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Note

Characterization of Lewis-acid transformation-products from 2,3-unsaturated relatives of methyl *N*-acetylneuraminate during attempted glycoside synthesis*

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We have earlier described the synthesis of several relatives of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid ("2,3-dehydro-NeuAc")** that are transition-state analogs for *Arthrobacter sialophilus* neuraminidase[1–4]. These included 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonic acid ("2,3-dehydro-4-epi-NeuAc")[2] and 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*manno*-non-2-en-4-ulosonic acid ("2,3-dehydro-4-keto-NeuAc")[1].

In a continuing effort to prepare mechanism-based affinity-labels for neuraminidase, these glycals were per-*O*-acetylated, and the acetates treated with stannic chloride or boron trifluoride etherate in the presence of nucleophiles, conditions that have been widely used for the synthesis of glycosides[5–11]. But, in contrast, ketosides of *N*-acetylneuraminic acid were not generated. The major reaction-products were found to be 2-methyl-(methyl 7,8,9-tri-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate)-[4,5-*d*]-2-oxazoline (**3**) (earlier found on treatment[2] of methyl *N*-acetylneuraminate with acetic anhydride plus sulfuric acid) and methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (**4**). A rationale to account for the nonformation of glycosides but, instead, of **3** and **4** is presented.

RESULTS AND DISCUSSION

Reaction of either **1** or **2** with boron trifluoride etherate and methanol for 20 h at 25° gave **3** in 88% yield (see Scheme 1). The reaction mixture was resolved by l.c. on a column of Partisil-10 silica gel with ethyl acetate as the solvent. The 100-MHz,

*Part 3 of a series on "Synthesis of Glycal Antagonists of Neuraminidase". For Part 2, see ref. 1.
**Convenient, but technically incorrect, semitrivial names commonly employed in the past for this compound include "2,3-dehydro-AcNeu" and "Neu-2-ene-5-Ac".

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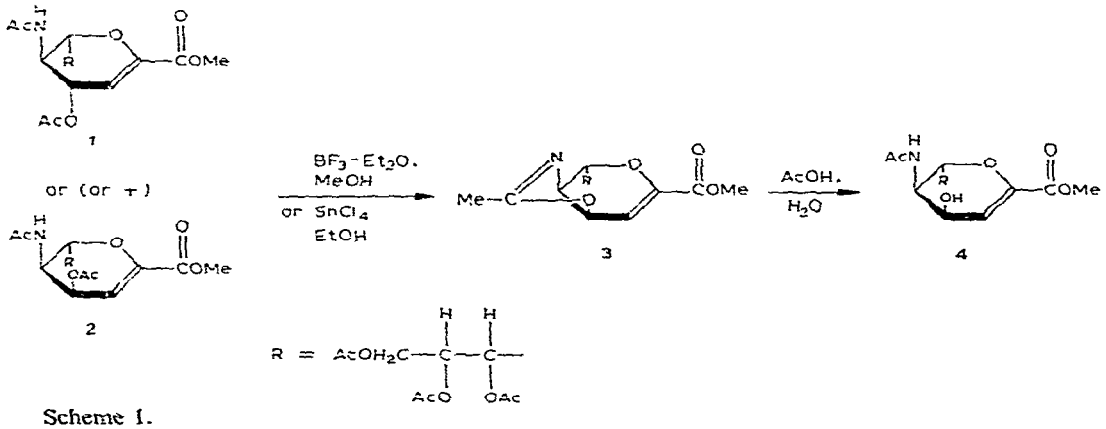
^{1}H-n.m.r. spectrum of compound **3** was identical to that reported for **3** formed in low yield from methyl *N*-acetylneuraminate by treatment with acetic anhydride–sulfuric acid[2]. The ^{13}C-n.m.r. spectrum of **3**, given here, is also consistent with the structure depicted.



Scheme 1.

Treatment of **1** or **2** with stannic chloride in ethanol for 6 h at 25° also gave **3** (92% yield). However, when the acid in the reaction mixture was slowly neutralized by the addition of sodium hydrogencarbonate, a second product, namely, **4**, was isolated. As **4** (yield 20–30%) was not formed when boron trifluoride was used as the Lewis acid, it appears probable that it was generated by hydrolysis of **3** during processing of the aqueous reaction-mixture. In conformity with this hypothesis, **4** was independently prepared from **3** by treatment with a mineral acid. The structure assigned to **4** is based on its 100-MHz, ^{1}H-n.m.r. spectrum, which exhibits a 4-hydroxyl proton as a broad doublet at δ 6.50 ($J_{4,OH} = J_{3,4} = 6$ Hz), exchanged with deuterium on addition of D_2O, and an alkenic proton on C-3 as a doublet at δ 6.25 ($J_{3,4} \sim 6$ Hz); and also on the presence of chemical shifts at 61.64 (C-4) and 110.38 p.p.m. (C-3) in its ^{13}C-n.m.r. spectrum. That **4** contains a free hydroxyl group in the axial orientation was confirmed by its acetylation with acetic anhydride–pyridine to yield **2**. Furthermore, *O*-deacetylation of **4** with sodium methoxide in anhydrous methanol gave "2,3-dehydro-4-epi-NeuAc methyl ester", thereby confirming the L-*glycero* configuration of C-4. Compound **3** was also prepared, in identical yield, from an unresolved mixture of **1** and **2**.

The generation of glycosides by the reaction of per-*O*-acetylated glycals with nucleophiles in the presence of a Lewis acid has been extensively studied[5–13]. The initial step in the formation of **3** is similar to that postulated by Grynkiewicz *et al.*[12], and involves the formation of the allyl cation from either **1** or **2**. However, **1** and **2** differ from the glycals usually studied (D-glucal[5–7] or D-galactal[7,12]), in that the former possess a 5-acetamido and a 2-methoxycarbonyl group. Thus, failure to detect glycosides on treatment of per-*O*-acetylated "2,3-dehydro-NeuAc" methyl ester with Lewis acids may, therefore, be primarily due to intramolecular attack of

the 5-acetamido function on the allyl cation, to generate the oxazolinium ion, which, on loss of a proton, yields **3**. It is conceivable that the findings described here for the attempted synthesis of NeuAc ketosides will be analogously encountered with other acetamido per-*O*-acetylated glycals.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover, capillary melting-point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 automatic polarimeter. Acetylated compounds were routinely purified by l.c. with a Perkin–Elmer 2/2 chromatograph and a column of Whatman Partisil-10 silica gel, with ethyl acetate as the solvent. Deacetylation was performed with sodium methoxide in methanol, and the reaction mixture was purified by l.c. in a reverse-phase column of Whatman Partisil-10–ODS-3, with water as the eluant. Silica gel P.F. 254 (E. Merck) was used for t.l.c., and compounds were detected either by their u.v. absorption at 254 nm, or by charring at 110° with 10% concentrated sulfuric acid in ethanol. Microanalyses were performed by Micro-Analysis, Inc., P.O. Box 5088, Wilmington, DE 19808, or by Galbraith Laboratories, Inc., P.O. Box 4187, Knoxville, TN 37921. ^{1}H-N.m.r. spectra were recorded with Varian A-60 and XL-100 spectrometers, and ^{13}C-n.m.r. spectra, at a frequency of 25.5 MHz, with a Varian XL-100 spectrometer. For ^{1}H-n.m.r. spectra, the chemical shifts are expressed in δ values relative either to internal tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-1-sulfonate. For ^{13}C-n.m.r. spectra, the chemical shifts are expressed in p.p.m. relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate. Mass spectra were recorded with a Finnegan-4000, GC/MS apparatus.

*2-Methyl-(methyl 7,8,9-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-talo-non-2-enonate)-[4,5-*d*]-2-oxazoline* (**3**) *and methyl 5-acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-talo-non-2-enonate* (**4**). — A solution of **1** plus **2** (1.0 g, 2.11 mmol) and dry ethanol (0.195 g, 4.24 mmol) in 1,2-dichloroethane (25 mL) was stirred at 25°, and anhydrous stannic chloride (1 mL) was slowly added. Absence of starting material after 6 h was revealed by t.l.c. (ethyl acetate, or 9:1 chloroform–methanol). The brown solution was made neutral with saturated, aqueous sodium hydrogencarbonate solution, chloroform was added, and the organic layer was washed with saturated brine, dried (anhydrous sodium sulfate), and evaporated, to give crude product (0.75 g); t.l.c. indicated two major products, **3** and **4**, and several, unidentified, minor products. The mixture was resolved by l.c. in a semi-preparative column of silica gel (ethyl acetate). The major product crystallized from 1:1 ethyl acetate–hexane, to give **3**, 0.540 g (63%); m.p. 82–83°, $[\alpha]_D^{22}$ −13.45° (*c* 1.1, chloroform). Its i.r., ^{1}H-n.m.r., and mass spectra were reported earlier[2]; ^{13}C-n.m.r. ($CDCl_3$): 171.06, 170.28, 170.10 (OAc), 167.66 (C=N), 162.41 (C-1), 147.75 (C-2), 108.00 (C-3), 77.25 (C-6), 72.64, 70.80, and 69.50 (C-7,8,9), 62.59 and 62.41 (C-4,5), 52.80 ($CO\text{-}O\text{-}CH_3$), 21.00 and 20.88 ($CO\text{-}CH_3$), and 14.35 p.p.m. ($N\text{-}CH_3$).

The formation of a second product, **4**, was dependent on the processing employed. On neutralization of acid by the slow addition of sodium hydrogencarbonate, variable amounts of **4** (20–30%) were obtained. If, however, the reaction mixture was rapidly mixed with the base, compound **4** was not observed. Compound **4** was isolated, and crystallized from 1:1 chloroform–hexane, to give 0.165 g (22%); m.p. 101–103°, $[\alpha]_D^{22}$ −34.14° (*c* 1.4 chloroform); m.s. (c.i., isobutane): *m/z* 432 $(M + 1)^+$; ^{1}H-n.m.r. (100 MHz, $CDCl_3$): δ 6.5 (bd, $J_{4,OH}$ ~6.0 Hz, OH-4), 6.25 (d, $J_{3,4}$ ~6.0 Hz, H-3), 5.48 (bd, $J_{7,8}$ ~5.0, $J_{6,7}$ ~0.8 Hz, H-7), 5.30 (m, $J_{8,9a}$ ~6.0, $J_{8,9b}$ ~2.0 Hz, H-8), 4.80 (dd, $J_{9a,9b}$ ~12.0 Hz, H-9a), 4.25 (m, H-4,5,6,9b), 3.80 (s, $-OCH_3$), 2.10 (ms, 3 OAc), and 1.95 (s, $NHCOCH_3$); ^{13}C-n.m.r. ($CDCl_3$); 175.18, 170.82 (3 OAc and NHAc), 162.88 (C-1), 145.44 (C-2), 110.38 (C-3), 73.46 (C-6), 72.35, 68.61, 62.76 (C-7,8,9), 61.64 (C-4), 52.77 ($CO\text{-}OCH_3$), 47.08 (C-5), 23.38 ($NHCOCH_3$), and 21.0 p.p.m. ($OCOCH_3$).

Anal. Calc. for $C_{18}H_{25}NO_{11} \cdot H_2O$: C, 48.10; H, 6.01; N, 3.12. Found: C, 48.43; H, 6.13; N, 3.06.

Compounds **3** and **4** could also be prepared directly from either **1** (0.152 g, 0.32 mmol) or **2** (0.160 g, 338 μmol), as described for their mixture. The products **3** (yield, ~60%) and **4** (yield, ~25%) had appropriate ^{1}H-n.m.r., ^{13}C-n.m.r., and i.r. spectra, and, in t.l.c., exhibited R_F values coincident with those of the authentic compounds.

Acetylation of **4** *to methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-talo-non-2-enonate* (**2**). — To a solution of **4** (50 mg, 116 μmol) in dry pyridine (2 mL) was added acetic anhydride (0.6 mL), and the mixture was stirred for 18 h at 25°, evaporated under diminished pressure, and the residue extracted with chloroform. Evaporation of the extract, and crystallization of the residue from 1:1 chloroform–hexane, gave **2**, 42 mg (78%); m.p. 95–97°, $[\alpha]_D^{30}$ −121.0° (*c* 1.0, chloroform); m.s. (c.i., isobutane): *m/z* 474 $(M + 1)^+$; ν_{max}^{KBr} 1740 (OAc and $COOCH_3$) and 1690 cm^{-1} (Amide I); ^{1}H-n.m.r. (100 MHz; $CDCl_3$): δ 6.20 (d, $J_{3,4}$ ~6.0 Hz, H-3), 5.60 (bd, $J_{5,NH}$ ~10.0 Hz, NH), 5.50 (dd, $J_{7,8}$ ~5.0, $J_{6,7}$ ~2.0 Hz, H-7), 5.30 (m, $J_{8,9a}$ ~6.0, $J_{8,9b}$ ~2.0 Hz, H-8), 5.18 (dd, $J_{3,4}$ ~6.0, $J_{4,5}$ ~4.0 Hz, H-4), 4.79 (dd, $J_{9a,9b}$ ~12.0 Hz, H-9a), 4.55 (dd, $J_{5,6}$ ~10.0 Hz, H-5), 4.30 (H-9b), 4.15 (H-6), 3.80 (s, OCH_3), 2.10 (ms, 4 OAc), and 1.95 (s, $NHCOCH_3$); ^{13}C-n.m.r. ($CDCl_3$): 170.79, 170.44, 170.08, 169.90, 169.55 (4 OAc and $NHCOCH_3$), 162.03 (C-1), 146.72 (C-2), 106.20 (C-3), 74.19 (C-6), 72.11, 68.11, 65.07 (C-7,8,9), 62.41 (C-4), 52.62 (CO_2CH_3), 44.57 (C-5), 23.18 ($NHCOCH_3$), and 20.92, 20.75 p.p.m. ($OCOCH_3$).

Anal. Calc. for $C_{20}H_{27}NO_{12} \cdot 0.5\ H_2O$: C, 49.74; H, 5.85; N, 2.90. Found: C, 49.55; H, 6.09; N, 2.92.

For comparative purposes, **2** was alternatively prepared by acetylation of[2] "methyl 2,3-dehydro-4-epi-*N*-acetylneuraminate". The t.l.c., and the i.r., ^{1}H-n.m.r., and ^{13}C-n.m.r. spectra were identical to those just reported for compound **2**.

Hydrolysis of oxazoline **3** *to* **4**. — To a solution of **3** (0.11 g, 266 μmol) in ethyl acetate (2 mL) were added water (2–3 drops) and acetic acid (1 drop), and the

mixture was stirred for 72 h at 25°; t.l.c. (ethyl acetate) then indicated the absence of starting material. The solvent was evaporated under diminished pressure, the residue extracted with chloroform, and the extract washed with water, dried (anhydrous sodium sulfate), and evaporated, to afford crude product which was resolved by l.c. in a semi-preparative column of silica gel (ethyl acetate), to give **4**, 0.101 g (88%).

Treatment of **1** *plus* **2** *with boron trifluoride etherate.* — To a solution of **1** plus **2** (0.473 g, 1 mmol) in dry benzene (20 mL) were added dry methanol (40 mg, 1.25 mmol) and boron trifluoride etherate (1 mL). The mixture was stirred for 20 h at 25°, whereupon analysis by t.l.c. (ethyl acetate) revealed only a small amount of starting material. After neutralization of the acid with sodium hydrogencarbonate, and addition of benzene, the benzene layer was separated, successively washed with water and saturated salt solution, dried, and evaporated. The crude products were separated in a semi-preparative column of Whatman Partisil-10 (ethyl acetate), to yield **3**, 0.362 g (87%). The second product, **4**, was present only in traces under these reaction conditions.

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