A biocatalytic approach to angiopterlactone B based on a chemo-inspired artificial in vitro metabolism

Alexander F. Kiefer,[+[b,c]] Yu-Chang Liu,[+[a,b]] Rebecca Gummerer,[[b]] Christina Jäger,[[a,b]] Jan Deska[[a,b]]*

[a] Dr. Yu-Chang Liu, MSc Christina Jäger, Prof. Dr. Jan Deska
University of Helsinki, Department of Chemistry, A.I. Virtasen aukio 1, 00560 Helsinki (Finland)
E-mail: jan.deska@helsinki.fi
Web: www.deskalab.com

[b] Dr. Alexander F. Kiefer, Dr. Yu-Chang Liu, MSc Rebecca Gummerer, MSc Christina Jäger, Prof. Dr. Jan Deska
Aalto University, Department of Chemistry, Kemistintie 1, 02150 Espoo (Finland)

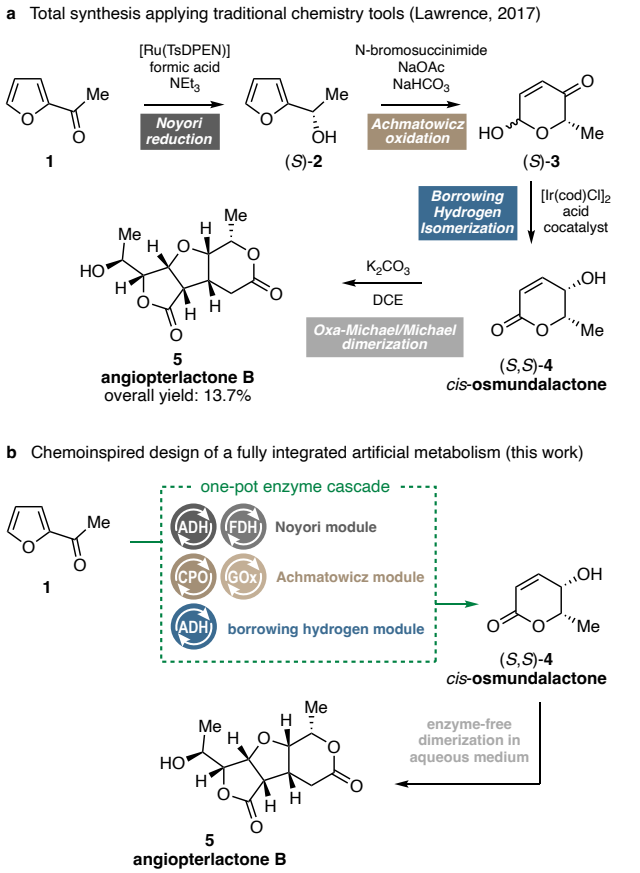
[c] Dr. Alexander F. Kiefer
Department of Chemistry, UF Scripps Biomedical Research, University of Florida, Jupiter, Florida 33458 (United States)

[+] These authors contributed equally to this work

Supporting information for this article is given via a link at the end of the document.

Abstract: *Nature's way to construct highly complex molecular entities with virtue as part of biosynthetic pathways is unmatched by any chemical synthesis. Yet, relying on a cascade of native enzymatic transformations to achieve a certain target structure, biosynthesis is also significantly limited in its scope. In this work, non-natural biocatalytic modules are successfully implemented into an artificial metabolism, combining the benefits of traditional retrosynthesis with the elegance and efficacy of biosynthetic networks. In a highly streamlined process, a total synthesis of the tricyclic angiopterlactone B is achieved operating entirely in an aqueous environment while relying on enzymes as reaction mediators.*

Enzymatic catalysis is nowadays often considered as the optimal template for the development of chemical processes, and imitations of the natural activation modes have led to numerous successful and broadly applied methodologies in the fields of organic and pharmaceutical chemistry.[1-3] Likewise, biocatalysis itself has been established as a powerful tool for the synthesis of organic compounds and today we can rely on an ever-growing toolbox that assists synthetic chemists to develop routes based on a biocatalytic retrosynthesis.[4] Nevertheless, two centuries of chemical research have not only led to biomimetic approaches to solve synthetic challenges but also created a parallel universe of powerful molecular transformations. In an attempt to reverse the biomimetics approach by imitating chemistry with biological tools, more recently, various research groups around the globe have started to investigate a portfolio of non-natural enzymatic reactions such as C-H bond insertions,[5] metathesis[6] and cyclopropanations[7]. Beyond the intriguing scientific question itself, the use of abiotic enzyme modules in enzyme cascades and thus the design of artificial pathways based on non-natural reactions offers the prospect to bring the biochemical machinery closer to the actual demands in academia and chemical industry. By arranging several enzymes in series, the overall efficiency of a multistep synthesis sequence can thus be significantly improved, since intermediate purification steps can be avoided, especially when dealing with unstable derivatives, while saving resources and time.[8-10] The specific aim of this study is showcase the enormous synthetic potential of implementing the extended reaction portfolio provided by abiotic biocatalysis modules, by presenting a streamlined multi-enzyme cascade towards the total synthesis of the structurally unique angiopterlactone B (Scheme 1).



Scheme 1. Total synthesis of angiopterlactone B: **a** traditional synthetic chemistry vs **b** integrated biocascade design.

The angiopterlactones were isolated during a screening campaign of bioactive plant compounds from the rhizome of Angiopteris caudatiformis, a fern species that grows primarily in Asia.[11] The tricyclic core structure of angiopterlactone B comprises seven stereogenic centers and may potentially be derived from the co-isolated minor compound angiopterlactone A through an intramolecular Michael addition. Furthermore, their bioactivity profile has not yet been fully elucidated, thus a flexible synthetic route to the natural product and its analogues would be highly desirable regarding structure-activity relationship studies to identify potential targets of this unexplored compound class.

Recently, the group of Lawrence introduced the first successful total synthesis of one of the lactones utilizing traditional synthetic transformations (Scheme 1a).[12,13] Taking the Lawrence route as an effective and highly elegant chemical blueprint, we present herein a streamlined, chemo-inspired artificial metabolism to convert simple biogenic furan to the highly complex target lactone 5 (Scheme 1b). In addition to native enzymatic conversions, a series of artificial modules for completely abiotic transformations, that is, an Achmatowicz ring expansion[14-17] and a stereoselective borrowing hydrogen redox isomerization,[18] are combined in an integrated process. As our investigation also underlines that the intermediate osmundalactone can be directly converted to the tricyclic angiopterlacone B in the aqueous reaction medium in a stereoselective manner, implications on the biosynthetic origin (genetically encoded vs spontaneous dimerization) can be derived. Overall, this case study introduces a highly attractive concept where traditional chemical retrosynthesis is translated in its entirety into an aqueous, enzyme-based cascade design. This integrated artificial metabolism matches the efficiency of its chemical blueprint with around 14% overall yield to angiopterlactone B while adding the intrinsic benefits of biocatalytic cascades such as improved step economy and benign reaction conditions.

For the effective translation of Lawrence's elegant and short synthesis route of angiopterlactone B into a fully enzyme-based scenario, generally, individual modules had already been developed over the past years. [19–22] Nevertheless, aiming for a true one-pot biocatalytic cascade, a number of restrictions and potential pitfalls have been identified (Figure 1). Therefore, the successful implementation of the artificial metabolism requires a very thorough understanding of catalytic parameters, interferences and dependencies which will be discussed in-depth in the following paragraphs.

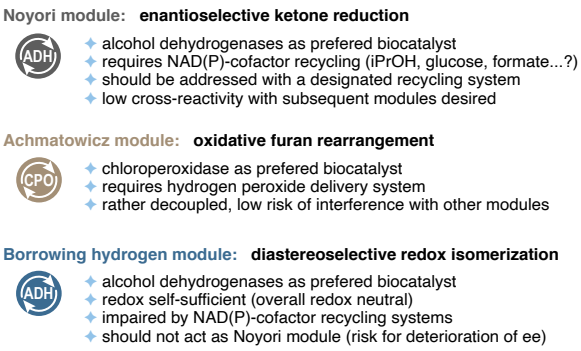
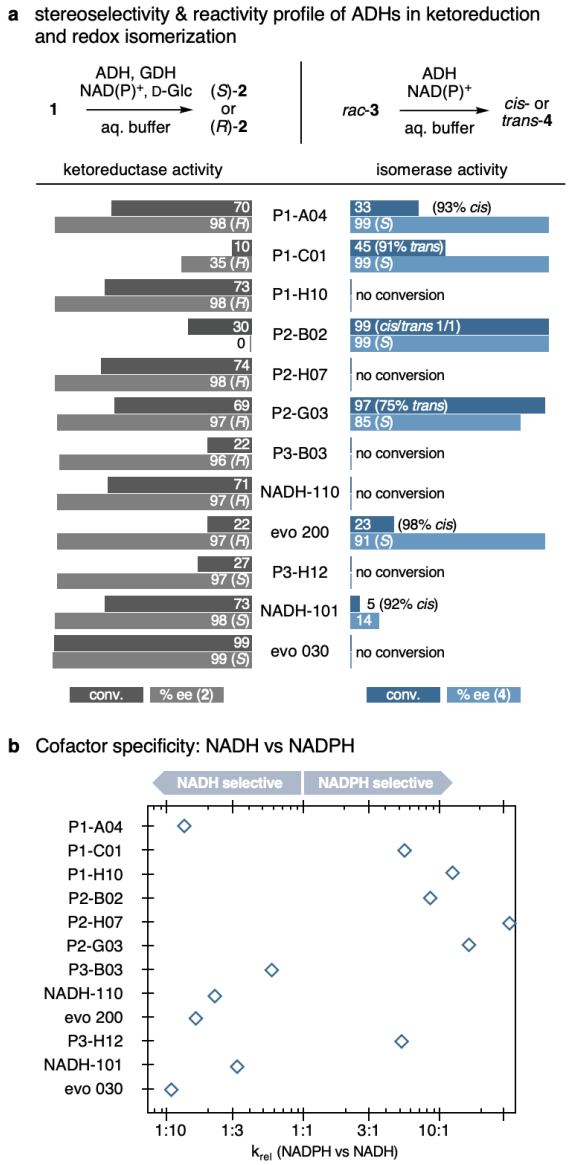


Figure 1. Key characteristics & challenges of individual modules

With the prerequisite of two dehydrogenase modules, the undesired interference between the Noyori module and the borrowing hydrogen module loomed as the major threat. Hence, we commenced our study with the identification and analysis of suitable biocatalysts for these two key reactions. Thus, both ketoreduction and redox isomerization were investigated, with the goal to identify suitable ADH couples where, in best case, the two candidates could operate exclusively on either of the reactions without cross-reactivity. As illustrated in Scheme 2a, from a set of 26 commercial biocatalysts taken from the ketoreductase screening kits of Codexis Inc. and evoCatal GmbH, a total of 12 enzymes showed significant activity in the reduction of acetylfuran **1** leading to the formation of the desired chiral secondary alcohol **2** in acceptable to excellent conversions (Table S1). Closer inspection showed that seven of the tested biocatalysts provided synthetically useful stereoinduction upon ketone reduction, resulting in the optically highly enriched alcohol **2** with an enantiomeric excess >97%. Alongside five R-selective ADHs (P_1-A_{04}: 98% ee, P_1-H_{10}: 98% ee, P_2-H_{07}: 98% ee, P_2-G_{03}: 97% ee, $NADH_{110}$: 97% ee), two enzymes with good enantiocomplementary S-selectivity ($NADH_{101}$: 98% ee, evo_{030}: 99% ee) could be identified (Scheme 2a left).

Far from optimal, the borrowing hydrogen activity was unfortunately also exclusively found in the same cluster of ADHs (Scheme 2a right, Table S2). However, among the six isomerization-active ones, three ADHs distinguished themselves with rather poor ketoreductase performance (P_1-C_{01}, P_2-B_{02} and evo_{200}) – a positive trait when it comes to the cascade design – while exhibiting good to excellent selectivity in the redox isomerization of rac-**3** to form osmundalactone **4** (91-99% ee). Here, P_1-C_{01} and evo_{200} operate via kinetic resolution, providing cis- or trans-osmundalactone selectively from racemic **3**, whereas P_2-B_{02} performs as unbiased

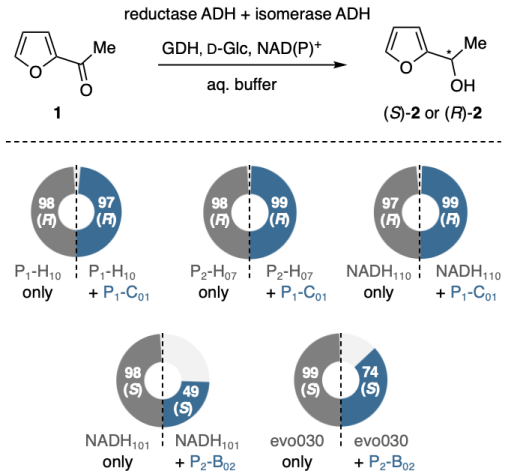


Scheme 2. Characterization of alcohol dehydrogenases to identify potentially productive binary combinations for the multi-enzyme cascades.

isomerization module that could be generally used for the synthesis of either of the osmundalactones, cis or trans. The same experiments revealed four isomerization-silent ADHs with otherwise excellent selectivities in the ketoreduction, making them strong contenders for the choice as Noyori module (P_1-H_{10}, P_2-H_{07}, $NADH_{110}$ and evo_{030}).

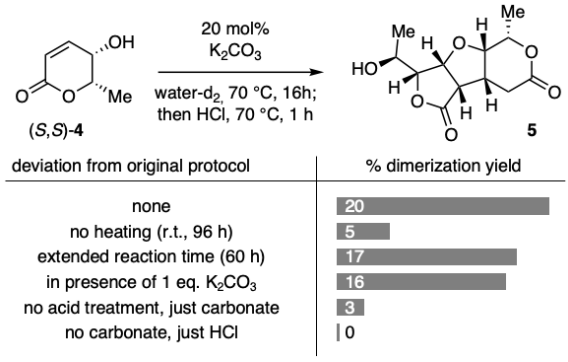
In order to gain further insights into potential ways to decouple the two dehydrogenase-mediated processes, the actual cofactor specificities of all relevant ADHs were also recorded (Scheme 2b, Table S3). Gratifyingly, the most promising ketoreductase modules all exhibited a strong NADH preference whereas the isomerase modules such as P_1-C_{01} and P_2-B_{02} showed high selectivity for NADPH as cofactor, offering an auspicious secondary feature that could help to operate reductive and redox-neutral dehydrogenase processes independently in the same reaction medium.

Before combining all biocatalytic sub-steps into a complete cascade consisting of reduction, oxidative ring expansion reaction and redox isomerization, we also had to further evaluate the influence of some of the preferred redox isomerases, P_1-C_{01} and P_2-B_{02}, on the enantioselectivity of the acetylfuran reduction (Scheme 3, Table S4). In the case of the reduction to (R)-furylethanol **2**, we barely encountered any effects on the enantioselectivity by the action of two ADHs in the cross-experiments (Figure 4). In the worst case, an ee value of >96% (P_1-H_{10}) was still obtained, whereas the best enantiomeric excess of >99% was observed in the reaction of $NADH_{110}$/P_1-C_{01}. Thus, all R-selective ADHs qualify for further investigation regarding the full enzyme cascade to form the osmundalactone (S,R)-**4**. When it comes to the S-selective reduction, a completely different picture is revealed. Here, the combination of two ADHs results in a drastic decline in enantiomeric excess. In the case of $NADH_{101}$/P_2-B_{02}, an ee value of only 49% was observed, which renders this combination useless for further studies. When evo030 is combined with P_2-B_{02}, an acceptable ee value about 74% is still achieved. Nevertheless, the prospect of a successful implementation in a genuine one-pot reaction to obtain the cis-osmundalactone (S,S)-**4** seemed to be far more challenging than for its trans counterpart. On the other hand, all identified modules would still allow to construct a one-pot case where the individual transformations were induced in a sequential manner.



Scheme 3. Assessment of the extent of undesired interference of the designated isomerase modules (P_1-C_{01} & P2-B_{02}) on the enantioselectivity of potential ketoreductase units.

As the biosynthesis of the angiopterlactones has so far not been elucidated, the question arises whether a particular enzyme would be responsible/necessary for the formation of the secondary metabolites, or if the dimerization occurred as a non-biosynthetic transformation inside the plant. Lawrence and co-workers could already show that minor amounts of the tricyclic angiopterlactone B would form with an inorganic base as catalyst even in aqueous media.[13] We envisaged that under those conditions, lactone hydrolysis may partially lead to ring-opened carboxylates and as a result to these low yields. To our very delight, slight variation of the aqueous dimerization protocol through addition of an acidic post-treatment led to an excellent yield of 20% of the tricyclic lactone. In order to decipher the tolerance of this method to various factors that are relevant for a potential implementation with the biocatalytic tools involved in the osmundalactone synthesis, a number of deviations were tested (Scheme 4). Firstly, dimerization did in fact also take place without heating, yet at a much lower rate yielding only 5% of **5** after three days. Nevertheless, this observation does support the assumption that at least the formation of angiopterlactone B could proceed in a spontaneous, non-biosynthetic manner in the plant too. Under no conditions, angiopterlactone A could be detected, not even in traces, which still leaves questions on their synthetic relationship. The extension of the reaction time to 60 h or an increase of the base concentration did not significantly affect the dimerization. Omission of either the basic or acidic part of the treatment on the other hand resulted only in traces of angiopterlactone B. Even though 20% yield may seem mediocre at first, the efficacy of this modified aqueous protocol keeps up with its chemical blueprint. In all cases, the main side products that prevent higher yields of the tricyclic addition product are the corresponding gamma-lactones derived from a ring contraction of **4**, identical to the observations made by Lawrence.



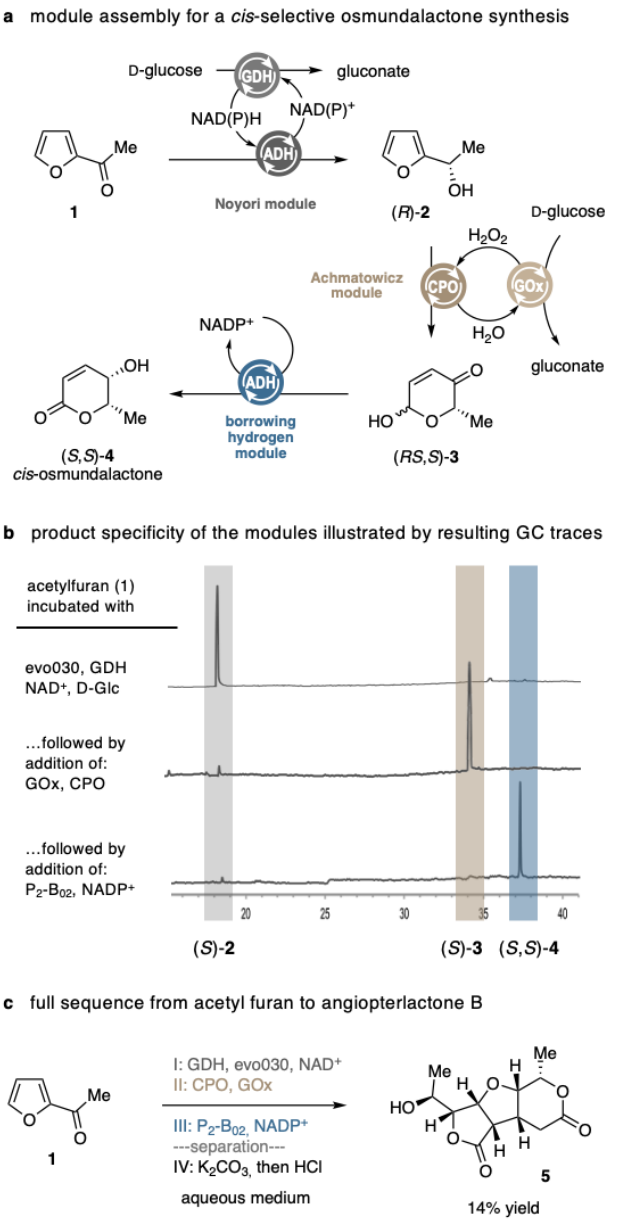
Scheme 4. Dimerization study in aqueous media

With all necessary tools mapped out, we were finally able to assemble the multi-enzymatic cascades that would lead to osmundalactone. Even though irrelevant for the angiopterlactone synthesis, we commenced this study with a brief investigation on the trans-configured osmundalactone (S,R)-**4**. We attempted a one-pot process consisting of three main enzymes, the combination of two ADHs as well as CPO and their two auxiliary enzymes GDH and GOx (Table S5). This biocatalytic cascade seemed to work rather well for P_1-H_{10} and P_2-H_{07} and, already after four hours, a 50% conversion was observed in both cases, as well as excellent enantio- and diastereoselectivity. Unfortunately, the formation of the 5-membered gamma-lactone was also observed to a certain extent. While the GDH recycling system operates both

on NADH and NADPH, formate dehydrogenase specifically recycles only NADH, and allows therefore to target NADH-dependent enzymes without much interference of NADPH-dependent ones. Consequently, when the Noyori module was changed from glucose- to formate-dependence, i.e. from GDH to the corresponding FDH recycling system, a conversion of almost 90% was achieved. In order to further optimize the protocol, we opted to run the enzymatic cascade via sequential addition of the individual components. To our delight, the exclusive formation of the desired trans- osmundalactone (S,R)-**4** was thus achieved with excellent enantio- and diastereoselectivity of >99% ee and >99% de. Most importantly, the trans-configured lactone (S,R)-**4** could be obtained on a preparative scale with a yield of 61% after a simple extraction from the five-enzyme one-pot reaction mixture. Considering the challenging cross-reactivity of ADHs for the cis-osmundalactone (S,S)-**4** production, and the initially anticipated FDH-coupled system proved to be ineffective and sluggish, giving only 32% of the lactone with more than 50% of remaining (S)-**2**. Opting for a sequential cascade reaction, however, we were also here able to obtain the desired cis-osmundalactone in a glucose-dependent system (GDH & GOx) by combining evo$_{030}$ and P$_2$-B$_{02}$ not just with exclusive selectivity (Scheme 5b) but on preparative scale in an excellent yield of 69%. While the initial two modules behaved robust in the sequential biocatalysis assembly, the final borrowing hydrogen isomerization turned out rather sensitive and extended reaction times led to a significant deterioration in yields and loss of diastereoselectivity (Table S6). It is therefore critically important to carefully monitor the reaction progress and isolate the osmundalactones without any delay to achieve the best results.

Combining the five-enzyme cascade with the carbonate-induced dimerization, a fully water-borne two-step procedure was created. Thus, the tricyclic target product could be obtained in 14% overall yield in a sequential fashion. The complete incorporation of all modules into a true one-pot is quite generally also challenged by the high load of nucleophilic additives and catalytic components that further suppress the dimerization. Future improvements will likely focus on compartmentalization techniques to separate the different catalytic and reaction components and mitigate unproductive mutual interference.

In summary, this study illustrates the enormous potential of biocatalytic cascade design in the context of organic synthesis of complex molecular architectures. By combining native and abiotic enzyme modules to an integrated metabolism-like network, great synergies are achieved, and the efficacy of the process can be vastly improved even compared to the already elegant blueprint that provided the retrosynthetic foundation. In addition to a finely adjusted network analysis of multiple biocatalytic entities, this work also sheds light on the pivotal dimerization process converting osmundalactone to the tricyclic angiopterlactone B, both in terms of its synthetic implementation and its biosynthetic mode of action, as we disclose an effective protocol in an aqueous reaction medium with high selectivity and good yields.



Scheme 5. Two-step route to angiopterlactone via streamlined multi-enzyme cascade and base-induced dimerization.

Acknowledgements

We gratefully acknowledge financial support by the European Research Council (ABIONYS, 865885), the Academy of Finland (298250 & 324976), and the Ruth & Nils-Erik Stenbäcks Stiftelse.

Keywords: artificial metabolism • biocatalysis • cascade design • natural product synthesis • angiopterlactone

[1] S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 88-119; *Angew. Chem.* **2021**, *133*, 89-123.

[2] J. M. Sperl, V. Sieber, *ACS Catal.* **2018**, *8*, 2385-2396.

[3] N. Losada-Garcia, Z. Cabrera, P. Urrutia, C. Garcia-Sanz, A. Andreu, J. M. Palomo, *Catalysts* **2020**, *10*, 1258-1277.

[4] a) N. J. Turner, E. O'reilly, *Nat. Chem. Biol.* **2013**, *9*, 285-288; b) R. O. M. A. de Souza, L. S. M. Miranda, U. T. Bornscheuer. *Chem. Eur. J.* **2017**, *23*, 12040-12063; c) M. Hönig, O. Sondermann, N. J. Turner, E. M. Carreira, *Angew. Chem. Int. Ed.* **2017**, *56*, 8942-8973; *Angew. Chem.* **2017**, *129*, 9068-9100; d) W. Finnigan, L. J. Hepworth, S. L. Flitsch, N. J. Turner, *Nat. Catal.* **2021**, *4*, 98-104.

[5] a) P. Dydio, H. M. Key, A. Nazarenko, J. Y.-E. Rha, V. Seyedkazemi, D. S. Clark, J. F. Hartwig, *Science* **2016**, *354*, 102-106; b) S. V. Athavale, S. Gao, A. Das, S. C. Mallojjala, E. Alfonzo, Y. Long, J. S. Hirschi, F. H. Arnold, *J. Am. Chem. Soc.* **2022**, *144*, 19097-19105; c) S. Chakrabarty, Y. Wang, J. C. Perkins, A. R. H. Narayan, *Chem. Soc. Rev.* **2020**, *49*, 8137-8155; d) S. V. Athavale, S. Gao, Z. Liu, S. C. Mallojjala, J. S. Hirschi, F. H. Arnold, *Angew. Chem. Int. Ed.* **2021**, *60*, 24864-24869; *Angew. Chem.* **2021**, *133*, 25068-25073.

[6] a) M. Jeschek, R. Reuter, T. Heinisch, C. Trindler, J. Klehr, S. Panke, T. R. Ward, *Nature* **2016**, *537*, 661-665; b) S. Fischer, T. R. Ward, A. D. Liang, *ACS Catal.* **2021**, *11*, 6343-6347.

[7] a) P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold, *Science* **2013**, *339*, 307-310; b) O. F. Brandenberg, C. K. Prier, K. Chen, A. M. Knight, Z. Wu, F. H. Arnold, *ACS Catalysis* **2018**, *8*, 2629-2634; c) A, Kunzendorf, G. Xu, M. Saifuddin, T. Saravanan, G. J. Poelarends, *Angew. Chem. Int. Ed.* **2021**, *60*, 24059-24063; *Angew. Chem.* **2021**, *133*, 24261-24265; d) B. J. Wittmann, A. M. Knight, J. L. Hofstra, S. E. Reismann, S. B. J. Kan, F. H. Arnold, *ACS Catal.* **2020**, *10*, 7112-7116.

[8] F. Xu, J. Y. L. Chung, J. C. Moore, Z. Liu, N. Yoshikawa, R. S. Hoerrner, J. Lee, M. Royzen, E. Cleator, A. G. Gibson, R. Dunn, K. M. Maloney, M. Alam, A. Goodyear, J. Lynch, N. Yasuda, P. N. Devine, *Org. Lett.* **2013**, *15*, 1342-1345.

[9] C. A. Martinez, S. Hu, Y. Dumond, J. Tao, P. Kelleher, L. Tully, *Org. Proc. Res. Devel.* **2008**, *12*, 392–398.

[10] M. A. Huffman, A. Fryszkowska, O. Alvizo, M. Borra-Garske, K. R. Campos, K. A. Canada, P. N. Devine, D. Duan, J. H. Forstater, S. T. Grosser, H. M. Halsey, G. J. Hughes, J. Jo, L. A. Joyce, J. N. Kolev, J. Liang, K. M. Maloney, B. F. Mann, N. M. Marshall, M. Mclaughlin, J. C. Moore, G. S. Murphy, C. C. Nawrat, J. Nazor, S. Novick, N. R. Patel, A. Rodriguez-Granillo, S. A. Robaire, E. C. Sherer, M. D. Truppo, A. M. Whittaker, D. Verma, L. Xiao, Y. Xu, H. Yang, *Science* **2020**, *368*, 1255-1259.

[11] Y. M. Yu, J. S. Yang, C. Z. Peng, V. Caer, P. Z. Cong, Z. M. Zou, Y. Lu, S. Y. Yang, Y. C. Gu, *J. Nat. Prod.* **2009**, *72*, 921–924.

[12] M. I. Thomson, G. S. Nichol, A. L. Lawrence, *Org. Lett.* **2017**, *19*, 2199-2201.

[13] T. K. Kotammagari, R. G. Gonnade, A. K. Bhattacharya, *Org. Lett.* **2017**, *19*, 3564-3567.

[14] J. R. Achmatowicz, P. Bukowski, B. Szechner, Z. Zwierzchowska, A. Zamojski, *Tetrahedron* **1971**, *27*, 1973-1996.

[15] P. S. Mahajan, V. T. Humne, S. B. Mhaske, *Curr. Org. Chem.* **2017**, *21*, 503–545.

[16] A. K. Ghosh, M. Brindisi, *RSC Advances* **2016**, *6*, 111564-111598.

[17] L. Liang, L.-D. Guo, R. Tong, *Acc. Chem. Res.* **2022**, *55*, 2326-2340.

[18] H.-Y. Wang, K. Yang, S. R. Bennett, S. Guo, W. Tang, *Angew. Chem. Int. Ed.* **2015**, *54*, 8756-8759; *Angew. Chem.* **2015**, *127*, 8880-8883.

[19] Y. C. Liu, C. Merten, J. Deska, *Angew. Chem. Int. Ed.* **2018**, *57*, 12151-12156; *Angew. Chem.* **2018**, *130*, 12328-12333.

[20] D. Thiel, D. Doknić, J. Deska, *Nat. Commun.* **2014**, *5*, 5278.

[21] D. Thiel, F. Blume, C. Jäger, J. Deska, *Eur. J. Org. Chem.* **2018**, 2717-2725.

[22] F. Blume, Y. C. Liu, D. Thiel, J. Deska, *J. Mol. Catal. B Enzym.* **2016**, *134*, 280-284.

[23] R. Wang, S. Park, *ChemCatChem* **2021**, *13*, 1898-1919.

[24] R. Noyori, M. Kitamura, T. Ohkuma, *Proc. Natl. Acad. Sci.* **2004**, *101*, 5356-5362.

[25] R. Patel, A. Banerjee, V. Nanduri, A. Goswami, F.T. Comezoglu, *J. Am. Oil Chem. Soc.* **2000**, *77*, 1015-1019.

[26] J. Liang, J. Lalonde, B. Borup, V. Mitchell, E. Mundorff, N. Trinh, D. A. Kochrekar, R. N. Cherat, G. Ganesh Pai, *Org. Proc. Res. Devel.* **2010**, *14*, 193-198.

[27] A. Berenguer-Murcia, R. Fernandez-Lafuente, *Curr. Org. Chem.***2010**, *14*, 1000-1021