Electronic Supplementary Material (ESI) for Green Chemistry.

A system for ω-transaminase mediated (*R*)-amination using L-alanine as amine donor

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SUPPORTING INFORMATION

Table of Contents

1. Gene sequence of the SeAlaR and PpAlaR

The coding sequence of the gene is given in capital letters, restriction sites are underlined and the N-terminal His-Tag is shown in italics.

Optimised sequence of the SeAlaR (pEG238):
catATGAGCGAAACCACCGCACGTCGTGATGCAGATGCAGTTCTGCGTGCACGTGCCGAAATTGATCTGGCAGCA
CTGCGTGCAAATGTTCGTGCCCTGCGTGAACGCGCACCGGGTGCAGCACTGATGGCAGTTGTTAAAGCAGATGCC
TATGGTCATGGTGCAATTCCGTGTGCCCGTGCAGCAGTTGCAGCGGGTGCAACCTGGCTGGGCACCGCAACACCG
CAAGAGGCACTGGCGCTGCGTGCAGCCGAACCGGGTCTGCCGGATGATGTTCGTATTATGTGTTGGCTGTGGACA
CCGGGTGGTCCGTGGCGTGAAGCAGTTGAAGCACGTCTGGATGTTAGCGTTAGCGCAATGTGGGCAATGGAAGAA
GTTACAGGCGCAGCACGCGCAGCCGGTGTTCCGGCACGTGTGCAGCTGAAAGCAGATACCGGTCTGGGTCGTGGT
GGTTGTCAGCCTGGTGCAGATTGGGAACGTCTGGTTGGTGCAGCCCTGCGTGCCGAAGAAGAAGGTCTGCTGCGC
GTTACAGGTCTGTGGTCACATTTTGCATGTGCAGATGAACCTGGTCATCCGAGCATTGCAGCACAGCTGACCCGT
TTTCGTGAAATGACCGCCTATGCAGAACAGCGTGGTCTGCGTCCGGAAGTTCGTCATATTGCAAATAGTCCGGCA
ACCCTGACCCTGCCTGATGCACATTTTGATCTGGTTCGTCCGGGTATTGCAATGTATGGTGTTAGCCCGAGTCCG
GAAATTGGTACACCGGCAGATTTTGGCCTGCGTCCTGTTATGACCCTGGCAGCAAGCCTGGCACTGGTTAAACAG
GTTCCGGGTGGCCATGGTGTGAGTTATGGTCATCATTATACCACTCCGGGTGAAACCACCCTGGGTCTGGTTCCG
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GTTGCAGGTCGTATTGCCATGGATCAGTTTGTTGTTGATCTGGGTGGTGATCGTCCGGAACCGGGTGCCGAAGCA
GTTCTGTTTGGTCCGGGTGATCGTGGTGAACCGACCGCAGAAGATTGGGCACAGGCAGCAGGCACCATTGCCTAT
GAAATTGTTACCCGTATTGGTAGCCGTGTTCCGCGTGTTTATGTTAATGAActcgag*caccaccaccaccaccac*

Optimised sequence of the PpAlaR (pEG236):
catATGCCGTTTCGTCGTACCCTGCTGGCAGCAAGCCTGGCACTGCTGATTACCGGTCAGGCACCGCTGTATGCA
GCACCGCCTCTGAGCATGGATAATGGCACCAATACCCTGACCGTTCAGAATAGCAATGCATGGGTTGAAGTTAGC
GCAAGCGCACTGCAGCATAATATTCGCACCCTGCAGGCAGAACTGGCAGGTAAAAGCAAACTGTGTGCAGTTCTG
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GTTGCAAGCAATGAAGAGGCACGTGTTGTTCGTGCAAGCGGTTTTACAGGTCAGCTGGTTCGTGTTCGTCTGGCA
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GTTGCACTGATGACCCATTTTGCAGTGGAAGATAAAGATGATGTTCGTAAAGGTCTGGCAGCCTTTAATGAACAG
ACCGATTGGCTGATTAAACATGCACGTCTGGATCGTAGCAAACTGACCCTGCATGCCGCAAATAGCTTTGCAACC
CTGGAAGTTCCGGAAGCCCGTCTGGATATGGTTCGTACCGGTGGTGCACTGTTTGGTGATACCGTTCCGGCACGT
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AATACACTGATGGTTGATGTGACCGATTTTCCGGATGTTAAAGGTGGTAATGAAGTTGTGCTGTTTGGAAAACAG
GCAGGCGGTGAAATTACCCAGGCAGAAATGGAAGAAATTAATGGTGCGCTGCTGGCCGATCTGTATACCGTTTGG
GGTAATAGCAATCCGAAAATTCTGGTTGATctcgag*caccaccaccaccaccac*

2. Background racemisation by an *E.coli* AlaR

Background racemisation using *E. coli* whole cells: In order to investigate the background racemisation mediated by the lyophilised cells containing the overexpressed ω-TA, the cells (20 mg) were rehydrated in sodium phosphate buffer (300 µL, 50 mM, pH 8) containing PLP (3.3 mM, 1 mM final concentration in the reaction) at 30°C and 120 rpm for 15 min. In parallel L-alanine (5 eq., 125 mM final concentration) was incubated in sodium phosphate buffer (400 µL, 50 mM, pH 8) at 30°C and 120 rpm for 15 min. After this initial incubation both mixtures were combined and the volume was adjusted to 1 mL by adding sodium phosphate buffer (50 mM, pH 8) containing NAD^+ (1 mM final concentration), ammonium formate (150 mM final concentration), FDH (20 U) and AlaDH (10 U). The mixture was incubated in the absence of ω-TA-substrate at 30 °C and 800 rpm. After 1, 2, 4, 6, 10 and 24 h samples were taken and processed as described in the experimental section of the main paper.

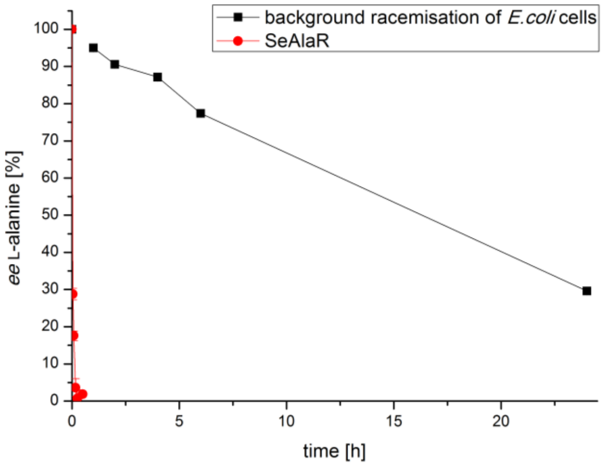


Fig. S1 Background racemisation of L-alanine (125 mM) over 24 h when incubated with *E. coli* whole cells containing the AT ω-TA (20 mg)(black). The racemisation using *E. coli* crude extract containing the SeAlaR (5mg) is plotted in red.

Figure S1 shows the racemisation of the *E. coli* cells not expressing the SeAlaR. The results clearly show that racemisation of L-alanine is catalysed by the *E. coli* cells. However, a comparison of the racemisation rate of the whole cells (black) with the SeAlaR catalysed racemisation (red) clearly shows that no efficient racemisation is catalysed by the *E. coli* background.

2.1 Purification of the ArR and AT ω-TA

Purification of the ArR ω-TA: For the disruption of the cells a 10 % (w/v) solution was prepared using an equilibration buffer (100 mM HEPES pH8, containing 0.5 mM PLP and 20 mM imidazole). The cells were disrupted by sonication (3 cycles of 1 min on ice, burst length 0.1 sec on and 0.4 off and duty cycle of 40%), and after each cycle the cells were kept on ice for 1 min. The disrupted cells were centrifuged at 13,000 rpm, and the supernatant was used in the purification procedure. For purification the crude extract was loaded onto a column containing 2 mL of NiNTA material (Macherey Nagel). After binding, the column was washed with 2 volumes of equilibration buffer. Next the protein was eluted using an imidazole concentration of 200 mM (100 mM HEPES pH8, containing 0.5 mM PLP and 200 mM imidazole). For desalting a PD10-desalting column (GE Healthcare) was used with 10 mM HEPES buffer at pH 8. The desalted protein was freeze dried and stored at -20.

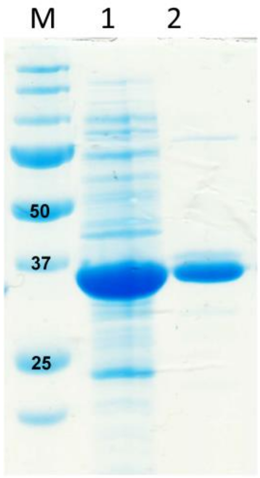


Fig. S2 Purification of the ArR ω-TA. Showing the crude extract (lane 1) and the purified protein (lane 2; 2.5 µg). M = precision plus protein all blue standard (Bio Rad).

Figure S2 shows the result of the purification indicating that the ArR ω-TA was purified to homogeneity (>95%). Both ω-TAs were purified following the outlined procedure but based on the higher specific activity further experiments addressing the background racemisation were performed using the purified ArR ω-TA.

2.2 Racemisation in the presents of the purified ArR ω-TA

Determination of the FDH and GDH activity: Activities of the formate dehydrogenase (FDH) and glucose dehydrogenase (GDH) were determined spectrophotometrically by measuring the initial velocity change at 340 nm. The assay was performed as follows: 980 µL of substrate solution [50 mM glucose (GDH) or 30 mM ammonium formate (FDH) in sodium phosphate buffer (50 mM, pH 8)] and 10 µL NAD^+ solution (100 mM in water) were mixed in a 1 mL cuvette. The reaction was started by the addition of 10 µL of enzyme solution, and production of NADH was monitored at 340 nm over a period of 1 min. One unit was defined as the amount of NADH (µmol) produced per unit of time (min) and per amount of lyophilised crude extract (mg).

Amination of 4-phenyl-2-butanone using purified ArR ω-TA and FDH and GDH for NADH recycling: The reactions were performed as follows: 4-phenyl-2-butanone (10 mM), co-substrate [30 mM ammonium formiate (FDH) or 50 mM glucose (GDH)], D- or L-alanine (100 mM), NAD^+ (1 mM) AlaDH (10 U) and GDH or FDH (17 or 3.4 U) were solved in sodium phosphate buffer (50 mM, pH 8) containing PLP (0.5 mM). Reactions were started by the addition of ArR ω-TA (2 mg) and incubated at 30°C and 800 rpm shaking. Samples were taken over a period of 4h and analysed as described in the experimental section of the main paper.

Racemisation of L-alanine in the presence of the individual enzymes: For determining the racemisation of L-alanine in the presence of the individual enzymes the following experiment was performed (1 mL): L-alanine (125 mM) was incubated in the presence of ammonium formiate (150 mM), PLP (1 mM) and NAD^+ (1 mM) in sodium phosphate buffer (50 mM, pH 8). Individually, FDH (5 mg), AlaDH (10 U), AT ω-TA (2 mg) and ArR ω-TA (2 mg) and no enzyme were added and the mixture was incubated at 30°C and 800 rpm shaking over 24 h. The racemisation of L-alanine was monitored as described in the experimental section of the main paper.

Table S1 Conversion of 4-phenyl-2-butanone using the purified ArR ω-TA, D- or L-alanine without the SeAlR as amine donor and different recycling enzymes.

	recycling enzyme[a]		conversion [%][b]	
	units	amount [mg]	D-alanine	L-alanine
FDH	3.7	5	81.5	6.8
GDH	3.7	1	74.3	0
GDH	17.0	5	90.0	5

[a] for both crude preparations were used

[b] conversions after 90 min

The results of the biotransformations using purified ArR ω-TA indicate that the activity with L-alanine is not dependent on the amount of ω-TA, as it was kept constant at 2 mg. The reason for the observed racemisation of L-alanine without the SeAlR is caused by the crude preparation of the recycling enzyme also produced in *E. coli*. Comparing the used FDH, which shows a rather low activity, with the GDH displaying a much higher activity the background activity can be significantly reduced in case of the GDH when adding similar units of both recycling enzymes. However, when also adding 5 mg of the GDH preparation, the racemisation rate is similar. Therefore, the activity of the ω-TAs with L-alanine is not due to acceptance of L-alanine as substrate but due to an AlR in the *E. coli* host. These findings were further proven by an additional experiment were the individual enzyme preparations (purified AlaDH, AT ω-TA, ArR ω-TA, crude FDH) were incubated with L-alanine. Here only the reaction containing the FDH showed racemisation of L-alanine over time and after 24 h an *ee*-value of 63.6% was reached. In summary the performed experiments could clearly show that the observed racemisation is caused by an AlaR in the *E. coli* background and not by a non-specific ω-TA.

2.3 Time course of the biotransformation of the amination of acetophenone using AT ω-TA

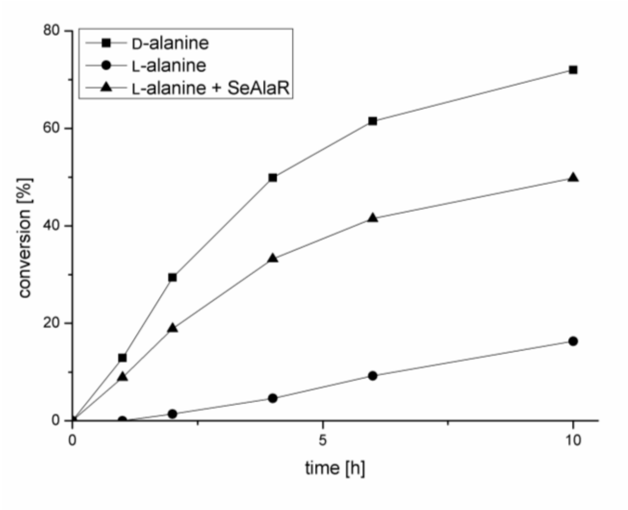
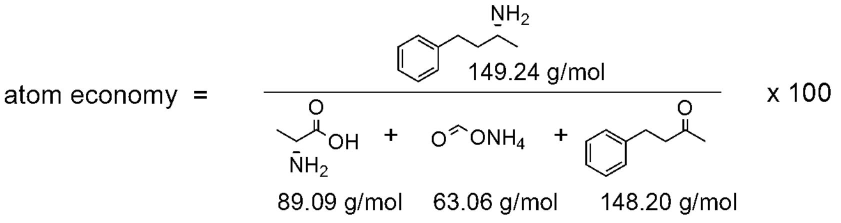


Fig. S3 Time study of the amination of acetophenone using the AT ω-TA with either D-alanine, L-alanine or racemic alanine derived by the SeAlaR as amine donor.

3. Atom economy calculations

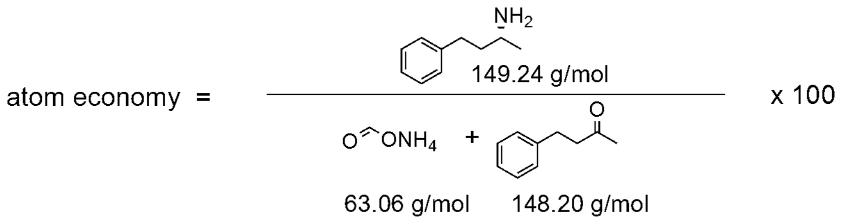
$$\text{atom economy} = \frac{\text{molecular mass of desired product}}{\text{molecular mass of all reactants}} \times 100$$

1) AlaDH/FDH system using D-alanine as amine donor



atom economy = **49.7%**

2) AlaDH/FDH/AlaR system using L-alanine after racemisation as amine donor



atom economy = **70.6%**

4. Environmental assessment using EATOS

The E-factor was calculated using the EATOS software tool (v1.1)[1,2] and results are summarised in figure S4. All parameters used for the calculation are given in a separate excel file.

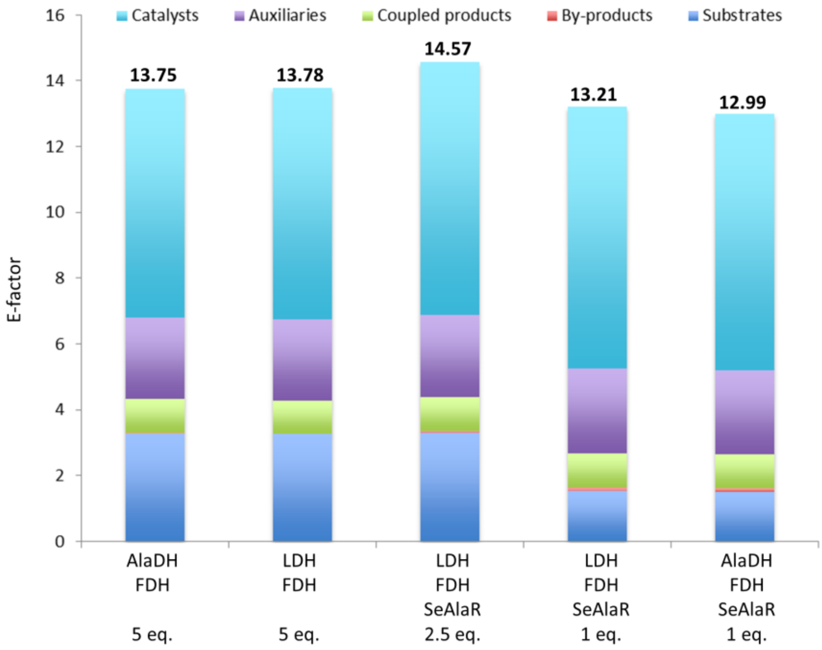


Fig. S4 Comparison of E-factors comparing the different biocatalytic procedures.

5. Literature

1. M. Eissen and J. O. Metzger, Chem.–Eur. J., 2002, 8, 3580–3585;
2 EATOS: Environmental Assessment Tool for Organic Syntheses, http://www.metzger.chemie.uni-oldenburg.de/eatos/english.htm