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Curr Top Med Chem. Author manuscript; available in PMC 2011 June 6.

Published in final edited form as:
Curr Top Med Chem. 2008 ; 8(6): 448–459.

Biosynthesis of Enediyne Antitumor Antibiotics

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Abstract

The enediyne polyketides are secondary metabolites isolated from a variety of Actinomycetes. All members share very potent anticancer and antibiotic activity, and prospects for the clinical application of the enediynes has been validated with the recent marketing of two enediyne derivatives as anticancer agents. The biosynthesis of these compounds is of interest because of the numerous structural features that are unique to the enediyne family. The gene cluster for five enediynes has now been cloned and sequenced, providing the foundation to understand natures' means to biosynthesize such complex, exotic molecules. Presented here is a review of the current progress in delineating the biosynthesis of the enediynes with an emphasis on the model enediyne, C-1027.

Keywords

Anticancer antibiotic; biosynthesis; C-1027; enediynes; maduropeptin; neocarzinostatin; polyketide synthase

INTRODUCTION

During the 1980s a new class of natural products named the enediynes was introduced with the structural elucidation of neocarzinostatin [1] and calicheamicin [2]. Since this time, thirteen enediynes have been structurally confirmed, which includes two probable enediynes isolated as inactive degradation products (Table 1; Fig. 1) [3–17]. All of the enediyne members share potent antibiotic and antitumor activities with cytotoxicity comparable to any known microbial metabolite [18]. Consequently, much effort has been put forth to develop the enediynes as anticancer agents.

Regardless of their phenomenal biological activity, members of the enediyne family display structural rarities that have hitherto been unseen in other natural products. All of the members share a characteristic unsaturated core containing two acetylenic groups conjugated to a double bond, harbored within a nine- or ten-membered ring termed the enediyne core. The majority of the ten-membered enediynes contain an allylic trisulfide that is directly implicated in triggering the generation of the reactive chemical species for their biological activity. Several of the ten-membered enediynes also contain unusual thiol-sugars. Finally, while mono-chlorinated aromatic moieties are common in enediyne

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structures, unique to the ten-membered enediyne calicheamicin is an iodinated orsellinic acid component that has been shown to directly aid in DNA-binding [19].

While the peripheral chemistry displayed for each enediyne fine-tunes the biological activity, it is the enediyne core that is the hallmark feature essential for their remarkably potent cytotoxicity. Upon an environmental trigger such as thiol activation or ultraviolet light, the enediyne core undergoes Bergman or Myers-Saito cyclization to yield a benzenoid diradical (Fig. 2). The diradical species is capable of abstracting hydrogen atoms from DNA, resulting in adverse cellular effects such as mutations and environmental trigger. C-1027 [20] and maduropeptin [21] are exceptionally reactive and have been shown to cycloaromatize without external activation. If they are indeed enediynes, the sporolides [11] and cyanospora-sides [12] represent the extreme case since neither is isolated in the enediyne form.

The biosynthesis of the enediynes is intriguing because of the plethora of unique chemical features incorporated into each molecule. The origin of the enediyne core was initially studied using isotope-labeling experiments by monitoring the production of neocarzinostatin [22], dynemicin [23], and esperamicin [24]. While the data unequivocally established acetate as a precursor unit, the results did not distinguish whether the enediyne core was constructed by the degradation of fatty acids or by de novo biosynthesis with a dedicated fatty acid or polyketide synthase (PKS). Since the time of the feeding experiments, the gene cluster for 5 enediynes, C-1027 [25], neocarzinostatin (NCS) [26], calicheamicin [27], dynemicin [28,29], and maduropeptin [63], have been cloned and characterized providing the foundation to investigate enediyne biosynthesis. Presented here are highlights of recent molecular and biochemical studies deciphering enediyne biosynthesis [30]. The reader is referred to other articles in this issue and a number of excellent reviews for information regarding total synthetic accomplishments [31–34], mechanisms of resistance to enediynes [35,36], and the biological activity of the enediyne family [37,38].

FINDING THE GENE CLUSTER

A distinguishing characteristic of the nine-membered enediyne family is the isolation of the molecule as a chromoprotein complex consisting of a binding protein, also known as the apo-protein, and a dissociable enediyne chromophore. The binding protein directs transport of the reactive enediyne chromophore to the extracellular environment [35,39], and is also essential for self-resistance by stabilizing the reactive enediyne chromophore. The latter function has been suggested from observations that the gene for the binding protein of C-1027 [40] and macromomycin [41] are constitutively expressed and, as previously mentioned, the 9-membered enediynes C-1027 and maduropeptin readily undergo cycloaromatization in the absence of the binding protein. Interestingly, no binding protein was found for N1999A2, which is the only 9-membered enediyne that has been isolated in the enediyne form without a binding protein [10].

Pioneering work on the isolation of C-1027 and NCS chromoprotein complexes established the amino acid sequence of the homologous binding proteins [35], which ultimately led to the cloning and the sequencing of the gene (*cagA* for C-1027 [42] and *ncsA* for NCS [43]). This locus was subsequently used, in combination with degenerate primers for a predicted dNDP-glucose-4,6-dehydratase gene ubiquitously found in pathways for the production of 6-deoxy aminosugars, as probes to identify and localize the entire gene cluster for C-1027 [25,44]. A different strategy was used to clone the gene cluster for the ten-membered enediyne calicheamicin since there was no luxury of a binding protein and its sequence [27]. By screening clones capable of conferring calicheamicin resistance and using PCR-based screens, followed up by DNA-shotgun sequencing, the calicheamicin gene cluster was

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localized and cloned. While numerous differences between the clusters exist, a unified biosynthetic scheme for the nine- and ten-membered enediynes was evident with the uncovering of a shared iterative type I PKS that is unique to the enediyne family, and many shared open reading frames are apparent [28]. The conserved architecture of enediyne biosynthetic gene clusters, featuring the enediyne PKS, formed the basis of several expedient strategies to clone additional enediyne biosynthetic gene clusters as exemplified by the NCS, maduropeptin, and dynemicin gene clusters *via* a PCR approach [28] and numerous yet-to-be characterized enediyne gene clusters from previously unknown producers *via* a genome scanning method [29].

GENERAL BIOSYNTHETIC STRATEGY

The C-1027 chromophore can be dissected into 4 biosynthetic building blocks: an enediyne core, a β-amino acid, a deoxy aminosugar, and a benzoxazolinate moiety (Fig. 3A). Sequencing of the gene cluster revealed homologs for a glycosyl transferase, an acyltransferase, and condensation enzymes, suggesting a convergent biosynthetic approach is used in enediyne assembly [25]. Similarly, the calicheamicin [27], NCS [26], and maduropeptin [63] gene clusters also contain open reading frames with analogous predictions, as shown in Fig. 3B for NCS. As discussed below, the initial enzymatic step(s) in every pathway for C-1027 have now been analyzed using a combination of *in vivo* gene inactivation and *in vitro* characterization of recombinant enzymes, unambiguously establishing the starting metabolite for each moiety and providing substantial evidence for a convergent approach in enediyne biosynthesis.

PKSE: AN ITERATIVE TYPE I PKS

Bacterial polyketide biosynthesis typically follows one of three paradigms: biosynthesis by a noniterative, modular PKS (type I), a multienzyme complex of iteratively acting PKS activities (type II), or homodimeric, iteratively acting condensing PKS without an acyl carrier protein (ACP) (type III) [45,46]. While these three PKS archetype have provided the molecular rationale to explain structural diversity among polyketides as well as have provided the basis for the discovery of novel natural products by genetic engineering, data from numerous genome-sequencing and secondary metabolite gene cluster-sequencing projects have now revealed alternative means to polyketide biosynthesis that extend beyond the classical paradigms. The enediyne PKS family represents one such extension.

To date, among the five gene clusters cloned only one type of PKS has been found, an iterative type I PKS. The gene cluster for C-1027 contains a single PKS that is shared by sequence homology and domain architecture among the enediyne family (Fig. 4A). Disruption of this PKS (SgcE) in the C-1027 producer abolished production of C-1027, which was restored by the introduction of a complementation plasmid containing *sgcE* under control of the constitutive *ermE** promoter [25]. Identical results have been obtained with the homologous neocarzinostatin PKS, NcsE [26], and in total the results provided definitive evidence that the enediyne core is produced by a polyketide pathway.

Bioinformatic analysis of this PKS family, PKSE, identified 4 domains in the following order (N- to C-terminus): a ketosynthase (KS), an acyltransferase (AT), a ketoreductase (KS), and dehydrogenase (DH), with closest sequence homology to polyunsaturated fatty acid (PUFA) synthases involved in the biosynthesis of docosahexaenoic acid in *Moritella marina* and eicosapentaenoic acid in *Shewanella* [47] (Fig. 4A). An internal region located between the AT and KR was initially proposed to be an ACP based on the exact architecture to PUFA synthases, although this region has no homology to any proteins in public databanks. The C-terminal domain based on structural modeling predictions, was proposed

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to be a phosphopantetheinyl transferase (PPTase) responsible for loading the phosphopantetheine (Ppant) cofactor to the ACP to initiate polyketide biosynthesis [29].

Amino acids essential for the function of individual domains were tested using the established *in vivo* system for the C-1027 producer and the *ΔsgcE* mutant by preparing point mutations of SgcE within the complementation construct [64]. Of the multiple mutations prepared, the results have established that S974, the serine originally hypothesized to be the site of Ppant modification within the ACP domain, and D1827, an aspartic acid proposed to be critical for PPT activity within the C-terminal PPT domain, both abolished the production of C-1027, consistent with the designation of the ACP and PPTase domains.

The PKSE was also analyzed using recombinant protein to further probe domain predictions. SgcE and NcsE are produced as soluble proteins in *E.coli*, and production of PKSE results in a yellow pigment associated with the protein and cell debris suggesting the protein is in the active form. The purified ~210 kDa protein was subjected to trypsin digestion and peptide mapping using liquid chromatography-fourier transform mass spectroscopy. As predicted S974 and the analogous residue of NcsE had +340 amu shifts consistent with modification by a Ppant group. Mutation of this serine eliminated the mass shift, as did mutations within the predicted PPTase domain. In contrast, all mutations outside the ACP and PPTase domains did not alter the observed Ppant mass shift. As expected, the S974A and D1827A point mutations also eliminated the production of the yellow pigment associated with the *sgcE* or *ncsE* gene product. In total the data is consistent with self-phosphopanetheinylation of a unique ACP, unambiguously establishing an ACP-dependent PKSE-catalyzed pathway for enediyne biosynthesis (Fig. 5A). While self-phosphopantetheinylation has been observed for a yeast fatty acid synthase [48], this is the first example of a bacterial PKS with such catalytic activity and domain organization [49], representing a new PKS paradigm.

OTHER ITERATIVE TYPE I PKS

In addition to the shared iterative type I PKS, PKSE, a second, separate family of iterative type I PKS is also found in the calicheamicin [27], NCS [26], and maduropeptin [62] gene clusters. The 3 PKSs have a minimum of 4 domains in the following order: KS, AT, DH, and ACP, and the domain organization is similar to AviM involved in the biosynthesis of the orsellinic acid of avilamycin in *S. viridochromogenes Tü57* (Fig. 4B) [50]. The calicheamicin gene cluster contains CalO5, presumed to be responsible for the production of the orsellinic acid moiety [27]. Within the maduropeptin gene cluster, a PKS (MadB) was discovered that is likely responsible for the biosynthesis of the 6-methylsalicyclic acid moiety [63]. Finally, the neocarzinostatin gene cluster contains a second iterative PKS, NcsB [28], and this PKS has already been shown to heterologously produce the precursor naphthoic acid of NCS [51]. Together, the family represents a separate class of iterative type I PKS that provides an alternative biosynthetic approach to monocyclic or bicyclic aromatic polyketides (Fig. 5B).

PATHWAYS OF C-1027 BIOSYNTHESIS

The majority of the biosynthetic investigations have focused on elucidating the initial steps of C-1027 biogenesis. An attractive feature of this enediyne is the robust genetic system that has been established for the producing organism *Streptomyces globisporus* [25,44]. Of the 56 open reading frames contained within the boundaries of the cluster, 30 have been successfully inactivated confirming their involvement in C-1027 production [Ben Shen, unpublished data]. While the majority solely abolished C-1027 production, several mutants accumulated new intermediates, and, in total, the gene inactivations have provided the preliminary rationale for *in vitro* examination of specific biosynthetic enzymes.

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Sequence analysis of a free-standing adenylation enzyme, SgcC1, suggests the β-amino acid moiety originates from L-α-tyrosine, which, through 5 steps, is converted to the final product (Fig. 6A). While the biosynthesis does indeed begin with L-α-tyrosine, the initial step is not catalyzed by SgcC1 but has been shown to be the conversion of L-α-tyrosine to (S)-β-tyrosine by an unprecedented aminomutase, SgcC4 [52,53]. It is the SgcC4 product that is the preferred substrate of SgcC1 that only loads β-tyrosine and other β-analogs, as opposed to any α-amino acid, to a free-standing peptidyl carrier protein (PCP), SgcC2 [54]. After covalent tethering to the PCP, a flavin-dependent halogenase SgcC3 incorporates chlorine followed by hydroxylation catalyzed by SgcC. While inactivation of *sgcC3* [54] and *sgcC* [25] resulted in the isolation of the deschloro- and deshydroxy-analogs of C-1027, suggesting these two steps may occur after incorporation to the enediyne core (i.e., the final steps in C-1027 maturation), we now have *in vitro* data supporting halogenation and hydroxylation occurring on the SgcC2 PCP-tethered β-tyrosine substrate. Finally, a type II condensation enzyme, SgcC5, incorporates the fully modified β-tyrosyl moiety into the enediyne core, although the precise timing of the coupling step relative to the final assembly of other moieties remains to be resolved.

The initial step in the biosynthesis of the deoxy aminosugar of C-1027 has been confirmed and the recombinant enzyme, SgcA1, characterized (Fig. 6B) [55]. SgcA1, similar to the large family of NDP-sugar synthases, utilizes dTTP and α-D-glucose to form dTDP-glucose. Inspired by the biochemical data, a significant yield improvement in C-1027 isolation was accomplished by overexpressing *sgcA1* and it's flanking region in the producer *S. globisporus* [55]. Subsequent steps in the deoxy aminosugar biosynthesis involve 5 additional enzymes, a dTDP-glucose-4,6-dehydratase (SgcA), a dTDP-4-keto-6-deoxyglucose epimerase (SgcA2), a C-methyl transferase (SgcA3), an amino transferase (SgcA4), an N-methyl transferase, and a glycosyl transferase, catalyzing conversions common for deoxy aminosugar biosynthesis in secondary metabolism [56].

The remaining moiety of C-1027 is the benzoxazolinate, an uncommon structural feature in natural products. A subcluster within the C-1027 locus contains homologs to anthranilate synthase components I and II (SgcD and SgcD1), suggesting the pathway begins from chorismate *via* the conversion to anthranilate. Production and purification of recombinant SgcD did not result in anthranilate synthase activity, but instead SgcD converts chorismate to 2-amino-2-deoxyisochorismate [65], which is the first half-reaction of anthranilate synthase I (Fig. 6C) [57]. Subsequently, an iron-sulfur, flavin-dependent dehy-drogenase, SgcG, has been shown to convert the SgcD product into 3-O-enolpyruvalan-thranilate. Final reactions include amide bond formation (SgcD5), hydroxylation (SgcD3), O-methylation (SgcD4), and CoA-activation (SgcH) and covalent attachment to the enediyne core (SgcD6), although the final steps await *in vitro* characterization.

Although differences will undoubtedly exist in the maturation process for assembly of other enediynes, the general biosynthetic strategy of C-1027 is likely shared among the family. Findings from studying the C-1027 biosynthetic machinery as a model system, therefore, should greatly facilitate the characterization of other enediyne biosynthetic pathways.

NEW PATHWAYS AND UNUSUAL CHEMISTRY

As expected from the exotic structures, many of the enzymatic steps in C-1027 and enediyne biosynthesis represent new types of chemical conversions or contain unexpected transformations and pathways. Firstly, PKSE represents a new architecture for a bacterial PKS family, and contains ACP and PPTase domains that do not have homology to proteins with the respective functions. The ability to heterologously express *sgcE* in *E. coli* in an active form has now set the stage to identify the first intermediate produced by PKSE and

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explore the activity and mechanism of iterative type I PKS, both of which are currently in progress. Furthermore, the results also laid the groundwork to explore the mechanism of triple bond formation, of which little is currently known [58].

Secondly, during the first step of the biosynthesis of the modified-β-amino acid, an aminomutase SgcC4 catalyzes amine migration. Unlike other aminomutases described to date, SgcC4 does not employ any of the following cofactors: cobalamin (or AdoMet) and an iron-sulfur cluster, pyridoxamine phosphate, or ATP [53]. Instead, SgcC4 is post-translationally modified to contain a 4-methylidenei-midazole-5-one (MIO), a cofactor that is necessary for phenylalanine or histidine ammonia lyase activity. However, SgcC4 extends ammonia lyase activity by catalyzing Michael addition of the released ammonia to the bound p-hydroxycinnamic acid intermediate to form (S)-β-tyrosine, representing a novel class of aminomutases [59].

Processing of (S)-β-tyrosine is carried out by a free-standing adenylation domain, SgcC1. While SgcC1 was predicted to activate L-α-tyrosine from sequence comparisons, activity tests with α-amino acids were to no avail [54]. After discovering the novel aminomutase, it became evident that SgcC1 activity was specific for S-β-tyrosine as we have now demonstrated [54]. Interestingly, while absolutely no α-amino acids are turned over by SgcC1, several β-tyrosine analogs are recognized and processed setting the stage to engineer new C-1027 analogs using a chemoenzymatic approach [66].

Finally, the discovery of SgcD activity represents the challenges that remain in analyzing sequence data for the purpose of pathway predictions. SgcD has lost the 2-amino-2-deoxyisochorismate lyase activity inherent in anthranilate synthase, a prediction not readily observed from sequence comparisons [65]. At least 4 different families of enzymes with sequence homology to anthranilate synthase are now known, suggesting the anthranilate synthase template is readily amenable to mutations that evolve new functions [60,61]. Furthermore, the tandem conversion by SgcD and SgcG represents yet another biosynthetic pathway that originates from chorismate, of which a minimum of 6 is currently known [62].

During the cloning and sequencing of the maduropeptin gene cluster, a new type of enediyne binding protein was identified [63]. The yellow pigment associated with expression of SgcE and NcsE was identified as a linear polyene; when *sgcE* or *ncsE* was coexpressed with the gene encoding a putative type II thioesterase found within all enediyne gene clusters, 1,3,5,7,9,11,13-pentadecaheptaene was isolated and spectroscopically characterized [64]. The X-ray crystal structure of the aminomutase SgcC4 was recently reported [68]. While SgcC1 adenylates several β-tyrosine analogs, kinetic analysis revealed SgcC1 is most efficient with (S)-β-tyrosine, suggesting (S)-β-tyrosine is the *in vivo* substrate [66]. The halogenase SgcC3 has been characterized as an FAD-dependent halogenase that regiospecifically chlorinates (S)-β-tyrosyl-*S*-SgcC2 [69]. Genome sequencing of the marine actinomycete *Salinispora tropica* revealed two independent enediyne PKS homologs suggesting this organism produces enediyne natural products [70]. Genetic engineering with the C-1027 producer has led to the biosynthesis of new enediyne analogs that have an altered ability to produce double-strand breaks and interstrand cross-links in cellular DNA and an altered cellular response to damage [71].

PROSPECTS FOR CLINICAL APPLICATIONS

Despite their remarkable potency as a family, only two second generation of enediynes have seen use in clinical settings: a polymer derivative of NCS (SMANCS®) marketed in Japan and an antibody conjugate of calicheamicin (Mylotarg®) used in the United States. So how can the information gathered from the biosynthetic studies presented here be applied to discovering enediynes with a better therapeutic index?

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First and foremost, new enediynes are continually being discovered (Table 1). We have used a comparative genomics approach based on the knowledge of a few known enediyne gene clusters to discover new potential enediynes [29]. Furthermore, even without using a genomics-guided approach, standard screens for bioactive metabolites have yielded shishijimicins A-C [16], uncialamycin [17], sporolides A and B [11], and cyanosporasides A and B [12]. Interestingly, these new enediynes were isolated from marine organisms, a diverse population that has generated substantial interest due to its rich, untapped source of natural products. Secondly, the producers for C-1027 [25] and NCS [26] are readily manipulated using genetic engineering, which has allowed the isolation of new enediyne analogues with altered reactivity that have improved bioactivity traits [67]. Finally, the biosynthetic studies discussed here have paved the way to rationally engineer new enediynes that are of great challenge to prepare by total synthesis. Our biosynthetic studies have also revealed that heterologous hosts such as *E. coli* are suitable for enediyne engineering since the prerequisite building blocks are abundant and because of the self-activating nature of the enediyne PKS, PKSE.

In total, the foundation for the discovery of the next generation of enediynes is now in place. Using a combination of synthetic and biosynthetic studies, large libraries of enediynes can be prepared using combinatorial biosynthesis and chemoenzymatic approaches. It is anticipated a fraction of these compounds will have the appropriate compromise of potency and cytotoxicity to be utilized as anticancer agents.

Acknowledgments

We acknowledge and are grateful for the work of Dr. Pieter C. Dorrestein and Prof. Niel L. Kelleher, University of Illinois at Urbana-Champaign, who performed FT-MS experiments with recombinant SgcE, NcsE, and SgcC2. We also acknowledge other contributors from the Shen Lab for the unpublished data, including Wen Liu, Jian Zhang, Shuangjun Lin, Wenli Li, and Jianhua Ju, other members of the Shen Lab whose contribution were cited in the references, and Prof. Y. Li, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijin, China for the wild-type C-1027 producing strain of *Streptomyces globisporus*. Enediyne biosynthesis described from the Shen Lab was supported in part by National Institute of Health grant CA78747. S.V.L is a recipient of an NIH postdoctoral fellowship CA1059845, and B.S is the recipient of an NIH Independent Scientist Award AI51689.

ABBREVIATIONS

ACP	Acyl carrier protein
AT	Acyltransferase
DH	Dehydratase
KR	Ketoreductase
KS	Ketosynthase
NCS	Neocarzinostatin
PCP	Peptidyl carrier protein
PKS	Polyketide synthase
Ppant	Phosphopantetheine
PPTase	Phosphopantetheinyl transferase
PUFA	Polyunsaturated fatty acid

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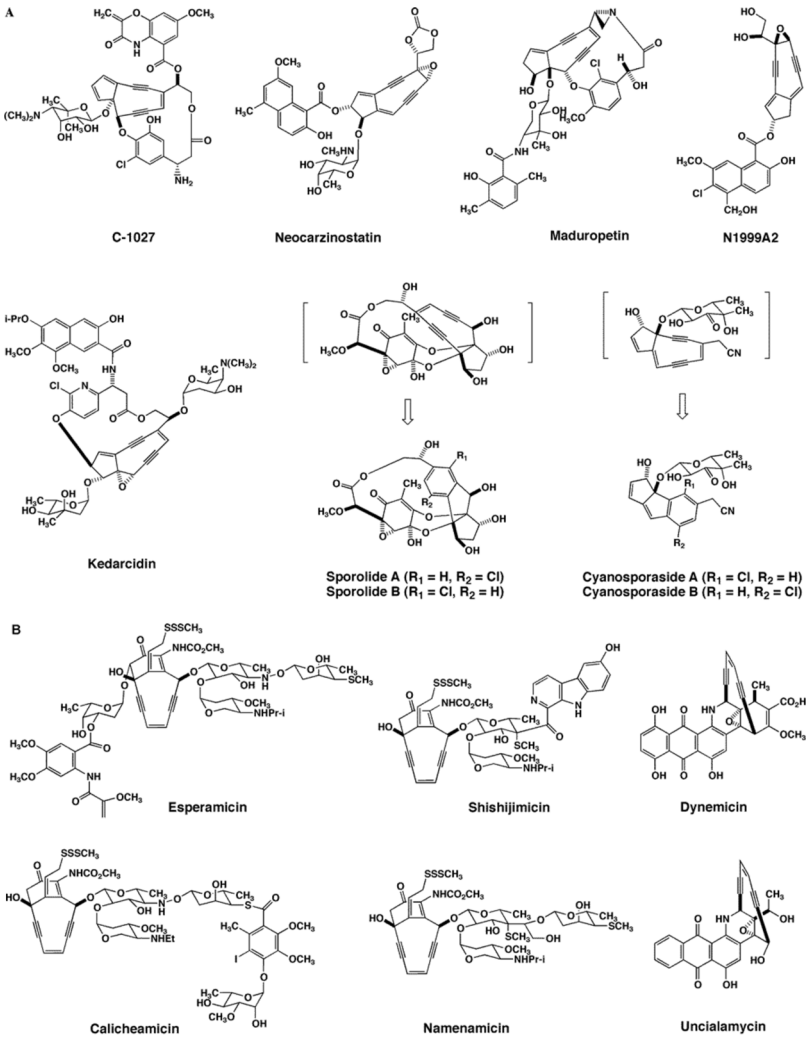

Fig. 1.

(**A**) Nine-membered enediynes and (**B**) ten-membered enediynes that have been structurally elucidated. The enediyne structures shown in brackets are hypothetical.

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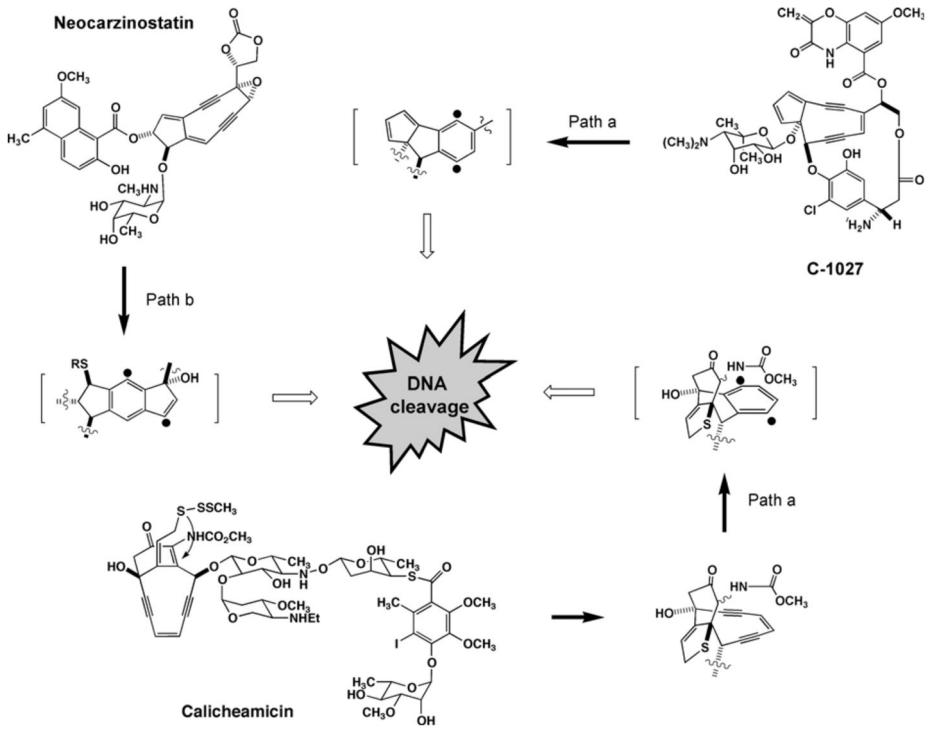

Fig. 2.

Enediyne activation *via* Bergman (path **a**) or Myers-Saito (path **b**) cyclization to yield a benzenoid diradical that causes the eventual DNA cleavage.

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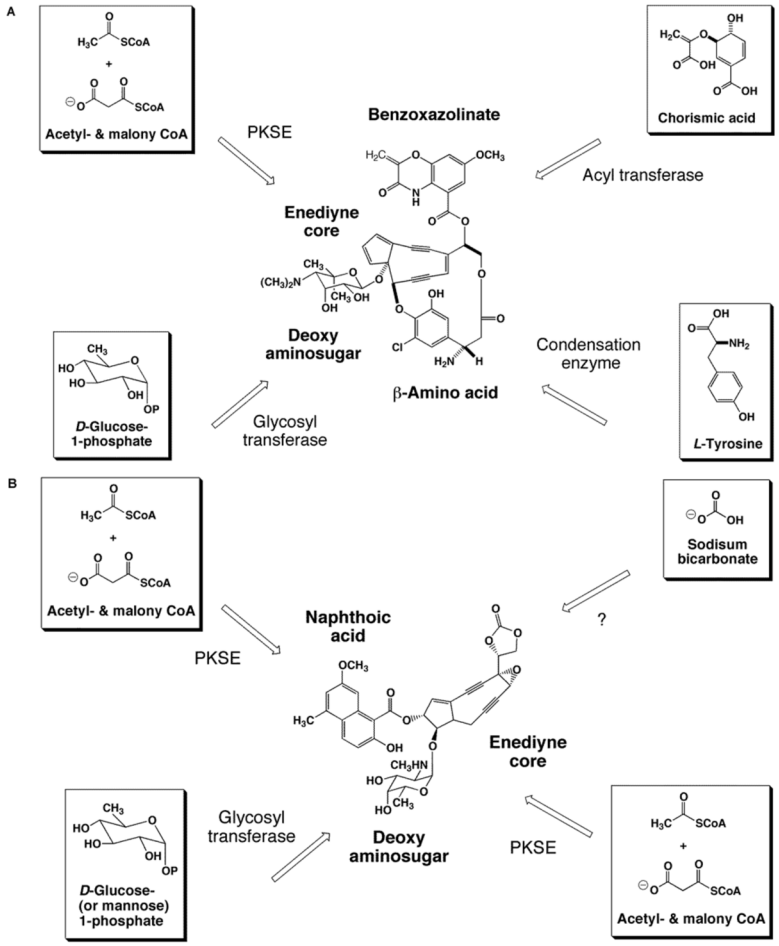

Fig. 3.

A model of convergent biosynthesis for the enediynes as exemplified by (**A**) C-1027 and (**B**) neocarzinostatin.

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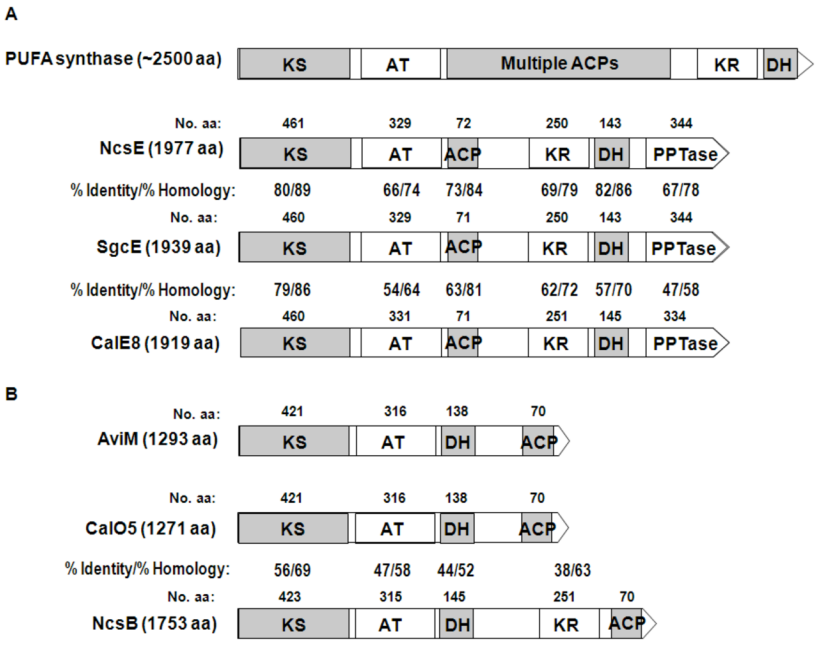

Fig. 4.

Architecture and domain organization of ierative type I PKSs found in enediyne gene clusters: (**A**) the enediyne PKSE and its relationship with PUFA synthase and (**B**) PKS for the aromatic polyketide moieties of enediynes and its comparison with orsellinic acid synthase AviM. ACP, acyl carrier protein, AT, acyl transferase; DH, dehydratase; KR, ketoreductase; KS, ketosynthase; PPTase, phosphopantetheinyl transferase.

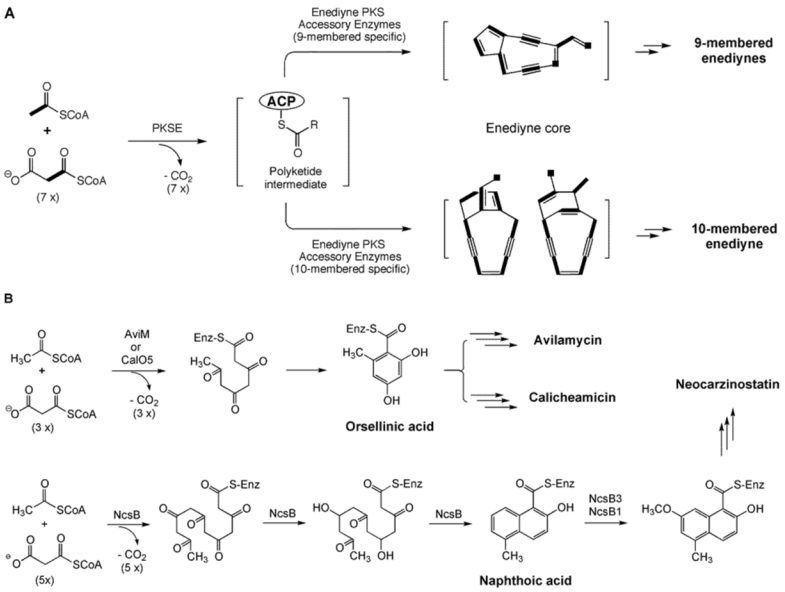

Fig. 5.

(**A**) Proposed pathway for biosynthesis of a polyunsaturated intermediate (structure unknown) from acyl CoA by PKSE and the subsequent transformation by enediyne PKS associated enzymes into putative nine- or ten-membered enediyne cores that are finally tailored to individual enediyne natural product. Atoms that were incorporated intact from acyl CoA precursors to the enediyne cores are shown in bold. (**B**) Proposed pathway for the biosynthesis of aromatic polyketides by iterative type I PKS as exemplified by AviM or CalO5 for orsellinic acid and by NcsB for a naphthoic acid.

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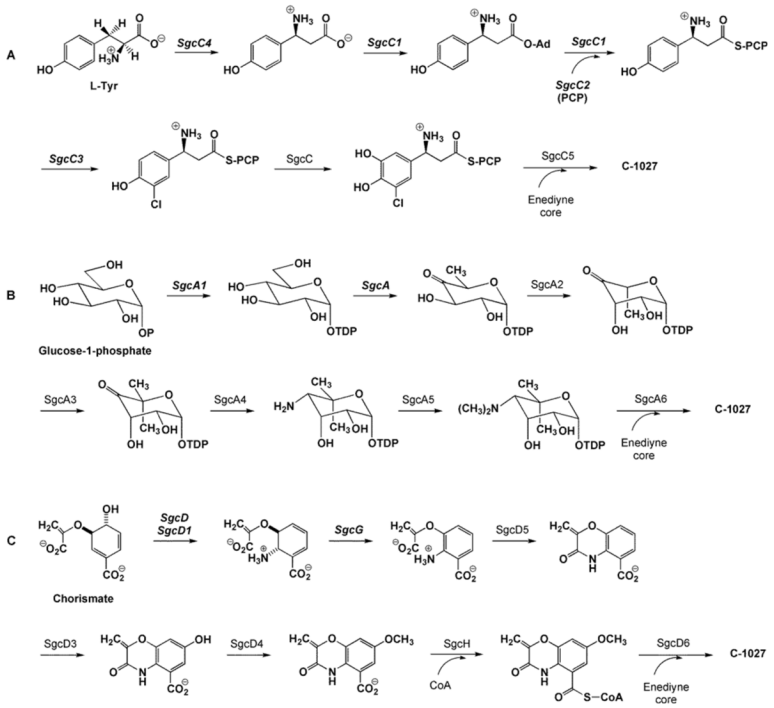

Fig. 6.

Proposed biosynthetic pathways of the peripheral moieties of C-1027: (**A**) β-amino acid, (**B**) deoxy aminosugar, and (**C**) benzoxazolinate. Recombinant enzymes that have been characterized experimentally *in vitro* are shown in bold-italics.

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Table 1

Enediyne Natural Products

Name	Producer	Year[a]	Ref.
Nine-Membered Category			
Auromomycin	*Streptomyces macromomyceticus*	1968	[3]
Largomycin	*Streptomyces pluricolorescens*	1970	[4]
Actinoxanthin	*Actinomyces globisporus*	1976	[5]
Sporamycin	*Streptosporangium pseudovulgare*	1978	[6]
Neocarzinostatin	*Streptomyces carzinostaticus*	1985	[1]
C-1027	*Streptomyces globisporus*	1991	[7]
Maduropeptin	*Actinomadura madurea*	1994	[8]
Kedarcidin	*Actinomycete L585-6*	1997	[9]
N1999A2	*Streptomyces sp. AJ9493*	1998	[10]
Sporolides A and B[b]	*Salinispora tropica*	2005	[11]
Cyanosporasides A and B[b]	*Salinispora pacifica*	2006	[12]
Ten-Membered Category			
Esperamicin	*Actinomadura verrucosospora*	1985	[13]
Calicheamicin	*Micromonospora echinospora ssp. calichensis*	1987	[2]
Dynemycin	*Micromonospora chersina*	1990	[14]
Namenamicin	*Polysyncraton lithostrotum*	1996	[15]
Shishijimicin	*Didemnum proliferum*	2003	[16]
Uncialamycin	Unknown[c]	2005	[17]

[a] Year in which the endiyne metabolites were reported or structure determined.

[b] Tricyclic core was proposed to be derived from an enediyne precursor based on chlorine substitution pattern after cycloaromatization.

[c] Similar to *Streptomyces cyanogenus* from 16S RNA sequencing.