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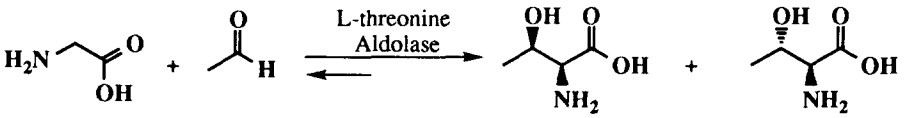
L-Threonine Aldolase in Organic Synthesis: Preparation of Novel β-Hydroxy-α-Amino Acids

Vassil P. Vassilev[a], Taketo Uchiyama[a], Tetsuya Kajimoto*[a], Chi-Huey Wong*[a,b]

[a] Frontier Research Program, The Institute of Physical and Chemical Research 2-1 Hirosawa, Wako City, 351-01, Japan,
[b] Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA.

Abstract: The L-threonine aldolase from *Candida humicola* was used in the synthesis of multifunctional β-hydroxy-α-amino acids. Of many aldehydes with different substituents tested as substrates for reaction with glycine, benzyloxy- and alkoxy- aldehydes are found to be good substrates, providing a new synthetic route to α-amino-β,ω-dihydroxy and α,ω-diamino-β-hydroxy acids. The enzymatic reactions are not stereospecific regarding the new stereocenter formed at the β-carbon, though they all give L-α-amino acids.

β-Hydroxy-α-amino acids are natural products and constituents of complex organic compounds with interesting biological properties[1,2]. 4-Hydroxy-L-threonine, for example, has been considered to be involved in the biosynthesis of vitamin B6[3] and also is a precursor of rizobitoxine - a potent inhibitor of pyridoxal-dependent enzymes[4]. 3,4,5-Trihydroxy-L-α-aminopentanoic acid is a key component of polyoxins. Our recent work on the development of carbohydrate mimetics using hydroxylated threonines and analogs as synthons prompts us to further exploit the synthetic utility of threonine aldolase.



L-threonine
7

:

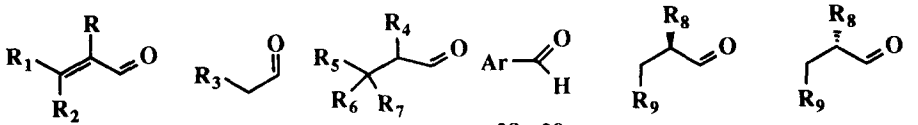
L-*allo*-threonine
93

Scheme 1

L-Threonine aldolases which catalyze the aldol cleavage of threonine to glycine and acetaldehyde in metabolic pathways (Scheme 1) also catalyze the aldol condensation using glycine as donor; however, the enzymes have not been widely used in organic synthesis, since Yamada succeeded in the isolation and crystallization of the enzyme from *Candida humicola* (AKU No 4586)[5,6].

We recently found[7] that the L-threonine aldolase purified from *Candida humicola* (*vide infra*) catalyzes the condensation of glycine and acetaldehyde to form a 93:7 mixture of L-*allo*-threonine and L-threonine. In fact the enzyme produced by *Candida humicola* accepts L-*allo*-threonine as a better substrate in the cleavage reaction; D-threonine and D-*allo*-threonine are not substrates. Preliminary screening indicates that the enzyme accepts a broad range of aldehydes (Fig.1) and the results are summarized in Table 1. Investigation of the stereochemistry of some products (**34-41**, Fig.2) indicates that the reactions are not stereospecific, giving a mixture of *erythro* and *threo* products[8]. To determine the stereochemistry of the newly formed α-amino center, compounds **36** and **40** were subjected to reaction with D- and L-amino acid oxidase [12μM substrate in 1mL of Tris. buffer (10mM, pH 8.5), 5U of amino acid oxidase, 30 °C, 62 h] and it was found that both compounds were substrates for L-amino acid oxidase (more than 90% of the substrates were consumed) but inactive toward D-amino acid oxidase, indicating the aldolase reactions give *S*-stereocenter at the α-position.

Fig.1 Aldehydes tested as substrates for L-threonine aldolase



1 - 7 **8 - 22** **23 - 26** **28 - 29** **30a, 31a, 32a, 33** **30b, 31b, 32b**

1: $R_1 = R_2 = R_3 = H$; **2:** $R_1 = R_3 = H$, $R_2 = C_6H_5$; **3:** $R_1 = R_3 = H$, $R_2 = Me$; **4:** $R_2 = R_3 = Me$, R = H; **5:** $R = R_2 = Me$, $R_1 = H$; **6:** $R = R_2 = H$, $R_1 = CH_3(CH_2)_{12}$; **7:** $R = R_2 = H$, $R_1 = EtOCO$; **8:** $R_3 = H$; **9:** $R_3 = OH$; **10:** $R_3 = Cl$; **11:** $R_3 = N_3$; **12:** $R_3 = C_6H_5SCH_2$; **13:** $R_3 = C_6H_5CH_2$; **14:** $R_3 = cis\text{-}CH_3(CH_2)_3CH{=}CH(CH_2)_8$; **15:** $R_3 = Et$; **16:** $R_3 = Me$; **17:** $R_3 = BnO$; **18:** $R_3 = BnOCH_2$; **19:** $R_3 = BnOCH_2CH_2$; **20:** $R_3 = BnOCH_2CH_2CH_2O$; **21:** $R_3 = PhtN$; **22** $R_3 = PhtNCH_2CH_2O$; **23:** $R_4 = R_5 = H$, $R_6 = Me$, $R_7 = C_6H_5S$; **24:** $R_4 = H$, $R_5 = R_6 = Me$, $R_7 = C_6H_5S$; **25:** $R_4 = R_5 = Me$, $R_6 = C_6H_5S$, $R_7 = H$; **26:** $R_4 = R_5 = H$, $R_6 = CH_3(CH_2)_{12}$, $R_7 = C_6H_5S$; **27:** $R_4 = R_5 = H$, $R_6 = C_6H_5S$, $R_7 = EtOCO$; **28:** $Ar = C_6H_5$; **29:** $Ar = 4\text{-}OH(C_6H_4)$; **30a,b:** $R_8 =$ OH, $R_9 = H$; **31a,b:** $R_8 = R_9 = OH$; **32a,b:** $R_8 = OH$, $R_9 = N_3$; **33:** $R_8 = R_9 = BnO$;

Table 1

Entry No	Substrate class [a]	Entry No	Substrate class	Entry No	Substrate class	Entry No	Substrate class	Entry No	Substrate class
1	+	**8**	+++	**15**	++(+)	**22**	+++(+)	**29**	++(+)
2	+[b]	**9**	+[d]	**16**	+	**23**	+(+)	**30a**	+
3	-[c]	**10**	+[d]	**17**	+++++	**24**	++	**30b**	+
4	-[c]	**11**	+++++	**18**	++++	**25**	(+)	**31a**	-
5	-[c]	**12**	+++++	**19**	++++	**26**	-	**31b**	-
6	-	**13**	++	**20**	++++	**27**	+(+)[d]	**32a or b**	-
7	-[c]	**14**	++(+)	**21**	++(+)	**28**	+++(+)	**33**	+

a-the substrate class was decided from TLC experiments, taken together with the isolated yield for some examples (Fig.2) under the reaction condition described. +++++ more than 75% yield. +++30-45% ++10-30%. +less than 5%. b-chemical reaction also occurs, but the R_f value for the enzymatic product(s) is different. c-chemical reaction only. d-more than one product detected.

The enzymatic reaction with azidoacetaldehyde **11** (Table 1) was achieved in a relatively good yield, 45-75%, to give mainly the *erythro* product[9]. Though hydroxyaldehydes are substrates for the enzyme, they generate a mixture of products which are difficult to isolate. It appears that hydroxyacetaldehydes interact with free amino groups and as a result crosslink proteins[10]. Protection of the secondary hydroxyl group only could not prevent the oligomerization of 2-*O*-benzylglyceraldehyde[11]. Dibenzyloxyprotected aldehyde **33** was, however, a good substrate. The other benzyloxy (or alkoxy) aldehydes (**17-21**) tested were also good substrates for the L-threonine aldolase, opening a new route to 4-hydroxy-L-threonine and 4-hydroxy-*allo*-threonine with suitably protected hydroxyl function at 4-position.

The yields of ω-benzyloxy-β-hydroxy-α-amino acids **36** and **37** are relatively good (78% and 53% respectively). It was observed that when an oxygen is at the β-position of the aldehyde the *erythro*/*threo* ratio is high, *e.g.* 92:8 for **36**. The ratio is reduced to 53:47, when the oxygen is in the γ-position. The β-

oxygen probably is important for achieving a high diastereoselectivity, while the position of the hydrophobic aromatic ring is probably not important. This hypothesis is supported by the diastereomeric ratio of 92:8 for another preparative scale synthesis of **38**. On the other hand **41**, in which the aromatic phtalimidoprotecting group is too far way from the carbonyl function, but its aldehyde precursor contains a β-oxygen, was obtained in a 86:14 diastereomeric mixture.

The reactivity of some substrates (**23-26**) is decreased when near to the carbonyl group is a bulky substituent. With benzaldehyde **28** as a substrate the enzymatic reaction exhibits a low (*erythro/threo* = 40/60) selectivity, similar to the reaction catalyzed by serine hydroxymethyltransferase[1]. When 4-hydroxybenzaldehyde **29** is used as a substrate the ratio is 30:70. Small substituents at α-carbon atom of the aldehydes seem to favour *erythro* configuration, while aromatic aldehydes give mainly the *threo* aldol products[7].

Similar to some other aldolases (RAMA for example)[12], L-threonine aldolase does not accept α,β-unsaturated aldehydes (**3-7**) as substrates. The thiophenol-derived aldehydes (**23-27**) are, however, good substrates, providing a new route to unsaturated amino acids. Another good substrate for the enzyme is 3-phenylthiopropanal **12** which gives product **39** with 80% yield. The phenylthiogroup thus provides an alternative for the synthesis of γ-unsaturated compounds, which may be useful for the synthesis of ceramide derivatives.

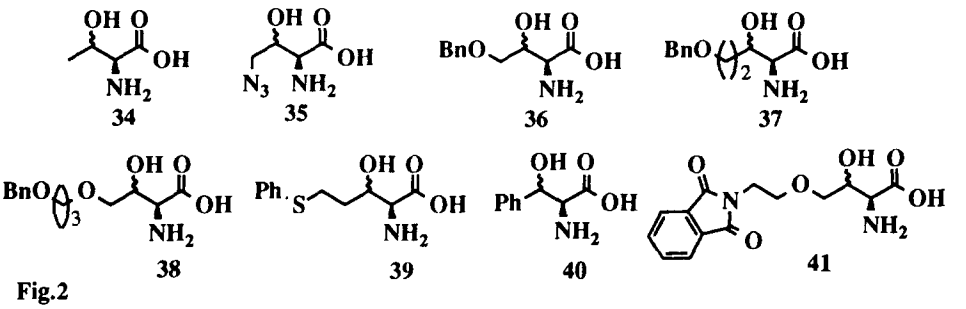


Fig.2

The synthesis of azidohydroxyamino acid **35** demonstrates the utility of the enzyme in producing highly functuionalized molecules. Compound **35** has four carbon atoms with four different functional groups and is a valuable intermediate for the synthesis of azasugar analogs[13]. Synthesis of **36** on gram scales was achieved and the product was recrystalized (ethanol:water = 1:1) and used in the synthesis of sialyl Lewis x mimetic[14].

General Procedure for the Enzymatic Reaction: To the enzyme solution, prepared according to the described procedure[5], containing about 100 units of L-threonine aldolase (based on L-threonine as a substrate) in 15mL of 20mM Tris.HCl, pH 6.3 was added 8.6 mg of pyridoxal-5-phosphate, 400 mg of KCl and glycine (7.5 g). An aldehyde was added (1 mmol in a total volume of 40mL) and the reaction mixture was gently shaken for 16h at 30°C. To the reaction mixture was then added EtOH until the final concentration of EtOH was 75-85% (v/v). After incubation for 4 to 16 h at 4 °C, filtration and repeating (if necessary) precipitation removed the excess of glycine. Further purification of the products could be achieved by flash chromatography, reverse phase or ion exchange procedures.

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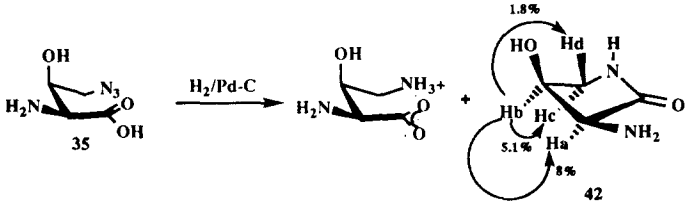
8. Data for **34-41** (Yield, *erythro/threo* ratio - solvent for ^{1}H NMR). **34:** 38%. L-*allo*-threonine : L-threonine - 93:7. The absolute stereochemistry of the producrs was determined by ^{1}H NMR (distinguish the diastereomers) and by measuring the optical rotation (distinguish L from D), compared to authentic samples. **35:** (45-75%, 70/30 to100/0, D_2O, HDO = 4.75 ppm), major isomer 4.16 (ddd, 1H, *J* = 4.3, 4.6 and 6.6 Hz; C<u>H</u>(OH)), 3.79 (d, 1H, *J* = 4.3 Hz; C<u>H</u>(NH_2)), 3.46 (d, 1H, *J* = 6.6 Hz; CH_2), 3.45 (d,1H, *J* = 4.6 Hz; CH_2).$[\alpha]_D$= + 6.96 (c=3.42, CH_3OH/H_2O - 1:1). **36** (78%, 92/8, CD_3OD/D_2O ~ 3/1) major isomer 7.29-7.38 (m, 5H), 4.55 (s, 2H), 4.24-4.29 (dd, 1H *J* = 4 .0 and 4.3 Hz), 3.84 (d, 1H *J* = 4.3 Hz), 3.70 (d, 2H, *J* = 4.0 Hz) $[\alpha]_D$=+20.1 (c=0.88 1N HCl). **37:** (53%, 53/47, CD_3OD/D_2O - 60/40) 7.30-7.37 (m, 5H), 4.53 and 4.54 (two s, 2H, $C_6H_5C\underline{H}_2O$-), 4.21-4.29 (m, 1H -C<u>H</u>(OH)-), 3.73-3.74 d, *J* = 2.7 and 3.53-3.55 d, *J* = 4.3 (1H, -C<u>H</u>(NH_2)-); 3.66-3.70 (m, 2H, -O<u>C</u>H_2<u>C</u>H_2-); 1.76-1-96 (m, 2H -$CH_2C\underline{H}_2$-). **38:** (45%, 92/8, $CDCl_3/CD_3OD$ 2:1) 7.29-7.33 (m, 5H), 4.52 (s, 2H), 4.16-4.18 (m,1H), 3.57-3.74 (m, 7H) 1.87-1.91 (m, 2H). **39:** (80%, 50/50, D_2O, HDO = 4.75ppm, isomeric mixture) 7.24-7.44 (m,5H), 4.20-4.30(m,1H); {3.77 (d, *J* = 3.6 Hz) and 3.53 (d, *J* = 5.0 Hz), 1H}, 3.17 (m,2H), 3.03 (m, 2H). **40:** 87%. 40/60. ^{1}H NMR is in agreement with previously reported[1]. In addition, a comercial DL-*threo*-β-phenylserine hydrate (Aldrich) was used as internal standard for NMR measurements and for the estimation of the yield. **41:** (10%, 86/14, D_2O/CD_3OD - 4:1), 7.75-7.77 (m, 4H), 3.81-3.86 (m, 3H), 3.62-3.64 (m, 4H), *E/T* determined in C_5D_5N/D_2O - 60/40.

9. The stereochemistry of **35** is presumed to be *erythro* by ^{1}H DIFNOE NMR experiment of **42**.



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