Synthesis and biological evaluation of sulfur isosteres of the potent influenza virus sialidase inhibitors 4-amino-4-deoxy- and 4-deoxy-4-guanidino-Neu5Ac2en

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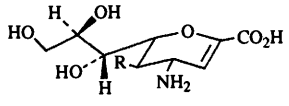
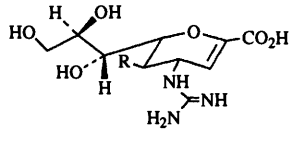
The synthesis of the thioisosteres of 4-amino-4-deoxy-Neu5Ac2en (16) and 4-deoxy-4-guanidino-Neu5Ac2en (17) has been achieved. These compounds have been found to be as bioactive as the corresponding oxygen analogues. The preparation of the key intermediate, 7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-2′-methyl-6-thio-(methyl D-*glycero*-D-*talo*-non-2-enonato)[5,4-*d*]oxazole 13, has been achieved from 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-3-thio-α,β-D-mannofuranose 8 in four steps in 43% overall yield.

Introduction

We have recently described the design, synthesis and biological evaluation of two potent *N*-acetylneuraminic acid-based inhibitors of influenza virus sialidase.[1–4] These compounds, 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (4-amino-4-deoxy-Neu5Ac2en, **1**) and 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*galacto*-non-2-enonic acid (4-deoxy-4-guanidino-Neu5Ac2en, **2**), both derivatives of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (Neu5Ac-2en, **3**), have significant *in vivo* activity and the latter compound is currently undergoing evaluation in human clinical trial studies as a drug candidate.[1,5,6] The success of these *N*-acetylneuraminic acid analogues as potential therapeutics has directed our attention to the synthesis of the corresponding thioisosteres, as these compounds may hold some biological advantage over their oxygen counterparts.



1 R = NHAc



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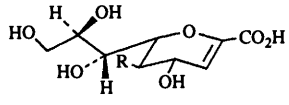
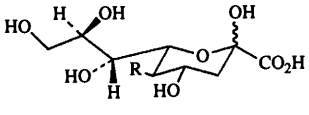


3 R = NHAc

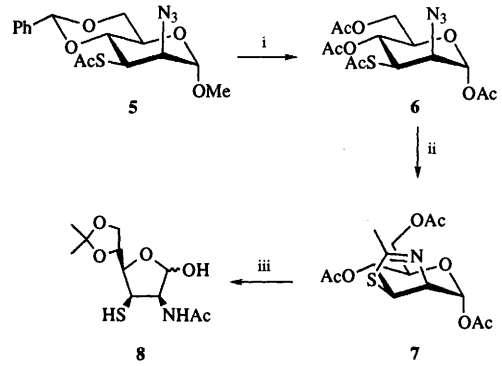


4 R = NHAc

In this paper, we present the synthesis and biological evaluation of the thioisosteres of compounds **1** and **2**. Compounds **1** and **2** have been synthesized[3] directly from *N*-acetylneuraminic acid (Neu5Ac, **4**), which is readily prepared *via* an enzyme-catalysed aldol condensation between 2-acetamido-2-deoxy-D-mannose and pyruvate. The thioisostere of Neu5Ac **4**, 5-acetamido-2,6-anhydro-3,5-dideoxy-6-thio-D-*glycero*-α,β-D-*galacto*-non-2-ulosonic acid (6-thio-Neu5Ac, **10**), is a known[7] compound and was prepared by a metal-catalysed aldol condensation between the appropriate precursor carbohydrate and oxalacetic acid. We thought that our strategy towards the synthesis of the thioisosteres of **1** and **2** should take advantage of the known compound **5** for the preparation of the key intermediate, 7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-2′-methyl-6-thio-(methyl D-*glycero*-D-*talo*-non-2-enonato)[5,4-*d*]-oxazole (4,5-oxazolino-6-thio-Neu5Ac2en, **13**), thereby paralleling our synthesis[3] of compounds **1** and **2**. We have found that these activated allylic oxazolines are very much susceptible to attack at the C-4 position on the sialic acid template by various nucleophiles, including sulfur- and nitrogen-based nucleophiles.[3]

Results and discussion

The synthesis of 6-thio-Neu5Ac **10** has been previously reported.[7] In this brief report, condensation of oxalacetic acid with thiofuranose **8** in the presence of Ni^{2+} (reaction pH 7.5–8) resulted in the formation of compound **10** in a relatively low isolated yield (24% based on **8**). We have prepared the known precursor thiofuranose **8** in six steps from methyl 3-*S*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-3-thio-α-D-mannopyranoside **5**, using a modification of the procedure reported by Brossmer and Mack[8] (Scheme 1).

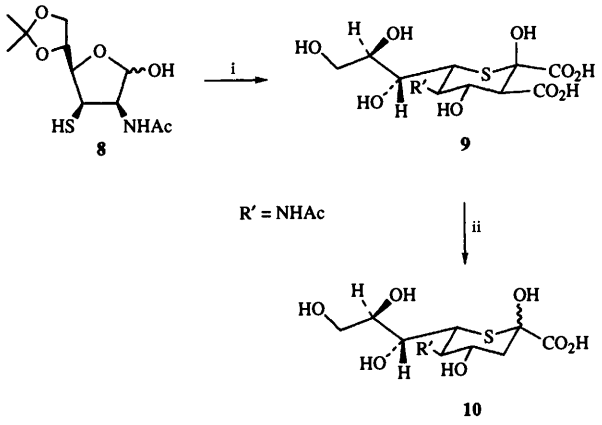


Scheme 1 *Reagents and conditions*: i, HOAc–water (1:1 v/v), 100 °C, 2 h; HOAc–Ac_2O–conc. H_2SO_4 (120:120:1 v/v), room temp., 20 h; ii, triphenylphosphine, CH_2Cl_2, −78 °C (2 h) to room temp. (18 h); iii, ref. 8

Although Brossmer and Mack[8] successfully prepared the thiazoline **7** by treatment of compound **5** with triphenylphosphine followed by acetolysis, we have found that the reaction of compound **5** with triphenylphosphine is somewhat capricious and only proceeded in low yield. We have discovered that by

reversing the order of the reactions, *i.e.* acetolysis of acetal **5** to give triacetate **6**, followed by reaction of compound **6** with triphenylphosphine, this problem was overcome and provided compound **7** in 31% yield (based on **5**).

In the light of a more recent report[9] on the synthesis of a deculopyranosonic acid analogue of *N*-acetylneuraminic acid, we decided to implement this procedure (Scheme 2) for the synthesis of compound **10** in multigram quantities and in good yield (52% based on **8**). Thus treatment of compound **8** with an ice-cooled slurry of oxalacetic acid, sodium hydroxide in water, and nickel acetate tetrahydrate (reaction pH 7–7.5) gave the diacid **9** in good yield (68%), which following decarboxylation at pH 6.0, led to the formation of compound **10** (77%).



Scheme 2 *Reagents and conditions*: i, oxalacetic acid, $Ni(OAc)_2 \cdot (H_2O)_4$, NaOH; ii, 0.1 M aq. Na_2HPO_4 (pH 6), 90 °C

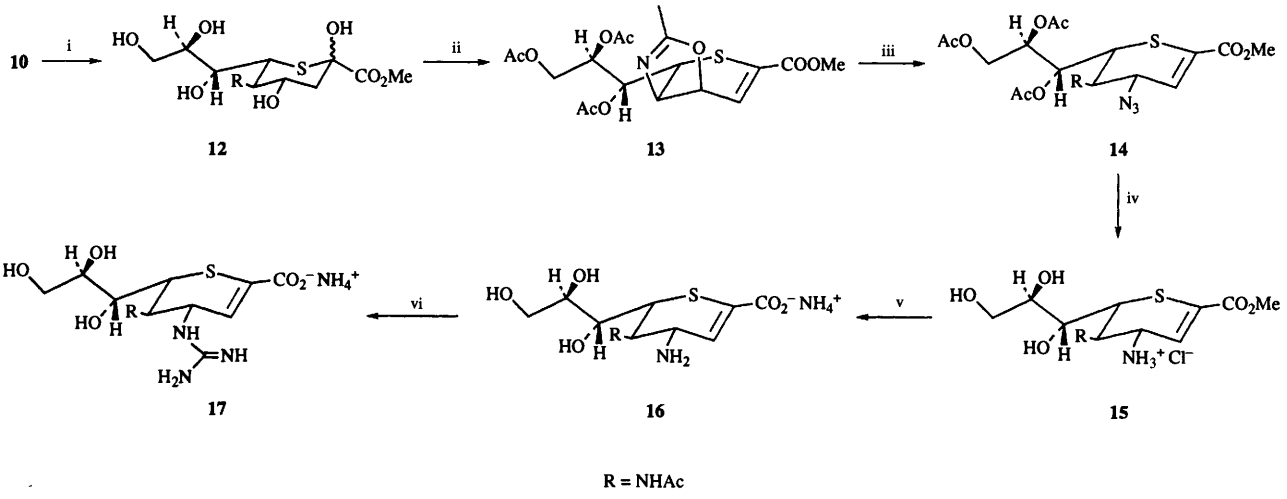
We have recently reported[3] on the stereospecific introduction of azide at C-4 of Neu5Ac2en *via* the oxazoline **11**. Hence the corresponding (hitherto unknown) oxazoline, 4,5-oxazolino-6-thio-Neu5Ac2en **13**, is pivotal in the further elaboration of this unsaturated 6-thio-*N*-acetylneuraminic acid series (Scheme 3).

The synthesis of this key intermediate **13** was achieved in a one-pot reaction from the methyl ester of 6-thio-Neu5Ac (compound **12**), prepared in 80% yield from compound **10** (see Experimental section), using conditions previously described.[10] Thus methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-6-thio-D-*glycero*-α,β-D-*galacto*-nonulosonate (6-thio-Neu5AcIMe, **12**) in glacial acetic acid was treated with conc. sulfuric acid and acetic anhydride at room temperature for 40 h. Following work-up with saturated aq. sodium hydrogen carbonate, compound **13** was isolated in 90% yield. The expected characteristic ^1H resonances for the olefinic 3-H and the oxazoline methyl protons were found at δ7.30 and 2.15, respectively. As anticipated, a doublet for 3-H was observed with a coupling of $J_{3,4} = 3.5$ Hz. We[11] and others[12] have found that this coupling is characteristic of a vinylic proton adjacent to an equatorially positioned proton in an unsaturated *N*-acetylneuraminic acid system.

Introduction of azide at C-4 of the oxazoline **13** was readily accomplished by the reaction of compound **13** with azidotrimethylsilane in 2-methylpropan-2-ol at approximately 80 °C for 48 h under similar conditions to those previously reported.[13] The desired methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-6-thio-D-*glycero*-D-*galacto*-non-2-enonate (4-azido-4-deoxy-6-thio-Neu5,7,8,9Ac$_4$2enIMe, **14**) was isolated in 66% yield. The reduction of azide **14** to give the corresponding de-*O*-acetylated 4-amino-4-deoxy derivative, methyl 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-6-thio-D-*glycero*-D-*galacto*-non-2-enonate (4-amino-4-deoxy-6-thio-Neu5Ac2enIMe) was only satisfactorily achieved utilising zinc dust and HCl at 75 °C. Under the reaction conditions the amine hydrochloride salt **15** was formed. This salt was readily converted into the deesterified free amino species **16** and was then isolated by resin chromatography in 66% yield. Interestingly, difficulties were encountered when attempts were made to reduce compound **14** using triphenylphosphine, hydrogen sulfide, or propane-1,3-dithiol, including the procedure previously employed for the corresponding oxygen isostere, *i.e.*, hydrogenation over palladium on carbon.[3]

The synthesis of the final target compound, 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-6-thio-D-*glycero*-D-*galacto*-non-2-enonic acid (4-deoxy-4-guanidino-6-thio-Neu5Ac2en, **17**), was achieved by treatment of an aqueous solution of amine **16** with the well known guanidinating reagent pyrazole-2-carboxamidine hydrochloride[13] in the presence of imidazole at 60 °C for 24 h. Following purification by chromatography, compound **17** was isolated in 55% yield.

Compounds **16** and **17** were evaluated for biological activity against influenza virus sialidase using a modified fluorometric assay[14] and found to have IC_{50} values of 1×10^{-6} M and 5×10^{-9} M, respectively, at a substrate concentration of 80 mM. The synthetic substrate used in the enzyme assay was 4-methylumbelliferyl-*N*-acetyl-α-D-neuraminic acid.[14] The



Scheme 3 *Reagents and conditions*: i, MeOH, H^+, room temp., 48 h; ii, HOAc–Ac_2O–conc. H_2SO_4 (10:10:1 v/v), room temp., 40 h; iii, $TMSN_3$, 2-methylpropan-2-ol, 80 °C, 48 h; iv, Zn, 2 M HCl, 75 °C, 45 min; v, NaOH (pH 13), room temp., 16 h; 1.5 M NH_4OH; vi, pyrazole-2-carboxamidine hydrochloride, imidazole, water, 60 °C, 24 h

nature of the reaction kinetics of compound **17** is under further investigation, as preliminary data suggest that it has unusual properties. Interestingly enough the corresponding oxygen isostere was found to be a slow binding inhibitor.[15,16]

In conclusion, the synthesis of the thioisosteres of 4-amino-4-deoxy-Neu5Ac2en and 4-deoxy-4-guanidino-Neu5Ac2en has been accomplished. The isosteres were found to be as bioactive as their oxygen counterparts as inhibitors against influenza virus sialidase. These two compounds may well provide further prospects for the development of potential anti-influenza drug candidates.

Experimental

Mps were determined on a Gallenkamp melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra (in ppm) were recorded using a Bruker AMX-300 spectrometer unless otherwise stated and the spectra were referenced using solvent residues. *J*-Values are given in Hz. Low-resolution (LR) and high-resolution (HR) fast-atom bombardment (FAB) mass spectra were obtained using a JEOL JMS-DX 300 mass spectrometer. IR spectra were recorded on a Hitachi 270-30 spectrophotometer. Optical rotations were measured at 25 °C using a JASCO DIP-370 polarimeter. $[\alpha]_D$-Values are given in 10^{-1} deg $cm^2\ g^{-1}$. Microanalyses were performed either by the Australian Microanalytical Service, Notting Hill, Victoria, or the Chemical and Microanalytical Service, Essendon, Victoria. Reactions were monitored by TLC on Kieselgel 60 F_{254} plate (Merck 5554) and detection of the spots was carried out by spraying with a 95% aq. ethanolic solution containing 5% H_2SO_4.

Methyl 3-*S*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-3-thio-α-D-mannopyranoside 5

This compound was prepared following literature methods[9] without modification. ^1H NMR spectral data are in accord with the literature[9]; $\delta_C(CDCl_3)$ 193.8, 137.0, 128.9, 128.1, 126.0, 102.0, 98.9, 75.0, 68.7, 65.5, 64.2, 55.0, 44.8 and 30.6.

1,4,6-Tri-*O*-acetyl-3-*S*-acetyl-2-azido-2-deoxy-α-D-mannopyranose 6

A mixture of compound **5** (17.70 g, 48.31 mmol) and 50% aq. acetic acid (200 cm^3) was heated at 100 °C for 2 h. The resulting solution was cooled and concentrated *in vacuo*. The residue was purified by passage through a short plug of silica gel: the by-products were removed by washing the plug with diethyl ether–hexanes (2 : 1); the desired diol was eluted with ethyl acetate as a pale yellow solid after removal of solvent (11.48 g, 91%).

A solution of the diol (11.25 g, 43.10 mmol) in glacial acetic acid (120 cm^3) and acetic anhydride (120 cm^3) was chilled to 0 °C prior to the addition of conc. sulfuric acid (1 cm^3) over a period of 30 min. The ice-bath was removed after 2 h and stirring was continued for 18 h. Sodium acetate (12 g) was added and, after 15 min, the reaction mixture was diluted with toluene (200 cm^3) and then evaporated to dryness. The residue was partitioned between diethyl ether (200 cm^3) and water (100 cm^3), the organic phase was washed with water (5 × 200 cm^3), dried, and concentrated to give **6** as a pale yellow gum which solidified under high vacuum (13.51 g, 81%). A small portion of crude compound **6** was recrystallized from ethyl acetate–hexanes, mp 87.5–88 °C; $[\alpha]_D$ +5 (*c* 1.07, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 2112, 1750, 1715, 1370 and 1225; $\delta_H(CDCl_3)$ 6.18 (1 H, s, 1-H), 5.19 (1 H, pseudo t, *J* 10.5 and 10.5, 4-H), 4.17–4.26 and 4.00–4.11 (4 H, m, 3-, 5-H and 6-H_2), 3.85 (1 H, d, *J* 1.3, 2-H), 2.38 (3 H, s, $SCOCH_3$) and 2.03, 2.09 and 2.21 (3 × 3 H, s, $OCOCH_3$); $\delta_C(CDCl_3)$ 193.8, 170.6, 169.3, 168.4, 90.0, 71.9, 64.1, 62.9, 62.0, 45.1, 30.6, 20.9, 20.6 and 20.5.

1,4,6-Tri-*O*-acetyl-2,3-dideoxy-2′-methyl-α-D-mannopyranoso[2,3-*d*]thiazole 7

A stirred solution of the azide **6** (13.50 g, 34.70 mmol) in dry CH_2Cl_2 (200 cm^3) was cooled to −78 °C before the slow addition of a solution of triphenylphosphine (9.09 g, 34.70 mmol) in dry CH_2Cl_2 (200 cm^3) over a 2 h period. The reaction mixture was allowed to warm up to room temperature and stirring was continued for 18 h. The resulting solution was concentrated before chromatographic separation of the thiazoline **7** from triphenylphosphine oxide [silica gel; ethyl acetate–hexanes (1 : 3 v/v)]. Needles of *product* **7** were obtained by recrystallization from ethyl acetate–hexanes (5.3 g, 44%) (Found: C, 48.8; H 5.6; N, 3.9. $C_{14}H_{19}NO_7S$ requires C, 48.77; H, 5.54; N, 4.06%); $[\alpha]_D$ −57 (*c* 1.22, $CHCl_3$); mp 139–140 °C (lit.,[9] 135 °C); δ_H data are consistent with the literature;[9] $\delta_C(CDCl_3)$ 170.6, 169.4, 168.4, 168.3, 91.1, 77.9, 69.9, 68.7, 62.4, 52.0, 20.8, 20.7 and 20.6.

2-Acetamido-2-deoxy-5,6-*O*-isopropylidene-3-thio-α,β-D-mannofurannose 8

A mixture of the triacetate **7** (10.00 g, 28.99 mmol) and Amberlite IRA-400 (OH^-) (10 g) in MeOH (250 cm^3) was stirred at room temperature for 48 h. The filtrate obtained after removal of resin was concentrated to give the corresponding triol, 2,3-deoxy-2′-methyl-α,β-D-mannopyranoso[2,3:*d*]thiazole, as a solid (5.4 g, 86%); δ_C[D_2O–CD_3OD (4 : 1 v/v)] 174.1, 98.6, 78.7, 71.4, 70.4, 61.8, 55.5 and 20.7. Alternatively, the triacetate **7** can be deacetylated with sodium methoxide, in similar yield, using the procedure of Brossmer and Mack.[8]

The triol, 2,3-dideoxy-2′-methyl-α,β-D-mannopyranoso[2,3-*d*]thiazole, was then converted into the title compound **8** following the method reported by Brossmer and Mack.[8] The ^1H NMR spectral data for thiol **8** are consistent with the literature.[9]

5-Acetamido-2,6-anhydro-3,5-dideoxy-6-thio-D-*glycero*-α,β-D-*galacto*-non-2-ulosonic acid 10 (refs. 7 and 8)

To an ice-cooled slurry of oxalacetic acid (3.75 g, 28.39 mmol) in water (13 cm^3) was added aq. sodium hydroxide (2.27 g, 56.75 mmol in 12 cm^3) resulting in a solution of pH 7–7.5. The oxalacetate solution and nickel diacetate tetrahydrate (0.94 g, 3.78 mmol) were added to compound **8** (3.42 g, 9.46 mmol) and the mixture was stirred at room temperature for 16 h. The solution was then stirred for 6 h at pH 3 (Amberlite IR-120, H^+-form). The resin was filtered off and the filtrate was lyophilized to a solid, which was purified by column chromatography [Amberlite IRA-400 ($HCOO^-$ form), elution with formic acid (2 M)] to afford the diacid **9** (2.38 g, 68%); $\delta_H(D_2O)$ 4.18 (1 H, pseudo t, *J* 10.5 and 10.5, 5-H), 4.10 (1 H, pseudo t, *J* 10.0 and 10.0, 4-H), 3.91 (1 H, m, 7-H), 3.76 (1 H, m, 9-H^a), 3.57–3.47 (3 H, m, 6-, 8-H and 9-H^b), 3.36 (1 H, d, *J* 10.2, 3-H) and 2.04 (3 H, s, $NHCOCH_3$); $\delta_C(D_2O)$ 178.8, 177.2 and 176.3 (carbonyls), 85.5 (C-2), 74.6 (C-7), 73.7 (C-4), 72.0 (C-8), 66.7 (C-9), 62.3 (C-3), 59.1 (C-5), 48.0 (C-6) and 25.8 ($NHCOCH_3$).

An aq. solution of the diacid **9** (2.38 g, 6.45 mmol in 200 cm^3) was adjusted to pH 6 with 0.1 M aq. Na_2HPO_4 and the mixture was heated at 90 °C for 5–6 h. The resulting solution was then chromatographed [Amberlite IRA-400 ($HCOO^-$ form) eluting successively with water (1000 cm^3) and HCO_2H (2 M)]. The latter fraction was lyophilized, to afford 6-thio-Neu5Ac **10** as a solid (1.62 g, 77%); $\delta_H(D_2O)$ 4.03 (1 H, pseudo t, *J* 10.3 and 10.3, 5-H), 3.92 (1 H, ddd, *J* 4.4, 11.0 and 11.0, 4-H), 3.83 (1 H, dd, *J* 1.5 and 9.2, 7-H), 3.75 (1 H, dd, *J* 5.0 and 10.0, 9-H^a), 3.60 (1 H, ddd, *J* 5.0, 5.8 and 9.2, 8-H), 3.54 (1 H, dd, *J* 5.8 and 10.1, 9-H^b), 3.42 (1 H, dd, *J* 1.5 and 9.9, 6-H), 2.46 (1 H, dd, *J* 4.4 and 13.3, 3-H^{eq}), 2.24 (1 H, dd, *J* 11.2 and 13.3, 3-H^{ax}) and 2.03 (3 H, s, $NHCOCH_3$); $\delta_C(D_2O)$ 177.6 (carbonyls), 83.8 (C-2), 73.4 (C-8), 71.3 and 70.9 (C-4 and -7), 63.2 (C-9), 58.4 (C-5), 47.2 (C-6), 46.9 (C-3) and 24.6 ($NHCOCH_3$).

Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-6-thio-D-*glycero*-β-D-*galacto*-non-2-ulosonate 12

Dry Dowex 50W-X8 (H^+) (2 cm^3) was added to a solution of the acid **10** (2.16 g, 6.65 mmol) in dry methanol (200 cm^3) and

the mixture was stirred at room temperature for 48 h. The resin was filtered off and the filtrate was concentrated to give the *ester* **12** as a solid (1.91 g, 85%); $\delta_H(D_2O)$ 4.02 (1 H, pseudo t, *J* 10.2 and 10.2, 5-H), 3.92 (1 H, ddd, *J* 4.4, 10.8 and 11.3, 4-H), 3.85–3.70 and 3.59–3.50 (8 H, m, 5-, 6-, 8-H, 9-H_2 and CO_2CH_3), 2.49 (1 H, dd, *J* 4.4 and 13.1, 3-H^{eq}), 2.24 (1 H, dd, *J* 11.3 and 13.1, 3-H^{ax}) and 2.03 (3 H, s, NHCOCH_3); $\delta_C(D_2O)$ 175.4 and 177.7 (carbonyls), 83.2 (C-2), 73.5, 71.1 and 71.0 (C-4, -7 and -8), 65.7 (C-9), 58.4 and 56.5 (C-5 and CO_2CH_3), 47.3 (C-6), 46.7 (C-3) and 24.7 (NHCOCH_3); *m/z* 340 (MH^+) (Found: $[M + H]^+$, 340.1075. $C_{12}H_{22}NO_8S$ requires *m/z*, 340.1066).

7,8,9-Tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-2′-methyl-6-thio-(methyl-D-*glycero*-D-*talo*-non-2-enonato)[5,4-*d*]oxazole 13

Conc. sulfuric acid (0.4 cm^3) and acetic anhydride (4.0 cm^3) were added to a solution of ester **12** (0.97 g, 2.87 mmol) in glacial acetic acid (4.0 cm^3), and the resultant solution was stirred at room temperature for 40 h.[10] The solution was then poured into stirred aq. saturated sodium hydrogen carbonate (pH 9), and stirred for 1 h before extraction with ethyl acetate (3 × 20 cm^3). The combined extracts were dried (Na_2SO_4) and concentrated to afford the oxazoline **13** as a syrup (1.20 g, 97%); 95% pure by ^1H NMR spectroscopy. This was used in the subsequent step without further purification. A *small sample* was purified (silica gel; ethyl acetate) for physical and spectroscopic characterization purposes; $[\alpha]_D$ −47 (*c* 1.37, $CHCl_3$); $\delta_H(CDCl_3)$ 7.20 (1 H, d, *J* 3.5, 3-H), 5.77 (1 H, dd, *J* 2.9 and 5.9, 7-H), 5.42 (1 H, ddd, *J* 3.2, 5.9 and 6.3, 8-H), 4.89 (1 H, dd, *J* 3.5 and 9.4, 4-H), 4.37 (1 H, dd, *J* 3.2 and 12.2, 9-H^a), 4.25 (1 H, dd, *J* 9.4 and 10.7, 5-H), 4.14 (1 H, dd, *J* 6.3 and 12.2, 9-H^b), 3.84 (3 H, s, CO_2CH_3), 2.75 (1 H, dd, *J* 2.9 and 10.7, 6-H) and 2.15, 2.11, 2.09 and 2.04 (4 × 3 H, s, NHCOCH_3, OCOCH_3, oxazoline CH_3); $\delta_C(CDCl_3)$ 170.5, 169.8, 169.5, 165.9 and 163.8 (N=C, carbonyls), 131.8 (C-2), 129.9 (C-3), 75.8 (C-4), 70.8 (C-8), 69.2 (C-7), 67.5 (C-5), 61.7 (C-9), 52.8 (CO_2CH_3), 47.0 (C-6) and 20.8, 20.6, 20.5 and 14.0 (NHCOCH_3 and OCOCH_3); *m/z* 430 (MH^+) (Found: $[M + H]^+$, 430.1189. $C_{15}H_{26}O_{12}S$ requires *m/z*, 430.1172).

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-6-thio-D-*glycero*-D-*galacto*-non-2-enonate 14

The oxazoline **13** (1.04 g, 2.42 mmol) was treated with azidotrimethylsilane (0.83 g, 7.20 mmol) in 2-methylpropan-2-ol (10 cm^3) at 75–80 °C for 48 h.[13] The solution was cooled, added to saturated aq. sodium hydrogen carbonate, and after 1 h, extracted with ethyl acetate (3 × 20 cm^3). The extracts were dried (Na_2SO_4) and then evaporated to give a syrup (1.01 g). Column chromatography on silica gel [ethyl acetate–hexanes (3:1 v/v)] afforded the *azide* **14** as a foam (0.75 g, 66%); ν_{max}(NaCl)/cm^{-1} 2104; $[\alpha]_D$ +73 (*c* 0.31, $CHCl_3$); $\delta_H(CDCl_3)$ 6.83 (1 H, d, *J* 3.9, 3-H), 6.01 (1 H, d, *J* 7.9, NH), 5.49 (1 H, dd, *J* 4.8 and 6.1, 7-H), 5.37 (1 H, ddd, *J* 3.7, 5.8 and 6.1, 8-H), 4.67 (1 H, dd, *J* 3.9 and 6.9, 4-H), 4.36 (1 H, dd, *J* 3.7 and 12.2, 9-H^a), 4.16 (1 H, dd, *J* 5.8 and 12.2, 9-H^b), 4.01 (1 H, ddd, *J* 6.4, 6.9 and 7.9, 5-H), 3.85 (1 H, dd, *J* 4.8 and 6.4, 6-H), 3.82 (3 H, s, CO_2CH_3) and 2.11, 2.10, 2.06 and 2.04 (4 × 3 H, s, NHCOCH_3, OCOCH_3); $\delta_C(CDCl_3)$ 170.6, 170.5, 169.9, 169.6 and 163.4 (carbonyls), 129.1 (C-2), 127.7 (C-3), 69.8 (C-8), 68.2 (C-7), 61.5 (C-9), 59.0 (C-4), 52.9 (CO_2CH_3), 46.8 (C-5), 43.4 (C-6) and 23.1, 20.7 and 20.5 (NHCOCH_3, OCOCH_3); *m/z* 473 (MH^+) (Found: $[M + H]^+$, 473.1339. $C_{18}H_{25}N_4O_9S$ requires *m/z*, 473.1342).

Ammonium 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-6-thio-D-*glycero*-D-*galacto*-non-2-enonate 16

A mixture of the azide **14** (126 mg, 0.27 mmol), zinc dust (70 mg, 1.07 mmol) and 2M HCl (2 cm^3) was heated at 75 °C for 45 min. The resulting solution was concentrated to dryness to yield the amine hydrochloride **15** as a straw-coloured foam (140 mg).

To an aq. solution of the crude amine hydrochloride **15** (140 mg in 5 cm^3) was added sodium hydroxide (to pH 13) and the solution was stirred at room temperature for 16 h. The pH was then adjusted to 7.5 using Dowex 50W-X8 (H^+) resin, the resin was filtered off, and the filtrate was purified on a Dowex 50W-X8 (H^+) column. The column was washed with water (200 cm^3) and was then eluted with 1.5 M NH_4OH. The latter fraction was lyophilized to afford the amine **16** as a foam (57 mg, 66%); $\delta_H(D_2O)$ 6.38 (1 H, br s, 3-H), 4.26–3.55 (7 H, m, 4-, 5-, 6-, 7-, 8-H, and 9-H_2) and 2.03 (3 H, s, NHCOCH_3); $\delta_C(D_2O)$ 177.4 and 172.4 (C-1, NHCOCH$_3$), 139.1 (C-2), 124.0 (C-3), 73.5 (C-7), 70.9 (C-8), 65.5 (C-9), 54.7 (C-4), 51.5 (C-5), 47.1 (C-6) and 24.8 (NHCOCH_3).

A small amount of the amine hydrochloride **15** was desalted (HPLC; Waters Millipore μBondapak C18 reversed phase; eluent: 0.1% aq. trifluoroacetic acid) to give the corresponding *acid*, for physical and spectroscopic characterization purposes; $[\alpha]_D$ +18 (*c* 1.41, $CHCl_3$); $\delta_H(D_2O)$ 6.61 (1 H, pseudo s, 3-H), 4.45 (1 H, pseudo t, *J* 10.0 and 10.0, 5-H), 4.29 (1 H, dd, *J* 2.5 and 9.4, 7-H), 3.94 and 3.86 (2 H, pseudo d, *J* 9.8 and 9.5, respectively, 4- and 6-H), 3.82 (1 H, pseudo d, *J* 11.3, 9-H^a), 3.72 (1 H, m, 8-H), 3.62 (1 H, dd, *J* 5.6 and 11.6, 9-H^b) and 2.09 (3 H, s, NHCOCH_3); *m/z* 307 (MH^+) (Found: $[M + H]^+$, 307.0974. $C_{11}H_{19}N_2O_6S$ requires *m/z*, 307.0964).

The amine hydrochloride **15** was also characterized as its pentaacetyl derivative. Thus the azide **14** (48 mg, 0.10 mmol) was treated with zinc dust and 2 M HCl as described above. The crude amine hydrochloride **15** was then treated with acetic anhydride (1 cm^3) in the presence of conc. sulfuric acid (0.1 cm^3) at room temperature for 16 h before being poured into saturated aq. sodium hydrogen carbonate. After extraction with acetonitrile (5 × 10 cm^3) and removal of solvent, an oil (39 mg) was obtained. Flash chromatography [ethyl acetate–methanol (19:1 v/v)] gave methyl 4,5-*bisacetamido*-7,8,9-*tri*-O-*acetyl*-2,6-*anhydro*-3,4,5-*trideoxy*-6-*thio*-D-glycero-D-galacto-*non*-2-*enonate* as a syrup (25 mg, 60%); $[\alpha]_D$ −13 (*c* 0.40, $CHCl_3$); $\delta_H(CDCl_3)$ 6.70 (1 H, d, *J* 3.0, 3-H), 6.41 and 6.16 (2 × 1 H, d, *J* 8.9 and 9.1, 2 × NH), 5.52 (1 H, dd, *J* 4.6 and 6.3, 7-H), 5.21 (1 H, ddd, *J* 3.5, 5.8 and 6.3, 8-H), 4.85 (1 H, ddd, *J* 3.0, 9.1 and 9.1, 4-H), 4.38 (1 H, m, 5-H), 4.37 (1 H, dd, *J* 3.5 and 12.4, 9-H^a), 4.15 (1 H, dd, *J* 5.8 and 12.4, 9-H^b), 3.80 (3 H, s, CO_2CH_3), 3.66 (1 H, dd, *J* 4.6 and 8.9, 6-H) and 2.11, 2.07, 2.06, 2.00 and 1.95 (5 × 3 H, s, NHCOCH_3, OCOCH_3); $\delta_C(CDCl_3)$ 171.0, 170.6, 169.9 and 163.6 (carbonyls), 128.1 (C-2), 70.3 (C-8), 67.6 (C-7), 61.6 (C-9), 52.9 (CO_2CH_3), 50.4 and 49.3 (C-4 and -5), 44.3 (C-6) and 23.0, 20.9, 20.7 and 20.5 (NHCOCH_3, OCOCH_3); *m/z* 489 (MH^+) (Found: $[M + H]^+$, 489.1542. $C_{20}H_{29}N_2O_{10}S$ requires *m/z*, 489.1543).

Ammonium 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-6-thio-D-*glycero*-D-*galacto*-non-2-enonate 17

To an aq. solution of the amine **16** (40 mg, 0.12 mmol in 1 cm^3) and imidazole (34 mg, 0.50 mmol) was added pyrazole-2-carboxamidine hydrochloride (34 mg, 0.25 mmol) and the resulting solution was stirred at 60 °C for 24 h.[13] The volatiles were removed *in vacuo* and the residue was chromatographed on silica gel [propan-2-ol–water (4:1 v/v)] to provide compound **17** as a solid after removal of solvent (25 mg, 55%); $\delta_H(D_2O)$ 6.31 (1 H, br s, 3-H), 3.86–3.51 (7 H, m, 4-, 5-, 6-, 7-, 8-H and 9-H_2) and 1.96 (3 H, s, NHCOCH_3); $\delta_C(D_2O)$ 177.4 and 173.1 (C-1, NHCOCH$_3$), 159.7 [C(NH$_2$)=NH], 137.6 (C-2), 130.0 (C-3), 73.8 (C-8), 71.4 (C-7), 65.8 (C-9), 56.2 (C-4), 53.2 (C-5), 47.1 (C-6) and 24.7 (NHCOCH_3); *m/z* 365 (M^+) and 349 $(M - NH_2)^+$ (Found: $[M - NH_2]^+$, 349.1164. $C_{12}H_{21}N_4O_6S$ requires *m/z*, 349.1182).

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