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ARTICLE

Amine dehydrogenases: Efficient biocatalysts for the reductive amination of carbonyl compounds

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Amines constitute the major targets for the production of a plethora of chemical compounds that have applications in the pharmaceutical, agrochemical and bulk chemical industries. However, the asymmetric synthesis of α-chiral amines with elevated catalytic efficiency and atom economy is still a very challenging synthetic problem. Here, we investigated the biocatalytic reductive amination of carbonyl compounds employing a raising class of enzymes for amine synthesis: the amine dehydrogenases (AmDHs). The three AmDHs from this study - operating in tandem with a formate dehydrogenase from *Candida boidinii* (Cb-FDH) for recycling of the nicotinamide coenzyme - performed the efficient amination of a range of diverse aromatic and aliphatic ketones and aldehydes with up to quantitative conversion and elevated turnover numbers (TONs). Moreover, the reductive amination of prochiral ketones proceeded with perfect stereoselectivity, always affording the (*R*)-configured amines with more than 99% enantiomeric excess. The most suitable amine dehydrogenase, the optimised catalyst loading and the required reaction time were determined for each substrate. The biocatalytic reductive amination with this dual-enzyme system (AmDH – Cb-FDH) possesses elevated atom efficiency as it utilizes the ammonium formate buffer as the source of both nitrogen and reducing equivalents. Inorganic carbonate is the sole by-product.

Introduction

Amines are the most widely used chemical intermediates for the production of active pharmaceutical ingredients, fine chemicals, agrochemicals and a significant number of bulk chemicals.[1,2] Nowadays, the production of amines in the laboratory, as well as in industrial scale, relies principally on the reductive amination of carbonyl containing compounds.[3] Nonetheless, the chemocatalytic reductive amination requires precious, and sometimes toxic, metal catalysts coordinated to sophisticated organic ligands that operate at high pressure of hydrogen gas. Furthermore, the overall process is quite lengthy as various protection and deprotection steps are involved. Finally, a follow-up recrystallization of the amine product is often needed in order to improve the enantiomeric excess, and the traces of heavy metals from the catalyst have to be removed to comply with legislative requirements. On the other hand, tremendous advancements in biocatalytic methods for chiral amines have been reached during the past two decades.[4] An industrially applied method is the kinetic resolution of racemic amines via the selective acylation catalysed by a lipase; however, this process is limited by a maximum of 50% theoretical yield.[5] Quantitative yield into enantiopure amines can be obtained through dynamic kinetic resolution (e.g. combining a hydrolase with a metal-catalyst)[6] or deracemisation and desymmetrisation (e.g. combining an amine oxidase with a chemical reducing reagent or artificial metal-enzymes or Pd nanoparticles)[7-16] Besides these earlier established methods, the arsenal of enzymes for asymmetric amine synthesis has been enriched, for instance encompassing wild-type as well as engineered ω-transaminases[17-22] However, the formal reductive amination of carbonyl compounds by ω-transaminases requires supra-stoichiometric amounts of an amine donor (e.g. 5 equivalents of alanine or ca. 10 equivalents of 2-propylamine) and strategies for shifting the unfavorable thermodynamic equilibrium. For example, the use of an additional enzyme for removing the co-product pyruvate[19] or special equipment for the selective evaporation of the co-product acetone.[21] Whilst the use of alternative amine donors has demonstrated to provide an improved thermodynamic driving force, these molecules are expensive and require multi-step chemical synthesis or generate co-products that polymerise and therefore complicate the work-up of the reaction.[23-25] More recently imine reductases have gained interest, but the substrate scope of these enzymes is practically limited to cyclic secondary imines.[26-30] Finally, other enzymes have been applied for chiral amine synthesis such as ammonia lyases,[31-36] Pictet-Spenglerases,[37-40] berberine bridge enzymes[41-43] and engineered P450 monooxygenases.[44-46]

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Electronic Supplementary Information (ESI) available: full list of the substrates with related structures, detailed descriptions of the expression and purification of the enzymes, full data sets and explanation for the biocatalytic reactions, NMR spectra, GC analytical methods and data as well as GC traces. See DOI: 10.1039/x0xx00000x

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However, all these last mentioned classes of enzymes are active on rather specific, yet valuable, families of substrates.

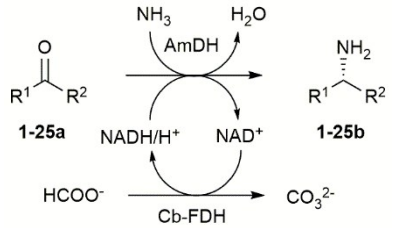
In this context, amine dehydrogenases (AmDHs) are a new class of enzymes that possess tremendous potential for the development of the next generation of processes for the synthesis of α-chiral amines.[47] The applicability of this class of enzymes in organic synthesis has been demonstrated in our notable biocatalytic dual-enzyme hydrogen-borrowing amination of alcohols.[48,49] In general, AmDHs catalyse the reductive amination of ketone and aldehyde substrates using NAD(P)H as the hydride source. The study and exploitation of AmDHs for biocatalytic processes is, however, underdeveloped. In particular, only one report on a natural occurring amine dehydrogenase has been reported about two decades ago but experiments have not been reproduced again.[50] As a consequence, a narrow panel of AmDHs have been created recently through protein engineering starting from wild-type amino acid dehydrogenases as scaffold[51-53] or DNA shuffling of first generation variants.[54] Although initial reaction rates have been determined for the amination of a limited number of ketones, a systematic investigation on the substrate acceptance, optimal reaction conditions as well as chemo- and stereoselectivity of the known AmDHs has not been undertaken until to date. This works aims at providing this knowledge and at showing the potential of AmDHs for the efficient asymmetric synthesis of α-chiral amines.

Results and discussion

Optimisation of the reaction conditions

In our previous study the most elevated reaction rates for the reductive amination of (*para*-fluorophenyl)acetone (**1a**), catalysed by the AmDH variant originated from the wild-type L-phenylalanine dehydrogenase from *Bacillus badius* (for the sake of clarity, referred in this work as Bb-PhAmDH), were observed in ammonium chloride and ammonium formate at pH between 8.2 and 8.8.[48] Besides the elevated activity and stability of the enzymes under the operational reaction conditions, another important and often neglected parameter to be considered for the reductive amination using AmDHs is the stability of the coenzyme NADH/NAD^+ in solution at more basic pH. In fact, in practical biocatalytic reactions, the coenzyme has to be applied in catalytic amounts and recycled at the expense of a sacrificial substrate such as, among the others, formate in combination with formate dehydrogenase (FDH, Scheme 1) or glucose in combination with glucose dehydrogenases (GDH, not shown).

Surprisingly, studies on the stability of nicotinamide coenzymes dissolved in aqueous buffers at different pH and temperature are only a few in the literature. Although NADH is reported to be fairly stable at basic pH, other publications suggest that the decomposition of NADH in solution occurs at ambient temperature in alkali [55] or even at nearly neutral pH with certain type of buffers.[56-58] Hence, in this study, the structural stability of the reduced form of the coenzyme, NADH, was monitored spectrophotometrically (λ 325 nm) over time in buffers at different pH (see Supporting Information S5.1). No significant decrease of the absorbance for NADH was detected in buffer at pH 6.5 for 24 h, indicating that the coenzyme is stable under this condition. At pH 8.8, the absorbance started to diminish smoothly after 5 h, but it was still two thirds of the initial value after 24 h. In contrast, NADH was significantly more unstable at pH 10. In fact, the absorbance decreased linearly and it was halved just after 2 h and depleted after 24 h. Finally, the absorbance of NADH in NaOH (0.1 N) was reduced to 20% of the initial value in 3 minutes and fully depleted within 1 h. These data showed that the ideal pH of 8.2 – 8.8 for the biocatalytic reductive amination might also be a consequence of a diminished stability of the nicotinamide coenzyme at higher values of pH. Hence, ammonium chloride and ammonium formate buffers at pH 8.5 – 8.7 were used for the continuation of our studies.



Scheme 1. Amine dehydrogenases catalyze the reductive amination of ketones and aldehydes (50 mM) to chiral amines. Catalytic amount of nicotinamide coenzyme (1 mM) is applied. The reducing equivalents as well as the nitrogen source are originated from the buffer of the reaction: ammonia/ammonium formate (pH 8.5, 1 M). The only by-products are water and carbonate.

In our previous study, we also determined that ca. 700 mM of ammonium cation/ammonia was required to achieve > 99% conversion, at 30 °C, for substrate **1a** (20 mM). In that case, NAD^+ was applied in catalytic amount (1 mM) and recycled using glucose (60 mM) and a commercial engineered GDH.[48] Nevertheless, the reaction with glucose as cosubstrate generates a stoichiometric amount of gluconic acid, hence reducing the atom economy of the reaction.[59] Furthermore, we had to employ a large amount of GDH (300 U mL^{-1}) for sustaining the amination in ammonium buffer (pH 8.7, > 700 mM), due to its mediocre stability under the reaction conditions. Consequently, in the present study, we envisaged the recycling system based on formate and FDH (recombinant enzyme from *Candida boidinii*)[60] to be the preferable alternative because formate was already present in the reaction buffer as counteranion of the ammonium species. Moreover, these new experiments showed that an extremely low amount of FDH (2.0 – 3.0 U mL^{-1}) was sufficient to obtain a quantitative amination. Therefore, we compared the performance of the reductive amination in the following cases: *i)* glucose/GDH (150 U) as system for the recycling of NADH in ammonium chloride buffer (pH 8.7, 1 M); *ii)* glucose/GDH (150 U) in ammonium formate (pH 8.5, 1 M) and *iii)* formate/FDH (purified, 14 μM equal to 1.5 U) in ammonium formate (pH 8.5, 1 M), (for details, Supporting Information S5.2 and S5.3). This investigation was extended to the three amine

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dehydrogenases that are available in our collection: *i)* the Bb-PhAmDH, *ii)* a variant originated from the L-phenylalanine dehydrogenase from *Rhodococcus sp.* M4 (in this work indicated as Rs-PhAmDH)[53] and *iii)* a previously described chimeric AmDH (in this work indicated as Ch1-AmDH)[48,54] obtained by domain shuffling of the Bb-PhAmDH with a variant from the leucine dehydrogenase from *Bacillus stearothermophilus*. The reductive aminations were carried out with the representative best substrate for each AmDHs, according to our own data and other data from literature (**1a**) for Bb-PhAmDH, 4-phenylbutan-2-one (**24a**) for Rs-PhAmDH and 2-heptanone (**10a**) for Ch1-AmDH). The initially tested reaction conditions were: substrate concentration (20 mM), NAD^+ concentration (1 mM), AmDH concentration (80 - 130 μM) and ammonium buffer (1 M), at 30 °C, for 24 h. Under these reaction conditions it was not possible to reach quantitative conversion (within 21 h) using the glucose/GDH recycling system in ammonium chloride buffer with any of the three AmDHs, despite the use of 3 equivalents of glucose (Table 1, entries 1, 5 and 9). Switching from ammonium chloride to formate and maintaining the same composition of the reaction mixture resulted in quantitative conversion for the amination of substrates **24a** and **10a** with Rs-PhAmDH and Ch1-AmDH (Table 1, entries 6 and 10). However, no improvement was observed in the case of Bb-PhAmDH (Table 1, entry 2). When the third, preferred, option with formate as cosubstrate was tested, all the amination reactions afforded the related product with >99% conversion (Table 1, entries 3, 7 and 11). Hence, we deduced that the employed FDH[60] can recycle NADH more efficiently than the GDH, likely due to its higher stability in ammonium/ammonia buffer at pH 8.5. It is notable that the stereoselective outcome of the reaction was perfect in all the cases (Table 1, > 99% (*R*)).

Aim at understanding the overall catalytic efficiency of the reductive amination under the optimized reaction conditions, we increased gradually the concentration of the substrate up to 50 mM and maintaining the same concentration of AmDH (80 - 130 μM), NAD^+ (1 mM), FDH (14 μM), ammonium buffer (1 M). The Rs-PhAmDH and Ch1-AmDH converted the related substrates **24a** and **10a** (50 mM), respectively, with > 99% conversion and perfect stereoselectivity (> 99% (*R*)) within 21 h (Supporting Information S5.4). In contrast, Bb-PhAmDH turned out to be a less efficient catalyst in this regard as the conversion of **1a** at 50 mM concentration slightly dropped to 88% within 21 h reaction time (Table 1 entry 4 and full data set in Supporting Information S5.4). Finally, the catalyst loading was reduced for the reductive amination employing Rs-PhAmDH and Ch1-AmDH. The amination of **24a** and **10a** (50 mM) proceeded quantitatively within 21 h using 50 μM of Rs-PhAmDH and 32 μM of Ch1-AmDH, respectively. The calculated turnover number (TON)[61] was equal or more than 1000 and therefore comparable, or even superior, to the values previously obtained for the amination of ketones in aqueous buffers with other enzymes such as ω-transaminases.[21,62,63] Moreover, compared to the bio-amination with ω-transaminases, AmDHs do not require an enantiopure amine donor (e.g. L- or D-alanine)[19] and inhibition phenomena are not observed (i.e. inhibition due to cosubstrate alanine and/or coproduct pyruvate).[64-66] As an additional parameter, the concentration of FDH could be lowered to only 9.5 μM, still providing the same conversion.

Table 1. Optimization of the reductive amination using AmDHs. The influence of the composition of the buffer solution, the enzyme loading and the substrate concentration was investigated.

Entry	Enzyme	Substrate	Substrate concentration [mM]	Enzyme concentration [μM]	coenzyme/buffer system	Conversion [%]	ee% (*R*)
1	Bb-PhAmDH	**1a**	20	115	GDH/NH_4Cl	79	>99
2	Bb-PhAmDH	**1a**	20	115	GDH/$HCOONH_4$	76	>99
3	Bb-PhAmDH	**1a**	20	115	Cb-FDH/$HCOONH_4$	>99	>99
4	Bb-PhAmDH	**1a**	50	115	Cb-FDH/$HCOONH_4$	88	>99
5	Rs-PhAmDH	**24a**	20	130	GDH/NH_4Cl	72	>99
6	Rs-PhAmDH	**24a**	20	130	GDH/$HCOONH_4$	>99	>99
7	Rs-PhAmDH	**24a**	20	130	Cb-FDH/$HCOONH_4$	>99	>99
8	Rs-PhAmDH	**24a**	50	50	Cb-FDH/$HCOONH_4$	>99	>99
9	Ch1-AmDH	**10a**	20	80	GDH/NH_4Cl	61	>99
10	Ch1-AmDH	**10a**	20	80	GDH/$HCOONH_4$	99	>99
11	Ch1-AmDH	**10a**	20	80	Cb-FDH/$HCOONH_4$	>99	>99
12	Ch1-AmDH	**24a**	50	32	Cb-FDH/$HCOONH_4$	98	>99

Reaction conditions: NAD+ (1 mM); recycling enzyme (GDH or Cb-FDH) in ammonium chloride buffer (1 M, pH 8.7) or ammonium formate buffer (1.005 M, pH 8.5); reaction volume 0.5 mL, temperature 30 °C, reaction time 24 h; agitation on an orbital shaker 190 rpm.

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Influence of the temperature and time studies

Under the selected reaction conditions (ammonium formate buffer pH 8.5, 1M; substrate concentration 50 mM; NAD^+ 1 mM; FDH 14 µM; varied concentration of AmDHs), we studied the influence of the temperature on the progress of the reductive amination. In fact, we postulated that an increase in the temperature might accelerate the kinetics of the reaction, whereas an excessive temperature may be detrimental for the stability of the enzymes.

The progress for the reductive amination of **1a** (50 mM) using Bb-PhAmDH (46 µM) showed a consistent increase in the reaction rate when the temperature was raised from 20 °C, to 30 °C and finally 40 °C (Figure 1A). It is interesting to note that, for this enzyme, the conversion increased almost linearly over time for every temperature tested. Furthermore, the final conversion (taken after 24 h) at 40 °C doubled the value observed at 20 °C (83% vs. 37%). Nonetheless, the progress of the reaction at 50 °C was worse than at 20 °C, leading to a mediocre conversion of 21 % after 24 h. A further increase of the temperature up to 60 °C provoked a complete loss of the enzymatic activity (Supporting Information S5.6). The lack of conversion at 60 °C cannot be attributed to the deactivation of the FDH or the decomposition of the coenzyme NAD since the reductive amination well performed up to 60 °C with Ch1-AmDH as the biocatalyst (Figure 1C). Furthermore, our data are in agreement with the profile of activity vs. stability of Bb-PhAmDH that shows a rapid denaturation of the enzyme above 50 °C.[52]

The reaction profiles for the reductive amination of substrates **24a** and **10a** (50 mM) with Rs-PhAmDH (48 µM) and Ch1-AmDH (33 µM) respectively, were significantly different from the previous one. For both Rs-PhAmDH and Ch1-AmDH, the conversions increased hyperbolically over time (Figure 1B and 1C). In particular, Rs-PhAmDH is an extremely active enzyme on its preferred substrate **10a**. Considering the first hour of the reaction, wherein the conversion correlated linearly with time, the maximum turnover frequency (TOF) was reached already at 20 °C. In particular the increase of the temperature in the range 20 °C, 30 °C, 40 °C and 50 °C led always to the same conversion after 1 h (varying from 80% - 83%). Quantitative conversion (>98%) of **24a** was obtained at 20 °C and 30 °C within 3 h (Figure 1B and Supporting Information S5.7). The efficiency at 40 °C was slightly lower as the reaction required 5 h to overcome 99% conversion. In contrast, the kinetics of the reaction was negatively influenced at 50 °C with a drop of the catalytic activity after 1 h. In fact, additional 29 h were required to increase the conversion from 83% (after 1h) to 98% (after 30 h) at this temperature. The activity of Rs-PhAmDH was affected at 60 °C as the conversion raised smoothly, reaching a maximum of 93% only after 30 h.

Conversely, the chimeric enzyme Ch1-AmDH performed the amination of **10a** almost equally well in the range of temperatures investigated that spans from 30 °C to 60 °C. In fact, after 5 h the conversion was above 90% for the aminations at 30 °C, 40 °C and 50 °C and reached 82% at 60 °C. The rate of the reductive amination, instead, was lower at 20 °C.

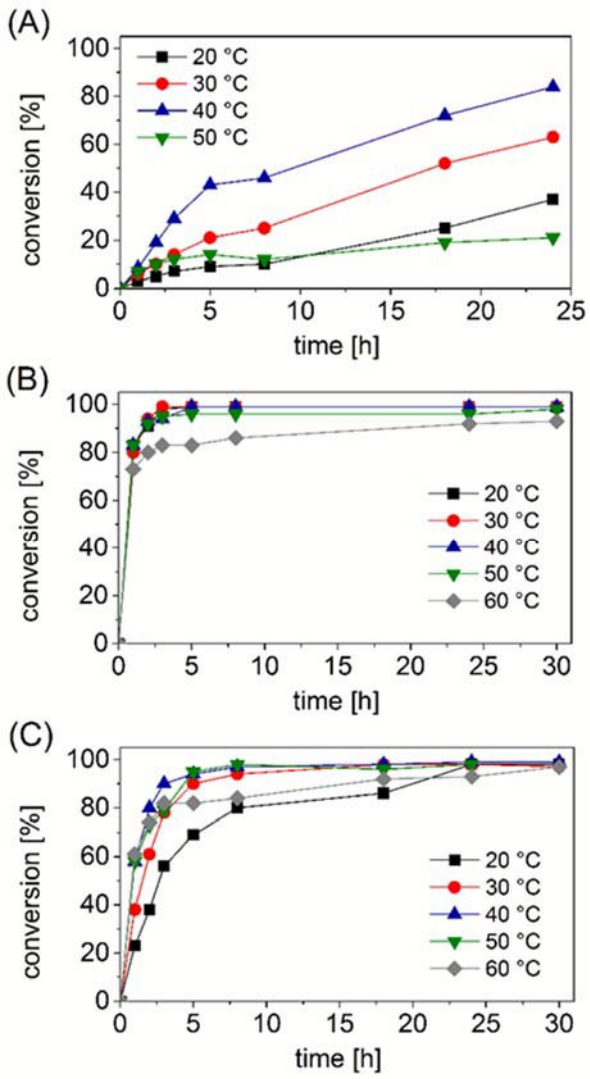

Figure 1. Progress of the reaction versus the time for the reductive amination of: A) **1a** using Bb-PhAmDH (46 µM), B) **24a** using by Rs-PhAmDH (48 µM), and C) **10a** using Ch1-AmDH (33 µM). The study was carried out at different temperatures: 20 °C (black), 30 °C (yellow), and 40 °C (blue), 50 °C (green), and 60 °C (red). Reaction conditions: 0.5 mL, 50 mM substrate, ammonium formate buffer (1.00 M, pH 8.5), FDH (14 µM).

Nevertheless, only with Ch1-AmDH, quantitative conversion (> 98%) was obtained at every temperature from 20 °C to 60 °C at the end of the reaction (30 h, Supporting Information S5.8). The Ch1-AmDH/Cb-FDH dual enzyme system for the reductive amination was instead inapplicable at 70 °C, albeit a mediocre conversion (8%) was observed at this temperature after 18 h. Our observation is in agreement with the previously determined half-life of 40 min for Ch1-AmDH at 70 °C.[54]
Regardless to the degree of conversion, the AmDH enzyme and the substrate employed, the enantiomeric excess was not affected by the reaction time or the temperature. The stereoselectivity remained always perfect (> 99% (*R*)).

Current substrate scope of the reductive amination using AmDHs

The initial reaction rates for the reductive amination of a limited number of carbonyl compounds and the oxidative deamination of a few amines catalysed by AmDHs have been previously measured.[51,52] However, a study describing the substrate scope of these enzymes for the organic synthesis of amines from prochiral ketones and aldehydes has not been published so far. Moreover, the information regarding the stereoselectivity for the amination with AmDHs is limited to a very few compounds. Therefore, in this research, we tested an extensive library of structurally diverse prochiral ketones such as phenylacetone derivatives and phenylacetaldehyde (**1-7a**), aliphatic methylketones and aldehydes (**8-13a**), acetophenone derivatives (**14-19a**) and, finally, a selection of more sterically demanding ketones (**20-25a**) (Figure 2). The substrate concentration was kept at 50 mM, whereas the amount of enzyme and the reaction time was varied in order to achieve the maximum efficiency (i.e. highest ratio [S]/[E] with highest conversion).

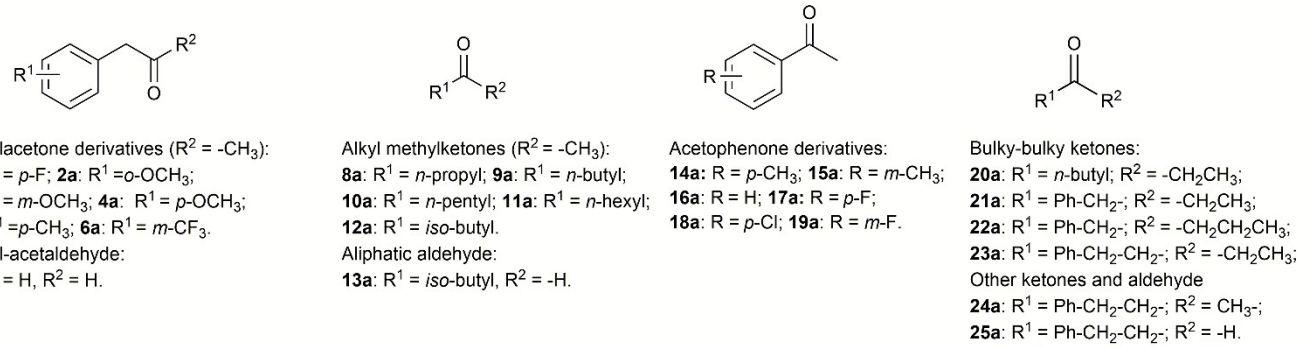

Figure 2. Substrate scope tested by amine dehydrogenases.

First, we examined the family of phenylacetone derivatives (Figure 2, Table 2). It was previously shown that Bb-PhAmDH accepts (*para*-fluorophenyl)acetone (**1a**) as the best substrate.[52] In our independent experiment (Table 2, entry 2), Bb-PhAmDH (50 µM) converted **1a** (50 mM) to 93% of the amine product **1b** within 48 h and perfect stereoselectivity (>99% (*R*)). Hence, it may be logical to assume that Bb-PhAmDH can also be a useful biocatalyst for the amination of other substituted phenylacetones. Indeed, Bb-PhAmDH converted *ortho*-, *meta*- and *para*- methoxy substituted phenylacetone derivatives (**2-4a**), but the conversion was mediocre (from 3% to 21%, Supporting Information Table S7) despite the reaction time was prolonged up to 48 h. Bb-PhAmDH also accepted (*para*-methyl)phenylacetone (**5a**, 7% conversion), whilst (*meta*-trifluoromethyl)phenylacetone (**6a**) was not converted at all (Supporting Information Table S7).

Table 2. Reductive amination of phenyl 2-propanone derivatives and phenyl-acetaldehyde employing AmDHs.

Entry	Nr.	Enzyme	Enzyme concentration [µM]	Time [h]	Conversion substrate [%]	ee% (*R*)
1	**1a**	Ch1-AmDH	30	24	93	>99
2	**1a**	Bb-PhAmDH	50	48	93	>99
3	**2a**	Ch1-AmDH	130	48	>99	>99
4	**3a**	Rs-PhAmDH	50	24	98	>99
5	**4a**	Rs-PhAmDH	50	24	>99	>99
6	**5a**	Rs-PhAmDH	130	48	98	>99
7	**6a**	Rs-PhAmDH	130	48	98	>99
8	**7a**	Bb-PhAmDh	50	48	34	n.a.

Reaction conditions: substrate 50 mM, AmDH 30-130 µM, Cb-FDH 14 µM, ammonium formate buffer (1.005 M, pH 8.5), T 30°C, agitation orbital shaker 190 rpm.
n.a not applicable

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Surprisingly the chimeric enzyme Ch1-AmDH, known to be active on aliphatic ketones[48] and acetophenone derivatives,[54] was a superior catalyst for the amination of **1a**. Compared to the amination with Bb-PhAmDH, Ch1-AmDH afforded the product **1b** with the same conversion but in half of the reaction time (24 h) and at a significantly lower enzyme loading (30 μM, Table 2, entry 1). Ch1-AmDH was also the best catalyst for the amination of **2a** that reached quantitative conversion in 48 h (Table 2, entry 3).

The third AmDH from this study, Rs-PhAmDH, has been developed and tested only for the reductive amination of 4-phenylbutan-2-one as substrate (**24a**, Table 5, entry 6).[53] In this study we disclosed that Rs-PhAmDH has a much wider substrate scope than expected and reported before. Interestingly Bb-PhAmDH and Rs-PhAmDH were engineered from their respective parent wild-type phenylalanine dehydrogenases by mutating similar positions in the active site (K78, N277 for Bb-PhAmDH and K66, S149 and N262 for Rs-PhAmDH). In fact, in the wild-type enzymes, the side chains of the K and N residues located in the active site generate hydrogen bond interactions with the oxygens of carboxylic moiety of the natural substrate. Furthermore, the two parent phenylalanine dehydrogenases have 34% identity. Despite these similarities, we have shown before (Figure 1) that the two variants have a different thermal stability and reactivity measured on their respective preferred substrate: Rs-PhAmDH can operate efficiently at 60 °C, whereas Bb-PhAmDH is completely deactivated above 50 °C. Table 2 reveals that Rs-PhAmDH is a superior biocatalyst than Bb-PhAmDH also in terms of substrate acceptance. In fact, Rs-PhAmDH was the optimal AmDH for the conversion of **3a**, **4a**, **5a** and **6a** affording the related amines in elevated conversion (more or equal to 98%) and excellent stereoselectivity (> 99% (*R*)), (Table 2, entries 4-7). On the other hand, Bb-PhAmDH proved to be still a useful catalyst for the amination of phenylacetaldehyde (**7a**), (Table 2, entry 8). In contrast, both Rs-PhAmDH and Ch1-AmDH were inactive on this type of aldehyde.

A similar scenario was revealed also in the case of the reductive amination of aliphatic ketones and aldehydes (Figure 2, Table 3). Initially, we investigated the less sterically demanding alkyl methylketones. Ch1-AmDH and Rs-PhAmDH were the most active biocatalysts on this family of substrates. Substrates (50 mM) bearing a medium length linear chain such as 2-hexanone (**9a**) and 2-heptanone (**10a**) were efficiently converted by Ch1-AmDH (30 μM) within 24 h (Table 3, entries 2 and 3). Ketones bearing a shorter linear chain (2-pentanone, **8a**) or a branched chain (4-methylpentan-2-one, **12a**) were converted with lower turnover numbers by Ch1-AmDH and, therefore, an elevated concentration of enzyme was necessary (130 μM, Table 3, entries 1 and 7). A ketone bearing a longer chain such as 2-octanone (**11a**) was a challenging substrate for Ch1-AmDH (Table 3, entry 5). Fortunately, Rs-PhAmDH seems to be a complementary enzyme in this respect: Rs-PhAmDH (50 μM) aminated 2-octanone (50 mM, **11a**) with elevated conversion (93%, table 3, entry 6). Notably, the enantiomeric excess was perfect for all the reductive aminations (>99% (*R*)).

Interestingly, Ch1-AmDH and Rs-PhAmDH rapidly converted 3-methylbutanal (**13a**, Table 3, entry 9 and 10) even though both enzymes were inactive on the arylaliphatic aldehyde **7a**. These data demonstrate that all the known AmDHs are capable of converting ketones and aldehydes although with different substrate scope.

Table 3. Reductive amination of aliphatic methyl ketones and aldehydes employing AmDHs.

Entry	Nr.	Enzyme	Enzyme concentration [μM]	Time [h]	Conversion substrate [%]	ee% (*R*)
1	**8a**	Ch1-AmDH	130	48	75	>99
2	**9a**	Ch1-AmDH	30	24	92	>99
3	**10a**	Ch1-AmDH	30	24	98	>99
4		Rs-PhAmDH	50	24	99	>99
5	**11a**	Ch1-AmDH	30	48	50	>99
6		Rs-PhAmDH	50	48	93	>99
7	**12a**	Ch1-AmDH	130	48	96	>99
8		Rs-PhAmDH	130	48	91	>99
9	**13a**	Ch1-AmDH	30	24	>99	n.a

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10	Rs-PhAmDH	130	48	99	n.a

Reaction conditions: 0.5 mL, 50 mM substrate, 30-130 μM enzyme, ammonium formate buffer (1.005 M, pH 8.5), 14 μM Cb-FDH.
n.a not applicable

Acetophenone derivatives (Figure 2) are discussed separately as the type and position of the substituents on the phenyl ring affect considerably the reactivity of these substrates, due to the existence of resonance and field effects.[67] This phenomenon is not always considered for enzymatic reactions, for which a low catalytic rate is often solely - and sometimes misleadingly - interpreted as the result of a poor affinity of the substrate to the active site of the enzyme or the intrinsic low enzymatic turnovers (k_{cat}). In contrast, a number of publications on the reactivity of acetophenone derivatives with other oxidoreductases such as alcohol dehydrogenases showed that resonance and field effects can play a major, and sometimes unexpected, role.[68-70]

In this regard, the chimeric Ch1-AmDH and Rs-PhAmDH turned out to be the most efficient enzymes for the amination of acetophenone derivatives (Table 4 and Supporting Information Table S8). The reductive amination of these substrates (50 mM) required generally a higher amount of enzyme (up to 130 μM) for obtaining moderate conversions. *para*-Methylacetophenone (**14a**) was the less converted substrate (9%, table 4, entry 1) whereas *meta*-fluoroacetophenone (**19a**) afforded the most elevated conversion (43%, table 4, entry 6). Along the series of acetophenone derivatives reported in table 4, the *para*-methyl substituent in **14a** is the one possessing the most intense electron donating character, while the *meta*-fluoro in **19a** is the strongest electron withdrawing substitutions. Furthermore *para*-hydroxy acetophenone, whose hydroxyl substituent has an even higher electron donating impact than a *para*-methyl,[67,68] was not converted at all (Supporting Information Table S6).

Although not based on a more rigorous determination of the initial reaction rates, this initial observation may suggest that the enzymatic reductive amination with AmDHs is favored by the delocalization of a higher partial positive charge on the reactive carbonyl carbon during the reaction. This assumption is corroborated by the fact that the same influence of the substituents was reveled for the reduction of acetophenone to alcohols with alcohol dehydrogenases (ADH). In fact, both ADHs and AmDHs belong to the class of the oxidoreductases (EC1) and share the same cofactor (NAD) and a similar reaction mechanism.[68-71] Additionally, none of the AmDHs from this study accepted *ortho*-methyl acetophenone as a substrate, indicating that steric effect might also play a significant role in the enzymatic reductive amination (Supporting Information Table S6).

Table 4. Reductive amination of acetophenone derivatives employing AmDHs.

Entry	Nr.	Enzyme	Enzyme concentration [μM]	Time [h]	Conversion substrate [%]	ee% (*R*)
1	**14a**	Ch1-AmDH	130	48	9	>99
2	**15a**	Ch1-AmDH	50	48	39	>99
3	**16a**	Ch1-AmDH	130	48	34	>99
4	**17a**	Ch1-AmDH	130	48	22	>99
5	**18a**	Rs-PhAmDH	100	48	33	>99
6	**19a**	Ch1-AmDH	130	48	43	>99

Reaction conditions: 0.5 mL, 50 mM substrate, 30-130 μM enzyme, ammonium formate buffer (1.005 M, pH 8.5), 14 μM Cb-FDH.

Finally, the reactivity of the AmDHs was investigated on more sterically demanding substrates. Bulky-bulky ketones (**20-23a**) (Figure 2) are challenging substrates for the amination catalysed by other enzymes such as ω-transaminases. Natural occurring ω-transaminases, that are suitable for applications in biotechnology, seem to accept mainly ketones possessing a bulky group on one side and a small methyl group on the other side.[19,20,72-77] Alternatively, wild-type ω-transaminases are active on bicyclic ketones.[78] To the best of our knowledge, only one scaffold from a wild-type ω-transaminase was engineered for accepting bulky-bulky substrates.[21,76]

Thus, we were intrigued to understand whether AmDHs also share the same limitation in relation to the range of ketones that can be converted. Interestingly, all the tested bulky-bulky ketones bearing the carbonyl moiety conjugated with the phenyl ring such as 1-phenylpropan-1-one, 1-phenylbutan-1-one and 1-phenylpentan-1-one were either not accepted or afforded mediocre conversions (Supporting Information Table S6). Conversely, when the carbonyl moiety was positioned further from the aromatic ring, the ketones (50 mM) were converted efficiently by Rs-PhAmDH (90-130 μM). For instance, 1-phenylbutan-2-one (**21a**), 1-phenylpentan-2-one (**22a**) and 1-phenylpentan-3-one (**23a**) afforded the amine product up to >99% conversion and perfect stereoselectivity (>99% (*R*)), (Table 5, entries 3, 4 and 5). In contrast, Bb-PhAmDH and Ch1-AmDH were poorly active on these substrates or not active at all (Supporting Information Table S7 and S8).

As an example of an aliphatic and more sterically demanding ketone, 3-heptanone (**20a**) was tested. In this case Ch1-AmDH

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was the most active enzyme (Table 5, entry 1) in agreement with the general trend for this enzyme (i.e. elevated conversions for aliphatic ketones, table 3).

For the sake of completeness, 4-phenyl-butan-2-one (**24a**) and an aldehyde such as hydrocinnamaldehyde (**25a**) were assayed for the reductive amination. Rs-PhAmDH (50 µM) was capable of quantitatively aminating **24a** at 50 mM scale within 24 h (Table 5, entry 6) with more than 99% ee. Rs-PhAmDH was also the optimal biocatalyst for the reduction of the aldehyde **25a** (96%, Table 5, entry 7). The Ch1-AmDH also quantitatively converted **25a**. However, surprisingly, besides the desired product 3-phenylpropan-1-amine **25b** (70%), the hydrocinnamic alcohol was obtained as side-product (30%). To the best of our knowledge, this is the only documented case wherein an amine dehydrogenase reduces a carbonyl compound leading to the formation of significant amount of alcohol as by-product.

Table 5. Reductive amination of bulky-bulky ketones employing AmDHs.

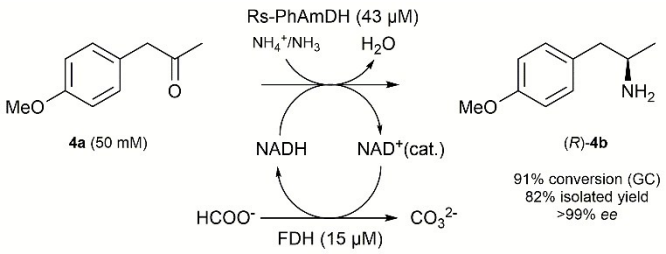
Entry	Nr.	Enzyme	Enzyme concentration [µM]	Time [h]	Conversion substrate [%]	ee% (*R*)
1	**20a**	Ch1-AmDH	90	48	57	>99
2		Rs-PhAmDH	100	48	32	>99
3	**21a**	Rs-PhAmDH	130	48	>99	>99
4	**22a**	Rs-PhAmDH	100	48	71	>99
5	**23a**	Rs-PhAmDH	100	48	87	>99
6	**24a**	Rs-PhAmDH	50	24	>99	>99
7	**25a**	Rs-PhAmDH	100	48	96	n.a.

Reaction conditions: 0.5 mL, 50 mM substrate, 30-130 µM enzyme, ammonium formate buffer (1.005 M, pH 8.5), 14 µM Cb-FDH.
n.a not applicable

Representative biocatalytic reductive amination in preparative scale

In order to ascertain that our optimized reaction conditions at analytical scale are applicable in preparative scale biocatalytic reactions, we attempted the asymmetric amination of (*para*-methoxy)phenylacetone (**4a,** 208 mg) using Rs-AmDH (Scheme 2).

The reaction was successfully performed with ca. 50 mM substrate (208 mg), 43 µM of Rs-AmDH, 15 µM FDH, 1 mM of NAD^+ in ammonium formate buffer (1 M, pH 8.5) at the temperature of 30 °C. The substrate was converted into the optically pure amine (*R*)-**4b** with 91% conversion and >99% ee within 24 h reaction time. After work-up, (*R*)-**4b** was isolated with 82% yield. The purity and authenticity of the product was confirmed by NMR and GC (Supporting Information S6). Amine (*R*)-**4b** was recently reported as an important building block for the synthesis of tacrine–selegiline hybrids that possess cholinesterase and monoamine oxidase inhibition activities for the treatment of Alzheimer's disease.[79]



Scheme 2. Preparative reductive amination of (*para*-methoxy phenyl)acetone (**4a**, 208 mg) using Rs-PhAmDH. Reaction conditions: ammonium formate buffer (1 M, pH 8.5), T 30 °C, agitation on an orbital shaker (190 rpm), reaction time 24 h.

Additionally, amine (R)-**4b** is the optically active intermediate for the synthesis of the blockbuster pharmaceutical formoterol [80, 81] sold under various trade names including Foradile and Oxeze.

Conclusions

In this work, we have shown that amine dehydrogenases hold the premise for the development of the next generation of chemical processes for the synthesis of α-chiral amines.[47] The applicability of amine dehydrogenases was demonstrated for the asymmetric amination of a range of structurally diverse prochiral ketones and aldehydes. The most suitable enzyme, the optimal catalyst loading and reaction times were determined for each substrate from this study. The majority of the substrates tested were aminated with elevated conversion and elevated TONs; moreover, all the α-chiral amine products were obtained with perfect optical purity (>99% *R*). This fact is of particular interest as ω-transaminases capable of giving access to (*R*)-configured amines are rare in nature. Only a very few (*R*)-selective ω-transaminases have been discovered [82,83] and only one enzyme was engineered to convert a specific bulky-bulky ketone with elevated stereoselectivity.[21,62]From this study, it became also evident that a single amine dehydrogenase capable of accepting a large variety of substrates is not available. For instance, the chimeric Ch1-AmDH is very active on aliphatic ketones and acetophenone derivatives whereas Rs-PhAmDH is an excellent biocatalyst for the amination of phenylacetone derivatives and more sterically demanding ketones. Additionally, the influence of the temperature on the biocatalytic reductive amination with

the three AmDHs variants was determined. In particular, a pH in the range of 8.2 – 8.8 is preferable as a consequence of the improved stability of the enzymes (AmDHs and FDH) and, especially, of the coenzyme (NAD). Finally, the optimised reaction parameters were applied to the synthesis of an important drug precursor on a scale of multi-hundreds of milligrams.

The reductive amination catalyzed by amine dehydrogenases operating in tandem with formate dehydrogenase possesses an elevated atom efficiency as the ammonium formate buffer is simultaneously the source nitrogen and reducing equivalents. Stoichiometric inorganic carbonate is the sole by-product. Additionally, the reductive amination catalyzed by AmDH / FDH is performed under atmospheric pressure. In contrast, the amination catalysed by ω-transaminases in industrial scale, in aqueous system and using 2-propylamine (ca. 10 equivalents) as amine donor requires a removal of the co-product acetone by employing reduced pressure and nitrogen sweep. Hence, besides the requirement of supra-stoichiometric amount of 2-propylamine and the generation of one equivalent of acetone, a significant amount of energy is consumed to operate at low pressure. We expect therefore that the herein described reductive amination will be applied increasingly in the future when new amine dehydrogenase variants possessing expanded substrate scope and complementary stereoselectivity will become available. Co-expression of AmDH/FDH in a single host organism and / or co-immobilisation of both enzymes will enhance the practical applicability of the biocatalytic process.[84-86]

Experimental

General

The AmDH variants and the Cb-FDH were expressed as recombinant enzymes in *E. coli* BL21 (DE3). Details are reported in the Supporting Information paragraph S4.

General optimized procedure for the biocatalytic reductive amination on analytical scale

The reactions were conducted in ammonium formate buffer (1.005 M, pH 8.5, final volume 0.5 mL) containing NAD^+ (final concentration 1 mM). Enzymes AmDH (30 - 130 μM) and Cb-FDH (14.1 μM) and the substrate (50 mM) were also added. The reactions were run at 30 °C in an incubator for 21 hours (190 rpm) or longer if required in selected cases. Work-up was performed by the addition of KOH (100 μL, 10 M) followed by the extraction with dichloromethane (600 μL). The water layer was removed after centrifugation and the organic layer was dried with $MgSO_4$. Conversion was determined by GC with an Agilent DB-1701 column. The enantiomeric excess of the amine product was determined after derivatization to acetamido. Derivatization of the samples was performed by adding 4-dimethylaminopyridine in acetic anhydride (40 μL of stock solution 50 mg mL^{-1}). The samples were shaken in an incubator at RT for 30 minutes. Afterwards water (300 μL) was added and the samples were shaken for additional 30 minutes. After centrifugation, the organic layer was dried with $MgSO_4$. Enantiomeric excess was determined by GC with a Variant Chiracel DEX-CB column. Details on the GC analysis and methods are reported in the Supporting Information paragraph S7.

Preparative biocatalytic reductive amination for the synthesis of (*R*)-4b

NAD^+ (final concentration 1 mM) was dissolved in ammonium formate buffer (30 mL, 1.005 M, pH 8.5) in a 50 mL round bottom flask. Ketone **4a** (195 μL, 1.27 mmol), FDH (233 μL from stock solution 80.7 mg mL^{-1}, final concentration 15 μM,), and Rs-PhAmDH (1.02 mL from stock solution 48.8 mg mL^{-1}, final concentration 43 μM) were added and the reaction mixture was shaken in an incubator at 30 °C for 24 hours. The progress of the reaction was monitored by TCL and GC. When quantitative conversion was achieved, the reaction mixture was acidified to pH 2-4 via addition of HCl (1M). The water layer was washed with methyl *tert*-butyl ether (15 mL) to remove any possible remaining ketone starting material. The pH of the water phase was increased to basic pH via KOH (10 M) while cooling in an ice bath. The water layer was extracted with methyl *tert*-butyl ether (2 x 15 mL). The organic fractions containing the amine product were combined and dried over $MgSO_4$. After filtration and evaporation of the solvent, the product was obtained in pure form. Column chromatography was not required. The authenticity of the product was confirmed by ^{1}H-NMR (Supporting Information Figure S4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 3.81 (s, 3H), 3.13 (m, 1H), 2.67 (dd, *J* = 13.4, 5.3 Hz, 1H), 2.47 (dd, *J* = 13.4, 8.1 Hz, 1H), 1.12 (d, *J* = 6.3 Hz, 3H).

Keywords

amine dehydrogenases, stereoselective reductive amination, biocatalysis,α-chiral amine synthesis

Competing financial interest

The authors declare to have no competing interests, or other interests that might be perceived to influence the results and/or discussion reported in this article.

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Notes and references

1. H. A. Wittcoff, B. G. Rueben and J. S. Plotkin, *Industrial Organic Chemicals, 2nd ed.*, Wiley-Iterscience, New York, 2004.

2. T. C. Nugent, ed., *Chiral amine synthesis: Methods, Developments and Applications*, Wiley-VCH, Weinheim, 2010.
3. T. C. Nugent and M. El-Shazly, *Adv. Synth. Catal.*, 2010, 352, 753-819.
4. S. K. Au, J. Groover, B. D. Feske and A. S. Bommarius, in *Organic Synthesis Using Biocatalysis, 1st Edition*, Elsevier, 2015, pp. 187-212.
5. F. Balkenhohl, K. Ditrich, B. Hauer and W. Ladner, *J. Prakt. Chem.*, 1997, 339, 381-384.
6. Y. Kim, J. Park and M.-J. Kim, *ChemCatChem*, 2011, 3, 271-277.
7. G. Li, J. Ren, P. Yao, Y. Duan, H. Zhang, Q. Wu, J. Feng, P. C. K. Lau and D. Zhu, *ACS Catal.*, 2014, 4, 903-908.
8. N. J. Turner, *Chem. Rev.*, 2011, 111, 4073-4087.
9. D. Ghislieri, A. P. Green, M. Pontini, S. C. Willies, I. Rowles, A. Frank, G. Grogan and N. J. Turner, *J. Am. Chem. Soc.*, 2013, 135, 10863-10869.
10. C. J. Dunsmore, R. Carr, T. Fleming and N. J. Turner, *J. Am. Chem. Soc.*, 2006, 128, 2224-2225.
11. R. Carr, M. Alexeeva, A. Enright, T. S. Eve, M. J. Dawson and N. J. Turner, *Angew. Chem. Int. Ed.*, 2003, 42, 4807-4810.
12. M. Alexeeva, A. Enright, M. J. Dawson, M. Mahmoudian and N. J. Turner, *Angew. Chem. Int. Ed.*, 2002, 114, 3309-3312.
13. V. Kohler, Y. M. Wilson, M. Durrenberger, D. Ghislieri, E. Churakova, T. Quinto, L. Knorr, D. Haussinger, F. Hollmann, N. J. Turner and T. R. Ward, *Nat. Chem.*, 2013, 5, 93-99.
14. T. Li, J. Liang, A. Ambrogelly, T. Brennan, G. Gloor, G. Huisman, J. Lalonde, A. Lekhal, B. Mijts, S. Muley, L. Newman, M. Tobin, G. Wong, A. Zaks and X. Zhang, *J. Am. Chem. Soc.*, 2012, 134, 6467-6472.
15. J. M. Foulkes, K. J. Malone, V. S. Coker, N. J. Turner and J. R. Lloyd, *ACS Catal.*, 2011, 1, 1589-1594.
16. V. Kohler, K. R. Bailey, A. Znabet, J. Raftery, M. Helliwell and N. J. Turner, *Angew. Chem. Int. Ed.*, 2010, 49, 2182-2184.
17. M. Fuchs, J. E. Farnberger and W. Kroutil, *Eur. J. Org. Chem.*, 2015, 6965-6982.
18. S. Mathew and H. Yun, *ACS Catal.*, 2012, 2, 993-1001.
19. D. Koszelewski, K. Tauber, K. Faber and W. Kroutil, *Trends Biotechnol.*, 2010, 28, 324-332.
20. D. Koszelewski, I. Lavandera, D. Clay, G. M. Guebitz, D. Rozzell and W. Kroutil, *Angew. Chem. Int. Ed.*, 2008, 47, 9337-9340.
21. C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman and G. J. Hughes, *Science*, 2010, 329, 305-309.
22. A. Cuetos, M. Garcia-Ramos, E. M. Fischereder, A. Diaz-Rodriguez, G. Grogan, V. Gotor, W. Kroutil and I. Lavandera, *Angew. Chem. Int. Ed.*, 2016, 55, 3144-3147.
23. B. Wang, H. Land and P. Berglund, *Chem. Commun,*, 2013, 49, 161-163.
24. A. P. Green, N. J. Turner and E. O'Reilly, *Angew. Chem. Int. Ed.*, 2014, 53, 10714-10717.
25. L. Martínez-Montero, V. Gotor, V. Gotor-Fernández and I. Lavandera, *Adv. Synth. Catal.*, 2016, 358, 1618-1624.
26. J. H. Schrittwieser, S. Velikogne and W. Kroutil, *Adv. Synth. Catal.*, 2015, 357, 1655-1685.
27. D. Wetzl, M. Berrera, N. Sandon, D. Fishlock, M. Ebeling, M. Muller, S. Hanlon, B. Wirz and H. Iding, *ChemBioChem*, 2015, 16, 1749-1756.
28. H. Li, Z.-J. Luan, G.-W. Zheng and J.-H. Xu, *Adv. Synth. Catal.*, 2015, 357, 1692-1696.
29. S. Hussain, F. Leipold, H. Man, E. Wells, S. P. France, K. R. Mulholland, G. Grogan and N. J. Turner, *ChemCatChem*, 2015, 7, 579-583.
30. F. Leipold, S. Hussain, D. Ghislieri and N. J. Turner, *ChemCatChem*, 2013, 5, 3505-3508.
31. F. Parmeggiani, S. L. Lovelock, N. J. Weise, S. T. Ahmed and N. J. Turner, *Angew. Chem. Int. Ed.*, 2015, 54, 4608-4611.
32. N. J. Weise, F. Parmeggiani, S. T. Ahmed and N. J. Turner, *J. Am. Chem. Soc.*, 2015, 137, 12977-12983.
33. S. L. Lovelock, R. C. Lloyd and N. J. Turner, *Angew. Chem. Int. Ed.*, 2014, 53, 4652-4656.
34. B. Wu, W. Szymanski, G. G. Wybenga, M. M. Heberling, S. Bartsch, S. de Wildeman, G. J. Poelarends, B. L. Feringa, B. W. Dijkstra and D. B. Janssen, *Angew. Chem. Int. Ed.*, 2012, 51, 482-486.
35. N. J. Turner, *Curr. Opin. Chem. Biol.*, 2011, 15, 234-240.
36. B. J. Verkuijl, W. Szymanski, B. Wu, A. J. Minnaard, D. B. Janssen, J. G. de Vries and B. L. Feringa, *Chem. Commun,*, 2010, 46, 901-903.
37. E.-M. Fischereder, D. Pressnitz and W. Kroutil, *ACS Catal.*, 2016, 6, 23-30.
38. F. Wu, H. Zhu, L. Sun, C. Rajendran, M. Wang, X. Ren, S. Panjikar, A. Cherkasov, H. Zou and J. Stockigt, *J. Am. Chem. Soc.*, 2012, 134, 1498-1500.
39. T. Pesnot, M. C. Gershater, J. M. Ward and H. C. Hailes, *Adv. Synth. Catal.*, 2012, 354, 2997-3008.
40. A. Bonamore, I. Rovardi, F. Gasparrini, P. Baiocco, M. Barba, C. Molinaro, B. Botta, A. Boffi and A. Macone, *Green Chem.*, 2010, 12, 1623.
41. J. H. Schrittwieser, B. Groenendaal, V. Resch, D. Ghislieri, S. Wallner, E. M. Fischereder, E. Fuchs, B. Grischek, J. H. Sattler, P. Macheroux, N. J. Turner and W. Kroutil, *Angew. Chem. Int. Ed.*, 2014, 53, 3731-3734.
42. J. H. Schrittwieser, V. Resch, J. H. Sattler, W. D. Lienhart, K. Durchschein, A. Winkler, K. Gruber, P. Macheroux and W. Kroutil, *Angew. Chem. Int. Ed.*, 2011, 50, 1068-1071.
43. A. Winkler, A. Lyskowski, S. Riedl, M. Puhl, T. M. Kutchan, P. Macheroux and K. Gruber, *Nat. Chem. Biol.*, 2008, 4, 739-741.
44. T. K. Hyster, C. C. Farwell, A. R. Buller, J. A. McIntosh and F. H. Arnold, *J. Am. Chem. Soc.*, 2014, 136, 15505-15508.
45. J. A. McIntosh, P. S. Coelho, C. C. Farwell, Z. J. Wang, J. C. Lewis, T. R. Brown and F. H. Arnold, *Angew. Chem. Int. Ed.*, 2013, 52, 9309-9312.
46. E. M. Brustad, *Nat. Chem. Biol.*, 2014, 10, 170-171.
47. B. M. Nestl, S. C. Hammer, B. A. Nebel and B. Hauer, *Angew. Chem. Int. Ed.*, 2014, 53, 3070-3095.
48. F. G. Mutti, T. Knaus, N. S. Scrutton, M. Breuer and N. J. Turner, *Science*, 2015, 349, 1525-1529.
49. J. B. Wang and M. T. Reetz, *Nat. Chem.*, 2015, 7, 948-949.
50. N. Itoh, C. Yachi and T. Kudome, *J. Mol. Cat. B: Enz.*, 2000, 10, 281-290.
51. M. J. Abrahamson, E. Vazquez-Figueroa, N. B. Woodall, J. C. Moore and A. S. Bommarius, *Angew. Chem. Int. Ed.*, 2012, 51, 3969-3972.
52. M. J. Abrahamson, J. W. Wong and A. S. Bommarius, *Adv. Synth. Catal.*, 2013, 355, 1780-1786.
53. L. J. Ye, H. H. Toh, Y. Yang, J. P. Adams, R. Snajdrova and Z. Li, *ACS Catal.*, 2015, 5, 1119-1122.
54. B. R. Bommarius, M. Schürmann and A. S. Bommarius, *Chem. Commun.*, 2014 50, 14953-14955.
55. O. H. Lowry, J. V. Passonneau and M. K. Rock, *J. Biol. Chem.*, 1961, 236, 2756-2759.
56. S. G. A. Alivisatos, F. Ungar and G. Abraham, *Nature*, 1964, 203, 973-975.
57. S. G. A. Alivisatos, F. Ungar and G. J. Abraham, *Biochemistry*, 1965, 4, 2616-2630.
58. L. Rover, J. C. B. Fernandes, G. d. O. Neto, L. T. Kubota, E. Katekawa and S. l. H. P. Serrano, *Anal. Biochem.*, 1998, 260, 50-55.
59. R. A. Sheldon, I. Arends and U. Hanefeld, *Green Chemistry and Catalysis*, Wiley-VCH Verlag GmbH & Co. KGaA, 2007.
60. H. Schutte, J. Flossdorf, H. Sahm and M.-R. Kula, *Eur. J. Biochem.*, 1976, 62, 151-160.
61. *The TON is here defined as the number of molecules of substrate converted per molecule of enzyme in the given reaction time.*
62. F. G. Mutti, J. Sattler, K. Tauber and W. Kroutil, *ChemCatChem*, 2011, 3, 109-111.
63. F. G. Mutti and W. Kroutil, *Adv. Synth. Catal.*, 2012, 354, 3409-3413.
64. J. S. Shin and B. G. Kim, *Biotechnol. Bioeng.*, 2002, 77, 832-837.
65. J.-S. Shin and B.-G. Kim, *Biosci. Biotechnol. Biochem.*, 2001, 65, 1782-1788.
66. J.-S. Shin and B.-G. Kim, *Biotechnol. Bioeng.*, 1999, 65, 206-211.
67. M. B. Smith and J. March, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, Sixth Edition*, John Wiley & Sons, Inc., 2007.
68. M. Vogl, R. Kratzer, B. Nidetzky and L. Brecker, *Org. Biomol. Chem.*, 2011, 9, 5863-5870.
69. M. L. Contente, I. Serra, L. Palazzolo, C. Parravicini, E. Gianazza, I. Eberini, A. Pinto, B. Guidi, F. Molinari and D. Romano, *Org. Biomol. Chem.*, 2016, 14, 3404-3408.
70. D. Zhu, B. E. Rios, J. D. Rozzell and L. Hua, *Tetrahedron: Asymmetry*, 2005, 16, 1541-1546.
71. C. Rodriguez, W. Borzecka, J. H. Sattler, W. Kroutil, I. Lavandera and V. Gotor, *Org. Biomol. Chem.*, 2014, 12, 673-681.
72. R. C. Simon, B. Grischek, F. Zepeck, A. Steinreiber, F. Belaj and W. Kroutil, *Angew. Chem. Int. Ed.*, 2012, 51, 6713-6716.
73. E. O'Reilly, C. Iglesias, D. Ghislieri, J. Hopwood, J. L. Galman, R. C. Lloyd and N. J. Turner, *Angew. Chem. Int. Ed.*, 2014, 53, 2447-2450.
74. D. Koszelewski, M. Göritzer, D. Clay, B. Seisser and W. Kroutil, *ChemCatChem*, 2010, 2, 73-77.
75. F. G. Mutti, C. S. Fuchs, D. Pressnitz, N. G. Turrini, J. H. Sattler, A. Lerchner, A. Skerra and W. Kroutil, *Eur. J. Org. Chem.*, 2012, 1003-1007.
76. F. G. Mutti, C. S. Fuchs, D. Pressnitz, J. H. Sattler and W. Kroutil, *Adv. Synth. Catal.*, 2011, 353, 3227-3233.
77. S. Schätzle, F. Steffen-Munsberg, A. Thontowi, M. Höhne, K. Robins and U. T. Bornscheuer, *Adv. Synth. Catal.*, 2011, 353, 2439-2445.
78. D. Pressnitz, C. S. Fuchs, J. H. Sattler, T. Knaus, P. Macheroux, F. G. Mutti and W. Kroutil, *ACS Catal.*, 2013, 3, 555-559.
79. C. Lu, Q. Zhou, J. Yan, Z. Du, L. Huang and X. Li, *Eur. J. Med. Chem.*, 2013, 62, 745-753.
80. F. Campos, M. P. Bosch and A. Guerrero, *Tetrahedron: Asymmetry*, 2000, 11, 2705-2717.
81. G. P. Anderson, *Life Sci.*, 1993, 52, 2145-2160.
82. A. Iwasaki, Y. Yamada, N. Kizaki, Y. Ikenaka and J. Hasegawa, *Appl. Microbiol. Biotechnol.*, 2006, 69, 499-505.
83. M. Hohne, S. Schatzle, H. Jochens, K. Robins and U. T. Bornscheuer, *Nat. Chem. Biol.*, 2010, 6, 807-813.
84. R. A. Sheldon and S. van Pelt, *Chem. Soc. Rev.*, 2013, 42, 6223-6235.
85. R. DiCosimo, J. McAuliffe, A. J. Poulose and G. Bohlmann, *Chem. Soc. Rev.*, 2013, 42, 6437-6474.
86. S. Cantone, V. Ferrario, L. Corici, C. Ebert, D. Fattor, P. Spizzo and L. Gardossi, *Chem. Soc. Rev.*, 2013, 42, 6262-6276.

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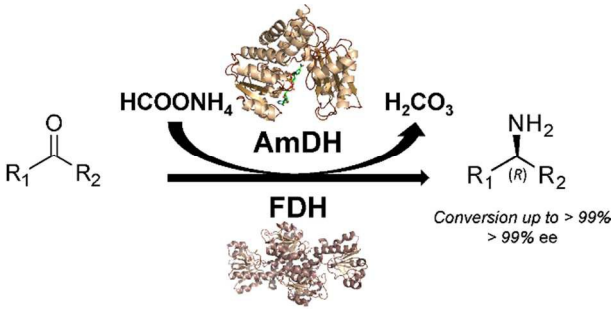

The optimised dual-enzyme (AmDH-FDH) reductive amination of a broad range of carbonyl compounds affords enantiopure amines with conversion up to 99% using ammonia as amine donor and formate as reducing reagent.