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Note

# Alkylation of *N*-acetylneuraminic acid at C-4

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For a number of years we have been involved in the development of influenza diagnostic agents. Our approach is based on the enzymatic cleavage of chromogenic ketosides of *N*-acetylneuraminic acid (Neu5Ac) by influenza virus sialidase, and as such, it requires the utilization of substrates which will exhibit specificity towards the viral enzyme.

The specificity of various sialidase substrates has been studied in the past, and it has already been reported [1] that 4-methoxy-Neu5Ac bound to fetuin showed resistance towards *Vibrio cholerae* sialidase, but was cleaved rapidly by fowl plague virus sialidase. It has also been shown that a 4-*O*-acetyl substitution in sialic acids blocks the action of bacterial sialidases for substrates containing these derivatives, while viral enzymes show significant activity [2]. In another study it was found that the sialidase of *Streptococcus sanguis*, from the human oral cavity, did not cleave 4-*O*-acetylated sialic acid derivatives [3].

4-Methoxy-Neu5Ac has been synthesized before by a multistep process [4,5], the main features of which are the conversion of a 3-acetamido-heptose [6] into the di-*O*-isopropylidene diethyl dithioacetal, followed by methylation and chain elongation by a Wittig reaction. This scheme produced two stereoisomers in equal proportions.

In an effort to simplify the synthesis of 4-methoxy-Neu5Ac, we were able to obtain it by direct alkylation at the C-4 position of a suitably protected Neu5Ac derivative. We also used the same alkylation method to obtain 4-ethoxy-Neu5Ac, the synthesis of which has never been described before.

Selective methylation of methyl (methyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-D-*glycero*-$\alpha$-D-*galacto*-2-nonulopyranosidonate) (**1**) [7] with 1.2 molar equiv of dimethyl sulfate in the presence of sodium hydride (80% dispersion in oil) for 20 min at 0°C, gave one major product, the 4-methoxy derivative **2**, which was isolated in 69%

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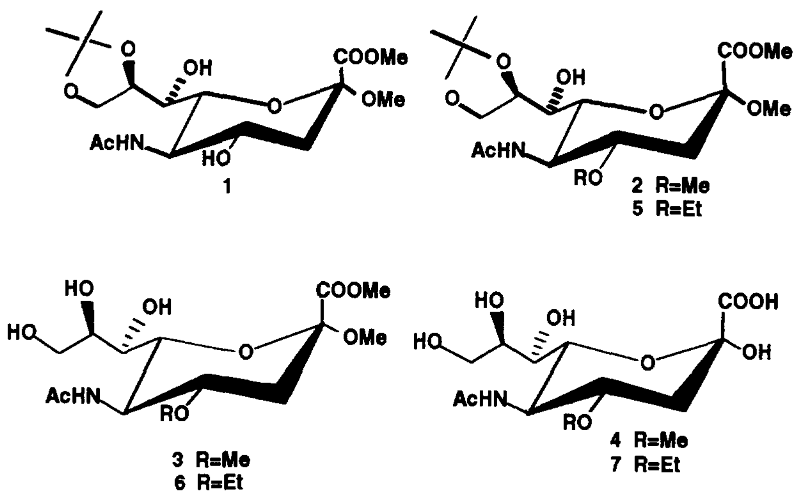

Scheme 1.

yield after column chromatography on silica gel. The structural assignment of **2** was based on its $^1$H NMR spectrum which showed the presence of one additional methoxy group ($\delta$ 3.34), microanalysis, and on the well-known reactivity of OH-4 in compound **1** [7,8].

Treatment of **2** with 80% aqueous acetic acid for 1 h at 85°C gave the methyl ester methyl ketoside **3** in 80% yield after column chromatography. Finally, treatment of **3** with M sodium hydroxide solution at room temperature, followed by acid hydrolysis with 0.025 M hydrochloric acid and Dowex 50 (H$^+$) resin at 100°C for 2 h, gave the practically pure 4-methoxy-Neu5Ac (**4**) in 88% yield. A small sample was purified by HPLC and crystallized from 2-propanol–water. The optical rotation value and the $^{13}$C NMR data are in good agreement with the literature data [4].

In a similar manner, alkylation of **1** with diethyl sulfate, in the presence of sodium hydride, gave the 4-ethoxy analog **5** as the major product. However, the alkylation in this case was sluggish and was carried out overnight at room temperature. Also, the yield was considerably lower than that of the 4-methoxy product **2** (42% after purification by column chromatography). Under more drastic conditions, several byproducts were obtained and the yield was even lower. The $^1$H NMR showed the expected signals of one ethoxy group [a triplet at $\delta$ 1.17 ($J$ 6.8 Hz) and a doublet of doublets at $\delta$ 3.40 ppm (partially overlapped with the ring protons)].

Hydrolysis of **5** with 80% aq acetic acid followed by chromatography, as already described, gave the 4-ethoxy ketoside **6** in 73% yield. Final deprotection of **6** was performed as just described and gave 4-ethoxy-Neu5Ac (**7**) in 80% yield. An analytically pure sample was obtained after HPLC purification and crystallization from 2-propanol water. The $^{13}$C NMR data are very similar to those found for the analogous 4-methoxy product (except the C-4 and the alkyl signals, as expected).

## 1. Experimental

*General methods.* — Optical rotations were measured with a Rudolph DS11 automatic polarimeter. NMR spectra were recorded with Jeol FX-90QM Multinuclear spectrometer. Melting points were determined with a Fisher–Johns melting point apparatus and are not corrected. Column chromatography was performed on silica gel, 70–230 mesh (Aldrich). Thin-layer chromatography was performed on Analtech Uniplate silica gel plates. HPLC purifications were performed on a Waters system and a $C_{18}$ reverse-phase column (2.2 × 25 cm) using 10–75% aq methanol gradient over 80 min. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

*Methyl(methyl 5-acetamido-3,5-dideoxy-4-O-methyl-8,9-O-isopropylidene-*D-glycero-α-D-galacto-*2-nonulopyranosidonate* (**2**). — A cold (ice bath) solution of **1** [7] (1.6 g) in MeCN (15 mL) was saturated with nitrogen. Sodium hydride (80% dispersion in oil, 245 mg) was added, and the mixture was stirred under nitrogen for 15 min. Dimethyl sulfate (0.8 mL) was then added and stirring was continued for an additional 30 min. The mixture was filtered through Celite and the filtrate was evaporated to give a syrupy residue which was chromatographed. Elution with 25:1 $CH_2Cl_2$–MeOH removed minor byproducts. Continued elution with the same solvent system afforded the homogeneous 4-methoxy product **2** (1.22 g, 69%). Crystallization from acetone–hexane afforded an analytical sample, mp 193–194°C (sin. 178°); $[\alpha]_D$ −41.5° (*c* 1.6, $CHCl_3$); $^1H$ NMR data: δ 3.82 (s, 3H, COOMe), 3.34 (s, 3H, 4-OMe), 3.29 (s, 3 H, 2-OMe), 2.69 (dd, 1 H, $J_{3eq,3ax}$ 13.3, $J_{3eq,4}$ 4.2 Hz, H-3*eq*), 2.09 (s, 3 H, NHAc), 1.78 (1 H, dd, $J_{3ax,4}$ 11.5 Hz, H-3*ax*), 1.38, 1.31 (2s, 6 H, $Me_2CO$). Anal. Calcd for $C_{17}H_{29}NO_9$: C, 52.16; H, 7.46; N, 3.57. Found: C, 52.03; H, 7.56; N, 3.56.

*Methyl (methyl 5-acetamido-3,5-dideoxy-4-O-methyl-*D-glycero-α-D-galacto-*2-nonulopyranosidonate)* (**3**). — The 4-methoxy product **2** (1.22 g) was treated with 80% aq AcOH (15 mL) for 1 h at 85°C. The mixture was evaporated, then water was evaporated from the residue, and the residue was chromatographed. Elution with 5:1 $CH_2Cl_2$–MeOH removed a minor byproduct. Continued elution with the same solvent mixture gave the partially deprotected product **3** (0.87 g, 80%). An analytically pure sample was obtained by crystallization from acetone mp 177–178°C, $[\alpha]_D$ −31.6° (*c* 1.8, MeOH). Anal. Calcd for $C_{14}H_{25}NO_9$: C, 47.88; H, 7.17; N, 3.98. Found: C, 47.68; H, 7.17; N, 3.85.

*5-Acetamido-3,5-dideoxy-4-O-methyl-*D-glycero-D-galacto-*nonulopyranosidonic acid (4-methoxy-N-acetylneuraminic acid)* (**4**). — The 4-methoxy-methyl ester methyl ketoside **3** (0.87 g) was treated with M NaOH solution (3 mL) in methanol (5 mL) and water (5 mL) for 1 h at room temperature. The mixture was neutralized with Dowex 50 ($H^+$) and the resin was filtered off and washed with MeOH. The filtrate was evaporated, and the residue was then treated with Dowex 50 ($H^+$) resin (1.5 g) in 0.025 mM HCl for 2 h at 100°C. The resin was filtered off, and the filtrate was evaporated. The residue was dried under vacuum to give the practically pure 4-methoxy-Neu5Ac (**4**) (708 mg, 88%). A small sample was purified by HPLC (retention time 17–20 min) and crystallized from 2-propanol–water; $[\alpha]_D$ −26.0° (*c* 1.9, water); lit[4] $[\alpha]_D$ −28.4°; mp: decomposition started at 160°C; (lit[4] 160°); $^{13}C$ NMR data ($D_2O$; 1,4-dioxane was used as the internal standard, δ 67.4 ppm): δ 175.5 (C=O), 173.9 (C-1), 96.2 (C-2), 76.7 (C-4), 71.4 (C-8), 71.0 (C-6), 69.1. (C-7), 64.0 (C-9), 57.2 ($OCH_3$), 51.2 (C-5), 36.6 (C-3), 22.9 ($CH_3NAc$); $^1H$ NMR ($D_2O$): δ 3.43 (s, 3 H, 4-OMe), 2.53 (dd, 1 H, $J_{3eq,3ax}$ 13.3, $J_{3eq,4}$ 4.5 Hz, H-3*eq*), 2.09 (s, 1 H, NHAc), 1.83 (dd, 1 H, $J_{3ax,4}$ 11.0 Hz, H-3*ax*).

*Methyl (methyl 5-acetamido-3,5-dideoxy-4-*O*-ethyl-8,9-*O*-isopropylidene-*D-glycero-α-D-galacto-*2-nonulopyranosidonate)* (**5**). — A cold (ice bath) solution of **1** [7] (1.69 g) in MeCN (10 mL) was saturated with nitrogen. Sodium hydride (80% dispersion in oil, 220 mg) was added, and the mixture was stirred for an additional 15 min. Diethyl sulfate (2.5 mL) was then added; and after 10 more min, the ice bath was removed and the mixture was stirred overnight at room temperature. It was filtered through Celite and the filtrate was evaporated. The residue was dried under vacuum and chromatographed. Fast-moving impurities were removed by elution with 25:1 $CH_2Cl_2$–MeOH. Continued elution with the same solvent system gave the 4-ethoxy derivative **5** (0.76 g, 42%). Crystallization from acetone–hexane afforded an analytical sample: mp 222–223°C (sin. 190°); $[\alpha]_D$ −33.8° (*c* 1.5, $CHCl_3$), $^1H$ NMR data ($CDCl_3$): δ 3.80 (s, 3H, COOMe), 3.29 (s, 3H, 2-OMe), 2.58 (dd, 1 H, $J_{3eq,4}$ 4.0, $J_{3eq,3ax}$ 12.5 Hz, H-3*eq*), 2.05 (s, 3 H, NHAc), 1.8 (dd, 1 H, $J_{3ax,4}$ 13.3 Hz, H-3*ax*), 1.37, 1.30 (2s, 6 H, $Me_2CO$), 1.17 (t, 3 H, *J* 9.0 Hz, Me-ethoxy). Anal. Calcd for $C_{18}H_{31}NO_9$: C, 53.32; H, 7.70; N, 3.34. Found: 53.21; H, 7.70; N, 3.45.

*Methyl (methyl 5-acetamido-3,5-dideoxy-4-*O*-ethyl-*D-glycero-α-D-galacto-*2-nonulopyranosidonate)* (**6**). — The 4-ethoxy product (**5**, 680 mg) was treated with 80% aq AcOH (10 mL) for 1 h at 90°C. The mixture was evaporated, then water was evaporated from the residue, and the residue was dried under vacuum and chromatographed. Elution with 5:1 $CH_2Cl_2$–MeOH removed a minor byproduct. Continued elution with the same solvent system gave the product **6** (450 mg, 73%). A small sample was crystallized from acetone: mp 194–195°C $[\alpha]_D$ −33.9° (*c* 1.5, MeOH); $^1H$ NMR ($D_2O$): δ 3.87 (s, 3 H, COOMe), 3.29 (s, 3 H, 2-OMe), 2.60 (dd, 1 H, $J_{3eq,4}$ 4.0, $J_{3eq,3ax}$ 12.5 Hz, H-3*eq*), 1.75 (dd, 1 H, $J_{3ax,4}$ 11.0 Hz, H-3*ax*), 1.15 (t, 3 H, J 9.0 Hz, Me-ethoxy). Anal. Calcd. for $C_{15}H_{27}NO_9$: C, 49.30; H, 7.44; N, 3.83. Found: C, 48.38; H, 7.66; N, 3.63.

*5-Acetamido-3,5-dideoxy-4-*O*-ethyl-*D-glycero-D-galacto-*2-nonulopyranosidonic acid (4-ethoxy-Neu5Ac)* (**7**). — The partially deprotected 4-ethoxy product (**6**, 450 mg) was treated with M NaOH solution (1 mL) in methanol (5 mL) and water (5 mL) for 1 h at room temperature. It was neutralized with Dowex 50 ($H^+$), and the resin was filtered off. The filtrate was evaporated, and the residue was then treated with Dowex 50 ($H^+$) (0.95 g) in 25 mM HCl (15 mL) at 90°C for 4 h. The resin was filtered off, and the filtrate was evaporated. The residue was dried under vacuum to give the practically pure 4-ethoxy-*N*-acetylneuraminic acid (**7**, 333 mg, 80%). An analytically pure sample was obtained after HPLC purification (retention time 24–27 min) and crystallization from 2-propanol–water; mp: decomposition started at 160°C, $[\alpha]_D$ −26.8° (*c* 1.3, water); $^{13}C$ NMR data ($D_2O$); 175.5 (C=O), 174.0 (C-1), 96.3 (C-2), 75.2 (C-4), 71.6 (C-8), 71.1 (C-6), 69.2 (C-7), 66.3 ($\mathit{C}H_2CH_3$), 64.1 (C-9), 51.5 (C-5), 37.6 (C-3), 22.9 ($CH_3NAc$), 15.5 ($CH_2$–$\mathit{C}H_3$). $^1H$ NMR data ($D_2O$): δ 2.02 (s, 3 H, NHAc), 2.52 (dd, 1 H, $J_{3eq,4}$ 4.0, 12.5 Hz, H-3*eq*) 1.72 (dd, 1 H, $J_{3ax,3eq}$ 12.5, $J_{3ax,4}$ 11.0 Hz, H-3*ax*), 1.12 (t, 3 H, *J* 9.0 Hz, Me-ethoxy).

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