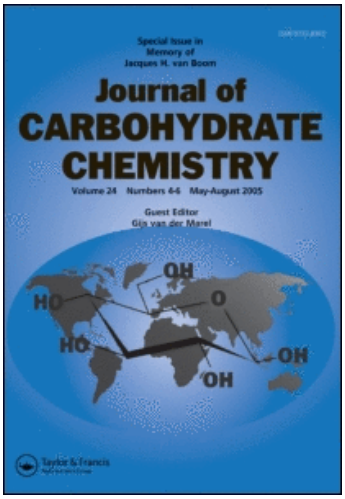

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Stereoselective Synthesis of 5'-*S*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-and-β-D-*Galacto*-2-nonulopyranosylonic Acid)-5'-thio Cytidixe

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STEREOSELECTIVE SYNTHESIS OF 5'-S-(5-ACETAMIDO-3,5-DIDEOXY-D-GLYCERO-α- AND -β-D-GALACTO-2-NONULOPYRANOSYLONIC ACID)-5'-THIO-CYTIDINE*

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ABSTRACT

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-glycero-β-D-galacto-2-nonulopyranosonate (1) underwent displacement with fluorine ion, to yield, after 2-S-acetylation, methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-S-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosonate (4), which was converted, by selective S-deacetylation, into the corresponding sodium salt (6). Condensation of the α- and β-sodium salts (5 and 6) with 2',3'-di-O-acetyl-N^4-benzoyl-5'-bromo-5'-deoxycytidine (9), derived from N^4-benzoylcytidine (7) in two steps, gave their respective coupled products. These were converted by O-deacetylation, N^4-debenzoylation, and hydrolysis of the methyl ester group, into the title compounds.

INTRODUCTION

Cytidine-5'-monophosphono-N-acetylneuraminic acid (CMP-β-Neu5Ac)[2] is known as a donor of sialic acid in the biosynthesis

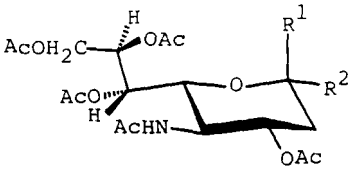
*Studies on the thioglycosides of N-acetylneuraminic acid, Part 3. For Part 2, see ref. 1. Presented at the 13th International Carbohydrate Symposium, Ithaca, New York, U.S.A., August 10-15, 1986.

of glycoconjugates, and contains a β-glycosidic linkage between the phosphate and the anomeric hydroxyl group at C-2 of the sialic acid. Sialyltransferases transfer sialic acids from CMP-β-Neu5Ac to sugar acceptors, to form α-glycosidic linkage. Analogs and derivatives of CMP-β-Neu5Ac are of interest as inhibitors and model substrates in studies of sialyltransferases. In previous papers,[1,3] we demonstrated the stereoselective and high yield synthesis of a series of alkyl α-thioglycosides of N-acetylneuraminic acid, and synthesis of disaccharides, 6-S-(N-acetyl-α-D-neuraminyl)-D-hexopyranosides. We now describe a stereoselective synthesis of 5'-S-(5-acetamido-3,5-dideoxy-D-glycero-α- or -β-D-galacto-2-nonulopyranosylonic acid)-5'-thiocytidine.

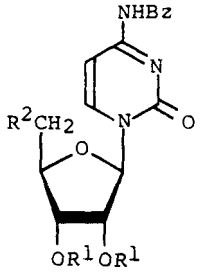
RESULTS AND DISCUSSION

For the synthesis of 5'-S-(5-acetamido-3,5-dideoxy-D-glycero-α- and -β-D-galacto-2-nonulopyranosylonic acid)-5'-thiocytidine, we employed the sodium salts (**5** and **6**) of α- and β-2-thio-Neu5Ac derivatives as the glycosyl donor, and as the acceptor, 2',3'-di-O-acetyl-N^4-benzoyl-5'-bromo-5'-deoxycytidine (**9**).

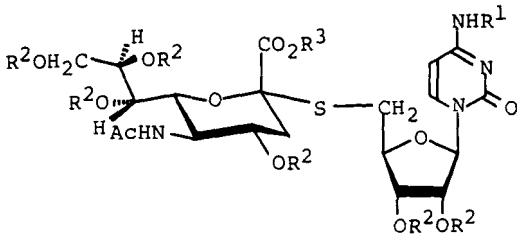
The α-anomer (**2**) was easily prepared from methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-glycero-β-D-galacto-2-nonulopyranosonate[4] (**1**), according to the procedure described in our previous paper[3] {$[\alpha]_D$ +30.5° (chloroform), lit. $[\alpha]_D$ -15.6°}. The β-anomer was prepared in the following way. Treatment of **1** with silver fluoride in dry acetonitrile in the dark gave methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-fluoro-2,3,5-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosonate[5] (**3**) in 93% yield, which was treated with thioacetic acid in the presence of boron trifluoride-diethyl ether complex in dry dichloromethane afforded crystalline methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-S-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosonate (**4**) in 72% yield. Significant signals in the ^{1}H-NMR spectrum were a one-proton doublet of doublets at δ 2.52 ($J_{3a,3e}$ 13.6, $J_{3e,4}$ 4.8 Hz, H-3e) and one-proton multiplet at δ 5.14 for H-4 proton, for the β-2-S-anomer [for the α-2-S-acetyl-anomer[3]: δ 2.63 (H-3e) and 4.89 (H-4); for the β-2-O-acetyl derivative[8]: δ 2.47 (H-3e) and 5.28 (H-4)], and a three



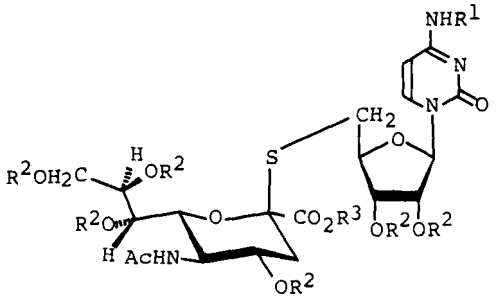
1 R^1 = Cl, R^2 = CO_2Me
2 R^1 = CO_2Me, R^2 = SAc
3 R^1 = CO_2Me, R^2 = F
4 R^1 = SAc, R^2 = CO_2Me
5 R^1 = CO_2Me, R^2 = SNa
6 R^1 = SNa, R^2 = CO_2Me



7 R^1 = H, R^2 = OH
8 R^1 = H, R^2 = Br
9 R^1 = Ac, R^2 = Br



10 R^1 = Bz, R^2 = Ac, R^3 = Me
11 R^1 = R^2 = H, R^3 = Me
12 R^1 = R^2 = R^3 = H



13 R^1 = Bz, R^2 = Ac, R^3 = Me
14 R^1 = R^2 = H, R^3 = Me
15 R^1 = R^2 = R^3 = H

proton singlet at δ 2.34 (S-acetyl). Other NMR data are given in the Experimental section, and are consistent with structure 4.

Selective bromination[6] of the primary hydroxyl group in N^4-benzoylcytidine[7] (7) with carbon tetrabromide in the presence of triphenylphosphine gave N^4-benzoyl-5'-bromo-5'-deoxycytidine (8), which was acetylated with acetic anhydride and pyridine to give 2',3'-di-O-acetyl-N^4-benzoyl-5'-bromo-5'-deoxycytidine (9).

S-Deacetylation of 2 or 4 with the amount of sodium methoxide calculated to be equivalent to 2 or 4 in dry methanol at -40 °C, respectively gave the sodium salts (5 and 6) of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α- and -β-D-galacto-2-nonulopyranosonate, which were used for the next reaction without further purification. Treatment of compound 5 with 9 in dry N,N-dimethylformamide (DMF) under nitrogen atmosphere at room temperature gave 2',3'-di-O-acetyl-N^4-benzoyl-5'-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-5'-thiocytidine (10) in 65% yield, after purification, on the basis of 2. The structure was unambiguously proved by ^{1}H-NMR spectroscopy. The NMR spectrum of 10 exhibited eight sharp singlets, each integrating for three protons, which demonstrated the presence of one methyl ester (δ 3.80), six O-acetyl (δ 2.19-2.03), and one N-acetyl group (δ 1.88); H-3e appeared at δ 2.77 ($J_{3a,3e}$ 12.5, $J_{3e,4}$ 4.8 Hz; Neu5Ac unit) as a doublet of doublets, and H-4 (Neu5Ac unit) at δ 4.97 as multiplet, indicating the α-glycosidic linkage.[1,3,9-11]

In a similar way, condensation of the sodium salt (6) of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosonate with 9 at 50 °C yielded 2',3'-di-O-acetyl-N^4-benzoyl-5'-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate)-5'-thiocytidine (13) in 91% yield significant; signals in the ^{1}H-NMR spectrum were a one-proton doublet of doublets for H-3e at δ 2.52 ($J_{3a,3e}$ 12.3, $J_{3e,4}$ 4.5 Hz; Neu5Ac unit) and H-4 (Neu5Ac unit) at δ 5.58-5.47 as multiplet, which are characteristic for the β-glycoside of Neu5Ac.[11-13] Other NMR data are consistent with structure 13.

Removal of O-acetyl and N^4-benzoyl groups in 10 or 13 with methanolic sodium methoxide gave 5'-S-(methyl 5-acetamido-3,5-

dideoxy-D-glycero-α- and -β-D-galacto-2-nonulopyranosylonate)-5'-thiocytidine (11 and 14) in quantitative yields, respectively. The observed chemical shifts and coupling constants for H-3e of each compound were δ 2.79 ($J_{3a,3e}$ 12.8, $J_{3e,4}$ 4.4 Hz) in 11 and δ 2.50 ($J_{3a,3e}$ 13.9, $J_{3e,4}$ 4.8 Hz) in 14, indicating respectively the configuration of the glycosidic linkage. Finally, hydrolysis of the methyl ester in 11 and 14 with 0.2M potassium hydroxide yielded the desired 5'-S-(5-acetamido-3,5-dideoxy-D-glycero-α- and -β-D-galacto-2-nonulopyranosylonic acid)-5'-thiocytidine (12 and 15) in quantitative yields, respectively.

EXPERIMENTAL

General Procedures. Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union MP-201 polarimeter at 25°C, and IR spectra were recorded with a Jasco IR-1 spectrophotometer. ^{1}H-NMR spectra were recorded at 270 MHz with a Jeol JNM-GX270 spectrometer, and the NMR data were confirmed by use of decoupling techniques. Preparative chromatography was performed on silica gel (Wako Co.; 200 mesh) with the solvent systems specified. Concentrations were conducted in vacuo.

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-fluoro-2,3,5-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (3). To a stirred solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-2,3,5-trideoxy-D-glycero-β-D-galacto-2-nonulopyranosonate[4] (1; 550 mg) in dry acetonitrile (2.5 mL) was added silver fluoride (150 mg), and the mixture was stirred for 3 h at room temperature in the dark; the progress of the reaction being monitored by TLC (ethyl acetate). The mixture was filtered through Celite (No. 545) and the residue washed with dichloromethane. The filtrate and washings were combined and concentrated to a syrup which was extracted with dichloromethane. The extract was washed with a saturated sodium thiosulfate solution, water, and a saturated sodium chloride solution, dried (sodium sulfate), and concentrated to a syrup, which was purified by chromatography on a column of silica gel (40 g) with

4:1 ethyl acetate-hexane, to give 3 (490 mg, 91.3%) as a colorless crystalline mass, which was recrystallized from ether-hexane; mp 45-47°, $[\alpha]_D$ -16.2° (c 0.72, dichloromethane); IR (Nujol): 3300 (NH), 1760 and 1230 (ester), and 1670 and 1550 cm^{-1} (amide); NMR data ($CDCl_3$): δ 5.67 (d, 1H, $J_{5,NH}$ 9.2 Hz, NH), 5.32 (2m, 2H, H-7,8), 5.22 (ddd, 1H, $J_{3a,4} = J_{4,5}$ = 9.2, $J_{3e,4}$ 5.5 Hz, H-4), 4.37 (dd, 1H, $J_{9,9'}$ 12.5 Hz, H-9), 4.24 (d, 1H, $J_{5,6}$ 11.0 Hz, H-6), and 4.12 (dd, 1H, H-9'), 4.11 (q, 1H, H-5), 3.85 (s, 3H, MeO), 2.70 (ddd, 1H, $J_{3a,3e}$ 13.9, $J_{3e,F}$ 8.8 Hz, H-3e), 2.15, 2.09, 2.05, 2.04 (4s, 12H, 4AcO) and 1.92 (s, 3H, AcN).

Anal. Calcd for $C_{20}H_{28}FNO_{12}$: C, 48.68; H, 5.71; N, 2.84. Found: C, 48.55; H, 5.81; N, 2.79.

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 (4). To a stirred solution of 3 (200 mg) in dichloromethane (5 mL) were added thioacetic acid (0.44 mL) and boron trifluoride-diethyl ether complex (0.15 mL) at room temperature, and the stirring was continued for 10 h. The mixture was extracted with dichloromethane, and the extract was washed with water, dried (sodium sulfate), and concentrated to a syrup. The product was purified by chromatography on a column of silica gel (30 g) with 4:1 ethyl acetate-hexane to give 4 (160 mg, 72%), which was crystallized from ether-hexane; mp 140-142°, $[\alpha]_D$ -77.4° (c 0.67, chloroform); IR (KBr): 3300 (NH), 1750 and 1240 (ester), and 1660 and 1570 cm^{-1} (amide); NMR ($CDCl_3$): δ 5.39 (dd, 1H, $J_{6,7}$ 2.2, $J_{7,8}$ 4.0 Hz, H-7), 5.35 (d, 1H, $J_{5,NH}$ 10.3 Hz, NH), 5.14 (ddd, 1H, $J_{3e,4}$ 4.8, $J_{4,5} = J_{3a,4}$ = 10.3 Hz, H-4), 5.00 (ddd, 1H, $J_{8,9}$ 7.0, $J_{8,9'}$ 2.2 Hz, H-8), 4.62 (dd, 1H, $J_{5,6}$ 10.3 Hz, H-6), 4.14 (dd, 1H, H-9), 4.09 (q, 1H, H-5), 3.84 (s, 3H, MeO), 2.52 (dd, 1H, $J_{3a,3e}$ 13.6 Hz, H-3e), 2.34 (s, 3H, AcS), 2.15, 2.07, 2.04, 2.03 (4s, 12H, 4AcO), and 1.89 (s, 3H, AcN).

Anal. Calcd for $C_{22}H_{31}NO_{13}S$: C, 48.08; H, 5.68; N, 2.55. Found: C, 48.13; H, 5.62; N, 2.43.

2',3'-Di-O-acetyl-N^4-benzoyl-5'-bromo-5'-deoxycytidine (9). To a solution of N^4-benzoylcytidine[7] (7; 1.0 g) in dry N,N-dimethylacetamide (13 mL) were added, with stirring, carbon tetrabromide (2.7 g) and triphenylphosphine (2.2 g) at 0°C, and the mixture was stirred for 3.5 h at room temperature; the progress of the reaction being

monitored by TLC (5:1 dichloromethane-methanol). Methanol (1 mL) was added to the mixture, and concentrated to a syrup, which was chromatographed on a column of silica gel (100 g) with chloroform and then 50:1 chloroform-methanol. The latter eluate gave N^4-benzoyl-5'-bromo-5'-deoxycytidine (**8**) as crystals, which was acetylated with acetic anhydride (2 ml) and pyridine (3 mL) at room temperature overnight, to afford **9** (620 mg, 51%) as an amorphous mass; mp 74-76°, $[\alpha]_D$ +23.2° (c 0.36, chloroform); IR (film): 3330 (NH), 1760 and 1240 (ester), 1630 and 1560 (amide), and 1680 cm^{-1} (-C=N); NMR ($CDCl_3$): δ 8.09, 7.58 (2d, 2H, $J_{5,6}$ 7.3 Hz, H-5,6), 7.94-7.48 (m, 5H, Ph), 6.20 (d, 1H, $J_{1',2'}$ 5.1 Hz, H-1), 5.50 (t, 1H, $J_{2',3'}$ 5.3 Hz, H-2), 5.40 (t, 1H, $J_{3',4'}$ 5.3 Hz, H-3), 4.44 (q, 1H, $J_{4',5'a} = J_{4',5'b}$ = 4.0 Hz, H-4'), 3.74 (dd, 1H, $J_{5'a,5'b}$ 11.5 Hz, H-5'a), 3.80 (dd, 1H, H-5'b), 2.11 and 2.09 (2s, 6H, 2AcO).

Anal. Calcd for $C_{20}H_{20}N_3O_7Br$: C, 48.59; H, 4.07; N, 8.50. Found: C, 48.47; H, 4.22; N, 8.43.

2',3'-Di-O-acetyl-N^4-benzoyl-5'-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl-onate)-5'-thiocytidine (**10**). To a stirred solution of **2**[3] (88 mg) in dry methanol (1 mL) was added a solution of sodium metal (3 mg) in dry methanol (1 mL) at -40°C; within 10 min, TLC (ethyl acetate) showed all of the starting material had been converted into the salt (5). The mixture was concentrated to an amorphous mass, which was dissolved in dry DMF (1 mL). To the stirred solution was added, at 0°C, a solution of **9** (100 mg) in dry DMF (1 mL), and the mixture was stirred overnight at room temperature under nitrogen atmosphere. The mixture was concentrated to a syrup, which was extracted with dichloromethane. The extract was washed with water, dried (sodium sulfate), and concentrated to a syrup, which was chromatographed on a column of silica gel (15 g) with (a) dichloromethane, (b) 150:1, and (c) 50:1 dichloromethane-methanol. The eluant (c) gave **10** (96 mg, 65%) as a syrup; $[\alpha]_D$ +41° (c 0.5, chloroform); IR (film): 3300 (NH), 1740 and 1240 (ester), 1660 (-C=N), and 1630 and 1560 cm^{-1} (amide); NMR ($CDCl_3$): Neu5Ac unit: δ 5.52 (d, 1H, $J_{5,NH}$ 9.5 Hz, NH), 5.40 (m, 1H, H-8), 5.34 (dd, 1H, $J_{6,7}$ 1.8, $J_{7,8}$ 9.2 Hz, H-7), 4.97 (m, 1H, H-4), 4.26 (dd, 1H, $J_{8,9'}$ 2.4, $J_{9,9'}$ 12.6 Hz, H-9'), 4.09 (dd, 1H, $J_{8,9}$ 5.0 Hz, H-9), 3.84 (dd, 1H, $J_{5,6}$ 11.0 Hz, H-6),

3.80 (s, 3H, MeO), 2.77 (dd, 1H, $J_{3a,3e}$ 12.5, $J_{3e,4}$ 4.8 Hz, H-3e), and 1.88 (s, 3H, AcN); cytidine unit: δ 8.16 (d, 1H, $J_{5,6}$ 7.3 Hz, H-6), 7.60 (d, 1H, H-5), 7.94-7.49 (m, 5H, Ph), 6.29 (d, 1H, $J_{1',2'}$ 5.9 Hz, H-1'), 5.44 (near t, 1H, $J_{2',3'}$ 5.9 Hz, H-2'), 5.26 (dd, 1H, $J_{3',4'}$ 4.4 Hz, H-3'), 4.32 (near t, 1H, H-4'), 3.23 (dd, 1H, $J_{4',5'b}$ 5.1, $J_{5'a,5'b}$ 12.6 Hz, H-5'b), and 3.09 (dd, 1H, $J_{4',5'a}$ 6.6 Hz, H-5'a); other groups: δ 2.19, 2.17, 2.12, 2.07, 2.04, and 2.03 (6s, 18H, 6AcO).

Anal. Calcd for $C_{40}H_{48}N_4O_{19}S$: C, 52.17; H, 5.25; N, 6.08. Found: C, 52.26; H, 5.34; N, 6.00.

5'-S-(Methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-5'-thiocytidine (11). To a solution of 10 (72 mg) in dry methanol (1 mL) was added a small amount of sodium methoxide at -10 °C, and the mixture was stirred for 1 h at room temperature; at that time, all of the starting material had been converted into 11. The mixture was treated with Amberlite IR-120 (H^+) resin to remove the base, and concentrated to give 11 as a syrup in quantitative yield, which showed a single spot in TLC; $[\alpha]_D$ +18.5° (c 0.5, methanol); IR (film): 3400-3300 (OH, NH), 1730 and 1200 (ester), 1660 (-C=N), 1630 and 1560 cm^{-1} (amide); NMR (1:1 D_2O-CD_3OD): Neu5Ac unit: δ 3.79 (s, 3H, MeO), 3.68 (m, 1H, H-4), 2.79 (dd, 1H, $J_{3a,3e}$ 12.8, $J_{3e,4}$ 4.4 Hz, H-3e), 1.99 (s, 3H, AcN), and 1.84 (t, 1H, $J_{3a,4}$ 12.8 Hz, H-3a); cytidine unit: δ 7.69 (d, 1H, $J_{5,6}$ 7.7 Hz, H-6), 6.08 (d, 1H, H-5), 5.80 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.28 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 4.4 Hz, H-3), 3.20 (dd, 1H, $J_{4',5'b}$ 3.3, $J_{5'a,5'b}$ 14.7 Hz, H-5'b), and 3.08 (dd, 1H, $J_{4',5'a}$ 4.8 Hz, H-5'a).

Anal. Calcd for $C_{21}H_{32}N_4O_{12}S$: C, 44.67; H, 5.71; N, 9.92. Found: C, 44.45; H, 5.87; N, 9.83.

5'-S-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-5'-thiocytidine (12). To a solution of 11 (25 mg) in 1,4-dioxane (1 mL) was added, with stirring, 0.2M potassium hydroxide (0.1 mL), and the mixture was stirred for 10 min at room temperature; the course of the reaction being monitored by TLC. The mixture was treated with Amberlite IR-120 (H^+) resin to remove the base, and the resin was filtered off, and washed with water. The filtrate and washings were combined, and lyophilized, to afford 12 as a syrup in quantitative yield, which showed a single spot in TLC;

$[\alpha]_D$ +21.9° (c 0.28, H_2O); IR (KBr): 3450-3350 (OH, NH), 1730 and 1700 (C=O), 1680 (-C=N), 1640 and 1560 cm^{-1} (amide); NMR (D_2O): δ 7.86, 6.19 (2d, 2H, -CH=CH-), 5.77 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 3.67 (m, 1H, H-4), 2.75 (dd, 1H, $J_{3a,3e}$ 12.8, $J_{3e,4}$ 4.4 Hz, H-3e), 1.96 (s, 3H, AcN), and 1.78 (t, 1H, $J_{3a,4}$ 12.3 Hz, H-3a).

Anal. Calcd for $C_{20}H_{30}N_4O_{12}S$: C, 43.63; H, 5.49; N, 10.18. Found: C, 43.68; H, 5.56; N, 9.97.

2',3'-Di-O-acetyl-N^4-benzoyl-5'-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl-onate)-5'-thiocytidine (13). To a stirred solution of 4 (135 mg) in dry methanol (2 mL) was added a solution of sodium metal (5.6 mg) in dry methanol (2 mL) at -40 °C; within 10 min, TLC (ethyl acetate) showed all of the starting material had been converted into the salt (6). The mixture was concentrated to an amorphous mass, which was dissolved in dry DMF (1 mL). To the stirred solution was added a solution of compound 9 (151 mg) in dry DMF (1 mL) at 0 °C, and the mixture was heated at 50 °C, with stirring, overnight under nitrogen atmosphere. The mixture was concentrated to a syrup, which was extracted with dichloromethane. The extract was washed with water, dried (sodium sulfate), and concentrated to a syrup, which was chromatographed on a column of silica gel (20 g) with (a) dichloromethane, (b) 150:1, and (c) 50:1 dichloromethane-methanol. The eluant (c) gave 13 (210 mg, 91%) as a syrup; $[\alpha]_D$ -8.4° (c 0.53, dichloromethane); IR (KBr): 3300 (NH), 1750 and 1240 (ester), and 1660 and 1550 cm^{-1} (amide); NMR ($CDCl_3$): Neu5Ac unit: δ 5.58-5.48 (m, 3H, H-4,7,8), 5.29 (d, 1H, $J_{5,NH}$ 10.3 Hz, NH), 4.93 (dd, 1H, $J_{8,9}$ 2.0, $J_{9,9'}$ 10.3 Hz, H-9'), 4.57 (dd, 1H, $J_{5,6}$ 10.5, $J_{6,7}$ 2.0 Hz, H-6), 4.18 (q, 1H, $J_{4,5}$ 10.3 Hz, H-5), 4.10 (dd, 1H, H-9), 3.81 (s, 3H, MeO), 2.54 (dd, 1H, $J_{3a,3e}$ 12.3, $J_{3e,4}$ 4.5 Hz, H-3e), and 1.90 (s, 3H, AcN); cytidine unit: δ 8.04-7.49 (m, 5H, Ph), 7.87 (d, 1H, $J_{5,6}$ 7.3 Hz, H-6), 7.51 (d, 1H, H-5), 7.33 (d, 1H, NH), 6.19 (d, 1H, $J_{1',2'}$ 5.1 Hz, H-1'), 5.41 (near t, 1H, $J_{2',3'}$ 5.5 Hz, H-2'), 5.22 (t, 1H, $J_{3',4'}$ 5.5 Hz, H-3'), 4.39 (q, 1H, $J_{4',5'a} = J_{4',5'b}$ = 5.0 Hz, H-4'), 3.21 (dd, 1H, $J_{5'a,5'b}$ 16.0 Hz, H-5'a), and 3.12 (dd, 1H, H-5'b); other groups: δ 2.16, 2.12, 2.11, 2.06, and 2.03(2) (5s, 18H, 6AcO).

Anal. Calcd for $C_{40}H_{48}N_4O_{19}S$: C, 52.17; H, 5.25; N, 6.08. Found: C, 52.30; H, 5.33; N, 6.13.

5'-S-(Methyl 5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate)-5'-thiocytidine (14). Compound 14 was obtained from 13 as crystals in quantitative yield, according to the procedure described for 11; mp 158-159°, $[\alpha]_D$ -64.1 (c 0.41, methanol); IR (KBr): 3400-3200 (OH, NH), 1730 and 1200 (ester), 1660 (-C=N), 1620 and 1560 cm^{-1} (amide); NMR (D_2O): δ 7.55, 6.03 (2d, 2H, -CH=CH-), 5.75 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1'), 3.75 (s, 3H, MeO), 2.50 (dd, 1H, $J_{3a,3e}$ 13.9, $J_{3e,4}$ 4.8 Hz, H-3e), and 2.00 (s, 3H, AcN).

Anal. Calcd for $C_{21}H_{32}N_4O_{12}S$: C, 44.67; H, 5.71; N, 9.92. Found: C, 44.65; H, 5.83; N, 9.85.

5'-S-(5-Acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonic acid)-5'-thiocytidine (15). Compound 15 was obtained from 14 as crystals in quantitative yield, according to the procedure described for 12; mp 209-211°, $[\alpha]_D$ -32.0° (c 0.5, 1:1 methanol-water); IR (KBr); 3430-3360 (OH, NH), 1730 and 1700 (C=O), 1680 (-C=N), 1640 and 1560 cm^{-1} (amide); NMR (D_2O): δ 7.81, 6.20 (2d, 2H, -CH=CH-), 5.76 (d, 1H, $J_{1',2'}$ 2.8 Hz, H-1'), 2.48 (dd, 1H, $J_{3a,3e}$ 13.9, $J_{3e,4}$ 4.8 Hz, H-3e), and 1.99 (s, 3H, AcN).

Anal. Calcd for $C_{20}H_{30}N_4O_{12}S$: C, 43.63; H, 5.49; N, 10.18. Found: C, 43.50; H, 5.72; N, 9.98.

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