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Synthesis of 5-*epi*Hydantocidin from D-Ribose

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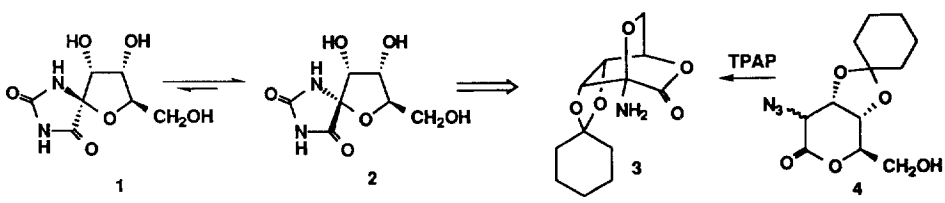
Abstract : Tetra-*n*-propyl ammonium perruthenate in the presence of morpholine-N-oxide induced the unanticipated transformation of the epimeric α-azido-1,4-lactones **4** into the bicyclic amine **3** and provided the key step in the synthesis of 5-*epi*hydantocidin **2**. Brief investigations into the acid catalysed equilibration of hydantocidin **1** and 5-*epi*hydantocidin **2** are described.

This paper reports the synthesis of the C-5 epimer **2** of hydantocidin **1**, *via* the unanticipated transformation of α-azido lactones **4** into the bicyclic amine **3** by treatment with a catalytic amount of tetra-*n*-propyl ammonium perruthenate (TPAP) in the presence of morpholine-N-oxide; the epimer **2** is thermodynamically more stable than the biologically more active natural product **1**, predominating in an equilibrium mixture in a ratio of approximately 4:1. Some aspects of this work, including the crystal structure of the bicyclic amine **3**, have been published in preliminary form. [1]



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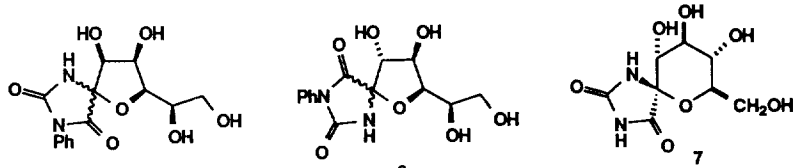

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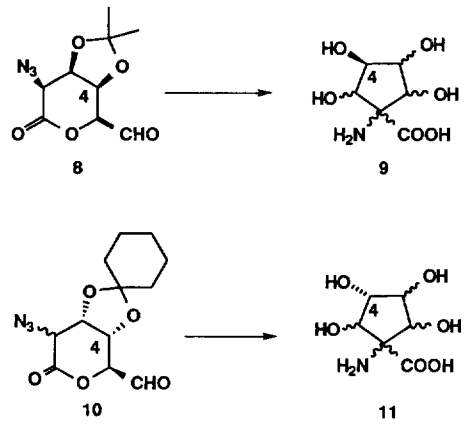
Many novel development products in agrochemistry only require very low application rates per hectare and several of these are based on homochiral natural products.[2] Hydantocidin **1**, first isolated from the fermentation broth of *Streptomyces hygroscopicus* SANK 63584 in 1991,[3,4] shows potent herbicidal activity against perennial plants that is almost equal to that of glyphosate[5] and has essentially no toxicity to micro-organisms or animals.[6] No proposal has yet been published for the mode of action of hydantocidin. The novel structure of this molecule, containing the first example of a spirohydantoin at the anomeric position of a sugar, provided a challenge for the synthesis both of the natural product itself[7] and of analogues. Spirohydantoins of all the diastereomeric pentoses,[8] of some deoxygenated pentoses[9] and of

mannofuranoses **5**[10] and glucofuranoses **6**[11] have been reported. Spirodiketopiperazines[12] and other spiro derivatives of furanoses[13,14] have been described. The potent inhibitor of glycogen phosphorylase **7** provides the first example of a pyranose containing a spirohydantoin,[15] an approach to a galactopyranose[16] analogue of hydantocidin has been indicated.

The azidolactone **8**[17] has proved a versatile intermediate for the controlled synthesis of six diastereomeric tetrahydroxylated cyclopentane α-amino acids **9** by a series of reversible aldol condensations which allow epimerisation of every centre of the original aldehyde **8**, other than at C4.[18] The original objective of the chemistry in this paper was the synthesis of the azidolactones **4** which on oxidation, as separate epimers or as a mixture, should provide the corresponding azidoaldehyde(s) **10** as suitable substrates for aldol cyclisations to give the enantiomeric highly functionalised cyclopentanes **11**. Again C-4 is the only carbon that cannot be epimerised by reversible aldol condensations from intermediates in principal derived from **10**. Accordingly, the azidoalcohols **4** were synthesised from D-ribose in order to develop the methodology for the synthesis of highly functionalised cyclopentanes.[19]



8 **9**

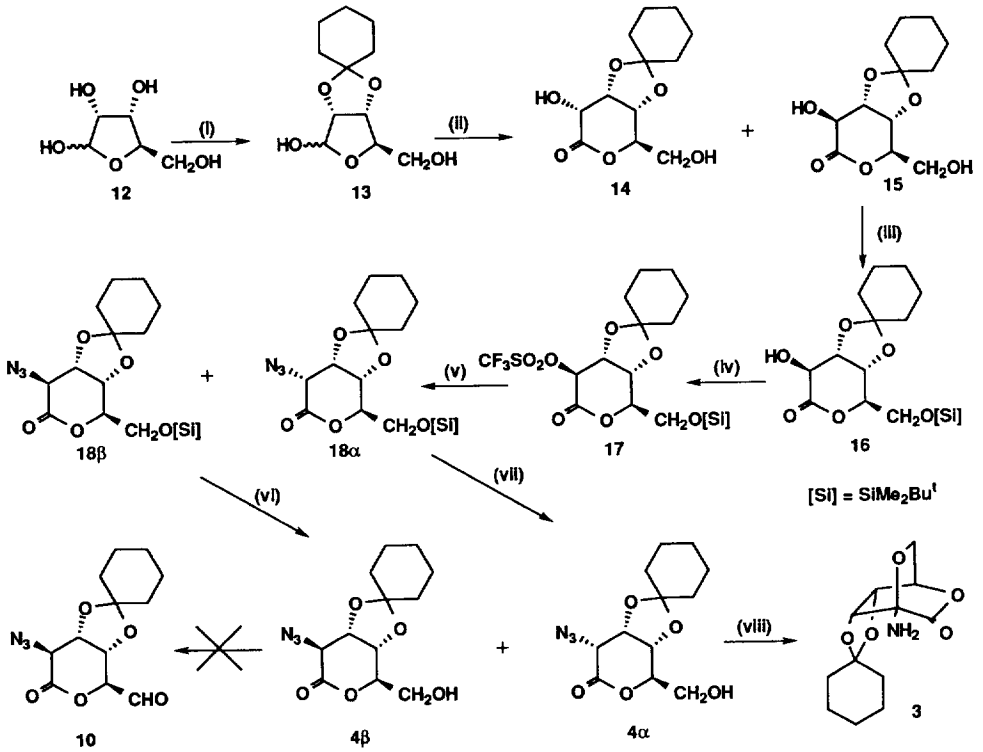
10 **11**

The synthetic route employed [Scheme 1] involved one carbon extension and δ-lactone formation from D-ribose via Kiliani ascension, followed by functional group manipulation and introduction of nitrogen at C-2. It is necessary to protect C-3 of D-ribose **12** so that a δ-lactone will be formed; if the C-4 alcohol of the heptonic acid produced by the Kiliani synthesis is free, a γ-lactone - rather than the required δ-lactone - will be formed. Kiliani reactions on protected sugars usually proceed in lower yields than on unprotected carbohydrates,[20] and the cyanohydrin chain extension on 2,3-isopropylidene ribose, which would produce an isopropylidene derivative corresponding to **10**, only proceeds in very low yield.[21] Accordingly, the cyanohydrin extension was carried out using the cyclohexylidene protecting group which proceeds in rather better yields.

2,3-*O*-Cyclohexylidene ribose[22] **13** was synthesised from D-ribose **12** as a precursor for the Kiliani ascension by treatment of **12** with cyclohexanone and camphor sulfonic acid, in 97% yield. Treatment of **13** with sodium cyanide followed by lactonisation with acetic acid produced the *altrono*lactone **15** as the major reaction product, together with traces of the *allono*lactone **14**. The diastereoselectivity in the Kiliani ascension of the cyclohexylidene derivative of ribose **13** is the same as that in the analogous chain extension of isopropylidene ribose in which the *altrono*-δ-lactone was also the major product.[21]

The major *altrono* product **15** was then selectively silylated at the primary position by treatment with *tert*-butyldimethylsilyl chloride in dimethylformamide at -20°C in the presence of imidazole to yield the silyl lactone **16** in 92% yield; trace amounts of disilylated material were also isolated. Esterification of the remaining free hydroxyl at C-2 in **15** with trifluoromethanesulfonic anhydride and pyridine in dichloromethane, quantitatively yielded the silyl triflate **17**. Introduction of azide at C-2 by treatment of **17** with sodium azide in dimethylformamide produced a mixture of epimeric *altrono* **18**β and *allono* **18**α silyl azides, which were easily separated by chromatography. The configuration of the azide functionality was

established by n.O.e. experiments which revealed enhancements (12% and 14%) between H-2 and H-5 only for the *altrono* isomer **18β** in which the two protons in question are found to both occupy flagpole positions in the most favourable boat conformation.

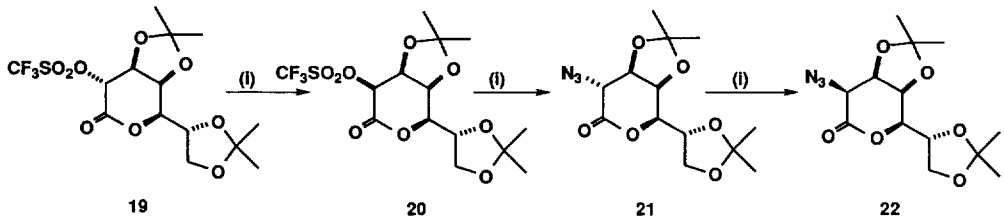


Scheme 1 (i) cyclohexanone, CSA, 97% (ii) NaCN, H_2O; then AcOH, 20% of **15**, 1% of **14** (iii) Me_2Bu^tSiCl, imidazole, DMF, -20°C, 92% (iv) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, -20°C, quantitative (v) NaN_3, DMF, room temp., 50% of **18β**, 25% of **18α** (vi) AcOH : H_2O, 4 : 1, 60°C, 76% of **4β**, 3% of **4α** (vii) AcOH : H_2O, 4 : 1, 60°C, 28% of **4β**, 47% of **4α** (viii) TPAP, NMO, MeCN, room temp., 63% from **4β**, 61% from **4α**.

Attempted desilylation of either **18β** or **18α** with tetrabutylammonium fluoride in tetrahydrofuran was found to cause extensive product decomposition; these δ-lactones were found to be sensitive to base. Removal of the silyl protecting group was finally achieved by treatment with aqueous acetic acid. However this reaction was accompanied in both cases by a small amounts of epimerisation of the azido substituent. Thus desilylation of *altrono* azide **18β** yielded mainly the *altrono* azidoalcohol **4β** (76%) together with some of the *allono* isomer **4α** (3%). Likewise, the *allono* azide **18α** yielded, as the major product, the *allono* alcohol **4α** (47%) together with a significant amount of the *altrono* isomer **4β** (28%). Thus there is significant interconversion between the epimers of **4** even under mild acid conditions.

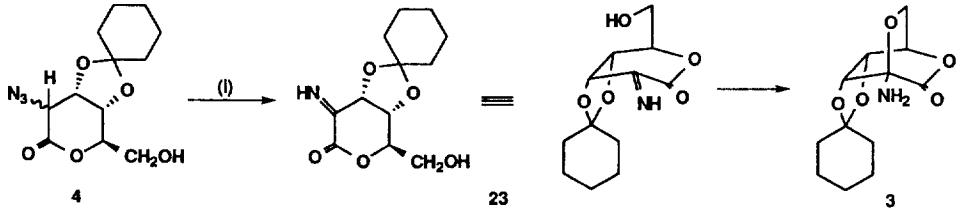
The behaviour of the azides **4**, in which the protected *cis* diol unit is *trans* to the adjacent carbon substituent on the δ-lactone is worth contrasting with the azides **21** and **22**, where the ketal unit is *cis* to the carbon side chain. Reaction of either of the triflates **19** or **20** with sodium azide in dimethylformamide at room temperature kinetically gave the same azide **21** which under the reactions conditions was isomerised

exclusively to the azide **22** [Scheme 2]; the apparent S_N2 displacement for the conversion of **19** into **22** proceeds mainly by an initial base catalysed epimerisation to the more stable isomer **20** which undergoes displacement by azide to give an S_N2 displacement forming the more stable azide **22**.[23] Both **21** and **22** react with aqueous acetic acid to lose the side chain acetonides to give the corresponding azidodiols with no loss of stereochemical integrity at the azide bearing carbon. It is apparent that whereas the all *cis*-azide **22** is much more thermodynamically more stable than **21**, the relative stablities of the epimers of **4** is more finely balanced and also that interconversion of the epimers can take place even in the presence of weak acid.



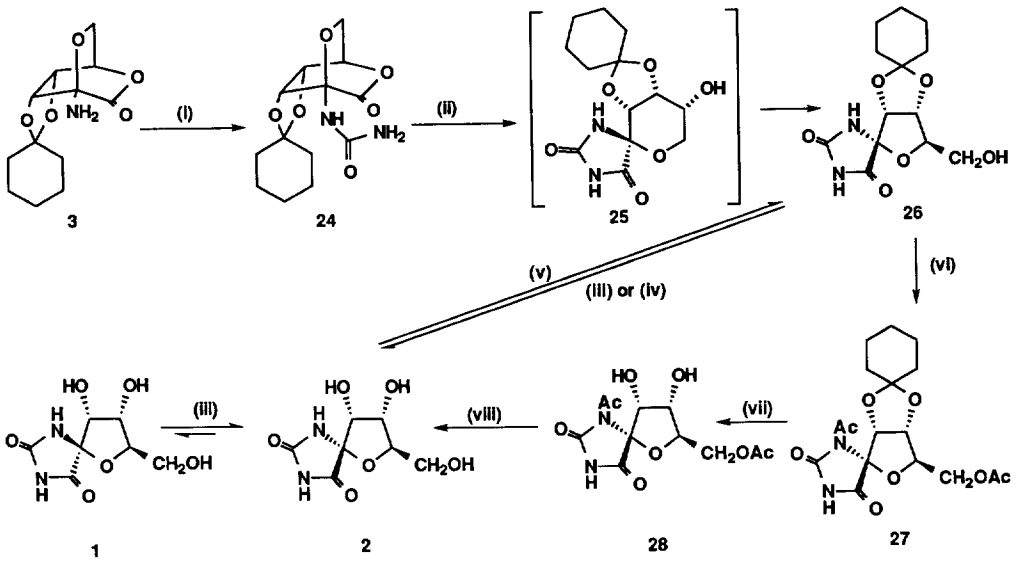
Scheme 2 (i) NaN_3, DMF, room temp.

A large number of attempts were made to find a suitable oxidising agent for the conversion of the alcohol functionality in either of the epimers of **4** into the corresponding aldehydes **10**; however, conditions that were studied gave neither any significant amount of **10**, nor of products that would have resulted from intramolecular aldol products derived from **10**. However, during these investigations, it was found that treatment of either **4**β or **4**α with a catalytic amount of TPAP[24] in the presence of morpholine-N-oxide (NMO) unexpectedly initiated a rapid reaction resulting in the formation of the bicyclic amine **3** as the sole reaction product in around 60% yield, regardless of which epimer of **4** was used as the substrate; the structure of **3** was established by single crystal X-ray analysis.[1] The TPAP-NMO mixture has not caused any oxidation and merely induced disproportionation of the azide functionality to produce nitrogen and an imine **23**; the hydroxyimine **23** underwent subsequent cyclisation to give the bicyclic amine **3** which, on this occasion, is relatively stable and easy to handle [Scheme 3].



Scheme 3 (i) TPAP, NMO, MeCN, room temp., 60%

In principal, either acid or base catalysis might also induce the transformation of the azide **4** to the imine **23**; low yields of such base catalysed transformations have been observed in δ-lactones with unprotected OH groups[11] but in the case of **4** no such reaction products could be identified. Other azidolactones do not give analogous products to **3** and this may be because the [2.2.2] bicyclic bridgehead amine in this case is stable relative to its (more reactive) open chain hydroxyimine isomer; it appears that the relative stereochemistry in the three oxygen bearing chiral centres makes this framework more stable and this feature has been exploited in the easy synthesis of related tetrahydropyran-1-carboxylate esters.[25]



Scheme 4 (i) KCNO, AcOH, 60°C, 76% (ii) KOBu^t, DMF, room temp., 61% over 2 steps from **3** (iii) TFA:H_2O, 2:3, room temp., 98% (iv) MeOH, Dowex 50W-X8(H^+), 40°C, 87% (v) cyclohexanone, conc. H_2SO_4, room temp., 94% (vi) Ac_2O, pyridine, DMAP, room temp., 72% (vii) TFA:H_2O, 2:3, room temp., 94% (viii) $N_2H_4.H_2O$, MeOH, room temp., 70%.

The bicyclic amine **3** is an ideal precursor for the construction of a spirohydantoin at the anomeric position of ribose since it contains all the correct stereochemical and functional group features of ribose as well as an amine and a reactive carbonyl group attached to C-1 of the sugar. Accordingly, the amine **3** was treated with potassium cyanate in acetic acid [Scheme 4] to yield the urea **24** in 76% yield. Cyclisation of **24** to a spirohydantoin, induced by treatment with potassium *tert*-butoxide in dimethylformamide, afforded the cyclohexylidene *epi*hydantocidin **26** as the sole reaction product in 61% yield over two steps from **3**. Presumably, initial nucleophilic attack by the urea nitrogen of **24** at the lactone carbonyl group would lead to a spirohydantoin of ribopyranose **25**; however, no pyranose isomer was isolated. Removal of a proton from the anomeric nitrogen in **25** would allow ring opening by loss of the primary C-7 alkoxide followed by ring closure of the secondary C-6 hydroxyl function to give the thermodynamically more stable ribofuranose structure in **26**. The cyclohexylidene group was then removed either by treatment with aqueous trifluoroacetic acid or with acidic ion exchange resin in methanol to produce 5-*epi*hydantocidin **2** in 98% and 87% yields, respectively. This material was found to possess identical 500 MHz ^1H and ^{13}C nmr spectra with spectra of authentic samples of 5-*epi*hydantocidin provided by Dr S. Mio of Sankyo Agricultural Research Laboratories and Dr S. Mirza of CIBA GEIGY.

It has been reported that significant epimerisation of the spirocentre of hydantocidin occurred during acidic conditions employed for the removal of a protecting group.[26] In order to validate the structural assignment of compound **26**, a series of interconversion experiments on **26** and **2** were undertaken. In particular, it was found that 5-*epi*hydantocidin **2** could be reconverted to **26** by treatment with cyclohexanone and sulfuric acid in 94% isolated yield. The original paper[26] also indicated that epimerisation of the spiro centre could be avoided by prior acetylation of the hydantoin ring. Acetylation of **26** with acetic anhydride and 4-(*N,N*-dimethylamino)pyridine (DMAP) in pyridine yielded the diacetate **27** in 72% yield.

Removal of the cyclohexylidene protecting group was than achieved by treatment with aqueous trifluoroacetic acid to yield the diol **28** in 94% yield. Final removal of the acetate groups by treatment with hydrazine monohydrate in methanol again yielded 5-*epi*hydantocidin **2** as the sole reaction product in 70% yield, with no sign of any hydantocidin **1** in the reaction mixture. Thus, in our hands, no epimerisation of the spiro centre took place during the final deprotection step of 5-*epi*hydantocidin **2**.

Pure samples of *epi*hydantocidin **2** and hydantocidin **1** were subjected to acidic conditions [40% aqueous trifluoroacetic acid] and the degree of interconversion of the two epimers monitored [Table]. These acidic conditions were simply those used for removal of the cyclohexylidene protecting group in the synthesis, but with prolonged reaction times - the deprotection of **26** to **2** is complete in 2 hours at room temperature.

Pure Starting Material		Ratios of products observed		
		hydantocidin **1**		5-*epi*hydantocidin **2**
hydantocidin **1**	(a)	1	:	2.2
	(b)	1	:	4
5-*epi*hydantocidin **2**	(a)	1	:	10
	(b)	1	:	4.5

Table. 40% aqueous trifluoroacetic acid room temperature for (a) 16 h (b) 32 h

The ratios of products were determined by integration of the H-2 signals, which are well separated and distinct for the two materials, in the 500 MHz nmr spectrum of the crude reaction mixture. Epimerisation is observed for both materials, though on a timescale appreciably longer than was employed for the protecting group removal - hence the lack of epimerisation observed therein. The equilibrium ratio of the two materials is approximately 4 : 1 in favour of 5-*epi*hydantocidin **2**.

In summary, this paper reports the synthesis of spirohydantoins of ribofuranose by a route that involves a novel bicyclic [2.2.2] lactone with an amine at the bridgehead position. Although in this case only a spirohydantoin of a furanose was isolated, such an approach may provide access to pyranose sugars with spiroderivatives at the anomeric position. The biological activity of both hydantocidin **1** and the glucopyranose analogue **7** make these compounds attractive targets for improved syntheses.

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Experimental. Melting points were recorded on a Kofler hot block and are corrected. ^1H nuclear magnetic resonance (δ_H) spectra were recorded on Bruker WH 300 (300 MHz) or Bruker AM 500 (500 MHz) spectrometers. ^{13}C Nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50.3 MHz) spectrometer and multiplicities were assigned using DEPT sequence. ^{13}C spectra run in D_2O were referenced to methanol (δ_C 49.6 ppm) as an internal standard. All chemical shifts are quoted on the δ-scale. Infra-red spectra were recorded on a Perkin-Elmer 1750 FT spectrophotometer. Mass spectra were recorded on VG Micromass 30F, ZAB 1F, Masslab 20-250 or Trio-1 GCMS (DB-5 column) spectrometers using

desorption chemical ionisation (NH_3, DCI), chemical ionisation (NH_3, CI), electrospray, or electron impact (EI), as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis service of the Dyson-Perrins laboratory. Thin layer chromatography (t.l.c.) was carried out on aluminium sheets coated with $60F_{254}$ silica or glass plates coated with silica Blend 41. Plates were developed using a spray of 0.2% w/v cerium (IV) sulfate and 5% w/v ammonium molybdate in 2M sulfuric acid, or 0.5% w/v ninhydrin in methanol. All solvents were removed *in vacuo*. Flash chromatography was carried out using Sorbsil C60 40/60 silica. CMAW (chloroform / methanol / acetic acid / water) used as an eluant was prepared in the following ratio ($CHCl_3$: MeOH : AcOH : H_2O, 60 : 30 : 3 : 5). Ion exchange columns were packed with 'Dowex' 50W-X8 in the H^+ form. Solvents and commercially available reagents were dried and purified before use according to standard procedures; dichloromethane was refluxed over and distilled from calcium hydride; methanol was distilled from magnesium methoxide; pyridine was distilled from, and stored over, potassium hydroxide; tetrahydrofuran was distilled, under nitrogen, from a solution dried with sodium in the presence of benzophenone. Hexane was distilled at 68 °C before use to remove involatile fractions.

2,3-*O*-Cyclohexylidene-D-ribose **13**. A mixture of D-ribose **12** (30.0 g, 0.2 mol) and *p*-toluenesulfonic acid (0.7 g, 3.7 mmol) was stirred in freshly distilled cyclohexanone (200 ml) under nitrogen. After 12 h, t.l.c. (ethyl acetate) indicated complete consumption of starting material (R_f 0.1), and the formation of a major product (R_f 0.7). Ethyl acetate (500 ml) was added and the mixture washed with sodium bicarbonate solution (300 ml), and water (300 ml). The organic extracts were dried (magnesium sulfate), filtered, the solvent removed and the residue purified by dry flash chromatography (chloroform : methanol, 20 : 1) to yield 2,3-*O*-cyclohexylidene-D-ribose **13** (44.5 g, 97%) as a colourless oil; $[\alpha]_D^{20}$ -20.0 (*c*, 1.1 in $CHCl_3$), [Lit[22] -20.8 (*c*, 1.01 in $CHCl_3$)]; δ_H ($CDCl_3$) 1.40-1.86 (10H, m, cyclohexylidene), 3.70-3.79 (2H, m, H-5, H-5'), 4.42 (1H, s, H-4), 4.58 (1H, dd, H-2, $J_{2,3}$ 5.9 Hz), 4.83 (1H, d, H-3), 5.43 (1H, d, H-1, $J_{1,2}$ 5.5 Hz); δ_C ($CDCl_3$) 23.5, 23.8, 24.8, 34.0, 35.7 (5 x t, cyclohexylidene), 63.4 (t, C-5), 81.2, 86.3, 87.8 (3 x d, C-2, C-3, C-4), 102.1 (d, C-1), 113.0 (s, cyclohexylidene).

3,4-*O*-Cyclohexylidene-D-*altrono*-1,5-lactone **15** and 3,4-*O*-cyclohexylidene-D-*allono*-1,5-lactone **14**. 2,3-*O*-Cyclohexylidene-D-ribose **13** (44.5 g, 0.19 mol) and sodium cyanide (8.53 g, 0.17 mol) were stirred together in water (500 ml) at room temperature overnight. The temperature of the reaction mixture was then raised to 70 °C and nitrogen was bubbled through the solution until evolution of ammonia had ceased (approx 6 h). At this point a test for cyanide was found to be negative and t.l.c. (ethyl acetate) indicated some starting material (R_f 0.7) together with a major product at the baseline. The mixture was allowed to cool to room temperature and then washed with ethyl acetate (4 x 400 ml) to remove unreacted starting material, which was reclaimed. The aqueous extract was adjusted to pH 7 by the addition of acetic acid, the solvent removed under reduced pressure and the residue co-evaporated with toluene (3 x 50 ml). Acetic acid (200 ml) was added and the mixture heated at 70 °C. After 1.5 h, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.7) and a minor product (R_f 0.5). The solvent was removed and the residue co-evaporated with toluene (2 x 30 ml). The residue was then shaken with ethyl acetate (300 ml) and water (300 ml) and the aqueous layer further extracted with ethyl acetate (2 x 200 ml). The combined organic extracts were dried (magnesium sulfate), filtered, and the solvent removed to produce a residue which was

purified by repeated flash chromatography (hexane : ethyl acetate, 1 : 1) to yield 3,4-*O*-cyclohexylidene-D-*altrono*-1,5-lactone **15** (7.92 g, 20% based on recovered starting material, R_f 0.7) as a white crystalline solid, m.p. 119-120 °C (ethyl acetate / methanol); $[\alpha]_D^{20}$ +80.4 (*c*, 1.02 in EtOH); ν_{max} (KBr) 3500-3200 (br, OH), 1773 (C=O) cm^{-1} ; δ_H (d_6 acetone) 1.40-1.70 (10H, m, cyclohexylidene), 3.72 (1H, dd, H-6, $J_{5,6}$ 4.9 Hz, $J_{6,6'}$ 12.6 Hz), 3.90 (1H, dd, H-6', $J_{5,6'}$ 2.1 Hz), 4.27 (1H, ddd, H-5, $J_{4,5}$ 9.4 Hz), 4.34 (1H, m, H-3, $J_{2,3}$ 6.9 Hz, $J_{3,4}$ 7.8 Hz), 4.42 (1H, dd, H-4), 4.55 (1H, d, H-2); δ_C (d_6 acetone) 24.2, 24.6, 25.6, 34.7, 37.4 (5 x t, cyclohexylidene), 61.5 (t, C-6), 71.0, 71.7, 78.3, 79.1 (4 x d, C-2, C-3, C-4, C-5), 112.9 (s, cyclohexylidene), 173.4 (s, C-1); *m/z* (NH_3, DCI) 276 (MNH_4^+, 100%), 259 (MH^+). (Found: C, 55.70; H, 7.26; $C_{12}H_{18}O_6$ requires: C, 55.81; H, 7.02%);

and 3,4-*O*-cyclohexylidene-D-*allono*-1,5-lactone **14** (389 mg, 1% based on recovered starting material, R_f 0.5) as a white crystalline solid, m.p. 128-130 °C (ethyl acetate / hexane); $[\alpha]_D^{20}$ -68.1 (*c*, 1.03 in EtOH); ν_{max} (KBr) 3400 (br, OH), 1751 (C=O) cm^{-1} ; δ_H (d_6 acetone) 1.38-1.63 (10H, m, cyclohexylidene), 2.97 (2H, s, OH), 3.87-3.90 (2H, m, H-6, H-6'), 4.47 (1H, br t, H-5), 4.70-4.86 (3H, m, H-2, H-3, H-4); δ_C (d_6 acetone) 24.2, 24.5, 25.6, 34.5, 36.8 (5 x t, cyclohexylidene), 63.0 (t, C-6), 68.0, 74.3, 76.7, 84.0 (4 x d, C-2, C-3, C-4, C-5), 111.0 (s, cyclohexylidene), 172.5 (s, C-1); *m/z* (NH_3, DCI) 276 (MNH_4^+), 259 (MH^+, 100%). (Found: C, 55.58; H, 6.94; $C_{12}H_{18}O_6$ requires: C, 55.81; H, 7.02%).

6-*O*-*tert*-Butyldimethylsilyl-3,4-*O*-cyclohexylidene-D-*altrono*-1,5-lactone **16**. 3,4-Cyclohexylidene-D-*altrono*-1,5-lactone **15** (4.72 g, 18.3 mmol) and imidazole (2.61 g, 38.4 mmol) were stirred under nitrogen in dry dimethylformamide (100 ml) at -20 °C. *tert*-Butyldimethylsilyl chloride (2.90 g, 19.2 mmol) was added and the mixture stirred for 2.5 h when t.l.c. (hexane : ethyl acetate, 1 : 1) showed complete consumption of starting material (R_f 0.2) and the formation of a major product (R_f 0.8), together with a minor product (R_f 0.9). The solvent was removed and dichloromethane (75 ml) was added. The mixture was shaken with water (75 ml), which was further extracted with dichloromethane (2 x 50 ml). The combined organic extracts were then dried (magnesium sulfate), filtered, the solvent removed, and the residue purified by flash chromatography (hexane : ethyl acetate, 3 : 1) to yield 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-D-*altrono*-1,5-lactone **16** (6.28 g, 92%) as a white crystalline solid, m.p. 74-75 °C (methanol / water); $[\alpha]_D^{20}$ +71.3 (*c*, 1.02 in $CHCl_3$); ν_{max} (KBr) 3400 (br, OH), 1767 (C=O) cm^{-1} ; δ_H ($CDCl_3$) 0.11 (6H, s, Me_2Si), 0.92 (9H, s, Bu^t), 1.40-1.76 (10H, m, cyclohexylidene), 3.36 (1H, d, OH, $J_{2,OH}$ 3.2 Hz), 3.88 (1H, dd, H-6, $J_{5,6}$ 4.5 Hz, $J_{6,6'}$ 12.0 Hz), 4.04 (1H, dd, H-6', $J_{5,6'}$ 2.0 Hz), 4.15 (1H, ddd, H-5, $J_{4,5}$ 9.0 Hz), 4.27-4.32 (1H, m, H-3), 4.36 (1H, dd, H-4, $J_{3,4}$ 7.8 Hz), 4.42 (1H, dd, H-2, $J_{2,3}$ 6.7 Hz); δ_C ($CDCl_3$) -5.6 (q, Me_2Si), 18.2 (s, Me_3CSi), 23.2, 23.7, 25.3 (3 x t, cyclohexylidene), 25.7 (q, Me_3C), 33.9, 36.6 (2 x t, cyclohexylidene), 61.8 (t, C-6), 69.7, 70.9, 77.1, 78.7 (4 x d, C-2, C-3, C-4, C-5), 113.0 (s, cyclohexylidene), 173.1 (s, C-1); *m/z* (NH_3, DCI) 390 (MNH_4^+, 100%), 373 (MH^+). (Found: C, 58.26; H, 8.90; $C_{18}H_{32}O_6Si$ requires: C, 58.03; H, 8.66%);

and a small amount of disilylated material (0.414 g, 5%) as a colourless oil; δ_H ($CDCl_3$) 0.09 (6H, s, Me_2Si), 0.14, 0.18 (6H, 2 x s, Me_2Si), 0.90, 0.95 (18H, 2 x s, 2 x Bu^t), 1.40-1.75 (10H, m, cyclohexylidene), 3.83 (1H, dd, H-6, $J_{5,6}$ 4.5 Hz, $J_{6,6'}$ 11.8 Hz), 3.99 (1H, dd, H-6', $J_{5,6'}$ 1.1 Hz), 4.08 (1H, m, H-5), 4.23-4.38 (3H, m); *m/z* (NH_3, DCI) 504 (MNH_4^+), 487 (MH^+).

6-*O*-*tert*-Butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-*O*-trifluoromethanesulfonyl-D-*altrono*-1,5-lactone **17**. 6-*O*-*tert*-Butyldimethylsilyl-3,4-*O*-cyclohexylidene-D-*altrono*-1,5-lactone **16** (1.53 g, 4.11 mmol) and dry pyridine (0.84 ml, 10.3 mmol) were stirred under nitrogen in dry dichloromethane (50 ml) at -20 °C. Trifluoromethanesulfonic anhydride (1.04 ml, 6.17 mmol) was added. After 10 min, t.l.c. (hexane : ethyl acetate, 3 : 1) indicated complete product formation (R_f 0.6) and further dichloromethane (20 ml) was added. The reaction mixture was shaken with water (30 ml, containing a few drops of 1M aqueous hydrochloric acid) and then washed with water (30 ml) and brine (30 ml). The organic extracts were then dried (magnesium sulfate), filtered, the solvent removed and the residue purified by flash chromatography (hexane : ethyl acetate, 7 : 1) to yield 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-*O*-trifluoromethanesulfonyl-D-*altrono*-1,5-lactone **17** (2.06 g, quantitative) as a white crystalline solid, m.p. 76-78 °C (methanol / water); $[\alpha]_D^{20}$ +19.1 (*c*, 1.1 in $CHCl_3$); ν_{max} (film) 1785 (C=O) cm^{-1}; δ_H ($CDCl_3$) 0.11 (6H, s, Me_2Si), 0.92 (9H, s, Bu^t), 1.41-1.77 (10H, m, cyclohexylidene), 3.90 (1H, dd, H-6, $J_{5,6}$ 4.0 Hz, $J_{6,6'}$ 11.9 Hz), 4.04 (1H, dd, H-6', $J_{5,6'}$ 2.2 Hz), 4.25 (1H, m, H-5), 4.51-4.56 (2H, m, H-3, H-4), 5.22 (1H, d, H-2, $J_{2,3}$ 7.0 Hz); δ_C ($CDCl_3$) -5.7 (q, Me_2Si), 18.2 (s, $Me_3\underline{C}Si$), 23.3, 23.6, 24.7 (3 x t, cyclohexylidene), 25.6 (q, $\underline{Me}_3C$), 34.1, 36.6 (2 x t, cyclohexylidene), 61.7 (t, C-6), 70.2, 73.9, 79.0, 82.0 (4 x d, C-2, C-3, C-4, C-5), 114.1 (s, cyclohexylidene), 164.3 (s, C-1); *m/z* (NH_3, DCI) 522 (MNH_4^+, 100%), 505 (MH^+). (Found: C, 45.19; H, 6.42; $C_{19}H_{31}O_8F_3SSi$ requires: C, 45.23; H, 6.19%).

2-Azido-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-deoxy-D-*altrono*-1,5-lactone **18β** and 2-Azido-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-deoxy-D-*allono*-1,5-lactone **18α**. 6-*O*-*tert*-Butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-*O*-trifluoromethanesulfonyl-D-*altrono*-1,5-lactone **17** (850 mg, 1.69 mmol) and sodium azide (329 mg, 5.06 mmol) were stirred at room temperature in dimethylformamide (10 ml). After 10 min t.l.c. (hexane : ethyl acetate, 5 : 1) indicated complete consumption of starting material (R_f 0.7), and the formation of two products (R_f 0.6 and R_f 0.5). The solvent was removed and ethyl acetate (50 ml) added. The mixture was then washed with water (50 ml), brine (50 ml), dried (magnesium sulfate), filtered, the solvent removed and the residue purified by flash chromatography (hexane : ethyl acetate, 7 : 1) to yield 2-azido-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-deoxy-D-*altrono*-1,5-lactone **18β** (340 mg, 50%, R_f 0.6) as a white crystalline solid, m.p. 67-68 °C (methanol / water); $[\alpha]_D^{20}$ +18.2 (*c*, 1.06 in $CHCl_3$); ν_{max} (KBr) 2125 (N_3), 1757 (C=O) cm^{-1} ; δ_H ($CDCl_3$) 0.11, 0.12 (6H, 2 x s, Me_2Si), 0.92 (9H, s, Bu^t), 1.41-1.74 (10H, m, cyclohexylidene), 3.89 (1H, dd, H-6, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 11.9 Hz), 4.03 (1H, dd, H-6', $J_{5,6'}$ 2.1 Hz), 4.15 (1H, ddd, H-5, $J_{4,5}$ 9.2 Hz), 4.27-4.30 (2H, m, H-2, H-3), 4.37 (1H, m, H-4); δ_C ($CDCl_3$) -5.6, -5.5 (2 x q, Me_2Si), 18.3 (s, $Me_3\underline{C}Si$), 23.3, 23.7, 24.8 (3 x t, cyclohexylidene), 25.7 (q, $\underline{Me}_3C$), 33.9, 36.7 (2 x t, cyclohexylidene), 61.9 (t, C-6), 62.4 (d, C-2), 70.0, 75.2, 79.0 (3 x d, C-3, C-4, C-5), 113.4 (s, cyclohexylidene), 167.4 (s, C-1); *m/z* (NH_3, DCI) 415 (MNH_4^+, 100%), 398 (MH^+), 370 (MH^+-N_2, 100%). (Found: C, 54.60; H, 7.97; N, 10.56; $C_{18}H_{31}O_5SiN_3$ requires: C, 54.38; H, 7.86; N, 10.57%).

n.O.e. Data. Irradiate δ 4.15 (H-5) ; enhancements : 3.89 (H-6, 3.4%), 4.03 (H-6', 3.4%), 4.28 (H-2, H-3, 14%). Irradiate δ 4.28 (H-2, H-3) ; enhancements : 4.15 (H-5, 12%), 4.37 (H-4, 7.9%).

and 2-azido-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-deoxy-D-*allono*-1,5-lactone **18α** (160 mg, 25%, R_f 0.5) as a white crystalline solid, m.p. 160-162 °C (ether / hexane); $[\alpha]_D^{20}$ -77.2 (*c*, 1.08 in $CHCl_3$); ν_{max} (KBr) 2115 (N_3), 1743 (C=O) cm^{-1} ; δ_H (d_6 benzene) -0.20, -0.19 (6H, 2 x s, Me_2Si), 0.75

(9H, s, Bu^t), 1.41-1.75 (10H, m, cyclohexylidene), 3.07 (1H, dd, H-6, $J_{5,6}$ 3.2 Hz, $J_{6,6'}$ 11.9 Hz), 3.34 (1H, dd, H-6', $J_{5,6'}$ 3.3 Hz), 4.14 (1H, dd, H-4, $J_{3,4}$ 7.3 Hz, $J_{4,5}$ 1.9 Hz), 4.23 (1H, dt, H-5), 4.44 (1H, d, H-2, $J_{2,3}$ 4.0 Hz), 4.51 (1H, dd, H-3); δ_C ($CDCl_3$) -5.9 (q, Me_2Si), 18.0 (s, $Me_3\underline{C}Si$), 23.5, 23.7, 24.8 (3 x t, cyclohexylidene), 25.7 (q, $\underline{Me}_3C$), 33.9, 36.0 (2 x t, cyclohexylidene), 58.4 (d, C-2), 64.9 (t, C-6), 73.7, 76.2, 82.8 (3 x d, C-3, C-4, C-5), 111.6 (s, cyclohexylidene), 167.4 (s, C-1); *m/z* (NH_3, DCI) 415 (MNH_4^+, 100%), 398 (MH^+), 370 (MH^+-N_2, 100%). (Found: C, 54.37; H, 7.93; N, 10.52; $C_{18}H_{31}O_5SiN_3$ requires: C, 54.38; H, 7.86; N, 10.57%).

n.O.e. Data. Irradiate δ 4.24 (H-5) ; enhancements : 3.07 (H-6, 7.8%), 3.34 (H-6', 6.7%). No enhancement seen to H-2. Irradiate δ 4.44 (H-2) ; enhancements : 4.51 (H-3). No enhancement seen to H-5. Irradiate δ 4.51 (H-3) ; enhancements : 4.44 (H-2, 7.2%).

2-Azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*altrono*-1,5-lactone **4β**. 2-Azido-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-deoxy-D-*altrono*-1,5-lactone **18β** (210 mg, 0.53 mmol) was stirred in acetic acid : water, 4 : 1 (10 ml) at 60 °C. After 5 h, t.l.c. (hexane : ethyl acetate, 1 : 1) indicated complete consumption of starting material and the formation of two products (R_f 0.5 and 0.3). The solvent was removed and the residue co-evaporated with toluene (2 x 5 ml). Ethyl acetate (10 ml) was added, the mixture preabsorbed onto silica and then purified by flash chromatography (hexane : ethyl acetate, 3 : 2) to yield 2-azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*altrono*-1,5-lactone **4β** (114 mg, 76%, R_f 0.5) as a white crystalline solid, m.p. 97-99 °C (ether / hexane); $[\alpha]_D^{20}$ +28.9 (*c*, 1.01 in $CHCl_3$); ν_{max} (KBr) 3472 (OH), 2119 (N_3), 1762 (C=O) cm^{-1} ; δ_H ($CDCl_3$) 1.43-1.78 (10H, m, cyclohexylidene), 3.87 (1H, dd, H-6, $J_{5,6}$ 4.6 Hz, $J_{6,6'}$ 12.8 Hz), 4.06 (1H, dd, H-6', $J_{5,6'}$ 2.6 Hz), 4.21 (1H, ddd, H-5, $J_{4,5}$ 9.4 Hz), 4.30-4.32 (2H, m, H-2, H-3), 4.42 (1H, m, H-4); δ_C ($CDCl_3$) 23.3, 23.7, 24.7, 33.9, 36.6 (5 x t, cyclohexylidene), 61.3 (t, C-6), 62.3 (d, C-2), 69.9, 75.2, 78.8 (3 x d, C-3, C-4, C-5), 113.8 (s, cyclohexylidene), 167.5 (s, C-1); *m/z* (NH_3, DCI) 301 (MNH_4^+, 100%), 284 (MH^+), 256 (MH^+-N_2, 100%); and 2-azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*allono*-1,5-lactone 4α (4 mg, 3%, R_f 0.3) identical in all respects to the material described below.

2-Azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*allono*-1,5-lactone **4α**. 2-Azido-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-cyclohexylidene-2-deoxy-D-*allono*-1,5-lactone **18α** (160 mg, 0.4 mmol) was stirred in acetic acid : water, 4 : 1 (10 ml) at 60 °C. After 15 h, t.l.c. (hexane : ethyl acetate, 1 : 1) indicated complete consumption of starting material and the formation of two products (R_f 0.5 and 0.3). The solvent was removed, the residue co-evaporated with toluene (2 x 5 ml) and then purified by flash chromatography (hexane : ethyl acetate, 2 : 1) to yield 2-azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*allono*-1,5-lactone **4α** (55 mg, 47%, R_f 0.3) as a white crystalline solid, m.p. 110-112 °C (ether / hexane); $[\alpha]_D^{20}$ -68.8 (*c*, 1.03 in EtOH); ν_{max} (KBr) 3438 (OH), 2116 (N_3), 1757 (C=O) cm^{-1} ; δ_H (d_6-acetone) 1.40-1.77 (10H, m, cyclohexylidene), 3.94 (2H, m, H-6, H-6'), 4.58 (1H, t, H-5, J 4.1 Hz), 4.67 (1H, d, H-2, $J_{2,3}$ 4.1 Hz), 4.79 (1H, d, H-4, $J_{3,4}$ 7.3 Hz), 4.97 (1H, dd, H-3); δ_C (d_6-acetone) 23.3, 23.6, 24.6, 33.4, 35.9 (5 x t, cyclohexylidene), 58.3 (d, C-2), 62.3 (t, C-6), 73.5, 76.1, 82.8 (3 x d, C-3, C-4, C-5), 110.4 (s, cyclohexylidene), 167.2 (s, C-1); *m/z* (NH_3, DCI) 301 (MNH_4^+, 100%), 284 (MH^+), 256 (MH^+-N_2, 100%). (Found: C, 51.00; H, 5.99; N, 14.70; $C_{12}H_{17}O_5N_3$ requires: C, 50.88; H, 6.05; N, 14.83%); and 2-azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*altrono*-1,5-lactone 4β (32 mg, 28%, R_f 0.5) identical in all respects to the material described above.

(1S,4R,7R,8S)-1-Amino-7,8-*O*-cyclohexylidene-7,8-dihydroxy-2,5-dioxa-bicyclo[2.2.2.]octan-6-one **3**.

Method 1. 2-Azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*altrono*-1,5-lactone **4β** (1.04 g, 3.67 mmol) and *N*-methylmorpholine-*N*-oxide (646 mg, 5.51 mmol) were stirred in dry acetonitrile (20 ml) at room temperature under nitrogen. Tetra-*n*-propylammonium perruthenate (65 mg, 0.18 mmol, 5%) was added and after 20 min t.l.c. (hexane : ethyl acetate, 1 : 1) indicated complete consumption of starting material (R_f 0.5) and the formation of a major product (R_f 0.1). The solvent was removed, the residue dissolved in a small volume of dichloromethane and purified by flash chromatography (ethyl acetate : hexane, 2 : 1) to yield (1S,4R,7R,8S)-1-amino-7,8-*O*-cyclohexylidene-7,8-dihydroxy-2,5-dioxa-bicyclo[2.2.2.]octan-6-one **3** (593 mg, 63%) as a white crystalline solid, m.p. 161-163 °C (ethyl acetate / hexane); $[\alpha]_D^{20}$ -54.2 (*c*, 1.1 in $CHCl_3$); ν_{max} (KBr) 3428, 3346 (NH), 1767 (C=O) cm^{-1} ; δ_H ($CDCl_3$) 1.36-1.67 (10H, m, cyclohexylidene), 3.89 (1H, d, H-3, $J_{3,3'}$ 10.6 Hz), 4.09 (1H, dd, H-3', $J_{3,4}$ 2.8 Hz), 4.38 (1H, d, H-7, $J_{7,8}$ 7.6 Hz), 4.49 (1H, dd, H-8, $J_{4,8}$ 2.1 Hz), 4.82 (1H, t, H-4); δ_C ($CDCl_3$) 23.4, 23.6, 24.8, 34.0, 35.3 (5 x t, cyclohexylidene), 63.7 (t, C-3), 73.2, 74.2, 77.1 (3 x d, C-4, C-7, C-8), 83.7 (s, C-1), 112.1 (s, cyclohexylidene), 169.2 (s, C-6); *m/z* (NH_3, DCI) 273 (MNH_4^+), 256 (MH^+, 100%). (Found: C, 56.78; H, 6.40; N, 5.33; $C_{12}H_{17}O_5N$ requires: C, 56.46; H, 6.71; N, 5.49%).

Method 2. 2-Azido-3,4-*O*-cyclohexylidene-2-deoxy-D-*allono*-1,5-lactone **4α** (648 mg, 2.29 mmol) and *N*-methylmorpholine-*N*-oxide (402 mg, 5.51 mmol) were stirred in dry acetonitrile (20 ml) at room temperature under nitrogen. Tetra-*n*-propylammonium perruthenate (40 mg, 0.11 mmol, 5%) was added and after 1 h t.l.c. (hexane : ethyl acetate, 1 :1) indicated complete consumption of starting material (R_f 0.4) and the formation of a major product (R_f 0.1). The solvent was removed, the residue dissolved in a small volume of dichloromethane and purified by flash chromatography (ethyl acetate : hexane, 2 : 1) to yield (1S,4R,7R,8S)-1-amino-7,8-*O*-cyclohexylidene-7,8-dihydroxy-2,5-dioxa-bicyclo[2.2.2.]octan-6-one **3** (355 mg, 61%), identical in all respects to the material described above.

(1S,4R,7R,8S)-7,8-*O*-Cyclohexylidene-7,8-dihydroxy-2,5-dioxa-1-ureido-bicyclo[2.2.2.]octan-6-one **24**.

(1S,4R,7R,8S)-1-Amino-7,8-*O*-cyclohexylidene-7,8-dihydroxy-2,5-dioxa-bicyclo[2.2.2.]octan-6-one **3** (160 mg, 0.63 mmol) and potassium cyanate (152 mg, 1.88 mmol) were stirred in acetic acid (10 ml) at 60 °C. After 1.5 h, t.l.c. (ethyl acetate) indicated complete consumption of starting material (R_f 0.5) and the formation of a major product (R_f 0.3). The solvent was removed, the residue co-evaporated with toluene (2 x 5 ml) and purified by flash chromatography (tetrahydrofuran) to yield (1S,4R,7R,8S)-7,8-*O*-cyclohexylidene-7,8-dihydroxy-2,5-dioxa-1-ureido-bicyclo[2.2.2.]octan-6-one **24** (142 mg, 76%) as a white crystalline solid, m.p. 258-262 °C (decomp, methanol); $[\alpha]_D^{20}$ -46.9 (*c*, 0.7 in MeOH); ν_{max} (KBr) 3439, 3390, 3347 (NH), 1780 (C=O), 1658 (urea) cm^{-1} ; δ_H (d_6-DMSO) 1.26-1.61 (10H, m, cyclohexylidene), 3.92 (2H, m), 4.65 (1H, dd, J 2.1 Hz, J' 7.7 Hz), 5.01 (1H, d, J 1.9 Hz), 5.56 (1H, d, J 7.7 Hz), 6.03 (2H, br s, NH_2), 6.92 (1H, s, NH); δ_C (d_6-DMSO) 23.5, 23.8, 24.7, 35.8, 36.4 (5 x t, cyclohexylidene), 63.0 (t, C-3), 72.6, 73.3, 74.2 (3 x d, C-4, C-7, C-8), 82.5 (s, C-1), 110.7 (s, cyclohexylidene), 157.3 (s, urea), 168.3 (s, C-6); *m/z* (NH_3, DCI) 299 (MH^+, 100%), 256. (Found: C, 52.03; H, 5.82; N, 9.19; $C_{13}H_{18}O_6N_2$ requires: C, 52.35; H, 6.08; N, 9.39%). In later experiments this material was used directly without isolation.

(2R,3R,4R,5R)-3,4-*O*-Cyclohexylidene-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxaspiro[4.4.]nonan-7,9-dione **26**. *Method 1*. (1S,4R,7R,8S)-1-Amino-7,8-*O*-cyclohexylidene-7,8-dihydroxy-2,5-dioxabicyclo[2.2.2.]octan-6-one **3** (340 mg, 1.3 mmol) and potassium cyanate (324 mg, 4.2 mmol) were stirred in acetic acid (20 ml) at 60 °C. After 1.5 h, t.l.c. (ethyl acetate) indicated complete consumption of starting material (R_f 0.5) and the formation of a major product (R_f 0.3). The solvent was removed, the residue co-evaporated with toluene (2 x 5 ml) and flushed through a short silica plug (eluant tetrahydrofuran). The solvent was then removed, the residue dissolved in dry dimethylformamide (10 ml) and stirred at room temperature under nitrogen. Potassium *tert*-butoxide (346 mg, 3.1 mmol) was added and after 10 min t.l.c. (ethyl acetate) indicated complete consumption of starting material (R_f 0.3) and the formation of a single product (R_f 0.8). Acetic acid (5 ml) was added, the solvent removed, the residue co-evaporated with toluene (2 x 5 ml), preabsorbed onto silica and purified by flash chromatography (ethyl acetate : hexane, 1 : 1) to yield (2R,3R,4R,5R)-3,4-*O*-cyclohexylidene-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxaspiro[4.4.]-nonan-7,9-dione **26** (243 mg, 61% over two steps) as a white foam; $[\alpha]_D^{20}$ -59.4 (*c*, 1.18 in $CHCl_3$); ν_{max} (film) 3370 (br, OH, NH), 1791, 1735 (hydantoin) cm^{-1}; δ_H ($CDCl_3$) 1.41-1.78 (10H, m, cyclohexylidene), 3.67 (1H, dd, H-1, $J_{1',2}$ 2.8 Hz, $J_{1',1''}$ 13.0 Hz), 3.87 (1H, dd, H-1'', $J_{1'',2}$ 1.6 Hz), 4.50 (1H, br s), 4.90 (1H, d, J 5.9 Hz), 5.02 (1H, d, J 5.9 Hz), 5.92 (1H, br s, NH), 8.14 (1H, br s, NH); δ_C ($CDCl_3$) 23.4, 23.8, 24.7, 33.5, 35.9 (5 x t, cyclohexylidene), 63.7 (t, C-1'), 80.7, 81.9, 85.2 (3 x d, C-2, C-3, C-4), 94.3 (s, C-5), 115.0 (s, cyclohexylidene), 155.4, 175.4 (2 x s, C-7, C-9); *m/z* (NH_3, DCI) 316 (M+NH4+, 100%), 299 (M+H+). (Found: C, 52.53; H, 6.39; N, 9.18; $C_{13}H_{18}O_6N_2$ requires: C, 52.35; H, 6.08; N, 9.39%).

Method 2. (2R,3S,4R,5R)-6,8-Diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxaspiro[4.4.]nonan-7,9-dione **2** (24 mg, 0.11 mmol) was stirred in cyclohexanone (3 ml) at room temperature. Concentrated sulfuric acid (one drop) was added and after 20 min t.l.c. (ethyl acetate) indicated the formation of a single product (R_f 0.8). Sodium bicarbonate (20 mg) and ethyl acetate (5 ml) were then added and the mixture stirred for a further 10 min. The crude reaction mixture was then filtered, the solvent removed and the residue purified by flash chromatography (ethyl acetate : hexane, 1 : 1) to yield (2R,3R,4R,5R)-3,4-*O*-cyclohexylidene-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxa-spiro[4.4.]nonan-7,9-dione **26** (31 mg, 94%) identical to the material described above.

(2R,3R,4R,5R)-2-(Acetoxymethyl)-6-*N*-acetyl-3,4-*O*-cyclohexylidene-6,8-diaza-3,4-dihydroxy-1-oxaspiro-[4.4.]nonan-7,9-dione **27**. (2R,3R,4R,5R)-3,4-*O*-Cyclohexylidene-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxaspiro[4.4.]nonan-7,9-dione **26** (80 mg, 0.27 mmol) was stirred in a mixture of dry pyridine (2 ml) and acetic anhydride (2 ml) at room temperature under nitrogen. DMAP (2 mg) was added and after 10 min t.l.c. (hexane : ethyl acetate, 1 : 1) indicated complete consumption of starting material (R_f 0.2) and the formation of a single product (R_f 0.5). The solvent was removed and ethyl acetate (15 ml) added. The mixture was then washed sequentially with 1M aqueous hydrochloric acid (10 ml), water (10 ml) and brine (10 ml). The organic extracts were then dried (magnesium sulfate), filtered, the solvent removed, and the residue purified by flash chromatography (hexane : ethyl acetate, 2 : 1) to yield (2R,3R,4R,5R)-2-(acetoxymethyl)-6-*N*-acetyl-3,4-*O*-cyclohexylidene-6,8-diaza-3,4-dihydroxy-1-oxaspiro[4.4.]nonan-7,9-dione **27** (74 mg, 72%), as a white crystalline solid, m.p. 135-136 °C (ether / hexane); $[\alpha]_D^{20}$ +36.8 (*c*, 0.53 in $CHCl_3$); ν_{max} (film) 3226 (br, NH), 1807 (hydantoin), 1762 (br, C=O) cm^{-1} ; δ_H ($CDCl_3$) 1.38-1.78 (10H, m, cyclohexylidene), 2.14, 2.56 (6H, 2 x s, 2 x Ac) 4.09 (1H, dd, H-1', $J_{1',2}$ 4.8 Hz, $J_{1',1''}$ 12.2 Hz),

4.60 (1H, dd, H-1'', $J_{1'',2}$ 3.4 Hz), 4.88-4.91 (2H, m, H-3, H-4), 4.99 (1H, m, H-2), 7.63 (1H, br s, NH); δ_C ($CDCl_3$) 20.8, 26.1 (2 x q, 2 x Ac), 23.3, 23.7, 24.7, 33.6, 34.2 (5 x t, cyclohexylidene), 63.6 (t, C-1'), 81.8, 82.9, 85.7 (3 x d, C-2, C-3, C-4), 96.0 (s, C-5), 118.3 (s, cyclohexylidene), 152.7 (s, hydantoin), 169.1, 170.1, 171.6 (3 x s, hydantoin, 2 x Ac); *m/z* (NH_3, DCI) 400 (MNH_4^+, 100%), 383 (MH^+). (Found: C, 53.43; H, 5.76; N, 7.08; $C_{17}H_{22}O_8N_2$ requires: C, 53.40; H, 5.80; N, 7.33%).

(2R,3S,4R,5R)-2-(Acetoxymethyl)-6-*N*-acetyl-6,8-diaza-3,4-dihydroxy-1-oxa-spiro[4.4.]nonan-7,9-dione **28.** (2R,3R,4R,5R)-2-(Acetoxymethyl)-6-*N*-acetyl-3,4-*O*-cyclohexylidene-6,8-diaza-3,4-dihydroxy-1-oxa-spiro[4.4.]nonan-7,9-dione **27** (71 mg, 0.19 mmol) was stirred in a mixture of trifluoroacetic acid and water, (2 : 3, 5 ml) at room temperature. After 20 min, t.l.c. (hexane : ethyl acetate, 1 : 1) indicated complete conversion to a single product (R_f 0.1). The solvent was removed, the residue co-evaporated with toluene (2 x 2 ml) and purified by flash chromatography (ethyl acetate) to yield (2R,3S,4R,5R)-2-(acetoxymethyl)-6-*N*-acetyl-6,8-diaza-3,4-dihydroxy-1-oxa-spiro[4.4.]nonan-7,9-dione **28** (53 mg, 94%) as a colourless hygroscopic oil; ν_{max} (film) 3270 (br, OH, NH), 1793 (hydantoin), 1762 (br, C=O) cm^{-1} ; δ_H (CD_3OD) 2.08, 2.15 (6H, 2 x s, 2 x Ac), 4.07 (1H, dd, H-1', $J_{1',2}$ 9.7 Hz, $J_{1',1''}$ 12.0 Hz), 4.22 (1H, m, H-2), 4.39 (1H, dd, H-1'', $J_{1'',2}$ 3.6 Hz), 4.45 (1H, m, H-3, $J_{3,4}$ 5.0 Hz), 5.19 (1H, d, H-4); δ_C (CD_3OD) 18.9, 19.2 (2 x q, 2 x Ac), 63.1 (t, C-1'), 70.7, 73.5, 81.4 (3 x d, C-2, C-3, C-4), 91.7 (s, C-5), 157.0 (s, hydantoin), 170.5, 171.4, 173.0 (3 x s, hydantoin, 2 x Ac); *m/z* (NH_3, DCI) 320 (MNH_4^+, 100%), 303 (MH^+).

(2R,3S,4R,5R)-6,8-Diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxa-spiro[4.4.]nonan-7,9-dione **2.**

Method 1. (2R,3R,4R,5R)-3,4-*O*-Cyclohexylidene-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxaspiro-[4.4.]nonan-7,9-dione **26** (61 mg, 0.2 mmol) was stirred in a mixture of trifluoroacetic acid and water (2 : 3, 5 ml) at room temperature. After 2 h, t.l.c. (ethyl acetate) indicated complete product formation (R_f 0.1). The solvent was removed, the residue co-evaporated with toluene (2 x 1 ml) and purified by flash chromatography (dichloromethane : methanol, 6 : 1) to yield (2R,3S,4R,5R)-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxa-spiro[4.4.]nonan-7,9-dione **2** (44 mg, 98%) as a white amorphous solid; $[\alpha]_D^{20}$ -7.0 (*c*, 0.52 in MeOH)[Lit[8] -11.0 (*c*, 0.3 in MeOH)]; ν_{max} (film) 3333 (br, OH, NH), 1783, 1736 (hydantoin) cm^{-1} ; δ_H (CD_3OD) 3.59 (1H, dd, H-1', $J_{1',2}$ 5.2 Hz, $J_{1',1''}$ 12.1 Hz), 3.66 (1H, dd, H-1'', $J_{1'',2}$ 4.3 Hz), 4.09 (1H, ddd, H-2, $J_{2,3}$ 3.2 Hz), 4.17 (1H, dd, H-3, $J_{3,4}$ 4.9 Hz), 4.25 (1H, d, H-4); δ_C (CD_3OD) 63.6 (t, C-1'), 73.1, 74.3, 87.1 (3 x d, C-2, C-3, C-4), 94.4 (s, C-5), 158.2, 175.7 (2 x s, C-7, C-9); *m/z* (NH_3, DCI) 236 (MNH_4^+, 100%), 219 (MH^+). (Found: C, 38.56; H, 4.63; N, 12.47; $C_7H_{10}O_6N_2$ requires: C, 38.54; H, 4.62; N, 12.84%).

Method 2. (2R,3R,4R,5R)-3,4-*O*-Cyclohexylidene-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxaspiro-[4.4.]nonan-7,9-dione **26** (33 mg, 0.11 mmol) and Dowex 50W-X8(H^+) ion exchange resin (30 mg) were stirred in methanol (3 ml) at 40 °C. After 16 h, t.l.c. (dichloromethane : methanol, 4 : 1) indicated the formation of a major product (R_f 0.3). The reaction mixture was filtered, the solvent removed and the residue purified by flash chromatography (dichloromethane : methanol, 4 : 1) to yield (2R,3S,4R,5R)-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxa-spiro[4.4.]nonan-7,9-dione **2** (16 mg, 66%, 87% based on recovered starting material, R_f 0.3) identical to the material described previously, together with unreacted staring material (8 mg, 24 %, R_f 0.8).

Method 3. (2R,3S,4R,5R)-2-(Acetoxymethyl)-6-*N*-acetyl-6,8-diaza-3,4-dihydroxy-1-oxa-spiro[4.4.]nonan-7,9-dione **28** (44 mg, 0.15 mmol) was stirred at room temperature in methanol (2 ml). Hydrazine monohydrate (18 µl, 0.36 mmol) was added and the reaction mixture stirred for 2 h after which time t.l.c. (ethyl acetate) indicated complete consumption of starting material (R_f 0.5) and formation of a single product (R_f 0.1). The solvent was removed and the residue purified by flash chromatography (dichloromethane : methanol, 6 : 1) to yield (2R,3S,4R,5R)-6,8-diaza-3,4-dihydroxy-2-(hydroxymethyl)-1-oxa-spiro[4.4.]nonan-7,9-dione **2** (22 mg, 70 %) identical to the material described previously.

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