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Use of a recycle-type SEC method as a powerful tool for purification of thiosialoside derivatives

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ABSTRACT

An efficient separation between fully acetylated thiosialoside methyl esters and fully acetylated Neu5Ac2en methyl esters was accomplished by means of a size-exclusion chromatography (SEC) method. Purity determinations and structural elucidation of the isolated compounds were performed by a combination of elemental analyses and spectroscopic analyses, including IR, ^{1}H, and ^{13}C NMR, and mass spectroscopic analyses.

1. Introduction

Much interest has been shown in the synthesis of sialyl oligosaccharides because of their importance in biological systems.[1] Therefore, much effort has been devoted to the development of sialic acid chemistry.[2] We have also been synthesizing sialyl oligosaccharides and their glycoclusters by chemical and enzymatic methods, and the glycoclusters have been used in the biochemical and biomedical fields.[3] In our ongoing study on the synthesis of the sialyl oligosaccharides having thioglycosidic linkages, we have encountered a problem in that an inseparable mixture of a desired sialyl derivative and the 2,3-didehydro sialic acid byproduct was 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 reversed-phase C_{18} column after deprotection of the product mixture.[4] 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 size of the products prompted us to consider size-exclusion chromatography (SEC), which separates molecules based on differences in molecular size. Consequently, SEC was chosen for separation of the products. This paper deals with a convenient method for removal of the 2,3-didehydro sialic acid byproduct in the reaction mixture by the SEC method.

2. Results and discussion

Scheme 1 summarizes production of the 2,3-didehydro sialic acid byproduct **3**[5] accompanied by thioacetate **2** as major product by simple S_N2 reaction of known anomeric chloride **1** and KSAc.[6] The reaction proceeds smoothly, but simultaneous elimination of HCl from **2** giving a side product **3** was often observed. After the usual workup, we tried to separate these mixtures by well-known procedures, including crystallization and normal-phase chromatography, but separation unfortunately failed. Both compounds had the same R_f on TLC, even when a variety of solvent systems as eluents were tested for TLC. In our previous study, a 5-azido sialic acid derivative did not have an amide proton at C-5 as a highly hydrophilic group, and it indeed showed excellent separation from side products after the O-glycosidation reaction.[7] However, in the present study, another direct method for the isolation of thiosialoside having both an *N*-acetamido group and an amide proton was needed.

Therefore, we next turned our attention to purification of sialooligosaccharide after forming their respective thioglycosides. Alkyl halide-type glycosides **4** and **6** as candidates for the thioglycosidation were synthesized from the corresponding 4-pentenyl glycosides in good yields by the previously reported method.[8] Scheme 2 shows the synthetic conversion of thioacetate **2** into the corresponding disaccharides having a newly formed interglycosidic sulfide linkage. Condensation of 6-bromo-6-deoxy-D-glucoside **4** with thioacetate **2**, after producing a thiolate anion by treatment of **2** with diethylamine,[9] followed by chromatographic purification using silica gel, gave a mixture of disaccharide **5** having an

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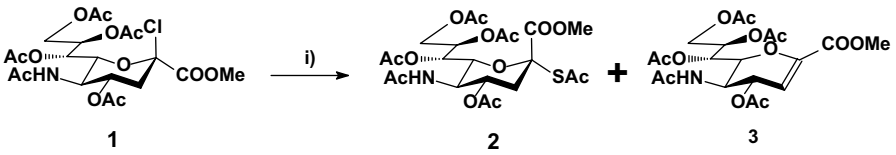
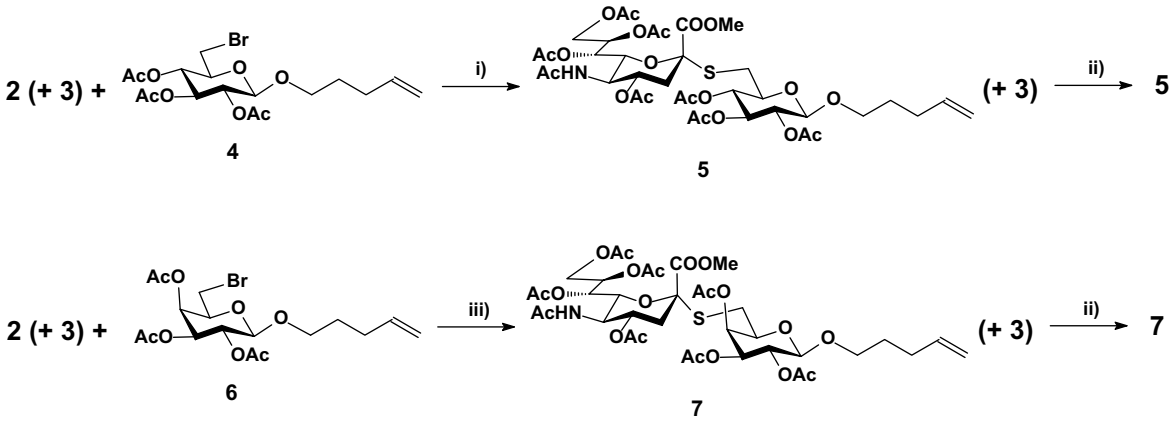


Scheme 1. Reagents and conditions: (i) KSAc (5 M excess), CH_2Cl_2, 0 °C→rt, 2 d.



Scheme 2. Reagents and conditions: (i) diethylamine (10 M excess vs Br), DMF, 0 °C→rt, overnight; (ii) purified by SEC; (iii) K_2CO_3, MeOH–DMF, 0 °C→rt, 2 d, then Ac_2O–pyridine, rt, overnight.

endo-thioglycosidic linkage and glycal **3**. Figure 1A shows the ^1H NMR spectrum of the mixture of **5** and **3**. Characteristic signals due to both compounds were observed. Since chromatographic purification of the products by silica gel as a solid phase adsorbent was unfortunately unsuccessful, another separation method was needed. Therefore, we turned to size-exclusion chromatography (SEC) for separation of the inseparable mixture, a process that is based on different molecular sizes of the compounds and does not involve physical adsorption. The inseparable mixture was subjected to a recycle-type SEC system using chloroform as the eluent.

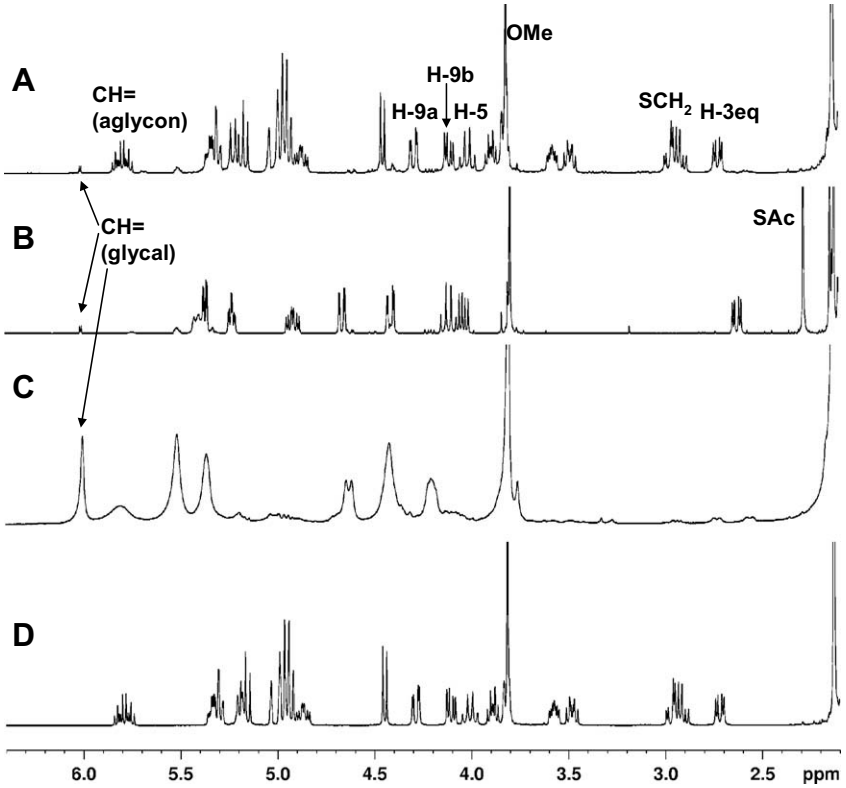


Figure 1. ^1H NMR spectra of (A) mixture of disaccharide **5** and glycal **3**, (B) thioacetate **2**, (C) glycal **3**, and (D) pure disaccharide **5**.

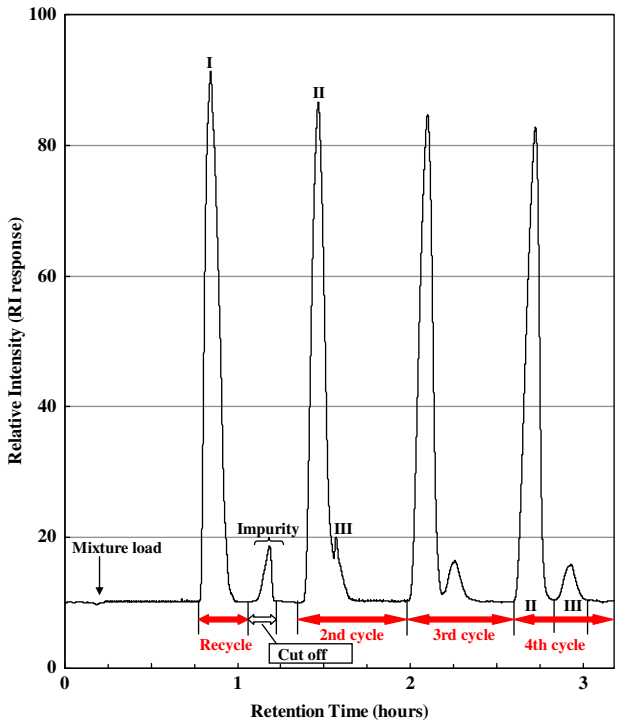


Figure 2. SEC profile of the separation of disaccharide **5** and glycal **3**. Elution conditions are described in Section 3.

The chromatographic profile of the separation of **5** and **3** is shown in Figure 2. The peak of the inseparable mixture by silica gel chromatography was initially one peak (I), which was gradually split into two peaks (II and 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 NMR spectroscopic analyses, the results of which are shown in Figure 1B–D. Since Figure 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%. Furthermore, a galactosyl bromide **6** was also used for the preparation of sialyl α-(2→6)-D-galactose analogue. The coupling reaction proceeded smoothly, and isolation by using the same procedure as that described for the preparation of **5** afforded pure **6** in 42.5% yield, the purity of which was determined by ^{1}H NMR spectroscopy. The results of the ^{1}H NMR analyses are summarized in Tables 1 and 2. The low yield of this condensation reaction was apparently caused by a β-elimination of the galactose residue under the basic reaction conditions, as well as by the relatively low reactivity of the galactosyl bromide due to steric hindrance of the 4-acetoxyl group that has an axial orientation.

Since separation of the sialyl disaccharide and glycal was successfully accomplished, our attention was then turned to the separation of the monosaccharidic thiosialoside from the product mixture after thioglycosidation, including the side product **3**. We previously reported glycopolymers having thiosialoside moieties as pendant-type epitopes, and the glycopolymers had inhibitory activities against sialidases of human influenza virus.[10] Therefore, the convenient separation method by SEC was applied to the preparation of the glycomonomers, and the synthetic scheme is shown in Scheme 3. Before the coupling reaction of **2**, bifunctional compound **9**, which has a leaving group and an azide moiety as a precursor of an amine, was prepared from a known azidoalcohol **8**[11] in 96.9% yield. Thus, the thioacetate **2**, including a small amount of **3**, was treated with a mesylate **9** having an azide at the ω-position in the presence of potassium carbonate to give crude products, which were first passed through a column of silica gel to remove undesired byproducts and decomposed compounds. The mixture of **9** and **3** was then applied to the recycle-type SEC apparatus to give a pure thiosialoside **9** in 72.0% yield. ^{1}H NMR spectral data for pure **9** are summarized in Tables 1 and 2.

In conclusion, the purification of thiosialosides has been efficiently demonstrated by means of the SEC method as a convenient and a useful tool. Therefore, this methodology is applicable for the separation of other thiosialoside mixtures, and synthetic studies that make use of this separation system are now underway. Results of further application of this methodology will be reported elsewhere.

Table 1
^{1}H NMR chemical shifts and multiplicities of Neu5Ac moieties of compounds **1**–**10**[a]

Compounds	Chemical shifts (δ), multiplicity					
	1	**2**	**3**[b]	**5**	**7**	**10**
H-9b	4.45, dd	4.41, dd	4.60, dd	4.29, dd	4.29, dd	4.30, dd
H-9a	4.08, dd	4.03, dd	4.20, dd	4.10, dd	4.14, dd	4.09, dd
H-8	5.18, ddd	5.23, ddd	5.37, ddd	5.34, ddd	5.3, m[c]	5.36, ddd
H-7	5.49, dd	5.37, dd	5.51, dd	5.29, dd	5.3, m[c]	5.31, br d
H-6	4.38, dd	4.66, dd	4.41, dd	3.82, dd	3.9, m[c]	3.81, br d
H-5	4.23, q	4.12, q	4.38, ddd	4.01, q	3.9, m[c]	4.04, q
H-4	5.24, ddd	4.91, ddd	5.52, dd	4.87, ddd	4.92, ddd	4.87, dt
H-3eq	2.79, dd	2.62, dd	6.00, d	2.72, dd	2.72, dd	2.73, dd
H-3ax	2.27, dd	1.89, m		ND[c]	ND[c]	2.00, t
Me (ester)	3.88, s	3.80, s	3.81, s	3.81, s	3.82, s	3.80, s
NH	5.81, d	5.44, s	5.55, d	5.19, d	5.22, d	5.17, d
NAc	1.91, s	1.88, s	1.94, s	1.87, s	1.80, s	1.88, s
OAc	2.13, s	2.15, s	2.13, s	ND[c]	ND[c]	2.16, s
	2.09, s	2.13, s	2.08, s			2.14, s
	2.06, s	2.04, s	2.07, s			2.04, s
	2.06, s	2.02, s	2.06, s			2.03, s

[a] In chloroform-*d* with TMS as the internal standard.
[b] Ref. 5.
[c] Overlapped and could not be determined.

Table 2
^{1}H NMR first-order coupling constants of the Neu5Ac moieties of compounds **1**–**10**[a]

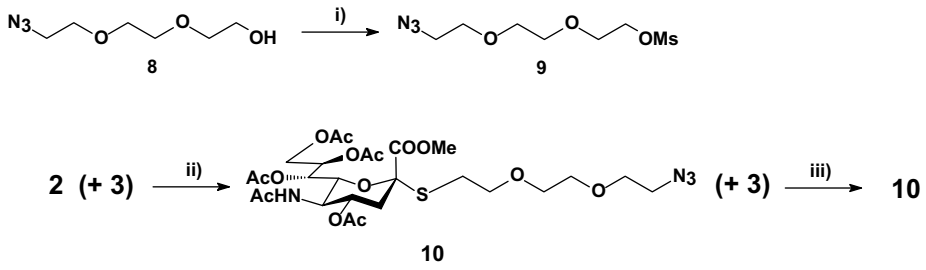
Compounds	First-order coupling constants (Hz)					
	1	**2**	**3**[b]	**5**	**7**	**10**
$J_{9a,9b}$	12.5	12.3	12.5	12.4	12.7	12.3
$J_{8,9b}$	6.3	4.9	7.0	5.2	4.0	4.8
$J_{8,9a}$	2.6	2.1	3.2	2.6	2.2	1.5
$J_{7,8}$	6.7	6.4	4.3	8.2	ND[c]	9.3
$J_{6,7}$	2.4	2.1	3.5	1.8	ND[c]	~1
$J_{5,6}$	10.8	11.0	8.9	10.3	ND[c]	10.3
$J_{4,5}$	10.7	11.0	7.0	10.3	11.4	10.7
$J_{3eq,4}$	4.3	4.6		4.6	4.6	4.5
$J_{3ax,4}$	10.7	11.0	3.4	ND[c]	ND[c]	12.4
$J_{3ax,3eq}$	13.9	13.1	—	12.7	12.8	12.7
$J_{5,NH}$	10.1	10.2	8.8	10.3	9.5	10.0

[a] In chloroform-*d* with TMS as the internal standard.
[b] Ref. 5.
[c] Overlapped and could not be determined.

3. Experimental

3.1. General methods

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Pyridine (Pyr) and *N*,*N*-dimethylformamide (DMF) were stored over molecular sieves (4 Å MS), and methanol (MeOH) was stored over 3 Å



Scheme 3. Reagents and conditions: (i) MsCl, pyridine, 0 °C, 4 h; (ii) K_2CO_3, MeOH–DMF, 0 °C→rt, overnight, then Ac_2O–pyridine, DMAP, rt, overnight.

MS before use. The optical rotations were determined with a JASCO DIP-1000 digital polarimeter. The IR spectra were obtained using a Shimadzu IR Prestage-21 spectrometer. The ^{1}H NMR spectra were recorded at 400 MHz with a Bruker DPX-400 or a Bruker DRX-400 spectrometer or at 200 MHz with a Varian Gemini-2000 spectrometer in chloroform-*d* ($CDCl_3$), including tetramethylsilane (TMS) as the internal standard. The internal standards used for ^{13}C NMR spectra were $CDCl_3$ (77.0 ppm) in $CDCl_3$. Ring-proton assignments in the ^{1}H NMR spectra were made by first-order analysis of the spectra and are supported by the results of homonuclear decoupling experiments and H–H or C–H COSY 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 (FABMS) spectra were recorded with a JEOL DX-303 spectrometer. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of Silica Gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For detection of the intermediates, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) MeOH–*p*-anisaldehyde–concd H_2SO_4 and heated for a few minutes (for carbohydrate) or (b) an aq solution of 5 wt % $KMnO_4$ and heated similarly (for detection of C=C double bonds). Column chromatography was performed on silica gel (Silica Gel 60; 63–200 μm, E. Merck). Flush column chromatography was performed on silica gel (Silica Gel 60, spherical neutral; 40–100 μm, E. Merck). 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 × 600 mm) and H-2002 columns (I.D., 20.0 mm × 500 mm).[12] 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. All extractions were concentrated below 45 °C under diminished pressure.

3.2. Synthesis

3.2.1. 4-Pentenyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-3,4,6-tri-*O*-acetyl-6-thio-β-D-glucopyranoside (5)

Diethylamine (12.9 mL, 124 mmol) was added dropwise to a mixture of 4-pentenyl 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-β-D-glucopyranoside **4** (5.42 g, 12.4 mmol) and methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**2**)[6] (10.2 g, 18.6 mmol) in DMF (25 mL) at 0 °C under Ar atmosphere, and the reaction mixture was stirred at room temperature for 10 h. The reaction mixture was concentrated in vacuo and the resulting residue was diluted with $CHCl_3$. The organic solution was successively washed with water and brine, dried over anhyd $MgSO_4$, filtered, and evaporated. The residue was chromatographed on silica gel (450 g) with 2:3→0:1 (v/v) toluene–EtOAc as the eluent to give an inseparable mixture **5** including a small amount of **3** (9.2 g) as a yellow foam, in which each compound has the same R_f on TLC. A portion of the inseparable mixture of **5** and **3** (ca. 500 mg) was applied to the SEC apparatus to afford pure **5** as a white foam. Total yield of pure **5** was 8.44 g (78.8%): R_f 0.56 [5:4:1 (v/v/v) $CHCl_3$–EtOAc–MeOH]; $[\alpha]_D^{24}$ −31.3° (*c* 1.07, $CHCl_3$); IR (KBr) 2955 (ν_{C-H}), 1748 ($\nu_{C=O}$), 1655 ($\nu_{C=O}$, amide I), 1541 (δ_{N-H}, amide II), 1223 (ν_{C-O}), 1038 (ν_{C-O-C}) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67 (m, 2H, CH_2), 1.99, 2.03, 2.03, 2.05, 2.07, 2.13, and 2.13 (each s, 21H, 7 $COCH_3$), 2.91 (dd 1H, $J_{5,6b}$ = 7.1 Hz and $J_{6a,6b}$ = 14.1 Hz, H-6b), 2.97 (dd 1H, $J_{5,6a}$ = 3.8 Hz, H-6a), 3.57 (m, 1H, H-5), 3.67 (m, 2H, OCH_2), 4.45 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.98 (m, 4 H, H-2, H-4, and $=CH_2$), 5.16 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3), and 5.79 (m, 1H, CH=), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.60, 20.63, 20.70, 20.72, 20.78, 20.80, 21.09, 23.16, 28.51, 29.81, 30.24, 37.99, 49.37, 52.96, 62.10, 67.30, 68.50, 69.14, 69.43, 71.41, 72.60, 72.91, 74.10, 77.21, 82.66, 100.62 (C-1), 115.02 (CH_2=), 137.81 (CH=), 169.32 (C=O), 169.26 (C=O), 169.50 (C=O), 169.79 (C=O), 170.08 (C=O), 170.12 (C=O), 170.32 (C=O), 170.60 (C=O), and 170.87 (C=O); FABMS calcd for [M+H$^+$]: 864.3. Found: *m*/*z* 864.6, [M+Na$^+$]: 886.3. Found: *m*/*z* 886.4.

Anal. Calcd for $C_{37}H_{53}NO_{20}S$: C, 51.44; H, 6.18; N, 1.62. Found: C, 51.26; H, 6.11; N, 1.52.

3.2.2. 4-Pentenyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-3,4,6-tri-*O*-acetyl-6-thio-β-D-galactopyranoside (7)

To a solution of 4-pentenyl 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-β-D-galactopyranoside (**6**) (145 mg, 0.322 mmol) and sialyl derivative **2**[6] (364 mg, 0.662 mmol) in 1:1 DMF–MeOH (1.4 mL) was added K_2CO_3 (91 mg, 0.658 mmol) portionwise at 0 °C under an Ar atmosphere, and the reaction mixture was stirred at room temperature for 45 h. When TLC indicated the end of the reaction, AcOH (76 μL, 1.24 mmol) was added to the reaction mixture at 0 °C. After concentration in vacuo, the resulting mixture was then treated with pyridine (4 mL) and Ac_2O (3 mL) at 0 °C, and the mixture was stirred at room temperature for 21 h. The reaction mixture was concentrated in vacuo, and the resulting residue was diluted with $CHCl_3$. The organic solution was successively washed with 1 M aq H_2SO_4, satd aq $NaHCO_3$, and brine, dried over anhyd $MgSO_4$, filtered, and the solvents were evaporated to yield a yellow foam (394 mg). Chromatographic purification of the residue on silica gel (20 g) with 15:14:1 (v/v/v) $CHCl_3$–EtOAc–MeOH as the eluent gave an inseparable mixture of **7** and **3** (264 mg), which was further purified by the SEC apparatus to give pure **7** (122 mg, 42.5%) as a white foam: R_f 0.55 [5:4:1 (v/v/v) $CHCl_3$–EtOAc–MeOH]; $[\alpha]_D^{25}$ −10.4° (*c* 1.09, $CHCl_3$); IR (KBr) 2957 (ν_{C-H}), 1748 ($\nu_{C=O}$), 1668 ($\nu_{C=O}$, amide I), 1549 (δ_{N-H}, amide II), 1225 (ν_{C-O}), 1057 (ν_{C-O-C}) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69 (m, 2H, CH_2), 1.97, 2.03, 2.04, 2.05, 2.14, 2.15, and 2.15 (each s, 21H, 7 $COCH_3$), 2.66 (dd 1H, $J_{5,6b}$ = 7.1 Hz and $J_{6a,6b}$ = 14.3 Hz, H-6b), 2.90 (dd 1H, $J_{5,6a}$ = 7.2 Hz, H-6a), 3.75 (m, 2H, OCH_2), 3.90 (m, 1H, H-5), 4.61 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.00 (m, 2H, $=CH_2$), 5.07 (dd, 1H, $J_{2,3}$ = 10.4 Hz and $J_{3,4}$ = 2.7 Hz, H-3), 5.17 (dd, 1H, H-2), 5.53

(d, 1H, H-4), and 5.80 (m, 1H, CH=), and other signals are summarized in Tables 1 and 2; ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.59, 20.68, 20.75, 20.78, 21.03, 23.19, 28.61, 29.71, 29.85, 38.04, 49.51, 53.10, 62.22, 66.78, 67.82, 68.29, 69.01, 69.17, 71.17, 72.24, 73.82, 83.72, 100.78 (C-1), 114.81 (CH_2=), 138.01 (CH=), 169.15 (C=O), 169.48 (C=O), 169.64 (C=O), 169.92 (C=O), 170.09 (C=O), 170.19 (C=O), 170.40 (C=O), 170.60 (C=O), and 170.81 (C=O); FABMS calcd for [M+H$^+$]: 864.3. Found: *m*/*z* 864.5, [M+Na$^+$]: 886.3. Found: *m*/*z* 886.4. Anal. Calcd for $C_{37}H_{53}NO_{20}S{\cdot}0.5\ H_2O$: C, 50.91; H, 6.24; N, 1.61. Found: C, 50.96; H, 6.09; N, 1.52.

3.2.3. 2-[2-(2-Azidoethoxy)ethoxy]ethyl methanesulfonate (9)

Known 2-[2-(2-azidoethoxy)ethoxy]ethanol (**8**)[11] (1.32 g, 7.53 mmol) in pyridine (10 mL) was treated with methanesulfonyl chloride (1.17 mL, 15.1 mmol) at 0 °C under an Ar atmosphere, and the reaction mixture was stirred at the same temperature for 4 h. To the mixture were added $CHCl_3$ and water, and the mixture was partitioned. The organic solution was successively washed with 1 M aq H_2SO_4, satd aq $NaHCO_3$, and brine, dried over anhyd $MgSO_4$, filtered, and the solvents were evaporated to yield pure **9** as a light-yellow syrup (1.85 g, 96.9%), which was used for the next reaction without further purification: R_f 0.65 (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.39 (t, 2H, *J* = 4.9 Hz, CH_2N_3), 3.67 (m, 6H, $3CH_2$), 3.79 (m, 2H, CH_2CH_2OMs), and 4.39 (m, 2H, CH_2OMs).

3.2.4. Methyl {2-[2-(2-Azidoethoxy)ethoxy]ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate (10)

To a solution of a known thioacetate **2**[6] (600 mg, 1.09 mmol) and a mesylate **9** (550 mg, 2.18 mmol) in MeOH (6.0 mL) was added K_2CO_3 (150 mg, 1.09 mmol) at 0 °C under an Ar atmosphere, and the reaction mixture was stirred at room temperature overnight. After consuming starting materials judged by TLC, the reaction mixture was treated with AcOH (0.125 mL, 2.18 mmol), and the mixture was concentrated. The residue was allowed to react with Ac_2O (2.06 mL, 21.8 mmol) in pyridine (6.0 mL) in the presence of 4-dimethylaminopyridine (DMAP) at 0 °C under Ar atmosphere, and the reaction mixture was stirred at room temperature overnight. The mixture was diluted with $CHCl_3$ and then poured into ice-cold water. The mixture was partitioned, and the organic layer was successively washed with 1 M aq H_2SO_4, satd aq $NaHCO_3$, and brine, dried over anhyd $MgSO_4$, filtered, and concentrated in vacuo. Chromatographic purification of the residue on silica gel with 1:3 (v/v) toluene–EtOAc as the eluent was followed by further purification using the SEC apparatus to give corresponding **10** (520 mg, 71.8%): R_f 0.40 [5:4:1 (v/v/v) $CHCl_3$–EtOAc–MeOH]; $[\alpha]_D^{27}$ +17.8° (*c* 1.00, $CHCl_3$); IR (KBr) 2959 (ν_{C-H}), 2870 (ν_{C-H}), 2104 ($\nu_{N=N=N}$), 1742 ($\nu_{C=O}$), 1655 ($\nu_{C=O}$, amide I), 1560 (δ_{N-H}, amide II), 1223 (ν_{C-O}), 1037 (ν_{C-O-C}) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.81 (dt, 1H, J_{vic} = 4.5, J_{gem} = 13.5 Hz, SCHb), 2.95 (dt, 1H, J_{vic} = 5.7 Hz, SCHa), 3.39 (t, 2H, *J* = 4.9 Hz, CH_2N_3), 3.67 (m, 8H, $4CH_2$), and other signals are summarized in Tables 1 and 2; FABMS calcd for [M+H$^+$]: 665.7. Found: *m*/*z* 665.2, [M+Na$^+$]: 687.7. Found: *m*/*z* 687.1. Anal. Calcd for $C_{26}H_{40}NO_{14}S$: C, 46.98; H, 6.07; N, 8.43. Found: C, 47.10; H, 6.06; N, 8.38.

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