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Bioproduction of Enantiopure (*R*)- and (*S*)-2-Phenylglycinols from Styrenes and Renewable Feedstocks

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Abstract. Enantiopure (*R*)- and (*S*)-2-phenylglycinols are important chiral building blocks for pharmaceutical manufacturing. Several chemical and enzymatic methods for their synthesis were reported, either involving multi-step synthesis or starting from a relatively complex chemical. Here, we developed one-pot simple syntheses of enantiopure (*R*)- and (*S*)-2-phenylglycinols from cheap starting materials and renewable feedstocks. Enzyme cascades consisting of epoxidation-hydrolysis-oxidation-transamination were developed to convert styrene **2a** to (*R*)- and (*S*)-2-phenylglycinol **1a**, with butanediol dehydrogenase for alcohol oxidation as well as BmTA and NfTA for (*R*)- and (*S*)-enantioselective transamination, respectively. The engineered *E. coli* strains expressing the cascades produced 1015 mg/L (*R*)-**1a** in >99% *ee* and 315 mg/L (*S*)-**1a** in 91% *ee*, respectively, from styrene **2a**. The same cascade also converted substituted styrenes **2b-k** and indene **2l** into substituted (*R*)-phenylglycinols **1b-k** and (1*R*, 2*R*)-1-amino-2-indanol **1l** in 95->99% *ee*. To transform bio-based *L*-phenylalanine **6** to (*R*)-**1a** and (*S*)-**1a**, (*R*)- and (*S*)-enantioselective enzyme cascades for decarboxylation-deamination-epoxidation-hydrolysis-oxidation-transamination were developed. The engineered *E. coli* strains produced (*R*)-**1a** and (*S*)-**1a** in high *ee* at 576 mg/L and 356 mg/L, respectively, from *L*-phenylalanine **6**, as the first synthesis of these compounds from a bio-based chemical. Finally, *L*-phenylalanine biosynthesis pathway was combined with (*R*)- or (*S*)-enantioselective cascade in one strain or coupled strains, to achieve the first synthesis of (*R*)-**1a** and (*S*)-**1a** from a renewable feedstock. The coupled strain approach enhanced the production, affording 274 and 384 mg/L (*R*)-**1a** and 274 and 301 mg/L (*S*)-**1a**, from glucose and glycerol, respectively. The developed methods could be potentially useful to produce these high-value chemicals from cheap starting materials and renewable feedstocks in a green and sustainable manner.

Keywords: Amino alcohols; Biocatalysis; Enantioselectivity; Enzyme catalysis; One-pot reaction; Renewable resources

Introduction

Enantiopure β-amino alcohols are important high value chemicals used in the preparation of pharmaceutics[1] such as randolazine,[2] nebivolol and metoprolol,[3] as a ligand for chiral catalysts in asymmetric synthesis, and as a chiral building block in the synthesis of other chiral compounds. Specifically, (*R*)-2-phenylglycinol **1a** is used in the synthesis of novel antibiotic pyrazolopyrimidines, EGFR inhibitor thienopyrimidines, and 3-phosphoinositide-dependent protein kinase-1 (PDK1) inhibitor.[1e] (*S*)-2-phenylglycinol **1a** is used in the synthesis of a neurotrophic agent, potent histone deacetylase inhibitors, novel anti-cancer asymmetric triplex metallohelices[1b] and as chiral ligands for asymmetric annulation or cyclization. Both (*R*)- and (*S*)-2-phenylglycinol **1a** are high value compounds with the price of $40-$100 per kg. Moreover, the substituted (*R*)-2-phenylglycinols **1b-lk** (Figure 1a) are also useful pharmaceutical intermediates, for instance, (*R*)-2-amino-2-(4-methoxyphenyl)ethanol **1k** and (1*R*,2*R*)-1-amino-2-indanol **1l** are used in the synthesis of agonists and Alk5 inhibitors to treat cancers.[4]

Several chemical methods were established for the synthesis of (*R*)- and (*S*)-**1a**; however, these methods have one or more disadvantages. For instance, methods for the reduction of *D*- or *L*-phenylglycine to (*R*)- or (*S*)-**1a** require expensive enantiopure substrates and utilizes hazardous chemicals.[5]

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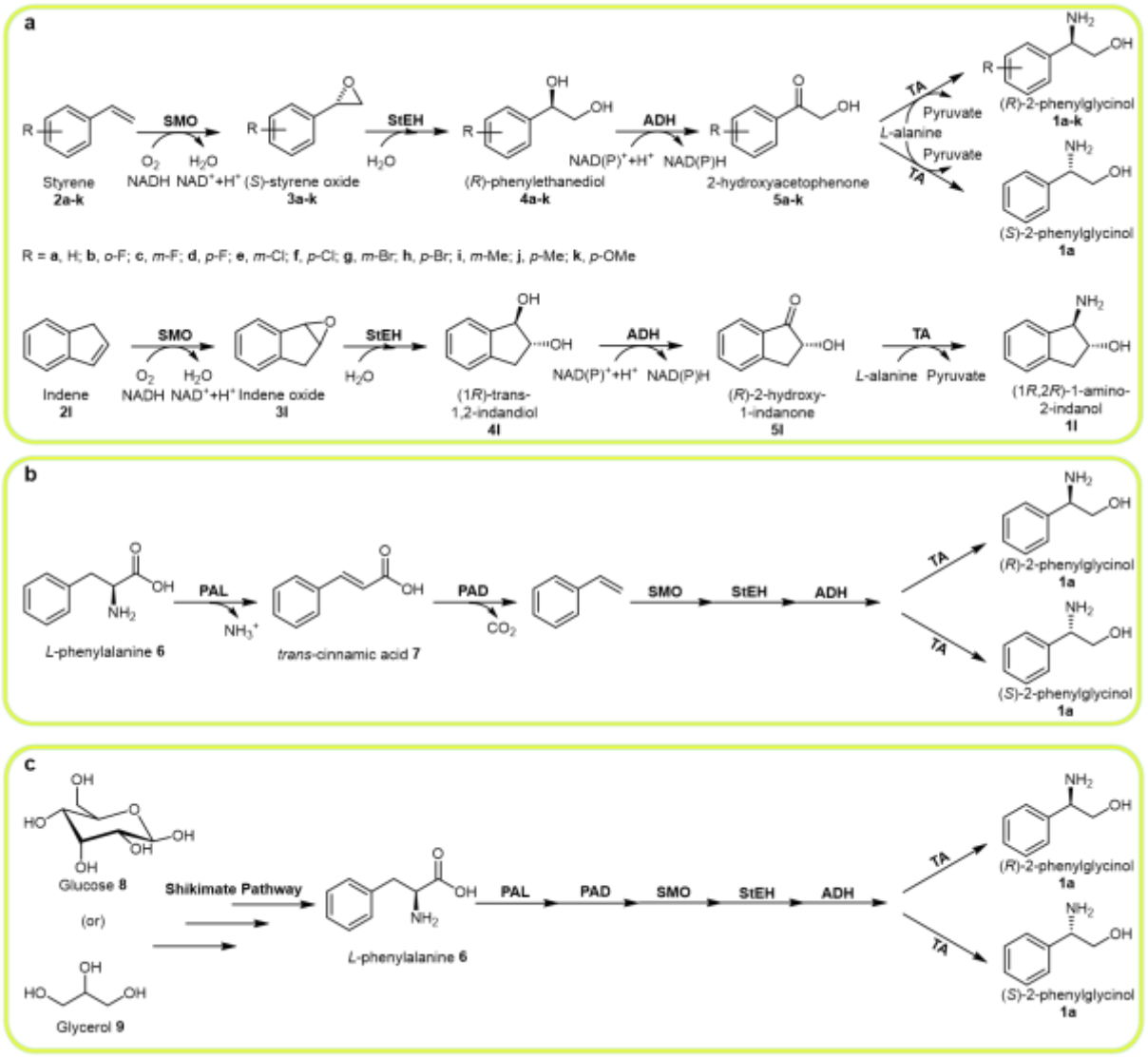

Figure 1: Production of enantiopure (*R*)-2-phenylglycinols **1a-l** and (*S*)-2-phenylglycinol **1a** from styrenes **2a-k**, indene **2l**, bio-based *L*-phenylalanine **6** and renewable feedstocks glucose **8** and glycerol **9**. (a) Artificial enzyme cascade for the conversion of styrene **2a-k**, indene **2l** to (*R*)-**1a-l** and (*S*)-**1a** *via* one-pot epoxidation-hydrolysis-oxidation-amination reaction. (b) Artificial enzyme cascade for the biotransformation of *L*-phenylalanine **6** to (*R*)- and (*S*)-**1a** *via* one-pot deamination-decarboxylation-epoxidation-hydrolysis-oxidation-amination reaction. (c) Conversion of glucose **8** and glycerol **9** to (*R*)- and (*S*)-**1a** *via* the combination of natural Shikimate pathway for the synthesis of **6** and the artificial enzyme cascade for the conversion of **6** to (*R*)- and (*S*)-**1a.** Enzymes involved are styrene monooxygenase (SMO), epoxide hydrolase (StEH), alcohol dehydrogenase (ADH), transaminase (TA), phenylalanine ammonia lyase (PAL) and phenylacrylic acid decarboxylase (PAD).

Methods for the kinetic resolution of racemic **1a** to obtain enantiopure (*R*)- or (*S*)-**1a** suffers from low theoretical yield (50%).[6] Enzyme catalysis is attractive due to high regio- and stereo-selectivity, mild reaction conditions, and environmental friendliness.[7] However, the enzymatic approaches developed for the synthesis of (*R*)- and (*S*)-**1a** also have drawbacks. For example, kinetic resolution of racemic **1a** using transaminase suffers from low theoretical yield (50%).[8] Amination of 2-hydroxyacetophenone **5a** using transaminase to give (*R*)-**1a** requires special substrate.[9] Recently, one-pot cascade biotransformation of styrene oxide **3a** to (*R*)-**1a**[10] and styrene oxides **3a**, **d**, **f**, **h** to (*S*)-**1a**, **d**, **f**, **h**[11] were reported, but needs to synthesize the starting material and solve the inhibition by the substrates. In this study, we are interested in developing the enzymatic method for the synthesis of enantiopure β-amino alcohols such as (*R*)-**1a** and (*S*)-**1a** from renewable feedstocks as well as (*R*)-**1a-l** and (*S*)-**1a** from cheap and easily available starting materials.

To reach our goal we are focusing on the development of one-pot cascade biotransformation which is an emerging technique for the production of high-value chemicals from cheap substrates and renewable feedstocks. This technique uses several enzymes in one-pot to perform multi-step reactions under mild reaction conditions, being of advantageous over traditional multi-step synthesis

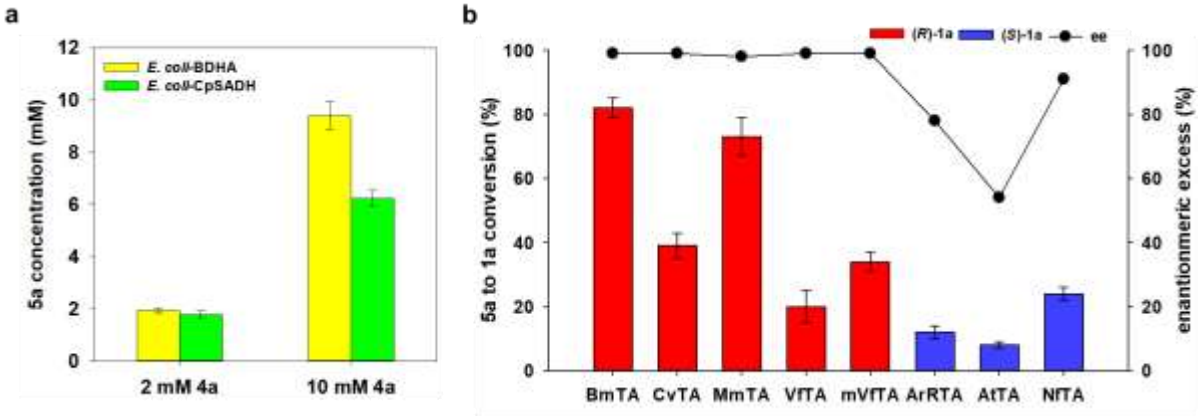

Figure 2: (a) Production of hydroxyacetophenone **5a** from 2 and 10 mM of (*R*)-phenylethanediol **4a** by *E. coli*-BDHA or *E. coli*-CpSADH (10 g cdw/L). (b) Screening of transaminases for the conversion of **5a** to (*R*)- or (*S*)- 2-phenylglycinol **1a** by biotransformation of 10 mM **5a** with *E. coli* strains expressing transaminases (20 g cdw/L) in phosphate buffer (200 mM, pH 8) containing 0.5% glucose and 200 mM amino donor. Biotransformations were performed in duplicates with error bars showing ± SD.

without product recovery in each step.[7c, 12] Several studies were reported on one-pot whole-cell biotransformation for the production of high-value chemicals such as enantiopure amino acids, α-hydroxy acids, amino alcohols and aroma chemicals from cheap and renewable substrates in an economical and sustainable manner.[12f, 13] Here we report the development of artificial enzyme cascades for the one-pot synthesis of enantiopure (*R*)-**1a-l** and (*S*)-**1a** from cheap substrate styrenes **2a-l**, further engineering of the cascade to convert bio-based *L*-phenylalanine **6** to (*R*)- and (*S*)-**1a**, and the combination of natural *L*-phenylalanine biosynthesis pathway with artificial enzyme cascade for the conversion of renewable feedstocks as glucose **8** and glycerol **9** to (*R*)- and (*S*)-**1a**.

Results and Discussion

Design of cascade biotransformation for the production of (*R*)- and (*S*)-2-phenylglycinol 1a from styrene 2a

To convert styrene **2a** to (*R*)- and (*S*)- 2-phenylglycinol **1a**, we designed artificial cascade with four enzymes for the epoxidation-hydrolysis-oxidation-transamination reactions (Figure 1a). Conversion of styrene **2a** to (*R*)-phenylethanediol **4a** by epoxidation-hydrolysis reaction were achieved by styrene monoxygenase (SMO) from *Pseudomonas* sp. and epoxide hydrolase (StEH) from *Solanum tuberosum*.[13f, 14] Conversion of **4a** to 2-hydroxyacetophenone **5a** by oxidation reaction could be achieved by the use of alcohol dehydrogenase. Amination of **5a** to (*R*)- and (*S*)-2-phenylglycinol **1a**, respectively, could be achieved by using (*R*) and (*S*)-enantioselective transaminases.

For the oxidation of (*R*)-phenylethanediol **4a** to 2-hydroxyacetophenone **5a**, butanediol dehydrogenase (BDHA) from *Bacillus subtilis* BGSC1A1 and secondary alcohol dehydrogenase (CpSADH) from *Candida parapsilosis* were examined. These enzymes were reported for the efficient production of hydroxy ketones.[12f, 13d, 15] The genes coding for BDHA and CpSADH were cloned in individual pET28a plasmid under the control of T7 promoter, and the two recombinant plasmids were transformed into separate *E. coli* T7 strains to obtain *E. coli*-BDHA expressing BDHA and *E. coli*-CpSADH expressing CpSADH, respectively. The resting cells of *E. coli*-BDHA and *E. coli*-CpSADH (10 g cdw/L) were evaluated for oxidation of 2 mM of **4a**, giving 95-100% conversion to **5a** for both cases (Figure 2a). The concentration of **4a** was increased to 10 mM, and *E. coli*-BDHA produced 9.4 mM **5a** with 94% conversion within 12 h, whereas *E. coli*-CpSADH produced only 6.24 mM of **5a** with 62% conversion at the same time. Therefore, BDHA was chosen as the enzyme for the oxidation of **4a** in the cascade biotransformations.

For the conversion of **5a** to (*R*)-**1a**, databases were searched for (*S*)-enantioselective transaminases with substrate preference similar to **5a** and simple amino donors such as glutamate, *L*-alanine and isopropylamine. Eight (*S*)-enantioselective transaminases such as HnTA, DpgTA, EeaTA, VfTA, CvTA, mVfTA[9], BmTA and MmTA were identified with substrate preference similar to **5a**. The genes coding for eight (*S*)-enantioselective transaminases were cloned in individual pRSFDuet-1 plasmid and transformed into *E. coli* T7 strains, each expressing one of eight different transaminases. 10 g cdw/L of resting cells of *E. coli* strains expressing eight transaminases were used for the biotransformation of 10 mM **5a** in KP buffer containing 0.5% glucose and 200 mM amino donor (*D*-, *L*-alanine, glutamate) for 24 h.

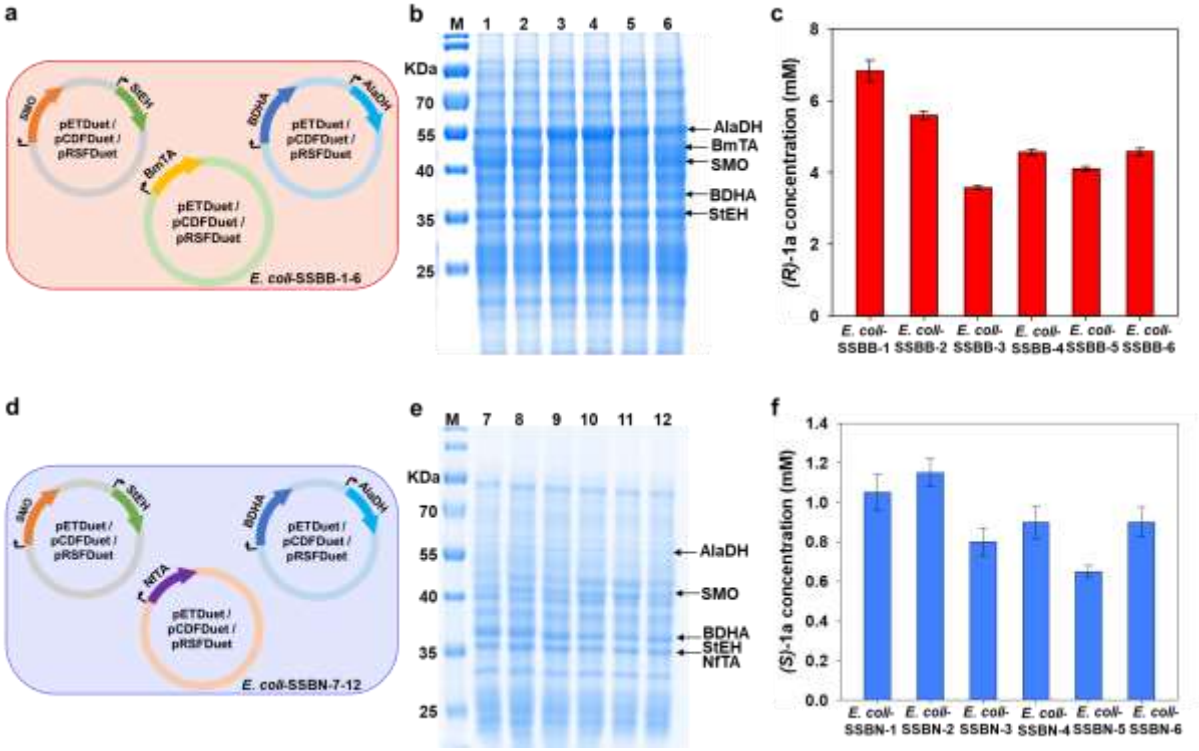

Figure 3: Cascade biotransformation of styrene **2a** to (*R*)- and (*S*)-**1a** using *E. coli* strains expressing the artificial enzyme cascade shown in Figure 1a. (a) *E.* coli-SSBB-1-6 harboring three expression plasmids with different copy numbers for the expression of SMO, StEH, BDHA, AlaDH and BmTA at various expression levels for producing (*R*)-**1a**. (b) SDS-PAGE analysis of crude extracts of *E. coli*-SSBB-1-6 showing the expression of enzymes for producing (*R*)-**1a**. Lane M, protein ladder; Lane 1-6, *E. coli*-SSBB-1-6. (c) Synthesis of (*R*)-**1a** from 10 mM **2a** by *E. coli*-SSBB-1-6 (20 g cdw/L) at 30°C for 24 h. (d) *E. coli*-SSBN-1-6 harboring three expression plasmids with different copy numbers to express SMO, StEH, BDHA, AlaDH and NfTA at various expression levels for producing (*S*)-**1a**. (e) SDS-PAGE analysis of crude extracts of *E. coli*-SSBN-1-6 showing the expression of enzymes for producing (*S*)-**1a**. Lane M, protein ladder; Lane 7-12, *E. coli*-SSBN-1-6. (f) Synthesis of (*S*)-**1a** from 5 mM **2a** by *E. coli*-SSBN-1-6 (20 g cdw/L) at 30°C for 48 h. Reaction conditions: Two-phase system of 2 mL phosphate buffer (200 mM, pH 8) and 2 mL *n*-hexadecane containing **2a**. Biotransformations were performed in duplicates with error bars showing ± SD.

As shown in Figure 2b, several transaminases were able to convert **5a** to (*R*)-**1a** and the best result was obtained from *E. coli*-BmTA expressing the transaminase from *Bacillus megaterium*. BmTA was able to convert 10 mM **5a** to (*R*)-**1a** with 82% efficiency and >99% *ee* with *L*-alanine as amino donor. BmTA was thus chosen for the artificial cascade for the production of (*R*)-**1a**. Similarly, (*R*)-enantioselective transaminases were searched in database and three transaminase candidates such as NfTA, ArRTA and AtTA were identified for the examination on the conversion of **5a** to (*S*)-**1a**. The selected transaminases were cloned in individual pRSFDuet-1 plasmid, and transformed into three different *E. coli* T7 strains. 10 g cdw/L of the resting cells of *E. coli* strains expressing three (*R*)-enantioselective transaminases were used for the biotransformation of 10 mM **5a** in KP buffer containing 0.5% glucose and 200 mM amino donor (*L*-alanine, isopropylamine) for 24 h. As shown in figure 2a, only *E. coli*-NfTA expressing transaminase from *Neosartora fischeri* was able to convert **5a** to (*S*)-**1a** with relatively high efficiency. NfTA was able to convert 10 mM **5a** to (*S*)-**1a** in 24% and 91% *ee* with *L*-alanine as amino donor. Therefore, NfTA was chosen for the artificial cascade for the production of (*S*)-**1a**.

Engineering of *E. coli* co-expressing 5 enzymes of the artificial cascade for the conversion of styrene 2a to (*R*)- and (*S*)-2-phenylglycinol 1a

For the efficient whole cell biotransformation of **2a** to (*R*)- and (*S*)-**1a** with a single strain, combinatorial approach was attempted for the expression of enzymes in the artificial cascade. Combinatorial approach facilitates to obtain maximum conversion efficiency by optimizing the expression level of multiple enzymes in the cascade. This could be achieved by expressing the enzymes of the artificial cascade from three plasmids (pCDFDuet-1, pETDuet-1 and pRSFDuet-1) with varying copy numbers of 10 to >100.[16] Different combinations of these plasmids could result in different expression levels of the enzymes.[14a, 17] Thus, SMO and StEH for the conversion of **2a** to **4a** were cloned together in

Table 1: Cascade biotransformation of styrenes **2a**-**2k** and indene **2l** to (*R*)-2-phenylglycinols-**1a**-**1k** and (1*R*,2*R*)-1-amino-2-indanol **1l** with resting cells of *E. coli*-SSBB-1

Substrate [a]	R	Substrate conc. [b] (mM)	Cell conc. (g cdw L^{-1})	Product	Conv. [c] (%)	*ee* [c] (%)
2a	H	10	20	**1a**	74	>99
2b	*o*-F	10	20	**1b**	57	>99
2c	*m*-F	10	20	**1c**	69	>99
2d	*p*-F	10	20	**1d**	73	99
2e	*m*-Cl	10	20	**1e**	56	>99
2f	*p*-Cl	5	25	**1f**	53	>99
2g	*m*-Br	5	25	**1g**	34	95
2h	*p*-Br	5	25	**1h**	22	>99
2i	*m*-Me	10	20	**1i**	67	>99
2j	*p*-Me	5	25	**1j**	57	>99
2k	*p*-OMe	5	25	**1k**	61	>99
2l	-	5	25	**1l**	24	>99

[a] Substrates **2a**-**2l** were converted to **1a**-**1l** by *E. coli* SSBB-1 (20 g cdw/L) at 30°C for 48 h in a two-phase system of 2 mL phosphate buffer (200 mM, pH 8) and 0.4 mL *n*-hexadecane containing **2a**-**2l**. [b] normalized to aqueous phase volume. [c] Concentration and enantiomeric excess of **1a**-**1l** were analysed using reverse phase HPLC with SB-C18 and Crownpak CR (+) chiral column, respectively. Biotransformations were performed in duplicates and the mean values were presented.

pCDFDuet-1, pETDuet-1 and pRSFDuet-1. BDHA for the conversion of **4a** to **5a** and alanine dehydrogenase[13f] for recycling amino donor (*L*-alanine) were cloned together in pCDFDuet-1, pETDuet-1 and pRSFDuet-1. BmTA for (*R*)-**1a** or NfTA for (*S*)-**1a** production from **5a** was cloned in pCDFDuet-1, pETDuet-1 and pRSFDuet-1. The recombinant pCDFDuet-1, pETDuet-1 and pRSFDuet-1 plasmids were transformed into twelve different *E. coli* strains so that all five enzymes are present in compatible plasmids in each *E. coli* strain (Figure 3a, 3d and Table S1). The *E. coli* strains containing the five-enzyme cascade for the conversion of **2a** to (*R*)-**1a** and (*S*)-**1a**, were named *E.* coli-SSBB-1-6 and *E. coli*-SSBN-1-6, respectively. SDS-PAGE analysis of *E.* coli-SSBB-1-6 and *E. coli*-SSBN-1-6 were performed, which confirmed the expression of all five enzymes in the artificial cascade (Figure 3b, 3e). The expression of BmTA was relatively higher in *E.* coli-SSBB-1 and *E.* coli-SSBB-2 compared to other strains.

One-pot biotransformation of styrene 2a to (*R*)- and (*S*)-2-phenylglycinol 1a with *E. coli* co-expressing 5 enzymes of artificial cascade

One-pot biotransformation of 10 mM **2a** to (*R*)-**1a** was performed with resting cells of *E.* coli-SSBB-1-6 (20 g cdw/L) at 30°C for 24 h. As shown in Figure 3c, difference in copy numbers of expression vectors showed huge variation in the synthesis of (*R*)-**1a** by *E.* coli-SSBB-1-6. *E. coli*-SSBB-1 (pCDF-SMO-StEH, pET-BDHA- AlaDH, pRSF-BmTA) was the best strain to produce 6.83 mM of (*R*)-**1a** (>99% *ee* and 68% conversion) from **2a** (Figure 3c). One-pot biotransformation of 5 mM **2a** to (*S*)-**1a** was performed with resting cells of *E.* coli-SSBN-1-6 (20 g cdw/L) at 30°C for 48 h. As shown in Figure 3f, *E. coli*-SSBN-2 (pCDF-SMO-StEH, pET-NFTA, pRSF-BDHA- AlaDH) showed the highest conversion of 23%. As NfTA was not efficient like BmTA, relatively low production of (*S*)-**1a** was observed in *E. coli*-SSBN-1-6. Evolution of NfTA and search for new *R*-selective transaminase for further improvement of (*S*)-**1a** production is under progress.

To further improve the conversion of **2a** to (*R*)-**1a** by *E.* coli-SSBB-1, the reaction conditions were optimized (Table S2). Firstly, the biotransformation of **2a** to (*R*)-**1a** was performed with *E.* coli-SSBB-1 (20 g cdw/L) with 0- or 100-mM supplementation of *L*-alanine. *E. coli* naturally produces *L*-alanine and *E.* coli-SSBB-1 express alanine dehydrogenase for recycling *L*-alanine using NH_3/NH_4Cl, therefore the supplementation of *L*-alanine might not be necessary. In fact, same conversion efficiency (68-69%) was obtained with or without *L*-alanine supplementation. Secondly, the organic phase *n*-hexadecane was replaced by ethyl oleate and the synthesis of (*R*)-**1a** from **2a** was evaluated. As *n*-hexadecane is obtained from petroleum, a greener and biocompatible organic phase such as ethyl oleate which can be obtained from biodiesel was tested. No significant difference was observed in the conversion (68-69%) suggesting that ethyl oleate could be used as a greener alternative. Further, the volume of organic phase (*n*-hexadecane or ethyl oleate) was reduced by changing the ratio of organic phase: phosphate buffer ratio from 1:1 to 0.2:1. Improved conversion of **2a** to (*R*)-**1a** was observed with both *n*-hexadecane: phosphate buffer and ethyl oleate: phosphate buffer in 0.2:1 ratio, respectively. 74% conversion of **2a** to (*R*)-**1a** was achieved in biotransformation with *n*-hexadecane: phosphate buffer in 0.2:1 ratio. Reduced volume of organic phase usage increased the conversion efficiency and could also contribute to reducing operation cost in large-scale production.

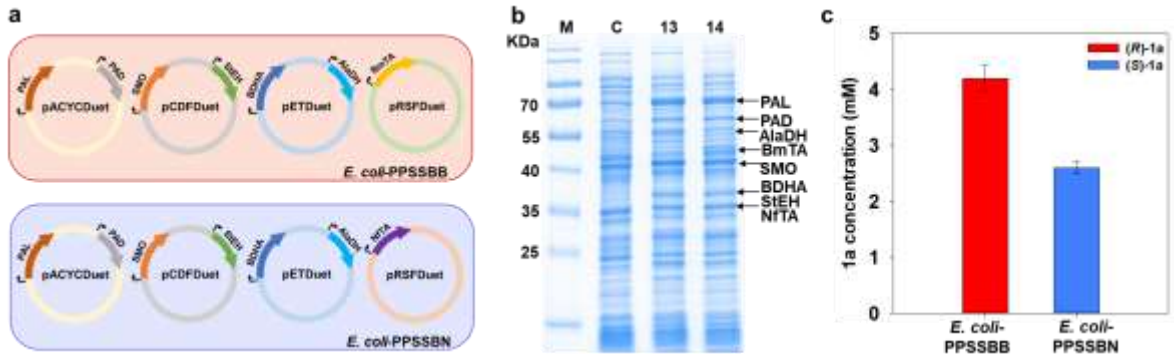

Figure 4: Cascade biotransformation of *L*-phenylalanine **6** to (*R*)- and (*S*)-**1a** using *E. coli* strains expressing the artificial enzyme cascade shown in Figure 1b. (a) *E. coli*-PPSSBB and *E. coli*-PPSSBN harboring four expression plasmids for the expression of PAL, PAD, SMO, StEH, BDHA, AlaDH and BmTA or NfTA for the production of (*R*)- or (*S*)-**1a**, respectively. (b) SDS-PAGE analysis of crude extracts of *E. coli*-PPSSBB and *E. coli*-PPSSBN showing the expression of enzymes for producing (*R*)- or (*S*)-**1a**. Lane M, protein ladder; Lane C, *E. coli* T7 (negative control); Lane 13, *E. coli*-PPSSBB; Lane 14, *E. coli*-PPSSBN. (c) Synthesis of (*R*)- and (*S*)-**1a** from 10 mM **6** by *E. coli*-PPSSBB and *E. coli*-PPSSBN (15 g cdw/L), respectively in a two-phase system of 5 mL phosphate buffer (200 mM, pH 8) containing **6** and 1 mL *n*-hexadecane at 30°C for 24 h. Biotransformations were performed in duplicates with error bars showing ± SD.

One-pot biotransformation of styrenes 2a-2k and indene 2l to (*R*)-phenylglycinols 1a-1k and (1*R*,2*R*)-1-amino-2-indanol 1l with *E. coli* co-expressing 5 enzymes of artificial cascade

Substituted (*R*)-phenylglycinols **1a-1k** and (1*R*,2*R*)-1-amino-2-indanol **1l** are also important building blocks in the synthesis of pharmaceutics. Therefore, *E. coli*-SSBB-1 cells were examined for the conversion of substituted styrenes **2a-k** and indene **2l** to (*R*)-**1a-1k** and (1*R*,2*R*)-1-amino-2-indanol **1l**. One-pot biotransformation was performed in the two-phase system with *n*-hexadecane and phosphate buffer (0.2:1) with 5-10 mM **2a-2l**, *E. coli*-SSBB-1 (20 g cdw/L) and the conversion to **1a-1l** were listed in Table 1. All the tested substrates (**2a-2k**) were converted to their respective (*R*)-phenylglycinols (**1a-1k**) and (1*R*,2*R*)-1-amino-2-indanol **1l** in 95->99% *ee*. Among the eleven substituted substrates, eight substrates were converted to their respective (*R*)-phenylglycinols with >50% conversion. Specifically, **2c**, **2d**, **2i**, **2k** showed >60% conversion. Thus, the developed artificial cascade is useful for the production of various (*R*)-2-phenylglycinols **1a-1k** and (1*R*,2*R*)-1-amino-2-indanol **1l**.

One-pot biotransformation of styrenes **2a**, **2d**, **2e**, **2j**, **2k** to (*R*)-2-phenylglycinols **1a**, **1d**, **1e**, **1j**, **1k** were scaled up. Preparative biotransformation was performed with resting cells of *E. coli*-SSBB-1 (25 g cdw/L) in 100 mL phosphate buffer with 20 mL of *n*-hexadecane containing **2a**, **2d**, **2e**, **2j**, **2k** (50 mM, normalized to aqueous phase volume). After reaction for 48 h at 30°C, (*R*)- **1a**, **1d**, **1e**, **1j** and **1k** were isolated from the aqueous phase in high purity with 59%, 50%, 37%, 32%, and 44% yield, respectively.

Design of cascade biotransformations and development of resting-cells bioprocess to convert bio-based *L*-phenylalanine 6 to (*R*)- and (*S*)-2-phenylglycinol 1a

For the conversion of bio-based *L*-phenylalanine **6** to enantiopure (*R*)- and (*S*)-**1a**, artificial enzyme cascade consisting of decarboxylation-deamination-epoxidation-hydrolysis-oxidation-transamination, Phenylalanine ammonia lyase (PAL) of *Arabidopsis thaliana* and phenylacrylic acid decarboxylase (PAD) of *Saccharomyces cerevisiae* were expressed together in the cascade for **2a** to (*R*)- and (*S*)-**1a** (Figure 1b). We previously proved that PAL and PAD efficiently produces **2a** from **6**.[13a, 13c, 14a, 18] Thus, PAL and PAD enzymes cloned in pACYCDuet-1 (pACYC-PAL-PAD)[13c] were co-expressed in *E. coli*-SSBB-1 (pCDF-SMO-StEH, pET-BDHA-AlaDH, pRSF-BmTA) and *E. coli*-SSBN-2 (pCDF-SMO-StEH, pET-NFTA, pRSF-BDHA-AlaDH) and the resulted recombinant strains were named as *E. coli*-PPSSBB and *E. coli*-PPSSBN, respectively (Figure 4a). SDS-PAGE analysis of the crude cell lysate of *E. coli*-PPSSBB and *E. coli*-PPSSBN was performed (Figure 4b), showing the expression of all seven enzymes of the artificial cascade in *E. coli*-PPSSBB and *E. coli*-PPSSBN.

Cascade biotransformation of 10 mM **6** to (*R*)- and (*S*)-**1a** was performed with the resting cells (20 g cdw/L) of *E. coli*-PPSSBB and *E. coli*-PPSSBN, respectively, in the two-phase system with *n*-hexadecane and phosphate buffer (0.2:1). After 24 h of reaction at 30°C, 576 mg/L (*R*)-**1a** and 356 mg/L (*S*)-**1a** was produced by *E. coli*-PPSSBB and *E. coli*-PPSSBN, respectively (Figure 4c). This is the first report on the synthesis of (*R*)- and (*S*)-**1a** from bio-based substrate **6**. Since the accumulation of intermediates such as **4a** and **5a** was observed, further enhancing the productivity might be achieved by improving the activity of the alcohol dehydrogenase and transaminase.

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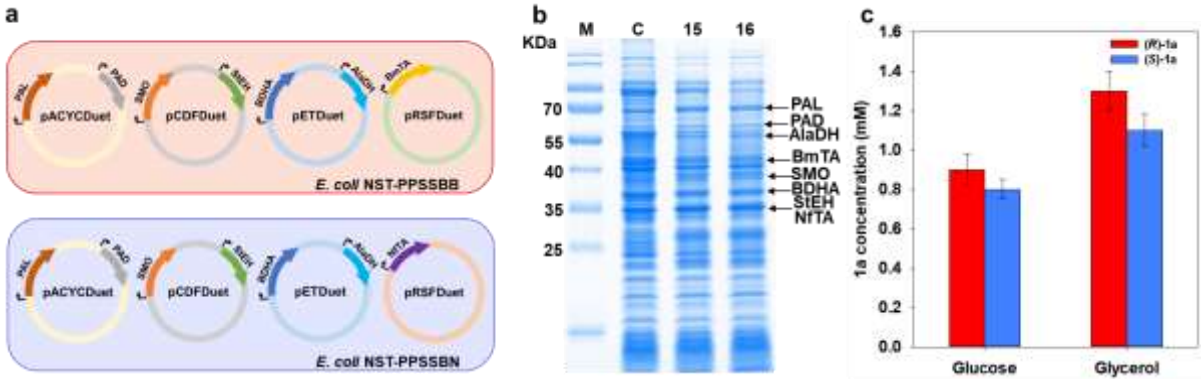

Figure 5: Fermentation of glucose **8** or glycerol **9** to (*R*)- and (*S*)-**1a** using *E. coli* NST74(DE3) strains expressing the artificial enzyme cascade shown in Figure 1c. (a) *E. coli* NST-PPSSBB and *E. coli* NST-PPSSBN harboring engineered Shikimate pathway and four expression plasmids for the expression of PAL, PAD, SMO, StEH, BDHA, AlaDH and BmTA or NfTA for the production of (*R*)- or (*S*)-**1a**, respectively. (b) SDS-PAGE analysis of crude extracts of *E. coli* NST-PPSSBB and *E. coli* NST-PPSSBN showing the expression of enzymes for the conversion of **6** to (*R*)- and (*S*)-**1a**. Lane M, protein ladder; Lane C, *E. coli* NST74(DE3); Lane 15, *E. coli* NST-PPSSBB; Lane 16, *E. coli* NST-PPSSBN. (c) Synthesis of (*R*)- and (*S*)-**1a** from 20 g/L **8** or **9** by *E. coli* NST-PPSSBB and *E. coli* NST-PPSSBN, respectively *via* fermentation with 50 mL M9 medium containing **8** or **9** and 10 mL *n*-hexadecane at 25°C for 24 h. Experiment was performed in duplicates with error bars showing ± SD.

Design of cascade biotransformations and development of growing-cells bioprocess to convert renewable feedstocks glucose 8 and glycerol 9 to (*R*)- and (*S*)-2-phenylglycinol 1a

E. coli naturally produces *L*-phenylalanine **6** by fermentation of renewable feedstocks such as glucose **8** and glycerol **9** through Shikimate pathway.[13b, 19] The natural *L*-phenylalanine biosynthesis pathway was combined with the above developed artificial enzyme cascade of converting **6** to (*R*)- or (*S*)-**1a**, to transform **8** and **9** to (*R*)- and (*S*)-**1a**, respectively. As the biosynthesis of **6** is strictly regulated in *E. coli* by feedback regulation[20], we engineered *E. coli* NST74 (DE3) containing natural *L*-phenylalanine biosynthesis pathway for the overproduction of **6** from **8** or **9** with relaxed feedback regulation. [13b, 18] This strain was used as the host. The plasmids pACYC-PAL-PAD, pCDF-SMO-StEH, pET-BDHA-AlaDH, pRSF-BmTA and pACYC-PAL-PAD, pCDF-SMO-StEH, pET-BDHA- AlaDH, pRSF-NfTA were transformed into *E. coli* NST74 (DE3) to produce *E. coli* NST-PPSSBB and *E. coli* NST-PPSSBN, respectively (Figure 5a). SDS-PAGE analysis was performed with the crude cell lysate of *E. coli* NST-PPSSBB and *E. coli* NST-PPSSBN (Figure 5b). The expression of all seven enzymes in the artificial cascade were confirmed in *E. coli* NST-PPSSBB and *E. coli* NST-PPSSBN.

The fermentation of **8** or **9** to (*R*)- and (*S*)-**1a** was performed with *E. coli* NST-PPSSBB and *E. coli* NST-PPSSBN, respectively, in 50 mL M9 media containing 10 g/L **8** or **9** and 10 mL *n*-hexadecane at 25°C for 24 h. *E. coli* NST-PPSSBB produced 123 and 178 mg/L (*R*)-**1a** from glucose **8** and glycerol **9**, respectively, while *E. coli* NST-PPSSBN produced 110 and 151 mg/L of (*S*)-**1a** from **8** and **9**, respectively (Figure 5c). This is the first report on the bioproduction of (*R*)- and (*S*)-**1a** from renewable feedstocks such as **8** and **9**.

Production of (*R*)- and (*S*)-2-phenylglycinol 1a from renewable feedstocks glucose 8 and glycerol 9 by coupling two strains expressing *L*-phenylalanine biosynthesis pathway and the artificial cascade

The fermentative synthesis of (*R*)- and (*S*)-**1a** from sugars **8**, **9** could burden the cells due to overexpression of several enzymes in a single strain. In addition, the supplementation of organic phase to growing cells and the accumulation of reaction intermediates could inhibit the cell growth thus reducing the fermentative synthesis of (*R*)- and (*S*)-**1a**. To overcome these issues, we coupled fermentation-biotransformation,[13a, 13b, 21] to fermentatively produce **6** from **8** or **9** and sequentially biotransform **6** to (*R*)- and (*S*)-**1a,** respectively.

Fermentative production of L-phenylalanine **6** from sugars **8**, **9** was performed by *E. coli* NST-Phe to reach high concentrations of **6**, followed by the one-pot biotransformation of **6** to (*R*)- and (*S*)-**1a** by the resting cells of *E. coli*-PPSSBB and *E. coli*-PPSSBN, respectively (Figure 6a). *E. coli* NST-Phe overexpressing five key enzymes of Shikimate pathway (AroG*, AroK, YdiB, PheA*, and TyrB) was employed to produce 67-80 mM **7**.[13a, 13b] Resting cells (20 g cdw/L) of *E. coli*-PPSSBB and *E. coli*-PPSSBN were added to the fermentation broth with biosynthesized **6**, to perform biotransformation of **6** to (*R*)- and (*S*)-**1a**, respectively. By coupled fermentation-biotransformation approach, 274 and 384 mg/L of (*R*)-**1a** were obtained from glucose **8** and glycerol **9**, respectively. For the synthesis of (*S*)-**1a**, the product titer of 274 and 301 mg/L were achieved from glucose **8** and glycerol **9**, respectively (Figure 6b).

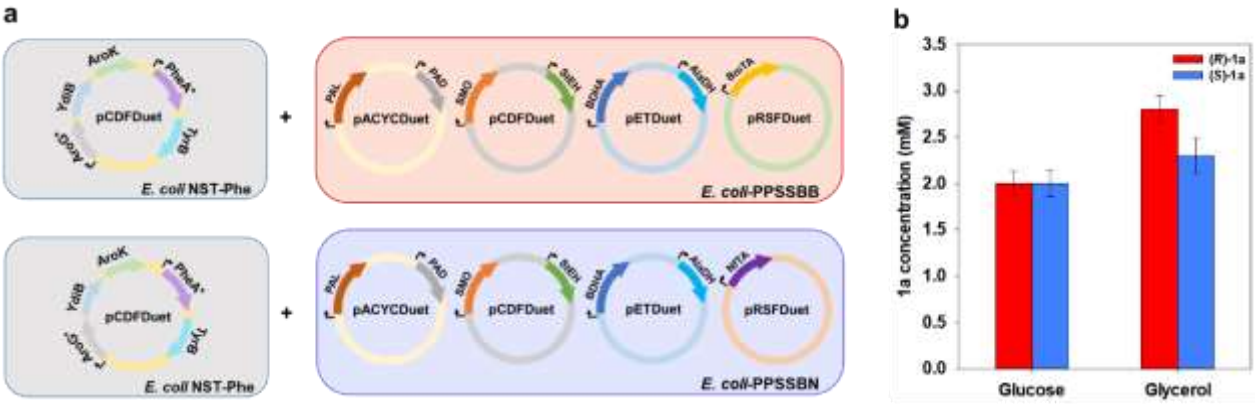

Figure 6: Coupled fermentation and biotransformation for the conversion of glucose **8** and glycerol **9** to (*R*)- and (*S*)-**1a** from. (a) *E. coli* NST-Phe harboring expression plasmid for the expression of key enzymes of Shikimate pathway for improved production of *L*-phenylalanine **6** was coupled with *E. coli*-PPSSBB and *E. coli*-PPSSBN harboring four expression plasmids for the expression of PAL, PAD, SMO, StEH, BDHA, AlaDH and BmTA or NfTA for the production of (*R*)- or (*S*)-**1a**, respectively from **8** and **9**. (b) Synthesis of (*R*)- and (*S*)-**1a** by *E. coli*-PPSSBB and *E. coli*-PPSSBN, respectively from biosynthesized **6** produced by the fermentation of **8** and **9** by *E. coli* NST-Phe. Reaction procedure: Fermentation was performed using growing cells of *E. coli* NST-Phe at 37°C for 24-28 h for the production of 67-80 mM **6** from **8** or **9**. Biotransformation was performed by *E. coli*-PPSSBB or *E. coli*-PPSSBN (20 g cdw/L) at 30°C for 24 h in a two-phase system containing 5 mL fermented mixture containing biosynthesized **6** (10 mM), phosphate buffer (200 mM, pH 8) and 5 mL *n*-hexadecane. Experiment was performed in duplicates with error bars showing ± SD.

Conclusion

We successfully designed and developed novel artificial enzyme cascades consisting of epoxidation-hydrolysis-oxidation-transamination for the one-pot conversion of styrene **2a** to enantiopure (*R*)- and (*S*)-2-phenylglycinol **1a**, respectively. The engineered *E. coli* strains expressing the developed enzyme cascade converted styrene **2a** to (*R*)-**1a** in >99% *ee* at 1015 mg/L and (*S*)-**1a** in 91% *ee* at 315 mg/L, respectively. The enzyme cascade was successfully applied for the biotransformation of substituted styrenes **2b-k** and indene **2l** to produce the corresponding (*R*)-phenylglycinols **1b-k** (95->99% *ee*) and (1*R*,2*R*)-1-amino-2-indanol **1l** (>99% *ee*), respectively, with >60% conversion of four substituted styrenes. While the syntheses of (*R*)-**1a** and (*S*)-**1a** from styrene **2a** were achieved for the first time *via* the developed cascade, the conversion of **2b-l** to (*R*)-**1b-l** represents the first cascade transformation to produce these chemicals.

We also successfully designed and developed novel artificial enzyme cascade consisting of decarboxylation-deamination-epoxidation-hydrolysis-oxidation-transamination for the conversion of bio-based *L*-phenylalanine **6** to (*R*)-**1a** and (*S*)-**1a**, respectively. The engineered *E. coli* cells expressing the cascade were able to transform *L*-phenylalanine **6** to (*R*)-**1a** and (*S*)-**1a** in high *ee* at 576 mg/L and 356 mg/L, respectively, demonstrating the first example of synthesis of (*R*)-**1a** and (*S*)-**1a** from an easily available bio-based chemical.

Combination of *L*-phenylalanine biosynthesis pathway with the developed artificial enzyme cascade to produce (*R*)-**1a** and (*S*)-**1a** was successfully demonstrated. Fermentative biotransformation with engineered *E. coli* cells expressing the *L*-phenylalanine biosynthesis pathway and the artificial cascade afforded (*R*)-**1a** at 123 and 178 mg/L and (*S*)-**1a** at 110 and 151 mg/L from glucose and glycerol, respectively. These results represent the first examples of synthesis of (*R*)-**1a** and (*S*)-**1a** from renewable feedstocks. Coupling of *E. coli* strain expressing *L*-phenylalanine biosynthesis pathway with *E. coli* strain expressing the artificial cascade enhanced the synthesis of (*R*)-**1a** and (*S*)-**1a** from glucose and glycerol, respectively, giving 274 and 384 mg/L (*R*)-**1a** and 274 and 301 mg/L (*S*)-**1a**, respectively. The developed strategies are potentially useful for producing high-value chemicals from cheap and renewable feedstocks.

Experimental Section

Recombinant strain construction

***E. coli* strain expressing enzymes for the cascade conversion of styrene 2a to (R)- and (S)-2-phenylglycinol 1a**

The genes coding for enzymes in the cascade (SMO, StEH, BdhA, AlaDH and BmTA or NfTA) were cloned into three expression plasmids (pETDuet, pCDFDuet and pRSFDuet) with different copy numbers and antibiotic resistance. The expression plasmids were transformed into *E. coli* T7 to obtain 12 different recombinant strains (*E. coli*-SSBB-1-6 and *E. coli*-SSBN-1-6) with varying expression levels of the enzymes in the artificial cascade (Table S1, Supporting information).

***E. coli* strain expressing enzymes for the cascade conversion of L-phenylalanine 6 to (R)- and (S)-2-phenylglycinol 1a**

E. coli-SSBB-1 and *E. coli*-SSBN-2 were further engineered for the cascade conversion of *L*-phenylalanine **6** to phenylglycinol **1a**. pACYCDuet expressing PAL and PAD from our previous study [13e] was transformed into *E. coli*-SSBB-1 (pCDF-SMO-StEH, pET-BDHA- AlaDH, pRSF-NFTA) and *E. coli*-SSBN-2 (pCDF-SMO-StEH,

pET-NFTA, pRSF-BDHA- AlaDH) to obtain *E. coli*-PPSSBB (produce (*R*)-**1a**) and *E. coli*-PPSSBN (produce (*S*)-**1a**), respectively.

***E. coli* strain expressing enzymes for the production of (*R*)- and (*S*)-2-phenylglycinol 1a from glucose 8 and glycerol 9**

For the synthesis of phenylglycinol **1a** from glucose **8** and glycerol **9**, *E. coli* NST74 (DE3) was used as expression strain which was previously engineered to produce *L*-phenylalanine.[18] pCDF-SMO-StEH, pET-BdhA-AlaDH, pRSF-BmTA/NfTA and pACYC-PAL-PAD were transformed into *E. coli* NST74 (DE3) to obtain *E. coli* NST-PPSSBB (produce (*R*)-**1a**) and *E. coli* NST-PPSSBN (produce (*S*)-**1a**), respectively.

General procedure for the cultivation of *E. coli* strains

The glycerol stock of recombinant *E. coli* was inoculated in 2 mL of LB medium with appropriate antibiotics and grown at 37°C and 220 rpm for 6-8 h. 1 mL of the culture was added to 50 mL of modified M9 medium with appropriate antibiotics (50 µg/mL kanamycin, 50 µg/mL chloramphenicol, 50 µg/mL streptomycin, and 100 µg/mL ampicillin) in 250 mL flask and grown at 37°C and 220 rpm. Antibiotics were reduced to 50% for strains containing more than two plasmids. At 2 h, 0.5 mM IPTG was added and the growth temperature was set at 22°C. After 16 h of growth at 22°C, the cells were harvested by centrifugation at 2680 × g for 10 min. The cell pellets were resuspended in appropriate volume of phosphate buffer and used for biotransformation.

Cascade conversion of styrene 2a to (*R*)- and (*S*)-2-phenylglycinol 1a

Biotransformation was performed at 30°C in a two-phase system with 2 mL suspension of *E. coli*-SSBB-1-6 and *E. coli*-SSBN-1-6 (20 g cdw/L) in phosphate buffer (200 mM, pH 8) with *n*-hexadecane containing **2a** (final conc., 10 mM for (*R*)-**1a** and 5 mM for (*S*)-**1a**). 2% glucose and 100 mM amino donor were added to the reaction at 0 h. Additional glucose (1%) and amino donor (100 mM) were added together at 12 and 20 h. The reaction was continued for 24 h and 48 h for the synthesis of (*R*)- and (*S*)-**1a**, respectively. (*R*)- and (*S*)-**1a** was present only in the aqueous phase and 50 µL of sample was taken to analyse its concentration using reverse phase HPLC.

General procedure for the cascade conversion of styrenes 2b-l to (*R*)-2-phenylglycinols 1b-l

Biotransformation was performed at 30°C in a two-phase system with 2 mL suspension of *E. coli*-SSBB-1 in phosphate buffer (200 mM, pH 8) with 0.4 mL *n*-hexadecane containing **2b-1** (final conc., 5-10 mM). 2% glucose and 100 mM amino donor were added to the reaction at 0 h. Additional glucose (1%) and amino donor (100 mM) were added together at 12 and 20 h. After 48 h of biotransformation, (*R*)-**1b-l** were present only in the aqueous phase and their concentration were analysed using reverse phase HPLC.

Preparative biotransformation of styrenes (2a, 2d, 2e, 2j, 2k) to produce (*R*)-2-phenylglycinols (1a, 1d, 1e, 1j, 1k)

Preparative biotransformation was performed at 30°C in a two-phase system with 100 mL suspension of *E. coli*-SSBB-1 (25 g cdw/L) in phosphate buffer (200 mM, pH 8) with 20 mL *n*-hexadecane containing 50 mM of **2a**, **2d**, **2e**, **2j** and **2k**. 2% glucose and 100 mM NH_3/NH_4Cl were added to the reaction at 0, 16 and 24 h. After 48 h of biotransformation, the aqueous phase was separated by centrifugation at 4200 × g for 10 min. The aqueous phase was saturated with NaCl, adjusted to pH<2 with HCl (10 M), and washed with ethyl acetate (2×30 ml) to remove trace *n*-hexadecane and other organic impurities. The aqueous phase was adjusted to pH>12 with NaOH (10 M), followed by extraction with ethyl acetate (3×100 ml). The organic phase was separated and dried over Na_2SO_4. After filtration, the organic phase was subjected to evaporation. The crude phenylglycinols was purified by flash chromatography on a silica gel column with CH_2Cl_2:MeOH:NH_3 (28% aqueous solution) of 100:10:1 as eluent (Rf ≈ 0.3–0.6 for (*R*)-**1a**, **1d**, **1e**, **1j** and **1k**). The collected fraction containing the product was dried over Na_2SO_4. After filtration, the organic solvent was removed by evaporation, and the product was dried under vacuum overnight to obtain pure product (>98% HPLC) (**1a**, 81 mg, 59% yield; **1d**, 78 mg, 50% yield; **1e**, 64 mg, 37% yield; **1j**, 49 mg, 32% yield; **1k**, 73 mg, 44% yield). The isolated product was confirmed by HPLC, ^{1}H-NMR and ^{13}C-NMR. **1a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.21 (m, 5H), 4.04 (dd, *J* = 8.4, 4.3 Hz, 1H), 3.73 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.56 (dd, *J* = 10.9, 8.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.57, 128.84, 127.75, 126.70, 68.02, 57.55. **1d**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.23 (m, 2H), 7.08 – 6.96 (m, 2H), 4.05 (dd, *J* = 8.2, 4.3 Hz, 1H), 3.70 (dd, *J* = 10.8, 4.3 Hz, 1H), 3.53 (dd, *J* = 10.8, 8.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.36 (d, *J* = 245.8 Hz), 128.33 (d, *J* = 8.0 Hz), 139.69, 115.66 (d, *J* = 21.3 Hz), 68.00, 56.85. **1e**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.14 (m, 4H), 4.03 (dd, *J* = 7.2, 4.0 Hz, 1H), 3.73 (dd, *J* = 10.6, 3.6 Hz, 1H), 3.53 (dd, *J* = 10.4, 8.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.03, 134.72, 130.10, 127.86, 126.96, 124.94, 68.03, 57.14. **1j**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (dd, *J* = 23.7, 8.1 Hz, 4H), 4.01 (dd, *J* = 8.4, 4.3 Hz, 1H), 3.71 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.54 (dd, *J* = 10.8, 8.5 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.53, 137.43, 129.51, 126.59, 68.02, 57.25, 21.24. **1k**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.20 (m, 2H), 6.92 – 6.84 (m, 2H), 4.00 (dd, *J* = 8.0, 4.1 Hz, 1H), 3.79 (s, 3H), 3.69 (dd, *J* = 10.8, 4.3 Hz, 1H), 3.59 – 3.48 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.03, 127.63, 114.05, 100.00, 67.86, 56.72, 55.30.

Cascade conversion of L-phenylalanine 6 to (*R*)- and (*S*)-phenylglycinol 1a

Biotransformation of *L*-phenylalanine **6** using *E. coli*-PPSSBB and *E. coli*-PPSSBN was performed at 30°C for 24 h for the synthesis of (*R*)- and (*S*)-**1a**, respectively. Reaction was performed in a two-phase system with 5 mL suspension of *E. coli*-PPSSBB or *E. coli*-PPSSBN (20 g cdw/L) in phosphate buffer (200 mM, pH 8) with 1 mL *n*-hexadecane. 10 mM **6**, 2% glucose and 100 mM NH_3/NH_4Cl were added to the reaction at 0 h. Additional glucose (1%) and NH_3/NH_4Cl (100 mM) were added together at 12 h. At 24 h, 50 µL of the aqueous phase was collected to quantify the concentration of (*R*)- or (*S*)-**1a** using reverse phase HPLC.

Fermentation of glucose 8 and glycerol 9 to produce (*R*)- or (*S*)-2-phenylglycinol 1a

E. coli NST-PPSSBB and *E. coli* NST-PPSSBN were inoculated, respectively in 2 mL LB medium containing appropriate antibiotics and grown at 37ºC, 220 rpm for 6-8 h. 1 mL of the culture of *E. coli* NST-PPSSBB or *E. coli* NST-PPSSBN was added to 50 mL of modified M9 medium with 20 g/L **8** or **9**, respectively, and appropriate antibiotics in 250 mL flask. After 2 h of growth at 37°C, 0.1 mM IPTG and 10 mL *n*-hexadecane was added and the growth temperature was set at 25°C. At 24 h, 50 µL of the aqueous phase was collected to quantify the concentration of (*R*)- or (*S*)-**1a** using reverse phase HPLC.

Coupled fermentation-biotransformation for the production of (*R*)- or (*S*)-2-phenylglycinol 1a from glucose 8 or glycerol 9

Fermentative production of *L*-phenylalanine **6** from **8** or **9** by *E. coli* NST-Phe was performed as mentioned previously [13b], to achieve 67-80 mM **6** and the fermentation culture containing biosynthesized **6** and *E.*

coli NST-Phe cells was stored at 4 °C or used directly in biotransformation. 5 mL of reaction mixture was prepared with fermentation broth containing biosynthesized **6** (final conc. 10 mM), phosphate buffer (200 mM, pH 8), 2% glucose, 100 mM NH_3/NH_4Cl. Resting cells of *E. coli* NST-PPSSBB or *E. coli* NST-PPSSBN (20 g cdw/L) and 1 mL of *n*-hexadecane were added and biotransformation was performed at 30°C, 220 rpm. Additional glucose (1%) and NH_3/NH_4Cl (100 mM) were added together at 12 h. At 24 h, 50 µL of the aqueous phase was collected to quantify the concentration of (*R*)- or (*S*)-**1a** using reverse phase HPLC.

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References

[1] a) M. Amat, J. Bosch, J. Hidalgo, M. Cantó, M. Pérez, N. Llor, E. Molins, C. Miravitlles, M. Orozco, J. Luque, *J. Org. Chem.* **2000**, *65*, 3074-3084; b) R. E. Lee, M. Protopopova, E. Crooks, R. A. Slayden, M. Terrot, C. E. Barry, *J. Comb. Chem.* **2003**, *5*, 172-187; c) R. Sakurai, K. Sakai, *Tetrahedron Asymmetry* **2003**, *14*, 411-413; d) D. J. Augeri, J. A. Robl, D. A. Betebenner, D. R. Magnin, A. Khanna, J. G. Robertson, A. Wang, L. M. Simpkins, P. Taunk, Q. Huang, *J. Med. Chem.* **2005**, *48*, 5025-5037; e) D. A. Erlanson, J. W. Arndt, M. T. Cancilla, K. Cao, R. A. Elling, N. English, J. Friedman, S. K. Hansen, C. Hession, I. Joseph, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3078-3083.

[2] O. K. Karjalainen, A. M. Koskinen, *Org. Biomol. Chem.* **2011**, *9*, 1231-1236.

[3] U. Kaulmann, K. Smithies, M. E. Smith, H. C. Hailes, J. M. Ward, *Enzyme Microb. Technol.* **2007**, *41*, 628-637.

[4] a) C. Gardelli, H. Wada, A. Ray, M. Caffrey, A. Llinas, I. Shamovsky, J. Tholander, J. Larsson, U. Sivars, L. Hultin, *J. Med. Chem.* **2018**, *61*, 5974-5987; b) S. W. C. Smith, Craig Alan; Dubose, Robert Finley, *Vol. US 2018-62651561*, **2019**; c) C. D. Dzierba, Y. Bi, B. Dasgupta, R. A. Hartz, V. Ahuja, G. Cianchetta, G. Kumi, L. Dong, S. Aleem, C. Fink, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1448-1452.

[5] a) Y. Cao, G. Yang, *J. Chem. Pharm.* **2016**, *8*, 139-143; b) A. Abiko, S. Masamune, *Tetrahedron Lett.* **1992**, *33*, 5517-5518.

[6] M. Periasamy, S. Sivakumar, M. N. Reddy, *Synthesis* **2003**, *13*, 1965-1967.

[7] a) C. T. Hou, *Handbook of industrial biocatalysis*, CRC press, **2005**; b) M. T. Reetz, *J. Am. Chem. Soc.* **2013**, *135*, 12480-12496; c) S. Gandomkar, A. Żądło- Dobrowolska, W. Kroutil, *ChemCatChem* **2019**, *11*, 225-243. d) F. Rudroff, M. D. Mihovilovic, H. Groger, R. Snajdrova, H. Iding, U. T. Bornscheuer, *Nat. Catal.* **2018**, *1*, 12-22. e) B. Hauer, *ACS Catal*. **2020**, *10*, 8418-8427.

[8] J.-D. Zhang, J.-W. Zhao, L.-L. Gao, H.-H. Chang, W.-L. Wei, J.-H. Xu, *J. Biotechnol.* **2019**, *290*, 24-32.

[9] A. Nobili, F. Steffen- Munsberg, H. Kohls, I. Trentin, C. Schulzke, M. Höhne, U. T. Bornscheuer, *ChemCatChem* **2015**, *7*, 757-760.

[10] Z. B. Sun, Z. J. Zhang, F. L. Li, Y. Nie, H. L. Yu, J. H. Xu, *ChemCatChem* **2019**, *11*, 3802-3807.

[11] J.-D. Zhang, X.-X. Yang, Q. Jia, J.-W. Zhao, L.-L. Gao, W.-C. Gao, H.-H. Chang, W.-L. Wei, J.-H. Xu, *Catal. Sci. Technol.* **2019**, *9*, 70-74.

[12] a) J. I. Ramsden, R. S. Heath, S. R. Derrington, S. L. Montgomery, J. Mangas-Sanchez, K. R. Mulholland, N. J. Turner, *J. Am. Chem. Soc.* **2019**, *141*, 1201-1206; b) P. N. Devine, R. M. Howard, R. Kumar, M. P. Thompson, M. D. Truppo, N. J. Turner, *Nat. Rev. Chem.* **2018**, *2*, 409-421; c H. B. Cui, L. Z. Xie, N. W. Wan, Q. He, Z. Li, *Green Chem.* **2019**, *21*, 4324-4328. d) J. H. Schrittwieser, S. Velikogne, M. l. Hall, W. Kroutil, *Chem. Rev.* **2018**, *118*, 270-348; e) S. Wu, Y. Zhou, Z. Li, *ChemComm.* **2019**, *55*, 883-896; f) S. Wu, Z. Li, *ChemCatChem* **2018**, *10*, 2164-2178. g) G. Morgado, D. Gerngross, T. M. Roberts, S. Panke, *Metab. Eng*. **2016**, 117-146. h) T. Narancic, S. A. Almahboub, K. E. O'Connor, *World J. Microbiol. Biotechnol*. **2019**, *35*, 67-11. i) H. Puetz, E. Puchľová, K. Vranková, F. Hollmann, *Catalysts* **2020**, *10*, 952-981. j) F.W. Krainer, A. Glieder, *Appl. Microbiol Biotechnol*. **2015**, *99*, 1611-1625.

[13] a) B. R. Lukito, B. S. Sekar, S. Wu, Z. Li, *Adv. Synth. Catal.* **2019**, *361*, 3560-3568; b) B. S. Sekar, B. R. Lukito, Z. Li, *ACS Sustain. Chem. Eng.* **2019**, *7*, 12231-12239; c) B. R. Lukito, S. Wu, H. J. J. Saw, Z. Li, *ChemCatChem* **2019**, *11*, 831-840; d) J. Zhang, S. Wu, J. Wu, Z. Li, *ACS Catal.* **2015**, *5*, 51-58; e) Y. Zhou, S. Wu, Z. Li, *Adv. Synth. Catal.* **2017**, *359*, 4305-4316; f) S. Wu, J. Liu, Z. Li, *ACS Catal.* **2017**, *7*, 5225-5233; g) P. H. Opgenorth, T. P. Korman, J. U. Bowie, *Nat. Chem. Biol.* **2016**, *12*, 393-395. h) J. Muschiol, C. Peters, N. Oberleitner, M. D. Mihovilovic, U. T. Bornscheuer, F. Rudroff, *Chem. Comm*. **2015**, *51*, 5798-5811. i) S. Wu, R. Snajdrova, J. Moore, K. Baldenius, U. T. Bornscheuer, *Angew. Chem. Int. Ed*. **2020**, DOI: 10.1002/anie.202006648.

[14] a) Y. Zhou, S. Wu, Z. Li, *Angew. Chem.* **2016**, *128*, 11819-11822; b) S. Wu, Y. Chen, Y. Xu, A. Li, Q. Xu, A. Glieder, Z. Li, *ACS Catal.* **2014**, *4*, 409-420.

[15] J. Liu, Z. Li, *Biotechnol. Bioeng.* **2019**, *116*, 536-542.

[16] N. H. Tolia, L. Joshua-Tor, *Nat. Methods* **2006**, *3*, 55-64.

[17] S. Wu, Y. Zhou, D. Seet, Z. Li, *Adv. Synth. Catal.* **2017**, *359*, 2132-2141.

[18] Y. Zhou, S. Wu, J. Mao, Z. Li, *ChemSusChem* **2018**, *11*, 2221-2228.

10.1002/adsc.202001322

[19] a) M. Weiner, C. Albermann, K. Gottlieb, G. A. Sprenger, D. Weuster-Botz, *Biochem. Eng. J.* **2014**, *83*, 62-69; b) Y. Liu, Y. Xu, D. Ding, J. Wen, B. Zhu, D. Zhang, *BMC Biotechnol.* **2018**, *18*, 5.

[20] S.-P. Liu, M.-R. Xiao, L. Zhang, J. Xu, Z.-Y. Ding, Z.-H. Gu, G.-Y. Shi, *Process Biochem.* **2013**, *48*, 413-419.

[21] Y. Zhou, B. S. Sekar, S. Wu, Z. Li, *Biotechnol. Bioeng.* **2020**, *117*, 2340-2350.

10.1002/adsc.202001322

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