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Terpene Cyclases

Terpenoid Biosynthesis Off the Beaten Track: Unconventional Cyclases and Their Impact on Biomimetic Synthesis

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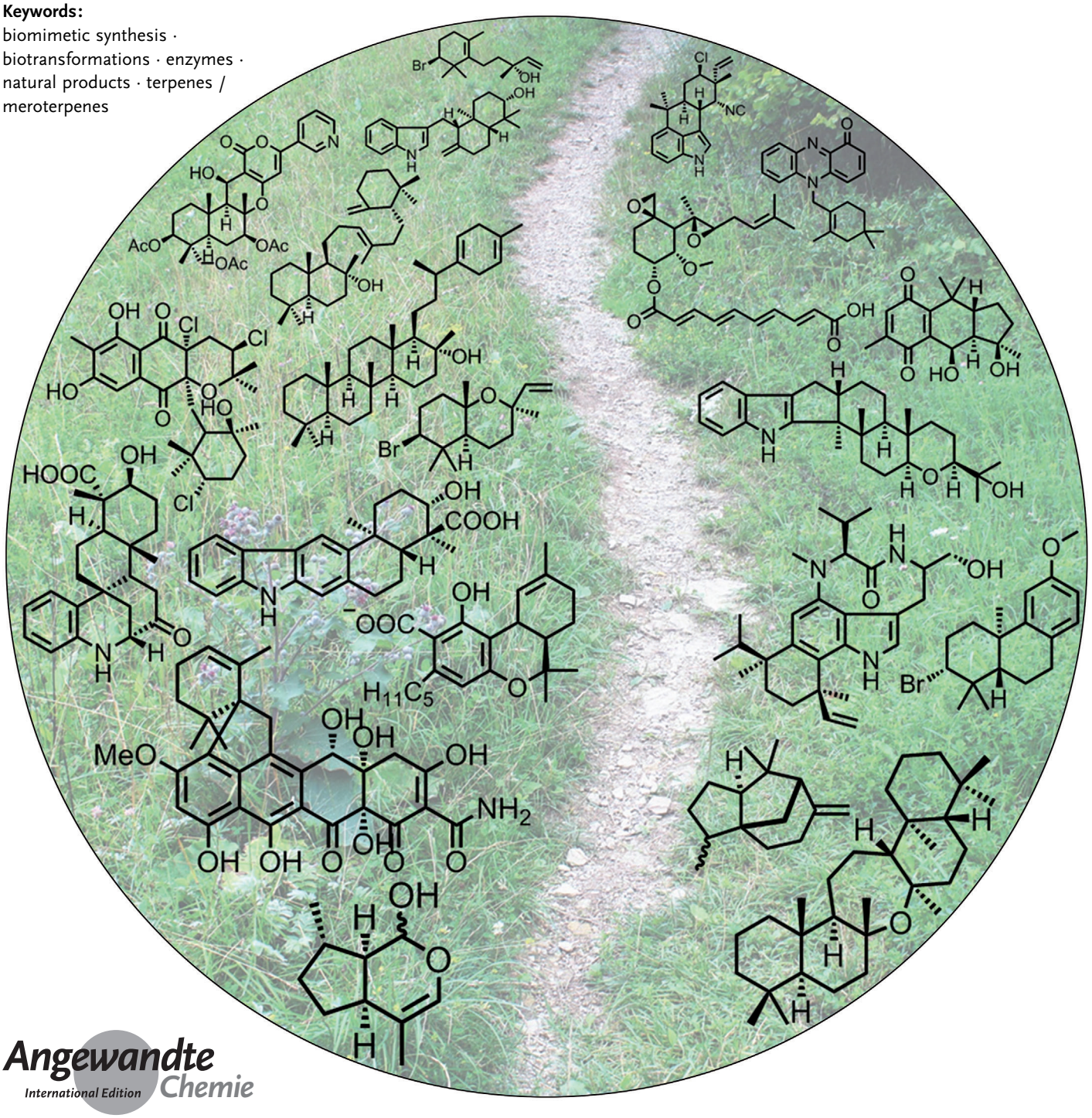

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Terpene and terpenoid cyclizations are counted among the most complex chemical reactions occurring in nature and contribute crucially to the tremendous structural diversity of this largest family of natural products. Many studies were conducted at the chemical, genetic, and biochemical levels to gain mechanistic insights into these intriguing reactions that are catalyzed by terpene and terpenoid cyclases. A myriad of these enzymes have been characterized. Classical textbook knowledge divides terpene/terpenoid cyclases into two major classes according to their structure and reaction mechanism. However, recent discoveries of novel types of terpenoid cyclases illustrate that nature's enzymatic repertoire is far more diverse than initially thought. This Review outlines novel terpenoid cyclases that are out of the ordinary.

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1. Introduction

The complexity of terpene natural products is highly intriguing, in particular if one considers that the tens of thousands of already known members of this largest family of natural products are derived from the same simple C_5 building blocks: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP).[1] These constituent C_5 units can be linked together and then be cyclized and/or rearranged in manifold ways, thereby leading to enormous structural diversity.[2] In particular, the highly variable cyclization patterns of terpene natural products contribute to this diversity and have propelled sophisticated studies since these cyclization reactions are, in part, outstandingly complex.[3] The transformation of the linear hydrocarbon chain of squalene into hopene, for example, leads to a pentacyclic ring structure through the formation of 13 covalent bonds and nine stereogenic centers.[3c] As a result of this complexity, terms such as "chemical wizardry" have been used to describe terpene and terpenoid cyclization.[4] In fact, nature does not depend on wizardry, but rather on dedicated enzymes called terpene and terpenoid cyclases (TCs; also referred to as terpene/terpenoid synthases) to facilitate these complex cyclization reactions. The classical TCs have been divided into two distinct classes, whose members differ in their substrate activation mechanisms as well as their protein folds.[2,3,5] Class I TCs trigger the formation of an allylic cation by loss of the terminal diphosphate group (Scheme 1 A2)[6] through the use of trinuclear metal clusters which are coordinated by conserved acidic and polar amino acids. In contrast, class II TCs initiate carbocation formation by general acid catalysis, through the use of a conserved aspartate residue as a Brønsted acid to protonate an isoprene/isoprenoid double bond (Scheme 1 B1) or an oxirane moiety (Scheme 1 B2) to generate a reactive intermediate.[2] This leads to a different direction of charge propagation along the polyisoprene chain in the subsequent cyclization cascade, which results in a head-to-tail cyclization for class II TCs after electrophilic activation of a terminal prenyl unit (head), or a tail-to-head cyclization for class I TCs after ionization of the allylic diphosphate ester bond (tail).[7] Notably, the reaction mechanism of class I TCs resembles the catalytic cycle of enzymes involved in elongation of the isoprenoid chain. These enzymes are called prenyl transferases (PTs), although the terms prenylprenyl transferases, isoprenyl diphosphate synthases, or prenyl elongases would be more accurate to distinguish them from enzymes that transfer (poly)isoprene moieties onto other types of molecules, such as aromatic compounds.[2] They catalyze the metal-triggered ionization of DMAPP or an analogous polyprenyl diphosphate to an allylic carbocation that can be attacked by the π bond of IPP, followed by the elimination of a proton to yield the elongated linear terpenoid (Scheme 1 A1).[3a] PTs can be subdivided into *cis*- and *trans*-isoprenyl diphosphate synthases according to the stereochemical outcome of their products.[8] In contrast, the carbocation in the reaction cycle of class I TCs reacts with a π bond of the same molecule; therefore, cyclizations catalyzed by class I TCs may be considered as the intramolecular equivalent of the intermolecular coupling reaction catalyzed by PTs (Scheme 1 A2).[1] This rationale of a close relationship between class I TCs and PTs is supported by the discovery that a chicken PT is capable of generating cyclic olefins as by-products when incubated with a farnesyl diphosphate (FPP) substrate, although in rates thousands of times slower than the condensation with IPP.[9] Since the overall protein folds of *trans*-isoprenyl diphosphate synthases and class I TCs, which have an α-bundle fold designated as "class I terpenoid synthase fold",[5] are also very similar and highly conserved, a divergent evolution from a common ancestral synthase is widely believed.[10] In stark contrast, class II TCs have an unrelated double α-barrel fold, designated as a "class II terpenoid synthase fold" that differs remarkably from the α-helical class I TCs.[3b,5] Despite their

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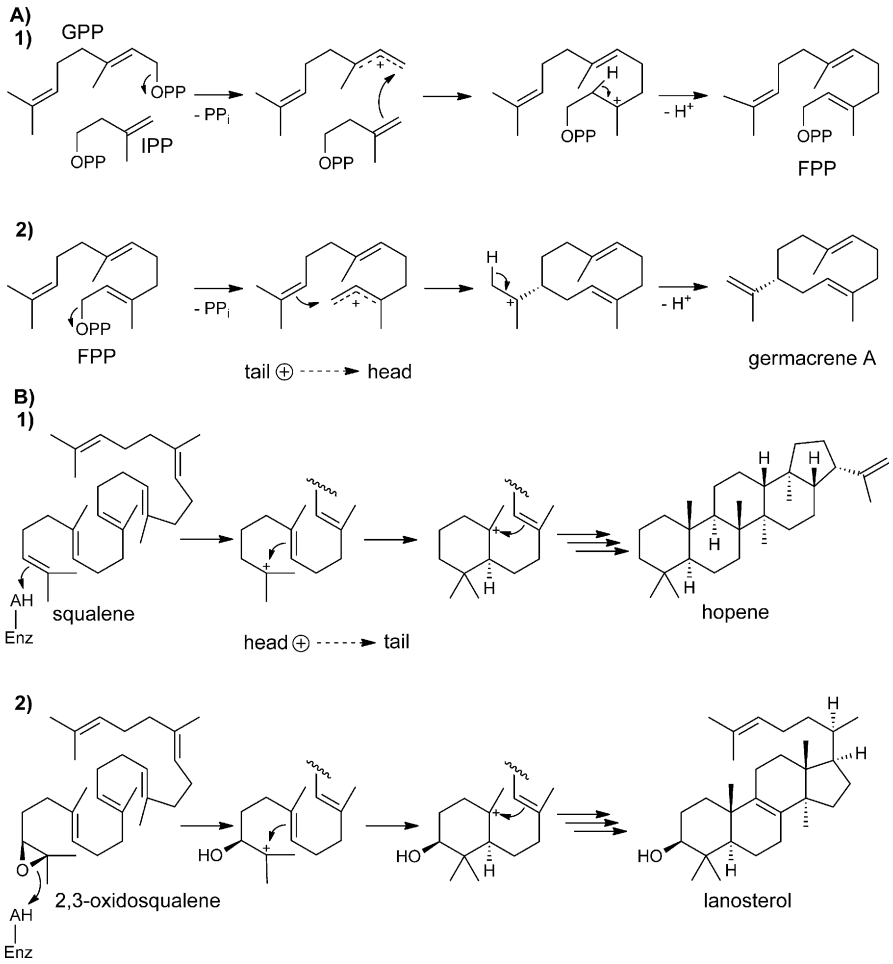

Scheme 1. Reactions catalyzed by terpene/terpenoid synthases. A) Reactions catalyzed by enzymes with a class I terpenoid synthase fold: 1) Farnesyl diphosphate synthase, representing a *trans*-isoprenyl diphosphate synthase[11] and 2) germacrene A synthase, representing a class I terpene cyclase.[12] B) Reactions catalyzed by enzymes with a class II terpenoid synthase fold: 1) Squalene-hopene cyclase, representing class II terpene cyclases that initiate cyclization by protonation of an isoprene double bond,[13] and 2) lanosterol synthase, representing a class II terpenoid cyclase that initiates cyclization by protonation of an oxirane moiety.[14] GPP = geranyl diphosphate; IPP = isopentenyl diphosphate; FPP = farnesyl diphosphate; PP_i = inorganic diphosphate.

different overall structure and different activation mechanisms, both enzyme classes are capable of triggering the formation of a highly reactive cationic intermediate, then serving as a template to position the flexible substrate chain correctly and protect the carbocation intermediates throughout the sequential cyclization reaction.[3a,b]

Although most TCs fall into this scheme, one may wonder whether more structurally unrelated enzyme classes exist that make use of the same mechanistic concepts. Indeed, recent discoveries of cryptic TCs revealed that this is exactly the case, and most surprisingly, it seems that the whole mechanistic repertoire of class I and class II TCs is imitated by structurally very distinct enzymes. Moreover, recent discoveries show that nature has invented further cyclization strategies to add structural diversity to the "terpenome". In the following sections various novel TCs will be introduced exemplarily. Finally, novel biomimetic synthetic approaches will be highlighted that enable synthetic chemists to emulate enzyme-catalyzed cyclization reactions.

2. Novel Terpene Cyclases from Bacteria, Fungi, and Plants

2.1. Terpenoid Cyclization That Resembles the Mechanism of Class I or Class II Terpene Cyclases

2.1.1. Terpenoid Cyclization Catalyzed by Vanadium-Dependent Haloperoxidases

Marine macroalgae are a rich source of halogenated cyclic terpene natural products such as the snyderols or (+)-3β-bromo-8-epicaparrapi oxide isolated from the red alga *Laurencia obtusa*.[15] Biosynthetic studies on brominated cyclic sesquiterpenes suggested that they are biosynthesized

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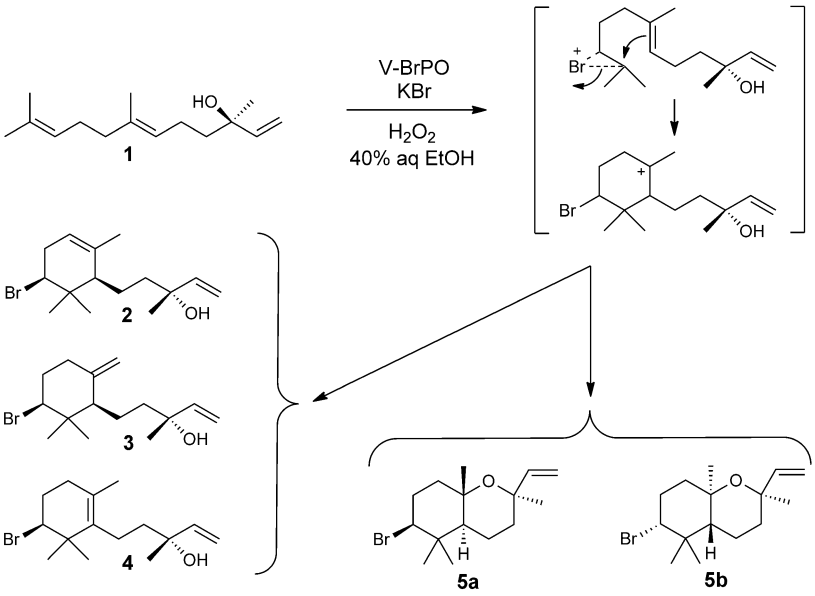
by a bromonium ion induced cyclization of an acyclic terpene precursor.[16] The discovery of haloperoxidases in many marine organisms provided for the first time potential catalysts, and indeed vanadium-dependent bromoperoxidases (V-BrPOs) from marine algae turned out to catalyze the bromonium ion initiated cyclization of terpenes and ethers in vitro.[17] In a chemoenzymatic approach, V-BrPOs isolated from the marine red algae *Corallina officinalis*, *Plocamium cartilagineum*, and *Laurencia pacifica* were shown to catalyze the cyclization of monoterpenes such as geraniol to cyclic products related in structure to larger bromocyclic terpene natural products such as the sesquiterpenes α-snyderol and β-snyderol.[17a] Although the resulting cyclic monoterpenes are not known as marine natural products, this study strongly suggests that V-BrPOs are generally involved in the biosynthesis of brominated cyclic terpene natural products. Finally, the role of V-BrPO in the biosynthesis of brominated cyclic sesquiterpenes was established by enzymatic conversion of the sesquiterpene (*E*)-(+)-nerolidol (**1**) into α-, β-, and γ-snyderol (**2**, **3**, **4**), as well as the bicyclic (+)-3β-bromo-8-epicaparrapi oxide (**5a**) and its corresponding diastereomer **5b**.[17b] The proposed mechanism assumes a selective bromination of the terminal olefinic bond that leads to a bromocarbenium ion intermediate. After subsequent attack by the electron-rich internal olefin, three different elimination reactions lead to **2**, **3**, and **4**, whereas nucleophilic trapping of the proposed bromocarbenium ion by the hydroxy group leads to **5a** and **5b** (Scheme 2).[17b]



Scheme 2. Proposed mechanism for the biosynthesis of α-, β-, and γ-snyderol (**2**, **3**, **4**), as well as (+)-3β-bromo-8-epicaparrapi oxide (**5a**) and its corresponding diastereomer **5b**, catalyzed by vanadium-dependent bromoperoxidases.[17b]

The three-dimensional structure of the V-BrPO from *Corallina officinalis* (CVBPO), one of the various V-BrPOs that was capable of catalyzing the above-mentioned cyclizations has been determined at 2.3 Å resolution.[18] Two enzyme subunits with 595 amino acid residues each form a dimer (Figure 1A). Six of these homodimers again form a dodecamer. The active site of each CVBPO subunit is located at the bottom of a deep funnel-shaped cavity that is lined with several hydrophobic patches and charged residues. Furthermore, the enzyme subunits have a binding site for divalent cations, which seem to be necessary to maintain the structure of the active-site cavity and for dimer interaction, but do not seem to be involved in the CVBPO reaction. The crystal was grown in the presence of inorganic phosphate that acts as a vanadate surrogate and competes with the latter for binding in the enzyme active site. Unlike the vanadate group, the phosphate group is not covalently bound to the active-site histidine residue (His551). The phosphate group is stabilized by hydrogen bonds with Ser483, the backbone of the Gly484 amide, and His485. Additionally, the phosphate group forms salt bridges with Lys398, Arg406, and Arg545 (Figure 1B). Vanadium, which is proposed to be covalently bound to His551, is likely coordinated by the protein in a trigonal bipyramidal coordination geometry, as observed in other vanadium-dependent haloperoxidases (V-HPOs).[19] Based on the protein structure[18] and studies on the mechanistic details of other V-HPOs,[19,20] a catalytic cycle can be proposed that is initiated by coordination of one equivalent of H_2O_2 to the vanadium center. Hydrogen bonding from the Lys398 side chain activates the vanadium-coordinated peroxide through charge separation and assists bromide oxidation. The oxidized bromine intermediate likely leaves the coordination sphere as hypobromous acid after protonation by an incoming water molecule,[20a] although other brominating species are conceivable.[19] This intermediate will react with a nucleophile to form the brominated compound or react with another equivalent of hydrogen peroxide to generate singlet oxygen and bromide in the absence of an appropriate substrate (Scheme 3).[19,20b]

The proposed mechanism of V-BrPOs for the cyclization of **1** via a bromocarbenium ion intermediate resembles that of class II TCs, such as the squalene-hopene cyclase, which triggers the stereospecific cyclization of squalene by the

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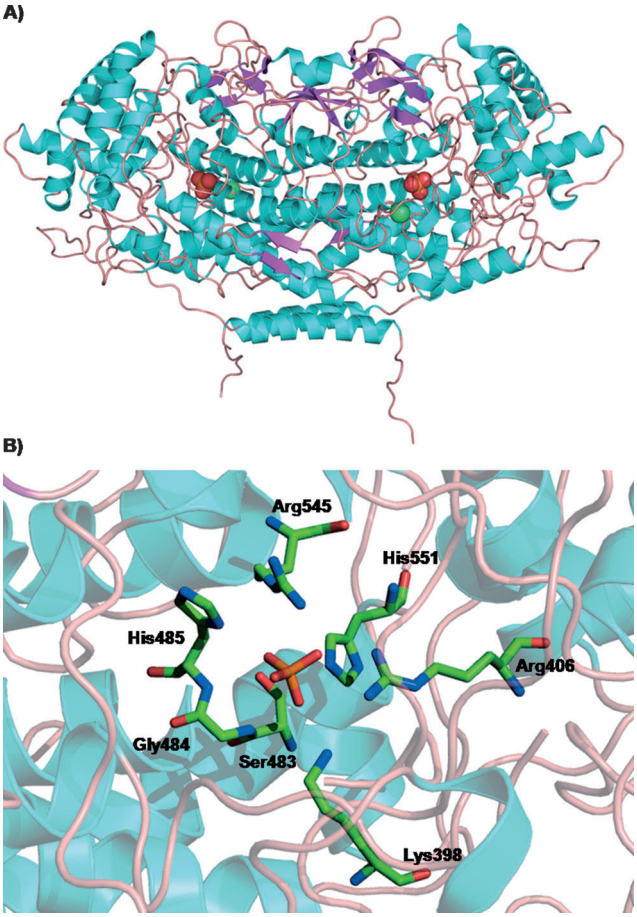
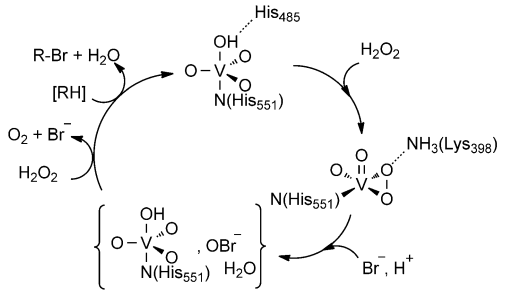


Figure 1. Structure of the vanadium-dependent bromoperoxidase from *Corallina officinalis* (CVBPO; PDB: 1QHB).[18] A) Ribbon diagram of the overall structure of a CVBPO homodimer; α helices are colored cyan, β strands magenta, and loops pink. Phosphate groups (P orange, O red) and divalent cations (Ca green) are shown in sphere representations. B) Diagram of active-site residues of CVBPO with a bound phosphate group in a stick representation (C green, N blue, P orange, O red). The diagrams were prepared with PyMol.[22]

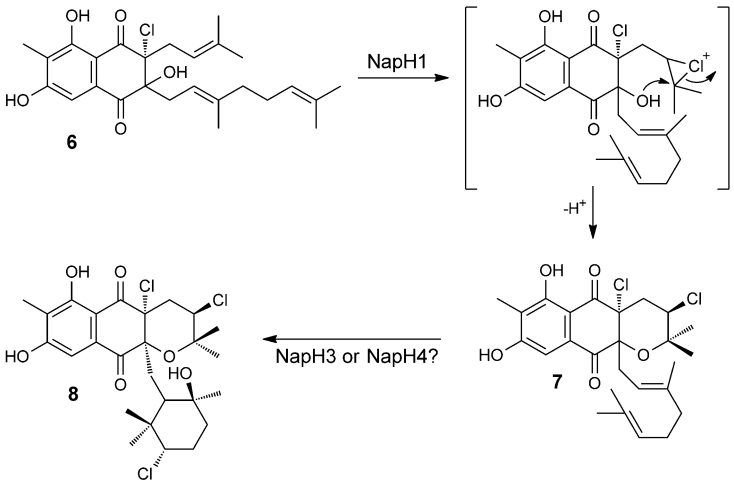


Scheme 3. Proposed catalytic cycle of the vanadium-dependent bromoperoxidase from *Corallina officinalis* (CVBPO). For details see the text. This scheme was adapted from Refs. [18–20].

protonation of the terminal olefin, followed by a successive cyclization cascade within the enzyme cavity.[3c,13] The active site of squalene-hopene cyclase is predominantly hydrophobic due to numerous aromatic residues that are hypothesized to stabilize carbocation intermediates and their flanking transition states through quadrupole–charge interactions.[21] Analogously to this scheme, Carter-Franklin and Butler suggested a cyclization along the hydrophobic substrate channel in V-BrPO after the asymmetric bromination of the terminal olefin.[17b]

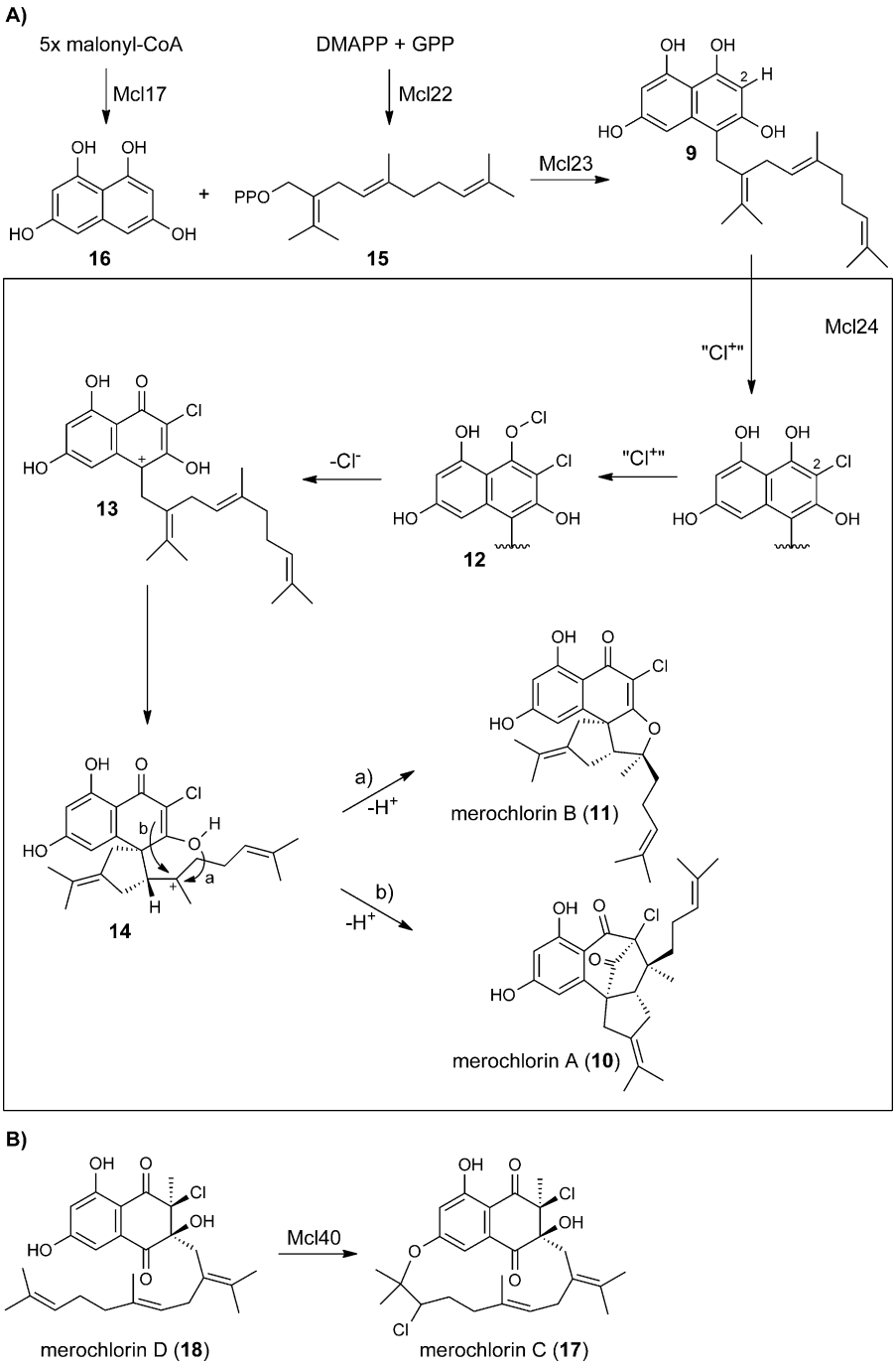
Interestingly, the napyradiomycin biosynthetic gene cluster (*nap*) from *Streptomyces aculeolatus* NRRL 18422 and from the marine sediment-derived *Streptomyces* sp. CNQ-525 encodes three putative vanadium-dependent chloroperoxidases (V-ClPOs) NapH1, NapH3, and NapH4, which have been suggested to take part in chloronium ion induced cyclization in the biosynthesis of chlorinated dihydroquinones such as **8**, which belongs to the napyradiomycin family (Scheme 4).[23] These compounds, which are partly derived from polyketides, are hybrid natural products of both terpenoid and nonterpenoid origin and are, therefore, classified as meroterpenoids. The first experimental evidence of the involvement of one of the V-ClPOs in the cyclization of the terpenoid portions of **8** came from the biochemical characterization of NapH1, which was shown in vitro to catalyze the stereoselective conversion of SF2415B1 (**6**) into SF2415B3 (**7**) though a chloroetherification reaction (Scheme 4).[24]

More experimental evidence regarding the involvement of bacterial V-ClPOs in the cyclization of complex meroterpenoids comes from studies on the biosynthesis of merochlorins.[25] Recently, Diethelm and co-workers showed that Mcl24, which is one of the two V-ClPOs encoded in the merochlorin biosynthetic gene cluster (*mcl*) from *Streptomyces* sp. strain CNH-189, can transform the triene pre-merochlorin (**9**) directly into merochlorin A (**10**) and B (**11**).[25a] However, the mechanism of the reaction seems to differ remarkably from that of other V-HPOs involved in terpenoid cyclization reactions. This transformation likely involves an unprecedented reaction sequence involving a chlorination



Scheme 4. Proposed biosynthetic pathway of the chlorinated dihydroquinone A80915A-D (**8**) starting from its precursor SF2415B1 (**6**)[23] via intermediate SF2415B3 (**7**), which is biosynthesized from **6** by the vanadium-dependent chloroperoxidase NapH1.[24]

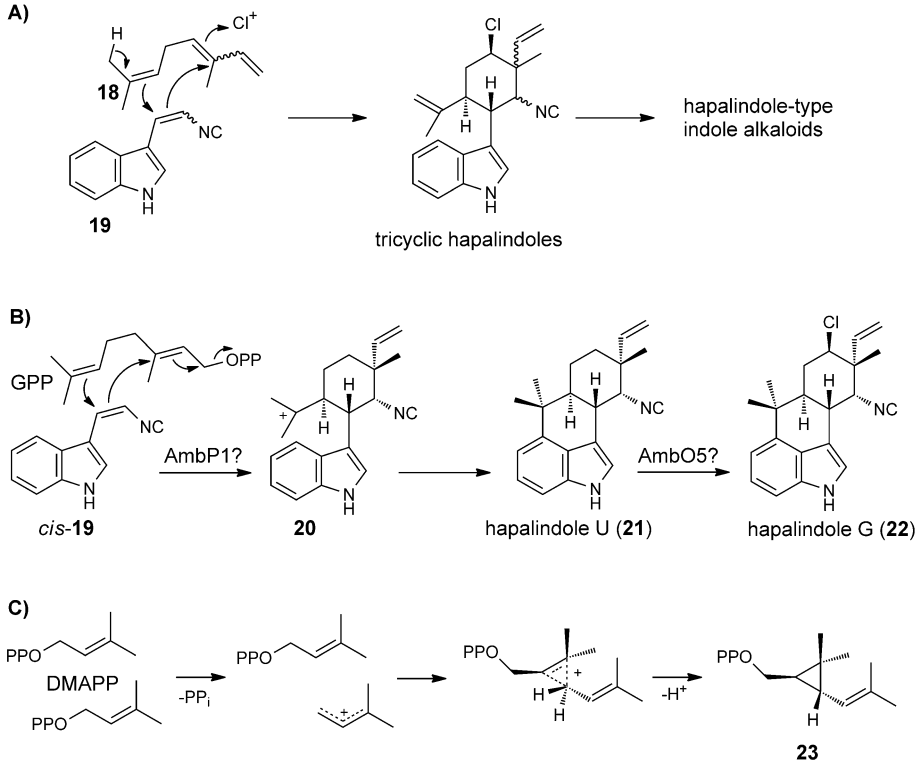
step followed by an oxidative dearomatization/terpene cyclization. The proposed reaction mechanism assumes a C2-selective chlorination of **9** followed by a second chlorination that yields the aromatic hypochlorite species **12**. Loss of chloride gives the benzylic carbocation **13**, which is prone to a cation-induced terpene cyclization via intermediate **14** (Scheme 5 A). The authors further developed a chemical synthesis that parallels the oxidative dearomatization/cyclization of the enzyme by using chlorination agents. The use of *N*-chlorosuccinimide in the presence of two equivalents of $i\text{Pr}_2\text{NH}$ appeared to be the best of the tested conditions, which led to the production of various merochlorin analogues together with **10** and **11** in a total yield of 30 % upon incubation with **9**. This observation tempted the authors to speculate whether an in situ generated chloramine intermediate may be the active oxidant, similar to that postulated for the flavin-dependent chlorinase RebH in rebeccamycin biosynthesis.[25a] With this key enzyme in hand, Teufel et al. succeeded in the one-pot enzymatic synthesis of **10** and **11**.[25c] In an in vitro reconstitution of the biosynthetic pathway to merochlorin A and B, the authors showed that Mcl24, the isosesquilavandulyl diphosphate (**15**) synthase Mcl22, the type III polyketide synthase Mcl17 that produces 1,3,6,8-tetrahydroxynaphthalene (THN; **16**), and the aromatic prenyltransferase Mcl23 are sufficient to synthesize **10** and **11** from DMAPP, GPP, and malonyl-CoA (Scheme 5 A).[25c] In vivo experiments suggest that the second V-ClPO encoded in the *mcl* biosynthetic gene cluster (Mcl40) is also likely involved in a terpene cyclization step during the biosynthesis of merochlorin C (**17**).[25b] A mutant that heterologously expressed a fragment of the *mcl* biosynthetic gene cluster that lacks *mcl40* accumulated **10**, **11**, and the previously unrecognized metabolite merochlorin D (**18**), but did not produce **17**, whereas a clone expressing a fragment that included *mcl40* was able to produce all the natural merochlorins. Therefore, it is highly conceivable that Mcl40 catalyzes the macrocyclization of **18** or a less-substituted derivative to form **17** or the corresponding derivative, similar to the ether ring formation catalyzed by NapH1 in napyradiomycin biosynthesis (Scheme 5 B).[25b]



Scheme 5. Proposed biosynthesis of merochlorins. A) Proposed biosynthesis of merochlorin A (**10**) and B (**11**) starting from DMAPP, GPP, and malonyl-CoA. Pre-merochlorin (**9**) is formed through prenylation of 1,3,6,8-tetrahydroxynapthalene (THN; **16**) with isosesquilavandulyl diphosphate (**15**).[25c] **9** is further cyclized by selective C2 chlorination, followed by a second chlorination to yield **12**, which is transformed into benzylic carbocation **13** after loss of chloride. This reactive carbocation is prone to a cation-induced terpene cyclization via **14** to yield **10** and **11**. This reaction sequence is catalyzed solely by V-ClPO Mcl24.[25a] B) Proposed biosynthesis of merochlorin C (**17**) starting from its putative precursor merochlorin D (**18**) catalyzed by the V-ClPO Mcl40.[25b]

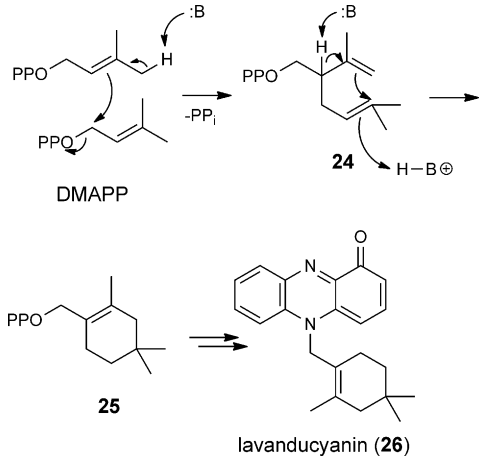
2.1.2. *Prenyltransferases that also Act as Cyclases*

Another case in which a chloronium ion catalyzed cyclization was suggested is the biosynthesis of hapalindole-type indole alkaloids, namely tetracyclic natural products bearing an isonitrile group.[26] The initial hypothesis on the generation of hapalindoles suggested a chloronium ion catalyzed cyclization cascade between the monoterpene β-ocimene (**18**) and vinyl isonitrile **19** to yield the tricyclic hapalindoles. These tricyclic scaffolds might be further transformed into other subfamilies of hapalindole-type indole alkaloids (Scheme 6A).[26,27] However, since not all hapalindole-type compounds are chlorinated, it was suggested that during the course of the cyclization reaction chloronium and proton ions compete in the active site of the condensing enzyme.[28] Hillwig et al. were the first to succeed in the identification of a biosynthetic gene cluster for hapalindole-type natural products, namely the ambiguine (*amb*) biosynthetic gene cluster from *Fischerella ambigua* UTEX1903.[26] Surprisingly, no chloroperoxidase-like enzyme is encoded in the pathway or in the UTEX1903 genome that could facilitate the proposed chloronium ion catalyzed cyclization cascade. Moreover, no apparent terpene synthase homologue to generate β-ocimene (**18**) was encoded in the *amb* gene cluster. Instead, three genes of the prenyltransferase superfamily were identified (*ambP1-3*) for which it was shown in vitro that *ambP2* encodes a GPP synthase and that *ambP3* encodes an aromatic prenyltransferase that catalyzes a further prenylation on the aromatic ring of hapalindole U (**21**), which is a prerequisite to form the pentacyclic ambiguines. Since none of the three members of the prenyltransferase superfamily was capable of producing β-ocimene (**18**) in vitro, the authors suggest that GPP might be the precursor of the isoprene portion of hapalindoles. Furthermore, it was suggested that AmbP1, which similar to AmbP3, is a homologue of bacterial aromatic prenyltransferases, likely plays a key role in the fusion of *cis*-**19** and GPP to form the tetracyclic scaffolds.[26] Taking this line of thought further, a scenario is conceivable in which AmbP1 catalyzes the formation of an allylic carbocation that can trigger the formation of the tricyclic intermediate **20**. The resulting carbocation can be quenched by the indole moiety to give access to the tetracyclic hapalindoles or by the direct elimination of a proton to give the tricyclic hapalindoles, which can be further processed to give **21**. This biosynthetic precursor can then be chlorinated by the proposed halogenase AmbO5 to yield **22** (Scheme 6B). Such a cyclization of two alkenes by a prenyltransferase would be somewhat reminiscent of chrysanthemyl diphosphate synthase (CPPase), which catalyzes the cyclopropanation between two molecules of DMAPP, after an initial dephosphorylation of one of the reaction partners, to give chrysanthemyl diphosphate (**23**; Scheme 6C).[29] Since CPPase and FPPase proteins, which are *trans*-isoprenyl diphosphate synthases, share high sequence similarity (75 % identity and 96 % similarity), it is believed that CPPase has evolved from a parental FPPase.[29,30] Proving that an aromatic prenyltransferase has also evolved to catalyze the cyclization of two alkenes would be highly intriguing from a mechanistic point of view.

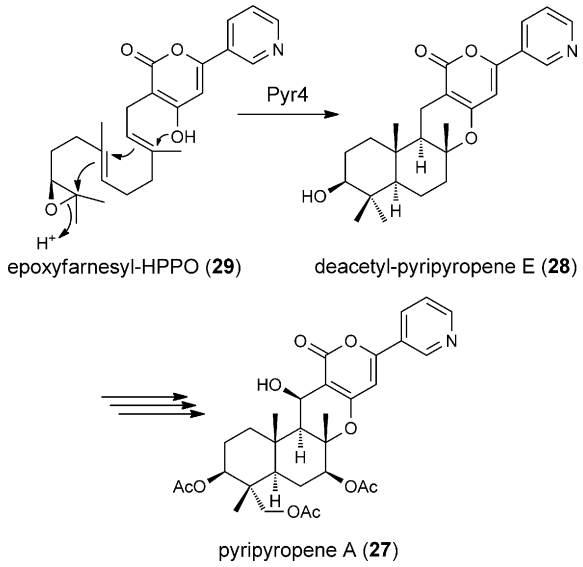


Scheme 6. A) Initial hypothesis on hapalindole biosynthesis.[26,27] B) Conceivable new biosynthetic route in the biosynthesis of hapalindole U (**21**) involving AmbP1 as a key catalyst in the formation of a cyclic scaffold, based on considerations of Hillwig et al.[26] C) Proposed pathway in the biosynthesis of chrysanthemyl diphosphate (**23**) by CPP synthase.[29]

Even more mechanistic parallels to the above-mentioned chrysanthemyl diphosphate synthase can be found in a very recent report on the biosynthesis of cyclolavandulyl diphosphate (**25**). Ozaki et al. showed that an unprecedented member of the *cis*-isoprenyl diphosphate synthase superfamily from the lavanducyanin producer *Streptomyces* sp. CL190 is responsible for the condensation and cyclization of two molecules of DMAPP after an initial dephosphorylation to yield **25**, which is a structural feature of lavanducyanin (**26**).[31] The proposed reaction mechanism assumes two reactions in which two molecules of DMAPP are condensed in an irregular non-head-to-tail fashion to yield lavandulyl



Scheme 7. Proposed reaction mechanism of cyclolavandulyl diphosphate synthase starting with the condensation of two molecules of DMAPP in an irregular non-head-to-tail fashion to yield lavandulyl diphosphate (**24**), which is subsequently cyclized to cyclolavandulyl diphosphate (**25**), a structural feature of lavanducyanin (**26**).[31]

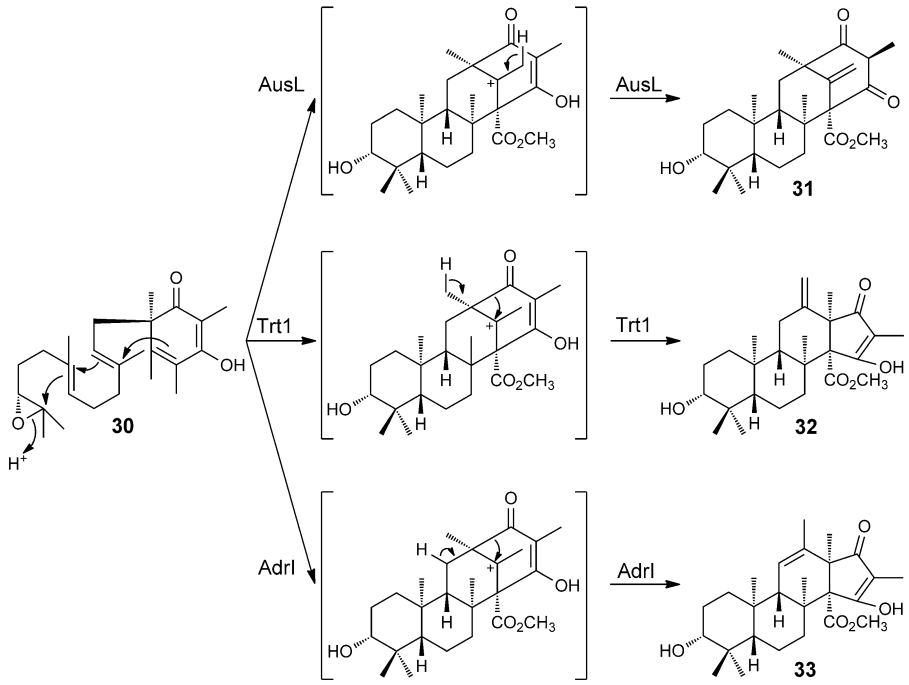


Scheme 8. Proposed mechanism for the cyclization of epoxyfarnesyl-HPPO (**29**) to deacetylpyripyropene E (**28**), which is the precursor of pyripyropene A (**27**), catalyzed by the novel membrane-bound meroterpenoid cyclase Pyr4.[33a]

diphosphate (**24**), which is subsequently cyclized to **25** (Scheme 7).[31] Notably, cyclolavandulyl diphosphate synthase is highly similar to undecaprenyl diphosphate synthase, which catalyzes the sequential *cis*-condensation of eight IPP units to FPP in the biosynthesis of undecaprenyl diphosphate.[32] Since *cis*-isoprenyl diphosphate synthases have a very distinct protein fold compared to *trans*-isoprenyl diphosphate synthases,[2] it seems that another unrelated prenyltransferase has evolved independently to act as a prenyltransferase and cyclase at the same time.

2.1.3. *A New Family of Meroterpene Cyclases*

All the above-mentioned TCs have in common that their annotation either gave a hint regarding their function, as for the haloperoxidases, or regarding their affiliation to terpene metabolism, as for the prenyltransferases which also act as cyclases. The next example will be a cryptic TC whose annotation initially gave no hint of catalytic activity or a role in terpene biosynthesis at all. In an elegant study, Itoh et al. identified biosynthetic genes for meroterpenoids from fungi, namely pyripyropene A (**27**) produced by *Aspergillus fumigatus* FO-1289 (Scheme 8).[33] Retrobiosynthetic analysis suggested that a fungal iterative type I polyketide synthase (PKS) that utilizes nicotinyl-CoA as a starter unit forms 4-hydroxy-6-(3-pyridinyl)-2*H*-pyran-2-one (HPPO), the α-pyrone core that is subsequently farnesylated by a prenyltransferase, followed by epoxidation of the terminal double bond. Protonation of the oxirane moiety followed by cyclization yields deacetylpyripyropene E (**28**), the precursor of pyripyropene A (**27**). This would resemble a head-to-tail cyclization strategy in analogy to

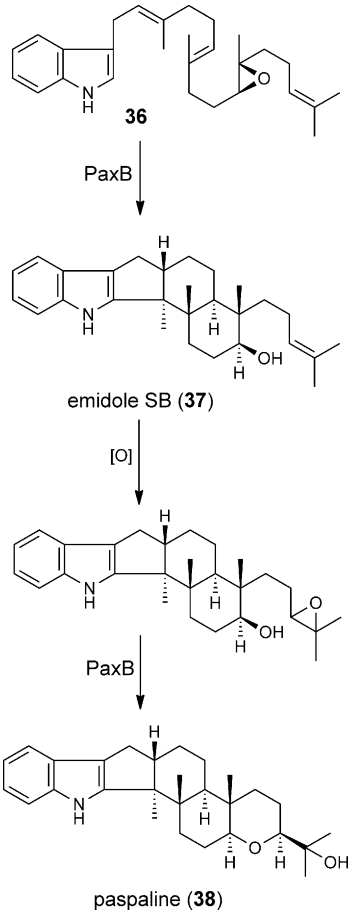


Scheme 9. Proposed mechanisms for the cyclization of epoxyfarnesyl-DMOA methyl ester (**30**) to protoaustinoid A (**31**), preterretonin A (**32**),[36] and andrastin E (**33**),[37] catalyzed by AusL, Trt1, and AdrI, respectively, depending on the position of proton abstraction from the shared tetracyclic carbocationic intermediate.

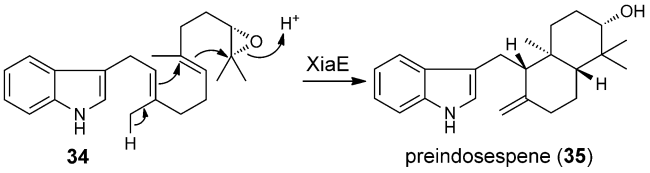
class II TCs, such as the lanosterol synthase, which initiate carbocation formation by protonation of a terminal oxirane moiety.[14] Therefore, a classical TC was considered to be among the key enzymes in pyripyropene biosynthesis. However, on the basis of homology, none of the genes in the proposed gene cluster appeared to encode a TC. It was then speculated that an epoxidase might catalyze an oxygen-triggered cyclization reaction. To test this hypothesis, the heterologous fungal host *A. oryzae* M-2-3 that expresses the proposed prenyltransferase as well as the proposed epoxidase was supplemented with HPPO, which afforded only the dihydroxyfarnesyl-substituted pyrone instead of the cyclized meroterpenoid. Thus, the epoxidase failed to catalyze the subsequent cyclization of the epoxide, which was prone to hydrolysis to the corresponding diol. As a consequence, there had to be a cryptic, as yet overlooked cyclase. Finally, the gene *pyr4*, annotated as "integral membrane protein", aroused attention because of the abundance of homologues in other presumed polyketide-derived meroterpenoid gene clusters from *A. terreus* and *A. fumigatus*, as well as in indoloditerpene biosynthetic gene clusters[34] and the biosynthetic gene cluster of the meroterpenoid quinone BE-40644 from the actinomycete *Actinoplanes* sp. strain A40644.[35] Pyr4 lacks the aspartate-rich motifs that are typically found among TCs. Moreover, the protein consists of only 242 amino acids and, therefore, was considerably smaller than any other known TC. A hydropathy plot predicted Pyr4 to comprise seven transmembrane helices, which would also be a novelty for TCs. Nevertheless, an in vitro reaction with Pyr4 embedded in microsomes, carried out with epoxyfarnesyl-HPPO (**29**) as a substrate, resulted in the formation of **28**. This outcome confirmed Pyr4 as a novel membrane-bound meroterpenoid cyclase (MTC) that appears to be the first member of a large family of MTCs.

Homologues of Pyr4 in other polyketide-derived meroterpenoid pathways have been shown to be involved in meroterpenoid cyclization. In a reconstitution attempt, the Pyr4 homologues Trt1 encoded in the biosynthetic gene cluster of terretonin, as well as AusL encoded in the biosynthetic gene cluster of austinol and dehydroaustinol, were shown to be MTCs.[36] In a very similar approach, AdrI was also shown to be involved as a MTC in the biosynthesis of andrastin A.[37] These compounds are derived from 3,5-dimethylorsellinic acid (DMOA) and FPP, and their biosyntheses share epoxyfarnesyl-DMOA methyl ester (**30**) as a common intermediate (Scheme 9). The different outcomes of the AusL, Trt1, and AdrI reactions, which yield protoaustinoid A (**31**), preterretonin A (**32**), and andrastin E (**33**), respectively, depends on the preference of the respective MTC for the position of proton abstraction from the shared tetracyclic carbocationic intermediate.

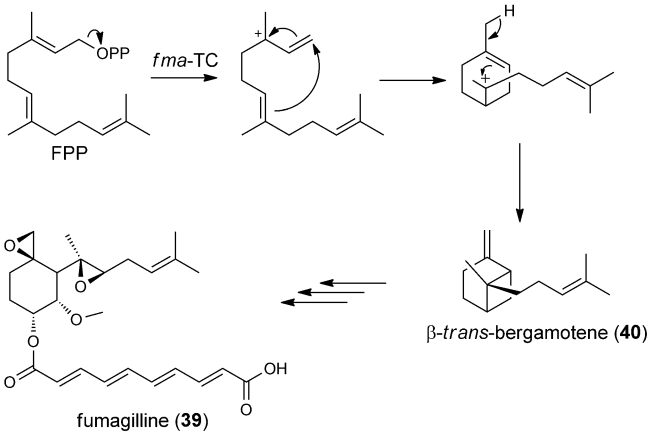
These examples underline the role of this novel family of TCs in the biosynthesis of polyketide-derived meroterpenoids



Scheme 11. Proposed mechanism for the biosynthesis of paspaline (**38**) by a stepwise epoxidation and cyclization mechanism catalyzed by PaxB.[40]



Scheme 10. Proposed mechanism for the cyclization of an indolylfarnesyl epoxide intermediate (**34**) to preindosespene (**35**), catalyzed by the bacterial indolosesquiterpenoid cyclase XiaE.[39]



Scheme 12. Proposed mechanism for the cyclization of farnesyl diphosphate (FPP) to β-*trans*-bergamotene (**40**), a precursor of fumagillin (**39**), catalyzed by the cryptic terpene cyclase *fma*-TC.[42]

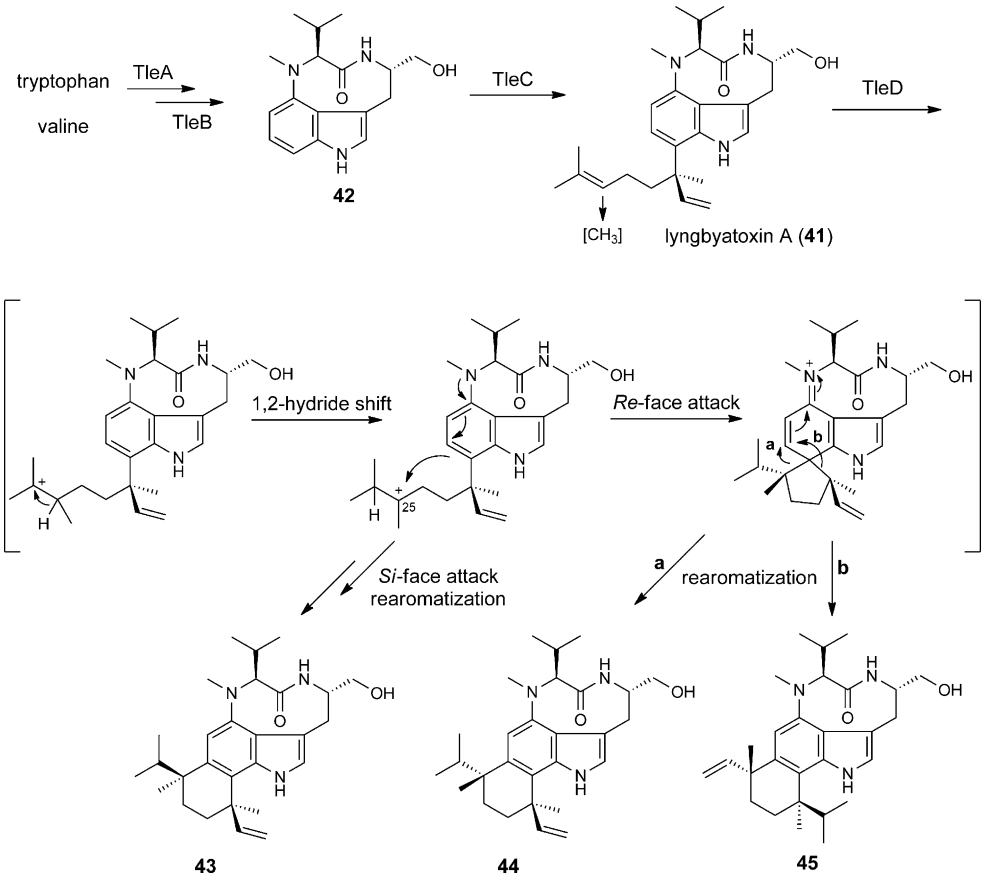
from fungi. However, the presence of Pyr4 homologues in indoloditerpene biosynthetic gene clusters suggests that these novel MTCs might accept more diverse substrates in terms of the nonterpenoid-derived portion and the length of the terpenoid chain. Furthermore, the occurrence of a Pyr4 homologue in the BE-40644 biosynthetic gene cluster from an actinomycete indicates that these enzymes might be widespread among different kingdoms of life. These conjectures are supported by a study on the biosynthesis of indolosesquiterpenes from endophytic streptomycetes isolated from mangroves.[38] Genetic analysis, gene-deletion experiments, as well as heterologous reconstitution experiments implied that the Pyr4 homologue XiaE is responsible for the cyclization of an indolylfarnesyl epoxide intermediate (**34**) to preindosespene (**35**; Scheme 10), thus making XiaE the first bacterial representative of this novel type of TC and the first functionally characterized member of this family that catalyzes the cyclization of indole terpenoids.[39]

Recently, in vivo experiments revealed that the Pyr4 homologue PaxB is capable of cyclizing the epoxidized prenyl indole **36** to yield emidole SB (**37**; Scheme 11). Furthermore, it was shown that PaxB can cyclize prenylindole, which is epoxidized at both terminal double bonds to yield paspaline (**38**), the core structure of paxilline.[40] These findings speak for a stepwise epoxidation and cyclization mechanism in the biosynthesis of paspaline and make PaxB the first characterized member of this family that cyclizes a diterpene portion of a meroterpenoid.

Previous groundbreaking studies on the biosynthesis of paspaline (**38**) revealed that *paxB* is among the four genes that are the minimum number of genes required to produce this indole diterpene.[41] However, the prenyltransferase PaxC was (mis)interpreted as the TC in the proposed biosynthetic model.[34,41] This suggestion was likely due to the close structural relationship between class I TCs and PTs with the class I terpenoid synthase fold mentioned in Section 1, although the mechanism of these enzymes that catalyze a tail-to-head cyclization after ionization of the allylic diphosphate ester bond can not be applied to the epoxidized 3-geranylgeranyl indole precursor. A secondary structure analysis suggested that PaxB might be an important substrate transporter.[41] This example showcases the pitfalls when relying on annotations based on known enzyme reactions and protein functions when investigating unknown reactions, and should encourage sometimes thinking outside the box.

2.1.4. *Convergent Evolution of Bergamotene Synthases*

Another case in which a homology search did not reveal an apparent TC is the biosynthesis of fumagillin (**39**), where the absence of a TC homologue in the entire genome of *A. fumigatus* Af293 hampered the identification of a biosynthetic gene cluster for many years.[42] Initially Lin et al. concluded that the gene cluster comprises a noncanonical TC that is concealed from search strategies based on homology, since the terpenoid portion of fumagillin is likely derived from the sesquiterpene β-*trans*-bergamotene (**40**), whose formation should require a TC.[42] Thus, other structural features of fumagillin (**39**) were brought into consideration to target a biosynthetic gene cluster, such as the dioic acid moiety and several oxidations, thus leading to a gene cluster named *fma*. Its identity was confirmed by genetic and biochemical studies. With the *fma* gene cluster identified, the nearby genes were inspected more closely for a potential TC. Attention was drawn to the predicted gene product Af520 (named *fma*-TC), which is likely comprised of six transmembrane helices and contains a PFAM01040 UbiA prenyl-



Scheme 13. Proposed biosynthesis of teleocidin B-1 (**43**), teleocidin B-4 (**44**), and des-*O*-methyl-olivoretin C (**45**) by the *C*-methyltransferase TleD starting from lyngbyatoxin A (**41**). For details, see the text.[44]

transferase (*p*-hydroxybenzoic acid oligoprenyltransferase) domain, given the structural relationship of *E. coli* UbiA prenyltransferase to class I TCs. Indeed, in vitro studies with *fma*-TC-containing microsomes unequivocally proved that this enzyme is a new membrane-bound TC that can convert FPP into the bicyclic terpene **40** (Scheme 12). This reaction depends strictly on Mg^{2+} ions, which suggests that the catalytic mechanism of *fma*-TC resembles that of class I TCs, that is the metal cluster dependent ionization of the allylic diphosphate ester to generate an allylic cation.[42] Notably, plants produce several bergamotenes using soluble TCs[43] and therefore *fma*-TC is a perfect example of the convergent evolution of apparently unrelated enzymes that synthesize similar products.[42]

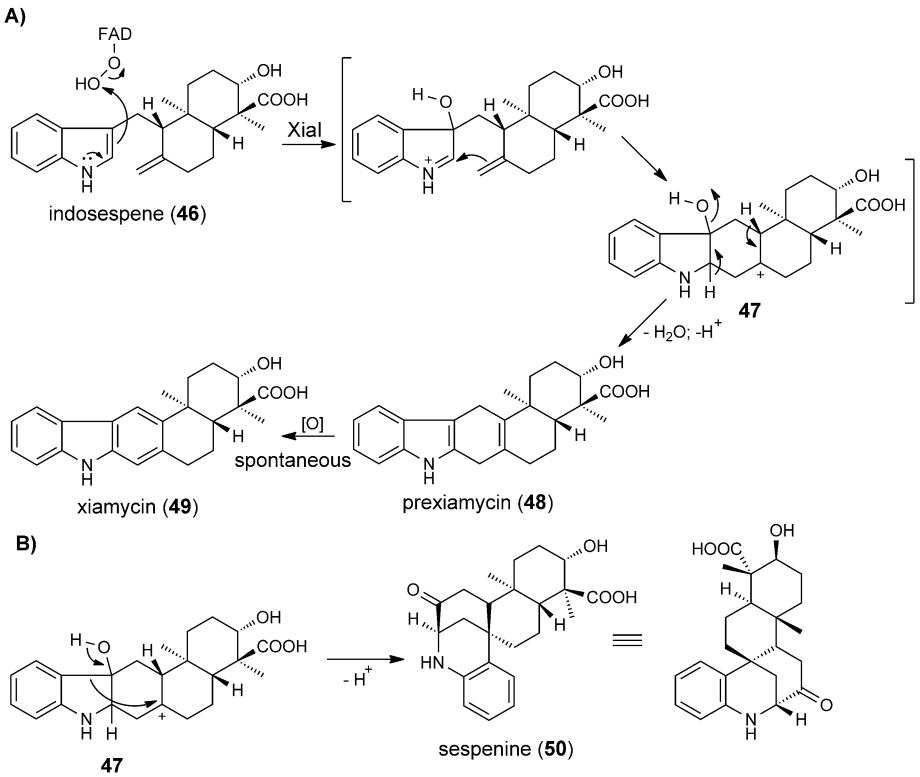
2.1.5. Terpenoid Cyclization Mediated by a Methyltransferase

A completely unprecedented cyclization reaction was found to take place during the biosynthesis of the protein kinase C activator teleocidin B. Initially, Awakawa et al. aimed to shed light on the late steps of teleocidin biosynthesis, which include the formation of an indole-fused six-membered ring, starting from a geranyl moiety.[44] At that time, it was already known that the biosynthesis machinery of lyngbyatoxin A (**41**), which is structurally related to teleocidin B, comprises a nonribosomal peptide synthetase (LtxA) and a cytochrome P450 monooxygenase (LtxB) that work together to transform L-valine and L-tryptophan into (−)-indolactam V (**42**). The indole ring of **42** is then further decorated with a geranyl moiety by an aromatic prenyltransferase (LtxC) to yield **41**, which was suggested as the precursor of teleocidin B on the basis of feeding experiments.[45] Notably, in contrast to teleocidin B, the structure of **41** lacks further methylation and cyclization of the geranyl moiety. A homology search by the authors led to them identifying a gene cluster (*tle*) in the genome of their teleocidin producer that is very similar to the *ltx* cluster, and its role in the biosynthesis of **41** was proven by heterologous expression. Since no genes related to a *C*-methyltransferase or a terpene cyclase could be found in the region adjacent to the *tle* cluster, the authors started to co-express six candidate methyltransferases with the *tle* genes in the heterologous host that produces **41**. The transformant harboring the *C*-methyltransferase gene *tleD* produced three new compounds that were identified as teleocidin B-1 (**43**), teleocidin B-4 (**44**), and des-*O*-methyl-olivoretin C (**45**; Scheme 13). In vitro analysis of TleD with **41** as a substrate confirmed its affiliation in the biosynthesis of **43**–**45**, and proved that a methyltransferase is sufficient to methylate and concomitantly cyclize the geranyl moiety of **41**. The authors suggest that TleD initiates the further cyclization of **41** by installing a methyl group at the geranyl moiety, thereby leading to a cationic species. After a 1,2-hydride shift, the resultant tertiary cation is prone to nucleophilic attack from the electron-rich indole to give a spiro-fused intermediate. This step therefore controls the stereochemical outcome at C25 by either a *Re*- or a *Si*-face spirocyclization. Two different C–C bonds of this intermediate can then migrate to give the structural backbones of **44** and **45**, driven by the rearomatization of the indole ring system.[44] The authors draw a parallel to class I TCs, but indeed it is more reminiscent of class II TCs that activate a terminal isoprenyl double bond prior to cyclization, with the difference being that the generation of the carbocation intermediate is facilitated by methylation instead of protonation and a 1,2-hydride shift occurs.

2.2. Terpenoid Cyclization by Unprecedented Cyclization Mechanisms

2.2.1. Meroterpene Cyclization Catalyzed by a Flavin Monooxygenase

All the TCs described in the preceding sections (with the exception of the V-ClPO Mcl24) catalyze reactions that are reminiscent of class I or class II TCs, although they differ



Scheme 14. A) Proposed mechanism for the further cyclization of indosespene (**46**) to prexiamycin (**48**) by a cryptic hydroxylation, catalyzed by the flavin-dependent monooxygenase Xial.[46] B) Proposed cyclization route in the biosynthesis of sespenine (**50**).[38b,39]

markedly in their protein structures. However, it seems that nature has invented further strategies to cyclize terpenoids, besides the reuse of approved cyclization mechanisms. This is indicated by some noteworthy reports of terpenoid cyclization catalyzed by oxidases. An interesting cyclization step catalyzed by a flavin-dependent oxidase, for example, is the further cyclization of the decalin ring system of indosespene (**46**) to the xiamycin ring system in indolosesquiterpene biosynthesis (Scheme 14 A). For this reaction step, a cryptic hydroxylation was proposed to set the stage for a ring annulation, which results in the intermediate carbenium ion **47** that reacts to form the xiamycin ring system through deprotonation, loss of one equivalent of water, and aromatization.[38b] This model was supported by deletion of *xiaF*, a gene coding for a flavin-dependent oxidase that was annotated as an indole oxygenase, which abolished xiamycin production.[39] An independent in vitro study using a XiaF homologue (XiaI; 99 % identity) of a marine-derived streptomycete that also produces xiamycin showed conversion of indosespene (**46**) into prexiamycin (**48**), which undergoes spontaneous oxidation to xiamycin (**49**; Scheme 14 A).[46]

A similar biosynthetic route was suggested for the biosynthesis of the related indolosesquiterpene sespenine (**50**), which was isolated together with xiamycin and indosespene. In this route, the hydroxylated carbenium ion **47** is a common intermediate that rearranges to the pentacyclic ring system with a central quaternary carbon atom (Scheme 14 B).[38b,39]

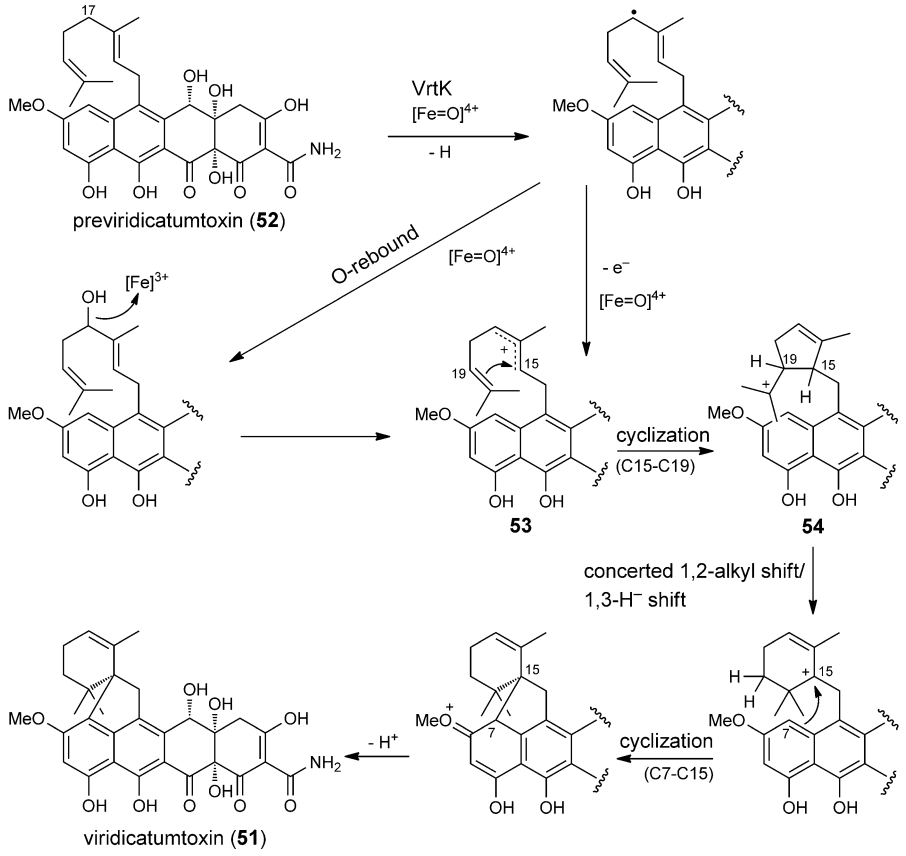
2.2.2. *Meroterpene Cyclization Catalyzed by a Cytochrome P450 Enzyme*

A cryptic hydroxylation that triggers cyclization of a prenyl side chain is also one suggestion in the biosynthesis of the tetracycline-like fungal meroterpenoid viridicatumtoxin (**51**). In this case, the cytochrome P450 enzyme VrtK was shown in vivo to catalyze the conversion of acyclic previridicatumtoxin (**52**) into **51** (Scheme 15).[47] To initiate the cyclization, C17-hydroxylation of **52** through a hydrogen abstraction/oxygen rebound mechanism was suggested, followed by dehydratation to form an allylic cation intermediate (**53**). However, since no C17-hydroxylated intermediate could be found in biotransformation assays, the dehydrogenation of **52** to a carbon radical, followed by electron transfer to the iron-heme center, thereby resulting in the formation of allylic cation **53**, was suggested as an alternative. The proposed formation of **53** would set the stage for further cyclization events. Based on isotope labeling studies with [^{13}C,^{2}H]-labeled mevalonates and quantum chemical modeling, Chooi et al. proposed the formation of a tertiary carbocation intermediate (**54**) by a C15–C19 ring closure. The intermediate then undergoes ring expansion through a concerted 1,2-alkyl shift/1,3-hydride shift, followed by attack of C7 on C15 to form the spirobicyclic ring system of **51** (Scheme 15).[47]

In 1962, Breslow speculated that a free-radical pathway could be responsible for the polycyclization of squalene in the biosynthesis of sterols, which fueled the successful synthetic exploitation of radical pathways in terpenoid polycyclization.[48] Later, however, oxidosqualene was confirmed as the biosynthetic intermediate and the nowadays broadly accepted mechanism involving a carbocation was proposed. This caused Breslow to state in retrospect that his model study was "of limited biochemical interest".[49] In light of VrtK being a novel TC that likely catalyzes the cyclization of the terpene moiety in the biosynthesis of **51** by a radical mechanism, this conclusion seems to be premature. However, strategies exploiting radical species should be kept in mind when planning synthetic polycyclizations of terpenes.

2.2.3. *Oxidative Cyclization in Δ^1-Tetrahydrocannabinolic Acid Biosynthesis*

The above outlined new TCs, as well as class I and class II TCs, have something in common: They all seem to



Scheme 15. Proposed mechanism for the cyclization of previridicatumtoxin (**52**) to viridicatumtoxin (**51**) catalyzed by the CYP450 enzyme VrtK. For details, see the text.[47]

exploit carbocation chemistry. From this perspective it is intriguing that an exception to this rule was discovered only recently in a protein study on the cyclization enzyme in the biosynthesis of Δ^1-tetrahydrocannabinol (Δ^1-THC), the principle psychoactive constituent of *Cannabis sativa* L.[50] An in vitro study by Taura et al. provided experimental evidence that cannabigerolic acid (**55**) is converted into Δ^1-tetrahydrocannabinolic acid (Δ^1-THCA, **56**), the precursor of Δ^1-THC, by an oxidative cyclization catalyzed by THCA synthase.[51] Shoyama et al. determined the structure of this flavoprotein at 2.75 Å resolution (Figure 2A).[52]

On the basis of mutational analysis and the crystal structure, a mechanism was proposed in which the N5 atom of FAD, which is covalently bound to His114 and Cys176, is suggested to work in concert with a tyrosine moiety (Tyr484) to catalyze the formation of a reactive *o*-quinone methide from **55** by a hydride transfer reaction. The active-site residues His292 and Tyr417 (Figure 2B) were suggested to assist by binding the substrate.[52] This oxidation step paves the way for an inverse electron-demand oxo-Diels–Alder reaction (Scheme 16).

2.2.4. Reductive Cyclization in Iridoid Biosynthesis

The cyclization route catalyzed by THCA synthase seems to be very exceptional for TCs. Nevertheless, similar cyclization schemes might be more widespread in nature. This is indicated by a study on an iridoid synthase from *Catharanthus roseus* that was reported shortly after that of the THCA synthase. The iridoid synthase catalyzes the formation of the signature iridoid ring system, composed of a cyclopentane ring fused to a tetrahydropyran ring.[53] Previous studies using crude plant extracts have indicated that the direct precursor of iridoids is 10-oxogeranial, which is converted into nepetalactol in a NADH/NADPH-dependent manner.[54] To narrow down the potential NAD(P)H-dependent enzymes, Gue-Flores et al. performed a co-expression analysis using an enzyme upstream of the cyclization step (geraniol 10-hydroxylase) as bait. The highest-ranking of these co-regulated NADPH-using enzymes turned out to be a progesterone-5β-reductase (P5βR) homologue, an enzyme normally involved in the reduction of a C–C double bond of progesterone in cardenolide biosynthesis.[55] In vitro studies with this enzyme showed the conversion of 10-oxogeranial (**57**) into *cis-trans*-nepetalactol (**58**), which is in equilibrium with its open dialdehyde forms (*cis-trans*-iridodials; **59a** and **59b**), in the presence of either NADH or NADPH. This result proved the role of iridoid synthase in the unusual reductive cyclization of 10-oxogeranial, for which a two-step mechanism was proposed. First, the enzyme catalyzes a 1,4-reduction similar to the proposed reaction of P5βR,[56] thereby yielding enol intermediate **60**, which can further react either through an inverse electron-demand oxo-Diels–Alder reaction that resembles the proposed reaction in Δ^1-THCA biosynthesis or an intramolecular Michael addition (Scheme 17A). To clarify which pathway is the right one, two substrate analogues, **61** and **62**, each of which disfavors one of the pathways, were synthesized and used for the enzymatic reaction in a follow-up study (Scheme 17B).[57] Strikingly,

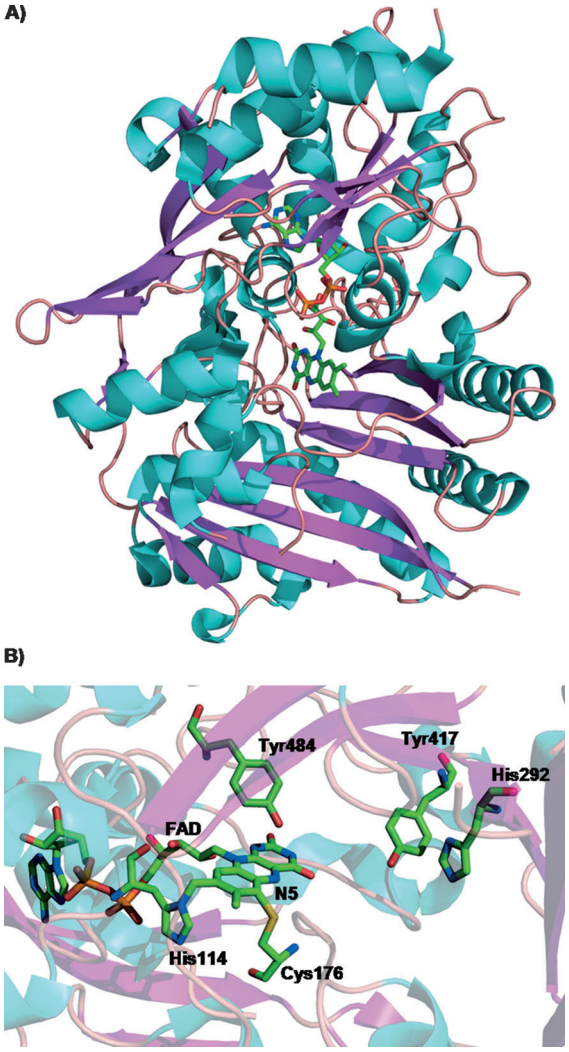
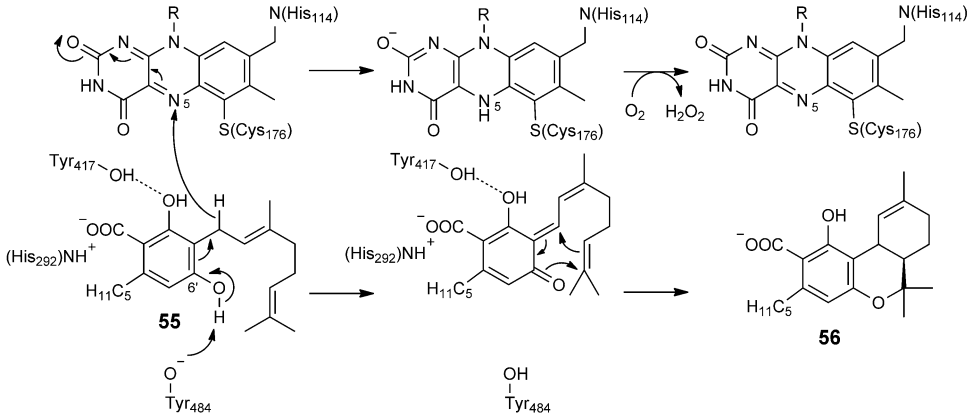


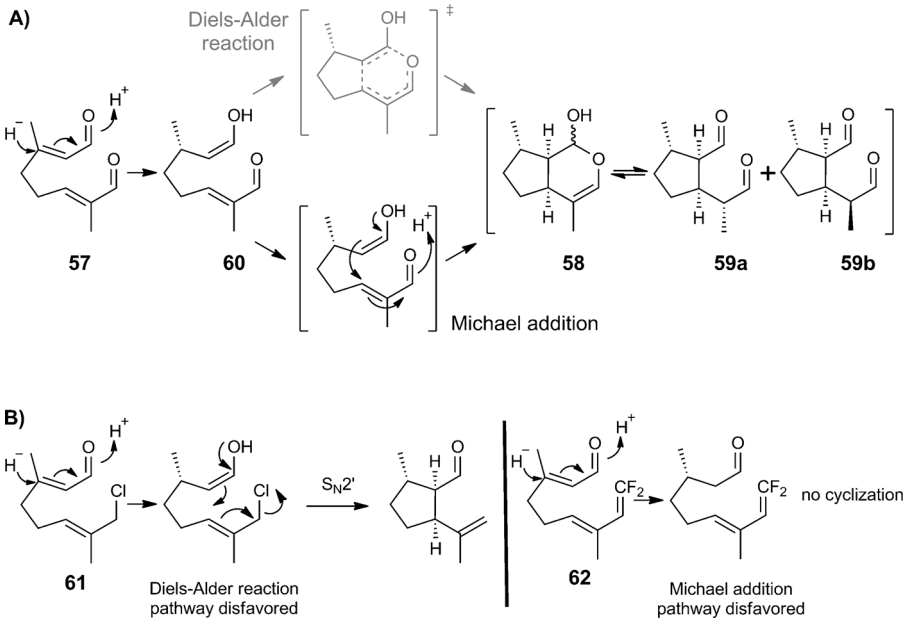
Figure 2. Structure of the Δ^1-tetrahydrocannabinolic acid synthase (THCA synthase; PDB: 3VTE).[52] A) Ribbon diagram of the overall structure of THCA synthase; α helices are colored cyan, β strands magenta, and loops pink. The FAD cofactor (C green, N blue, O red, P orange) is shown in a stick representation. B) Diagram of the active-site residues of THCA synthase in a stick representation (C green, N blue, O red, P orange, S yellow); the FAD cofactor is covalently bound to His114 and Cys176. The diagrams were prepared with PyMol.[22]

only the chlorinated analogue **61** was found to cyclize in a S_N2' reaction analogous to the proposed Michael addition, whereas in the case of **62** the reaction stopped after the initial reduction step. From their findings the authors conclude that the enzyme follows a Michael reaction pathway.

These findings raise the question whether this mechanism might be another common scheme in terpenoid cyclization. Notably, the formation of the reactive intermediate is achieved through reduction instead of oxidation as in Δ^1-THCA biosynthesis. This enzyme together with THCA synthase are striking examples of nature's ingenuity in the biosynthesis of cyclized terpenoids.[53,58]



Scheme 16. Proposed mechanism for the oxidative cyclization of cannabigerolic acid (**55**) to Δ^1-tetrahydrocannabinolic acid (**56**) catalyzed by THCA synthase.[52]

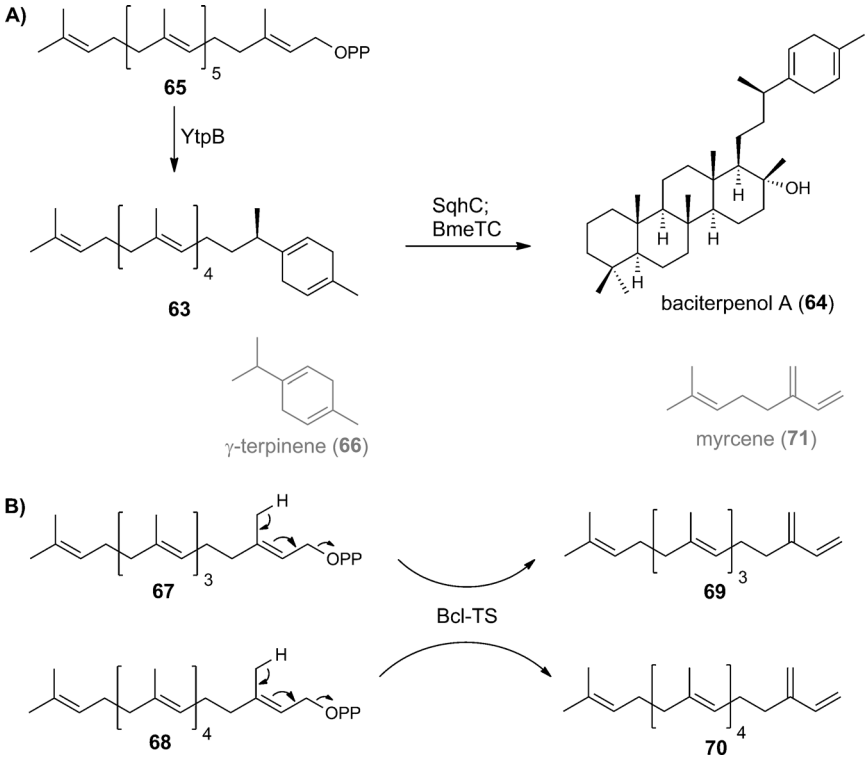


Scheme 17. Reductive cyclization of 10-oxogeranial (**57**) in iridoid biosynthesis. A) Originally proposed mechanisms for the cyclization of **57** to *cis-trans*-nepetalactol (**58**), which is in equilibrium with its open dialdehyde forms (*cis-trans*-iridodials **59a** and **59b**), catalyzed by iridoid synthase.[53] B) Additional mechanistic studies using substrate analogues **61** and **62**. Only substrate **61**, which strongly disfavors a Diels–Alder reaction, is cyclized.[57]

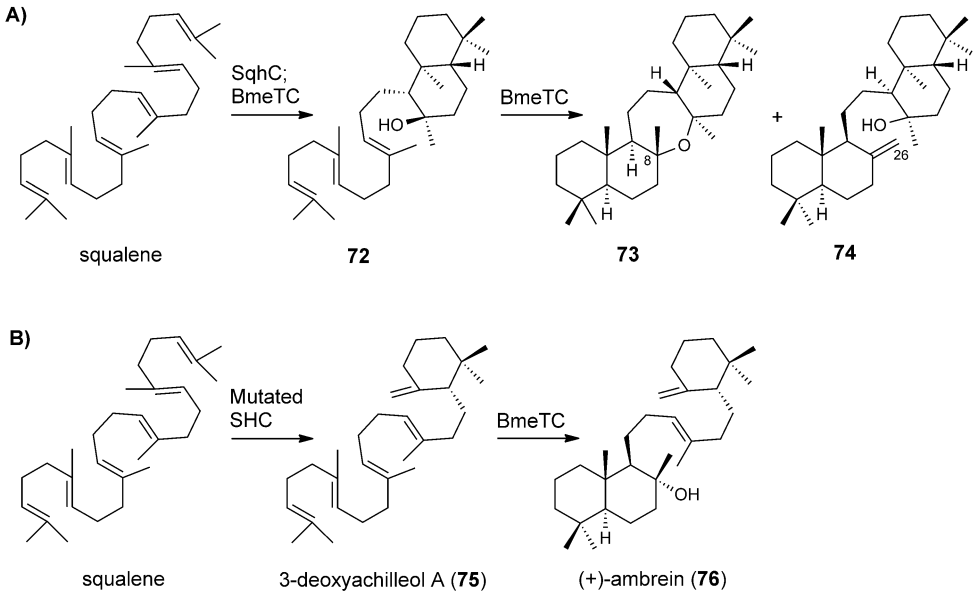
2.3. Terpenoid Cyclization without a Proposed Mechanism

Sato and co-workers made a huge effort to study the biosynthesis of mono- and pentacyclic C_{35} terpenes such as tetraprenyl-β-curcumene (**63**) and baciterpenol A (**64**) from *Bacillus subtilis* (for which the authors suggest the term "sesquarterpenes") by screening 2514 gene-disrupted strains of *B. subtilis* provided by the National BioResource Project (NIG, Japan).[59] This was necessary because no suitable cyclase candidates could be found around the genes coding for a heterodimeric heptaprenyl diphosphate synthase, the enzymes that supply the pathway with the terpenoid precursor heptaprenyl diphosphate (**65**). The screening considered all "functional unknown proteins" and "conserved hypothetical proteins" consisting of more than 180 and 240 amino acids, respectively, that were conserved among the genomes of several bacteria that produce monocyclic C_{35} terpenes, but not in those of nonproducers of the same genus. The search could thus be narrowed down to 49 strains and analysis of their lipid fractions revealed that only the mutant with disrupted *ytpB* was not able to produce monocyclic sesquarterpenes anymore. In vitro studies with purified YtpB finally confirmed that this enzyme is a tetraprenyl-β-curcumene synthase, which converts **65** into tetraprenyl-β-curcumene (**63**; Scheme 18A). A mechanism for this cyclization reaction was not proposed, since the primary structure of YtpB has no similarity to that of any other known TC.[59] However, the outcome of the reaction is reminiscent of the cyclization of GPP to γ-terpinene (**66**; Scheme 18) catalyzed by a classical class I TC,[60] and therefore mechanistic parallels appear plausible. Interestingly, the tetraprenyl-β-curcumene synthase homologue Bcl-TS from *B. clausii* was shown in vitro to catalyze the acyclic conversion of geranylfarnesyl diphosphate (**67**) and hexaprenyl diphoshate (**68**) into β-geranylfarnesene (**69**) and β-hexaprene (**70**; Scheme 18B).[61] This connection shows analogy to enzymes with the class I terpenoid synthase fold, since homologues of class I TCs also catalyze the conversion of polyprenyl diphosphates into acyclic terpenes such as myrcene (**71**; Scheme 18) catalyzed by myrcene synthase.[62] Further enzyme studies will likely shed light on detailed reaction mechanisms and may reveal whether or not there are indeed parallels to the above-mentioned terpene synthases. Compound **63** is then further cyclized to the pentacyclic scaffold of baciterpenol A (**64**) by SqhC, which is similar to squalene-hopene cyclase.[59]

The evolutionary proximity of SqhC to squalene-hopene cyclase inspired Sato et al. to incubate recombinant SqhC with squalene, which resulted in the formation of the bicyclic triterpene **72**,[63] a natural product from the fern *Polypodium formosana*[64] (Scheme 19A). Whilst the authors could not detect **72** in vivo in *Bacillus subtilis* they found it in *B. mega-*



Scheme 18. Proposed biosynthetic pathway for A) the YtpB-catalyzed conversion of heptaprenyl diphosphate (**65**) into tetraprenyl-β-curcumene (**63**), which is further cyclized to baciterpenol A (**64**),[59] and B) the Bcl-TS-catalyzed conversion of geranylfarnesyl diphosphate (**67**) and hexaprenyl diphosphate (**68**) into β-geranylfarnesene (**69**) and β-hexaprene (**70**), respectively.[61] γ-Terpinene (**66**) and myrcene (**71**) are shown for comparison.

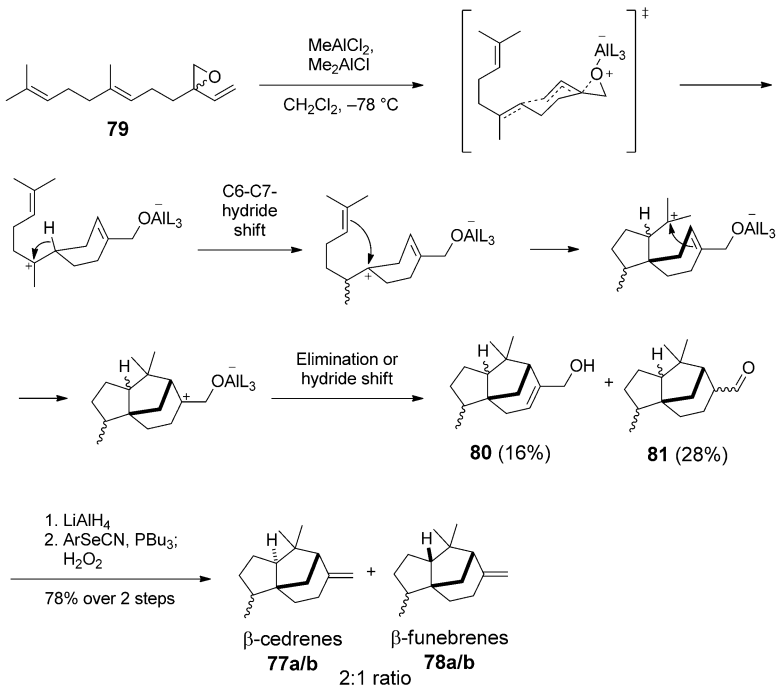


Scheme 19. A) Cyclization of squalene to **72** catalyzed by SqhC or its homologue BmeTC from *B. megaterium*.[63] **72** can be further cyclized by BmeTC to an onoceranoxide (**73**), as well as triterpene **74**.[65] B) Access to (+)-ambrein (**76**) from squalene via the unnatural monocyclic triterpene 3-deoxyachilleol A (**75**) by using a mutated squalene-hopene cyclase (SHC) and the tetraprenyl-β-curcumene cyclase BmeTC.

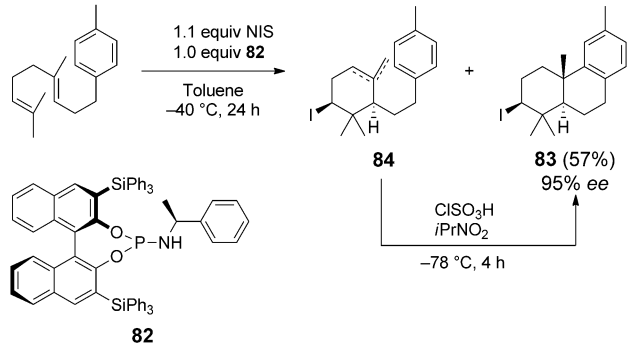
terium and proved that the SqhC homologue BmeTC from *B. megaterium* is also capable of producing **72** from squalene (Scheme 19A) as well as being active in the cyclization of tetraprenyl-β-curcumene.[63] In addition, Ueda et al. showed that **72** can be further cyclized by the same enzyme to an onoceranoxide **73**,[65] which was also previously isolated from a fern,[66] as well as to the novel triterpene **74**.[65] Therefore, BmeTC is a bifunctional triterpene/sesquarterpene cyclase that can convert tetraprenyl-β-curcumene into baciterpenol A (**64**; Scheme 18A), but also squalene into onoceroids through cyclization at both termini (Scheme 19A). Since **73** and **74** could also be detected in *B. megaterium* in vivo, both compounds are natural products and the first onoceroids of bacterial origin.[65]

The mechanism of the cyclization of **63** to **64** might well be in analogy to that catalyzed by squalene-hopene cyclase, where a terminal double bond gets protonated and leads to a cyclization cascade that is in the end quenched by a water molecule. Notably, for squalene-hopene cyclase it has been reported that the positive charge can be neutralized by the addition of water to form hopanol, besides the direct deprotonation of the polycyclic intermediate to afford hopene.[3c] However, the cyclization reactions leading to the onoceroids appear to be exceptional. The authors speculate for good reasons that squalene is cyclized to the bicyclic intermediate **72**, which is released from the active site, turned, and again taken up by the active site to catalyze the polycyclization of the other hemisphere of **72**. The positive charge of the resulting C8 carbocation could then be neutralized by nucleophilic addition of an intramolecular hydroxy group (leading to **73**) or the deprotonation of H26 (leading to **74**), respectively (Scheme 19A).[65] Such a scenario would be unprecedented for terpene cyclases.

Finally, the enzymatic promiscuity of BmeTC was probed in the same study. It was tested whether the enzyme is capable of further cyclizing the non-natural monocyclic triterpene 3-deoxyachilleol A (**75**). Indeed,



Scheme 20. Tail-to-head cyclization of racemic vinyl epoxide **79** by using a nondissociating Lewis acid. The strong coordination of the Lewis acid to the alcohol allows unhindered charge propagation to afford the tricyclic products **80** and **81**, which can be converted into β-cedrenes (**77a/b**) and β-funebrenes (**78a/b**).[7]



Scheme 21. Enantioselective iodocyclization using *N*-iodosuccinimide (NIS) and the chiral phosphoramidite **82**. The undesired olefin **84** could be converted into the tricyclic terpenoid **83** using $ClSO_3H$.[76]

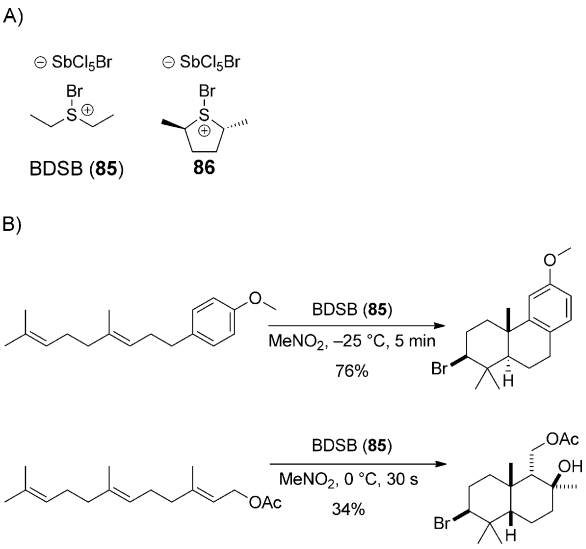
BmeTC catalyzed the cyclization of **75** in the same manner as the cyclization of squalene to **72**, thereby yielding (+)-ambrein (**76**), which is a major constituent of ambergris, a secretion of the digestive system of sperm whales and one of the most valuable animal perfumes (Scheme 19B). Compound **75** was synthesized enzymatically from squalene by using mutated squalene-hopene cyclase from *Alicyclobacillus acidocaldarius*. This enzymatic strategy, which was performed with cell-free *E. coli* extracts containing the overexpressed recombinant enzymes, required only two steps from squalene to (+)-ambrein (**76**) compared to 19–35 steps for the reported total syntheses.[65]

3. Novel Biomimetic Synthetic Approaches

As a consequence of its unique ability to form multiple rings and stereogenic centers simultaneously, the biosynthesis of terpenoids has strongly influenced the synthetic community since the pioneering work of Stork and Eschenmoser.[49] Several successful attempts have been made to mimic biosynthetic pathways by using various agents to trigger the cyclization of linear precursors, such as Brønsted acids, Lewis acids, UV light,[67] transition metals such as Ir,[68] Pt,[69] and Au,[70] and also more exotic materials such as zeolites[71] and antibodies.[72] As most of these methods are already well established and have been reviewed,[49,73] this section will focus on more recent developments in the synthetic field.

Interestingly, although both tail-to-head and head-to-tail cyclizations are used in nature by class I and class II terpene cyclases, respectively, chemists strongly favor tail-to-head cyclizations because charge propagation following a head-to-tail pathway is often stopped in organic solvents by E1 or S_N1 reactions before complete cyclization.[7] Only recently, Pronin and Shenvi showed that nondissociating Lewis acids are required to allow nonstop charge propagation[7]—a strategy that mimics the precisely controlled localization of catalytic residues in the active site of class II terpene cyclases. This concept allowed for the synthesis of a mixture of β-cedrenes (**77a/b**) and β-funebrenes (**78a/b**) starting from racemic vinyl epoxide **79** (Scheme 20).

Vinyl epoxide **79** was obtained in four steps from geranyl bromide.[7] A screening of various Lewis acids for the cyclization cascade clearly showed that alkyl aluminum



Scheme 22. Evaluation of the novel brominating agent BDSB (**85**). A) Structure of BDSB and the simple chiral derivative **86**.[77] B) Examples showing the potential of BDSB for fast, mild, and selective racemic bromocyclizations.[77]

chlorides gave the best results, leading prominently to the cedrene/funebrene folding pattern. The resulting cation was quenched either by elimination to give the allylic alcohol **80** or by a hydride shift to give the aldehyde **81**. The latter could be transformed in two steps into a 2:1 mixture of β-cedrenes **77a/b** and β-funebrenes **78a/b**. This new method allowed for the first synthesis of a member of the funebrene class of terpenes[7] and demonstrates that also neglected reaction strategies bear great potential to access difficult scaffolds.

3.1. Cyclizations Triggered by Halonium Ions

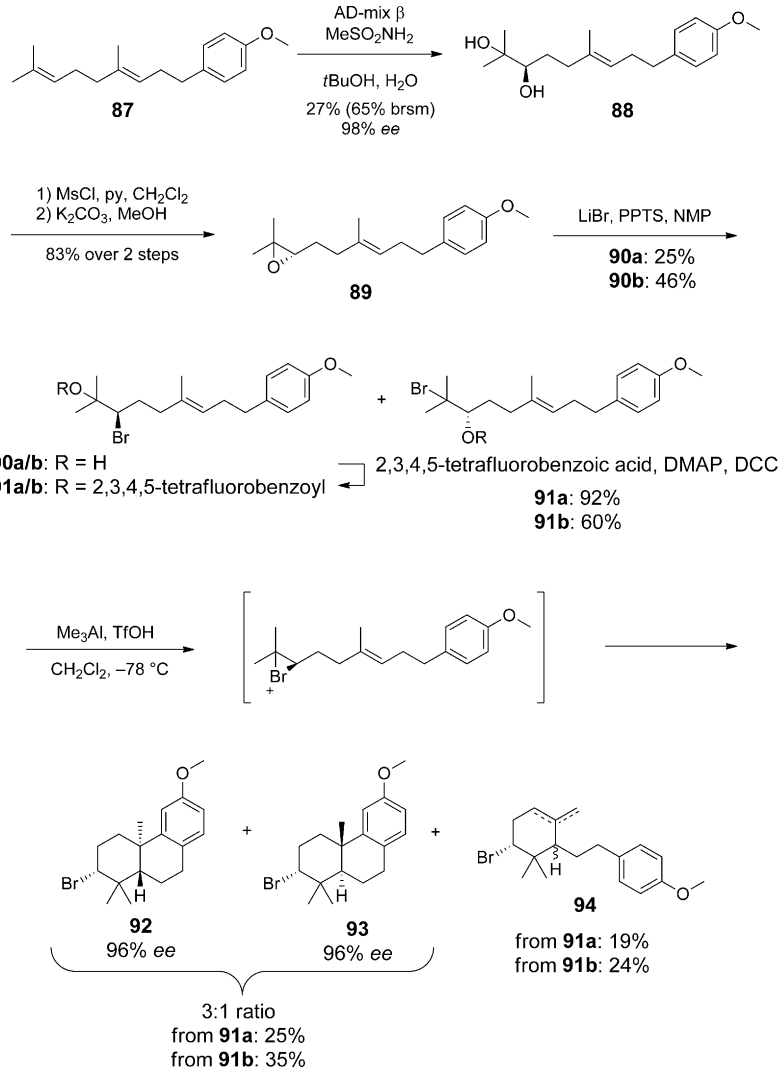
The use of halonium ions for triggering cyclizations is a long-known method, but has suffered for a long time from a lack of enantioselective strategies.[74] Although it was an important tool to control relative stereochemistry in the middle of the last century, it was soon outperformed by powerful enantioselective functionalizations such as epoxidations, aziridinations, and dihydroxylations.[74] The basis for enantioselective, possibly even catalytic, halogenations is being established only now.[74,75]

In 2007, Sakakura et al. reported the first enantioselective halocyclization of a polyprenoid.[76] By employing asymmetric phosphoramidites such as **82** as nucleophilic promoters, they achieved an enantiomeric excess up to 95% in the synthesis of tricyclic terpenoid **83** when using *N*-iodosuccinimide (NIS; Scheme 21).

The iodocyclizations typically produced undesired *endo*- or *exo*-olefins such as **84** as side products in a typical ratio of 3:7 for **84**/**83**. These compounds arise from premature quenching of the cation before nucleophilic attack of the aromatic system. Fortunately, it was possible to convert this side product into the desired tricyclic terpenoid **83** by using $ClSO_3H$. Motivated by the outstanding results for enantioselective iodocyclizations, the authors moved on to bromocyclizations. Although attempts to use *N*-bromosuccinimide as the halogenating agent gave the expected product in good yields, the enantiomeric excess dropped to a mere 36%. In conclusion, this method provides a very promising starting point, but successful examples for the cyclization of electron-poor polyprenoids[77] as well as applications to the total synthesis of a natural product are still missing.

As brominated and chlorinated natural products greatly outnumber iodinated natural products, it is highly important to expand the known method to other halogens. Recent publications have concentrated on the development of new catalysts and brominating agents, such as phosphite-urea derivatives[78] or BDSB (**85**; Scheme 22A).[77] BDSB, although being achiral, allows very mild, fast, and selective cation-π cyclizations (Scheme 22B); its usefulness has been additionally supported by several racemic total syntheses.[77] The simple chiral analogue **86** has been developed to tackle the missing enantioselectivity. Unfortunately, however, initial experiments did not show any enantiomeric excess.[77] Future experiments will have to determine the precise mechanistic course to evaluate the potential of an asymmetric version.

A different strategy than using novel bromating agents was followed by Braddock et al., who reported the first enantiospecific polyene cyclization with an enantiopure bromonium ion.[79] The chiral information on geranyl derivative **87** was introduced by Sharpless asymmetric dihydroxylation to give diol **88**, followed by interconversion into epoxide **89** and ring opening with lithium bromide to afford



Scheme 23. Enantioselective bromocyclization by the first enantiopure bromonium ion. The bromonium ion is generated in situ from the tetrafluorobenzoyl esters **91a/b**, which converge to the same bromonium ion.[79] brsm = based on recovered starting material; DCC = dicyclohexylcarbodiimide; DMAP = 4-(dimethylamino)pyridine; Ms = mesyl; NMP = *N*-methyl-2-pyrrolidone; PPTS = pyridinium *p*-toluenesulfonate; Py = pyridine; TfOH = trifluoromethanesulfonic acid.

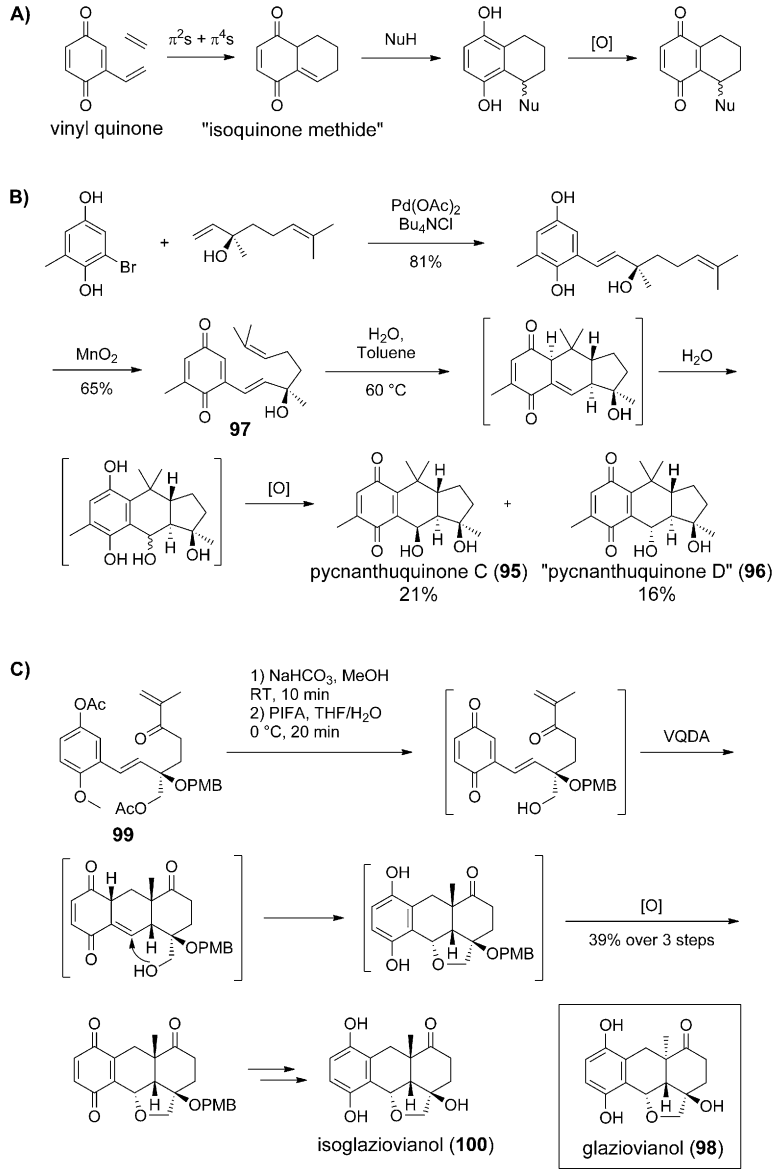
isomeric bromohydrins **90a/b** (Scheme 23). After activation of these precursors with tetrafluorobenzoic acid to yield **91a/b**, the authors carried out a cyclization using dimethylaluminium triflate to give a mixture of diastereomeric terpenoids **92** and **93** as a result of different conformations during the cyclization. In addition, olefin **94** was obtained as a side product from the premature quenching of the carbocation (as seen in Scheme 21). More importantly, however, both compounds had an enantiomeric excess of 96 %, thereby underlining the potential of this method. However, this example also shows that it is very difficult to control the folding during cyclization outside of the active site of an enzyme to prevent the formation of unwanted diastereomers. In the future, additional advancements and exemplary applications of this method are needed to make sufficient use of enantiopure bromonium ions.

Finally it should be mentioned that methods for fluorocyclizations are being developed and improved steadily,[80] but because of the scarcity of fluorinated natural products they are not discussed here further.

3.2. Diels–Alder Reactions of Vinyl Quinones

An interesting novel biosynthetic pathway for the cyclization of meroterpenoid quinones based on retrosynthetic considerations—the vinyl quinone Diels–Alder (VQDA) reaction—was proposed by Trauner and co-workers in 2010 (Scheme 24A).[81] It is built on the hypothesis that vinyl quinones can act as electron-deficient dienes in Diels–Alder reactions, reacting as the dienophile with the electron-rich trisubstituted olefins from terpene side chains. They demonstrated the validity of their model with a short, protecting-group-free synthesis of pycnanthuquinone C (**95**) along with the unknown diastereomer **96** (Scheme 24B).[81] Most interestingly, the VQDA transformation of the key intermediate **97** also proceeded in citrate-phosphate buffer at room temperature, albeit in very low yields of 8 %. As this study supports the idea that the VQDA cascade also occurs in nature, they propose that **96** likely exists in nature as well and should accordingly be named pycnanthuquinone D.

Afterwards, Trauner and co-workers turned to the closely related glaziovianol family.[82] Glaziovianol (**98**) is composed of a central 6-6-6 ring system instead of the 6-6-5 system of the pycnanthuquinones. The required protected precursor **99** was constructed in three steps from known starting materials. After deprotection and oxidation, the resulting vinyl quinone directly underwent a VQDA reaction at room temperature before it could be isolated. Surprisingly, however, it turned out after cleavage of the PMB group and reduction to the hydroquinone that the obtained product was the wrong diastereomer, (−)-isoglaziovianol (**100**), as a result of an *endo*- instead of an *exo*-transition state during the Diels–Alder reaction (Scheme 24C). Attempts to overcome this hurdle were unsuccessful, thus leaving open questions whether a VQDA cascade would occur spontaneously in biosynthetic pathways or would require enzymatic catalysis. Finally, it is also conceivable that isoglaziovianol (**100**) is actually a natural product which epimerizes spontaneously to glaziovianol (**98**)

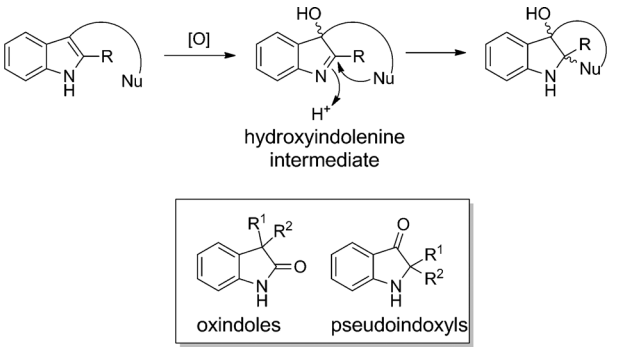


Scheme 24. Biomimetic application of the VQDA reaction for the synthesis of quinone meroterpenoid natural products. A) General principle of the VQDA reaction. The intermediary isoquinone methide can be trapped by nucleophiles. B) Biomimetic synthesis of pycnanthuquinone C (**95**) by a VQDA reaction of vinyl quinone **97**.[81] The intermediate is quenched by H_2O and oxidizes spontaneously to pycnanthuquinone C (**95**) as well as the diastereomer **96**. C) Attempted biomimetic synthesis of glaziovianol (**98**).[82] Surprisingly, the VQDA cascade provided the isomeric terpenoid isoglaziovianol (**100**), which was unknown before. PIFA = phenyliodine bis(trifluoroacetate); PMB = *para*-methoxybenzyl.

through a photochemical reaction.[82] Additional studies are required to clarify the biosynthetic relevance of the VQDA reaction.

3.3. Oxidative Cyclizations of Indoloterpenoids

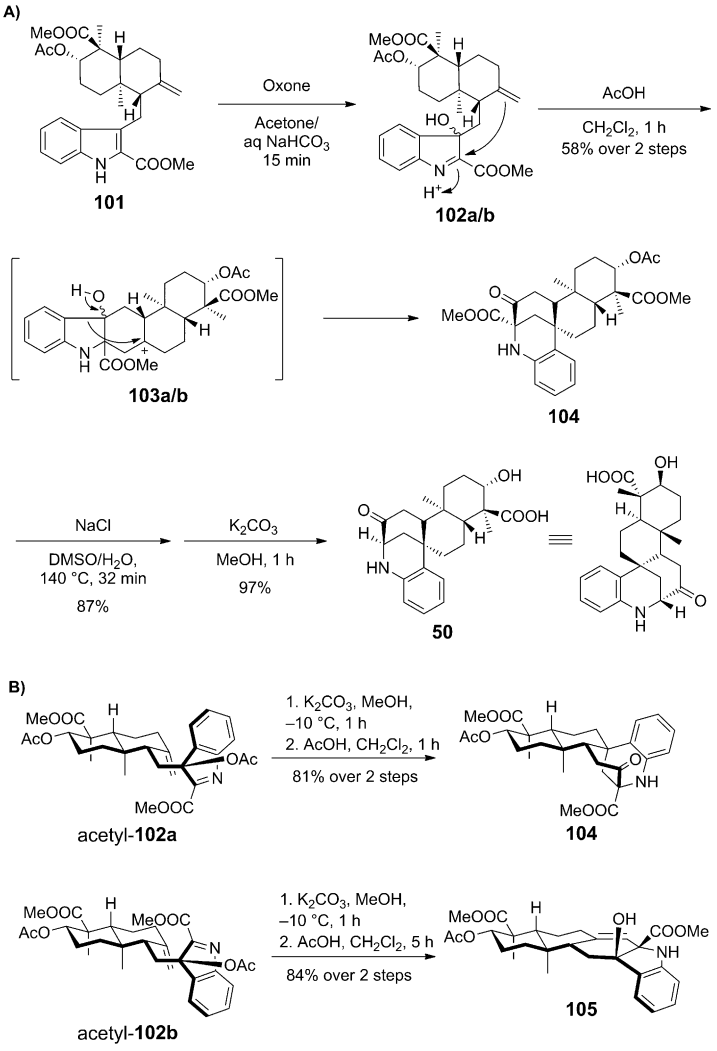
As discussed in Section 2.2.1, indoloterpene cyclizations can be triggered by oxidation of the indole ring system to give an intermediary hydroxyindolenine that can be attacked by intramolecular nucleophiles (Scheme 25). It is important to highlight that this intermediate is very prone to pinacol-like rearrangements to afford oxindoles and pseudoindoxyls. As such, it is the common biosynthetic precursor of spirocyclic prenylated diketopiperazines.[83]



Scheme 25. Oxidative cyclization of indoloterpenes by nucleophilic attack of an intramolecular nucleophile on the hydroxyindolenine intermediate. Alternatively, or additionally, pinacol-like rearrangements to afford oxindoles and pseudoindoxyls are often encountered.

In the case of sespenine (**50**), the nucleophile for the oxidative cyclization is the *exo*-methylene group of the cyclic sesquiterpene moiety of indosespene—formally an aza-Prins reaction. This consideration was recently successfully employed for the first total synthesis of sespenine (Scheme 26 A).[84] Initial attempts with various oxidizing agents showed that strictly biomimetic conditions only gave low yields of sespenine (< 21 %), because the lack of a C2 substituent in indole promoting oxidative cleavage of the pyrrole ring. As a consequence of their convergent synthesis route, this problem could easily be solved by using methoxycarbonyl-substituted indole derivative **101**, which was obtained in 12 steps. Subsequent oxidation with oxone gave an inseparable mixture of epimeric hydroxyindolenines **102 a/b**. This mixture was then treated to slightly acidic conditions at room temperature to trigger the cyclization cascade to generate the pentacyclic intermediary cations **103 a/b** and furthermore to the protected sespenine derivative **104**, which was smoothly converted into sespenine (**50**) in two additional steps. Notably, although hydroxyindolenines **102 a/b** can theoretically rearrange to give oxindoles and pseudoindoxyls, no such compounds have been identified.

To study the mechanistic process of the cyclization in more detail, it was necessary to separate the two epimers **102 a/b**. This was achieved by preparing the acetyl derivatives (acetyl-**102 a/b**) followed by HPLC purification. Interestingly, both epimers showed different reaction modes when subjected to the cyclization conditions. Only the acetyl-**102 a** epimer was smoothly converted into the sespenine-like rearrangement product **104**. In contrast, no pinacol-like reaction occurred in the case of acetyl-**102 b**; the intermediate carbocation was simply quenched by proton elimination to afford cyclohexene derivative **105**, which resembles the carbon skeleton of prexiamycin (**48**; Scheme 26 B). Based on this finding, the authors concluded that the biosynthetic divergence between xiamycin (**49**) and sespenine (**50**) is not random, but predefined by the stereochemistry of the preceding hydroxyindolenine (Scheme 14). The authors, therefore, not only provided the first synthesis of sespenine and thus confirmed the structural assignments, they also demonstrated how



Scheme 26. Bio-inspired synthesis of sespenine (**50**). A) Key transformations for the acid-catalyzed oxidative cyclization. B) Different reaction modes of epimeric hydroxyindolenines acetyl-**102 a/b**. The intermediary cation can either be quenched by a pinacol-like rearrangement to afford **104** or by simple proton elimination to give **105**.[84]

synthetic organic chemistry can be an important tool to elucidate biosynthetic pathways, even up to intricate stereochemical details. In addition, this example again underlines that stereochemistry plays a pivotal role in the fate of carbocations and can lead to completely different scaffolds.

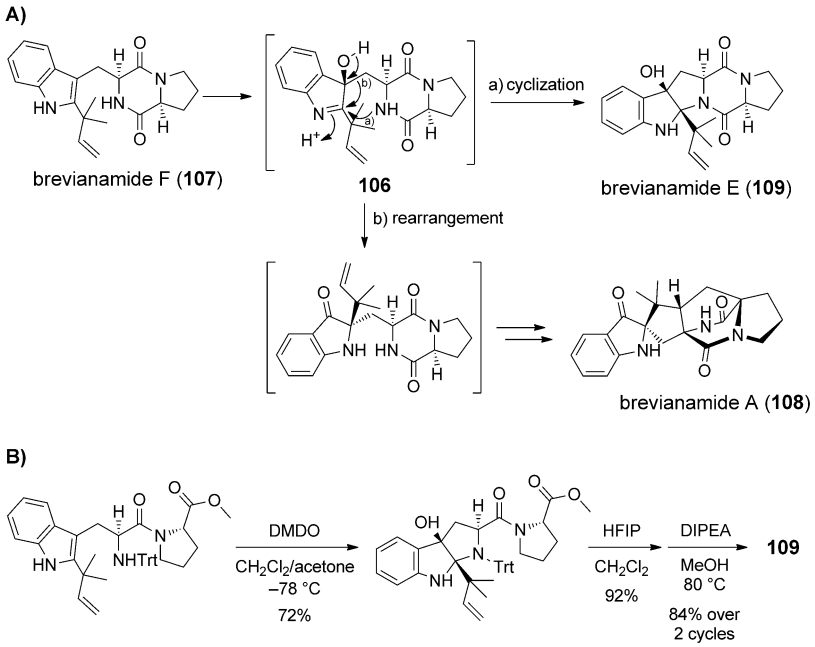
Similar oxidative pathways are not only known for indoloterpenes, but also for prenylated indole alkaloids. An example is the brevianamides, a group of diketopiperazines from *Penicillium brevicompactum*.[85] As proposed by Sanz-Cervera et al., the central hydroxyindolenine intermediate **106**, which is derived from brevianamide F (**107**), can undergo either a pinacol-type rearrangement, comparable to the sespenine biosynthesis, to afford brevianamide A (**108**) or can be trapped by a nitrogen nucleophile to generate brevianamide E (**109**) (Scheme 27 A).

Although this biosynthetic model has never been verified biochemically, it was supported by a stereoselective synthesis of brevianamide E (**109**) by using dimethyldioxirane (DMDO) as the oxidizing agent (Scheme 27 B).[86] Further support comes from a study on the biosynthesis of structurally related notoamides, where the FAD-dependent oxidase NotB was shown to catalyze a 2,3-oxidation of indole that sets the stage for an N-C ring closure and a pinacol-like rearrangement after ring-opening of the epoxyindole intermediate.[87] A recent synthetic study of the structurally related spirocyclic citrinalin and cyclopiamine families also investigated different possibilities to achieve stereoselective indole oxidations and regioselective rearrangements.[88] These examples underline the broad spectrum of oxidative chemistry involving indole derivatives, where nucleophiles of all kind can be used to form complex polycyclic molecules. It is left to the creativity of the reader to think of possibilities of how the cyclization modes presented in this Review can be expanded to other nucleophiles, and thus to advance the chemistry for the formation of carbocycles and heterocycles.

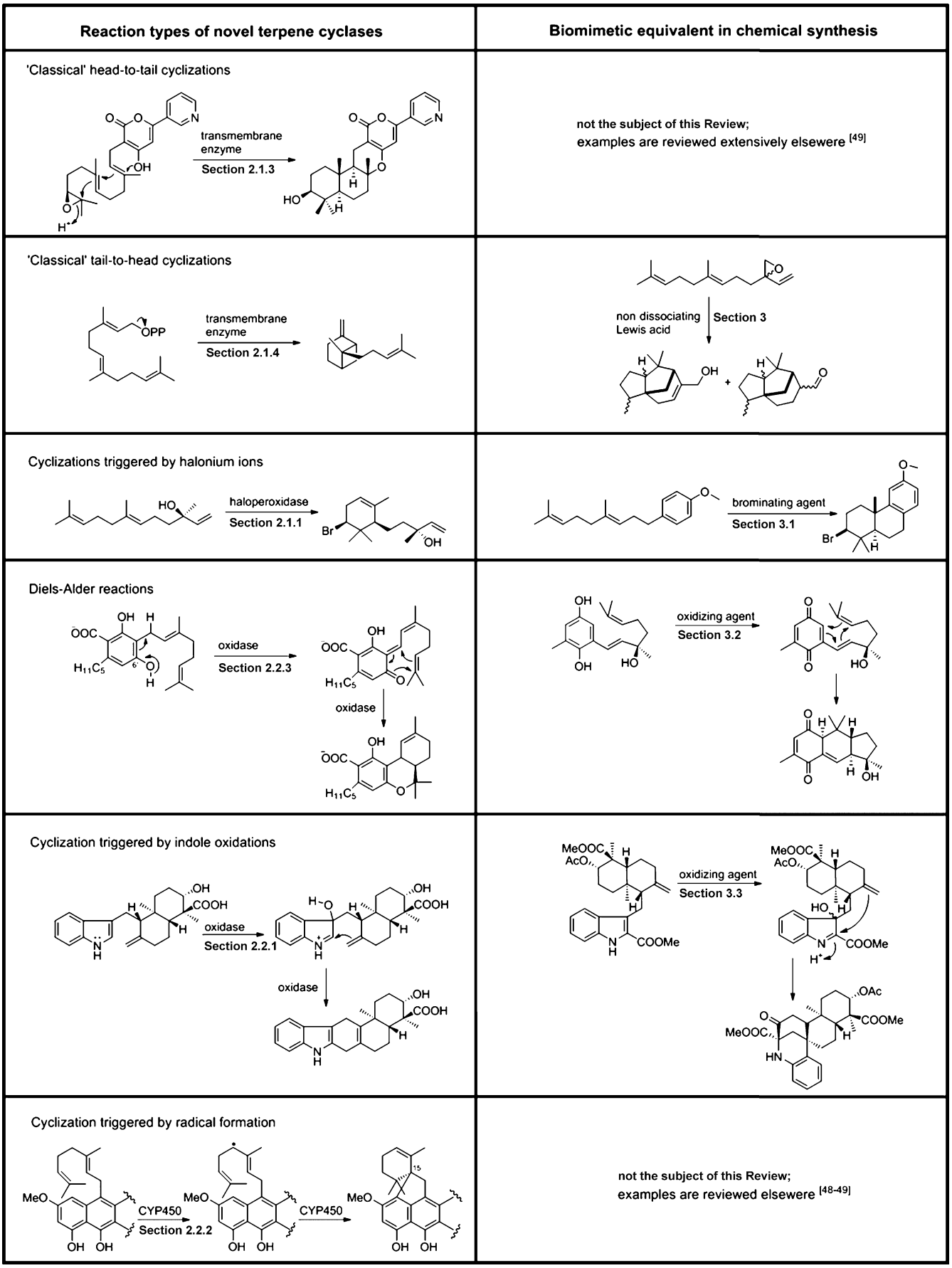
4. Summary and Outlook

Taking all this together, a common scheme exists for terpene/terpenoid cyclization in which similar reactive intermediates are formed that are prone to further cyclization by very distinct enzymes in many different ways. Considering this diversity, it can be assumed that there are plenty of unknown TCs to discover. Nevertheless, the discovery of novel TCs from different kingdoms of life has so far already brought many chances. Since, for example, Pyr4 homologues are highly conserved among many meroterpene biosynthetic gene clusters, such new TC enzymes might be powerful tools for genome mining approaches to access new compounds. On the other hand, their discovery should encourage researchers not to just rely on homology searches of characterized enzymes but to search actively for new TCs, if apparent TCs cannot be found in the biosynthetic gene clusters of cyclized terpenoids. A great challenge will be the further investigation of the precise reaction mechanisms of the novel TCs. Detailed mechanistic insights of the cyclization reactions might be of high value when it comes to engineering the biosynthetic pathway to generate "unnatural" natural products. Since conventional class I and class II TCs have been shown to be easily manipulated in terms of their cyclization outcome by alteration of distinct amino acids,[89] a greater understanding of the underlying mechanisms of novel TCs might be a valuable key to the rational design of proteins and compounds. Last but not least, more detailed knowledge of novel TCs would be of utmost interest concerning the evolution of these enzymes. The homology of Pyr4 homologues to transporters, for example, raised the question whether these proteins are also involved in transport: Crawford and Clardy speculated that these MTCs might work as efflux proteins whilst catalyzing the cyclization reaction.[33b] Although such a scenario seems contradictory if one considers that these cyclization products in part undergo substantial post-cyclization modifications, which are likely executed in the cell, such exciting questions should be addressed in the future.

However, new TCs and their mechanisms of action are not only interesting from an enzymatic/biosynthetic point of view. For synthetic chemists, who may employ strategies to emulate enzyme-catalyzed reactions, novel TCs harbor great potential. This is highlighted by the recent bio-inspired/biomimetic syntheses that are summarized in Scheme 28. These already achieved synthe-



Scheme 27. Oxidative pathways of prenylated indole alkaloids of the brevianamide family. A) Proposed biosynthetic pathways diverging at hydroxyindolenine **106** to afford cyclic brevianamide E (**109**) or pseudoindoxyl brevianamide A (**108**). B) First stereoselective oxidative cyclization towards brevianamide E (**109**);[85] DIPEA = *N*,*N*-diisopropylethylamine; DMDO = dimethyldioxirane; HFIP = hexafluoroisopropanol; Trt = triphenylmethyl.



Scheme 28. Reaction types of novel TCs that were already used in synthetic chemistry. CYP450 = cytochrome P450.

ses, together with the unprecedented reaction types of novel TCs such as prenyltransferases (Section 2.1.2) or methyltransferases (Section 2.1.5), will hopefully stimulate more breakthroughs in this synthetic field.

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