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Amine transaminase engineering for spatially bulky substrate acceptance

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Abstract Amine transaminase (ATA) catalysing stereoselective amination of prochiral ketones is an attractive alternative to transition metal catalysis. As wild-type ATAs accept only non-sterically hindered ketones, efforts to widen the substrate scope to more challenging targets are of general interest. We recently designed ATAs to accept aromatic and thus planar bulky amines, via a sequenced based motif that supports the identification of novel enzymes. However, these variants were not active against 2,2-dimethyl-1-phenyl-propan-1-one, which carries a spatially bulky *tert*-butyl substituent adjacent to the carbonyl function. Here, we report on a differentiated solution for this type of substrate. The evolved ATAs can perform asymmetric synthesis of the respective (*R*)-amine with high conversions using either alanine or isopropylamine as amine donors.

Introduction

Amine transaminases (ATAs) are a promising alternative to transition-metal catalysis for the stereoselective synthesis of chiral amines.[1] Transaminases are pyridoxal-5'-phosphate (PLP)-dependent transferases that catalyse the amino group transfer to ketones or aldehydes, using a donor amine, usually D- or L-alanine or isopropylamine.[2] If prochiral ketones are applied for synthesis, amine transaminases perform an asymmetric amino group transfer providing optical pure amines, which makes ATAs a valuable tool for the synthesis of chiral key intermediates for pharmaceutically active compounds.[1b, 1c] However, the major challenge to date is their limited substrate scope. Transaminases in general are homodimeric proteins comprising two active sites each of them located at the interface of the two subunits. Thus, each of the two active sites is surrounded by amino acid residues from both monomers encompassing a large and a small binding pocket. In most cases, the small binding pocket is restricted to accommodate only small moieties such as methyl groups.[2a, 3] Accordingly, protein engineering is required to expand the active site for larger substrates to enable biosynthesis of chiral amines of high pharmaceutical or synthetic interest. In the past decade, many groups succeeded to raise ATAs to the spotlight of organic synthesis. The most prominent past example of protein engineering of ATAs was the synthesis of (*R*)-sitagliptin. Merck & Co. and Codexis introduced 27 mutations in a fold class IV TA which exhibited minimal, but detectable activity towards prositagliptin.[1c] This engineering effort led to the development of an industrial process. In a more recent and broad study, we could identify a sequence based motif comprising four core mutations in ATAs of fold class I that enable them to accept a set of bulky aromatic and thus planar ketones. We exhibited their catalytic potential by performing preparative asymmetric synthesis of the corresponding amines, leading to high isolated yields and excellent optical purities.[1b] The sequence motif was demonstrated to be transferable to fold-class I ATAs with at least 70 % sequence identity to the *Ruegeria* sp. TM1040 enzyme (denoted as 3FCR in the PDB) by incorporating the motif in previously non-characterized putative ATAs. All synthetic variants that were expressed as soluble protein were proved to exhibit activity towards the interrogated bulky amines (as verified for kinetic resolutions), which underlines the generality of our sequence motif.[1b]

The motif we suggested provides a toolbox of ATAs for aromatic and aliphatic substrates that have planar bulky substituents, but these variants are not efficient catalysts for ketones with spatial bulkiness. Recently, we provided a solution for the asymmetric synthesis of a bridged bicyclic amine, the first that was ever reported.[4] The engineering was based on the same scaffold, but the positions targeted were different, showing that spatially bulky substrates require a different approach.

In the current study we investigated the acceptance of the ketone 2,2-dimethyl-1-phenyl-propan-1-one (**1**), to synthesize the (*R*)-2,2-dimethyl-1-phenylpropan-1-amine (**2**), as shown in Scheme 1. This substrate is of interest, due to its *tert*-butyl substituent in addition to the aromatic ring. Our group investigated previously the acceptance of ketone **1** by the ATA from *Vibrio fluvialis* (VibFlu) in an extensive engineering work, and several variants were identified that could perform the asymmetric synthesis.[5] Just recently, another example was published were VibFlu was engineered to catalyze the synthesis of (*S*)-1-(1,1´-biphenyl-2-yl)ethanamine.[6]

The ATA from *Ruegeria* sp. TM1040 (3FCR)[7] that has been used in the present study differs significantly from VibFlu (~33 % sequence identity) and in our previous work[1b] we could show that the evolution of the two ATAs of the same fold class I is

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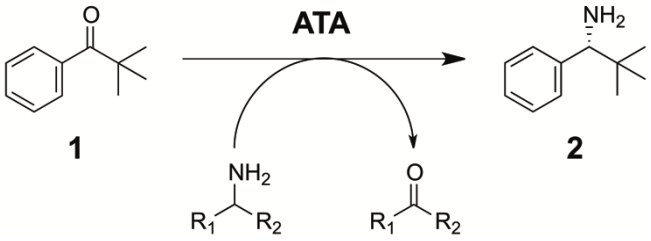
different: Although several of the mutations present in a sequence motif discovered by us in the 3FCR enzyme scaffold are already present in the wild-type of VibFlu; the latter did not exhibit any detectable activity against any of the bulky planar substrates studied.

Thus, to understand the engineering of the enzymes and the features that enable the acceptance of this challenging substrate, herein we suggest another engineering solution based on the 3FCR scaffold, and with hindsight we provide a comparison of the most prominent variants of the two protein scaffolds with little homology to each other to understand the binding and conversion of this specific target compound to facilitate the engineering of ATA acceptance of further bulky ketones of this type.



Scheme 1. Asymmetric synthesis of (*R*)-2,2-dimethyl-1-phenylpropan-1-amine (**2**) via amino group transfer to 2,2-dimethyl-1-phenyl-propan-1-one (**1**) catalysed by ATAs. Isopropylamine (R_1=R_2=–CH_3) or alanine (R_1=–CH_3 , R_2=–COOH) served as amine donors.

Results and Discussion

Protein engineering for acceptance of amine 2. The wild-type 3FCR has a narrow substrate scope with low activities even for standard substrates such as 1-phenylethylamine. For this reason, our engineering efforts started with the most prominent variant (3FCR_Y59W/T231A) identified in our previous study[1b] as it enabled the acceptance of planar bulky substrates. However, this variant did not exhibit any detectable activity towards amine **2** in kinetic resolution reactions. According to an initial *in silico* analysis, we identified that the position Y87 is an important target, as it seems to block the entrance for the sterically demanding *tert*-butyl moiety. We selected hydrophobic residues for this position, as they need to coordinate a hydrophobic group. The variants 3FCR_Y59W/Y87V/T231A and 3FCR_Y59W/Y87L/T231A exhibited for the first time activity towards the desired amine (Table 1). These findings were quite interesting as first of all the 3FCR_Y59W/Y87F/T231 variant – developed in our previous work for activity towards aromatic bulky substrates[1b] – was not active, supporting our theory that a different engineering approach is needed. More interestingly, the leucine variant was more active than the valine mutant. This is striking, as one would expect that smaller residues would be preferred, in order to accommodate such a sterically challenging substrate. However, it seems that the hydrophobicity of the residue plays a role. Smaller residues may provide more space; however, this extra space might be occupied by water molecules and thus the diffusion of hydrophobic substrates in the binding pocket is disfavoured. On the contrary, when the mutation provides just enough space for the binding, then hydrophobic interactions between position 87 and the substrate can take place, leading to higher specific activity.

Our rational analysis on the quinonoid intermediate did not suggest any other position of interest. In this scaffold – for which we observed the first measurable activity – we incorporated the mutation L382M that was identified as beneficial in our previous work dealing with a bridged bicyclic amine.[4] In parallel, we performed error-prone PCR, using the variant 3FCR_Y59W/Y87L/T231A as a template. After screening about 5000 colonies of three different ep-PCR libraries with our solid phase assay,[8] using amine **2** as amine donor, we identified a variant with increased activity in kinetic resolution mode (Table 1). The variant 3FCR_Y59W/Y87L/T231A/P281S/G429A exhibited almost 3-fold increased activity compared to the template, 3FCR_Y59W/Y87L/T231A, just by the incorporation of two mutations. Position 429 was identified to be located close to the large binding pocket, thus we focused on this mutation. Indeed, the mutation P281S seems to have a negative effect on the specific activity (Table 1) and thus it was discarded. When the mutation G429A was incorporated into the 3FCR_Y59W/Y87L/T231A/L382M scaffold, the effect was almost the same; the mutation G429A led to a 4-fold increase (Table 1). As the mechanism of the effect of the mutation at position 429 on the specific activity of the ATA is not clear, and the epPCR random mutagenesis might not provide the best mutation at a given position, we performed a saturation mutagenesis at position 429, but no variant better than G429A was found (data not shown).

Table 1. Specific activity (mU/mg) of 3FCR variants towards *rac*-amine **2** (1 mM) determined in kinetic resolution mode using pyruvate as amine acceptor (2 mM) at 30°C, pH 9.0 (CHES, 50 mM).

Variant	Activity (mU/mg)
3FCR_Y59W/T231A	n.a.
3FCR_Y59W/Y87F/T231A	n.a.
3FCR_Y59W/Y87V/T231A	6.5 ± 0.4
3FCR_Y59W/Y87L/T231A	11.5 ± 0.8
3FCR_Y59W/Y87L/T231A/L382M	19.0 ± 1.9
3FCR_Y59W/Y87L/T231A/P281S/G429A	32.4 ± 0.1
3FCR_Y59W/Y87L/T231A/G429A	44.8 ± 0.9
3FCR_Y59W/Y87L/T231A/L382M/G429A (3FCR_WLAMA)	77.1 ± 5.5

n.a.: not active or below detection limit (<1 mU/mg)

In parallel to the protein engineering experiments, we investigated the synthetic usefulness of the identified variants. First, we performed a preparative scale experiment (100 mg) in kinetic resolution mode, using the 3FCR_Y59W/F87L/T231A mutant. The findings were striking, as the isolated residual enantiomer of amine **2** (38 mg) had (*S*)-configuration with 96.2% ee. This shows that the enzyme did not prefer the (*S*)-enantiomer for this specific compound as one would have assumed for an enzyme of fold class I. With this new elucidated result, we performed another round of *in silico* analysis, where we docked the *tert*-butyl moiety in the large binding pocket, a conformation that is in line with the preferentially converted (*R*)-enantiomer. In this position the hydrophobic group interacts with

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the positions 58, 59, 85, 231 and 422. Based on the best variant created so far (3FCR_WLAMA, Figure 1) further saturation mutagenesis libraries were designed. Position 59 was fully saturated using a NNK library, while L58 was mutated to alanine, valine, methionine, isoleucine and cysteine to provide more space and potentially trigger specific interactions with the substrate in the large binding pocket. At position H85, two hydrophobic residues (valine or leucine) were incorporated. Nevertheless, these rational variants did not exhibit any improved activity (data not shown).

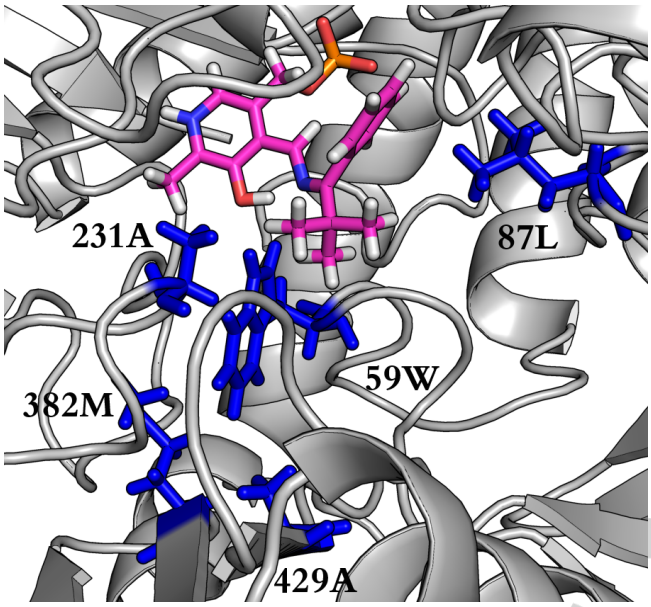

Figure 1. 3FCR_WLAMA with the quinonoid of 2,2-dimethyl-1-phenyl-propan-1-one **1**. The side chains of the engineered positions are colored in blue.

Next, all interesting variants were applied in analytical scale asymmetric synthesis reactions, which is the desired mode as it theoretically can yield 100% product. We used (L)-alanine ((*S*)-alanine) or isopropylamine as amine donors. Despite the low activity in kinetic resolutions, all variants were active in asymmetric synthesis. Interestingly, the use of L-alanine as amine donor, using the lactate dehydrogenase/glucose dehydrogenase cascade for the removal of pyruvate,[9] led to full conversion after 42 h. Isopropylamine was also accepted as amine donor, albeit at lower conversions (Table 2). As expected from the kinetic resolution experiments, the variant 3FCR_WLAMA exhibited the highest conversion rates. In order to increase the productivity of the best variant, we incorporated the mutation Y152F, as in previous studies it was related to significant stabilization of the enzyme under the experimental conditions.[1b] Nevertheless, this mutation did not provide any benefit for the current reaction, while it also decreased the specific activity towards the desired amine.

Table 2. Asymmetric synthesis of (*R*)-amine **2** catalysed by the most interesting variants. Reaction conditions: 4 mM ketone (**1**), HEPES buffer (50 mM, pH 8.0), 5 % (v/v) DMSO, 1.35 mg/mL ATA, 1 mM PLP at 30°C and 600 rpm. As amine donor 200 mM IPA or 200 mM L-alanine were applied. In case of L-alanine, 150 mM D-glucose, 45 U/mL LDH, 15 U/mL GDH and 1 mM NADH were added. Samples were taken after 42 h incubation at 30 °C and 600 rpm.

Variant	Conversion (ee) [%] (w/ L-alanine)	Conversion (ee) [%] (w/ IPA)
3FCR_Y59W/Y87L/T231A	100 (>99)	22 ± 2.3 (n.d.)
3FCR_Y59W/Y87L/T231A/ L382M	100 (>99)	38 ± 1.4 (>99)
3FCR_Y59W/Y87L/T231A/ G429A	100 (>99)	45 ± 0.8 (>99)
3FCR_Y59W/Y87L/T231A/ L382M/G429A (3FCR_WLAMA)	100 (>99)	71 ± 3.5 (>99)
3FCR_Y59W/Y87L/Y152F/ T231A/L382M/G429A	100 (>99)	40 ± 2.8 (>99)

Furthermore, we compared our most active 3FCR variants under identical conditions with the two most active VibFlu variants.[5] These were the VibFlu_H1_A, which was reported to be the most active variant in kinetic resolution using pentanal as amine acceptor and VibFlu_H3_RAV, which was reported to be the only variant that reached full conversion in asymmetric synthesis of 10 mM ketone **1** using alanine as amine donor, however with a significantly higher protein load (3.8-4.6 mg/mL).[5] In direct comparison, the variants of 3FCR reached significantly higher conversions than the variants of VibFlu (Table 3).

Table 3. Comparison of the most active 3FCR variants with the most active variants of VibFlu in analytical scale asymmetric synthesis reactions using alanine or isopropylamine as amine donors. Reaction conditions: 4 mM ketone (**1**), HEPES buffer (50 mM, pH 8.0), 5 % (v/v) DMSO, 1 mM PLP at 30°C and 600 rpm. In case of isopropylamine as amine donor 0.725 mg/mL ATA and 200 mM isopropylamine were applied. In case of alanine as amine donor, 0.25 mg/mL ATA, 200 mM L-alanine, 150 mM D-glucose, 45 U/mL LDH, 15 U/mL GDH and 1 mM NADH were applied. Samples were taken after 23 hours.

Variant	Conversion [%] (w/ L-alanine)	Conversion [%] (w/ Isopropylamine)
VibFlu_H3_RAV	24 ± 2	3 ± 0.4
VibFlu_H1_A	0 ± 0	17 ± 1
3FCR_WLAMA	**75 ± 7**	**28 ± 1**
3FCR_WLAMA/152F	69 ± 4	15 ± 1.7

Additionally, we were interested to compare the stability of the 3FCR_WLAMA to the 3FCR variant including mutation 152F that was previously reported to stabilize the 3FCR scaffold. Therefore we prepared asymmetric synthesis reactions without pre-incubation and with a 6 hours pre-incubation of the solution before the reaction was started by addition of the keto substrate. Both scaffolds lose some of their activity during the 6 hours of incubation with 200 mM isopropylamine resulting in 4 % lower

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conversion for the variant with Y152F and 8 % lower conversion for the 3FCR_WLAMA compared to the conversion without pre-incubation. However, the 3FCR_WLAMA still provides the highest overall conversions despite 6 hours of pre-incubation (Table 4).

Table 4. The effect of mutation Y152F on the 3FCR_WLAMA stability was evaluated in terms of conversion (%) after 42 h reaction time with and without previous 6 hours pre-incubation (one sample at 30°C and 600 rpm without isopropylamine and another sample with 200 mM isopropylamine). Final reaction conditions: 4 mM ketone (**1**), HEPES buffer (50 mM, pH 8.0), 5 % (v/v) DMSO, 1 mM PLP, 200 mM IPA and 1.15 mg/mL TA at 30°C, 600 rpm for 42 h.

Conditions	3FCR_WLAMA	3FCR_WLAMA/Y152F
No pre-incubation	43 ± 2.5 %	30 ± 1.9 %
Pre-incubation without IPA (6 h)	41 ± 2.0 %	30 ± 2.0 %
Pre-incubation with IPA (6 h)	35 ± 0.0 %	26 ± 1.8 %

Bioinformatic and structural comparison of active variants of 3FCR and VibFlu. We now identified two different solutions to synthesize (*R*)-2,2-dimethyl-1-phenylpropan-1-amine using engineered ATAs: one is based on the 3FCR scaffold and one on the well-studied VibFlu scaffold. Hence, it would be interesting to analyze in detail, which mutations lead to active enzyme variants in the two ATA-scaffolds. VibFlu and 3FCR share only 33% sequence identity and only 54% sequence similarity. However, the structures of them (PDB-codes: 3FCR and 4E3Q) are quite similar as the alignment of their monomers results in an RMSD of only 1.154 Å. Moreover, most of the residues that are in a radius of 10 Å from the enamine nitrogen atom of the external aldimine of PLP and the substrate and thus are supposed to form the active-site are conserved. Aligning the dimeric structures of the 3FCR_WLAMA and the VibFlu wild-type ATA returned 49 amino acids with the above-mentioned criteria, from which 25 amino acids were identical (51%) and 32 amino acids were similar (65%). Notably, both wild-type enzymes did not accept the spatially bulky amine **2**.

The best variants of VibFlu identified by Genz et al. included the mutations L56V, W57C, F85V, V153A (H3_RAV) and L56V, W57F, F85V, V153A, R415C (H1_A),[5] which correspond to the positions L58, W59, L87, S155 and R420 in the 3FCR_WLAMA. It is interesting to note that in the study of Genz *et al.* the library design theoretically comprised 16 384 different combinatorial variants including wild-type amino acid codons at each position, but in fact only 2240 variants needed to be screened to find improved variants. Position L58 was already targeted in 3FCR in the current study and did not result in any improved activity. An interesting position in 3FCR is residue 59: in VibFlu at this position a cysteine was found as beneficial mutation. Interestingly, in 3FCR the Y59W mutation was found to be the best, the residue already present in wild-type VibFlu. The saturation of this position in either the 3FCR_Y59W/Y87L/T231A or the 3FCR_WLAMA did not identify any residue resulting in higher activity than tryptophan. The most important mutation however was at position 87. Mutations Y87L or Y87V were the ones that created activity for amine **2** in the first place in the 3FCR_59W/231A variant. In VibFlu this mutation is not crucial, as there are variants that accept the amine without the mutation F85V, although the variants that included this mutation led to higher conversion in asymmetric synthesis. So, although the side chain of the position is not directly interacting with the *tert*-butyl moiety of the interrogated compound in the quinonoid intermediate, an aliphatic hydrophobic side chain is required. This can be related to the hydrophobicity of the microenvironment and the diffusion of the substrate to the large binding pocket. Finally, mutation V153A was incorporated in the mutants of VibFlu by Genz et al. based on literature reports.[10] The effect seems to be specific for this template, as the residue is in the second sphere of interactions and does not interact directly to the quinonoid.[10a] Indeed, preparation of the alanine mutation at the corresponding position in 3FCR (3FCR_WLAMA/S155A) reduced the activity in kinetic resolution (data not shown). Three out of the five mutations identified in our best variant 3FCR_WLAMA (Y59W, T231A, L382M) are already present in the wild-type VibFlu. The mutation G429A is also a second shell residue, and seems to be specific for the 3FCR scaffold.

From this analysis it can be seen that despite the structural similarity and the conservation of the residues that constitute the active site, there is no motif that can be easily identified. The most crucial position for substrates with a *tert*-butyl substitutent is position Y87(3FCR)/F85(VibFlu) as its mutation to aliphatic hydrophobic residues had a beneficial effect in both enzymes.

Conclusions

The amine transaminase of *Ruegeria* sp. TM1040 (3FCR) was engineered to enable the synthesis of the spatially bulky amine (*R*)-2,2-dimethyl-1-phenylpropan-1-amine. Although the wild-type enzyme did not exhibit any detectable activity towards this substrate, the incorporation of five mutations led to the acceptance of the corresponding bulky ketone and allowed the asymmetric synthesis of (*R*)-amine **2**, even with isopropylamine as amine donor. We could show that these mutations are different from the ones required to convert planar bulky compounds, and hence a different protein-engineering strategy was needed. The most critical mutation that enabled the acceptance of the bulky substrate was Y87L, a position that was also important for VibFlu; thus this seems to be a critical residue for the identification of ATAs that are active towards compounds with a tertiary carbon substituent.

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Experimental Section

Materials. Ketone **1** and amine **2** – as racemic mixture as well as its individual enantiomers – were made available by F. Hoffmann-La Roche. All other reagents were of analytical grade. Recombinant expressions of the ATA from *Ruegeria* sp. TM1040 (3FCR) and all variants were performed as described previously.[1b] The glycine oxidase was produced as described previously.[8]

Directed evolution libraries – point and site-saturation mutagenesis. For the preparation of error-prone PCR libraries the gene of the respective template of the ATA from *Ruegeria* sp. TM1040 was amplified using the GeneMorph II Random Mutagenesis Kit from Agilent Technologies according to the manual instructions and using the flanking primers as described before with 40 ng, 50 ng and 60 ng of plasmid DNA template.[4] All other variants were prepared using a modified version of the QuikChange PCR method.[4] All subsequent steps were performed as described before.[4]

Protein expression and purification. Protein expression and purification was performed as described previously.[4]

Kinetic resolution experiments. For determination of specific activities and for screening libraries in crude lysate a direct spectrophotometric assay was applied as described previously.[1b] Formation of the corresponding ketone (**1**) was monitored at the optimum wavelength at 245 nm (extinction coefficient: 7953 $M^{-1}cm^{-1}$). Saturation libraries based on the 3FCR_59W/87L/231A were screened in crude lysate using the glycine oxidase assay as described previously.[4]

Solid-phase assay screening of error-prone PCR libraries. The ep-PCR libraries were screened using the glycine oxidase solid-phase assay and all subsequent steps were performed as described before.[4] The assay plates contained 4.44 mM of racemic 2,2-dimethyl-1-phenylpropan-1-amine and 10 mM glyoxylate as transaminase substrates.

Asymmetric synthesis experiments. These were performed in analytical scale in a total volume of 0.5 or 1 mL in glass vials using a shaker from Eppendorf. Detailed final concentrations and conditions are given in the main text. For HPLC analysis 65 µL of the sample were mixed with 65 µL acetonitrile including 0.1% diethylamine that was passed through a tip filter before injection into HPLC. For chiral analysis a 150 µL sample was used for extraction as described previously.[5] Chiral GC-analysis was performed as described previously.[5] In GC the ketone eluted after 35.26 min, the (*S*)-amine eluted after 35.9 min and the (*R*)-amine after 37.58 min. Conversions were determined by HPLC analysis on a Luna C8 150x4.6 mm column (Phenomenex) using a gradient over 25 min at 1 mL/min flow rate, oven temperature 45°C with acetonitrile and water containing 0.1% trifluoroacetic acid and detection at 210 nm. 0–4 min: 10% acetonitrile, 90% water, 8–12 min: 60% acetonitrile, 40% water, 12–25 min: 10% acetonitrile and 90% water. A standard curve from 0–4 mM amine (**2**) was recorded and returned a slope of 10 094 443 AU/mM amine (**2**) for an injection volume of 15 µL. In achiral HPLC the amine peak eluted after 9.38 min. For each reaction with full conversion, it was certified via GC that the entire ketone was consumed.

Preparative scale preparation of (*S*)-amine 2. In 89.75 mL reaction buffer (TRIS 50 mM pH 8.5; 1 mM PLP) 129 mg sodium pyruvate and the purified solution of mutant transaminase 3FCR_Y59W/Y87L/T231A (18.5 ml, protein 2.47 mg/ml) were added subsequently and stirred for 5 minutes at 30°C. The reaction was started by the addition 100 mg racemic amine **2** dissolved in 1 ml DMSO. After 48 h and a conversion of roughly 55 area% (IPC-HPLC), the reaction was acidified to pH 2.0 to precipitate the enzymes and was stirred 20 minutes. Subsequently, the reaction mixture was filtrated through 25 g filter aid (Dicalite) bed and subsequently the filter cake was washed with deionized water and 50 mL methyl *tert*-butyl ether (MTBE). After phase separation the aqueous phase was extracted with 50 ml MTBE to remove the 2,2-dimethyl-1-phenyl-propan-1-one (**1**). The combined organic phases were dried over anhydrous $MgSO_4$, filtrated and evaporated under vacuum at 40°C yielding 25 mg (26.5%; chiral HPLC: 99 area%) 2,2-dimethyl-1-phenyl-propan-1-one (**1**) as yellow vicious oil. The pH of the aqueous phase was adjusted to 12 using 2 N NaOH and subsequently extracted twice with 50 ml MTBE. The combined organic phases were dried over anhydrous $MgSO_4$, filtrated and evaporated under vacuum at 40°C yielding 38 mg (40%) (*S*)-2,2-dimethyl-1-phenyl-propan-1-amine (**2**) as yellow oil.

Chiral HPLC: 98.1 area% ((*S*)-enantiomer), 1.9 area % ((*R*)-enantiomer) [220 nm; Chiracel OD-3R; 150*4.6 mm, 3 µm, flow 1.0 ml, 25°C, A: 50% acetonitrile (ACN), B: 50% 6.3 g ammonium formate in 950 ml H_2O : 50 ml ACN]; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.27 - 7.31 (m, 4 H), 7.24 (dt, J=6.0, 2.6 Hz, 1 H), 3.71 (s, 1 H), 0.91 (s, 9 H); GC-MS: 162 (M).

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Keywords: amine transaminase • asymmetric synthesis • enzyme catalysis • protein engineering

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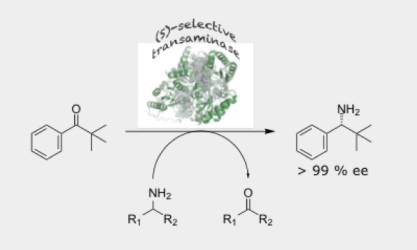
FULL PAPER

An amine transaminase was evolved to accept the spatially bulky 2,2-dimethyl-1-phenyl-propan-1-one, which carries a *tert*-butyl substituent adjacent to the carbonyl function. The evolved ATAs are able to perform asymmetric synthesis of the respective (*R*)-amine at high conversions and excellent stereoselectivities with either alanine or isopropylamine as donors.



Martin S. Weiß, Ioannis V. Pavlidis, Paul Spurr, Steven P. Hanlon, Beat Wirz, Hans Iding, Uwe T. Bornscheuer**

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Amine transaminase engineering for spatially bulky substrate acceptance

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