

## Total Synthesis of Trehalase Inhibitor, Trehazolin

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The total synthesis of trehalase inhibitor, trehazolin has been accomplished by coupling the optically active aminocyclopentanepentaol with  $\alpha$ -D-glucopyranosylisothiocyanate derivative, followed by subsequent oxazoline-ring formation and removal of the protecting groups, thereby confirming its absolute configuration.

In 1991 trehazolin 1, a potent inhibitor against trehalase, was isolated by Ando *et al.*<sup>1)</sup> from the culture broth of *Micromonospora* strain SANK 62390, and it was shown most likely to be identical to trehalostatin previously isolated by Murao *et al.*<sup>2-4)</sup> from *Amycolatopsis trehalostatica*. The two structures, being only epimeric at C-4', have been proposed by two groups, and the correct one 1 has finally been established by an unambiguous synthesis<sup>5)</sup> of the branched aminocyclopentanepentaol moiety 3 as the penta-*N,O*-acetyl derivative 2 and comparison of its physical and spectroscopic data with those of the equivalent derivative obtained from 1.

In this communication, we wish to report the first complete synthesis of 1 and its diastereoisomer 10 by coupling of DL-(1,3/2,4,5)-5-amino-1-*C*-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol (3), obtained by treatment of 2<sup>5)</sup> with 2 M hydrochloric acid, and 2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosylisothiocyanate (4),<sup>6)</sup> followed by formation of the oxazoline ring with mercuric oxide and removal of the protecting groups.

Thus, reaction of the racemic amine 3 with the isothiocyanate 4 in a mixture of *N,N*-dimethylformamide (DMF) and methanol afforded a 93% yield of a diastereoisomeric mixture of the thiourea derivatives<sup>7)</sup> 5a and 5b: IR (neat) 1540  $\text{cm}^{-1}$  (NH). Without separation, the mixture was successively treated with mercuric oxide in diethyl ether, resulting in a simultaneous ring-closure through attack of the neighbouring *cis*-hydroxy group to give rise to an inseparable mixture (96%) of the  $\alpha$ -glucosylamino-oxazolines<sup>7)</sup> 6a and 6b: IR (neat) 1670  $\text{cm}^{-1}$  (C=N), which was treated with acetic anhydride in pyridine to convert to the tetra-*N,O*-acetyl derivatives 7a,b. Similar



acetylation in the presence of 4-dimethylaminopyridine (DMAP) gave penta-*N,O*-acetyl derivatives **8a,b**. The diastereoisomeric mixture was readily separable by a silica gel chromatography to afford **7a** (47%),  $[\alpha]_D^{24} +100^\circ$  (c 2.5,  $\text{CHCl}_3$ ), and **7b** (47%),  $[\alpha]_D^{24} +29^\circ$  (c 2.5,  $\text{CHCl}_3$ ). Compounds **8a** and **8b** were also separated and obtained in 40 and 42% yields, respectively. Removal of the acyl groups of **7a** or **8a** was readily effected by treatment with methanolic sodium methoxide to give the tetraol **6a**<sup>7)</sup> (100%).

*O*-Debenzylation of **6a** was then carried out in liquid ammonia with sodium at  $-78^\circ$  to give the crude inhibitor **1** that was isolated as the octa-*N,O*-acetyl derivative **9a** (77%),  $[\alpha]_D^{25} +104^\circ$  (c 1.7,  $\text{CHCl}_3$ ), the  $^1\text{H-NMR}$  spectrum of which was identical with that reported for the equivalent derivative<sup>8)</sup> derived<sup>1)</sup> from natural **1**. *N,O*-Deacetylation of **9a** with methanolic sodium methoxide in methanol proceeded cleanly at room temperature to afford, after elution from a column of Dowex 50W-X2 ( $\text{H}^+$ ) resin with aqueous 4% ammonia, the free base **1**,  $[\alpha]_D^{23} +105^\circ$  (c 0.36,  $\text{H}_2\text{O}$ ), in 71% yield, the  $^1\text{H NMR}$  spectrum of which was superimposable on that of an authentic sample,<sup>1)</sup>  $[\alpha]_D^{25} +99.5^\circ$  (c 0.41,  $\text{H}_2\text{O}$ ), of trehalosin. Likewise, the diastereoisomer **10**,  $[\alpha]_D^{25} +63^\circ$  (c 0.40,  $\text{H}_2\text{O}$ ), of **1** was prepared from **6b** through the octa-*N,O*-acetyl derivative **9b**,<sup>7)</sup>  $[\alpha]_D^{25} +30^\circ$  (c 1.6,  $\text{CHCl}_3$ ), obtained from the tetraol **6b**.

Biological assay<sup>9)</sup> of the synthetic **1** and **10** showed inhibitory activity  $IC_{50}$  11.6 and 35.9 ng/ml, respectively, against porcine trehalase (*cf.* an authentic sample of **1**:  $IC_{50}$  9.39 ng/ml). Very interestingly, the diastereoisomer **10** was shown to possess about 30% of activity.

Concerning the position of the C=N bond in **1**, there has so far been no firm spectroscopic evidence to differentiate between the two tautomers. In fact, the tautomers of **6a** or **6b** seem to be rapidly interchangeable at room temperature, considering from a pH-dependent property of its  $^1\text{H NMR}$  spectrum. Therefore, it remains unknown which structure plays a role as the inhibitor.

Attempts to establish the absolute configuration of **1** have been carried out by optical resolution of the alcohol **11**<sup>5)</sup> as the (*S*)-(+)-*O*-acetylmandelate **12** and conversion into (*S*)-2-acetamido-1,4-butanediol diacetate (**13**),  $[\alpha]_D^{29} -42.7^\circ$  (c 0.9,  $\text{CHCl}_3$ ), by the sequence of reaction: deoxygenation via the methylthiothiocarbonate, periodate oxidation after de-*O*-ketallization followed by reduction with sodium borohydride and acetylation. This compound was shown to be identical to an authentic sample,  $[\alpha]_D^{32} -42.3^\circ$  (c 1,  $\text{CHCl}_3$ ), derived by acetylation of the amino alcohol<sup>10)</sup> obtained from L-aspartic acid by three-step reaction. Compound **1** could similarly be synthesized by use of the optically active **3** derived from **11**, establishing the absolute configuration as **1** depicted.

In summary, the present communication described the first total synthesis of trehazolin 1 and determination of its absolute configuration.

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- 7) All new compounds were characterized by 270 MHz  $^1\text{H}$  NMR and IR, spectroscopic and elemental analyses. Selected  $^1\text{H}$  NMR (270 MHz) data for 5a,b (in  $\text{CDCl}_3$ )  $\delta$  7.80-7.72 (2 H, 2 d, 2 NH), 6.74-6.65 (2 H, 2 br s, 2 NH). For 6a,b ( $\text{CDCl}_3$ )  $\delta$  5.40 (1 H, d,  $J_{1,2}$  4 Hz, 1-H), 5.28 (1 H, d,  $J_{1,2}$  5.1 Hz, 1-H). For 10 (in  $\text{D}_2\text{O}$ )  $\delta$  5.25 (1 H, d,  $J_{1,2}$  5.9 Hz, 1-H), 5.22 (1 H, dd,  $J_{1,2}$  8.4,  $J_{2,3}$  1.1 Hz, 2'-H), 4.43 (1 H, d, 1'-H), 4.25 (1 H, dd,  $J_{3,4}$  3.7 Hz, 3'-H), 3.90 (1 H, d, 4'-H), 3.72-3.48 (5 H, m, 2, 6, and 6'-H), 3.52 (1 H, dd,  $J$  8.4 and 9.9 Hz, 3-H or 4-H), 3.42-3.36 (1 H, m, 5-H), 3.30 (1 H, dd,  $J$  8.8 and 9.9 Hz, 4-H or 3-H).
- 8) Two isomeric octa-*N,O*-acetyl derivatives were initially prepared by Murao *et al.*<sup>4)</sup> by treatment of trehalostatin with acetic anhydride in pyridine. On the other hand, one octa-*N,O*-acetyl derivative was reported to be obtained from trehazolin 1 by Ando *et al.*,<sup>1)</sup> and it was identified with 9a obtained here. Compound 9a is most likely to be identical to one of the two described by Murao *et al.*, judging from the  $^1\text{H}$  NMR spectroscopic data.<sup>4)</sup>
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