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Biocatalytic Asymmetric Synthesis of N-Aryl-functionalized Amino Acids and Substituted Pyrazolidinones

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ABSTRACT

N-arylated α -amino acids and pyrazolidin-3-ones are widely being used as chiral building blocks for pharmaceuticals and agrochemicals. Here we report a biocatalytic route for the asymmetric synthesis of various N-arylated aspartic acids applying ethylenediamine-N,Ndisuccinic acid lyase (EDDS lyase) as biocatalyst. This enzyme shows a broad substrate scope, enabling the addition of a variety of arylamines to fumarate with high conversions, yielding the corresponding N-arylated aspartic acids in good isolated yields and with high enantiomeric excess (ee >99%). Furthermore, we developed a chemoenzymatic method towards synthetically challenging chiral 2-aryl-5-carboxylpyrazolidin-3-ones, using arylhydrazines as bisnucleophilic donors in the EDDS lyase-catalyzed hydroamination of fumarate followed by an acid-catalyzed intramolecular amidation, achieving good overall yields and high optical purity (ee >99%). In addition, we successfully combined the EDDS lyase-catalyzed hydroamination and acid-catalyzed cyclization steps in one pot, thus providing a simple chemoenzymatic cascade route for synthesis of enantiomerically pure pyrazolidin-3-ones. Hence, these biocatalytic methods provide convenient alternative routes to important chiral Narylated aspartic acids and difficult 2-aryl-5-carboxylpyrazolidin-3-ones.

KEYWORDS

Asymmetric synthesis, biocatalysis, EDDS lyase, unnatural amino acids, pyrazolidinones, cascade synthesis.

INTRODUCTION

Optically pure functionalized α -amino acids are highly valuable as tools for biological research and as chiral building blocks for pharmaceuticals, nutraceuticals, and agrochemicals.¹⁻³ In particular, N-arylated α -amino acids are part of the core structures of a number of medicinally important agents, such as fibrinogen receptor antagonist Lotrafiban (1, Figure 1a)⁴ and protein kinase C activator Indolactam-V (2, Figure 1a).^{5,6} Despite their broad applications, the direct synthesis of chiral N-arylated α -amino acids remains a challenge. Current chemical strategies for the synthesis of enantioenriched N-arylated α -amino acids and their esters are mainly based on extending the existing free amino group of the C α -stereocentre through Cu-catalyzed Ullmann-type coupling reactions⁶⁻⁹, Pd-catalyzed N-arylation¹⁰⁻¹³, and hypervalent iodine chemistry (Figure 1a).¹⁴ However, they are limited by their poor atom economy, the use of heavy metals, and harsh reaction conditions that may result in partial or complete racemization of the α -stereocentre. Biocatalysis provides a valuable alternative route to chiral unnatural amino acids.^{15–18} Previously reported enzymatic asymmetric synthesis of N-alkylfunctionalized α -amino acids were primarily based on two types of carbon-nitrogen bondforming reactions: (i) conjugate addition of amines to the double bond of α , β -unsaturated acids catalyzed by various types of carbon-nitrogen lyases, including aspartate ammonia lyases (aspartases)^{19,20}, methylaspartate ammonia lyases (MALs)^{21,22}, and the recently reported ethylenediamine-N,N-disuccinic acid lyase (EDDS lyase)^{23,24}; and (ii) reductive amination of α -keto acids with amines catalyzed by a number of oxidoreductases, such as reductive aminase (RedAm)²⁵, opine dehydrogenases (OpDHs)^{26,27}, *N*-methyl-amino acid dehydrogenases $(NMAADHs)^{28,29}$, ketimine reductases $(KIREDs)^{29,30}$, and Δ^1 -pyrroline-5-carboxylate

reductases (P5CRs)^{29,31}. However, to the best of our knowledge, no enzymatic route has been reported for the synthesis of *N*-aryl-functionalized α -amino acids. Thus, the development of an efficient and sustainable biocatalytic methodology to enantiomerically pure *N*-arylated α -amino acid derivatives would be particularly desirable.

Pyrazolidin-3-ones and related five-membered dinitrogen-fused heterocycles are widely found as core framework in dyes, agrochemicals, and pharmaceutically active molecules, such as the very first synthetic analgesic and antipyretic drug Phenazone (**3**, Figure 1b)³², lipoxygenase inhibitor Phendione (**4**, Figure 1b)³³, and anti-Alzheimer agents (**5**, Figure 1b).³⁴ In addition, chiral pyrazolidine-3-ones can also function as efficient catalysts in promoting Diels-Alder reactions³⁵, and catalyzing kinetic resolution of secondary alcohols and axially chiral biaryl compounds.³⁶ Due to their broad application in drug development, as well as in synthetic methodologies, several chemical methods have been developed for the synthesis of enantiomerically pure pyrazolidinones and related heterocycles, including chemical^{35,37} or kinetic resolution³⁸, 1,3-dipolar cycloaddition³⁹⁻⁴¹, Lewis acid catalyzed conjugate addition⁴², and Pd-catalyzed asymmetric hydrogenation.⁴³ However, creating a biocatalytic methodology as alternative route to chiral pyrazolidinones is an as yet unmet challenge.

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Figure 1. Synthetic strategies for preparation of noncanonical amino acids that are part of the core structures of biologically active compounds. (**a**) Biologically active molecules containing an *N*-arylated amino acid (left panel), and previous synthetic strategies and our biocatalytic strategy towards *N*-arylated aspartic acids (right panel). (**b**) Biologically active molecules containing pyrazolidine-3-one (left panel), and established synthetic strategies and our chemoenzymatic route towards pyrazolidine-3-ones (right panel).

The enzyme ethylenediamine-N,N-disuccinic acid lyase (EDDS lyase), from *Chelativorans sp.* BNC1, naturally catalyzes a reversible two-step sequential addition of ethylenediamine to two molecules of fumarate providing (*S*, *S*)-EDDS as final product (Figure 2a).⁴⁴ We recently demonstrated that EDDS lyase could accept a wide variety of amino acids with terminal amino groups for regio- and stereoselective addition to fumarate, providing the natural product aspergillomarasmine A and various related aminocarboxylic acids (Figure 2b).²³ In addition, EDDS lyase could also accept a number of (hetero)cycloalkyl-substituted amines, allowing the asymmetric synthesis of (*S*)-*N*-cycloalkyl-substituted aspartic acids (Figure 2b).²⁴ Therefore, the remarkably broad nucleophile scope of EDDS lyase prompted us to further explore the less nucleophilic arylamines as novel non-natural substrates in the asymmetric hydroamination of fumarate, which would enable the production of chiral *N*-arylated aspartic acids as the corresponding enzymatic products (Figure 2c). Moreover, we envisioned that chiral

pyrazolidine-3-ones could be constructed by using arylhydrazines as bisnucleophilic donors in the EDDS lyase-catalyzed regio- and stereoselective addition to fumarate followed by a simple intramolecular amidation (Figure 2c).

Herein, we report a biocatalytic methodology for the synthesis of optically pure (*S*)-*N*-arylated aspartic acids in high conversions and isolated yields. Moreover, an efficient one-pot two-step chemoenzymatic route towards chiral pyrazolidine-3-ones has been developed. These strategies highlight a highly regio- and stereoselective hydroamination step catalyzed by EDDS lyase, offering alternative synthetic choices to prepare chiral *N*-arylated α -amino acids as well as chiral pyrazolidine-3-ones.



Figure 2. Overview of reactions catalyzed by EDDS lyase. (a) Natural reaction catalyzed by EDDS lyase. (b) EDDS lyase-catalyzed asymmetric addition of amino acids and (hetero)cycloalkyl-substituted amines to fumaric acid to yield complex aminocarboxylic acids and *N*-cycloalkyl-substituted aspartic acids, respectively. (c) Exploring the arylamines and arylhydrazines scope of EDDS lyase for the (chemo)enzymatic asymmetric synthesis of *N*-arylated aspartic acids and pyrazolidinones, respectively.

RESULTS

Biocatalytic synthesis of N-arylated aspartic acids

In contrast to aliphatic amines, aromatic amines are challenging substrates for biocatalytic addition reactions due to their relatively low nucleophilicity. Our previous study demonstrated that EDDS lyase could accept glycine as an unnatural substrate, facilitating the low nucleophilic α -amino group of glycine to function as the nucleophile in the amination of fumarate (6).²³ This prompted us to start our investigation by testing aniline (7a, Table 1, entry 1) as potential substrate in the EDDS lyase-catalyzed biotransformation. Remarkably, aniline (7a) was efficiently converted by purified EDDS lyase to afford *N*-phenyl-substituted aspartic acid (8a), with high conversion (91%) and good isolated yield (80%) using only 0.05 mol% biocatalyst loading under optimized conditions (Table 1). To determine the stereochemistry of enzymatic product 8a, HPLC analysis on a chiral stationary phase was conducted by using chemically prepared authentic standards with known (*R/S*)- and (*S*)-configuration (Figure S89). This analysis revealed that product 8a was present as a single (*S*)-configured enantiomer with excellent enantiomeric excess (*ee* >99%, Table 1, entry 1).

Next, the substrate scope was investigated by examining a panel of electronically diverse substituted anilines and heteroarylamines as unnatural substrates in the EDDS lyase-catalyzed amination of fumarate, as monitored by ¹H NMR spectroscopy (Table S1). We were pleased to find that EDDS lyase displayed a broad arylamine substrate scope which was, as expected, affected by the electron-withdrawing/donating nature, position and bulkiness of the substituents on the aromatic ring (Table 1 and Table S1). Clearly, substitution of the aromatic ring at the *ortho*-position was not tolerated by the enzyme, for which only *o*-fluoroaniline (**7b**) gave a very

low conversion (6%, Table 1 and Table S1). Impressively, arylamines with *meta*-substituents, including *m*-fluoroaniline (7c), *m*-toluidine (7d) and *m*-methoxyaniline (7e), were efficiently accepted by EDDS lyase providing the respective products 8c-e (Table 1, entry 3-5). High conversions (87-97%), good isolated product yields (53-84%), and excellent stereoselectivity (ee >99%) were observed (Table 1, entry 3-5). Similarly, para-substituted arylamines, such as *p*-fluoroaniline (7f), *p*-toluidine (7g), *p*-methoxyaniline (7h), *p*-ethylaniline (7i), *m*,*p*dimethylaniline (7i), and *p*-carboxylaniline (7k), were also well accepted by the enzyme, giving high to excellent conversions (75-95%) and yielding the corresponding amino acids 8f-k (34-85% isolated yields) as the (S)-configured enantiomers with >99% ee (Table 1, entry 6-11). Noteworthy, para-halogenated anilines (71-n) were also processed to deliver chiral (S)-Nhaloarylaspartic acids (81-n) with 82-96% conversions and 52-69% isolated yields (ee >99% in all cases, Table 1, entry 12-14), leaving the halogens available for potential downstream synthetic manipulation. The larger nucleophile *p*-isopropylaniline (70) was a poor substrate for EDDS lyase, resulting in low conversion (17%, Table 1, entry 15). Arylamines bearing a strong electron-withdrawing group (such as *p*-nitro or *p*-CF₃) or electron-deficient heteroarylamines (such as pyridine-2-amine, pyridine-4-amine, and thiazol-2-amine) were not accommodated as substrates by EDDS lyase, most likely due to their diminished nucleophilicity (Table S1).

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Entry	Arvlamine	Product	R	Time	Conv. ^b	ee^d	
				[h]	(yield ^c) [%]	[%]	
1	7a	8 a	Н	48	91 (80)	>99 (<i>S</i>) ^f	
2	7b	8b	o-F	72	6 (n.d. ^{<i>e</i>})		
3	7c	8c	<i>m</i> -F	48	91 (53)	>99 (S) ^g	
4	7d	8d	<i>m</i> -Me	24	97 (84)	>99 (<i>S</i>) ^g	
5	7e	8e	<i>m</i> -OMe	48	87 (53)	>99 (<i>S</i>) ^g	
6	7f	8f	<i>p</i> -F	24	92 (85)	>99 (<i>S</i>) ^f	
7	7g	8g	<i>p</i> -Me	48	75 (57)	>99 (<i>S</i>) ^f	
8	7h	8h	<i>p</i> -OMe	48	92 (75)	>99 (<i>S</i>) ^f	
9	7i	8i	<i>p</i> -Et	48	76 (53)	>99 (<i>S</i>) ^g	
10	7j	8j	<i>m,p</i> -diMe	72	90 (70)	>99 (<i>S</i>) ^g	
11	7k	8k	<i>p</i> -СО ₂ Н	24	95 (34)	>99 (<i>S</i>) ^g	
12	71	81	<i>p</i> -Cl	48	96 (63)	>99 (S) ^g	
13	7m	8m	<i>p</i> -Br	48	95 (69)	>99 (<i>S</i>) ^f	
14	7n	8n	p-I	48	82 (52)	>99 (<i>S</i>) ^g	
15	70	80	<i>p</i> -iPr	72	17 (n.d. ^e)		

Table 1. Enzymatic synthesis of (*S*)-*N*-arylated aspartic acids.

^aConditions and reagents: the reaction mixture (15 mL) consisted of fumaric acid (**6**, 50 mM), arylamine substrates **7a-o** (10 mM) and purified EDDS lyase (0.05 mol% based on arylamine) in buffer (50 mM NaH₂PO₄/NaOH, pH 8.5), with 5% DMSO as cosolvent at room temperature. A 5-fold excess of **6** (rather than an excess of amine) was used to drive amine **7** to completion, simplifying product purification and preventing enzyme inhibition by a high concentration of amine substrate. ^bConversions were determined by comparing ¹H NMR signals of substrates and corresponding products. ^cIsolated yield after cation-exchange chromatography. ^dEnantiomeric excess (*ee*) was determined by HPLC on a chiral stationary phase using racemic standards. ^eNot determined owing to low conversion, the product formation was confirmed by comparison of ¹H NMR data of a crude reaction mixture to that of chemically prepared reference compound. ^fThe absolute configurations of **8a**, **8f-h** and **8m** were determined by chiral HPLC using chemically synthesized authentic standards with known (*R/S*) and (*S*) configuration respectively. ^gThe absolute configurations of **8c-e**, **8i-l** and **8n** were tentatively assigned the (*S*)-configuration

based on analogy and according to chiral HPLC data.

Chemoenzymatic synthesis of chiral pyrazolidine-3-ones

Enantioselective conjugate addition of bisnucleophilic donors (such as hydrazines) to electronpoor acceptors provides convenient access to valuable small-ring heterocycles with potential pharmaceutical applications.^{32-34,42} Encouraged by the exquisite stereoselectivity of the EDDSlyase-catalyzed biotransformation accepting a broad range of arylamines (7, Table 1), we further questioned whether bisnucleophilic arylhydrazines (9, Table 2) could be processed as substrates by this enzyme in the amination of fumarate (6). The corresponding enzymatic products, N-(arylamino)aspartic acids (10), are not only valuable scaffolds in their own right, but they also could serve as chiral precursors for the preparation of synthetically challenging chiral pyrazolidine-3-ones (11) through an acid-catalyzed cyclization reaction (Table 2). Remarkably, phenylhydrazine (9a), as the first chosen potential bisnucleophilic substrate, was efficiently converted by EDDS lyase (0.1 mol%) to afford the single product N-(phenylamino)aspartic acid (10a), as ascertained by ¹H NMR in comparison with a chemically prepared authentic standard. In the enzymatic semipreparative synthesis (0.20 mmol scale) of compound 10a, excellent conversion (94%) and good isolated yield (80%, 36 mg) were achieved (Table 2, entry 1). Note that it is necessary to perform the enzymatic reaction under anaerobic conditions, otherwise the substrate phenylhydrazine could be oxidized by molecular oxygen and thus lead to diminished conversion. Subsequently, the enzymatic product 10a was cyclized smoothly under optimized conditions (1 M HCl, reflux for 3 h)³⁷, affording the desired heterocycle 2-phenyl-5-carboxylpyrazolidin-3-one (11a, 71% isolated yield) without racemization of the potentially sensitive C α stereogenic center (*ee* >99%, Table 2, entry 1). The

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chemoenzymatically prepared heterocycle **11a** was identified as the (*S*)-configured enantiomer by chiral HPLC analysis (Figure S102).

To further illustrate the synthetic usefulness of this chemoenzymatic method, we first determined that EDDS lyase has a broad substrate scope with respect to arylhydrazines (9), enabling the addition of various arylhydrazines to fumarate (Table S2). Pleasingly, several arylhydrazines with an ortho-substituent (o-fluoro, 9b) or meta-substituent (such as m-fluoro, *m*-methyl, and *m*-chloro, **9c-e**) were efficiently converted by EDDS lyase giving the respective enzymatic products 10b-e with excellent conversions (92-98%) and good isolated yields (76-85%, Table 2, entry 2-5). Notably, a number of arylhydrazines containing para-substituents, such as *p*-fluoro, *p*-chloro, *p*-bromo, *p*-cyano, *p*-methyl, and *p*-carboxyl (9f-k), were also well accepted by the enzyme giving the corresponding N-(arylamino)aspartic acids (10f-k) in high to excellent conversions (71-97%) and 52-89% isolated yields (Table 2, entry 6-11). However, p-(methoxyphenyl)hydrazine (91) was not well accepted by EDDS lyase with unsatisfactory conversion (28%, Table 2, entry 12); the reason for the low conversion of this substrate is not known. Typically, arylhydrazines containing a strong electron-withdrawing group at the aromatic ring (namely *p*-nitro or *p*-CF₃), or a bulky naphthalen-2-yl group, failed to undergo the EDDS-lyase-catalyzed hydroamination reaction (Table S2).

With the precious enzymatically prepared intermediates (**10b-k**) in hand, we subsequently performed the acid-catalyzed cyclization reaction to achieve the target 2,5-disubstituted pyrazolidin-3-one products. Remarkably, all the intermediates (**10b-k**) could be cyclized smoothly under the optimized conditions to provide the desired pyrazolidin-3-ones (**11b-k**) with good isolated yield (46-80%, Table 2, entry 2-11). Moreover, all the tested

chemoenzymatic products (**11b-k**) were assigned the (*S*)-configuration, with excellent enantiomeric excess (ee > 99%, Table 2, entry 2-11), using chiral HPLC analysis (Figures S103-S111). As such, we have established an efficient two-step chemoenzymatic route towards chiral 2-aryl-5-carboxylpyrazolidin-3-ones (**11a-k**) with good overall yields and excellent stereoselectivity (ee > 99%).

 Table 2. Chemoenzymatic synthesis of chiral pyrazolidine-3-ones.



		R	First enzymatic step		Second cyclization step			
Entry	Aryl hydrazine		Intermediate	Conv. ^b (Yield ^c) [%]	Product	Yield ^c [%]	ee ^e [%]	Overall yield ⁱ
1	9a	Н	10a	94 (80)	11a	71	>99 (<i>S</i>) ^f	57
2	9b	<i>o</i> -F	10b	98 (81)	11b	46	>99 (<i>S</i>) ^g	37
3	9c	<i>m</i> -F	10c	98 (83)	11c	73	>99 (<i>S</i>) ^f	61
4	9d	<i>m</i> -Me	10d	92 (76)	11d	62	>99 (<i>S</i>) ^f	47
5	9e	<i>m</i> -Cl	10e	98 (85)	11e	67	>99 (<i>S</i>) ^f	57
6	9f	<i>p</i> -F	10f	85 (52)	11f	59	>99 (<i>S</i>) ^f	31
7	9g	<i>p</i> -Cl	10g	80 (68)	11g	80	>99 (<i>S</i>) ^f	54
8	9h	<i>p</i> -Br	10h	91 (81)	11h	76	>99 (<i>S</i>) ^f	62
9	9i	<i>p</i> -CN	10i	92 (70)	11i	57	>99 (<i>S</i>) ^g	40
10	9j	<i>p</i> -Me	10j	71 (63)	11j	58	>99 (<i>S</i>) ^f	37
11	9k	<i>p</i> -СО ₂ Н	10k	97 (89)	11k	69	n.d. ^h	61
12	91	<i>p</i> -OMe	101	28 (n.d. ^d)	111			

^aConditions and reagents: the reaction mixture (20 mL) consisted of fumaric acid (6, 50 mM), arylhydrazine substrates **9a-1** (10 mM) and purified EDDS lyase (0.1 mol% based on arylhydrazine, the amount of applied enzyme was chosen such that reactions were completed within 24-96 h) in degassed buffer (50 mM

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NaH₂PO₄/NaOH, pH 8.5) under argon atmosphere at room temperature (24 h for **10k**; 48 h for **10a**, **10c**, **10e**, and **10h-i**; 96 h for **10b**, **10d**, **10f-g**, **10j** and **10l**). ^bConversions were determined by comparing ¹H NMR signals of substrates and corresponding products. ^cIsolated yield after purification. ^dNot determined owing to low conversion, the product formation was confirmed by comparison of ¹H NMR data of a crude reaction mixture to that of chemically prepared reference compound. ^eEnantiomeric excess (*ee*) was determined by HPLC on a chiral stationary phase using chemically synthesized racemic standards. ^fThe absolute configurations of **11a**, **11c-h**, and **11j** were determined by chiral HPLC using authentic standards with known (*R/S*) and (*S*) configuration. ^gThe absolute configurations of **11b** and **11i** were tentatively assigned the (*S*)-configuration based on analogy and according to chiral HPLC data. ^hThe *ee* value was not determined. ⁱIsolated yield over two steps.

One-pot chemoenzymatic synthesis of chiral pyrazolidin-3-ones

Having established a stepwise chemoenzymatic route towards chiral pyrazolidin-3-ones (11), we sought to combine the EDDS lyase-catalyzed biotransformation and acid-catalyzed cyclization into one pot (Figure 3). In order to achieve high overall yield, as well as to effect the second cyclization step in the one-pot synthesis of pyrazolidin-3-ones (11), high conversion of the starting arylhydrazine substrates **9** in the first enzymatic step is required, preventing it from reacting with the intermediate **10** during the subsequent acid-promoted amidation step. Toward this end, the substrate phenylhydrazine (**9a**) that could be efficiently converted by EDDS lyase with 94% conversion was chosen for our initial investigation to provide the corresponding intermediate **10a** (Table 2 and Figure 3). Without any purification, intermediate **10a** was subjected to cyclization in the same pot, to which fuming hydrochloric acid (HCl) was added to adjust the final concentration of HCl to 1 M, providing full conversion for the second cyclization step after heating to reflux for 3 h. Pleasingly, the one-pot chemoenzymatically prepared product (*S*)-2-phenyl-5-carboxylpyrazolidin-3-one (**11a**) was isolated with good overall yield (68%) and excellent optical purity (*ee* >99%, Figure 3).

To further demonstrate the synthetic usefulness of this one-pot two-step chemoenzymatic strategy, we selected five other starting arylhydrazines (**9c-e** and **9h-i**, Table 2), which proved to be well accepted as substrates by EDDS lyase. The corresponding chemoenzymatically prepared (*S*)-pyrazolidin-3-one derivatives (**11c-e** and **11h-i**) were obtained with good overall isolated yields (61-70%) and excellent enantiopurity (*ee* >99% in all cases, Figure 3). Therefore, this one-pot chemoenzymatic synthesis route provides a simplified practical procedure towards optically pure pyrazolidin-3-ones with higher overall isolated yields.



Figure 3. One-pot two-step chemoenzymatic synthesis of chiral pyrazolidin-3-ones. Reagents and reaction conditions: ^aarylhydrazine substrates **9** (10 mM), fumaric acid (**6**, 50 mM), and purified EDDS lyase (0.1 mol% based on arylhydrazine) in degassed buffer (50 mM NaH₂PO₄/NaOH, pH 8.5) under argon atmosphere at room temperature (48 h for **9a**, **9c**, **9e** and **9h-i**; 96 h for **9d**). ^b1 M HCl, reflux for 3 h under nitrogen atmosphere. ^cIsolated yield over two steps. ^dEnantiomeric excess (*ee*) was determined by HPLC on a chiral stationary phase using chemically synthesized racemic standards. ^cThe absolute configuration of the one-pot chemoenzymatic products **11a**, **11c-e** and **11h** were determined by chiral HPLC analysis using authentic standards with known (*R/S*)

and (*S*) configuration. The absolute configuration of product **11i** was tentatively assigned the (*S*)-configuration based on analogy and according to chiral HPLC data.

DISCUSSION

In contrast to previously reported chemical synthesis strategies for preparation of enantioenriched N-arylated α -amino acids, such as metal-catalyzed N-arylation⁷⁻¹³ or hypervalent iodine chemistry¹⁴, which mainly depend on extending the free amino group of the starting chiral α -amino acids (or their esters), our biocatalytic method starts with a prochiral α,β -unsaturated acid (fumarate) and creates the C α -stereocentre of the target N-arylated amino acids in a single asymmetric step with excellent stereocontrol (Figure 1a). We demonstrated that EDDS lyase shows a broad scope of anilines, enabling the addition of a variety of aromatic amines (7a-n) to fumarate, yielding optically pure (ee >99%) (S)-N-arylated aspartic acids (8a**n**) with high conversions and in good isolated yields (Table 1). Furthermore, we discovered that EDDS lyase can accept a wide range of arylhydrazines (9a-k) in the hydroamination of fumarate, yielding the corresponding N-(arylamino)-substituted aspartic acids (10a-k) with high conversions and in good isolated yields (Table 2). Subsequently, these enzymatic products (11a-k) could undergo a smooth acid-catalyzed cyclization to give the synthetically challenging chiral (S)-pyrazolidin-3-one derivatives **11a-k** with excellent enantiomeric excess (*ee* >99%, Table 2). In addition, we successfully combined the EDDS lyase-catalyzed biotransformation and acid-catalyzed cyclization into one pot, thus providing a rather simple two-step chemoenzymatic route for the rapid synthesis of optically pure pyrazolidin-3-ones with good overall isolated yields (Figure 3).

Enantioselective addition of ammonia or amines to appropriate α,β -unsaturated carboxylic acids catalyzed by carbon-nitrogen lyases represents an attractive strategy for the synthesis of chiral unnatural amino acids. This enzymatic strategy makes use of readily available prochiral α,β -unsaturated acids as starting substrates without a requirement for cofactor recycling, circumvents tedious steps of protecting or activating carboxylic groups, gives 100% theoretical yield, and normally provides high stereoselectivity under mild and potentially green reaction conditions. Several synthetically useful carbon-nitrogen lyases, such as aspartate ammonia lyases (DALs)^{16,17,20,45}, methylaspartate ammonia lyases (MALs)^{16,17,21,46}, phenylalanine ammonia lyases (PALs)^{16,17} and phenylalanine aminomutases (PAMs)^{16,17}, were successfully used in the synthesis of optically pure unnatural α - or β -amino acids. However, with the exception of an engineered mutant of MAL (MAL-Q37A), which accepts various alkylamines as substrates in the addition to mesaconate²¹, these enzymes display a rather limited nucleophile scope. In contrast, EDDS lyase has a very broad nucleophile scope, accepting a wide variety of structurally distinct amines for stereoselective addition to fumarate, providing enzymatic access to various aminocarboxylic acids including the natural products toxin A, aspergillomarasmine A and aspergillomarasmine B²³, N-cycloalkyl-substituted aspartic acids²⁴, as well as difficult *N*-arylated aspartic acid derivatives and substituted pyrazolidin-3-ones (this study, Figure 2). As such, EDDS lyase nicely complements the biocatalytic toolbox for the preparation of noncanonical amino acids. In future work, we will focus our attention on extending the electrophile scope of EDDS lyase, which was found to be highly specific for fumarate with other α,β -unsaturated carboxylic acids (including crotonic acid, mesaconic acid, itaconic acid, or 2-pentenoic acid) not being accepted as alternative electrophiles,⁴⁴ by computational design

and structure-guided protein engineering.

METHODS

Enzymatic synthesis of (S)-N-arylated aspartic acids (8a-n)

Enzyme expression and purification were performed according to procedures described elsewhere (see Supporting Information).^{23,44} The reaction mixture (15 mL) consisted of fumaric acid (50 mM) and an arylamine substrate (**7a-n**, 10 mM) in 50 mM NaH₂PO₄-NaOH buffer (pH 8.5) with 5% DMSO as cosolvent. The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (0.05 mol%). The reaction mixture was then incubated at room temperature from 24 h to 72 h (Table 1). After completion of the reaction, the enzyme was inactivated by heating to 70 °C for 10 min. The progress of the enzymatic reaction was monitored by ¹H NMR spectroscopy by comparing signals of substrates and corresponding products.

The amino acid products were purified by cation-exchange chromatography. For a typical purification procedure, the precipitated enzyme was removed by filtration (pore diameter $0.45 \,\mu$ m). The filtrate was washed with ethyl acetate (10 mL x 3) to remove the remaining amines. The aqueous layer was acidified with 1 M HCl to pH=1 and loaded slowly onto a cation-exchange column (5 g of Dowex 50W X8 resin, 100-200 mesh), which was pretreated with 2 M aqueous ammonia (5 column volumes), 1 M HCl (3 column volumes) and finally water (5 column volumes). The column was washed with water (3 column volumes) to remove the remaining fumaric acid and eluted with 2 M aqueous ammonia until the desired product was collected. The ninhydrin-positive fractions were collected, concentrated under vacuum and lyophilized to provide the desired products (**8a-n**) as ammonium salts.

One-pot chemoenzymatic synthesis of pyrazolidine-3-ones (11)

Step 1: The reaction mixture (20 mL) consisted of fumaric acid (50 mM) and an arylhydrazine substrate (9, 10 mM) in 50 mM NaH₂PO₄-NaOH degassed buffer (pH 8.5) under argon atmosphere. The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (0.1 mol%). The reaction mixture was then incubated at room temperature from 48 h to 96 h (Figure 3). The progress of the enzymatic reaction was monitored by ¹H NMR spectroscopy

by comparing signals of substrates and corresponding products. Without purification of the enzymatic product **10**, the reaction mixture was subjected to the next step immediately.

Step 2: To the stirred reaction mixture from the previous step was added 1.6 mL of fuming hydrochloric acid dropwise under cooling (ice-bath). After 5 min, the reaction mixture was heated to reflux for 3 h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was allowed to cool down to room temperature. The reaction mixture was extracted with EtOAc (20 mL x 3), and washed with brine (30 mL). The solvent was evaporated to provide crude product **11**, which was purified by C18 column chromatography (5% to 50% CH₃CN in H₂O as the eluent).

ASSOCIATED CONTENT

Supporting Information

This information is available free of charge on the ACS Publications website. Detailed experimental procedures, ¹H NMR and ¹³C NMR spectra illustrating chemical structures, and chiral HPLC analysis for (chemo)enzymatic products (PDF).

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Notes

The authors declare no competing financial interest.

Data availability

All data are available from the corresponding author upon reasonable request. Correspondence and requests for materials should be addressed to G.J.P.

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REFERENCES

- Sonke, T.; Kaptein, B.; Schoemaker, H. E. Use of Enzymes in the Synthesis of Amino Acids. In Amino Acids, Peptides and Proteins in Organic Chemistry. Hughes, A. B., Ed.; Wiley-VCH, 2009, Vol. 1, 77–117.
- Blaskovich, M. A. T. Unusual Amino Acids in Medicinal Chemistry. J. Med. Chem. 2016, 59, 10807–10836.
- Chattopadhyay, S.; Raychaudhuri, U.; Chakraborty, R. Artificial Sweeteners A Review. J. Food Sci. Technol. 2014, 51, 611–621.
- Miller, W. H.; Ku, T. W.; Ali, F. E.; Bondinell, Wrt al. E.; Calvo, R. R.; Davis, L. D.; Erhard, K. F.; Hall, L. B.; Huffman, W. F.; Keenan, R. M.; Kwon, C.; Newlander, K. A.; Ross, S. T.; Samanen, J. M.; Takata, D. T.; Yuan, C.-K. Enantiospecific Synthesis of SB 214857, a Potent, Orally Active, Nonpeptide Fibrinogen Receptor Antagonist. *Tetrahedron Lett.* 1995, *36*, 9433–9436.
- (5) Kozikowski, A. P.; Wang, S.; Ma, D.; Yao, J.; Ahmad, S.; Glazer, R. I.; Bogi, K.; Acs, P.; Modarres, S.; Lewin, N. E.; Blumberg, P. M. Modeling, Chemistry, and Biology of the Benzolactam Analogues of Indolactam V (ILV). 2. Identification of the Binding Site of the Benzolactams in the CRD2 Activator-Binding Domain of PKCδ and Discovery of an ILV Analogue of Improved Isozyme Selectivity. *J. Med. Chem.*, **1997**, *40*, 1316–1326.
- (6) Haynes-Smith, J.; Diaz, I.; Billingsley, K. L. Modular Total Synthesis of Protein Kinase C
 Activator (-)-Indolactam V. Org. Lett. 2016, 18, 2008–2011.
- (7) Ma, D.; Cai, Q. Copper/Amino Acid Catalyzed Cross-Couplings of Aryl and Vinyl Halides with Nucleophiles. *Acc. Chem. Res.* 2008, *41*, 1450–1460.
- Ma, D.; Zhang, Y.; Yao, J.; Wu, S.; Tao, F. Accelerating Effect Induced by the Structure of α-Amino Acid in the Copper-Catalyzed Coupling Reaction of Aryl Halides with α-Amino Acids. Synthesis of Benzolactam-V8. *J. Am. Chem. Soc.* **1998**, *120*, 12459–12467.

- (9) Sharma, K. K.; Sharma, S.; Kudwal, A.; Jain, R. Room Temperature *N*-Arylation of Amino Acids and Peptides Using Copper (I) and β-Diketone. *Org. Biomol. Chem.* 2015, *13*, 4637–4641.
 - King, S. M.; Buchwald, S. L. Development of a Method for the *N*-Arylation of Amino Acid
 Esters with Aryl Triflates. *Org. Lett.* 2016, *18*, 4128–4131.
 - (11) Hammoud, H.; Schmitt, M.; Blaise, E.; Bihel, F.; Bourguignon, J.-J. *N*-Heteroarylation of Chiral α-Aminoesters by Means of Palladium-Catalyzed Buchwald–Hartwig Reaction. *J. Org. Chem.* 2013, 78, 7930–7937.
 - (12) Ma, F.; Xie, X.; Ding, L.; Gao, J.; Zhang, Z. Palladium-Catalyzed Coupling Reaction of Amino Acids (Esters) with Aryl Bromides and Chlorides. *Tetrahedron* 2011, 67, 9405–9410.
 - (13) Dominguez-Huerta, A.; Perepichka, I.; Li, C.-J. Catalytic *N*-Modification of α-Amino Acids and Small Peptides with Phenol under Bio-Compatible Conditions. *Commun. Chem.* 2018, *1*, 45.
- McKerrow, J. D.; Al-Rawi, J. M. A.; Brooks, P. Use of Diphenyliodonium Bromide in the Synthesis of Some *N*-Phenyl α-Amino Acids. *Synth. Commun.* 2010, *40*, 1161–1179.
- (15) Xue, Y.-P.; Cao, C.-H.; Zheng, Y.-G. Enzymatic Asymmetric Synthesis of Chiral Amino Acids. Chem. Soc. Rev. 2018, 47, 1516–1561.
- (16) Almhjell, P. J.; Boville, C. E.; Arnold, F. H. Engineering Enzymes for Noncanonical Amino Acid Synthesis. *Chem. Soc. Rev.* 2018, 47, 8980–8997.
- Parmeggiani, F.; Weise, N. J.; Ahmed, S. T.; Turner, N. J. Synthetic and Therapeutic Applications of Ammonia-Lyases and Aminomutases. *Chem. Rev.* 2018, *118*, 73–118.
- (18) Hyslop, J. F.; Lovelock, S. L.; Watson, A. J. B.; Sutton, P. W.; Roiban, G.-D. *N*-Alkyl-α Amino Acids in Nature and Their Biocatalytic Preparation. *J. Biotechnol.* 2019, 293, 56–65.
- de Villiers, M.; Puthan Veetil, V.; Raj, H.; de Villiers, J.; Poelarends, G. J. Catalytic
 Mechanisms and Biocatalytic Applications of Aspartate and Methylaspartate Ammonia Lyases.
 ACS Chem. Biol. 2012, 7, 1618–1628.
- (20) Weiner, B.; Poelarends, G. J.; Janssen, D. B.; Feringa, B. L. Biocatalytic Enantioselective Synthesis of *N*-Substituted Aspartic Acids by Aspartate Ammonia Lyase. *Chem. A Eur. J.* **2008**, *14*, 10094–10100.
- (21) Raj, H.; Szymański, W.; de Villiers, J.; Rozeboom, H. J.; Veetil, V. P.; Reis, C. R.; de Villiers, 20

1 ว		
2 3 4		M.; Dekker, F. J.; de Wildeman, S.; Quax, W. J.; Thunnissen, AM. W. H.; Feringa, B. L.;
5 6		Janssen, D. B.; Poelarends, G. J. Engineering Methylaspartate Ammonia Lyase for the
7 8		Asymmetric Synthesis of Unnatural Amino Acids. Nat. Chem. 2012, 4, 478-484.
9 10	(22)	Puthan Veetil, V.; Raj, H.; de Villiers, M.; Tepper, P. G.; Dekker, F. J.; Quax, W. J.;
11 12		Poelarends, G. J. Enantioselective Synthesis of N-Substituted Aspartic Acids Using an
13		Engineered Variant of Methylaspartate Ammonia Lyase. ChemCatChem 2013, 5, 1325–1327.
15	(23)	Fu, H.; Zhang, J.; Saifuddin, M.; Cruiming, G.; Tepper, P. G.; Poelarends, G. J.
16 17		Chemoenzymatic Asymmetric Synthesis of the Metallo-β-lactamase Inhibitor
18 19		Aspergillomarasmine A and Related Aminocarboxylic Acids. Nat. Catal. 2018, 1, 186–191.
20 21	(24)	Zhang, J.; Fu, H.; Tepper P. G.; Poelarends, G. J. Biocatalytic Enantioselective
22 23		Hydroaminations for Production of <i>N</i> -Cycloalkyl-Substituted L-Aspartic Acids Using Two C-
24 25		N Lyases Adv Synth Catal 2019 361 2433–2437
26 27	(25)	Aleku G A · France S P · Man H · Mangas-Sanchez I · Montgomery S I · Sharma M ·
28	(23)	Leinold E : Hussain S : Grogan G : Turner N L A Reductive Aminase from Asnergillus
30		Ourrage Nat Chem 2017 0 061 060
32	(26)	Vete V. Vemede U. Asono V. Storocolective Semthesis of Onine Teme Secondam Amine
33 34	(20)	Kato, F., Famada, H., Asano, F. Stereoserective Synthesis of Opine-Type Secondary Annue
35 36		Carboxylic Acids by a New Enzyme Opine Denydrogenase Use of Recombinant Enzymes. J.
37 38		<i>Mol. Catal. B Enzym.</i> 1996 , <i>1</i> , 151–160.
39 40	(27)	Chen, H.; Collier, S. J.; Nazor, J.; Sukumaran, J.; Smith, D.; Moore, J. C.; Hughes, G.; Janey,
41		J.; Huisman, G. W.; Novick, S. J.; Agard, N. J. Engineered Imine Reductases and Methods for
43		the Reductive Amination of Ketone and Amine Compounds. 2016, U.S. Patent 9,487,760.
44 45	(28)	Muramatsu, H.; Mihara, H.; Kakutani, R.; Yasuda, M.; Ueda, M.; Kurihara, T.; Esaki, N.
46 47		Enzymatic Synthesis of N-Methyl-L-phenylalanine by a Novel Enzyme, N-Methyl-L-amino
48 49		Acid Dehydrogenase, from Pseudomonas Putida. Tetrahedron: Asymmetry 2004, 15, 2841-
50 51		2843.
52 53	(29)	Hyslop, J. F.; Lovelock, S. L.; Sutton, P. W.; Brown, K. K.; Watson, A. J. B.; Roiban, GD.
54 55		Biocatalytic Synthesis of Chiral N-Functionalized Amino Acids. Angew. Chemie Int. Ed. 2018,
56 57		57, 13821–13824.
58	(30)	Hallen, A.; Cooper, A. J. L.; Smith, J. R.; Jamie, J. F.; Karuso, P. Ketimine Reductase/CRYM
60		Catalyzes Reductive Alkylamination of α -Keto Acids, Confirming Its Function as an Imine
		24

Reductase. Amino Acids 2015, 47, 2457-2461.

- (31) Fujii, T., Mukaihara, M., Agematu, H. & Tsunekawa, H. Biotransformation of L-Lysine to L-Pipecolic Acid Catalyzed by L-Lysine 6-Aminotransferase and Pyrroline-5-carboxylate Reductase. *Biosci. Biotechnol. Biochem.* 2002, *66*, 622–627.
- (32) Brune, K. The Early History of Non-Opioid Analgesics. Acute Pain 1997, 1, 33–40.
- (33) Cucurou, C.; Battioni, J. P.; Thang, D. C.; Nam, N. H.; Mansuy, D. Mechanisms of Inactivation of Lipoxygenases by Phenidone and BW755C. *Biochemistry* 1991, *30*, 8964– 8970.
- (34) Kosower, E. M.; Hershkowitz, E. Isr. Patent ISXXAQ IL 94658. 1994, [*Chem. Abstr.* 122, 214077 (1994)].
- (35) Gould, E.; Lebl, T.; Slawin, A. M. Z.; Reid, M.; Smith, A. D. Structural Effects in
 Pyrazolidinone-Mediated Organocatalytic Diels–Alder Reactions. *Tetrahedron* 2010, *66*, 8992–9008.
- Ma, G.; Deng, J.; Sibi, M. P. Fluxionally Chiral DMAP Catalysts: Kinetic Resolution of Axially Chiral Biaryl Compounds. *Angew. Chemie Int. Ed.* 2014, *53*, 11818–11821.
- (37) Melgar-Fernández, R.; González-Olvera, R.; Vargas-Caporali, J.; Pérez-Isidoro, R.; Juaristi, E.
 Resolution of 5-Oxo-1-phenylpyrazolidine-3-carboxylic Acid and Synthesis of Novel
 Enantiopure Amide Derivatives. *ARKIVOC* 2010, *8*, 55-75.
- (38) Wang, M.; Huang, Z.; Xu, J.; Chi, Y. R. *N*-Heterocyclic Carbene-Catalyzed [3+4]
 Cycloaddition and Kinetic Resolution of Azomethine Imines. *J. Am. Chem. Soc.* 2014, *136*, 1214–1217.
- (39) Hashimoto, T.; Maruoka, K. Recent Advances of Catalytic Asymmetric 1,3-Dipolar Cycloadditions. *Chem. Rev.* 2015, *115*, 5366–5412.
- (40) Hesping, L.; Biswas, A.; Daniliuc, C. G.; Mück-Lichtenfeld, C.; Studer, A. Stereoselective Lewis Base Catalyzed Formal 1,3-Dipolar Cycloaddition of Azomethine Imines with Mixed Anhydrides. *Chem. Sci.* 2015, *6*, 1252–1257.
- (41) He, L.; Liu, L.; Han, R.; Zhang, W.; Xie, X.; She, X. Thermal 1,3-Dipolar Cycloaddition
 Reaction of Azomethine Imines with Active Esters. *Org. Biomol. Chem.* 2016, *14*, 6757–6761.
- (42) Sibi, M. P.; Soeta, T. Enantioselective Conjugate Addition of Hydrazines to α,β-Unsaturated Imides. Synthesis of Chiral Pyrazolidinones. J. Am. Chem. Soc. 2007, 129, 4522–4523.

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- (43) Chen, Z.-P.; Chen, M.-W.; Shi, L.; Yu, C.-B.; Zhou, Y.-G. Pd-catalyzed Asymmetric Hydrogenation of Fluorinated Aromatic Pyrazol-5-ols via Capture of Active Tautomers. *Chem. Sci.* 2015, *6*, 3415–3419.
 - (44) Poddar, H.; de Villiers, J.; Zhang, J.; Puthan Veetil, V.; Raj, H.; Thunnissen, A.-M. W. H.;
 Poelarends, G. J. Structural Basis for the Catalytic Mechanism of Ethylenediamine-*N*,*N*[']disuccinic Acid Lyase, a Carbon-Nitrogen Bond-forming Enzyme with a Broad Substrate
 Scope. *Biochemistry* 2018, *57*, 3752–3763.
 - (45) Li, R.; Wijma, H. J.; Song, L.; Cui, Y.; Otzen, M.; Tian, Y. E.; Du, J.; Li, T.; Niu, D.; Chen, Y;
 Feng, J.; Han J.; Chen H.; Tao Y.; Janssen D. B.; Wu B. Computational Redesign of Enzymes
 for Regio- and Enantioselective Hydroamination. *Nat. Chem. Biol.* 2018, *14*, 664–670.
 - (46) Fu, H.; Zhang, J.; Tepper, P. G.; Bunch, L.; Jensen, A. A.; Poelarends, G. J. Chemoenzymatic Synthesis and Pharmacological Characterization of Functionalized Aspartate Analogues as Novel Excitatory Amino Acid Transporter Inhibitors. *J. Med. Chem.* 2018, *61*, 7741–7753.

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