

## MULTIPLE REACTIONS FOR THE ASYMMETRIC SYNTHESIS OF UNUSUAL AMINO ACIDS

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Key Words: Desymmetric hydrolysis, Asymmetric hydroxylation, Dynamic kinetic resolution.

Unusual amino acids, such as  $\gamma$ -amino butyric acid (GABA) derivatives and hydroxy amino acids, are known to have worthwhile physiological activities on the mammals or are used as chiral building blocks in the chemical synthesis of other compounds. So far, we have been screening microbial enzymes in order to develop novel biocatalysts useful for various kinds of bioproduction process. In the course of the screening, a variety of enzymes were found to catalyze unique reactions acting to amino acids and the related compounds. Therefore, we suitably combined these biocatalysts and chemical reactions to make multiple reaction systems, by which several unusual amino acids were obtained with high steric purity.

Optically active dicarboxylic acid monoamides (chiral half amides) such as (*R*)-3-(4-chlorophenyl)-glutaric acid monoamide [(*R*)-CGM] are easily converted into corresponding chiral 3-substituted GABA derivatives by a coupling of Hofmann rearrangement. Some chiral 3-substituted GABA derivatives are used as important medicinal drugs. Chiral half amides are available via desymmetric hydrolysis of prochiral cyclic imides. For the reactions, stereoselective imide-hydrolyzing enzymes (imidases) are necessary to obtain chiral half amides effectively. Then we searched microbial imidases for desymmetric hydrolyze of several cyclic imides. As the result of the microbial screening, *Burkholderia phytofirmans* DSM17436 was found to have the imidase activity hydrolyzing a cyclic imide, 3-(4-chlorophenyl)glutarimide [CGI], into (*R*)-CGM with 97.5% ee. An imidase of *B. phytofirmans* was purified through a classical column chromatographic separation method. The identified enzyme, BpIH, has similarity with an allantoinase of *Pseudomonas fluorescens* and a 5-benzyl-2,4-thiazolidindione-hydrolyzing enzyme of *Brevibacterium linens* C-1. Consequently, bioconversion of prochiral CGI into (*R*)-CGM was achieved with a yield of 99% and 99% ee with the recombinant *E. coli* cells expressing BpIH.

(2*R*)-Hydroxyamino acids are naturally-occurring but trace constituent amino acids. Their bioactivities are little known because of difficulty in selective preparation of their stereoisomers with multiple chiral centers. So we developed multi-enzymatic reaction systems to obtain enough amount of (2*R*)-hydroxyamino acids to be investigated as to their bioactivity. One of the systems was composed of *N*-succinyl-L-amino acid 3-hydroxylase (SadA) of *Burkholderia ambifaria* AMMD, *N*-succinyl-amino acid racemase (NSAR) of *Chloroflexus aurantiacus*, and *N*-succinyl-D-amino acid desuccinylase (DSA) of *Cupriavidus* sp. P4-10-C. In this system, several aliphatic (2*R*)-3-hydroxyamino acids were formed with high optical activity via successive reaction of asymmetric hydroxylation and dynamic kinetic resolution. Currently, (2*R*,3*R*)-3-hydroxyleucine was successfully produced from *N*-succinyl-L-leucine with a yield of 76% and 88% *de*.

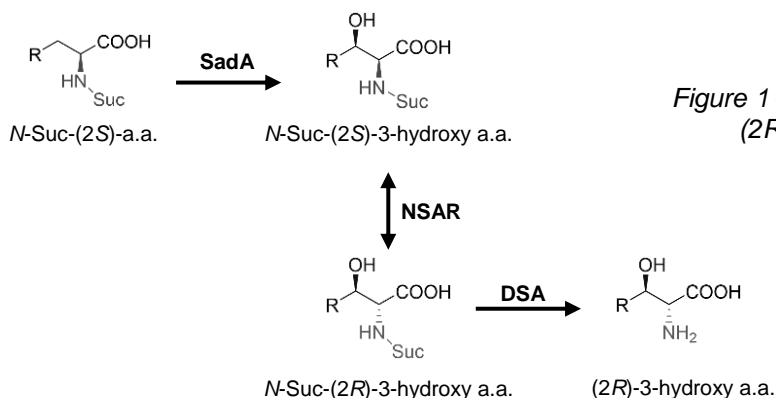


Figure 1 – Multiple reaction to produce (2*R*)-3-hydroxyamino acids