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Total Synthesis and Trehalase-Inhibitory Activity of Trehalostatin and Its Diastereoisomer

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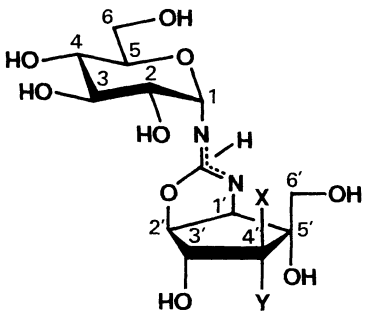
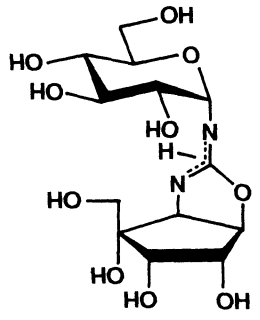
Complete synthesis of both diastereoisomeric structures, initially proposed for the trehalase inhibitor trehalostatin, and their biological assay have been carried out. The synthetic compounds were found to lack an inhibitory activity against trehalase, suggesting that the inhibitor would possess convincingly its 4'-epimeric structure later assigned for trehazolin.

In 1990, trehalostatin **1**, a potent and specific inhibitor against blowfly trehalase, was isolated by Murao *et al.*[1] from the culture broth of *Amycolatopsis trehalostatica*, and the structure[2] was proposed as depicted mainly on the basis of the ^{1}H-NMR spectroscopic data. Later, Ando *et al.*[3] reported the isolation of trehalase inhibitor trehazolin **2** from the culture broth of *Micromonospora* strain SANK 62390, and showed it to be rather identical to **1** by comparison of physical and spectroscopic data. They, however, assigned a different structure to it. Very recently, conclusive synthesis[4] of the aminocyclitol moiety of **2**, followed by a total synthesis[5,6] of the whole molecule of the inhibitor, established the structure proposed for **2** and its absolute configuration as depicted. Therefore, the question still remains unanswered whether compounds **1** and **2** are identical or the former is in fact the 4'-epimer of **2**.

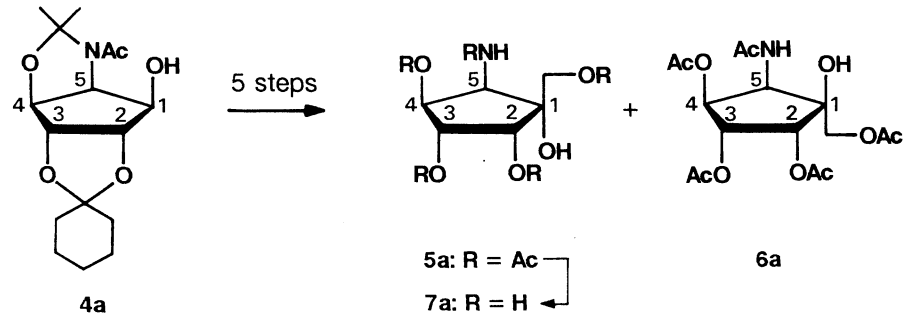


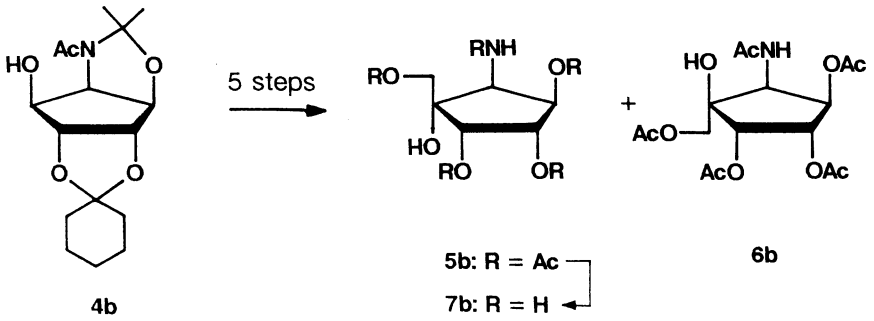
Trehalostatin 1: X = H, Y = OH

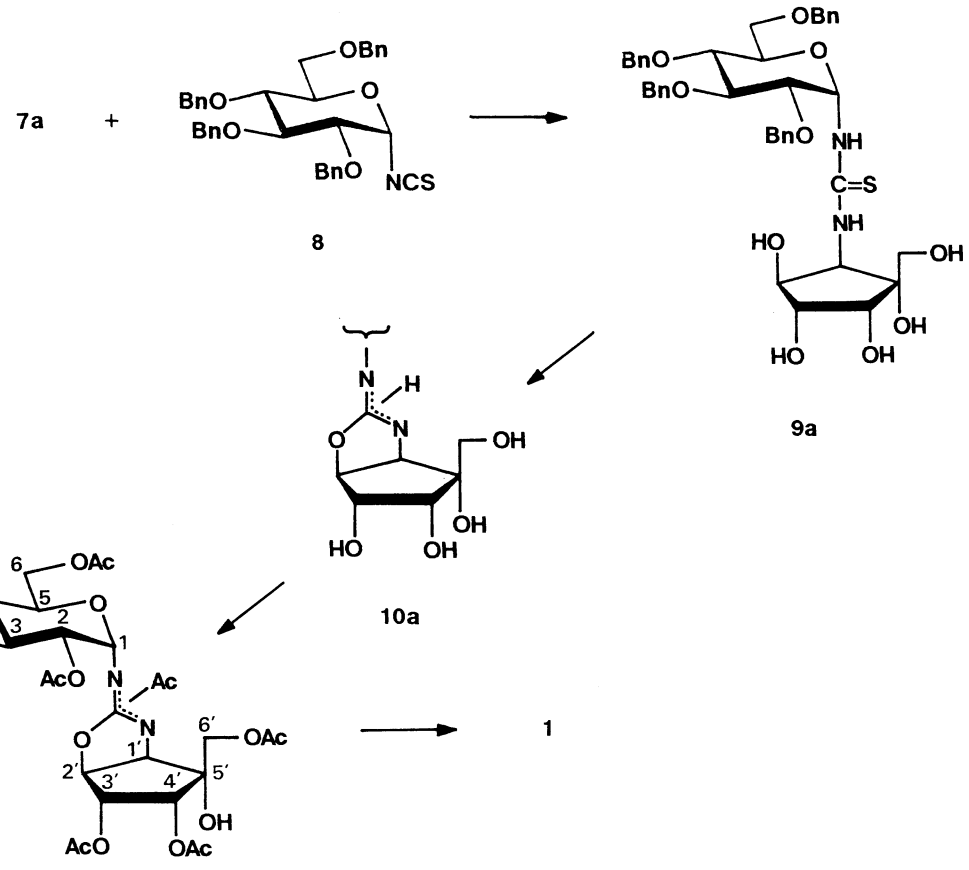
Trehazolin 2: X = OH, Y = H



Trehalostatin diastereoisomer 3


NAc
OH
5 steps
RO
RNH
OR
AcO
AcNH
OAc
4a
5a: R = Ac
7a: R = H
6a


AcN
HO
5 steps
RO
RNH
OR
AcO
AcNH
OAc
4b
5b: R = Ac
7b: R = H
6b


7a
+
OBn
BnO
NCS
8
NH
C=S
OH
HO
9a
10a
Ac
OAc
11a
1

This communication describes a complete synthesis of **1** and its diastereoisomer **3** *via* an unambiguous route similar to that[5] for **2** and evaluation of their inhibitory activity against silkworm trehalase.

The aminocyclitol moiety of **1** was first prepared from *O*-cyclohexylidene-*N,O*-isopropylidene derivative of (1,4,5/2,3)-5-acetamido-1,2,3,4-cyclopentanetetrol.[4] Thus, optical resolution[5] of the racemic compound was carried out by chromatographic separation of their (*S*)-acetylmandelates, giving the 1*R*-isomer **4a**, $[\alpha]_D^{29}$ +49° (*c* 1.2, $CHCl_3$) and 1*S*-isomer **4b**, $[\alpha]_D^{28}$ -45° (*c* 1.1, $CHCl_3$), in 41 and 45% yields, respectively. Compound **4a** was converted into (1*R*)-(1,2,3/4,5)- (**5a**) and (1*S*)-(1,4,5/2,3)-5-amino-1-*C*-hydroxymethyl-1,2,3,4-cyclopentanetetrol penta-*N,O*-acetates (**6a**) in 21 and 19% over-all yields, following essentially the five-step sequence[4] employed for the racemic compounds. Similarly, the antipodes **5b** and **6b** were obtained from **4b**. Hydrolysis of **5a** and **5b** with 2 mol dm^{-3} hydrochloric acid at 80 °C, followed by purification by a column of Dowex 50W-X2 (H^+) resin with aq. 5% ammonia afforded the respective free amines **7a**, $[\alpha]_D^{23}$ -10° (*c* 0.8, H_2O), and **7b**, $[\alpha]_D^{22}$ +9° (*c* 1.0, H_2O), quantitatively.

Coupling of **7a** and 1.2 molar eq. of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosylisothiocyanate[7] (**8a**) in aq. 75% *N,N*-dimethylformamide for 4 h at room temperature afforded a thiourea[8] **9a**, $[\alpha]_D^{20}$ +138° (*c* 1.0, $CHCl_3$), IR (neat) 1540 cm^{-1} (NH), in 91% yield. Treatment of **9a** with excess of mercury(II) oxide in acetone-ether (1:6) for 23 h resulted in a ready formation of oxazoline ring to give an isourea **10a**, $[\alpha]_D^{24}$ +39° (*c* 0.9, $CHCl_3$), IR (neat) 1665 cm^{-1} (C=N), quantitatively. Deblocking of the benzyl ether groups of **10a** was effected by treatment with sodium in liquid ammonia to give the free base **1**, which, after conventional acetylation, was purely isolated as a sole octa-*N,O*-acetyl derivative **11a** (69% over-all yield). De-*N,O*-acetylation of **11a** with methanolic sodium methoxide afforded **1**, $[\alpha]_D^{22}$ +92° (*c* 0.6, H_2O), as a syrup in a quantitative yield. Interestingly, the ^1H NMR spectrum (270 MHz, D_2O) of **1** was rather similar to that[5] of **2**. Therefore, it might have been very difficult to differentiate between two structures **1** and **2** only on the basis of ^1H NMR spectroscopic data.

Likewise, the diastereoisomer **3** , $[\alpha]_D^{22}$ +117° (*c* 0.8, H_2O), was prepared in three steps (**9b**→**10b**→**11b**→**3**) in 69% over-all yield, starting from the thiourea **9b** obtained, in 90% yield, by coupling of **7b** and **8.**

Biological assay[9] of the synthetic **1** and **3** showed no inhibitory activity against silkworm trehalase at the final concentration of 100 μg/ml (*cf.* synthetic **2**[5]: IC_{50} 0.016 μg/ml). Consequently, the present results clearly suggest that the structure **1** or **3** initially proposed for trehalostatin is incorrect and should probably be revised to the structure **2**, being identical to that of trehazolin.[10]

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References

1) S. Murao, T. Sakai, H. Gibo, T. Nakayama, and T. Shin, *Agric. Biol. Chem.*, **55**, 895 (1991).
2) T. Nakayama, T. Amachi, S. Murao, T. Sakai, T. Shin, H. Komura, P. M. Kenny, M. Zagorski, T. Iwashita, and K. Nomoto, Annual Meeting of the Agricultural Chemical Society of Japan,

March 31, 1991 (Kyoto); Abstract 3Ap14, p. 183; 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. For convenience, the structure of trehalostatin **1** depicts one of the diastereoisomers, the absolute configuration of which is related to that of trehazolin **2**.

3) O. Ando, H. Satake, K. Itoi, A. Sato, M. Nakajima, S. Takahashi, H. Haruyama, Y. Ohkuma, T. Kinoshita, and R. Enokita, *J. Antibiot.*, **44**, 1165 (1991).

4) S. Ogawa, C. Uchida, and Y. Yuming, *J. Chem. Soc., Chem. Commun.*, **1992**, 886; S. Ogawa and C. Uchida, *J. Chem. Soc., Perkin Trans. 1*, **1992**, 1939.

5) C. Uchida and S. Ogawa, 62th Symposium on Organic Synthesis, Japan, November 6, 1992 (Tokyo); Abstract 2-16, pp. 154-157 ; S. Ogawa and C. Uchida, *Chem. Lett.*, **1993**, 173. The absolute configuration of **4a** was clearly established by converting it into (*S*)-2-acetamido-1,4-butanediol diacetate.

6) Y. Kobayashi, H. Miyazaki, and M. Shiozaki, *J. Am. Chem. Soc.*, **111**, 10065 (1992).

7) M. J. Canarasa, P. Fernandez-Resa, M. T. Garcia-Lopez, F. G. de las Heras, P. P. Mendez-Castrillon, and A. S. Feelix, *Synthesis*, **1984**, 509.

8) All new compounds were characterized by 270 MHz ^{1}H NMR and IR spectrometric, and elemental analyses. Selected ^{1}H NMR data for **10a** (in $CDCl_3$) δ 5.33 (1 H, br s, 1 H), 4.89 and 4.77 (each 1 H, ABq, J_{gem} 11 Hz, PhC$\underline{H}_2$), 4.83 (1 H, d, $J_{1',2'}$ 8.1 Hz, 2'-H), 4.76 and 4.54 (each 1 H, ABq, J_{gem} 11.3 Hz, PhC$\underline{H}_2$), 4.62 and 4.58 (each 1 H, ABq, J_{gem} 11 Hz, PhC$\underline{H}_2$), 4.44 and 4.40 (each 1 H, ABq, J 11.7 Hz, PhC$\underline{H}_2$), 4.41 (1 H, d, 1'-H), 4.01 (1 H, d, $J_{3',4'}$ 3.6 Hz, 3'-H). For **11a** (in $CDCl_3$) δ 5.58 (1 H, d, $J_{1,2}$ 4 Hz, 1-H), 5.51—5.46 (2 H, m, 3'-H, 4'-H), 5.40 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 9.7 Hz, 3-H), 5.07 (1 H, dd, $J_{4,5}$ 9.9 Hz, 4-H), 5.06 (1 H, dd, $J_{1,2}$ 4 Hz, 2-H), 4.99—4.90 (2 H, m, 1'-H, 2'-H), 4.30 (1 H, ddd, $J_{5,6a}$ 1.8, $J_{5,6b}$ 4.8 Hz, 5-H), 4.22 (1 H, dd, J_{gem} 12.5 Hz, 6b-H), 4.09 (1 H, dd, 6a-H), 4.10 and 3.95 (each 1 H, ABq, J_{gem} 11.7 Hz, 6',6'-H), 3.58 (1 H, br s, OH), 2.66 (3 H, s, NAc), 2.14, 2.11, 2.10, 2.06, 2.03, 2.004, and 2.002 (each 3 H, 7 s, 7 Ac). For **1** (in D_2O) δ 5.17 (1 H, d, $J_{1,2}$ 5.1 Hz, 1-H), 4.85 (1 H, dd, $J_{1',2'}$ 8.4, $J_{2',3'}$ 1.2 Hz, 2'-H), 4.25 (1 H, d, 1'-H), 4.06 (1 H, dd, $J_{3',4'}$ 5.1 Hz, 3'-H), 3.80 (1 H, d, 4'-H), 3.67 (1 H, dd, $J_{5,6a}$ 2.7, J_{gem} 13 Hz, 6a-H), 3.58 (1 H, dd, $J_{2,3}$ 8.8 Hz, 2-H), 3.66 and 3.50 (each 1 H, ABq, J_{gem} 12.1 Hz, 6',6'-H), 3.58 (1 H, dd, $J_{5,6b}$ 5.9 Hz, 6b-H), 3.50 (1 H, dd, $J_{3,4}$ 9.9 Hz, 3-H), 3.42 (1 H, ddd, 5-H), 3.25 (1 H, dd, 4-H). For **3** (in D_2O) δ 5.13 (1 H, d, $J_{1,2}$ 5.1 Hz, 1-H), 4.78 (1 H, dd, $J_{1',2'}$ 8.2, $J_{2',3'}$ 1.1 Hz, 2'-H), 4.19 (1 H, d, 1'-H), 4.00 (1 H, dd, $J_{3',4'}$ 5.1 Hz, 3'-H), 3.76 (1 H, d, 4'-H), 3.56 (1 H, dd, $J_{2,3}$ 9.4 Hz, 2-H), 3.58—3.44 (5 H, m, 3-H, 6,6-H, 6',6'-H), 3.39 (1 H, ddd, $J_{4,5}$ 10.1, $J_{5,6}$ 2.8 Hz, 5-H), 3.24 (1 H, dd, $J_{3,4}$ 8.6 Hz, 4-H).

9) Dr. Shuji Takahashi, personal communication.

10) It is now very difficult to get each authentic sample for direct comparison, because of a minute quantity of natural trehalostatin.

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