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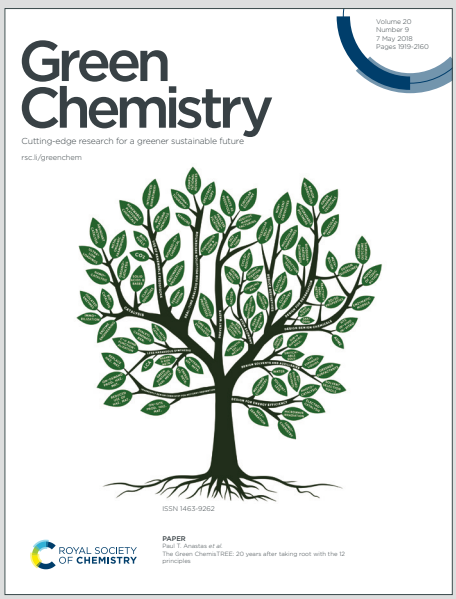

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ARTICLE

A biocatalytic redox cascade approach for one-pot deracemization of carboxyl-substituted tetrahydroisoquinolines by stereoinversion

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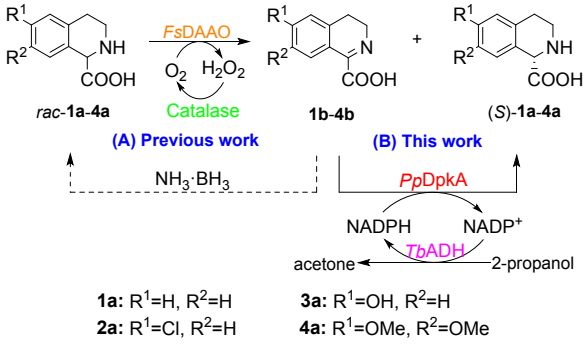
Optically pure 1,2,3,4-tetrahydroisoquinolines carboxylic acids are important chiral building blocks in the pharmaceutical and fine chemical industries. However, the existing chemo-enzymatic deracemization method employing D-amino acid oxidase from *Fusarium solani* M-0718 (*Fs*DAAO) suffers from the requirement for a large excess of nonselective chemical reducing agent. To explore an alternative method, we envisaged a concurrent biocatalytic oxidation and reduction cascade in one pot. Herein, we report a novel biocatalytic route for the asymmetric reduction of 3,4-dihydroisoquinoline-1-carboxylic acids employing Δ^1-piperidine-2-carboxylate/Δ^1-pyrrolidine-2-carboxylate reductase from *Pseudomonas putida* KT2440 (*Pp*DpkA) as biocatalyst, yielding the corresponding (*S*)-1-carboxyl-substituted tetrahydroisoquinolines with high conversions and enantiomeric excess (>99% *ee*). By combining *Fs*DAAO and *Pp*DpkA in one pot, a fully biocatalytic method was demonstrated for the deracemization of a range of racemic 1-carboxyl substituted tetrahydroisoquinolines to produce the corresponding (*S*)-enantiomers with >99% conversions and >99% *ee*. Furthermore, preparative-scale biotransformation of racemic 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid gave the the (*S*)-enantiomer with 89% isolated yield and >99% *ee*. Taken together, we provide an enantioselective biocatalytic redox cascade method for the one-pot synthesis of enantiopure 1,2,3,4-tetrahydroisoquinoline carboxylic acids.

Introduction

Optically pure 1,2,3,4-tetrahydroisoquinolines (THIQs) constitute a class of privileged structural moieties that exist in a variety of drugs and natural isoquinoline alkaloids with diverse biological activities.[1,2] Compared with chemical synthetic methodologies generally involving toxic organic reagents or transition metal catalysts,[3-9] elegant and environmentally friendly enzymatic methods for the preparation of chiral THIQs, especially 1-substituted THIQs, have aroused widespread attention in recent years. Several oxidoreductase-mediated biocatalytic approaches have been investigated for the preparation of chiral 1-alkyl-, 1-benzyl-, or 1-aryl-substituted THIQs.[10-15] For those carboxyl-substituted THIQs, we recently developed a chemo-enzymatic approach to (*S*)-enantiomers employing D-amino acid oxidase from *Fusarium solani* M-0718 (*Fs*DAAO) (Scheme 1A).[16]

Although this system provided an effective route to (*S*)-1,2,3,4-tetrahydroisoquinoline carboxylic acids, it suffered from the requirement for a large excess of nonselective chemical reducing agent such as ammonia-borane ($NH_3{\cdot}BH_3$). We envisaged that we might be able to couple the *Fs*DAAO-catalyzed oxidation of racemic substrates with a (*S*)-selective reductase-mediated asymmetric reduction of dihydroisoquinoline carboxylic acids in one pot, thus providing a "greener" alternative method for the production of (*S*)-enantiomers (Scheme 1B). Theoretically, the use of an (*S*)-selective reductase would lead to a more efficient deracemization process than the reported chemo-enzymatic approach, because no reduction to the (*R*)-enantiomer would occur. Compared with the *Fs*DAAO-$NH_3{\cdot}BH_3$ cascade, the addition of $NH_3{\cdot}BH_3$ and the time-consuming and yield-reducing



Scheme 1 One-pot enantioselective synthesis of (*S*)-Carboxyl substituted THIQs via (A) previously reported chemo-enzymatic deracemization or (B) multi-enzymatic deracemization demonstrated in this work.

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removal of this chemical reducing agent would be avoided in this multi-enzyme system. Deracemization of racemic mixtures through such concurrent biocatalytic oxidation and reduction cascade is an attractive approach and has been widely applied in the synthesis of chiral *sec*-alcohols,[17,18], 2-hydroxy acids,[19-21] amino acids,[22-25] and amines.[26-30] Recently, the deracemization of 1-methyl-1,2,3,4-tetrahydroisoquinoline by combining an engineered amine oxidase (AO) with artificial transferhydrogenase (ATHase) has been studied.[31,32] Despite the advantages and attractiveness, this system was not suitable for the preparation of chiral carboxyl-substituted THIQs because of the limitation of substrate specificities of these two enzymes. To date, no general comparable biocatalytic approach for the synthesis of chiral carboxyl-substituted THIQs has been reported.

In this study, we successfully construct a multi-enzymatic cascade for the deracemization of racemic 1-carboxyl-substituted THIQs in one pot (Scheme 1B). To achieve this, a panel of diverse imine-reducing enzymes for the reduction of model substrate 3,4-dihydroisoquinoline-1-carboxylic acid (**1b**) was cloned and evaluated. The Δ^1-piperidine-2-carboxylate reductase/Δ^1-pyrrolidine-2-carboxylate reductase from *Pseudomonas putida* KT2440 (termed *Pp*DpkA) was achieved with highest activity and excellent enantioselectivity. By combining *Fs*DAAO with *Pp*DpkA in one pot, a range of racemic 1-carboxyl substituted THIQs were transformed to the corresponding (*S*)-enantiomers. Therefore, this system provides a novel and sustainable method for the efficient synthesis of optically pure carboxyl-substituted THIQs.

Results and discussion

Enzyme exploitation for the asymmetric reduction of 3,4-dihydroisoquinoline-1-carboxylic acid

The asymmetric reduction of these sterically hindered dihydroisoquinoline carboxylic acids to the corresponding (*S*)-enantiomers with excellent enantioselectivity is critical to the proposed biocatalytic cascade. Unfortunately, no applicable enzyme has been reported for this process to date. Although various microbial imine reductases (IREDs) have been successfully employed for the reduction of analogues, such as 1-methyl-3,4-dihydroisoquinoline,[12,33-36] previous applications of these enzymes mainly focused on the analogous non-carboxylate imines and iminium ions.[37,38] Interestingly, another class of natural imine-reducing enzymes such as bacterial Δ^1-piperidine-2-carboxylate (Pip2C) reductase (EC 1.5.1.21) and Δ^1-pyrrolidine-2-carboxylate (Pyr2C) reductases (EC 1.5.1.1) are known to catalyze the reduction of Pip2C or Pyr2C to L-pipecolic acid or L-proline.[39] The most prominent and best characterized members are those from *Pseudomonas* spp. (also known as DpkAs or *N*-methyl-L-amino acid dehydrogenases), which are biologically involved in the catabolism of D-lysine and D-proline.[40,41] DpkAs as imine-reducing biocatalysts have been reported to exhibit a narrow substrate scope.[38] Nevertheless, considering the structural characteristics of the model substrate **1b**, which also bears a carboxylic acid substituent on the imine carbon atom, we speculated that DpkAs might be potential enzyme toolbox for our reduction process.

To search for an ideal biocatalyst, a genome-mining strategy was adopted. A mini-library of DpkAs was constructed using the protein-protein basic local alignment search tool (BLAST) with the amino acid sequence of *Pp*DpkA as the template. According to the sequence identity (43%–87%), three candidate enzymes were selected (Supporting Information, Table S1). These four enzyme genes were cloned and overexpressed in *E. coli* BL21 (DE3). To investigate the expression patterns, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. As shown in Figure S2, these four enzymes were successfully expressed in a soluble form. The imine reduction activity of these enzymes toward substrate **1b** was evaluated at 30 °C and pH 8.0 using crude extract. As shown in Table 1, all of the tested enzymes exhibited significant activities toward the substrate and produced the corresponding (*S*)-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (**1a**) with excellent enantioselectivity (>99% enantiomeric excess (*ee*)). Among them, *Pp*DpkA, which displayed the highest activity of 31.07±0.91 U/mL, was selected for further research.

Table 1 Evaluation of enzymatic imine reduction activity toward **1b**

Entry	Enzyme	Activity (U/mL)[a]	*ee* (%)[b] /Config.[c]
1	*Pp*DpkA	31.07±0.91	>99% (*S*)
2	*Pe*DpkA	17.65±0.21	>99% (*S*)
3	*Pf*DpkA	16.09±0.65	>99% (*S*)
4	*Pa*DpkA	0.36±0.01	>99% (*S*)

[a] Enzyme activity was determined by monitoring the decrease in the absorbance of NADPH at 340 nm.

[b] The product *ee* was determined by chiral HPLC.

[c] Configuration was determined by comparison with literature data or by analogue.

Purification and characterization of *Pp*DpkA

The recombinant *Pp*DpkA with a His-tag at the *N*-terminal was purified using nickel-affinity chromatography, and analyzed by SDS-PAGE (Supporting Information, Figure S3A), indicating a subunit size of approximately 40 kDa. The enzymatic properties of *Pp*DpkA were then investigated using compound **1b** as the substrate. *Pp*DpkA exhibited maximal activity at pH 6.5 and 42% relative activity at pH 8.0 (Supporting Information, Figure S3B). The enzyme was stable from pH 6.5 to 8.5 (retained more than 90% activity) for 24 h at 4 °C (Supporting Information, Figure S3C). The specific activities from 20 to 55 °C were then measured. The enzyme showed maximal activity at 40 °C and 80% relative activity at 30 °C (Supporting Information, Figure S3D). The thermostability of *Pp*DpkA was assessed at 30, 40, and 50 °C (Supporting Information, Figure S3E). The half-lives of the enzyme were calculated to be 52 h at 30 °C, 21 h at 40 °C and 0.4 h at 50 °C, indicating relative good thermal stability under mild reaction conditions. The kinetic parameters of purified *Pp*DpkA toward substrate **1b** were determined at pH 8.0 and 30

°C, indicating a catalytic efficiency constant (*k*cat/*K*m) of 759.84 s^{-1} mM^{-1} (Table 2).

Table 2 Kinetic parameters of *Pp*DpkA[a]

Sub.	*K*m (mM)	k_{cat} (s^{-1})	k_{cat}/*K*m (s^{-1} mM^{-1})
1b	0.77±0.02	587.52±5.88	759.84

[a] The assay was performed using the HPLC method, and each value represents mean±standard deviation of three measurements.

Evaluation of the feasibility and compatibility of the proposed biocatalytic cascade

Constructing an efficient concurrent multi-enzymatic cascade in one pot was considered to be rather challenging due to the different reaction conditions suitable for individual biotransformation. Furthermore, the combination of reactions may lead to the enzyme activity inhibition by reaction intermediates.[42,43] Hence, it is extremely important and necessary to take each reaction into full consideration and identify compatible conditions.

To investigate the feasibility of the proposed deracemization, we initially performed the *Fs*DAAO-*Pp*DpkA cascade reaction in one pot without cofactor regeneration system. Considering the effect of pH and temperature on enzymatic activity and stability, a compromised mild reaction condition (pH 8.0 and 30 °C) was adopted. Biotransformation reaction was performed in 1 mL of Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0) that contained *rac*-**1a** (10 mM), purified *Fs*DAAO (0.1 mg/mL, 2 U/mL), flavine adenine dinucleotide (FAD) (100 μM), catalase (200 U/mL), an excess of purified *Pp*DpkA (10 U/mL) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (8 mM). After incubation for 1 h, chiral HPLC results revealed that the concentration of (*S*)-**1a** was indeed improved, which is the prerequisite for the one-pot simultaneous cascade. However, 60% of (*R*)-**1a** still existed in the solution. This result was inconsistent with the previously described kinetic resolution,[16] in which (*R*)-**1a** (5 mM) could be completely oxidized by purified *Fs*DAAO (2 U/mL) in 1 h. We speculated that the activity of *Fs*DAAO may have been inhibited by the reduction process, which subsequently resulted in low efficiency in the oxidation process.

To identify the problem, the specific activity of *Fs*DAAO was separately determined with different concentrations (0–10 mM) of NADPH and $NADP^+$. As shown in Figure S4C, no significant activity inhibition was observed with $NADP^+$. However, only 38% of relative activity was retained in the presence of 8 mM NADPH (Figure S4B), suggesting that the relative high concentration of NADPH was the predominant reason for the low efficiency of the *Fs*DAAO-catalyzed oxidation. Fortunately, the presence of low concentrations of NADPH (0–0.5 mM) had no negative effect on the specific activity of *Fs*DAAO.

Given the high cost of NADPH and the negative effect of this cofactor at relatively high concentrations on *Fs*DAAO, cofactor regeneration was essential for our deracemization reaction. Considering the product separation and the activity of the enzyme used for NADPH regeneration, an alcohol dehydrogenase from *Thermoanaerobacter brockii* (*Tb*ADH)[44]/isopropanol regeneration system was selected. To access the potential effect of this cofactor regeneration system on *Fs*DAAO and *Pp*DpkA, the specific activities of these two enzymes were separately examined in the presence of different concentrations (0–100 mM) of isopropanol and co-product acetone. As shown in Fig. S3 and S4, the activities of these two enzymes were not affected.

Enantioselective synthesis of (*S*)-1a employing the *Pp*DpkA-*Tb*ADH system

Based on these results, the asymmetric reduction of substrate **1b** was then performed using purified *Pp*DpkA. A control experiment without cofactor regeneration system was initially conducted. The cofactor NADPH was supplied at a sufficient concentration (8 mM). Substrate **1b** (5 mM) was transformed into (*S*)-**1a** at a high conversion (>99%) and >99% *ee* within 1 h (Table 3, entry 1). Then, we applied *Pp*DpkA for the reduction of substrate **1b** in combination with the *Tb*ADH/isopropanol regeneration system. A relatively low concentration of $NADP^+$ (5 μM) was used. The chiral HPLC analysis showed that (*S*)-**1a** was obtained with excellent enantioselectivity (>99% *ee*). However, the conversion of substrate **1b** only reached 93% at 1 h (Table 3, entry 2). To further improve the efficiency, we increased the amount of *Tb*ADH. As a result, *Pp*DpkA (2 U/mL) and *Tb*ADH (5 U/mL) were combined and afforded high conversion (>99%) of substrate **1b** to (*S*)-**1a** with excellent enantioselectivity (>99% *ee*) within 1 h (Table 3, entry 4).

Table 3 Asymmetric reduction of **1b** to (*S*)-**1a** employing *Pp*DpkA[a]

Entry	*Pp*DpkA (U/mL)[b]	*Tb*ADH (U/mL)[c]	Cofactor (μM)	Conv. (*c*) (%)[d]	*ee* (%)[e]/ Config.[e]
1	2	/	NADPH (8000)	>99%	>99 (*S*)
2	2	1	$NADP^+$ (5)	93%	>99 (*S*)
3	2	3	$NADP^+$ (5)	96%	>99 (*S*)
4	2	5	$NADP^+$ (5)	>99%	>99 (*S*)

[a] Reaction conditions (1ml): substrate **1b** (5 mM), purified *Pp*DpkA (2 U/mL), purified *Tb*ADH (1–5 U/mL), cofactor NADPH (8000 μM) or $NADP^+$ (5 μM), and isopropanol (6 mM) in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0). Reactions were incubated at 30 °C with 600 rpm shaking for 1 h.

[b] One unit (U) was defined as the amount of enzyme that converted 1 μmol of NADPH to $NADP^+$ per minute using **1b** as substrate at pH 8.0 and 30 °C.

[c] One unit (U) was defined as the amount of enzyme that converted 1 μmol of $NADP^+$ to NADPH per minute using isopropanol as substrate at pH 8.0 and 30 °C.

[d] Conversion (*c*) of **1b** to (*S*)-**1a** was determined by HPLC analysis.

[e] The *ee* was determined by chiral HPLC analysis, and configuration was determined by comparison with literature data or by analogue.

One-pot synthesis of (*S*)-carboxyl-substituted THIQs via a multi-enzymatic cascade

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Since the kinetic resolution of *rac*-**1a** employing *Fs*DAAO and the asymmetric synthesis of (*S*)-**1a** using the *Pp*DpkA-*Tb*ADH system have been demonstrated, we sought to develop a novel method for the synthesis of (*S*)-**1a** by combining these two reactions in one pot.

Table 4 Deracemization of *rac*-**1a**–**4a** employing *Fs*DAAO-*Pp*DpkA cascade [a]

Entry	Sub. (mM)	*Fs*DAAO (U/mL) [b]	*Pp*DpkA (U/mL) [c]	*Tb*ADH (U/mL) [d]	$NADP^+$ (μM)	Time (h)	Conv.[e] (*C*) (%)	*ee* (%)[f]/ Config. [f]
1	**1a** (10)	2	2	5	5	1	>99	>99 (*S*)
2	**1a** (50)	10	10	25	25	1	>99	>99 (*S*)
3	**1a** (100)	20	20	50	50	6	>95	>99 (*S*)
4	**2a** (10)	2	40	5	5	1	>99	>99 (*S*)
5	**3a** (10)	2	40	5	5	1	>99	>99 (*S*)
6	**4a** (5)	2	40	5	5	5	>99	>99 (*S*)

[a] Reaction conditions (1ml): racemic substrates (5-100 mM), purified *Fs*DAAO (2–20 U/mL), FAD (100 μM), catalase (100 times the activity of *Fs*DAAO), purified *Pp*DpkA (2–40 U/mL), $NADP^+$ (5–50 μM), purified *Tb*ADH (5–50 U/mL), and isopropanol (1.2 equivalents of **1b**–**4b**) in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0). Reactions were shaken at 30 °C, 600 rpm.

[b] One unit (U) was defined as the amount of enzyme that catalyzed the oxidation of 1 μmol (*R*)-**1a** per minute at pH 8.0 and 30 °C.

[c] One unit (U) was defined as the amount of enzyme that converted 1 μmol of NADPH to $NADP^+$ per minute using **1b** as substrate at pH 8.0 and 30 °C.

[d] One unit (U) was defined as the amount of enzyme that converted 1 μmol of $NADP^+$ to NADPH per minute using isopropanol as substrate at pH 8.0 and 30 °C.

[e] Conversion (*C*) of (*R*)-enantiomer to (*S*)-enantiomer was determined by chiral HPLC analysis. *C* = [(the residual (*S*)-**a** - the initial (*S*)-**a**)/the initial (*R*)-**a**] × 100%.

[f] The *ee* was determined by chiral HPLC analysis, and configuration was determined by comparison with literature data or by analogue.

To realize this process, we began our synthesis with a low concentration of substrate *rac*-**1a** (10 mM). Biotransformation was performed by combining purified *Fs*DAAO, *Pp*DpkA, and *Tb*ADH in one pot. Based on the feasibility and compatibility analysis, $NADP^+$ (5 μM) and isopropanol (6 mM) were used for the cofactor regeneration, which had no negative effect on the activity of *Fs*DAAO or *Pp*DpkA. Catalase was utilized to decompose the H_2O_2 generated by *Fs*DAAO. Chiral HPLC analysis revealed that (*S*)-**1a** was obtained with high conversion (>99%) and excellent enantioselectivity (>99% *ee*) within 1 h (Table 4, entry 1).

Encouraged by this result, we further investigated the application of this cascade with higher substrate concentrations. As expected, 50 mM of *rac*-**1a** was completely deracemized within 1 h (Table 4, entry 2). When the substrate concentration was 100 mM, the reaction efficiency decreased, which was due to the slight insolubility of **1a** at this high concentration. Prolonging the reaction time to 6 h, chiral HPLC analysis revealed that 95% of (*R*)-**1a** was transformed to (*S*)-**1a** with >99% *ee* (Table 4, entry 3).

Having established the *Fs*DAAO-*Pp*DpkA cascade in one pot, we were curious to explore whether this system would be applicable to derivatives of **1a**. The *Fs*DAAO-catalyzed kinetic resolution of racemic substrates **2a**–**4a** with different substituent groups (Cl, OH, OMe) on phenyl ring has been demonstrated.[16] However, the feasibility of the asymmetric reduction procedure was not certain. To investigate the substrate tolerance of *Pp*DpkA for phenyl substituted dihydroisoquinoline-1-carboxylic acids (**2b**–**4b**), step-by-step sequential reactions were performed. The purified *Fs*DAAO-catalyzed kinetic resolution was carried out separately at analytical scale. Upon completion of the reaction, the solution was incubated at 99 °C for 10 min to denature the enzymes. The purified *Pp*DpkA and NADPH were then added to the reaction mixture at 30 °C. After incubation for 1 h, chiral HPLC analysis revealed that the concentrations of (*S*)-**2a**–**4a** were significantly increased, and no (*R*)-enantiomers were observed. Our preliminary results demonstrated that compounds **2b**–**4b** could be reduced by *Pp*DpkA with excellent enantioselectivity (>99% *ee*), which indicated a broad and remarkable complementary substrate scope to IREDs and ATHase.[45]

Subsequently, the two reactions were performed simultaneously in one pot. As shown in Table 4, the racemic substrates **2a**–**4a** were successfully transformed into the corresponding (*S*)-enantiomers with high conversions (up to >99%) and excellent enantioselectivity (>99% *ee*) in 1 or 5 h. Therefore, the presented cascade successfully extended the biocatalytic toolbox for the synthesis of chiral THIQs.

Preparative-scale deracemization of *rac*-1a

To further access the synthetic potential of the *Fs*DAAO-*Pp*DpkA cascade, the one-pot deracemization of substrate **1a** (50 mM) was performed on a preparative scale. The reaction process was monitored by chiral HPLC. As shown in Fig. 1, the amount of (*R*)-**1a** decreased rapidly in the first 2 h, and the conversion of (*R*)-**1a** to (*S*)-**1a** reached 98% at 2.5 h. In the next 2.5 h, complete deracemization was achieved with >99% conversion and >99% *ee*. Finally, (*S*)-**1a** was obtained with good isolated yield (89%) and excellent enantioselectivity (>99% *ee*). In comparison with the previously described chemo-enzymatic deracemization, this multi-enzymatic cascade was more efficient owing to the direct reduction of **1b** to (*S*)-**1a**. Noteworthily, the removal of large excesses of the chemical reducing agent was circumvented,

which not only saves resources and operation time but also enables significant improvement of the yield.

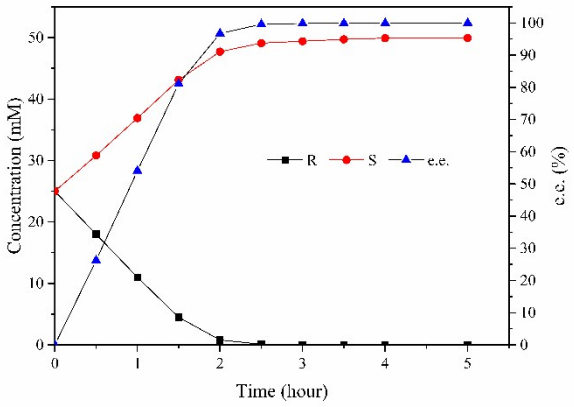

Fig. 1 The time-course study of multi-enzymatic deracemization of *rac*-**1a**.

Conclusions

In summary, we have shown the first application of DpkAs for the asymmetric reduction of 3,4-dihydroisoquinoline-1-carboxylic acids with high activity and excellent enantioselectivity, yielding the synthetically challenging (*S*)-1-carboxyl-substituted THIQs. By combining *Fs*DAAO and *Pp*DpkA in one pot, we developed a novel fully biocatalytic cascade approach for the deracemization of a range of racemic carboxyl-substituted THIQs to the corresponding (*S*)-enantiomers with high conversions (up to >99%) and excellent enantioselectivity (>99% *ee*). The cofactor NADPH regeneration system was essential for this process. Preparative-scale deracemization of substrate **1a** was demonstrated with good isolated yield (89%) and excellent enantioselectivity (>99% *ee*). Compared to the chemo-enzymatic method, the non-selective chemical reducing agent was replaced with environmentally benign biocatalysts in this multi-enzymatic cascade, thereby providing an alternative promising and sustainable route to the synthesis of optically pure carboxyl-substituted THIQs. Owing to the broad substrate scope of *Fs*DAAO and *Pp*DpkA, we anticipate that this approach might be further applied for the preparation of other pharmaceutically important chiral building blocks.

Experimental

Chemicals

Cofactor FAD, formaic acid (FA), trifluoroacetic acid (TFA) were purchased from Aladdin (Shanghai, China). Cofactor $NADP^+$ and NADPH were purchased from Bontac Bio-engineering Co., Ltd. (Shenzhen, China). Tryptone and yeast extract were obtained from Oxoid (Basingstoke, England). Substrates racemic 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrochloride (*rac*-**1a**) and (*R*)-**1a** were provided by Tongli Biomedical Co., Ltd. Substrates racemic 6-chloro-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrochloride (*rac*-**2a**) and racemic 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrochloride (*rac*-**4a**) were purchased from BOC sciences (Shirley, NY). Substrate racemic 6-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (*rac*-**3a**) was purchased from J&K scientific Ltd (Beijing, China). Substrate racemic 1-phenyl-1,2,3,4-tetrahydroisoquinoline was purchased from TCI (Shanghai) Development co., Ltd (China). Methanol (MeOH) and methyl cyanide (MeCN), diethyl amine (DEA) for HPLC, catalase from bovine liver, isopropyl-β-D-thiogalactopyranoside (IPTG), and kanamycin were purchased from Sigma-Aldrich (Munich, Germany). Genome DNA extraction kit, plasmid purification kit, DNA gel extraction kit, restriction endonucleases, T4 DNA ligase, and PrimerSTAR MAX DNA polymerase were purchased from Takara (Beijing, China). All other reagents and compounds used were of analytical grade and commercially available.

Cloning, expression, purification and characterization of enzymes

*Fs*DAAO, DpkAs and *Tb*ADH genes were cloned and overexpressed in *E. coli* BL21 (DE3). Their activities were detected using HPLC method or monitoring the changes in the absorbance of NADPH at 340 nm. Recombinant enzymes were purified by His-tag affinity chromatography. The enzymatic properties were characterized. All these methods were described in the Supporting Information in detail.

Analytical-scale asymmetric reduction of 1b and deracemization of *rac*-1a–4a

For the asymmetric reduction of substrate **1b**, a typical 1 mL reaction mixture contained substrate **1b** (5 mM), purified *Pp*DpkA (2 U/mL), purified *Tb*ADH (1–5 U/mL), cofactor NADPH (8 mM) or $NADP^+$ (5 μM), and isopropanol (6 mM) in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0). Reactions were incubated at 30 °C with 600 rpm shaking for 1 h.

The analytical-scale deracemization of racemic substrates were performed at pH 8.0 and 30 °C. Unless stated otherwise, a typical 1 mL reaction mixture contained substrate *rac*-**1a**–**4a** (5–100 mM), purified *Fs*DAAO (2–20 U/mL), FAD (100 μM), catalase (100 times the activity of *Fs*DAAO), purified *Pp*DpkA (2–40 U/mL), $NADP^+$ (5–50 μM), purified *Tb*ADH (5–50 U/mL), and isopropanol (1.2 equivalents of **1b**-**4b**) in Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 8.0).

Preparative-scale deracemization of *rac*-1a

The reaction was carried out in a 50 mL three-necked flask equipped with a magnetic stirrer. The *rac*-**1a** hydrochloride salt (214 mg, 1 mmol) were dissolved in 8 mL water. The pH was carefully adjusted to a value of pH 8.0 with ammonium hydroxide. Isopropanol (0.6 mmol) and $NADP^+$ (0.5 μmol) were added to the substrate solution. The lyophilized *E.coli* cells containing *Fs*DAAO or *Pp*DpkA or *Tb*ADH from 200 or 50 or 200 mL ferments was resuspended in 12.5 or 5 or 10 mL water, and the crude lysate was prepared by sonication and centrifugation. 5 or 1 or 6 mL crude lysate and 50 mg catalase were added to the substrate solution. The pH was kept at 8.0. The reactor was kept at 30 °C through a thermostatic water bath. The reaction was monitored by chiral HPLC. Upon the completion of reaction,

the proteins were removed through ultrafiltration. The solution were then evaporated off under vacuum, and the residue was washed with warm water. The solution was concentrated so that compound (*S*)-**1a** was recrystallized as white solid.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1. M. E. Welsch, S. A. Snyder and B. R. Stockwell, *Curr. Opin. Chem. Biol.*, 2010, **14**, 347-361.
2. I. P. Singh and P. Shah, *Expert. Opin. Ther. Pat.*, 2017, **27**, 17-36.
3. N. Kawai, R. Abe, M. Matsuda and J. Uenishi, *J. Org. Chem.*, 2011, **76**, 2102-2114.
4. S. Kotha, D. Deodhar and P. Khedkar, *Org. Biomol. Chem.*, 2014, **12**, 9054-9091.
5. W. Liu, S. Liu, R. Jin, H. Guo and J. Zhao, *Org. Chem. Front.*, 2015, **2**, 288-299.
6. I. Bułyszko, M. Chrzanowska, A. Grajewska and M. D. Rozwadowska, *Eur. J. Org. Chem.*, 2015, 383-388.
7. N. Kawai, N. Hoshiya, K. Kurata, K. Inoue, K. Nishimura and J. Uenishi, *Synthesis*, 2015, **47**, 1238-1244.
8. Y. Ji, L. Shi, M. W. Chen, G. S. Feng and Y. G. Zhou, *J. Am. Chem. Soc.*, 2015, **137**, 10496-10499.
9. M. Chrzanowska, A. Grajewska and M. D. Rozwadowska, *Chem. Rev.*, 2016, **116**, 12369-12465.
10. S. Gandomkar, E.-M. Fischereder, J. H. Schrittwieser, S. Wallner, Z. Habibi, P. Macheroux and W. Kroutil, *Angew. Chem. Int. Ed.*, 2015, **54**, 15051-15054.
11. J. Zhu, H. Tan, L. Yang, Z. Dai, L. Zhu, H. Ma, Z. Deng, Z. Tian and X. Qu, *ACS Catal.*, 2017, **7**, 7003-7007.
12. H. Li, P. Tian, J. H. Xu and G. W. Zheng, *Org. Lett.*, 2017, **19**, 3151-3154.
13. J. H. Schrittwieser, B. Groenendaal, S. C. Willies, D. Ghislieri, I. Rowles, V. Resch, J. H. Sattler, E.-M. Fischereder, B. Grischek, W.-D. Lienhart, N. J. Turner and W. Kroutil, *Catal. Sci. Technol.*, 2014, **4**, 3657-3664.
14. G. Li, P. Yao, R. Gong, J. Li, P. Liu, R. Lonsdale, Q. Wu, J. Lin, D. Zhu and M. T. Reetz, *Chem. Sci.*, 2017, **8**, 4093-4099.
15. G. A. Aleku, J. Mangas-Sanchez, J. Citoler, S. P. France, S. L. Montgomery, R. S. Heath, M. P. Thompson and N. J. Turner, *ChemCatChem*, 2018, **10**, 515-519.
16. S. Ju, M. Qian, G. Xu, L. Yang and J. Wu, *Adv. Synth. Catal.*, 2019, **361**, 3191-3199.
17. C. V. Voss, C. C. Gruber, K. Faber, T. Knaus, P. Macheroux and W. Kroutil, *J. Am. Chem. Soc.*, 2008, **130**, 13969-13972.
18. C. V. Voss, C. C. Gruber and W. Kroutil, *Angew. Chem. Int. Ed.*, 2008, **47**, 741-745.
19. Y.-P. Xue, Y.-G. Zheng, Y.-Q. Zhang, J.-L. Sun, Z.-Q. Liu and Y.-C. Shen, *Chem. Commun.*, 2013, **49**, 10706-10708.
20. B. Sheng, J. Xu, Y. Ge, S. Zhang, D. Wang, C. Gao, C. Ma and P. Xu, *ChemCatChem*, 2016, **8**, 2630-2633.
21. Y. P. Xue, H. Zeng, X. L. Jin, Z. Q. Liu and Y. G. Zheng, *Microb. Cell Fact.*, 2016, **15**, 162. View Article Online DOI: 10.1039/C9GC02795E
22. Z. Findrik and D. Vasic-Racki, *Biotechnol. Bioeng.*, 2007, **98**, 956-967.
23. S. YM, M. S, B. HS, K. YH, L. SH, K. BG and Y. H, *Org. Biomol. Chem.*, 2012, **10**, 2482-2485.
24. F. Khorsand, C. D. Murphy, A. J. Whitehead and P. C. Engel, *Green Chem.*, 2017, **19**, 503-510.
25. C. Walton, F. Parmeggiani, J. Barber, J. Mccann, N. J. Turner and R. Chica, *ChemCatChem*, 2018, **10**, 470-474.
26. D. Koszelewski, D. Pressnitz, D. Clay and W. Kroutil, *Org. Lett.*, 2009, **11**, 4810-4812.
27. K. Dominik, G. Barbara, S. M. Glueck, K. Wolfgang and F. Kurt, *Chem. Eur. J.*, 2011, **17**, 378-383.
28. G. Shin, S. Mathew, M. Shon, B. G. Kim and H. Yun, *Chem. Commun.*, 2013, **49**, 8629-8631.
29. E. O'Reilly, C. Iglesias and N. J. Turner, *ChemCatChem*, 2014, **6**, 992-995.
30. R. S. Heath, M. Pontini, S. Hussain and N. J. Turner, *ChemCatChem*, 2016, **8**, 117-120.
31. V. Kohler, Y. M. Wilson, M. Durrenberger, D. Ghislieri, E. Churakova, T. Quinto, L. Knorr, D. Haussinger, F. Hollmann, N. J. Turner and T. R. Ward, *Nat. Chem.*, 2013, **5**, 93-99.
32. Y. Okamoto, V. Köhler and T. R. Ward, *J. Am. Chem. Soc.*, 2016, **138**, 5781-5784.
33. S. Hussain, F. Leipold, H. Man, E. Wells, S. P. France, K. R. Mulholland, G. Grogan and N. J. Turner, *ChemCatChem*, 2015, **7**, 579-583.
34. G. A. Aleku, H. Man, S. P. France, F. Leipold, S. Hussain, L. Toca-Gonzalez, R. Marchington, S. Hart, J. P. Turkenburg, G. Grogan and N. J. Turner, *ACS Catal.*, 2016, **6**, 3880-3889.
35. H. Li, G.-X. Zhang, L.-M. Li, Y.-S. Ou, M.-Y. Wang, C.-X. Li, G.-W. Zheng and J.-H. Xu, *ChemCatChem*, 2016, **8**, 724-727.
36. G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan and N. J. Turner, *Nat. Chem.*, 2017, **9**, 961-969.
37. G. Grogan and N. J. Turner, *Chem. Eur. J.*, 2016, **22**, 1900-1907.
38. J. Mangas-Sanchez, S. P. France, S. L. Montgomery, G. A. Aleku, H. Man, M. Sharma, J. I. Ramsden, G. Grogan and N. J. Turner, *Curr. Opin. Chem. Biol.*, 2017, **37**, 19-25.
39. J. H. Schrittwieser, S. Velikogne and W. Kroutil, *Adv. Synth. Catal.*, 2015, **357**, 1655–1685.
40. M. Goto, H. Muramatsu, H. Mihara, T. Kurihara, N. Esaki, R. Omi, I. Miyahara and K. Hirotsu, *J. Biol. Chem.*, 2005, **280**, 40875-40884.
41. H. Muramatsu, H. Mihara, R. Kakutani, M. Yasuda, M. Ueda, T. Kurihara and N. Esaki, *J. Biol. Chem.*, 2005, **280**, 5329-5335.
42. J. Muschiol, C. Peters, N. Oberleitner, M. D. Mihovilovic, U. T. Bornscheuer and F. Rudroff, *Chem. Commun.*, 2015, **51**, 5798-5811.
43. J. H. Schrittwieser, S. Velikogne, M. Hall and W. Kroutil, *Chem. Rev.*, 2018, 118, 270-348.
44. M. Peretz, O. Bogin, S. Tel-Or, A. Cohen, G. Li, J.-S. Chen and Y. Burstein, *Anaerobe*, 1997, **3**, 259-270.
45. D. Gamenara and P. Dominguez de Maria, *Org. Biomol. Chem.*, 2014, **12**, 2989-2992.

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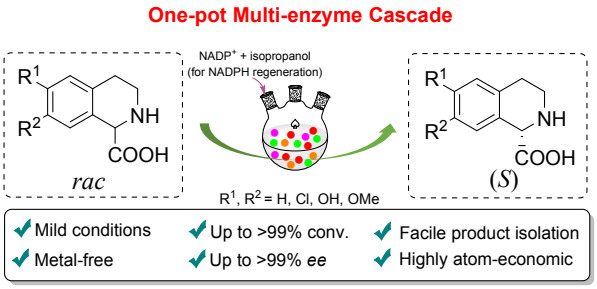

We describe a biocatalytic redox cascade reaction for one-pot enantioselective synthesis of (*S*)-carboxyl substituted tetrahydroisoquinolines.