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Reinvigorating the Chiral Pool: Chemoenzymatic Approaches to Complex Peptides and Terpenoids

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CONSPECTUS

Biocatalytic transformations that leverage the selectivity and efficiency of enzymes represent powerful tools for the construction of complex natural products. Enabled by innovations in genome mining, bioinformatics, and enzyme engineering, synthetic chemists are now more than ever able to develop and employ enzymes to solve outstanding chemical problems – one of which is the reliable and facile generation of stereochemistry within natural product scaffolds. In recognition of this unmet need, our group has sought to advance novel chemoenzymatic strategies to both expand and reinvigorate the chiral pool. Broadly defined, the chiral pool comprises cheap, enantiopure feedstock chemicals that serve as popular foundations for asymmetric total synthesis. Among these building blocks, amino acids and enantiopure terpenes, whose core structures can be mapped onto several classes of structurally and pharmaceutically intriguing natural products, are of particular interest to the synthetic community.

In this Account, we summarize recent efforts from our group in leveraging biocatalytic transformations to expand the chiral pool, as well as efforts toward the efficient application of these transformations in natural products total synthesis – the ultimate testing ground for any novel methodology. First, we describe several examples of enzymatic generation of noncanonical amino acids as means to simplify the synthesis of peptide natural products. By extracting amino acid hydroxylases from native biosynthetic pathways, we obtain efficient access to hydroxylated variants of proline, lysine, arginine, and their derivatives. The newly-installed hydroxyl moiety then becomes a chemical handle that can facilitate additional complexity generation, thereby expanding the pool of amino acid-derived building blocks available for peptide synthesis. Next, we present our efforts in enzymatic C–H oxidations of diverse terpene scaffolds, in which traditional chemistry can be combined with strategic applications of biocatalysis to selectively and efficiently derivatize several commercial terpenoid skeletons. The synergistic logic of this approach enables a small handful of synthetic intermediates to provide access to a plethora of terpenoid natural product families. Taken together, these findings demonstrate the advantages of applying enzymes in total synthesis in conjunction with established methodologies, as well as toward the expansion of the chiral pool to enable facile incorporation of stereochemistry during synthetic campaigns.

Graphical Abstract

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Author Contribution

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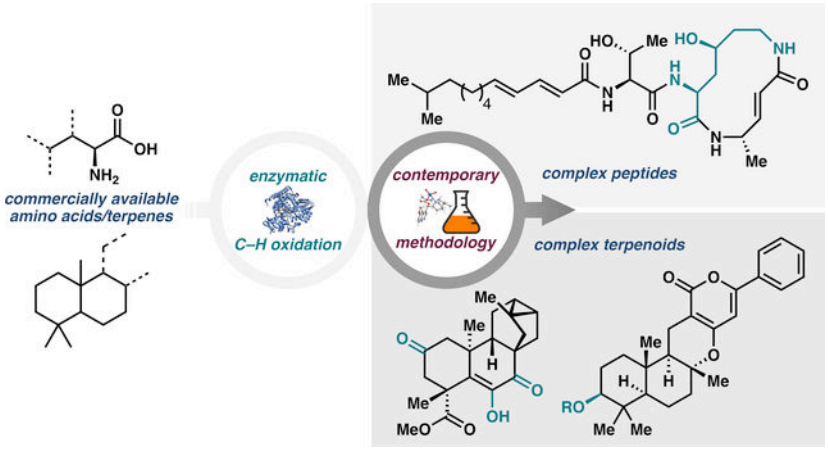

1. INTRODUCTION

The regio-, chemo-, and stereoselective functionalization of C–H bonds represents a powerful paradigm for the construction and derivatization of complex natural products.[5] Given the ubiquity of C–H bonds in organic molecules, the ability to chemically differentiate them to facilitate oxidations, fragment couplings, and other transformations stands as a holy grail in synthetic chemistry. Despite the ongoing expansion of the synthetic repertoire, selective C–H functionalization remains a significant challenge, especially in complex settings.

Recent advances in genome mining and microbial genetics have granted unprecedented access to biosynthetic enzymes, and the ability to heterologously express and isolate them with relative ease has rendered biocatalysis more feasible than ever before.[6] Recognizing the inherent potential therein, synthetic chemists have begun to apply enzymes in methodology and synthesis.[7] However, the field is still very much in its infancy, and the biocatalytic utility of many enzyme superfamilies, such as Fe- and α-ketoglutarate-dependent dioxygenases (Fe/αKGs), remains largely untapped, especially for natural product synthesis.[8] Responding to the demand for widely applicable C–H functionalization strategies, our group has sought to leverage biocatalytic methods for selective C–H oxidation en route to a variety of natural products in the past few years. During this time, contemporaneous efforts from other research groups, such as Narayan,[7a] Stoltz,[7c] Sherman,[7d] Fasan[7e] and Moore,[8a] have also provided significant contributions to address various unmet knowledge gaps in the field.

To date, we have focused on the biocatalytic oxidation of readily available compounds as a means to prepare building blocks and synthetic intermediates that are otherwise challenging to construct; this can be viewed as a reinvigoration of the chiral pool approach to natural product synthesis (Figure 1). Drawn upon for decades as a source of inexpensive stereochemical information, the chiral pool comprises a diverse array of enantiopure feedstock chemicals that can be easily converted into useful starting materials or chiral catalysts/reagents.[9] Many terpenes and amino acids are commercially available in various stereoforms and thus represent important members of the chiral pool. Much effort has been devoted to selectively derivatizing these compounds, but overriding the innate reactivity of

their scaffolds remains a difficult undertaking in organic synthesis.[9,10] To bolster the traditional synthetic toolbox, we have advanced a number of biocatalytic methodologies to functionalize amino acid and terpene building blocks, ultimately with an eye toward natural products synthesis.

2. AMINO ACID FUNCTIONALIZATION

Amino acids represent a highly useful class of organic small molecules, finding practical application as pharmaceuticals and small molecule probes, among many others.[10] The twenty canonical amino acids, so called due to their incorporation into proteins during translation, are commercially available and provide traditional methodology and synthesis with valuable sources of stereochemical information. Conversely, noncanonical amino acids (ncAAs) trace their biological origins to post-translational modifications in protein biosynthesis or to secondary metabolic pathways.[11] Given that the presence of ncAAs in small molecules can influence biological activity, such compounds are often attractive drug or probe candidates.[4,12] The synthetic community has devoted significant effort to establishing concise routes to ncAAs, ultimately in pursuit of rapid access to ncAA-containing natural products and drug scaffolds. Due to the innate chemical challenges posed by amino acids, including the presence of free amino and carboxylate moieties and potentially reactive side chains, as well as the need to set one or more stereogenic centers, construction of ncAAs from existing amino acids or by de novo synthesis remains difficult.[11]

Therefore, our group has turned to Nature for inspiration, noting that nonribosomal peptide biosynthesis often utilizes hydroxylation as a gateway transformation to synthesize ncAAs.[12] We looked to reproduce this process *in vitro* by (1) directly access hydroxylated amino acid building blocks and (2) preparing additional ncAAs by utilizing the newly-introduced alcohol as a chemical handle for further complexity generation. In the following section, we describe our exploration of biocatalytic hydroxylation as an effective means to derivatize amino acids, as well as our applications of these methods toward syntheses of ncAA-containing natural products.

a. HYDROXYLATION AS A GATEWAY

At the outset of our group, we were aware of several proposed biogeneses of 4-methylproline in nonribosomal peptides that invoked iterative oxidation of leucine, intramolecular amine condensation, and subsequent reduction of the cyclic species.[13] Inspired by Nature's strategy, we sought to replicate and subsequently improve upon this sequence in the flask, first using Fe/αKGs to hydroxylate amino acid scaffolds and then converting the resulting alcohol into other useful functional groups – either via biocatalytic or chemical means – to facilitate more diverse transformations.

In 2015, the leucine δ-hydroxylase GriE was implicated in the biosynthesis of griselimycin[14] – a peptide natural product containing two 4-methylproline motifs – and was later found to work in tandem with zinc-dependent dehydrogenase GriF to effect iterative leucine oxidation and imine formation.[15] Looking to obtain a robust biocatalyst for preparative-scale leucine hydroxylation, we acquired pure GriE from heterologous

expression in high yield (*ca.* 100 mg from 1 L of culture) and subjected a large panel of amino acids to hydroxylation in the presence of αKG, ascorbate, $FeSO_4$, and O_2.[1] GriE readily converted leucine to the corresponding δ-hydroxylated product with complete regio- and diastereoselectivity and high total turnover number (TTN). Several other amino acids were also accepted as substrates, exclusively yielding δ-hydroxylation with complete diastereoselectivity in nearly all cases (Figure 2A). The impressive promiscuity of GriE is complemented by excellent scalability, as reactions in GriE-containing lysate could be run at high substrate concentrations (up to 50 mM in leucine) on gram scale with no decrease in conversion. Other amino acid substrates proceeded in high conversion on 100–200 mg scale, further validating the utility of GriE. During this study, reactions conducted in lysate were found to be more scalable and convenient than with purified enzyme, simply requiring sonication of resuspended cells followed by addition of the appropriate substrates and cofactors (αKG, Fe^{2+} and ascorbic acid for Fe/αKGs, NAD(P)H for P450s). Subsequent work from our lab has predominantly employed lysate for scaled-up reactions.

We then sought to implement GriE toward the synthesis of manzacidin C (**11**), a densely functionalized alkaloid natural product from *Hymeniacidon* sp.[16] A two-step process has been reported to convert lactone **10** to manzacidin C, but efficient, step-economic access to **10** has yet to be achieved.[17] We proposed a formal synthesis of **10**, wherein biocatalytic hydroxylation would introduce a primary alcohol at C5 and facilitate lactone formation via routine intramolecular cyclization. In light of the substrate-activity relationship of GriE, we envisioned that a masked amine derivative of leucine could be submitted to hydroxylation and later revealed as the amine.

Thus, treatment of leucine with tetrabutylammonium decatungstate (TBADT) and azide **6** under photocatalytic conditions gave azidoleucine **7**,[18] which was subjected to reaction with GriE to deliver the desired hydroxylated product **8** with >95% conversion. A telescoped hydrogenation/dual Boc protection/selective lactonization procedure then afforded lactone **10** in 41% yield over two steps (Figure 2B). Given the aforementioned two-step elaboration of **10** to the natural product, our route represents a five-step formal synthesis of manzacidin C and a drastic improvement in step economy over prior approaches.[19] This improvement, coupled with absolute regio- and stereocontrol, underscores the capability of enzymatic C–H functionalization to streamline synthetic efforts. At the time of publication, this work also comprised the first use of an Fe/αKG-dependent enzyme in natural product synthesis.

During the characterization of GriE, we discovered that GriE also performs iterative δ-oxidation on γ-methylleucine, which led us to investigate the use of GriE to construct various proline derivatives. Leucine and several related analogues were submitted to a two-step, one-pot sequence of GriE-catalyzed oxidation followed by *in situ* imine reduction with $NH_3{\cdot}BH_3$, which provided proline analogues **14a**–**e** in high yields and with complete stereocontrol (Figure 2C). This highly efficient protocol stands in contrast to existing chemical methods, which often lack stereocontrol at C4 and require several functional group interconversions. A similar method was devised to access 3-hydroxy-3-methylproline (**18**) from isoleucine using the Fe/αKGs UcsF and GetF,[20] thereby demonstrating the broad applicability of this strategy and laying the groundwork for access to 3-hydroxy-3-methylproline-containing natural products (Figure 3A).

To highlight the synthetic utility of our approach, we devised a total synthesis of cavinafungin B (**22**), an antiviral lipopeptide natural product containing 4-methylproline.[21] Having already obtained access to 4-methylproline via action of GriE and subsequent imine reduction (Figure 2B), we performed Fmoc protection of the free amine and executed a solid-phase peptide synthesis (SPPS) campaign featuring an AT(Boc)G-Rink resin linkage. After four rounds of successive PyAOP/NMM-mediated peptide coupling and piperidine-mediated Fmoc deprotection, a sequence of oleic acid coupling, global deprotection, and resin cleavage ultimately gave cavinafungin B in 37% yield over 10 steps (Figure 3C).

Beyond facilitating lactone formation or iterative oxidation, hydroxylation can also serve as a gateway to other functional groups, as illustrated by our synthesis of tambromycin (**37**),[22] a cytotoxic peptide produced by several *Streptomyces* strains (Scheme 1).[23] Comprising four modified amino acid monomers, tambromycin derives its name from the presence of tambroline, a rare pyrrolidine-containing ncAA originating biogenically from lysine. This biosynthetic proposal and previous synthetic efforts toward similar compounds led us to attempt hypoiodite-based C–H amination at the β-position of lysine to deliver the pyrrolidine ring. However, this strategy proved unsuccessful.

We turned instead to KDO1, an Fe/αKG reported to catalyze β-hydroxylation of lysine,[24] envisioning that the pyrrolidine motif could be constructed through a stereocontrolled displacement of the hydroxylysine β-OH by its ε-amine. Initial heterologous expression of KDO1 provided insufficient yield of soluble enzyme, but following co-expression of the molecular chaperones GroES/GroEL,[8c,25] reaction with KDO1 allowed for hydroxylation of 4.1 g of lysine from 1 L of expression culture, giving >99% conversion to **24** at high (35 mM) substrate concentration. Following routine protecting group introductions, **25** was converted to sulfamidate **26**, which was heated in DMA to cleanly give protected tambroline **27**.

Next, a C6-selective C–H borylation/halogenation sequence was chosen to construct the 3,4,6-trisubstituted indole motif of tambromycin.[26] Thus, treatment of **31** with B_2Pin_2 and catalytic $[Ir(cod)OMe]_2$ followed by chlorination with $CuCl_2$ gave indole **32**, which was quickly converted to acid **33** after N1 methylation and ester hydrolysis. The remainder of the synthesis followed summarily from elaboration of **33** and tambroline **27**, wherein a series of peptide couplings and functional group interconversions gave tambromycin after the liberation of the terminal carboxylic acid from the methyl ester. Out of this work emerged the first total synthesis of tambromycin, empowered by harnessing two C–H functionalization methods in tandem: namely, a gram scale procedure for biocatalytic implementation of KDO1 to hydroxylate the β position of lysine and a modular chemocatalytic approach to synthesize 3,4,6-trisubstituted indoles.

b. HYDROXYLATION AS A DESTINATION

We have also sought to leverage biocatalysis to access natural products that themselves contain hydroxylated ncAAs. In particular, 4-hydroxylysine, 4-hydroxycitrulline, and 4-hydroxyarginine are found in several nonribosomal peptides, though the current chemical state-of-the-art is unable to selectively and efficiently access such functionalities. Thus, we have developed biocatalytic procedures for hydroxylation of the parent amino acids and

applied them to the total syntheses of several natural products and derivatives thereof, namely cepafungin I and GE81112 B1.

The syrbactins comprise a family of peptide-polyketide natural products that share a common 12-membered unsaturated macrolactam and display potent inhibitory activity against the eukaryotic 20S proteasome core particle.[27] Given the prior success of proteasome inhibitors in the treatment of multiple myeloma,[28] the syrbactins are attractive candidates for anticancer applications. Among the family members, cepafungin I (**38**) was reported to display the highest potency with an IC_{50} of 4 nM against purified yeast 20S proteasome.[29] We noted that the glidobactin/cepafungin biosynthetic gene cluster features the enzyme GlbB,[30] which belongs to the PF10014 Fe/αKG family and is likely responsible for formation of the 4-hydroxylysine motif. Motivated to fill a gap in the lysine derivatization literature,[31] we sought to characterize GlbB and evaluate its utility as a hydroxylation biocatalyst.

In vitro assay with purified GlbB revealed that the enzyme could hydroxylate the γ-position of lysine with high turnover number (5900 TTN) and excellent diastereoselectivity (>99:1).[32] Leucine (γ-hydroxylation) and methionine (S-oxidation) were also accepted as substrates, giving 310 and 330 TTN, respectively (Figure 4A). We next investigated the large-scale preparation of 4-hydroxylysine to ensure adequate material supply for the synthesis of cepafungin I. Co-expression of GlbB with GroES/GroEL[25] increased yield of soluble enzyme such that 6–7 g of lysine could be fully converted to **40** with 1 L of lysate. To access a suitable cyclization precursor, Weinreb amide **44** was used in an $AlMe_3$-mediated coupling with lactone **43**, providing dipeptide **45** in 90% yield. Upon reduction of the amide and olefination of the resulting aldehyde, enoate **46** was subjected to global deprotection and treated with DMTMMT to effect macrocyclization.[33] The macrolactam core (**47**) was coupled with acid **48** to give cepafungin I in 9 linear steps and 7.9% overall yield (Figure 2B).[4]

By biocatalytically expanding the amino acid chiral pool, our route allows for concise introduction of the secondary alcohol to the cepafungin macrolactam, thereby addressing the main shortcoming of prior approaches to the natural product. This strategy also enabled biological investigations into cepafungin I through the synthesis of a chemoproteomic probe and several analogues (Figure 4C).[4] Competitive proteomic profiling experiments with cepafungin I showed remarkable selectivity for 20S proteasome subunits PSMB2 and PSMB5 with minimal off-target activity. Further PSMB5 inhibitory assays with synthetic analogues suggest the importance of the macrocyclic secondary alcohol and the degree of chain unsaturation of the lipid tail, as analogues varying in these key motifs showed drastically attenuated potency relative to the parent natural product.

In addition to the syrbactins, we also set our sights on the GE81112s, an intriguing family of hydroxylated tetrapeptide natural products (Figure 5A).[34] Notable for their dense functionalities and unique antibacterial activity, the GE81112s consist largely of noncanonical amino acids and function as inhibitors of prokaryotic translation. With the exception of the 3-hydroxypipecolic acid unit, which is formed via hydroxylation by the Fe/αKG GetF,[35] little is known about the biogenesis of the GE81112 ncAA fragments. Previous

studies into the GE81112 biosynthetic gene cluster uncovered a dedicated nonribosomal peptide synthase (NRPS) and several tailoring enzymes, including the aforementioned GetF and a second Fe/αKG GetI.[36] Initially proposed to catalyze β-hydroxylation of 2-chlorohistidine,[35] GetI represented a potentially useful biocatalyst to generate ncAAs and aid in the synthesis of GE81112 B1.

Surprisingly, neither 2-chlorohistidine nor histidine was converted to its corresponding hydroxylation product in reaction with GetI.[37] However, the high sequence homology shared by GetI with OrfP[38] and VioC,[39] two previously characterized Fe/αKG arginine hydroxylases, led us instead to examine citrulline (**55**) as a substrate. To our delight, LCMS analysis revealed a monohydroxylated product, and subsequent NMR analysis confirmed its identity as 4-hydroxycitrulline (**56**). At this stage, GetI was noted to exhibit comparable activity on α-amino-δ-carbamoylhydroxyvaleric acid and low levels of hydroxylation activity on arginine. Given that no dedicated arginine γ-hydroxylase was known at the time of this work, this discovery inspired an enzyme engineering campaign to develop such enzyme. Using homology models of OrfP and VioC, we performed sequential site-directed mutagenesis on GetI and obtained variant QDPYF, which was capable of converting arginine to 4-hydroxyarginine with 94 TTN. Preparative scale reaction with *E. coli* lysates expressing GetI QDPYF afforded nearly complete conversion, and we successfully implemented this process in a brief synthesis of a dipeptide fragment of enduracidin.[37]

We next targeted the chemoenzymatic synthesis of GE81112 B1 (**52**),[40] which comprises 3-hydroxypipecolic acid (AA1), 4-hydroxycitrulline (AA2), 2-aminohistidine (AA3), and β-hydroxy-2-chlorohistidine (AA4) (Figure 5B). Chemical construction of these monomers is nontrivial, as a previous synthesis of GE81112 A required 7–8 steps to make each fragment and suffered from poor stereocontrol.[41] A strategy was thus devised that would showcase the strengths of both biocatalysis and contemporary chemical methodology: Specifically, enzymatic hydroxylations with GetF and GetI were proposed to enable construction of AA1 and AA2, whereas traditional chemistry could grant access to AA3 and AA4.

For the preparation of AA1, co-expression of GetF with GroES/GroEL[8c,25] delivered complete conversion of pipecolic acid (**53**) to 3-hydroxypipecolic acid on 500 mg scale. Upon treatment with Boc_2O, protected monomer **65** was obtained in 74% yield over two steps. Toward AA2, GetI facilitated gram-scale conversion of citrulline to 4-hydroxycitrulline and provided, after subsequent protecting group adjustments, fragment **66** in four steps and 41% overall yield. Unmasking of the primary amine **67** from **66** was followed by routine coupling with **65** to give dipeptide **68** after saponification.

Preparation of AA3 proceeded via two-step functionalization of Boc-His-OMe (**69**), wherein C2 azotisation of its imidazole ring was followed by saponification of the methyl ester to reveal acid **70**. Construction of AA4 proved more challenging, as several attempts at an asymmetric aldol reaction were met with failure. Finally, upon optimization of the steric environment, reaction of titanium enolate **71** and aldehyde **72** gave the desired adduct as a single diastereomer in 59% yield after methanolysis. Treatment with aqueous ammonium sulfide cleanly provided the desired amine **73**, which was coupled with acid **70** and deprotected under buffered conditions to deliver dipeptide **74**.

With **68** and **74** in hand, the corresponding peptide coupling reaction proceeded uneventfully to afford the desired protected tetrapeptide in 66% yield as a single diastereomer. The use of H_2 and PtO_2 cleanly reduced the azo moiety of AA3 to the corresponding amine, and after ester hydrolysis and global deprotection we successfully isolated the pure TFA salt of GE81112 B1 in 11 steps (longest linear sequence) (Scheme 2). Importantly, our synthesis features an ideal combination of biocatalytic and chemical approaches, wherein the strengths of each paradigm are leveraged to streamline construction of the complex GE81112 scaffold. This route has also enabled synthesis of several analogues, facilitating structure-activity relationship studies and identification of the key pharmacophores of GE81112 B1.[40]

3. TERPENE FUNCTIONALIZATION

In stark contrast to amino acids, terpenes consist primarily of hydrocarbons in varying degrees of saturation and possess few other functional groups. Despite this, the terpene chiral pool is exceptionally diverse and has provided materials for many total syntheses of terpene-derived natural products.[9b] Indeed, as sources of carbon framework and stereochemical information, enantiopure terpenes are broadly employed as cheap synthetic building blocks and are generally available in both stereoisomers. Our lab has been increasingly interested in a number of these inexpensive terpene building blocks; namely, sclareolide (**89**), sclareol (**90**), and stevioside (**121**). Available in kilogram quantities for ca. $1/g, these compounds have begun to see application in chiral pool terpene synthesis,[42] though their broad use remains limited due to a general inability to regio- and stereoselectively derivatize different positions on their skeletons. Eager to demonstrate the potential of these compounds, our group has leveraged biocatalysis to functionalize positions previously inaccessible to traditional chemistry, thereby reinvigorating these molecules for use in chiral pool synthesis.

a. SCLAREOL AND SCLAREOLIDE OXIDATION: MEROTERPENOIDS

Meroterpenoids constitute a large family of natural products that arise from mixed terpenoid-polyketide biosynthetic pathways (Figure 6A).[43] Two subclasses are the α-pyrone meroterpenoids,[44] which contain a C3-oxidized drimane unit coupled to a polyketide pyrone, and the meroditerpenoids,[45] which bear a C3-oxidized *ent*-isocopolane motif appended to various aromatic moieties. Traditional synthetic approaches to the C3-oxidized terpene core rely on polyene cyclization from polyprenyl-derived epoxides[46] or elaboration of the Wieland-Miescher ketone[46] but often suffer from poor stereoselectivity and synthetic efficiency. Moreover, as meroterpenoid natural products possess diverse biological activities, there is considerable demand for concise and modular routes to these compounds and analogues thereof.

We thus sought to implement enzymatic C–H oxidation to access the core terpenoid skeleton and to leverage recent advances in radical chemistry to introduce the polyketide unit. Retrosynthetically, the α-pyrone meroterpenoids could be traced to sclareolide, and the meroditerpenoids could arise in a similar fashion from sclareol (Figure 6B).[2] Radical transformations would then effect conjugation of the pendant aromatic functionalities or late-stage modification of the skeleton. We hoped to employ two common intermediates to

construct eight oxidized meroterpenoids, thereby highlighting the potential of an expanded chiral pool, wherein increased utility of key starting materials empowers modular synthesis of a number of complex scaffolds.

At this stage, we were aware of a number of P450 monooxygenases reported to catalyze C3 hydroxylation of sclareolide, [48] though their use was limited to 50 mg scale reactions performed under high substrate dilution. A brief enzyme engineering campaign was conducted, wherein several variants of $P450_{BM3}$ were tested for activity on sclareol and sclareolide. Out of this screen emerged BM3 MERO1, which selectively hydroxylated C3 of sclareolide to >95% conversion and exhibited low levels of analogous activity on sclareol. Importantly, hydroxylation of sclareolide was amenable to large scale, consistently giving 80–90% conversion and 60–70% isolated yield of **91**. Moreover, the use of phosphite dehydrogenase Opt13 alongside BM3 MERO1 allowed for substoichiometric use of NADPH, greatly decreasing cost of material preparation.[49]

Having established the utility of BM3 MERO1, we next sought to access aldehyde **92** as the branching point toward the α-pyrone meroterpenoids. Thus, a four-step tailoring sequence was executed to fragment the lactone moiety of **91** and give **92** in 54% overall yield. A screen of Lewis and Brønsted acids identified phosphoric acid **95** as a suitable catalyst to append the aromatic pyrone moieties (**96**–**98**) in a formal [3 + 3] transformation. This strategy enabled access to **99** and **101**, whereas **104** followed from initial dehydration of the tertiary alcohol to enal **93**. En route to meroterpenoids **102**, **103**, and **105**, chemoselective hydrogen atom transfer (HAT) reduction proved the only viable method to regio- and stereoselectively convert the C9–C11 alkene to the desired hydrogenated products. Lastly, the unique oxidation pattern of **106** required elaboration of **93** to acid chloride **94**, followed by Friedel–Crafts acylation and cyclization/reduction for the completion of its synthesis.

In tandem with the above efforts, we also pursued syntheses of several C3-oxidized meroditerpenoids. Due to the poor activity of BM3 MERO1 on sclareol, the skeleton was elaborated in 5 steps to acid **107**, and each intermediate was subjected to enzymatic hydroxylation with BM3 MERO1 and other $P450_{BM3}$ mutants. Acid **107** showed the highest activity, and after further alanine screening we identified variant MERO1 L75A, which provided 62% isolated yield of the C3-hydroxylated species **108** on gram scale. Hydroxylated acid **108** was then transformed to iodide **109**, which served as the divergency point in the series. Treatment of **109** with catalytic Ni(II) and partners **111** and **112** successfully afforded the desired coupling products, which each required two further steps to complete the synthesis of taondiol (**113**) and chevalone A (**115**), respectively.

Alternatively, access to decaturin E (**118**) and stypodiol (**120**) proceeded from diene **110** – obtained via *t*BuOK-mediated elimination of iodide **109** – through a single-electron-transfer-based (SET) [3 + 2] transformation. Whereas ceric ammonium nitrate (CAN) effected coupling of diene **110** and pyrone **116** to deliver decaturin E, phenol **117** required deployment of electrochemical methodology.[50] With coupled product **119** in hand, we turned again to selective HAT reduction, followed by BBr_3-mediated demethylation to give stypodiol in 58% yield over two steps (Scheme 3B). Overall, this work demonstrates the efficient merger of enzymatic oxidations and radical-based methodology toward several

meroterpenoid natural products and provides the foundation for the synthesis of other drimane-containing structures.

b. STEVIOSIDE DERIVATIZATION: *ENT*-KAURANE, *ENT*-ATISANE, *ENT*-TRACHYLOBANE DITERPENOIDS

Given the success of our meroterpenoid campaign, we looked to extend this paradigm of chiral pool synthesis to the *ent*-kaurane, *ent*-atisane, and *ent*-trachylobane diterpenoids.[51] Arising from unique carbocationic rearrangements of *ent*-copalyl pyrophosphate,[52] these terpenoid families share many structural features but differ in the architecture of their C and D rings. Furthermore, family members display a wide range of biological activities,[52] making them appealing targets for medicinal chemistry evaluation and chemical probe development. Previous semisynthetic studies[53] inspired us to examine the *ent*-kaurane diterpene stevioside (**121**) as a potential starting point for our endeavor. At $0.65/g, stevioside is available in bulk quantities and can be readily converted to the aglycones steviol (**122**) and isosteviol (**137**), though synthetic methodologies to selectively functionalize its *ent*-kaurane skeleton are exceedingly limited. Thus, we sought to develop a biocatalytic C–H oxidation program that would afford rapid access to not only the *ent*-kauranes, but also the *ent*-atisanes and *ent*-trachylobanes through manipulation of the C and D rings.

At the outset, it was crucial to identify enzymes that could selectively oxidize the A, B, and C rings of steviol and related structures. Previous collaboration with the Shen lab in the characterization of the platensimycin biosynthetic pathway (Figure 7), as well as our aforementioned work with $P450_{BM3}$ variants, revealed several potential enzymes for this purpose.[54] After a comprehensive screening campaign, three of these enzymes – $P450_{BM3}$ variant BM3 MERO1 M177A, the Fe/αKG PtmO6, and the chimeric P450 PtmO5-RhFRed – emerged as promising biocatalysts to effect selective hydroxylation of the A, B, and C rings, respectively, of steviol and *ent*-kaurenoic acid (Figure 7).[3] Critically, each enzyme was amenable to preparative scale and accepted a range of substrates en route to *ent*-kaurane, *ent*-atisane, and *ent*-trachylobane natural products.

With three efficient terpene hydroxylases in hand, we first pursued divergent syntheses of mitrekaurenone (**126**), fujenoic acid (**128**), and pharboside aglycone (**129**), each of which would require only B ring oxidation (Scheme 4A). Starting from *ent*-kaurenoic acid (**123**), we performed C7 hydroxylation with PtmO6 to obtain secondary alcohol **124** in high yield as a single diastereomer. Toward mitrekaurenone, **124** was oxidized to ketone **125** and submitted to α-oxidation to effect intramolecular lactonization, giving **126** in five steps and 36% overall yield. Alternatively, it was found that ketone **125** could be oxidized by PtmO6 to C6-alcohol **127**, which was then treated successively with $NaIO_4$ and DMP to afford fujenoic acid in seven steps and 26% overall yield. Finally, access to pharboside aglycone came in three steps from secondary alcohol **124**, featuring methyl esterification, dehydration, and dual dihydroxylation.

To further demonstrate the utility of our approach, we targeted rosthornin B (**134**) and C (**133**) and fischericin B (**136**), *ent*-kauranes containing oxidations on multiple rings (Scheme

4A). Stevioside was first deglycosylated to steviol, which was then subjected to a two-step oxidation sequence involving enzymatic installation of the C7 alcohol with PtmO6 and subsequent PDC oxidation to ketone **130**. Gratifyingly, PtmO5-RhFRed acted on **130**, selectively introducing the C11 alcohol to give **131**, which was elaborated in two further steps to intermediate **132**. Following installation of the D-ring enone, synthesis of rosthornin C was completed, and rosthornin B could be accessed via acetylation of the C19 alcohol. Fischericin B, on the other hand, lacks any oxidation on its B ring. Starting again from steviol, a three-step sequence was employed to (1) hydroxylate at C11 with PtmO5-RhFRed, (2) form the corresponding ether, and (3) esterify the C19 carboxylate to give **135** in 65% overall yield. Completion of the synthesis proceeded via selective tailoring steps and HAT-based functionalization at C20, furnishing fischericin B in nine total steps and 25% overall yield.

We next sought to employ a carbocation rearrangement strategy to access the alternative C and D ring architectures of *ent*-atisane and *ent*-trachylobane natural products (Scheme 4B).[52] In contrast to the *ent*-kaurane series, entry to these scaffolds began with isosteviol (**137**), obtained in a single step from stevioside. Stereo- and regioselective hydroxylation at C12 was accomplished in excellent yield with PtmO5-RhFRed to give secondary alcohol **138**, which was then treated with trifluoromethanesulfonic acid to effect rearrangement to *ent*-atisane tetracycle **139**. Further validating this approach, **139** could be converted to *ent*-trachylobane **140** in two additional steps. Thus, the *ent*-atisane and *ent*-trachylobane architectures could be accessed in two and four steps, respectively, from isosteviol, ultimately enabled by biocatalytic C–H hydroxylation.

Using **139** as a springboard to *ent*-atisane natural products, we targeted spiramilactone C (**143**), which contains further B ring oxidation, and cochleareine (**146**), which contains further A ring oxidation (Scheme 4B). Our strategy delivered late-stage synthetic intermediates **142** and **145**, each of which contains the requisite functionalities to be elaborated to their corresponding target natural products. Analogously, intermediate **140** served as a gateway to the mitrephorone family of *ent*-trachylobane natural products (Scheme 4C). First, construction of mitrephorone C (**148**) was initiated by a series of enzymatic and chemical oxidations of **140**, wherein BM3 MERO1 M177A and PtmO6 oxidized at C2 and C7, respectively, providing diketone **147** after reaction with PDC. At this juncture, we leveraged the promiscuous C6-hydroxylation activity of PtmO6 to install an α-hydroxyketone that was treated sequentially with diazomethane and DMP to effect methyl esterification and enone formation, yielding mitrephorone C in nine steps and 12% overall yield. Analogously, mitrephorones A and B were accessed by C7 hydroxylation of **140** with PtmO6 to give alcohol **149**. PDC oxidation and a second PtmO6-catalyzed hydroxylation delivered an α-hydroxyketone that was elaborated in similar fashion to mitrephorone B (**150**). Serendipitously, it was discovered that **150** could undergo autooxidation to mitrephorone A (**151**) over several days, thus completing the synthesis of the mitrephorone family of natural products. Overall, our strategy toward these natural products not only represents a highly efficient, modular synthesis of various terpene scaffolds, but also lays the groundwork for combining biocatalytic and chemical oxidation methodologies in total synthesis.

4. CONCLUSIONS

Inspired by cutting-edge chemical methodology and by Nature's enduring biosynthetic logic, the above work represents a synthetic paradigm that is distinct from but also complementary to other approaches in chemical synthesis. In particular, the use of enzymatic C–H oxidation to uniquely modify commercial starting materials and provide access to a larger variety of enantiopure building blocks reinvigorates the chiral pool approach to synthesis and, in turn, streamlines synthetic access to a wide variety of natural products. Excitingly, the number of characterized and uncharacterized biosynthetic enzymes is ever growing, providing ample opportunity for biocatalyst discovery and development to further expand this strategy. Though the synthetic repertoire is more complete today than ever before, we hope others are encouraged to view biocatalysis-enabled expansion of the chiral pool as an increasingly viable solution to current and future problems in total synthesis.

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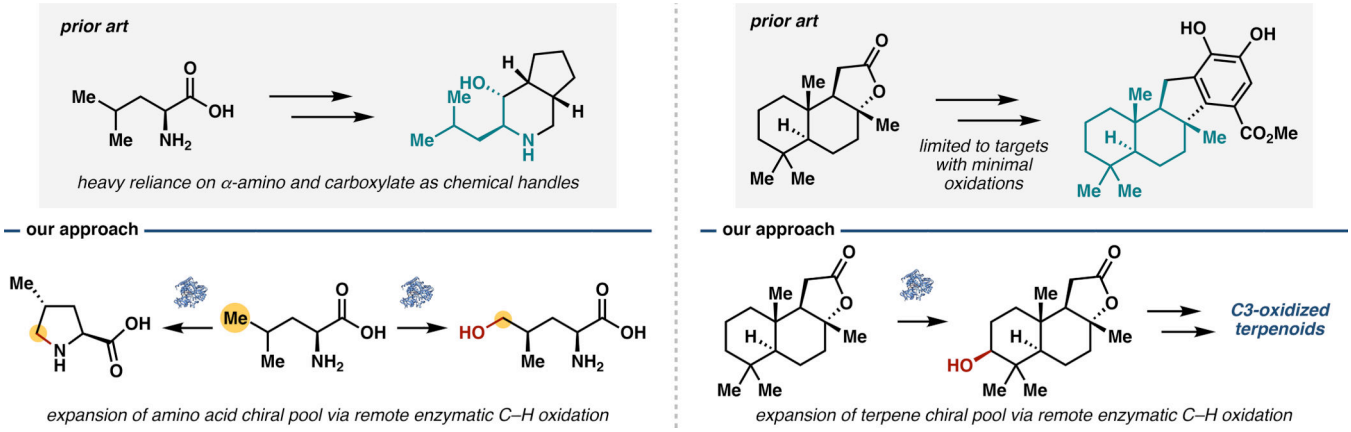


Figure 1.

Comparison between prior applications of amino acid and terpenes in chiral pool synthesis and the approaches advanced by our group.

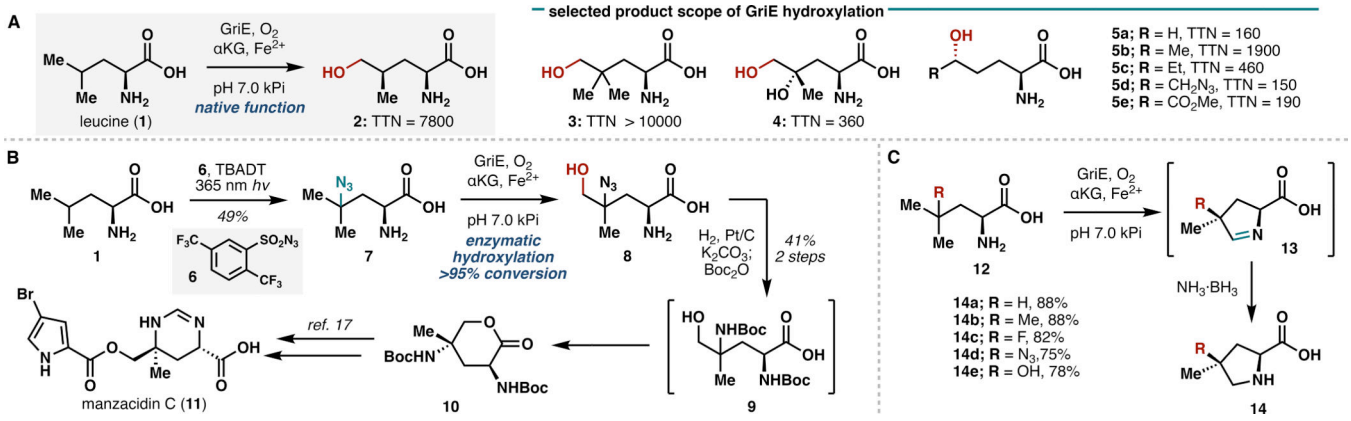


Figure 2.

(A) Native function and substrate scope of GriE. (B) Use of GriE in the chemoenzymatic synthesis of manzacidin C (**11**). (C) Synthesis of various proline analogues via GriE-catalyzed iterative C5 oxidation.

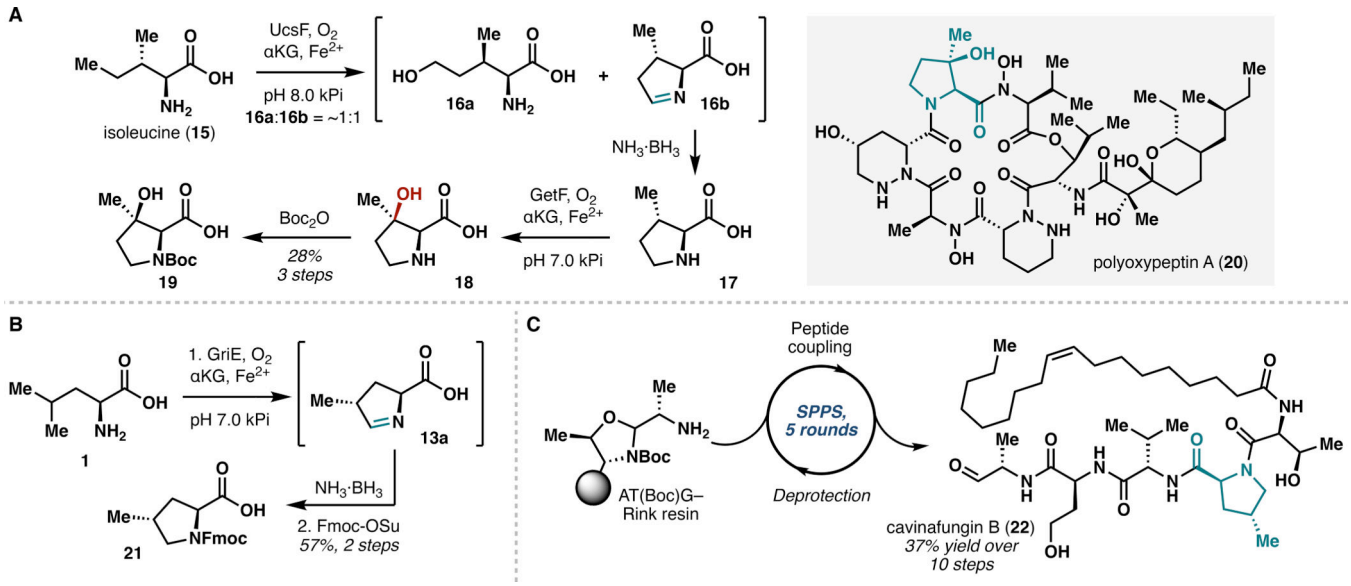


Figure 3.

(A) Chemoenzymatic synthesis of 3-hydroxy-3-methylproline (**18**) utilizing UcsF and GetF. (B) GriE-enabled synthesis of Fmoc-protected 4-methylproline monomer **21**. (C) One-pot solid-phase peptide synthesis of cavinafungin B (**22**).

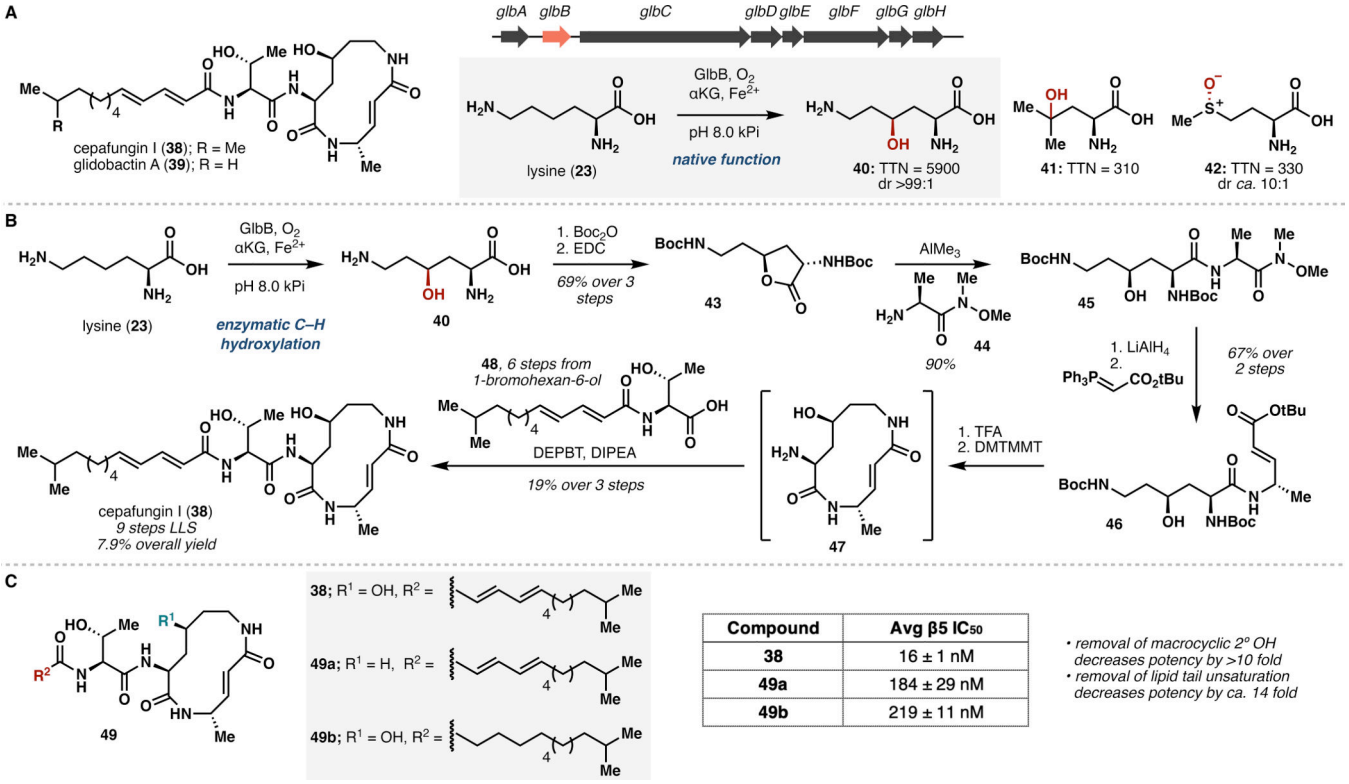


Figure 4.

(A) Characterization of lysine hydroxylase GlbB and selected substrate scope. (B) Nine-step total synthesis of cepafungin I empowered by lysine hydroxylation with GlbB. (C) PSMB5 inhibitory assay results with synthetic analogues of **38**.

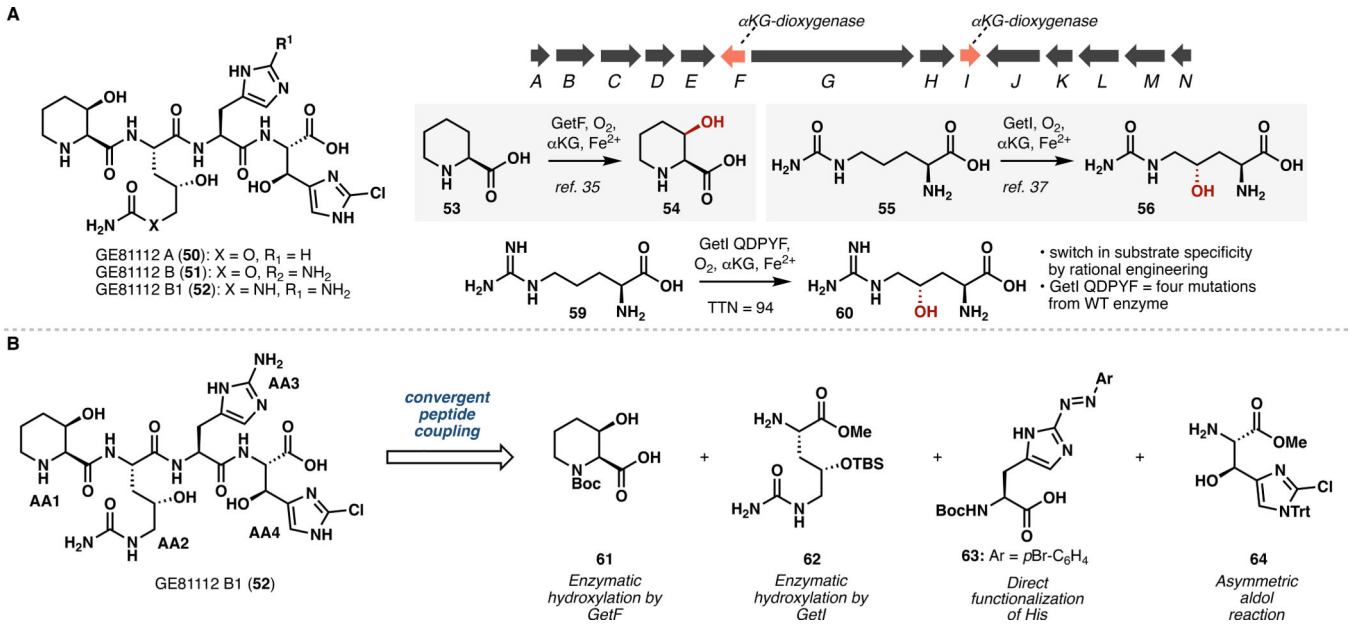


Figure 5.

(A) Characterization and rational engineering of GetI. (B) Retrosynthetic analysis of GE81112 B1.

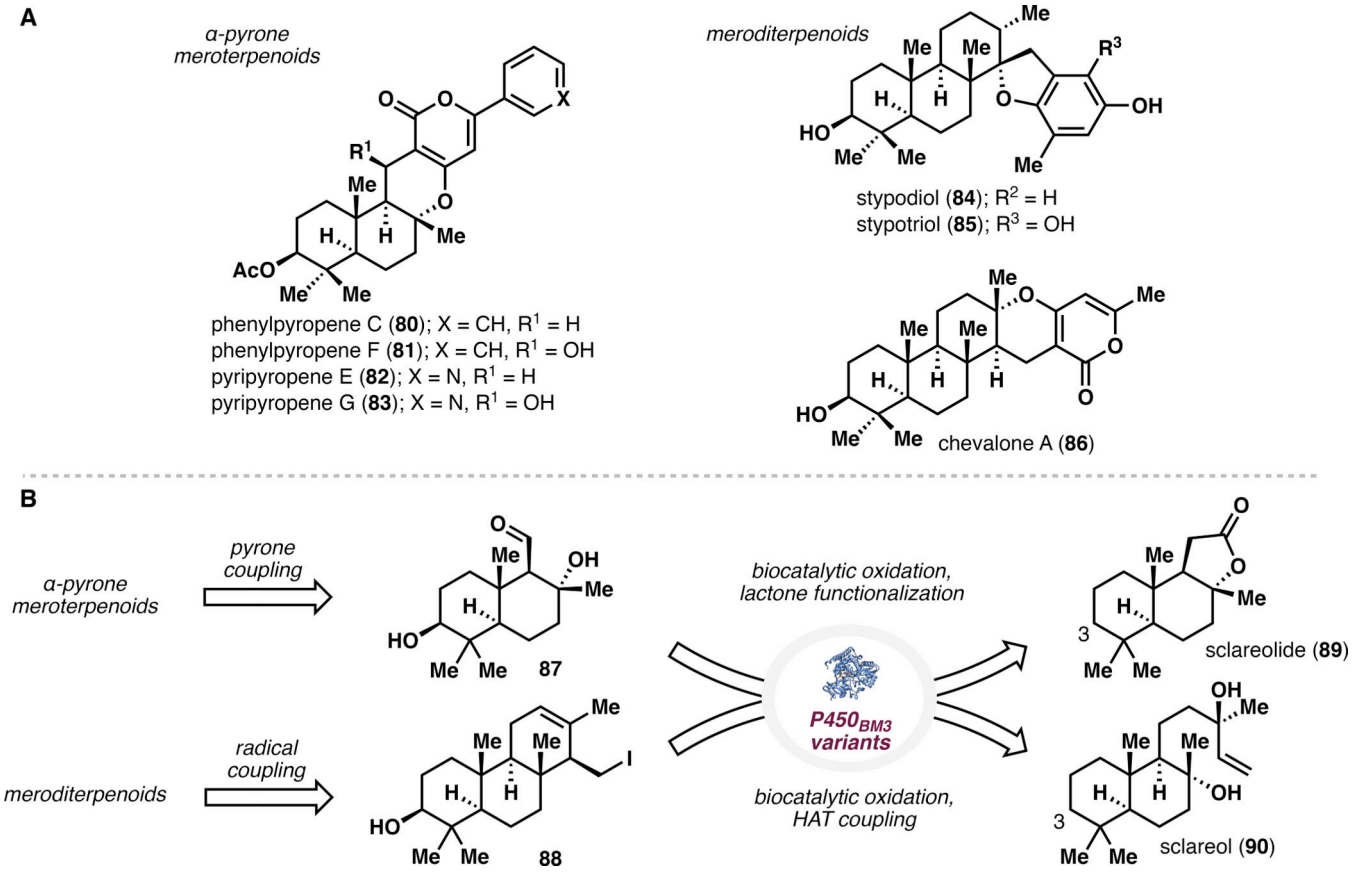


Figure 6.

(A) Representative α-pyrone meroterpenoids and meroditerpenoids. (B) Retrosynthetic analysis of the two meroterpenoid families.

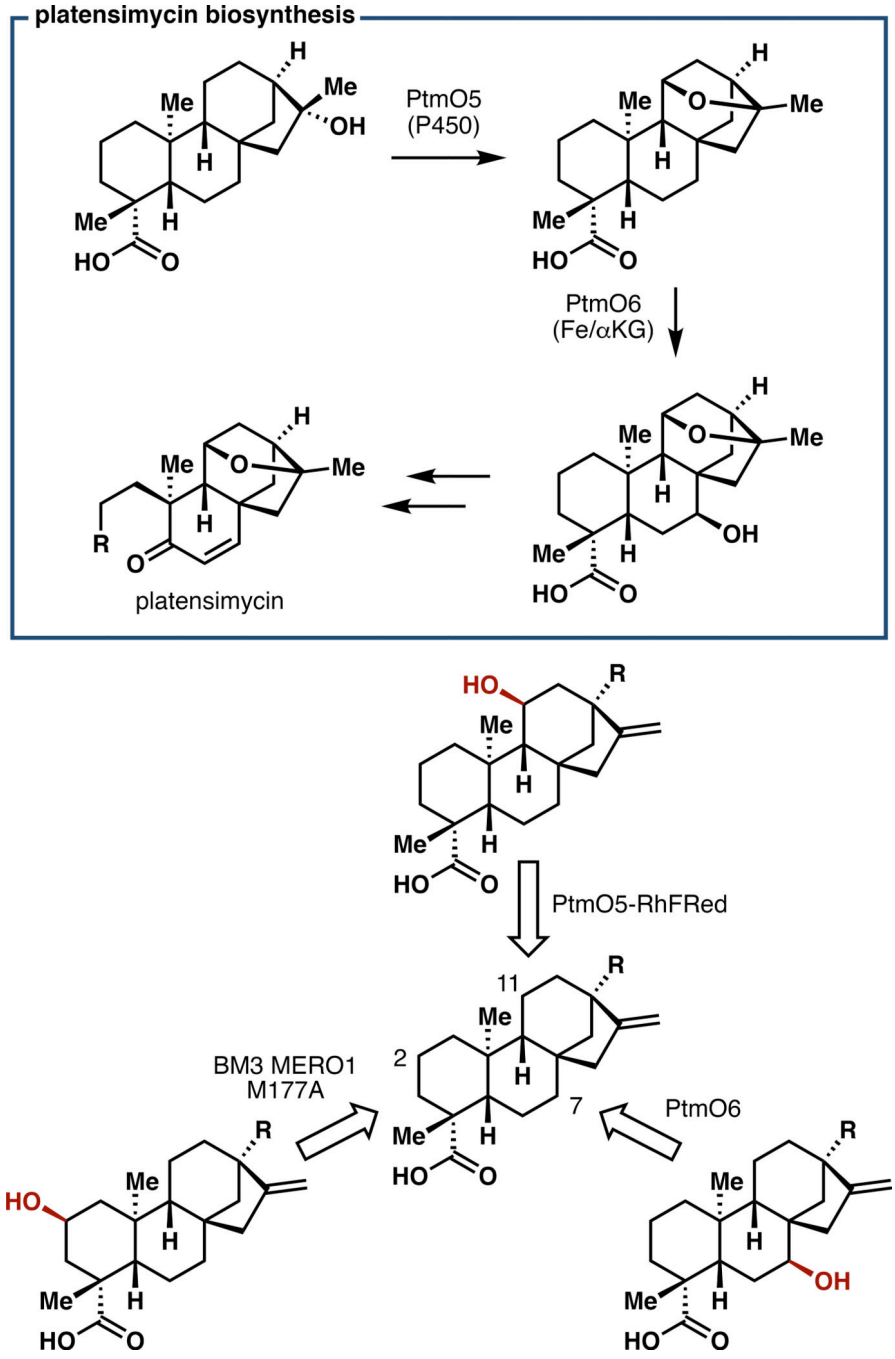
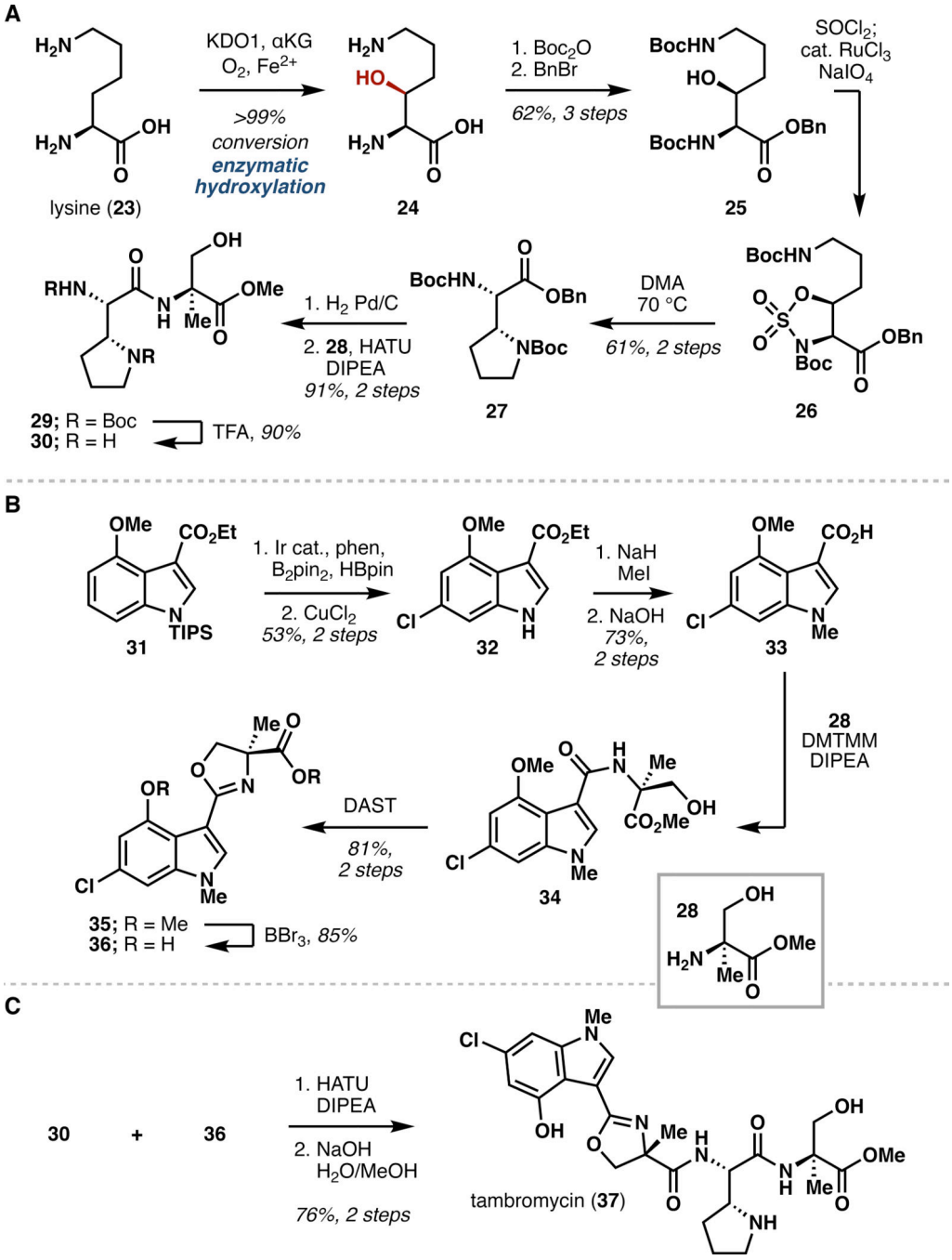


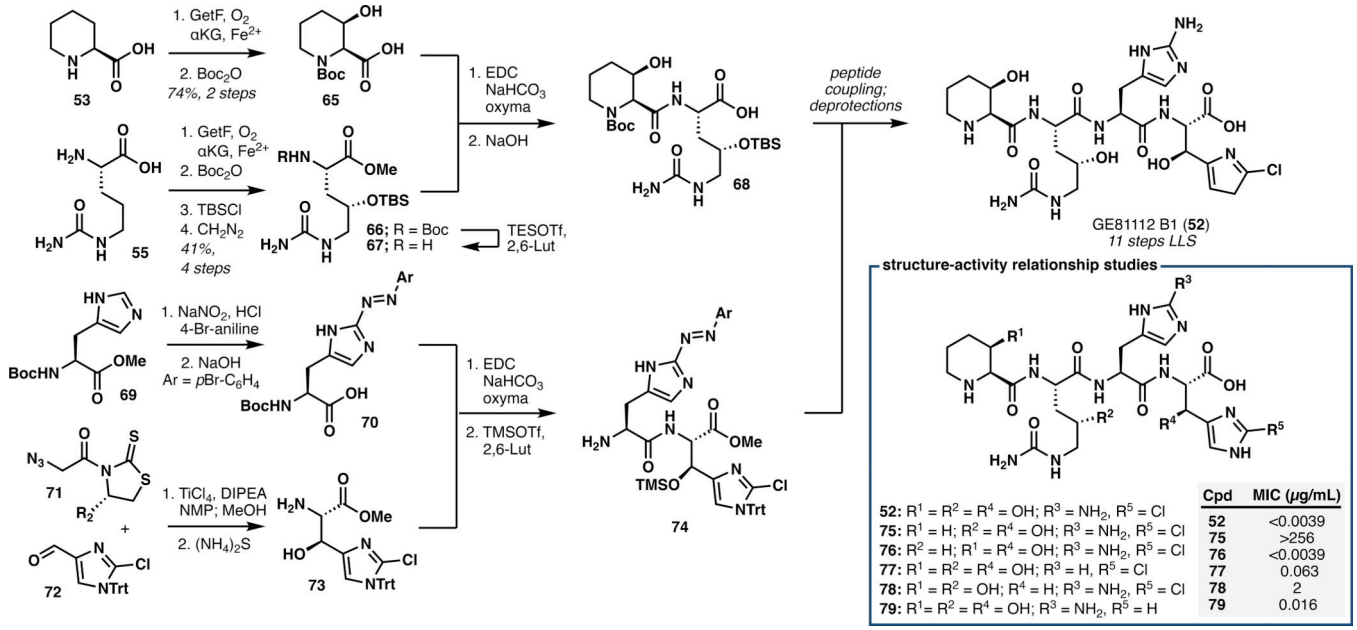
Figure 7.

Snapshot of platensimycin biosynthesis involving hydroxylases PtmO5 and PtmO6; proposed application of biocatalytic oxidation to the steviol skeleton.



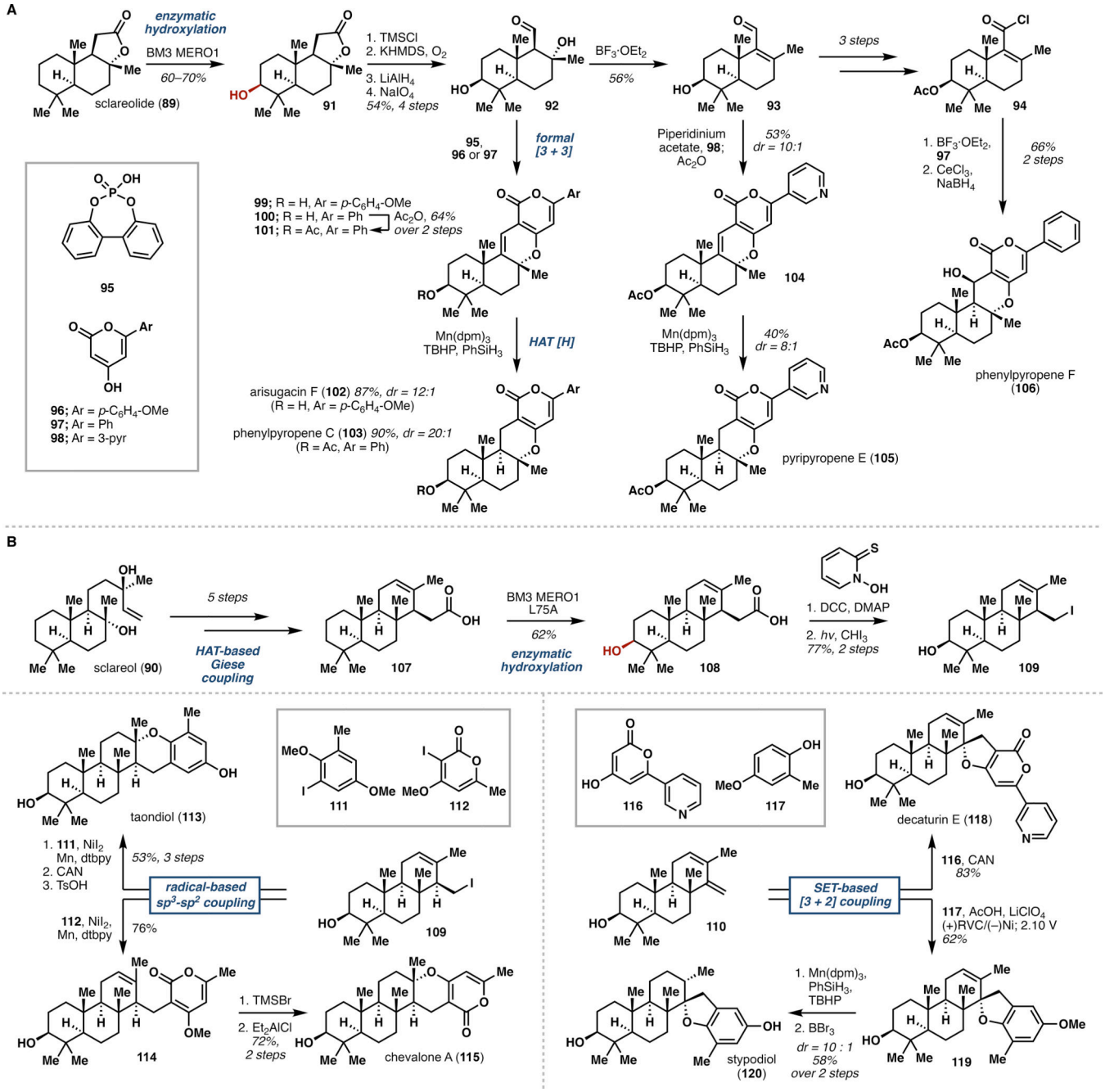
Scheme 1.

Total Chemoenzymatic Synthesis of Tambromycin (**37**)



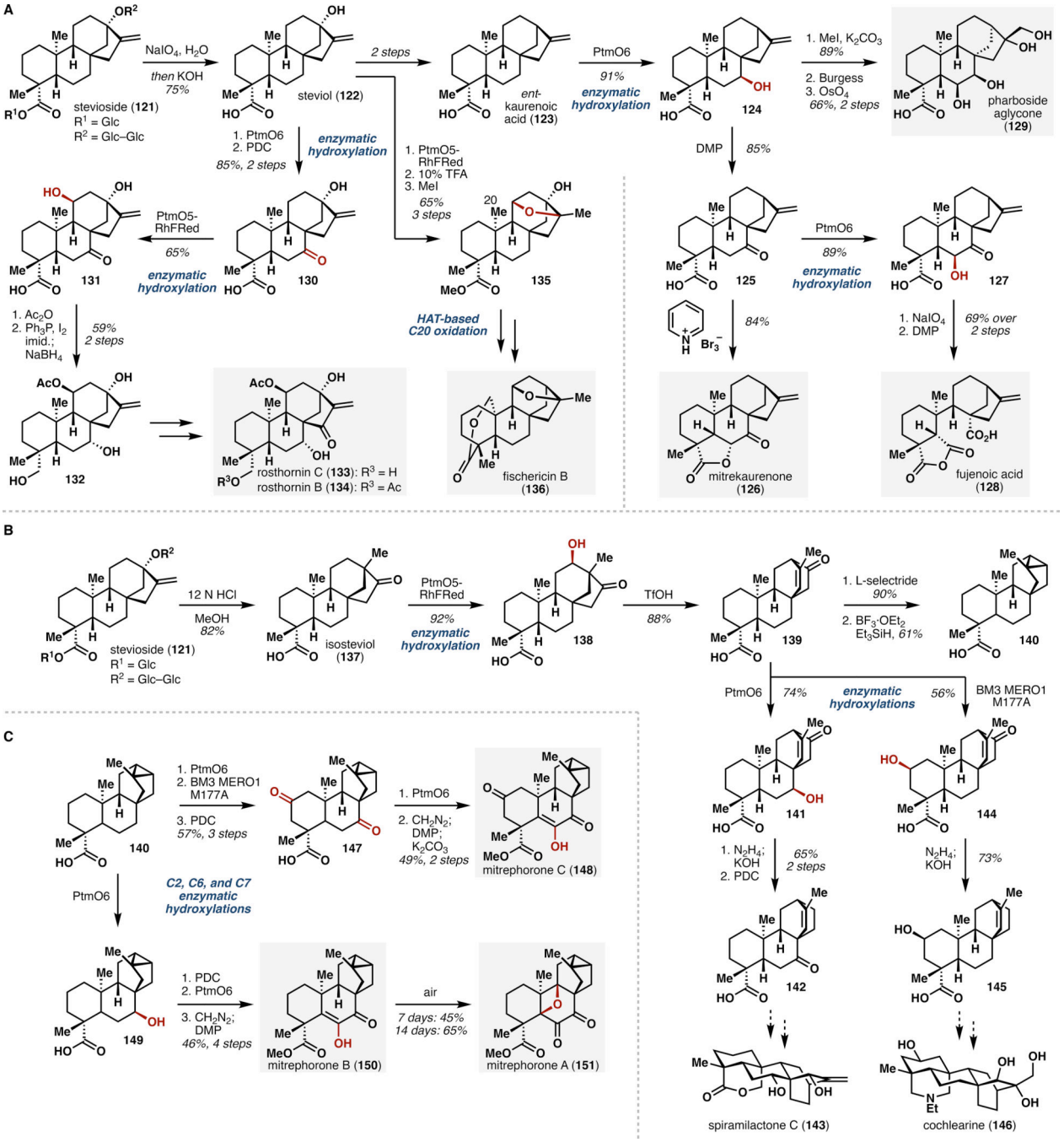
Scheme 2.

Total Chemoenzymatic Synthesis of GE81112 B1 and Structure-Activity Relationship of Synthetic Analogues.



Scheme 3.

Enzymatic Hydroxylation in (A) Total Synthesis of α-Pyrone Meroterpenoids from Sclareolide and (B) Total Synthesis of Meroditerpenoids from Sclareol.



Scheme 4.

Applications of Enzymatic C–H Oxidation in (A) Total Synthesis of *ent*-Kaurane Diterpenes. (B) Carbocationic Rearrangement of the *ent*-Kaurane Skeleton and Synthetic Studies Toward *ent*-Atisane Natural Products. (C) Total Synthesis of *ent*-Trachylobane Natural Products.