

Cascade Reactions

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Whole-Cell-Catalyzed Multiple Regio- and Stereoselective Functionalizations in Cascade Reactions Enabled by Directed Evolution

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Abstract: Biocatalytic cascade reactions using isolated stereoselective enzymes or whole cells in one-pot processes lead to value-added chiral products in a single workup. The concept has been restricted mainly to starting materials and intermediate products that are accepted by the respective wild-type enzymes. In the present study, we exploited directed evolution as a means to create *E. coli* whole cells for regio- and stereoselective cascade sequences that are not possible using man-made catalysts. The approach is illustrated using P450-BM3 in combination with appropriate alcohol dehydrogenases as catalysts in either two-, three-, or four-step cascade reactions starting from cyclohexane, cyclohexanol, or cyclohexanone, respectively, leading to either (*R,R*)-, (*S,S*)-, or *meso*-cyclohexane-1,2-diol. The one-pot conversion of cyclohexane into (*R*)- or (*S*)-2-hydroxycyclohexanone in the absence of ADH is also described.

Retrosynthetic analysis based on man-made reagents, catalysts, and synthons is a routine technique used by organic chemists when planning the preparation of structurally simple or complex compounds.^[1] Biocatalytic retrosynthesis^[2] exploiting enzyme mixtures, whole cells, or enzymes in combination with transition-metal catalysts has not reached a similar state of maturity.^[3] The design of such artificial reaction sequences is different from metabolic engineering as this approach is independent of naturally occurring pathways. The former generally involve wild-type (WT) enzymes,^[3] which restricts their scope. Fortunately, advanced methods in directed evolution provide a means to lift the traditional limitations of enzyme catalysis, such as insufficient stereo- and regioselectivity.^[4] Herein, we applied directed evolution to achieve cascade reactions that are not readily amenable to state-of-the-art synthetic transition-metal catalysis or organocatalysis.

We chose cyclohexane **1** as the model starting material, a compound that traditionally requires harsh conditions for

oxidative functionalization. Our approach utilizes an inexpensive starting material derived from petro-chemical sources and is therefore different from using renewable feedstocks such as glucose, which would require metabolic pathway engineering. Four different whole cells were envisioned, each leading to one of the three possible stereoisomeric forms of 1,2-cyclohexanediol, namely (*R,R*)-**5**, (*S,S*)-**5**, (*S,R*)-**5** (*meso*), or (*R,S*)-**5** (also *meso*), in a multi-step sequence (Figure 1). To construct *R,R*-selective whole cells, the plan was to use a single mutant of an appropriate cytochrome P450 monooxygenase (CYP)^[5] to regio- and enantioselectively access acyloin (*R*)-**4** as the primary product followed by reduction with *R*-selective alcohol dehydrogenase (ADH) to form (*R,R*)-**5**. The advantages of a single CYP mutant for the first three C–H activating steps include simplicity and minimal cell stress. Enantiomeric (*S,S*)-**5** as well as the *meso* stereoisomer of **5** could be targeted analogously.

We first focused on cyclohexanone **3** as the starting substrate and designed whole cells for its conversion into the final stereoisomeric diols **5**. Thereafter, the plan was to extend the cascade sequence in a stepwise fashion, specifically by starting from cyclohexanol **2** and finally from cyclohexane **1**. This bottom-up approach was thought to constitute a useful guide in the construction of the final four-step sequence **1** → **2** → **3** → **4** → **5**. The self-sufficient P450-BM3 monooxygenase from *Bacillus megaterium*^[5,6] was chosen as the catalyst. WT P450-BM3 with its large binding pocket shows very poor activity towards cyclohexane **1**,^[6b] probably owing to its small size. A variant was recently generated by protein engineering to accept this substrate with formation of **2**, which was further oxidized to **3** with an ADH.^[7] Owing to the aforementioned reasons, this alternative route to **3** is not suitable for our goal,

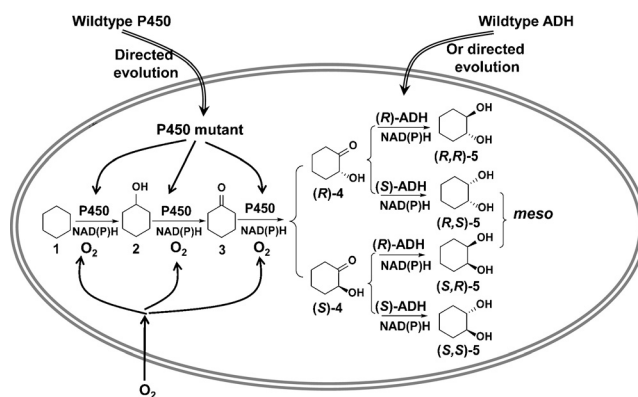


Figure 1. Construction of *E. coli* whole cells for producing either (*R,R*)-**5**, (*S,S*)-**5**, or *meso*-**5**, respectively.

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in addition to the anticipated problems with the final ADH-catalyzed steps.

As the gene mutagenesis method for evolving P450-BM3 mutants for the hydroxylation of cyclohexanone **3**, we chose structure-guided saturation mutagenesis at sites lining the enzyme binding pocket.^[4c,8] To minimize screening, we tested triple code saturation mutagenesis (TCSM) utilizing three amino acids as the reduced amino acid alphabet (in addition to WT) at medium-sized randomization sites, also in combination with iterative saturation mutagenesis (ISM).^[4c,9] WT P450-BM3 does not accept **3**, nor do any of the variants evolved earlier for α -hydroxylation of structurally different ketones.^[10]

Based on the crystal structure of P450-BM3,^[6a] at least two dozen residues lining the binding pocket can be chosen for saturation mutagenesis as shown by previous studies.^[11] Following these guidelines and exploratory mutagenesis experiments, which included NNK-based randomization at selected amino acid positions (see the Supporting Information for details), three amino acids were chosen for TCSM, followed by ISM. Accordingly, we first docked substrate **3** into the crystal structure of P450-BM3.^[6a] Figure 2A features the arrangement in which the substrate is positioned closest to the catalytically active high-spin species heme-Fe=O (compound **I**). None of the C–H entities of **3** are close enough for smooth hydroxylation, which explains the inertness of this substrate. Eight residues lining the binding pocket were chosen as potential randomization candidates, which were grouped into two four-residue sites, A (L75/L181/I263/A264) and B (V78/A82/A328/T438). We analyzed all exploratory data (see Tables S1 and S2) and designed a triple code consisting of asparagine, isoleucine, and phenylalanine (N-I-F) for combinatorial randomization at sites A and B (Figure 2A). This entailed the screening of only 552 and 736 transformants, respectively, for 95% library coverage. The best variants from library A proved to be L181F (e.r. = 80:20 in favor of (*R*)-**4**) and L181F/I263N (e.r. = 22:78 in favor of (*S*)-**4**; Figure 2B). Library B was more productive (Figure 2B), the best variants being V78I/A82I/A328N (e.r. = 89:11 in favor of (*R*)-**4**) and V78I/A82F/A328F/T438I (e.r. =

11:89 in favor of (*S*)-**4**). Further hits of comparable quality are shown in the Supporting Information (see Table S3). The genes of the best variants from library A were then used as templates for N-I-F based saturation mutagenesis at site B (ISM scheme A $\rightarrow$ B), and the analogous procedure based on pathway B $\rightarrow$ A was also explored (see Table S4). The best results, with e.r. = 95:5 (or 5:95) for both *R* and *S* selectivity, originate from ISM scheme A $\rightarrow$ B (Figure 2C). This ratio corresponds to $\Delta\Delta G^\ddagger = 7.3 \text{ kJ mol}^{-1}$ relative to a 50:50 ratio. Kinetic characterization of the two best variants and coupling efficiencies are shown in the Supporting Information, Table S5. We also performed docking computations to gain insight into the origin of the regio- and stereoselectivity (Figure S1).

To extend the cascade sequence from **3** to the stereoisomeric cyclohexane-1,2-diols **5** (Figure 1), we considered 2,3-butanediol dehydrogenases from different sources (Table S6) because they catalyze the structurally related oxidation of 2,3-butanediol with acyloin formation or the reverse reaction.^[12] We initially tested two stereocomplementary enzymes, BDHA and BUDC, as catalysts in the oxidative kinetic resolution of *rac*-**5**. Gratifyingly, they proved to be effective, leading to selectivity factors of $E > 100$ in favor of (*R*)- and (*S*)-**4**, respectively, a sign that genetic optimization is not necessary. With *meso*-**5** as the substrate, BDHA provided (*S*)-**4** with e.r. = 3:97 whereas BUDC favored the formation of (*R*)-**4** (e.r. = 97:3). We also tested L-2,3-butanediol dehydrogenase (LBDHA), but it showed no appreciable enantioselectivity in the oxidative kinetic resolution of *rac*-**5** (formation of almost racemic **4**). However, when *meso*-**5** was subjected to oxidation, it proved to be selective for (*R*)-**4** (e.r. = 95:5). Thus directed evolution was not necessary.

With these results in hand, we first designed whole cells for cascade reactions starting from **3** with final formation of cyclohexane-1,2-diols **5**. The *E. coli* host cells were transformed with one plasmid carrying both the P450-BM3 mutant and alcohol dehydrogenase (see Figure S2A), which resulted in six different whole cells based on different combinations of two P450s (P450ATC06 and P450ATD04) and three ADHs (BDHA, BUDC, and LBDHA). For comparison, *E. coli* cells

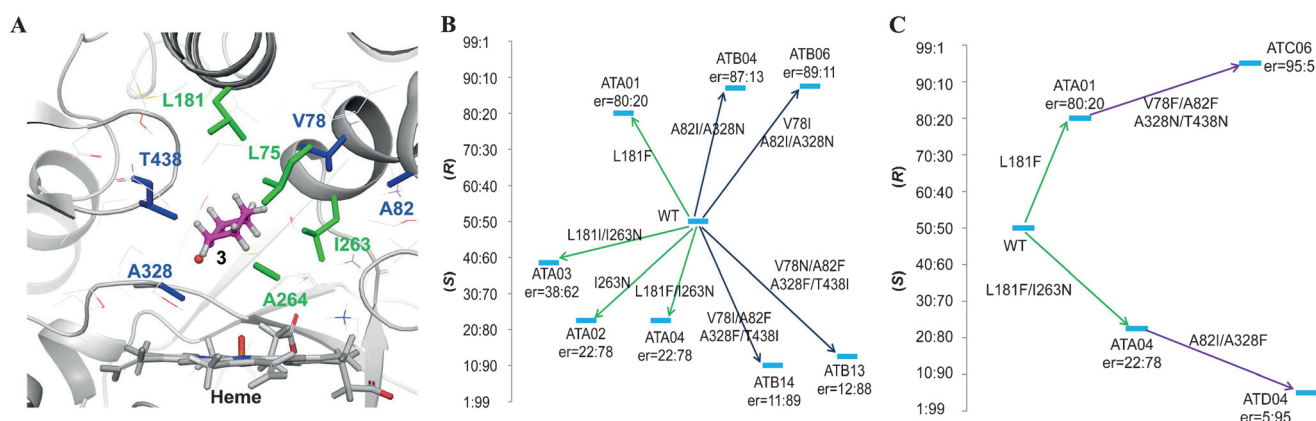


Figure 2. A) Binding pocket of P450-BM3 harboring cyclohexanone **3** closest to heme-Fe=O, featuring two four-residue randomization sites A (green) and B (blue). B) Typical P450-BM3 variants from libraries A (green arrows) and B (blue arrows) showing activity as well as regio- and enantioselectivity in the reaction of **3** into **4**. C) Best ISM pathways for evolving *R*- and *S*-selective variants. Green arrow: variants originating from initial library A; purple arrow: improved *R*- or *S*-selective variants resulting from ISM at site B.

with two plasmid systems showed very poor protein expression for P450 (see Figures S2 B and S2 C). The designed whole cells with one plasmid system were then tested in the conversion of **3** into **5**. Under these conditions, the cofactor is provided by the *E. coli* host cells using glucose as an energy source. As shown in Table 1, all three stereoisomeric diols **5** were obtained with high enantio- and diastereoselectivity on an optional basis. Thus the *E. coli* (P450ATC06 + BDHA) cells that combine the *R*-selective P450-BM3 mutant and *R,R*-selective BDHA provided (*R,R*)-**5** (Table 1, entry 1) whereas *E. coli* (P450ATD04 + BDHA) or *E. coli* (P450ATC06 + BUDC) containing the *S/R*-selective P450-BM3 mutant and the *R,R/S,S*-selective ADH favored *meso*-**5**, the former being more selective (Table 1, entries 2 and 3). The exclusive formation of (*S,S*)-**5** was achieved by *E. coli* (P450ATD04 + BUDC) co-expressing the *S*-selective P450-BM3 mutant and the *S,S*-selective BUDC (Table 1, entry 4). (*S,S*)-**5** was also obtained using the *E. coli* (P450ATD04 + LBDHA) strain that harbors the *S*-selective P450-BM3 mutant and LBDHA (Table 1, entry 6). This ADH is known to accept (*S*)-2-hydroxybutanone with formation of (*S,S*)-2,3-butanediol.^[12c] The remaining combination of P450ATC06 with LBDHA failed to show acceptable levels of enantio- and diastereoselectivity. In control experiments, it was shown that both BUDC and LBDHA catalyze the reduction of **3**, whereas BDHA does not. Therefore, a trace amount of **2** was detected in the whole-cell systems that contained BUDC or LBDHA. At this point, mixtures of two *E. coli* cells, one harboring a P450-BM3 mutant and the other one an ADH, or two cell lysates, were tested, but all led to unacceptable results (20–50 % conversion; see the Supporting Information for details).

Next, we tested whether the two best P450-BM3 mutants ATC06 and ATD04 were capable of catalyzing the sequence **2**→**3**→**4** (see Table S7), which proved to be the case.

Surprisingly, whereas *meso*-**5** was formed in the case of mutant ATC06, it was not detected when **3** was used as the substrate in the respective control experiment. This indicates that the ATC06 variant is also able to catalyze the direct hydroxylation of **2** into *meso*-**5**. The best whole cells were then tested in the three-step cascade sequence **2**→**3**→**4**→**5**. Using the best cell systems, it was possible to obtain all three stereoisomeric cyclohexane-1,2-diols **5** as the major products with high enantio- and diastereoselectivity (Table 2). In contrast, *E. coli* (P450ATC06 + BDHA) cells gave a mixture of *trans*- and *meso*-**5** with reduced selectivity (85:15) owing to the competing direct oxidation of **2** into *meso*-**5**.

Finally, the utility of the two best mutants P450ATC06 and P450ATD04 was tested in the cascade sequence of **1**→**2**→**3**→**4**. Unfortunately, only low conversions into **3** were observed (Table S8). This may come as a surprise but we postulated that these P450-BM3 variants, although highly regio- and stereoselective, are not active enough to smoothly catalyze all three reactions **1**→**2**→**3**→**4**. Indeed, the *E. coli* whole cells (Table 1) failed to function well in the longer cascade sequence **1**→**2**→**3**→**4**→**5**. We therefore turned our attention to the mutants generated in the initial library (Table S1), and discovered that the two enantiocomplementary P450 mutants A82F and A82F/A328F catalyze the sequence **1**→**2**→**3**→**4** with good selectivity (Table S8). These variants are considerably more active than ATC06 and ATD04 in the hydroxylation of **3**, although a little less selective. Consequently, four additional whole cells were engineered according to the best combinations. These were then used in the cascade sequences starting from **1** with final formation of cyclohexane-1,2-diols **5** (Table 3). Good results were obtained in the case of the enantiomeric diols (*R,R*)- and (*S,S*)-**5**, these being the major products with enantiomeric ratios ranging between 96:4 and > 99:1 and diastereoselectivities of 85–93 %. When aiming for

Table 1: Conversion of **3** into stereoisomeric diols **5** using *E. coli* cells co-expressing P450-BM3 mutants and ADHs.

Entry	Catalyst	Conv. [%] ^[a]	<i>trans</i> / <i>meso</i>	e.r. [%] ^[c]	Favored stereoisomer	Product distribution ^[b] [%]		
						2	4	5
1	<i>E. coli</i> (P450ATC06 + BDHA)	90	98:2	> 99:1	(<i>R,R</i>)	0	1	99
2	<i>E. coli</i> (P450ATD04 + BDHA)	98	5:95	— ^[d]	<i>meso</i>	0	1	99
3	<i>E. coli</i> (P450ATC06 + BUDC)	84	8:92	— ^[d]	<i>meso</i>	17	1	82
4	<i>E. coli</i> (P450ATD04 + BUDC)	98	95:5	> 99:1	(<i>S,S</i>)	3	0	97
5	<i>E. coli</i> (P450ATC06 + LBDHA)	91	51:49	— ^[d]	N.A.	29	2	69
6	<i>E. coli</i> (P450ATD04 + LBDHA)	97	96:4	98:2	(<i>S,S</i>)	8	1	91

[a] Conditions: 10 mM substrate, 30 °C, 200 rpm, 5 h. The conversion was determined by GC analysis and is based on the amount of converted substrate. For detailed conditions see the Supporting Information. [b] Relative amounts based on the peak areas in the GC chromatograms.

[c] Determined for *trans*-cyclohexane-1,2-diols **5**. [d] Values not determined. N.A. = not available.

Table 2: Conversion of **2** into stereoisomeric diols **5** using *E. coli* cells co-expressing P450-BM3 mutants and ADHs.

Entry	Catalyst	Conv. [%] ^[a]	<i>trans</i> / <i>meso</i>	e.r. [%] ^[c]	Favored stereoisomer	Product distribution [%] ^[b]		
						3	4	5
1	<i>E. coli</i> (P450ATC06 + BDHA)	96	85:15	> 99:1	(<i>R,R</i>)	4	1	95
2	<i>E. coli</i> (P450ATD04 + BDHA)	20	4:96	— ^[d]	<i>meso</i>	35	0	65
3	<i>E. coli</i> (P450ATC06 + BUDC)	97	19:81	— ^[d]	<i>meso</i>	3	1	96
4	<i>E. coli</i> (P450ATD04 + BUDC)	99	95:5	> 99:1	(<i>S,S</i>)	2	1	97
5	<i>E. coli</i> (P450ATD04 + LBDHA)	99	96:4	97:3	(<i>S,S</i>)	2	0	98

[a] Conditions: 5 mM substrate, 30 °C, 200 rpm, 5 h. The conversion was determined by GC analysis and is based on the amount of converted substrate. For detailed conditions see the Supporting Information. [b] Relative amounts based on the peak areas in the GC chromatograms.

[c] Determined for *trans*-cyclohexane-1,2-diols **5**. [d] Values not determined.

Table 3: Conversion of **1** into stereoisomeric diols **5** using *E. coli* cells co-expressing P450-BM3 mutants and ADHs.

Entry	Catalyst	Conv. [%] ^[a]	<i>trans</i> / <i>meso</i>	e.r. [%] ^[c]	Favored stereoisomer	Product distribution [%] ^[b]			
						2	3	4	5
1	<i>E. coli</i> (P450A82F + BDHA)	72	88:12	> 99:1	(<i>R,R</i>)	5	15	3	77
2	<i>E. coli</i> (P450A82F/A328F + BDHA)	77	20:80	— ^[d]	<i>meso</i>	16	19	0	65
3	<i>E. coli</i> (P450A82F/A328F + BUDC)	83	85:15	> 99:1	(<i>S,S</i>)	7	23	0	70
4	<i>E. coli</i> (P450A82F/A328F + LBDHA)	87	93:7	96:4	(<i>S,S</i>)	3	15	0	82

[a] Conditions: 5 mM substrate, 25 °C, 200 rpm, 8 h. The conversion was determined by GC analysis and is based on the amount of converted substrate. For detailed conditions see the Supporting Information. [b] Relative amounts based on the peak areas in the GC chromatograms. [c] Values determined for *trans*-cyclohexane-1,2-diols **5**. [d] Values not determined.

meso-**5**, a diastereoselectivity of 80 % was achieved. Several reactions were scaled up; the optimal workup procedure involved the use of *n*-butanol in the extraction step (see the Supporting Information for details), which led to yields of isolated products of 29–66 % (Table S15).

In conclusion, we have shown that the use of directed evolution enables the creation of *E. coli* whole cells for synthetically challenging cascade reactions that are not possible with wild-type enzymes or man-made catalysts. In terms of method development in directed evolution, it also shows that recently reported triple code saturation mutagenesis (TCSM)^[9] is effective for mechanistically and structurally more complex enzymes such as P450 monooxygenases. Starting from the achiral substrates cyclohexane, cyclohexanol, or cyclohexanone, cyclohexane-1,2-diols as functionalized products were shown to be accessible in three different stereoisomeric forms. The cascade sequence can also be terminated at the stage of (*R*)- or (*S*)-**4**, acyloins that are likewise value-added compounds.^[13] Numerous other enzyme types await consideration when designing bacterial or yeast whole cells for synthetically useful stereoselective cascade sequences requiring only a single workup.

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Keywords: cascade reactions · C–H activation · directed evolution · enantioselectivity · P450 monooxygenase

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- [1] a) E. J. Corey, *The Logic of Chemical Synthesis*, Wiley, New York, **1995**; b) C. Grondal, M. Jeanty, D. Enders, *Nat. Chem.* **2010**, *2*, 167–178; c) K. C. Nicolaou, J. S. Chen, *Chem. Soc. Rev.* **2009**, *38*, 2993–3009; d) *Catalytic Cascade Reactions* (Eds.: P.-F. Xu, W. Wang), Wiley, New York, **2014**.
- [2] N. J. Turner, E. O'Reilly, *Nat. Chem. Biol.* **2013**, *9*, 285–288.
- [3] a) *Enzyme Catalysis in Organic Chemistry* (Eds.: K. Drauz, H. Gröger, O. May), Wiley-VCH, Weinheim, **2012**; b) P. Both, H. Busch, P. P. Kelly, F. G. Mutti, N. J. Turner, S. L. Flitsch, *Angew. Chem. Int. Ed.* **2016**, *55*, 1511–1513; *Angew. Chem.* **2016**, *128*, 1533–1536; c) T. Bayer, S. Milker, T. Wiesinger, F. Rudroff, M. D. Mihovilovic, *Adv. Synth. Catal.* **2015**, *357*, 1587–1618; d) F. G. Mutti, T. Knaus, N. S. Scrutton, M. Breuer, N. J. Turner, *Science* **2015**, *349*, 1525–1529; e) L. Skalden, C. Peters, J. Dickerhoff, A. Nobili, H. J. Joosten, K. Weisz, M. Hohne, U. T. Bornscheuer, *ChemBioChem* **2015**, *16*, 1041–1045; f) S. Riva, W.-D. Fessner, *Cascade Biocatalysis*, Wiley-VCH, Weinheim, **2014**; g) S. K. Wu, Y. Z. Chen, Y. Xu, A. T. Li, Q. S. Xu, A. Glieder, Z. Li, *ACS Catal.* **2014**, *4*, 409–420; h) H. Gröger, W. Hummel, *Curr. Opin. Chem. Biol.* **2014**, *19*, 171–179; i) R. Agudo, M. T. Reetz, *Chem. Commun.* **2013**, *49*, 10914–10916; j) E. Ricca, B. Brucher, J. H. Schrittwieser, *Adv. Synth. Catal.* **2011**, *353*, 2239–2262; k) S. K. Wu, Y. Zhou, T. W. Wang, H. P. Too, D. I. C. Wang, Z. Li, *Nat. Commun.* **2016**, *7*, 11917.
- [4] a) A. S. Bommarius, *Annu. Rev. Chem. Biomol.* **2015**, *6*, 319–345; b) C. A. Denard, H. Q. Ren, H. M. Zhao, *Curr. Opin. Chem. Biol.* **2015**, *25*, 55–64; c) M. T. Reetz, *Angew. Chem. Int. Ed.* **2011**, *50*, 138–174; *Angew. Chem.* **2011**, *123*, 144–182; d) E. M. Brustad, F. H. Arnold, *Curr. Opin. Chem. Biol.* **2011**, *15*, 201–210; e) C. Jäckel, D. Hilvert, *Curr. Opin. Biotechnol.* **2010**, *21*, 753–759; f) N. J. Turner, *Nat. Chem. Biol.* **2009**, *5*, 568–574; g) S. Lutz, U. T. Bornscheuer, *Protein Engineering Handbook*, Wiley-VCH, Weinheim, **2009**.
- [5] a) H. M. Girvan, A. W. Munro, *Curr. Opin. Chem. Biol.* **2016**, *31*, 136–145; b) P. R. Ortiz de Montellano, *Chem. Rev.* **2010**, *110*, 932–948.
- [6] a) D. C. Haines, D. R. Tomchick, M. Machius, J. A. Peterson, *Biochemistry* **2001**, *40*, 13456–13465; b) A. Glieder, E. T. Farinas, F. H. Arnold, *Nat. Biotechnol.* **2002**, *20*, 1135–1139.
- [7] S. Staudt, E. Burda, C. Giese, C. A. Muller, J. Marienhagen, U. Schwaneberg, W. Hummel, K. Drauz, H. Gröger, *Angew. Chem. Int. Ed.* **2013**, *52*, 2359–2363; *Angew. Chem.* **2013**, *125*, 2415–2419.
- [8] Z. Sun, Y. Wikmark, J. E. Bäckvall, M. T. Reetz, *Chem. Eur. J.* **2016**, *22*, 5046–5054.
- [9] a) Z. Sun, R. Lonsdale, L. Wu, G. Li, A. T. Li, J. Wang, J. Zhou, M. T. Reetz, *ACS Catal.* **2016**, *6*, 1590–1597; b) Z. Sun, R. Lonsdale, A. Ilie, G. Li, J. Zhou, M. T. Reetz, *ACS Catal.* **2016**, *6*, 1598–1605.
- [10] R. Agudo, G. D. Roiban, R. Lonsdale, A. Ilie, M. T. Reetz, *J. Org. Chem.* **2015**, *80*, 950–956.
- [11] a) S. Kille, F. E. Zilly, J. P. Acevedo, M. T. Reetz, *Nat. Chem.* **2011**, *3*, 738–743; b) C. J. C. Whitehouse, S. G. Bell, L. L. Wong, *Chem. Soc. Rev.* **2012**, *41*, 1218–1260; c) R. Bernhardt, V. B. Urlacher, *Appl. Microbiol.* **2014**, *98*, 6185–6203; d) R. Fasan, *ACS Catal.* **2012**, *2*, 647–666.
- [12] a) Y. J. Yan, C. C. Lee, J. C. Liao, *Org. Biomol. Chem.* **2009**, *7*, 3914–3917; b) J. D. Zhang, T. T. Xu, Z. Li, *Adv. Synth. Catal.* **2013**, *355*, 3147–3153; c) S. Ui, Y. Takusagawa, T. Sato, T. Ohtsuki, A. Mimura, M. Ohkuma, T. Kudo, *Lett. Appl. Microbiol.* **2004**, *39*, 533–537.
- [13] P. Hoyos, J. V. Sinisterra, F. Molinari, A. R. Alcantara, P. D. De Maria, *Acc. Chem. Res.* **2010**, *43*, 288–299.

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