DOI: 10.1002/cbic.200800546

The Structural Diversity of Acidic Lipopeptide Antibiotics

Matthias Strieker and Mohamed A. Marahiel*[a]

Acidic lipopeptide antibiotics are a new class of potent antibiotics, which includes daptomycin, A54145, calcium-dependent antibiotics (CDAs), friulimicins/amphomycins, laspartomycin/glycinocins and others. The importance of this novel class is exemplified by the success story of the clinically approved daptomycin, which is used for the treatment of skin infections and bacteremia caused by multidrug-resistant bacteria, including methicillin-resistant Staphylococcus aureus *and vancomycin-resistant* enterococci*. The potency of acidic lipopeptides is inherent in their chemical structure. The nonribosomally synthesized peptide cores consist of eleven to 13 amino acids, which are rigidified by the formation of a ten-membered ring. An N-terminal fatty acid, which facilitates insertion into the lipid bilayer of bacterial membranes, completes the structure. All these antibiotics contain multiple nonproteinogenic amino acids as well as different lipid tails; this yields remarkable structural diversity. This review summarizes the observed structural variety through a detailed description of the composition of the acidic lipopeptides. Furthermore, engineering approaches towards novel lipopeptides are presented. Recent discoveries in the field of tailoring enzymes, which enable structural plurality mainly by amino and fatty acid precursor biosynthesis, are highlighted.*

Introduction

There has been a steady rise in the prevalence of multidrug-resistant, especially Gram-positive, and other pathogens. Furthermore, concerns were expressed about the clinical effectiveness of glycopeptides in treating infections due to *Staphylococcus aureus*.[1] Thus, there is an urgent need for new antibiotics with a novel mode of action. Since the 1960s, new antibiotics have largely been developed by making minor modifications to existing drug classes, in particular, penicillins, cephalosporins, macrolides and quinolones, so that activity against bacteria that have become resistant to the previous generation of drugs is recovered. In fact, daptomycin (Cubicin, Cubist Pharmaceuticals) is the first nontopically used natural antibiotic in a new structural class, namely the acidic lipopeptides, that has entered clinical use since then.[2]

Daptomycin was discovered in the early 1980s.[3] The United States FDA approved daptomycin for the treatment of serious skin and soft tissue infections in 2003 and for methicillin-resistant *S. aureus* (MRSA) infections of the bloodstream (bacteremia) in 2006.[4] The A21978C complex to which daptomycin belongs is produced through the action of nonribosomal peptide synthetases (NRPSs)[5–8] in *Streptomyces roseosporus*.[3] Daptomycin is a macrocyclic compound comprising 13 amino acids, including three with D-stereochemistry and a linear *n*-decanoyl fatty acid tail. The macrocycle contains ten residues, and the terminal kynurenine (Kyn13) is connected to the hydroxyl group of Thr4 by an ester bond to form a macrolactone.

In addition to daptomycin, the best known representative of the acidic lipopeptide antibiotics, there are a number of other ten-membered cyclic lipopeptides. These natural products are also secondary metabolites produced by soil bacteria and include the depsipeptides, the calcium-dependent antibiotics (CDAs) produced by the model streptomycete *Streptomyces coelicolor* A(3)2[9] and A54145 produced by *Streptomyces fradiae*[10] (Table 1).[11] Other cyclic lipopeptides antibiotics produced by actinomycetes include friulimicin[12] produced by *Actinoplanes friuliensis*[13] and the amphomycins,[14] laspartomycins[15] and glycinocins.[16] Within these lipopeptides, the positioning of D-amino acids or achiral amino acids is conserved, as is the Asp-X-Asp-Gly motif in the macrocycle. Some of the amino acids are heavily modified during biosynthesis. Tailoring enzymes,[23,24] which include oxygenases, oxidases, methyltransferases and others are responsible for these modifications, and thus, provide a large number of nonproteinogenic amino

Table 1. Important producer strains of acidic lipopeptide antibiotics[a,b]

Compound	Producer
A-1437	*Actinoplanes* sp. (= *Actinoplanes friuliensis* DSM 7358)[17]
A21978C/ daptomycin	*S. roseosporus* NRRL11379[3]
A54145	*S. fradiae* NRRL18158[10]
	S. refuineus spp. *thermotolerans*[18]
amphomycin	*S. canus* ATCC 12237[14]
aspartocin	*S. griseus* var. *spiralis* ATCC13733[19]
	S. violaceus var. *aspartocinius* ATCC 13734[19]
CDA	*S. coelicolor* A3(2)[9]
	S. violaceoruber Kutner 673[9]
	S. lividans[20]
friulimicin	*A. friuliensis* DSM 7358[12]
glycinocin	*Actinomycete* AW 998[16]
laspartomycin	*S. viridochromogens* ATCC 29814[15]
parvuline	*S. parvulus* var. *parvuli* NRRL 5740[21]
tsushimycin	*S. griseoflavus* ATCC 21139[22]

[a] Please note this table does not list all acidic lipopeptide producers, only those that are related to this review. [b] Table drawn on the basis of Baltz et al.[11]

[a] *M. Strieker, Prof. Dr. M. A. Marahiel*
Chemistry and Biochemistry Department, Philipps-University Marburg
Hans-Meerwein-Strasse, 35032 Marburg (Germany)
Fax: (+49) 6421-282-2191
E-mail: marahiel@staff.uni-marburg.de

acids and a fascinating structural diversity. The inherent pool of unusual amino and fatty acid building blocks of the acidic lipopeptide antibiotics can be used for the manipulation of their biosynthesis. By employing biocombinatorial or other engineering approaches, the generation of structurally distinct or hybrid compounds is possible. This is an important aspect, because resistance to daptomycin in S. *aureus* and *Enterococcus* spp. during prolonged treatment has already been observed.[25]

In this article we present an overview of the acidic lipopeptide structures, with focus on the common and unusual building blocks of each molecule. Approaches to enhance the structural plurality by genetic engineering, chemoenzymatic and mutasynthesis methods are traced, and in the last part of this review, recent advances in the field of the precursor and building block biosynthesis are discussed in detail.

Structures of Acidic Lipopeptide Antibiotics

In addition to their cyclic nature, a key structural feature of lipopeptide antibiotics is the long-chain fatty acid, which is invariably attached to the macrocyclic peptide core. All members of this class of antibiotics are produced by NRPSs with variation of the fatty acid tail. Straight- and branched-chain fatty acids that significantly differ in the degree of saturation and oxidation state are frequently found and contribute to the high structural diversity of this class of compounds (Figure 1). In particular, the lipid portion impacts the biological properties of these molecules; antimicrobial behavior and toxicity are dramatically affected by the nature of the incorporated fatty acid group.[27] The length of the fatty acid chain is variable and ranges from 6–16 carbons. CDA has the shortest fatty acid, an epoxidized hexanoic acid, which in contrast to other lipopeptides with their complex fatty acid mixtures, is invariant. The longest fatty acid tail, containing 16 carbon atoms, is found in glycinocin B (for a more comprehensive review on acidic lipopeptide structures, refer to ref. [11]). An overview of the acidic lipopeptides can be found in Figure 1, where all the variants are listed.

Cyclic lipodepsipeptides

A21978C/daptomycin

The peptide core of the A21978C complex (Figure 1, **2**–**4**), produced by *Streptomyces roseosporus*, consists of 13 amino acids. The characteristic ten-membered ring is built by macrolactonization between Thr4 and the C-terminal Kyn13.[3] Other nonproteinogenic and D-amino acids are present in A21978C, namely D-Asn2,[28] ornithine (Orn6), D-Ser11 and 3-methylglutamic acid (MeGlu12). The most famous representative of the A21978C complex, daptomycin (Figure 1, **1**), was originally found as a minor component,[3] but showed superior biological activity compared with other members of the A21978C family.[27] Thus, the fermentation process was optimized to yield high-quantity daptomycin production by feeding decanoic acid.[29] Although the complex was known since the early 1980s, the biosynthetic gene cluster was not described before 2005. It encodes for three NRPSs: DptA, DptBC and DptD. The catalytic domains are predicted to couple five, six and two amino acids, respectively, with three epimerization domains present, consistent with the three D-amino acids found in the antibiotic.[28]

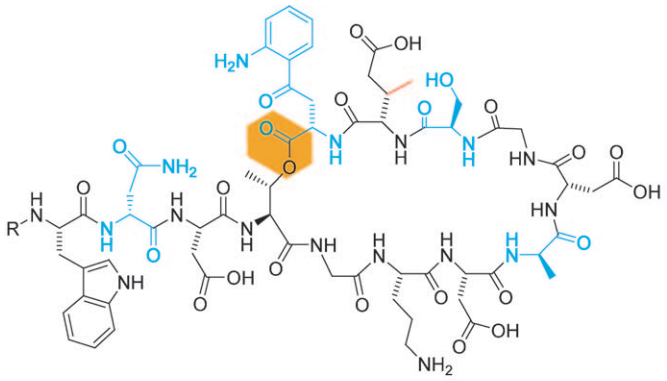
A54145

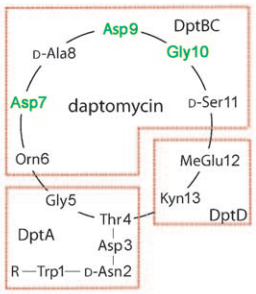
Streptomyces fradiae produces the lipopeptide A54145, a complex antibiotic mixture active against Gram-positive bacteria.[30] The identities of the acyl side chains were established as *iso*-decanoyl (Figure 1, **5**, **8**, **12**), *n*-decanoyl (Figure 1, **6** and **7**) and *anteiso*-decanoyl (Figure 1, **9**–**11**).[31] Seven of the 13 amino acids are nonproteinogenic, including three D-configured peptide building blocks. The six amino acids D-Glu2, 3-hydroxy-L-asparagine (hAsn3), sarcosine (Sar5), D-Lys8, 3-methoxyAsp (MeO-Asp9) and D-Asn11 are found in all A54145s, whereas MeGlu12 is only observed in **7**–**9** and **11**. The higher complexity of A54145 compared to that of A21978C is also due to the variation at position 13, where either Ile (**5**–**8**, **10** and **11**) or Val (**9** and **12**) is incorporated. The assumption that D-amino acids are present at positions two, seven and eleven was made after the biosynthetic gene cluster was characterized and three epimerization domains were found in the corresponding modules.[32] Four NRPSs are also encoded by the biosynthetic gene cluster—LptA, LptB, LptC and LptD—responsible for the incorporation of five, two, four and two amino acids, respectively, into the growing peptide chain.

Calcium-dependent antibiotics (CDAs)

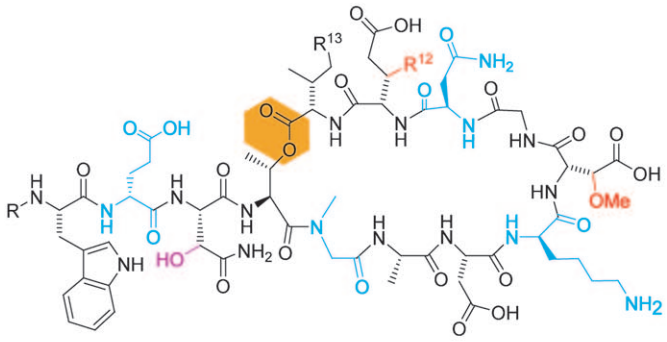
In 1983, Hopwood et al.[9] isolated a substance from *S. coelicolor* A3(2) fermentations that inhibited the growth of Gram-positive bacteria in the presence of Ca^{II} ions. Because of this activity, the substance was named CDA. Originally four (CDA1b, CDA2b, CDA3b and CDA4b) of the nine currently known CDAs were structurally characterized (Figure 1, **13**–**16**).[33] Macrolactonization of the CDAs occurs through the side chain of Thr2 and the C-terminal Trp11. The exocyclic tail only consists of one additional amino acid, and the fatty acid acyl

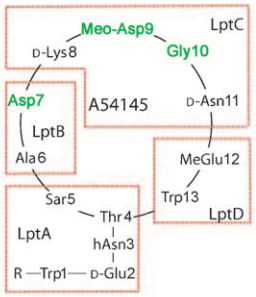
Figure 1. Comprehensive overview of the chemical structures of the acidic lipopeptide antibiotics. The cyclic depsipeptides A21978C/daptomycin, A54145, CDA and the cyclic peptides friulimicins, laspartomycins and glycinocins are shown. The macrolactonization site is shaded in orange, and the macrolactamization is shaded in rose. D- and unusual amino acids are highlighted in blue, and sites where tailoring enzymes act are in red. Next to the structure, the peptide cores are represented schematically. The position of the conserved Asp-X-Asp-Gly-motif is accentuated in green. The red dashed-line boxes resemble the NRPSs responsible for the incorporation of the framed amino acid(s) (if known). Next to the schemes, the observed variants of the lipopeptide antibiotics are listed. Abbreviations: Orn, ornithine; MeGlu, 3-methyl-glutamic acid; Kyn, kynurenine; hAsn, 3-hydroxyasparagine; Sar, Sarcosine; MeOAsp, 3-methoxyAsp; Hpg, 4-hydroxyphenylglycine; phAsn, 3-phosphohydroxyasparagine; ΔTrp, 2′,3′-dehydrotryptophan; Dab, diaminobutyric acid; Pip, pipecolinic acid; Dap, diaminopropionic acid. This figure is influenced by refs. [11] and [26].



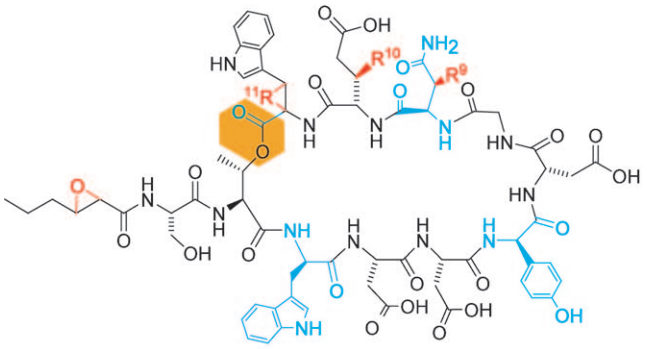


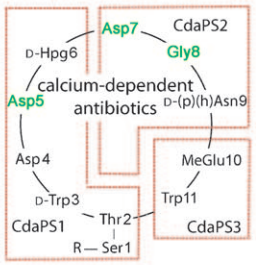
compound		R
daptomycin	**1**	*n*-decanoyl
A21978C$_1$	**2**	*anteiso*-undecanoyl
A21978C$_2$	**3**	*iso*-dodecanoyl
A21978C$_3$	**4**	*anteiso*-tridecanoyl



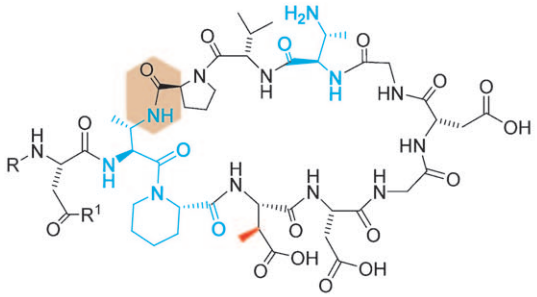


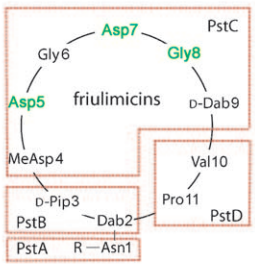
factor		R	R^{12}	R^{13}
A54145 A	**5**	*iso*-decanoyl	H	Me
A54145 A$_1$	**6**	*n*-decanoyl	H	Me
A54145 B	**7**	*n*-decanoyl	Me	Me
A54145 B$_1$	**8**	*iso*-decanoyl	Me	Me
A54145 C	**9**	*anteiso*-undecanoyl	Me	H
A54145 D	**10**	*anteiso*-undecanoyl	H	Me
A54145 E	**11**	*anteiso*-undecanoyl	Me	Me
A54145 F	**12**	*iso*-decanoyl	H	H



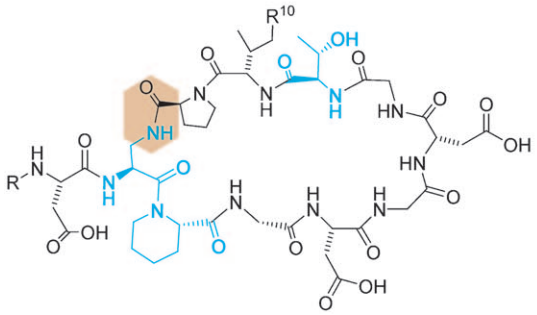


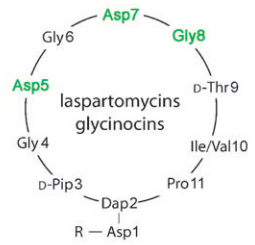
compound		R^9	R^{10}	R^{11}
CDA1b	**13**	OPO_3H_2	H	H, H
CDA2b	**14**	OPO_3H_2	Me	H, H
CDA3b	**15**	OH	H	H, H
CDA4b	**16**	OH	Me	H, H
CDA2a	**17**	OPO_3H_2	Me	π-bond
CDA3a	**18**	OH	H	π-bond
CDA4a	**19**	OH	Me	π-bond
CDA5a	**20**	H	H	π-bond
CDA6a	**21**	H	Me	π-bond





compound		R	R^1
A-1437 A/parvuline	**22**	Δ3-*iso*-tridecenoyl	OH
A-1437 B/tsushimycin	**23**	Δ3-*iso*-tetradecenoyl	OH
A-1437 E/amphomycin	**24**	Δ3-*anteiso*-tridecenoyl	OH
A-1437 G/aspartocin	**25**	Δ3-*anteiso*-pentadecenoyl	OH
friulimicin A	**26**	Δ3-*iso*-tridecenoyl	NH_2
friulimicin B	**27**	Δ3-*iso*-tetradecenoyl	NH_2
friulimicin C	**28**	Δ3-*anteiso*-tridecenoyl	NH_2
friulimicin D	**29**	Δ3-*anteiso*-pentadecenoyl	NH_2
amphomycin/ parvuline minor component	**30**	Δ3-*iso*-dodecenoyl	OH





compound		R	R^1
glycinocin A/laspartomycin C	**31**	Δ2-*iso*-pentadecenoyl	Me
glycinocin B	**32**	Δ2-*iso*-hexadecenoyl	Me
glycinocin C	**33**	Δ2-*iso*-tetradecenoyl	Me
glycinocin D	**34**	Δ2-*iso*-pentadecenoyl	H

chain is invariant and exclusively found to be 2,3-epoxy-hexanoyl. Nonproteinogenic amino acids can be found for the first four characterized CDAs at positions nine and ten. Asn9 is either hydroxylated (Figure 1, **15** and **16**), or in a subsequent reaction, phosphorylated (Figure 1, **13** and **14**) at the β position. The tenth residue, Glu10, is methylated in two cases (**14** and **16**). The C terminal Trp was found to be a third site of amino acid modification by Hojati et al.[34] and led to the discovery of three new CDA variations that each contain *Z*-2′-3′-dehydro-Trp (ΔTrp, Figure 1, **17**–**19**). In the same work, the organization of the biosynthetic gene cluster was determined. It revealed three NRPSs—CDA peptide synthetases 1–3—which are responsible for the incorporation of six, three and two amino acids, respectively. Recently, two further CDA derivatives have been isolated from genetically manipulated *S. coelicolor*, in which the *asnO* and *hasP* genes were knocked out.[35] AsnO[36] is an iron(II)/α-ketoglutarate (αKG) oxygenase, which hydroxylates L-Asn at the β position (also, see the section on tailoring enzymes), and *hasP* is a putative phosphotransferase. Accordingly, CDA variants produced by this strain lack the phosphohydroxy group at Asn9 (Figure 1, **20** and **21**). CDA is also produced in some closely related *Streptomyces* species (Table 1).[12,20]

Cyclic Lipopeptides

The following antibiotics also consist of a ten-membered ring, but are true peptides in that the macrocycles are formed by macrolactamization. This family of undecapeptide antibiotics can be further divided into two subgroups, the amphomycins/friulimicins and the laspartomycins/glycinocins.[11] All representatives of this subclass are very similar. They differ only at one position in the peptide core and in the branching of the fatty acids.

Amphomycins/friulimicins

Since the discovery of amphomycin,[14] similar or identical antibiotics have been reported under different names; this has caused significant confusion in this substance class. In 2000, Wink et al.[12] reported the structural elucidation of eight bioactive lipopeptides isolated from *Actinoplanes friuliensis*. That work and an extensive review by Baltz et al.[11] clarified the former uncertainties in the literature. These lipopeptides can be divided into the friulimicins and the A-1437 compounds. The peptide core of A-1437s (Figure 1, **22**–**25**) is rigidified through a linkage between diaminobutyrate (Dab2) and Pro11. An exocyclic Asp residue with different fatty acid substituents completes the structures. The study of Wink et al.[12] revealed that the different A-1437s are identical to previously described[11,14,19,21,22] lipopeptide antibiotics (see Figure 1 for details). The friulimicins A–D (Figure 1, **26**–**29**) only differ in the exocyclic amino acid, which is Asn1 instead of Asp1. Unusual amino acids, such as L-threo and D-erythreo-2,3-Dab, D-pipecolinic acid (D-Pip) as well as L-threo-3-methyl-Asp (MeAsp) are found at positions two, nine, three and four in the peptide core, respectively. Later studies revealed that the minor friulimicin components contain Asp4 instead of MeAsp4.[37] Initially, only a fragment of the friulimicins gene cluster was known,[37] but the entire gene cluster has been described recently.[13] It shows an unusual organization; the first peptide-synthetase-encoding gene, *pstA*, is divided from the next synthetase, *pstB*, by nine genes. The gene products PstC and PstD complete the assembly line. PstA, PstB , PstC and PstD have one, two, six and two adenylation (A) domains, respectively, responsible for the incorporation of the corresponding number of amino acids into the peptide core. The presence of two epimerization domains is consistent with the observed D-Dab9 and D-Pip3 residues.

Laspartomycins/glycinocins

Laspartomycin was originally isolated and characterized as a lipopeptide antibiotic related to amphomycin in 1968,[15] but its molecular weight and structure remained unknown. Novel purification methods led to the determination of the structure of the major component, laspartomycin C (Figure 1, **31**).[38] Compared with **22**–**30**, diaminopropionate (Dap_2) facilitates ten-membered ring formation rather than Dab_2. In addition, the fatty acid side chain is 2,3-unsaturated. Furthermore, the second D-amino acid is D-Thr9 instead of D-Dab9. Gly4 replaces the MeAsp4 residue, and Ile substitutes the Val residue at position ten. Two additional peptides were isolated during laspartomycin C purification, but these species were not structurally characterized.[38]

Glycinocin A, a compound of the glycinocins family (Figure 1, **31**–**34**) was shown to have an identical structure to that of laspartomycin C (**31**).[16] Glycinocin B (**32**) and C (**33**) have the same peptide core as that of **31** and differ only in the length of the fatty acid tail. Glycinocin D (**34**), on the other hand, has the same lipid part as does **31**, but instead of having Ile at position eleven, it has Val. To the best of our knowledge, no biosynthetic gene cluster for either laspartomycin or glycinocin production is known.

Approaches towards Novel Lipopeptides

Combinatorial biosynthesis

Daptomycin (**1**) is the only approved drug for clinical use within the acidic lipopeptide antibiotics, and Friulimicin B (**27**, MerLion Pharmaceuticals) is in phase I clinical trials. Due to their structural diversity resulting in various antimicrobial activities, the acidic lipopeptides have the potential for the manufacture of combinatorial antibiotics with altered activity.[39,40] The daptomycin producer *Streptomyces roseosporus* is amendable to molecular genetic manipulations, and the gene cluster has been cloned, sequenced[28] and expressed in *Streptomyces lividans*.[20] The potential of genetically manipulated *S. roseosporus* to produce hybrid lipopeptides has been extensively studied.[41–43] Miao et al.[41] applied four strategies in the genetic manipulation of *S. roseosporus* to biocombinatorially synthesize lipopeptides by multiple and individual NRPS module exchange, resulting in modifications within the lipid chain and

amino acids. In prior work, the manipulation of *Streptomyces roseosporus* by conjugal, *E. coli*-derived plasmid DNA was described.[44] This technique, which can be used to integrate plasmid DNA site-specifically[45] into the chromosomal DNA of *Streptomyces* species, was used for multimodule swapping and slightly modified for module exchange.

As stated above, the daptomycin gene cluster contains three NRPS-encoding genes (*dptA, dptBC* and *dptD*).[28] DptD is responsible for the incorporation of MeGlu12 and Kyn13 into the peptide core. The last NRPS subunits of the related lipopeptides A54145 and CDA (LptD and CDAPS3) also incorporate the penultimate amino acid, MeGlu, and the last amino acid, Ile/Val, for LptD as well as Trp in the case of CDAPS3. DptD modules 12 and 13 were exchanged with the corresponding *lptD* and *cdaPS3* modules for the biosynthesis of new hybrid lipopeptides.[42] In both cases, hybrid lipopeptides were generated, with Ile13 and Trp13, respectively, instead of Kyn13. LptD complementation yielded 25% of the original production and 50% of CDAPS3.

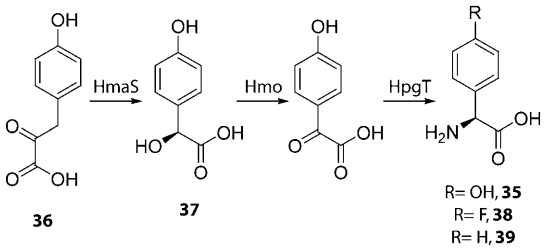
Module exchanges at positions D-Ala8 and D-Ser11 with different combinations of corresponding A54145 modules (D-Lys8 and D-Asn11) in the DptBC NRPS subunit and the combination with DptD substitutions produced additional novel derivatives of daptomycin.[43] To increase the possible outcome in terms of novel lipopeptides, the *dptD* substitutions were introduced with and without the *dptI* gene, which was shown to be essential for the Glu12 methylation[46] (also, see the section on tailoring enzymes). In total, 30 different hybrid pathways were generated. From the theoretically possible 90–120 novel hybrid molecules, 60 lipopeptides were detected by mass analysis. The maximum yield was 50% of the A21978C_1 control, and some of the hybrid NRPSs were found to produce truncated products or failed to produce lipopeptides entirely. Single amino acid changes at positions 12 and 13 showed less in vitro activity.

To determine the influence of interdomain linkers in terms of efficient hybrid lipopeptide production, module exchanges at nucleotide sequences encoding interpeptide linkers in *dptD* were carried out.[47] Amino acid substitutions, deletions or insertions in the intermodule linker had no negative effects on lipopeptide yields. Hybrid DptD subunits were generated by fusing the twelfth (MeGlu12) module to the last modules of *cdaPS3* (Trp) or *lptD* (Ile/Val). By inserting these module genes into the flexible linker between the peptidyl carrier protein (PCP) and condensation domain (C), recombinants were gained, which produced hybrid lipopeptides with Trp13 and Ile13/Val13 at levels comparable to those of A21978C. The substitution of the *dtpD* Kyn13 C-A didomain with the A54145 D-Asn11 C-A didomain, leaving the PCP-thioesterase (TE) linker of *dptD* intact, yielded recombinants, which produced compounds containing D-Asn13 in moderate yields.[47]

Mutasynthesis

Mutasynthesis usually refers to blocking a biosynthetic pathway by deleting a specific gene encoding an enzyme in the pathway. The resulting mutant, which is unable to produce downstream intermediates and products, is then fed an analogue of one of the intermediates, which can be incorporated into the pathway leading to the biosynthesis of a natural product analogue with modified functionality. Mutasynthesis has been successfully applied in the field of secondary metabolite biosynthesis to obtain natural products with different structures.[48,49] Two examples from the Micklefield group exemplify how this approach to structurally diverse natural products was applied on acidic lipopeptide, namely CDA, biosynthesis.

The nonproteinogenic 4-hydroxyphenylglycine (**35**, Hpg, Scheme 1) is incorporated into CDAs **13**–**21** (Figure 1). The Hpg synthesis requires HmaS, a 4-hydroxymandelate synthase,



Scheme 1. Hpg biosynthesis in *Streptomyces coelicolor*. In the mutasynthesis approach towards diverse CDA analogues, the hydroxymandelate synthase gene *hmaS* was knocked out.[34]

to convert 4-hydroxyphenylpyruvate (**36**) to 4-hydroxymandelate (**37**).[34] Two additional enzymes, the 4-hydroxymandelate oxidase (Hmo) and the 4-hydroxyphenyl-glycine transaminase (HpgT), complete Hpg biosynthesis (Scheme 1).

Hojati et al.[34] deleted the *hmaS* gene of the CDA biosynthetic gene cluster, generating a mutant unable to synthesize CDA. Feeding with the 4-fluoro (**38**) and 4-dehydroxy (**39**) analogues resulted in the biosynthesis of the corresponding analogues of CDA. Antibiotic activity was restored in these analogues but not quantified.

The second approach targeted fatty acid incorporation. A mutasynthesis strategy was developed that enabled the directed biosynthesis of CDAs with alternative fatty acid moieties.[50] This relies on the key Ser→Ala point mutation in the PCP of module 1 of the CdaPS1, which prevents the phosphopantetheinylation and subsequent accumulation of the *N*-epoxyhexanoyl-L-serinyl-S-PCP intermediate;[6,7] this allows for the incorporation of exogenously supplied, synthetic *N*-acyl-L-serinyl *N*-acetylcysteamine (*N*-acyl-SNAC) thioester analogues. The SNAC moiety mimics the PCP domain and is a valuable tool for the characterization of polyketide synthase and NRPS enzymes in vivo and in vitro.[26,51,52] The authors tested C5, C6, C7 and C10 analogues and gained pentanoyl and hexanoyl CDAs in detectable yields.[65]

Chemoenzymatic synthesis

Chemoenzymatic synthesis combines organic synthesis with natural product biosynthetic enzymes. This approach was successfully applied to the synthesis of analogues of daptomycin, CDA, A54145 and combinations thereof.[26,53–55]

For the chemoenzymatic synthesis of nonribosomal peptides, the last catalytic domain of the assembly line, a TE, is used. In many NRPSs, the TE is a peptide cyclase that catalyzes the release of the mature peptide chain from the assembly line by intramolecular cyclization. Often, the TE-encoding gene fragment can be cloned and expressed in *E. coli*. Subsequently, the 30 kDa recombinant TEs catalyze the macrocyclization of activated thioester substrates, mimicking the PCP-bound linear peptide. The peptides can be synthesized through solid-phase peptide synthesis (SPPS).[26]

Grünewald et al.[53] applied this method to the generation of CDA and daptomycin derivatives. The recombinant CDA cyclase efficiently catalyzed ring formation from linear peptidyl thioester substrates based on a sequence analogous to that of natural CDA. The cyclase catalyzes the formation of two regioisomeric macrolactones, which arise from the simultaneous nucleophilic attack of the two adjacent Thr2 and Ser1 residues. The substitution of either of these residues by alanine led to the selective formation of a decapeptide or undecapeptide lactone ring. Despite this relaxed regioselectivity, the CDA TE showed strong stereoselectivity; hence, only L-Ser or L-Thr was accepted for enzyme-mediated cyclization. Another finding was that the elongation of the fatty acyl group of the thioester substrate from acyl to hexanoyl yielded solely the decapeptide lactone with a significantly improved cyclization/hydrolysis ratio. In a later study, Grünewald et al.[54] used the CDA TE to generate daptomycin derivatives. Linear CDA undecapeptide thioesters with single exchanges at six daptomycin-specific residues were successfully cyclized. The simultaneous incorporation of all six of these residues into the peptide backbone and the elongation of the N terminus of CDA by two residues yielded a daptomycin derivative that lacked only the β-methyl group of MeGlu for synthetic reasons. Bioactivity studies of these chemoenzymatically generated lipopeptides revealed the important role of nonproteinogenic amino acids and Ca^{II}, as expected. Single substitution of the four acidic residues in the peptide backbone to Asn or Gln suggested that only Asp7 and Asp9 were essential for antimicrobial potency. These findings might explain the conserved Asp-X-Asp-Gly motif.

In a more recent study, Kopp et al.[55] used the TEs from A54145 and daptomycin NRPSs to catalyze the macrocyclization of linear daptomycin and A54145 peptides. Derivatives of these acidic lipopeptides were generated as well as hybrid molecules of both compounds. The bioactivity of these cyclic lipopeptides was also examined and revealed new insights into the structure-activity relationship of the acidic lipopeptide family. Thereby, the general importance of the two conserved Asp residues was confirmed. Compared with authentic daptomycin, the chemoenzymatic variant showed approximately sixfold reduced bioactivity due to the incorporation of Glu12 rather than MeGlu12, resembling the results from Grünewald et al.[54] and Nguyen et al.[43] Furthermore, the A54145 TE was able to catalyze both macrolactonization and macrolactamization. It was found that the exocyclic amino acids Trp1, D-Glu2 and hAsn3 were not important for antimicrobial behavior. Furthermore, different ring sizes were gained by shifting the Thr4 residue to positions three and five, in the linear substrate to gain nine- and eleven-membered rings, respectively. Thus, this method provides a rapid approach towards diverse lipopeptides.

Tailoring Enzymes

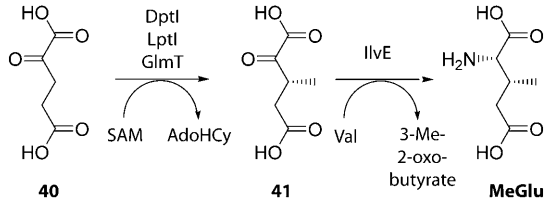
Tailoring enzymes are often found within NRPS gene clusters. These enzymes catalyze the formation of nonproteinogenic amino acids or enhance the structural diversity of secondary metabolites by post-assembly-line functionalization. These structural changes improve the biological activity of secondary metabolites and provide the producer with an evolutionary advantage by, for instance, making the antibiotic more stable to protease cleavage by the target strains. Tailoring events are ubiquitous in nonribosomal peptide synthesis and can take place on amino acids bound to the PCP domain of the assembly line in *cis* by the corresponding catalytic domains integrated into the NRPS or in *trans*.[23] The cyclization of cysteine to thiazoline during bacitracin biosynthesis[56] and halogenation of Thr during syringomycin E[57] biosynthesis are noteworthy examples of how tailoring affects activity and stability, respectively. In contrast, to vancomycin-type antibiotics, in which tailoring events mainly take place in a post-assembly-line fashion,[58] recent studies on the origin of nonproteinogenic amino acids in acidic lipopeptides suggest that pre-assembly-line modifications, that is, amino and fatty acid building block biosynthesis, are more common for this antibiotic class. Altogether, twelve nonproteinogenic amino acids are present in the acidic lipopeptides (Figure 1). Six of these (Hpg, Kyn, Dab, Dap, Pip and Orn) have been extensively discussed before[11] and will not be covered in this review. Instead, our focus will be on recent studies addressing methylation, hydroxylation and dehydrogenation events as well as on the epoxidation of the hexanoic acid tail of CDA.

Methylation

One common feature of daptomycin, A54145 and CDA is the presence of a β-methylated Glu residue at the same relative ring position. Whereas CDA and A54145 lipopeptides are produced as mixtures of compounds containing MeGlu or Glu,[30,32] the fermentation of *Streptomyces roseosporus* results exclusively in MeGlu-containing peptides.[46] As mentioned earlier, the MeGlu-containing acidic lipopeptides exhibit a higher bioactivity than do the Glu-containing analogues.[30,46,54,55,59]

Following the determination of the CDA biosynthetic gene cluster,[34] a gene, *sco3215*, was predicted to encode an *S*-adenosyl methionine (SAM)-dependent glutamate-3-methyl-transferase (GlmT). Genes homologous to *glmT* are found in the daptomycin (*dptI*)[46] and A54145 (*lptI*)[32] pathway. It was observed previously that the fermentation of deletion mutants of *S. coelicolor* (Δ*glmT*) and *S. roseosporus* (Δ*dptGHIJ*) led to the production of CDA and daptomycin analogues containing exclusively Glu instead of MeGlu.[46,59] The complementation of the Δ*dptGHIJ* mutant by *dptI* or *glmT* and the complementation of Δ*glmT* with synthetic MeGlu restored the biosynthesis of the MeGlu-containing compounds.[46,59] These results

showed that GlmT, DptI and LptI are methyltransferases involved in the biosynthesis of MeGlu residues in CDA, daptomycin and A54145. By the characterization of recombinant GlmT, DptI and LptI, Mahlert et al.[60] determined the substrate specificity of the methyltransferases. Combined with the in vivo results,[59] the complete mechanistic details of the biosynthesis of MeGlu and its incorporation into the acidic lipopeptides are now available (Scheme 2).



Scheme 2. Mechanism of MeGlu precursor synthesis. The SAM-dependent methyltransferases DptI, LptI and GlmT transfer a methyl group to αKG (**40**) leading to 3-methyl-2-oxoglutarate (**41**). Subsequent amino group transfer from valine to **41** catalyzed by branched chain aminotransferases yields the MeGlu.

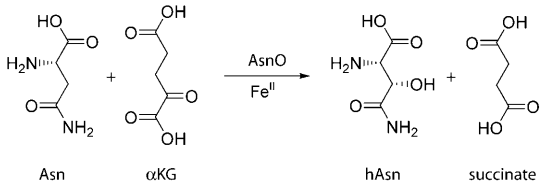
It was shown that GlmT, DptI and LptI are indeed all SAM-dependent methyltransferases.[60] But instead of catalyzing the methylation of Glu directly, all these enzymes act exclusively on αKG (**40**), leading to 3-methyl-2-oxoglutarate (**41**). Together with a branched-chain aminotransferase (IlvE) from the primary metabolism of *S. coelicolor*, the mechanism was established. The coupling of the methylation and transamination reaction led to the synthesis of MeGlu starting from αKG. The subsequent activation and incorporation of MeGlu by the adenylation domain of the tenth module of the CDA and the twelfth module of the daptomycin/A54145 NRPS will lead to the corresponding acidic lipopeptide antibiotics. For compounds **5**, **6**, **10**, **12**, **13**, **15**, **18** and **20**, Glu instead of MeGlu is found, and this is most likely due to the relaxed substrate specificity of the corresponding adenylation domain. However, this hypothesis has not yet been proven.

The methylation of an Asp residue observed at position four of the amphomycins/friulimicins **22**–**30** was not fully examined biochemically, but there is genetic evidence that this methyl group results from the mutase-catalyzed rearrangement of Glu into MeAsp.[37] This notion was validated by in the vitro activity of the gene products predicted to show the described mutase activity in the friulimicin pathway, GlmA and GlmB, which were expressed heterologously in *S. lividans*. The disruption of these genes in *A. friuliensis* caused a 60% reduction in the yield of MeAsp-A54145. Whether this is due to a leaky recombinant phenotype or to production by an alternative pathway is unclear.[37]

Hydroxylation

Hydroxylated asparagines are found at position nine in the peptide core of some CDAs (Figure 1, **15**, **16**, **18** and **19** and subsequently phosphorylated **13**, **14** and **17**) and at position three of A54145 (Figure 1, **5**–**12**). Additionally, a methoxylated Asp is found at position nine in A54145. For the latter modification, a mechanism was proposed by a sequence analysis of the genes from the A54145 biosynthesis cluster.[32] The gene *lptK* was annotated as an *O*-methyltransferase because it had high sequence homology to known enzymes, and *lptJ* was annotated as a regulatory protein with sequence homology to the putative regulatory enzyme SyrP, which is a gene product of the syringomycin biosynthetic gene cluster in *Pseudomonas syringae*.[61] The SyrP sequence was recently re-examined and showed high homology to iron(II)/αKG-dependent hydroxylases. The biochemical characterization of SyrP revealed hydroxylation activity on Asp bound to the PCP of the eighth module of the syringomycin NRPS.[62] Therefore, a possible MeOAsp precursor pathway is that LptJ hydroxylates Asp at the β position, while tethered to the PCP, followed by *O*-methylation through LptK catalysis.

The hydroxylation of the asparagine residue in the CDAs is carried out by the enzyme AsnO,[36] an iron(II)/αKG-dependent oxygenase. These enzymes couple the oxidative decarboxylation of αKG to succinate with the generation of a reactive iron(IV)-oxo species, which can abstract a hydrogen from an aliphatic carbon to yield hydroxyl and substrate radicals. Subsequent radical recombination gives the hydroxylated product.[63] The *asnO* gene was amplified from chromosomal *S. coelicolor* DNA and heterologously expressed in *E. coli*. The recombinant enzyme was extensively characterized biochemically and shown to specifically act on free L-asparagine (Scheme 3), which was subsequently activated, epimerized and



Scheme 3. Mechanism of hAsn formation during CDA and A54145 biosynthesis. AsnO hydroxylates Asn, coupled with the oxidative decarboxylation of αKG to succinate.

incorporated into the CDA peptide chain. Δ*asnO S. coelicolor*[35] was found to produce CDAs **20** and **21** with Asn instead of hAsn. This is most likely due to a relaxed substrate specificity of the adenylation domain of the ninth module of the CDA peptide synthetase. Compounds **20** and **21** were isolated in poor yield which suggests that hAsn is the preferred substrate of the adenylation domain.

It is postulated that the observed phosphorylation of the hAsn residue in the CDAs **12**, **14** and **17** is carried out by the gene product of *hasP*. The *hasP* gene product exhibits moderate sequence similarity with the spectinomycin phosphotransferase (SpcN),[64] which is involved in the phosphorylation of an aminoglycoside antibiotic; hasP is, therefore, likely to encode the required 3-hydroxyasparagine phosphotransferase.

The X-ray structure elucidation of AsnO with a bound hydroxylated asparagine (PDB ID: 2OG7) facilitated the determination of the stereochemistry of hAsn, which was found to be *trans* configured.[36] The AsnO structure in complex with the products of the reaction, namely succinate and hAsn, allowed for the identification of the product-binding residues, which should be identical to the substrate-binding residues. The Asp side chain of the AsnO residue 241 was found to bind the carbamide function of the Asn substrate. By changing this residue to Asn, the AsnO variant D241N was able to hydroxylate stereospecifically Asp to yield L-*trans*-hydroxyaspartate (hAsp).[65] The described work shows how modifying enzymes can be manipulated to yield more structural diversity in terms of precursor synthesis.

For A54145, the gene *lptL* within the biosynthetic gene cluster[32] of *S. fradiae* shares high sequence homology with AsnO, and therefore, the gene product should also catalyze hAsn precursor formation in the same manner as does AsnO. Thus, *lptL* was cloned and expressed in *E. coli*, and the recombinant enzyme was able to stereospecifically hydroxylate L-asparagine with αKG as the co-substrate (M. Strieker, unpublished results).

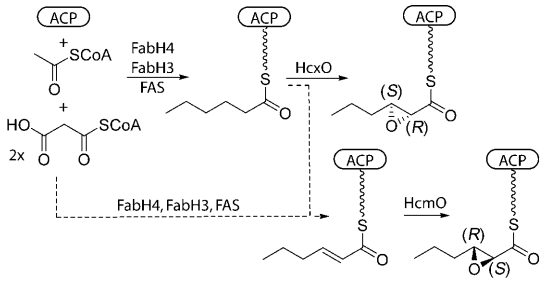
Dehydrogenation

The a-series of the CDAs (**17**–**21**) exhibit a C-terminal *Z*-2′S-3′S-dehydrotryptophan (ΔTrp) residue.[34] Little is known about the nature of the enzymes involved in the dehydrogenation of amino acid residues during NRPS biosynthesis. Nevertheless, related enzymes activities have been characterized, namely the tryptophan side chain oxidase from *Pseudomonas*[66] and the L-tryptophan-2′-3′-oxidase from *Chromobacterium violaceum*.[67] No gene was identified within or outside the CDA biosynthetic cluster whose product shares sequence homology to any of these enzymes. Amir-Heidari et al.[68] indirectly supported the stereochemical course of the Trp dehydrogenation. By feeding a Trp auxotroph *S. coelicolor* strain with synthetic C3′-deuterated tryptophans, it was possible to follow the fate of the C3′ deuterium labels during CDA biosynthesis using mass spectroscopy. The possibility of hydroxylation followed by dehydration was ruled out because no hydroxylated Trp-containing intermediates were observed. It is suggested that the dehydrogenation of Trp during CDA biosynthesis takes place directly, as in the case of L-tryptophan-2′-3′-oxidase from *Chromobacterium violaceum*.[67] This oxidase was shown to catalyze the dehydrogenation of *N*-Boc-L-Trp to the ΔTrp derivative by abstracting the C3′-*proS*-proton with *syn* stereochemistry,[69] which was also observed in the feeding experiments. The unidentified enzyme responsible for ΔTrp formation during CDA biosynthesis should, therefore, follow the logic of the L-tryptophan-2′-3′-oxidase.

Fatty acid tailoring

The mechanism of the incorporation of 2,3-epoxyhexanoic acid as an invariant fatty acid tail in the CDAs (Figure 1, **13**–**21**) was determined recently by Kopp et al.[70] It was found that the tailoring of the unique 2,3-epoxyhexanoyl moiety of CDA is mediated by acyl carrier protein (ACP). It was shown that a new type of FAD-dependent oxidase (HxcO) with intrinsic enoyl-ACP epoxidase activity and a second enoyl-ACP epoxidase (HcmO) are responsible for the generation of the epoxidized fatty acid tail. HxcO and HcmO were predicted to be a fatty acid oxidase and epoxidase, respectively, both acting on acyl-CoA substrates.[33] Nevertheless, the experiments by Kopp et al.[70] with a set of acyl-CoA, acyl-ACP and chemoenzymatically derived CDA substrates demonstrated that only ACP-tethered fatty acids were accepted by HxcO and HcmO. In addition to the physiological substrate, hexanoyl-*S*-ACP, HxcO accepted a range of ACP-bound 4–10-carbon substrate analogues. In contrast, HcmO epoxidation activity showed more restricted substrate specificity and was limited to hex-2-enoyl- and crotonyl-*S*-ACP substrates. The characterization of the putative dehydrogenase, HxcO, showed the time-dependent formation of 2,3-epoxyhexanoyl-*S*-ACP and minor amounts of the expected hex-2-enoyl-*S*-ACP product. Using chiral reagents for amide ligation, the configurations of the epoxides generated were determined.[70] The epoxide generated by HxcO was (2*R*,3*S*)-configured, and the HcmO product had the opposite configuration based on HPLC analysis.

As two epoxidases displaying different stereochemistry were found within the CDA gene cluster, one can speculate about the molecular logic underlying the CDA epoxyhexanoic acid biosynthesis. It was suggested[70] that hexanyol-ACP is produced by FabH4 and FabH3, putative β-ketoacyl-ACP synthases encoded within the *fab* operon,[34] together with enzymes from primary metabolism. Subsequently, fatty acid tailoring by HxcO occurs on the ACP-bound hexanoyl moiety and results in the (2*R*,3*S*)-2,3-epoxyhexanoyl product. Alternatively, the hex-2-enoyl-ACP, either a side product of HxcO or derived from primary metabolism, can be epoxidized directly by HcmO during fatty acid synthesis on the ACP (Scheme 4).



Scheme 4. Proposed mechanism of the fatty acid tailoring events leading to 2,3-epoxyhexanoyl, the fatty acid tail of the CDAs (see the text for details).

Based on the finding that two epoxidases exist in the CDA cluster, it is suggested that HxcO has gained its epoxidation activity during evolution. HcmO and HxcO produce the two possible 2,3-epoxyhexanoyl enantiomers, but it remains unclear if both enantiomers are transferred from the ACP to the first module of the CDA peptide synthetase.

Outlook

Since daptomycin made its way into US clinics in 2003, a great deal of research has been carried out in the field of acidic lipopeptide antibiotics. The findings revealed the potential to fully understand and manipulate the molecular mechanisms underlying the remarkable structural diversity exhibited by these compounds.

With this information, engineering approaches to yield novel antimicrobial agents were successfully applied. The examples of the biocombinatorial synthesis of hybrid acidic lipopeptides show that, in general, it is possible to engineer NRPS assembly lines. This approach, however, is time-consuming, "wild-type" yields are rarely obtained and no hybrid has exhibited better bioactivity than that of its parent compound. Nevertheless, this branch of NRPS research is very young, and little is known about the mechanisms that provide a functional NRPS assembly line. Therefore, the presented efforts are remarkable findings and might facilitate more efficient biocombinatorial syntheses of NRPS products in vivo in the future. The possible combination of mutasynthesis and biocombinatorial approaches might also prove useful. Together with work covering NRPS interdomain and interpeptide docking sites[47,71,72] and on interactions modulating conformational switches[73] as well as the recent 3D structural elucidation of an NRPS termination module,[74] biocombinatorial methods might become more feasible soon. Chemoenzymatic approaches have the advantage that they are quick and can be employed to generate large libraries of hybrid lipopeptides in a reasonable time span; however, they are also inefficient due to the hydrolysis of the activated TE substrates. Once a hybrid molecule with improved bioactivity is found by this method, one could employ more efficient biocombinatorial approaches.

Furthermore, detailed mechanisms have been proposed through the biochemical, microbial and/or bioinformatical characterization of precursor syntheses carried out by modifying enzymes. More knowledge in this field is necessary to provide insights into how nature's unique ability to generate structural diversity can be adopted to build novel antibiotic building blocks. Further research routes towards new lipopeptides, together with the elucidation of the structural diversity-causing tailoring steps, is necessary to yield potent antimicrobial agents, as increased resistance is only a question of time.

Acknowledgements

We gratefully acknowledge the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support. We thank Dr. Elizabeth M. Nolan for valuable comments on the manuscript and, together with Verena Helmetag, for carefully proofreading the manuscript.

Keywords: acidic lipopeptides · antibiotics · daptomycin · NRPS · peptides · tailoring enzymes

[1] D. A. Enoch, J. M. Bygott, M. L. Daly, J. A. Karas, *J. Infect.* **2007**, *55*, 205–213.
[2] A. Raja, J. LaBonte, J. Lebbos, P. Kirkpatrick, *Nat. Rev. Drug Discovery* **2003**, *2*, 943–944.
[3] M. Debono, M. Barnhart, C. B. Carrell, J. A. Hoffmann, J. L. Occolowitz, B. J. Abbott, D. S. Fukuda, R. L. Hamill, K. Biemann, W. C. Herlihy, *J. Antibiot.* **1987**, *40*, 761–777.
[4] R. Sauermann, M. Rothenburger, W. Graninger, C. Joukhadar, *Pharmacology* **2008**, *81*, 79–91.
[5] K. J. Weissmann, R. Müller, *ChemBioChem* **2008**, *9*, 826–848.
[6] M. A. Fischbach, C. T. Walsh, *Chem. Rev.* **2006**, *106*, 3468–3496.
[7] R. Finking, M. A. Marahiel, *Annu. Rev. Microbiol.* **2004**, *58*, 453–488.
[8] C. T. Walsh, *Science* **2004**, *303*, 1805–1810.
[9] J. H. Lakey, E. J. Lea, B. A. Rudd, H. M. Wright, D. A. Hopwood, *J. Gen. Microbiol.* **1983**, *129*, 3565–3573.
[10] L. D. Boeck, H. R. Papiska, R. W. Wetzel, J. S. Mynderse, D. S. Fukuda, F. P. Mertz, D. M. Berry, *J. Antibiot.* **1990**, *43*, 587–593.
[11] R. H. Baltz, V. Miao, S. K. Wrigley, *Nat. Prod. Rep.* **2005**, *22*, 717–741.
[12] W. Aretz, J. Meiwes, G. Seibert, G. Vobis, J. Wink, *J. Antibiot.* **2000**, *53*, 807–815.
[13] C. Müller, S. Nolden, P. Gebhardt, E. Heinzelmann, C. Lange, O. Puk, K. Welzel, W. Wohlleben, D. Schwartz, *Antimicrob. Agents Chemother.* **2007**, *51*, 1028–1037.
[14] B. Heinemann, M. A. Kaplan, R. D. Muir, I. R. Hooper, *Antibiot. Chemother.* **1953**, *3*, 1239–1242.
[15] H. Naganawa, M. Hamada, K. Maeda, Y. Okami, T. Takeushi, H. Umezawa, *J. Antibiot.* **1968**, *21*, 55–62.
[16] F. Kong, G. T. Carter, *J. Antibiot.* **2003**, *56*, 557–564.
[17] L. Vertesy, E. Ehlers, H. Kogler, M. Kurz, J. Meiwes, G. Seibert, M. Vogel, P. Hammann, *J. Antibiot.* **2000**, *53*, 816–827.
[18] C. Farnet, A. Staffa, E. Zazopoulos, *PCT Int. Appl.* WO03060127A2, **2003**.
[19] A. J. Shay, J. Adam, J. H. Martin, W. K. Hausmann, P. Shu, N. Bohonos, *Antibiot. Annu.* **1960**, *7*, 194–198.
[20] J. Penn, X. Li, A. Whiting, S. K. Wrigley, M. Latif, T. Gibson, C. Silva, J. E. Davies, V. Miao, P. Brian, R. H. Baltz, *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 121–128.
[21] E. Toth-Sarudy, I. Horvath, J. Gyimesi, I. Ott, L. Alfoldi, J. Berdy, I. Koczka, V. Scholtz, V. Szell, E. Laszio, US Pat. 3,798,129, **1974**.
[22] J. I. Shoji, S. Kozuki, S. Okamoto, R. Sakazaki, H. Otsuka, *J. Antibiot.* **1968**, *21*, 439–443.
[23] C. T. Walsh, H. Chen, T. A. Keating, B. K. Hubbard, H. C. Losey, L. Luo, C. G. Marshall, D. A. Miller, H. M. Patel, *Curr. Opin. Chem. Biol.* **2001**, *5*, 525–534.
[24] S. A. Samel, M. A. Marahiel, L.-O. Essen, *Mol. Biosyst.* **2008**, *4*, 387–393.
[25] C. I. Montero, F. Stock, P. R. Murray, *Antimicrob. Agents Chemother.* **2008**, *52*, 1167–1170.
[26] J. Grünewald, M. A. Marahiel, *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 121–146.
[27] M. Debono, B. J. Abbott, R. M. Molloy, D. S. Fukuda, A. H. Hunt, V. M. Daupert, F. T. Counter, J. L. Ott, C. B. Carrell, L. C. Howard, L. D. Boeck, R. L. Hamill, *J. Antibiot.* **1988**, *41*, 1093–1105.
[28] V. Miao, M.-F. Coëffet-LeGal, P. Brian, R. Brost, J. Penn, A. Whiting, S. Martin, R. Ford, R. Parr, M. Bouchard, C. J. Silva, S. K. Wrigley, R. H. Baltz, *Microbiology* **2005**, *151*, 1507–1523.
[29] F. M. Huber, R. L. Pieper, A. J. Tietz, *J. Biotechnol.* **1988**, *7*, 283–292.
[30] F. T. Counter, N. E. Allen, D. S. Fukuda, J. N. Hobbs, J. Ott, P. W. Ensminger, J. S. Mynderse, D. A. Preston, C. Y. E. Wu, *J. Antibiot.* **1990**, *43*, 616–622.
[31] D. S. Fukuda, R. H. Du Bus, P. J. Baker, B. M. Berry, J. S. Mynderse, *J. Antibiot.* **1990**, *43*, 594–600.
[32] V. Miao, R. Brost. J. Chapple, K. She, M. F. Gal, R. H. Baltz, *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 129–140.
[33] C. Kempter, D. Kaiser, S. Haag, G. Nicholson, V. Gnau, T. Walk, K. H. Gierling, H. Decker, H. Zahner, G. Jung, J. W. Metzger, *Angew. Chem.* **1997**, *109*, 510–513; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 498–501.
[34] Z. Hojati, C. Milne, B. Harvey, L. Gordon, M. Borg, F. Flett, B. Wilkinson, P. J. Sidebottom, B. A. Rudd, M. A. Hayes, C. P. Smith, J. Micklefield, *Chem. Biol.* **2002**, *9*, 1175–1187.
[35] J. M. Neary, A. Powell, L. Gordon, C. Milne, F. Flett, B. Wilkinson, C. P. Smith, J. Micklefield, *Microbiology* **2007**, *153*, 768–776.

[36] M. Strieker, F. Kopp, C. Mahlert, L.-O. Essen, M. A. Marahiel, *ACS Chem. Biol.* **2007**, *2*, 187–196.
[37] E. Heinzelmann, S. Berger, O. Puk, B. Reichenstein, W. Wohlleben, D. Schwartz, *Antimicrob. Agents Chemother.* **2003**, *47*, 447–457.
[38] D. B. Borders, R. A. Leese, H. Jarolmen, N. D. Francis, A. A. Fantini, T. Falla, J. C. Fiddes, A. Aumelas, *J. Nat. Prod.* **2007**, *70*, 443–446.
[39] R. H. Baltz, *Nat. Biotechnol.* **2006**, *24*, 1533–1540.
[40] R. H. Baltz, *Curr. Top. Med. Chem.* **2008**, *8*, 618–638.
[41] R. H. Baltz, P. Brian, V. Miao, S. K. Wrigley, *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 66–74.
[42] V. Miao, M.-F. Coëffet-Le Gal, K. T. Nguyen, P. Brian, J. Penn, A. Whiting, J. Steele, D. Kau, S. Martin, R. Ford, T. Gibson, M. Bouchard, S. K. Wrigley, R. H. Baltz, *Chem. Biol.* **2006**, *13*, 269–276.
[43] K. T. Nguyen, D. Ritz, J.-Q. Gu, D. Alexander, M. Chu, V. Miao, P. Brian, R. H. Baltz, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17462–17467.
[44] M. A. McHenney, R. H. Baltz, *Microbiology* **1996**, *142*, 2363–2373.
[45] M. Bierman, R. Logan, K. O'Brien, E. T. Seno, R. N. Rao, B. E. Schoner, *Gene* **1992**, *116*, 43–49.
[46] K. T. Nguyen, D. Kau, J. Q. Gu, P. Brian, S. K. Wrigley, R. H. Baltz, V. Miao, *Mol. Microbiol.* **2006**, *61*, 1294–1307.
[47] S. Doekel, M.-F. Coëffet-Le Gal, J. Q. Gu, M. Chu, R. H. Baltz, P. Brian, *Microbiology* **2008**, *154*, 2872–2880.
[48] J. Kennedy, *Nat. Prod. Rep.* **2008**, *25*, 25–34.
[49] A. Kirschning, F. Taft, T. Knobloch, *Org. Biomol. Chem.* **2007**, *5*, 3245–3259.
[50] A. Powell, M. Borg, B. Amir-Heidari, J. M. Neary, J. Thirlway, B. Wilkinson, C. P. Smith, J. Micklefield, *J. Am. Chem. Soc.* **2007**, *129*, 15182–15191.
[51] J. W. Trauger, R. M. Kohli, H. D. Mootz, M. A. Marahiel, C. T. Walsh, *Nature* **2000**, *407*, 215–218.
[52] J. R. Jacobsen, C. R. Hutchinson, D. E. Cane, C. Khosla, *Science* **1997**, *277*, 367–369.
[53] J. Grünewald, S. A. Sieber, M. A. Marahiel, *Biochemistry* **2004**, *43*, 2915–2925.
[54] J. Grünewald, S. A. Sieber, C. Mahlert, U. Linne, M. A. Marahiel, *J. Am. Chem. Soc.* **2004**, *126*, 17025–17031.
[55] F. Kopp, J. Grünewald, C. Mahlert, M. A. Marahiel, *Biochemistry* **2006**, *45*, 10474–10481.
[56] D. Konz, A. Klens, K. Schörgendorfer, M. A. Marahiel, *Chem. Biol.* **1997**, *4*, 927–937.
[57] F. H. Vaillancourt, J. Yin, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 10111–10116.
[58] B. K. Hubbard, C. T. Walsh, *Angew. Chem. Int. Ed.* **2003**, *42*, 730—765; *Angew. Chem.* **2003**, *115*, 752–789.
[59] C. Milne, A. Powell, J. Jim, M. Al Nakeeb, C. P. Smith, J. Micklefield, *J. Am. Chem. Soc.* **2006**, *128*, 11250–11259.
[60] C. Mahlert, F. Kopp, J. Thirlway, J. Micklefield, M. A. Marahiel, *J. Am. Chem. Soc.* **2007**, *129*, 12011–12018.
[61] J. H. Zhang, N. B. Quigley, D. C. Gross, *Appl. Environ. Microbiol.* **1997**, *63*, 2771–2778.
[62] G. M. Singh, P. D. Fortin, A. Koglin, C. T. Walsh, *Biochemistry* **2008**, *47*, 11310–11320.
[63] R. P. Hausinger, *Crit. Rev. Biochem. Mol. Biol.* **2004**, *39*, 21–68.
[64] D. Lyutzkanova, J. Distler, J. Altenbuchner, *Microbiology* **1997**, *143*, 2135–2143.
[65] M. Strieker, L.-O. Essen, C. T. Walsh, M. A. Marahiel, *ChemBioChem* **2008**, *9*, 374–376.
[66] K. Takai, O. Hayaishi, *Methods Enzymol.* **1987**, *142*, 195–217.
[67] R. Genet, P.-H. Bénetti, A. Hammadi, A. Ménez, *J. Biol. Chem.* **1995**, *270*, 23 540–23 545.
[68] B. Amir-Heidari, J. Thirlway, J. Micklefield, *Org. Lett.* **2007**, *9*, 1513–1516.
[69] M. E. Gustafson, D. Miller, P. J. Davis, J. P. Rosazza, C. J. Chang, H. G. Floss, *J. Chem. Soc. Chem. Commun.* **1977**, 842–843.
[70] F. Kopp, U. Linne, M. Oberthür, M. A. Marahiel, *J. Am. Chem. Soc.* **2008**, *130*, 2656–2666.
[71] M. Hahn, T. Stachelhaus, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15585–15590.
[72] M. Hahn, T. Stachelhaus, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 275–280.
[73] A. Koglin, M. R. Mofid, F. Löhr, B. Schäfer, V. V. Rogov, M.-M. Blum, T. Mittag, M. A. Marahiel, F. Bernhard, V. Dötsch, *Science* **2006**, *312*, 273–276.
[74] A. Tanovic, S. A. Samel, L.-O. Essen, M. A. Marahiel, *Science* **2008**, *321*, 659–663.

Received: August 13, 2008
Published online on January 20, 2009

