# Applications of L-*allo*-threonine aldolase in synthesis of glycoconjugate precursors

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*Frontier Research Program*

Threonine aldolases have been applied to the synthesis on preparative scales of complex aminoacids, which are valuable precursors to azasugars, ceramide derivatives and polyoxins. We report here some applications of L-*allo*-threonine aldolase from *Candida humicola* for the synthesis of precursors to some important glycoconjugate analogs.

Threonine aldolases which naturally generate glycine and acetaldehyde from threonine in metabolic pathways also catalyze aldol condensation using glycine as an enolate source. However, the enzymes are not widely used in organic synthesis since Yamada succeeded in crystallization of the enzyme from *Candida humicola* (ATCC No. 14438).[1] We present here some application of L-*allo*-threonine aldolase from the same source,[2] which was reported[1] as L-threonine aldolase. The enzyme is specific for L-amino acids (Fig. 1), but does not show enantioselectivity when L- and D-hydroxyaldehydes are used in the aldol reaction.[2,3]

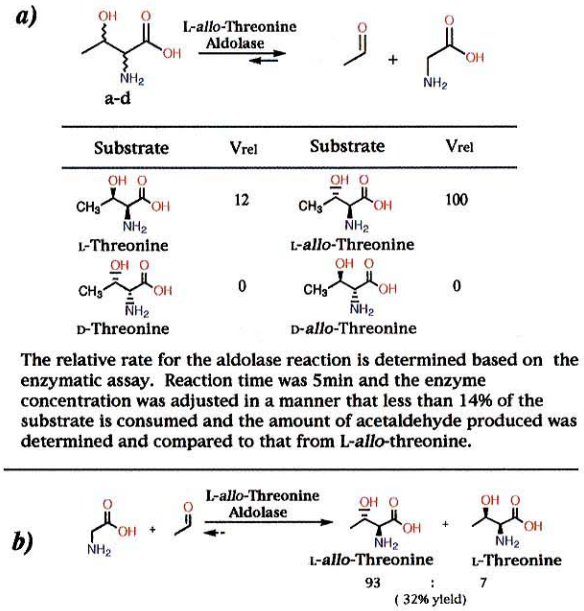


Fig. 1. Relative rate (*a*) and stereoselectivity (*b*) for the aldolase reaction.

L-*allo*-Threonine aldolase requires as a cofactor pyridoxal phosphate (PLP).[4] We recently found that L-threonine aldolase roughly purified from *Candida humicola* (*vide infra*) catalyzes the condensation of glycine and acetaldehyde to afford L-*allo*-threonine. The enzyme produced by *Candida humicola* accepts L-*allo*-threonine as a better substrate. It accepts as substrates only L-hydroxyaminoacids at a rate more than eight times in favour for the *erythro* configuration (Fig. 1, *a*). D-aminoacids are not substrates. These facts explain why the enzyme produces L-*allo*-threonine and *erythro* configuration, when small substituents at $\alpha$-position to the carbonyl group exist.

A successful enzymatic reaction with azidoacetaldehyde **10** (Fig. 2) was achieved with relatively good yields: 45–75%. Attempts to obtain different diastereomeric ratio by changing the ratio of the reactants and adding additional enzyme after several hrs during the reaction, did not give products probably because it is practically impossible to prevent the aldehyde decomposition. The synthesis of azidohydroxyaminoacids demonstrates the capability of L-*allo*-threonine aldolase of producing highly functionalized molecules, which contain four carbon atoms and four different functional groups and are valuable intermediates for azasugar analogs.

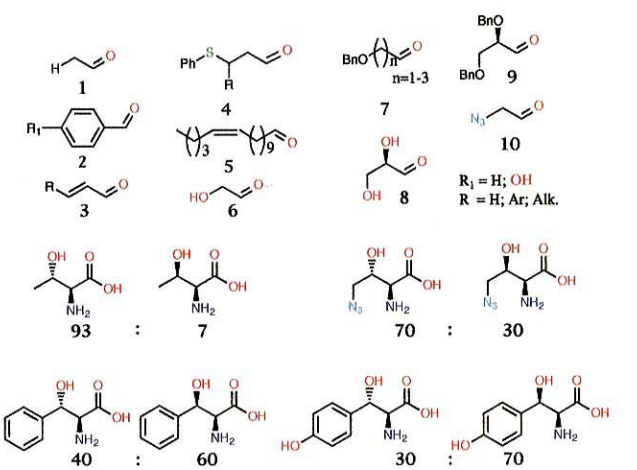


Fig. 2. The structure of aldehydes tested as substrates and the stereochemistry of some products.

Similar to some aldolases [Rabbit Muscle Aldolase (RAMA) for example][5] L-*allo*-threonine aldolase does not accept as substrates $\alpha,\beta$-unsaturated aldehydes. While no condensation product from $\alpha,\beta$-unsaturated aldehydes and glycine was observed, 3-phenylthiopropanal (**4**, R=H) was a good substrate for L-*allo*-threonine aldolase to give a product in 80% yield. The phenylthio group could be easily eliminated to af-

ford $\alpha,\beta$-unsaturated compound. This strategy could provide a new synthetic route for the synthesis of ceramide derivatives.

The reactivity of the substrates decreases in some cases when a bulky group is placed at $\alpha$-position to the carbonyl group.

With benzaldehyde as a substrate L-*allo*-threonine aldolase exhibits low selectivity (poor *threo-erythro* discrimination) and the ratio *threo/erythro* is 60 : 40.

When 4-hydroxybenzaldehyde is used as a substrate the ratio is 70 : 30. Small atoms and functional groups at $\alpha$-carbon of aldehydes (hydrogen, azido group) seem to favor erythro configuration, while aromatic aldehydes give aldol products with *threo* stereochemistry.

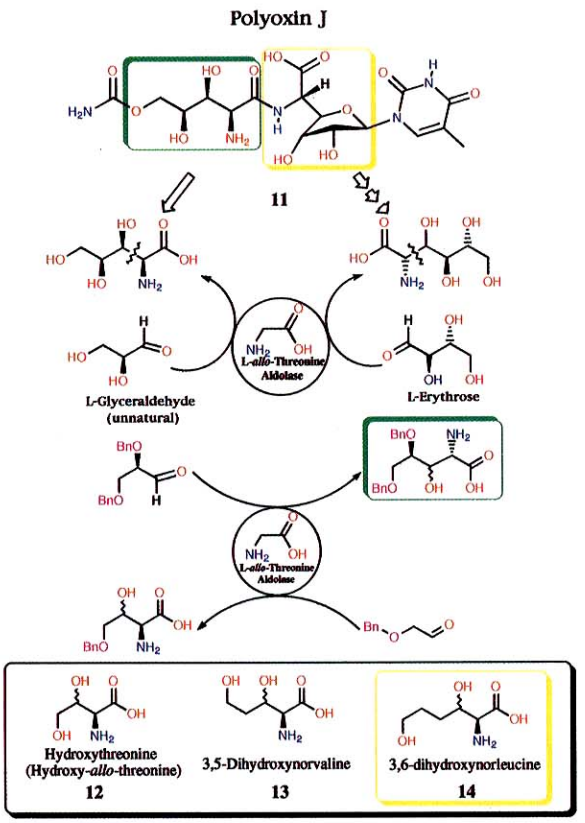


Fig. 3. Possible application of L-*allo*-threonine aldolase for the synthesis of Polyoxin analogs.

When hydroxyacetaldehyde **6** was used the reaction mixture became dark brown and an exhausted purification procedure was necessary. The poor yield could not be overcome by adding an additional quantity of the aldehyde. It appears that the hydroxyacetaldehyde interacts with free aminogroups and as a result to crosslink and denaturate the proteins.[6] After protection of the $\alpha$-hydroxyl group, however, the obtained benzyloxyacetaldehyde became an excellent substrate providing a new route to hydroxythreonine 12, which is a precursor of rizobitoxine - a potent inhibitor of pyridoxal-dependent enzymes.[7]

Probably the same factors are responsible for the negative (for now) results with unprotected glyceraldehyde. When the same protecting group applied to D-glyceraldehyde, the protected aldehyde became a substrate (Fig. 3, the yield not optimized), providing a new way to a component of Polyoxins (Fig. 3, **11**), a family of antibiotics, which act as competitive inhibitors of the enzyme chitin synthase, thereby blocking in the biosynthesis of chitin in cell wall assembly.[8] Using benzyloxyprotected aldehydes as substrates, the enzymatic reactions provide new building blocks for glycopeptidolipids, e.g. dihydroxynorvaline **13** and dihydroxynorleucine **14**.

We gratefully appreciate the financial support from the Ministry of Education, Science, and Culture - Japan and thank Dr. M. Shimizu and H. Yamada for supplying *Candida humicola* (ATCC No. 14438).

## References

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