Synthesis of 6-, 7- and 8-carbon sugar analogues of potent anti-influenza 2,3-didehydro-2,3-dideoxy-*N*-acetylneuraminic acid derivatives

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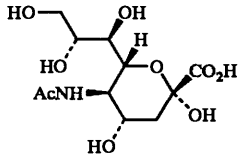
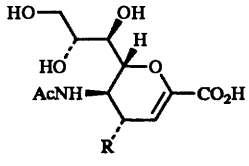
Analogues of the potent anti-influenza A and B compound, 4-guanidino-Neu5Ac2en, are described in which the stereochemically demanding C-6-glycerol side-chain is truncated. Syntheses of the one- and two-carbon side-chain analogues, of both 4-guanidino- and 4-amino-Neu5Ac2en, are presented, as well as the syntheses of analogues lacking any side-chain. Whilst complete removal of the C-6 side-chain abolishes activity, a stepwise increase in inhibition of influenza neuraminidase and influenza A and B in cell culture with increasing C-6 chain length is observed. The one-carbon, hydroxymethyl derivative retains significant activity to represent a suitable lead in the search for neuraminidase inhibitors of reduced stereochemical demand and synthetic complexity.

In the preceding paper[1] we describe the synthesis of 4-guanidino-Neu5Ac2en, a 4-substituted neuraminic acid derivative which is currently under development as a potential drug for the prophylaxis and treatment of disease caused by the influenza A and B viruses. As part of our studies to understand the structure–activity relationships in this class we have prepared a series of analogues to examine the contribution made by the components of the glycerol side-chain to the overall profile.

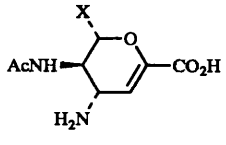
Molecular-modelling studies[2] using GRID[3] calculations suggested there were no specific interactions between the C-6 stereospecific triol side-chain of these molecules and the influenza neuraminidase (NA) enzyme. Analogues **1** and **2** were synthesized in order to probe this hypothesis. Removal of the triol side-chain may free the synthesis from the restrictions of using the expensive Neu5Ac as starting material, or from the stereospecific chemistry involved in building up the chiral side-chain from simpler and cheaper molecules.



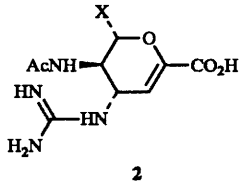
Neu5Ac



Neu5Ac2en R = OH
4-Amino-Neu5Ac2en R = NH_2
4-Guanidino-Neu5Ac2en R = $NHC(NH)NH_2$



1
a X = H
b X = CH_2OH
c X = $CH(OH)CH_2OH$

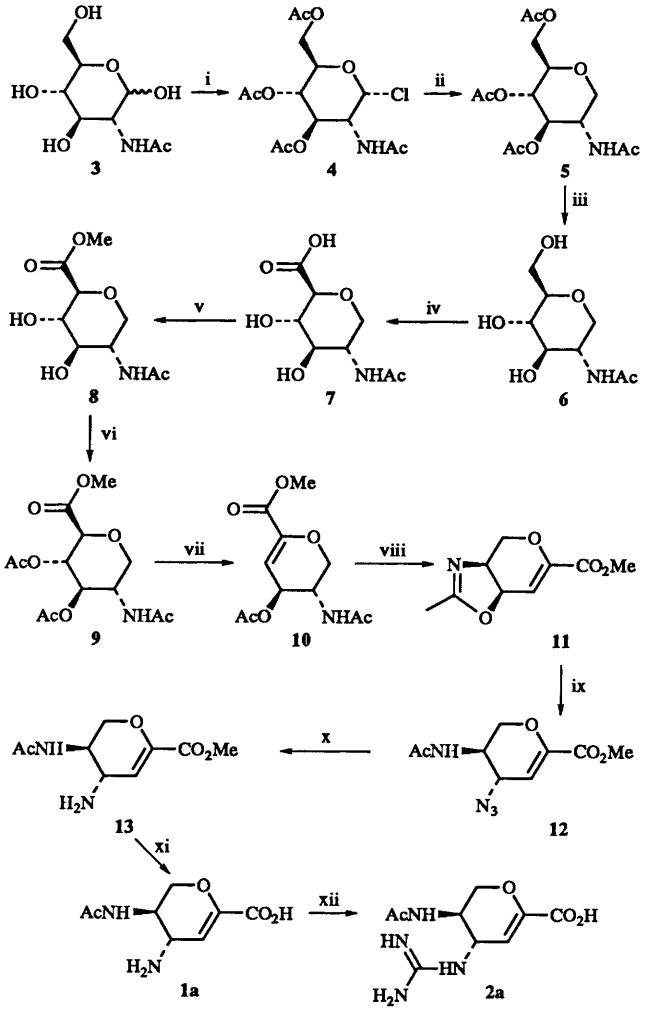


2
a X = H
b X = CH_2OH
c X = $CH(OH)CH_2OH$

Results and discussion

Analogues of 4-amino- and 4-guanidino-Neu5Ac2en lacking any C-6 side-chain were synthesized according to Scheme 1. *N*-Acetylglucosamine **3** was used to prepare the tri-*O*-acetyl-1-chloro derivative **4** by the procedure of Horton.[4] The main side-product was the 1-*O*-acetyl derivative formed in 4% (isolated) yield, but this was easily separated by flash chromatography on silica gel.† Free-radical-initiated dehalogenation of chloride **4** furnished the crystalline tetrahydropyran compound **5** in good yield (80%). The two 1-H protons resonated 1 ppm apart. After removal of acetyl protecting groups to give triol **6** by using 1% sodium in methanol, selective oxidation of the primary alcohol to give the acid **7** was successfully effected[5] in almost quantitative yield by using oxygen gas with a catalytic amount of platinum in the presence of aq. sodium hydrogen carbonate in water. On repetition the reaction time varied from 2 h to 20 h presumably dependent on the rate of oxygen flow and the efficiency of stirring relative to the scale of reaction. No epimerisation at C-2 (adjacent to the newly formed acid) was apparent, a single compound being produced. Esterification of the acid **7** was subsequently carried out by treatment with Dowex (H^+) resin in methanol. All attempts to obtain a crystalline form of the ester **8** were unsuccessful. Following acetylation of the two secondary hydroxy groups by using acetic anhydride in pyridine, elimination of the resulting protected compound **9** across the C-2–C-3 bond was achieved by refluxing with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in chloroform. The α,β-unsaturated product **10** showed only one O-acetyl resonance and a downfield shift in the 3-H resonance (from δ 5.23 to δ 6.20). This was converted by reaction[1] with trimethylsilyl triflate (TMSOTf) into the oxazoline **11** obtained as a gum in good yield (98%). No attempt at further purification was made. The crude product was subjected to nucleophilic attack at C-4 by hydrazoic acid, generated *in situ* by the reaction[1] of azidotrimethylsilane with *tert*-butyl alcohol at 80 °C. The azide moiety was successfully incorporated in the pseudo-equatorial α-configuration evidenced by the ^1H NMR spectrum. The small coupling constant (4 Hz) for 3-H due to the coupling with the pseudo-axial 4-H results from a dihedral angle close to 90°. β-Epimers have a larger coupling constant for 3-H due to an acute dihedral angle with the pseudo-equatorial 4-H. These characteristic coupling constants are inferred from

† The 1-chloro compound was found to be unstable when kept on silica for long periods.



Scheme 1 *Reagents:* i, AcCl; ii, Bu_3SnH, AIBN, toluene; iii, Na, MeOH; iv, Pt, O_2, water; v, Dowex H^+, MeOH; vi, Ac_2O, pyridine; vii, DBU, $CHCl_3$; viii, TMSOTf, MeCN; ix, $TMSN_3$, Bu^{t}OH; x, H_2, Pd/C, 1,4-dioxane–water; xi, aq. Et_3N; xii, AIMSA, aq. K_2CO_3

similar observations for the parent Neu5Ac2en analogues. Hydrogenation of the azide **12** to give the amine **13** proved problematic. Under the conditions employed, hydrogen with a catalytic amount of 10% Pd/C, there was a competitive reduction of the double bond. Hence, the reaction was halted well before all the azide had reacted and the amine **13** was obtained in only 19% yield after chromatography. This was then deprotected to give the target amino acid **1a** as the partial triethylamine salt, requiring no further purification.

Reaction of amine **1a** with aminoiminomethanesulfonic acid[6] (AIMSA) under basic conditions effected introduction of the guanidino functionality. Recrystallisation of the product obtained from Dowex (H^+) ion-exchange chromatography was unsuccessful when using water, propan-2-ol, ethanol and methanol as solvents, even though the compound with a triol side-chain at C-6 is crystalline under similar conditions. Analytically pure guanidino compound **2a** was obtained as the trifluoroacetate salt after preparative HPLC. The stereochemistry at C-4 of amino compound **1a** and guanidino compound **2a** were again inferred from the ^1H NMR coupling constants of the vinylic 3-H proton.

The 4-amino and 4-guanidino compounds (**1b** and **2b**, respectively) possessing a hydroxymethyl group at the C-6 position were also synthesized. In order to simplify the chemistry as much as possible, the approach taken was to cleave two carbons from the appropriate parent neuraminic acid derivative, preferably without any protection required. Such a strategy has been used[7] in looking at the binding of the C-6-truncated forms of sialylglycoproteins to influenza virus haemagglutinin. Thus, the C-6 side-chain of 4-amino-Neu5Ac2en was cleaved with two mole equivalents of sodium periodate and the resulting aldehyde was reduced with excess of sodium boranuide to the hydroxymethyl derivative **14**, which was purified by Dowex (OH^-) ion-exchange chromatography. Interestingly, in this compound the amine group was concomitantly formylated. The formyl group was cleaved by using HCl in methanol and, owing to the resulting formation of the methyl ester of the carboxylic acid group (as observed by ^1H NMR examination of the crude acid-treated product), this was subsequently treated with aq. triethylamine. The product was purified by Dowex (H^+) ion-exchange chromatography. This, and the formyl intermediate, both contained an impurity from which the desired product could not be freed. Similar aldehydes have been isolated and used previously without such problems,[8] but it could not be ruled out[9] that this impurity was due to epimerisation at C-6 of the intermediate aldehyde under the basic reducing conditions.

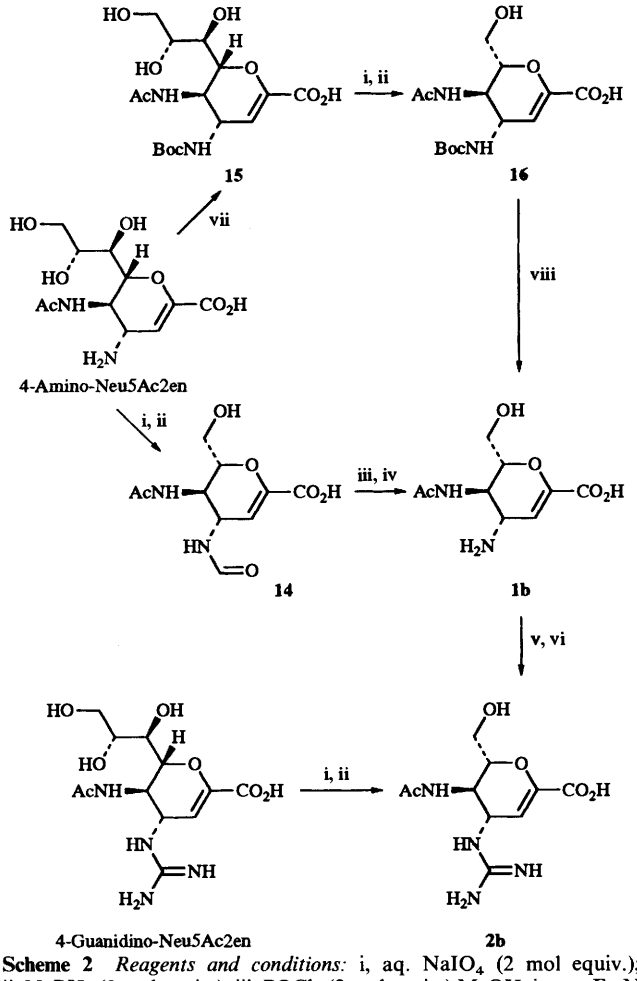
In an attempt to control the sequence of reactions more closely and thereby avoid formation of the impurity, the amine functionality was protected to give compound **15** prior to the oxidation/reduction step. In this way the C-6-truncated protected amino derivative **16** was obtained as a single compound in good yield and in crystalline form without the need for chromatography. This compound showed none of the contaminant previously observed without amine protection, and represents a versatile synthetic intermediate for the exploration of additional analogues. It was found that the *tert*-butoxycarbonyl (Boc) group is cleaved on heating of compound **16** in water to give pure crystalline truncated amino derivative **1b**.

Attempts to convert the amino derivative **1b** into the 4-guanidino derivative **2b** by using the AIMSA methodology (Scheme 2) gave impure material which resisted attempts at purification by ion-exchange chromatography and crystallisation. This target was obtained most conveniently (Scheme 2) directly from the parent guanidino compound **2a** by the oxidation/reduction protocol, analytically pure compound being obtained in modest (47%) yield following ion-exchange [Dowex (H^+)] chromatography and crystallisation.

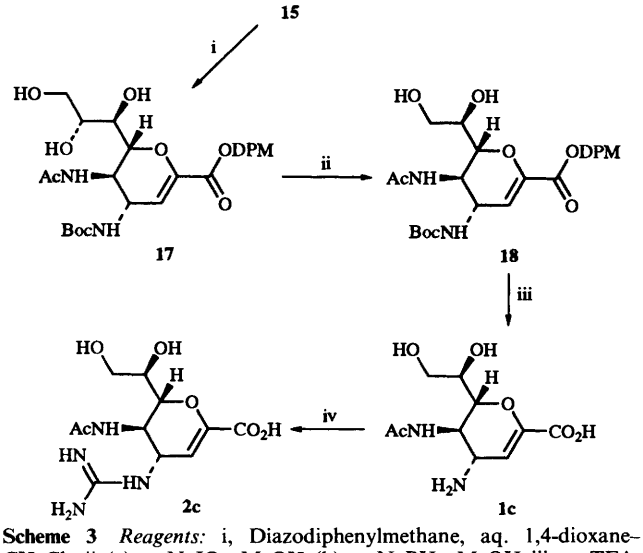
The 4-amino and 4-guanidino compounds possessing the intermediary length 2-carbon C-6-side-chain, analogues **1c** and **2c**, respectively, were obtained (Scheme 3) by using a slightly modified route; Boc protection was again used to mask the 4-amino group whilst, in order to simplify handling of intermediates, the acid functionality was protected as its diphenylmethyl (DPM) ester. Thus, Boc-protected intermediate **15** was treated with diazodiphenylmethane at 21 °C for 3 days to give the fully protected DPM ester **17**. This was subjected to the oxidation/reduction methodology in which only 1.1 mole equivalents each of $NaIO_4$ and $NaBH_4$ were used. The presence of protecting groups allowed purification by chromatography on silica to give the 2-carbon truncated intermediate **18**. Concomitant removal of both the DPM ester and Boc protecting groups with aq. trifluoroacetic acid (TFA) afforded the amino acid derivative **1c** in very high yield (94% from **18**) as its TFA salt. This was converted in the usual manner into the 4-guanidino derivative **2c** which was purified by preparative HPLC.

The target compounds **1** and **2** were tested *in vitro* against isolated neuraminidase enzyme and influenza virus,[10] and their IC_{50}-values were compared with those of the parent neuraminic acid analogues (Table 1).

The data for compounds **1a** and **2a** indicate that complete removal of the triol side-chain results in loss of virtually all



Scheme 2 *Reagents and conditions:* i, aq. $NaIO_4$ (2 mol equiv.); ii, $NaBH_4$ (9 mol equiv.); iii, $POCl_3$ (2 mol equiv.) MeOH; iv, aq. Et_3N (10 mol equiv.); v, NaOH (pH 9); vi, AIMSA (3.4 mol equiv.), aq. K_2CO_3 (3.3 mol equiv.); vii, $(Boc)_2O$, aq. Na_2CO_3; viii, water, reflux



Scheme 3 *Reagents:* i, Diazodiphenylmethane, aq. 1,4-dioxane–CH_2Cl_2; ii, (a) aq. $NaIO_4$, MeOH; (b) aq. $NaBH_4$, MeOH; iii, aq. TFA; iv, AIMSA, aq. K_2CO_3

neuraminidase inhibitory, and influenza A and B plaque-reducing activity. Thus, whilst our computational studies suggest no major *specific* interaction of this moiety with the enzyme, it must fulfil a vital role in binding. It is possible that the hydroxy groups undergo a water-mediated interaction with the enzyme in this region of the active site or play an entropic role through the displacement of water. Compounds **1b** and **2b** possessing a simple hydroxymethyl functionality at C-6 also show much reduced activity relative to the parents, but compound **2b** is still a potent inhibitor (equivalent in activity to Neu5Ac2en) of both isolated NA enzyme and influenza viruses A and B *in vitro*. It therefore represents a suitable lead in the search for NA inhibitors of reduced stereochemical demand and synthetic complexity.

Compounds **1c** and **2c** show inhibitory activity intermediary to that of the simple CH_2OH side-chain compounds and the parent Neu5Ac2en analogues; thus, each hydroxymethylene unit contributes significantly to the binding of 4-amino- and 4-guanidino-Neu5Ac2en to the enzyme active site.

Table 1 Enzyme inhibition and anti-viral data

Compound	IC_{50}NA[a] (μmol dm^{-3})	IC_{50}FluA[b] (μg cm^{-3})	IC_{50}FluB[c] (μg cm^{-3})
Neu5Ac2en	8.6	12	4.8
4-Amino-Neu5Ac2en	0.32	1.5	0.065
4-Guanidino-Neu5Ac2en	0.005	0.023	0.005
1a	> 1000	*d*	*d*
2a	130	> 100	48
1b	270	> 100	19
2b	9.2	17	1.9
1c	13	3.8	24
2c	0.55	< 0.1	2.1

[a] NA = isolated neuraminidase enzyme inhibitory assay. [b,c] Flu A and B = *in vitro* influenza virus A and B inhibitory assay as determined by plaque reduction.[10] [d] Not determined.

Experimental

Mps were determined in open capillaries and those using a Mettler FP51 automatic melting point apparatus are expressed in °C as $M^x y$ where x = rate of temperature rise (°C min^{-1}) and y = the starting temperature. ^{1}H NMR spectra were run on a Bruker 250 MHz spectrometer with Me_4Si as internal standard; coupling constants (J) are quoted in Hz. IR spectra were obtained using a Nicolet 5SxC FT-IR spectrometer. Optical rotations were measured ($[\alpha]_D$-units are 10^{-1} deg cm^2 g^{-1}) using apparatus supplied by Optical Activity Ltd., England. Preparative silica column chromatography was performed on Merck 9385 silica under flash conditions. GLC analysis was performed by using a column derivatised to 5% with methylphenyl silicone (temperature: 50–325 °C; gradient: 10 °C min^{-1}; carrier: He gas). HPLC analysis was conducted as follows: Column 1: S5-ODS2, with acetonitrile in water as eluent and a flow of 2.0 cm^3 min^{-1}. Detection was by UV at 210 nm; column 2: Hypersil SAS, with 10% acetonitrile–0.05 mol dm^{-3} $NH_4H_2PO_4$ as eluent and a flow of 1.0 cm^3 min^{-1}. Detection was by UV at 235 nm; column 3: Dynamax C18 + guard with 5% acetonitrile + 0.1% TFA–water + 0.1% TFA and a flow of 1 cm^3 min^{-1}. Detection was by UV at 230 nm. Preparative HPLC used (unless otherwise stated) column type 1, solvent acetonitrile–water with 5–25% TFA, flow 40 cm^3 min^{-1} detection UV, 230 nm. Capillary zone electrophoresis (CZE) analysis was performed using a 50 μm fused silica column, 72 cm total length, UV detector (210 nm), 50 cm distant, 20 kV at 30 °C, pH 7 (attained by 50 mmol dm^{-3} phosphate + 50 mmol dm^{-3} borate buffer).

Enzyme and virus inhibitory assays were conducted as previously described.[10]

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride 4

Dried (1 mm Hg, 22 °C, 24 h) 2-acetamido-2-deoxy-D-glucose **3** (60 g, 0.27 mol) was added to vigorously stirred acetyl chloride

(120 cm^3, excess). The resulting suspension was stirred under dry conditions (calcium chloride tube) for 16 h, at 22 °C. The amber solution obtained was diluted with dry chloroform (480 cm^3) and poured into a stirred mixture of ice (480 g) and water (120 cm^3). The organic layer was separated, and neutralised by being stirred with ice in saturated aq. sodium hydrogen carbonate (490 cm^3) before being dried over $MgSO_4$ (30 g). The entire washing procedure was completed within 15 min at 0 °C. The solution was concentrated under reduced pressure at 50 °C to ~75 cm^3, and dry diethyl ether (600 cm^3) was added to initiate crystallisation. After 60 h the solid was filtered off, washed with dry diethyl ether (150 cm^3) and dried to give a crystalline product (77.66 g). This was purified by flash chromatography [dichloromethane–ethyl acetate (2:1, then 1:1)]. Evaporation of fractions containing the first eluted species gave the title compound **4** as a foam (41.85 g, 43.7%); mp ($M^2{}_{70}$) 114.6; $[\alpha]_D^{20}$ +107 (*c* 1.1, $CHCl_3$) (Found: C, 46.0; H, 6.3; H, 3.8. $C_{14}H_{20}ClNO_8$ requires C, 45.97; H, 5.51; N, 3.83%); $\delta_H(CDCl_3)$ 6.20 (1 H, d, *J* 3, 1-H), 5.90 (1 H, d, *J* 8, NH), 5.38–5.19 (2 H, m, 3- and 4-H), 4.53 (1 H, m, 2-H), 4.32–4.10 (3 H, m, 5-H and 6-H_2), 2.12 (3 H, s, OAc), 2.06 (6 H, s, OAc) and 2.0 (3 H, s, NH*Ac*).

Evaporation of fractions containing the second eluted product gave a foam, consistent with the 1-*O*-acetyl compound: $\delta_H(CDCl_3)$ 6.20 (1 H, d, *J* 3.75, 1-H), 5.77 (1 H, d, NH), 5.23 (2 H, m, 3- and 4-H), 4.50 (1 H, m, 2-H), 4.25 and 4.05 (2 H, m, 6-H_2), 4.00 (1 H, m, 5-H), 2.20, 2.11, 2.10 and 2.05 (12 H, 4 s, 4 × OAc) and 1.95 (3 H, s, NAc).

2-Acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-glucitol 5

A stirred solution of chloride **4** (9.1 g, 0.025 mol) in dry toluene (150 cm^3) was degassed with nitrogen for 30 min. Tributyltin hydride (8.7 g, 0.03 mol) was added followed by azoisobutyronitrile (AIBN) (815 mg) and the solution was heated at reflux under nitrogen for 1 h 20 min. The reaction mixture was evaporated to dryness and purified by flash chromatography. Elution with dichloromethane–ethyl acetate (1:1, then 1:3), then ethyl acetate, followed by ethyl acetate–methanol (10:1) furnished the title compound **5** as a foam (6.59 g, 80%); mp 158–160 °C; $[\alpha]_D^{21}$ +4.9 (*c* 1.03, $CHCl_3$) (Found: C, 50.0; H, 6.3; N, 3.8. $C_{14}H_{21}NO_8$ requires C, 50.75; H, 6.39; N, 4.23%); $\nu_{max}(CHBr_3)/cm^{-1}$ 3418 (NH), 1738, 1678 (C=O) and 1237; $\delta_H(CDCl_3)$ 5.70 (1 H, d, *J* 7.5, NH), 5.09 (1 H, d, *J* 10, 3-H), 4.95 (1 H, d, *J* 10, 4-H), 4.25–4.10 (4 H, m, 1-H^b, 2-H and 6-H_2), 3.55 (1 H, m, 5-H), 3.16 (1 H, dd, *J* 12.5, 1-H^a), 2.13–2.03 (9 H, 3 s, 3 × OAc) and 1.95 (3 H, s, NH*Ac*).

2-Acetamido-1,5-anhydro-2-deoxy-D-glucitol 6

A solution of compound **5** (24.17 g, 0.073 mol) in methanol (100 cm^3) was stirred with exclusion of moisture with 1% sodium in methanol (36.80 cm^3). A solid immediately precipitated out; this was filtered off, washed with the minimum amount of methanol, and dried *in vacuo* to give the *title compound* **6** as a crystalline solid (11.67 g, 78%); a further crop was obtained from the mother liquors (1.33 g, 9%); mp 206.3 °C; $[\alpha]_D^{21}$ +10.3 (*c* 0.58, water) (Found: C, 46.7; H, 6.9; N, 6.5. $C_8H_{14}NO_5$ requires C, 46.83; H, 6.88; N, 6.83%); $\nu_{max}(Me_2SO)/cm^{-1}$ 3334 and 3232 (OH, NH), 1668 (C=O), 1549, 1371 and 1303; $\delta_H(D_2O)$ 4.00–3.20 (8 H, m) and 2.01 (3 H, s, NH*Ac*); *m/z* 206 (MH^+).

5-Acetamido-2,6-anhydro-5-deoxy-L-*gulo*-hexonic acid 7

A solution of compound **6** (3.722 g, 0.018 mol) and sodium hydrogen carbonate (2.59 g, 0.031 mol) in water (180 cm^3) was treated with platinum [obtained by hydrogenation of platinum(IV) oxide (0.6 g) for 5 h]. Oxygen was bubbled through the vigorously stirred mixture for 6 h at 90 °C. The catalyst was removed by filtration through Kieselguhr and the filtrate was acidified using Dowex 50W × 8 (16–40 mesh) resin, H^+-form. After 0.5 h the resin was filtered off and the filtrate was concentrated under reduced pressure to furnish the *title compound* **7** as a foam (3.86 g, 97%); $[\alpha]_D^{21}$ −8.41 (*c* 0.36, water); $\nu_{max}(Me_2SO)/cm^{-1}$ 3293 (OH, NH), 1728 and 1640 (C=O), 1557 and 1097; $\delta_H(D_2O)$ 4.00–3.50 (5 H, m), 3.30 (1 H, t, *J* 10, 6-H^a) and 2.0 (3 H, s, NH*Ac*) [Found: (M^+ + 1), 220.081 743. $C_8H_{14}NO_6$ requires *m/z*, 220.082 112]; GLC 96% pure.

5-Acetamido-2,6-anhydro-5-deoxy-L-*gulo*-hexonic acid methyl ester 8

A solution of compound **7** (12.27 g, 56 mmol) in methanol (250 cm^3) was stirred with Dowex 50W × 8 (16–40 mesh) resin (12 g; H^+-form, methanol-washed) at ambient temperature for 66 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give the *title compound* **8** as an off-white foam (10.02 g, 77%); $\delta_H(D_2O)$ 4.03–3.85 (3 H, m, 2- and 5-H, 6-H^b), 3.82 (3 H, s, CO_2Me), 3.64–3.56 (2 H, m, 3- and 4-H), 3.38–3.28 (1 H, m, 6-H^a) and 2.01 (3 H, s, NH*Ac*) [Found: (M^+ + 1), 234.097 773. $C_{19}H_{16}NO_6$ requires *m/z*, 234.097 762]; HPLC 90% pure.

5-Acetamido-3,4-di-*O*-acetyl-2,6-anhydro-5-deoxy-L-*gulo*-hexonic acid methyl ester 9

A solution of compound **8** (10.02 g, 0.043 mol) in dry pyridine (40 cm^3)–acetic anhydride (20 cm^3) was stirred with exclusion of moisture at 21 °C for 16 h. The reaction mixture was then evaporated to dryness and the residue was purified by flash chromatography [ethyl acetate–cyclohexane (4:1) followed by ethyl acetate]. The appropriate fractions were combined and concentrated to 30 cm^3. The *title compound* **9** slowly crystallised at 21 °C, as a solid (4.62 g, 33%), mp 152.4 °C; $[\alpha]_D^{20}$ +16 (*c* 1.1, $CHCl_3$) (Found: C, 49.2; H, 6.3; N, 4.5. $C_{13}H_{19}NO_8$ requires C, 49.20; H, 6.03; N, 4.41%); $\delta_H(CDCl_3)$ 5.82 (1 H, *J* 7.5, NH), 5.23 (1 H, d, *J* 8, 3-H), 4.98 (1 H, d, *J* 8, 4-H), 4.31 (1 H, dd, J_1 5, J_2 8, 6-H^b), 4.20 (1 H, m, 5-H), 4.02 (1 H, d, *J* 7.5, 2-H), 3.76 (3 H, s, CO_2Me), 3.30 (1 H, dd, J_1 8, J_2 2.5, 6-H^a), 2.07 (6 H, 2 s, 2 × OAc) and 1.95 (3 H, s, NH*Ac*); *m/z* 318 (MH^+) and 335 (MNH_4^+).

5-Acetamido-4-*O*-acetyl-2,6-anhydro-3,5-dideoxy-L-*threo*-hex-2-enonic acid methyl ester 10

A solution of compound **9** (2.704 g, 8.5 mmol) in dry chloroform (40 cm^3), under nitrogen, was treated with DBU (5.1 cm^3, 34 mmol). The resulting mixture was heated at reflux for 1.5 h and was then allowed to cool to 21 °C. The solution was washed sequentially with 2 mol dm^{-3} hydrochloric acid (2 × 40 cm^3), water (50 cm^3) and brine (60 cm^3), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give an off-white foam. The crude product was purified by flash chromatography (gradient elution with 0–3% methanol in chloroform) to afford the *title compound* **10** as a gum (2.35 g, quant.), $[\alpha]_D^{21}$ +212.2 (*c* 0.49, $CHCl_3$); $\delta_H(CDCl_3)$ 6.20 (1 H, dd, J_1 5, J_2 1, 3-H), 5.69 (1 H, br, NH), 5.03 (1 H, m, 4-H), 4.33–4.10 (3 H, m, 5-H and 6-H_2), 3.83 (3 H, s, CO_2Me), 2.07 (3 H, s, OAc) and 1.98 (3 H, s, NH*Ac*) [Found: (M^+ + 1), 258.097 015. $C_{11}H_{16}NO_6$ requires *m/z*, 258.097 762]; HPLC 93% pure.

Methyl (3a*S*,7a*R*)-2-methyl-3a,7a-dihydro-4*H*-pyrano[3,4-*d*]-oxazole-6-carboxylate 11

A solution of compound **10** (4.68 g, 17.9 mmol) in dry acetonitrile (80 cm^3) under nitrogen was treated with TMSOTf (3.63 cm^3, 18.8 mmol). The resulting solution was heated at 50 °C for 1 h. The solution was allowed to cool to 21 °C and then was poured into ice-cold, saturated aq. sodium hydrogen carbonate (60 cm^3) containing extra sodium hydrogen carbonate (6 g). After being stirred for 5 min the mixture was

extracted with cold ethyl acetate (2 × 100 cm^3). The combined organic extracts were washed with brine (120 cm^3), dried over $MgSO_4$, filtered, and evaporated to give the title compound **11** as a pale yellow gum (3.47 g, 98%); $\nu_{max}(Me_2SO)/cm^{-1}$ 1736 (C=O), 1668 (C=C, C=N), 1439, 1388, 1300, 1278 and 1153; $\delta_H(CDCl_3)$ 6.23 (1 H, d, *J* 3.75, 3-H), 4.94 (1 H, dd, J_1 3.75, J_2 5, 4-H), 4.31–4.18 (2 H, m, 6-H[b] and 5-H), 3.88–3.78 (4 H, m, CO_2Me and 6-H[a]) and 2.0 (3 H, s, Me).

5-Acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-L-*threo*-hex-2-enonic acid methyl ester 12

Azidotrimethylsilane (3.4 cm^3, 24 mmol) was added dropwise (during 1 h) to a stirred solution of compound **11** (1.581 g, 8 mmol) in dry 2-methylpropan-2-ol at 80 °C. TLC analysis showed incomplete reaction and so further azidotrimethylsilane (1 cm^3) was added dropwise after 4 h and again (2.4 cm^3, 48 mmol total) an hour later. The mixture was stirred at 80 °C for a further 3 h and then at 21 °C for 16 h, then was poured into ice-cold, saturated aq. sodium hydrogen carbonate (100 cm^3) containing extra sodium hydrogen carbonate (7 g) and was stirred for 15 min. The mixture was extracted with cold ethyl acetate (3 × 50 cm^3), and the combined organic extracts were washed with brine (70 cm^3), dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by flash chromatography [ethyl acetate–cyclohexane (3:2) followed by ethyl acetate–cyclohexane (2:1) and then neat ethyl acetate, changing to ethyl acetate–methanol (10:1)] to furnish the *title compound* **12** as a gum (1.02 g, 53%); $[\alpha]_D^{21}$ +304.9 (*c* 1.06, $CHCl_3$); $\nu_{max}(CHBr_3)/cm^{-1}$ 3422 (NH), 2100 (N_3), 1733 and 1676 (C=O), 1507, 1263 and 1236; $\delta_H(CDCl_3)$ 6.14 (1 H, dd, J_1 2, J_2 4, 3-H), 5.83 (1 H, d, *J* 7, NH), 4.29–3.98 (4 H, m, 4- and 5-H and 6-H_2), 3.86 (3 H, s, CO_2Me) and 1.90 (3 H, s, NH*Ac*); *m/z* 241 (MH^+), 213 $(MH - N_2)^+$ and 198 $(MH - N_3)^+$ [Found: (M^+ + 1), 241.092 800. $C_9H_{13}N_4O_4$ requires *m/z* 241.093 680); HPLC (column 2) 96% pure.

5-Acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-L-*threo*-hex-2-enonic acid methyl ester 13

A solution of compound **12** (843 mg, 3.5 mmol) in 1,4-dioxane (15 cm^3)–water (15 cm^3) was hydrogenated over 10% Pd/C (90 mg) at ambient temperature for 1.6 h. The reaction mixture was filtered through Kieselguhr and the filtrate was evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography (gradient elution with 0–15% methanol in chloroform) to obtain the *title compound* **13** as a pale yellow solid (146 mg, 19%) (Found: C, 49.8; H, 6.7; N, 13.0. $C_9H_{14}N_2O_4$ requires C, 50.05; H, 6.59; N, 13.08%); $\nu_{max}(CHBr_3)/cm^{-1}$ 3423 (NH), 1729 (C=O ester), 1669 (C=O amide), 1511, 1437, 1309 and 1262; $\delta_H(CDCl_3)$ 6.12 (1 H, d, *J* 5, 3-H), 5.72 (1 H, br, NH), 4.15 (2 H, s, 6-H_2), 3.95 (1 H, m, 5-H), 3.85 (3 H, s, CO_2Me), 3.40 (1 H, m, 4-H) and 1.98 (3 H, s, NH*Ac*); *m/z* 215 $(MH)^+$.

5-Acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-L-*threo*-hex-2-enonic acid 1a

A suspension of compound **13** (123 mg, 0.57 mmol) in water (1.2 cm^3) was treated with triethylamine (0.42 cm^3, excess). The resulting solution was stirred at ambient temperature for 4 h and was then concentrated under reduced pressure to give a gum. This was taken up in water (5 cm^3) and the mixture was stirred with charcoal (Norrits ultra, 2 mg) for 10 min. The now colourless solution was filtered through Kieselguhr, the Kieselguhr was washed thoroughly, and the combined filtrate and washings were freeze-dried to give the *title compound* **1a** as a pale yellow solid (106 mg, 88%) (Found: C, 42.9; H, 6.7; N, 12.3. $C_8H_{12}N_2O_4$ requires C, 43.2; H, 7.0; N, 12.3%); $\nu_{max}(Me_2SO)/cm^{-1}$ 3248 (OH, NH), 1583 (C=O), 1463, 1377 and 1281; $\delta_H(D_2O)$ 5.75 (1 H, d, *J* 4.0, 3-H), 4.30–4.10 (3 H, m, 5-H and 6-H_2), 3.91 (1 H, t, *J* 4.0, 4-H) and 2.02 (3 H, s, NH*Ac*); CZE 100% pure.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-L-*threo*-hex-2-enonic acid 2a

A solution of compound **1a** (70 mg, 0.345 mmol) in water (0.5 cm^3) was treated with 0.1 mol dm^{-3} sodium hydroxide (3.45 cm^3) at 21 °C. The solution was then warmed to 35 °C and treated with potassium carbonate (10.4 mg) followed by AIMSA[6] (9.3 mg) every 0.5 h for 8 h (total 16 additions, 1.2 mmol each). The reaction mixture was then stirred at ambient temperature for 16 h, diluted with water (1 cm^3) filtered, and the solid was washed with water (1 cm^3). The filtrate and washings were combined and applied to a column of Dowex 50W × 8 (16–40 mesh, H^+-form) (37 cm^3). Elution with water (270 cm^3) followed by 0.6 mol dm^{-3} aq. triethylamine (550 cm^3) and then evaporation of the appropriate fractions gave the crude product as an off-white powder. Purification was achieved by preparative HPLC on a C_{18} 'Dynamax microsorb' column and elution with 5% acetonitrile containing 0.1% TFA at a flow rate of 1 cm^3 min^{-1}, UV detection at 230 nm. The required fractions were concentrated under reduced pressure and then freeze dried to furnish the *title compound* **2a** as a powder (40 mg, 27%) (Found: C, 35.4; H, 4.2; N, 13.75. $C_9H_{14}N_4O_4$ requires C, 35.26; H, 4.22; N, 13.76%); $\nu_{max}(Me_2SO)/cm^{-1}$ 1692 and 1653 (C=O) 1549 and 1199; $\delta_H(D_2O)$ 5.95 (1 H, dd, J_1 5, J_2 1.25, 3-H), 4.35 (1 H, m, 5-H), 4.15–3.80 (3 H, m, 4-H, 6-H_2) and 2.03 (3 H, s, NH*Ac*); HPLC (column 3) 99% pure.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-formamido-L-*arabino*-hept-2-enonic acid 14

4-Amino-Neu5Ac2en (871 mg, 3.0 mmol) was dissolved in water (45 cm^3) and the solution was treated with sodium metaperiodate (1.41 g, 6.59 mmol). This mixture was stirred in the dark at 20 °C for 1 h, diluted with water (45 cm^3), and then treated with aq. sodium boranuide (1.04 g, 27 mmol in 45 cm^3) by dropwise addition over a period of 10 min. This mixture was stirred at 20 °C for 1 h. pH was adjusted to 4 by addition of glacial acetic acid (~2 cm^3). The whole was then freeze-dried and the solid was redissolved in water (170 cm^3) and loaded onto a column of ion-exchange resin (Dowex 2 × 8, OH^--form) (30 g). This was washed with water (850 cm^3), then eluted with 1 mol dm^{-3} AcOH. Fractions containing the UV-active species [R_f 0.25 on silica, butan-1-ol–AcOH–water (3:1:1)] were combined, evaporated under reduced pressure (T < 50 °C) to a volume of 100 cm^3, then freeze-dried to give the *title compound* **14** as a crispy solid (551 mg, 71%) (Found: C, 43.6; H, 6.0; N, 9.65. $C_{10}H_{14}N_2O_6 \cdot 1.5H_2O \cdot 0.15Et_3N$ requires C, 43.57; H, 6.45; N, 10.02%); λ_{max}(water)/nm 234; $\nu_{max}(Me_2SO)/cm^{-1}$ 3500, 1714, 1675 and 1546; $\delta_H(D_2O)$ 8.12 [1 H, s, *H*C(O)NH], 5.85 (1 H, d, *J* 2.5, 3-H), 4.85 (1 H, m, 4-H), 4.20–4.00 (2 H, m, 5- and 6-H), 3.90–3.70 (2 H, m, 7-H_2) and 2.00 (3 H, s, Ac); $\delta_C(Me_2SO)$ 171.6 (MeCO), 164.8 (C-1), 162.7 (HCONH), 147.5 (C-2), 109.9 (C-3), 80.7 (C-4), 61.8 (C-7), 48.6 (C-6), 46.8 (C-5) and 24.1 (Me); *m/z* (CI) 259 (MH^+) and 276 (MNH_4^+); *m/z* (FAB) 259 (MH^+); HPLC (column 1) 90% pure.

5-Acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-L-*arabino*-hept-2-enonic acid 1b

Compound **14** (500 mg, 1.80 mmol) was dissolved in methanol (46 cm^3), and treated dropwise over a period of 5 min with phosphoryl trichloride (34 cm^3, 3.67 mmol). This mixture was stirred at 20 °C for 3 h. Solvent was removed under reduced pressure to give a brown gum, which was dissolved in water (10 cm^3) and the mixture was treated with triethylamine (2.6 cm^3, 10.5 mequiv.); this mixture was stirred at 20 °C for 3.5 h. Solvent was evaporated under reduced pressure and the resulting gum was dissolved in water (50 cm^3). This solution

was applied to a column of ion-exchange resin [Dowex 50W × 8 (H^+), 190 cm^3] and washed with water (1.4 dm^3). Elution with 0.6 mol dm^{-3} Et_3N (~ 1.5 dm^3) and evaporation under reduced pressure of the appropriate fractions gave the title compound **1b** as an off-white, crispy foam (320 mg, 77%); $\nu_{max}(Me_2SO)/cm^{-1}$ 3400, 1714, 1666, 1602, 1551, 1373 and 1263; $\delta_H(D_2O)$ 5.70 (1 H, d, *J* 2.5, 3-H), 4.25–4.10 (3 H, m, 4-, 5- and 6-H), 3.95–3.75 (2 H, m, 7-H_2) and 2.10 (3 H, s, Ac); $\delta_C(D_2O)$ 188.6 (C=O Ac), 174.8 (CO_2H), 150.5 (C-2), 100.0 (C-3), 77.0 (C-4), 60.0 (C-7), 49.9 (C-6), 46.0 (C-5) and 22.0 (Me); *m/z* 187 ($MH^+ - CO_2$), 231 (MH^+) and 248 (MNH_4^+); CZE 89.6% pure, contains 10.4% impurity.

5-Acetamido-2,6-anhydro-4-(*tert*-butoxycarbonylamino)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid 15

A suspension of 4-amino-Neu5Ac2en (0.29 g, 1 mmol) in methanol (1.5 cm^3) was treated with a solution of di-*tert*-butyl dicarbonate (240 mg, 1.1 mmol) in methanol (0.5 cm^3), followed by aq. sodium hydrogen carbonate (176 mg, 2.1 mmol in 1.5 cm^3). The resulting solution was stirred at 20 °C for 45 min, then was evaporated under reduced pressure. The residue was taken up in water and washed with diethyl ether; the aqueous solution was stirred with Dowex 50W × 8 (H^+) until acidic. This mixture was filtered and freeze-dried to give the *title compound* **15** as a low-density solid (289 mg, 74%), mp 210 (decomp); $[\alpha]_D^{21}$ +21.6 (*c* 0.83, MeOH) (Found: C, 46.9; H, 6.9; N, 6.7. $C_{16}H_{26}N_2O_9 \cdot H_2O$ requires C, 47.06; H, 6.91; N, 6.86%); λ_{max}(EtOH)/nm 233 (ε 6309); ν_{max}(Nujol)/cm^{-1} 3331, 2949, 2854, 1689 and 1461; $\delta_H(D_2O)$ 5.95 (1 H, s, 3-H), 4.50–4.35 (2 H, m, 5- and 6-H), 4.15 (1 H, t, *J* 10, 4-H), 3.90 and 3.65 (4 H, m, 7- and 8-H and 9-H_2), 2.02 (3 H, s, Ac) and 1.40 (9 H, s, Bu^t); *m/z* (TSP +ve) 391 (MH^+), 335 ($MH^+ - Bu^t$) and 291 ($MH^+ - Bu^tOCO$); CZE 98.6% pure.

5-Acetamido-2,6-anhydro-4-(*tert*-butoxycarbonylamino)-3,4,5-trideoxy-L-*arabino*-hept-2-enonic acid 16

Compound **15** (7.02 g, 18 mmol) was dissolved in water (300 cm^3), and the solution was treated portionwise with sodium metaperiodate (8.46 g, 39.5 mmol) and stirred at 20 °C for 45 min. Aq. sodium boranuide (6.24 g, 162 mmol in 240 cm^3) was added dropwise during 20 min and the mixture was stirred for 1 h. Acetic acid was added to adjust the pH to 4, and the resulting solution was concentrated to 300 cm^3 and the pH was adjusted to 1 with 2 mol dm^{-3} HCl. This mixture was stored at 2 °C for 16 h and the resulting crystals were filtered off, washed with a little water, and dried over P_2O_5. The filtrate was concentrated to ~ 70 cm^3 to give a second crop (combined yield of *title compound* **16**, 5.78 g, 97%); $[\alpha]_D^{20}$ +43.95 (*c* 0.5, MeOH) (Found: C, 48.1; H, 6.8; N, 7.9. $C_{14}H_{22}N_2O_7 \cdot H_2O$ requires C, 48.27; H, 6.94; N, 8.04%); λ_{max}(EtOH)/nm 236 (ε 6670); ν_{max}(Nujol)/cm^{-1} 3613, 3487, 3343, 3300, 2926, 2853, 1714, 1687, 1462 and 1377; $\delta_H[(CD_3)_2SO]$ 12.85 (1 H, br d, CO_2H), 8.00 (1 H, d, *J* 7.5, $NHCO_2$) 7.07 (1 H, d, *J* 8, N*H*COAc), 5.65 (1 H, d, *J* 2.25, 3-H), 4.70 (1 H, br d, OH), 4.32 (1 H, t, *J* 7.5, 4-H), 3.80 (2 H, m, 5- and 6-H), 3.50 (2 H, m, 7-H_2), 1.85 (3 H, s, Ac) and 1.40 (9 H, s, Bu^t); *m/z* (LSIMS +ve) 331 (MH^+), 353 (MNa^+), 275 ($MH^+ - Bu^t$), 231 ($MH^+ - Bu^tOCO$) and 214 ($MH^+ - NHCO_2Bu^t$); CZE 97.6% pure.

Amine 1b from carbamate 16

Compound **16** (821 mg, 2.48 mmol) was boiled in water (130 cm^3) for 1 h. On cooling, crystallisation gave some recovered starting material as large needles (216 mg, 26%). Freeze-drying of the mother liquors followed by trituration of the resulting solid with methanol gave the title compound **1b** as small needles (196 mg, 33%); mp > 250 °C (decomp.) (Found: C, 46.4; H, 5.9; N, 11.9. $C_9H_{14}N_2O_5$ requires C, 46.95; H, 6.13; N, 12.17%); CZE 97% pure.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-L-*arabino*-hept-2-enonic acid 2b

4-Guanidino-Neu5Ac2en (507 mg, 1.75 mmol) was dissolved in water (25 cm^3) and the solution was treated with sodium metaperiodate (0.824 g, 3.84 mmol) at 20 °C. After being stirred in the dark for 1 h, the solution was diluted with water (25 cm^3) and treated with aq. sodium boranuide (0.608 g, 15.7 mmol in 25 cm^3) in a dropwise manner over a period of 10 min. This mixture was then stirred at 20 °C for 1 h. Adjustment to pH 4 with acetic acid (glacial) was followed by freeze-drying to give a solid. This was taken up in water (30 cm^3) and applied to a column of ion-exchange resin [Dowex 50W × 8 (H^+), 30 cm^3]. The column was washed with water (200 cm^3), then was eluted with 0.6 mol dm^{-3} aq. triethylamine. Appropriate fractions were combined, and evaporated under reduced pressure. The residue was repeatedly co-evaporated with water to give a solid (0.33 g), which was dissolved in water (6 cm^3), the solution was warmed (45 °C) and propan-2-ol (15 cm^3) was added to the swirled mixture. Crystallisation was observed on cooling of the solution. The crystals were filtered off, washed with (3:1) propan-2-ol–water, then were dried at 60 °C under vacuum, to give the *title compound* **2b** as fine needles (0.226 g, 47%), mp > 240 °C (decomp.); $[\alpha]_D^{21}$ +15.1 (*c* 0.53, water) (Found: C, 41.1; H, 5.9; N, 19.2. $C_{10}H_{16}N_4O_5 \cdot H_2O$ requires C, 41.37; H, 6.25; N, 19.30%); λ_{max}(EtOH)/nm 233 (ε 4964); $\nu_{max}(Me_2SO)/cm^{-1}$ 3327, 3214, 3127, 3046, 2983, 1673, 1648, 1600, 1596 and 1392; $\delta_H(D_2O)$ 5.68 (1 H, d, *J* 2, 3-H), 4.32 (1 H, m, 4-H), 4.22 (1 H, m, 6-H), 4.12 (1 H, m, 5-H), 3.80 (2 H, m, 7-H_2) and 2.02 (3 H, s, Me); $\delta_C(D_2O)$ 177.2 (C=O Ac), 171.6 (CO_2H), 159.5 [NHC(=NH)NH_2], 151.2 (C-2), 105.6 (C-3), 79.3 (C-6), 62.8 (C-7), 52.2 (C-4), 50.5 (C-5) and 24.3 (Me); CZE 99.1% pure.

5-Acetamido-2,6-anhydro-4-(*tert*-butoxycarbonylamino)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid benzhydryl ester 17

Compound **15** (10 g, 34.4 mmol) was dissolved in 1,4-dioxane (60 cm^3)–water (30 cm^3). To this at 21 °C was added a solution of diazodiphenylmethane in dichloromethane (45 cm^3; 0.965 mol dm^{-3}). This mixture was stirred at 21 °C for 3 days. The organic phase was separated and evaporated to dryness. The resulting solid was treated with anhydrous diethyl ether and the mixture filtered. The solid was washed with diethyl ether several times, then dried to give the *title compound* **17** (13.8 g, 72%) (Found: C, 59.2; H, 6.5; N, 4.7. $C_{29}H_{36}N_2O_9 \cdot 1.6H_2O$ requires C, 59.49; H, 6.75; N, 4.78%); ν_{max}(Nujol)/cm^{-1} 3322br, 2955, 2924, 2853, 1716, 1689, 1653, 1521, 1456, 1367, 1249 and 1164; $\delta_H[(CD_3)_2SO]$ 8.10 (1 H, br d, NH), 7.25–7.50 (10 H, m, Ph), 7.15 (1 H, br d, NH), 6.90 (1 H, s, C*H*Ph_2), 5.90 (1 H, s, 3-H), 4.7–4.6 (1 H, m, 6-H), 4.50 (1 H, t, *J* 9, OH), 4.35 (1 H, t, *J* 5.8, 4-H), 4.10 (1 H, d, *J* 11, 5-H), 3.95 and 3.70 (4 H, m, 7- and 8-H, and 9-H_2), 1.90 (3 H, s, Ac) and 1.40 (9 H, s, Bu^t); HPLC 99.2% pure.

5-Acetamido-2,6-anhydro-4-(*tert*-butoxycarbonylamino)-3,4,5-trideoxy-D-*galacto*-oct-2-enonic acid benzhydryl ester 18

Compound **17** (2.0 g, 3.6 mmol) was dissolved in methanol (80 cm^3), and water (5 cm^3) was added until precipitation was observed. To this mixture at 0 °C was added sodium metaperiodate (770 mg) and the mixture was stirred for 1 h before being filtered, and the filtrate was evaporated under reduced pressure. The solid residue was treated with methanol (60 cm^3) and to this suspension was added water (10 cm^3), followed by sodium boranuide (150 mg, 4.0 mmol). After 3 h the mixture was acidified with 1 mol dm^{-3} HCl and evaporated. The solid residue was purified by flash chromatography (4% methanol in dichloromethane) to give the *title compound* **18** as a foam (1.38 g, 73%) (Found: C, 60.8; H, 6.7; N, 5.01. $C_{28}H_{34}N_2O_8 \cdot 0.67H_2O$ requires C, 60.75; H, 6.74; N, 5.06%);

$\nu_{max}(Me_2SO)/cm^{-1}$ 3326, 3232, 2977, 1732, 1704, 1652 and 1589; $\delta_H[(CD_3)_2SO]$ 8.10 (1 H, d, *J* 7.7, NH), 7.25–7.50 (10 H, m, Ph), 7.12 (1 H, d, *J* 9.5, NH), 6.90 (1 H, s, Ph_2CH), 5.90 (1 H, d, *J* 2, 3-H), 4.65 (2 H, m, 5- and 6-H), 4.45 (1 H, br, OH), 3.95 (2 H, m, 4-H and OH), 3.5 (3 H, m, 7-H and 8-H), 2.90 (3 H, s, Ac) and 1.4 (9 H, s, Bu^t); *m/z* 527 (MH^+) and 549 (MNH_4^+).

5-Acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*galacto*-oct-2-enonic acid–trifluoroacetic acid complex 1c

Compound **18** (150 mg, 0.576 mmol) was dissolved at 0 °C in a TFA–water mixture (4 cm^3 of a 5:2 mixture) and the mixture was then allowed to warm to 21 °C over a period of 4 h. The mixture was treated with diethyl ether (25 cm^3) and the organic phase was extracted with water. The combined aqueous solutions were freeze-dried to give the *title compound* **1c** as a pale yellow solid (70 mg, 94%) (Found: C, 36.5; H, 4.7; N, 6.7. $C_{10}H_{16}N_2O_6 \cdot C_2HF_3O_2 \cdot H_2O$ requires C, 36.74; H, 4.88; N, 7.14%); $\nu_{max}(MeOH)/cm^{-1}$ 3500–3100br, 3079, 2947, 1607, 1556, 1203 and 1139; $\delta_H(D_2O)$ 5.95 (1 H, s, 3-H), 4.35 (1 H, t, *J* 8.5, 5-H), 4.25 (2 H, m, 4- and 6-H), 3.90 (1 H, t, *J* 6.5, 7-H), 3.75 (2 H, d, *J* 6.5, 8-H_2) and 2.10 (3 H, s, Ac); HPLC 93% pure.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*galacto*-oct-2-enonic acid–trifluoroacetic acid complex 2c

A solution of compound **1c** (104 mg, 0.4 mmol) in water (5 cm^3) was treated with aq. potassium carbonate (0.4 cm^3 of a 110 mg cm^{-3} solution). To this were then added AIMSA (20 mg) and aq. potassium carbonate (0.1 cm^3) to a total of 16 additions over a period of 2 days. This aqueous solution was then purified by preparative HPLC (S5-ODS2 column, 5–25% TFA in acetonitrile–water gradient, 40 cm^3 min^{-1}) to give, after evaporation and freeze-drying of appropriate fractions, the *title compound* **2c** as a solid (67 mg, 36%) (Found: C, 33.9; H, 4.55; N, 12.1. $C_{11}H_{18}N_4O_6 \cdot 1.2C_2HF_3O_2 \cdot 1.6H_2O$ requires C, 34.40; H, 4.83; N, 11.97%); $\nu_{max}(MeOH)/cm^{-1}$ 3079, 2822, 2448, 2301, 1919, 1702, 1415, 1299, 1186, 1115 and 1093; $\delta_H(D_2O)$ 6.00 (1 H, d, *J* 2.5, 3-H), 4.5 (1 H, m, 5-H), 4.30 (2 H, m, 4- and 6-H), 3.95 (1 H, t, *J* 6.5, 7-H), 3.75 (2 H, d, *J* 6.5, 8-H_2) and 2.05 (3 H, s, Ac); *m/z* 303 (MH^+).

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