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DOI: 10.1002/anie.200806121

Polyketide Biosynthesis

The Biosynthetic Logic of Polyketide Diversity

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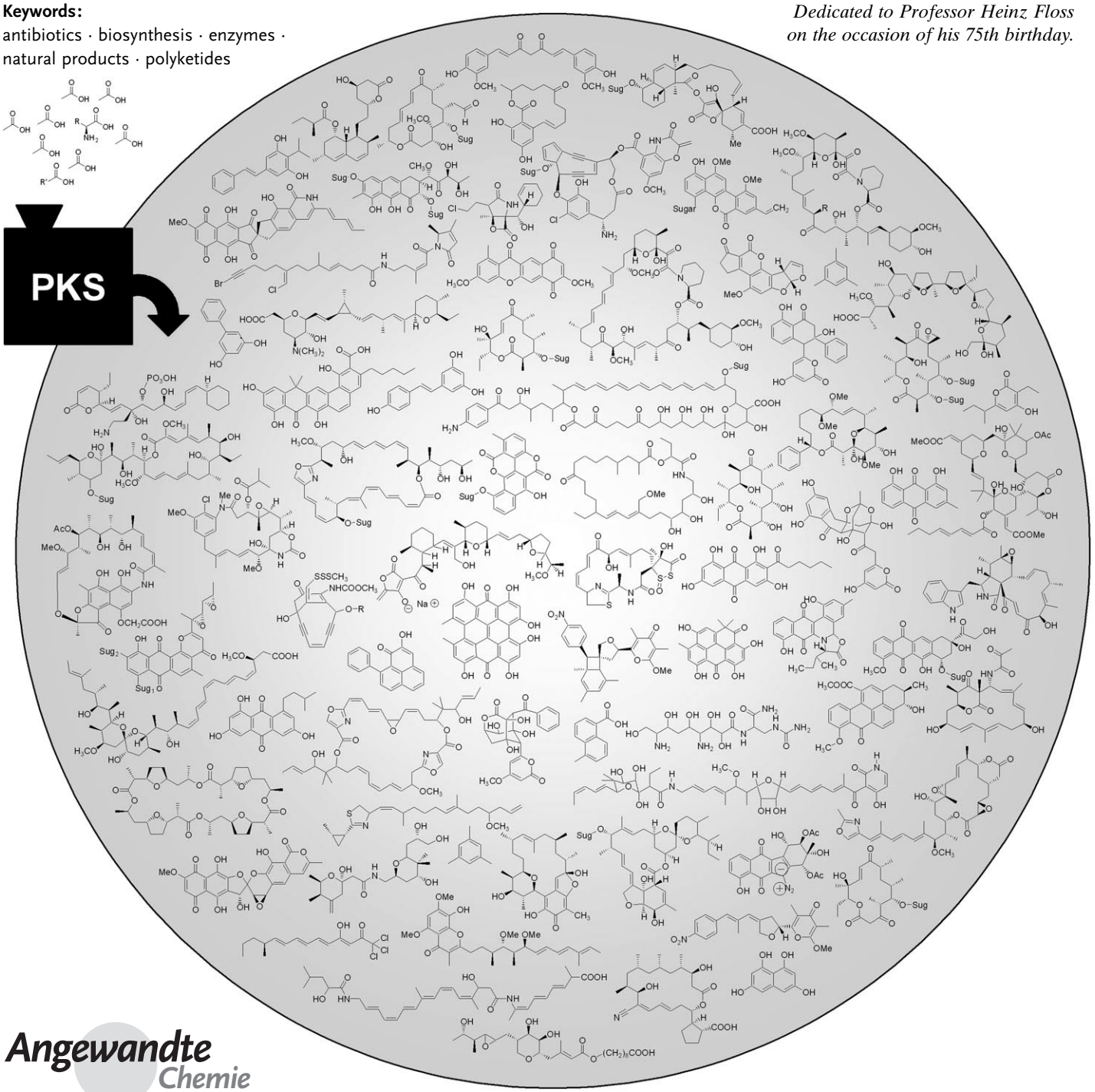
Keywords:
antibiotics · biosynthesis · enzymes · natural products · polyketides

Dedicated to Professor Heinz Floss on the occasion of his 75th birthday.



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Polyketides constitute one of the major classes of natural products. Many of these compounds or derivatives thereof have become important therapeutics for clinical use; in contrast, various polyketides are infamous food-spoiling toxins or virulence factors. What is particularly remarkable about this heterogeneous group of compounds comprising of polyethers, polyenes, polyphenols, macrolides, and enediynes is that they are mainly derived from one of the simplest building blocks available in nature: acetic acid. Investigations at the chemical, genetic, and biochemical levels have shed light on the biosynthetic programs that lead to the large structural diversity of polyketides .This review highlights recently unveiled biosynthetic mechanisms to generate highly diverse and complex molecules.

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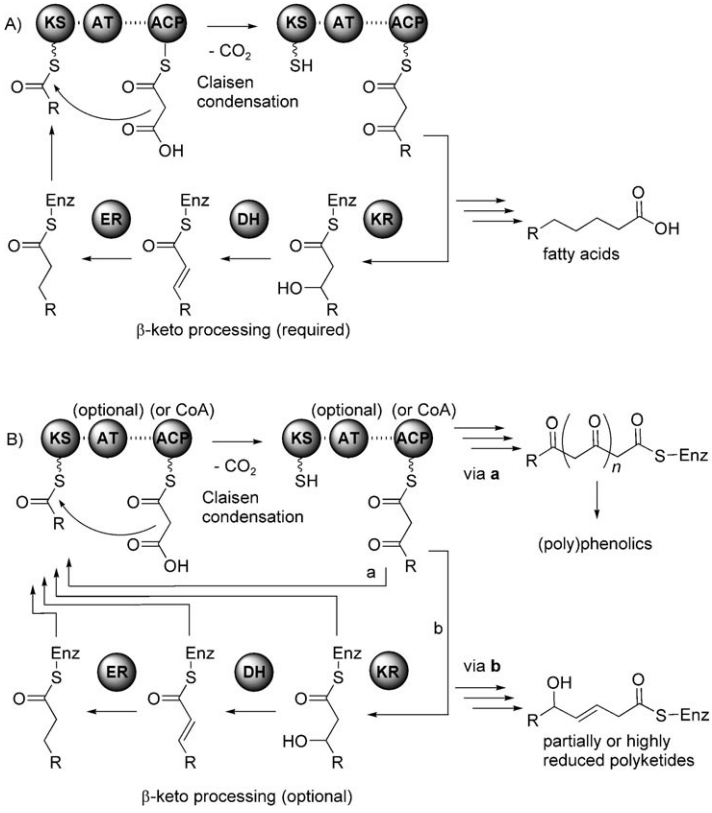
1. Polyketides and the Basic Programming

Polyketides represent a highly diverse group of natural products having structurally intriguing carbon skeletons which comprises polyphenols, macrolides, polyenes, enediynes, and polyethers. Although their exact roles in their original biological contexts are not known in all cases, it is believed that they function as pigments, virulence factors, infochemicals, or for defense. From a pharmacological point of view, polyketides are an important source of novel therapeutics. In particular, they are used in medicine mainly as antibiotics, immunosuppressants, antiparasitics, cholesterol-lowering, and antitumoral agents.[1] The highly complex structures and the strong pharmacological relevance of these compounds have triggered an immense endeavor to gain synthetic access to the natural products and derivatives thereof. Whereas the total chemical synthesis of polyketides is highly challenging, it is remarkable that their vast structural and functional diversity results from the controlled assembly of some of the simplest biosynthetic building blocks: acetate and propionate. Since the first biosynthetic considerations by Collie, who coined the term polyketide, and the 'acetogenin' hypothesis of Barton,[2] nature's virtuosity of linking and tailoring simple carboxylic acid monomers has become a fascinating interdisciplinary area of research. With the advent of molecular techniques,[3] it has now become possible to gain a better understanding of the biosynthetic logic of polyketide diversity. On the basis of this growing body of knowledge, polyketide biosynthesis pathways may be manipulated or redesigned for the production of novel drug candidates.[4–7]

1.1. Mechanisms of Chain Assembly

Polyketide biosynthesis has much in common with fatty acid biosynthesis. Not only are they alike in the chemical mechanisms involved in chain extension but also in the common pool of simple precursors employed, such as acetyl-coenzyme A (CoA) and malonyl-CoA (MCoA) units.[8] In general, both polyketides and fatty acids are constructed by repetitive decarboxylative Claisen thioester condensations of an activated acyl starter unit with malonyl-CoA-derived extender units (Scheme 1).[9] Typically, this process involves a β-ketoacylsynthase (KS), an optional (malonyl)acyl transferase (MAT/AT), and a phosphopantethienylated acyl carrier protein (ACP) or



Scheme 1. Basic mechanisms involved in fatty acid (A) and polyketide (B) biosynthesis. Enz = enzyme.

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coenzyme A (CoA), which serves as an anchor for the growing chain. After every chain elongation, the β-oxo functionality is processed by a ketoreductase (KR), dehydratase (DH), and an enoyl reductase (ER), which yields a fully saturated acyl backbone. However, polyketide biosynthesis deviates in many ways from fatty acid biosynthesis. Polyketide synthases (PKSs) clearly differ from fatty acid synthases (FASs) not only in their ability to use a broader range of biosynthetic building blocks but also in the formation of various chain lengths. Most importantly, whereas FAS typically catalyze a full reductive cycle after each elongation, in polyketide biosynthesis the reductive steps are optional; they can be partly or fully omitted before the next round of elongation, thus giving rise to a more complex pattern of functionalization (Scheme 1). Nonetheless, in both pathways the elongation/reduction cycles are repeated until a defined chain length is obtained, and finally the thioester-bound substrates are released from the enzyme complex. The primary products may then be subjected to additional modifications. Despite striking similarities in their enzymology in chain propagation, PKSs and FASs[9] are different and constitute a metabolic branch point between primary and secondary metabolism. Both pathways may have diverged at an early stage during evolution. Even so, in this context it may be interesting to note that PKSs may be involved in the biosynthesis of microbial polyunsaturated fatty acids[10,11] as well as mycobacterial cell wall lipids.[12]

1.2. Architectures of PKSs

On the basis of the architecture and mode of action of the enzymatic assembly lines, PKSs are classified into various types (Table 1).[3,13] As in FAS nomenclature, type I refers to linearly arranged and covalently fused catalytic domains within large multifunctional enzymes, whereas the term type II indicates a dissociable complex of discrete and usually monofunctional enzymes. Furthermore, a third group of multifunctional enzymes of the chalcone synthase type is denoted as type III PKSs. Apart from the structures of the enzymes or enzyme complexes, the PKSs are also categorized as iterative or noniterative, that is, whether or not each KS domain catalyzes more than one round of elongation. Noniterative type I PKSs, such as the archetypal erythromycin PKS, 6-deoxyerythronolide (6-dEB, **1**) synthase (DEBS)[14] are giant multimodular megasynthases which are mainly found in prokaryotes (Scheme 2).[14] Only recently, noniterative PKSs have also been found in protozoans, such as dinoflagellates.[15–17] A set of KS, AT, and ACP domains, as well as optional β-keto processing domains constitute a module, and generally, each module is responsible for only a single elongation cycle. The number of the modules thus correlates with the number of extension cycles executed by the PKS, and the presence of KR, DH, and ER domains defines the degree of β-keto processing.[18–20] The one-to-one correspondence of PKS architecture and metabolite structure is known as the principle of colinearity. The canonical rule not only serves for rational reprogramming of complex polyketide biosynthesis by genetic manipulations,[21] but also allows the prediction of metabolite structures from enzyme architectures and vice versa. Some bacterial modular type I PKSs do not fit into this scheme as modules may be used more than once or may be skipped, or the module architecture simply does not fit with the resulting metabolite structure. The latter are well represented in a subclass called trans-AT PKS where the modules lack individual AT domains. As opposed to the standard cis-AT PKS, the trans-AT PKS ACPs are loaded by stand-alone ATs.[22,23]

Iterative type I PKSs such as the lovastatin (**2**) synthase are a hallmark of fungal polyketide biosynthesis (Scheme 3).[24,25] According to the presence or absence of β-keto processing domains, fungal PKSs are classified as nonreducing (NR), partially reducing (PR), or highly reducing (HR) PKSs. Although the multidomain enzymes usually act in an iterative fashion, the degree of reduction can vary in each extended unit. KR, DH, ER, and even methyl transferase (MT) domains are optionally used in every extension round, thereby setting the substitution pattern. The factors governing this variability are largely unknown. Related bacterial (monomodular) iterative type I PKSs are scarce, and they are exclusively involved in the formation of small aromatic compounds or polyenes (including enediynes; see Section 3.6.2).

Table 1: Survey of the types of PKSs.[a]

PKS type	Building blocks	Organisms
Modular type I (non-iterative); subtypes: cis-AT, trans-AT	ACP, various extender units; (in situ methylation possible)	Bacteria, (protists)
Iterative type I subtypes: NR-, PR-, HR-PKS	ACP, only malonyl-CoA extenders (in situ methylation possible)	Mainly fungi, some bacteria
(Iterative) type II	ACP, only malonyl-CoA extenders	Exclusively bacteria
(Iterative) type III	Acyl-CoA, only malonyl-CoA extenders*	Mainly plants, some bacteria and fungi
PKS-NRPS hybrid	ACP, malonyl-CoA, amino acids	Bacteria (modular) fungi (iterative)

[a] Two exceptions reported (see Section 2.2.2).

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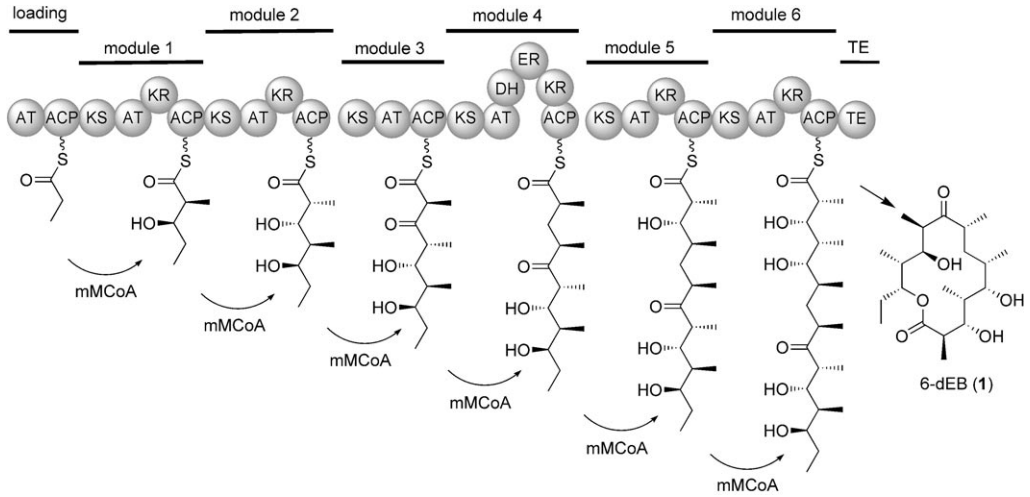
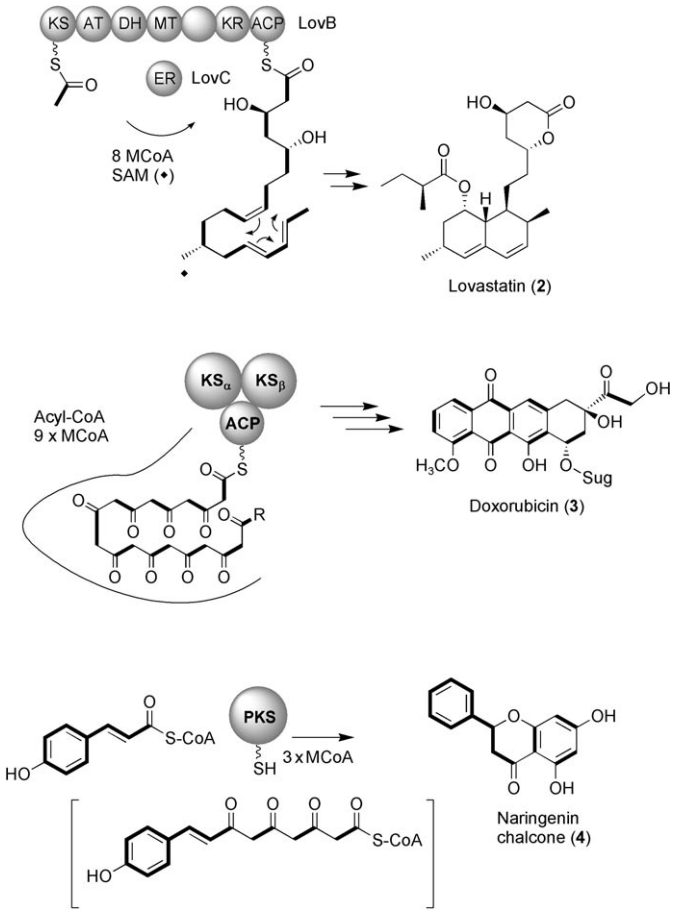


Scheme 2. The deoxyerythronolide-B-synthase (DEBS) required for erythromycin biosynthesis as an example of a modular type I PKSs.



Scheme 3. Examples of iterative PKSs involved in the biosynthesis of lovastatin (fungal iterative type I PKS), doxorubicin (bacterial type II PKS), and naringenin chalcone (plant type III PKS, chalcone synthase). Sug = sugar, SAM = *S*-adenosylmethionone.

In prokaryotes, iterative type II PKS systems are much more common, and also restricted to these organisms. In these systems, a minimal set of iteratively used enzymes, each expressed from a distinct gene, is required for polyketide assembly (e.g. doxorubicin (**3**)). This "minimal PKS" consists of two ketosynthase units (KS_α and KS_β, or chain length factor (CLF)) and an ACP, which serves in tethering the growing polyketide chain. Additional PKS subunits, including ketoreductases, cyclases (CYC), and aromatases (ARO) define the folding pattern of the nascent poly-β-keto intermediate.[26–28] Type II PKSs are mainly found in actinomycetes, and only two examples from Gram-negative bacteria are known.[29,30]

Type III PKSs are known from the well-studied family of plant chalcone/stilbene synthases (CHS/STS) which produce compounds such as naringenin chalcone (**4**). These enzymes are multifunctional in selecting the starter unit (e.g. *p*-coumaroyl-CoA), assembling the chain, and promoting its folding. Whereas type III PKSs have long been found in plants, during the last decade numerous related enzymes have been discovered from bacteria,[31,32] and, more recently, also from fungi.[33] In stark contrast to bacterial modular type I PKSs, but in analogy to FASs, the length of the polyketide backbone formed by an iterative PKS is apparently dictated by the size of the cavity within the ketosynthase component (or the entire complex).

In some cases, modules from type I PKSs are linked to nonribosomal peptide synthetase (NRPS) modules,[20] which results in the production of polyketide–peptide hybrid metabolites. Furthermore, various mixed polyketide pathways have been found, such as type III/type I, type I/type II, and FAS/PKS hybrids.

1.3. The Stages of Polyketide Diversification

The impressive diversity of polyketide structures results from a number of programmed events that occur before, during, and after chain assembly (Scheme 4). In all cases, the primary determinants are the type and number of biosynthetic building blocks employed.[34] β-Keto processing reactions and the resulting stereochemistry, as well as other in situ reactions, such as α- and β-alkylations, define the basic substitution pattern. After the synthesis of the polyketide

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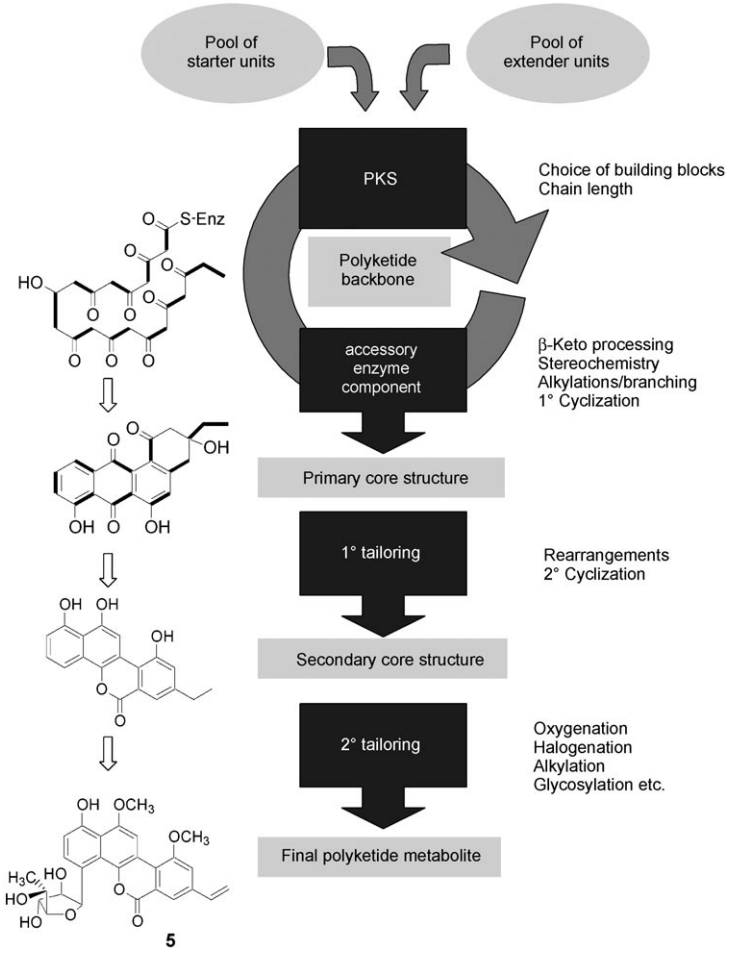


Scheme 4. Diversification levels in polyketide biosynthesis exemplified by the model of the gilvocarcin (**5**) pathway.

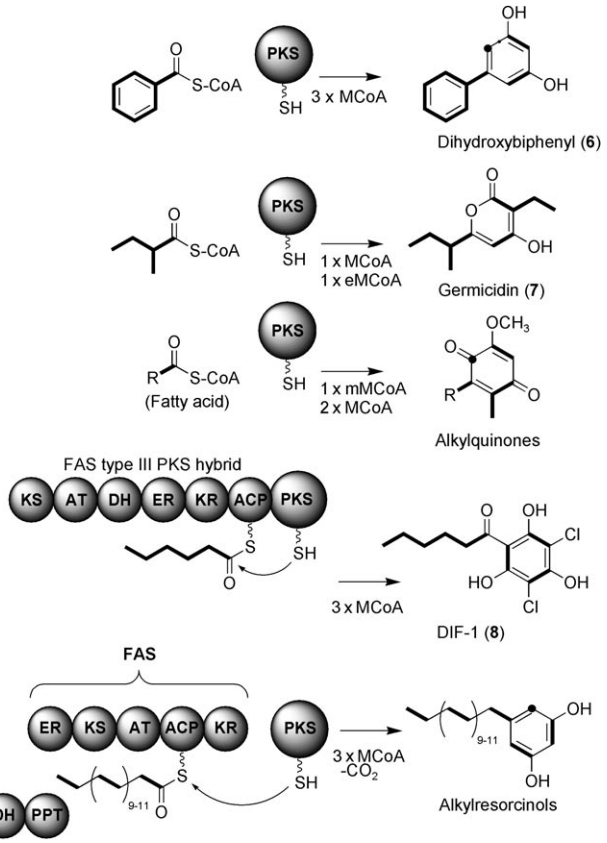
backbone is finished, the chain is released from the PKS by lactonization,[35] hydrolysis, other nucleophilic attacks (see Section 3.5), or reductive release,[36] and a subsequent core cyclization may occur. The resulting carbon skeleton can undergo secondary cyclizations, C–C cleavage, and rearrangement reactions which finally give rise to novel carba- and heterocycles. Finally, a broad range of tailoring reactions may decorate the polyketide structure.[37] Common biotransformations are C, O, and N glycosylations, alkylations, acyl transfers, hydroxylations, and epoxidations. Other known modifications involve halogenation, transamination, nitrile formation, and desaturation to yield alkynes.

2. Diversity of Aromatic Polyketides

2.1. Non-acetate Starter Units Used for Polyphenol Diversification

In most cases polyphenols derive from an acetyl primed polyketide chain, which is typically formed by decarboxylation of an activated malonyl unit. However, there are many examples from all types of iterative PKSs that utilize non-acetate starter units. Although the enzymology can be quite different, there are striking analogies in the various strategies to activate and load the primary building block.

Type III PKSs utilize various starter units such as hydroxy-substituted and nonsubstituted cinnamoyl (e.g., in CHS, STS) and benzoyl (e.g., in biphenyl synthase[38]) units, as well as fatty acids that are mainly activated as CoA thioesters (Scheme 5). The starter unit selection relies on spatial



Scheme 5. Utilization of non-acetate acyl-CoA starter units by type III PKS.

constraints of the substrate binding tunnel of the homodimeric protein.[39–41] Of particular importance is the Cys-His-Asn catalytic triad located in the active site cavity, which is connected to the CoA binding tunnel and binds to the starter unit through a thioester link to the Cys moiety.[40] In various cases, structure-guided mutagenesis resulted in a broadened substrate specificity.[42]

An "orphan" (or cryptic) type III PKS gene was found in the genome of *S. coelicolor* and is involved in the germicidin (**7**) biosynthesis.[43] The bacterial type III PKS also accepts branched fatty acid starters; it is the first member of this enzyme family which utilizes an extender unit other than malonyl-CoA (MCoA): In vitro studies showed that ethylmalonyl-CoA (eMCoA) is accepted as an extender molecule.[43] The second example demonstrating the utilization of type III PKS extender units other than malonyl-CoA has been reported by Horinouchi and co-workers. SrsA from *Streptomyces griseus* produces alkyl-substituted resorcinols, pyrones, and quinones from acyl-CoA units of various chain

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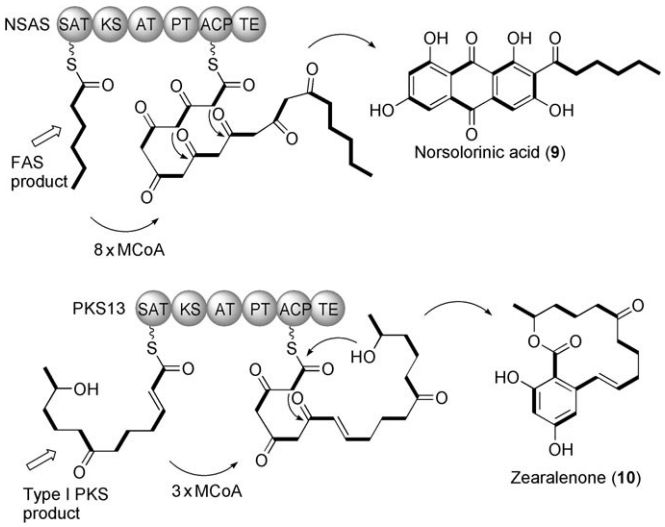
lengths, such as malonyl-CoA (MCoA) and methylmalonyl-CoA (mMCoA).[44] The factors controlling the selection of these unusual building blocks are yet to be determined. Whereas type III PKS starter units are usually provided as CoA thioesters, there are reports on the direct transfer of fatty acids from FAS to the type III PKS active site. A remarkable example is a type I FAS/type III PKS hybrid synthase involved in the biosynthesis of the differentiation-inducing factor, DIF-1 (**8**), of the slime mould *Dictyostelium discoideum*.[45] A similar scenario has been discovered by Horinouchi and co-workers in the context of phenolic lipid biosynthesis in the nitrogen-fixing soil bacterium *Azotobacter vinelandii*. In vitro experiments demonstrated that the fatty acid starter molecules are generated from an unusual type I FAS and are directly transferred to the type III PKS.[46] The resulting alkylresorcinols and related pyrones can replace membrane phospholipids, and are required for differentiation of the bacteria into metabolically dormant cysts under adverse environmental conditions.[47] A fungal type III PKS from *Neurospora crassa* accepts a number of aliphatic CoA thioesters, having chain lengths from 4 to 20 carbon atoms long, as starters and produces a variety of resorcinols and pyrones in vitro.[46,48]

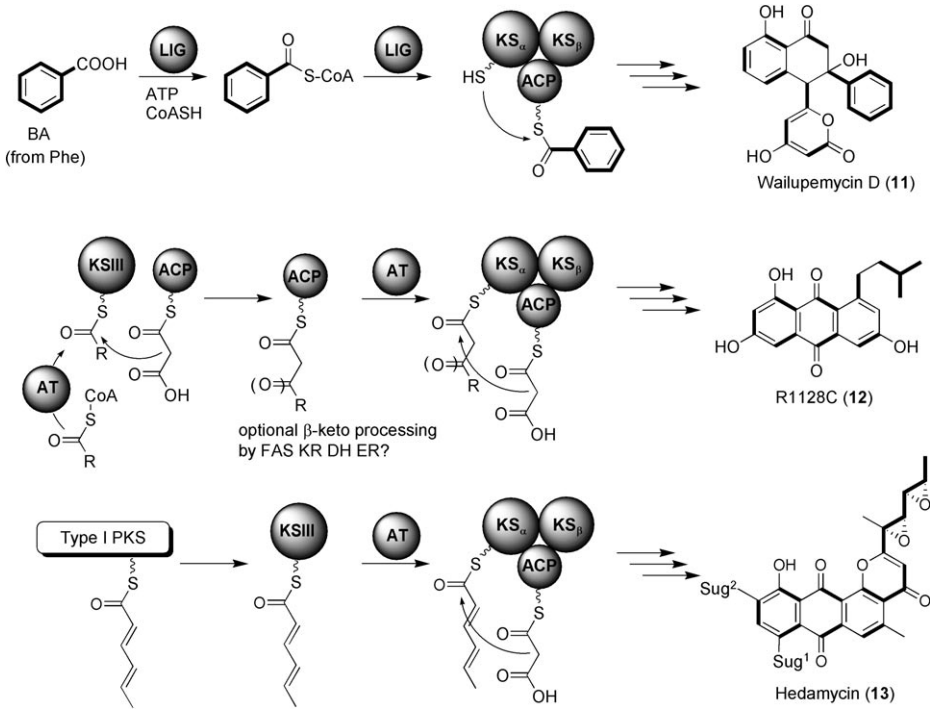
The priming of the fungal iterative type I PKS was a riddle until recently, when Townsend and co-workers systematically deconstructed fungal PKS domains and identified a starter acyl transferase (SAT) domain.[49,50] As shown for the hexanoate-primed norsolorinic acid (**9**) synthase (NSAS) this N-terminal domain is substrate specific and loads the starter molecule onto the PKS (Scheme 6). An alternative priming mechanism was observed in the pathway leading to zearalenone (**10**), a mycotoxin from *Gibberella zeae* which causes hyperestrogenic syndrome in animals.[51,52] Here, fungal HR-PKS and NR-PKS are capable of enzymatic teamwork. Tang and co-workers unveiled, by in vitro work, that the resorcilic acid-forming NR-PKS (PKS13) is primed with a reduced polyketide starter produced by a HR-PKS (PKS4). Surprisingly, PKS13 can also interact with the *Echerichia coli* fatty acid biosynthetic machinery and can be primed with alternative fatty-acyl ACPs.[53]

Non-acetate starter units that are known to be employed by type II PKS include propionate (anthracyclines), malonamate (tetracyclines), and a set of short linear and branched fatty acids, such as butyryl, valeryl, or 4-methylvaleryl starters (e.g., frenolicin and the R1128 complex).[54] In many cases, the biosynthetic logic behind the loading of alternative starters has been investigated and employed for pathway engineering.[55–57] The aryl side chains of various biosynthetically related antibiotics produced by *Streptomyces maritimus*, enterocin, and the wailupemycins, result from the incorporation of a rare phenylalanine-derived benzoyl starter unit (Scheme 7).[58–60] Moore and co-workers found that the priming of the type II PKS is reminiscent of that observed in NRPS pathways: the free acid is first adenylated, then activated as its CoA thioester, and transfered onto the ACP. All steps are catalyzed by a single ligase, EncN.[61] The blockage of primer biosynthesis and attachment allowed the first mutasynthesis of type II PKS products.[62]

The loading of short chain fatty acids typically involves a ketoacylsynthase component (KS III), homologues of which are known from bacterial fatty acid biosynthesis, for example, FabH from *E. coli*, which is required for the selection of the



Scheme 6. Priming of the iterative fungal type I PKSs by the starter acyl transferase (SAT) domain.



Scheme 7. Strategies for priming the type II PKS with non-acetate starter units. LIG = ligase.

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starter and the first elongation step. KS III shares functional similarity with type III PKSs as they feature the highly conserved Cys-His-Asn catalytic triad responsible for priming/transacylation, decarboxylation, and condensation. Homologues of these enzymes are characteristic for most aromatic polyketide pathways which are initiated with non-acetate starter units, such as those giving rise to the anthracyclines, R1128, and frenolicin.[54] More recent examples for the utilization of short fatty acid starters are the hedamycin (**13**),[63] fredericamycin,[64], benastatin,[65] and alnumycin[66] biosynthetic pathways. Additional ACP and AT components may be involved in tethering and transferring the acyl units, respectively, but they are not found in all cases. Furthermore, the newly generated β-keto group may or may not undergo a full reduction, which is likely mediated through cross talk with FAS enzymes.

Thorson and co-workers have implicated a novel priming mechanism in the hedamycin (**13**) biosynthetic pathway. Analysis of the biosynthetic gene cluster suggested that the pluramycin-type antibiotic is assembled from a hexadiene starter provided by an iterative type I PKS (Scheme 7). An additional ketosynthase (KSIII, HedS) and a putative acyl transferase (HedF) might assist in the priming process.[63]

The KS III component also exerts some control over primer selection, as shown in the benastatin (**14**) pathway (Scheme 8). The biosynthesis of these potent glutathione S-transferase inhibitors and inducers of apoptosis starts with a hexanoate unit. In the absence of the KS III, various analogues having modified side chains are produced. If a shorter fatty acid (butyrate) starter is incorporated, the length of the polyketide backbone is increased, resulting in the formation of an extended hexacyclic ring system (**15**) reminiscent of intermediates in the griseorhodin and fredericamycin biosynthetic pathways.[65,67]

2.2. Getting Polyphenols into Shape

Polyphenols result from directed cyclocondensations of poly-β-keto intermediates or only partially reduced polyketide chains produced by iterative PKSs. Despite variation in their architecture, the enzymes responsible for the biosynthesis of polyphenols are capable of generating and stabilizing the highly reactive intermediates through preorganization of their folding mode. To avoid spontaneous aldol chemistry and to direct the cyclizations into defined reaction channels, particular enzymatic functions are essential. The situation has become more apparent since the discovery of designated cyclization cavities (in type III PKSs, see Section 2.2.2), product template (PT) domains (in iterative fungal PKSs, see Section 2.2.3), or discrete accessory components (cyclases and aromatases, in type II PKSs, see Section 2.2.4) which function in a chaperone-like manner and direct the growing chain into a particular reaction channel.

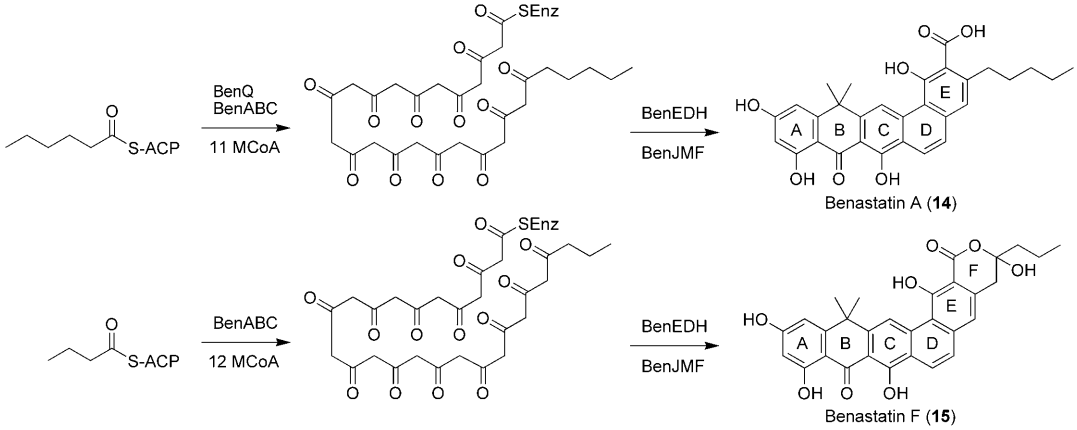
2.2.1. Divergent Plant, Fungal, and Bacterial Polyketide Folding Modes

One of the great mysteries in aromatic polyketide biosynthesis is the molecular basis for diverging cyclization patterns in plants, fungi, and bacteria.[68] Isotope labeling experiments revealed that Gram-positive bacteria (such as *Streptomyces* spp.) typically construct polyphenols in which the first rings (resulting from the "bend") are composed of three intact acetate units. In contrast, most (but not all) fungal polyphenols result from an F-type folding wherein analogous rings are composed of two intact acetate units and two partial acetate units. The diverging folding modes were illustrated by Bringmann et al. who investigated the biosynthesis of chrysophanol (**16**), a pigment and chemical defense agent. In eukaryotes (fungi, higher plants, and insects) chrysophanol is formed by the folding mode F (for fungi). In actinomycetes, by contrast, the cyclization proceeds through mode S (for *Streptomyces*) (Scheme 9).[69]

Whereas this comparative biosynthetic study highlights that a polyketide can be assembled by more than one polyketide folding mode, the enzymatic factors governing the F- or S-mode cyclizations remain elusive.

2.2.2. Aromatic Polyketides from the Type III PKS/Chalcone Synthase Superfamily

The all-in-one multifunctional type III PKS enzymes select the starter unit, govern the polyketide assembly, and catalyze specific cyclization reactions. Usually, only small benzol or naphthol rings are formed. The first enzymes



Scheme 8. Expansion of a polyphenol ring system by chain elongation. BenQ = KS III, BenABC = minimal PKS (min PKS), BenEDHJMF = cyclase and modifying enzyme.

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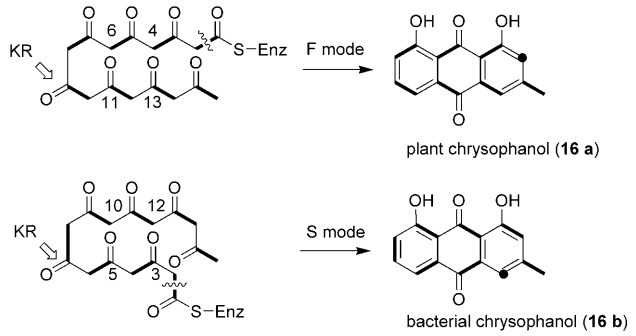
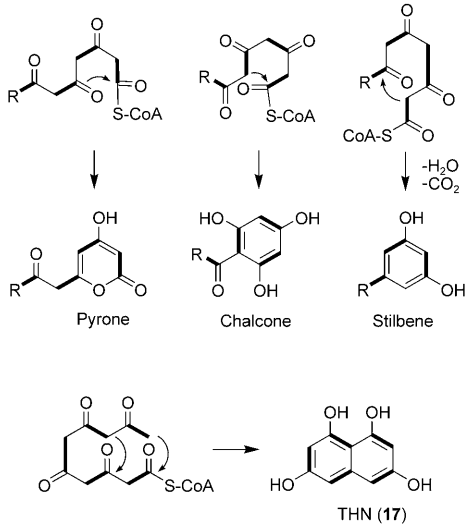


Scheme 9. Different folding modes observed in anthraquinone biosynthetic pathways.

constituting this family are the plant-specific 2-pyrone synthases (2-PSs), which form the triketide methylpyrone from an acetyl-CoA starter molecule and two malonyl-CoA units, and chalcone synthases (CHSs), which produce the tetraketide chalcone from *p*-coumaroyl-CoA and three malonyl-CoA units with subsequent Claisen ester condensation. Notably, plant stilbene synthases (STSs) employ the same precursor but catalyze an aldol-type cyclization (Scheme 10). Structural studies revealed that the chain length and cyclization modes are defined by enzyme cavities and active site architectures.[70]

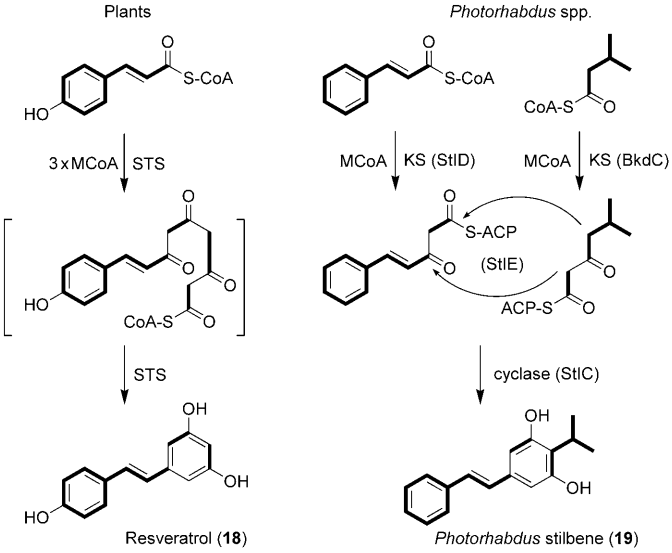


Scheme 10. Principle cyclization reactions observed in plant type III PKSs and bacterial type III PKSs (THN).

These three principal avenues give rise to the large family of plant pyrones and phenylpropanoids such as naringenin chalcone and resveratrol.[71] A more recent example of plant type III PKSs is the chromone pentaketide PKS from aloe.[72] Surprisingly, site directed mutagenesis yielded a synthase that produces longer chains (octaketides) that undergo spontaneous cyclization to give known bacterial polyketide shunt products.[73]

Elucidation of the crystal structure of tetrahydroxynaphthol (THN (**17**)) synthase, the first type III PKS from a bacterium (*Streptomyces coelicolor*),[74] revealed that the cavity for chain elongation and cyclization is extended compared to that of the plant homologues (Scheme 10).[40] Interestingly, THN is also known as a fungal metabolite, but the enzymology of its formation is dramatically different, as an iterative type I PKS is involved, and the hexaketide chain is derived from degradation of a heptaketide precursor.[74]

Another biosynthetic convergence of PKS metabolites has been observed in plants and entomopathogenic bacterial nematode symbionts. Bacteria of the genus *Photorhabdus* can assemble stilbenes in a radically different way compared to plants (Scheme 11). In a convergent biosynthesis two discrete



Scheme 11. Biosynthesis of stilbenes in plants and bacteria.

ketosynthases first elongate cinnamoyl, for example, using isovaleryl units, which then undergo a cyclase-catalyzed condensation. The resulting stilbenes (e.g., **19**) are multifunctional as they not only act as antibiotics and inhibitors of the insect immune system, but may also serve as a signal between taxonomic kingdoms, which is required for normal growth and development of the nematode hosts.[75]

The convergent biosynthesis of a phenolic compound from a diketide and a second thioester component is reminiscent of recently identified plant type III PKSs which produce diketides and catalyze a head-to-head condensation of CoA thioesters (Scheme 12). An important example is the type III PKS leading to diarylheptanoids (**20**) and phenylphenalenones (**21**) in *Wachendorfia thyrsiflora*, which has been studied by Schneider, Schroeder, and co-workers.[76] The formation of a diarylheptanoid (**22**) from two 4-coumaroyl-CoA units and one malonyl-CoA (MCoA) was also shown in vitro using a type III PKS (curcuminoid synthase) from *Oryza sativa*.[77] By analogy, gingerol (**23**) could be produced by the same general mechanisms, although the PKS has yet to be identified.[78]

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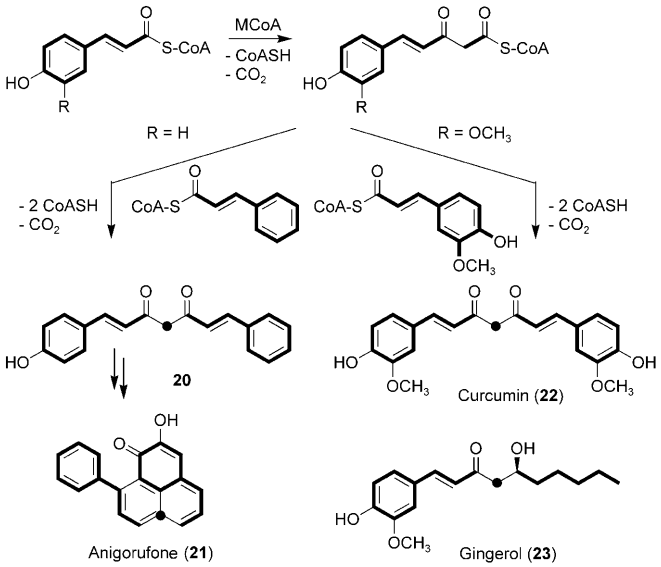


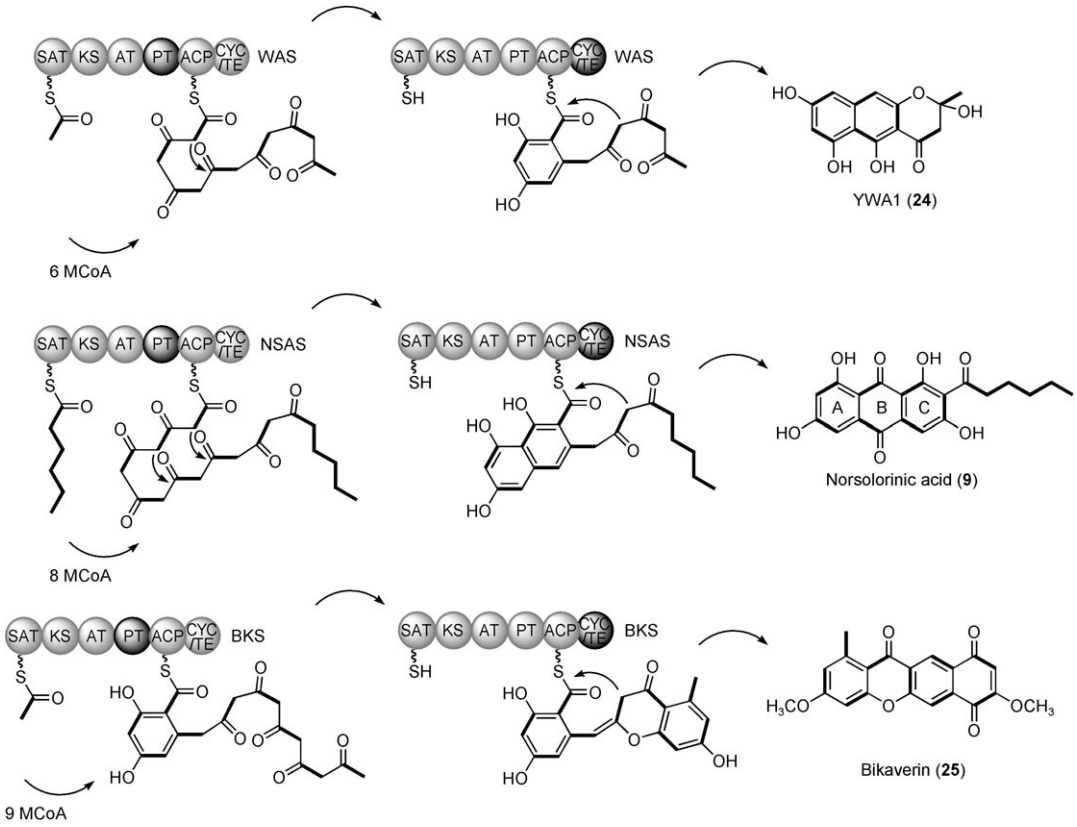
Scheme 12. Model of the diarylheptanoid (curcumin) and phenylphenalenone (anigorufone) biosyntheses and, by analogy to the gingerol biosynthesis.

2.2.3. *Control of Fungal Polyphenol Biosynthesis*

Townsend and co-workers have recently provided the stepping stone to understanding fungal polyketide cyclization. By using bioinformatic deconstruction of the multifunctional proteins, they have identified a product template (PT) domain that is relevant for the correct cyclization of ACP-bound polyketide intermediates.[79] The sequence variations in PT domains from various PKSs, for example, the yWA1 (**24**) synthase (WAS), norsolorinic acid (**9**) synthase (NSAS), and bikaverin (**25**) synthase (BKS), correlate with the chain length of their products, thus suggesting that cavities of different sizes are provided (Scheme 13). The function of PT was experimentally proven in the aflatoxin biosynthesis wherein it drives aromatization to irreversibly form rings A and B (Scheme 13) and to increase the flux of the norsoloronic acid precursor from the PKS enzyme.[79] Ebizuka and co-workers showed previously that the final cyclization to yield YWA1 (**24**) is accomplished by a Claisen ester cyclization catalyzed by a cyclase/thioesterase (CYC/TE) domain.[80] The essential role of the C-terminal thioesterase/Claisen cyclase (CYC/TE) domain for the final cyclization has been shown in vitro using wild type and a mutated version of the bikaverin synthase from *Gibberella fujikuroi*.[81] Complementation of the mutant PKS4 with a stand-alone CYC/TE domain restored the regioselective cyclization steps.

2.2.4. *Orchestrating Polyphenol Biosynthesis through Type II PKS Multienzyme Complexes*

The range of basic phenolic ring structures generated by type III and iterative type I PKSs is rather limited (Scheme 15). Exclusively linear mono- to tetracyclic aromatic compounds are obtained, which is likely because of the spatial restrictions in the plant type III PKS substrate channels or in



Scheme 13. Control of polyphenol biosynthesis in fungi, which is mediated by product template (PT) and cyclase/thioesterase (CYC/TE) domains.

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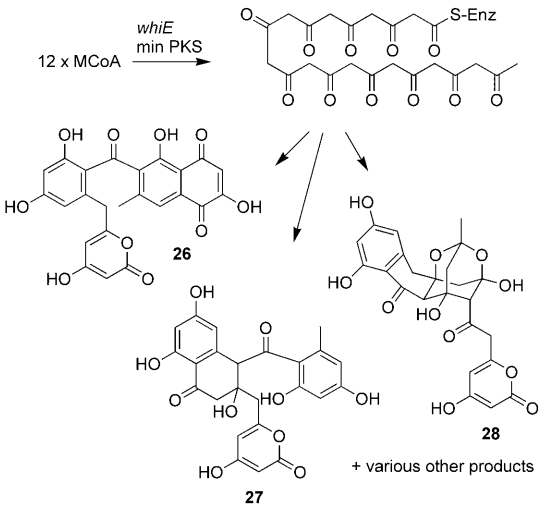
the cavities of the PT domains of fungal PKS. In contrast, there is much higher diversity in the ring topologies of bacterial metabolites. Possible explanations are that a) the polyketide chains are longer and b) the type II PKS multienzyme complexes, with the participation of up to three cyclases, have a greater flexibility in shaping the polyketide backbone.

The chain length of polyketides synthesized by type II PKSs usually ranges between 16 (octaketides, such as actinorhodin), 20 (decaketides, such as tetracenomycin), and 24 (dodecaketides, such as pradimicin) units. The longest chains occur in the griseorhodin (tridecaketide), benastatin (tetradecaketide), and fredericamycin (pentadecaketide) pathways. In type II PKS complexes, chain length is largely controlled by the KS_β subunit, which is also termed the chain length factor (CLF), but it appears that the entire complex has an impact on the size of the metabolite.[82,83] However, the importance of the CLF in controlling chain length[84] has been shown by various in vivo and in vitro experiments. Furthermore, the chain length is defined by "measuring" (i.e. the size of the enzyme cavities), not by "counting".[85] Recent structural modeling studies suggested that the "gatekeeper" amino acid residues defining chain length are located at the interface of the KS_α/KS_β heterodimer.[83,84]

Khosla, Stroud, and co-workers solved the first crystal structure of a KS_α/KS_β heterodimer and demonstrated that the nascent, highly reactive polyketide intermediate is stabilized by the PKS.[86] However, to direct a controlled cyclization of the poly-β-keto chains into defined polyphenol structure, cyclases and aromatases are needed. All cyclases, although quite heterogeneous in sequence and structure, function in a chaperone-like manner and catalyze specific aldol condensations; aromatases support the cyclodehydration process. Through systematic gene inactivation, recombination, and in vitro enzymatic studies, a large body of knowledge has been obtained on how bacterial PKSs generate polyphenolic ring topologies using cyclases.[28] In the absence of these enzymes or when an incomplete or mismatched set of enzymes is present, the polyketide chain undergoes random cyclizations. This process has been impressively demonstrated for the *whiE Streptomyces coelicolor* pigment synthase, which yields numerous structurally intriguing shunt products (**26**–**28**) such as a substituted dioxoadamantane **28** (Scheme 14).[87]

Various investigations indicate that the first cyclization (i.e. C9–C14 or C7–C12) is—at least in part—controlled by the PKS.[26–28] Furthermore, the presence or absence of a KR has some impact on the preformation of a bend in the carbon chain. Notably, if present, the primary KR, which acts on the nascent chain and has an impact on the first cyclization, exclusively reduces the C9-position. Through targeted knockout and coexpression, cyclase functions could be attributed to individual cyclization steps, such as second and third ring formation, and so forth (Scheme 15). Typical primary products of type II PKSs, resulting from the concerted action of PKS, KR, and cyclases, are polyphenols that can be classified as the linear tetracyclines, anthracyclines, benzoisochromanequinones, tetracenomycins, aureolic acids, and the angular angucyclines, as well as a group of pentangular polyphenols.



Scheme 14. Examples of polyketides resulting from the spontaneous cyclization of a poly-β-keto intermediate. min PKS = minimal PKS.

Whereas it has been possible to redirect the polyketide cyclization mode in some cases,[88,89] apparently there are design rules or particular structural restraints which cause some incompatibility between the types of PKSs and cyclases.[90] Nonetheless, it is remarkable that bacterial cyclases can affect the cyclization of polyketides produced by (mutated) fungal PKSs. The addition of the first ring aromatase/cyclase from the griseusin PKS and the second ring cyclase from the tetracycline pathway (OxyN) to the bikaverin PKS resulted in the formation of anthraquinone products. These experiments impressively show how fungal PKSs can be complemented with in trans-acting domains and tailoring enzymes even from distinct families of PKSs.[81]

In bacteria the basic ring systems are almost exclusively formed by a U-shaped folding of the poly-β-keto intermediates. Consequently, only a limited number or modes of cyclizations is realized, and virtually all polyphenols have a linear or angular architecture. Clear exceptions from this biosynthetic scheme are the pentacyclic polyphenols resistomycin (**29**) and resistoflavin. The multiple perifused rings result from an unparalleled S-shaped folding, cyclization of a decaketide,[91] and hydroxylation.[92] Recent biochemical studies support a model in which the "discoid" ring system is shaped by a cagelike multienzyme complex and not by sequentially acting cyclases.[89] Although conceivable, the geminal bismethylation does not contribute to the formation of the perifused polyphenol.[93] More recently, an S-shape folding pattern has been found to take place in the formation of the naphthoyl residue of azinomycin (**30**; Scheme 16).[94] Here, a bacterial iterative type I PKS is capable of promoting the rare cyclocondensations.

2.2.5. *Derailment of Aromatic Polyketide Cyclization (Favorskiiase)*

In virtually all type II polyketide pathways, cyclases mediate polyketide cyclization to generate aromatic ring systems, but none of these enzymes are involved in the biosynthesis of the antibiotic enterocin and related benzoyl-

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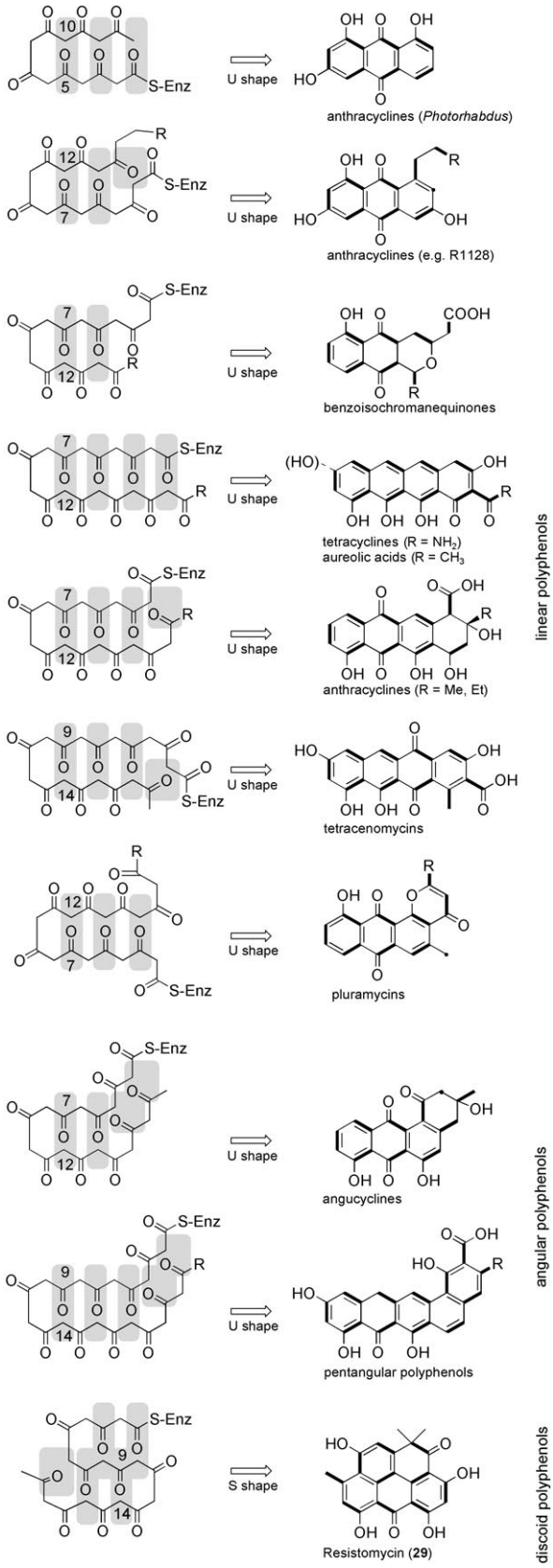
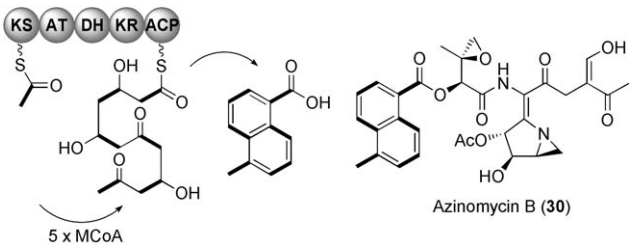
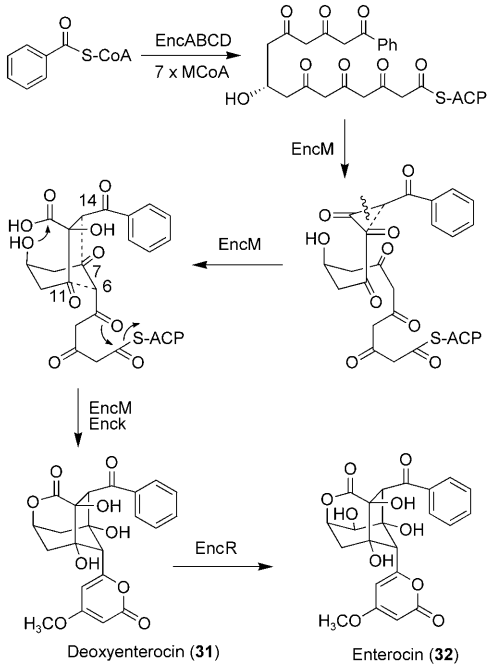


Scheme 15. Overview of the polyketide cyclization patterns of the bacterial type II PKS products.



Scheme 16. S-shaped cyclization of a hexaketide by a bacterial iterative type I PKS.

primed polyketides in the marine-derived bacterium *Streptomyces maritimus*.[58,95,96] Moore and co-workers demonstrated, by using in vivo and in vitro experiments, that in lieu of cyclases, a rare oxygenase (EncM) is the key to structural diversity,[97] and it also controls the overall shape of the cage molecule enterocin.[98] EncM catalyzes a remarkable reaction sequence which is reminiscent of a Favorskii-type rearrangement. The presumed substrate of EncM is a linear C9-reduced octaketide which is oxidized at C12 to form a trione intermediate (Scheme 17). Furthermore, EncM promotes two aldol condensations, one between C6 and C11 and one between C7 and C14, thus defining the absolute configuration of the product. Finally, EncM participates in two heterocycle-forming reactions during the formation of desmethyl-5-deoxyenterocin.[98] The final hydroxylation step from 5-deoxyenterocin to enterocin is catalyzed by EncR, a cytochrome P-450 monooxygenase.[58] The enterocin biosynthetic pathway was the first to be fully reconstituted in vitro as an enzymatic total synthesis.[99]



Scheme 17. The biosynthetic pathway to enterocin which requires a 'Favorskiiase', EncM. EncABC = minimal PKS, EncD = KR, EncK = MT, EncR = oxgenase.

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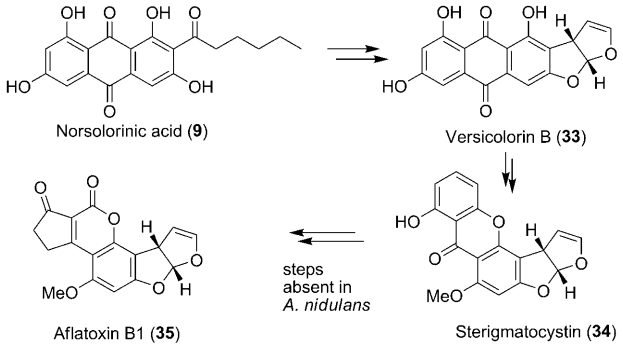
2.3. Polyphenol Ring Extensions, Rearrangements, and Heterocyclizations

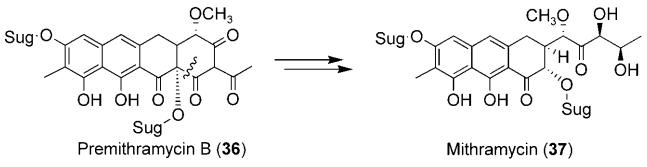
On the basis of the set of carbocycles produced by polyketide folding and condensation, enzymatic transformations may lead to a variety of modified ring structures. Frequently observed biosynthetic strategies to access such secondary polyketide cores are ring extensions through couplings or condensations, additional cyclizations of protruding carbon chains or substituents, C–C bond cleavages, and subsequent rearrangements of the skeleton. A particularly stunning example for a global rearrangement of a primary polyketide skeleton is the stepwise transformation of the pigment norsolorinic acid into the mycotoxins sterigmatocystin (**34**) and aflatoxin B1 (**35**) by the fungus *Aspergillus flavus* (Scheme 18).[100] Although not all steps have been elucidated in detail, this sequence demonstrates the impact of oxygenases on the final polyketide structure.



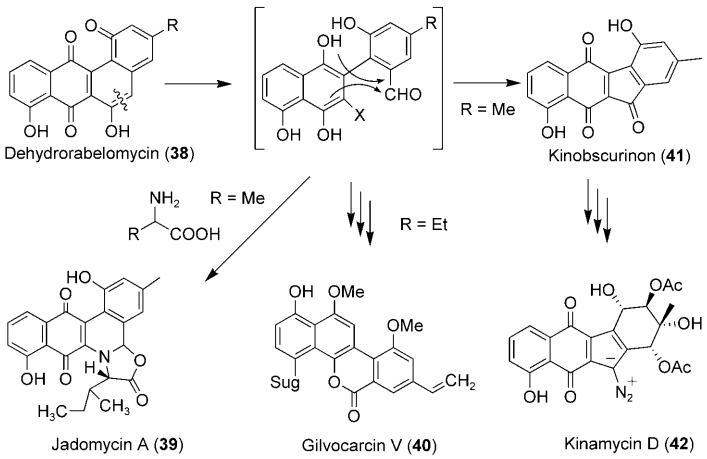
Scheme 18. Oxidative rearrangement of norsolorinic acid into the fungal toxins sterigmatocystin and aflatoxin B1 in *Aspergillus flavus*.

The initial step of bacterial polyphenol diversification through rearrangements typically involves an oxidative C–C bond cleavage, often catalyzed by Baeyer–Villiger-oxygenases (BVOs). One of the best studied BVOs catalyzes the D-ring cleavage of premithramycin B (**36**) in the biosynthesis of mithramycin (**37**) (Scheme 19).[101] Surprisingly, inactivation of one of the early-acting oxygenases yielded a tetracyclic shunt product with a five-membered D ring.[102]

A large number of different ali- and heterocyclic metabolites is derived from angucyclic polyphenol precursors (Scheme 20). The initial C-ring cleavage is also a key step in the pathways leading to the antibacterial jadomycins and antitumoral gilvocarcins from *Streptomyces venezuelae* and



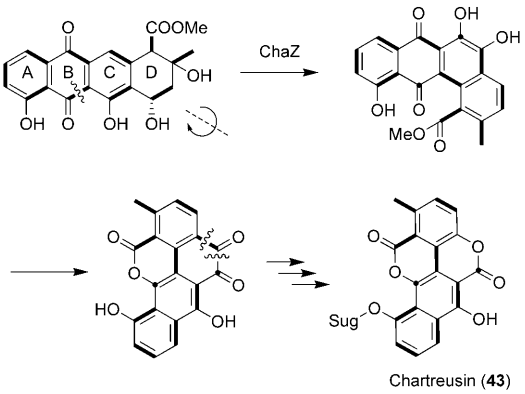
Scheme 19. Oxidative ring cleavage by a Baeyer–Villigerase in the mithramycin biosynthesis.



Scheme 20. Oxidative rearrangements of angucyclines leading to structural diversity. X = unknown.

Streptomyces griseoflavus, respectively. Rohr and co-workers have elucidated such multistep angucycline rearrangements and have demonstrated the participation of multioxygenase complexes.[103–105] In the gilvocarcin V (**40**) biosynthesis, a new ring is formed through lactonization, whereas in the jadomycin A (**39**) biosynthesis amino acids are incorporated to yield the N-heterocycle.[106] Another possibility has been proposed for the biosynthesis of the kinamycins, a group of unusual diazonium antibiotics from *Streptomyces murayamaensis*. By using isotope labeling and genetic studies it was concluded that the angucyclic dehydrorabelomycin (**38**) is transformed into kinobscurinone (**41**), and possibly involved a BVO.[107]

The unusual pentacyclic aglycone of chartreusin (**43**) from *Streptomyces chartreusis* is structurally related to gilvocarcin and also represents a highly efficient DNA intercalator.[108] Chartreusin biosynthesis involves the unprecedented rearrangement of a linear ring system, as proven by genetic inactivation and identification of an anthracyclic precursor. According to the current biosynthetic model,[109] a quinone C–C bond is cleaved by a BVO and a new C–C bond is formed between the carbonyl group and the unsubstituted carbon atom of the C ring (Scheme 21). After a rearrange-



Scheme 21. Oxidative rearrangement of an anthracycline into the dioxabenzo[a]pyrene framework of chartreusin. ChaZ = oxygenase.

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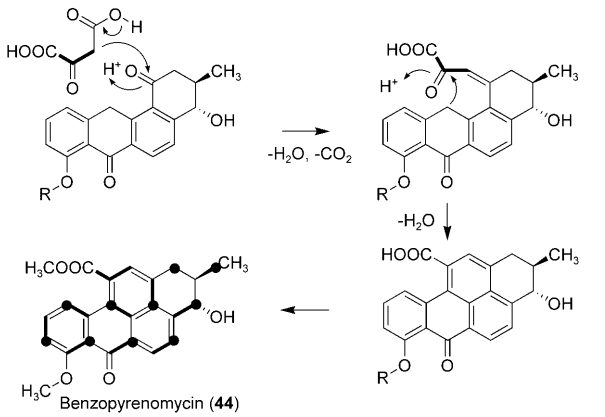
ment cascade, which remains hypothetical, a putative oxabenzo[a]pyrene dione is formed. A dioxygenase then disrupts the dione moiety and yields the dioxabenzo[a]pyrene ring system of the chartreusin aglycone.[109] In this context, notably, only recently was the first benzo[a]pyrene natural product, benzopyrenomycin (**44**), identified from a culture of a *Streptomyces lavendulae* strain (Scheme 22). The presence

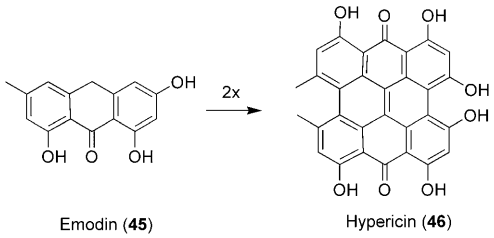


Scheme 22. Proposed formation of benzopyrenomycin from an angucyclic precursor.

of angucyclic congeners and the similarity in their substitution pattern suggest that the benzopyrene scaffold results from the condensation of an angucyclic anthrone precursor with a four-carbon building block such as oxaloacetate.[110]

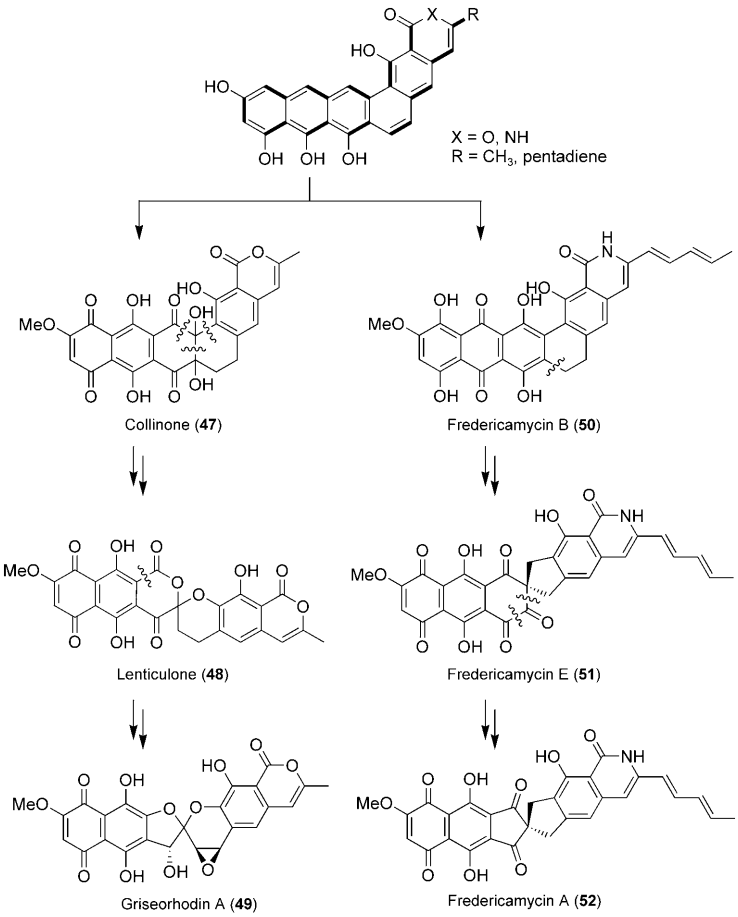
Polyketide folding patterns leading to such multicyclic perifused ring systems are not yet known. However, individual perifused polyphenols can be generated by aryl couplings. Such reactions are possibly promoted by laccaselike enzymes.[111] A famous example for a pathway involving multiple aryl couplings is the biosynthesis of the photosensitizer hypericin (**46**; Scheme 23). The enzymology behind its formation has not yet been elucidated despite recent progress in detecting enzyme candidates in St. John's wort.[112,113] Notably, the higly reactive benzylic position of the anthrones is prone to spontaneous radical-mediated dimerization, as demonstrated for related polyphenols.[114]

Complex oxidative rearrangement processes are involved in the pathways leading to the structurally intriguing spiro compounds griseorhodin A (**49**) and fredericamycin A (**52**), which share early biosynthetic steps in the formation of the pentangular core structure (Scheme 24).[115] Li and Piel have identified the gene cluster coding for the griseorhodin biosynthesis in a marine *Streptomyces* sp.[116] A biosynthetic model was proposed on the basis of putative gene functions and a shunt product or intermediate, collinone (**47**), was obtained by expressing incomplete pathway genes involved in the formation of the related compound rubromycin,[117] and the detection of lenticulone (**48**) from an



Scheme 23. Model of hypericin formation from two emodin units as an example for extending ring systems by aryl couplings.

engineered oxygenase mutant.[118] Accordingly, four carbon–carbon bonds are consecutively cleaved en route to the final spiro compound. The carbaspiro metabolite fredericamycin A (**52**) from *Streptomyces griseus* results from an equally sophisticated albeit different process as proposed by Shen and co-workers on the basis of gene cluster analyses. The structure elucidation of plausible pathway intermediates, such as fredericamycin E (**51**), sheds more light on the rearrangement step. The final steps possibly involve a benzylic acidlike rearrangement.[119]



Scheme 24. A model for the biosynthesis of the spiro polyphenols griseorhodin A (**49**) and fredericamycin A (**52**).

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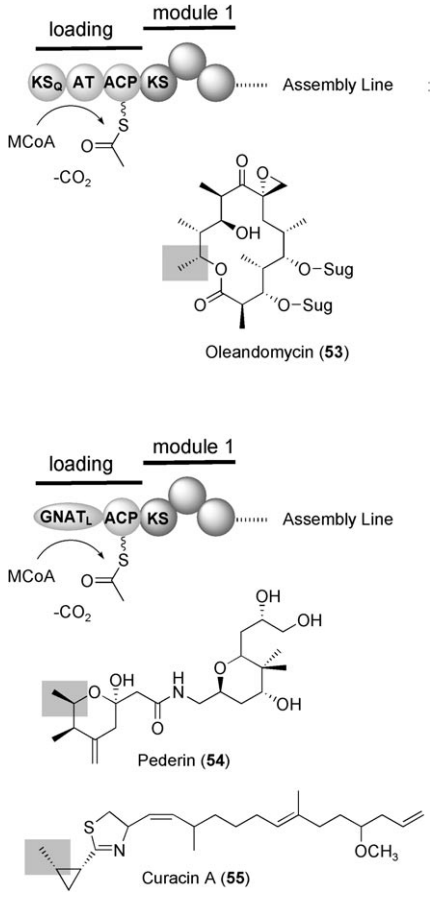
3. Diversity of Complex Polyketides

3.1. Loading Mechanisms and Rare Starter Units

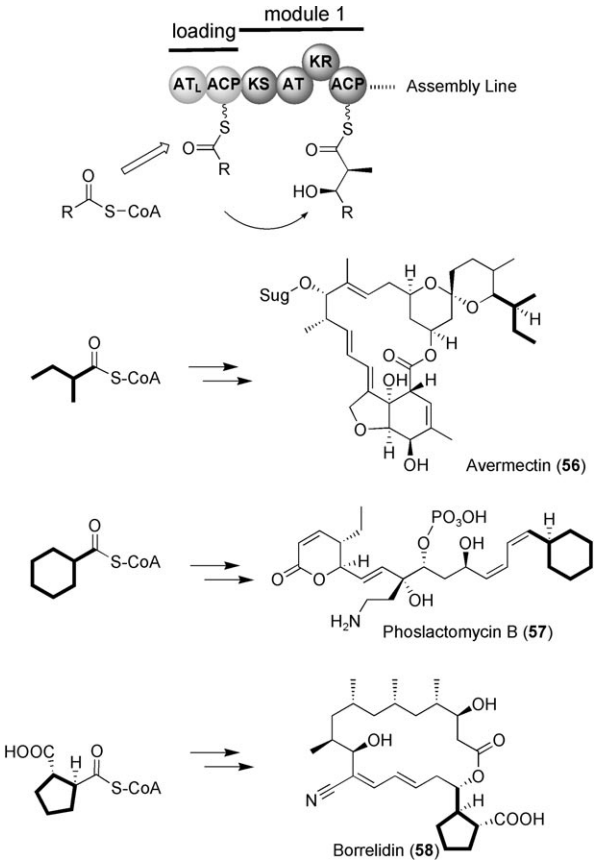
According to the textbook model, complex polyketides are mainly derived from acetate/malonate and propionate/methylmalonate, and polyketide biosynthesis is usually initiated by the loading of acetyl-CoA onto the synthase. However, there are in fact numerous alternative starter units and also various strategies for their activation and loading.[54] The choice of the starter unit is governed by the substrate specificity of a distinct loading module. Most modular PKSs house a KS_Q domain at the N-terminus, in which the active site cysteine (VDTACSSS) has been mutated into a glutamine (Q) residue (Scheme 25). These loading domains load malonyl units onto the PKS and catalyze their decarboxylation to yield an ACP thioester.[120] This priming mechanism is widespread amongst modular PKSs, such as the tylosin, pikromycin, and oleandomycin (**53**) PKSs.[121]



Scheme 25. Priming of a PKS with acetyl starters by KS_Q or GNAT domains.

More recently, an alternative strategy for initiating polyketide biosynthesis using an acetate unit has been reported. Piel and co-workers observed a GCN5-related *N*-acetyltransferase (GNAT) domain in the context of the pederin (**54**) biosynthesis, which might be involved in PKS priming.[122] Sherman, Smith, and co-workers showed, by using in vitro studies and solving the X-ray crystallographic structure, that a homologous GNAT domain of the curacin (**55**) PKS exhibits a bifunctional decarboxylase/S-acetyltransferase activity and directs acetyl transfer onto an adjacent loading-ACP (ACP_L).[123] This scenario seems to be widespread among PKSs of the trans-AT family.

The loading of propionyl-CoA as in the erythromycin biosynthetic pathway requires a designated N-terminal loading didomain comprised of a loading acyltransferase (AT_L) and an ACP. In the same fashion, alternative starter units which are presented as CoA thioesters are loaded onto their respective PKSs. For example, isovaleryl-CoA is loaded by the modular PKS involved in the biosynthesis of the important antiparasitic agent avermectin (**56**) in *Streptomyces avermitilis* (Scheme 26).[124] Similarly, the phoslactomycin (**57**) PKS is primed with a cyclohexanoyl-CoA starter,[125] and the angiogenesis inhibitor borrelidin (**58**) is derived from *trans*-cyclohexane-1,2-dicarboxylate.[126]



Scheme 26. Priming of a PKS with non-acetate CoA thioesters by an AT_L-ACP loading didomain.

The architectures of the loading modules can deviate and are not always discernible. In the myxobacterial PKSs involved in the myxothiazol[127] and soraphen (**59**)[128] biosyntheses, two ATs are directly adjacent to each other such that it was not obvious which AT domain promoted loading the

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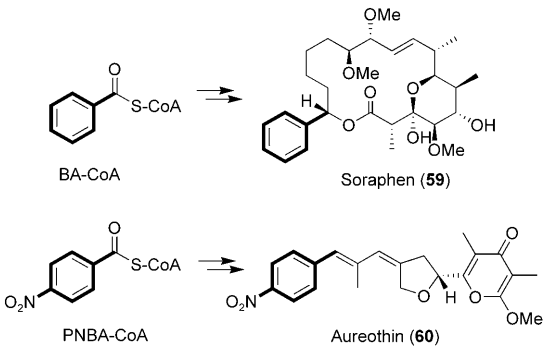
starter unit (isovaleryl-CoA or benzoyl-CoA) and ACP malonylation. Studies by Leadlay and co-workers showed that by transplanting the AT into the erythromycin PKS, the first AT is an AT_L.[128] In contrast to the examples above, the aureothin (**60**) PKS from *Streptomyces thioluteus*, which is primed with a rare *p*-nitrobenzoyl-CoA (PNBA) starter (Scheme 27),[129,130] lacks a designated N-terminal AT domain.

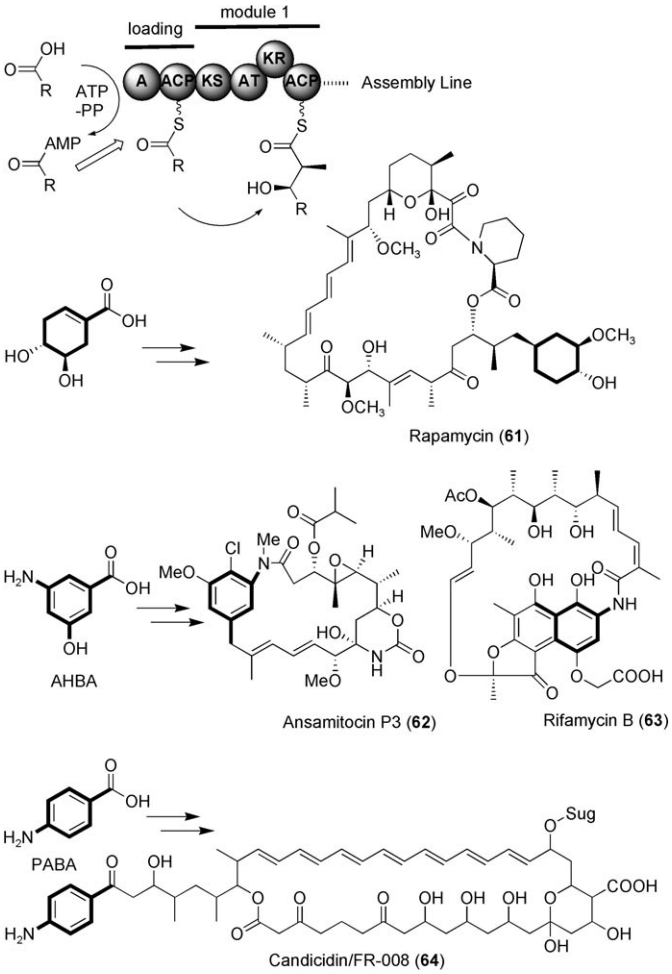


Scheme 27. Complex polyketides resulting from priming modular PKSs with benzoyl-CoA thioesters.

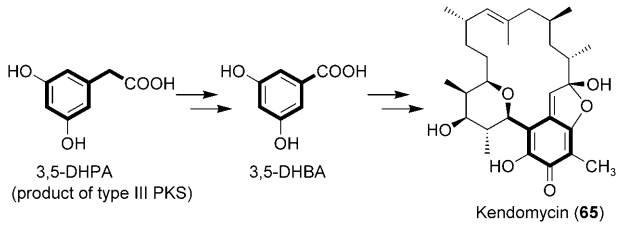
Finally, if the starter unit is provided as the free acid, it can be activated and loaded by a nonribosomal peptide synthetase (NRPS)-like adenylation and thiolation (A-ACP) loading didomain (Scheme 28). The prototypes for this priming strategy are the rifamycin and rapamycin (**61**) PKSs. Polyketides belonging to the rapamycin (**61**)/FK506 (**68**) family of immunosuppressants are derived from dihydroxycyclohexene carboxylic acid.[131] Floss, Staunton, Leadlay, and co-workers found that the loading modules bear additional ER domains which reduce the double bond after the starter unit has been loaded. The antibiotic rifamycin (**63**) from the bacterium *Amycolatopsis mediterranei* is derived from 3-amino-5-hydroxybenzoic acid (AHBA), which is transformed into the aminonaphthol moiety.[132] Floss, Leistner, and co-workers demonstrated that AHBA is also employed and activated in the same fashion as in the PKS pathway to access the antitumoral agent ansamitocin (**62**) in the bacterium *Actinosynnema pretiosum*.[133] The loading of a *p*-aminobenzoate (PABA) unit onto an A-ACP module has been observed for the biosynthesis of the polyene macrolide aglycones of the antifungal agents candicidin and FR-008 (**64**).[134,135]

Recently, Wenzel et al. identified the molecular basis for the biosynthesis of the antibiotic kendomycin (**65**) from *Streptomyces violaceoruber* (Scheme 29).[136] The unusual ansa compound is assembled from a 3,5-dihydroxybenzoate (3,5-DHBA) starter unit that derives from the type III PKS product 3,5-dihydroxyphenylacetate (3,5-DHPA) and is loaded onto the A-ACP didomain of the modular type I PKS.

Knowledge of the mechanisms of the starter unit supply, activation, and loading has paved the way to enlarging the range of starter units. Through genetic engineering (e.g., swapping loading domains) or complementation of a mutant with non-natural starter unit surrogates (mutasynthesis) many novel polyketide derivatives have been generated.[137] One of the most important examples is the genetically engineered biosynthesis of the avermectin derivative doramectin, which



Scheme 28. Activation and loading of non-acetate carboxylic acid starter units. PP = diphosphate, ATP = adenosine triphosphate.



Scheme 29. The biosynthesis of kendomycin by a type III/type I hybrid pathway.

is a highly potent anthelmintic agent in clinical use.[138] Other successful mutasynthesis approaches yielded novel biologically active analogues of aureothin,[139] rapamycin,[140,141] borrelidin,[142] myxalamid,[143] and ansamitocin.[144]

3.2. Pool of Alternative Extender Units and Other Building Blocks

Modular PKSs found in bacteria usually employ malonyl-CoA (MCoA) or methylmalonyl-CoA (mMCoA) building blocks for chain extension. The resulting metabolites are either nonsubstituted or show a methyl branch at the

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α position to the carbonyl group. The selection of the type of extender is governed by the specificity motifs of the AT domain, as demonstrated by successful domain mutagenesis and swapping experiments.[145] Notably, α-methyl branches may also be introduced through α-methylation by a designated methyltransferase domain in the PKS module. This occurence is often observed in bacterial trans-AT type I PKSs, but can also apply to complex fungal PKSs.[25]

In bacterial polyketide biosynthesis the utilization of extender units other than MCoA or mMCoA is only rarely observed. An exception is the extended 2-ethylmalonyl-CoA (eMCoA) unit, which accounts for the two-carbon side chains of the antibiotic niddamycin (**66**) from *Streptomyces caelestis*[146] and the immunosuppressant FK520 (ascomycin (**67**)) from *Streptomyces hygroscopicus* var. *ascomyceticus*.[147] Other complex polyketides which are (as other extender units are present) composed of eMCoA units are tylosin, concanamycin (**70**), and kirromycin (**69**). The latter is particularly intriguing as the MCoA, mMCoA, and eMCoA extenders are selected by stand-alone trans-AT entities that need to interact with the correct modules of the PKS.[148]

The eMCoA and related 2-alkylmalonate units are biosynthesized from substituted acryloyl-CoA precursors by a crotonase-catalyzed reduction/carboxylation.[149] By analogy, the pool of fatty acids could provide a range of alternative extenders, and indeed the structures of various bacterial metabolites hint at the incorporation of eMCoA homologues, which could account for even longer side chains as found in the FK520 homologue FK506 (**68**), featuring a propenyl side chain (Figure 1).

Apart from the alkylated malonyl building blocks, a variety of heterosubstituted malonyl derivatives are incorporated into complex polyketide structures. Labeling studies revealed that 1,3-bis(phosphoglycerate) derived extension units give rise to hydroxy and methoxy substitutions in various antibiotics, such as ansamitocin P-3 (**62**) and soraphen A (**59**),[150] FK520 (**67**),[151] and concanamycin (**70**).[152] The rare methoxymalonyl (moM) extender has been grafted into the erythromycin backbone by swapping the DEBS AT6 domain with the AT8 domain from the FK520 biosynthetic gene cluster of *Streptomyces hygroscopicus* and co-expression of a subcluster required for moM-CoA biosynthesis.[153]

Hydroxymalonylate (hoM) and aminomalonate (aM) are two additional type I PKS extender units which have been discovered by Handelsman, Thomas, and co-workers in the context of the zwittermicin A (**71**) biosynthesis.[154] Zwittermicin is a highly functionalized antibiotic produced by *Bacillus cereus*. An analysis of the zwittermicin biosynthesis gene cluster in conjunction with protein mass spectrometry revealed that glycolyl and ethanolamine units are incorporated as hoM- and aM-ACP, repectively. Whereas hoM-ACP is formed by analogy to moM-ACP, and aM-ACP is derived from serine (Scheme 30).[155]

The repertoire of extender units has been additionally expanded through the discovery of a chloroethylmalonate building block in the bio-

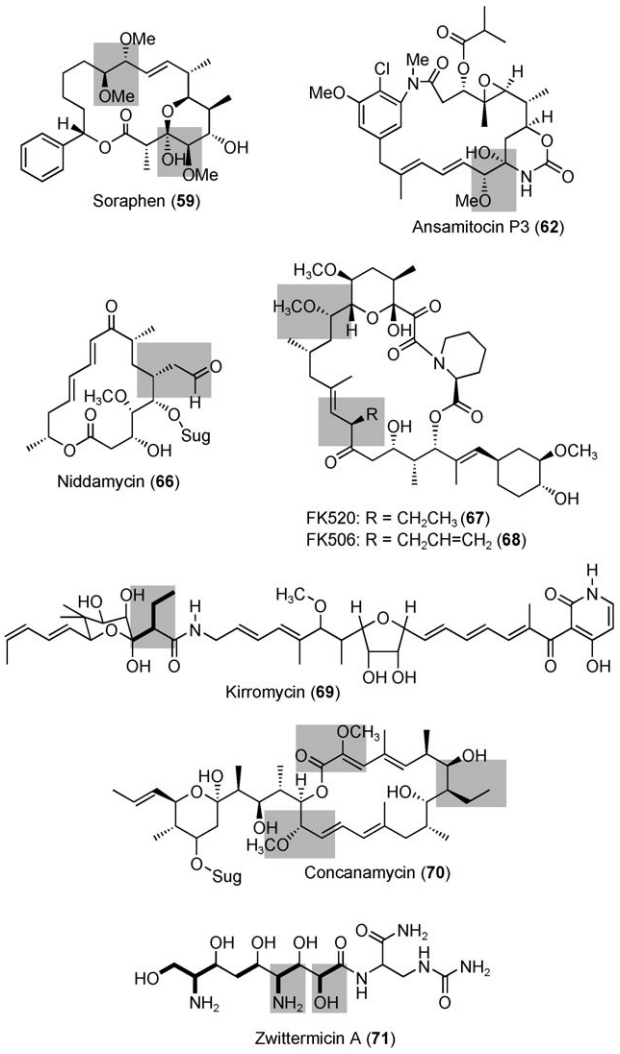
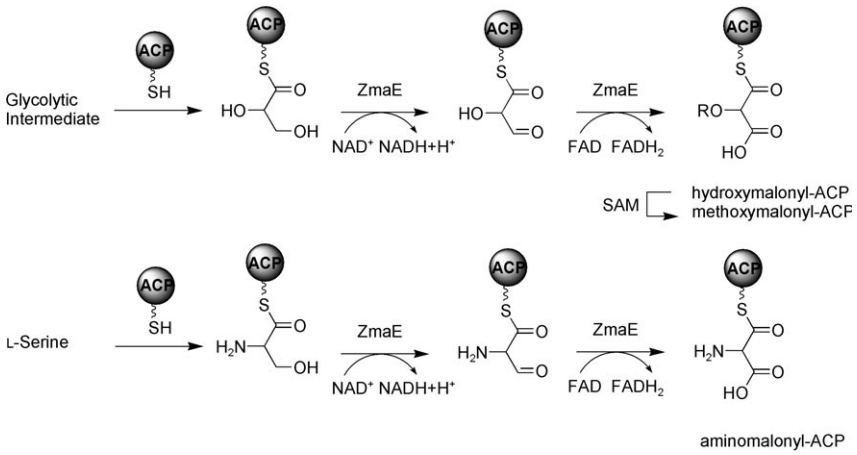


Figure 1. Structures of complex polyketides resulting from the incorporation of unusual malonyl-derived extender units (as other extender units are present). The aforementioned units are highlighted with a grey background.



Scheme 30. The biosynthesis of hydroxy-, methoxy-, and aminomalonyl-ACP extender units. ZmaE = oxygenase.

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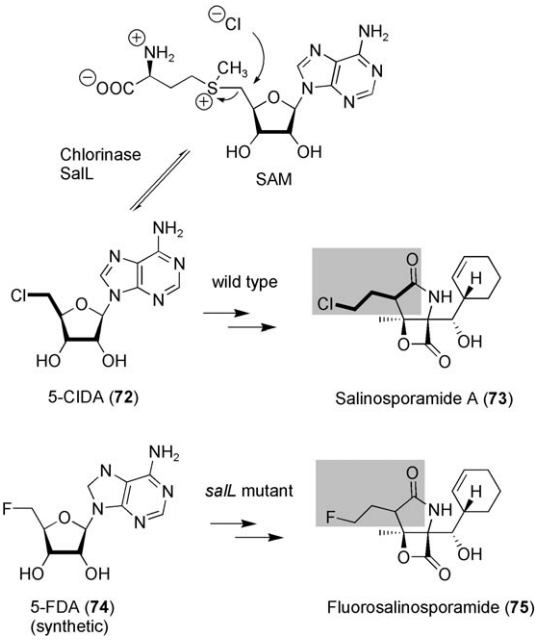
synthesis of salinosporamide A (**73**) (Scheme 31), a chlorinated natural product from the marine bacterium *Salinispora tropica*. Moore and co-workers found that the potent proteasome inhibitor and anticancer agent is assembled by a



Scheme 31. Biosynthetic origin of the chloroethylmalonyl extender unit in the salinosporamide biosynthetic pathway, and mutasynthesis of fluorosalinosporamide. Grey box: highlights parts of the molecules derived from halogenated extender units.

PKS/NRPS hybrid, and surprisingly, that the chlorobutyryl moiety originates from the pentose portion of SAM.[156,157] The finding that chlorinase SalL plays a crucial role in halogenating SAM to generate 5-chlorodeoxyadenosine (5-ClDA (**72**)) led to the first rational mutasynthesis using an alternative extender unit. A mutant devoid of SalL is incapable of producing salinosporamide A (**73**), but can be supplemented with synthetic 5-fluoro-5-deoxyadenosine (5-FDA (**74**)) to yield a fluorosubstituted analogue **75** having potent antitumor activity.[157] To date, the biosynthesis and incorporation of seven different malonyl-derived extender units has been reported (Figure 2). These extender units offer unique possibilities for pathway engineering as they allow the incorporation of heterofunctionalities into polyketide structures.

A novel polyketide extender unit and polyketide off-loading mechanism has been identified in pathways leading to polyketides of the (spiro)tetronate family, such as the antibiotic tetronomycin (**76**)[158] and the antitumor agent chlorothricin (**77**)[159,160] from various *Streptomyces* species. Leadlay, Spencer, and co-workers demonstrated, by in vitro enzyme assays and mass spectroscopy studies, that an FkbH-like protein transfers a phosphoglycerate unit onto designated ACPs from the tetronomycin synthase (Scheme 32).[161] According to independent biosynthetic proposals from the research groups of Leadlay, Tang, and Liu, glyceryl-S-ACP

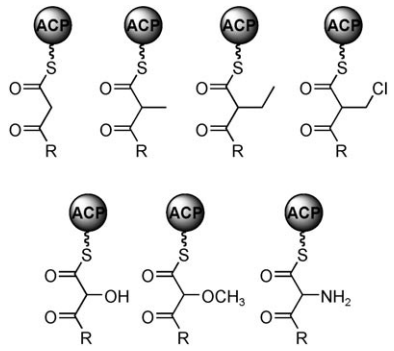
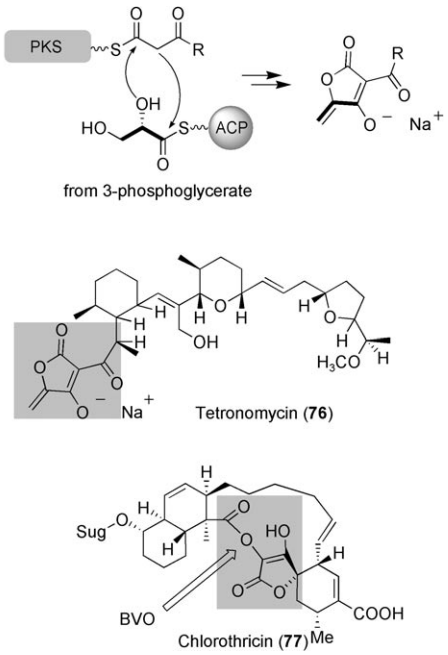


Figure 2. Set of extender units known to be employed by modular PKSs.



Scheme 32. Incorporation of a terminal glycerate-derived three-carbon unit into the tetronomycin and chlorothricin structures (the tetronate unit is disrupted by a BVO). Grey box: highlights the incoorporated glycerate.

serves as the final three-carbon unit which is annealed onto the polyketide chain with concomitant release of the metabolite from the modular PKS.[158–160]

In addition to the malonyl and glycerate extenders, a series of other building blocks can be incorporated into polyketides, mainly by using the NRPS biosynthetic logic. Important examples of products having PKS/NRPS-derived substructures are the serine- or cysteine-derived oxazolyl and thiazolyl groups as in rhizoxin[162] and epothilone,[163] respectively, or the pipecolyl moiety of FK506.[164] If the NRPS portion is found at the N-terminus, the PKS is typically primed with an amino acid.[54] Conversely, when it is located at the C-terminus, the polyketide is off-loaded and morphed, for example, into tetramic acids[165] and pyridones.[148,166,167]

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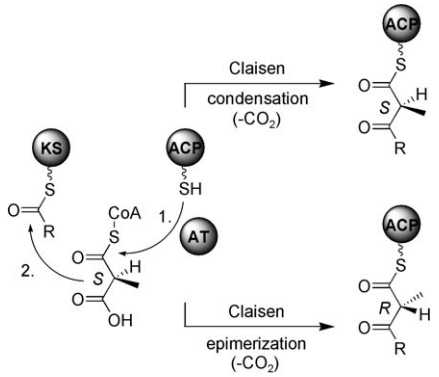
3.3. Control of Stereochemistry in Complex Polyketides

Despite the vast number of possible products arising from permutations of all the configurations and substitution patterns of complex polyketide chains, natural products exhibit similar stereochemical patterns. This observation is referred to as Celmer's rule and suggests that the requisite PKSs have evolved from the same precursor and share the same enzyme mechanisms. Over the past years, fundamental insights into the underlying principles of polyketide stereochemistry have been acquired.

3.3.1. Configuration of α-Branching Substituents

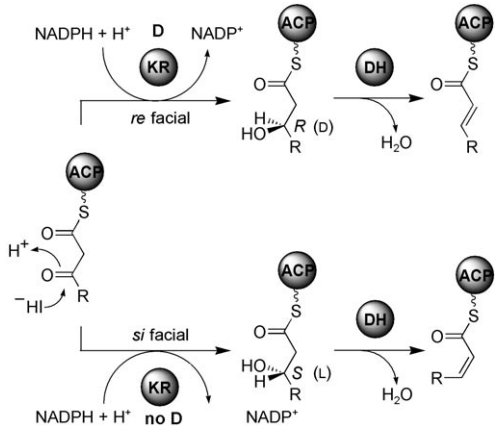
(2*S*)-mMCoA is used as a substrate by the AT and loaded onto the ACP in DEBS,[168] and is assumed for other modular PKSs by extrapolation. However, after condensation the 2-methyl-ketoacyl thioester can be epimerized into the 2*R* isomer (Scheme 33). Work by Weissman et al. implicated the KS as the seat for epimerization;[169] other suggestions (e.g., the KR or DH) have been made since. Khosla and co-workers demonstrated that the DEBS AT domains do not influence epimerization of the (2*S*)-mMCoA extender units.[170]



Scheme 33. Retention or inversion of (2*S*)-methylmalonyl-CoA during polyketide chain extension.

3.3.2. Stereochemistry of Ketoreduction

The bioinformatic analysis by Caffrey established some design rules for β-keto processing. The KR specificity can be predicted from the presence or absence of an LDD motif upstream from the conserved GVxHxA motif, and additional indicative residues.[171] Reid et al. focused on the key aspartic acid (D) moiety.[172] The finding that conserved amino acid residues correlate with the ketoreductase stereospecificity has been experimentally demonstrated by mutagenesis and genetic engineering experiments using KR domains from the erythromycin[173,174] and tylosin[175,176] PKSs. Leadlay and co-workers were also able to evaluate models of stereochemical control in the KR domains by using high throughput mutagenesis.[177] As a rule of thumb, a D in the KR motif leads to D-3-hydroxy substituents (Scheme 34).



Scheme 34. Stereochemistry of ketoreduction and double-bond formation.

3.3.3. E versus Z Double-Bond Formation

In the subsequent β-keto processing step, the dehydratase-catalyzed *anti* elimination of water usually leads to desaturation having a *trans* geometry. A *cis*-configured double bond, as in rifamycin is rather rare and could, in principle, arise from a variety of mechanisms.[178] Cane, Khosla, and co-workers have shown that the inactivation of a DH domain of module two of the pikromycin PKS yields a D-3-hydroxy moiety in lieu of the *trans* double bond.[179] Conversely, the L-3-hydroxy-substituted thioester is the speculated intermediate in the KR-DH domains generating *cis* double bonds.[171] Reynolds and co-workers addressed this issue in the biosynthesis of the phoslactomycins, which are antitumoral phosphatase inhibitors having a characteristic conjugated *cis* diene. Feeding experiments showed that only *cis*-configured intermediate surrogates are accepted, ruling out an isomerization domain in subsequent modules.[180] Taken together, these data suggest that the enzymatic dehydration of D-3-hydroxyacyl moieties yields *trans* double bonds, whereas the L isomers give rise to *cis* double bonds. Notably, the *cis*-configurated α,β-double bond at the lactone moiety of phoslactomycin (**57**) is introduced as a post-PKS reaction.[181] Other examples in which modular PKSs generate multiple *cis* double bonds, are the processing lines yielding the myxobacterial metabolites chivosazol A (**78**)[182] and disorazol A1 (**79**; Figure 3).[183] However, it should be mentioned that the predicted stereochemistry of the reduction has been found, in many cases, to not correlate with the ultimate double bond stereochemistry, thus raising the question of how the double bond stereochemistries are established, or whether the coded residues are truly predictive.

3.3.4. Stereochemical Course of Enoyl Reduction

The molecular basis for the stereochemical course of enoyl reduction has for some time remained unclear. Only recently, conserved motifs have been identified which correlate to the amino acid sequence of ER stereochemistry.[184] Leadlay and co-workers compared ER sequences across a broad range of sources (i.e. including macrolide-producing

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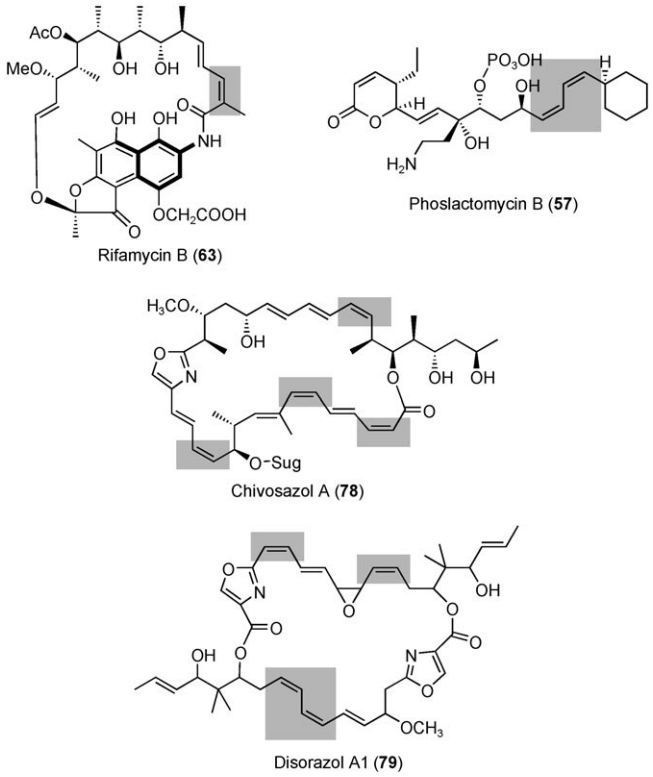
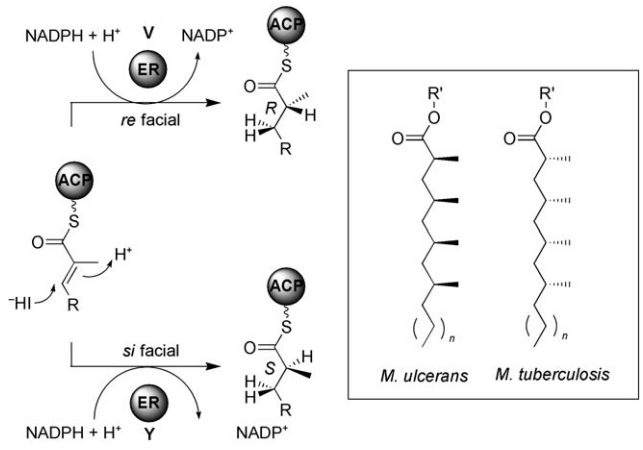


Figure 3. Examples of complex polyketides featuring *cis* double bonds (grey background).

PKSs such as DEBS, OLEA, etc.) involved in the formation of enantiomeric methyl-branched polyketides which are components of mycobacterial cell wall lipids[12,185] (Scheme 35). They noted the occurrence of a conserved tyrosine (Y) in the vicinity of the NADPH binding motif (consensus sequence HAAAGGVGMA) of ER domains producing a (2*S*)-methyl branch. Conversely, ER domains that yield a (2*R*)-methyl branch feature other amino acids, mostly valine (V), at this position. With this knowledge, the ER of a model PKS derived from the erythromycin megasynthase was mutated (Tyr to Val), which indeed resulted in a switch in the methyl branch configuration in the product from *S* to *R*. However, the reverse mutation (Val to Tyr) at this position in an *R*-specific ER from the rapamycin PKS was insufficient to achieve a switch to *S*. Apparently, additional residues also participate in stereocontrol of the 1,4-nucleophilic hydride attack.[184]



Scheme 35. Left: stereospecificity in enoyl reduction; right: substructures of enantiomeric methyl-branched polyketide components of cell wall lipids in *Mycobacterium* spp.

The fundamental advances in understanding the stereochemical course of polyketide assembly and β-keto processing in bacterial PKSs greatly contribute to the rational genetic engineering of complex polyketide biosynthesis pathways.[5,186] Moreover, these design rules support the elucidation of absolute configurations in bacterial polyketide metabolites by in silico predictions.[187] However, it should be emphasized that knowledge of the cryptic programming of highly reducing, iterative fungal PKSs lags far behind.[25]

3.4. β-Branching Mechanisms

As depicted in Section 2, substituted malonyl extender units or SAM α-methylation reactions give rise to alkyl branches at carbon atoms corresponding to former C2-positions. In several polyketide pathways, alkyl branches with one or two acetate-derived carbon atoms at positions corresponding to former acetyl carboxyl groups (C1) have been observed. These substituents could not result from the incorporation of noncanonical extenders or by alkylation using electrophiles. Instead, investigations at the genetic and biochemical levels have revealed that such β branches are typically introduced by using a biosynthetic scheme that resembles mevalonate biosynthesis.[188]

3.4.1. Isoprenoid Logic

In virtually all gene clusters coding for the biosynthesis of polyketides with β-alkyl branches, a set of genes coding for 3-hydroxy-3-methylglutaryl-CoA (HMG) synthase (HCS) and enoyl-CoA hydratase (ECH, or crotonase) homologues as well as free-standing KS and ACP domains can be found. These novel domains were first identified in the context of the pederin (**80**) biosynthetic gene analyses.[122] Enzymatic in vitro studies with distinct acyl-ACPs of the bacillaene (**81**)[189] and curacin (**82**)[190] PKSs demonstrated that the β substituents result from a HCS-mediated aldol addition of free-standing acetyl-ACP with the β-ketoacyl ACP, and subsequent ECH-catalyzed Grob fragmentation (Scheme 36).

Several pieces of evidence showed that the β-branching process takes place during and not after chain elongation. The deletion of the β-branch genes of the myxovirescin (**83**)[191–193] and mupirocin (pseudomonic acid (**84**))[194–196] biosynthetic pathways resulted in either abolished or derailed production. Piel and co-workers determined the exact masses of the bacillaene (**81**) intermediates from a blocked TE mutant and thus deduced the precise timing of β branching.[197] Likewise, bioinformatic analysis of the trans-AT KS specificities allows the prediction of β-branching events.[198]

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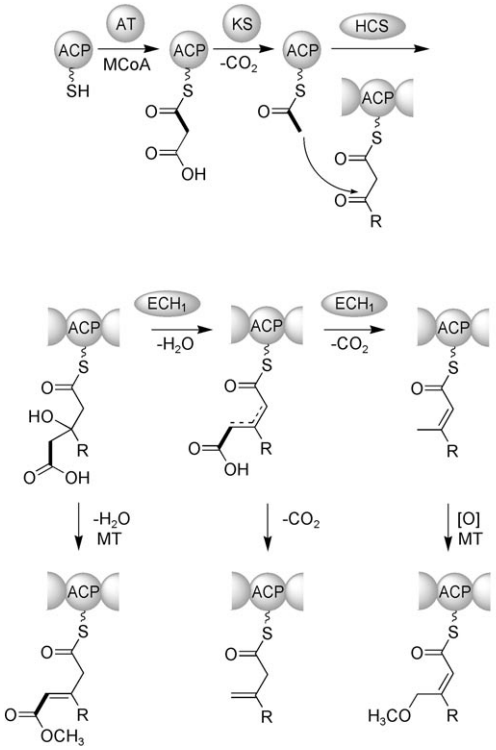


Scheme 36. β Branching of a polyketide chain by an isoprenoidlike mechanism. HCS = HMG-CoA synthase, ECH = enoyl-CoA hydratase.

Through the downstream processing of the acetate unit, the side chain can be elaborated into a variety of functionalities (Figure 4, Scheme 36). From genetic analyses it appears plausible that a variety of intriguing structural units result from β branching and additional processing. β,γ-Dehydration and methylation would yield the acrylic ester side chain in the antitumor agent bryostatin (**85**) from a microbial symbiont of the marine bryozoan *Bugula neritina*.[199,200] The β-methyl branch can be additionally hydroxylated and methylated to produce the methoxymethyl group observed in myxovirescin A (TA; **83**),[192,193] whereas an exomethylene group could result from alternative dehydration/decarboxylation or double bond migration in the pederin/onnamide pathways.[22,122,201] Sherman, Gerwick, and co-workers have shown that the successive dehydration and decarboxylation of (*S*)-HMG-ACP yields a 3-methylcrotonyl-ACP intermediate in the curacin (**82**) biosynthetic pathway. However, the final transformation of this presumed intermediate into the cyclopropyl ring in curacin A needs to be clarified.[190,202] Likewise, the mechanisms involved in the formation of the rare vinyl chloride residue of jamaicamide (**86**)[203] or the dithiolactone side chain in the leinamycin (**87**) biosyntheticpathway[204] have not yet been elucidated.

Notably, the isoprenoid branching is not restricted to acetate units. The research groups of Müller and Walsh independently showed, by using in vivo and in vitro experiments, that the ethyl β branch in myxovirescin results from the HCS-mediated incorporation of a methylmalonyl-derived propionate building block (Scheme 37).[192,205]

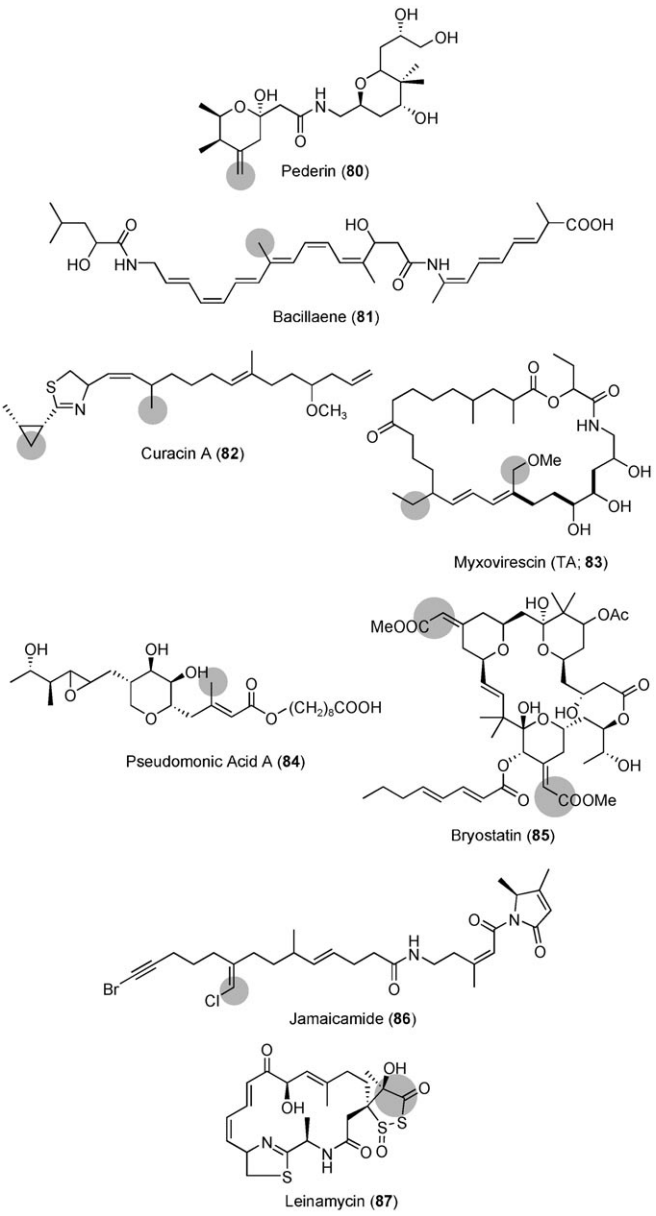
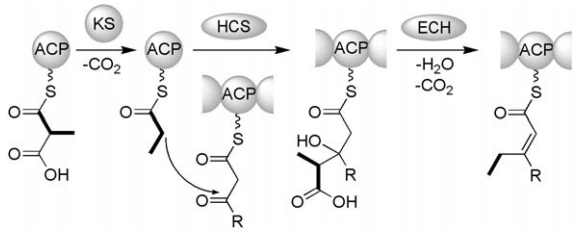


Figure 4. Polyketide structures with unusual substitutions resulting from isoprenoid-like β-branching events. (grey background)



Scheme 37. Incorporation of a β-ethyl branch in the myxovirescin biosynthetic pathway.

3.4.2. β Branching By a Michael-Type Conjugate Addition

The δ-lactone β branch in rhizoxin (**88**), the antimitotic agent and phytotoxin produced by bacterial endosymbionts of

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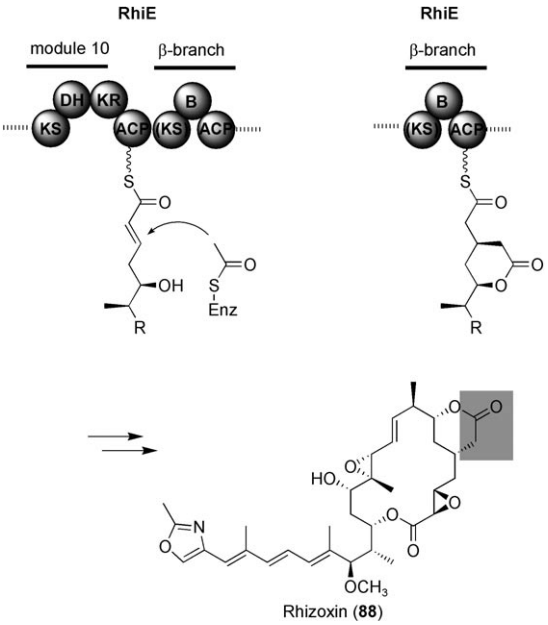
the fungus *Rhizopus microsporus*[206,207] and by *Pseudomonas fluorescens*,[208] is governed by a completely different mechanism. Firstly, the typical isoprenoid branching gene cassette cannot be found in the rhizoxin biosynthesis gene cluster;[162] and secondly, the architecture of the PKS module 10 translates into a double bond, not the keto group of the intermediate.[162,198] Recent mutagenesis experiments and isolation of pathway intermediates revealed that the β branch is introduced by a novel mechanism involving a conjugate addition of an acetate building block to the enzyme-bound enoyl moiety, possibly mediated by a branching domain (B) (Scheme 38).[209]

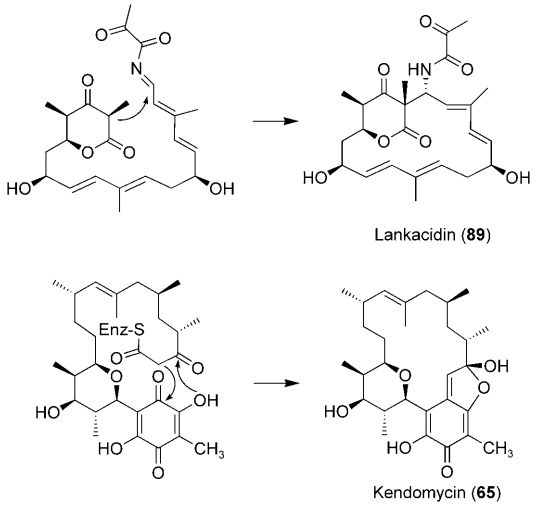


Scheme 38. A model for a Michael-type β-branching mechanism in the rhizoxin biosynthetic pathway.

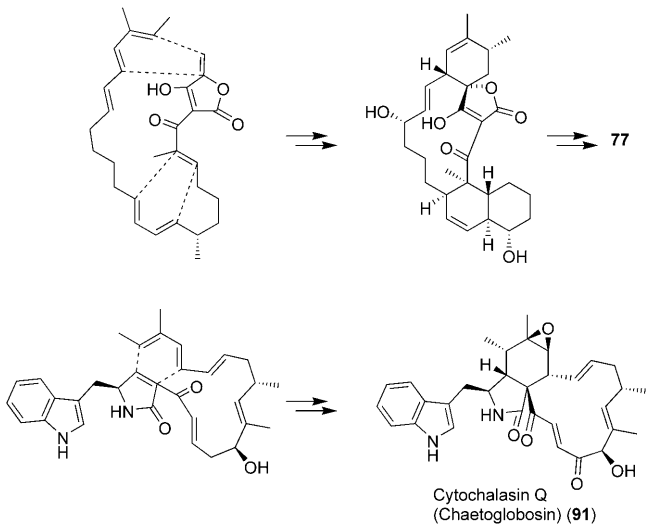
3.5. Chain Release and Primary (Macro)Cyclizations

After the polyketide chain has reached its final length it is released from the PKS to yield either a linear or a cyclized metabolite. Whereas oxidative or reductive release mechanisms are rare, the thioester is typically cleaved by hydrolysis or attack by nucleophiles. Both hydrolysis and macrocyclization are usually catalyzed by a thioesterase domain.[35,210] The cyclization processes studied in more detail are those of the final lactonizations in the erythromycin[211] and picromycin/methymycin[212,213] biosynthetic pathways. Stroud, Khosla, and co-workers found a substrate channel in the DEBS TE which passes through the entire protein and an active site Asp-His-Ser triad which is shielded from external water, thus favoring macrolactone formation over hydrolysis.[211] Boddy and co-workers proposed, on the basis of TE protein structures, that hydrophobic interactions between the binding cavity and substrate drive substrate specificity and thus define the size of the macrolactone.[214]

Unusual thioesterases that lead to linear products have been identified in the biosynthetic machineries leading to the polyethers nanchangmycin[215,216] and monensin.[217] An alternative cyclization mechanism has been proposed for the synthesis of macrocyclic antibiotic lankacidin (**89**) in *Streptomyces rochei* (Scheme 39).[218] Preliminary data from mutational analyses suggested that the unusual skeleton is formed by a Knoevenagel-type attack onto an imine. Similarly, a β-ketothioester intermediate could attack a quinone carbonyl group to produce the kendomycin (**65**) framework.[136] Diels–Alder-type macrocyclizations were proposed for the chlorothricin (**77**)[159] and cytochalasin Q (**91**)[219] biosynthetic pathways (Scheme 40).



Scheme 39. Hypothetical alternative macrocyclizations in the lankacidin and kendomycin biological pathways.



Scheme 40. Polyketide macrocyclizations by Diels–Alder reactions.

3.6. Ali- and Heterocyclizations of the Polyketide Backbone

Many complex polyketide structures are endowed with additional small- or medium-sized ali- and heterocycles which

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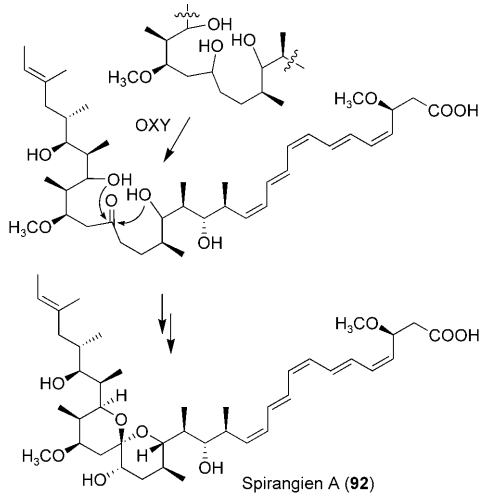
lend rigidity to the polyketide core structure. Whereas oxirane rings typically arise from cytochrome P450 monooxygenase-catalyzed epoxidations of *cis* or *trans* double bonds, there are elaborate biosynthetic strategies to generate larger O-heterocycles or carbacyclic substructures.

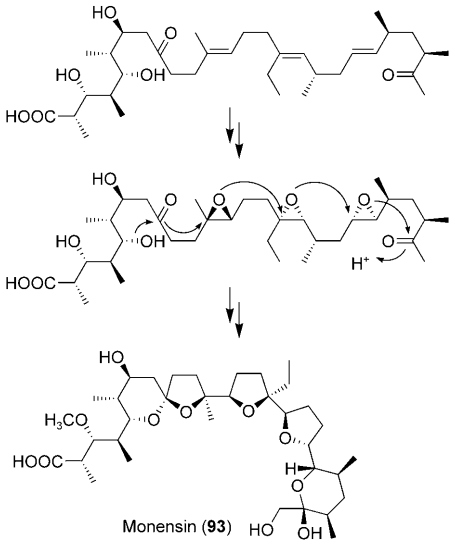
3.6.1. O-Heterocycles

In macrolides pyran or tetrahydrofuran (THF) rings can result from spontaneous monoacetal formation, which can be reversible, as in candicidin (**64**) and concanamycin (**70**). Müller and co-workers found that the formation of the stable spiroketal moiety in spirangien (**92**), a cytotoxic metabolite from *Sorangium cellulosum*, requires a cytochrome P450 monooxygenase-mediated oxygenation (Scheme 41).[220]



Scheme 41. Example of a spiroketal formation in complex polyketides.

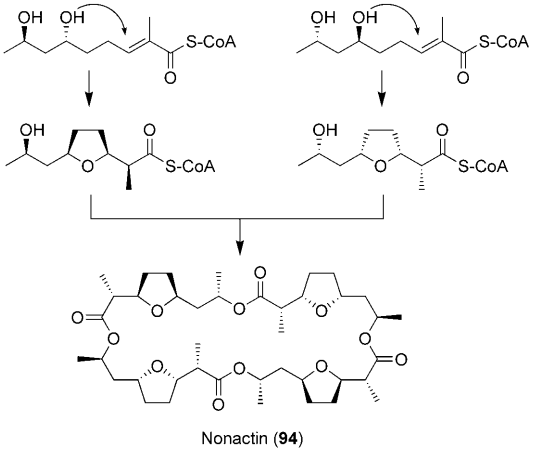
An alternative strategy towards medium-sized O-heterocycles is the nucleophilic ring-opening of epoxides, which has been implicated in the biosynthesis of polyethers such as monensin (**93**),[221–223] nigericin,[224] nanchangmycin,[225] and tetronomycin (**76**).[158] From analyses of the monensin biosynthetic gene cluster and the characterization of biosynthetic intermediates obtained from block mutants, Spencer, Leadlay, and co-workers concluded that a polyepoxide intermediate undergoes a concerted zipper-type cyclization reaction (Scheme 42).[221–223] The sequence is initiated by the nucleophilic attack of a hydroxy group onto a carbonyl moiety, forming a semiacetal hydroxy group, which then attacks the adjacent epoxy moiety; the electrons are passed along the preorganized polyketide chain. The same principal route is likely to be found in the biosynthesis of the structurally intriguing polyether ladders found in marine toxins such as maitotoxin.[226] It appears that the stereochemistry of the epoxidation is uniform and defines the overall absolute configuration of the molecules. Whereas one would expect that these processes generally obey Baldwin's rules, in the lasalocid biosynthesis a disfavored polyether ring closure has been observed.[227]



Scheme 42. The biosynthesis of the polyether monensin by a zipper reaction.

The THF rings present in nonactin (**94**), the *meso* macrotetrolide produced by *Streptomyces griseus*, are formed by an alternative mechanism. Independent studies by the research groups of Priestley and Shen revealed that the ionophore is assembled by an unusual type II PKS[228,229] and that the THF rings of the building blocks are formed by PKS (NonS)-catalyzed conjugate addition of the hydroxy groups onto the acryloyl moiety (Scheme 43).[230,231] A similar mechanism has been postulated for the formation of the pyran rings of tetronomycin and ambruticin (see Section 3.6.2).

The formation of the THF moiety of aureothin deviates from these precedents, as a single cytochrome P450 monooxygenase (AurH) is sufficient for the heterocyclization of deoxyaureothin. In vivo and in vitro studies showed that AurH sequentially installs both C–O bonds and defines the absolute stereochemistry in the first hydroxylation (Scheme 44).[232,233] This rare bifunctional biocatalyst may



Scheme 43. The formation of THF rings in macrotetrolide (nonactin) biosynthesis.

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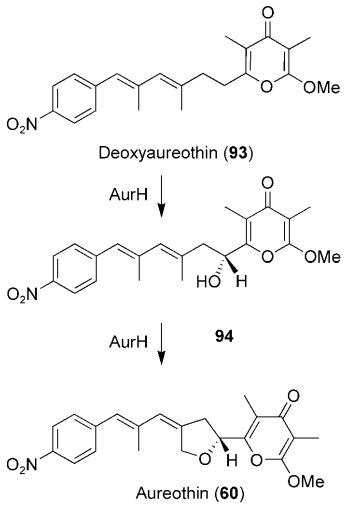


Scheme 44. The sequential THF ring formation for the biosynthesis of aureothin catalyzed by a single cytochrome P450 monooxygenase.

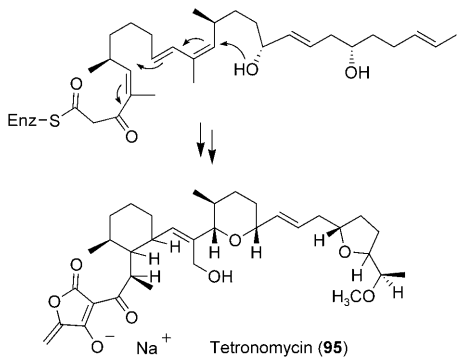
also be used for transforming synthetic polyketide surrogates.[234]

The mechanisms of various other heterocyclization reactions remain to be elucidated, for example, tetrahydropyran formation in the pederin (**80**) and bryostatin (**85**) biosynthetic pathways, which might be catalyzed by unusual twin DH domains.[122,200]

3.6.2. Small- and Medium-Sized Carbacycles

Various complex polyketides have alicycles integrated into their core structure. These alicycles can arise from electrocyclic rearrangements[235] (also see Section 3.5) or Diels–Alder cycloadditions[236] (e.g. chlorothricin (**77**)). Other possibilities are radical reactions or, as proposed for the tetronomycin biosynthesis, a conjugate addition, which may initiate a zipper reaction (Scheme 45).

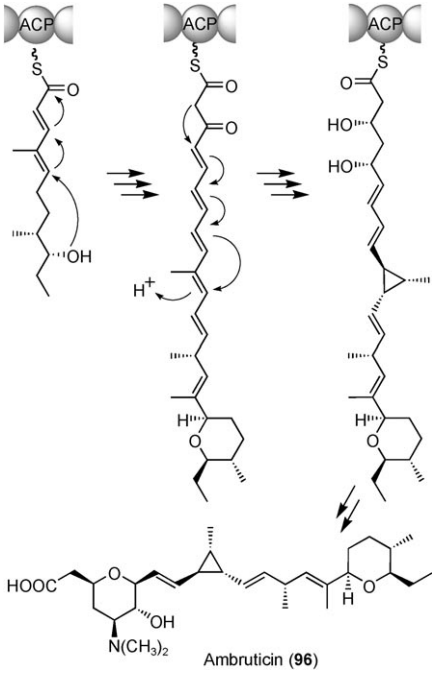
From analysis of the assembly line for the antifungal agent ambruticin in *Sorangium cellulosum*, an unusual reaction sequence was proposed by Reeves and co-workers. After formation of the polyene chain, a global double bond migration takes place which includes the formation of a cyclopropane ring (Scheme 46). One of the downstream steps



Scheme 45. The formation of a cyclohexane ring as part of a zipper reaction

is reminiscent of a Favorskii rearrangement, wherein the C1 carbon atom of a putative intermediary cyclopropanone is excised from the chain.[237]

The antitumor metabolites calicheamicin (**97**) and C-1027 (**98**) from soil bacteria feature structurally unique bicyclic enediyne pharmacophores (Scheme 47). Elucidation of the



Scheme 46. The key steps in the hypothetical pathway to ambruticin.

parent biosynthetic gene clusters by the research groups of Shen and Thorson revealed that their biosynthesis involves bacterial iterative type I PKSs.[238,239] This genetic information could be successfully employed for mining other bacterial genomes for related biosynthetic machineries and metabolites,[240] and cloning of the related neocarcinostatin[241] and dynemicin[242] biosynthetic gene clusters; however, the detailed mechanism for enediyne formation has remained unclear. Only recently, two independent reports from the research groups of Shen and Liang revealed that enediynes derive from polyene precursors.[243,244] The polyene is potentially elaborated into a macrocycle by means of a putative cyclase, and then, an as yet to be characterized acetylase, would catalyze additional desaturation to yield the alkyne groups. The resulting enediynes intercalate into chromosomal DNA and lead to double-strand scission through a Bergman-type cyclization into aryl diradicals.[245] Notably, this is an alternative pathway to that of the type III/type II PKSs used to generate aromatic polyketide structures.

3.7. Diversification Through Noncolinearity in Modular PKSs: Iteration, Skipping, and Polyene Splicing

In contrast to iterative PKSs—such as fungal type I and bacterial types II and III PKSs from plants, bacteria, and

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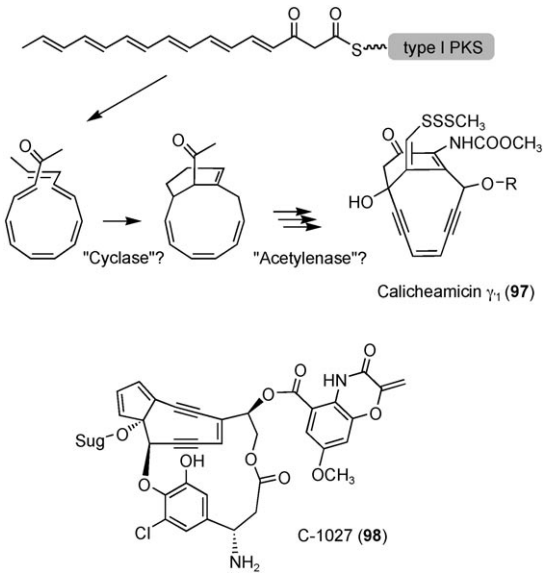


Scheme 47. The biosynthesis of enediyne carbacycles from a linear polyene precursor.

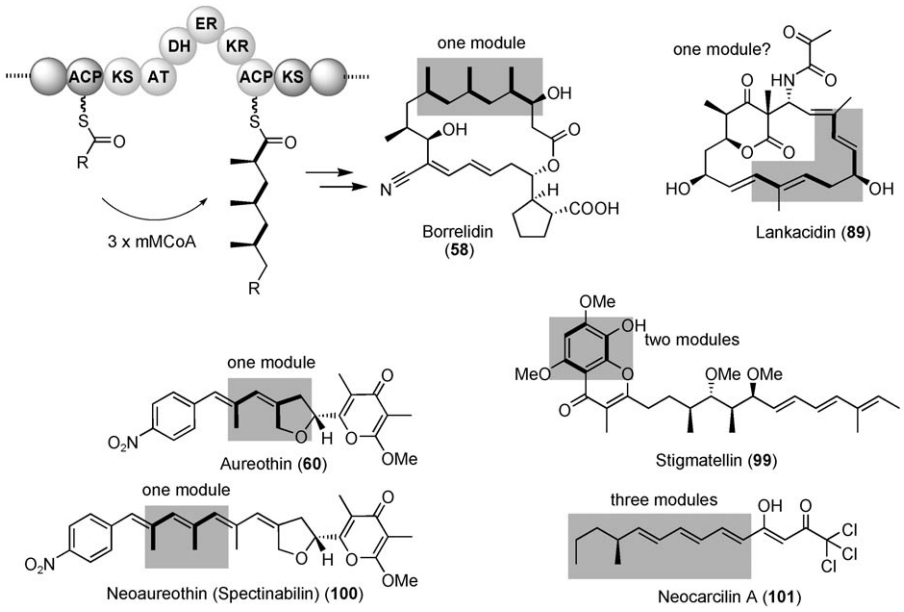
fungi—modular PKSs are typically co-linear with the metabolites produced. However, there is a growing number of exceptions.[246] Biosynthetic machineries have been identified in which modules are skipped or iteratively used, that is individual domains are not functional or seem to catalyze reactions in downstream or upstream modules, and in various assembly lines (e.g. mupirocin) no clear correlation can be made between the PKS architecture and product. The trans-AT PKSs may be considered as the largest class of noncanonical PKS variants.

3.7.1. *"Stuttering" and Programmed Iteration*

After the initial discovery that modular PKSs may erratically produce longer chains by using individual modules twice ("stuttering"),[247] several modular PKSs, which appear to be programmed for such iterative use (Scheme 48) have been discovered.[246] A comparison of the deduced PKS and the metabolite structure, indicated that in the stigmatellin (**99**) biosynthetic pathway one of the last modules is apparently used twice.[248] Functional analyses of the borrelidin (**58**)[126] and aureothin (**60**)[129] biosynthetic pathways provided the first direct evidence for the programmed iterative usage of modular type I PKSs in bacteria. AurA from the aureothin biosynthetic pathway catalyses two rounds of elongation and β-keto processing,[249] whereas BorA5 from the borrelidin biosynthetic pathway proceeds through three interations.[250] Other examples of iterative modular PKSs include the neoaureothin (**100**) neocarzilin (**101**),[251] lankacidin (**89**),[252,253] and DKxanthene[254] PKSs. Interestingly, a pikromycin PKS module may act iteratively when taken out of its natural context.[255]

3.7.2. *Skipping and Flexibility in Chain Release*

In sharp contrast to the iterative use of one or more modules, skipping a module results in shortened polyketide chains. An important example is the pikromycin (**103**) PKS from *Streptomyces venezuelae*, which is uniquely capable of generating 12- and 14-membered ring macrolactones by premature release and cyclization of the polyketide chain (Scheme 49).[256] Sherman and co-workers found that the formation of ring-contracted product methymycin (**102**) relies on the transfer of the hexaketide-ACP intermediate from the penultimate module to the ACP of the terminal module before release and cyclization by the terminal thioesterase domain.[257,258] This finding is in accord with the investigations by Leadlay and co-workers on the skipping process in a hybrid PKS which provided strong evidence for ACP-to-ACP chain transfer.[259]



Scheme 48. Complex polyketides resulting from the programmed iteration in bacterial modular PKSs. Grey box: highlights parts of the molecules resulting from an interation.

3.7.3. *Polyene Splicing*

It tempting to speculate that many polyketides ,differing only in the size of the backbone, share a common origin and that only the programmed number of elongation steps is variable. The same was assumed for the pyrone metabolites neoaureothin (**100**), aureothin (**60**), and orinocin (**104**; Scheme 50). Whereas this holds true for the aureothin and neoaureothin assembly lines, genetic and chemical analyses revealed that orinocin, unexpectedly, does not result from a truncated thiotemplate system or from module skipping. In fact, orinocin is only formed under the influence of light, and

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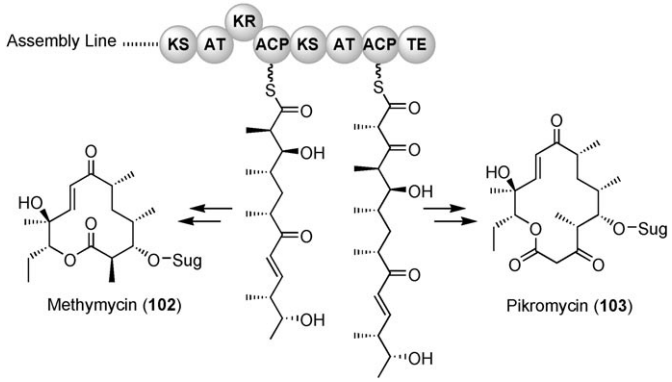
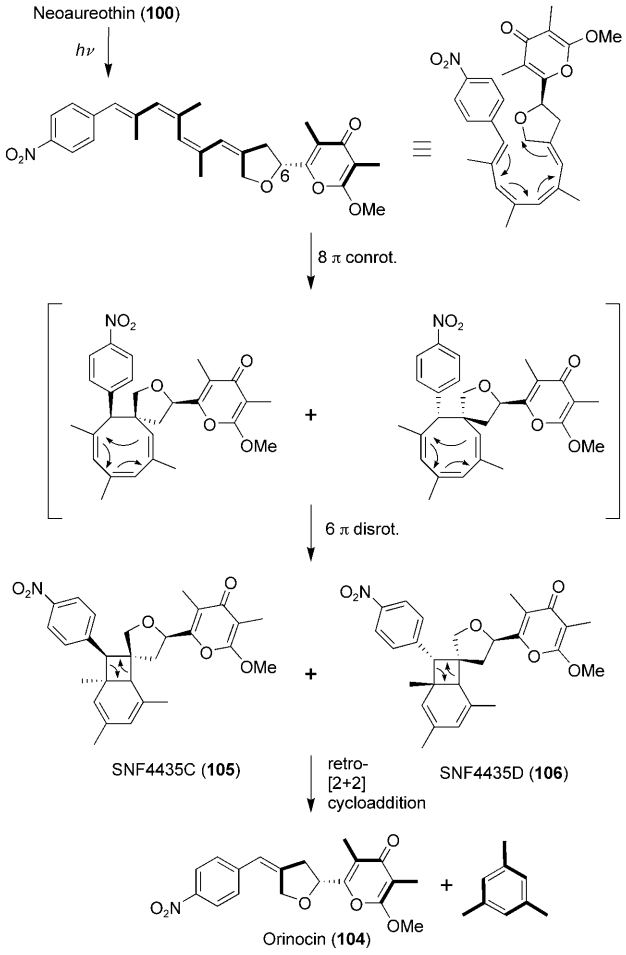


Scheme 49. The formation of alternatively sized macrolactone rings by skipping modules in the pikromycin/methymycin biosynthetic pathway.



Scheme 50. The formation of orinocin and mesitylene through photo-induced polyene splicing.

it is possible to obtain this compound through irradiation of the polycyclic immunosuppressants SNF4435C/D (**105**/**106**; Scheme 50). The latter are formed by a light-induced electrocyclic rearrangement cascade starting with the *E* to Z isomerization of the triene. As a final step, SNF4435C/D undergoes a light-mediated retro [2+2] cycloaddition to yield orinocin and mesitylene.[260] Although this mechanism does not require enzyme catalysis, the neoaureothin pathway provides built-in diversity. One can imagine that various other polyketides are prone to such polyene splicing reactions to yield truncated carbon skeletons and aromatic compounds such as xylene, toluene, and others.

4. Concluding Remarks

The above-mentioned repertoire of biosynthetic assembly lines highlight nature's impressive strategies to produce structurally sophisticated compounds from a pool of simple building blocks and a toolbox of biocatalysts. In particular, it is overwhelming to imagine the evolutionary processes and to note how finely tuned—and at the same how flexible—these biosynthetic machineries have become. On the basis of the knowledge acquired it is possible to rationally engineer polyketide biosynthetic pathways, and it is conceivable that novel insights and techniques will allow design of such pathways in the laboratory. From a synthetic point of view, there is a lot chemists can learn from the concerted action of enzyme catalysis. The synthesis of complex molecules in the laboratory can greatly benefit from adopting enzymelike processing lines. There will be exciting times ahead when this knowledge is routinely applied to chemoenzymatic synthesis and to the genetic engineering of novel biologically active compounds.

Received: December 16, 2008

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