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Note

Synthesis of *N*-acetyl-9-*S*-acetyl-9-thioneuraminic acid, *N*-acetyl-9-thioneuraminic acid, and their methyl α-glycosides

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Thio sugars frequently exhibit biological properties which differ widely from those of the oxygen-containing parent compounds. For instance, they were found to act as inhibitors of certain enzymes and transport proteins that require the normal sugar derivative [1].

We are interested in the synthesis and biological activities of sialic acids with distinct oxygen atoms replaced by sulfur. Thus, we previously prepared *N*-acetyl-6-thioneuraminic acid, with sulfur in place of the ring oxygen [2]. Remarkably, the corresponding α-glycoside was not a substrate for *Vibrio cholerae* sialidase, although the β-glycoside showed inhibition of the enzyme. Further, replacement of the amide oxygen by sulfur led to novel *N*-thioacylated amino sugars [3] and *N*-thioacetylneuraminic acids [4], which possess biological potential. For instance, compared to the parent compound, *N*-thioacetylneuraminic acid methyl α-glycoside interacted more strongly with influenza A virus hemagglutinin [5], and *N*-acetyl-9-deoxy-9-thioacetamidoneuraminic acid, when transferred to the host cell surface, prevented influenza C virus infection [6].

We now report the synthesis of sialic acids bearing sulfur at the terminal carbon atom. *N*-Acetyl-9-thioneuraminic acid and its methyl α-glycoside were obtained from the corresponding 9-acetylthio compounds, which, as such, are of biological interest. As an example, the latter is a close analog of *N*-acetyl-9-*O*-acetylneuraminic acid, the natural influenza C virus receptor-dominant group [7].

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The most direct access to thio sugars starts from the free hydroxyl function that is substituted in a one-pot reaction by an acetylthio group, as in the Mitsunobu reaction [8]. In a second step the thiolester can easily be cleaved to afford the thiol. For *N*-acetylneuraminic acid, the OH-9 position is by far the least hindered one. For this reason, protection of the other hydroxyl groups may not be necessary. More considerable difficulties can arise from the anomeric centre and the carboxyl group. Therefore, we first employed the methyl α-glycoside of methyl *N*-acetylneuraminate and obtained the respective 9-acetylthio derivative. As expected, cleavage of the ester concomitantly hydrolysed the thiolester with partial formation of the disulfide. Reduction and reacetylation were then necessary steps. Cleavage of the glycoside was not straightforward owing to the lability of the compound. Taken together, this multi-step reaction sequence turned out to be tedious and low-yielding.

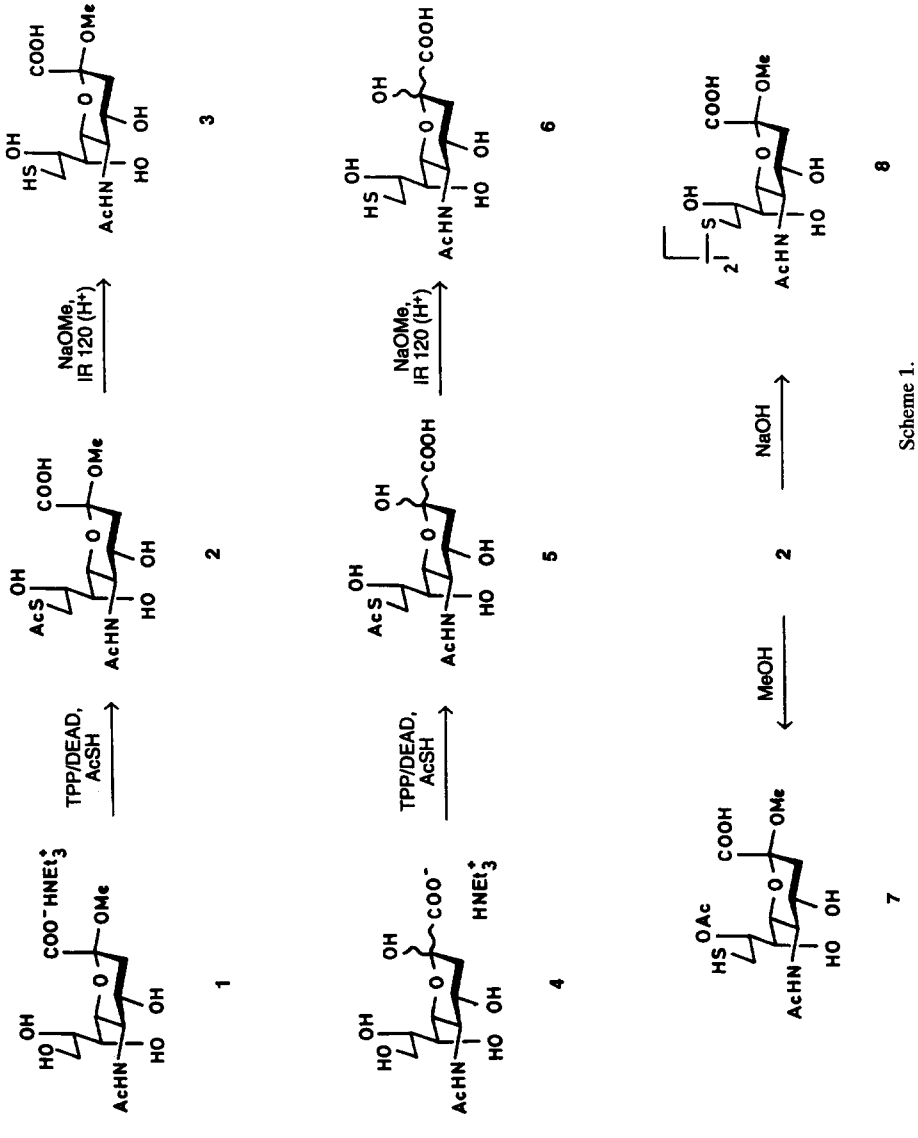
To circumvent the difficulties mentioned above, we then employed as substrates in the Mitsunobu reaction (a) the methyl α-glycoside of *N*-acetylneuraminic acid, and (b) free *N*-acetylneuraminic acid.

Under typical Mitsunobu conditions, we treated the preformed adduct of triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD) in THF with a mixture of the sugar and thioacetic acid in DMF [9]. First, we used the methyl α-glycoside of *N*-acetylneuraminic acid (**1**, Scheme 1 [10]). To achieve sufficient solubility, the free acid was transformed into the triethylammonium salt. The DMF solution was added at 0°C and the resulting pale-yellow solution was allowed to warm up to room temperature. After 3 h, TLC indicated the disappearance of **1** and the formation of less polar thioacetate **2**, both positive by UV and nitroprusside/ammonia reagent. The reaction was quenched by addition of a few drops of methanol followed by removal of excess reagent by extraction with ethyl acetate–diethyl ether. Lyophilization then gave crude methyl α-glycoside of *N*-acetyl-9-*S*-acetyl-9-thioneuraminic acid (**2**) in 87% yield. As a byproduct, *N*-acetyl-8-*O*-acetyl-9-thioneuraminic acid methyl α-glycoside (**7**, vide infra) was obtained ($\sim$ 5%). Attempts to purify **2** by anion-exchange chromatography resulted in extensive decomposition of the labile thiolester. However, column chromatography on silica gel employing 10:1:2 *n*-butanol–acetic acid–water as the eluent afforded pure **2** in 78% yield.

The ^{1}H NMR spectrum of **2** (Table 1) showed the methyl protons of *S*-acetyl groups at 2.41 ppm as well as a characteristic upfield shift [11] of protons H-9 and H-9′ (3.47

Table 1
^{1}H NMR data [a] for 9-thio sialic acids **2**, **3**, **5**, **6**, **7**, and **8**

	Chemical shifts (δ in ppm)											
	H-3$_{ax}$	H-3$_{eq}$	H-4	H-5	H-6	H-7	H-8	H-9	H-9′	NC(O)CH$_3$	OCH$_3$	SC(O)CH$_3$
2	1.72	2.67	3.74 [b]	3.84 [c]	3.84 [c]	3.49	4.00	3.47	3.05	2.04	3.37	2.41
3	1.74	2.67	3.75	3.85 [d]	3.85 [d]	3.58	3.93	2.97	2.69	2.04	3.39	—
5β [e]	1.89	2.32	4.08	3.94	4.07	3.50	3.89	3.46	3.04	2.07	—	2.41
5α [e]	1.72	2.72	[f]	[f]	[f]	[f]	[f]	[f]	[f]	2.06	—	2.41
6β [e]	1.87	2.30	4.053	3.92	4.054	3.58	3.81	2.94	2.69	2.05	—	—
6α [e]	1.69	[g]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	2.03	—	—
7 [h]	1.56	2.59	3.62	3.84	4.05	3.89	5.15	3.12	2.90	2.05	3.29	—
8 [i]	1.67	2.70	3.70 [j]	3.83 [k]	3.83 [k]	3.59	4.14	3.31	2.84	2.04	3.37	—


TPP/DEAD, AcSH
NaOMe, IR 120 (H+)
TPP/DEAD, AcSH
NaOMe, IR 120 (H+)
MeOH
NaOH

Scheme 1.

and 3.05 ppm vs. 3.89 and 3.65 ppm of **1**, [12]). More significantly, in the ^{13}C NMR spectrum (Table 2) the signal of C-9 appeared at 33.9 ppm in comparison to ~ 64 ppm for C-9 carrying a hydroxyl group [13]. The signal at 201.8 ppm indicated the presence of the thiolester carbon [14] in **2**.

For *S*-deacetylation, a solution of **2** in methanol was treated under strictly oxygen-free conditions with methanolic methoxide for 2 h. After addition of Amberlite IR 120 (H^+) cation exchange resin, filtration, and freeze-drying, *N*-acetyl-9-thioneuraminic acid methyl α-glycoside (**3**) was obtained in an almost quantitative yield. Dimer **8**, which may occur as a byproduct in small quantities, can be removed by silica gel chromatography. Although **3** proved to be more stable than **2**, purification by anion-exchange chromatography failed. As expected, in TLC with detection by nitroprusside reagent, thiol **3** spontaneously gave a red colour. The IR spectrum of **3** showed the characteristic SH absorption at 2567 cm^{-1}. As observed for the *S*-acetyl compound **2**, but even more pronounced, NMR spectra of **3** revealed a strong upfield shift of protons H-9 and H-9′ (2.97 and 2.69 ppm, respectively) as well as of carbon C-9 (29.0 ppm) due to the shielding effect of the sulfur atom at C-9.

Treatment of **2** with aqueous sodium hydroxide under aerobic conditions led to the formation of dimer **8**. The disulfide structure was deduced from the mass spectrum, which contained an appropriate molecular ion. Further, no IR absorption due to a sulfhydryl group occurred and detection of **8** by nitroprusside colour reagent failed. In the ^1H NMR spectrum, a slight downfield shift of the 9-methylene protons of **8** in comparison with those of **2** proved the presence of the disulfide dimer [15].

When **2** was kept in methanol for a few days, acetyl group migration from S-9 to O-8 occurred. The newly formed compound, *N*-acetyl-8-*O*-acetyl-9-thioneuraminic acid (**7**) was found to possess a free SH group, and the ^1H NMR spectrum showed an *O*-acetyl methyl signal at 2.17 ppm and the downfield shifted signal of H-8 (5.15 ppm vs. 4.00 ppm in **2**); **7** was proved to be identical with the above mentioned byproduct from the Mitsunobu reaction.

The results obtained with the methyl α-glycoside of *N*-acetylneuraminic acid (**1**) prompted us to explore whether even the free sugar would be a suitable candidate in the Mitsunobu reaction. Thus, the triethylammonium salt of *N*-acetylneuraminic acid (**4**) was reacted with thioacetic acid and the Mitsunobu adduct (TPP/DEAD) in the same way as glycoside **1**. As observed for **1**, the reaction was complete after 3 h. Work-up as described for **2** afforded, after lyophilization, crude *N*-acetyl-9-*S*-acetyl-9-thioneuraminic acid (**5**) as a colourless powder. Pure **5** crystallized from 50:1:2 *n*-butanol–acetic acid–water [55%, mp 148°C (dec)]. A second crop afforded another 15% of crystalline **5**. Column chromatography of the mother liquor gave, after lyophilization, additional 15% of **5** and ~ 10% of *N*-acetyl-8-*O*-acetyl-9-thioneuraminic acid.

Treatment of **5** with sodium methoxide afforded, after Na^+/H^+ exchange and freeze-drying, *N*-acetyl-9-thioneuraminic acid (**6**) in 98% yield. *N*-Acetyl-8-*O*-acetyl-9-thioneuraminic gave, as expected, the same product. As observed for glycosides **2** and **3**, the characteristic features in the NMR spectra of **5** and **6** were the upfield shift of protons H-9 and H-9′ by ~ 0.5 ppm (for thiolester **5**) to ~ 1.0 ppm (for thiol **6**) as well as the upfield shift of carbon C-9 by ~ 35 ppm. The free sialic acids **5** and **6** mainly exist in the β-form (~ 94%) with the carboxylic acid function in the equatorial position.

Table 2
^{13}C NMR data [a,b] for 9-thio sialic acids **2**, **3**, **5**, and **6**

	Chemical shifts (δ in ppm)													
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	$NC(O)CH_3$	$NC(O)CH_3$	OCH_3	$SC(O)CH_3$	$SC(O)CH_3$
2 [a]	172.2	100.2	39.9	68.3 [c]	52.8	71.5 [c]	70.1 [c]	73.6 [c]	33.9	175.8	23.0	52.5	201.8	30.9
3 [b]	172.4	100.3	40.0	68.4 [c]	52.9	71.0 [c]	70.9 [c]	73.3 [c]	29.0	175.9	23.1	52.5	—	—
5 [b,d]	174.0	96.2	39.9	67.6 [c]	53.2	71.4 [c]	69.6 [c]	71.5 [c]	34.3	175.7	23.0	—	201.7	30.9
6 [a,d]	174.3	96.2	39.8	67.6 [c]	53.1	70.9 [c]	70.5 [c]	71.3 [c]	28.9	175.7	23.0	—	—	—

[a] Recorded at 90.6 MHz in D_2O.
[b] Recorded at 75.5 MHz in D_2O.
[c] Assignments may be interchanged.
[d] Data for the β-anomer.

The 9-thio analogs were studied in a number of biological tests. Glycosides **2** and **3** were cleaved by *Arthrobacter ureafaciens* sialidase at a rate comparable to *N*-acetylneuraminic acid methyl α-glycoside. According to TLC the products were identical with *N*-acetyl-9-*S*-acetyl-9-thioneuraminic acid (**5**) and *N*-acetyl-9-thioneuraminic acid (**6**), respectively, obtained by the direct synthetic route. Furthermore, **5** and **6** turned out to be good substrates for *Escherichia coli* *N*-acylneuraminate pyruvate lyase (Neu5Ac aldolase). In each case, TLC showed less polar substances, being apparently *N*-acetyl-6-*S*-acetyl-6-thiomannosamine (from **5**) and *N*-acetyl-6-thiomannosamine (from **6**). During enzymatic incubation, *S*-acetyl compounds **2** and **5** partially decomposed.

Interestingly, *N*-acetyl-9-*S*-acetyl-9-thioneuraminic acid (**5**) and *N*-acetyl-9-thioneuraminic acid (**6**) can be readily activated by CMP-sialate synthase from bovine brain and transferred onto glycoconjugates as has been demonstrated earlier with other 9-substituted sialic acids [16]. This will be reported in detail elsewhere.

In summary, we have developed a simple, high-yielding Mitsunobu reaction-based two-step synthesis that, remarkably, is compatible with the free carboxyl and unmasked anomeric center of Neu5Ac. The thiol group in **3** and **6** is very reactive and allows many conversions to biological interesting compounds.

1. Experimental

General methods.—Optical rotations were measured with a Perkin–Elmer 241 polarimeter after 24 h, keeping at ambient temperature (*c* 0.5, H_2O). UV spectra were recorded in aq solution with a Hitachi U-2000 spectrophotometer. IR spectra were run on a Bruker IFS 66 spectrometer. ^1H NMR spectra were recorded in D_2O at 30°C with a Bruker AM 360 spectrometer. Chemical shifts are reported in ppm relative to HOD (4.76 ppm). Data are given in Tables 1 and 3. ^{13}C NMR spectra were measured in D_2O at 30°C with a Bruker AC 300 and AM 360 spectrometer. The reference was CD_3OD

Table 3
Coupling constants [a] for compounds **2**, **3**, **5**, **6**, **7**, **8**

	First-order coupling constants (J in Hz)									
	$J_{3ax,3eq}$	$J_{3ax,4}$	$J_{3,eq,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$
2	−12.7	~11.7	4.5	[l]	[l]	[m]	8.8	3.1	7.6	−14.2
3	−12.6	11.7	4.6	~9.9	[l]	[m]	8.9	2.8	7.1	−14.2
5β	−13.0	11.6	4.9	9.9	10.6	0.8	9.3	3.2	7.3	−14.2
5α	−12.7	~11.6	4.9	[f]	[f]	[f]	[f]	[f]	[f]	[f]
6β	−13.0	11.6	4.8	10.0	10.4	1.1	9.1	3.0	6.7	−14.2
6α	~−13.0	~11.6	[g]	[f]	[f]	[f]	[f]	[f]	[f]	[f]
7	−12.2	12.0	4.4	~10.0	10.5	1.3	8.4	3.4	5.6	−14.6
8	−12.5	~11.8	4.6	[l]	[l]	~1.2	~8.6	2.5	~8.6	−14.1

[a] Recorded at 360 MHz in D_2O. [b] 3.70–3.78 (m). [c] 3.81–3.87 (m). [d] 3.82–3.89 (m). [e] Ratio α:β ~ 6:94. [f] Signals could not be identified. [g] Signal was hidden by H-9′ of the β-anomer. [h] Methyl of $OC(O)CH_3$ appeared at 2.17 ppm. [i] Dimer; each signal corresponds to two protons. [j] 3.66–3.74 (m). [k] 3.78–3.87 (m). [l] First-order interpretation was not possible. [m] H-7 appeared as broadened d.

(49.0 ppm). For data, see Table 2. FABMS (matrix: glycerol; ion energy 15 kV) were recorded on a MAT 95 mass spectrometer (Finnigan MAT, Bremen, Germany). TLC was performed on aluminium sheets coated with Silica Gel 60 F_{254} (Merck) using the solvent combinations 1:1 EtOAc–MeOH (A), 4:1:2 *n*-butanol–AcOH–water (B), and others specifically mentioned. Compounds were detected by UV light, if possible, and by spraying TLC plates with 2 M H_2SO_4 and charring at 200°C for a few minutes. For discrimination between SH and SAc compounds, nitroprusside reagent [17] was used. Thiolesters gave the colour reaction only after exposure of TLC to ammonia vapour. Column chromatography was performed on Silica Gel Merck 60 (70–230 mesh). *Arthrobacter ureafaciens* sialidase (EC 3.2.1.18) was purchased from Boehringer Mannheim (Germany), Neu5Ac aldolase of *Escherichia coli* (EC 1.4.3.3) from Serva (Heidelberg, Germany).

*Methyl 5-acetamido-9-*S*-acetyl-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**2**).—TPP (236 mg, 0.90 mmol) was dissolved in THF (2.5 mL). DEAD (0.14 mL, 0.90 mmol) was added dropwise at 0°C and, after 10 min at this temperature, a solution of **1** (212 mg, 0.50 mmol) and thioacetic acid (0.14 mL, 1.97 mmol) in dry DMF (1.5 mL) was added. The mixture was allowed to warm up to room temperature and, after 3 h, TLC (A) showed complete conversion to **2**. MeOH (0.05 mL) was added and the solution stirred for 0.5 h. The solvents were removed under reduced pressure and the residue was taken up in 1:1:1 Et_2O–EtOAc–H_2O (6 mL). The aqueous phase was extracted twice with 1:1 Et_2O–EtOAc (4 mL each). Residual organic solvents were removed from the aq. phase under reduced pressure followed by passing the solution through a column of Amberlite IR 120 (H^+) resin at 4°C. Subsequent lyophilization gave crude **2** (177 mg, 87% for **2** · 1.5 H_2O) as a pale yellow powder. Column chromatography on silica gel with 15:1:2 *n*-butanol–AcOH–H_2O afforded pure **2** (159 mg, 78% for **2** · 1.5 H_2O); R_f 0.35 (A), 0.32 (B); $[\alpha]_D^{20}$ + 26.9°; λ_{max} 231.0 nm (ϵ_{mM} 5.15). Negative FABMS: *m/z* 380 [69%, $(M-H)^-$], 320 [100%]. Anal. Calcd for $C_{14}H_{23}NO_9S \cdot 1.5\ H_2O$ (408.42): C, 41.17; H, 6.42; N, 3.43; S, 7.85%. Found: C, 40.82; H, 6.68; N, 3.39; S, 7.69%.

Methyl 5-acetamido-3,5-dideoxy-9-thio-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**3**).—To an ice-cooled solution of **2** (100 mg, 0.245 mmol) in MeOH (8 mL), 1 N NaOMe in MeOH (0.5 mL) was added under oxygen-free conditions. The solution was kept for 2 h at 0°C, then neutralized with Amberlite IR 120 (H^+) and filtered. After addition of water (3 mL), the solution was concentrated under vacuum to ~ 0.5 mL and passed at 4°C through a column of Amberlite IR 120 (H^+). Freeze-drying gave **3** (84 mg, 96% for **3** · H_2O) as a white amorphous solid; R_f 0.32 (B), 0.33 (100:1:10 *n*-propanol–AcOH–H_2O; cf. $R_{f,2}$ 0.30); $[\alpha]_D^{20}$ +9.2°; ν_{max} (KBr) 2567 cm^{-1}. Negative FABMS: *m/z* 338.3 [100%, $(M-H)^-$]. Anal. Calcd for $C_{12}H_{21}NO_8S \cdot H_2O$ (357.38): C, 40.33; H, 6.49; N, 3.92; S, 8.97%. Found: C, 40.39; H, 6.44; N, 3.68; S, 9.15%.

*5-Acetamido-9-*S*-acetyl-3,5-dideoxy-9-thio*-D-glycero-D-galacto-*2-nonulopyranosonic acid* (**5**).—A solution of **4** (205 mg, 0.50 mmol) and thioacetic acid (0.14 mL, 1.97 mmol) in dry DMF (3.0 mL) was added at 0°C to a solution of TPP (236 mg, 0.90 mmol) and DEAD (0.14 mL, 0.90 mmol) in THF (2.5 mL), cf. the preparation of **2**. Work-up of the reaction mixture was performed as described for **2**. From the solution (50:1:2 *n*-butanol–AcOH–H_2O) of the lyophilized material, **5** crystallized sponta-

neously (101 mg, 55%, mp 148°C (dec)). The second crop gave another 28 mg (15%). From the mother liquor remaining, **5** (29 mg, 15% for **5** · 1.5 H_2O, after lyophilization) and *N*-acetyl-8-*O*-acetyl-9-thioneuraminic acid (R_f 0.27 (B), ~ 18 mg, 9%) were obtained by silica gel chromatograhy (50:1:2 to 10:1:2 *n*-butanol–AcOH–H_2O). R_f 0.19 (A), 0.23 (B); $[\alpha]_D^{20}$ + 7.8°. Negative FABMS: *m/z* 366 [100%, $(M-H)^-$]. Anal. Calcd for $C_{13}H_{21}NO_9S$ (crystals, 367.37): C, 42.50; H, 5.76; N, 3.81; S, 8.73%. Found: C, 42.80; H, 5.88; N, 3.72; S, 8.67%. Anal. Calcd for $C_{13}H_{21}NO_9S \cdot 1.5\ H_2O$ (lyophilizate, 394.39): C, 39.59; H, 6.13; N, 3.55; S, 8.13%. Found: C, 39.69; H, 5.86; N, 3.42; S, 7.98%.

5-Acetamido-3,5-dideoxy-9-thio-D-glycero-D-galacto-*2-nonulopyranosonic acid* (*9-Thio-Neu5Ac*, **6**).—The reaction of **5** (100 mg, 0.25 mmol) yielding **6** was carried out as described for the synthesis of **3**. Final lyophilization afforded **6** (85 mg, 98% for **6** · H_2O) as a white powder. R_f 0.18 (B); $[\alpha]_D^{20}$ −13.0°; ν_{max} (KBr) 2568 cm^{-1} (SH). Negative FABMS: *m/z* 324.3 [100%, $(M-H)^-$]. Anal. Calcd for $C_{11}H_{19}NO_8S \cdot H_2O$ (343.36): C, 38.48; H, 6.16; N, 4.08; S, 9.34%. Found: C, 38.15; H, 5.81; N, 4.45; S, 9.15%.

*Methyl 5-acetamido-8-*O*-acetyl-3,5-dideoxy-9-thio*-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**7**).—To a solution of compound **2** (50 mg, 0.12 mmol) in MeOH (3 mL), triethylamine (~ 17 μL, ~ 0.12 mmol) was added. After keeping the mixture for 14 days at room temperature, TLC (A) showed three well-separated spots due to **2** (R_f 0.35), **7** (R_f 0.30), and **8** (R_f 0.05). Using 30:2:5 *n*-butanol–AcOH–H_2O for TLC, **7** had R_f 0.31, **2** R_f 0.17, and **8** R_f 0.04. Silica gel chromatography with this solvent combination furnished **7** (22 mg, 47% for **7** · 0.5 H_2O) as a white powder. $[\alpha]_D^{20}$ +5.9°; ν_{max} (KBr) 2575 cm^{-1} (SH). Anal. Calcd for $C_{14}H_{23}NO_9S \cdot 0.5\ H_2O$ (390.41): C, 43.07; H, 6.20; N, 3.59; S, 8.21%. Found: C, 42.59; H, 6.10; N, 3.22; S, 8.03%.

9,9-Dithiobis(methyl 5-acetamido-3,5,9-trideoxy-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid)* (**8**).—To a solution of **2** (20 mg, 0.049 mmol) in MeOH (1 mL), 0.1 N NaOH (2 mL) was added at ambient temperature. After 24 h, TLC (A) indicated the reaction to be complete. The mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered, and concentrated. Purification on DEAE-Sephadex A-25 (HCO_3^-) with 0.05 M NH_4HCO_3 as the eluent gave, after thrice repeated freeze-drying, **8** as its diammonium salt. Transformation into the free dicarboxylic acid was achieved by passing the aq solution of the salt through a column of Amberlite IR 120 (H^+) at 4°C and subsequent lyophilization. Yield 9.1 mg (53%, for **8** · 1.5 H_2O); R_f 0.06 (A), 0.24 (5:2 *n*-propanol–H_2O); $[\alpha]_D^{20}$ +74.3°. Negative FABMS: *m/z* 675 [49%, $(M-H)^-$], 338 [100%, (monomer–H)$^-$]. Anal. Calcd for $C_{24}H_{40}N_2O_{16}S_2 \cdot 1.5\ H_2O$ (703.73): C, 40.96; H, 6.16; N, 3.98; S, 9.11%. Found: C, 40.50; H, 6.17; N, 4.05; S, 7.94%.

Sialidase experiments.—Glycoside **2**, **3**, and Neu5Ac-2αMe as the reference (~ 2 mg each, ~ 5.5 μmol) was dissolved in water (30 μL) and the solution was brought to pH ~ 5 by addition of 0.1 N NaOH (~ 50 μL). Sodium acetate buffer (0.1 N, 50 μL) and 30 mU of *Arthrobacter ureafaciens* sialidase were added, and the mixture was incubated at 37°C. After 7 h, TLC (B) showed Neu5Ac-2αMe to be completely transformed into Neu5Ac, whereas the ratios **2**/**5** (R_f 0.32 vs. 0.23) and **3**/**6** (R_f 0.32 vs. 0.18) were ~ 1:2 to ~ 1:3. For *S*-acetyl compounds **2** and **5**, acetyl migration occurred as the main side reaction.

Aldolase experiments.—The solution of **5**, **6**, and Neu5Ac as the reference (~ 2.5 mg each, ~ 7.5 μmol) in water (40 μL) was brought to pH ~ 7 by addition of 0.1 N NaOH (~ 70 μL), and 50 mU of Neu5Ac aldolase were added. After 4 h at 37°C, according to TLC (B) ~ 80% of Neu5Ac was cleaved ($R_{f,\text{Neu5Ac}}$ 0.13 vs. $R_{f,\text{ManNAc}}$ 0.50), ~ 50% of **5** (R_f 0.23) was split (*N*-acetyl-6-*S*-acetyl-6-thiomannosamine, R_f 0.69), and from **6** ~ 50% of *N*-acetyl-6-thiomannosamine (R_f 0.58) was formed. In the case of **5**, TLC showed additional spots due to *N*-acetyl-8-*O*-acetylneuraminic acid and *N*-acetyl-6-thiomannosamine.

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