

Synthesis of the α -Methyl Ketoside of 5-Amino Neuraminic Acid Methyl Ester and Its Corresponding 5-Myristoyl and 5-Cyclopropanoyl Derivative

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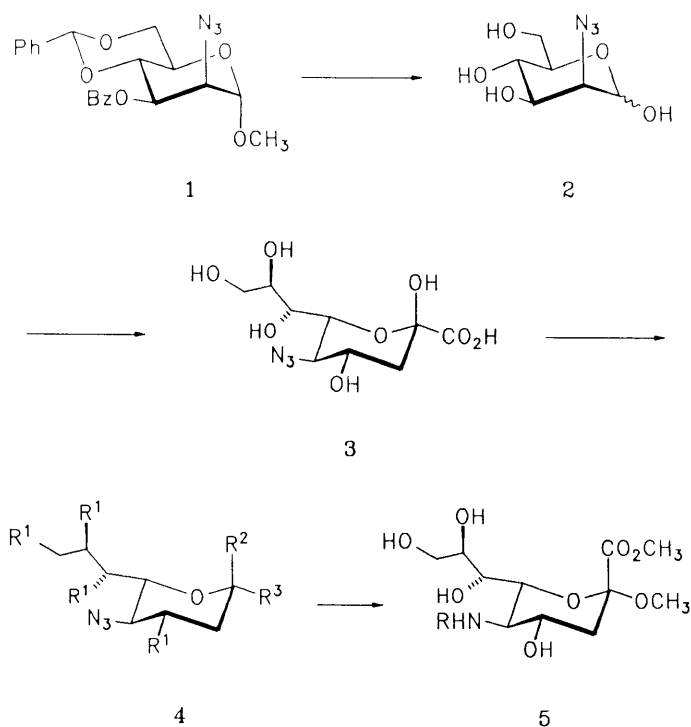
This communication describes the first synthesis of the α -methyl ketoside of an *N*-unprotected neuraminic acid in the form of its methyl ester (Neu5NH₂1Me- α -2Me) (**5a**). This compound is a valuable intermediate for the synthesis of unnatural

N-substituted sialic acids. Syntheses of Neu5myristoyl1Me- α -2Me (**5b**) and Neu5cyclopropanoyl1Me- α -2Me (**5c**) are presented.

Sialic acids are involved in a large variety of cellular functions as components of glycoproteins, gangliosides and polysaccharides^{1,2}. They are essential parts of the carbohydrate structures of mammalian cell membranes and are important in molecular recognition. We are particularly interested in their role in the infection of mammalian cells by influenza virus. The initial event in infection involves binding of the membrane haemagglutinin (HA) of the virus to the surface of the cells³. Terminal sialic acid glycosides of cell-surface glycoproteins and glycolipids and the viral HA are the only components necessary for this interaction between virus and cell.

After the crystal structure of the HA-sialic acid complex became available^{4,5}, several programs have been started with the object of modifying 5-acetylneuraminic acid in order to increase its affinity to the recognition site of the viral haemagglutinin. Successful synthesis of compounds capable of binding to the sialic acid recognition site of HA with high affinity would suggest possible approaches to the development of drugs against influenza that would work by blocking the binding of virus to cell. A number of research groups have synthesized sialic acids with structural variations in the side chain (mainly to investigate their behaviour toward CMP-sialate synthase)^{6,7}. Many of these compounds have also been screened for binding to HA, but no strongly binding materials have been discovered. We have been particularly interested in neuraminic acids with new substituents in the C-5 position. Therefore, we focused our work on neuraminic acids having small residues at the C-5 position and those substituted with fatty acids. The former were interesting because the crystal structure suggested unoccupied space in the vicinity of Trp-153 in the complex of HA with *N*-acetyl neuraminic acid. The latter were to investigate the effect of non-specific hydrophobic interactions between the alkyl chains and non-polar regions of the HA close to the sialic acid binding site. Because binding to haemagglutinin shows an absolute requirement for a α -glucoside structure⁴, we have developed a practical synthesis of a chemically stable sialic acid intermediate as an α -anomer with a free amino function at C-5.

Due to the ease of its transformation to free amines, the azide group represents a valuable intermediate functionality. An enzymatic approach using *N*-acetylneuraminate pyruvate lyase (E.C. 1.4.3.3) to 5-azidoneuraminic acid seemed to be particularly appropriate. Our own investigations, as well as those by Augé et al., had already shown that 2-azido-2-deoxymannopyranose is a good substrate for the *N*-acetylneuraminate pyruvate lyase^{8,9}.



4	R ¹	R ²	R ³	5	R
a	OH	OH	CO ₂ Me	a	H
b	OAc	OAc	CO ₂ Me	b	myristoyl
c	OAc	Cl	CO ₂ Me	c	cyclopropanecarbonyl
d	OAc	CO ₂ Me	OMe		

Employing a synthetic strategy developed independently from that of Augé et al., we obtained free 2-azidomannopyranose **2** in 45% yield after treatment of the 2-azido- α -mannopyranoside **1** with sodium methoxide followed by neutralization with a weakly acidic ion exchange resin and reaction with 6 M hydrogen chloride/acetic acid solution. The enzyme-catalyzed condensation to the 5-azidoneuraminic acid (**3**) was carried out with 10 equivalents of sodium pyruvate in 5 mM tris buffer at pH 7.5, containing 0.1% sodium azide, at 37 °C. In analogy to a procedure of Vasella et al.¹⁰ we produced the methyl ester **4a** by stirring Neu5Az **3** in methanol in the presence of a catalytic amount of trifluoroacetic acid. The subsequent reaction with acetic anhydride/DMAP in pyridine led to the pentaacetyl derivative **4b**. Chloride **4c** was prepared from the acetyl compound **4b** by treatment of **4b** at -40 °C in dry ether with anhydrous hydrogen chloride. This reaction furnished **4c** in an almost quantitative yield. The conversion of **4c** into the α -ketoside **4d** was accomplished in dry methanol using catalysis by freshly prepared silver carbonate¹¹ in 83% yield. From the α -anomer **4d**, we obtained the desired α -methyl ketoside **5a** of neuraminic acid with an unprotected amino group as its methyl ester in more than 90% yield after deacylation and reduction with hydrogen and palladium on carbon. The reduction step could also be performed with 1,3-propanedithiol in the presence of triethylamine in comparable yields^{12a}. This latter system is much milder than the palladium-catalyzed hydrogenolysis and permits certain useful selective reactions (e.g. reduction of azides to free amines in the presence of a Cbz-protected amine^{12b}). The final acylation of the free amine group was carried out either according to standard procedures in pyridine with catalysis by DMAP or after conversion of the acylation reagent into the corresponding *N*-hydroxysuccinimide ester. The *N*-myristoyl and the *N*-cyclopropanoyl derivatives of neuraminic acid and other *N*-acyl analogs of this substance are currently being tested for their activity in binding to HA.

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Experimental

Chemicals were purchased from Aldrich and were reagent grade. Solvents were dried and distilled before use. Biochemicals were purchased from Sigma. Neu5Ac aldolase (E.C. 1.4.3.3.) was obtained from Toyobo. Analytical thin-layer chromatography was performed on Merck plates, Kieselgel 60 F₂₅₄, having 0.25 mm layer thickness. Flash chromatography was performed using Merck Kieselgel 60 with 0.04–0.063 mm thickness. Compounds that were not visualized by UV were detected by treatment with a solution of *p*-anisaldehyde in a mixture of ethanol/sulfuric acid/acetic acid or by spraying with a solution of 3% Ce(SO₄)₂ in 2 N H₂SO₄ followed by heating to 200 °C. ¹H-NMR spectra were recorded on Bruker AM 300, AM 400 or AM 500 spectrometers.

Methyl 2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- α -D-mannopyranoside (**1**) was obtained in four steps starting from α -methyl glucopyranoside according to literature procedures^{13–16}.

2-Azido-2-deoxymannopyranose (2): A solution of 7.4 g (18 mmol) methyl 2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene- α -2-deoxy-D-man-

nopyranoside (**1**) in a mixture of 150 ml of methanol and 50 ml of 1,2-dichloromethane was treated with 35 ml of a 1 M sodium methoxide solution at 40 °C. After 3 h the solution was cooled to 5 °C, quenched by addition of 20 ml of a weakly acidic ion exchange resin (Amberlite CG-50, capacity 3.5 meq/ml) and stirred for 1 h. The reaction mixture was filtered to afford a solution, which was concentrated and co-evaporated three times with water to remove the methyl benzoate. To the resulting oil, a mixture of 98.5 ml of acetic acid, 65 ml of concentrated HCl and 32 ml of water was added and the solution was heated to 45–50 °C. After 72 h the reaction mixture was cooled to room temperature and evaporated under high vacuum to give a residue, which was purified by flash chromatography [150 g of silica gel, solvent: toluene/ethanol (4:1)] to afford 1.66 g (45%) of 2-azidomannose **2** as a yellow foam. The 500-MHz ¹H-NMR spectrum of this material corresponded with that reported in ref.⁹; [α]_D²⁰ = 24.4 (*c* = 5 in methanol; ref.⁹ [α]_D²⁰ = 14.4 (*c* = 5 in methanol)).

5-Azido-3,5-dideoxy- β -D-glycero-D-galacto-2-nonulopyranosidonic Acid (Neu5Az, 3): Sodium pyruvate (4.74 g, 43 mmol) was dissolved in 15 ml of tris buffer (50 mM in tris and 15.6 mM in sodium azide, pH 7.5) and treated with a solution of 885 mg (4.3 mmol) of 2-azido-2-deoxymannose (**2**) in 6 ml of the same buffer solution. The pH of the reaction mixture was adjusted to pH 7.5 with 1 N sodium hydroxide solution. Addition of a suspension of 20 U of Neu5Ac aldolase (E.C. 1.4.3.3) in 1 ml of buffer initiated the conversion at 37 °C. The reaction was monitored by ¹H NMR until signals characteristic for the starting material had completely disappeared. For purification, the whole solution was loaded onto an ion exchange column containing 150 ml of Bio Rad AG 1-X2 (100–200 mesh, formate form, capacity 0.6 meq/ml), washed with water and was then eluted with a volume of 1.3 l of a 0–1.5 M formic acid gradient. Fractions containing the product, detected by TLC [solvent: butanol/acetic acid/water (8:3:3); *R*_f = 0.26], were collected and concentrated under high vacuum to dryness to give 896 mg (70%) of Neu5Az (**3**) as a colorless sirup. The ¹H-NMR data agreed with those in ref.¹; [η]_D²⁰ = -54.8 (*c* = 5 in H₂O).

5-Azido-3,5-dideoxy- β -D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (Neu5Az1Me, 4a): To a solution of 540 mg (1.85 mmol) of Neu5Az **3** in 50 ml of dry methanol was added 100 μ l of trifluoroacetic acid. After stirring the reaction mixture for 24 h, the solvent was removed under reduced pressure to give crude **4a** as a foam. The product was purified by chromatography on a flash column [50 g of silica gel, solvent: ethyl acetate/methanol (9:1)] to separate the ester from more polar impurities. Concentration of the product-containing fractions gave 551 mg of **4a** (1.69 mmol, 91%) as a white microcrystalline powder, after recrystallization from ethyl acetate/methanol, small white needles, m.p. 167 °C. — TLC with ethyl acetate/methanol (9:1); *R*_f = 0.37. — ¹H NMR (400 MHz, D₂O HDO): δ = 1.92 (dd, 1H, 3-H_{ax}), 2.28 (dd, 1H, 3-H_{eq}), 3.53 (dd, 1H, 5-H), 3.65 (dd, 1H, 9-H₃), 3.74 (m, 1H, 8-H), 3.82 (s, 3H, CO₂CH₃), 3.84 (m, 2H, 7-, 9-H₅), 3.96 (dd, 1H, 6-H), 4.1 (m, 1H, 4-H); *J*(3_{ax},3_{eq}) = -12 Hz, *J*(3_{ax},4) = 12 Hz, *J*(3_{eq},4) = 4.5 Hz, *J*(4,5) = 10 Hz, *J*(5,6) = 10 Hz, *J*(6,7) = 1 Hz, *J*(8,9_a) = 5 Hz, *J*(8,9_b) = 2 Hz, *J*(9_a,9_b) = -11.5 Hz. — MS (FAB, 70 eV): *m/z* (%) = 308 (14.5) [M⁺ + 1], 290 (28) [M⁺ - N₂], 217 (100) [M⁺ - C₃O₃H₆], 109 (95).

C₁₀H₁₇N₃O₈ (307.2) Calcd. C 39.09 H 5.57 N 13.67
Found C 38.69 H 5.65 N 13.16

2,4,7,8,9-Penta-*O*-acetyl-5-azido-3,5-dideoxy- β -D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (Neu5Az2,4,7,8,9Ac₅1Me, 4b): To a solution of 100 mg (0.32 mmol) of Neu5Az1Me (**4a**) in 2 ml of pyridine at 5 °C, was added 1 ml of acetic anhydride.

The mixture was warmed to room temperature and stirred until TLC (ethyl acetate/methanol, 9:1; $R_f = 0.84$) showed the disappearance of **4a**. After evaporation of the solvent, the crude material was purified by flash chromatography [25 g of silica gel, hexane/ethyl acetate (1:1)] to yield **4b** (185 mg, 0.35 mmol, 92%) as a colorless foam. — TLC with ethyl acetate/methanol (9:1); $R_f = 0.84$. — $^1\text{H NMR}$ (400 MHz, $\text{CDCl}_3/\text{CHCl}_3$): $\delta = 1.92$ (dd, 1H, 3- H_{ax}), 2.07, 2.1, 2.14, 2.17, 2.22 (5 s, $5 \times 3\text{H}$, COCH_3), 2.67 (dd, 1H, 3- H_{eq}), 3.37 (dd, 1H, 5-H), 3.77 (dd, 1H, 6-H), 3.8 (s, 3H, CO_2CH_3), 4.21 (dd, 1H, 9- H_a), 4.42 (dd, 1H, 9- H_b), 5.22 (m, 2H, 4-, 8-H), 5.54 (dd, 1H, 7-H); $J(3_{ax}, 3_{eq}) = -13$ Hz, $J(3_{ax}, 4) = 11$ Hz, $J(3_{eq}, 4) = 4.5$ Hz, $J(4, 5) = 9.5$ Hz, $J(5, 6) = 9.5$ Hz, $J(6, 7) = 1.5$ Hz, $J(7, 8) = 6.5$ Hz, $J(8, 9_a) = 5$ Hz, $J(8, 9_b) = 2.5$ Hz, $J(9_a, 9_b) = -12.5$ Hz. — MS (FAB, 70 eV); m/z (%) = 458 (11) [$\text{M}^+ - \text{C}_2\text{H}_3\text{O}_2$], 398 (56) [$\text{M}^+ - \text{C}_4\text{H}_4\text{O}_2$], 196 (100), 154 (68).

$\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_{13}$ (517.4) Calcd. C 46.42 H 5.25 N 8.12
Found C 46.63 H 5.34 N 7.79

Methyl 4,7,8,9-tetra-O-Acetyl-5-azido-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (Neu5Az4,7,8,9Ac,1Me- α -2Me, 4d): The pentaacetyl compound **4b** (185 mg, 0.35 mmol) was dissolved in 15 ml of dry ether and cooled to -40°C . The mixture was then treated with a stream of anhydrous HCl gas (generated from ammonium chloride and sulfuric acid) for 60 min. The flask was stoppered tightly and the solution was allowed to reach room temperature. After 12 h, the glycosyl chloride **4c** was isolated as a light yellow oil by evaporation of the solvent with an aspirator and drying the residue under high vacuum. To the crude material were added dry methanol (10 ml) and freshly prepared silver carbonate (400 mg, 1.44 mmol) and the mixture was stirred for 5 h. Filtration through celite, removal of the solvent under reduced pressure and flash chromatography [20 g of silica gel, hexane/ethyl acetate (2.5:1)] resulted in 158 mg (0.32 mmol, 83%) of the α -methyl ketoside **4d** as a white foam. — TLC with hexane/ethyl acetate (1:1); $R_f = 0.56$. — $^1\text{H NMR}$ (500 MHz, $\text{CDCl}_3/\text{CHCl}_3$): $\delta = 1.74$ (dd, 1H, 3- H_{ax}), 2.08, 2.12, 2.17, 2.21 (4 s, $4 \times 3\text{H}$, COCH_3), 2.71 (dd, 1H, 3- H_{eq}), 3.22 (dd, 1H, 5-H), 3.87 (dd, 1H, 6-H), 4.23 (dd, 1H, 9- H_a), 4.33 (dd, 1H, 9- H_b), 4.84 (ddd, 1H, 4-H), 5.44 (m, 1H, 8-H), 5.54 (dd, 1H, 7-H); $J(3_{ax}, 3_{eq}) = -12.5$ Hz, $J(3_{ax}, 4) = 12$ Hz, $J(3_{eq}, 4) = 4.5$ Hz, $J(4, 5) = 10.5$ Hz, $J(5, 6) = 10.5$ Hz, $J(6, 7) = 1.5$ Hz, $J(7, 8) = 9$ Hz, $J(8, 9_a) = 4$ Hz, $J(8, 9_b) = 2.5$ Hz, $J(9_a, 9_b) = -12.5$ Hz. — $^{13}\text{C NMR}$ (125.7 MHz, $\text{CDCl}_3/\text{CHCl}_3$): $\delta = 20.74, 20.85, 21.01$ (3c, CH_3CO), 37.4 (C-3), 52.37, 52.77 (2C, C-5, OCH_3), 60.03, 61.97 (2C, C-9, CO_2CH_3), 67.84, 70.99, 71.44 (4C, C-4, -6, -7, -8), 98.67 (C-2), 167.6, 169.6, 169.74, 169.93, 170.71 (5C, CH_3CO , C-1). — MS (FAB, 70 eV); m/z (%) = 398 (38.5) [$\text{M}^+ - \text{C}_3\text{H}_5\text{O}_2$], 217 (100).

$\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_{12}$ (489.4) Calcd. C 46.62 H 5.56 N 8.58
Found C 47.09 H 5.65 N 8.12

Methyl 5-Amino-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (Neu5NH₂1Me- α -2Me, 5a): — *Reduction of 4d with H₂/Pd/C:* The α -methyl ketoside **4d**, 130 mg (0.26 mmol), was dissolved in 25 ml of dry methanol and treated with 90 μl of 1 M sodium methoxide solution (90 μmol). After stirring for about 12 h, TLC [ethyl acetate/methanol (9:1)] indicated the complete disappearance of the starting material. Methoxide was removed by column filtration and the solvent was evaporated under reduced pressure. Dry methanol (10 ml) and 10 mg of catalyst (10% Pd on activated carbon) were added, the flask was fitted with a balloon of hydrogen, evacuated and purged three times with hydrogen. After 3 h, the solution was filtered through celite, concentrated and purified by flash chromatography on 15 g of silica gel [solvent: ethyl acetate/methanol (1:1)] to afford 72 mg (94%) of

pure 5-amino- α -methyl ketoside **5a**. — TLC with butanol/acetone/water (4:5:1); $R_f = 0.38$. — $^1\text{H NMR}$ (500 MHz, $\text{D}_2\text{O}/\text{H}_2\text{O}$): $\delta = 1.78$ (dd, 1H, 3- H_{ax}), 2.68 (dd, 1H, 3- H_{eq}), 2.84 (dd, 1H, 5-H), 3.42 (s, 3H, OCH_3), 3.58 (ddd, 1H, 8-H), 3.73 (dd, 1H, 7-H), 3.76 (dd, 1H, 9- H_a), 3.88 (dd, 1H, 6-H), 3.93 (s, 3H, CO_2CH_3), 3.93–3.96 (m, 2H, 4-, 9- H_b); $J(3_{ax}, 3_{eq}) = -12$ Hz, $J(3_{ax}, 4) = 12$ Hz, $J(3_{eq}, 4) = 4.5$ Hz, $J(4, 5) = 10$ Hz, $J(5, 6) = 10$ Hz, $J(6, 7) = 1.5$ Hz, $J(7, 8) = 10$ Hz, $J(8, 9_a) = 6.5$ Hz, $J(8, 9_b) = 4$ Hz, $J(9_a, 9_b) = -11.5$ Hz. — MS (FAB, 70 eV); m/z (%) = 296 (47) [$\text{M}^+ + 1$], 264 (8) [$\text{M}^+ - \text{CH}_3\text{O}$], 217 (43), 109 (53), 91 (100).

$\text{C}_{17}\text{H}_{21}\text{NO}_8$ (295.2) Calcd. C 44.74 H 7.16 N 4.74
Found C 44.25 H 7.16 N 4.90

Reduction of 4d with 1,3-Propanedithiol: To a solution of 20 mg (62.5 μmol) of **4d** (deacetylated according to the procedure outlined above) in 2 ml of dry methanol, 34.6 μl (250 μmol) of triethylamine and 25 μl (250 μmol) of 1,3-propanedithiol were added. The reaction mixture was stirred for 24 h at room temp., evaporated to dryness and purified by chromatography on a flash column (5 g of silica gel) with a gradient of ethyl acetate/methanol. The yield of **5a** was 16.6 mg (90%). The analytical data were indistinguishable from those obtained from the product of the hydrogen reduction.

Methyl 3,5-Dideoxy-5-myristoyl- α -D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (Neu5myr1Me- α -2Me, 5b): *N*-hydroxysuccinimide (23 mg, 200 μmol) and 30 μl (215 μmol) of triethylamine were dissolved in 1 ml of dry chloroform. Myristoyl chloride (54.3 μl , 200 μmol) was added at 0°C and the solution was stirred for 1 h. After the addition of sodium bicarbonate (10 mg) and stirring for 30 min at room temp., the mixture was filtered and concentrated in vacuum. The resulting residue was chromatographed on silica gel (6 g) with ethyl acetate as eluant. Fractions containing *N*-myristoylsuccinimide ester were collected, the solvent was removed and the resulting white powder dried in high vacuum. For the acylation of **5a**, the succinimide was then dissolved in 2.5 ml of dry pyridine and 40 mg (135 μmol) of the amine **5a** was added. After stirring the reaction mixture for 40 h at room temp., TLC [butanol/acetone/water (4:5:1)] showed that acylation was complete. The pyridine was removed and the residue was loaded on a flash column (15 g of silica gel). Elution with a gradient of ethyl acetate/methanol resulted in 41.7 mg (61%) of **5b** as a white powder; m.p. 146°C (dec.). — TLC with ethyl acetate/methanol (9:1); $R_f = 0.52$. — $^1\text{H NMR}$ (500 MHz, $\text{CDCl}_3/\text{CHCl}_3$): $\delta = 0.9$ (t, 3H, CH_3), 1.28 (m, 22H, $11 \times \text{CH}_2$), 1.9 (dd, 1H, 3- H_{ax}), 2.27 (t, 2H, RCH_2CONH), 2.83 (dd, 1H, 3- H_{eq}), 3.44 (dd, 1H, 6-H), 3.55 (dd, 1H, 7-H), 3.62 (ddd, 1H, 4-H), 3.76 (dd, 1H, 9- H_a), 3.86 (m, 4H, 5-H, COOCH_3), 3.94 (ddd, 1H, 9- H_b), 4.01 (ddd, 1H, 8-H); $J(3_{ax}, 3_{eq}) = -12.7$ Hz, $J(3_{ax}, 4) = 12.7$ Hz, $J(3_{eq}, 4) = 4.5$ Hz, $J(4, 5) = 10.5$ Hz, $J(5, 6) = 9.8$ Hz, $J(6, 7) = 1.8$ Hz, $J(7, 8) = 8.6$ Hz, $J(8, 9_a) = 5.5$ Hz, $J(8, 9_b) = 3.5$ Hz, $J(9_a, 9_b) = -11.3$ Hz. — MS (FAB, 70 eV); m/z (%) = 475 (70) [$\text{M}^+ - \text{CH}_3\text{O}$], 457 (100) [$\text{M}^+ - \text{CH}_3\text{O} - \text{H}_2\text{O}$], 229 (72).

$\text{C}_{25}\text{H}_{47}\text{NO}_9$ (505.6) Calcd. C 59.38 H 9.36 N 2.77
Found C 58.66 H 9.27 N 2.11

Methyl 5-Cyclopropanecarbonyl-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (Neu5cycloprop1Me- α -2Me, 5c): To a mixture of 40 mg (135 μmol) of the α -methyl ketoside **5a** in 2 ml of pyridine, 1.1 equiv. of cyclopropanecarbonyl chloride and a catalytic amount of DMAP were added at 10°C . The solution was stirred at room temperature until TLC [butanol/acetone/water (4:5:1)] showed the complete disappearance of the starting material. The mixture was evaporated to dryness and the resulting acylated neuraminic acid purified by flash chromatography on 15 g of silica gel with ethyl acetate/methanol (20:1) to yield

29.3 mg (81 μmol , 60%) of **5b** as a white powder; m.p. 127°C. — TLC in ethyl acetate/methanol (9:1): $R_f = 0.21$. — $^1\text{H NMR}$ (400 MHz, $\text{D}_2\text{O}/\text{H}_2\text{O}$): $\delta = 0.85$ (m, 4H, 2 CH_2), 1.59 (m, 1H, RRCHCONH), 1.77 (dd, 1H, 3- H_{ax}), 2.66 (dd, 1H, 3- H_{eq}), 3.35 (s, 3H, OCH_3), 3.53 (d, 1H, 7-H), 3.62 (dd, 1H, 9- H_a), 3.81 (m, 8H, 4-, 5-, 6-, 8-H, 9- H_b , COOCH_3); $J(3_{\text{ax}}, 3_{\text{eq}}) = -12$ Hz, $J(3_{\text{ax}}, 4) = 12$ Hz, $J(3_{\text{eq}}, 4) = 4$ Hz, $J(7, 8) = 9$ Hz, $J(8, 9_a) = 5.5$ Hz, $J(8, 9_b) = 8.5$ Hz, $J(9_a, 9_b) = -11.5$ Hz. — MS (FAB, 70 eV): m/z (%) = 364 (38) [$\text{M}^+ + 1$], 332 (19) [$\text{M}^+ - \text{CH}_4\text{O}$], 314 (23) [$\text{M}^+ - \text{CH}_4\text{O} - \text{H}_2\text{O}$], 217 (78), 109 (100).

$\text{C}_{15}\text{H}_{25}\text{NO}_9$ (363.3) Calcd. C 49.58 H 6.93 N 3.85
Found C 48.89 H 7.11 N 3.81

CAS Registry Numbers

1: 129213-72-5 / **2**: 97604-58-5 / **3**: 123457-69-2 / **4a**: 129151-82-2
4b: 126695-28-1 / **4d**: 129151-83-3 / **5a**: 129311-59-7 / **5b**: 129151-84-4 / **5c**: 129151-85-5 / myristoyl chloride: 112-64-1 / cyclopropanecarbonyl chloride: 4023-34-1 / sodium pyruvate: 113-24-6
Neu5Ac aldolase: 9000-88-8

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