Synthesis of C-4-disubstituted analogues of *N*-acetylneuraminic acid

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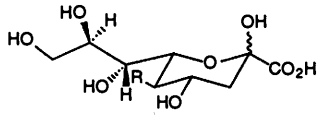
The synthesis of some novel C-4-disubstituted analogues of *N*-acetylneuraminic acid has been achieved by nucleophilic opening of the epoxide ring in the acetyl-4,4′-anhydroneuraminic acid derivatives 6 and 7. This has provided the azide 8, the methyl ether 9, the nitrile 10 and the chloride 11 of the 8,9-isopropylidenated β-methyl ketoside of methyl *N*-acetylneuraminate in moderate to good yields.

Introduction

N-Acetylneuraminic acid (Neu5Ac, **1**) and related sialic acids are widely distributed in living systems, often occurring as the terminal sugar of oligosaccharides associated with glycoconjugates including glycolipids and glycoproteins.[1,2] A number of these glycoconjugates have been implicated in complex and diverse functions such as cell-recognition and immunologic events.[1,3] The modulation of cell-surface sialic acid expression is achieved by a variety of enzymes. For example, sialidases cleave terminal sialic acid residues from sialoglycoconjugates and this process is thought to be important in revealing other recognition sites.[4] One example of this process is the action of *Vibrio cholerae* sialidase on gangliosides, which reveals recognition sites for cholera toxin.[5] Our interest in this particular enzyme and other sialic acid-recognising proteins has given rise to the synthesis of structurally modified Neu5Ac analogues.



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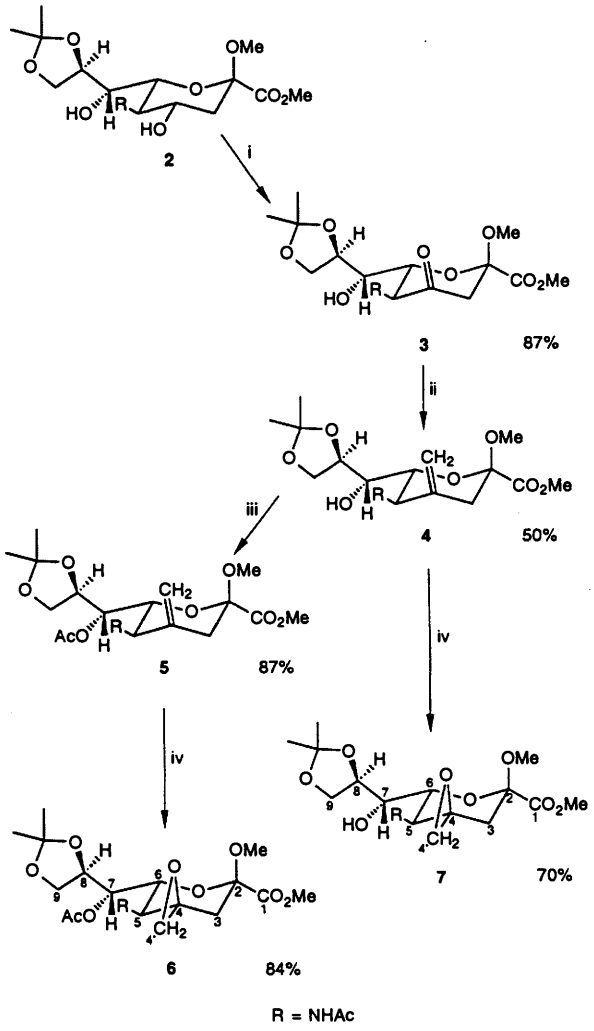
R = NHAc

Although there have been a large number of structurally modified sialic acids reported in the literature, including C-4-substituted analogues of *N*-acetylneuraminic acid,[6–11] there have been few reported cases of C-4-disubstituted Neu5Ac analogues.[12,13] We thought that investigation into the synthesis of these disubstituted analogues would provide valuable biological probes which may serve to expand our understanding of the ability of sialidases, and indeed other *N*-acetylneuraminic acid-recognising proteins, to cope with branching at this position.

Results and discussion

In order to synthesize C-4-disubstituted Neu5Ac analogues which retain a hydroxy group at C-4, as in the parent compound Neu5Ac **1**, we decided that one of the best approaches would involve nucleophilic opening of the epoxide ring of the 4,4′-anhydro-Neu5Ac derivatives **6** or **7** (Scheme 1).

A key precursor in the synthesis of the epoxides **6** and **7** is the well known ketone **3**.[7] Thus, selective oxidation at C-4 of methyl (methyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **2**[14] with pyridinium dichromate (PDC)–acetic anhydride[15] afforded ketone **3** in 87% yield. This yield was an improvement on that reported



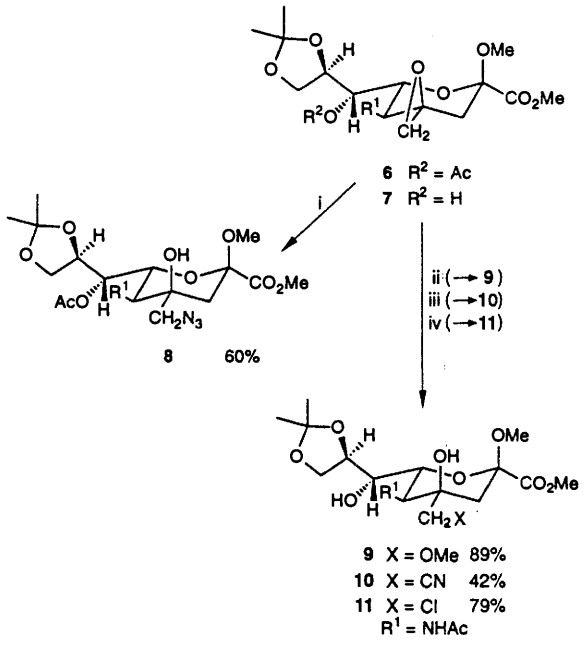
Scheme 1 *Reagents and conditions:* i, PDC, Ac_2O, CH_2Cl_2, room temp.; ii, Cp_2ZrCl_2, Zn, CH_2I_2, THF, 0 °C to room temp.; iii, Ac_2O, pyridine, room temp.; iv, MCPBA, CH_2Cl_2, 0 °C

using ruthenium tetraoxide[7] and was significantly better than that reported using either pyridinium chlorochromate[14] or pyridinium fluorochromate.[14] Methylenation of the ketone **3** was achieved by treatment with zirconocene dichloride–zinc–diiodomethane in tetrahydrofuran (THF) and provided the alkene **4** in 50% yield according to the procedure of Hartmann *et al.*[13] These authors observed that the ketone **3** readily enolised, thus making olefination by classical Wittig chem-

istry difficult or impossible. The alkene **4** was also acetylated as reported[13] to provide the 7-*O*-acetate **5**.[13]

Epoxidation of the olefinic double bond of compound **5** by treatment with *m*-chloroperbenzoic acid (MCPBA) provided a single product, one of the two possible isomers of the epoxide **6**, in 84% yield. Analogous epoxidation of alkene **4** with MCPBA furnished the epoxide **7** in 70% yield (*cf.* 73% overall yield of epoxide **6** from alkene **4**). Epoxide **7** was characterised as the 7-*O*-acetate by acetylation with Ac_2O–pyridine. The ^1H NMR spectrum of the protected epoxide thus formed was identical, not unexpectedly, with that of epoxide **6**. Nuclear Overhauser effect (NOE) difference spectroscopy supported the assigned structure of compound **6**. On irradiation of the 4′-H proton at δ 2.63 a positive NOE was observed to the N–H and 5-H protons; however, no NOE was observed to the 6-H proton.

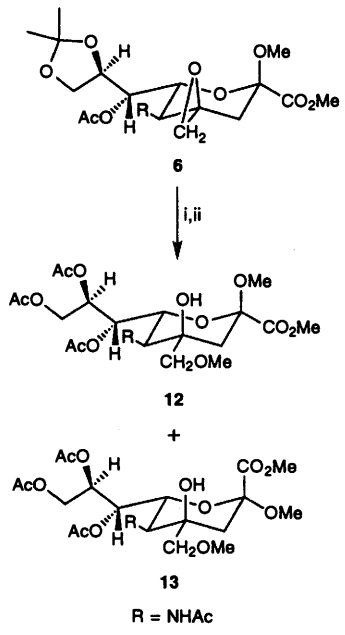
Both epoxides **6** and **7** proved to be ideal intermediates for the synthesis of C-4-disubstituted derivatives of the *N*-acetylneuraminic acid template. Thus nucleophilic opening of both epoxides was investigated with a number of nucleophiles to obtain four hitherto unknown C-4-disubstituted compounds (Scheme 2).



Scheme 2 *Reagents and conditions:* i, **6**; LiN_3, acetone, 56 °C; ii, **6**; NaOMe, MeOH, 65 °C; iii, **7**; KCN, MeOH, room temp.; iv, **7**; NH_4Cl, $Ti(OPr^i)_4$, DMSO, room temp.

Nucleophilic opening of epoxide **6** with lithium azide was readily achieved and provided the azide **8** in 60% isolated yield. The C-4 hydroxy group of this compound has been found, not unexpectedly, to be rather resistant to acetylation under standard conditions (Ac_2O–pyridine). Similar nucleophilic opening of epoxide **6** with sodium methoxide in methanol under reflux occurred with concomitant 7-de-*O*-acetylation to afford the methyl ether **9** in 86% yield. Interestingly, preliminary results (^1H NMR, data not shown) indicated that treatment of epoxide **6** with MeOH in the presence of an acidic resin (Dowex 50WX8, H^+) under reflux followed by acetylation (Ac_2O–pyridine) of the crude products provided a 3:1 mixture of the β and α anomers **12** and **13** (Scheme 3). This anomerisation process is a direct consequence of the reaction conditions. Once again, it is worth noting that the C-4 tertiary hydroxy group did not acetylate under these conditions. ^1H NMR spectroscopy indicated the presence of four acetates for both anomers and a singlet at δ 3.76 in the spectrum of compound **13** which disappeared on D_2O exchange.

Nucleophilic opening of epoxide **7** also provided two novel disubstituted analogues. Introduction of a nitrile group at



Scheme 3 *Reagents and conditions:* i, Dowex 50WX8 (H^+), MeOH, 65 °C; ii, Ac_2O, pyridine, room temp.

C-4′ was readily achieved under standard conditions. Thus treatment of epoxide **7** with potassium cyanide at room temperature afforded the nitrile **10** in moderate yield. Similar ring opening of epoxide **7** with chloride ion might be expected to be less easy due to the poor nucleophilic nature of Cl^-. Reaction of epoxide **7** with 2 mole equivalents of NH_4Cl in dimethyl sulfoxide (DMSO)[16] at room temperature afforded only starting material after 3 h. Addition of 1.5 mole equivalents of $Ti(OPr^i)_4$ to the reaction[16] afforded the chloride **11** and some epoxide **7** as indicated by TLC after a further 1.5 h. Allowing the reaction to continue for another 14 h did not increase the ratio of product to starting material. Purification by chromatography on silica gel provided the chloride **11** in 50% yield (79% based on consumed **7**).

In all of the above cases only a single isomer was detected for each ring-opening reaction where nucleophilic attack had occurred at the less hindered carbon of the epoxide moiety. Analysis of the ^{13}C (JMOD) NMR spectra for the disubstituted analogues **8**, **10** and **11** displays resonances due to C-4′ at δ_C 57.0, 29.0 and 48.6 respectively. The alternative structural isomers which have a hydroxymethyl group at the C-4 equatorial position would be expected to display resonances due to C-4′ at $\delta_C \sim 70$.[17] In addition, the resonances due to C-4 for analogues **8**, **9**, **10** and **11** were consistent and were found at δ_C 73.2, 71.6, 70.0 and 72.1 respectively. All of these observations lend support to the proposal that nucleophilic attack occurs exclusively at C-4′ for both epoxides **6** and **7** resulting in the formation of products **8**–**11**. In the case of the methyl ether **9** it is unclear, based on the spectral data obtained, as to which one of the two possible isomers is formed. However, on the basis of the results observed above, one would expect nucleophilic attack to have occurred at the less hindered carbon of the epoxide moiety of **6** resulting in the formation of compound **9** as shown in Scheme 2.

The preparation of the C-4-disubstituted compounds **8**–**11** has been achieved in moderate to good yields from the novel epoxides **6** and **7**. The adopted synthetic strategy provides a host of valuable biological probes for the investigation of *N*-acetylneuraminic acid-recognising proteins. The use of this methodology for the synthesis of a wider range of *N*-acetylneuraminic acid analogues is currently under investigation.

Experimental

General

Mps were determined on a Gallenkamp melting point apparatus and are uncorrected. ^1H and ^{13}C (JMOD) NMR spectra were recorded using a Brüker AMX-300 spectrometer. Chemical shifts were referenced to tetramethylsilane (0.0 ppm) as internal standard and *J*-values are given in Hz. NOE difference spectra were recorded using a Brüker DRX-500 spectrometer. Mass spectra were obtained using a JEOL JMS-DX 300 mass spectrometer. Optical rotations were obtained using a JASCO DIP-370 polarimeter and $[\alpha]_D$-values are quoted in 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded using a Hitachi 270-30 spectrophotometer as KBr disks unless specified otherwise. Microanalyses were performed by Chemical and Micro Analytical Services Pty. Ltd., Belmont, Victoria.

Methyl (methyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-β-D-*manno*-2,4-nonudiulopyranosid)onate 3

To a mixture of PDC (98%; 1.65 g, 4.30 mmol) and acetic anhydride (1.75 cm^3, 18.5 mmol) in CH_2Cl_2 (15 cm^3) was added a solution of the alcohol **2** (2.32 g, 6.15 mmol) in CH_2Cl_2 (20 cm^3). The resulting suspension was stirred at room temperature for 1.5 h, at which time the reaction was quenched by the addition of propan-2-ol (2 cm^3) and concentrated under reduced pressure. Toluene was co-distilled from the residue three times, affording a dark brown gum, which was dissolved in CH_2Cl_2 and applied to a short column of florisil. Thorough elution with CH_2Cl_2 followed by EtOAc and evaporation of the solvents afforded a pale green foam. Further purification of the crude product by flash chromatography (silica gel; EtOAc) afforded the ketone **3** (2.02 g, 87%) as an amorphous mass. Spectroscopic data of product **3** were in good agreement with those previously reported.[7]

Methyl (methyl 5-acetamido-3,4,5-trideoxy-8,9-*O*-isopropylidene-4-*C*-methylene-β-D-*manno*-2-nonulopyranosid)onate 4

This compound was prepared in 50% yield according to the method of Hartmann *et al.*[13] Spectroscopic data of product **4** were in good agreement with those previously reported.[13]

Methyl (methyl 5-acetamido-7-*O*-acetyl-3,5-trideoxy-8,9-*O*-isopropylidene-4-*C*-methylene-β-D-*manno*-2-nonulopyranosid)onate 5

This compound was prepared in 87% yield according to the method of Hartmann *et al.*[13] Spectroscopic data of product **5** were in good agreement with those previously reported.[13]

Methyl (methyl 5-acetamido-7-*O*-acetyl-4,4′-anhydro-3,5-dideoxy-4-*C*-hydroxymethyl-8,9-*O*-isopropylidene-β-D-*glycero*-D-*talo*-2-nonulopyranosid)onate 6

A solution of the alkene **5** (380 mg, 0.915 mmol) and MCPBA (85%; 280 mg, 1.38 mmol) in CH_2Cl_2 (8 cm^3) was stirred at 0 °C for 3 h. The reaction mixture was then diluted with EtOAc (40 cm^3) and the organic layer was washed with saturated aq. $NaHCO_3$ (2 × 10 cm^3). Subsequent drying of the organic layer over Na_2SO_4 and concentration under reduced pressure yielded a foam. Purification of the crude product on silica gel (EtOAc) afforded the *epoxide* **6** as an amorphous solid (331 mg, 84%); mp 134–138 °C; $[\alpha]_D^{26}$ −34.7 (*c* 1.39, $CHCl_3$) (Found: C, 53.05; H, 7.1; N, 3.0. $C_{19}H_{29}NO_{10}$ requires C, 52.9; H, 6.8; N, 3.25%); ν_{max}/cm^{-1} 1744, 1662, 1538, 1440, 1372, 1262, 1216, 1164, 1094, 1048 and 1024; $\delta_H(CDCl_3)$ 1.33 and 1.38 (2 × 3 H, 2 × s, CMe_2), 1.91 (1 H, d, $J_{3a,3e}$ 14.6, H^a-3), 1.92 (3 H, s, AcN), 2.14 (3 H, s, AcO), 2.47 (1 H, d, $J_{3e,3a}$ 14.6, H^e-3), 2.50 (1 H, d, $J_{4'a,4'b}$ 4.1, H^a-4′), 2.63 (1 H, d, $J_{4'b,4'a}$ 4.1, H^b-4′), 3.35 (3 H, s, OMe), 3.82 (3 H, s, CO_2Me), 3.94 (1 H, dd, $J_{9a,9b}$ 8.8, $J_{9a,8}$ 6.9, H^a-9), 4.07 (1 H, dd, $J_{9b,9a}$ 8.8, $J_{9b,8}$ 6.1, H^b-9), 4.14 (1 H, dd, $J_{6,5}$ 10.8, $J_{6,7}$ 2.3, H-6), 4.39 (1 H, ddd, $J_{8,9a}$ 6.9, $J_{8,7}$ 6.7, $J_{8,9b}$ 6.1, H-8), 4.57 (1 H, dd, $J_{5,6}$ 10.8, $J_{5,NH}$ 10.3, H-5), 5.22 (1 H, d, $J_{NH,5}$ 10.3, NH) and 5.24 (1 H, dd, $J_{7,8}$ 6.7, $J_{7,6}$ 2.3, H-7); $\delta_C(CDCl_3)$ 20.9 [OC(O)*Me*], 22.9 [NC(O)*Me*], 25.4, 26.6 (C*Me*$_2$), 38.7 (C-3), 43.9 (C-5), 47.2 (C-4′), 51.6 (OMe), 52.6 (CO_2*Me*), 55.2 (C-4), 66.5 (C-9), 69.7 (C-7), 70.1 (C-6), 74.0 (C-8), 98.4 (C-2), 108.9 (*C*Me$_2$), 167.8 (C-1) and 170.3 and 170.5 [O*C*(O)Me/N*C*(O)Me]; FABMS: *m/z* 432 [(MH$^+$), 100%], 400 (100), 342 (100), 223 (70), 198 (65) and 138 (80) (Found: [M + H]$^+$, 432.1890. $C_{19}H_{30}NO_{10}$ requires *m/z*, 432.1870).

Methyl (methyl 5-acetamido-4,4′-anhydro-3,5-dideoxy-4-*C*-hydroxymethyl-8,9-*O*-isopropylidene-β-D-*glycero*-D-*talo*-2-nonulopyranosid)onate 7

A solution of the alkene **4** (450 mg, 1.21 mmol) in CH_2Cl_2 (10 cm^3) was treated with MCPBA (73%; 430 mg, 1.82 mmol) and stirred at 0 °C for 5 h. The reaction was then diluted with EtOAc (40 cm^3) and the organic layer was washed with saturated aq. $NaHCO_3$ (2 × 10 cm^3). Subsequent drying of the organic layer over Na_2SO_4 and concentration under reduced pressure yielded a foam. Purification of the crude product on silica gel (EtOAc) afforded the *epoxide* **7** as a solid (326 mg, 70%); $\delta_H(CDCl_3)$ 1.31 and 1.38 (2 × 3 H, 2 × s, CMe_2), 1.92 (1 H, d, $J_{3a,3e}$ 14.8, H^a-3), 2.02 (3 H, s, AcN), 2.48 (1 H, d, $J_{3e,3a}$ 14.8, H^e-3), 2.57 (1 H, d, $J_{4'a,4'b}$ 3.9, H^a-4′), 2.79 (1 H, d, $J_{4'b,4'a}$ 3.9, H^b-4′), 3.37 (3 H, s, OMe), 3.46 (1 H, dd, $J_{7,8}$ 8.3, $J_{7,6}$ 1.5, H-7), 3.79 (3 H, s, CO_2Me), 3.87 (1 H, dd, $J_{6,5}$ 10.7, $J_{6,7}$ 1.5, H-6), 4.04 (1 H, dd, $J_{9a,9b}$ 8.7, $J_{9a,8}$ 5.4, H^a-9), 4.17 (1 H, dd, $J_{9b,9a}$ 8.7, $J_{9b,8}$ 6.2, H^b-9), 4.37 (1 H, ddd, $J_{8,7}$ 8.3, $J_{8,9b}$ 6.2, $J_{8,9a}$ 5.4, H-8), 4.49 (1 H, dd, $J_{5,6}$ 10.7, $J_{5,NH}$ 9.2, H-5) and 5.61 (1 H, d, $J_{NH,5}$ 9.2, NH); $\delta_C(CDCl_3)$ 22.8 [NC(O)*Me*], 25.3, 26.9 (C*Me*$_2$), 38.7 (C-3), 44.4 (C-5), 47.1 (C-4′), 51.4 (OMe), 52.4 (CO_2*Me*), 55.0 (C-4), 67.7 (C-9), 70.3, 70.5 and 74.1 (C-6, -7, -8), 98.1 (C-2), 108.9 (*C*Me$_2$), 168.1 (C-1) and 172.0 [N*C*(O)Me]; FABMS: *m/z* 390 [(MH$^+$), 100%], 358 (100) and 300 (76) (Found: [M + H]$^+$, 390.1780. $C_{17}H_{28}NO_9$ requires *m/z*, 390.1764).

Methyl (methyl 5-acetamido-7-*O*-acetyl-4-*C*-azidomethyl-3,5-dideoxy-8,9-*O*-isopropylidene-β-D-*glycero*-D-*talo*-2-nonulopyranosid)onate 8

To a mixture of the epoxide **6** (60 mg, 0.14 mmol) and LiN_3 (10 mg, 0.20 mmol) was added anhydrous acetone (6.0 cm^3) and the suspension was stirred under reflux for 2.5 h. Acetone was removed by concentration under reduced pressure and the residue was dissolved in EtOAc (30 cm^3). The organic layer was washed with a 1:1 mixture of saturated aq. NaCl and water (10 cm^3) twice and dried over Na_2SO_4. Subsequent evaporation of the mixture under reduced pressure yielded a crude product, which was purified on silica gel (EtOAc–hexane, 4:1) to afford the *azide* **8** as a foam (40 mg, 60%), $[\alpha]_D^{25}$ −24.6 (*c* 0.99, $CHCl_3$) (Found: C, 47.7; H, 6.5; N, 11.65. $C_{19}H_{30}N_4O_{10}$ requires C, 48.1; H, 6.4; N, 11.8%); ν_{max}/cm^{-1} (NaCl) 2104, 1746, 1700, 1664, 1546, 1528, 1370, 1260, 1218, 1170, 1144, 1064 and 1032; $\delta_H(CDCl_3)$ 1.32 and 1.39 (2 × 3 H, 2 × s, CMe_2), 1.89 (1 H, d, $J_{3a,3e}$ 14.7, H^a-3), 1.99 (3 H, s, AcN), 2.12 (3 H, s, AcO), 2.45 (1 H, d, $J_{3e,3a}$ 14.7, H^e-3), 2.96 (1 H, d, $J_{4'a,4'b}$ 12.8, H^a-4′), 3.36 (1 H, d, $J_{4'b,4'a}$ 12.8, H^b-4′), 3.39 (3 H, s, OMe), 3.84 (3 H, s, CO_2Me), 3.91 (1 H, dd, $J_{9a,9b}$ 8.6, $J_{9a,8}$ 7.0, H^a-9), 4.00–4.06 (3 H, m, H-6, H^b-9 and OH), 4.12 (1 H, dd, $J_{5,6}$ 10.6, $J_{5,NH}$ 9.9, H-5), 4.37 (1 H, ddd, $J_{8,7}$ 7.4, $J_{8,9a}$ 7.0, $J_{8,9b}$ 6.2, H-8), 5.20 (1 H, dd, $J_{7,8}$ 7.4, $J_{7,6}$ 2.2, H-7) and 5.57 (1 H, d, $J_{NH,5}$ 9.9, NH); $\delta_C(CDCl_3)$ 21.0 [OC(O)*Me*], 23.1 [NC(O)*Me*], 25.5 and 26.6 (C*Me*$_2$), 38.6 (C-3), 47.9 (C-5), 51.7 (OMe), 52.8 (CO_2*Me*), 57.0 (C-4′), 66.7 (C-9), 68.5, 69.7 and 73.8 (C-6, -7, -8), 73.2 (C-4), 99.1 (C-2), 108.9 (*C*Me$_2$), 167 (C-1) and 170.1 and 170.5 [O*C*(O)Me/N*C*(O)Me]; FABMS: *m/z* 475 [(MH$^+$), 99%], 443 (63), 425 (100), 385 (43) and 367 (24) (Found: [M + H]$^+$, 475.2044. $C_{19}H_{31}N_4O_{10}$ requires *m/z*, 475.2040).

Methyl (methyl 5-acetamido-3,5-dideoxy-4-*C*-methoxymethyl-8,9-*O*-isopropylidene-β-D-*glycero*-D-*talo*-2-nonulopyranosid)-onate 9

A solution of the epoxide **6** (50 mg, 0.12 mmol) in anhydrous MeOH (4.0 cm^3) was treated with methanolic NaOMe (2.0 M; 0.10 cm^3, 0.20 mmol) and heated under reflux for 2 h. Excess of NaOMe was neutralised by bubbling CO_2 gas through the solution for *ca.* 30 min. The solution was then concentrated under reduced pressure and the residue was partitioned between EtOAc (30 cm^3) and water (10 cm^3). The organic layer was separated, and washed with water (10 cm^3) followed by saturated aq. NaCl (10 cm^3). Subsequent drying of the organic layer over Na_2SO_4 and evaporation of the mixture under reduced pressure afforded the *methyl ether* **9** as an amorphous solid (42 mg, 86%); recrystallisation (EtOAc–hexane) afforded needles; mp 170–172 °C; $[\alpha]_D^{27}$ −45.1 (*c* 1.11, $CHCl_3$) (Found: C, 50.9; H, 7.8; N, 3.2. $C_{18}H_{31}NO_{10}$ requires C, 51.3; H, 7.4; N, 3.3%); ν_{max}/cm^{-1} 3480, 3352, 1736, 1638, 1522, 1452, 1380, 1370, 1288, 1252, 1214, 1168, 1144, 1066 and 1048; $\delta_H(CDCl_3)$ 1.31 and 1.39 (2 × 3 H, 2 × s, CMe_2), 2.07 (3 H, s, AcN), 2.15 (1 H, d, $J_{3a,3e}$ 14.7, H^a-3), 2.20 (1 H, d, $J_{3e,3a}$ 14.7, H^e-3), 3.19 (1 H, d, $J_{4'a,4'b}$ 9.5, H^a-4'), 3.26 (1 H, d, $J_{4'b,4'a}$ 9.5, H^b-4'), 3.32, 3.38 (2 × 3 H, 2 × s, 2 × OMe), 3.46 (1 H, ddd, $J_{7,8}$ 8.5, $J_{7,OH}$ 4.8, $J_{7,6}$ 1.4, H-7), 3.74 (1 H, dd, $J_{6,5}$ 10.8, $J_{6,7}$ 1.4, H-6), 3.80 (3 H, s, CO_2Me), 4.02 (1 H, dd, $J_{9a,9b}$ 8.6, $J_{9a,8}$ 5.5, H^a-9), 4.15 (1 H, dd, $J_{5,6}$ 10.8, $J_{5,NH}$ 8.9, H-5), 4.17 (1 H, dd, $J_{9b,9a}$ 8.6, $J_{9b,8}$ 6.2, H^b-9), 4.38 (1 H, ddd, $J_{8,7}$ 8.5, $J_{8,9b}$ 6.2, $J_{8,9a}$ 5.5, H-8), 4.49 (1 H, d, $J_{OH,7}$ 4.8, 7-OH) and 6.04 (1 H, d, $J_{NH,5}$ 8.9, NH); $\delta_C(CDCl_3)$ 22.9 [NC(O)*Me*], 25.4 and 27.0 (CMe_2), 38.6 (C-3), 47.8 (C-5), 51.5 (OMe), 52.6 (CO_2Me), 59.5 (CH_2OMe), 68.0 (C-9), 68.8, 70.5 and 74.0 (C-6, -7, -8), 71.6 (C-4), 75.8 (C-4'), 99.3 (C-2), 108.9 (CMe_2), 167.5 (C-1) and 172.1 [*C*(O)Me]; FABMS: *m/z* 422 [(MH^+), 100%], 390 (100), 372 (89), 332 (48) and 314 (49) (Found: $[M + H]^+$, 422.2042. $C_{18}H_{32}NO_{10}$ requires *m/z*, 422.2026).

Methyl (methyl 5-acetamido-4-*C*-cyanomethyl-3,5-dideoxy-8,9-*O*-isopropylidene-β-D-*glycero*-D-*talo*-2-nonulopyranosid)onate 10

A solution of the epoxide **7** (100 mg, 0.257 mmol) in anhydrous MeOH (5.0 cm^3) was treated with KCN (84 mg, 1.3 mmol) and stirred at room temperature for 18 h. MeOH was removed under reduced pressure and the residue was taken up in EtOAc–MeOH (1:1) and applied to a short column of silica gel. Elution with EtOAc provided the *nitrile* **10** as needles (45 mg, 42%), mp 165–171 °C; $[\alpha]_D^{25}$ −46.7 (*c* 1.65, $CHCl_3$) (Found: C, 50.9; H, 6.8. $C_{18}H_{28}N_2O_9 \cdot 0.5H_2O$ requires C, 50.8; H, 6.9%); ν_{max}/cm^{-1} 3444br, 3348br, 2244, 1742, 1634, 1528, 1438, 1372, 1264, 1208, 1164, 1138, 1104, 1064 and 1026; $\delta_H(CDCl_3)$ 1.31 and 1.38 (2 × 3 H, 2 × s, CMe_2), 2.03 (1 H, d, $J_{3a,3e}$ 14.4, H^a-3), 2.11 (3 H, s, AcN), 2.44 (1 H, d, $J_{4'a,4'b}$ 16.8, H^a-4'), 2.51 (1 H, d, $J_{3e,3a}$ 14.4, H^e-3), 2.70 (1 H, d, $J_{4'b,4'a}$ 16.8, H^b-4'), 3.41 (3 H, s, OMe), 3.41–3.45 (1 H, m, H-7), 3.74 (1 H, dd, $J_{6,5}$ 10.6, $J_{6,7}$ 1.4, H-6), 3.82 (3 H, s, CO_2Me), 4.01 (1 H, dd, $J_{9a,9b}$ 8.6, $J_{9a,8}$ 5.2, H^a-9), 4.05 (1 H, dd, $J_{5,6}$ 10.6, $J_{5,NH}$ 9.3, H-5), 4.16 (2 H, dd, $J_{9b,9a}$ 8.6, $J_{9b,8}$ 6.2, H^b-9, OH), 4.34 (1 H, ddd, $J_{8,7}$ 8.7, $J_{8,9b}$ 6.2, $J_{8,9a}$ 5.2, H-8), 4.40 (1 H, s, OH) and 6.08 (1 H, d, $J_{NH,5}$ 9.3, NH); $\delta_C(CDCl_3)$ 23.0 [NC(O)*Me*], 25.3 and 27.1 (CMe_2), 29.0 (C-4'), 40.3 (C-3), 50.6 (C-5), 51.7 (OMe), 52.9 (CO_2Me), 67.9 (C-9), 69.2, 70.4 and 73.9 (C-6, -7, -8), 70.0 (C-4), 98.7 (C-2), 109.1 (CMe_2), 116.0 (CN), 166.8 (C-1) and 172.4 [*C*(O)Me]; FABMS: *m/z* 417 [(MH^+), 54%], 385 (96), 367 (17), 341 (17), 327 (80), 309 (37) and 101 (100) (Found: $[M + H]^+$, 417.1875. $C_{18}H_{29}N_2O_9$ requires *m/z*, 417.1873).

Methyl (methyl 5-acetamido-4-*C*-chloromethyl-3,5-dideoxy-8,9-*O*-isopropylidene-β-D-*glycero*-D-*talo*-2-nonulopyranosid)-onate 11

A solution of the epoxide **7** (0.10 g, 0.26 mmol) and NH_4Cl (28 mg, 0.52 mmol) in DMSO (5.0 cm^3) was stirred for 3 h at room temperature, prior to the addition of $Ti(OPr^i)_4$ (0.12 cm^3, 0.39 mmol), and stirring was continued for a further 15 h. DMSO was evaporated off under high vacuum and the residue was taken up in a mixture of CH_2Cl_2 (50 cm^3) and water (10 cm^3). The aqueous layer was separated and extracted twice with CH_2Cl_2 (50 cm^3). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to afford a syrup. Purification of the crude product by column chromatography on silica gel (EtOAc) gave unchanged epoxide **7** (38 mg) and compound **11** (54 mg, 50%; 79% based on consumed **7**) as a syrup; crystallisation (EtOAc) afforded *compound* **11** as needles; mp 155–159 °C; $[\alpha]_D^{25}$ −40.6 (*c* 1.26, $CHCl_3$) (Found: C, 47.9; H, 6.8; N, 2.9. $C_{17}H_{28}ClNO_9$ requires C, 47.95; H, 6.6; N, 3.3%); ν_{max}/cm^{-1} 3472, 3340, 1736, 1642, 1526, 1438, 1370, 1290, 1260, 1218, 1170, 1148, 1114, 1060 and 1034; $\delta_H(CDCl_3)$ 1.31 and 1.39 (2 × 3 H, 2 × s, CMe_2), 2.08 (3 H, s, AcN), 2.15 (1 H, d, $J_{3a,3e}$ 14.6, H^a-3), 2.33 (1 H, d, $J_{3e,3a}$ 14.6, H^e-3), 3.40 (3 H, s, OMe), 3.43–3.48 (3 H, m, H-7 and H_2-4'), 3.75 (1 H, dd, $J_{6,5}$ 10.6, $J_{6,7}$ 1.4, H-6), 3.82 (3 H, s, CO_2Me), 4.01 (1 H, dd, $J_{9a,9b}$ 8.6, $J_{9a,8}$ 5.5, H^a-9), 4.17 (1 H, dd, $J_{9b,9a}$ 8.6, $J_{9b,8}$ 6.2, H^b-9), 4.23 (1 H, dd, $J_{5,6}$ 10.6, $J_{5,NH}$ 9.1, H-5), 4.29 (1 H, s, OH), 4.34–4.41 (2 H, m, H-8, OH) and 5.99 (1 H, d, $J_{NH,5}$ 9.1, NH); $\delta_C(CDCl_3)$ 23.0 [NC(O)*Me*], 25.3 and 27.1 (CMe_2), 38.5 (C-3), 48.3 (C-5), 48.6 (C-4'), 51.6 (OMe), 52.7 (CO_2Me), 68.0 (C-9), 69.1, 70.4 and 74.0 (C-6, -7, -8), 72.1 (C-4), 99.0 (C-2), 109.0 (CMe_2), 167.2 (C-1) and 172.1 [*C*(O)Me]; FABMS: *m/z* 428 [$(MH + 2)^+$, 24%], 426 [(MH^+), 66], 396 (39), 394 (100), 376 (46), 336 (52) and 318 (44) (Found: $[M + H]^+$, 426.1518. $C_{17}H_{29}ClNO_9$ requires *m/z*, 426.1531).

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References

1 A. P. Corfield and R. Schauer, *Sialic Acids, Chemistry, Metabolism and Function*, in *Cell Biology Monographs*, ed. R. Schauer, Springer Verlag, Wien, 1982, vol. 10, p. 195; R. Schauer, *Adv. Carbohydr. Chem. Biochem.*, 1982, **40**, 131.

2 M. J. Horowitz and W. Pigman, *The Glycoconjugates*, Academic Press, New York, 1978, vols. I and II: H. Tuppy and A. Gottschalk, *Glycoproteins: Their Composition, Structure and Function*, ed. A. Gottschalk, Elsevier, London, 1972, p. 403.

3 W. Reutter, E. Köttgen, C. Bauer and W. Gerok, *Sialic Acids, Chemistry, Metabolism and Function*, in *Cell Biology Monographs*, ed. R. Schauer, Springer Verlag, Wien, 1982, vol. 10, p. 263; R. W. Jeanloz and J. F. Codington, *Biological Roles of Sialic Acids*, ed. A. Rosenberg and C. L. Schengrund, Plenum Press, New York, 1976, p. 201.

4 R. Schauer, *Trends Biochem. Sci.*, 1985, **10**, 357.

5 J. E. Galen, J. M. Ketley, A. Fasano, S. H. Richardson, S. S. Wasserman and J. B. Kaper, *Infect. Immun.*, 1992, **60**, 406.

6 M. Hartmann, R. Christian and E. Zbiral, *Liebigs Ann. Chem.*, 1990, 83.

7 M. Hartmann and E. Zbiral, *Monatsh. Chem.*, 1989, **120**, 899.

8 M. von Itzstein, B. Jin, W.-Y. Wu and M. Chandler, *Carbohydr. Res.*, 1993, **244**, 181; M. Chandler, M. J. Bamford, R. Conroy, B. Lamont, B. Patel, V. K. Patel, I. P. Steeples, R. Storer, N. G. Weir, M. Wright and C. Williamson, *J. Chem. Soc., Perkin Trans. 1*, 1995, 1173.

9 M. von Itzstein, B. Jin and W.-Y. Wu, *Carbohydr. Res.*, 1994, **259**, 300.

10 D. R. Groves, J. C. Wilson and M. von Itzstein, *Aust. J. Chem.*, 1995, **48**, 1217.

11 S. Ciccotosto and M. von Itzstein, *Tetrahedron Lett.*, 1995, **36**, 5405.

12 M. Hartmann and E. Zbiral, *Liebigs Ann. Chem.*, 1991, 795.

13 M. Hartmann, R. Christian and E. Zbiral, *Monatsh. Chem.*, 1991, **122**, 111.

14 H. H. Brandstetter and E. Zbiral, *Liebigs Ann. Chem.*, 1983, 2055.
15 F. Andersson and B. Samuelsson, *Carbohydr. Res.*, 1984, **129**, C1.
16 M. Caron and K. B. Sharpless, *J. Org. Chem.*, 1985, **50**, 1557.
17 R. M. Silverstein, G. C. Bassler and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, Wiley, New York, 4th edn., 1981, pp. 261, 268.

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