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Charting the Evolution of Chemoenzymatic Strategies in the Syntheses of Complex Natural Products

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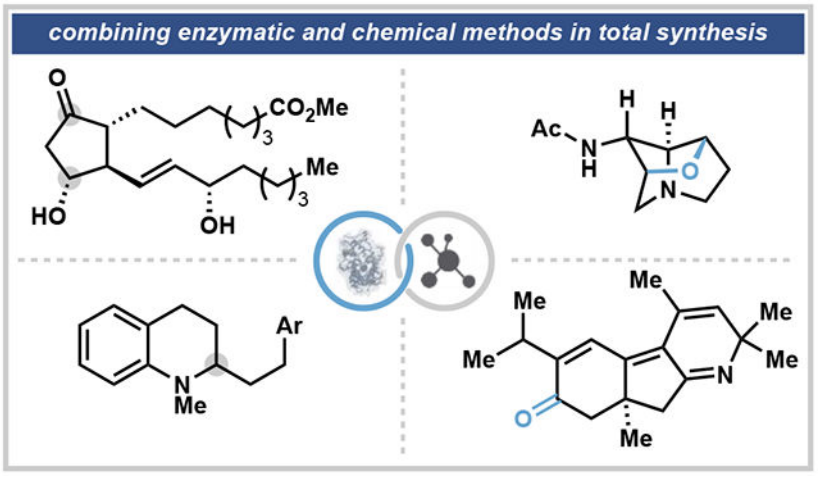
Abstract

Bolstered by recent advances in bioinformatics, genetics, and enzyme engineering, the field of chemoenzymatic synthesis has enjoyed a rapid surge in popularity and utility. This Perspective explores the integration of enzymes into multi-step chemical syntheses, highlighting the unique potential of biocatalytic transformations to streamline the synthesis of complex natural products. In particular, we identify four primary conceptual approaches to chemoenzymatic synthesis and illustrate each with a number of landmark case studies. Future opportunities and outstanding challenges are also discussed.

Graphical Abstract



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INTRODUCTION

At the time of publication, we celebrate the 195th anniversary of Friedrich Wöhler's synthesis of urea,[1] which many regard as the inception of contemporary organic synthesis.[2] Over the past two centuries, practitioners have developed powerful methodologies, synthesized valuable compounds, and driven innovation throughout the chemical sciences, cementing chemical synthesis as an essential subdiscipline of organic chemistry. The initial question, "Can we make it?", has gradually – but inevitably – given way to "How *well* can we make it?"[3] Though certain chemical entities remain difficult to access, organic synthesis is now primarily focused on efficiency, economy, and modularity.[4]

Molecules obtained from Nature (i.e., natural products) have always been a crucial source of inspiration for chemists, and the field of organic synthesis is indelibly intertwined with their study.[5] Indeed, while the structural characterization of complex natural products served as an early motivation for synthetic chemists,[6] bioactive natural products yield valuable blueprints for pharmaceutical endeavors to this day.[7] Equally intriguing, however, are the enzymes that constitute the biosynthetic pathways to such molecules.[8] Recent breakthroughs in genomics, bioinformatics, and molecular biology have enabled powerful techniques for biosynthetic analysis and enzyme engineering,[9] thereby providing chemists an unprecedented opportunity to apply enzymes in total synthesis.[10] As Nature's potent catalysts, enzymes are capable of unparalleled regio-, chemo-, and enantioselectivity across a wide range of substrates and reactions.[11] Many of these transformations are exceedingly difficult – if not impossible – to replicate in the flask. Importantly, enzymes are also amenable to directed evolution,[12] which can generate biocatalysts with vastly improved or "new-to-nature" activity.[13] Thus, by combining the state-of-the-art in chemical and biocatalytic methodology, practitioners of chemoenzymatic synthesis are uniquely positioned to implement advances in both fields toward concise synthesis of complex natural products.[14]

To this end, there exist several different frameworks for the integration of biocatalysis with chemical synthesis. Herein, we seek to trace the development of enzyme-assisted or -enabled synthesis over the years and to classify these endeavors into four main approaches, in which enzymes are used

- **To provide enantioenriched starting materials or intermediates:** In this approach, enzymes play a "support role" in multi-step synthesis campaigns, typically performing kinetic resolutions to generate chiral compounds. Notably, the overall logic of the route remains unchanged. That is, though a racemic intermediate might be targeted with the intent of conducting biocatalytic resolution, the enzymatic step does not influence the broader synthetic design.
- **To enable the evaluation of biosynthetic hypotheses:** This approach provides an alternative to genetics-based investigation of biosynthetic pathways. More specifically, rather than conducting genetic knockout or inactivation experiments, researchers can access biosynthetic intermediates by chemical (or chemoenzymatic) synthesis. The intermediates can then be used to probe

subsequent biosynthetic transformations, thereby enabling the characterization of putative or poorly-understood enzymes.

- **To motivate retrosynthetic disconnections with known enzymatic reactions:** In contrast to the first approach, enzymatic reactions can also serve as inspiration for synthetic designs. As the biocatalytic toolbox has expanded and diversified, an increasing number of syntheses are driven by the application of a particular enzymatic disconnection as a simplifying transform ("T-goal", a term coined by Corey).[15] Unlike Approach 1, the deliberate incorporation of an enzymatic T-goal influences the broader synthetic design, resulting in a retrosynthesis that is orthogonal to those relying on purely chemical transforms.
- **To motivate retrosynthetic disconnections by filling gaps in current methodology:** Distinct from Approach 3, this approach entails proposing a retrosynthetic disconnection *prior* to obtaining a suitable enzyme for the corresponding forward transformation. More now than ever, advances in genetic technology enable the discovery and evolution of enzymes that can perform roles traditionally occupied by (or currently inaccessible to) chemical methodology. Note that the initial disconnection need not be selected with a biocatalytic approach in mind, though this is often the case.

As a disclaimer, this Perspective is not meant to be an exhaustive survey of the literature, nor a definitive categorization of chemoenzymatic strategies for natural product synthesis. Instead, we present several cases that illustrate not only these four approaches, but also the progress achieved by practitioners of chemoenzymatic synthesis and the obstacles yet to be overcome. Our discussion will exclude total biocatalytic synthesis (or total biosynthesis), which has been reviewed elsewhere.[16]

APPROACH 1: TO PROVIDE ENANTIOENRICHED STARTING MATERIALS OR INTERMEDIATES

The synthetic community has developed many technologies to introduce, adjust, or otherwise control absolute stereochemistry in complex chemical environments[17] – for example by using asymmetric, transition metal-catalyzed transformations[18] or starting from cheap, readily-available enantiopure starting materials (i.e., the chiral pool).[19] Another technique employed for this purpose is kinetic resolution, which exploits differential reaction rates between two enantiomers to give enantioenriched compounds.[20] Upon the introduction of a suitable chiral catalyst or reagent to a racemic mixture, one enantiomer will react much faster than the other. The results are (1) enantiopure product derived from the quicker-reacting enantiomer, and (2) an enriched sample of the unreacted starting material enantiomer, each of which is useful depending on the application. Furthermore, racemic materials can be cheaper and easier to access than chiral alternatives. This, along with the intrinsic value of enantiopure products, mitigates to an extent the drawback of 50% maximum theoretical yield.

Enzymes are particularly suited for kinetic resolution, as the chiral environment created by the active site usually favors one enantiomer over the other and leads to the preferential

consumption of the “matched” isomer.[21] To this end, kinetic resolution with lipases and acylases was the predominant implementation of biocatalysis in chemical synthesis in the 90s and early-to-mid 00s.[22] Biocatalysts are also capable of desymmetrizing *meso* compounds, enabling methods that go beyond the 50% maximum yield. It should be noted that our discussion excludes enzymatic dynamic kinetic resolution, which has recently been reviewed separately.[23] Crucially, in this Approach, the retrosynthetic disconnections and forward synthetic logic remain unaltered, as myriad enzyme varieties and substrates are reported in the literature. Thus, the enzyme plays a brief yet vital supporting role, providing access to asymmetric compounds from achiral precursors. Three examples of this approach are discussed herein.

1.1. Williams’ Synthesis of Tetrazomine.

Isolated from soil bacteria in 1991, tetrazomine (**1**) features a pentacyclic core characteristic of the quinocarcins[24] but also bears an unusual 3-hydroxypipecolic acid motif (Figure 1A).[25] Neither absolute nor relative stereochemistry of this amino acid moiety was assigned in the isolation study.[26] Noting both the structural uncertainty and promising bioactivity of **1**, Scott and Williams undertook a total synthesis of tetrazomine.[27–29] Their strategy featured an azomethine ylide [1,3]-dipolar cycloaddition to furnish a tetracyclic intermediate (**2**), while the enantiopure 3-hydroxypipecolic acid **3** would be accessed enzymatically and appended late-stage (Figure 1A).

Synthesis of the pentacyclic core commenced from *o*-anisaldehyde (**4**), which was converted to racemic amine **5** in six steps and 46% overall yield (Figure 1B). Derivatization with bromoacetaldehyde (**6**) and Fmoc-sarcosine-Cl delivered amide **7**, which was elaborated to bicycle **8** in five steps. The cyclization to **9** was mediated by silver(I) trifluoroacetate in the presence of TFA (trifluoroacetic acid) and TFAA (trifluoroacetic acid anhydride). This tricyclic intermediate was then exposed to NBS in refluxing chloroform to form an iminium ion, which upon deprotonation yielded the desired azomethine ylide. Trapping with *tert*-butyl acrylate (**10**) completed the [1,3]-dipolar cycloaddition and afforded a 3.9:1 mixture of adducts **11** and **12**. The undesired adduct (**11**) was epimerized and reduced to alcohol **13** in four steps, whereas **12** required only deprotection to reach the same alcohol, which was converted to aniline **14** in three additional steps.

In parallel, Scott and Williams enacted a chemoenzymatic synthesis of chiral pipecolic acid derivative **3** inspired by prior work from Toyooka.[30] Starting from picolinic acid **15**, exhaustive hydrogenation furnished racemic *cis*-3-hydroxypipecolic acid, which was protected as the Fmoc derivative and esterified to **17**. Treatment with lipase PS and vinyl acetate in diisopropyl ether over 3.5 days effected a kinetic resolution to give nearly quantitative yield (46%) of enantiopure **18**. Four additional steps generated acid chloride **19** with 98:2 er and ~62% overall yield.

En route to **1**, **14** and **19** were coupled in the presence of 4-dimethylaminopyridine (DMAP) to supply a 1:1 mixture of optically active diastereomers (Figure 1D). The crude mixture was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to unmask the amine and provide separable diastereomers **20** and **21**, which were taken independently through the

final steps to deliver **1** and **22**. Scott and Williams extended their approach to synthesize deoxypipecolic-acid-containing derivatives (**23** and **24**), which demonstrated heightened biological activity compared to **1** (Figure 1E).[29a,b] Despite playing a seemingly trivial role in the overall synthesis, the biocatalytic resolution employed *en route* to **19** proved instrumental to the assignment of the correct configuration of **1**.

1.2. Enzymatic Resolutions en Route to the Prostaglandins.

The prostanoids have long attracted the attention of synthetic chemists and biologists due to their roles in inflammatory response and lipid signaling cascades,[31] and numerous synthetic strategies toward prostanoid natural products have been developed over the decades.[32] In particular, the 1,3-cyclopentanedione subunits in these molecules offer an ideal testbed for biocatalytic desymmetrization with lipases or esterases. Alternatively, the cyclic core can derive from linear precursors that themselves arise from biocatalytic resolution. As several excellent reviews examine the use of enzymes in this context,[33] we will focus on two applications of enzymatic resolution in total synthesis: Mori's synthesis of punaglandin 4 (**25**),[34] and Johnson's synthesis of prostaglandin E_1 (PGE_1, **26**) (Figure 2A).[35]

Mori and coworkers sought to employ porcine pancreatic lipase (PPL) to desymmetrize a crucial chlorinated cyclopentene intermediate toward punaglandin 4 (**25**), a prostanoid that features a unique chlorinated core and exhibits potent antitumor activity.[36] First, the racemic acetate **28** was obtained from **27** (readily available from furfural via Piancatelli rearrangement) through acetylation and chlorination (Figure 2B). Luche reduction then delivered a mixture of *cis* and *trans* diastereomers that were protected as TBS ether **29**, the precursor for enzymatic resolution. Subjecting all four isomers of **29** to PPL in buffered phosphate solution afforded 25% yield of the desired enantiomer of *cis*-**30**, which underwent subsequent oxidation to β-chloroketone **31**. After constructing cyclopentenone **32** in six steps from **31**, a second acyclic chain (**33**) was appended via aldol condensation. The synthesis concluded with a three-step sequence of deprotection and acetylation to provide enantiopure **25**.

PGE_1 (**26**) has long been implemented in various medical applications, earning a place on the World Health Organization's Model List of Essential Medicines.[37] In 1993, Johnson and coworkers reported a chemoenzymatic formal synthesis of PGE_1 that leveraged two biocatalytic transformations to enable a three-component coupling strategy for prostaglandin assembly.[35] The cyclopentanone core originated from *meso*-diol **34** (readily available from cyclopentadiene via photooxygenation), which was desymmetrized by treatment with isopropenyl acetate and SP-435, an acrylic-supported lipase B of fungal origin[38] (Figure 2C) to provide acetate **35** in 50% yield and >98% ee, along with the undesired diacetate product. Acetate **35** was further elaborated to α-iodoketone **36** in four steps. Similarly, enantiopure **38** was obtained by action of SP-435 with **37** and became vinyl stannane **39** in three steps. The key three-component coupling featured palladium-catalyzed α-alkylation of α-iodoketone **36** with alkyl boron **40** to furnish cyclopentenone **41**, a known precursor to PGE_1 (Figure 2D). Conjugate addition with a vinyl cuprate derived from **39** and global deprotection yielded PGE_1 methyl ester (**43**).

1.3. White's Synthesis of Byssochlamic Acid.

Isolated in the 1930s from *Byssochlamys fulva*,[39] byssochlamic acid (**44**) is nonadride natural product comprising a nine-membered macrocycle flanked by two five-membered anhydrides.[40] Select members of the nonadride family possess hepatotoxic properties and display powerful inhibition of Ras farnesyltransferase.[41] This combination of unique structural features and potent bioactivity has made them appealing synthetic targets.[42–48]

Adapting the strategy from their first synthesis,[49] White and coworkers envisioned a stereoselective route to (+)-byssochlamic acid that invoked [2+2] photoaddition-cycloreversion of a diolide intermediate rendered asymmetric by pig liver esterase (PLE) (Figure 3A).[49] Racemic 4-ethylcyclohexanone (**45**) was subjected to a three-step sequence that yielded cyclopentene **46** (Figure 3B). This compound proved highly amenable to stereoselective hydrolysis with PLE, which provided enantiopure mono acid **47** in 98% yield. In the first-generation route, **47** was carried forward to monoacid **48** with two further steps.

Synthesis of the cyclobutene coupling partner commenced from anhydride **49** (Figure 3B). Photoaddition of 1-pentene (**50**) and basic hydrolysis yielded a diacid that was treated sequentially with diazomethane and DBU to give diester **51**. This intermediate was also accepted by PLE, furnishing the corresponding monoacid **52**. Here, although the enzyme differentiated the two esters relative to the propyl tail, it did not distinguish between the two enantiomers and delivered racemic **52**. This result proved inconsequential as prior studies suggested that the propyl substituent could be epimerized later. Monoacid **52** was then elaborated to monoprotected diol **53** in four steps before a sequence of coupling and deprotection with **48** and **53** provided diolide **54**, the precursor for photoaddition-cycloreversion.

Despite numerous attempts, no reaction occurred upon irradiation of **54**. Thus, White and coworkers devised a second-generation route that inverted the orientation of the ester linkages. Monoacid **47** was converted to monoprotected diol **55**, whereas diester **51** was transformed to *bis*(methylthio)methyl ester **56** (Figure 3C). This diester was subjected to biocatalytic hydrolysis with PLE, which gave a racemic 7:1 mixture of monoacids favoring the desired compound **57**. With **55** and **57** in hand, the authors reapplied their earlier procedure to obtain the alternative diolide **58**. Irradiation of **58** followed by heating in toluene led to successful photoaddition-cycloreversion and gave a 1:1 mixture of lactones **61** and **62**, which was converted to (+)-byssochlamic acid (**44**) in five additional steps.[49]

APPROACH 2: TO ENABLE THE EVALUATION OF BIOSYNTHETIC HYPOTHESES

Elucidation of natural product biogenesis is a powerful tool that enriches not only synthetic biology but also expands the toolbox of chemical and chemo-enzymatic synthesis. Understanding the biosynthetic machinery and the sequence that a natural product is put together allows pathway engineering for scaffold diversification and the generation of analogue libraries that are valuable for biological studies.[50] Additionally, it provides

a ripe avenue for enzyme discovery as well as a means for uncovering new chemical reactivity. In a traditional pathway elucidation, the biosynthetic gene cluster is first identified bioinformatically and confirmed through gene inactivation[51] or cloned and heterologously expressed in a non-native producer.[52] The functions of individual enzymes are then explored through knockout/inactivation of its encoding gene and structural elucidation of the accumulated product. This is typically followed by *in vitro* enzymatic assay with the putative pathway intermediate for activity confirmation. Relying on gene disruption for the biosynthetic assembly of natural product precursors for enzymatic assays may be inefficient due to the polar effect. Since all enzymes in the pathway are encoded for in the same gene cluster, disrupting one gene in the sequence could downregulate downstream genes thereby obstructing the assembly of intermediates towards the secondary metabolite. As a workaround, chemical/chemo-enzymatic synthesis could be implemented. This approach also has the advantage of potentially being more scalable as well as offering the possibility of isotope labelling to facilitate mechanistic investigations

2.1. Biosynthetic Investigation of Chlorinated Naphthoquinone Meroterpenoids.

The Moore and George groups have capitalized on the above strategy for studying the biogenetic basis of meroterpenoids originating from 1,3,6,8-tetrahydroxynaphthalene.[53] The naphterpins and marinones are polyketide-terpene hybrids that possess a highly functionalized hydroxynaphthoquinone core with a cyclized geranyl or farnesyl side chain (Figure 4). Labelling experiments indicated the provenance of the hydroxynaphthoquinone system from 1,3,6,8-tetrahydroxynaphthalene[54] but it was unclear how alkylation occurred at the less nucleophilic C3 position and how the oxygen atom migrated from C3 to C2. Their studies of the merochlorins and napyromycins identified two vanadium-dependent chloroperoxidases, Mcl24 and NapH3, which induce a unique α-hydroxyketone rearrangement for alkyl migration from C4 to C3, generating the 6,8-dihydroxynapthoquinone scaffold.[55] Having unraveled this reactivity pattern, the authors were able to propose a pathway for the formation of the naphterpins and marinones (Figure 4).[56] They next performed a total biomimetic chemical synthesis to establish the viability of the hypothesized reactivity pattern. Starting from protected tetrahydroxynaphthalene **67,** they performed C4-geranylation/farnesylation using the appropriate carbonate precursor under optimized Tsuji–Trost coupling conditions. After oxidative dearomatization with $Pb(OAc)_4$, dichlorination and deprotection, the α-hydroxyketone was taken to thermal rearrangement to induce prenyl group migration and furnish intermediates **72a** and **b**. Epoxidation and reductive oxirane opening transposed the hydroxyl group from C3 to C2. Finally, the hexahydrobenzopyran was constructed through desaturation, double-bond isomerization, and intramolecular hetero-Diels–Alder.

Having generated this library of potential marinone/naphterpin precursors, the authors were able to investigate the function of two vanadium-dependent chloroperoxidases, MarH1 and MarH3, from the putative biosynthetic gene cluster. MarH1 was first tested with alkylated tetrahydroxynaphthalenes **77a** and **77b**; exposing each to one equivalent of hydrogen peroxide yielded dearomatized products **78a** and **78b**, respectively. Introducing additional hydrogen peroxide induced further mono-chlorination at C2. Formation of the dichlorinated species was not possible using MarH1 even in the presence of excess oxidizing agent.

Subjecting **79a/79b** to MarH3 resulted in the installation of the second halogen and induced the α-hydroxyketone rearrangement. This approach supports the hypothesis that nature adopts the same strategy to construct the core of the marinones and naphterpins as that used for the assembly of merochlorins and napyromycins. Additionally, their total biomimetic synthesis suggests the likelihood that cryptic halogenations are essential for epoxide formation and subsequent oxidation state transposition.

2.2. Mechanistic Elucidation of Ether Bond Formation in Loline Biosynthesis.

The loline alkaloids are potent insecticides synthesized by symbiotic fungi to protect their cool-season grass host from herbivory. Though the tricyclic alkaloids are toxic to invertebrate herbivores, they are completely innocuous to mammals, making them attractive herbicides for the agriculture industry.[57] Curiosity for the genesis of the strained tricycle has driven biosynthetic studies to identify the mechanism for ether installation. Although ether linkages are usually constructed from pre-oxidized precursors,[58] feeding experiments with deuterium-labeled putative biosynthetic intermediates by the Grossman and Schardl groups provided strong evidence that etherification does not follow the typical biosynthetic logic and that only one enzyme is involved in the transformation (Figure 5A). The biosynthesis of these natural products commences with the union of (*L*)-proline and (*L*)-*O*-acetyl-homoserine.[59] Oxidative decarboxylation to the imine, decarboxylation of the 3' position and ring closure constructs the bicyclic system. Since it was found that nature does not perform pre-functionalization prior to etherification, enzymatic C–H activation was invoked. A non-heme dioxygenase, LolO, was identified as a likely candidate for this oxidation and its role in the loline pathway was probed through RNA interference experiments.[60] Disruption of *lolO* shut down loline formation and accumulated N-acetylamino pyrrolizidine (AcAP), which was found to possess *exo*- stereoconfiguration through comparison with synthetically assembled (±)-*exo*-1-acetamidopyrrolizidine. The reference material was constructed by diastereoselective reduction of 1-oximinopyrrolizidine, generated from **88** through key Dieckmann cyclization and condensation with hydroxylamine, and subsequent acetylation (Figure 5B).

A thorough mechanistic campaign was undertaken in collaboration with the Bollinger and Krebs groups to further understand this unique double oxidation process.[61] *In vitro* assays of purified LolO with AcAP under varied α-ketoglutarate concentrations showed that 2-*endo*-hydroxyl-AcAP **91** initially forms and cyclizes in the presence of excess oxidant, supporting LolO's involvement in both C–H oxidation events (Figure 5B). To further reinforce the notion that the first C–H activation occurs at C2, the authors resorted to deuterium kinetic isotope effect (KIE) using AcAPs labelled either at C7 (**92**) or C2 (**94**) (Figure 6A). Freeze-quench Mössbauer experiments revealed that 2-[$^2H_2$]-AcAP **94** undergoes significantly slower decay of the ferryl complex than the non-labelled isotopolog or **92**. Furthermore, a KIE of 23 was measured for 2-[$^2H_2$]-AcAP and 1 for 7-[$^2H_2$]-AcAP, confirming that C2 is the initial site of activation. To differentiate the mechanism by which dehydrogenation takes place between a stereoretentive radical-rebound or that resulting from a radical-polar crossover sequence, feeding experiments with precursors of *cis*-labelled (**98** and **102**) and *trans*-labelled (**97** and **100**) AcAPs were conducted (Figure 6C). The logic behind this is that identifying the intermediates that retain their deuterium should disclose the facial selectivity

of each C–H abstraction event. Preparation of C3 deuterated prolines, **97** and **98**, was achieved using the appropriate isomer of 3-hydroxyproline through tosylation and reduction with $NaBD_4$ (Figure 6B). Aspartic acid labeling was accomplished chemoenzymatically using aspartase B. M+2 peak enrichment observed from cultures fed with (3R)-3-[^{2}H]Asp (**102**) and *cis*-3-deuteroproline (**98**) confirmed that both C–H activations occur from the *endo* face, resulting in retention in stereochemistry. This study highlights the added benefit of using chemical or chemoenzymatic synthesis as a starting point for biosynthetic and enzymological studies with purified enzymes as it can provide ready access to isotopologs to answer in-depth mechanistic questions.

2.3. Mechanistic Study of Polyether Cyclization in Lasalocid Biosynthesis.

Cyclic polyether natural products epitomize the unique ability of enzymes to exert excellent stereochemical control over their reactions.[62] In 1983, Cane, Celmer, and Westley proposed a common biogenetic basis for actinomycetes ionophore polyethers.[63] They postulated that the cyclic ether scaffolds originate from *E*-polyene precursors through enzymatic epoxidation and sequential S_N2 ring openings. Oikawa and Oguri set out to investigate the activity of Lsd19, the putative epoxide hydrolase from lasalocid A biosynthesis, thereby directly testing the Cane–Celmer–Westley hypothesis.[64] At the time of publication, studies of the biosynthetic pathway of monensin A revealed that ether oxygens at C16, 20, and 25 originate from O_2 (Figure 7A).[65] Furthermore, gene disruption of the putative epoxidase, MonCl, accumulated *E*-polyene **110**.[66,67] These results provided preliminary support for the involvement of a polyepoxide intermediate that arises from stereospecific epoxidation. To demonstrate enzymatic conversion of the polyepoxide, the researchers conducted *in vitro* assay of the function of Lsd19. Prelasalocid A was assembled using key *anti*-aldol and *B*-alkyl Suzuki–Miyaura coupling (Figure 7B).[68] Auxiliary directed *syn*-aldol of butanoic amide onto 2-methacrolein and subsequent diastereoselective hydroboration with 9-BBN primed the system for *B*-alkyl Suzuki coupling with (*E*)-iodo olefin **114**. After auxiliary removal and conversion to the ethyl ketone, aldol addition using conditions reported by Paterson formed benzyl protected prelasalocid A (**118**).[69] Epoxidation with Shi's catalyst generated diepoxide **120** in a 3:1 diastereomeric ratio. After TES deprotection, attempts at global benzyl removal induced spontaneous cyclization to give a mixture of **123** and isolasalocid A (**124**), which proceeded via kinetically favored 5-*exo*-tet ring opening, and was believed to be promoted by the carboxylic acid moiety. To prevent this non-enzymatic cyclization cascade, hydrogenolysis was conducted under reduced reaction time and the crude mixture containing 90% **122** and 3% **121** was directly subjected to purified Lsd19. Stepwise cyclization was observed; **123** first formed, and was detected on the LC-MS trace, followed by anti-Baldwin 6-*endo*-tet ring closure. X-ray structure analysis and alanine screening revealed that each epoxide opening event occurs at a specific domain of Lsd19; Lsd19A induces 5-*exo*-tet cyclization while Lsd19B forms the tetrahydropyran ring.[70] These results evince the role of **121** as a biosynthetic intermediate, as initially proposed by Cane and coworkers, and showcase Lsd19's exemplary regiochemical control to bias the cyclization, which would otherwise be energetically disfavored, to the natural product.

2.4. Preparation of Non-Natural Meroterpenoids via Terpene Cyclase Catalysis.

Terpenes have been a topic of great interest in the synthetic community not only for their wide range of biological activity but also their vast structural diversity. Starting with simple and structurally similar precursors, different terpene synthases (TS)[71,72] can mold their substrates into different conformational arrangements, controlling their cyclization fate to provide products with markedly varied core scaffolds and substitution patterns. To gain insights about the promiscuity of fungal TS and their potential utility in chemo-enzymatic synthesis, the Porco and Abe groups collaborated to prepare a library of non-natural meroterpenoid precursors and subjected them to reactions with nine different fungal TS (Figure 8).[73] Here, a decision to chemically synthesize the TS precursors was made as these substrates can only be produced biosynthetically in low concentrations and may be vulnerable to hydrolysis in the presence of endogenous hydrolases. Having previously established methodology to selectively dearomatize dimethyl orsellinic acid **126a** and its methyl ester derivative **126b** at the 5’ and 3’ positions by adjusting the leaving group on the allyl partner, the researchers constructed a collection of these derivatives while varying the alkene geometry or epoxide stereochemistry.[74] Interestingly, dearomatized dimethyl orsellinic ester was widely accepted by the enzymes, with only three out of the nine TS not displaying notable conversion, since some of their native substrates possess significantly different polyketide structures. Although most TS displayed great tolerance towards unnatural alkene geometry, epoxide recognition was crucial for enzyme activity as only Trt1 and AdrI were able to convert substrates with unnatural stereochemistry at the epoxide. Most enzymes orient these non-native systems in the active site with the same conformation as their natural substrates. For example, Pyr4 situates the A-B ring of **135** in a chair-chair conformation, generating the *trans*,*trans*-tricyclic system similar to pyripyropene E. A surprising result from this study came from the products formed by AndB, the TS from the anditomin pathway. While substrate **139**, substituted at the 3’ position and decorated with the *E*,*E*-diene, formed the expected tetracyclic product **144**, **137** bearing an alternative *E*,*Z*-diene produced a unique 6–5/5–6 bicyclic **145** not yet identified in nature.

APPROACH 3: TO MOTIVATE RETROSYNTHETIC DISCONNECTIONS WITH KNOWN ENZYMATIC REACTIONS

Enzymatic catalysis greatly expands the reaction reservoir of organic chemistry and opens avenues for method development of enzyme-mediated modifications. Enzymes can induce asymmetric and site-selective functionalization on a variety of substrate scaffolds without protecting groups, offering complementary methods to traditional chemical approaches. As such, conscious incorporation of enzymatic transformations in synthetic planning can modify the choice of strategic disconnections, sometimes defying common chemical logic, to provide novel routes and shorter and more efficient syntheses. A main feature that distinguishes this Approach from Approach 1 is the intentional use of an enzymatic reaction as the key step in the synthetic design, which consequently leads to different strategic disconnections compared to a conventional total synthesis. Within the scope of this Approach, designing a transform-guided enzyme-mediated synthesis commences with the identification of an enzymatic transformation that has been previously implemented on the substrate of interest or closely related structures to establish a specific structural motif. This

is then coupled with further tailoring steps using chemical or enzymatic means to complete the target molecule. In *The Logic of Chemical Synthesis*,[15] Corey and Cheng stress the importance of using a multistrategic approach in devising retrosynthetic pathways, including the combination of transform-goal (T-goal) and structure-goal (S-goal) strategies. In light of the potentially narrow substrate scope of a given enzymatic reaction, the combined use of T-goal and S-goal strategies (i.e., applying a known enzymatic reaction on a known substrate or closely-related substrates) is all the more important in chemoenzymatic synthesis as it will minimize the failure risk associated with the route. Examples provided below highlight the successful manifestation of this idea in complex molecule synthesis.

3.1. Synthesis of Pancratistatin via Enzyme-Catalyzed Oxidative Dearomatization.

Pancratistatin (**154**) is a highly oxygenated amaryllidaceae alkaloid with potent anti-cancer activity. Shortly after its discovery in 1984, multiple total syntheses of **154** were disclosed.[75] In their approach,[76] the Hudlicky group exploited toluene dioxygenase as a key catalyst to access the densely-substituted cyclohexane ring with the appropriate stereochemistry. The researchers reasoned that utilizing a system with a built in *syn*-diol function would direct the facial selectivity of an epoxidation or an equivalent transformation enabling subsequent ring openings to properly situate the *anti*-stereocenters. In the late 1960s, the Gibson group elucidated the dihydrodiol oxidative degradation pathway of toluene in *Pseudomonas putida* and identified *P. putida* 3D variant which effectively processes toluene and other aromatic derivatives to *syn*-1,2-dihydroxylcyclohexadiene without further oxidation.[77] Whole cell or recombinant oxidation with toluene dioxygenase (TDO), as showcased by Gibson, have found vast application in the total synthesis of oxygenated natural products and carbohydrates.[78] This system and variants thereof accept a variety of substituents and aromatic scaffolds and are able to transform halobenzene substrates.[79] With respect to Hudlicky's pancratistatin synthesis, bromobenzene was chosen as a starting point and was asymmetrically converted to its 2,3-*syn*-diol counterpart by exposure to TDO (Figure 9). Acetonide protection, Cu-mediated aziridination, and dehalogenation sets the stage for 1,2-*anti* aziridine ring opening. This was achieved by using higher order cuprate reagent **148** prepared from directed metalation of the corresponding aryl carboxamide. Cyclization to the lactam presented significant challenges due to the steric bulk around the carboxyl group and the rigid acetonide-bearing cyclohexene scaffold. As a workaround, the researchers resorted to a protecting group swap from tosyl to Boc and epoxidation prior to amide formation. Acetal hydrolysis of **150** enabled directed epoxidation under Sharpless conditions. Finally, Boc deprotection, lactamization, epoxide opening and debenzylation completed the synthesis of **154**.

3.2. Chemoenzymatic Synthesis of Tetrahydroquinoline Natural Products.

Method development for the asymmetric preparation of pharmaceutically relevant 1,2,3,4-tetrahydroquinolines (THQs) is a ripe area of research. Although many chemical methods exist that generate asymmetrically functionalized THQs, they utilize expensive transition metals and high hydrogen pressures,[80] or are limited to 2-aryl or poly-substituted THQs.[81] With the advent of enzyme engineering, researchers can hijack enzymes and evolve them to accept foreign substrates or induce abiotic transformations. This section showcases the

implementation of these two strategies to stereoselectively produce 2-alkyl THQs and their application in the chemoenzymatic synthesis of cuspareine.

Taking inspiration from Turner's deracemization of amines through selective oxidation with mono-amine oxidase (MAO) and subsequent chemical or biocatalyzed reduction,[82] the Lau, Wu and Zhu groups sought to develop an efficient dynamic-kinetic resolution of 2-alkyl THQs. By employing cyclohexylamine oxidase (CHAO) from *Brevibacterium oxidans* and *in situ* reduction with BH_3•NH_3, 2-methyltetrahydroquinoline undergoes resolution in 98% ee to yield 76% of the (*R*)-enantiomer.[83] Unfortunately, the enzyme could not tolerate more sterically demanding substitutions at C2. To address this issue, the researchers performed directed evolution around the enzyme active site.[84] Nine variants displayed enhanced activity over wild-type and accepted substrates with varying chain length, branching and substitutions including isopropyl, cyclopropyl, allyl and benzyl. The same protocol was then mapped onto racemic des-methyl cuspareine **155** to convert this direct precursor to a single enantiomer (Figure 10A). Subjecting **155** *to E. coli* harboring CHAO-Y459T and BH_3•NH_3 transformed the racemic alkaloid to the (*R*)-enantiomer in 91% yield and with 94% ee.

Both enantiomers of cuspareine (**158**) were synthesized by the Arnold group using their sp^3 C–H diazo-insertion with evolved Fe-P411 enzymes (Figure 10B).[85] Aiming to expand enzymatic C–H alkylation chemistry, the researchers utilized Fe-heme systems to abiotically form carbene species that can stereoselectively insert into a C–H bond. This approach greatly expanded the alkylation repertoire to alkyls bearing useful functionalities including esters, Weinreb amides, and ketones. A directed evolution campaign produced variant P411-CHF that is able to perform C–H functionalization at benzylic, allylic, and propargylic positions with excellent regio- and stereo-selectivity and delivers **160** from **159** with 2,150 TTN and 96:4 er. With respect to arylamine substrates, the authors observed a surprising complete preference for insertion at the α-amino position over benzylic C–H bonds. Capitalizing on this unexpected result, direct insertion onto N-methyl tetrahydroquinoline **156** forms (*R*)-**157** or (*S*)-**157** with 73:27 er and 91:9 er respectively using P411-CHF (*R*-selective) or P411-gen5 (*S*-selective), a variant formed in the earlier stages of the evolution campaign. With practically the entire framework of cuspareine in place, the authors were able to convert these intermediates to either enantiomer of the natural product in three steps.

3.3. Divergent Synthesis of Meroterpenoids via Biocatalytic Oxidation of Sclareolide.

3-hydroxy-drimane is a prevalent motif among polyketide sesquiterpenoids. Their biosynthetic assembly commences with the merger of a polyketide fragment with a polyisoprene pyrophosphate unit, followed by stereoselective epoxidation and subsequent TS mediated oxirane activation, which induces the requisite polyene cyclization cascade (c.f. Approach 2.4), and results in the 3-hydroxy-drimane subunit. Although a simple task to nature, preparing this motif synthetically is not trivial.[87] In 2012, the Baran group demonstrated the use of sclareolide as a common building block to access various quinone sesquiterpenoids by manipulation of the lactone moiety.[87] Since sclareolide lacks a C3 oxidation, the approach is restricted to accessing systems bearing no oxidations on the decalin core. Inspired by this strategy, the Renata group envisaged the use of enzymatic oxidation to selectively install a C3 hydroxyl group on sclareolide to enable efficient

access to diverse 3-hydroxy-drimane containing natural products (Figure 11).[88] Fasan and coworkers previously identified mutein II-H8 capable of hydroxylating sclareolide at C3, albeit on a small scale (50 mg) and under high dilution conditions (1mM overall substrate concentration).[89] To enhance the reaction scalability, the Renata group examined a small library of $P450_{BM3}$ variants that closely resemble II-H8, while adopting Fasan's protocol of using thermostabilized phosphate dehydrogenase Opt13 for NADPH recycling. BM3 MERO1, containing several mutations with respect to II-H8's parent (9–10A), enabled gram-scale formation of **161** in 60–70% yield. This compound became a direct precursor for the synthesis of a variety of fungal merosesquiterpenoids including pyripyropene E, 5-deoxyterreulactone C, phenylpyropene C, and arisugacin F. First, the lactone moiety was converted to key ß-hydroxyaldehyde in 4 steps. Formal [3+3]-coupling with the appropriate hydroxy-α-pyrone in the presence of biphenyl phosphoric acid **168** followed by hydrogen atom transfer (HAT) reduction with Shenvi's catalyst forged phenylpyropene C (**173**) and arisugacin F (**174**). [3+3]-Cyclization towards pyripyropene E necessitated the use of enal **163** as coupling partner, which was accessed from **162** through dehydration with boron trifluoride. 5-Deoxyterreulactone C (**172**) was produced through Mukaiyama hydration and subsequent Swern oxidation of dehydro-arisugacin F (**171**). This strategy was also expanded to encompass the enzymatic C3 oxidation of a sclareol derivative to enable access to four different meroditerpenoids in 10–12 steps (not shown).

3.4. Chemoenzymatic Synthesis and Stereochemical Reassignment of Trichoflectin.

In pursuit of achieving selective oxidative dearomatization of resorcinol-type scaffolds, the Narayan group first fingerprinted three known flavin-dependent monooxygenases, of which AzaH and TropB functionalize the C3 position of resorcinol derivatives generating the (*R*)-product, and SorbC forms (*S*)-α-quinol at C5 (Figure 12).[90] Building upon this discovery, a sequence similarity network (SSN) of the FAD dependent monooxygenase superfamily (pfam01494) was constructed to expand the pool of available biocatalysts for reactions with complimentary selectivity profile. This analysis allowed the researchers to home in on 1211 putative resorcinol dearomatizing enzymes, among which AfoD was chosen for activity profiling due to its location within the SSN.[91] Exposure of **175** to AfoD imparted C3 functionalization similar to AzaH but with opposite facial selectivity. Sequence alignment analysis provided insights into key residues responsible for substrate positioning and the resulting facial selectivity. (*R*)-Selective enzymes bear Phe/Trp and Tyr at conserved residues 118 and 237 respectively, while these residues are switched in (*S*)-selective systems. The importance of the 118 Tyr was further consolidated by observing roughly a 1:1 er using mutant AfoD Y118F. Although this model provides a convenient tool for stereoselectivity prediction, it is worth noting that some enzymes, including AzaH and SorbC, operate with a different mode for substrate positioning.[92]

With stereodivergent enzymes identified, the method was then mapped onto the synthesis of bioactive azaphilone natural products, which can have (*R*)- or (*S*)-configuration at the C7 position. Towards trichoflectin, the authors first identified a common intermediate that can be accepted by both AfoD and AzaH, and possesses functional handles for further downstream manipulations. Enone **177** displayed 83% and 95% conversion with AfoD and AzaH, respectively, and was the divergence point in the syntheses. (*R*)-**178** was generated

using AzaH in 96% yield and with >99% ee. Knoevenagel condensation with acylketene **179** completed (*S*)-trichoflectin. (*R*)-**180** was accessed through the same sequence using AfoD. Surprisingly, the measured optical rotation sign of (*S*)-trichoflectin was opposite to the reported value. X-ray crystallography and comparison of calculated and measured CD spectra of (*S*)-**180** confirmed the absolute (*S*)-configuration of the synthetic sample and enabled the stereochemical reassignment of the natural product. This case study fully captures the essence of Approach 3 and highlights the synergy of bioinformatics and substrate profiling to expand the scope of a biocatalytic reaction, and divergently access different natural products of the same family.

APPROACH 4: TO MOTIVATE RETROSYNTHETIC DISCONNECTIONS BY FILLING GAPS IN CURRENT METHODOLOGY

In contrast to hybrid T-goal/S-goal approaches described in Approach 4, one could also develop “top-down” strategies for natural product chemoenzymatic synthesis by proposing biocatalytic disconnections for which no direct or close precedents have been reported. Here, the practitioner makes an educated guess for the biocatalytic disconnection based on known reactivity trends or reported substrate scope of a given enzyme. This approach is inherently riskier due to the uncertainties surrounding the biocatalytic step and a proper risk and reward assessment is warranted before proceeding with the route. Enzymes can exhibit high substrate specificity and low promiscuity. Although two compounds may share many structural features, an enzyme might only be able to accept one but not the other depending on the recognition elements needed for reaction. In a less extreme case, the enzyme’s activity is partially compromised due to minor changes in the substrate structure and further engineering for activity improvement is needed. In this approach, some form of screening will likely be needed to identify a suitable enzyme/substrate pair that fits within the proposed synthetic approach. Undoubtedly, this approach will be greatly enabled by the existence of homologous enzymes or known engineered variants to serve as potential workarounds. In this context, enzymes whose promiscuity and evolvability are well-documented or those that have been widely engineered for a variety of applications, such as $P450_{BM3}$, have seen a number of successful applications in natural product synthesis.[88,93] Beyond “brute force” screening, one could also develop a discovery workflow to identify candidate enzymes for the proposed disconnection, as illustrated in Lei’s synthesis of chalcomoracin discussed later in the section.[94]

4.1. Synthesis of Nigelladine via Late-Stage Enzymatic C–H Oxidation.

In 2017, the Stoltz and the Arnold groups completed the first enantioselective synthesis of nigelladine A (**191**) (Figure 13).[93] The synthesis features the use of a late-stage enzymatic C–H functionalization with an engineered cytochrome P450 to realize regio- and chemoselective allylic oxidation among four oxidizable positions. Retrosynthetically, imine **190** was considered as a viable precursor for late-stage C–H oxidation, and **190** was traced back to vinyl bromide **187** through a cross-coupling disconnection. The forward synthesis commenced with 1,3-cyclohexanedione (**181**), which was subjected to a Stork–Danheiser transposition to afford **183**. Following conversion to hydrazone **184**, acylation and methylation were performed to furnish β-ketoester **185**, setting the stage for the

key quaternary center formation. Toward this goal, palladium-mediated asymmetric allylic alkylation was applied to generate the desired enone **186** with high enantioselectivity and excellent yield.[95,96] Tsuji–Wacker reaction, intramolecular aldol and α-bromination followed to provide vinyl bromide **187**. A Suzuki cross-coupling with boronic ester **188** was next performed, followed by deprotection with TFA, which also led to spontaneous imine formation along with isomerization to give **190**. Performing regio- and chemoselective oxidation on **190** turned out to be challenging with chemical methods as there are four reactive positions to distinguish. Most chemical allylic oxidation conditions failed to provide desirable results. SeO_2 preferentially oxidized at C1, Pd/peroxide oxidation mostly gave C10-oxidized product and Cr oxidation generated an isomerized product. While Rh oxidation conditions favored C7 position, the isolated yields were low. The authors thus sought to achieve the oxidation through biocatalytic means. $P450_{BM3}$ variants were selected as suitable candidate enzymes as they are renowned for their substrate promiscuity and exceptional selectivity in C–H oxidations.[97] The enzyme screening was performed with a small library of previously reported $P450_{BM3}$ variants.[98] Though wild-type $P450_{BM3}$ showed some selectivity for C7, the best variant found was 8C7, that contains L75A and L181A mutations. The variant favored oxidation at C7 over other positions in a 2.8:1 ratio, which translates to superior chemo- and regioselectivity and improved yield in comparison to traditional chemical methods. Routine oxidation with DMP completed the synthesis of **191**.

4.2. Diels–Alderase Identification Toward Prenylflavonoid Synthesis.

In addition to enzyme engineering, identifying new enzyme is a viable alternative to discover powerful enzymatic transformations to enable efficient chemoenzymatic synthesis. Prenylflavonoid Diels–Alder-derived natural products from mulberry (*Moraceae*) plants[99] possess a unique methylcyclohexene motif and exhibit various biological activities.[100,101] Lei group completed the first asymmetric total synthesis of four of the family members, kuwanons I and J and brosimones A and B (**192**-**195**) (Figure 14A).[101d] Their synthesis featured a boron-catalyzed[102] asymmetric Diels–Alder cycloaddition between **196** and **197** to provide two endo-/exo- diastereomers with high enantioselectivity. Though both endo- and exo-D–A products could be converted to kuwanon I and J, the route was beset by the lack of endo/exo selectivity in the Diels–Alder step.

The authors subsequently sought to figure out which enzyme(s) catalyze the biosynthetic D–A reactions in the native mulberry plant.[103,104] However, plant enzyme identification is challenging since many genes accounting for secondary metabolite biosynthesis are not organized in gene clusters.[105] There is also no precedent for an intermolecular Diels–Alderase, which further complicated their search for candidate enzymes. To circumvent these issues, a novel identification method was developed featuring biosynthetic intermediate probe (BIP)-based strategy. They first used *M. alba* leaf lysate, which was assayed by incubating with morachalcone A (**200**) and moracin C (**201**), and observed the natural product chalcomoracin (**198**) as the major product along with an unstable side product (**199**). This observation indicated an oxidase and a Diels–Alderase could be involved (Figure 14B). To enrich the target protein(s), sequential fractionation and chromatographic separations were performed, which resulted in an 11-fold increase of the

production of **198**. They then initiated the BIP-based target identification using diazirine **205** (Figure 13C). Incubation of **205** and **201** resulted in the production of adduct **209**. Based on this result, BIP **205** was subsequently used in photoaffinity labelling in the SEC fraction. Upon ultraviolet irradiation, the diazirine motif decomposes to a carbene species which binds covalently to potential target protein(s), including the putative Diels–Alderase. Subsequent pull-down assay was performed to collect the enriched target proteins, which eventually led to the characterization of two putative enzymes MaMO and MaDA. The former was confirmed as an oxidase which converts **201** to **199**, whereas the latter was found to be the first stand-alone intermolecular Diels–Alderase. Product **198**, generated from MaDA-catalyzed [4+2] cycloaddition from **199** and **200**, was obtained exclusively in the *endo* configuration in comparison to the chemically-synthesized adduct, highlighting the extraordinary selectivity of the enzymatic process. The authors further extended the utility of MaDA in late-stage D–A reactions with other substrates. MaDA was proven to be promiscuous for dienes **210**–**214** (Figure 15) but has strict preference with respect to the dienophile. This late-stage enzymatic D–A resulted in efficient access (up to 1873 total turnover number) to natural products guangsangon E (**216**), kuwanon E (**217**), kuwanon J (**193**), deoxyartonin I (**219**), and 18″-O-methylchalcomoracin (**218**).

4.3. Synthesis of Mitrephorone C via Sequential Enzymatic Oxidations.

The *ent*-kaurane, *ent*-atisane, and *ent*-trachylobane diterpenoids are biosynthetically-related diterpenoid families that differ primarily in their C/D-ring architectures.[106,107] Aside from their intriguing structural features, family members exhibit a wide range of biological activities and thus have drawn attention from synthetic communities in recent years.[108,109] Inspired by previous semisynthetic efforts,[110] the Renata lab chose stevioside (**223**) as a starting point for a divergent synthesis of highly oxidized members of these diterpenoid families.[111] Although steviol and isosteviol are readily available from stevioside, the lack of functional handles in the A, B, and C rings makes their remote functionalization with chemical methods challenging. To address this issue, the Renata lab conceived a hybrid oxidative approach for site-selective oxidations of steviol derivatives by combining both enzymatic and chemical C–H functionalizations for expedient scaffold modification. In collaboration with the Shen lab, three oxygenases, PtmO5, PtmO3, and PtmO6, were characterized from platensimycin biosynthesis and demonstrated as useful and effective biocatalysts for selective C- and B-ring oxidations of steviol and its derivatives (Figure 16A).[112] With biocatalysts for selective B- and C-ring hydroxylation, they next sought to identify a suitable enzyme for A-ring oxidation. In previous studies, $P450_{BM3}$ variants were found to exhibit not only exceptional activity in selective A-ring oxidation of decalin-containing terpenes, but also outstanding substrate promiscuity.[88,89,93,113] Following a brief screening of alanine-scanning derivatives of $P450_{BM3}$ on **220**, variant BM3 MERO1 M177A was noted to provide excellent selectivity and conversion for the production of **221**. Further investigation on M177A confirmed its substrate promiscuity, allowing similar A-ring hydroxylation on additional steviol derivatives. The ability to achieve site-selective A-, B-, and C-ring oxidations inspired the authors to design an efficient semisynthetic access to mitrephorone C (Figure 16B). Starting from isosteviol (**224**), treatment with PtmO5-RhFRed[114] – a self-sufficient version of PtmO5 – installed a hydroxyl group in the C ring to deliver **225**. Subsequent use of trifluoromethanesulfonic acid (TfOH) effected a

Wagner–Meerwein rearrangement to afford **226**, which contains the *ent*-atisane [2,2,2] C- and D-rings. Two additional reductive steps were designed to generate the *ent*-trachylobane product **227**, which served as a starting point for further oxidative modifications. Upon sequential treatment with BM3 MERO1 M177A and PtmO6, two hydroxylations took place and the resulting product was further subjected to PDC oxidation to afford diketone **228**. They then took advantage of the C6 hydroxylation capability of PtmO6 to convert **228** to the corresponding α-hydroxyketone, which was subsequently treated with diazomethane and DMP. Finally, the synthesis of mitrephorone C (**229**) was achieved after spontaneous tautomerization.

CONCLUSION

On the allure of chemical synthesis, R. B. Woodward once[115] said, “The structure known, but not yet accessible by synthesis, is to the chemist what the unclimbed mountain, the uncharted sea, the untilled field, the unreached planet, are to other men… The unique challenge which chemical synthesis provides for the creative imagination and the skilled hand ensures that it will endure as long as men write books, paint pictures, and fashion things which are beautiful, or practical, or both.” In the same lecture series, he also mused, “Not infrequently, Nature is more knowledgeable and artful than the chemist, and devises combinations between, or transformations of, reacting molecules which the designer had not anticipated at all.” The rapid pace of development in genomics and enzymology in the last few decades has led us to the point where we can not only dissect and understand the transformations that Nature employs to produce complex molecules, but also manipulate them to develop useful and enabling biocatalytic reactions. When appropriately designed and executed, the combination of enzymatic catalysis and traditional synthetic chemistry can provide a powerful means for addressing important chemical and biological questions. The above case studies illustrate the benefits of this combination not only for elucidating biosynthetic pathways, but also for facilitating efficient natural product synthesis. From the standpoint of synthetic strategy development, the recent explosion of new enzyme discovery has reshaped the role of enzymatic catalysis in multi-step synthetic sequences, from playing a supporting part in chiral building block preparation to serving as a “centerpiece”. This evolution has also been accompanied by a notable rise in the applications of more specialized biosynthetic enzymes in synthetic work. Furthermore, there has also been an increased interest in employing this strategy in the preparation of natural product analogs for useful structure-activity relationship profiling, which is reviewed in depth elsewhere.[116]

Despite its rapid rise in popularity, the field of chemoenzymatic synthesis still faces a number of outstanding challenges. Firstly, the gap between the initial enzyme discovery and its biocatalytic application in organic synthesis may require non-trivial effort to address. Initial enzyme discovery from secondary metabolite biosynthesis typically places little emphasis on reaction efficiency, practicality, and scalability, which are key parameters for synthetic applications. In the event that the initial activity of the wild-type enzyme is not high enough to support synthetic work, significant optimization of the reaction parameters, homolog screening, and/or enzyme engineering may be needed. For synthetic applications with non-native substrates, it is difficult to predict a priori whether a particular enzyme would display sufficiently broad substrate promiscuity. While emerging enzyme engineering

and screening techniques may provide effective tools for addressing these challenges,[117] the readers are cautioned to not treat this method as a universal panacea that would work in all cases.

As illustrated herein, most chemoenzymatic synthesis campaigns emphasize on the use of natural enzymatic reactions from biosynthetic pathways. On the other hand, there has been a nascent push toward expanding biocatalysis by developing enzymatic transformations that are new to nature,[118] either by challenging natural enzymes with stimuli not encountered in nature or by constructing artificial metalloenzymes.[119] As an emerging research area, this approach is still overlooked in the design of chemoenzymatic routes and, to the best of our knowledge, has only featured in one natural product synthesis. Taking these methods into consideration during synthetic planning would allow for a greater variety of chemoenzymatic strategies that can be developed and put into practice. The extension of chemoenzymatic route design to synthetic molecules belonging to non-natural product chemical space also presents a non-trivial challenge. Arguably, enzymatic reactions discovered from natural product biosyntheses are more relevant to the synthesis of natural products themselves than of man-made small molecules, which often reside in orthogonal chemical space. A recent “pressure test” conducted by DARPA[120] revealed that it is exceedingly difficult to develop a biotechnological platform to produce tetrahydrofuran due to its lack of structural similarity to natural products. Even though biocatalytic processes are commonly employed in the pharmaceutical industry for the synthesis of key chiral building blocks, they mostly rely on well-established enzymes such as ketoreductases, transaminases and imine reductases, for which commercial screening libraries already exist. In contrast, the use of more specialized secondary metabolite enzymes in the preparation of man-made small molecules likely entails reactions with substrates that do not resemble any intermediates from these pathways and will require extensive screening and engineering efforts.

Finally, enzymatic reactions require specific biochemical knowledge and technical knowhow, which discourages their adoption by many non-specialists. Interdisciplinary collaborations[93] between synthetic chemistry and biocatalysis laboratories that leverage their respective strengths will mitigate this issue and advance both subfields in a synergistic fashion. Concurrently, the development of a biocatalytic reaction database or integration of biocatalysis in retrosynthesis planning tools would be highly beneficial to the synthetic community. An online automated biocatalytic cascade design tool, RetroBioCat,[121] was recently established, which offers potential biocatalytic routes to user-specified targets based on known reactions. While this platform is presently limited to purely biocatalytic routes, an iteration that allows it to interface with chemical reaction databases or traditional retrosynthesis software would be a welcome development. This, along with a conscious effort to introduce concepts of enzymatic catalysis in organic chemistry courses,[122] is anticipated to result in more incorporation of chemoenzymatic paradigms in complex molecule synthesis in the future.

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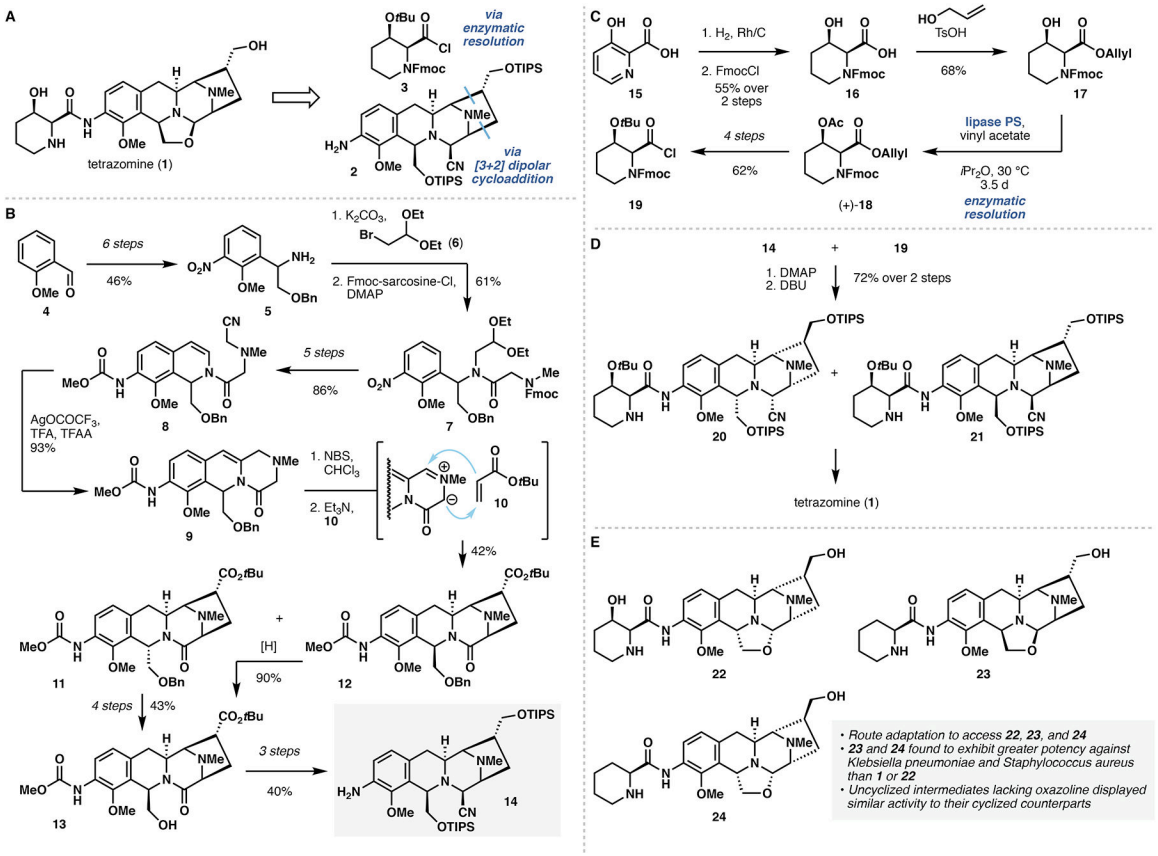


Figure 1.

William's total synthesis of tetrazomine. (A) Structure of tetrazomine (**1**) and general retrosynthetic strategy. (B) Synthesis of aniline **14** featuring 1,3-dipolar cycloaddition. (C) Chemoenzymatic synthesis of **19** via enzymatic kinetic resolution of racemic allyl ester **17**. (D) Coupling of aniline **14** and acid chloride **19** and elaboration to tetrazomine (**1**). (E) Analogs of **1** synthesized using the same general strategy. Note that enzymatic resolution was not required to construct **23** and **24**.

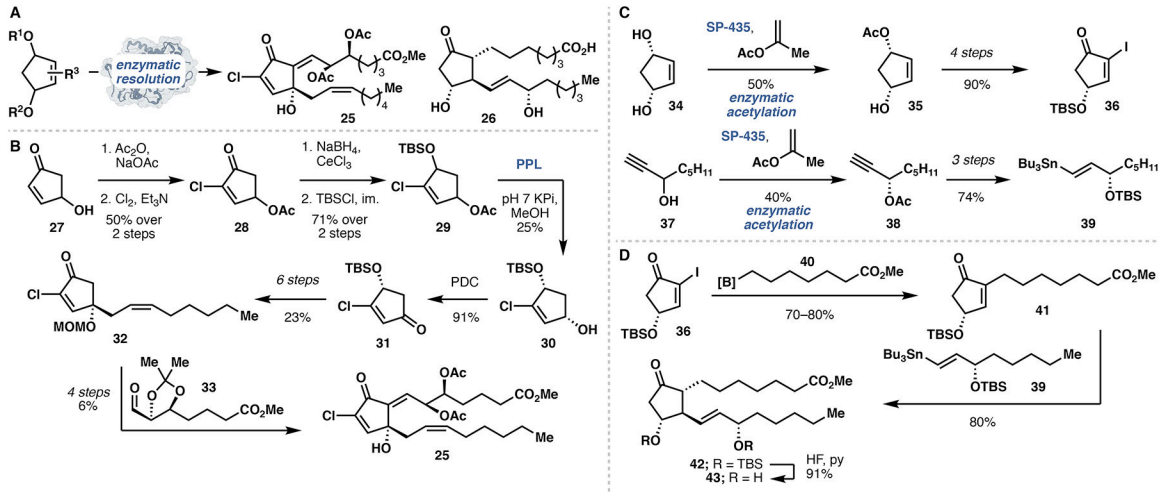


Figure 2.

Enzymatic resolution in the synthesis of prostanoid natural products. (A) General strategy deployed to synthesize punaglandin 4 (**25**) and PGE_1 (**26**). (B) Mori's total synthesis of punaglandin 4 (**25**). (C) Enzymatic acetylations used to access two key fragments in Johnson's formal synthesis of PGE_1 (**26**). (D) Three-component, two-step coupling used to access a precursor of PGE_1.

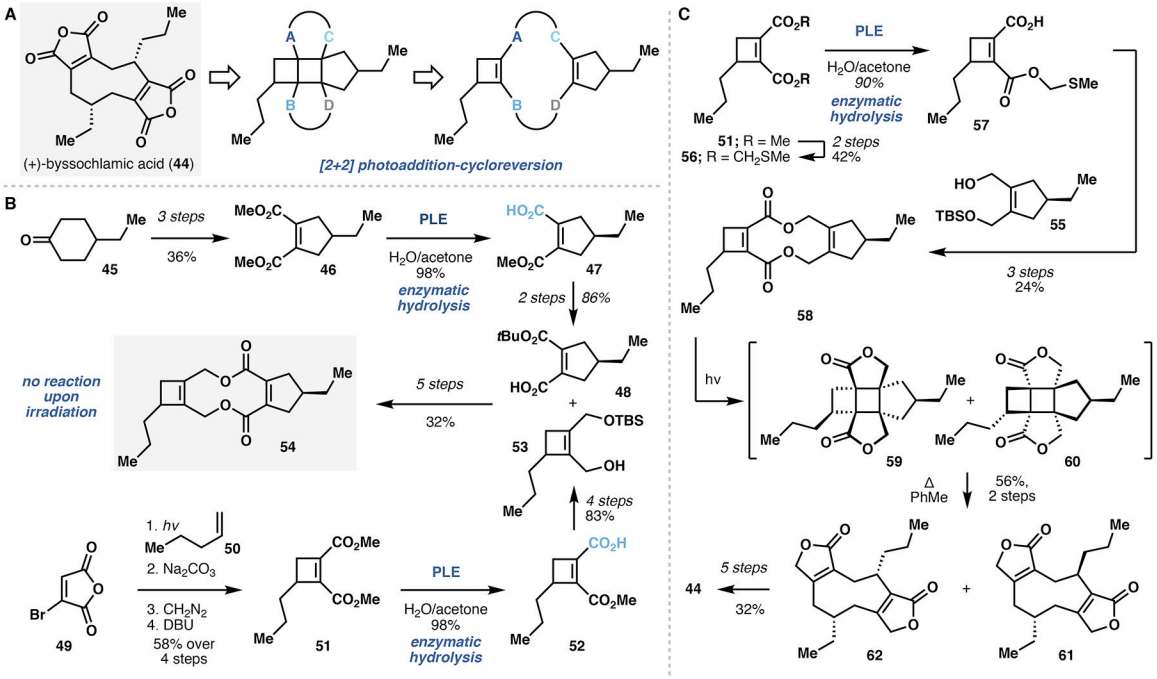


Figure 3.

White's enantiospecific chemoenzymatic synthesis of (+)-byssochlamic acid (**44**). (A) Structure of **44** and White's proposed [2+2] photoaddition-cycloreversion strategy. (B) First-generation chemoenzymatic route toward (+)-byssochlamic acid, in which the esters are appended to the cyclopentene ring. (C) Second-generation route and execution of the photoaddition-reversion approach.

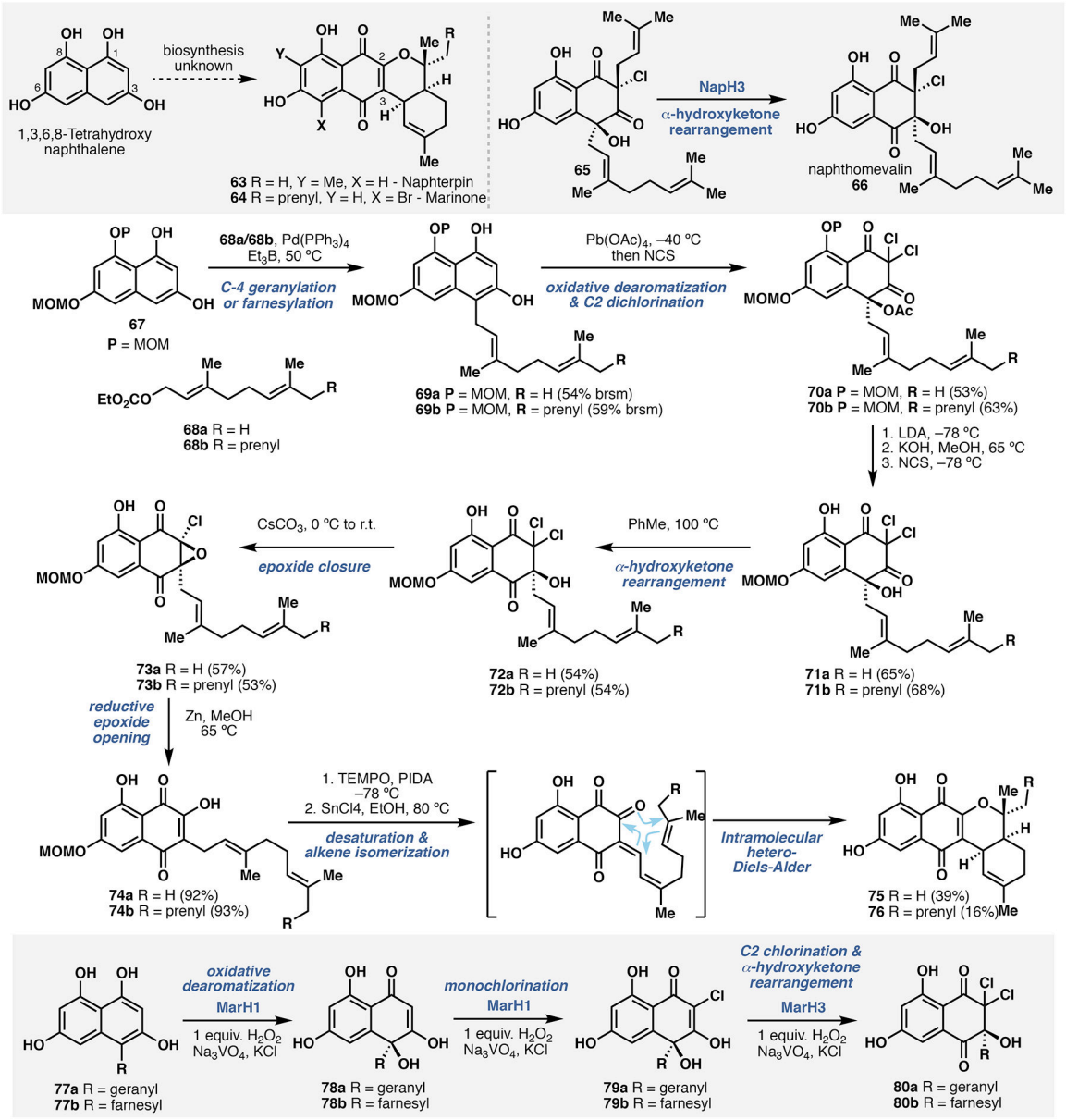


Figure 4.

The total biomimetic synthesis of debromomarinone and 7-demethylnaphterpin, and *in vitro* characterization of MarH1 and MarH3 towards the investigation of marinone and naphterpin biosynthesis.

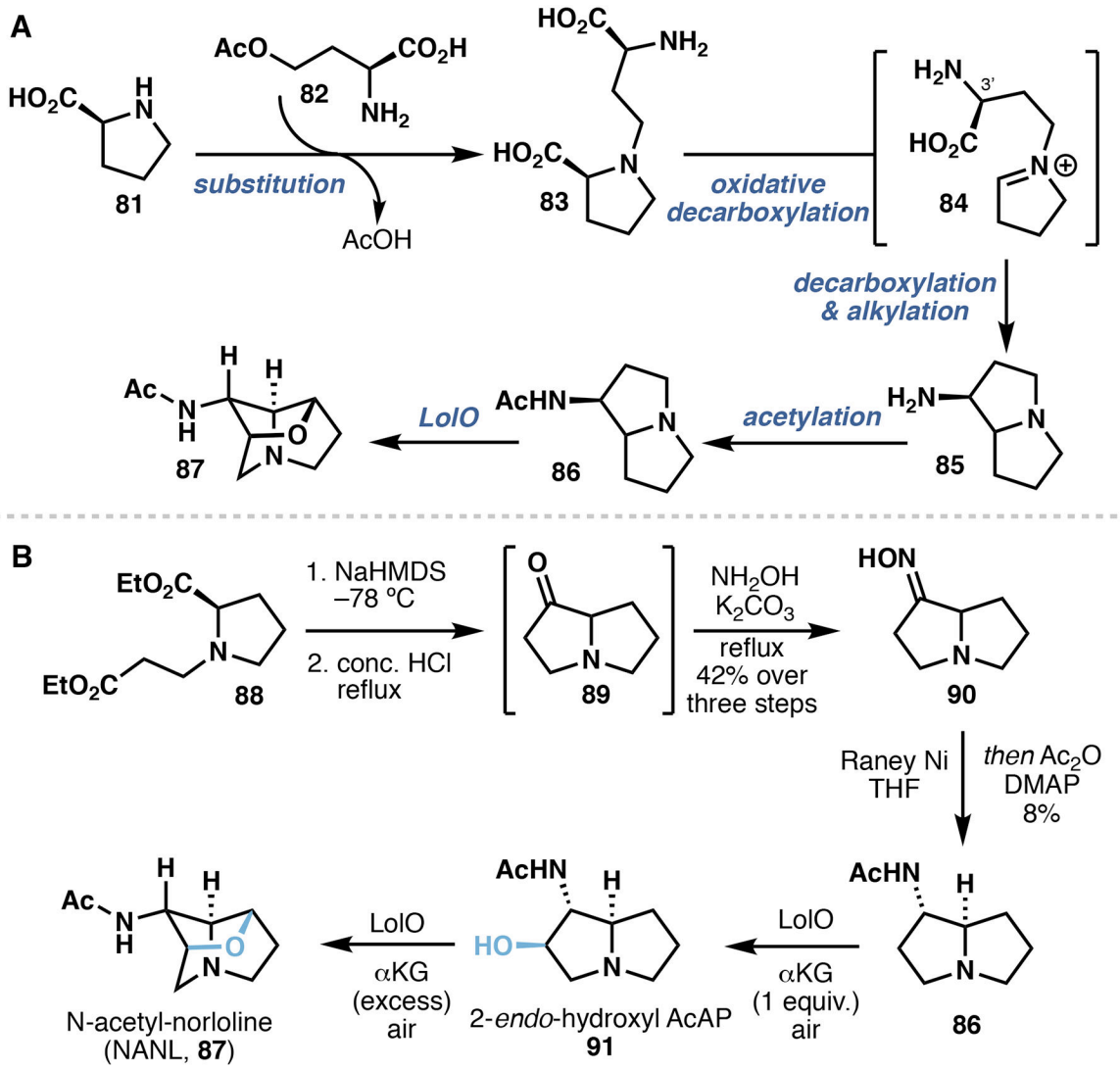


Figure 5.

(A) The biosynthetic assembly of loline tricyclic core scaffold. (B) Synthetic preparation of (±)-*exo*-1-acetamidopyrrolizidine and LolO's stepwise C–H oxidation for ether bridge construction.

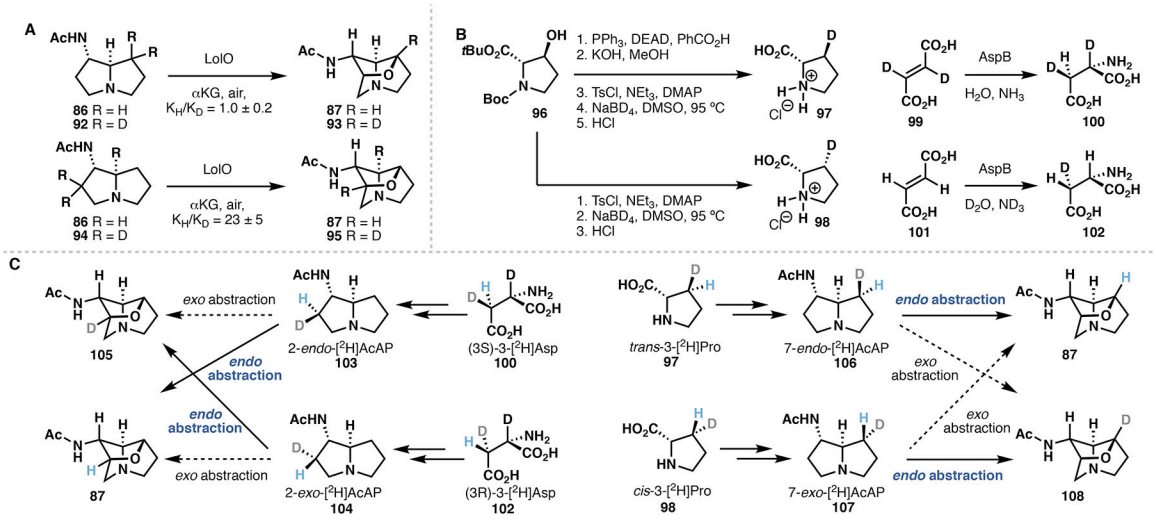


Figure 6.

(A) Investigation of the regioselectivity of LolO through deuterium kinetic isotope effect studies, (B) preparation of deuterium-labelled mechanistic probes, and (C) determination of the facial selectivity of LolO's C–H abstraction events through feeding of properly deuteruo-labelled intermediates.

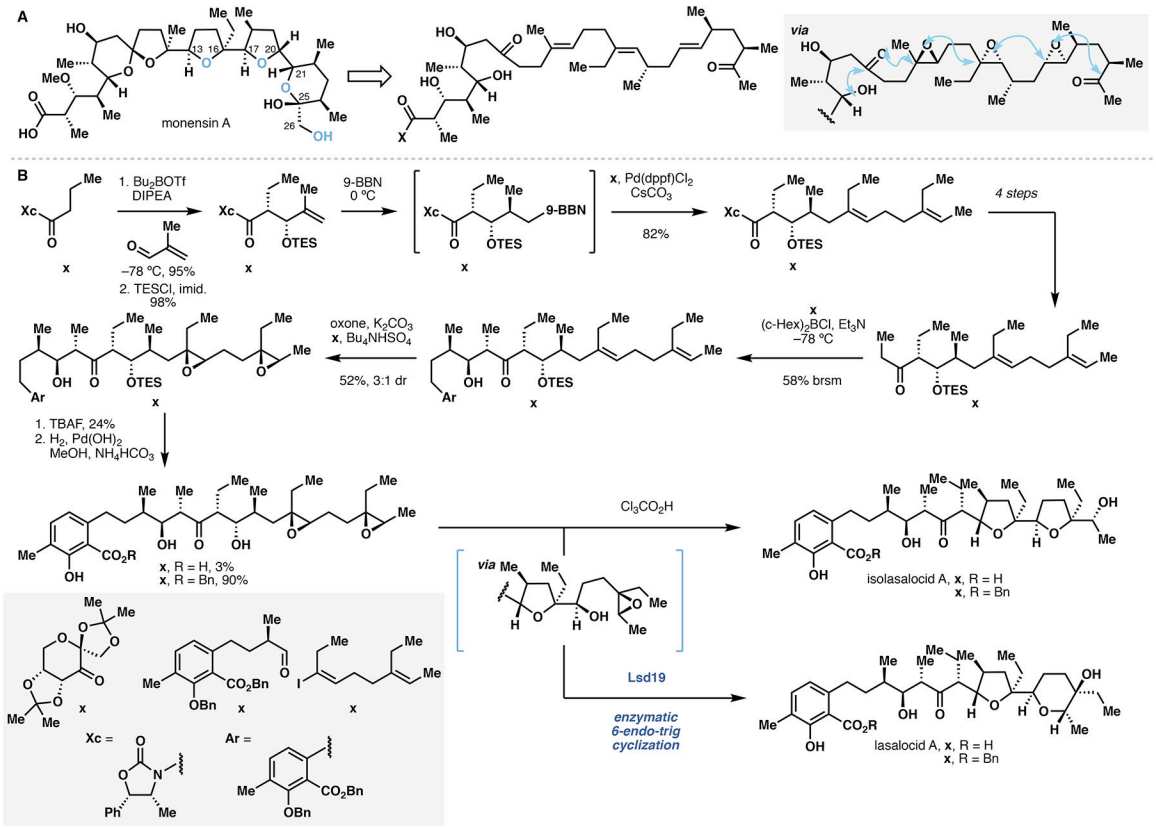


Figure 7.

(A) Proposed biosynthesis of monensin A. (B) Oikawa and Oguri's *in vitro* assay of Lsd19's activity to test Cane and coworkers' hypothesis of the intermediacy of a polyepoxide intermediate in the assembly of the polyether function in lasalocid A.

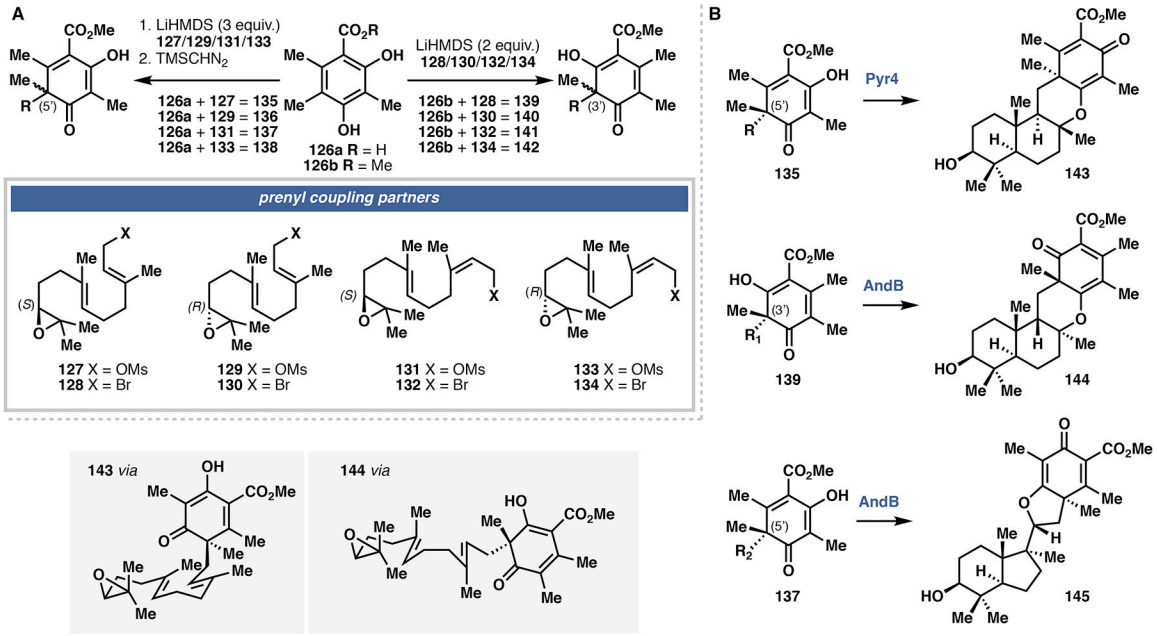


Figure 8.

Porco and Abe's investigation of the promiscuity of fungal terpene synthases. (A) Synthesis of polyene cyclization precursors. (B) Selected examples of product diversity arising from the enzymatic cyclizations.

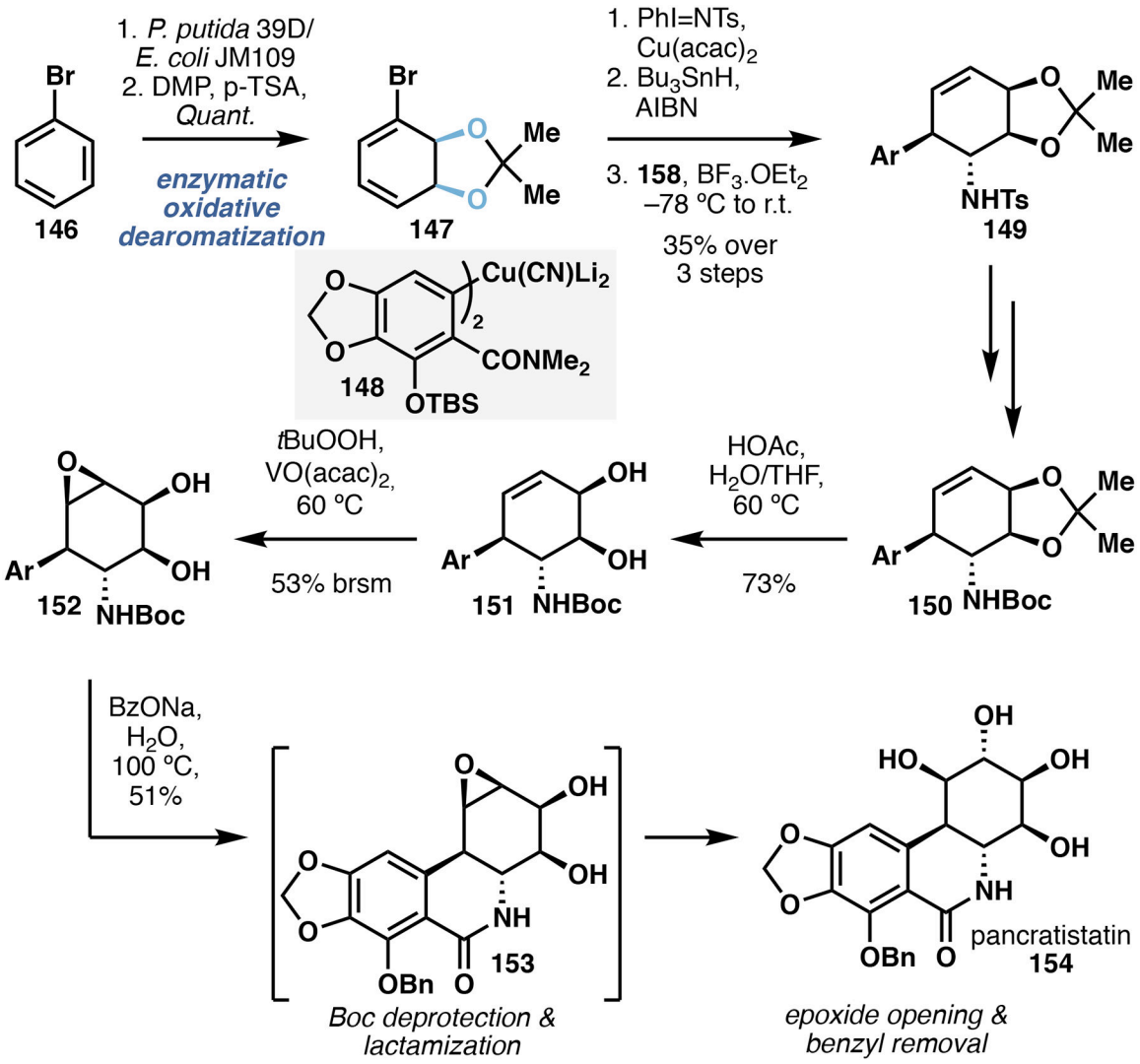


Figure 9.

Hudlicky's synthesis of pancratistatin utilizing toluene dioxygenase to form key intermediate **147** for the asymmetric formation of the core tetrahydroxylated scaffold.

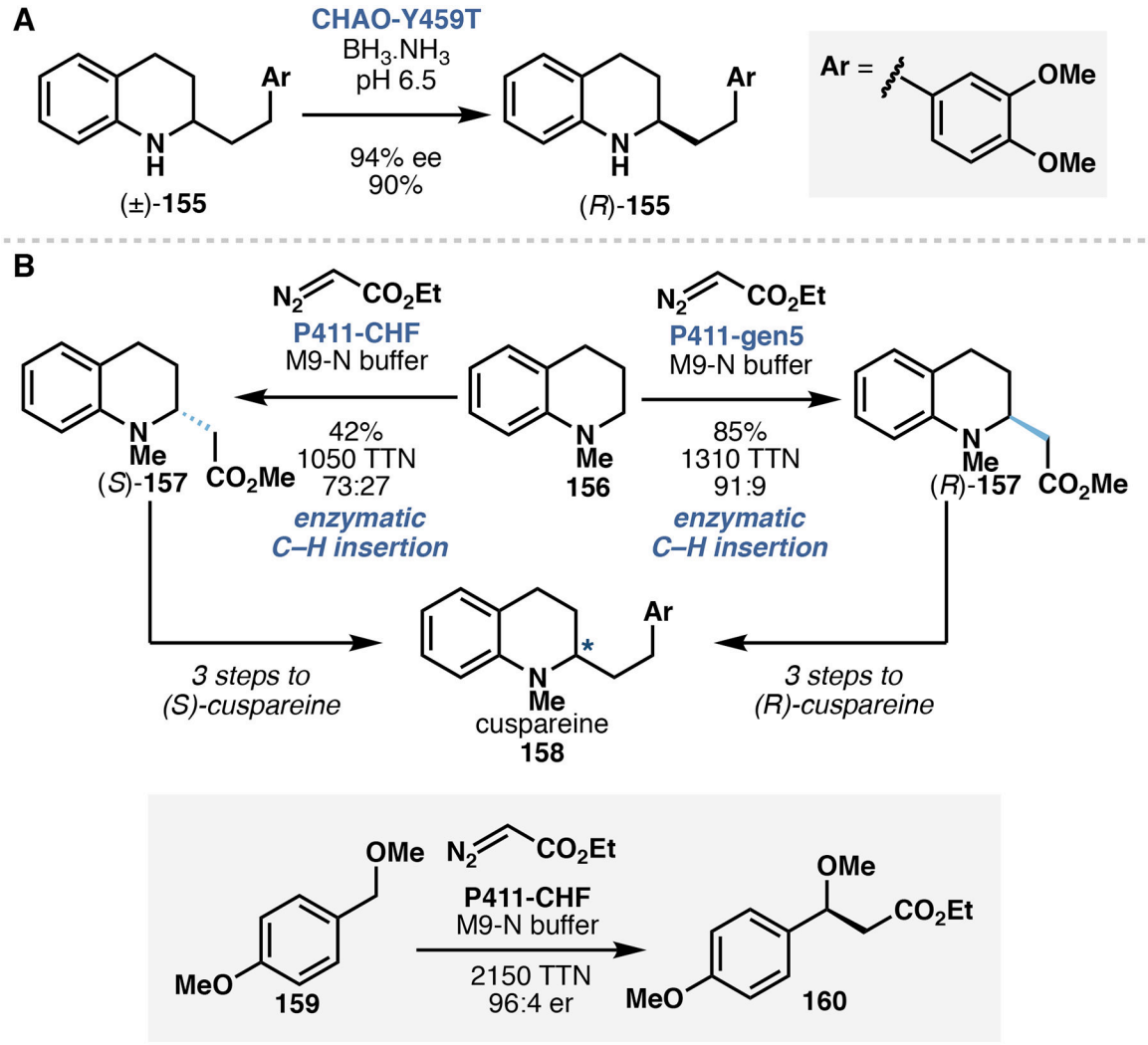


Figure 10.

(A) Lau, Wu and Zhu's oxidative kinetic resolution of 2-alkyl THQs, and (B) Arnold's diazo P411 C–H insertion, and their implementation in the synthesis of cuspareine.

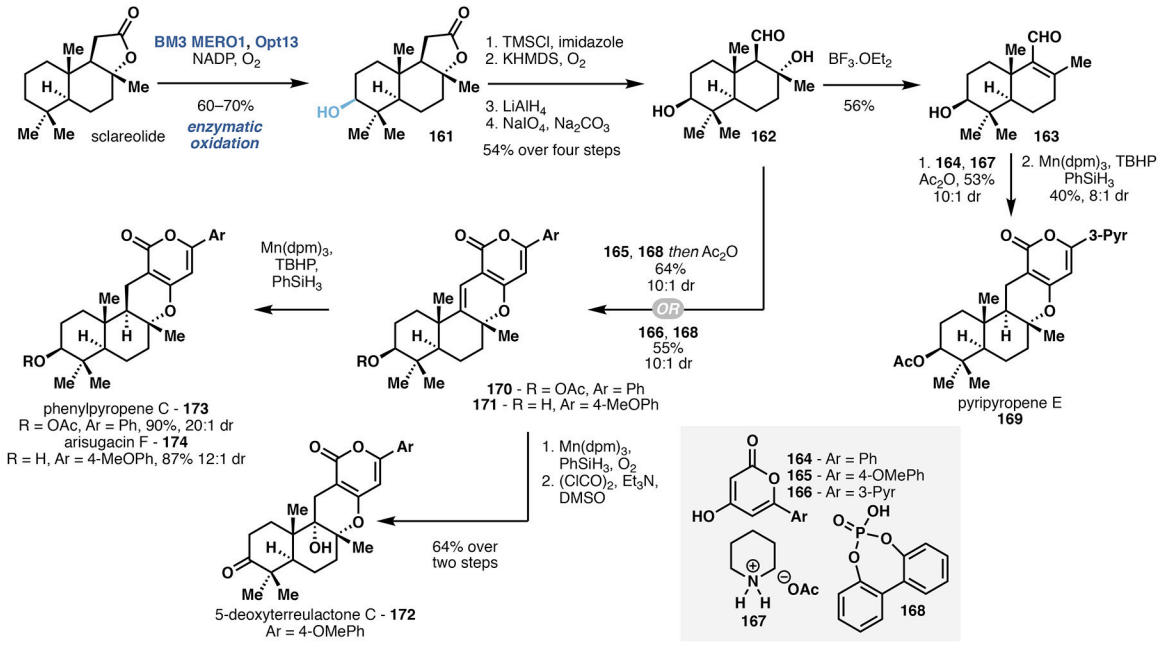


Figure 11.

Renata's C-3 oxidation of sclareolide enables divergence to a variety of merosesquiterpenoids via subsequent key radical-based manipulations.

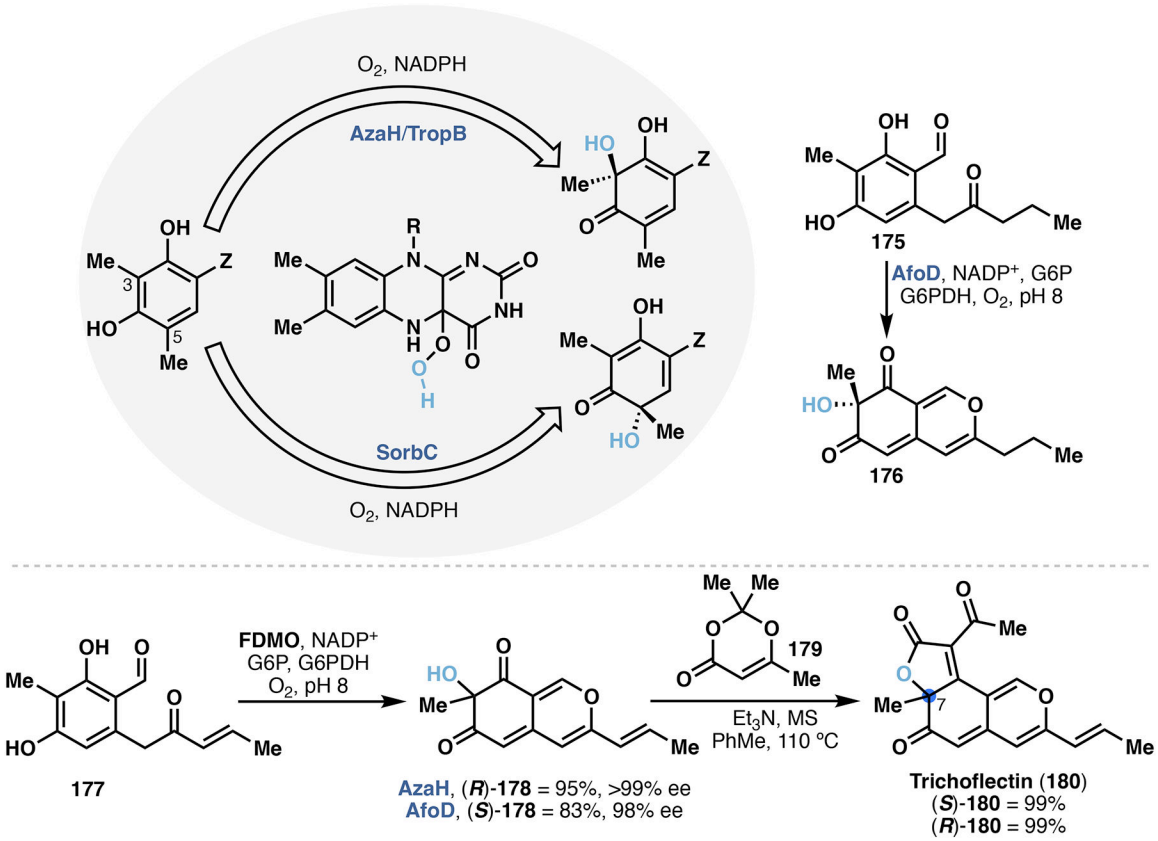
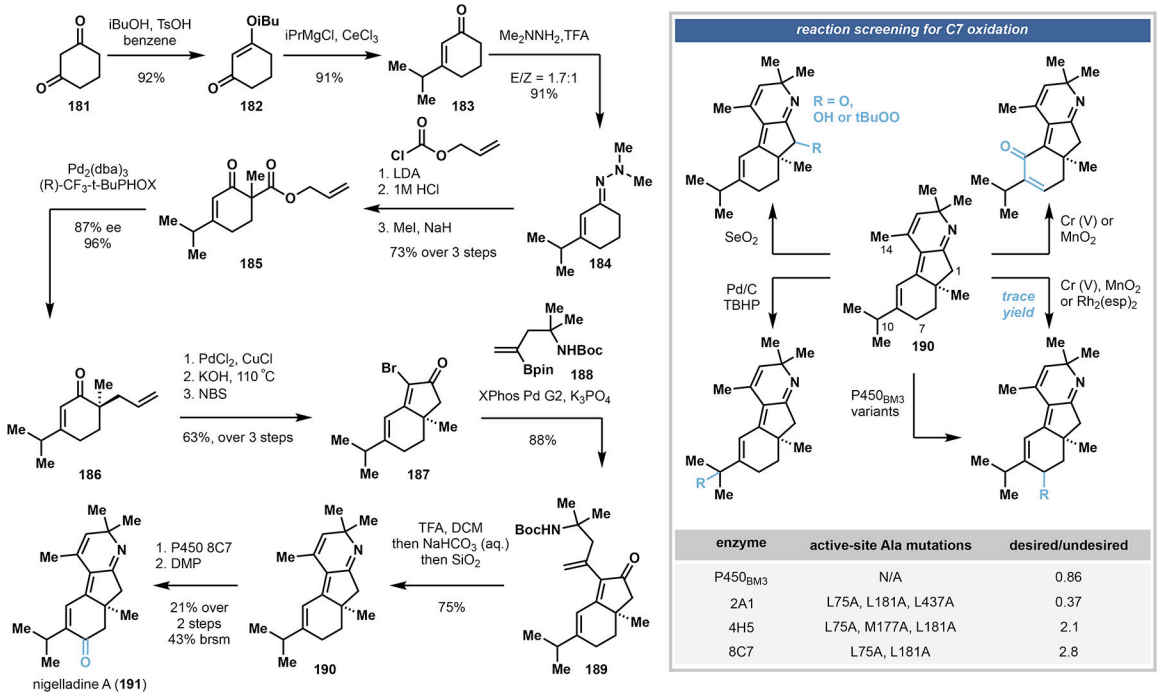


Figure 12.

Narayan's enzymatic toolbox for the oxidative dearomatization of resorcinol derivatives, and their application in the total synthesis of (*R*)- and (*S*)-trichoflectin.



enzyme	active-site Ala mutations	desired/undesired
$P450_{BM3}$	N/A	0.86
2A1	L75A, L181A, L437A	0.37
4H5	L75A, M177A, L181A	2.1
8C7	L75A, L181A	2.8

Figure 13.

Chemoenzymatic synthesis of nigelladine A enabled by the discovery of a $P450_{BM3}$ variant for site-selective oxidation at C7.

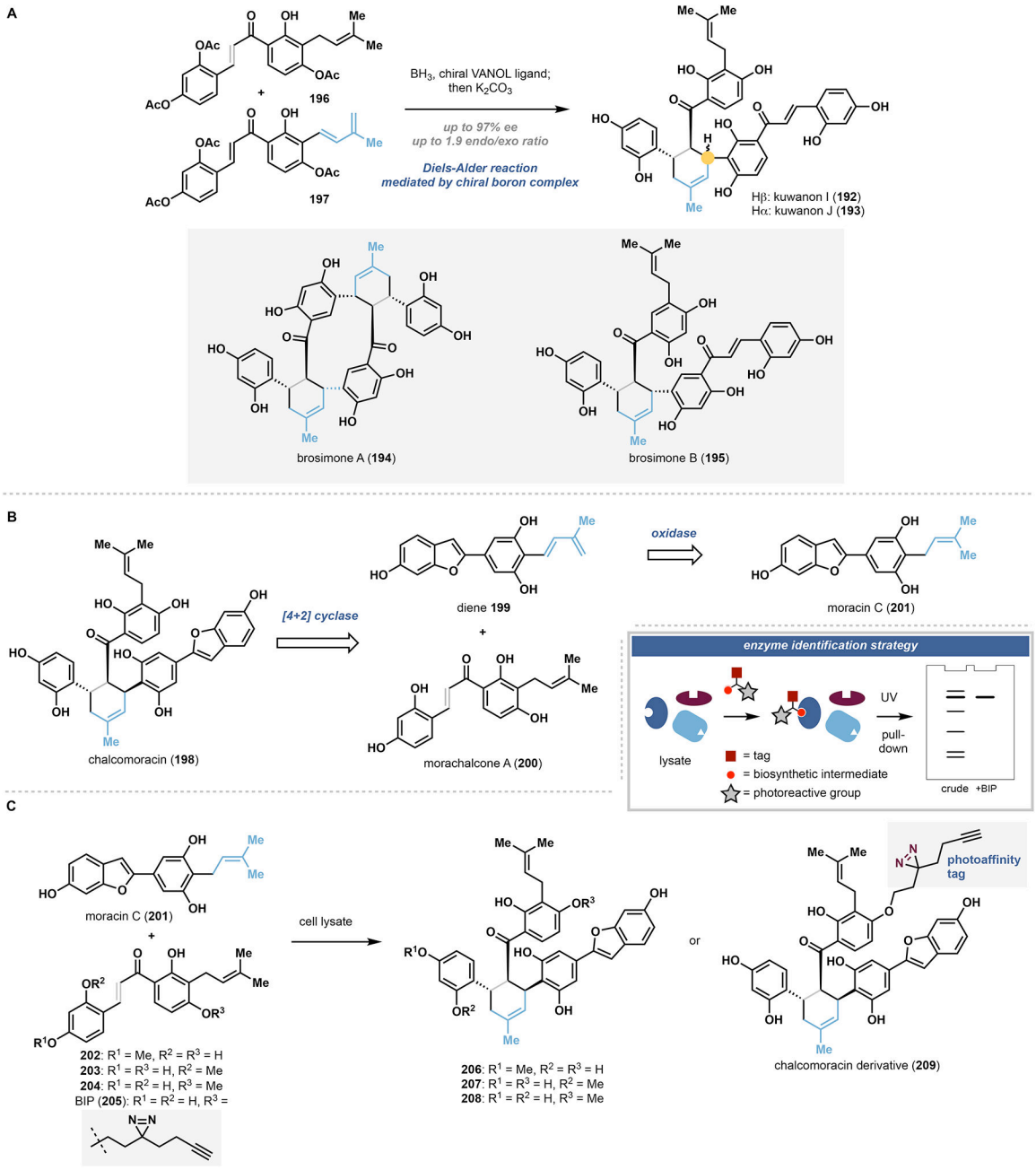


Figure 14.

(A. Lei's synthesis of kuwanons I and J featuring enantioselective Diels–Alder reaction mediated by a chiral boron Lewis acid. (B) Proposed biosynthesis of chalcomoracin. (C) Probe development and synthesis for the identification of key enzymes in the pathway.

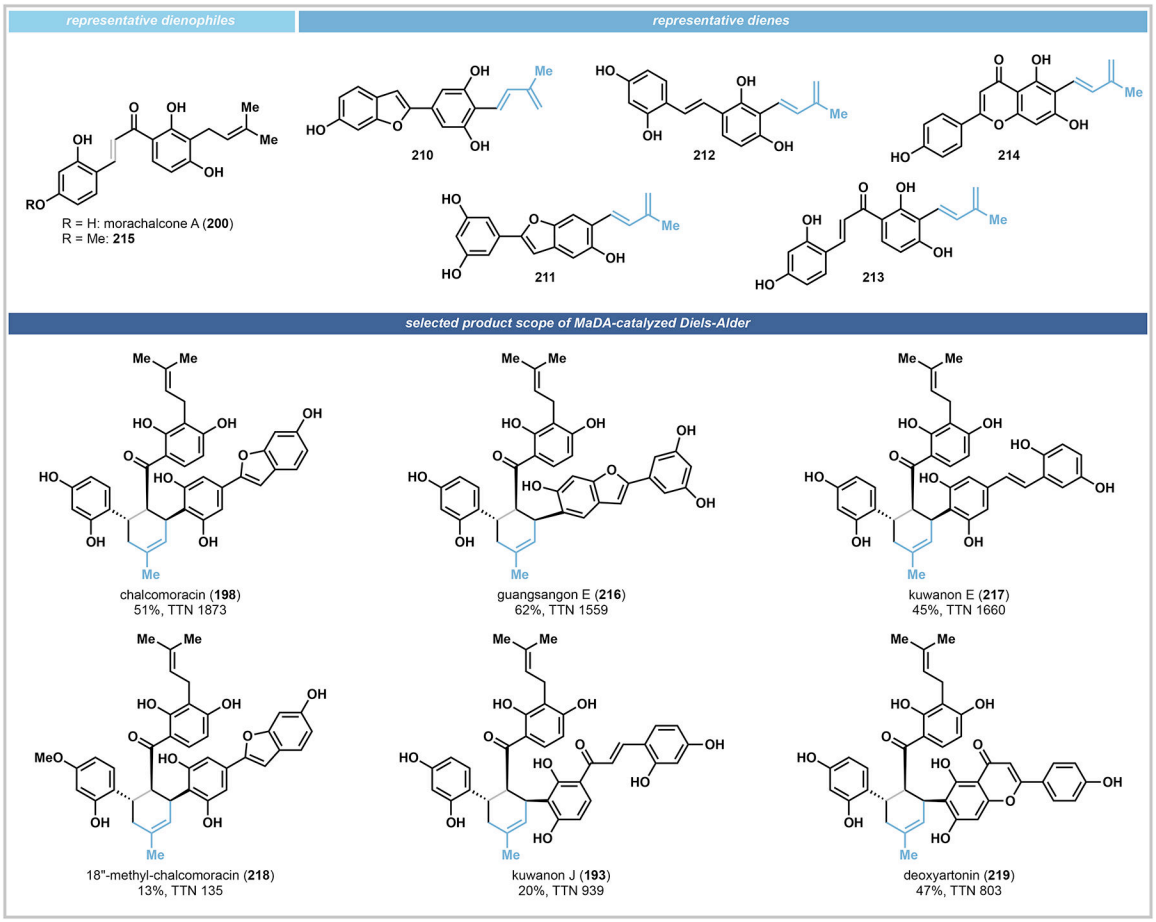


Figure 15.
Selected substrate and product scope of MaDA-catalyzed Diels–Alder.

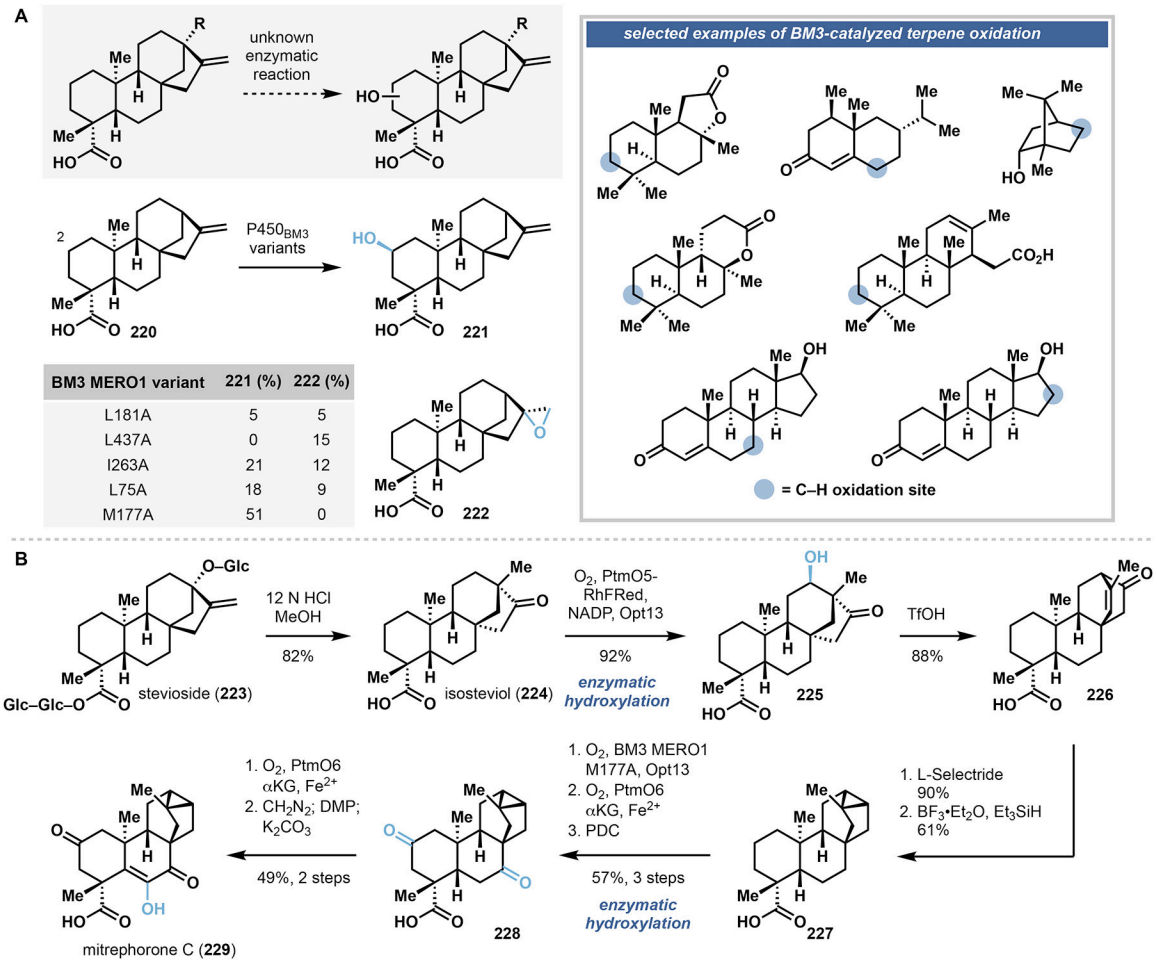


Figure 16.

(A) Screening of $P450_{BM3}$ variants for site-selective C2-oxidation on **220**. (B) Application of the reaction in the synthesis of mitrephorone C.