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Note

Acetylation of *N*-acetylneuraminic acid and its methyl ester

ALBERTO MARRA AND PIERRE SINAŸ*

Ecole Normale Supérieure, Laboratoire de Chimie, UA 1110, 24, Rue Lhomond, F-75231 Paris 05 (France)

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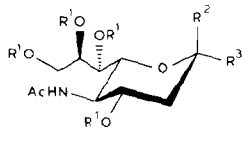
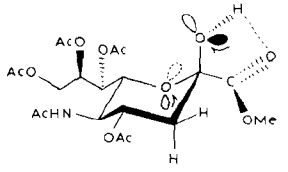
The literature on the acetylation of the methyl ester of *N*-acetylneuraminic acid (**1**) is confusing and this note is intended to clarify the situation.

In 1966, Kuhn *et al.*[1] reported that acetylation of **1** with acetic anhydride in the presence of a catalytic amount of aqueous 60% perchloric acid at 40° for 2.5 h gave 61% of crystalline methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (m.p. 156–157°). However, Baggett and Marsden[2] reported that this reaction gave the crystalline 4,7,8,9-tetra-acetate **3**. In spite of this important observation, the method of Kuhn *et al.*[1] has been referred to frequently for the preparation of the penta-acetate[3–8].



1 R^1 = H, R^2 = OH, R^3 = COOMe
2 R^1 = Ac, R^2 = OAc, R^3 = COOMe
3 R^1 = Ac, R^2 = OH, R^3 = COOMe
4 R^1 = Ac, R^2 = COOMe, R^3 = OAc
6 R^1 = Ac, R^2 = Cl, R^3 = COOMe



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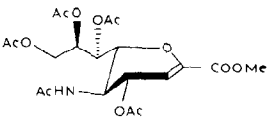

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When we repeated the experiment of Kuhn *et al.*[1] at either 40° or 20°, crystalline **3** was obtained, but the melting point (147–148°) was at variance with that (m.p. 174–175°) reported by Baggett and Marsden[2]. No trace of the penta-acetates **2** or **4** was detected by t.l.c. The ^{1}H-n.m.r. (400 MHz) spectra fully confirmed the structure of **3** (see Experimental). The existence of a long-range (W) coupling of HO-2 with H-3*a* is consistent[9] with a β configuration in which HO-2, C-2, C-3, and H-3*a* are coplanar (**5**), as favored by the exo-anomeric effect. The unusual upfield

*Author for correspondence.

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TABLE I

SELECTED CHEMICAL SHIFTS (δ, P.P.M.) AND J VALUES (Hz)

	Ref.[a]	**3**			**2**		**4**	
δ_{H-4}	14	5.26[b]	5.22[c]	5.04[d]	5.17[b]	5.26[c]	5.12[b]	5.02[c]
$J_{7,8}$	14	4.2[b]	5.6[c]	4.6[d]	3.8[b]	5.3[c]	n.d.[e]	7.0[c]
$\Delta\delta_{(H-9a-H-9b)}$	21	0.79[b]	0.48[c]	0.41[d]	0.62[b]	0.38[c]	0.53[b]	0.30[c]

[a]References deal with use of the corresponding data as criteria for the assignment of the anomeric configurations. [b]In C_6D_6. [c]In $CDCl_3$. [d]In $(CD_3)_2SO$. [e]Not determined in C_6D_6.

displacement of the signal for H-3*e* (in $CDCl_3$ or C_6D_6) may be due to a particular orientation of the methoxycarbonyl group, as a consequence of intramolecular hydrogen bonding with HO-2. This shift was not observed for a solution in $(CD_3)_2SO$, since the intramolecular hydrogen bond would be disrupted.

Acetylation of *N*-acetylneuraminic acid by acetic anhydride–pyridine[10,11] followed by esterification with diazomethane has been reported[12–14] to be the method of choice for the preparation of **2**, but, surprisingly, this compound has been neither fully characterized nor its anomeric configuration ascertained. Repetition of this work gave 94% of amorphous **2**. In order to establish unambiguously the anomeric purity of **2**, a selective synthesis of the unknown α-acetate **4** was devised. Compound **2** was converted into the glycosyl chloride **6**, which was treated with cesium acetate in acetonitrile to give a mixture of **4** and the glycal derivative **7** which could not be fractionated by chromatography. Treatment of the mixture with sodium metaperiodate in the presence of a catalytic amount of ruthenium trichloride[15] destroyed **7**, and **4** was obtained in a one-pot operation (65% from **2**). The $[\alpha]_D$ values of **2** (−32°) and **4** (+13°) and their ^{1}H-n.m.r. spectra (Table I) accord with the assigned anomeric configurations.

Kuhn *et al.*[1] suggested that acetylation of **1** with acetic anhydride–pyridine gave an anomeric mixture of **2** and **4**, but the mixture was not analyzed. Using these conditions, Baggett and Marsden[2] reported the formation of the crystalline tetra-acetate **3** (48%), Sharma and Eby[16] reported that the penta-acetate was formed, but the physical properties reported appeared to be those of the tetra-acetate **3** (m.p. 157–158°, $[\alpha]_D^{24}$ −3.4°), and Warner and Lee[17] also reported the formation of amorphous penta-acetate. When we applied this procedure* to **1**, 97% of an ~7:2 mixture of **2** and **4** was obtained which was fractionated easily by column chromatography. No trace of the tetra-acetate was detected by t.l.c. Under similar conditions, **3** was converted into an ~8:1 mixture of **2** and **4**.

We hope that this work will prevent further erroneous statements on the molecular structure of acetylated derivatives of *N*-acetylneuraminic acid.

*Various modifications in the literature[2,16,17] gave similar results.

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi Model 510 capillary apparatus and are uncorrected. Optical rotations were measured at 20 ±2° with a Perkin–Elmer Model 241 polarimeter. Elemental analyses were performed at the University Pierre et Marie Curie (Paris VI). ^{1}H-N.m.r. spectra were recorded for solutions in the stated solvent (internal Me_4Si) with a Bruker AM-400 spectrometer. ^{13}C-N.m.r. spectra (100.57 MHz) were recorded for solutions in $CDCl_3$, adopting δ 77.0 for the central line of $CDCl_3$. Assignments were aided by the J-MOD technique[18,19]. Reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck), using ethyl acetate, 6:1 toluene–methanol, or 20:1 chloroform–methanol, and detection by charring with sulphuric acid. Flash column chromatography[20] was performed on Silica Gel 60 (230–400 mesh, Merck).

*Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-*D*-glycero-*D*-galacto-2-nonulopyranosonate* (**3**). — To a stirred, warmed (40°) solution of acetic anhydride (1.44 g) and aqueous 60% perchloric acid (10 μL) was added **1** (440 mg, 1.36 mmol) during 30 min in small portions. The mixture was stirred for 2 h at 40°, then cooled to room temperature, diluted with cold water (20 mL), saturated with ammonium chloride, and extracted with dichloromethane (3 × 80 mL). The combined extracts were washed with saturated aqueous sodium hydrogencarbonate (20 mL) and water (20 mL), dried ($MgSO_4$), and concentrated to give a white solid. Crystallization from ethyl acetate–hexane gave **3** (502 mg, 75%) as white needles, m.p. 147–148°, $[\alpha]_D$ −2.1° (*c* 1, chloroform); lit.[2] m.p. 174–175° (from ether), $[\alpha]_D$ −3.1° (*c* 1, chloroform); N.m.r. data: ^{1}H (C_6D_6), δ 5.64 (dd, 1 H, $J_{6,7}$ 2.3, $J_{7,8}$ 4.2 Hz, H-7), 5.61 (ddd, 1 H, $J_{8,9a}$ 2.0, $J_{8,9b}$ 7.6 Hz, H-8), 5.26 (ddd, 1 H, $J_{3a,4}$ 10.8, $J_{3e,4}$ 5.7, $J_{4,5}$ 10.5 Hz, H-4), 5.02 (dd, 1 H, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.80 (d, 1 H, $J_{3a,OH}$ ~1.0 Hz, OH), 4.78 (d, 1 H, $J_{5,NH}$ 10.2 Hz, NH), 4.54 (ddd, 1 H, $J_{5,6}$ 10.8 Hz, H-5), 4.26 (dd, 1 H, H-6), 4.23 (dd, 1 H, H-9b), 3.28 (s, 3 H, MeO), 2.25 (ddd, 1 H, $J_{3a,3e}$ 12.8 Hz, H-3*a*), 2.19 (dd, 1 H, H-3*e*), 1.92, 1.85, 1.70, 1.63, and 1.60 (5 s, 15 H, 5 Ac); ^{1}H ($CDCl_3$), δ 5.71 (m, 1 H, NH), 5.36 (dd, 1 H, $J_{6,7}$ 1.5, $J_{7,8}$ 5.6 Hz, H-7), 5.25 (ddd, 1 H, $J_{8,9a}$ 2.4, $J_{8,9b}$ 7.5 Hz, H-8), 5.22 (ddd, 1 H, $J_{3a,4}$ 11.4, $J_{3e,4}$ 5.4, $J_{4,5}$ 9.5 Hz, H-4), 4.51 (dd, 1 H, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.47 (d, 1 H, $J_{3a,OH}$ ~0.8 Hz, OH), 4.21–4.13 (m, 2 H, H-5,6), 4.03 (dd, 1 H, H-9b), 3.86 (s, 3 H, MeO), 2.26 (ddd, 1 H, $J_{3a,3e}$ 12.8 Hz, H-3*a*), 2.19 (dd, 1 H, H-3*e*), 2.15, 2.11, 2.03, 2.02, and 1.91 (5 s, 15 H, 5 Ac); ^{1}H [$(CD_3)_2SO$], δ 7.81 (d, 1 H, $J_{5,NH}$ 9.8 Hz, NH), 7.30 (bs, 1 H, $J_{3a,OH}$ ~0.5 Hz, OH), 5.26 (dd, 1 H, $J_{6,7}$ 2.4, $J_{7,8}$ 4.6 Hz, H-7), 5.09 (ddd, 1 H, $J_{8,9a}$ 2.5, $J_{8,9b}$ 7.3 Hz, H-8), 5.04 (ddd, 1 H, $J_{3a,4}$ 11.6, $J_{3e,4}$ 4.8, $J_{4,5}$ 10.2 Hz, H-4), 4.44 (dd, 1 H, $J_{9a,9b}$ 12.2 Hz, H-9a), 4.19 (dd, 1 H, $J_{5,6}$ 10.6 Hz, H-6), 4.03 (dd, 1 H, H-9b), 3.85 (ddd, 1 H, H-5), 3.70 (s, 3 H, MeO), 2.18 (dd, 1 H, $J_{3e,3a}$ 12.6 Hz, H-3*e*), 1.74 (ddd, 1 H, H-3*a*), 2.00, 1.99, 1.98, 1.91, and 1.67 (5 s, 15 H, 5 Ac); ^{13}C, δ 171.43, 171.04, 170.72, 170.30, 170.12, and 168.93 (6 C=O), 94.84 (C-2), 72.07, 71.32, 69.12, and 68.3 (C-4,6,7,8), 62.50 (C-9), 53.18 (CH_3O), 49.04 (C-5), 36.11 (C-3), 22.93, 20.93, and 20.75 (3 CH_3CO), 20.65 (2 CH_3CO).

Anal. Calc. for $C_{20}H_{29}NO_{13}$: C, 48.88; H, 5.85; N, 2.95. Found: C, 49.04; H, 6.04; N, 2.87.

If the reaction was carried out for 2 h at room temperature (after an initial 30 min at 40°), similar results were obtained.

*Methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-β-*D*-glycero-*D*-galacto-2-nonulopyranosonate* (**2**). — To a stirred, ice-cooled suspension of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (2.0 g, 6.5 mmol) in pyridine (25 mL) was added acetic anhydride (30 mL). The mixture was stirred overnight at room temperature, then concentrated, and toluene was evaporated from the residue to leave crude 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid, a solution of which in 2:1 methanol–ether (30 mL) was treated with ethereal diazomethane at 0°. After the starting material had disappeared (t.l.c.; AcOEt–AcOH–H_2O, 5:2:2), the solution was concentrated, and the residue was eluted from a column of silica gel with 60:1 chloroform–methanol to give **2** (3.26 g, 94%), $[\alpha]_D$ −32° (*c* 1, chloroform). N.m.r. data: ^1H (C_6D_6), δ 5.62 (dd, 1 H, $J_{6,7}$ 2.3, $J_{7,8}$ 3.8 Hz, H-7), 5.46 (ddd, 1 H, $J_{8,9a}$ 2.3, $J_{8,9b}$ 7.6 Hz, H-8), 5.17 (ddd, 1 H, $J_{3a,4}$ 11.7, $J_{3e,4}$ 4.8, $J_{4,5}$ 10.5 Hz, H-4), 5.07 (dd, 1 H, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.58 (d, 1 H, $J_{5,NH}$ 10.2 Hz, NH), 4.47 (ddd, 1 H, $J_{5,6}$ 10.5 Hz, H-5), 4.45 (dd, 1 H, H-9b), 4.08 (dd, 1 H, H-6), 3.45 (s, 3 H, MeO), 2.57 (dd, 1 H, $J_{3a,3e}$ 13.4 Hz, H-3*e*), 1.95 (dd, 1 H, H-3*a*), 1.90, 1.84, 1.75, 1.72, 1.63, and 1.61 (6 s, 18 H, 6 Ac); ^1H ($CDCl_3$), δ 5.38 (dd, 1 H, $J_{6,7}$ 1.5, $J_{7,8}$ 5.3 Hz, H-7), 5.33 (m, 1 H, NH), 5.26 (m, 1 H, $J_{3a,4}$ 11.5, $J_{3e,4}$ 5.0 Hz, H-4), 5.08 (ddd, 1 H, $J_{8,9a}$ 2.6, $J_{8,9b}$ 6.6 Hz, H-8), 4.50 (dd, 1 H, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.17–4.08 (m, 2 H, H-5,6), 4.12 (dd, 1 H, H-9b), 3.80 (s, 3 H, MeO), 2.55 (dd, 1 H, $J_{3a,3e}$ 13.5 Hz, H-3*e*), 2.10 (dd, 1 H, H-3*a*), 2.15, 2.14, 2.07, 2.04, 2.03, and 1.90 (6 s, 18 H, 6 Ac); ^{13}C, δ 170.80, 170.46, 170.20, 170.19, 170.13, 168.13, and 166.24 (7 C=O), 97.39 (C-2), 72.72, 71.37, 68.26, and 67.71 (C-4,6,7,8), 62.04 (C-9), 53.06 (CH_3O), 49.05 (C-5), 35.82 (C-3), 22.97, 20.75, 20.70, 20.64, 20.62, and 20.57 (6 CH_3CO).

Anal. Calc. for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.63. Found: C, 49.31; H, 6.04; N, 2.59.

*Methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-α-*D*-glycero-*D*-galacto-2-nonulopyranosonate* (**4**). — To a cooled (0°) solution of **2** (533 mg, 1 mmol) in acetyl chloride (10 mL) was added an ice-cold saturated solution of hydrogen chloride in acetyl chloride (5 mL). The mixture was kept overnight at room temperature, then concentrated, and toluene was evaporated from the residue to leave crude glycosyl chloride **6**. A solution of **6** in anhydrous acetonitrile (10 mL) was stirred with anhydrous cesium acetate (290 mg, 1.5 mmol) overnight at room temperature, then concentrated, diluted with dichloromethane (150 mL), washed with water (30 mL), and concentrated. A catalytic amount of ruthenium trichloride hydrate (~3 mg) was added to a vigorously stirred biphasic solution of the residue and sodium metaperiodate (214 mg, 1 mmol) in carbon tetrachloride (4 mL), acetonitrile (4 mL), and water (6 mL). After 5 min at room temperature, the yellow mixture was diluted with dichloromethane (150 mL), washed with water (30 mL),

dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel with ethyl acetate to give **4** (346 mg, 65% from **2**), $[\alpha]_D$ +13° (*c* 1, chloroform). N.m.r. data: ^1H (C_6D_6), δ 5.69–5.64 (m, 2 H, H-7,8), 5.12 (ddd, 1 H, $J_{3a,4}$ 11.7, $J_{3e,4}$ 4.8, $J_{4,5}$ 10.4 Hz, H-4), 4.94 (dd, 1 H, $J_{5,6}$ 11.0, $J_{6,7}$ 1.6 Hz, H-6), 4.90 (m, 1 H, H-9a), 4.89 (d, 1 H, $J_{5,NH}$ 10.3 Hz, NH), 4.58 (ddd, 1 H, H-5), 4.37 (m, 1 H, H-9b), 3.43 (s, 3 H, MeO), 2.68 (dd, 1 H, $J_{3a,3e}$ 13.0 Hz, H-3*e*), 2.09 (dd, 1 H, H-3*a*), 2.01, 1.95, 1.76, 1.67, 1.65, and 1.61 (6 s, 18 H, 6 Ac); ^1H ($CDCl_3$), δ 5.39 (d, 1 H, $J_{5,NH}$ 10.5 Hz, NH), 5.38 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 7.0 Hz, H-7), 5.20 (ddd, 1 H, $J_{8,9a}$ 2.5, $J_{8,9b}$ 5.7 Hz, H-8), 5.02 (ddd, 1 H, $J_{3a,4}$ 12.0, $J_{3e,4}$ 4.7, $J_{4,5}$ 10.3 Hz, H-4), 4.70 (dd, 1 H, $J_{5,6}$ 10.7 Hz, H-6), 4.36 (dd, 1 H, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.16 (ddd, 1 H, H-5), 4.06 (dd, 1 H, H-9b), 3.76 (s, 3 H, MeO), 2.57 (dd, 1 H, $J_{3a,3e}$ 13.2 Hz, H-3*e*), 2.08 (dd, 1 H, H-3*a*), 2.14, 2.05, 2.04, and 1.90 (4 s, 12 H, 4 Ac). 2.10 (s, 6 H, 2 Ac); ^{13}C, δ 170.81, 170.68, 170.30, 168.49, and 168.22 (5 C=O), 170.00 (2 C=O), 95.41 (C-2), 73.84, 70.14, 68.45, and 67.43 (C-4,6,7,8), 62.17 (C-9), 52.81 (CH_3O), 48.88 (C-5), 36.80 (C-2), 23.06 and 20.66 (2 CH_3CO), 20.78 (2 CH_3CO), 20.71 (2 CH_3CO).

Anal. Calc. for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.63. Found: C, 49.47; H, 5.91; N, 2.49.

Acetylation of **1**. — Compound **1** (323 mg, 1 mmol) at ~0° was stirred with anhydrous pyridine (4 mL) and acetic anhydride (4.5 mL) for 4 h at 0° and for 48 h at room temperature, then concentrated, and toluene was evaporated from the residue. The crude product was eluted from a column of silica gel with 40:1 toluene–methanol to give **4** (112 mg, 21%) and then **2** (405 mg, 76%).

Acetylation of **3**. — Treatment of **3** (246 mg, 0.5 mmol) with pyridine (6 mL) and acetic anhydride (5 mL) for ~14 h at room temperature gave, after work-up and purification as reported for the acetylation of **1**, **4** (29 mg, 11%) and **2** (230 mg, 86%).

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