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Synthesis of 7-*O*-Acetyl-*N*-acetylneuraminic Acid Derivative[1]

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Selective protection of the C-4, C-8, and C-9 hydroxy groups of methyl (methyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosid)onate followed by acetylation of the C-7 hydroxy group gave a 7-*O*-acetyl-*N*-acetylneuraminic acid derivative.

Keywords——7-*O*-acetyl-*N*-acetylneuraminic acid derivative; sialic acid; partially *O*-acylated sialic acid; methyl 7-*O*-acetyl-*N*-acetylneuraminate α-methyl glycoside; selective acetylation

Sialic acids, which have important biological activities, are widely distributed in animal cells in various forms.[2] The occurrence of partially *O*-acylated sialic acids has been investigated by Schauer *et al.*[3,4] These compounds have significant effects on functions such as complement activation and antigenicity.[5] We have been interested in various *O*-acylated sialic acids as new blockers of sialidases, and as starting materials for the synthesis of other sialic acids.[6,7]

Previously, we reported on the synthesis of various 9-*O*-acyl- and 4-*O*-acetyl-*N*-acetylneuraminic acid derivatives.[8] However, efficient synthesis of 7-*O*-acetyl-*N*-acetylneuraminic acid is more difficult because the 7-OH group of sialic acid is less reactive owing to steric hindrance. In this paper, we wish to report a concise synthesis of α-methyl glycoside of methyl 7-*O*-acetyl-*N*-acetylneuraminate.

N-Acetylneuraminic acid has five hydroxy groups at the C-2, C-4, C-7, C-8, and C-9 positions in its molecule. It was found by Schauer *et al.*[4] that the most reactive position to acetylation was C-9 and the next most reactive position was C-4. For selective acetylation of 7-OH, the more reactive hydroxy groups of *N*-acetylneuraminic acid must be protected.

First, we tried to synthesize the 7-*O*-acetyl derivative from methyl *N*-acetylneuraminate (**1**) as a starting material. Treatment of **1** with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate gave the crystalline 8,9-*O*-isopropylidene derivative (**2**) in 80% yield.[9,10] Treatment of **2** with *tert*-butyldimethylsilyl chloride and imidazole in *N*,*N*-dimethylformamide (DMF) gave the crystalline 4-*O*-*tert*-butyldimethylsilyl derivative (**3**) in 50% yield.[10-12] However, acetylation of **3** with 1.5 molar eq of acetic anhydride in pyridine at 0 °C gave the undesired 2-*O*-acetyl derivative (**4**). Thus, the 7-OH group of **3** was less reactive to acetylation than 2-OH owing to the steric hindrance of the dimethyldioxolane moiety.

The α-methyl glycoside of methyl *N*-acetylneuraminate was used as a starting material. Treatment of the α-methyl glycoside of methyl *N*-acetylneuraminate (**5**) with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate gave the crystalline 8,9-*O*-isopropylidene derivative (**6**) in 70% yield.[9-11] Treatment of **6** with *tert*-butyldimethylsilyl chloride and imidazole in DMF gave the crystalline 4-*O*-*tert* butyldimethylsilyl derivative (**7**) in 50% yield.[11-13] The 7-*O*-acetyl derivative (**8**) was obtained as crystals by acetylation of **7**, which was deprotected with 80% acetic acid at 60 °C to give methyl (methyl 5-acetamido-7-*O*-acetyl-D-*glycero*-α-D-*galacto*-nonulopyranosid)onate

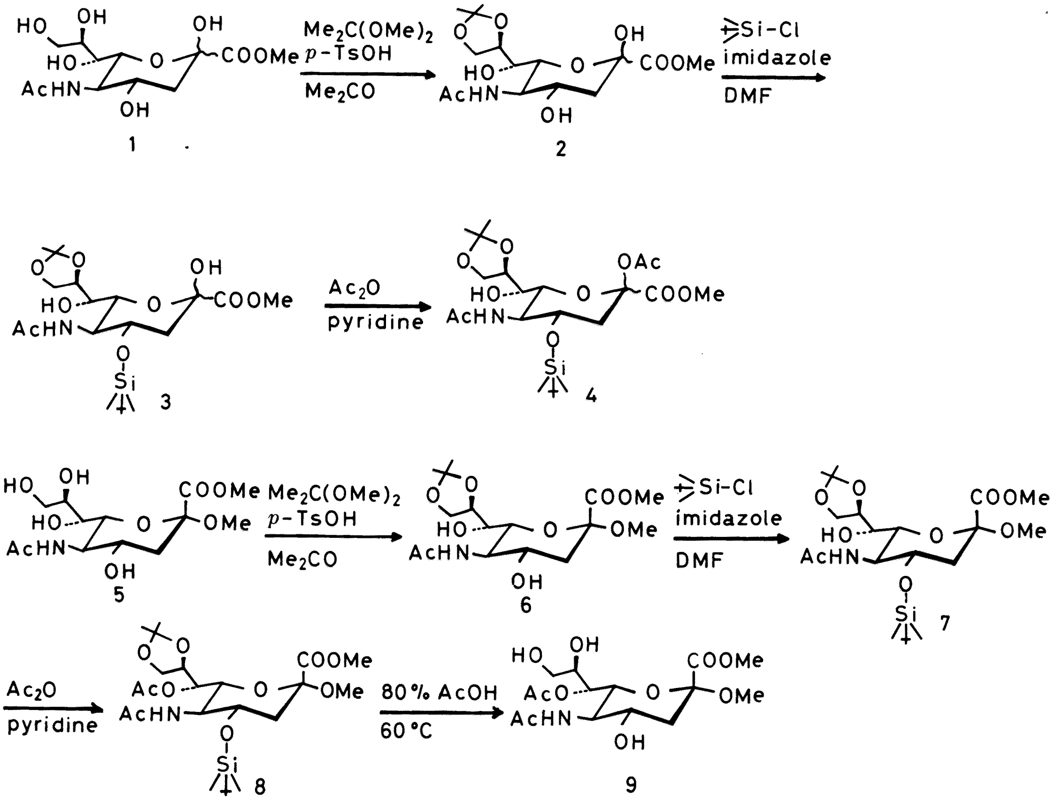

Chart 1

(9) in 94% yield.

Experimental

Melting points were determined in capillary tubes, and are uncorrected. Optical rotations were measured with a JASCO-JIP-4 polarimeter. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian XL-400 spectrometer using tetramethylsilane as an internal standard in $CDCl_3$ and CD_3OD.

Methyl 5-Acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-*tert*-butyldimethylsilyl-D-*glycero*-D-*galacto*-nonulopyranosonate (3)——Imidazole (85 mg, 1.25 mmol) and *tert*-butyldimethylsilyl chloride (75 mg, 0.5 mmol) were added to a solution of **2** (90 mg, 0.25 mmol) in DMF (5 ml) at 0 °C. The solution was stirred at room temperature for 1 h, and then poured into 100 ml of ice water under stirring. The solution was extracted with ether, and the ether solution was washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to a syrup. Recrystallization from ether gave pure **3** (60 mg, 50%, mp 189—191 °C) as colorless needles. $[\alpha]_D^{22}$ $-18.4°$ ($c=1$, MeOH). MS *m/z*: 477 (M^+), 462 (M^+-15). IR ν_{max}^{KBr} cm^{-1}: 1750 (CO), 1620, 1570 (CONH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.80 (3H, s, COOMe), 0.07, 0.09 (each 3H, s, Si-Me), 0.86 (9H, s, Si-CMe_3), 1.30 1.37 (each 3H, s, CMe_2), 5.41 (1H, d, $J_{5,NH}=7.0$ Hz, NH), 2.30 (3H, s, NHAc), 2.10 (1H, d, $J_{3ax,3eq}=0$, $J_{3ax,4}=8.0$ Hz, H-3_{ax}), 2.10 (1H, d, $J_{3eq,3ax}=0$, $J_{3eq,4}=8.0$ Hz, H-3_{eq}), 3.40 (1H, dd, $J_{6,7}=8.0$, $J_{7,OH}=4.5$ Hz, H-7), 3.83—4.18 (6H, m). *Anal.* Calcd for $C_{21}H_{39}NO_9Si$: C, 52.81; H, 8.23; N, 2.93. Found: C, 52.73; H, 8.33; N, 2.77.

Methyl 5-Acetamido-2-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-*tert*-butyldimethylsilyl-D-*glycero*-D-*galacto*-nonulopyranosonate (4)——Acetic anhydride (15 mg, 0.15 mmol) was added to a solution of **3** (50 mg, 0.1 mmol) in dry pyridine (3 ml) at 0 °C and the mixture was stirred for 3 h, then poured into 100 ml of ice-water under stirring. The solution was extracted with chloroform. The chloroform solution was washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to a syrup. Recrystallization from ether gave pure **4** (39 mg, 75%, mp 190—193 °C (dec.)) as colorless needles. $[\alpha]_D^{27}$ $-39°$ ($c=1$, MeOH). MS *m/z*: 504 (M^+-15). IR ν_{max}^{KBr} cm^{-1}: 1750 (CO), 1690, 1530 (CONH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.80 (3H, s, COOMe), 0.10, 0.10 (each 3H, s, Si-Me), 0.87 (9H, s, Si-CMe_3), 1.29, 1.36 (6H, s, CMe_2), 5.40 (1H, d, $J_{NH,5}=8.0$ Hz, NH), 1.84 (3H, s, OAc), 2.04 (3H, (3H, s, NHAc), 1.82 (1H, dd, $J_{3ax,3eq}=15.0$, $J_{3ax,4}=11.0$ Hz, H-3_{ax}), 2.40 (1H, dd, $J_{3eq,3ax}=15.0$, $J_{3eq,4}=6.0$ Hz, H-3_{eq}), 4.14 (1H, dt, $J_{3ax,4}=11.0$, $J_{3eq,4}=6.0$, $J_{4,5}=11.0$ Hz, H-4), 3.80 (1H, dt, $J_{4,5}=11.0$, $J_{5,6}=11.0$, $J_{5,NH}=8.0$ Hz, H-5), 3.75 (1H, dd, $J_{5,6}=11.0$, $J_{6,7}=1.0$ Hz, H-6), 3.50 (1H, dd, $J_{6,7}=1.0$, $J_{7,8}=8.0$ Hz, H-7), 4.15 (1H, dt, $J_{7,8}=8.0$, $J_{8,9}=6.0$, $J_{8,9'}=6.0$ Hz, H-8), 3.96 (1H, dd, $J_{8,9}=6.0$, $J_{9,9'}=8.0$ Hz, H-9), 4.09 (1H, dd, $J_{8,9'}=6.0$, $J_{9,9'}=8.0$ Hz, H-9′). *Anal.* Calcd for $C_{23}H_{41}NO_{10}Si$: C, 53.16; H, 7.95; N, 2.70. Found: C, 53.24; H, 7.75; N, 2.48.

Methyl (Methyl 5-Acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-nonulopyranosid)onate (6)——2,2-Dimethoxypropane (37 mg, 0.36 mmol) and *p*-toluenesulfonic acid monohydrate (5 mg) were added to a stirred suspension of **5** (100 mg, 0.3 mmol) in acetone (50 ml). The reaction mixture was stirred at room temperature for 1 h. The resulting clear solution was treated with Dowex-1 (OH^-) anion exchange resin to remove the acid catalyst and the resin was filtered off and washed with acetone. The combined filtrate was evaporated under reduced pressure to a syrup. Recrystallization from ether gave pure **6** (78 mg, 70%, mp 166—167 °C) as colorless needles. Further purification of **6** was done by repeated recrystallization from ethyl acetate–hexane until the oserved melting point value became constant. (ref. 8, 181 °C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.80 (3H, s, COOMe), 3.38 (3H, s, OMe), 2.06 (3H, s, NAc), 5.84 (1H, d, $J_{5,NH}=8.0$ Hz, NH), 1.36, 1.40 (each 3H, s, CMe_2), 1.83 (1H, dd, $J_{3eq,3ax}=13.0$, $J_{3ax,4}=11.0$ Hz, H-3_{ax}), 2.69 (1H, dd, $J_{3eq,3ax}=13.0$, $J_{3eq,4}=4.5$ Hz, H-3_{eq}), 3.70 (1H, m, H-4), 3.86 (1H, q, $J_{5,NH}=8.0$, $J_{5,4}=10.0$, $J_{5,6}=10.0$ Hz, H-5), 3.50 (1H, dd, $J_{5,6}=10.0$, $J_{6,7}=1.2$ Hz, H-6), 3.57 (1H, dt, $J_{6,7}=1.2$, $J_{7,8}=6.5$, $J_{7,OH}=6.5$ Hz, H-7), 4.29 (1H, q, $J_{8,9}=6.5$, $J_{8,9'}=6.5$, $J_{7,8}=6.5$ Hz, H-8), 4.06 (1H, dd, $J_{8,9}=6.5$, $J_{9,9'}=8.5$ Hz, H-9), 4.12 (1H, dd, $J_{8,9'}=6.5$, $J_{9,9'}=8.5$ Hz, H-9′).

Methyl (Methyl 5-Acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-*tert*-butyldimethylsilyl-D-*glycero*-α-D-*galacto*-nonulopyranosid)onate (7)——Imidazole (70 mg, 1 mmol) and *tert*-butyldimethylsilyl chloride (62 mg, 0.4 mmol) were added to a solution of **6** (78 mg, 0.2 mmol) in DMF (5 ml) at 0 °C. The solution was stirred at room temperature for 1 h, and then poured into 100 ml of ice water under stirring. The solution was extracted with ether. The ether solution was washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to a syrup. Recrystallization from ether gave pure **7** (49 mg, 50%, mp 176—177 °C) as colorless needles. $[\alpha]_D^{22}$ $-10°$ ($c=1$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1750 (CO), 1650, 1540 (CONH). MS *m/z*: 490 (M^+-1), 476 (M^+-15). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.80 (3H, s, COOMe), 3.40 (3H, s, OMe), 2.02 (3H, s, NAc), 0.09, 0.10 (each 3H, s, Si-Me), 0.87 (9H, s, Si-CMe_3), 1.36, 1.39 (each 3H, s, CMe_2), 1.82 (1H, dd, $J_{3ax,3eq}=13.0$, $J_{3ax,4}=11.0$ Hz, H-3_{ax}), 2.59 (1H, dd, $J_{3ax,3eq}=13.0$, $J_{3eq,4}=4.0$ Hz, H-3_{eq}), 4.32 (1H, q, $J_{7,8}=6.5$, $J_{8,9}=6.5$, $J_{8,9'}=6.5$ Hz, H-8), 4.10 (1H, dd, $J_{8,9}=6.5$, $J_{9,9'}=8.8$ Hz, H-9), 4.12 (1H, dd, $J_{8,9'}=6.5$, $J_{9,9'}=8.8$ Hz, H-9′). *Anal.* Calcd for $C_{22}H_{41}NO_9Si$: C, 53.75; H, 8.41; N, 2.85. Found: C, 53.93; H, 8.55; N, 2.85.

Methyl (Methyl 5-Acetamido-7-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-*tert*-butyldimethylsilyl-D-*glycero*-α-D-*galacto*-nonulopyranosid)onate (8)——Acetic anhydride (12 mg, 0.12 mmol) was added to a solution of **7** (40 mg, 0.08 mmol) in dry pyridine (3 ml) at 0 °C. The mixture was stirred at room temperature for 10 h, and poured with stirring into 100 ml of ice water. The solution was extracted with chloroform, washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to a syrup. Recrystallization from ether gave pure **8** (33 mg, 80%, mp 175—176 °C) as colorless needles. $[\alpha]_D^{27}$ $+17.6$ ($c=1$, $CHCl_3$), MS *m/z*: 517 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1770, 1750 (CO), 1650, 1560 (CONH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.83 (3H, s, COOMe), 3.35 (3H, s, OMe), 1.92 (3H, s, NHAc), 2.16 (3H, s, OAc), 1.34, 1.35 (each 3H, s, CMe_2), 0.04 (3H, s, Si-Me), 0.85 (9H, s, Si-CMe_3), 1.70 (1H, br t, $J_{3ax,3eq}=12.6$, $J_{3ax,4}=10.5$ Hz, H-3_{ax}), 2.58 (1H, dd, $J_{3eq,4}=5.4$, $J_{3eq,3ax}=12.6$ Hz, H-3_{eq}, 4.10 (1H, dt, $J_{4,3ax}=10.5$, $J_{4,3eq}=5.4$, $J_{4,5}=9.5$ Hz, H-4), 3.18 (1H, br q, $J_{4,5}=9.5$, $J_{5,6}=9.5$, $J_{5,NH}=9.5$ Hz, H-5), 4.18 (1H, dd, $J_{5,6}=10.8$, $J_{6,7}=1.5$ Hz, H-6), 5.42 (1H, dd, $J_{7,8}=3.3$, $J_{6,7}=1.5$ Hz, H-7), 4.36 (1H, dt, $J_{7,8}=3.3$, $J_{8,9}=6.6$, $J_{8,9'}=6.6$ Hz, H-8), 4.09 (1H, dd, $J_{9,9'}=14.1$, $J_{8,9}=6.6$ Hz, H-9), 4.11 (1H, dd, $J_{9,9'}=14.1$, $J_{8,9'}=6.6$ Hz, H-9′), 5.37 (1H, d, $J_{5,NH}=8.1$ Hz, NH). *Anal.* Calcd for $C_{24}H_{43}NO_{10}Si$: C, 54.01; H, 8.12; N, 2.62. Found: C, 54.29; H, 8.19; N, 2.55.

Methyl (Methyl 5-Acetamido-7-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosid)onate (9)——A solution of **8** (100 mg, 0.19 mmol) in 80% acetic acid (5 ml) was stirred for 3 h at 60 °C. The mixture was evaporated under reduced pressure to give pure **9** (65 mg, 94%) as a syrup. ^{1}H-NMR (400 MHz, CD_3OD) δ: 3.85 (3H, s, COOMe), 3.35 (3H, s, OMe), 2.07 (3H, s, OAc), 1.89 (3H, s, NAc), 2.63 (1H, dd, $J_{3eq,3ax}=13.0$, $J_{3eq,4}=4.5$ Hz, H-3_{eq}), 1.72 (1H, dd, $J_{3ax,3eq}=13.0$, $J_{3ax,4}=12.0$ Hz, H-3_{ax}), 3.47 (1H, ddd, $J_{4,3ax}=12.0$, $J_{4,3eq}=4.5$, $J_{4,5}=10.5$ Hz, H-4), 3.78 (1H, t, $J_{5,4}=10.5$, $J_{5,6}=10.5$ Hz, H-5), 3.87 (1H, dd, $J_{6,5}=10.5$, $J_{6,7}=2.0$ Hz, H-6), 4.97 (1H, dd, $J_{7,6}=2.0$, $J_{7,8}=9.0$ Hz, H-7), 3.97 (1H, ddd, $J_{8,7}=9.0$, $J_{8,9}=7.0$, $J_{8,9'}=3.0$ Hz, H-8), 3.43 (1H, dd, $J_{9,9'}=12.0$, $J_{9,8}=7.0$ Hz, H-9), 3.57 (1H, dd, $J_{9,9'}=12.0$, $J_{9',8}=3.0$ Hz, H-9′).

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