CHEMISTRY
A EUROPEAN JOURNAL

ChemPubSoc Europe

DOI: 10.1002/chem.201202666

Artificial Multi-Enzyme Networks for the Asymmetric Amination of *sec*-Alcohols

Katharina Tauber,[a] Michael Fuchs,[a] Johann H. Sattler,[a] Julia Pitzer,[a] Desiree Pressnitz,[a, b] Dominik Koszelewski,[a, b] Kurt Faber,[a] Jan Pfeffer,[c] Thomas Haas,[c] and Wolfgang Kroutil*[a]

Abstract: Various artificial network designs that involve biocatalysts were tested for the asymmetric amination of *sec*-alcohols to the corresponding α-chiral primary amines. The artificial systems tested involved three to five redox enzymes and were exemplary of a range of different *sec*-alcohol substrates. Alcohols were oxidised to the corresponding ketone by an alcohol dehydrogenase. The ketones were subsequently aminated by employing a ω-transaminase. Of special interest were redox-neutral designs in which the hydride abstracted in the oxidation step was reused in the amination step of the cascade. Under optimised conditions up to 91% conversion of an alcohol to the amine was achieved.

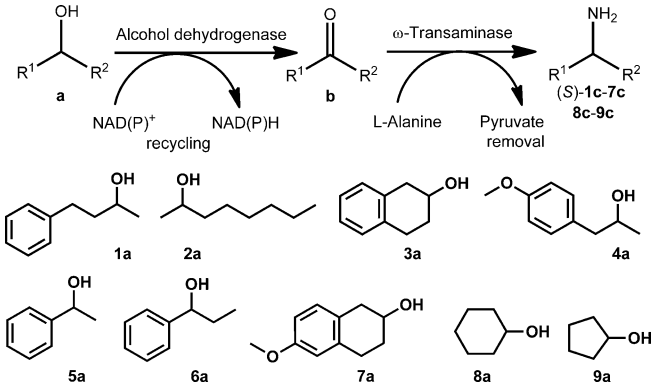
Keywords: alcohols · amines · domino reactions · enzymes · oxidation

Introduction

Metabolic pathways in microorganisms have successfully been engineered to enhance the production of natural target metabolites[1] or to produce novel metabolites by introducing foreign enzymes into organisms.[2] α-Chiral amines represent an important functional group in the pharmaceutical and agrochemical industry.[3] Unfortunately, microorganisms are not known to transform commonly available *sec*-alcohol moieties to the corresponding desired primary amines, as recently described for metal catalysts.[4,5] Consequently, a novel artificial reaction pathway had to be designed from scratch and various network possibilities were investigated in vitro.

Results and Discussion

To achieve the formal substitution of an alcohol by an amine moiety a linear pathway that encompassed two sequential steps was chosen as the base for a cascade: 1) in the first step the alcohol was oxidised by an alcohol dehydrogenase[6] (ADH) to the corresponding ketone, which was then 2) aminated by a ω-transaminase[7] (ω-TA; Scheme 1).[8,9]



Scheme 1. Basic reaction sequence for the amination of *sec*-alcohols.

For both steps additional cofactors/cosubstrates were required, whereby for the oxidation step redox equivalents had to be supplied in the form of nicotinamide adenine dinucleotide phosphate ($NAD(P)^+$), which had to be recycled; for the transamination step, L-alanine was chosen as the amine donor, whereby the formed coproduct pyruvate had to be removed to shift the equilibrium to the product side.[7c] Furthermore, the chiral centre of the *sec*-alcohol had to be considered, thus the stereopreference and selectivity of the ADH employed had to match the absolute configuration of the substrate: for instance, in the case of racemic alcohols, both enantiomers should be oxidised, which might require two enantio-complementary ADHs. However, the stereo-

[a] K. Tauber, M. Fuchs, J. H. Sattler, J. Pitzer, D. Pressnitz, Dr. D. Koszelewski, Prof. K. Faber, Prof. W. Kroutil
Department of Chemistry
Organic and Bioorganic Chemistry, University of Graz
Heinrichstrasse 28, 8010 Graz (Austria)
Fax: (+43) 316-380-9840
E-mail: Wolfgang.Kroutil@uni-graz.at

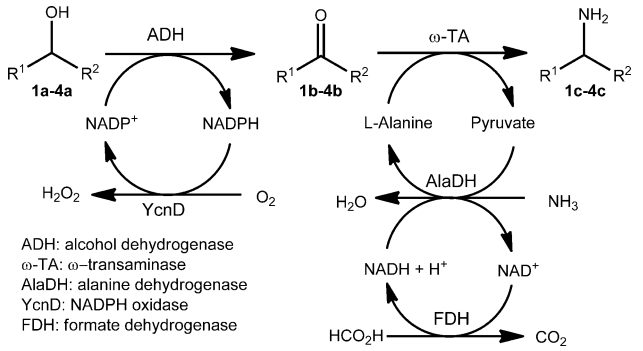
[b] D. Pressnitz, Dr. D. Koszelewski
ACIB GmbH, c/o Heinrichstrasse 28
8010 Graz (Austria)

[c] J. Pfeffer, T. Haas
Evonik Industries AG
CREAVIS Technologies & Innovation
Paul-Baumann-Strasse 1, 45772 Marl (Germany)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201202666.

chemistry of the product amine was controlled exclusively by ω-transaminase.

As a first approach, an artificial catalyst cascade was designed, whereby the oxidation and the amination steps were independent with respect to the recycling of the cofactor and cosubstrate (Scheme 2).



Scheme 2. Amination of *sec*-alcohols employing redox-independent oxidation and amination steps.

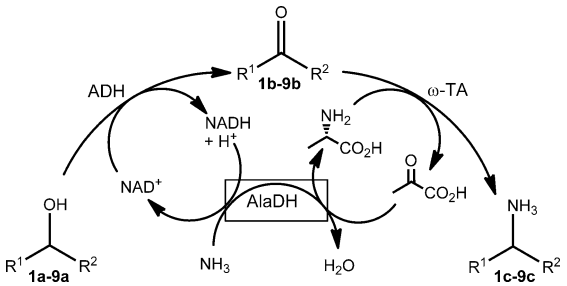
Pyruvate was recycled to alanine by an NADH-dependent alanine dehydrogenase from *Bacillus subtilis*[10] (AlaDH); consequently, the ADH-catalysed oxidation step had to be $NADP^+$-dependent to avoid short-circuits between the two cofactor recycling systems that run simultaneously beside each other (Scheme 2). For the recycling of $NADP^+$, an NADPH oxidase from *Bacillus subtilis*[11] (YcnD) was employed. For the recycling of NADH, a commercial formate dehydrogenase (FDH) was used. Testing this cascade concept with various substrates led indeed to amine formation by employing the crude enzyme preparations (Table 1). Since racemic alcohols were employed in combination with one enantioselective ADH, the amine formation was limited theoretically by 50%, since a perfect enantioselective ADH could oxidise only 50% of the substrate.

Table 1. Amination of *sec*-alcohols by means of the biocatalyst network of Scheme 2.[a]

Substrate	ADH	ωTA[b]	Ketone **b**[c] [%]	(*S*)-Amine **c**[d] [%]
rac-**1a**[d]	LB-ADH	BM	5	32
rac-**2a**	ADH-005	BM	5	23[e]
rac-**3a**	LB-ADH	ArS	1	10[f]
rac-**4a**	LB-ADH	BM	2	24

[a] Reaction conditions: phosphate buffer (50 mM, pH 7.5), NADH (0.5 mM), NADPH (0.5 mM), L-alanine (5 equiv), ammonium formate (4 equiv), pyridoxal-5′-phosphate (PLP, 1 mM), transaminase (in lyophilised *E. coli* cells, 20 mg), AlaDH (crude, 0.63 U, 50 μL), ADH [ADH-005: 77.5 U, 50 μL, Codexis; (*R*)-selective LB-ADH from *Lactobacillus brevis*, 0.13 U, 50 μL, Codexis], NADPH-oxidase YcnD (1.67 U, 10 μL), FDH (Codexis, 10 U), substrate (50 mM); 1 mL total volume, 30 °C and 450 rpm for 24 h using an Eppendorf Thermomixer Comfort. [b] BM = transaminase from *Bacillus megaterium*,[12,13] ArS = *Arthrobacter* sp.[12,14] [c] Composition measured by GC-FID analysis, peak areas. [d] NADH (1 mM), NADPH (1 mM), purified AlaDH (0.16 U). [e] 94% *ee*. [f] > 99% *ee*.

Encouraged by these results, a redox-neutral catalyst network[15–17] that consisted of only three enzymes (instead of five) and avoided external redox reagents, thereby reducing the complexity of the system, was envisioned (Scheme 3).



Scheme 3. Redox-neutral catalyst network for amination of *sec*-alcohols consuming ammonium as sole reagent.

We call the system a 'network' because the individual reaction steps of the cascade are interlinked not only by means of the transformed substrate, but also through the cofactors and cosubstrates. The idea was to reuse the hydride abstracted in the oxidation step for the formal reductive amination step. This was achieved by employing NADH-dependent ADHs in the oxidation step; the NADH formed during oxidation could serve as reducing agent for the recycling of the amine-donor alanine by reductive amination of pyruvate, thereby again providing NAD^+ for the subsequent oxidation cycle. In contrast to the first approach, the oxidation is directly linked to the reductive amination, thereby giving a redox-neutral process at the expense of ammonia as the only reagent (Scheme 2).

A first test that employed crude enzyme preparations led to promising amine formation (20% of (*S*)-**1a** was converted). During optimisation studies, 5 equiv of L-Ala proved to be sufficient, as no increase of amine formation was found at higher concentrations. Additional increases in ammonium concentration should show a similar beneficial effect on the equilibrium. Indeed, higher conversions to the amine were observed by applying 4 equiv of ammonium chloride or ammonium formate; higher concentrations, however, led to lower conversions. Very low concentrations of pyridoxal-5′-phosphate (PLP, 0.3 mM) led to good conversions, whereas higher amounts (3 mM) lowered the conversion.[18] The same effect was observed for the cofactor NADH. Whereas low amounts of NADH (0.1 mM) were beneficial, higher amounts (2.5 mM) retarded the system remarkably. The pH optimum was determined to be between 7 and 7.5, which is the usual optimum of the ADHs employed. To increase substrate solubility, different organic solvents (e.g., 1,2-dimethoxyethane, or DMSO) were tested, but no major positive effect was observed, whereas better mixing (450 rpm instead of 120 rpm, 1.5 mm radius) improved the results.

Under optimised conditions, amine formation was further increased to 64 and 55% for the nonchiral substrates **8a** and **9a** (Table 2).

Table 2. Redox-neutral amination of *sec*-alcohols employing the concept of Scheme 3.[a]

Substrate	ADH[b]	ωTA[c]	Ketone **b**[d] [%]	Amine **c**[d] [%]
(*S*)-**1a**	ADH-A	Vf	25	47[b]
rac-**1a**[e,l]	ADH-A[f] and ADH-007	BM	35	50
(*S*)-**2a**	ADH-A[f]	CV	32	54[j]
rac-**3a**	ADH-A	ArS	5	9[k]
rac-**4a**	ADH-A	BM	10	31
(*S*)-**5a**	ADH-A[f]	Vf	47	25[i]
rac-**6a**	ADH-A[f]	BM	13	28
rac-**7a**	ADH-A	ArS	<1	10
8a[g,h]	ADH-A	BM	–	55
9a[g,h]	ADH-A and ADH-007	BM	14	64

[a] Reaction conditions: phosphate buffer (50 mM, pH 7.5), NADH (1 mM), L-alanine (5 equiv), ammonium chloride (4 equiv), PLP (1 mM), transaminase (lyophilised cells containing overexpressed enzyme, 20 mg), AlaDH (0.62 U, crude, 50 μL), ADH (ADH-A thermally treated, 2.11 U, 50 μL; ADH-007, 1.2 U, 10 mg, Codexis), substrate (50 mM); 1 mL total volume, 30 °C and 450 rpm for 24 h using an Eppendorf Thermomixer Comfort. [b] ADH-A = (*S*)-selective NADH-ADH from *Rhodococcus ruber* DSM 44541;[19] ADH 007 = (*R*)-selective NADH-ADH, Codexis. [c] BM = transaminase from *Bacillus megaterium*, ArS = *Arthrobacter* sp., Vf = *Vibrio fluvialis*,[12,20] CV = *Chromobacterium violaceum*.[12,21] [d] Composition measured using GC-FID, peak areas. [e] 1 mM NAD^+. [f] ADH-A thermally treated (4.23 U, 100 μL). [g] purified AlaDH (0.16 U, 10 μL). [h] L-Alanine (10 equiv), ammonium chloride (2 equiv). [i] 98 % *ee* (*S*). [j] 78 % *ee* (*S*). [k] > 99 % *ee* (*S*). [l] Reaction performed at 20 °C.

Starting from optically pure (*S*)-**1a** with ADH-A gave 47 % of amine **1c**. A similar conversion (50 %) was obtained from *rac*-**1a** by using a combination of (*R*)- and (*S*)-selective ADHs. In general, it was observed that amine formation went hand-in-hand with high ketone formation, thus indicating that unwanted side reactions took place (not depicted in Scheme 2; see below). Interestingly, a decrease in *ee* of the amine over time was also observed, which could most likely be attributed to constantly running back-and-forth reactions as in an equilibrating system.[15e]

Following the reaction over time for (*S*)-**2a** revealed that after six hours the reaction slowed down, whereby 10 % of the alcohol still remained, which did not further decrease (Figure 1). Nevertheless, the ketone was still slowly transformed to the amine. Amine conversion stopped or reached equilibrium at 60 % amine formation.

The scalability of the reaction was shown for (*S*)-**2a** (65 mg) at 10 mL total volume by employing ADH-A and BM-ωTA. In this setup, 43 % (*S*)-amine was obtained (19 % ketone) as determined by GC. After extraction and crystallisation as the corresponding HCl salt, 35 % (*S*)-**2c** was obtained (30 mg, 94 % *ee*).

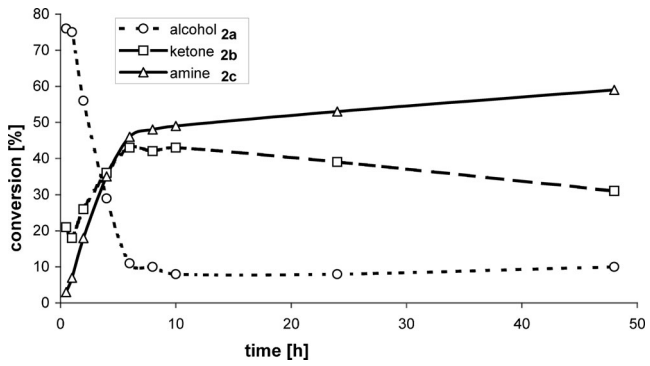


Figure 1. Time course of the redox-neutral catalyst network employing (*S*)-**2a** as substrate.

To gain a better understanding of the network system of Scheme 3, the experiment was repeated for substrate (*S*)-**1a** by employing purified enzymes. In this case, only 11 % amine formation (Table 3, entry 1) was found, whereas the experiment with crude enzyme preparations led to 47 % amine formation by using comparable enzyme activities (Table 2). To exclude the possibility that the type of buffer had any negative influence,[22] the experiment was repeated in piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer, thus leading to comparable results. In the previous experiments that employed crude enzyme preparations, a significant amount of ketone was formed. However, according to the amount of NAD^+ added, a maximum of 2–4 % of ketone could be formed. Therefore the additional redox equivalents were suspected to be provided by NADH-oxidases in the crude enzyme preparations derived from the *E. coli* host cells, which oxidised NADH to NAD^+ before its hydride was consumed in the reductive amination (Scheme 4a).

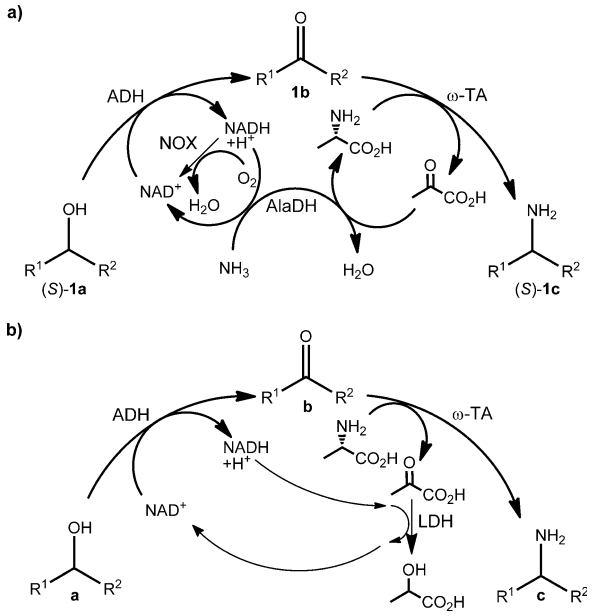
Table 3. Variation of the catalyst network in Scheme 4 for amination of (*S*)-**1a** by employing purified enzymes (ADH-A, AlaDH, ωTA-Vf).[a]

Entry	Additional enzyme	Ketone **1b** [%][b]	Amine **1c** [%][b]
1	–	5	11
2	NOX-2[c]	12	34
3	NOX-2 5×[c]	48	45
4	LDH[d]	10	58
5	LDH 2×[d]	14	71

[a] General reaction conditions: ωTA from *Vibrio fluvialis* (purified, lyophilised powder, 20 mg), AlaDH from *Bacillus subtilis* (purified, 0.16 U, 10 μL), ADH-A from *Rhodococcus ruber* (purified, 0.72 U, 50 μL), **1a** (50 mM); phosphate buffer (50 mM, pH 7.5), NADH (0.5 mM), NAD^+ (0.5 mM), L-alanine (5 equiv), ammonium chloride (4 equiv), PLP (1 mM), 1 mL total volume, 30 °C and 450 rpm for 24 h using an Eppendorf Thermomixer Comfort. [b] Determined by GC-FID, peak areas. [c] NADH oxidase from *Streptococcus mutans*, 0.48 and 2.42 U, respectively (10 μL, 50 μL). [d] LDH from rabbit muscle, 1655 and 3310 U (5 and 10 mg, respectively, Jülich). In the case of LDH, no ammonium chloride or AlaDH were added.

To verify this hypothesis, the NADH-oxidase NOX-2 from *Streptococcus mutans*[23] was tested in the reaction cascade with purified enzymes, which led indeed to improved amine formation of up to 45 % but also, as expected, to increased ketone formation (12–48 %, Table 3, entries 2 and 3).

In another attempt, the network was redesigned in such a way that pyruvate was not recycled to alanine but reduced to lactate by a lactate dehydrogenase (LDH) at the expense of the hydride (NADH) gained in the oxidation step (Scheme 4b). This network was still redox-neutral; however, the



Scheme 4. Variations of the catalyst network for amination of *sec*-alcohols: a) NADH-oxidase (NOX-2) leads to internal recycling of NAD^+; b) lactate dehydrogenase (LDH) for removal of pyruvate.

overall reaction now resembled the transformation of alcohol plus alanine to amine plus lactate. By contrast, in the original version (Scheme 3), alcohol plus ammonium were transformed to amine plus water.

This variation of the redox-neutral catalyst network that involved LDH led to high amine formation (up to 71%) with purified enzymes (Table 3, entries 4 and 5). Consequently, the network that involved LDH was tested for the crude enzyme preparations for selected substrates (Table 4). All substrates tested were transformed with significant improved conversions and reached up to 91% conversion for substrate **8a** (Table 4, entry 5). Ketone formation was elevated again due to the NADH oxidases present in *E. coli* host cells.

Table 4. Redox-neutral amination of sec-alcohols employing lactate dehydrogenase, crude ADH-A and crude ω-TAs (Scheme 4b).[a]

Entry	Substrate	ωTA	Ketone **b** [%][b]	Amine **c** [%][b]	*ee* of (*S*)-amine **c** [%]
1	(*S*)-**1a**	BM	26	63	20
2	(*S*)-**1a**	Vf	27	64	88
3	(*S*)-**2a**	BM	27	48	96
4	(*S*)-**2a**	Vf	31	64	96
5	**8a**	BM	<1	91	n.a.[c]
6	**8a**	Vf	2	54	n.a.[c]
7	**9a**	Vf	44	30	n.a.[c]
8	**9a**	BM	10	85	n.a.[c]

[a] General reaction conditions: ωTA (lyophilised whole cells, 20 mg), ADH-A from *Rhodococcus ruber* (ADH-A thermally treated, 4.22 U, 100 μL); LDH from rabbit muscle (3310 U, 10 mg), substrate (50 mM); phosphate buffer (50 mM, pH 7.5), NADH (0.5 mM), NAD^+ (0.5 mM), L-alanine (5 equiv), PLP (1 mM), 1 mL total volume, 30°C and 450 rpm for 24 h using an Eppendorf Thermomixer Comfort in 1.5 mL reaction tubes in the horizontal position. [b] Determined by GC-FID analysis, peak areas. [c] Not applicable.

Conclusion

A redox-neutral catalyst network for the transformation of *sec*-alcohols to the corresponding primary amines was successfully accomplished. In an ideal system, any NADH-oxidase activity has to be minimised, which makes the application of *E. coli* as host difficult due to the NADH oxidases present.[24] The best results were obtained using a lactate dehydrogenase to remove pyruvate, since the highest amounts of amine were observed with this system. Employing an amino acid dehydrogenase to recycle pyruvate back to alanine showed certain advantages, since it requires only ammonia and water is the sole byproduct. This process represents a cost-efficient and redox-neutral, benign route to highly valuable α-chiral amines. Additionally, the transformation of an alcohol to an amine with inversion of configuration by the appropriate choice of stereo-complementary enzymes can be seen as a reagent-saving alternative to Mitsunobu protocols, thus avoiding triphenylphosphane and the associated purification issues.[25]

Experimental Section

Materials and methods: Substrates and reference materials were purchased from commercial sources: **1a**, (*S*)-**1a**, **1b**, **1c**, **2a**, **2b**, **2c**, (*R*)-**2c**, **3b**, **4b**, **5b**, **5c**, (*S*)-**5c**, **6b**, **6c**, **9a**, **9b** and **9c** from Sigma Aldrich; (*S*)-**2c**, (*S*)-**5a**, **6a**, **8a**, **8b** and **7c** from Fluka; (*S*)-**1c**, (*R*)-**1c**, **3a**, **7b** and (*S*)-**2a** from Lancaster; and **3c** from BASF. Compounds **4a** and **7a** were synthesised starting from the ketone.[26] All other chemicals were purchased from Sigma Aldrich or Fluka and used as received. Solvents were obtained from Roth.

Enzymes were purchased from Codexis (ADH 005, ADH 007, FDH) and previously Jülich Fine Chemicals (LDH, L-lactate dehydrogenase from rabbit muscle, now Codexis, #20.10, 331 U mg^{-1}) or overexpressed in *E. coli* BL 21 (DE3) cells and used as lyophilised cells (ωTA-BM, ωTA-Vf, ωTA-AT-S, ωTA-CV),[12] crude extract (AlaDH) or purified as described (ADH-A,[27] YcnD[11]) or by using His-tag (NOX, AlaDH). Alcohol dehydrogenase 007: 0.12 U mg^{-1}, #42.10. In the case of ADH-LB,[28] a crude extract of the wild-type enzyme from *Lactobacillus brevis* was employed. L-Lactate dehydrogenase from rabbit muscle was commercially available from Jülich Fine Chemicals, now Codexis, #20.10, 331 U mg^{-1}.

General procedure for the cascade with independent cofactor regeneration: First a stock solution that contained phosphate buffer (50 mM, pH 7.5), NADH (0.5 mM), NADPH (0.5 mM), L-alanine (5 equiv), ammonium formate (4 equiv), PLP (1 mM), alanine dehydrogenase (cell-free crude extract, 50 μL per sample, 0.63 U), FDH (10 U mL^{-1}), YcnD (purified 10 μL per sample, 1.67 U) and transaminase (20 mg cells per sample) was prepared. Stock solutions (950 μL) were distributed onto Eppendorf vials (2 mL) and ADH (ADH-005, 77.5 U, 50 μL, Jülich Fine Chemicals; or LB-ADH from *Lactobacillus brevis*, crude, 0.13 U, 50 μL) and the substrate (50 mM) were added. All samples were incubated for 24 h at 30°C and 450 rpm using an Eppendorf Thermomixer Comfort instrument.

Workup: The reaction was stopped by the addition of NaOH (100 μL, 10 M) and ethyl acetate (0.9 mL). The solution was mixed thoroughly and centrifuged for 5 min at 13000 rpm. Then the organic layer was separated and dried over sodium sulfate, which was then removed by centrifugation (3 min, 13000 rpm; Heraeus Biofuge pico). The supernatant was subjected to gas chromatography/flame ionization detector (GC-

FID) analysis and the products were identified by comparison to authentic reference material.

Derivatisation for determination of enantiomeric excess: A solution of DMAP (5–10 mg) in acetic anhydride (100 μL) was added to the samples in AcOEt. Then they were shaken at 21 °C and 150 rpm for 3 h. Water was added (500 μL, to destroy excess amounts of acetic anhydride) and the samples were shaken for 3 h at 21 °C. For workup the samples were centrifuged (3 min, 13000 rpm; Heraeus Biofuge pico) and the organic phase was dried over sodium sulfate.

General procedure for cascade with internal cofactor regeneration: A stock solution that contained phosphate buffer (50 mM, pH 7.5), NADH (1 mM), L-alanine (5 or 10 equiv), ammonium chloride (4 equiv), pyridoxal phosphate (1 mM), alanine dehydrogenase (0.62 U, crude cell-free extract, 50 μL per sample) and transaminase (lyophilised cells 20 mg per sample) was prepared. Stock solutions (950–900 μL) were distributed onto Eppendorf vials (2 mL) and ADH (ADH-A thermally treated, 2.11 U, 50 μL; ADH-007, 1.2 U, 10 mg, Codexis) and substrate (50 mM) were added. All samples were incubated for 24 h at 30 °C (for samples that contained ωTA-CV: 20 °C) and 450 rpm using an Eppendorf Thermomixer Comfort. Workup was carried out as described above.

General procedure for the cascade that employed LDH recycling: A stock solution that contained phosphate buffer (50 mM, pH 7.5), NADH (0.5 mM), NAD^+ (0.5 mM), L-alanine (5 equiv), LDH (5–10 mg), transaminase (20 mg purified lyophilised enzyme) and ADH-A (100 μL) was charged with the substrate (50 mM). All samples were incubated for 24 h at 30 °C and 450 rpm using an Eppendorf Thermomixer Comfort. Workup was carried out as described above.

Determination of conversion: The determination of the conversion was carried out by GC (Varian 3800 using hydrogen as carrier gas) analysis on an achiral stationary phase using a 14% cyanopropylphenyl-phase capillary column (J&W Scientific DB-1701; 30 m × 0.25 mm, 0.25 μm film), with an injection and detection temperature of 220 and 250 °C, respectively, and a 20:1 split ratio.

Temperature program for 1, 2, 5 and 6: 70 °C, hold 1 min, 10 °C min^{-1} to 90 °C, hold 2 min and 20 °C min^{-1} to 160 °C, hold 4 min. Retention times [min]: **1a** 10.1, **1b** 9.8, **1c** 9.2; **2a** 6.0, **2b** 5.8, **2c** 4.8; **5a** 7.7, **5b** 7.5, **5c** 6.8; **6a** 8.7, **6b** 8.6, **6c** 7.9.

Temperature program for 3 and 4: 90 °C, hold for 2 min, 10 °C min^{-1}, to 180 °C, hold for 2 min. Retention times [min]: **3a** 11.2, **3b** 10.7, **3c** 10.2; **4a** 11.0, **4b** 11.2, **4c** 10.4.

Temperature program for 7: 110 °C, hold 0 min, 10 °C min^{-1} to 200 °C, hold 8 min. Retention times [min]: **7a** 11.1, **7b** 10.9, **7c** 10.1.

Temperature program for 8 and 9: 50 °C, hold 2 min, 10 °C min^{-1} to 90 °C, hold 2 min and 20 °C min^{-1} to 160 °C, hold 0 min. Retention times [min]: **8a** 6.3, **8b** 6.6, **8c** 5.0; **9a** 4.6, **9b** 4.7, **9c** 3.4. Amines and ketones were identified by co-injection with commercially available reference material.

Determination of enantiomeric excess: Values of *ee* were measured by GC analysis on a chiral stationary phase using a β-cyclodextrin capillary column (CP-Chirasil-DEX CB, 25 m × 0.32 mm, 0.25 μm film), split ratio 10:1. The temperature of the injector and detector for chiral measurements were 180 and 250 °C, respectively.

Temperature program for 1, 2 and 5: 70 °C, hold 2 min, 15 °C min^{-1} to 130 °C, hold 2 min and 10 °C min to 160 °C, hold 5 min. Retention times [min]: (*S*)-**1c** 21.3, (*R*)-**1c** 21.5; (*S*)-**2c** 14.0, (*R*)-**2c** 14.5; (*S*)-**1c** 21.3, (*R*)-**1c** 21.5; (*S*)-**5c** 17.3, (*R*)-**5c** 17.5.

Temperature program for 3: 100 °C, hold 2 min, 5 °C min^{-1} to 130 °C, hold 2 min and 10 °C min^{-1} to 180 °C, hold 10 min. Retention times [min]: (*S*)-**3c** 17.1, (*R*)-**3c** 17.3. Amines were identified by co-injection with derivatised commercially available reference material.

Acknowledgements

The research that led to these results received funding from Evonik Industries AG and the German Federal Ministry of Education and Research (BMBF: project BioxAmine). J.H.S. received funding from the European Union Seventh Framework Programme FP7/2007-2013 under grant agreement number 245144 (AmBioCas). D.P. acknowledges the Federal Ministry of Economy, Family and Youth (BMWFJ), the Federal Ministry of Traffic, Innovation and Technology (bmvit), the Styrian Business Promotion Agency (SFG), the Standortagentur Tirol and ZIT, the Technology Agency of the City of Vienna, through the COMET-Funding Program managed by the Austrian Research Promotion Agency FFG.

[1] a) J. W. Lee, D. Na, J. M. Park, J. Lee, S. Choi, S. Y. Lee, *Nat. Chem. Biol.* **2012**, *8*, 536–546; b) J. D. Keasling, *Science* **2010**, *330*, 1355–1358; c) G. Stephanopoulos, *Science* **2007**, *315*, 801–804; d) V. F. Wendisch, M. Bott, B. J. Eikmanns, *Curr. Opin. Microbiol.* **2006**, *9*, 268–274.

[2] a) W. S. Glenn, E. Nims, S. E. O'Connor, *J. Am. Chem. Soc.* **2011**, *133*, 19346–19349; b) J. W. Lee, H. U. Kim, S. Choi, J. Yi, *Curr. Opin. Biotechnol.* **2011**, *22*, 758–767; c) Sang Yup Lee K. L. J. Prather, C. H. Martin, *Curr. Opin. Biotechnol.* **2008**, *19*, 468–474.

[3] a) T. B. Nguyen, H. Bousserouel, Q. Wang, F. Guéritte, *Adv. Synth. Catal.* **2011**, *353*, 257–262; b) A. Henseler, M. Kato, K. Mori, T. Akiyama, *Angew. Chem.* **2011**, *123*, 8330–8333; *Angew. Chem. Int. Ed.* **2011**, *50*, 8180–8183; c) V. N. Wakchaure, J. Zhou, S. Hoffmann, B. List, *Angew. Chem.* **2010**, *122*, 4716–4718; *Angew. Chem. Int. Ed.* **2010**, *49*, 4612–4614; d) R. I. Storer, D. E. Carrera, Y. Ni, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2006**, *128*, 84–86; e) T. C. Nugent, M. El-Shazly, *Adv. Synth. Catal.* **2010**, *352*, 753–819; f) T. C. Nugent in *Chiral Amine Synthesis: Methods, Developments and Applications* (Ed.: T. C. Nugent), Wiley-VCH, Weinheim, **2010**, pp. 225–245; g) T. Sun, G. Hou, M. Ma, X. Zhang, *Adv. Synth. Catal.* **2011**, *353*, 253–256; h) X.-Y. Zhou, M. Bao, Y.-G. Zhou, *Adv. Synth. Catal.* **2011**, *353*, 84–88; i) H. U. Blaser, F. Spindler, M. Studer, *Appl. Catal. A* **2001**, *221*, 119–143.

[4] Selected recent examples: a) M. Lafrance, M. Roggen, E. M. Carreira, *Angew. Chem.* **2012**, *124*, 3527–3530; *Angew. Chem. Int. Ed.* **2012**, *51*, 3470–3473; b) S. Imm, S. Bähn, M. Zhang, L. Neubert, H. Neumann, F. Klasovsky, J. Pfeffer, T. Haas, M. Beller, *Angew. Chem.* **2011**, *123*, 7741–7745; *Angew. Chem. Int. Ed.* **2011**, *50*, 7599–7603; c) D. Pingen, C. Müller, D. Vogt, *Angew. Chem.* **2010**, *122*, 8307–8310; *Angew. Chem. Int. Ed.* **2010**, *49*, 8130–8133; d) R. Kawahara, K.-i. Fujita, R. Yamaguchi, *J. Am. Chem. Soc.* **2010**, *132*, 15108–15111; e) O. Saidi, A. J. Blacker, M. M. Farah, S. P. Marsden, J. M. J. Williams, *Angew. Chem.* **2009**, *121*, 7511–7514; *Angew. Chem. Int. Ed.* **2009**, *48*, 7375–7378.

[5] First reports on metal-catalysed amination of alcohols: a) R. Grigg, T. R. B. Mitchell, S. Sutthivaiyakit, N. Tongpenyai, *J. Chem. Soc. Chem. Commun.* **1981**, 611–612; b) Y. Watanabe, Y. Tsuji, Y. Ohsugi, *Tetrahedron Lett.* **1981**, *22*, 2667–2670.

[6] a) F. Hollmann, I. W. C. E. Arends, K. Buehler, A. Schallmey, B. Bühler, *Green Chem.* **2011**, *13*, 226–265; b) T. Matsuda, R. Yamanaka, K. Nakamura, *Tetrahedron: Asymmetry* **2009**, *20*, 513–557; c) K. Goldberg, K. Schroer, S. Lütz, A. Liese, *Appl. Microbiol. Biotechnol.* **2007**, *76*, 237–248; d) K. Edegger, H. Mang, K. Faber, J. Gross, W. Kroutil, *J. Mol. Catal. A* **2006**, *251*, 66–70; e) W. Kroutil, H. Mang, K. Edegger, K. Faber, *Adv. Synth. Catal.* **2004**, *346*, 125–142.

[7] For recent reviews on ω-transaminases, see: a) M. S. Malik, E.-S. Park, J.-S. Shin, *Appl. Microbiol. Biotechnol.* **2012**, *94*, 1163–1171; b) S. Mathew, H. Yun, *ACS Catal.* **2012**, *2*, 993–1001; c) P. Tufvesson, J. Lima-Ramos, J. S. Jensen, N. Al-Haque, W. Neto, J. M. Woodley, *Biotechnol. Bioeng.* **2011**, *108*, 1479–1493; d) D. Koszelewski, K. Tauber, K. Faber, W. Kroutil, *Trends Biotechnol.* **2010**, *28*, 324–332; e) N. J. Turner, M. Truppo in *Chiral Amine Synthesis: Methods, Developments and Applications* (Ed.: T. C. Nugent), Wiley-VCH, Weinheim, **2010**, pp. 431–459; f) H. C. Hailes, P. A. Dalby, G. J. Lye, F. Baganz, M. Micheletti, N. Szita, J. M. Ward, *Curr. Org. Chem.* **2010**, *14*, 1883–1893; g) J. Ward, R. Wohlgemuth, *Curr. Org. Chem.* **2010**, *14*, 1914–1927; h) M. Höhne, U. T. Bornscheuer, *ChemCat Chem* **2009**, *1*, 42–51.

[8] Redox-neutral biocatalytic transformations of alcohols to amines have only been reported for α-hydroxy acids to the corresponding α-amino acids that involve amino acid dehydrogenases and alcohol dehydrogenases: a) transformation of mandelic acid to L-phenylglycine: V. Resch, W. M. F. Fabian, W. Kroutil, *Adv. Synth. Catal.* **2010**, *352*, 993–997; b) transformation of lactate to alanine: C. Wandrey, E. Fiolitakis, U. Wichmann, *Ann. N. Y. Acad. Sci.* **1984**, *434*, 091–094; as well as for *prim*-alcohols: c) J. H. Sattler, M. Fuchs, K. Tauber, F. G. Mutti, K. Faber, J. Pfeffer, T. Haas, W. Kroutil, *Angew. Chem. Int. Ed.* **2012**, *51*, 9156–9159.

[9] *prim*-Alcohols to amines were transformed in a cascade that consisted of an alcohol oxidase and a ω-transaminase: M. Fuchs, K. Tauber, J. Sattler, H. Lechner, J. Pfeffer, W. Kroutil, K. Faber, *RSC Adv.* **2012**, *2*, 6262–6265.

[10] F. G. Mutti, C. S. Fuchs, D. Pressnitz, N. G. Turrini, J. H. Sattler, A. Lerchner, A. Skerra, W. Kroutil, *Eur. J. Org. Chem.* **2012**, 1003–1007.

[11] a) C. V. Voss, C. C. Gruber, K. Faber, T. Knaus, P. Macheroux, W. Kroutil, *J. Am. Chem. Soc.* **2008**, *130*, 13969–13972; b) A. Morokutti, A. Lyskowski, S. Sollner, E. Pointner, T. B. Fitzpatrick, C. Kratky, K. Gruber, P. Macheroux, *Biochemistry* **2005**, *44*, 13724–13733.

[12] D. Koszelewski, M. Göritzer, D. Clay, B. Seisser, W. Kroutil, *ChemCatChem* **2009**, *2*, 73–77.

[13] R. L. Hanson, B. L. Davis, Y. Chen, S. L. Goldberg, W. L. Parker, T. P. Tully, M. A. Montana, R. N. Patel, *Adv. Synth. Catal.* **2008**, *350*, 1367–1375.

[14] S. Pannuri, S. V. Kamat, A. R. M. Garcia, (Cambrex North Brunswick Inc.) WO 2006/063336 A2, **2006**.

[15] Redox-neutral sequential cascades that exclusively involve enzymes have been reported in Ref. [8] and for the combination of an alcohol dehydrogenase and an enoate reductase: a) S. Gargiulo, D. J. Opperman, U. Hanefeld, I. W. C. E. Arends, F. Hollmann, *Chem. Commun.* **2012**, *48*, 6630–6632; b) B. Boonstra, D. A. Rathbone, C. E. French, E. H. Walker, N. C. Bruce, *Appl. Environ. Microbiol.* **2000**, *66*, 5161–5166; the combination of an alcohol dehydrogenase and a Baeyer–Villiger mono-oxygenase: c) R. Gagnon, G. Grogan, S. M. Roberts, R. Villa, A. J. Willetts, *J. Chem. Soc. Perkin Trans. 1* **1995**, 1505–1511; d) A. J. Willetts, C. J. Knowles, M. S. Levitt, S. M. Roberts, H. Sandey, N. F. Shipston, *J. Chem. Soc. Perkin Trans. 1* **1991**, 1608–1610; the combination of two alcohol dehydrogenases to achieve racemisation: e) C. C. Gruber, B. M. Nestl, J. Gross, P. Hildebrandt, U. T. Bornscheuer, K. Faber, W. Kroutil, *Chem. Eur. J.* **2007**, *13*, 8271–8276.

[16] For a recent review on cascades with redox steps, see: J. H. Schrittwieser, J. Sattler, V. Resch, F. G. Mutti, W. Kroutil, *Curr. Opin. Chem. Biol.* **2011**, *15*, 249–256.

[17] Selected recent reviews on biocatalytic cascades: a) E. Ricca, B. Brucher, J. H. Schrittwieser, *Adv. Synth. Catal.* **2011**, *353*, 2239–2262; b) N. J. Turner, *Curr. Opin. Chem. Biol.* **2010**, *14*, 115–121; c) *Multi-Step Enzyme Catalysis* (Ed.: E. Garcia-Junceda), 1st ed., Wiley-VCH, Weinheim, **2008**; d) F. Hollmann, K. Hofstetter, A. Schmid, *Trends Biotechnol.* **2006**, *24*, 163–171.

[18] K. E. Cassimjee, C. Branneby, V. Abedi, A. Wells, P. Berglund, *Chem. Commun.* **2010**, *46*, 5569–5571.

[19] a) K. Edegger, C. C. Gruber, T. M. Poessl, S. R. Wallner, I. Lavandera, K. Faber, F. Niehaus, J. Eck, R. Oehrlein, A. Hafner, W. Kroutil, *Chem. Commun.* **2006**, 2402–2404; b) B. Kosjek, W. Stampfer, M. Pogorevc, W. Goessler, K. Faber, W. Kroutil, *Biotechnol. Bioeng.* **2004**, *86*, 55–62.

[20] J.-H. Seo, D. Kyung, K. Joo, J. Lee, B.-G. Kim, *Biotechnol. Bioeng.* **2011**, *108*, 253–263.

[21] U. Kaulmanna, K. Smithies, M. E. B. Smith, H. C. Hailes, J. M. Ward, *Enzyme Microb. Technol.* **2007**, *41*, 628–637.

[22] a) S. Schätzle, F. Steffen-Munsberg, A. Thontowi, M. Höhne, K. Robins, U. T. Bornscheuer, *Adv.Synth. Catal.* **2011**, *353*, 2439–2445; b) K. E. Cassimjee, M. S. Humble, V. Miceli, C. G. Colomina, P. Berglund, *ACS Catal.* **2011**, *1*, 1051–1055.

[23] M. Higuchi, M. Shimada, J. Matsumoto, Y. Yamamoto, A. Rhaman, Y. Kamio, *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1603–1607.

[24] a) S. Kerscher, S. Dröse, V. Zickermann, U. Brandt, *Results Probl. Cell Differ.* **2008**, *45*, 185–222; b) G. Unden, J. Bongaerts, *Biochim. Biophys. Acta Bioenerg.* **1997**, *1320*, 217–234.

[25] a) K. C. K. Swamy, N. N. B. Kumar, E. Balaraman, K. V. P. P. Kumar, *Chem. Rev.* **2009**, *109*, 2551–2651; b) D. L. Hughes, R. A. Reamer, J. J. Bergan, E. J. J. Grabowski, *J. Am. Chem. Soc.* **1988**, *110*, 6487–6491; c) O. Mitsunobu, *Synthesis* **1981**, *1*, 1–28; d) E. Ślusarska, A. Zwierzak, *Liebigs Ann. Chem.* **1986**, 402–405; e) O. Mitsunobu, M. Yamada, *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380–2382.

[26] N. Becker, E. M. Carreira, *Org. Lett.* **2007**, *9*, 3857–3858.

[27] K. Tauber, M. Hall, W. Kroutil, W. M. F. Fabian, K. Faber, S. M. Glueck, *Biotechnol. Bioeng.* **2011**, *108*, 1462–1467.

[28] W. Hummel, *Adv. Biochem. Eng./Biotechnol.* **1997**, *58*, 145–184.

Received: July 26, 2012
Revised: December 19, 2012
Published online: January 22, 2013

