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Synthesis of Fluorescent 4-Methyl-7-thiocoumaryl *S*-Glycosides of Sialic Acid

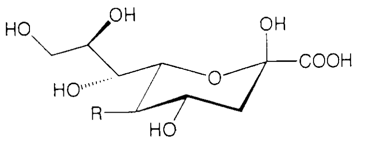
Makoto TANAKA,[a] Toshitsugu KAI,[a] Xue-Long SUN,[a] Hiroaki TAKAYANAGI,[a]
Yutaka UDA,[b] and Kimio FURUHATA*,[a]

School of Pharmaceutical Sciences, Kitasato University,[a] 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan and Department of Health Chemistry, Niigata College of Pharmacy,[b] 5-9-12, Kamishin'ei-cho, Niigata, Niigata 950-21, Japan. Received April 6, 1995; accepted June 28, 1995

Condensation of 4-methyl-7-thiocoumarin sodium salt with methyl 5-*N*-acetyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (2), methyl 5-*N*-(*O*-acetylglycolyl)-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,6-trideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (11), and methyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-2,3-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (14) under Williamson reaction conditions gave the corresponding α-glycosides in good yields. Deprotection of these α-glycosides gave three new fluorogenic substrates, the 4-methylcoumarin-7-yl *S*-glycosides of *N*-acetylneuraminic acid, *N*-glycolylneuraminic acid, and 3-deoxy-D-glucose-D-*galacto*-2-nonulopyranosonic acid (KDN). Furthermore, we have developed a facile method for preparation of benzyl 5-amino-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (7), a key intermediate for the synthesis of *N*-glycolylneuraminic acid.

Key words *N*-acetylneuraminic acid; *N*-glycolylneuraminic acid; KDN; sialic acid; 7-mercapto-4-methylcoumarin; fluorogenic substrate

Neuramidase activity has been detected on the surface of bacterial and viral outer membranes. This enzymatic activity is involved in the hydrolytic processing of terminal sialic acid units of glycoproteins and glycolipids.[1] In studies of sialic acid, the aryl α-glycosides of sialic acid are useful fluorogenic substrates for tracing and quantifying sialidase (neuramidases EC 3.2.1.18) in biological materials.[2-4]



R = CH_3CONH : *N*-Acetylneuraminic Acid
R = $HOCH_2CONH$: *N*-Glycolylneuraminic Acid
R = HO : KDN

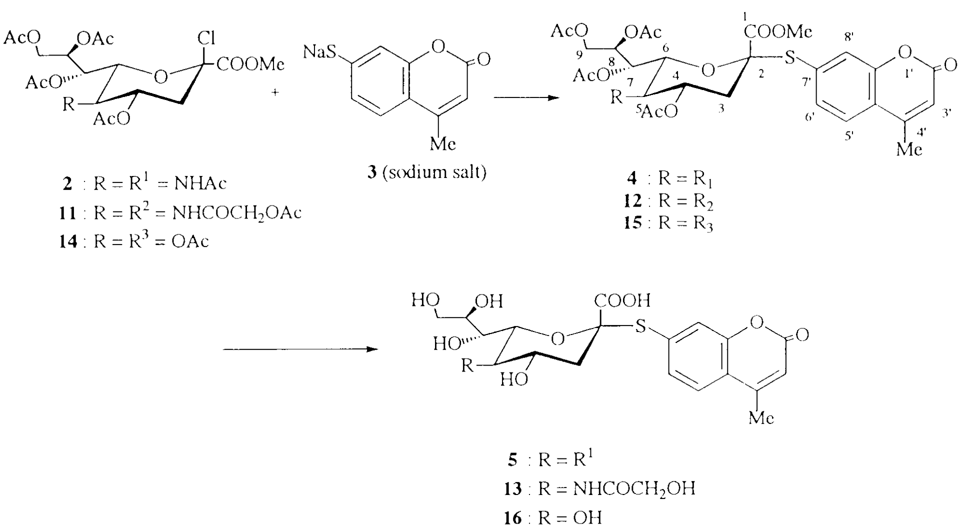
Fig. 1

Many biological functions of sialosylglycoconjugates have been reported.[5-8] As an aid to such investigations, we now report the synthesis of 4-methylcoumarin-7-yl α-*S*-glycosides of *N*-acetyl-neuraminic acid, *N*-glycolylneuraminic acid, and 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (KDN) as new luorogenic substrates for neuramidase. The structures of the synthesized derivatives were elucidated mainly on the basis of the 300 MHz proton nuclear magnetic resonance (^1H-NMR) spectra.The proton signal assignments were based on published data.[7,9-11]

Results and Discussion

The synthesis of 4-methyl-7-thiocoumarin was accomplished by the hydrolysis of the thiocarbamate as reported.[12,13]

Sodium 7-hydroxy-4-methylcoumarate was allowed to react with dimethylthiocarbamoyl chloride in dry aceto-



2 : R = R^1 = NHAc
11 : R = R^2 = $NHCOCH_2OAc$
14 : R = R^3 = OAc

3 (sodium salt)

4 : R = R_1
12 : R = R_2
15 : R = R_3

5 : R = R^1
13 : R = $NHCOCH_2OH$
16 : R = OH

Chart 1

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* To whom correspondence should be addressed.

Table 1. ^{1}H-NMR Spectral Data for **4**,[a] **5**,[b] **12**,[a] **13**,[b] **15**,[a] and **16**[b]

Compound	Chemical shifts (δ)												
	Sialic acid moiety										NHAc		
	3-H_{ax}	3-H_{eq}	4-H	5-H	6-H	7-H	8-H	9-H	9-H′	NH	(or $COCH_2$)	OAc	CO_2Me
4	2.00	2.81	4.85	3.97	3.92	5.28	5.21	4.14	4.34	5.40	1.88	1.99, 2.01, 2.03, 2.15	3.64
5	1.88	2.83	3.62	3.83	3.46	3.44	3.65	3.49	3.70	—	1.93	—	
12	2.01	2.85	4.95	3.96	3.99	5.22 (2H)		4.16	4.35	5.87	4.29 4.58	2.00, 2.01, 2.04, 2.16 × 2	3.68
13	1.95	2.86	3.80	3.97	3.62	3.56	3.68	3.57	3.75	—	4.51 (2H)	—	
15	2.02	2.90	4.89	4.80	3.97	5.31	5.23	4.18	4.33	—	—	2.00 × 3 2.01, 2.03	3.69
16	1.85	2.76	3.58	3.53	3.38	3.74	5.23	3.55	3.72	—	—	—	

Compound	Spin couplings (Hz)											
	Sialic acid moiety											
	$J_{3ax,3eq}$	$J_{3ax,4}$	$J_{3eq,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9a}$	$J_{8,9b}$	$J_{9a,9b}$	$J_{5,NH}$	J_{NCOCHH}
4	12.5	11.5	4.5	10.0	10.5	1.5	8.0	5.0	3.0	12.5	10.0	—
5	12.5	11.5	4.5	10.0	10.5	1.5	9.0	5.5	2.5	11.5	—	—
12	13.0	11.5	5.0	10.0	10.5	1.5		4.5	2.0	12.5	9.5	15.0
13	13.0	11.0	4.5	10.0	10.0	1.5	9.0	6.0	2.5	11.5	—	0
15	13.0	11.6	4.9	10.0	10.0	1.9	8.0	8.0	4.6	12.5	—	—
16	12.0	11.0	3.0	10.0	10.0	1.5	9.0	2.0	0.5	13.0	—	—

Table 2. ^{13}C-NMR Spectral Data for **5**, **13**, and **16**

Carbon	Compound		
	5	**13**	**16**
C-1	174.3	173.9	174.2
C-2	89.8	89.3	89.6
C-3	42.5	42.3	42.1
C-4	70.8	70.4	72.7
C-5	54.2	53.8	72.1
C-6	77.9	77.7	79.0
C-7	70.7	70.7	70.4
C-8	74.6	74.5	74.8
C-9	65.2	65.2	65.3
5-N$\underline{C}$OCH	177.6	178.4	—
5-NCO$\underline{C}$H	24.7	63.6	—
C-2′	166.1	165.8	165.9
C-3′	117.1	117.2	117.1
C-4′	123.5	123.5	123.4
4′-Me	20.6	20.6	20.5
C-4a′	158.0	157.9	157.9
C-5′	128.0	128.0	128.0
C-6′	134.5	134.5	134.4
C-7′	154.3	154.3	153.4
C-8′	126.2	126.1	126.0
C-1a′	135.9	135.6	135.9

Recorded in D_2O at 75 MHz.

nitrile at room temperature to yield 7-[(*N*,*N*-dimethylthiocarbamoyl)oxy]-4-methyl-coumarin, which was then thermally rearranged into 7-[(*N*,*N*-di-methylcarbamoyl)-thio]-4-methylcoumarin. Alkaline hydrolysis of this compound with sodium methoxide in methanol resulted in the formation of 7-mercapto-4-methylcoumarin (**3**) in excellent yield. The structure of **3** was elucidated from the ^{1}H-NMR spectrum in comparison with that of 4-methyl-7-hydroxycoumarin. The observed chemical shift of δ 3.00 is characteristic of thiophenol protons.

Glycosylation reaction of methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosyl chlorid)onate (**2**) with the sodium salt of **3** in *N*,*N*-dimethylformamide (DMF) under Williamson reaction conditions gave methyl (4′-methyl-coumarin-7′-yl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**4**) in 60% yield. Compound **4** was subjected to saponification with 1 N sodium hydroxide, the reaction mixture was de-ionized with Dowex-50 (H^+) to give 4′-methylcoumarin-7′-yl 5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**5**). The structures were elucidated on the basis of the ^{1}H-NMR and ^{13}C-NMR spectra (Tables 1 and 2). Consistent patterns were noted in the ^{1}H-NMR spectra for **4**, suggesting the presence of a 4-methylcoumarin structure and a fully protected *N*-acetylneuraminic acid moiety.

Empirical studies of the sialic acid derivatives indicated that for α-anomers the chemical shift of 3-H_{eq} varies between δ 2.6 and δ 2.9.[9,11] The chemical shift of δ 2.81 was therefore considered to be that of the α-anomer. The structure of the saponification product **5** was also in full agreement with the ^{1}H-NMR data.

As an extension of our synthetic studies of *S*-glycosides of sialic acid, we have synthesized 4′-methylcoumarin-7′-yl 5-*N*-glycolyl-2,3,5-trideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**13**).

Several methods are available for preparing *N*-glycolylneuraminic acid,[14-16] and furthermore, we have established the feasibility for synthesizing of the partially protected *N*-glycolylneuraminic acid derivatives from *N*-acetylneuraminic acid derivatives as starting materials.

The *N*-acetyl group in benzyl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonoic acid (**6**) was hydrolyzed with barium hydroxide to afford crude **7**

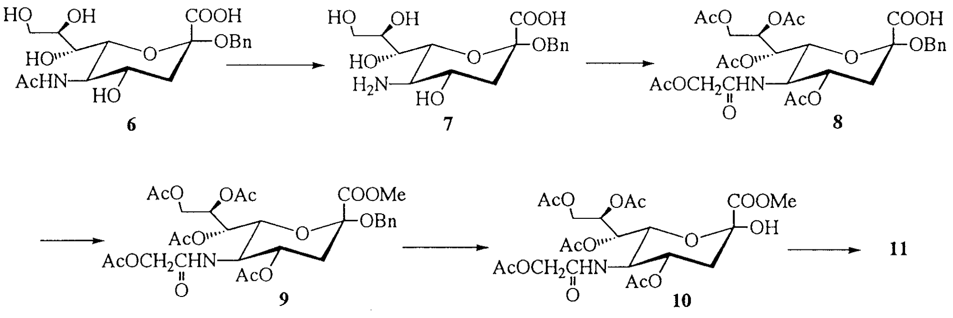

Chart 2

in 92% yield with more than 92% purity (^{1}H-NMR). It was recrystallized from isopropyl alcohol to give pure **7** at (mp 186°C) in 62% yield as needles; **7** had previously been obtained as an amorphous solid by Byramova *et al.*[16] Treatment of **7** with acetoxyacetyl chloride gave the *N*-(*O*-acetylglycolyl) derivative of **7**, which was used for the next acetylation step without isolation. Acetylation was carried out by treatment with acetic anhydride and pyridine to give benzyl 5-*N*-(*O*-acetylglycolyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**8**) in 75% yield. After esterification of **8** with diazomethane in ether, the product **9** was subjected to catalytic hydrogenation over palladium on charcoal to afford methyl 5-*N*-(*O*-acetylglycolyl)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosidonate (**10**) in good yield. The chloride (**11**) was directly prepared by treatment of **10** with a solution of acetyl chloride in acetic acid saturated with hydrogen chloride gas. The structure of **11** was elucidated by ^{1}H-NMR comparison with **10**.

Condensation of **11** with the sodium salt of **3** in DMF gave methyl (4′-methylcoumarin-7′-yl 5-*N*-(*O*-acetylglycolyl)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**12**) in 88% yield. Compound **12** was deprotected with 1 N sodium hydroxide to give 4′-methylcoumarin-7′-yl 5-*N*-glucolyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**13**) in 90% yield. The structures of **12** and **13** were established unambiguously on the basis of elemental analysis and spectral properties. The assignment of the ^{1}H- and ^{13}C-NMR signals of **12** and **13** was achieved by comparison with that of **4** and **5**, respectively (Tables 1 and 2).

On the other hand, KDN *S*-glucoside, methyl (4′-methylcoumarin-7′-yl 4,5,7,8,9-penta-*O*-acetyl-2,3-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**15**) was also synthesized by condensation of methyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl chlorid)onate (**14**)[4] with the sodium salt of **3**. Removal of the protective groups on **15** gave 4′-methylcoumarin-7′-yl 3-deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**16**) in 90% yield. The structures of **15** and **16** were confirmed by comparison of the ^{1}H-NMR spectra of the corresponding *S*-glycosides (**4**, **5**, **12**, **13**) and their *O*-glycosides with those published earlier.[4]

In conclusion, we have developed a facile method for the synthesis of three new fluorogenic substrates, 4-methylcoumarin-7-yl *S*-glycosides of sialic acid (**5**, **13**, **16**). The biological activities of these compounds are under investigation.

Experimental

Melting points were measured with a Yamato melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Thin layer chromatography (TLC) was performed on silica gel (Merck) plates, and spots were detected by spraying with 5% sulfuric acid solution. Infrared (IR) spectra were recorded as KBr disks with a JASCO A-2 spectrometer. Ultraviolet (UV) spectra were measured in methanol solution with Hitachi 340 istrument. The NMR spectra were measured in $CDCl_3$ or D_2O with tetramethylsilane (TMS) as an internal standard, with a Varian VXR-300 spectrometer. Column chromatography was conducted on Silica gel 60 (70—230 mesh, Merck).

7-Mercapto-4-methylcoumarin (3) *N*,*N*-Dimethylthiocarbamoyl chloride (6.2 g, 0.26 mol) was added to suspension of 7-hydroxy-4-methylcoumarin sodium salt (10.0 g, 50.5 mmol) in dry acetonitrile (80 ml) and the mixture was stirred for 4 h at room temperature, and then filtered. The filtrate was concentrated by evaporation. The residual syrup was purified by recrystallization with ethyl acetate–isopropyl ether (1 : 2) to give 7-[(*N*,*N*-dimethylthiocarbamoyl)oxy]-4-methylcoumarin (10.6 g, 80%) as colorless needles, mp 208—209 °C. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 2.25 (3H, d, $J=1.0$ Hz, 4-Me), 3.28 (3H, s, NMe), 3.35 (3H, s, NMe), 6.15 (1H, d, $J=1.0$ Hz, 3-H), 6.90 (2H, m, 6-H, 8-H), 7.50 (1H, d, $J=9.0$ Hz, 5-H).

This compound (5 g, 19 mmol) was fused at 240 °C for 1 h. The reaction products was purified by recrystallization from ethyl acetate–isopropyl ether to give 7-[(*N*,*N*-dimethylcarbamoyl)thio]-4-methylcoumarin (4.8 g, 96%) as colorless needles, mp 159—160 °C. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 2.30 (3H, d, $J=1.0$ Hz, 4-Me), 2.95 (6H, s, NMe_2), 6.27 (H, d, $J=1.0$ Hz), 7.40 (2H, m, 6-H, 8-H), 7.55 (1H, d, $J=8.0$ Hz, 5-H).

A 28% sodium methoxide–methanol solution (1 ml) was added to a solution of the above compound (263 mg, 1 mmol) in methanol (30 ml), and the mixture was kept for 16 h at room temperature, then neutralized with Dowex-50 (H^+) resin. The filtrate and washings were combined and evaporated to dryness. The residue was purified by recrystallization with ethyl acetate to give 4-methyl-7-thiocoumarin (**3**, 144 mg, 75%) as slightly yellowish needles, mp 138—139 °C. *Anal.* Calcd for $C_{10}H_8O_2S$: C, 62.48; H, 4.20. Found: C, 62.40; H, 4.25. UV λ_{max} nm (ε): 325 (19223) sh, 298 (11747) sh, 224 (16019). IR ν_{max} cm^{-1}: 1750, 1630, 1605, 1560. ^{1}H-NMR (90 MHz, DMSO-d_6) δ: 2.41 (3H, d, $J=1.0$ Hz, 4-Me), 3.00 (1H, s, SH), 6.30 (1H, d, $J=1.0$ Hz, 3-H), 7.52 (2H, m, 6-H, 8-H), 7.75 (1H, d, $J=9.0$ Hz, 5-H).

Sodium Salt of 3 A solution of **3** (0.38 g, 2 mmol) in methanol (20 ml) was added to 0.1 N sodium hydroxide (22 ml). The resultant clear solution was concentrated to about 10 ml *in vacuo*. The product crystallized spontaneously to give the sodium salt of **3** (0.32 g, 75%) as yellow prisms, mp 157—158 °C. *Anal.* Calcd for $C_{10}H_7NaO_2S$: C, 56.07; H, 3.29. Found: C, 56.13; H, 3.30. ^{1}H-NMR (90 MHz, DMSO-d_6) δ: 2.41 (3H, br s, 4-Me), 3.00 (1H, s, SH), 6.30 (1H, br s, 3-H), 7.52 (2H, br s, 6-H, 8-H), 7.75 (1H, d, $J=9.0$ Hz, 5-H).

Methyl (4′-Methylcoumarin-7′-yl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (4) A mixture of **2** (3 g, 5.3 mmol) and the sodium salt of **3** (1.3 g, 6 mmol) in DMF (10 ml) was stirred for 16 h at room temperature, and evaporated *in vacuo*. Water (50 ml) was added to the oily residue and water-soluble materials were removed by filtration. The residue was purified by silica-gel column chromatography with ethyl acetate–*n*-hexane (4 : 1) to give **4** (2.3 g, 60%) as a colorless powder, $[\alpha]_D^{18}$ $+2.4°$ ($c=1$, MeOH). *Anal.* Calcd for $C_{30}H_{35}NO_{14}S$: C, 54.41; H, 5.30; N, 2.10. Found: C, 54.48; H, 5.33; N, 2.06. IR ν_{max} cm^{-1}: 1755, 1770, 1675, 165, 1610, 1555. NMR data are given in Tables 1 and 2.

4′-Methylcoumarin-7′-yl 5-Acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (5) A solution of **4** (0.67 g, 1 mmol) in methanol (10 ml) was added to 1 N sodium hydroxide (10 ml). The reaction mixture was stirred for 16 h at room temperature, and then diluted with water and de-ionized with Dowex-50 (H^+) resin. The filtrate was lyophilized to give **5** (0.44 g, 90%) as a colorless powder, $[\alpha]_D^{18}$ $+63°$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{21}H_{25}NO_{10}S$: C, 52.17; H, 5.21; N, 2.90. Found: C, 52.33; H, 5.26; N, 2.78. IR ν_{max} cm^{-1}: 3380, 1730, 1610, 1570. UV λ_{max} nm (ε): 320 (9820), 295 (8310), 212 (21150). NMR data are given Tables 1 and 2.

Benzyl 5-Amino-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (7) A solution of **6** (2.0 g, 5 mmol) in 5% barium hydroxide (40 ml) was heated for 10 h at 90 °C. The mixture was brought to pH 6.0 by addition of 1 N sulfuric acid at 0 °C and filtered through Celite. The Celite was washed with water, and the combined filtrates were brought to pH 10 by addition of 1 N NaOH and evaporated to dryness *in vacuo* at 60 °C. The residue was dissolved in water (10 ml) and the solution was filtered. The filtrate was passed through IRC-50 (H^+) resin (20 ml) which was washed with water (40 ml), then the eluate and washings were combined and lyophilized to give crude **7** (1.6 g, 92%). The residue was recrystallized from isopropyl alcohol to give pure **7** (1.1 g, 62%) as colorless needles, mp 186—187 °C, $[\alpha]_D^{18}$ $-42°$ ($c=1$, H_2O). (ref. $[\alpha]_D^{20}$ $-41°$ ($c=0.5$, H_2O)).[16] *Anal.* Calcd for $C_{16}H_{23}NO_8$: C, 53.77; H, 6.94; N, 3.92. Found: C, 53.71; H, 7.02; N, 3.88. IR ν_{max} cm^{-1}: 3380, 2960, 1610, 1550. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.61 (1H, t, $J=12.0$ Hz, 3-H_{ax}), 2.72 (1H, dd, $J=4.5$, 12.0 Hz, 3-H_{ax}), 3.64 (1H, ddd, $J=12.0$, 10.0, 5.0 Hz, 4-H), 3.14 (1H, t, $J=10.0$ Hz, 5H), 3.90 (1H, dd, $J=10.0$, 2.0 Hz, 6-H), 3.70 (1H, dd, $J=9.0$, 2.0 Hz, 7-H), 3.76 (1H, ddd, $J=2.0$, 5.5, 2.5 Hz, 8-H), 3.62 (1H, dd, $J=11.5$, 5.5 Hz, 9-H), 3.78 (1H, dd, $J=11.5$, 2.5 Hz, 9-H′), 4.44 (1H, d, $J=11.0$ Hz, –CH–Ph), 4.64 (1H, d, $J=11.0$ Hz, –CH′–Ph), 7.72 (5H, m, phenyl group).

Benzyl 5-*N*-(*O*-Acetylglycolyl)-4,7,8,9-Tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (8) Acetoxyacetyl chloride was added to a solution of **7** (1.07 g, 3 mmol) in methanol–water (1 : 1, 50 ml) and triethylamine (4 ml) at 0 °C. The mixture was stirred for 3 h at room temperature, and evaporated to dryness *in vacuo*. The redidue was acetylated by using acetic anhydride (50 ml) and pyridine (50 ml) for 3 h at room temperature. The mixture was poured into ice water, and extracted with ethyl acetate. The extract was washed with 1 N hydrochloric acid and water, and dried over sodium sulfate, filtered and evaporated dryness. The residue was purified by recrystallization from ethyl acetate to give **8** (1.41 g, 75%) as colorless needles, mp 186—187 °C. $[\alpha]_D^{18}$ $-9.4°$ ($c=1$, MeOH). *Anal.* Calcd for $C_{28}H_{35}NO_{15}$: C, 53.77; H, 6.94; N, 3.92. Found: C, 53.75; H, 6.99; N, 3.86. IR ν_{max} cm^{-1}: 3360, 1750, 1715, 1565. ^{1}H-MNR (300 Hz, $CDCl_3$) δ: 2.04 (1H, t, $J=12.0$ Hz, 3-H_{ax}), 2.04, 2.06, 2.09, 2.14, 2.15 (each 3H, s, OAc), 2.75 (1H, dd, $J=13.0$, 5.0 Hz, 3-H_{eq}), 5.15 (1H, ddd, $J=12.0$, 10.5, 5.0 Hz, 4-H), 4.19 (1H, dt, $J=11.5$, 10.5 Hz, 5-H), 4.30 (1H, dd, $J=10.5$, 2.0 Hz, 6-H), 5.34 (1H, dd, $J=6.5$, 2.0 Hz, 7-H), 5.44 (1H, dt, $J=6.5$, 2.0 Hz, 8-H), 4.14 (1H, dd, $J=12.5$, 6.5 Hz, 9-H), 4.45 (1H, dd, $J=12.5$, 2.0 Hz, 9-H′), 4.56 (1H, d, $J=11.0$ Hz, –CH–Ph), 4.83 (1H, d, $J=11.0$ Hz, –CH′–Ph), 7.42 (5H, m, phenyl group), 4.34 (1H, d, $J=15.0$ Hz, 5-NCOCH), 4.57 (1H, d, $J=15.0$ Hz, 5-NCOCH′), 6.57 (1H, d, $J=10.0$ Hz, NH).

Methyl 5-*N*-(*O*-Acetylglycolyl)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (10) An ethereal solution of diazomethane (prepared from *N*-methyl-*N*-nitrosourea (1 g) and 40% potassium hydroxide) was added to a solution of **8** (1.25 g, 2 mmol) in methanol–ether (1 : 1, 5 ml) at 0 °C. The mixture was kept at 0 °C for 1 h and then evaporated *in vacuo*. The residue was crystallized from isopropyl ether to give methyl ester of **8** as colorless needles (mp 95—96 °C). This methyl ester was dissolved in methanol and reduced with hydrogen gas over 10% palladium-on-charcoal (0.1 g) for 2 h at room temperature. The solution was filtered through Celite, and evaporated to dryness. The residue was purified by silica gel column chromatography with chloroform–methanol (50 : 1) to give **10** (1.06 g, 96%) as a colorless powder. $[\alpha]_D^{18}$ $-8.0°$ ($c=1$, MeOH). *Anal.* Calcd for $C_{22}H_{31}NO_{15}$: C, 48.09; H, 5.69; N, 2.55. Found: C, 48.04; H, 5.72; N, 2.48. IR ν_{max} cm^{-1}: 3370, 2970, 1760, 1700, 1550. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.25 (1H, t, $J=13.0$ Hz, 3-H_{ax}), 2.00, 2.02, 2.09, 2.12, 2.16 (each 3H, s, OAc), 2.18 (1H, dd, $J=13.0$, 5.5 Hz, 3-H_{eq}), 5.30 (1H, ddd, $J=5.5$, 10.5, 12.5 Hz, 4-H), 4.15(1H, q, $J=10.0$ Hz, 5-H), 4.22 (1H, dd, $J=10.5$, 2.0 Hz, 6-H), 5.27 (1H, dd, $J=6.0$, 2.0 Hz, 7-H), 5.24 (1H, ddd, $J=6.0$, 5.0, 2.0 Hz, 8-H), 4.01 (1H, br dd, $J=12.5$, 5.0 Hz, 9-H), 4.45 (1H, dd, $J=12.5$, 2.0 Hz, 9-H′), 3.86 (3H, s, COOMe), 4.32 (1H, d, $J=15.0$ Hz, 5-NCOCH), 4.58 (1H, br d, $J=15.0$ Hz, 5-NCOCH′), 6.18 (1H, br d, $J=10.0$ Hz, NH), 3.85 (1H, s, 2-OH).

Methyl 5-*N*-(*O*-Acetylglycolyl)-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,6-trideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (11) A solution of **10** (0.55 g, 1 mmol) in acetyl chloride (5 ml) and acetic acid (10 ml) was placed in a pressure-protected glass cylinder, which was immersed in an ice bath, and the solution was saturated with dry hydrogen chloride gas. The tube was stoppered tightly and left overnight at room temperature. The reaction solution was carefully transferred in a larger vessel and concentrated *in vacuo* at 20 °C to a syrup. This was dissolved in dry ether and evaporated to dryness to give **11** (0.56 g) as a colorless powder. This sample was directly used in the next step without purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.25 (1H, dd, $J=11.0$, 13.5 Hz, 3-H_{ax}), 2.76 (1H, dd, $J=4.5$, 13.5 Hz, 3-H_{eq}), 2.00, 2.02, 2.03, 2.08, 2.15 (each 3H, s, OAc), 3.85 (3H, s, COOMe), 4.04 (1H, dd, $J=6.0$, 12.5 Hz, 9-H), 4.18 (1H, q, $J=10.0$ Hz, 5-H), 4.30 (1H, d, $J=15.0$ Hz, NCOCH), 4.40 (1H, dd, $J=2.5$, 12.5 Hz, 9-H′), 4.39 (1H, dd, $J=2.5$, 11.0 Hz, 6-H), 4.56 (1H, d, $J=15.0$ Hz, NCOCH′), 5.14 (1H, dt, $J=2.5$, 6.0 Hz, 8-H), 5.40 (1H, dd, $J=2.5$, 6.0 Hz, 7-H), 5.42 (1H, dt, $J=10.5$, 4.5 Hz, 4-H), 6.24 (1H, d, $J=10.0$ Hz, NH).

Methyl 4′-Methylcoumarin-7′-yl 5-*N*-(*O*-Acetylglycolyl)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (12) A mixture of **11** (0.57 g, 1 mmol) and the sodium salt of **3** (0.26 g, 1.2 mmol) in DMF (10 ml) was stirred overnight at room temperature. The reaction mixture was poured into water (100 ml) and extracted with ethyl acetate. The extracts were successively washed with water, 3% sodium hydrogen carbonate and water. The ethyl acetate solution was dried over sodium sulfate and filtered, and the filtrate was evaporated to dryness. The residue was purified on a column of silica gel with chloroform–methanol (50 : 1) to give **12** (0.61 g, 88%) as a colorless powder, $[\alpha]_D^{18}$ $+17.0°$ ($c=1$, MeOH). *Anal.* Calcd for $C_{32}H_{37}NO_{16}S$: C, 55.56; H, 5.39; N, 2.03. Found: C, 55.28; H, 5.44; N, 2.10. IR ν_{max} cm^{-1}: 3380, 2980, 1755, 1630, 1610, 1540. NMR data are given in Tables 1 and 2.

4′-Methylcoumarin-7′-yl 5-*N*-Glycolyl-2,3,5-trideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (13) A solution of **12** (0.50 g, 0.72 mmol) in methanol (10 ml) was added to 1 N sodium hydroxide (10 ml). The reaction mixture was stirred for 16 h at room temperature, and then diluted with water and de-ionized with Dowex-50 (H^+) resin. The filtrate was lyophilized to give **13** (0.45 g, 90%) as a yellowish powder, $[\alpha]_D^{18}$ $+122°$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{21}H_{25}NO_{11}S$: C, 50.50; H, 5.05; N, 2.80. Found: C, 50.39; H, 5.12; N, 2.65. IR ν_{max} cm^{-1}: 3380, 2940, 1725, 1700, 1610, 1555. UV λ_{max} nm (ε): 320 (8980), 297 (7810), 217 (19510). NMR data are givens in Tables 1 and 2.

Methyl (4′-Methylcoumarin-7′-yl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (15) A mixture of **14** (0.51 g, 1 mmol) and the sodium salt of **3** (0.26 g, 1.2 mmol) in DMF (10 ml) was stirred overnight at room temperature. The reaction mixture was poured into water (100 ml) and extracted with ethyl acetate. The extracts were successively washed with water, 3% sodium hydrogen carbonate and water. The ethyl acetate solution was dried over sodium sulfate and filtered, and the filtrate was evaporated to dryness. The residue was purified on a column of silica gel with chloroform–methanol (100 : 1) to give **15** (0.45 g, 70%) as a colorless powder. $[\alpha]_D^{18}$ $-2.4°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{30}H_{34}NO_{13}S$: C, 55.55; H, 5.28; N, 2.16. Found: C, 55.64; H, 5.30; N, 2.11. IR ν_{max} cm^{-1}: 3050, 1750, 1640, 1615, 1560. NMR data are given in Tables 1 and 2.

4′-Methylcoumarin-7′-yl 3-Deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (16) A solution of **15** (0.32 g, 0.5 mml) in methanol (10 ml) was added to 1 N sodium hydroxide (10 ml). The reaction mixture was stirred for 4h at room temperature, and then diluted with water and de-ionized with Dowex-50 (H^+) resin. The filtrate was lyophilized to give **16** (0.20 g, 90%) as a slightly yellowish powder, $[\alpha]_D^{18}$ $+25°$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{19}H_{22}O_{10}S$: C, 51.58; H, 5.01. Found: C, 51.50; H, 5.12. IR ν_{max} cm^{-1}: 3450, 2960, 1730, 1635, 1615,

1560. UV λ_{max} nm (ε): 230 (9220), 295 (7370), 214 (19050). NMR data are given in Tables 1 and 2.

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