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Biocatalytic Formal Anti-Markovnikov Hydroamination and Hydration of Aryl Alkenes

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ABSTRACT: Biocatalytic anti-Markovnikov alkene hydroamination and hydration were achieved based on two concepts involving enzyme cascades: epoxidation-isomerization-amination for hydroamination and epoxidation-isomerization-reduction for hydration. An *Escherichia coli* strain co-expressing styrene monooxygenase (SMO), styrene oxide isomerase (SOI), ω-transaminase (CvTA), and alanine dehydrogenase (AlaDH) catalyzed the hydroamination of 12 aryl alkenes to give the corresponding valuable terminal amines in high conversion (many ≥ 86%) and exclusive anti-Markovnikov selectivity (> 99 : 1). Another *E. coli* strain co-expressing SMO, SOI, and phenylacetaldehyde reductase (PAR) catalyzed the hydration of 12 aryl alkenes to the corresponding useful terminal alcohols in high conversion (mostly ≥ 80%) and very high anti-Markovnikov selectivity (> 99 : 1). Importantly, SOI was discovered for stereoselective isomerization of a chiral epoxide to a chiral aldehyde, providing some insights on enzymatic epoxide rearrangement. Harnessing this stereoselective rearrangement, highly enantioselective anti-Markovnikov hydroamination and hydration were demonstrated to convert α-methylstyrene to the corresponding (*S*)-amine and (*S*)-alcohol in 84-81% conversion with 97-92% *ee*, respectively. The biocatalytic anti-Markovnikov hydroamination and hydration of alkenes, utilizing cheap and non-toxic chemicals (O_2, NH_3, and glucose) and cells, provide an environmentally friendly, highly selective, and high-yielding synthesis of terminal amines and alcohols.

KEYWORDS: alkenes, anti-Markovnikov, biocatalysis, enzyme cascades, hydration, hydroamination, Meinwald rearrangement, whole-cell biotransformation

INTRODUCTION

Anti-Markovnikov addition to alkenes provides a facile access to various terminal functionalized chemicals, with huge potential for industrial applications.[1,2] The two most important anti-Markovnikov reactions are hydroamination and hydration to produce terminal amines and alcohols, respectively, which was listed as one of ten challenges in industrial catalysis.[2] Despite many developments on these reactions over the years, green and efficient methods are still highly wanted. Anti-Markovnikov hydroamination with CuH or acridinium photoredox catalyst was recently reported,[3–5] but requiring expensive hydride reagent or a long reaction time. Anti-Markovnikov hydroamination of unactivated olefins with iridium photocatalyst was reported very recently.[6] Current hydroamination processes mainly use primary or secondary amines to produce secondary or tertiary hydroamination products, while they are difficult in using NH_3 to generate primary amines. On the other hand, anti-Markovnikov hydration of alkene is very challenging. Formal hydration methods were thus developed, such as multi-step hydroboration-oxidation or oxidation-hydrolysis-reduction.[7,8] However, these methods either requires borane reagents, or needs high loading of toxic precious metal catalysts. Very recently, light-mediated anti-Markovnikov hydration was reported with acridinium photoredox catalyst with a hydrogen atom donor, but it requires a long reaction time with a TON of 230.[9]

Biocatalysis is a useful tool for chemical synthesis in a greener and more sustainable way.[10–15] Nature provides ammonia-lyases and aminomutases for hydroamination of α,β-unsaturated acids to give amino acids.[16–18] However, no biocatalytic method has been described for hydroamination of unfunctionalized alkenes to produce amines. On the other hand, some hydratases were known for hydration of C=C bonds conjugated to carbonyl groups.[19] Terpene hydratases,[20] oleate hydratase[21] and decarboxylases[22] were reported for hydrating terpenes, 1-

decene and hydroxystyrenes, respectively. However, they show Markovnikov selectivity. There is a lack of a biocatalytic method for anti-Markovnikov hydration of unfunctionalized alkenes to alcohols.

Here, we present two novel biocatalytic methods for formal anti-Markovnikov hydroamination and hydration of alkenes, respectively. The methods utilize one-pot cascade biotransformation[23–40] and involve two enzyme cascades: epoxidation-isomerization-amination for hydroamination and epoxidation-isomerization-reduction for hydration (Scheme 1).

RESULTS AND DISCUSSION

Selection of Enzymes for Target Reactions. To demonstrate the concept, we selected the transformation of styrenes **1** to phenethylamines **4** and phenethyl alcohols **5** as the model reactions. While styrene is a simple and easily available alkene, the products are very useful with many synthetic applications (Table S1 in Supporting Information). Styrene monooxygenase (SMO)[41-43] is a well-studied and efficient enzyme, thus it was select selected for the epoxidation of **1** to (*S*)-**2**. For isomerization of (*S*)-**2** to **3**, a unique styrene oxide isomerase (SOI)[39, 44-46] was chosen. A ω-transaminase (ω-TA)[47-50] and an alanine dehydrogenase (AlaDH)[51] were selected for the amination of **3** to **4**. Combining these four enzymes together enables the formal anti-Markovnikov alkene hydroamination. On the other hand, formal anti-Markovnikov alkene hydration could be achieved by combining alcohol dehydrogenase (ADH) or aldehyde reductase (AR)[52] (for reducing **3** to **5**) with SMO and SOI. The enzymes were co-expressed in whole cells for practical biotransformation,[53] because 1) SMO in whole cells produced much higher concentration of (*S*)-**2** (36.3 g L^{-1})[42] than that produced by SMO *in vitro* (0.16 g L^{-1});[43] 2) SOI is

an integral membrane protein;[44-46] 3) the cofactor NAD(P)H could be regenerated by cellular metabolism of glucose.

Genetic Engineering of Biocatalysts. The SOI gene (*styC*)[44] from *Pseudomonas* sp. VLB120 was synthesized and cloned together with the SMO genes (*styAB*)[28] to give plasmid pRSF-StyABC. For hydroamination, CvTA from *Chromobacterium violaceum*[48] is active for amination of **3a** to **4a** (Figure S1). The genes of CvTA and AlaDH from *Bacillus subtilis*[51] were constructed with pRSF-StyABC to give pRSF-StyABC-CvTA-AlaDH (Figure S2). Hydroamination of **1a** with *E. coli* T7 Express host harboring this plasmid gave 20% byproduct **5a** (Figure S3), due to the competing reduction by native ADHs and reductases in the host. A new *E. coli* RAREΔMao strain, which was engineered by deletion of *maoA-padA* genes from *E. coli* RARE strain[54] (already deleted 6 ADH genes), was used as the host, and the formation of **4a** is much less (3%, Figure S3). To optimize the co-expression of the four enzymes, the StyABC and CvTA-AlaDH was split into two plasmids to generate 12 different strains (Figure S4). The strain containing pACYC-StyABC and pCDF-CvTA-AlaDH (Figure 1a) gave the best biotransformation result of **1a** to **4a** (Figure S5), thus it was named *E. coli* (StyABC-CvTA-AlaDH) strain and used for hydroamination.

For hydration, we tested several ADHs and ARs for the reduction of **3a** to **5a**, and the phenylacetaldehyde reductase (PAR) from tomato *Solanum lycopersicum*[52] was found to be the best (Figure S6). Thus, the PAR gene was engineered with StyABC to give plasmid pRSF-StyABC-PAR. The recombinant plasmid was transformed into *E. coli* T7 Express cells to give *E. coli* (StyABC-PAR) for hydration of **1** to **5** (Figure 1b).

Hydroamination of Styrene Derivatives. Hydroamination of **1a** at 50–100 mM was performed with resting cells of *E. coli* (StyABC-CvTA-AlaDH), and high conversion to **4a** was achieved for 75 mM **1a** (Figure S7). The time course of hydroamination of 80 mM **1a** was investigated (Figure 1c): **1a** was quickly converted to **4a** in the first 4 h, and a small amount of **2a** (up to 8 %) and **3a** (up to 2 %) was accumulated initially and then converted. At the end of reaction (10 h), the amine (**4a**) was produced in 93 % conversion. Noteworthly, byproduct (**5a**) was detected in trace amount (0.6%), indicating the very high chemoseletivity. In addition, another isomer of **4a**, 1-phenyethylamine, was not observed, demonstrating the excellent anti-Markovnikov regioselectivity.

To explore the substrate scope of hydroamination, aryl alkenes (**1a**-**1l**) were transformed with the resting cells of *E. coli* (StyABC-CvTA-AlaDH) (Table 1). Styrene derivatives with a fluoro substituent (**1b**, **1c**, **1d**), a methyl substituent (**1i**, **1j**) or a methoxy substituent (**1k**, **1l**) were converted to the corresponding phenethylamines **4** in very high conversion of 93–99 %. Chloro substituted styrenes (**1e**, **1f**) and bromo substituted styrenes (**1g**, **1h**) were also converted to the corresponding terminal amines **4** in good to moderate conversion of 86–45 %. The chemoselectivity of amine over alcohol is also very high (all > 20 : 1, Figure S46–S57), and the anti-Markovnikov regioselectivity is excellent (all > 99 : 1, Figure S20–S31).

Hydration of Styrene Derivatives. Biocatalytic hydration of **1a** at 60–80 mM was carried out with resting cells of *E. coli* (StyABC-PAR), and high conversion to **5a** (>90 %) was obtained with 60 mM **1a** in organic phase (Figure S8). The detail time course of hydration of 60 mM **1a** is shown in Figure 1d: **1a** was quickly converted to **5a** in the initial 3 h, and only a very small amount of **2a** (0.3 %) and **3a** (3 %) were accumulated at initial 1 h. At end of reaction (8 h), the desired **5a** was formed in 93 % conversion with little **1a** (2 %) was remained. Very importantly,

another isomer of **5a**, 1-phenyethanol, was not observed, proving the exclusive anti-Markovnikov regioselectivity.

The scope of hydration was explored by subjecting 12 aryl alkenes (**1a**-**1l**) to the resting cells of *E. coli* (StyABC-PAR) (Table 2). Many substituted 2-phenylethanols were produced in very high conversion (90–99 %), including fluoro substituted **5b**, **5c**, **5d**, methyl substituted **5i**, **5j**, and methoxy substituted **5k**, **5l**. 2-Phenylethanols with a chloro (**5e**, **5f**) or bromo (**5g**, **5h**) substituent were formed in good conversion of 89–60 %. Importantly, the anti-Markovnikov regioselectivity for all the hydration was excellent (> 99 : 1, Figure S33–S44).

Stereoselective Isomerization of an Epoxide to an Aldehyde. To establish enantioselective hydroamination and hydration, we chose **1m** as the model substrate (Scheme 1b). As SMO was known for epoxidation of **1m** to (*S*)-**2m**[43] [we further validated the production of (*S*)-**2m** in 98% *ee* with the SMO in our system, Figure S9], the key for the enantioselective cascades is the isomerization of (*S*)-**2m**. *E. coli* (SOI) was then used to isomerize (*S*)-**2m** to give (*S*)-**3m** in 91 % *ee* at 15-30 min (Figure 2a). Slow decrease of the *ee* was observed due to the racemization via tautomerization in aqueous buffer.[55–57] To confirm that the stereoselective isomerization of (*S*)-**2m** is catalyzed by SOI, the reaction of (*S*)-**2m** was carried out by using *E. coli* cells without SOI (control), which did not produce **3m** (Figure S10). Furthermore, isomerization of (*S*)-**2m** was performed with partially purified SOI, which gave (*S*)-**3m** in 95% *ee* in 15 min (Figure S11). In comparison, chemical Meinwald rearrangement of (*R*)-**2m** gave the chiral aldehyde in very low *ee*.[58] Further investigation of the conversion of *rac*-**2m** with *E. coli* (SOI) gave nearly racemic **3m** [6 % *ee* (*R*), Figure S12], indicating that the isomerization of (*R*)-**2m** produced (*R*)-**3m**. Kinetic resolution of *rac*-**2m** (20 mM) with *E. coli* (SOI) was then performed (Figure S12): the *ee* of (*S*)-**2m** was 10% at 77% conversion, and the *ee* of (*S*)-**2m** reached 18% at 91% conversion,

which corresponds to an E value of 1.2. This suggests that SOI slightly prefers the isomerization of (*R*)-**2m** over the isomerization of (*S*)-**2m**; on the other hand, the *ee* of (*R*)-**3m** reached 6% at 35% conversion at 15 min and decreased gradually to nearly zero. A previously proposed mechanism of SOI involves following steps: enzymatic protonation induces the opening of the epoxide ring and the formation of a benzyl cation intermediate; it was subjected to proton abstraction to give an enol, which undergos tautomerization to an aldehyde (Figure S13a).[45] We found now that SOI is stereoselective, thus, If SOI follows this mechanism, (*S*)-**2m** will be firstly converted to a planar enol intermediate which requires asymmetric tautomerization to give (*S*)-**3m**. We think, the following mechanism of SOI is also possible: enzymatic Brønsted acid (glutamic acid or aspartic acid residue) protonates the epoxide and induces a C-O cleavage to form a similar benzyl cation intermediate; a unique stereoselective 1,2-hydrogen shift (H-shift) takes place to give the chiral aldehyde (*S*)-**3m** (Figure S13b). Currently, we are investigating the catalytic mechanism of SOI.

Enantioselective hydroamination. To achieve enantioselective hydroamination, the last step in the cascade should be fast and enantioselective towards (*S*)-**3m**. For asymmetric hydroamination of **1m**, transaminases CvTA, HnTA from *Hyphomonas neptunium*,[49] and VfTA from *Vibrio fluvialis*[50] were examined for the transamination of *rac*-**3m** (Table S2). While CvTA and VfTA are highly active for transamination of *rac*-**3m** with *R*-selectivity, HnTA was less active but showing the desired *S*-selectivity towards *rac*-**3m**. *E. coli* (StyABC-HnTA) and *E. coli* (StyABC-VfTA-AlaDH) were thus engineered (Figure S14, S15). Biotransformation of **1m** with *E. coli* co-expressing SMO, SOI, and different ω-TA (and AlaDH) gave (*S*)-**4m** in 76% conversion with 51 % *ee,* 8% conversion with 95 % *ee*, and 81% conversion with 70 % *ee*, respectively (Table 3, entry 1-3). Considering both conversion and product *ee*, VfTA was

selected for directed evolution[59–63] to invert its *R*-enantioselectivity to *S*-enantioselective towards *rac*-**3m**: 12 residues (Figure S16) near the substrate binding site of VfTA were subjected to iterative saturation mutagenesis[64] to give $VfTA_{DM}$ (L56A/I259T) as a highly *S*-enantioselective mutant (Table S3). Docking of (*R*)- or (*S*)-**4m** in the substrate binding pockets of VfTA and $VfTA_{DM}$ explained the reverse of enantiopreference (Figure S17). *E. coli* (StyABC-$VfTA_{DM}$-AlaDH) was then engineered (Figure S18) for hydroamination of **1m**, producing (*S*)-**4m** in 92 % *ee* and 84 % conversion (entry 4). During the reaction, (*S*)-**3m** was accumulated only in a small amount due to the fast conversion of (*S*)-**3m** to (*S*)-**4m,** thus significantly reducing the racemization (*S*)-**3m**; and (*S*)-**4m** was produced continuously in high *ee* (Figure 2b). The biocatalytic hydroamination is much more enantioselective than the CuH-catalyzed method, which produced *N, N*-dibenzyl (*S*)-**4m** in 52% *ee* from **1m**.[4]

Enantioselective hydration. For asymmetric hydration of **1m**, PAR and horse liver ADH (HLADH, isoenzyme E)[55] were studied for the reduction of *rac*-**3m** (Table S4). While PAR was almost non-enantioselective towards *rac*-**3m**, HLADH was highly *S*-enantioselective. *E. coli* (StyABC-HLADH) was engineered to co-express SMO, SOI and HLADH (Figure S16). Biotransformation of **1m** with *E. coli* (StyABC-PAR) gave (*S*)-**5m** in 87 % *ee* and 85 % conversion (Table 3, entry 5), whereas the same hydration of **1m** with *E. coli* (StyABC-HLADH) afforded (*S*)-**5m** in 97 % *ee* and 81 % conversion (entry 6). In comparison with the asymmetric hydroboration-oxidation method [(*S*)-**5m** in 66 % *ee* from **1m**],[7] the biocatalytic asymmetric hydration is much more stereoselective.

Preparative Biotransformation. Hydroamination of **1a**, **1d**, **1j**, and **1k** (2.5 mmol each) was performed with *E. coli* (StyABC-CvTA-AlaDH) in a 60-mL system to give the corresponding phenethylamines in 83-95 % conversion. Extraction and flash chromatography purification

afforded **4a**, **4d**, **4j**, and **4k** in 78, 68, 71, 82 % isolated yield, respectively. This is corresponding to 1.7–2.1 mmol product per gram of dry cells. **1a**, **1d**, **1j**, and **1k** (2.5 mmol each) were transformed with *E. coli* (StyABC-PAR) in a 100-mL system to produce the corresponding 2-phenylethanols in 87-99 % conversion. Extraction and flash chromatography purification offered **5a**, **5d**, **5j**, and **5k** in 83, 74, 66, 78 % isolated yield, respectively. This is corresponding to 1.6–2.1 mmol product per gram of dry cells. Preparative asymmetric hydroamination of **1m** (0.5 mmol) was demonstrated with *E. coli* (StyABC-VfTA$_{TM}$-AlaDH) to give (*S*)-**4m** in 65 % isolated yield and 90 % *ee.* Preparative asymmetric hydration of **1m** (0.5 mmol) was performed with *E. coli* (StyABC-HLADH) to give (*S*)-**5m** in 59 % isolated yield and 96 % *ee*.

CONCLUSION

Biocatalytic formal anti-Markovnikov hydroamination and hydration of aryl alkenes were achieved via two enzyme cascades: epoxidation-isomerization-amination for hydroamination and epoxidation-isomerization-reduction for hydration. *E. coli* cells co-expressing 3-4 enzymes of the cascades catalyzed the hydroamination and hydration of 12 aryl alkenes, respectively, to give the corresponding terminal amines and terminal alcohols in high conversion with excellent chemoselectivity and anti-Markovnikov regioselectivity. Furthermore, highly enantioselective hydroamination and hydration were also successfully demonstrated, offering a new biocatalytic access to chiral amines and chiral alcohols. In addition, SOI was proven to be stereoselective, shedding some light on enzymatic epoxide rearrangement and opening the possibility of using this enzyme for enantioselective synthesis. To the best of our knowledge, this is the first biocatalytic anti-Markovnikov hydroamination and hydration of unfunctionalized alkenes. The biocatalytic anti-Markovnikov hydroamination and hydration with *E. coli* cells utilize cheap and

non-toxic reagents (O_2, NH_3, and glucose) and cells (biodegradable) to provide a green, highly selective, and high-yielding synthesis of terminal amines and alcohols. Currently, we are working on exploring and engineering SOI for converting other epoxides, to apply the biocatalytic cascades for anti-Markovnikov hydroamination and hydration of other alkenes.

EXPERIMENTAL SECTION

General Procedure for Culturing *E. coli* Cells for Biotransformation. *E. coli* strain was initially inoculated in LB medium (1 mL) with kanamycin (50 mg L^{-1}) [for *E. coil* (StyABC-PAR), *E. coil* (StyABC-HLADH)], a mixture of chloramphenicol (50 mg L^{-1}) and streptomycin (50 mg L^{-1}) [for *E. coil* (StyABC-CvTA-AlaDH)] or a mixture of chloramphenicol (50 mg L^{-1}) and kanamycin (50 mg L^{-1}) [for *E. coil* (StyABC-HnTA), *E. coil* (StyABC-VfTA-AlaDH), *E. coil* (StyABC-$VfTA_{DM}$-AlaDH)]. The cells grew for 8–10 h at 37 °C, and then transferred to a 250-mL tri-baffled flask with 50 mL M9 medium supplemented with glucose (20 g L^{-1}), yeast extract (6 g L^{-1}) and appropriate antibiotics. The cells continued to grow at 37 °C and 250 rpm for about 2 h to reach an OD_{600} of 0.6, and then IPTG (0.5 mM final concentration) was added to induce the enzyme expression. The cells further grew at 22 °C overnight (12–13 h) and they were harvested by centrifugation (4000 g, 10 min).

Hydroamination of 1 to 4 with *E. coli* (StyABC-CvTA-AlaDH). The cell pellets of *E. coli* (StyABC-CvTA-AlaDH) were resuspended in NaP buffer (sodium phosphate, 200 mM, pH 8, 2% glucose, 200 mM NH_3/NH_4Cl) to a cell density of 10 g cdw L^{-1}. To a shaking flask (100 mL) containing 2 mL of cell suspension, 2 mL *n*-$C_{16}H_{34}$ containing substrate **1a-1l** (20-80 mM) was added. The reaction was incubated in a shaking incubator at 250 rpm and 30 °C for 10-24 h.

For determining the reaction time curve (Figure 1c), 80 μL samples of the two phases were taken out at 1, 2, 4, 6, and 10 h. 50 μL of *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 950 μL EtOAc [with internal standard: 2 mM benzyl alcohol (BA)], and analyzed by GC-FID for measuring the concentration of **1a**, **2a**, **3a**, and **5a**. 20 μL aqueous supernatant was isolated by centrifugation (12000 g, 3 min), mixed with acid solution (0.5% TFA) and 500 μL ACN (with 2 mM BA), and then analyzed by HPLC for measuring the concentration of **4a** and **5a**. For determining the substrate scope (Table 1), 200 μL samples of the two phase were taken out at the end of reaction. 100 μL *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 900 μL EtOAc (with 2 mM BA), and analyzed by GC-FID for measuring the concentration of **1a-1l** and **5a-5l**. 100 μL of aqueous supernatant was isolated by centrifugation (12000 g, 3 min), mixed with 400 μL acid solution (0.5% TFA) and 500 μL ACN (with 2 mM BA), and then analyzed by HPLC for measuring the concentration of **4a-4l** and **5a-5l**. At the end of the reaction, the aqueous phase was subjected to centrifugation (12000 g, 3 min). 500 μL supernatant was isolated and adjusted to pH > 13 with NaOH solution (10 M). Then 500 μL EtOAc was used to extract **4a-4l** and other amines and analyzed by GC-FID for determining the regioselectivity.

Hydration of 1 to 5 with *E. coli* (StyABC-PAR). The cell pellets of *E. coli* (StyABC-PAR) were resuspended in KP buffer (potassium phosphate, 200 mM, pH 8, 2% glucose) to a cell density of 10 g cdw L^{-1}. To a shaking flask (100 mL) containing 2 mL cell suspension, 2 mL *n*-$C_{16}H_{34}$ containing substrate **1a-1l** (20-60 mM) was added. The reaction was incubated in a shaking incubator at 250 rpm and 30 °C for 8 h. For determining the reaction time curve (Figure 1d), 80 μL samples of the two phases were taken out at 0.5, 1, 1.5, 2, 3, 5, and 8 h. 50 μL *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 950 μL EtOAc (with 2 mM

BA), and analyzed by GC-FID for measuring the concentration of **1a**, **2a**, **3a**, and **5a**. 20 μL aqueous supernatant was isolated by centrifugation (12000 g, 3 min), mixed with 480 μL acid solution (0.5% TFA) and 500 μL ACN (with 2 mM BA), and then analyzed by HPLC for measuring the concentration of **5a**. For determining the substrate scope (Table 2), 200 μL samples of the two phases were taken out at the end of reaction. 100 μL *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 900 μL EtOAc (with 2 mM BA), and analyzed by GC-FID for measuring the concentration of **1a-1l** and **5a-5l** and determining the regioselectivity. 100 μL aqueous supernatant was isolated by centrifugation (12000 g, 3 min), mixed with 400 μL acid solution (0.5% TFA) and 500 μL ACN (with 2 mM BA), and then analyzed by HPLC for measuring the concentration of **5a-5l**.

Asymmetric Hydroamination of 1m. The cell pellets of *E. coli* (StyABC-CvTA-AlaDH), *E. coli* (StyABC-HnTA), *E. coli* (StyABC-VfTA-AlaDH), or *E. coli* (StyABC-$VfTA_{DM}$-AlaDH) were resuspended in KP buffer (200 mM, pH 8, 2% glucose, 200 mM NH_3/NH_4Cl) (200 mM D-alanine instead of NH_3/NH_4Cl for reaction with HnTA) to a cell density of 20 g cdw L^{-1}. To a shaking flask (100 mL) containing 2 mL of cell suspension, 2 mL *n*-$C_{16}H_{34}$ containing 10 mM substrate **1m** (for reactions with CvTA and VfTA) or 1 mL *n*-$C_{16}H_{34}$ containing 10 mM substrate **1m** (for reactions with HnTA and $VfTA_{DM}$) was added. The reaction was incubated in a shaking incubator at 250 rpm and 30 °C for 24 h. For determining the end point data (Table 3), 200 μL samples of the two phases were taken out at 24 h. 100 μL *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 900 μL EtOAc (with 2 mM BA), and analyzed by GC-FID for measuring the concentration of **1m**, **2m**, **3m** and **5m**. 100 μL aqueous supernatant was isolated by centrifugation (12000 g, 3 min), mixed with 900 μL acid solution (0.5% TFA), and then analyzed by chiral HPLC for measuring the concentration and *ee* of **4m**. The aqueous

phase was subjected to centrifugation (12000 g, 3 min). 500 μL supernatant was isolated and adjusted to pH ≥ 13 with NaOH solution (10 M). Then 500 μL EtOAc was used to extract amines and analyzed by GC-FID for determining the regioselectivity. For determining the reaction time curve (Figure 2b), 40 μL samples of organic phase and 80 μL samples of aqueous phase were taken out at 1, 2, 5, 10, and 24 h. 20 μL *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 200 μL EtOAc (with 2 mM BA), and analyzed by GC-FID for measuring the concentration of **1m**-**3m**. 50 μL aqueous supernatant was isolated by centrifugation (12000 g, 3 min), mixed with 450 μL acid solution (0.5% TFA), and then analyzed by chiral HPLC for measuring the concentration and *ee* of **4m**.

Asymmetric Hydration of 1m. The cell pellets of *E. coli* (StyABC-PAR) or *E. coli* (StyABC-HLADH) were resuspended in KP buffer (200 mM, pH 8, 2% glucose) to a cell density of 15 g cdw L^{-1}. To a shaking flask (100 mL) containing 2 mL cell suspension of *E. coli* (StyABC-PAR), 2 mL *n*-$C_{16}H_{34}$ containing substrate **1m** (10 mM) was added. To a shaking flask (100 mL) containing 2 mL cell suspension of *E. coli* (StyABC-HLADH), 40 μL substrate **1m** (250 mM in EtOH) was added to reach a final concentration of 5 mM. The reaction was incubated in a shaking incubator at 250 rpm and 30 °C for 24 h. At the end of reaction, 300 μL samples of the two phases were taken out. 100 μL *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 900 μL EtOAc (with 2 mM BA), and analyzed by GC-FID for measuring the concentration of **1m**, **2m**, **3m** and **5m**. Another 100 μL *n*-$C_{16}H_{34}$ was isolated by centrifugation (12000 g, 3 min), mixed with 900 μL *n*-hexane, and analyzed by chiral HPLC for determining the *ee* of **5m**. 100 μL aqueous supernatant was isolated by centrifugation (12000 g, 3 min), mixed with 400 μL acid solution (0.5% TFA) and 500 μL ACN (with 2 mM BA), and then analyzed by HPLC for measuring the concentration of **5m**.

Preparative Hydroamination. The cell pellets of *E. coli* (StyABC-CvTA-AlaDH) were resuspended in NaP buffer (200 mM, pH 8, 2% glucose, 200 mM NH_3/NH_4Cl) to form a 50-mL suspension (20 g cdw L^{-1}) in a tri-baffled flask (250 mL). 10 mL of *n*-$C_{16}H_{34}$ containing substrate **1a**, **1d**, **1j**, or **1k** (2.5 mmol each) was added into the flask to start the reaction at 250 rpm and 30 °C. To monitor the reaction, samples were taken out at different time. Extra glucose (0.5%) and NH_3/NH_4Cl (50 mM) were added into the system at 8 h. At the end of reaction (24 h), the mixture was adjusted to pH ≤ 2 with concentrated HCl, and the *n*-$C_{16}H_{34}$ and the cells were removed by centrifugation (4000 g, 15 min). The aqueous solution was collected and washed with 50 mL of *n*-hexane to remove organic impurities and residues of *n*-$C_{16}H_{34}$. The aqueous solution was adjusted to pH ≥ 12 with NaOH solution (10 M), then saturated with NaCl, and extracted with EtOAc (50 mL) for 3 times. The EtOAc was combined and dried by adding anhydrous Na_2SO_4. The EtOAc was evaporated by using a rotary evaporator. The crude product was purified by flash chromatography on a silica gel column with CH_2Cl_2: MeOH: triethylamine of 100: 5: 1 as the eluent ($R_f \approx 0.3$). The collected fractions were subjected to GC-FID analysis to confirm the purity. The organic solvent in the desired fractions was removed by evaporation and vacuum pumping.

Phenethylamine 4a was obtained as light yellow oil: 236 mg, 78% yield from **1a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24–7.11 (m, 5H), 2.90 (t, *J* = 6.8 Hz, 2H), 2.68 (t, *J* = 6.8 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.6, 127.8, 127.4, 125.2, 42.4, 38.8 ppm.

4-Fluorophenethylamine 4d was obtained as light yellow oil: 237 mg, 68% yield from **1d**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.20–6.89 (m, 4H), 2.87 (t, *J* = 6.8 Hz, 2H), 2.65 (t, *J* = 6.8 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.5 (d, *J* = 242 Hz), 134.3 (d, *J* = 3 Hz), 129.1 (d, *J* = 8 Hz), 114.2 (d, *J* = 21 Hz), 42.5, 38.0 ppm.

4-Methylphenethylamine 4j was obtained as light yellow oil: 240 mg, 71% yield from **1j**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18–7.00 (m, 4H), 2.87 (t, *J* = 6.8 Hz, 2H), 2.64 (t, *J* = 6.8 Hz, 2H), 2.25 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.6, 134.6, 128.1, 127.7, 42.6, 38.6, 20.0 ppm.

3-Methoxyphenethylamine 4k was obtained as light yellow oil: 309 mg, 82% yield from **1k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.19–7.12 (m, 1H), 6.73–6.67 (m, 3H), 3.72 (s, 3H), 2.90 (t, *J* = 6.8 Hz, 2H), 2.66 (t, *J* = 6.8 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.7, 140.3, 128.4, 120.2, 113.6, 110.4, 54.1, 42.3, 38.8 ppm.

The cell pellets of *E. coli* (StyABC-VfTA$_{DM}$-AlaDH) were resuspended in KP buffer (200 mM, pH 8, 2% glucose, 200 mM NH_3/NH_4Cl) to form a 10-mL suspension (20 g cdw L^{-1}) in a flask (250 mL). 5 mL *n*-$C_{16}H_{34}$ containing substrate **1m** (0.05 mmol) was added into the flask to start the reaction at 250 rpm and 30 °C. 10 parallel experiments were performed together. To monitor the reaction, samples were taken out at different time. Extra glucose (0.5%) and NH_3/NH_4Cl (50 mM) were added into the system at 5 h. At the end of reaction (24 h), the reaction mixture was combined, adjusted to pH ≤ 2 with concentrated HCl, and the *n*-$C_{16}H_{34}$ and the cells were removed by centrifugation (4000 g, 15 min). The aqueous solution was collected and washed with 50 mL of *n*-hexane to remove organic impurities and residues of *n*-$C_{16}H_{34}$. The aqueous solution was adjusted to pH ≥ 12 with NaOH solution (10 M), then saturated with NaCl, and extracted with EtOAc (100 mL) for 3 times. The EtOAc was combined and dried by adding anhydrous Na_2SO_4. The EtOAc was evaporated by using a rotary evaporator. The crude product was purified by flash chromatography on a silica gel column with CH_2Cl_2: MeOH: triethylamine of 100: 5: 1 as the eluent ($R_f \approx 0.3$). The collected fractions were subjected to GC-FID analysis to

confirm the purity. The organic solvent of the desired fractions was removed by evaporation and vacuum pumping.

(*S*)-β-methylphenethylamine 4m was obtained as light yellow oil: 45 mg, 67% yield from **1m**, 91% *ee*, $[\alpha]_D^{20}$: –31° (*c* 1.0, $CHCl_3$) {literature[65] $[\alpha]_D^{20}$: –35.3° (*c* 0.5, $CHCl_3$), 95% *ee*}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.19 (m, 5H), 2.87–2.60 (m, 3H), 1.25 (d, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 144.9, 128.6, 127.4, 126.4, 49.4, 43.4, 19.3 ppm.

Preparative Hydration. The cell pellets of *E. coli* (StyABC-PAR) were suspended in KP buffer (200 mM, pH 8, 2% glucose) to form a 50-mL suspension (20 g cdw L^{-1}) in a tri-baffled flask (500 mL). 50 mL *n*-$C_{16}H_{34}$ containing substrate **1a**, **1d**, **1j**, or **1k** (2.5 mmol each) was added into the flask to start the reaction at 250 rpm and 30 °C. To monitor the reaction, samples were taken out at different time. Extra glucose (0.5%) was added into the system at 8 h. At the end of reaction (24 h), the cells were removed by centrifugation (4000 g, 15 min). The *n*-$C_{16}H_{34}$ was extracted with water (50 mL) for 5 times. The water and aqueous solution were combined, saturated with NaCl, and extracted with EtOAc (100 mL) for 3 times. The EtOAc was combined and dried by adding anhydrous Na_2SO_4. The EtOAc was evaporated by using a rotary evaporator. The crude product was purified by flash chromatography on a silica gel column with *n*-hexane: EtOAc of 5: 1 as the eluent ($R_f \approx 0.3$). The collected fractions were subjected to GC-FID analysis to confirm the purity. The organic solvent of the desired fractions was removed by evaporation and vacuum pumping.

Phenethyl alcohol 5a was obtained as colorless oil: 253 mg, 83% yield from **1a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.21 (m, 5H), 3.84 (t, *J* = 6.6 Hz, 2H), 2.86 (t, *J* = 6.6 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.7, 129.2, 128.8, 126.7, 63.9, 39.4 ppm.

4-Fluorophenethyl alcohol 5d was obtained as colorless oil: 259 mg, 74% yield from **1d**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26–6.97 (m, 4H), 3.83 (t, *J* = 6.6 Hz, 2H), 2.83 (t, *J* = 6.6 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.9 (d, *J* = 243 Hz), 134.4 (d, *J* = 3 Hz), 130.6 (d, *J* = 8 Hz), 115.5 (d, *J* = 21 Hz), 63.8, 38.5 ppm.

4-Methylphenethyl alcohol 5j was obtained as colorless oil: 225 mg, 66% yield from **1j**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25–7.11 (m, 4H), 3.83 (t, *J* = 6.6 Hz, 2H), 2.82 (t, *J* = 6.6 Hz, 2H), 2.32 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 136.2, 135.5, 129.5, 129.1, 64.0, 38.9, 21.2 ppm.

3-Methoxyphenethyl alcohol 5k was obtained as colorless oil: 297 mg, 78% yield from **1k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26–7.21 (m, 1H), 6.83–6.77 (m, 3H), 3.85 (t, *J* = 6.4 Hz, 2H), 3.80 (s, 3H), 2.84 (t, *J* = 6.6 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.0, 140.3, 129.8, 121.5, 115.0, 112.0, 63.7, 55.4, 39.4 ppm.

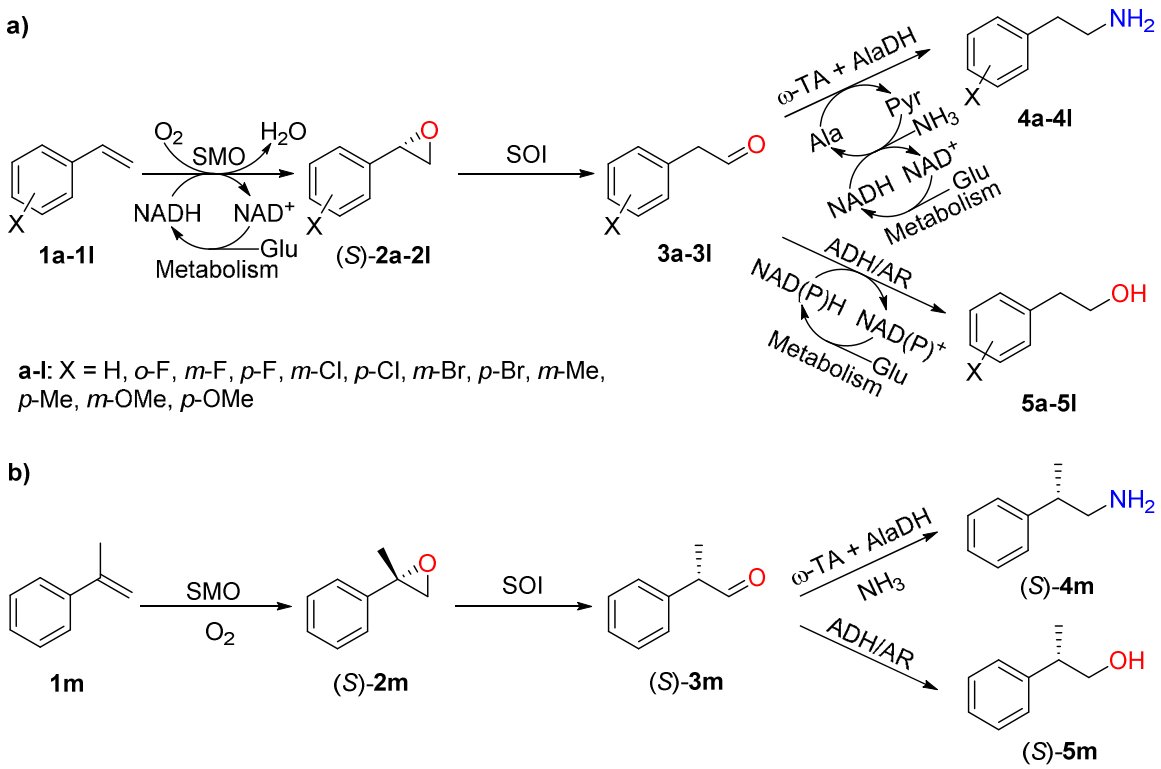
The cell pellets of *E. coli* (StyABC-HLADH) were suspended in KP buffer (200 mM, pH 8, 2% glucose) to form a 10-mL suspension (20 g cdw L^{-1}) in a flask (250 mL). 200 μL substrate **1m** in EtOH (250 mM, **1m** amount 0.05 mmol) was added into the flask to start the reaction at 250 rpm and 30 °C. 10 parallel experiments were performed together. To monitor the reaction, samples were taken out at different time. Extra glucose (0.5%) was added into the system at 5h. At the end of reaction (24 h), the reaction mixture was combined and subjected to centrifugation (4000 g, 15 min) to remove the cells. The supernatant was saturated with NaCl, and extracted with EtOAc (50 mL) for 3 time. The EtOAc was combined and dried by adding anhydrous Na_2SO_4. The EtOAc was evaporated by using a rotary evaporator. The crude product was purified by flash chromatography on a silica gel column with *n*-hexane: EtOAc of 5: 1 as the

eluent ($R_f \approx 0.3$). The collected fractions were subjected to GC-FID analysis to confirm the purity. The organic solvent of the desired fractions was removed by evaporation and vacuum pumping.

(*S*)-2-phenyl-1-propanol 5m was obtained as colorless oil: 40 mg, 59% yield from **1m**, 96% *ee*, $[\alpha]_D^{20}$: –12° (*c* 1.0, $CHCl_3$) {literature[66] $[\alpha]_D^{22}$: –11.6° (*c* 0.21, $CHCl_3$), 95% *ee*}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.23 (m, 5H), 3.70 (d, *J* = 6.8 Hz, 2H), 2.95 (sex, *J* = 6.8 Hz, 1H), 1.28 (d, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 143.7, 128.6, 127.5, 126.7, 68.7, 42.5, 17.6 ppm.

SCHEMES

Scheme 1. a) Formal anti-Markovnikov hydroamination and hydration of aryl alkenes using O_2 and NH_3 via epoxidation-isomerization-amination cascade, and using O_2 via epoxidation-isomerization-reduction cascade, respectively. b) Enantioselective hydroamination and hydration of α-methylstyrene via the enzyme cascades.



SMO = styrene monooxygenase, SOI = styrene oxide isomerase, ω-TA = ω-transaminase, AlaDH = L-alanine dehydrogenase, ADH = alcohol dehydrogenase, AR = aldehyde reductase.

FIGURES

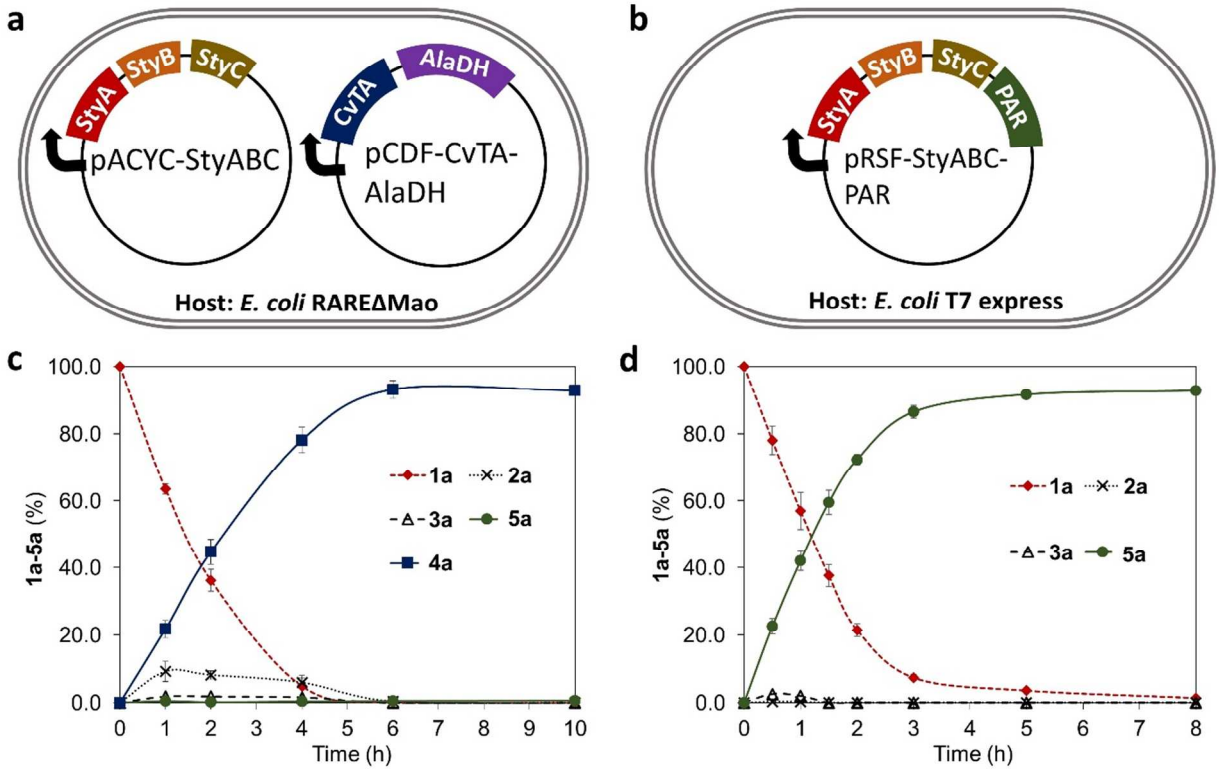


Figure 1. a) *E. coli* (StyABC-CvTA-AlaDH) co-expressing SMO, SOI, CvTA and AlaDH for hydroamination. b) *E. coli* (StyABC-PAR) co-expressing SMO, SOI and PAR for hydration. c) Hydroamination of 80 mM styrene **1a** to 2-phenylethylamine **4a** with *E. coli* (StyABC-CvTA-AlaDH) cells (10 g cdw L^{-1}) in NaP buffer (200 mM, pH 8, 2 % glucose, 200 mM NH_3/NH_4Cl) and *n*-$C_{16}H_{34}$ (1:1) at 30 °C. 1% Glucose and 100 mM NH_3/NH_4Cl were added at 4 h. d) Hydration of 60 mM styrene **1a** to 2-phenylethanol **5a** with *E. coli* (StyABC-PAR) cells (10 g cdw L^{-1}) in KP buffer (200 mM, pH 8, 2 % glucose) and *n*-$C_{16}H_{34}$ (1:1) at 30 °C.

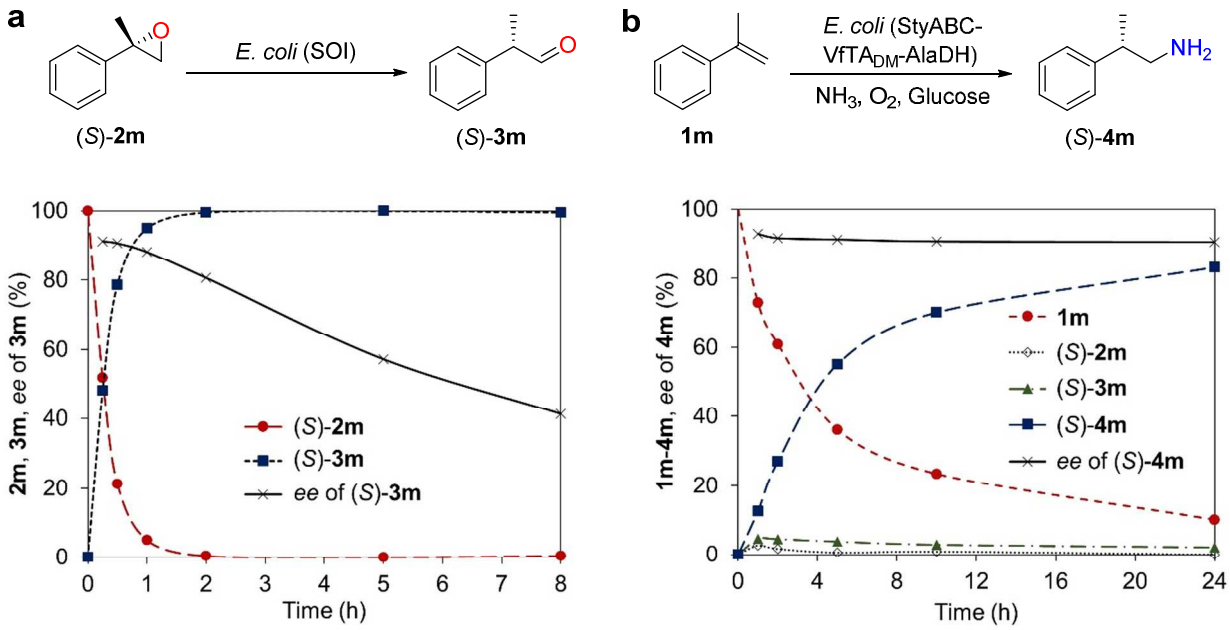
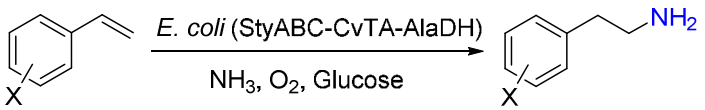


Figure 2. a) Enantioselective conversion of 20 mM (*S*)-**2m** to (*S*)-**3m** with *E. coli* (SOI) cells (1 g cdw L^{-1}) in KP buffer (200 mM, pH 8) and *n*-$C_{16}H_{34}$ (1:1) at 30 °C. b) Asymmetric formal hydroamination of 5 mM **1m** to (*S*)-**4m** with *E. coli* (StyABC-VfTA$_{DM}$-AlaDH) cells (20 g cdw L^{-1}) in KP buffer (200 mM, pH 8, 2 % glucose, 200 mM NH_3/NH_4Cl) and *n*-$C_{16}H_{34}$ (2:1) at 30 °C.

TABLES.

Table 1. Anti-Markovnikov hydroamination of styrenes with *E. coli* (StyABC-CvTA-AlaDH).[a]

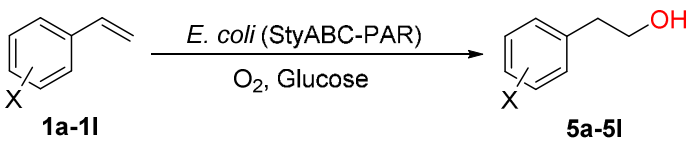


1a-1l **4a-4l**

Sub.	X	Conv. to **4** (%) [b]	Chemoselectivity **4** : **5** [b]	Regioselectivity 2-NH_2 : 1-NH_2 [b]
1a	H	98	96 : 4	> 99 : 1
1b	*o*-F	94	99 : 1	> 99 : 1
1c	*m*-F	> 99	99 : 1	> 99 : 1
1d	*p*-F	96	98 : 2	> 99 : 1
1e	*m*-Cl	86	98 : 2	> 99 : 1
1f	*p*-Cl	76	98 : 2	> 99 : 1
1g	*m*-Br	45	97 : 3	> 99 : 1
1h	*p*-Br	60	98 : 2	> 99 : 1
1i	*m*-Me	93	97 : 3	> 99 : 1
1j	*p*-Me	99	98 : 2	> 99 : 1
1k	*m*-OMe	> 99	99 : 1	> 99 : 1
1l	*p*-OMe	94	96 : 4	> 99 : 1

[a] 20 mM **1** was used under the same reaction condition of Figure 1c for 10 h, except 24 h for **1e**-**1h**. [b] Determined by HPLC and GC analysis.

Table 2. Anti-Markovnikov hydration of styrenes with *E. coli* (StyABC-PAR).[a]

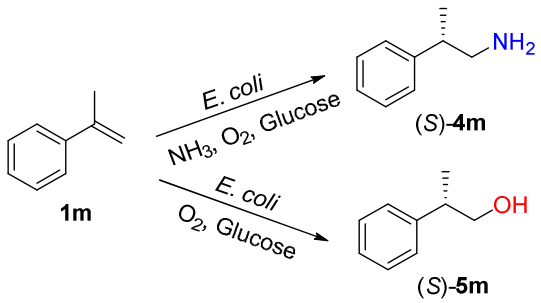


1a-1l **5a-5l**

Sub.	X	Conv. to **5** (%) [b]	Regioselectivity 2-OH : 1-OH [b]
1a	H	> 99	> 99 : 1
1b	*o*-F	90	> 99 : 1
1c	*m*-F	94	> 99 : 1
1d	*p*-F	98	> 99 : 1
1e	*m*-Cl	89	> 99 : 1
1f	*p*-Cl	78	> 99 : 1
1g	*m*-Br	83	> 99 : 1
1h	*p*-Br	60	> 99 : 1
1i	*m*-Me	99	> 99 : 1
1j	*p*-Me	99	> 99 : 1
1k	*m*-OMe	> 99	> 99 : 1
1l	*p*-OMe	94	> 99 : 1

[a] 20 mM **1** was used under the same reaction condition of Figure 1d for 8 h. [b] Determined by HPLC and GC analysis.

Table 3. Asymmetric anti-Markovnikov hydroamination and hydration of **1m**.[a]



Entry	Catalyst	Product	Conv. to prod. (%) [b]	Regioselectivity [b]	*ee* (%) [c]
1	*E. coli* (StyABC-CvTA-AlaDH)	(*S*)-**4m**	76	> 99 : 1	51
2	*E. coli* (StyABC-HnTA)	(*S*)-**4m**	8	> 99 : 1	95
3	*E. coli* (StyABC-VfTA-AlaDH)	(*S*)-**4m**	81	> 99 : 1	70
4	*E. coli* (StyABC-VfTA$_{DM}$-AlaDH)	(*S*)-**4m**	84	> 99 : 1	92
5	*E. coli* (StyABC-PAR)	(*S*)-**5m**	85	99 : 1	87
6	*E. coli* (StyABC-HLADH)	(*S*)-**5m**	81	> 99 : 1	97

[a] 5-10 mM **1m** was used for biotransformation with *E. coli* cells for 24 h (see experimental section for details). [b] Determined by HPLC and GC analysis. [c] Determined by chiral HPLC analysis.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Supporting tables, supporting figures, additional experimental section, GC chromatograms, HPLC chromatograms, and NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Beller, M.; Seayad, J.; Tillack, A.; Jiao, H. *Angew. Chem. Int. Ed.* **2004**, *43*, 3368–3398.

(2) Haggin, J. *Chem. Eng. News* **1993**, *71* (22), 23–27.

(3) Müller, T. E.; Hultzsch, K. C.; Yus, M.; Foubelo, F.; Tada, M. *Chem. Rev.* **2008**, *108*, 3795–3892.

(4) Zhu, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **2014**, *136*, 15913–15916.

(5) Nguyen, T. M.; Manohar, N.; Nicewicz, D. A. *Angew. Chem. Int. Ed.* **2014**, *53*, 6198–6201.

(6) Musacchio, A. J.; Lainhart, B. C.; Zhang, X.; Naguib, S. G.; Sherwood, T. C.; Knowles, R. R. *Science* **2017**, *355*, 727–730.

(7) Gonzalez, A. Z.; Román, J. G.; Gonzalez, E.; Martinez, J.; Medina, J. R.; Matos, K.; Soderquist, J. A. *J. Am. Chem. Soc.* **2008**, *130*, 9218–9219.

(8) Dong, G.; Teo, P.; Wickens, Z. K.; Grubbs, R. H. *Science* **2011**, *333*, 1609–1612.

(9) Hu, X.; Zhang, G.; Bu, F.; Lei, A. *ACS Catal.* **2017**, *7*, 1432–1437.

(10) Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. *Nature* **2012**, *485*, 185–194.

(11) Reetz, M. T. *J. Am. Chem. Soc*. **2013**, *135*, 12480–12496.

(12) Huisman, G. W.; Collier, S. J. *Curr. Opin. Chem. Biol.* **2013**, *17*, 284–292.

(13) *Science of Synthesis: Biocatalysis in Organic Synthesis*; Faber, K., Fessner, W.-D., Turner, N., Eds.; Thieme: Stuttgart, 2014.

(14) Nestl, B. M.; Hammer, S. C.; Nebel, B. A.; Hauer, B. *Angew. Chem. Int. Ed.* **2014**, *53*, 3070–3095.

(15) Bommarius, A. S. *Annu. Rev. Chem. Biomol. Eng.* **2015**, *6*, 319–345.

(16) Heberling, M. M.; Wu, B.; Bartsch, S.; Janssen, D. B. *Curr. Opin. Chem. Biol.* **2013**, *17*, 250–260.

(17) Lovelock, S. L.; Lloyd, R. C.; Turner, N. J. *Angew. Chem. Int. Ed.* **2014**, *53*, 4652–4656.

(18) Weise, N. J.; Parmeggiani, F. S.; Ahmed, T.; Turner, N. J. *J. Am. Chem. Soc.* **2015**, *137*, 12977–12983.

(19) J. Jin, U. Hanefeld, *Chem. Commun.* **2011**, *47*, 2502–2510.

(20) Nestl, B. M.; Geinitz, C.; Popa, S.; Rizek, S.; Haselbeck, R. J.; Stephen, R.; Noble, M. A.; Fischer, M. P.; Ralph, E. C.; Hau, H. T.; Man, H.; Omar, M.; Turkenburg, J. P.; van Dien, S.; Culler, S. J.; Grogan, G.; Hauer, B. *Nat. Chem. Biol.* **2017**, *13*, 275–281.

(21) Demming, R. M.; Otte, K. B.; Nestl, B. M.; Hauer, B. *ChemCatChem* **2017**, *9*, 758–766.

(22) Wuensch, C.; Gross, J.; Steinkellner, G.; Gruber, K.; Glueck, S. M.; Faber, K. *Angew. Chem. Int. Ed.* **2013**, *52*, 2293–2297.

(23) Simon, R. C.; Richter, N.; Busto, E.; Kroutil, W. *ACS Catal.* **2014**, *4*, 129–143.

(24) Muschiol, J.; Peters, C.; Oberleitner, N.; Mihovilovic, M.; Bornscheuer, U.; Rudroff, F. *Chem. Commun.* **2015**, *51*, 5798–5811.

(25) France, S. P.; Hepworth, L. J.; Turner, N. J.; Flitsch, S. L. *ACS Catal.* **2017**, *7*, 710–724.

(26) Schrittwieser, J. H.; Velikogne, S.; Hall, M.; Kroutil, W. *Chem. Rev.* **2017**, DOI: 10.1021/acs.chemrev.7b00033.

(27) Voss, C. V.; Gruber, C. C.; Faber, K.; Knaus, T.; Macheroux, P.; Kroutil, W. *J. Am. Chem. Soc.* **2008**, *130*, 13969–13972.

(28) Wu, S.; Chen, Y.; Xu, Y.; Li, A.; Xu, Q.; Glieder, A.; Li, Z. *ACS Catal*. **2014**, *4*, 409–420.

(29) Mutti, F. G.; Knaus, T.; Scrutton, N. S.; Breuer, M.; Turner, N. J. *Science* **2015**, *349*, 1525–1529.

(30) Zhang, J.; Wu, S.; Wu, J.; Li, Z. *ACS Catal*. **2015**, *5*, 51–58.

(31) Dennig, A.; Busto, E.; Kroutil, W.; Faber, K. *ACS Catal.* **2015**, *5*, 7503–7506.

(32) Both, P.; Busch, H.; Kelly, P. P.; Mutti, F. G.; Turner, N. J.; Flitsch, S. L. *Angew. Chem. Int. Ed.* **2016**, *55*, 1511–1513.

(33) Wu, S.; Zhou, Y.; Wang, T.; Too, H. P.; Wang, D. I. C.; Li, Z. *Nat. Commun*. **2016**, *7*, 11917.

(34) Busto, E.; Simon, R. C.; Richter, N.; Kroutil, W. *ACS Catal*. **2016**, *6*, 2393–2397.

(35) France, S. P.; Hussain, S.; Hill, A. M.; Hepworth, L. J.; Howard, R. M.; Mulholland, K. R.; Flitsch, S. L.; Turner, N. J. *ACS Catal.* **2016**, *6*, 3753–3759.

(36) Zhou, Y.; Wu, S.; Li, Z. *Angew. Chem. Int. Ed.* **2016**, *55*, 11647–11650.

(37) Li, A.; Ilie, A.; Sun, Z.; Lonsdale, R.; Xu, J. H.; Reetz, M. T. *Angew. Chem. Int. Ed.* **2016**, *55*, 12026–12029.

(38) Oberleitner, N.; Ressmann, A. K.; Bica, K.; Gärtner, P.; Fraaije, M. W.; Bornscheuer, U. T.; Rudroff, F.; Mihovilovic, M. D. *Green Chem*. **2017**, *19*, 367–371.

(39) Wu, S.; Zhou, Y.; Seet, D.; Li, Z. *Adv. Synth. Catal.* **2017**, *359*, 2132–2141.

(40) Schmidt, S.; de Almeida, T. P.; Rother, D.; Hollmann, F. *Green Chem.* **2017**, *19*, 1226–1229.

(41) Panke, S.; Witholt, B.; Schmid, A. Wubbolts, M. G. *Appl. Environ. Microbiol.* **1998**, *64*, 2032–2043.

(42) Kuhn, D.; Kholiq, M. A.; Heinzle, E.; Bühler, B.; Schmid, A. *Green Chem.* **2010**, *12*, 815–827.

(43) Paul, C. E.; Tischler, D.; Riedel, A.; Heine, T.; Itoh, N.; Hollmann, F. *ACS Catal.* **2015**, *5*, 2961–2965.

(44) Itch, N.; Hayashi, K.; Okada, K.; Ito, T.; Mizuguchi, N. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 2058–2062.

(45) Miyamoto, K.; Okuro, K.; Ohta, H. *Tetrahedron Lett.* **2007**, *48*, 3255–3257.

(46) Oelschlägel, M.; Gröning, J. A.; Tischler, D.; Kaschabek, S. R.; Schlömann, M. *Appl. Environ. Microbiol.* **2012**, *78*, 4330–4337.

(47) Koszelewski, D.; Tauber, K.; Faber, K.; Kroutil, W. *Trends Biotechnol.* **2010**, *28*, 324–332.

(48) Kaulmann, U.; Smithies, K.; Smith, M. E.; Hailes, H. C.; Ward, J. M. *Enzyme Microb. Technol.* **2007**, *41*, 628–637.

(49) Höhne, M.; Schätzle, S.; Jochens, H.; Robins, K.; Bornscheuer, U. T. *Nat. Chem. Biol.* **2010**, *6*, 807–813.

(50) Shin, J. S.; Yun, H.; Jang, J. W.; Park, I.; Kim, B. G. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 463–471.

(51) Koszelewski, D.; Lavandera, I.; Clay, D.; Guebitz, G. M.; Rozzell, D.; Kroutil, W. *Angew. Chem. Int. Ed.* **2008**, *47*, 9337–9340.

(52) Tieman, D. M.; Loucas, H. M.; Kim, J. Y.; Clark, D. G.; Klee, H. J. *Phytochemistry* **2007**, *68*, 2660–2669.

(53) Schrewe, M.; Julsing, M. K.; Bühler, B.; Schmid, A. *Chem. Soc. Rev.* **2013**, *42*, 6346–6377.

(54) Kunjapur, A. M.; Tarasova, Y.; Prather, K. L. *J. Am. Chem. Soc.* **2014**, *136*, 11644–11654.

(55) Giacomini, D.; Galletti, P.; Quintavalla, A.; Gucciardo, G.; Paradisi, F. *Chem. Commun.* **2007**, 4038–4040.

(56) Könst, P.; Merkens, H.; Kara, S.; Kochius, S.; Vogel, A.; Zuhse, R.; Holtmann, D.; Arends, I.W.; Hollmann, F. *Angew. Chem. Int. Ed.* **2012**, *51*, 9914–9917.

(57) Fuchs, C. S.; Hollauf, M.; Meissner, M.; Simon, R.C.; Besset, T.; Reek, J. N.; Riethorst, W.; Zepeck, F.; Kroutil, W. *Adv. Synth. Catal.* **2014**, *356*, 2257–2265.

(58) Ertürk, E.; Göllü, M.; Demir, A. S. *Tetrahedron* **2010**, *66*, 2373–2377.

(59) Romero, P. A.; Arnold, F. H. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 866–876.

(60) Reetz, M. T. *Angew. Chem. Int. Ed.* **2011**, *50*, 138–174.

(61) Denard, C. A.; Ren, H.; Zhao, H. *Curr. Opin. Chem. Biol.* **2013**, *25*, 55–64.

(62) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. *Science* **2010**, *329*, 305–309.

(63) Nobili, A.; Steffen-Munsberg, F.; Kohls, H.; Trentin, I.; Schulzke, C.; Höhne, M.; Bornscheuer, U. T. *ChemCatChem* **2015**, *7*, 757–760.

(64) Reetz, M. T.; Carballeira, J. D. *Nat. Protoc.* **2007**, *2*, 891–903.

(65) Liu, M.; Kong, D.; Li, M.; Zi, G.; Hou, G. *Adv. Synth. Catal.* **2015**, *357*, 3875–3879.

(66) Friest, J. A.; Maezato, Y.; Broussy, S.; Blum, P.; Berkowitz, D. B. *J. Am. Chem. Soc.* **2010**, *132*, 5930–5931.

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