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One-Pot Enantioselective Synthesis of D-Phenylglycines from Racemic Mandelic Acids, Styrenes, or Biobased L-Phenylalanine *via* Cascade Biocatalysis

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Abstract. Enantiopure D-phenylglycine and its derivatives are an important group of chiral amino acids with broad applications in pharmaceutical industry. However, the existing synthetic methods for D-phenylglycine mainly rely on toxic cyanide chemistry and multistep processes. To provide green and safe alternatives, we leveraged cascade biocatalysis for one-pot synthesis of D-phenylglycine from racemic mandelic acid, styrene, and biobased L-phenylalanine, respectively. Recombinant *Escherichia coli* (LZ110) was engineered to coexpress four enzymes to catalyze 3-step reactions in one pot, transforming mandelic acid (210 mM) to give enantiopure D-phenylglycine in 29.5 g L^{-1} (195 mM) with 93% conversion. Using the same whole-cell catalyst, other 12 D-phenylglycine derivatives were also produced from the corresponding mandelic acid derivatives in high conversion (58-94%) and very high *ee* (93-99%). *E. coli* (LZ116) expressing seven enzymes was constructed for the transformation of styrene to enantiopure D-phenylglycine in 80% conversion via one-pot 6-step cascade biotransformation. 12 Substituted D-phenylglycines were also produced from the corresponding styrene derivatives in high conversion (45-90%) and very high *ee* (92-99%) via the same cascade reactions. A nine-enzyme-expressing *E. coli* (LZ143) was engineered to transform biobased L-phenylalanine to enantiopure D-phenylglycine in 83% conversion via one-pot 8-step transformation. Preparative biotransformation was also demonstrated. The high-yielding synthetic methods use cheap and green reagents (ammonia, glucose, and/or oxygen), and *E. coli* whole-cell catalysts, thus providing green and useful alternative methods for manufacturing D-phenylglycine.

Keywords: Amino acids; Biotransformations; Cascade reactions; Enzyme catalysis; Green chemistry

Introduction

Enantiopure D-amino acids are a group of very important chiral chemicals for manufacturing pharmaceuticals and other bioactive compounds.[1] For instance, D-phenylglycine **1a** is consumed at several thousand tons per year for the synthesis of β-lactam antibiotics (ampicillin and cephalexin)[2] and also used for the preparation of an HCV NS5A inhibitor[3a] and a dual inhibitor of phosphoinositide-dependent kinase-1 and aurora kinase A.[3b] 4-Fluoro-D-phenylglycine **1d** is a chiral building block for a potent h5-HT_{1D} receptor agonist[4a] and HCV NS5B polymerase inhibitors.[4b] 4-Chloro-D-phenylglycine **1f** is a key chiral synthon for a macrocyclic Hedgehog pathway inhibitor[5a] and a potent morpholinone MDM2 inhibitor.[5b] The conventional process to manufacture D-phenylglycine is via Strecker reaction of benzaldehyde with highly toxic cyanide,[2a,6] followed by diastereomeric crystallization with D-camphorsulfonic acid to give 68% yield.[7] Recently, asymmetric Strecker reaction was reported for the synthesis of enantiopure α-amino acids from *N*-substituted imines without the resolution step, but it requires complicated and costly protection/deprotection steps with low atom efficiency.[8] For example, asymmetric hydrocyanation of *N*-benzhydryl-protected benzylimine with a thiourea-derived catalyst gave *N*-benzhydryl-protected phenylglycinonitrile in 98% *ee*,[8b] but it needs further hydrolysis and deprotection steps to form D-phenylglycine.

Recently, enzyme catalysis has become a useful tool for enantioselective synthesis.[9] Kinetic resolution of *N*-acetylphenylglycine or phenylglycine methyl ester with acylase or lipase to give D-phenylglycine are well known but suffering from the 50% maximum yield.[10] Dynamic kinetic resolution (DKR) of phenylglycine methyl ester with lipase and

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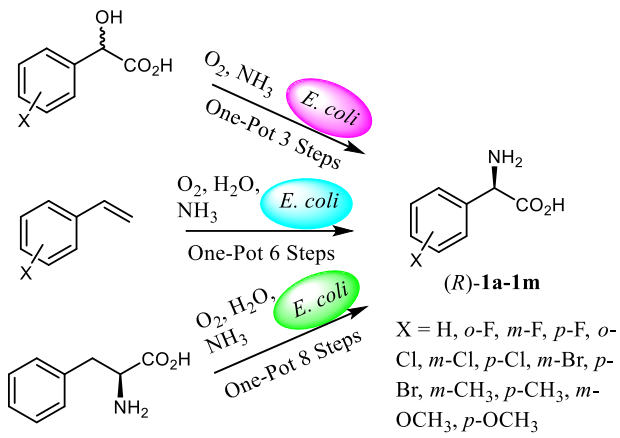


Scheme 1. One-pot synthesis of D-phenylglycines (*R*)-**1a-1m** from racemic mandelic acids, styrenes, or L-phenylalanine via cascade biocatalysis with recombinant *E. coli* cells containing the necessary enzymes.

pyridoxal gave D-phenylglycine amide in 88% *ee* at 85% conversion.[11a] DKR of phenylglycine amide with racemase and amidase gave D-phenylglycine in 88% *ee* at 99% conversion.[11b] DKR of phenylglycinonitrile with nitrilase at alkaline condition gave D-phenylglycine in 95% *ee* at 81% conversion.[11c] Clearly, the product *ee* in these processes was not high enough for direct application. On the other hand, the hydantoinase process was developed to produce enantiopure D-phenylglycines in 100% conversion from racemic hydantoins by hydantoinase, carbamoylase, and racemase.[2a,6,12] However, the substrate 5-phenylhydantoin has to be synthesized by Bucherer–Bergs reaction with benzaldehyde, urea, and highly toxic cyanide, and the low solubility of 5-phenylhydantoin in aqueous solution resulted in low overall reaction rate.[13] Coupling D-selective α-transaminase and *R*-selective ω-transaminase gave enantiopure D-phenylglycine from a primary amine and relative expensive phenylglyoxylic acid.[14] Therefore, highly efficient green methods for the synthesis of enantiopure D-phenylglycine are highly wanted. Moreover, in all chemical and enzymatic methods described above, cyanide was unavoidably used for the synthesis of either substrates or products. Thus, developing a cyanide-free and sustainable process is of great importance. In a recent attempt, D-phenylglycine was fermentatively produced from glucose, but in a low concentration mixed with a large amount of L-phenylalanine, thus requiring difficult and costly isolation and generating much waste.[15]

We aimed to achieve green and efficient synthesis of D-phenylglycine **1** from racemic mandelic acid, styrene, or L-phenylalanine via one-pot cascade biocatalysis (Scheme 1). Racemic mandelic acid is a cheap and easily available substrate, thus its conversion to (*R*)-**1** could be highly attractive. While styrene is a very cheap hydrocarbon, its own synthesis and conversion to (*R*)-**1** are free of cyanide. On the other hand, L-phenylalanine is an easily available fermentation product from renewable feedstock in a large amount, thus its conversion to (*R*)-**1** could provide sustainable synthesis of new biobased chemicals. In each of these synthetic routes (Figure 1a, 2a, 3a), the involved multistep reactions are performed in one pot, which can avoid the tedious and costly intermediate isolation and waste generation.[16] Here, we report the engineering of three recombinant *E. coli* strains coexpressing 4-9 enzymes as efficient biocatalysts for the corresponding purposed one-pot enantioselective syntheses, and the high-yielding production of enantiopure D-phenylglycines from the three substrates, respectively, by whole-cell based one-pot cascade biotransformation.

Results and Discussion

Enantioselective conversion of racemic mandelic acids to D-phenylglycines

Selection of enzymes, engineering of a host, and construction of recombinant *E. coli* strains. Previously, L-phenylglycine was produced from racemic mandelic acid via a 3-step enzyme cascade with mandelate racemase, D-mandelate dehydrogenase, and L-amino acid dehydrogenase.[17] However, attempt to produce D-phenylglycine failed: initial test of several commercially available D-amino acid dehydrogenases could not produce D-phenylglycine,[17a] and an engineered broad-range D-amino acid dehydrogenase showed no activity for the formation of D-phenylglycine.[18] To provide access to the important D-phenylglycine (Figure 1a), we employed a unique D-phenylglycine aminotransferase (DpgAT) from *Pseudomonas stutzeri* ST-201[19] for the last amination step. The gene of DpgAT was synthesized and cloned to give pRSF-*dpgat* plasmid. *E. coli* BL21 (DE3) containing pRSF-*dpgat* was engineered and the whole cells were used to convert phenylglyoxylic acid **2a** to give D-phenylglycine **1a** in 70% *ee*. The product *ee* is not high probably due to the endogenous L-α-transaminases in the *E. coli* BL21 (DE3) host transformed **2a** to (*S*)-**1a**. A new *E. coli* DL39 (DE3) host was engineered by integration of λDE3 prophage into the *E. coli* DL39 strain,[20] which has mutations in three α-transaminase genes *ilvE*, *tyrB*, and *aspC*. *E. coli* DL39 (DE3) expressing DpgAT produced (*R*)-**1a** in 99% *ee* from **2a** (Figure S1 in the Supporting Information). Thus *E. coli* DL39 (DE3) was used as the expression host throughout this study.

DpgAT catalyzes the conversion of **2a** to (*R*)-**1a** by using L-glutamate as the amine donor to form α-ketoglutarate. To enhance the productivity, we utilized the glutamate dehydrogenase (GluDH) to regenerate L-glutamate from α-ketoglutarate and ammonia.

For the oxidation of (*S*)-mandelic acid **3a**, a hydroxy acid oxidase (HMO) was used in previous studies,[21] yet it was not very efficient and required

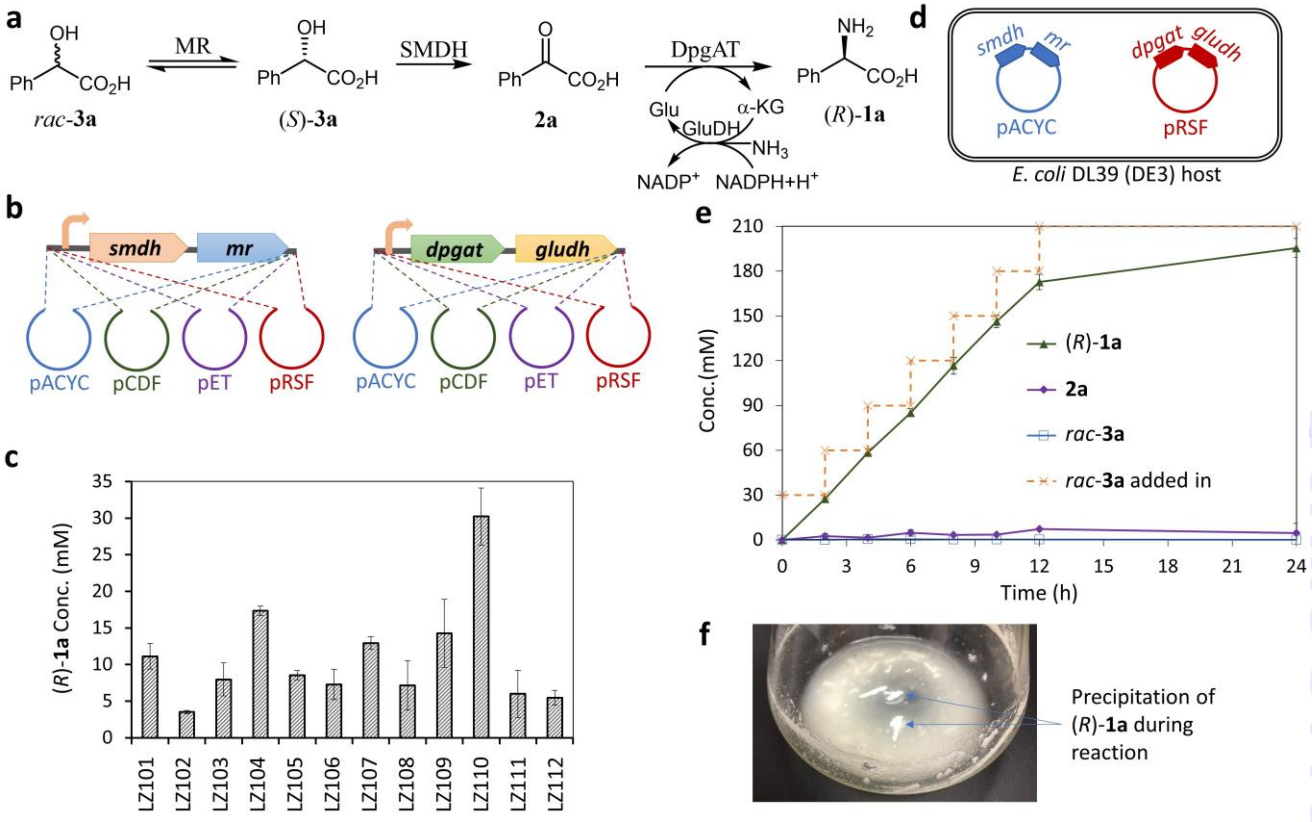


Figure 1. (a) Cascade biotransformation of *rac*-**3a** to (*R*)-**1a** with (*S*)-mandelate dehydrogenase (SMDH), mandelate racemase (MR), D-phenylglycine aminotransferase (DpgAT) and glutamate dehydrogenase (GluDH). (b) Engineering of recombinant *E. coli* by combination of SMDH-MR and DpgAT-GluDH modules on four compatible plasmids, pACYC, pCDF, pET and pRSF, respectively. (c) Screening of *E. coli* strains (LZ101-112) cells (10 g cdw L^{-1}) for one-pot production of (*R*)-**1a** from *rac*-**3a** (30 mM) in 2 h. (d) Construction of *E. coli* (LZ110). (e) Time course of one-pot production of (*R*)-**1a** from *rac*-**3a** using *E. coli* (LZ110) cells (15 g cdw L^{-1}) with step-wise substrate feeding in KP buffer containing glucose and NH_3/NH_4Cl. (f) Precipitation of (*R*)-**1a** in the reaction buffer during the biotransformation. Error bars in (c) and (e) represent the standard deviation of triplicated biotransformation.

catalase to remove the toxic H_2O_2. Here, we cloned a membrane-associated, FMN-dependent (*S*)-mandelate dehydrogenase (SMDH)[22] from *Pseudomonas putida* ATCC 12633 for the oxidation of (*S*)-**3a** to **2a**. SMDH showed much higher productivity than HMO (Figure S2 in the Supporting Information). Therefore, SMDH was chosen for the second step of the reaction. The mandelate racemase (MR)[23] from the same *P. putida* strain was cloned in *E. coli* and employed for racemization of **3a** with high efficiency.

To achieve the balanced enzyme expression in an *E. coli* strain, we divided the four enzymes into two enzyme modules, SMDH-MR and DpgAT-GluDH, and each enzyme module was constructed on four plasmids, pACYC, pCDF, pET, and pRSF (Figure 1b). To obtain the best single *E. coli* strain as the catalyst for the cascade biotransformation, the four plasmids of SMDH-MR and the four plasmids of DpgAT-GluDH were combinatorially transformed in *E. coli* DL39 (DE3) host to give twelve strains *E. coli* (LZ101-LZ112), each coexpressing SMDH, MR, DpgAT, and GluDH.

One-pot synthesis of D-phenylglycine 1a from racemic mandelic acid 3a with *E. coli* coexpressing 4 enzymes. The resting cells of the twelve strains *E. coli* (LZ101-LZ112) were examined for biotransformation of 30 mM *rac*-**3a** in potassium phosphate (KP) buffer containing NH_3 and glucose. The overall cascade reaction requires 1 mole of NADPH (by GluDH) for producing 1 mole of (*R*)-**1a**. Thus, glucose (2%) was added to regenerate NADPH via cellular metabolism. As shown in Figure, 1c, all twelve strains successfully produced (*R*)-**1a** in 2 h. The *E. coli* (LZ110) containing pACYC-*smdh-mr* and pRSF-*dpgat-gludh* (Figure 1d) gave quantitative conversion to (*R*)-**1a**, thus it was selected for further investigation.

Biotransformation of *rac*-**3a** in 50 mM with *E. coli* (LZ110) gave a very low amount of (*R*)-**1a** (15%) with significant accumulation of **2a** (85%), suggesting the inhibition of **2a**. Further experiment using 30-50 mM **2a** as substrate revealed that higher than 40 mM of **2a** significantly inhibited the transamination to (*R*)-**1a** (Figure S3 in the Supporting Information). To tackle this issue, we exploited the step-wise addition of substrate *rac*-**3a** into the reaction system. The best result was depicted in Figure 1e: 30 mM *rac*-**3a** was fed in every two hours for 7 times, and 195 mM (29.5 g L^{-1}) of enantiopure (*R*)-**1a** was produced in 24 h, representing 93% conversion from *rac*-**3a** (210 mM). Interestingly, (*R*)-**1a** precipitated in the reaction buffer during the biotransformation (Figure 1f). Clearly, this *in situ* precipitation is highly wanted in bioprocess to avoid

the product inhibition and facilitate the product recovery.

The one-pot 3-step cascade utilizes easily available racemic mandelic acid as substrate, cheap and green O_2, NH_3, and glucose as reagents, and gives enantiopure product in high yield. With *in situ* precipitation, (*R*)-**1a** could be produced in up to 30 g L^{-1}, representing one of the most efficient examples of biocatalytic conversion of -OH group to -NH_2 group.[24]

One-pot synthesis of substituted D-phenylglycines 1b-1m from substituted racemic mandelic acids 3a-3m with *E. coli* (LZ110). To explore the application scope of the 3-step cascade, the resting cells of *E. coli* (LZ110) were applied to convert 12 different substituted racemic mandelic acids **3b-3m** to (*R*)-**1b-1m**. As listed in Table 1, 5 substituted D-phenylglycines, **1i**, **1j**, **1k**, **1l**, **1m**, were produced in enantiopure form (≥ 99% *ee*) with high conversion (80-91%). Other 5 D-phenylglycines, **1b**, **1c**, **1d**, **1f**, **1g**, were produced in very high *ee* (95-98%) and high conversion (73-94%). (*R*)-**1e** was formed in very high *ee* (97%) with moderate conversion (58%), probably due to the low activity of SMDH on (*S*)-**3e**. (*R*)-**1h** was formed in slightly lower *ee* (93%) and high conversion (81%), possibly due to the less satisfactory enantioselectivity of DpgAT on **2h**. These results demonstrated that the 3-step enzyme cascade route could be generally applicable to produce many useful D-phenylglycine derivatives from racemic mandelic acids.

Table 1. One-pot synthesis of (*R*)-**1b-1m** from *rac*-**3b-3m** via cascade biocatalysis with recombinant *E. coli* (LZ110) cells.[a]

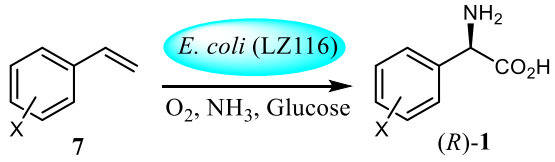
rac-**3**	X	Sub. conc. [mM]	(*R*)-**1**	Conv. [%][b]	Prod. *ee* [%][c]
3b	*o*-F	20	**1b**	89	98
3c	*m*-F	20	**1c**	86	95
3d	*p*-F	20	**1d**	94	97
3e	*o*-Cl	10	**1e**	58	97
3f	*m*-Cl	20	**1f**	88	95
3g	*p*-Cl	10	**1g**	73	98
3h	*m*-Br	10	**1h**	81	93
3i	*p*-Br	10	**1i**	90	99
3j	*m*-CH_3	20	**1j**	91	99
3k	*p*-CH_3	20	**1k**	90	99
3l	*m*-OCH_3	10	**1l**	80	99
3m	*p*-OCH_3	20	**1m**	86	99

[a] Reaction conditions: *rac*-**3b-3m** (10-20 mM) and *E. coli* (LZ110) cells (15 g cdw L^{-1}) in 2mL KP buffer (400 mM, pH 8.0) containing glucose (2% w/v) and NH_3/NH_4Cl (200 mM, pH 8.0) at 30 °C for 24 h. [b] Determined by HPLC analysis. [c] Measured by chiral HPLC analysis.

Enantioselective conversion of styrenes to D-phenylglycines

Selection of enzymes and construction of *E. coli* strains. Styrene is manufactured at a very large scale and low price by dehydrogenation of ethylbenzene. Direct production of (*R*)-**1a** from styrene **7a** could provide an economic and cyanide-free synthesis. Based on the above 2-step cascade from (*S*)-**3a** to (*R*)-**1a** (Figure 1a), additional four enzymes were used to convert **7a** to (*S*)-**3a** (Figure 2a). Styrene monooxygenase (SMO), epoxide hydrolase (SpEH) and alcohol dehydrogenase (AlkJ) and aldehyde dehydrogenase (EcALDH) were employed for the conversion of styrene **7a** to (*S*)-**3a**.[21a] The SMDH, DpgAT, and GluDH were used for the conversion of (*S*)-**3a** to (*R*)-**1a**. To achieve the balanced enzyme expression in *E. coli* strain, we divided the 7 enzymes into three enzyme modules, SMO-SpEH, AlkJ-EcALDH, and DpgAT-GluDH-SMDH, and each enzyme module was constructed on four plasmids, pACYC, pCDF, pET, and pRSF (Figure 2b). The combination of plasmids of SMO-SpEH, AlkJ-EcALDH, and DpgAT-GluDH-SMDH in *E. coli* DL39 (DE3) host gave 24 strains *E. coli* (LZ113-LZ136), each coexpressing the 7 enzymes.

Table 2. One-pot synthesis of (*R*)-**1b-1m** from **7b-7m** via cascade biocatalysis with recombinant *E. coli* (LZ116) cells.[a]



7	X	Sub. conc. [mM]	(*R*)-**1**	Conv. [%][b]	Prod. *ee* [%][c]
7b	*o*-F	10	**1b**	82	99
7c	*m*-F	20	**1c**	88	97
7d	*p*-F	20	**1d**	80	99
7e	*o*-Cl	10	**1e**	49	97
7f	*m*-Cl	20	**1f**	75	94
7g	*p*-Cl	20	**1g**	86	99
7h	*m*-Br	10	**1h**	50	92
7i	*p*-Br	10	**1i**	45	99
7j	*m*-CH_3	20	**1j**	86	98
7k	*p*-CH_3	20	**1k**	90	99
7l	*m*-OCH_3	10	**1l**	87	97
7m	*p*-OCH_3	20	**1m**	90	96

[a] Reaction conditions: **7b-7m** (10-20 mM) and *E. coli* (LZ116) cells (15 g cdw L^{-1}) in two-liquid phase system consisting of 0.4 mL EO and 2 mL KP buffer (400 mM, pH 8.0) containing glucose (0.5%) and NH_3/NH_4Cl (100 mM, pH 8.0) at 30 °C for 24 h. Additional glucose (1%) and NH_3/NH_4Cl (100 mM) was added at 10 h. [b] Determined by HPLC analysis. [c] Measured by chiral HPLC analysis.

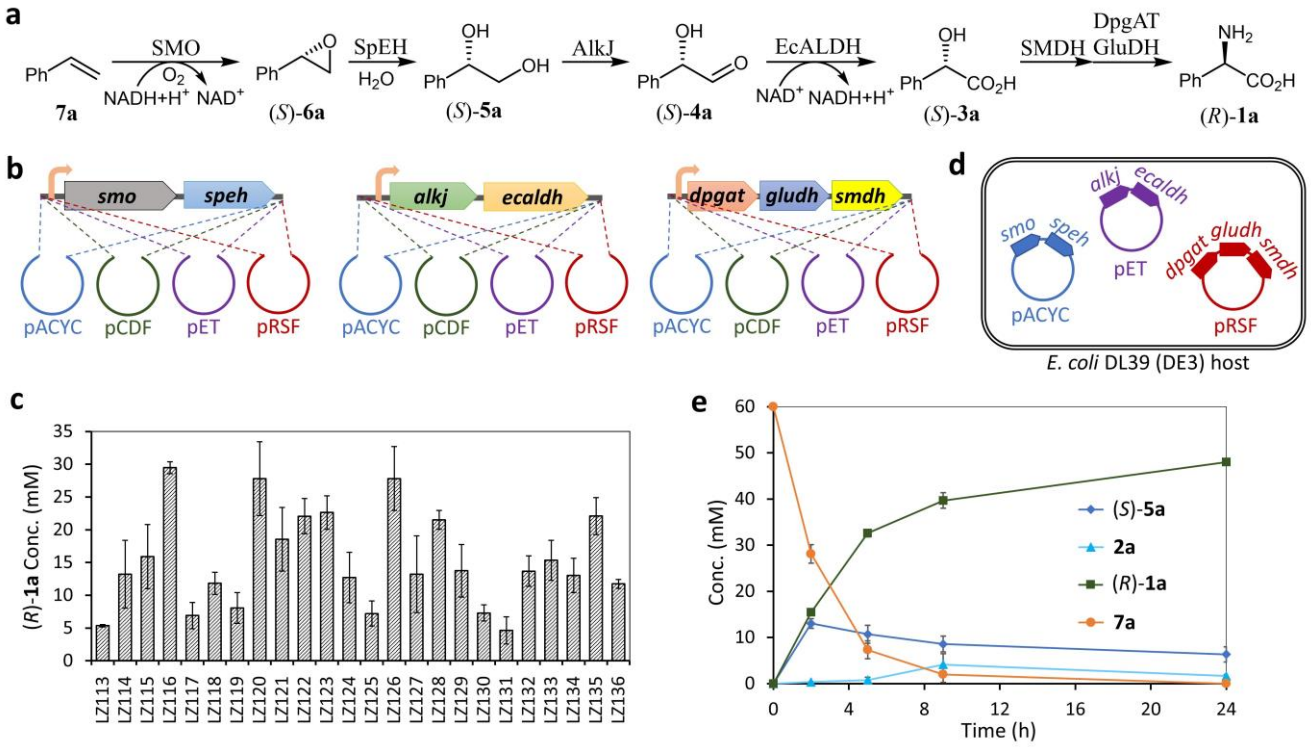


Figure 2. (a) Cascade biotransformation of **7a** to (*R*)-**1a** with styrene monooxygenase (SMO), epoxide hydrolase (SpEH), alcohol dehydrogenase (AlkJ), aldehyde dehydrogenase (EcALDH), SMDH, DpgAT, and GluDH. (b) Engineering of recombinant *E. coli* by combination of SMO-SpEH, AlkJ-EcALDH, and DpgAT-GluDH-SMDH modules on four compatible plasmids, pACYC, pCDF, pET and pRSF, respectively. (c) Screening of *E. coli* (LZ113-136) cells (10 g cdw L^{-1}) for one-pot production of (*R*)-**1a** from **7a** (30 mM) in 24 h. (d) Construction of *E. coli* (LZ116). (e) Time course of one-pot production of (*R*)-**1a** from **7a** (60 mM) using *E. coli* (LZ116) cells (15 g cdw L^{-1}) in a two-liquid phase system of EO and KP buffer containing glucose and NH_3/NH_4Cl. Error bars in (c) and (e) represent the standard deviation of triplicated biotransformation.

One-pot synthesis of D-phenylglycine 1a from styrene 7a with *E. coli* coexpressing 7 enzymes. Cascade biotransformation of 30 mM **7a** was performed with resting cells of *E. coli* (LZ113-LZ136) in a two-liquid phase system of ethyl oleate (EO)[25] and KP buffer containing NH_3 and glucose. EO serves as a reservoir for hydrophobic substrates **7** and intermediate **6** to reduce the toxicity. Glucose was added to regenerate NADPH for the cascade reaction [1 mole of NADPH required for producing 1 mole of (*R*)-**1a**] via cellular metabolism. As shown in Figure 2c, (*R*)-**1a** was produced by all 24 strains in 24 h. Among the strains, the *E. coli* (LZ116) containing pACYC-*smo-speh*, pET-*alkj-ecaldh* and pRSF-*dpgat-gludh-smdh* (Figure 2d) gave the highest conversion to (*R*)-**1a**, thus it was selected as the best catalyst for this cascade biotransformation. Under the optimized conditions, 60 mM of **7a** was reacted with resting cells of *E. coli* (LZ116) to give 48 mM (7.3 g L^{-1}) of enantiopure (*R*)-**1a** in 80% conversion in 24 h (Figure 2e). A small amount of **5a** (6 mM) and **2a** (2 mM) were accumulated at the end of the reaction.

One-pot synthesis of substituted D-phenylglycines 1b-1m from substituted styrenes 7b-7m with *E. coli* (LZ116). 12 Substituted styrenes **7b-7m** (10-20 mM) were subjected to the resting cells of *E. coli* (LZ116) to explore the substrate scope (Table 2). 5 Substituted D-phenylglycines, **1b**, **1d**, **1g**, **1j**, and **1k**, were produced in excellent *ee* (98-99%) and high conversion (80-90%). (*R*)-**1c**, (*R*)-**1f**, (*R*)-**1l**, and (*R*)-**1m**, were also formed in very high *ee* (94-97%) and high conversion (75-90%). Moderate conversion (45-50%) was observed for *ortho*-chloro substituted (*R*)-**1e** (97% *ee*) and bromo substituted (*R*)-**1h** (92% *ee*) and (*R*)-**1i** (99% *ee*), probably due to the low activity of SMO on the corresponding alkene substrates (**7e**, **7h**, **7i**) with a *ortho*- or bulky substituent. These results demonstrated the general applicability of this 6-step enzyme cascade for synthesis of D-phenylglycine derivatives.

In comparison with the 3-step cascade from mandelic acid, the product concentration of this 6-step cascade from styrene is lower, probably due to the moderate efficiency of styrene epoxidation with SMO and diol oxidation with AlkJ.[20a] Nevertheless, the successful production of D-phenylglycine from styrene in high conversion by using O_2, NH_3, and glucose demonstrated a totally new one-pot enantioselective synthesis of this important class of chiral compounds without involving the cyanide chemistry. We believe that cascade biocatalytic oxidation could be a general tool for cyanide-free production of carboxy acids.

Enantioselective conversion of L-phenylalanine to D-phenylglycine

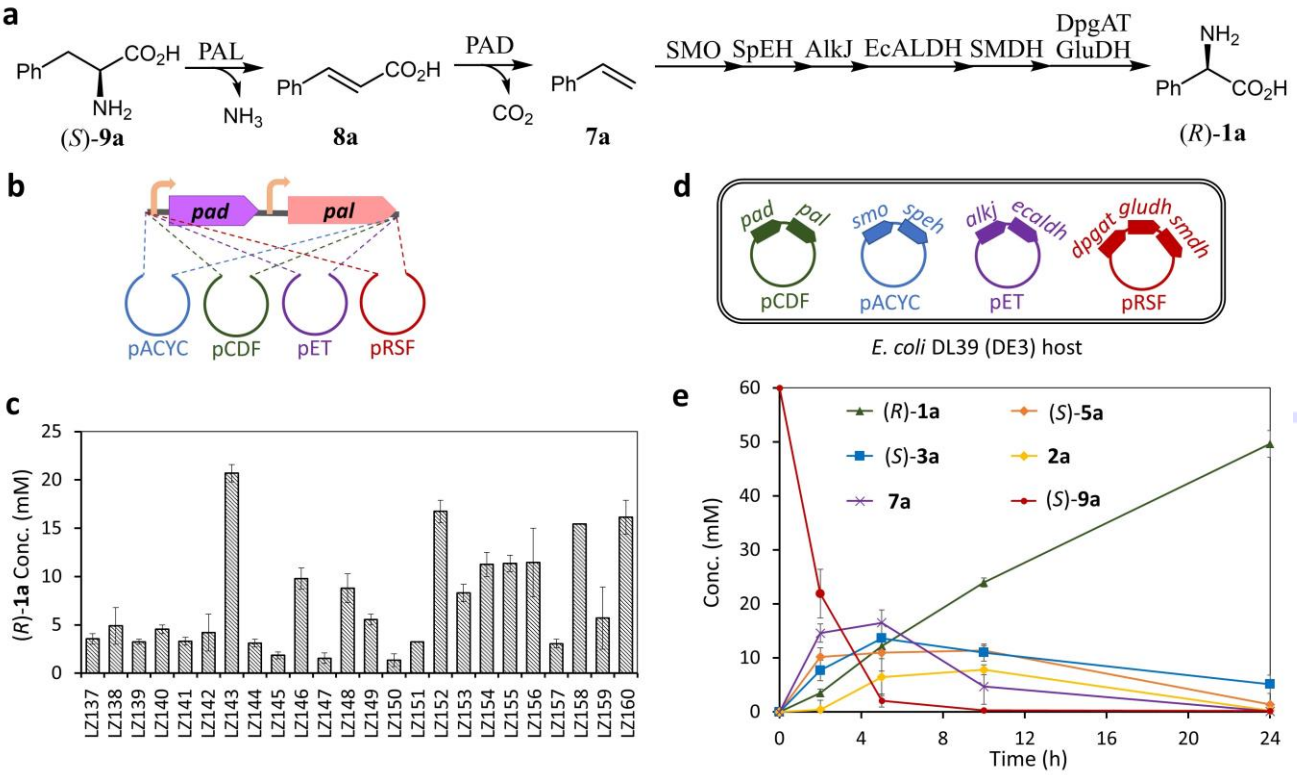


Figure 3. (a) Cascade biotransformation of (*S*)-**9a** to (*R*)-**1a** with phenylalanine ammonia lyase (PAL), pheylacrylic acid decarboxylase (PAD), SMO, SpEH, AlkJ, EcALDH, SMDH, DpgAT, and GluDH. (b) PAD-PAL module on four compatible plasmids, pACYC, pCDF, pET and pRSF, respectively. (c) Screening of *E. coli* (LZ137-160) cells (10 g cdw L^{-1}) for one-pot production of (*R*)-**1a** from (*S*)-**9a** (30 mM) in 24h. (d) Construction of *E. coli* (LZ143). (e) Time course of one-pot production of (*R*)-**1a** from (*S*)-**9a** (60 mM) using *E. coli* (LZ143) cells (15 g cdw L^{-1}) in a two-liquid phase system of EO and KP buffer containing glucose and NH_3/NH_4Cl. Error bars in (c) and (e) represent the standard deviation of triplicated biotransformation.

Selection of enzymes and construction of *E. coli* strains. An important trend in chemical synthesis is to use renewable starting material.[26] While mandelic acid and styrene are mainly synthesized from petroleum-based materials, many biogenic L-amino acids are currently manufactured from sugars by microbial fermentation. L-Phenylalanine (*S*)-**9a** is one of them, and its cascade biotransformation to (*R*)-**1a** could provide a sustainable synthetic route. Building-up on the above 6-step cascade from **7a** to (*R*)-**1a** (Figure 2a), two more enzymes, phenylalanine ammonia lyase (PAL) and phenylacrylic acid decarboxylase (PAD),[21b] were used to convert (*S*)-**9a** to **7a** (Figure 3a). The PAL-PAD module was constructed on the four plasmids (Figure 3b), which were then transformed into *E. coli* (LZ112-LZ136) cells to give 24 strains *E. coli* (LZ137-LZ160). Each of *E. coli* (LZ137-LZ160) coexpressed the 9 enzymes, PAD, PAL, SMO, SpEH, AlkJ, EcALDH, DpgAT, GluDH, and SMDH.

One-pot synthesis of D-phenylglycine 1a from L-phenylalanine 9a with *E. coli* coexpressing 9 enzymes. The 24 *E. coli* (LZ137-LZ160) strains were examined for cascade biotransformation of 30 mM (*S*)-**9a** in a two-liquid phase system of KP buffer containing glucose and EO, which serves as a reservoir for toxic intermediate **7a**. A small amount of glucose was added to regenerate NADPH for the cascade reaction [1 mole of NADPH required for producing 1 mole of (*R*)-**1a**] via cellular metabolism. As shown in Figure 3c, (*R*)-**1a** was produced from (*S*)-**9a** by all 24 strains in 24 h. The *E. coli* (LZ143) with pCDF-*pad-pal*, pACYC-*smo-speh*, pET-*alkj-ecaldh*, and pRSF-*dpgat-gludh-smdh* (Figure 3d) clearly outperformed other 23 strains in conversion to (*R*)-**1a**, thus it was chosen for further study. Under the optimized reaction conditions, 60 mM of (*S*)-**9a** was transformed by resting cells of *E. coli* (LZ143) to give 50 mM (7.5 g L^{-1}) of enantiopure (*R*)-**1a** in 83% conversion in 24 h (Figure 3e). Only a small amount of **5a** (1 mM) and **3a** (5 mM) were left at the end of the reaction. In comparison with the 6-step cascade from **7a**, this 8-step cascade from (*S*)-**9a** achieved the similar high product concentration and conversion, demonstrating the high efficiency of a long non-natural enzyme cascade/pathway.

Preparation of D-phenylglycine 1a and D-4-fluorophenylglycine 1d by cascade biocatalysis.

To demonstrate the synthetic utility of the three types of whole-cell based cascade biocatalysis, preparative biotransformation was performed on a 40-100 mL-scale in shaking flasks (Table 3). *E. coli* (LZ110) (20 g cdw L^{-1}) was applied to convert *rac*-**3a** (200 mM) and *rac*-**3d** (40 mM) into enantiopure (*R*)-**1a** and (*R*)-

Table 3. Preparation of (*R*)-**1a** and (*R*)-**1d** from different substrates via one-pot cascade biocatalysis with *E. coli* (LZ110), *E. coli* (LZ116), or *E. coli* (LZ143).[a]

Sub.	Sub. Conc. [mM]	Biocatalyst	Reaction volume [mL	Prod.	Conv. [%][d]	Isol. Yield [%][e]	Prod. *ee* [%][f]
rac-**3a**	200	*E. coli* (LZ110)	40[b]	(*R*)-**1a**	93	71	99
rac-**3d**	60	*E. coli* (LZ110)	40[b]	(*R*)-**1d**	78	52	99
7a	60	*E. coli* (LZ116)	120[c]	(*R*)-**1a**	83	62	99
7d	30	*E. coli* (LZ116)	120[c]	(*R*)-**1d**	85	46	99
(*S*)-**9a**	60	*E. coli* (LZ143)	96[c]	(*R*)-**1a**	81	53	99

[a] General reaction conditions: *E. coli* resting cells (20 g cdw L^{-1}), glucose, NH_3/NH_4Cl, 30 °C, 24 h (see experiment part for details). [b] KP buffer (400 mM, pH 8.0). [c] KP buffer (400 mM, pH 8.0) and EO (5: 1). [d] Determined by HPLC analysis. [e] Simple precipitation without optimization. [f] Measured by chiral HPLC analysis.

1d in 93% and 78% conversion in 24 h, respectively. The products were easily recovered by the following simple precipitation procedure without optimization: acidification of the reaction mixture with HCl, centrifugation to remove the cells, neutralization of the supernatant with NaOH to precipitate the product, and filtration. Finally, enantiopure (*R*)-**1a** and (*R*)-**1d** were obtained in 71% and 52% isolated yield, respectively.

For the cascade biocatalysis from styrene, **7a** (60 mM) and **7d** (30 mM) were transformed with *E. coli* (LZ116) (20 g cdw L^{-1}) to enantiopure (*R*)-**1a** and (*R*)-**1d** in 83% and 85% conversion in 24 h, respectively. The products were isolated in 62% and 46% yield after the similar workup, respectively.

For the cascade biotransformation from biobased L-phenylalanine, enantiopure (*R*)-**1a** was produced in 81% conversion from (*S*)-**9a** (60 mM) after reaction with *E. coli* (LZ143) (20 g cdw L^{-1}) for 24 h. Following the similar workup procedure, enantiopure (*R*)-**1a** was obtained in 53% isolated yield. These preparative biotransformations demonstrated the synthetic potential of these biocatalytic one-pot cascades.

Conclusion

Highly efficient, enantioselective, and green synthesis of D-phenylglycine **1a** in high *ee* and high yield from racemic mandelic acid **3a** was demonstrated for the first time via one-pot 3-step cascade biotransformation. An L-α-transaminase-deficient *E. coli* (LZ110) strain coexpressing 4 enzymes (MR, SMDH, DpgAT, and GluDH) in double plasmids was engineered as a highly productive catalyst for the cascade reactions. Whole-cell based biotransformation of easily available *rac*-**3a** with non-toxic reagents (NH_3 and glucose) gave enantiopure (*R*)-**1a** in 29.5 g L^{-1} with 93% conversion, providing a green and productive method for practical manufacturing of D-phenylglycine. Enantioselective synthesis of D-phenylglycine **1a** in high *ee* and high yield from very cheap hydrocarbon styrene **7a** was also demonstrated for the first time via one-pot 6-step cascade biotransformation. *E. coli* (LZ116) strain overexpressing 7 enzymes (SMO, SpEH, AlkJ, EcALDH, SMDH, DpgAT and GluDH) in 3 plasmids was developed as an efficient catalyst for this type of transformation. By using the *E. coli* cells as catalysts, enantiopure (*R*)-**1a** was successfully produced from **7a** in 80% conversion. This one-pot synthesis starting from styrene and using O_2, NH_3, and glucose provides a green and efficient synthesis of D-phenylglycine and also a cyanide-free synthesis of both substrate and product. Enantioselective synthesis of D-phenylglycine **1a** in high *ee* and high yield from biobased L-phenylalanine **9a** was demonstrated for the first time via one-pot 8-step cascade biotransformation. Engineering of *E. coli* (LZ143) strain coexpressing 9 enzymes (PAL, PAD, SMO, SpEH, AlkJ, EcALDH, SMDH, DpgAT and GluDH) in 4 plasmids gave a powerful catalyst for the cascade reactions. Whole-cell based biotransformation afforded enantiopure (*R*)-**1a** in 83% conversion from biobased (*S*)-**9a**. This synthesis starts from a renewable substrate, uses O_2, NH_3, and glucose, and gives rise to a cyanide-free and sustainable synthesis of D-phenylglycine. The one-pot 3-step and 6-step cascade biotransformations were generally applicable to produce different D-phenylglycine derivatives (*R*)-**1b-1h** in high *ee* with high conversion from the corresponding mandelic acids **3b-3m** and styrenes **7b-7m**, respectively. Representative preparation examples [(*R*)-**1a** and (*R*)-**1d]** with all three one-pot syntheses were demonstrated by whole-cell biotransformation, followed by simple product isolation via precipitation. This study highlights the great potential of engineering cascade biocatalysis for one-pot synthesis of important non-natural chiral chemicals in a green, safe, sustainable, enantioselective, and efficient manner.

Experimental Section

Genetic engineering of recombinant plasmids

Genetic engineering of plasmids containing *dpgat-gludh*: The gene of DpgAT was synthesized and amplified using primers "ACTGTCATGAGTATTCTGAACGACT ACAAGC" and "ACTGAGATCTTTAGGATTGATTAC CACTCAGGTTG" with Phusion DNA polymerase. The PCR product was double-digested with BspHI and BglII, and then ligated to the NcoI and BglII digested pRSFduet-1 vector with T4 DNA ligase. The ligation product was transformed into *E. coli* T7 Expression competent cells to give pRSF-*dpgat*. The gene of GluDH was amplified from

the plasmid from previous research[21a] using primers "ACTGAGATCTTAAGGAGATATATAATGGATCAG ACATATTCTCTGGAGTC" and "ACTGCTCGAGTTA AATCACACCCTGCGCCAGCATC" with Phusion DNA polymerase. The PCR product was double-digested with BglII and XhoI, and then ligated to the BglII and XhoI digested pRSF-*dpgat* with T4 DNA ligase. The ligation product was transformed into *E. coli* T7 Expression competent cells to give pRSF-*dpgat-gludh*. The *dpgat-gludh* was sub-cloned to the other three vectors by the following procedure. *dpgat-gludh* was amplified with primers "ACTGTCATGAGTATTCTGAACGACTACAA GC" and "ACTGGCTCGAGTTAAATCACACCCTGCGC CAGCATC", digested with BspHI and XhoI, and then ligated to NcoI and XhoI digested pACYCduet-1, pCDFduet-1, and pETduet-1. The transformation of these products gave pACYC-*dpgat-gludh*, pCDF-*dpgat-gludh*, and pET-*dpgat-gludh*, respectively.

Genetic engineering of the plasmids containing *smdh-mr*: SMDH was amplified from the genome of *Pseudomonas putida* ATCC 12633[23] using primers "ACTGTCATGAGCCAGAATCTCTTTA ACGTTG" and "ACTGGAATTCTCATGCGTGTGTTCCTTTACCAAT G" with Phusion DNA polymerase. The PCR product was double-digested with BspHI and EcoRI, and then ligated to the NcoI and EcoRI digested pRSFduet-1 vector with T4 DNA ligase. The ligation product was transformed into *E. coli* T7 Expression competent cells to give pRSF-*smdh*. The XhoI restriction site inside *smdh* was removed by the following procedure. pRSF-*smdh* was subjected to mutagenesis by cyclic PCR with primers "TCGCTTTAG CTCGTGCAGCAACCAAGG" and "CCTTGGTTGCTG CACGAGCTAAAGCGA", then the PCR product was digested with DpnI and transformed into *E. coli* T7 Expression competent cells to give pRSF-*smdh*(A288T). The gene of MR was amplified using primers "ACTG GAATTCTAAGGAGATATATAATGAGTGAAGTACT GATTACCGGCC" and "ACTGCTCGAGTTACACCA GATATTTCCCGATTTCT" with Phusion DNA polymerase. The PCR product was double-digested with EcoRI and XhoI, and then ligated to the EcoRI and XhoI digested pRSF-*smdh*(A288T) with T4 DNA ligase. The ligation product was transformed into *E. coli* T7 Expression competent cells to give pRSF-*smdh-mr*. The *smdh-mr* was sub-cloned to the other three vectors by the following procedure. *smdh-mr* was amplified with primers "ACTGTCATGAGCCAGAATCTCTTTAACGTTG" and "ACTGCTCGAGTTACACCAGATATTTCCCGATTTC T", digested with BspHI and XhoI, and then ligated to NcoI and XhoI digested pACYCduet-1, pCDFduet-1, and pETduet-1. The transformation of these products gave pACYC-*smdh-mr*, pCDF-*smdh-mr*, and pET-*smdh-mr*, respectively.

Genetic engineering of plasmids containing *dpgat-gludh-smdh*: *dpgat-gludh* was amplified with primers "ACTGTCATGAGTATTCTGAACGACTACAAGC" and "ACTGGGTACCTTAAATCACACCCTGCGCCAGCAT C", digested with BspHI and KpnI, and then ligated to NcoI and KpnI digested pRSFduet-1. The transformation of these products gave pRSF-*dpgat-gludh*(KpnI) with KpnI and XhoI restriction sites after the *gludh*. The gene of SMDH was amplified from the pRSF-*smdh*(A288T) using primers "ACTGGGTACCTAAGGAGATATATAATGAG CCAGAATCTCTTTAACGTTG" and "ACTGCTCGAG TCATGCGTGTGTTCCTTTACCAATG" with Phusion DNA polymerase. The PCR product was double-digested with KpnI and XhoI, and then ligated to the KpnI and XhoI digested pRSF-*dpgat-gludh*(KpnI) with T4 DNA ligase. The ligation product was transformed into *E. coli* T7 Expression competent cells to give pRSF-*dpgat-gludh-smdh*. The *dpgat-gludh-smdh* was amplified with primers "ACTGTCATGAGTATTCTGAACGACTACAAGC" and "ACTGCTCGAGTCATGCGTGTGTTCCTTTACCAAT G", digested with BspHI and XhoI, and then ligated to NcoI and XhoI digested pACYCduet-1, pCDFduet-1, and pETduet-1. The transformation of these products gave pACYC-*dpgat-gludh-smdh*, pCDF-*dpgat-gludh-smdh*, and pET-*dpgat-gludh-smdh*, respectively.

Other plasmids: The plasmids from previous research were directly used in this study without modification: four plasmids containing *smo-speh* (pACYC-*smo-speh*, pCDF-*smo-speh*, pET-*smo-speh*, pRSF-*smo-speh*),[21a] four plasmids containing *alkj-ecaldh* (pACYC-*alkj-ecaldh*, pCDF-*alkj-ecaldh*, pET-*alkj-ecaldh*, pRSF-*alkj-ecaldh*),[21a] and four plasmids containing PAD-PAL (pACYC-*pad-pal*, pCDF-*pad-pal*, pET-*pad-pal*, pRSF-*pad-pal*).[21b]

Genetic Engineering of recombinant *E. coli* strains

Engineering of *E. coli* DL39 (DE3) host: *E. coli* DL39 strain[20] with the genome type *λ⁻, aspC13, fnr-25, rph-1, ilvE12, tyrB507* was purchased from the Coli Genetic Stock Center, Yale University (CGSC#: 6913). *E. coli* DL39 (DE3) strain was engineered by using a λDE3 lysogenization kit (Merck) according to the standard protocol of the kit with the following steps: 1. grow the *E. coli* DL39 strain on LB supplemented with maltose, $MgSO_4$; 2. infect the cells with λDE3 phage lysate, helper phage lysate and selection phage lysate; 3. transfer the cells to normal LB plate to culture single colonies. 4. verification of λDE3 lysogens with the T7 tester phage lysate.

Engineering of *E. coli* (LZ101-112) strains: each of *smdh-mr* plasmids (pACYC-*smdh-mr*, pCDF-*smdh-mr*, pET-*smdh-mr*, and pRSF-*smdh-mr*) and each of *dpgat-gludh* plasmids (pACYC-*dpgat-gludh*, pCDF-*dpgat-gludh*, pET-*dpgat-gludh*, and pRSF-*dpgat-gludh*) were co-transformed into competent cells of *E. coli* DL39 (DE3) to give the 12 *E. coli* strains coexpressing SMDH, MR, DpgAT and GluDH. The details were provided in Table S1 Supporting Information.

Engineering of *E. coli* (LZ113-136) strains: each of *smo-speh* plasmids (pACYC-*smo-speh*, pCDF-*smo-speh*, pET-*smo-speh*, and pRSF-*smo-speh*), each of *alkj-ecaldh* plasmids (pACYC-*alkj-ecaldh*, pCDF-*alkj-ecaldh*, pET-*alkj-ecaldh*, and pRSF-*alkj-ecaldh*), and each of *dpgat-gludh-smdh* plasmids (pACYC-*dpgat-gludh-smdh*, pCDF-*dpgat-gludh-smdh*, pET-*dpgat-gludh-smdh*, and pRSF-*dpgat-gludh-smdh*) were co-transformed into competent cells of *E. coli* DL39 (DE3) to give the 24 *E. coli* strains coexpressing SMO, SpEH, AlkJ, EcALDH, SMDH, DpgAT and GluDH. The details were provided in Table S1 in the Supporting Information.

Engineering of *E. coli* (LZ137-160) strains: the *E. coli* (LZ113-136) strains were cultured and made into competent cells with $CaCl_2$ treatment. One of *pad-pal* plasmids (pACYC-*pad-pal*, pCDF-*pad-pal*, pET-*pad-pal*, pRSF-*pad-pal*) was transformed into competent cells of *E. coli* (LZ113-136) to give the 24 *E. coli* strains coexpressing PAL, PAD, SMO, SpEH, AlkJ, EcALDH, SMDH, DpgAT and GluDH. The details were provided in Table S1 Supporting Information.

Procedure for culturing *E. coli* and biotransformation

General procedure for culturing *E. coli* cells: *E. coli* strain (LZ101-160) was inoculated from the cell stock in deep freezer to 1 mL LB medium containing a mixture of antibiotic (50 mg L^{-1} chloramphenicol, 50 mg L^{-1} streptomycin, 100 mg L^{-1} ampicillin, 50 mg L^{-1} kanamycin) and grew at 37 °C for 8–10 h. The culture was inoculated into 50 mL M9 medium containing glucose (20 g L^{-1}), yeast extract (6 g L^{-1}), and antibiotics in a 250-mL tri-baffled flask. The cells continued to grow at 37 °C and 300 rpm for about 2 h to reach an OD_{600} of 0.6-0.8, followed by the addition of IPTG to final concentration of 0.5 mM to induce the protein expression. The cells further grew at 22 °C overnight (12–13 h) to reach late

exponential phase, and they were harvested by centrifugation (4000 rpm, 10 min). The cell pellets were resuspended in KP buffer to the desired density as resting cells for biotransformation.

General procedure for biotransformation of *rac*-2-hydroxy-2-phenylacetic acid 3 to (*2R*)-amino(phenyl)acetic acid 1: freshly prepared *E. coli* (LZ101-112) cells were resuspended in KP buffer (400 mM, pH 8.0) containing glucose (2%) and NH_3/NH_4Cl (200 mM, pH 8.0). Stock solutions of **3a**-**3m** (200 mM) in the same KP buffer were prepared. Suspension of cells and solution of **3a**-**3m** were added into a 100-mL flask to form a 2-mL system with cell density of 10-15 g cdw L^{-1} and substrate concentration of 10-30 mM. The reaction mixture was incubated at 30 °C and 250 rpm. For the time curve of (*R*)-**1a** production (Figure 1e), 0.06 mmol of *rac*-**3a** was added at 0, 2, 4, 6, 8, 10, and 12 h. Additional 0.5% of glucose and 100 mM of NH_3/NH_4Cl was fed at 2, 4, 6, 8, 10, and 12 h. 50 μL of reaction samples were taken at 2, 4, 6, 8, 10, 12, and 24 h. For the screening of the best strain (Figure 1c), 100 μL of reaction samples were taken at 2 h. For the testing of substrate scope (Table 1), 100 μL of reaction samples were taken at 24 h. The reaction samples were mixed with equal volume of 1 M HCl solution (50-100 μL) and subjected to centrifugation (12000 rpm, 3 min). The supernatant (50-100 μL) was mixed with TFA solution (0.5%, 450-400 μL) and acetonitrile (containing 2 mM benzyl alcohol, 500 μL) to prepare the samples for HPLC analysis.

General procedure for biotransformation of styrene 7 to (*2R*)-amino(phenyl)acetic acid 1: freshly prepared *E. coli* (LZ113-136) cells were resuspended in KP buffer (400 mM, pH 8.0) containing glucose (0.5%) and NH_3/NH_4Cl (100 mM, pH 8.0). Stock solutions of **7a**-**7m** (50-150 mM) in EO were prepared. Suspension of cells was added into a 100-mL flask to form a 2-mL system with cell density of 10-15 g cdw L^{-1}, and 0.4 mL of EO containing **7a**-**7m** was added to reach a substrate concentration of 10-30 mM. The reaction mixture was incubated at 30 °C and 250 rpm. Additional glucose (1%) and NH_3/NH_4Cl (100 mM) was added at 10 h. For the time curve of (*R*)-**1a** production (Figure 2e), 100 μL of reaction samples were taken at 2, 5, 9, and 24 h. For the screening of the best strain (Figure 2c) and testing of substrate scope (Table 2), 100 μL of reaction samples were taken at 24 h. The reaction samples were mixed with equal volume of 1 M HCl solution (100 μL) and subjected to centrifugation (12000 rpm, 3 min). The aqueous solution (100 μL) was mixed with TFA solution (0.5%, 400 μL) and acetonitrile (containing 2 mM benzyl alcohol, 500 μL) to prepare the samples for HPLC analysis. For the time curve of (*R*)-**1a** production (Figure 2e), 50 μL of EO samples were mixed with EtOAc (containing 2 mM benzyl alcohol, 950 μL) to prepare the samples for GC analysis.

General procedure for biotransformation of (*S*)-2-amino-3-phenylpropanoic acid 9a to (*2R*)-amino(phenyl)acetic acid 1a: freshly prepared *E. coli* (LZ137-160) cells were resuspended in KP buffer (400 mM, pH 8.0) containing glucose (0.5%). Stock solutions of (*S*)-**9a** (150 mM) in the same KP buffer was prepared. Suspension of cells and solution of (*S*)-**9a** were added into a 100-mL flask to form a 2-mL system with cell density of 10-15 g cdw L^{-1} and substrate concentration of 30-60 mM. And 0.4 mL of EO was added to the system. The reaction mixture was incubated at 30 °C and 250 rpm. Additional glucose (2%) and NH_3/NH_4Cl (200 mM) was added at 10 h. For the time curve of (*R*)-**1a** production (Figure 3e), 100 μL of reaction samples were taken at 2, 5, 10, and 24 h. For the screening of the best strain (Figure 3c), 100 μL of reaction samples were taken at 24 h. The reaction samples were mixed with equal volume of 1 M HCl solution (100 μL) and subjected to centrifugation (12000 rpm, 3 min). The aqueous solution (100 μL) was mixed with TFA solution (0.5%, 400 μL) and acetonitrile (containing 2 mM benzyl alcohol, 500 μL) to prepare the samples for HPLC analysis. For the time curve of (*R*)-**1a** production (Figure 3e), 50 μL of EO samples were mixed with EtOAc (containing 2 mM benzyl alcohol, 950 μL) to prepare the samples for GC analysis.

Preparation of (*2R*)-amino(phenyl)acetic acid 1a from *rac*-2-hydroxy-2-phenylacetic acid 3a and (*2R*)-amino(4-fluorophenyl)acetic acid 1d from *rac*-(4-fluorophenyl)(hydroxy)acetic acid 3d by cascade biotransformation with *E. coli* (LZ110)

E. coli (LZ110) cells were resuspended in 40 mL of KP buffer (400 mM, pH 8.0) containing glucose (2%) and NH_3/NH_4Cl (200 mM, pH 8.0) to reach a final density of 20 g cdw L^{-1} in a 250-mL flask with baffles. The reaction mixture was incubated at 30 °C and 250 rpm with the following substrate feeding procedure: for the synthesis of (*R*)-**1a**, 1.2 mmol of *rac*-**3a** was added at 0, 2, 4, 6, 8, and 10 h, and 0.8 mmol) of *rac*-**3a** was added at 12 h (total 8 mmol, 1217 mg, of *rac*-**3a** added). Additional 0.5% (w/v) of glucose and 100 mM of NH_3/NH_4Cl was fed at 2, 4, 6, 8, 10, and 12 h. For the synthesis of (*R*)-**1d**, 1.2 mmol of *rac*-**3d** was added at 0 and 6 h (total 2.4 mmol, 408 mg, of *rac*-**3d** added). Additional 1% of glucose and 100 mM of NH_3/NH_4Cl was fed at 6 h. The reaction proceeded for 24 h, and then HCl was added to reach pH of 1 to dissolve the precipitated (*R*)-**1a** and (*R*)-**1d**. The mixture was subjected to centrifugation (3200 *g*, 15 min), and the clear aqueous solution was collected, while the cells were washed with HCl solution (0.01 M). The aqueous solution was combined and neutralized to pH = 7 with NaOH solution (10 M). Then, the aqueous mixture was concentrated to about 10 mL with rotary evaporator. The precipitated products were separated by filtration and carefully washed with cold water and ethanol. The products were dried under vacuum overnight.

D-Phenylglycine **1a** was isolated as white solid (859 mg, 71% yield) in 99% *ee*. ^{1}H NMR (400 MHz, in D_2O with 2% H_2SO_4): δ = 7.40–7.35 (m, 5H), 5.07 (s, 1H) ppm. ^{13}C NMR (100 MHz, in D_2O with 2% H_2SO_4): δ = 170.7, 131.4, 130.3, 129.7, 128.0, 56.5 ppm. $[\alpha]_D^{20}$: −152° (*c* 1.0, 1 M HCl), {reported $[\alpha]_D^{20}$: −157° (*c* 1.0, 1 M HCl)[27a]}.

D-4-Fluorophenylglycine **1d** was isolated as white solid (211 mg, 52% yield) in 99% *ee*. ^{1}H NMR (400 MHz, in D_2O with 2% H_2SO_4): δ = 7.39–7.35 (m, 2H), 7.12–7.08 (m, 2H), 5.09 (s, 1H) ppm. ^{13}C NMR (100 MHz, in D_2O with 2% H_2SO_4): δ = 170.6, 163.4 (d, *J* = 245.9 Hz), 130.4 (d, *J* = 9.0 Hz), 127.5 (d, *J* = 3.4 Hz), 116.5 (d, *J* = 22.0 Hz), 55.8 ppm. $[\alpha]_D^{20}$: −137° (*c* 1.0, 1 M HCl), {reported $[\alpha]_D^{22}$: −135° (*c* 0.5, 1 M HCl) 98% *ee*[27b]}.

Preparation of (*2R*)-amino(phenyl)acetic acid 1a from styrene 7a and (*2R*)-amino(4-fluorophenyl)acetic acid 1d from 1-fluoro-4-vinylbenzene 7d by cascade biotransformation with *E. coli* (LZ116)

E. coli (LZ116) cells were resuspended in 100 mL of KP buffer (400 mM, pH 8.0) containing glucose (0.5%) and NH_3/NH_4Cl (100 mM, pH 8.0) to reach a final density of 20 g cdw L^{-1} in a 500-mL flask with baffles. 20 mL of ethyl oleate (EO) were added to form the second phase. 6 mmol (625 mg) of **7a** or 3 mmol (366 mg) of **7d** was added into the system to start the reaction at 30 °C and 250 rpm for 24 h. Additional 1% of glucose and 200 mM of NH_3/NH_4Cl was added at 10 h. At the end of the reaction, the reaction mixture was acidified with HCl to pH = 1, and then subjected to centrifugation (3200 *g*, 15 min). The cells were washed with HCl solution (0.01 M). The aqueous solution was combined and washed with cyclohexane (20 mL) to remove the hydrophobic impurities. The aqueous solution was neutralized to pH = 7 with NaOH solution (10 M), and concentrated to about 20 mL with rotary evaporator. The precipitated products were separated by filtration and carefully washed with cold water and ethanol. The products were dried under vacuum overnight.

D-Phenylglycine **1a** was isolated as white solid (562 mg, 62% yield) in 99% *ee*. ^{1}H NMR (400 MHz, in D_2O with 2% H_2SO_4): δ = 7.41–7.36 (m, 5H), 5.08 (s, 1H) ppm. ^{13}C NMR (100 MHz, in D_2O with 2% H_2SO_4): δ = 170.8,

131.4, 130.3, 129.7, 128.0, 56.5 ppm. $[\alpha]_D^{20}$: −150° (*c* 1.0, 1 M HCl), {reported $[\alpha]_D^{20}$: −157° (*c* 1.0, 1 M HCl)[27a]}.

D-4-Fluorophenylglycine **1d** was isolated as white solid (233 mg, 46% yield) in 99% *ee*. ^{1}H NMR (400 MHz, in D_2O with 2% H_2SO_4): δ = 7.39–7.36 (m, 2H), 7.13–7.09 (m, 2H), 5.09 (s, 1H) ppm. ^{13}C NMR (100 MHz, in D_2O with 2% H_2SO_4): δ = 170.6, 163.4 (d, *J* = 245.7 Hz), 130.4 (d, *J* = 8.9 Hz), 127.4 (d, *J* = 3.0 Hz), 116.6 (d, *J* = 22.1 Hz), 55.8 ppm. $[\alpha]_D^{20}$: −138° (*c* 1.0, 1 M HCl), {reported $[\alpha]_D^{22}$: −135° (*c* 0.5, 1 M HCl) 98% *ee*[27b]}.

Preparation of (*2R*)-amino(phenyl)acetic acid 1a from (*S*)-2-amino-3-phenylpropanoic acid 9a by cascade biotransformation with *E. coli* (LZ143)

E. coli (LZ143) cells were resuspended in 80 mL of KP buffer (400 mM, pH 8.0) containing glucose (0.5%) to reach a final density of 20 g cdw L^{-1} in a 500-mL flask with baffles. 16 mL of EO was added to form the second phase. 4.8 mmol (793 mg) of (*S*)-**9a** was added into the system to start the reaction at 30 °C and 250 rpm. Additional 2% of glucose and 200 mM of NH_3/NH_4Cl was added at 10 h. The reaction was stopped at 24 h. The same downstream processing to the preparation of (*R*)-**1a** from **7a** was carried out.

D-Phenylglycine **1a** was isolated as white solid (385 mg, 53% yield) in 99% *ee*. ^{1}H NMR (400 MHz, in D_2O with 2% H_2SO_4): δ = 7.40–7.35 (m, 5H), 5.07 (s, 1H) ppm. ^{13}C NMR (100 MHz, in D_2O with 2% H_2SO_4): δ = 170.8, 131.4, 130.3, 129.7, 128.0, 56.5 ppm. $[\alpha]_D^{20}$: −151° (*c* 1.0, 1 M HCl), {reported $[\alpha]_D^{20}$: −157° (*c* 1.0, 1 M HCl)[27a]}.

Contents in supporting information

Chiral HPLC chromatograms (Figure S4-S16), NMR spectra (Figure S17-S21), list of strains (Table S1), chemicals, biochemicals, medium, analytical methods, source and sequence of genes.

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