Synthesis of the potent influenza neuraminidase inhibitor 4-guanidino Neu5Ac2en. X-Ray molecular structure of 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*erythro*-L-*gluco*-nononic acid

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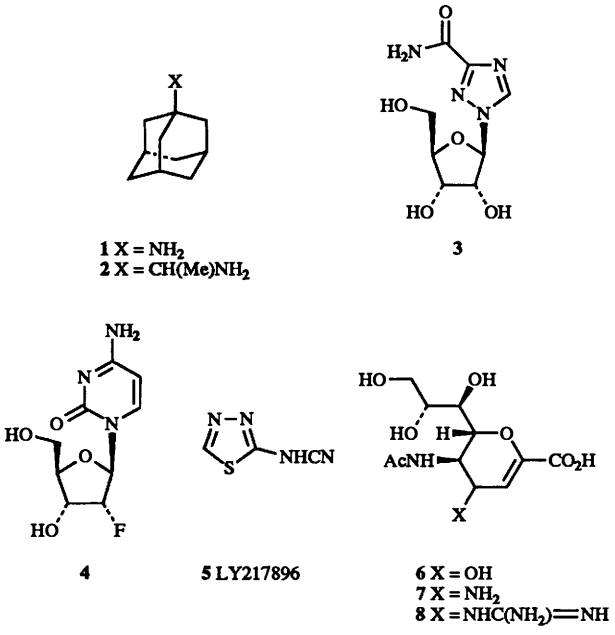
An efficient and high-yielding synthesis of 4-guanidino Neu5Ac2en, the potent anti-influenza A and B compound, is described. The route exploits a stereospecific introduction of the key nitrogen functionality at C-4 *via* an oxazoline intermediate. Three different methods for the final-step conversion of the 4-amino into 4-guanidino derivatives are described. To explore the structure–activity relationship in this region of the molecule, a series of substituted guanidino derivatives were synthesized and their activity is described.

Influenza viruses are the most serious viral cause of respiratory illness both in terms of morbidity and mortality,[1] and influenza is a disease of immense economic importance. In 1918–1919 influenza is thought to have been responsible for the deaths of 20 million people. Since then, less serious pandemics have occurred every 10–20 years and are the result of introduction of a new subtype as a consequence of the ability of the virus to modify its surface antigens. Influenza A is the most significant in terms of epidemic disease and even in the absence of major epidemics remains a significant cause of illness and mortality. A second human strain, influenza B, is not associated with any of the major pandemics but contributes significantly to the overall extent of the disease.

Influenza is essentially an uncontrolled disease. Although vaccines are available and are used, their efficacy is limited. The antigenic variability of the virus has hampered production of a viable vaccine and precluded effective control. Current vaccines are unlikely to be effective against a new pandemic strain whereas a good antiviral agent would have enormous potential in such situations. Amantadine **1** and its analogue rimantadine **2** remain the only compounds licensed for the prophylaxis and treatment of influenza A infection. These compounds are believed to exert their antiviral activity as a result of blocking the ion-channel function of the virus protein M2.[2] Neither compound has activity against influenza B viruses which do not possess the M2 protein and the clinical utility of the more extensively studied amantadine has been limited by side-effects and the rapid emergence of resistant strains.[3] There is a clear need for a safe, clinically effective agent active against both influenza A and B.

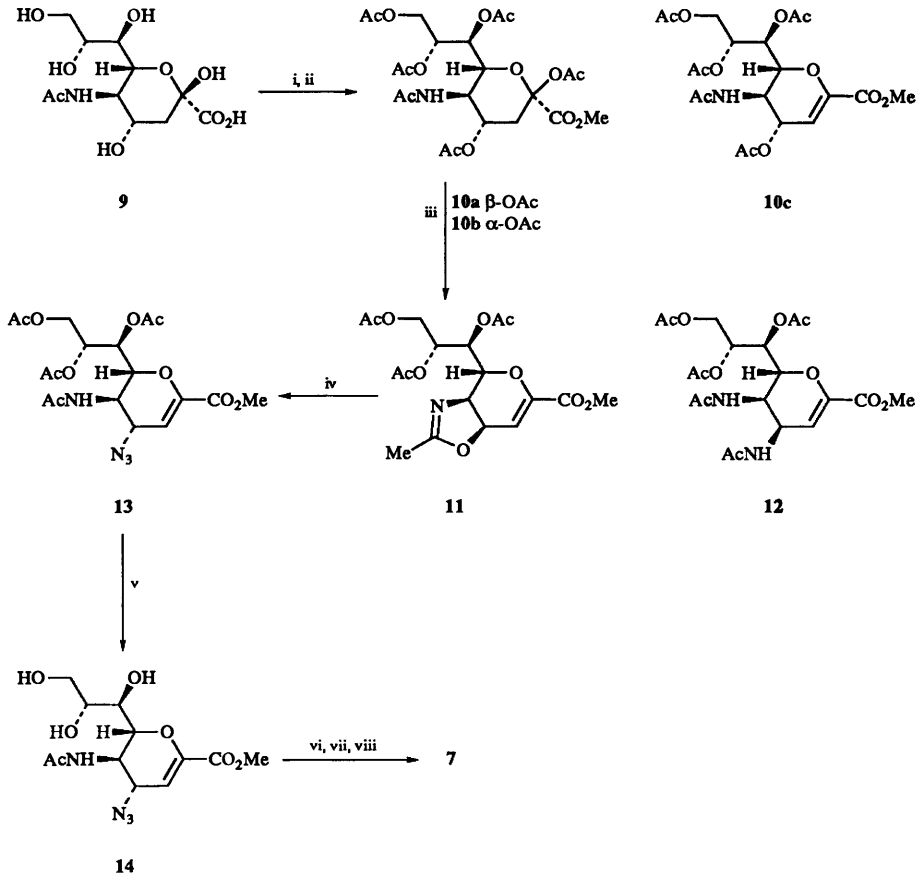
A number of compounds directed towards a variety of molecular targets have been identified as potential leads in the search for such an agent. These include a number of nucleoside analogues, most notably ribavirin **3**[4] and a fluorinated cytidine analogue **4**.[5] A thiadiazole derivative LY217896 **5**[6] has also received considerable attention. Definitive information on the mechanism(s) of action is not available for any of these compounds but they appear to target intracellular events. None of these compounds has reached the market as a treatment for influenza, although ribavirin is licensed for the treatment of respiratory syncytial virus infection in hospitalized infants.

The two surface glycoproteins of influenza virus, haemagglutinin (HA) and neuraminidase (NA), also represent potential targets for antiviral action.[7] A variety of compounds have been



reported to inhibit the action of influenza virus NA and of these the most interesting and most extensively studied have been 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid† **6** and the corresponding *N*-trifluoroacetyl analogue.[8] Although effective against both influenza A and B *in vitro* they showed no selectivity for the viral enzymes over those isolated from other sources[9] and were not effective in animal models when administered systemically.[7b] Recently, the determination of the crystal structures of glycoproteins HA[10] and NA[11] has allowed, in the latter case, for a process of rational design to identify more potent and specific neuraminic acid-based inhibitors which have proved to be effective *in vivo* after intranasal administration.[12] The 4-amino **7** and particularly the 4-guanidino compound **8** are the most exciting anti-influenza compounds to emerge so far. In this and the following papers we describe our contributions to the development of synthetic

† 2,3-Didehydro-2-deoxy-*N*-acetylneuraminic acid is also known as ddNANA and Neu5Ac2en.



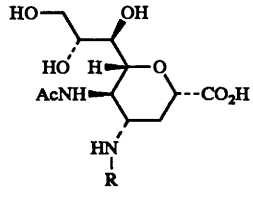
Scheme 1 *Reagents and conditions:* i, MeOH, HCl (gas); ii, Ac_2O, pyridine, 4-(dimethylamino)pyridine; iii, trimethylsilyl trifluoromethanesulfonate, ethyl acetate; iv, trimethylsilyl azide, Bu^{t}OH, 80 °C; v, MeONa, MeOH; vi, Et_3N, water; vii, Lindlar catalyst, H_2, water; viii, Dowex 2 × 8 resin

routes to these compounds and a variety of structural analogues.

Results and discussion

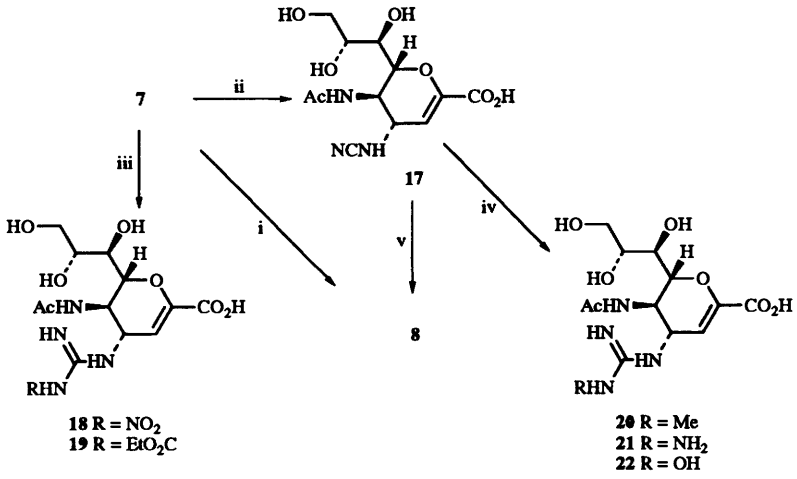
The most direct approach to the synthesis of Neu5Ac2en analogues is from the commercially available *N*-acetylneuraminic acid **9**. Synthesis of compounds **7** and **8** therefore requires introduction of the 2,3 double bond and in particular a stereospecific introduction of a nitrogen-based C-4 substituent (Scheme 1). *N*-Acetylneuraminic acid **9** was esterified using methanolic HCl to give the methyl ester in high yield. Alternatively, Dowex 50W × 8 resin in methanol, as has been previously reported,[13] could be used. The penta-acetoxy compound **10a** could be prepared from this by using an excess of acetic anhydride at 50 °C with perchloric acid catalysis. The crude product thus obtained appeared by NMR spectroscopy to be a mixture of compound **10a**, its anomeric isomer **10b**, and the unsaturated derivative **10c**. It has also been reported[14] that at lower reaction temperatures the 4,7,8,9-tetra-acetoxy derivative having a free anomeric hydroxy group is also produced. We found it was possible to avoid these problems and to obtain pure compound **10a** by using acetic anhydride in pyridine with 4-(dimethylamino)pyridine catalysis. Preparation of the oxazoline **11** was then achieved by using a modification of the procedure described by Zbiral and co-workers.[15] Hence, treatment of compound **10a** with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in ethyl acetate at 52 °C gave the oxazoline **11** in high yield. When acetonitrile was used as the solvent we found that the oxazoline **11** was contaminated with ~ 10% of the corresponding Ritter product **12**.

While a number of methods for the preparation of 4-azidoneuraminic acids have been reported[15,16] we found that reaction of the oxazoline **11** with trimethylsilyl azide in *tert*-butyl alcohol at 80 °C was superior, giving the azido compound **13** stereoselectivity. Liberation of the 4-amino function required the selective reduction of the azido group in the presence of the double bond. This was achieved by first gaining water solubility by removal of the acetate protecting groups with catalytic sodium methoxide in methanol to afford triol **14**, followed by hydrolysis of the ester by using triethylamine in water. Hydrogenolysis with a Lindlar catalyst then gave the triethylamine salt of free amine **7** which was desalted using Dowex 2 × 8 ion-exchange resin to give the free amino acid **7**. When palladium on carbon was used as the catalyst we found that over-reduction occurred, to give the fully saturated compound **15**. The carboxylic acid residue of compound **15** was shown to have an equatorial orientation by X-ray spectroscopy (Fig. 1).



15 R = H
16 R = –C(=NH)NH$_2$

Amino acid **7** was efficiently converted (Scheme 2) into the 4-guanidino compound **8** by reaction with aminoiminomethanesulfonic acid (AIMSA),[17] the latter synthesized according to the method of Miller and Bischoff.[18] Treatment of compound



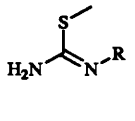
Scheme 2 *Reagents:* i, (a) AIMSA (3.5 mol equiv.), NaOH (1 mol equiv.), K_2CO_3 (3.5 mol equiv.), water; or (b) **25** (3.3 mol equiv.), Et_3N (3.3 mol equiv.), MeOH; ii, CNBr (1.1 mol equiv.), NaOAc (2.2 mol equiv.), MeOH; iii, R = NO_2: **23** (3.3 mol equiv.), Et_3N (3.3 mol equiv.), MeOH; R = EtO_2C: **24** (4 mol equiv.), Et_3N (4 mol equiv.), MeOH; iv, R = Me: $MeNH_2$ (10 mol equiv.), EtOH; R = NH_2: NH_2NH_2 (10 mol equiv.), MeOH; R = OH: NH_2OH (10 mol equiv.), MeOH; v, NH_4OCHO (6 mol equiv.), NH_4OH (xs)

7 with 3 mol equiv. each of AIMSA and potassium carbonate in a portionwise manner over a period of 8 h allowed isolation in reasonable yield (48%) of the crystalline guanidine **8** following ion-exchange chromatography. In a similar manner reaction of the saturated derivative **15** with AIMSA gave the corresponding compound **16**. In order to explore the optimal nature of the substituent at C-4, a series of substituted 4-guanidino compounds were also synthesized. In the course of this work insight was gained into alternative methods of synthesis of the parent 4-guanidino compound.

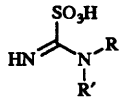
In the synthesis of analogues of compound **8** with modified guanidino functionality at C-4, two main approaches were taken (Scheme 2). In the first, 4-amino compound **7** was used as starting material, whilst the second required synthesis of the intermediate cyanamide derivative **17**. The nitro- and ethoxycarbonyl-substituted guanidines **18** and **19**, respectively, were obtained from compound **7** by use of the appropriate methylisothioureas. Thus, reaction of compound **7** with *S*-methyl-N^3-nitroisothiourea **23**[19] or N^3-ethoxycarbonyl-*S*-methylisothiourea **24**[20] in the presence of triethylamine in water afforded the desired compounds **18** and **19**, respectively, following ion-exchange or silica gel chromatography.

Interestingly, reaction of compound **7** with N^3-acetyl-*S*-methylisothiourea **25**[21] under similar conditions gave only the parent guanidine compound **8** in 27% yield following crystallization. This was presumably formed due to the instability of the *N*-acetyl derivative. (This is consistent with the failure to effect direct acetylation of compound **7**.) Treatment of compound **7** with N^3-cyano- **26**[22] or N^3-methyl- **27**[23] isothiourea resulted in no observed reaction; it is apparent that only the most reactive isothioureas, N-substituted with electron-withdrawing groups, are able to react with amino acid **7**. Use of the more reactive sulfonic acid derivatives **28** and **29** also proved to be unsuccessful.

The range of accessible, substituted guanidine analogues of



23 R = NO_2
24 R = CO_2Et
25 R = Ac
26 R = CN
27 R = Me



28 R = Me, R′ = H
29 R = R′ = Me

Table 1 Flu NA, and Flu A and B plaque-reducing activities of 4-(substituted guanidino) derivatives of Neu5Ac2en compared to the parent 4-amino- and 4-guanidino-Neu5Ac2en (assays were conducted as previously described)[12]

Compound	IC_{50} NA (μmol dm^{-3})	IC_{50} FluA Pl (μg cm^{-3})	IC_{50} FluB Pl (μg cm^{-3})
7	0.32	1.5	0.065
8	0.005	0.023	0.005
18 (N^3-nitro)	2.7	30.0	24.0
19 (N^3-ethoxycarbonyl)	15.0	1.7	0.6
20 (N^3-methyl)	12.0	3.0	ND[a]
21 (N^3-amino)	3.7	2.9	3.0
22 (N^3-hydroxy)	1.6	0.14	< 0.1

[a] ND = not done.

compound **8** was extended according to the methodology shown in Scheme 2. Cyanamide derivative **17** was obtained by treatment of amino acid **7** with cyanogen bromide (1.1 mol equiv.) in the presence of sodium acetate in methanol. A simple selective precipitation procedure was sufficient to free the material from the bulk of impurities, and the somewhat moisture sensitive material was used directly.

Reaction of the cyanamide **17** with methylamine, hydrazine or hydroxylamine in methanol gave the corresponding *N*-methyl-, *N*-amino- or *N*-hydroxy-guanidino derivatives **20**, **21** or **22**, respectively; the products precipitated out from the reaction mixture and were easily filtered off. Use of cyanamide, a less potent nucleophile, gave none of the desired *N*-cyanoguanidino derivative.

The parent guanidino derivative **8** was also accessible through cyanamido intermediate **17**. Treatment of compound **17** with ammonium hydroxide and ammonium formate at 85 °C followed by purification of the product by ion-exchange chromatography and crystallization gave compound **8** in 36% yield.

The data for inhibition of influenza neuraminidase by the substituted guanidino derivatives compared with those of the parent guanidino and amino derivatives are shown in Table 1.

None of the compounds is as effective as the parent guanidine **8** either in NA enzyme inhibition or in Flu A or B inhibition in whole cell assays. This observation is in general agreement with data for substituted 4-amino derivatives.[24] The reduced activities of *N*-nitro- **18** and *N*-ethoxycarbonyl- **19** derivatives may be explained by a reduction in pK_a by the strongly electron-

withdrawing nitro and ethoxycarbonyl groups. All groups investigated will have some steric demand as well as the facility to disrupt what is likely to be an extensive H-bonding network between compound **8** and the enzyme active site.

Within the series of substituted guanidino analogues presented here the more lipophilic N^3-ethoxycarbonyl- and N^3-methyl-guanidino derivatives **19** and **20**, respectively, are the least enzyme inhibitory. Interestingly, in the whole cell assay, the N^3-ethoxycarbonyl derivative is one of the best of the substituted guanidine derivatives at inhibiting influenza. The most active of the substituted-guanidine series is the N^3-hydroxy derivative which is as good as 4-aminoNeu5Ac2en **7** in both enzyme and whole cell inhibitory activity. Of the substituent groups examined, the hydroxy group is likely to disrupt the properties of the parent guanidine functionality to the smallest degree.

Experimental

Mps were determined using a Mettler FP51 automatic melting point apparatus and are expressed as M^xy 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. IR spectra were obtained using a Nicolet 5SXC FT-IR spectrometer. Optical rotations were measured using apparatus supplied by Optical Activity Ltd., England; $[\alpha]_D$-units are 10^{-1} deg cm^2 g^{-1}. Preparative silica column chromatography was run on Merck 9385 silica under flash conditions. GLC analysis was on a column derivatized to 5% with methylphenyl silicone (temperature: 50–325 °C. Gradient: 10 °C min^{-1}. Carrier: helium gas). HPLC analysis: Column 1: S5-ODS2. Eluent acetonitrile in water, flow 2.0 cm^3 min^{-1}; detection UV, 210 nm, temp.: ambient. Column 2: Hypersil SAS. Eluent 10% acetonitrile–0.05 mol dm^{-3} $NH_4H_2PO_4$, flow 1.0 cm^3 min^{-1}; detection UV, 235 nm. Column 3: Dynamax C_{18} + guard. Eluent 5% acetonitrile + 0.1% trifluoroacetic acid (TFA)–water + 0.1% TFA; flow 1 cm^{-3} min^{-1}; detection UV, 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. CZE (capillary electrophoresis) analysis on 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). *N*-Acetylneuraminic acid was obtained from the Shin Nippon Yakrugyo Co. Ltd. and was found to contain 2.44% water. Dowex 50W × 8 was purified prior to use by being washed with three bed volumes of water followed by three volumes of methanol. All solvents were of Analar purity and unless otherwise stated were used as supplied without further purification.

5-Acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosonic acid methyl ester

A stirred suspension of *N*-acetylneuraminic acid (250.0 g) in methanol (3.5 dm^3) was treated with a 2.2 mol dm^{-3} solution of hydrochloric acid in methanol (36.8 cm^3, 80.8 mmol). The resulting mixture was stirred at 50 °C for 2.5 h. The resulting clear solution was allowed to cool and was then concentrated under reduced pressure at 48–50 °C (rotary evaporator) to ~1.5 dm^3. Ethyl acetate (2 dm^3) was then added and the mixture was reconcentrated, during which crystallization occurred to give a thick suspension. When the volume was ~1.5 dm^3 additional ethyl acetate was added (1 dm^3) and the mixture was again concentrated to ~1.5 dm^3; this process was repeated once more and the resulting suspension was diluted with ethyl acetate (1 dm^3) and cooled in an ice–water-bath for 1.5 h. Filtration and washing of the filter with ethyl acetate (3 × 500 cm^3) then gave the *title compound*, which was dried *in vacuo* at 21 °C to give a solid (245.12 g, 93.8%) [single spot by TLC, R_f 0.3 on silica gel butan-1-ol–acetic acid–water (3:1:1), visualized with cerum(IV) sulfate spray], mp 174.2 °C; $[\alpha]_D^{22}$ −31.73 (*c* 0.01, MeSO); $\delta_H(D_2O)$ 3.96 (2 H, m, 4- and 6-H), 3.84 (1 H, m, 5-H), 3.75 (3 H, s, CO_2Me), 3.73 (1 H, m, 9-H^a), 3.64 (1 H, m, 8-H), 3.53 (1 H, dd, *J* 12 and 6, 9-H^b), 3.46 (1 H, d, *J* 10, 7-H), 2.22 (1 H, dd, *J* 13 and 5, 3-H^a), 1.99 (3 H, s, NAc) and 1.83 (1 H, dd, *J* 13 and 12, 3-H^b); ν_{max}(KBr)/cm^{-1} 3495, 3439, 3335, 3279, 1741, 1628 and 1551 [Found: (M + 1), 324.129669. $C_{12}H_{22}NO_9$ requires *m/z*, 324.129 457] (Found: C, 42.0; H, 6.75; N, 4.1. $C_{12}H_{21}NO_9 \cdot H_2O$ requires C, 42.23; H, 6.79; N, 4.10%).

Methyl (3aR,4R,7aR)-2-Methyl-4-[(1'S,2'R)-1',2',3'-triacetoxypropyl]-3a,7a-dihydro-4H-pyrano [3,4-d]oxazole-6-carboxylate 11

A suspension of 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonic acid methyl ester (43.53 g, 134.64 mmol) in pyridine (218 cm^3) was treated with DMAP (435 mg, 3.56 mmol). The resulting mixture was cooled in an ice–water-bath while acetic anhydride (127 cm^3) was added dropwise over a period of 15 min. After being stirred for 18 h at 21 °C the mixture was concentrated under reduced pressure at ~4 mmHg (oil-pump) to give an orange oil. This was dissolved in ethyl acetate (220 cm^3) and washed sequentially with 2 mol dm^{-3} hydrochloric acid (2 × 88 cm^3), saturated aq. sodium hydrogen carbonate (3 × 88 cm^3), and finally brine (88 cm^3). The organic layer was then dried ($MgSO_4$), and evaporated under reduced pressure at 48–50 °C (rotary evaporator) to give a straw coloured syrup (72.64 g). This was redissolved in warm ethyl acetate (360 cm^3) and the solution was then cooled to 30 °C while TMSOTf (76 cm^3, 0.39 mol) was then added dropwise during 10 min with stirring (magnetic stirrer) of the mixture under an inert atmosphere of nitrogen. After the addition was complete the temperature was raised to 52 °C over a period of 20 min. After 2.5 h at this temperature the reaction mixture was allowed to cool and was poured into a vigorously stirred mixture of ice-cold saturated aq. sodium hydrogen carbonate (360 cm^3) and solid sodium hydrogen carbonate (100 g). Owing to the acid lability of the oxazoline care was taken to ensure the solution remained basic (pH > 7.5 as measured by universal indicator paper). After *ca.* 10 min the solution was filtered and the aqueous phase was separated, and extracted with ethyl acetate (2 × 180 cm^3). The combined organic layers were concentrated to approximately half the original volume, when a precipitate was removed and discarded by filtration. The filtrate was then evaporated to leave an amber gum. This was dissolved in hot propan-2-ol (50 cm^3) which, on cooling in an ice–water-bath, deposited crystals. The mixture was filtered and the filter was washed with a mixture of diisopropyl ether and propan-2-ol (2:1; 2 × 50 cm^3) to give the title compound **11** after being dried *in vacuo* at 40 °C (34.4 g, 61.7%) (single spot by TLC, R_f 0.55 on silica gel with ethyl acetate, visualize with UV and cerium sulfate spray), mp 92.1 °C; $[\alpha]_D^{22}$ −10.63 (*c* 0.005, Me_2SO); $\delta_H(CDCl_3)$ 6.35 (1 H, d, *J* 4, 7-H), 5.63 (1 H, dd, *J* 6 and 2.5, 1'-H), 5.45 (1 H, ABX system, *J* 6, 6 and 2.5, 2'-H), 4.82 (1 H, dd, *J* 8 and 4, 7a-H), 4.60 (1 H, dd, *J* 12 and 2.5, 3'-H^a), 4.20 (1 H, dd, *J* 12 and 6, 3'-H^b), 3.95 (1 H, dd, *J* 10 and 8, 3a-H), 3.80 (3 H, s, CO_2Me), 3.43 (1 H, dd, *J* 10 and 2.5, 4-H) and 2.16–2.0 (12 H, each s, 3 × OAc and oxazoline Me); ν_{max}(KBr)/cm^{-1} 2987, 1745 and 1665 [Found: (M + 1)$^+$, 414.139 557. $C_{18}H_{24}NO_{10}$ requires *m/z*, 414.140 021] (Found: C, 52.0; H, 5.6; N, 3.5. $C_{18}H_{23}NO_{10}$ requires C, 52.30; H, 5.61; N, 3.39%).

4,5-Diacetamido-7,8,9-tri-O-acetyl-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonic acid methyl ester 12

δ_H(400 MHz; $CDCl_3$) 6.22 and 6.21 (each 1 H, each s, each NH),

6.04 (1 H, m, 3-H), 5.47 (1 H, t, 7-H), 5.28 (1 H, m, 8-H), 4.79 (1 H, m, 4-H), 4.74 (1 H, m, 9-H^a), 4.41 (1 H, m, 5-H), 4.21 (1 H, m, 6-H), 4.19 (1 H, d, 9-H^b), 3.80 (3 H, s, Me), 2.01–2.12 (12 H, each s, each Me) and 1.92 (3 H, s, Me) [Found: $(M + 1)^+$, 473.177 696. $C_{20}H_{29}N_2O_{11}$ requires *m/z*, 473.177 135].

5-Acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid methyl ester 13

A stirred solution of the oxazoline **11** (600 g, 1.45 mol) in *tert*-butyl alcohol (4.5 dm^3) containing azidotrimethylsilane (289 cm^3, 2.18 mol) under nitrogen, was heated to reflux on a steam-bath. A hot-water condenser was used to prevent any possible condensation of hydrazoic acid. After 10.5 h the reaction mixture was allowed to cool overnight. Aq. sodium nitrite (120 g in 600 cm^3) was then added. 6 Mol dm^{-3} hydrochloric acid was then added dropwise over a period of 1 h to give vigorous evolution of gases. Ethyl acetate (3 dm^3) and water (3 dm^3) were then added and the organic layer was separated off and washed with water (2 × 5 dm^3). The combined aqueous layers were back-extracted with ethyl acetate (1 dm^3) and the combined organic layers were washed successively with 6% aq. sodium hydrogen carbonate (2 × 3 dm^3) followed by brine (3 dm^3). Aqueous residues were then destroyed according to the published procedure.[25] The combined organic extracts were then dried ($MgSO_4$), and evaporated under reduced pressure at 48–50 °C (rotary evaporator) to give an oil. This was dissolved in propan-1-ol (600 cm^3) and treated dropwise with water (2 dm^3) added over a period of 1 h. The resulting crystalline solid was filtered off, and washed with water (2 × 700 cm^3) to give the *title compound* **13** after being dried *in vacuo* at 42 °C for 24 h (523 g, 76%) [single spot by TLC R_f 0.34 on silica gel with acetone–chloroform (1:3), visualize with UV and cerium(IV) sulfate spray], mp 93.6 °C; $[\alpha]_D^{22}$ +59.55 (*c* 0.01, Me_2SO); $\delta_H[(CD_3)_2SO]$ 8.15 (1 H, d, *J* 9, NH), 5.85 (1 H, d, *J* 2.5, 3-H), 5.35 (1 H, dd, *J* 6 and 2, 7-H), 5.25 (1 H, ddd, *J* 6.5, 6 and 3, 8-H), 4.46 (1 H, dd, *J* 12 and 3, 9-H^a), 4.35 (1 H, dd, *J* 10.5 and 2, 6-H), 4.30 (1 H, dd, *J* 9 and 2.5, 4-H), 4.09 (1 H, dd, *J* 12 and 6.5, 9-H^b), 4.05 (1 H, ddd, *J* 10.5, 9 and 9, 5-H), 3.75 (3 H, s, CO_2Me), 2.02 (9 H, each s, 3 × OAc) and 1.8 (3 H, s, NAc); ν_{max}(KBr)/cm^{-1} 2107, 1753, 1732 and 1661 (Found: C, 45.7; H, 5.5; N, 11.9. $C_{18}H_{24}N_4O_{10}\cdot H_2O$ requires C, 45.57; H, 5.52; N, 11.81%).

5-Acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid methyl ester 14

To a stirred solution of triacetate **13** (114.72 g, 251.5 mmol) in anhydrous methanol (350 cm^3) under nitrogen was added solid sodium methoxide (683 mg, 12.6 mmol) and the resulting mixture was stirred at 21 °C for 2.5 h. Dowex 50 × 8 (H^+) resin (~23 g) was then added to adjust the pH to 7 as indicated by universal indicator paper and solvent was removed by filtration. The resin was further washed with methanol (3 × 55 cm^3) and the combined filtrates were concentrated under reduced pressure (rotary evaporator) at 48–50 °C until crystallisation occurred. Ethyl acetate was then added (375 cm^3) and the thick suspension was stirred at 21 °C for 2 h. The mixture was then filtered and the filter was washed with ethyl acetate (2 × 230 cm^3) to give the *title compound* **14** (59.16 g, 71.2%) after drying *in vacuo* [single spot by TLC, R_f 0.025, on silica gel with MeOH–$CHCl_3$ (1:19), visualize with UV and cerium(IV) sulfate], mp 157.9 °C; $[\alpha]_D^{22}$ +62.26 (*c* 0.007, Me_2SO); $\delta_H[(CD_3)_2SO]$ 8.35 (1 H, d, *J* 9, NH), 5.85 (1 H, d, *J* 2.5, 3-H), 4.64 (1 H, d, *J* 5, OH), 4.60 (1 H, d, *J* 6, OH), 4.50 (1 H, dd, *J* 9 and 2.5, 4-H), 4.34 (1 H, t, *J* 6, OH), 4.15 (1 H, d, *J* 11, 6-H), 3.95 (1 H, ddd, *J* 11, 9 and 9, 5-H), 3.75 (3 H, s, CO_2Me), 3.65 (2 H, m, 8-H and 9-H^a), 3.44 (2 H, m, 7-H and 9-H^b) and 1.95 (3 H, s, NAc); ν_{max}(KBr)/cm^{-1} 3260, 3183, 3079 and 2918 (Found C, 42.9; H, 5.4; N, 16.55. $C_{12}H_{18}N_4O_7\cdot 0.67H_2O$ requires C, 42.86; H, 5.59; N, 16.55%).

5-Acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid 7

The ester **14** (35 g, 0.1 mol) was dissolved in water (250 cm^3) and treated with triethylamine (65 cm^3). The resulting mixture was stirred under nitrogen at 21 °C. After 7 h, TLC [ethyl acetate–propan-2-ol–water (5:2:1)] showed no starting material. The reaction mixture was then evaporated under reduced pressure to give a brown syrup [single spot by TLC, R_f 0.06, with ethyl acetate–propan-2-ol–water (5:2:1)]. This was transferred to a 0.5 dm^3 3-necked, round-bottomed flask containing water (200 cm^3) and equipped with a gas inlet tube extending below the level of liquid, and a gas outlet adapter. Lindlar catalyst (7 g) was then added and the flask was flushed with nitrogen. Hydrogen gas was then bubbled through the vigorously stirred solution for *ca.* 21 h. The reaction mixture was then filtered through Celite, and the filter-bed was washed with water (190 cm^3). The combined filtrates were then evaporated under reduced pressure at 48–50 °C (rotary evaporator) to give the triethylamine salt of acid **7**. This was desalted on a column (5.5 cm diam.) of Dowex 2 × 8 (Cl^-) resin (350 g) [washed with water (2 dm^3), and converted into its hydroxide form with 2 mol dm^{-3} aq. sodium hydroxide (5.25 dm^3), followed by a final wash with water until the eluent was neutral]. After addition of the above salt in water (~800 cm^3) over a period of 1.5 h the column was eluted with water (1.4 dm^3), followed by 1 mol dm^{-3} aq. acetic acid (5 dm^3). Like fractions were combined and treated with Norit Ultra SX+ charcoal. After 0.5 h the solution was filtered and the filtrate was freeze-dried to give a foam. This was crystallized from aq. propan-2-ol to give *title compound* **7** (16.1 g, 55.5% overall yield from **14**) [single spot on TLC, R_f 0.16, with silica gel, butan-1-ol–water–acetic acid (3:1:1)]; $[\alpha]_D^{22}$ 18.04 (*c* 0.01, Me_2SO); $\delta_H(D_2O)$ 5.60 (1 H, d, *J* 2, 3-H), 4.25 (2 H, m, 5- and 6-H), 4.04 (1 H, m, 4-H), 3.85 (1 H, m, 8-H), 3.80 (1 H, dd, *J* 12 and 3, 9-H^a), 3.58 (1 H, m, 9-H^b), 3.58 (1 H, m, 7-H) and 2.1 (3 H, s, NAc); ν_{max}(KBr)/cm^{-1} 3268, 3038, 2886 and 1582 (Found: C, 40.4; H, 6.8; N, 8.5. $C_{11}H_{18}N_2O_7\cdot 2H_2O$ requires C, 40.49; H, 6.80; N, 8.59%).

5-Acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*erythro*-L-*gluco*-nononic acid 15

A 250 cm^3 3-necked, round-bottomed flask, fitted with gas inlet and outlet tubes, was charged with a solution of azide **14** (2 g, 6.84 mmol) in distilled water (90 cm^3) followed by 10% palladium on carbon catalyst (0.24 g). The system was purged with nitrogen gas prior to the addition of a slow stream of hydrogen gas. After 24 h the system was purged again with nitrogen and the reaction mixture was filtered through a plug of Celite. The filtrate was then evaporated under reduced pressure at a water-bath temperature of 48 °C to give a foam, which crystallized on storage. Recrystallization from water–propan-2-ol (40: 90 cm^3) gave the title compound **15** as a solid (0.95 g, 47.5%), δ_H(400 MHz; D_2O) 4.05 (2 H, m, 2- and 5-H), 3.80 (2 H, m, 7- and 9-H^a), 3.65 (1 H, m, 6-H), 3.60–3.50 (2 H, m, 4- and 8-H), 3.45 (1 H, m, 9-H^b), 2.40 (1 H, m, 3-H^a), 2.00 (3 H, s, NAc) and 1.70 (1 H, m, 3-H^b) (Found: C, 38.9; H, 7.5; N, 8.25. $C_{11}H_{20}N_2O_7\cdot 3H_2O$ requires C, 38.15; H, 7.57; N, 8.09%).

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*galacto*-non-2-enonic acid 8

(i) From amine 7 using AIMSA. Compound **7** (2.5 g, 8.0 mmol) was dissolved in water (33.5 cm^3) and the stirred solution was treated dropwise with aq. sodium hydroxide (8 cm^3 of a 1 mol dm^{-3} solution). This mixture was then warmed to 40 °C and stirred while potassium carbonate (245 mg) followed by AIMSA (215 mg) were added. Subsequent additions were made

every 30 min to a total of 16 additions [total quantities: K_2CO_3 (3.9 g, 28.1 mmol); AIMSA (3.5 g, 27.9 mmol)]. The turbid mixture was then stirred at 20 °C for 16 h before being diluted with water (42 cm^3) and filtered. The filtrate was applied to a column of Dowex 50W × 8 (H^+) resin (850 cm^3) and washed with water (6 dm^3). The column was then eluted with 0.6 mol dm^{-3} aq. triethylamine and the fractions containing the UV-active species were combined, evaporated under reduced pressure, and coevaporated with water (5 × 85 cm^3). The residue was taken up in water (46 cm^3) warmed to 50 °C and the mixture was filtered. The filtrate was diluted with propan-2-ol (112 cm^3) and warmed with swirling until crystallization began. The crystal was then stirred at 20 °C for 16 h, and the white solid was filtered off to give the title compound **8** (1.28 g, 48%), mp >240 °C (decomp.); $[\alpha]_D^{20}$ +41.0 (*c* 0.9, water); $\delta_H(D_2O)$ 5.53 (1 H, d, 3-H), 4.50–4.38 (2 H, 2 dd, 4- and 6-H), 4.21 (1 H, dd, 5-H), 4.00–3.88 (2 H, dd + ddd, 9-H^a and 8-H), 3.70–3.62 (2 H, 2 dd, 9-H^b and 7-H) 2.05 (3 H, s, Ac); $\delta_C(D_2O)$ 177.3 (C=O, Ac), 172.1 (C-1), 159.9 (guanidine), 152.1 (C-2), 106.8, (C-3), 78.3 (C-6), 72.6 (C-8), 71.0 (C-7), 65.9 (C-9), 54.0 (C-4), 50.6 (C-5) and 24.8 (Me); $\nu_{max}(Me_2SO)/cm^{-1}$ 3332, 1676, 1600, 1560, 1394, 1322 and 1281; *m/z* 333 $(MH)^+$; λ_{max}(water)/nm 235 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 199) (Found: C, 41.0; H, 5.7; N, 16.0. $C_{12}H_{20}N_4O_7 \cdot 0.9H_2O$ requires C, 41.35; H, 6.30; N, 16.08%); CZE 96.9% pure.

(ii) From amine 7 using N^3-acetyl-*S*-methylisothiourea 25. Compound **7** (201 mg, 0.69 mmol) as a solution in methanol (2.1 cm^3) was treated with triethylamine (0.196 cm^3, 1.38 mmol) followed by compound **25** (197 mg, 0.76 mmol) and the mixture was stirred at 50 °C for 4 h, then at 21 °C for 16 h. This process was repeated twice more with 1.1 mol equiv. each of triethylamine and reagent **25**, and then the whole was evaporated under reduced pressure and purified as above to give the title compound **8** as a crystalline solid (35 mg, 27%).

(iii) From nitrile 17 (see below). Cyanamide derivative **17** (1.0 g, 3.17 mmol) and ammonium formate (1.17 g, 18.6 mmol) were dissolved in 0.880 s.g. ammonium hydroxide (20 cm^3) and the mixture was heated at 90 °C for 4.25 h. After cooling, the mixture was diluted to 50 cm^3 with water and purified as above to give the title compound **8** as a crystalline solid (0.223 g, 36%).

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*erythro*-L-gluco-nononic acid 16

A solution of compound **15** (107 mg, 0.32 mmol) in water (4.3 cm^3) was treated with a slow stream of hydrogen gas. After 3 h the reaction mixture was purged of hydrogen, diluted with water (10 cm^3), and filtered through Celite. The filter was further washed with water (10 cm^3) and the combined filtrates were evaporated under reduced pressure at a water-bath temperature lower than 50 °C to give the *title compound* **16** as a foam (100 mg, 93%), mp >230 °C (decomp.); $[\alpha]_D^{20}$ +0.0135 (*c* 0.53, water); $\delta_H(D_2O)$ 1.65 (1 H, ddd, *J* 12.5, 12.5 and 12.5, 3-H^a), 2.40 (1 H, m, 3-H^b), 3.50–4.10 (8 H, m, 2-, 4-, 5-, 6-, 7- and 8-H and 9-H_2); ν_{max}(Nujol)/cm^{-1} 3312, 2953, 2924, 2853, 1647 and 1591 (Found: C, 37.6; H, 6.8; N, 14.35. $C_{12}H_{22}N_4O_7 \cdot 2.8H_2O$ requires C, 37.45; H, 7.23; N, 14.56%).

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-(N^3-nitroguanidino)-D-*glycero*-D-*galacto*-non-2-enonic acid 18

Compound **7** (200 mg, 0.689 mmol) was dissolved in absolute methanol (2.7 cm^3) by the addition of triethylamine (0.098 cm^3, 0.69 mmol). *S*-Methyl-N^3-nitroisothiourea **23**[7] (103 mg, 0.757 mmol) was then added. The stirred mixture was warmed to 40 °C under nitrogen for 2 h, then was stirred at 21 °C for 16 h. The addition of reagent, with stirring and heating, was repeated twice more. The mixture was then filtered and the solid was washed with methanol. Combined filtrate and washings were evaporated and the residue was purified by ion-exchange chromatography [Dowex 50W × 8 (H^+) resin, and elution with water]. Appropriate fractions were combined and freeze-dried. The residue was triturated with warm water (4 cm^3) and the *off-white solid* **18** was filtered off, and dried under high vacuum (50 mg, 19%), mp >230 °C (decomp.); δ_H 5.95 (1 H, d, *J* 2, 3-H), 4.80 (1 H, m, 4-H), 4.45 (1 H, d, *J* 10, 6-H), 4.25 (1 H, t, *J* 10, 5-H), 3.85–4.00 (2 H, m, 9-H^a and 8-H), 3.60–3.75 (2 H, m, 9-H^b and 7-H) and 2.00 (3 H, s, Ac); $\nu_{max}(Me_2SO)/cm^{-1}$ 1260 (NO_2); λ_{max}(water)/nm 272 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 405) (Found: C, 34.8; H, 5.1; N, 17.0. $C_{12}H_{19}N_5O_9 \cdot 2H_2O$ requires C, 34.86; H, 5.61; N, 16.95%); CZE, >91% pure.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-[N^3-(ethoxycarbonyl)guanidino]-D-*glycero*-D-*galacto*-non-2-enonic acid 19

Compound **7** (200 mg, 0.69 mmol) was dissolved in methanol (2.7 cm^3) by the addition of triethylamine (0.098 cm^3, 0.69 mmol). To this was added N^3-ethoxycarbonyl-*S*-methylisothiourea **24** (112 mg, 0.69 mmol) and the resulting solution was heated at 50 °C for 5 h and was then stirred at 21 °C for 16 h. This process was repeated three more times and the mixture was stirred at 50 °C for 3 days. The whole was then evaporated to dryness and the residue was redissolved in methanol (4 cm^3) and purified by preparative TLC [butan-1-ol–acetic acid–water (3:1:1)]. Appropriate fractions (R_f 0.3) were removed from silica by being stirred in methanol. The suspension was filtered to remove silica, then was evaporated to give a gum. This was triturated with ethyl acetate, and the mixture was stirred vigorously for 1 h. Filtration and drying under high vacuum gave the title compound **19** as an off-white solid (85 mg, 31%), mp 232 °C (decomp.); $[\alpha]_D^{21}$ +19.3 (*c* 0.40, water); $\delta_H(D_2O)$ 5.62 (1 H, d, *J* 2, 3-H), 4.62 (1 H, m, 4-H), 4.45–4.25 (4-H, m, 5- and 6-H and OCH_2), 3.99–3.87 (2 H, m, 9-H^a and 8-H), 3.69–3.60 (2 H, m, 9-H^b and 7-H), 1.95 (3 H, s, Ac) and 1.28 (3 H, t, *J* 5, CH_3); ν_{max}(Nujol)/cm^{-1} 2953, 2924, 2853, 1581, 1461 and 1377; *m/z* 405 (MH^+) and 427 (MNa^+); λ_{max}(EtOH)/nm 227 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 244); CZE, >92% pure.

5-Acetylamino-2,6-anhydro-4-cyanoamino-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid 17

Compound **7** (3.0 g, 10.35 mmol) was suspended in methanol (37.5 cm^3) and to this was added sodium acetate (1.89 g, 23.1 mmol). Severe caking was observed, and this made stirring difficult. To the mixture was added dropwise at 21 °C, with exclusion of moisture, and slowly over a period of 3.5 h, a solution of cyanogen bromide (1.14 g, 10.8 mmol) in methanol (150 cm^3). Stirring gradually became easier until a readily stirred suspension was obtained. The mixture was then stirred at 21 °C for 44 h. The small amount of remaining solid was filtered off and the solvent was evaporated off to give an orange–brown foam. This was taken up in methanol (125 cm^3) and the solution was stirred rapidly. To this was added propan-2-ol (130 cm^3) dropwise. The resulting precipitate was filtered off, washed with propan-2-ol–methanol (3:2), and the combined filtrate and washings were evaporated to give the title compound **17** as a pale yellow foam (3.48 g, quant.), $\delta_H(D_2O)$ 5.65 (1 H, s, 3-H), 4.30 (1 H, d, *J* 10, 4-H), 4.18–3.75 (4 H, m, 5-, 6-, 7- and 8-H), 3.65 (2 H, m, 9-H) and 2.08 (3 H, s, Ac); ν_{max}(KBr)/cm^{-1} 3300 and 2224 (CN).

General procedure for synthesis of substituted guanidines from compound 17

Compound **17** (500 mg, 1.59 mmol) was dissolved in dry methanol 20 cm^3) and treated with a nucleophile (excess). The solution was then stirred at 21 °C for 18 h. The precipitate was filtered off and purified further as described below.

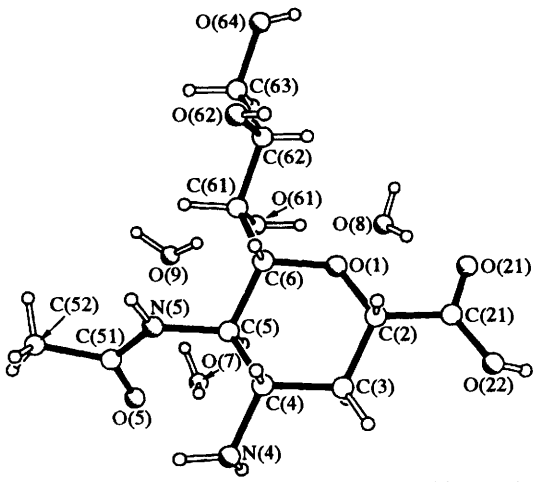


Fig. 1 X-ray molecular structure of 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*erythro*-D-*gluco*-nononic acid

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-(N^3-methylguanidino)-D-*glycero*-D-*galacto*-non-2-enonic acid 20

The precipitate (127 mg, 23%) obtained by treatment of compound **17** with methylamine (1.93 cm^3, 15.85 mmol) of a 33% w/w solution in ethanol) according to the general procedure was recrystallized from a mixture of water (1.4 cm^3)–propan-2-ol (6.9 cm^3) to give, in two crops, the *title compound* **20** as a solid (77 mg, 14%), mp > 180 °C; $\delta_H(D_2O)$ 5.62 (1 H, d, J 2, 3-H), 4.46 (1 H, dd, J_1 10, J_2 2, 4-H), 4.38 (1 H, d, J 10, 6-H), 4.24 (1 H, dd, J_1 10, J_2 10, 5-H), 3.98–3.90 (2 H, m, 7- and 8-H), 3.70–3.60 (2 H, m, 9-H_2), 2.83 (3 H, s, NMe) and 2.01 (3 H, s, Ac); ν_{max}(Nujol)/cm^{-1} 2953, 2923, 2853, 1633, 1463 and 1376; λ_{max}(water)/nm 235 (ε/dm^3 mol^{-1} cm^{-1} 198) (Found: C, 43.7; H, 6.6; N, 15.5. $C_{13}H_{22}N_4O_7 \cdot 0.6H_2O$ requires C, 43.72; H, 6.54; N, 15.69%); CZE 97.7% pure.

5-Acetamido-4-(N^3-aminoquanidino)-2,6-anhydro-3,4-5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid 21

The precipitate obtained by treatment of nitrile **17** with anhydrous hydrazine (0.5 cm^3, 15.9 mmol) according to the general procedure was taken up in water (3.2 cm^3). To this was added propan-2-ol (8.1 cm^3) with swirling and warming. The crystallized material was filtered off, and dried under high vacuum to give the *title compound* **21** (0.127 g, 23%) as a solid, mp > 180 °C; $\delta_H(D_2O)$ 5.62 (1 H, d, J 2, 3-H), 4.47 (1 H, dd, J_1 10, J_2 2, 4-H), 4.39 (1 H, d, J 10, 6-H), 4.25 (1 H, dd, J_1 10, J_2 10, 5-H), 3.99–3.85 (2 H, m, 7- and 8-H), 3.69–3.60 (2 H, m, 9-H_2) and 2.03 (3 H, s, Ac); ν_{max}(Nujol)/cm^{-1} 3234, 2952, 1685, 1667, 1619 and 1571; λ_{max}(water)/nm 234 (ε/dm^3 mol^{-1} cm^{-1} 206) (Found: C, 40.8; H, 5.8; N, 19.8. $C_{12}H_{21}N_5O_7 \cdot 0.2H_2O$ requires C, 41.06; H, 6.15; N, 19.96%); CZE, >97% pure.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-(N^3-hydroxyguanidino)-D-*glycero*-D-*galacto*-non-2-enonic acid 22

A solution of hydroxylamine in methanol was generated by addition of sodium carbonate (0.835 g, 7.9 mmol) to a solution of hydroxylamine hydrochloride (1.1 g, 15.85 mmol) (dried under high vacuum) in anhydrous methanol (20 cm^3). The mixture was stirred for 15 min under nitrogen and the solid was then filtered off. The filtrate was used as the nucleophile solution in the general method to give the title compound **22** without further purification as a solid, $\delta_H(D_2O)$ 5.62 (1 H, d, J 2, 3-H), 4.48 (1 H, dd, J_1 10, J_2 2, 4-H), 4.38 (1 H, d, J 10, 6-H), 4.27 (1 H, dd, J_1 10, J_2 10, 5-H), 3.98–3.86 (2 H, m, 7- and 8-H), 3.70–3.60 (2 H, m, 9-H_2) and 2.02 (3 H, s, Ac); ν_{max}(Nujol)/cm^{-1} 3238, 3088, 3024, 1626, 1554, 1457, 1402, 1376 and 1322; λ_{max}(water)/nm 234 (ε/dm^3 mol^{-1} cm^{-1} 154) (Found: C, 31.6; H, 4.8; N, 12.4; Cl, 12.4. $C_{12}H_{20}N_4O_8 \cdot 1.6NaCl$ requires C, 31.97; H, 4.69; N, 12.43; Cl, 12.58%); CZE 97.9% pure.

X-Ray experimental data for 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*erythro*-L-*gluco*-nononic acid 15

Crystal data. $C_{11}H_{20}N_2O_7 \cdot 3H_2O$, M = 346.33, Orthorhombic, a = 6.661(8), b = 9.365(8), c = 27.08(3) Å, V = 1689(5) $Å^3$ (by least-squares refinement on diffractometer angles for 14 automatically centred reflections, λ = 1.541 78 Å). Space group $P2_12_12_1$ (No. 19), Z = 4, D_c = 1.36 g cm^{-3}, $F(000)$ = 744, μ(Cu-Kα) = 1.00 mm^{-1}. The compound was crystallized from Pr^iOH–water.

Data collection and processing. Three-dimensional, room-temperature (295 K) (X-ray data were collected on a Siemens R3m/V diffractometer with monochromatized Cu-Kα X-radiation $2\theta/\omega$ mode with scan range (ω) 1–14° plus Kα separation and a variable scan speed (1.95–14.65° min^{-1}). 1396 Reflections were measured ($3 < 2\theta < 115°$, min hkl 0, 0, 0; max hkl 8, 11, 30) of which 1372 were unique [$R(\sigma)$ = 0.002, Friedel opposites merged] and 1220 had $L > 3\sigma(L)$. Two control data monitored every 98 reflections showed no appreciable decay during 17.6 h of exposure of the crystal to X-rays.‡

Structure analysis and refinement. Direct methods resulted in the location of all the non-hydrogen atoms. Full-matrix least-squares refinement was employed with anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms were refined in riding mode. Individual weights were applied according to the scheme $w = [\sigma^2(F_o) + 0.0009|F_o|^2]^{-1}$ and refinement converged at R = 0.039, Rw = 0.042, goodness-of-fit = 1.40. Maximum and mean shift/error in final cycle of refinement was 0.082 and 0.002, respectively. The final electron-density-difference synthesis showed no peaks >0.26 or holes < −0.20 e $Å^{-3}$. All computations were carried out using the SHELXTL PLUS (μ-VAX II) system of programs.‡ An X-ray molecular structure of compound **15** is given in Fig. 1.

Acknowledgements

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‡ *Supplementary publication.* Tables of atomic coordinates and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre. See Instructions for Authors (1995), in the January issue.

§ G. M. Sheldrick, SHELXTL PLUS – Release 4.11/V (Copyright 1990 Siemens Analytical X-ray Instr., Inc.).

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