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Chemoenzymatic Synthesis of All Four Diastereomers of 2,6-Disubstituted Piperidines through Stereoselective Monoamination of 1,5-Diketones

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Abstract: The regioselectivity of various enantiocomplementary ω-transaminases was evaluated for the stereoselective monoamination of designated 1,5-diketones; excellent conversions, enantio- and regioselectivities were observed. The resulting amino-ketones underwent spontaneous intramolecular ring closure to afford Δ1-piperideines, which served as precursors for the *cis*- and *anti*-piperidine scaffold as demonstrated for the synthesis of the alkaloids dihydropinidine and *epi*-dihydropinidine. Key to the success of accessing the *trans*-piperidines was a Lewis acid mediated conformational change of the Δ1-piperideines in the reduction step. Thus, all four diastereomers of 2,6-disubstituted piperidines could successfully be prepared.

Keywords: alkaloids · chiral amines · piperidines · regioselectivity · ω-transaminases

Introduction

Biocatalysis has emerged as a powerful tool for the synthesis of chiral key intermediates, pharmaceuticals and complex natural products.[1] The main advantage of biocatalysis is the ability to use mild reaction conditions to afford the products in a chemo-, regio- and stereoselective manner.[2] For instance, several biocatalytic methods have been established to access optically pure amines and derivate that play a pivotal role as building blocks for pharmaceutical drugs, agrochemicals, natural products and as chiral ligands.[3] Typical enzymes employed are hydrolases,[2c,4] monoamine oxidases,[5] and pyridoxal-5′-phosphate (PLP)-dependent transaminases.[6] The latter might be used in two complementary ways: either in the asymmetric reductive amination of a prochiral ketone[7] or in a kinetic resolution of racemic amines.[8] Although the substrate scope as well as the stereoselectivity of ω-transaminases (ω-TA) were investigated in detail, their regioselectivity was exploited only recently.[9] Regioselective reactions are highly desirable transformations in organic synthesis that can be used to differentiate between two or more chemically identical reactive centers,[10] avoiding additional chemical operations and reaction steps as well as sophisticated protection strategies. Protecting-group-free strategies are considered superior and have received reasonable recognition;[11,12] recent examples include the regioselective amination of, for example, alkanes,[13] allylic systems,[14] and indoles.[15]

Chiral piperidines and their derivate are common key structural elements in the scaffold of several alkaloids and other natural products.[16] Considerable efforts have been devoted to their stereoselective synthesis, nevertheless, there remains a demand for more efficient and/or alternative methods.[17] Based on our previous investigations on the biocatalytic regioselective monoamination of selected 1,5-diketones **1**,[9] we report here on the regioselectivity of further ω-transaminases (ω-TAs) including one ω-TA designed for industrial purposes that accepts 2-propylamine as amine donor; furthermore, the concept is extended to obtain all four diastereomers of 2,6-disubsituted piperidines **6** (Scheme 1).

Results and Discussion

For our studies, we focused on various aliphatic, cyclic, and aromatic diketones **1a–e** (Scheme 1), which were synthesized as reported earlier.[9] In addition to ω-TAs originating from *Chromobacterium violaceum*,[18a] *Bacillus megaterium*,[8e] *Vibrio fluvialis*,[18c] (*R*)-*Arthrobacter*,[7b] *Hyphomonas neptunium*[7b,e] and *Aspergillus terreus*,[7b,e] further ω-TAs were tested that originated from *Pseudomonas fluorescens*,[18b] *Paracoccus denitrificans*,[18d] and *Arthrobacter citreus*[18e] for the monoamination of nonane-2,6-dione (**1a**) as model substrate (50 mM, Table 1). To evaluate and compare the reaction conditions with previous data, the asymmetric reductive amination employing ω-TAs was performed with

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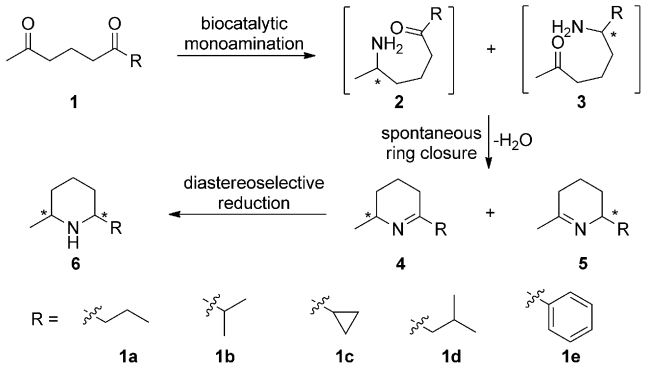


Scheme 1. Chemoenzymatic synthesis of 2,6-disubstituted piperidines **6** through regioselective monoamination of diketones **1** in the key step; spontaneous ring closure of the monoamino ketones **2** or **3** afford Δ1-piperedeines **4** or **5**, which are reduced chemically in a diastereoselective fashion.

alanine as amine donor using an alanine dehydrogenase (AlaDH) or a lactate dehydrogenase (LDH) for removal of pyruvate to shift the reaction to the product amine side. All the tested ω-TAs readily accepted substrate **1a** employing the AlaDH-system, leading to full conversion. The regioselectivity was, in general, perfect, affording only a single regioisomer (**4a**); in the case of the ω-TA from *P. denitrificans* (entry 4) and for *V. fluvialis* (entry 3) a minor amount of regioisomer **5a** (5%) was also observed. Notably, the optical purity of the obtained regioisomer **4a** was, in all cases, perfect (>99% *ee*).

The substrate scope of the enzymes from *P. denitrificans*, *P. fluorescens*, and *A. citreus* was investigated in a next step, testing diketones **1b–e**. In all cases the *S*-selective ω-TAs afforded cyclic imines **4b–e** with perfect regio- and excellent stereoselectivity at high conversions (Table 2). For instance, substrate **1b** was transformed by all enzymes with high conversions (ranging from 68 to 97%) with perfect regio- and stereoselectivity (>99%; Table 2, entry 1–3). The ω-TA from *P. denitrificans* also showed perfect regioselectivity for substrates **1b–e** (Table 2, entries 1, 4, 7, and 10), which is in contrast to the results obtained with substrate **1a** (Table 1, entry 4).

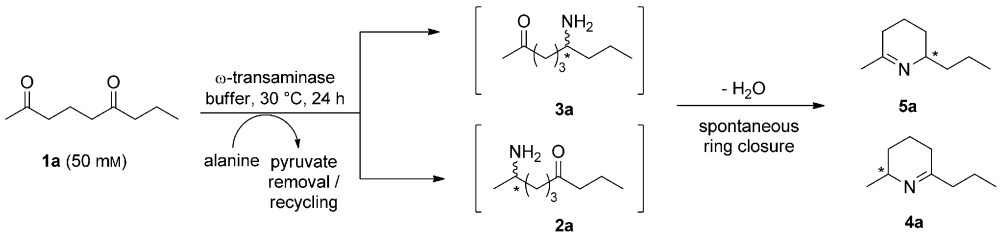
Excellent conversions, regioselectivity and *ee* values were also obtained for substrates **1c** and **1d**. Diketone **1e**, possessing a phenyl group adjacent to one carbonyl moiety, was converted into imine **4e** by *P. denitrificans* with decreased optical purity (87% *ee*) at low conversion (Table 2,

Table 2. Regioselective asymmetric reductive amination of 1,5-diketones **1b–e** employing various *S*-selective ω-transaminases.[a]

Entry	Substrate	ω-Transaminase	Conv. [%]	**4** [%]	*ee* **4** [%]	**5** [%]
1	**1b**	*P. denitrificans*	77	77	>99 (*S*)	<0.1
2	**1b**	*P. fluorescens*	68	68	>99 (*S*)	<0.1
3	**1b**	*A. citreus*	97	97	>99 (*S*)	<0.1
4	**1c**	*P. denitrificans*	82	82	>99 (*S*)	<0.1
5	**1c**	*P. fluorescens*	55	55	>99 (*S*)	<0.1
6	**1c**	*A. citreus*	>99	>99	>99 (*S*)	<0.1
7	**1d**	*P. denitrificans*	84	84	98 (*S*)	<0.1
8	**1d**	*P. fluorescens*	88	88	>99 (*S*)	<0.1
9	**1d**	*A. citreus*	>99	>99	>99 (*S*)	<0.1
10	**1e**[b]	*P. denitrificans*	4	4	87 (*S*)	<0.1
11	**1e**[b]	*P. fluorescens*	92	92	98 (*S*)	<0.1
12	**1e**[b]	*A. citreus*	90	90	98 (*S*)	<0.1

[a] Conversions/compositions and *ee* values were measured by GC-FID analysis. Reaction conditions: Diketone **1b–e** (50 mM), lyophilized *E. coli* cells containing the overexpressed ω-TA (20 mg), PLP (1 mM), NAD^+ (1 mM), L-alanine (10 equiv), 24 h, 30°C, AlaDH system (12 U AlaDH, 11 U FDH, 150 mM ammonium formate). [b] 1,2-Dimethoxyethane (DME; 5 vol%) was added.

Table 1. Monoamination of nonane-2,6-dione (**1a**).[a]

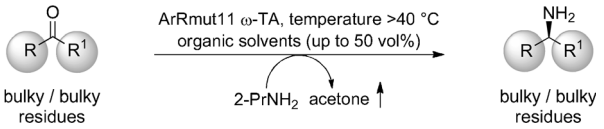


Entry	ω-Transaminase	AlaDH-system				LDH-system				Ref.
		Conv. [%]	**4a** [%]	*ee* **4a** [%]	**5a** [%]	Conv. [%]	**4a** [%]	*ee* **4a** [%]	**5a** [%]	
1	*Chromobacterium violaceum*	>99	>99	>99 (*S*)	<0.1	93	93	>99 (*S*)	<0.1	[9]
2	*Bacillus megaterium*	>99	83[b]	>99 (*S*)	<0.1	98	98	>99 (*S*)	<0.1	[9]
3	*Vibrio fluvialis*	>99	93	>99 (*S*)	7	98	92	>99 (*S*)	6	[9]
4	*Paracoccus denitrificans*	>99	83[b]	>99 (*S*)	5	96	91	>99 (*S*)	5	this study
5	*Pseudomonas fluorescens*	>99	91	>99 (*S*)	<0.1	78	78	>99 (*S*)	<0.1	this study
6	*Arthrobacter citreus*	>99	>99	>99 (*S*)	<0.1	>99	>99	>99 (*S*)	<0.1	this study
7	(*R*)-*Arthrobacter*	>99	87[b]	>99 (*R*)	<0.1	63	63	>99 (*R*)	<0.1	[9]
8	*Aspergillus terreus*	>99	>99	>99 (*R*)	<0.1	56	56	>99 (*R*)	<0.1	[9]
9	*Hyphomonas neptunium*	>99	89[b]	>99 (*R*)	<0.1	78	78	>99 (*R*)	<0.1	[9]

[a] Conversions/compositions and *ee* values were determined by GC-FID analysis. Reaction conditions: Diketone **1a** (50 mM), lyophilized *E. coli* cells containing the overexpressed ω-TA (20 mg), PLP (1 mM), NAD^+ (1 mM), D- or L-alanine (10 equiv), 24 h, 30°C, AlaDH system (12 U AlaDH, 11 U FDH, 150 mM ammonium formate) or LDH system (90 U LDH, 15 U GDH, 10 equiv D- or L-alanine, 150 mM glucose). [b] An unidentified side product was detected by GC analysis; consequently, the percentage of **4a** and **5a** did not sum up to the percentage of the conversion.

entry 10); nevertheless, the ω-TAs from *P. fluorescens* and *A. citereus* yielded imine **4e** in almost enantiopure form (98% *ee*) at high conversions (92 and 90%, respectively; Table 2, entries 11 and 12).

To access the opposite enantiomers of **4a–e**, the round-11 variant of the *R*-selective ω-TA originating from *Arthrobacter* (ArRmut11)[7h] was investigated. This aminotransferase was especially engineered for amination of sterically hindered ketones, giving access to sitagliptin on an industrial scale (Scheme 2). ArRmut11 tolerates elevated tempera-



Scheme 2. Formal asymmetric reduction of sterically demanding ketones using an engineered ω-TA (ArRmut 11) and tolerated reaction conditions.

tures (>40 °C), organic solvents up to 50 vol% and, most important, 2-propylamine as an alternate achiral amine donor instead of alanine.

The initially tested reaction conditions (1 M 2-$PrNH_2$ as amino-source, pH 11.0 and 45 °C) led to a spontaneous intramolecular enamine-mediated ring-closure/elimination sequence that rendered these conditions impractical for our purposes. Consequently, the amount of 2-$PrNH_2$ was decreased (0.5 M) and the pH value adjusted to pH 7.5. When performing the reactions at 30 °C, the conversions varied between 65 and 91% for diketones **1a–e**, resulting in perfect stereo- and regioselectivity (>99%; Table 3, entries 1–4).

Table 3. Regioselective asymmetric reductive amination of 1,5-diketones **1a–e** employing ω-TA round-11 mutant ArRmut11 at different temperatures.[a]

Entry	Temp. [°C]	Substrate	Conv. [%]	**4** [%]	*ee* **4** [%]	**5** [%]
1	30	**1a**	91	91	>99 (*R*)	<0.1
2	30	**1b**	65	65	>99 (*R*)	<0.1
3	30	**1c**	86	86	>99 (*R*)	<0.1
4	30	**1d**	96	96	>99 (*R*)	<0.1
5	30	**1e**	55	55	95 (*R*)	<0.1
6	45	**1a**	98	98	>99 (*R*)	<0.1
7	45	**1b**	96	96	>99 (*R*)	<0.1
8	45	**1c**	99	99	>99 (*R*)	<0.1
9	45	**1d**	99	99	>99 (*R*)	<0.1
10	45	**1e**	64	61	81 (*R*)	<0.1

[a] Conversions/compositions and *ee* values were measured by GC-FID analysis. Reaction conditions: Diketone **1a–e** (50 mM) in DMSO (10 vol%), lyophilized *E. coli* cells containing the overexpressed ω-TA ArRmut11 (20 mg), PLP (1 mM), 2-$PrNH_2$ (0.5 M), pH 7.5.

Only Δ1-piperideine **4e** (phenyl) was obtained with decreased optical purity (95% *ee*), whereas perfect regioselectivity was still maintained (Table 3, entry 5).

Raising the temperature to 45 °C resulted in enhanced conversions (more than 96%) for the aliphatic and cyclic ketones without a decrease in the regio- or stereoselectivity (Table 3, entries 6–9). Although the conversion for substrate **1e** could be enhanced to 64%, a significant drop of the optical purity was observed from 95 to 81% *ee* (Table 3, entry 10). Nevertheless, ArRmut11 turned out to be a promising *R*-selective amination catalyst even for ketones containing a small and a bulky substituent, allowing the reaction to be run at elevated temperatures in the presence of organic solvents and using achiral 2-$PrNH_2$ as amine donor.

To set up the second chiral center to access 2,6-disubstituted piperidines **6a** through diastereoselective reduction, sufficient starting material was required; therefore, the biotransformation was carried out on a preparative scale (78 mg **1a**, 0.5 mmol) using ω-TAs with opposite stereopreference: (*R*)-*Arthrobacter* sp. for the *R*-configured and *C. violaceum* for the *S*-configured enantiomer of **4a**. The reactions were complete after 26 h, as judged by GC, yielding the enantiopure stereoisomers (>99% *ee* in both cases) at >99% conversion. Compound **4a** was readily reduced without further purification through hydrogenation with Pd/C as catalyst, affording the *cis*-piperidines **6a** as reported previously.[9] The same procedure was also applied for the preparation of *cis*-piperidine (2*S*,6*S*)-**6b**, for which also the crystal structure of the corresponding hydrochloride could be solved (Figure 1).

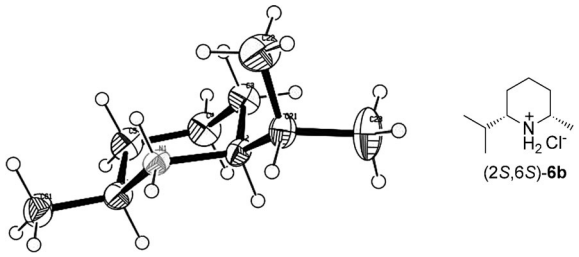
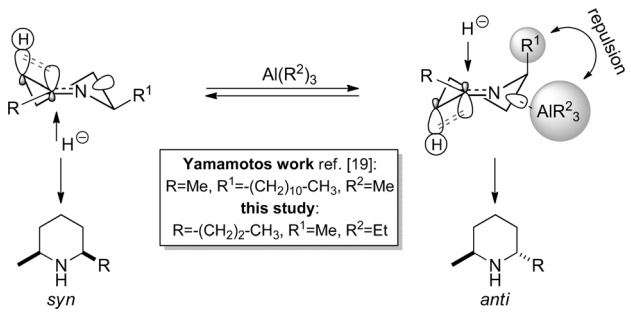


Figure 1. Stereoscopic ORTEP plot of the HCl salt of (2*S*,6*S*)-**6b**. Ellipsoid set at 50% probability. The chlorine atom was omitted for clarity.

Extensive optimization of a published diastereoselective reduction protocol was required to access the *anti*-diastereomers:[19] A Lewis acid mediated conformational change could be triggered by coordination of the Lewis acid (AlR_3) to the nitrogen of the C=N imino bond (Scheme 3); this leads to charge-transfer stabilization during the nucleophilic addition in which the methyl residue occupies the axial position due to steric repulsion. As a result, the hydride prefers an antiperiplanar top-side attack at the imino-function, yielding the desired *anti*-diastereomers.

Notably, because the trialkylaluminum-mediated conformational change with subsequent reduction has been reported only for similar but not identical compounds,[19a,20a] various reaction conditions had to be tested to obtain high *anti*-diastereoselectivity (Table 4 and the Supporting Information). For example, when the reduction of imine **4a** was performed with $LiAlH_4$ in the absence of any Lewis acid, a strong preference for the *syn*-diastereomer was observed: d.r. (*syn*/*anti*) = 98:2 (Table 4, entry 1), which was in agree-



Scheme 3. Adapted mechanistic rational for the diastereoselective reduction leading to the *syn*- and *anti*-diastereomers according to Yamamoto and co-workers.[19]

Table 4. Diastereoselective reduction of cyclic imine **4a** under various reaction conditions.[a]

Entry	Solvent	Lewis acid ([equiv])	Reducing agent ([equiv])	Reaction conditions	**6a** d.r. (*syn*/*anti*)[b]
1	Et_2O	–	$LiAlH_4$ (5)	−78 °C, 3 h	98:2
2	THF	Me_3Al in heptane (10)	$LiAlH_4$ (10)	−78 °C, 2 h	40:60
3	THF	Me_3Al in heptane (7)	$LiAlH_4$ (7)	−78 °C, 3 h	40:60
4	THF	Me_3Al in heptane (10)	DiBAlH (10)	−78 °C, 3 h	99:1
5	THF	iBu_3Al in heptane (10)	$LiAlH_4$ (10)	−78 °C, 3 h	99:1
6	THF	Et_2OEtAl in toluene (10)	$LiAlH_4$ (10)	−78 °C, 2 h	31:69
7	THF	Et_3Al in toluene (10)	$LiAlH_4$ (10)	−78 °C, 2 h	24:76
8	THF	Et_3Al in hexane (10)	$LiAlH_4$ (10)	−78 °C, 2 h	27:73
9	THF	Et_3Al in hexane (10)	$LiAlH_4$ (5)	−78 °C, 2 h	16:84

[a] Due to the instability of compound **4a**, the enzymatic reductive amination was performed immediately before the reduction. Reaction conditions: diketone **1a** (78 mg, 0.5 mmol, 50 mM), ω-TA from *C. violaceum* or (*R*)-*Arthrobacter* sp., PLP (1 mM), NAD^+ (1 mM), L-alanine (10 equiv), NH_4HCOO (150 mM), 11 U FDH, 12 U AlaDH; 26 h, 30 °C, 120 rpm followed by extraction with Et_2O and careful evaporation (650 mbar, 35 °C). The residue was diluted with the solvent indicated and cooled to −78 °C. The yields depended on the work-up procedure but were always in excess of 60 %. [b] The d.r. was determined by GC-FID and ^1H NMR analyses of the crude reaction mixture.

ment with theory. The established reaction conditions adapted from literature (Me_3Al–$LiAlH_4$)[19a] led to the desired *anti*-isomers, however, with only moderate stereoselectivity (d.r. (*syn*/*anti*) = 40:60; Table 4, entries 2 and 3).

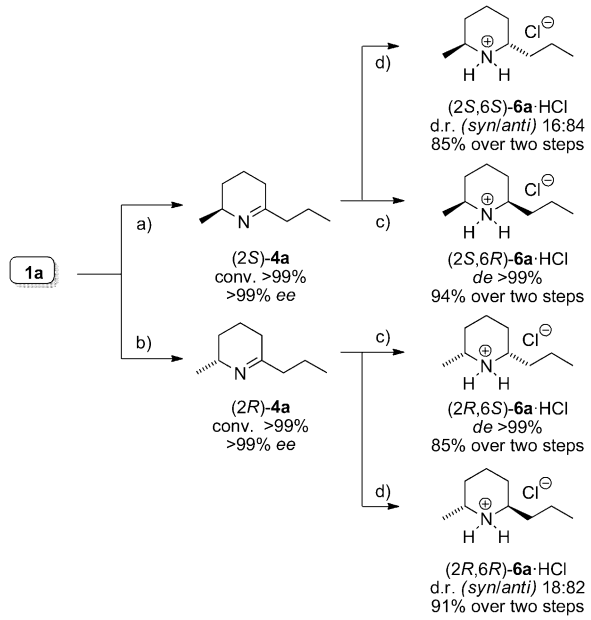
We assumed that the lack of steric repulsion of the residue R^1 (in our study CH_3 in contrast to n-$C_{11}H_{23}$ in Yamamoto's work) with the coordinating Lewis acid Me_3Al is responsible for the diminished stereoselectivity. However, the use of a more bulky reducing agent such as diisobutylaluminum hydride (DIBAL-H) (Table 4, entry 4) also failed to induce *anti*-stereoselectivity; only the *syn*-isomer could be detected by GC and ^1H NMR spectroscopic analysis of the crude reaction mixture. Increasing the size of the Lewis acid (iBu_3Al instead of Me_3Al) afforded exclusively the *syn*-diastereomer as the sole product, probably due to the lack of coordination to the nitrogen atom (Table 4, entry 5). Promising results were obtained when Et_2OEtAl was applied; in this case, the *anti*-isomer was obtained in a good ratio of 31:69 (Table 4, entry 6). Further optimization studies were conducted, and the best results were obtained with 10 equiv of Et_3Al and 5 equiv $LiAlH_4$, which afforded the *anti*-diastereomer finally in a ratio of d.r. (*syn*/*anti*) = 16:84 (Table 4, entry 9). It was found that the amount and type of solvent as well as the order of addition has a significant influence on the d.r., and a large excess of reagents were crucial for high stereocontrol, as was also previously reported by other groups[20a] (see also the Supporting Information).

Finally, the overall chemoenzymatic synthetic concept was demonstrated for the synthesis of all four stereoisomers of **6a**, thus including the natural alkaloids dihydropinidine (*cis*-isomers) and *epi*-dihydropinidine (*trans*).[21] (+)-Dihydropinidine (2*S*,6*R*)-**6a** is a potential antifeedant against the pine weevil *Hylobius abietis*, whereas (−)-*epi*-dihydropinidine (2*S*,6*S*)-**6a** acts against the eastern spruce budworm; both compounds have been the target of numerous synthetic efforts resulting in a range of different synthetic strategies. For instance, elegant asymmetric syntheses were reported based on chiral auxiliaries and chiral precursors,[20] whereas racemic syntheses were commonly realized through reductive amination, reduction of the related pyridine, rearrangement of *N*-acyl lactams, or transition-metal-catalyzed intramolecular hydroamination.[22] Although some syntheses are highly sophisticated, long reaction sequences (up to 14 steps),[20f,k] the use of protecting groups or stoichiometric amount of auxiliaries[20c,h] and also low levels of induced chirality hampered their overall efficiency.

By applying the presented chemoenzymatic approach, all four diastereomers of **6a** were obtained within just three steps in a linear sequence. The *syn*-diastereomer (2*S*,6*R*)-**6a**, for example, was obtained in diastereomerically pure form with an overall yield of 71 %, whereas the *anti*-isomer (2*S*,6*S*)-**6a** was obtained with 69 % yield with a d.r. of 16:84 in favor of the *anti*-isomer (Scheme 4). This approach represents, for the first time, a convenient access to all diastereomers by the appropriate choice of the ω-TA with highest overall yield to date.

Conclusions

Enantiocomplementary ω-transaminases were investigated for the successful regioselective monoamination of selected 1,5-diketones. The investigated ω-TAs converted the substrates, in general, with perfect regio- and stereoselectivity, leading to the designated amino ketones, which underwent (spontaneous) intramolecular ring closure. The resulting Δ1-piperideines were then further converted into the corresponding *syn*- as well as *anti*-diastereomers, employing stereodivergent chemical reduction conditions. The chemoenzymatic approach was applied for the synthesis of all four diastereomers of the alkaloids dihydropinidine and *epi*-dihydropinidine, representing the shortest and highest yielding route to date. Key to the *anti*-diastereomers was a Lewis acid mediated change in conformation during the reduction step.



Scheme 4. Chemoenzymatic synthesis of all four stereoisomers of the alkaloids dihydropinidine and *epi*-dihydropinidine **6a**. Reagents and conditions: a) Diketone **1a** (78 mg, 0.5 mmol, 50 mM), ω-TA from *C. violaceum*, PLP (1 mM), NAD^+ (1 mM), L-alanine (10 equiv), NH_4HCOO (150 mM), 11 U FDH, 12 U AlaDH; 26 h, 30 °C, 120 rpm; b) conducted as for (a) but with (*R*)-ω-TA from *Arthrobacter* sp. and D-alanine; c) Pd/C, H_2, 4 h, 22 °C; d) Et_3Al in hexane (5.0 mmol), $LiAlH_4$ in THF (2.5 mmol), THF, −78 °C, 2 h.

Experimental Section

All starting materials were obtained from commercial suppliers and were used as received unless stated otherwise. The reactions were carried out with standard Schlenk techniques under an Ar/N_2 atmosphere using oven-dried (120 °C) glassware. Solvents were dried and purified by conventional methods prior to use. Preparative chromatographic separations were performed by column chromatography on Merck silica gel 60 (0.063–0.200 μm). Solvents for flash chromatography (petroleum ether/ethyl acetate) were distilled before use; petroleum ether refers to the fraction boiling between 60–90 °C. TLC was carried out with precoated aluminum sheets (TLC Silica gel 60 F254, Merck) with detection by UV (254 nm) and/or by staining with anisaldehyde solution [4-anisaldehyde (1.00 g), conc. H_2SO_4 (2.00 mL), EtOH (100 mL)] or cerium molybdenum solution [phosphomolybdic acid (25 g), $Ce(SO_4)_2{\cdot}H_2O$ (10 g), conc. H_2SO_4 (60 mL), H_2O (940 mL)]. Optical rotation was measured at 20 °C with a PerkinElmer Polarimeter 341 against the sodium D-line. GC-MS spectra were recorded with an Agilent 7890A GC-system, equipped with an Agilent 5975C mass selective detector and a HP-5 MS column [30 m × 0.25 mm × 0.25 μm; helium as carrier gas (flow = 0.55 mL min^{-1})]. ^1H and ^{13}C NMR spectra were recorded at 20 °C with a 300 Bruker NMR unit; chemical shifts are given in ppm relative to Me_4Si (^1H: $\delta = 0.0$ ppm) or relative to the resonance of the solvent $CDCl_3$ (^1H: $\delta = 7.26$ ppm; ^{13}C: $\delta = 77.0$ ppm).

L-Lactate dehydrogenase from rabbit muscle (lyophilized powder, 136 U mg^{-1} protein [one unit will reduce 1.0 μmol of pyruvate to L-lactate per min at pH 7 at 25 °C], catalogue no. 61309) was purchased from Sigma–Aldrich. Glucose dehydrogenase (lyophilized powder, 25 U mg^{-1} [one unit will oxidize 1 μmol β-D-glucose to D-glucono-δ-lactone per min at pH 8.0 and 37 °C], catalogue no. B-4) was purchased from X-zyme (Düsseldorf, Germany). Formate dehydrogenase from *Candida boidinii* (aqueous buffer solution with glycerol, 215 U ml^{-1} [one unit will oxidize 1.0 μmol of formate to CO_2 per min at pH 7.6 at 37 °C], catalogue no. FDH 002 [lyophilized powder 2.2 U mg^{-1}]) and β-NAD-free acid were purchased from Codexis.

Lyophilized *E. coli* cells containing overexpressed ω-transaminases were prepared as previously reported.[7a,b,g] Purified recombinant L-alanine dehydrogenase was prepared as described recently.[7b]

Biotransformations (AlaDH-system; analytical scale): Lyophilized cells of *E. coli* containing the corresponding overexpressed ω-transaminase (20 mg) were rehydrated in a potassium phosphate buffer (pH 7.0, 100 mM) containing PLP (1 mM), NAD^+ (1 mM), ammonium formate (150 mM), FDH (11 U), Ala-DH (12 U), and D- or L-alanine (250 mM for 25 mM substrate and 500 mM for 50 mM substrate) at RT for 30 min. The substrate was added afterwards and the reductive amination was carried out at 30 °C in an Eppendorf orbital shaker (650 rpm) for 24 h. After the period of time, the reaction was stopped by the addition of saturated Na_2CO_3 (200 μL) and vigorous shaking. The residue was extracted with EtOAc (2 × 500 μL), the combined organic layers were dried over $MgSO_4$, and an aliquot was withdrawn for further analysis.

Biotransformations (LDH-system, analytical scale): Lyophilized cells of *E. coli* containing the corresponding overexpressed ω-transaminase (20 mg) were rehydrated in a potassium phosphate buffer (pH 7.0, 100 mM) containing PLP (1 mM), NAD^+ (1 mM), glucose (150 mM), LDH (90 U), GDH (15 U), and D- or L-alanine (250 mM for 25 mM substrate and 500 mM for 50 mM substrate) at RT for 30 min. The substrate was then added and the reductive amination was carried out at 30 °C in an Eppendorf orbital shaker (650 rpm) for 24 h. After the period of time, the reaction was stopped by addition of saturated Na_2CO_3 (200 μL) and vigorous shaking. The residue was extracted with small amounts of EtOAc (2 × 500 μL) and the combined organic layers were dried over $MgSO_4$ and an aliquot was withdrawn for further analysis.

Biotransformations on preparative scale (representative procedure): Lyophilized cells of *E. coli* containing overexpressed ω-TA from either *Chromobacterium violaceum* or (*R*)-*Arthrobacter* sp. (225 mg) were rehydrated in a KPi buffer (10 mL, pH 7.0, 100 mM) containing PLP (1 mM), NAD^+ (1 mM), ammonium formate (150 mM), FDH (11 U), Ala-DH (12 U) and D-/L-alanine (500 mM) at 22 °C for 30 min. The corresponding substrate **1a–e** (0.5 mmol) was added and the reaction was shaken for 26 h at 30 °C. Saturated Na_2CO_3 solution was added (1.00 mL) and the reaction mixture was extracted with EtOAc, toluene or Et_2O (4 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and converted without further purification.

(2*S*)-2-Methyl-6-propyl-2,3,4,5-tetrahydropyridine [(2*S*)-4a]: The product was obtained in >99% conversion with an optical purity of >99% *ee* as determined by GC analysis. Product **4a** could be characterized when using Et_2O for extraction, followed by careful concentration with a rotary evaporator (650 mbar, 35 °C). ^1H NMR (300 MHz, $CDCl_3$): $\delta =$ 0.80 (t, $^3J_{9,8} = 7.4$ Hz, 3H; 9-H), 1.08 (d, $^3J_{Me,2} = 6.4$ Hz, 3H; Me at C-2), 1.45 (m_c, 4H; 4-H and 8-H), 1.60 (m_c, 2H; 3-H), 1.98 (m_c, 2H; 5-H), 2.04 (t, $^3J_{7,8} = 7.4$ Hz, 2H; 7-H), 3.34 ppm (m_c, 1H; 2-H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.0$ (C-9), 18.5 (C-4), 20.5 (C-8), 23.7 (Me at C-2), 28.6 (C-5), 29.3 (C-3), 42.9 (C-7), 52.8 (C-2), 171.6 ppm (C-6). GC-MS (EI+, 70 eV): *m/z* (%): 139 [M^+] (22), 124 [$C_8H_{14}N^+$] (46), 111 [$C_7H_{13}N^{\cdot+}$] (58), 96 [$C_6H_{10}N^+$] (100). Determination of conversion by means of achiral GC analysis: column HP-5 (Agilent); carrier gas = helium; 60 °C to 150 °C at 5 °C min^{-1}; $t_R = 10.08$ (**4a**), 10.82 (**5a**), 18.85 min (**1a**). Determination of enantiomeric excess by means of chiral GC analysis (see below): column DEX-CB (Agilent; CP-Chirasil); carrier gas = helium; 80 °C (5 min isotherm) to 90 °C at 0.5 °C min^{-1}; $t_R = 11.05$ [(2*R*)-**4a**], 11.45 min [(2*S*)-**4a**].

(2*R*)-2-Methyl-6-propyl-2,3,4,5-tetrahydropyridine [(2*R*)-4a]: The product was obtained in 97% conversion with an optical purity of >99% *ee* as determined by chiral and achiral GC (see above). Analytical data are in full agreement with those reported for its enantiomer.

(2*S*)-6-Ispropyl-2-methyl-2,3,4,5-tetrahydropyridine [(2*S*)-4b]: The product was obtained in >99% conversion with an optical purity of >99% *ee* as determined by GC. GC-MS (EI+, 70 eV): *m/z* (%): 139 [M^+] (26), 124 [$C_8H_{14}N^+$] (100), 96 [$C_6H_{10}N^+$] (36). Determination of conversion by means of achiral GC analysis: column HP-5 (Agilent); carrier gas = helium; 80 °C to 180 °C at 10 °C min^{-1}; $R_t = 3.22$ (**4b**), 5.19 min (**1b**). De-

termination of enantiomeric excess by means of chiral GC analysis (see below): column DEX-CB (Agilent; CP-Chirasil); carrier gas = helium; 80 °C (10 min isotherm) to 90 °C at 0.5 °C min^{-1}; t_R = 7.18 [(2*R*)-**4b**], 7.95 min [(2*S*)-**4b**].

(2*S*)-6-Cyclopropyl-2-methyl-2,3,4,5-tetrahydropyridine [(2*S*)-4c]: The product was obtained in more than 99% conversion with an optical purity of >99% *ee* as determined by GC. GC-MS (EI+, 70 eV): *m*/*z* (%): 137 [M^+] (44), 122 [$C_8H_{12}N^+$] (100), 41 [$C_3H_5^+$] (18). Determination of conversion by means of achiral GC analysis: column HP-5 (Agilent); carrier gas = helium; 100 °C to 200 °C at 10 °C min^{-1}; t_R = 3.88 (**4c**), 6.75 min (**1c**). Determination of enantiomeric excess by GC analysis on chiral phase: column DEX-CB (Agilent; CP-Chirasil); carrier gas = helium; 80 °C (10 min isotherm) to 90 °C at 0.5 °C min^{-1}; t_R = 15.63 [(2*R*)-**4c**], 16.45 min [(2*S*)-**4c**].

(2*S*)-6-Isobutyl-2-methyl-2,3,4,5-tetrahydropyridine [(2*S*)-4d]: The product was obtained in >99% conversion with an optical purity of >99% *ee* as determined by GC. GC-MS (EI+, 70 eV): *m*/*z* (%): 154 [M^+] (2), 138 [$C_9H_{16}N^+$] (28), 111 [$C_7H_{13}N^+$] (65), 96 [$C_6H_{10}N^+$] (100). Determination of conversion by GC analysis on an achiral phase: column HP-5 (Agilent); carrier gas = helium; 100 °C to 200 °C at 10 °C min^{-1}; t_R = 3.06 (**4d**), 4.69 min (**1d**). Determination of enantiomeric excess by GC on a chiral phase: column DEX-CB (Agilent; CP-Chirasil); carrier gas = helium; 80 °C (10 min isotherm) to 90 °C at 0.5 °C min^{-1}; t_R = 13.65 [(2*R*)-**4d**], 14.25 min [(2*S*)-**4d**].

(2*R*)-2-Methyl-6-phenyl-2,3,4,5-tetrahydropyridine [(2*R*)-4e]: ω-TA from (*R*)-*Arthrobacter* (225 mg) and D-alanine as amino source were used. The product was obtained in >99% conversion with an optical purity of >99% *ee* as determined by GC. GC-MS (EI+, 70 eV): *m*/*z* (%): 173 [M^+] (61), 172 [$C_{12}H_{15}N^+$] (100), 158 [$C_{11}H_{12}N^+$] (22), 104 [$C_7H_6N^+$] (80), 77 [$C_6H_5^+$] (20). Determination of conversion by GC analysis on an achiral phase: column HP-5 (Agilent); carrier gas = helium; 100 °C to 250 °C at 10 °C·min^{-1}; t_R = 7.68 (**4e**), 9.05 min (**1e**). Determination of enantiomeric excess by GC analysis on achiral phase: column DEX-CB (Agilent; CP-Chirasil); carrier gas = helium; 100 °C to 150 °C at 2.5 °C min^{-1}; t_R = 16.22[(2*R*)-**4e**], 16.53 min [(2*S*)-**4e**].

Diastereoselective Reductions (general procedure for a *syn*-selective approach): A crude solution of the biotransformation containing the corresponding cyclic imine **4a**–**e** was treated with palladium on activated charcoal (10% wt^{-1}). The mixture was stirred vigorously and a stream of hydrogen was bubbled through the solution for 4 h. After full conversion of the starting material was detected by GC or GC-MS analysis, the solution was filtered through a small plug of Celite 545 and cooled to 0 °C. Etherial HCl solution was added dropwise (ca. 5 equiv), the solvent was removed and the precipitate was collected. If necessary, all products could be recrystallized from pure $CHCl_3$.

(2*S*,6*R*)-2-Methyl-6-propyldihydropiperidine·Hydrochloride (−)-Dihydropinidine ([2*S*,6*R*]-6a·HCl): The product was obtained as a colorless solid in the form of its HCl salt in 94% yield (82 mg, 0.47 mmol, *de* > 99%). Obtained spectroscopic data were in full agreement with those previously reported.[20a] M.p. 243–245 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.85 (t, $^3J_{9,8}$ = 7.2 Hz, 3H; 9-H), 1.28–1.48 (m, 3H; 8-H and 4-H$_a$), 1.50 (d, $^3J_{Me,2}$ = 6.2 Hz, 3H; Me at C-2), 1.52–1.65 (m, 2H; 5-H$_a$), 1.68–1.78 (m, 3H; 3-H and 7-H$_a$), 1.80–1.94 (m, 2H; 4-H$_b$ and 5-H$_b$), 1.96–2.10 (m, 1H; 7-H$_b$), 2.88 (m$_c$, 1H; 6-H), 3.05 (m$_c$, 1H; 2-H) 9.02 ppm (br. d, 2H; NH_2^+). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.8 (C-9), 18.8 (C-4), 19.5 (Me at C-2), 22.9 (C-4), 27.5 (C-5), 30.7 (C-3), 35.2 (C-7), 54.5 (C-6), 58.3 ppm (C-2). GC-MS (EI+, 70 eV): *m*/*z* (%): 98 [$C_6H_{12}N^+$] (100). $[\alpha]_D^{20}$ = −12.2 (*c* 0.5, EtOH, *de* > 99%); literature value for the opposite enantiomer:[20d] $[\alpha]_D^{20}$ = +12.4 (*c* 0.1, EtOH, *de* > 99%).

(2*R*,6*S*)-2-Methyl-6-propyldihydropiperidine·Hydrochloride ([2*R*,6*S*]-6a·HCl): The product was obtained as a colorless solid in the form of its HCl salt in 85% yield (75 mg, 0.43 mmol, *de* > 99%). Spectroscopic data were in full agreement with those reported for its enantiomer. $[\alpha]_D^{20}$ = + 12.1 (*c* 0.9, EtOH, *de* > 99%); literature value:[20d] $[\alpha]_D^{20}$ = +12.4 (*c* 0.1, EtOH, *de* > 99%).

(2*S*,6*S*)-2-Methyl-6-propyldihydropiperidine·Hydrochloride (−)-*epi*-dihydro-pinidine ([2*S*,6*S*]-6a·HCl): Due to the instability of imine **4a**, the biotransformation was performed with ω-TA from *C. violaceum* on a preparative scale (78 mg, 0.5 mmol) directly before the reduction (see above). After extraction with Et_2O (4 × 5 mL), the organic layer was dried over $MgSO_4$, filtered, and carefully concentrated with a rotary evaporator (35 °C, 650 mbar). The residue was then diluted with anhydrous THF (10 mL) and cooled to −78 °C before Et_3Al (1 M in hexane, 5.0 mL, 5 mmol) was added dropwise over 10 min. The mixture was stirred for an additional 10 min before $LiAlH_4$ (2 M in THF, 1.25 mL, 2.5 mmol) was added dropwise. After stirring for 3 h at −78 °C, saturated Rochells salt solution (30 mL) was added and the mixture was warmed to RT. When the phases resolved, the aqueous phase was extracted with MTBE (4 × 10 mL) and the combined organic layers were dried over $MgSO_4$. Precipitation with etherial HCl solution afforded the product in the form of its HCl salt in 91% yield over two steps (81 mg, 0.46 mmol, d.r. (*syn*/*anti*) 18:82). Recrystallization from $CHCl_3$ gave diastereomerically pure *epi*-dihydropinidine as a colorless solid. Spectroscopic data were full in agreement with those previously reported.[20a] M.p. 165–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, $^3J_{9,8}$ = 7.3 Hz, 3H; 9-H), 1.32–1.43 (m, 2H; 8-H), 1.46 (d, $^3J_{Me,2}$ = 6.7 Hz, 3H; Me at C-2), 1.54–1.76 (m, 5H; 4-H and 5-H$_a$ and 7-H$_a$), 1.85–2.02 (m, 3H; 3-H$_b$ and 5-H$_b$ and 7-H$_b$), 3.27 (br. s, 1H; 6-H), 3.52 (br. s, 1H; 2-H), 9.28 ppm (br. s, 2H; NH_2^+). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.9 (C-9), 16.9 (Me at C-2), 17.5 (C-4), 19.1 (C-8), 26.4 (C-7), 19.0 (C-5), 32.9 (C3), 48.0 (C-6), 51.6 ppm (C-2). GC-MS (EI+, 70 eV): *m*/*z* (%): 98 [$C_6H_{12}N^+$] (100). $[\alpha]_D^{30}$ = −3.9 (*c* 0.89, EtOH, *de* > 99%); literature value:[20a] $[\alpha]_D^{25}$ = −3.61 (*c* 0.166, 96% EtOH, *de* > 99%).

(2*R*,6*R*)-2-Methyl-6-propyldihydropiperidine·Hydrochloride ([2*R*,6*R*]-6a·HCl): The product was obtained as a colorless solid in the form of its HCl salt in 85% yield (75 mg, 0.43 mmol, *de* > 99%) after the biotransformation with (*R*)-*Arthrobacter* sp. and subsequent reduction. The crude product was recrystallized from $CHCl_3$. Spectroscopic data were full in agreement with those reported for its enantiomer. $[\alpha]_D^{30}$ = +3.7 (*c* 0.7, EtOH, *de* > 99%); literature value for the opposite enantiomer:[20d] $[\alpha]_D^{25}$ = −3.61 (*c* 0.166, 96% EtOH, *de* > 99%).

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Biocatalysis

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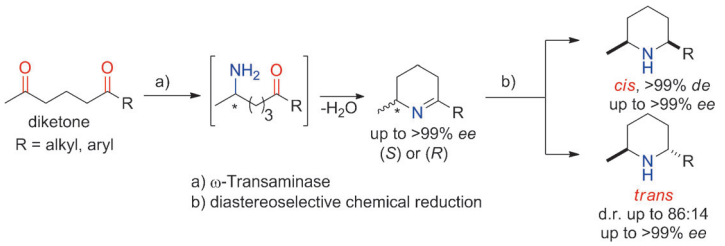
Chemoenzymatic Synthesis of All Four Diastereomers of 2,6-Disubstituted Piperidines through Stereoselective Monoamination of 1,5-Diketones



Being selective: By applying either an *S*- or *R*-stereoselective ω-transaminase, the (*S*)- or (*R*)-Δ1-piperideines were accessible through regioselective monoamination of 1,5-diketones (see scheme). Diastereoselective reduction of the optically pure Δ1-piperideines gave access to all four diastereomers of 2,6-dialkyl piperidines.

