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Recent Developments of Cascade Reactions Involving ω-Transaminases

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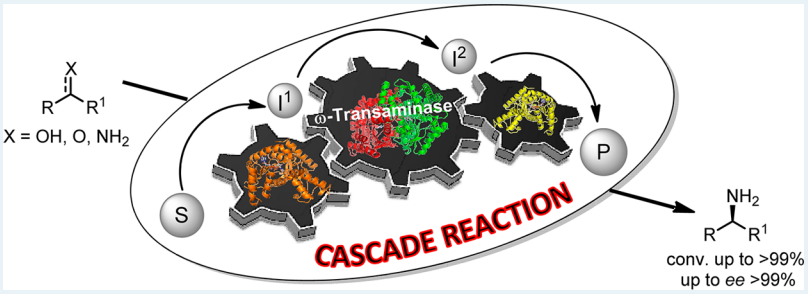

ABSTRACT: Enzymatic cascade reactions experience tremendous attention by cutting short conventional step-by-step synthesis in a highly efficient and elegant fashion. Focusing on ω-transaminases, this review provides an overview of different biocatalytic strategies to afford a variety of (chiral) amines employing diverse cascade systems: Cascades to shift the reaction equilibrium as well as cascades for the amination of alcohols and nonactivated C−H bonds are discussed. Cascades enable the deracemization of *rac*-amines, other ones involve biocatalyzed C−C bond formation and C−C bond hydrolysis. Finally, the potential of spontaneous ring closure reactions initiated by ω-transaminases is illustrated.

KEYWORDS: *ω-transaminase, cascade reaction, chiral amines, functional group interconversion, reductive amination*

1. INTRODUCTION

1.1. Biocatalysis and Cascades. The shortage of resources and the increasing awareness of environmental aspects have changed the major focus of contemporary chemical research to provide any desired target molecules not only via an economical but also via an environmental benign synthetic route. Moreover, legislative regulations regarding process-, safety-, and quality-improvements together with required cost-, time-, and energy-reductions force the search for novel sophisticated methods. Catalytic procedures replacing stoichiometric amounts of reagents have consequently emerged as popular strategies; however, further optimization with respect to ecological aspects are still desired.[1]

Biocatalysis covers several of these aspects and has gained therefore enhanced attention from both, academia[2−6] and industry;[7−9] enzymes usually display a high stereo- and regioselectivity, a high turnover rate, and allow to run the reactions under mild and benign reaction conditions, that is, at temperatures between 20 and 50 °C, often in buffer at physiological pH. Furthermore, enzymes are readily tunable by rational protein design allowing the creation of tailored catalysts for designated reaction conditions (e.g., elevated temperature and/or pH and/or stability toward organic cosolvents).[10−14] These and many more features have made them attractive alternatives to pure chemical processes, not only for pharmaceutical purposes[15−18] but also increasingly for natural product synthesis.[19,20] As all biocatalysts originate from a similar environment, they are mostly compatible with each other at defined operation conditions. This opens the possibility to combine several enzymes classes in vitro facilitating a one pot cascade reaction without intermediate isolation. Such reaction sequences enable to shorten the reaction time, avoid elaborate protecting group manipulations, and reduce waste since less solvents and chemicals are needed (e.g., for the purification). Thus, such enzymatic cascade reactions are intrinsically green.[21,22] The concept to replace multiple single-step operations by an enzymatic one-pot cascade has opened novel opportunities and reached an extraordinary level of efficiency:[23−28] for example, highly functionalized molecules can be quickly constructed from readily available starting materials and the isolation of labile intermediates circumvented; thermodynamically nonfavored reactions can be driven to completion if the intermediates are constantly removed from the equilibrium.

This review provides an overview about various cascade techniques and successful application of multiple-enzyme cascades involving ω-transaminases (ωTAs). This includes chemoenzymatic- and multienzyme systems but also spontaneous transformations initiated by ωTAs.

1.2. Classification of Cascade Reactions. The term "cascade" is often used in a rather broad sense and is applied sometimes for "tandem-" and/or "domino-" reactions equally.[29]

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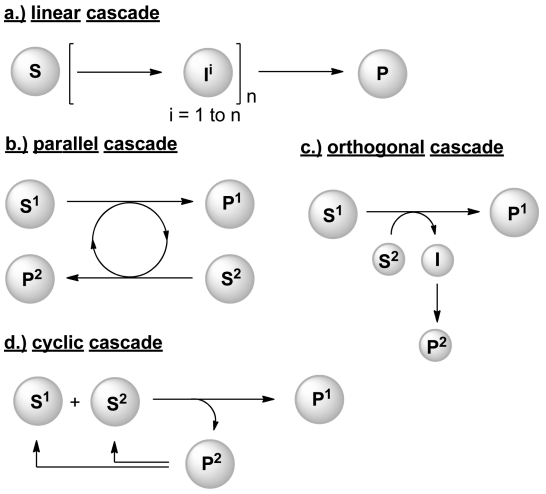
However, as in biocatalytical cascade reaction usually several independent reactions proceed simultaneously a further classification is appropriate; according to previous suggestions[23,27] enzymatic cascades illustrated within this article will be divided into four basic concepts, namely, linear-, parallel-, orthogonal-, and cyclic cascades (Scheme 1). The most common system consists of consecutive transformations in one-pot (Scheme 1, a) whereby (i) each step is catalyzed by an enzyme, or (ii) biocatalyzed steps are followed by spontaneous reactions or (iii) enzymatic transformations and chemical steps (organo- or metal catalyzed, chemical transformations) alternate. We will refer to such a cascade as *linear cascade*, whereby a starting material or substrate (S) is converted via one or several (reactive) intermediates (I^i), which shall not be isolated, to the product (P). This cascade has a practical advantage as the storage and handling of toxic, unstable and explosive intermediates can be avoided. An example would be the transformation of a racemic mixture into one single enantiomer by deracemization.[30−33] Notably, no distinction between a process in which the reagents and catalyst are present from the outset or added sequentially will be made in this article for simplicity.

Scheme 1. Overview of Different Types of Enzymatic Cascades[a]



[a] *S* = starting material; *I* = intermediate, *P* = product.

Parallel cascades are probably the most common type of cascades applied in the field of redox biocatalysis; the product formation is coupled with a second parallel reaction proceeding simultaneously and providing for instance the reducing equivalents (Scheme 1, b). A typical example is the cofactor recycling of NAD(P)H for oxidoreductases. Another example are coupled processes yielding two products simultaneously due to concomitant transformation. Very closely related to *parallel cascades* are *orthogonal cascades* (Scheme 1, c): a cosubstrate S^2 leads to an intermediate I which is further converted to a coproduct P^2. This system enables for instance, to shift equilibria toward product formation or to minimize enzyme inhibition by an intermediate I.

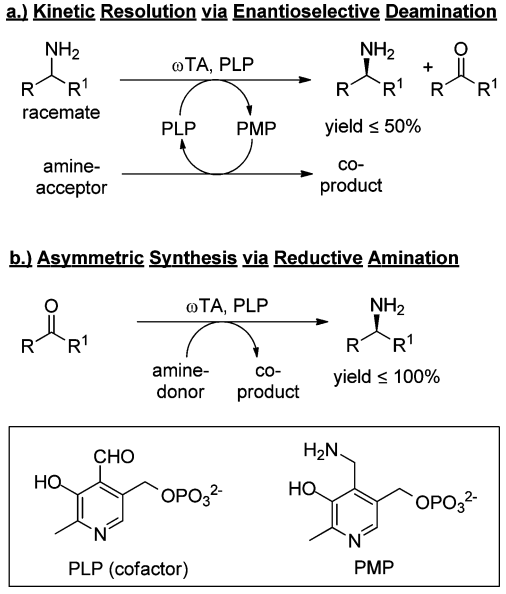
The main characteristic in a *cyclic cascade* is that a formed product (P^2) is converted back to one or both starting materials (Scheme 1, d). For instance two starting materials (S^1 and S^2 which can be either two separate substrates but also two enantiomers) are converted into one product P^1 whereas the coproduct P^2 is transformed back to the starting material. This concept has been used for stereoinversion.[31,33]

In the examples given later on, various basic types of cascades mentioned above will be combined. For the linear cascades involving ωTAs, also orthogonal and parallel cascade system are often required to remove an inhibiting coproduct and/or to shift the equilibrium. Consequently because of the more supporting function of orthogonal/parallel cascades this class of cascades will be treated first after the following introduction to ωTAs.

2. ω-TRANSAMINASES

ω-Transaminases (ωTAs) (E.C. 2.6.1.X) are pyridoxal-5′-phosphate (PLP) depended enzymes which have been proven to be remarkable efficient for the synthesis of (chiral) amines.[34−38] The cofactor PLP serves thereby as molecular shuttle for ammonia and electrons, transferring an amino group between a suitable amine donor and an acceptor (ketone or aldehyde). As PLP gets recycled, PLP is required only in catalytic amounts. Based on the starting material (amine or ketone) the ωTAs can be utilized in two basic reaction types, namely, the enantioselective oxidative deamination (Scheme 2, a) and the stereoselective reductive amination (Scheme 2, b). In the first case kinetic resolution takes place, while in the second case the prochiral starting ketone is transformed creating the chiral center in an asymmetric synthesis. The reductive amination is favored since the product can be obtained in up to 100% yield in contrast to only 50% via kinetic resolution.

Scheme 2. Basic Transformations Employing ω-Transaminases (ωTAs) to Provide Chiral Amines



Nevertheless, each method has its advantages and drawbacks. For example, the kinetic resolution requires a single enzyme and is in general thermodynamically favored if the amine acceptor is pyruvate. On the other hand, the enantiomeric excess (*ee*) of the amine depends on the conversion, and only 50% yield can be reached at maximum in case of high enantioselectivity (E >100).[39] The asymmetric reductive amination conversely allows converting prochiral ketones and aldehydes in quantitative yield but suffers mostly from an

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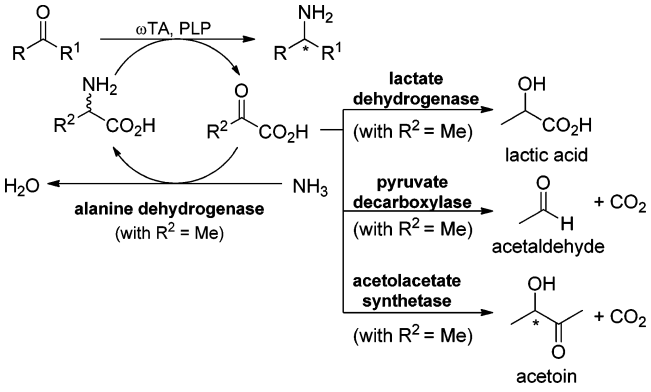
unfavorable equilibrium which lies on the side of the starting materials. However, this issue can be circumvented either by utilizing a large excess of amine donor and/or removal of the coproduct, for example, using of a multienzyme network. Artificial multienzyme networks were recently successfully expressed in a single host.[40−42]

3. ORTHOGONAL AND PARALLEL CASCADES

3.1. Enzyme Cascades to Shift the Equilibrium. To use ωTAs efficiently for the asymmetric reductive amination of ketones the thermodynamic barrier has to be overcome to shift the equilibrium toward product formation. Various well working methods are available like (i) removing volatile coproducts (e.g., acetone, propanone)[43−46] or (ii) the application of designated amine donors,[47,48] (iii) multienzyme networks (Scheme 3) as well as (iv) nonenzymatic spontaneous

Scheme 3. Overview of Selected Enzyme-Coupled Techniques to Shift the Equilibrium Towards Product Formation in ωTAs Catalyzed Reactions

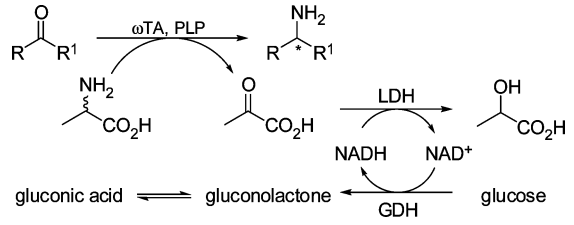


reactions. The most common cascades will be briefly discussed hereafter and their potential illustrated. In all examples the cofactor PLP/PMP recycling as already depicted in Scheme 2a is omitted; this subcascade will not be drawn anymore for the sake of clarity.

Lactate Dehydrogenase (LDH). When a lactate dehydrogenase (LDH) was first combined with a ωTA to remove pyruvate from the reaction,[49] the NAD(P)H cofactor recycling system for the LDH was omitted; thus stoichiometric amounts of NAD(P)H were required. Using the (*S*)-selective ωTA originating from *Vibrio fluvialis*[50] the conversion of acetophenone to 1-phenylethylamine was investigated. The cell-free extract afforded enantiopure (*S*)-1-phenylethylamine with only 0.56% conversion employing a 10-fold excess of alanine, whereas in the presence of the LDH a distinct enhancement was observed: the conversion could be improved 10-fold to 5.8%. The applicability of the system was demonstrated some years later by the incorporation of a glucose dehydrogenase (GDH) for recycling of the cofactor nicotinamide NADH (Scheme 4); this enabled to use this method for asymmetric synthesis on routine to afford the corresponding amines with high conversions.[51]

This ωTA/LDH/GDH coupled system has then been systematically investigated by various research groups providing a series of (pharmacologically) valuable amines. By employing either a (*R*)- respective (*S*)-selective ωTA several aliphatic and aromatic amines have become easily accessible, usually with excellent conversion and high optical purity (Figure 1).[52−56]

Scheme 4. LDH/GDH Multi-Enzyme Network to Shift the Equilibrium Towards Product Formation[a]



[a]LDH = lactate dehydrogenase, GDH = glucose dehydrogenase.

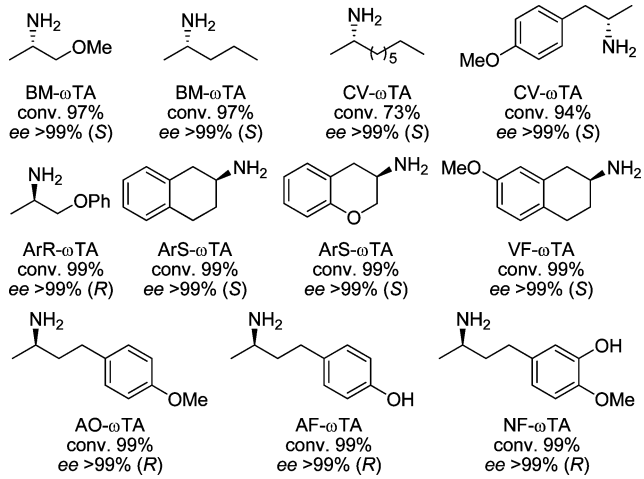
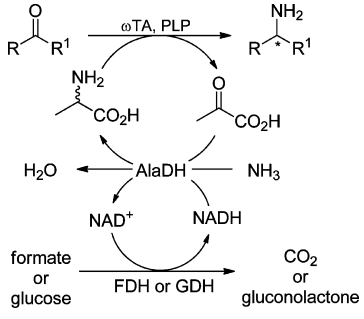


Figure 1. Selection of chiral amines produced by reductive amination of the corresponding ketone employing ωTAs in combination with LDH for pyruvate removal. BM = *Bacillus megaterium*,[57] CV = *Chromobacterium violaceum*,[58] ArR = *Arthrobacter* sp.,[53] VF = *Vibrio fluvialis*,[50] ArS = *Arthrobacter citreus*,[59] AO = *Aspergillus oryzae*,[54] AF = *Aspergillus fumigatus*,[54] NF = *Neosartorya fischeri*.[54]

Alanine Dehydrogenase (AlaDH). Another approach represents the combination of ωTAs with an alanine dehydrogenase (AlaDH). In this case, pyruvate is not removed but recycled back to the amine donor alanine at the expense of ammonia and a cheap reducing agent like formate or glucose (Scheme 5).[60] The NADH required for the reductive amination of pyruvate to alanine is recycled by well-established techniques integrating either a formate- (FDH) or glucose dehydrogenase (GDH) in the network. The feasibility has been demonstrated several times,[53,55,56,61] providing various amines usually with

Scheme 5. Shifting the Equilibrium in ωTA Catalyzed Reactions via Alanine Dehydrogenase (AlaDH) in Combination with Formate- or Glucose-Dehydrogenase (FDH/GDH)



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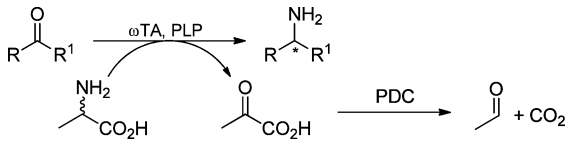
excellent conversion. A limitation of the system is that currently only an L-AlaDH was applied, thus only L-alanine could be recycled. In case of using D-alanine as amine donor the system worked like the LDH system to remove pyruvate, although some (*R*)-selective ω-TAs were noticed to accept also L-alanine.[60]

Pyruvate Decarboxylase (PDC). An alternative enzymatic approach to remove pyruvate represents the incorporation of a pyruvate decarboxylase (PDC); pyruvate is thereby converted to acetaldehyde and CO_2 which are both easily removed from the reaction media making this system consequently irreversible (Scheme 6).[62] This orthogonal cascade comprises only two enzymes in total and no additional NADH recycling is required.

Scheme 6. Irreversible Orthogonal Cascade Using ω-TAs in Combination with Pyruvate Decarboxylases (PDCs) to Shift the Equilibrium Towards Product Formation



The efficiency was verified for designated cyclic and aromatic ketones in a comparative study: pyruvate was removed by either the PDC- or the LDH-system. It was found that the conversion and the *ee* were comparable (Figure 2). However,

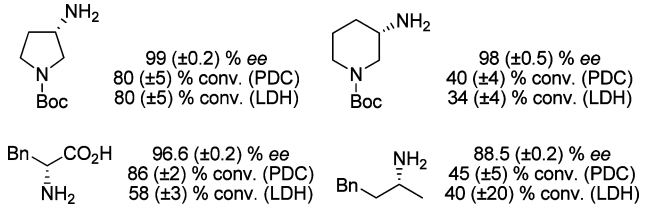


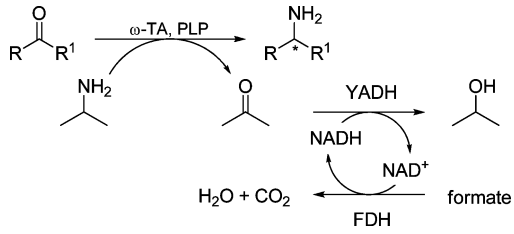
Figure 2. Comparison of cofactor removal techniques via PDC and LDH. PDC = pyruvate decarboxylase, LDH = lactate dehydrogenase.

two issues needed to be considered when using this system: first, acetaldehyde was found to inactivate selected enzymes[63] and second, acetaldehyde might get aminated by the ω-TA yielding ethanamine as an unwanted side product thereby decreasing the overall efficiency of the reaction.

Alcohol Dehydrogenase (ADH). While in the former methods exclusively alanine was employed as amine source, an alternative cascade was established by using 2-propanamine as cheap and achiral amine donor.[64] Equilibrium displacement was enabled by removing the coproduct acetone employing a yeast alcohol dehydrogenase (YADH) from *Saccheromyces cerevisiae* in combination with NADH recycling (Scheme 7.).[65] The ADH features a very narrow substrate spectrum and is therefore unable to accommodate medium- or large groups (>C3). Thus, discrimination between the substrate for the ω-TA and acetone was achieved enabling an efficient use of this technique as shown for various phenethyl-amine-derivatives.

Furthermore, enzymatic cascades were employed in the kinetic resolution of racemic amines.[66−69] As already mentioned the kinetic resolution of racemic amines using ω-TAs is a well-established methodology but frequently substrate as well as product inhibition were found to limit the reaction with respect to productivity and optical purity of

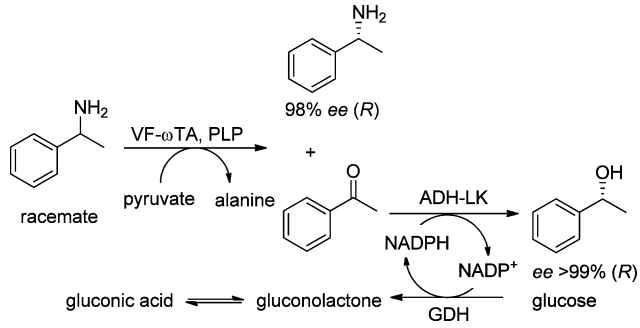
Scheme 7. Removal of Acetone by an Alcohol Dehydrogenase (ADH) to Shift the Equilibrium Using 2-PrNH$_2$ as Amine Donor[a]



[a]YADH= Yeast alcohol dehydrogenase.

the desired product.[70] Therefore, strategies to couple the kinetic resolution with other enzymatic transformations are potent routes to avoid such inhibition issues. For example, inhibition by a product could be eliminated by its removal by an oxidoreductase (Scheme 8).[71] More precisely, the ω-TA

Scheme 8. Simultaneous Synthesis of (*R*)-1-Phenylethanol and (*R*)-1-Phenylethylamine Using a One-Pot One-Step Cascade[a]



[a]VF = ω-TA originating from *Vibrio fluvialis*, ADH-LK = alcohol dehydrogenase originating from *Lactobacillus kefir*.

originating from *Vibrio fluvialis*[50] catalyzed deamination step was combined with a reduction of the formed ketone using a NADPH-dependent alcohol dehydrogenase from *Lactobacillus kefir* (ADH-LK),[72] thereby removing the ketone from the reaction media. As a first step the very efficient cosubstrate-dependent NADPH recycling system using 2-propanol was investigated. However, based on a severe inhibition of the ω-TA with 2-propanol a third enzyme, a GDH, was introduced in the cascade to catalyze the required cofactor-recycling. Finally, the combination of a ω-TA, ADH, and GDH facilitated the simultaneous production of two valuable products with high conversion an excellent enantioselectivity E >200. In a strict sense this cascade is actually a linear cascade but should be mentioned here as a method to improve conversion fitting to the other methods in this section.

3.2. Parallel Enzyme Cascade for Providing a Suitable Amine Donor. The combination of two transaminases (α and ω) was established for the amination of a α-keto acid at the expense of 2-propanamine (Scheme 9).[73] The overall reaction is thermodynamically favored. However, the challenge was that the enzymes appropriate for the amination of the desired α-keto acids did not accept 2-propanamine as amine donor. Consequently a proper shuttle substrate was required which can be used as amine donor for the αTA catalyzed amination and its corresponding deaminated derivative should act as

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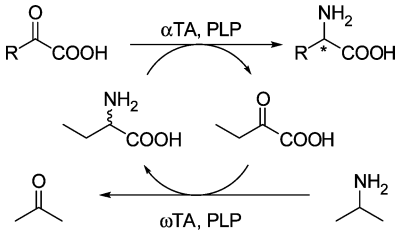
Scheme 9. Production of Unnatural Amino Acids by Combining a αTA and a ωTA

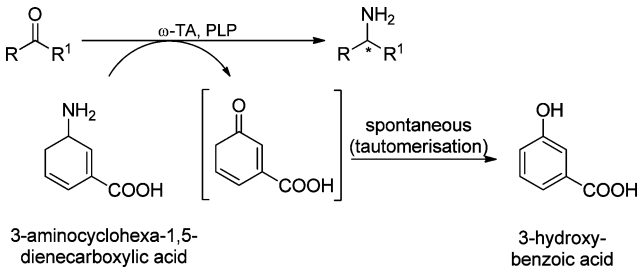


amine acceptor for the ωTA catalyzed reaction. It turned out that that L-homoalanine and its deaminated derivate 2-oxobutyric acid were the best shuttle pair.

The combination of either a branched chain transaminase (BCTA from *E. coli*) with an (*S*)-selective ωTA (OATA from *O. anthropi*)[74] facilitated access to L-amino acids, while a D-amino-acid transaminase (DATA, *Bacillus sphaericus*) with a highly evolved (*R*)-selective ωTA (ArRmut)[45] gave access to the other the D-enantiomer. Several valuable and bioactive products as for instance L-trimethylpyruvic acid (used for HIV treatment), L-norvaline (employed as ACE inhibitor), D-phenylglycine (acts as antibiotic) as well as D-phenylalanine (precursor for antidiabetic drug) were obtained (Table 1). Notably, the scalability of the cascade was also demonstrated by the successful preparative-scale syntheses of L-*tert*-leucine (1.37 g, 70% yield, >99% *ee*) and D-phenylglycine (2.02 g, 89% yield, >99% *ee*).

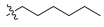
3.3. Nonenzymatic Methods to Shift the Equilibrium Toward Product Formation. The application of amine donors such as 2-propan- and butanamine is highly attractive because of the low price and the volatility of the formed byproducts (acetone and butanone).[43,44] However, these donors are limited to a restricted number of ωTAs being able to accept them. The limitation can be circumvented either by, for example, rational protein design or reaction engineering yielding very appealing single-enzyme systems.[45,47,48] Alternative amine donors include 1-phenylethylamine and derivatives thereof but also more complex amines as for example an amino-cyclohexadien-derivative (Scheme 10). While 1-phenylethylamine was found to support the amination due to thermodynamics[75] the formal intermediate coproduct ketone resulting from the commercial available amino-cyclohexadien-derivative undergoes a spontaneous irreversible tautomerization to 3-hydroxybenzoic acid shifting thereby the equilibrium toward product formation. This system requires only one enzyme and avoids expensive cofactors such as NADH but suffers from the rather expensive amine donor which has to be added in equimolar amounts.[76]

Scheme 10. Equilibrium Displacement Due to Spontaneous Tautomerization of the Intermediate Co-Product



3.4. Orthogonal Enzyme Cascade Leading to Two Valuable Products. The dual preparation of two valuable products was shown by connecting the kinetic resolution of racemic amines with the simultaneous production of the unnatural amino acid L-homoalanine.[77] In an orthogonal cascade a threonine deaminase (TD from *E. coli*) catalyzed the oxidative deamination of L-threonine producing 2-

Table 1. Selected Examples of the Coupled BCTA/OATA As Well As DATA/ArR$_{mut}$ Cascade for the Preparation of Unnatural Amino Acids (Scheme 9)[a]

entry	R-COOH	cascade	time [h]	conv. [%]	*ee* [%]
1		BCTA/OATA	9	99	>99 (L)
2		DATA/ArRmut	3	98	>99 (D)
3		DATA/ArRmut	7	96	>99 (D)
4		BCTA/OATA	9	97	>99 (L)
5		DATA/ArRmut	5	96	>99 (D)
6		DATA/ArRmut	7	99	>99 (D)
7		BCTA/OATA	18	94	>99 (L)
8		BCTA/OATA	5	98	>99 (L)
9		DATA/ArRmut	5	99	>99 (D)
10		BCTA/OATA	12	95	>99 (L)
11		DATA/ArRmut	12	97	>99 (D)
12		DATA/ArRmut	3	99	>99 (D)
13		BCTA/OATA	18	94	>99 (L)

[a]Origin of TAs: BCTA = *Escherichia coli*, OATA = *Ochrobacterium anthropi*, DATA = *Bacillus sphaericus*, ArRmut = variant[45] of the *Arthrobacter* sp. ArR ωTA.

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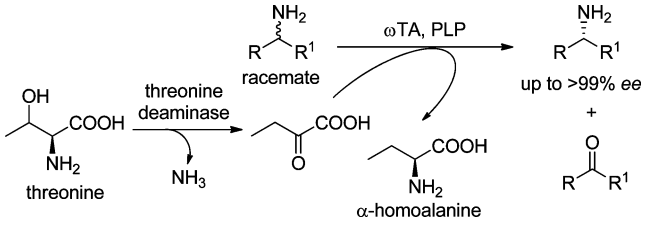
oxobutyrate as amine acceptor. The latter was consumed by the (*S*)-selective ωTA originating from *Ochrobacterium anthropi*[74] giving L-homoalanine and the deaminated ketone as well as the optically enriched remaining amine (Scheme 11).[78,79]

Scheme 11. Simultaneous Production of (*R*)-amine and L-Homoalanine Coupling the ωTA from *O. anthropi*[74] with the Threonine Deaminase (TD) from *E. coli*



The general applicability was demonstrated for a variety of different racemic amines using L-threonine as the amino-acceptor precursor to afford a variety of chiral amines at excellent conversions and in high optically purity (Table 2).

Table 2. Kinetic Resolution of Different Amines Using the ωTA/TD Orthogonal Cascade and L-Threonine as Precursor for the Amine Acceptor (Scheme 11)[a]

entry	R	R^1	t [h]	conv. [%]	*ee* [%]
1	C_6H_5	CH_3	3	49.9	>99 (*R*)
2	*p*-F-C_6H_4	CH_3	3.5	50.5	>99 (*R*)
3	C_6H_5	CH_2CH_3	30	50.1[b]	>99 (*R*)
4	$C_6H_5(CH_2)_2$	CH_3	5	50.9	>99 (*R*)
5	CH_2CH_3	CH_3	8	50.2	>99 (*R*)
6	cyclopropyl	CH_3	8	50.1	>99 (*R*)
7	$CH_3(CH_2)_2$	CH_3	9	50.5	>99 (*R*)
8	$CH_3(CH_2)_5$	CH_3	24	50.2[b]	>99 (*R*)
9	CH_3OCH_2	CH_3	9	50.6	>99 (*R*)
10	$HOCH_2$	CH_3	30	40.8[b]	66 (*R*)

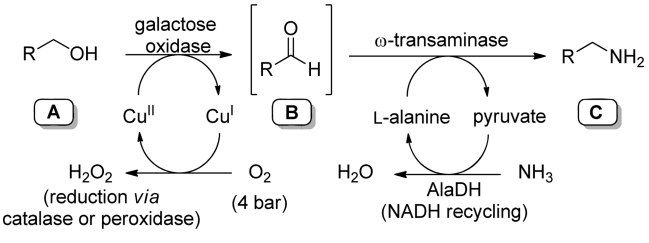
[a]Origin of enzymes: ωTA = *O. anthropi*, TD = threonine deaminase from *E. coli*. [b]2.7 times more ωTA was used compared to the other experiments.

The concept was also extended using an (*R*)-selective ωTA originating from *Aspergillus terreus*[80] for the production of the (*S*)-amine and D-homoalanine.

4. LINEAR CASCADES

4.1. Functional Group Interconversions (FGIs): from Alcohols to Amines. A number of metal-catalyzed methodologies have been developed to access amines from the corresponding alcohols[81] since alcohol is mostly easily accessible. Furthermore, the alcohol and the amine are in the same oxidation state, thus in an ideal situation the transformation would not require any additional reducing or oxidation reagents if the reaction can follow a borrowing hydrogen mechanism.[82,83] Chemical methods require harsher conditions compared to enzymes; additionally transition metals are required as catalyst.

Scheme 12. One-Pot Two Steps Oxidation-Transamination Cascade to Provide Primary Amines from the Corresponding Alcohol[a]



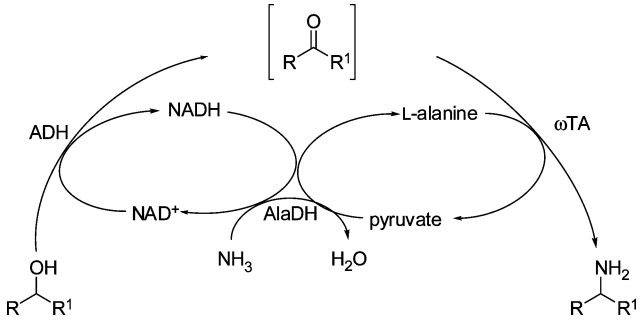
[a]AlaDH = Alanine dehydrogenase.

Table 3. Scope of the Oxidation-Amination Cascade (Scheme 12) Involving the Galactose Oxidase from *Fusarium* NRRL 2903 and ωTAs from *Vibrio fluvialis* (VF) and *Paracoccus denitrificans* (PD)[a]

entry	R	ωTA	**A** [%]	**B** [%]	**C** [%]
1	C_6H_5	VF	<1	<1	>99
2	*m*-Cl-C_6H_4	VF	<1	4	96
3	*m*-Me-C_6H_4	PD	<1	<1	>99
4	*m*-OMe-C_6H_4	VF	<1	19	81
5	*p*-OMe-C_6H_4	PD	<1	32	68
6	*p*-OMe-C_6H_4	PD	16	2	82
7	3-phenylallyl	PD	8	<1	92

[a]**A** = alcohol, **B** = aldehyde, **C** = amine.

Scheme 13. Redox-Neutral Cascade for the Transformation of Primary (R = H) or Secondary (R ≠ H) Alcohols into the Corresponding Amines[a]



[a]ADH = alcohol dehydrogenase; AlaDH = alanine dehydrogenase.

Consequently, the biocatalytic amination of alcohols would represent an excellent alternative. However, no enzyme is known to catalyze the direct amination of an alcohol; consequently various multienzyme systems have been designed to convert primary alcohols to the corresponding amines. All methods are based on alcohol oxidation to the corresponding ketone/aldehyde intermediate which is aminated subsequently by a ωTA. For instance, the chemoselective O_2-dependent galactose oxidase from *Fusarium* NRRL 2903[84,85] oxidizes the alcohol to the corresponding aldehyde at the expense of molecular oxygen. The intermediate aldehyde is subsequently aminated by the ωTA giving the terminal amine (Scheme 12).[86]

Different ωTAs were tested for the amination whereby ωTAs originating from *Vibrio fluvialis* (VF-ωTA)[50] or *Paracoccus denitrificans* (PD-ωTA)[78] led to highest conversions. Replacing ammonium formate with ammonium chloride as nitrogen source allowed the cascade to proceed smoothly, isolating finally benzylamine as the sole product. With optimized conditions the scope of the methodology was studied on a 50 mM substrate concentration leading to excellent results for the amination of various benzylic alcohols (Table 3, entries 1–

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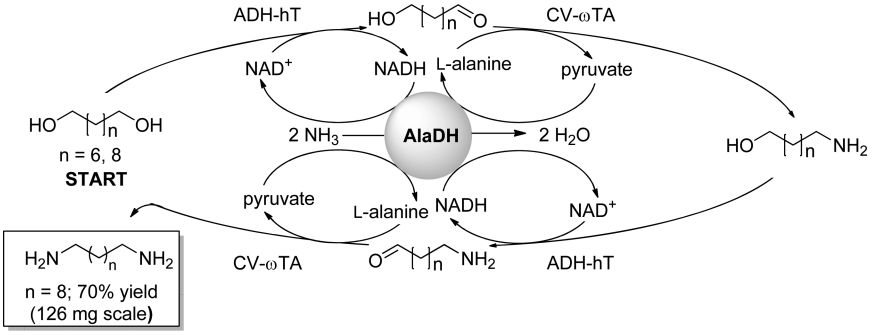
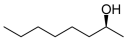
Scheme 14. Redox-Neutral Cascade for the Diamination of Designated 1,ω-diols (Octane-1,8-diol *n* = 6, decane-1,10-diol *n* = 8)[a]



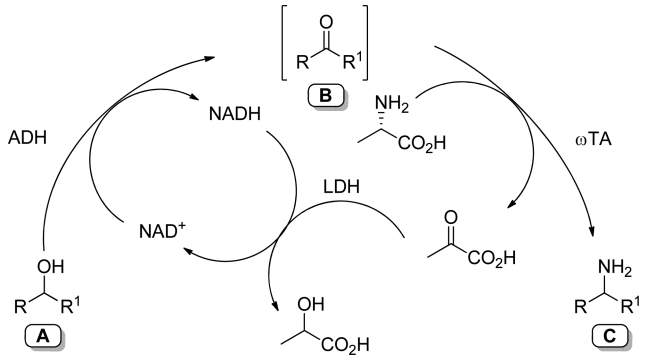
[a]ADH-hT = alcohol dehydrogenase originating from *Bacillus stearothermophilus*; CV = ω-transaminase originating from *Chromobacterium violaceum*; AlaDH = alanine dehydrogenase.

Table 4. Redox-Neutral Cascade for the Amination of Achiral, Enantiopure As Well As Racemic Alcohols Applying the System Depicted in Scheme 10[a]

entry	substrate	ADH	ωTA	ketone [%]	amine [%]	ee_{Amine} [%]
1		ADH-A	BM	--	55	n. a.
2		ADH-A/ ADH 007	BM	14	64	n. a.
3		ADH-A	CV	32	54	78 (*S*)
4		ADH-A	VF	25	47	n. r.
5		ADH-A	VF	47	25	98 (*S*)
6		ADH-A/ ADH 007	BM	35	50	n. r.

[a]n. r.: not reported. n. a.: not applicable. ADH-*A* = alcohol dehydrogenase from *Rhodococcus ruber*; BM = ωTA from *Bacillus megaterium*, CV = ωTA from *Chromobacterium violaceum*, VF = ωTA from *Vibrio fluvialis*.

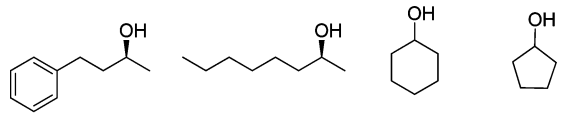
Scheme 15. Redox-Neutral Network Using Lactate Dehydrogenase (LDH) to Remove Pyruvate



6). Noteworthy, even the sensitive cinnamyl alcohol (*E*)-3-phenyl-2-en-1-ol was transformed with excellent conversion to the corresponding amine without isomerization of the double bond (entry 7). The resulting amine was used for the synthesis of the antifungal compound Naftifine, demonstrating the practical applicability of this methodology.[86] Overall, five enzyme-catalyzed steps were combined within one-pot in an efficient concurrent cascade.

The necessity of external oxidizing and reducing agents (O_2, formate, glucose) in the system above led to the search for an alternative system without external redox-reagents, especially since generally the amine is thermodynamically favored over the alcohol. Consequently a redox-self-sufficient multienzyme

Table 5. Redox-Neutral Amination Cascade of Secondary Alcohols Using an Alcohol Dehydrogenase from *Rhodococcus ruber* (ADH-A) and Various ωTAs in Combination with a Lactate Dehydrogenase (LDH) for NADH Regeneration (Scheme 13)[a]



I II III IV

entry	alcohol	ωTA	ketone **B** [%]	amine **C** [%]	*ee* [%]
1	I	VF	27	64	88
2	II	VF	31	64	96
3	III	BM	<1	91	n. a.
4	IV	BM	10	85	n. a.

[a]n. a.: not applicable.

cascade was established, thus a redox-cascade not requiring external redox-reagents. In this system, an ADH catalyzes the oxidation of the primary alcohol to the corresponding aldehyde producing one equivalent of the reduced cofactor NADH (Scheme 13).[87] The ωTA consumes one equivalent of L-alanine for the amination of the aldehyde producing one equivalent of pyruvate. Both steps are interconnected by an AlaDH[53,88] which catalyzes the regeneration of L-alanine from pyruvate at the expenses of ammonia as well as the NADH produced in the oxidation step yielding only one molecule of water as byproduct. For simplicity reasons, primary alcohols were

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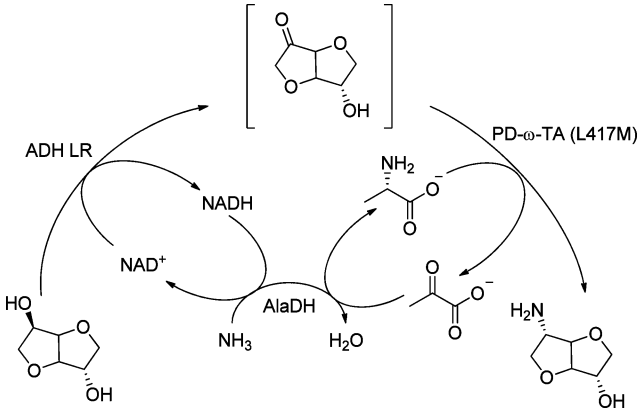
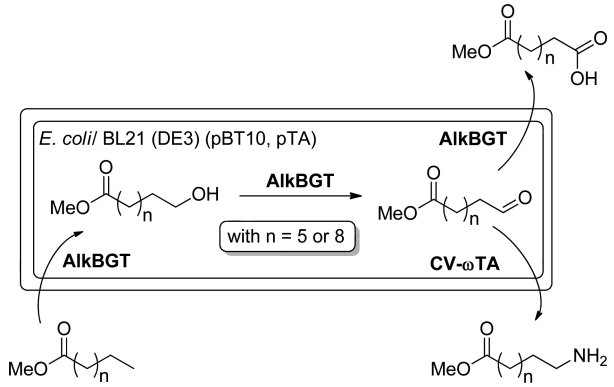
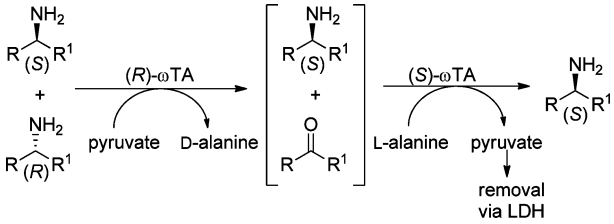
Scheme 16. Redox Neutral Cascade for the Mono-Amination of Isosorbide Using an ADH from *Leifsonia aquatica* (ADH LR) and a Variant of *Paracoccus denitrificans* (PD-ωTA)



Scheme 17. Three-Step Cascade Catalyzed by an Oxygenase (AlkBGT) and a ωTA (Originating from *Chromobacterium violaceum*) co-Expressed in *E. coli* for the Amino Functionalization of Octane- and Dodecanoic Acid Methyl Ester, Respectively

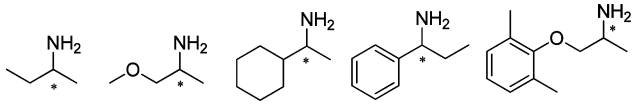


Scheme 18. Deracemization of Racemic Amines Catalyzed by Stereocomplementary ωTAs via a Deamination/Amination Cascade



selected to demonstrate the viability of this redox-neutral cascade.[89] Best results for the oxidation step were obtained with the ADH from *Bacillus stearthermophilus* (ADH-hT)[90,91] while the ωTAs from *Chromobacterium violaceum* (CV-ωTA) and *Arthrobacter citreus* (ArS-ωTA) were found to be the optimal for the amination. The cascade (50 mM substrate concentration) was performed with a set of aliphatic, benzyl, and cinnamyl alcohols. Excellent results were obtained for 1-hexanol and 3-phenyl-1-propanol leading to the exclusive formation of the corresponding amines. Activated alcohols such as benzyl or cinnamyl alcohols afforded excellent conversions although

Table 6. One-Pot Two-Step Deracemization of Pharmacologically Relevant Amines[a]



I II III IV V

entry	amine	1st ωTA	2nd ωTA	*c* [%]	*ee* [%]
1	I	ATA-117	ATA-113	>99	>99 (*S*)
2	I	ATA-114	ATA-117	>99	>99 (*R*)
3	II	ATA-117	ATA-113	>99	>99 (*S*)
4	II	ATA-114	ATA-117	>99	96 (*R*)
5	III	ATA-117	ATA-113	62	>99 (*S*)
6	III	ATA-114	ATA-117	88	>99 (*R*)
7	IV	ATA-117	ATA-113	82	>99 (*S*)
8	IV	ATA-114	ATA-117	72	>99 (*R*)
9	V	ATA-117	ATA-113	98	>99 (*S*)
10	V	ATA-113	ATA-117	97	>99 (*R*)

[a]The abbreviation ATA (amino transferase) was introduced by Codexis for its commercial ωTAs.

significant amounts of the intermediate aldehyde (13−30%) were also detected.

The applicability of the multienzyme network has also been demonstrated for the diamination of 1,ω-diols.[87] The cascade provides straightforward access to 1,ω-diamines which are valuable building blocks for the preparation of polyamide polymers. In the case of 1,ω-diols, two redox-neutral oxidation-amination processes are taking place; thus four sequential enzymatic steps are required to afford the aimed 1,ω-diamine (Scheme 14). The diamination was studied with two model substrates, octane-1,8-diol and decane-1,10-diol. Because of their low solubility in water, a cosolvent was added to increase the solubility of the diol. After optimization the 1,ω-diamines were obtained with almost full conversion (98%) without detecting traces of the intermediate aldehyde or overoxidation products. The diamination of decane-1,10-diol was performed on a preparative scale (126 mg) leading to 70% isolated yield.

The direct amination of secondary alcohols is a more challenging transformation; however, it provides access to valuable α-chiral amines. Considering the possibilities of the above-mentioned redox-neutral amination cascade, the concept was further extended for the amination of secondary alcohols (Scheme 13 with R ≠ H).[89] In this case, the complexity of the biocatalytic network increases making necessary a careful analysis of each step for a successful transformation. For instance, the enantiopreference of the ADH needs to match with the absolute configuration of the starting material; thus for racemic alcohols the use of two stereocomplementary ADHs would be required to achieve full conversion; alternatively a single ADH transforming both enantiomers would be desirable. Moreover, the use of a NAD^+-dependent ADHs is essential to allow the internal recycling of the cofactor by the NADH-dependent AlaDH.[53,88] On the other hand the optical purity of the final product is exclusively controlled by the applied ωTA. Consequently the following catalysts were used for the amination of *sec*-alcohols: For the oxidation the (*S*)-selective ADH-A from *Rhodococcus rubber*[92,93] and the (*R*)-selective ADH007 (Codexis) were found to be the optimal catalysts. Concerning the reductive amination, the (*S*)-selective CV-ωTA, Vf-ωTA, and a ωTA from *Bacillus megaterium* (BM-ωTA) were identified as the most appropriate catalysts. The

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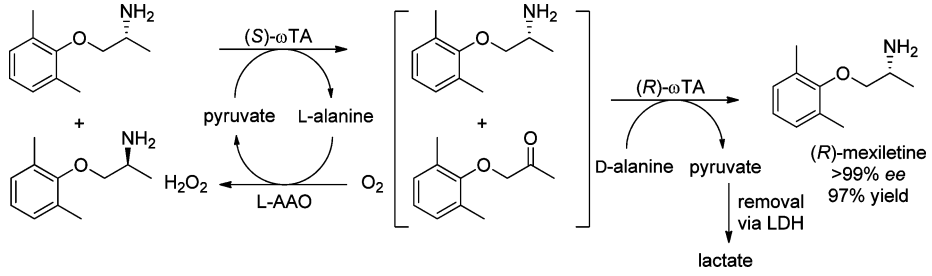
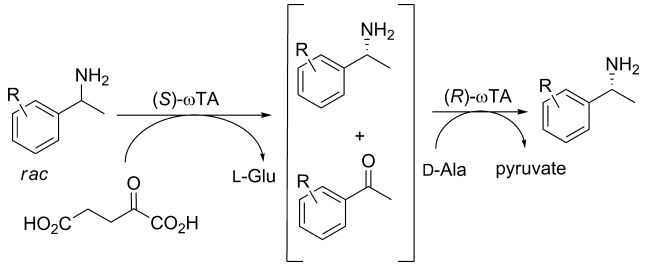
Scheme 19. Deracemization of Mexiletine through a Deamination/Amination Sequence Employing Two Enantiocomplementary ωTAs[a]



[a]LDH = lactate dehydrogenase; AAO = amino acid dehydrogenase.

Scheme 20. Concurrent One-Pot and One-Step Deracemization Cascade of Aromatic Amines Combining Sterecomplementary ωTAs Simultaeneously[a]



[a]L-Glu = L-glutamic acid, D-Ala = D-alanine.

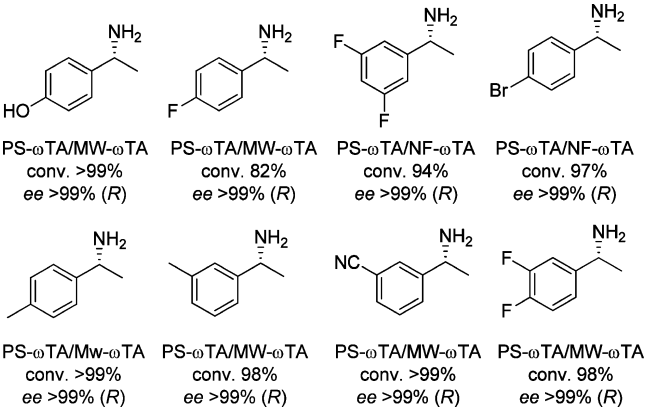
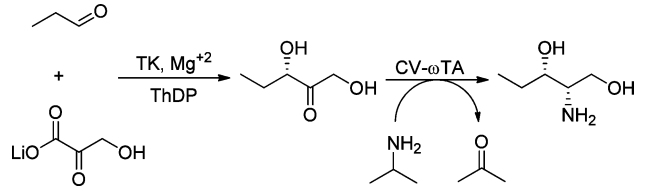


Figure 3. Selection of amines obtained in a one-pot deracemization cascade using the simultaneous combination of an (*S*)- and an (*R*)-selective ωTAs. ωTAs originating from PS = *Polaromonas species,* NF = *Neorsatoya fischeri,* and MF = *Mycobacterium vanbaadenii*..

Scheme 21. Two-Step Synthesis of (2*S*,3*S*)-2-Aminopentane-1,3-diol Combining a Transketolase (TK) with a ω-TA



combination of both steps led to a promising amine formation [20% for (*S*)-4-phenylbutan-2-ol] which stimulated optimization of the cascade. The cascade was finally tested for a broad range of nonchiral and chiral substrates. Good conversions were detected thereby for achiral substrates such as cyclohexanol or cyclopentanol (55% and 64% respectively, Table 4, entries 1−2). However, the bioamination of chiral (*S*)-1-octanol led to only moderate amounts of the corresponding octyl-2-amine (entry 3). Experiments indicated that the amination of *sec*-alcohols via the redox-neutral cascade depicted in Scheme 13 will not go to completion or close to completion because of thermodynamic reasons, which was in contrast to the results observed for primary alcohols. Furthermore, the amine was recovered in lower *ee* (78%) compared with the previous results obtained in the ωTA amination of the ketone octane-2-one in which the *ee* was >99%. This result was attributed to the continuous back- and forward-reactions of the equilibrating system leading to racemization of the amine.

In the previous cascade the overall reaction transformed alcohol plus ammonia to amine plus water. To improve the amination of secondary alcohols, the network was redesigned to allow an alternative NADH recycling and changing the overall reaction equation to the transformation of alcohol plus alanine giving amine plus lactate (Scheme 15).[89] Thus, in this case pyruvate was transformed to lactate by an LDH instead of L-alanine by the AlaDH. The redox-neutral character of the cascade was still maintained, since the hydride abstracted in the oxidation was used for the reduction of pyruvate. The redesign led to substantially improved amine formation (up to 96% conversion, Table 5). Moreover, for chiral substrates the (*S*)-amines were obtained with excellent optical purity at reasonable conversion (entries 1−2).

Related to this redox-neutral oxidation-amination cascade, the amination of isosorbide, an enatiomerically pure bulk chemical obtained from renewable sources, has been reported.[94] Different biocatalysts were assayed for each step finding ADH from *Leifsonia aquatica* (ADH-LR)[95] and a variant of a ωTA originating from *Paracoccus denitrificans* (PD-ωTA)[78] as the best catalysts for the cascade. The transformation gave the monoaminated (2*S*,5*S*)-aminoalcohol with 7% conversion (Scheme 16).

4.2. Amination of Nonactivated C−H Bonds. Recently, an in vivo one-pot three step cascade was reported for the ω-amino functionalization of fatty acids. The reaction sequence can be seen as an extension of the previous chapter, inserting a hydroxylation step of the terminal CH_3-moiety before the cascade for the amination of an alcohol. The cascade was realized by coexpressing two enzymes in an *E. coli* strain,[42] namely, an alkane monoxygenase from *Pseudomonas putida* (AlkBGT) and the ωTA originating from *Chromobacterium violaceum*. The oxygenase catalyzes the hydroxy functionalization of the terminal alkane as well as the subsequent oxidation of the alcohol to the corresponding aldehyde at the expense of

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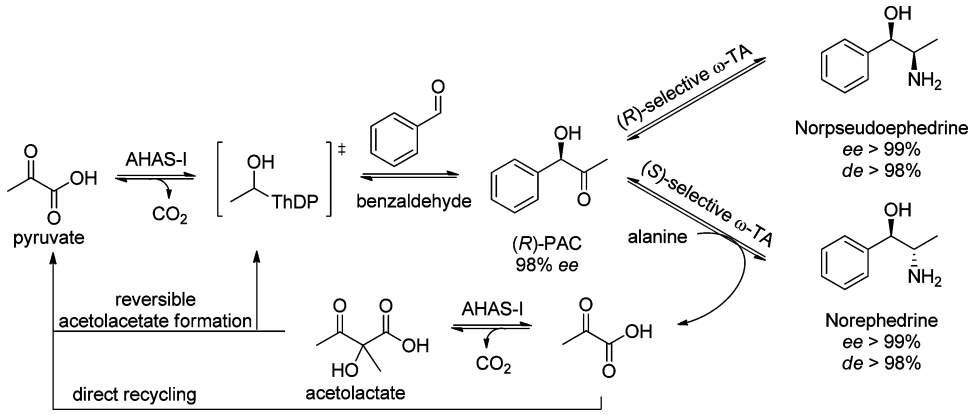
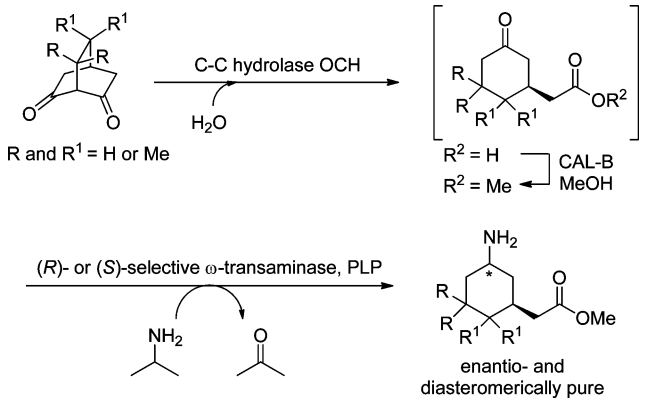
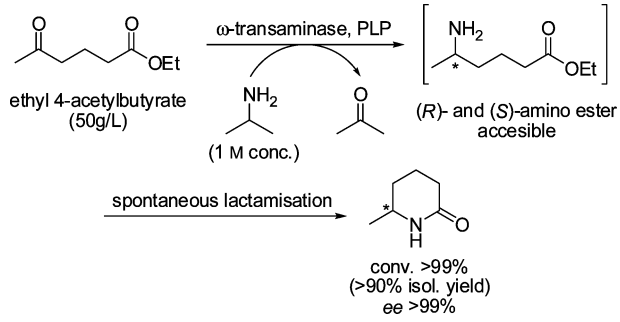
Scheme 22. Synthesis of Norephedrine (NP) and Norpseudoephedrine (NPE) by the Combination of a Thiamine-Diphosphate-Dependent (ThDP) Acetohydroxyacid Synthase I (AHAS-I) with Enantiocomplementary *ω*-Transaminases in a One-Pot Cascade Performed in a Stepwise Fashion



Scheme 23. Linear Three-Step Cascade to Provide 3-Substituted Cyclohexylamine Derivatives Employing Two Hydrolases and a *ω*TA



Scheme 24. *ω*TA Initiated Amination-Lactamization Yielding a *δ*-Lactam



molecular oxygen. The reductive amination of the aldehyde was catalyzed by the *ω*TA. This three step whole-cell cascade was successfully performed with octane- and dodecanoic acid methyl ester (0.5−2.9 mM) as starting materials, affording the corresponding *ω*-amino esters, however, along with the overoxidized side products (Scheme 17). The described example to coexpress the enzymes of interest in one host for an artificial cascade in a single cell can be expected to gain more significance in the future, and further examples are expected to be published in the near future.[40,41]

4.3. Deracemization of Amines using Enantiocomplementary *ω*-Transaminases. In a deracemization process a racemic substrate is transformed ideally to an optically pure product in 100% yield and optical purity.[30−33,96] This strategy is in general recommended for amines if the synthesis of the racemic amine is easier than of the corresponding ketone, or the corresponding ketone is not stable. The deracemization cascade involving *ω*TAs proceeds in two reaction steps: first the enantioselective oxidative deamination affords the prochiral ketone (50%) along with the enantiopure amine (50%) in a kinetic resolution; in the second step, the stereoselective amination of the ketone with a stereocomplementary *ω*TA provides the desired amine with theoretically 100% yield (Scheme 18).[97−99]

Table 7. Results of the Studied Three-Steps Cascade Reaction Giving Access to 3-Substituted Cyclohexylamine Derivatives[b]

entry	substrate	hydrolysis	esterification	amination	conv. [%]	*ee* [%]	*de* [%]	product
1		OCH	CAL-B	VF-ωTA	71	>99	>99	(1*S*,3*S*)
				ArR_{mut}	91	>99	>99	(1*S*,3*R*)
2		OCH	CAL-B	VF-ωTA	25[a]	>99	>99	(1*S*,5*R*)
				ArR_{mut}	69[a]	>99	>99	(1*S*,5*R*)
3		OCH	chemical	VF-ωTA	>99[a]	>99	>99	(1*S*,5*S*)
				ArR_{mut}	>99[a]	>99	>99	(1*S*,5*R*)

[a]Conversions refer to the amination reaction only. [b]OCH = C−C hydrolase originating from *Rhodococcus* sp.; CAL-*B* = hydrolase originating from *Candida antarctica*, VF = *ω*TA originating from *Vibrio fluvialis*.

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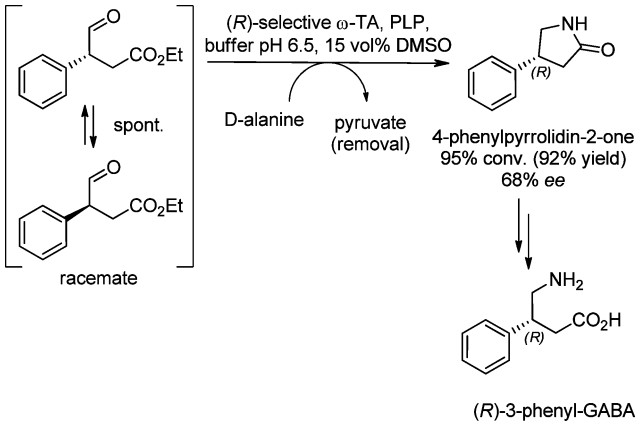
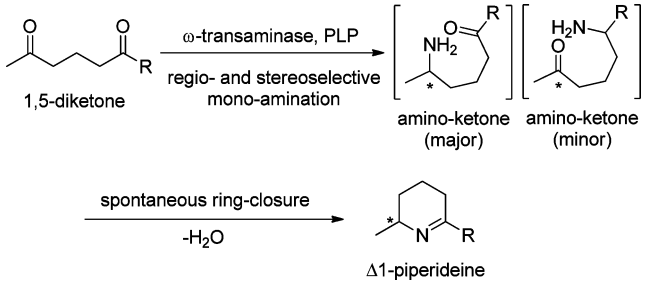
Scheme 25. Synthesis of 4-Phenyl-pyrrolidin-2-one via a ωTA Triggered Amination-Lactamization



Scheme 26. Linear Amine-Imination Cascade of 1,5-Diketones with a Regioselective Monoamiation As Key Step



The one-pot stepwise deracemization methodology was successfully demonstrated for the preparation of a broad panel of pharmacologically active amines. By selecting the appropriate combination of enzymes the (*R*)- or the (*S*)-enantiomers were successfully prepared in conversions up to 99% in enantiopure form (Table 6).[97] In a first study, stoichiometric amounts of pyruvate were employed for the deamination step leading to the accumulation of L-alanine.

Alternatively to minimize the amount of pyruvate needed and the amount of L-alanine formed, L-alanine was recycled to pyruvate using an L-amino acid oxidase (L-AAO).[100] The improved deracemization cascade was finally applied for the synthesis of both enantiomers of the antiarrhythmic drug mexiletine, both obtained in enantiopure form and 97% isolated yield (Scheme 19).[99]

However, the main limitation of the concept is the interference between the two stereocomplementary ωTAs leading to a decrease in the *ee* of the final amine when performing the reaction in a one-pot one-step manner. This limitation has been overcome by heat inactivation of the first enzyme although the economic viability of the approach is low considering the cost of ωTAs. An alternative method has been developed thereafter based on the immobilization of the first enzyme. Consequently, the ωTA of the first step was removed by filtration respective centrifugation after the kinetic resolution and reused several times. The improved cascade enabled the synthesis of the (*S*)-amine of mexiletine in 95% isolated yield and enantiopure form.[101]

Recently, a general method for deracemization of amines was developed in which the deamination and amination steps take place simultaneously.[102] The success was based on the selection of an appropriate amine acceptor for the (*S*)-selective ωTA whereby this amine acceptor should not be transformed by the (*R*)-selective ωTA. Finally α-ketoglutaric acid turned out to be suited best because of its low price and excellent reactivity (Scheme 20).

Deracemization of different aromatic amines was carried out at 10 mM concentration combining the (*S*)-selective ωTA from *Polaromonas species* (PS-ω-TA) with the (*R*)-selective ωTAs from *Neorsatoya fischeri* (NF-ω-TA) or *Mycobacterium vanbaadenii* (MV-ωTA). The (*R*)-amines were isolated in enantiopure form and excellent conversion after 24 h of reaction time (Figure 3).

4.4. Cascade Reactions Combining ω-Transaminases and Biocatalysts for C−C Bond Formation and Breakage. In this section selected examples are shown combining ωTAs with transferases, hydrolases, and lyases involved in C−C bond formation or cleavage. Starting with transferases, a ωTA was combined with a thiamine-diphosphate dependent (ThDP) transketolase (TK).[103,104] The sequence comprises the stereoselective C−C bond formation catalyzed by a variant of *E. coli* transketolase (TK)[105] followed by the ωTA-catalyzed reductive amination of the intermediate acyloine. 2-Propanamine was employed as amine donor to yield (2*S*,3*S*)-2-aminopentane-1,3-diol successfully (Scheme 21). Such vicinal amino-alcohols are valuable intermediates for the pharmaceutical industry as also demonstrated for aromatic amino-alcohols.[106]

Both steps were optimized separately prior to combination; this included not only reaction but also protein-engineering of the TK to accept propanal as substrate as well as the identification of a proper ωTA by bioinformatic strategies.[104]

Table 8. Scope of the Linear Regioselective Monoamination Cyclization Cascade[a]

entry	R	ωTA	conv. [%]	*ee* [%]
1		CV	>99	>99 (*S*)
2		AT	>99	>99 (*R*)
3		VF	>99	>99 (*S*)
4		HN	>99	>99 (*R*)
5		ArS	97	>99 (*S*)
6		AT	93	>99 (*R*)
7		BM	>99	>99 (*S*)
8		HN	>99	>99 (*R*)
9		PF	>99	98 (*S*)
10		ArR	>99	>99 (*R*)

[a]Origin of ωTAs: CV = *Chromobacterium violaceum*, AT = *Aspergillus terreus*, HN = *Hyphomonas neptunium*, ArS = *Arthrobacter citreus*, PF = *Pseudomonas fluorescens*, ArR = (*R*)-selective *Arthrobacter* sp.

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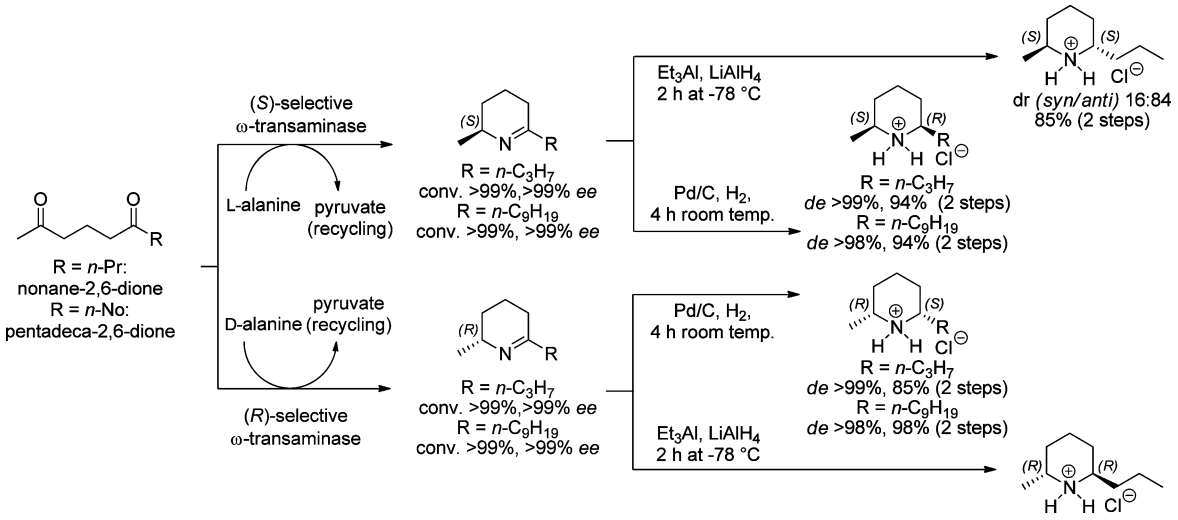
Scheme 27. Chemoenzymatic Synthesis of the Natural Alkaloids Dihydropinidine (R = n-C_3H_7, *cis*), *epi*-Dihydropinidine (R = n-C_3H_7, *trans*), and Isosolenopsin (R = n-C_9H_{19}, *cis*) with an ωTA Catalyzed Regioselective Monoamination as Key Step



The cascade was finally performed in two sequential steps, since the ωTA was also found to catalyze the amination of the TK's substrate propanal. However, in a preparative scale reaction (300 mg) the final product could be isolated in only 8.7% yield. Even though the TK catalyzed C−C bond formation displayed suitable conversions (up to 23%) the reductive amination was identified to be the bottleneck of the cascade reaction; further optimization especially with respect to the reductive amination should facilitate a more efficient process.

Another cascade offering an alternative route to enantio- and diastereomerically pure aryl-amino-alcohols is the combination of a ThDP-dependent lyase in combination with enantiocomplementary ωTAs.[107] The linear cascade facilitates an easy access to norephedrine (NE) and norpseudoephedrine (NPE) from cheap starting materials within only two steps (Scheme 22). The intermediate (*R*)-phenylacetylcarbinol ((*R*)-PAC) is produced via carboligation of pyruvate and benzaldehyde in almost optically pure form (*ee* 98%)[108] employing the acetohydroxyacid synthase I (AHAS-I) from *E. coli*. In the second step (*R*)-PAC is directly aminated by either a (*R*)- or (*S*)-selective ωTA to yield the desired ephedrine derivatives NE respective NPE. Extensive process optimizations were conducted to yield the desired products finally with very good conversions (between 85 and 96%). Furthermore, since the coproduct of the reductive amination (pyruvate) is the starting material for AHAS-I in the first reaction the coproduct could by recycled in a "recycling cascade".[107] Overall, the established one-pot cascade performed in a stepwise fashion allows the preparation of valuable aryl-amino-alcohols NE and NPE in excellent conversion and high optical purity.

In another example a series of chiral 3-subsituted cyclohexylamine derivatives were accessed by combining two hydrolases with ωTAs (Scheme 23).[109] The linear three-step cascade employed a β-diketone hydrolase [6-oxocamphor hydrolase (OCH) originating from *Rhodococcus* sp.][110−112] for the stereoselective hydrolysis of a C−C bond followed by a lipase catalyzed esterification [*Candida antarctica* lipase B (CAL-B)] and an asymmetric amination by either the (*S*)-selective VF-ωTA or the (*R*)-selective variant of the *Arthrobacter* sp. ArR ωTA (ArR_{mut}).[45] Within this study various bicyclic β-diketones were successfully converted to the corresponding cyclohexylamines (Table 7):[113] while the stereochemical outcome was throughout excellent (*ee* and *de* > 99% in all cases) the conversions in the asymmetric reductive amination depended on the substrate, consequently it varied from 25→99%. Notably, while the first two reactions (hydrolysis and esterification) with designated cyclic β-diketones (R and R^1 = H; entry 1 Table 5 and R and R1 = Me, entry 3) were performed in one-pot, the esterification for entry 2 (R = Me) needed to be performed by chemical means because of lack of acceptance by the ωTA.

Only in the case of entry 1 with R and R^1 = H was it possible to perform the third step, the amination reaction, in a cascade fashion. In this case the intermediate keto-ester could be aminated using the same reaction media after a simple filtration step. For the amination of the two other substrates the reaction media had to be changed from an organic- to a buffered system to facilitate the production of the final product. Consequently, the described transformations with respect to entry 2 and 3 cannot be defined as a true cascade.

4.5. Linear Cascades Involving Spontaneous Cyclization Initiated by ω-Transaminases. Six membered heterocyclic compounds can be found in a vast number of bioactive compounds and a variety of pharmaceuticals. Such scaffolds have been accessed by highly sophisticated chemical approaches but also using biocatalysts.[114] One example represents the transaminase catalyzed amination-lactamization cascade: In this study, ethyl 4-acetyl butyrate was used as model-substrate which was converted to the corresponding amino ester which subsequently underwent a spontaneous ring closure reaction. Depending on the choice of the ωTA both enantiomers were accessible at high substrate concentrations (50 g/L) in excellent conversion and stereocontrol (Scheme 24).[115] A related sequence was also recently employed for the synthesis of dual orexin receptor antagonist on a kilogram-scale.[116]

This concept (amination-lactamization cascade) has been successfully applied for the chemoenzymatic synthesis of the pharmaceutically relevant γ-aminobutyric acid (GABA):[51] Starting from the racemic aromatic aldehyde-ester, a ωTA catalyzed dynamic kinetic resolution afforded the analogues amino-ester which cyclized to 4-phenylpyrrolidin-2-one (Scheme 25).[117] Even though the obtained enantiomeric excess was only moderate as its best (68% *ee*), it represents a significant improvement in comparison to previous approaches.

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A further variation closely related has been elaborated to provide 2,6-disubstituted piperidines, a heterocylic ring-structure prominent in numerous synthetic protocols and common in multiple natural products.[114,118] In contrast to the concepts already presented, this linear reaction sequence consists of an amine-imination cascade as the starting materials are 1,5-diketones rather than keto- or aldehyde-esters. The enzymatic amination proceeds almost exclusively at the sterically less demanding (ω-1)-ketone (with R >Me) affording in general one enantiopure regioisomer which cyclized instantly to the Δ1-piperideine (Scheme 26 and Table 8).[119−121]

A detailed investigation demonstrated that the regioselective monamination is not a special feature of only one particular enzyme but a general one. Hence, various aliphatic, cyclic, and aromatic 1,5-diketones were converted by several enantiocomplementary biocatalysts to the analogues Δ1-piperideine with perfect optical purity (Table 8).

The regioselective monoamination-cyclization cascade was applied in the chemoenzymatic synthesis of the alkaloids dihydropinidine, *epi*-dihydropinidine and isosolenopsin (Scheme 27). Transformation of the respective 1,5-diketone (nonane-2,6-dione respective pentadeca-2,6-dione) afforded the Δ1-piperideins at full conversion and perfect stereoselectivity, respectively. The *cis*-diasteromers of the natural products (dihydropinidine and isosolenopsin)[119,120] were obtained via hydrogenation whereas the *anti*-isomers through a Lewis-mediated conformational change during the reduction with LAH.[121]

5. CONCLUSION

The combination of enzymes in a cascade allows performing several reactions in one pot and thereby turning synthetic strategies more efficient. ω-Transaminases (ωTAs) have been identified during the past decade as an excellent catalyst to prepare optically pure α-chiral primary amines. So it is not surprising that more and more examples came up applying ωTAs also in various types of cascade sequences. One purpose of selected cascades is to shift the asymmetric amination reaction of ketones to the side of the product: although established procedures are available, further developments can be expected on this challenging topic. For the combination of ωTAs in linear cascades with other enzymes there is probably no limit to imagination; if the reactions involved are reversible, the only limitation might be thermodynamics to achieve high conversions for a given design of a cascade. This limitation can only be overcome be redesign of the cascade, thus by switching, for example, to alternative reagents. One challenge for cascades is currently the fine-tuned coexpression of the enzymes in a single host. This will turn cascades applicable for efficient synthesis on large scale by minimizing costs.

The cascades allow setting up transformations for which no enzyme is available, like the amination of alcohols or deracemization. Nature provides a tool kit of enzymes which can be exploited to create novel cascades to short cut the synthetic routes of valuable target molecules.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AADH = amino acid dehydrogenase; ADH = alcohol dehydrogenase; AHAS-I = acetohydroxyacid synthase I from *E. coli*; AlaDH = alanine dehydrogenase; AlkBGT = alkane monoxygenase from *Pseudomonas putida*; BCTA = branched chain transaminase; Bn = Benzyl; Boc = *tert*-butyl-oxycarbonyl; BVMO = Baeyer−Villigermonooxygenase; E = enantioselectivity; DATA = D-amino transaminase; FDH = formate dehydrogenase; GDH = glucose dehydrogenase; LDH = lactate dehydrogenase; MBA = methyl benzyl amine; OCH = oxocamphor hydrolase from *Rhodococcus* sp; PDC = pyruvate decarboxylase; PMP = pyridoxamine-5′-phosphate; PLP = pyridoxal-5′-phosphate; αTA = α-transaminase; ωTA = ω-transaminase; TD = threonine deaminase; ThDP = thiamine diphosphate; TK = transketolase; YADH = yeast alcohol dehydrogenase

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