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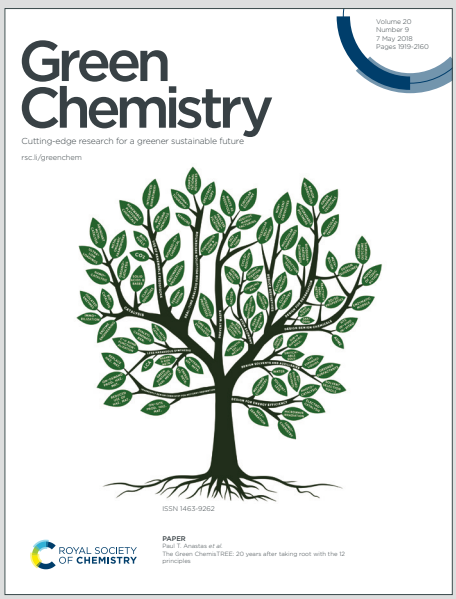

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COMMUNICATION

Cascade bio-hydroxylation and dehalogenation for one-pot enantioselective synthesis of optically active β-halohydrins from halohydrocarbons

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Stereoselective hydroxylation and enantioselective dehalogenation cascade reaction was developed for the synthesis of optically active β-haloalcohols from halohydrocarbons. This cascade system employed P450 and halohydrin dehalogenase as two compatible biocatalysts, allowing a straightforward, greener and efficient access to β-halohydrins with excellent enantioselectivities (98-99%).

It is well known that enantiopure β-halohydrins are particularly interesting as synthons for the preparation of a large number of bioactive products including natural products, agrochemicals and pharmaceuticals.[1] Optically active 2-chloro-1-phenylethanol, for instance, is the key precursor for the synthesis of *anti*-depressants α- or β-adrenergic drugs such as Tomoxetine, Fluoxetine and Nisoxetine.[2] They also can be easily converted to the corresponding chiral epoxides with controlled stereochemistry,[3] which opens a large spectrum for further synthetic applications. In addition, growing methods have been developed for the transformations of enantiopure halohydrins into a broad range of functional groups to construct various useful chiral organic compounds, including aminoalcohols,[4] azidoalcohols,[5] hydroxynitriles,[6] and 1, 2-diols,[7] which provides a powerful strategy for asymmetric synthesis.

Even though some methods access to enantiopure β-halohydrins have been developed, especially *via* transition metal catalyzed asymmetric transfer hydrogenation of the corresponding β-halo-ketones,[3b, 8] it is still a challenge to synthesize these enantiopure β-halohydrins with more greener and atom economic strategies. In the past decades, many biocatalytic routes have been emerged to synthesize the enantioenriched β-halohydrins due to their high selectivity, mild reaction conditions and environmental compatibility.[9] Until now, four biocatalytic methods have been reported to prepare enantioenriched β-halohydrins that are summarized as follows: (I) carbonyl reductases (KREDs)[10] and (II) alcohol dehydrogenases (ADHs)[11] catalyzed asymmetric reduction of prochiral haloketones; (III) lipases[12] and (IV) halohydrin dehalogenases (HHDHs)[13] catalyzed kinetic solution of racemic halohydrins. Some of these methods have been exploited and applied to synthesize halohydrins with excellent yield, enantiomeric excesses (*ee*) and a broad substrate scope.[14] However, it should be noted that all these methods mentioned above absolutely require prior oxygen-functionalization at the target C-H bonds to form the carbonyl or hydroxyl group substituted precursors (Fig. 1).

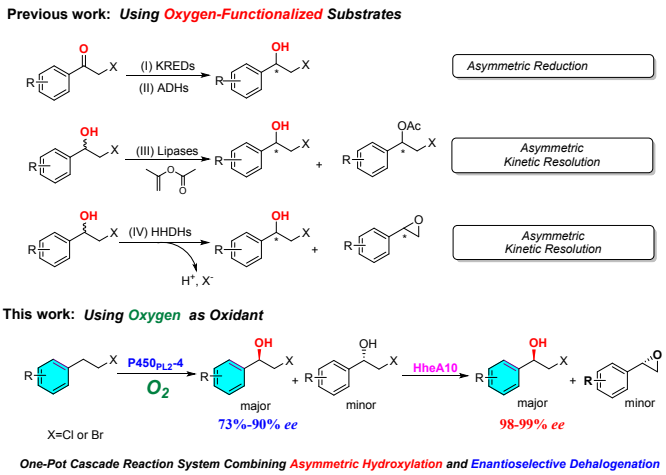

Fig. 1 Biocatalytic approaches to enantiopure β-halohydrins.

Cytochrome P450 monooxygenases (P450s) are the most versatile enzymes and capable of catalyzing a wide range of synthetically challenging oxidation reactions such as hydroxylation,[15] sulfoxidation,[16] and C-H amination.[17] P450s are able to introduce an oxygen atom into a C-H bond under normal pressure and room temperature. We therefore were interested

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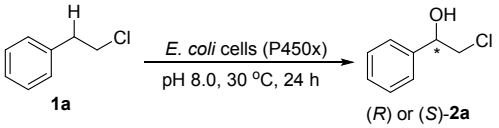
in the synthesis of enantiopure β-halohydrins through P450-catalyzed direct asymmetric hydroxylation of prochiral halohydrocarbons. Herein, enantioenriched β-halohydrins **2a**-**2m** were synthesized from the corresponding halohydrocarbons **1a**-**1m** through $P450_{PL2}$-4 catalyzed asymmetric hydroxylation. In addition, the *ee* values were improved up to 98-99% by developing a one-pot cascade biocatalysis using $P450_{PL2}$-4 and halohydrin dehalogenase HheA10 (Fig. 1).

Previously, we have expressed several P450s from strain *Parvibaculum lavamentivorans* DS-1 and constructed a series of recombinant *Escherichia coli* strains harboring P450 enzyme and redox partner Fdx-Fdr (ferredoxin-ferredoxin reductase).[18] Additionally, we have obtained another thirteen *E. coli* strains containing P450pyr mutants and the corresponding Fdx-Fdr from professor Li group.[19] With these P450 biocatalysts in hand (Table S1), we initially examined their catalytic activity and stereoselectivity using 2-chloroethyl)benzene (**1a**) as a model substrate. Biocatalytic reactions were carried out using recombinant *E. coli* cells as catalyst without addition of any exogenous cofactor. After incubation at 30 °C for 24 h, the yield and *ee* of the product 2-chloro-1-phenlyehthanol (**2a**) were determined using chiral HPLC (Table 1). The result indicated that all the *E. coli* strains containing $P450_{PL2}$ or $P450_{PL7}$ exhibited hydroxylation activity and *R* stereoselectivity to **1a**, which produced **2a** in 17-49% yields and up to 76% *ee* (Table 1, entries 1-10). According to our previous study of sulfoxidation reactions, hydroxylation activity of these P450 strains was also dependent on the redox partner.[18] For the same P450, the Fdx2-Fdr or Fdx4-Fdr redox partner gave the relative higher yields than other redox partners (Table S1-S2). Surprisingly, most of P450pyr mutants could not convert **1a** to **2a**, except for P450pyr-M4, P450pyr-M6 and P450pyr-M9 (Table 1, entries 12-14). These three P450pyr variants showed the opposite stereoselectivity to $P450_{PL}$ strains, which produced (*S*)-**2a** with the *ee* value up to 90%. However, their yields were much lower than that of $P450_{PL}$ strains. As we known, P450pyr is able to catalyze terminal-selective hydroxylation of non-activated C-H bonds and has been successfully engineered for the subterminal hydroxylation of alkanes with excellent regio- and enantioselectivity.[15a,20] Herein, we tested thirteen P450pyr mutants containing 1-6 mutations and found three variants could convert **1a** to **2a**, catalyzing the hydroxylation of activated C-H bond at subterminal position. Interestingly, the best active mutant *E. coli* P450pyr-M4 (N100S/F430I) for subterminal hydroxylation of propylbenzene almost lost hydroxylation activity,[15a] because the yield was not detectable under normal reaction conditions (Table 1, entry 11). The F430I mutation also existed in the best enantioselective P450pyr-M6, and its role in stereocontrol of *S*-selective hydroxylation of propylbenzene has been explained by molecular dynamics and docking simulation.[15a] What needs to be emphasized is that all the *E. coli* strains in the absence of P450 and Fdx-Fdr genes were also carried out as controls, and no hydroxylation activity was observed (Table S2, entries 24-27).

Table 1. Screening of P450 strains for asymmetric hydroxylation of **1a**.



Entry[a]	Biocatalyst	Yield **2a** (%)[b]	ee **2a** (%)[b]	Conf.[c]
1	$P450_{PL2}$-1	48	75	*R*
2	$P450_{PL2}$-2	26	76	*R*
3	$P450_{PL2}$-3	33	77	*R*
4	$P450_{PL2}$-4	49	77	*R*
5	$P450_{PL2}$-5	20	77	*R*
6	$P450_{PL7}$-1	48	77	*R*
7	$P450_{PL7}$-2	32	76	*R*
8	$P450_{PL7}$-3	33	76	*R*
9	$P450_{PL7}$-4	49	76	*R*
10	$P450_{PL7}$-5	17	76	*R*
11	P450pyr-M2	n.d.	n.d.	n.d.
12	P450pyr-M4	trace	35	*S*
13	P450pyr-M6	17	90	*S*
14	P450pyr-M9	8	57	*S*

[a] Reactions were carried out in 5 mL PBS buffer (50 mM, pH 8.0) containing 2 mM of substrate **1a** and 10 g cdw/L of recombinant *E. coli* cells. [b] Yield and *ee* were measured by chiral HPLC analysis after reaction for 24 h, see Table S2 for details. [c] Absolute configuration was confirmed using commercial (*R*)-**2a** and (*R*,*S*)-**2a** as references. n.d. = not detected.

Subsequently, the strains $P450_{PL2}$-4 and P450pyr-M6 were selected for the optimization of reaction conditions. Though the P450pyr-M6 exhibited excellent *S* enantioselectivity toward **1a** (entry 11, Table 1), its catalytic activity was really low which gave only 37% yield of **2a** after reaction optimization (data not shown). Herein, we only discussed the optimization results of strain $P450_{PL2}$-4 (Fig. 2). The results in Fig. 2A indicated that the yield was dramatically influenced by the cell density. Increasing cell density from 5 to 30 g cdw/L improved the yield, while further increasing cell density to 50 g cdw/L by no means obtained a higher yield. The highest yield was found at pH 8.5, which was illustrated by Fig. 2B. In addition, both the yield and *ee* were influenced by reaction temperature (Fig. 2C). The highest *ee* was found at 20 °C, while the corresponding yield was only 40% of that obtained at 35 °C. With the increase of temperature from 35 to 50 °C, the *ee* slowly reduced to 73% and the yield significantly decreased to 16%. To sum up, the reaction conditions were set at pH 8.5 and 35 °C using 30 g cdw/L of recombinant *E. coli* cells.

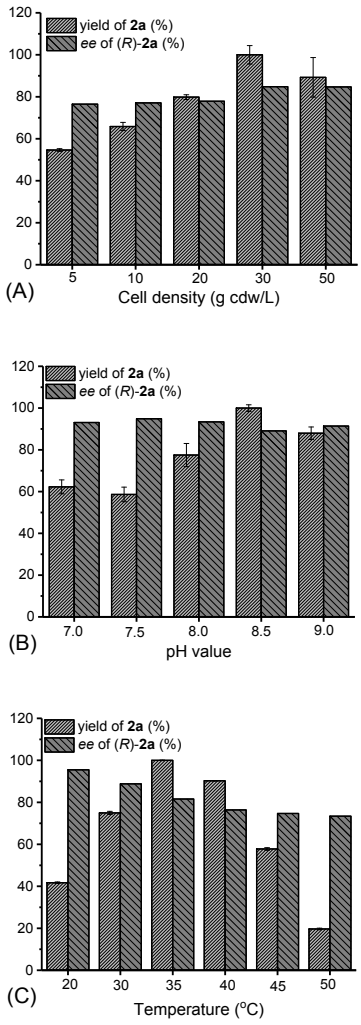
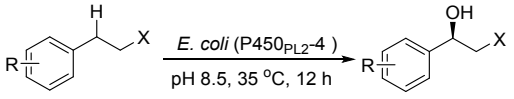


Fig. 2 Conditions optimization for the *E. coli* ($P450_{PL2}$-4) catalyzed asymmetric hydroxylation of **1a**: cell density (A); reaction pH (B); reaction temperature (C). (see Table S3-S5 for details).

Under the optimized conditions, the substrate scope was investigated with various chlorohydrocarbons and bromohydrocarbons. As shown in Table 2, substrates with diverse *ortho*-, *meta*-, and *para*-substituted group on phenyl ring, such as F, Cl, Br and methyl, were found to be suitable for the biotransformation, generating *β*-halohydrin products in 16-81% yields with moderate *ee*. Among *ortho*-, *meta*-, and *para*-substituted halohydrocarbons, the *ortho*-substituted derivatives were not suitable substrates for hydroxylation by $P450_{PL2}$-4 (entry 2 and 8) and gave very low yields (<5%). These results revealed that the steric size of the *ortho*-substituted group had great effect on the activity, which was also in agreement with the report results of P450 BM3[21] and P450tol for benzylic hydroxylation of aromatic hydrocarbons.[22] The substitution at the *ortho*-position might have steric effect on the oxygen attack from the ferryl species (cytochrome P450 Compound I),[23] and reduces oxidation activation efficiency at the benzylic C-H bond. Interestingly, the yields of most chlorohydrins with either substituted or non-substituted group were generally higher than that of the corresponding bromohydrins. These results might be caused from that the higher electronegativity of chloro group leading the benzylic C-H bond easier to accept oxygen. A decrease in activity was also observed in the wake of decrease in electron-withdrawing capability of substituent on phenyl (Table 2, entry 5 *vs* 6). Most chlorohydrins exhibited the similar *ee* values to the corresponding bromohydrins. It was worth noting that almost all the tested halohydrocarbons yielded the corresponding *R* halohydrins with 80-90% *ee*, and only **2d** and **2k** showed >90% *ee*.

Table 2. Asymmetric hydroxylation of pro-chiral halohydrocarbons **1a-1m** using *E. coli* ($P450_{PL2}$-4) cells.



1a-1m **2a-2m**

Entry[a]	R	X	Subs.	Prod.	Yield **2** (%)[b]	*ee* (*R*)-**2** (%)[b]
1	H	Cl	**1a**	**2a**	80	82
2	*o*-CH_3	Cl	**1b**	**2b**	*trace*	n.d.
3	*m*-CH_3	Cl	**1c**	**2c**	80	85
4	*p*-CH_3	Cl	**1d**	**2d**	10	99
5	*p*-F	Cl	**1e**	**2e**	81	87
6	*m*-Br	Cl	**1f**	**2f**	29	84
7	H	Br	**1g**	**2g**	75	82
8	*o*-CH_3	Br	**1h**	**2h**	*trace*	n.d.
9	*m*-CH_3	Br	**1i**	**2i**	35	90
10	*p*-CH_3	Br	**1j**	**2j**	36	80
11	*p*-F	Br	**1k**	**2k**	26	95
12	*p*-Cl	Br	**1l**	**2l**	22	80
13	*m*-Br	Br	**1m**	**2m**	16	88

[a] All the reactions were performed in 5 mL PBS buffer (50 mM, pH 8.5) containing 2 mM of substrate **1a-1m** and 30 g cdw/L of *E. coli* ($P450_{PL}$2-4) cells; [b] The yield and *ee* of halohydrins were determined by chiral HPLC analysis after incubation at 35 °C for 12 h. Subs.=substrate; Prod.=product; n.d.= not detected.

Recent years, biocatalytic cascades have been rapidly developed and lead to the generation of complex valuable chemicals from simple precursors.[24] The P450s have also been used to develop cascade reactions for multiple biotransformation reactions.[25] Recently, we have expressed and characterized a novel HHDH (HheA10) from *Tsukamurella* sp. 1534, which exhibited high *S* enantioselectivity toward *β*-halohydrins.[26] Inspired by the combination of sequential biocatalytic reactions, we attempted to improve the *ee* of halohydrins **2a-2m** by consumption of the *S* isomer halohydrins with the HheA10. With this idea in mind, a "one-pot" biocatalytic cascade reaction was constructed using $P450_{PL2}$-4 and HheA10 (Fig. 3). In the first step, halohydrocarbon was catalyzed by $P450_{PL2}$-4 to generate major *R* and minor *S* halohydrin with moderate *ee* (*R* isomer). Subsequently, the

HheA10 converted the minor *S* halohydrin into *S* epoxide. As a result, the *ee* of *R* halohydrin increased to a higher value.

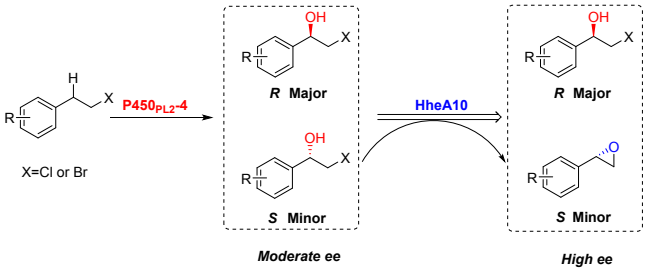
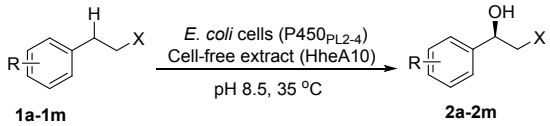


Fig. 3 The hydroxylation-dehalogenation cascade process for one-pot enantioselective synthesis of chiral β-haloalcohols.

To avoid the excessive dehalogenation of *R* halohydrin in the second step, the cell-free extract of HheA10 was added after hydroxylation for 8 h by *E. coli* cells ($P450_{PL2}$-4). As we have known, HHDH was more active to bromohalohydrin than chlorohalohydrin. Consequently, the dehalogenation reaction times in the cascade process for bromohydrocarbons and chlorohalohydrins were 1 h and 4 h, respectively. The yield and *ee* of β-halohydrin products **2a-2m** were determined and showed in Table 3 (except **2b** and **2h** with low activity). As we expected, the increase of *ee* was observed for the halohydrins product (such as entry 1, Table 3 *vs* entry 1, Table 2), and all the tested halohydrins were generated in >98% *ee*. The yields of some halohydrins reduced slightly, which might result from the conversion of *S* isomer and the excessive conversion of *R* isomer in the dehalogenation process. In general, the optical purity of β-halohydrins could be improved by using the hydroxylation-dehalogenation cascade strategy in a short time. This strategy might be more effective than obtaining a stereoselectivity-improved P450 variant via a complicated and time-consuming engineering process, especially for the asymmetric hydroxylation reaction. More importantly, P450-catalyzed C-H direct hydroxylation process shows greener access, which do not need the preoxidation treatment at the target C-H bond.

Table 3. Synthesis of optically active β-halohydrins **2** via one-pot cascade biocatalysis.



Entry[a]	R	X	Subs.	Prod.	Yield (%)[b]	2	*ee* (*R*)-**2** (%)[b]
1	H	Cl	**1a**	**2a**	85		98
2	*o*-CH_3	Cl	**1b**	**2b**	n.d.		n.d.
3	*m*-CH_3	Cl	**1c**	**2c**	45		99
4	*p*-CH_3	Cl	**1d**	**2d**	5		99
5	*p*-F	Cl	**1e**	**2e**	62		99
6	*m*-Br	Cl	**1f**	**2f**	41		99
7	H	Br	**1g**	**2g**	46		99
8	*o*-CH_3	Br	**1h**	**2h**	n.d.		n.d.
9	*m*-CH_3	Br	**1i**	**2i**	31		99
10	*p*-CH_3	Br	**1g**	**2g**	12		98
11	*p*-F	Br	**1k**	**2k**	28		99
12	*p*-Cl	Br	**1l**	**2l**	23		99
13	*m*-Br	Br	**1m**	**2m**	24		99

[a] All the reactions were performed in 5 mL PBS buffer (50 mM, pH 8.5) containing 2 mM of substrate **1a-1m** and 30 g cdw/L of *E. coli* cells ($P450_{PL}$2-4). After reaction at 35 °C for 8 h, 2 mL cell-free extract of HheA10 were added, proceeding for another 3 h (**1a-1f**) or 1 h (**1g-1m**). [b] The yield and *ee* values were determined by chiral HPLC analysis. Subs.=substrate; Prod.=product; n.d.= not detected.

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In summary, we developed a direct and greener route for the synthesis of enantioenriched β-halohydrins via $P450_{PL2}$-4-catalyzed asymmetric hydroxylation of halohydrocarbons at benzylic C-H bonds. In addition, a hydroxylation-dehalogenation enzymatic cascade reaction was constructed by using the $P450_{PL2}$-4 and HheA10, which produced β-halohydrins products in excellent *ee*. This synthetic method uses oxygen as an oxidant, avoids the use of oxygen-functionalized substrates, and achieves the excellent product *ee* by a biocatalytic process in "one-pot".

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 H. Hamada, T. Miura, H. Kumobayashi, T. Matsuda, T. Harada and K. Nakamura, *Biotechnol. Lett.,* 2001, **23**, 1603; (b) S. Eirik, H. Jarle, V. Anders and A. Thorleif, *Eur. J. Org. Chem.,* 2004, **2004**, 1239; (c) J. Y. L. Chung, R. Cvetovich, J. Amato, J. C. McWilliams, R. Reamer and L. DiMichele, *J. Org. Chem.,* 2005, **70**, 3592; (d) M. C. Bryan, D. J. Burdick, B. K. Chan, Y. Chen, S. Clausen, J. Dotson, C. Eigenbrot, R. Elliott, E. J. Hanan, R. Heald, P. Jackson, H. La, M. Lainchbury, S. Malek, S. E. Mann, H. E. Purkey, G. Schaefer, S. Schmidt, E. Seward, S. Sideris, S. Wang, I. Yen, C. Yu and T. P. Heffron, *ACS Med. Chem. Lett.,* 2016, **7**, 100. (e) T. d. Fonseca, L. D. Lima, M. d. de Oliveira, T. L. de Lemos, D. Zampieri, F. Molinari and M. C. de Mattos, *Eur. J. Org. Chem.,* 2018, **2018**, 2110.

2 D. Zhu, C. Mukherjee and L. Hua, *Tetrahedron: Asymmetry,* 2005, **16**, 3275.

3 (a) P. Besse, T. Sokoltchik and H. Veschambre, *Tetrahedron: Asymmetry,* 1998, **9**, 4441; (b) T. Hamada, T. Torii, K. Izawa, R. Noyori and T. Ikariya, *Org. Lett.,* 2002, **4**, 4373; (c) R. Imashiro and M. Seki, *J. Org. Chem.,* 2004, **69**, 4216; (d) T. Poessl, B. Kosjek, U. Ellmer, C. Gruber, K. Edegger, K. Faber, P.

Hildebrandt, U. Bornscheuer and W. Kroutil, *Adv. Synth. Catal.,* 2005, **347**, 1827; (e) K. Wu, H. Wang, L. Chen, H. Fan, Z. Zhao and D. Wei, *Appl. Microbiol. Biot.,* 2016, **100**, 8757.

4 (a) S. P. Hameed, M. Chinnapattu, G. Shanbag, P. Manjrekar, K. Koushik, A. Raichurkar, V. Patil, S. Jatheendranath, S. S. Rudrapatna, S. P. Barde, N. Rautela, D. Awasthy, S. Morayya, C. Narayan, S. Kavanagh, R. Saralaya, S. Bharath, P. Viswanath, K. Mukherjee, B. Bandodkar, A. Srivastava, Panduga, V. J. Reddy, K. R. Prabhakar, A. Sinha, B. Jiménez-Díaz, M. M. S. Martínez, I. Angulo-Barturen, S. Ferrer, L. M. Sanz, F. J. Gamo, S. Duffy, V. M. Avery, P. A. Magistrado, A. K. Lukens, D. F. Wirth, D. Waterson, V. Balasubramanian, P. S. Iyer, S. Narayanan, V. Hosagrahara, V. K. Sambandamurthy and S. Ramachandran, *J. Med.Chem.* 2014, **57**, 5702; (b) A. Murugan, V. K. Kadambar, S. Bachu, M. R. Reddy, V. Torlikonda, S. G. Manjunatha, S. Ramasubramanian, S. Nambiar, G. P. Howell and J. Withnall, *Tetrahedron Lett.,* 2012, **53**, 5739.

5 (a) T. Ohkuma, K. Tsutsumi, N. Utsumi, N. Arai, R. Noyori and K. Murata, *Org. Lett.,* 2007, **9**, 255; (b) L. S. Campbell-Verduyn, W. Szymański, C. P. Postema, R. A. Dierckx, P. H. Elsinga, D. B. Janssen and B. L. Feringa, *Chem. Commun.,* 2010, **46**, 898.

6 (a) I. Kévin, S. Jérémie, R. Pascal, B. Jean-François and M. Angela, *Eur. J. Org. Chem.,* 2014, **2014**, 4099; (b) S. Jérémy, I. Kévin, P. Julien, F. Gilles, R. Pascal, V. Arnaud, B. Jean-François and M. Angela, *Adv. Synth. Catal.,* 2013, **355**, 3613.

7 J. Barluenga, J. Flórez and M. Yus, *J. Chem. Soc., Chem. Commun.,* 1982, **0**, 1153.

8 (a) C. Yin, W. Wu, Y. Hu, X. Tan, C. You, Y. Liu, Z. Chen, X.-Q. Dong, X. Zhang, *Adv. Synth. Catal.,* 2018, **360**, 2119; (b) T. Touge, T. Hakamata, H. Nara, T. Kobayashi, N. Sayo, T. Saito, Y. Kayaki and I. Takao, *J. Am. Chem. Soc.,* 2011, **133**, 14960; (c) Y. Yamato, T. Taichiro, N. Hideki, M. Kazuhiko, F. Mitsuhiko, K. Yoshihito and I. Takao, *Adv. Synth. Catal.,* 2018, **360**, 568.

9 (a) A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts and B. Witholt, *Nature,* 2001, **409**, 258; (b) J. Tao and J.-H. Xu, *Curr. Opin. Chem. Biol.,* 2009, **13**, 43; (c) R. A. Sheldon and J. M. Woodley, *Chem. Rev.,* 2018, **118**, 801; (d) Z.-M. Wu, R.-C. Zheng, X.-L. Tang and Y.-G. Zheng, *App. Microbiol. Biot.* 2017, **101**, 1953.

10 (a) Y. Xie, J.-H. Xu and Y. Xu, *Bioresource Technol.,* 2010, **101**, 1054; (b) Y. Xie, J.-H. Xu, W.-Y. Lu and G.-Q. Lin, *Bioresource Technol.,* 2009, **100**, 2463; (c) S.-Y. Chen, C.-X. Yang, J.-P Wu, G. Xu and L.-R. Yang, *Adv. Synth. Catal.,* 2013, **355**, 3179; (d) F. Qin, B. Qin, T. Mori, Y. Wang, L. Meng, X. Zhang, X. Jia, I. Abe and S. You, *ACS Catal.,* 2016, **6**, 6135; (e) X. Chen, H. Zhang, J. Feng, Q. Wu and D. Zhu, *ACS Catal.,* 2018, **8**, 3525.

11 (a) N. Itoh, K. Isotani, M. Nakamura, K. Inoue, Y. Isogai and Y. Makino, *Appl. Microbiol. Biot.,* 2012, **93**, 1075; (b) J. Mangas-Sánchez, E. Busto, V. Gotor-Fernández, F. Malpartida and V. Gotor, *J. Org. Chem.,* 2011, **76**, 2115; (c) D. Zhu, B. A. Hyatt and L. Hua, *J. Mol. Catal. B-Enzym.,* 2009, **56**, 272.

12 (a) I. M. Ferreira, R. H. V. Nishimura, A. B. A. Souza, G. C. Clososki, S. A. Yoshioka and A. L. M. Porto, *Tetrahedron Lett.,* 2014, **55**, 5062; (b) A. Träff, K. Bogár, M. Warner and J.-E. Bäckvall, *Org. Lett.,* 2008, **10**, 4807; (c) O. Pàmies and J.-E. Bäckvall, *J. Org. Chem.,* 2002, **67**, 9006.

13 (a) M. Majerić Elenkov, L. Tang, B. Hauer and D. B. Janssen, *Org. Lett.,* 2006, **8**, 4227; (b) R. M. Haak, C. Tarabiono, D. B. Janssen, A. J. Minnaard, J. G. de Vries and B. L. Feringa, *Org. Biomol. Chem.,* 2007, **5**, 318; (c) A. Westerbeek, J. G. E. van Leeuwen, W. Szymanski, B. L. Feringa and D. B. Janssen, *Tetrahedron*, 2012, **68**, 7645; (d) H. Arabnejad, M. Dal Lago, P. A. Jekel, R. J. Floor, A-M. W. H. Thunnissen, A. C. Terwisscha van Scheltinga, H. J. Wijma and D. B. Janssen, *Protein Eng. Des. Sel.*, 2017, **30**, 175.

14 (a) W. Borzęcka, I. Lavandera and V. Gotor, *J. Org. Chem.,* 2013, **78**, 7312; (b) G.-C. Xu, H.-L. Yu, X.-Y. Zhang and J.-H. Xu, *ACS Catal.,* 2012, **2**, 2566; (c) Y.-P. Shang, Q. Chen, X.-D. Kong, Y.-J Zhang, J.-H. Xu and H.-L. Yu, *Adv. Synth. Catal.,* 2017, **359**, 426.

15 (a) Y. Yang, J. Liu and Z. Li, *Angew. Chem. Int. Ed.,* 2014, **53**, 3120; (b) S. Kille, F. E. Zilly, J. P. Acevedo and M. T. Reetz, *Nat. Chem.,* 2011, **3**, 738; (c) E. Weber, A. Seifert, M. Antonovici, C. Geinitz, J. Pleiss and V. B. Urlacher, *Chem. Commun.,* 2011, **47**, 944.

16 (a) P. Gao, A. Li, H. H. Lee, D. I. C. Wang and Z. Li, *ACS Catal.,* 2014, **4**, 3763; (b) J.-D. Zhang, A.-T. Li, Y. Yang and J.-H. Xu, *Appl. Microbiol. Biot.,* 2010, **85**, 615.

17 (a) C. K. Prier, R. K. Zhang, A. R. Buller, S. Brinkmann-Chen and F. H. Arnold, *Nat. Chem.,* 2017, **9**, 629; (b) T. K. Hyster, C. C. Farwell, A. R. Buller, J. A. McIntosh and F. H. Arnold, *J. Am. Chem. Soc.,* 2014, **136**, 15505; (c) A. McIntosh, J. P. S. Coelho, C. C. Farwell, Z. J. Wang, J. C. Lewis, T. R. Brown and F. H. Arnold, *Angew. Chem. Int. Ed.,* 2013, **52**, 9309; (d) R. Singh, M. Bordeaux and R. Fasan, *ACS Catal.,* 2014, **4**, 546.

18 K. Wu, L. Tang, H. Cui, N. Wan, Z. Liu, Z. Wang, S. Zhang, B. Cui, W. Han and Y. Chen, *ChemCatChem,* 2018, **10**, 5410.

19 J. B. van Beilen, E. G. Funhoff, A. van Loon, A. Just, L. Kaysser, M. Bouza, R. Holtackers, M. Röthlisberger, Z. Li and B. Witholt, *Appl. Environ. Microb.,* 2006, **72**, 59.

20 W. Zhang, W. L. Tang and Z. Li, *Adv. Synth. Catal.,* 2010, **352**, 3380.

21 K. Neufeld, J. Marienhagen, U. Schwaneberg and J. Pietruszka, *Green Chem.,* 2013, **15**, 2408.

22 A. Li, S. Wu, J. P. Adams, R. Snajdrova and Z. Li, *Chem. Commun.,* 2014, **50**, 8771.

23 J. Rittle and M. T. Green, *Science,* 2010, **330**, 933.

24 (a) S. P. France, L. J. Hepworth, N. J. Turner and S. L. Flitsch, *ACS Catal.,* 2017, **7**, 710; (b) J. H. Schrittwieser, S. Velikogne, M. Hall and W. Kroutil, *Chem. Rev.,* 2018, **11**, 270; (c) J. M. Sperl, V. Sieber, *ACS Catal.,* 2018, **8**, 2385.

25 (a) R. Agudo and M. T. Reetz, *Chem. Commun.,* 2013, **49**, 10914; (b) A. Li, A. Ilie, Z. Sun, R. Lonsdale, J.-H. Xu and M. T. Reetz, *Angew. Chem. Int. Edit.,* 2016, **55**, 12026; (c) M. Tavanti, F. Parmeggiani, J. R. G. Castellanos, A. Mattevi and N. J. Turner, *ChemCatChem,* 2017, **9**, 3338.

26 N. Wan, J. Tian, H. Wang, M. Tian, Q. He, R. Ma, B. Cui, W. Han and Y. Chen, *Bioorg. Chem.,* 2018, **81**, 529.