Enzyme Kits to Facilitate the Integration of Biocatalysis into Organic Chemistry – First Aid for Synthetic Chemists

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First Aid Kits are collections of the most important medical equipment required for quick medical assistance. Similarly, enzyme kits can provide a proficient, ready- and easy-to-use collection of biocatalysts that can be applied with high reproducibility. In this article, we illustrate how kits of oxy-functionalisation enzymes could operate as synthetic 'First Aid' for chemists working on complex natural product total synthesis in an early- or late-stage fashion, as well as in lead diversification in drug discovery processes. We reason that enzyme kits could catalyse the integration of biocatalysis into (synthetic) organic chemistry and describe how we envision their future application.

Introduction

Historically, the invention of the "First Aid Kit" dates back to the late 18th century and was grounded on the incentive to gather the most important medical equipment required for quick medical assistance. This simple but ingenious idea to provide quick access to an essential and easy-to-use toolbox was transferred from medicine also to other scientific fields such as biocatalysis.[1,2]

Nowadays, proficient enzyme kits from different enzyme classes, including hydrolases, oxidoreductases, lyases, halogenases, and C–C bond forming enzymes, are commercially available.[3] They offer collections of well-studied, homologous enzymes or mutant libraries in order to rapidly identify a suitable biocatalyst for highly selective and reproducible conversion of targeted substrates under defined conditions.[4]

In the past decade, profound changes in custom paradigms of synthetic chemistry were initiated by the urgent need for sustainable industrial processes in light of the approaching climate crisis,[5] but were also fuelled by the continuous interest in the synthesis of increasingly complex natural product analogues and important industrial sectors such as drug discovery programmes.[6,7] More refined synthetic strategies such as protecting group-free[8] and biomimetic total syntheses,[9] diversity-oriented compound generation,[10,11] inert C–H activation,[12,13] early- or late-stage modifications,[14,15] and biocatalysis in general,[16,17] are boosting the synthetic elegance by reducing the number of steps required, increasing the atom economy, and ultimately making chemical reactions more environmentally friendly and sustainable.[18] The recent Nobel prizes in chemistry for 'asymmetric organocatalysis'[19,20] in 2021[21] and for the 'directed evolution of enzymes'[22,23] in 2018[24] symbolise such efforts.

Biocatalysis was fostered by enzyme discovery (*e.g.*, the recent discovery of synthetically useful acyltransferases,[25] imine reductases,[26,27] reductive aminases,[28] and Fe(II)/α-ketoglutarate-dependent halogenases (αKGDs)[29,30]), directed evolution,[1,31] enzymatic cascades (allowing (chemo-) enzymatic total syntheses[32,33]) and metabolic engineering.[34,35] These advancements continually pressure organic chemists to implement aforementioned strategies for the handling of complex natural products because biocatalysis enables sustainable modifications with nature-like efficiency.[36,37] In parallel, protein engineering is transitioning from directed evolution towards more rational engineering, which involves smarter mutant libraries[38,39] or computational methods[40,41] (*e.g.*, machine-learning tools[42–44] and AlphaFold[45,46]). Ultimately, it seems inevitable that chemical catalysis will need to integrate biocatalysts[17] in order to allow synthesis and modification of natural products with increasing complexity in a similarly efficient and sustainable manner as in natural biosynthesis. However, this is still a distant scenario and one requires suitable tools to overcome existing barriers.

Therefore, in this article we aim to promote the use of enzyme kits – especially as synthetic "First Aid Kits" for chemists – by briefly showcasing their rarely explored potential in the synthesis and modification of complex molecules. Importantly, enzyme kits can be easily and reproducibly applied, even by scientists unexperienced in biocatalysis. The principle of synthetic "First Aid" by enzyme kits is illustrated by three examples in Figure 1:

A) Regiospecific early- and late-stage functionalisation in total synthesis by screening enzymes or mutant libraries[47,48]
B) One-step late-stage functionalisation at different positions by a single promiscuous enzyme or mutant libraries[49]
C) Chemoenzymatic late-stage diversification of substrates by mutant libraries and subsequent chemical modifications[50]

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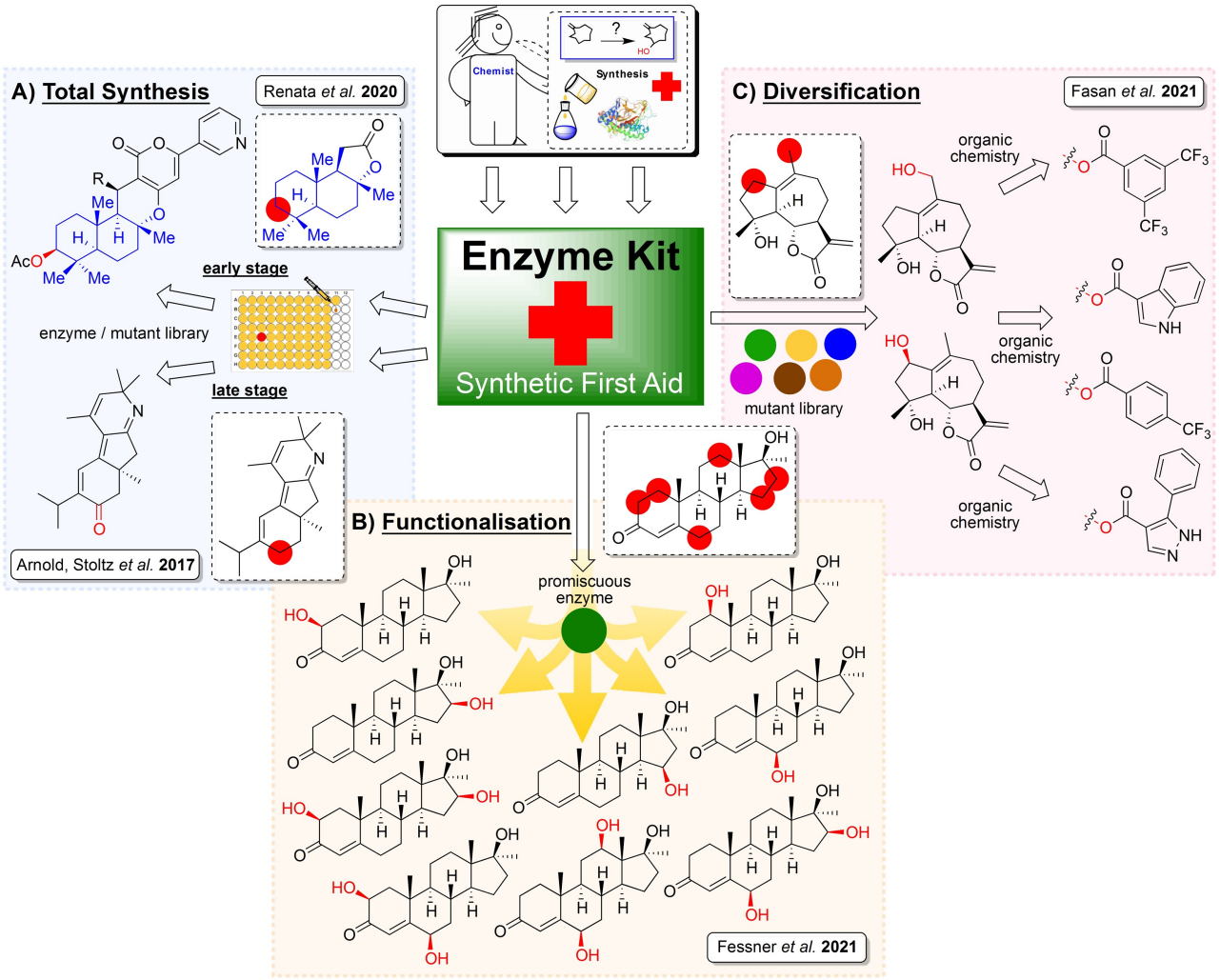


Figure 1. Enzyme kits as synthetic 'First Aid' for chemists: Three examples of enzymatic oxyfunctionalisation of complex natural products employing enzyme kits are illustrated, which enable **A)** completion of a total synthesis by selective functionalisation at an early or late stage[47,48] or **B)** functionalisation at various positions to produce a number of functionalised compounds using a single promiscuous enzyme,[49] and **C)** diversification of a molecular scaffold by enzymatic functionalisation at selected positions using mutant libraries and subsequent chemical modification in order to generate compound libraries.[50]

Using the example of oxyfunctionalisation enzymes such as cytochrome P450 monooxygenases (P450s),[51,52] unspecific peroxygenases (UPOs),[53,54] flavin-dependent monooxygenases,[55,56] Rieske dioxygenases,[57,58] and αKGDs,[59,60] this perspective complements recent cutting-edge biocatalysis review articles[2,14,16,17,61,62] in order to aid the transition towards sustainable chemistry by wide-spread combinations of chemo- and biocatalysis.[63–66] Many of the here presented examples do not involve commercial enzyme kits yet, but rather self-generated mutant libraries. However, these might as well serve as commercial enzyme kits in the future. Notably, we intend to advertise the use of biocatalysis as a realistic alternative complementary to conventional chemical catalysis.

Enzyme Kits to Aid Integration of Biocatalysis into Synthetic Organic Chemistry

Articles assessing the successes[36,37,61,67–69] and limitations[65,70–72] of biocatalysts in synthetic and industrial[73–77] chemistry are rapidly accumulating. These show that biocatalysis is increasingly integrated into chemical syntheses at an intellectual level. For example, the concept of biocatalytic retrosynthesis was introduced to incorporate biocatalysts into the ubiquitous planning strategy of synthetic chemists[78–80] including computer-guided tools.[81,82] On the other hand, the implementation of biocatalytic retrosynthesis occurred surprisingly late, considering that much earlier textbooks aimed at providing chemistry-educated scientists facile access to biocatalytic tools.[83–85] In addition, only theoretical aspects of biocatalysis are usually taught in chemistry degrees at university as a complementary, albeit elective, subject.[86]

Therefore, synthetic chemists often lack practical experience with enzymes, although biocatalysis is merely a subclass of both homo- and heterogeneous catalysis.[87] Likewise, biochemists are not well trained in chemical syntheses, hence biocatalysis needs chemists to cover the fundamental understanding of molecular reactivity and stability to design efficient synthetic routes. On a practical level, implementation efforts can already be seen in the increasingly used term 'chemoenzymatic (total) synthesis' in the literature[15,63–66,88] and the one-pot combination of both chemo- and biocatalysis.[89] Nevertheless, these examples are still scarce and generally involve multidisciplinary scientists or cooperation between groups.

In principle, bioinformatics,[90] protein engineering, as well as DNA sequencing and DNA synthesis make enzymes nowadays accessible to chemists.[17] However, limiting factors for chemists are clearly DNA manipulation (molecular biology), host cultivation and recombinant protein expression and purification. These are techniques that differ significantly from the reaction set-up, synthetic planning and chemical handling that synthetic chemists are used to. Hence, substantially different expertise (*i.e.*, molecular biology, microbiology, and enzyme engineering) and laboratory equipment for genetic engineering work are required, and this is often not available in a classic organic synthesis group. More importantly, even biocatalysis research labs are usually too specialised to have all the above-mentioned skills and protein engineering projects often fill entire PhD theses. Clearly, such expertise in biotechnology cannot be expected from chemistry labs.

Therefore, enzymes should be as available as chemical reagents. We believe that enzyme kits have the potential to fill this gap and unify the two fields (Table 1). These kits should provide chemists with ready-to-use enzymes. If applied frequently, as alternative synthetic tools, they would drastically expand catalytic information about these commercially available enzymes.

The advantage of supplying and employing libraries of enzymes at the same, optimal conditions provides a factor of highly reproducible catalytic activity, allowing the accumulation of information in databases to depict a vast portfolio of substrate scope. In turn, this knowledge might help to decrease the estimated amount of unreported test reactions and give value to negative results. Moreover, concurrent expansion of bioinformatic storage and analysis tools would help to complete gaps on enzyme activity in publicly available databases, in terms of individual substrate scope and functionality by sequence-guided prediction. Noteworthy, the exact extent of newly explored reactivity will depend on how experiments will be designed to uncover novel promiscuous functions or unpredictable products. On the other hand, enzyme kits will help biochemists to focus on enzymes that turn out to be synthetically useful, or find a good starting enzyme for protein engineering by accessing the collected information on catalytic activity towards derivatives. In the following sections, examples will be given to illustrate how the application of enzyme kits is envisioned as a useful tool for synthetic chemists.

Enzymes for the Total Synthesis of Complex Natural Products

For decades, the total synthesis of natural products was considered to be the masterpiece of organic chemistry,[92] forged by the desire for greater efficiency.[93] Even today this area of chemistry is still providing a hub for innovation by exploring novel methodologies and strategies in context of important topics such as drug discovery.[94–96] Many famous scientists are known in the field, such as Woodward, Corey, and Nicolaou, to name but a few, with important contributions such as the concept of retrosynthesis.[97]

Of the numerous approaches for conducting a total synthesis, many were inspired by natural biosynthesis.[6] For example, the Baran group introduced the biomimetic concept of 'two-phase' total syntheses of the terpenes **1** and **3**–**6** in 2009 (Figure 2A),[98] in order to make use of nature's highly efficient approach of accessing maximum diversity of natural products in a minimum number of steps by the modification of common structural scaffolds (**3**; Figure 2B).[99] Starting with a

Table 1. Benefits and current limitations of enzyme kits for the implementation of biocatalysis in synthetic organic chemistry.

	Benefits	Limitations
Commercial enzyme kits	Ready-to-use High reproducibility Optimal conditions Substrate scope and stereoselectivity known for benchmark substrates/enzymes	Often high prices Little control over kit design Potential stability issues upon extended storage or reconstitution of lyophilised powder Limited number of enzyme families Company secrecy about kit information
Novelty gained by the application of enzyme kits	Synthetically useful alternative to chemical methods Sustainable chemistry (e.g., no (transition) metal catalysts, no hazardous solvents (as water is considered benign[91])) Greater synthetic elegance (e.g., several steps (cascades) under similar conditions, better atom economy, no protecting group chemistry)	Low availability of enzymes and their (super-)families
Utilisation of enzyme kits	Biocatalytic applications in synthetic routes Easy handling Rapid identification of starting points (e.g., for enzyme engineering, large-scale synthesis) Collection of reproducible results in databases	Screening effort required for kit application Low reaction scale (0.01–1 mg) depending on the enzyme (class); but often with the option for up-scaling (1 mg–1 g)

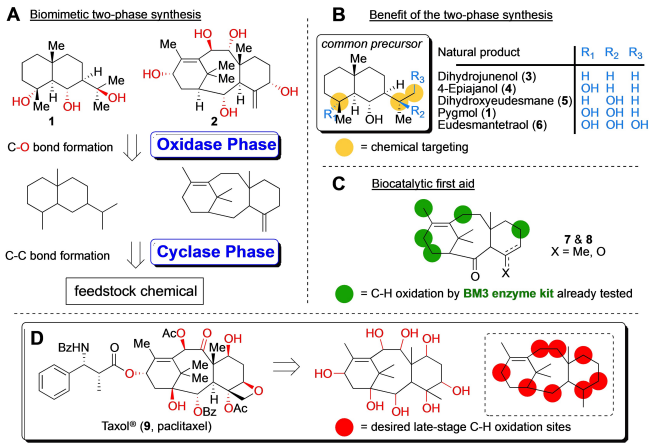


Figure 2. The synthetic two-phase strategy suits the application of oxygenases for late-stage functionalisation. **A**: Retrosynthetic planning of the biomimetic two-phase total synthesis of terpenes **1** and **3**–**6**,[98] as well as **2**[106] based on the cyclase and oxidase phases.[100] Most of the hydroxyl functional groups (red) are introduced in a synthetic late-stage fashion. **B**: Benefit of the two-phase synthesis is the common precursor **3**, which allows the synthesis of derivatives by site-selective functionalisation to different extents.[98] **C**: Biocatalytic first aid for the late-stage C–H oxidation (green circles) of **7** and **8** by a P450-BM3 mutant library of the Wong group based on preliminary data.[104] **D**: In principle, the structure of taxol® (**9**, paclitaxel) could be synthesised by eight late-stage hydroxylations of the hydrocarbon skeleton (red circles) followed by chemical modifications. P450-BM3: self-sufficient P450 monooxygenase from *Bacillus megaterium*, recombinantly expressed in *Escherichia coli*.

chemical cyclase phase to build up the target compound's hydrocarbon skeleton employing C–C bond-forming reactions, Baran introduced functionality in the following chemical oxidase phase *via* sequential, site-selective C–H activation of unactivated (sp^3-hybridised) positions.[100] Such late-stage functionalisation often renders protecting groups unnecessary.

Ever since the concept was initiated, the Baran group established it in several excellent total syntheses ranging from **2** up to Taxol® (**9**, paclitaxel) in 2020 as the taxane with the highest oxidation level.[100] However, they admitted that specific access by chemical synthesis "will likely never be competitive with a fully enzymatic approach".[101] Other groups also picked up this concept during the parallel rise and exploration of powerful C–H activation strategies (for the oxidase phase).[12,13] Therefore, the synthetic chemistry society attempted to implement biomimetic strategies in the quest for efficiency and sustainability.[9] Nevertheless, selective C–H functionalisation in the presence of other functionalities still remains very challenging, demanding reactivity differences, directing groups or intramolecular reactions.[102,103]

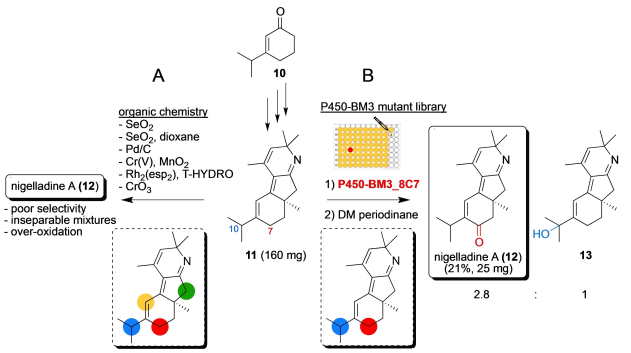
Therefore, the complementary implementation of nature's toolbox, which is highly optimised for site-selective C–H activation in one step at various inert positions, could be used more frequently for targets hardly accessible by chemical means. Hydroxylation enzymes such as cytochrome P450 monooxygenases, which are found in all kingdoms of life and participate in the biosynthesis of many natural products or the degradation of xenobiotics (*e.g.*, in the liver), are renowned for such abilities.[88,103]

"Synthetic First Aid": Late-stage biocatalytic oxyfunctionalisation

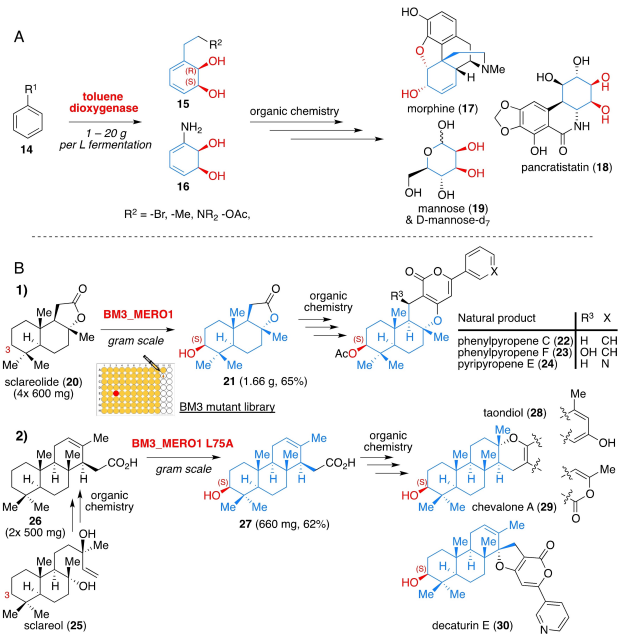
Preliminary data for the enzymatic late-stage functionalisation of taxanes such as **7** and **8** were generated in a collaborative doctoral thesis by the Robertson (synthetic chemistry) and Wong (biocatalysis) groups.[104] A small mutant library of the P450 monooxygenase CYP102A1 (P450-BM3) from *Bacillus megaterium* from the Wong group was able to access five positions of **7** and **8** or their derivatives (Figure 2C). The bacterial P450-BM3 is soluble, easily expressed in *Escherichia coli*, and self-sufficient by nature, which means that the haem domain is fused to the required redox partner in one continuous polypeptide chain, allowing the highest reported catalytic activity of any P450.[105] In some cases, even dihydroxylated products of **7** and **8** were observed, indicating the possibility for targeting two positions in one step. Although only two of the targeted positions ultimately require hydroxylation for the synthesis of the end product **9** (Figure 2D), the ability to target a number of positions (simultaneously) in a one-step and late-stage fashion under mild conditions is clearly valuable.

Notably, any of the isomers at any particular position on the hydrocarbon skeleton are in principle accessible by enzyme engineering. Moreover, additional modifications found in the final target **9**, such as the acetylation of newly installed hydroxyl groups in the Taxol scaffold, might be achieved by enzymatic one-pot cascades that combine monooxygenases and acyltransferases.[25,107]

A collaborative study by the Stoltz (synthetic chemistry) and Arnold (biocatalysis) groups is a beautiful example of how enzymes could be introduced in a 'two-phase' total synthesis *via* enzyme kits. Having synthesised precursor compound **11**, consisting of a hydrocarbon skeleton with a single imine functional group, they only needed an allylic oxidation at position 7 *via* post-synthetic C–H activation as the oxidase phase to obtain the product nigelladine A (**12**; Scheme 1A)[47]. However, the lack of success by a large number of tested chemical conditions due to the less favourable hydrogen abstraction at the sp^3-hybridised C7 in the presence of a neighbouring isopropyl substituent (sp^3-hybridised C10) called for a different approach. A focused mutant library of P450-BM3 was screened for C7-hydroxylation activity (Scheme 1B). The engineered variants had previously been generated for promiscuous activity towards larger substrates by the Arnold group.[108] Several variants, and even wild-type P450-BM3 itself, were identified as positive hits, producing **12** and **13** with different degrees of regioselectivity towards C7. Ultimately, variant P450-BM3_8C7, which exhibited a preference towards C7 over C10 in a 2.8:1 ratio, was selected for the preparative scale (160 mg) reaction, with 21% yield. This study is a great example of biocatalytic "First Aid" in form of enzyme kits to help complete a synthesis, and a milestone for the "chemoenzymatic collaboration" between groups in the two fields.



Scheme 1. The use of an enzyme library provides synthetic 'First Aid' in the total synthesis of nigelladine A (**12**).[47] **A**: Various attempted conditions of traditional synthetic methods achieved oxidation only with poor regioselectivity, yielding inseparable mixtures of mono- or poly-oxidised products. **B**: Screening of a previously generated P450-BM3 mutant library led to the identification of variant P450-BM3_8C7 exhibiting regioselectivity of 2.8 : 1 for oxidation at position 7 (red; **12**) as compared to hydroxylation at position 10 (blue; **13**).



Scheme 2. The use of an enzyme library enabled synthetic 'precaution' in the total synthesis of meroterpenoids. **A**: The principle of early-stage biocatalytic supply of unconventional building blocks in an enantioselective manner is illustrated by the use of toluene dioxygenase, which stereospecifically prepares the essential structural arene *cis*-dihydrodiol motifs (**15**, **16**) of the final natural product (*e.g.*, **17**–**19**).[115] **B**: Screening of a self-generated P450-BM3 mutant library allowed the identification of variants that catalyse the required 3-hydroxylation of **1)** sclareolide (**20**) and **2)** sclareol derivative **26** at an early stage of the synthesis of several natural meroterpenoids.[48]

"Synthetic precaution": Early-stage biocatalytic oxyfunctionalisation

During the 'second wave of biocatalysis' in the 1990s,[1] enzymes or microorganisms were frequently applied for the resolution of racemic mixtures or for asymmetric synthesis. They enabled the straightforward preparation of high-value enantiomerically pure building blocks, which were challenging to obtain by chemical means, from available chemical feedstocks at an early stage of a (total) synthesis.

This concept was realised, for example, for the preparation of various unconventional arene dihydrodiol derivatives (**15**, **16**) from toluene derivatives (represented by generic structure **14**) *via* toluene dioxygenase-catalysed stereospecific *cis*-dihydroxylation (red).[109–111] An equivalent chemical synthesis of precursors **15** and **16** is still not available.[112] The enzymatic reaction can be executed on a 1–20 g/L scale reaction *via* fermentation of *E. coli* strains expressing toluene dioxygenase.[111] Natural products of several classes (**17**–**19**; Scheme 2A), such as a number of morphine alkaloids targeted by the Hudlicky group,[113–117] could be assembled efficiently using this chemoenzymatic, early-stage building block synthesis.[111]

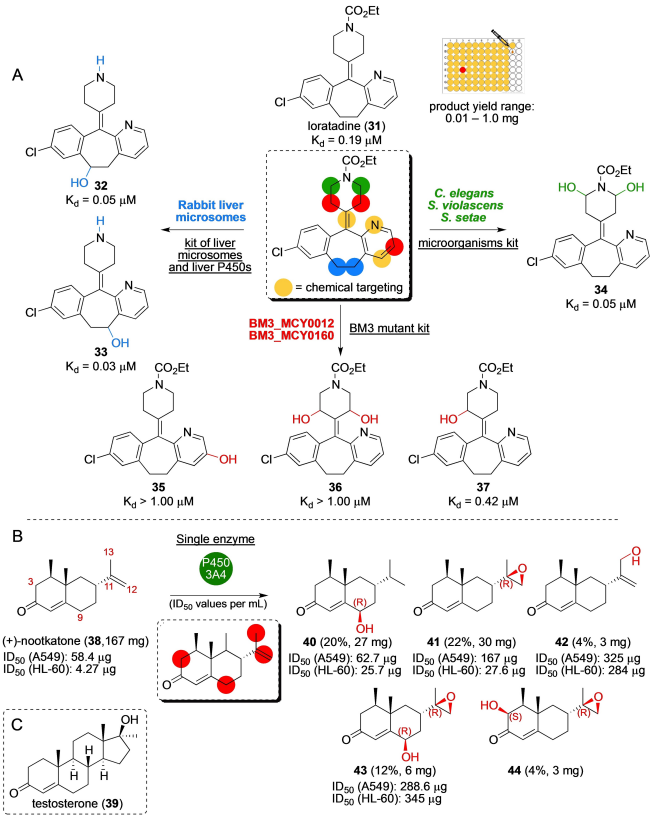
In a similar manner, the Renata group was working on the total synthesis of various complex natural products (**22**–**24**, **28**–**30**) in a chemoenzymatic manner to showcase the power of early-stage integration of biocatalysts for the simplification and improvement of previous routes.[15,59,64,102,118,119] For example, in 2020,[48] they attempted to use sclareol (**25**) as the starting point in the total synthesis of meroterpenoids by hydroxylation at the inert 3 position. Screening an engineered P450 enzyme library allowed the identification of variants that exclusively targeted the desired position in **25**, albeit with rather low conversion.[118] Hence, they structurally modified **25** to identify other useful intermediates that would better fit the enzymes' geometry (*e.g.*, active-site, access tunnel) and thus lead to better conversion. This so-called substrate engineering to derivatives **20** and **26** enabled the gram-scale early-stage modification of the essential building blocks. Subsequently, they were able to successfully synthesise three structurally diverse meroterpenoids each (**22**–**24** and **28**–**30**). It is a highly effective strategy to execute synthetic 'precaution' by preparing the most challenging motifs of structurally complex natural products at an early stage, circumventing the necessity of synthetic first aid, especially if it concerns a commonly occurring motif (blue; Scheme 2) in natural products.

Enzymes for the Late-stage Diversification of Complex Lead Compounds

The aforementioned biomimetic 'two-phase' synthesis is in line with nature's evolutionary logic of generating a maximum diversity of natural products time- and material-efficiently *via* a mutual structural scaffold using promiscuous enzymes. The selective chemical manipulation, especially of complex natural products, is a notoriously challenging task[120] considering that the degree of functionalisation in mutual precursors represents a risky balance between the need for polarity, which dictates reactivity, and the absence of alternative reactive functional groups. However, the ability to modify natural products is of supreme importance for drug discovery and development programmes in the pharmaceutical industry because of the

need to adjust a lead compound's properties.[103,121–123] Only recently, an article by Stoltz *et al.* called the topic of late-stage diversification for the manipulation of complex molecules a "motivational force in organic synthesis",[10] since successful and comprehensive diversification demands larger amounts of late-stage material and thus continuous refinement of the preparative route. The natural ability of many enzymes to proficiently modify natural products in the presence of other reactive functional groups in one step makes them the ideal candidates for this task.

In order to rapidly evaluate the easily accessible chemical space, efficient C–H activation tools are most desirable, especially when synthetically challenging sites are targeted. Importantly, alcohol functional groups are highly desired considering that they increase polarity, thereby decreasing lipophilicity and often enhancing metabolic stability, as well as installing both a hydrogen bond acceptor and donor at the same time, which results in better affinity to the biological target.[124] Furthermore, once a hydroxyl group is present, it can be modified by additional transformations such as methylation, to further alter the properties of the compound. Therefore, oxyfunctionalisation enzymes such as P450s are key elements in the toolbox of pharmaceutical companies for the improvement of lead compound properties.[103,120]



Scheme 3. (Chemo-) enzymatic late-stage functionalisation of lead drugs (**A**) or natural products (**B**, **C**) for exploring chemical space of candidate derivatives. **A**: The drug loratadine (**31**) was diversified by several chemical and biocatalytic late-stage functionalisation techniques *via* C–H activation.[125] Some of the products (**32**–**37**) formed by screening commercial enzyme libraries (i.e., a BM3 mutant kit, microorganism kit, and a kit containing liver microsomes and recombinant liver P450s) are shown. Some of the products had better human histamine H1 receptor dissociation constants than the precursor **31**. **B**: Human P450 3A4 enabled the derivatisation of various natural product such as the antileukemic (+)-nootkatone (**38**) or testosterone (**39**) to form compound libraries of functionalised metabolites in one step.[49] In a previous study, some of the produced analogues (**40**–**44**) had shown better or equal anti-proliferative properties.[126]

Enzymatic late-stage functionalisation of natural products

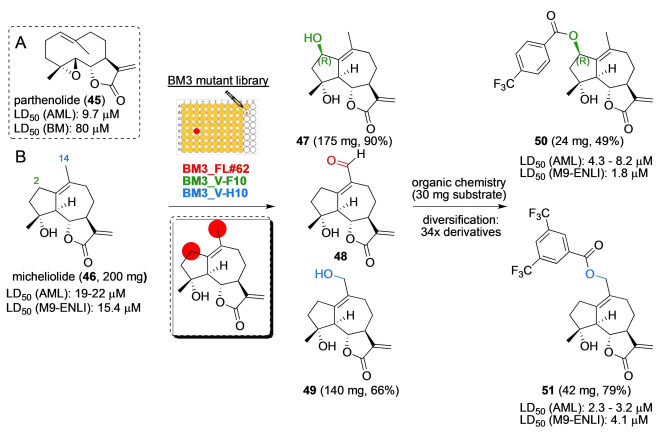
In 2020, Lall *et al.* demonstrated comprehensive chemo- and enzymatic late-stage functionalisation of the potent antihistamine drug loratadine (**31**; Scheme 3A).[125] On the one hand, the lead compound was exposed to chemical conditions of electrolysis, electrochemical trifluoromethylation, biomimetic metalloporphyrin oxidation (BMO) and alkylation using alkyl sulfinate salts (Baran Diversinates). On the other hand, **31** underwent biocatalytic functionalisation by screening three commercially available enzyme kits: P450-BM3 mutants (Codexis), liver microsomes (Corning, Pfizer, and XenoTech), recombinant liver P450s (PanVera), as well as microorganisms (various culture collection facilities, etc.). Scaling of their reaction with the most diversifying options to obtain up to 1 mg of each functionalised product allowed subsequent characterisation and biological testing. Only a fraction of those reported are shown in Scheme 3A. Some of the obtained compounds (**32**–**37**) possessed better human histamine H_1 receptor dissociation constants than **31**. The three kits had a 46% product similarity, and fifteen of the produced metabolites were isolated. Noteworthy, such a diverse biocatalytic panel thoroughly targeted a large part of the substrate scaffold, while chemical C–H activation tools were rather restricted in site-selectivity, albeit producing more exotic derivatives such as ring-expansions by a House-Meinwald rearrangement. Clearly, biocatalytic hydroxylation at synthetically challenging sites and, for example, chemical (fluoro-) alkylation can go hand-in-hand to produce potent lead candidates that would be rather impossible to produce by either strategy on its own.

Very recently, a commercial *P. pastoris* strain expressing human liver P450 3A4 (Bisy GmBH) was examined for a similar purpose by Fessner *et al.*[49] This highly promiscuous monooxygenase substantially functionalised the molecular scaffold of different types of natural products such as (+)-nootkatone (**38**; Scheme 3B) or testosterone (**39**; Scheme 3C) to generate compound mixtures of more than five (**40**–**44**) and ten products (Figure 1C), respectively. The whole-cell biotransformations were carried out at preparative scale, albeit with low yields (4–22%) due to the large number of individual products, making proficient purification techniques necessary. Metabolite **44** had never been reported previously. However, some of the other functionalised derivatives (**40**–**43**) had shown better or equally good antiproliferative activity towards two tested human cancer cell lines.[126] Hence, a single enzyme was sufficient for thorough

diversification of natural products of different classes. Other human liver P450s, such as P450 2D6, have similarly broad substrate scope and can complement the diversification potential of P450 3A4 in a human liver enzyme kit.[127] In addition, these human P450s produce authentic human metabolites and thus allow the evaluation of a compound's metabolic fate in the body. In comparison, time-consuming enzyme engineering would be necessary to obtain a mutant library of P450-BM3 that would enable a similar degree of derivatisation of **39**, as shown by a recent study by the Wong group.[128] Therefore, both enzymatic strategies can be used for the rapid generation of diverse compound libraries for subsequent chemical derivatisation.

Chemoenzymatic late-stage diversification of natural products

The Fasan group published intriguing examples of natural product functionalisation with subsequent chemical derivatisation. They used enzyme libraries based on P450-BM3 to diversify terpenes.[129–131] For example, in 2014,[129] the group started to enzymatically functionalise the antileukemic natural product parthenolide (**45**; Scheme 4A) for subsequent chemical coupling to substituted benzoyl moieties. Encouraged by better LD_{50} values against human cancer cell lines, they later expanded their library of analogues of **45** *via* chemical O–H acylation, alkylation, carbamoylation, and carbene insertion.[132,133] More recently, they went on a similar quest with the sesquiterpene lactone micheliolide (**46**), another natural product displaying antileukemic activity (Scheme 4B).[50] Using the same P450-BM3 variant (FL#62) from previous studies on **45** as the starting point, they observed functionalisation of **46**, albeit with poor selectivity, affording products **47**–**49**, which were not accessible by chemical means. The P450-BM3 mutant kit they employed yielded variants V–F10 and V–H10, which selectively functionalised the scaffold at C2 and C14 at preparative scale, respectively. This late-stage functionalisation allowed further diversification *via* chemical means to generate a large panel of analogues to be tested for antileukemic activity in five human cancer cell lines. Several derivatives possessed enhanced potency relative to **46**, with the most potent agents, **50** and **51**, shown in Scheme 3. Clearly, this study showcased the power of chemoenzymatic synthesis for the production of lead natural product analogues displaying promising bioactivity.



Scheme 4. (Chemo-) enzymatic late-stage diversification of natural products for drug discovery. **A**: The antileukemic natural product parthenolide (**45**) had been diversified using a P450-BM3 mutant enzyme kit in a study by the Fasan group in 2014.[129] **B**: Similarly, the antileukemic micheliolide (**46**) was synthesised from **45** by the same group. An enzyme kit containing P450-BM3 mutants enabled the efficient functionalisation to allow facile synthetic follow-up modification (**47**–**49**).[50] The two analogues **50** and **51** possessed better antileukemic potency than both **45** and **46**.

Future Outlook for Enzyme Kits

Most of the examples used throughout this article, except for those in Scheme 3, did not even use commercial enzyme kits, but instead depended on generating mutant libraries or obtaining such libraries from collaborators. Some of those defined sets of mutants are proficient enough that they could be transformed into commercially available enzyme kits by academic labs, non-profit organisations, or companies. For this, it becomes very important to select mutants that are (i) stable, (ii) active and (iii) functionally diverse in order to embody a useful kit that minimises (screening) effort, but maximises (scientific) gain. In addition, the diversity of enzyme kits based on oxyfunctionalising enzymes is still limited. The only frequently used enzyme superfamily of this category is that of cytochrome P450 enzymes, which again is almost exclusively based on the self-sufficient, bacterial P450-BM3 – well known for high turnover efficiencies, stability, self-sufficiency, and for being easy to engineer.[105,134] Only human liver P450s are used occasionally because of their high relevance for human drug metabolism. However, some studies also proved their utility for synthetic modifications.[127]

Consequently, the recent release of the first enzyme kits consisting of UPOs (AminoVerse, EvoEnzymes) demonstrates the steady advancement of the field of commercial enzyme kits.[135–138] The research on UPOs is still in its infancy, but they generally possess promiscuous substrate scopes, require only H_2O_2 as co-substrate, and often possess good organic solvent resistance, making their application more straightforward.[51,53,54,139] In addition, an αKGDs kit could be available soon, judging from the current frequency of natural product modification success stories in the literature,[59,60,119] and the patent application by the company Codexis some years ago.[30,140]

In general, enzyme kits of other classes such as hydrolases (*e.g.*, lipases and esterases) and ketoreductases have existed for decades[1,2] and are commercially available from many different companies.[3,4] Furthermore, enzyme kits of yet less well investigated classes such as methyltransferases[141–143] for the selective alkylation (*e.g.*, methylation and carboxymethylation) of natural products of lead drugs should be highly valuable for synthetic (pharmaceutical) chemists,[144–146] especially since the discovery of the 'magic methyl effect'.[147,148]

In parallel, proficient options to execute transition metal and organocatalysis for C–H activation are often either easy to synthesise or commercially available at low price. In Figure 3,[11] biocatalytic examples applied to the natural products (+)-artemisinin (**51**; all R = H)[130] and **20**[48] are compared to renowned chemical catalysts and plain oxidising agents.[149–155] Notably, instead of protein engineering, derivatisation can be varied by the metal, modification of the ligand(s) or even a plain change in reagents (Figure 3A 4–6). On the other hand, these chemical catalysts are limited to reactivity differences, directing groups or intramolecular reactions for the C–H bond functionalisation of complex natural products,[15] clearly highlighting the need for complementary application of bio- and chemocatalysis.

Therefore, in combination with efficient enzyme engineering strategies to cover vast amounts of sequence space, oxyfunctionalisation enzyme kits have the potential to induce a paradigm shift in the collaboration between biocatalysis and synthetic organic chemistry. Eventually, continuously refined enzyme kits might even enable modification (*e.g.*, hydroxylation) of every single C–H position of key motif structures (*e.g.*, of APIs) using enzyme variants in a regio- and stereoselective manner. Reetz and Roiban expressed a similar goal in 2015 in the context of mutagenesis methods.[156] Assuming a certain degree of enzymatic promiscuity towards functionalised substrates, the preparation of mono-, di-, … poly-hydroxylated products in any combination would be plausible in a matrix-like scheme.[99]

Optimally, such strategy would be aided by computational predictions nurtured by machine learning technology, grounding its calculations on the database of information collected from the continuous use of enzyme kits: Hence, optimal variants for the catalysis of new reactions could be predicted. Such thought is not entirely unrealistic considering the machine learning capabilities applied in biocatalysis nowadays.[38,43,157] While highly reliable structure predictions are already possible from the sequence alone,[45,46,158] the design of novel proteins with desired structures by sequence predictions is currently being established[40,41] – thus accurate sequence-guided prediction of functionality might not be far off.[45,159] Hence, we are confident that we will see more and more milestones as well as fully enzymatic (total) syntheses[32,33] with the help of enzyme kits.

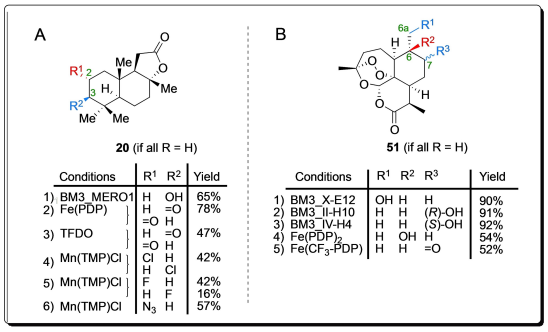


Figure 3. Drawing parallels between enzyme kits and commercial transition metal catalysts.[11] Natural products **20** and (+)-artemisinin (**51**) are functionalised either by P450-BM3 mutants,[48,130] transition metal catalysts, or an oxidising agent.[149–155]

Conclusion

This perspective themed enzyme kits as synthetic 'First Aid' for chemists, which we illustrated using several examples of major applications (Figure 1): Simplification of total syntheses and facile chemoenzymatic diversification of natural products *via* early- or late-stage functionalisation. Enzymes are highly proficient and selective tools for inert C–H activation and can thereby help to significantly reduce the number of synthetic steps and allow several of these to proceed simultaneously under the same conditions, improve the atom economy, and ultimately increase the synthetic elegance by making unique chemical connections that are unavailable in conventional chemistry.

At the same time, enzyme kits could act as 'First Aid' for biocatalysis by facilitating the integration of enzymes as homo- or heterogeneous catalysts into the chemical toolbox. As ready- and easy-to-use collections of biocatalysts that can be applied with high reproducibility, commercial enzyme kits soften barriers for application by chemists inexperienced in biocatalysts. We hope that enzyme kits will catalyse the unification of bio- and chemocatalysis.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: biocatalysis · early-stage functionalisation · enzyme kits · late-stage functionalisation · lead diversification

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