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Enzymatic C–H functionalizations for natural product synthesis

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Direct functionalization of C–H bond is rapidly becoming an indispensible tool in chemical synthesis. However, due to the ubiquity of C–H bonds, achieving site-selective functionalization remains an arduous task, especially on advanced synthetic intermediates or natural products. In contrast, Nature has evolved a multitude of enzymes capable of performing this task with extraordinary selectivity, and the use of these enzymes in organic synthesis may provide a viable solution to contemporary challenges in site-selective functionalization of complex molecules. This review covers recent applications of enzymatic C–H functionalization strategies in natural product synthesis, both in the context of key building block preparation and late-stage functionalization of advanced synthetic intermediates.

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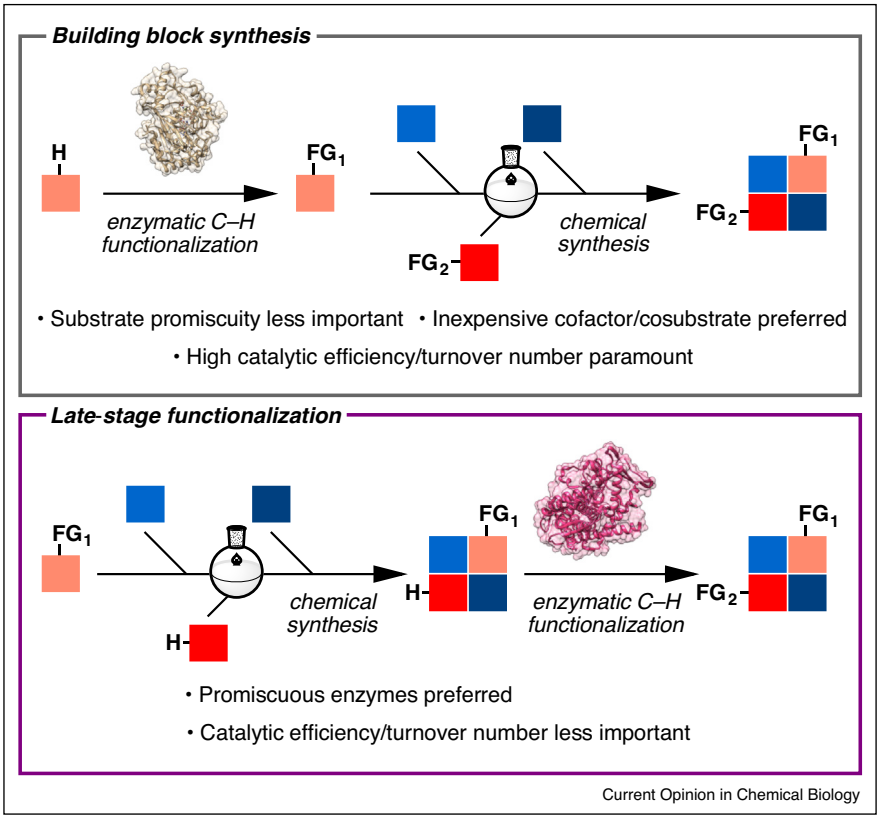
Introduction

Direct functionalization of C–H bond constitutes a highly powerful strategy for the synthesis of organic compounds [1]. Under this paradigm, an inert C–H bond is viewed as a functional handle for the construction of a new C–C or C–X (X = heteroatom) bond in one step. This approach stands in stark contrast to traditional multistep sequences, which entail independent preparation of prefunctionalized substrates and their use in subsequent C–C or C–X (X = heteroatom) bond formation step. Thus, judicious application of C–H functionalization in complex molecule synthesis offers numerous strategic benefits that ultimately will allow chemical synthesis to be performed with greater efficiency [2,3].

The field of natural product synthesis is often regarded as the ultimate proving ground for new synthetic methods. Unsurprisingly, the number of total syntheses featuring C–H functionalization as the key step has risen dramatically in the past decade. Despite these successes, achieving chemo and regioselective C–H bond functionalization remains a formidable challenge, especially in the context of complex synthetic intermediates which contain numerous C–H bonds with similar bond energies. Successful applications of C–H bond functionalization in natural product synthesis typically rely on the use of intramolecular reactions [4,5], preinstalled directing groups [6] or innate reactivity differences within the molecular framework of interest [7]. Meanwhile, case studies that demonstrate catalyst-controlled selectivity remain rare [8]. In contrast, natural product biosynthesis pathways are replete with tailoring enzymes capable of performing different types of C–H bond functionalization—including hydroxylation [9•,10•], halogenation [11•,12•], alkylation [13•], and desaturation—with exquisite selectivity profiles unmatched by conventional small-molecule catalysts.

Aided by advances in microbial genetics and enzyme engineering, practitioners of organic chemistry and biocatalysis have recently begun to explore the synthetic utility of these catalysts for the synthesis of medicinally relevant molecules and natural products [14]. The application of this strategy can manifest in either early-stage building block synthesis or late-stage functionalization. The former refers to the use of enzymatic functionalization in the upstream portion of a synthetic route to produce a key intermediate, which undergoes subsequent chemical transformations *en route* to the molecular target. In this case, as the enzyme at hand needs to deliver ample quantities of a particular product for downstream manipulation, high catalytic efficiency/turnover number is paramount. In late-stage functionalization, an advanced intermediate is chemically synthesized and submitted to an enzymatic C–H functionalization step to afford the target natural product. Thus, promiscuous enzymes that can accept different advanced intermediates are typically preferred even if their catalytic efficiency is not particularly high. Herein, we highlight recent applications of biocatalytic C–H functionalization in natural product total synthesis, making clear distinction between examples of building block synthesis and late-stage functionalization for pedagogical purposes (Figure 1).

Figure 1



Schematic illustration of biocatalytic C–H functionalization for building block synthesis and late-stage modification in multi-step synthesis.

Building block synthesis

As outlined above, building block synthesis requires highly efficient enzymes that utilize inexpensive cosubstrates and/or cofactors so that the desired products can be obtained in the most practical and economical manner. Given these criteria, members of the iron and α-ketoglutarate-dependent dioxygenase (Fe/αKG) superfamily are excellent candidates, as they do not require dedicated reductase partners or expensive cofactors. Since 2016 our laboratory has conducted a series of proof-of-concept studies to examine the biocatalytic potential of these enzymes in the preparation of key building blocks, particularly in the context of nonribosomal peptide and alkaloid syntheses.

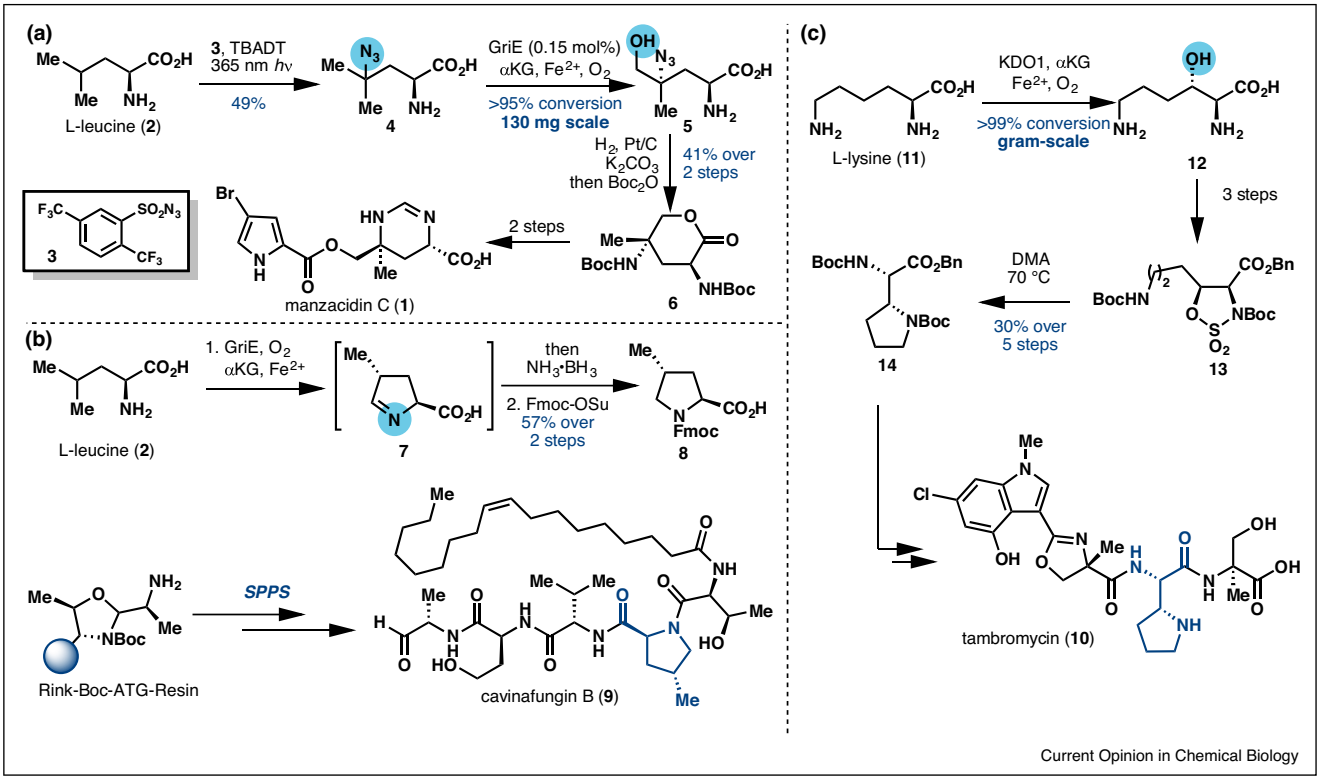
Manzacidin C and cavinafungin B

Isolated from the Okinawan sponge *Hymeniacidon* sp. in 1991, manzacidin C (**1**) is a bromopyrrole alkaloid that contains a unique tetrahydropyrimidine motif [15]. After the first reported total synthesis in 2000 [16–21], numerous synthetic approaches to **1** have been developed. In 2018, our laboratory reported a formal synthesis of **1** (Figure 2A) featuring a remote C–H hydroxylation catalyzed by an Fe/αKG leucine 5-hydroxylase GriE [22••] from the griselimycin biosynthesis [23]. By exploiting the substrate promiscuity of GriE, azidoleucine (**4**, prepared via photocatalytic azidation of L-leucine) could be selectively hydroxylated at the δ position in >95% conversion on 130 mg scale. A one-pot hydrogenation, Boc protection, and intramolecular cyclization provided lactone **6**, which is an advanced intermediate (two steps away from **1**) from a previous synthesis [16]. This route represents one of the shortest approaches to **1** and illustrates the simplifying power of biocatalytic C–H hydroxylation in complex molecule synthesis. We also found that GriE could catalyze iterative C5 oxidation of L-leucine at high enzyme concentration (Figure 2B) to give the corresponding imine (**7**), which was reduced with $NH_3{\cdot}BH_3$ in one-pot to yield (2*S*,4*R*)-4-methylproline. Utilizing this method, protected (2*S*,4*R*)-4-methylproline **8** was prepared from L-leucine in 57% overall yield on 100 mg scale. With **8** in hand, the first synthesis of cavinafugin B (**9**), an antiviral aldehyde lipopeptide isolated from *C. cavincola* [24], was completed in 10 steps and 37% overall yield using Fmoc-based solid-phase peptide synthesis (SPPS) [25].

Tambromycin

Isolated from several *Streptomyces* strains, tambromycin (**10**) is a nonribosomal peptide natural product with antiproliferative activity against cancerous B-cell and T-cell

Figure 2



(a) Formal synthesis of manzacidin C featuring remote hydroxylation of **4** with the Fe/αKG GriE. **(b)** Chemoenzymatic cascade with GriE for the preparation of protected (2*S*,4*R*)-4-methylproline (**8**) in the synthesis of cavinafungin B. **(c)** Selective C3 hydroxylation of L-lysine with the Fe/αKG KDO1 for the preparation of protected tambroline monomer (**14**) in the total synthesis of tambromycin.

lines [26]. Structurally, tambromycin contains a trisubstituted indole fragment, a methyloxazoline moiety and an unusual pyrrolidine-containing amino acid named tambroline. Our laboratory recently developed a chemoenzymatic synthesis of **10** (Figure 2C) by enlisting a biocatalytic C–H functionalization approach to construct the tambroline monomer [27••]. Utilizing L-lysine (**11**) as the starting material, regioselective and stereoselective C3 hydroxylation employing an Fe/αKG lysine hydroxylase KDO1 [28] produced 3-hydroxylysine (**12**) on multigram scale. Subsequent three-step transformation of this intermediate generated the corresponding sulfamidate **13**, which was heated at 70 °C in DMA for 24 h to form protected tambroline (**14**). In parallel, a chemocatalytic C–H borylation [29] was devised to prepare the trisubstituted indole fragment. With the combination of chemocatalytic and enzymatic C–H functionalization, the total synthesis of tambromycin was completed in 10 steps (longest linear sequence) with 2.4% overall yield.

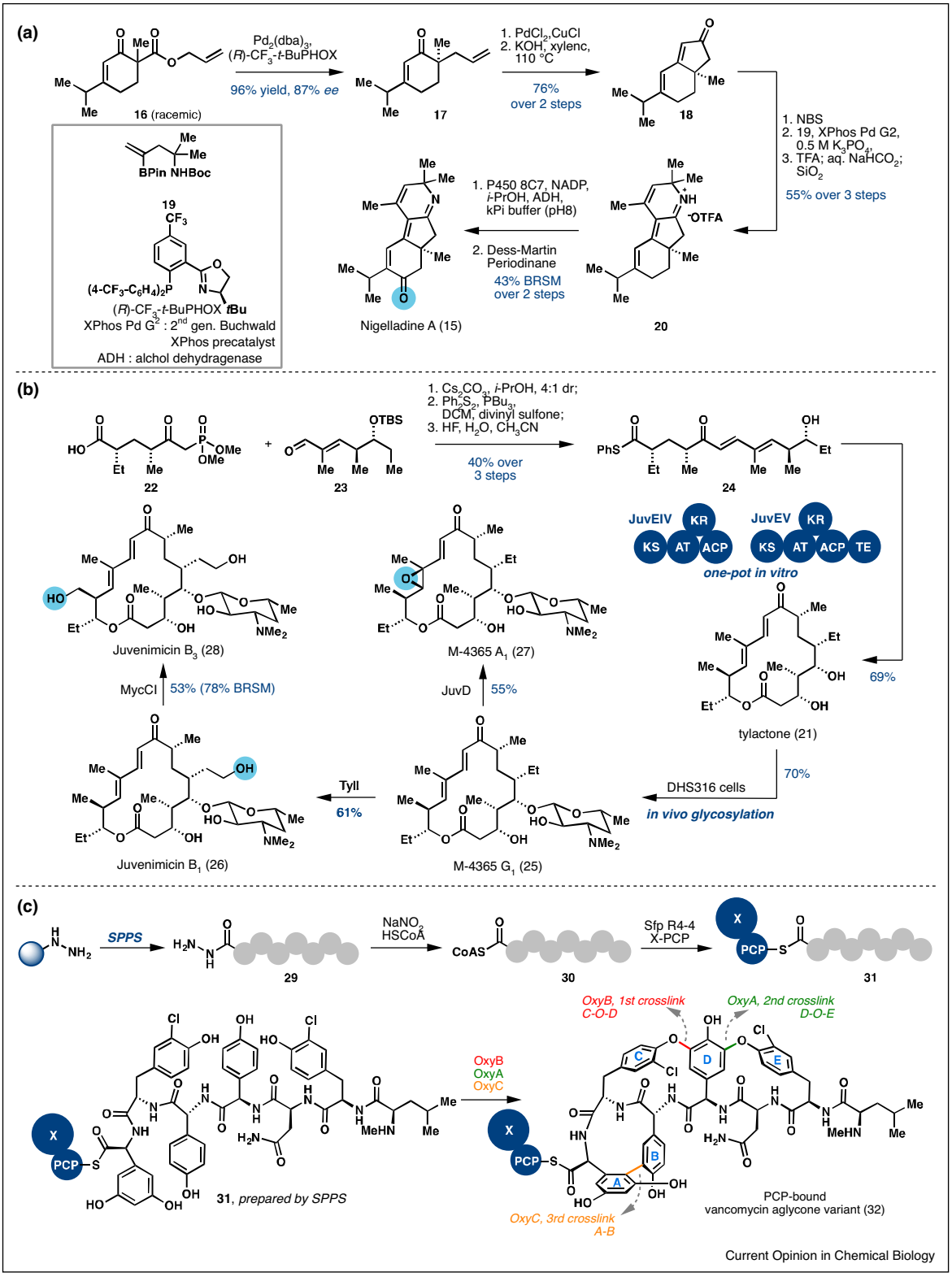
Late-stage functionalization

To allow flexibility in synthesis design, enzymes utilized in late-stage functionalization should ideally be able to accept a range of structurally related advanced intermediates as substrates. Furthermore, substrate promiscuity is a desirable trait if a late-stage enzymatic functionalization is to be employed in subsequent analogue development for medicinal chemistry exploration. Exemplified by the versatile $P450_{BM3}$ [30], members of the cytochrome P450 superfamily have garnered significant attention from the synthetic community due to their substrate and catalytic promiscuity and thus are generally viewed as well suited for applications in late-stage functionalization. In the past few years, several P450s have been utilized in late-stage biocatalytic oxidation *en route* to complex natural products.

Nigelladine A

The first enantioselective total synthesis of nigelladine A (**15**), a highly conjugated norditerpene alkaloid isolated from the *Nigella glandulifera* plant [31], was achieved chemoenzymatically (Figure 3A) by the Arnold and Stoltz groups through a P450-catalyzed allylic oxidation of a late-stage imine intermediate [32••]. The enantiopure enone **17** was synthesized from racemic **16** in three steps through the use of Tsuji–Trost asymmetric allylic alkylation [33], Tsuji–Wacker oxidation [34], and Robinson annulation. Subsequent bromination, Suzuki coupling, and

Figure 3



(a) Application of late-stage biocatalytic oxidation with an engineered $P450_{BM3}$ 8C7 in the total synthesis of nigelladine A. **(b)** Late-stage oxidative diversification of M-4365 G1 with the P450s TylI, MycCl, and JuvD. **(c)** Oxidative phenol couplings catalyzed by the P450s OxyA, OxyB, and OxyC in the chemoenzymatic synthesis of vancomycin aglycone.

condensation completed the construction of the tricyclic skeleton in 55% yield. Disappointingly, various traditional allylic oxidation conditions gave a mixture of inseparable regioisomers or overoxidized byproducts. The selectivity issue was addressed by the use of $P450_{BM3}$ variant 8C7, which was previously evolved for regioselective deprotection of methoxymethyl-protected glycosides [35]. Employing 8C7, the desired hydroxylation product could be obtained with 2.8:1 regioselectivity on 160 mg scale. Subsequent DMP oxidation gave nigelladine A in 43% yield over two steps based on recovered **20**.

The juvenimicins

Tylosin and juvenimicins are a family of potent antibiotics featuring a 16-membered macrolide tylactone (**21**) [36]. In 2017, Sherman and coworkers reported chemoenzymatic total syntheses of tylactone and the juvenimicins (Figure 3B) by late-stage polyketide assembly, tailoring, and C–H functionalizations [37••]. The two enantiopure fragments **22** and **23**, prepared via Evans' and Myers' chiral auxiliary methodologies respectively [38,39], underwent Horner–Wadsworth–Emmons olefination, thioesterification, and desilylation to afford the key hexaketide intermediate **24** in 32% yield over three steps. Subsequent one-pot *in vitro* reaction catalyzed by the P450s JuvEIV and JuvEV introduced the final 4 carbon atoms and forged the desired macrocycle, affording tylactone, the corresponding aglycone of the juvenimicins in 69% yield. Further feeding of tylactone into DHS316, a mutated *Streptomyces venezuelae* strain [40,41], produced M-4365 G_1 (**25**) in more than 60% yield on 100 mg scale. With a large amount of **25** in hand, a divergent enzymatic synthesis of other juvenimicin family members was achieved via late-stage biotransformations with P450 oxygenases TylI, JuvD, and MycCI.

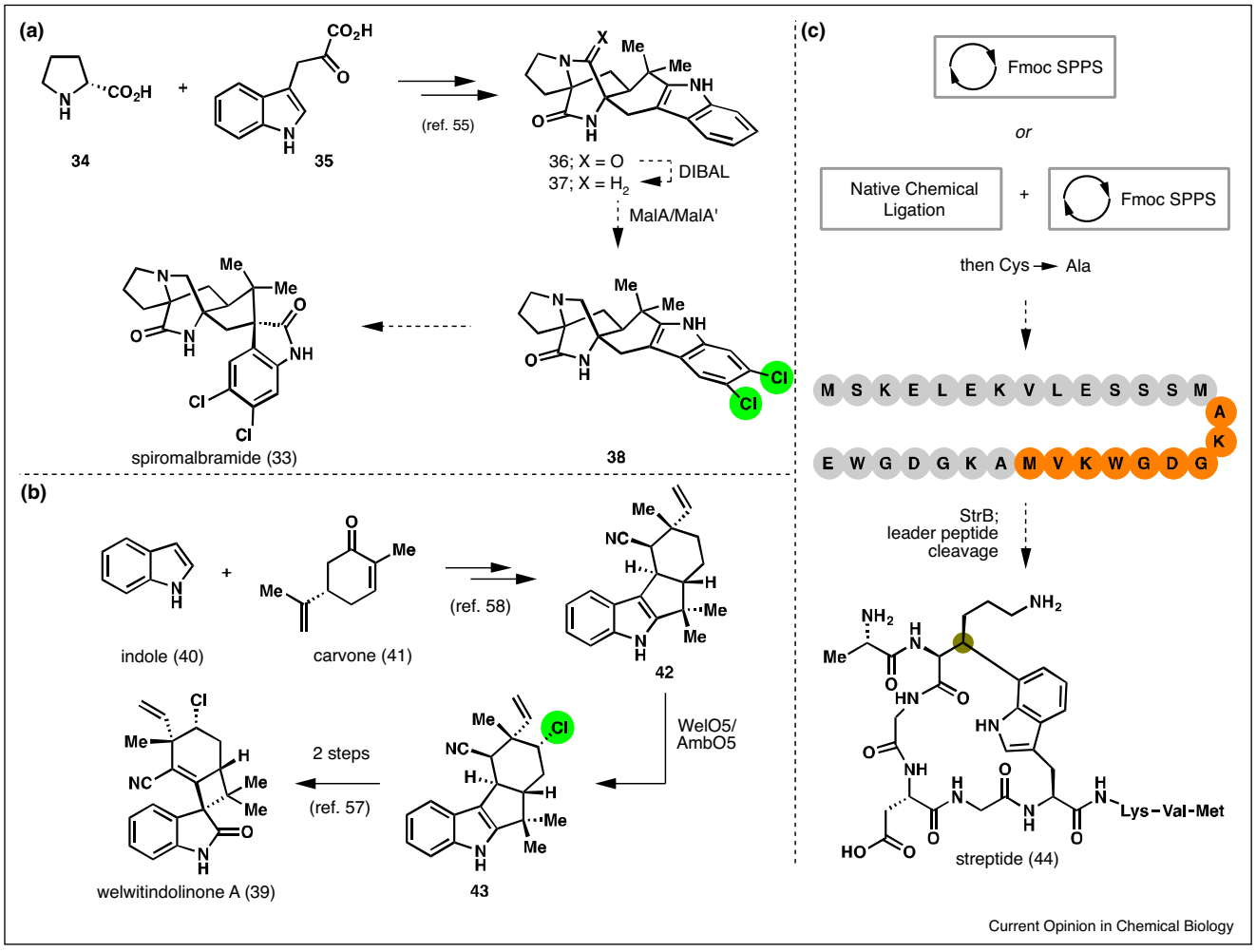
Vancomycin

The glycopeptide vancomycin is used as a drug of last resort to treat serious bacterial infections [42]. Structurally, it is a rigid heptapeptide with three macrocyclic rings, a biaryl linkage and two aryl ether crosslinks. Because of the structural complexity and outstanding clinical value, vancomycin has attracted much attention of synthetic chemists [43], culminating in three total syntheses in the late 1990s [44–50]. In 2018, Seyedsayamdost *et al.* reported a chemoenzymatic synthetic approach (Figure 3C) towards vancomycin aglycone variants [51••]. A 7mer substrate **30** was prepared by SPPS, followed by thioesterification with coenzyme A and pantetheinylation with an X-domain peptidyl carrier protein (X-PCP) to give precursor **31**. Treatment of **31** with three P450s, OxyA, OxyB, and OxyC installed the three synthetically challenging aromatic crosslinks via a sequence consisting of: first, C–O–D aryl ether bond formation by OxyB, second, D–O–E aryl ether bond formation by OxyA, and third, A–B biaryl linkage formation by OxyC. In addition, the synthesis of a thioamide-containing analogue via the same method suggested the potential of this biocatalytic cascade in the creation of vancomycin analogue libraries.

Future directions and outlook

The case studies outlined above suggest that the use of enzymatic C–H functionalization in natural product synthesis has mainly revolved around hydroxylation chemistry. For the field to continue to flourish, it is crucial that we begin tapping into a wider range of biocatalytic transformations. In the last decade, significant progress has been made in the discovery, characterization, and engineering of various enzyme families that catalyze other types of C–H functionalization, including halogenation [10•,11•] and alkylation [12•]. Despite early work by Kirschning on the ansamitocins [52•] and recent success of alkene/arene halofunctionalization by Moore [53•], the use of enzymatic C–H halogenation in chemoenzymatic total synthesis has remained underexplored. Cursory examination of synthetic strategies to access certain natural product motifs, however, quickly reveals the decided advantage of applying these transformations in chemoenzymatic synthesis. For example, many prenylated indole and monoterpene indole alkaloids contain distinct halogenation patterns that are nontrivial to introduce via traditional chemical methods. As an alternative, one can envision an alternative strategy involving late-stage enzymatic halogenation on advanced synthetic intermediates, which in turn can be accessed using established chemical methods. This strategy can be implemented in the synthesis of spiromalbramide (**33** Figure 4A), a dichlorinated spirooxindole alkaloid from *Malbranchea graminicola* [54], by first targeting the construction [55] of the nonhalogenated precursor (premalbrancheamide, **37**). Subjecting **37** to enzymatic halogenation with MalA [56] would furnish the corresponding dichlorinated product, which can in turn be oxidized using established procedures to generate the spirooxindole motif [57]. A similar strategy can also be conceived to access welwitindolinone A (**39**) via enzymatic chlorination of synthetic [58] 12-*epi*-fischerindole U (**42**) with the halogenase WelO5/AmbO5 [59], followed by spirooxindole ring formation (Figure 4B). In the same vein, enzymatic C–H alkylation can be used to construct otherwise challenging C–C bonds in natural product synthesis. While radical SAM enzymes have gained notoriety due to the general sensitivity of the Fe–S cluster, we believe that in certain cases, their use in chemoenzymatic synthesis can be strategically enabling. One potential application can be found in the topological problem presented by the streptide family of natural product [60], which contains a highly unique Lys-Trp crosslink. Here, one can conceive a chemoenzymatic approach towards streptide (**44**) involving solid-phase synthesis or a combination of solid-phase synthesis and native chemical ligation to assemble the linear precursor peptide, which can subsequently be cyclized through the use of StrB [61], the native radical SAM enzyme within the streptide biosynthesis pathway (Figure 4C).

Figure 4



(a) Proposed use of late-stage enzymatic chlorination for the chemoenzymatic synthesis of spiromalbramide. **(b)** Proposed use of late-stage enzymatic chlorination for the chemoenzymatic synthesis of welwitindolinone A. **(c)** Proposed application of StrB in late-stage Lys–Trp crosslinking for the chemoenzymatic synthesis of streptide.

The examples presented in this review serve to highlight the power of enzymatic C–H functionalization in solving challenging problems in natural product synthesis. These developments notwithstanding, we believe that we are nowhere close to realizing the full potential of this platform. Advances in sequencing technology have provided an abundance of genomic data that is now at our disposal. New techniques in DNA synthesis [62] and protein and metabolic engineering [63,64] also hold promise in accelerating the discovery of new, synthetically useful C–H functionalization biocatalysts. These developments will facilitate more widespread incorporation of enzymatic C–H functionalization in natural product synthesis and will enable the invention of creative biocatalytic retrosynthetic disconnections that will bring us closer to achieving 'ideality' in synthesis [65].

Conflict of interest statement

Nothing declared.

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