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Deracemisation of α-Chiral Primary Amines by a One-Pot, Two-Step Cascade Reaction Catalysed by ω-Transaminases

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Dedicated to Kalle Hult on the occasion of his 65th birthday

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Racemic α-chiral primary amines were deracemised to optically pure amines in up to >99 % conversion and >99 % *ee* within 48 h. The deracemisation was a result of a stereoinversion of one amine enantiomer; the formal stereoinversion was achieved by a one-pot, two-step procedure: in the first step, kinetic resolution of the chiral racemic amine was performed by employing a ω-transaminase to yield an intermediate ketone and the remaining optically pure amine; in the second step, the ketone intermediate was stereoselectively transformed into the amine by employing alanine as the amine donor and a ω-transaminase displaying opposite stereopreference than the ω-transaminase in the first step. In the second step, lactate dehydrogenase was used to remove the side product pyruvate to shift the unfavourable reaction equilibrium to the product side. Depending on the order of the enantiocomplementary enzymes employed in the cascade, the (*R*), as well as the (*S*), enantiomer was accessible.

Introduction

Optically active amines are required for the preparation of a broad range of biologically active compounds showing various pharmacological properties.[1,2] Subsequently, chiral amines have been used as resolving agents, chiral auxiliaries and building blocks in the synthesis of neurological, cardiovascular, immunological, antihypertensive, anti-infective and antiemetic drugs.[3] In most cases, the pharmacological activities of these amines are related to the configuration of the stereogenic centre.[4,5] For example, (*R*)-4-phenylbutan-2-amine (**1g**) is a precursor of the antihypertensive dilevalol,[6] and (*S*)-*sec*-butylamine (**1b**), (*S*)-1-methoxy-2-propanamine (**1c**) and (*S*)-1-cyclohexylethylamine (**1d**) are precursors of inhibitors of tumour necrosis factor-α(TNF-α).[7] Furthermore, 1-phenyl-1-propylamine (**1f**) is a precursor of corticotropin releasing factor type-1 receptor antagonist, a potent antidepressant agent.[8] Finally, mexiletine (**1h**) is an orally effective antiarrhythmic,[9] antimyotonic[10] and analgesic[11] agent in its racemic form and is available for clinical use as the racemate (Figure 1). Mexiletine undergoes stereoselective disposition in vivo associated with the selective binding of the (*R*)-mexiletine to a cardiac sodium channel and the higher antiarrhythmic activity of this enantiomer.[12,13] Consequently, there is a need for efficient methods to obtain the desired (*R*) or (*S*) enantiomer in optically pure form starting from easily accessible substrates. Resolution of some of these amines has been carried out by fractional crystallisation or distillation of the diastereomeric salts,[14,15] chromatographic separation of diastereomeric amides,[16] microbial hydrolysis of an *N*-acyl derivative[17–19] or by enantioselective acylation of racemic amines catalysed by lipase B from *Candida antarctica*.[20–22] Furthermore, ω-transaminases received recently increased attention for (i) the kinetic resolution of racemic amines and (ii) the asymmetric amination of the corresponding ketones.[23–34]

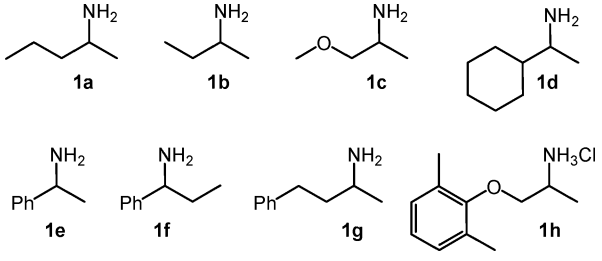

Figure 1. Racemic amines **1a**–**h** chosen for the biocatalytic one-pot, two-step deracemisation protocol employing two ω-transaminases.

Additionally, protocols like dynamic kinetic resolution of amines[35–37] and cyclic deracemisation[38] for the deracemisation of racemic amines to yield optically pure products in

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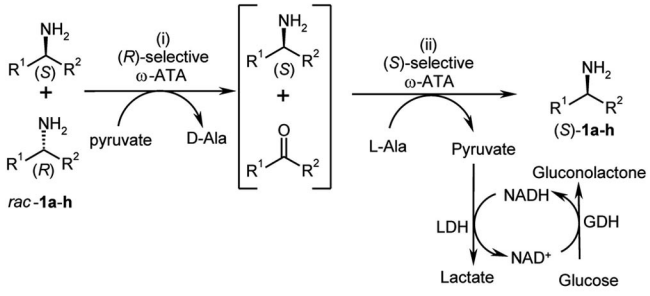
100% yield and >99%*ee* have been developed. Because in various cases the racemic amine is more readily available than the corresponding ketone, deracemisation will gain increased attention; for example, the corresponding ketones of **1d** and **1h** are not commercially available. Therefore, we envisaged a two-step, one-pot process as outlined in Scheme 1 that consists of (i) a kinetic resolution and (ii) a stereoselective amination. The advantage of this concept is to avoid the limitation of kinetic resolution (50% conversion) giving quantitative yield of optically pure amine starting with racemic amines.

Results and Discussion

For the deracemisation of amines by a two-step process as outlined in Scheme 1 a kinetic resolution step is required first, followed by a reductive amination step.



Scheme 1. One-pot, two-step synthesis procedure towards enantioenriched amines employing two ω-transaminases (ω-ATAs) with opposite stereopreference. Removal of pyruvate was performed by its reduction employing lactate dehydrogenase (LDH) to shift the equilibrium to the product side.

Testing first the kinetic resolution for various amines, three commercial ω-transaminases (ATA-113, ATA-114 and ATA-117) were chosen as catalysts (Table 1). The ω-transaminases catalysed this reaction efficiently, giving the amines with up to >99%*ee* (Table 1).

For a number of substrate/enzyme combinations, the formal ideal 50% conversion barrier was surpassed, indicating that the ω-transaminases possess for these amines a nonperfect enantioselectivity. For instance, in the case for which a limiting amount of pyruvate was used, the enantiomeric excess at 50% conversion was not perfect (Table 1, Entries 2 and 4). However, because the ketone will be transformed back to the remaining amine enantiomer in the second step of the one-pot transformation, the goal of the first step is complete removal of the "wrong" enantiomer. The most important point, however, was that transaminases ATA-113 and ATA-114 showed (*S*) preference, whereas ATA-117 displayed (*R*) preference; thus, enantiocomplementary enzymes were available. This is actually the basis for the complete deracemisation sequence.

Having identified the suitable conditions for the kinetic resolution of the desired amines we turned our attention to couple the first step (i.e., kinetic resolution) with the stereoselective amination to achieve a deracemisation reaction sequence without separation of intermediate mixtures (Scheme 1). Shifting the ketone–amine equilibrium to the amine side is a challenge with ω-transaminases, especially when using an amino acid like alanine as an amino donor, as in this case the equilibrium is on the side of the substrates (ketone, alanine) and not on the side of the products (amine, pyruvate).[23] To shift the equilibrium[29,31] the pyruvate formed was removed by reduction by using lactate dehydrogenase (LDH) in a coupled reaction system. The stereoselectivity for the reductive amination of the commercial ketones was already previously reported.[24]

Table 1. Kinetic resolution of amines **1** catalysed by commercially available ω-transaminases.

Entry	Substrate	ATA[a]	*c* [%][b]	*ee* [%][c]
1	**1a**	117	57	>99 (*S*)
2	**1a**	117	50[d]	94 (*S*)
3	**1a**	113	88	>99 (*R*)
4	**1a**	113	51[d]	87 (*R*)
5	**1a**	114	86	>99 (*R*)
6	**1b**	117	52	>99 (*S*)
7	**1b**	113	62	>99 (*R*)
8	**1b**	114	58	>99 (*R*)
9	**1c**	117	76	99 (*S*)
10	**1c**	117	65[e]	97 (*S*)
11	**1c**	113	79	87 (*R*)
12	**1c**	114	96	96 (*R*)
13	**1d**	117	51	>99 (*S*)
14	**1d**	113	50	>99 (*R*)
15	**1d**	114	49	98 (*R*)
16	**1e**	117	50	>99 (*S*)
17	**1e**	113	51	>99 (*R*)
18	**1e**	114	53	>99 (*R*)
19	**1f**	117	50	>99 (*S*)
20	**1f**	113	48	98 (*R*)
21	**1f**	114	49	99 (*R*)
22	**1g**	117	50	>99 (*S*)
23	**1g**	113	93	35 (*R*)
24	**1g**	114	61	>99 (*R*)
25	**1h**	117	61	>99 (*S*)
26	**1h**	113	60	>99 (*R*)
27	**1h**	114	43	29 (*R*)

[a] Reaction conditions: amine **1** (50 mM), sodium pyruvate (50 mM), ω-transaminase (6 mg), phosphate buffer (100 mM, pH 7.0, 1 mM PLP), shaking at 30 °C for 24 h. [b] Determined by GC. [c] Determined by GC analysis on a chiral phase. [d] A 25 mM solution of sodium pyruvate was employed. [e] 30 mM.

The reaction sequence was performed in a way that after the kinetic resolution the second ω-transaminase with opposite stereopreference was added together with the corresponding alanine enantiomer. Because the two ω-transaminases should also have different stereopreference for the alanine enantiomers, we speculated that this approach should work. However, as can be seen from the results (Table 2), the optical purity of the final product was moderate, although exclusively amine could be detected in almost all cases. The reason for this was that the ω-transaminases also accept, to a certain extent, the opposite alanine enantiomer, meaning that the ω-transaminase of the first step also catalysed the amination reaction although at a reduced rate. Overall, this led to a diminished *ee* value. The same was observed when DMSO was added in the second step, al-

though DMSO partially inhibited ω-transaminase ATA-113, whereas the activity of ATA-117 stayed untouched (Table 2, Entries 1 and 3 vs. 2 and 4).

Table 2. Two-step procedure catalysed by various commercially available ω-transaminases.

Entry	Substrate	ATA[1]/ ATA[2][a]	DMSO [%v v^{-1}]	*c* [%][b]	*ee* [%][c]
1	**1a**	117/113	–	>99	77 (*S*)
2	**1a**	117/113	10	>99	75 (*S*)
3	**1a**	113/117	–	>99	29 (*R*)
4	**1a**	113/117	10	>99	56 (*R*)
5	**1b**	117/113	–	>99	88 (*S*)
6	**1b**	117/113	10	96	86 (*S*)

[a] Order of addition of ω-transaminase; in the case of ATA-117, D-Ala was used in the amination reaction and L-Ala was used with ATA-113 and ATA-114. [b] Determined by GC. [c] Determined by GC analysis on a chiral phase.

To solve this problem, heat treatment was introduced between the two steps. Thus, after the kinetic resolution, the sample was kept at 75 °C for 30 min before the enzyme required for the second step was added. Although destruction of the enzyme may lead to an expensive process, this trick enabled efficient one-pot, two-step deracemisation leading to optically enriched and even optically pure (*R*)- or (*S*)-amines with very high conversion (Table 3). From all eight substrates **1a**–**h** only the (*S*) enantiomer of **1g** was obtained with a low *ee* value. For all other cases, the *ee* value was at least 96% [(*R*)-**1c**], otherwise the value was >99% for both enantiomers.

Table 3. Two-step procedure with the addition of heat to 75 °C after the first step catalysed by various commercially available ω-transaminases.

Entry	Substrate	ATA[1]/ATA[2][a]	*c* [%][b]	*ee* [%][c]
1	**1a**	117/113	99	>99 (*S*)
2	**1a**	113/117	82	>99 (*R*)
3	**1a**	114/117	87	>99 (*R*)
4	**1b**	117/113	>99	>99 (*S*)
5	**1b**	113/117	>99	98 (*R*)
6	**1b**	114/117	>99	>99 (*R*)
7	**1c**	117/113	>99	>99 (*S*)
8	**1c**	113/117	>99	86 (*R*)
9	**1c**	114/117	>99	96 (*R*)
10	**1d**	117/113	62	>99 (*S*)
11	**1d**	113/117	86	>99 (*R*)
12	**1d**	114/117	88	>99 (*R*)
13	**1e**	117/113	60	>99 (*S*)
14	**1e**	113/117	80	>99 (*R*)
15	**1e**	114/117	84	>99 (*R*)
16	**1f**	117/113	82	>99 (*S*)
17	**1f**	113/117	75	97 (*R*)
18	**1f**	114/117	72	>99 (*R*)
19	**1g**	117/113	>99	5 (*S*)
20	**1g**	113/117	96	97 (*R*)
21	**1g**	114/117	>99	>99 (*R*)
22	**1h**	117/113	>99	>99 (*S*)
23	**1h**	113/117	81	>99 (*R*)

[a] Order of addition of ω-transaminase; in the case of ATA-117, D-Ala was used in the amination reaction and L-Ala was used with ATA-113 and ATA-114. [b] Determined by GC. [c] Determined by GC analysis on a chiral phase.

Depending on the order of addition of the ω-transaminases, either the (*R*) or the (*S*) enantiomer was accessible. Thus, just by switching the order, the other enantiomer was obtained.

Employing the optimised conditions, a preparative transformation of 50 mg of *rac*-**1d** at 20 mM substrate concentration yielded (*S*)-**1d** at >99% conversion after 48 h with >99%*ee* and 92% isolated yield. Transformation of 50 mg of racemic 1-(2,6-dimethylphenoxy)-2-propanamine (**1h**) led to optically pure amine (*S*)-**1h** with complete conversion, >99%*ee* and 95% isolated yield.

Conclusions

In summary, a one-pot, two-step deracemisation cascade leading to optically pure pharmacologically relevant amines through kinetic resolution and subsequent stereoselective amination catalysed by two enantiocomplementary ω-transaminases was described. The resulting amines were obtained with up to quantitative conversion and excellent enantioselectivities (up to >99%*ee*).

Experimental Section

Amines **1a**–**h** and ketones, as well as solvents (DMSO), were purchased from Sigma–Aldrich (Vienna, Austria) or BASF (Ludwigshafen, Germany) and used as received unless otherwise stated. All chemicals used were of analytical grade. ω-Transaminases ATA-113, 114 and 117 (transaminase ATA-113, 102907WW, 0.46 U mg^{-1}; transaminase ATA-117, 102907WW, 1.9 U mg^{-1}; transaminase ATA-114, 1091108MW, 2.7 U mg^{-1}) and lactate dehydrogenase mix (LDH, PRM-102, 101807KVP, mixture of lactate dehydrogenase, glucose dehydrogenase, glucose, NAD^+) were obtained from Codexis Inc. One unit of ω-transaminase was defined as the amount of enzyme that catalyses the formation of 1 μmol acetophenone from α-methylbenzylamine at pH 9.0 at 22 °C.

Kinetic Resolution: All biocatalytic reactions were performed at 30 °C for 24 h in sodium phosphate buffer (100 mM, pH 7) containing pyridoxal-5′-phosphate monohydrate (1 mM) in a 2-mL eppendorf tube. The reaction buffer (1 mL) was mixed with ω-transaminase (6 mg) and sodium pyruvate (50 mM). The reaction mixture contained 50 mM of corresponding amine **1**. The conversion to ketone **2** was measured by GC chromatography. The reaction was stopped by adding NaOH (200 μL, 10 N), followed by extraction with ethyl acetate (600 μL, 2×). Organic phase was dried with anhydrous Na_2SO_4.

Analysis of Optical Purity of Products: The enantiomeric excess of amines **2a**–**g** was analysed by gas chromatography on a chiral phase after derivatisation to the acetoamides, which was performed by adding a DMAP and a 20-fold excess of acetic acid anhydride. Amine **2h** was analysed after derivatisation to trifluoroacetamide, which was performed by adding a 20-fold excess of trifluoroacetic acid anhydride. After washing with water and drying with anhydrous Na_2SO_4 the *ee* value of the derivatised compound was measured.[24,25]

Representative Example for Amination with Heating at 75 °C: After the kinetic resolution step (24 h), the mixture was kept at 75 °C for 30 min, cooled to room temperature and LDH-mix (40 mg, 1 mM NAD^+, glucose, lactate dehydrogenase, glucose dehydrogenase) was

added (to remove pyruvate from the first step), and the mixture was shaken for 1 h at 30 °C. L- or D-Alanine (5 equiv., 250 mM) and the second ω-transaminase (6 mg, Table 3) were added, and the mixture was shaken at 30 °C. After 24 h, 200 μL 10 N NaOH and 600 μL EtOAc were added. The organic phase was dried (Na_2SO_4) and analysed by GC chromatography.[24,25]

Supporting Information (see footnote on the first page of this article): Preparative transformations, determination of absolute configuration and (chiral) analytics are reported.

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