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

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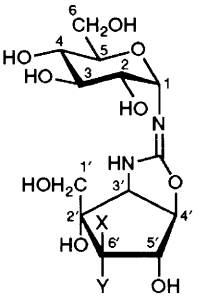
The respective aminocyclitol moieties **3a** and **4a** of trehalostatin **1** and trehazolin **2**, potent trehalase inhibitors, are synthesized as the *N,O*-pentaacetyl derivatives **3b** and **4b** in racemic form, in order both to establish the structure of the inhibitor and to provide a synthon useful for its total synthesis.

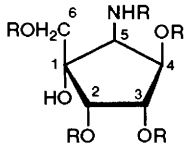
In 1991, trehalostatin **1**, a potent and specific inhibitor against blowfly trehalase, was isolated by Murao *et al.*[1] from culture broth of *Amycolatopsis trehalostatica*, and the structure was assigned[2] as depicted, mainly on the basis of the ^{1}H NMR spectral data.

Very recently, Ando *et al.*[3] reported the isolation of trehalase inhibitor trehazolin **2** from culture broth of *Micromonospora*, strain SANK 62390, and showed it to be identical to **1**. They however proposed a different structure, the configuration at C-4′ being opposite to that of **1**, on the basis of the results observed in the ^{1}H–^{1}H NOE and ^{1}H–^{13}C long range coupling experiments of **2** and its *O*-heptaacetate.

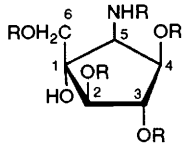
In connection with our synthetic studies[4] on glycoside hydrolases, with the aim of totally synthesizing **1** and **2**, we



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



3a; R = H
3b; R = Ac



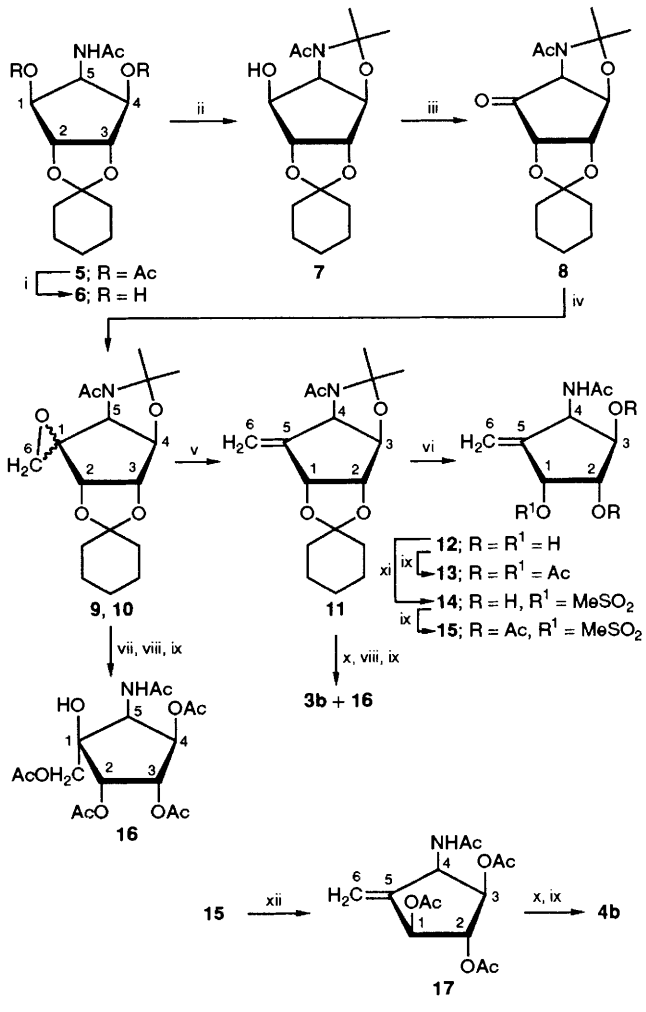
4a; R = H
4b; R = Ac

therefore first needed to prepare the respective aminocyclitol moieties **3a** and **4a** of **1** and **2** in a racemic form without ambiguity, thereby possibly establishing the correct structure of the inhibitor.

In this communication, syntheses of the *N,O*-pentaacetyl derivatives **3b** and **4b** have been successfully carried out by use of the starting compound, (1,4,5/2,3)-5-aminocyclopentane-1,2,3,4-tetraol derivative[5] **5**, which was obtained[6,7] as the minor product (~10% overall yield) by 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 dimethyl sulfoxide–diethyl ether afforded‡ a mixture of two isomeric spiro epoxides [**9** (1,4,5/2,3)- and **10** (1,2,3/4,5)-; 87%]. In the ^{1}H NMR spectra (270 MHz, $CDCl_3$), the epoxide protons appear 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, to give the sole *N,O*-pentaacetyl derivative **16** (97%) with (1,4,5/2,3)-configuration. Selective ring opening of **10** at C-1 may be explained by assuming the assistance of the neighbouring *N*-acetyl group. Therefore, an attempt was made to convert the spiro epoxide to the *exo*-alkene§ **11**, which was expected to be oxidized with osmium tetraoxide to afford the two isomers. Thus, treatment of the mixture of **9** and **10** with trimethyl phosphite gave the alkene **11** (53%), *cis*-hydroxylation of which 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, the *N,O*-pentaacetates **3b** (43%) and **16** (37%).

On the other hand, synthesis of compound **4b** was attempted by initial conversion of the configuration at C-1 of **11**. Thus, acid hydrolysis of **11** gave the triol **12** (~100%), the allylic hydroxy of which was selectively sulfonylated with 1.3 mol equiv. of methylsulfonyl chloride in pyridine (0 °C) to afford the 1-methanesulfonate **14**† that was converted into the acetate **15** (52%). The ^{1}H NMR spectrum (270 MHz, D_2O) of



Scheme 1 *Reagents and conditions*: i, NaOMe–MeOH, room temp., 2 h; ii, $Me_2C(OMe)_2$, *p*-TsOH·H_2O, DMF, 50 °C, 2 h; iii, PCC, CH_2Cl_2, room temp., 1 h; iv, CH_2N_2, dimethyl sulfoxide–diethyl ether, room temp., 20 h; v, $P(OMe)_3$, 130 °C, 51 h; vi, aq. 80% AcOH, 80 °C; vii, NaOAc, aq. 80% DMF, 120 °C, 24 h; viii, aq. 2 mol dm^{-3} HCl, 80 °C, 3 h; ix, Ac_2O, pyridine, room temp., 2 h; x, OsO_4 (0.2 equiv., 0.05 mol dm^{-3} solution in Bu^{t}OH), MNO (3 equiv.), aq. 80% acetone, 24 h; xi, $MeSO_2Cl$ (1.3 equiv.), pyridine, 0 °C, 2 h; xii, NaOAc, DMF, 80 °C, 17 h

† Selected ^{1}H NMR spectral data for **8**: δ_H (270 MHz, $CDCl_3$) (*inter alia*) 1.47–1.69 (16 H, m, C_6H_{10} and CMe_2), 2.21 (3 H, s, Ac), 4.44 (1 H, dd, $J_{2,3}$ 5.5 and $J_{3,4}$ 1.0 Hz, 3-H), 4.59 (1 H, dd, $J_{4,5}$ 4.8 Hz, 4-H), 4.62 (1 H, d, 5-H) and 4.74 (1 H, d, 2-H). For **11**: δ_H (90 MHz, $CDCl_3$) (*inter alia*) 1.25–1.72 (16 H, m, C_6H_{10} and CMe_2), 2.15 (3 H, s, Ac), 4.41 (1 H, d, $J_{4,5}$ 4.8 Hz, 4-H), 4.79 (1 H, d, 5-H), 4.48 and 4.96 (each 1 H, 2 d, $J_{2,3}$ 5.7 Hz, 2- and 3-H) and 5.27 and 5.54 (each 1 H, 2 d, J_{gem} 2.5 Hz, 2 × 6-H). For **13**: δ_H (270 MHz, $CDCl_3$) (*inter alia*) 2.05, 2.07, 2.09 and 2.10 (each 3 H, 4 s, 4 × Ac), 5.28 (1 H, dd, $J_{1,2}$ 4.6 and $J_{2,3}$ 3.1 Hz, 2-H), 5.30 (1 H, dd, $J_{3,4}$ 3.7 Hz, 3-H), 5.33–5.40 (3 H, m, 4-H and 2 × 6-H), 5.52 (1 H, d, $J_{4,NH}$ 8.4 Hz, NH) and 5.77 (1 H, dd, $J_{1,6}$ 2.2 Hz, 1-H). For **14**: δ_H (270 MHz, D_2O, acetone) (*inter alia*) 4.40 (1H, dd, $J_{1,2}$ 4.4 and $J_{2,3}$ 5.5 Hz, 2-H), 4.09 (1 H, dd, $J_{3,4}$ 6.2 Hz, 3-H), 5.32 (1 H, $J_{1,6a}$ 1.8 Hz, 1-H), 5.42 (1 H, dd, $J_{6,6}$ 1.7 Hz, 6a-H) and 5.5 (1 H, $J_{4,6b}$ 2.6 Hz, 6b-H). For **17**: δ_H (270 MHz, $CDCl_3$) (*inter alia*) 2.07, 2.10 and 2.12 (3, 6 and 3 H, 3 s, 4 × Ac), 5.11 (1 H, dd, $J_{2,3}$ 2.9 and $J_{3,4}$ 5.7 Hz, 3-H), 5.16 (1 H, dd, $J_{1,2}$ 3.5 Hz, 2-H), 5.22 (1 H, ddd, $J_{4,6a}$ 2.6 and $J_{4,NH}$ 8.4 Hz, 4-H), 5.30 (1 H, dd, $J_{6,6}$ 2.2 Hz, 6a-H), 5.33 (1 H, dd, $J_{1,6b}$ 2.2 Hz, 6b-H), 5.48 (1 H, dd, 1-H) and 5.67 (1 H, d, NH).

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

§ Wittig alkenation of **8** resulted in an elimination reaction, mainly giving rise to a conjugate enone.

Table 1 ^{1}H NMR spectral data[a] (400 MHz, $CDCl_3$) of the *N,O*-pentaacetates **3b**, **4b** and **16**

	Compound		
Proton	**3b**	**4b**	**16**
2-H	5.37 (*J* 5.6)	5.30 (*J* 6.4)	5.28 (*J* 4.9)
3-H	5.33 (*J* 5.6, 3.9)	5.26 (*J* 6.4, 4.4)	5.51 (*J* 4.9, 5.4)
4-H	5.39 (*J* 3.9, 8.1)	5.40 (*J* 4.4, 8.3)	5.38 (*J* 5.4, 8.8)
5-H	4.83 (*J* 8.1, 7.8)	4.76 (*J* 8.3, 7.8)	4.78 (*J* 8.8, 9.3)
6a-H	4.19 (*J* 12.2)	4.29 (*J* 12.2)	4.29 (*J* 12.2)
6b-H	4.07 (*J* 12.2)	4.14 (*J* 12.2)	4.10 (*J* 12.2)
NH	5.94 (*J* 7.8)	5.89 (*J* 7.8)	6.12 (*J* 9.3)
Ac	2.150	2.134	2.114
	2.147	2.129[b]	2.107
	2.13	2.09	2.08
	2.08	2.07	2.04
	2.07		2.03

[a] Chemical shifts (δ_H) are given relative to Me_4Si as references. Coupling constants are indicated in Hz. Peaks of tertiary hydroxy groups were not observed. [b] Peak of two acetoxymethyl groups.

14 revealed a doublet of doublets (δ 5.32, *J* 4.4 and 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, **14** gave only the elimination product, however, **15** afforded after acetylation the substitution product **17**† (51%), which was different from the *N,O*-tetraacetate **13**† derived from **12**. Similar osmium oxidation of **17** at room temperature proceeded almost selectively through a rear-side attack of the reagent to give¶ after acetylation the *N,O*-pentaacetate **4b** (71%).

The ^{1}H NMR spectral data (400 MHz, $CDCl_3$; Table 1) of **3b**, **4b** and **16**, combined with the NOE experiment, were fully consistent with the structures that were deduced unambiguously from the reaction sequence. The NOE were observed between 2-H and 6-H, 3-H and 6-H, and 4-H and 5-H in **3b**. On the other hand, in **4b**, NOE between 2-H and 5-H, and 4-H and 5-H were observed. In **16**, the NOE between 1-HO and 2-H, 2-H and 3-H, and 4-H and 5-H were observed.

The $J_{2,3}$ and $J_{4,5}$ of **3b** and **4b** suggested that the cyclopentane rings adopt the distorted half-chair conformation. This stereochemical preference would be preserved to a considerable extent in the cyclitol portion. Acetylation of the inhibitor afforded the *N,O*-heptaacetyl derivative[2], in which the $J_{5'6'}$ changed from 4.8 to 8.5 Hz. These results might be better explained[3] by assuming further conformational distortion of the cyclopentane ring with the 5′-H,6′-H *anti*-configuration. The NOE between 5′-H and 6′-H observed[2] is seemingly consistent with 5′-H,6′-H *syn*-configuration, however, the long range coupling[2] between 3′-H and 6′-H, and 4′-H and 6′-H rather supports the structure **2**. Furthermore, the observed[3] NOE between 4′-H and 6′-H, can only be interpreted by the 4′-H,6′-H *syn*-configuration, thereby strongly supporting the same structure.

The final determination of the structure **2** has been successfully carried out by identification of the configuration of **4b** with that of the equivalent derivative of the aminocyclitol moiety, isolable by degradation of trehazolin, by comparison of the ^{1}H NMR spectra (400 MHz, $CDCl_3$). A total synthesis of the inhibitor using **4a** is on the way in our laboratory.

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¶ The 1-epimer of **4b** was isolated as the minor product (<10%) under optimized conditions: ^{1}H NMR spectral data: δ_H (270 MHz, $CDCl_3$) (*inter alia*) 2.03, 2.08, 2.11 and 2.12 (3, 6, 3 and 3 H, 4s, NAc and 4 × OAc), 3.96 (2 H, s, 6-H), 4.78 (1 H, dd, $J_{4,5}$ 8.2 and $J_{5,NH}$ 9 Hz, 5-H), 5.25 (1 H, dd, $J_{3,4}$ 3.3 Hz, 4-H), 5.27 (1 H, d, $J_{2,3}$ 7.2 Hz, 2-H), 5.37 (1 H, dd, 3-H) and 5.94 (1 H, d, NH). NOEs were observed between 2- and 5-H, 4- and 5-H, and 6- and 5-H.

References

1 S. Murao, T. Sakai, H. Gibo, T. Nakayama and T. Shin, *Agric. Biol. Chem.*, 1991, **55**, 895.
2 T. Nakayama, T. Amachi, S. Murao, T. Sakai, T. Shin, P. T. M. Kenny, T. Iwashita, M. Zagorski, H. Komura and K. Nomoto, *J. Chem. Soc., Chem. Commun.*, 1991, 919.
3 O. Ando, H. Satake, K. Itoi, A. Sato, M. Nakajima, S. Takahashi, H. Haruyama, Y. Ohkuma, T. Kinoshita and R. Enokita, *J. Antibiot.*, 1991, **44**, 1165.
4 S. Ogawa and Y. Yuming, *J. Chem. Soc., Chem. Commun.*, 1991, 890.
5 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.
6 S. J. Angyal, M. E. Tate and S. D. Gero, *J. Chem. Soc.*, 1961, 4116.
7 S. J. Angyal and S. D. Gero, *Aust. J. Chem.*, 1965, **18**, 1973; R. Ahluwalia, S. J. Angyal and B. M. Luttrell, *Aust. J. Chem.*, 1970, **23**, 1819.
8 T. Suami, K. Tadano, S. Nishiyama and F. W. Lichtenthaler, *J. Org. Chem.*, 1973, **38**, 3691.