Synthesis of Trehazolin Analogues containing Modified Aminocyclitol Moieties

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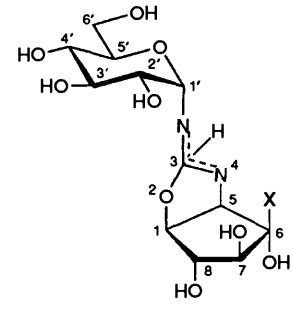
In order to elucidate structure–activity relationships of the trehalase inhibitor trehazolin **1**, three analogues: deoxytrehazolin D-**2**, and de(hydroxymethyl)trehazolin D-**3** and its diastereoisomer L-**3** were synthesized by coupling of the sugar isothiocyanate **18** with the newly prepared aminocyclopentanetetraols D-**10**, D-**26** and L-**26**, followed by cyclisation to give the isourea, and deprotection. In addition, an attempt was made to synthesize the analogue **4**, the aminocyclitol moiety being replaced with the 5a-carba sugar, validamine. A biological assay showed that enantiomers D-**3** and L-**3** are strong inhibitors of trehalase, and, interestingly, the latter isomer, having the unnatural-type structure, is ~50 times more potent.

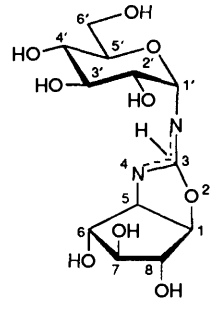
Trehazolin **1** was isolated in 1991 by Ando *et al.*[1] from the culture broth of *Micromonospora* strain SANK 62390, and was shown to exhibit very potent and specific inhibitory activity against trehalase *in vitro* but not *in vivo* assay. In connection with our synthetic studies[2] on glycoside hydrolase inhibitors, the four analogues of compound **1** have therefore been synthesized and subjected to bioassay, in order to elucidate the structure–activity relationship of this kind of inhibitor.

The stereochemistry of the hydroxy groups on the cyclopentane ring of trehazolin **1** has been shown to be important for it to exhibit activity, as exemplified by the complete inactivity of the synthesized 7-epimer.[3,4] Therefore, the aminocyclitol moiety, trehazolamine, 1D-(1,3/2,4,5)-5-amino-1-*C*-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol, has first been chemically modified. Thus, three analogues with modifications to the 6-hydroxymethyl function have been prepared: the deoxy compound D-**2**,† and de(hydroxymethyl) D-**3** and its diastereoisomer L-**3**. The present results contribute to the elucidation of the functions of the hydroxymethyl and tertiary hydroxy groups.

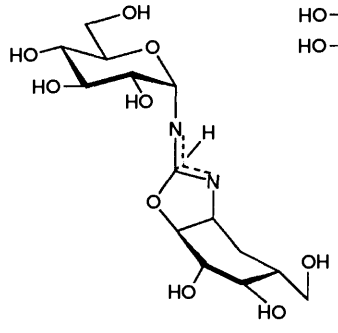
In addition, an attempt was made to replace the aminocyclitol part of compound **1** with validamine[5] **35**, 5a-carba-α-D-glucopyranosylamine, providing the analogue **4**, a structural mimic of α,α-trehalose. Since compound **35** seems to correspond to trehazolamine from the structural viewpoint, this work might explain the role of the branched-chain structures as well as the ring size of the aminocyclitol moiety.



1 X = CH_2OH (Trehazolin)
D-**2** X = Me
D-**3** X = H



L-**3**



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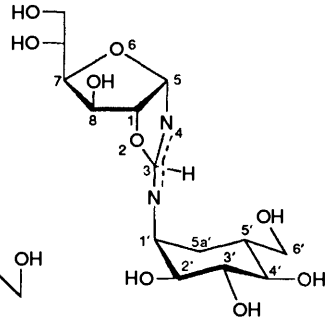

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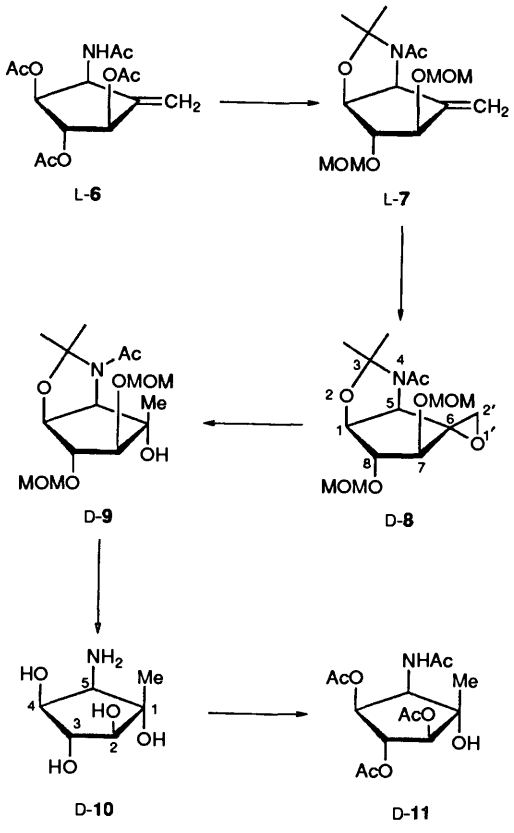
Results and Discussion

Synthesis of the Deoxy Derivative D-**2** *of Trehazolin* **1**.—Construction of the trehazolin structure was conventionally carried out[4,6] by coupling of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl isothiocyanate[7] **18** with free aminocyclitol D-**10**, followed by treatment with yellow mercury(II) oxide and O-debenzylation.

The aminocyclitol moiety, 1D-(1,3/2,4,5)-5-amino-1-methylcyclopentane-1,2,3,4-tetraol‡ D-**10** was synthesized from 1L-(1,3,4/2)-4-acetamido-1,2,3-tri-*O*-acetyl-5-methylenecyclopentane-1,2,3-triol[4,9] L-**6** (Scheme 1). Since direct peracid oxidation of the *exo*-methylene group of compound L-**6** was shown to give selectively the undesired β-spiro epoxide,[9] it was first converted into the new bicyclic derivative L-**7** (61%), the functional groups being protected by methoxymethyl (MOM) and *N*,*O*-isopropylidene groups, since it was expected that their stereoelectronic effects would contribute to alter the reaction course of the epoxidation. Oxidation of compound L-**7** with *m*-chloroperbenzoic acid (MCPBA), as had been expected, proceeded preferentially to give the desired α-spiro epoxide D-**8** (80%). The alcohol D-**9**, readily obtained (73%) by reductive cleavage of epoxide D-**8** with lithium triethylboranuide ($LiBHEt_3$) in tetrahydrofuran (THF), was demonstrated to be a 2:3 mixture of two alcohols on the basis of the ^1H NMR spectrum (270 MHz; $CDCl_3$) measured under ordinary condition. However, hydrolysis of the mixture of the alcohol(s) D-**9** with hydrochloric acid gave, after purification by a column of acidic resin with ammonia, a single amino alcohol D-**10**

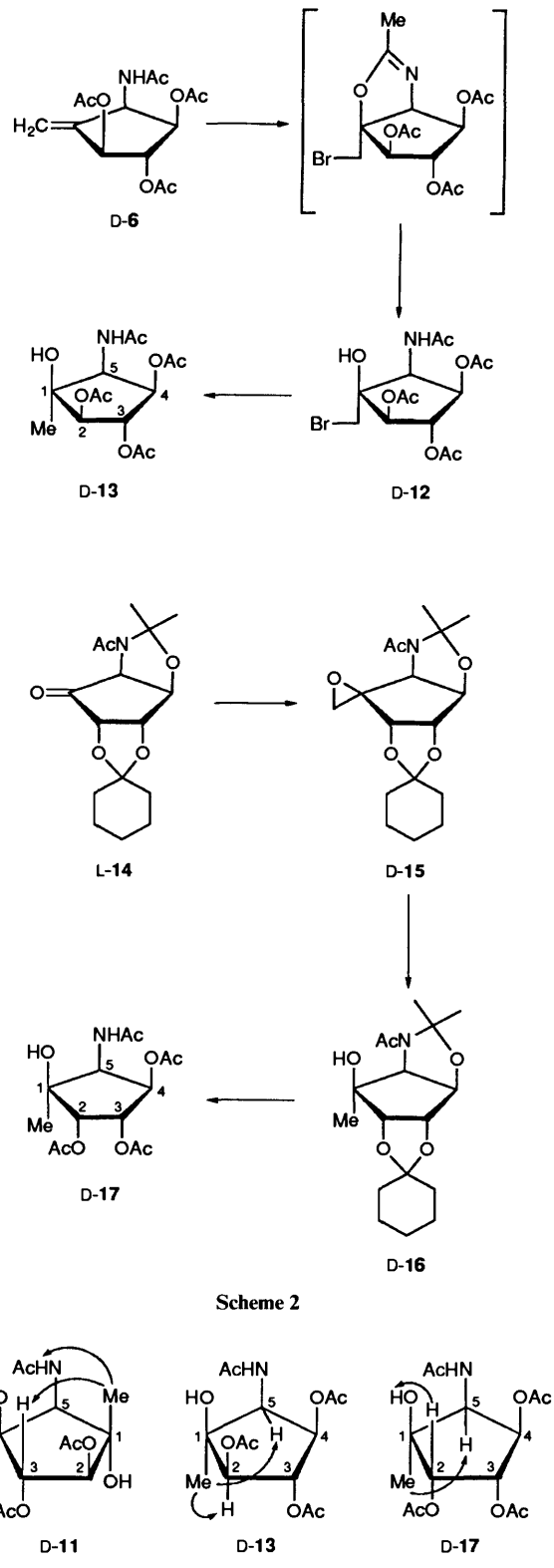
† The D-,L-notation of the compound-numbers **2**, **3**, **19**–**21** and **32**–**34** refers only to that of the absolute configuration of the cyclitol moiety.
‡ In this paper, nomenclature of cyclitols follows IUPAC-IUB 1973 recommendations for cyclitols (ref. 8).



Scheme 1 MOM = methoxymethyl

(94%), which was further characterised by conversion into the sole tetra-*N*,*O*-acetyl derivative D-**11** (82%). In order to confirm the structure of product D-**11**, its 1-epimer D-**13** was also prepared, from compound D-**6*** by the following sequence. Treatment of compound D-**6** with *N*-bromosuccinimide (NBS) proceeded through neighbouring participation of the acetamido group to give the β-bromo alcohol D-**12** (69%) selectively (Scheme 2). Debromination of compound D-**12** with tributyltin hydride afforded the isomeric tetra-*N*,*O*-acetyl derivative D-**13** (93%). An NOE experiment on isomers D-**11** and D-**13** supported the structures assigned. In the former, NOEs were observed between the methyl protons, and amido proton (2.8%) and 3-H (10.6%), respectively. In the latter, there were observed NOEs between the methyl protons, and 2-H (12%) and 5-H (10.3%), respectively (Fig. 1).

Two structures possibly assigned to the ring-opening product D-**9** on the basis of ^{1}H NMR spectroscopy therefore seemed to be attributable to those of rotamers, existing due to the restricted rotation of the acetamido group at room temperature. In fact, the ^{1}H NMR spectrum measured in [$^2H_6$]dimethyl sulfoxide ([$^2H_6$]DMSO) at 70 °C revealed compound D-**9** to be a single compound. These finding suggested that we reinvestigate the structure of the spiro epoxide D-**15** obtained by treatment of the ketone L-**14** with diazomethane. Compound D-**15** was previously stated[9] to consist of an inseparable 2:3 mixture of the isomeric spiro epoxides on the basis of its ^{1}H NMR spectrum, although it gave practically only a single ring-opening product when treated with acetate ion. The ^{1}H NMR spectrum of epoxide D-**15** in [$^2H_6$]DMSO at 70 °C finally showed it to be a single compound. For confirmation of the



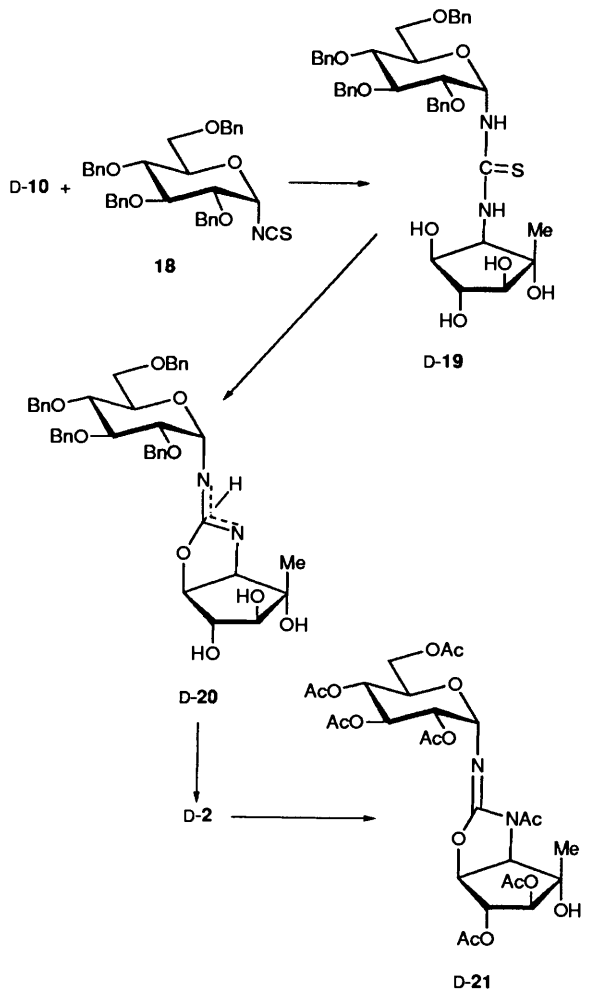
Scheme 2

Fig. 1 NOE experiments on isomers D-**11**, D-**13** and D-**17**

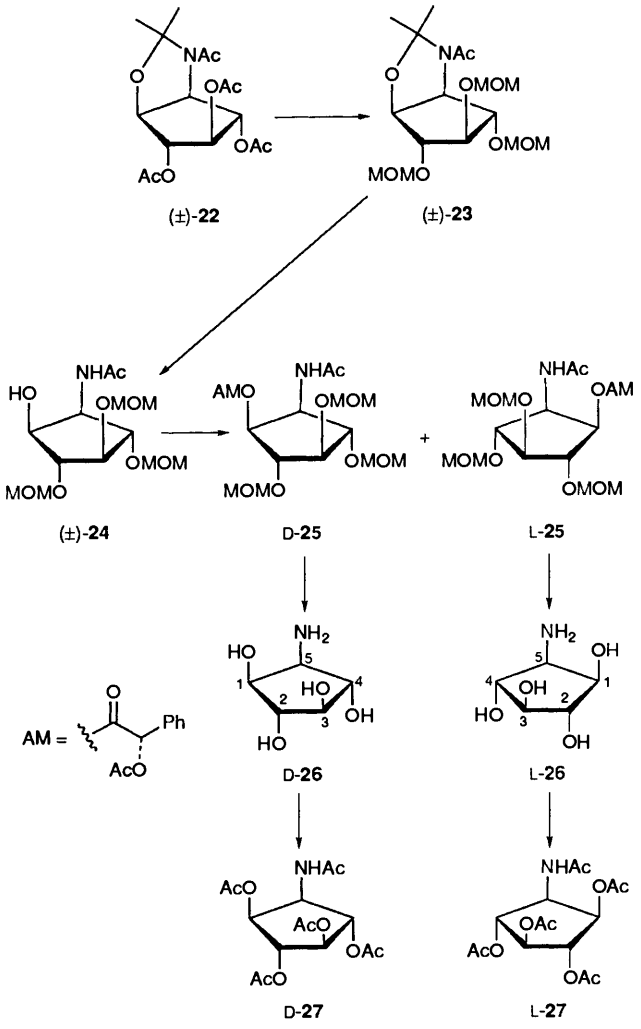
structure, epoxide D-**15** was subjected to a similar reduction with lithium triethylboranuide (⟶ D-**16**, 96%). Conventional hydrolysis of tricycle D-**16** afforded, after acetylation, the tetra-*N*,*O*-acetyl derivative D-**17** (62%), the structure of which was established by an NOE experiment. Thus, NOEs were observed both between 2-H and the OH proton (3.4%) and between 5-H and the methyl protons (11.6%).

Coupling of the amino alcohol D-**10** and the isothiocyanate

* For convenience, a synthesis of the reference compounds D-**13** and D-**17** for structural confirmation has been carried out starting from the enantiomers D-**6** and L-**14**, respectively.



Scheme 3

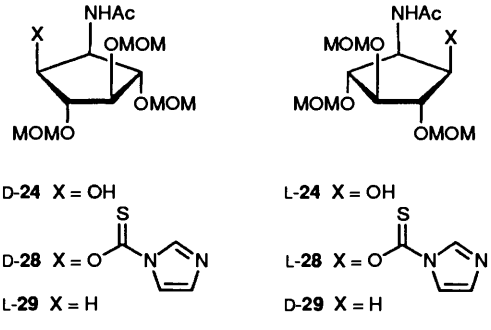


Scheme 4 For convenience, the structures of the racemic compounds (±)-**22**–(±)-**24** depict only one of the respective enantiomers

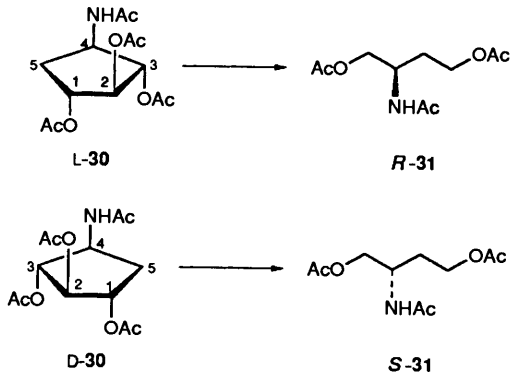
18 was conducted in 75% aq. *N,N*-dimethylformamide (DMF) for 23 h at room temperature gave the thiourea D-**19** (~ 100%), which was treated with an excess of yellow mercury(II) oxide in diethyl ether for 24 h at room temperature to afford the isourea D-**20** (93%) (Scheme 3). Deprotection of the benzyl ether groups was effectively carried out with sodium in liquid ammonia for 15 min at −78 °C to give, after purification by a column of Dowex 50W-X2 (H^+) resin with ammonium hydroxide as eluent, the trehazolin analogue D-**2** (90%). The ^1H NMR spectra of compound D-**2** and its hepta-*N,O*-acetyl derivative D-**21** fully supported the structures assigned.

Synthesis of the De(hydroxymethyl) Derivatives D-**3** *and* L-**3** *of Trehazolin* **1**.—An attempt was first made to resolve the known racemic *N,O*-isopropylidene derivative[9,10] (±)-**22** of (±)-(1,3,5/2,4)-5-acetamido-2,3,4-tri-*O*-acetylcyclopentane-1,2,3,4-tetraol by converting it into the diastereoisomeric esters of an optically active acid. Thus, compound (±)-**22** was O-deacetylated and again protected with MOM groups [⟶ (±)-**23** (94%)] (Scheme 4). O-Deisopropylidenation of (±)-**23** with aq. acetic acid [⟶ (±)-**24** (64%)] followed by esterification with (*S*)-*O*-acetylmandelic acid gave a mixture of the (*S*)-*O*-acetylmandelates, which were easily separable by silica gel column chromatography to give enantiomers D-**25** (48%) and L-**25** (49%). Compounds D-**25** and L-**25** were deprotected to give quantitatively the respective amino alcohols D-**26** and L-**26**, which were further characterised by conversion into the penta-*N,O*-acetyl derivatives D-**27** (91%) and L-**27** (91%).

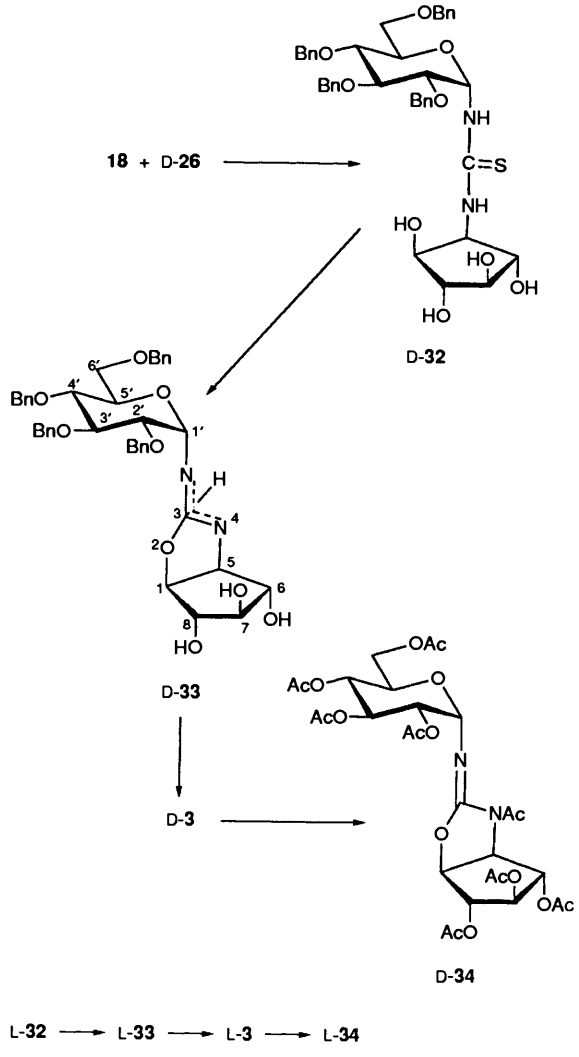
The absolute configurations of compounds D- and L-**25** were then established by correlating them to the optically active 2-aminobutane-1,4-diol[11] derived from aspartic acid. Zemplén O-deacylation of compound D-**25** gave the acetamide D-**24** (97%), whose 1-hydroxy group was removed through a thionocarbamate intermediate by Barton's procedure[12] to give the deoxy derivative [⟶ D-**28** (60%) ⟶ L-**29** (53%)]. Deprotection of triether L-**29** followed by acetylation gave the tetra-*N,O*-acetyl derivative L-**30** (~ 100%). This compound was O-deacylated, the resulting triol was oxidised with excess of sodium periodate, and the dialdehyde obtained was reduced



with sodium boranuide followed by acetylation, to give the known crystalline (*R*)-2-acetamido-1,4-di-*O*-acetylbutane-1,4-diol[4] (*R*-**31**, 64%) (Scheme 5). Likewise, the enantiomer D-**29**



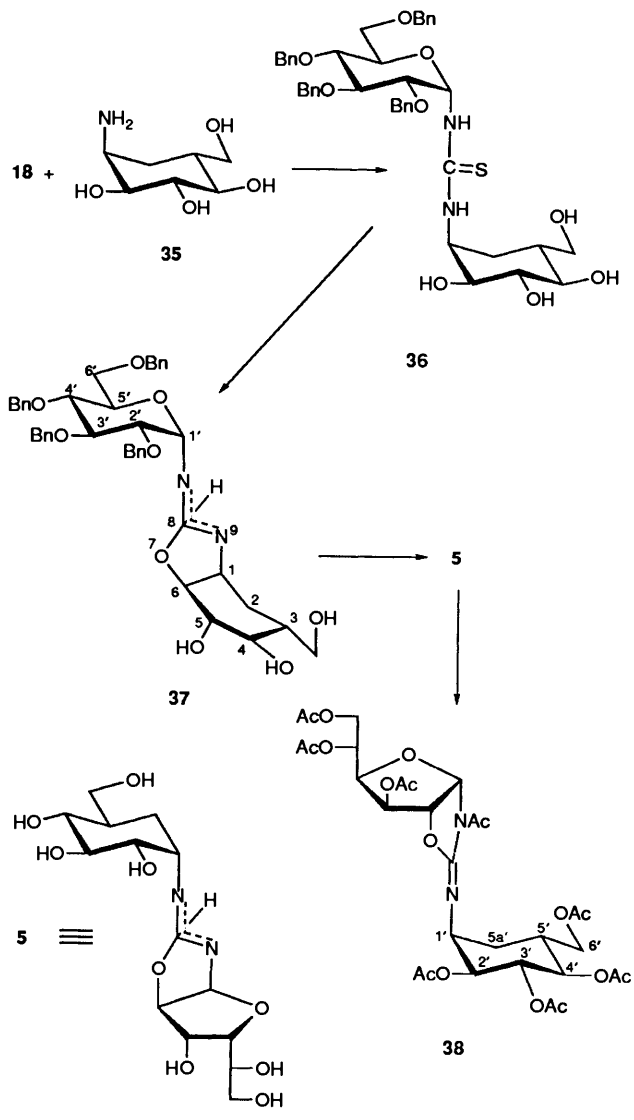
Scheme 5



Scheme 6

obtained (26% overall yield) from L-**25** by the three-step reaction sequence was converted into the enantiomeric triacetyl derivative *S*-**31**[4] (64%) *via* triacetate D-**30**. These experiments convincingly established the absolute stereochemistry of tetraols D-**26** and L-**26**.

Similar coupling of the isothiocyanate **18** and the amino alcohol D-**26** gave the thiourea D-**32** (93%), which was similarly converted into the cyclic isourea D-**33** (~100%) (Scheme 6). Conventional O-debenzylation afforded the trehazolin analogue D-**3** (96%), which was further characterised as the octa-*N*,*O*-acetyl derivative D-**34**. Likewise, the diastereoisomer L-**3** was synthesized in 91% overall yield by the reaction



Scheme 7

sequence (L-**32** ⟶ L-**33** ⟶ L-**3**), starting from the thiourea L-**32** (97%) obtained from isothiocyanate **18** and amino tetraol L-**26**. The ^{1}H NMR spectra of the octa-*N*,*O*-acetyl derivatives D-**34** and L-**34** of tetraol L-**3** were consistent with the proposed structures.

Replacement of the Aminocyclitol Part of Trehazolin **1** *with Validamine*.—Potent trehalase inhibitors, validoxylamine A[13] and its dihydro derivative,[14] 5a-carbatrehalose bonded by way of an imino-linkage, possess analogous structures to that of substrate α,α-trehalose. By structural analogy, the aminocyclitol moiety of trehazolin **1**, including the cyclic isourea structure, would obviously correspond to the other α-D-glucopyranosyl residue when it acts as an enzyme inhibitor. Very recently, 5'a-carbatrehazolin,[15] the D-glucopyranose residue of trehazolin **1** being replaced with the corresponding 5a-carba-D-glucopyranose, was shown to possess potent inhibitory activity comparable to that of the parent trehazolin **1**. Therefore, replacement of the aminocyclitol moiety of trehazolin **1** by the 5a-carba sugar residue might well give analogues having such a structure based on **4**.

Coupling of the isothiocyanate **18** with validamine[3] **35** gave the thiourea **36** (93%), treatment of which with yellow mercury(II) oxide afforded the cyclic isourea **37** (95%), whose ^{1}H NMR spectral data supported its assigned structure where

Table 1 Inhibitory activity of compounds D-**2**, D-**3**, L-**3** and **5** against some sugar hydrolases

Compound	Enzyme [Inhibitory activity (IC_{50})/μg cm^{-3}]		
	Trehalase (silkworm)	Trehalase (porcine)	Isomaltase (rat)
D-**2**	> 100	1.6	23
D-**3**	2.8	0.256	17
L-**3**	0.059	0.005	7
5	> 100	65	> 100

the cyclic isourea is attaching to the validamine moiety which exists in the half-chair form (Scheme 7). O-Debenzylation of compound **37** with sodium in liquid ammonia readily gave the free base (86%), which was further characterised as the octa-*N*,*O*-acetyl derivative **38** (90%). The ^{1}H NMR spectrum of the free base isolated, however, was not consistent with the proposed structure **4**. Thus, the signal of the proton coupled with the anomeric proton (δ 5.82) appeared unexpectedly lowfield (δ 4.88), being assignable to the signal of the proton attached to the cyclic isourea. These data satisfied structure **5**, which seemed to be formed by the preferential rearrangement of the isourea ring located on the cyclopentane of the sugar portion, accompanied by rearrangement of the glycosylamine and its ring contraction: the pyranoid ring (7-oxa-9-azabicyclo[4.3.0]nonane) ⟶ the furanoid ring (2,6-dioxa-4-azabicyclo[3.3.0]octane). In fact, the ^{1}H NMR spectrum of compound **38** indicated the presence of the intact validamine residue and the glucofuranosyl structure, thereby agreeing with the structure assigned. Under basic conditions, such a rearrangement is likely to occur between a cyclic isourea and a neighbouring *cis*-hydroxy group when the steric requirement is satisfied as seen in the trehazolin analogues.[4] The crude free base obtained by O-debenzylation of compound **37**, without purification by a column of acidic resin, was directly acetylated to give mainly compound **38**. TLC analysis of the crude products, however, revealed the presence of a small amount of another octa-*N*,*O*-acetyl derivative, probably of compound **4**, whose ^{1}H NMR spectrum roughly supported the structure proposed. From consideration of its behaviour on TLC, compound **4** was not stable, especially under basic conditions, and readily changed to isomer **5**. Compound **5** is thought to be a 5a′-carbatrehazolin[15] analogue having the structurally related hexofuranose ring instead of trehazolamine.

Biological Assay.—The trehazolin analogues D-**2**, D-**3**, L-**3** and **5** were chiefly subjected to bioassay for trehalase-inhibitory activity (listed in Table 1). They completely lack inhibitory activity against α-amylase, β-glucosidase, α-mannosidase, maltase and sucrase. The inhibitory activity is rather specific and is retained to a considerable extent in both the de(hydroxymethyl) derivatives D-**3** and L-**3**, compared with the parent trehazolin **1**, indicating that the cyclopentane-1,2,3-triol structure with all-*trans* stereochemistry is essential for exhibiting the activity, *i.e.* the important sites for interaction with the enzymes. It is very interesting to note that compound L-**3**, having the unnatural absolute structure, possesses inhibitory activity against silkworm trehalase at least one third of that of trehazolin **1** * and is about 50 times more potent than the natural-type D-**3**. On the other hand, it may be assumed that, in compound D-**2**, the presence of the *C*-methyl function at C-6 would hinder the cyclopentane part in binding the active site of the enzyme through stereoelectronic effects, seemingly resulting in a significant decrease in activity. These results might support the idea that the hydroxymethyl function of trehazolin **1** plays a role in enhancing the binding of the cyclopentanetriol moiety to the enzyme. The above assumption would partly be justified in future by our ongoing chemical modifications of trehazolin **1**, for example, through removal of each of the three ring hydroxy groups and/or conversion of each of their configurations in turn.

* Trehazolin has been shown to possess inhibitory activity (IC_{50} 0.016–0.030 μg cm^{-3}) against silkworm trehalase, the values being somewhat dependent on the conditions used.

Experimental

M.p.s were determined on a MEL-TEMP capillary melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter, and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded for solutions in deuteriochloroform (standard: Me_4Si), hexadeuteriodimethyl sulfoxide (standard: Me_4Si), or dideuterium oxide (standard: acetone) with a JEOL GSX-270 (270 MHz) instrument, and *J* values are given in Hz. IR spectra were measured with a JASCO IR-810 or Hitachi FTS-65 spectrometer. High-resolution mass spectra were measured with a JEOL JMS-DX-302 spectrometer (EI method at 70 eV). TLC was performed on silica gel 60 F-254 (E. Merck, Darmstadt). The silica gel used for column chromatography was Wakogel C-300 (Wako Junyaku Kogyo Co., Osaka, Japan; 300 mesh) or silica gel 60 KO 70 (Katayama Kagaku Kogyo Co., Osaka, Japan). Organic solutions were dried over anhydrous Na_2SO_4 or $MgSO_4$, and concentrated at < 45 °C under diminished pressure.

3,4-N,O-*Isopropylidene Derivative* L-**7** *of* 1L-(1,3,4/2)-4-*Acetamido*-1,2-*di*-O-*methoxymethyl*-5-*methylenecyclopentane*-1,2,3-*triol*.—1L-(1,3,4/2)-4-Acetamido-1,2,3-tri-*O*-acetylcyclopentane-1,2,3-triol[5,7] L-**6** (114 mg, 0.367 mmol) was treated with a catalytic amount of NaOMe in MeOH (2.5 cm^3) for 35 min at room temperature. After neutralisation with Amberlite IR 120B (H^+) resin, the mixture was evaporated to give a crude triol (65.3 mg), which was N,O-isopropylidenated with 2,2-dimethoxypropane (226 mm^3, 1.83 mmol, 5 mol equiv.) and a catalytic amount of toluene-*p*-sulfonic acid (PTSA) in DMF (1.5 cm^3) for 6 h at room temperature. The reaction mixture was neutralised with $NaHCO_3$ and evaporated. The residue was extracted with $CHCl_3$ and the extracts were evaporated to give a syrupy residue (85.2 mg). The residue was treated with a solution of diisopropylethylamine (767 mm^3, 4.40 mmol, 12 mol equiv.) and chloromethyl methyl ether (168 mm^3, 2.20 mmol, 6 mol equiv.) in CH_2Cl_2 (3 cm^3) for 5 h at reflux temperature. After cooling, the reaction mixture was diluted with EtOAc (30 cm^3) and the solution was washed successively with 1 mol dm^{-3} HCl (20 cm^3), saturated aq. $NaHCO_3$ (20 cm^3) and water (20 cm^3 × 2), dried, and evaporated. The residual product was chromatographed on a column of silica gel (5 g) with acetone–toluene (1:4, v/v) as eluent to afford *compound* L-**7** (70.4 mg, 60.9%) as a syrup (Found: C, 56.7; H, 8.4; N, 4.4. $C_{15}H_{25}NO_6$ requires C, 57.1; H, 8.0; N, 4.4%); $[\alpha]_D^{25}$ +34.7 (*c* 1.40, $CHCl_3$); ν_{max}(neat)/cm^{-1} 1650 (NAc); δ_H(270 MHz; $CDCl_3$) 5.40 and 5.15 (each 1 H, dd, $J_{1,6}$ 2.2, $J_{4,6}$ 2.2, 6-H_2), 4.88 and 4.76

(each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.78 and 4.75 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.59 (1 H, ddd, J 2.2, 2.2 and 5.9), 4.52 (1 H, ddd, J 2.2, 2.2 and 5.9), 4.36 (1 H, d, J 5.9), 3.95 (1 H, d, J 5.9), 3.44 and 3.43 (each 3 H, 2 s, 2 × *Me*OCH$_2$), 2.18 (3 H, s, Ac) and 1.62 and 1.56 (each 3 H, 2 s, CMe$_2$).

(1S,5R,6R,7S,8R)-N-*Acetyl*-7,8-*di*(*methoxymethoxy*)-3,3-*dimethylspiro*{2-*oxa*-4-*azabicyclo*[3.3.0]*octane*-6,2′-1′-*oxacyclopropane*}D-**8**.—The *exo*-alkene L-**7** (70.4 mg, 0.223 mmol) was treated with a solution of 70% MCPBA (275 mg, 1.12 mmol, 5 mol equiv.) in 1,2-dichloroethane (2 cm^3) in the presence of a phosphate buffer solution (2 cm^3; 1 mol dm^{-3} Na_2HPO_4–1 mol dm^{-3} NaH_2PO_4, 1:1, v/v) for 10.5 h at room temperature in the dark. The mixture was poured into saturated aq. $NaHCO_3$ (20 cm^3) and the solution was extracted with $CHCl_3$ (30 cm^3 × 3). The extracts were dried and evaporated. The residual product was purified by a column of a silica gel (5 g) with acetone–toluene (1:5, v/v) to give the *epoxide* D-**8** (59.3 mg, 80.1%) as a syrup (Found: C, 54.0; H, 7.5; N, 4.1. $C_{15}H_{25}NO_7$ requires C, 54.4; H, 7.6; N, 4.2%); $[\alpha]_D^{24}$ −23.2 (*c* 1.21, $CHCl_3$); ν_{max}(neat)/cm^{-1} 1650 (NAc); δ_H(270 MHz; $CDCl_3$) 4.80 and 4.76 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.70 and 4.62 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.48 (1 H, d, J 6.6), 4.44 (1 H, d, J 6.6), 4.27 (1 H, d, J 7.0), 4.08 (1 H, d, J 7.0), 3.43 and 3.36 (each 3 H, 2 s, 2 × *Me*OCH$_2$), 3.08 and 2.78 (each 1 H, ABq, J_{gem} 5.1, 2′-H$_2$), 2.07 (3 H, s, Ac) and 1.64 and 1.54 (each 3 H, 2 s, CMe$_2$).

4,5-N,O-*Isopropylidene Derivative* D-**9** *of* 1D-(1,3/2,4,5)-5-*Acetamido*-2,3-di-O-*methoxymethyl*-1-*methylcyclopentane*-1,2,3,4-*tetraol*.—To a solution of the epoxide D-**8** (59.3 mg, 0.179 mmol) in THF (2 cm^3) was added a 1 mol dm^{-3} THF solution of lithium triethylboranuide (305 mm^3, 0.305 mmol, 1.7 mol equiv.) at 0 °C under Ar. The reaction mixture was stirred for 3.3 h at the same temperature. To the mixture was added 28% aq. hydrogen peroxide (1 cm^3), and it was extracted with $CHCl_3$ (30 cm^3 × 3) and the extract was dried and evaporated. The residue was chromatographed on a column of silica gel (5 g) with acetone–toluene (1:4, v/v) as eluent to give *compound* D-**9** (43.3 mg, 72.5%) as a syrup (Found: C, 54.4; H, 8.1; N, 4.5. $C_{15}H_{27}NO_7$ requires C, 54.0; H, 8.2; N, 4.2%); $[\alpha]_D^{25}$ +8.2 (*c* 0.98, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3400 (OH), 1650 and 1630 (NAc); δ_H(270 MHz; [$^2H_6$]DMSO; 70 °C) 4.99 (1 H, s, OH), 4.79 and 4.69 (each 1 H, ABq, J_{gem} 6.2, MeOCH_2), 4.66 and 4.64 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.33 (1 H, dd, $J_{3,4}$ 1.9, $J_{4,5}$ 7.7, 4-H), 4.25 (1 H, d, $J_{4,5}$ 7.7, 5-H), 3.92 (1 H, d, $J_{2,3}$ 8.8, 2-H), 3.71 (1 H, dd, $J_{2,3}$ 8.8, $J_{3,4}$ 1.9, 3-H), 3.33 and 3.30 (each 3 H, 2 s, 2 × *Me*OCH$_2$), 2.12 (3 H, s, Ac), 1.56 and 1.39 (each 3 H, 2 s, CMe$_2$) and 1.02 (3 H, s, Me).

1D-(1,3/2,4,5)-5-*Amino*-1-*methylcyclopentane*-1,2,3,4-*tetraol* D-**10**.—Compound D-**9** (43.3 mg, 0.130 mmol) was treated with 2 mol dm^{-3} HCl (2 cm^3) for 12 h at 80 °C. The mixture was evaporated and the residue was purified by a column of Dowex 50W-X2 (H^+) resin (2 cm^3) with 5% aq. NH_3 to give the amino alcohol D-**10** (20.0 mg, 94.3%) as a syrup, $[\alpha]_D^{27}$ −5.4 (*c* 0.92, water); ν_{max}(neat)/cm^{-1} 3400 (OH and NH_2); δ_H(270 MHz; D_2O) 3.78 (1 H, dd, $J_{3,4}$ 2.8, $J_{4,5}$ 8.1, 4-H), 3.57–3.46 (2 H, m, 2- and 3-H), 2.91 (1 H, d, $J_{4,5}$ 8.1, 5-H) and 0.93 (3 H, s, Me).

1D-(1,3/2,4,5)-5-*Acetamido*-2,3,4-*tri*-O-*acetyl*-1-*methylcyclopentane*-1,2,3,4-*tetraol* D-**11**.—The amino alcohol D-**10** (20.0 mg, 0.123 mmol) was treated with acetic anhydride (1 cm^3) in pyridine (1 cm^3) for 3 h at room temperature. The mixture was evaporated and the residue was chromatographed on a column of silica gel (1 g) with acetone–toluene (1:2, v/v) as eluent to afford the *tetra*-N,O-*acetyl derivative* D-**11** (33.3 mg, 82.0%) as crystals, m.p. 180–181 °C (from EtOH) (Found: C, 51.0; H, 6.2; N, 4.0. $C_{14}H_{21}NO_8$ requires C, 50.8; H, 6.4; N, 4.2%); $[\alpha]_D^{25}$ +2.2 (*c* 1.67, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3380 (OH and NH), 1740 (OAc), 1660 (NAc) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 5.79 (1 H, br d, $J_{5,NH}$ 7.7, NH), 5.24 (1 H, d, $J_{2,3}$ 7.0, 2-H), 5.20 (1 H, dd, $J_{3,4}$ 2.9, $J_{4,5}$ 7.3, 4-H), 5.05 (1 H, dd, $J_{2,3}$ 7.0, $J_{3,4}$ 2.9, 3-H), 4.60 (1 H, dd, $J_{4,5}$ 7.3, $J_{5,NH}$ 7.7, 5-H), 4.52 (1 H, br s, OH), 2.15, 2.12, 2.091 and 2.086 (each 3 H, 4 s, 4 × Ac) and 1.22 (3 H, s, Me).

1D-(1,2,4,5/3)-5-*Acetamido*-2,3,4-*tri*-O-*acetyl*-1-(*bromomethyl*)*cyclopentane*-1,2,3,4-*tetraol* D-**12**.—To a solution of the *exo*-alkene D-**6**[7] (47.4 mg, 0.151 mmol) in 75% aq. THF (2 cm^3) was added NBS (53.9 mg, 0.303 mmol, 2 mol equiv.) at 0 °C, and the mixture was stirred for 4 h at room temperature. The reaction mixture was diluted with saturated aq. $Na_2S_2O_3$ (3 cm^3) and the products were extracted with $CHCl_3$ (30 cm^3 × 3). The organic layer was dried, and evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (5 g) with acetone–toluene (2:5, v/v) as eluent to afford the *bromohydrin* D-**12** (42.8 mg, 69.0%) as a syrup [Found: $(M + H)^+$, 410.0455. $C_{14}H_{21}BrNO_8$ requires *m/z*, 410.0451]; $[\alpha]_D^{23}$ −4.5 (*c* 0.81, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3350 (OH and NH), 1650 (NAc) and 1520 (NH); δ_H(270 MHz; $CDCl_3$) 5.96 (1 H, d, $J_{5,NH}$ 9.9, NH), 5.36 (1 H, dd, $J_{2,3}$ 7.3, $J_{3,4}$ 3.0, 3-H), 5.22 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 8.1, 4-H), 4.82 (1 H, dd, $J_{4,5}$ 8.1, $J_{5,NH}$ 9.9, 5-H), 3.45 and 3.41 (each 1 H, ABq, J_{gem} 11.0, 6-H$_2$), 3.00 (1 H, s, OH) and 2.14, 2.13, 2.08 and 2.04 (each 3 H, 4 s, 4 × Ac).

1D-(1,2,4,5/3)-5-*Acetamido*-2,3,4-*tri*-O-*acetyl*-1-*methylcyclopentane*-1,2,3,4-*tetraol* D-**13**.—To a solution of the bromohydrin D-**12** (19.9 mg, 0.0490 mmol) and a catalytic amount of azoisobutyronitrile (AIBN) in toluene (1 cm^3) was added Bu_3SnH (79.1 mm^3, 0.294 mmol, 6 mol equiv.) at reflux under Ar. The mixture was stirred for 20 min under reflux. Evaporation of the solvent gave an oily residue, which was purified by column chromatography on silica gel (2 g) with acetone–toluene (2:3, v/v) as eluent to give *compound* D-**13** (17.1 mg, 92.5%) as a syrup (Found: C, 50.8; H, 6.4; N, 4.2. $C_{14}H_{21}NO_8$ requires C, 50.5; H, 6.4; N, 4.0%); $[\alpha]_D^{24}$ +19.2 (*c* 0.86, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3390 (OH and NH), 1650 (NAc) and 1530 (NH); δ_H(270 MHz; $CDCl_3$) 6.04 (1 H, d, $J_{5,NH}$ 9.9, NH), 5.34 (1 H, dd, $J_{2,3}$ 7.2, $J_{3,4}$ 3.3, 3-H), 5.23 (1 H, dd, $J_{3,4}$ 3.3, $J_{4,5}$ 8.4, 4-H), 5.09 (1 H, d, $J_{2,3}$ 7.2, 2-H), 4.54 (1 H, dd, $J_{4,5}$ 8.4, $J_{5,NH}$ 9.9, 5-H), 2.18 (1 H, s, OH), 2.14 2.10, 2.06 and 2.05 (each 3 H, 4 s, 4 × Ac) and 1.23 (3 H, s, Me).

2,3-O-*Cyclohexylidene*-4,5-N,O-*isopropylidene Derivative* D-**16** *of* 1D-(1,4,5/2,3)-5-*Acetamido*-1-*methylcyclopentane*-1,2,3,4-*tetraol*.—To a solution of the spiro epoxide D-**15** (39.7 mg, 0.123 mmol) in THF (1.5 cm^3) was added a 1 mol dm^{-3} THF solution of lithium triethylboranuide (270 mm^3, 0.269 mmol, 2.2 mol equiv.) at 0 °C. The mixture was stirred for 2 h at 0 °C and quenched by addition of 28% aq. H_2O_2 (1 cm^3). The mixture was poured into water (10 cm^3) and extracted with $CHCl_3$ (20 cm^3 × 3). The extracts were dried and evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (2 g) with acetone–toluene (1:5, v/v) as eluent to afford *compound* D-**16** (38.3 mg, 96.0%) as a syrup (Found: M^+, 325.1906. $C_{17}H_{27}NO_5$ requires *M*, 325.1889); $[\alpha]_D^{27}$ −21.7 (*c* 2.05, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3400 (OH) and 1630 (NAc); δ_H (270 MHz; $CDCl_3$) 4.65 (1 H, d, J 5.3), 4.44 (1 H, d, J 5.3), 4.43 (1 H, d, J 5.1), 4.27 (1 H, d, J 5.1), 2.33 (1 H, s, OH), 2.17 (3 H, s, Ac) and 1.80–1.38 (19 H, m, C_6H_{10}, CMe$_2$ and Me).

1D-(1,4,5/2,3)-5-*Acetamido*-2,3,4-*tri*-O-*acetyl*-1-*methylcyclopentane*-1,2,3,4-*tetraol* D-**17**.—Compound D-**16** (38.3 mg, 0.118 mmol) was treated with 2 mol dm^{-3} HCl (1 cm^3) for 4 h at 80 °C. The crude amino alcohol obtained was acetylated conventionally and the product was purified by chromatography on

silica gel (2 g) with acetone–toluene (1 : 1, v/v) as eluent to give the *tetra*-N,O-*acetyl derivative* D-**17** (24.0 mg, 61.5%) as crystals, m.p. 188–189 °C [from EtOH–toluene (1 : 3, v/v)] (Found: C, 50.9; H, 6.9; N, 4.1. $C_{14}H_{21}NO_8$ requires C, 50.8; H, 6.4; N, 4.2%); $[\alpha]_D^{26}$ +24.1 (*c* 1.20, $CHCl_3$); ν_{max}(KBr disk)/cm^{-1} 3370 (OH and NH), 1750 (OAc), 1660 (NAc) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 6.19 (1 H, br d, $J_{5,NH}$ 9.5, NH), 5.54 (1 H, dd, $J_{2,3}$ 4.8, $J_{3,4}$ 4.8, 3-H), 5.35 (1 H, dd, $J_{3,4}$ 4.8, $J_{4,5}$ 8.9, 4-H), 5.17 (1 H, d, $J_{2,3}$ 4.8, 2-H), 4.63 (1 H, dd, $J_{4,5}$ 8.9, $J_{5,NH}$ 9.5, 5-H), 3.41 (1 H, br s, OH), 2.12, 2.09, 2.05 and 2.03 (each 3 H, 4 s, 4 × Ac) and 1.27 (3 H, s, Me).

N-[(1R)-(1,3,5/2,4)-2,3,4,5-*Tetrahydroxy*-2-*methylcyclopentyl*]-N′-(2,3,4,6-*tetra*-O-*benzyl*-α-D-*glucopyranosyl*)*thiourea* D-**19**.—A mixture of the free base D-**10** (16.3 mg, 0.0999 mmol) and the glucopyranosyl isothiocyanate[7] **18** (75.5 mg, 0.130 mmol, 1.3 mol equiv.) in 75% aq. DMF (3 cm^3) was stirred for 22.5 h at room temperature. Evaporation of a solvent gave a syrupy residue, which was chromatographed on a column of silica gel (7 g) with EtOAc–hexane (1 : 3, v/v) ⟶ EtOH–toluene (1 : 10, v/v) as eluent to give the *thiourea* D-**19** (74.4 mg, 100%) as a syrup (Found: C, 66.1; H, 6.8; N, 3.7. $C_{41}H_{48}N_2O_9S$ requires C, 66.1; H, 6.5; N, 3.8%); $[\alpha]_D^{26}$ +136 (*c* 1.94, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3300 (OH and NH) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 7.95 (1 H, br d, $J_{1,NH}$ 5.2, NH), 7.33–7.07 (20 H, m, 4 × Ph), 6.75 (1 H, br s, N′H), 5.98 (1 H, br s), 5.31 (1 H, br s), 5.14 (1 H, br s), 4.87 (11 H, m), 4.16 (1 H, br s), 3.97 (1 H, br d, *J* 8.1) and 3.80–3.51 (8 H, m).

(1S,5R,6S,7S,8S)-6-*Methyl*-3-(2′,3′,4′,6′-*tetra*-O-*benzyl*-α-D-*glucopyranosylamino*)-2-*oxa*-4-*azabicyclo*[3.3.0]*oct*-3-*ene*-6,7,8-*triol* D-**20**.—The thiourea D-**19** (66.3 mg, 0.0890 mmol) was stirred and treated with four portions of yellow HgO (each 57.8 mg, 0.267 mmol, 3 mol equiv., total 231 mg, 1.07 mmol, 12 mol equiv.) in diethyl ether (2.5 cm^3) for 24 h at room temperature. The reaction mixture was filtered through a bed of Celite and washed thoroughly with ethanol. The filtrate and washings were combined and evaporated to give the *isourea* D-**20** (59.0 mg, 93.2%) as a syrup (Found: C, 69.6; H, 6.7; N, 3.8. $C_{41}H_{46}N_2O_9$ requires C, 69.3; H, 6.5; N, 3.9%); $[\alpha]_D^{27}$ +68.7 (*c* 0.76, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3350 (OH and NH) and 1660 (C=N); δ_H(270 MHz; $CDCl_3$) 7.34–7.10 (20 H, m, 4 × Ph), 5.46 (1 H, br s, 1′-H), 4.90 and 4.76 (each 1 H, ABq, J_{gem} 11.0, PhCH_2), 4.78 and 4.45 (each 1 H, ABq, J_{gem} 11.0, PhCH_2), 4.69 (1 H, dd, $J_{1,5}$ 8.4, $J_{1,8}$ 1.8, 1-H), 4.59 (2 H, s, PhCH_2), 4.56 and 4.41 (each 1 H, ABq, J_{gem} 12.5, PhCH_2), 4.29 (1 H, d, $J_{1,5}$ 8.4, 5-H), 3.76–3.53 (11 H, m, 7-, 8-, 2′-, 3′-. 4′-, 5′-H, 6′-H_2 and 3 × OH) and 1.25 (3 H, s, Me).

(1S,5R,6S,7S,8S)-3-(α-D-*Glucopyranosylamino*)-6-*methyl*-2-*oxa*-4-*azabicyclo*[3.3.0]*oct*-3-*ene*-6,7,8-*triol* D-**2**.—To a mixture of sodium (174 mg, 7.55 mmol, 100 mol equiv.) in liquid ammonia (5 cm^3) was added a solution of the isourea **20** (53.7 mg, 0.0756 mmol) in THF (1.5 cm^3). The mixture was stirred for 15 min at −78 °C and then an excess of NH_4Cl (606 mg, 11.3 mmol, 150 mol equiv.) was added. Spontaneous evaporation of ammonia gave a residue, which was dissolved in water (5 cm^3) and washed with $CHCl_3$ (5 cm^3 × 3). The water layer was charged on a column of Dowex 50W-X2 (H^+) resin (20 cm^3) and the column was washed with water (100 cm^3). Elution of the column with 0.5 mol dm^{-3} aq. NH_3 gave the free base D-**2** (23.8 mg, 89.8%) as an amorphous powder, $[\alpha]_D^{22}$ +95.2 (*c* 0.64, water); ν_{max}(KBr disk)/cm^{-1} 3450 (OH and NH) and 1660 (C=N); δ_H(270 MHz; D_2O) 5.20 (1 H, d, $J_{1',2'}$ 4.8, 1′-H), 4.60 (1 H, dd, $J_{1,5}$ 9.5, $J_{1,8}$ 3.7, 1-H), 4.09 (1 H, d, $J_{1,5}$ 9.5, 5-H), 3.82 (1 H, dd, $J_{1,8}$ 3.7, $J_{7,8}$ 8.2, 8-H), 3.66 (1 H, dd, $J_{5',6'}$ 2.8, J_{gem} 12.8, 6′-H), 3.65 (1 H, d, $J_{7,8}$ 8.2, 7-H), 3.61 (1 H, dd, $J_{1',2'}$ 4.8, $J_{2',3'}$ 10.3, 2′-H), 3.59 (1 H, dd, $J_{5',6'}$ 4.4, J_{gem} 12.8, 6′-H), 3.51 (1 H, dd, $J_{2',3'}$ 10.3, $J_{3',4'}$ 8.8, 3′-H), 3.42 (1 H, ddd, $J_{4',5'}$ 9.9, $J_{5',6'}$ 2.8 and 4.4, 5′-H), 3.26 (1 H, dd, $J_{3',4'}$ 8.8, $J_{4',5'}$ 9.9, 4′-H) and 0.94 (3 H, s, Me).

(1S,5R,6S,7S,8R)-4-N,7-O,8-O-*Triacetyl*-3-(2′,3′,4′,6′-*tetra*-O-*acetyl*-α-D-*glucopyranosylimino*)-2-*oxa*-4-*azabicyclo*[3.3.0]-*octane*-6,7,8-*triol* D-**21**.—The free base **2** (13.8 mg, 0.0394 mmol) was acetylated with acetic anhydride in pyridine conventionally. The crude product was chromatographed on a column of silica gel (1 g) with acetone–toluene (1 : 4, v/v) as eluent to give the *acetamido hexaacetate* D-**21** (18.4 mg, 72.4%) as a syrup (Found: C, 50.0; H, 6.1; N, 4.3. $C_{27}H_{36}N_2O_{16}$ requires C, 50.3; H, 5.6; N, 4.4%); $[\alpha]_D^{26}$ +120 (*c* 0.92, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3500 (OH), 1750 (OAc), 1700 (C=N and NAc); δ_H(270 MHz; $CDCl_3$) 5.58 (1 H, d, $J_{1',2'}$ 4.3, 1′-H), 5.45 (1 H, dd, $J_{2',3'}$ 10.3, $J_{3',4'}$ 9.5, 3′-H), 5.42 (1 H, d, $J_{7,8}$ 9.2, 7-H), 5.13 (1 H, dd, $J_{1,8}$ 3.3, $J_{7,8}$ 9.2, 8-H), 5.09 (1 H, dd, $J_{3',4'}$ 9.5, $J_{4',5'}$ 9.9, 4′-H), 5.06 (1 H, dd, $J_{1',2'}$ 4.3, $J_{2',3'}$ 10.3, 2′-H), 4.78 (1 H, d, $J_{1,5}$ 9.5, 5-H), 4.72 (1 H, dd, $J_{1,5}$ 9.5, $J_{1,8}$ 3.3, 1-H), 4.29–4.09 (3 H, m, 5′-H and 6′-H_2), 3.71 (1 H, s, OH), 2.70, 2.11, 2.099, 2.095, 2.04, 2.01 and 1.98 (each 3 H, 7 s, 7 × Ac) and 1.18 (3 H, s, Me).

1,5-N,O-*Isopropylidene Derivative* (±)-**23** *of* DL-(1,3,5/2,4)-5-*Acetamido*-2,3,4-*tri*-O-(*methoxymethyl*)*cyclopentane*-1,2,3,4-*tetraol*.—To a solution of the 1,5-*N*,*O*-isopropylidene derivative (±)-**22** of DL-(1,3,5/2,4)-5-acetamido-2,3,4-tri-*O*-acetylcyclopentane-1,2,3,4-tetraol[9,10] in CH_2Cl_2 (12 cm^3) were added *N*,*N*-diisopropylethylamine (8.6 cm^3, 50.9 mmol, 18 mol equiv.) and chloromethyl methyl ether (1.9 cm^3, 25.5 mmol, 9 mol equiv.), and the mixture was stirred for 4 h under reflux. The cooled reaction mixture was diluted with $CHCl_3$ (150 cm^3) and washed successively with 1 mol dm^{-3} HCl (100 cm^3), saturated aq. $NaHCO_3$ (100 cm^3) and water (100 cm^3 × 2). The organic layer was dried and evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (35 g) with acetone–toluene (1 : 4, v/v) as eluent to afford the *tris*(*methoxymethyl ether*) (±)-**23** (961 mg, 93.6%) as prisms, m.p. 45.0–46.0 °C (from EtOH) (Found: C, 52.5; H. 7.7; N, 4.0. $C_{16}H_{29}NO_8$ requires C, 52.9; H, 8.0; N, 3.9%); ν_{max}(neat)/cm^{-1} 1660 (NAc); δ_H(270 MHz; $CDCl_3$) 4.89 and 4.68 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.80 and 4.70 (each 1 H, ABq, J_{gem} 6.8, MeOCH_2), 4.74 and 4.71 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.37 (1 H, d, *J* 5.9), 4.27 (1 H, dd, *J* 5.5 and 5.5), 4.15–4.08 (3 H, m), 3.41, 3.40 and 3.39 (each 3 H, 3 s, 3 × *Me*OCH$_2$), 2.23 (3 H, s, Ac) and 1.69 and 1.54 (each 3 H, 2 s, CMe_2).

DL-(1,3/2,4,5)-5-*Acetamido*-1,2,3-*tri*-O-(*methoxymethyl*)-*cyclopentane*-1,2,3,4-*tetraol* (±)-**24**.—The tris(methoxymethyl ether) (±)-**23** (849 mg, 2.34 mmol) was treated with 60% aq. AcOH (15 cm^3) for 2 h at 60 °C, and was then evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (30 g) with acetone–toluene (1 : 1, v/v) as eluent to afford *compound* (±)-**24** (482 mg, 63.8%) as crystals, m.p. 83.5–85.0 °C (from ethyl acetate) (Found: C, 48.2; H, 7.6; N, 4.3. $C_{13}H_{25}NO_8$ requires C, 48.3; H, 7.8; N, 4.3%); ν_{max}(neat)/cm^{-1} 3300 (OH and NH), 1640 (NAc) and 1560 (NH); δ_H(270 MHz; $CDCl_3$) 6.29 (1 H, br d, $J_{5,NH}$ 5.7, NH), 4.83–4.71 (6 H, m, 3 × MeOCH_2), 4.20–4.10 (2 H, m), 4.02–3.97 (2 H, m), 3.82 (1 H, ddd, *J* 1.5, 4.2 and 6.3), 3.43, 3.392 and 3.387 (each 3 H, 3 s, 3 × *Me*OCH$_2$), 3.37 (1 H, br s, OH) and 2.03 (3 H, s, Ac).

1D-D-**25** *and* 1L-(1,3,5/2,4)-5-*Acetamido*-1-O-[(2S)-2-*acetoxy*-2-*phenylacetyl*]-2,3,4-*tri*-O-(*methoxymethyl*)*cyclopentane*-1,2,3,4-*tetraol* L-**25**.—To a mixture of the alcohol (±)-**24** (550 mg, 1.70 mmol), 4-(dimethylamino)pyridine (DMAP) (41.6 mg, 0.340 mmol, 0.2 mol equiv.) and (*S*)-(+)-*O*-acetylmandelic acid (397 mg, 2.04 mmol, 1.2 mol equiv.) in CH_2Cl_2 (10 cm^3) was added a solution of dicyclohexylcarbodiimide (DCC) (409 mg, 2.04 mmol, 1.2 mol equiv.) in CH_2Cl_2 (8 cm^3) at 0 °C, and it

was stirred for 10 min at the same temperature. After addition of hexane (50 cm^3), the mixture was filtered through a bed of Celite, and the filtrate was diluted with EtOAc (50 cm^3), washed successively with 1 mol dm^{-3} HCl (80 cm^3), saturated aq. $NaHCO_3$ (80 cm^3) and water (80 cm^3 × 2) and dried, and evaporated. The syrupy residue was chromatographed on a column of silica gel (40 g) with acetone–toluene (1 : 5, v/v) as eluent to afford, first, the L-*acetylmandelate* L-**25** (417 mg, 49.1%) as a syrup (Found: C, 55.2; H, 6.3; N, 2.8. $C_{23}H_{33}NO_{11}$ requires C, 55.3; H, 6.7; N, 2.8%); $[\alpha]_D^{20}$ +108 (*c* 0.95, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3300 (NH), 1750 (C=O), 1660 (NAc) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 7.52–7.38 (5 H, m, Ph), 5.94 [1 H, s, Ph(OAc)C*H*CO], 5.30 (1 H, d, $J_{5,NH}$ 8.4, NH), 5.16 (1 H, ddd, $J_{1,2}$ 1.0, $J_{1,3}$ 1.0, $J_{1,5}$ 5.3, 1-H), 4.76 and 4.72 (each 1 H, ABq, J_{gem} 6.8, MeOCH_2), 4.71 and 4.66 (each 1 H, ABq, J_{gem} 7.0, MeOCH_2), 4.71 and 4.64 (each 1 H, ABq, J_{gem} 7.0, MeOCH_2), 4.46 (1 H, ddd, $J_{1,5}$ 5.3, $J_{4,5}$ 9.5, $J_{5,NH}$ 8.4, 5-H), 3.99 (1 H, ddd, $J_{1,3}$ 1.0, $J_{2,3}$ 3.4, $J_{3,4}$ 5.9, 3-H), 3.95 (1 H, dd, $J_{1,2}$ 1.0, $J_{2,3}$ 3.4, 2-H), 3.83 (1 H, dd, $J_{3,4}$ 5.9, $J_{4,5}$ 9.5, 4-H), 3.40, 3.36 and 3.32 (each 3 H, 3 s, 3 × *Me*OCH$_2$) and 2.23 and 1.65 (each 3 H, 2 s, 2 × Ac).

The second fraction gave the D-*acetylmandelate* D-**25** (405 mg, 47.6%) as a syrup (Found: C, 55.0; H, 6.4; N, 2.9%); $[\alpha]_D^{20}$ −5.1 (*c* 0.92, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3300 (NH), 1740 (C=O), 1660 (NAc) and 1550 (NH); δ_H(270 MHz; $CDCl_3$) 7.51–7.38 (5 H, m, Ph), 6.01 (1 H, d, $J_{5,NH}$ 8.4, NH), 5.80 [1 H, s, Ph(OAc)C*H*CO], 5.18 (1 H, ddd, $J_{1,2}$ 1.0, $J_{1,3}$ 1.0, $J_{1,5}$ 4.8, 1-H), 4.76 and 4.70 (each 1 H, ABq, J_{gem} 6.8, MeOCH_2), 4.66 and 4.65 (each 1 H, ABq, J_{gem} 6.3, MeOCH_2), 4.60 (1 H, dd, $J_{1,5}$ 4.8, $J_{5,NH}$ 8.4, 5-H), 4.58 and 4.54 (each 1 H, ABq, J_{gem} 7.7, MeOCH_2), 4.03–3.94 (2 H, m, 3- and 4-H), 3.72 (1 H, dd, $J_{1,2}$ 1.0, $J_{2,3}$ 2.9, 2-H), 3.37, 3.31 and 3.24 (each 3 H, 3 × *Me*OCH$_2$) and 2.21 and 1.95 (each 3 H, 2 s, 2 × Ac).

1D-(1,3,5/2,4)-5-*Aminocyclopentane*-1,2,3,4-*tetraol* D-**26**.—The acetylmandelate D-**25** (144 mg, 0.288 mmol) was treated with 2 mol dm^{-3} HCl (3 cm^3) for 5 h at 80 °C. After removal of the reagent, chromatography of the product with a column of Dowex 50W-X2 (H^+) resin (4 cm^3) with 2 mol dm^{-3} aq. NH_3 gave the amino alcohol D-**26** (43.0 mg, 100%) as a syrup, $[\alpha]_D^{24}$ −6.8 (*c* 1.12, water); ν_{max}(neat)/cm^{-1} 3350 (OH and NH_2); δ_H(270 MHz; D_2O) 3.75 (1 H, dd, *J* 7.3 and 4.4), 3.63–3.46 (3 H, m) and 2.93 (1 H, dd, *J* 7.3 and 6.6, 5-H).

1L-(1,3,5/2,4)-5-*Aminocyclopentane*-1,2,3,4-*tetraol* L-**26**.—The acetylmandelate L-**25** (150 mg, 0.300 mmol) was treated, as in the preparation of compound D-**26**, to give the amino alcohol L-**26** (44.8 mg, 100%) as a syrup, $[\alpha]_D^{25}$ +6.0 (*c* 1.78, water). The ^{1}H NMR data were identical with those of the enantiomer D-**26**.

1D-(1,3,5/2,4)-5-*Acetamido*-1,2,3,4-*tetra*-O-*acetylcyclopentane*-1,2,3,4-*tetraol* D-**27**.—The amino alcohol D-**26** (49.4 mg, 0.331 mmol) was acetylated conventionally and the crude product was chromatographed on a column of silica gel (3 g) with acetone–toluene (1 : 3, v/v) as eluent to afford the *penta*-N,O-*acetyl derivative* D-**27** (108 mg, 90.8%) as a syrup (Found: C, 49.8; H, 6.2; N, 4.1. $C_{15}H_{21}NO_9$ requires C, 50.1; H, 5.9; N, 3.9%); $[\alpha]_D^{23}$ −13.9 (*c* 0.95, $CHCl_3$). The ^{1}H NMR data were identical with those of the racemate.[10]

1L-(1,3,5/2,4)-5-*Acetamido*-1,2,3,4-*tetra*-O-*acetylcyclopentane*-1,2,3,4-*tetraol* L-**27**.—The amino alcohol L-**26** was similarly converted into the *penta*-N,O-*acetyl derivative* L-**27** (100 mg, 90.5%) as a syrup (Found: C, 50.0; H, 6.3; N, 4.0%); $[\alpha]_D^{22}$ +10.4 (*c* 0.96, $CHCl_3$). The ^{1}H NMR data were identical with those of the enantiomer D-**27**.

1D-(1,3,5/2,4)-5-*Acetamido*-2,3,4-*tri*-O-(*methoxymethyl*)-*cyclopentane*-1,2,3,4-*tetraol* D-**24** *by Zemplén Deacylation*.—A solution of the acetylmandelate D-**25** (405 mg, 0.810 mmol) in CH_2Cl_2 (8 cm^3) was treated with 1 mol dm^{-3} methanolic sodium methoxide (1 cm^3) for 15 min at room temperature. The reaction mixture was diluted with $CHCl_3$ (100 cm^3) and washed with water (50 cm^3). The water layer was extracted with $CHCl_3$ (100 cm^3 × 3) and the extracts were dried. Evaporation of the solvent gave a residue, which was chromatographed on a column of silica gel (8 g) with acetone–toluene (1 : 1, v/v) as eluent to afford the *alcohol* D-**24** (253 mg, 96.5%) as a syrup (Found: C, 48.3; H, 7.5; N, 4.4. $C_{13}H_{25}NO_8$ requires C, 48.3; H, 7.8; N, 4.3%); $[\alpha]_D^{21}$ −31.4 (*c* 1.03, acetone). The ^{1}H NMR data were identical with those of the racemate (±)-**24**.

1L-(1,3,5/2,4)-5-*Acetamido*-2,3,4-*tri*-O-(*methoxymethyl*)-*cyclopentane*-1,2,3,4-*tetraol* L-**24** *by Zemplén Deacylation*.—The acetylmandelate L-**25** (417 mg, 0.835 mmol) was treated as described above to give the *alcohol* L-**24** (252 mg, 93.5%) as a syrup (Found: C, 48.2; H, 7.5; N, 4.4%); $[\alpha]_D^{21}$ +34.0 (*c* 0.99, acetone). The ^{1}H NMR data were identical with those of the racemate (±)-**24**.

1D-(1,3,5/2,4)-5-*Acetamido*-1-O-(*imidazolyl*-1-*thiocarbonyl*)-2,3,4-*tri*-O-(*methoxymethyl*)*cyclopentane*-1,2,3,4-*tetraol* D-**28**.—The alcohol D-**24** (92.1 mg, 0.285 mmol) was treated with 1,1′-thiocarbonyldiimidazole (152 mg, 0.855 mmol, 3 mol equiv.) in THF (4 cm^3) for 1.5 h under reflux. Removal of the solvent gave a syrupy residue, which was chromatographed on a column of silica gel (5 g) with acetone–toluene (1 : 2, v/v) as eluent to afford the *xanthate* D-**28** (74.0 mg, 59.9%) as a syrup (Found: C, 47.3; H, 6.6; N, 9.8. $C_{17}H_{27}N_3O_8S$ requires C, 47.1; H, 6.3; N, 9.7%); $[\alpha]_D^{25}$ −63.1 (*c* 0.77, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3280 (NH), 1660 (NAc) and 1560 (NH); δ_H(270 MHz; $CDCl_3$) 8.35, 7.63 and 7.05 (each 1 H, 3 br s, imidazole), 6.26 (1 H, d, $J_{5,NH}$ 7.0, NH), 5.90 (1 H, dd, $J_{1,2}$ 1.9, $J_{1,5}$ 2.6, 1-H), 4.82 and 4.73 (each 1 H, ABq, J_{gem} 7.0, MeOCH_2), 4.78 and 4.74 (each 1 H, ABq, J_{gem} 7.0, MeOCH_2), 4.74 and 4.70 (each 1 H, ABq, J_{gem} 7.0, MeOCH_2), 4.65 (1 H, ddd, $J_{1,5}$ 2.6, $J_{4,5}$ 2.6, $J_{5,NH}$ 7.0, 5-H), 4.16 (1 H, dd, $J_{1,2}$ 1.9, $J_{2,3}$ 3.4, 2-H), 4.12–4.07 (2 H, m, 3- and 4-H), 3.404, 3.398 and 3.37 (each 3 H, 3 s, 3 × *Me*OCH$_2$) and 1.97 (3 H, s, Ac).

1L-(1,3,5/2,4)-5-*Acetamido*-1-O-(*imidazol*-1-*ylthiocarbonyl*)-2,3,4-*tri*-O-(*methoxymethyl*)*cyclopentane*-1,2,3,4-*tetraol* L-**28**.—The alcohol L-**24** (47.4 mg, 0.147 mmol) was similarly converted into the *xanthate* L-**28** (34.7 mg, 54.6%) as a syrup (Found: C, 47.4; H, 6.5; N, 9.7%); $[\alpha]_D^{25}$ +73.7 (*c* 0.98, $CHCl_3$). The ^{1}H NMR data were identical with those of the enantiomer D-**28**.

1L(1,3/2,4)-4-*Acetamido*-1,2,3-*tri*-O-(*methoxymethyl*)*cyclopentane*-1,2,3-*triol* L-**29**.—To a solution of AIBN (1.6 mg, 0.009 28 mmol, 0.1 mol equiv.) and Bu_3SnH (74.8 mm^3, 0.278 mmol, 3 mol equiv.) in toluene (0.5 cm^3) was added a solution of the xanthate D-**28** (40.2 mg, 0.0928 mmol) in toluene (1.5 cm^3) under Ar. The mixture was stirred for 30 min under reflux. After cooling, it was diluted with $CHCl_3$ (30 cm^3) and washed with water (10 cm^3). The aqueous layer was thoroughly extracted with $CHCl_3$ (30 cm^3 × 2), and the combined organic solution was dried and evaporated. The syrupy residue was chromatographed on a column of silica gel (2 g) with acetone–toluene (1 : 2, v/v) as eluent to afford *compound* L-**29** (15.2 mg, 53.1%) as a syrup (Found: C, 50.6; H, 8.6; N, 4.9. $C_{13}H_{25}NO_7$ requires C, 50.8; H, 8.2; N, 4.6%); $[\alpha]_D^{26}$ −47.3 (*c* 1.23, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3280 (NH), 1650 (NAc) and 1560 (NH); δ_H(270 MHz; $CDCl_3$) 5.93 (1 H, d, $J_{4,NH}$ 4.4, NH), 4.75 and 4.71 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.73 and 4.72 (each 1 H, ABq, J_{gem} 6.6, MeOCH_2), 4.67 and 4.63 (each 1 H, ABq, J_{gem} 7.3, MeOCH_2), 4.25 (1 H, dddd, $J_{3,4}$ 4.4, $J_{4,5}$ 4.4 and 4.4, $J_{4,NH}$ 4.4, 4-H), 4.08–4.03 (2 H, m, 1- and 2-H), 3.76 (1 H, dd, $J_{2,3}$ 4.4, $J_{3,4}$

4.4, 3-H), 3.39, 3.38 and 3.37 (each 3 H, 3 s, 3 × *Me*OCH$_2$), 2.30 (1 H, ddd, $J_{1,5}$ 2.7, $J_{4,5}$ 4.4, J_{gem} 8.2, 5-H), 1.97 (3 H, s, Ac) and 1.87 (1 H, ddd, $J_{1,5}$ 4.4, $J_{4,5}$ 4.4, J_{gem} 8.2, 5-H).

1D-(1,3/2,4)-4-*Acetamido*-1,2,3-*tri*-O-(*methoxymethyl*)*cyclopentane*-1,2,3-*triol* D-**29**.—The xanthate L-**28** (46.3 mg, 0.107 mmol) was similarly converted into the *compound* D-**29** (16.9 mg, 51.5%) as a syrup (Found: C, 50.6; H, 8.1; N, 4.6%); $[\alpha]_D^{26}$ +46.7 (*c* 1.46, $CHCl_3$). The ^{1}H NMR data were identical with those of the enantiomer L-**29**.

1L-(1,3/2,4)-4-*Acetamido*-1,2,3-*tri*-O-*acetylcyclopentane*-1,2,3-*triol* L-**30**.—Compound L-**29** (24.6 mg, 0.0800 mmol) was hydrolysed with 2 mol dm^{-3} HCl (1 cm^3) and was then acetylated with acetic anhydride (0.5 cm^3) in pyridine (0.5 cm^3) conventionally. Chromatography of silica gel (1.5 g) with acetone–toluene (1:3, v/v) gave the *tetra*-N,O-*acetyl derivative* L-**30** (24.1 mg, 100%) as a syrup (Found: C, 51.5; H, 6.7; N, 4.5. $C_{13}H_{19}NO_7$ requires C, 51.8; H, 6.4; N, 4.7%); $[\alpha]_D^{27}$ −12.9 (*c* 1.01, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3300 (NH), 1740 (OAc), 1660 (NAc) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 6.03 (1 H, d, $J_{4,NH}$ 6.6, NH), 5.20 (1 H, ddd, $J_{1,2}$ 3.7, $J_{2,3}$ 6.2, $J_{2,5}$ 1.3, 2-H), 5.13–5.06 (2 H, m, 1- and 3-H), 4.42 (1 H, dddd, $J_{3,4}$ 6.6, $J_{4,5}$ 7.2 and 7.8, $J_{4,NH}$ 6.6, 4-H), 2.34 (1 H, dddd, $J_{1,5}$ 2.4, $J_{2,5}$ 1.3, $J_{4,5}$ 7.8, J_{gem} 14.8, 5-H), 2.10, 2.08 and 1.96 (3, 6 and 3 H, 3 s, 4 × Ac) and 2.07–1.94 (1 H, m, 5-H).

1D-(1,3/2,4)-4-*Acetamido*-1,2,3-*tri*-O-*acetylcyclopentane*-1,2,3-*triol* D-**30**.—Compound D-**29** (25.9 mg, 0.0843 mmol) was similarly converted into the *tetra*-N,O-*acetyl derivative* D-**30** (23.7 mg, 93.3%) (Found: C, 51.6; H, 6.5; N, 4.8%); $[\alpha]_D^{25}$ +14.1 (*c* 1.19, $CHCl_3$). The ^{1}H NMR data were identical with those of the enantiomer L-**30**.

(2R)-2-*Acetamido*-1,4-*di*-O-*acetylbutane*-1,4-*diol* (R)-**31**.—Compound L-**30** (20.2 mg, 0.0670 mmol) was treated with 1 mol dm^{-3} methanolic sodium methoxide (1 cm^3). After neutralisation with Amberlite IR 120B (H^+) resin, the mixture was evaporated to give the crude triol (11.4 mg), which was oxidised with $NaIO_4$ (83.5 mg, 0.401 mmol, 6 mol equiv.) in water (1 cm^3) for 2 h at room temperature. The reaction mixture was neutralised with aq. $NaHCO_3$, saturated with NaCl, and extracted with THF (25 cm^3 × 5). The extracts were dried over $MgSO_4$ and evaporated. The resulting dialdehyde was treated with $NaBH_4$ (49.2 mg, 1.34 mmol, 20 mol equiv.) in methanol (1 cm^3) for 1 h at room temperature. The mixture was neutralised with AcOH and evaporated to give a crude diol, which was acetylated conventionally. Chromatography on silica gel (1 g) with acetone–toluene (1:3, v/v) afforded the tri-*N*,*O*-acetyl derivative (*R*)-**31** (9.9 mg, 64.3%) as crystals, m.p. 118–119 °C (from EtOH) (Found: C, 51.7; H, 7.8; N, 5.8. Calc. for $C_{10}H_{17}NO_5$: C, 51.9; H, 7.4; N, 6.1%); $[\alpha]_D^{28}$ +40.2 (*c* 0.50, $CHCl_3$). The ^{1}H NMR data were identical with those of an authentic sample.[4]

(2S)-2-*Acetamido*-1,4-*di*-O-*acetylbutane*-1,4-*diol* (S)-**31**.—Compound D-**30** (19.4 mg, 0.0644 mmol) was similarly converted into the tri-*N*,*O*-acetyl derivative (*S*)-**31** (9.6 mg, 64.2%), m.p. 113–114 °C (from EtOH) (Found: C, 51.5; H, 7.7; N, 5.9%); $[\alpha]_D^{25}$ −40.8 (*c* 0.48, $CHCl_3$). The ^{1}H NMR data were identical with those of an authentic sample.[4]

N-[(1D)-(1,2,4/3,5)-2,3,4,5-*Tetrahydroxycyclopentyl*]-N′-(2′,3′,4′,6′-*tetra*-O-*benzyl*-α-D-*glucopyranosyl*)*thiourea* D-**32**.—A mixture of the amino alcohol D-**26** (42.6 mg, 0.286 mmol) and the isothiocyanate **18** (191 mg, 0.327 mmol, 1.14 mol equiv.) in 75% aq. DMF (5 cm^3) was stirred for 2 h at room temperature, and was then evaporated. The residue was chromatographed on a column of silica gel (8 g) with EtOAc–hexane (1:2, v/v) ⟶ EtOH–toluene (1:7, v/v) as eluent to give the *thiourea* D-**32** (194 mg, 92.7%) as a syrup (Found: C, 65.3; H, 6.5; N, 3.9. $C_{40}H_{46}N_2O_9S$ requires C, 65.7; H, 6.3; N, 3.8%); $[\alpha]_D^{26}$ +104 (*c* 1.57, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3320 (OH and NH) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 7.82 (1 H, br s, NH), 7.42–7.01 (20 H, m, 4 × Ph), 6.97 (1 H, br s, N′H), 5.65 (1 H, br s, 1′-H), 5.21 (1 H, br s, OH), 5.05–4.19 (11 H, m, 1-H, 2 × OH and 4 × PhCH_2), 4.12–3.43 (10 H, m, 2-, 3-, 4-, 5-, 2′-, 3′-, 4′- and 5′-H and 6′-H_2) and 2.58 (1 H, br s, OH).

N-[1L-(1,2,4/3,5)-2,3,4,5-*Tetrahydroxycyclopentyl*]-N′-(2′,3′,4′,6′-*tetra*-O-*benzyl*-α-D-*glucopyranosyl*)*thiourea* L-**32**.—The amino alcohol L-**26** (40.0 mg, 0.268 mmol) and isothiocyanate **18** (185 mg, 0.318 mmol, 1.19 mol equiv.) were similarly treated to afford the *thiourea* L-**32** (190 mg, 96.9%) as a syrup (Found: C, 65.5; H, 6.2; N, 3.9%); $[\alpha]_D^{26}$ +67.1 (*c* 1.64, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3320 (OH and NH) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 7.95 (1 H, br s, NH), 7.35–7.07 (20 H, m, 4 × Ph), 6.72 (1 H, br s, N′H), 5.26 (1 H, br s, 1′-H), 5.03 (1 H, s, OH), 4.88 and 4.73 (each 1 H, ABq, J_{gem} 11.0, PhCH_2), 4.76 and 4.42 (each 1 H, ABq, J_{gem} 11.0, PhCH_2), 4.63 and 4.57 (each 1 H, ABq, J_{gem} 11.9, PhCH_2), 4.45 and 4.37 (each 1 H, ABq, J_{gem} 11.4, PhCH_2), 4.35 (1 H, br s, OH), 4.23 (1 H, br s, OH), 4.15 (1 H, br s, 1-H), 3.92–3.55 (9 H, m, 2-, 3-, 4-, 5-, 2′-, 3′-, and 5′-H and 6′-H_2), 3.35 (1 H, dd, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.2, 4′-H) and 2.44 (1 H, br s, OH).

(1S,5S,6S,7R,8S)-3-(2′,3′,4′,6′-*Tetra*-O-*benzyl*-α-D-*glucopyranosylamino*)-2-*oxa*-4-*azabicyclo*[3.3.0]*oct*-3-*ene*-6,7,8-*triol* D-**33**.—The thiourea D-**32** (192 mg, 0.263 mmol) was treated with yellow HgO (171 mg, 0.789 mmol, 3 mol equiv.) in acetone–diethyl ether (3.5 cm^3; 1:6, v/v) for 3 h at room temperature. In addition, after 3, 10 and 23 h, further aliquots of yellow HgO (171 mg, 0.789 mmol) were added to the reaction mixture, and the mixture was further stirred for 6 h at the same temperature. The reaction mixture was filtered through a bed of Celite and washed with EtOH. The combined filtrate and washings were evaporated to afford the *cyclic isourea* D-**33** (183 mg, 100%) as a syrup (Found: C, 68.8; H, 6.5; N, 4.2. $C_{40}H_{44}N_2O_9$ requires C, 69.0; H, 6.4; N, 4.0%); $[\alpha]_D^{27}$ +60.1 (*c* 1.25, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3350 (OH and NH) and 1670 (C=N); δ_H(270 MHz; $CDCl_3$) 7.40–7.01 (20 H, m, 4 × Ph), 5.39 (1 H, br s, 1′-H), 4.88 and 4.74 (each 1 H, ABq, J_{gem} 10.8, PhCH_2), 4.74 and 4.44 (each 1 H, ABq, J_{gem} 11.2, PhCH_2), 4.61 (1 H, br d, $J_{1,5}$ 8.6, 1-H), 4.60 and 4.55 (each 1 H, ABq, J_{gem} 11.0, PhCH_2), 4.55 and 4.38 (each 1 H, ABq, J_{gem} 11.7, PhCH_2), 4.96–4.36 (2 H, m, 2 × OH), 4.14 (1 H, br d, $J_{1,5}$ 8.6, 5-H) and 3.94–3.49 (10 H, m, 6-, 7-, 8-, 2′-, 3′-, 4′- and 5′-H, 6′-H_2 and OH).

(1R,5R,6R,7S,8R)-3-(2′,3′,4′,6′-*Tetra*-O-*benzyl*-α-D-*glucopyranosylamino*)-2-*oxa*-4-*azabicyclo*[3.3.0]*oct*-3-*ene*-6,7,8-*triol* L-**33**.—The thiourea L-**32** (166 mg, 0.228 mmol) was similarly converted into the *isourea* L-**33** (158 mg, 99.3%) as a syrup (Found: C, 68.6; H, 6.4; N, 4.1%); $[\alpha]_D^{27}$ +55.6 (*c* 1.02, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3350 (OH and NH) and 1650 (C=H); δ_H(270 MHz; $CDCl_3$) 7.43–7.00 (20 H, m, 4 × Ph), 5.38 (1 H, br s, 1′-H), 4.92–4.24 (2 H, m, 2 × OH), 4.86 and 4.73 (each 1 H, ABq, J_{gem} 11.0, PhCH_2), 4.71 and 4.39 (each 1 H, ABq, J_{gem} 10.8, PhCH_2), 4.64 (1 H, br d, $J_{1,5}$ 8.0, 1-H), 4.54 and 4.51 (each 1 H, ABq, J_{gem} 10.1, PhCH_2), 4.53 and 4.31 (each 1 H, ABq, J_{gem} 12.1, PhCH_2), 4.13 (1 H, br d, $J_{1,5}$ 8.0, 5-H) and 4.00–3.26 (10 H, m, 6-, 7-, 8-, 2′-, 3′-, 4′- and 5′-H, 6′-H_2 and OH).

(1S,5S,6S,7R,8S)-3-(α-D-*Glucopyranosylamino*)-2-*oxa*-4-*azabicyclo*[3.3.0]*oct*-3-*ene*-6,7,8-*triol* D-**3**.—To a mixture of sodium (167 mg, 7.25 mmol, 100 mol equiv.) in liquid NH_3 (~5 cm^3)

was added a solution of the isourea D-**33** (50.5 mg, 0.0725 mmol) in THF (1.5 cm^3) at −78 °C. The mixture was stirred for 10 min at −78 °C, and was then quenched by addition of excess of NH_4Cl (581 mg, 10.9 mmol, 150 mol equiv.). Ammonia was removed by spontaneous evaporation, and the residue was diluted with water (5 cm^3) and washed with $CHCl_3$ (5 cm^3 × 2). The aqueous layer was charged on a column of Dowex 50W-X2 (H^+) resin (30 cm^3) which was then washed with water and eluted with 0.5 mol dm^{-3} aq. NH_3 to give the free base D-**3** (23.3 mg, 95.6%) as a powder, $[\alpha]_D^{26}$ +85.0 (*c* 0.49, water); ν_{max}(KBr disk)/cm^{-1} 3420 (OH and NH) and 1660 (C=N); δ_H(270 MHz; D_2O) 5.20 (1 H, d, $J_{1',2'}$ 5.1, 1'-H), 4.61 (1 H, dd, $J_{1,5}$ 9.7, $J_{1,8}$ 5.3, 1-H), 3.96 (1 H, ddd, $J_{1,5}$ 9.7, $J_{5,6}$ 3.5, $J_{5,7}$ 1.5, 5-H), 3.82 (1 H, dd, $J_{1,8}$ 5.3, $J_{7,8}$ 8.8, 8-H), 3.69–3.49 (2 H, m, 6- and 7-H), 3.67 (1 H, dd, $J_{5',6'}$ 2.2, J_{gem} 11.6, 6'-H), 3.63 (1 H, dd, $J_{1',2'}$ 5.1, $J_{2',3'}$ 9.9, 2'-H), 3.59 (1 H, dd, $J_{5',6'}$ 5.5, J_{gem} 11.6, 6'-H), 3.51 (1 H, dd, $J_{2',3'}$ 9.9, $J_{3',4'}$ 8.8, 3'-H), 3.42 (1 H, ddd, $J_{4',5'}$ 9.9, $J_{5',6'}$ 2.2 and 5.5, 5'-H) and 3.27 (1 H, dd, $J_{3',4'}$ 8.8, $J_{4',5'}$ 9.9, 4'-H).

(1R,5R,6R,7S,8R)-3-(α-D-*Glucopyranosylamino*)-2-*oxa*-4-*azabicyclo*[3.3.0]*oct*-3-*ene*-6,7,8-*triol* L-**3**.—O-Debenzylation of the isourea L-**33** (56.3 mg, 0.0808 mmol) was similarly conducted to give the free base L-**3** (24.9 mg, 91.6%) as a powder, $[\alpha]_D^{26}$ +101.9 (*c* 0.59, water); ν_{max}(KBr disk)/cm^{-1} 3420 (OH and NH) and 1660 (C=N); δ_H(270 MHz; D_2O) 5.19 (1 H, d, $J_{1',2'}$ 5.1, 1'-H), 4.62 (1 H, dd, $J_{1,5}$ 9.9, $J_{1,8}$ 5.5, 1-H), 3.95 (1 H, ddd, $J_{1,5}$ 9.9, $J_{5,6}$ 4.2, $J_{5,7}$ 2.0, 5-H), 3.81 (1 H, ddd, $J_{1,8}$ 5.5, $J_{6,8}$ 2.0, $J_{7,8}$ 7.1, 8-H), 3.68–3.50 (2 H, m, 6- and 7-H), 3.67 (1 H, dd, $J_{5',6'}$ 2.6, J_{gem} 13.0, 6'-H), 3.62 (1 H, dd, $J_{1',2'}$ 5.1, $J_{2',3'}$ 8.8, 2'-H), 3.60 (1 H, dd, $J_{5',6'}$ 4.6, J_{gem} 13.0, 6'-H), 3.51 (1 H, dd, $J_{2',3'} = J_{3',4'}$ = 8.8, 3'-H), 3.41 (1 H, ddd, $J_{5',6'}$ 2.6 and 4.6, $J_{4',5'}$ 9.9, 5'-H) and 3.27 (1 H, dd, $J_{3',4'}$ 8.8, $J_{4',5'}$ 9.9, 4'-H).

(1S,5R,6S,7R,8R)-4-N,6-O,7-O,8-O-*Tetraacetyl*-3-(2',3',4',6'-*tetra*-O-*acetyl*-α-D-*glucopyranosylimino*)-2-*oxa*-4-*azabicyclo*-[3.3.0]*octane*-6,7,8-*triol* D-**34**.—The free base D-**3** (9.3 mg, 0.0258 mmol) was acetylated with acetic anhydride (1 cm^3) in pyridine (1 cm^3) for 3 h at room temperature. The crude product was chromatographed on a column of silica gel (1 g) with acetone–toluene (1 : 4, v/v) as eluent to afford the *octaacetyl derivative* D-**34** (17.0 mg, 91.4%) as a syrup (Found: C, 49.8; H, 5.5; N, 3.9. $C_{28}H_{36}N_2O_{17}$ requires C, 50.0; H, 5.4; N, 4.2%); $[\alpha]_D^{26}$ +90.3 (*c* 0.83, $CHCl_3$); ν_{max}(neat)/cm^{-1} 1750 and 1720 (OAc) and 1695 (C=N and NAc); δ_H(270 MHz; $CDCl_3$) 5.57 (1 H, d, $J_{1',2'}$ 4.0, 1'-H), 5.41 (1 H, dd, $J_{2',3'}$ 10.1, $J_{3',4'}$ 9.5, 3'-H), 5.32 (1 H, ddd, $J_{1,8}$ 3.1, $J_{7,8}$ 4.4, $J_{6,8}$ 1.1, 8-H), 5.24 (1 H, ddd, $J_{7,8}$ 4.4, $J_{6,7}$ 4.4, $J_{5,7}$ 0.9, 7-H), 5.18 (1 H, ddd, $J_{5,6}$ 2.2, $J_{6,7}$ 4.4, $J_{6,8}$ 1.1, 6-H), 5.10 (1 H, dd, $J_{3',4'}$ 9.5, $J_{4',5'}$ 9.5, 4'-H), 5.06 (1 H, dd, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.1, 2'-H), 4.89 (1 H, dd, $J_{1,5}$ 8.1, $J_{1,8}$ 3.1, 1-H), 4.83 (1 H, ddd, $J_{1,5}$ 8.1, $J_{5,6}$ 2.2, $J_{5,7}$ 0.9, 5-H), 4.32 (1 H, ddd, $J_{4',5'}$ 9.5, $J_{5',6'}$ 1.8 and 4.4, 5'-H), 4.24 (1 H, dd, $J_{5',6'}$ 4.4, J_{gem} 12.1, 6'-H), 4.07 (1 H, dd, $J_{5',6'}$ 1.8, J_{gem} 12.1, 6'H) and 2.64, 2.12, 2.11, 2.10, 2.04, 2.03, 2.00 and 1.99 (each 3 H, 8 s, 8 × Ac).

(1R,5S,6R,7S,8S)-4-N,6-O,7-O,8-O-*Tetraacetyl*-1-(2',3',4',-6'-*tetra*-O-*acetyl*-α-D-*glucopyranosylimino*)-2-*oxa*-4-*azabicyclo*-[3.3.0]*octane*-6,7,8-*triol* L-**34**.—The free base L-**3** (11.0 mg, 0.0327 mmol) was similarly converted into the *octaacetyl derivative* L-**34** (22.0 mg, 100%) as a syrup (Found: C, 49.9; H, 5.6; N, 4.1%); $[\alpha]_D^{26}$ +39.8 (*c* 1.17, $CHCl_3$); ν_{max}(neat)/cm^{-1} 1750 and 1715 (OAc) and 1695 (C=N and NAc); δ_H(270 MHz; $CDCl_3$) 5.58 (1 H, d, $J_{1',2'}$ 4.4, 1'-H), 5.45 (1 H, dd, $J_{2',3'}$ 10.3, $J_{3',4'}$ 9.5, 3'-H), 5.41 (1 H, ddd, $J_{5,6}$ 3.8, $J_{6,7}$ 5.7, $J_{6,8}$ 0.9 6-H), 5.29 (1 H, ddd, $J_{1,7}$ 0.8, $J_{6,7}$ 5.7, $J_{7,8}$ 5.7, 7-H), 5.18 (1 H, ddd, $J_{1,8}$ 3.2, $J_{6,8}$ 0.9, $J_{7,8}$ 5.7, 8-H), 5.10 (1 H, dd, $J_{1',2'}$ 4.4, $J_{2',3'}$ 10.3, 2'-H), 5.09 (1 H, dd, $J_{3',4'}$ 9.5, $J_{4',5'}$ 9.5, 4'-H), 4.90 (1 H, ddd, $J_{1,5}$ 9.0, $J_{1,7}$ 0.8, $J_{1,8}$ 3.2, 1-H), 4.83 (1 H, dd, $J_{1,5}$ 9.0, $J_{5,6}$ 3.8, 5-H), 4.28 (1 H, ddd, $J_{4',5'}$ 9.5, $J_{5',6'}$ 2.4 and 4.5, 5'-H), 4.20 (1 H, dd, $J_{5',6'}$ 4.5, J_{gem} 12.1, 6'-H), 4.09 (1 H, dd, $J_{5',6'}$ 2.4, J_{gem} 12.1, 6'-H) and 2.62, 2.13, 2.11, 2.09, 2.08, 2.05, 2.04 and 2.01 (each 3 H, 8 s, 8 × Ac).

N-[(1S)-(1,2,4/3,5)-2,3,4-*Trihydroxy*-5-(*hydroxymethyl*)-*cyclohexyl*]-N'-(2',3',4',6'-*tetra*-O-*benzyl*-α-D-*glucopyranosyl*)-*thiourea* **36**.—A mixture of (1R)-(1,3,4/2,6)-4-amino-6-(hydroxymethyl)cyclohexane-1,2,3-triol [5] (validamine) **35** (74.4 mg, 0.420 mmol) and the isothiocyanate **18** (165 mg, 0.284 mmol) in 75% aq. DMF (8 cm^3) was stirred for 40 min at room temperature. The solution was evaporated to give a residue, which was chromatographed on a column of silica gel (10 g) with EtOAc–hexane (1 : 3, v/v) ⟶ EtOH–toluene (1 : 5, v/v) as eluent to afford the *thiourea* **36** (200 mg, 92.8%) as a syrup (Found: C, 66.2; H, 6.5; N, 3.8. $C_{42}H_{50}N_2O_9S$ requires C, 66.5; H, 6.6; N, 3.7%); $[\alpha]_D^{21}$ +111 (*c* 0.80, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3320 (OH and NH) and 1540 (NH); δ_H(270 MHz; $CDCl_3$) 7.59 (1 H, br s, NH or N'H), 7.38–7.10 (20 H, m, 4 × Ph), 6.64 (1 H, br s, N'H or NH), 4.93–4.43 (9 H, m, 1'-H and 4 × PhCH_2), 3.76–3.16 (13 H, m, 1-, 2-, 3-, 4-, 2', 3', 4' and 5'-H, 5-CH_2OH and 6'-H_2 and OH) and 1.86–0.86 (4 H, m, 5-H, 6-H_2 and OH). Signals due to two OH groups were not observed.

(1S,3R,4R,5S,6S)-3-*Hydroxymethyl*-8-(2',3',4',6'-*tetra*-O-*benzyl*-α-D-*glucopyranosylamino*)-7-*oxa*-9-*azabicyclo*[4.3.0]*non*-8-*ene*-4,5-*diol* **37**.—To a stirred solution of the thiourea **36** (150 mg, 0.198 mmol) in diethyl ether (6 cm^3) was added yellow HgO (129 mg, 0.594 mmol, 3 mol equiv.) at room temperature. In addition, after 2.5, 7.5, 22.5 and 32.5 h, aliquots (129 mg, 0.594 mmol) of yellow HgO were added to the mixture, which was then stirred for 13.5 h at the same temperature. The reaction mixture was filtered through a bed of Celite and was washed with ethanol. The filtrate and washings were combined and evaporated to give the *isourea* **37** (136 mg, 94.8%) as a syrup (Found: C, 69.3; H, 7.0; N, 3.5. $C_{42}H_{48}N_2O_9$ requires C, 69.6; H, 6.7; N, 3.9%); $[\alpha]_D^{21}$ +96.8 (*c* 1.00, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3350 (OH and NH) and 1660 (C=N); δ_H(270 MHz; $CDCl_3$) 7.38–7.04 (20 H, m, 4 × Ph), 5.85 (1 H, br s, OH), 5.52 (1 H, br s, 1'-H), 5.01–3.16 (20 H, m, 1-, 4-, 5-, 6-, 2'-, 3'-, 4'-, and 5'-H, CH_2OH, 6'-H_2 and 4 × PhCH_2), 1.98–0.85 (3 H, m, 2-H_2 and 3-H). Signals due to two OH groups and the NH group were not observed.

(1S,5S,7R,8S,1''R)-3-(5a'-*Carba*-α-D-*glucopyranosylamino*)-7-(1'',2''-*dihydroxyethyl*)-2,6-*dioxa*-4-*azabicyclo*[3.3.0]*oct*-3-*en*-8-*ol* **5**.—To a mixture of sodium (226 mg, 9.82 mmol, 100 mol equiv.) in liquid ammonia (~8 cm^3) was added a solution of the isourea **37** (71.1 mg, 0.0981 mmol) in THF (4 cm^3) at −78 °C. After 15 min, an excess of NH_4Cl (786 mg, 14.7 mmol, 150 mol equiv.) was added to the mixture and ammonia was removed by spontaneous evaporation. The residue was diluted with water (3 cm^3) and washed with $CHCl_3$ (1 cm^3 × 3). The aqueous layer was charged on a column of Dowex 50W-X2 (H^+) resin (25 cm^3) and eluted with 0.5 mol dm^{-3} aq. ammonia to give the free base **5** (30.6 mg, 85.7%) as a powder, $[\alpha]_D^{28}$ + 54.9 (*c* 0.20, water); ν_{max}(KBr disk)/cm^{-1} 3450 (OH and NH) and 1650 (C=N); δ_H(270 MHz; D_2O) 5.82 (1 H, d, $J_{1,5}$ 4.8, 5-H), 4.88 (1 H, d, $J_{1,5}$ 4.8, 1-H), 4.29 (1 H, br s, 8-H), 3.89 (1 H, ddd, $J_{1',2'}$ 4.2, $J_{1',5a'\text{-eq}}$ 3.0, $J_{1',5a'\text{-ax}}$ 3.4, 1'-H), 3.79 (1 H, ddd, $J_{7,1''}$ 7.3, $J_{1'',2''}$ 2.6 and 2.9, 1''-H), 3.65 (1 H, dd, $J_{1'',2''}$ 2.6, J_{gem} 12.1, 2''-H), 3.59 (1 H, dd, $J_{5',6'}$ 4.3, J_{gem} 10.3, 6'-H), 3.55 (1 H, m, 7-H), 3.51 (1 H, dd, $J_{1',2'}$ 4.2, $J_{2',3'}$ 9.9, 2'-H), 3.50 (1 H, dd, $J_{5',6'}$ 5.9, J_{gem} 10.3, 6'-H), 3.48 (1 H, dd, $J_{1'',2''}$ 2.9, J_{gem} 12.1, 2''-H), 3.33 (1 H, dd, $J_{2',3'}$ 9.9, $J_{3',4'}$ 9.2, 3'-H), 3.15 (1 H, dd, $J_{3',4'}$ 9.2, $J_{4',5'}$ 10.3, 4'-H), 1.79 (1 H, ddd, $J_{1',5a'\text{-eq}}$ 3.0, $J_{5',5a'\text{-eq}}$ 3.2, J_{gem} 11.4, 5a'-eq-H), 1.58 (1 H, m, 5'-H) and 1.33 (1 H, ddd, $J_{1',5a'\text{-ax}}$ 3.4, $J_{5',5a'\text{-ax}}$ 12.2, J_{gem} 11.4, 5a'-ax-H).

(1S,5S,7R,8S,1″R)-8-*Acetoxy*-4-N-*acetyl*-7-(1″,2″-*diacetoxyethyl*)-3-(2′,3′,4′,6′-*tetra*-O-*acetyl*-5a′-*carba*-α-D-*glucopyranosylimino*)-2,6-*dioxa*-4-*azabicyclo*[3.3.0]*octane* **38**.—The free base **5** (30.0 mg, 0.0827 mmol) was acetylated conventionally, and the product was chromatographed on a column of a silica gel (3 g) with acetone–toluene (1 : 4, v/v) to give the *octaacetyl derivative* **38** (52.0 mg, 89.8%) as a syrup (Found: C, 51.7; H, 6.1; N, 4.0. $C_{30}H_{40}N_2O_{17}$ requires C, 51.4; H, 5.8; N, 4.0%); $[\alpha]_D^{27}$ +99.3 (*c* 0.35, $CHCl_3$); ν_{max}(neat)/cm^{-1} 1745 (OAc) and 1695 (NAc and C=N); δ_H(270 MHz; $CDCl_3$) 6.39 (1 H, d, $J_{1,5}$ 4.8, 5-H), 5.479 (1 H, d, $J_{7,8}$ 2.6, 8-H), 5.476 (1 H, dd, $J_{2',3'}$ 10.3, $J_{3',4'}$ 9.5, 3′-H), 5.26 (1 H, ddd, $J_{7,1''}$ 12.5, $J_{1'',2''}$ 2.6 and 6.2, 1″-H), 5.05 (1 H, dd, $J_{3',4'}$ 9.5, $J_{4',5'}$ 11.0, 4′-H), 4.93 (1 H, dd, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.3, 2′-H), 4.65 (1 H, d, $J_{1,5}$ 4.8, 1-H), 4.58 (1 H, dd, $J_{7,8}$ 2.6, $J_{7,1''}$ 12.5, 7-H), 4.26 (1 H, ddd, $J_{1',2'}$ 3.7, $J_{1',5a'\text{-eq}}$ 3.1, $J_{1',5a'\text{-ax}}$ 3.5, 1′-H), 4.12 (1 H, dd, $J_{5',6'}$ 5.5, J_{gem} 11.4, 6′-H), 4.11 (1 H, dd, $J_{1'',2''}$ 6.2, J_{gem} 12.1, 2″-H), 4.10 (1 H, dd, $J_{1'',2''}$ 2.6, J_{gem} 12.1, 2″-H), 3.93 (1 H, dd, $J_{5',6'}$ 3.3, J_{gem} 11.4, 6′-H), 2.66, 2.10, 2.07, 2.05, 2.04, 2.01, 1.99 and 1.98 (each 3 H, 8 s, 8 × Ac) and 2.58–1.67 (3 H, m, 5′-H and 5a′-H_2).

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