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RESEARCH ARTICLE

A simple biosystem for the high-yielding cascade conversion of racemic alcohols to enantiopure amines

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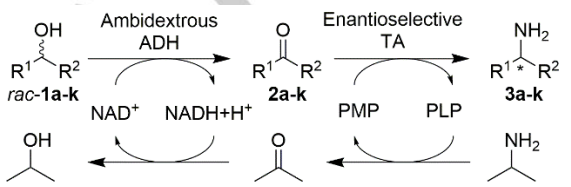
Abstract: The amination of racemic alcohols to produce enantiopure amines is an important green chemistry reaction for pharmaceutical manufacturing, requiring simple and efficient solutions. Here we developed a novel concept and the simplest system for ADH-TA-catalyzed cascade reaction to aminate racemic alcohols, which utilizes an ambidextrous ADH to oxidize a racemic alcohol, an enantioselective transaminase to convert the ketone intermediate to chiral amine, and isopropylamine to recycle PMP and NAD^+ cofactors via the reversed cascade reactions. The concept was proven by using an ambidextrous CpSADH-W286A engineered from (*S*)-enantioselective CpSADH as the first example of evolving ambidextrous ADHs, an enantioselective BmTA, and isopropylamine. A biosystem containing isopropylamine and the cells of *E. coli* (CpSADH-W286A/BmTA) expressing the two enzymes was developed for the amination of racemic alcohols to produce eight useful and high-value (*S*)-amines in 72-99% yield and 98-99% *ee*, providing with a simple and practical solution to this type of reaction.

Introduction

The conversion of easily available racemic alcohols to chiral amines in high yield and high *ee* is of great importance in pharmaceutical manufacturing,[1] since enantiopure amines are often useful and valuable pharmaceutical intermediates.[2] The conventional chemical methods for the amination of racemic alcohols are classic two-step oxidation and amination reactions, often relying on the use of toxic metal catalysts and reagents, high temperatures, and elevated pressures with poor atom efficiency and unsatisfied product *ee* and yield.[3] Thus far, this type of transformation has remains as a key challenging green chemistry reaction in pharmaceutical manufacturing.[4]

Enzymes can catalyze the amination of alcohols to chiral amines *via* cascade reactions in one pot with *non*-toxicity and mild conditions, thus providing with a greener alternative.[5] The cascade reactions are usually achieved by the oxidation of alcohols with alcohol dehydrogenases (ADHs) and the amination of ketones with amine dehydrogenases (AmDHs), reductive aminases (RedAms), or ω-transaminases (TAs).[6] The practical application of these cascade reactions encounter two challenging problems: the simple and easy recycling of the co-factors involved in the oxidation and amination steps; and the lack of ambidextrous ADHs for the oxidation of both enantiomers of the racemic alcohol.

The ADH-AmDH and ADH-RedAm cascades require the regeneration of $NAD(P)^+$ for the oxidation and NAD(P)H for the amination in one pot. An elegant concept using hydrogen-



a: R^1 = Ph R^2 = Me
b: R^1 = *p*-ClPh R^2 = Me
c: R^1 = *p*-FPh R^2 = Me
d: R^1 = *p*-BrPh R^2 = Me
e: R^1 = *m*-CH_3Ph R^2 = Me
f: R^1 = *m*-ClPh R^2 = Me
g: R^1 = Ph R^2 = Et
h: R^1 = Ph R^2 = *n*-Pr
i: R^1 = $PhCH_2CH_2$ R^2 = Me
j: R^1 = $PhCH_2$ R^2 = Me
k: R^1 = $CH_3(CH_2)_5$ R^2 = Me

Scheme 1. A simple biosystem for the high-yielding cascade conversion of racemic alcohols **1** to enantiopure amines **3** by using two enzymes, an engineered ambidextrous ADH for alcohol oxidation and an enantioselective TA for transamination, as well as isopropylamine as the only "coupled substrate" for the reversed cascade reactions to regenerate PMP and NAD^+.

borrowing cascades was developed for the self-regeneration of NAD^+ and NADH during the reactions.[6a] Another approach is much more complicated: ADH and AmDH (or RedAm) utilized two different cofactors, such as NAD^+ and NADPH, and each of the cofactors was regenerated with its own regeneration system by using co-substrate or co-enzyme.[6b, 6c] On the other hand, the ADH-TA cascades are performed in a different manner, requiring two different types of co-factors $NAD(P)^+$ and PMP. The biocatalytic system is thus complex, involving an ADH and a $NAD(P)^+$-regenerating system for the alcohol oxidation and an TA and a PMP-regenerating system for the transamination. For the second reaction step, a co-substrate is needed for the generation of PMP, and the resulted co-product had to be removed to achieve high amine synthesis yields in the reversible transamination reaction.[6d, 7]

Naturally occurring ADHs are usually highly enantioselective[8] and thus incapable of oxidizing both enantiomers of a racemic alcohol with high conversion. With racemic alcohols as the substrates, most approaches had to employ two enantiocomplementary ADHs for the oxidation of the two alcohol enantiomers, hence making the above mentioned

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cascade systems more complicated. Thus far, only two examples involving the use of a single ADH for the oxidation of racemic alcohols have been reported: *Sphingobium yanoikuyae* ADH or *Thermoanaerobacter ethanolicus* secondary ADH mutant was combined with a RedAm in a hydrogen-borrowing cascade to convert racemic 4-phenyl-2-butanol **1i** to the corresponding (*R*)-amine **3i** in 38-42% *ee* and 52-84% yield;[6e] *Streptomyces coelicolor* carbonyl reductase was combined with an AmDH for the amination of racemic 2-pentanol and 3-methyl-2-butanol to produce the corresponding (*R*)-amines in 93-94% yield and >99% *ee*.[9] To the best of our knowledge, there is no report of ADH-TA cascades employing an ambidextrous ADH for the oxidation of a racemic alcohol.

Here we report a simple biosystem consisting of only two enzymes and one co-substrate for the high-yielding ADH-TA catalyzed cascade conversion of racemic alcohols to chiral amines with cofactor recycling. The novel concept was achieved by utilizing an engineered ambidextrous ADH for the oxidation of a racemic alcohol to give the corresponding ketone, an enantioselective TA for the amination of the ketone to produce the enantiopure amine, and isopropylamine as the co-substrate for the reversed casade reactions to regenerate PMP and NAD^+ (Scheme 1). We also report the first success on the engineering of ambidextrous ADHs *via* directed evolution for the full oxidation of racemic alcohols with unique substrate scope.

Results and Discussion

Design of a simple biosystem for the amination of *rac*-alcohols to enantiopure amine with an ADH, a TA, and isopropylamine

To demonstrate the concept, the easily available racemic alcohols **1** were selected as the substrates, and the useful and valuable enantiopure amines (*S*)-**3** were chosen as the target product. These enantiopure amines are highly valuable compounds in the pharmaceutical industry. For instance, (*S*)-**3a-h** are useful precursors for the production of (*S*)-Rivastigmine (a cholinesterase inhibitor for treating Alzheimer's disease)[10] while (*S*)-**3j** is commercially available as Adderall™ (a drug commonly prescribed for the treatment of attention-deficit hyperactivity disorder in children).[11] An enzymatic cascade coupling an ambidextrous ADH with an enantioselective TA was designed to catalyze the cascade reactions, and isopropylamine was used as the co-substrate for the reversed casade reactions to regenerate PMP and NAD^+ (Scheme 1).

Since many enantioselective TAs were reported for the transamination, we first screen a panel of TAs collected in our lab for the transamination of ketone **2a** (10 mM) to amine **3a** with isopropylamine as the amine donor. *Bacillus megaterium* ω-transaminase (BmTA)[12] was found to be the most efficient one, achieving a conversion of 72% to give (*S*)-**3a** in 99% *ee* (Supporting Figure S3). Therefore, BmTA was chosen for the transamination step in the designed cascade reactions.

The first step of the reaction in the designed cascade is the full oxidization of racemic benzylic alcohol **1a** to give the corresponding ketone **2a**, Thus far, no enzyme has been reported for the ambidextrous oxidation of racemic benzylic alcohols. Therefore, the key task for the designed simple biosystem for the cascade reactions is the development of ambidextrous ADHs for the first reaction step.

Engineering of ambidextrous ADHs for the oxidation of racemic 1a to ketone 2a

Directed evolution is a useful technique for engineering enzymes,[13] yet it has not been applied in evolving ambidextrous ADHs. A highly (*S*)-enantioselective *Candida parapsilosis* secondary alcohol dehydrogenase (CpSADH)[14] was selected as the target enzyme for the engineering, since it catalyzed the asymmetric reduction of a broad variety of ketones[15] including benzylic ketones **2a**, **2d**, **2e**, and **2f** to give benzylic alcohols (*S*)-**1a**, (*S*)-**1d**, (*S*)-**1e**, and (*S*)-**1f** with high enantioselectivity. While the oxidation of *rac*-**1a** with CpSADH gave the enantioselectivity factor (*E*) of 120, our engineering goal is to generate a mutant with *E* of < 5 for this oxidation (Supporting Figure S7a).

Benzylic alcohol (*S*)-**1a** was docked into the crystal structure of CpSADH (PDB ID: 3WLF),[15b] and seven amino acids (H49, L55, W116, L119, T158, F285, W286) within 5 Å of the substrate in enzyme catalytic pocket were chosen (Supporting Figure S5) for iterative saturation mutagenesis (ISM) to generate a moderate size of mutant library.[16] A high-throughput colorimetric screening assay based on the detection of NADH formation was conducted in 96-well microtiter plates for the oxidation of (*R*)-**1a** and (*S*)-**1a**, respectively, in the presence of NAD^+ (Supporting Figure S7b), with the measurment at 580 nm using a microplate reader. The OD_{580} ratio for the oxidatioon of (*R*)- and (*S*)-**1a** ($OD_{580,R/S}$) was calculated for each mutant to estimate the enzyme enantioselectivity. Out from 1316 mutants in the first round of evolution, two ambidextrous enzymes were identified: CpSADH-W286A had an $OD_{580,R/S}$ of 0.78 with a specific activity of 109 and 72 U/mg protein for the oxidation of (*S*)-**1a** and (*R*)-**1a**, respectively; CpSADH-W286G had an $OD_{580,R/S}$ of 1.66 with a specific activity of 22.2 and 32.5 U/mg protein for the oxidation of (*S*)-**1a** and (*R*)-**1a**, respectively. In comparison, CpSADH had an $OD_{580,R/S}$ of 0.02 with a specific activity of 1560 U/mg protein for the oxidation of (*S*)-**1a** and nearly no detectable activity for the oxidation of (*R*)-**1a**.

The Michaelis-Menten kinetics and enantioselectivities of the CpSADH mutants W286A and W286G for the oxidation of (*S*)-**1a** or (*R*)-**1a** to **2a** were investigated by using purified enzymes (>95% purity) from the cells of recombinant *E. coli* strains expressing his-tagged CpSADH-W286A or CpSADH-W286G (Supporting Information; Supporting Table S1; Supporting Figure S1; Supporting Figure S2). While no **2a** was detected in the oxidation of (*R*)-**1a** with the wild type CpSADH, CpSADH-W286A and CpSADH-W286G catalyzed the oxidation of (*R*)-**1a** with K_M of 0.979 and 14.1 mM and k_{cat} of 0.596 and 2.22 s^{-1}, respectively (Figure 1a). Both W286A and W286G mutants could also catalyzed the oxidation of (*S*)-**1a**, giving higher K_M (0.557 and 14.3 mM, respectively) and lower k_{cat} (0.582 and 0.551 s^{-1}, respectively) than the wild type CpSADH (K_M of 0.084 mM and k_{cat} of 2.60 s^{-1}). *E* values for W286A and W286 G mutants were calculated as 1.71 (*S*) and 4.08 (*R*), respectively. Thus, CpSADH-W286A and CpSADH-W286G mutants are ambidextrous enzymes (*E*<5) for the oxidation of both enantiomers of racemic **1a**.

To explore the relationship between the enzyme structure and enantioselectivity, (*R*)-**1a** and (*S*)-**1a** were docked into the X-ray structure of CpSADH (PDB ID: 3WLF)[46] and the structure models of CpSADH-W286A and CpSADH-W286G established by

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using the X-ray structure of CpSADH as template, respectively. For wild type CpSADH, the distance between the benzylic carbon of (*S*)-**1a** and NAD^+ was 3.5 Å, enabling hydride transfer for the oxidation of (*S*)-**1a** (Figure 1b); and the benzylic carbon in (*R*)-**1a** faced away from NAD^+ with a distance of 4.1 Å, making the oxidation of (*R*)-**1a** very difficult (Figure 1c). For CpSADH-W286A, the bulky tryptophan (W) at position 286 was replaced by the small alanine (A), thus enlarging the substrate binding pocket. The distance between the benzylic carbon of (*S*)-**1a** and NAD^+ remains 3.5 Å (Figure 1d), and the distance of 3.7 Å between the benzylic carbon of (*R*)-**1a** and NAD^+ molecule was observed (Figure 1e), which enables the oxidation of both (*S*)-**1a** and (*R*)-**1a**. The docking results of (*S*)-**1a** and (*R*)-**1a** in the CpSADH-W286G showed a similar situation as in CpSADH-W286A (Supporting Figure S6), giving the distance between the benzylic carbon of (*S*)-**1a** or (*R*)-**1a** and NAD^+ of 3.6 Å or 3.7 Å with the ability of oxidizing both enantiomers of **1a**.

a)

Sub.	Para-meters	WT	W286A	W286G
(*S*)-**1a**	K_M (mM)	0.084	0.557	14.3
	k_{cat} (s^{-1})	2.60	0.582	0.551
	k_{cat}/K_M (s^{-1} mM^{-1})	31.1	1.05	0.0385
(*R*)-**1a**	K_M (mM)	-	0.979	14.1
	k_{cat} (s^{-1})	0	0.596	2.22
	k_{cat}/K_M (s^{-1} mM^{-1})	0	0.609	0.157
	E	Very high(*S*)	1.71(*S*)	4.08(*R*)

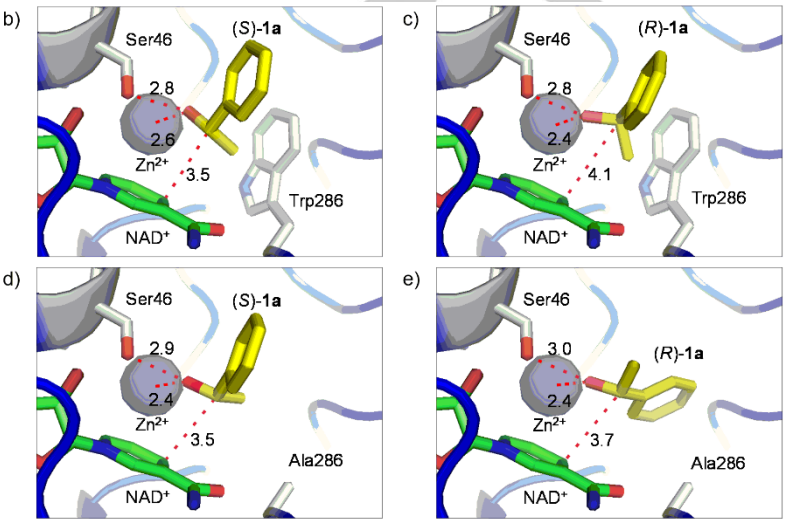

Figure 1. (a) Kinetic data and enantioselectivity of the engineered ambidextrous CpSADH variants, W286A and W286G, in comparison with the wild type enzyme. (b-c) Docking of (b) (*S*)-**1a** and (c) (*R*)-**1a** in the crystal structure of CpSADH**.** (d-e) Docking of (d) (*S*)-**1a** and (e) (*R*)-**1a** in the structure model of CpSADH-W286A. Distances between atoms are shown in red broken lines, substrate (*S*)-**1a** and (*R*)-**1a** are shown as yellow sticks, zinc atom is shown as a grey sphere, and NAD^+ is shown as green sticks.

High-yielding oxidation of *rac*-alcohols to produce ketones using ambidextrous CpSADH-W286A and CpSADH-W286G

The purified CpSADH-W286A and CpSADH-W286G were examined for the oxidation of racemic alcohols **1a-k** to produce ketones **2a-k** and compared with CpSADH. The *E* values and specific activities were given in Figure 2a-b. For the oxidation of eight alcohols (**1a**, **1b**, **1c**, **1e**, **1f**, **1i**, **1j**, and **1k**), wild type CpSADH exhibited *E* of 120, 61, 275, 146, 84, 101, 32, and 65, respectively, while CpSADH-W286A and CpSADH-W286G gave *E* of 3, 17, 2, 1, 3, 10, 4, and 1 and *E* of 2, 5, 2, 3, 18, 11, 2, and 1, respectively. Thus, the engineered enzyme mutants are also able to oxidize both enantiomers of these racemic alcohols. The specific activities of CpSADH-W286A and CpSADH-W286G for the ambidextrous oxidation of these alcohols (**1a**, **1b**, **1c**, **1e**, **1f**, **1i**, **1j**, and **1k)** were 433, 499, 578, 645, 192, 615, 276, 816 U/mg protein and 280, 525, 454, 491, 142, 527, 171, 840 U/mg protein, respectively, which were similar to those of the wild type CpSADH for the enantioselective oxidation (540, 526, 658, 582, 412, 573, 375, and 770 U/mg protein, respectively).

The bulky alcohols **1g** and **1h** were poor substrates for CpSADH with very low oxidation activity of 33 and 52 U/mg, respectively (Figure 2b). Interestingly, **1g** and **1h** were easily oxidized by the W286A and W286G mutants with high activities of 446 and 620 U/mg protein and 474 and 685 U/mg protein, respectively. This could be due to the enlarged substrate binding pockets of the two mutants which allowed the easy binding of bulky alcohols **1g** and **1h**, whereas the smaller binding pocket of the wild type enzyme makes the acess of these alcohols to the catalytic center very difficult. The mutants could also perform the ambidextrous oxidation of **1g** and **1h**, with CpSADH-W286A showing *E* of 3 and 2 and CpSADH-W286A exhibiting *E* of 6 and 1, respectively.

CpSADH-W286A was chosen for further development due to its better activity than CpSADH-W286G for the ambidextrous oxidation of racemic alcohol (Supporting Table S2). The oxidation of *rac*-**1a** by purified CpSADH-W286A with catalytic amounts of NAD^+ was studied in the presence of acetone or purified NADH oxidase (NOX) for cofactor recycling, respectively. In the case of using acetone (Supporting Figure S8a), 89% conversion of 20 mM *rac*-**1a** to **2a** were achieved with a TTN of 1790 for NADH recycling (Supporting Table S3). When CpSADH-W286A was coupled with NOX (Supporting Figure S8b), a much higher TTN of 9970 could be obtained, together with a higher conversion of >99%. This is possibly due to the more efficient regeneration of NAD^+ *via* the irreversible conversion of molecular oxygen to water by NOX. The *E. coli* strain co-expressing CpSADH-W286A and NOX was engineered (Supporting Information), and whole cells were used for the practical synthesis of ketones **2a-k** from racemic alcohols **1a-k**. As shown in Figure 2c, oxidation of **1a**, **1b**, **1c**, **1d**, **1e**, **1f**, **1g**, **1h**, **1j**, and **1k** gave very high conversions of 99, 94, 99, 92, 98, 99, 91, 98, 97, 99, and 99%, respectively.

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These ketones are useful compounds for pharmaceutical[17] and flavor industries.[18]

The simple preparation of ketones from racemic alcohols was demonstrated on a 100 mL scale (Supplementary Table S5). 20-30 mM racemic **1a**, **1c**, **1e**, and **1k** were converted into ketones **2a**, **2c**, **2e**, and **2k** with 97, 98, 100, and 99% conversion, respectively, by using *E. coli* (CpSADH-W286A/NOX) cells. The products were easily recovered by extracting the reaction mixture with *n*-hexane and evaporation of the organic solvent at reduced pressure. Pure ketones **2a**, **2c**, **2e**, and **2k** were obtained in 76, 84, 79, and 82% yields, respectively. The structures were fully characterized by ^{1}H-NMR, ^{13}C-NMR, and HRMS (Supporting Information). These results demonstrate the general applicablity of the engineered enzyme mutant for the high-yielding synthesis of ketones from the corresponding racemic alcohols.

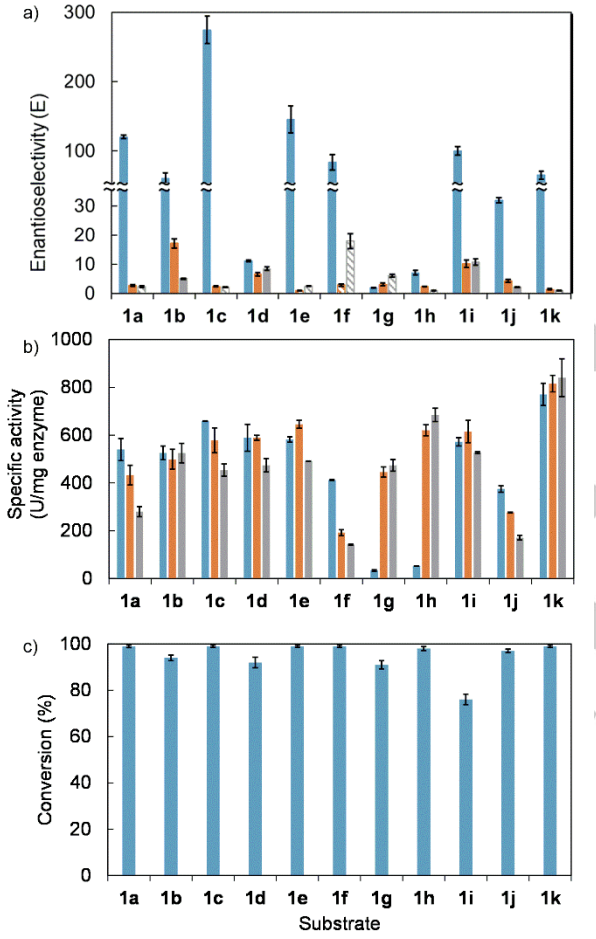

Figure 2. (a) Enantioselectivity factor (*E*) of wild type CpSADH (■), CpSADH-W286A (■), and CpSADH-W286G (■) for the oxidation of racemic alcohols **1a-k** to ketones **2a-k**. Solid bars indicate (*S*)-enantioselectivity, striped bars indicate (*R*)-enantioselectivity. The absolute stereochemistry was established by chiral HPLC or GC analysis using (*S*)-**1a** as standard or following the reported methods for **1b-1j**. (b) Specific activity of wild type CpSADH (■), CpSADH-W286A (■), and CpSADH-W286G (■) for the oxidation of racemic alcohols **1a-k** to ketones **2a-k**. Biotransformations were carried out with 5 mM racemic alcohols **1a-k**, 5 mM NAD^+, and 50 μg/mL purified CpSADH, CpSADH-W286A, or CpSADH-W286G at 30 °C for 1 h. (c) Conversion of 20 mM racemic alcohols **1a-k** to ketones **2a-k** with 10 g cdw/L *E.* coli (CpSADH-W286A/NOX) cells at 30 °C for 24 h. Data are the mean values of triplicate experiments with standard deviations shown as error bars.

High-yielding amination of *rac*-alcohol to produce enantiopure amine with an ADH, a TA, and isopropylamine: proof of concept

With the engineered ambidextrous CpSADH-W286A and the enantioselective BmTA, we examined the designed system consisting of these two enzymes and isopropylamine as the sole co-substrate for the amination of *rac*-alcohol **1a** to produce enantiopure amine (*S*)-**3a** with the recycling of PMP and NAD^+ (Scheme 1). 5 mM *rac*-**1a** were treated with purified CpSADH-W286A, BmTA, isopropyl-amine, and 0.05 mM NADH, in the presence or absence of 0.1 mM ketone **2a** (Figure 3a). Without ketone **2a**, nearly no amine **3a** is formed. In this case, no acetone is produced, thus no NAD^+ can be generated for the alcohol oxidation reaction. In the presence of **2a**, the transamination reaction happened and producd acetone which was used to regenerate NAD^+ by CpSADH-W286A for alcohol oxidation. As a result, amine (*S*)-**3a** could be produced in 83%, with the recycling of both PMP and NAD^+ for at least 83 times.

This new concept was fully proven by the amination of 10 mM racemic **1a** with CpSADH-W286A, BmTA, 0.05 mM NAD^+, and 0.8 M isopropylamine to give amine (*S*)-**3a** in >99% *ee* with 88% conversion. The recycling of both PMP and NAD^+ was sucessful and achieved for at least 88 times. The concentration of the intermediate ketone **2a** remained low (≤5%) in the reaction course (Figure 3b), suggesting the efficient amination reaction with BmTA. This is possibly caused by shifting the equalibrium *via* the removal of acetone co-product through the conversion to isopropanol with CpSADH-W286A. Thus, the concept of using a novel simple biosystem containing only an ADH, a TA, and isopropylamine for high-yielding amination of *rac*-alcohol to produce enantiopure amine was sucessfully demonstrated.

In comparison, the use of CpSADH-W286A and NOX for the oxidation of *rac*-**1a** (10 mM) with NAD^+ recycling as well as BmTA and isopropylamine for the amination of **2a** with PMP recycling gave (*S*)-**3a** with 78% conversion and 99% *ee* (Supporting Figure S9a). Following the reaction time course, the accumulation of the intermediate ketone **2a** was observed and reached 18% at 24 h (Supporting Figure S9b). This is possibly caused by the accumulation of acetone in the reaction system leading to unfavorable thermodynamic equilibrium for the transamination of **2a** to **3a**.[13] Therefore, our new system consisting of an ADH, a TA, and isopropylamine is not only much simpler than the traditional system, but also higher yielding with less intermediate accumulation.

High-yielding amination of *rac*-alcohols to produce (*S*)-amines using *E. coli* cells co-expressing CpSADH-W286A and BmTA in the presence of isopropylamine

The concept was used to develop an even more practical version of the biosystem which contains *E. coli* cells co-expressing ADH-TA and isopropylamine for the conversion of racemic alcohols to enantiopure amines. In such a system, the intracellular PLP and NADH are ultilized for the reactions and recycling, without the need of adding PLP and NADH to the reaction system. Moreover, the easily available cells are efficient catalysts, without the needs of using the two purified enzymes. The recombinant *E. coli* co-expressing CpSADH-W286A and BmTA was constructed (Supporting Information), and the *E. coli* cells were prepared and used for bioconversion of 5 mM racemic alcohols **1a-k** in the presence of isopropylamine to prepare the corresponding enantiopure (*S*)-amines **3a-k** (Table 1; For

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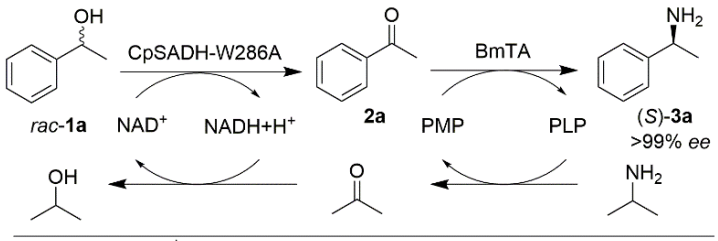
absolute stereochemistry and *ee* determination, see Supporting Figures S40-49 and S53). Amination of racemic alcohols **1a**, **1b**, **1c**, **1e**, **1f**, **1g**, **1i**, and **1j** gave (*S*)-amines **3a**, **3b**, **3c**, **3e**, **3f**, **3g**, **3i**, and **3j** in excellent *ee* (99-98%) with high conversion (79, 79, 77, 94, 84, 89, 72, and 99%, respectively). Thus, the engineered *E. coli* (CpSADH-W286A/BmTA) cells together with isopropylamine are highly efficient biosystem for the amination of racemic alcohols **1** to produce enantiopure amines (*S*)-**3** in high yield and high *ee*. As an exception, the amination of racemic alcohol **1h** gave only 4% of amine (*S*)-**3h**. Here, CpSADH-W286A could fully oxidize racemic alcohol **1h** to ketone **2h**, but ketone **2h** was shown to be a poor substrate for BmTA. A solution to enhance the yield of (*S*)-**3h** is to engineer a more active TA for the cascade reaction.

The practice of the whole cell system in synthetic application was demonstrated in the preparative biotransformation of racemic alcohol **1a**, **1e**, **1g**, and **1j** to produce the corresponding amines (*S*)-**3a**, (*S*)-**3e**, (*S*)-**3g**, and (*S*)-**3j** (Table 1, entries 2, 7, 10, and 14). Biotransformation of 10 mM racemic alcohols was conducted with *E. coli* (CpSADH-W286A/BmTA) cells and isopropylamine on a 100 mL-scale, the reaction mixtures were extracted with *n*-hexane, and the solvent was removed by evaporation to give (*S*)-amines **3a**, **3e**, **3g**, and **3j** in high *ee* (>99% *ee*) with an isolated yield of 77%, 75%, 74%, and 57%, respectively. The products have high purity and their structures were identified by ^{1}H-NMR, ^{13}C-NMR, and HRMS (Supporting Information). Thus, the catalytic system consisting of microbial cells expressing an ambidextrous ADH and an enantioselective TA and isopropylamine is simple and useful for the one-pot and green amination of racemic alcohols to synthesize enantiopure amines in high ee and high yield.

a)



rac-**1a** (mM)	NAD$^+$ (mM)	NADH (mM)	**2a** (mM)	Product distribution **1a** (%)	**2a** (%)	**3a** (%)
5	0	0.05	0	95	1	4
5	0	0.05	0.1	1	16	83
10	0.05	0	0	7	5	88

b)

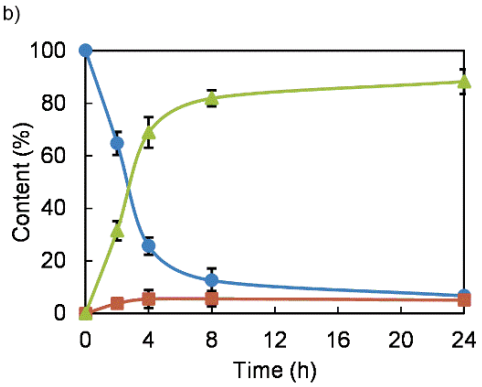


Figure 3. (a) Coupling of an ambidextrous ADH and an enantioselective TA for the high conversion of racemic alcohol to enantiopure amine with isopropylamine as co-substrate for both transamination and NAD$^+$ regeneration for alcohol oxidation. (b) Time course of the cascade biotransformation of 10 mM *rac*-**1a** (●) to (*S*)-**3a** (▲) via ketone intermediate **2a** (■) in potassium phosphate buffer (0.1 M, pH 8) containing 1 mg/mL purified CpSADH-W286A, 5 mg/mL purified BmTA, 0.8 M isopropylamine, 1 mM PLP, and 0.05 mM NAD$^+$ at 30 °C for 24 h. Data are the mean values of triplicate experiments with standard deviations shown as error bars.

Table 1. Cascade biotransformation of racemic alcohols **1a-k** to (*S*)-amines **3a-k** with *E. coli* (CpSADH-W286A/BmTA) using isopropylamine as the co-substrate for the amination and for the cofactor regeneration for alcohol oxidation.

Entry[a]	Racemic alcohol **1**	Alcohol concentration (mM)	Time (h)	Volume (mL)	(*S*)-Amine **3**	*ee* (%)[b]	Conversion (%)[c]	Isolated yield (%)[d]
1	**1a**	5	21	5	**3a**	>99 (*S*)	79	-
2	**1a**	10	22	100	**3a**	>99 (*S*)	81	77
3	**1b**	5	22	5	**3b**	98 (*S*)	79	-
4	**1c**	5	22	5	**3c**	>99 (*S*)	77	-
5	**1d**	5	22	5	**3d**	84 (*S*)	69	-
6	**1e**	5	22	5	**3e**	>99 (*S*)	94	-
7	**1e**	10	22	100	**3e**	>99 (*S*)	84	75
8	**1f**	5	6	5	**3f**	>99 (*S*)	84	-
9	**1g**	5	4	5	**3g**	>99 (*S*)	89	-
10	**1g**	10	4	100	**3g**	>99 (*S*)	86	74
11	**1h**	5	22	5	**3h**	6 (*S*)	4	-
12	**1i**	5	22	5	**3i**	>99 (*S*)	72	-
13	**1j**	5	4	5	**3j**	>99 (*S*)	>99	-
14	**1j**	10	4	100	**3j**	>99 (*S*)	97	57
15	**1k**	5	22	5	**3k**	93 (*S*)[e]	75[f]	-

[a] Biotransformation of 5-10 mM racemic alcohols **1a-k** to (*S*)-amines **3a-k** was performed with 5 g cdw/L of *E. coli* (CpSADH-W286A/BmTA) cells in the presence of 1.2 M isopropylamine and 1 mM PLP at 30 °C for 24 h. [b] *ee* of **3a-j** were determined by chiral HPLC. [c] Concentrations of **3a-j** were determined by HPLC analysis. [d] Isolated yield was calculated based on the pure product obtained by extraction with *n*-hexane and evaporation of organic solvent. [e] *ee* of **3k** was determined by chiral GC. [f] Concentration of **3k** was determined by GC analysis.

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Conclusion

A simple and efficient cascade reaction system for the conversion of racemic alcohols to enantiopure amines was developed, with a novel concept of using an ambidextrous ADH and an enantioselective TA as the only two ezymes for the target reaction and isopropylamine as the only one co-substrate for the regeneration of the two co-factors PMP and NAD^+ via reversed cascade reactions. The novel concept was successfully proven by utilizing a newly engineered ambidextrous ADH (CpSADH-W286A) for the oxidation of a racemic alcohol, an enantioselective transaminase (BmTA) for the amination of the ketone intermediate to produce chiral amine product, and isopropylamine for co-factors regeneration. It has also been achieved by using a biosystem containing the cells of recombinant *E. coli* (CpSADH-W286A/BmTA) expressing the ADH and the TA, together with isopropylamine. The use of biosystem containing *E. coli* (CpSADH-W286A/BmTA) cells allows for the production of eight useful and high-value (*S*)-amines **3a-c, 3e-g, 3i-j** in 72-99% yield and 98-99% *ee* from the corresponding easily available racemic alcohols, respectively. This biocatalytic system is the simplest one for ADH-TA catalyzed amination of racemic alcohols, enables high-yielding production of the corresponding chiral amines with high *ee* in a sustainable manner, and provides with a useful solution to this challenging green chemistry reaction in pharmaceutical synthesis. Two new ambidextrous ADHs (CpSADH-W286A and CpSADH-W286G) were successfully evolved from the highly (*S*)-enantioselective CpSADH, being able to oxidize both enantiomers of racemic alcohols **1a-k** to give the corresponding ketones **2a-k** with high conversions. To the best of our knowledge, this represents the first example of engineering an ambidextrous ADH *via* directed evolution. The success was accomplished by mutating a large amino acid (tryptophan) in the binding pocket into a smaller one (alanine or glycine), thus widening the binding pocket of the enzyme. This could be a general principle for the future engineering of ambidextrous enzymes to accept both enantiomers of substrates. CpSADH-W286A and CpSADH-W286G show unique substrate specificity, being the only available ADHs for the high-yielding oxidation of racemic benzylic alcohols and also capable of oxidizing other types of racemic alcohols with high conversion. This significantly expands the substrate scope of the biooxidation of racemic alcohols and opens new possibility of using ambidextrous alcohol oxidation enzymes for cascade reactions in green chemical synthesis.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Alcohol amination • Alcohol dehydrogenase • Biocatalysis • Cascade biotransformation • Directed evolution

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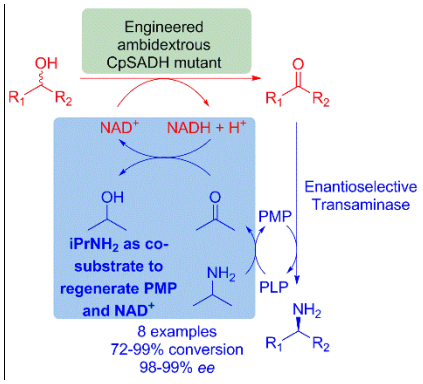

New concept, simple system, and high-yielding: A novel cascade reaction system was developed to convert *rac*-alcohols to chiral amines in high *ee* and high yield by combining an ambidextrous alcohol dehydrogenase (ADH) with a transaminase and using isopropylamine as co-substrate to regenerate PMP and NAD^+, wherein the ambidextrous ADHs were engineered for the first time by directed evolution to achieve full oxidation of *rac*-alcohols to ketones.

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