Biosynthetic Diels–Alder Reactions

Chemistry and Biology of Biosynthetic Diels–Alder Reactions

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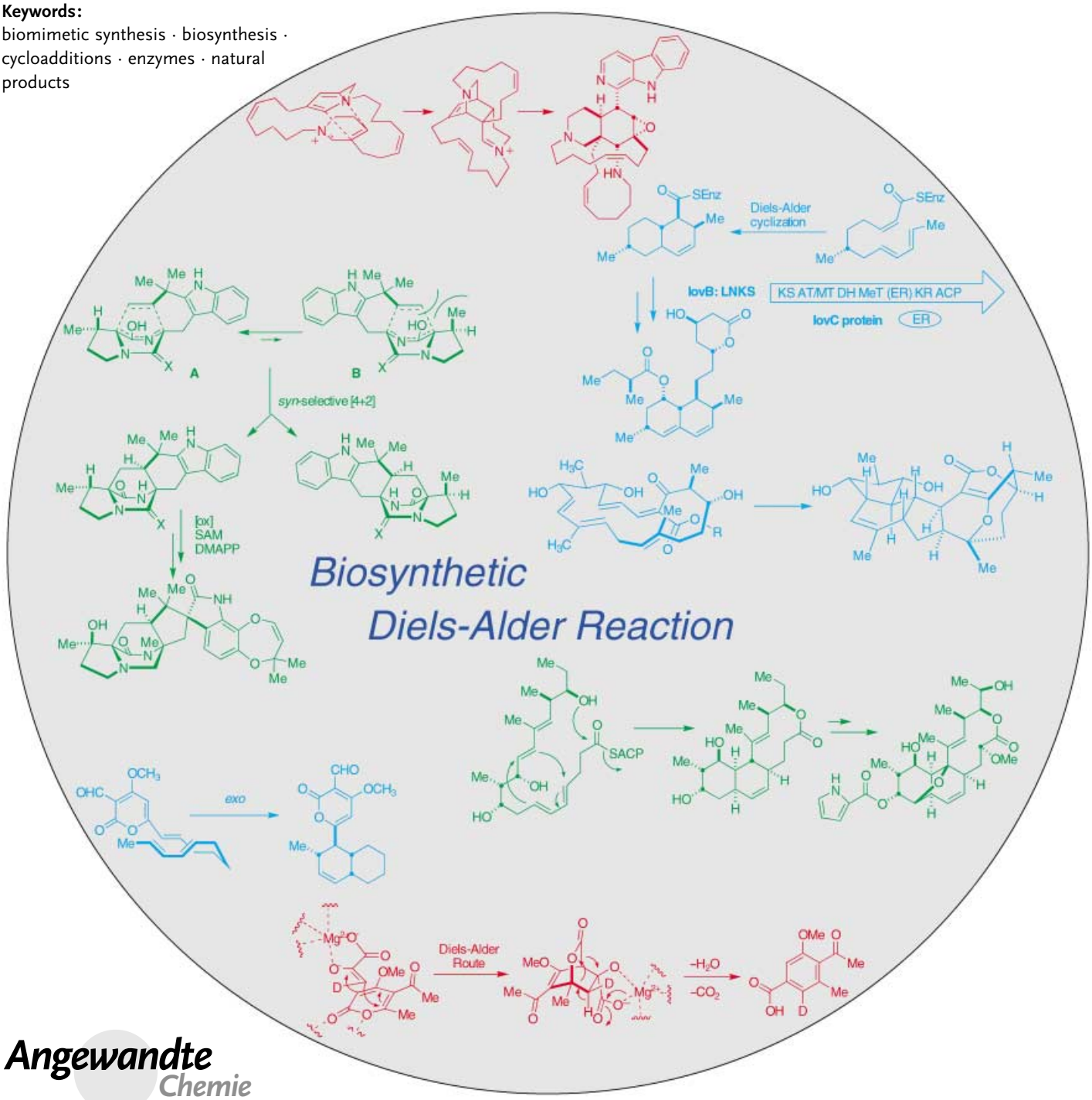

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Nature's repertoire of biosynthetic transformations has recently been recognized to include the Diels–Alder cycloaddition reaction. Evidence now exists that there are enzymes that mediate the Diels–Alder reaction in secondary metabolic biosynthetic pathways. 2002 marked the 100th anniversary of Alder's birth and 75 years since the discovery of the Diels–Alder reaction. It would appear that living systems discovered and made use of this ubiquitously useful ring-forming reaction eons ago for the construction of complex natural products. In this Review an overview is given of all of the known classes of natural products (polyketides, isoprenoids, phenylpropanoids, alkaloids) that have been speculated to arise by a biological Diels–Alder reaction.

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1. Introduction

The Diels–Alder reaction is a powerful reaction for the formation of carbon–carbon bonds in synthetic organic chemistry which allows facile, stereospecific entry into six-membered ring systems.[1] The structures of various secondary metabolites have led to an array of provocative proposals which suggest that nature might also make use of this valuable reaction.[2] An intriguing aspect of many of these biosynthetic proposals involves the possibility of enzymatic catalysis of the [4+2] cycloaddition, which would accommodate the stereochemistry extant in the respective natural product. Enzymes generally catalyze reactions by stabilizing the structure and charge of the developing transition state. For most reactions subject to this stratagem of catalysis, both the starting substrate and the product differ significantly from the transition state with respect to structure. Both the product and the starting substrate must bind to the enzyme less tightly than the transition-state structure for catalysis to occur. The transition state in the Diels–Alder reaction is highly ordered and closely resembles the structure of the product. In other words, an enzyme that was designed to stabilize the transition-state structure for this reaction would be expected to be inhibited by the product (by tight binding) and turnover (thus, catalysis) would be precluded. Alternatively, the free energy of activation can be lowered by raising the ground-state energy of the reactants. This might be accomplished in the Diels–Alder reaction by the introduction of torsional strain into the dienophile or diene components, but it is difficult to find solid precedent for this strategy in the literature. The prospect of discovering a Diels–Alderase is therefore especially enticing to mechanistic enzymologists, since it could represent a new mechanism of catalysis in nature.

Until recently, the existence of a Diels–Alderase has remained elusive, but catalysis of the Diels–Alder cycloaddition reaction by biomolecules has indeed been realized. Hilvert et al. first reported the catalysis of a Diels–Alder reaction by an antibody in 1989.[3] Monoclonal antibodies were raised against hapten **1** (Scheme 1), which resembles the transition state of the Diels–Alder reaction between tetrachlorothiophene dioxide (**3**) and *N*-ethylmaleimide (**4**). The antibody catalyzed the Diels–Alder reaction by binding the diene **3** and dienophile **4** in a reactive conformation, thus lowering the entropy of activation. The problem of product inhibition was overcome by the extrusion of SO_2 from the labile cycloadduct to give a product **5** that did not resemble **1** and, thus, catalyst turnover was not impeded.

Braisted and Schultz used an alternative approach to overcome the difficulty of product inhibition in Diels–Alder catalysis by an antibody (Scheme 1).[4] They used an ethano bridge to lock the cyclohexene ring of the hapten **6** into a conformation resembling the proposed transition state **7** for the Diels–Alder reaction bewteen the acyclic diene **8** and the dienophile **9**. The authors argue that the product **10** prefers a twisted chair conformation relative to the rigid boat conformation induced by the bicyclo[2.2.2] hapten **6** that allows for release from the antibody combining site. Subsequent structural elucidation of the complex formed between this catalytic antibody and the hapten revealed the presence of 89 van der Waals interactions and two hydrogen bonds between the antibody and its hapten. These interactions apparently activate the dienophile and control the relative geometries of the bound substrates.[5]

Another type of biomolecule used to catalyze the Diels–Alder reaction is ribonucleic acid (RNA).[6] However, the mechanism of catalysis is radically different from that of catalytic antibodies. Since RNA Diels–Alderase activity is reliant on base specificity and the coordination of a transition metal, the mode of catalysis is more likely akin to Lewis acid catalysis of the Diels–Alder reaction.

The role of protein organization in natural systems and the possible mechanism of catalysis has long been a subject of debate, and was rekindled by the recent characterization of two naturally occurring potential Diels–Alderases.[7,8] The isolation of these enzymes also

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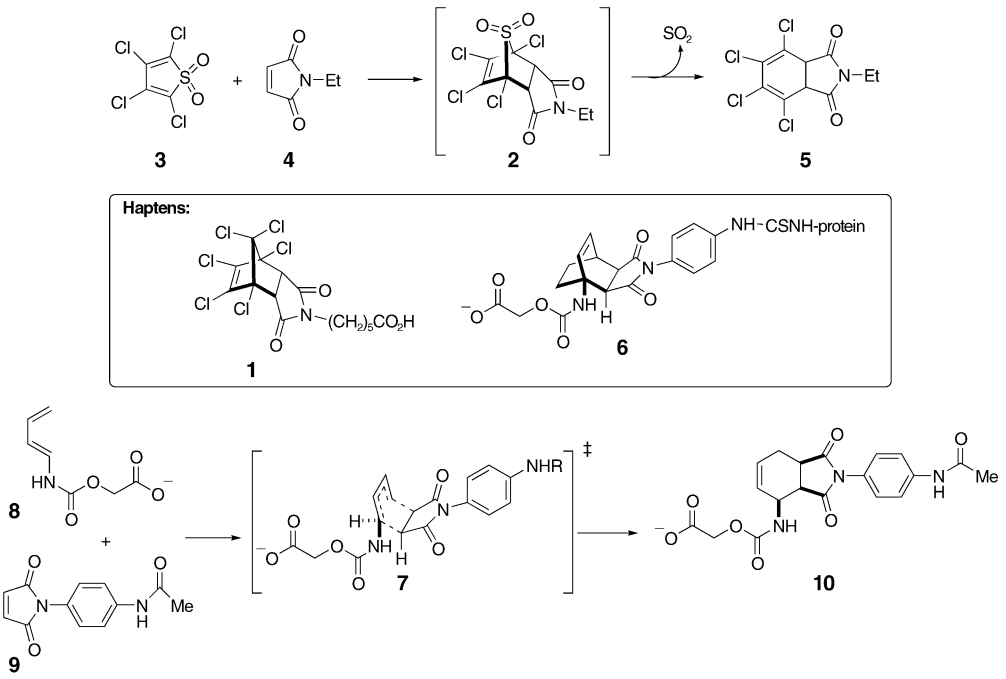

Scheme 1. Antibody catalysis of Diels–Alder reactions.[3,4]

establishes the Diels–Alder reaction as a viable biosynthetic transformation.

This Review is intended to provide an overview of the natural products that have been proposed in the literature to be constructed biosynthetically by a Diels–Alder reaction, both catalyzed and uncatalyzed. Where available, the biosynthetic studies pertaining to these substances to probe these questions are summarized. Although there are countless structures that can formally be envisioned to arise by a [4+2] cycloaddition, this Review is limited to those natural products that have been described in the literature as putative Diels–Alder cycloadducts. The Review is organized into classes of compounds based on their biosynthetic derivations: polyketides (acetate), isoprenoids (mevalonate), phenylpropanoids (shikimic acid), and alkaloids (amino acids). In many cases, this segregation is superficial, since many compounds are often of mixed biosynthetic origins (for example, cytochalasans, pycnidione, and brevianamides).

2. Polyketides

Since polyketides are derived from acetate, these compounds are particularly well-suited for isotopic labeling studies. For example, isotopically labeled precursors, such as ^{13}C-acetate, are readily accessible, comparatively cheap, and are often readily incorporated into the corresponding secondary metabolite in feeding experiments. Thus, it is hardly surprising that a large portion of the experimental evidence for the Diels–Alder reaction in nature has been obtained for this class of compounds. In fact, both lovastatin and macrophomic acid, for which reported Diels–Alderase enzymes have been isolated,[7,8] are classified as polyketides.

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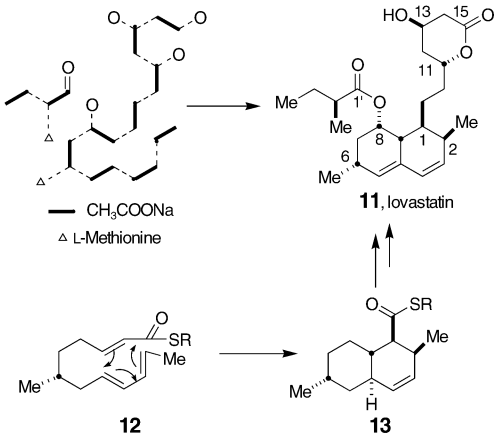
Emily Stocking was born in Oakland, California in 1970. She obtained a B.A.S. in Chemistry and Biology at the University of California at Davis and in 1993 she took a position at the NIH Stable Isotopes Resource at Los Alamos National Laboratory. In 1994 she joined the research group of Professor R. M. Williams at Colorado State University. Her doctoral studies included the total synthesis of the indole alkaloid VM55599 and studies on the biosynthesis of paraherquamide A. Since completion of her degree in 2001, she has worked at Johnson and Johnson Pharmaceutical Research and Development in La Jolla, California.

2.1. Decalin Polyketides

2.1.1. Lovastatin (Mevinolin)

Lovastatin (**11**), also known as mevinolin, has received significant attention in the literature, since it is a potent inhibitor of cholesterol biosynthesis in humans and has become a clinically useful and very successful drug. Feeding experiments on the producing fungal strain *Aspergillus terreus* (ATCC 20542) with [1-^{13}C], [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,1-$^{18}O_2$]-, [1-^{13}C,2-$^{2}H_3$]-, and [2-^{13}C,2-$^{2}H_3$]acetates established that lovastatin is comprised of a C_{18} unit and a C_4 unit constructed through the head-to-tail attachment of acetate (Scheme 2).[9]



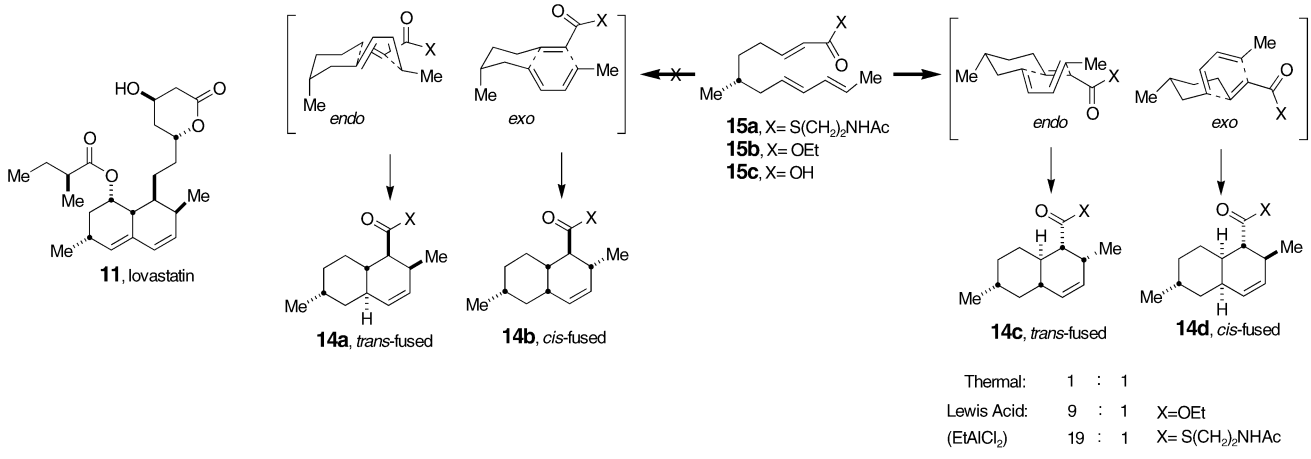
Scheme 2. Origin of the carbon atoms of lovastatin (**11**) and proposed biogenesis.[9,10]

Interestingly, of the five oxygen atoms in lovastatin, only the oxygen atom attached at C11 could definitively be assigned as a derivative of acetate. In addition, it was discovered that the two methyl groups at C2 and C6 are derived from *S*-adenosylmethionine (SAM), as shown by feeding experiments with [$^{13}CH_3$]methionine.

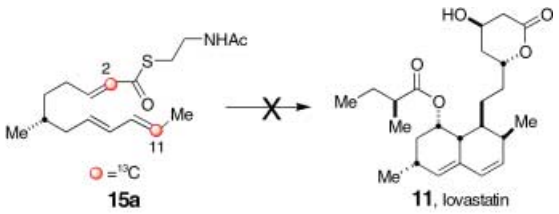
Re-examination of the origin of the oxygen atoms in lovastatin (**11**) was made possible by the subculture selection of a new strain of *Aspergillus terreus* (MF 4845), which increased the production of **11** to 200 mg per litre of culture.[10] Fermentation of *Aspergillus terreus* in the presence of an $^{18}O_2$-enriched atmosphere showed the oxygen atom at C8 was derived from molecular oxygen. A separate feeding experiment with [1-^{13}C,1-$^{18}O_2$]acetate indicated the oxygen atoms at C1′, C11, C13, and C15 are derived from acetate. The results of these feeding experiments as well as the previous experiments led to the biosynthetic hypothesis outlined in Scheme 2.[9,13] Vederas and co-workers speculated that the enzymes involved in the biosynthesis of lovastatin (**11**) are similar to those involved in the biosynthesis of fatty acids.[9] They proposed that condensation of acetate units (from malonate) could produce a triene **12** that would undergo an *endo*-selective Diels–Alder cycloadditon to the decalin **13**.

The first test of this hypothesis was a synthesis of the analogue **14** through laboratory Diels–Alder cyclizations of the thioester **15a**, ethyl ester **15b**, and acid **15c** both thermally and with a Lewis acid catalyst (Scheme 3).[11] There was a 1:1 ratio of the *endo*(**14c**):*exo*(**14d**) products in the thermal cyclization, presumably through a chairlike transition state with the methyl side chain disposed in a pseudo-equatorial manner. However, no *endo* product **14a** corresponding to the stereochemistry of **11** was observed (with a pseudo-axial methyl side chain in the transition state). The Lewis acid catalyzed Diels–Alder reaction gave the same two products as the thermal reaction but in a 9:1 *endo*:*exo* ratio for **15b** and a 19:1 ratio for **15a**. The absence of product **14a** in the laboratory cyclizaton suggests that the Diels–Alder cyclization in the biogenesis of **11** could be enzymatic.

Feeding experiments were performed under a variety of conditions on *Aspergillus terreus* (MF4845) with **15a** doubly labeled with ^{13}C at C2 and C11 to test for Diels–Alder activity in vivo (Scheme 4).[11] This doubly labeled precursor readily degrades by β oxidation to smaller building blocks (for example, acetate). Incorporation of the intact precursor would lead to adjacent ^{13}C labels, which would be perceived as carbon–carbon coupling in the ^{13}C NMR spectrum. However, feeding experiments with [2,11-$^{13}C_2$]-**15a** did not reveal detectable ^{13}C–^{13}C coupling in the ^{13}C NMR spectra. Appa-



Scheme 3. Synthesis of the decalin **14** though an in vitro Diels–Alder cyclization of triene **15**. Thermally: toluene, 160 °C, 4 days; Lewis acid catalyzed: 0.9 equiv $EtAlCl_2$, RT, 3 h.[11]

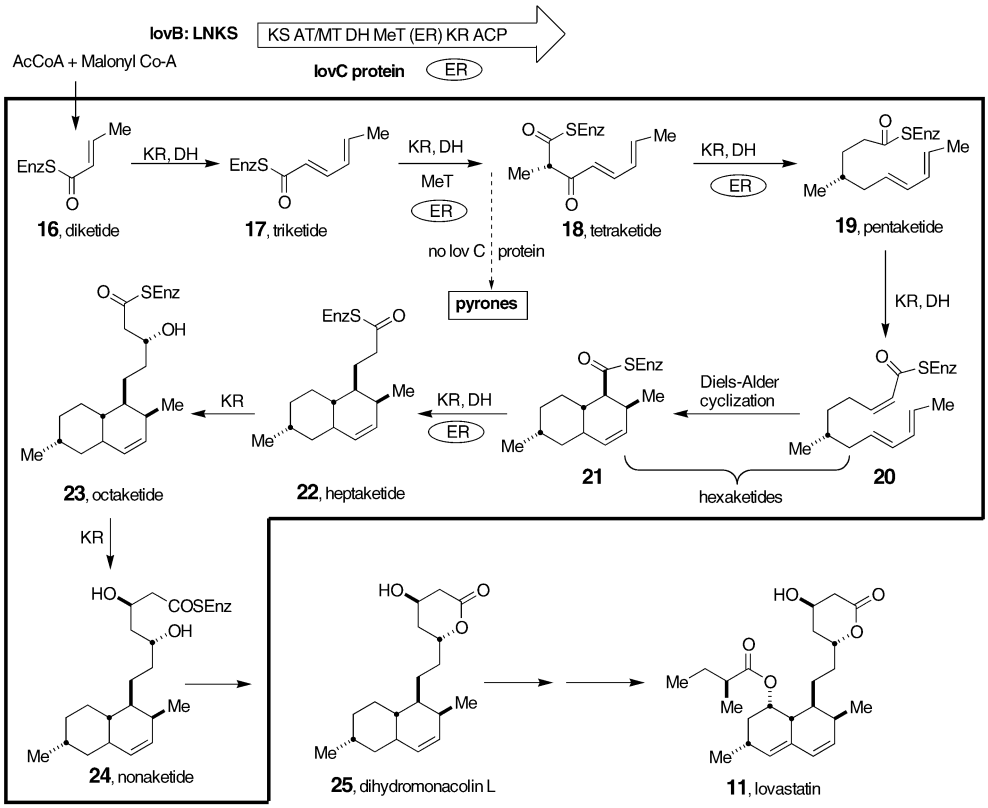


Scheme 4. Feeding experiment with [2,11-$^{13}C_2$]-**15a** in the investigation of the Diels–Alder activity of *A. terreus* (MF4845).[11]

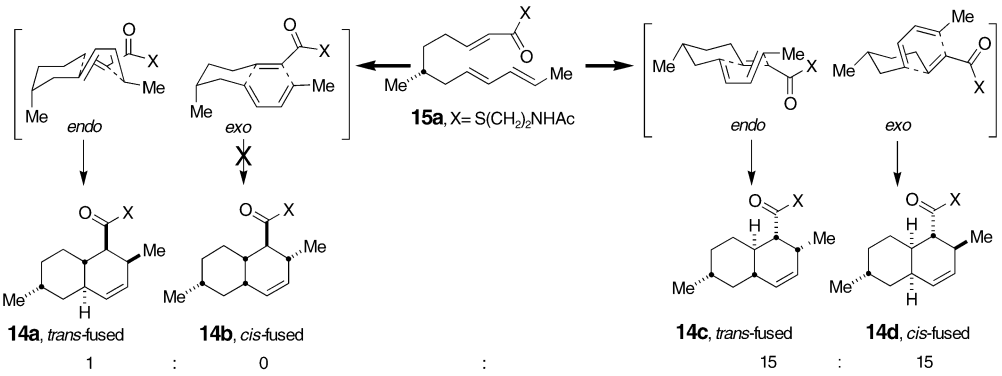
rently, **15a** was catabolized before it could undergo cycloaddition.

A complete outline of the proposed biosynthesis of **11** including the role of the *lovB* and *lovC* genes is shown in Scheme 5. Vederas, Hutchinson, and co-workers demonstrated that dihydromonacolin L (**25**), an established intermediate in the biosynthesis of lovastatin (**11**),[12] was formed in a heterologous host, *Aspergillus nidulans*, containing the *lovB* and *lovC* genes from *Aspergillus terreus*.[13] In addition, expression of the lovB protein (lovastatin nonaketide synthase, LNKS) in the absence of lovC protein led to truncated pyrones because of the inefficient enoyl reduction at the tetraketide stage. These results were interpreted as supporting the notion of catalytic Diels–Alder activity for LNKS.[7,13]

The enzymatic activity of LNKS was tested on **15a**, the analogue of the proposed cycloaddition precursor. The *N*-acetylcysteamine (NAC) ester **15a** (Scheme 6) was added to an aqueous buffered solution containing pure homogenous LNKS protein.[7] The *endo*-Diels–Alder product **14a**, which had the same stereochemistry observed in **11**, was obtained along with the non-enzymatic products **14c** and **14d** (**14a**:**14c**:**14d** = 1:15:15). The *cis*-fused *exo*-product **14b** was not observed under any conditions. When **15a** was added to thermally denatured LNKS, adducts **14c** and **14d** were formed, but **14a** was not detected. Cycloadducts **14c** and **14d** result from a transition state with the C6 methyl group in a sterically favored pseudo-equatorial arrangement. However, the transition state leading to **14a** requires a more crowded pseudo-axial disposition of the methyl group at C6. Thus, it seems the function of LNKS is to bind the substrate in a conformation that resembles the *endo* transition state that leads to **14a** (analogous to the mode of action of catalytic antibodies). In addition, hydrogen bonding of the carbonyl oxygen atom within the active site of LNKS would make the dienophile more electron deficient, thus resembling Lewis acid catalysis of laboratory Diels–Alder reactions. Since the product **14a** was not obtained in the presence of denatured LNKS, the asymmetric induction of the Diels–Alder reaction cannot be caused by nonspecific binding of a chiral protein.[14] Therefore, LNKS represents the first naturally occurring Diels–Alderase enzyme to be purified to homogeneity.[7]



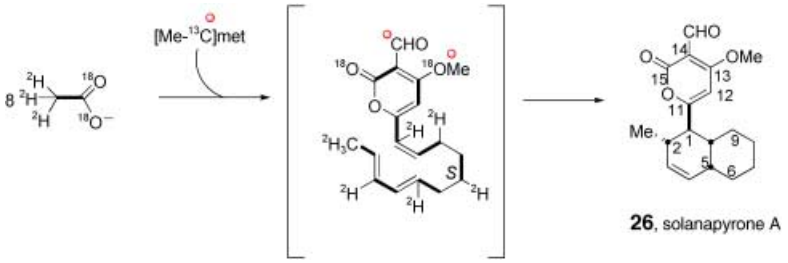
Scheme 5. Proposed biosynthetic pathway for lovastatin (**11**). The boxed region shows reactions catalyzed with LNKS and the lovC protein. The domains for the LNKS and the lovC protein were assigned from sequence homology to other polyketide synthase (PKS) proteins.[7] KR = keto reductase, DH = dehydratase, MeT = methyltransferase, ER = enoyl reductase, KS = β-ketoacyl synthase, ACP = acyl carrier protein, AT/MT = acetyl/malonyltransferase.



Scheme 6. Synthesis of **14** though an in vitro enzymatic Diels–Alder cyclization.[7]

2.1.2. *Solanapyrones*

Another decalin polyketide thought to arise through a [4+2] cycloaddition is solanapyrone, a phytotoxin produced by the pathogenic fungus, *Alternaria solani*.[15] A series of feeding experiments with singly and multiply labeled acetate and [$^{13}CH_3$]methionine revealed the origin of all the carbon atoms in solanapyrone A (**26**).[16] The results are summarized in Scheme 7. An upfield shift of the C13 and C15 signals in the ^{13}C NMR spectra after feeding experiments with [1-^{13}C,1-^{18}O]acetate excluded the possibility that the polyketide is derived by oxidative scission of a longer precursor and indicates that C15 is the terminal carbon atom of the polyketide. Feeding experiments with [1-^{13}C,2-$^{2}H_3$]- and [2-^{13}C,2-$^{2}H_3$]acetate established the fate of the hydrogen atoms and proved that the *pro-S* hydrogen atom is retained by the enoyl reductase. The stereochemistry at C1, C2, C5, and C10, the location of the double bond, and the stereochemistry at C5 and C7 with the acetate deuterium atoms are consistent with a Diels–Alder construction of the decalin ring system.



Scheme 7. Incorporation of labeled acetate and methionine into solanapyrone A (**26**).[16]

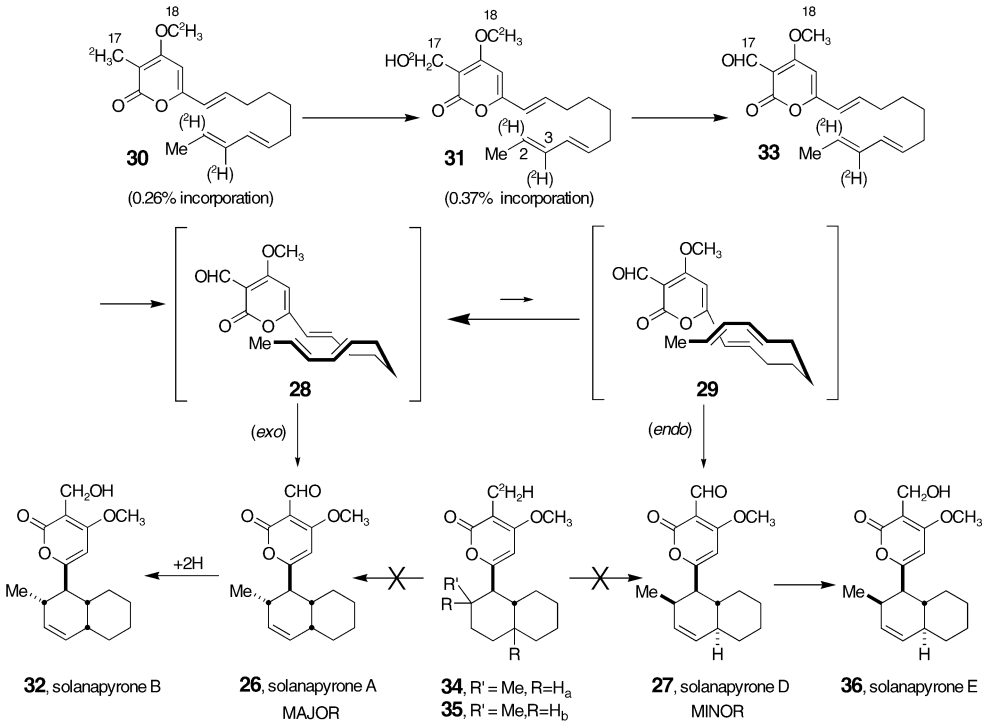
The isolation of the minor metabolite solanapyrone D (**27**), a diastereomer of **26**, provided additional support for the Diels–Alder reaction in the biosynthetic pathway.[17] Rotation about the C5–C6 bond in the Diels–Alder reaction could give rise to either the *exo*- or *endo*-cycloaddition products (Scheme 8). Dreiding models indicate a similar stability of the two transition states **28** and **29**, but a biomimetic synthesis of **26** provided a 2:1 product ratio in favor of the *endo* adduct **27**.[18] Since the *exo* product is the major isomer observed in the natural system, this was the first indication that the reaction might be enzyme-mediated.

Evidence for the biosynthetic Diels–Alder reaction in the biosynthesis of solanapyrone was obtained when the achiral deuterated trienes **30** and **31** were incorporated in vivo into **26** and **32**.[19,20] Incorporation of the precursor **30** indicated loss of deuterium at C17. The ratio of the integration for the signals of deuterium at C17 to deuterium at C18 in the ^{2}H NMR spectrum changed from 2:3 in **30** to a ratio of 1:4.3 for **26** and 1:5.1 for **32**. Observation of the same deuterium ratio for **26** and **32** indicates that **26** is reduced to **32** and that the two triene precursors **30** and **31** are oxidized to the same intermediate, presumably the C17 aldehyde prosolanopyrone II (**33**). Feeding experiments could not be performed with **33** because it was so reactive and underwent spontaneous *endo* cyclization in aqueous conditions.

The reactivity of the aldehyde **33** makes it a likely candidate as a direct substrate for the Diels–Alder cyclization (Scheme 8). Incorporation of the [$^{2}H_7$]-**31** with an essentially unchanged deuterium ratio demonstrated that the diene/dienophile precursor was incorporated intact. Additionally, the labeled compounds **34** and **35** were not incorporated into **26** or **27**, which indicates that the Diels–Alder reaction probably occurs after oxidation.

Enzymatic activity was found in a cell-free extract of *Alternaria solani*, which catalyzed the conversion of **33** into solanapyrones A (**26**) and D (**27**) in 25% yield with a ratio of the *exo* to *endo* cycloadduct of 53:47 .[21] A control experiment with denatured enzyme provided a 3:97 ratio of the *exo* to *endo* cycloadducts with only 10% consumption of starting material. The observed stereoselectivity in the cell-free extract was interpreted as being indicative of enzymatic activity.

Conversion of **31** with the crude enzyme preparation was accomplished in 25% yield (19% **26** and **27**, 6% **33**) with an *exo*/ *endo*-cycloaddition ratio of 85:15 and an optical purity (for **26**) of 99% *ee*. The optical purity of **26** produced from **33** was 92% *ee*. Since this value is lower than that obtained from **31**, it seems that a single enzyme catalyzing the oxidation and cycloaddition is responsible for producing the optically pure solanapyrones found in the natural system. Further proof of



Scheme 8. Incorporation of deuterated trienes into solanapyrones A (**26**) and D (**27**). The thioacetal of **27** is the major product (2:1) in the laboratory synthesis.[19,20]

this sequence of events was obtained when the enzymatic cycloaddition reaction of **31** was suppressed in the absence of oxygen (argon atmosphere).

2.1.3. *Nargenicin*

Nargenicin (**37**), a polyketide antibiotic isolated from *Nocardia argentinensis*, contains a macrocyclic lactone fused to a *cis*-octahydronaphthaline (octalin) ring system that is derived biosynthetically from five acetate and four propionate units.[22] Feeding experiments with [1-$^{18}O_2$,1-^{13}C]acetate and [1-$^{18}O_2$,1-^{13}C]propionate indicated that the oxygen atoms at C1 and C11 were derived from acetate while the oxygen atoms at C9 and C17 were derived from propionate (Scheme 9, inset). In accord with these results, incubation of *N. argentinensis* with ^{13}C-labeled acetate and propionate in an $^{18}O_2$-enriched atmosphere indicated that the two ether oxygen atoms at C2 and at C13 and the hydroxy group at C18 are derived from molecular oxygen.[23]

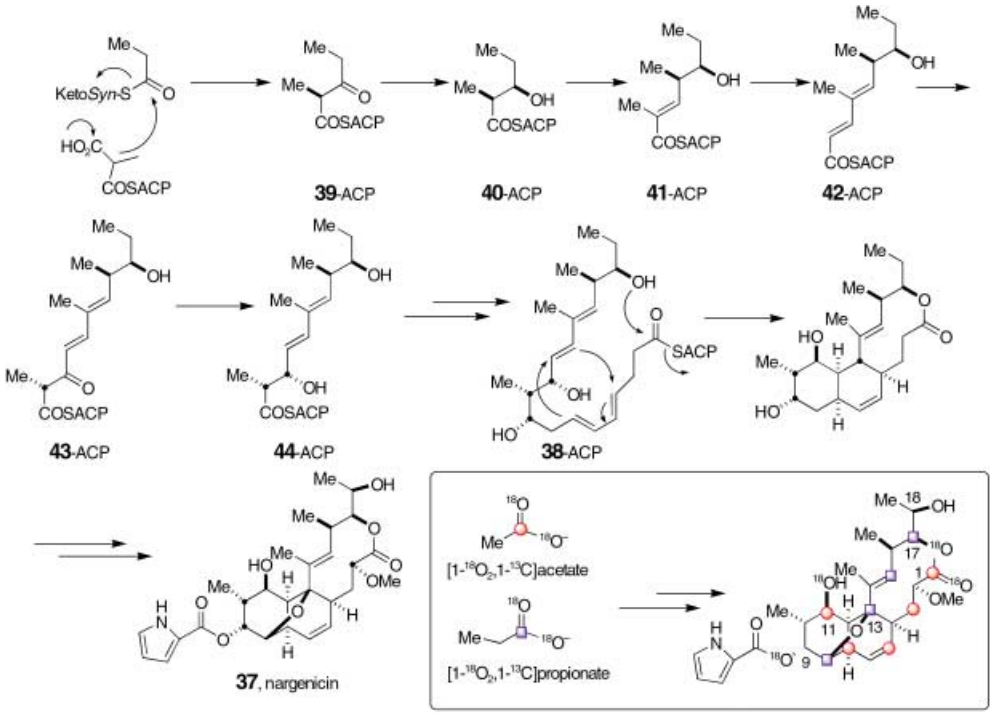
Since the oxygen atom at C13 is not derived from propionate, this implies that the C4–C13 bond is not formed by an aldol-type condensation. Instead, a Diels–Alder cyclization was invoked through the intermediacy of the triene **38** (Scheme 9). By the incorporation of the ^{13}C- and/or ^{2}H-labeled NAC esters of the precursors **40**, **41**, **42**, and **44** (Scheme 9), Cane et al. demonstrated that the stereochemistry and level of oxidation are set prior to chain elongation.[24] The incorporation of [$^{13}C_2$]-**44** also further supports the notion that the *cis*-octalin ring system is generated through a Diels–Alder cycloaddition.[24]

2.1.4. *Betaenone B*

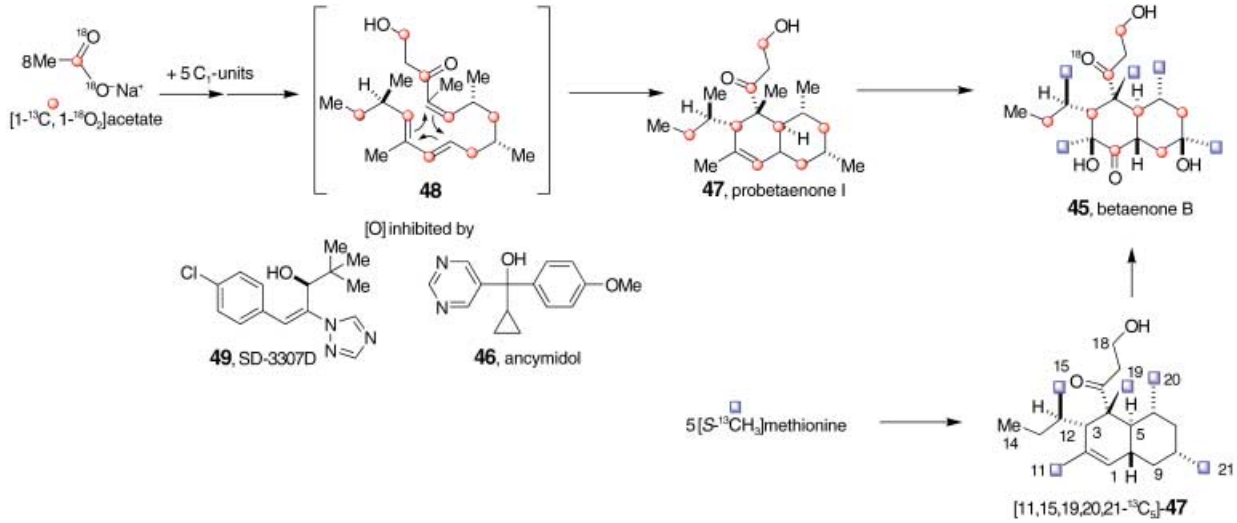
Betaenone B (**45**) is a phytotoxin from *Phoma betae* (a fungus) that causes a leaf spot disease on sugar beet. Feeding experiments with [1-^{13}C,1-$^{18}O_2$]acetate indicated that only the oxygen atom at C16 of **45** is derived from acetate (Scheme 10).[25] The absence of an ^{18}O-induced isotopic shift in the signal corresponding to C18 could indicate that the oxygen atom is derived from molecular oxygen or it could indicate a "washing out" of the label by exchange with water.

When a P450 inhibitor, ancymidol (**46**), was added to cultures of *P. betae*, the production of betaenone B (**45**) was suppressed in proportion to the amount of inhibitor added. In addition, a new deoxygenated metabolite, probetaenone I (**47**), was isolated which was proposed to be a biosynthetic precursor of **45** through the intramolecular Diels–Alder reaction of the projected intermediate **48**.[25]

Probetaenone I (**47**) was later proven to be a precursor to **45** (Scheme 10). Separate feeding experiments of *Phoma betae* with [1-^{14}C]acetate, [1,2-$^{13}C_2$]acetate, and [*S*-$^{13}CH_3$]methionine in the presence of the P450 inhibitor SD-3307D (**49**) provided labeled **47**. Subsequent feeding experiments with each labeled probetaenone I (**47**) displayed incorporation into **45** (6.02% incorporation from [1-^{14}C]acetate, 19.1% enrichment from [1,2-$^{13}C_2$]acetate, and 9.6% enrichment from [*S*-$^{13}CH_3$]methionine).[26] Synthesis of **47** through an intramolecular Diels–Alder reaction confirmed the structure and provided credence for the proposed biosynthetic pathway.[27]



Scheme 9. Proposed biosynthesis of nargenicin (**37**).[23,24d]

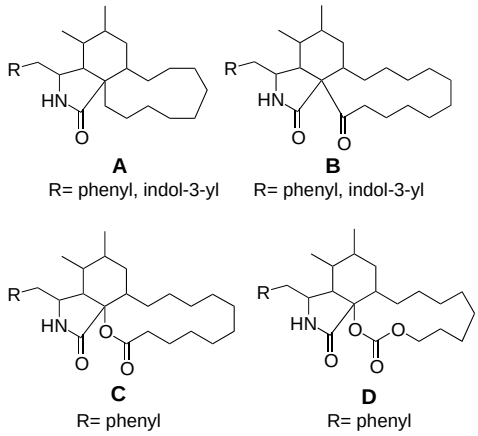


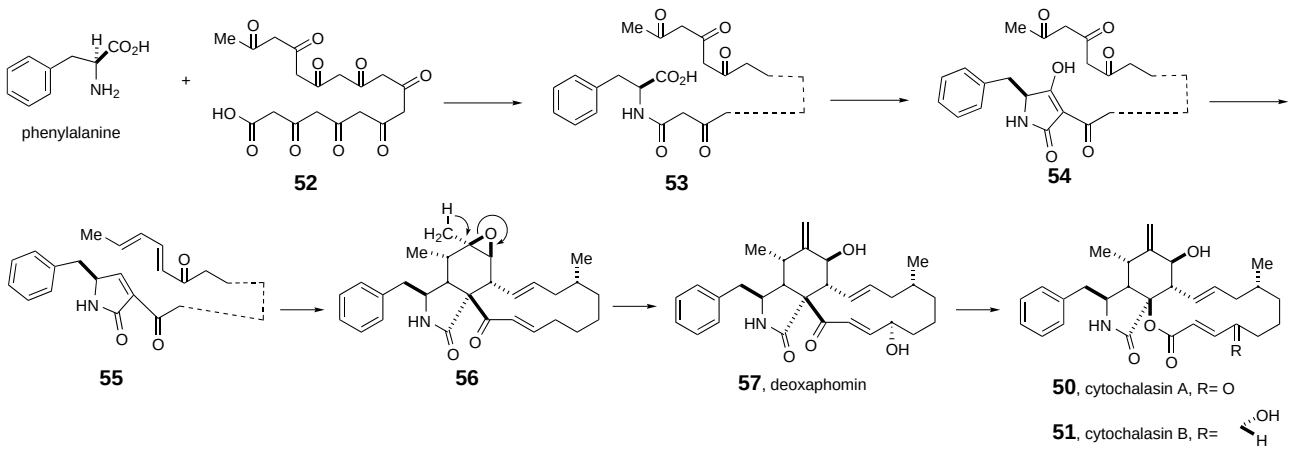
Scheme 10. Biosynthetic studies on betaenone B (**45**).[25,26]

2.2. Macrocyclic Polyketides

2.2.1. Cytochalasans

The cytochalasans are a large family of macrocyclic polyketides that possess cytostatic activity.[28] To date, approximately 60 natural products belonging to this class of mycotoxins have been isolated.[29] Structurally, the cytochalasans are characterized by a highly substituted perhydroisoindole group fused to a macrocyclic ring to give the four basic skeletal structures **A**–**D**. The majority of the macrocycles are carbocyclic, but the macrocycle can also be a lactone or cyclic carbonate.





Scheme 11. Proposed biosynthesis of cytochalasins A (**50**) and B (**51**).[28]

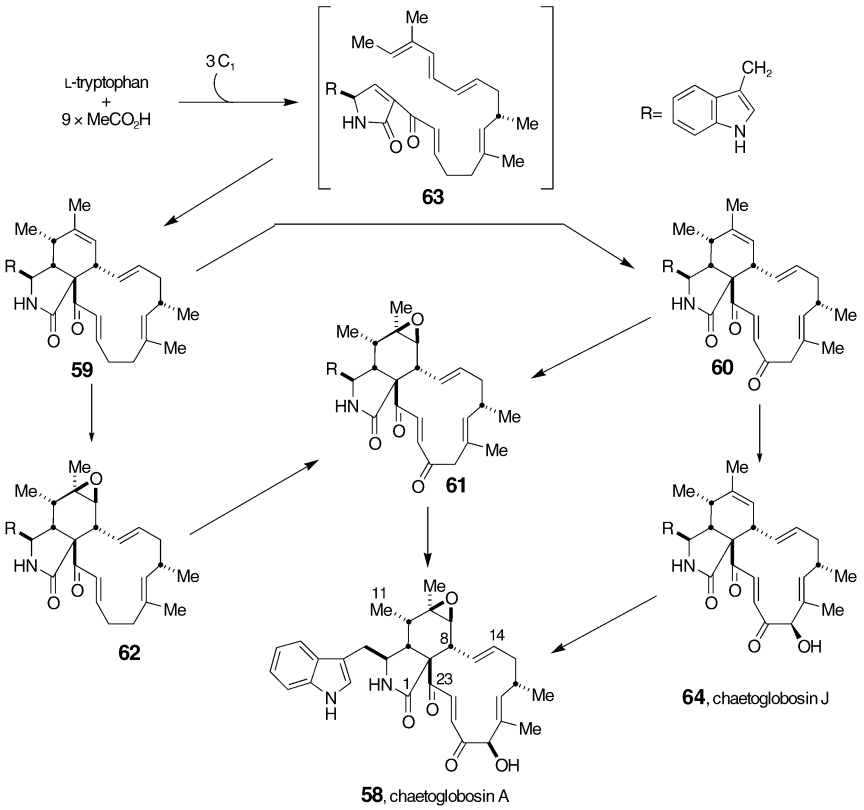
Feeding experiments have established acetate,[30,31] propionate,[30,31] methionine,[31,32] phenylalanine,[33] and tryptophan[31] as biosynthetic precursors to the cytochalasans. The perhydroisoindole group of cytochalasin A (**50**) and B (**51**) is thought to arise through an *endo*-selective intramolecular Diels–Alder reaction (Scheme 11).[28] Incorporation of deoxaphomin (**57**) into **51** indicates that oxidation to the macrolide occurs after the putative Diels–Alder cyclization and implies there is a common biosynthetic pathway for the cytochalasans.[34]

Indirect evidence for the Diels–Alder-mediated biosynthesis of the cytochalasins was obtained by feeding[35] and inhibition[36] experiments with *Chaetomium subaffine*, which produces chaetoglobosin A (**58**, Scheme 12). A feeding experiment with [1-^{13}C,2-$^{2}H_3$]acetate showed retention of the deuterium labels at C11, C8, and C14.[35] Retention of deuterium at C8 and C14 precludes formation of the perhydroisoindole and macrocycle through a proposed formation of a carbon–carbon bond in which a carbonyl group is located at C14.[28] A feeding experiment with [1-^{13}C,1-$^{18}O_2$]acetate established that the oxygen atoms at C1 and C23 originate from the acetate, while incubation in an $^{18}O_2$-enriched atmosphere displayed an upfield shift of the C6, C7, and C20 signals in the NMR spectrum.[35] An inhibition experiment with the cytochrome P450 inhibitor metapyrone led to the formation of the metabolites **59**–**62** (with **59** as the major product).[36] These results led to the biosynthetic proposal for the formation of **58** outlined in Scheme 12. An intramolecular Diels–Alder reaction of the putative hexaene **63** would provide **59**, which could then undergo a stepwise oxidation to provide **58**.

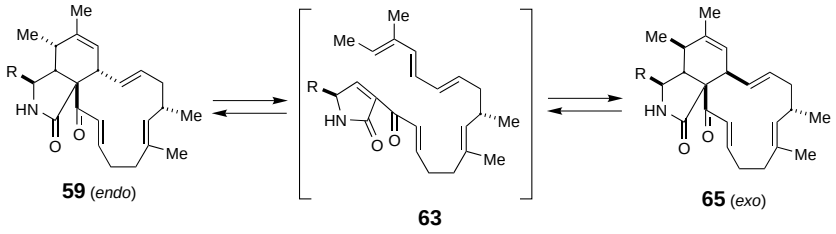
The possibility for enzymatic involvement in the proposed Diels–Alder cyclization of the cytochalasans was evidenced by the retro-Diels–Alder reaction of **59** (Scheme 13). Instead of forming the expected triene **63**, pyrolysis (180 °C, sealed tube) of **59** produced equal amounts of starting material and the diastereomer **65**.[35] The lack of stereoselectivity in the thermal Diels–Alder reaction supports the hypothesis that an enzyme preorganizes the substrate conformation to favor the *endo*-transition state in the biological system, which results in exclusive formation of **59**.

2.2.2. *Cochleamycins*

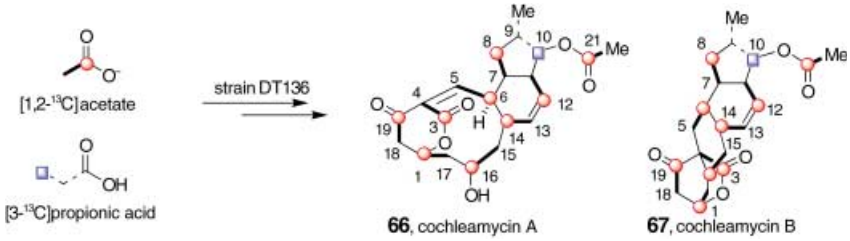
The polyketide origin of cochleamycins A (**66**) and B (**67**), produced by *Streptomyces* sp. strain DT136, was determined from feeding experiments with [1-^{13}C]acetate, [2-^{13}C]acetate,



Scheme 12. Proposed biosynthesis of chaetoglobosin A (**58**).[35]



Scheme 13. Retro-Diels–Alder reaction of the chaetoglobosin A precursor **59**.[35]



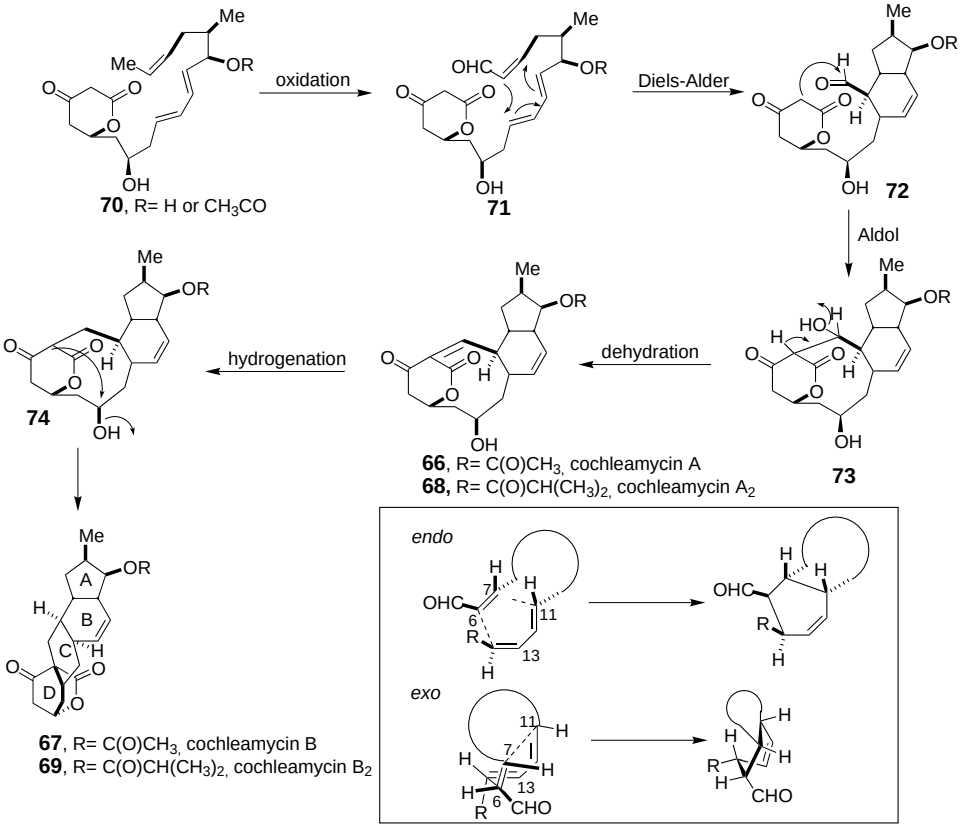
Scheme 14. Incorporation of acetate and propionic acid into cochleamycin A (**66**) and B (**67**).[37]

[1,2-$^{13}C_2$]acetate, and [3-^{13}C]propionic acid (Scheme 14).[37] Based on these results, the biosynthesis shown in Scheme 15 was proposed.

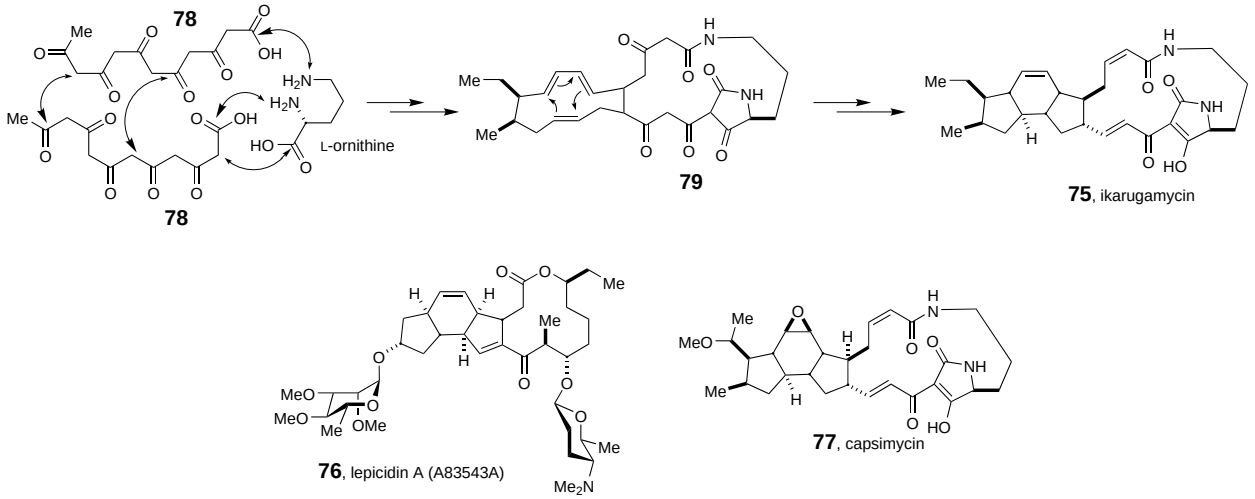
Oxidation of the allylic methyl group in the proposed intermediate **70** followed by an intramolecular Diels–Alder reaction and aldol condensation could lead to the formation of **66**. Formation of **67** is thought to arise from reductive transannular cyclization at the C4- and C16-positions of **66** accompanied by elimination of the hydroxy group at C16. The desired stereochemistry for the intramolecular Diels–Alder reaction at the AB- and BC-ring junctures can be obtained by *endo* addition of the *trans*-olefin at the C6-position to the 11-*trans*-13-*cis*-diene, or by the *exo* addition of the *trans* olefin to the 11-*cis*,13-*trans*-diene (Scheme 15, inset).

2.2.3. *Ikarugamycin*

Ikarugamycin (**75**)[38] is a member of a small family of macrocyclic antibiotics produced by *Streptomyces phaeochromogenes* var. *ikaruganensis* Sakai, which possess an unusual perhydro-*as*-indacene ring system. Other members of this family include lepicidin A (**76**, A83543A)[39] and capsimycin (**77**).[40] The structure and stereochemistry of ikarugamycin were determined by Ito and Hirata in 1972.[38a,b] They proposed that ikarugamycin was biosynthesized from two hexaacetate units **78** and L-ornithine, and that the decahydroindacene skeleton



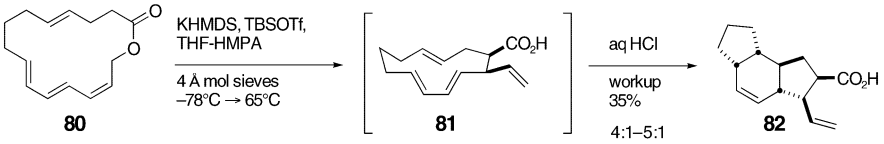
Scheme 15. Proposed biosynthetic pathway of cochleamycins A (**66**) and B (**67**).[37]



Scheme 16. Proposed biosynthesis of ikarugamycin (**75**) and structures of related natural products lepicidin A (**76**) and capsimycin (**77**).[38–40]

was synthesized through a transannular Diels–Alder reaction of **79** (Scheme 16).

Roush and Works modeled this system with a functionalized cyclododecatriene **81** (Scheme 17).[41] Stereoselective enolate Claisen ring contraction of lactone **80** provided the desired triene **81** in situ. Heating the reaction mixture to 65 °C overnight provided a mixture of the Diels–Alder cycloadducts **82** and a second diastereomer (4:1–5:1). Evans and Black pursued an alternate route for the synthesis of the decahydro-*as*-indacene skeleton of **76**, in which they utilized an intramolecular Diels–Alder reaction in conjuction with an aldol reaction.[42] Additional biosynthetic studies on this interesting family of natural products have not yet appeared.



Scheme 17. Biomimetic synthesis of the perhydro-*as*-indacene ring system of **75**. KHMDS = potassium hexamethyldisilazide, TBS = tributylsily, Tf = trifluoromethanesulfonyl, HMPA = hexamethyl phosphoramide.[41]

2.3. Perhydroindane Polyketides

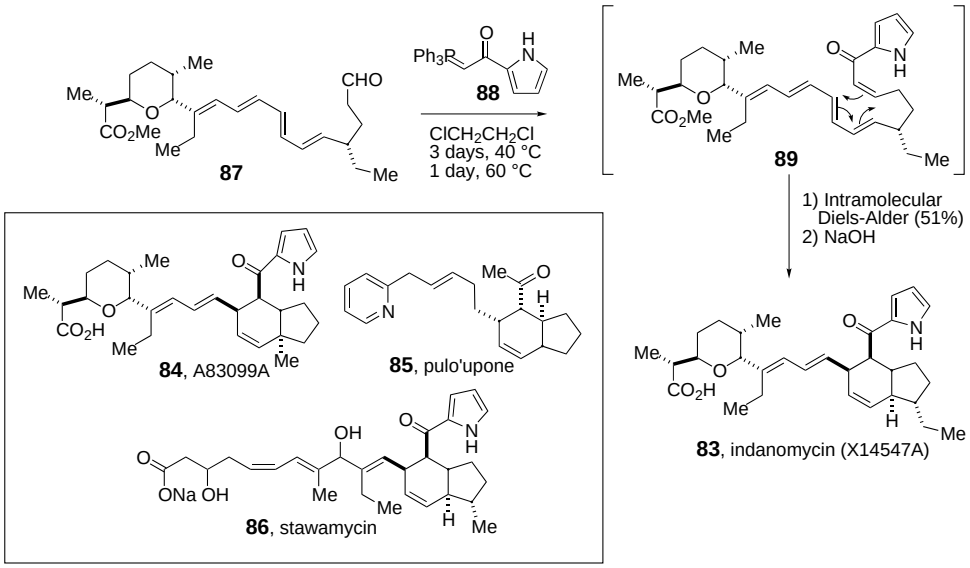
Indanomycin (**83**, X14547A)[43] is a member of a small family of polyketides produced by *Streptomyces antibioticus* which contain a perhydroindane skeleton. Other members of this family include the antibiotic A83099A (**84**), which is produced by *Streptomyces setonii*,[44] the marine natural product pulo'upone (**85**), which is produced by the mollusk *Philinopsis speciosa*,[45] and stawamycin (**86**), produced by a *Streptomyces* sp. Tularik 8349.[46] Roush et al. hypothesized that the biosynthesis of **83** might involve an intramolecular Diels–Alder reaction via a pentaene intermediate such as **89**.[47] Based on this hypothesis, the total synthesis of indanomycin was completed by using an intramolecular [4+2] cycloaddition of **89** as the key step (Scheme 18).[47c] The isomerically pure product was obtained in 51 % yield with 5 % of a mixture of *cis*-fused products and 5 % of the C10,C11-*Z* isomer.

2.4. Other Polyketides

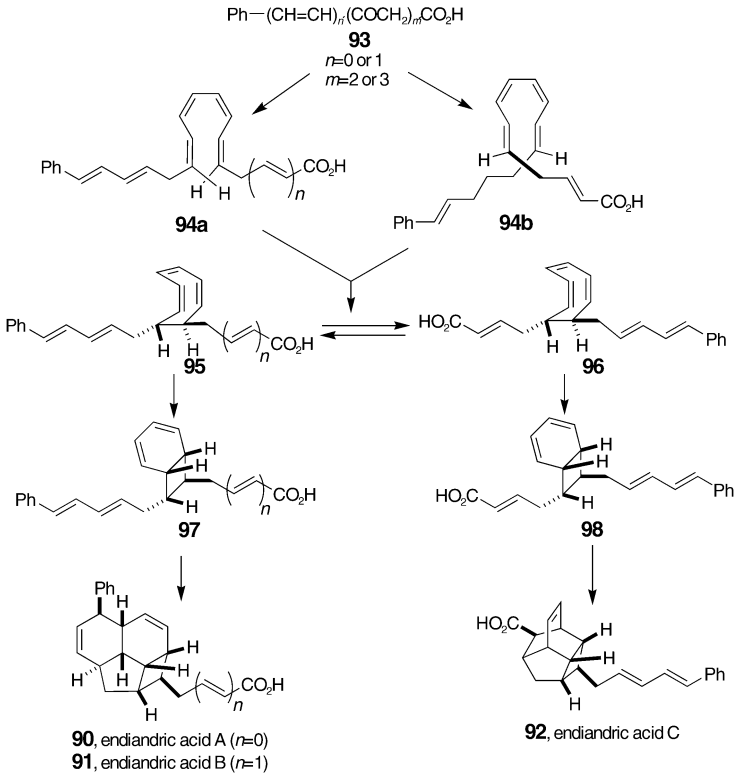
2.4.1. Endiandric Acids

The endiandric acids are isolates from the leaves of the Australian plant *Endiandra introrsa* (*Lauraceae*).[48] Since endiandric acid A (**90**) and B (**91**) occur together with endiandric acid C (**92**), and as these compounds are isolated in racemic form, Bandaranayake et al. postulated there was a unified biogenesis involving a series of electrocyclizations from an achiral precursor (Scheme 19).[49] They proposed that a polyketide of type **93** might lead to a phenylpolyene acid with a central conjugated tetraene unit. An 8π conrotatory electrocyclization of the all-*cis* tetraene **94a** or the *trans*,*cis*,*cis*,*trans* isomer **94b**, followed by a 6π disrotatory electrocyclization and finally an intramolecular Diels–Alder (4πs+2πs) cycloaddition would provide **90** and **91** or **92**, respectively.

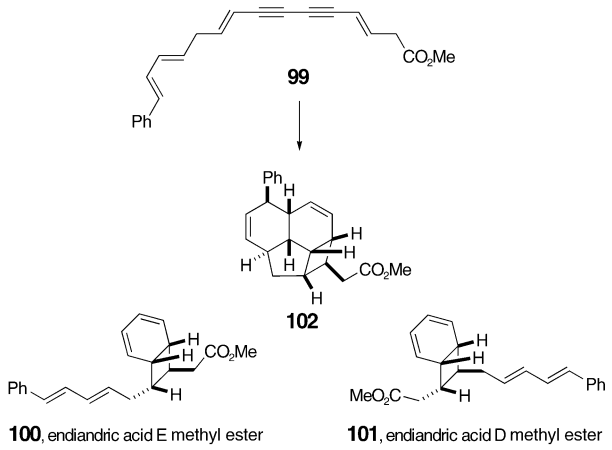
Nicolaou et al. explored the feasibility of the proposed pathway though a biomimetic synthesis. Stepwise stereocontrolled syntheses of endiandric acids A–D (**90**–**92**, **101**) were first completed to determine if the proposed sequence of events was viable.[50] Next, they completed a one-pot electrocyclic cascade reaction.[51] Hydrogenation of the acyclic precursor **99** (Scheme 20) with Lindlar's catalyst provided the methyl esters of endiandric acids E and D (**100** and **101**), while brief heating of the reaction provided the methyl ester (**102**) of endiandric acid A. An analogue of **99** with an elongated chain was used to synthesize **91** and **92** along with



Scheme 18. Biomimetic total synthesis of indanomycin (**83**).[47c] The inset shows the structures of related natural products A83099A (**84**),[44] pulo'upone (**85**),[45] and stawamycin (**86**).[46]



Scheme 19. Proposed biosynthesis of endiandric acids A–C (**90**–**92**).[49]

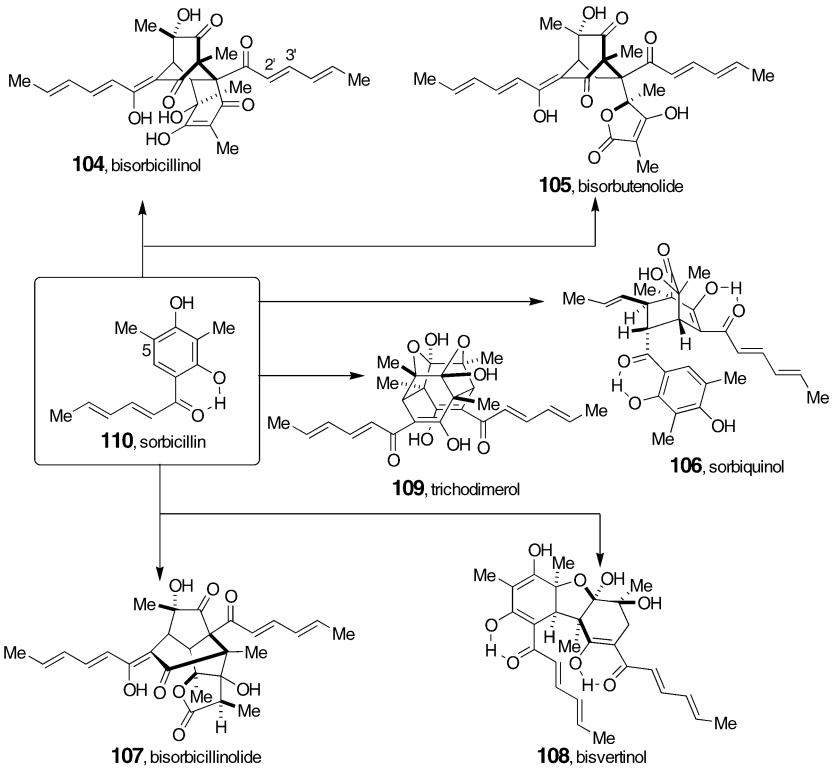


Scheme 20. Biomimetic synthesis of the endiandric acids.[51]

the unnatural endiandric acids G–F. These syntheses validate the biosynthetic hypothesis of Bandaranayake et al. and indicate that these electrocyclic reactions are not enzymatically catalyzed. In addition, the syntheses of the unnatural endiandric acids E–G may help in the identification of these compounds in the natural system.

2.4.2. *Bisorbicillinoids*

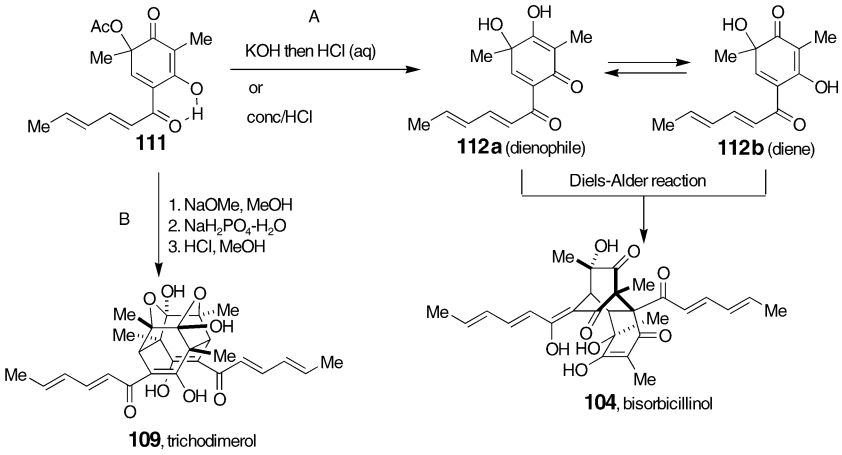
The bisorbicillinoids (**103**–**109**) are a growing family of mycotoxins that are proposed to arise from a common biosynthetic precursor, sorbicillin (**110**, Scheme 21). The 2′,3′-dihydro derivative of biscorbicillinol (**104**), the first member of the bisvertinoquinol (**103**) class of compounds to be isolated, was postulated to be a Diels–Alder adduct of two different quinols derived from the the co-metabolites **110** and 2′,3′-dihydrosorbicillin through enantioselective oxidation of C5.[52] This route was postulated because the structure of **103** is consistent with a spontaneous *endo*-selective Diels–Alder reaction. However, the variations in the sorbyl and dehydrosorbyl side chains mean that four Diels–Alder adducts are possible. Only one optically active bisvertinoquinol-type product was observed in the cultures, which



Scheme 21. Biomimetic total syntheses of bisorbicillinol mycotoxins from the same precursor **110**.[55]

suggests that chain differentiation occurs after the Diels–Alder reaction and that **103** is not an artifact of isolation.

A similar biosynthetic Diels–Alder proposal has been made by Abe, Murata, and Hirota for bisorbicillinol (**104**), which could form bisorbutenolide (**105**) through an anionic casade reaction.[53] Sorbiquinol (**106**) has also been postulated to arise from a [4+2] cycloaddition. However, for **106**, the Diels–Alder reaction would occur between the C2′–C3′ double bond of the sorbicillin (**110**) side chain as the dienophile and enantioselectively oxidized sorbicillin as the diene.[54] Alternatively, the biosyntheses of bisorbicillinolide (**107**) and trichodimerol (**109**) can be rationalized as products of an oxidation-Michael-ketalization cascade.[55]



Scheme 22. Biomimetic total syntheses of bisorbicillinol (**104**) by Nicolaou et al. (Path A)[55a,b] and of trichlorodimerol (**109**) by Barnes–Seeman and Corey (Path B).[55c]

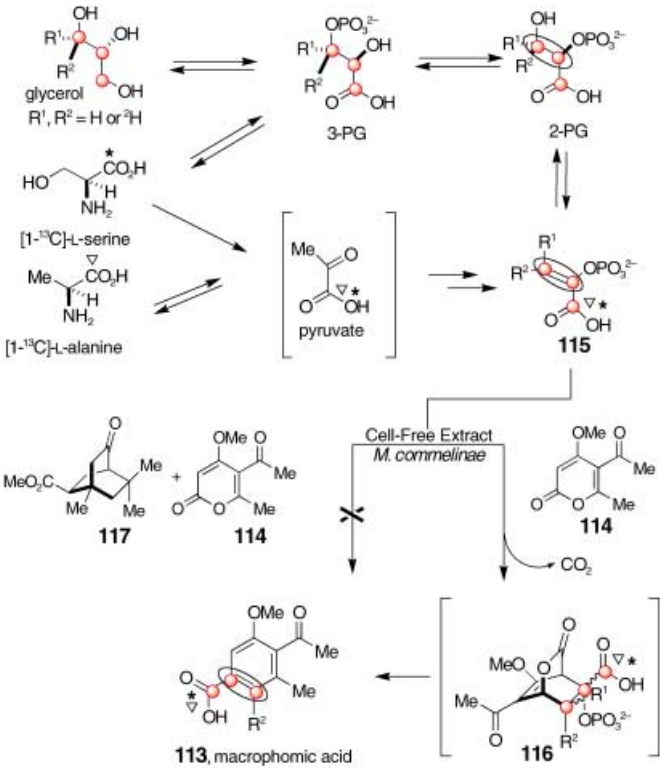
In support of the proposed biosynthetic pathways, two research groups independently and concomitantly completed the biomimetic total syntheses of bisorbicillinol (**104**) and trichodimerol (**109**, Scheme 22).[55] Nicolaou et al. reported that basic or acidic hydrolysis of the acetoxy functionality of **111** provided the quinols **112a** and **112b** which spontaneously formed the Diels–Alder cycloadduct **104** (path A in Scheme 22)[55a,b] Four stereogenic centers were created in the Diels–Alder reaction with complete regio- and diastereocontrol. Additionally, the quinol intermediate **112b** has recently been identified as a metabolite of the bisorbicillinoid producing fungus *Trichoderma* sp. USF-2690.[56]

Barnes–Seeman and Corey used the same acetoxydiene as the Nicolaou lab, but instead found that careful neutralization of the methoxide hydrolysis of **111** with NaH_2PO_4 (Path B in Scheme 22) followed by treatment with methanolic HCl provided trichodimerol (**109**) in 10% yield.[55c]

2.4.3. *Macrophomic Acid*

Macrophomic acid (**113**) is a fungal metabolite isolated from *Macrophoma commelinae*. Sakurai et al. established that macrophomic acid is derived from an unidentified C3 unit and the 2-pyrone **114** with loss of CO_2 and an unidentified C_3 unit.[57] Subsequent work on the biosynthesis of macrophomic acid revealed incorporation of [1-^{13}C]-L-alanine, [1-^{13}C]-L-serine, [U-^{13}C]glycerol, (1*RS*,2*S*)-[1-^{2}H]glycerol, and (1*RS*,2*R*)-[1-^{2}H]glycerol.[58] Based on these experiments, Oikawa et al. proposed the biosynthetic pathway outlined in Scheme 23 with phosphoenolpyruvate (**115**) as the C_3 unit. In practice, incubation of a cell-free extract of *M. commelinae* with **114** and **115** led to the enzymatic formation of **113**.[58]

The proposed biosynthetic pathway for macrophomic acid entails an inverse-electron-demand Diels–Alder reaction of **114** and the dienophile **115**. The intermediate **116** is transformed to **113** by successive retro-Diels–Alder reaction and *syn* elimination of phosphoric acid. To test this hypothesis, an analogue **117** of the putative bicyclic intermediate **116** was synthesized and incubated with the cell-free extract of



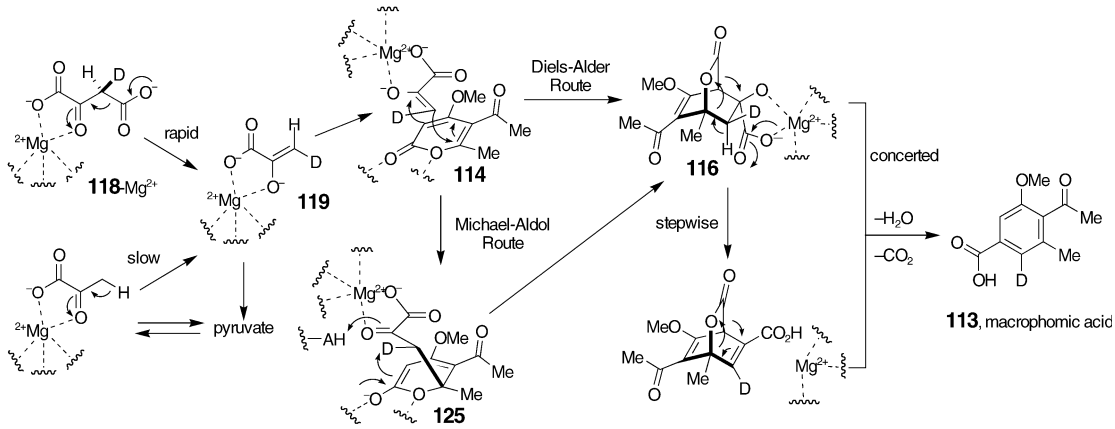
Scheme 23. Original biosynthetic proposal for macrophomic acid (**113**).[58] 3-PG = glycerin-3-phosphate.

M. commelinae, **114**, and **115**. The analogue **117** inhibited the formation of **113** (IC_{50} value 200 μM).[58]

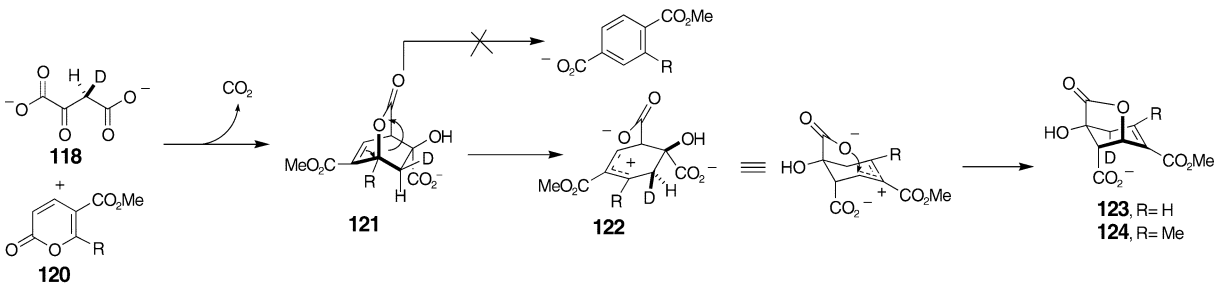
A recent re-examination of the origin of the C_3 unit led to the discovery that oxaloacetate (**118**) is a more efficient and direct precursor to **113**. The Mg^{2+}-dependent enzyme macrophomate synthase was isolated and purified by using oxaloacetate as the sole substrate for the C3 unit.[59,60] This single enzyme, a homodimer of a 36-kDa protein, was found to catalyze a five-step transformation involving two decarboxylations, two C–C bond-forming reactions, and a dehydration.[8]

Oikawa and co-workers speculate that the C3 unit might still be an enol pyruvate (**119**), the product of oxaloacetate decarboxylation. To test this, macrophomate synthase was incubated with **118** in the absence of **114** in a lactate dehydrogenase coupled assay.[8] Rapid formation of pyruvate was observed. However, in a competition experiment, **114** was found to inhibit the conversion of **118** into pyruvate. This observation indicates that the enzymatic product of oxaloacetate decarboxylation is not hydrolyzed and undergoes further reaction with **114** (Scheme 24).

Incubation of macrophomate synthase with oxaloacetate (**118**) and 2-pyrones lacking a C4 substituent, such as methyl coumalate (**120**), result in the formation of aberrant bicyclic compounds such as **123** and **124** (Scheme 25).[8,61] The location of the double bond and absence of an oxygen functionality at C5 suggests that the proposed intermediate **121** undergoes allylic rearrangement and subsequent re-lactonization. The aberrant cycloadduct **123** may be formed instead of the



Scheme 24. Revised proposal for the biosynthesis of **113**.[8]

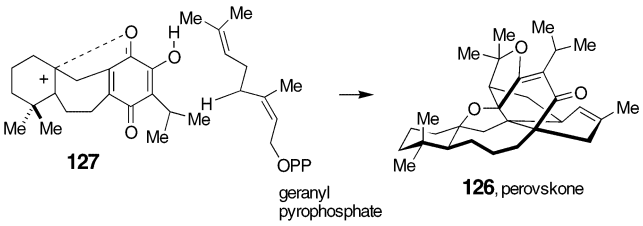


Scheme 25. Proposed mechanism for the formation of side products by a cycloaddition catalyzed by macrophomate synthase.[8]

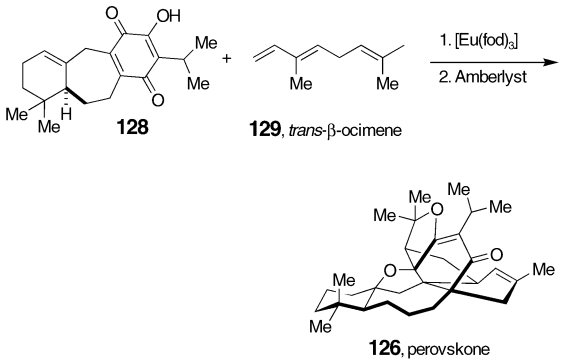
benzoate because the lack of a C4 substituent causes improper interaction between the catalytic residue and the elimination groups or because the C4 substituent interrupts attack of the carboxylate group on the carbocation. In either case, the driving force for the rearrangement is probably the release of steric strain of **121**. Deuterium labeling experiments revealed the pro-*R* position of adduct **124** is retained, which indicates that the first decarboxyation step provides the *Z* enolate.[8] The stereochemistry of the decarboxylation reaction is consistent with the known enzyme, phosphoenolpyruvate carboxylase.

Two possible routes, a stepwise Michael–aldol reaction or a concerted Diels–Alder reaction, can account for C–C bond formation by macrophomate synthase (Scheme 24).[8] In the Michael–aldol reaction, attack of the enolate on **114** would provide the first C–C bond and stabilize the negative charge on the 2-pyrone. Subsequently, the enolate could attack the newly formed carbonyl group to afford the bicyclic intermediate **116**. However, an intermediate in which only a single C–C bond has been formed, such as **125**, has not been observed for reactions catalyzed by macrophomate synthase. In the second case, a Diels–Alder cyclization may resemble an antibody-catalyzed Diels–Alder reaction. The bicyclic intermediate **116** in the macrophomate synthase catalyzed reaction could be stabilized by the groups used for recognition of the enolate and **114**.

Support for the Diels–Alder proposal was proffered from a known example of a [4+2] cycloaddition of a 2-pyrone and an equivalent of pyruvate enolate.[62] Oikawa and co-workers interpreted the high stereospecificity observed in aberrant cyclization products from the macrophomate synthase catalyzed cyclization as being consistent with a concerted mechanism. However, since the "normal" reaction products from the synthase are achiral, this is highly speculative. Nevertheless, more information will be needed to determine if the C–C bond-forming reactions of macrophomate synthase indeed arise from a concerted Diels–Alder reaction that is enzyme-mediated.



Scheme 26. Early biosynthetic proposal for perovskone (**126**).[63]



Scheme 27. Biomimetic total synthesis of perovskone (**126**).[64] fod = 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octandionate.

3. Isoprenoids

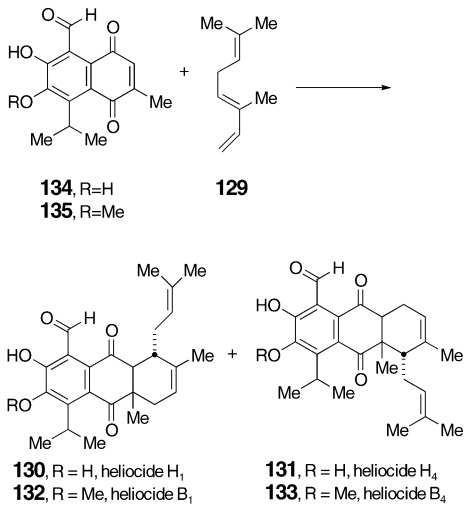
3.1. Derivatives of Myrcene and trans-β-Ocimene

3.1.1. Perovskone

The terpenes myrcene and *trans*-β-ocimene are often utilized as dienes in the construction of Diels–Alder-derived natural products. Perovskone (**126**) is a triterpene isolated from *Perovskia abrotanoides*. Initially, it was thought that **126** was constructed from an icetexone precursor **127** and geranyl pyrophosphate (Scheme 26).[63] A [4+2] cycloaddition route from **128** and *trans*-β-ocimene **129** was later proposed and this concept was used to complete a biomimetic total synthesis (Scheme 27).[64]

3.1.2. Heliocides

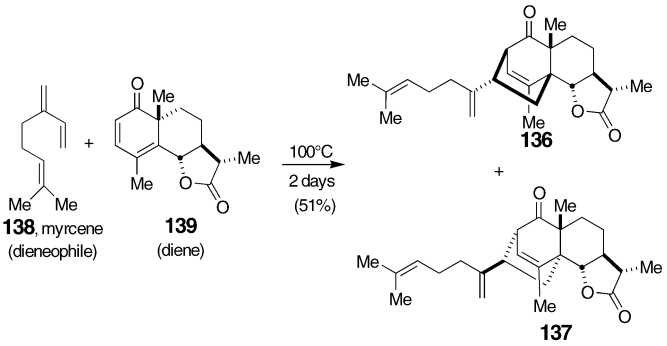
Heliocides H_1 (**130**), H_4 (**131**), B_1 (**132**), and B_4 (**133**) are proposed to be derived from *trans*-β-ocimene (**129**) and hemigossypolone (**134**) or its methyl ether derivative (**135**). Heliocides B_1 (**132**) and B_4 (**133**) were synthesized in a 3:1 ratio by a biomimetic [4+2] cycloaddition; this is the same ratio observed in the isolation of the natural products (Scheme 28).[65] While incorporation studies have not been reported, the biomimetic synthesis shown in Scheme 28 provides indirect, provocative evidence for the postulated biosynthesis.



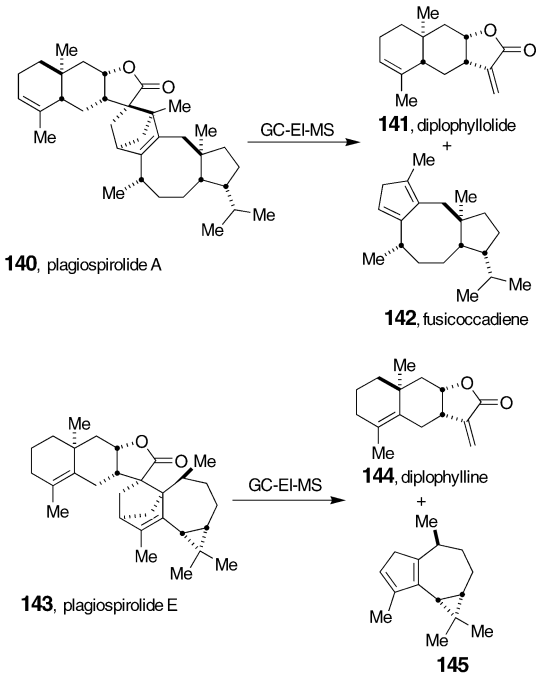
Scheme 28. Biomimetic synthesis of heliocides B_1 (**132**) and B_4 (**133**).[65]

3.1.3. Eudesmanolides

The eudesmanolides **136** and **137** were isolated from the aerial parts of *Artemisia herba-alba*.[66] They are formally derived through an inverse-electron-demand Diels–Alder reaction between myrcene (**138**) as the dienophile and **139** as the dieneone. The synthesis of **136** and **137** was accomplished in a 1:1 ratio by heating **138** and **139** to 100 °C (Scheme 29). Since the conditions required for the synthesis of **136** and **137** are so harsh, it is unlikely that they are artifacts of isolation.



Scheme 29. Biomimetic synthesis of eudesmanolide adducts **136** and **137**.[66]



Scheme 30. Retro-Diels–Alder fragmentation of plagiospirolide A (**140**) and plagiospirolide E (**143**).[67,68]

3.2. α-exo-Methylene-γ-lactones

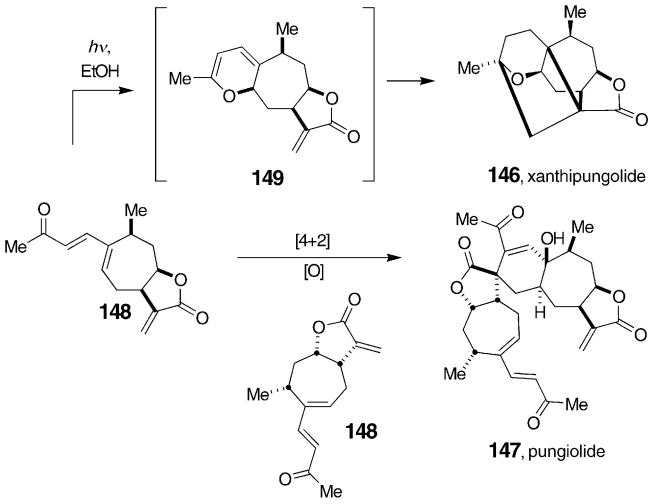
3.2.1. Plagiospirolides

GC-EIMS analysis was carried out on plagiospirolide A (**140**) to enable the structures of the spiroterpenoids isolated from the Panamanian liverwort *Plagiochila moritziana* to be determined. Diplophyllolide (**141**) and fusicoccadiene (**142**) were detected, possibly resulting from a retro-Diels–Alder reaction, and both substances were isolated from extracts of *Plagiochila moritziana*.[67] Further isolations of *Plagiochila moritziana* provided plagiospirolide E (**143**). Again, GC-EIMS provided potential retro-Diels–Alder products—diplophylline (**144**) isolated from *Plagiochila moritziana* and the diene (**145**, Scheme 30).[68]

Since a synthetic Diels–Alder route to the related triterpenes from *Helenium autumnale* required harsh conditions and gave low yields of a mixture of isomers,[69] it is unlikely that **140** is an artifact of isolation. In addition, since no other diastereomers of **140** were found in *P. moritizana* cultures, it is possible that the putative biosynthetic Diels–Alder reaction is enzymatic.

3.2.2. Xanthipungliolide, Pungiolide, and Others

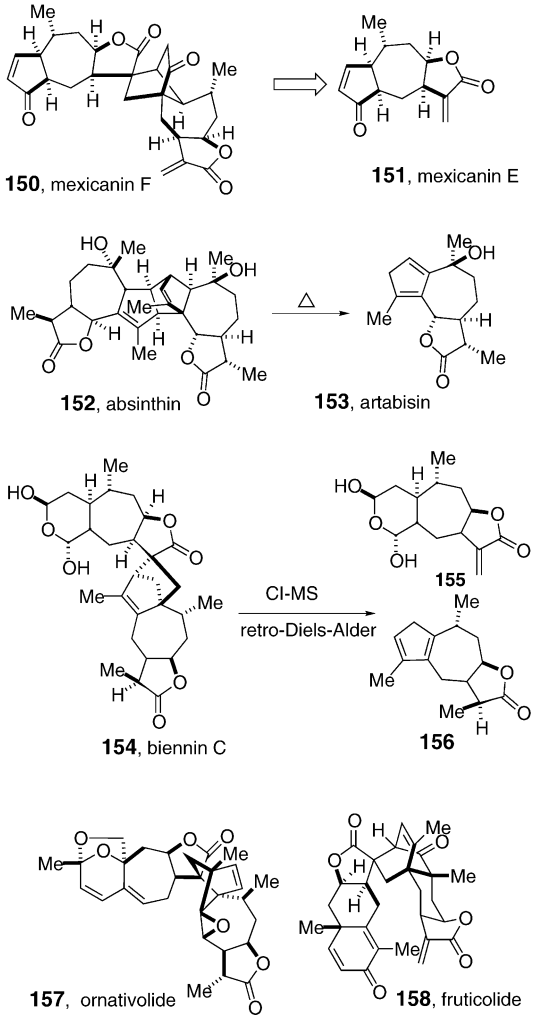
Besides the plagiospirolides, there are a number of putative Diels–Alder adducts derived from α-*exo*-methylene-γ-lactones. The species *Xanthium pungens* produces both xanthipungolide (**146**) and pungiolide (**147**).[70] Both substances were proposed to be biosynthetic derivatives of xanthanolide **148** (Scheme 31). It was proposed that an electrocyclic reaction of **148** forms **149** which is then followed by an intramolecular Diels–Alder reaction in the biogenesis of **146**. This proposal was supported by the synthesis of **146** from **148**, accomplished by irradiation of **148** in ethanol. The biosynthesis of the dimer **147** is thought to arise from an intermolecular Diels–Alder reaction of **148** followed by an oxidation.



Scheme 31. Proposed biosynthesis of xanthipungolide (**146**) and pungiolide (**147**).[70]

Mexicanin F (**150**), from *Helenium mexicanin*, is thought to arise from the co-metabolite mexicanin E (**151**).[71] Heating the dimeric sesquiterpene lactone absinthin (**152**) gives the

monomer artabisin (**153**).[72] From its fragmentation pattern in the CI mass spectrum, the biogenesis of biennin C (**154**) was proposed to occur from an intermolecular cycloaddition of the monomers **155** and **156**.[73] Ornativolide A (**157**)[74] and fruticolide (**158**)[75] could also be [4+2] cycloadducts derived from α-*exo*-methylene-γ-lactones (Scheme 32).

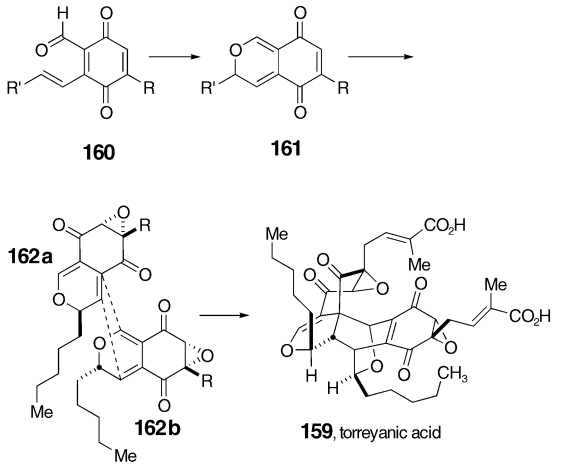


Scheme 32. Diels-Alder cycloadducts derived from α-*exo*-methylene-γ-lactones.[71–75]

3.3. Homodimer Terpenoids

3.3.1. Torreyanic Acid

Torreyanic acid (**159**) is a cytotoxin isolated from the endophytic fungus *Pestalotiopsis microspora*.[76] This substance possesses an unusual dimeric quinone structure that was postulated to arise from a Diels–Alder cycloaddition of two diastereomeric monomers. A proposed biosynthetic pathway might involve the following: a) electrocyclic ring closure of achiral **160** to form racemic **161**; b) enzymatic oxidation to generate the diastereomers **162a** and **162b**; and c) a [4+2] cycloaddition to produce **159** (Scheme 33). A

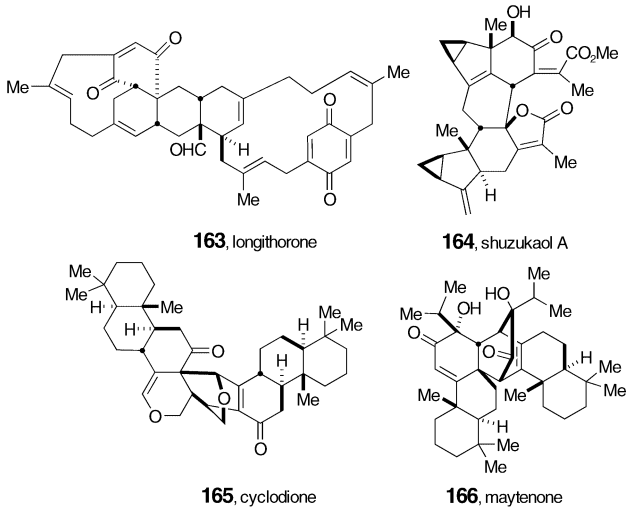


Scheme 33. Proposed biosynthesis of torreyanic acid (**159**).[76] R = $CH_2CH{=}C(CH_3)COOH$, R′ = C_5H_{11}.

biomimetic total synthesis of **159** was recently completed, which employed the [4+2] dimerization of diastereomeric monomers.[77]

3.3.2. Longithorone and Other Homodimer Terpenoids

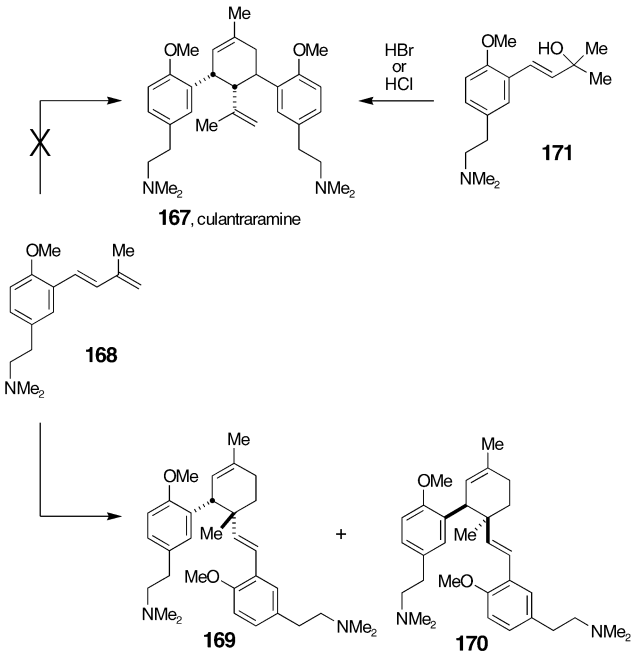
There are a number of examples of terpenoid homodimers that might arise through a [4+2] cycloaddition. A recent example of a Diels–Alder-cyclized quinone dimer is longithorone (**163**), isolated from a marine tunicate.[78] Other examples include shizukaol A (**164**),[79] cyclodione (**165**),[80] and maytenone (**166**; Scheme 34).[81]



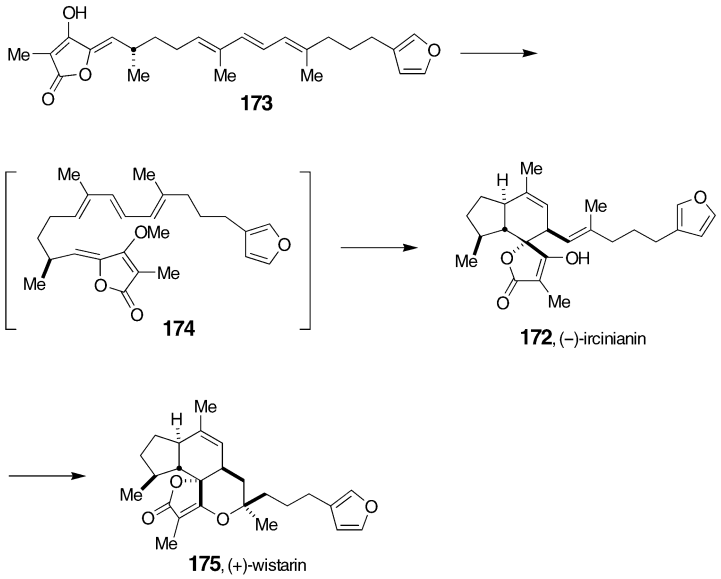
Scheme 34. Homodimeric terpenes as possible Diels–Alder cycloadducts.[78–81]

3.3.3. Culantraramine

Caution needs to be taken when considering the biosynthesis of these dimers; for example, culantraramine (**167**) could be considered as a natural Diels–Alder cycloadduct.[82] However, when the proposed precursor **168** was allowed to



Scheme 35. Biosynthetic studies on culantraramine (**167**).[82]



Scheme 36. Proposed biosynthesis of ircinianin (**172**) and wistarin (**175**).[85]

stand in xylene at room temperature for 10 days, the cycloadducts **169** and **170** were obtained, not the natural product **167** (Scheme 35). On the other hand, when **171** was treated with acid, the product **167** was formed at room temperature within 30 minutes. Thus, it seems that the biosynthesis of **167** does not occur though a "true" Diels–Alder cyclization, but perhaps through a nonsynchronous cation–diene [4+2] cycloaddition.

3.4. Other Isoprenoids

3.4.1. Ircinianin and Wistarin

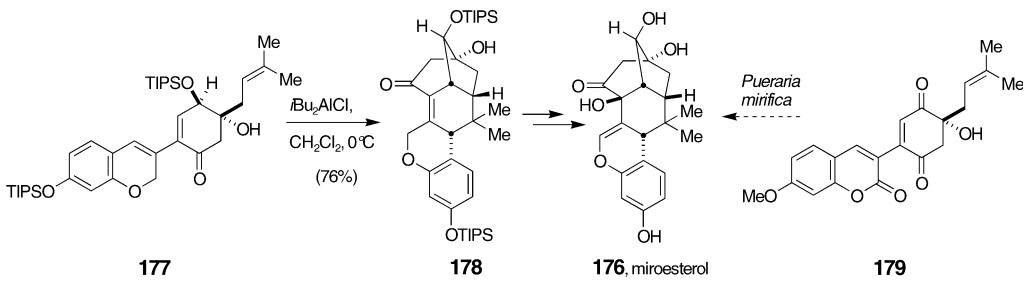
Ircinianin (**172**) is a sesterterpene isolated from the marine sponge *Ircinia wistarii*. It was postulated to arise from a [4+2] cycloaddition of the linear tetraene (**173**).[83] Both the racemate and the (−) isomer of **172** have been synthesized utilizing this approach.[84] Wistarin (**175**) is a tetracyclic isomer of tricyclic **172**. Interestingly, both the (+) and (−) isomers of **175** have been isolated, but only one enantiomer of **172** has been isolated (Scheme 36).[85] This observation could be considered as evidence that the formation of **175** is mediated by enzyme catalysis.

3.4.2. Miroesterol

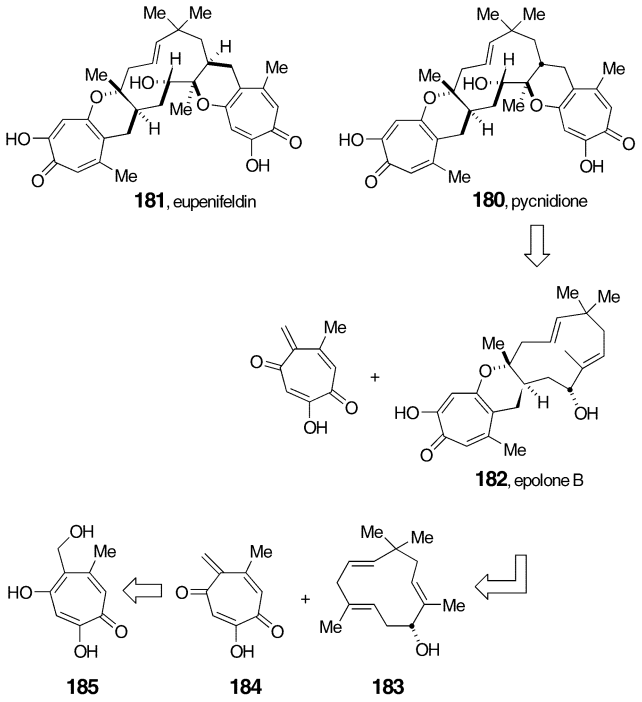
Miroesterol (**176**) is an estrogenic phenol isolated from the Thai medicinal plant *Pueraria mirifi*ca. A key step in the first total synthesis of this compound by Corey and Wu was the Lewis acid catalyzed cyclization of the tricyclic ketone **177** to form **178** (Scheme 37).[86] This reaction can be regarded as a transannular double cation-olefin cyclization or as a Lewis acid catalyzed, inverse-electron-demand intramolecular Diels–Alder reaction. Interestingly, during the course of the total synthesis of **176**, approximately 1 mg of **179** was also isolated from extracts of *P. mirifi*ca. It is thus possible that **179** is a biosynthetic precursor to **176**, in which case **176** may arise through an inverse-electron-demand Diels–Alder cycloaddition.

3.4.3. Pycnidione and Others

Pycnidione (**180**),[87] eupenifeldin (**181**),[88] and epolone B (**182**)[89] are a group of recently isolated fungal metabolites that possess identical tropolone rings attached to a sesqui-



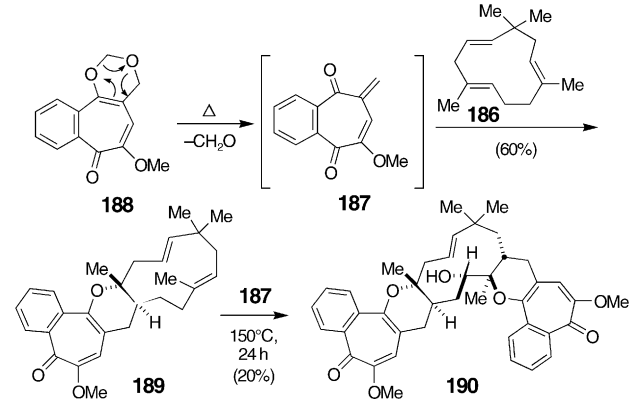
Scheme 37. Key step in the total synthesis of miroesterol (**176**).[86] TIPS = triisopropylsilyl.



Scheme 38. Proposed biosynthesis of epolone B (**182**) and pycnidione (**180**).[90]

terpene backbone. Biosynthetically, these compounds are proposed to arise from a hetero-Diels–Alder reaction of the C11-hydroxylated humulene **183** and quinone methide tropolone **184** (Scheme 38). The quinone methide **184** may in turn be generated by dehydration of the trihydroxy species **185**. Cai et al. suggested epolone B (**182**) might be a biosynthetic precursor to pycnidione (**180**) through a second hetero-Diels–Alder reaction.[89]

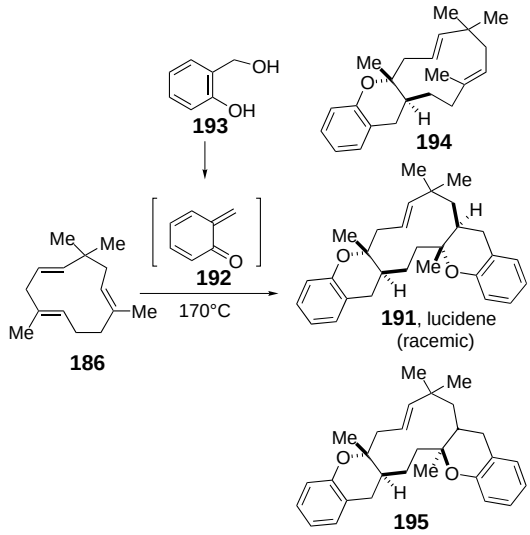
To test this hypothesis, a model study was performed using humulene (**186**) and the benzotropolone **187** (Scheme 39). The benzotropolone **187** was formed from a thermal retro-Diels–Alder reaction of **188**.[90] In situ trapping with humulene (**186**) afforded the Diels–Alder cycloadduct **189**, which is analogous to epolone B (**182**). Addition of an excess of **187** at 150 °C gave **190** as a 1:1 mixture of diastereomers. Since the



Scheme 39. Biomimetic synthesis of an analogue of epolone B **189**[90] and the pycnidione **190**.

naturally occurring Diels–Alder cycloadducts **180**–**182** are enantiomerically pure, there is a possibility that the addition of the tropolone is enzymatically catalyzed in the natural system.

A structurally similar compound, lucidene (**191**), has been isolated in racemic form from the root bark of *Uraria lucida*.[91] It has been proposed as the product of a double [4+2] cycloaddition of *o*-benzoquinone methide (**192**) and α-humulene (**186**), which is also a co-metabolite. A biomimetic synthesis provided the natural product **191** as well as the monoadduct **194** and isolucidene (**195**).[92] Unlike compounds **180**–**182**, lucidene (**191**) is not optically active, thus it most likely arises from a non-enzymatic Diels–Alder reaction (Scheme 40).



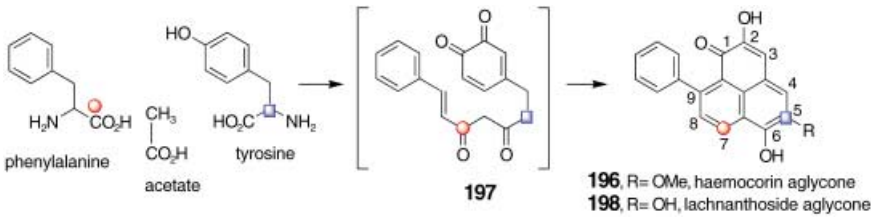
Scheme 40. Biomimetic total synthesis of lucidene (**191**).[92]

4. Phenylpropanoids

4.1. Intramolecular Cycloadducts

4.1.1. Phenylphenalenones

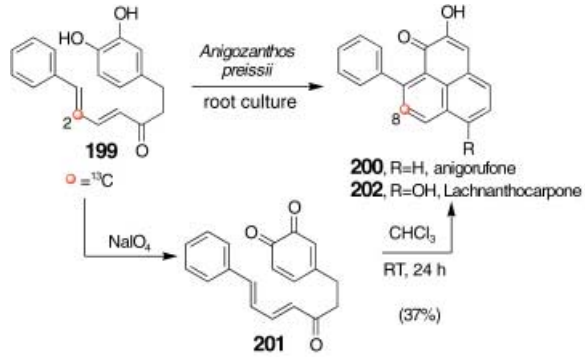
Phenylphenalenones are characteristic pigments found in the monocotyledon family *Tinctoria*. An early study on the biosynthesis of these compounds indicated that [2-^{14}C]tyrosine was incorporated specifically at C5 of the haemocorin aglycone (**196**).[93] A biosynthetic pathway was proposed (Scheme 41) that involves condensation of one molecule each of phenylalanine and tyrosine (or the metabolic equivalent) with one molecule of acetic acid and loss of a carboxy group to provide a diarylheptanoid intermediate **197**. This intermediate could then cyclize, possibly through a Diels–Alder cycloaddition, to provide the phenylphenalenone ring system. Further evidence for this pathway was the specific incorporation of [1-^{13}C]phenylalanine at C7 of the lachnanthoside aglycone (**198**).[94] Although phenylalanine and tyrosine were found to be precursors to the phenylphenalenones, other shikimate-derived phenylpropanoids, such as cinnamic



Scheme 41. Proposed biosynthesis of the phenylphenalenones.[97]

acid and coumaric acid, have also been determined to be precursors.[95,96]

It was not until 1995 that experimental evidence for the intermediacy of a diarylheptanoid in phenylphenalenone biosynthesis was obtained. Höschler and Schneider showed that the Diels–Alder precursor **199** was specifically incorporated into anigorufone (**200**) from feeding experiments with the cultured roots of *Anigozanthos preissii* (Scheme 42).[96] An earlier synthetic study showed that after oxidation with



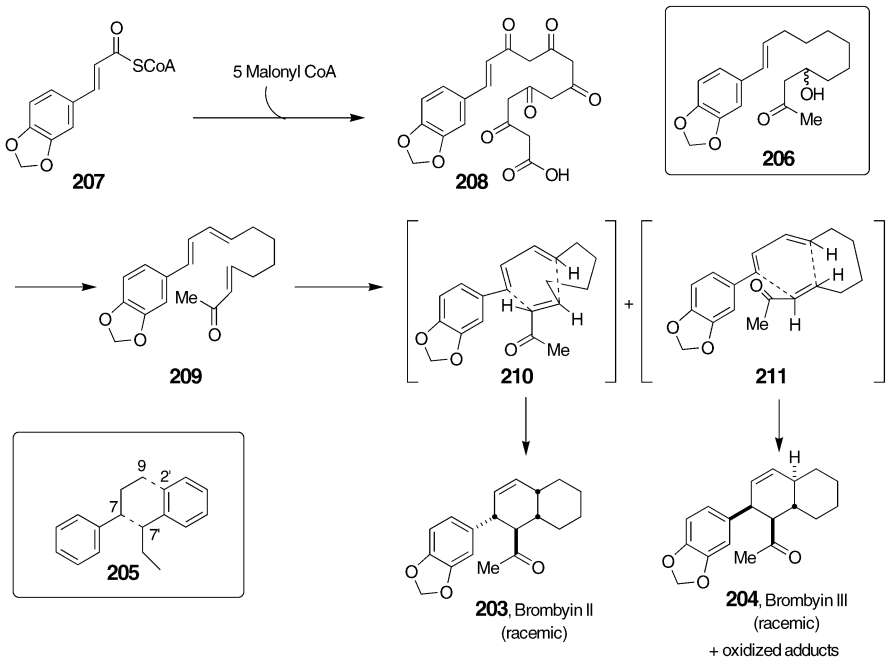
Scheme 42. Synthesis and biosynthesis of phenylphenalenones **200** and **202**.[96,97]

$NaIO_4$, unlabeled **199** was converted into lachnanthocarpone (**202**) spontaneously at room temperature through an intramolecular Diels–Alder cycloaddition.[97] Thus, the Diels–Alder cyclization leading to the phenylphenalenone ring system appears to be nonenzymatic.

4.1.2. *Brombyins*

The brombyins are novel decalin derivatives from the Australian tree *Brombya platynema* which are produced in nature in racemic form.[98] Although the metabolites **203** and **204** could be biogenetically derived from the oxidative coupling of two cinnamic acid residues (the 9-2′-, 7-7′-positions in **205**, Scheme 43), this seems unlikely because of the perhydrogenated nature of one of the six-membered rings and the lack of optical activity of the natural products.

Instead, the isolation of the intermediate **206** led to the hypothesis of the linkage of a single C_6C_3 moiety **207** with an acetate chain to give an intermediate such as **208** (Scheme 43). A spontaneous intramolecular Diels–Alder cyclization of intermediate **209** could lead to two racemic products corresponding to **203** and **204**.



Scheme 43. Proposed biosynthesis of the brombyins.[98]

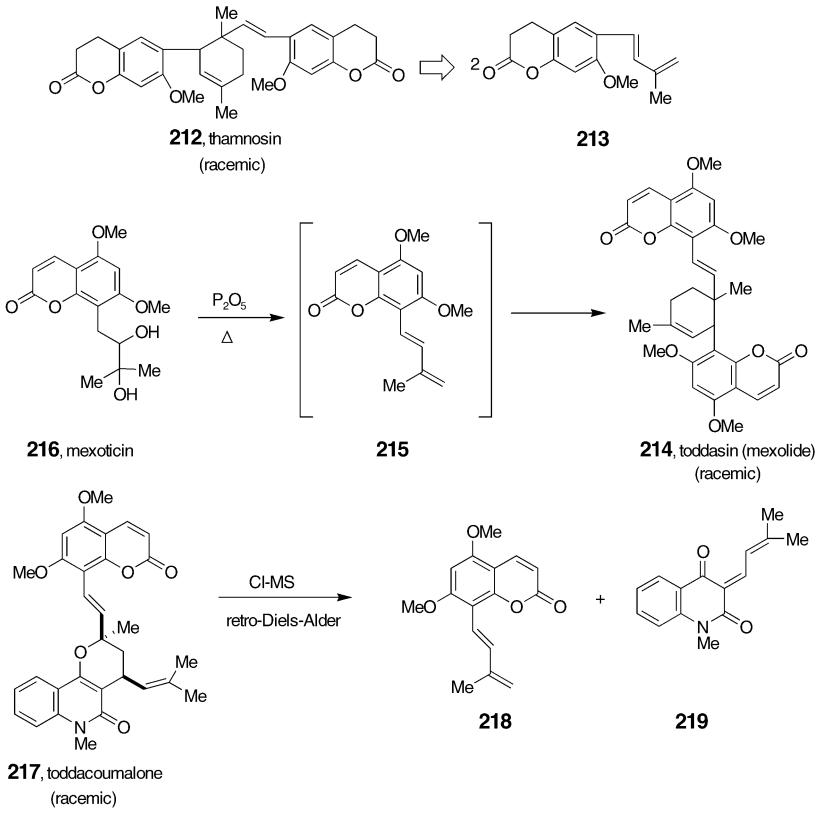
4.2. *Intermolecular Dimeric Cycloadducts*

4.2.1. *Dimeric Coumarins*

Another group of phenylpropanoid Diels–Alder adducts is represented by the dimeric coumarins (Scheme 44). The first dicoumarin discovered, thamnosin (**212**), was postulated to arise from the Diels–Alder cycloaddition of two molecules of the monomer **213**.[99] Later, the dicoumarin toddasin (**214**, mexolide) was isolated from two different sources, *Toddalia asiatica* and *Murraya exotic*a.[100,101] EI mass spectrometry of **214** led to the formation of the retro-Diels–Alder fragment **215**.[100] Treatment of mexoticin (**216**), a co-metabolite of **214** in *Murraya exotica*, with P_2O_5 in refluxing xylenes led to the formation of **214**, presumably through the dehydration product **215**.[101] Toddacoumalone (**217**) was the first example of a mixed coumarin dimer. The CI mass spectrum of **217** showed the presence of protonated ions corresponding to the coumarin **218** and the quinolone **219**.[102]

4.2.2. *Kuwanon J and Chalcomoracin*

Other phenylpropanoids that are reportedly derived from a biological Diels–Alder cyclization are the metabolites kuwanon J (**220**) and chalcomoracin (**221**) from *Morus alba* L. Selection of callus cultures from *Morus alba* that



Scheme 44. Diels–Alder cycloaddition to give the coumarin dimers and their fragmentation through a retro-Diels–Alder reaction.[99–102]

efficiently produce Diels–Alder-type adducts (ca. 100 times more of **220** and **221** than the intact plant) allowed biosynthetic studies to be performed on these compounds.[103] Feeding experiments with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate revealed that **220** and **221** are formed from the condensation of two cinnamoylpolyketide-derived skeletons **222** (see Scheme 45).

The arylbenzofuran skeleton of **220** is apparently formed from a novel type of cyclization of the cinnamoylpolyketide **222** followed by decarboxylation to give **223**.[103] Interestingly, [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]acetate were not incorporated into the prenyl groups of **220** and **221**, thus indicating that the isoprene groups are not derived through the usual mevalonate pathway.[103,104] Incorporation of [3-^{13}C]-L-phenylalanine and [3-^{13}C]-L-tyrosine into both halves of **220** and **221** suggests a common biosynthetic route to the cinnamoylpolyketide skeleton via *p*-coumarate.[105]

Addition of the *O*-methylated chalcone **229** to *Morus alba* cell cultures resulted in the formation of **230** as well as the *O*-methyl derivatives of kuwanon J (**231**) and chalcomoracin (**232**).[106] The structure of **230** indicates that prenylation occurs after aromatization of the cinnamoyl polyketide. Subsequent addition of **230** to *M. alba* cell cultures resulted in the formation of **231** and **232.** This result strongly suggests that, in the natural system, one molecule of the prenylated chalcone is recognized as the dienophile (**225**) while another prenylated chalcone, after dehydrogenation, acts as the diene (**226** or **228**). Compounds **231** and **232** are optically active and possess the same configuration as **220** and **221**, which suggests that the condensation reaction between these partners is enzymatic.[106] Close examination of the Diels–Alder-type adducts after [2-^{13}C]acetate feeding experiments with *M. alba* revealed that the adducts kuwanon V (**233**) and mulberrofuran (**234**) had a higher enrichment factor (24 and 22%, respectively) than either **220** or **221** (4 and 17%, respectively).[107] This result suggests that **220** and **221** are formed by hydroxylation of **233** and **234**.

Biotransformation experiments with *M. alba* cell cultures were also used to determine the structure of the Diels–Alder adduct artonin (**235**, Scheme 46).[108] Since **235** is only a minor metabolite from the root bark of *Artocarpus heterophyllus* (0.7 mg), structure determination was difficult. The co-occurrence of artocarpesin (**236**) in the same plant led to the proposed biogenesis and structure shown in Scheme 46. To confirm this proposal, **236** was added to a *M. alba* cell culture. Work-up provided an aberrant metabolite (8 mg, 3.5×10^{-6}%) that had an identical mass and ^{1}H NMR spectrum as **235**. These results indicate that in *M. alba* cultures, **236** reacted as a diene and **237**, which is produced in the cells, acted as a dienophile in the formation of the putative cycloadduct.

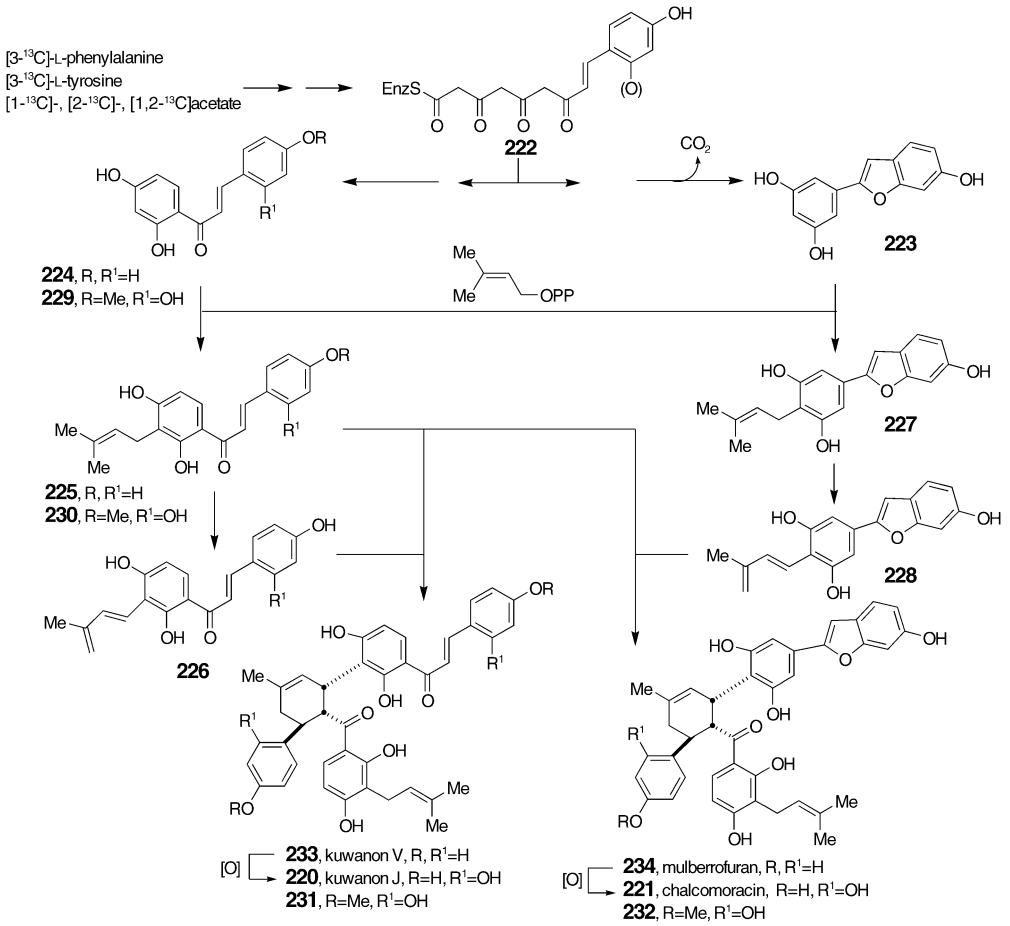
4.2.3. Asatone

Asatone (**238**) is a neolignan isolated from the stems and rhizomes of *Asarum teitonese* Hayata.[109] The base peak in the mass spectrum of **238** was observed at half the molecular weight, which is consistent with a retro-Diels–Alder fragmentation. The skeleton of **238** is comprised of two C_6C_3 units, which biosynthetically can be envisioned as enzymatically oxidized 4-allyl-2,6-dimethoxyphenol (**239**, Scheme 47). The optically inactive dienone **240** can then dimerize to provide **238** through an intermolecular Diels–Alder reaction. In fact, anodic oxidation of **239** in methanol provided a mixture of **240** and **238**. **240** was quantitatively converted into asatone (**238**) upon standing at room temperature.[110] The related lignans heterotropatrione (**241**) and isoheterotropatrione (**242**) were postulated to be the Diels–Alder adducts of **238** and **240**.[111]

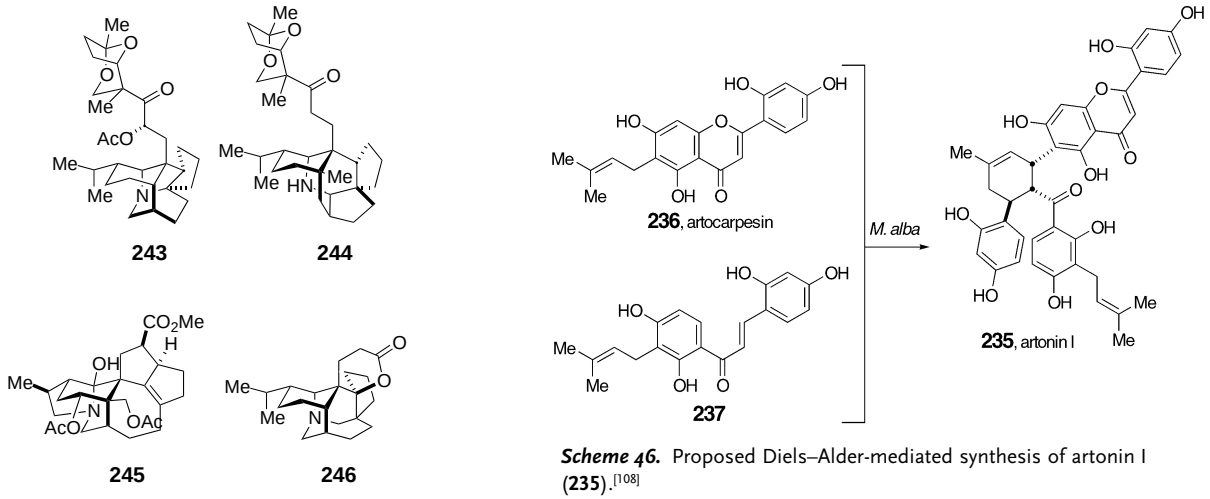
5. Alkaloids

5.1. Daphniphyllum Alkaloids

The daphniphylline alkaloids are a growing class of polycyclic natural products that were first isolated in 1909 from the deciduous tree Yuzurha (*Daphniphyllum macropodum*). The four different skeletal classes of daphniphylline alkaloids are represented by daphnipylline (**243**), secodaphniphylline (**244**), yuzurimine (**245**), and daphnilactone A



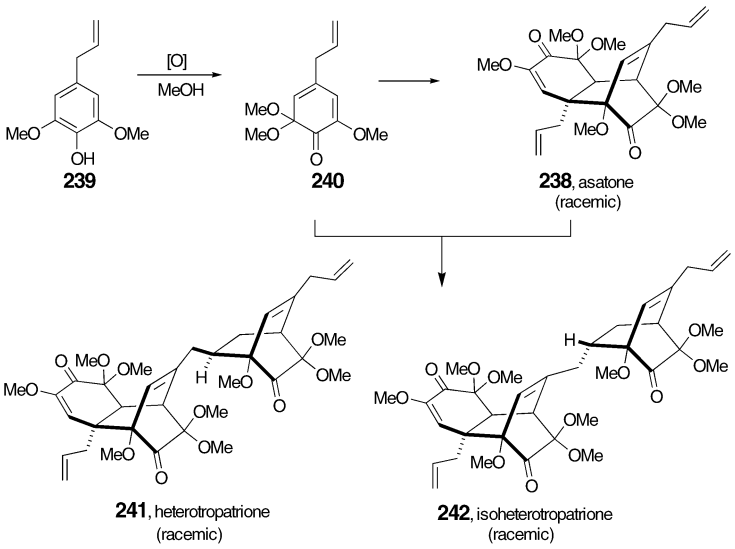
Scheme 45. Proposed biosynthesis of kuwanon J (**220**) and chalcomoracin (**221**).[106]



Scheme 46. Proposed Diels–Alder-mediated synthesis of artonin I (**235**).[108]

(**246**). Early work on the biosynthesis of daphniphylline (**243**) established its mevalonate origin via a squalene-like intermediate.[112] Later, Ruggeri and Heathcock devised a biosynthetic proposal for the construction of the complex polycyclic ring systems of the daphniphyllum alkaloids through a hetero-Diels–Alder cyclization (see Scheme 48).[113,114]

They proposed that the squalene-derived dialdehyde **247** might condense with pyridoxamine to provide the azadiene **248**. A prototropic shift in **248** would give **249**, which upon nucleophilic addition of an amine (possibly from lysine) would furnish the enamine **250**. An intramolecular enamine/enal cyclization of **250** would afford a bicyclic dihydropyran dervative **251**. A process of proton-mediated addition and elimination would then provide the dihydropyridine derivative **255**. A catalyzed intramolecular hetero-Diels–Alder reaction of **255** would give the tetrahydropyridine **256**.



Scheme 47. Biosynthesis of asatone (**238**) and the heterotropatriones **241** and **242**.[110,111]

Subsequent enelike cyclization of **256** would give the pentacyclic compound proto-daphniphylline (**257**), a proposed precursor to daphniphylline.

To explore the proposed biosynthesis, Heathcock et al. completed a biomimetic total synthesis of **257** (see Scheme 49).[115] The synthesis utilizes a one-pot procedure that was also used to synthesize five daphniphyllum alkaloids.[115,116] Oxidation of the 1,5-diol **258** to the dialdehyde **259** was accomplished through a Swern oxidation. The crude reaction mixture was treated with ammonia followed by acetic acid and ammonium acetate to provide the azadiene **261**. An intramolecular Diels–Alder reaction furnished the imine **256**. Heating the acetic acid solution of the imine then facilitated an intramolecular aza–Prins cyclization and gave **257**.

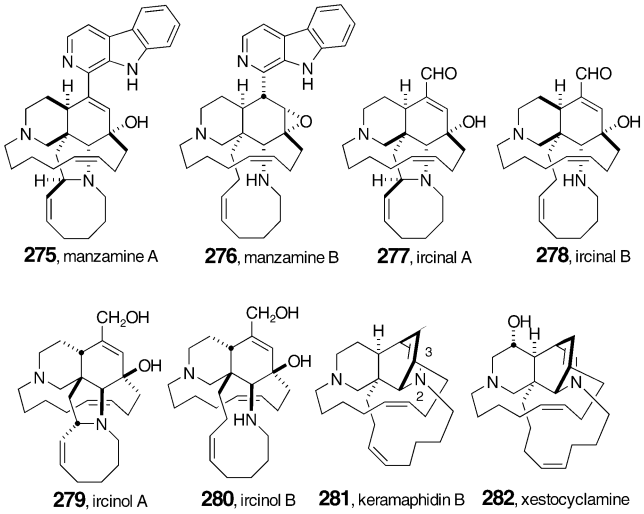
5.2. Indole Alkaloids

5.2.1. Iboga/Aspidosperma Alkaloids

The iboga and aspidosperma alkaloids are perhaps the most well-known examples of natural products potentially arising from a biosynthetic Diels–Alder reaction, and yet there is still no definitive proof for this biosynthetic pathway. By 1970, Scott had elucidated a significant portion of the biosynthetic pathway through hydroponic feeding experiments with *Vinca rosea* shoots.[117] These results along with chronological isolation studies led to the proposed biosynthetic pathway outlined in Scheme 50. The intermediacy of **271** was invoked to explain the incorporation of stemmadenine (**272**) into both catharanthine (**273**, iboga skeleton) and into vindoline (**274**, aspidosperma skeleton). Scott proposed that heterolytic ring opening and concomitant dehydration of stemmadenine (**272**) would lead to the formation of dehydrosecodine (**271**), which could undergo two possible [4+2] cycloadditions. If the 2-dihydropyridine system of **271** behaved as a dienophile, then **274** would be produced. If, on the other hand, the 2-dihydropyridine ring of **271** reacted as a diene, then **273** would be formed. However, with the exception of biomimetic syntheses,[118] no direct biosynthetic evidence for this provocative postulate has been obtained.

5.2.2. Manzamine Alkaloids

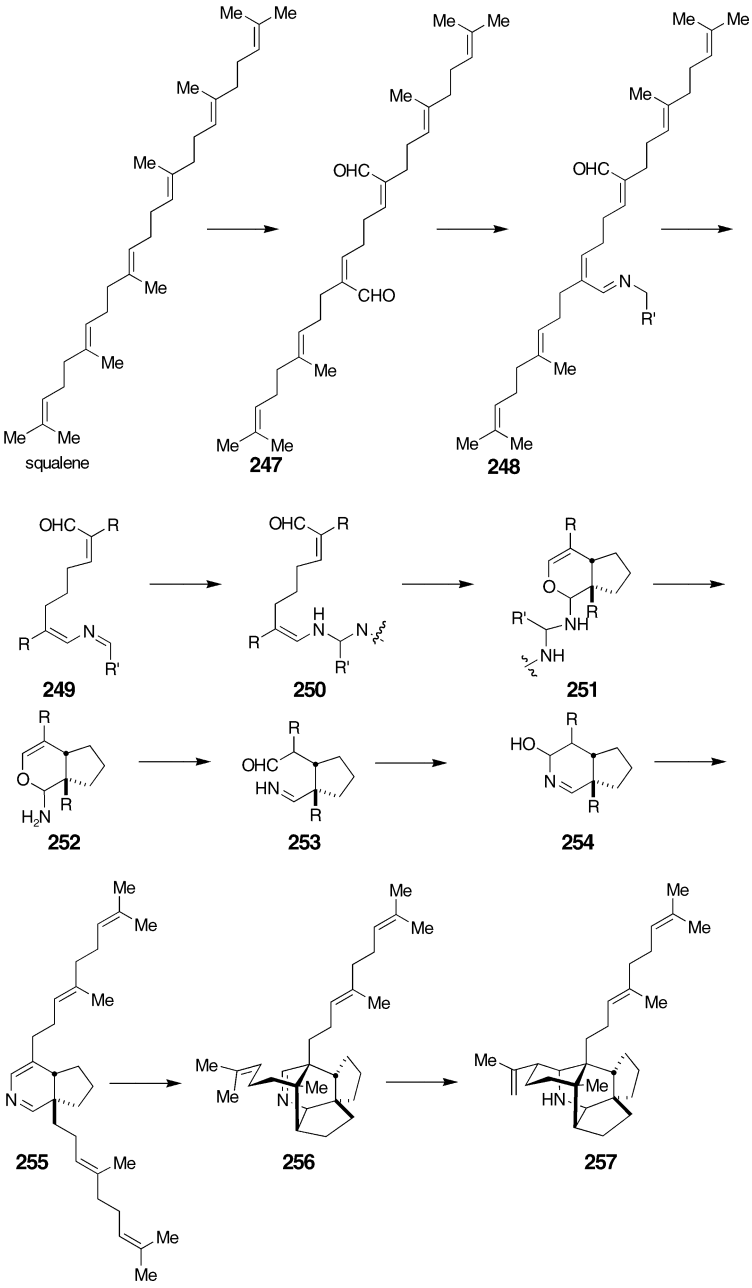
The manzamines are a growing group of cytotoxic marine sponge alkaloids that possess unusual polycyclic diamine skeletons. Among this group are manzamine A (**275**) and B (**276**),[119] ircinal A (**277**) and B (**278**),[120] ircinol A (**279**) and B



(**280**),[121] keramaphidin B (**281**),[122] xestocyclamine (**282**),[123] and ingenamine.[124] The ircinals **277** and **278** were proposed to be biosynthetic precursors to the manzamines **275** and **276**.[120] In fact, **277** was chemically transformed to **275** through a Pictet–Spengler cyclization with tryptamine and subsequent oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[120] Ircinols **279** and **280** are antipodes of the alcoholic forms of **277** and **278** and represent the first alkaloids in this class of compounds to possess the opposite absolute configuration to that of the manzamines.[119] Keramaphidin B (**281**) was also postulated as a manzamine biosynthetic precursor through formation of an ircinal from hydrolysis the N2–C3 bond of the imino form of **281**.[122]

In 1992, Baldwin and Whitehead outlined an elegant unified biogenesis for the manzamines (Scheme 51).[125] Manzamine B (**276**) was envisioned to be derived from four building blocks: ammonia, the C_{10} unit **283**, a C_3 unit (acrolein), and tryptophan. The key step for the proposed biogenesis was an intramolecular *endo*-Diels–Alder cycloaddition of a partially reduced bispyridinium species (**284**→**285**→**286**). The intermediacy of **284** was supported by the isolation of the bispyridinium macrocycles cyclostellettamines A–F from the marine sponge *Stelletta maxima*.[126] Later, Baldwin et al. expanded their biosynthetic proposal to include **281**.[127]

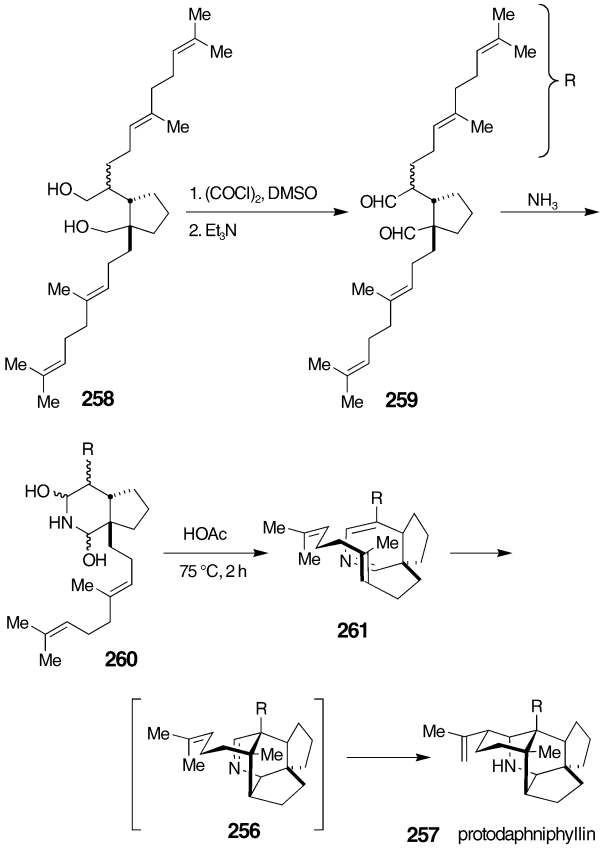
Baldwin, et al., completed a biomimetic total synthesis of keramaphidin B (**281**) by dissolving the proposed intermediate **284** in a methanol/Tris buffer solution followed by



Scheme 48. Proposed biosynthesis of the daphniphyllum alkaloids.[113,114]

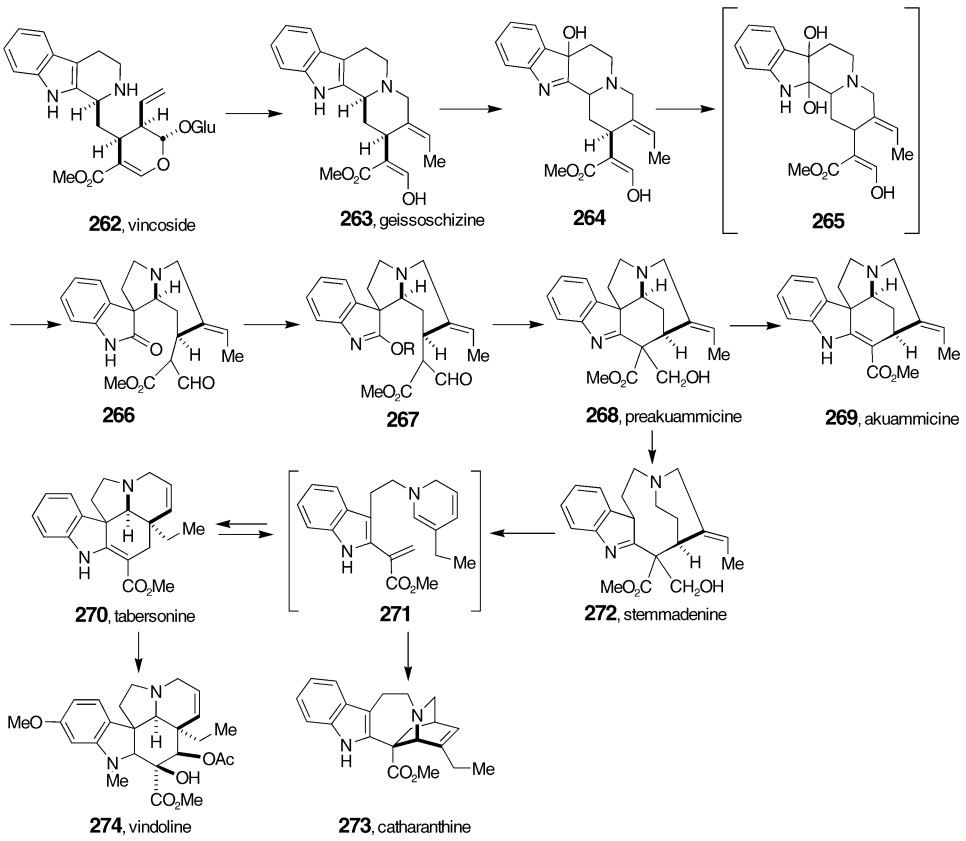
reduction with $NaBH_4$ to provide a small amount of **281** (Tris = tris(hydroxymethyl)aminomethane).[128] The low yield of **281** was rationalized as a reflection of the inclination of intermediate **285** to disproportionate. The researchers further argued that in vivo, a Diels–Alderase could limit the conformational mobility of the substrate, which would not only minimize the change in entropy toward the transition state but would also obviate the intrinsic problem of disproportionation.

Marazano and co-workers proposed an alternate route for the biosynthesis of the manzamines in 1998.[129] They suggested that the biosynthetic Diels–Alder reaction could involve a substituted 5-amino-2,4-pentadienal as the diene thereby avoiding the problem of disproportionation and implementing less ring strain during the [4+2] cycloadditon. In this proposal, the building blocks of malondialdehyde (instead of acrolein), ammonia, and an appropriate unsaturated dialdehyde would generate the macrocycle **290**. From **290**, two possible routes were proposed (Scheme 52). Analogous to the model of Baldwin et al., reductive cyclization of **290** would lead to the dihydropyridinium species **291**. Diels–Alder cycloaddition followed by reduction of **292** would directly produce the proposed manzamine precursor, aldehyde **288**. Alternatively, direct cyclization of **290** to give intermediate **293**, followed by reduction of the resulting imine functionality and cyclization would provide the enamine **294**, which presumably could lead to manzamine B (**276**). The occurrence of the proposed enamine **294** was justified by the isolation of the manzamine dimer, kauluamine, isolated from an Indonesian sponge.[130]

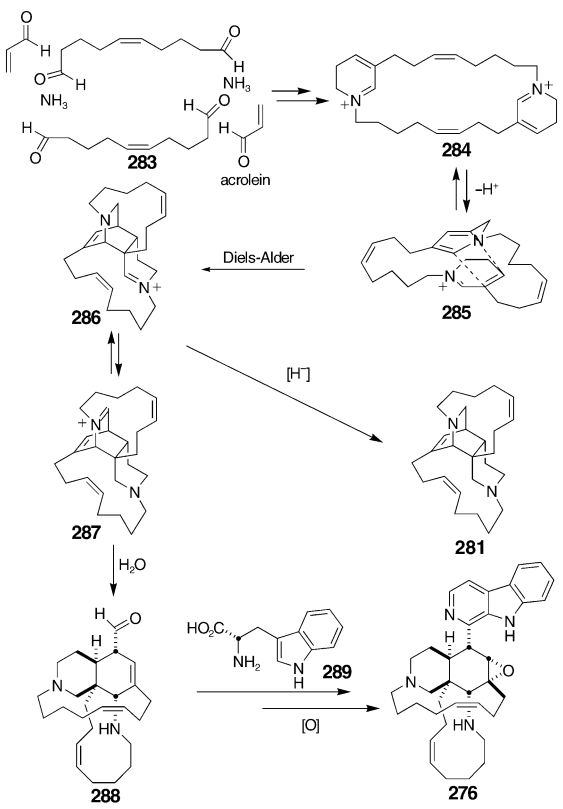


Scheme 49. Biomimetic total synthesis of protodaphniphylline (**257**).[115]

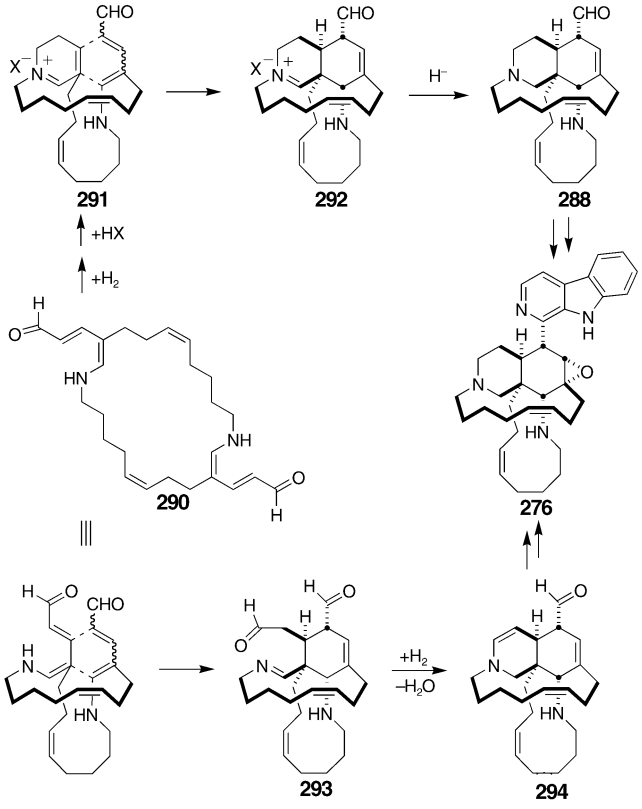
To test the first of these alternate biosynthetic routes, Marazano and co-workers performed a model synthesis as shown in Scheme 53. However, the cycloaddition of the salt **295** with **296** only produced the amino ester **297**. Presumably intramolecular hydride transfer occurred to give **298** followed by hydrolysis of the resultant imine **299**.



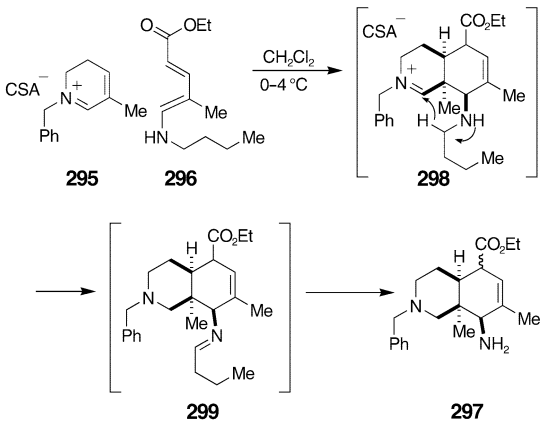
Scheme 50. Proposed biosynthesis of the iboga and aspidosperma alkaloids.[117]



Scheme 51. Proposed biosynthesis by Baldwin et al. of manzamine B (**276**).[127]



Scheme 52. Proposed biosynthesis by Marazano and co-workers of manzamine B (**276**).[129a]

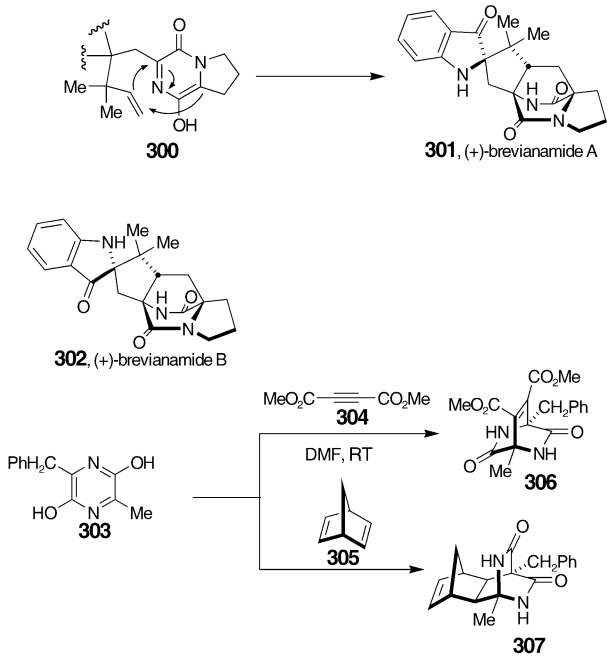


Scheme 53. Model study for the proposed biosynthetic Diels–Alder cycloaddition of the manzamine alkaloids.[129b] CSA = camphorsulfonic acid.

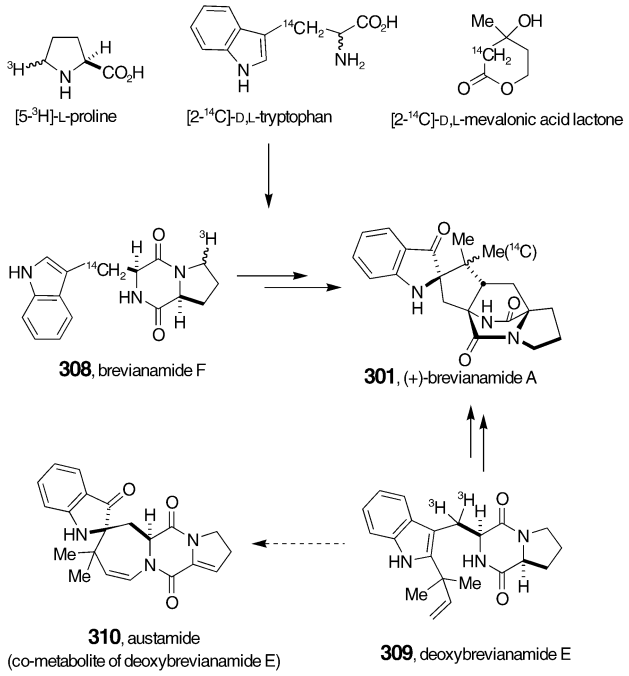
5.2.3. *Brevianamides*

Birch, Wright, and Russel first isolated the fungal metabolite brevianamide A (**301**) from *Penicillium brevicompactum* as well as other minor metabolites including brevianamide B (**302**).[131] In 1970, Sammes proposed that the unique bicyclo[2.2.2]diazaoctan ring system of brevianamide A originated from a hetero-Diels–Alder cycloaddition reaction from **300**.[132] This hypothesis was evaluated experimentally by treating the model dihydroxypyrazine **303** with dimethyl acetylenedicarboxylate (**304**) and with norbornadiene (**305**) to provide the Diels–Alder cycloadducts **306** and **307**, respectively (Scheme 54).

Early radiolabeling and feeding experiments performed by Birch and co-workers indicated that tryptophan, proline, and mevalonic acid were precursors to brevianamide A (Scheme 55).[133] This same study also revealed the incorporation of isotopically labeled brevianamide F (**308**) into **301** in significant radiochemical yield. From these results, it was postulated that the reverse-prenylated intermediate deoxybrevianamide E (**309**) was a biosynthetic precursor. Deoxybrevianamide E (**309**) was isolated from the austamide (**310**) producing fungus, *Aspergillus ustus*, but it has not been detected as a free metabolite from brevianamide-producing cultures. In 1993 Williams and co-workers performed feeding experiments with [8-$^3H_2$]-**309** that provided strong experimental evidence that **309** was a biosynthetic precursor to **301** and **302**.[134]

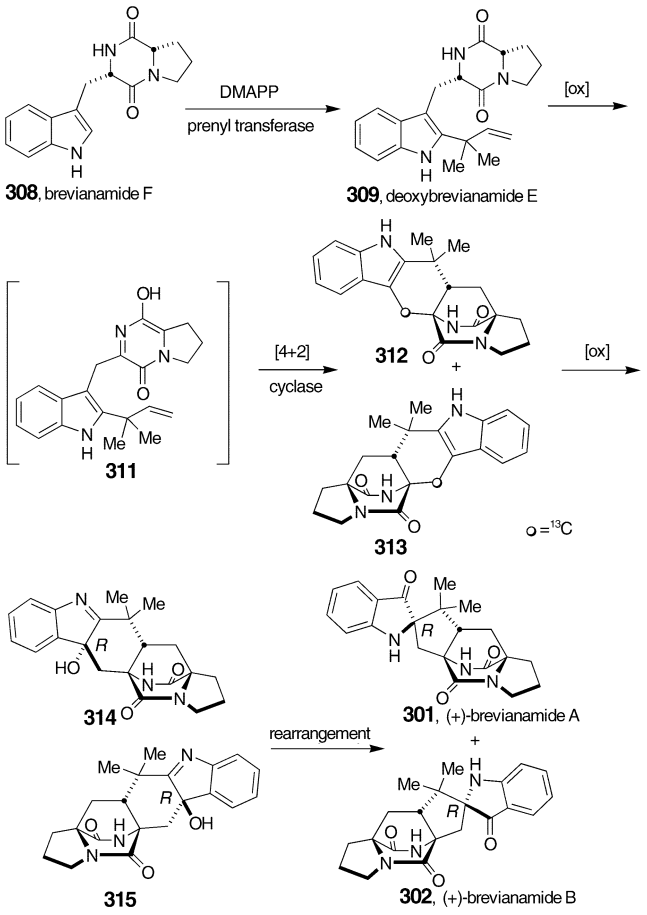


Scheme 54. Proposed and a model study by Porter and Sammes of the biosynthesis of brevianamide A (**301**).[132]



Scheme 55. Biosynthetic studies on the brevianamides.[133,134]

Williams and co-workers completed the first asymmetric total synthesis of (−)-brevianamide B (**302**), which revealed an unusual enantiomorphic relationship between **301** and **302** with respect to the bicyclo[2.2.2]diazaoctan nucleus.[135] Based on these results, the biogenesis outlined in Scheme 56 was postulated. According to this proposal, two-electron oxidation of **309** would yield the azadiene **311**, which would suffer intramolecular hetero-Diels–Alder cycloaddition to form the enantiomeric hexacyclic cycloadducts **312** and **313**. Finally, *R*-selective oxidation of the indole at the 3-position and a pinacol-type rearrangement would provide **301** and **302**. Feeding experiments were performed with *Penicillium brevicompactum* using the proposed synthetic ^{13}C-labeled intermediates **312** and **313**, yet no detectable incorporation was observed.[134] In addition, efforts to identify compounds **312** and **313** as natural metabolites of *Penicillium brevicompactum* failed to produce any evidence for these substances. Although these results do not rigorously exclude the biosynthetic

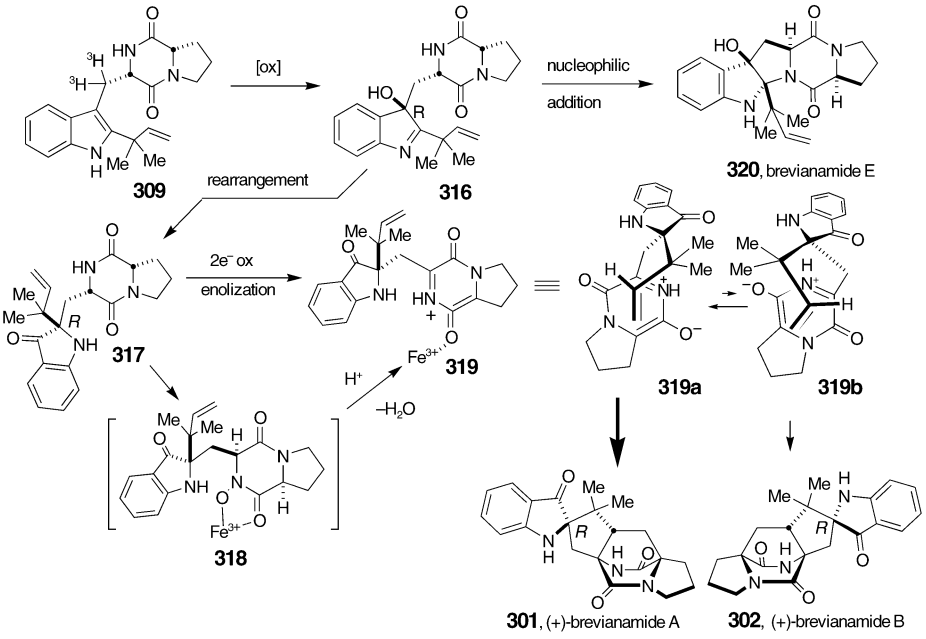


Scheme 56. Initial proposal by Williams et al. for the biosynthesis of brevianamides.[135] DMAPP = allyldimethyl diphosphonate.

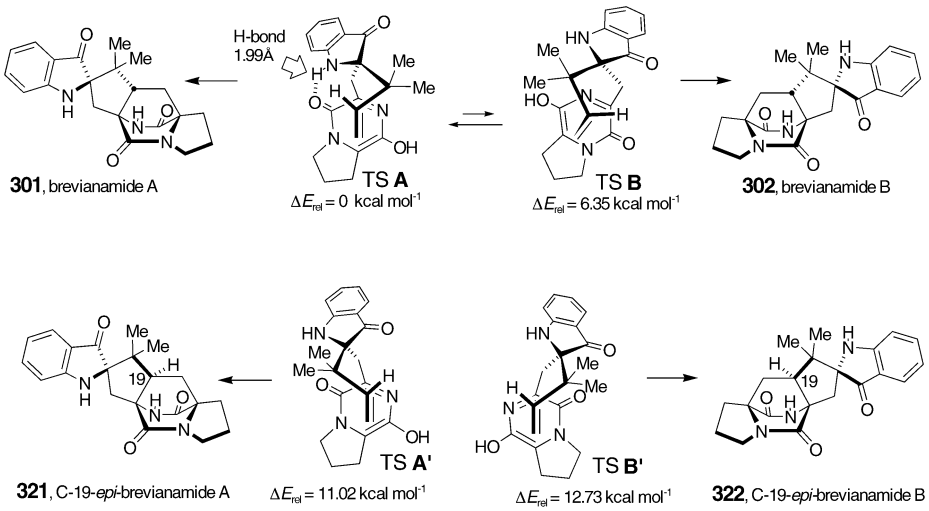
intermediacy of **312** or **313**, it led to the proposal of an alternate biosynthetic pathway as illustrated in Scheme 57.[134]

In the new proposal, **309** was envisaged to undergo oxidation to the hydroxyindolenine **316** and pinacol rearrangement to the indoxyl **317** before forming the requisite azadiene **319** through two-electron oxidation and enolization. The intermediacy of **316** was supported by the isolation of the co-metabolite brevianamide E (**320**), which was shown to be a shunt metabolite formed by irreversible nucleophilic ring closure via **316**. It was demonstrated that [8-$^3H_2$]-**309** was incorporated into **320** in high radiochemical yield (38.5% specific incorporation).[134] However, tritium-labeled **320** when re-fed to cultures of *P. brevicompactum* resulted in no significant radiochemical labeling of either brevianamide A or B, thus indicating that brevianamide E is indeed a shunt metabolite. Based on these findings, it was speculated that the hydroxyindolenine **316** could suffer two fates: 1) irreversible ring closure to brevianamide E or 2) pinacol-type rearrangement to **317** leading ultimately to brevianamides A and B. The natural products **301** and **302** would thus arise from the putative intramolecular hetero-Diels–Alder cycloaddition of the azadiene **319**. For this hypothesis to be valid, **319** must form a major conformer **319a** that results in the formation of **301** and a minor conformer **319b** produces **302**. It is also possible that **316** is oxidized to the corresponding azadiene prior to the pinacol-type rearrangement, wherein the intramolecular Diels–Alder reaction would give the two diastereomeric hydroxyindolenine precursors to **301** and **302**. This possibility has not yet been experimentally tested.

Ab initio calculations were carried out to determine if there was a conformational predilection of the azadiene **319**.[136] The potential energy barriers for the four possible diastereomeric transtion-state structures **A**, **B**, **A′**, and **B′** were calculated (6-31G*/3-21G; Scheme 58). The potential energy barrier for **A** was determined to be 38.68 kcal mol^{-1} and the potential energy barriers for **B**, **A′**, and **B′** were higher by 6.35, 11.02, and 12.73 kcal mol^{-1}, respectively. While the transition-state structures **A** and **B** lead to the observed biosynthetic products **301** and **302**, respectively, the transition-state structures **A′** and **B′** lead to diastereomers **321** and **322**, respectively, which are unknown as natural products. The positioning of the vinyl group in relation to the azadiene system may cause the difference in energy between the four transition states, and the difference between **A** and **B** was rationalized by the capacity of **A** to access an intramolecular hydrogen bond between the indoxylamino group and the oxygen atom of the amide carbonyl group. This ab initio study is consistent with the observed product ratios of **301** and **302**



Scheme 57. Revised biosynthetic proposal for the brevianamides.[134]

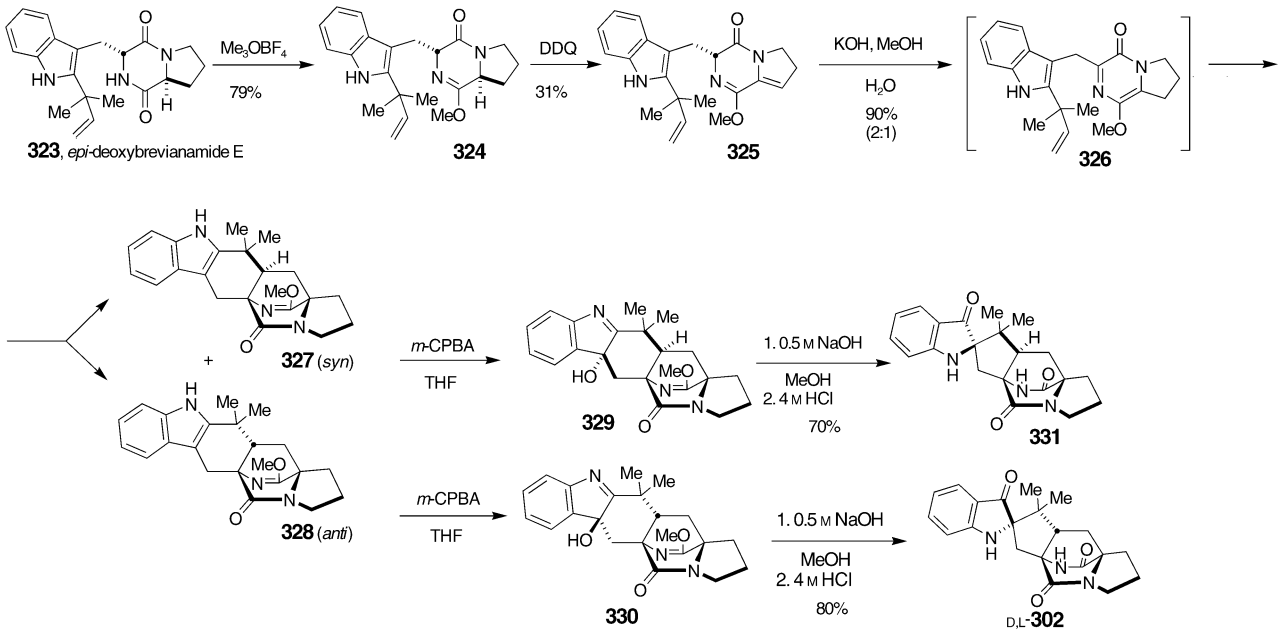


Scheme 58. Calculation of the energy barriers of the four possible transition states (TS) of the Diels–Alder cyclization of the brevianamides.[136]

and supports the proposal of an intramolecular Diels–Alder cycloaddition of the proposed key biosynthetic intermediate **319**. However, the issue of enzymatic catalysis or protein organization of the pretransition state conformers remains unsolved.

Although the biogenesis of the brevianamides was first postulated to occur through a biosynthetic Diels–Alder cycloaddition in 1970 by Porter and Sammes,[132] there was very little published data on the reactivity of such azadienic systems until recently.[137] To expore the feasibility of an intramolecular [4+2] reaction for the construction of the bicyclo[2.2.2]diazaoctan, a biomimetic total synthesis of brevianamide B (**302**) was completed by Williams and coworkers (Scheme 59).[138] Treatment of *epi*-deoxybrevianamide E (**323**) with trimethyloxonium tetrafluoroborate provided the lactim ether **324**. Subsequent oxidation with DDQ gave the azadiene **325**, which cyclized spontaneously upon tautomerization under aqueous basic conditions to furnish the racemic, diastereomeric cycloadducts **327** and **328** in a 2:1 ratio (90% combined yield) favoring the unnatural *syn* product **327**.

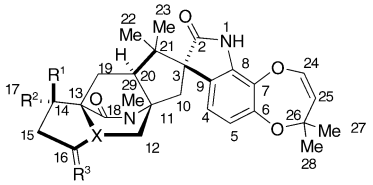
Separate diastereoselective oxidations of **327** and **328** with *m*-CPBA provided the hydroxyindolenines **329** and **330**, respectively. Finally, base-catalyzed pinacol-type rearrangements and removal of the lactim ethers provided racemic C19-*epi*-brevianamide A (**331**) and racemic brevianamide B (**302**). This study demonstrated that the core bicyclo[2.2.2]diazaoctane can indeed arise through an intramolecular Diels–Alder cyclization of the unactivated isoprene-derived dienophile and an azadiene system structurally and electronically similar to that proposed for the biosynthesis in water under ambient conditions. However, the stereoselectivity in the biosynthetic system which exclusively favors formation of the *anti* product, was not mirrored in the laboratory cyclization which favored the *syn configuration* at C19. These results raise the possibility of protein organization of the pretransition state conformations of the substrate, but leave uncertainty as to the oxidation state of the indole moiety (indole, hydroxyindolenine, or indoxyl).



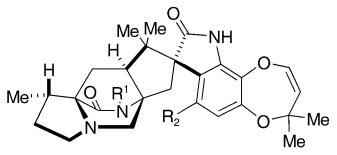
Scheme 59. Biomimetic synthesis of brevianamide B (**302**).[138] DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, *m*-CPBA = *m*-chloroperbenzoic acid.

5.2.4. *Paraherquamides*

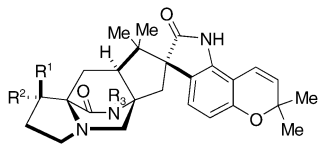
The paraherquamides (**332**–**344**) are a group of heptacyclic mycotoxins isolated from various *Penicillium* sp. and *Aspergillus* sp. Structurally, the paraherquamides are similar to the brevianamides, and vary with respect to the substitution and oxygenation in the proline and the prenylated oxindole rings. The first member of this family of spirooxindoles to be discovered was paraherquamide A (**332**), isolated in 1980 from *Penicillium paraherquei*.[139] Subsequently, paraherquamides A–G (**332**–**335, 338, 341, 342**) were isolated from *Penicillium charlesii* (*fellutanum*).[140] Paraherquamides A (**332**), E (**338**), F (**341**), and G (**342**) were also isolated from a *Penicillium* sp. (IMI332995) found in the soil of Kemer, Turkey. Several related compounds, including VM55595 (**343**), VM55596 (**336**), VM55597 (**337**), and VM55599 (**344**), were also isolated from this strain.[141] VM55599 (**344**) is the only member of the family that contains an indole ring instead of an oxindole ring. The most recent additions to the paraherquamide family are SB203105 (**339**) and SB200437 (**340**), which were isolated in 1998 from an *Aspergillus sp.* (IMI337664).[142]



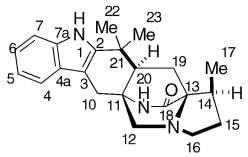
332, (–)-paraherquamide A, R^1 = OH, R^2 = Me, R^3 = H_2, X = N
333, (–)-paraherquamide B, R^1 = H, R^2 = H, R^3 = H_2, X = N
334, (–)-paraherquamide C, R^1 = R^2 = CH_2, R^3 = H_2, X = N
335, (–)-paraherquamide D, R^1 = O, R^2 = CH_2, R^3 = H_2, X = N
336, VM55596, R^1 = OH, R^2 = Me, R^3 = H_2, X = N^+-O^-
337, VM55597, R^1 = OH, R^2 = Me, R^3 = O, X = N



338, paraherquamide E (VM54159), R^1 = Me, R^2 = H
339, SB203105, R^1 = Me, R^2 = OH
340, SB200437, R^1 = H, R^2 = H



341, paraherquamide F (VM55594), R^1 = H, R^2 = Me, R^3 = Me
342, paraherquamide G (VM54158), R^1 = OH, R^2 = Me, R^3 = Me
343, VM55595, R^1 = H, R^2 = Me, R^3 = H



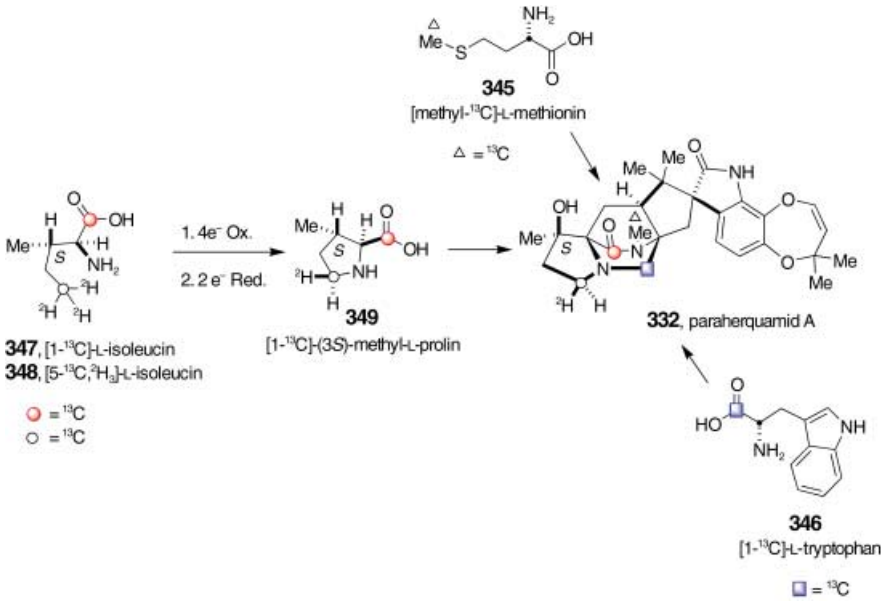
344, VM55599

Through feeding experiments, Williams and co-workers determined that L-methionine, L-tryptophan, and L-isoleucine were the proteinogenic amino acid building blocks, which give rise to paraherquamide A (**332**, Scheme 60).[143] Incorporation of isotopically labeled L-isoleucine revealed that it was the source of the unusual nonproteinogenic amino acid β-methylproline (which is later converted into β-methyl-β-hydroxyproline) through a four-electron oxidation/two-electron reduction sequence with retention of the *pro-S* hydrogen atom at C16 (paraherquamide numbering).[143,144] Additional feeding experiments with [$^{13}C_2$]acetate and [$^{13}C_6$]glucose revealed the mevalonate origin of the isoprene moieties (C19–C23 and C24–C28) of **332**.[145]

Interestingly, the feeding experiments indicated that *Penicillium fellutanum* constructs each isoprene-derived quaternary center in **332** by disparate stereochemical pathways. The quaternary center in the dioxepin ring (C24–C28) was found to be formed in a completely stereospecific manner. However, scrambling of the ^{13}C label into both of the *gem*-dimethyl groups was observed in the quaternary center of the bicyclo[2.2.2]diazaoctane portion (C19–C23), thus indicating that the stereochemical integrity of the acetate-derived ^{13}C label in the dimethylallyl pyrophosphate moiety was sacrificed in the construction of this quaternary center.

Williams and co-workers proposed that a "reverse" prenyltransferase catalyzes a nonface-selective intermolecular S_N2'-type addition of the dimethylallyl pyrophosphate moiety to the 2-position of the tryptophan-derived indole ring, thus scrambling the *Z*-^{13}C label in the isoprene moiety (Scheme 61). Analogous to the proposed biosynthesis for the brevianamides, it was anticipated that this reverse-prenylated moiety **351** would undergo a [4+2] cycloaddition reaction across the α carbon atoms of L-tryptophan and (3*S*)-methyl-L-proline and eventually lead to paraherquamide A (**332**).

As in the case of the brevianamides, it was unclear whether oxidation of the tryptophan moiety occurred before or after the putative hetero-Diels–Alder reaction. Isolation of the hexacyclic indolic metabolite VM55599 (**344**) from the paraherquamide-producing *Penicillium* sp. (IMI332995) suggested that the tryptophan oxidations occur after the construction of the bicyclo[2.2.2] ring system. However, the relative stereochemistry of C14 and C20 in **344**, as determined by Everett and co-workers by extensive ^1H NMR NOE data, was found to be opposite to that found in **332**.[141] If the β-methylproline ring of **344** was derived, as in the case of **322**, from (*S*)-isoleucine, then cycloaddition would have to occur from the seemingly more hindered face of the azadiene system (**B** in Scheme 62) with the methyl group of the β-methylproline ring *syn* to the bridging isoprene moiety. If, on the other hand, cycloaddition occurs with the methyl group of the β-methylproline ring *anti* to the bridging isoprene unit (**A** in Scheme 62), then an intermediate would be formed which could lead to all of the paraherquamides containing a β-methylproline moiety. Indirect support for the hypothesis that

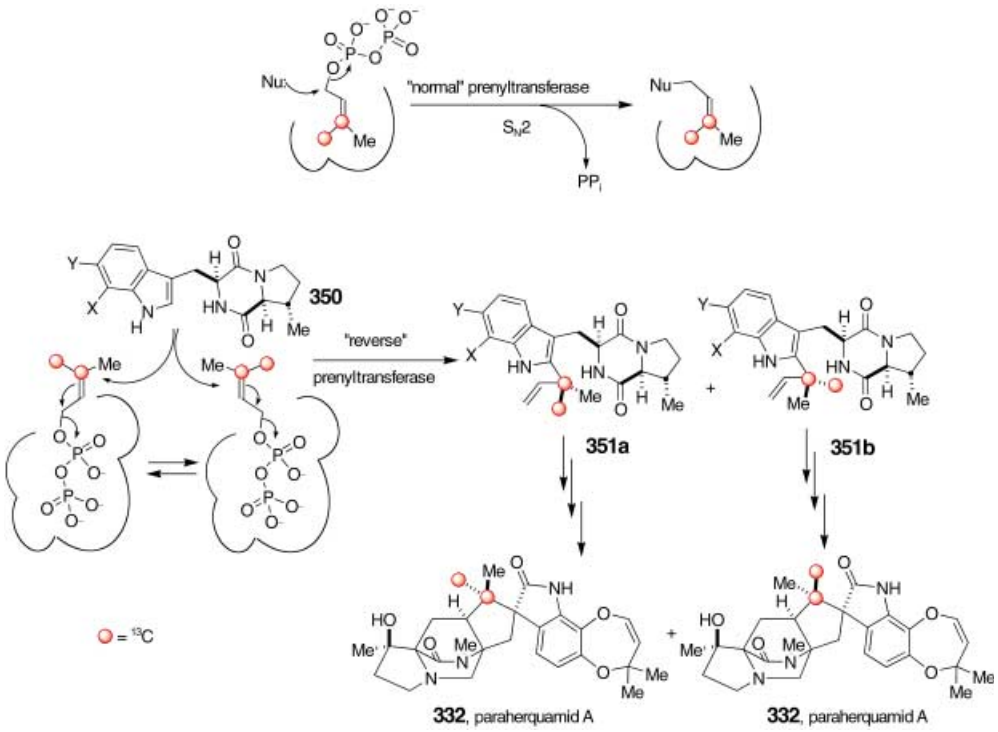


Scheme 60. Incorporation of amino acids into paraherquamide A (**332**).[143]

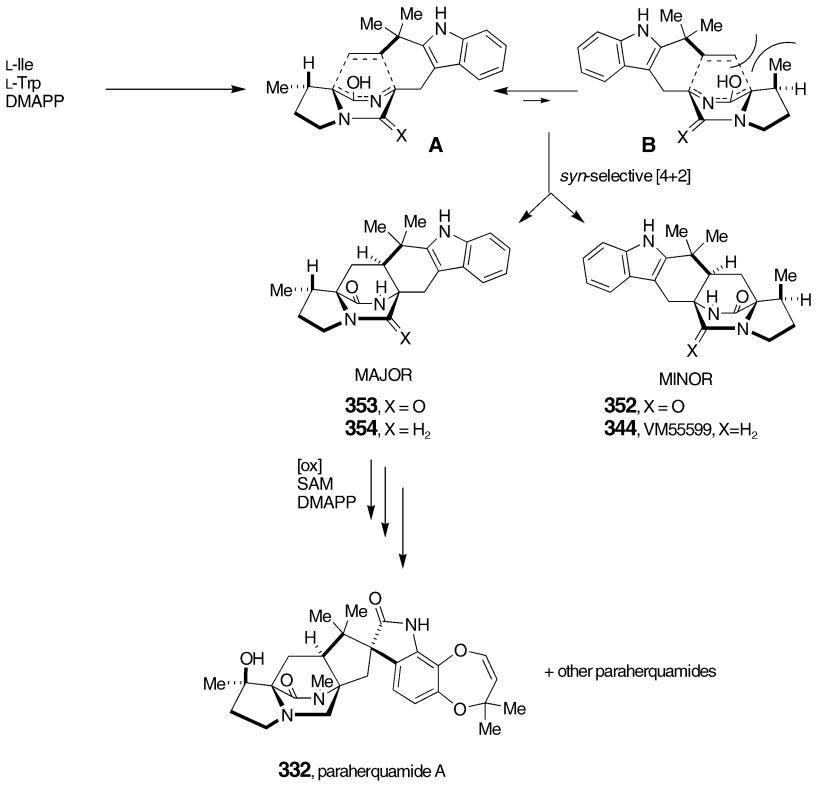
the major cycloaddition pathway passes through conformer **A** was based on the small amount of **344** isolated from *Penicillium* sp. (IMI332995) cultures (**344**:**332** ca. 1:600).[146]

To determine if **344** was an intermediate in the biosynthesis of **332**, as initially postulated by Everett and co-workers, Williams and co-workers prepared [$^{13}C_2$]-(±)-**344**, the oxidized form of [$^{13}C_2$]-(±)-**352** as well as the alleged ^{13}C-labeled paraherquamide progenitors **353** and **354**, and examined these substances as potential pathway metabolites in *Penicillium fellutanum*.[147] Syntheses of these labeled substrates were accomplished through a biomimetic intramolecular Diels–Alder cycloaddition strategy as illustrated in Scheme 63.[146,147] Interestingly, the laboratory cycloaddition slightly favored (1.47:1) the approach of the dienophile from the same face as the methyl group in the β-methyl proline ring, which leads to the relative stereochemistry of **344**. Additionally, the ratio of the *anti* to *syn* isomers at C20 was approximately 1:2.4 in favor of the natural configuration. As in the related cycloaddition reaction of **326** (Scheme 59) the poor facial bias of the laboratory Diels–Alder reaction strongly hints that protein organization of the pretransition state conformers might be operative in the biosynthesis of paraherquamide A.

Feeding experiments with the ^{13}C-labeled cycloadducts revealed no incorporation in (±)-**344**, its oxidized counterpart (±)-**352**, or the diketopiperazine (±)-**353** (Scheme 64). However, significant incorporation of (±)-**354**, the C14 epimer of **344**, into paraherquamide A (**332**) was observed by ^{13}C NMR spectroscopy and from analysis of the electrospray mass spectrum (0.72% incorporation). These results indicate that the formation of the bicyclo[2.2.2]diazaoctane ring system occurs at the stage of the nonoxidized



Scheme 61. Proposed mechanism for the attachment of the C19–C23 segment in paraherquamide A (**332**).[145]

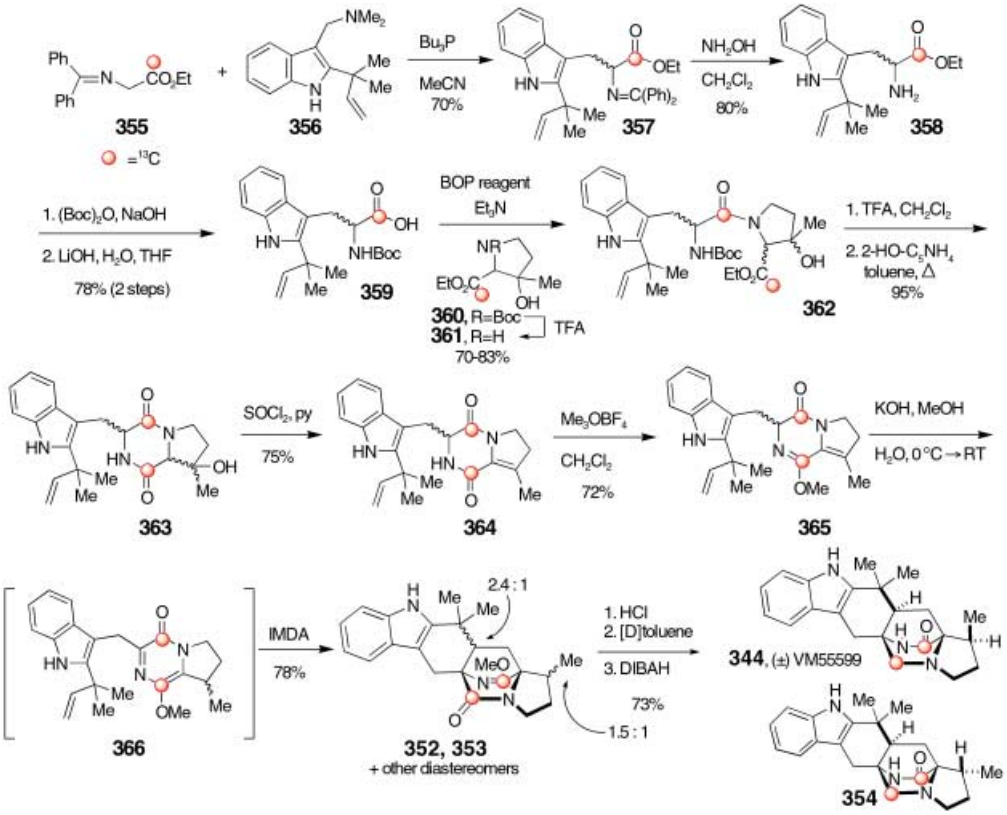


Scheme 62. Proposed unified biogenesis of paraherquamide A (**332**) and VM55599 (**344**).[146]

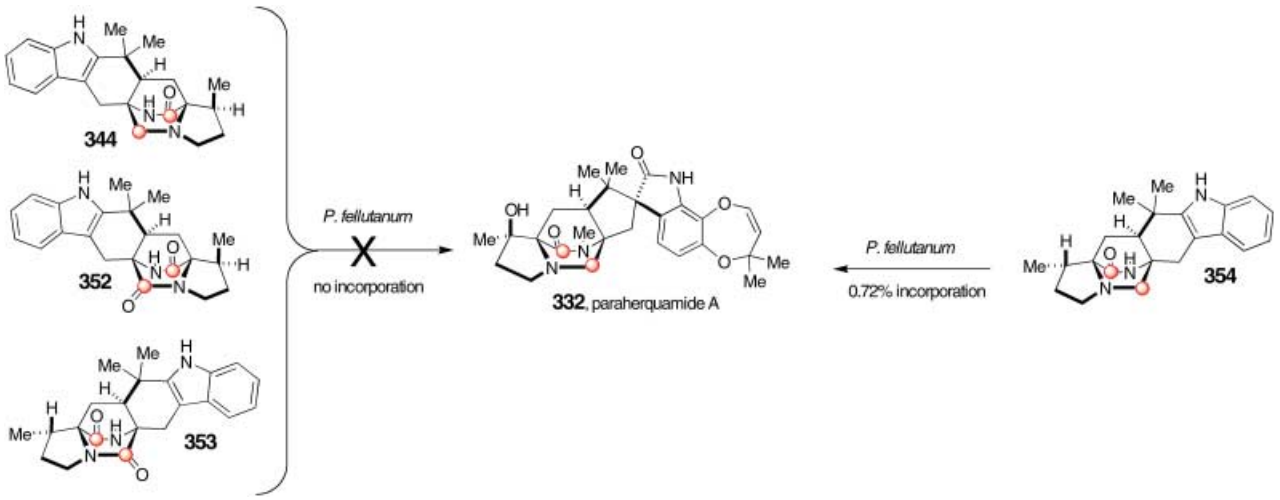
tryptophanyl moiety (namely, indolyl). These results provide additional evidence that **344** is a minor shunt metabolite of the paraherquamide pathway. Moreover, these results document the intermediacy of an advanced metabolite **354**, potentially formed by an intramolecular hetero-Diels–Alder cycloaddition, which contains the core structural elements of the paraherquamide framework prior to a series of oxygenation reactions.

Very recently, Sanz-Cervera and Williams completed an asymmetric biomimetic total synthesis of (−)-**344** that served to unambiguously establish the absolute stereochemistry of this substance (Scheme 65).[148] As predicted by these authors in the unified biogenesis proposal depicted in Scheme 62, **344** retains the (*S*)-Ile stereochemistry in the β-methylproline ring and consequently, has a bicyclo[2.2.2]-diazaoctane ring system that is enantiomorphic to that embedded in the paraherquamides.

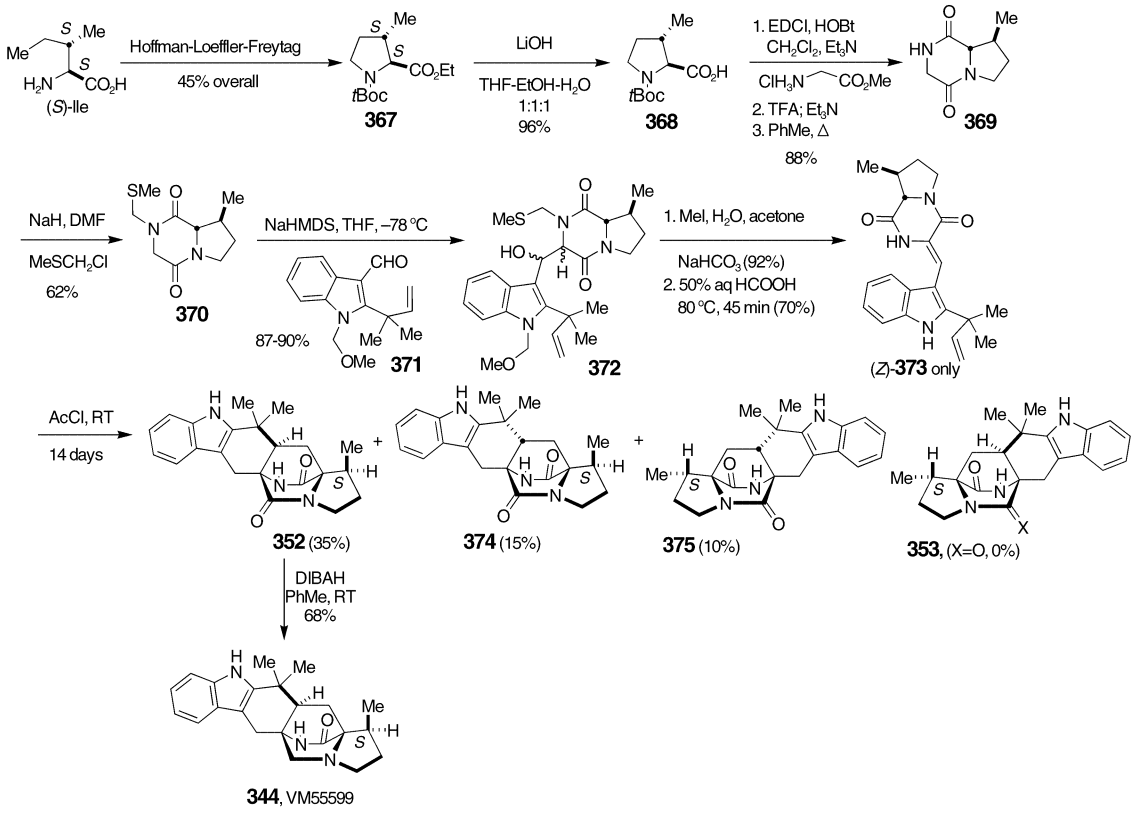
It was quite surprising to observe that cycloadduct **353**, which contains the relative and absolute stereochemistry of the



Scheme 63. Biomimetic synthesis of [$^{13}C_2$]-(±)-**344** and other potential intermediates in the biosynthesis of **332**.[146,147] Boc = *tert*-butoxycarbonyl, BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, DIBAH = diisobutylaluminum hydride. The numbers 2.4:1 and 1.5:1 on **352**, **353** indicate the *syn*/*anti* relationship between C20 and the cyclic amino acid residue and between the methyl groups that are pointing up and down, respectively.



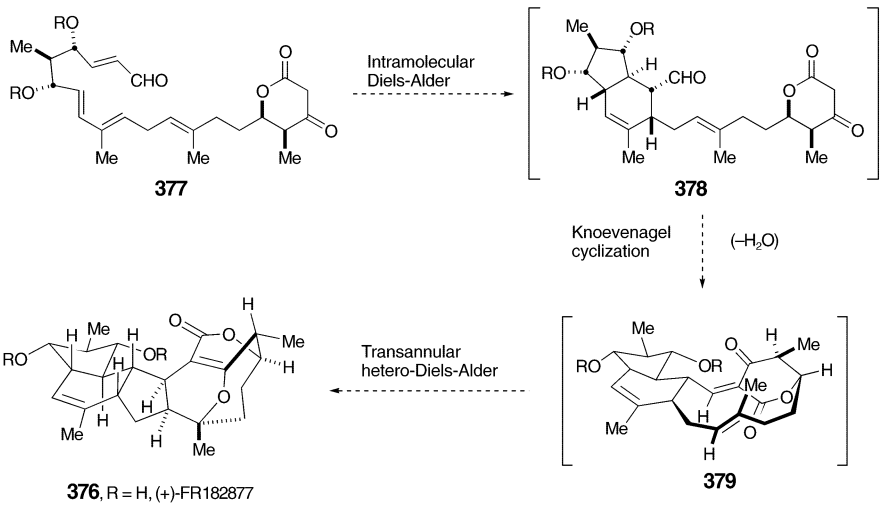
Scheme 64. Feeding experiments with ^{13}C-labeled indolic progenitor candidates of paraherquamide A (**332**).[146] EDCI = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide, HOBt = 1-hydroxy-1*H*-benzotriazole, NaHMDS = sodium hexamethyldisilazanide.



Scheme 65. Asymmetric biomimetic synthesis of **344** and establishment of the absolute stereostructure.[148]

paraherquamides, was not detected from the cycloaddition reaction. The cycloadducts obtained in the previously reported racemic synthesis gave (as lactim ethers) compounds stereochemically corresponding to **352**:**374**:**375**:**353** in a ratio of 3.7:1.6:1:2.6. In the present case, the ratio is 3.5:1.5:1:0. In the biological system, the diastereochemical distribution is expressed as > 600:1 (corresponding to **332**:**344**) as evidenced by the complete lack of natural metabolites that would arise from substances containing the stereochemistry imbedded in either **374** or **375**. The laboratory intramolecular Diels–Alder cycloadditions described in Schemes 63 and 65 again demonstrate an unexpected proclivity for the formation of the relative stereochemistry of **344**. This result is in sharp contradiction to the stereochemical preference expressed in nature. Although the oxidation state of the putative azadiene species (**A**/**B**, X = O or H_2 in Scheme 62) in the biological system currently remains uncertain. The contrast between the two biomimetic laboratory cycloaddition reactions and that

postulated to occur in the biosynthetic constructions strongly implicates protein organization of the precyclization substrate conformation to greatly favor formation of the paraherquamide stereochemistry.



Scheme 66. Biogenesis of FR182877 (**376**) initially proposed by Sorensen and co-workers.[151a]

6. Addendum

Since submission of this manuscript, several recent papers appeared on the biomimetic total synthesis of FR182877 (**376**) and longithorone (**163**). As these works have significant implications concerning the biosynthesis of these natural products, the key findings will be briefly described.

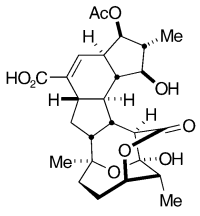
FR182877

Two impressive biomimetic total syntheses of FR182877 (**376**) have appeared[149,150] that provide experimental support for a provocative biogenetic proposal originally suggested by Sorensen and co-workers in 1999.[151] The construction of FR182877 (formerly known as WS9885B) could arise by a cascade of cyclization reactions (Scheme 66) involving: a) an intramolecular Diels–Alder cycloaddition from the polyketide **377** to **378,** b) an intramolecular Knoevenagel cyclization (to **379**), and c) a transannular hetero-Diels–Alder cycloaddition to directly furnish **376**.

In 2001, Sorensen and co-workers suggested a slight revision of this elegant biogenesis wherein the related polyketide substrate **380** would undergo an intramolecular Knoevenagel cyclization (to **381**). Successive transannular Diels–Alder and transannular hetero-Diels–Alder cycloadditions then directly furnished **376** (Scheme 67).[151b]

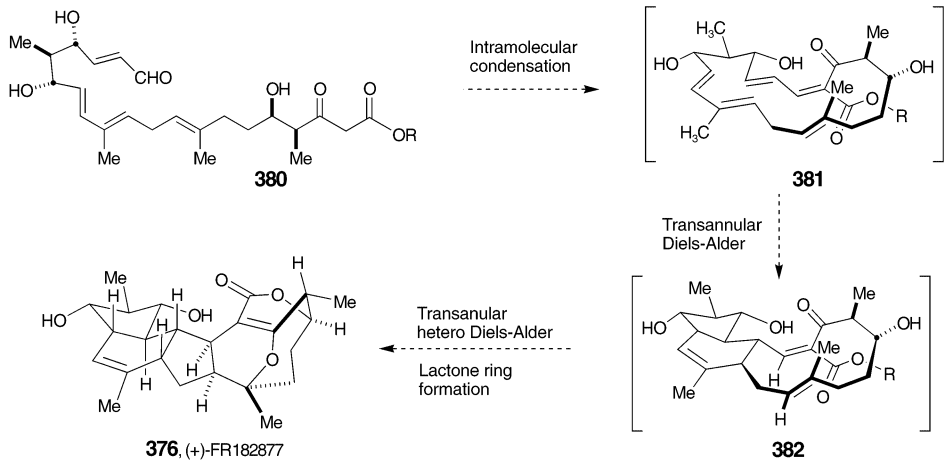
This strategy inspired a biomimetic total syntheses of this natural product[149] that lends strong, albeit indirect, experimental support for the biogenetic hypothesis. The key features of the total synthesis of (+)-FR182877 by Sorensen and co-workers are illustrated in Scheme 68.

Evans and Starr also reported a biomimetic cyclization cascade to (−)-**376** (Scheme 69).[150] This study also confirmed the absolute configuration of this natural product.[152] The biosynthesis of the related natural product hexacyclinic acid (**390**)[153] may arise from an alternative *exo* conformer of a related polyketide-derived macrocycle.

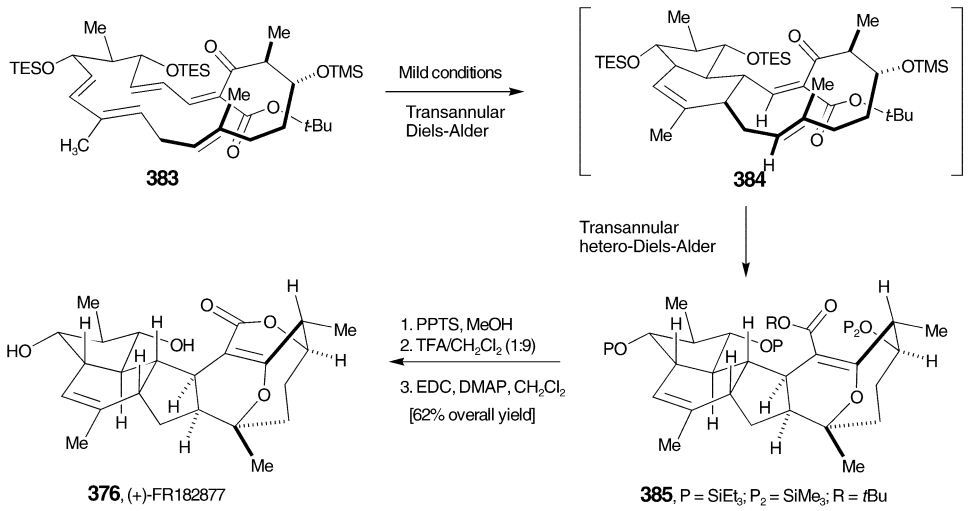


Longithorone

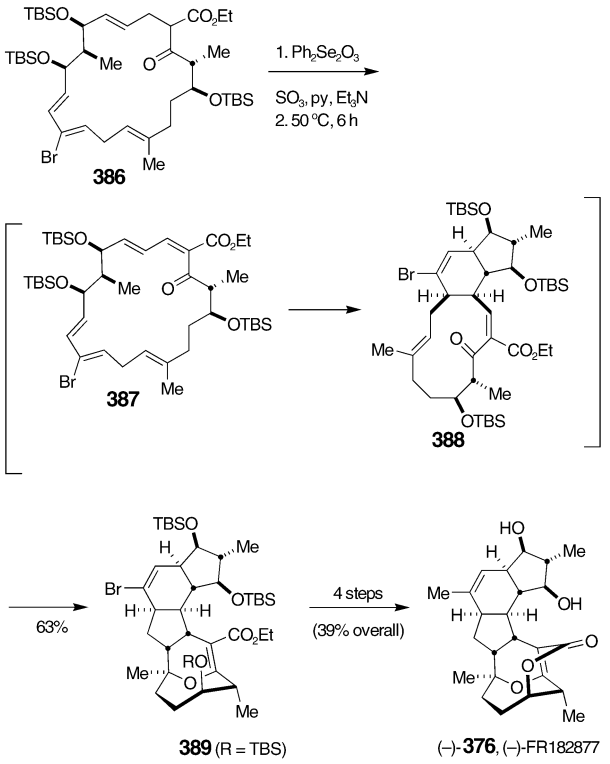
Shair and co-workers recently completed an elegant and impressive biomimetic total synthesis of longithorone (**163**). These researchers exploited an interesting chirality-transfer strategy that involved the use of stereogenic centers to control the atropisomerism followed by removal of the stereogenic centers, and, transfer of the atropisomerism chirality back to the stereogenic centers.



Scheme 67. Revised biogenesis of **376** proposed by Sorensen and co-workers.[151b]

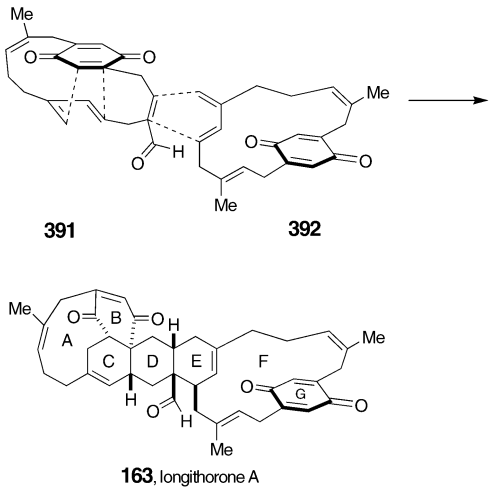


Scheme 68. Biomimetic total synthesis of (+)-**376** by Sorensen and co-workers.[149] DMAP = dimethylaminopyridine, EDC = 1,2-dichloroethane, PPTS = pyridinium *p*-toluenesulfonate, TES = triethylsilyl.



Scheme 69. Biomimetic total synthesis of (−)-**376** by Evans and Starr.[150] TBS = *tert*-butyldimethylsilyl.

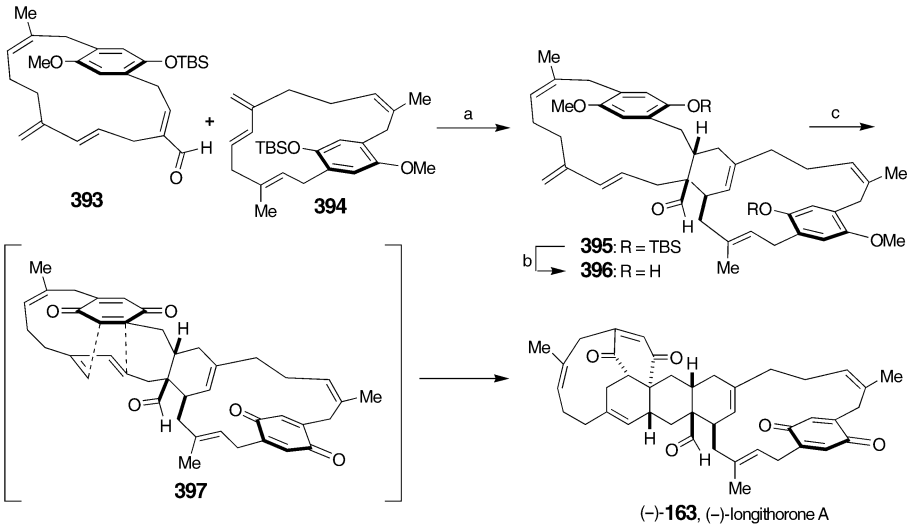
Schmitz and co-workers proposed a provocative biogenesis of longithorone wherein an intermolecular Diels–Alder reaction between [12]paracyclophanes **391** and **392** form ring E and a subsequent transannular intramolecular Diels–Alder reaction across **391** forms rings A, C, and D (Scheme 70).[78a] Shair and co-workers have capitalized on this hypothesis in a beautiful total synthesis of (−)-**163** (Scheme 71).[78b]



Scheme 70. Biogenesis of longithorone A (**163**) as proposed by Schmitz and co-workers.[78a]

7. Summary

The rapidly accumulating body of literature in this field that has been summarized in this Review suggests that nature indeed utilizes the Diels–Alder construction to generate a complex array of natural products. In many cases, such as in the endiandric acids,[49] lucidene (**191**),[92] and asatone (**238**),[110] current experimental evidence argues that the putative biosynthetic Diels–Alder cyclization reactions are not enzyme-mediated, but occur spontaneously in the producing organism in a stereorandom fashion and give rise to racemic products. For the natural products that are enantiomerically pure, there is growing evidence that the Diels–Alder reactions might be enzyme-mediated. The experimental evidence for enzyme involvement is, however, circumstantial for virtually all of these systems. For example, the biomimetic laboratory cyclizations of the epolone B (**182**)[90] and VM55599 (**344**)[146] systems were not stereoselective,



Scheme 71. Biomimetic total synthesis of (−)-**163** by Shair and co-workers.[78b] a) Me_2AlCl, CH_2Cl_2, −20 °C (70%, diastereomeric ratio 1:1.4); b) TBAF, THF, 0 °C; c) PhI(O), MeCN-H_2O, 0→25 °C (90%, 2 steps).

which indicates that there may be some protein organization of the pretransition state conformations in the natural system.[154] The three systems in which the strongest and most direct experimental support for the existence of a Diels–Alderase resides are: a) the enzymatic activity observed for cell-free extracts of *Alternaria solani*[21] that leads to the production of solanapyrone A (**11**), b) lovastatin nonaketide synthase[7] that leads to the production of lovastatin, and c) macrophomate synthase[8] that leads to the production of macrophomic acid (**113**). Despite the impressive and difficult experimental work that the authors of these publications have gathered, in the quest for proving the existence of a Diels–Alderase, rigorous proof that the purified or partially purified (in the case of solanapyrone) proteins are catalyzing (that is, accelerating the rates of these reactions relative to the uncatalyzed systems) the pericyclic Diels–Alder reaction remains to be rigorously established. A great deal of difficult biophysical and kinetic work needs to be done on these systems to ascertain if true catalysis occurs, as measured by the classical tests of rate acceleration and stereoselectivity versus the substrate conversions to product in the absence of enzyme.

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