Hydrogen-Borrowing Alcohol Bioamination with Coimmobilized Dehydrogenases

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Hydrogen-Borrowing Alcohol Bioamination with Coimmobilized Dehydrogenases

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The amination of alcohols is an important transformation in chemistry. The redox-neutral (i.e., hydrogen-borrowing) asymmetric amination of alcohols is enabled by the combination of an alcohol dehydrogenase (ADH) with an amine dehydrogenase (AmDH). In this work, we enhanced the efficiency of hydrogen-borrowing biocatalytic amination by co-immobilizing both dehydrogenases on controlled porosity glass Fe^{III} ion-affinity beads. The recyclability of the dual-enzyme system was demonstrated (5 cycles) with total turnover numbers of >4000 and >1000 for ADH and AmDH, respectively. A set of (*S*)-configured alcohol substrates was aminated with up to 95% conversion and $>99\%$ *ee* (*R*). Preparative-scale amination of (*S*)-phenylpropan-2-ol resulted in 90% conversion and 80% yield of the product in 24 h.

α-Chiral amines are major synthetic targets for the production of a vast number of chemical compounds.[1] Chemical and enzymatic methods are currently widely applied in industry for the conversion of prochiral ketones into optically active amines.[2] Conversely, efficient catalytic strategies for the direct and stereoselective amination of alcohols are still in demand. In this context, hydrogen-borrowing processes are of high interest because of elevated atom efficiency.[3] Our group and others recently developed the dual-enzyme, asymmetric hydrogen-borrowing amination of alcohols.[4] In this reaction, an alcohol dehydrogenase (ADH) and an amine dehydrogenase (AmDH) operate in tandem and consume ammonia and generate water as the only byproduct.

Immobilization of enzymes can confer advantageous properties such as enhanced thermal and mechanical stability, the possibility to recover and recycle the biocatalyst, and tolerance for wider reaction conditions.[5] Enzyme immobilization techniques include encapsulation in (in)organic microporous structures, attachment on functionalized supports, cross-linking, and coordination through ionic interactions.[5,6]

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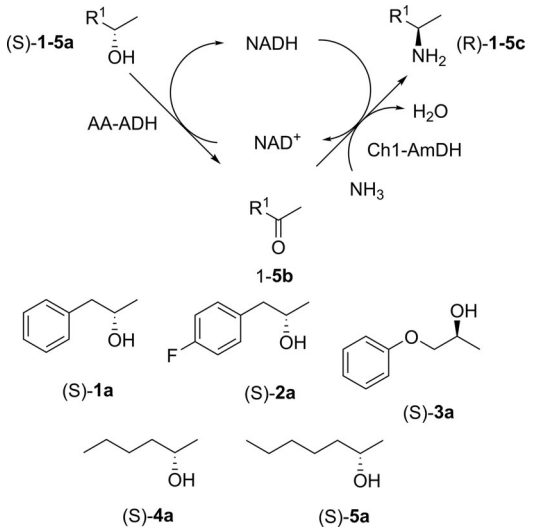
Supporting Information (general information, materials, expression and purification of enzymes, and analytics) and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/cctc.201701366.

Immobilization through ionic interactions can be performed by affinity binding between enzymes and metal ions such as Fe^{3+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+}.[7] Various enzymes with a fused polyhistidine chain (His-tag) have been employed on modified support materials in batch and flow synthesis.[8] A method, based on metal-ion affinity, for the specific binding of His-tagged enzymes on controlled porosity glass (CPG) was recently developed (commercial name: EziG). In previous studies, this method permitted to minimize enzyme leaching and loss of activity with several enzymes, including lipases, transaminases, Baeyer–Villiger monooxygenases, flavine reductases, and alanine dehydrogenases.[8c,9] Enzyme immobilization on CPG-derivatized metal-ion affinity particles offers several advantages over other methods such as: one, improved physicochemical stability in organic and aqueous media ($pH<10$); two, shorter times for immobilization (under optimized conditions, 20–30 min instead of many hours); three, low resistance to mass transfer of substrate and product; four, enzymatic activity upon immobilization is often quantitatively retained; five, selective binding of the target enzyme(s) from a crude lysate, hence avoiding pre-purification; six, general applicability to diverse enzyme classes; seven, the possibility to recycle the carrier material after loss of enzymatic activity.[8c,9] Some of these advantages compensate the fact that the manufacturing costs of CPG carriers are higher than those of organic polymer (plastic) materials, depending on the application (for detailed discussion, see the Supporting Information, Section S12). Conversely, encapsulation (or entrapment) and noncovalent immobilization often suffer from enzyme leakage, whereas covalent enzyme immobilization can lead to random attachment between the enzyme surface and the functional groups on the support, potentially decreasing the activity.[5]

In this study, we co-immobilized an alcohol dehydrogenase from *Aromatoleum aromaticum* (AA-ADH)[10] and a chimeric amine dehydrogenase (Ch1-AmDH)[11] on CPG metal-ion affinity beads (EziG Fe-amber)[8c] to perform the hydrogen-borrowing amination of a panel of (*S*)-configured alcohols [i.e., (*S*)-**1**–**5 a**] with ammonia (Scheme 1). The selected reaction proceeds with perfect inversion of configuration.[4a] Thus, it exemplifies a highly atom-efficient alternative to amination by the Mitsunobu reaction.[12] The performance of the heterogeneous immobilized dual-enzyme system was optimized in terms of loadings of the catalysts, molar ratio between ADH and AmDH, total amount of enzymes per mass of affinity beads, and substrate concentration.

EziG Fe^{III} ion affinity beads can be used to bind selectively His-tagged enzymes from crude enzyme cell extracts. However, in this study, we decided to use purified enzymes so that estimation of turnover numbers (TONs) could be done with ex-



Scheme 1. Hydrogen-borrowing amination of alcohols by using co-immobilized AA-ADH and Ch1-AmDH on EziG Fe-Amber ion-affinity beads.

Table 1. Summary of experiments with co-immobilized AA-ADH and Ch1-AmDH on EziG Fe-Amber ion-affinity beads (50 $mg_{enzyme}\,g_{beads}^{-1}$).[a]

Entry	ADH [nmol]	AmDH [nmol]	(*S*)-**1a** [mM]	Conv.[b] [%]	TON[c] ADH	TON[c] AmDH
1	35	11	20	90.4 ± 0.2	259 ± 1	788 ± 1
2	3.5	23	20	93.7 ± 0.4	2688 ± 12	408 ± 2
3	3.5	11	20	40.8 ± 7.5	1194 ± 219	363 ± 67
4	3.5	23	30	80.6 ± 6.5	3541 ± 285	538 ± 43
5	3.5	23	50	45.7 ± 3.7	3346 ± 271	508 ± 41

[a] For detailed data, see the Supporting Information. Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), 4 °C, 120 rpm, 2–3 h. Reaction conditions: NH_4Cl (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), (*S*)-**1a**, 30 °C, 170 rpm, 48 h. In every experiment, the yield of immobilization was > 99% (measured by Bradford assay). The data represent the average of three independent experiments with the standard deviation ($n = 3$). [b] Obtained percentage of amine product **1c**. [c] TON is defined as μmol of converted substrate per μmol of enzyme.

treme accuracy, as the concentrations of the biocatalysts would be exactly known. The enzymes AA-ADH and Ch1-AmDH were expressed with an N-terminal His_6-tag and were purified by Ni^{2+} affinity chromatography. The concentrations of both enzymes were determined spectrophotometrically at $\lambda = 280$ nm (see Section S3). Optimization of the conditions for co-immobilization of the enzymes with EziG Fe-Amber affinity beads (50 $mg_{enzymes}\,g_{beads}^{-1}$) was conducted with a AA-ADH/AmDH molar ratio of 23:35. The progress of the immobilization was monitored over time through determination of the residual concentrations of the enzymes in solution (i.e., Bradford assay, see Section S5). Complete immobilization of both enzymes was reached within 2 h under the optimized conditions: Tris/HCl (1 mL, 50 mM, pH 8), 4 °C, orbital mixing (120 rpm).

In our previous study on dual-enzyme hydrogen-borrowing amination by using free enzymes in solution, we used 33 nmol of AA-ADH and 63 nmol of Bb-PhAmDH[11b,13] to convert (*S*)-**1a** (20 mM, 0.5 mL) into (*R*)-**1c** with a maximum conversion of approximately 93% (in NH_3/NH_4Cl buffer 2 M, pH 8.7).[4a] That corresponds to TONs of 303 and 159 for the ADH and AmDH, respectively. In a recent publication from our group, we showed that a maximum conversion of approximately 93–95% was fixed by the thermodynamics of the system, under the mentioned reaction conditions.[14]

Aiming at assessing and optimizing the efficiency of the asymmetric hydrogen-borrowing amination in a co-immobilized ADH/AmDH system, we performed several sets of experiments. In these experiments, we applied reaction conditions typical for hydrogen-borrowing amination with free enzymes in solution.[4a] In the first set, the amount of AA-ADH was kept constant to a nonlimiting value (35 nmol), whereas the amount of Ch1-AmDH was varied (2.3–46 nmol). We performed the reactions with co-immobilized enzymes (total loading 50 $mg_{enzymes}\,g_{beads}^{-1}$) in NH_4Cl buffer (2 M, pH 8.7, 0.5 mL) containing NAD^+ (1 mM) and (*S*)-**1a** (20 mM). The highest TON obtained was 788 ± 1 for Ch1-AmDH by using 11 nmol of enzyme, which correlated to a conversion above 90% (Table 1, entry 1; Figure 1a and Table S2). In the second set, the amount of AmDH was kept constant to an estimated nonlimiting value (23 nmol), whereas the amount of ADH was varied (3.5–70 nmol). The lowest amount of AA-ADH (3.5 nmol) was sufficient to reach the maximum conversion. The estimated TON for AA-ADH was 2688 ± 12 (Table 1, entry 2; Figure 1b and Table S3). Control experiments with the use of non-immobilized AA-ADH and Ch1-AmDH provided similar results, which demonstrated that immobilization on EziG Fe-Amber did not reduce the activity of the enzymes (Section S7).

Combining the optimum concentrations of co-immobilized ADH/AmDH (total loading 50 $mg_{enzymes}\,g_{beads}^{-1}$) obtained from the previous sets of experiments (AA-ADH: 3.5 nmol, Ch1-AmDH: 11 nmol; 0.5 mL reaction) led to moderate conversions (from 34 to 49%) for the amination of (*S*)-**1a** at 20 mM (Table 1 entry 3; Table S5, entry 1). A gradual increase in the concentration of (*S*)-**1a** in the range of 20 to 100 mM produced a further decrease in the TONs for both ADH and AmDH (Figure S2 and Table S5). Finally, the optimal conditions in terms of productivity of the system were found upon using a 23:3.5 nmol ratio of AmDH/ADH for the amination on 0.5 mL scale. The calculated TONs were again 2676 ± 72 and 406 ± 11 for AA-ADH and Ch1-AmDH, respectively (Table S6, entry 1). The productivity of the system with the same molar ratio of ADH/AmDH was tested with an increased substrate concentration (20–100 mM). The best performance was revealed between 30 and 50 mM (Table 1 entries 4 and 5; Table S6). The highest TONs were 3541 ± 285 and 538 ± 43 for AA-ADH and Ch1-AmDH, respectively (Table 1, entry 4). Furthermore, we demonstrated the practical applicability of the system by performing a preparative-scale amination of (*S*)-**1a** (20 mM, 50 mg), which resulted in 90% conversion and 80% yield of the product within 24 h and > 99% *ee* (Section S10).

To assess and improve the volumetric productivity, we performed the co-immobilization at different total amounts of enzymes: 10, 20, 30, 50, 70, and 100 mg total enzyme per gram of beads. Even at the highest loading of 100 $mg_{enzymes}\,g_{beads}^{-1}$, we did not observe a negative impact on the performance of

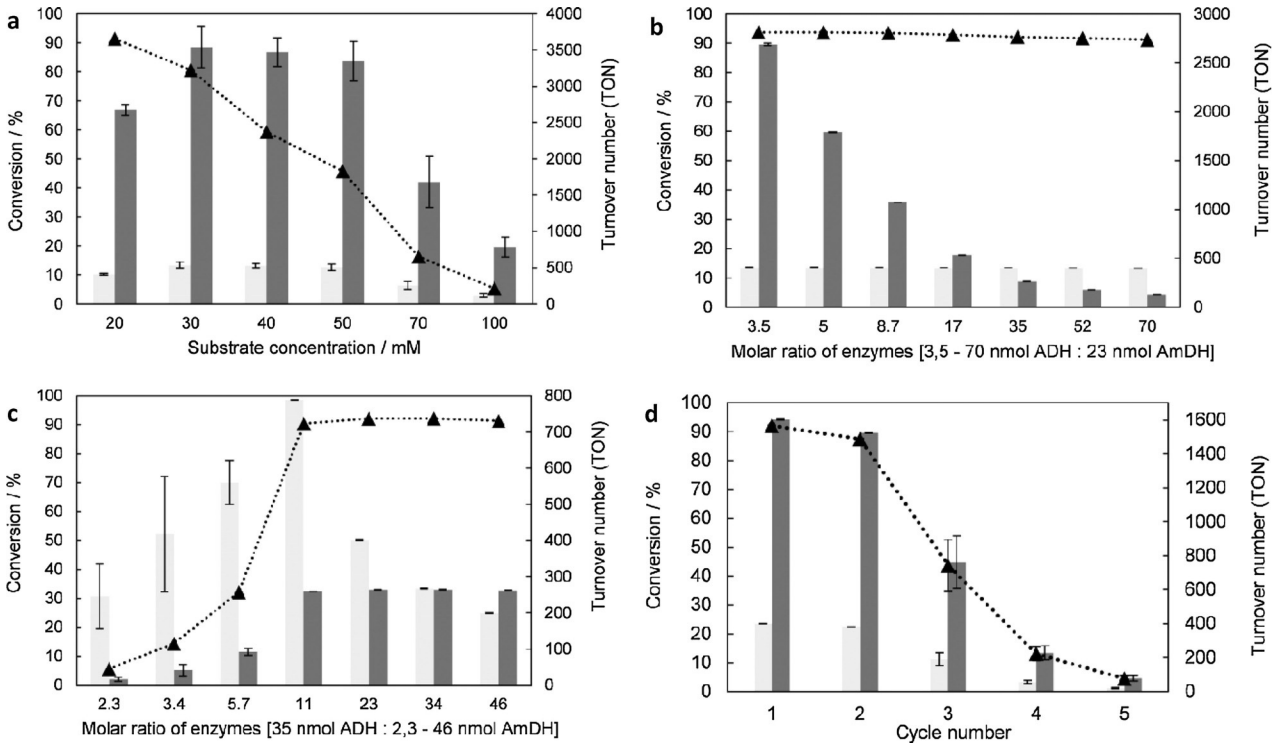


Figure 1. Studies on the co-immobilization and recycling of AmDH and ADH on EziG Fe-Amber ion-affinity beads (50 $mg_{enzyme}\,g_{beads}^{-1}$). Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), 4 °C, 120 rpm, 2–3 h. General reaction conditions: NH_4Cl buffer (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), 30 °C, 170 rpm orbital shaker (panels a–c) or 700 rpm Eppendorf thermomixer (panel d), 48 h single cycle (panels a–c) or 24 h per cycle (panel d). Conversion of (*S*)-**1a** (if not specified 20 mM) into (*R*)-**1c** (black triangles), TON_{ADH} (dark gray bars), and TON_{AmDH} (light gray bars) are shown for: a) influence of the amount of immobilized Ch1-AmDH (2.3–46 nmol) at constant amount of immobilized ADH (35 nmol), b) influence of the amount of immobilized AA-ADH (3.5–70 nmol) at constant amount of immobilized Ch1-AmDH (23 nmol), and c) influence of substrate concentration (20–100 mM). d) Recycling of co-immobilized AA-ADH and Ch1-AmDH (8.7:23 molar ratio). The data represents the average of three experiments and error bars the standard deviation ($n=3$).

the system, as the conversions and TONs were unaltered (Section S9 and Figure S3).

As proof of principle, we also demonstrated that the co-immobilized dual-enzyme system could be recycled and reused. Applying consecutive cycles of 24 h, partial activity was retained up to five cycles (Figure 1 d and Section S11). The calculated total turnover number (TTN) (i.e., the sum of the TONs from each cycle) were 4195 ± 220 and 1049 ± 55 for immobilized ADH and AmDH, respectively.

Finally, the optimized reaction conditions for the amination of (*S*)-**1a** were applied for the conversion of aliphatic- and phenyl-substituted alcohol substrates (*S*)-**2**–**5a** (Table 2). Conversions varied from moderate up to 95%. Enantiomeric excess values were always perfect (>99% *R*).

In summary, we improved the applicability of asymmetric hydrogen-borrowing alcohol bioamination by co-immobilizing an alcohol dehydrogenase (ADH) with an amine dehydrogenase (AmDH) on controlled porosity glass (CPG)-Fe^{III} ion-affinity beads (EziG). Conversions (up to 95%) and enantiomeric excess values (>99% *R*) were comparable to those of reactions performed with isolated enzymes in solution. Notably, recyclability of the dual-enzyme system was demonstrated, which led to total turnover numbers that were improved approximately 2 to 15-fold over those of previous studies with free enzymes in solution[4a] and control experiments from this work. Admittedly, the substrate concentration is currently below the requirements for industrial application. Furthermore, recyclability is limited. One major issue was found to be product inhibition observed in AmDH-catalyzed reactions.[15] However, the use of aqueous–organic biphasic media enabled the reductive amination of ketones with AmDHs at a concentration of 400 mM and 96% conversion.[15a] Hence, future work must focus on evaluating diverse CPG carriers possessing different polymeric films (from hydrophobic to hydrophilic) and on improving the stability of enzymes in aqueous–organic media. This strategy might

Table 2. Hydrogen-borrowing amination of alcohols (*S*)-**2**–**5a** with co-immobilized AA-ADH (8.7 nmol) and Ch1-AmDH (23 nmol) on EziG Fe-Amber ion-affinity beads (50 $mg_{enzyme}\,g_{beads}^{-1}$).[a]

Entry	Substrate	Conv.[b] [%]	*ee* [%]	TON[c] ADH	TON[c] AmDH
1	(*S*)-**2a**	95.0 ± 0.2	>99	2183 ± 4	826 ± 2
2	(*S*)-**3a**	27.7 ± 1.1	>99	649 ± 24	241 ± 9
3	(*S*)-**4a**	82.4 ± 11	>99	1894 ± 254	716 ± 96
4	(*S*)-**5a**	94.5 ± 0.3	>99	2173 ± 6	822 ± 2

[a] Immobilization conditions: Tris/HCl (50 mM, pH 8, 1 mL), 4 °C, 120 rpm, 2–3 h. Reaction conditions: NH_4Cl (2 M, pH 8.7, 0.5 mL), NAD^+ (1 mM), substrate (20 mM), 30 °C, 170 rpm, 48 h. Data represent the average of two independent experiments with absolute difference between them ($n=2$). [b] Obtained percentage of amine product. [c] TON is defined as μmol of converted substrate per μmol of enzyme.

permit to tune the compatibility between carrier and dehydrogenases depending on the reaction media and conditions. Another option is to extend the hydrogen-borrowing amination to a subsequent biocatalytic step, which would allow for the in situ removal of the amine product and hence solve the issue of product inhibition along with shifting the thermodynamic equilibrium of the reaction. Thus, apparent kinetics and actual TTNs might increase significantly. Finally, the possibility to conduct the hydrogen-borrowing amination with co-immobilized dehydrogenases in flow reactors might already increase the longevity and productivity of the system.

Experimental Section

General procedure for immobilization of enzymes

Ch1-AmDH and AA-ADH (molar ratio 23:3.5) were combined in Tris/HCl (1 mL, 50 mM, pH 8) at 4 °C and EziG Fe-Amber beads (40 mg, 50 $mg_{enzyme}\,g_{beads}^{-1}$) were added. The suspension was mixed at 120 rpm (orbital shaker) at 4 °C for 2–3 h. Bradford assay was used to confirm full immobilization. The beads carrying the immobilized enzymes were collected by centrifugation (12000 rpm, 4 °C, 2 min). The immobilized enzymes were either stored at 4 °C or used directly for the reaction.

General procedure for biocatalytic reactions with immobilized enzymes

EziG beads carrying the immobilized enzymes (20 mg, dry weight) were suspended in NH_4Cl buffer (0.5 mL, 2 M, pH 8.7) containing NAD^+ (1 mM). Then, substrate (*S*)-**1**–**5a** (20 mM, 1.37 μL) was added. The reaction was incubated at 30 °C and 170 rpm (orbital shaker) or 700 rpm (Eppendorf thermomixer) for 24 or 48 h. At the end of the reaction, the beads were separated by centrifugation. The aqueous phase was treated with KOH (100 μL, 10 M) and was then extracted with EtOAc (2×500 μL). The organic layer was dried with $MgSO_4$. Conversion and the enantiomeric excess value were determined by GC-FID.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: alcohols · amination · biocatalysis · enzyme immobilization · hydrogen borrowing

[1] a) H. A. Wittcoff, B. G. Rueben, J. S. Plotkin, *Industrial Organic Chemicals, 2nd ed.*, Wiley-Interscience, New York, **2004**; b) M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Kesseler, R. Sturmer, T. Zelinski, *Angew. Chem. Int. Ed.* **2004**, *43*, 788–824; *Angew. Chem.* **2004**, *116*, 806–843.

[2] a) C. Wang, J. Xiao in *Stereoselective Formation of Amines, Vol. 343* (Eds.: W. Li, X. Zhang), Springer, Berlin, Heidelberg, **2014**, pp. 261–282;b) *Chiral amine synthesis: Methods, Developments and Applications* (Ed.: T. C. Nugent), Wiley-VCH, Weinheim, **2010**; c) S. K. Au, J. Groover, B. D. Feske, A. S. Bommarius in *Organic Synthesis Using Biocatalysis, 1st ed.*, (Eds.: A. Goswami, J. Stewart), Elsevier, Amsterdam, **2015**, pp. 187–212.

[3] a) R. Grigg, T. R. B. Mitchell, S. Sutthivaiyakit, N. Tongpenyai, *J. Chem. Soc. Chem. Commun.* **1981**, 611–612; b) S. Bähn, S. Imm, L. Neubert, M. Zhang, H. Neumann, M. Beller, *ChemCatChem* **2011**, *3*, 1853–1864; c) S. Imm, S. Bahn, L. Neubert, H. Neumann, M. Beller, *Angew. Chem. Int. Ed.* **2010**, *49*, 8126–8129; *Angew. Chem.* **2010**, *122*, 8303–8306; d) C. Gunanathan, D. Milstein, *Angew. Chem. Int. Ed.* **2008**, *47*, 8661–8664; *Angew. Chem.* **2008**, *120*, 8789–8792; e) D. Pingen, O. Diebolt, D. Vogt, *ChemCatChem* **2013**, *5*, 2905–2912; f) D. Pingen, C. Muller, D. Vogt, *Angew. Chem. Int. Ed.* **2010**, *49*, 8130–8133; *Angew. Chem.* **2010**, *122*, 8307–8310; g) O. Saidi, A. J. Blacker, M. M. Farah, S. P. Marsden, J. M. Williams, *Angew. Chem. Int. Ed.* **2009**, *48*, 7375–7378; *Angew. Chem.* **2009**, *121*, 7511–7514; h) Y. Zhang, C. S. Lim, D. S. Sim, H. J. Pan, Y. Zhao, *Angew. Chem. Int. Ed.* **2014**, *53*, 1399–1403; *Angew. Chem.* **2014**, *126*, 1423–1427; i) N. J. Oldenhuis, V. M. Dong, Z. Guan, *J. Am. Chem. Soc.* **2014**, *136*, 12548–12551.

[4] a) F. G. Mutti, T. Knaus, N. S. Scrutton, M. Breuer, N. J. Turner, *Science* **2015**, *349*, 1525–1529; b) F.-F. Chen, Y.-Y. Liu, G.-W. Zheng, J.-H. Xu, *ChemCatChem* **2015**, *7*, 3838–3841; c) S. L. Montgomery, J. Mangas-Sanchez, M. P. Thompson, G. A. Aleku, B. Dominguez, N. J. Turner, *Angew. Chem. Int. Ed.* **2017**, *56*, 10491–10494; *Angew. Chem.* **2017**, *129*, 10627–10630.

[5] a) R. A. Sheldon, *Adv. Synth. Catal.* **2007**, *349*, 1289–1307; b) O. Barbosa, C. Ortiz, A. Berenguer-Murcia, R. Torres, R. C. Rodrigues, R. Fernandez-Lafuente, *Biotechnol. Adv.* **2015**, *33*, 435–456; c) N. R. Mohamad, N. H. Marzuki, N. A. Buang, F. Huyop, R. A. Wahab, *Biotechnol. Biotechnol. Equip.* **2015**, *29*, 205–220; d) M. L. E. Gutarra, L. S. M. Miranda, R. O. M. A. De Souza in *Organic Synthesis using Biocatalysis*, (Eds.: A. Goswami, J. Stewart), Elsevier, Amsterdam, **2016**, pp. 99–126; e) R. C. Rodrigues, C. Ortiz, A. Berenguer-Murcia, R. Torres, R. Fernandez-Lafuente, *Chem. Soc. Rev.* **2013**, *42*, 6290–6307; f) P. Zucca, E. Sanjust, *Molecules* **2014**, *19*, 14139–14194; g) R. Huang, S. Wu, A. Li, Z. Li, *J. Mater. Chem. A* **2014**, *2*, 1672–1676; h) R. Huang, M. Wu, M. J. Goldman, Z. Li, *Biotechnol. Bioeng.* **2015**, *112*, 1092–1101; i) H. Ren, Y. Zhang, J. Su, P. Lin, B. Wang, B. Fang, S. Wang, *J. Biotechnol.* **2017**, *241*, 33–41; j) W. Neto, M. Schürmann, L. Panella, A. Vogel, J. M. Woodley, *J. Mol. Catal. B* **2015**, *117*, 54–61; k) J. C. Y. Wu, C. H. Hutchings, M. J. Lindsay, C. J. Werner, B. C. Bundy, *J. Biotechnol.* **2015**, *193*, 83–90; l) H. Mallin, U. Menyes, T. Vorhaben, M. Hohne, U. T. Bornscheuer, *ChemCatChem* **2013**, *5*, 588–593; m) J. Britton, C. L. Raston, G. A. Weiss, *Chem. Commun.* **2016**, *52*, 10159–10162.

[6] J. C. S. dos Santos, O. Barbosa, C. Ortiz, A. Berenguer-Murcia, R. C. Rodrigues, R. Fernandez-Lafuente, *ChemCatChem* **2015**, *7*, 2413–2432.

[7] a) J. Porath, J. A. N. Carlsson, I. Olsson, G. Belfrage, *Nature* **1975**, *258*, 598–599; b) C. Ljungquist, A. Breitholtz, H. Brink-Nilsson, T. Moks, M. Uhlen, B. Nilsson, *Eur. J. Biochem.* **1989**, *186*, 563–569.

[8] a) J. B. Yang, K. F. Ni, D. Z. Wei, Y. H. Ren, *Biotechnol. Bioprocess Eng.* **2015**, *20*, 901–907; b) A. K. Vahidi, Y. Yang, T. P. N. Ngo, Z. Li, *ACS Catal.* **2015**, *5*, 3157–3161; c) K. E. Cassimjee, M. Kadow, Y. Wikmark, M. S. Humble, M. L. Rothstein, D. M. Rothstein, J. E. Backvall, *Chem. Commun.* **2014**, *50*, 9134–9137; d) M. Planchestainer, M. L. Contente, J. Cassidy, F. Molinari, L. Tamborini, F. Paradisi, *Green Chem.* **2017**, *19*, 372–375; e) J. Liu, B. Q. W. Pang, J. P. Adams, R. Snajdrova, Z. Li, *ChemCatChem* **2017**, *9*, 425–431.

[9] K. E. Cassimjee, J. E. Bäckvall, (Novitas, Stockholm) WO2015/115993, **2015**.

[10] H. W. Höffken, M. Duong, T. Friedrich, M. Breuer, B. Hauer, R. Reinhardt, R. Rabus, J. Heider, *Biochemistry* **2006**, *45*, 82–93.

[11] a) B. R. Bommarius, M. Schurmann, A. S. Bommarius, *Chem. Commun.* **2014**, *50*, 14953–14955; b) T. Knaus, W. Böhmer, F. G. Mutti, *Green Chem.* **2017**, *19*, 453–463.

[12] K. C. Swamy, N. N. Kumar, E. Balaraman, K. V. Kumar, *Chem. Rev.* **2009**, *109*, 2551–2651.

[13] M. J. Abrahamson, J. W. Wong, A. S. Bommarius, *Adv. Synth. Catal.* **2013**, *355*, 1780 – 1786.
[14] T. Knaus, L. Cariati, M. F. Masman, F. G. Mutti, *Org. Biomol. Chem.* **2017**, *15*, 8313 – 8325.
[15] a) A. Pushpanath, E. Siirola, A. Bornadel, D. Woodlock, U. Schell, *ACS Catal.* **2017**, *7*, 3204 – 3209; b) S. K. Au, B. R. Bommarius, A. S. Bommarius, *ACS Catal.* **2014**, *4*, 4021 – 4026.

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