

## COMMUNICATION

View Article Online  
View Journal



Cite this: DOI: 10.1039/d5ob01304f

Received 9th August 2025,  
Accepted 4th September 2025

DOI: 10.1039/d5ob01304f

rsc.li/obc

## Biocatalytic synthesis of $\beta$ -hydroxy tryptophan regioisomers

Samantha K. Bruffy, Hunter Samuel,<sup>†</sup> Holly A. Weilbaker and Andrew R. Buller \*

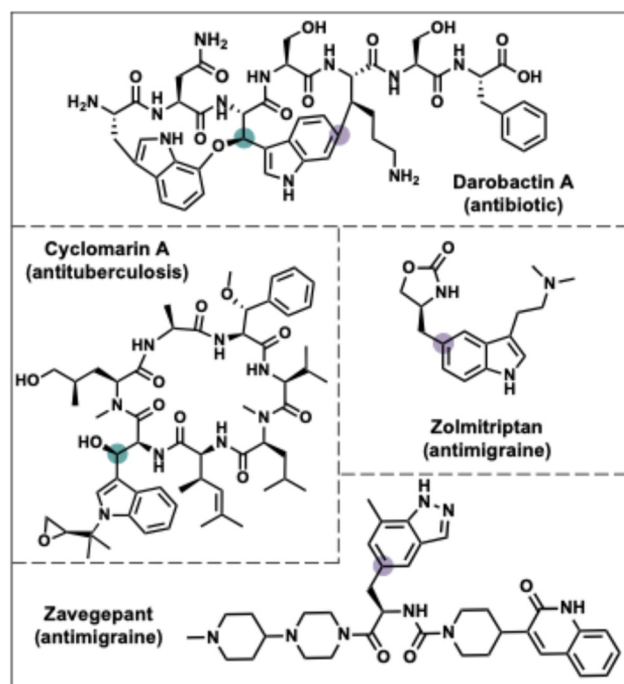
**We report a biocatalytic strategy to access novel  $\beta$ -hydroxy L-tryptophan regioisomers using an L-threonine transaldolase. We show that classical threonine aldolases cannot perform this transformation, highlighting the unique reactivity of transaldolases. By coupling turnover to byproduct removal, high isolated yields of up to 90% were achieved. This reaction enables a multi-enzyme cascade to synthesize diverse non-canonical analogs of L-tryptophan.**

Amino acids are among nature's most versatile chemical building blocks, forming the basis of all proteins and featuring prominently in natural products. Beyond the 20 proteinogenic amino acids, both natural and synthetic non-canonical amino acids (ncAAs) have garnered significant interest for their ability to impart unique physicochemical and structural properties to peptides and proteins.<sup>1,2</sup> Specifically, analogs of L-tryptophan (L-Trp) are attractive due to the electron-rich character of the indole ring. These analogs have been widely employed as fluorescent and biochemical probes,<sup>3</sup> and often serve as precursors to compounds that exhibit diverse pharmacological activities (Fig. 1).<sup>4</sup>

There have been major advances in the use of engineered Trp synthase to access substituted L-Trps from their corresponding indole.<sup>5–9</sup> Analogs bearing a stereogenic methyl group at C $\beta$  have also been synthesized.<sup>10,11</sup> However, current methods cannot tolerate alternative orientations of the indole side chain and non-alkyl groups at C $\beta$ . Access to  $\beta$ -hydroxy ( $\beta$ -OH) L-Trp is desirable as the stereocenter can engage in H-bonding while simultaneously imposing conformational constraints on the sidechain.<sup>12</sup> Modifying the orientation of the indole ring has also been shown to alter molecular properties,<sup>13,14</sup> exemplified by the use of L-Trp regioisomers in FDA-approved antimigraine drugs zolmitriptan and zavegepant.

Despite the appeal of these complementary approaches, access to  $\beta$ -OH L-Trps has remained limited to a single regioisomer, in which the  $\beta$ -hydroxyalkyl chain is installed at the C3 position of the indole ring. The lack of methods to prepare  $\beta$ -OH L-Trp regioisomers represents a gap in the current synthetic repertoire. Traditional synthetic approaches to  $\beta$ -OH L-Trp involve derivatization of the native amino acid scaffold,<sup>15</sup> or nucleophilic addition of indole derivatives into serine-derived aldehydes.<sup>16</sup> Although such methods could be adapted to obtain the regioisomers, the need for multiple purification steps and the use of toxic reagents hinders utility.

Enzymatic strategies for  $\beta$ -OH amino acid synthesis have primarily leveraged pyridoxal 5'-phosphate (PLP)-dependent



**Fig. 1** Bioactive compounds containing  $\beta$ -OH L-Trp residues (blue) or analogs linked via the benzene core of a heterocycle (purple).

Department of Chemistry, The University of Wisconsin-Madison, Madison, WI 53706, USA. E-mail: arbuller@wisc.edu

<sup>†</sup>Present address: School of Dental Medicine, Rutgers University, Newark, NJ 07103, USA.



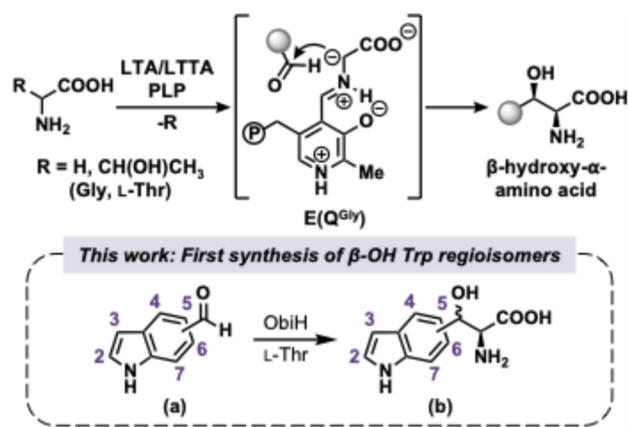


Fig. 2 PLP-dependent aldolases catalyze the formation of  $\beta$ -OH amino acids via an aldol-like addition. This work expands the substrate scope of L-threonine transaldolase ObiH to include indole carboxaldehydes for the synthesis of novel  $\beta$ -OH L-Trp regioisomers.

aldolases, such as L-threonine aldolases (LTAs) and transaldolases (LTTAs), which mediate the formation of carbon-carbon bonds with high stereocontrol.<sup>17,18</sup> These mechanistically related enzymes form a resonance-stabilized glycyI quinonoid nucleophile, ( $E(Q^{Gly})$ ), that performs an aldol addition into diverse carbonyl electrophiles (Fig. 2). LTAs generate a nucleophile by reversible deprotonation of glycine (Gly), necessitating an excess of Gly to overcome unfavorable thermodynamics.<sup>19</sup> In contrast, LTTAs generate the nucleophile exclusively through retro-aldol cleavage of a  $\beta$ -OH amino acid side chain, and protonation of the nucleophile is intrinsically slow.<sup>20</sup> However, neither LTA nor LTTA enzymes have been utilized to access  $\beta$ -OH L-Trps. Expanding the substrate scope of this class of biocatalysts to include indole-containing aldehydes could enable a streamlined and sustainable approach to synthesizing  $\beta$ -OH L-Trp and its regioisomers.

We began our investigation by evaluating whether LTAs or LTTAs exhibit activity on indole carboxaldehydes. LTA from *Thermotoga maritima* (*Tm*LTA) is a well-characterized, thermostable enzyme known to react with a range of aldehydes.<sup>21,22</sup> However, when we conducted analytical-scale reactions with 10 mM indole-5-carboxaldehyde (**5a**), 1.0 M Gly, and 0.1 mol% *Tm*LTA, only trace formation of  $\beta$ -OH L-Trp regioisomer **5b** was observed after 5 h (Fig. 3A, entry 1). In contrast, analogous conditions using ObiH, an LTTA from the obafluorin biosynthesis pathway,<sup>23,24</sup> and 100 mM L-Thr afforded **5b** in 17% yield (Fig. 3A, entry 2). These results reinforce our recent finding that ObiH generates a more versatile nucleophile than *Tm*LTA,<sup>25</sup> and consequently, ObiH was selected for further reaction development.

To assess the substrate scope of ObiH, we tested its activity on a panel of indole carboxaldehydes (Fig. 3B). Consistent with our previous report,<sup>26</sup> ObiH showed no detectable activity with indole-3-carboxaldehyde (**3a**), precluding the formation of the canonical  $\beta$ -OH L-Trp isomer (**3b**). Similarly, indole-2-carboxaldehyde (**2a**) was unreactive under standard conditions.

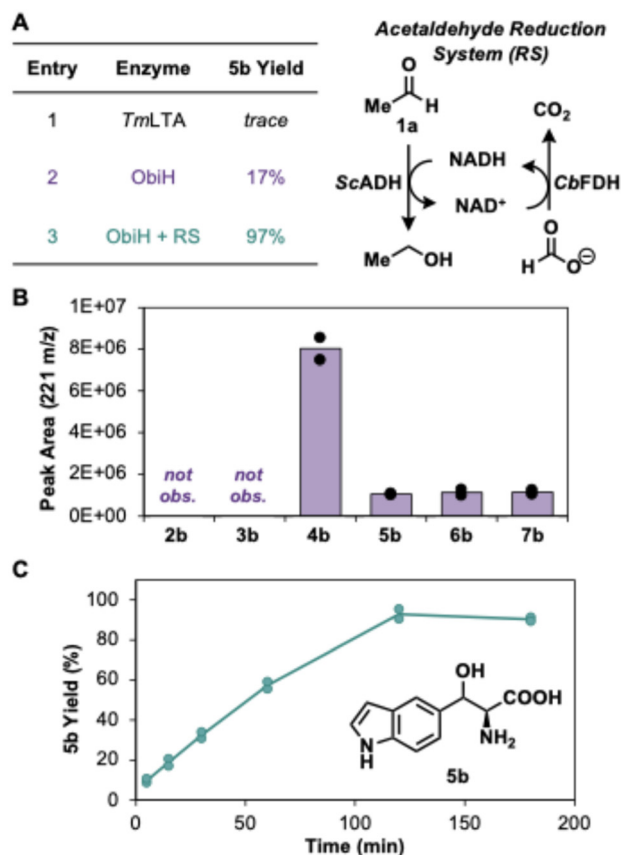


Fig. 3 (A) Effects of different Thr (trans)aldolases on the synthesis of **5b**. Acetaldehyde reduction system (RS) adapted from Xu and co-workers.<sup>28</sup> (B) Analytical substrate scope of ObiH with diverse indole carboxaldehydes. (C) Product formation over time for **5b** after reaction optimization. See SI for individual reaction components.

Positioning the formyl group around the benzene ring, however, afforded amino acid formation. Reactions involving indole-6-carboxaldehyde (**6a**) and indole-7-carboxaldehyde (**7a**) resulted in yields similar to **5b**. Notably, reactions with indole-4-carboxaldehyde (**4a**) afforded near-quantitative yields, highlighting that bulky aryl groups can be well-tolerated by ObiH.

To further increase the yield of **5b–7b**, we explored alternative methods to drive product formation. Acetaldehyde (**1a**) is generated as a by-product of  $E(Q^{Gly})$  formation and is a competitive inhibitor of ObiH, with a dissociation constant ( $K_D$ ) of  $\sim 400 \mu\text{M}$ .<sup>20</sup> Previous studies by us and others have used enzymatic cascades to remove **1a** from LTTA reactions, leading to improvements in yields with benzaldehyde analogs.<sup>27–29</sup> These reduction systems (RS) typically include an alcohol dehydrogenase (ADH), which reduces **1a** into ethanol. A formate dehydrogenase (FDH) regenerates the nicotinamide adenine dinucleotide (NAD) cofactor from formate and releases  $\text{CO}_2$  as a thermodynamic driving force (Fig. 3A). We opted to use commercially available ADH from *Saccharomyces cerevisiae* (*Sc*ADH) and an FDH from *Candida boidinii* (*Cb*FDH) heterologously expressed in *E. coli* prepared as clarified lysate. When we introduced this RS to reactions with **5a**, a  $\sim 6$ -fold improvement in



yield to 97% was observed (Fig. 3A, entry 3). Monitoring reaction progress over time indicated that the reaction approaches quantitative yields within 2 h at 0.1 mol% ObiH and 10 equiv. of L-Thr (Fig. 3C).

With optimized conditions established, we conducted preparative-scale reactions at a 0.5 mmol scale using 25 mM indole carboxaldehydes and 0.01 mol% ObiH (Fig. 4A). Reactions proceeded smoothly and the high aqueous solubility of the  $\beta$ -OH L-Trp regioisomers impeded purification by reverse-phase chromatography. Therefore, *N*-Boc protection was used to enable isolation of **4b–7b** (forming **Boc-4b–7b**). **Boc-4b** was isolated in 56% yield with slight enrichment for the native (2*S*,3*R*) 'syn' diastereomer, corresponding to ~5800 turnovers. Similarly, **Boc-5b** and **Boc-6b** were isolated in 47% and 52% yield, respectively. **Boc-7b** was isolated in 49% yield as an equal mixture of diastereomers. Reactions with **4a–6a** could be driven to >80% yield by increasing catalyst concentration to 0.1 mol% ObiH (Fig. 4A). However, this change had only a marginal effect on reactions with **7a**. In all cases, the dr of the products from the two sets of reaction conditions was unchanged.

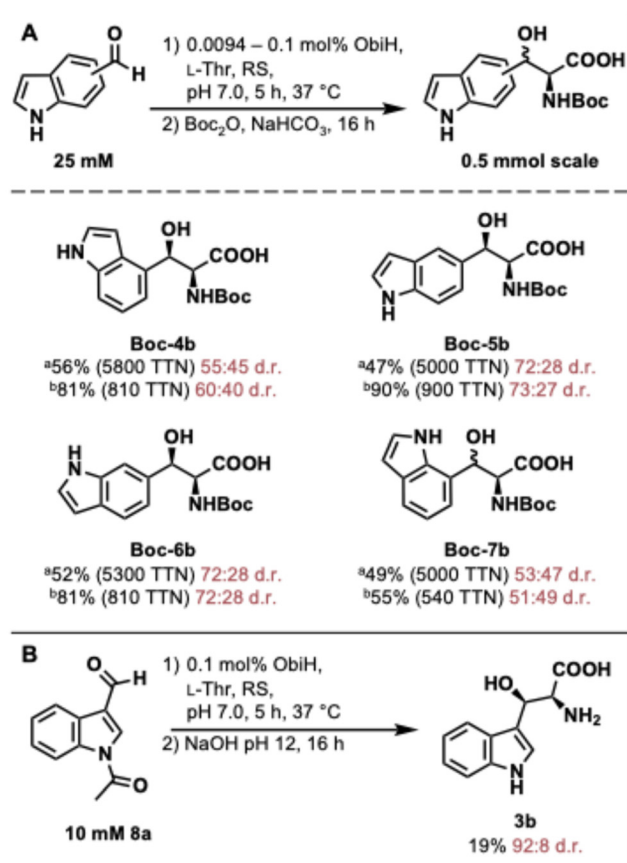
The low diastereoselectivity observed here is consistent with previous findings of ObiH with aryl carboxaldehydes.<sup>26</sup> While

access to both diastereomers may be beneficial for medicinal chemistry applications, enhanced selectivity towards a specific isomer could likely be achieved through a concerted directed evolution campaign.<sup>30</sup> Previously, Xi *et al.* engineered ObiH for higher diastereoselectivity with electron-deficient aryl aldehydes.<sup>30</sup> We tested if the resultant triple mutant (ObiH<sup>TQT</sup>: C57Q, H69T, F70T) also had higher activity with the bulky, electron-rich aldehydes used here. However, when used with **5a**, this variant had only a marginal impact on diastereoselectivity (see SI), indicating that more specific enzyme engineering is necessary to substantially enhance selectivity for these substrates. Despite these challenges, this report represents the first isolation of these  $\beta$ -OH L-Trp regioisomers, enabling access for further study.

Encouraged by our success in isolating the C4–C7 substituted regioisomers, we re-visited ObiH's apparent lack of reactivity with **3a**. We hypothesize that activity is impeded by decreased electrophilicity of the formyl group due to resonance donation of the lone pair electrons of indole N1. If so, this electronic effect may be minimized by modification of N1 with a  $\pi$ -withdrawing group (Fig. 4B). To our delight, reactions with *N*-acetylindole-3-carboxaldehyde (**8a**) resulted in formation of the corresponding amino acid. Preparative-scale synthesis and isolation of *N*<sup>1</sup>-acetyl- $\beta$ -OH L-Trp, however, yielded a mixture of both acetylated and free  $\beta$ -OH L-Trp (**3b**), resulting from spontaneous deacetylation under the reaction conditions. To fully liberate **3b**, we subjected the purified mixture to basic conditions adapted from Previero and coworkers.<sup>31</sup> This process afforded **3b** in 19% yield and good diastereoselectivity. The lower yield may be attributed to many factors, including the low solubility and instability of the acetylated indole aldehyde substrate in aqueous conditions. We envision that a similar approach could be used for **2a**, but it was not tested here.

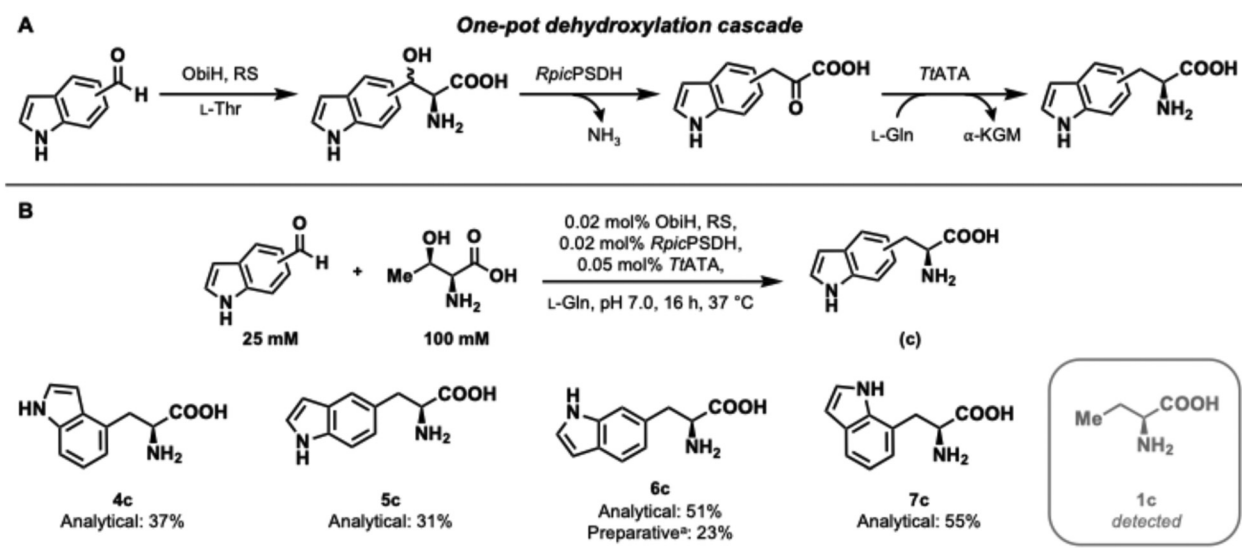
With a route to generate  $\beta$ -OH L-Trp regioisomers established, we next sought to explore their potential as intermediates in biocatalytic cascades. We envisioned the corresponding (non-hydroxylated) L-Trp isomers could be directly accessed through downstream removal of the  $\beta$ -hydroxy stereocenter. Given the general propensity of medicinal chemists to explore effects of regioisomerism, and the limited synthetic routes to L-Trp regioisomers,<sup>13,32–34</sup> this strategy offers a complementary enzymatic approach to existing methods. Recently, multi-enzyme cascades have been developed that remove the  $\beta$ -OH group from LTA and LTTA products.<sup>22,35,36</sup> These cascades rely on a dehydratase to form the corresponding  $\alpha$ -keto acid and either a transaminase or amino acid dehydrogenase to reinstall the  $\alpha$ -amino stereocenter. We opted to use phenylserine dehydratase from *Ralstonia picketti* (RpPSDH) and aromatic L-amino-acid transaminase from *Thermus thermophilus* (TtATA) as we had previously shown these enzymes are expressed to high titer in *E. coli* and tolerate bulky, heterocyclic-aryl substrates (Fig. 5A).<sup>22</sup>

We assessed the one-pot dehydroxylation cascade for synthesis of L-Trp regioisomers (**4c–7c**) on analytical-scale using previously described conditions (Fig. 5B).<sup>22</sup> L-Glutamine (L-Gln) was chosen as the artificial amine donor to drive the transamination reaction.<sup>37</sup> Reactions involving 25 mM alde-



**Fig. 4** Preparative-scale synthesis of (A) C4–C7 substituted and (B) C3 substituted  $\beta$ -OH L-Trp regioisomers. Product purity and dr was assessed via <sup>1</sup>H NMR. <sup>a</sup>The reaction was performed with ~0.1 mol% ObiH. <sup>b</sup>The reaction was performed with ~0.01 mol% ObiH.





**Fig. 5** (A) Proposed enzymatic cascade to access dehydroxylated L-Trp regioisomers. (B) Analytical substrate scope of dehydroxylation cascade.  
<sup>a</sup> The reaction was performed at 0.5 mmol scale with 0.04 mol% ObiH, 0.04 mol% RpicPSDH, 0.05 mol% TtATA, and 200 rpm shaking.

hyde, 0.02 mol% ObiH, 0.02 mol% RpicPSDH, 0.05 mol% TtATA afforded **4c–7c** in 37%, 31%, 51%, and 55% yield, respectively. One possible explanation for the modest yields is background activity of RpicPSDH on L-Thr, which is present in superstoichiometric amounts.<sup>22,36</sup> The dehydration of L-Thr leads to the formation of α-ketobutyric acid, which can compete with the desired product for transamination. Indeed, varying amounts of dehydroxylated L-Thr product, α-homoalanine (**1c**), was detected alongside desired L-Trp products (Fig. S1).

To test the scalability of this cascade, we performed a 0.5 mmol scale reactions with substrate **6a**. We increased ObiH and RpicPSDH loadings to 0.04 mol%, and the reaction was shaken at 200 rpm to improve mass transfer. The desired product **6c** was isolated in 23% yield without the need of a protecting group. While these conditions may serve to access molecules on scales used by medicinal chemists, further optimization of the dehydratase specificity, enzyme stoichiometry, and reaction timing may reduce side-product formation and enhance overall efficiency for access to higher product quantities. Nonetheless, these results highlight the potential of biocatalytic cascades for generating L-Trp isomers and provide a strong foundation for future development.

## Conclusions

In summary, we have developed a biocatalytic platform for the synthesis of β-OH L-Trp regioisomers in high yields from commercially available starting materials. We show how a classical Thr aldolase cannot meet this challenge due to thermodynamic constraints, which can be overcome by using a Thr transaldolase instead. This strategy is compatible with enzymatic cascades, enabling access to dehydroxylated L-Trp deriva-

tives. The modularity of this approach facilitates the generation of structurally diverse non-canonical L-Trp analogs that could be extended to other functionalized indole carboxaldehydes, providing a versatile route to pharmacologically relevant compounds. To our knowledge, this study represents the first report of a biocatalytic strategy for accessing both β-OH and dehydroxylated L-Trp regioisomers.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

All data that support the findings of this study have been included as part of the SI.

Supplementary information is available. See DOI: <https://doi.org/10.1039/d5ob01304f>.

## Acknowledgements

This work was supported by the Office of the Vice Chancellor for Research and Graduate Education at the University of Wisconsin–Madison (UW–Madison) with funding from the Wisconsin Alumni Research Foundation and the National Institutes of Health (NIH) R35GM153276 to A. R. B. Through acceptance of NIH federal funding, the NIH has been given a right to make this manuscript publicly available in PubMed Central upon the Official Date of Publication. We thank Merck & Co., Inc. and Dr J. McIntosh for the generous gift of reagents and fruitful discussion. The Bruker AVANCE III-500 NMR spectrometers were purchased by the UW–Madison Department of



Chemistry with a portion of a generous gift from P. J. and M. M. Bender. We thank the remaining Buller buddies for their support.

## References

- 1 M. A. T. Blaskovich, *J. Med. Chem.*, 2016, **59**, 10807–10836.
- 2 J. L. Hickey, D. Sindhikara, S. L. Zultanski and D. M. Schultz, *ACS Med. Chem. Lett.*, 2023, **14**, 557–565.
- 3 R. W. Sinkeldam, N. J. Greco and Y. Tor, *Chem. Rev.*, 2010, **110**, 2579–2619.
- 4 Y. Imai, K. J. Meyer, A. Iinishi, Q. Favre-Godal, R. Green, S. Manuse, M. Caboni, M. Mori, S. Niles, M. Ghiglieri, C. Honrao, X. Ma, J. J. Guo, A. Makriyannis, L. Linares-Otoya, N. Böhringer, Z. G. Wuisan, H. Kaur, R. Wu, A. Mateus, A. Typas, M. M. Savitski, J. L. Espinoza, A. O'Rourke, K. E. Nelson, S. Hiller, N. Noinaj, T. F. Schäberle, A. D'Onofrio and K. Lewis, *Nature*, 2019, **576**, 459–464.
- 5 M. J. Corr, D. R. M. Smith and R. J. M. Goss, *Tetrahedron*, 2016, **72**, 7306–7310.
- 6 A. R. Buller, S. Brinkmann-Chen, D. K. Romney, M. Herger, J. Murciano-Calles and F. H. Arnold, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 14599–14604.
- 7 D. K. Romney, J. Murciano-Calles, J. E. Wehrmüller and F. H. Arnold, *J. Am. Chem. Soc.*, 2017, **139**, 10769–10776.
- 8 A. D. McDonald, P. M. Higgins and A. R. Buller, *Nat. Commun.*, 2022, **13**, 5242.
- 9 P. J. Almhjell, C. E. Boville and F. H. Arnold, *Chem. Soc. Rev.*, 2018, **47**, 8980–8997.
- 10 M. Herger, P. Van Roye, D. K. Romney, S. Brinkmann-Chen, A. R. Buller and F. H. Arnold, *J. Am. Chem. Soc.*, 2016, **138**, 8388–8391.
- 11 C. E. Boville, R. A. Scheele, P. Koch, S. Brinkmann-Chen, A. R. Buller and F. H. Arnold, *Angew. Chem., Int. Ed.*, 2018, **57**, 14764–14768.
- 12 L. Nicke, P. Horx, R. Müller, S. Els-Heindl and A. Geyer, *ChemBioChem*, 2021, **22**, 330–335.
- 13 Y. Nakata, J. Fu, H. Itoh, S. Panthee, H. Hamamoto, K. Sekimizu and M. Inoue, *Chem. – Eur. J.*, 2023, **29**, e202301225.
- 14 X. Han, R. L. Civiello, C. M. Conway, D. A. Cook, C. D. Davis, R. MacCi, S. S. Pin, S. X. Ren, R. Schartman, L. J. Signor, G. Thalody, K. A. Widmann, C. Xu, P. V. Chaturvedula, J. E. MacOr and G. M. Dubowchik, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 4723–4727.
- 15 D. Crich and A. Banerjee, *J. Org. Chem.*, 2006, **71**, 7106–7109.
- 16 P. Barbie and U. Kazmaier, *Org. Biomol. Chem.*, 2015, **13**, 9267–9275.
- 17 M. A. Jones, N. D. Butler, S. R. Anderson, S. A. Wirt, I. Govil, X. Lyu, Y. Fang and A. M. Kunjapur, *Commun. Biol.*, 2023, **6**, 929–912.
- 18 K. Fesko, *Appl. Microbiol. Biotechnol.*, 2016, **100**, 2579–2590.
- 19 T. Kimura, V. P. Vassilev, G. J. Shen and C. H. Wong, *J. Am. Chem. Soc.*, 1997, **119**, 11734–11742.
- 20 P. Kumar, A. Meza, J. M. Ellis, G. A. Carlson, C. A. Bingman and A. R. Buller, *ACS Chem. Biol.*, 2021, **16**, 86–95.
- 21 S. F. Beaudoin, M. P. Hanna, I. Ghiviriga and J. D. Stewart, *Enzyme Microb. Technol.*, 2018, **119**, 1–9.
- 22 S. H. Young, J. S. Andon, J. Lim, M. C. Franke, D. J. Weix, T. Wang and A. R. Buller, *ChemCatChem*, 2025, **17**, e202401958.
- 23 T. A. Scott, D. Heine, Z. Qin and B. Wilkinson, *Nat. Commun.*, 2017, **8**, 15935–15946.
- 24 J. E. Schaffer, M. R. Reck, N. K. Prasad and T. A. Wencewicz, *Nat. Chem. Biol.*, 2017, **13**, 737–744.
- 25 S. K. Bruffy, A. Meza, J. Soler, T. J. Doyon, S. H. Young, J. Lim, K. G. Huseeth, P. H. Willoughby, M. Garcia-Borràs and A. R. Buller, *Nat. Chem.*, 2024, **16**, 2076–2083.
- 26 T. J. Doyon, P. Kumar, S. Thein, M. Kim, A. Stitgen, A. M. Grieger, C. Madigan, P. H. Willoughby and A. R. Buller, *ChemBioChem*, 2022, **23**, e202100577.
- 27 L. Xu, L.-C. Wang, B.-M. Su, X.-Q. Xu and J. Lin, *Bioresour. Technol.*, 2020, **310**, 123439–123448.
- 28 L. Xu, L. C. Wang, B. M. Su, X. Q. Xu and J. Lin, *Bioorg. Chem.*, 2021, **110**, 104766.
- 29 Y. Xiu, G. Xu and Y. Ni, *Syst. Microbiol. Biomanuf.*, 2022, **2**, 705–715.
- 30 Z. Xi, J. Rao, X. Zhang, Z. Liu, M. Zheng, L. Li, W. Zhang, Y. Xu and R. Zhang, *ACS Catal.*, 2024, **14**, 10462–10474.
- 31 A. Previero, M. A. Coletti-Previero and J. C. Cavadore, *Biochim. Biophys. Acta*, 1967, **147**, 453–461.
- 32 S. Dachwitz, B. Scharkowski and N. Sewald, *Chem. – Eur. J.*, 2021, **27**, 18043–18046.
- 33 P. R. Carlier, P. C. H. Lam and D. M. Wong, *J. Org. Chem.*, 2002, **67**, 6256–6259.
- 34 R. C. Glen, G. R. Martin, A. P. Hill, R. M. Hyde, P. M. Woollard, J. A. Salmon, J. Buckingham and A. D. Robertson, *J. Med. Chem.*, 1995, **38**, 3566–3580.
- 35 W. Song, J. H. Wang, J. Wu, J. Liu, X. L. Chen and L. M. Liu, *Nat. Commun.*, 2018, **9**, 3818.
- 36 S. R. Anderson, M. R. Gopal, A. P. Spangler, M. A. Jones, D. R. Wyllis and A. M. Kunjapur, *ChemCatChem*, 2025, **17**, e202401979.
- 37 E. Heuson, F. Charmantray, J. L. Petit, V. de Berardinis and T. Gefflaut, *Adv. Synth. Catal.*, 2019, **361**, 778–785.

