Synthesis of Aminocyclitol Moieties of Trehalase Inhibitors, Trehalostatin and Trehazolin. Confirmation of the Correct Structure of the Inhibitor

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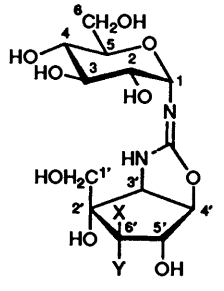
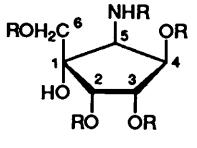
The respective aminocyclitol moieties **3a** and **4a** of trehalostatin **1** and trehazolin **2**, potent trehalase inhibitors, have been synthesized as the penta-*N,O*-acetyl derivatives **3b** and **4b** by racemic modification. The latter was shown to be identical to the equivalent derivative obtained from **2** on the basis of the ^{1}H NMR spectra, confirming the correct structure of the inhibitor.

In 1990, trehalostatin **1**, a potent and specific inhibitor against blowfly trehalase, was isolated by Murao *et al.*[1,2] from the culture broth of *Amycolatopsis trehalostatica*, and the structure[1] initially proposed was recently revised[3] as depicted below†, mainly on the basis of the ^{1}H NMR spectroscopic data.

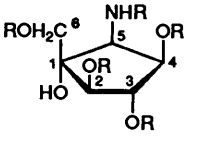
Recently, Ando *et al.*[4] reported the finding of the trehalase inhibitor trehazolin **2** from the culture broth of *Micromonospora*, strain SANK 62390, and showed it to be identical to **1** by



1 X = H, Y = OH(Trehalostatin)
2 X = OH, Y = H(Trehazolin)



3a R = H
3b R = Ac



4a R = H
4b R = Ac

comparison of physical and spectroscopic data. They, however, assigned a different structure to it, the configuration at C-6′ being opposite to that proposed[3] for **1**, on the basis of the ^{1}H NMR spectroscopic data observed in the ^{1}H–^{1}H NOE and ^{1}H–^{13}C long range-coupling experiments of **2** and its hepta-*O*-acetate.

In connection with our synthetic studies[5] on glycoside hydrolases, for the purpose of totally synthesizing **1** and **2**, and analogues thereof, we first needed to prepare the respective aminocyclitol moieties **3a** and **4a** of **1** and **2** in a racemic modification without ambiguity, thereby allowing the possibility of establishing the correct structure of the inhibitor.

In this paper we describe unambiguous syntheses of the penta-*N,O*-acetyl derivatives **3b** and **4b** which have been successfully carried out starting from DL-(1,4,5/2,3)-5-amino-1,2,3,4-cyclopentantetrol derivative‡ **5**, which was obtained[6,7] as the minor product (~5% overall yield) from the base-catalysed nitromethane cyclization reaction of the dialdehyde derived from 1,2-*O*-cyclohexylidene-*myo*-inositol.[8]

O-Deacetylation (**5**→**6**, ~100%), followed by treatment with 2,2-dimethoxypropane in *N,N*-dimethylformamide (DMF) gave the *N,O*-isopropylidene derivative **7** (~100%). The 1-hydroxy function of **7** was readily oxidized with pyridinium chlorochromate (PCC) to give the ketone **8** (~100%), reaction of which with diazomethane§ in dimethylsulfoxide–diethyl ether afforded a 2:3 mixture (87%) of two isomeric spiro epoxides: DL-1,7-anhydro-(1,4,5/2,3) **9** and (1,2,3/4,5)-5-acetamido-1-*C*-hydroxymethylcyclopentane-1,2,3,4-tetrol triacetate **10**. In the ^{1}H NMR spectra (270 MHz; $CDCl_3$), the epoxide protons appeared as two AB-quartets at δ 3.20 and 2.89 (J_{gem} 5.9 Hz), and 3.04 and 2.91 (J_{gem} 4.4 Hz), respectively. Without separation, the mixture was directly subjected to cleavage of the epoxide ring with sodium acetate in aqueous DMF, followed by conventional acetylation, giving a sole penta-*N,O*-acetyl derivative **11** (97%). Selective ring opening of **10** may be explained by assuming the assistance of the neighbouring *N*-acetyl group at C-1, suggesting that **11** possesses the (1,4,5/2,3)-configuration. Therefore, an attempt was made to convert the spiro epoxide into the *exo*-alkene¶ **12**, which was expected to be oxidized with osmium tetraoxide to afford the two isomers unselectively. Thus, treatment of the mixture of **9** and **10** with trimethylphosphite gave the alkene **12** (53%), together with the α,β-unsaturated ketone **13** (11%): ν_{max}/cm^{-1} 1740. *cis*-Hydroxylation of **12** with OsO_4 in aqueous 80% acetone in the presence of 4-methylmorpholine *N*-oxide (MNO) at 50 °C gave, after successive acid hydrolysis and acetylation, and fractionation by chromatography on silica gel, the penta-*N,O*-acetates **3b** (53%) and **11** (45%).

The ^{1}H NMR spectroscopic data (400 MHz; $CDCl_3$; Table 1) of **3b** and **11** combined with the NOE experiments, were consistent with the structures which were deduced from the reaction sequence. Thus, NOEs were observed between 2-H and 6-H, 3-H and 6-H, and 4-H and 5-H in **3b**, and between 1-OH and 2-H, 2-H and 3-H, and 4-H and 5-H in **11**.

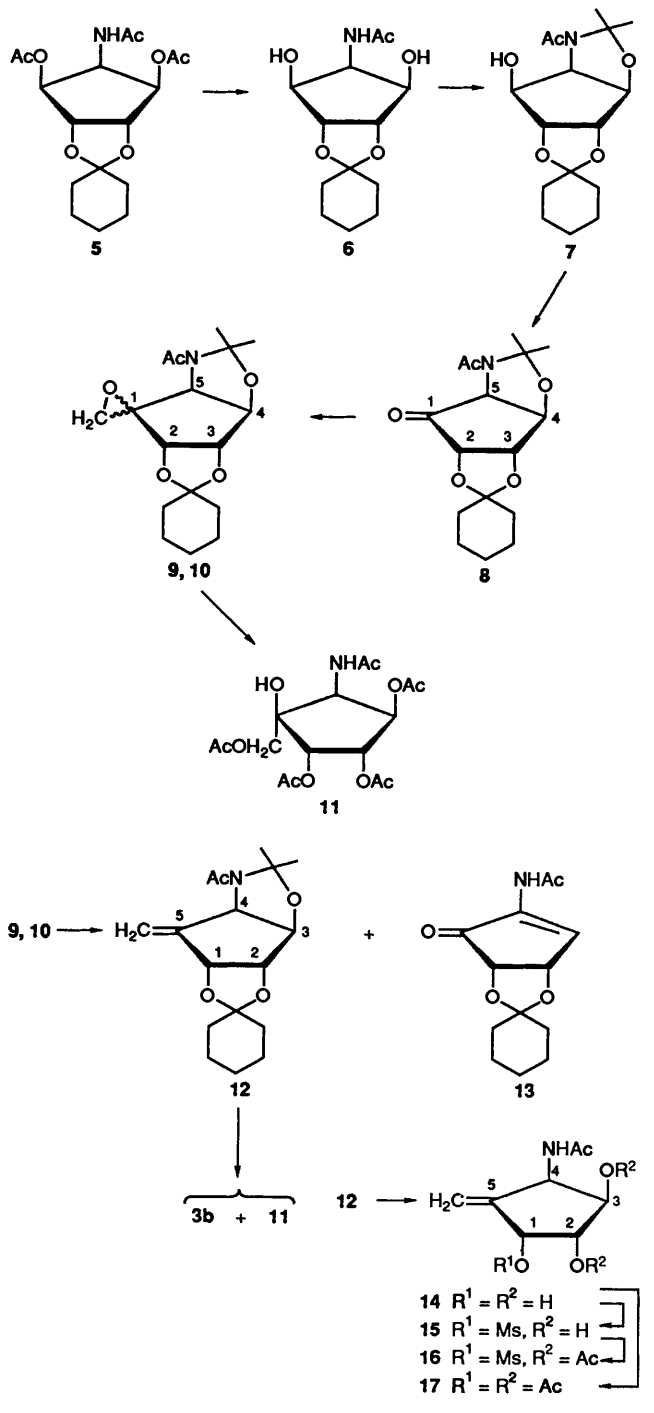
On the other hand, synthesis of compound **4b** was attempted

† For convenience, the structural formulae of the compounds **3b**, **4b**–**20** synthesized in this paper depict only one of the respective enantiomers.

‡ In this paper, nomenclature of cyclitols follows IUPAC-IUB 1973 Recommendations for Cyclitol [Pure Appl. Chem., 1974, 37, 285]. The stereochemical feature of cyclitols is shown by a fractional notation whereby numerals in the numerator denote hydroxy or other groups above the plane of the ring while numerals in the denominator denote hydroxy or other groups below that plane.

§ Reaction of the corresponding *N,O*-carbonyl derivative with diazomethane resulted in a selective spiro epoxidation, giving selectively the (1,4,5/2,3)-isomer in low yield, along with the ring-expansion product.

¶ Wittig olefination of **8** resulted in elimination reaction, giving rise to a conjugate enone **13** mainly.

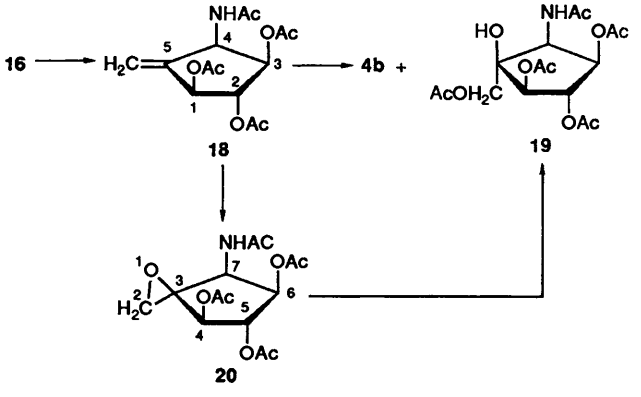


by initial conversion of the configuration at C-1 of **12**. Thus, acid hydrolysis of **12** gave the triol **14** (~100%), the allylic hydroxy group of which was selectively sulfonylated with 1.3 mol equiv. of mesyl chloride in pyridine (0 °C) to afford the 1-mesate **15** which was converted into the acetate **16** (65%). The ^{1}H NMR spectrum (270 MHz; D_2O) of **15** revealed a doublet of doublets (δ 5.32, J 4.4, J 1.8 Hz) due to 1-H, in which a long range coupling with the *exo*-methylene proton was observed. On treatment with an excess of sodium acetate in DMF, **15** gave a complex mixture containing mainly elimination products, however, **16** afforded, after acetylation, the substitution product **18** (60%), which was different from the tetra-*N*,*O*-acetate **17** derived from **14**. Similar OsO_4 oxidation of **18** at room temperature proceeded almost selectively through a rear-side attack of the reagent to give, after acetylation, the penta-*N*,*O*-acetate **4b** (89%) and the epimer **19** (9%). The ^{1}H NMR

Table 1 ^{1}H NMR spectroscopic data[a] (270 or 400 MHz; $CDCl_3$) of the penta-*N*,*O*-acetates **3b**, **4b**, **11** and **19**

	δ_H			
Proton	**3b**[b]	**4b**[b]	**11**[b]	**19**[c]
2-H	5.37	5.30	5.28	5.27
3-H	5.33	5.26	5.51	5.37
4-H	5.39	5.40	5.38	5.25
5-H	4.83	4.76	4.78	4.78
6a-H	4.19	4.29	4.29	3.96
6b-H	4.07	4.14	4.10	3.96
NH	5.94	5.89	6.12	5.94
Ac	2.07	2.07	2.03	2.03
	2.08	2.09	2.04	2
	2.13	2.129[d]	2.08	.08[d]
	2.147	2.134	2.107	2.11
	2.150		2.114	2.12
J	Coupling constant (Hz)			
$J_{2,3}$	5.6	6.4	4.9	7.2
$J_{3,4}$	3.9	4.4	5.4	3.3
$J_{4,5}$	8.1	8.3	8.8	8.2
$J_{6,6}$	12.2	12.2	12.2	—
$J_{5,NH}$	7.8	7.8	9.3	9.0

[a] Chemical shifts (δ_H) are given relative to Me_4Si as references. Peaks of tertiary hydroxy groups were not observed. [b] Measured at 400 MHz. [c] Measured at 270 MHz. [d] Peak of two acetoxymethyl groups.



spectroscopic data (Table 1) of **4b** and **19** were fully consistent with the structures which were deduced unambiguously from the reaction sequence. In **4b**, NOEs were observed between 2-H and 5-H, and 4-H and 5-H. In **19**, NOEs were observed between 2-H and 5-H, 4-H and 5-H, and 6-H and 5-H.

Furthermore, epoxidation of **18** gave a single spiro epoxide **20** (64%), which was transformed into **19** (83%) exclusively by treatment with sodium acetate followed by acetylation. In the ^{1}H NMR spectrum of **20**, NOEs were observed between each of the epoxide-methylene protons and 4-H, and 7-H, supporting the structure depicted. The amide function seems to facilitate the sterically hindered up-side attack of the peracid.

The $J_{2,3}$ and $J_{4,5}$ values of **3b** and **4b** suggested that the cyclopentane rings adopt the distorted half-chair conformation. That stereochemical preference would be preserved to a considerable extent in the cyclitol portion of the inhibitor. Acetylation of the inhibitor afforded the hepta-*N*,*O*-acetyl derivative,[3] with which the $J_{5',6'}$ changed from 4.8 to 8.5 Hz. These results would be better explained[4] by assuming further conformational distortion of the cyclopentane ring with the 5'-H,6'-H *anti*-configuration. The NOE observed between 5'-H and 6'-H[3] is seemingly consistent with the 5'-H,6'-H *syn*-

configuration, however, the long range couplings[2] between 3′-H and 6′-H, and 4′-H and 6′-H rather support the structure **2**. Moreover, the NOE observed[4] between 4′-H and 6′-H, can only be interpreted by the 4′-H,6′-H *syn*-configuration, thereby strongly supporting the structure **2**.

The final discrimination between two structures **1** and **2** has been successfully conducted by identification of the configuration of **4b** with that of the equivalent derivative of the aminocyclitol moiety, isolated by degradation of trehazolin **2**, by comparison* of the ^{1}H NMR spectra (400 MHz; $CDCl_3$). A total synthesis of the inhibitor using **4a** is in progress in our laboratory.

Experimental

M.p.s were determined on a MEL-TEMP capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured in deuteriochloroform or deuterium oxide solution with a JEOL JNM-EX 90 (90 MHz), JNM-GX 270 FT (270 MHz), or JNM-GX 400 FT (400 MHz) instruments, and *J*-values are given in Hz. IR spectra were measured with a Jasco A-202 spectrometer or Hitachi FTS-65. TLC was performed on Silica gel 60 F-254 (E. Merck, Darmstadt). The silica gel used for column chromatography was Wakogel C-300 (Wako Co. Osaka, Japan; 300 mesh).

2,3-O-*Cyclohexylidene*-1,5-N,O-*isopropylidene Derivative* **7** *of* DL-(1,4,5/2,3)-5-*Acetamidocyclopentane*-1,2,3,4-*tetrol*.—To a solution of the tri-*N*,*O*-acetate[6-8] **5** (294 mg, 0.82 mmol) in methanol (5 cm^3) was added 1 mol dm^{-3} methanolic sodium methoxide (1 cm^3), and the mixture was stirred for 1 h at room temperature and then neutralized with Amberlite IR 120B (H^+) resin. Removal of the solvent gave a crystalline diol **6** (225 mg, 100%), which was dissolved in DMF (4 cm^3). Then, 2,2-dimethoxypropane (0.5 cm^3, 4.14 mmol) and a catalytic amount of toluene-*p*-sulfonic acid monohydrate were added to the solution, which was stirred for 2 h at 50 °C. After neutralization with $NaHCO_3$, the solution was evaporated and the residue was extracted with $CHCl_3$. The extracts were evaporated and the residue was crystallized from EtOH to give the alcohol **7** (270 mg, ~100%), m.p. 183–184 °C (Found: C, 61.8; H, 7.9; N, 4.4. $C_{16}H_{25}NO_5$ requires C, 61.7; H, 8.1; N, 4.5%); ν_{max}(KBr)/cm^{-1} 3350 (OH), 1620 and 1630 (NAc); δ_H(270 MHz; $CDCl_3$) 4.74 and 4.70 (each 2 H, 2 d, $J_{2,3}$ 5.5, 2 and 3-H), 4.49 (1 H, d, $J_{1,5}$ 5.1, 1-H), 4.43 (1 H, dd, $J_{4,5}$ 5.0, 5-H), 4.32 (1 H, d, 4-H), 3.06 (1 H, br s, OH), 2.10 (3 H, s, Ac) and 1.67–1.52 (16 H, m, CMe_2 and C_6H_{10}).

2,3-O-*Cyclohexylidene*-4,5-N,O-*isopropylidene Derivative* **8** *of* DL-(2,3/4,5)-5-*Acetamido*-2,3,4-*trihydroxycyclopentan*-1-*one*.—A mixture of compound **7** (258 mg, 0.83 mmol) and 4 Å molecular sieves (500 mg) in dichloromethane (4 cm^3) was treated with pyridinium chlorochromate (PCC) (536 mg, 2.49 mmol) for 1 h at room temperature. The mixture was charged onto a column of silica gel (10 g) and eluted with diethyl ether. Removal of the solvent gave the ketone **8** (266 mg, 100%) as crystals, m.p. 128–130 °C (from EtOH) (Found: C, 62.2; H, 7.3; N, 4.5. $C_{16}H_{25}NO_5$ requires C, 62.1; H, 7.5; N, 4.5%); ν_{max}(neat, $CHCl_3$)/cm^{-1} 1770 (ketone) and 1660 (NAc); δ_H(270 MHz; $CDCl_3$) 4.47 (1 H, d, $J_{2,3}$ 5.5, 2-H), 4.62 (1 H, d, $J_{4,5}$ 4.8, 5-H), 4.59 (1 H, dd, $J_{3,4}$ 1.0, 4-H), 4.44 (1 H, dd, 3-H), 2.21 (3 H, s, Ac) and 1.67–1.51 (16 H, m, CMe_2 and C_6H_{10}).

DL-(1,4,5/2,3)-5-*Acetamido*-1-*acetoxymethyl*-2,3,4-*tri*-O-*acetylcyclopentane*-1,2,3,4-*tetrol* **11**.—A solution of the ketone **8** (266 mg, 0.83 mmol) in dimethyl sulfoxide (DMSO) (4 cm^3) was treated with diazomethane in diethyl ether for 19 h at room temperature. The mixture was diluted with water and extracted with diethyl ether. The organic layers were combined, dried, and evaporated to give a crystalline residue, which was chromatographed on a column of silica gel (10 g) with butane-2-one–toluene (1/3, v/v) to give a mixture (234 mg, 87%) of diastereoisomeric spiro-epoxides **9** and **10**; δ_H(270 MHz; $CDCl_3$) 3.20 and 2.89 (0.4 H, ABq, *J* 5.9) and 3.04 and 2.91 (0.6 H, ABq, *J* 4.4) (epoxide protons).

Without separation, the mixture was dissolved in 80% DMF (5 cm^3), to which was added NaOAc (475 mg, 5.79 mmol), and the mixture was stirred for 19 h at 120 °C and then evaporated. The residue was extracted with $CHCl_3$. Removal of the solvent gave a residue which was hydrolysed with 2 mol dm^{-3} HCl for 5 h at 80 °C and then acetylated with acetic anhydride in pyridine at room temperature. The crude product was purified by a column of alumina with $CHCl_3$ to give the penta-*N*,*O*-acetate **11** (306 mg, 100% from **9** and **10**) as plates, m.p. 120–122 °C (from EtOH) (Found: C, 49.0; H, 5.7; N, 3.5. $C_{16}H_{23}NO_{10}$ requires C, 49.4; H, 6.0; N, 3.6%), ν_{max}(KBr)/cm^{-1} 3440 and 3380 (OH and NH), 1740 (OAc) and 1660 (NAc); ^{1}H NMR spectroscopic data are listed in Table 1.

1,2-O-*Cyclohexylidene*-3,4-N,O-*isopropylidene Derivative* **12** *of* DL(1,2/3,4)-4-*Acetamido*-5-*methylenecyclopentane*-1,2,3-*triol and* 2,3-O-*Cyclohexylidene Derivative* **13** *of* (4SR,5SR)-2-*Acetamido*-4,5-*dihydroxycyclopent*-2-*en*-1-*one*.—A mixture (240 mg, 0.74 mmol) of the epoxides **9** and **10** and trimethylphosphite (1.8 cm^3, 14.8 mmol) was heated for 51 h at 130 °C in a sealed tube, and then cooled to room temperature. Removal of trimethylphosphite by co-evaporation with toluene gave a syrupy residue, which was chromatographed on a column of silica gel with butan-2-one–toluene (1/7, v/v) as eluent to give, first, the enone **13** (21 mg, 11%) as plates, m.p. 128–130 °C (from EtOH) (Found: C, 61.8; H, 6.5; N, 5.4. $C_{13}H_{17}NO_4$ requires C, 62.1; H, 6.8; N, 5.6%); ν_{max}(KBr) cm^{-1} 3450 (NH), 1740 (α,β-unsaturated ketone) and 1680 (NAc); δ_H(90 MHz; $CDCl_3$) 7.75 (1 H, d, $J_{3,4}$ 3.0, 4-H), 7.42 (1 H, br s, NH), 5.35 (1 H, dd, $J_{2,3}$ 5.2, 3-H), 4.53 (1 H, d, 2-H), 2.16 (3 H, s, NAc) and 1.72–1.20 (10 H, m, C_6H_{10}).

The second fraction gave the *exo*-olefin **12** (120 mg, 53%) as plates, m.p. 91–93 °C (from EtOH) (Found: C, 66.5; H, 8.1; N, 4.5. $C_{17}H_{25}NO_4$ requires C, 66.4; H, 8.2; N, 4.6%); ν_{max}(KBr)/cm^{-1} 1660 (NAc); δ_C(270 MHz; $CDCl_3$) 5.63 and 5.27 (each 1 H, 2 d, $J_{4,6}$ 2.6 and 2.2, 6-H), 4.97 (1 H, d, $J_{1,2}$ 5.5, 1-H), 4.79 (1 H, ddd, $J_{3,4}$ 4.8, 4-H), 4.51 (1 H, d, 2-H), 4.41 (1 H, d, 3-H), 2.19 (3 H, s, Ac) and 1.67–1.56 (16 H, m, CMe_2 and C_6H_{10}).

Osmylation of **12**. *Preparation of* DL-(1,2,3/4,5)-5-*Acetamido*-1-*acetoxymethyl*-2,3,4-*tri*-O-*acetylcyclopentane*-1,2,3,4-*tetrol* **3b** *and* **11**.—To a solution of the *exo*-olefin **12** (20 mg, 0.064 mmol) in aq. 80% acetone (0.7 cm^3) were added 0.05 mol dm^{-3} OsO_4 in Bu^{t}OH (0.26 cm^3, 0.013 mmol) and NMO (23 mg, 0.19 mmol), and the mixture was stirred for 48 h at 50 °C in the dark. After treatment with $NaHSO_3$ (53 mg, 0.51 mmol) for 1 h at room temperature, the solution was dried (Na_2SO_4) and filtered through a bed of Celite. Evaporation of the solvent gave a syrupy residue, which was then treated with 2 mol dm^{-3} HCl for 3 h at 80 °C, followed by conventional acetylation. The mixture was evaporated and the residue was chromatographed on a column of silica gel (1 g) with acetonitrile–toluene (1/2, v/v) as eluent to give, first, the penta-*N*,*O*-acetate **11** (11 mg, 45%), identical to the compound obtained before in all respects.

The second fraction gave the penta-*N*,*O*-acetate **3b** (13 mg, 53%) as a syrup (Found: C, 49.6; H, 5.9; N, 3.5. $C_{16}H_{23}NO_{10}$ requires C, 49.4; H, 6.0; N, 3.6%); ν_{max}(neat, $CHCl_3$)/cm^{-1} 3350 (OH and NH), 1750 (OAc) and 1670 (NAc); ^{1}H NMR spectroscopic data are listed in Table 1.

* S. Takahashi, personal communication.

DL-(1,2/3,4)-4-*Acetamido*-1-O-*methanesulfonyl*-5-*methylene*-*cyclopentane*-1,2-3-*triol* **15**.—The *exo*-olefin **12** (10 mg, 0.033 mmol) was dissolved in aq. 80% acetic acid, and the mixture was stirred for 24 h at 80 °C. Removal of the solvent gave a syrupy triol **14**, which was treated with methanesulfonyl chloride (4 mm^3, 0.056 mmol) in pyridine (0.7 cm^3) for 1 h at 0 °C. The mixture was evaporated and the residue was chromatographed on a column of silica gel (2 g) with EtOH–toluene (1/5, v/v) as eluent to give the mesate **15** (4 mg, 49%) as a syrup (High-resolution mass spectrum, Found: M^+H; 266.0672. $C_9H_{16}NO_6S$ requires *m/z*; 266.0699); ν_{max}(neat, MeOH)/cm^{-1} 1640 (NAc); δ_H(270 MHz; D_2O) 5.55 (1 H, dd, $J_{4,6a}$ 2.6, J_{gem} 1.7, 6a-H), 5.42 (1 H, dd, $J_{1,6b}$ 1.8, 6b-H), 5.32 (1 H, dd, $J_{1,2}$ 4.4, 1-H), 4.09 (1 H, dd, $J_{2,3}$ 5.5, $J_{3,4}$ 6.2, 3-H) and 4.04 (1 H, dd, 2-H).

DL-(1,2/3,4)-4-*Acetamido*-2,3-*di*-O-*acetyl*-1-O-*methanesulf*-*onyl*-5-*methylenecyclopentane*-1,2,3-*triol* **16**.—To a solution of the triol **14** (53 mg, 0.28 mmol) in pyridine (1 cm^3) was added methanesulfonyl chloride (29 mm^3, 0.37 mmol), and the mixture was stirred at 0 °C. After 2 h, acetic anhydride (0.5 cm^3) was added to the mixture, and it was then stood for 1.5 h at room temperature. The mixture was evaporated and the residue was chromatographed on a column of silica gel with acetone–toluene (1/2, v/v) as eluent to afford the tri-*N*,*O*-acetate **16** (65 mg, 65%) as crystals, m.p. 150–152 °C (from acetone–toluene) (Found: C, 44.4; H, 5.2; N, 3.9. $C_{13}H_{19}NO_8S$ requires C, 44.7; H, 5.5; N, 4.0%); ν_{max}(KBr)/cm^{-1} 3440 (NH), 1760 (Ac) and 1640 (NAc); δ_H(270 MHz; $CDCl_3$) 5.67–5.64 (2 H, m), 5.53–5.50 (2 H, m), 5.34–5.30 (3 H, m), 3.06 (3 H, s, Ms) and 2.14, 2.11 and 2.05 (each 3 H, 3 s, 3 Ac).

DL-(1,3,4/2)-4-*Acetamido*-1,2,3-*tri*-O-*acetyl*-5-*methylene*-*cyclopentane*-1,2,3-*triol* **18**.—A mixture of the mesate **16** (60 mg, 0.17 mmol) and NaOAc (84 mg, 1.03 mmol) in DMF (1.5 cm^3) was stirred for 19 h at 80 °C, and then evaporated. The residue was chromatographed on a column of silica gel (2 g) with acetone–toluene (1/2, v/v) as eluent to give the compound **18** (32 mg, 60%) as crystals, m.p. 145–147 °C (from acetone–toluene) (Found: C, 53.5; H, 5.8; N, 4.6. $C_{14}H_{19}NO_7$ requires C, 53.7; H, 6.1; N, 4.5%); ν_{max}(KBr)/cm^{-1} 3280 (NH), 1750 and 1740 (Ac) and 1650 (NAc); δ_H(270 MHz; $CDCl_3$) 5.67 (1 H, d, $J_{4,NH}$ 8.4, NH), 5.48 (1 H, dd, $J_{1,2}$ 3.5, $J_{1,6}$ 2.2, 1-H), 5.33 and 5.30 (each 1 H, 2 dd, $J_{4,6}$ 2.6, J_{gem} 2.2, 6-H), 5.22 (1 H, ddd, $J_{3,4}$ 5.7, 4-H), 5.16 (1 H, dd, $J_{2,3}$ 2.9, 2-H), 5.11 (1 H, dd, 3-H) and 2.12, 2.10 and 2.07 (3, 6 and 3 H, 3 s, 4 Ac).

DL-(1,2/3,4)-4-*Acetamido*-1,2,3-*tri*-O-*acetyl*-5-*methylene*-*cyclopentane*-1,2,3-*triol* **17**.—To a solution of the triol **14** (6 mg, 0.029 mmol) in pyridine (0.5 cm^3) was added acetic anhydride (0.3 cm^3), and the mixture was stirred for 2 h at room temperature. Evaporation of the reaction mixture gave the residue, which was purified on a column of alumina with $CHCl_3$. The elution was concentrated to give the compound **17** (8 mg, 83%) as crystals, m.p. 128–130 °C (from acetone–toluene) (Found: C, 53.8; H, 5.8; N, 4.4. $C_{14}H_{19}NO_7$ requires C, 53.7; H, 6.1; N, 4.5%); ν_{max}(KBr)/cm^{-1} 1750 (Ac) and 1640 (NAc); δ_H(270 MHz; $CDCl_3$) 5.77 (1 H, dd, $J_{1,2}$ 4.6, $J_{1,6}$ 2.2, 1-H), 5.52 (1 H, d, $J_{4,NH}$ 8.4, NH), 5.40–5.33 (3 H, m, 4 and 6-H), 5.30 (1 H, dd, $J_{2,3}$ 3.1, $J_{3,4}$ 3.7, 3-H), 5.28 (1 H, dd, 2-H) and 2.10, 2.09, 2.07 and 2.05 (each 3 H, 4 s, 4 Ac).

DL-(1,3/2,4,5)- **4b** *and* DL-(1,2,4,5/3)-5-*Acetamido*-1-*acetoxy*-*methyl*-2,3,4-*tri*-O-*acetylcyclopentane*-1,2,3,4-*tetrol* **19**.—To a solution of the compound **18** (8 mg, 0.026 mmol) was added 0.05 mol dm^{-3} OsO_4 in Bu^{t}OH (0.1 cm^3, 0.0052 mmol) and NMO (9 mg, 0.077 mmol), and the mixture was stirred for 19.5 h at room temperature in the dark. After the usual work-up, the products were acetylated conventionally and separated by a column of silica gel (1 g) with acetone–toluene (1/2, v/v) as eluent to give, first, the compound **4b** (9 mg, 94%) as plates, m.p. 170–171 °C (from EtOH) (Found: C, 49.1; H, 5.8; N, 3.6. $C_{16}H_{23}NO_{10}$ requires C, 49.4; H, 6.0; N, 3.6%); ν_{max}(neat, $CHCl_3$)/cm^{-1} 3370 (OH and NH), 1740 (Ac) and 1670 (NAc); ^1H NMR spectroscopic data are listed in Table 1.

The second fraction gave compound **19** (1 mg, 9%) as crystals, m.p. 155–158 °C (acetone–toluene) (Found: C, 49.2; H, 5.7; N, 3.6); ν_{max}(neat, $CHCl_3$)/cm^{-1} 3360 (OH and NH), 1740 (Ac) and 1670 (NAc); ^1H NMR spectroscopic data are listed in Table 1.

(3SR,4RS,5SR,6RS,7SR)-7-*Acetamido*-4,5,6-*triacetoxy*-1-*oxaspiro*[2.4]*heptane* **20**.—To a solution of the compound **18** (11 mg, 0.036 mmol) in 1,2-dichloroethane (0.7 cm^3) was added *m*CPBA (*m*-chloroperbenzoic acid) (27 mg, 0.11 mmol) in the presence of phosphate buffer (pH 8), and the mixture was stirred for 10.5 h at room temperature in the dark. The reaction mixture was diluted with $CHCl_3$, washed with saturated aq. $Na_2S_2O_3$, saturated aq. $NaHCO_3$, and water, dried and evaporated. The product was chromatographed on a column of silica gel (0.5 g) with acetone–toluene (1/2, v/v) as eluent to give the epoxide **20** (8 mg, 64%) as crystals, m.p. 114–118 °C (from acetone–toluene) (Found: C, 50.7; H, 5.6; N, 4.1. $C_{14}H_{19}NO_8$ requires C, 51.1; H, 5.8; N, 4.3%); ν_{max}(neat; $CHCl_3$)/cm^{-1} 3350 (NH), 1750 (Ac) and 1670 (NAc); δ_H(270 MHz; $CDCl_3$) 5.59 (1 H, d, $J_{7,NH}$ 9.2, NH), 5.26 (1 H, dd, $J_{4,5}$ ~1, $J_{5,6}$ 2.9, 5-H), 5.21–5.17 (2 H, m, 4 and 6-H), 5.00 (1 H, dd, $J_{6,7}$ 5.1, 7-H), 2.98 and 2.68 (3ach 1 H, ABq, J_{gem} 4.8, 2-H) and 2.14, 2.12, 2.09 and 2.00 (each 3 H, 4 s, 4 Ac).

A mixture of the epoxide **20** (8 mg, 0.023 mmol) and NaOAc (11 mg, 0.14 mmol) in aq. 80% DMF (0.7 cm^3) was stirred for 20 h at 120 °C, evaporated, and acetylated with acetic anhydride in pyridine. Evaporation of a solvent gave the residue, which was chromatographed on a column of silica gel (1 g) with acetone–toluene (1/2, v/v) as eluent to give the pentaacetate (7 mg, 83%), identical to compound **19** obtained before in all respects.

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