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Scalable biocatalytic oxygenation of aliphatic amine derivatives using a class I unspecific peroxygenase

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artUPO is revealed as an effective biocatalyst for the regioselective oxygenation of cycloalkylamines to form cyclic amino ketones on preparative scale. Furthermore, amino alcohols can be formed via the selective C–H oxygenation of isopropyl-tethered amine derivatives. Enantiomerically enriched amino alcohols are also accessible using a kinetic resolution approach.

N-Heterocycles feature prominently in biologically active compounds and FDA approved pharmaceuticals.^{1–3} New methods to prepare oxygenated N-heterocycles *via* oxidative processes are therefore of value, both for organic synthesis and the study of metabolites. In this context, the selective oxygenation of saturated N-heterocycles presents a significant challenge, as they usually have several potential reaction sites with similar susceptibility to oxygenation.

Biocatalysis has thus been investigated for its utility in preparing oxygenated N-heterocycles *via* C–H oxygenation. Early studies at Upjohn in the late 1960s focused on the use of whole-cell preparations of filamentous fungi such as *Beauveria bassiana* (formerly *Sporotrichum sulfurescens*),^{4–6} this work was further developed by the groups of Roberts/Willetts⁷ and Flitsch.⁸ In these cases, various N-heterocycles were hydroxylated at different positions, assisted by derivatisation of the N atom, which was thought to assist substrate recognition by the cytochromes P450 (P450s) in the cells. However, in many cases mixtures of products formed, presumably as a consequence of the activity of multiple P450s within the organism (*e.g.* Scheme 1A, eqn (1)). The heterologous expression of isolated microbial P450s has allowed greater specificity to be achieved; *e.g.* Li/Witholt and co-workers exploited the P450_{Pyr} from *Sphingomonas* sp. HXN-200 for the regio- and enantio-selective hydroxylation of pyrrolidinones (*e.g.* Scheme 1A, eqn (2)).^{9,10} More recently, Wong and co-workers showed that variants of

the well-studied P450 BM3 from *Bacillus megaterium* deliver selectively hydroxylated products of a range of N-heterocycles with high conversions and enantioselectivity (*e.g.* Scheme 1A, eqn (3)).^{11,12}

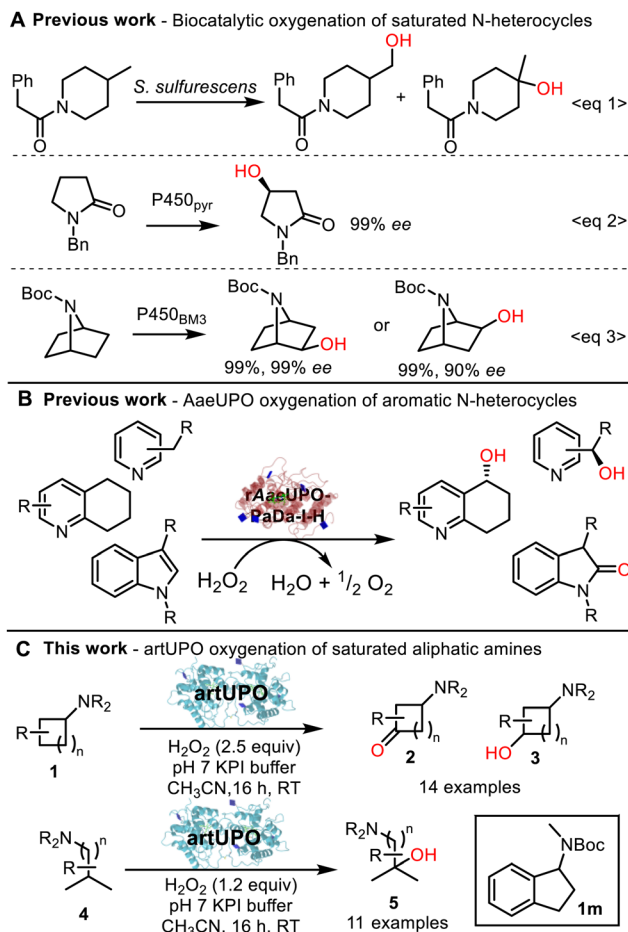
Despite their high selectivity, P450s present challenges; for example, the requirement for an exogenous reductant (NADPH) and electron transport proteins or domains to channel reducing equivalents from the cofactor to the heme to create the reactive oxygenating intermediate compound I.¹³ While these problems can be addressed to an extent through the use of whole cell catalysts, the use of microbe-free biocatalysts for selective oxygenations would be advantageous.

In this context, unspecific peroxygenases (UPOs) have assumed a role as alternatives to P450s for scalable biocatalytic oxygenations.^{14–16} UPOs are heme oxygenases that are secreted by fungi and easily prepared at scale using heterologous expression hosts such as *Pichia pastoris*.¹⁷ They depend only upon the addition of hydrogen peroxide (H₂O₂) to generate compound I and can consequently be used as off-the-shelf lyophilised powders, obviating the need for facilities for the growth and containment of microorganisms. Following early demonstrations of their activity on a variety of small molecules, including aliphatics,¹⁸ aromatic hydrocarbons¹⁹ and common drugs,²⁰ their suitability for scalable oxygenations was recently demonstrated with a multi-hectogram scale oxygenation of cyclohexane.²¹ In previous reports from our groups, we have shown that UPOs can carry out selective oxygenations of terpenes,²² toluenes²³ and allylic alcohols²⁴ on up to 1 g scales, and are also capable of ‘promiscuous’ reactions such as halogenations,²⁵ Achmatowicz reactions²⁶ and even cyclopropanations.²⁷

In our previous work we also focused on the oxygenation of aromatic N-heterocycles, such as pyridine and indole derivatives, using a lyophilized preparation of a UPO variant from *Agrocybe aegerita* (rAaeUPO-PaDa-I-H, Scheme 1B).²⁸ This new report focuses on extending the capability of UPOs to perform scalable oxygenations of non-aromatic cyclic and acyclic aliphatic amine derivatives, using the class I UPO, artUPO (Scheme 1C).²⁹ First, the regioselective biocatalytic conversion

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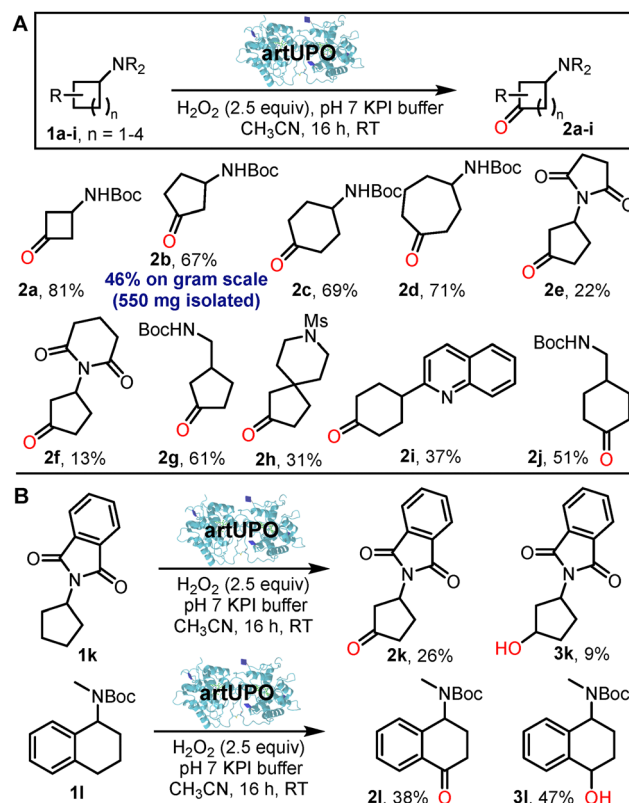
Scheme 1 UPO mediated oxygenation reactions of N-heterocycles. (A) Oxygenation of saturated N-heterocycles. (B) Oxygenation of aromatic N-heterocycles. (C) Oxygenation of saturated aromatic amines.

of cyclic aliphatic amines **1** into cyclic ketones **2** and alcohols **3** was explored. The selective C–H oxygenation of isopropyl groups on amine derivatives **4** was also examined, enabling the formation of tertiary alcohols **5**. Finally, the generation of enantiomerically enriched amino alcohols has been demonstrated, using a kinetic resolution approach from chiral amine **1m**.

We began by exploring the oxygenation of cyclic aliphatic amines, with initial experiments performed using cyclobutylamine derivatives (e.g. *tert*-butyl cyclobutylcarbamate **1a**). All biotransformations were performed in pH 7 KPi aqueous buffer, with 20% acetonitrile and H₂O₂ as stoichiometric oxidant, as similar conditions have been shown to enable a variety of UPO-mediated oxygenations.^{22–28} Two UPOs, the class I artUPO²⁹ and class II rAaeUPO–PaDa-I-H³⁰ were screened for activity initially, but only artUPO displayed the desired activities and was subsequently used for all biotransformations. This behaviour is consistent with previous observations of terpene biotransformations²² in which the ability of artUPO to accept larger substrates was attributed to it having a less sterically restricted active site²⁹ than rAaeUPO–PaDa-I-H. artUPO was prepared by fermentation in *Pichia pastoris* and added to reactions as the crude liquid secretate as previously

described.^{22,27,29} Different H₂O₂ loadings (1.0–2.5 equivalents) and eight cyclobutylamine substrates with different amine protecting groups/substituents were also screened (see SI Section S2.13). Superior conversion was observed using Boc-protected cyclobutylamine **1a** as substrate. Notably, in contrast to our earlier work on N-heterocycle oxygenations,²⁸ the initial results provided little evidence of oxygenation to form alcohols. Instead, cyclobutanone derivatives (e.g. **2a**) formed as the major product; ketone formation presumably arises from initial oxygenation to form an alcohol, followed by fast oxidation *in situ*.²³ Amino ketone derivatives are important building blocks in organic synthesis, and commonly found in bioactive molecules.^{31,32} We therefore decided to target the synthesis of amino ketone products, and found that using 2.5 equivalents of H₂O₂ promoted the desired oxygenation most effectively.

Attention now turned to performing preparative scale biotransformations (Scheme 2). First, *tert*-butyl cyclobutylcarbamate **1a** was converted into amino ketone **2a** with 81% isolated yield as a single regioisomer. Homologous 5-, 6- and 7-membered ring systems were also transformed, with amino ketones **2b–2d** isolated in good yields, all as single regioisomers. Reactions were typically performed using 0.2 mmol of the amine **1**, but to demonstrate their scalability, a 1-gram transformation of cyclopentyl derivative **1b** was performed and delivered 550 mg of product **2b**.



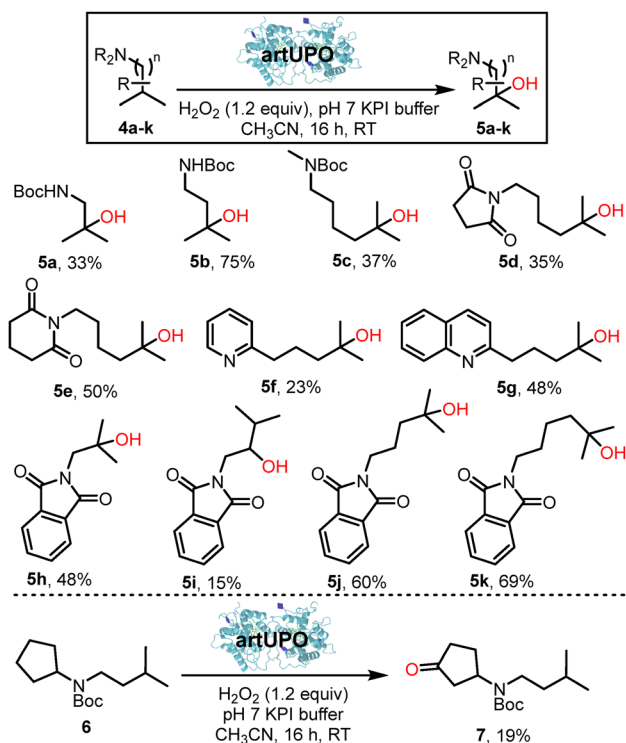
Scheme 2 Biocatalytic synthesis of cyclic amino ketones via the artUPO mediated oxygenation of cyclic amine derivatives to form (A) cyclic ketones and (B) cyclic ketones and alcohols. See SI Section S2.10 for a general synthetic protocol.



Cyclopentanone formation was also achieved using substrates with cyclic imide substituents, albeit in reduced yields (**2e** and **2f**). Syntheses of cyclopentanone and cyclohexanone derivatives were also demonstrated on substrates containing more remote NBoc groups (to form **2g** and **2j**), a spirocyclic *N*-mesyl system (**2h**) and a quinoline (**2i**). In all cases, the ketone was obtained as a single regioisomer, with oxygenation taking places at the methylene furthest from the substituted amine. Substrates **1k** and **1l** (Scheme 2B) were the only systems in which amino alcohol products (**3k** and **3l**) were formed alongside the amino ketones (**2k** and **2l**).

We next explored the oxygenation of isopropyl groups on linear aliphatic substrates to form amino alcohols. In our previous work, we showed that UPOs can promote selective and gram-scale C–H oxygenation of the isopropyl group of menthol.²² In this study, we explored whether preparative scale oxygenation could be performed on a wider array of isopropyl-containing substrates, containing amine derivatives or aza-heterocycles. Similar reaction conditions were used as for ketone formation above, but as only a single oxidation event was needed in this series, H₂O₂ loading was lowered to 1.2 equivalents accordingly (Scheme 3).

First, C–H oxygenation of the isopropyl group of *N*-Boc substituted linear alkanes was tested, and tertiary alcohol products were formed in 33–75% isolated yield (**5a–5c**). Similarly, selective oxygenation was also achieved using substrates substituted with cyclic imides (**5d** and **5e**), pyridine (**5f**) and quinoline (**5g**) groups. Interestingly, neither benzylic oxidation,

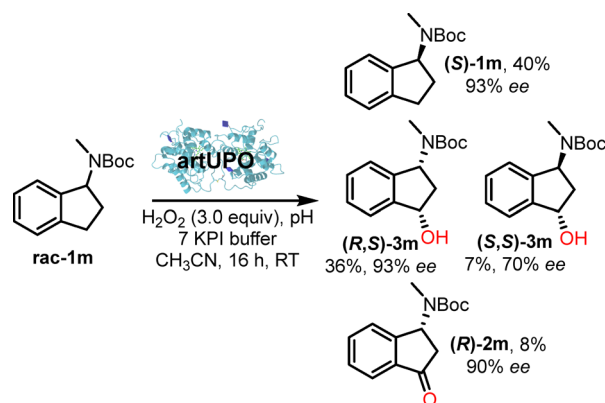


Scheme 3 Biocatalytic synthesis of amino alcohol derivatives via artUPO mediated oxygenation of isopropyl-tethered amines. See SI Section S2.11 for a general synthetic protocol.

nor *N*-oxide formation, were competing reactions for either the pyridine or quinoline substrates. Four homologous phthalimide-containing substrates were then tested, and in this series, the tertiary alcohol products were obtained in three out of four cases (**5h**, **5j** and **5k**), in 48–69% isolated yields. Isopentyl substrate **4i** was anomalous; lower conversion was observed, and unexpectedly, a secondary alcohol product **5i** was formed, *via* C–H oxygenation of the methylene adjacent to the isopropyl. Finally, substrate **6**, containing both a cyclic alkane and an isopropyl group, was tested. The yield was relatively low in this case, but the only product isolated was cyclopentanone **7**, indicating preferential cyclopentane oxygenation over the isopropyl group.

One of the most powerful features of biocatalysis is the ability to generate products with high enantioselectivity. As most products generated in this study are achiral this ability was not utilised hitherto. However, benzannulated substrate **rac-1m** is an interesting exception, being both chiral (racemic) and also possessing a benzylic site available for oxygenation. UPOs are well-capable of effecting the chemo- and enantioselective oxygenation of benzylic C–H bonds to form chiral alcohols; indeed, ethyl benzene oxygenation is commonly used to benchmark the activity and enantioselectivity of UPOs.³³ We therefore reasoned that oxygenation to form enantiomerically enriched amino alcohols should be possible with this substrate, *via* a kinetic resolution approach. Following optimisation (targeting $\approx 50\%$ conversion of **rac-1m**, see SI, Section S2.12), suitable conditions were found and used in the preparative scale kinetic resolution of **rac-1m** (Scheme 4).

artUPO has a clear preference to accept the (*R*)-enantiomer of **1m**, evidenced by the fact that the unreacted starting material was recovered in 40% isolated yield and enriched to 93% ee in favour of the (*S*)-enantiomer (**S-1m**). The absolute stereochemistry of (*S*)-**1m** was assigned by comparison to literature optical rotation data.^{34,35} As hoped, artUPO mediated oxygenation to form amino alcohol derivatives was achieved, with (*R,S*)-**3m** and (*S,S*)-**3m** isolated as an inseparable mixture in 43% combined yield, and with 93% ee and 70% ee respectively. The relative stereochemical assignments of (*R,S*)-**3m** (*cis*) and (*S,S*)-**3m** (*trans*) were made based on ¹H NMR data; literature data show a clear trend in which the benzyl alcohol



Scheme 4 artUPO mediated enantioselective oxygenation of **rac-1m** via a kinetic resolution approach.



C–H signal appears at higher chemical shift for the *trans*-isomer than the *cis*- in a series of closely related compounds, with the same trend seen in our products.³⁶ A small amount of over-oxidation was also observed, with (*R*)-**2m** isolated in 8% yield and 90% ee. Reduction of (*R*)-**2m** with NaBH₄ resulted in clean conversion into (*R,S*)-**3m**, confirmed by chiral HPLC (see SI Section S4). This result confirmed that both (*R*)-**2m** and (*R,S*)-**3m** were derived from the same enantiomer of **1m**, and given their 44% combined yield, their precursor logically must be (*R*)-**1m**, thus enabling the assignment of the absolute stereochemistry of (*R*)-**2m** and (*R,S*)-**3m**. The absolute stereochemical assignment of the minor alcohol product (*S,S*)-**3m** is less clear. We are confident in assigning its relative stereochemistry to be *trans* as outlined above. The absolute assignment of (*S,S*)-**3m** is more tentative, based partly on the overall mass balance of the kinetic resolution, but also based on the expectation that the UPO would more likely promote benzylic oxygenation with the same sense of stereochemistry for both enantiomers of *rac*-**1m**, and form the (*S*)-alcohol product in each case.

In summary, the utility of artUPO to perform preparative scale C–H oxygenation reactions has been extended to encompass the synthesis of amino ketone and amino alcohol derivatives from a range of nitrogen-containing hydrocarbons. Thus, the ever-growing toolbox of synthetically useful UPO-mediated transformations is expanded, further enhancing the reputation of UPOs as biocatalysts with great potential for the selective, safe and scalable oxidation reactions.

JL designed experiments and JC, WPU and GG designed and supervised experiments. JL and KC performed experiments. WPU and GG wrote the manuscript with contributions from all authors.

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Conflicts of interest

There are no conflicts to declare.

Data availability

Experimental procedures, optimisation details, spectroscopic data, HPLC data and NMR spectra.

Supplementary information is available. See DOI: <https://doi.org/10.1039/d5cc03882k>

Notes and references

- C. M. Marshall, J. G. Federice, C. N. Bell, P. B. Cox and J. T. Njardarson, *J. Med. Chem.*, 2024, **67**, 11622–11655.
- E. Vitaku, D. T. Smith and J. T. Njardarson, *J. Med. Chem.*, 2014, **57**, 10257–10274.
- P. Das, M. D. Delost, M. H. Qureshi, D. T. Smith and J. T. Njardarson, *J. Med. Chem.*, 2019, **62**, 4265–4311.
- R. A. Johnson, M. E. Herr, H. C. Murray and G. S. Fonken, *J. Org. Chem.*, 1968, **33**, 3187–3195.
- R. A. Johnson, M. E. Herr, H. C. Murray, L. M. Reineke and G. S. Fonken, *J. Org. Chem.*, 1968, **33**, 3195–3201.
- R. A. Johnson, H. C. Murray, L. M. Reineke and G. S. Fonken, *J. Org. Chem.*, 1969, **34**, 2279–2284.
- N. Floyd, F. Munyemana, S. M. Roberts and A. J. Willetts, *J. Chem. Soc., Perkin Trans. 1*, 1993, 881–882.
- S. J. Aitken, G. Grogan, C. S.-Y. Chow, N. J. Turner and S. L. Flitsch, *J. Chem. Soc., Perkin Trans. 1*, 1998, 3365–3370.
- Z. Li, H.-J. Feiten, D. Chang, W. A. Duetz, J. B. van Beilen and B. Witholt, *J. Org. Chem.*, 2001, **66**, 8424–8430.
- W. Zhang, W. L. Tang, Z. Wang and Z. Li, *Adv. Synth. Catal.*, 2010, **352**, 3380–3390.
- Y. Li and L. L. Wong, *Angew. Chem., Int. Ed.*, 2019, **58**, 9551–9555.
- Y. Zhang, Z. Xiong, Y. Li, M. Wilson, K. E. Christensen, E. Jaques, P. Hernández-Lladó, J. Robertson and L. L. Wong, *Nat. Synth.*, 2022, **1**, 936–945.
- J. Rittle and M. T. Green, *Science*, 2010, **330**, 933–937.
- Y. Wang, D. Lan, R. Durrani and F. Hollmann, *Curr. Opin. Chem. Biol.*, 2017, **37**, 1–9.
- M. Hobisch, D. Holtmann, P. Gomez de Santos, M. Alcalde, F. Hollmann and S. Kara, *Biotechnol. Adv.*, 2021, **51**, 107615.
- D. T. Monterrey, A. Menés-Rubio, M. Keser, D. Gonzalez-Perez and M. Alcalde, *Curr. Opin. Green Sustainable Chem.*, 2023, **41**, 100786.
- P. Molina-Espeja, S. Ma, D. M. Mate, R. Ludwig and M. Alcalde, *Enzyme Microb. Technol.*, 2015, **73–74**, 29–33.
- S. Peter, M. Kinne, X. Wang, R. Ullrich, G. Kayser, J. T. Groves and M. Hofrichter, *FEBS J.*, 2011, **278**, 3667–3675.
- P. Molina-Espeja, M. Cañellas, F. J. Plou, M. Hofrichter, F. Lucas, V. Guallar and M. Alcalde, *ChemBioChem*, 2016, **17**, 341–349.
- P. Gomez de Santos, F. V. Cervantes, F. Tieves, F. J. Plou, F. Hollmann and M. Alcalde, *Tetrahedron*, 2019, **75**, 1827–1831.
- T. Hilberath, R. van Oosten, J. Victoria, H. Brasselet, M. Alcalde, J. M. Woodley and F. Hollmann, *Org. Proc. Res. Dev.*, 2023, **27**, 1384–1389.
- B. Melling, T. Mielke, A. C. Whitwood, T. J. C. O’Riordan, N. Mulholland, J. Cartwright, W. P. Unsworth and G. Grogan, *Chem. Catal.*, 2024, **4**, 100889.
- B. Pogrányi, T. Mielke, J. Cartwright, W. P. Unsworth and G. Grogan, *ChemCatChem*, 2024, **16**, e202400702.
- J. Li, C. Duran, B. Pogrányi, K. A. S. Cornish, J. Cartwright, S. Osuna, W. P. Unsworth and G. Grogan, *Angew. Chem., Int. Ed.*, 2025, **64**, e202422241.
- V. Barber, T. Mielke, J. Cartwright, A. Díaz-Rodríguez, W. P. Unsworth and G. Grogan, *Chem. – Eur. J.*, 2024, **30**, e202401706.
- B. Pogrányi, T. Mielke, A. Díaz Rodríguez, J. Cartwright, W. P. Unsworth and G. Grogan, *Org. Biomol. Chem.*, 2024, **22**, 6149–6155.
- J. Li, K. A. S. Cornish, B. Pogrányi, B. Melling, J. Cartwright, W. P. Unsworth and G. Grogan, *Org. Biomol. Chem.*, 2025, **23**, 4897–4901.
- B. Pogrányi, T. Mielke, A. Díaz-Rodríguez, J. Cartwright, W. P. Unsworth and G. Grogan, *Angew. Chem., Int. Ed.*, 2023, **62**, e202214759.
- W. X. Q. Robinson, T. Mielke, B. Melling, A. Cuetos, A. Parkin, W. P. Unsworth, J. Cartwright and G. Grogan, *ChemBioChem*, 2023, **24**, e202200558.
- H. E. Bonfield, K. Mercer, A. Diaz-Rodríguez, G. C. Cook, B. S. J. McKay, P. Slade, G. M. Taylor, W. X. Ooi, J. D. Williams, J. P. M. Roberts, J. A. Murphy, L. Schmermund, W. Kroutil, T. Mielke, J. Cartwright, G. Grogan and L. J. Edwards, *ChemPhotoChem*, 2020, **4**, 45–51.
- L. A. T. Allen, R.-C. Raclea, P. Natho and P. J. Parsons, *Org. Biomol. Chem.*, 2021, **19**, 498–513.
- W.-W. Ding, Z.-Y. He, M. Sayed, Y. Zhou, Z.-Y. Han and L.-Z. Gong, *Nat. Synth.*, 2024, **3**, 507–516.
- M. Kluge, R. Ullrich, K. Scheibner and M. Hofrichter, *Green Chem.*, 2012, **14**, 440–446.
- C. K. Blasius, N. F. Heinrich, V. Vasilenko and L. H. Gade, *Angew. Chem., Int. Ed.*, 2020, **59**, 15974–15977.
- V. N. Wakchaure, P. S. J. Kaib, M. Leutzsch and B. List, *Angew. Chem., Int. Ed.*, 2015, **54**, 11852–11856.
- F. Luan, M. N. D. S. Cordeiro, N. Alonso, X. García-Mera, O. Caamaño, F. J. Romero-Duran, M. Yañez and H. González-Díaz, *Bioorg. Med. Chem.*, 2013, **21**, 1870–1879.

