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# Further Chemical Modification of Trehalase Inhibitor Trehazolin: Structure and Inhibitory–Activity Relationship of the Inhibitor

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**Abstract**—Eight analogues of trehazolin were synthesized and tested for trehalase inhibitors. Deoxygenation of the cyclopentanepolyol moiety all decreased the inhibitory activity. Epimerization at the branching point of the cyclopentane ring did not so affect the potency. The cyclic isourea part was shown to be replaced with guanidine structure with a considerable decrease of activity. The 6'-fluoro-6'-deoxy derivative was still a strong inhibitor. It seems that trehazolin strictly mimics the substrate α,α-trehalose and any structural change and/or removal of the hydroxyl functions appreciably influence its potency. The present results led to finding 5-aminocyclopentane-1,2,3,4-tetraols to be new lead compounds for glycohydrolase inhibitors.

## Introduction

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*. Interests in the structure and inhibitory–activity relationship of this unique inhibitor have so far prompted us to synthesize systematically 15 structural analogues of **1**, which contain the chemically modified cyclitol[2] or sugar portions.[3] Their distinct difference in biological properties have given much knowledge concerning the structure–activity relationship, furnishing useful hints to generate new types of lead compounds for the development of other glycosidase inhibitors.[4,5]

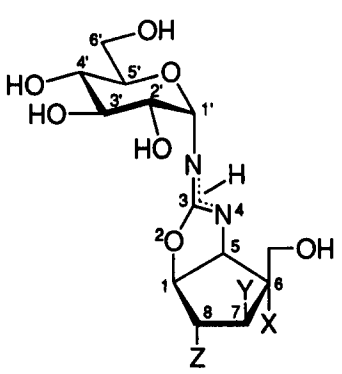
In this final paper of the series, in order to afford further insight into a role of the cyclitol moiety, its three deoxy (**2–4**), one deoxy-epi (**5**) and one epi analogues (**8**) of **1** have been synthesized according to the established procedure[6] (Scheme 1). On the other hand, in order to elucidate importance of the transitory charge distribution part, the cyclic isourea structure, attempts have been made to replace it with a more basic function, synthesizing the cyclic guanidine analogue **10**. Reaction of the diamine **21** and the sugar isothiocyanate[7] **23** readily gave thioureas. Under cyclization conditions it produced first a single product which however quickly gave rise to a complex mixture of compounds, probably due to the anomerization. Therefore, the guanidine analogue **11** having 5a-carba-α-D-glucopyranose residue was prepared instead. Furthermore, a synthesis of the analogue **12** , the cyclopentane ring of which is replaced with a cyclohexane ring having appropriate functionality and stereochemistry was carried out. However, since the analogue containing α-D-glucopyranose residue was shown[2] not to exist in a desired form because of their ready isomerization to the more stable furanoid-type tautomer, the analogues **13** the sugar portion of which was substituted by the 5a-carba-sugar was prepared. Finally, to demonstrate the essential role of the α-D-glucopyranose moiety, especially the importance of the 6-hydroxyl group involved in its binding to the active site of enzymes, the 6'-deoxy-6'-fluoro derivative **14** has been synthesized, thereby comparing its activity with that of the 6'-deoxy analogue.[3]

## Results and Discussion

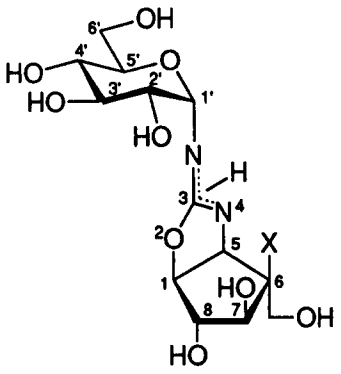
### *Synthesis of three deoxy derivatives 16–18 and epimer 19 of trehazolamine (15)*

All aminocyclopentanepolyols used in this paper (Scheme 2) have been originally synthesized starting from the *O*-cyclohexylidene derivatives of 5-acetamidocyclopentane-1,2,3,4-tetraol.[†3] Treatment of the *N,O*-isopropylidene derivative[2] **25** of 1L-(1,3,4/2)-4-acetamido-1,2-di-*O*-(methoxymethyl)-5-methylenecyclopentane-1,2,3-triol with NBS in aqueous THF gave a crude bromide **26**, which without isolation was debrominated with tributyltin hydride in the presence of AIBN in toluene to give, after chromatography, two alcohols **27** (59%) and **28** (13%). The alcohol **27** was deprotected with 2 M hydrochloric acid to give, after purification by a column of Dowex 50W-X2 ($H^+$) resin with M aq. ammonia, the amino alcohol **16** (93%), which was further characterized as the penta-*N,O*-acetyl derivative **16a**. Similarly, the free base **19** and the acetyl derivative **19a** were prepared from **28**. The characteristic differences observed between **16a** and **19a** in the $^1H$ NMR spectra was the signals due to 5-H,

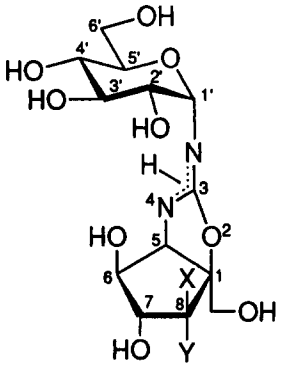
[†]In this paper, nomenclature of aminocyclitols follows IUPAC-IUB 1973 recommendations for cyclitols (*Pure Appl. Chem.* **1974**, *37*, 285).



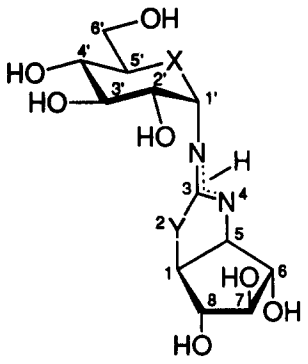
**1** X = Y = Z = OH
**2** X = H, Y = Z = OH
**3** X = Z = OH, Y = H
**4** X = Y = OH, Z = H



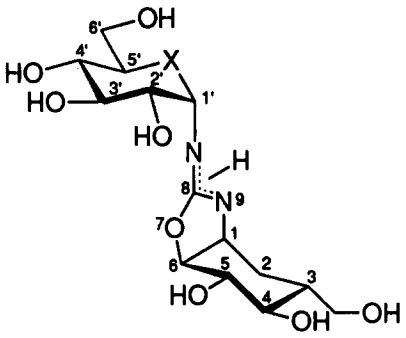
**5** X = H
**6** X = OH



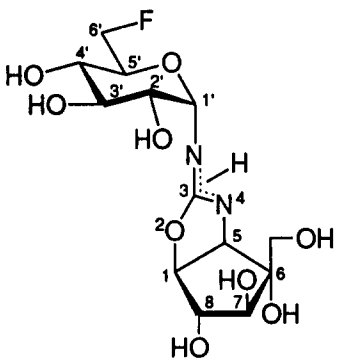
**7** X = H, Y = OH
**8** X = OH, Y = H



**9** X = Y = O
**10** X = O, Y = NH
**11** X = $CH_2$, Y = NH



**12** X = O
**13** X = $CH_2$



**14**

**2a, 5a, 6a, 13a**: octa-*N,O*-acetyl

**3a, 4a, 14a**: hepta-*N,O*-acetyl

**8a, 11a** : nona-*N,O*-acetyl

**Scheme 1.** Compound **11a** is an ~1:2.5 mixture of two isomers in regard to location of the N-acetyl group.

which appeared at δ 2.80 and 2.39 with spacings of $J_{4,5}$ = 6.2 and 10.6 Hz, respectively. These data would support the assigned structures, which were finally established by NOE experiment as depicted in Scheme 3.

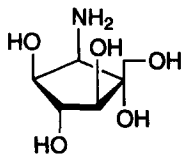
The deoxy derivatives **17** and **18** were prepared starting from penta-*N,O*-acetyltrehazolamine[6] (**29**) (Scheme 4). Isopropylidenation of the *N*-acetyltrehazolamine (**30**) derived from **29** with 2,2-dimethoxypropane in DMF in the presence of *p*-toluenesulfonic acid gave the di-*N,O*:*O,O*-isopropylidene derivative **31** (89%), which was characterized as the diacetate **32**. Selective benzoylation of **31** was effected by treatment with benzoyl chloride (2 molar equiv.) at 0 °C to give exclusively the 3-benzoate (**33**) (~100%). The selectivity may be attributed to the steric effect. Treatment of **33** with 1,1'-thiocarbonyldiimidazole in THF gave the thionocarbamate **34** (~100%), which was treated with tributyltinhydride and AIBN to afford the 2-deoxy derivative **38** (82%) of **29**. Hydrolysis of **38** with 2 M hydrochloric acid and purification by a resin column gave the free base **17** (89%), which was further characterized as the tetra-*N,O*-acetyl derivative **17a** (91%).

On the other hand, the alcohol **33** was protected as the methoxymethyl ether (**35**) (~100%), which was then *O*-deacylated to give the alcohol **36** (96%). Compound **36** was likewise deoxygenated through the thionocarbonate **37** (→ **39**, 84%) followed by hydrolysis, to afford the 3-deoxy derivative **18** (~100%) of **29**. This compound was characterized as the tetra-*N,O*-acetyl derivative **18a**.

*Synthesis of 1-epitrehazolamine*† *(20)*

The optically active epitrehazolamine (**20**) was prepared

†We here propose naming aminocyclitol **20**, the 1-epimer of **15**, 'epitrehazolamine'.



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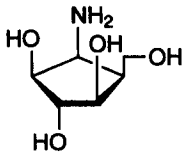

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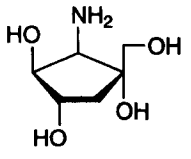

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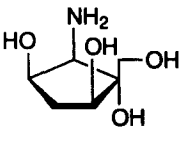

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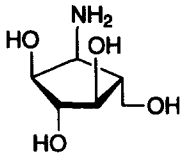

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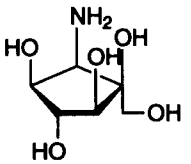

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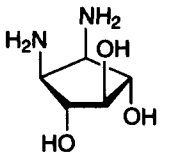

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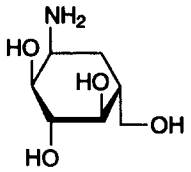

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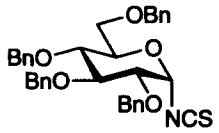

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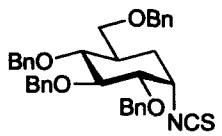

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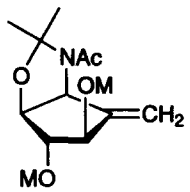
Scheme 2.



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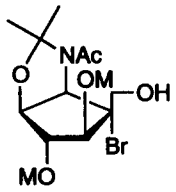

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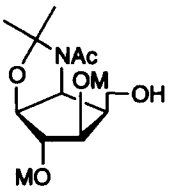

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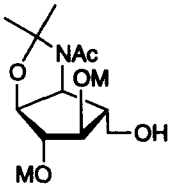

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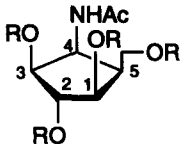
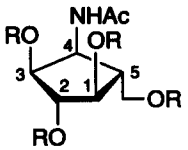
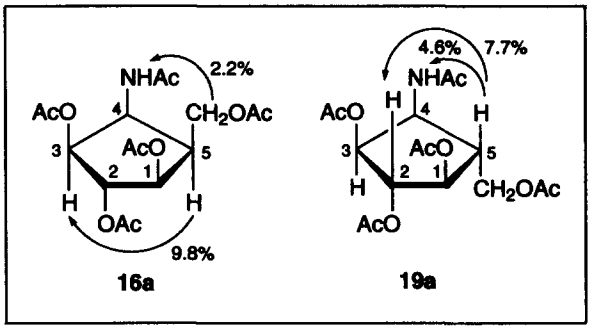


16 R = H
16a R = Ac



19 R = H
19a R = Ac




16a 19a

M = $MeOCH_2-$

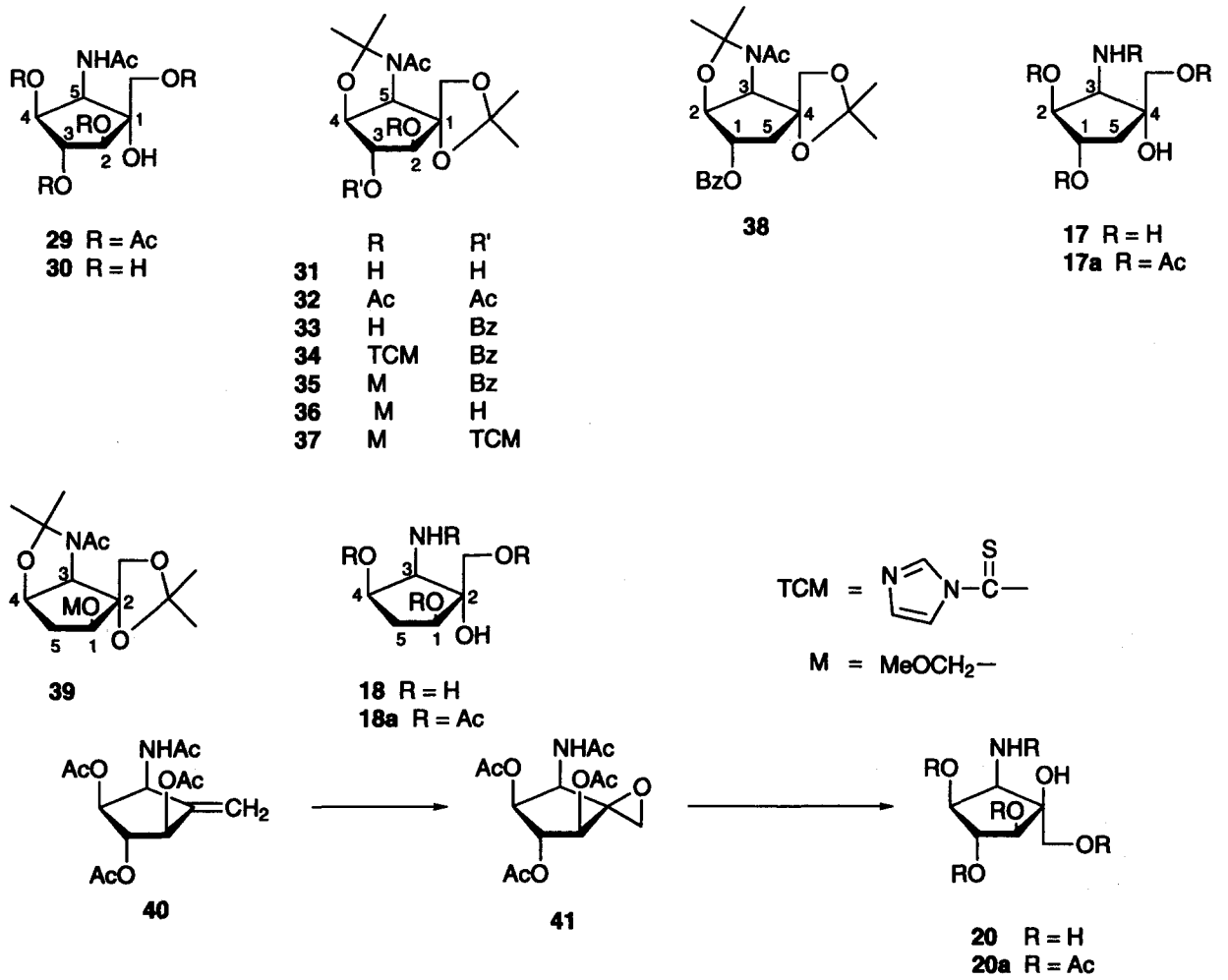
Scheme 3.

from the known 1L-(1,3,4/2)-5-acetamido-1,2,3-tri-*O*-acetyl-5-methylenecyclopentan-1,2,3-triol[6] (**40**) through peracid oxidation (→ the spiroepoxide **41**, 87%), substitution with an acetate ion in aq. DMF, followed by acetylation, (→ the penta-*N*,*O*-acetyl derivative **20a**, 65%) and hydrolysis (→ the free base **20**, ~100%) (Scheme 4).

*Synthesis of (1,3/2,4,5)-4,5-diaminocyclopentane-1,2,3-triol (21)*

The 4,5-*cis*-diaminocyclopentane-1,2,3-triol (**21**) with *meso* (1,3/2,4,5)-configuration was chosen for preparation of the cyclic guanidine analogue of **1**, because there is no need for optical resolution. Furthermore, since the corresponding trehazolin analogue[2] **9** and its diastereoisomer composed of the cyclic isourea is a strong trehalase inhibitor, a biological activity observed for the guanidine analogue **11** may conceivably indicate whether a cyclic guanidine structure exhibits the potency similarly as the cyclic isourea.

Oxidation of the racemic tris(methoxymethyl) derivative[2] **42** with Jones' reagent gave the ketone **43** which was subsequently transformed into the oximes **44** (a mixture of




Scheme 4.

the *E*- and *Z*-isomers). Without isolation, **44** was hydrogenated in ethanol containing acetic anhydride in the presence of freshly prepared Raney nickel to give rise to two isomers of the di-*N*-acetyl derivatives **45** (17%) and **46** (27%). Differentiation of two compounds could be readily done on the basis of the $^1$H NMR spectra. Thus, the isomer revealing the symmetric pattern of the spectrum was assigned to **45** and the other unsymmetric to **46**. Deprotection followed by acetylation afforded the penta-*N*,*O*-acetyl derivatives **21a** (85%) and **47** (81%), respectively. Acid hydrolysis followed by purification on a resin column **21a** produced the free base **21** (92%). Alternatively, the alcohol **42** was mesylated and the sulfonate **48** obtained was allowed to react with an azide anion to produce a single azide **49** (76%), which was converted into **47** (86%) by reduction in the presence of acetic anhydride, thereby verifying the structure of **46** (Scheme 5).

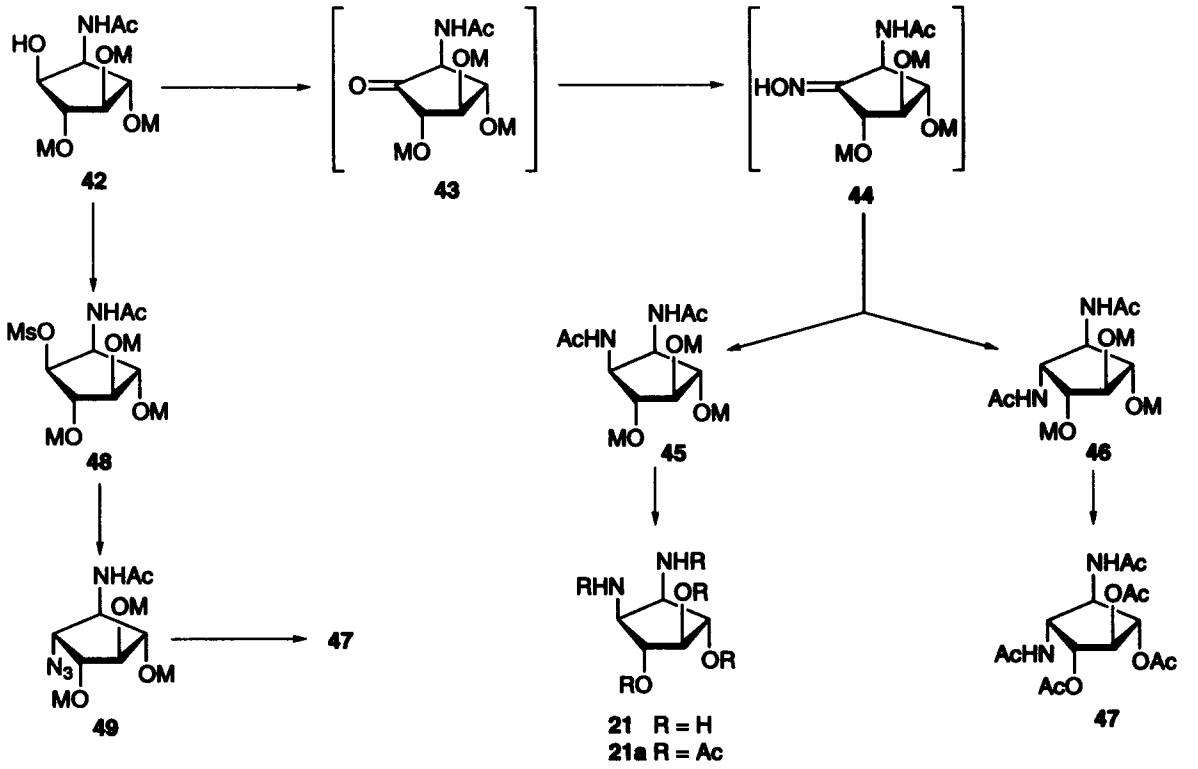
*Synthesis of trehazolin analogues 2–5 and 8*

Preparation of the trehazolin analogues **2–5** and **8** was carried out[6] conventionally. Coupling of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosylisothiocyanate (**23**) with the corresponding aminocyclitols **16–20** gave the thioureas **50–54**, cyclization of which with an excess of yellow mercury(II) oxide provided the cyclic isoureas **55–60**. Deblocking of the benzyl ether groups under Birch reduction conditions afforded free trehazolin analogues **2–5** and **8** (Scheme 6). Their structures were assigned on the basis of the $^1$H NMR spectra, together with characterization of the corresponding per-*N*,*O*-acetyl derivatives **2a–5a** and **8a**.

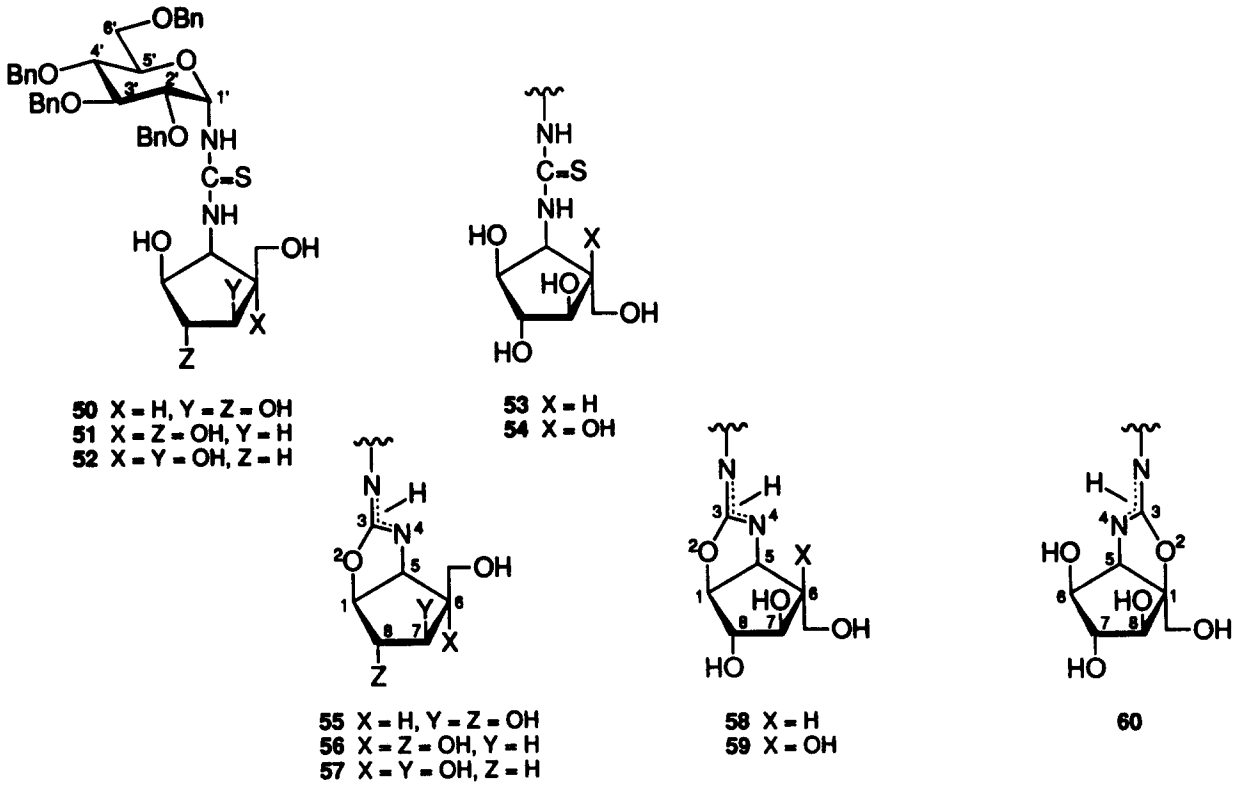
In the case of **54**, cyclization gave as expected an inseparable mixture of two tautomers **59** and **60** quantitatively. The mixture was deprotected to afford analogues **6** and **8**, which were finally separated as the per-*N*,*O*-acetyl derivatives **6a** (19%) and **8a** (81%). Deacylation of **8a** with methanolic sodium methoxide[6] gave pure **8** (97%), however **6a** gave mainly **8** through isomerization under basic conditions. The equilibrium apparently favors the cyclic isourea comprised of the tertiary hydroxyl group.[6]

*Synthesis of trehazolin analogues 11 and 13 containing 5a-carba-α-D-glucopyranose residues*

Since the usual cyclization of the thioureas derived from the *meso*-diamine **21** and the true-sugar isothiocyanate **23** resulted in formation of a sole but very labile isourea, the 5a-carba-glucosyl congener instead has been made as a target compound for estimating biochemical property of the




Scheme 5. Compounds 42–49 are racemic. For convenience, the formulae depict only one of the respective enantiomers.




Scheme 6.

cyclic guanidine. The 5a'-carbatrehazolin[8] has previously been shown to possess inhibitory activity almost compatible with that of trehazolin. Thus, reaction of **21** and 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-glucopyranosylisothiocyanate[8] (**24**) gave a diastereoisomeric mixture of the thioureas **61** and **62**, which was easily cyclized with yellow mercury(II) oxide to give the cyclic guanidine **63**. Similar deprotection afforded the guanidine analogue **11**, which on acetylation gave a mixture of two isomeric octa-*N*,*O*-acetyl derivatives **11a** with respect to location of the *N*-acetyl group. The $^1$H NMR spectrum showed it to be *ca* 1:2.5 mixture.

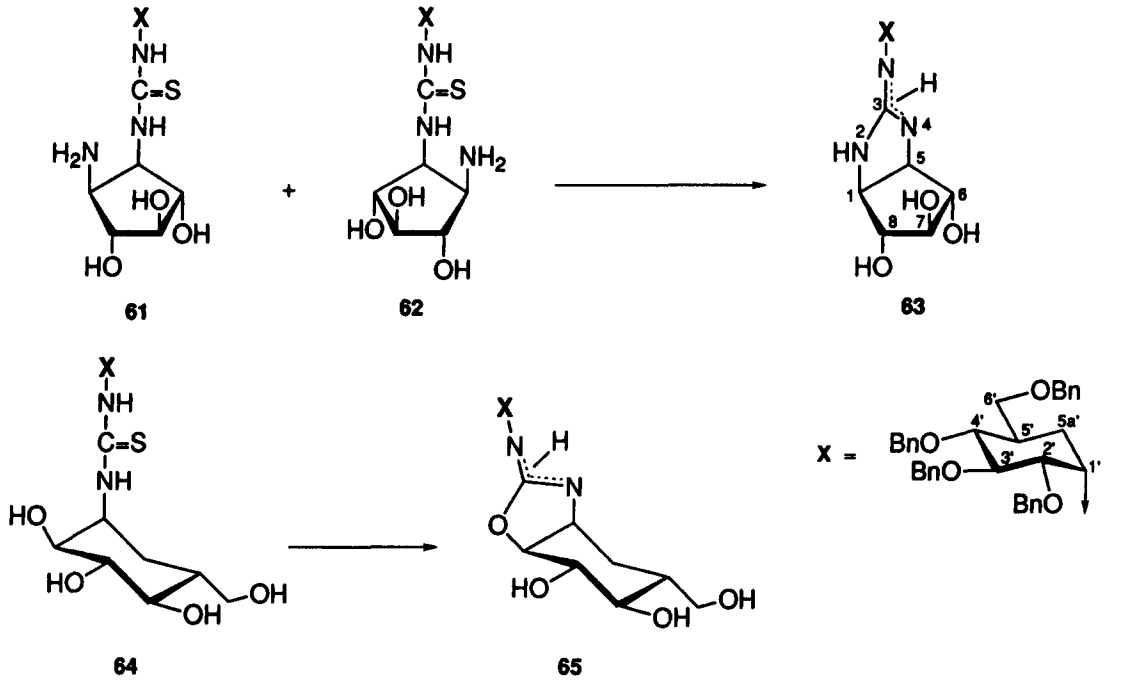
Furthermore, we were interested in inhibitory potency of a cyclohexane analogue, e.g. dihydrovalidoxylamine A,[9] mimicking the α,α-trehalose, the analogue composed of two 5a-carba-α-D-glucopyranose residues linked by way of an isourea ring has been synthesized starting from the coupling product **64** (67%) of validamine[10] **22** and the isothiocyanate **24** . Likewise cyclization of **64** gave the cyclic isourea **65** (89%), which on deprotection afforded the analogue **13**. The structure was confirmed by converting it to the octa-*N*,*O*-acetyl derivative **13a** (Scheme 7).

*Synthesis of 6'-deoxy-6'-fluoro derivative 14 of trehazolin*

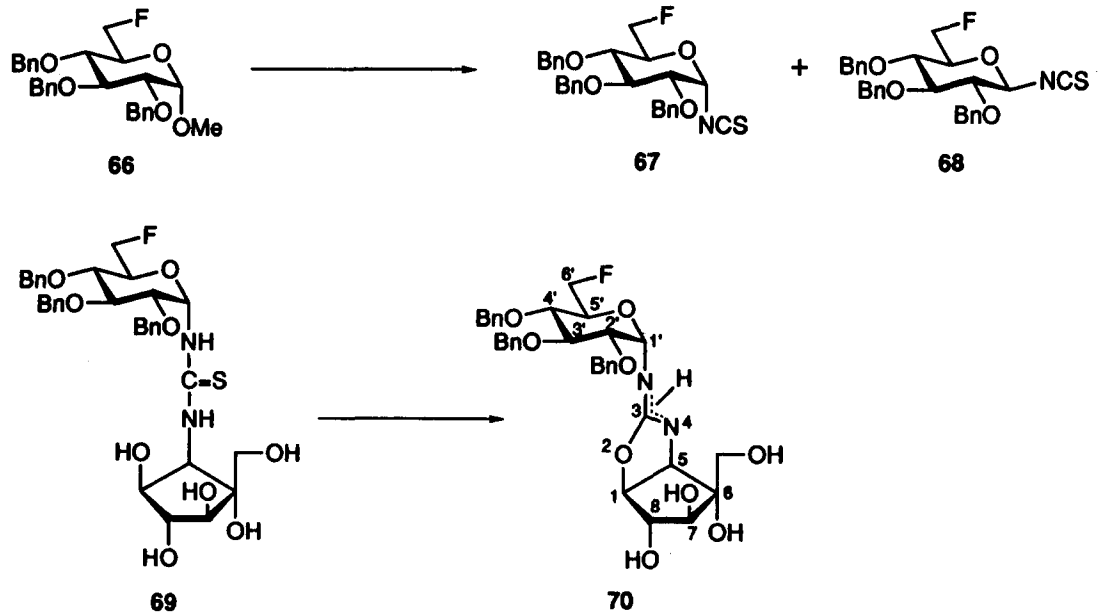
2,3,4-Tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranosyl-isothiocyanate (**67**) was prepared in the usual manner[3] starting from methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranoside[11] (**66**). The α- (**67**) and β-anomers (**68**) were obtained in the yields of 27 and 23%. Coupling of **15** and **67** proceeded smoothly to give the thiourea **69** (93%), which on cyclization afforded the isourea **70** (98%). Deprotection gave the analogue **14** (86%), the structure of which was confirmed as the hepta-*N*,*O*-acetyl derivative **14a** (92%) (Scheme 8).

*Biological assay*

Listed in Table 1 were the inhibitory activity ($IC_{50}$) against silkworm trehalase of the analogues **2–5**, **8**, **11**, **13** and **14**,




Scheme 7.




Scheme 8.

prepared in this study, together with the reference compounds: trehazolin[6] (**1**), and the analogues[6] **7** and **9**, and the diastereoisomers[3] thereof. Removal of each hydroxyl function of the 6,7,8-trihydroxy-2-oxa-4-azabicyclo-[3.3.0]octane moiety decreased more or less the inhibitory potency similarly as in the case of deoxygenation of the sugar part.[2] Trehalase from silkworm is known to be very selective and specific in recognizing the substrate α,α-trehalose. All three 6-, 7- and 8-hydroxyl groups, including the 6-hydroxylmethyl, are therefore considered to be topologically very important in mimicking those of the α-D-glucopyranose residue of the substrate, when binding to the active site of the enzyme. In fact, although the analogue **9** possesses a strong potency, the 7-epimer,[6] possibly thought to correspond with disaccharide α-Glc*p*(1→1)α-Gal*p* on the basis of the structure model, was shown to lose all potency. Interestingly, epimerization at the C-6 branching point of the cyclopentane ring as in the case of **7** and **8** seemed not to render such strong effects on the potency. The cyclic isourea structure seemed to be more appropriate for binding to the active site than the guanidine. The 6'-deoxy-6'-fluoro analogue **14** fairly restores the parent's potency compared to the 6'-deoxy analogue ($IC_{50}$ $7.9 \times 10^{-6}$ M), indicating that the α-D-glucopyranosyl residue plays a role just in binding the aglycone pocket of the active site of the enzyme. On the other hand, the cyclopentane part containing the cyclic isourea acts as the mimic of the postulated charge-distributed flattened half chair transition state related to the glycone part of trehalose during hydrolysis. It would be easily accepted that these parts cannot be replaced by the cyclohexane ring as shown in **13**.

**Table 1**. Inhibitory activity ($IC_{50}$) of trehazolin analogues[a] against silkworm trehalase

| Compound | Inhibitory activity [$IC_{50}$(M)] |
|---|---|
| Trehazolin (**1**) | $4.9 \times 10^{-8}$ |
| Diastereoisomer of **1** | $1.2 \times 10^{-6}$ |
| 6-Deoxy **2** | $2.1 \times 10^{-5}$ |
| 7-Deoxy **3** | $6.3 \times 10^{-5}$ |
| 8-Deoxy **4** | $>2.7 \times 10^{-4}$ |
| 6-Deoxy-6-epi **5** | $>2.7 \times 10^{-4}$ |
| 6,7-Diepi **7** | $9.2 \times 10^{-7}$ |
| Diastereoisomer of **7** | $2.6 \times 10^{-5}$ |
| 6-Epi **8** | $3.0 \times 10^{-7}$ |
| 6-Dehydroxymethyl **9** | $7.8 \times 10^{-6}$ |
| Diastereoisomer of **9** | $1.6 \times 10^{-7}$ |
| Cyclic guanidine **11** | $5.9 \times 10^{-5}$ |
| Validoxylamine-type **13** | $>5.0 \times 10^{-4}$ |
| 6'-Deoxy-6'-fluoro **14** | $2.3 \times 10^{-7}$ |

[a]The diastereoisomers contain the corresponding enantiomeric cyclopentanepolyol moieties.

These results thus provided further insight on a structure–inhibitory activity relationship of trehazolin analogues. According to the above assumption, some stereoisomers of 5-aminocyclopentane-1,2,3,4-tetraols and their branched chain derivatives were expected to possess inhibitory potency against glycohydrolases, and several compounds were promptly tested[4] for inhibitors. Among 13 *N*-phenyl cyclic isoureas, the isomers derived from epitrehazolamine (**20**) have so far been shown to be extremely potent against baker's yeast α-glucosidase. Therefore, the present studies have led to discovery of a new lead compound for development of the glycohydrolase and glycosyltransferase inhibitors.

## Experimental

### *General procedure*

Melting points were determined on a MEL-TEMP capillary melting point apparatus and are uncorrected. Optical rotations were measured with JASCO DIP-370 polarimeter. Silica gel column chromatography was performed on silica gel 200–300 mesh (Wakogel C-300, Wako Junyaku Kogyo Co., Osaka), and analytical TLC was performed on silica gel 60 F-254 (E. Merck, Darmstadt). $^1$H NMR spectra (270 MHz) were recorded on JEOL GSX-270 instrument and $^{13}$C NMR spectra (67.5 MHz) were measured with the same instrument. Chemical shifts are expressed as δ values with reference to $Me_4Si$ (δ 0.00) in $CDCl_3$, acetone-$d_6$, and DMSO-$d_6$, and reference to acetone (δ 2.08) or *t*-BuOH (δ 1.22) in $D_2O$, respectively. IR spectra were recorded on JASCO IR-810 or Hitachi FTS-65 spectrometer. Solutions were dried over anhydrous $Na_2SO_4$ and concentrated at < 45 °C under diminished pressure.

### *4,5-N,O-Isopropylidene derivative **26** of 1D-(1,2,4,5/3)-5-acetamido-1-bromo-1-hydroxymethyl-2,3-di-O-methoxymethyl-1,2,3,4-cyclopentanetriol*

To a solution of the *exo*-olefin[2] **25** (123 mg, 0.390 mmol) in 75% aq. THF (3 mL) was added NBS (417 mg, 2.34 mmol, 6 equiv.) at 0 °C, and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with saturated aq. $Na_2S_2O_3$ (10 mL) and the products were extracted with $CHCl_3$ (30 mL × 3). The organic layer was dried and evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (10 g) with acetone:toluene (1:4, v/v) as eluent to afford the bromohydrine (**26**) (200 mg) contaminated with succinimide; $R_f$ 0.41 (acetone:toluene, 1:2), $^1$H NMR ($CDCl_3$) δ 4.95 and 4.73 (each 1H, ABq, $J_{gem}$ = 6.6 Hz, C$\underline{H_2}$OCH$_3$), 4.78 and 4.74 (each 1H, ABq, $J_{gem}$ = 6.6 Hz, C$\underline{H_2}$OCH$_3$), 4.78 (1H, *d*, *J* = 6.6 Hz, 2- or 5-H), 4.43 (1H, *J* = 2.4 and 9.0 Hz, 3- or 4-H), 4.32 (1H, *dd*, *J* = 2.4 and 7.5 Hz, 3 or 4-H), 4.15 (1H, *dd*, $J_{6,OH}$ = 7.1 and 7.8 Hz, OH), 4.06 (1H, *d*, *J* = 8.8 Hz, 2- or 5-H), 3.95 (1H, *dd*, $J_{gem}$ = 12.1, $J_{6,OH}$ = 7.1 Hz, 6-H), 3.79 (1H, *dd*, $J_{gem}$ = 12.1, $J_{6,OH}$ = 7.8 Hz, 6-H), 3.46 and 3.42 (each 3H, 2*s*, 2 × CH$_2$OC$\underline{H_3}$ ), 2.21 (3H, *s*, Ac), 1.87 and 1.54 (each 3H, 2*s*, CMe$_2$). The crude product was used for the next step.

### *3,4-N,O-Isopropylidene derivatives **27** and **28** of respective 1L-(1,3,4/2,5)- and (1,3,4,5/2)-4-acetamido-5-hydroxymethyl-1,2-di-O-methoxymethyl-1,2,3-cyclopentanetriols*

To a solution of the crude bromohydrine **26** in toluene (4 mL) were added $Bu_3SnH$ (0.39 mL, 1.46 mmol, 3 equiv.)

and a catalytic amount of AIBN. The mixture was stirred for 20 min at refluxing temperature and evaporated to give an oily residue, which was chromatographed on a column of silica gel (10 g) with acetone:toluene (1:2, v/v) as eluent to afford, first, the (1,3,4/2,5)-alcohol **27** (24.0 mg, 13.2%) as a syrup; $R_f$ 0.21 (acetone:toluene; 1:2), $[\alpha]_D^{23}$ +43.5° (*c* 0.86; $CHCl_3$); IR (neat) ν 3450 (OH) and 1640 (NAc) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, at 70 °C) δ 4.68–4.61 (5H, *m*, 2- or 5-H and 2 × $C\underline{H}_2OCH_3$), 4.35 (1H, *d*, *J* = 5.5 Hz, 2- or 5-H), 4.25 (1H, *dd*, *J* = 5.9 and 7.0 Hz, 1- or 4-H), 3.96–3.91 (2H, *m*, 1- or 4-H and OH), 3.66–3.50 (2H, *m*, 2 × 6-H), 3.31 and 3.29 (each 3H, 2*s*, 2 × $CH_2OC\underline{H}_3$), 2.07 (3H, *s*, Ac), 2.03 (1H, *m*, 5-H), 1.55 and 1.45 (each 3H, 2*s*, $CMe_2$). Anal. calcd for $C_{15}H_{27}NO_7$: C, 54.04; H, 8.16; N, 4.20. Found: C, 53.97; H, 8.54; N, 4.09.

The second fractions gave the (1,3,4,5/2)-alcohol **28** (107 mg, 58.5%) as a syrup; $R_f$ 0.16 (acetone:toluene, 1:2), $[\alpha]_D^{23}$ +25.6° (*c* 1.47, $CHCl_3$); IR (neat) ν 3400 (OH) and 1630 (NAc) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, at 70 °C) δ 4.64 (4H, *s*, 2 × $C\underline{H}_2OCH_3$), 4.40 (1H, *dd*, $J_{1,2}$ = 7.9, $J_{1,5}$ = 6.6 Hz, 1-H), 4.30 (1H, *dd*, $J_{2,3}$ = 1.9, $J_{3,4}$ = 7.7 Hz, 3-H), 4.09 (1H, *dd*, $J_{6,OH}$ = 4.4 and 4.8 Hz, OH), 4.02 (1H, *dd*, $J_{3,4}$ = 8.1, $J_{4,5}$ = 6.6 Hz, 4-H), 3.89 (1H, *dd*, $J_{1,2}$ = 7.9, $J_{2,3}$ = 1.9 Hz, 2-H), 3.68 (1H, *ddd*, $J_{5,6}$ = 6.8, $J_{gem}$ = 13.2, $J_{6,OH}$ = 4.4 Hz, 6-H), 3.44 (1H, *ddd*, $J_{5,6}$ = 7.7, $J_{gem}$ = 13.2, $J_{6,OH}$ = 4.8 Hz, 6-H), 3.31 and 3.29 (each 3H, 2*s*, 2 × $CH_2OC\underline{H}_3$), 2.49 (1H, *dddd*, $J_{1,5}$ = 6.6, $J_{4,5}$ = 6.6, $J_{5,6}$ = 6.8 and 7.7 Hz, 5-H), 2.02 (3H, *s*, Ac), 1.57 and 1.39 (each 3H, 2*s*, $CMe_2$). Found: C, 53.95; H, 8.44; N, 4.15.

*1L-(1,3,4,5/2)-4-Amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (16)*

The alcohol **27** (76.1 mg, 0.228 mmol) was treated with 2 M aq. HCl (3 mL) for 2 h at 80 °C. Evaporation of a solvent gave a syrupy residue, which was purified by a column of Dowex 50W-X2 ($H^+$) resin (1 mL) with 1 M aq. $NH_4OH$ to give the aminoalcohol **16** (34.7 mg, 93.1%) as a syrup, $R_f$ 0.44 ($H_2O$:acetonitrile, 1:4), $[\alpha]_D^{27}$ +25.9° (*c* 1.14; water); IR (neat) ν 3400 (OH and $NH_2$) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. *t*-BuOH) δ 4.02 (1H, *dd*, $J_{1,2}$ = 3.5, $J_{1,5}$ = 7.0 Hz, 1-H), 3.99 (1H, *dd*, $J_{1,2}$ = 3.5, $J_{2,3}$ = 5.9 Hz, 2-H), 3.89 (1H, *dd*, $J_{2,3}$ = 5.9, $J_{3,4}$ = 5.7 Hz, 3-H), 3.85 (1H, *dd*, $J_{5,6}$ = 7.3, $J_{gem}$ = 11.8 Hz, 6-H), 3.79 (1H, *dd*, $J_{5,6}$ = 5.5, $J_{gem}$ = 11.8 Hz, 6-H), 3.39 (1H, *dd*, $J_{3,4}$ = 5.7, $J_{4,5}$ = 5.5 Hz, 4-H), 2.33 (1H, *dddd*, $J_{1,5}$ = 7.0, $J_{4,5}$ = 5.5, $J_{5,6}$ = 7.3 and 7.6 Hz, 5-H).

*1L-(1,3,4,5/2)-4-Acetamido-5-acetoxymethyl-1,2,3-tri-O-acetyl-1,2,3-cyclopentanetriol (16a)*

The free base **16** (13.3 mg, 0.0815 mmol) was treated with acetic anhydride (0.5 mL) in pyridine (1 mL) for 2 h at room temperature. Removal of a solvent gave a syrupy residue, which was chromatographed on a column of silica gel (2 g) with acetone:toluene (1:3, v/v) as eluent to give the acetate **16a** (17.3 mg, 56.8%) as a syrup; $R_f$ 0.46 (acetone:toluene, 1:1), $[\alpha]_D^{24}$ +37.9° (*c* 0.83; acetone); IR (neat) ν 3400 (NH), 1750 (OAc), 1660 (NAc) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.71 (1H, *d*, $J_{4,NH}$ = 9.5 Hz, NH), 5.24 (1H, *dd*, $J_{1,2}$ = 2.2, $J_{1,5}$ = 6.2 Hz, 1-H), 5.20 (1H, *dd*, $J_{1,2}$ = 2.2, $J_{2,3}$ = 4.6 Hz, 2-H), 5.17 (1H, *dd*, $J_{2,3}$ = 4.6, $J_{3,4}$ = 4.4 Hz, 3-H), 4.88 (1H, *ddd*, $J_{3,4}$ = 4.4, $J_{4,5}$ = 5.9, $J_{4,NH}$ = 9.5 Hz, 4-H), 4.22 (1H, *dd*, $J_{5,6}$ = 7.0, $J_{gem}$ = 11.7 Hz, 6-H), 4.12 (1H, *dd*, $J_{5,6}$ = 6.4, $J_{gem}$ = 11.7 Hz, 6-H), 2.80 (1H, *dddd*, $J_{1,5}$ = 6.2, $J_{4,5}$ = 5.9, $J_{5,6}$ = 6.4 and 7.0 Hz, 5-H), 2.13, 2.09, 2.07, 2.06 and 2.03 (each 3H, 5*s*, 5Ac). Anal. calcd for $C_{16}H_{23}NO_9$: C, 51.47; H, 6.21; N, 3.75. Found: C, 51.45; H, 6.58; N, 3.88.

*1L-(1,3,4/2,5)-4-Amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (19)*

The alcohol **28** (29.8 mg, 0.0894 mmol) was treated with 2 M aq. HCl (2 mL) for 3.5 h at room temperature, and the mixture was evaporated to give a syrupy residue, which was purified by a column of Dowex 50W-X2 ($H^+$) resin (2 mL) with 1 M aq. $NH_4OH$ as eluent to afford the aminoalcohol **19** (14.0 mg, 95.9%) as a syrup, $R_f$ 0.34 (AcOH:$H_2O$:acetonitrile, 1:4:10), $[\alpha]_D^{22}$ +7.5° (*c* 0.48; 50% aq. MeOH); IR (neat) ν 3350 (OH and $NH_2$) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref *t*-BuOH) δ 3.84 (1H, *dd*, $J_{1,2}$ = 6.6, $J_{2,3}$ = 5.7 Hz, 2-H), 3.81 (1H, *dd*, $J_{2,3}$ = 5.7, $J_{3,4}$ = 7.0 Hz, 3-H), 3.75 (1H, *dd*, $J_{5,6}$ = 4.6, $J_{gem}$ = 11.2 Hz, 6-H), 3.69 (1H, *dd*, $J_{5,6}$ = 5.4, $J_{gem}$ = 11.2 Hz, 6-H), 3.64 (1H, *dd*, $J_{1,2}$ = 6.6, $J_{1,5}$ = 8.3 Hz, 1-H), 3.16 (1H, *dd*, $J_{3,4}$ = 7.0, $J_{4,5}$ = 7.0 Hz, 4-H), 1.81 (1H, *dddd*, $J_{1,5}$ = 8.3, $J_{4,5}$ = 7.0, $J_{5,6}$ = 4.6 and 5.4 Hz, 5-H).

*1L-(1,3,4/2,5)-4-Acetamido-5-acetoxymethyl-1,2,3-tri-O-acetyl-1,2,3-cyclopentanetriol (19a)*

The free base **19** (14.0 mg, 0.0858 mmol) was acetylated conventionally. Chromatography of silica gel (1 g) with acetone:toluene (1:3, v/v) gave the acetate **19a** (21.8 mg, 68.1%) as a syrup ; $R_f$ 0.20 (acetone:toluene, 1:2), $[\alpha]_D^{21}$ −15.3° (*c* 1.09; $CHCl_3$); IR (neat) ν 3300 (NH), 1750 (OAc), 1690 (NAc) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.59 (1H, *br d*, $J_{4,NH}$ = 9.5 Hz, NH), 5.10 (1H, *dd*, $J_{1,2}$ = 4.1, $J_{2,3}$ = 2.9 Hz, 2-H), 5.07 (1H, *dd*, $J_{2,3}$ = 2.9, $J_{3,4}$ = 5.5 Hz, 3-H), 5.05 (1H, *dd*, $J_{1,2}$ = 4.1, $J_{1,5}$ = 6.7 Hz, 1-H), 4.59 (1H, *ddd*, $J_{3,4}$ = 5.5, $J_{4,5}$ = 10.6, $J_{4,NH}$ = 9.5 Hz, 4-H), 4.21 (1H, *dd*, $J_{5,6}$ = 4.2, $J_{gem}$ = 11.7 Hz, 6-H), 4.15 (1H, *dd*, $J_{5,6}$ = 5.0, $J_{gem}$ = 11.7 Hz, 6-H), 2.39 (1H, *dddd*, $J_{1,5}$ = 6.7, $J_{4,5}$ = 10.6, $J_{5,6}$ = 4.2 and 5.0 Hz, 5-H), 2.14, 2.10, 2.08, 2.07 and 2.01 (each 3H, 5*s*, 5Ac). Anal. calcd for $C_{16}H_{23}NO_9$: C, 51.47; H, 6.21; N, 3.75. Found: C, 51.75; H, 6.46; N, 3.92.

*1,6-O-:4,5-N,O-Diisopropylidene derivative 31 of 1D-(1,3/2,4,5)-5-acetamido-1-hydroxymethyl-1,2,3,4-cyclopentanetetraol*

Penta-*N*,*O*-acetyltrehazolamine (**29**) (292 mg, 0.749 mmol) was de-*O*-acetylated with 1 M methanolic NaOMe (0.5 mL) in MeOH (5 mL) for 1 h at room temperatue. The reaction mixture was neutralized with Amberlite IR 120B ($H^+$) and the resin was filtered off. The solution was evaporated to give the syrupy *N*-acetyl derivative **30** (161 mg), $R_f$ 0.14 (MeOH:$CHCl_3$, 1:4), which was treated with 2,2-dimethoxypropane (2.7 mL, 21.9 mmol, 30 equiv.) and a catalytic amount of *p*-toluenesulfonic acid monohydrate in DMF (3 mL) for 45 h at room temperature. After neutralization with $NaHCO_3$, the mixture was

evaporated to give a residue, which was diluted with $CHCl_3$ (30 mL) and then the salt was filtered off. The solution was evaporated to afford a syrupy residue, which was treated with AcOH (0.3 mL) in MeOH (4 mL) for 44 h at room temperature. The solution was concentrated to give a syrupy residue, which was chromatographed on a column of silica gel (7 g) with acetone:toluene (1:2, v/v, including 1% triethylamine) as eluent to afford the diisopropylidene derivative **31** (196 mg, 89.1%) as crystals, $R_f$ 0.38 (acetone:toluene, 1:1), mp 171–172 °C (from toluene); $[\alpha]_D^{25}$ –28.9° (*c* 0.81; $CHCl_3$); IR (neat) ν 3400 (OH) and 1630 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 4.58 (1H, *br d*, $J_{4,5}$ = 5.5 Hz, 4- or 5-H), 4.41 (1H, *br d*, $J_{4,5}$ = 5.5 Hz, 4- or 5-H), 4.33 and 4.04 (each 1H, ABq, $J_{gem}$ = 10.1 Hz, 2 × 6-H), 4.11 (1H, *br s*, 2-H), 3.96 (1H, *br d*, $J_{3,OH}$ = 8.8 Hz, 3-H), 2.88 (1H, *br d*, $J_{3,OH}$ = 8.8 Hz, OH), 2.57 (1H, *br s*, OH), 2.23 (3H, *s*, Ac), 1.64, 1.52, 1.47 and 1.44 (each 3H, 4*s*, $2CMe_2$). Anal. calcd for $C_{14}H_{23}NO_6$: C, 55.80; H, 7.69; N, 4.65. Found: C, 55.44; H, 7.97; N, 4.69.

*1,6-O-:4,5-N,O-Diisopropylidene derivative 32 of 1D-(1,3/2,4,5)-5-acetamido-2,3-di-O-acetyl-1-hydroxymethyl-1,2,3,4-cyclopentanetetraol*

The diol **31** (15.2 mg, 0.0504 mmol) was acetylated conventionally. Column chromatography of silica gel (1 g) with acetone:toluene (1:5, v/v) gave the tri-N,O-acetyl derivative **32** (19.4 mg, ~100%) as a syrup; $R_f$ 0.75 (acetone:toluene, 1:2), $[\alpha]_D^{24}$ –7.0° (*c* 0.97; $CHCl_3$); IR (neat) ν 1750 (OAc) and 1650 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.46 (1H, *d*, $J_{2,3}$ = 7.3 Hz, 2-H), 4.93 (1H, *dd*, $J_{2,3}$ = 7.3, $J_{3,4}$ = 0.7 Hz, 3-H), 4.58 (1H, *d*, $J_{4,5}$ = 6.4 Hz, 5-H), 4.39 (1H, *dd*, $J_{4,5}$ = 6.4, $J_{3,4}$ = 0.7 Hz, 4-H), 4.17 and 3.98 (each 1H, ABq, $J_{gem}$ = 9.5 Hz, 2 × 6-H), 2.26, 2.12 and 2.09 (each 3H, 3*s*, 3Ac), 1.72, 1.51, 1.38 and 1.34 (each 3H, 4*s*, $2CMe_2$). Anal. calcd for $C_{18}H_{27}NO_8$: C, 56.09; H, 7.06; N, 3.63. Found: C, 55.67; H, 7.40; N, 3.61.

*1,6-O-:4,5-N,O-Diisopropylidene derivative 33 of 1D-(1,3/2,4,5)-5-acetamido-3-O-benzoyl-1-hydroxymethyl-1,2,3,4-cyclopentanetetraol*

To a solution of the diol **31** (77.1 mg, 0.256 mmol) in pyridine (2 mL) was added BzCl (0.59 mL, 0.512 mmol, 2 equiv.) at 0 °C, and the mixture was stirred for 30 min at 0 °C. The reagent was quenched with MeOH (1 mL) and the mixture was evaporated to give a residue, which was diluted with $CHCl_3$ (35 mL) and washed with satd aq. $NaHCO_3$ (10 mL). The organic layer was dried and evaporated to afford a syrupy residue, which was chromatographed on a column of silica gel (5 g) with acetone:toluene (1:5, v/v) to give the benzoate **33** (104 mg, ~100%) as a syrup; $R_f$ 0.68 (acetone:toluene, 1:1), $[\alpha]_D^{24}$ –20.4° (*c* 1.27; $CHCl_3$); IR (neat) ν 3370 (OH), 1720 (OBz) and 1630 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 8.07–8.04, 7.64–7.59 and 7.50–7.44 (2, 1 and 2H, 3*m*, Ph), 5.07 (1H, *d*, $J_{2,3}$ = 5,1 Hz, 3-H), 4.61 (1H, *d*, $J_{4,5}$ = 6.2 Hz, 4-H), 4.57 (1H, *d*, $J_{4,5}$ = 6.2 Hz, 5-H), 4.35 and 4.00 (each 1H, ABq, $J_{gem}$ = 9.3 Hz, 2 × 6-H), 4.26 (1H, *br d*, $J_{2,3}$ = 5.1 Hz, 2-H), 3.42 (1H, *br s*, OH), 2.28 (3H, *s*, Ac), 1.72, 1.54, 1.48 and 1.44 (each 3H, 4*s*, $2CMe_2$). Anal. calcd for $C_{21}H_{27}NO_7$: C, 62.20; H, 6.71; N, 3.45. Found: C, 62.51; H, 6.76; N, 3.44.

*1,6-O-:4,5-N,O-Diisopropylidene derivative 34 of 1D-(1,3/2,4,5)-5-acetamido-3-O-benzoyl-1-hydroxymethyl-2-O-(1-imidazolyl)thiocarbonyl-1,2,3,4-cyclopentanetetraol*

To a solution of the benzoate **33** (92.7 mg, 0.229 mmol) in THF (2 mL) was added 1,1'-thiocarbonyldiimidazole (286 mg, 1.91 mmol, 7 equiv.) at room temperature, and the mixture was stirred for 24 h at reflux temperature. The mixture was evaporated to give a syrupy residue, which when chromatographed on a column of silica gel (5 g) with acetone:hexane (1:3, v/v) gave the thionocarbamate **34** (118 mg, ~100%) as a syrup; $R_f$ 0.25 (acetone:hexane, 1:3, twice irrigation), $[\alpha]_D^{22}$ –60.2° (*c* 0.98; $CHCl_3$); IR (neat) ν 1720 (OBz) and 1650 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 8.39, 7.67 and 7.05 (each 1H, 3 *br s*, imidazole), 8.05-8.02, 7.64–7.59 and 7.50–7.26 (2, 1 and 2H, 3*m*, Ph), 6.48 (1H, *d*, $J_{2,3}$ = 7.0 Hz, 2-H), 5.29 (1H, *dd*, $J_{2,3}$ = 7.0, $J_{3,4}$ = 0.7 Hz, 3-H), 4.83 (1H, *d*, $J_{4,5}$ = 6.2 Hz, 5-H), 4.62 (1H, *dd*, $J_{3,4}$ = 0.7, $J_{4,5}$ = 6.2, Hz, 4-H), 4.35 and 4.13 (each 1H, ABq, $J_{gem}$ = 9.5 Hz, 2 × 6-H), 2.31 (3H, *s*, Ac), 1.78, 1.56, 1.40 and 1.26 (each 3H, 4*s*, $2CMe_2$). HRMS calcd for $C_{25}H_{30}N_3O_7S$ $(M + H)^+$: m/z 516.1805. Found: m/z 516.1804.

*2,3-N,O-:4,6-O-Diisopropylidene derivative 38 of 1D-(1,4/2,3)-3-acetamido-1-O-benzoyl-4-hydroxymethyl-1,2,4-cyclopentanetriol*

To a solution of a catalytic AIBN and $Bu_3SnH$ (0.19 mL, 0.687 mmol, 3 equiv.) in toluene (0.5 mL) was added the thionocarbamate **34** (118 mg, 0.229 mmol) in toluene (2 mL) and the mixture was stirred for 10 min at reflux temperature. After cooling to room temperature, the mixture was evaporated to give an oily residue, which was purified by preparative TLC with acetone:toluene (1:5, v/v) to afford the deoxy derivative **38** (72.9 mg, 81.8%) as a syrup; $R_f$ 0.56 (acetone:toluene, 1:3), $[\alpha]_D^{23}$ –39.3° (*c* 0.93; $CHCl_3$); IR (neat) ν 1720 (OBz) and 1650 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 8.08–8.04, 7.63–7.56 and 7.49–7.43 (2, 1 and 2H, 3*m*, Ph), 5.30 (1H, *dd*, $J_{1,5}$ = 5.9 and 4.0 Hz, 1-H), 4.67 (1H, *d*, $J_{2,3}$ = 5.5 Hz, 2-H), 4.52 (1H, *d*, $J_{2,3}$ = 5.5 Hz, 3-H), 4.07 and 3.85 (each 1H, ABq, $J_{gem}$ = 9.3 Hz, 2 × 6-H), 2.59 (1H, *dd*, $J_{1,5}$ = 5.9, $J_{gem}$ = 14.3 Hz, 5-H), 2.25 (3H, *s*, Ac), 2.23 (1H, *dd*, $J_{1,5}$ = 4.0, $J_{gem}$ = 14.3 Hz, 5-H), 1.63, 1.55, 1.43 and 1.34 (each 3H, 4*s*, $2CMe_2$). Anal. calcd for $C_{21}H_{27}NO_6$: C, 64.77; H, 6.99; N, 3.60. Found: C, 64.37; H, 7.23; N, 3.64.

*1D-(1,4/2,3)-3-Amino-4-hydroxymethyl-1,2,4-cyclopentanetriol (17)*

The deoxy derivative **35** (72.9 mg, 0.187 mmol) was treated with 2 M aq. HCl (2 mL) for 13 h at 80 °C, and the mixture was evaporated to give a syrupy residue, which was purified by a column of Dowex 50W-X2 ($H^+$) resin (3 mL) with 1 M aq. $NH_4OH$ as eluent to afford the aminoalcohol **17** (27.2 mg, 89.2%) as a syrup, $R_f$ 0.65 (AcOH:$H_2O$:1-propanol, 1:1:3), $[\alpha]_D^{23}$ –15.2° (*c* 1.36; water); IR (neat) ν 3350 (OH and $NH_2$) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. *t*-BuOH) δ 4.19 (1H, *dd*, $J_{1,2}$ = 11.0, $J_{2,3}$ = 5.5

Hz, 2-H), 4.14 (1H, *ddd*, $J_{1,2}$ = 11.0, $J_{1,5}$ = 4.8 and 8.4 Hz, 1-H), 3.62 and 3.51 (each 1H, ABq, $J_{gem}$ = 12.1 Hz, 2 × 6-H), 3.25 (1H, *d*, $J_{2,3}$ = 5.5 Hz, 3-H), 2.29 (1H, *dd*, $J_{1,5}$ = 8.4, $J_{gem}$ = 15.0 Hz, 5-H), 1.51 (1H, *dd*, $J_{1,5}$ = 4.8, $J_{gem}$ = 15.0 Hz, 5-H).

*1D-(1,4/2,3)-3-Acetamido-4-acetoxymethyl-1,2-di-O-acetyl-1,2,4-cyclopentanetriol (17a)*

The aminocyclitol **17** (23.2 mg, 0.142 mmol) was acetylated conventionally. Chromatography of silica gel (2 g) with acetone:toluene (1:2, v/v) gave the acetate **17a** (43.0 mg, 91.3%) as a syrup; $R_f$ 0.33 (acetone:toluene, 1:1), $[\alpha]_D^{23}$ +45.3° (*c* 1.48; $CHCl_3$); IR (neat) ν 3360 (OH and NH), 1730 and 1720 (OAc), 1650 (NAc) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.14 (1H, *d*, $J_{3,NH}$ = 5.5 Hz, NH), 5.29 (1H, *dd*, $J_{1,2}$ = 1.5, $J_{2,3}$ = 6.2 Hz, 2-H), 5.05 (1H, *ddd*, $J_{1,2}$ = 1.5, $J_{1,5}$ = 3.7 and 7.3 Hz, 1-H), 4.65 (1H, *s*, OH), 4.59 (1H, *dd*, $J_{2,3}$ = 6.2, $J_{3,NH}$ = 5.5 Hz, 3-H), 1.99 and 1.93 (each 1H, ABq, $J_{gem}$ = 11.5 Hz, 2 × 6-H), 2.48 (1H, *dd*, $J_{1,5}$ = 7.3, $J_{gem}$ = 15.4 Hz, 5-H), 2.15, 2.11, 2.09 and 2.06 (each 3H, 4*s*, Ac), 1.96 (1H, *dd*, $J_{1,5}$ = 3.7, $J_{gem}$ = 15.4 Hz, 5-H). Anal. calcd for $C_{14}H_{21}NO_8$: C, 50.75; H, 6.39; N, 4.23. Found: C, 50.99; H, 6.48; N, 4.04.

*1,6-O-:4,5-N,O-Diisopropylidene derivative 35 of 1D-(1,3/2,4,5)-5-acetamido-3-O-benzoyl-1-hydroxymethyl-2-O-methoxymethyl-1,2,3,4-cyclopentanetetraol*

To a solution of the benzoate **33** (104 mg, 0.256 mmol) in $CH_2Cl_2$ (3 mL) was added *N,N*-diisopropylethylamine (0.72 mL, 4.10 mmol, 16 equiv.) and chloromethyl methyl ether (0.15 mL, 2.05 mmol, 8 equiv.) and the mixture was stirred for 23 h at reflux temperature. The reaction mixture was diluted with $CHCl_3$ (50 mL) and washed with 1 M aq. HCl (20 mL), satd aq. $NaHCO_3$ (20 mL) and water (20 mL × 2) and dried. The solution was evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (5 g) with acetone:toluene (1:5, v/v) as eluent to afford the compound **35** (115 mg, ~100%) as a syrup; $R_f$ 0.46 (ethanol:toluene, 1:10), $[\alpha]_D^{18}$ –61.7° (*c* 0.82; $CHCl_3$); IR (neat) ν 1720 (OBz) and 1660 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 8.10–8.06, 7.62–7.57 and 7.50–7.43 (2, 1 and 2H, 3*m*, Ph), 5.10 (1H, *dd*, $J_{2,3}$ = 7.0, $J_{3,4}$ = 0.7 Hz, 3-H), 4.79 and 4.76 (each 1H, ABq, $J_{gem}$ = 7.1 Hz, $C\underline{H}_2OCH_3$), 4.53 (1H, *d*, $J_{4,5}$ = 6.6 Hz, 5-H), 4.44 (1H, *dd*, $J_{3,4}$ = 0.7, $J_{4,5}$ = 6.6 Hz, 4-H), 4.32 (1H, *d*, $J_{2,3}$ = 7.0 Hz, 2-H), 4.27 and 4.06 (each 1H, ABq, $J_{gem}$ = 9.3 Hz, 2 × 6-H), 3.30 (3H, *s*, $CH_2OC\underline{H}_3$), 2.29 (3H, *s*, Ac), 1.76, 1.52, 1.45 and 1.43 (each 3H, 4*s*, $2CMe_2$). Anal. calcd for $C_{23}H_{31}NO_8$: C, 61.46; H, 6.95; N, 3.12. Found: C, 61.20; H, 7.25; N, 3.12.

*1,6-O-:4,5-N,O-Diisopropylidene derivative 36 of 1D-(1,3/2,4,5)-5-acetamido-1-hydroxymethyl-2-O-methoxymethyl-1,2,3,4-cyclopentanetetraol*

To a solution of the benzoate **35** (112 mg, 0.248 mmol) in $CH_2Cl_2$ (2 mL) was added 1 M methanolic NaOMe (0.2 mL) at room temperature, and the mixture was stirred for 30 min at room temperature. The mixture was diluted with $CHCl_3$ (40 mL), washed with water (10 mL) and dried. The solution was evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (4 g) with acetone:hexane (1:3, v/v) to give the alcohol **36** (82.1 mg, 95.8%) as a syrup; $R_f$ 0.23 (acetone:toluene, 1:3), $[\alpha]_D^{22}$ –63.4° (*c* 1.01; $CHCl_3$); IR (neat) ν 3440 (OH) and 1650 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 4.84 and 4.74 (each 1H, ABq, $J_{gem}$ = 7.0 Hz, $C\underline{H}_2OCH_3$), 4.43 (1H, *d*, $J_{4,5}$ = 6.8 Hz, 5-H), 4.35 (1H, *dd*, $J_{3,4}$ = 1.1, $J_{4,5}$ = 6.8 Hz, 4-H), 4.11 and 3.95 (each 1H, ABq, $J_{gem}$ = 9.2 Hz, 2 × 6-H), 3.93 (1H, *d*, $J_{3,OH}$ = 1.8 Hz, OH), 3.76 (1H, *ddd*, $J_{2,3}$ = 7.7, $J_{3,4}$ = 1.1, and $J_{3,OH}$ = 1.8 Hz, 3-H), 3.65 (1H, *d*, $J_{2,3}$ = 7.7 Hz, 2-H), 3.50 (3H, *s*, $CH_2OC\underline{H}_3$), 2.28 (3H, *s*, Ac), 1.72, 1.51, 1.44 and 1.39 (each 3H, 4*s*, $2CMe_2$). Anal. calcd for $C_{16}H_{27}NO_7$: C, 55.64; H, 7.88; N, 4.06. Found: C, 55.58; H, 8.21; N, 4.11.

*1,6-O-:4,5-N,O-Diisopropylidene derivative 37 of 1D-(1,3/2,4,5)-5-acetamido-1-hydroxymethyl-3-O-(1-imidazolyl)thiocarbonyl-2-O-methoxymethyl-1,2,3,4-cyclopentanetetraol*

To a solution of the alcohol **36** (72.6 mg, 0.210 mmol) in THF (2 mL) was added 1,1'-thiocarbonyldiimidazole (187 mg, 1.05 mmol, 5 equiv.), and the mixture was stirred for 3 h at reflux temperature. The reaction mixture was evaporated to give a syrupy residue, which was purified by preparative TLC with acetone:hexane (1:3, v/v) to give the thionocarbamate **37** (95.8 mg, ~100%) as a syrup; $R_f$ 0.53 (acetone:toluene, 1:1), $[\alpha]_D^{24}$ –59.6° (*c* 1.31; $CHCl_3$); IR (neat) ν 1650 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 8.36, 7.66 and 7.06 (each 1H, 3*br s*, imidazole), 5.62 (1H, *d*, $J_{2,3}$ = 5.5 Hz, 3-H), 4.79 and 4.74 (each 1H, ABq, $J_{gem}$ = 6.8 Hz, $C\underline{H}_2OCH_3$), 4.56 (1H, *d*, $J_{4,5}$ = 6.0 Hz, 4-H), 4.52 (1H, *d*, $J_{4,5}$ = 6.0 Hz, 5-H), 4.28 (1H, *d*, $J_{2,3}$ = 5.5 Hz, 2-H), 4.27 and 4.06 (each 1H, ABq, $J_{gem}$ = 9.5 Hz, 2 × 6-H), 3.33 (3H, *s*, $CH_2OC\underline{H}_3$), 2.27 (3H, *s*, Ac), 1.73, 1.52, 1.45 and 1.43 (each 3H, 4*s*, $2CMe_2$). HRMS, calcd for $C_{20}H_{29}N_3O_7S$ ($M^+$): m/z 455.1727. Found: m/z 455.1757.

*2,6-O-:3,4-N,O-Diisopropylidene derivative 39 of 1L-(1,3,4/2)-3-acetamido-1-O-methoxymethyl-2-hydroxymethyl-1,2,4-cyclopentanetriol*

To a solution of a catalytic AIBN and $Bu_3SnH$ (0.15 mL, 0.531 mmol, 3 equiv.) in toluene (0.5 mL) was added the thionocarbamate **37** (80.6 mg, 0.177 mmol) in toluene (1.5 mL) and the mixture was stirred for 10 min at reflux temperature. After cooling to room temperature, the mixture was evaporated to give an oily residue, which was chromatographed on a column of silica gel (4 g) acetone:toluene (1:6, v/v) as eluent to afford the deoxy derivative **39** (49.0 mg, 84.0%) as a hygroscopic syrup; $R_f$ 0.39 (acetone:toluene, 1:4), $[\alpha]_D^{18}$ –25.1° (*c* 1.02, toluene); IR (neat) ν 1650 (NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 4.72 and 4.65 (each 1H, ABq, $J_{gem}$ = 6.8 Hz, $C\underline{H}_2OCH_3$), 4.58 (1H, *ddd*, $J_{3,4}$ = 5.9, $J_{4,5}$ = 1.8 and 5.9 Hz, 4-H), 4.28 and 3.99 (each 1H, ABq, $J_{gem}$ = 9.7 Hz, 2 × 6-H), 4.24 (1H, *d*, $J_{3,4}$ = 5.9 Hz, 3-H), 4.00 (1H, *dd*, $J_{1,5}$ = 6.2 and 6.6 Hz, 1-H), 3.39 (3H, *s*, $CH_2OC\underline{H}_3$), 2.88 (1H, *ddd*, $J_{1,5}$ = 6.6, $J_{4,5}$ = 5.9, $J_{gem}$ = 14.7 Hz, 5-H), 2.24 (3H, *s*, Ac), 1.92 (1H, *ddd*, $J_{1,5}$ = 6.2, $J_{4,5}$ = 1.8, $J_{gem}$ = 14.7 Hz, 5-H), 1.66, 1.51 and 1.43 (3, 3 and 6H, 3*s*, $2CMe_2$). Anal. calcd for $C_{16}H_{27}NO_6$:

C, 58.34; H, 8.26; N, 4.25. Found: C, 57.86; H, 8.75; N, 4.19.

*1D-(1,3,4/2)-3-Amino-2-hydroxymethyl-1,2,4-cyclopentanetriol (18)*

The deoxy derivative **39** (49.0 mg, 0.149 mmol) was treated with 2 M aq. HCl (1 mL) for 5 h at 80 °C, and the mixture was evaporated to give a syrupy residue, which was purified by a column of Dowex 50W-X2 ($H^+$) resin (2.5 mL) with 1 M aq. $NH_4OH$ as eluent to afford the aminoalcohol **18** (24.3 mg, ~100%) as a syrup, $R_f$ 0.62 (AcOH:$H_2O$:1-propanol, 1:1:3), $[\alpha]_D^{23}$ –2.0° (*c* 0.74; water); IR (neat) ν 3350 (OH and $NH_2$) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. *t*-BuOH) δ 4.31 (1H, *ddd*, $J_{3,4}$ = 8.1 and 5.5, $J_{4,5}$ = 5.9 Hz, 4-H), 3.97 (1H, *dd*, $J_{2,3}$ = 5.5 and 7.3 Hz, 2-H), 3.82 (2H, *s*, 2 × 6-H), 3.09 (1H, *d*, $J_{4,5}$ = 5.9 Hz, 5-H), 2.62 (1H, *ddd*, $J_{2,3}$ = 7.3, $J_{3,4}$ = 8.1, $J_{gem}$ = 14.7 Hz, 3-H), 1.59 (1H, *ddd*, $J_{2,3}$ = 5.5, $J_{3,4}$ = 5.5, $J_{gem}$ = 14.7 Hz, 3-H).

*1D-(1,3,4/2)-3-Acetamido-2-acetoxymethyl-1,4-di-O-acetyl-1,2,4-cyclopentanetriol (18a)*

The aminocyclitol **18** (18.0 mg, 0.110 mmol) was acetylated conventionally. Chromatography of silica gel (2 g) with acetone:toluene (1:2, v/v) gave the acetate **18a** (33.7 mg, 92.3%) as a syrup; $R_f$ 0.36 (acetone:toluene, 1:1), $[\alpha]_D^{23}$ +14.6° (*c* 1.69; $CHCl_3$); IR (neat) ν 3400 (OH and NH), 1740 and 1730 (OAc), 1660 (NAc) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.01 (1H, *d*, $J_{3,NH}$ = 8.2 Hz, NH), 5.30 (1H, *ddd*, $J_{3,4}$ = 6.6, $J_{4,5}$ = 3.7 and 7.7 Hz, 4-H), 5.10 (1H, *dd*, $J_{1,5}$ = 4.8 and 7.7 Hz, 1-H), 4.59 (1H, *dd*, $J_{3,4}$ = 6.6, $J_{3,NH}$ = 8.2 Hz, 3-H), 4.34 and 4.27 (each 1H, ABq, $J_{gem}$ = 11.9 Hz, 2 × 6-H), 4.33 (1H, *s*, OH), 2.86 (1H, *ddd*, $J_{1,5}$ = 7.7, $J_{4,5}$ = 7.7, $J_{gem}$ = 15.8 Hz, 5-H), 2.10, 2.09, 2.08 and 2.05 (each 3H, 4*s*, Ac), 1.75 (1H, *ddd*, $J_{1,5}$ = 4.8, $J_{4,5}$ = 7.7, $J_{gem}$ = 15.8 Hz, 5-H). Anal. calcd for $C_{14}H_{21}NO_8$: C, 50.75; H, 6.39; N, 4.23. Found: C, 51.03; H, 6.62; N, 4.25.

*(3S,4R,5S,6R,7S)-7-Acetamido-4,5,6-tri-O-acetyl-1-oxaspiro[2.4]heptane-4,5,6-triol (41)*

The exo-olefin[6] **40** (21.7 mg, 0.0725 mmol) was treated with 70% *m*CPBA (53.4 mg, 0.218 mmol, 3 equiv.) in 1,2-dichloroethane (2 mL) in the presence of phosphate buffer (2 mL) for 26 h at room temperature in the dark. The mixture was diluted with $CHCl_3$ (30 mL), washed with satd aq. $Na_2S_2O_3$ (5 mL) and satd aq. $NaHCO_3$ (5 mL) and dried. The solution was evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (1 g) with acetone:toluene (1:2, v/v) as eluent to afford the epoxide **41** (19.8 mg, 86.5%) as a syrup; $R_f$ 0.41 (ethanol:toluene, 1:5, twice irrigation), $[\alpha]_D^{27}$ –63.7° (*c* 0.96; $CHCl_3$). The $^1H$ NMR data was identical with that of a racemic compound.[12] Anal. calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 51.61; H, 5.82; N, 4.20.

*1L-(1,2,4,5/3)-5-Acetamido-1-acetoxymethyl-2,3,4-tri-O-acetyl-1,2,3,4-cyclopentanetetraol (20a)*

To a solution of the epoxide **41** (19.8 mg) in 80% aq. DMF (1 mL) was added NaOAc (30.9 mg, 0.377 mmol, 6 equiv.), and the mixture was stirred for 20 h at 120 °C. The solution was evaporated to give a residue, which was acetylated conventionally. After evaporation of a solvent, column chromatography of silica gel (1 g) with acetone:toluene (1:2, v/v) as eluent to give the penta-*N*,*O*-acetyl derivative **20a** (16.0 mg, 65.3 %) as a syrup. The data of acetate were identical with those of an authentic compound.[5]

*1L-(1,2,4,5/3)-5-Amino-1-hydroxymethyl-1,2,3,4-cyclopentanetetraol (epitrehazolamine) (20)*

The acetate **20a** (42.5 mg, 0.109 mmol) was treated with 2 M aq. HCl (2 mL) for 2 h at 80 °C. The mixture was evaporated to give a residue, which was purified by a column of Dowex 50W-X2 ($H^+$) resin (1 mL) with 1 M aq. $NH_4OH$ as eluent to afford epitrehazolamine (**20**) (19.7 mg, ~100%) as a syrup, $R_f$ 0.38 ($H_2O$:acetonitrile, 1:4), $[\alpha]_D^{23}$ –3.8° (*c* 0.98; water); IR (neat) ν 3350 (OH and $NH_2$) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 3.96 (1H, *dd*, $J_{2,3}$ = 8.1, $J_{3,4}$ = 4.8 Hz, 3-H), 3.87 (1H, *dd*, $J_{3,4}$ = 4.8, $J_{4,5}$ = 7.7 Hz, 4-H), 3.69 (1H, *d*, $J_{2,3}$ = 8.1 Hz, 2-H), 3.51 (2H, *s*, 2 × 6-H), 3.27 (1H, *d*, $J_{4,5}$ = 7.7 Hz, 5-H).

*(1,3/2,4,5)- 45 and DL-(1,3,4/2,5)-4,5-Diacetamido-1,2,3-tri-O-(methoxymethyl)-1,2,3-cyclopentanetriols (46)*

To a solution of the alcohol **42** (1.00 g, 3.09 mmol) in acetone (12 mL) was added Jones' reagent (2.67 M, 2.5 mL) and the mixture was stirred for 2 h at room temperature. After cooling to 0 °C, 2-propanol (1 mL) was added to the mixture and then an insoluble material was removed by filtration through a Celite bed. The filtrate was diluted with chloroform (150 mL) and washed with water. The water layer was extracted with chloroform thoroughly, and the combined extracts were dried and evaporated. The residual ketone **43** was dissolved in methanol (12 mL) and treated with hydroxylamine hydrochloride (645 mg, 9.3 mmol, 3 molar equiv.) and magnesium sulfate (800 mg) for 2 h at room temperature. The reaction mixture was diluted with chloroform (150 mL) and washed with water, dried and evaporated. Without purification, a crude oxime **44** obtained was hydrogenated in ethanol (12 mL) containing acetic anhydride (1.5 mL) in the presence of freshly prepared Raney nickel T-4 (1 mL) in the initial hydrogen pressure of 2.9 kg $m^{-2}$ for 13 h at room temperature. The catalyst was removed by filtration and the filtrate was evaporated. Chromatography of the residue on a silica gel column (50 g) with acetone:toluene (1:1, v/v) → ethanol:toluene (1:4, v/v) as eluent afforded, first, the amide **46** (304 mg, 26.9%) as crystals, $R_f$ 0.42 (ethanol:toluene, 1:4), mp 180–181 °C (from ethanol:hexane) ; IR (KBr disk) ν 3435 (NH), 1650 (NAc) and 1560 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.48 and 6.15 (each 1H, 2*br d*, *J* = 8.1 Hz, 2 × NH), 4.76 and 4.67 (each 1H, ABq, *J* = 6.8 Hz, $CH_3OC\underline{H}_2$), 4.71 and 4.67 (each 1H, ABq, *J* = 7.0 Hz, $CH_3OC\underline{H}_2$), 4.70 (2H, *s*, $CH_3OC\underline{H}_2$), 4.43 (1H, *ddd*, $J_{4,5}$ = 11.7, *J* = 8.1, *J* = 8.1 Hz, 4- or 5-H), 4.24 (1H, *ddd*, $J_{4,5}$ = 11.7, *J* = 8.1, *J* = 4.8 Hz, 4- or 5-H), 4.00 (1H, *d*, *J* = 3.3 Hz, 2-H), 3.94 (1H, *d*, *J* = 4.8 Hz, 1- or 3-H), 3.80 (1H, *dd*, *J* = 8.1, *J* = 3.3 Hz, 1- or

3-H), 3.39, 3.38 and 3.36 (each 3H, 3H, 3 × $CH_3OCH_2$), 1.99 and 1.98 (each 3H, 2*s*, 2 × Ac). Anal. calcd for $C_{15}H_{28}N_2O_8$: C, 49.44; H, 7.75; N, 7.69. Found: C, 49.25; H, 8.00, N, 7.45.

The second fraction gave the amide **45** (176 mg, 16.6%) as crystals, $R_f$ 0.20 (ethanol:toluene, 1:4), mp 147–148 °C (from ethanol:hexane); IR (KBr disk) ν 3435 (NH), 1660 (NAc) and 1565 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.21 (2H, *br s*, $J$ = 4.1 Hz, 2 × NH), 4.77 and 4.71 (3 and 6H, 2*s*, $CH_3OCH_2$), 4.29 (2H, *dd*, $J_{1,5(3,4)}$ = 6.4, $J_{4,NH(5,NH)}$ = 4.1 Hz, 4- and 5-H), 4.05 (2H, *dd*, $J_{1,2(2,3)}$ = 6.6, $J_{1,5(3,4)}$ = 6.4 Hz, 1- and 3-H), 3.95 (1H, *t*, $J_{1,2(2,3)}$ = 6.6 Hz, 2-H), 3.39 and 3.36 (3 and 6H, 2*s*, 3 × $CH_3OCH_2$), 1.99 (6H, *s*, 2 × Ac). Anal. found: C, 49.17, H, 8.07, N, 7.49.

*(1,3/2,4,5)-4,5-Diamino-1,2,3-cyclopentanetriol (21)*

A mixture of the amide **45** (162 mg, 0.444 mmol) was heated with 2 M hydrochloric acid (4 mL) for 3 h at 80 °C, and then evaporated to dryness. Chromatography of the residue on a column of Dowex 50W-X2 ($H^+$) resin (7 mL) with 2.5 M aqueous ammonia as eluent to give the amine **21** (60.6 mg, 92.1%) as a colorless syrup, $R_f$ 0.55 (AcOH: $H_2O$:1-propanol, 1:1:3); IR (neat) ν 3320 (OH and $NH_2$) $cm^{-1}$, $^1H$ NMR ($D_2O$) δ 3.66–3.50 (3H, *m*, 1-, 2- and 3-H) and 3.04 (2H, *d*, $J_{1,5(3,4)}$ = 5.5 Hz, 4- and 5-H).

*(1,3/2,4,5)-1,2,3-Tri-O-acetyl-4,5-diacetamido-1,2,3-cyclopentanetriol (21a)*

A mixture of the amine **21** (50.1 mg, 0.338 mmol), acetic anhydride (1 mL) and pyridine (1 mL) was stirred for 2 h at room temperature and then evaporated. The residue was chromatographed on a silica gel column (8 g) with acetone: toluene (1:1, v/v) as eluent to give the penta-*N,O*-acetyl derivative **21a** (103 mg, 84.6%) as crystals, mp 199–200 °C (from ethanol); $R_f$ 0.15 (acetone:toluene, 1:1); IR (KBr disk) ν 3435 (NH), 1735 (OAc), 1665 (NAc) and 1545 (NH) $cm^{-1}$, $^1H$ NMR ($CDCl_3$) δ 6.37 (2H, *br s*, $J_{4,NH(5,NH)}$ = 5.9 Hz, 2 × NH), 5.66 (2H, *dd*, $J_{1,2(2,3)}$ = 7.6, $J_{1,5(3,4)}$ = 6.4 Hz, 1- and 3-H), 5.28 (1H, *t*, $J_{1,2(2,3)}$ = 7.6 Hz, 2-H), 4.16 (2H, *dd*, $J_{1,5(3,4)}$ = 6.4, $J_{4,NH(5,NH)}$ = 5.9 Hz, 4- and 5-H) and 2.07 and 2.00 (9H and 6H, 2*s*, 5 × Ac). Anal. calcd for $C_{15}H_{22}N_2O_8$ requires C, 50.28; H, 6.19; N, 7.82. Found: C, 50.29; H, 6.33; N, 7.80.

*DL-(1,3,4/2,5)-1,2,3-Tri-O-acetyl-4,5-diacetamido-1,2,3-cyclopentanetriols (47)*

A mixture of the amide **46** (78.3 mg, 0.215 mmol) and 2 M hydrochloric acid (2 mL) was stirred for 4 h at 80 °C, and then evaporated to dryness. The residue was treated with acetic anhydride (1 mL) and pyridine (1 mL) for 2 h at room temperature. The product was chromatographed on a silica gel (4 g) with ethanol:toluene (1:5, v/v) as eluent to give the penta-*N,O*-acetyl derivative **47** (62.3 mg, 81%) as crystals, $R_f$ 0.14 (acetone:toluene, 1:1), mp 250–251 °C (from ethanol) , IR (KBr, disk) ν 3277 (NH), 1765, 1740 (OAc), 1655 (NAc) and 1560 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.38 and 6.36 (each 1H, 2*br d*, $J$ = 8.2, $J$ = 7.3 Hz, 2 × NH), 5.12 (1H, *dd*, $J$ = 5.5, $J$ = 1.3 Hz, 1- or 3-H), 5.10 (1H, *dd*, $J$ = 8.2, $J$ = 4.6 Hz, 1- or 3-H), 5.01 (1H, *dd*, $J$ = 4.6, $J$ = 1.3 Hz, 2-H), 4.54 (1H, *ddd*, $J_{4,5}$ = 12.1, $J$ = 8.2, $J$ = 8.2 Hz, 4- or 5-H), 4.46 (1H, *ddd*, $J_{4,5}$ = 12.1, $J$ = 7.3, $J$ = 5.5 Hz, 4- or 5-H), 2.15, 2.101, 2.098, 1.98 and 1.96 (each 3H, 5*s*, 5 × Ac). Anal. calcd for $C_{15}H_{22}N_2O_8$: C, 50.28; H, 6.19; N, 7.82. Found: C, 50.04; H, 6.45; N, 7.68.

*DL-(1,3,5/2,4)-5-Acetamido-1-O-mesyl-2,3,4-tri-O-(methoxymethyl)-1,2,3,4-cyclopentanetetraol (48)*

To a solution of the alcohol **42** (39.1 mg, 0.12 mmol) in pyridine (1 mL) was added mesyl chloride (28 μL, 0.363 mmol, 3 molar equiv.), and the mixture was stirred for 2 h at room temperature and then evaporated. Chromatography of the residue on a silica gel column (1.5 g) with acetone:toluene (1:3, v/v) as eluent gave the mesylate **48** (48.5 mg, ~100%) as crystals, $R_f$ 0.49 (acetone:toluene, 1:1), mp 99–101 °C (from ethanol), IR (KBr disk) ν 3435 (NH), 1645 (NAc) and 1560 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.24 (1H, *br d*, $J_{5,NH}$ = 8.1 Hz, NH), 5.03 (1H, *d*, $J_{1,5}$ = 4.8 Hz, 1-H), 4.78–4.68 (6H, *m*, 3 × $CH_3OCH_2$), 4.40 (1H, *ddd*, $J_{1,5}$ = 4.8, $J_{4,5}$ = 6.6, $J_{5,NH}$ = 8.1 Hz, 5-H), 4.10 (1H, *br s*, 2-H), 4.01 (1H, *dd*, $J_{2,3}$ = 3.1, $J_{3,4}$ = 6.6 Hz, 3-H), 3.96 (1H, *dd*, $J_{3,4}$ = 6.6, $J_{5,NH}$ = 8.1 Hz, 5-H), 3.42, 3.39 and 3.38 (each 3H, 3*s*, 3 × $CH_3OCH_2$), 3.06 (3H, *s*, Ms), 2.03 (3H, *s*, Ac). Anal. calcd for $C_{14}H_{27}NO_{10}S$: C, 41.89; H, 6.78; N, 3.49. Found: C, 41.48, 7.15; N, 3.45.

*DL-(1,3,4/2,5)-5-Acetamido-4-azido-1,2,3-tri-O-methoxymethyl-1,2,3-cyclopentanetriol (49)*

A mixture of the mesylate **48** (50.7 mg, 0.126 mmol), sodium azide (82.1 mg, 1.26 mmol, 10 molar equiv.) and 90% aqueous DMF (1 mL) was stirred for 2 days at 120 °C. After cooling, the mixture was dissolved in water (10 mL) and extracted with chloroform (20 mL × 3). The extracts were dried and evaporated to dryness. Chromatography of the residue on a silica gel column (1.5 g) with acetone:toluene (1:4, v/v) as eluent to give the azide **49** (33.4 mg, 75.9%) as a syrup, $R_f$ 0.40 (acetone:toluene, 1:2), IR (neat) ν 3280 (NH), 2110 ($N_3$), 1660 (NAc) and 1560 (NH) $cm^{-1}$, $^1H$ NMR ($CDCl_3$) δ 5.93 (1H, *br d*, $J_{5,NH}$ = 7.7 Hz, NH), 4.78 and 4.73 (each 1H, ABq, $J_{gem}$ = 6.6 Hz, $CH_3OCH_2$), 4.72 and 4.69 (each 1H, ABq, $J_{gem}$ = 6.8 Hz, $CH_3OCH_2$), 4.71 and 4.68 (each 1H, ABq, $J_{gem}$ = 7.0 Hz, $CH_3OCH_2$), 4.27 (1H, *ddd*, $J_{5,NH}$ = 7.7, $J$ = 8.4, $J$ = 7.0 Hz, 5-H), 4.07–4.02 (2H, *m*, 2- and 3-H), 3.93 (1H, *dd*, $J$ = 7.0, $J$ = 3.7 Hz, 1- or 4-H), 3.75 (1H, *dd*, $J$ = 8.4, $J$ = 4.8 Hz, 1- or 4-H), 3.44, 3.38 and 3.37 (each 3H, 3*s*, 3 × $CH_3OCH_2$) and 2.03 (3H, *s*, Ac). Anal. calcd for $C_{13}H_{21}N_4O_7$: C, 44.82; H, 6.94; N, 16.08. Found: C, 44.81; H, 7.20; N, 16.10.

A solution of **49** (31.4 mg, 0.0901 mmol) in ethanol (1 mL) containing acetic anhydride (43 μL, 0.451 mmol, 5 equiv.) was hydrogenated in the presence of Raney nickel T-4 for 30 min under the atmospheric hydrogen pressure. The catalyst was removed by filtration and the filtrate was evaporated. The product was purified by a silica gel column to give the penta-*N,O*-acetyl derivative **47** (28.2 mg, 86.0%) as crystals, identical with the compound obtained before.

*N-[(1S)-(1,2,4,5/3)-5-Hydroxymethyl-2,3,4-trihydroxycyclopentyl]-N'-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)thiourea (50)*

The aminoalcohol **16** (8.5 mg, 0.0521 mmol) was coupled with 2,3,4,6-tetra-*O*-benzyl-α-D-glucosylisothiocyanate[7] **(23)** (38.7 mg, 0.0677 mmol, 1.3 equiv.) in 75% aq. THF (2 mL) for 24 h at room temperature. The reaction mixture was evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (2 g) with EtOAc:toluene (1:3, v/v) → EtOH:toluene (1:5, v/v) as eluent to afford the thiourea **50** (26.4 mg, 68.0%) as a hygroscopic syrup; $R_f$ 0.42 (EtOH:toluene, 1:5), $[\alpha]_D^{24}$ +91.9° (*c* 1.32; $CHCl_3$); IR (neat) ν 3300 (OH and NH) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.70 (1H, *d*, $J_{1,NH}$ = 7.7 Hz, NH), 7.31–7.10 (20H, *m*, 4Ph), 6.74 (1H, *br s*, N'H), 5.26 (1H, *br d*, $J_{1',2'}$ = 4.8 Hz, 1'-H), 4.93 (1H, *br s*, 1-H), 4.90 and 4.76 (each 1H, ABq, $J_{gem}$ = 11.0 Hz, $PhCH_2$), 4.80 and 4.45 (each 1H, ABq, $J_{gem}$ = 12.0 Hz, $PhCH_2$), 4.63 and 4.59 (each 1H, ABq, $J_{gem}$ = 12.5 Hz, $PhCH_2$), 4.46 and 4.40 (each 1H, ABq, $J_{gem}$ = 11.9 Hz, $PhCH_2$), 3.95–3.45 (9H, *m*, 2, 3, 4, 2 × 6, 3', 5' and 2 × 6'-H), 3.83 (1H, *br s*, OH), 3.69 (1H, *dd*, $J_{1',2'}$ = 4.8, $J_{2',3'}$ = 9.5 Hz, 2'-H), 3.57 (1H, *br s*, OH), 3.45 (1H, *br s*, OH), 3.37 (1H, *dd*, *J* = 8.4 and 9.5 Hz, 4'-H), 2.64 (1H, *br s*, 5-H), 1.87 (1H, *br s*, OH). Anal. calcd for $C_{41}H_{48}N_2O_9S$: C, 66.11; H, 6.49; N, 3.76. Found: C, 65.71; H, 6.69; N, 3.75.

*(1S,5S,6S,7R,8S)-3-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo-[3.3.0]oct-3-ene-7,8-diol (55)*

The thiourea **50** (25.1 mg, 0.0337 mmol) was treated with three portions of yellow HgO (65.7 mg, 0.303 mmol, 9 equiv.) in diethyl ether (2 mL) for 20 h at room temperature. The mixture was filtered through a bed of Celite and the Celite bed was washed with EtOH. The filtrate and washings were combined and evaporated to give the cyclic isourea **55** (24.0 mg, ~100%) as a syrup; $R_f$ 0.38 (EtOH:toluene, 1:5), $[\alpha]_D^{24}$ +99.5° (*c* 1.20; $CHCl_3$); IR (neat) ν 3350 (OH and NH), 1670 (C=N), and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.32–7.24 (20H, *m*, 4Ph), 5.36 (1H, *br s*, 1'-H), 4.90 and 4.75 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, $PhCH_2$), 4.78 and 4.46 (each 1H, ABq, $J_{gem}$ = 11.2 Hz, $PhCH_2$), 4.67 (1H, *br d*, *J* = 8.4 Hz, 1-H), 4.63 (1H, *br d*, *J* = 8.4 Hz, 5-H), 4.55 and 4.41 (each 1H, ABq, $J_{gem}$ = 11.4 Hz, $PhCH_2$), 4.59 and 4.50 (each 1H, ABq, $J_{gem}$ = 11.6 Hz, $PhCH_2$), 4.25 (1H, *br d*, *J* = 12.9 Hz, 9-H), 4.10 (1H, *br dd*, *J* = 5.1 and 12.9 Hz, 9-H), 3.95 (1H, *br d*, *J* = 3.9 Hz, 7-H), 3.71 (1H, *br s*, OH), 3.77–3.57 (9H, *m*, 8-, 2'-, 3'-, 4'-, 5'-, 2 × 6'-H and 2 × OH), 2.17 (1H, *br s*, 6-H). Anal. calcd for $C_{41}H_{46}N_2O_9$: C, 69.27; H, 6.79; N, 3.93. Found: C, 69.28; H, 6.52; N, 3.94.

*(1S,5S,6S,7R,8S)-3-(α-D-Glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-7,8-diol (6-deoxytrehazolin) (2)*

To a blue mixture of sodium (78.6 mg, 3.42 mmol, 100 equiv.) and liquid ammonia (*ca* 5 mL) was added a solution of the isourea **55** (24.3 mg, 0.0324 mmol) in THF (1 mL) at –78 °C and the mixture was stirred for 15 min at the same temperature. Powdered $NH_4Cl$ (274 mg, 5.13 mmol, 150 equiv.) was added to the reaction mixture and then ammonia was allowed to evaporate spontaneously. The residue obtained was diluted with water (5 mL) and washed with $CHCl_3$ (5 mL × 3). The water layer was charged on to a column of Dowex 50W-X2 ($H^+$) resin (15 mL) and eluted with 0.5 M aq. $NH_4OH$ to give the free base **2** (9.3 mg, 72.7%) as a white solid, $R_f$ 0.45 (AcOH:$H_2O$:acetonitrile, 1:4:10), $[\alpha]_D^{21}$ +132° (*c* 0.47; water); IR (neat) ν 3410 (OH and NH), 1670 (C=N) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 5.14 (1H, *d*, $J_{1',2'}$ = 5.1 Hz, 1'-H), 4.73 (1H, *d*, $J_{1,5}$ = 7.3 Hz, 1-H), 4.45 (1H, *dd*, $J_{1,5}$ = 7.3, $J_{5,6}$ = 7.3 Hz, 5-H), 4.11 (1H, *s*, 8-H), 4.01 (1H, *d*, $J_{6,7}$ = 4.0 Hz, 6-H), 3.77 (1H, *dd*, $J_{6,9}$ = 7.0, $J_{gem}$ = 10.9 Hz, 9-H), 3.72 (1H, *dd*, $J_{6,9}$ = 7.7, $J_{gem}$ = 10.9 Hz, 9-H), 3.66 (1H, *dd*, $J_{5,6'}$ = 2.6, $J_{gem}$ = 12.8 Hz, 6'-H), 3.59 (1H, *dd*, $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 8.4 Hz, 2'-H), 3.58 (1H, *dd*, $J_{5,6'}$ = 5.1, $J_{gem}$ = 12.8 Hz, 6'-H), 3.50 (1H, *dd*, $J_{2',3'}$ = 8.4, $J_{3',4'}$ = 9.9 Hz, 3'-H), 3.42 (1H, *ddd*, $J_{4',5'}$ = 8.8, $J_{5,6'}$ = 2.6 and 5.1 Hz, 5'-H), 3.25 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 8.8 Hz, 4'-H), 2.27 (1H, *dddd*, $J_{5,6}$ = 7.3, $J_{6,7}$ = 4.0, $J_{6,9}$ = 7.0 and 7.7 Hz, 6-H).

*(1S,5S,6S,7R,8S)-6-Acetoxymethyl-4,7,8-tri-N,O,O-acetyl-3-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-imino-2-oxa-4-azabicyclo[3.3.0]octane-7,8-diol (2a)*

6-Deoxytrehazolin (**2**) (9.3 mg, 0.0248 mmol) was acetylated with acetic anhydride and pyridine overnight at room temperature. Chromatography of the crude product on a column of silica gel (1 g) with acetone:toluene (1:4, v/v) as eluent gave the octa-*N,O*-acetyl derivative **2a** (17.0 mg, 99.4%) as a syrup; $R_f$ 0.32 (acetone:toluene, 1:3), $[\alpha]_D^{23}$ +103° (*c* 0.85; $CHCl_3$); IR (neat) ν 1750, 1740 and 1720 (OAc) and 1690 (C=N and NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.56 (1H, *d*, $J_{1',2'}$ = 4.2 Hz, 1'-H), 5.41 (1H, *dd*, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 9.9 Hz, 3'-H), 5.24 (1H, *ddd*, $J_{1,7}$ = 1.1, $J_{6,7}$ = 4.2, $J_{7,8}$ = 2.3 Hz, 7-H), 5.20 (1H, *dd*, $J_{1,8}$ = 1.1, $J_{7,8}$ = 2.3 Hz, 8-H), 5.15 (1H, *dd*, $J_{1,5}$ = 7.7, $J_{5,6}$ = 7.3 Hz, 5-H), 5.10 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 9.9 Hz, 4'-H), 5.07 (1H, *dd*, $J_{1',2'}$ = 4.2, $J_{2',3'}$ = 10.0 Hz, 2'-H), 4.70 (1H, *ddd*, $J_{1,5}$ = 7.7, $J_{1,7}$ = 1.1, $J_{1,8}$ = 1.1 Hz, 1-H), 4.36 (1H, *dd*, $J_{gem}$ = 11.5, $J_{6,9}$ = 5.1 Hz, 9-H), 4.23 (1H, *dd*, $J_{5',6'}$ = 4.4, $J_{gem}$ = 12.5 Hz, 6'-H), 4.07 (1H, *dd*, $J_{gem}$ = 11.5, $J_{6,9}$ = 8.4 Hz, 9-H), 4.03 (1H, *dd*, $J_{5,6'}$ = 2.2, $J_{gem}$ = 12.5 Hz, 6'-H), 2.66, 2.11, 2.09, 2.04, 2.03, 2.00 and 1.97 (3, 3, 6, 3, 3, 3 and 3H, 7*s*, 8Ac), 2.48 (1H, *dddd*, $J_{5,6}$ = 7.3, $J_{6,7}$ = 4.2, $J_{6,9}$ = 5.1 and 8.4 Hz, 6-H). Anal. calcd for $C_{29}H_{38}N_2O_{17}$: C, 50.73; H, 5.58; N, 4.08. Found: C, 51.07; H, 5.95; N, 3.90.

*N-[(1R)-(1,5/2,4)-2-Hydroxymethyl-2,4,5-trihydroxycyclopentyl]-N'-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-thiourea (51)*

The aminoalcohol **17** (15.6 mg, 0.0956 mmol) was allowed to couple with the isothiocyanate **23** (72.3 mg, 0.124 mmol, 1.3 equiv.) in 75% aq. DMF (1.5 mL) for 15 h at room temperature. The crude product was chromatographed on a column of silica gel (7 g) with EtOAc:hexane (1:2, v/v) → EtOH:toluene (1:15, v/v) as eluent to afford the thiourea **51** (67.5 mg, 94.8%) as a syrup; $R_f$ 0.40 (EtOH:toluene, 1:5), $[\alpha]_D^{20}$ +143° (*c* 0.90; $CHCl_3$); IR (neat) ν 3340 (OH and NH) and 1530 (NH)

$cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.36–7.07 (20H, *m*, 4Ph), 6.71 (1H, *d*, $J_{1',N'H}$ = 3.3 Hz, N'H), 5.04–4.96 (3H, *m*, 1, 1'-H and OH), 4.92 and 4.77 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, PhC$\underline{H}_2$), 4.81 and 4.42 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, PhC$\underline{H}_2$), 4.68 and 4.60 (each 1H, ABq, $J_{gem}$ = 11.5 Hz, PhC$\underline{H}_2$), 4.47 and 4.42 (each 1H, ABq, $J_{gem}$ = 12.4 Hz, PhC$\underline{H}_2$), 4.38 (1H, *s*, OH), 4.12–4.08 (2H, *m*, 4-H and OH), 3.95 (1H, *dd*, *J* = 4.4 and 11.1 Hz, 5-H), 3.85 (1H, *ddd*, $J_{4',5'}$ = 10.3, $J_{5',6'}$ = 7.0 and 1.5 Hz, 5'-H), 3.76 (1H, *dd*, $J_{2',3'}$ = 9.5, $J_{3',4'}$ = 8.4 Hz, 3'-H), 3.67 (1H, *dd*, $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 9.5 Hz, 2'-H), 3.58 (1H, *dd*, $J_{5',6'}$ = 1.5, $J_{gem}$ = 10.6 Hz, 6'-H), 3.40 and 3.17 (each 1H, ABq, $J_{gem}$ = 10.3 Hz, 2 × 6-H), 3.39 (1H, *dd*, $J_{5',6'}$ = 7.0, $J_{gem}$ = 10.6 Hz, 6'-H), 3.15 (1H, *dd*, $J_{3',4'}$ = 8.4, $J_{4',5'}$ = 10.3 Hz, 4'-H), 2.43 (1H, *dd*, $J_{3',4'}$ = 5.3, $J_{gem}$ = 15.4 Hz, 3-H), 2.07 (1H, *br s*, OH), 1.80 (1H, *d*, $J_{gem}$ = 15.4 Hz, 3-H). Anal. calcd for $C_{41}H_{48}N_2O_9S$: C, 66.11; H, 6.50; N, 3.76. Found: C, 66.03; H, 6.70; N, 3.57.

*(1S,5R,6R,8S)-3-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]-oct-3-ene-6,8-diol (**56**)*

The thiourea **51** (63.2 mg, 0.0848 mmol) was treated with three portions of yellow HgO (165 mg, 0.762 mmol, 9 equiv.) in diethyl ether (1 mL) for 28 h at room temperature. The mixture was filtered through a bed of Celite and the filtrate was evaporated to give the cyclic isourea **56** (60.3 mg, ~100%) as a syrup; $R_f$ 0.32 (acetone:toluene, 1:1), $[\alpha]_D^{23}$ +50.2° (*c* 0.87, $CHCl_3$); IR (neat) ν 3340 (OH and NH), 1660 (C=N) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.31–7.07 (20H, *m*, 4Ph), 5.65 (1H, *d*, $J_{1',2'}$ = 5.5 Hz, 1'-H), 4.92 (1H, *d*, $J_{1,5}$ = 7.7 Hz, 1-H), 4.89 and 4.76 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, PhC$\underline{H}_2$), 4.76 and 4.44 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, PhC$\underline{H}_2$), 4.62 and 4.55 (each 1H, ABq, $J_{gem}$ = 11.5 Hz, PhC$\underline{H}_2$), 4.57 and 4.41 (each 1H, ABq, $J_{gem}$ = 12.1 Hz, PhC$\underline{H}_2$), 4.49 (1H, *d*, $J_{1,5}$ = 7.7 Hz, 5-H), 4.28–3.41 (3H, *m*, 3OH), 4.10 (1H, *d*, $J_{7,8}$ = 4.8 Hz, 8-H), 3.91–3.57 (5H, *m*, 3', 4', 5' and 2 × 6'-H), 3.87 and 3.53 (each 1H, ABq, $J_{gem}$ = 11.5 Hz, 2 × 9-H), 3.80 (1H, *dd*, $J_{1',2'}$ = 5.5, $J_{2',3'}$ = 9.5 Hz, 2'-H), 1.69 (1H, *d*, $J_{gem}$ = 14.3 Hz, 7-H), 1.53 (1H, *dd*, $J_{7,8}$ = 4.8, $J_{gem}$ = 14.3 Hz, 7-H). Anal. calcd for $C_{41}H_{46}N_2O_9$: C, 69.28; H, 6.52; N, 3.94. Found: C, 69.67; H, 6.94; N, 4.00.

*(1S,5R,6R,,8S)-3-(α-D-Glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,8-diol (7-deoxytrahazolin) (**3**)*

To a mixture of sodium (111 mg, 4.83 mmol, 100 equiv.) and liquid ammonia (*ca* 5 mL) was added the isourea **56** (34.3 mg, 0.0483 mmol) in THF (1 mL) at –78 °C and the mixture was stirred for 15 min at the same temperature. The mixture was processed in the usual manner. The crude product was purified by a column of Dowex 50W-X2 ($H^+$) resin (20 mL) with 0.25 M aq. $NH_4OH$ as eluent to give the free base **3** (16.6 mg, 91.7%) as a white solid, $R_f$ 0.50 (AcOH:$H_2O$:acetonitrile, 1:4:10), $[\alpha]_D^{21}$ +120° (*c* 0.83, water); IR (neat) ν 3390 (OH and NH), 1660 (C=N), and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 5.15 (1H, *d*, $J_{1',2'}$ = 5.1 Hz, 1'-H), 4.89 (1H, *d*, $J_{1,5}$ = 7.0 Hz,1-H), 4.21 (1H, *dd*, $J_{1,5}$ = 7.0, $J_{5,7}$ = 0.9 Hz, 5-H), 4.19 (1H, *br dd*, $J_{7,8}$ = 5.3 and 0.9 Hz, 8-H), 3.66 (1H, *dd*, $J_{5',6'}$ = 2.4, $J_{gem}$ = 12.3 Hz, 6'-H), 3.60 (1H, *dd*, $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 9.7 Hz, 2'-H), 3.57 (1H, *dd*, $J_{5',6'}$ = 4.9, $J_{gem}$ = 12.3 Hz, 6'-H), 3.56 and 3.47 (each 1H, ABq, $J_{gem}$ = 12.1 Hz, 2 × 9-H), 3.49 (1H, *dd*, $J_{2',3'}$ = 9.7, $J_{3',4'}$ = 8.8 Hz, 3'-H), 3.41 (1H, *ddd*, $J_{4',5'}$ = 9.9, $J_{5',6'}$ = 2.4 and 4.9 Hz, 5'-H), 3.25 (1H, *dd*, $J_{3',4'}$ = 8.8, $J_{4',5'}$ = 9.9 Hz, 4'-H), 1.79 (1H, *dd*, $J_{7,8}$ = 5.3, $J_{gem}$ = 14.4 Hz, 7-H), 1.68 (1H, *ddd*, $J_{5,7}$ = 0.9, $J_{7,8}$ = 0.9, $J_{gem}$ = 14.4 Hz, 7-H).

*(1S,5R,6R,8S)-6-Acetoxymethyl-4,6-di-N,O-acetyl-3-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)imino-2-oxa-4-azabicyclo[3.3.0]octane-6,8-diol (**3a**)*

7-Deoxytrehazolin (**3**) (16.6 mg, 0.0443 mmol) was acetylated conventionally. Chromatography of silica gel (1 g) with acetone:toluene (1:4, v/v) gave the hepta-*N,O*-acetyl derivative **3a** (28.6 mg, ~100%) as a syrup, $R_f$ 0.43 (acetone:toluene, 1:2); $[\alpha]_D^{22}$ +121° (*c* 1.43; $CHCl_3$); IR (neat) ν 3490 (OH), 1760, 1750, 1740, 1730, 1720, and 1710 (OAc) and 1695 (C=N and NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.58 (1H, *d*, $J_{1',2'}$ = 4.4 Hz, 1'-H), 5.40 (1H, *dd*, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 9.5 Hz, 3'-H), 5.24 (1H, *ddd*, $J_{1,8}$ = 4.0, $J_{7,8}$ = 7.3 and 1.1 Hz, 8-H), 5.07 (1H, *dd*, $J_{3',4'}$ = 9.5, $J_{4',5'}$ = 10.3 Hz, 4'-H), 5.06 (1H, *dd*, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 10.3 Hz, 2'-H), 4.87 (1H, *d*, $J_{1,5}$ = 11.0 Hz, 5-H), 4.83 (1H, *dd*, $J_{1,5}$ = 11.0, $J_{1,8}$ = 4.0 Hz, 1-H), 4.30 (1H, *ddd*, $J_{4',5'}$ = 10.3, $J_{5',6'}$ = 2.2 and 4.8 Hz, 5'-H), 4.21 (1H, *dd*, $J_{5',6'}$ = 4.8, $J_{gem}$ = 12.1 Hz, 6'-H), 4.09 (1H, *dd*, $J_{5',6'}$ = 2.2, $J_{gem}$ = 12.1 Hz, 6'-H), 3.99 and 3.92 (each 1H, ABq, $J_{gem}$ = 11.5 Hz, 2 × 9-H), 3.56 (1H, *s*, OH), 2.65, 2.12, 2.10, 2.06, 2.04, 2.002 and 1.996 (each 3H, 7*s*, 7Ac), 2.62 (1H, *dd*, $J_{7,8}$ = 7.3, $J_{gem}$ = 14.3 Hz, 7-H), 2.12–2.00 (1H, *m*, 7-H). Anal. calcd for $C_{27}H_{36}N_2O_{16}$: C, 50.31; H, 5.63; N, 4.35. Found: C, 49.99; H, 5.79; N, 4.02.

*N-[(1R)-(1,3,5/2)-2-Hydroxymethyl-2,3,5-trihydroxycyclopentyl]-N'-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-thiourea (**52**)*

The aminoalcohol **18** (13.1 mg, 0.0802 mmol) was coupled with the isothiocyanate **23** (56.9 mg, 0.0978 mmol, 1.22 equiv.) in 75% aq. DMF (2 mL) for 7 h at room temperature. The product was chromatographed on a column of silica gel (5 g) with EtOAc:hexane (1:2, v/v) → EtOH:toluene (1:20, v/v) as eluent to afford the thiourea **52** (57.9 mg, 96.8%) as a syrup; $R_f$ 0.50 (EtOH:toluene, 1:5), $[\alpha]_D^{23}$ +136° (*c* 1.30; $CHCl_3$); IR (neat) ν 3310 (OH and NH) and 1530 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.78 (1H, *d*, $J_{1,NH}$ = 7.0 Hz, NH), 7.36–7.10 (20H, *m*, 4Ph), 6.68 (1H, *d*, $J_{1',N'H}$ = 2.2 Hz, N'H), 5.10 (1H, *dd*, $J_{1',2'}$ = 4.8, $J_{1',N'H}$ = 2.2 Hz, 1'-H), 4.91 and 4.79 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, PhC$\underline{H}_2$), 4.80 and 4.47 (each 1H, ABq, $J_{gem}$ = 10.6 Hz, PhC$\underline{H}_2$), 4.67 and 4.62 (each 1H, ABq, $J_{gem}$ = 11.7 Hz, PhC$\underline{H}_2$), 4.62 (1H, *s*, OH), 4.47 (2H, *s*, PhC$\underline{H}_2$), 4.41 (1H, *dd*, $J_{1,5}$ = 5.1, $J_{1,NH}$ = 7.0 Hz, 1-H), 4.19–4.12 (2H, *m*, 3- and 5-H), 3.85–3.52 (6H, *m*, 2 × 6-, 3'-, 5'-, 2 × 6'-H), 3.69 (1H, *dd*, $J_{1',2'}$ = 4.8, $J_{2',3'}$ = 9.5 Hz, 2'-H), 3.45 (1H, *dd*, *J* = 9.9 and 8.8 Hz, 4'-H), 3.31 (1H, *br s*, OH), 3.27 (1H, *br s*, OH), 2.86 (1H, *br s*, OH), 2.29 (1H, *ddd*, *J* = 5.1 and 5.5, $J_{gem}$ = 15.0 Hz, 4-H), 1.92 (1H, *br d*, $J_{gem}$ = 15.0 Hz, 4-H).

Anal. calcd for $C_{41}H_{48}N_2O_9S$: C, 66.11; H, 6.50; N, 3.76. Found: C, 66.26; H, 6.67; N, 3.63.

*(1R,5R,6R,7S)-3-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7-diol (57)*

The thiourea **52** (54.4 mg, 0.0730 mmol) was similarly treated with yellow HgO (total 190 mg, 0.876 mmol, 12 equiv.) in diethyl ether (1 mL) for 31 h at room temperature. The mixture was filtered through a bed of Celite and evaporated to give the cyclic isourea **57** (50.8 mg, 97.9%) as a hygroscopic syrup; $R_f$ 0.41 (EtOH:toluene, 1:5), $[\alpha]_D^{20}$ +64.5° (*c* 0.73; $CHCl_3$); IR (neat) ν 3350 (OH and NH), 1660 (C=N) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.29–7.10 (20H, *m*, 4Ph), 5.32 (1H, *d*, $J_{1',2'}$ = 4.8 Hz, 1'-H), 4.92 (1H, *br s*, $J_{1,5}$ = 7.3 Hz, 1-H), 4.90 and 4.74 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, PhC$H_2$), 4.78 and 4.45 (each 1H, ABq, $J_{gem}$ = 10.4 Hz, PhC$H_2$), 4.62 and 4.53 (each 1H, ABq, $J_{gem}$ = 11.7 Hz, PhC$H_2$), 4.51–4.37 (1H, *m*, OH), 4.67 and 4.39 (each 1H, ABq, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.24–3.50 (2H, *m*, 2 × OH), 4.13 (1H, *d*, $J_{1,5}$ = 7.3 Hz, 5-H), 4.06 (1H, *d*, $J_{gem}$ = 12.1 Hz, 9-H), 3.86–3.49 (7H, *m*, 7-, 9-, 2'-, 3'-, 5'- and 2 × 6'-H), 3.40 (1H, *dd*, *J* = 9.2 and 9.5 Hz, 4'-H), 2.10 (1H, *br ddd*, *J* = 4.8 and 5.1, $J_{gem}$ = 15.4 Hz, 8-H), 1.71 (1H, *br d*, $J_{gem}$ = 15.4 Hz, 8-H). Anal. calcd for $C_{41}H_{46}N_2O_9$: C, 69.28; H, 6.52; N, 3.94. Found: C, 68.84; H, 6.73; N, 3.77.

*(1R,5R,6R,7S)-3-(α-D-Glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7-diol (8-deoxytrehazolin) (4)*

To a blue mixture of sodium (76.4 mg, 3.32 mmol, 100 equiv.) and liquid ammonia (*ca* 5 mL) was added the isourea **57** (23.6 mg, 0.0332 mmol) in THF (1 mL) at –78 °C and the mixture was stirred for 15 min at the same temperature. The product was purified on a column of Dowex 50W-X2 ($H^+$) resin (20 mL) with 0.25 M aq. $NH_4OH$ as eluent to give the free base **4** (12.4 mg, ~100%) as a white solid, $R_f$ 0.49 (AcOH:$H_2O$:acetonitrile, 1:4:10), $[\alpha]_D^{21}$ +131° (*c* 0.62; water); IR (neat) ν 3440 (OH and NH), 1660 (C=N) and 1560 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 5.16 (1H, *d*, $J_{1',2'}$ = 5.1 Hz, 1'-H), 5.15 (1H, *dd*, $J_{1,5}$ = 7.3, $J_{1,8}$ = 5.9 Hz, 1-H), 4.05 (1H, *d*, $J_{1,5}$ = 7.3 Hz, 5-H), 3.97 (1H, *d*, $J_{7,8}$ = 4.6 Hz, 7-H), 3.79 and 3.68 (each 1H, ABq, $J_{gem}$ = 12.1 Hz, 2 × 9-H), 3.67 (1H, *dd*, $J_{5,6}$ = 2.6, $J_{gem}$ = 12.3 Hz, 6'-H), 3.60 (1H, *dd*, $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 9.9 Hz, 2'-H), 3.58 (1H, *dd*, $J_{5',6'}$ = 4.8, $J_{gem}$ = 12.3 Hz, 6'-H), 3.51 (1H, *dd*, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 8.8 Hz, 3'-H), 3.42 (1H, *ddd*, $J_{4',5'}$ = 9.9, $J_{5',6'}$ = 2.6 and 4.8 Hz, 5'-H), 3.26 (1H, *dd*, $J_{3',4'}$ = 8.8, $J_{4',5'}$ = 9.9 Hz, 4'-H), 2.26 (1H, *ddd*, $J_{1,8}$ = 5.9, $J_{7,8}$ = 4.6, $J_{gem}$ = 16.1 Hz, 8-H), 2.03 (1H, *d*, $J_{gem}$ = 16.1 Hz, 8-H).

*(1R, 5R, 6R, 7S)-6-Acetoxymethyl-4, 7-di-*N,O*-acetyl-3-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)imino-2-oxa-4-azabicyclo[3.3.0]octane-6,7-diol (4a)*

8-Deoxytrehazolin (**4**) (12.4 mg, 0.0331 mmol) was acetylated conventionally. Chromatography on a column of silica gel (1 g) with acetone:toluene (1:4, v/v) gave the hepta-*N,O*-acetyl derivative **4a** (18.3 mg, 85.9%) as a syrup; $R_f$ 0.41 (acetone:toluene, 1:2), $[\alpha]_D^{21}$+125° (*c* 0.92; $CHCl_3$); IR (neat) ν 3460 (OH), 1740 and 1710 (OAc) and 1690 (C=N and NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.54 (1H, *d*, $J_{1',2'}$ = 4.2 Hz, 1'-H), 5.42 (1H, *dd*, $J_{2',3'}$ = 10.1, $J_{3',4'}$ = 9.9 Hz, 3'-H), 5.10 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 9.9 Hz, 4'-H), 5.08–4.99 (2H, *m*, 1- and 7-H), 5.02 (1H, *dd*, $J_{1',2'}$ = 4.2, $J_{2',3'}$ = 10.1 Hz, 2'-H), 4.81 (1H, *d*, $J_{1,5}$ = 7.3 Hz, 5-H), 4.42 and 4.24 (each 1H, ABq, $J_{gem}$ = 12.3 Hz, 2 × 9-H), 4.36 (1H, *ddd*, $J_{4',5'}$ = 9.9, $J_{5',6'}$ = 2.2 and 4.0 Hz, 5'-H), 4.24 (1H, *dd*, $J_{5',6'}$ = 4.0, $J_{gem}$ = 12.5 Hz, 6'-H), 4.02 (1H, *dd*, $J_{5',6'}$ = 2.2, $J_{gem}$ = 12.5 Hz, 6'-H), 3.29 (1H, *br s*, OH), 2.68, 2.08, 2.07, 2.04, 2.00, 1.99 and 1.98 (each 3H, 7*s*, 7Ac), 2.49 (1H, *ddd*, *J* = 4.8 and 5.7, $J_{gem}$ = 15.8 Hz, 8-H), 2.23 (1H, *br d*, $J_{gem}$ = 15.8 Hz, 8-H). Anal. calcd $C_{27}H_{36}N_2O_{16}$: C, 50.31; H, 5.63; N, 4.35. Found: C, 50.27; H, 5.76; N, 4.08.

*N-[(1S)-(1, 2, 4/3, 5)-5-Hydroxymethyl-2, 3, 4-trihydroxycyclopentyl]-*N'*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)thiourea (53)*

The aminoalcohol **19** (9.5 mg, 0.0582 mmol) was coupled with α-glucosylisothiocyanate (**23**) (44.1 mg, 0.0757 mmol, 1.3 equiv.) in 75% aq. THF (2 mL) for 4 h at room temperature. The product was chromatographed on a column of silica gel (3 g) with EtOAc:toluene (1:3, v/v) → EtOH:toluene (1:15, v/v) as eluent to afford the thiourea **53** (33.1 mg, 76.4%) as a syrup; $R_f$ 0.40 (EtOH:toluene, 1:5), $[\alpha]_D^{20}$ +125° (*c* 1.65; $CHCl_3$); IR (neat) ν 3350 (OH and NH) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.70 (1H, *d*, $J_{1,NH}$ = 8.1 Hz, NH), 7.33–7.06 (20H, *m*, 4Ph), 6.77 (1H, *br s*, N'H), 5.42 (1H, *br s*, 1'-H), 5.09 (1H, *br s*, OH), 4.85 and 4.72 (each 1H, ABq, $J_{gem}$ = 10.8 Hz, PhC$H_2$), 4.74 and 4.53 (each 1H, ABq, $J_{gem}$ = 11.0 Hz, PhC$H_2$), 4.63 and 4.41 (each 1H, ABq, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.49 and 4.38 (each 1H, ABq, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.65–4.35 (3H, *m*, 1-H and 2 × OH), 3.94–3.43 (11H, *m*, 2-, 3-, 4-, 2× 6-, 2'-, 3'-, 4'-, 5'- and 2 × 6'-H), 2.15 (1H, *br s*, OH), 1.92 (1H, *br dd*, *J* = 9.4 and 9.9 Hz, 5-H). Anal. calcd for $C_{41}H_{48}N_2O_9S$: C, 66.11; H, 6.50; N, 3.76. Found: C, 65.77; H, 6.61; N, 3.64.

*(1S,5S,6R,7R,8S)-3-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo-[3.3.0]oct-3-ene-7,8-diol (58)*

The thiourea **53** (33.1 mg, 0.0444 mmol) was similarly treated with yellow HgO (total 144 mg, 0.667 mmol, 15 equiv.) in diethyl ether (1.5 mL) for 27 h at room temperature. The mixture was filtered through a bed of Celite and the filtrate was evaporated to give the cyclic isourea **58** (30.4 mg, 96.2%) as a syrup; $R_f$ 0.30 (EtOH: toluene, 1:5), $[\alpha]_D^{22}$ +65.1° (*c* 1.52; $CHCl_3$); IR (neat) ν 3320 (OH and NH), 1660 (C=N) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.31–7.06 (20H, *m*, 4Ph), 5.48 (1H, *br s*, 1'-H), 4.91–4.49 (3H, *m*, 3 × OH), 4.89 and 4.75 (each 1H, ABq, $J_{gem}$ = 11.0 Hz, PhC$H_2$), 4.75 and 4.47 (each 1H, ABq, $J_{gem}$ = 10.6 Hz, PhC$H_2$), 4.59–4.53 (1H, *m*, 1-H), 4.57 (2H, *s*, PhC$H_2$), 4.57 and 4.41 (each 1H, ABq, $J_{gem}$ = 11.9 Hz, PhC$H_2$), 4.19 (1H, *br dd*, $J_{1,5}$ = 8.0, $J_{5,6}$ = 8.0 Hz, 5-H), 3.96 (1H, *br dd*, $J_{1,8}$ = 4.0, $J_{7,8}$ = 7.1 Hz, 8-H), 3.86–3.58

(9H, *m*, 7-, 2 × 9-, 2'-, 3'-, 4'-, 5'- 2 × 6'-H), 1.83 (1H, *br s*, 6-H). Anal. calcd for $C_{41}H_{46}N_2O_9$: C, 69.28; H, 6.52; N, 3.94. Found: C, 69.15; H, 6.83; N, 3.81.

*(1S,5S,6R,7R,8S)-3-(α-D-Glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-7,8-diol (6-deoxy-6-epitrehazolin) (5)*

To a mixture of sodium (98.3 mg, 4.28 mmol, 100 equiv.) and liquid ammonia (*ca* 5 mL) was added the isourea **58** (30.4 mg, 0.0428 mmol) in THF (1 mL) at –78 °C and the mixture was stirred for 20 min at the same temperature. The reaction mixture was processed in the usual manner. The product was purified on a column of Dowex 50W-X2 ($H^+$) resin (25 mL) with 0.5 M aq. $NH_4OH$ as eluent to give the free base **5** (10.9 mg, 72.7%) as a white solid: $R_f$ 0.20 (AcOH:$H_2O$:acetonitrile, 1:2:10), $[\alpha]_D^{21}$ +135° (*c* 0.55; water); IR (KBr disk) ν 3400 (OH and NH), 1690 (C=N), and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 5.20 (1H, *d*, $J_{1',2'}$ = 5.1 Hz, 1'-H), 4.57 (1H, *dd*, $J_{1,5}$ = 9.3, $J_{1,8}$ = 5.3 Hz, 1-H), 4.02 (1H, *dd*, $J_{1,5}$ = 9.3, $J_{5,6}$ = 7.0 Hz, 5-H), 3.90 (1H, *dd*, $J_{1,8}$ = 5.3, $J_{7,8}$ = 8.6 Hz, 8-H), 3.67 (1H, *dd*, $J_{6,9}$ = 3.3, $J_{gem}$ = 12.5 Hz, 9-H), 3.66–3.56 (4H, *m*, 7-, 9-, and 2 × 6'-H), 3.63 (1H, *dd*, $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 10.3 Hz, 2'-H), 3.52 (1H, *dd*, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 9.0 Hz, 3'-H), 3.43 (1H, *ddd*, $J_{4',5'}$ = 9.9, $J_{5',6'}$ = 2.1 and 4.5 Hz, 5'-H), 3.27 (1H, *dd*, $J_{3',4'}$ = 9.0, $J_{4',5'}$ = 9.9 Hz, 4'-H), 1.75 (1H, *m*, 6-H).

*(1S,5S,6R,7R,8S)-6-Acetoxymethyl-4,7,8-tri-*N,O,O-*acetyl-3-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-imino-2-oxa-4-azabicyclo[3.3.0]octane-7,8-diol (5a)*

6-Deoxy-6-epitrehazolin (**5**) (10.9 mg, 0.0311 mmol) was acetylated conventionally. Chromatography on a column of silica gel (2 g) with acetone:toluene (1:5, v/v) gave the octa-*N,O*-acetyl derivative **5a** (20.8 mg, 97.2%) as a syrup; $R_f$ 0.25 (acetone:toluene, 1:4), $[\alpha]_D^{22}$+119° (*c* 0.80; $CHCl_3$); IR (neat) ν 1750 and 1720 (OAc) and 1690 (C=N and NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.59 (1H, *d*, $J_{1',2'}$ = 4.4 Hz, 1'-H), 5.42 (1H, *dd*, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 9.5 Hz, 3'-H), 5.22 (1H, *dd*, $J_{1,8}$ = 1.9, $J_{7,8}$ = 3.7 Hz, 8-H), 5.13 (1H, *dd*, $J_{6,7}$ = 3.7, $J_{7,8}$ = 3.7 Hz, 7-H), 5.10 (1H, *dd*, $J_{3',4'}$ = 9.5, $J_{4',5'}$ = 9.9 Hz, 4'-H), 5.06 (1H, *dd*, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 10.3 Hz, 2'-H), 4.78 (1H, *dd*, $J_{1,5}$ = 8.1, $J_{5,6}$ = 3.3 Hz, 5-H), 4.74 (1H, *dd*, $J_{1,5}$ = 8.1, $J_{1,8}$ = 1.9 Hz, 1-H), 4.35–4.27 (1H, *m*, 5'-H), 4.32 (1H, *dd*, $J_{6,9}$ = 7.0, $J_{gem}$ = 11.7 Hz, 9-H), 4.28 (1H, *dd*, $J_{6,9}$ = 6.2, $J_{gem}$ = 11.7 Hz, 9-H), 4.24 (1H, *dd*, $J_{5',6'}$ = 4.4, $J_{gem}$ = 12.3 Hz, 6'-H), 4.06 (1H, *dd*, $J_{5',6'}$ = 2.0, $J_{gem}$ = 12.3 Hz, 6'-H), 2.67–2.59 (1H, *m*, 6-H), 2.63, 2.12, 2.11, 2.09, 2.03, 2.02, 2.00 and 1.99 (each 3H, 8*s*, Ac). Anal. calcd for $C_{29}H_{38}N_2O_{17}$: C, 50.73; H, 5.58; N, 4.08. Found: C, 50.94; H, 5.83; N, 4.52.

N-*[(1R)-(1,2,3,5/4)-2-Hydroxymethyl-2,3,4,5-tetrahydroxycyclopentyl]-*N'-*(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)thiourea (54)*

The epitrehazolamine **20**[6] (37.9 mg, 0.212 mmol) was allowed to couple with the isothiocyanate **23** (138 mg, 0.237 mmol, 1.12 equiv.) in 75% aq. DMF (1.5 mL) for 7 h at room temperature. The product was chromatographed on a column of silica gel (12 g) with EtOAc:hexane (1:2, v/v) → EtOH:toluene (1:12, v/v) as eluent to afford the thiourea **54** (136 mg, 84.3%) as a syrup; $R_f$ 0.37 (EtOH:toluene, 1:5), $[\alpha]_D^{22}$ +144° (*c* 0.70; $CHCl_3$); IR (neat) ν 3340 (OH and NH) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.65 (1H, *br d*, $J_{1,NH}$ = 7.0 Hz, NH), 7.27–7.06 (20H, *m*, 4Ph), 5.46 (1H, *br s*, 1'-H), 4.89–3.35 (24H, *m*, 1-, 3-, 4-, 5-, 2 × 6-, 2'-, 3'-, 4'-, 5'-, 2 × 6'-H, 4 × PhC$\underline{H}_2$, and 4 × OH), 2.04 (1H, *br s*, OH). Anal. calcd for $C_{41}H_{48}N_2O_{10}S$: C, 64.72; H, 6.36; N, 3.68. Found: C, 64.38; H, 6.06; N, 3.80.

*Mixture of (1S,5R,6S,7S,8R)-3-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (59) and (1S,5R,6S,7R,8S)-3-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)amino-1-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (60)*

The thiourea **54** (111 mg, 0.116 mmol) was similarly treated with yellow HgO (total 379 mg, 1.39 mmol, 12 equiv.) in acetone:diethyl ether (1 mL, 1:6, v/v) for 25 h at room temperature. The mixture was filtered through a bed of Celite and the filtrate was evaporated to give a mixture of two isomeric cyclic isoureas **59** and **60** (84.0 mg, ~100%) as a syrup; $R_f$ 0.18 and 0.31 (EtOH:toluene, 1:5), IR (neat) ν 3380 (OH and NH), 1650 (C=N) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.50 (0.2 H, *d*, $J_{1',2'}$ = 4.5 Hz, 1'-H), 5.43 (0.8 H, *d*, $J_{1',2'}$ = 4.5 Hz, 1'-H). Anal. calcd for $C_{41}H_{46}N_2O_9$: C, 67.75; H, 6.38; N, 3.85. Found: C, 67.83; H, 6.28; N, 3.48.

*Mixture of (1S,5R,6S,7S,8R)-3-(α-D-glucopyranosyl)-amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (6) and (1S,5R,6S,7R,8S)-3-(α-D-glucopyranosyl)amino-1-hydroxymethyl-2-oxa-4-azabicyclo-[3.3.0]oct-3-ene-6,7,8-triol (8)*

To a mixture of sodium (226 mg, 11.6 mmol, 100 equiv.) and liquid ammonia (*ca* 5 mL) was added the mixture of the isoureas **59** and **60** (84.0 mg, 0.116 mmol) in THF (2 mL) at –78 °C and the mixture was stirred for 15 min at the same temperature. The mixture was processed in the usual manner, and the product was purified on a column of Dowex 50W-X2 ($H^+$) resin (55 mL) with 0.25 M aq. $NH_4OH$ as eluent to give a mixture of two free bases **6** and **8** (45.1 mg, ~100%) as white solids, $R_f$ 0.39 (AcOH:$H_2O$:acetonitrile, 1:4:10), IR (KBr disk) ν 3380 (OH and NH), 1650 (C=N) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 4.25 (0.3 H, *d*, $J_{1,5}$ = 9.5 Hz, 5-H), 4.02 (0.7 H, *d*, $J_{5,7}$ = 6.2 Hz, 5-H).

*(1S,5R,6S,7S,8R)-6-Acetoxymethyl-4,7,8-tri-*N,O,O-*acetyl-3-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-imino-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (6a) and (1S,5R,6S,7R,8S)-1-acetoxymethyl-4,6,7,8-tetra-*N,O,O,O-*acetyl-3-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)imino-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (8a)*

The mixture of isoureas **6** and **8** (45.1 mg, 0.115 mmol)

was acetylated conventionally. Chromatography of silica gel (5 g) with acetone:toluene (1:4, v/v) gave, first, the nona-*N,O*-acetyl derivative **8a** (69.7 mg, 81.0%) as a syrup; $R_f$ 0.35 (acetone:toluene, 1:3), $[\alpha]_D^{22}$ +45.5° (*c* 1.55; $CHCl_3$); IR (neat) ν 1760, 1750, 1740, 1730, and 1720 (OAc) and 1690 (C=N and NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.63 (1H, *d*, $J_{1',2'}$ = 4.4 Hz, 1'-H), 5.52 (1H, *ddd*, $J_{5,6}$ = 7.7, $J_{6,7}$ = 8.1, $J_{6,8}$ = 0.7 Hz, 6-H), 5.50 (1H, *dd*, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 9.9 Hz, 3'-H), 5.38 (1H, *dd*, $J_{6,7}$ = 8.1, $J_{7,8}$ = 9.2 Hz, 7-H), 5.31 (1H, *dd*, $J_{6,8}$ = 0.7, $J_{7,8}$ = 9.2 Hz, 8-H), 5.09 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 10.3 Hz, 4'-H), 5.08 (1H, *dd*, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 10.3 Hz, 2'-H), 4.98 (1H, *d*, $J_{5,6}$ = 7.7 Hz, 5-H), 4.31 (1H, *ddd*, $J_{4',5'}$ = 10.3, $J_{5',6'}$ = 2.6 and 4.8 Hz, 5'-H), 4.22 (1H, *dd*, $J_{5',6'}$ = 4.8, $J_{gem}$ = 12.3 Hz, 6'-H), 4.20 and 4.10 (each 1H, ABq, $J_{gem}$ = 12.1 Hz, 2 × 9-H), 4.10 (1H, *dd*, $J_{5',6'}$ = 2.6, $J_{gem}$ = 12.3 Hz, 6'-H), 2.64, 2.16, 2.15, 2.08, 2.07, 2.05 and 2.03 (3, 3, 3, 3, 6, 6 and 3H, 7*s*, 9Ac). Anal. calcd for $C_{31}H_{40}N_2O_{19}$: C, 50.00; H, 5.41; N, 3.76. Found: C, 49.77; H, 5.37; N, 3.64.

The second fractions gave the octa-*N,O*-acetyl derivative **6a** (15.4 mg, 19.0%) as a syrup; $R_f$ 0.23 (acetone:toluene, 1:3), $[\alpha]_D^{20}$ +117° (*c* 0.77, $CHCl_3$); IR (neat) ν 3450 (OH), 1750 and 1720 (OAc) and 1690 (C=N and NAc) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.58 (1H, *d*, $J_{1',2'}$ = 4.2 Hz, 1'-H), 5.48 (1H, *dd*, $J_{2',3'}$ = 10.1, $J_{3',4'}$ = 9.7 Hz, 3'-H), 5.38 (1H, *dd*, $J_{1,8}$ = 2.2, $J_{7,8}$ = 7.7 Hz, 8-H), 5.34 (1H, *d*, $J_{7,8}$ = 7.7 Hz, 7-H), 5.11 (1H, *dd*, $J_{3',4'}$ = 9.7, $J_{4',5'}$ = 9.7 Hz, 4'-H), 5.04 (1H, *dd*, $J_{1',2'}$ = 4.2, $J_{2',3'}$ = 10.1 Hz, 2'-H), 4.99 (1H, *d*, $J_{1,5}$ = 8.4 Hz, 5-H), 4.68 (1H, *dd*, $J_{1,5}$ = 8.4, $J_{1,8}$ = 2.2 Hz, 1-H), 4.29 (1H, *ddd*, $J_{4',5'}$ = 9.7, $J_{5',6'}$ = 3.8 and 4.2 Hz, 5'-H), 4.27 (1H, *dd*, $J_{5',6'}$ = 4.2, $J_{gem}$ = 13.6 Hz, 6'-H), 4.23 and 4.15 (each 1H, ABq, $J_{gem}$ = 11.4 Hz, 2 × 9-H), 4.07 (1H, *dd*, $J_{5',6'}$ = 3.8, $J_{gem}$ = 13.6 Hz, 6'-H), 2.90 (1H, *s*, OH), 2.67, 2.11, 2.10, 2.03, 2.01 and 1.98 (3, 9, 3, 3, 3 and 3H, 6*s*, 8Ac). Anal. calcd for $C_{29}H_{38}N_2O_{18}$: C, 49.57; H, 5.45; N, 3.99. Found: C, 49.63; H, 5.62; N, 3.69.

*(1S,5R,6S,7R,8S)-3-(α-D-Glucopyranosyl)amino-1-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (6-epitrehazolin) (8)*

The nona-acetate **8a** (28.8 mg, 0.0387 mmol) was treated with methanolic NaOMe in MeOH (1 mL) for 1 h at −15 °C. The mixture was charged on to a column of Dowex 50W-X2 ($H^+$) resin (2 mL), which was then eluted with 0.125 M aq. $NH_4OH$ to give the free base **8** (14.6 mg, 96.7%) as a white solid, $[\alpha]_D^{20}$ +135° (*c* 0.40; water); IR (neat) ν 3400 (OH and NH), 1660 (C=N) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 5.28 (1H, *d*, $J_{1',2'}$ = 5.1 Hz, 1'-H), 4.02 (1H, *d*, $J_{5,6}$ = 6.2 Hz, 5-H), 3.75 and 3.52 (each 1H, ABq, $J_{gem}$ = 12.6 Hz, 2 × 9-H), 3.73–3.43 (5H, *m*, 6-, 7-, 8- and 2 × 6'-H), 3.64 (1H, *dd*, $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 9.9 Hz, 2'-H), 3.54 (1H, *dd*, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 9.9 Hz, 3'-H), 3.48 (1H, *ddd*, $J_{4',5'}$ = 8.8, $J_{5',6'}$ = 2.4 and 4.6 Hz, 5'-H), 3.29 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 8.8 Hz, 4'-H).

*Mixture of (1S,5R,6S,7S,8R)-3-(α-D-glucopyranosyl)-amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (6) and (1S,5R,6S,7R,8S)-3-(α-D-glucopyranosyl)amino-1-hydroxymethyl-2-oxa-4-azabicyclo-[3.3.0]oct-3-ene-6,7,8-triol (8)*

The octa-acetate **6a** (13.6 mg, 0.0194 mmol) was similarly treated with methanolic NaOMe in MeOH (1 mL) for 1 h at −15 °C. The mixture was charged on to a column of Dowex 50W-X2 ($H^+$) resin (2 mL), which was eluted with 0.125 M aq. $NH_4OH$ to give a 1:1.8 mixture of two isoureas **6** and **8** (6.7 mg, 88.2%) as white solids, $^1H$ NMR ($D_2O$, ref. acetone) δ 4.25 (*d*, $J_{1,5}$ = 9.5 Hz, 5-H): 4.02 (*d*, $J_{5,6}$ = 6.2 Hz, 5-H) = 1:1.8.

*A mixture of N-[(1R)- **61** and (1S)-(1,3,5/2,4)-5-amino-2,3,4-trihydroxycyclopentyl]-N-(2,3,4,6-tetra-O-benzyl-5a-carba-α-D-glucopyranosyl)thioureas (62)*

To a solution of **21** (21.1 mg, 0.142 mmol, 1.1 equiv.) in water (0.3 mL) was added dropwise a solution of 5a-carba-α-D-glucopyranosylisothiocyanate (**24**) (74.3 mg, 0.128 mmol) in DMF (2.7 mL) for 1.5 h, and then the mixture was stirred for 17 h at room temperature and evaporated. The residue was chromatographed on a silica gel column (5 g) with EtOH:toluene (1:8, v/v) as eluent to give a mixture of the thioureas **61** and **62** (68.0 mg, 73.0%) as syrups: $R_f$ 0.29 (EtOH:toluene, 1:5); IR(neat) ν 3320 (OH and NH) and 1540 (NH) $cm^{-1}$. Anal. calcd for $C_{41}H_{49}N_3O_7S$: C, 67.65; H, 6.79; N, 5.77. Found: C, 67.40; H, 6.88; N, 5.41.

*(1S,5R,6S,8R)-3-(2,3,4,6-Tetra-O-benzyl-5a-carba-α-D-glucopyranosyl)imino-2,4-diazabicyclo[3.3.0]octan-6,7,8-triol (63)*

To a solution of the mixture (108 mg, 0.149 mmol) of **61** and **62** in diethyl ether (2 mL) was added yellow mercuric(II) oxide (96.5 mg, 0.447 mmol, 3 equiv.) and it was stirred at room temperature. After each 12 h and 27 h, 3 equiv-portions of HgO (totally 290 mg, 9 equiv.) were added and the mixture was treated for totally 23 h. The mixture was filtered through a bed of Celite and evaporated to dryness. The residue was chromatographed on a silica gel column (2 g) with EtOH:toluene (1:8, v/v) → 3% triethylamine:methanol as eluent to give the cyclic guanidine **63** (89.6 mg, 87%) as a syrup; $R_f$ 0.21 and 0.24 (AcOH:EtOH:toluene, 1:2:8); $^{13}C$ NMR ($CDCl_3$) δ 179.40, 159.76, 138.77, 138.65, 138.34, 137.68, 128.66, 128.26, 127.68, 80.57, 77.29, 77.12, 37.29, 29.23. 29.08, 24.99. $^1H$ NMR ($CDCl_3$) δ 7.25–7.20 (20H, *m*, 4 × Ph), 6.05 (4H, *br s*, 2 × NH and 2 × OH), 4.82 and 4.74 (each 1H, ABq, *J* = 11.2 Hz, $PhC\underline{H}_2$), 4.78 and 4.45 (each 1H, ABq, *J* = 11.0 Hz, $PhC\underline{H}_2$), 4.65 and 4.60 (each 1H, ABq, *J* = 12.2 Hz, $PhC\underline{H}_2$), 4.31 and 4.23 (each 1H, ABq, *J* = 12.1 Hz, $PhC\underline{H}_2$), 4.01–3.25 (12H, *m*), 2.11–1.92 (2H, *m*, 5' and 5'a-eq-H) and 1.54 (1H, *br t*, 5'a-ax-H). HRMS, calcd for $C_{41}H_{48}N_3O_7$ $(M+H)^+$: m/z 694.3493. Found: m/z 694.3491; Anal. calcd for $C_{41}H_{47}N_3O_7 \cdot 0.5H_2CO_3$ requires C, 68.77; H, 6.67; N, 5.80. Found: C 68.39; H, 6.87; N, 5.67.

*(1,5,7/6,8)-3-(5a-Carba-α-D-glucopyranosyl)imino-2,4-diazabicyclo[3.3.0]octan-6,7,8-triol acetate (11)*

To liquid ammonia (5 mL) containing sodium (200 mg, 8.6 mmol, 100 equiv.) was added a solution of **63** (60 mg,

0.086 mmol) in THF (1.5 mL) and the mixture was stirred for 15 min at –78 °C. After addition of methanol, ammonia was removed by spontaneous evaporation at room temperature. The residue was dissolved in water (5 mL), washed with chloroform and evaporated. Crude compound was chromatographed on a column of cellulose (Funacel 5 g) with AcOH:$H_2O$:acetonitrile (1:2:10, v/v) as eluent to give an AcOH salt of **11** as a white solid, $R_f$ 0.35 (cellulose TLC, AcOH:$H_2O$:acetonitrile, 1:2:10), $[\alpha]_D^{27}$ +25° (*c* 0.73; $CHCl_3$); IR (KBr disk) ν 3350 (OH and NH), 1670 (C=N) and 1560 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 4.22 (2H, *br s*, 1- and 5-H), 3.98–3.88 (3H, *m*, 6-, 8- and 1'-H), 3.77 (1H, *dd*, $J_{6,7} = J_{7,8}$ = 7.9 Hz, 7-H), 3.71–3.58 (3H, *m*, 2'- and 6'-H), 3.45 (1H, *dd*, $J_{2',3'}$ = 9.5, $J_{3',4'}$ = 9.9 Hz, 3'-H), 3.28 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 9.2 Hz, 4'-H), 1.97–1.85 (1H, *m*, 5'a-eq-H), 1.89 (3H, *s*, Ac), 1.80–1.65 (1H, *m*, 5'-H), 1.55 (1H, *br t*, *J* = 13.6 Hz, 5'a-ax-H); $^{13}C$ NMR (67.5 MHz, $D_2O$, ref. $CH_3CN$) δ 182.27, 159.15, 80.33, 80.27, 78.86, 75.15, 73.22, 72.87, 62.62, 54.24, 39.27, 28.84, 24.03. HR FAB MS: calcd for $C_{13}H_{24}N_3O_7$ $(M+H)^+$: m/z 334.1614. Found: m/z 334.1622.

*Mixture of (1,5,7/6,8)-2-*N*-**11a** and 4-*N*,6-*O*,7-*O*,8-*O*-tetraacetyl-3-(2,3,4,6-tetra-*O*-acetyl-5a-carba-α-D-glucopyranosyl)imino-2,4-diazabicyclo[3.3.0]octan-6,7,8-triol (**11'a**)*

The AcOH salt **11** (8.9 mg, 0.023 mmol) was treated with acetic anhydride (0.5 mL) and pyridine (0.5 mL) for 30 h at room temperature. The product was chromatographed on a silica gel column (1 g) with acetone:toluene (1:4, v/v) as eluent to give a mixture (14.5 mg, 96%) of the octa-*N*,*O*-acetyl derivatives **11a** and **11'a**; $R_f$ 0.29 (acetone: toluene, 1:2), IR (neat) ν 3550 (NH), 1750 and 1740 (OAc), 1680 (NAc and C=N) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) (*intra alia*) the major compound: δ 5.53 (1H, *dd*, $J_{2',3'} = J_{3',4'}$ = 9.9 Hz, 3'-H) and 2.61 (3H, *s*, NAc); the minor compound: 5.68 (1H, *dd*, $J_{2',3'} = J_{3',4'}$ = 9.9 Hz, 3'-H), 2.63 (3H, *s*, NAc). The spectrum revealed the products to be about 2.5:1 mixture of the isomers in regard to the location of the *N*-acetyl group. Anal. calcd for $C_{29}H_{39}N_3O_{15}$: C, 52.02; H, 5.87; N, 6.28. Found: C, 51.66; H, 5.77; N, 5.86.

*N-(5a-Carba-α-D-glucopyranosyl)-N'-(2,3,4,6-tetra-O-benzyl-5a-carba-α-D-glucopyranosyl)thiourea (**64**)*

To a solution of validamine[10] (**22**) (74.4 mg, 0.420 mmol) in water (1 mL) was added a solution of 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-glucopyranosylisothiocyanate[8] (**24**) (172 mg, 0.297 mmol) in THF (2 mL) and the mixture was stirred for 6 days at room temperature and then evaporated. Chromatography of the residue on a column of silica gel (15 g) with ethyl EtOAc:hexane (1:3, v/v) → EtOH:toluene (1:20, v/v) as eluent gave the thiourea **64** (149 mg, 66.5%) as a syrup; $R_f$ 0.37 (EtOH:toluene, 1:5); $[\alpha]_D^{26}$ +45° (*c* 1.7; MeOH); IR (neat) ν 3320 (OH and NH) and 1550 (NH) $cm^{-1}$; $^1H$ NMR (acetone-$d_6$) δ 7.58 (1H, *br s*, NH or NH'), 7.47–7.26 (20H, *m*, 4 × Ph), 6.99 (1H, *br s*, NH or NH'), 5.31 (1H, *br s*, 1 or 1'-H), 4.98–4.39 (10H, *m*, 1- or 1'-H, 4 × Ph and OH), 4.25 (1H, *br s*, OH), 4.15 (1H, *br s*, OH), 3.87–3.36 (11H, *m*), 2.30–1.36 (6H, *m*, 5-, 2 × 5a-, 5'- and 2 × 5'a-H). Anal. calcd for $C_{43}H_{52}N_2O_8S$ requires C, 68.23; H, 6.92; N, 3.70. Found: C, 67.99; H, 7.15; N, 3.63.

*(1S,3R,4R,5S,6S)-8-(2,3,4,6-Tetra-O-benzyl-5a-carba-α-D-glucopyranosyl)amino-3-methyl-7-oxa-9-azabicyclo-[4.3.0]non-8-ene-4,5-diol (**65**)*

To a solution of the thiourea **64** (99.9 mg, 0.132 mmol) in acetone:diethyl ether (3 mL, 2:1, v/v) was added yellow mercury(II) oxide (85.4 mg, 0.396 mmol, 3 equiv.) and the mixture was stirred for 7.5 h. The mixture was then treated as in the preparation of **63** with totally 256 mg (9 equiv.) of HgO for 23 h. The mixture was filtered through a Celite bed and evaporated. Chromatography on silica gel (2 g) with EtOH:toluene (1:5, v/v) containing 1.5% triethylamine as eluent gave the isourea **65** (85.1 mg, 89.2%) as a syrup; $R_f$ 0.22 (EtOH:toluene, 1:5), $[\alpha]_D^{25}$ +62.1° (*c* 1.29; MeOH); IR (neat) ν 3330 (OH and NH) and 1660 (C=N) $cm^{-1}$, $^1H$ NMR ($CDCl_3$) δ 7.33–7.15 (20H, *m*, 4 × Ph), 4.91 and 4.77 (each 1H, ABq, *J* = 11.0 Hz, PhC$\underline{H}_2$), 4.84 and 4.49 (each 1H, ABq, *J* = 10.8 Hz, PhC$\underline{H}_2$), 4.67 and 4.56 (each 1H, ABq, *J* = 11.4 Hz, PhC$\underline{H}_2$), 4.41 (2H, *s*, PhC$\underline{H}_2$), 4.25 (1H, *dd*, $J_{1,6} = J_{5,6}$ = 7.3 Hz, 6-H), 4.11 (1H, *ddd*, $J_{1',2'} = J_{1',5'a} = J_{1',5'a}$ = 3.1 Hz, 1'-H), 4.00 (1H, *ddd*, $J_{1,2}$ = 2.1, $J_{1,2}$ = 4.8, $J_{1,6}$ = 7.3 Hz, 1-H), 3.75–3.22 (11H, *m*), 3.26 (1H, *dd*, *J* = 9.9 and 9.9 Hz, 4- or 4'-H), 2.37 (1H, *ddd*, $J_{1',5'a\text{-}ax} = J_{1',5'a\text{-}eq}$ = 3.1, $J_{gem}$ 13.9 Hz, 5'a-eq-H), 2.07–1.77 (3H, *m*, 2-, 3- and 5'-H), 1.45–1.26 (2H, *m*, 2- and 5'a-ax-H). Anal. calcd for $C_{43}H_{50}N_2O_8$: C, 71.45; H, 6.95; N, 3.88. Found: C, 71.68; H, 6.93; N, 3.53.

*(1S,3R,4R,5S,6S)-8-(5a-Carba-α-D-glucopyranosyl)-amino-3-hydroxymethyl-7-oxa-9-azabicyclo[4.3.0]non-8-ene-4,5-diol (**13**)*

To liquid ammonia (5 mL) containing sodium (177 mg, 7.7 mmol, 100 equiv.) was added dropwise a solution of the isourea **65** (55.7 mg, 0.077 mmol) in THF (1.5 mL) at –78 °C and then the mixture was stirred for 15 min at the same temperature. The mixture was allowed to stand at room temperature and ammonia was removed by spontaneous evaporation. The residue was dissolved in water (5 mL) and washed with chloroform (3 mL × 3). The water layer was transferred on a column of Dowex 50W-X2 ($H^+$) resin (25 mL) and eluted with 0.5 M aq. ammonia to give the isourea **13** (27.1 mg, 97.1%) as a white solid, $R_f$ 0.18 (AcOH:$H_2O$:acetonitrile, 1:2:10); $[\alpha]_D^{25}$ +88.8° (*c* 1.36; water); IR (KBr disk) ν 3365 (OH and NH), 1655 (C=N) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 4.37 (1H, *dd*, $J_{1,6} = J_{5,6}$ = 7.3 Hz, 6-H), 4.02 (1H, *ddd*, $J_{1,2}$ = 2.3 and 4.4, $J_{1,6}$ = 7.3 Hz, 1-H), 3.85 (1H, *ddd*, $J_{1',2'}$ = 4.4, $J_{1',5'a\text{-}ax}$ = 2.9, $J_{1',5'a\text{-}eq}$ = 3.0 Hz, 1'-H), 3.63–3.48 (4H, *m*, 9- and 2 × 6'-H), 3.49 (1H, *dd*, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 9.9 Hz, 2'-H), 3.42 (1H, *dd*, $J_{4,5}$ = 9.5, $J_{5,6}$ = 7.3 Hz, 5-H), 3.36 (1H, *dd*, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 9.0 Hz, 3'-H), 3.15 (2H, *dd*, *J* = 9.0 and 10.3 Hz, 4 and 4'-H), 1.97 (1H, *ddd*, $J_{1,2}$ = 2.3, $J_{gem}$ = 14.3, $J_{2,3}$ = 2.3 Hz, 2-H), 1.80 (1H, *ddd*, $J_{1',5'a\text{-}eq}$ = 2.9, $J_{1',5'a\text{-}eq}$ = 3.0, $J_{gem}$ = 14.3 Hz, 5a-eq-H), 1.68–1.54 (2H, *m*, 3- and 5'-H), 1.52 (1H, *ddd*, $J_{1,2}$ = 4.4, $J_{gem}$ = 14.3 Hz, 2-H), 1.33 (1H, *ddd*, $J_{1',5'a\text{-}ax}$ = 2.9, $J_{5',5'a\text{-}ax}$ = 14.3 Hz, 5'a-ax-H).

*(1S,3R,4R,5S,6S)-3-Acetoxymethyl-4,5,9-*N,O,O*-triacetyl-8-(2,3,4,6-tetra-O-acetyl-5a-carba-α-D-glucopyranosyl)-imino-7-oxa-9-azabicyclo[4.3.0]nonane-4,5-diol (**13a**)*

The isourea **13** (27.1 mg, 0.0748 mmol) was acetylated conventionally. The product was chromatographed on a silica gel column (2 g) with acetone:toluene (1:5) as eluent to give the octa-*N,O*-acetyl derivative **13a** (47 mg, 90%) as a syrup; $R_f$ 0.29 (acetone:toluene, 1:4), $[\alpha]_D^{25}$ +119° (*c* 1.0; $CHCl_3$); IR (neat) ν 1740 (OAc) and 1680 (NAc and C=N) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.49 (1H, *dd*, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 9.5 Hz, 3'-H), 5.15 (1H, *dd*, $J_{4,5}$ = 6.4, $J_{5,6}$ = 5.7 Hz, 5-H), 5.05 (1H, *dd*, $J_{3',4'}$ = 9.5, $J_{4',5'}$ = 11.0 Hz, 4'-H), 4.92 (1H, *dd*, $J_{3,4}$ = 8.8, $J_{4,5}$ = 6.4 Hz, 4-H), 4.89 (1H, *dd*, $J_{1',2'}$ = 3.3, $J_{2',3'}$ = 10.3 Hz, 2'-H), 4.63 (1H, *dd*, $J_{1,2}$ = 4.4 and 4.4, $J_{1,6}$ = 7.5 Hz, 1-H), 4.47 (1H, *dd*, $J_{1,6}$ = 7.5, $J_{5,6}$ = 5.7 Hz, 6-H), 4.21 (1H, *ddd*, $J_{1',2'}$ = 3.3, $J_{1',5'a\text{-}eq}$ = 4.9, $J_{1',5'a\text{-}ax}$ = 2.6 Hz, 1'-H), 4.15 (1H, *dd*, $J_{5',6'}$ = 4.2, $J_{gem}$ = 11.4 Hz, 6'-H), 4.09 (1H, *dd*, $J_{3,10}$ = 4.8, $J_{gem}$ = 11.4 Hz, 10-H), 4.00 (1H, *dd*, $J_{3,10}$ = 4.4, $J_{gem}$ = 11.4 Hz, 10-H), 3.91 (1H, *dd*, $J_{5',6'}$ = 3.3, $J_{gem}$= 11.4 Hz, 6'-H), 2.64, 2.10, 2.06, 2.05, 2.03, 1.99 and 1.98 (3, 3, 6, 3, 3 and 3H, 7*s*, 8 × Ac), 2.50–2.38 (1H, *m*, 5'-H), 2.37 (1H, *dd*, $J_{1,2}$ = 4.4, $J_{gem}$ = 15.2, $J_{2,3}$ = 4.4 Hz, 2-H), 2.13–1.91 (1H, *m*, 3-H), 1.93–1.82 (2H, *m*, 2- and 5'a-eq-H), 1.70 (1H, *ddd*, $J_{1',5'a\text{-}ax}$ = 2.6, $J_{5',5'a\text{-}ax}$ = 15.0, $J_{gem}$ = 15.0 Hz, 5'a-ax-H). Anal. calcd for $C_{31}H_{42}N_2O_{16}$: C, 53.29; H, 6.06; N, 4.01. Found: C, 53.27; H, 6.17; N, 4.04.

*2,3,4-Tri-*O*-benzyl-6-deoxy-6-fluoro-α- (**67**)and β-D-glucopyranosylisothiocyanate (**68**)*

The methyl glycoside[11] **66** (1.12 g, 2.40 mmol) was treated with 2 M aq. HCl (30 mL) in AcOH (120 mL) for 24 h at 80 °C. The mixture was diluted with ether (300 mL) and washed with satd aq. $NaHCO_3$ (100 mL × 25) and dried. The solution was evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (60 g) with EtOAc:hexane (1:10, v/v) gave a mixture of α- and β-glucose derivatives (510 mg, 47.2%) as syrups. The mixture was acetylated conventionally and chromatographed on a column of silica gel (40 g) with EtOAc:hexane (1:8, v/v) as eluent to give 1-*O*-acetyl derivatives (540 mg, 96.8%) as syrups. The mixture of α- and β-acetate (540 mg) was treated with 1,4-dioxane (12 mL) saturated with HCl for 1 h at 40 °C. Removal of a solvent and HCl gave crude α-glycosyl chloride, of which solution was added to a suspension of KSCN (318 mg, 3.27 mmol, 3 equiv.), *n*-$Bu_4NBr$ (351 mg, 1.09 mmol, 1 equiv.), and MS 4A (350 mg) in $CH_3CN$ (15 mL) at room temperature. The mixture was stirred for 5 h at reflux temperature. The reaction mixture was filtered through a bed of Celite, and the bed was washed with EtOAc. The filtrate was evaporated to give a residue, which was chromatographed on a column of silica gel (50 g) with EtOAc:hexane (1:15, v/v) as eluent to afford, first, the α-isothiocyanate **67** (145 mg, 27.0%) as a syrup; $R_f$ 0.51 (EtOAc:hexane, 1:15, triple irrigation), $[\alpha]_D^{27}$ +125° (*c* 1.64; $CHCl_3$); IR (neat) ν 2010 (N=C=S) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.41–7.23 (15H, *m*, 3 × Ph), 5.41 (1H, *d*, $J_{1,2}$ = 4.0 Hz, 1-H), 4.96 and 4.84 (each 1H, ABq, $J_{gem}$ = 11.0 Hz, PhC$\underline{H}_2$), 4.89 and 4.61 (each 1H, ABq, $J_{gem}$ = 10.6 Hz, PhC$\underline{H}_2$), 4.78 and 4.68 (each 1H, ABq, $J_{gem}$ = 11.7 Hz, PhC$\underline{H}_2$), 4.62 (1H, *ddd*, $J_{5,6}$ = 2.6, $J_{gem}$ = 10.6, $J_{6,F}$ = 46.9 Hz, 6-H), 4.52 (1H, *ddd*, $J_{5,6}$ = 1.5, $J_{gem}$ = 10.6, $J_{6F}$ = 48.2 Hz, 6-H), 3.90 (1H, *dd*, $J_{2,3}$ = 9.5, $J_{3,4}$ = 9.2 Hz, 3-H), 3.76 (1H, *dddd*, $J_{4,5}$ = 9.9, $J_{5,6}$ = 1.5 and 2.6, $J_{5,F}$ = 30.8 Hz, 5-H), 3.67 (1H, *dd*, $J_{1,2}$ = 4.0, $J_{2,3}$ = 9.5 Hz, 2-H), 3.59 (1H, *dd*, $J_{3,4}$ = 9.2, $J_{4,5}$ = 9.9 Hz, 4-H). Anal. calcd for $C_{28}H_{28}FNO_4S$: C, 68.13; H, 5.72; N, 2.84. Found: C, 68.10; H, 5.89; N, 2.86.

The second fractions gave the β-isothiocyanate **68** (121 mg, 22.5%) as crystals, $R_f$ 0.40, mp 109–110 °C (from hexane); $[\alpha]_D^{27}$ −10.6° (*c* 1.12; $CHCl_3$); IR (neat) ν 2010 (N=C=S) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.38–7.23 (15H, *m*, 3 × Ph), 4.92 and 4.62 (each 1H, ABq, $J_{gem}$ = 10.6 Hz, PhC$\underline{H}_2$), 4.90 (2H, *s*, PhC$\underline{H}_2$), 4.87 and 4.82 (each 1H, ABq, $J_{gem}$ = 8.4 Hz, PhC$\underline{H}_2$), 4.83 (1H, *d*, $J_{1,2}$ = 8.4 Hz, 1-H), 4.61 (1H, *ddd*, $J_{5,6}$ = 1.8, $J_{gem}$ = 10.6, $J_{6,F}$ = 48.0 Hz, 6-H), 4.56 (1H, *ddd*, $J_{5,6}$ = 3.3, $J_{gem}$ = 10.6, $J_{6,F}$ = 47.3 Hz, 6-H), 3.68 (1H, *dd*, $J_{3,4}$ = 8.8, $J_{4,5}$ = 8.8 Hz, 4-H), 3.63 (1H, *dd*, $J_{2,3}$ = 9.3, $J_{3,4}$ = 8.8 Hz, 3-H), 3.53 (1H, *dd*, $J_{1,2}$ = 8.4, $J_{2,3}$ = 9.3 Hz, 2-H), 3.42 (1H, *dddd*, $J_{4,5}$ = 8.8, $J_{5,6}$ = 1.8 and 3.3, $J_{5,F}$ = 29.7 Hz, 5-H). Anal. Found: C, 68.00; H, 5.84; N, 2.84.

N-*[(1R)-(1,3,5/2,4)-2-Hydroxymethyl-2,3,4,5-tetrahydroxycyclopentyl]*-N'-*(2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranosyl)thiourea (**69**)*

Trehazolamine (**15**) (22.6 mg, 0.126 mmol) was allowed to couple with the isothiocyanate **67** (78.6 mg, 0.159 mmol, 1.26 equiv.) in 75% aq. THF (2 mL) for 3 h at room temperature. The reaction mixture was evaporated to give a syrupy residue, which was chromatographed on a column of silica gel (6 g) with EtOAc:hexane (1:10, v/v) →EtOH:toluene (1:18, v/v) as eluent to afford the thiourea **69** (78.9 mg, 93.0%) as a syrup; $R_f$ 0.49 (EtOH:toluene, 1:5), $[\alpha]_D^{24}$ +177° (*c* 1.18; $CHCl_3$); IR (neat) ν 3320 (OH and NH) and 1540 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.81 (1H, *br s*, NH), 7.37–7.10 (16H, *m*, N'H and 3 × Ph), 5.37 (1H, *br s*, 1'-H), 4.90–4.35 (15H, *m*), 4.20–4.02 (2H, *m*), 3.87–3.43 (5H, *m*). Anal. calcd for $C_{34}H_{41}FN_2O_9S{\cdot}0.5H_2O$: C, 59.90; H, 6.21; N, 4.11. Found: C, 59.79; H, 6.06; N, 4.16.

*(1S,5R,6R,7S,8R)-3-(2,3,4-Tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (**70**)*

The thiourea **69** (23.6 mg, 0.0351 mmol) was treated with five portions of yellow HgO (each 22.7 mg, 0.105 mmol, 3 equiv., total 114 mg, 0.525 mmol, 15 equiv.) in diethyl ether (2 mL) for 44 h at room temperature. The mixture was filtered through a bed of Celite and the filtrate was evaporated to give the isourea **70** (22.0 mg, 98.2%) as a syrup; $R_f$ 0.31 (EtOH:toluene, 1:5), $[\alpha]_D^{23}$ +68.1° (*c* 1.10; $CHCl_3$); IR (neat) ν 3360 (OH and NH), 1660 (C=N) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.38–7.12 (15H, *m*, 3 × Ph), 5.34 (1H, *br s*, 1'-H), 4.97–4.26 (15H, *m*), 4.18–3.40 (8H, *m*). Anal. calcd for $C_{34}H_{39}FN_2O_9{\cdot}H_2O$: C, 62.18; H, 6.29; N, 4.27. Found: C, 62.34; H, 6.29; N, 4.20.

*(1S,5R,6R,7S,8R)-3-(6-Deoxy-6-fluoro-α-D-glucopyranosyl)amino-6-hydroxymethyl-2-oxa-4-azabicyclo[3.3.0]oct-3-ene-6,7,8-triol (6'-deoxy-6'-fluorotrehazolin) (**14**)*

To a blue mixture of sodium (79.1 mg, 3.44 mmol, 100 equiv.) and liquid ammonia (*ca* 5 mL) was added the isourea **70** (22.0 mg, 0.0344 mmol) in THF (1.5 mL) at –78 °C, and the mixture was stirred for 15 min at the same temperature. After addition of $NH_4Cl$ (221 mg, 4.13 mmol, 120 equiv.), the mixture was evaporated spontaneously at room temperature. The residue obtained was diluted with water (5 mL) and washed with $CHCl_3$ (3 mL × 3). The water layer was charged on to a column of Dowex 50W-X2 ($H^+$) resin (20 mL), and the column was washed with water and then eluted with 0.5 M aq. $NH_4OH$ to give the free base **14** (10.9 mg, 85.8%) as an amorphous powder, $R_f$ 0.54 (AcOH:$H_2O$:acetonitrile, 1:4:10), $[\alpha]_D^{22}$ +84.5° (*c* 0.545; water); IR (KBr disk) ν 3350 (OH and NH), 1660 (C=N) and 1550 (NH) $cm^{-1}$; $^1H$ NMR ($D_2O$, ref. acetone) δ 5.22 (1H, *d*, $J_{1',2'}$ = 5.1 Hz, 1'-H), 4.82 (1H, *dd*, $J_{1,5}$ = 8.4, $J_{1,8}$ = 2.6 Hz, 1-H), 4.55 (1H, *ddd*, $J_{5',6'}$ = 3.3, $J_{gem}$ = 10.6, $J_{6',F}$ = 35.5 Hz, 6'-H), 4.47 (1H, *ddd*, $J_{5',6'}$ = 1.5, $J_{gem}$ = 10.6, $J_{6',F}$ = 49.5 Hz, 6'-H), 4.22 (1H, *d*, $J_{1,5}$ = 8.4 Hz, 5-H), 4.06 (1H, *dd*, $J_{1,8}$ = 2.6, $J_{7,8}$ = 4.8 Hz, 8-H), 3.81 (1H, *d*, $J_{7,8}$ = 4.8 Hz, 7-H), 3.67–3.47 (1H, *m*, 5'-H), 3.66 and 3.57 (each 1H, ABq, $J_{gem}$ = 12.1 Hz, C<u>H</u>$_2$OH), 3.63 (1H, *dd*, $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 9.9 Hz, 2'-H), 3.54 (1H, *dd*, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 9.9 Hz, 3'-H), 3.37 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 8.8 Hz, 4'-H).

*(1S,5R,6R,7S,8R)-6-Acetoxymethyl-4,7,8-tri-*N,O,O*-acetyl-3-(2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-α-D-glucopyranosyl)imino-2-oxa-4-azabicyclo[3.3.0]octane-6,7,8-triol (**14a**)*

The free base **14** (5.1 mg, 0.0138 mmol) was acetylated conventionally. Chromatography of silica gel (0.5 g) with acetone:toluene (1:5, v/v) gave the hepta-*N,O*-acetyl derivative **14a** (8.4 mg, 92.3%) as a syrup; $R_f$ 0.53 (acetone:toluene, 1:2), $[\alpha]_D^{21}$ +97.7° (*c* 0.42; $CHCl_3$); IR (neat) ν 3480 (OH), 1745 (OAc) and 1695 (NAc and C=N) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.61 (1H, *d*, $J_{1',2'}$ = 4.2 Hz, 1'-H), 5.54 (1H, *d*, $J_{7,8}$ = 8.8 Hz, 7-H), 5.46 (1H, *dd*, $J_{1,8}$ = 3.3, $J_{7,8}$ = 8.8 Hz, 8-H), 5.43 (1H, *dd*, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 9.9 Hz, 3'-H), 5.07 (1H, *dd*, $J_{3',4'}$ = 9.9, $J_{4',5'}$ = 9.7 Hz, 4'-H), 5.06 (1H, *dd*, $J_{1',2'}$ = 4.2, $J_{2',3'}$ = 10.3 Hz, 2'-H), 4.90 (1H, *d*, $J_{1,5}$ = 9.9 Hz, 5-H), 4.80 (1H, *dd*, $J_{1,5}$ = 9.9, $J_{1,8}$ = 3.3 Hz, 1-H), 4.43 (2H, *dd*, $J_{5',6'}$ = 3.7, $J_{6,F}$ = 46.9 Hz, 2 × 6'-H), 4.37–4.22 (1H, *m*, 5'-H), 4.18 and 3.88 (each 1H, ABq, $J_{gem}$ = 11.5 Hz, C<u>H</u>$_2$OAc), 3.73 (1H, *s*, OH), 2.67, 2.10, 2.09, 2.07, 2.05, 2.00 and 1.98 (each 3H, 7*s*, 7 × Ac).

Anal. calcd for $C_{27}H_{34}FN_2O_{16}$: C, 49.02; H, 5.18; N, 4.23. Found: C, 49.21; H, 5.60; N, 4.14.

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## References and Notes

1. Ando, O.; Satake, H.; Ito, K.; Sato, A.; Nakajima, M.; Takahashi, S.; Haruyama, H.; Ohkuma, Y.; Kinoshita, R.; Enokita, R. *J. Antibiot.* **1991**, *44*, 1165.

2. Uchida, C.; Kitahashi, H.; Yamagishi, T.; Iwaisaki, Y.; Ogawa, S. *J. Chem. Soc., Perkin Trans 1* **1994**, 2775.

3. Uchida, C.; Kitahashi, H.; Watanabe, S.; Ogawa, S. *J. Chem. Soc., Perkin Trans 1* **1995**, 1707.

4. Uchida, C.; Kimura, H.; and Ogawa, S., *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2643.

5. Kobayashi, Y.; Shiozaki, M.; Ando, O. *J. Org. Chem.* **1995**, *60*, 2570 and references cited therein.

6. Uchida, C.; Yamagishi, T.; Ogawa, S. *J. Chem. Soc., Perkin Trans 1*, **1994**, 589.

7. Camarasa, M. J.; Fernandez-Resa, P.; Garcia-Lopez, M. T.; De Las Heras, F. G.; Mendez-Castrillon, P. P.; Feelix, A. S. *Synthesis*, **1984**, 509.

8. Uchida, C.; Ogawa, S. *Carbohydr. Lett.* **1994**, *1*, 77.

9. Ogawa, S.; Sato, K.; Miyamoto, Y. *J. Chem. Soc., Perkin Trans 1* **1993**, 691.

10. Ogawa, S.; Iwasawa, Y.; Nose, T.; Suami, T.; Oba, S.; Ito, M.; Saito, Y. *J. Chem. Soc., Perkin Trans 1* **1985**, 903.

11. Kovac, P.; Yeh, H. J. C.; Jung, G. L. *J. Carbohydr. Chem.* **1987**, *6*, 423.

12. Ogawa, S.; Uchida, C. *J. Chem. Soc., Perkin Trans 1* **1992**, 1939.