Intramolecular cyclizations of polyketide biosynthesis: mining for a "Diels–Alderase"?

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Despite the large number of naturally occurring metabolites existing for which enzymatic Diels–Alder reactions have been proposed as a key biosynthetic step, the actual number of enzymes thus far identified for these transformations is incredibly low. Even for those few enzymes identified, there is currently little biochemical or mechanistic evidence to support the label of a "Diels–Alderase." For several families of polyketide metabolites, the transformation in question introduces a rigid, cross-linked scaffold, leaving the remaining peripheral modifications and polyketide processing to provide the variation among the related metabolites. A detailed understanding of these modifications—how they are introduced and the tolerance of enzymes involved for alternate substrates—will strengthen biosynthetic engineering efforts toward related designer metabolites. This review addresses intramolecular cyclizations that appear to be consistent with enzymatic Diels–Alder transformations for which either the responsible enzyme has been identified or the respective biosynthetic gene cluster for the metabolite in question has been elucidated.

Introduction

Examination of a mere handful of naturally occurring molecules allows one to appreciate the remarkable degree of structural diversity and complexity resulting from enzymatic transformations. The capability of enzymes to introduce structural modifications with precise regiospecificity and stereospecificity is without parallel in the discipline of synthetic chemistry. A diverse array of reaction types can be effected through enzymatic catalysis, including nucleophilic displacement, carbocation-mediated cyclization and rearrangement, hydroxylation and halogenation at unactivated hydrocarbons, and oxidative cross-linking. Although rare, there are those reactions proceeding through a pericyclic reaction mechanism; well-characterized examples from shikimic acid metabolism include the chorismate mutase-catalyzed Claisen rearrangement[1] and the conversion of isochorismate to salicylate and pyruvic acid by isochorismate-pyruvate lyase.[2] Enzyme-independent photoinduced electrocyclic rearrangements have also been established as pertinent steps in the biosynthesis of the immunosuppressants SNF4435C and SNF4435D.[3]

Developed in 1928, the Diels–Alder reaction is a powerful tool available to the modern synthetic chemist.[4] In the concerted [4 + 2] cycloaddition of a 1,3-diene and an electron-deficient alkene, two carbon–carbon bonds of a cyclohexene ring and up to four stereocenters are forged. Lewis acid catalysts have been developed that facilitate remarkable control over the regiochemistry and stereochemistry of the reaction.[5] Given the versatility of enzymatic catalysis and occurrence of enzyme-catalyzed pericyclic rearrangements, it is entirely possible that Nature could also orchestrate this transformation. Despite a number of metabolites for which a Diels–Alder cycloaddition has been proposed as a likely event, very few enzymes responsible for these transformations have actually been biochemically characterized. To date, only two enzymes have been purified to homogeneity and demonstrated to catalyze reactions wherein the substrates and products are *consistent* with a Diels–Alder cycloaddition: lovastatin nonaketide synthase (LovB) and macrophomate synthase, which construct the core framework of the cholesterol-lowering agent lovastatin **1** and the fungal metabolite macrophomic acid **2**, respectively (Fig. 1).[6,7]

Artificially selected biocatalysts have demonstrated capabilities in this arena: selection of catalytic antibodies and ribozymes catalyzing Diels–Alder reactions support the feasibility for an

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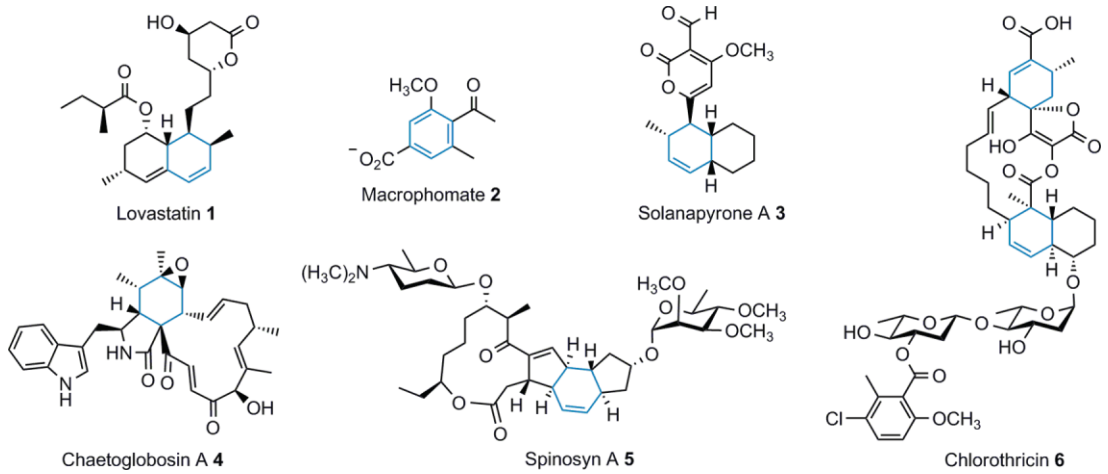

Fig. 1 Examples of naturally occurring metabolites for which biosynthetic Diels–Alder transformations have been proposed. The rings indicated in blue are those occurring from the cyclizations discussed in this review.

analogous naturally occurring-enzyme.[8–14] Since the Diels–Alder reaction proceeds through a late transition state with a structure resembling that of the product, it was anticipated that the catalytic antibodies and ribozymes generated for this reaction type would be subject to product inhibition. Indeed, such inhibition is observed in the RNA-catalyzed Diels–Alder reactions.[8,13] In the Diels–Alderase catalytic antibodies isolated, however, there were no reports of product inhibition. In one approach, the reaction product adopted a conformation distinct from that of the hapten used to raise the antibody.[9,10] Another tactic was to engineer the cyclization product to spontaneously extrude sulfur dioxide, generating a final structure substantially different from that of the transition state.[11] Product inhibition of a catalytic antibody was absent even when the hapten closely mimicked the actual Diels–Alder adduct.[15] Crystal structures of two catalytic antibodies both reveal hydrophobic binding sites that, for the large part, rely upon van der Waals interactions and proximity effects to promote the reaction.[16,17] In both cases, the dienophile was bound to the antibody by π-stacking and further activation is achieved through a hydrogen bond to the dienophile carbonyl donated by an asparagine side chain common to the two antibodies.[16,17] The structural studies of these artificial enzymes may ultimately provide insight into at least one possible mode of catalysis for a naturally occurring Diels–Alderase.

There are numerous examples of natural products for which the structures are highly suggestive of intramolecular cyclizations in which a [4 + 2] cycloaddition may be a biosynthetically reasonable step, a small sampling of these is shown in Fig. 1. Our current understanding of any one of these carbon–carbon bond forming cyclizations is minimal at best, and less so for those which lack an assigned enzyme to mediate the transformation. Even the aforementioned LovB and macrophomate synthase still lack experimental support for the involvement of a Diels–Alder mechanism. The remaining discussion in this review will highlight polyketide metabolites supposedly arising from the action of a "Diels–Alderase" in which either the genes needed for their biosynthesis have been identified or the transformation in question has been demonstrated by the use of cell-free studies. Although the exact cyclases still need further characterization for most of the following cases, insight into the respective transformations is provided by biochemical, genetic, or bioinformatic analysis.

Polyketide synthases

A striking fraction of the naturally occurring molecules for which a biosynthetic "Diels–Alderase" has been proposed are polyketide (or hybrid polyketide-nonribosomal peptide) metabolites of bacterial or fungal origin.[18,19] This family is a rich source of biologically active agents for agrochemical applications and for both veterinary and human medicine. Polyketides are biosynthesized using enzymatic logic comparable to that utilized for fatty acid biosynthesis.[20,21] The polyketide backbone is constructed two carbon atoms at a time by a decarboxylative thiol-Claisen reaction of acyl and malonyl thioesters to form the extending carbon–carbon bond (Fig. 2).[20] The primary function of both prokaryotic and fungal PKS systems is often the assembly of a linear carbon skeleton that can be further modified by additional tailoring enzymes, such as hydroxylation, epoxidation, and glycosylation. In the cases highlighted in this review, the polyketide carbon backbone is presented to a putative "Diels–Alderase" for an intramolecular cyclization. These cyclases introduce cross-links into the molecular framework of the metabolite, providing both conformational rigidity and a platform for peripheral modifications.

Prokaryotic type I modular polyketide synthases (PKSs) are large, multimodular, and often multisubunit megasynthases. Three core domains must be present for polyketide extension: a ketosynthase (KS), an acyl-CoA transferase (AT), and an acyl carrier protein (ACP).[20] The ACP is posttranslationally modified with a phosphopantetheinyl cofactor that is employed for the covalent sequestration of substrates and the nascent polyketide as thioester intermediates. The appropriate extension unit, usually a structural derivative of malonyl-CoA, is selected by the AT, which then catalyzes a net transthioesterification to generate the acyl-*S*-ACP. The final core domain, the KS, catalyzes the decarboxylative condensation between its upstream and downstream acyl-*S*-ACP substrates to form a β-keto-*S*-ACP intermediate. This product can either serve immediately as a substrate for the next round of

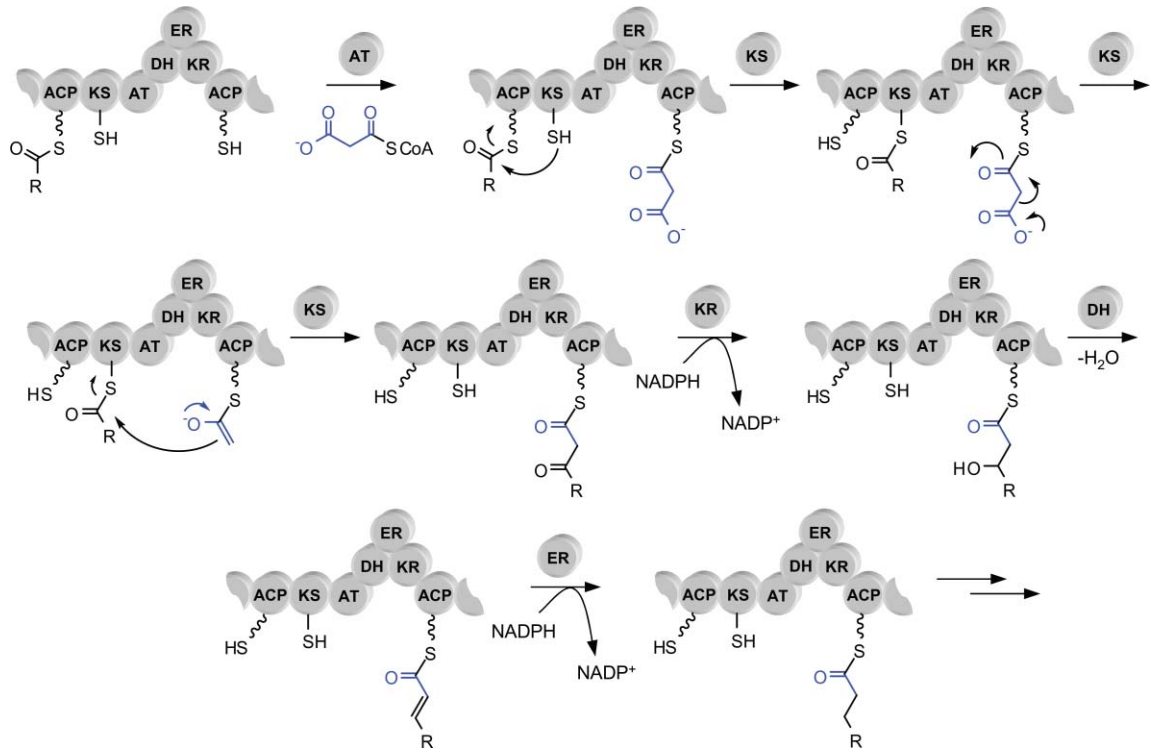

Fig. 2 General mechanism of chain extension by a type I modular PKS.

elongation or it can be reduced to the alcohol, dehydrated to form an alkene, or then reduced a second time to the alkane by the sequential action of three non-essential domains: the ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER).[20] For a canonical type I modular PKS, one module is in place for every two-carbon extender unit incorporated into the polyketide, and the oxygenation and oxidation pattern of the polyketide product is dictated by the non-essential domains present in each module.[20,21]

The fungal type I iterative PKS differs in that a single module is utilized repeatedly for each two-carbon extension of the nascent polyketide.[22] Many of the polyketides originating from the fungal iterative type I PKS systems are fully oxidized, generating polycyclic aromatic products.[22] There are also numerous iterative type I PKS systems that produce partially reduced polyketides, for which the oxygenation patterns and oxidation state of each two carbon addition varies within the product, according to the specific synthase.[22] The mechanisms guiding the timing and specificity of the reductive and dehydration steps for the iterative type I systems have yet to be fully understood.

Macrophomate synthase

While not formally catalyzing an intramolecular cross-linking reaction, macrophomate synthase (MPS) is the most extensively studied of all the putative Diels–Alderases. Conflicting positions surround the mechanism for MPS, an unusual enzyme catalyzing a total of four chemical steps that couple 2-pyrone **7** and oxaloacetate **8** with two decarboxylations and two carbon–carbon bond formations (Fig. 3).[23] The final step of this sequence

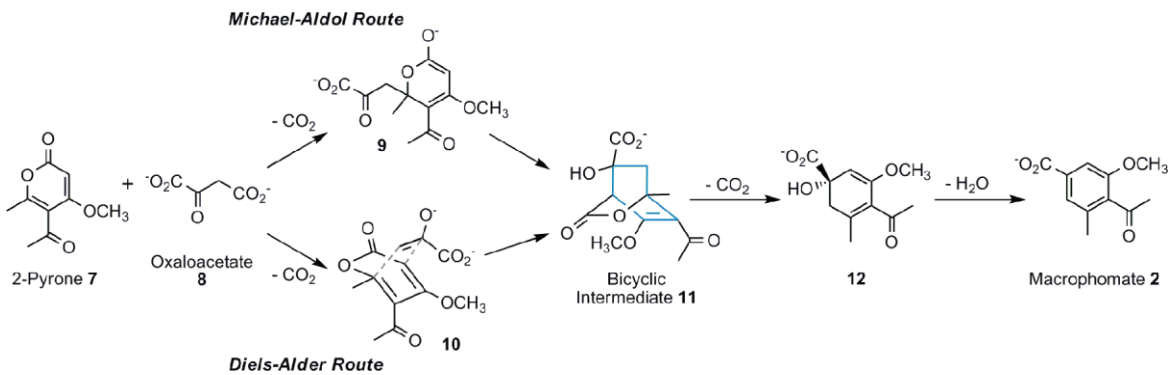

Fig. 3 Mechanisms proposed for macrophomic acid synthase. The top path represents the Michael–aldol mechanism and the bottom route represents the Diels–Alder mechanism.

is a spontaneous dehydration of **12** to provide macrophomate **2**.[24] Initial reports of this enzyme following its identification and characterization proposed a Diels–Alder mechanism as a key step for these transformations.[25,26] Mixed quantum and molecular mechanics (QM/MM) simulations of MPS, however, support a stepwise sequence of a Michael addition followed by an aldol reaction.[27] The crystal structure of MPS has been solved, and is similar in tertiary structure to that of 2-dehydro-3-deoxygalactarate (DDG) aldolase, despite weak amino acid sequence identity (20%).[26,28,29] Both MPS and DDG aldolase are Mg^{2+}-dependent enzymes possessing a $(\beta/\alpha)_8$-barrel fold, and both enzymes generate the pyruvate enolate in the active site; MPS by decarboxylation of oxaloacetate and DDG aldolase by the deprotonation of pyruvate. Although DDG aldolase directs attack of the enolate upon the electron-deficient aldehyde carbonyl of tartronic semialdehyde, it was initially proposed that MPS utilizes the pyruvate enolate to form bicyclic intermediate **11** *en route* to macrophomic acid by either an inverse electron demand Diels–Alder reaction[30]or a sequential Michael–aldol mechanism.[23,25] Hilvert *et al.* demonstrated that, like DDG aldolase, once MPS generates the pyruvate enolate in the active site it efficiently mediates an aldol reaction with a variety of aldehyde substrates.[31] Together with the QM/MM simulations, the demonstrated aldolase activity of MPS certainly suggests that the stepwise Michael–aldol pathway may in fact be the most likely mechanism employed by this enzyme.

Solanapyrones

The fungi *Alternaria solani* (*A. solani*) and *Ascochyta rabei* produce solanapyrones A–D (**3**, **15–18** Fig. 4), a series of polyketide phytotoxins.[32] Through the work of careful isotopically labeled precursor incorporation studies, it was determined that the decalin ring system observed in the solanapyrones is derived from an achiral triene.[33] A crude enzyme preparation from *A. solani* catalyzed the oxidation and cyclization of prosolanapyrone II **13** to generate solanapyrones A and D (**3** and **16**) with an 85 : 15 ratio, directing formation of the *exo*-adduct **3** (solanapyrone A) as the major isomer.[34,35] No cyclization of prosolanapyrone II **13** was detected in the absence of the enzyme preparation.[34,35] In contrast, nonenzymatic cyclization of prosolanapyrone III **14** provided a 3 : 97 ratio of solanapyrone A to solanapyrone D, the major isomer resulting from the *endo* transition state (solanapyrone D **16**).[34] When prosolanapyrone III **14** is presented to the enzyme preparation, the ratio of enzymatically generated solanapyrone A to D (**3** : **16**) increased to 87 : 13 to favor the *exo* adduct, as was observed for prosolanapyrone II **13**.[34]

On the basis of the isotope incorporation studies, it has been proposed that the pyrone alcohol at C-17 of prosolanapyrone II **13** must first be oxidized to the aldehyde prior to the proposed Diels–Alder cyclization, providing prosolanapyrone III **14**. The dienophile of prosolanapyrone III **14** (an aldehyde) is more electron deficient than that present in prosolanapyrone II **13** (an alcohol), and inherently a better substrate for a normal electron demand [4 + 2] cycloaddition, supporting the biosynthetic pathway proposed by Oikawa and coworkers. By this logic, the alcohol substituents observed in solanapyrones B and E (**15** and **17**) most likely result from the re-reduction of C-17 following formation of the decalin system, rather than from the direct cyclization of prosolanapyrone II **13**.[33] This reversible oxidation and reduction at C-17 was utilized to assist with partial purification of the postulated solanapyrone synthase.[35,36] The enzyme, or one of the enzymes, required for the conversion of prosolanapyrone II **13** to solanapyrone A **3** is oxygen dependent and appears to utilize a covalently-bound FAD cofactor.[35,36]

There remain several unanswered questions regarding this remarkable transformation, including the actual role the oxidizing enzyme plays in directing the cycloaddition. The facile nonenzymatic cyclization of prosolanapyrone III **14** in aqueous buffer suggests that the cyclase serves as a chaperone to guide the stereochemical outcome of the cyclization rather than serving as a true enzymatic catalyst. An additional point remaining to be addressed with the solanapyrone system is whether the conversion of prosolanapyrone II **13** to solanapyrones A and D (**3** and **16**) requires one or two enzymes to effect oxidation of C-17 and the subsequent cyclization.

Lovastatin and equisetin

An enzymatic Diels–Alder reaction has been suggested in the biosynthesis of the cholesterol-lowering agent lovastatin **1** (Fig. 5A), produced by *Aspergillus terreus* ATCC 20542 (*A. terreus*). The decalin ring system in lovastatin and its precursor dihydromonacolin L **21** is the product of a 335 kDa type I iterative PKS, LovB (lovastatin nonaketide synthase), and a *trans*-acting enoyl-*S*-ACP reductase, LovC.[6,37] The LovB/LovC product highlights the remarkable regioselectivity concerning the dehydration and enoyl-*S*-ACP reduction that must occur upon extension of a polyketide chain by an iterative type I PKS (Fig. 5A). Heterologous co-expression of LovB and LovC in *Aspergillus nidulans* is sufficient to generate dihydromonacolin

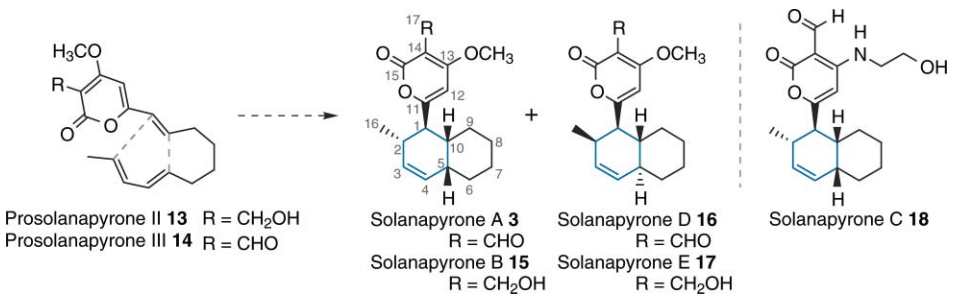

Fig. 4 Solanapyrones and the proposed mechanism accounting for decalin ring formation.

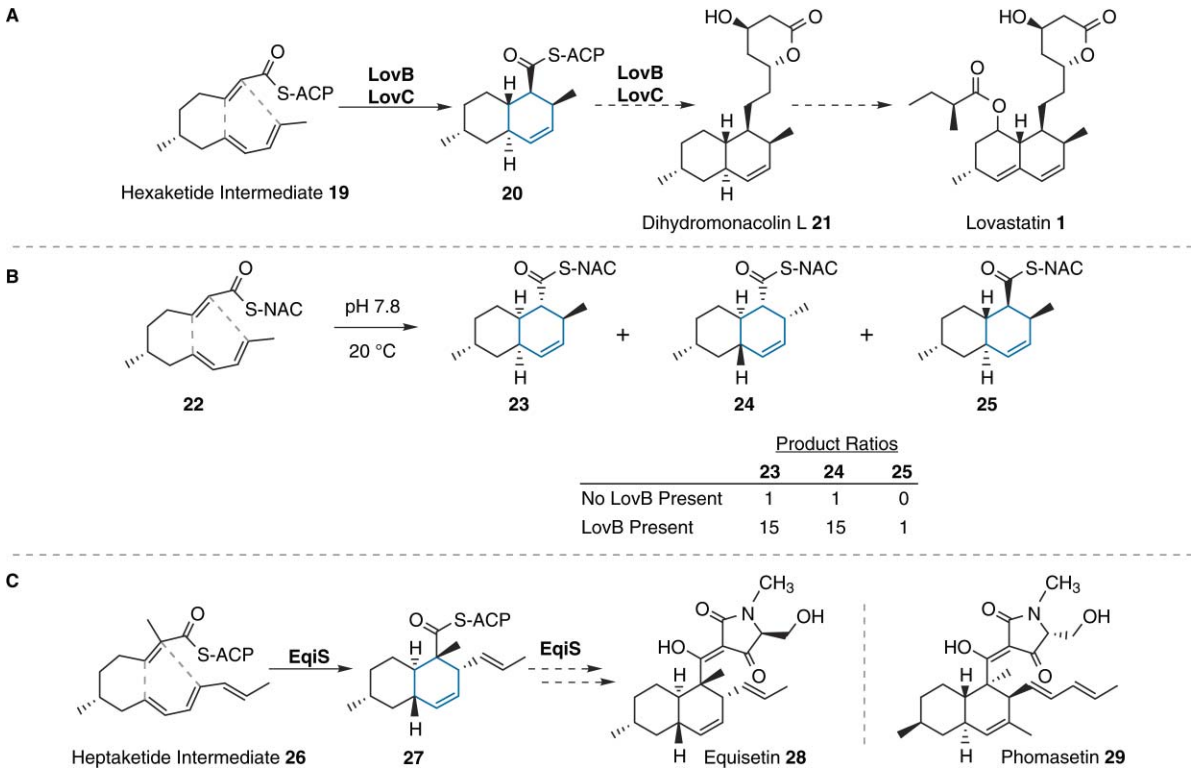

Fig. 5 (**A**) Key steps proposed for the biosynthetic pathway of lovastatin. (**B**) Cyclization of the LovB substrate analog in the presence and absence of enzyme (S-NAC = *S*-*N*-acetylcysteamine). (**C**) Equisetin, phomasetin, and the proposed intramolecular cyclization in the biosynthesis of equisetin.

L **21**, the first intermediate containing the *trans*-decalin system.[37] The presence of LovC is essential for proper processing of the nascent polyketide intermediates, but is not necessary for *trans*-decalin ring formation, as demonstrated by the LovB-catalyzed conversion of a substrate analog.[6] The actual substrate for LovB is believed to be the PKS-tethered hexaketide intermediate **19**.[37] Presentation of a hexaketide-*S*-*N*-acetylcysteamine **22** to purified LovB (Fig. 5B) established that this enzyme alone can catalyze formation of the *trans*-decalin **25**, identical in stereochemistry to dihydromonacolin L, at a k_{cat} of 0.073 ± 0.001 min^{-1}.[37] A diminished ability of LovB to efficiently recognize and process an *N*-acetylcysteamine analog of a protein-bound intermediate may very well explain the low turnover and inefficient utilization of this substrate analog, even if it does correctly portray the chain length of the nascent polyketide. Despite success with purification of this megasynthase, the large molecular weight of this protein and the protein-bound substrate both are considerable challenges for a more detailed biochemical characterization. Recent expression of the KS-AT didomain of LovB confirmed the malonyl-CoA specificity for initiation and elongation units to this enzyme.[38] It is possible that reconstitution of a dissected LovB, as was recently performed for the aflatoxin PKS,[39] will permit a more detailed query into the exact nature of this intramolecular cyclization.

Equisetin **28** and its enantiomeric homolog phomasetin **29** are tetramic acid-containing fungal metabolites that possess a *trans*-decalin scaffold (Fig. 5C).[40–43] The equisetin biosynthetic gene cluster in *Fusarium heterosporum* revealed EqiS, a hybrid iterative type I PKS-nonribosomal peptide synthetase (NRPS), and the requirement of EqiS for equisetin production was confirmed through disruption mutagenesis.[44] The domain organization of EqiS resembles that of LovB and CheA (discussed below for the cytochalasins).[37,45] The cyclase activity for both EqiS and LovB have yet to be assigned to a distinct domain of their respective megasynthases, but it is likely that similar mechanisms are utilized by the two to construct the *trans*-decalin, despite subtle differences in the predicted substrate. LovB could act upon a hexaketide intermediate **19**, while EqiS must recognize a cyclization substrate at least at the heptaketide stage **26**, having undergone an additional two-carbon extension cycle.

The opposing stereochemical outcomes following formation of the *trans*-decalin in dihydromonacolin L **21**, equisetin **28**, and phomasetin **29** is another intriguing aspect of this group of metabolites (Fig. 5). The dihydromonacolin/lovastatin substrate analog **22** undergoes a spontaneous Diels–Alder cyclization in aqueous buffer to yield only the *cis*-decalin **23** (an *exo* adduct) and the diastereomeric *trans*-decalin **24** (an *endo* adduct) at a 1 : 1 ratio (Fig. 5B).[6] The dihydromonacolin L stereochemistry in **25** was a minor product from the LovB-containing reaction, accounting for 3.2% of the cycloaddition product mixture, but was not observed upon omission of LovB from the assay mixture, supporting its requirement for the intramolecular cyclization *in vivo* (Fig. 5B).[6] The syntheses of equisetin employing a transannular Diels–Alder reaction to construct the *trans*-decalin scaffold yield predominantly the *endo* adduct containing the stereochemistry of the naturally occurring metabolite.[46–48] The similarity among the proposed cyclization substrates and the predominant stereochemistry resulting from nonenzymatic cyclizations suggest LovB and EqiS, at the very least, contain a binding pocket to direct the stereochemical outcome of the reaction. Details about the factors controlling these differences among dihydromonacolin L,

equisetin, and phomasetin will not likely be revealed until after the domain responsible for the cyclization is identified and the structure of at least one of these cyclization catalysts is solved.

Cytochalasins

The cytochalasins are a diverse group of fungal polyketide-nonribosomal peptide metabolites unified by the presence of an isoindolone ring fused to either a carbocyclic, lactone-containing, or a carbonate-containing macrocycle **4**, **30–32** (Fig. 6A).[49,50] The amino acid incorporated varies according to the cytochalasin in question: those derived from phenylalanine, tryptophan, and leucine have been observed.[50–52] Most notable for their cytotoxic properties, an impressive range of biological activities has been reported for members of the cytochalasin family that also includes antibiotic and antiretroviral properties.[51–53] The chaetoglobosin A biosynthetic gene cluster of *Pencillium expansum* was recently identified, including CheA, a hybrid fungal iterative type I PKS-NRPS. CheA bears similarity to megasynthases required for biosynthesis of the putative Diels–Alder adducts lovastatin and equisetin (LovB and EqiS, respectively), and to those required for production of mycotoxins fusarin (FusA) and tenellin (TENS).[37,44,54,55] As observed for EqiS and LovB, the enoyl reductase for chaetoglobosin, CheB, acts *in trans* with CheA to generate a nonaketide intermediate. The additional gene products in this cluster are predicted to encode two cytochrome P450s (CheD and CheG) and a FAD-dependent monooxygenase (CheA), all three of which are implicated in post-assembly line oxidative tailoring of chaetoglobosin.[45] The NRPS module of CheA is thought to activate tryptophan and generate an amide linkage with the nonaketide, and an analogous role is proposed for the EqiS NRPS module.[44,45] The polyketide–amino acid intermediate is thought to be reductively released to provide the aminoaldehyde **33**. The defining isoindolone ring is proposed to arise from a two-step process. First, a Knoevenagel condensation of **33** could generate the pyrrolinone intermediate **34** (Fig. 6B). Next, the pyrrolinone ring of **34** is positioned for a [4 + 2] cycloaddition to generate the isoindolone-fused macrocycle **35**.[45] The exact species or domain of CheA responsible for the proposed Diels–Alderase activity is currently undetermined, as it is for LovB and EqiS.

Various syntheses of the cytochalasins based upon the above proposal do incorporate an intramolecular Diels–Alder reaction in the construction of the core isoindolone ring system. The reaction conditions used in the cycloaddition, however, require that the amide nitrogen of the pyrrolinone precursor must be acylated in order to prevent unproductive migration of the double bond to the enol tautomer and to ensure presentation of the appropriate dienophile.[56–58] Certainly, the enzyme effecting this reaction will need to prevent this from occurring if a Diels–Alder mechanism is utilized. The macrocyclic ring characterizing this family of metabolites formed from these biomimetic synthesis is often produced as a mixture of stereochemical products resulting from both *endo*- and *exo*- transition states.[57,59–61] Elucidation of the true substrate for the cyclization leading to the cytochalasin scaffold will shed light on the type of reaction needed to direct isoindolone formation.

Spinosyn

The spinosyns (Fig. 7A) possess a 22-membered tetracyclic macrolide decorated with a D-forosamine and a permethylated rhamnose residue, produced by *Saccharopolyspora spinosa* (*S. spinosa*) and *Saccharopolyspora pogona* (*S. pogona*).[62,63] A mixture of spinosyns A and D, **5** and **36**, is marketed by Dow Agrosciences as the relatively non-toxic (at least to vertebrates!) and environmentally friendly insecticidal agent spinosad in the Tracer® and Naturalyte® lines of insecticides.[64] Spinosad selectively excites the insect nervous system, ultimately leading to paralysis and death.[64] The spinosyn biosynthetic gene cluster spans a 74 kb region in the *S. spinosa* chromosome, including the five genes encoding the subunits of a modular type I PKS.[65,66] The butenyl-spinosyns, *e.g.* **37**, from *S. pogona* are closely related, resulting from a nearly identical biosynthetic gene cluster with the exception of an additional extension module present in the first PKS subunit to produce the butenyl side chain.[67] A remarkable feature of the spinosyns is the presence of three carbon–carbon

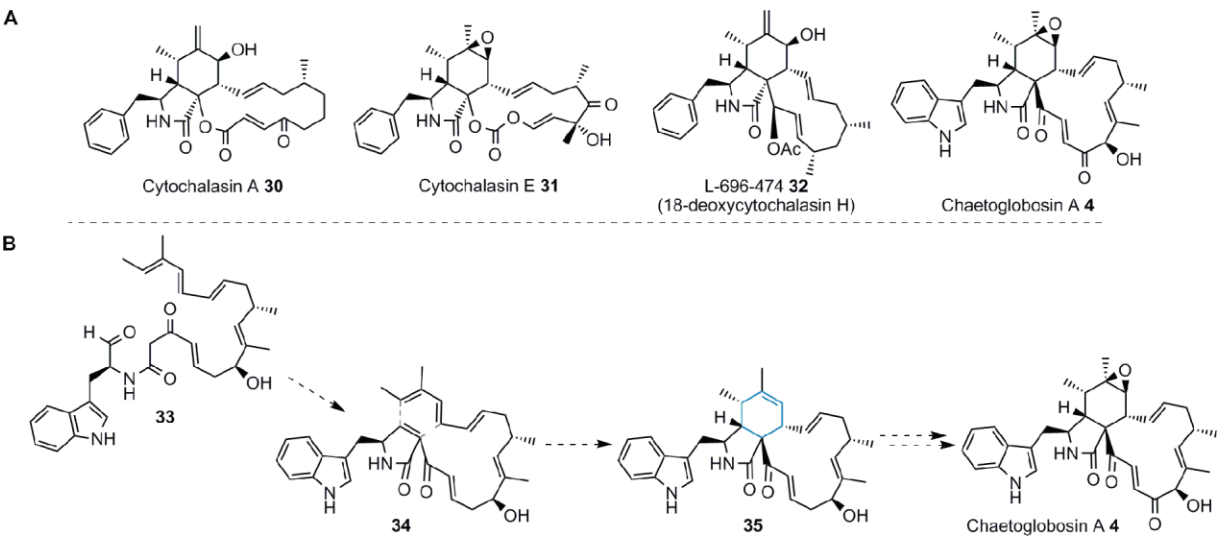

Fig. 6 (**A**) Examples of cytochalasin antibiotics. (**B**) Proposed mechanism for the cyclization of chaetoglobosin A.

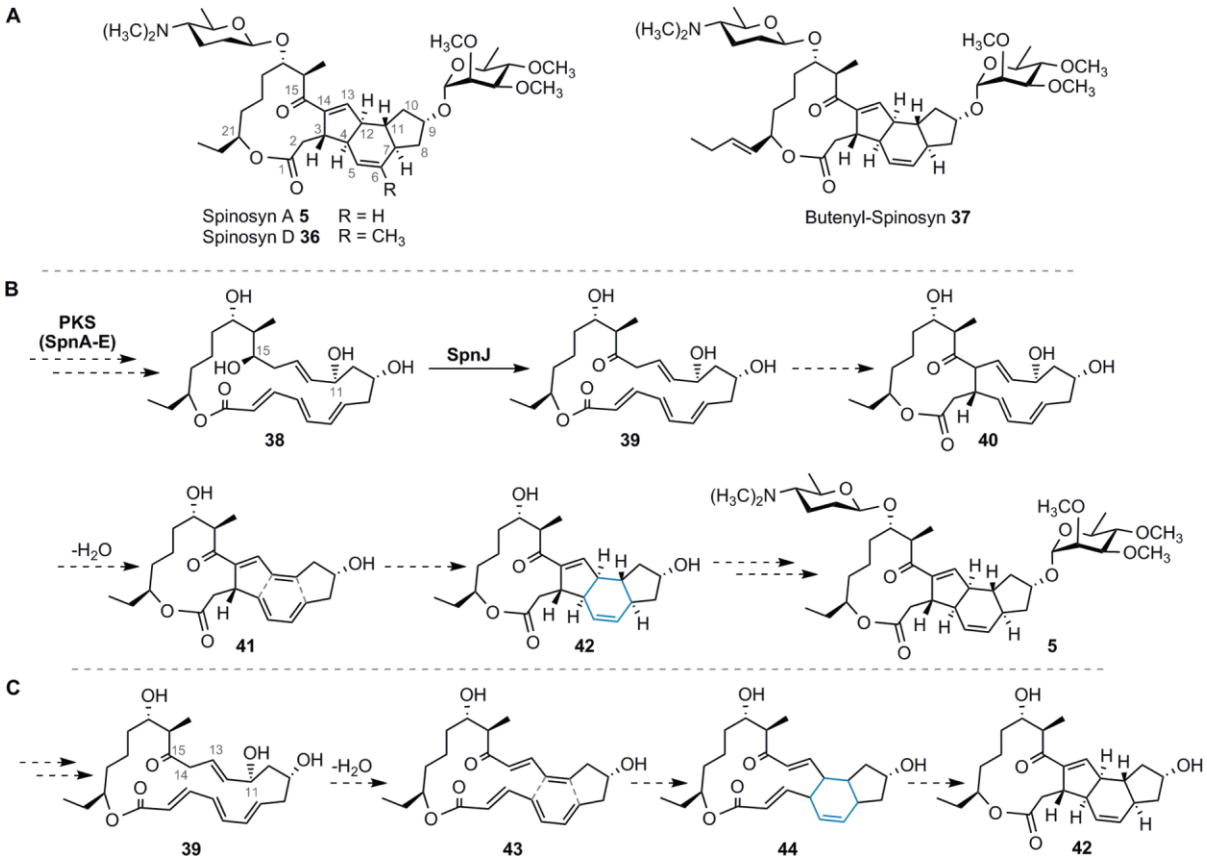

Fig. 7 (**A**) Spinosyns A and D and butenyl-spinosyn. (**B**) One possible sequence of biosynthetic steps leading to the tetracyclic spinosyn aglycone. (**C**) An alternate proposal for spinosyn biosynthesis.

bonds originating from a series of cross-linking reactions, the final two of which have been postulated to occur by a [4 + 2] cycloaddition (Fig. 7B).[66] It should be noted that although the isolated spinosyns include a ketone at C-15, the corresponding module of the spinosyn PKS appears to include a catalytically active KR domain (KR4), suggesting that the initial product released from the PKS machinery contains the hydroxyl group.[66] The re-oxidation of the C-15 alcohol to a ketone is required both to present an appropriate dienophile for the proposed Diels–Alder cyclization and to account for the final product (Fig. 7B).

Heterologous expression of the loading module and the first four extension modules of the *S. spinosa* spinosyn PKS in *Saccharopolyspora erythraea* led to production of a lactone metabolite confirming KR4 is indeed active: a hydroxyl group is present in the pentaketide product corresponding to reduction by KR4, and this, in turn, corresponds to the spinosyn C-15.[68] On this basis, Martin *et al.* suggested the flavin-dependent dehydrogenase encoded by *spnJ* is responsible for the post-assembly line oxidation at C-15. Indeed, complete conversion of the macrolactone C-15 alcohol of **38** to the ketone **39** was demonstrated with purified SpnJ.[69] Failure of SpnJ to oxidize a corresponding *S*-NAC analog of a putative PKS-bound intermediate is consistent with release of the reduced, non-bridged macrolactone prior to the intramolecular cyclizations assembling the spinosyn tetracyclic scaffold.[69] The post-PKS introduction of the C-15 ketone sets the stage for two events. First, abstraction of the C-14 proton facilitates a 1,4-addition and formation of the first carbon-carbon bond between C-3 and C-14 in **40**, followed by abstraction of the second C-14 proton, eliminating water and presenting the dienophile in a proposed bicyclic intermediate **41**.[68] Finally, a biosynthetic [4 + 2] cycloaddition has been proposed to provide the tetracyclic aglycone **42** that defines the spinosyns.

Roush *et al.* incorporated a transannular Diels–Alder reaction in the synthesis of spinosyn A, and offered an alternative proposal for the sequence of cross-linking events (Fig. 7C).[70,71] Following SpnJ-catalyzed oxidation to the C-15 ketone, dehydration of the C-11 alcohol **39** could generate the dienone **43** suitable for an intramolecular Diels–Alder reaction. The final transannular reaction is poised for an enzymatic version of the Morita–Baylis–Hillman reaction; attack by an enzyme active site nucleophile at C-13 of **44** would lead to an enzyme bound intermediate for the final cross-linking reaction. A second deprotonation at C-14 then permits release of the tetracyclic product **42** from the enzyme.[70] In the biomimetic synthesis of spinosyn A based upon this alternate proposal, the major diastereomers resulting from these two synthetic steps harbor identical stereochemistry at the ring junctions to that observed in the natural product.[70,71]

The enzymes encoded within the *S. spinosa* and *S. pogona* spinosyn biosynthetic gene clusters proffer little insight into the mechanisms guiding this cross-bridging cascade, although the gene products of *spnF*, *spnL*, and *spnM* have been implicated on the basis of genetic disruption experiments.[66] SpnF and SpnL have 34% sequence identity to each other, and both have some similarity to *O*-methyltransferases. It should be noted that neither of these

proteins are likely to serve as a functional methyltransferase since both lack the conserved motifs characteristic of this enzyme family.[72] Disruption of either *spnF* or *spnL* abrogates spinosyn production without affecting aglycone glycosylation in cell-based bioconversion assays, suggesting SpnF and SpnL act prior to these tailoring steps.[66] SpnM, a third possible candidate, is marginally similar to hypothetical proteases and lipases, but lacks the typically conserved active site serine nucleophile that is characteristic of these hydrolases.[66,73] Insertional mutagenesis of *spnM* severely hampered spinosyn production; only a trace amount of antibiotic was detected in the fermentation broth of the mutant, while the ability to effectively convert the aglycone to spinosyn was abolished.[66] These gene products are the most likely candidates for the cross-linking reactions and the modifications preceding them that produce the spinosyn aglycone. The exact sequence of events (including the order of ring formations), the role of each enzyme, and the mechanisms guiding tetracycle formation awaits further biochemical characterization.

Spirotetronates

The spirotetronate antibiotics chlorothricin **6**, kijanimicin **45**, and tetrocarcin A **46** are glycosylated polyketide metabolites possessing a variety of biological activities that include antibacterial, anticancer, and antimalarial properties (Fig. 8A).[74–77] The biogenesis of these metabolites has been suggested to involve not just one, but two [4 + 2] cycloadditions resulting from either enzymatic or nonenzymatic processes. The first possible intramolecular cyclization would install the *trans*-decalin, following an *endo* transition state, whereas the second introduces the spiro-fusion between the tetronic acid and the cyclohexene ring as an *exo* adduct.

Chlorothricin, the first reported spirotetronate, was isolated from *Streptomyces antibioticus* Tü99 and later from *Streptomyces* sp. A7361 during a screen for natural product inhibitors of mevalonate-mediated cholesterol biosynthesis.[75,78] In addition to the typical spirotetronate framework (the *trans*-decalin and the spiro-fusion between a cyclohexene and a tetronic acid), chlorothricin is further distinguished by a 2-methoxy-5-chloro-6-methylsalicylyl substituent present on one of the deoxysugar residues.[79] This 6-methylsalicylyl moiety is typically derived from an iterative type I PKS, irrespective of whether the producing species is a eukaryote or prokaryote. Identification of ChlB1, an iterative type I PKS, and its co-localization near genes encoding modular type I PKS subunits permitted identification of the chlorothricin biosynthetic gene cluster.[80,81] The cluster spans roughly 100 kb, harboring modular type I PKS genes responsible for construction of the pentacyclic chlorothricolide aglycone and the carbohydrate substituents. The discovery of the chlorothricin biosynthetic gene cluster was quickly followed by identification of the biosynthetic loci for two other metabolites of this family, tetrocarcin A and kijanimicin.[82,83] Typically, the genes encoding PKS subunits are organized co-linearly on the chromosome, but a noncanonical genetic organization of the PKS-encoding genes is observed for all three spirotetronates. Kijanimicin and tetrocarcin A are the most dramatic: in both systems, one of the PKS subunit-encoding genes is out of order and physically separated from the others by approximately 16 kb.

Analysis of the gene products present in the biosynthetic loci within this family of antibiotics does not reveal any obvious candidates for the proposed Diels–Alder reactions. For kijanimicin and the others, it has been suggested that either the penultimate or the final module of the corresponding PKS facilitates the first intramolecular cyclization to produce the *trans*-decalin system

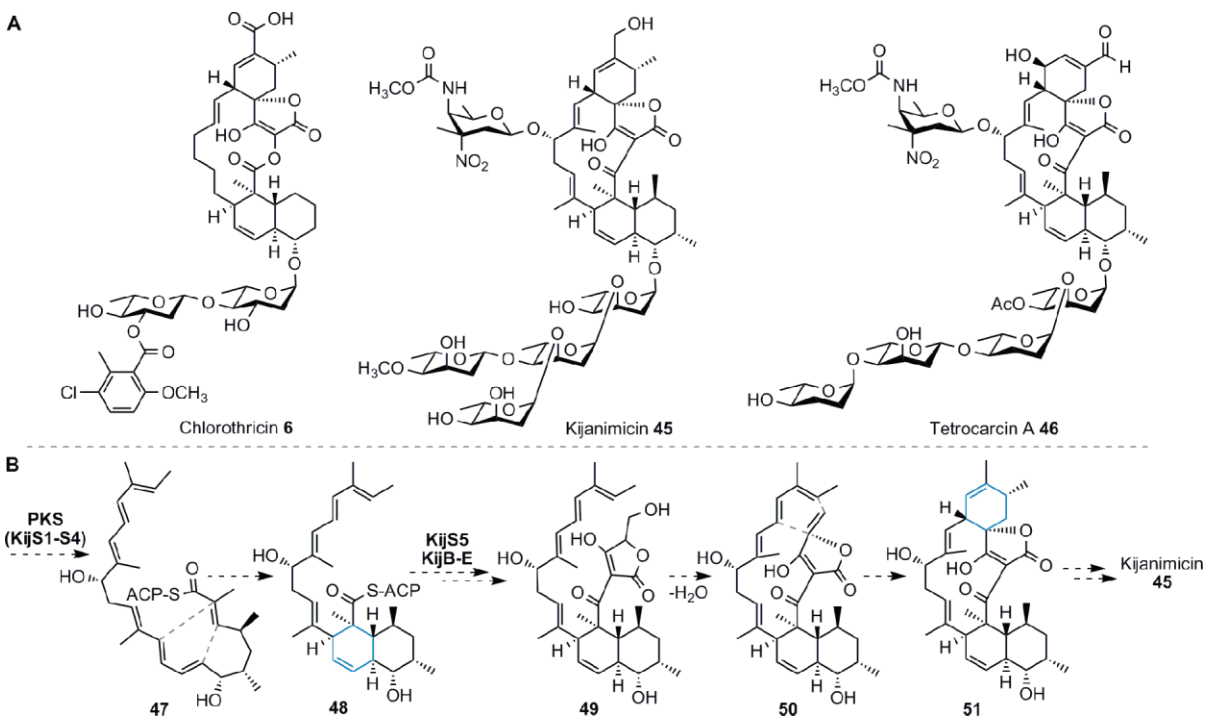

Fig. 8 (**A**) The spirotetronate antibiotics chlorothricin, kijanimicin, and tetrocarcin A. (**B**) Proposed [4 + 2] cycloadditions in the biosynthesis of spirotetronate antibiotics.

of **48** (Fig. 8B), but the *cis*- or *trans*-acting enzyme mediating that reaction is not clearly identifiable.[80,83] The remaining carbon backbone of kijanimicin and related metabolites is assembled from one additional ketide extension by KijS5 followed by attachment of 1,3-bisphosphoglycerate and cyclization by KijB-E to provide **49**.[83,84] Both tetrocarcin A and chlorothricin clusters contain homologs of these enzymes that are needed for installation of the tetronic acid.[80,82] KijA is proposed to introduce the kijanimicin spirotetronic acid: this gene product has sequence similarity to FAD-dependent JadH from the jadomycin biosynthetic pathway, a bifunctional dehydratase and oxygenase.[85] Homologs of KijA were present within the chlorothricin (ChlE1 and ChlE3) and tetrocarcin (TcaE1) gene clusters. It is proposed that KijA, like JadH, could facilitate a dehydration of **49** to produce the dienophile **50** followed by an adventitious and proximity-driven Diels–Alder cyclization while in the KijA active site to produce the spirotetronic acid moiety in **51**.[83] If the role of KijA is to mediate the intramolecular cyclization of kijanimicin, TcaE1 and either ChlE1 or ChlE3 would likely catalyze similar reactions for tetrocarcin A and chlorothricin, respectively. These proposed activities for the spirotetronate PKS and tailoring enzymes await experimental confirmation.

Synthetic strategies to assemble the spirotetronate aglycone utilized Diels–Alder reactions to assemble both the *trans*-decalin and the spiro ring systems.[86–89] In one example, synthesis of the chlorothricin aglycone employed remarkably selective tandem inter- and intramolecular Diels–Alder reactions to simultaneously install seven stereocenters. This approach does successfully model the biosynthetic proposal wherein the *trans*-decalin ring follows an *endo*-selective intramolecular Diels–Alder reaction, and the spirotetronate-cyclohexene an *exo*-transition state.[86,88,90]

Outlook

Until recently, it was presumed that most peripheral modifications to the polyketide and nonribosomal peptide backbones occurred following liberation of a full-length intermediate from the requisite megasynthase. There are, however, exceptions to this presumption. The naphthalene ring of rifamycin is a product of an intramolecular cyclization upon the backbone of a nascent polyketide intermediate.[91,92] Additional co-assembly line modifications have been implicated in the biogenesis of other polyketides following biochemical and/or genetic examination of the biosynthetic pathways, and the polyether ionophores nanchangmycin and mupirocin provide such an example. Heterologously expressed and purified NanE and MonCII thioesterases efficiently hydrolyze acyl-*S*-NAC analogs of polyether-containing intermediates, suggesting the epoxidations and cyclizations may occur upon a megasynthase-tethered polyketide prior to release.[93,94] This tactic has also been observed in NRPS assembly line biosynthesis. The rigid aglycone scaffold characteristic of the vancomycin-type glycopeptide antibiotics results from cytochrome P450-catalyzed oxidative cross-linking of phenolic side chains upon a nascent peptidyl-*S*-carrier protein substrate.[95,96] Several of the transformations of the polyketide metabolites discussed here are also likely to occur upon intermediates sequestered by the megasynthase. The examples of co-assembly line modifications occurring while substrates are covalently bound to a carrier protein continue to grow in number. This implies the preferred biosynthetic route for a cross-linked metabolite in the absence of biochemical or genetic characterization of a pathway cannot be conclusively delineated.

The debate surrounding whether or not naturally occurring "Diels–Alderases" exist has been, until now, largely limited to biomimetic proposals and syntheses concerning biosynthetic pathways. The [4 + 2] cycloaddition is invoked for numerous metabolites not only of the polyketide family but also for isoprenoid, nonribosomal peptide, and alkaloid metabolites.[18,19] The reduced cost of genome sequencing has permitted a rapidly expanding collection of bacterial and fungal biosynthetic gene clusters and improved the ease with which they are identified. Whole-genome scanning to locate a biosynthetic locus within a bacterium or fungus is now a highly effective approach to rapidly narrow the search.[97,98] Immense progress in the techniques available for the biochemical characterization of PKS and NRPS machinery permit insight into modifications occurring along the assembly lines.[39,99] Advances such as these have brought the broader biosynthetic community beyond speculation of proposed enzyme-catalyzed pericyclic reactions to a point that the proposed biochemistry can now be interrogated.

A majority of the cases discussed here and elsewhere still do require elucidation of the true substrate for the intramolecular cyclizations in question. As this process unfolds for each metabolite and each transformation, it may or may not fall in line with the intermediates consistent with initial proposals for a pericyclic reaction. Certainly, identification of a bona-fide "Diels–Alderase" would be an exciting addition to our knowledge of Nature's biosynthetic toolkit. Rigorous mechanistic studies will be necessary to establish whether or not the title of a "Diels–Alderase" can be applied with confidence. Regardless of the mechanisms employed to construct the elaborate ring systems discussed in this review, each of these transformations is likely to proceed through a fascinating mechanism and all exhibit the remarkable capabilities that define enzymatic catalysis.

The natural products in which these highlighted ring systems occur possess a remarkable range of biological properties including cholesterol-lowering, antibacterial, and anticancer properties. The cytochalasins and spirotetronate antibiotics are two striking examples of metabolic families unified by the presence of a common structural scaffold, yet distinct medicinal properties are possessed by individual members. Any effort toward biosynthetic engineering of these metabolites must factor in not only the various tailoring modifications needed to tune a given agent to its optimal biological activity and potency, but also whether or not such modifications occur upon a carrier protein-bound substrate. Successes with glycorandomization, mutasynthesis, and precursor-directed biosynthesis, among other strategies, are encouraging as methods that have emerged to generate alternate products.[20,100,101] As attention turns toward architecturally intricate metabolites such as those highlighted in this article, the ability of the tailoring enzymes to recognize and process alternate substrates must be considered. A clear understanding of how these enzymes effect their respective reactions, concerted or stepwise, complete with inherent substrate specificities and tolerances, will greatly enhance the biosynthetic engineer's ability direct the production of designer metabolites.

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References

1 Y. M. Chook, H. Ke and W. N. Lipscomb, *Proc. Natl. Acad. Sci. U.S.A.*, 1993, **90**, 8600–8603.
2 M. S. DeClue, K. K. Baldridge, D. E. Künzler, P. Kast and D. Hilvert, *J. Am. Chem. Soc.*, 2005, **127**, 15002–15003.
3 M. Muller, B. Kusebauch, G. Liang, C. M. Beaudry, D. Trauner and C. Hertweck, *Angew. Chem. Int. Ed. Engl.*, 2006, **45**, 7835–7838.
4 O. Diels and K. Alder, *Justus Liebigs Ann. Chem.*, 1928, **460**, 98–122.
5 K. C. Nicolaou, S. A. Snyder, T. Montagnon and G. Vassilikogiannakis, *Angew. Chem. Int. Ed. Engl.*, 2002, **41**, 1668–1698.
6 K. Auclair, A. Sutherland, J. Kennedy, D. J. Witter, J. P. Van, den Heever, C. R. Hutchinson and J. C. Vederas, *J. Am. Chem. Soc.*, 2000, **122**, 11519–11520.
7 K. Watanabe, H. Oikawa, K. Yagi, S. Ohashi, T. Mie, A. Ichihara and M. Honma, *J. Biochem.*, 2000, **127**, 467–473.
8 J. J. Agresti, B. T. Kelly, A. Jäschke and A. D. Griffiths, *Proc. Natl. Acad. Sci. U.S.A.*, 2005, **102**, 16170–16175.
9 A. C. Braisted and P. G. Schultz, *J. Am. Chem. Soc.*, 1990, **112**, 7430–7431.
10 V. E. Gouverneur, K. N. Houk, B. de Pascual-Teresa, B. Beno, K. D. Janda and R. A. Lerner, *Science*, 1993, **262**, 204–208.
11 D. Hilvert, K. W. Hill, K. D. Nared and M.-T. M. Auditor, *J. Am. Chem. Soc.*, 1989, **111**, 9261–9262.
12 C. J. Suckling, M. C. Tedford, L. M. Bence, J. I. Irvine and W. H. Stimson, *J. Chem. Soc. Perkin Trans. 1*, 1993, 1925–1929.
13 T. M. Tarasow, S. L. Tarasow and B. E. Eaton, *Nature*, 1997, **389**, 54–57.
14 J. T. Yli-Kauhaluoma, J. A. Ashley, C.-H. Lo, L. Tucker, M. M. Wolfe and K. D. Janda, *J. Am. Chem. Soc.*, 1995, **117**, 7041–7047.
15 C. J. Suckling, M. C. Tedford, L. M. Bence, J. I. Irvine and W. H. Stimson, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 49–52.
16 F. E. Romesberg, B. Spiller, P. G. Schultz and R. C. Stevens, *Science*, 1998, **279**, 1929–1933.
17 J. Xu, Q. Deng, J. Chen, K. N. Houk, J. Bartek, D. Hilvert and I. A. Wilson, *Science*, 1999, **286**, 2345–2348.
18 H. Oikawa and T. Tokiwano, *Nat. Prod. Rep.*, 2004, **21**, 321–352.
19 E. M. Stocking and R. M. Williams, *Angew. Chem. Int. Ed. Engl.*, 2003, **42**, 3078–3115.
20 C. Khosla, Y. Tang, A. Y. Chen, N. A. Schnarr and D. E. Cane, *Annu. Rev. Biochem.*, 2007, **76**, 195–221.
21 K. J. Weissman and P. F. Leadlay, *Nat. Rev. Microbiol.*, 2005, **3**, 925–936.
22 R. J. Cox, *Org. Biomol. Chem.*, 2007, **5**, 2010–2026.
23 H. Oikawa, K. Watanabe, K. Yagi, S. Ohashi, T. Mie, A. Ichihara and M. Honma, *Tetrahedron Lett.*, 1999, **40**, 6983–6986.
24 J. M. Serafimov, T. Westfeld, B. H. Meier and D. Hilvert, *J. Am. Chem. Soc.*, 2007, **129**, 9580–9581.
25 K. Watanabe, T. Mie, A. Ichihara, H. Oikawa and M. Honma, *J. Biol. Chem.*, 2000, **275**, 38393–38401.
26 T. Ose, K. Watanabe, T. Mie, M. Honma, H. Watanabe, M. Yao, H. Oikawa and I. Tanaka, *Nature*, 2003, **422**, 185–189.
27 C. R. W. Guimarães, M. Udier-Blagovic and W. L. Jorgensen, *J. Am. Chem. Soc.*, 2005, **127**, 3577–3588.
28 T. Ose, K. Watanabe, M. Yao, M. Honma, H. Oikawa and I. Tanaka, *Acta Crystallogr. D Biol. Crystallogr.*, 2004, **60**, 1187–1197.
29 T. Izard and N. C. Blackwell, *EMBO J.*, 2000, **19**, 3849–3856.
30 K. Afarinkia, V. Vinader, T. D. Nelson and G. H. Posner, *Tetrahedron*, 1992, **48**, 9111–9171.
31 J. M. Serafimov, D. Gillingham, S. Kuster and D. Hilvert, *J. Am. Chem. Soc.*, 2008, **130**, 7798–7799.
32 H. Oikawa, T. Yokota, T. Abe, A. Ichihara, S. Sakamura, Y. Yoshizawa and J. C. Vederas, *J. Chem. Soc. Chem. Comm.*, 1989, 1282–1284.
33 H. Oikawa, Y. Suzuki, A. Naya, K. Katayama and A. Ichihara, *J. Am. Chem. Soc.*, 1994, **116**, 3605–3606.
34 H. Oikawa, K. Katayama, Y. Suzuki and A. Ichihara, *J. Chem. Soc. Chem. Comm.*, 1995, 1321–1322.
35 K. Katayama, T. Kobayashi, H. Oikawa, M. Honma and A. Ichihara, *Biochim. Biophys. Acta*, 1998, **1384**, 387–395.
36 K. Katayama, T. Kobayashi, M. Chijimatsu, A. Ichihara and H. Oikawa, *Biosci. Biotechnol. Biochem.*, 2008, **72**, 604–607.
37 J. Kennedy, K. Auclair, S. G. Kendrew, C. Park, J. C. Vederas and C. R. Hutchinson, *Science*, 1999, **284**, 1368–1372.
38 S. M. Ma and Y. Tang, *FEBS J.*, 2007, **274**, 2854–2864.
39 J. M. Crawford, P. M. Thomas, J. R. Scheerer, A. L. Vagstad, N. L. Kelleher and C. A. Townsend, *Science*, 2008, **320**, 243–246.
40 H. R. Burmeister, G. A. Bennett, R. F. Vesonder and C. W. Hesseltine, *Antimicrob. Agents Chemother.*, 1974, **5**, 634–639.
41 R. F. Vesonder, L. W. Tjarks, W. K. Rohwedder, H. R. Burmeister and J. A. Laugal, *J. Antibiot.*, 1979, **32**, 759–761.
42 S. B. Singh, D. L. Zink, M. A. Goetz, A. W. Dombrowski, J. D. Polishook and D. J. Hazuda, *Tetrahedron Lett.*, 1998, **39**, 2243–2246.
43 N. J. Phillips, J. T. Goodwin, A. Fraiman, R. J. Cole and D. G. Lynn, *J. Am. Chem. Soc.*, 1989, **111**, 8223–8231.
44 J. W. Sims, J. P. Fillmore, D. D. Warner and E. W. Schmidt, *Chem. Commun.*, 2005, 186–188.
45 J. Schümann and C. Hertweck, *J. Am. Chem. Soc.*, 2007, **129**, 9564–9565.
46 L. T. Burke, D. J. Dixon, S. V. Ley and F. Rodriguez, *Org. Biomol. Chem.*, 2005, **3**, 274–280.
47 L. T. Burke, D. J. Dixon, S. V. Ley and F. Rodriguez, *Org. Lett.*, 2000, **2**, 3611–3613.
48 K. Yuki, M. Shindo and K. Shishido, *Tetrahedron Lett.*, 2001, **42**, 2517–2519.
49 M. Binder and C. Tamm, *Angew. Chem. Int. Ed. Engl.*, 1973, **12**, 370–380.
50 S. Sekita, K. Yoshihira, S. Natori and H. Kuwano, *Tetrahedron Lett.*, 1973, **14**, 2109–2112.
51 K. Gebhardt, J. Schimana, A. Hölztzel, K. Dettner, S. Draeger, W. Beil, J. Rheinheimer and H.-P. Fielder, *J. Antibiot.*, 2004, **57**, 707–714.
52 R. Liu, Q. Gu, W. Zhu, C. Cui, G. Fan, Y. Fang, T. Zhu and H. Liu, *J. Nat. Prod.*, 2006, **69**, 871–875.
53 A. W. Dombrowski, G. F. Bills, G. Sabnis, L. R. Koupal, R. Meyer, J. G. Ondeyka, R. A. Giacobbe, R. L. Monaghan and R. B. Lingham, *J. Antibiot.*, 1992, **45**, 671–678.
54 K. L. Eley, L. M. Halo, Z. Song, H. Powles, R. J. Cox, A. M. Bailey, C. M. Lazarus and T. J. Simpson, *ChemBioChem*, 2007, **8**, 289–297.
55 Z. Song, R. J. Cox, C. M. Lazarus and T. J. Simpson, *ChemBioBhem*, 2004, **5**, 1196–1203.
56 S. A. Harkin, O. Singh and E. J. Thomas, *J. Chem. Soc. Perkin Trans. 1*, 1984, 1489–1499.
57 E. J. Thomas, *Acc. Chem. Res.*, 1991, **24**, 229–235.
58 W. Vedejs and R. C. Gadwood, *J. Org. Chem.*, 1978, **43**, 376–378.
59 G. Stork and E. Nakamura, *J. Am. Chem. Soc.*, 1983, **105**, 5510–5512.
60 S. A. Harkin, R. H. Jones, D. J. Tapolczay and E. J. Thomas, *J. Chem. Soc. Perkin Trans. 1*, 1989, 489–497.
61 A. P. Craven, H. J. Dyke and E. J. Thomas, *Tetrahedron*, 1989, **48**, 2417–2429.
62 H. A. Kirst, K. H. Michel, J. W. Martin, L. C. Creemer, E. H. Chio, R. C. Yao, W. M. Nakatsukasa, L. D. Boeck, J. L. Occolowitz, J. W. Paschal, J. B. Deeter, N. D. Jones and G. D. Thompson, *Tetrahedron Lett.*, 1991, **32**, 4839–4842.
63 P. Lewer, D. R. Hahn, L. L. Karr, P. R. Graupner, J. R. Gilbert, T. Worden, R. Yao, and D. W. Norton, US Patent No. 6,455,504, 2002.
64 T. C. Sparks, G. D. Thompson, H. A. Kirst, M. B. Hertlein, J. S. Mynderse, J. R. Turner and T. V. Worden, *Meth. Biotechnol.*, 1999, **5**, 171–188.
65 C. Waldron, K. Madduri, K. Crawford, D. J. Merlo, P. Treadway, M. Broughton and R. H. Baltz, *Antonie Van Leeuwenhoek*, 2000, **78**, 385–390.
66 C. Waldron, P. Matsushima, P. R. Rosteck, Jr., M. C. Broughton, J. Turner, K. Madduri, K. P. Crawford, D. J. Merlo and R. H. Baltz, *Chem. Biol.*, 2001, **8**, 487–499.
67 D. R. Hahn, G. Gustafson, C. Waldron, B. Bullard, J. D. Jackson and J. Mitchell, *J. Ind. Microbiol. Biotechnol.*, 2006, **33**, 94–104.
68 C. J. Martin, M. C. Timoney, R. M. Sheridan, S. G. Kendrew, B. Wilkinson, J. C. Staunton and P. F. Leadlay, *Org. Biomol. Chem.*, 2003, **1**, 4144–4147.
69 H. J. Kim, R. Pongdee, Q. Wu, L. Hong and H. W. Liu, *J. Am. Chem. Soc.*, 2007, **129**, 14582–14584.

70 D. J. Mergott, S. A. Frank and W. R. Roush, *Proc. Natl. Acad. Sci. U.S.A.*, 2004, **101**, 11955–11959.
71 S. M. Winbush, D. J. Mergott and W. R. Roush, *J. Org. Chem.*, 2008, **73**, 1818–1829.
72 R. M. Kagan and S. Clarke, *Arch. Biochem. Biophys.*, 1994, **310**, 417–427.
73 L. Brady, A. M. Brzozowski, Z. S. Derewenda, E. Dodson, G. Dodson, S. Tolley, J. P. Turkenburg, L. Christiansen, B. Huge-Jensen, L. Norskov, L. Thim and U. Menge, *Nature*, 1990, **343**, 767–770.
74 A. C. Horan, M. C. Shearer, V. Hedge, M. L. Beyazova, B. C. Brodsky, A. King, R. Berrie, K. Cardaci and M. Nimeck, *J. Antibiot.*, 1997, **50**, 119–125.
75 W. Keller-Schierlein, R. Muntwyler, W. Pache and H. Zahner, *Helv. Chim. Acta*, 1969, **52**, 127–142.
76 F. Tomita and T. Tamaoki, *J. Antibiot.*, 1980, **33**, 940–945.
77 J. A. Waitz, A. C. Horan, M. Kalyanpur, B. K. Lee, D. Loebenberg, J. A. Marquez, G. Miller and M. G. Patel, *J. Antibiot.*, 1981, **34**, 1101–1106.
78 A. Kawashima, Y. Nakamura, Y. Ohta, T. Akama, M. Yamagishi and K. Hanada, *J. Antibiot.*, 1992, **45**, 207–212.
79 R. Muntwyler and W. Keller-Schierlein, *Helv. Chim. Acta.*, 1972, **55**, 2071–2094.
80 X.-Y. Jia, Z.-H. Tian, L. Shao, X.-D. Qu, Q.-F. Zhao, J. Tang, G.-L. Tang and W. Liu, *Chem. Biol.*, 2006, **13**, 575–585.
81 L. Shao, X.-D. Qu, X.-Y. Jia, Q.-F. Zhao, Z.-H. Tian, M. Wang, G.-L. Tang and W. Liu, *Biochem. Biophys. Res. Commun.*, 2006, **345**, 133–139.
82 J. Fang, Y. Zhang, L. Huang, X. Jia, Q. Zhang, X. Zhang, G. Tang and W. Liu, *J. Bacteriol.*, 2008, **190**, 6014–6025.
83 H. Zhang, J. A. White-Phillip, C. E. Melancon, H. J. Kwon, W. L. Yu and H. W. Liu, *J. Am. Chem. Soc.*, 2007, **129**, 14670–14683.
84 Y. Sun, H. Hong, F. Gillies, J. B. Spencer and P. F. Leadlay, *ChemBioChem*, 2008, **9**, 150–156.
85 Y. H. Chen, C. C. Wang, L. Greenwell, U. Rix, D. Hoffmeister, L. C. Vining, J. Rohr and K. Q. Yang, *J. Biol. Chem.*, 2005, **280**, 22508–22514.
86 W. R. Roush and R. J. Sciotti, *J. Am. Chem. Soc.*, 1994, **116**, 6457–6458.
87 W. R. Roush, M. L. Reilly, K. Koyama and B. B. Brown, *J. Org. Chem.*, 1997, **62**, 8708–8721.
88 W. R. Roush and R. J. Sciotti, *J. Am. Chem. Soc.*, 1998, **120**, 7411–7419.
89 K. Takeda, E. Kawanishi, H. Nakamura and E. Yoshii, *Tetrahedron Lett.*, 1991, **32**, 4925–1928.
90 W. R. Roush and B. B. Brown, *J. Org. Chem.*, 1992, **57**, 3380–3387.
91 J. Xu, E. Wan, C.-J. Kim, H. G. Floss and T. Mahmud, *Microbiology*, 2005, **151**, 2515–2528.
92 T.-W. Yu, Y. Shen, Y. Doi-Katayama, L. Tang, C. Park, B. S. Moore, C. R. Hutchinson and H. G. Floss, *Proc. Natl. Acad. Sci. U.S.A.*, 1999, **96**, 9051–9056.
93 B. M. Harvey, H. Hong, M. A. Jones, Z. A. Hughes-Thomas, R. M. Goss, M. L. Heathcote, V. M. Bolanos-Garcia, W. Kroutil, J. Staunton, P. F. Leadlay and J. B. Spencer, *ChemBioChem*, 2006, **7**, 1435–1442.
94 T. Liu, D. You, C. Valenzano, Y. Sun, J. Li, Q. Yu, X. Zhou, D. E. Cane and Z. Deng, *Chem. Biol.*, 2006, **13**, 945–955.
95 B. Hadatsch, D. Butz, T. Schmiederer, J. Steudle, W. Wohlleben, R. Süssmuth and E. Stegmann, *Chem. Biol.*, 2007, **14**, 1078–1089.
96 K. Woithe, N. Geib, K. Zerbe, D. B. Li, M. Heck, S. Fournier-Rousset, O. Meyer, F. Vitali, N. Matoba, K. Abou-Hadeed and J. A. Robinson, *J. Am. Chem. Soc.*, 2007, **129**, 6887–6895.
97 B. Ostash, A. Saghatelian and S. Walker, *Chem. Biol.*, 2007, **14**, 257–267.
98 E. Zazopoulos, K. Huang, A. Staffa, W. Liu, B. O. Bachmann, K. Nonaka, J. Ahlert, J. S. Thorson, B. Shen and C. M. Farnet, *Nat. Biotechnol.*, 2003, **21**, 187–190.
99 P. C. Dorrestein, S. B. Bumpus, C. T. Calderone, S. Garneau-Tsodikova, Z. D. Aron, P. D. Straight, R. Kolter, C. T. Walsh and N. L. Kelleher, *Biochemistry*, 2006, **45**, 12756–12766.
100 R. P. McGlinchey, M. Nett, A. S. Eustaquio, R. N. Asolkar, W. Fenical and B. S. Moore, *J. Am. Chem. Soc.*, 2008, **130**, 7822–7823.
101 C. Zhang, B. R. Griffith, Q. Fu, C. Albermann, X. Fu, I.-K. Lee, L. Li and J. S. Thorson, *Science*, 2006, **313**, 1291–1294.