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ω-Transaminases for the amination of functionalised cyclic ketones

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The potential of a number of enantiocomplementary ω-transaminases (ω-TAms) in the amination of cyclic ketones has been investigated. After a preliminary screening of several compounds with increasing complexity, different approaches to shift the equilibrium of the reaction to the amine products were studied, and reaction conditions (temperature and pH) optimised. Interestingly, 2-propylamine as an amine donor was tolerated by all five selected ω-TAms, and therefore used in further experiments. Due to the higher conversions observed and interest in chiral amines studies then focused on the amination of α-tetralone and 2-methylcyclohexanone. Both ketones were aminated to give the corresponding amine with at least one of the employed enzymes. Moreover, the amination of 2-methylcyclohexanone was investigated in more detail due to the different stereoselectivities observed with TAms used. The highest yields and stereoselectivities were obtained using the ω-TAm from *Chromobacterium violaceum* (CV-TAm), producing 2-methylcyclohexylamine with complete stereoselectivity at the (1*S*)-amine position and up to 24 : 1 selectivity for the *cis* : *trans* [(1*S*,2*R*) : (1*S*,2*S*)] isomer.

Introduction

Single isomer chiral amines are one of the most common functional groups found in natural products and pharmaceutical compounds including, sitagliptin the antihyperglycemic drug for the treatment of diabetes,[1] the antihypertensive dilevalol,[2] and (*S*)-rivastigmine used for the treatment of Alzheimer's disease.[3,4] Enantiopure amines are therefore highly desirable building blocks for the development of new pharmaceuticals. The frequent occurrence of such chiral amines has also highlighted interest in their preparation as synthons for use in the pharmaceutical sector. For instance, the aminotetraline motif is present in a variety of pharmaceuticals, such as the antidepressants sertraline and norsertraline with an α-aminoteraline core,[5–7] and rotigotine a treatment for Parkinson's disease containing a β-aminotetraline core unit.[8,9]

Traditionally single isomer chiral amines are generated from racemic mixtures using crystallisation methods, or they can be synthesised using chiral auxiliaries.[7,10–12] In addition more recently a variety of organocatalytic, metal-dependent as well as chemo-enzymatic dynamic kinetic resolution methods have been developed to produce enantiopure amines.[13–15] The requirement for metals in some of these systems such as lipase-catalysed dynamic kinetic resolutions is however a major backdraw when considering the sustainability of the process.[14] An alternative method to generate enantiopure amines that is currently attracting significant interest is the use of ω-transaminases (ω-TAms).[16–20] Despite the improved sustainability with this biocatalytic approach, one problem has been the issue of shifting the reaction equilibrium towards the amine product. However, in recent years efforts have been focussed on the development of methods to overcome this unfavourable equilibrium, *via* the chemical or enzymatic removal of the co-product or use of an excess of amine donor.[1,21–30] The incorporation of enzymatic cascades has been particularly successful, including reuse of the co-product in a multi-enzymatic cascade with a carboligation step.[31] Several of these studies used ω-TAms for the preparation of pharmaceutical intermediates or bioactive compounds,[24,25,27–36] and have also lead to an industrial process.[1]

Here we describe the use of several ω-TAms in the asymmetric amination of several cyclic substrates. Moreover, different methods to shift the equilibrium towards the desired amine product were compared and reaction parameters optimised with the model compound cyclohexanone **1**. The amination of two selected substrates was then investigated in further detail, to establish the different stereoselectivities of the ω-TAms used.

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Results and discussion

Our aim was to test a panel of promising and recently reported ω-TAms against a range of functionalised cyclic ketones, including sterically challenging substrates, to determine substrate type acceptance by the selected ω-TAms. These were (*S*)-ω-TAms from *Chromobacterium violaceum* DSM30191 (CV-TAm),[37] *Vibrio fluvialis* (Vf-TAm),[38] *Klebsiella pneumoniae* KPN_0799 (Kp-TAm),[39] and *Pseudomonas putida* PP_3718 (Pp-TAm).[36] Also the (*R*)-ω-TAms from *Mycobacterium vanbaalenii* (Mv-TAm),[40] and a variant from *Arthrobacter* sp. (ArRMut11).[1] CV-TAm and Vf-TAm were selected as they have been used with a range of acyclic substrates and cyclic compounds such as cyclohexanone,[19,20,31,37,38,41,42] while Pp-TAm has recently been reported to accept dopamine[36] and Kp-TAm[39] was selected as a promising TAm from screening our UCL TAm library. Mv-TAm has been used almost exclusively with ketones/amines as acyclic moietes,[40,43,44] and ArRMut11 with 1,3-ketoamides to generate the (*R*)-functionality in sitagliptin as well as for example bicyclic tetralone and chromone substrates and a carbazolamine.[1,28,34,45]

Ten ketone substrates **1–10** were selected, which would generate the corresponding amines **1a–10a**, including cyclohexanone **1** and cyclopentanone **5** to determine the influence of ring size, as well as diketones (**2**,**6**), α,β-unsaturated ketones (**4**,**7**,**9**), α-tetralone **8** and ketones with α-methyl groups (**3**, and camphor **10**). Initial assays used the ω-TAms (crude cell lysates) and either (*R*)- or (*S*)-α-methylbenzylamine (MBA) **11** as the amine donor, depending on the selectivity of the transaminase, with substrates **1–10**: the product acetophenone was detected by HPLC at 254 nm (Fig. 1).[37] This preliminary assay method highlighted substrates for further investigation. Control reactions were performed in the absence of amine acceptor and low levels of acetophenone were detected which were subtracted from assay results with amine acceptor present. The results indicated that several ketones showed good levels of conversion with the ω-TAms selected, particularly CV-TAm, Pp-TAm and ArRMut11. Cyclohexanone **1** was the best cyclic substrate for most ω-TAms with conversions of up to 40%. Interestingly, while the substitution at the α-position on the six-membered ring in 2-methylcyclohexanone **3** was particularly well tolerated with only slightly lower conversions, the presence of a conjugated C=C double bond led to significantly less activity with **4**. A similar reactivity pattern was observed with the five-membered rings: while cyclopentanone **5** was readily accepted by several ω-TAms, the corresponding enone **7** had negligible reactivity with all the ω-TAms used. This presumably reflects the modified steric demands and reactivity in the α,β-enones and less electrophilic carbonyl moiety. The diketones **2** and **6**, and bicyclic compound **10** had negligible levels of acceptance. The bicyclic systems α-tetralone **8** (Kp-TAm, ArRMut11) and 8a-methyl-3,4,8,8a-tetrahydro-1,6-(1*H*,7*H*)-naphthalenedione **9** were accepted with conversions at levels of 5–10% (ArRMut11), with even lower conversions for several of the other TAms. Tetralone **8** has previously been used as a substrate with ArRMut11 together with co-product removal to shift the equilibrium toward the desired amine, so these results using two TAms and no co-product removal were promising.[28,45] The reaction of ArRMut11 with **9** has also recently been reported, and although products were observed by LC-MS analysis no products could be isolated.[30] With the exception of the Vf-TAm which showed in general poor activity

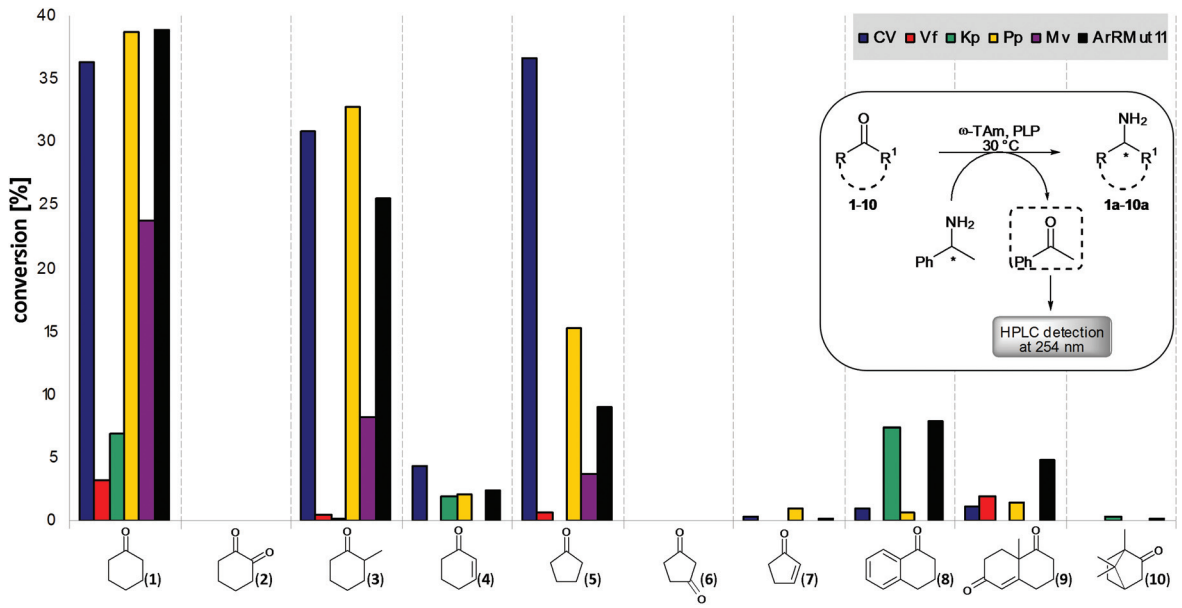
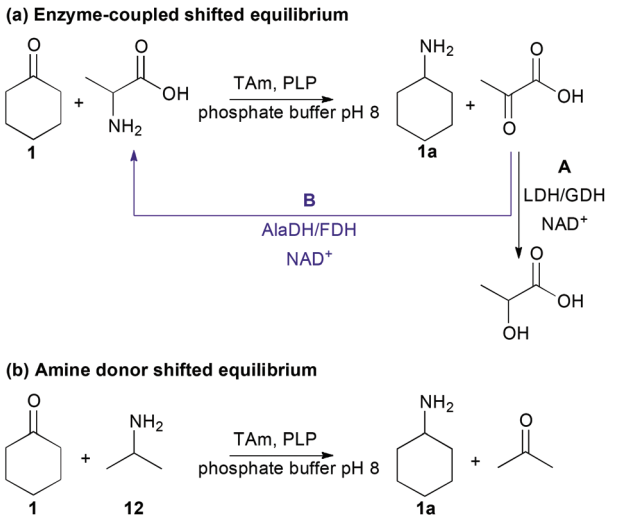


Fig. 1 Initial screening results giving conversions for a range of cyclic ketone substrates employing the selected ω-TAms. (*R*)- or (*S*)-MBA **11** were used as amine donors and the product acetophenone was detected by HPLC analysis (see Scheme). All reactions were performed in triplicate and standard deviations were less than 10%.

with cyclic substrates, the five other ω-TAms were studied in more detail, initially with ketone **1**.

To facilitate a better comparability of the different TAm enzymes, the specific activity of crude cell extracts in the amination of **1** was investigated. In addition, freeze dried samples of clarified lysate were prepared as convenient preparations for storage and usage. The highest activity was obtained using crude cell extracts of CV-TAm and ArRMut11, with 0.58 U mg^{-1} and 0.39 U mg^{-1} of total protein, respectively (see Experimental). After lyophilisation the activity was decreased in most cases, though not significantly, with residual activities in the range of 55–100%. For this reason and ease of usage lyophilised cell extracts were used in all of the following studies.

To identify optimal reaction conditions, as well as investigating the reaction temperature and pH, a study comparing the use of MBA **11** to both enzyme-coupled and excess amine donor methods to shift the equilibrium towards the product amine was conducted using cyclohexanone **1** (Scheme 1). Two enzyme-coupled systems were used (Scheme 1a), where L- or D-alanine was used as the amine donor and the co-product pyruvate was removed by either a L-lactate dehydrogenase (LDH) (Scheme 1a **A**) or recycled by an alanine dehydrogenase (AlaDH) for (*S*)-selective ω-TAms (Scheme 1a **B**).[18,28,46] The nicotinamide cofactor (NADH) required was recycled by employing standard techniques using formate dehydrogenase (FDH) or glucose dehydrogenase (GDH).[18,28,46] An alternative enzyme-independent method was investigated using 2-propylamine (isopropylamine, IPA) **12** as the amine donor which generates acetone as a co-product (Scheme 1b).[1,37,42]



Scheme 1 Methods used to shift the TAm reaction equilibrium toward the desired amine product, using **1** as the ketone acceptor. (a) Enzyme-coupled shifted equilibrium; (b) amine donor shifted equilibrium.

Most enzymatic reactions proceeded with good conversions of up to 95% (Scheme 1, Table 1), especially compared to the conversions observed in the initial screening experiments using MBA **11** (7–39%), confirming the benefits of using shifting systems in ω-TAm reactions. Interestingly, four of the ω-TAm reactions showed similar or higher conversions using the IPA (**12**)-amine donor reaction systems compared to the use of enzyme coupled-systems. Only the Mv-TAm showed a slightly lower conversion (39%) compared to the AlaDH/FDH-system (50%). In general, this broad acceptance of IPA **12** as amine donor was unexpected since IPA does not appear to be an amine donor for many ω-TAms.[47] To date, only a few have demonstrated high tolerance towards IPA **12** such as the engineered variant ArRMut11, and also CV-TAm which was used in the synthesis of (2*S*,3*S*)-2-aminopentane-1,3-diol, facilitating the use of this low cost amine donor to shift the equilibrium towards the product.[1,42] The observation that all five of the ω-TAms investigated can be used with IPA **12** is notable, especially with respect to the applicability of these enzymes, due its low cost and more facile optimisation of reaction condition since only one enzyme is required. Moreover, the highly volatile co-product acetone can be readily removed, as recently demonstrated in the synthesis of sitagliptin.[1]

Another interesting observation was that for the Kp-TAm and ArRMut11 no conversions were observed employing the enzyme-coupled systems, indicating that alanine was not accepted as an amine donor. While in engineering the *Arthrobacter* sp. ω-TAm to accept high IPA (**12**) and co-solvent concentrations the ability to use alanine has been lost, for the native Kp-TAm enzyme not to accept alanine as an amine donor is

Table 1 A comparison of the different methods used to shift the equilibrium toward the product amine **1a** and conversions observed after 24 h using **1** as substrate at pH 8, 30 °C

TAm	**MBA 11** amine donor (Fig. 1) conv. (%)	**A** LDH/GDH (Scheme 1a) conv. (%)	**B** AlaDH/FDH (Scheme 1a) conv. (%)	**IPA 12** amine donor (Scheme 1b) conv. (%)
CV-TAm	36	92	88	94
Kp-TAm	7	0	0	22[a]
Pp-TAm	39	91	88	95
Mv-TAm	24	31[a]	50[a]	39[a]
ArRMut11	39	0	0	93

[a] 48 h reaction time. Reactions were performed in triplicate with standard deviations of less than 10%. Product **1a** was detected by GC analysis for methods **A**, **B** and IPA **12**, and acetophenone was detected by HPLC as previously using MBA **11** as the amine donor (MBA **11** was not used in a large excess as high numbers of equivalents have been found to have a detrimental effect on the transaminase reaction).[42]

unexpected. However, since Kp is a member of the Class III transaminases and there is variable use of α-amino acids amongst the Class III transaminases, this finding is not too unusual.[16]

All further experiments were conducted using the IPA **12** shifting system because of the advantages outlined above. The amination of **1** was then performed at two different pHs and temperatures typically used in transamination reactions (Table 2). Optimal conditions which were used for further experiments were pH 8 and 30 °C for CV-TAm and Pp-TAm, pH 8 and 45 °C for Kp-TAm and Mv-TAm, while the best conditions for ArRMut11 were at pH 10 and 30 °C.

The amination of **1**, **3** and **8**, to give **1a**, **3a** and **8a** was then studied in more detail as initial experiments indicated reasonable levels of conversion (Table 3): compound **8a** is an important chiral product and the potential to establish two stereogenic centres in **3a** in a single step is particularly interesting. Ketones **1** and **3** were well accepted by the TAm enzymes, however ketone **8** was only accepted by ArRMut11 to give exclusively the α-aminotetraline (*R*)-**8a**, as determined by chiral GC analysis. This was consistent with previous reports using this ω-TAm with substrate **8**.[28,45] Only traces of **8a** were observed with Kp-TAm and Mv-TAm (Table 3).

Table 2 The pH and temperature optimisation of the TAm reaction using **1**, and IPA **12** as the amine donor equilibrium shifting method, to give **1a** after 24 h

TAm	pH 8, 30 °C conv. (%)	pH 8, 45 °C conv. (%)	pH 10, 30 °C conv. (%)	pH 10, 45 °C conv. (%)
CV-TAm	94	91	0	n.d.
Kp-TAm	5	18	8	14
Pp-TAm	94	10	0	n.d.
Mv-TAm	17	41	7	27
ArRMut11	93	n.d.	94	90

n.d. – not determined. Conversions were determined in triplicate with errors below 10%. Product **1a** detected by GC analysis.

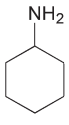
Amination of the α-substituted ketone **3** involves a dynamic kinetic resolution due to the chiral α-methyl group. Ketone **3** was accepted by all the selected ω-TAms, but conversion yields varied from 8% to 91%. Analysis of the products by GC indicated that CV-TAm preferentially gave *cis*-**3a**, while the *trans*-**3a** isomer was formed preferentially by Pp-TAm and ArRMut11-TAm.

A more detailed study of the amination of **3** was therefore performed, in order to evaluate the full product stereochemistry with the three most productive ω-TAms (Table 4). Samples were taken after 2 h, 4 h, 24 h and 48 h and conversions, *cis* : *trans* ratios and enantioselectivities were determined by GC analysis (Table 4). The data confirmed the stereoselectivities observed before (Table 3), but additionally by monitoring the amination over a period of time it became apparent that some selectivities decreased with increasing conversions/time. While CV-TAm was very selective for generating the *cis*-isomer the other enzymes seem to show lower selectivities. For example the ArRMut11 only showed a strong preference towards the formation of the *trans*-isomer at very low conversions (6% conversion and a *cis : trans* ratio of 20 : 80) while at a conversion of 20% the *cis : trans* ratio had increased to 40 : 60.

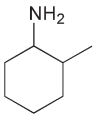
The absolute configurations of the **3a** stereoisomers were determined using known (*R*)- and (*S*)-selective TAms (ArRMut11 and CV-TAm respectively) with (2*R*)-**3** and racemic **3**. The amine products **3a** from the four reactions were then correlated to the isomeric amine products by chiral GC-analysis to establish the stereochemical outcome of the reactions.

In all cases the amine was formed in exceptionally high stereoselectivities (>99% ee) while the variable *cis* : *trans* ratios resulted from the ability of the ω-TAms to distinguish between the stereocentre at the α-methyl position. Racemic samples of **3** were used in all experiments, other than when establishing absolute configurations. For reactions where high conversions and/or high diastereomeric ratios were observed, some racemisation at the α-carbon of **3** will have occurred at pH 8 used with CV-TAm and Pp-TAm and pH 10 with ArRMut11. Such

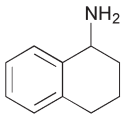
Table 3 ω-TAm catalysed amination of cyclic ketones **1**, **3**, and **8** using IPA **12** as the amine donor to give **1a**, **3a**, and **8a**



1a



3a



8a

TAm	Conv. (%)	Conv. (%)	Amine config.	*cis : trans* ratio	Conv. (%)	Amine config.
CV-TAm	94	58	(1*S*)	88 : 12	0	—
Kp-TAm	18	8	(1*S*)	63 : 37	1	n.d.
Pp-TAm	94	90	(1*S*)	43 : 57	0	—
Mv-TAm	41	10	(1*R*)	53 : 47	2	n.d.
ArRMut11	94	91	(1*R*)	36 : 64	19	(*R*)

n.d. – not determined. The reactions were performed under the optimised conditions in triplicate with a standard deviation of under 10%. Products detected by GC and chiral GC analysis. Conversions were determined after 24 h (**1a**), 48 h (**3a**) and 144 h (**8a**).

Table 4 Transamination of **3** to **3a**-isomers using IPA **12** as the amine donor, and conversions, *cis* : *trans* ratios and ees over a reaction time of 48 h

TAm	Time (h)	Conv. (%)	*cis*:*trans* ratio	*cis ee* (%)	*trans ee* (%)
CV-TAm	2	23	96 : 4		
	4	33	93 : 7	All	All
	24	47	88 : 12	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
	48	58	88 : 12		
Pp-TAm	2	31	61 : 36		
	4	49	54 : 46	All	All
	24	90	42 : 58	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
	48	90	43 : 57		
ArRMut11	2	6	20 : 80		
	4	20	40 : 60	All	All
	24	76	38 : 62	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
	48	91	36 : 64		

Conversions and *cis* : *trans* ratios were detected by achiral GC, while ee-values were determined using chiral GC; results were determined in triplicate with a standard deviation of less than 10%.

dynamic asymmetric transaminations involving α-substituted ketones have received little attention in the literature to date. Recently they have been described with an α-substituted ketone possessing a large α-phenethylether group.[33] However here we have the much less sterically differentiating methyl group at the α-position where notably for CV-TAm excellent ees and high diastereoselectivities were observed. The change in *cis*/*trans* product ratio over time probably reflects the consumption of the prefered isomer of **3** at shorter reaction times. Notably, for CV-TAm a high preference was observed for the (2*R*)-methyl group.

In light of the high stereoselectivities for CV-TAm, docking calculations were performed in order to gain insights into the stereopreference observed. Calculated binding affinities of all four PLP-imine quinonoid intermediates of the reaction were determined and results are summarised in Table 5.

Interestingly, the best calculated relative affinity (~−8 kcal mol^{-1}) was observed with the (*R*)-configured ligand, which is preferentially transformed by the CV-TAm (Table 5, entries 1 and 2). In contrast, the minor product of the transamination reaction (1*S*,2*S*)-**3a** had a lower calculated affinity (−6.4 kcal mol^{-1}, entries 3 and 4). Thus the trend generally matched the observed experimental data. For more insights, both equatorial quinonoid intermediates (*R*)-(entry 1) and (*S*)-(entry 3) were evaluated in more detail after modelling into the active site using the holo structure of CV-TAm (4AH3).[48] As shown in Fig. 2, the position of the six-membered ring varied depending on the methyl group stereochemistry. Moreover, the docked structures show that the (2*R*)-quinonoid (green), better fits the space available in the binding pocket (indicated in grey).

Table 5 Calculated relative binding affinities of the quinonoid intermediates (with the methyl group both axial and equatorial)

Entry	Configuration (methyl)[a]	Final conformation of methyl group	Relative binding affinity (kcal mol^{-1})
1	*R*	Equatorial	−8.1
2	*R*	Axial	−7.9
3	*S*	Equatorial	−6.4
4	*S*	Axial	−6.4

[a] The PLP structure in the docking experiments was overlayed with the PLP as reported in the crystal structure.

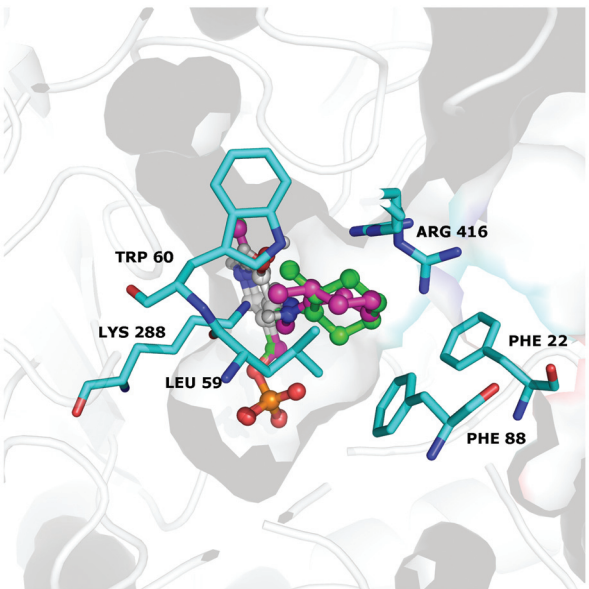


Fig. 2 Docking of (2*S*) (pink) and (2*R*) (green) quinonoid intermediate into the active site of CV-TAm (4AH3). The binding pocket is indicated as grey shadow, and amino acids involved in the transamination reaction are labelled.

However, apart from slightly better positioning of the (2*R*)- *vs.* the (2*S*)-quinonoid no additional steric factors could be determined to explain the stereochemistries observed. In general, the residue Lys288 is known to play a crucial role in the catalytic mechanism of TAms.[48,49] When no substrate is in the active site it forms a Schiffs base with the PLP cofactor, and during the reaction the amine donor replaces the Lys288, which is released as a consequence and changes its position. It is therefore possible that the dynamic repositioning of Lys288 further influences the stereopreference observed.

Conclusions

Several (*S*)- and (*R*)-ω-transaminases have been investigated for the transamination of a range of cyclic ketones. In a preliminary screen suitable TAms were identified to transform the substrates of interest and the amine donor 2-propylamine **12** was accepted by all five selected ω-TAms for further study. The amination of cyclohexanone **1**, 2-methylcyclohexanone **3**, and α-tetralone **8** were studied in more detail: yields of up to 94% were achieved with selected transaminases. When using 2-methylcyclohexanone **3** enantio- and diastereoselectivities were investigated. The highest selectivities were obtained when using CV-TAm: (1*S*)-2-methylcyclohexylamine was formed with complete selectivity at the amine position and up to 24 : 1 diastereoselectivity for the *cis* (1*S*,2*R*) isomer. This high reaction selectivity is extremely interesting as it enables the cyclic amine **3a** containing two-defined chiral centres to be formed in one step from racemic starting material. Such dynamic asymmetric transaminations with α-ketones have received little attention to date, and the high selectivities achieved by CV-TAm with α-substituted substrates will be explored in future work with CV-mutants.

Experimental

General

All starting materials were obtained from commercial suppliers and used as received unless otherwise stated. DNA-modifying enzymes were obtained from Thermo scientific (Germany) or New England Biolabs (USA). The enzymes used for shifting the equilibrium were commercially available from: L-lactate dehydrogenase from rabbit muscle (Sigma-Aldrich, Austria), glucose dehydrogenase (X-zyme, Germany), and formate dehydrogenase from *Candida boidinii* (Codexis, USA). The L-alanine dehydrogenase was prepared as reported previously.[46] (*R*)- and (*S*)-**8a** are commercially available. (*R*)-**3** was prepared as previously described.[50]

Cloning of TAm genes in suitable expression vectors

A synthetic gene for the previously reported ω-TAm from *Arthrobacter* sp. variant (ArRMut11)[1] was designed, as codon-optimised genes (DNA 2.0, U.S.A.), and subsequently cloned into the expression vector pET29a (Invitrogen, Germany) using standard techniques. Additionally, plasmids containing codon-optimised genes of the ω-TAms from *Chromobacterium violaceum*,[37] *Vibrio fluvialis*,[38] *Pseudomonas putida* (Pp-TAm),[36] and *Klebsiella pneumoniae*[39] were used. In addition, the ω-TAm from *Mycobacterium vanbaalenii* Mvan4516 (Mv-TAm) (gene bank accession no. 119958286; ABM15291)[40] was amplified from genomic DNA using the primers listed below and standard PCR procedures. Restriction sites were introduced to enable the cloning in the desired expression vector pET29a (Table 6).

Table 6 Sequence of the primers used for the amplification of Kp-TAm and Mv-TAm

Name	Sequence	Restriction site
Kp_forward	CATATGACACTGGACGATCTCGC	*Nde*I
Kp_reverse	CTCGAGTTCGCTAAAAAATGTTTC	*Xho*I
Mv_forward	ATATACATATGGGCATTG ACACCGGAACCTCGAATCTC	*NdeI*
Mv_reverse	ATAGCGGCCGCTCAG TACTGGATCGCTTCGATCAG	*NotI*

Restriction sites used for cloning are underlined.

Heterologous enzyme expression and preparation

The ω-TAms from *Chromobacterium violaceum* and *Vibrio fluvialis* were expressed as described previously.[37,38] All other ω-TAms were expressed in *E. coli* BL21(DE3)pLysS in lysogeny broth medium containing ampicillin (100 mg L^{-1}). Cultures were grown at 37 °C until an OD_{600} of 0.5-0.7 was reached. Enzyme expression was induced by addition of isopropyl-β-D-thiogalactopyranoside (0.5 mM), and the temperature was reduced to 25 °C. After 16–20 h cells were harvested by centrifugation and stored at −20 °C. To prepare cell free crude extract cells (20% v/w) were suspended in HEPES buffer (100 mM, pH 7.5) containing pyridoxal-5-phosphate (PLP) (0.5 mM), and disrupted by ultra sonication (2 × 1 min, 40% output). The crude extract was cleared by centrifugation (20 min, 16.000*g*) and either directly used or freeze dried and stored at −20 °C.

General procedure for TAm screening

For the HPLC-based TAm screening (*R*)- or (*S*)-α-methylbenzylamine (MBA) **11** (25 mM) and pyridoxal-5-phosphate (PLP) (0.5 mM) were dissolved in HEPES buffer (100 mM, pH 8). *E. coli* crude extract (12.5 μL) containing the overexpressed ω-TAm were added to 212.5 μL of this solution. The reaction was started by the addition of 25 μL of substrate in DMSO (final concentration 10 mM, 10 vol%). After an incubation for 21 h at 30 °C and 180 rpm the reaction was stopped by the addition of 250 μL of acetonitrile containing 0.2% TFA. Denaturated protein was removed by centrifugation, and the supernatant was analysed by HPLC (Agilent) using a Discovery®Bio Wide Pore C18 column (Supelco, 25 × 4.6 mm, 10 μm beads)

with UV detection at 254 nm. Concentrations of acetophenone were determined using a linear gradient: 30%–60% B over 10 min (A = water, B = acetonitrile, both containing 0.1% TFA). The acetophenone produced eluted at a retention time of 8.6 min.

Determination of initial rates of the five selected TAms

Initial rates were determined using the HPLC-based method described above using cyclohexanone **1** as the substrate. Cyclohexanone (10 mM), (*R*)- or (*S*)-MBA **11** (10 mM) and PLP (1 mM) were solved in sodium phosphate buffer (100 mM, pH 8). The reaction was started by addition of 100 μL of enzyme solution (crude cell extract or freeze dried cells). Samples were taken after 5 min, 10 min, 15 min, and 30 min, and the linear slope was used for the calculation of initial rates and specific activities (Table 7).

General procedure for the TAm reactions using different equilibrium shifting systems

Transamination reactions using the (a) AlaDH/FDH, (b) LDH/GDH or (c) 2-propylamine IPA **12** system were performed as follows: (a) substrate (20 mM), D- or L-alanine (200 mM), ammonium formate (60 mM), PLP (1 mM), NAD^+ (0.5 mM), formate dehydrogenase (11 U) and L-alanine dehydrogenase (12 U) were dissolved in sodium phosphate buffer (1 mL, 100 mM, pH 8); (b) substrate (20 mM), D- or L-alanine (200 mM), glucose (60 mM), PLP (1 mM), NAD^+ (0.5 mM), glucose dehydrogenase (30 U) and L-lactate dehydrogenase (90 U) were dissolved in sodium phosphate buffer (1 mL, 100 mM, pH 8); (c) substrate (20 mM), IPA **12** (200 mM), and PLP (1 mM) were dissolved in sodium phosphate buffer (1 mL, 100 mM, pH 8 or 10). All biotransformations were started by the addition of lyophilised *E. coli* crude cell extract containing the overexpressed ω-TAm corresponding to an activity of 0.5 U in the amination of cyclohexanone (CV-TAm, Pp-TAm and ArRMut11 see Table 1), while the maximum amount of 10 mg was added in the case of the Kp-TAm and Mv-TAm. The mixture was incubated at 30 °C or 45 °C and 800 rpm in a thermoshaker (Eppendorf, Germany).

Samples were taken at different time points, and the reaction was stopped by addition of 10 vol% of $NaHCO_3$, and extracted with ethyl acetate (2 × 500 μL). Conversions to the amine were measured by GC (Agilent 7890 A system equipped with a FID detector). The biotransformations of cyclohexanone **1** and 2-methylcyclohexanone **3** were monitored using an Agilent DB-1701 column (30 m, 0.25 mm, 0.25 μm) using the following temperature programmes: **A** 60 °C, hold for 5 min, 15 °C min^{-1} to 150 °C; retention times cyclohexylamine **1a** 6 min and cyclohexanone **1** 7.9 min. **B** 60 °C, hold for 5 min, 5 °C min^{-1} to 80 °C, 60 °C min^{-1} to 250 °C; retention times *trans*-**3a** 5.9 min, *cis*-**3a** 6.4 min and **3** 8.4 min. Conversions for α-tetralone **8** were determined using an Agilent HP5 (30 m, 0.32 mm, 0.25 μm) and the following programme: 120 °C, 5 °C min^{-1} to 160 °C, 50 °C min^{-1} to 300 °C, retention times 1-aminotetraline **8a** 4.4 min and α-tetralone **8** 4.7 min.

Table 7 Specific activities of selected TAms using cyclohexanone **1** as substrate and (*R*)- or (*S*)-MBA **11** as amine donor

TAm	Crude cell extract [U mg^{-1} total protein]	Freeze dried cell extract [U mg^{-1} total protein]
CV-TAm	0.58	0.32
Kp-TAm	n.d.	0.008
Pp-TAm	0.13	0.11
Mv-TAm	0.006	0.006
ArRMut11	0.39	0.24

n.d. – not determined.

Determination of enantiomeric purity

The enantiomeric exessess of amines **3a** and **8a** were determined by GC using a modified β-cyclodextrin column (CP-Chirasil-DEX CB, 25 m, 0.32 mm, 0.25 μm) after derivatisation to the corresponding trifuoroacetamides. For the derivatisation trifluoroacetic anhydride (5 μL) was added to the extracted sample, and after an incubation (800 rpm) at 30 °C the reaction was quenched with $NaHCO_3$ and the ee was analysed. Temperature programme for **3a**: 100 °C hold 2 min, 2 °C min^{-1} to 135 °C, 25 °C min^{-1} to 180 °C. Retention times: (1*S*,2*R*)-**3a** 5.5 min, (1*R*,2*S*)-**3a** 5.7 min, (1*S*,2*S*)-**3a** 6.7 min and (1*R*,2*R*)-**3a** 7 min. Temperature programme for **8a**: 120 °C, 5 °C min^{-1} to 160 °C, 20 °C min^{-1} to 180 °C. Retention times: (*S*)-**8a** 8.2 min and (*R*)-**8a** 8.5 min.

Docking studies

For docking experiments the holo crystal structure of the ω-TAm from *C. violaceum* was used (pdb: 4AH3). The apo-structure of 4AH3 as well as the structure of the quinonoid intermediate[51] of all conformers were generated with Maestro and the energy optimisation of the ligands was performed using the MacroModel "Minimization" followed by a "Conformational Search" (all Schrödinger LLC). The two conformations with the lowest energy were used for the docking experiments. Optimisation of the protein structure after removal of the bound PLP was performed with autodock tools v 1.5.6. Docking calculations were performed using AutoDock Vina[52] with a $x = 24/y = 24/z = 24$ grid box centred on $x = 4.8$, $y = -0.5$, $z = 7.4$. Lowest energy clusters were selected and visualised using pymol v0.99rc6.

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