Chiral Amines

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Formal Asymmetric Biocatalytic Reductive Amination**

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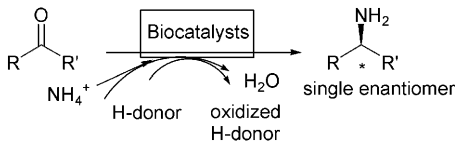
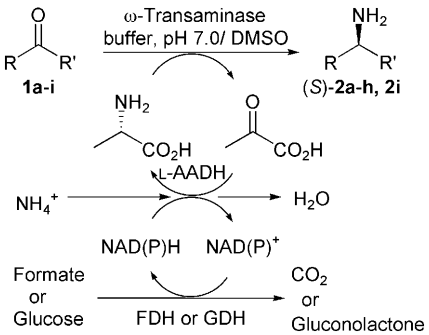
Asymmetric methods to prepare optically active α-chiral primary amines are highly demanded in asymmetric synthesis owing to the biological/pharmacological activity of many amines.[1] Various techniques have been reported, such as asymmetric 1,2-addition to imines and asymmetric amination of α,α-disubstituted aldehydes,[2] transformation of allylic alcohols into amines,[3] (dynamic) kinetic resolution,[4] and cyclic deracemization[5] employing racemic amines as substrates. Asymmetric reductive amination of ketones has been investigated with transition-metal catalysts[6] and organocatalysts,[7] as well as via sulfinyl imine intermediates.[8] Although tremendous progress in organo/metal catalysis has been achieved for the asymmetric reductive amination of ketones to access α-chiral amines, improved protocols are still required that are simple, green, and economically viable and that lead to high enantiomeric excesses.

Biocatalytic reductive amination or transamination is well established for accessing α-amino acids from the corresponding α-keto carboxylic acids.[9] However, the situation is different for primary amines that are not adjacent to a carbonic acid moiety. ω-Transaminases[4a–c,10] have recently received attention for the preparation of such α-chiral unprotected amines. ω-Transaminases are employed mainly in one way, namely for the kinetic resolution of racemic chiral amines;[11] only a few reports deal with asymmetric synthesis[12] by starting from a prochiral ketone, probably due to problems in shifting the equilibrium to the product side, as well as due to the moderate stereoselectivity of the employed ω-transaminases. These asymmetric synthetic processes usually require at least stoichiometric amounts of an amine donor (for example, alanine). The latter leads to a side product (pyruvate), which has to be removed during the transformation by using, for instance, pyruvate decarboxylase[12a] or lactate dehydrogenase.[12c] Additionally, limitations due to inhibition by the product amine and by pyruvate have been reported. An ideal process would use ammonium as the amine donor, together with a cheap reducing agent (for example, formate, hydrogen, or glucose; see Scheme 1). Even fewer reports employing biocatalysts can be found in the literature for such a reductive amination of *ketones* (excluding α-keto carbonic acids and aldehydes) by employing an enzyme, nicotinamide adenine dinucleotide (phosphate) in the reduced form (NAD(P)H), and ammonia.[13] Unfortunately, to the best of our knowledge, no enzyme catalyzing this reaction has been identified by DNA sequencing.



Scheme 1. Ideal reductive amination of ketones to form α-chiral amines at the expense of glucose or formate as a reducing agent.

Therefore, we envisaged a cascade that emulates the reaction scheme outlined in Scheme 1. For this purpose, we combined three enzymes: 1) an ω-transaminase transfers the amino group from alanine to the substrate to be converted (Scheme 2), to give the desired amine and pyruvate; 2) an amino acid dehydrogenase (for example, alanine dehydrogenase) recycles alanine from pyruvate by consuming ammonium and NAD(P)H; 3) finally, the cofactor is recycled[14] by using standard methods (for example, formate dehydrogenase and formate, glucose dehydrogenase and glucose).[15] In this concept, alanine is not consumed but recycled.



Scheme 2. Outline of the formal reductive amination concept for the preparation of optically pure amines. DMSO: dimethylsulfoxide; AADH: α-amino acid dehydrogenase; NAD(P)$^+$: nicotinamide adenine dinucleotide (phosphate) in the oxidized form; FDH: formate dehydrogenase; GDH: glucose dehydrogenase.

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In a first experiment, we tried this approach by using acetophenone as the substrate and commercial ω-transaminase from *Vibrio fluvialis*; however, we could not detect any conversion (< 1 %) into the desired amine.

Therefore, we moved back one step and searched for better suitable ω-transaminases. We tested 19 commercially available ω-transaminases for the transamination of 2-pentanone (**1a**) by employing alanine (5 equiv) as the amine donor and lactate dehydrogenase for the removal of pyruvate. Only two ω-transaminases, ATA-113 and ATA-117, showed high activity and stereoselectivity for the transamination of this ketone. ATA-113 led to the *S* enantiomer (> 99 % *ee*) and ATA-117 led to the *R* enantiomer (> 99 % *ee*). In the absence of the lactate dehydrogenase, no measurable conversion was detected; this can be attributed to the nonfavored equilibrium between pyruvate/amine and alanine/ketone, which is strongly on the side of alanine/ketone.[12a]

Having identified a suitable ω-transaminase, we tried the reductive amination concept (Scheme 2) on 2-pentanone (**1a**) at a preparative concentration (50 mM) by employing ATA-113 with L-alanine dehydrogenase and cofactor recycling. To increase the solubility of the lipophilic substrates in aqueous solution, DMSO (15 % v/v) was added. The reaction was started with just half an equivalent of L-alanine. To our delight, a conversion value of 56 % clearly indicated that the L-alanine was recycled;[16] however, the reaction time was rather long (68 h), whereby we identified the ω-transaminase as catalyzing the rate-limiting reaction step. To decrease the reaction time, either a larger amount of ω-transaminase or a larger amount of alanine could be used. Since the latter option seemed to be cheaper, we measured the conversion at various L-alanine concentrations (Figure 1).

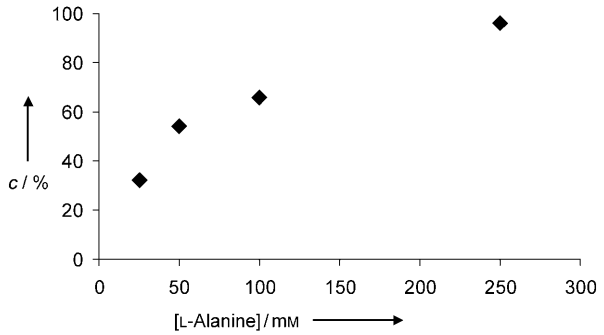
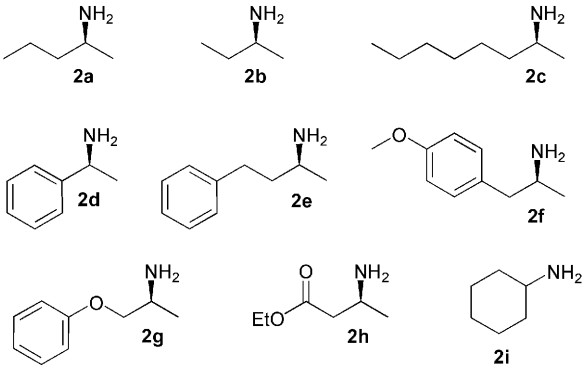


Figure 1. Conversion (*c*) of 2-pentanone (**1a**; 50 mM) into the corresponding amine after 24 h at various L-alanine concentrations by a formal reductive amination according to Scheme 2.

As expected, higher concentrations of L-alanine led to a faster total reaction. Since alanine is cheap, is internally recycled in the system, and does not interfere with the other enzymes in the system, we used it in our further experiments at a concentration of 250 mM.

To show the applicability of this concept, various ketones, **1a**–**1i**, were successfully transformed into the corresponding (*S*)-amines (Scheme 3) by employing ω-transaminase ATA-113 (Table 1). Interestingly, the optical purity slightly decreased for (*S*)-**2a** by using the reductive amination concept (*ee* 93 %), whereas optically pure amine (*ee* > 99 %) was obtained in the experiment employing lactate dehydrogenase for pyruvate removal. On the other hand, ketones 2-butanone (**1b**) and 2-octanone (**1c**) were converted into optically pure (*S*)-**2b** and (*S*)-**2c** (*ee* > 99 %), respectively.



Scheme 3. Amines **2a–i** were prepared by formal asymmetric reductive amination from the corresponding ketones.

Table 1: Formal biocatalytic reductive amination according to Scheme 2 by employing ω-transaminase ATA-113.

Substrate	Product	*c* [%][a]	*ee* [%][b]
1a	(*S*)-**2a**	92	93
1b	(*S*)-**2b**	> 99	> 99
1c[c]	(*S*)-**2c**	89	> 99
1d	(*S*)-**2d**	6	> 99
1e[c]	(*S*)-**2e**	> 99	34
1f[c]	(*S*)-**2f**	> 99	> 99
1g[c]	(*S*)-**2g**	50	86
1h	(*S*)-**2h**	> 99	94
1i[c]	**2i**	> 99	n.a.[d]

[a] The reaction was performed by employing ATA-113, L-alanine dehydrogenase, formate dehydrogenase, NAD^+, and L-alanine (see the Supporting Information); conversions were determined after 24 h by GC analysis. [b] The enantiomeric excess was measured, after derivatization, by GC analysis with a chiral stationary phase. [c] 2.76 U of ω-transaminase were applied. [d] n.a.: not applicable.

For aromatic and aryl-alkyl ketones, the conversion and optical purity varied. For instance, acetophenone (**1d**) was reductively aminated with complete stereoselectivity (*ee* > 99 %). 4-Phenyl-2-butanone (**1e**) was transformed very efficiently (> 99 % conversion), although with reduced stereoselectivity (34 %). In the case in which only one methylene unit was between the carbonyl moiety and the aromatic system (**1f**), complete stereoselectivity was again observed (*ee* > 99 %).

Chiral β-amino acid derivatives are valuable building blocks for the synthesis of numerous biologically active compounds, such as β-peptides, β-lactam antibiotics, and other drugs.[17] Thus, formal reductive amination of acetoacetate **1h** yielded the enantioenriched β-amino ester (*S*)-**2h** with complete conversion (> 99 %, 94 % *ee*). Additionally, the cyclic ketone **1i** was fully converted, a result indicating the high flexibility of the utilized enzyme to accept different types of substrates.

To access the opposite *R* enantiomer, one can imagine the same concept but employing the *R*-stereoselective ω-transaminase ATA-117 in combination with D-alanine and a D-alanine dehydrogenase. As a further option, we found that ATA-117 not only accepted D-alanine but also L-alanine. To our delight, the optical purity was not affected by switching the enantiomer of the amine donor. For instance, ATA-117 reduced 2-butanone (**1a**) into (*R*)-2-butylamine ((*R*)-**2a**) with > 99% *ee* by using D- or L-alanine; however, the reaction rate was 20 times slower with L-alanine than with D-alanine. Nevertheless, as a demonstration, the formal reductive amination of ketone **1g** by employing *R*-selective ATA-117, L-alanine, and L-alanine dehydrogenase gave enantiomerically pure (*R*)-1-phenoxy-2-propylamine (**2g**; > 99% *ee*, 46% conversion), which shows that ATA-117 possesses excellent stereoselectivity with this substrate. Amine (*R*)-**2g** is a valuable building block for the synthesis of several antiepileptic agents.[18]

Finally, we applied this transformation concept on a preparative scale. Thus, 100 mg of (4-methoxyphenyl)acetone (**1f**) were transformed into amine (*S*)-**2f** (98% *ee*) with complete conversion, within 24 h, and with 98% yield of isolated product. Optically active amine **2f** is an important building block in the synthesis of biologically active formoterol.[19]

In summary, we have reported a concept for a biocatalytic asymmetric reductive amination of ketones that leads to unprotected α-chiral primary amines.[20] The concept is highly flexible and offers the possibility of accessing both enantiomers by choosing the appropriate enzymes. In contrast to the asymmetric Leuckart–Wallach type reaction,[6h] the biocatalytic variant does not lead to any side products, for example, through amide formation. To the best of our knowledge, no enzyme for the reductive amination of ketones (not conjugated to carboxylic acids and aldehydes) has been identified by DNA sequencing; however, since amine formation has been observed in microorganisms,[13] it can be speculated that the cell machinery can perform this type of reaction through a related process, such as that described here. The proposed biocatalytic concept expands the toolkit for biocatalytic asymmetric synthesis[9b,21] to include the transformation of ketones into enantioenriched unprotected amines in aqueous solution under air at 30 °C, at the expense of ammonium and a cheap reducing agent, such as formate.

Experimental Section

Representative example of the preparation of (*S*)-amines: (4-methoxyphenyl)acetone (**1f**; 100 mg, 0.61 mmol) was transformed in phosphate buffer (17 mL, 100 mM, pH 7.0, 1 mM NAD^+, 1 mM PLP) containing a crude preparation of ω-transaminase ATA-113 (18.4 U, 40 mg, Codexis), L-alanine (272 mg, 3.05 mmol), L-alanine dehydrogenase (41 U, 48.09 U mg^{-1}; Fluka, no. A7653-100UN), ammonium formate (115 mg, 1.83 mmol), formate dehydrogenase (200 μL, 40 U, 200 U mL^{-1}; Jülich, now Codexis, no. 24.11), and DMSO (15% v/v) at 30 °C. After 24 h, the conversion was > 99%, the pH value of the mixture was adjusted to pH 1 with HCl (5 M), and any possible remaining ketone was extracted 5 times with dichloromethane (5 × 10 mL). The pH value was adjusted to pH 12 and the amine was extracted 4 times with dichloromethane (4 × 10 mL). The solvent of the combined organic phases was evaporated under reduced pressure and the amine was purified by flash column chromatography (hexane/ethyl acetate and methanol) to yield (*S*)-**2f** (98.6 mg, 98% yield; 98% *ee*): $[\alpha]_D^{20} = +27.8$ (c = 0.7, $CHCl_3$; literature value:[22] + 35.2, *c* = 0.95, $CHCl_3$, for the *S* enantiomer, 98% *ee*); the ^1H NMR spectrum was in agreement with that in the literature.[18c]

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