Isoprenoid-like alkylations in polyketide biosynthesis

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Polyketides are secondary metabolites biosynthesized by the iterative Claisen condensation of malonate units. Despite utilizing only a small set of biochemical transformations, the polyketide biosynthetic machinery yields products of striking structural complexity and diversity. Recently, a new polyketide alkylation pathway was characterized that allows access to "β-branched" structures. This *Highlight* will describe this alkylation sequence, with special emphasis on its parallels to isoprenoid biosynthesis from primary metabolism and the scope of structures accessible *via* this pathway.

Introduction

Polyketides are oligomeric secondary metabolites generated by enzymes known as polyketide synthases (PKSs). The therapeutic importance of polyketides—examples of which include the antibiotic erythromycin, the anticancer agent discodermolide, and the antilipidemic agent lovastatin—and the intriguing assembly-line-like organization of many PKSs have made polyketide synthesis perhaps the most-studied secondary metabolic pathway in nature.[1]

The PKS biosynthetic machinery is divided into modules, each responsible for incorporation of a single polyketide extender unit that often takes the form of a malonyl unit (Fig. 1). Each module, in turn, is divided into domains responsible for specific chemical transformations during monomer incorporation. First, thiolation (T) domains, also known as acyl carrier proteins (ACPs), are post-translationally phosphopantetheinylated and serve as attachment points to tether monomers and the growing polyketide to the synthase. Each T domain is loaded with a monomer derived from its coenzyme A (CoA) thioester by action of an acyltransferase (AT) domain, and a ketosynthase (KS) domain catalyzes the decarboxylative Claisen condensation of a nucleophilic malonyl-*S*-T domain thioester with the upstream acyl-*S*-T domain thioester, extending the growing polyketide chain by a C_2 unit. PKS modules may also contain optional tailoring domains such as ketoreductases, dehydratases, and enoyl reductases that further process the initially formed β-ketothioester, allowing access to a diverse library of polyketide structures. As a consequence of this elongation strategy, carbon atoms within a polyketide can be assigned in an alternating pattern as α or β according to their identities in the respective β-ketothioester intermediates.

Polyketide alkylation strategies

Many polyketides possess α-branches, which can be incorporated by two mechanisms. First, because the α-carbons correspond to C2 of the polyketide extender units, incorporation of C2-substituted monomers will yield α-branched polyketide products. This is the most commonly observed strategy for polyketide alkylation, and α-methyl,[2] ethyl,[3] methoxy,[4] hydroxy,[5] and amino[5] braches are known in polyketides. Alternatively, the α-carbons of β-ketothioesters have nucleophilic character, and tailoring domains known as methyltransferases (MT) can utilize the electrophilic methyl source *S*-adenosylmethionine (SAM) to generate α-methylated polyketides.[6]

β-Branching in polyketides

Unlike the nucleophilic α-position, the β-position is electrophilic and therefore requires a nucleophilic alkyl source. Though less common than their α-branched counterparts, β-branched polyketides are known, and inspection of their biosynthetic gene clusters reveals a conserved protein cassette that several researchers speculated was responsible for β-branch incorporation.[7–11] These proposals were later validated, and by early 2008, twelve polyketides with β-substitution that apparently arises by action of this cassette had been reported (Fig. 2).

The first β-branch pathway to be fully reconstituted *in vitro* was obtained from the *pksX*[12] cluster in *B. subtilis*.[13] At the time of this work, the small-molecule product of the cluster was unknown, but subsequent identification of bacillaene as the *pksX* product confirmed that β-alkylation occurred at the position predicted by the *in vitro* experiments.[14]

The core machinery required for β-branch incorporation includes five proteins: (i) a free-standing T domain; (ii) a free-standing KS domain that lacks a conserved cysteine residue required for carbon–carbon bond formation; (iii) an HMG-CoA synthase (HCS) homolog; (iv) an enoyl-CoA hydratase (ECH) homolog that serves as a dehydratase; and (v) a second ECH that serves as a decarboxylase. In addition, many of the β-branched polyketide biosynthetic clusters possess runs of two or three consecutive T domains in their assembly lines as well as free-standing AT domains. The converse correlation is also true; the β-branching cassette appears to be a hallmark of "AT-less" polyketide biosynthetic clusters, which often contain non-standard domain organizations.[15]

In bacillaene, β-branch incorporation begins with the loading of the free-standing T domain AcpK with a malonyl unit,

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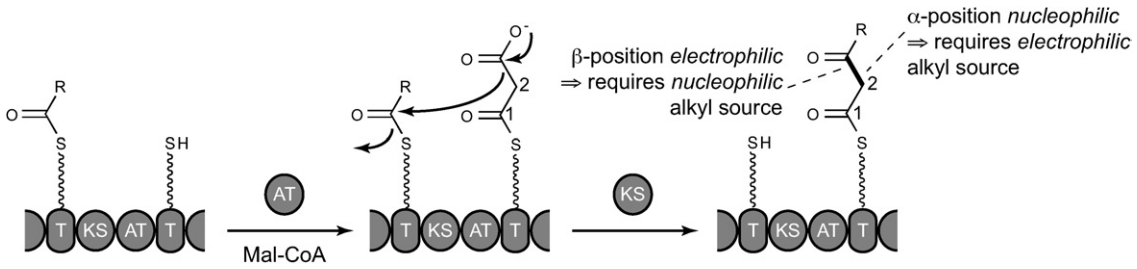


Fig. 1 A single round of ketide extension yields a β-ketothioester.

catalyzed by the AT PksC, followed by decarboxylation to generate acetyl (Ac)-*S*-AcpK, catalyzed by the KS PksF (Fig. 3). Ac-*S*-AcpK serves as the acetyl nucleophile donor in the key β-branch-incorporating step, with the HMG-CoA synthase homolog PksG catalyzing aldol attack of the acetyl enolate onto the assembly line-tethered β-ketothioester to generate an HMG-*S*-T thioester. The HMG-*S*-T thioester is then sequentially dehydrated and decarboxylated by the enoyl-CoA hydratase homologs PksH and PksI to yield the β-methylated intermediate.

Convergence with isoprenoid biosynthesis

The above "canonical" PKS β-methylation pathway represents a convergence between the mevalonate pathway for isoprene biosynthesis from primary metabolism and polyketide biosynthesis from secondary metabolism. It is instructive to consider the parallels between these two metabolic strategies during each stage of β-branch installation.

Provision of electrophiles and nucleophiles

The β-ketothioester electrophiles from both the isoprene and polyketide pathways arise from Claisen condensations. Though formally the isoprene nucleophile Ac-CoA could serve as the nucleophile in the polyketide β-methylation pathway, the β-branching machinery utilizes a T domain-tethered acetyl nucleophile, generated by a malonyl transfer/decarboxylation sequence. By tethering the malonyl unit to a protein scaffold, the β-branching pathway effectively diverts a portion of malonyl-CoA from primary metabolism toward polyketide β-alkylation. One can speculate that the use of the more complex T domain takes advantage of the increased information encoded in the protein's structure, allowing the β-branching pathway to control reactivity *via* non-covalent interactions of the catalytic machinery with the T domain scaffolds.

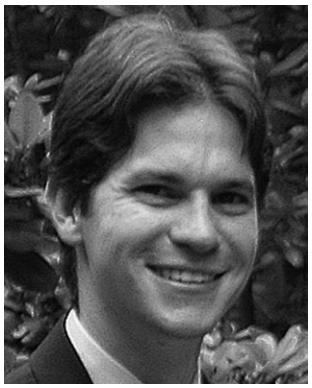
β-Carbon–carbon bond formation

β-Carbon–carbon bond formation in both polyketide biosynthesis and isoprenoid biosynthesis occurs by transfer of an acetyl unit from a thiol scaffold to a conserved cysteine residue in the HCS, though the exact identity of the labeled residue in the polyketide HCS has only been inferred.[16] In the key parallel step between isoprenoid and β-branch incorporation, the HCS then catalyzes the aldol attack of the acetyl enolate on the electrophilic β-ketothioester, forming the critical β-carbon–carbon bond and yielding an initial bis-thioester. Biochemical experiments utilizing acetoacetyl (Acac)-*S*-T as a model substrate revealed that the polyketide HCS is not sensitive to β-ketothioester structure.[13,17] Presumably, protein–protein interactions between the HCS and electrophile-bearing T-domain scaffold determine HCS reactivity and not the identity of β-ketoacyl electrophile itself. The acyl–enzyme thioester linkage is finally selectively hydrolyzed to yield the HMG thioester. In polyketide β-branch incorporation, the bis-thioester is hydrolyzed such that the growing ketide intermediate remains tethered to the assembly line, allowing subsequent ketide extension (Fig. 4).

HMG processing

The next stage is to process the HMG thioester to the β-branched product. In isoprenoid biosynthesis, HMG processing begins with thioester reduction by two hydride equivalents, generating a primary alcohol and cleaving the acyl unit from its thiol scaffold (Fig. 5a). This cleavage from the thiol scaffold in a polyketide context would be catastrophic, severing the polyketide intermediate from the assembly line and preventing further extension. A more immediate consequence of the thioester reduction is to decrease the acidity of the proton α to the former thioester. As a result, the tertiary hydroxyl must be activated as a leaving group to allow decarboxylation *via* a simple 1,2-elimination; only the Δ^3 olefin isomer can be accessed *via* this reaction.

The requirement for the growing polyketide to maintain a stable linkage to its thiol scaffold mandates an alternative strategy for HMG processing. In order to facilitate decarboxylation of the HMG-*S*-T, the polyketide pathway takes advantage of the thioester to acidify the α-protons and allow dehydration (Fig. 5b). The resulting olefin provides a low energy path for



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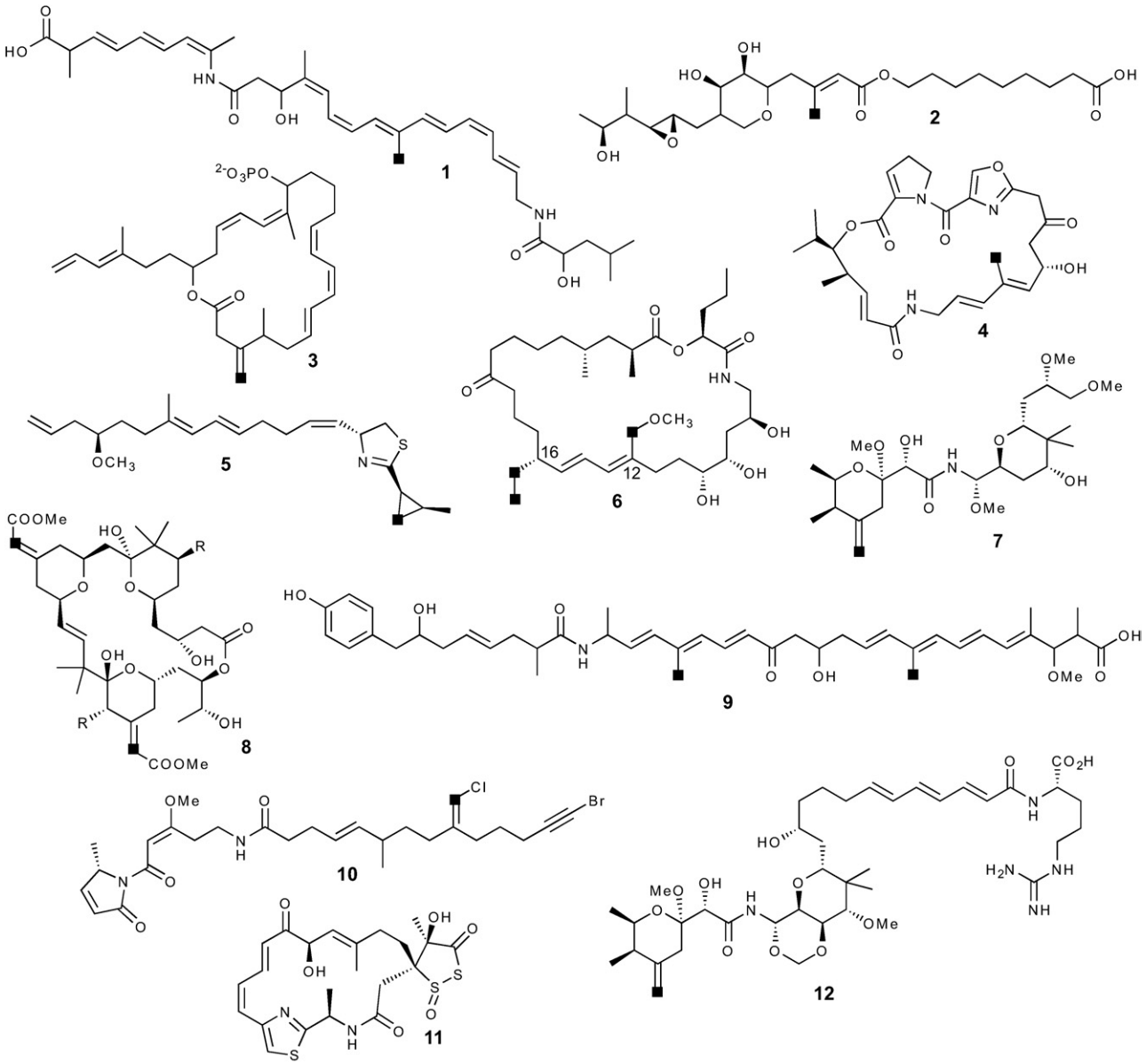

		AT	KS	T	HCS	ECH	ECH	T T
1	Bacillaene	PksC	PksF	AcpK	PksG	PksH	PksI	PksL
2	Mupirocin	MmpIII	MupG	Macp15	MupH	MupJ	MupK	MmpI
3	Difficidin	DifA	—	DifC	DifN	—	DifO	DifL
4	Virginiamycin M	VirI	VirB	*	VirC	VirD	VirE	VirA
5	Curacin	—	CurC	CurB	CurD	CurE	CurF	CurA(T3)
6	Myxovirescin	TaVC	TaK	TaBE	TaCF	TaX	TaY	—
7	Pederin	PedD	PedM	PedN	PedP	PedL	PedI	PedI
8	Bryostatin	BryP	BryQ	—	BryR	—	—	—
9	Thailandamide	BTHII1675	1671	1672	1670	1669	1668	1673
10	Jamaicamide	—	JamG	JamH	JamI	JamJ	JamC	JamE(T3)
11	Leinamycin	LnmG	—	LnmL	LnmM	LnmF	—	LnmJ
12	Onnamide	—	—	—	OnnA	—	—	OnnB(T3)

Fig. 2 Structures of β-branched polyketides, with β-branches denoted by black squares. The identities of β-branch-incorporating proteins are shown below. In the case of virginiamycin M (**4**), an orf homologous to a T domain can be identified between *virA* and *virB*; the original report did not include this orf. Clusters that contain triplets of T domains are denoted with "T3". The exact location of the β-branch in leinamycin (**11**) is not known, although it is likely that it is incorporated in the spiro-fused five-membered heterocycle.

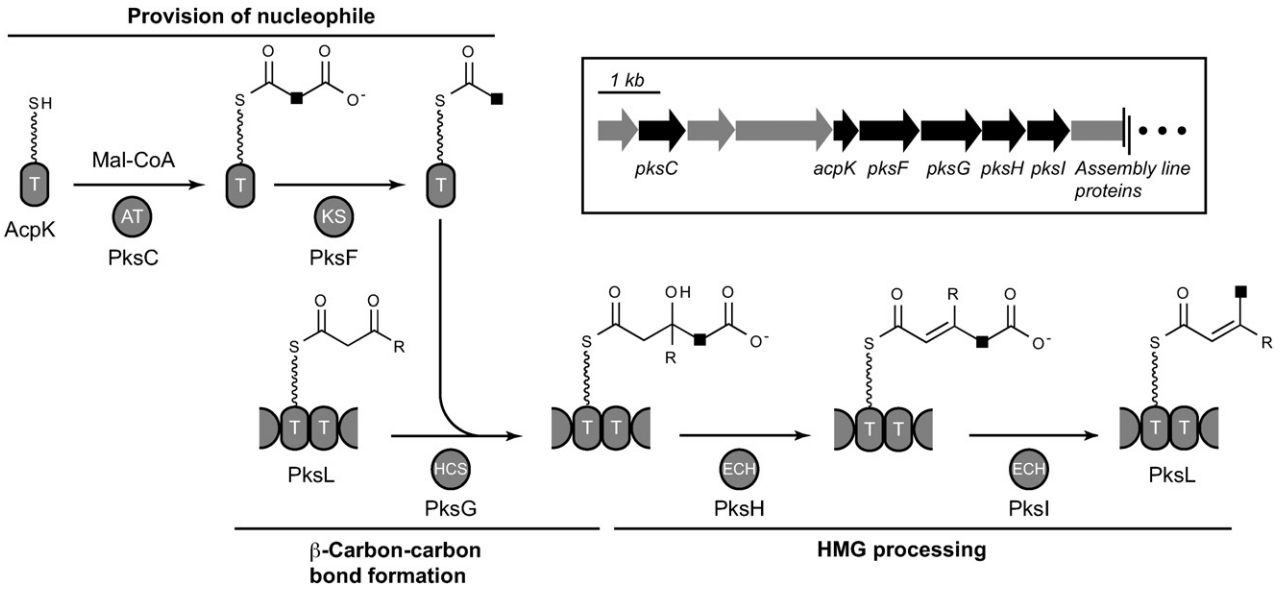

Fig. 3 β-Branch incorporation in bacillaene. (Inset) A portion of the *pksX* gene cluster from *B. subtilis*. Genes involved in β-branch incorporation are shown in black.

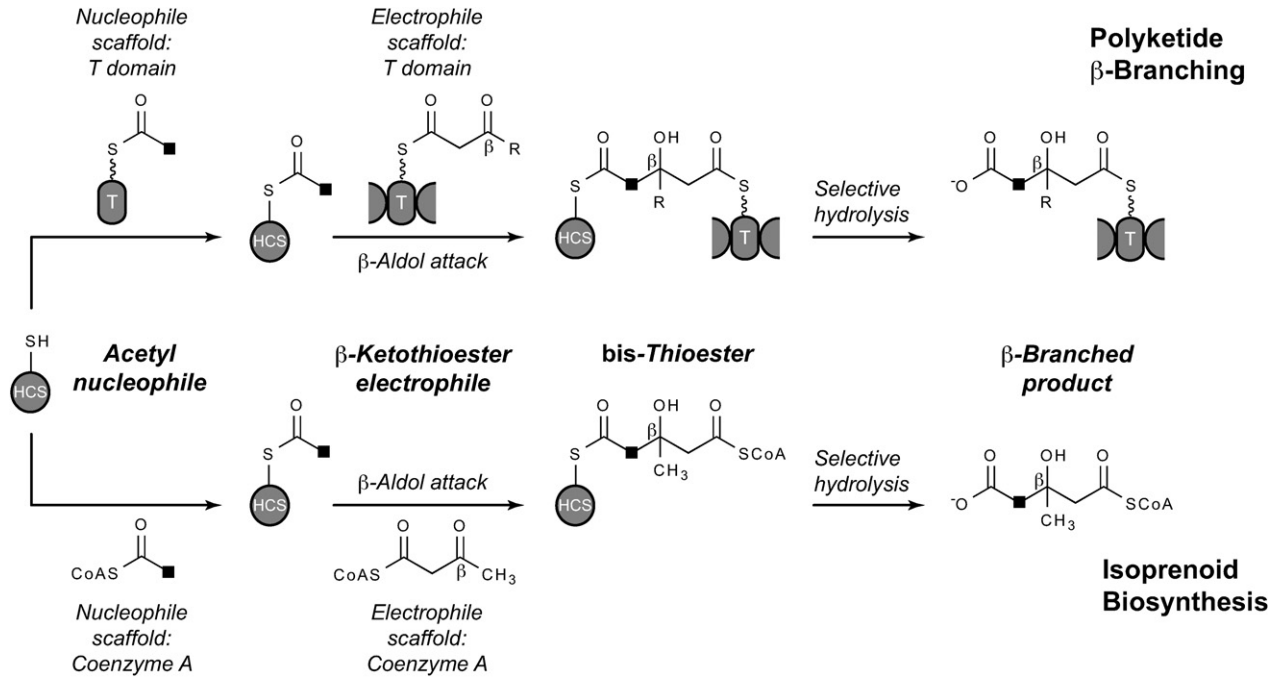

Fig. 4 Comparison of β-carbon–carbon bond formation in (top) the polyketide β-branching pathway and (bottom) canonical isoprenoid biosynthesis. Both pathways involve the aldol attack of acetyl nucleophiles on β-ketothioester electrophiles to yield bis-thioesters. The two pathways diverge in the identities of the electrophile- and nucleophile-bearing scaffolds.

decarboxylation *via* formation of the vinylogous enolate, which can be reprotonated at either the α-position, to yield the Δ^3 olefin isomer, or the γ-position, to yield the Δ^2 olefin isomer. In all systems studied thus far, the decarboxylating ECH produces the Δ^2 isomer. In the case of pederin/onnamide, which possesses a Δ^3 β-olefin, a third ECH is encoded in the cluster, perhaps to catalyze the isomerization of an initially generated Δ^2 olefin.[11,18]

The polyketide HMG-processing sequence has been most thoroughly studied in the curacin pathway.[19] In this system, dehydration and decarboxylation are catalyzed by CurE and a domain within the assembly-line protein CurF [denoted CurF(ECH)], respectively. CurE and CurF(ECH) process HMG-CoA thioesters as well as HMG-*S*-T thioesters, though CurF(ECH) preferred T domain-linked substrates 20-fold over CoA-linked substrates. The reactivity of these enzymes towards

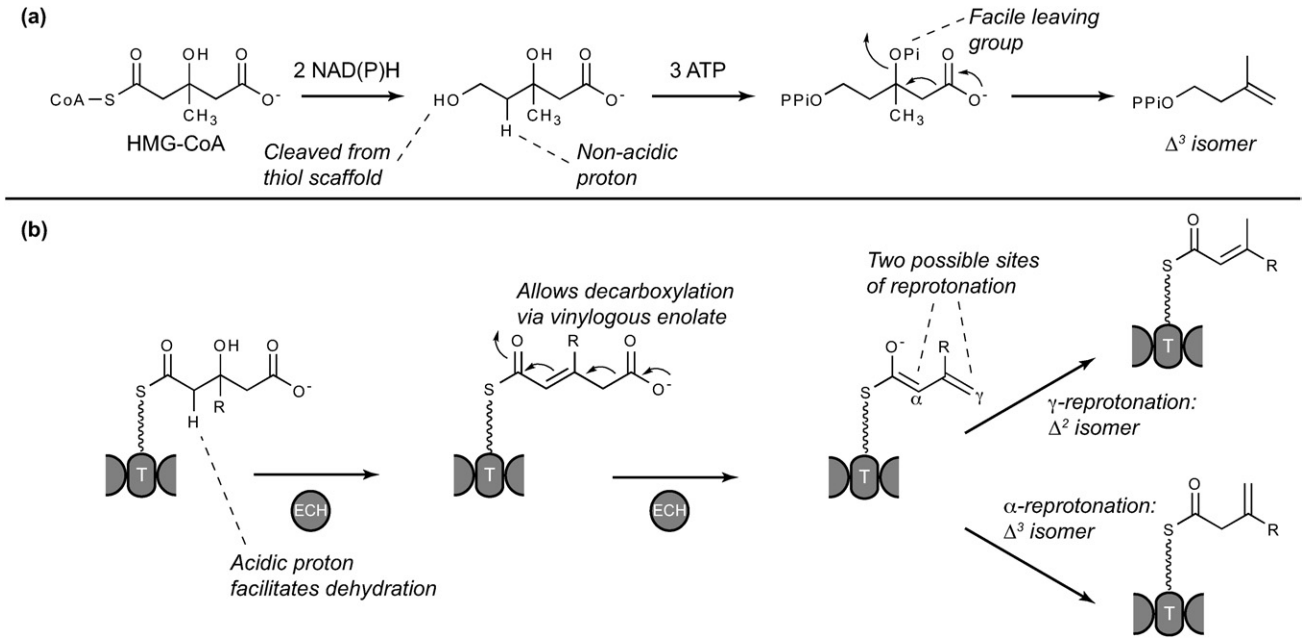

Fig. 5 Comparison of HMG processing (a) in the mevalonate pathway of isoprene biosynthesis and (b) polyketide β-branch incorporation.

the CoA thioesters suggests that CurE and CurF(ECH) recognize the phosphopantetheinyl arm of the substrate, and not necessarily the T domain scaffold, in accord with the differing selectivity requirements of the HCS and ECH in the β-branch-incorporation pathway. Because β-ketothioesters exist during every round of ketide extension, the HCS must recognize and interact only with β-branch-acceptor T domains within the assembly line, whereas because HMG-*S*-T thioesters only exist after reaction with the HCS, the committed β-branching step is upstream of the ECH-catalyzed reactions and the ECHs can be less selective. These experiments determined that CurE prefers *S*-HMG thioesters as substrates over *R*-HMG thioesters, implying that the product of the HCS step in curacin is the *S*-isomer.

The final step in β-branch incorporation is decarboxylation of the methylglutaconyl intermediate to the C_5 β-methylated thioester, catalyzed by CurF(ECH). To further probe the HMG processing sequence, the crystal structure of CurF(ECH) domain was solved to 1.85 Å, providing several insights into the mechanism of decarboxylation.[20] The authors propose that His240 interacts with the substrate carboxylate, Ala78 and Gly118 provide an oxyanion hole to stabilize the enolate generated by decarboxylation, and Lys86 reprotonates at the γ-position, yielding the Δ^2 thioester selectively. Finally, the authors propose that replacement of a basic residue that interacts with a phosphate in CoA with a bulky Tyr residue in CurF(ECH) shifts the substrate preference from CoA thioesters to T domain thioesters, though some reactivity with CoA thioesters is maintained.

Extension

In the polyketide case, because the key thioester linkage to the assembly line has been maintained, the β-branched ketide intermediate can directly serve as the electrophile in the KS-catalyzed Claisen condensation with the downstream nucleophilic malonyl unit regardless of the olefin isomer formed after decarboxylation (Fig. 6).

In isoprenoid biosynthesis, the isoprenyl pyrophosphate unit serves as both electrophile and nucleophile in subsequent reactions. However, in order to act as an electrophile, the Δ^3 isoprenyl unit must first be converted to the Δ^2 isomer, allowing formation of the stabilized allyl cation upon loss of the pyrophosphate, in contrast to the unstable primary cation that would be formed from the Δ^3 isomer. The allylic cation intermediate possesses two electrophilic reactive sites, allowing access to "normal" or "reverse" prenylated products.

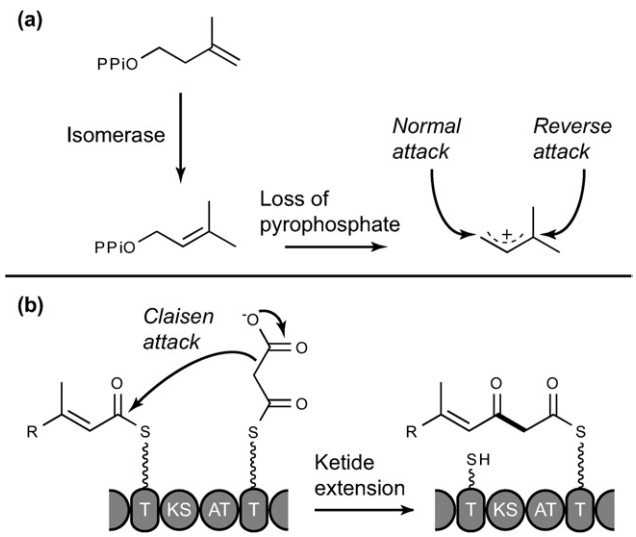

Fig. 6 Comparison of extension strategies of β-branched intermediates (a) in canonical isoprenoid biosynthesis and (b) in polyketide biosynthesis.

Variations in the β-branching pathway

The above pathway only allows access to β-methylated polyketides, but several polyketides possess more complex β-substituents, including ethyl,[21] methoxymethyl,[21] and cyclopropane branches.[9] These structures can be accessed by variation of the electrophile structure, nucleophile structure, or HMG processing sequence, as well as by elaboration of a β-methyl precursor.

Alternative electrophile

Frequently, the initially generated β-ketothioester undergoes ketoreduction and dehydration during ketide extension. The resulting α,β-unsaturated thioester can serve as an alternative β-electrophile for β-branch installation. The rhizoxin biosynthetic pathway contains an unprecedented "β-domain" that is proposed to exploit the β-electrophilicity of such T domain-tethered α,β-unsaturated thioesters, catalyzing the Michael attack of an acetyl enolate equivalent (Fig. 7a).[22] Ultimately, the β-acetyl branch incorporated by this uncharacterized β-domain forms a δ-lactone. It is noteworthy that there are no ECHs in the rhizoxin pathway; because the electrophile is the Michael-type acceptor, the initial β-adduct cannot be further processed by dehydration.

Alternative nucleophile

The C16 ethyl branch in myxovirescin is incorporated by utilization of an alternative nucleophile, and its installation has been partially reconstituted *in vitro* (Fig. 7b).[17] In the myxovirescin biosynthetic gene cluster, the HCS homolog TaF catalyzes the attack of a propionyl enolate, delivered by the free-standing T domain TaE, to a T domain-tethered β-ketothioester. The resulting γ-methyl-HMG derivative is dehydrated and decarboxylated by the two ECH homologs in the myxovirescin cluster TaXY to yield the β-ethyl branch. An AT/KS pair to generate the propionyl-*S*-TaE nucleophile could not be identified within the cluster, suggesting that this activity is encoded elsewhere in the genome. Independent genetic experiments have confirmed the role of TaEF in the generation of the β-ethyl branch in myxovirescin.[23,24]

Alternative processing

A third strategy to vary the β-branch structure is to vary the HMG-processing sequence. In the case of bryostatin, two β-methoxyacylidene moieties are proposed to be incorporated by a pathway similar to that described above (Fig. 7c).[25] In this

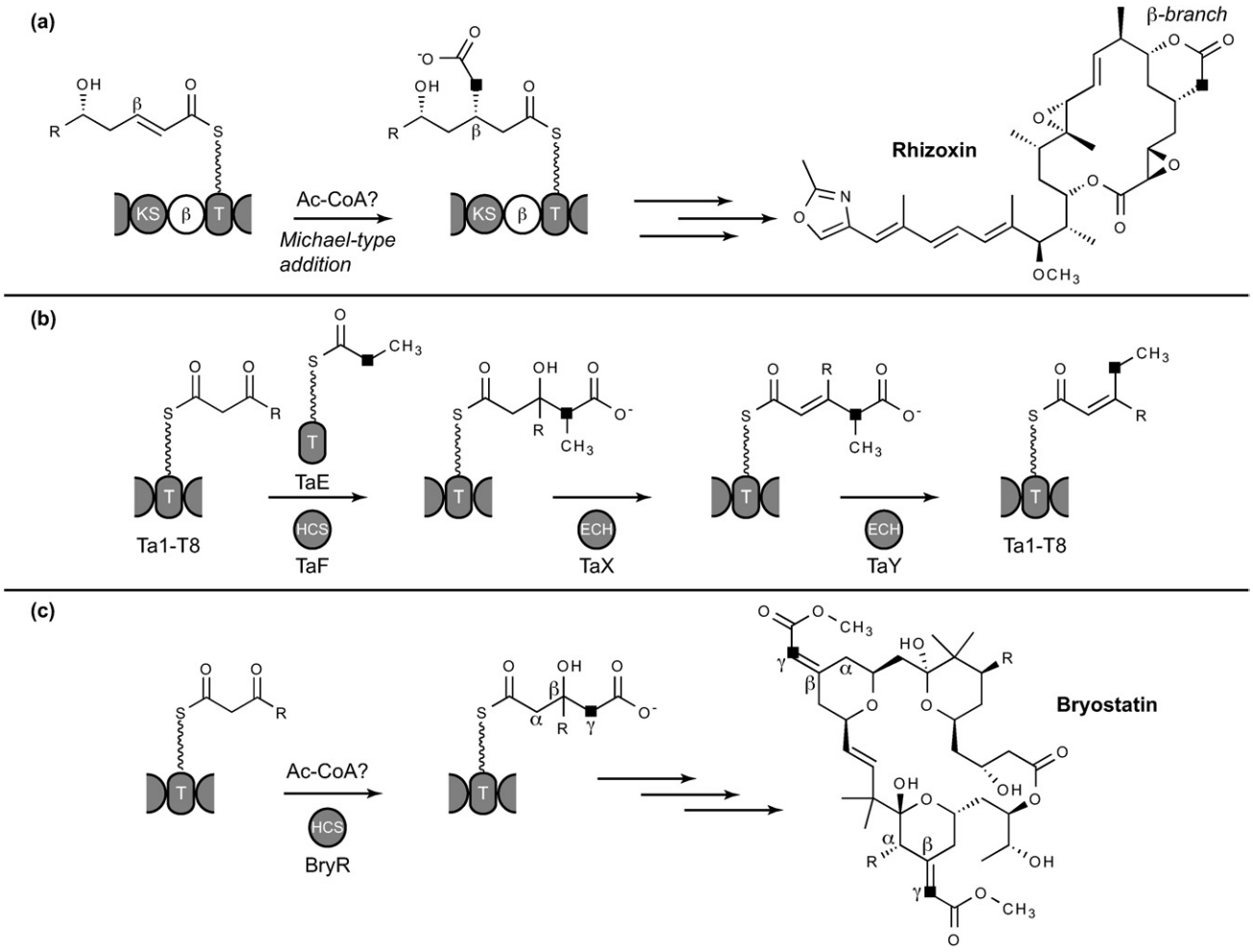

Fig. 7 Variations in polyketide β-branch incorporation. (a) An α,β-unsaturated thioester is utilized as a Michael-type electrophile during the biosynthesis of rhizoxin. (b) Replacing the acetyl enolate with a propionyl enolate in the myxovirescin biosynthesis allows access to the C16 β-ethyl branch. (c) The methoxyacylidene branches in bryostatin are accessed by varying the processing sequence of the HMG-thioester.

pathway, the HCS homolog BryR catalyzes the attack of an acetyl nucleophile likely provided by acetyl-CoA on an assembly-line-tethered β-ketothioester. Instead of undergoing a full dehydration/decarboxylation sequence, the resulting HMG analog undergoes β,γ-dehydration—the opposite regioisomer observed in the canonical pathway—thereby precluding thioester-assisted decarboxylation. Notably, there is no obvious candidate within the bryostatin cluster to catalyze this dehydration. The acid is then methylated, generating the methyl ester observed in the final product.

Methyl derivatization

A fourth strategy to achieve alternative β-branch structures is derivatization of the β-methyl branch subsequent to its incorporation. Such a pathway has been genetically confirmed during C12 β-methoxymethylation in myxovirescin. Analysis of culture extracts from mutant strains lacking the cytochrome P450 TaH or the *O*-methyltransferase TaQ revealed that the C12 β-methoxymethyl branch arises from sequential hydroxylation and methylation of a C12 β-methyl precursor.[26] This model was corroborated by the *in vitro* reconstitution of C12 β-methylation.[17] Indeed, myxovirescin variants possessing C12 β-methyl and β-hydroxymethyl branches are known in nature, suggesting that the myxovirescin β-methyl tailoring enzymes may be inefficient.[27]

Role of tandem T domains

A commonly observed property of the β-branch-encoding gene clusters is the presence of runs of two or three consecutive thiolation domains in the associated assembly lines. Furthermore, these tandem T domains are often located in modules on which β-branch incorporation is predicted to occur, suggesting that this unusual domain architecture may have some role in recruiting the *trans*-acting β-branch biosynthetic machinery to the appropriate site on the assembly line. Indeed, a set of tandem T domains in PksL was demonstrated *in vitro* to serve as the β-branch-acceptor scaffold during bacillaene biosynthesis, though there was no apparent preference of the *trans*-acting enzymes toward substrates tethered to one domain or the other.[13] Additionally, CurF(ECH)-catalyzed decarboxylation was reconstituted on a T domain excised from a triplet of consecutive T domains in the curacin assembly line.[20]

The role of tandem T domains was directly addressed in the mupirocin assembly line, which possesses two separate runs of two and three consecutive T domains.[28] Individual and pairwise in-frame deletion of T domains, along with generation of mutant strains in which the phosphopantetheinylated serine residues within the T domains were individually replaced by alanines, led to a model in which the multiple T domains act to increase biosynthetic flux through the pathway. The authors of this study explicitly proposed the possible role of the additional T domain in a T–T doublet as a way-station for β-branch incorporation. It should be pointed out that tandem T domains are not unique to β-branched polyketides; other tandem T domains are observed in the naphthopyrone,[29] sterigmatocystin[30] and albicidin[31] clusters, as well as in polyunsaturated fatty acid biosynthetic clusters where up to nine consecutive T domains have been observed.[32] There are no tandem T domains in the myxovirescin or bryostatin clusters, and in the case of bacillaene, a second set of tandem T domains do not play a role in β-branch incorporation. Taken together, the above observations reveal that the correlation of tandem T domains with β-branch sites is not absolute, and a unifying biochemical model for the presence of these runs of T domains has yet to be satisfactorily delineated.‡

Branch selectivity in myxovirescin

Myxovirescin (**6**) is unique among known β-branch-containing products in that it possesses two structurally distinct β-branches: a C12 β-methoxymethyl (produced by hydroxylation and *O*-methylation of a C12 β-methyl precursor) and C16 β-ethyl. The myxovirescin cluster also contains a partially duplicated β-branch-incorporating cassette, with two copies of the AT, free-standing T, and HCS homolog, but only a single copy of the KS-like decarboxylase and a single pair of ECH homologs. Myxovirescin thus provides an ideal system in which to study the selective recognition of β-branch-incorporating enzymes for one another (Fig. 8).[17]

First, the selectivities of the AT domains (TaV_C and TaV_N) toward their malonyl and T-domain substrates were tested biochemically. These experiments demonstrated that TaV_C selectively utilizes malonate (as expected for generation of the C12 β-methyl precursor), but loads both T domains (TaB and TaE) non-selectively. TaK decarboxylates malonyl and methylmalonyl substrates linked to TaB, but shows no activity toward any of the malonated TaE derivatives tested. The TaV_N substrates could not be identified, and as there are no other candidate AT or KS domains in the myxovirescin cluster, the source of the nucleophile for the β-ethylation pathway remains mysterious. Genetic

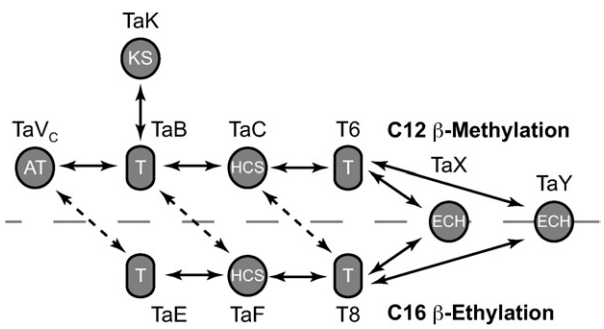

Fig. 8 Map of productive *in vitro* interactions among β-branch-incorporating proteins in the myxovirescin biosynthetic cluster. Proteins involved in incorporation of the β-methyl precursor to the C12 β-methoxymethyl branch are above the dashed line; proteins involved in incorporation of the C16 β-ethyl branch are below the dashed line. Physiologically relevant interactions are shown with solid double-headed arrows; interactions observed *in vitro* that are not thought to be physiologically relevant are shown with dotted double-headed arrows. An acyltransferase and decarboxylating ketosynthase to yield Prop-*S*-TaE have not been identified.

‡ While this *Highlight* was under review the following report examining the role of tandem T domains in polyunsaturated fatty acid biosynthesis was published: H. Jiang, R. Zirkle, J.G. Metz, L. Braun, L. Richter, S. G. Van Lanen and B. Shen, *J. Am. Chem. Soc.*, 2008, **130**, 6336–6337.

experiments revealed that TaK is not absolutely necessary for formation of myxovirescin.[24]

The key selectivity-determining event occurs at the HCS-catalyzed step. By linking the nucleophile donor scaffold and its acyl payload with the β-branch acceptor scaffold, the HCS interactions determine the identity and location of β-branches in the polyketide. To probe the interactions between the nucleophile donor T domains TaB and TaE, and the HCS homologs TaC and TaF, the relevant single knockout strains were generated in the myxovirescin producer *Myxococcus xanthus*. The phenotypes of the △*taB* and △*taC*, and △*taE* and △*taF* strains were identical, demonstrating that TaC utilizes TaB as a nucleophile scaffold selectively, and TaF utilizes TaE.[23,24] Additionally, the △*taF* mutant generated the known C16 β-methyl congener of myxovirescin, suggesting that the TaEF pair is responsible for the C16 β-ethylation and furthermore that TaC can complement TaF.[27]

Biochemical experiments corroborate the above interpretations.[17] In *in vitro* experiments, TaC utilizes only TaB as a nucleophile scaffold; in contrast, the Ac-TaF enzyme intermediate is formed when TaF is incubated with Ac-*S*-TaB, though no further reaction is observed. Presumably, the lack of acetyl transfer from Ac-TaF is a result of utilizing an acetyl nucleophile instead of the physiological propionyl nucleophile. The complementation of TaF by TaC was also confirmed; TaC can transfer acetyl nucleophiles from TaB to its own as well as TaF's β-branch-acceptor T domain, though it does not recognize non-β-branch-acceptor T domains. In contrast, TaF is only reactive toward its own β-branch-acceptor T domain.

Finally, only a single pair of ECH homologs is found in the myxovirescin biosynthetic cluster. They are biochemically promiscuous, both in terms of T domain scaffold and small-molecule HMG substrate, processing both the HMG-like methyl and ethyl branch precursors tethered to either T-domain scaffold.[17]

These experiments have begun to tease out the selectivity determinants of the β-branch-incorporating machinery. However, until the machinery responsible for the generation of propionyl-*S*-TaE is discovered, it will be impossible to account completely for β-branch selectivity in myxovirescin.

Conclusions

The recent discovery of isoprenoid-like polyketide β-branch-incorporating enzymes adds to our understanding of the relationship between primary and secondary metabolic logics. A pattern in many secondary metabolic pathways is their close convergence with primary metabolic pathways. For example, polyketide biosynthesis from secondary metabolism is closely related to fatty acid biosynthesis from primary metabolism, utilizing functionally equivalent domains to achieve identical transformations.

In many cases, however, the secondary metabolic pathways have a greater degree of biochemical potential than their primary metabolic counterparts. For example, although they are biosynthesized using the same set of transformations, polyketides display a markedly greater degree of structural diversity than fatty acids. β-Branch formation represents an additional example of this biosynthetic theme. As we have seen, it is closely related to isoprenoid biosynthesis, exploiting the obligatory generation of a β-electrophile during ketide extension to intersect with polyketide biosynthetic pathways, yet allows access to structures more complex than the simple β-methylated isoprene units.

A second lesson in the relationship between primary and secondary metabolism can be inferred by further inspection of the structure and biosynthetic gene clusters of β-branched polyketides. β-Branched polyketides are over-represented among the *trans*-AT class of polyketides,[33] which are biosynthesized by a patchwork collection of biosynthetic machineries assembled from multiple sources.[15] The ability to generate such chimeric assembly lines highlights the biochemical flexibility and opportunism of secondary metabolic pathways. Perhaps the key insight of the β-branch-containing clusters is the ingenuity of evolution to cobble together biochemical reactive pathways from multiple metabolic strategies. A challenge to those who wish to understand secondary metabolite biosynthesis is to elucidate how these strikingly hybrid pathways can come together on a molecular scale.

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References

1 M. A. Fischbach and C. T. Walsh, *Chem. Rev.*, 2006, **106**, 3468–3496.
2 S. Donadio, M. J. Staver, J. B. McAlpine, S. J. Swanson and L. Katz, *Science*, 1991, **252**, 675–679.
3 W. S. Jung, S. K. Lee, J. S. Hong, S. R. Park, S. J. Jeong, A. R. Han, J. K. Sohng, B. G. Kim, C. Y. Choi, D. H. Sherman and Y. J. Yoon, *Appl. Microbiol. Biotechnol.*, 2006, **72**, 763–769.
4 S. C. Wenzel, R. M. Williamson, C. Grünanger, J. Xu, K. Gerth, R. A. Martinez, S. J. Moss, B. J. Carroll, S. Grond, C. J. Unkefer, R. Müller and H. G. Floss, *J. Am. Chem. Soc.*, 2006, **128**, 14325–14336.
5 Y. A. Chan, M. T. Boyne, A. M. Podevels, A. K. Klimowicz, J. Handelsman, N. L. Kelleher and M. G. Thomas, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 14349–14354.
6 D. A. Miller, C. T. Walsh and L. Luo, *J. Am. Chem. Soc.*, 2001, **123**, 8434–8435.
7 Y. Paitan, E. Orr, E. Z. Ron and E. Rosenberg, *Microbiology (Reading, U. K.)*, 1999, **145**(Pt 11), 3059–3067.
8 A. K. El-Sayed, J. Hothersall, S. M. Cooper, E. Stephens, T. J. Simpson and C. M. Thomas, *Chem. Biol.*, 2003, **10**, 419–430.
9 Z. Chang, N. Sitachitta, J. V. Rossi, M. A. Roberts, P. M. Flatt, J. Jia, D. H. Sherman and W. H. Gerwick, *J. Nat. Prod.*, 2004, **67**, 1356–1367.
10 D. J. Edwards, B. L. Marquez, L. M. Nogle, K. McPhail, D. E. Goeger, M. A. Roberts and W. H. Gerwick, *Chem. Biol.*, 2004, **11**, 817–833.
11 J. Piel, D. Hui, G. Wen, D. Butzke, M. Platzer, N. Fusetani and S. Matsunaga, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 16222–16227.
12 A. M. Albertini, T. Caramori, F. Scoffone and C. Scotti, *Microbiology (Reading, U. K.)*, 1995, **141**(Pt. 2), 299–309.
13 C. T. Calderone, W. E. Kowtoniuk, N. L. Kelleher, C. T. Walsh and P. C. Dorrestein, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 8977–8982.
14 R. A. Butcher, F. C. Schroeder, M. A. Fischbach, P. D. Straight, R. Kolter, C. T. Walsh and J. Clardy, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 1506–1509.

15 T. Nguyen, K. Ishida, H. Jenke-Kodama, E. Dittmann, C. Gurgui, T. Hochmuth, S. Taudien, M. Platzer, C. Hertweck and J. Piel, *Nat. Biotechnol.*, 2008, **26**, 225–233.
16 A. M. Haapalainen, G. Merilainen and R. K. Wierenga, *Trends Biochem. Sci.*, 2006, **31**, 64–71.
17 C. T. Calderone, D. F. Iwig, P. C. Dorrestein, N. L. Kelleher and C. T. Walsh, *Chem. Biol.*, 2007, **14**, 835–846.
18 J. Piel, D. Butzke, N. Fusetani, D. Hui, M. Platzer, G. Wen and S. Matsunaga, *J. Nat. Prod.*, 2005, **68**, 472–479.
19 L. Gu, J. Jia, H. Liu, K. Håkansson, W. H. Gerwick and D. H. Sherman, *J. Am. Chem. Soc.*, 2006, **128**, 9014–9015.
20 T. W. Geders, L. Gu, J. C. Mowers, H. Liu, W. H. Gerwick, K. Håkansson, D. H. Sherman and J. L. Smith, *J. Biol. Chem.*, 2007, **282**, 35954–35963.
21 W. Trowitzsch, V. Wray, K. Gerth and G. Höfle, *J. Chem. Soc.*, 1982, **104**, 1340–1342.
22 L. P. Partida-Martinez and C. Hertweck, *ChemBioChem*, 2007, **8**, 41–45.
23 V. Simunovic and R. Müller, *ChemBioChem*, 2007, **8**, 497–500.
24 V. Simunovic and R. Müller, *ChemBioChem*, 2007, **8**, 1273–1280.
25 S. Sudek, N. B. Lopanik, L. E. Waggoner, M. Hildebrand, C. Anderson, H. Liu, A. Patel, D. H. Sherman and M. G. Haygood, *J. Nat. Prod.*, 2007, **70**, 67–74.
26 V. Simunovic, J. Zapp, S. Rachid, D. Krug, P. Meiser and R. Müller, *ChemBioChem*, 2006, **7**, 1206–1220.
27 W. Trowitzsch-Kienast, K. Schober, V. Wray, K. Gerth, H. Reichenbach and G. Höfle, *Liebigs Ann. Chem.*, 1989, 345–355.
28 A. S. Rahman, J. Hothersall, J. Crosby, T. J. Simpson and C. M. Thomas, *J. Biol. Chem.*, 2005, **280**, 6399–6408.
29 I. Fujii, A. Watanabe, U. Sankawa and Y. Ebizuka, *Chem. Biol.*, 2001, **8**, 189–197.
30 J. H. Yu and T. J. Leonard, *J. Bacteriol.*, 1995, **177**, 4792–4800.
31 G. Huang, L. Zhang and R. G. Birch, *Microbiology*, 2001, **147**(Pt. 3), 631–642.
32 J. G. Metz, P. Roessler, D. Facciotti, C. Levering, F. Dittrich, M. Lassner, R. Valentine, K. Lardizabal, F. Domergue, A. Yamada, K. Yazawa, V. Knauf and J. Browse, *Science*, 2001, **293**, 290–293.
33 G. L. Tang, Y. Q. Cheng and B. Shen, *Chem. Biol.*, 2004, **11**, 33–45.