Advanced Synthesis & Catalysis

Accepted Article

Title: Donor Amine Salt-Based Continuous in situ-Product Crystallization in Amine Transaminase-Catalyzed Reactions

Authors: Dennis Hülsewede, Jan-Niklas Dohm, and Jan von Langermann

To be cited as: *Adv. Synth. Catal.* 10.1002/adsc.201900217

Link to VoR: http://dx.doi.org/10.1002/adsc.201900217

10.1002/adsc.201900217

UPDATE

DOI: 10.1002/adsc.201((will be filled in by the editorial staff))

Donor Amine Salt-Based Continuous *in situ*-Product Crystallization in Amine Transaminase-Catalyzed Reactions.

Dennis Hülsewede, Jan-Niklas Dohm, Jan von Langermann*

University of Rostock, Institute of Chemistry, Biocatalytic Synthesis Group, Albert-Einstein-Str. 3A, 18059 Rostock, Germany
Fax: (+49)-(0)381-498-6452; phone: (+49)-(0)381-498-6456; e-mail: jan.langermann@uni-rostock.de

Received: ((will be filled in by the editorial staff))

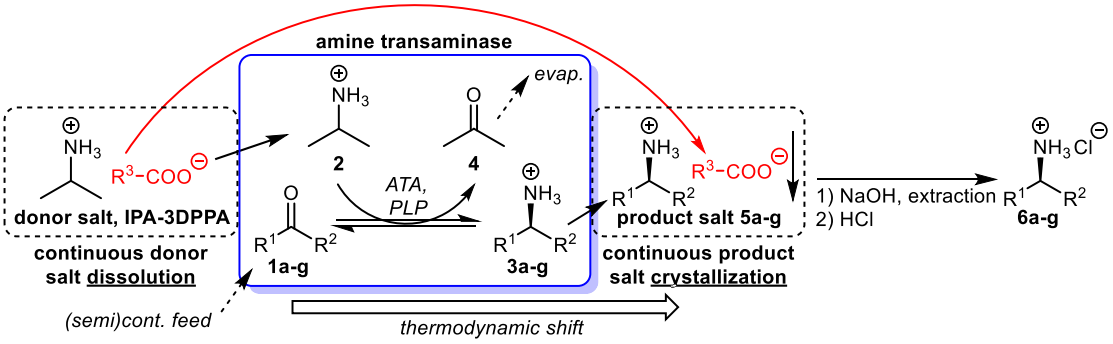
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201######.((Please delete if not appropriate))

Abstract. The unfavorable reaction equilibrium of transaminase-catalyzed reactions is a major challenge for the efficient biocatalytic synthesis of chiral amines. In this study the synthetic utilization of a salt-based, continuous *in situ*-product crystallization is described to overcome the thermodynamic limit in amine transaminase-reactions using only the commonly used amine donor isopropylamine. The simultaneous dissolution of isopropylammonium 3,3-diphenylpropionate (donor salt) in combination with the crystallization of the product salt facilitates a thermodynamic shift of the continuous amine transaminase-catalyzed reaction. The main process necessity is a lower product salt solubility in comparison to the applied donor salt. This concept facilitates a stoichiometric use of isopropylamine in combination with a significantly lowered concentration of the amines in solution.

Keywords: biocatalysis; *in situ*-product removal; crystallization; amine; salts

Transaminases (TAs) (E.C. 2.6.1.X) catalyze the selective transfer of an amino group from an amine donor to an amine acceptor using pyridoxal-5'-phosphate (PLP) as a cofactor.[1,2] The ability of transaminases to synthesize optically pure amines/ amino acids is a major advantage over conventional (transition) metal-catalyzed options and thus a powerful tool for industrial applications.[3] Herein the synthesis of chiral amines can be achieved either by kinetic resolution of a racemic amine mixture or asymmetric synthesis from prochiral carbonyl substrates using donor amines, e.g. alanine or isopropylamine.[4] The direct asymmetric synthesis of amines is typically preferred due to the theoretical maximum yield of 100%.[5]

However, a major challenge is often the unfavorable reaction equilibrium of transaminase-catalyzed reactions towards the desired products.[6] A conventional solution for this drawback is the use of significant excesses of the chosen amine donor, but maintaining the quaternary and tertiary protein structure under such non-physiological conditions (incl. high product concentration) becomes easily a significant problem.[2] Alternatively, selective (bio)catalytic cascades can be used to remove by-products from the reaction equilibrium, which similarly pushes the reaction equilibrium towards the product side.[7] A well-known example is the use of the donor amine alanine in combination with a lactate dehydrogenase and a glucose dehydrogenase.[8] Here the byproduct pyruvate is reduced to lactate, while the desired equilibrium shift is eventually achieved by the glucose dehydrogenase-based cofactor regeneration system. Unfortunately, these cascade options require additional substrates and even entire biocatalytic systems, which itself may cause



Scheme 1. Schematic representation of the continuous donor salt-dissolution and product salt-crystallization within an amine transaminase-catalyzed reaction system. R^1 = aliphatic/aromatic, R^2 = CH_3, R^3 = CH_2-$CH(Ph)_2$.

secondary issues with the transaminase-catalyzed reaction itself and will always lower overall atom efficiency of the entire process and increase costs.

A powerful alternative is the use of membrane-assisted *in situ*-product removal (ISPR)-techniques, which target the removal of the product amine from its reaction solution.[9,10] Rehn *et al.* introduced an approach involving a supported liquid membrane (SLM) with undecane, which used an alkaline reactor phase and an acidic stripping phase for product isolation. The presented system achieved an impressive final product concentration of 1 mol/L, but the limited lifetime of the SLM was identified as a potential issue for industrial applications. Interestingly, the authors similarly propose the application of a continuous process, which includes a continuous harvesting of the product from the reaction solution.[10]

A further synthetic approach is the use of tailor-made amine donors, which undergo spontaneous secondary reactions after deamination and thus shift the reaction equilibrium, e.g. via dimerization and oxidation.[11] Although this represents a valuable approach, a varying amount of amine byproducts is always obtained, which have to be removed and discarded as waste.

As an alternative we've recently introduced the use of *in situ*-product crystallization (ISPC) of the product salt to shift the transaminase reaction equilibrium.[12,13] The apparent equilibrium shift is obtained by salt formation of the product with a specifically chosen counter-ion, which eventually crystallizes from solution. Product salt isolation is then easily achieved by a simple filtration step. However, a noticeable limitation of the presented technique is still the inability to reach full or even very high conversions since a residual solubility of the product salt is unavoidable. In addition, a 2.5-fold excess of the donor amine is still required to push the reaction to the product side.

With this study we aim to expand the recently introduced ISPC-methodology to achieve full conversion in amine transaminase-catalyzed reactions with only a stoichiometric amount of the donor amine isopropylamine. We propose the application of a continuous fed batch-type ISPC-process, which requires the presence of the *donor salt* isopropylammonium 3,3-diphenylpropionate (IPA-3DPPA) (Figure 1).

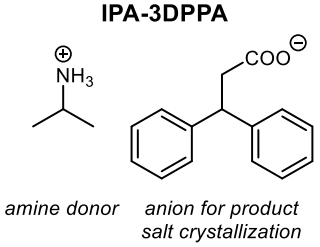


Figure 1. Chemical structure of the applied donor salt isopropylammonium 3,3-diphenylpropionate (IPA-3DPPA).

This represents a strong contrast to our earlier study, which aimed at specifically avoiding the presence of this unwanted donor salt since it reduces the purity of the crystallized product amine salt. The main purpose of this donor salt in the process concept is a continuous feed of isopropylamine (IPA, donor amine) and the required counter-ion 3,3-diphenylpropionate (3DPPA, for product amine crystallization) (Scheme 1). This is achieved by the continuous dissolution of the donor salt throughout the entire enzymatic process, which constantly feeds equimolar amounts of both reactants into the aqueous solution. The donor salt exhibits a moderate solubility in the aqueous solution, while any excess of solid IPA-3DPPA remains suspended in the aqueous reaction mixture without a direct, potentially harmful interaction with the amine transaminase reaction system (Figure 2). The application of this salt solves the low solubility issue of 3,3-diphenylpropionic acid, which is a main limiting parameter for the subsequent product salt formation. In addition, the undesired pH-shift by the acid is circumvented avoiding high buffer concentrations to compensate this effect.

During the continuous fed-batch process product salt crystallization occurs continuously in parallel and eventually enriches as the second suspended solid phase. In the shown concept, the mother liquor is recycled throughout the process, in contrast to discarding it as waste. The continuous process also does not require a significant excess of the donor amine to push the reaction equilibrium towards the product side.

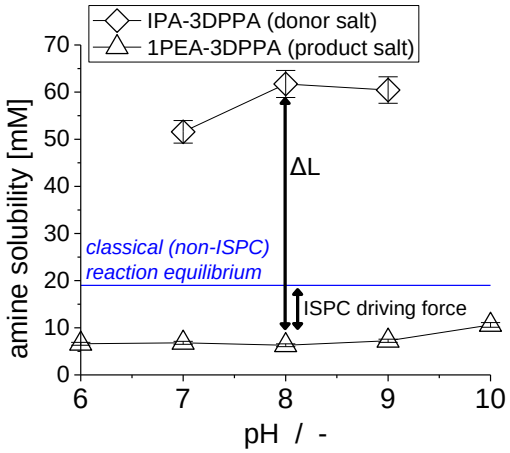


Figure 2. pH-dependency of salt solubilities; exemplary product salt: (*S*)-1-phenyl ethyl ammonium 3,3-diphenylpropionate (1PEA-3DPPA, **5a**). Conditions of non-ISPC-based batch reaction equilibrium: 100 mM acetophenone, 250 mM isopropylamine, 30 °C, 100 mM phosphate buffer pH 7.5.

Throughout the entire synthesis process both concentrations of donor and product amine remain constant in a steady state equilibrium with the suspended solid salts, which is only controlled by their respective solubilities. The amount of undissolved and thus suspended donor and product

salts will instead change continuously due to the constant dissolution and *in situ*-product crystallization. These interactions can be considered as two pH-dependent secondary equilibria, which will overlap with the amine transaminase-catalyzed reaction. As shown in Figure 2, the solubility of the product salt **5a** is significantly lower than the donor salt IPA-3DPPA over a broad pH range, indicated by the solubility difference ΔL ($\Delta L = L_{donor\ salt} - L_{product\ salt}$). Importantly, product salt solubility has to be below the original amine transaminase-based product concentration in solution (indicated by blue line) to force product salt crystallization, which represents the driving force of the apparent reaction equilibrium shift. If both salt solubilities are too similar the purity of the product salt will be affected. With the herein applied amine transaminase from *Silicibacter pomeroyi* (*Sp*ATA) the combined optimal pH for this ISPC-system was found to be at 7.5 (Figure 3).

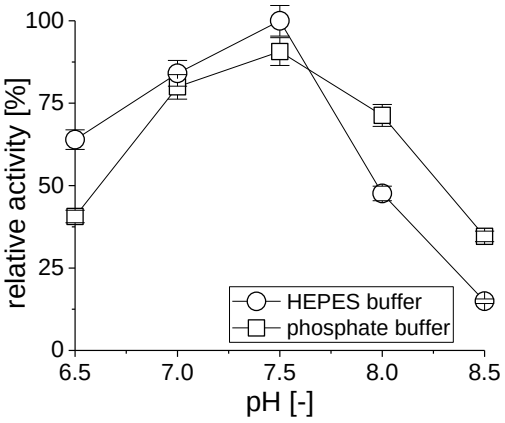


Figure 3. pH-dependency of the ISPC-based *Sp*ATA-catalyzed reaction; 30 °C, 100 mM acetophenone, 125 mM IPA-3DPPA, 125 mM IPA, 200 mM buffer, 0.5 U/mL *Sp*ATA, 2 mM PLP, t=22h. Relative activity is normalized to pH 7.5 = 100%.

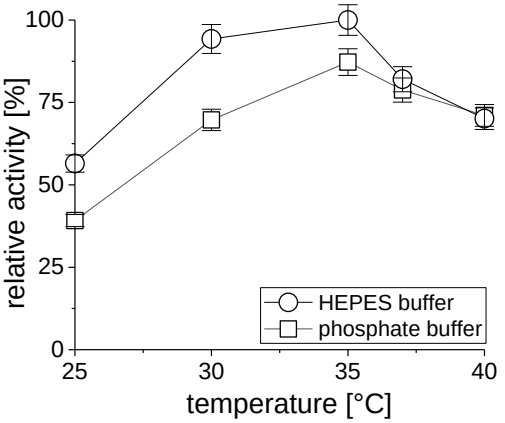


Figure 4. Temperature-dependency of the ISPC-based *Sp*ATA-catalyzed reaction; 100 mM acetophenone, 125 mM IPA-3DPPA, 125 mM IPA, 25 mM buffer pH 7.5, 0.5 U/mL *Sp*ATA, 2 mM PLP, t=22h. Relative activity is normalized to 35 °C = 100%.

Relatively strong reductions of enzymatic activity are visible below pH 6.5 and above pH 8. This relatively narrow window is mostly determined by the pH-optimum of the applied enzyme, but also influenced by the pH-dependent solubility behavior of both salts. Slight differences were found between the common HEPES and phosphate buffer systems. It should be noted that the presence of the applied buffer salts also increases the solubilities of both salts, based on the uncommon ion-effect, basically lowering the performance of the ISPC-based reaction system. The buffer capacity was therefore limited to 25 mM throughout this study to prevent any negative effects. Even with the lowest buffer capacity no pH shift was observed in this study. In addition, a similar effect is also present for reaction temperature, whereas an optimum of 35 °C was found (Figure 4). Herein the temperature range overlaps with the increasing salt solubility at higher temperatures. Consequently, the choice of the buffer salts and the pH has a significant effect on the ISPC-process and in this study HEPES-buffer was found to be the best choice (Figure 5).

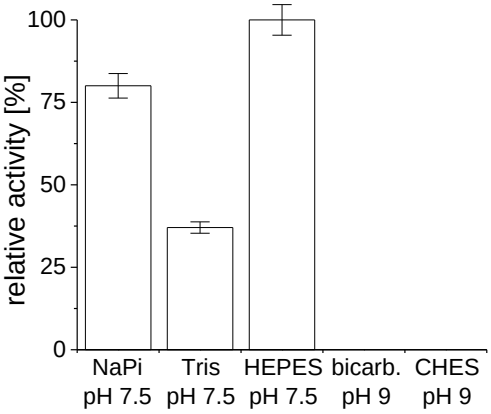


Figure 5. Effect of the applied buffer system on the ISPC-based *Sp*ATA-catalyzed reaction; 30 °C, 100 mM acetophenone, 125 mM IPA-3DPPA, 125 mM IPA, 25 mM buffer pH 7.5, 0.5 U/mL *Sp*ATA, 2 mM PLP, t=22h

Phosphate and TRIS buffer showed moderate to strong reductions of enzyme activity, which is mostly based on the (conventional) effect of different buffer salts towards the enzyme itself. CHES and bicarbonate buffer systems are clearly not applicable due to their high pH.

A further improvement of the ISPC-process was achieved by the use of additional isopropylamine in the reaction mixture. This strategy is based on the relatively high K_M-value of isopropylamine for many amine transaminases, which is hardly reached by the solubility limit of the applied donor salt IPA-3DPPA alone. A maximum of process performance was found using an additional concentration of 100 mM isopropylamine (Figure 6).

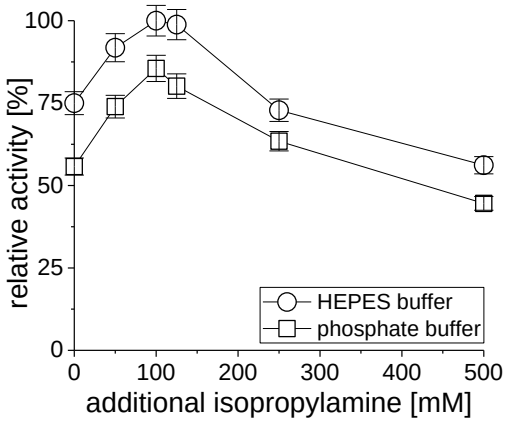


Figure 6. Effect of additional isopropylamine on the ISPC-based *Sp*ATA-catalyzed reaction; 30 °C, 100 mM acetophenone, 125 mM IPA-3DPPA, 25 mM buffer pH 7.5, 0.5 U/mL *Sp*ATA, t=22h.

Even higher isopropylamine concentration yield an increasing reduction of enzymatic activity based on LE CHATELIER'S principle, or in this case the so-called common ion-effect, since the additional amount of isopropylamine results in a re-crystallization of IPA-3DPPA. This effectively lowers the concentration of 3DPPA in solution, which itself gradually reduces crystallization of the product salt from solution. However, higher IPA-concentrations are not required by design since a high conversion towards the product amine (within the aqueous solution) is not required by the applied ISPC-process (see also Figure 2).

With the shown optimizations the presented donor amine salt-based ISPC-process was eventually performed continuously for substrate **1b** at improved reaction conditions (Figure 7).

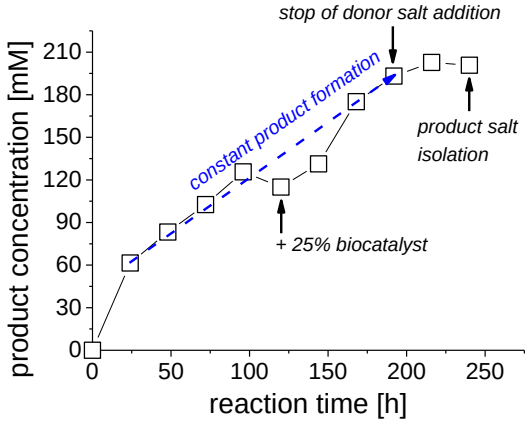


Figure 7. preparative continuous ISPC-process for the synthesis of (*S*)-1-(3-methoxyphenyl)ethylamine 3,3-diphenylpropionate (*S*)-**5b**. Conditions: 25 mM HEPES buffer pH 7.5, 100 mM 3'-methoxyacetophenone **1b** (re-adjusted every 24h), 125 mM IPA-3DPPA (re-adjusted every 24h), 100 mM additional IPA, 30 °C, 12 U/mL *Sp*ATA, 5 mL PLP, 1 h partial acetone removal every 24h, the blue line presents a guide for the continuous product salt formation.

Substrate concentration and the solid donor amine content were periodically re-adjusted to their original values (100 mM **1b** and 125 IPA-3DPPA) after each cycle to ensure identical process conditions throughout the entire reaction time. After 120h an additional 3 U/mL *Sp*ATA (+25%) were added to compensate for losses of enzymatic activity.

This fed batch process allowed a constant product formation beyond the original thermodynamic equilibrium, as highlighted by the blue line in Figure 2. After an initial fast increase in product formation, which is similar to conventional batch reactions, the space-time yield remained constant at ca. 4 g/(l·d), due to the continuous *in situ*-product removal. Noteworthy, the presented ISPC-process can be further optimized by increasing the amount of applied biocatalyst, which was not the aim of this study. The reaction byproduct acetone was periodically evaporated to avoid accumulation in the reaction solution. A major advantage of the shown ISPC-concept is that the formed acetone content does not have to be fully removed to facilitate a full conversion in solution.[14] A partial acetone removal is still sufficient since pure product formation is achieved in the crystallized solid phase (solid product salt), while in solution an incomplete reaction is present (see also Scheme 1). Significant losses of 3-methoxyacetophenone due to unwanted evaporation were not observed, but this will become more substantial with other, more volatile substrates.

Based on IPA-consumption, solely through IPA-3DPPA addition, an impressive isolated yield of 91.5 % of **5b** was achieved (see also Supplementary information), which is significantly higher than a conventional batch process without any ISPC (only 19%, see also Figure 2) and close to the proposed full conversion. The additional excess of 125 mM IPA (≙ 1.25 eq) remains also constant throughout the entire reaction in solution (not consumed) and is thus fully recycled after in every cycle. The shown process was eventually stopped by ceasing the addition of solid donor salt into the reaction suspension after the 8th cycle. Subsequently all remaining solid donor salt is removed due to the ongoing conversion, which only left the desired solid product salt as a single solid phase. The pure product salt is then easily filtered off and subjected to further downstream-processing. Crystalline purity was checked by X-ray powder diffraction, XRPD to ensure the absence of IPA-3DPPA (see Supplementary information).

Noteworthy, the process can in theory be continued indefinitely, but will be eventually limited by the amount of formed solid product salt. This issue can be easily solved by periodic filtration steps to reduce the amount of its solid phase. In this case donor salt addition has to be stopped temporarily as described above to allow solid product salt as the only solid phase. After filtration of the produce salt new donor salt and substrate can be re-applied to the

remaining mother liquor to resume the presented continues ISPC process.

In summary, the presented alternative application of a solid donor amine salt IPA-3DPPA as a continuous ISPC-process facilitates three significant advances over a conventional amine donor usage. First, a constant isopropylamine and 3DPPA concentration is achieved throughout the entire reaction time, which is solely based on the solubility limit of the donor salt IPA-3DPPA. A constant addition and control of two separate IPA- and 3DPPA streams into the ISPC-process is not required. Any removal of IPA-3DPPA by the biocatalytic reaction and *in situ*-crystallization is autonomously compensated by the dissolution of excess solid donor salt. Second, the use of this continuously operated design requires in theory only 1 mol donor amine for the synthesis of 1 mol product amine, which represents a lower E-factor (environmental factor) and a huge improvement over the conventional use of isopropylamine excesses. Moreover, the presented continuous ISPC-process closes the gap to tailor-made donor amines or complex biocatalytic cascades to overcome the unfavorable reaction equilibrium. Third, the combined amine concentration of donor and product amine remains significantly lower in solution, which is beneficial to ensure high catalyst activity and stability. The majority of both amines are only present as suspended solid salts.

The presented process can be further improved by a direct separation of both salts in divided reaction vessels, which will allow a direct continuous production of the product salt in pure form.

Experimental Section

Chemicals

All chemicals were obtained from Acros, TCI Chemicals, Aldrich, Alfa Aesar and ABCR and used as received. Deionized water was used throughout this study.

Enzymes

E. coli BL21(DE3) strains, containing the amine transaminase gene from *Silicibacter pomeroyi* were precultivated in LB-Media (5 g/L yeast extract, 10 g/L tryptone, and 10 g/L NaCl), with 0,1 mg/ml ampicillin as antibiotics at 37°C and 180 rpm overnight. The preculture was transferred the next day to 500 mL of LB-medium, with 0,1 mg/ml ampicillin and shaken at 37 °C. After an OD600 ≥ 0.6 was reached, 0.5 mM IPTG was added to the culture broth and shaken at 20 °C and 160 rpm for 12 h. The obtained cells were subsequently harvested by centrifugation, washed with phosphate buffer (50mM, pH 7.5) and lyophilized. Activity was tested via the amine transaminase assay, as described below. Typically, 0.2-0.3 U/mg were obtained.

Amine transaminase activity assay

Catalytic activity was measured at a wave length of 245 nm using the spectrophotometer Specord 200 from Analytik Jena (Jena, Germany) and the extinction coefficient of acetophenone: 11.852 $(mM \cdot cm)^{-1}$. Composition of the assay: 250 µL buffer solution, 250 µL 10 mM (*S*)-1-phenylethylamine in buffer solution, 250 µL 10 mM sodium pyruvate in buffer solution and 250 µL biocatalyst sample with suspended cells in buffer solution with 0.1 mM pyridoxal phosphate. All measurements were measured against a reference solution, whereas the biocatalyst solution was replaced with 200 µL buffer solution and 50 µL of 10 mM pyridoxal phosphate in buffer solution. Buffer solution: 50 mM phosphate buffer pH 8 with 0.25% DMSO.

General reaction procedure

A typical reaction mixture was prepared by adjusting 125 mM IPA-3DPPA and 125 mM IPA in 5 mL 25 mM HEPES buffer pH 7.5 and adjusting the pH with conc. H_3PO_4. Afterwards 2 mM PLP, 100 mM substrate and 0.5 U/ml lyophilized cells were added and the resulting mixture shaken horizontally at 150 rpm at 30 °C. Samples (1 mL) were taken directly from the suspension and analyzed as described below. During continuous reactions (20 mL reaction volume, see also Supplementary information), samples were taken and analyzed to determine and eventually re-adjust the substrate and IPA-3DPPA concentration to their original values. If required, full consumption of IPA-3DPPA was achieved (control via x-ray powder diffraction, XRPD) by stopping the addition of solid IPA-3DPPA. The remaining solid (mostly product salt) was filtered off, washed with 10 mL MTBE and dried.

Sampling

Samples (500 µL) were taken periodically and thoroughly mixed by a vortex mixer with 50 µL conc. NaOH to quench the reaction and increase pH. Afterwards 500 µL MTBE were added, mixed again by a vortex mixer and the solution was centrifuged (2 min, 3000 rpm) to improve phase separation. 200 µL were taken from the organic layer, combined with 50 µL of a 25 mM n-decane solution in MTBE (internal standard) and subsequently analyzed by gas chromatography.

Chromatography

Conversion was measured with a Trace 1310 gas chromatograph by Thermo Scientific (Dreieich, Germany), equipped with a 1300 flame ionization detector and a Agilent Capillary HP-5 19091J-433 (0.25 mm x 30 m x 0.25 µm). *n*-Decane was used as internal standard in all measurements. Temperatures of injector and detector were set to 250 °C. Enantiomeric excesses of the obtained amines were measured by High Performance Liquid Chromatography (HPLC) as described earlier.[12]

Solubility measurements

Solubility data were measured with 10 mL 100 mM phosphate buffer and an excess of the respective solid salt to create a stable suspension. The resulting mixture was shaken at 300 rpm and 30 °C for 7 days and the pH re-adjusted daily, if required. Afterwards the resulting mixture was filtered to obtain a saturated crystal-free solution. For 1PEA-3DPPA solubility a 500 µl sample was drawn and analyzed via GC as mentioned above. For IPA 3DPPA analysis a sample was diluted (typically 20x), the absorbance of 3DPPA at 249 nm measured and compared against a calibration curve of 3DPPA in 100 mM phosphate buffer at the identical pH (3DPPA-concentration range: 3 – 0.5 mM, 1 cm cuvette path length).

XRPD

Solid samples were measured via x-ray powder diffraction (XRPD) to discriminate the solid composition of donor salt and/or product salt. Powder x-ray diffraction data were collected on a Stoe Stadi-P with germanium-monochromatised Cu-Kα-radiation (λ=1.5418 Å) in horizontal transmission/Debey-Scherrer geometry. The x-rays were detected with a position-sensitive detector in the

2Θ range from 5 to 100 °. The 40 kV high voltage and 40 mA current were generated by a Seifert high voltage generator (ID 3003). The equipment was controlled and the raw data were handled with the software STOE WinXPOW (version 2.25, 2009). The position of the 2Theta and ω-circle were adjusted with the (111)-reflex of crystalline silicon (2Theta=28.44°). All samples were measured as flat preparation in poly acetate foils with minimum amount of silicone-based grease. The sample was spinned around its center during the measurement and the ω-circle was spinned as well with ½ 2Theta.

Acknowledgements

The authors thank Prof. Dr. Udo Kragl for his ongoing support, Dr. Dirk Michalik for his assistance with NMR measurements and Prof. M. Köckerling and Niels Ole Giltzau for their assistance with XRPD-measurements. Funding by German Federal Ministry of Education and Research (BMBF – Bundesministerium für Bildung und Forschung; project number: 031A123) and German Research Foundation (DFG – Deutsche Forschungsgemeinschaft; LA 4183/1-1) is gratefully acknowledged.

References

[1] a) A. Gomm, E. O'Reilly, *Curr. Opin. Chem. Biol.* **2018**, *43*, 106–112; b) S.-M. Dold, C. Syldatk, J. Rudat in *Green biocatalysis* (Ed.: R. N. Patel), John Wiley & Sons, Hoboken, NJ, **2016**; c) F. Steffen-Munsberg et al., *Biotechnol. Adv.* **2015**, *33*, 566–604; d) S. Mathew, H. Yun, *ACS Catal.* **2012**, *2*, 993–1001; e) P. K. Mehta, T. I. Hale, P. Christen, *Eur. J. Biochem.* **1993**, *214*, 549–561; f) M. Patil, G. Grogan, A. Bommarius, H. Yun, *Catalysts* **2018**, *8*, 254;

[2] F. Guo, P. Berglund, *Green Chem.* **2017**, *19*, 333–360.

[3] a) E. E. Ferrandi, D. Monti, *World J. Microbiol. Biotechnol.* **2017**, *34*, 13; b) A. A. Desai, *Angew. Chem. Int. Ed.* **2011**, *50*, 1974–1976; c) S. A. Kelly, S. Pohle, S. Wharry, S. Mix, C. C. R. Allen, T. S. Moody, B. F. Gilmore, *Chem. Rev.* **2018**, *118*, 349–367; d) M. S. Malik, E.-S. Park, J.-S. Shin, *Appl. Microbiol. Biotechnol.* **2012**, *94*, 1163–1171;

[4] M. Fuchs, J. E. Farnberger, W. Kroutil, *Eur. J. Org. Chem.* **2015**, *2015*, 6965–6982.

[5] D. Koszelewski, K. Tauber, K. Faber, W. Kroutil, *Trends Biotechnol.* **2010**, *28*, 324–332.

[6] a) M. T. Gundersen, R. Abu, M. Schürmann, J. M. Woodley, *Tetrahedron: Asymmetry* **2015**, *26*, 567–570; b) M. Voges, R. Abu, M. T. Gundersen, C. Held, J. M. Woodley, G. Sadowski, *Org. Process Res. Dev.* **2017**, *21*, 976–986; c) P. Tufvesson, J. Lima-Ramos, J. S. Jensen, N. Al-Haque, W. Neto, J. M. Woodley, *Biotechnol. Bioeng.* **2011**, *108*, 1479–1493; d) P. Tufvesson, M. Nordblad, U. Krühne, M. Schürmann, A. Vogel, R. Wohlgemuth, J. M. Woodley, *Org. Process Res. Dev.* **2015**, *19*, 652–660; e) Y. Satyawali, E. Ehimen, L. Cauwenberghs, M. Maesen, P. Vandezande, W. Dejonghe, *Biochem. Eng. J.* **2017**, *117*, 97–104;

[7] a) M. Höhne, U. T. Bornscheuer, *ChemCatChem* **2009**, *1*, 42–51; b) K. E. Cassimjee, C. Branneby, V. Abedi, A. Wells, P. Berglund, *Chem. Commun. (Cambridge, U. K.)* **2010**, *46*, 5569–5571; c) R. C. Simon, N. Richter, E. Busto, W. Kroutil, *ACS Catal.* **2014**, *4*, 129–143;

[8] D. Koszelewski, I. Lavandera, D. Clay, D. Rozzell, W. Kroutil, *Adv. Synth. Catal.* **2008**, *350*, 2761–2766.

[9] a) G. Rehn, P. Adlercreutz, C. Grey, *J. Biotechnol.* **2014**, *179*, 50–55; b) T. Börner, G. Rehn, C. Grey, P. Adlercreutz, *Org. Process Res. Dev.* **2015**, *19*, 793–799;

[10] G. Rehn, B. Ayres, P. Adlercreutz, C. Grey, *J. Mol. Catal. B: Enzym.* **2016**, *123*, 1–7.

[11] a) J. L. Galman, I. Slabu, N. J. Weise, C. Iglesias, F. Parmeggiani, R. C. Lloyd, N. J. Turner, *Green Chem.* **2017**, *19*, 361–366; b) A. Gomm, W. Lewis, A. P. Green, E. O'Reilly, *Chem. Eur. J.* **2016**, *22*, 12692–12695; c) A. P. Green, N. J. Turner, E. O'Reilly, *Angew. Chem. Int. Ed.* **2014**, *53*, 10714–10717; d) S. E. Payer, J. H. Schrittwieser, W. Kroutil, *Eur. J. Org. Chem.* **2017**, *2017*, 2553–2559;

[12] D. Hülsewede, M. Tänzler, P. Süss, A. Mildner, U. Menyes, J. von Langermann, *Eur. J. Org. Chem.* **2018**, *18*, 2130–2133.

[13] D. Hülsewede, L.-E. Meyer, J. von Langermann, *Chem. Eur. J.* **2019**, *25*, 4871–4884.

[14] P. Tufvesson, C. Bach, J. M. Woodley, *Biotechnol. Bioeng.* **2014**, *111*, 309–319.

10.1002/adsc.201900217

UPDATE

Donor Amine Salt-Based Continuous *in situ*-Product Crystallization in Amine Transaminase-Catalyzed Reactions.

Adv. Synth. Catal. **Year**, *Volume*, Page – Page

Dennis Hülsewede, Jan-Niklas Dohm, Jan von Langermann*