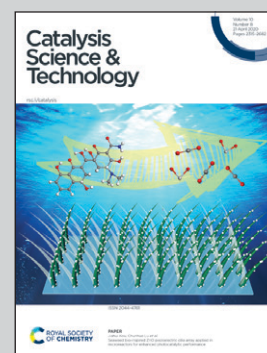


Showcasing research from Professor Gao-Wei Zheng's laboratory, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China.

Development of an engineered thermostable amine dehydrogenase for the synthesis of structurally diverse chiral amines

Using directed evolution, we have engineered a thermostable amine dehydrogenase based on a phenylalanine dehydrogenase that catalyses direct reductive amination of ketones and functionalized hydroxy ketones with ammonia or primary amines to diverse chiral primary amines, secondary amines and amino alcohols.

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## Development of an engineered thermostable amine dehydrogenase for the synthesis of structurally diverse chiral amines†

Lei Liu,<sup>a</sup> Dong-Hao Wang,<sup>a</sup> Fei-Fei Chen,<sup>a</sup> Zhi-Jun Zhang,<sup>a</sup> Qi Chen,<sup>a</sup>  
Jian-He Xu,<sup>a</sup> Zhi-Long Wang<sup>b</sup> and Gao-Wei Zheng<sup>\*a</sup>

Amine dehydrogenases (AmDHs) are emerging as a class of attractive biocatalysts for synthesizing chiral amines via asymmetric reductive amination of ketones with inexpensive ammonia as an amino donor. However, the AmDHs developed to date exhibit limited substrate scope. Here, using directed evolution, we engineered a GkAmDH based on a thermostable phenylalanine dehydrogenase from *Geobacillus kaustophilus*. The newly developed AmDH is able to catalyze reductive amination of a diverse set of ketones and functionalized hydroxy ketones with ammonia or primary amines with up to >99% conversion, thus accessing structurally diverse chiral primary and secondary amines and chiral vicinal amino alcohols, with excellent enantioselectivity (up to >99% ee) and releasing water as the sole by-product.

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Chiral amines are an important class of intermediates for the synthesis of numerous pharmaceutical drugs, bioactive compounds, and agrochemicals.<sup>1</sup> They are also extensively used as resolving agents in kinetic resolution reactions.<sup>2</sup> Therefore, the synthesis of such molecules continues to be of great interest for chemists. Traditional synthesis methods, however, rely heavily on precious transition metal catalysts and other reagents well known to be environmentally harmful, and have the additional downside of often failing to generate optically pure reaction products.

Advances in protein engineering have enabled the emergence of biocatalysis as a powerful alternative method for the synthesis of chiral compounds in the chemical and pharmaceutical industries.<sup>3–8</sup> To date, numerous biocatalytic processes have been developed for the synthesis of chiral amines; for example, the (dynamic) kinetic resolution of racemic amines catalyzed by amine oxidases<sup>9–12</sup> and lipases,<sup>13,14</sup> asymmetric reduction of imines catalyzed by (artificial) imine reductases,<sup>15–20</sup> (reductive) amination of ketones catalyzed by transaminases,<sup>21–24</sup> reductive aminases<sup>20,25,26</sup> and amine dehydrogenases,<sup>27–33</sup> direct C–H amination of alkane by cytochrome P450 monooxygenases,<sup>34,35</sup> and a series of elegant multi-enzymatic cascade reactions.<sup>36–41</sup>

In particular, a recently developed class of engineered enzymes, amine dehydrogenases (AmDHs), are now appreciated as attractive biocatalysts for the synthesis of chiral primary amines using simple and green approaches; they can use inexpensive free ammonia as an amino donor for the reductive amination of ketones. Moreover, only water is generated as a by-product in this process. Three AmDHs were initially created by Bommarius and co-workers through directed evolution of naturally occurring leucine dehydrogenases and phenylalanine dehydrogenases.<sup>32,42</sup> Using the same approach, Li and co-workers subsequently developed an AmDH based on *Rhodococcus* phenylalanine dehydrogenase, which displayed detectable activity towards the bulky 4-phenyl-2-butanone, a challenging substrate for these enzymes.<sup>31</sup> More recently, Schell and co-workers also developed a thermostable CtAmDH by directed evolution of a *Caldalkalibacillus thermarum* phenylalanine dehydrogenase, and demonstrated an efficient biphasic system for the amination of phenoxy-2-propanone.<sup>30</sup> In addition, Vergne-Vaxelaire and Grogan identified a family of native AmDHs for reduction amination of ketones.<sup>27</sup>

Notably, by coupling the engineered AmDHs with alcohol dehydrogenases (ADHs), both the Turner group and our group constructed elegant dual-enzyme ‘hydrogen-borrowing’ cascade systems for the production of chiral amines from available racemic alcohols in a green manner.<sup>41,43</sup> Mutti and coworkers performed a detailed investigation of AmDH-catalyzed reductive amination that considered substrate acceptance, optimal reaction conditions, and stereoselectivity.<sup>44</sup> Very recently, we further engineered AmDHs through mutation of two key residues surrounding

<sup>a</sup> State Key Laboratory of Bioreactor Engineering, Shanghai Collaborative Innovation Center for Biomanufacturing, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, P. R. China.  
E-mail: gaoweizheng@ecust.edu.cn; Fax: (+86) 21 6425 0840

<sup>b</sup> State Key Laboratory of Microbial Metabolism, School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, P. R. China

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the substrate-binding pocket which affect the binding of bulky aliphatic ketones.<sup>29</sup> The resultant AmDH mutants displayed broad substrate scopes, and are capable of accepting previously inaccessible bulky substrate ketones like 2-heptanone and 2-octanone.

Despite recent progress with AmDHs, the enzymes were mainly used for the synthesis of chiral primary amines *via* reductive amination of ketones with ammonia. Until recently, Mutti and co-workers reported the synthesis of chiral secondary amines from ketones and achiral organic amino donors using AmDHs.<sup>28,45</sup> However, few examples showed that AmDHs could have been utilized for asymmetric reductive amination of functionalized aromatic ketones (*e.g.*, aromatic hydroxy ketones).<sup>46</sup> Herein, we performed directed evolution of a thermostable phenylalanine dehydrogenase from *Geobacillus kaustophilus* to develop an AmDH that possesses a broad substrate scope. The resulting *GkAmDH* can catalyze the synthesis of structurally diverse chiral primary and secondary amines from ketones with ammonia or organic amines and chiral vicinal amino alcohols through amination of hydroxy ketones with ammonia (Scheme 1).

Initially, four phenylalanine dehydrogenases, which were identified from different microorganisms, were used as starting templates for the development of AmDHs by introducing the corresponding double mutations of K78S/N276L to alter substrate specificity.<sup>27–33</sup> Among the engineered AmDHs, *GkAmDH* (based on *Geobacillus kaustophilus* phenylalanine dehydrogenase) displayed the highest amination activity (6.0 U mg<sup>−1</sup> protein) towards the model substrate 4-fluorophenylacetone (**1**) (Table S2†). The optimal pH and temperature of *GkAmDH* were 9.0 and 55 °C, respectively (Fig. S1†). Analysis of kinetic parameters showed that *GkAmDH* gave a *K*<sub>M</sub> of 7.8 mM and *k*<sub>cat</sub> of 6.0 s<sup>−1</sup> towards ketone **1**, values comparable to those reported for *BbAmDH* and *CtAmDH*.<sup>30,32</sup> Its melting temperature (*T*<sub>m</sub>) reached 71.5 °C (Table S3†), suggesting that it is a thermostable biocatalyst for reductive amination. Moreover, we observed that its

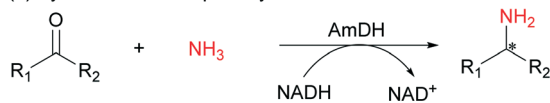
amination activity increased linearly with increases in the NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> concentration ranging from 0 to 5.0 M, and reached 18.0 U mg<sup>−1</sup> towards substrate **1** at 5.0 M (Fig. S2†).

With this promising enzyme in hand, we subsequently explored its amination activity towards a series of ketones using ammonia as an amino donor. As shown in Fig. 1, *GkAmDH* exhibited amination activities towards all tested substrates **1–12**, including aromatic ketones with a range of substitutions on their aromatic rings and aliphatic ketones with different carbon chain lengths, as well as the challenging 4-phenyl-2-butanone (**8**). *GkAmDH* exhibited higher activities towards **1–4**, which bear electron-withdrawing substituents on their benzene rings, than towards **6** and **7** with electron-donating substituents, clearly suggesting that electron-withdrawing substituents may activate the carbonyl carbon. Moreover, *GkAmDH* displayed similar activities towards **1–3**, suggesting that the substituent's position on the benzene ring had no apparent effect on the enzyme's activity.

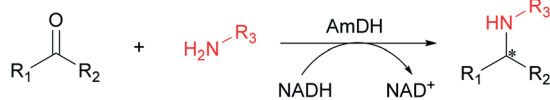
We next attempted the reductive amination of ketones **1–12** in 5 M NH<sub>3</sub>·H<sub>2</sub>O/NH<sub>4</sub>Cl buffer (pH 9.0) using *GkAmDH* combined with glucose dehydrogenase (GDH) used for the regeneration of NADH. However, we found that high concentrations of NH<sub>4</sub><sup>+</sup> caused poor GDH activity (Fig. 2a). We therefore examined another system based on formate dehydrogenase (FDH) with NH<sub>3</sub>·H<sub>2</sub>O/NH<sub>4</sub>COOH buffer (Fig. 2b). Consequently, use of a high concentration of NH<sub>3</sub>·H<sub>2</sub>O/NH<sub>4</sub>COOH buffer had no apparent deleterious effects on FDH activity. We therefore used an optimized FDH-based regeneration system (5 M, pH 9.0) in subsequent reductive amination reactions.

Using the optimized reaction system, analytical scale reductive amination reactions of various ketones **1–12** with ammonia were carried out using the *GkAmDH*/FDH coupling system in NH<sub>3</sub>·H<sub>2</sub>O/NH<sub>4</sub>COOH buffer (5 M, pH 9.0): *GkAmDH* exhibited great reactivity towards ketones **1–6** (Table 1), findings consistent with our results for specific activity (Fig. 1). 50 mM substrates **1–6** were converted with up to 99% conversions with low enzyme loading. For the challenging substrates **7–11**, good-to-excellent conversions were also achieved using 10 g L<sup>−1</sup> *GkAmDH*. Only for substrate **12**, *GkAmDH* exhibited poor conversion (<5%), suggesting that excessively long alkyl chains can hinder substrate access to

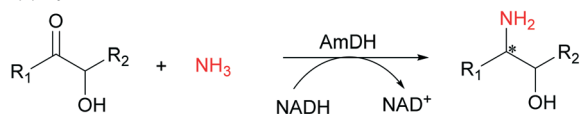
(a) Synthesis of chiral primary amines



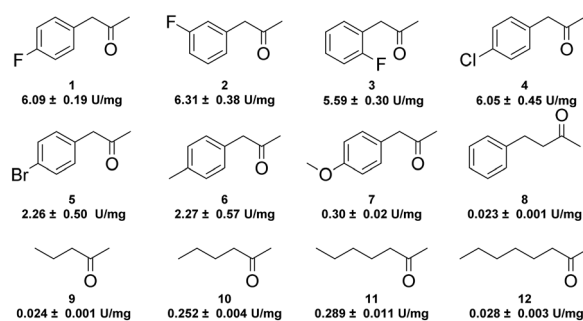
(b) Synthesis of chiral secondary amines



(c) Synthesis of chiral vicinal amino alcohols

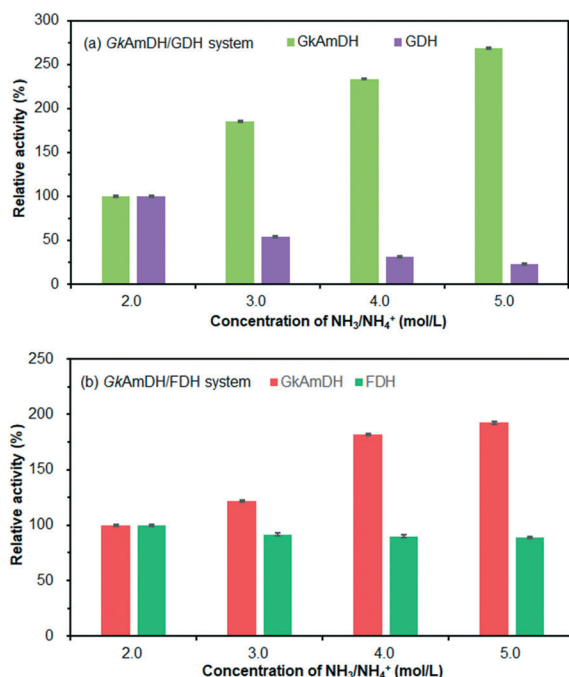


**Scheme 1** Synthesis of chiral primary and secondary amines and chiral vicinal amino alcohols by AmDH-catalyzed asymmetric reductive amination of ketones and hydroxy ketones with ammonia or organic amines. AmDH, amine dehydrogenase.



**Fig. 1** Specific activities (U mg<sup>−1</sup> protein) of *GkAmDH* towards various ketones using ammonia as an amino donor.





**Fig. 2** The relative activity of *GkAmDH* and *GDH* in  $\text{NH}_3\cdot\text{H}_2\text{O}/\text{NH}_4\text{Cl}$  buffer (a) and *GkAmDH* and *FDH* in  $\text{NH}_3\cdot\text{H}_2\text{O}/\text{NH}_4\text{COOH}$  buffer (b). The activity of the enzymes in buffer containing 2 M  $\text{NH}_3/\text{NH}_4^+$  was normalized as 100%.

the *GkAmDH* active site. Furthermore, *GkAmDH* displayed excellent stereoselectivity for all the tested substrates, and yielded (*R*)-configuration chiral primary amines with up to 99% enantiomeric excess (ee). These results highlight the

**Table 1** Reductive amination of ketones **1–12** with ammonia using a *GkAmDH*/FDH coupling system in 5 M  $\text{NH}_3\cdot\text{H}_2\text{O}/\text{NH}_4\text{COOH}$  buffer<sup>a</sup>

Substrate	Substrate concn. (mM)	<i>GkAmDH</i> (mg mL <sup>-1</sup> )	Conv. (%)	ee (%)
1	50	1	99	>99
2	50	1	98	>99
3	50	1	98	>99
4	50	1	91	>99
5	50	3	98	>99
6	50	3	99	>99
7	50	10	99	>99
8	50	10	85	98
9	10	10	77	>99
10	10	10	99	>99
11	10	10	98	>99
12	10	10	<5	>99

<sup>a</sup> Reaction conditions: 0.5 mL reaction mixture containing  $\text{NH}_3\cdot\text{H}_2\text{O}/\text{NH}_4\text{COOH}$  (5 M  $\text{NH}_4^+$ , pH 9.0), 10–50 mM substrate, 1 mM  $\text{NAD}^+$ , 10 mg mL<sup>-1</sup> FDH (lyophilized cell-free extract) and *GkAmDH* (lyophilized cell-free extract) shaken at 1000 rpm at 40 °C for 24 h. Conversions and enantiomeric excess were determined *via* GC and chiral GC, respectively.

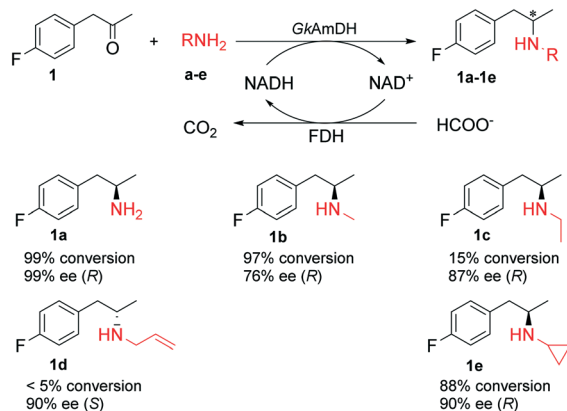
strong potential of *GkAmDH* as a biocatalyst for the synthesis of chiral primary amines.

Recently, a reductive aminase *AspRedAm* and several imine reductases have been described for the synthesis of chiral secondary amines *via* reductive amination of ketones with primary amines.<sup>19,26,47,48</sup> To explore the possibility of using AmDHs for the synthesis of chiral secondary amines, reductive amination of the model substrate **1** with four primary amines **b–e** was attempted with *GkAmDH*. Consequently, *GkAmDH* can reduce the imines formed with primary amines **b** and **e** with **1** at 97% conversion and 88% conversion, respectively. In contrast, *GkAmDH* only provided 15% and <5% conversions in the reduction of **1** with ethylamine **c** and allylamine **d**, highlighting the substantial impact of the amino donors on *GkAmDH*'s activity (Scheme 2). The corresponding chiral secondary amines were produced with good stereoselectivity (78–90% ee). Interestingly, a reversed stereoselectivity was observed for **1d**, perhaps owing to the rotation of the carbon–carbon bond connecting the phenyl and carbon of the carbonyl group. Recently, Mutti and co-workers reported AmDH-catalyzed coupling amination of ketones with primary amines in which up to 43% of the substrate was converted into the (*R*)-selective secondary amines with up to 72% ee.<sup>45</sup> Taken together, these results demonstrate that AmDHs possess the unique ability to reduce the imines formed from both inorganic ammonia and organic amines with ketones for the preparation of optically pure primary and secondary amines.

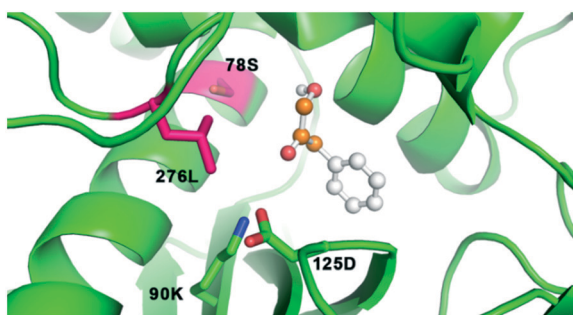
Encouragingly, we observed that *GkAmDH* showed amination activity towards aromatic hydroxy ketones **13** and **14** (3.50 U mg<sup>-1</sup> and 2.06 U mg<sup>-1</sup>, respectively). Docking analysis showed that the hydroxy ketone **14** is accommodated into the binding pocket of *GkAmDH* in a reasonable conformation in which the terminal hydroxyl group points towards the binding residues S78 and L276, while the carbonyl group points to the two catalytic residues K90 and D125, thereby generating satisfactory amination activity (Fig. 3).

We also detected the activity of *GkAmDH* for aliphatic hydroxy ketones **15** and **16** (0.39 U mg<sup>-1</sup> and 0.04 U mg<sup>-1</sup>, respectively) (Table 2). The results indicate that AmDHs are able to catalyze the reduction of imines formed from hydroxy ketones with inexpensive ammonia to access vicinal amino alcohols. These amino alcohols are extensively exploited as privileged scaffolds in pharmaceutically active molecules and natural products,<sup>49</sup> such as norephedrine and norpseudoephedrine, which are used as amphetamine pharmaceuticals in most countries.<sup>50</sup> In addition, they also serve as important chiral auxiliaries or ligands in asymmetric synthesis reactions.<sup>51,52</sup>

Seeking to develop AmDHs with higher activity towards hydroxy ketones, we undertook double site combinational mutagenesis at two key residues S78 and L276 affecting substrate specificity using *GkAmDH* as the template. After screening separately using hydroxy ketones **13–16**, the best mutants displayed further increased activity towards the corresponding substrate (Table 2). To investigate whether



**Scheme 2** *GkAmDH*-catalyzed reduction of imines formed from ketone **1** with primary amines for the synthesis of chiral secondary amines. Reaction conditions: 40 mg mL<sup>-1</sup> *GkAmDH* (lyophilized cell-free extract), 20 mg mL<sup>-1</sup> *FDH* (lyophilized crude cell-free extract), 10 mM substrate **1**, 1 mM NAD<sup>+</sup>, amino donor (2.5 M, pH 9.0), 0.5 mL total volume, 40 °C, 1000 rpm, 24 h.



**Fig. 3** The docking model of substrate **14** into the binding pocket of *GkAmDH*. S78 and L276 are two key residues for altering substrate specificity and K90 and D125 are the catalytic residues.

chiral vicinal amino alcohols could be achieved using the newly developed AmDHs, we next performed the reductive amination of **14** on a preparative scale as a representative example. Using the best AmDH<sub>K78S/N276T</sub>, 35 mM **14** was smoothly converted to (*S*)-2-amino-3-phenylpropanol ((*S*)-**14a**) in 41% isolated yield and >99% ee. This result showcases the potential of the newly developed AmDH enzymes for the preparation of chiral vicinal amino alcohols, especially for aromatic chiral vicinal amino alcohols.

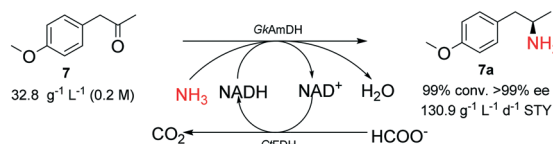
Finally, ketone **7** was aminated with ammonia on a 100 mL scale (Scheme 3). The preparative reductive amination of **7** (200 mM, 5% (v/v) DMSO) was performed in NH<sub>3</sub>·H<sub>2</sub>O/NH<sub>4</sub>COOH buffer (5 M, pH 9.0) and the pH was maintained at 9.0 by titrating 5 M NH<sub>3</sub>·H<sub>2</sub>O during the reaction course. Consequently, 99% of the substrate was converted into the corresponding chiral primary amine product (*R*)-4-methoxyamphetamine ((*R*)-**7a**) within 6 h, corresponding to a calculated space-time yield of 130.9 g L<sup>-1</sup> d<sup>-1</sup>. After normal workup, the product was prepared with 43% isolated yield and >99% ee.

We also performed the reductive coupling of ketone **1** (10 mM, 1% (v/v) DMSO) with methylamine **b** on a 50 mL scale.

**Table 2** The amination activity (U mg<sup>-1</sup>) of the AmDHs towards hydroxy ketones **13-16**<sup>a</sup>

Substrate	Specific activity of <i>GkAmDH</i> <sub>K78S/N276L</sub>	The best AmDH	Specific activity (U mg <sup>-1</sup> )
<b>13</b>	3.50 ± 0.01	AmDH <sub>K78S/N276C</sub>	9.35 ± 0.08
<b>14</b>	2.06 ± 0.08	AmDH <sub>K78S/N276T</sub>	4.59 ± 0.37
<b>15</b>	0.39 ± 0.01	AmDH <sub>K78T/N276L</sub>	0.43 ± 0.01
<b>16</b>	0.04 ± 0.01	AmDH <sub>K78T/N276C</sub>	0.07 ± 0.01

<sup>a</sup> Activity was measured in NH<sub>3</sub>·H<sub>2</sub>O/NH<sub>4</sub>COOH buffer (2 M, pH 9.0) containing 0.2 mM NADH and 10 mM substrate (except 1 mM for substrate **14**) at 40 °C.



**Scheme 3** Synthesis of 4-methoxyamphetamine (**7a**) catalyzed by the *GkAmDH*/*FDH* system on a 100 mL scale.

The 98% conversion was reached in 12 h, and the corresponding chiral secondary amine product **1b** was obtained with 45% yield and 70% ee.

In summary, the present study developed a thermostable *GkAmDH* by engineering a phenylalanine dehydrogenase from *Geobacillus kaustophilus*. The newly developed *GkAmDH* not only catalyzes the reductive amination of ketones with inexpensive ammonia and primary amines to chiral primary and secondary amines, but also can mediate the bioamination of ketones **13-16** with ammonia to access chiral vicinal amino alcohols. Using *GkAmDH*, a wide range of chiral primary and secondary amines and chiral vicinal amino alcohol **14a** were synthesized with up to >99% conversion and up to >99% ee, generating water as the sole by-product. These results demonstrate extensive applications of AmDHs for the synthesis of structurally diverse chiral amines using a simple and green approach.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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## Notes and references

- 1 M. E. Welsch, S. A. Snyder and B. R. Stockwell, *Curr. Opin. Chem. Biol.*, 2010, **14**, 347–361.
- 2 T. C. Nugent, *Chiral amine synthesis: methods, developments and applications*, John Wiley & Sons, Weinheim, Germany, 2010.
- 3 O. Kuchner and F. H. Arnold, *Trends Biotechnol.*, 1997, **15**, 523–530.
- 4 U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore and K. Robins, *Nature*, 2012, **485**, 185–194.
- 5 J. M. Woodley, *Curr. Opin. Chem. Biol.*, 2013, **17**, 310–316.
- 6 Z. Chen and A. P. Zeng, *Curr. Opin. Biotechnol.*, 2016, **42**, 198–205.
- 7 G. W. Zheng and J. H. Xu, *Curr. Opin. Biotechnol.*, 2011, **22**, 784–792.
- 8 G. Grogan, *Curr. Opin. Chem. Biol.*, 2018, **43**, 15–22.
- 9 D. Ghislieri, A. P. Green, M. Pontini, S. C. Willies, I. Rowles, A. Frank, G. Grogan and N. J. Turner, *J. Am. Chem. Soc.*, 2013, **135**, 10863–10869.
- 10 T. Li, J. Liang, A. Ambrogelly, T. Brennan, G. Gloor, G. Huisman, J. Lalonde, A. Lekhal, B. Mijts, S. Muley, L. Newman, M. Tobin, G. Wong, A. Zaks and X. Y. Zhang, *J. Am. Chem. Soc.*, 2012, **134**, 6467–6472.
- 11 G. Y. Li, P. Y. Yao, R. Gong, J. L. Li, P. Liu, R. Lonsdale, Q. Q. Wu, J. P. Lin, D. M. Zhu and M. T. Reetz, *Chem. Sci.*, 2017, **8**, 4666.
- 12 S. Nakano, K. Yasukawa, T. Tokiwa, T. Ishikawa, E. Ishitsubo, N. Matsuo, S. Ito, H. Tokiwa and Y. Asano, *J. Phys. Chem. B*, 2016, **120**, 10736–10743.
- 13 L. Munoz, A. M. Rodriguez, G. Rosell, M. P. Bosch and A. Guerrero, *Org. Biomol. Chem.*, 2011, **9**, 8171–8177.
- 14 M. T. Reetz and K. Schimossek, *Chimia*, 1996, **50**, 668–669.
- 15 J. Mangas-Sanchez, S. P. France, S. L. Montgomery, G. A. Aleku, H. Man, M. Sharma, J. I. Ramsden, G. Grogan and N. J. Turner, *Curr. Opin. Chem. Biol.*, 2017, **37**, 19–25.
- 16 H. Li, P. Tian, J. H. Xu and G. W. Zheng, *Org. Lett.*, 2017, **19**, 3151–3154.
- 17 H. Li, G. X. Zhang, L. M. Li, Y. S. Ou, M. Y. Wang, C. X. Li, G. W. Zheng and J. H. Xu, *ChemCatChem*, 2016, **8**, 724–727.
- 18 H. Li, Z. J. Luan, G. W. Zheng and J. H. Xu, *Adv. Synth. Catal.*, 2015, **357**, 1692–1696.
- 19 P. Matzel, M. Gand and M. Höhne, *Green Chem.*, 2017, **19**, 385–389.
- 20 S. C. Cosgrove, A. Brzezniak, S. P. France, J. I. Ramsden, J. Mangas-Sanchez, S. L. Montgomery, R. S. Heath and N. J. Turner, *Methods Enzymol.*, 2018, **608**, 131–149.
- 21 A. Gomm and E. O'Reilly, *Curr. Opin. Chem. Biol.*, 2018, **43**, 106–112.
- 22 I. Slabu, J. L. Galman, R. C. Lloyd and N. J. Turner, *ACS Catal.*, 2017, **7**, 8263–8284.
- 23 C. E. Paul, M. Rodriguez-Mata, E. Busto, I. Lavandera, V. Gotor-Fernandez, V. Gotor, S. Garcia-Cerrada, J. Mendiola, O. de Frutos and I. Collado, *Org. Process Res. Dev.*, 2014, **18**, 788–792.
- 24 J. Rudat, B. R. Brucher and C. Syldatk, *AMB Express*, 2012, **2**, 11.
- 25 G. A. Aleku, J. Mangas-Sanchez, J. Citoler, S. P. France, S. L. Montgomery, R. S. Heath, M. P. Thompson and N. J. Turner, *ChemCatChem*, 2018, **10**, 515–519.
- 26 G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan and N. J. Turner, *Nat. Chem.*, 2017, **9**, 961–969.
- 27 O. Mayol, K. Bastard, L. Beloti, A. Frese, J. P. Turkenburg, J. L. Petit, A. Mariage, A. Debar, V. Pellouin, A. Perret, V. de Berardinis, A. Zaparucha, G. Grogan and C. Vergne-Vaxelaire, *Nat. Catal.*, 2019, **2**, 324–333.
- 28 V. Tseliou, T. Knaus, M. F. Masman, M. L. Corrado and F. G. Mutti, *Nat. Commun.*, 2019, **10**, 3717.
- 29 F. F. Chen, G. W. Zheng, L. Liu, H. Li, Q. Chen, F. L. Li, C. X. Li and J. H. Xu, *ACS Catal.*, 2018, **8**, 2622–2628.
- 30 A. Pushpanath, E. Siirola, A. Bornadel, D. Woodlock and U. E. Schell, *ACS Catal.*, 2017, **7**, 3204–3209.
- 31 L. J. Ye, H. H. Toh, Y. Yang, J. P. Adams, R. Snajdrova and Z. Li, *ACS Catal.*, 2015, **5**, 1119–1122.
- 32 M. J. Abrahamson, J. W. Wong and A. S. Bommarius, *Adv. Synth. Catal.*, 2013, **355**, 1780–1786.
- 33 M. J. Abrahamson, E. Vazquez-Figueroa, N. B. Woodall, J. C. Moore and A. S. Bommarius, *Angew. Chem., Int. Ed.*, 2012, **51**, 3969–3972.
- 34 C. K. Prier, R. J. K. Zhang, A. R. Buller, S. Brinkmann-Chen and F. H. Arnold, *Nat. Chem.*, 2017, **9**, 629–634.
- 35 P. Dydio, H. M. Key, H. Hayashi, D. S. Clark and J. F. Hartwig, *J. Am. Chem. Soc.*, 2017, **139**, 1750–1753.
- 36 J. H. Schrittwieser, S. Velikogne, M. Hall and W. Kroutil, *Chem. Rev.*, 2017, **118**, 270–348.
- 37 S. P. France, S. Hussain, A. M. Hill, L. J. Hepworth, R. M. Howard, K. R. Mulholland, S. L. Flitsch and N. J. Turner, *ACS Catal.*, 2016, **6**, 3753–3759.
- 38 H. D. Peng, E. M. Wei, J. L. Wang, Y. N. Zhang, L. Cheng, H. M. Ma, Z. X. Deng and X. D. Qu, *ACS Chem. Biol.*, 2016, **11**, 3278–3283.
- 39 F. Parmeggiani, S. L. Lovelock, N. J. Weise, S. T. Ahmed and N. J. Turner, *Angew. Chem., Int. Ed.*, 2015, **54**, 4608–4611.
- 40 S. L. Montgomery, J. Mangas-Sanchez, M. P. Thompson, G. A. Aleku, B. Dominguez and N. J. Turner, *Angew. Chem., Int. Ed.*, 2017, **56**, 10491–10494.
- 41 F. F. Chen, Y. Y. Liu, G. W. Zheng and J. H. Xu, *ChemCatChem*, 2015, **7**, 3838–3841.
- 42 B. R. Bommarius, M. Schurmann and A. S. Bommarius, *Chem. Commun.*, 2014, **50**, 14953–14955.
- 43 F. G. Mutti, T. Knaus, N. S. Scrutton, M. Breuer and N. J. Turner, *Science*, 2015, **349**, 1525–1529.
- 44 T. Knaus, W. Bohmer and F. G. Mutti, *Green Chem.*, 2017, **19**, 453–463.
- 45 V. Tseliou, M. F. Masman, W. Bohmer, T. Knaus and F. G. Mutti, *ChemBioChem*, 2019, **20**, 800–812.
- 46 F. F. Chen, S. C. Cosgrove, W. R. Birmingham, J. Mangas-Sanchez, J. Citoler, M. P. Thompson, G. W. Zheng, J. H. Xu and N. J. Turner, *ACS Catal.*, 2019, **9**, 11813–11818.
- 47 P. N. Scheller, M. Lenz, S. C. Hammer, B. Hauer and B. M. Nestl, *ChemCatChem*, 2015, **7**, 3239–3242.

- 48 T. Huber, L. Schneider, A. Präg, S. Gerhardt, O. Einsle and M. Müller, *ChemCatChem*, 2014, **6**, 2248–2252.
- 49 S. C. Bergmeier, *Tetrahedron*, 2000, **56**, 2561–2576.
- 50 M. Yakoot, *J. Pharmacol. Pharmacother.*, 2012, **3**, 4–6.
- 51 D. J. Ager, I. Prakash and D. R. Schaad, *Chem. Rev.*, 1996, **96**, 835–875.
- 52 Q. Y. Tan, X. Q. Wang, Y. Xiong, Z. M. Zhao, L. Li, P. Tang and M. Zhang, *Angew. Chem., Int. Ed.*, 2017, **56**, 4829–4833.