VIP *Very Important Paper*

Recent Applications of Carbon-Nitrogen Lyases in Asymmetric Synthesis of Noncanonical Amino Acids and Heterocyclic Compounds

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Carbon-nitrogen (C–N) lyases are enzymes that normally catalyze the cleavage of C–N bonds. Reversing this reaction towards carbon-nitrogen bond formation can be a powerful approach to prepare valuable compounds that could find applications in everyday life. This review focuses on recent (last five years) applications of native and engineered C–N lyases, either as stand-alone biocatalysts or as part of multienzymatic and chemoenzymatic cascades, in enantioselective synthesis of noncanonical amino acids and dinitrogen-fused heterocycles, which are useful tools for neurobiological research and important synthetic precursors to pharmaceuticals and food additives.

1. Carbon-Nitrogen Lyases and Biocatalytic Hydroamination of α,β-Unsaturated Carboxylic Acids

Lyases are a class of enzymes that cleave covalent bonds (C–C, C–O, C–N, C–S and others) through elimination reactions, yielding double bonds or ring structures in the resulting products. Carbon-nitrogen (C–N) lyases (EC 4.3.X.X) selectively catalyze C–N bond cleavage.[1] They have been isolated and characterized from different prokaryotic and eukaryotic sources, playing roles in various physiological activities such as nitrogen metabolism, amino acid metabolism, and biosynthesis of natural products.[1–3] Based on the chemical nature of their products, C–N lyases are further divided into four subclasses, which are ammonia-lyases (4.3.1.X), amidine/amide-lyases (4.3.2.X), amine-lyases (4.3.3.X) and others (4.3.99.X), showing rich diversity in structural and mechanistic features. The well-studied biotechnologically relevant C–N lyases such as aspartate ammonia lyase (aspartase), 3-methylaspartate ammonia lyase (MAL), and histidine and phenylalanine ammonia lyase (HAL and PAL) belong to three different protein superfamilies, namely aspartase/fumarase superfamily, enolase superfamily, and 4-methylideneimidazole-5-one (MIO) cofactor dependent enzyme family, respectively. In recent years, several new C–N lyases were identified, like nitrosuccinate lyase[4] and choline trimethylamine-lyase,[5] expanding our knowledge of C–N lyases regarding reactions, structures and catalytic mechanisms.

C–N lyases have shown great potential as biocatalysts for the synthesis of optically pure (un)natural amino acids through asymmetric hydroamination of α,β-unsaturated mono- or dicarboxylic acids (Figure 1).[3,6] C–N lyase-catalyzed hydroamination reactions employ ubiquitous α,β-unsaturated acids as starting materials, require no external cofactors and are atom-economic and highly selective, providing a complementary strategy to form chiral amino acids, lining up with other biocatalytic approaches (Figure 1).[7]

Early in the 1970s, ammonia-lyases, such as aspartase and PAL, were exploited in reverse to synthesize their natural substrates L-aspartic acid and L-phenylalanine starting from fumaric acid and cinnamic acid, respectively.[8] In recent years, C–N lyases with desired biocatalytic profiles (substrate scope, activity, selectivity) have been obtained by discovery or engineering. The rapidly expanding toolbox of C–N lyases enables enzymatic production of a broad range of unnatural amino acids, including substituted aspartic acids, β-substituted α- and β-alanines, with most reactions having no counterparts in organic chemistry (Tables 1 and 2).[6] Besides, multienzymatic and chemoenzymatic cascades containing C–N lyases have been developed, giving access to complex bioactive molecules in a sustainable and step-economic manner. C–N lyases provide an alternative approach to synthesize valuable amino acids and offer more options for biocatalytic retrosynthesis.

L-Aspartic acid derivatives are particularly important molecules because of their diverse biological activities and wide-ranging applications in pharmaceutical and nutraceutical fields

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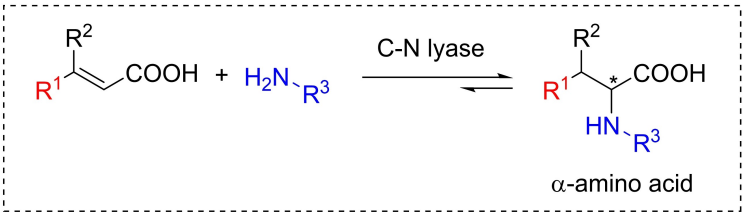
Table 1. Synthesis of optically pure α-amino acids using C–N lyases.



R^1	R^2	R^3	C–N lyase	Products	Ref.
COOH	H	H, Me, OH, OMe, NH_2	AspB	aspartate and its derivatives	[9]
COOH	H, Me, Et, Pr, *i*Pr, Cl	H, Me, Et, OH, OMe, NH_2	CtMAL	aspartate and its derivatives	[10,11]
COOH	H, alkyl, aryl, alkoxy, aryloxy, alkylthio, arylthio	H	CtMAL-L384A	3-substituted aspartates	[12–14]
COOH	H, Me	alkyl	CtMAL-Q73A	N-substituted aspartates	[12,15]
COOH	H	alkyl aryl arylamino	EDDS lyase	N-substituted aspartates	[16–21]
Ph, aryl	H	H	RgPAL, AvPAL, PcPAL	phenylalanine, β-aryl α-alanines	[6,22–26]

(Figure 2). Three types of C–N lyases, aspartase, MAL and ethylenediamine-*N,N*′-disuccinic acid lyase (EDDS lyase), have been used for biocatalytic preparation of L-aspartic acid derivatives. Here we focus on EDDS lyase and MAL, providing a review on their structural and mechanistic characteristics and recent applications in asymmetric synthesis of important biologically active compounds comprising an L-aspartic acid moiety.

2. Ethylenediamine-*N,N*′-Disuccinic Acid (EDDS) Lyase

2.1. Properties, structure and catalytic mechanism

EDDS lyase is an amine-lyase, which naturally catalyzes two successive steps of reversible deamination of (*S,S*)-EDDS to fumaric acid and ethylenediamine via the intermediate *N*-(2-aminoethyl)aspartic acid (AEAA; Figure 3). (*S,S*)-EDDS is a metal-

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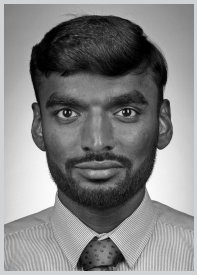
Mohammad Zainal Abidin obtained his M.Sc. in Biotechnology from the University of Gadjah Mada, Indonesia. He recently completed his Ph.D. research at the University of Groningen, The Netherlands, under the supervision of Prof. Dr. Gerrit J. Poelarends. He worked on the asymmetric synthesis of noncanonical amino acids and substituted pantothenic acids by using single-step biotransformations and multienzymatic synthesis. He is currently a lecturer at the University of Gadjah Mada at Yogyakarta, Indonesia.



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Gerrit Poelarends received his Ph.D. from the University of Groningen, The Netherlands, and carried out postdoctoral research at the University of Texas at Austin, USA. He is currently a Professor of Pharmaceutical Biotechnology at the University of Groningen. His research interests include the development of new enzymes and multienzymatic cascades for the production of pharmaceuticals and fine chemicals. This spans the discovery to the exploitation of promiscuous enzyme activities to create biocatalysts for new-to-nature reactions, making use of laboratory evolution strategies to improve the properties of enzymes for industrial applications.

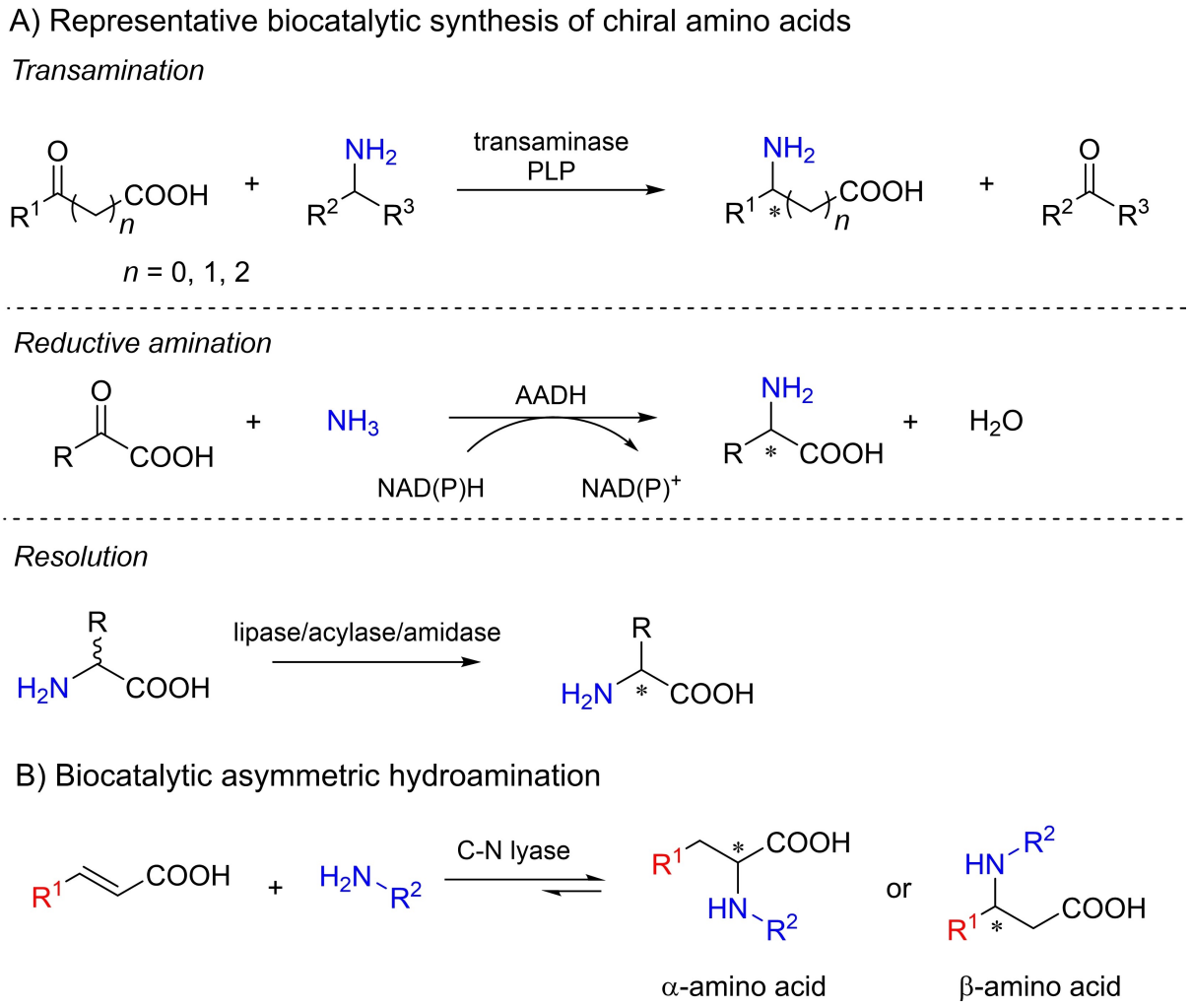
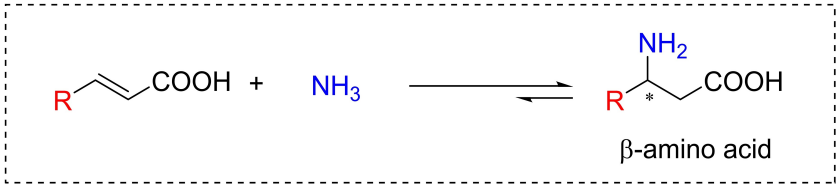


Figure 1. A) Biocatalytic synthetic approaches for chiral (un)natural amino acids; B) C–N lyase-catalyzed synthesis of (un)natural amino acids by asymmetric hydroamination of α,β-unsaturated carboxylic acids.

Table 2. Synthesis of optically pure β-amino acids using C–N lyases.



R	Biocatalyst	Products	Ref.
–Me	AspB variants	(*R*)-β-aminobutanoic acid	[27]
–Et		(*R*)-β-aminopentanoic acid	
$–CONH_2$		(*S*)-β-asparagine	
–Ph		(*S*)-β-phenylalanine	
–Ph, aryl	PAM	β-aryl β-alanines	[6,28]

chelating compound widely used in industry for a wide range of applications, such as soil remediation, paper manufacturing, waste water treatment and so on. (*S,S*)-EDDS was isolated as a secondary metabolite from the actinomycete *Amycolatopsis japonicum*, hypothetically serving as a scavenger of zinc (zincophore) and its bioproduction was strictly repressed by zinc.[29,30] Recently, the biosynthetic gene cluster of (*S,S*)-EDDS was uncovered by using unique bioinformatics approaches by elucidating the zinc-responsive regulation mechanism.[31] The microbial biodegradation of (*S,S*)-EDDS undergoes another set of reactions different from those involved in its biosynthesis. The biodegradation of (*S,S*)-EDDS was first observed in some

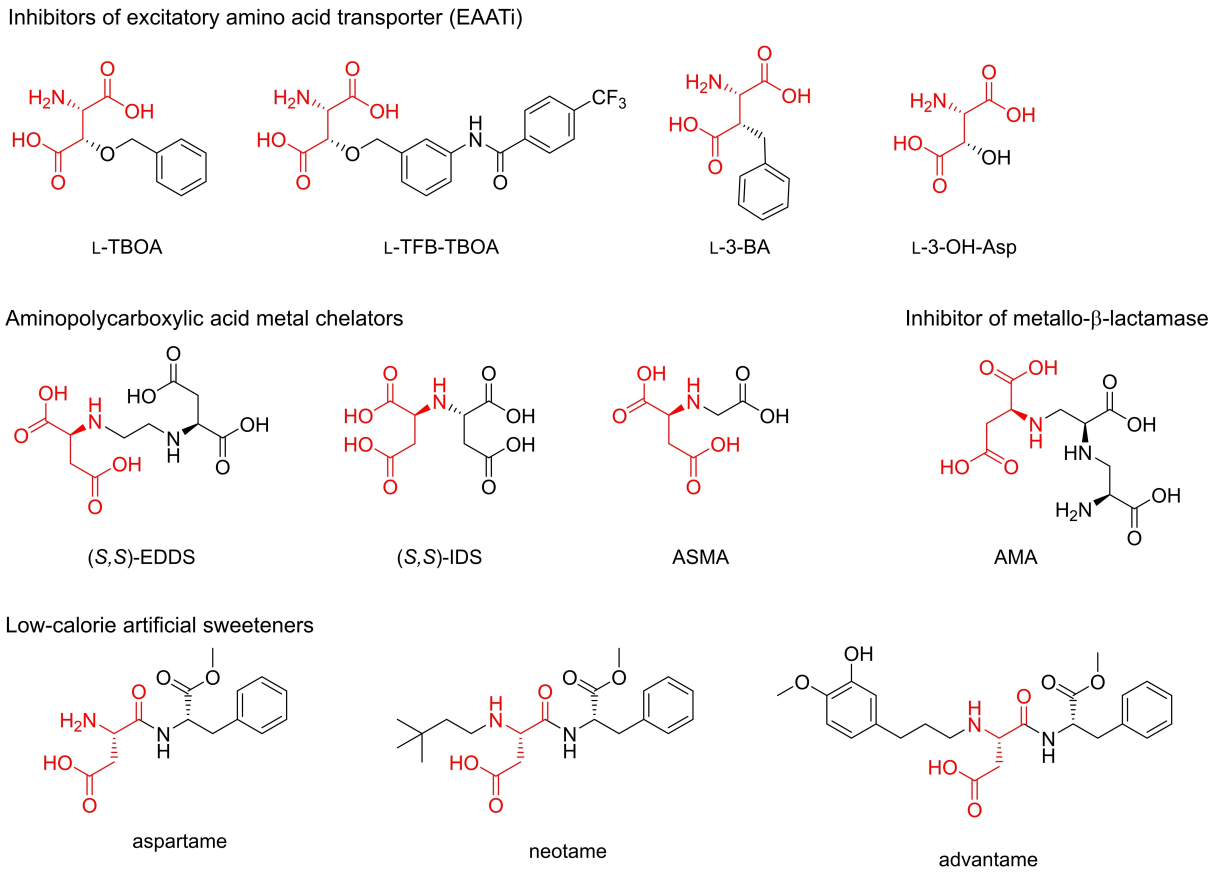


Figure 2. Representative bioactive molecules and natural products containing an L-aspartic acid moiety.

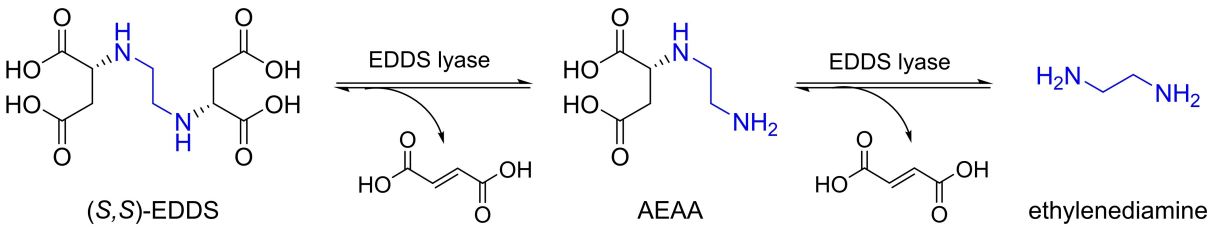


Figure 3. The EDDS-lyase-catalyzed reversible deamination of (*S,S*)-EDDS to fumaric acid and ethylenediamine via AEAA serves as the first step in the bacterial biodegradation of (*S,S*)-EDDS.

microorganisms isolated from soil and sludge from various sources in the late 1990s, revealing a lyase that supposedly initiated the breakdown of (*S,S*)-EDDS to fumarate and AEAA or ethylenediamine through a nonhydrolyzing cleavage reaction.[32–34] These micro-ogranisms were exploited for the production of (*S,S*)-EDDS and related aminopolycarboxylic acids from fumarate and diamines on a gram scale.[34–37] EDDS lyase was first isolated from *Brevundimonas* sp. TN3 in 2001, and was recombinantly expressed and used for (*S,S*)-EDDS production.[38] Yet, the detailed biochemical properties and mechanistic and structural features have remained unexplored since then.

Poelarends and co-workers recently identified an EDDS lyase from *Chelativorans* sp. BNC1 by using a BLAST search with the TN3 EDDS lyase gene as the query (79% identity), and subsequently investigated the structure and catalytic mechanism of an EDDS lyase for the first time.[18] The EDDS lyase from strain BNC1 catalyzes the reversible deamination of (*S,S*)-EDDS with maximum activity at 60 °C and pH 8.0. Except for its natural substrates, it also accepts ammonia and various mono- and diamines in the addition to fumaric acid.

Crystal structures of EDDS lyase in unliganded form and those bound with fumarate, AEAA and (*S,S*)-EDDS were solved by X-ray diffraction analysis.[18] EDDS lyase is a homotetramer (56 kDa per subunit) sharing the characteristic tertiary and quaternary structural features of members of the aspartase/fumarase superfamily.[2] Interestingly, the catalytically essential SS-loop of EDDS lyase shows little movement upon substrate binding, which is different from the flexible SS-loop undergoing

an open/closed conformational change upon substrate binding as observed in most aspartase/fumarase superfamily members. EDDS lyase has a composite active site located at the interface of three subunits, with most residues forming the α- and β-carboxylate binding pockets being highly conserved.[18] The amine binding site is less conserved, and rich in polar and charged side chains making it quite hydrophilic. Residues Asn288 and Asp290 form water-mediated hydrogen-bond interactions with the distal amino group of the bound (*S,S*)-EDDS, appearing to play important roles in binding and positioning of the natural substrate. Ser280 was proposed to serve as the crucial base catalyst, positioned close to the Cβ carbon with a proper orientation for proton abstraction to start the deamination reaction.

Based on the crystal structures and previous mechanistic studies of aspartase/fumarase superfamily members, a general base-catalyzed, sequential two-step deamination mechanism was proposed.[18] The Ser280 oxyanion first abstracts the proton from Cβ of (*S,S*)-EDDS, leading to formation of an enediolate intermediate, which is stabilized by interactions with residues from the β-carboxylate binding pocket (Ser111, Arg112, Ser281). The collapse of the enediolate intermediate leads to cleavage of the C–N bond, releasing AEAA and fumarate. AEAA will re-bind in the active site, allowing another round of deamination to give ethylene diamine and fumarate as final products.

2.2. Synthetic applications

(*S,S*)-EDDS is a widely used metal-chelating compound in industry as well as a pharmaceutically active compound serving as an inhibitor of Zn-dependent enzymes like phospholipase C (PLC)[29] and metallo-β-lactamase.[39] Consequently, EDDS degrading microorganisms and the purified EDDS lyase have been exploited for the production of (*S,S*)-EDDS[37,38] and related metal chelators (e.g., phenylenediamine-,[36] propanediamine-, and cyclohexylenediamine-*N,N′*-disuccinic acid[35]) starting from fumarate and diamines. Suzuki and co-workers first reported the production of (*S,S*)-EDDS at multigram scale by incubating *Acidovorax* sp. TNT149 cells with fumarate and ethylene diamine at optimum pH (7.5) and temperature (35 °C).[37]

Recently, EDDS lyase from *Chelativorans* sp. BNC1 was used for chemoenzymatic synthesis of the natural products aspergillomarasmine A (AMA), aspergillomarasmine B (AMB) and toxin A, as well as related aminocarboxylic acids with excellent regio- and stereoselective control (Figure 4).[16] AMA is a potent inhibitor of metallo-β-lactamases (including the notorious NDM-1 and VIM-2) with a low IC_{50} value at micromolar concentration. It received extensive academic attention yet remained challenging to synthesize with correct 2*S*,2′*S*,2″*S* configuration. Toxin A is the direct biosynthetic precursor to AMA and its analogue AMB. EDDS lyase was incubated with fumaric acid and a series of retrosynthetically designed amine substrates in aqueous solution at pH 8.5–9 and 25 or 37 °C. AMA, AMB, toxin A and their derivatives (Figures 4 and 5) were obtained with excellent stereoselectivity (*ee* > 99%, *de* > 98%) and in moderate to good yields. Based on the biocatalytic synthesis of toxin A, a one-pot two-step chemoenzymatic approach was developed for rapid and efficient synthesis of AMB and its derivatives (Figure 5).[16] Compared to previous chemocatalytic methods, this (chemo) enzymatic approach significantly reduced the total number of synthetic steps for AMA, AMB, and toxin A, showing great potential for efficient practical synthesis of complex amino (poly)carboxylic acids.

In addition, a series of aminocarboxylic acid analogues of (*S,S*)-EDDS was synthesized by the addition of various diamine substrates to fumaric acid catalyzed by EDDS lyase.[21] The enzyme was shown to accept various (substituted) diamines with two to four atoms between the two amino groups, giving good conversions (47–83%) and yielding the corresponding aminocarboxylic acid products (Figure 5) with moderate to good isolated yield. Like AMA, AMB and their derivatives, (*S,S*)-EDDS and several of its analogues demonstrated effective inhibition of the bacterial metallo-β-lactamase NDM-1, displaying a strong correlation between the inhibitory potency of the compounds and their ability to bind zinc.[21]

Moreover, EDDS lyase from *Chelativorans* sp. BNC1 was also employed for enantioselective synthesis of complex *N*-cycloalkyl substituted L-aspartic acids, which are structurally distinct from

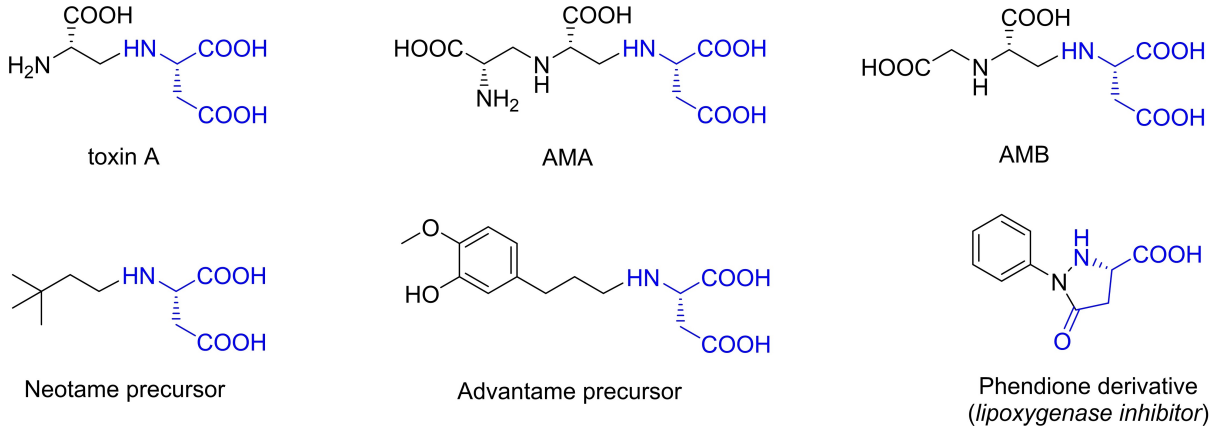


Figure 4. Complex biologically active compounds obtained from enzymatic and chemoenzymatic synthesis procedures involving EDDS lyase and its variants.

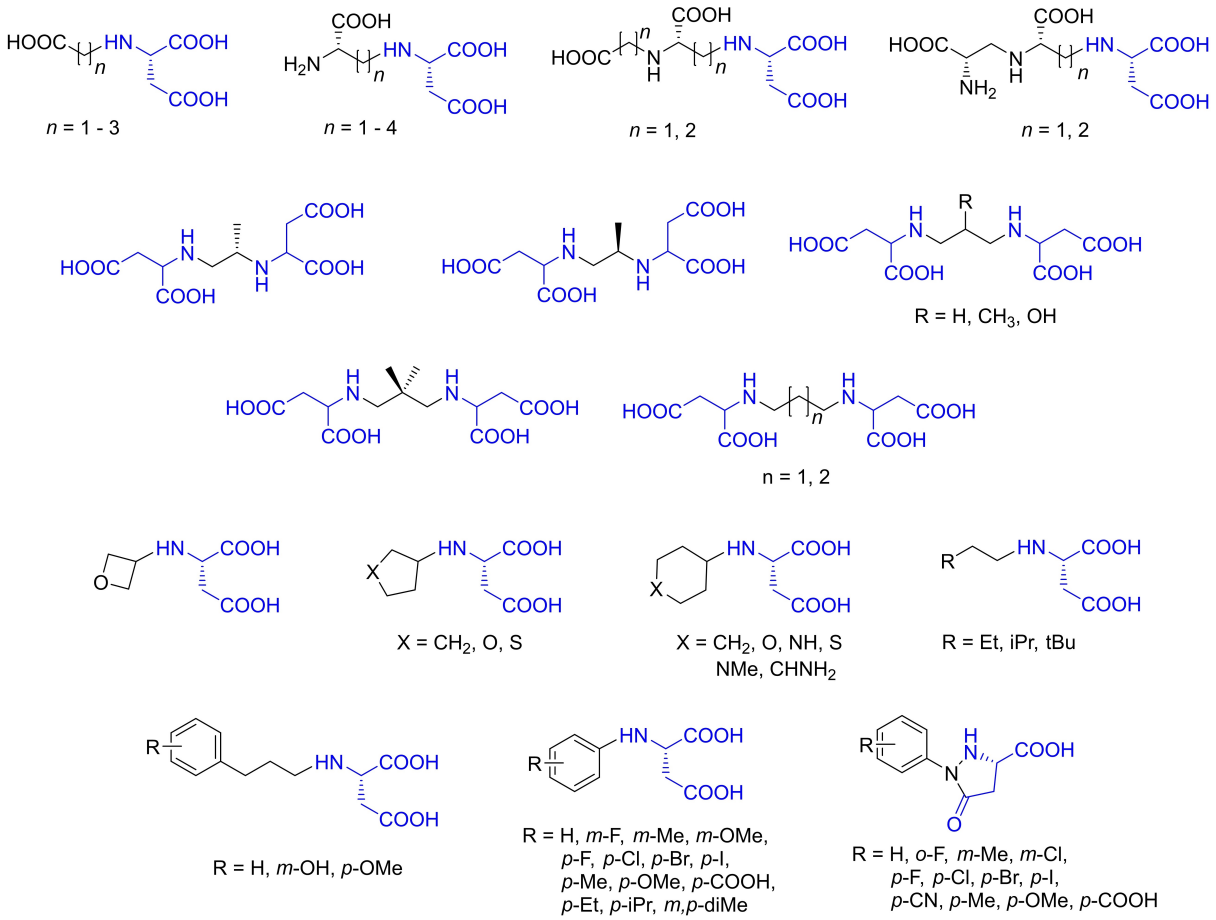


Figure 5. Examples of noncanonical amino acids obtained by preparative (chemo)enzymatic synthesis using EDDS lyase and its variants as biocatalysts.

its natural substrates (Figure 5).[17] Reactions were performed with fumaric acid, various homo- and heterocycloalkyl amines (comprising four-, five- and six-membered rings) and EDDS lyase at pH 8.5 and room temperature. EDDS lyase exhibited broad substrate promiscuity, accepting a variety of cycloalkylamines in hydroamination of fumarate to give the corresponding *N*-cycloalkyl-L-aspartic acids with excellent enantioselectivity (*ee* > 99%).

Another synthetic application of EDDS lyase was demonstrated by the biocatalytic asymmetric synthesis of various *N*-arylated L-aspartic acids and the chemoenzymatic synthesis of challenging 2-aryl-5-carboxylpyrazolidin-3-ones (Figure 5).[19] Especially, pyrazolidinones are widely found as core structures in pharmaceutically active molecules including lipoxygenase inhibitors (Figure 4) and anti-Alzheimer agents.[19] EDDS lyase accepted a broad range of arylamines and arylhydrazines as substrates for addition to fumarate, giving the corresponding *N*-arylated L-aspartic acids and *N*-(arylamino)aspartic acids, respectively, with good isolated yields and excellent enantiopurity. Furthermore, a one-pot chemoenzymatic synthesis route combining the EDDS lyase mediated biotransformation with an acid-catalyzed cyclization step was developed, giving rise to a series of chiral pyrazolidin-3-one derivatives (Figure 5), with 61–70% isolated yield and >99% *ee*.[19] This chemoenzymatic methodology enables an efficient, step-economic alternative process to this important class of drug precursors.

In a recent study, an engineered variant of EDDS lyase was successfully applied as biocatalyst for the efficient asymmetric synthesis of precursors to the important dipeptide sweeteners neotame and advantame.[20] These dipeptides are *N*-substituted derivatives of aspartame (Figure 2), having improved sweetness, that were recently approved as sugar substitutes in a broad range of food products. Although wild-type EDDS lyase can be used for the enantioselective hydroamination of fumarate to produce *N*-(3,3-dimethylbutyl)-L-aspartic acid and *N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-aspartic acid (Figure 4), which are the synthetic precursors to neotame and advantame, respectively (Figure 2), reaction times were very long (7 days, using 0.15 mol% catalyst loading). Therefore, to enhance the hydroamination activity of EDDS lyase, enzyme engineering based on the structure of EDDS lyase in complex with its natural substrate (*S,S*)-EDDS was applied. Two residues (Asp290 and Tyr320) that were presumed to position the amine substrate in the active site were selected for mutagenesis. A highly efficient EDDS lyase variant (D290M/Y320M) was obtained with an 1140-fold improvement in activity over the wild-type enzyme, allowing the selective synthesis of the neotame and advantame precursors, as well as related compounds, with excellent

enantioselectivity (>99% ee) and high conversion, requiring only a few hours reaction time instead of 7 days, while using even lower biocatalyst loadings (0.05 mol%).[20] The development of such an effective biocatalyst opens up new opportunities to develop practical multienzymatic processes for the more sustainable and step-economic synthesis of an important class of food additives.

3. Methylaspartate Ammonia Lyase (MAL)

3.1. Properties, structure and catalytic mechanism

Identified from several facultative anaerobic bacteria, the MAL enzyme forms part of the glutamate catabolic pathway and reversibly deaminates L-*threo*- and L-*erythro*-3-methylaspartate to give mesaconate.[9,40] The two best studied MAL enzymes were isolated from *Clostridium tetanomorphum* (CtMAL) and *Citrobacter amalonaticus* (CaMAL), with the first crystal structures reported in 2002.[41,42] The overall structure of the homodimeric MAL (45 kDa per subunit) resembles that of members of the enolase superfamily, sharing the characteristic TIM barrel fold (eightfold α/β barrel). MAL requires K^+ and Mg^{2+} for optimal activity, and the catalytic mechanism of MAL is proposed to involve general base-catalyzed proton abstraction resulting in the formation of an enolate anion intermediate, which is stabilized by interactions with the active site Mg^{2+} ion and side chains of amino acid residues.[9,41] Based on structural work and mutagenesis studies, residues Lys331 and His194 have been identified as the *S*- and *R*-specific base catalysts, respectively.[43]

3.2. Synthetic applications

Based on its natural substrate promiscuity, CtMAL has been used for the asymmetric synthesis of L-aspartic acid derivatives with small N- and C_β-substitutions.[6,9] The scope of biocatalytic applications was greatly enlarged by structure-guided engineering of CtMAL, which led to an engineered variant (MAL-Q73A) with broad nucleophile scope, accepting linear and cycloalkylamines, and an engineered variant (MAL-L384A) with wide electrophile scope, accepting non-native fumarate derivatives with alkyl, aryl, alkoxy, aryloxy, alkylthio, and arylthio substitutions at the C-2 position.[12] By using these engineered MAL enzymes, a diverse collection of *N*- and C_β-substituted L-aspartic acids (Figure 6) were synthesized by asymmetric hydroaminations with moderate to good stereoselectivity.[12,15] In another study, a variant of CtMAL (MAL-H194A) with strongly enhanced diastereoselectivity, which was obtained by mechanism-based

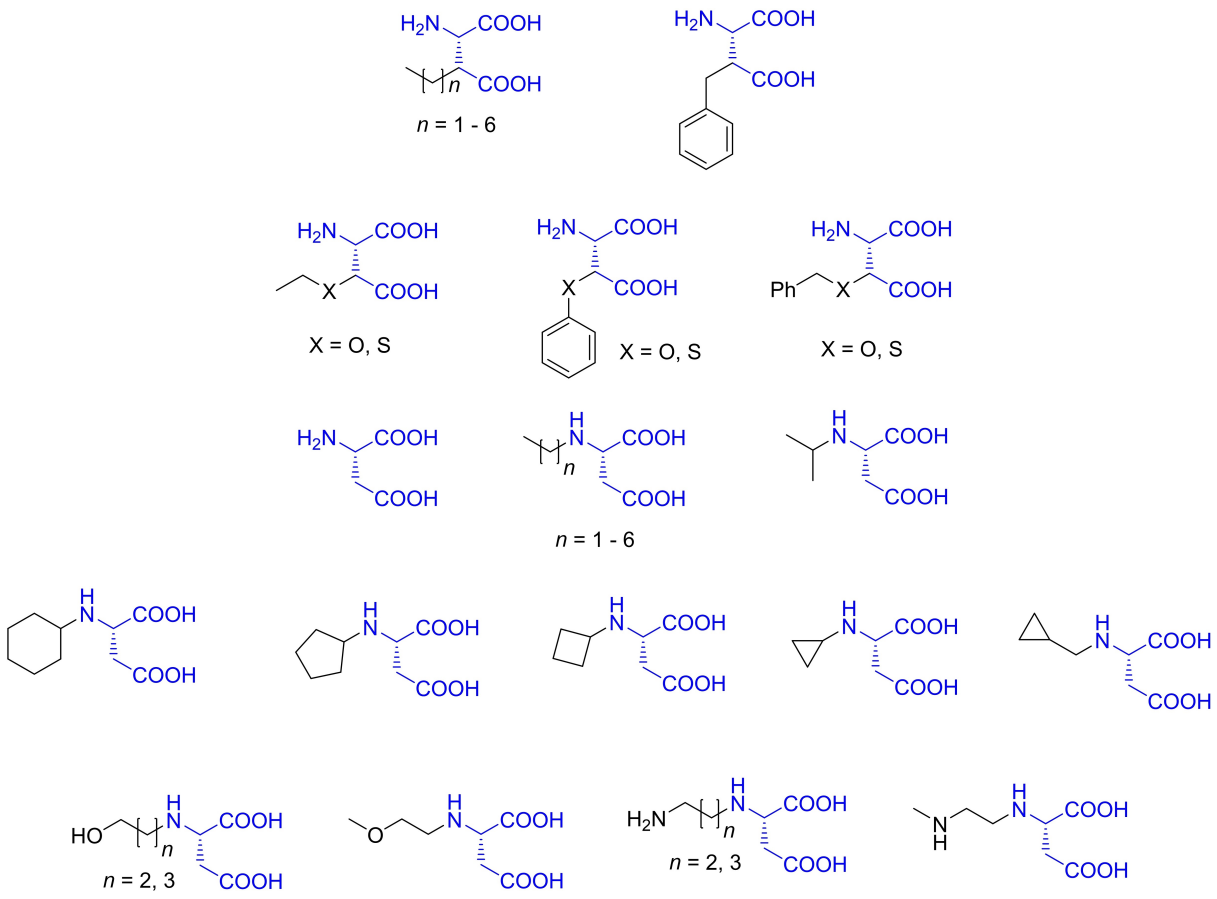


Figure 6. Examples of noncanonical amino acids obtained by preparative (chemo)enzymatic synthesis using MAL and its variants as biocatalysts.

engineering, has been exploited for asymmetric synthesis and kinetic resolution of various 3-substituted aspartic acids.[43,44]

Inspired by the expanded scope towards 2-substituted fumarate derivatives, MAL variants have been exploited in chemoenzymatic synthesis of excitatory amino acid transporter (EAAT) inhibitors that are extremely difficult to synthesize chemically.[13,14,45] Located in the membranes of mammalian neurons and surrounding glial cells, EAATs are responsible for regulating the concentration of the excitatory neurotransmitter glutamate in the synaptic cleft. Inhibitors of EAATs are therefore useful tools to study the precise functions of these transporters in glutamatergic neurotransmission and in neurological disorders related to extracellular glutamate accumulation. For example, L-*threo*-3-benzyloxyaspartate (L-TBOA, Figures 2 and 7) is a widely used nonselective inhibitor of EAATs, the chemical synthesis of which is highly challenging (11 steps in total with an overall yield of $<1\%$). By using an engineered variant of MAL as biocatalyst, de Villiers and co-workers developed an elegant three-step chemoenzymatic synthesis route to L-TBOA and several ring-substituted derivatives.[13] 2-Benzyloxyfumarate and a series of derivatives with F, CH_3 and CF_3 groups at the *ortho*, *meta* and *para* position of the aromatic ring, which were obtained by two-step chemical synthesis from dimethyl acetylenedicarboxylate, were incubated with ammonia in the presence of the MAL-L384A or MAL-L384G variant at room temperature and pH 9.0. Reactions achieved $>89\%$ conversion within 24 h, with products L-TBOA and seven analogues purified with good yield (57–78%) and identified as the desired L-*threo* isomers with excellent stereoselectivity (*ee* $>99\%$; *de* $>95\%$; Figure 7).

The synthetic usefulness of MAL variants for preparation of EAAT blockers was further extended by synthesizing a series of L-TBOA derivatives whose pharmacological properties were evaluated.[14] Catalyzed by MAL-L384A, aspartate derivatives with (cyclo)alkyloxy and (hetero)aryloxy substituents at the C-3 position were prepared by hydroamination of the corresponding fumarate derivatives (prepared from dimethyl acetylenedicarboxylate) with excellent stereoselectivity (Figure 7). These derivatives showed potent inhibitory activities towards EAAT1-4 subtypes with IC_{50} values ranging from micro- to nanomolar concentrations.[14]

Based on the synthesis of L-TBOA, the chemoenzymatic preparation of another potent and widely used nontransportable EAAT inhibitor, (L-*threo*)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]aspartate (L-TFB-TBOA, Figure 7) was achieved with excellent stereochemical control and a dramatically reduced number of synthetic steps.[45] L-TBOA was first synthesized by the MAL-L384A-catalyzed hydroamination of 2-benzyloxyfumarate, and subsequently used as starting material for debenzylation and protection (three steps), yielding the key intermediate dimethyl (L-*threo*)-*N*-Boc-3-hydroxyaspartate (71–73% yield). This chiral intermediate was subsequently subjected to O-alkylation and global deprotection, which gave rise to L-TFB-TBOA at multigram scale with 6% overall yield in nine steps (in comparison, chemical synthesis takes 20 steps). This method provides a convenient strategy to produce L-aspartic acid derivatives with large aryloxy substituents at the C3 position (Figure 7), enabling stereoselective preparation of four L-TFB-TBOA derivatives with strong inhibitory activities for EAAT1-4 subtypes (IC_{50} values range from 5 to 530 nM).[14] This strategy was later exploited for the design and preparation of novel photo-controlled glutamate transporter inhibitors by functionalization of L-TBOA with a photoswitchable azobenzene moiety (azo-TBOAs).[46] Remarkably, (L-*threo*)-*trans*-3-(3-((4-(methoxy)phenyl)diazenyl)benzyloxy) aspartate (*p*-MeO-azo-TBOA) showed good photochemical properties, reversibly switched from the *trans* to *cis* isomer in response to irradiation, with the

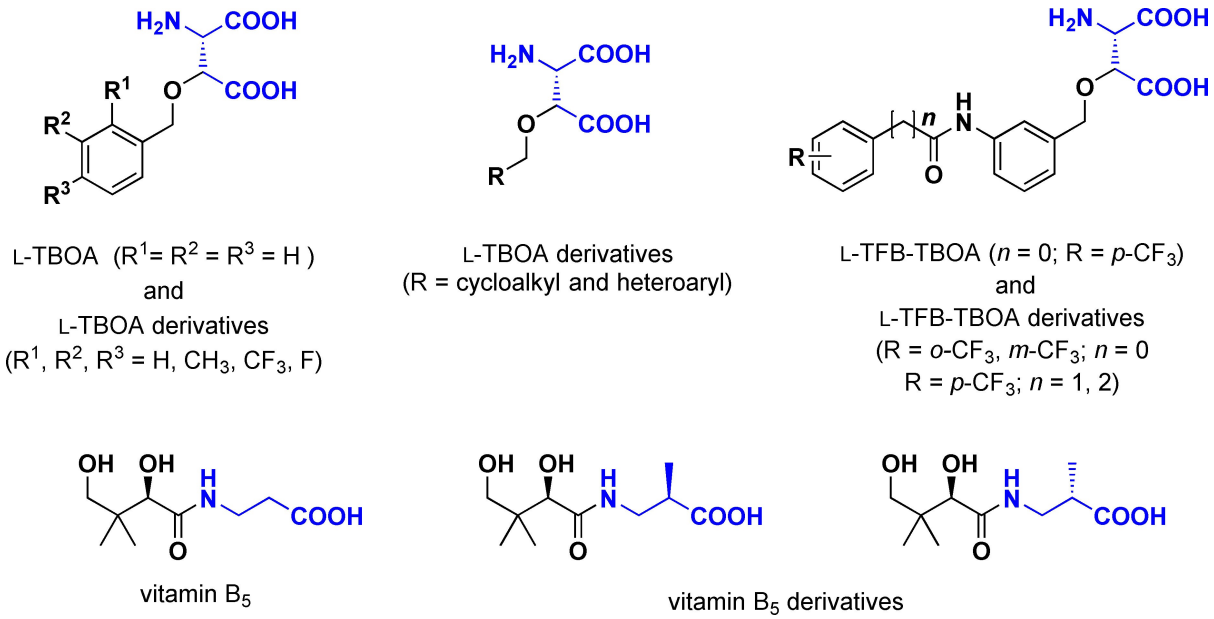


Figure 7. Complex biologically active compounds obtained from multienzymatic and chemoenzymatic synthesis procedures involving MAL and its variants.

isomers showing a 3.6-fold difference in inhibitory activity towards the prokaryotic transporter Glt_{Tk}.[46]

Moreover, MAL and its H194 A variant were used to form linear cascades of three different enzymes in one pot to build an *in vitro* artificial pantothenate biosynthesis pathway for the production of vitamin B_5 [(*R*)-pantothenic acid] and its derivatives (Figure 7).[47] Vitamin B_5 is the biosynthetic precursor of coenzyme A, and its derivatives serve as important synthetic precursors to antimicrobial pantothenamides. A one-pot, three-step enzymatic cascade was developed, consisting of asymmetric hydroamination (catalyzed by MAL or MAL-H194A), α-decarboxylation (catalyzed by an appropriate decarboxylase) and condensation (catalyzed by pantothenate synthase) reactions. Starting from achiral fumarate or mesaconate, ammonia and (*R*)-pantoate, vitamin B_5 and both diastereoisomers of α-methyl-substituted vitamin B_5 (Figure 7) were produced in good isolated yield and with excellent stereoselectivity (> 99 % *ee*).

4. Concluding Remarks

Asymmetric hydroamination of α,β-unsaturated carboxylic acids by C–N lyases is an attractive biocatalytic strategy to synthesize optically pure amino acids. These enzymes have been applied in single-step biotransformations as well as in multienzymatic and chemoenzymatic cascade syntheses of complex molecules with biological activity. Particularly, MAL and EDDS lyase are promising biocatalysts because they display high activity, excellent regio- and stereoselectivity, and good evolvability, providing great potential for practical synthesis of various noncanonical amino acids and (di)nitrogen-fused heterocycles (Figures 4–7), which are useful tools for neurobiological research and important synthetic precursors to pharmaceuticals and food additives. Even though C–N lyases are promising industrial biocatalysts, more development is required to expand the reaction scope, allowing the connection of a wider variety of structurally diverse building blocks through C–N links, and for robustness and scalability.

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Conflict of Interest

The authors declare no conflict of interest.

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