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Note

Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*manno*-non-2-en-4-ulosonate*

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We have recently described a facile synthesis of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (2,3-dehydro-NeuAc), which also leads to the formation of the diastereomer 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonic acid (2,3-dehydro-4-epi-NeuAc)[1]. Each of these compounds, and their methyl esters, is a competitive inhibitor of *Arthrobacter sialophilus* neuraminidase[1,2]. In a continuing effort to define the role of the 4-hydroxyl group of sialic acid analogs for neuraminidase binding and catalysis, we now report the preparation of methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*manno*-non-2-en-4-ulosonate (4-keto-2,3-dehydro-NeuAc Me ester) and a determination of its relative effectiveness as an enzyme inhibitor.

To facilitate the oxidation of 2,3-dehydro-NeuAc Me ester (**1**) in chloroform, this compound was treated with chlorotriphenylmethane in dry pyridine to give the 9-trityl ether **3** in 81% yield. Oxidation of **3** by manganese dioxide in dry chloroform gave, after resolution by l.c. and crystallization, the 4-keto derivative **6** (80%). The ^{1}H-n.m.r. spectrum (100 MHz) of **6** showed a singlet at δ 6.35 for H-3 in contrast to the doublet at δ 5.90 ($J_{3,4}$ ~2.5 Hz) shown by **3**. The presence of a carbonyl carbon atom (191.90 p.p.m.) in the ^{13}C-n.m.r. spectrum, and the carbonyl absorption at 1680 cm^{-1} in the i.r. spectrum of **6** were also consistent with the assigned enone structure. Detritylation with 70% acetic acid at 50–60° followed by resolution of the product by l.c., and crystallization gave 2,3-dehydro-4-keto-NeuAc Me ester (**8**) in 21% yield.

Compounds **1** and **2** were also converted into their 9-bis(4-methoxyphenyl)-phenylmethyl ethers as these substituted trityl derivatives are known[3] to be cleaved efficiently by the action of mild acid at room temperature. Oxidation of **4** or **5** with manganese dioxide gave **7**, which had similar physicochemical characteristics as **6**. In line with an earlier, comparable study[4], compound **5** required more prolonged

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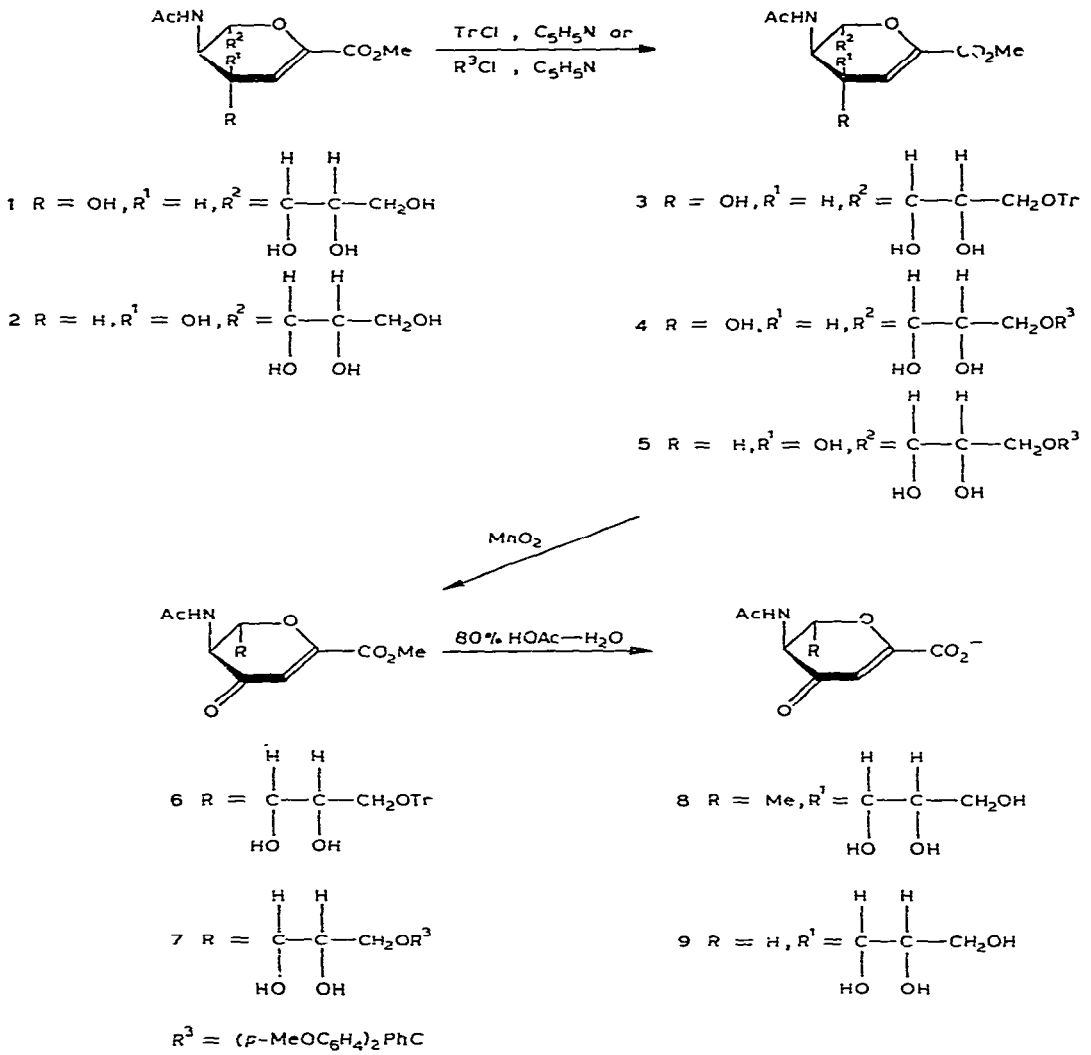
and drastic reaction conditions to undergo oxidation, because of the quasi-axial orientation of the oxidized group. Removal of the protecting group from **7** by treatment with 80% aqueous acetic acid for 30 min at 25° and resolution by l.c. gave **8** (19%). Thus, the hoped-for improvement in overall yield was not achieved.

Both 2,3-dehydro-4-keto-NeuAc (**9**) and its methyl ester **8** are competitive inhibitors of *Arthrobacter* neuraminidase. Their K_I values are 6.10×10^{-5} and 1.78×10^{-5}M, respectively. These values are smaller than those we reported[1] earlier for 2,3-dehydro-4-epi-NeuAc and its methyl ester, suggesting that the enones bind more strongly to the enzyme. Thus these findings provide encouragement for the possible use of such compounds or derivatives thereof as site-directed, affinity reagents for neuraminidases.



EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover capillary apparatus and are uncorrected. ^{1}H-N.m.r. spectra were recorded with Varian A-60 and XL-100 spectrometers. ^{13}C-N.m.r. spectra were measured at a frequency of 25.2 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 to 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 obtained by using a Finnegan-4000 with a solid probe. Chemical ionization employed methane gas. I.r. spectra were obtained with a Perkin–Elmer 137-B spectrometer. Silica gel P.F. 254 (E. Merck) was used for t.l.c. Compounds were detected either by their u.v. absorption (254 nm) or by charring at 110° after spraying with 10% concentrated sulfuric acid in ethanol. Compounds were routinely purified by l.c. with a Perkin–Elmer 2/2 instrument with a Whatman Partisil-10-silica gel column and ethyl acetate as solvent or a Whatman Partisil-10-ODS-3 reversed-phase column with water–methanol as solvent. Optical rotations were recorded with a Perkin–Elmer Model 141 automatic polarimeter. Microanalyses were performed by Micro-Analysis Inc., P.O. Box 5088, Wilmington, DE 19808.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-9-O-trityl-*D-glycero-D-galacto-*non-2-en-onate* (**3**). — A suspension of **1** (0.10 g, 0.328 mmol) in dry pyridine (5 mL) was stirred at 25° and chlorotriphenylmethane (0.224 g, 0.8 mmol) was added. After 48 h, t.l.c. in 7:3 chloroform–methanol revealed the absence of starting material. Pyridine was removed by evaporation of toluene from the product. A solution of the residue in chloroform was washed with water and dried with anhydrous sodium sulfate. Evaporation gave a mixture that was resolved by l.c. on a semipreparative column of silica gel (ethyl acetate). The preponderant product was **3** (0.145 g 81%), m.p. 140–142° (from 7:3 ethyl acetate–hexane); ^{1}H-n.m.r. (100 MHz, $CDCl_3$): δ 7.35 (m, aromatic protons), 6.55 (d, $J_{5,NH}$ ~5 Hz, NH), 5.90 (d, $J_{3,4}$ ~2.5 Hz, H-3), 4.50 (b, H-6), 4.35 (m, H-5), 4.15 (b, H-8), 3.85 (s, CO_2Me), 3.45 (m, H-7 and H-9), and 2.10 (s, NHAc).

Anal. Calc. for $C_{31}H_{33}NO_8$: C, 67.97; H, 6.08; N, 2.56. Found: C, 67.61; H, 5.85; N, 2.48.

*Methyl 5-Acetamido-2,6-anhydro-3,5-dideoxy-9-O-trityl-*D-manno-*non-2-en-4-ulosonate* (**6**). — To a solution of **3** (0.075 g, 0.137 mmol) in dry chloroform (10 mL) was added an excess of active manganese dioxide (0.5 g, 5.7 mmol). The mixture was stirred at 25°. After 4 h, t.l.c. with 9:1 ethyl acetate–hexane revealed the absence of starting material. The manganese dioxide was removed by filtration through a pad of Celite and the filtrate was evaporated to dryness. The products were separated by l.c. on a semi-preparative column of silica gel (7:3 ethyl acetate–hexane) to give **6** (0.06 g, 80%), m.p. 128–130° (3:7 ethyl acetate–hexane), $[\alpha]_D^{28}$ +29.5° (*c* 1.0, chloroform); ν_{max}^{KBr} 1720 (Co_2Me and OAc), 1680 (C=C-C=O), 1650 (NHCO I),

and 1520 cm^{-1} (NHCO II): *m/e*: 468 ($M - CO_2CH_3 + H_2O$)$^+$, 272 ($M - CH_2OTr$)$^+$, 243 [base peak, $M - CO_2CH_3$ + Tr and Tr (M^+)]$^+$ and 212 [M — CHOH(CHOH)-CH_2OTr]$^+$; ^{1}H-n.m.r. (100 MHz, $CDCl_3$): δ 7.34 (m, aryl), 6.34 (d, J ~5.6 NH), 6.26 (s, H-3), 4.94 (dd, $J_{5,6}$ ~9.5, J ~5.7 Hz, H-5), 4.75 (d, H-6), 4.15 (m, H-8), 3.85 (s, Me), 3.63 (d, $J_{7,8}$ ~9.0 Hz, H-7), 3.57 (dd, $J_{8,9A}$ ~3.8, $J_{9A,9B}$ ~12.0 Hz, H-9A), 3.43 (q, $J_{8,9B}$ ~2.8 Hz, H-9B), and 2.08 (s, COMe); ^{13}C-n.m.r. (p.p.m.): 191.9 (C-4), 173.8 (Ac), 160.13 (C-1), 144.1 (C-2), 128.9, 128.07, 127.3 (aryl), 106.74 (C-3), 82.35 (C-6), 69.22 (C-7), 68.91 (C-8), 65.20 (C-9), 53.27 (OCH_3), 51.97 (C-5), and 22.77 (CH_3).

Anal. Calc. for $C_{31}H_{31}NO_8$: C, 68.22; H, 5.73; N, 2.57. Found: C, 68.08; H, 5.68; N, 2.52.

Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-D-manno-*non-2-en-4-ulosonate* (**8**) *from* **6**. — A suspension of **6** (75 mg, 0.137 mmol) in aqueous 80% acetic acid (2.5 mL) was heated to 50–60°. After 2 h, the mixture was evaporated, the residue triturated with water, and the mixture filtered. The aqueous solution was subjected to l.c. on a Whatman Partisil-10-ODS-3 reversed-phase column. The major product (**8**) was eluted with 19:1 (v/v) water–methanol and lyophilized to give **8** as a white solid (9 mg, 21%); m.p. 137–139° (1:1 methanol–ether), $[\alpha]_D^{28}$ —48.0° (*c* 0.65, methanol); ν_{max}^{KBr} 1725 (COMe and OAc), 1690 (C=C-C=O), 1650 (NHCO I), and 1515 cm^{-1} (NHCO II); *m/e* (c.i.): 304 (M + 1), 332 (M + 29), and 344 (M + 41); ^{1}H-n.m.r. (100 MHz, D_2O): δ 6.35 (s, H-3), 4.90 (d, $J_{5,6}$ ~10.0 Hz, H-5), 4.55 (dd, $J_{6,7}$ ~1.0 Hz, H-6), 3.90 (s, OMe), 3.85 (dd, $J_{8,9A}$ ~3.5, $J_{9A,9B}$ ~11.0 Hz, H-9A), 3.70 (m, H-8), 3.6 (m, H-9B and H-7), and 2.10 (s, NHAc).

Anal. Calc. for $C_{12}H_{17}NO_8 \cdot H_2O$: C, 44.86; H, 5.95; N, 4.35. Found: C, 44.48; H, 5.92; N, 4.12.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-9-*O-*bis(4-methoxyphenyl)phenyl-methyl*-D-glycero-D-galacto-*non-2-en-onate* (**4**). — This compound was prepared from **1**, essentially as described for **3**, in 71% yield, m.p. 120–123° (from 1:1 chloroform–hexane), $[\alpha]_D^{28}$ +55.6° (*c* 0.5, chloroform); ^{1}H-n.m.r. (100 MHz, $CDCl_3$); δ 7.5–6.3 (m, aryl), 6.0 (d, $J_{3,4}$ ~2.4 Hz, H-3), 3.8 (ms, OMe), and 1.9 (s, NHAc).

Anal. Calc. for $C_{33}H_{37}NO_{10} \cdot 1.5\ H_2O$: C, 62.45; H, 6.35; N, 2.20. Found: C, 62.82; H, 6.44; N, 1.98.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-9-*O-*bis(4-methoxyphenyl)phenyl-methyl*-D-glycero-D-talo-*non-2-en-onate* (**5**) was prepared from **2** (0.234 g, 0.69 mmol) and isolated analogously to give 0.321 g (69%); m.p. 133–135° (from ethyl acetate–hexane), $[\alpha]_D^{28}$ —40.2° (*c* 1.0, chloroform); ^{1}H-n.m.r. (100 MHz, $CDCl_3$): δ 7.50–6.85 (m, aryl), 6.05 (d, $J_{3,4}$ ~5.5 Hz, H-3 proton), 3.9–3.8 (ms, OMe), and 1.95 (s, NHAc).

Anal. Calc. for $C_{33}H_{37}NO_{10} \cdot 0.5\ H_2O$: C, 64.27; H, 6.21; N, 2.27. Found: C, 64.47; H, 6.30; N, 2.17.

*Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-9-*O-*bis(4-methoxyphenyl)phenyl-methyl*-D-manno-*non-2-en-4-ulosonate* (**7**). — This compound was prepared from **4**, essentially as described for **6**, in 83% yield; syrup; $[\alpha]_D^{28}$ +16.8° (*c* 0.50, chloro-

form); ν_{max}^{KBr} 1730 (CO_2Me and OAc) and 1690 cm^{-1} (C=C-C=O); ^{1}H-n.m.r. (100 MHz, $CDCl_3$); δ 8.75–6.7 (m, aryl), 6.40 (s, H-3), 3.9–3.6 (ms, OMe), and 1.90 (s, NHAc). Similar treatment of methyl 5-acetamido-2,6-anhydro-2,3,5-trideoxy-9-*O*-bis(4-methoxyphenyl)phenylmethyl-D-*manno*-non-2-en-4-ulosonate (**7**) (the 4-epimer of **4**) gave **7** in 85% yield.

Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-D-manno-*non-2-en-4-ulosonate* (**8**). — De-etherification of **7** was performed by using 80% acetic acid for 30 min at 25° to give **8** in 19% yield. This product gave a ^{1}H-n.m.r. spectrum identical to that of **7**.

5-Acetamido-2,6-anhydro-3,5-dideoxy-D-manno-*non-2-en-4-ulosonic acid* (**9**). — Compound **8** (1 mg) was dissolved in water (0.40 mL) and the pH was adjusted to 9.0 with M sodium hydroxide. The mixture was kept overnight at 0°, and then made neutral with Dowex 50W-X8 (H^+). Analysis by t.l.c. in 4:1 chloroform–methanol showed a single sulfuricacid-reactive spot, R_F 0.21 (**9**). No evidence for residual **8** (R_F 0.38) was found. This solution was used directly in enzyme studies.

Enzyme studies. — A homogeneous preparation of *A. sialophilus* neuraminidase was prepared as described previously[5]. Inhibition studies were performed as recommended by Segel[6] in 10mM citrate–phosphate buffer, pH 6.0, with sialylactose as substrate (K_M, 0.90mM). These assay conditions have been described earlier[7]. The K_I values were calculated from secondary plots of K_M app *vs.* inhibitor concentration. K_M values were determined from enzyme-reaction velocities at 37° for six substrate concentrations, ranging from 0.3 to 2 K_M, and four inhibitor concentrations ranging from 0 to 2 K_I.

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