salt to alkyl bromide **8β** was completely accomplished. On the other hand, the conversion of α-ammonium salt gave a mixture of alkyl chloride **7α** and alkyl bromide **8α**. Although chloride **7α** was still obtained, the ratio of chloride in the product was decreased. A lower temperature was also examined, but the reaction did not proceed.

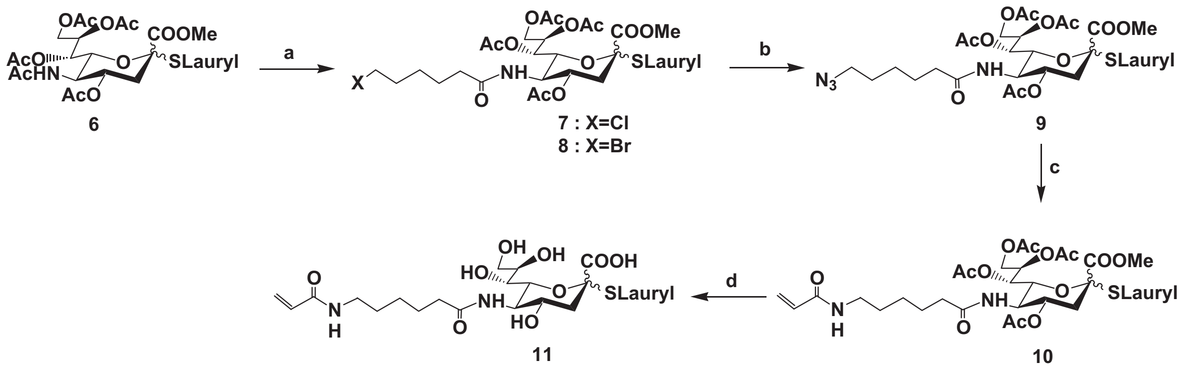
Leaving groups of **7** and **8**, chloride and bromide, were quantitatively displaced by an azido group to afford azide **9**. This reaction proceeded as a usual S_N2 mechanism, and IR spectroscopic analysis showed that the azide derivative had strong absorbance around 2100 cm^{-1} due to stretching vibrations of the cumulated double bond of an N═N═N group. Azide **9** was deprotected under Zemplén conditions and then hydrogenated in the presence of Pd $(OH)_2/C$ under a H_2 atmosphere to yield the corresponding amine, which was further N-acryloylated with acryloyl chloride under Schotten–Baumann conditions followed by reacetylation to give sialic acid derivative **10** having a longer aliphatic spacer-arm. Transesterification followed by treatment with aq NaOH of compound **10** yielded water-soluble compound **11** in 69% yield.

2.3. Polymerization of C-5-modified sialic acid derivatives

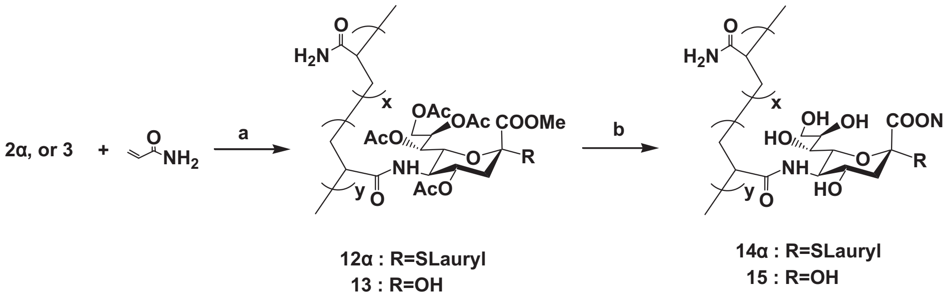
Since we accomplished synthesis of polymerizable sialic acid derivatives, our attention was turned to construction of a series of sialic acid polymers. At first, we planned to use a water-soluble glycomonomer as a candidate for radical polymerization. Thus, protected derivatives having a C═C double bond at the appropriate position were deprotected and then polymerized in water with acrylamide using ammonium peroxodisulfate (APS) as an initiator and *N*,*N*,*N′*,*N′*-tetramethylethylenediamine (TEMED) as a catalyst.[16] However, all attempts using this commonly used procedure gave insoluble gel-state products. Strong hydrogen-bonding formation or crosslinking formation between linear polymers during radical polymerization could take place. Thus, we needed to execute removal of protection after polymerization. Following a new synthetic strategy, acetylated glycomonomers **2α**, **3**, **10α** and **10β** were polymerized with various ratios of acrylamide in DMF in the presence of APS and TEMED to give corresponding glycopolymers **12α**, **13**, **16α** and **16β**, respectively (Schemes 3 and 4). Due to the inherent solubility of glycopolymers depending on the sugar-acrylamide unit ratio, the glycopolymers were purified in two different ways. Ethyl acetate-soluble glycopolymers were purified by gel filtration (Sephadex LH-20) with ethyl acetate as the eluent and deprotected with 1 M MeONa in MeOH, followed by hydrolysis using 0.2 M aq NaOH. On the other hand, ethyl acetate-insoluble glycopolymers were directly treated with 1 M NaOMe in MeOH without chromatographic purification, followed by hydrolysis using 0.2 M aq NaOH. All solutions of the corresponding deprotected polymers were purified by dialysis against distilled water followed by lyophilization to yield white powdery glycopolymer **14α**, **15**, **17α** and **17β**, respectively (Table 1). The



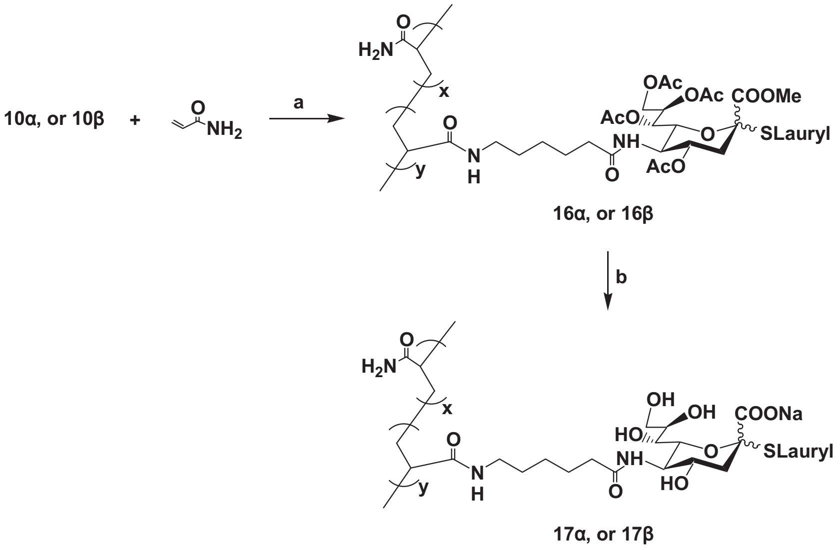
Scheme 1. Reagents and conditions: (a) (i) MeONa, MeOH, rt, overnight; (ii) $MeSO_3H$, MeOH, 60 °C, overnight; (iii) acryloyl chloride, Et_3N, MeOH, 0 °C; (iv) DMAP, Ac_2O, Pyr., rt, overnight (54% in four steps); (b) NIS, H_2O–acetone (1:9), rt, 1 h (quant.); (c) MeONa, MeOH, rt, 1 h, then 0.2 M aq NaOH (quant.); (d) MeONa, MeOH, rt, 1 h, then, 0.05 M aq NaOH (quant.).



Scheme 2. Reagents and conditions: (a) (i) MeONa, MeOH, rt, 1 h; (ii) $MeSO_3H$, MeOH, 60 °C, overnight; (iii) 6-bromohexanoyl chloride, Et_3N, MeOH, −15 °C; (iv) DMAP, Ac_2O, Pyr., rt, overnight (**6α**→**7α**+**8α**; 40% in four steps, **6β**→**7β**+**8β**; 51% in four steps); (b) NaN_3, DMF, 60 °C, 1 h (**7α**+**8α**→**9α**; quant., **7β**+**8β**→**9β**; 97%); (c) (i) MeONa, MeOH, rt, overnight; (ii) H_2, Pd(OH)/C, MeOH, rt, overnight; (iii) acryloyl chloride, Et_3N, MeOH, 0 °C; (iv) DMAP, Ac_2O, Pyr., rt, overnight (**9α**→**10α**; 34% in four steps, **9β**→**10β**; 76% in four steps); (d) MeONa, MeOH, rt, 1 h, then 0.2 M aq NaOH (**10α**→**11α**; 69%).



Scheme 3. Reagents and conditions: (a) TEMED, APS, DMF, 60 °C; (b) 1 M MeONa in MeOH, rt, 3 h, then 0.2 M aq NaOH, rt, 1 day.



Scheme 4. Reagents and conditions: (a) TEMED, APS, DMF, 60 °C; (b) 1 M MeONa in MeOH, rt, 3 h, then 0.2 M aq NaOH, rt, 1 day.

use of dialysis, instead of neutralization, enabled the sodium salts to be left in sialic acid residues, and it induced solubility for water.

2.4. Inhibitory assays against influenza viruses

Given the success of syntheses of the glycomonomers and glycopolymers modified at the C-5 position, our interest was aimed at evaluating the glycocompounds as anti-influenza viral agents. Thus, hemagglutination inhibition (HAI) assays and neuraminidase inhibition (NAI) assays were performed by means of the previously reported method.[17] In order to assess glycomonomers and glycopolymers for the biological assays, three types of influenza viruses were used. Two were human influenza viruses, A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2), and one was an avian influenza virus, A/DK/HK/313/78 (H5N3). HAI assays were initially carried out, and the results are summarized in Tables 2 and 3. As shown in Tables 2 and 3, the glycomonomers and glycopolymers containing long hydrophobic moieties at the anomeric position (**4α**, **11α**, **14α**,

Table 1
Glycopolymers

Compound	Polymer composition[a] [sugar:acryl amide]	Mw[b] (kDa)	Mw/Mn[b]
14α	1:11	49	1.33
	1:4	11	1.38
	1:1	14	1.30
15	1:13	300	1.99
	1:3	23	1.99
17β	1:56	106	1.42
	1:10	50	1.47
	1:4	44	1.17
17α	1:10	100	2.40

[a] Polymer compositions of sugar unit:acrylamide unit were estimated on the basis of the results of ^{1}H NMR.

[b] The weight-average molecular weights were estimated by size-exclusion chromatography in aqueous NaCl solution using tandem-bonded Shodex SB-802.5 and SB-804 columns. Calibration curves were obtained using pullulan standards (5.9, 11.8, 22.8, 47.3, 112, 212, 404, and 788 kDa; Shodex P-82).

Table 2
IC$_{50}$ of hemagglutination inhibition (HAI) activities of glycomonomers (μM)

Compound	Influenza virus subtype		
	A/PR/8/34 (H1N1)	A/Aichi/2/68 (H3N2)	A/DK/HK/313/78 (H5N3)
4α	7.81	3.90	ND[a]
5	>250	>250	>250
11α	7.81	7.81	ND[a]

[a] ND means not detectable.

Table 3
IC$_{50}$ of hemagglutination inhibition (HAI) activities of glycopolymers (μM)

Compound	Polymer composition [sugar:acryl amide]	Influenza virus subtype		
		A/PR/8/34 (H1N1)	A/Aichi/2/68 (H3N2)	A/DK/HK/313/78 (H5N3)
14α	1:11	15.62	15.62	250
	1:4	15.62	15.62	ND[*]
	1:1	31.25	7.81	ND
15	1:13	>250	>250	>250
	1:3	>250	>250	>250
17β	1:56	31.25	31.25	125
	1:10	62.5	62.5	250
	1:4	15.62	15.62	ND[*]
17α	1:10	7.81	7.81	ND[*]

[*] ND means not detectable.

17α, and **17β**) showed potent inhibitory activities. On the other hand, the glycomonomer and glycopolymer containing a hydroxyl group at the anomeric position (**5** and **15**) did not show any inhibitory activities under 250 μM. Glycopolymer **17α** inhibited hemagglutination more strongly than glycopolymer **17β** because influenza viruses recognize α-linked sialic acid residues in nature. We presumed that the steric difference had an effect on the ability of **17β** as an inhibitor. Tables 2 and 3 also showed that the IC$_{50}$ value of glycomonomer **11α** having a hydrophobic chain is nearly equal to the IC$_{50}$ value of glycopolymer **17α**. Surprisingly, the IC$_{50}$ value of glycomonomer **4α** having a hydrophobic chain is even stronger than that of glycopolymer **14α**.

Next, NAI assays were carried out, and the results are summarized in Table 4. Glycomonomers **4α**, **5**, **11α** showed potent inhibitory activities against both human influenza viruses, A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2), and the avian influenza virus, A/DK/HK/313/78 (H5N3). The results suggested that highly hydrophobic glycomonomers play a role as potent inhibitors for NA.

Table 4
EC$_{50}$ of neuraminidase inhibition (NAI) activities of glycomonomers (μM)

Compound	Influenza virus subtype		
	A/PR/8/34 (H1N1)	A/Aichi/2/68 (H3N2)	A/DK/HK/313/78 (H5N3)
4α	1347.06 ± 332.34	428.02 ± 48.24	533.13 ± 26.88
5	2481.00 ± 348.59	1081.10 ± 51.36	608.60 ± 98.14
11α	742.96 ± 331.39	839.70 ± 62.05	101.68 ± 101.68

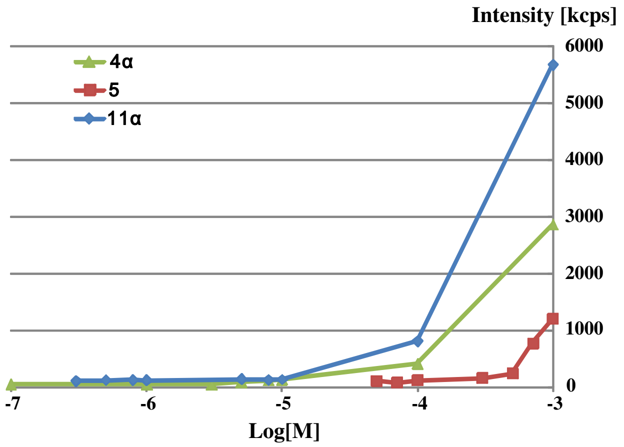


Figure 1. Results of dynamic light scattering (DLS) measurement of glycomonomers.

2.5. Critical micelle concentration determinations using dynamic light scattering

The glycomonomers having hydrophobic moieties at the anomeric position showed potent inhibitory activities as strong as those of their glycopolymers. A monomeric glycosubstrate for biological investigation generally shows weak binding affinities, and clustering sugar moieties by covalent bonds is required to enhance affinities. Since monomeric glycomonomers had activity similar to that of the glycopolymers in this case, we speculated that the glycomonomers function as polymeric compounds. To examine the effect of glycomonomers, critical micelle concentrations were therefore determined by means of dynamic light scattering (DLS). The results, summarized in Figure 1, indicate that glycocluster effect is caused by micelle formation of hydrophobic-type glycomonomers. This phenomenon is strongly related to the results of biological activities, and clustering the monomeric glycomonomers by hydrophobic interaction increases binding affinities between glycomonomers and influenza viruses.

3. Conclusion

In summary, we have reported the synthesis of sialic acid derivatives modified at the C-5 position and their polymers. At the polymerization step, the fully protected glycomonomers were directly polymerized in order to avoid aggregation of the products. After deprotection, biological evaluations of the activities of glycopolymers **14α**, **15**, **17α**, **17β** and glycomonomers **4α**, **5**, **11α** against HAs and NAs of human influenza viruses (H1N1, H3N2) and avian influenza virus (H5N3) were examined. Glycomonomers (**4α**, **11α**) and glycopolymers (**14α**, **17α**, and **17β**) showed inhibitory activities against HAs. Furthermore, glycomonomers **4α**, **11α** containing long hydrophobic chains at the anomeric position showed potent HAI and NAI activities.

4. Experimental section

4.1. Materials and methods

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Pyridine (Pyr), dimethylformamide (DMF), and 1,4-dioxane (THF) were stored over molecular sieves (MS4 Å), and methanol (MeOH) was stored over MS3 Å before use. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. The IR spectra were obtained using a JASCO FT/IR-300E spectrophotometer. The ^{1}H NMR spectra were recorded at 400 MHz with a Bruker AM-400 spectrometer, a Bruker DPX-400 spectrometer or at 200 MHz with a Varian Gemini-2000 spectrometer in chloroform-*d*, deuterium oxide. The ^{13}C NMR spectra were recorded at 50.3 or 100.6 MHz using the same instruments. Tetramethylsilane (TMS) and $CHCl_3$ (7.26 ppm for ^{1}H or 77.0 ppm for ^{13}C) were used as internal standards. Ring-proton assignments in NMR were made by first-order analysis of the spectra and were supported by the results of homonuclear decoupling experiments. Elemental analyses were performed with a Fisons EA1108 on samples extensively dried at 50–60 °C over phosphorus pentoxide for 4–5 h. Fast atom bombardment mass spectra (FAB MS) were recorded with a JEOL JMS-HX110 spectrometer. Matrix-assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF-MS) were obtained by using a Buruker Autoflex spectrometer. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of Silica Gel 60 F_{254} (layer thickness, 0.25 mm; E. Merck, Darmmstadt, Germany). For detection of the intermediates, TLC sheets were dipped in (a) a solution of 85:10:5 (v/v/v) MeOH–*p*-anisaldehyde–concentrated sulfuric acid and heated for a few minutes (for carbohydrate), (b) an aq solution of 5 wt % potassium permanganate and heated similarly (for C=C double bond), or (c) an ethanolic solution of 7% phosphomolybdic acid and heated similarly (for organic compound). Column chromatography was performed on silica gel (Silica Gel 60; 63–200 μm, E. Merck). Flash column chromatography was performed on silica gel (Silica Gel 60, spherical neutral; 40–100 μm, E. Merck). All extractions were concentrated below 45 °C under diminished pressure.

4.1.1. Methyl (dodecyl-*N*-acrylamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-galacto-2-nonulopyranosid)onate (2α)

To a solution of acetate **1α** (1.36 g, 2.01 mmol) in dry MeOH (14 mL), sodium methoxide (NaOMe) (43.0 mg, 0.80 mmol) was added. The solution was stirred for 1 h at room temperature under an argon atmosphere. After neutralization with amberlite IR-120B (H^+ form) and filtration, the solvent was evaporated under diminished pressure. The residue was dissolved in dry MeOH (10 mL). Methanesulfonic acid (260 μL, 4.00 mmol) was added to the solution. The reaction mixture was heated under reflux overnight under an argon atmosphere. After cooling to room temperature, the mixture was neutralized with amberlite IRA-93ZU (OH^- form) and filtered. The solvent was evaporated under diminished pressure. The residue was dissolved in dry MeOH (5 mL), and the solution was cooled to 0 °C. Under an argon atmosphere, triethylamine (3.92 mL, 28.2 mmol) was added to the stirred solution, and then acryloyl chloride (1.14 mL, 14.1 mmol) was dropwise added to the stirred solution. After completion of the reaction judged by TLC, the solvent was removed in vacuo. The residue was dissolved in Pyr (9 mL). Dimethylaminopyridine (DMAP) (86.0 mg, 0.70 mmol) and acetic anhydride (Ac_2O) (3.00 mL, 35.2 mmol) were added to the solution at 0 °C, and the reaction mixture was stirred overnight. The solution was concentrated in vacuo and co-evaporated with toluene. The residue was diluted by $CHCl_3$, transferred into a separating funnel, and washed successively with distilled water, 1 M aq HCl, and then satd aq $NaHCO_3$. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and concentrated. Purification by column chromatography on silica gel (hexane/EtOAc 5:6) yielded compound **2α** (742 mg, 54%): R_f 0.74 (EtOAc); $[\alpha]_D^{31}$ −25.4° (*c* 1.27, $CHCl_3$); IR (KBr) 3246 ($\nu_{\mathrm{N-H}}$), 2956 ($\nu_{\mathrm{C-H}}$, alkane), 1744 ($\nu_{\mathrm{C=O}}$), 1660 ($\nu_{\mathrm{C=O}}$; amide I), 1547 ($\delta_{\mathrm{N-H}}$; amide II) cm^{-1}; ^{1}H NMR δ (400 MHz, $CDCl_3$) 6.18 (d, 1H, J_{trans} = 17.1 Hz, –$\mathrm{CH{=}C}H_2$), 5.98 (dd, 1H, J_{Cis} = 10.4 Hz, –$\mathrm{C}H\mathrm{{=}CH_2}$), 5.64 (d, 1H, –$\mathrm{CH{=}C}H_2$), 5.44 (d, 1H, $J_{\mathrm{NH,5}}$ = 10.0 Hz, N*H*), 5.37 (m, 1H, H-8), 5.31 (dd, 1H, $J_{7,8}$ = 6.9 Hz, $J_{7,6}$ = 2.1 Hz, H-7), 4.95 (ddd, 1H, $J_{4,3e}$ = 4.3 Hz, $J_{4,3a}$ = 11.0 Hz, $J_{4,5}$ = 11.0 Hz, H-4), 4.34 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, $J_{9a,8}$ = 2.6 Hz, H-9a), 4.12 (m, 2H, H-5,H-9b), 3.91 (dd, 1H, $J_{6,5}$ = 10.7 Hz, $J_{6,7}$ = 2.0 Hz, H-6), 3.81 (s, 3H, $\mathrm{COOC}H_3$), 2.75 (tt, 1H, H-3e), 2.74 (m, 1H, $\mathrm{SC}H_2\mathrm{CH_2}$), 2.54 (m, 1H, $\mathrm{SC}H_2\mathrm{CH_2}$), 2.12 (s, 6H, 2Ac), 2.03, 1.99 (2s, 6H, 2Ac), 1.99 (m, 1H, H-3a), 1.49 (m, 2H, $\mathrm{SCH_2C}H_2$), 1.25 (s, 18H, $9\mathrm{C}H_2$), 0.87 (t, 3H, $\mathrm{CH_2C}H_3$); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 170.96, 170.58, 170.10, 170.04, 168.49, 165.54, 130.26, 127.11, 83.17, 74.16, 69.55, 68.96, 67.56, 62.19, 52.88, 49.52, 38.08, 31.87, 29.62, 29.58, 29.55, 29.45, 29.30, 29.18, 28.89, 28.78, 22.64, 21.12, 20.82, 20.75, 20.72, 14.07; FAB MS Calcd for $[M+H^+]$: 688.8. Found: *m/z* 688.5.

Anal. Calcd for $C_{33}H_{53}O_{12}NS$: C, 57.62; H, 7.77; N, 2.04. Found: C, 57.53; H, 7.81; N, 1.96.

4.1.2. Methyl-*N*-acrylamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-galacto-2-nonulosaminate (3α)

To a solution of compound **2α** (3.00 g, 4.36 mmol) in distilled water and acetone (1:9, v/v, 15 mL), *N*-iodosuccinimide (NIS) (1.96 g, 8.72 mmol) was added and stirred for 1 h at room temperature. The reaction mixture was washed with distilled water, satd aq $NaHCO_3$, 10% aq $Na_2S_2O_3$, and then with brine. The organic layer was dried over anhyd $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (toluene/EtOAc, 1:2, v/v) to provide compound **3** in quantitative yield: R_f 0.53 (EtOAc); $[\alpha]_D^{30}$ −2.14° (*c* 1.13, $CHCl_3$); IR (KBr) 3399 ($\nu_{\mathrm{O-H}}$), 3252 ($\nu_{\mathrm{N-H}}$), 2974 ($\nu_{\mathrm{C-H}}$), 1736, 1717 ($\nu_{\mathrm{C=O}}$), 1653 ($\nu_{\mathrm{C=O}}$, amide I), 1628 ($\nu_{\mathrm{C=C}}$), 1551 ($\delta_{\mathrm{N-H}}$, amide II); ^{1}H NMR δ (400 MHz, $CDCl_3$) 6.40 (d, 1H, $J_{\mathrm{NH,5}}$ = 9.18 Hz, N*H*), 6.20 (d, 1H, J_{trans} = 17.0 Hz, –$\mathrm{CH{=}C}H_2$), 6.04 (dd, 1H, –$\mathrm{C}H\mathrm{{=}CH_2}$), 5.63 (d, 1H, J_{Cis} = 10.3 Hz, –$\mathrm{CH{=}C}H_2$), 5.38 (t, 1H, H-7), 5.26 (m, 2H, H-4, H-8), 4.64 (dd, 1H, $J_{9a,9b}$ = 12.25 Hz, $J_{9a,8}$ = 2.10 Hz, H-9a), 4.26 (m, 2H, H-6, H-5), 4.04 (m, 1H, H-9b), 3.85 (s, 3H, $\mathrm{COOC}H_3$), 2.23 (m, 2H, H-3e, H-3a), 2.16, 2.10, 2.02, 1.97 (s, 12H, 4Ac); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 171.60, 171.17, 170.80, 170.06, 168.96, 165.69, 130.49, 126.66, 94.91, 72.16, 71.47, 69.06, 68.51, 62.57, 53.25, 49.17, 36.15, 20.99, 20.75, 20.70; FAB MS Calcd for $[M+H^+]$: 504.5. Found: *m/z* 504.3.

Anal. Calcd for $C_{21}H_{29}O_{13}N$: C, 50.10; H, 5.81; N, 2.78. Found: C, 50.20; H, 5.78; N, 2.94.

4.1.3. Dodecyl-*N*-acrylamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-galacto-2-nonulopyranosaminic acid (4α)

To a solution of compound **2α** (60.0 mg, 87.2 μmol) in dry MeOH (0.6 mL) was added NaOMe (2.00 mg, 37.0 μmol). The solution was stirred for 1 h at room temperature under an argon atmosphere. Then, 0.2 M aq NaOH (4 mL) was added to the solution. The solution was stirred for 3 days at room temperature. After neutralization with amberlite IR-120B (H^+ form) followed by filtration, the filtrate was lyophilized to furnish compound **4α** in quantitative yield: R_f 0.60 [65:25:4 (v/v/v) $CHCl_3$–MeOH–H_2O]; IR (KBr) 3333 ($\nu_{\mathrm{O-H}}$), 2924 ($\nu_{\mathrm{C-H}}$), 1716 ($\nu_{\mathrm{C=O}}$), 1655 ($\nu_{\mathrm{C=O}}$; amide I), 1626 ($\nu_{\mathrm{C=C}}$), 1557 ($\delta_{\mathrm{N-H}}$; amide II) cm^{-1}; ^{1}H NMR δ (400 MHz, D_2O) 6.24 (br m, 2H, –$\mathrm{CH = C}H_2$, –$\mathrm{C}H\mathrm{ = CH_2}$), 5.79 (br s, 1H, –$\mathrm{CH = C}H_2$), 4.18 (br m, 4H, H-4, H-5, H-8, H-9a), 4.06 (br m, 2H, H-6, H-7), 3.98 (br m, 1H, H-9b), 3.04 (br s, 1H, H-3e), 3.04 (br s, 1H, SCH_2CH_2), 2.91 (br s, 1H, SCH_2CH_2), 2.21 (br s, 1H, H-3a), 1.94 (br s, 2H, SCH_2CH_2), 1.61 (br s, 18H, 9 CH_2), 1.18 (br s, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, D_2O) 174.84, 169.32, 130.04, 127.88, 75.03, 71.47, 68.24, 62.68, 58.08, 51.97, 40.96, 36.16, 31.87, 29.77, 29.64, 29.44, 29.35, 29.30, 28.99, 22.51, 13.76; FAB MS Calcd for [$M+Na^+$]: 550.2. Found: *m/z* 550.4.

4.1.4. *N*-Acrylamido-3,5-dideoxy-D-*glycero*-D-galacto-2-nonulosaminic acid (5)

To a solution of compound **3** (52.0 mg, 103 μmol) in dry MeOH (0.5 mL) was added NaOMe (4.00 mg, 74.0 μmol) and the solution was stirred for 1 h at room temperature under an argon atmosphere. Then 0.05 M aq NaOH (3 mL) was added to the solution and the solution was further stirred overnight at room temperature. After neutralization with amberlite IR-120B (H^+ form), the solvent was lyophilized to furnish compound **5** in quantitative yield: R_f 0.59, 0.50 [3:3:1 (v/v/v) $CHCl_3$–MeOH–H_2O]; IR (KBr) 3383 (ν_{O-H}), 2924 (ν_{C-H}), 1771, 1732 ($\nu_{C=O}$), 1657 ($\nu_{C=O;}$ amide I), 1626 ($\nu_{C=C}$), 1545 (δ_{N-H}; amide II) cm^{-1}; ^1H NMR δ (400 MHz, D_2O) 6.23 (m, 2H, $-CH=CH_2$, $-CH=CH_2$), 5.79 (m, 1H, $-CH=CH_2$), 4.39 (dd, 1H, $J_{6,5}$=8.0 Hz, H-6), 4.12 (m, 1H, H-4), 4.04 (m, 1H, H-5), 3.84 (dd, 1H, H-7), 3.76 (dd, 1H, H-9a), 3.62 (ddd, 1H, H-8), 3.54 (dd, 1H, H-9b), 2.32 (dd, 1H, H-3e), 1.91 (dd, 1H, $J_{3a,4}$=12.4 Hz, H-3a); ^{13}C NMR δ (100.6 MHz, D_2O) 169.69, 168.16, 129.62, 128.34, 80.35, 80.00, 73.51, 73.42, 73.24, 69.37, 66.88, 63.35; FAB MS Calcd for [M^+]: 321.1. Found: *m/z* 321.4.

4.1.5. Methyl (dodecyl-*N*-6-bromohexylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-galacto-2-nonulopyranosid) onate (8α)

To a solution of compound **6** (5.13 g, 7.59 mmol) in dry MeOH (51 mL) was added NaOMe (164 mg, 3.03 mmol) and the solution was stirred for 1 h at room temperature under an argon atmosphere. After neutralization with amberlite IR-120B (H^+ form) and filtration, the solvent was evaporated under diminished pressure. The residue was dissolved in dry MeOH (30 mL), and the solution was cooled to −15 °C. Triethylamine (2.10 mL, 15.1 mmol) was added to the stirred solution under an argon atmosphere, and then 6-bromohexanoyl chloride (1.20 mL, 7.82 mmol) in diethyl ether (4.8 mL) was dropwise added to the stirred solution. After completion of the reaction, the solvent was removed in vacuo. The residue was dissolved in Pyr (49 mL). Ac_2O (14.3 mL, 151.6 mmol) was added to the solution at 0 °C, and the reaction mixture was stirred overnight. The solution was concentrated in vacuo and co-evaporated with toluene. The residue was diluted by $CHCl_3$, transferred into a separating funnel, and washed successively with ice-water, 1 M aq H_2SO_4, satd aq $NaHCO_3$, and brine, and then dried over anhyd $MgSO_4$, filtered, and concentrated. Purification of the residual syrup by column chromatography on silica gel (hexane/EtOAc 1:1 v/v) yielded a mixture of chloride **7α** and bromide **8α** (2.48 g, 40%): R_f 0.74 [1:8 (v/v) MeOH–$CHCl_3$]; IR (KBr) 3368 (ν_{N-H}), 2953 (ν_{C-H}), 1748 ($\nu_{C=O}$), 1651 ($\nu_{C=O}$, amide I), 1557 (δ_{N-H}, amide II) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 5.35 (m, 1H, *H-8*), 5.31 (dd, 1H, $J_{7,6}$ = 2.00 Hz, $J_{7,8}$ = 8.09 Hz, H-7), 5.17 (d, 1H, $J_{NH,5}$ = 10.01 Hz, N*H*), 4.87 (ddd, 1H, $J_{4,3a}$ = $J_{4,5}$ = 10.07 Hz, $J_{4,3e}$ = 4.76 Hz, H-4), 4.31 (dd, 1H, $J_{9a,9b}$ = 12.41 Hz, $J_{9a,8}$ = 2.50 Hz, H-9a), 4.12 (dd, 1H, H-9b), 4.09 (m, 1H, H-5), 3.84 (dd, 1H, $J_{6,7}$ = 2.02 Hz, $J_{6,5}$ = 10.85 Hz, H-6), 3.81 (s, 3H, $COOCH_3$), 3.53 (t, 0.8H, $-CH_2Cl$), 3.40 (t, 1.2H, $-CH_2Br$), 2.73 (dd, 1H, H-3e), 2.73 (m, 1H, SCH_2CH_2), 2.53 (m, 1H, SCH_2CH_2), 2.16, 2.14, 2.05, 2.02 (4s, 12H, 4Ac), 2.10 (m, 1H, H-3a), 1.98 (m, 2H, $-NHCOCH_2-$), 1.86 (m, 2H, $-CH_2CH_2Br$), 1.56 (m, 2H, $-NHCOCH_2CH_2(CH_2)_3Br$), 1.49 (m, 2H, SCH_2CH_2), 1.43 (m, 2H, $-NHCO(CH_2)_2CH_2(CH_2)_2Br$), 1.25 (m, 18H, $9CH_2$), 0.88 (t, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 172.73, 170.92, 170.64, 170.12, 170.09, 168.53, 83.18, 74.16, 69.64, 68.87, 67.39, 62.16, 52.93, 49.27, 38.13, 36.38, 33.55, 32.38, 31.92, 29.67, 29.64, 29.60, 29.49, 29.36, 29.24, 29.21, 28.93, 28.82, 27.66, 26.39, 24.33, 22.69, 21.18, 21.07, 20.94, 20.88, 20.80, 14.20; FAB MS Calcd for [$M+H^+$]: 811.8. Found: *m/z* 810.2.

4.1.6. Methyl (dodecyl-*N*-6-bromohexylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-β-D-*glycero*-D-galacto-2-nonulopyranosid) onate (8β)

To a solution of compound **6** (4.15 g, 6.14 mmol) in dry MeOH (41 mL) was added NaOMe (138 mg, 2.55 mmol) and the solution was stirred for 1 h at room temperature under an argon atmosphere. After neutralization with amberlite IR-120B (H^+ form) and filtration, the solvent was evaporated under diminished pressure. The residue was dissolved in dry MeOH (30 mL), and the solution was cooled to −15 °C. Triethylamine (1.95 mL, 14.0 mmol) was added to the stirred solution under an argon atmosphere, and then 6-bromohexanoyl chloride (1.41 mL, 9.21 mmol) in diethyl ether (4.0 mL) was dropwise added to the stirred solution. After completion of the reaction, the solvent was removed in vacuo. The residue was dissolved in Pyr (39 mL). Ac_2O (12.0 mL, 127 mmol) was added to the solution at 0 °C, and the reaction mixture was stirred overnight. The solution was concentrated in vacuo and co-evaporated with toluene. The residue was diluted by $CHCl_3$, transferred into a separating funnel, and washed successively with ice-water, 1 M aq H_2SO_4, satd aq $NaHCO_3$, and brine, and then dried over anhyd $MgSO_4$, filtered, and concentrated. Purification of the residual syrup by column chromatography on silica gel (hexane/EtOAc 1:1 v/v) yielded a mixture of chloride **7β** and bromide **8β** (2.51 g, 51%): R_f 0.73 [1:2 (v/v) *n*-hexane–EtOAc]; $[\alpha]_D^{32}$ −41.3° (*c* 0.67, $CHCl_3$); IR (KBr) 3370 (ν_{N-H}), 2926 (ν_{C-H}), 1748 ($\nu_{C=O}$), 1659 ($\nu_{C=O}$, amide I), 1537 (δ_{N-H}, amide II) cm^{-1}; ^1H NMR δ (400 MHz, $CDCl_3$) 5.41 (t, 1H, $J_{7,8}$ = 2.33 Hz, H-7), 5.39 (d, 1H, $J_{NH,5}$ = 10.22 Hz, N*H*), 5.28 (ddd, 1H, $J_{4,3a}$ = $J_{4,5}$ = 10.96 Hz, $J_{4,3e}$ = 4.78 Hz, H-4), 5.09 (m, 1H, *H-8*), 4.82 (dd, 1H, $J_{9a,9b}$ = 12.36 Hz, $J_{9a,8}$ = 2.04 Hz, H-9a), 4.34 (dd, 1H, $J_{6,7}$ = 2.06 Hz, $J_{6,5}$ = 10.46 Hz, H-6), 4.18 (dd, 1H, $J_{9b,9a}$ = 12.37 Hz, $J_{9b,8}$ = 8.29 Hz, H-9b), 4.09 (q, 1H, $J_{5,NH}$ = $J_{5,4}$ = $J_{5,6}$ = 10.32 Hz, H-5), 3.81 (s, 3H, $COOCH_3$), 3.55 (t, 0.6H, $-CH_2Cl$)3.41 (t, 1.4H, $-CH_2Br$), 2.52 (dd, 1H, H-3e), 2.51 (m, 2H, SCH_2CH_2), 2.10 (m, 3H, H-3a, $-NHCOCH_2-$), 2.13, 2.07, 2.04, 2.01 (4s, 12H, 4Ac), 1.85 (m, 2H, $-CH_2CH_2Br$), 1.55 (m, 4H, $-NHCOCH_2CH_2(CH_2)_3Br$, SCH_2CH_2), 1.41 (m, 2H, $-NHCO(CH_2)_2CH_2(CH_2)_2Br$), 1.25 (m, 18H, $9CH_2$), 0.88 (t, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 172.71, 170.85, 170.82, 170.39, 170.07, 168.41, 84.78, 72.99, 72.31, 69.24, 68.56, 62.55, 52.81, 49.35, 37.24, 36.29, 33.44, 32.40, 31.86, 29.61, 29.53, 29.46, 29.30, 29.14, 29.04, 28.44, 27.65, 24.34, 22.63, 20.99, 20.88, 20.72, 14.07; FAB MS Calcd for [$M+H^+$]: 811.8. Found: *m/z* 810.2.

Anal. Calcd for $C_{36}H_{60}Br_1N_1O_{12}S_1$: C, 53.33; H, 7.46; N, 1.73. Found: C, 53.35; H, 7.50; N, 1.76.

4.1.7. Methyl (dodecyl-*N*-6-azidehexylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-galacto-2-nonulopyranosid) onate (9α)

A mixture of compound **7α** and compound **8α** (1.08 g, 1.33 mmol) was dissolved in DMF (24 mL). Sodium azide (597 mg, 9.18 mmol) was added to the solution under an argon atmosphere, and the solution was stirred for 1 h at 60 °C. After cooling, the reaction mixture was diluted by $CHCl_3$, transferred into a separating funnel, and washed with distilled water and brine. The organic layer was dried over anhyd $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on silica gel (hexane/acetic ether 1:1 v/v) afforded compound **9α** (2.37 g, quant.): R_f 0.74 [1:8 (v/v) MeOH–$CHCl_3$]; IR (neat) 3344 (ν_{N-H}), 2926

(ν_{C-H}), 2097 (ν_{N3}), 1742 ($\nu_{C=O}$), 1659 ($\nu_{C=O}$, amide I), 1531 (δ_{N-H}, amide II) cm^{-1}; ^{1}H NMR δ (400 MHz, $CDCl_3$) 5.36 (m, 1H, *H-8*), 5.30 (dd, 1H, $J_{7,6}$ = 2.02 Hz, $J_{7,8}$ = 6.24 Hz, H-7), 5.17 (d, 1H, $J_{NH,5}$ = 10.01 Hz, N*H*), 4.87 (ddd, 1H, $J_{4,3a}$ = $J_{4,5}$ = 11.07 Hz, $J_{4,3e}$ = 4.29 Hz, H-4), 4.33 (dd, 1H, $J_{9a,9b}$ = 12.44 Hz, $J_{9a,8}$ = 2.59 Hz, H-9a), 4.13 (dd, 1H, $J_{8,9b}$ = 5.22 Hz, H-9b), 4.09 (m, 1H, $J_{5,NH}$ = $J_{5,4}$ = $J_{5,6}$ = 10.36 Hz, H-5), 3.85 (dd, 1H, $J_{6,5}$ = 13.79 Hz, $J_{6,7}$ = 2.02 Hz, H-6), 3.82 (s, 3H, COOCH_3), 3.28 (t, 2H, –CH_2N_3), 2.75 (m, 1H, SCH_2CH_2), 2.73 (dd, 1H, H-3e), 2.54 (m, 1H, SCH_2), 2.11 (t, 2H, –NHCOCH_2–), 1.99 (dd, 1H, H-3a), 2.17, 2.14, 2.05, 2.03 (4s, 12H, 4Ac), 1.59 (m, 4H, –NHCOCH_2CH_2CH_2CH_2CH_2N_3), 1.52 (m, 2H, SCH_2CH_2), 1.35 (m, 2H, –NHCO(CH_2)$_2$CH_2(CH_2)$_2N_3$), 1.26 (m, 18H, 9CH_2), 0.89 (t, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 172.68, 170.85, 170.57, 170.07, 170.03, 168.50, 83.16, 74.15, 69.62, 68.87, 67.39, 62.15, 52.88, 51.17, 49.26, 38.11, 36.37, 31.88, 29.63, 29.60, 29.56, 29.45, 29.31, 29.19, 28.90, 28.78, 28.48, 26.21, 24.67, 22.65, 21.12, 20.86, 20.83, 20.73, 14.08; FAB MS Calcd for [M+Na$^+$]: 795.4. Found: *m/z* 795.4.

4.1.8. Methyl (dodecyl-*N*-6-azidehexylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-β-D-*glycero*-D-galacto-2-nonulopyranosid) onate (9β)

A mixture of compound **7β** and compound **8β** (1.08 g, 1.33 mmol) was dissolved in DMF (11 mL). Sodium azide (253 mg, 3.89 mmol) was added to the solution under an argon atmosphere, and the solution was stirred for 1 h at 60 °C. After cooling, the reaction mixture was diluted by $CHCl_3$, transferred into a separating funnel, and washed with distilled water and brine. The organic layer was dried over anhyd $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on silica gel (hexane/acetic ether 1:1 v/v) afforded compound **9β** (972 mg, 97%): R_f 0.73 [1:2 (v/v) *n*-hexane–EtOAc]; IR (neat) 3374 (ν_{N-H}), 2926 (ν_{C-H}), 2097 (ν_{N3}), 1732 ($\nu_{C=O}$), 1651 ($\nu_{C=O}$, amide I), 1531 (δ_{N-H}, amide II) cm^{-1}; ^{1}H NMR δ (400 MHz, $CDCl_3$) 5.44 (d, 1H, $J_{NH,5}$ = 10.29 Hz, N*H*), 5.42 (t, 1H, $J_{7,8}$ = $J_{7,6}$ = 2.43 Hz, H-7), 5.28 (ddd, 1H, $J_{4,3a}$ = $J_{4,5}$ = 10.99 Hz, $J_{4,3e}$ = 4.61 Hz, H-4), 5.09 (m, 1H, *H-8*), 4.82 (dd, 1H, $J_{9a,9b}$ = 12.35 Hz, $J_{9a,8}$ = 2.14 Hz, H-9a), 4.34 (dd, 1H, $J_{6,7}$ = 2.17 Hz, $J_{6,5}$ = 10.50 Hz, H-6), 4.18 (dd, 1H, $J_{9b,9a}$ = 12.34 Hz, $J_{9b,8}$ = 8.33 Hz, H-9b), 4.09 (q, 1H, $J_{5,NH}$ = $J_{5,4}$ = $J_{5,6}$ = 10.32 Hz, H-5), 3.81 (s, 3H, COOCH_3), 3.41 (t, 2H, –CH_2N_3), 2.60–2.44 (m, 3H, H-3e, SCH_2CH_2), 2.20–2.00 (m, 3H, H-3a, –NHCOCH_2(CH_2)$_4N_3$), 2.13, 2.07, 2.04, 2.01 (4s, 12H, 4Ac), 1.63–1.31 (m, 8H, –NHCOCH_2(CH_2)$_3CH_2N_3$, SCH_2CH_2), 1.25 (m, 18H, 9CH_2), 0.88 (t, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 172.78, 170.91, 170.87, 170.46, 170.14, 168.46, 84.81, 73.03, 72.35, 69.26, 68.58, 62.60, 52.88, 51.20, 49.40, 37.27, 36.38, 31.92, 29.66, 29.64, 29.59, 29.51, 29.34, 29.20, 29.09, 28.54, 28.49, 26.24, 24.75, 22.69, 21.07, 21.04, 20.90, 20.78, 14.13; FAB MS Calcd for [M+Na$^+$]: 795.4. Found: *m/z* 795.4.

4.1.9. Methyl (dodecyl-*N*-6-acryloylaminohexylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-galacto-2-nonulopyranosid) onate (10α)

Compound **9α** (1.71 g, 2.21 mmol) was dissolved in MeOH (17 mL). NaOMe (48.0 mg, 889 μmol) was added, and the mixture was stirred overnight at room temperature. After neutralization with Amberlite IR-120B (H$^+$ form) followed by filtration, the solvent was evaporated under diminished pressure. The residue was dissolved in dry MeOH (13 mL). Pd$(OH)_2$ on charcoal (235 mg, 2.21 mmol) was added to the solution and stirred vigorously overnight at room temperature under a hydrogen atmosphere. The reaction was monitored by TLC. After completion of the reaction, the suspension was filtered through activated carbon and evaporated. The residue was dissolved in MeOH (8 mL). Triethylamine (1.23 mL, 8.84 mmol) was added to the solution under an argon atmosphere followed by dropwise addition of acryloyl chloride (359 μL, 4.42 mmol) in 1,4-dioxane (1.4 mL). Upon completion of the reaction on TLC, the reaction mixture was filtered and concentrated. The residue was dissolved in Pyr (17 mL). Ac_2O (4.20 mL, 44.5 mmol) was added dropwise to the solution at 0 °C. After stirring overnight at room temperature, the mixture was evaporated in vacuo. The residue was diluted by $CHCl_3$, transferred into a separating funnel, and washed successively with ice-water, 1 M aq H_2SO_4, satd aq $NaHCO_3$, and brine, and then dried over anhyd $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography on silica gel (acetic ether) yielded compound **10α** (610 mg, 34%): R_f 0.43 [8:1 (v/v) $CHCl_3$–MeOH]; $[\alpha]_D^{32}$ +20.4° (*c* 1.27, $CHCl_3$); IR (KBr) 3258 (ν_{N-H}), 2926 (ν_{C-H}), 1744 ($\nu_{C=O}$), 1650 ($\nu_{C=O}$, amide I), 1624 ($\nu_{C=C}$), 1560 (δ_{N-H}, amide II) cm^{-1};^{1}H NMR δ (400 MHz, $CDCl_3$) 6.30 (t, 1H, –N*H*COCH=CH_2), 6.29 (dd, 1H, J_{trans} = 17.02 Hz, J_{gem} = 1.40 Hz, –CH=CH_2), 6.12 (dd, 1H, J_{Cis} = 10.17 Hz, –C*H*=CH_2), 5.63 (dd, 1H, –CH=CH_2), 5.55 (d, 1H, $J_{NH,5}$ = 8.73 Hz, N*H*), 5.34 (m, 1H, H-8), 5.29 (dd, 1H, $J_{7,8}$ = 7.82 Hz, $J_{7,6}$ = 1.82 Hz, H-7), 4.88 (ddd, 1H, $J_{4,3e}$ = 4.50 Hz, $J_{4,3a}$ = $J_{4,5}$ = 11.00 Hz, H-4), 4.34 (dd, 1H, $J_{9a,9b}$ = 12.39 Hz, $J_{9a,8}$ = 2.52 Hz, H-9a), 4.11 (dd, 1H, $J_{9b,8}$ = 5.60 Hz, H-9b), 4.08 (m, 1H, H-5), 3.87 (dd, 1H, $J_{6,5}$ = 10.73 Hz, $J_{6,7}$ = 1.62 Hz, H-6), 3.80 (s, 3H, COOCH_3), 3.38 (m, 2H, –CH_2NHCOCH=CH_2), 2.72 (dd, 1H, H-3e), 2.74 (m, 1H, SCH_2CH_2), 2.75 (m, 1H, SCH_2CH_2), 2.08 (t, 2H, –NHCOCH_2(CH_2)$_4$NHCO–), 2.08 (m, 1H, H-3a), 2.15, 2.15, 2.03, 2.01 (4s, 12H, 4Ac), 1.53 (m, 2H, SCH_2CH_2), 1.52 (m, 4H, –NHCOCH_2C$H_2$$CH_2CH_2$$CH_2$NHCO–), 1.33 (m, 2H, –NHCO(CH_2)$_2$-CH_2(CH_2)$_2$NHCO–), 1.25 (m, 18H, 9CH_2), 0.88 (t, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 173.01, 170.83, 170.54, 170.44, 170.05, 168.48, 165.64, 130.93, 126.13, 83.12, 74.12, 69.67, 68.97, 67.70, 62.16, 52.84, 49.15, 39.02, 38.07, 36.08, 31.85, 29.60, 29.57, 29.54, 29.43, 29.28, 29.17, 29.15, 28.88, 28.77, 28.74, 26.03, 24.40, 22.62, 21.09, 20.90, 20.86, 20.70, 14.06; FAB MS Calcd for [M+H$^+$]: 802.0. Found: *m/z* 801.5.

Anal. Calcd for $C_{39}H_{64}N_2O_{13}S$: C, 58.48; H, 8.05; N, 3.50. Found: C, 58.30; H, 8.06; N, 3.35.

4.1.10. Methyl (dodecyl-*N*-6-acryloylaminohexylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-β-D-*glycero*-D-galacto-2-nonulopyranosid) onate (10β)

Compound **9β** (619 mg, 801 μmol) was dissolved in MeOH (6 mL). NaOMe (21.0 mg, 389 μmol) was added, and the mixture was stirred overnight at room temperature. After neutralization with Amberlite IR-120B (H$^+$ form) followed by filtration, the solvent was evaporated under diminished pressure. The residue was dissolved in dry MeOH (4 mL). Pd$(OH)_2$ on charcoal (80.0 mg, 752 μmol) was added to the solution and stirred vigorously overnight at room temperature under a hydrogen atmosphere. The reaction was monitored by TLC. After completion of the reaction, the suspension was filtered through activated carbon and evaporated. The residue was dissolved in MeOH (5 mL). Triethylamine (836 μL, 6.00 mmol) was added to the solution under an argon atmosphere followed by dropwise addition of acryloyl chloride (84.0 μL, 3.00 mmol) in 1,4-dioxane (400 μL). Upon completion of the reaction on TLC, the reaction mixture was filtered and concentrated. The residue was dissolved in Pyr (5 mL). Ac_2O (1.40 mL, 14.8 mmol) was added dropwise to the solution at 0 °C. After stirring overnight at room temperature, the mixture was evaporated in vacuo. The residue was diluted by $CHCl_3$, transferred into a separating funnel, and washed successively with ice-water, 1 M aq H_2SO_4, satd aq $NaHCO_3$, and brine, and then dried over anhyd $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography on silica gel (acetic ether) yielded compound **10β** (122 mg, 25%): R_f 0.46 [8:1 (v/v) $CHCl_3$–MeOH]; $[\alpha]_D^{32}$ −42.0° (*c* 1.27, $CHCl_3$); IR (KBr) 3252 (ν_{N-H}), 2928 (ν_{C-H}), 1748 ($\nu_{C=O}$), 1655 ($\nu_{C=O}$, amide I), 1626 ($\nu_{C=C}$), 1553 (δ_{N-H}, amide II) cm^{-1}; ^{1}H

NMR δ (400 MHz, $CDCl_3$) 6.48 (t, 1H, $-NHCOCH{=}CH_2$), 6.30 (dd, 1H, J_{trans} = 16.99 Hz, J_{gem} = 1.60 Hz, $-CH{=}CH_2$), 6.15 (dd, 1H, J_{Cis} = 10.17 Hz, $-CH{=}CH_2$), 5.91 (d, 1H, $J_{NH,5}$ = 10.07 Hz, NH), 5.62 (dd, 1H, $-CH{=}CH_2$), 5.42 (t, 1H, $J_{7,8} = J_{7,6}$ = 2.30 Hz, H-7), 5.29 (ddd, 1H, $J_{4,3e}$ = 4.66 Hz, $J_{4,3a} = J_{4,5}$ = 10.95 Hz, H-4), 5.08 (m, 1H, H-8), 4.83 (dd, 1H, $J_{9a,9b}$ = 12.39 Hz, $J_{9a,8}$ = 2.21 Hz, H-9a), 4.37 (dd, 1H, $J_{6,5}$ = 10.49 Hz, $J_{6,7}$ = 2.08 Hz, H-6), 4.19 (dd, 1H, $J_{9b,9a}$ = 12.34 Hz, $J_{9b,8}$ = 8.34 Hz, H-9b), 4.09 (m, 1H, $J_{5,NH} = J_{5,6} = J_{5,4}$ = 10.37 Hz, H-5), 3.81 (s, 3H, $COOCH_3$), 3.34 (m, 2H, $-CH_2NHCOCH{=}CH_2$), 2.52 (m, 1H, H-3e), 2.52 (m, 2H, SCH_2CH_2), 2.10 (m, 2H, $-NHCOCH_2(CH_2)_4NHCO-$), 2.09 (m, 1H, H-3a), 2.15, 2.05, 2.04, 2.00 (4s, 12H, 4Ac), 1.56 (m, 2H, SCH_2CH_2), 1.52 (m, 4H, $-NHCOCH_2CH_2CH_2CH_2CH_2NHCO-$), 1.31 (m, 2H, $-NHCO(CH_2)_2CH_2(CH_2)_2NHCO-$), 1.25 (m, 18H, $9CH_2$), 0.88 (t, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 173.03, 170.89, 170.81, 170.46, 170.37, 168.41, 165.61, 130.96, 126.05, 84.74, 73.09, 72.27, 69.27, 68.84, 62.53, 52.79, 49.29, 39.04, 37.20, 36.07, 31.84, 29.58, 29.57, 29.51, 29.44, 29.27, 29.12, 29.03, 28.77, 28.43, 26.02, 24.50, 22.61, 20.94, 20.85, 20.80, 20.70, 14.05; FAB MS Calcd for $[M+H^+]$: 802.0. Found: *m/z* 801.4.

Anal. Calcd for $C_{39}H_{64}N_2O_{13}S$: C, 58.48; H, 8.05; N, 3.50. Found: C, 58.29; H, 8.09; N, 3.56.

4.1.11. Dodecyl-*N*-6-acryloylaminohexylamino-3,5-dideoxy-2-thio-D-*glycero*-D-galacto-2-nonulopyranosaminic acid (11α)

To a solution of compound **10α** (50.0 mg, 62.4 μmol) in dry MeOH (0.5 mL) was added NaOMe (1.30 mg, 24.0 μmol) and the solution was stirred for 1 h at room temperature under an argon atmosphere. Then 0.2 M aq NaOH (2 mL) was added to the solution and the solution was stirred for 3 days at room temperature. After neutralization with amberlite IR-120B (H^+ form), the solvent was lyophilized to furnish compound **11α** (27.0 mg, 69%.): R_f 0.58 [65:25:4 (v/v/v) $CHCl_3$–MeOH–H_2O]; IR (KBr) 3372 (ν_{O-H}), 2924 (ν_{C-H}), 1715 ($\nu_{C=O}$), 1651 ($\nu_{C=O;}$ amide I), 1626 ($\nu_{C=C}$), 1553 (δ_{N-H}; amide II) cm^{-1}; ^1H NMR δ (400 MHz, D_2O) 6.21 (br m, 2H, $-CH{=}CH_2$, $-CH{=}CH_2$), 5.72 (br d, 1H, $-CH{=}CH_2$), 3.86 (br m, 4H, H-4, H-5, H-8, H-9a), 3.64 (br m, 3H, H-6, H-7, H-9b), 3.23 (br s, 2H, $-CH_2NHCOCH{=}CH_2$), 2.81 (m, 1H, H-3e), 2.81 (br s, 1H, SCH_2CH_2), 2.67 (br s, 1H, SCH_2CH_2), 2.30 (br s, 2H, $-NHCOCH_2(CH_2)_4NHCO-$), 1.86 (br s, 1H, H-3a), 1.60 (br s, 2H, SCH_2CH_2), 1.60 (br s, 4H, $-NHCOCH_2CH_2CH_2CH_2CH_2NHCO-$), 1.53 (br s, 2H, $-NHCO(CH_2)_2CH_2(CH_2)_2NHCO-$), 1.26 (br s, 18H, 9 CH_2), 0.85 (br s, 3H, CH_2CH_3); ^{13}C NMR δ (100.6 MHz, D_2O) 174.20, 168.24, 130.29, 127.11, 63.30, 52.50, 39.90, 36.68, 32.17, 30.14, 29.70, 29.37, 28.04, 25.71, 25.64, 22.82, 14.05; FAB MS Calcd for $[M+Na^+]$: 663.3. Found: *m/z* 663.3.

4.1.12. Radical polymerization of glycomonomer (14α, 15, 17α, 17β)

An appropriate amount of a monomer (**2α**, **3**, **10α**, or **10β**) and acrylamide were dissolved in DMF. The solution was degassed using an aspirator. TEMED and APS were added under an argon atmosphere, and the reaction mixture was stirred. The obtained glycopolymer was purified in two different ways depending on its solubility. The EtOAc-soluble glycopolymer was purified by gel filtration (Sephadex LH-20) with EtOAc, and then the glycopolymer was dissolved in 1 M NaOMe in MeOH and stirred overnight at room temperature. On the other hand, the EtOAc-insoluble glycopolymer was directly treated with 1 M NaOMe in MeOH without purification and stirred overnight at room temperature. Both the EtOAc-soluble and -insoluble glycopolymers were neutralized with acidic ion-exchange resin and filtration. The filtrate was purified by dialysis (Seamless Cellulose Tubing 36/32) against distilled water and lyophilized to furnish the corresponding copolymer (**14α**, **15**, **17α**, or **17β**).

4.2. Viruses

Human influenza viruses A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2) and avian influenza virus A/DK/HK/313/78 (H5N3) were cultured in 11-day-old embryonic chicken eggs, purified, and concentrated as described previously.[16] The virus suspensions in saline were used as the source of hemagglutinin and neuraminidase.

4.3. Hemagglutination inhibition (HAI) assay

The HAI assay was carried out using 96-well microtiter plates. Phosphate buffer saline (pH 6.5) containing 0.01% gelatin was used as a dilution buffer. Guinea pig erythrocytes were used as indicator cells. Virus suspension (2^2 HA units in 25 μL of PBS) was added to each well containing an inhibitor in twofold serial dilutions with the dilution buffer. The plates were incubated for 1 h at 4 °C. After 0.05 mL of 0.5% (v/v) chicken erythrocytes in PBS had been added to the plates, the plates were kept at 4 °C for 1 h. The maximum dilution of the samples showing complete inhibition of hemagglutination was defined as the hemagglutination inhibition titer.

4.4. Neuraminidase inhibition (NAI) assay

Influenza A virus suspension (HAU = 26) and appropriate amounts of inhibitors and 4-MU-Neu5Ac were mixed in 20 mM acetate buffer (pH 5.2), and the mixture was adjusted to 0.4 mM of final concentration of 4-MU-Neu5Ac and total volume of 20 μL. The reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 1 mL of 100 mM carbonate buffer (pH 10.7). The fluorescence of released 4-methylumbelliferone (4-MU) was measured using a fluorescence spectrophotometer (Hitachi F-4010) with excitation at 355 nm and emission at 460 nm.

4.5. Dynamic light scattering (DSL) measurement

A Zetasizer Nano ZS (Spectris Co., Ltd) equipped with a 4.0 mW He–Ne laser (λ = 633 nm) and a backscatter detector at a fixed angle of 173° were used for the measurement. All DLS measurements were performed in millipore water at 20 °C. The instrument recorded the intensity autocorrelation function using a digital correlator with minimum sampling time of 25 ns.

Acknowledgment

We are grateful to Snow Brand Milk Products Co., Ltd for providing the sialic acid and Spectris Co., Ltd for providing a chance to measure DLS using a Zetasizer Nano ZS.

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