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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  $\gamma$ -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 immunosuppressant. Copyright © 1996 Elsevier Science Ltd

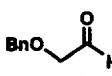
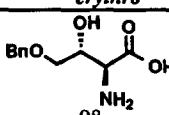
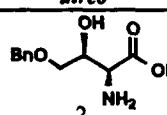
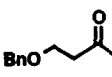
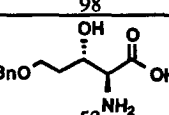
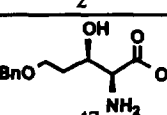
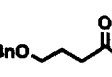
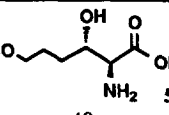
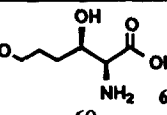
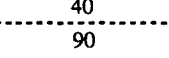
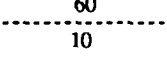
Recently we have reported that L-threonine aldolase from *Candida humicola* (AKU 4586) catalyzes the aldol condensation of glycine and an aldehyde to give a  $\beta$ -hydroxy-L- $\alpha$ -amino acid in good yield and high *erythro*/*threo* selectivity when an oxygen or nitrogen atom exists at  $\alpha$ -position of the substrate aldehyde, e.g.  $\alpha$ -benzyloxyacetaldehyde (**1**) and  $\alpha$ -azidoacetaldehyde.<sup>1</sup> Here, we report that the *erythro*/*threo* ratio can be controlled by using either the kinetic or thermodynamic reaction condition. This phenomenon was found in the case where  $\gamma$ -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.<sup>2</sup>

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 immunosuppressants, mycestericins and ISP-I (myriocin, thermozyiocidin), 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<sup>2</sup> reported ( $IC_{50}=16$  nM).

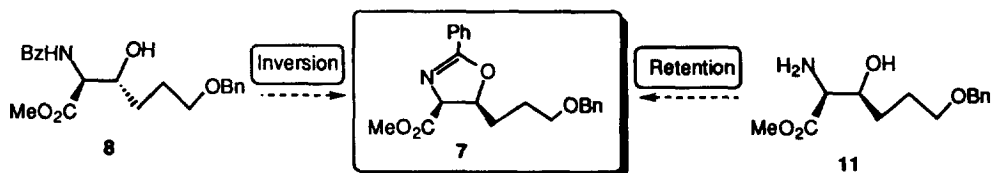
Table 1 L-Threonine Aldolase Catalyzed Reaction with Glycine

Substrates	Products		Yield
	<i>erythro</i>	<i>threo</i>	
 1	 98	 2	88 %
 4	 53	 47	53 %
 2	 40	 60	70 %
	 90	 10	18 %

The L-threonine aldolase catalyzed aldol condensation of  $\gamma$ -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.

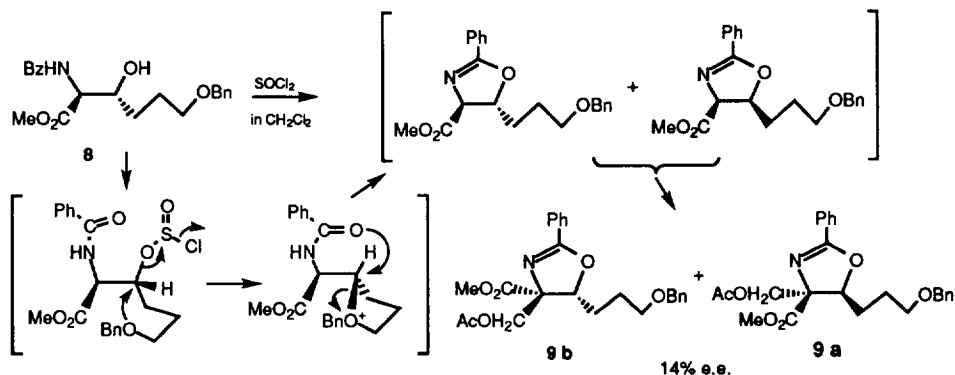
Both of *erythro* (5) and *threo* (6) isomers were investigated as starting material for the synthesis of oxazoline derivative 7 (Scheme 1). Using the *erythro* isomer requires a retention reaction to prepare 7, while using the *threo* isomer requires an inversion reaction.

Scheme 1 Synthesis of Oxazoline Derivative (7)



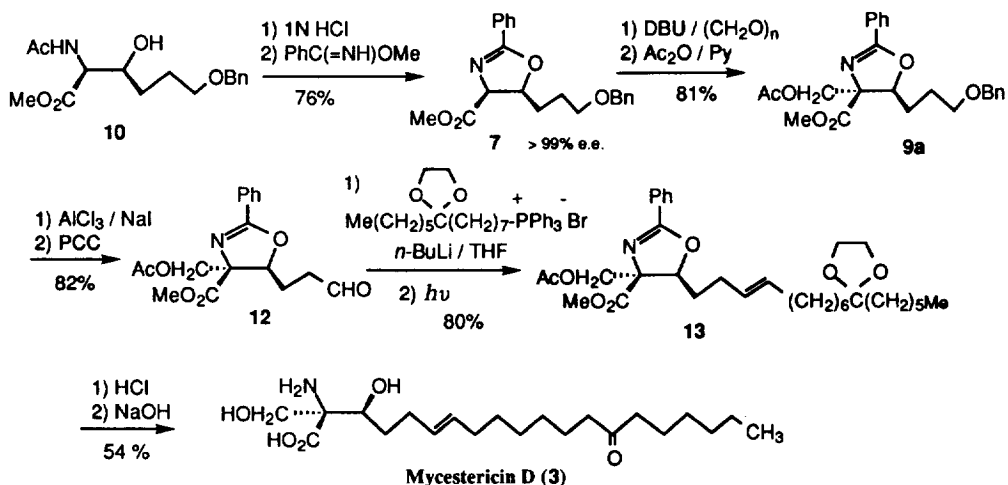
When the *threo* isomer (6) was used as the starting material (Scheme 2), the amino and the carboxy groups were protected with benzoyl and methyl groups, respectively, to afford compound 8 in 99% ee. After treating 8 with thionyl chloride in dichloromethane,<sup>3</sup> the aldol condensation with formaldehyde in the presence of DBU followed by acetylation gave a mixture of 9a and 9b with 14% e.e.<sup>4</sup> 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.<sup>5</sup> After cleaving the benzyl group of 9a with aluminum chloride - sodium iodide,<sup>6</sup> the generated alcohol was oxidized with PCC to aldehyde 12. Wittig reaction of 12 with C<sub>6</sub>H<sub>13</sub>C(OCH<sub>2</sub>CH<sub>2</sub>O)(CH<sub>2</sub>)<sub>6</sub>CH=PPH<sub>3</sub> afforded the fully protected *cis*-mycestericin D. After photoisomerization from *cis*-isomer to 13<sup>7</sup> and deprotection, mycestericin D (3) was obtained. All of the physical data (<sup>1</sup>H-NMR, IR, FAB-MS, m.p., [α]<sub>D</sub>) of the synthesized and natural mycestericin D were identical.

#### Acknowledgement

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- The value of e.e. was determined by chiral HPLC [DAICEL CHIRALCEL OD (hexane:*i*-PrOH = 9 : 1)]
- Compound 9a: [α]<sub>D</sub> - 50.4 (CHCl<sub>3</sub>, c=1.83), <sup>1</sup>H-NMR(CDCl<sub>3</sub>) 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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- Compound 13: [α]<sub>D</sub> - 49.3 (CHCl<sub>3</sub>, c=0.46), <sup>1</sup>H-NMR(CDCl<sub>3</sub>) 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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