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Asymmetric synthesis of chiral halogenated amines using amine transaminases

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Abstract: Amine transaminases (ATA) are versatile and industrially relevant biocatalysts, catalyzing the transfer of an amine group from a donor to an acceptor molecule. Asymmetric synthesis from a prochiral ketone is the most preferred route to the desired amine product, since it is obtainable at a theoretical yield of 100 %. In addition to the requirement of active and enantioselective ATAs, also the choice of a suitable amine donor is important to save costs and to avoid additional enzymes for shifting the equilibrium and/or recycling of cofactors. In this work, we identified suitable (*R*)- and (*S*)-ATAs from *Aspergillus fumigatus* and *Silicibacter pomeroyi*, respectively to afford a set of halogen-substituted derivatives of brominated or chlorinated 1-phenyl-2-propanamine, 4-phenylbutan-2-amine and 1-(3-pyridinyl)ethanamine. Optimization of the donor-acceptor-ratio enabled the application of isopropylamine as amine donor resulting in high conversion and amines with 73-99 %*ee*.

Optically pure amines are important and versatile building blocks for the pharmaceutical and agrochemical industry. Chemical synthesis strategies are usually waste intensive and the formation of chiral amines requires the use of transition metals and chiral auxiliaries.[1] Enzymatic routes to chiral compounds are an environmentally benign alternative, because of their mild operation conditions and intrinsic selectivity of the biocatalysts.
One strategy to access primary chiral amines is the utilization of amine transaminases (ATA). ATAs are pyridoxal-5'-phosphate (PLP)-dependent enzymes and belong to the PLP fold classes I or IV. They catalyze the transfer of a primary amine group from a donor molecule to an acceptor molecule via a Ping-Pong-Bi-Bi-mechanism using PLP as co-factor. The substrate recognition is ensured by two binding pockets: a small one only able to accommodate a methyl group, and a large one with space for e.g., a phenyl or a carboxyl moiety. An asymmetric synthesis is the most convenient and economically favored route to a target amine since it starts from the prochiral ketone and results in the desired chiral product with a theoretical yield of 100 %.[2–4] The first asymmetric synthesis of (*S*)-1-phenylethylamine (1-PEA) utilizing the ATA from *Vibrio fluvialis* JS17 was reported by Shin *et al.* applying several amine donors, e.g. 1-PEA, 1-aminoindane or 1-aminotetralin.[5] Those amine donors are favored in a thermodynamic manner since the corresponding ketones show a drastically lower reactivity considering the reverse reaction, which makes them interesting for a biocatalytic approach.[5–8] However, the use of alanine – the natural amine donor – is one of the most common strategies for the amination of the target ketones.[9–11] The major drawback is the necessity of the removal of the co-product pyruvate by additional enzymes, since a large excess of alanine does not lead to a thermodynamic more favorable situation.[12] For this purpose pyruvate decarboxylase[2], alanine dehydrogenase or most commonly the combination of lactate and glucose dehydrogenase (LDH/GDH-system)[13,14] were applied, in order to drive the desired transamination reaction to completeness. More recently, aliphatic diamines were reported as effective amine donors.[15–20]
An interesting alternative as amine donor is isopropylamine (IPA) since it is cheap, achiral and the by-product acetone can be stripped off the reaction continuously as demonstrated on industrial scale for the synthesis of (*S*)-sitagliptin using an engineered (*R*)-ATA.[21] The only downside of this system is the necessity of a large IPA excess to drive the equilibrium to the desired product side, which might hamper the stability and activity of the ATAs.[9,12,19,21]

To the best of our knowledge there are only a few reports dealing with the acceptance of halogenated ketones by amine transaminases.[15–17,19,20,22–31] For instance, Slabu *et al.*,[19] Cassimjee *et al.*[22] and Paul *et al.*[24] investigated halogenated acetophenone derivatives. López-Iglesias *et al.*[27] successfully produced fluorinated 1-(3-pyridinyl)-ethanamine and Meadows *et al.*[28] as well as Frodsham *et al.*[29] reported the synthesis of (*S*)-1-(5-fluoropyrimidin-2-yl)-ethylamine. Gomm *et al.*,[15] Green *et al.*[16] and Martinez-Montero *et al.*[17] subjected 4-fluoro-phenylacetone to an asymmetric synthesis of the corresponding amine. Finally Schmidt *et al.*[30] focused on halogenated propargyl amies. However, the compounds described in this work were not yet investigated. We thus herein report the asymmetric synthesis of chlorinated and brominated 1-phenyl-2-propanamine, 4-phenylbutan-2-amine or 1-(3-pyridinyl)-ethanamine derivatives (Scheme 1) using different ATAs and isopropylamine as amine donor.

The choice of suitable amine transaminases was mainly guided by literature search, where similar, but usually non-halogenated ketones served as substrates.[9,10,13,19,23,24,32–35] For instance, ATA-117 was used to make (*R*)-1-(4-methoxy-phenyl)propan-2-amine[13], the ATA from *Chromobacterium violaceum* (Cvi-TA) catalyzed the conversion of 1-(4-methoxyphenyl)propan-2-one and 3-acetylpyridine[33], the ATAs from *Silicibacter pomeroyi*[32] (Spo-TA) and *Aspergillus fumigatus* (Afu-TA)[35] were utilized for the synthesis of both enantiomers of 4-phenylbutane-2-amine. Hence, we chose for our investigations the (*S*)-selective ATAs Spo-TA, Cvi-TA and due to structural similarities to the enzymes mentioned above, the ATA from *Vibrio fluvialis* (Vfl-TA) as well as the (*R*)-selective ATA from *Aspergillus fumigatus* (Afu-TA).[32,36–38] Of particular interest was the reported IPA acceptance by Spo-TA and Afu-TA.[39]

In order to get additional indicators for successful catalysis, docking experiments with representatives of the three substrate groups (Scheme 1) were performed *in silico* to strengthen the choice of focused ATAs. The quinonoid intermediates of **1b**, **9b** and **12b** were docked into the active sites of Spo-TA (PDB ID

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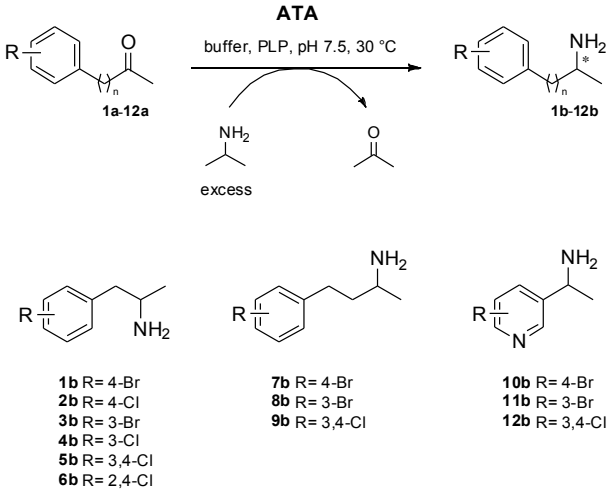
3HMU), Afu-TA (PDB ID 4CHI), Cvi-TA (PDB ID 4A6T) and Vfl-TA (PDB ID 4E3Q). The result showed a plausible coordination of all docked intermediate complexes for every considered ATA (Figure S1, Supporting Information).



Scheme 1. Amine products (bottom) obtained by the asymmetric synthesis (top) from the corresponding prochiral ketones (**1a**-**12a**) using isopropylamine as amine donor and different amine transaminases.

For preliminary test purposes, we decided to make the reaction with 1-(4-bromophenyl)propan-2-one **1a** as a model reaction to investigate several reaction conditions. Initially, we tested the ATAs for activity towards **1a** using various amine donors. By the application of the alanine/LDH/GDH system or providing 1-PEA in a slight excess (5-fold), very good conversion of **1a** to **1b** was observed using Cvi-TA (LDH/GDH system: 99 %, no conversion with 1-PEA), Afu-TA (LDH/GDH system: 99 % and 1-PEA: 98 %) and Spo-TA (1-PEA: 95 %; Table S1, Supporting Information). Vfl-TA did not convert **1a** at all. However, as 1-PEA is rather cost-intensive[6] and the alanine/LDH/GDH system requires the involvement of additional enzymes, isopropylamine as donor was investigated (especially the donor-acceptor-ratio) using the model substrate 1-(4-bromophenyl)propan-2-one **1a** with Spo-TA and Afu-TA. In case of Spo-TA a relative low 10-fold excess of IPA was already sufficient to observe high conversion (>80 %). The reaction with Afu-TA reached the same value only at 1 M IPA (a 200-fold excess; Figure S5, Supporting Information). Consequently, Spo-TA and Afu-TA were used for the asymmetric synthesis of all substrates using IPA as amine donor (Scheme 1, Table 1). Both, Spo-TA and Afu-TA showed excellent conversions (>90 %) with minor exceptions (**8a**–**10a** and **12a**, ~78–88 % conv.). The optical purity of the chiral amine products were predominantly excellent (>95 %*ee*, with two exceptions: amines **7b** and **9b**, 73–80 %*ee*, Table 1) as determined by chiral gas chromatography (GC) analysis (see Supporting Information for chromatograms and retention times, Table S4). Preparative scale reactions (50 or 100 mg **1a**) using Spo-TA or Afu-TA confirmed the identity of 1-(4-bromophenyl)-2-propanamine **1b** after GCMS analysis, ^{1}H- and ^{13}C-NMR spectroscopy (Supporting Information). The absolute configuration of each enantiomer was verified by literature data using optical rotation values.[40]

Thus, we demonstrated the enzymatic synthesis of a set of halogen-substituted chiral benzylic amines derived from 1-phenyl-2-propanamine, 4-phenylbutan-2-amine or 1-(3-pyridinyl)-ethanamine with isopropylamine as a cheap and convenient amine donor. The use of the ATAs from *Silicibacter pomeroyi* (Spo-TA) and *Aspergillus fumigatus* (Afu-TA) enabled mostly quantitative conversion of compounds **1a**–**12a** yielding the desired amines with excellent optical purity (mostly >99 %*ee*).

Table 1. Conversion and enantiomeric excess values of the amine products **1b**–**12b** obtained by asymmetric synthesis using IPA as donor and crude cell lysate containing overexpressed Spo-TA (0.75 U) or Afu-TA (0.5 U).

Substrate	Spo-TA			Afu-TA		
	c [%][a,b]		ee_P [%][b]	*c* [%][a,b]		ee_P [%][b]
1a	95.6	± 3.0	>99	96.2	± 0.3	>99
2a	96.6	± 0.8	>99	96.1	± 0.4	>99
3a	91.4	± 0.4	98	95.0	± 0.3	>99
4a	97.6	± 1.3	>99	95.9	± 0.5	>99
5a	96.3	± 2.9	98	93.4	± 1.1	>99
6a	97.1	± 2.2	>99	91.1	± 0.4	>99
7a	93.3	± 2.8	80	93.9	± 0.4	>99
8a	95.9	± 1.9	95	84.5	± 4.1	>99
9a	88.0	± 6.0	73	95.6	± 0.7	>99
10a	88.8	± 5.0	>99	78.4	± 2.3	>99
11a	93.9	± 4.7	>99	91.4	± 0.4	>99
12a	90.7	± 8.3	>99	86.4	± 2.9	>99

[a] Reaction conditions: HEPES buffer (50 mM) pH 7.5, 5 mM ketone, isopropylamine (0.25 M for Spo-TA, 1 M for Afu-TA) , 10 % DMF, 30°C, 18 h. [b] Conversion and enantiomeric excess were determined via chiral GC analysis using a Hydrodex-ß-TBDAc column (Macherey & Nagel). All measurements were performed in triplicates.

Experimental Section

All chemicals were purchased either from Sigma Aldrich (Darmstadt, Germany), Roth (Karlsruhe, Germany), Acros/Thermofisher Scientific (Waltham, USA) or Enamine (Monmouth, USA) in analytical grade.

Enzyme expression and cell lysis

Genes encoding the ATAs from *Silicibacter pomeroyi* and *Aspergillus fumigatus* were subcloned into the pET22b vector with a C-terminal His-tag and transformed into *E. coli* BL21 (DE3) as described previously.[32] ATAs from *Chromobacterium violaceum* and *Vibrio fluvialis* were available in a pET24b and pET28a vector containing a C-terminal or N-terminal His-tag, respectively. The protein expression was done in TB media with 100 µg/mL ampicillin or 50 µg/mL kanamycin at 160 rpm and 20°C. After the optical density at 600 nm (OD_{600}) reached 0.7, expression was induced by adding 0.2 mM isopropyl β-D-1-thiogalactopyranoside (final concentration). After 18 h the cultures were centrifuged (4,000 x *g*, 15 min, 4 °C) and washed with lysis buffer (HEPES (50 mM pH 7.5), 0.1 mM PLP, 300 mM NaCl). Cell disruption was performed via sonication using the Bandelin Sonoplus HD 2070 (8 min, 50 % pulsed cycle, 50 % power) on ice followed by centrifugation in order to remove cell debris (12,000 x *g*, 45 min, 4 °C, Sorvall centrifuge). The supernatant containing the crude ATA was stored at 4 °C until use.

Determination of transaminase activity

The characterization of the ATAs was done via the acetophenone assay according to Schätzle *et al.*[41] with slight modifications. As all used ATAs showed their optimum at basic pH values, the assay was performed in CHES buffer (50 mM pH 9). In the reaction solution the concentrations of the amine donor ((*R*)- or (*S*)-1-PEA) and the acceptor pyruvate was set to 1.25 mM in 0.25 % (v/v) DMSO. Briefly, 10 µL of a pre-diluted ATA solution was mixed with buffer and the reaction was initiated by the addition of the reaction solution. The formation of acetophenone was quantified at 245 nm using the Tecan Infinite M200 Pro (Crailsheim, Germany) at 30 °C. One unit (U) of ATA activity was defined as the formation of 1 µmol acetophenone per minute (ε= 12 $mM^{-1}cm^{-1}$). All measurements were performed in triplicates.

Asymmetric synthesis of the chiral amines 1b**–**12b

Biotransformations of ketones **1a**–**12a** were performed in triplicates and in 0.25 mL scale using 1.5 mL glass vials at 30 °C and 950 rpm shaking. The reaction mixtures contained 50 µL ATA crude lysate (9–14 U/mL), 5 mM ketone, 10 % DMF as co-solvent, 250 mM IPA in HEPES 50 mM pH 7.5–8. After 24 h incubation, the reaction was quenched by adding 3 M NaOH (resulting in pH ≥13). Samples for gas chromatography (GC) analysis were taken immediately after this quenching.

GC analysis

Samples of 30–40 µL were taken for chiral GC analysis and extracted with 250 µL ethyl acetate containing 1 mM 4'-iodoacetophenone as internal standard for quantification. The organic layers were dried over anhydrous $MgSO_4$ and derivatized with *N*-Methyl-bis-trifluoroacetamide (MBTFA) by adding 7.5 µL of the commercial stock solution to 100 µL of the organic layer and incubation at 60 °C for 30 min. Afterwards, the samples were analyzed immediately using the chiral Hydrodex-ß-TBDAc column (Macherey & Nagel). For the analysis of all ketones the following temperature gradient program was established: initial temperature 80 °C, kept for 10 min, linear gradient to 175 °C with a slope of 4 °C min^{-1}, kept for 13 min, linear gradient to 220 °C with a slope of 20 °C min^{-1}, kept for 5 min. The conversion for each compound was determined by quantification of substrate consumption via calculation of the response factor. Each sample included an internal standard and was set in relation to control experiments in each batch experiment with known substrate concentration. The linearity of the response factor over a certain range was verified via a calibration curve. The chiral analysis of the amine products was either done as described above or with the following temperature profile: 60 °C, kept for 35 min, linear gradient to 165 °C with a slope of 2 °C min^{-1}, kept for 20 min, linear gradient to 220 °C with a slope of 5 °C min^{-1}, kept for 10 min.

Preparative scale synthesis of 1b

The conversion of 1-(4-bromophenyl)propan-2-one **1a** to 1-(4-bromophenyl)-2-propanamine **1b** was performed in preparative scale with crude cell lysate containing Spo-TA or Afu-TA, respectively. The following conditions were applied: 50 mg **1a** (using Spo-TA) or 100 mg **1a** (using Afu-TA) were added to an Erlenmeyer flask and mixed with DMF (12 % final concentration), HEPES buffer (50 mM final, pH 7.5) and isopropylamine (850 mM final concentration). The pH was adjusted with aqueous HCl. In the end 15 vol% of crude cell lysate was added, which led to a final working volume of 30 mL in case of Spo-TA and 50 mL in case of Afu-TA. The reaction mixture was incubated for 72 h at 30 °C under agitation. For the quantification of the conversion, samples were taken and extracted as described above. The reaction was stopped when no further conversion was observed during reaction monitoring (for Spo-TA 98 %, for Afu-TA 62 %). The following reaction workup was done as followed: After acidification with 3 M aq. HCl to pH 1, an extraction with 2x 30 mL ethyl acetate was performed in a separation funnel. The aqueous layer was basified afterwards with 3 M aq. NaOH solution to a pH of 11–12 and extracted 5 times with 15 mL methyl *tert*-butyl ether. The combined organic layers were washed with 2x 15 mL saturated brine solution, dried over anhydrous $MgSO_4$ and evaporated under vacuum. The consistency of the amine product was a yellow oil. Each product was confirmed via GCMS and 10-12 mg each were subjected to ^{1}H- and ^{13}C-NMR spectroscopy. A racemic standard was synthesized for comparison reasons according to literature[42] (^{1}H- and ^{13}C-NMR spectra, see Supporting Information).

The absolute configuration was determined via optical rotation data using the Polar-L polarimeter from IBZ Messtechnik. A solution of each enantiomer in chloroform was prepared (concentration as indicated). The specific optical rotation data of each enantiomer were as followed and could be verified with literature.[40]
(*S*)-1-(4-bromophenyl)-2-propanamine (*S*)-**1b**, 25 % isolated yield (not optimized), >99 %*ee*, $[\alpha]^{20}_{D}$ = +20.8 (c =0.9, $CHCl_3$).
(*R*)-1-(4-bromophenyl)-2-propanamine (*R*)-**1b**, 27 % isolated yield (not optimized), >99 %*ee*, $[\alpha]^{20}_{D}$ = -25.9 (c =1.2, $CHCl_3$).

Bioinformatic analyses

Docking studies were done using *YASARA Structure* (v.17.1.28).[43] The quinonoid intermediate (the reactive intermediate in the ATA reaction) consisting of PLP and **1b**/**9b**/**12b** was modelled by a combination of *ChemDraw*® (v.11) and *YASARA* followed by energy minimization. Prior to the docking experiments a refinement of the lowest energy ligand conformation and all used crystal structures from the PDB database was performed with the built in macro (YAMBER3 force field at 298 K for 500 ps, standard settings). Since Spo-TA (PDB ID 3HMU) was crystallized in the apo-form, the position of the PLP co-factor had to be determined first as its position was crucial for the visual evaluation of the docking result (mainly by superposing the docked ligand-receptor complex with the refined, PLP containing crystal structure and checking the coverage of the pyridine ring of the PLP). A PDB-BLAST search was done, revealing the best result in sequence identity of 53 % with the ATA from *Chromobacterium violaceum* (PDB ID 4A6T, for BLAST result see Supporting Information). Both structures were superimposed and the position of the PLP was transferred from one structure to another. Because of plausibility reasons the surrounding binding residues of the phosphate group and the nitrogen of the PLP pyridine ring were confirmed with literature data.[44] The applied docking method was the implemented *AutoDock VINA* algorithm with standard settings, which means 100 runs in total and subsequent clustering to give distinct complex conformations. The output was evaluated visually. Usually the best conformation revealed by this way was one of those with the highest binding energy and lowest dissociation constant according to the docking log file. All illustrations were made with *Pymol* (v.1.7).

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[1] M. Höhne, U. T. Bornscheuer, *ChemCatChem* **2009**, *1*, 42–51.
[2] M. Höhne, S. Kühl, K. Robins, U. T. Bornscheuer, *ChemBioChem* **2008**, *9*, 363–365.
[3] A. Iwasaki, Y. Yamada, Y. Ikenaka, J. Hasegawa, *Biotechnol Lett* **2003**, *25*, 1843–1846.
[4] A. Iwasaki, Y. Yamada, N. Kizaki, Y. Ikenaka, J. Hasegawa, *Appl Microbiol Biotechnol* **2006**, *69*, 499–505.
[5] J. S. Shin, B. G. Kim, *Biotechnol. Bioeng.* **1999**, *65*, 206–11.

[6] P. Tufvesson, M. Nordblad, U. Krühne, M. Schürmann, A. Vogel, R. Wohlgemuth, J. M. Woodley, *Org. Process Res. Dev.* **2015**, *19*, 652–660.
[7] J.-H. Seo, D. Kyung, K. Joo, J. Lee, B.-G. Kim, *Biotechnol. Bioeng.* **2011**, *108*, 253–63.
[8] U. Kaulmann, K. Smithies, M. E. Smith, H. C. Hailes, J. M. Ward, *Enzyme Microb. Technol.* **2007**, *41*, 628–637.
[9] F. G. Mutti, C. S. Fuchs, D. Pressnitz, J. H. Sattler, W. Kroutil, *Adv. Synth. Catal.* **2011**, *353*, 3227–3233.
[10] W. Kroutil, E.-M. Fischereder, C. S. Fuchs, H. Lechner, F. G. Mutti, D. Pressnitz, A. Rajagopalan, J. H. Sattler, R. C. Simon, E. Siirola, *Org. Process Res. Dev.* **2013**, *17*, 751–759.
[11] A. Nobili, F. Steffen-Munsberg, H. Kohls, I. Trentin, C. Schulzke, M. Höhne, U. T. Bornscheuer, *ChemCatChem* **2015**, *7*, 757–760.
[12] D. Koszelewski, K. Tauber, K. Faber, W. Kroutil, *Trends Biotechnol.* **2010**, *28*, 324–32.
[13] D. Koszelewski, I. Lavandera, D. Clay, D. Rozzell, W. Kroutil, *Adv. Synth. Catal.* **2008**, *350*, 2761–2766.
[14] P. Tufvesson, J. Lima-Ramos, J. S. Jensen, N. Al-Haque, W. Neto, J. M. Woodley, *Biotechnol. Bioeng.* **2011**, *108*, 1479–1493.
[15] A. Gomm, W. Lewis, A. P. Green, E. O'Reilly, *Chem.- Eur. J.* **2016**, *22*, 12692–12695.
[16] A. P. Green, N. J. Turner, E. O'Reilly, *Angew. Chem. Int. Ed.* **2014**, *53*, 10714–10717.
[17] L. Martinez-Montero, V. Gotor, V. Gotor-Fernández, I. Lavandera, *Adv. Synth. Catal.* **2016**, *358*, 1618–1624.
[18] S. E. Payer, J. H. Schrittwieser, W. Kroutil, *Eur. J. Org. Chem.* **2017**, *2017*, 2553–2559.
[19] I. Slabu, J. L. Galman, C. Iglesias, N. J. Weise, R. C. Lloyd, N. J. Turner, *Catal. Today* **2017**.
[20] J. L. Galman, I. Slabu, N. J. Weise, C. Iglesias, F. Parmeggiani, R. C. Lloyd, N. J. Turner, *Green Chem.* **2017**, *19*, 361–366.
[21] C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, *Science* **2010**, *329*, 305–309.
[22] K. E. Cassimjee, M. S. Humble, H. Land, V. Abedi, P. Berglund, *Org. Biomol. Chem.* **2012**, *10*, 5466–5470.
[23] M. Svedendahl, C. Branneby, L. Lindberg, P. Berglund, *ChemCatChem* **2010**, *2*, 976–980.
[24] C. E. Paul, M. Rodriguez-Mata, E. Busto, I. Lavandera, V. Gotor-Fernández, V. Gotor, S. Garcia-Cerrada, J. Mendiola, Ó. de Frutos, I. Collado, *Org. Process Res. Dev.* **2014**, *18*, 788–792.
[25] S.-W. Han, E.-S. Park, J.-Y. Dong, J.-S. Shin, *Adv. Synth. Catal.* **2015**, *357*, 2712–2720.
[26] A. Cuetos, M. Garcia-Ramos, E.-M. Fischereder, A. Diaz-Rodriguez, G. Grogan, V. Gotor, W. Kroutil, I. Lavandera, *Angew. Chem. Int. Ed.* **2016**, *128*, 3196–3199.
[27] M. López-Iglesias, D. González-Martínez, M. Rodríguez-Mata, V. Gotor, E. Busto, W. Kroutil, V. Gotor-Fernández, *Adv. Synth. Catal.* **2017**, *359*, 279–291.
[28] R. E. Meadows, K. R. Mulholland, M. Schürmann, M. Golden, H. Kierkels, E. Meulenbroeks, D. Mink, O. May, C. Squire, H. Straatman, *Org. Process Res. Dev.* **2013**, *17*, 1117–1122.
[29] L. Frodsham, M. Golden, S. Hard, M. N. Kenworthy, D. J. Klauber, K. Leslie, C. Macleod, R. E. Meadows, K. R. Mulholland, J. Reilly, *Org. Process Res. Dev.* **2013**, *17*, 1123–1130.
[30] N. G. Schmidt, R. C. Simon, W. Kroutil, *Adv. Synth. Catal.* **2015**, *357*, 1815–1821.
[31] M. L. Contente, M. Planchestainer, F. Molinari, F. Paradisi, *Org Biomol Chem* **2016**, *14*, 9306–9311.
[32] F. Steffen-Munsberg, C. Vickers, A. Thontowi, S. Schätzle, T. Tumlirsch, M. Svedendahl Humble, H. Land, P. Berglund, U. T. Bornscheuer, M. Höhne, *ChemCatChem* **2013**, *5*, 150–153.
[33] A. K. Holzer, K. Hiebler, F. G. Mutti, R. C. Simon, L. Lauterbach, O. Lenz, W. Kroutil, *Org. Lett.* **2015**, *17*, 2431–2433.
[34] D. Koszelewski, M. Göritzer, D. Clay, B. Seisser, W. Kroutil, *ChemCatChem* **2010**, *2*, 73–77.
[35] S. Schätzle, F. Steffen-Munsberg, A. Thontowi, M. Höhne, K. Robins, U. T. Bornscheuer, *Adv. Synth. Catal.* **2011**, *353*, 2439–2445.
[36] M. Höhne, S. Schätzle, H. Jochens, K. Robins, U. T. Bornscheuer, *Nat. Chem. Biol.* **2010**, *6*, 807–813.
[37] M. Thomsen, L. Skalden, G. J. Palm, M. Höhne, U. T. Bornscheuer, W. Hinrichs, *Acta Crystallogr. D Biol. Crystallogr.* **2014**, *70*, 1086–1093.
[38] L. Skalden, M. Thomsen, M. Höhne, U. T. Bornscheuer, W. Hinrichs, *FEBS J.* **2015**, *282*, 407–415.
[39] H. Mallin, M. Höhne, U. T. Bornscheuer, *J. Biotechnol.* **2014**, *191*, 32–7.
[40] L. Munoz, A. M. Rodriguez, G. Rosell, M. P. Bosch, A. Guerrero, *Org. Biomol. Chem.* **2011**, *9*, 8171–8177.
[41] S. Schätzle, M. Höhne, E. Redestad, K. Robins, U. T. Bornscheuer, *Anal. Chem.* **2009**, *81*, 8244–8248.
[42] R. Laufer, G. Ng, Y. Liu, N. K. B. Patel, L. G. Edwards, Y. Lang, S.-W. Li, M. Feher, D. E. Awrey, G. Leung, *Bioorg. Med. Chem.* **2014**, *22*, 4968–4997.
[43] E. Krieger, G. Koraimann, G. Vriend, *Proteins Struct. Funct. Bioinforma.* **2002**, *47*, 393–402.
[44] M. S. Humble, K. E. Cassimjee, M. Haakansson, Y. R. Kimbung, B. Walse, V. Abedi, H.-J. Federsel, P. Berglund, D. T. Logan, *FEBS J.* **2012**, *279*, 779–792.

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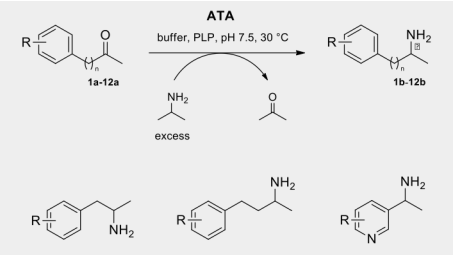
The asymmetric synthesis of chiral brominated and chlorinated 1-phenyl-2-propanamine, 4-phenyl-butan-2-amine and 1-(3-pyridinyl)-ethanamine derivatives is presented. Using suitable (*R*)- and (*S*)-selective ATAs from *Aspergillus fumigatus* and *Silicibacter pomeroyi* a set of halogen-substituted amines was obtained at high conversion (74–99%) with mostly excellent optical purity (73–99%*ee*) using isopropylamine as amine donor.



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Asymmetric synthesis of chiral halogenated amines using amine transaminases