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New enzymes to prepare amino acid precursors to pharmaceuticals and food additives

Jielin Zhang

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“We take a handful of sand from the endless landscape of awareness around us and call that handful of sand the world.”

Robert M. Pirsig

Zen and the Art of Motorcycle Maintenance

Paranimfen Lieuwe Biewenga

Haigen Fu

Table of Contents

Aim and outline of this thesis

L-aspartic acid derivatives are unnatural amino acids with a broad range of applications in neurobiological research and the synthesis of pharma- and nutraceuticals. Although carbon-nitrogen bond-forming C-N lyases are attractive enzymes to prepare such compounds, only two C-N lyases known as aspartate ammonia lyase (aspartase) and 3-methylaspartate ammonia lyase (MAL) have been carefully explored for their usefulness in the synthesis of difficult L-aspartic acid derivatives. Unfortunately, the substrate scope of these enzymes is rather limited, with low or no reactivity for desired non-native substrates. Expanding the toolbox of C-N lyases for amino acid synthesis by enzyme discovery and engineering is thus highly interesting. Another field with great potential is the use of C-N lyases in multienzymatic and chemoenzymatic cascades, allowing the construction of artificial metabolic pathways for the more sustainable and step-economic synthesis of complex amino acid molecules starting from simple and cheap building blocks.

The work described in this thesis aimed to expand the biocatalytic applications of C-N lyases for asymmetric synthesis of important amino acid precursors to biologically active compounds and food additives. For this, the MAL enzyme and a newly identified ethylenediamine-*N,N'*-disuccinic acid lyase **(**EDDS lyase), as well as engineered variants of both these C-N lyases, were investigated for their usefulness in the selective (cascade) synthesis of difficult aminocarboxylic acid products.

Chapter 1 gives a brief overview of the properties of EDDS lyase and MAL, including their biochemical, structural and mechanistic features and biocatalytic applications to prepare unnatural amino acids.

In **Chapter 2**, a chemoenzymatic methodology for asymmetric synthesis of the fungal natural products aspergillomarasmine A (AMA), aspergillomarasmine B (AMB), toxin A and related aminocarboxylic acids is reported. AMA is a potent inhibitor of *metallo*-β-lactamases, with great pharmaceutical potential in battling bacterial resistance to β-lactam antibiotics. This step-economic (chemo)enzymatic route towards AMA, AMB, and related aminocarboxylic acids highlights a highly regio- and stereoselective C-N bond forming step catalyzed by EDDS lyase.

Our knowledge of EDDS lyase was broadened by the identification and structural characterization of EDDS lyase from *Chelativorans* sp. BNC1 (**Chapter 3**). The determined crystal structures of EDDS lyase in unliganded and substrate- and product-bound forms not only support a general base-catalyzed deamination mechanism characteristic for members of the aspartase/fumarase superfamily, but also provide structural basis for future enzyme engineering.

The potential for biocatalytic application of EDDS lyase and MAL-Q73A was further demonstrated by the enantioselective synthesis of *N*-substituted L-aspartic acid derivatives with diverse homo- and heterocycloalkyl substituents (**Chapter 4**). Another example is given in **Chapter 5**, which describes the engineering of an improved EDDS lyase variant for efficient enantioselective production of *N*-(3,3-dimethylbutyl)-L-aspartic acid and *N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-aspartic acid, important precursors to the artificial dipeptide sweeteners neotame and advantame, respectively.

Chapter 6 describes the development of a one-pot three-step enzymatic cascade for stereoselective synthesis of vitamin B_5 [(*R*)-pantothenic acid] and both diastereoisomers of α-methyl-substituted vitamin B_5, which are valuable precursors to promising antimicrobials against *Plasmodium falciparum* and multidrug-resistant *Staphylococcus aureus*, using a C-N lyase (MAL), an appropriate decarboxylase, and pantothenate synthetase enzymes.

Lastly, **Chapter 7** provides a summary of the work presented in this thesis, concluding remarks, and some perspectives for future research.