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,N'-disuccinic acid lyase (EDDS lyase) as a 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 toward the synthetically challenging chiral 2-aryl-5-carboxylypyrazolidin-3-ones, using aryldrazines as bis-nucleophilic 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 enantioselectively pure pyrazolidin-3-ones. Hence, these biocatalytic methods provide convenient alternative routes to important chiral N-arylated aspartic acids and difficult 2-aryl-5-carboxylypyrazolidin-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 the fibrinogen receptor antagonist Lotrafiban (1, Figure 1a)5,6 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 free amino group of the Cu stereocenter through Cu-catalyzed Ullmann-type coupling reactions,6−9 Pd-catalyzed N-arylation,10−13 and hypervalent iodine chemistry (Figure 1a).14 However, these strategies 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-stereocenter. Biocatalysis provides a valuable alternative route to chiral unnatural amino acids.15−18 Previously reported enzymatic asymmetric synthesis of N-alkyl-functionalized α-amino acids were primarily based on two types of carbon–nitrogen bond-forming 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 (aspar- tases),19,20 methaspartate 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-methylamino acid dehydrogenases (NMAADHs),28,29 ketimine reductases (KIREDs),30,31 and Δ1-pyrroline-5-carboxylate reductases (PSCRs).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 enantioselectively pure N-aryl-α-amino acid derivatives would be particularly desirable.

Pyrazolidin-3-ones and related five-membered dinitrogen-fused heterocycles are widely found as the core framework in dyes, agrochemicals, and pharmaceutically active molecules, such as the very first synthetic analgesic and antipyretic drug Phenazon (3, Figure 1b),32 lipoxigenase inhibitor Phendione (4, Figure 1b),33 and anti-Alzheimer agents (5, Figure 1b).34 In addition, chiral pyrazolidin-3-ones can also function as efficient catalysts in promoting Diels–Alder reactions35 and catalyze the 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 resolution, \(^{38}\) 1,3-dipolar cycloaddition, \(^{39-41}\) Lewis acid catalyzed conjugate addition, \(^{42}\) and Pd-catalyzed asymmetric hydrogenation. \(^{43}\) However, creating a biocatalytic methodology as an alternative route to chiral pyrazolidinones is an as yet unmet challenge.

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 the 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

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 toward N-arylated aspartic acids (right panel); (b) biologically active molecules containing pyrazolin-3-one (left panel), and established synthetic strategies and our chemoenzymatic route toward pyrazolidin-3-ones (right panel).

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) exploration of the arylamine and arylhydrazine scope of EDDS lyase for the (chemo)enzymatic asymmetric synthesis of \(N\)-arylated aspartic acids and pyrazolidinones, respectively.
product aspergillomarasmine A and various related amino-carboxylic acids (Figure 2b). 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). 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 pyrazolidin-3-ones could be constructed by using arylhydrazines as bis-nucleophilic 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 toward chiral pyrazolidin-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 pyrazolidin-3-ones.

## 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). This prompted us to start our investigation by testing aniline (7a, Table 1, entry 1) as a 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 the 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 configurations (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, entries 3–5). High conversions (87–97%), good isolated product yields (53–84%), and excellent stereoselectivity (ee > 99%) were observed (Table 1, entries 3–5). Similarly, para-substituted arylamines, such as p-fluoroaniline (7f), p-toluidine (7g), p-methoxyaniline (7h), p-ethylaniline (7i), m,p-dimethylaniline (7j), 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 in >99% ee (Table 1, entries 6–11). It is worth noting that para-halogenated anilines (7l–n) were also processed to deliver chiral (S)-N-haloarylaspatic acids (8l–n) with 82–96% conversions and 52–69% isolated yields (ee > 99% in all cases, Table 1, entries 12–14), leaving the halogens available for potential downstream synthetic manipulation. The larger nucleophile p-isopropylaniline (7o) was a poor substrate for EDDS lyase, resulting in low conversion (17%, Table 1, entry 15). Arylamines bearing a strongly electron withdrawing group (such as p-nitro or p-CF3) or electron-deficient heteroarylamines (such as pyridin-2-

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*Conditions 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 and EDDS lyase to a crude reaction mixture was used to drive amine 7 to completion, simplifying product purification and preventing enzyme inhibition by a high concentration of amine substrate. Conversions were determined by comparing 1H NMR signals of substrates and corresponding products. Not determined owing to low conversion. The enantiomeric excess (ee) was determined by HPLC on a chiral stationary phase using racemic standards. Not determined owing to low conversion. The product formation was confirmed by comparison of 1H NMR data of a crude reaction mixture to those of a chemically prepared reference compound. The absolute configurations of 8a–f and 8i–m were determined by chiral HPLC using chemically synthesized authentic standards with known R/S and S configurations, respectively. The absolute configurations of 8c–e and 8n were tentatively assigned by the configuration on the basis of analogy and according to chiral HPLC data.

Table 1. Enzymatic Synthesis of (S)-N-Arylated Aspartic Acids

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amine, pyridin-4-amine, and thiazol-2-amine) were not accommodated as substrates by EDDS lyase, most likely due to their diminished nucleophilicity (Table S1).

**Chemoenzymatic Synthesis of Chiral Pyrazolidin-3-ones.** Enantioselective conjugate addition of bis-nucleophilic donors (such as hydrazines) to electron-poor acceptors provides convenient access to valuable small-ring heterocycles and olds in their own right but they also can be served as chiral precursors for the preparation of synthetically challenging chiral pyrazolidin-3-ones (11) through an acid-catalyzed cyclization reaction (Table 2).

Remarkably, phenylhydrazine (9a), as the first chosen potential bis-nucleophilic 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), affording the desired heterocycle 2-phenyl-5-carboxylpyrazolidin-3-one (11a, 71% isolated yield) without racemization of the potentially sensitive Cα stereogenic center (ee > 99%, Table 2, entry 1). The enantioselectively 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, entries 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, entries 6–11). However, p-(methoxycarbonyl)hydrazine (9l) 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 strongly 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).

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**Table 2. Chemoenzymatic Synthesis of Chiral Pyrazolidin-3-ones**

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*Conditions 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 NaH₂PO₄/NaOH, pH 8.5) under an argon atmosphere at room temperature (24 h for 10a, 48 h for 10b-e; 96 h for 10b,f,g,l). Conversions were determined by comparing ^1H NMR signals of substrates and corresponding products. Isolated yield after purification. Not determined owing to low conversion. The product formation was confirmed by comparison of ^1H NMR data of a crude reaction mixture to those of a chemically prepared reference compound. Enantiomeric excess (ee) was determined by HPLC on a chiral stationary phase using chemically synthesized racemic standards. The absolute configurations of 11a-c,h-l were determined by chiral HPLC using authentic standards with known R/S and S configurations. The ee value was not determined. *Isolated yield over two steps.*
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 of 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, entries 2−11). Moreover, all of the tested chemoenzymatic products (11b−k) were assigned the S configuration, with excellent enantiomeric excess (ee > 99%, Table 2, entries 2−11), using chiral HPLC analysis (Figures S103−S111). As such, we have established an efficient two-step chemoenzymatic route toward chiral 2-aryl-5-carboxylpyrazolidin-3-ones (11a−k) with good overall yields and excellent stereoselectivity (ee > 99%).

**One-Pot Chemoenzymatic Synthesis of Chiral Pyrazolidin-3-ones.** Having established a stepwise chemoenzymatic route toward 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 a 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,h,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,h,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 toward optically pure pyrazolidin-3-ones with higher overall isolated yields.

**DISCUSSION**

In contrast to previously reported chemical synthesis strategies for preparation of enantioenriched N-arylated α-amino acids, such as metal-catalyzed N-arylation14−15 or hypervalent iodine chemistry,16 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 Cα stereocenter of the target N-arylated α-amino acids in a single asymmetric step with excellent stereocontrol (Figure 1a). We demonstrated that EDDS lyase shows a broad scope of amines, 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-(arylamo)-substituted aspartic acids (10a−k) with high conversions and in good isolated yields (Table 2). Subsequently, these enzymatic products (10a−k) could undergo a smooth acid-catalyzed cyclization to give the synthetically challenging chiral (S)-pyrazolidin-3-one derivatives 11a−k with excellent enantio-meric 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 the 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 the 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 mesaconate,21 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 B,23 N-cycloalkyl-substituted aspartic acids,25 and 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, and 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 the 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 (0.1 mol %). The reaction mixture was then incubated at room temperature from 24 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 the 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 μm). The filtrate was washed with ethyl acetate (10 mL × 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 Pyrazolidin-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 an 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 to 96 h (Figure 3). The progress of the enzymatic reaction was monitored by 1H NMR spectroscopy by comparing the 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 with cooling (ice bath). After 5 min, the reaction mixture was heated to reflux for 3 h under a nitrogen atmosphere. After completion of the reaction, the reaction mixture was cooled to room temperature. The reaction mixture was extracted with EtOAc (20 mL × 3), and washed with brine (30 mL). The solvent was evaporated to provide crude product 11, which was purified by C18 column chromatography (5−50% CH2CN in H2O as the eluent).

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

■ ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.9b01748.

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.

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