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Kinetic and Thermodynamic Control of L-Threonine Aldolase Catalyzed Reaction and Its Application to the Synthesis of Mycestericin D.

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Abstract : L-Threonine aldolase catalyzes the aldol condensation of γ-benzyloxybutanal and glycine with high *erythro/threo* selectivity under a kinetically controlled condition. The *erythro* product was used in the synthesis of mycestericin D, a potent immunosupressant. Copyright © 1996 Elsevier Science Ltd

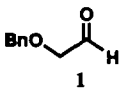
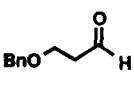
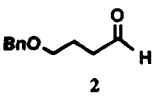
Recently we have reported that L-threonine aldolase from *Candida humicola* (AKU 4586) catalyzes the aldol condensation of glycine and an aldehyde to give a β-hydroxy-L-α-amino acid in good yield and high *erythro/threo* selectivity when an oxygen or nitrogen atom exists at α-position of the substrate aldehyde, *e.g.* α-benzyloxyacetaldehyde (**1**) and α-azidoacetaldehyde.[1] Here, we report that the *erythro/threo* ratio can be controlled by using either the kinetic or thermodynamic reaction condition. This phenomenum was found in the case where γ-benzyloxybutanal (**2**) was used as an acceptor substrate. The kinetically controlled process was applied to the synthesis of a key intermediate used in the synthesis of mycestericin D (**3**), a new lipid isolated from *Mycelia sterilia* with novel type of immunosuppressive activity.[2]

As shown in Table 1, the *erythro/threo* ratio of the aldolase products can be manipulated under a kinetic or thermodynamic process. As predicted from the result obtained previously, the longer distance between the benzyl ether group and the aldehyde group gave a lower *erythro/threo* selectivity under the thermodynamic condition. When the condensation of **2** and glycine was carried out in a short reaction time (15 min.), a high *erythro/threo* ratio was, however, obtained.

We have found that the aldolase products are useful for the synthesis of ceramide-related structures. Of particular interest are the syntheses of the potent immunosupressants, mycestericins and ISP-I (myriocin, thermozymocidin), recently

isolated from the culture broth of *Mycelia sterilia* (ATCC 20349) and *Isaria sinclairii* (ATCC 24400) respectively. The modes of action of these new ceramides are different from that of cyclosporin A and FK506. Mycestericin D (3) is one of the most active mycestericins[2] reported (IC_{50}=16 nM).

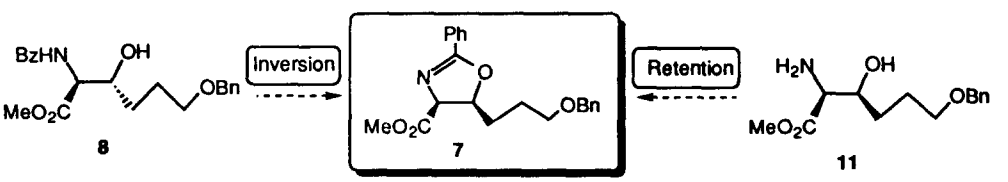
Table 1 L-Threonine Aldolase Catalyzed Reaction with Glycine

Substrates		Products *erythro*		Products *threo*	Yield
 1	15 hrs	98	:	2	88 %
 4	15 hrs	53	:	47	53 %
 2	15 hrs	40	:	60	70 %
	15 min	90	:	10	18 %

The L-threonine aldolase catalyzed aldol condensation of γ-benzyloxybutanal (2) and glycine can be taken as a key step for the synthesis of 3. As mentioned above, the aldol reaction under the kinetically controlled condition gave mainly the *erythro* product (5), while under a thermodynamically controlled condition gave a 6 : 4 (*threo* : *erythro*) mixture. In addition, the *threo* isomer (6) was purely obtained by digesting the thermodynamic mixture with the aldolase. Therefore, either the *erythro* (5) or the *threo* (6) isomer can be prepared on a preparative scale as a starting material by the L-threonine aldolase catalyzed reaction.

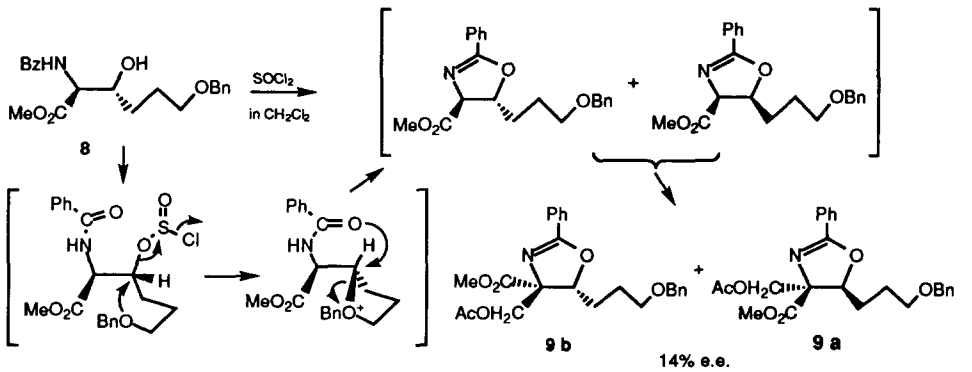
Both of *erythro* (5) and *threo* (6) isomers were investigated as starting material for the synthesis of oxazoline derivative 7 (Scheme 1). Using the *erythro* isomer requires a retention reaction to prepare 7, while using the *threo* isomer requires an inversion reaction.

Scheme 1 Synthesis of Oxazoline Derivative (7)



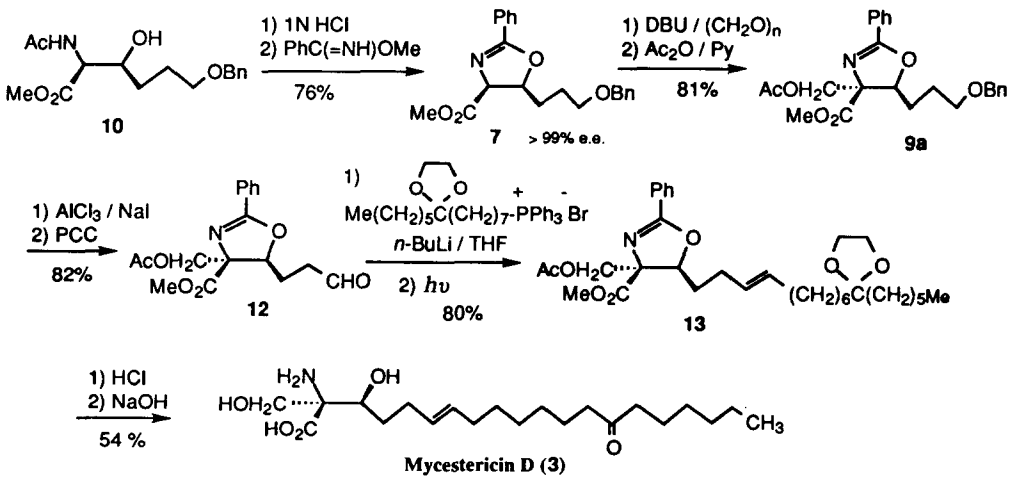
When the *threo* isomer (**6**) was used as the starting material (Scheme 2), the amino and the carboxy groups were protected with benzoyl and methyl groups, respectively, to afford compound **8** in 99% ee.. After treating **8** with thionyl chloride in dichloromethane,[3] the aldol condensation with formaldehyde in the presence of DBU followed by acetylation gave a mixture of **9a** and **9b** with 14% e.e..[4] This decrease of optical purity during the reactions was considered to be caused by the participation of the benzyloxy group as shown in Scheme 2.

Scheme 2 Presumed Mechanism of Racemization



The L-*erythro* predominant product (**5**) was then derived to methyl *N*-acetylamino ester (**10**) with acetic anhydride and diazomethane, and the *erythro*/*threo* mixture of **10** was easily separated by silica gel chromatography at this stage. As shown in Scheme 3, the *N*-acetyl group was deprotected with 1*N* hydrochloric acid to give compound **11**, and the

Scheme 3 Synthetic Route of Mycestericin D



regenerated amine and the hydroxyl group on the neighbouring carbon were protected with benzimidate to afford **7**.

Stereoselective addition to formaldehyde in the presence of DBU followed by acetylation yield **9a**.[5] After cleaving the benzyl group of **9a** with aluminum chloride - sodium iodide,[6] the generated alcohol was oxidized with PCC to aldehyde **12**. Wittig reaction of **12** with $C_6H_{13}C(OCH_2CH_2O)(CH_2)_6CH{=}PPh_3$ afforded the fully protected *cis*-mycestericin D. After photoisomerization from *cis*-isomer to **13**[7] and deprotection, mycestericin D (**3**) was obtained. All of the physical data (^{1}H-NMR, IR, FAB-MS, m.p., $[\alpha]_D$) of the synthesized and natural mycestericin D were identical.

Acknowledgement

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4. The value of e.e. was determined by chiral HPLC [DAICEL CHIRALCEL OD (hexane:*i*-PrOH = 9 : 1)]
5. Compound **9a**: $[\alpha]_D$ - 50.4 ($CHCl_3$, *c*=1.83), ^{1}H-NMR($CDCl_3$) δ 7.80 - 7.97(2H, m), 7.54 - 7.26(8H, m), 4.57(1H, dd, *J* = 8.8, 4.6), 4.54(1H, d, A part of AB, *J* = 11.3), 4.51(2H, s), 4.39 (1H, d, B part of AB, *J* = 11.3), 3.77(3H, s), 3.56 - 3.49(2H, m), 2.03(3H, s), 1.94 - 1.71(4H, m).
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7. Compound **13**: $[\alpha]_D$ - 49.3 ($CHCl_3$, *c*=0.46), ^{1}H-NMR($CDCl_3$) δ 8.02 - 7.99(2H, m), 7.55 - 7.40(3H, m), 5.50(1H, dt, *J* = 15.4, 6.4), 5.38(1H, dt, *J* = 15.4, 6.5), 4.55(1H, dd, *J* = 10.0, 3.6), 4.54, 4.39(each 1H, d, AB type, *J* = 11.2), 3.92(4H, s), 3.78(3H, s), 2.30 - 2.16(2H, m), 2.05(3H, s), 1.99(2H, br q, *J* = 6.7), 1.73 - 1.56(6H, m), 1.29 - 1.24(16H, m), 0.88(3H, t, *J* = 6.7).

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