REVIEW

Structural diversity, biosynthesis, and biological functions of lipopeptides from *Streptomyces*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Streptomyces are ubiquitous in terrestrial and marine environment, where they display a fascinating metabolic diversity. As a result, these bacteria are a prolific source of active natural products. One important class of these natural products is the nonribosomal lipopeptides, which have diverse biological activities and play important roles in the lifestyle of *Streptomyces*. The importance of this class is highlighted by the use of related antibiotics in the clinic, such as daptomycin (tradename Cubicin). By virtue of recent advances spanning chemistry and biology, significant progress has been made in biosynthetic studies on the lipopeptide antibiotics produced by *Streptomyces*. This review will serve as a comprehensive guide for researchers working in this multidisciplinary field, providing a summary of recent progress regarding the investigation of lipopeptides from *Streptomyces*. In particular, we highlight the structures, properties, biosynthetic mechanisms, chemical and chemoenzymatic synthesis, and biological functions of lipopeptides. In addition, the application of genome mining techniques to *Streptomyces* that have led to the discovery of many novel lipopeptides is discussed, further demonstrating the potential of lipopeptides from *Streptomyces* for future development in modern medicine.

1. Introduction

1.1 *Streptomyces*

Streptomyces are Gram-positive bacteria that display filamentous growth as well as spore generation. They possess a similar hyphae diameter and type-I cell wall to bacteria, which includes peptidoglycan with the L-form of diaminopimelic acid as the main component.[1] *Streptomyces* is the largest genus in the actinobacteria phylum, with almost 700 species formally reported and correctly identified from both terrestrial and marine habitats.[2, 3] Among them, *Streptomyces coelicolor* A3(2), *S. avermitilis*, and *S. griseus* are examples of well-studied strains in use for antibiotic production.[4]

In-depth investigations regarding the morphological differentiation and regulatory mechanisms of secondary metabolism in *Streptomyces* provide a solid platform for creating an efficient microbial drug manufacturing route using these bacteria. *Streptomyces* are notable for their complicated and fungal-like life cycles, and undergo apparent morphological and physiological differentiations. The production of secondary metabolites such as antibiotics occurs during the transition from substrate mycelium to aerial hyphae.[4] In this regard, *Streptomyces* are one of the most important sources for production of modern pharmaceuticals, contributing almost half of all antibiotics,[3] and it has been suggested that around two thirds of the antibiotics in nature are produced by *Streptomyces* and proximate actinomycetes. Through advances in DNA sequencing technology, large-scale genome mining has revealed that *Streptomyces* possess the ability to produce abundant unknown secondary metabolites that await further exploration.[2, 5] Today, many valuable secondary metabolites from *Streptomyces* are used in medicine, agriculture, animal husbandry and industry. For example, erythromycin, tetracycline, streptomycin, and rifamycin are all examples used for medical and veterinary purposes, tylosin is used as a growth promoter for animals, and avermectin is used as an anti-parasitic drug for livestock. In addition, *Streptomyces* is also widely used in the production of industrial enzymes, such as amylase, chitinase, cellulase, keratinase, pectinase, xylanase, and other extracellular hydrolytic enzymes.[6, 7]

Streptomyces possess a large genome, typically much larger than is found in other prokaryotes. The genome size of *S. coelicolor* A3(2) is around 8.6 Mb, making it twice the size of the genome of *E. coli* and ~75% of the size of the eukaryote yeast (approximately 12 Mb). *Streptomyces* species contain a unique linear bacterial chromosome, and this feature plays an important role during the strain evolution. The chromosome of *Streptomyces* consists of the essential "core region" in the genome centre and flexible "arm regions" at either end. The presence of terminal inverted repeats (TIRs) at the chromosome

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

ends causes the chromosomal terminus to be less conserved, and TIRs are more likely to experience gene loss or rearrangement throughout *Streptomyces'* genetic development.[5, 8] This structural adaptability of the chromosome in the telomeric region plays an important role during *Streptomyces* evolution, with more than half of secondary metabolite related genes found in gene clusters in the telomeric regions. For example, *S. coelicolor* A3(2) contains 23 gene clusters (accounting for 5% of all genes) and *S. avermitilis* contains 30 gene clusters (6.6% of all genes), which were believed to be acquired through horizontal gene transfer.[9, 10]

Streptomyces is a powerful chassis for natural product production and genetic manipulation. On the one hand, flexible regions of *Streptomyces* can be modified, for example by deletion of large segments to simplify modified strains with less energy consumption and a shorter life cycle.[11, 12] On the other hand, by optimising the expression of the genes for precursor biosynthesis, cell growth and resistance, modified strains can provide a genetically stable platform for the efficient biosynthesis of microbial drugs. *Streptomyces* genomes have been rationally modified to establish an optimal surrogate host for efficient production of antibiotics: for example, in *S. avermitilis*, the engineering of the sigma factor *hrdB* enhanced the expression of pathway-specific regulator AveR, and led to over 50% increase in the yield of avermectin B1a.[13] Many lipopeptides have been produced by heterologous expression in a *Streptomyces* host, which will be discussed in the subsequent section.

Streptomyces represent a promising platform for recombinant protein production. Given that *Streptomyces* naturally secrete multiple hydrolytic enzymes to degrade complex organic substrates, makes them ideal for this purpose.[14, 15] *Streptomyces* have also been developed both as a cell-free toolkit and as cell-free protein synthesis (CFPS) systems, showing great potential for characterising DNA/enzyme elements, activating desired biosynthetic pathways and other synthetic biology applications.[16-18] The exploration and establishment of a standardised library of functional and regulatory elements from *Streptomyces* (including the initiation/extension module, post-modification enzymes, and global transcriptional regulators) provide additional tools for efficient assembly and engineering of biosynthetic pathways of microbial drugs in synthetic systems.[19]

1.2 Lipopeptides

Lipopeptides are a class of compounds consisting of a hydrophilic polypeptide core and at least one hydrophobic fatty acyl chain. They exhibit a wide range of biological activities, such as antibacterial, antiviral, antitumor and immunosuppressant activities, and have excellent potential as pharmaceutical agents. Pharmaceutical lipopeptides offer distinct benefits including tolerance for human administration, ready accessibility for structural modification and an ability to access protein targets. These favourable traits make lipopeptide agents popular in the pharmaceutical and biotechnology industries.[20, 21] In addition, lipopeptides have been effectively demonstrated in roles such as biocontrol, as biosurfactants, for microbial oil recovery and environmental management, amongst others.[22]

Lipopeptides are mostly produced by bacteria from a variety of species, including *Myxobacteria*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Cyanobacteria* and *Streptomyces*; some fungi and plants can also produce lipopeptides.[22-27] *Streptomyces* are one of the most important sources of lipopeptides, with the exploration of such lipopeptides greatly contributing to the development of modern antibiotics for therapeutic use. Arguably the most important lipopeptide from *Streptomyces* is daptomycin (tradename Cubicin), a calcium-dependent cyclic lipopeptide produced by *S. roseosporus*, which was approved by the Food and Drug Administration (FDA) in 2003 for the treatment of serious blood and skin infections caused by Gram-positive microorganisms.[28] Daptomycin currently remains an important antibiotic in clinical use.

Biosynthesis of Lipopeptides. In general, lipopeptides are biosynthesised in three steps: (1) generation of the lipid moiety, (2) assembly of lipopeptidyl chain and (3) post-assembly line modifications. The characteristic fatty acyl chain is typically derived either from primary metabolism or is assembled by a dedicated polyketide synthase (PKS), while the lipopeptidyl chain is assembled by the actions of nonribosomal peptide synthetases (NRPSs) or NRPS/PKS hybrid machineries. In addition, further enzymes can be involved in generation of precursor substrates for lipopeptidyl assembly or modifications after assembly is complete. Here, we described the general mechanisms of lipopeptide biosynthesis.

Assembly of the Peptidyl Chain. Many peptides natural products are biosynthesized by non-ribosomal peptide synthetases (NRPSs), which are large multi-modular enzymes typically organized into modules of repeating catalytic domains. A canonical NRPS consists of adenylation (A), condensation (C), thiolation (T) and thioesterase (TE) domains, with the C-A-T architecture representing the minimal module for peptide extension by one amino acid (AA).[29] In terms of peptide assembly, A domains select specific amino acids from the cellular pool and activate them as aminoacyl-AMPs with consumption of ATP. The aminoacyl-moiety is then transferred to the terminal thiol group of the 4'-phosphopantetheine arm of a neighbouring T domain, with C domains then catalysing the nucleophilic attack of the amine of a downstream donor aminoacyl-T to the upstream T domain-bound peptide chain, thus generating an amide bond together with substrate transfer onto the acceptor T domain. This mechanism requires the

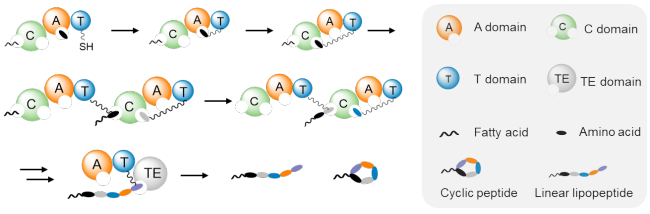

Figure 1. General scheme for lipopeptide biosynthesis.

carrier protein T domain to be highly dynamic, with an extension module T domain required to interact with at least three other catalytic domains.[30, 31] Repetition of this minimal C-A-T modular architecture then leads to the biosynthesis of a peptide whose length typically corresponds to the number of modules within the assembly line. The final module of an NRPS assembly line often contains a TE domain, which is responsible for releasing the peptidyl chain from the machinery. TE-mediated chain release can lead to alternative products, including hydrolysis to produce linear peptides and intramolecular macrocyclization to generate cyclic peptides. Cyclic peptides possess greater stability against protease, which is an important consideration in the development of NRPS-derived medicines (**Figure 1**).[32] In addition to A, C and T domains, epimerisation (E), methyltransferase (MT), formylation (F), heterocyclisation (Cyc), reductase (Re) and oxidation (Ox) domains can also be added to minimal C-A-T modules, adding yet further structural diversity to the peptide products of such NRPS assembly lines. For example, in some NRPS modules, C domains are substituted or supplemented with Cyc domains, which perform both condensation and cyclization functions. In such domains, cysteine, serine, or threonine acceptor substrates can be cyclized into thiazoline, oxazoline or methyloxazoline moieties. Addition of Ox domains then enables the further conversion of these heterocycles into thiazoles or oxazoles.[29]

Amino acid residues, as the basic building block of peptides, play important roles in determining the property of the lipopeptide natural products. Proteinogenic amino acids are basic building blocks for proteins and most peptides. In addition to modifying domains embedded in NRPS machineries, the complexity of NRPs can be further increased by the recruitment of nonproteinogenic building blocks and the post-assembly modifications. The peptide backbone of lipopeptides from *Streptomyces* usually contains many nonproteinogenic amino acids, making them a highly diverse group of secondary metabolites. These types of unusual residues are often generated by the activity of tailoring enzymes, *e.g.* hydroxylases, halogenases, dehydrogenases, P450 monooxygenases and aminotransferases.[29, 33, 34] As such, hydroxylation is the most common post-modification seen with amino acid building blocks in lipopeptides, typically occurring at the β-position.[35] The hydroxyl groups installed in this way can serve as important handles for post-assembly modification, such as glycosylation, methylation *etc*.[29, 36]

Loading of the Starter Unit. In NRPS assembly lines, C domains play the crucial role of peptidyl chain extension. Based on differences in substrate specificity and catalytic functions, C domains have been classified into five subtypes: $^{L}C_{L}$ domains, that catalyse a peptide bond between two L-configured amino acids in their donor/ acceptor substrates; $^{D}C_{L}$ domains that generate an amide bond between a D-configured amino acid donor and an L-amino acid acceptor; starter C domains (Cs domain or lipoinitiation domains) that acylate the first amino acid with a fatty acyl chain; Cyc domains that can catalyse both peptide bond formation and subsequent cyclisation of cysteine, serine or threonine residues; and dual E/C domains that catalyse both epimerisation and condensation reaction during NRPs biosynthesis.[37] Beyond these core roles, C domains have been implicated in diverse biosynthetic processes, including β-lactam formation and alkene insertion (**Figure 2**).[38] The flexible nature of C domains in tolerating active site modifications plays an important role in supporting the range of catalysis seen by C domains, whilst their role as proof-reading domains appears limited to specific examples related to amino acid modification during peptide assembly.[39] C domains also play important roles in recruiting *trans*-acting enzymes to NRPS assembly lines to modify carrier protein bound substrates, including the X-domain from glycopeptide antibiotic biosynthesis and I-domains that recruit β-hydroxylating enzymes.[40-42] As there are excellent review articles on C domains,[43, 44] we will not extensively review C domains but rather focus on recent studies concerning C domains in lipopeptide biosynthesis.

The lipopeptide products of NRPS assembly contain an N-terminal acyl chain, which is incorporated during peptide biosynthesis by lipoinitiation that is catalysed by a Cs domain in the first NRPS module. Here, the Cs domain catalyses the conjugation of the fatty acyl moiety with the first amino acid of the peptidyl backbone in the lipopeptide. In the biosynthesis of some lipopeptides, such as surfactin and calcium-dependent

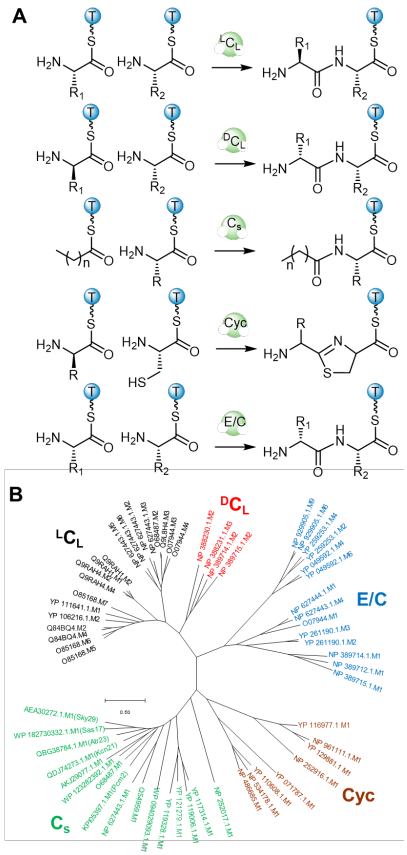

Figure 2. Types of condensation domains. Reactions catalysed by different C domains (A) and the phylogenetic tree of C domains (B).

antibiotics (CDA), fatty acyl chains are accepted by the Cs domain as fatty acyl-CoAs, whilst in other pathways the fatty acid is presented as a fatty acyl-T (**Figure 2**).[39]

The classical Cs domain consists – as with all C-domains – of two subdomains possessing a chloramphenicol acetyl-transferase (CAT) fold and that together adopt a "V-shaped" configuration. The catalytic core of C domains includes the central "HHxxDG" motif, along with binding sites for donor and acceptor substrates (**Figure 3**).[45, 46] To date, Cs domains involved in the biosynthesis of CDA, daptomycin, and surfactin have been well-characterised. Marahiel *et al.* identified that the Cs domain in surfactin biosynthesis shows clear substrate selectivity towards the acyl substrate of this domain, catalysing the transfer of CoA-activated 3-hydroxy fatty acids at its donor site with significant substrate specificity.[47] Likewise, the Cs domain in the glidobactin biosynthetic machinery has a significant substrate preference for long-chain fatty acids.[48] The Cs domain from CDA biosynthesis catalyses amide bond formation between the donor 2,3-epoxyhexanoyl-T and a T-bound serine accepter substrate. The co-crystal structure of an engineered CDA-C1 domain with a covalently linked acceptor showed that the H157 and S386 residues in the active site form hydrogen bonds with this substrate mimic, with residue S309 also interacting with the substrate.[49] In the A54145 machinery, the equivalent H143 residue of the Cs domain LptA-C1 was also found to be crucial for nucleophilic attack on the thioester carbonyl group of the donor substrate, supporting the mechanism postulated for CDA. Two single mutants (A152G and A386S) in Lpt-C1 decreased specificity toward the natural C_{10} fatty acid substrate but led to increased activity for nonnatural C_9 and C_{12} fatty acyl substrates, showing that active site mutations in Cs domains can change their selectivity for the acyl donor (**Figure 3**).[50]

The biosynthesis of lipopeptides commonly produces small amounts of congeners that only differ from the major lipopeptide in their fatty acid composition are usually produced by the same NRPS assembly line.[28] Altering the N-terminal fatty acid chain can improve the properties of lipopeptides, although routes that rely on semi-synthesis or the supplementation of fatty acid precursors are often complicated or inefficient. In 2021, the Bian group demonstrated how Cs domain exchange can be used to selectively alter the fatty acid chains present in lipopeptides. They identified two types of lipopeptides, rhizomide and holrhizin, which contain different lengths of fatty acid chains in *Burkholderia cepacia*. The Cs domain from their biosynthesis share high homology but accept fatty acids of different length (C_2 vs C_8).[46] The crystal structure of the Cs domain from rhizomide biosynthesis revealed several crucial residues impacting the selectivity of fatty acid chains and the role of the conserved H140 residue in stabilizing substrates. This work also demonstrated the generation of rhizomide derivatives acylated with fatty acids of specific lengths, which was made possible by targeted protein engineering.[46]

Beyond simple linear acyl chains, cinnamoyl-containing lipopeptides (CCLPs), such as WS9326A and skyllamycin, contain an unusual aromatic cinnamic acid moiety at the N-terminus. Similar compounds acylated by an aryl acid include JBIR-78/95, enterobactin, actinomycin and bacillibactin.[43, 51-53] The presence of this unusual acyl moiety implies that such Cs domains can be highly selective for such aryl acids, although the source of this selectivity is unclear. Sequence alignment and phylogenetic analysis has shown that the Cs domains for CCLP biosynthesis are located in a relatively independent evolutionary branch that differs from those found in daptomycin, surfactin and related lipopeptide biosyntheses (**Figure 3**). Further analysis of substrate recognition and specificity of Cs domains in CCLP biosynthesis will doubtless prove important for the future development of lipopeptide antibiotics containing such aryl acid moieties.

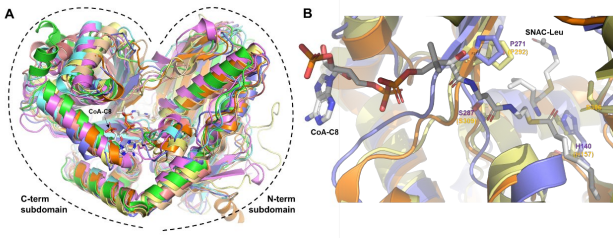

Figure 3. Substrate binding model and structural alignment of Cs domains. (A) The superimposition of Cs domains from seven NRPSs, including RzmA (PDB:7C1H) in blue, CDA-C1 (PDB: 4JN3) in yellow, VibH (PDB: 1L5A) in green, SrfA-C (PDB: 2VSQ) in magentas, TycC (PDB: 2JGP) in cyans, EntF (PDB: 5JA1) in tints, RzmA (PDB:7C1S) in orange, and LgrA (PDB: 6MFZ) in light orange. The ligand C8-CoA and Leu-SNAC (colored by element) was displayed from the protein complex 7C1S. (B) The enlarged view of the comparison of the binding pocket between 4JN3, 7C1H and 7C1S. The substrate ligands C8-CoA and SNAC-Leu in 7C1S and the key amino acids involved into the lipoinitiation were displayed.

Biosynthesis of fatty acid chains. The presence of a fatty acyl (FA) moiety is the characteristic difference between lipopeptides and other non-ribosomal peptides and has significant impact on their biological properties. The FA group modulates the polarity of the peptide and protects the peptide from degradation.[29] In addition, the FA has been shown to influence the chemoselectivity, regioselectivity, and kinetics of recombinant TE-mediated macrocyclisation during lipopeptide biosynthesis.[54] To date, introducing alternative fatty acid groups via mutasynthesis has been the main strategy for modulating the biological properties of lipopeptides.[55]

The origins of FA chains in lipopeptides differs depending on the specific biosynthesis pathway. In most cases, FAs are derived from primary metabolism and are synthesised by fatty acid synthases (FAS). However, in some lipopeptides the FAs are formed by specific polyketide synthases (PKSs). For example, the polyunsaturated FA in myxochromides, lipopeptides produced by myxobacteria, are synthesised by an iterative type I PKS.[56, 57] In the cinnamoyl-containing lipopeptides skyllamycin and WS9326, the cinnamic acid moieties are generated by type II PKSs. The FA can also undergo additional modifications, including β-hydroxylation, amination, epoxidation, and dehydrogenation (e.g. the acyl-CoA dehydrogenase LipB formation of an unusual Δ*cis3* double bond in the friulimicin FA chain),[58] further diversifying this important moiety (**Figure 4**).

The mechanisms of FA activation can also depend upon the specific lipopeptide biosynthesis pathway. In the biosynthesis of some lipopeptides such as daptomycin, FAs are activated by an independent fatty acyl-AMP ligase (FAAL) and loaded onto a free-standing T domain, with the Cs domain then accepting the FA-T to acylate the first amino acid residue. In the biosynthesis of lipopeptides such as surfactin and calcium-dependent antibiotics (CDA), FAs are instead activated by fatty acyl-CoA ligases (FACL) and covalently attached to coenzyme A. These FA-CoAs are then accepted as donor substrates by the Cs domain. For FAs synthesised by iterative type I PKSs or type II PKSs, the former mechanism (FA-T) is used (**Figure 4**).

2. Identification and biosynthesis of lipopeptides from *Streptomyces*

In recent decades, significant progress has been achieved in understanding the biosynthesis of lipopeptides. This section will focus on the discovery and biosynthesis of lipopeptides from *Streptomyces*. Based on the chemical structures, we have classified lipopeptides into six types: cyclic lipopeptides, linear

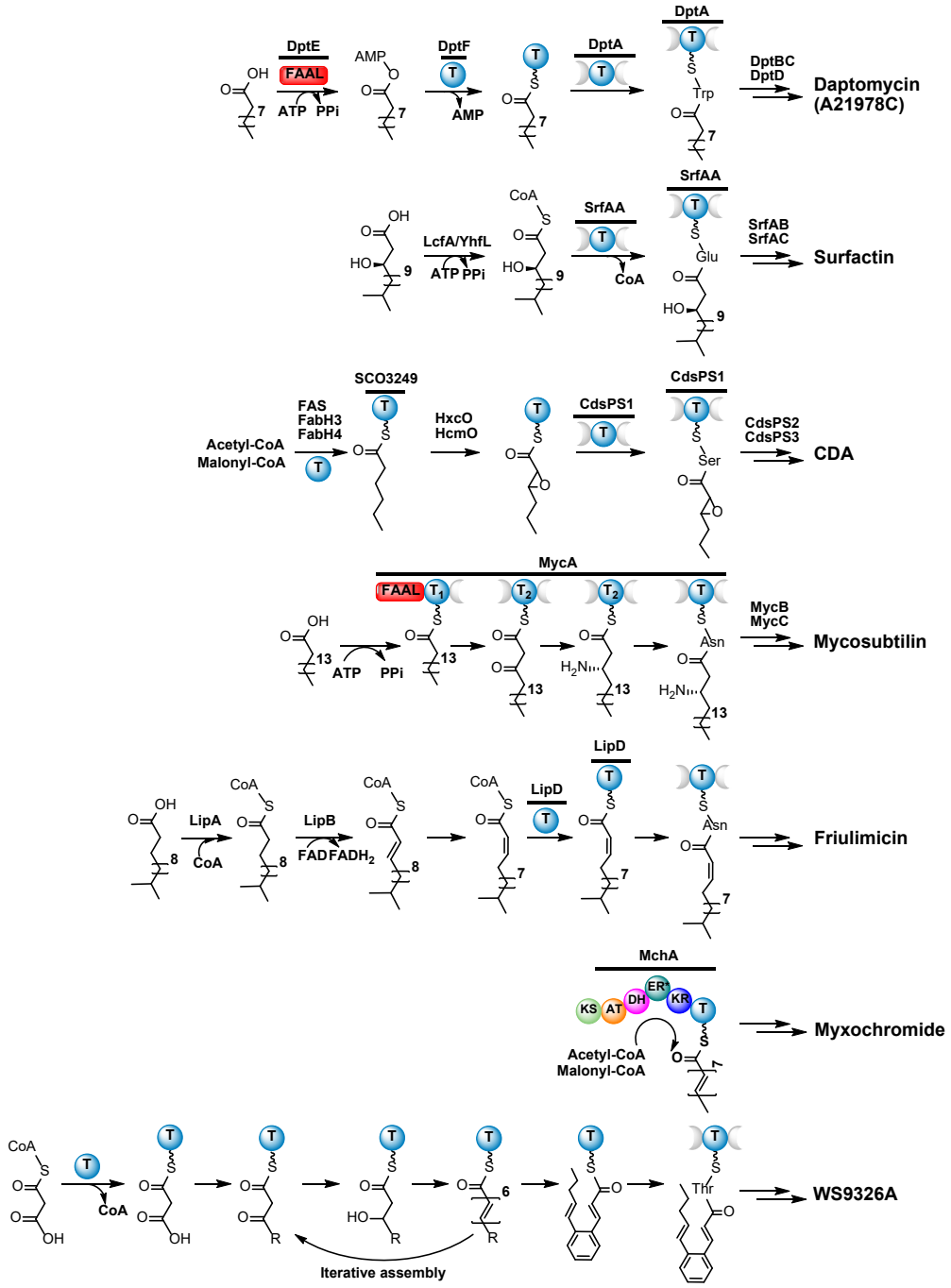

Figure 4. Representative biosynthetic pathways of fatty acid chains found in different lipopeptides.

lipopeptides, lipoglycopeptides, nucleoside-lipopeptides, PK/RiPP lipopeptides and cinnamoyl-containing lipopeptides. Lipopeptides discovered by heterologous expression of BGC in *Streptomyces* will be discussed separately.

2.1 Cyclic lipopeptides

A21978C and daptomycin. The calcium-dependent antibiotics (CDAs) from *Streptomyces* are a recognised source of antibacterial agents and include daptomycin, A54145, CDA and amphomycin. Structurally, CDAs are N-acylated cyclic peptides that are specific for calcium ion chelation. Most CDAs share the positioning of amino acids, especially the conserved Asp-X-Asp-Gly motif needed for calcium binding and antibacterial activity.[59] Here, we will discuss the biosynthesis of daptomycin, A54145, and CDA from *Streptomyces*, focusing on their assembly, regulation and combinatorial biosynthesis.

A21978C is a class of acidic lipopeptides with activity against-Gram-positive bacteria produced by *S. roseosporus* NRRL 11379; they consist of 13 AAs and a FA.[60] Six AAs in the structure of A21978C are non-proteinogenic, including D-ornithine (Orn), D-Ala, D-Ser, L-methylglutamate (MeGlu), L-kynurenine (Kyn) and D-Asn. A cyclic polypeptide of 10 AAs is formed via an ester bond between Kyn and L-Thr, leaving a linear peptide consisting of the 3 N-terminal AAs and FA. A21978C$_{1–3}$ contain C$_{11}$-C$_{13}$ branched FAs, while the C$_{10}$ FA form of A21978C was named daptomycin (**Figure 5**).[61] Daptomycin was approved in 2003 for the treatment of infections caused by Gram-positive pathogens, displaying excellent antimicrobial activity. The gene cluster encoding daptomycin biosynthesis (*dpt*) was identified in *S. roseosporus* NRRL 11379.[62] The biosynthetic gene cluster (BGC) of A21978C$_{1–3}$ and daptomycin contains three NRPS-encoding genes, *dptA*, *dptBC* and *dptD*, with other genes involved in precursor formation, regulation and modification (**Figure 6**).[63] Two discrete proteins (DptE and DptF) are involved FA incorporation: DptE (an acyl-CoA ligase) catalyses FA activation and coupling in an ATP dependent manner to the DptF carrier protein, with a preference for branched mid- to long-chain FAs. DptF then delivers the FA to the Cs domain of the initiating NRPS module of DptA, which then acylates L-Trp-T$_1$. The thioesterase domain in DptD catalyses cyclisation between the side chain of Thr4 and the Kyn13 C-terminus, forming the ten-membered depsipeptide.[63] The SAM-dependent methyltransferase (MTase) DptI is involved in the formation of MeGlu, catalysing the methylation of α-ketoglutarate to generate (3*R*)-3-methyl-2-oxoglutarate, that is transformed into MeGlu via transamination (**Figure 6**).[64]

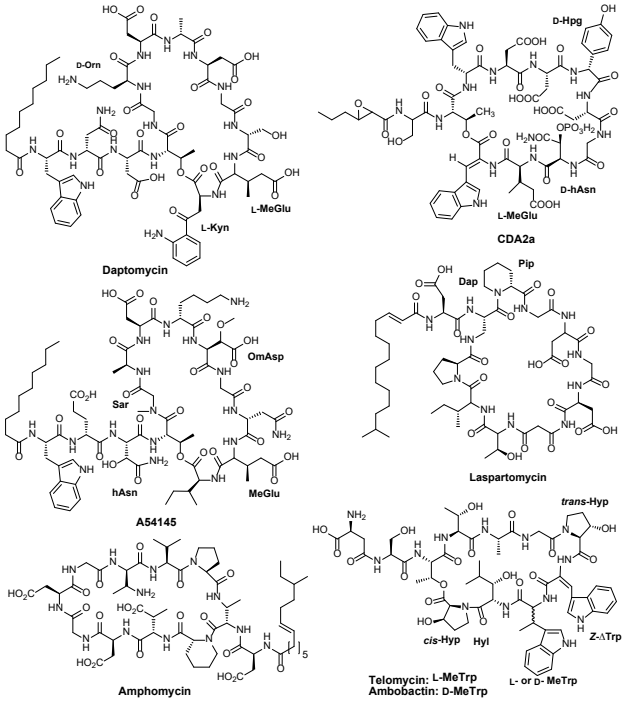

Figure 5. Chemical structures of cyclic lipopeptides from *Streptomyces* (I).

Second-generation derivatives of daptomycin remain in clinical development. Around 30 derivatives have been obtained using combinatorial biosynthesis by the substitution of subunits within the NRPS, including individual domains and multi-domain modules.[65, 66] Unfortunately, none of the hybrid daptomycin derivatives generated by combinatorial biosynthesis have been shown to be superior to daptomycin. Instead, the assembly of novel derivatives based on the peptide core of daptomycin has helped to explore a deeper understanding of the structure-activity relationships (SAR) of this antibiotic. SAR investigations suggest that AAs present at Asp7 and Asp9 are particularly important for the antibacterial potency of daptomycin, whilst Asp3, Thr4, mGlu12 and Kyn13 are required for optimal activity.[66, 67] Charge has also been identified as an important feature when designing lipopeptides for improved bioactivity.[68]

Calcium-dependent antibiotic (CDA). In 1983, a substance named calcium-dependent antibiotic (CDA) was identified from *S. coelicolor* A3(2) that showed antibacterial activity and channel-forming characteristics only in the presence of calcium.[69] Discovery of a series of CDA derivatives revealed that CDA comprises a 10-membered cyclic depsipeptide core with an exocyclic Ser residue at its N-terminus linked with a 2,3-epoxyhexanoyl FA unit.[70, 71] CDA contains four non-proteinogenic AAs: D-4-hydroxyphenylglycine (D-Hpg), mGlu, (*Z*)-2,3-dehydrotryptophan, and D-3-hydroxyasparagine (D-hAsn) (**Figure 5**).

The 82 kb BGC of CDA encodes three NRPS enzymes (CdaPS1, CdaPS2 and CdaPS3) and additional enzymes related to precursor synthesis and post-assembly modifications.[71] Hpg, lacking a β-carbon,[72] is formed from 4-hydroxyphenylpyruvate by sequential oxidative decarboxylation, oxidation and transamination catalysed by 4-hydroxymandelate synthase (HmaS), 4-hydroxymandelate oxidase (HmO) and 4-hydroxyphenylglycine aminotransferase (HpgT), respectively.[71] Asparagine oxygenase (AsnO) and 3-hydroxyasparagine phosphotransferase (HasP) are implicated in the post-assembly modification of Asn to generate D-phAsn.[71] Methyltransferase GlmT is homologous to DptI in daptomycin biosynthesis, suggesting a role in mGlu formation.[73] D-3-hydroxyasparagine (D-hAsn) is formed by the non-heme Fe^{2+}/α-ketoglutarate-dependent oxygenase AsnO that stereospecifically generates hydroxyasparagine prior to incorporation into the CDA

peptide.[74] In engineered Δ*asnO* mutants of *S. coelicolor* A3(2), new Asn-containing CDAs were produced that retain calcium-dependent antimicrobial activity.[75] The structure of CDA2a, CDA3a and CDA4a include a (*Z*)-2,3-dehydrotryptophan (*Z*-ΔTrp) residue. This dehydrogenated residue is found in other lipopeptides, such as telomycin and jahnellamides, although the formation of *Z*-Δ*Trp* has yet to be clearly delineated.[76, 77]

Five genes are involved in the biosynthesis of the unusual epoxide-containing FA, 2,3-epoxyhexanoic acid, including *hcmO*, *fabH4*, *hxcO*, *fabF3*, *acp* (**Figure 6**). Hexanoxyl-T is biosynthesised by *fabF3* and *fabH4* encoded β-ketoacyl-T synthase II, whilst HcmO (hexenoyl-CoA monooxygenase) and HxcO (hexanoyl-CoA oxidase) desaturate and epoxidise the hexanoyl FA.[68] The side chain length of the 2,3-epoxyhexanoyl FA is determined by the specificity of the KAS-II enzyme (FabF3),

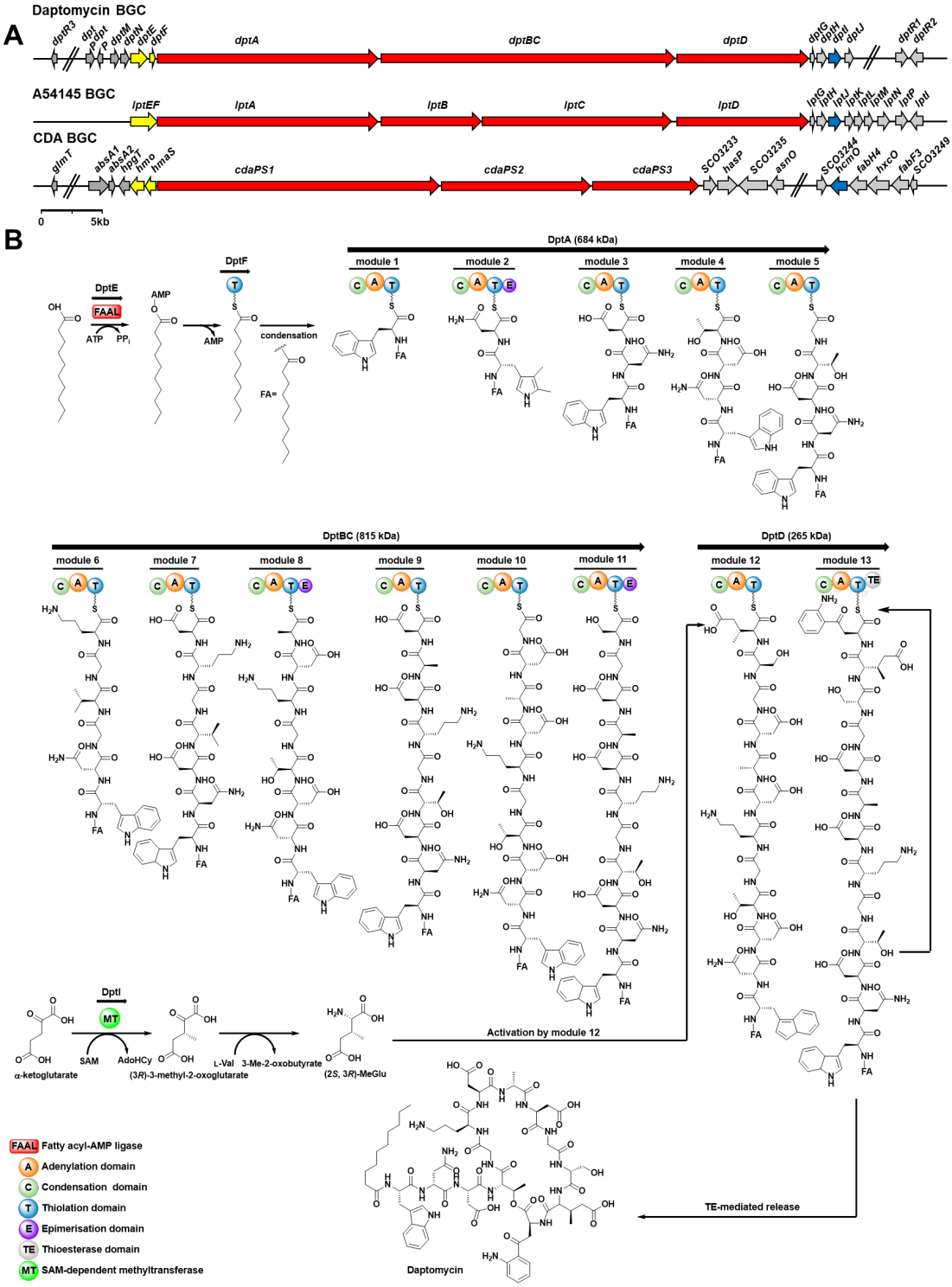

Figure 6. Biosynthetic gene clusters of daptomycin, A54145 and CDA (A) and the assembly pathway of daptomycin (B).

with mutation of Phe107 in FabF3 (SCO3248) leading to novel CDA derivatives bearing truncated (C_4) FA chains.[78]

A54145. A54145 (factor B1) comprises a group of Ca^{2+}-dependent cyclic lipodepsipeptides isolated from *S. fradiae* NRRL 18160 that exhibited growth-activity towards Gram-positive pathogens.[79] The structures of A54145 resemble daptomycin and CDA with a ten-membered cyclic depsipeptide and FA. A54145 contains 13 AAs with four nonproteinogenic residues: hAsn, sarcosine (Sar), 3-*O*-methyl-aspartic acid (OmAsp) and MeGlu. Members of A54145 can differ at AA 12 (Glu or MeGlu) and 13 (Val or Ile) in the depsipeptide ring as well as the FA (n-decanoyl, 8-methylnonanoyl or 8-methyldecanoyl chains) (**Figure 5**).

The biosynthesis of A54145 was elucidated by Baltz *et al*.[80] The core of A54145 is synthesised by four NRPSs encoded by *lptA*, *lptB*, *lptC* and *lptD* (**Figure 6**). The genes *lptEF*, upstream of the *lpt* NRPS genes, encode a protein containing acyl-CoA ligase and T domains that transfer the FA from FA-CoA thioesters.[80] The methyltransferase LptK is implicated in the synthesis of OmAsp after hydroxylation by the hydrolase LptL, which shares high similarity with AnsO from CDA biosynthesis. The *lptI* gene encodes a methyltransferase homologous with GlmT/DptI, and generates of MeGlu via the same mechanism.[73] The MT domain embedded in NRPS module 5 is anticipated to *N*-methylate Gly and generate the unusual residue Sar, whilst the two NRPS proteins LptB and LptC are analogs to the DptBC module in daptomycin NRPS.[80] The detailed biosynthetic mechanisms of A21978C, A54145 and CDA have been thoroughly reviewed by Baltz,[28, 68] and are thus only briefly mentioned in this review.

Amphomycin and related compounds. Amphomycin was identified in 1953 by Heinemann and co-workers and is believed to be the first cyclic lipopeptide reported from *Streptomyces* (**Figure 5**).[81] Subsequently, several amphomycin-like lipopeptides were reported from other *Streptomyces* strains. Structural elucidation of these peptides remained unsolved for decades, finally revealing that several differently named lipopeptides share the same structure, confusing their naming. Crystallomycin was reported in 1957 by Gause, which indicated a very similar structure to amphomycin. The producer of crystallomycin, *Actinomyces violaceoniger* INA 00887, was recently defined as *S. griseorubens* based on 16S rRNA analysis. Reanalysis of crystallomycin using modern analytic techniques uncovered two major components, Cryst-1 and Cryst-2, identical with aspartocin B and C, respectively.[82]

The aspartocins were initially discovered from *S. griseus* var. *spiralis* and *S. violaceus* in 1959,[83] although their structures were not elucidated until 2009.[84] The structures of aspartocin B and C were found to be identical with tsushimycin A and B, respectively, which were identified from *Streptomyces* strain Z-r237.[85-87] Both compounds are highly active against Gram-positive bacteria, including MRSA, in the presence of calcium.[82]

In 1960 Inoue *et al.* reported the isolation of glumamycin from *S. zaomyceticus* No. 7548, which showed inhibition activity to antibiotic-resistant *Micrococcus aureus*, *Bacillus subtilis* and other Gram-positive bacteria.[88] Structural elucidation revealed high similarity in the structures of glumamycin and amphomycin.[89-91] *S. zaomyceticus* No. 7548 is also the producer of zaomycin – an amphomycin similar lipopeptide discovered from *Streptomyces* sp. in 1954,[92] but no further information concerning this compound has been reported. All amphomycin-related lipopeptides consist of a 10-membered peptide ring and an exocyclic residue attached to a FA. Early analysis of the amphomycin FA indicated the presence of 3-anteisotridecenoic acid,[93] whilst in 2000, 3 amphomycin derivatives (A-1437 B, E and G) containing Δ3-isotetradecenoic acid, Δ3-anteisotridecenoic acid or Δ3-anteisopentadecenoic acid were isolated from *Actinoplanes friuliensis* HAG 010964.[94] In 2014, Yang and co-workers isolated two analogues of amphomycin, named aspartocin D and aspartocin E, from *S. canus* strain FIM0916;[95] these share the same core as amphomycin but contain Δ3-isoundecenoic acid and Δ3-isododecenoic acid, respectively. The biological activity of all amphomycins is restricted to Gram-positive bacteria in a calcium dependent manner.[95]

Laspartomycin. Laspartomycin was isolated from *S. viridochrontogenes* in 1968 and displayed activity against Gram-positive bacteria and fungi,[96] although the structure remained obscure until 2007.[97] The major component, laspartomycin C, was revealed as an 11-membered peptide core linked to a 2,3-unsaturated C_{15} FA. 10 AAs form the cyclic core in these peptides, generated via an amide bond between L-2,3-diaminopropionic acid (Dap) and L-Pro (**Figure 5**). Related compounds detected differ from laspartomycin C in the length of the FA. The 60 kb BGC of laspartomycin consists of 21 genes, in which *lpmA-D* encode four NRPSs responsible for the biosynthesis of the peptide backbone.[98] The genes *orf21*, *orf22* and *orf24* in the *lpm* gene cluster encode an acyl-CoA ligase, acyl-CoA dehydrogenase, and an T domain, respectively. Orf21 and Orf24 are believed to perform FA activation and loading, whilst Orf22 is anticipated to generate the double bond in the FA. The nonproteinogenic AA pipecolic acid (Pip) is putatively formed by a lysine cyclodeaminase encoded by *orf31*, and Dap by a pyridoxal-5'phosphate-dependent cysteine synthase and an ornithine cyclodeaminase (these are encoded by *svB* and *svK* located elsewhere in the genome).[98]

Telomycin and ambobactin. Telomycin (TEM) is a cyclic depsipeptide initially isolated from an unidentified *Streptomyces*, and later was found to be produced by *S. canus* ATCC 12646 and *Micromonospora schwarzwaldensis* HKI0641.[99-103] Whilst not isolated in acylated form, the biosynthesis of telomycin involves *N*-terminal acylation that is removed as a part of the maturation process (hence its inclusion in this section, *vide infra*). Telomycin displays activity against Gram-positive bacteria but less so towards Gram-negative bacteria. Structural analysis showed that telomycin is composed of a nine-membered depsipeptide ring with two exocyclic AAs.[104-106] Telomycin contains 5 non-AAs: β-OH-Leu10, *trans* 3-OH-Pro7, *cis*-3-OH-Pro11, *Z*-ΔTrp8, and methyl-Trp9. Among these residues, three are hydroxylated: β-OH-Leu10, *trans* 3-

OH-Pro7 and *cis*-3-OH-Pro11, whilst the lactone ring is formed between Thr1 and the carboxyl group of *cis*-Hyp3 (**Figure 5**). A telomycin-like lipopeptide, ambobactin, was isolated from *S. ambofaciens* F3 and was shown to be active against both Gram-positive and Gram-negative bacteria.[107] The structure of ambobactin differs from telomycin in the configuration of the β-MeTrp (D- in ambobactin).

The 80.5 kb BGC of telomycin (*tem*) was identified in the genome of *S. canus* ATCC 12646 and encodes three NRPSs and thirty-one other enzymes related to regulation, FA ligation, structural modification, and transport.[108] The gene *tem18* encodes a FAAL that is anticipated to activate the FA and transfer it to a free-standing T domain, Tem19, which is accepted by the NRPS. The P450 monooxygenase encoded by *tem23* catalyses β-hydroxylation of Leu and generates Hyl, while another P450, Tem29, is responsible for the *trans*-3-hydroxylation of the Pro7 residue (*trans*-Hyp3). *Cis*-Hyp3 is postulated to be formed by Tem32, a proline hydroxylase. Methyltransferase Tem27 and dehydrogenase Tem12 are putatively involved in the generation of β-MeTrp and Δ-Trp, respectively, but their roles require characterisation. After assembly, the FA-containing precursor is hydrolysed by the deacylase *tem25*, yielding mature telomycin. Deletion of modification genes has led to production of a series of telomycin derivatives, while deletion of *tem25* accumulated acylated precursor compounds. Interestingly, the FA-containing precursor displayed higher antibacterial activity than mature telomycin.[108]

Cyclodysidins. Cyclodysidins A-D are cyclic lipopeptides produced by *Streptomyces* strain RV15 which was isolated from a marine sponge (**Figure 7**).[109] Cyclodysidin D was also identified from *Streptomyces* sp. IB 2014/I/78-8, from a cave ecosystem.[110] The cyclodysidins comprise 7 AAs plus a β-amino FA, and are differentiated by the length of the FA. Their bioactivities have not been described to date.

Viennamycins. Viennamycins A and B were isolated from *Streptomyces* sp. S4.7, which is a rhizospheric bacteria of *Leontopodium nivale*.[111] The viennamycins consist of 12 AAs and a (2*Z*,4*E*)-8-methylnona-2,4-dienoic acid (MNDA) moiety. The cyclic peptide core is formed via lactonisation between L-Pro and L-Thr (**Figure 7**). Although no obvious bioactivity was observed for viennamycins, an unusual cysteic acid (Cya) moiety was found in viennamycin A. The 57 kb BGC of viennamycins (vie) encoding 13 proteins was identified from the genome of *Streptomyces* sp. S4.7. Analysis of the BGC suggest that the unique Cya residue is synthesised by VieA, a cysteate synthase, while the lipid chain MNDA is synthesised via dehydrogenation (by VieG) and reduction (by VieH) of 8-methylnonanoic acid.[111]

Cystargamide. Cystargamide was initially isolated from the actinomycete *Kitasatospora cystarginea*.[112] Its analog cystargamide B was recently identified from *Streptomyces* sp. PB013.[113] Both cystargamides are comprised of a 6-membered cyclic peptide, including a rare 5-hydroxytryptophan (L-Htrp) residue, and a 2,3-epoxy FA. The cystargamides differ in the configuration of the Hpg residue in the structure (L-configured in cystargamide A, D- in cystargamide B) (**Figure 7**). Although no antibacterial activity has been reported, cystargamide B has shown serine protease inhibiting activity to the NS2B/NS3 complex from dengue virus.[113]

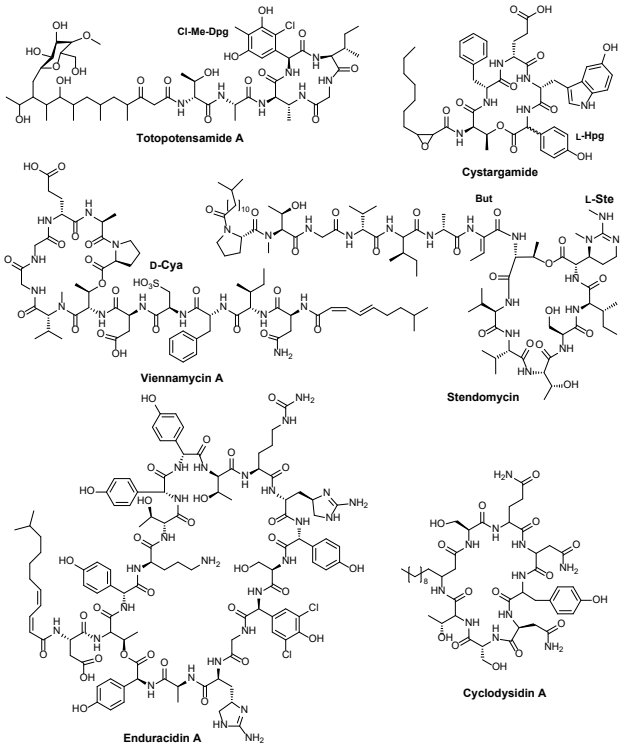

Figure 7. Chemical structures of cyclic lipopeptides from *Streptomyces* (II).

Stendomycins. The stendomycins are antifungal lipopeptides isolated from *S. endus* in 1963.[114] They consist of 14 AAs and a branched-chain FA of different lengths.[115-119] Impressively, 10 of the 14 AAs are nonproteinogenic, including MeThr, D-Ala, dehydrobutyrine (But), stendomycidine (L-Ste), two species of *allo*-isoleucine (D-*a*lle), *allo*-threonine (D-*a*Thr) and D-Val. The 7-membered lactone ring is formed between D-*a*Thr and L-Ste (**Figure 7**). In 2011, stendomycin was rediscovered from *S. hygroscopicus* ATCC 53653 and marine *Streptomyces* sp. CNQ27, together with 5 new derivatives.[120] A candidate BGC for stendomycin, consisting of five NRPS genes and fourteen other genes, was identified from the genome of *S. hygroscopicus* ATCC 53653. Analysis suggests that L-Ste is derived from arginine via dehydrogenation and intramolecular cyclisation followed by *N*-methylations.[120] An antibiotic named pantomycin was isolated from *S. hygroscopicus* NRRL 2751 in 1979 and showed antifungal, antibacterial and antiviral activities.[121] Recent structural elucidation has revealed that pantomycin is in fact identical to stendomycin.[122]

Enduracidins. Enduracidin A and B are produced by *S. fungicidicus* No. B 5477 and *S. atrovirens* MGR140 and exhibit potent antibacterial activity towards Gram-positive bacteria.[123-126] Enduracidin is similar to ramoplanin, a lipoglycopeptide from *Actinoplanes* ATCC 33076.[127] Both compounds contain 17 AAs, 16 of which forming a cyclodepsipeptide, whilst their sequences

differ (**Figure 7**). Enduracidins contain two enduracididine moieties and a citrulline residue, with the 2 D-mannose groups found in ramoplanin absent.[128] In addition, the length of the FAs in the enduracidins differ to that found in ramoplanin. The 84 kb BGC encoding the enduracidins was identified from the genome of *S. fungicidicus* and contains four NRPS encoding genes, *endA-D* responsible for the assembly of the lipopeptide. Four genes (*orf35*, *orf39*, *orf44*, and *orf45*) are proposed to be involved in the biosynthesis of the FA, with Hpg putatively formed by Orf23, Orf25 and Orf29. Orf23 and Orf25 share homology to pyruvate dehydrogenase and 4-hydroxyphenylpyruvate dioxygenase, while Orf29 possesses the function of both an FMN-dependent β-hydroxyacid dehydrogenase and a PLP-dependent class I and II aminotransferase; the gene *orf30* encodes a halogenase which is anticipated to generate the 3,5-dichloro-L-4-Hpg moiety. The formation of enduracididine and citrulline remains to be elucidated. In terms of regulation, Orf22 and Orf42 are positive regulators, with high production titer of enduracidins achieved by overexpressing Orf22 and Orf42.[129]

Totopotensamides. Totopotensamides A and B were initially isolated from *Streptomyces* sp. 1053U.I.1a.1b and comprise 6 AAs and a 17-carbon FA (**Figure 7**).[130] The four-membered cyclic peptide contains an unusual 4-chloro-6-methyl-5,7-dihydroxyphenylglycine (Cl-Me-Dpg) residue, and a 4-*O*-methylglucose unit was also found linked the FA of totopotensamide A. Recently, the totopotensamides were also obtained from *S. pactum* SCSIO 02999 through activation of the silent *tot* gene cluster. The new sulfonate-containing totopotensamide C, was obtained from these studies.[131] In the 34 proteins encoded by the 92 kb *tot* gene cluster, the two PKSs TotA1 and TotA2 synthesise the polyketide acyl chain, whilst three NRPS genes are responsible for the assembly of the peptide. From analysis of the cluster, the four enzymes TotC1 (type III PKS), TotC2 (enoyl-CoA dehydratase), TotC3 (enoyl-CoA dehydrogenase) and TotC4 (transaminase) are proposed to transform four malonyl-CoA units into 3,5-dihydroxyphenylglycine, which is then subsequently modified into Cl-Me-Dpg by TotH (halogenase) and TotM (methyltransferase) on the assembly line.[132] The glucosyl group in totopotensamide A is introduced by the glycosyltransferase TotG, while the sulfonation of totopotensamide C is performed by the sulfotransferase TotS.[131]

Neopeptins. The neopeptins are cyclic lipopeptides consisting of an FA and 9 AAs, which were isolated from *Streptomyces* sp. K-71O;[133, 134] neopeptins A, B and C differ in their FA (**Figure 8**). Neopeptins showed remarkable antifungal activity, due to inhibition of glucans synthesis in cell wall biosynthesis, leading to the swelling of pathogenic fungal mycelium.[134] Neopeptins A and B also displayed potent therapeutic ability and protective activity against powdery mildew disease.[135, 136]

Arylomycins. The arylomycins are a class of macrocyclic lipohexapeptide antibiotics bearing a saturated FA attached to the N-terminus and containing a unique C-C biaryl bridge between *N*-methy-L-4-hydroxyphenylglycine (MeHpg5) and Tyr7.[137] Arylomycins A and B were initially isolated from the fermentation broth of *Streptomyces* sp. TÜ 6075 (**Figure 8**).[137] Arylomycin B is yellow, which is caused by the nitro substituent on Tyr7, whilst also exhibiting higher antibacterial activity than arylomycin A.[138] In 2004, a number of lipoglycopeptides were discovered by Eli Lilly during screening of SPase inhibitors; these feature glycosylation modifications of the macrocyclic core of arylomycin, and were named the arylomycin C series.[139] Arylomycin D was discovered by Merck and was found to be uniquely active against *S. aureus*; it contains a sulfate moiety linked to the macrocyclic core and a lipopeptide tail. The antibacterial activity of arylomycin D is related to the special FA present in this molecule.[140] Several derivatives of arylomycin A as well as the corresponding BGC have been identified from *S. roseosporus* NRRL 15998,[141] with three NRPS enzymes (AryABD) responsible for the assembly of the peptide core of the arylomycins. A cytochrome P450 protein (AryC) was recently confirmed to generate the biaryl bond, in a process that occurs after cleavage of the peptide from the NRPS.[142]

K97-0239s and Enamidonin. K97-0239A and K97-0239B are cyclic lipopeptides isolated from *Streptomyces* sp. K97-0239, consisting of a unique imidazolidinone-containing tetrapeptide core attached to a C_{14} unsaturated FA (**Figure 8**). The K97-0239s were shown to inhibit the formation of macrophage foam cell and the number and size of lipid droplets in macrophages, decreasing the synthesis of cholesterol esters and triacylglycerols and blocking the development of atherosclerosis. K97-0239s also showed antibacterial activity against some Gram-positive bacteria, such as *B. subtilis* and *S.*

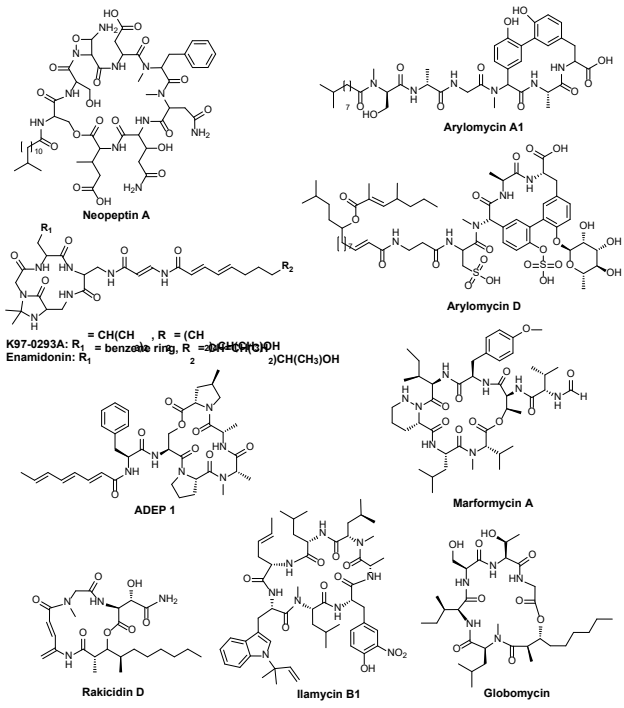

Figure 8. Chemical structures of cyclic lipopeptides from *Streptomyces* (III).

aureus.[143] No putative gene cluster has been reported for these lipopeptides to date. Enamidonin was isolated from *Streptomyces* in soil samples,[144] and is highly similar to K97-0239B. The difference between these compounds is one less double bond present in the FA of K97-0239B compared to that found in enamidonin. In 2018, two novel enamidonin analogues with different tetrapeptide backbones were isolated from *Streptomyces* sp. KCB14A132.[145] Enamidonin possesses antibacterial activity against *B. subtilis*, while the two new analogues exhibit broader antibacterial activities against Gram-positive bacteria including *B. subtilis*, MRSA, quinolone-resistant *S. aureus* and *Enterococcus faecalis*.[145] The dimethylimidazolidinone residue present in these molecules was found to be essential for their antibacterial activity.

ADEPs. The acyldepsipeptides (ADEPs) are antibiotics produced by *S. hawaiiensis* NRRL 15010,[146, 147] possessing potent antibacterial activity against various Gram-positive bacteria including multidrug-resistant *Staphylococcus* and *Streptococcus*.[146, 148] ADEP1 consists of a cyclic pentapeptide (Phe-L-Ser-Pro-L-Ala-Ala-MePro) attached to a phenylalanine triene side chain (**Figure 8**). The ADEP BGC (*ade*) was annotated in the strain *S. hawaiiensis* NRRL 15010, comprising genes encoding two NRPSs (*adeG* and *adeH*), a type II PKS responsible for assembling the polyene side chain, and a caseinolytic protease P (ClpP) that serves as accessory resistance factor.[149] Two genes (*adeA* and *adeB*) encoding a leucine hydroxylase and a alcohol dehydrogenase are predicted to be involved into the biosynthesis of 4-methylproline. A cluster-associated clpP homolog, *clpPADEP*, was identified as an acyl depsipeptide (ADEP) resistance gene, which constitutes a novel bacterial resistance factor that is necessary for conferring high-level ADEP resistance.[149] The biosynthesis of the diene or triene FA of ADEPs is performed by the polyketide synthase; the *ade* BGC contains a type II PKS for the biosynthesis of the triene side chain. This is highly similar to the PKSs present in skyllamycin, simocyclinones, and ishiagamide biosyntheses.

Marformycins. Marformycins (A-F) are cyclic heptadepsipeptides extracted from the fermentation broth of the deep South China Sea-derived strain *S. drozdowiczii* SCSIO 10141.[150] They show selective anti-infective activity against *Micrococcus luteus*, *Propionibacterium acnes* and *Propionibacterium granulosum*, with no cytotoxicity observed toward human tumour cells.[150] Their structures contain a N-terminally formylated side chain and 7 AAs including 5 non-proteinogenic residues (piperazic acid, *O*-methyl-D-Tyr, D-*a*Ile/ D-Val, L-*a*Ile/L-Val, and *N*-methyl-Val) (**Figure 8**). The 45 kb BGC of the marformycins encodes 20 proteins, of which six are NRPS enzymes (MfnCDEFKL), a methionyl-tRNA formyltransferase MfnA responsible for the formylation of the N-terminus of the peptide chain,[151] a SAM-dependent *O*-methyltransferase MfnG that generates *O*-Me-Tyr, a P450 MfnN that hydroxylates piperazine acid,[151] a pyridoxal 5'-phosphate linked aminotransferase (MfnO) and an isomerase (MfnH) that transforms L-Ile into L-*a*Ile.[152] MfnM acts as a positive regulator in the biosynthesis of marformycins and the ABC transporter MfnR enables extracellular transport of these peptides.[153]

Ilamycins. These pentapeptides were isolated in 1962 from *Streptomyces* sp. No. A-165-Z1, and were active against Mycobacteria.[154, 155] In recent years, new derivatives have been isolated from *S. atratus* SCSIO ZH16 or mutated strains.[156, 157] Structural studies have shown that 4 of the 7 AAs residues are nonproteinogenic: L-2-amino-4-hexenoic acid (L-AHA), a derivative of L-Trp, a derivative of L-*N*-Me-Leu and a modified L-Tyr. The amino group of the L-Trp residue is linked to various prenyl groups, and the L-Tyr residue is modified with a nitro-group in most ilamycins (**Figure 8**). Ilamycins C1/C2, E1/E2 and P demonstrate activity against human cancer cells, while the ilamycins D, E1/E2, F, J and L are potently active against mycobacteria.[156, 157] The 57.1 kb PKS/NRPS hybrid gene cluster encoding the ilamycins was identified from the genome of *S. atratus* SCSIO ZH16. In this cluster, the PKS IlaE is proposed to synthesize hexenoic acid, which is transformed into l-AHA by a P450 (IlaD), an aminotransferase (IlaH) and a type II thioesterase (IlaF). An aromatic prenyltransferase (IlaO) is proposed to generate the *N*-isopentene-Trp residue, while a further P450 (IlaR) catalyses isopentane epoxidation. A nitric oxide synthase (IlaM) and additional P450 (IlaN) are believed responsible for the generation of L-3-nitro-Tyr, whilst yet another P450 IlaL is proposed to generate the carboxylate moiety found the L-N-Me-Leu residue at a late stage of ilamycin biosynthesis.[156]

Rakicidins. These cyclic lipopeptides are produced by *Micromonospora* sp. (rakicidins A-B, E and G-I),[158, 159] and *Streptomyces* sp. (rakicidins C, D and F).[160-162] With the exception of rakicidin C, they are cytotoxic to certain cell lines and display activity against anaerobic bacteria. Rakicidin A is inhibitory against the growth of chronic myelogenous leukemia stem cells, where the diene moiety is necessary for activity.[163, 164] Structurally-related lipopeptides include vinylamycin, microtermolide A and BE-43547,[165-167] with these compounds all composed of a tripeptide and FA (**Figure 8**). The 4-amido-2,4-pentadienoate (APDA) moiety found within these peptides is rarely found in other natural products. Based on bioinformatic analysis, APDA-containing compounds are synthesised by a NRPS/PKS hybrid. The FA is incorporated by an initiating FACL domain and extended with six carbons by the actions of two PKS modules. The APDA moiety is putatively formed from a KS-catalysed condensation reaction between L-Ser and malonate, followed by ketoreduction and dehydration.[167, 168]

Globomycins. Globomycin is a cyclic depsipeptide first discovered in 1978 and produced by four different strains isolated from soil samples: *S. halstedii* No. 13912, *Streptoverticillium cinnamoneum* No. 15037, *S. neohygroscopicus* subsp. *globomyceticus* No. 15631 and *S. hagronensis* No. 17834.[169, 170] Globomycin was named for the formation of spherical spheroplast by *E. coli* in its presence.[170] These lipopeptides are composed of 5 AAs (*N*-Me-L-*a*Leu, L-*a*Ile, L-Ser, L-*a*Thr, Gly) and a 3-hydroxy-2-methyl FA of varying

lengths (**Figure 8**).[171] They exhibit moderate activity against Gram-negative bacteria,[171] with activity sensitive to the length of the FA (longer chains showing stronger activity).[172] Unexpectedly, a synthetic globomycin analogue with the longest FA showed antibacterial activity against Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA).[173, 174]

Azinothricin and related lipopeptides. Azinothricin is a hexadepsipeptide identified in 1985 from *Streptomyces* sp. X-14950,[175] which was found to be active against Gram-positive bacteria. Subsequently, several structurally related compounds were isolated and structurally characterized, including A83586C,[176] IC101,[177] pipalamycin,[178] GE3, L-156,602,[179] diperamycin,[180] variapeptin,[181] citropeptin,[182] verucopeptin,[183] kettapeptin,[184] the aurantimycins[185] and the polyoxypeptins (**Figure 9**).[186] Except for the antitumor antibiotic verucopeptin that was isolated from *Actinomadura verrucosospora* Q886-2, all other compounds were identified from *Streptomyces*. These 'azinothricin family' compounds are composed of a cyclic hexadepsipeptide featuring 2 piperazic acid moieties and a FA. Although not all these compounds have been biosynthetically investigated, studies on polyoxypeptin, aurantimycin and verucopeptin biosynthesis have revealed that they are synthesised by PKS/NRPS hybrid assembly lines.[186-188] The FAs found in these compounds are generated by PKS assembly lines, while NRPSs machineries generate the hexapeptide; the piperazic acid residues are putatively generated by hydroxylation and intramolecular cyclisation of ornithine.

Fengycins and Iturins. *Bacillus* are known for the production of bioactive lipopeptides exhibiting surfactant and antimicrobial activities, including the fengycins, iturins and surfactins, amongst others (**Figure 9**).[189] Recently fengycin and iturin were identified from *Streptomyces*, with iturin A6 produced by marine *Streptomyces* sp. SSA 13 demonstrating potent antitumor activities.[190, 191] Surfactins, iturins, and fengycins were found to be active against a variety of plant pathogens, making them efficient crop-protection agents. Their antimicrobial activities are mainly mediated by permeabilising cellular membranes.[192, 193]

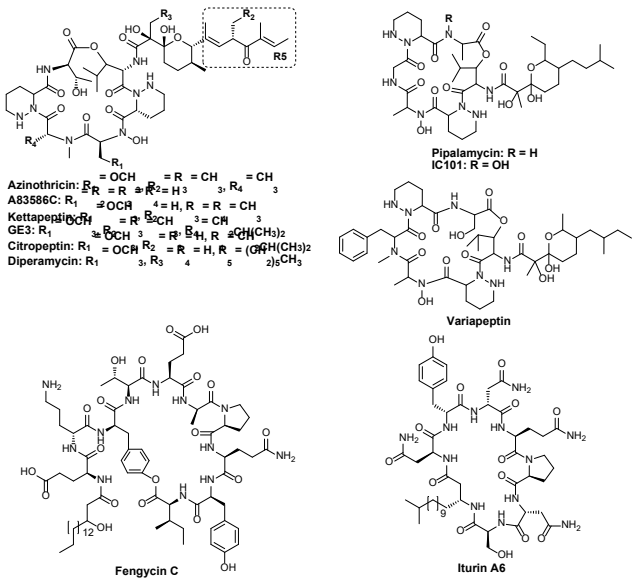

Figure 9. Chemical structures of azinothricin family antibiotics, fengycin and iturin.

2.2 Linear acyldepsipeptides

Sarpeptins. These linear lipopeptides produced by *Streptomyces* sp. KO7888 were obtained by overexpression of a regulatory gene *speR* in the sarpeptin (*spe*) BGC.[194] Both sarpeptin A and B consist of an (8-methyl-)-2,4-decadienoic acyl moiety and 7 AAs, including a 3-hydroxy aspartic acid amide (OH-Asp-$CONH_2$) residue at the C-terminus (**Figure 10**). The FA is putatively generated from (8-methyl-) decadienoic acid by two acyl-CoA dehydrogenases (*speF* and *speG*). In a deviation from the heptapeptide core, the *spe* gene cluster contains three NRPS genes, *speABC*, which theoretically synthesise a tridecapeptide. No antimicrobial activity has been reported for the sarpeptins, and the hypothesis that sarpeptins are hydrolytic products of a precursor requires further investigation.[194]

Rotihibins. Rotihibin A and B are linear lipopeptides isolated from *S. graminofaciens* 3C02.[195, 196] Their structures consist of a 2-decenoic acid FA and a peptide comprising 6 AAs, of which the L-*a*Thr, L-Dab, Orn, D-Cit, β-hydroxyasparagin (L-HyAsn) / β-hydroxyaspartic acid (L-HyAsp) and asparaginol (L-Asnol) are all non-proteinogenic. Rotihibins A and B differ in the presence of HyAsp or HyAsn at position 5 of the peptide (**Figure 10**). Whilst no antimicrobial activity or cytotoxicity was detected for these peptides, rotihibin A showed significant growth inhibiting activity against plants.[197] Mechanistic investigations using *Arabidopsis thaliana* revealed that rotihibin A is an inhibitor of the target of rapamycin (TOR) signaling.[198] Recently, two novel rotihibins C and D, possessing a Thr3 rather than Ser3, were isolated from *S. scabies* RL-34 and 87-22. These also showed inhibiting activity on plant growth and effects on photosystem II.[199] The BGC of rotihibins C and D has been identified in *S. scabies* genome,[199] where it encodes 14 biosynthetic genes including the NRPS RthA that possesses five modules and is responsible for the synthesis of the peptide. As the A domain of module 2 is missing, Thr2 is proposed to be transferred by another NRPS RthB, which contains a free-standing A-T

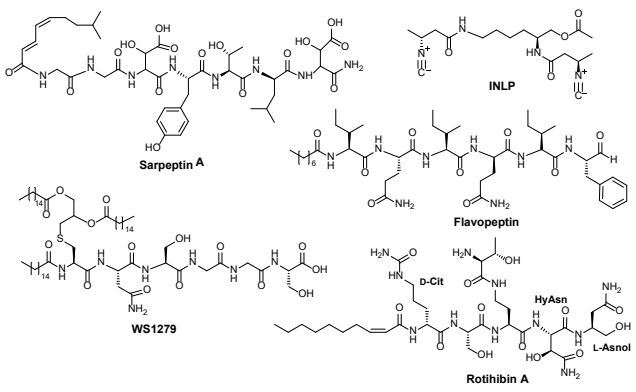

Figure 10. Chemical structures of linear acyldepsipeptides.

didomain. The L-*α*Thr residue attached on the side chain of Dab is also putatively loaded by RthB with the aid of the Type II-like thioesterase RthD. The assembly machinery in RthA ends with a reductase domain, which reduces the terminal Asn into the corresponding alcohol. HyAsn is hypothesised to be formed by L-asparagine oxygenase (AsnO) RthM, while Dab is putatively synthesised by diaminobutyrate-2-oxoglutarate transaminase RthN.[199]

Flavopeptins. These linear lipopeptide aldehydes were identified from *Streptomyces* sp. NRRL-F6652 through a proteomics guided method and demonstrated inhibitory activity towards cysteine proteases as well as the human 20S proteasome.[200] The flavopeptins are composed of a C_6-C_{10} FA and a hexapeptide that displays an uncommon C-terminal aldehyde (**Figure 10**). Analysis of the 22.6 kb NRPS gene cluster, identified from the genome of *S. flavogriseus* ATCC 33331 (a relative strain of *Streptomyces* sp. NRRL-F6652), suggests that the flavopeptins are synthesised by two NRPS proteins, FlavA and FlavB. The atypical aldehyde group is putatively generated by the NAD(P)H-dependent reductase domain at the end of biosynthetic assembly line.[200]

WS1279. WS1279 is a mixture of linear lipopeptides isolated from *S. willmorei* No. 1279 that exhibit immunostimulatory activity.[201-203] These compounds are composed of 3 FAs and 6 AAs. An unusual *S*-[2,3-bis(palmitoyloxy)propyl] acyl FA is attached to the amino group of L-Cys, while the other two FA are linked to hydroxyl groups of this propyl moiety (**Figure 10**). The biosynthetic pathway of WS1279 has not been identified, and thus the biosynthetic mechanism of the unusual *S*-[2,3-bis(palmitoyloxy)propyl] moiety remains to be elucidated.

Isonitrile lipopeptides. The isonitrile lipopeptides (INLPs) are a unique class of lipopeptides (**Figure 10**). Their BGC are widespread in the group of pathogenic mycobacteria and actinobacteria.[204] The 5 conserved biosynthetic enzymes responsible for their biosynthesis (*scoA-E* from *S. coeruleorubidus*) were cloned and expressed in *E. coli*, yielding two new lipopeptides, INLP2 and the deacetylated INLP1. Structurally, these compounds are similar to known isonitrile antibiotics SF2768 and SF2369.[205] The putative biosynthetic pathway for INLP has been reported, with the assembly line commencing with the activation of crotonic acid by ScoC that is subsequently loaded onto a T domain (ScoB) for further processing.[206] ScoD is a thioesterase homologue that catalyses the Michael addition of glycine to the α, β-unsaturated fatty acyl-ScoB to yield an *N*-carboxymethyl-3-aminoacyl-ScoB. ScoE catalyses the subsequent reaction to afford an α, β-isonitrile moiety through oxidative decarboxylation.[206] The isonitrile-modified FA is then covalently attached to both amino groups of lysine incorporated by the NRPS ScoA, where the Cs domain is predicted to catalyse multiple *N*-acylation between the two isonitrile-modified FAs and the T-tethered Lys.

2.3 Lipoglycopeptides

Some microbes produce complex glycopeptide antibiotics (GPAs) consisting of a heptapeptide scaffold that forms a polycyclic core through oxidative cross-linking of multiple aromatic AAs. Based on intramolecular crosslinking patterns and post-synthesis modifications, the GPAs are classified into five types (Types I - V).[207, 208] Vancomycin, teicoplanin, and three semisynthetic GPAs, telavancin, dalbavancin and oritavancin, are representative GPAs currently used in clinic for the treatment of Gram-positive bacterial infections. In addition to the glycosylated heptapeptide core, teicoplanin, telavancin, dalbavancin and oritavancin contain a lipophilic group that provides additional antimicrobial activity.

Since the discovery of vancomycin in *S. orientalis* (reclassified as *Amycolatopsis orientalis*) in 1953, more than 100 related (lipo)glycopeptides and derivatives have been identified from Actinomyces.[209] These (lipo)glycopeptides are differentiated by the amino acid residues of the polypeptide chain as well as the number and position of side chain crosslinks, halogen atoms, glycosylation and lipoylation. The (lipo)glycopeptides produced by *Streptomyces* include A35512,[210, 211] avoparcin,[212] mannopeptins,[213] OA-7653,[214, 215] A41030,[216, 217]A47934,[218] complestatin,[219] chloropeptins,[220-223] mannopeptimycins,[224] gausemycins,[225] pekiskomycin[226] amongst others. Due to the length limitation of this review, we will not discuss their biosynthetic mechanisms here. Researchers interested in lipoglycopeptides are suggested to read the reviews written by Nicolaou,[209] Hubbard,[227] Zhao[228] and Cryle.[207]

2.4 Nucleoside-lipopeptides

Liposidomycin, caprazamycin and A-90289. This is a group of structurally-related liponucleoside antibiotics isolated from *Streptomyces*. They feature a uridine moiety, a diazepanone ring, an amino-deoxyribose and FA (**Figure 11**). Liposidomycin was first isolated in 1985 from *S. griseosporeus* and displays inhibitory activity towards bacterial peptidoglycan synthesis.[229] Caprazamycins, which possess anti-tuberculosis activity, were isolated from culture broth of *Streptomyces* sp. MK730–62F2,[230-232] while the A-90289s were identified from *Streptomyces* sp. SANK 60405 via screening of translocase I inhibiting activity. The biosynthesis of liposidomycin, caprazamycin and A-90289 were comparably reviewed by Gust *et al.*[233, 234] Briefly, UMP is transformed to uridine-5'-aldehyde, which is conjugated with a glycyl or an amino group, yielding 5'-C-glycyluridine or 5'-amino-5'-deoxyuridine. A 3-amino-3-carboxypropyl group is then attached to the amino group of glycine followed by hydroxylation at the 2'-C position. The 5'-amino-5'-deoxyuridine is hydrolyzed and phosphorylated, and the amino-deoxyribose moiety is then transferred to 2-OH-3-amino-3-carboxypropyl-glycyluridine. *N*-methylation occurs on 3-amino-3-carboxypropane and glycine, with the two moieties then cyclised, generating the caprazol scaffold with a characteristic diazepanone ring; the FA and 3-methyl-glutarate moieties are attached sequentially. The liposidomycins and A-90289s are further modified by sulfation, with the A-90289s and caprazamycins also decorated with methyl-rhamnose units.

Muraymycins. This family of unusual nucleoside-lipopeptides isolated from *Streptomyces* sp. NRRL 30471[235, 236] shows activity against Gram-positive bacteria and a permeable strain of *E. coli*.

The muraymycins are complex, comprising a peptide and an uracil-uronic acid-ribofuranoside group linked to an aminopropane moiety (**Figure 11**). The uridyl nucleoside scaffold is similar to liposidomycin, caprazamycin and A-90289. The muraymycin peptide consists of a valine urea moiety, an epicapreomycidine residue and a Leu; based on the modification of Leu, the muraymycins can be classified into four types (A-D). A guanidino or hydroxyguanidino FA is attached to the hydroxyleucine residue of muraymycin A, while the hydroxyl group of OH-Leu in muraymycin B is modified via addition of a branched FA.

The 43.4 kb BGC of muraymycins, encoding 33 proteins, was identified from the genome of *Streptomyces* sp. NRRL 30471.[237] The uracil-uronic acid is proposed to be dehydrogenated by an acyl-CoA dehydrogenase (Mur22) and attached to a glycyl group by a serine hydroxymethyl transferase (Mur17). Based on characterization of the homologue LipK in A-90289 biosynthesis, Mur17 appears to be an L-threonine: uridine-5'-aldehyde transaldolase.[238] The 1-uracil-6-diaminopropane uronic acid scaffold is formed via transfer of 3-amino-3-carboxypropyl group and decarboxylation by an aminotransferase (Mur24) and a decarboxylase (Mur23). An aminotransferase (Mur20), a pyrimidine-nucleoside phosphorylase (Mur26) and a nucleotidyltransferase (Mur18) are proposed to convert nucleoside to dNDP-5-aminoribose. The aminoribose moiety is then conjugated to the 1-uracil-6-diaminopropane uronic acid group by a putative glycosyltransferase (Mur19). The peptide scaffold of the muraymycins is assembled by NRPS enzymes (Mur12-Mur14, Mur21, Mur25 and Mur27). Recently, Mur22 was characterised as an FAD-dependent dehydrogenase: a cyclase that generates the epicapreomycidine residue via dehydrogenation and intramolecular cyclisation of L-Arg, while Mur15 was confirmed as a nonheme Fe^{2+}- and αKG-dependent dioxygenase, generating β-OH-Leu by C_β hydroxylation.[239] Interestingly, modifications of L-Arg and Leu occur after NRPS-mediated peptide chain extension. Since no lipase was found in the *mur* gene cluster, addition of the FA remains unknown.

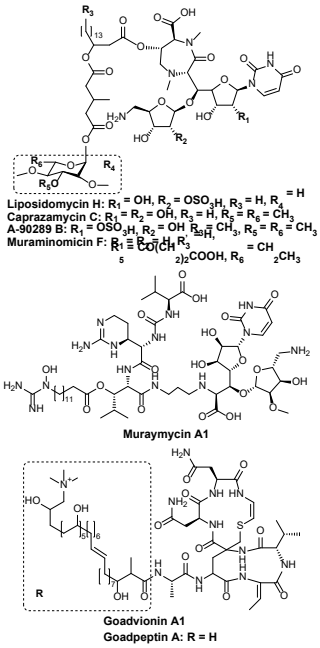

Figure 11. Representative chemical structures of nucleoside-lipopeptides and PKS/RiPP lipopeptides.

2.5 PKS/RiPP lipopeptides

Lipopeptides from *Streptomyces* can also be formed via ribosomally synthesised and post-translationally modified peptides (RiPP) pathways.[240] The variety of these lipopeptides and their biosynthesis has been gradually uncovered thanks to a wealth of recent genome sequencing data.

Goadvionins and Goadpeptins. Goadvionins are PKS/RiPP hybrid lipopeptides discovered in 2020 from *Streptomyces* sp. TP-A0584.[241] They are classified as lipolanthine, consisting of a C_{32} FA and an 8-membered RiPP with an avionin scaffold (**Figure 11**). Goadvionins showed antibacterial activity against Gram-positive bacteria, including *Streptomyces*, *S. aureus* and *B. subtilis*.[241] The goadvionins BGC comprises 22 genes encoding type-I PKS, FAS, RiPPs, and regulators, amongst others. The precursors of the RiPPs, goadpeptin A and B, are encoded by *gdvA* and *gdvB*, respectively, with GdvD and GdvKC then responsible for the generation of avionin scaffold. A rare 5'-trimethylammonium-containing valeric acid moiety is generated from lysine by GdvMT, GdvY and GdvJ, which is subsequently incorporated by FASs (GdvFB, GdvFG, GdvFZ, GdvF1) to generate a C_{29} FA. After extension with a propionyl group catalysed by GdvPKS, the trimethylammonium-containing C_{32} FA is generated (**Figure 12**). Condensation of the PKS and RiPP moieties is catalysed by the acyltransferase GdvG, which belongs to the GNAT superfamily. The resultant compounds are further modified by tailoring reactions to generate the mature goadvionins. *In vitro* reconstitution has shown that GdvG does not recognize FA-CoA as a substrate, demonstrating strict selectivity for a FA-binding T domain (cACP-GdvPKS). Bioinformatic analysis indicates that GdvG homologues flanked by PKS/fatty acid and RiPP biosynthesis genes are widely distributed in microbial species, suggesting

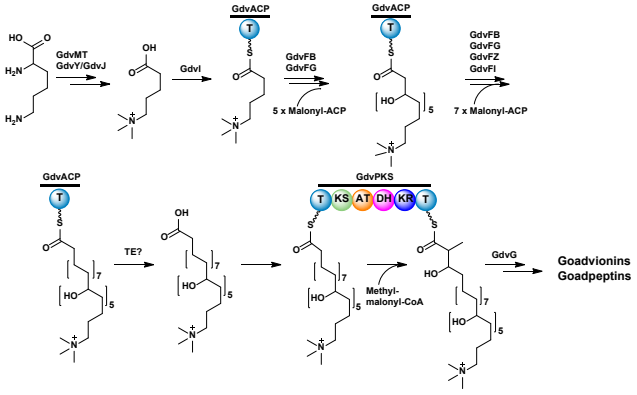

Figure 12. Biosynthetic pathway of the lipid chain in goadvionins and goadpeptins.

that FA transfer by GdvG-like enzymes is a general strategy in the PKS/RiPP hybrid lipopeptide biosynthesis.[241]

2.6 Cinnamoyl-containing lipopeptides (CCLPs)

CCLPs are a family of a cyclopeptides bearing a specific cinnamoyl FA. To date, CCLPs have been identified from a range of microorganisms, including WS9326s, mohangamides, skyllamycins, coprisamides, atratumycins, atrovimycin, cinnapeptin, kitacinnamycins, nyuzenamides and cipralphelin (**Figure 13**). Except for the kitacinnamycins from *Kitasatospora* sp. CGMCC 16924 and cipralphelin from *Penicillium brevicompactum* FKJ-0123,[242, 243] all CCLPs were isolated from *Streptomyces*. CCLPs are synthesised by type II PKS/NRPS hybrid machineries, with characteristic cinnamoyl FA biosynthesised by a type II PKS. Cinnamoyl FAs are formed from a T domain-bound polyene chain, with subsequent generation of the aromatic ring via intramolecular cyclization catalysed by a stereospecific enzyme. Subsequently, the T-bound cinnamoyl FA is incorporated by the NRPS machinery.

WS9326. WS9326A and WS9326B were identified from *S. violaceusniger* No. 9326 through screening of a tachykinin antagonist,[244-246] and contain a cyclic heptapeptide attached to a cinnamoyl FA (**Figure 13**). Three analogues, WS9326C, D and E, were isolated from fermentation broth of *Streptomyces* sp. 9078 by inhibition screening of an asparaginyl-tRNA synthetase from the *Brugia malayi* parasite.[247] WS9326A functions as a transcriptional inhibitor of gene pfoA in *Clostridium perfringens*, while WS9326B was observed to reduce the toxicity of *S. aureus* to human corneal epithelial cells.[248] *S. asterosporus* DSM 41452 produces analogues WS9326F and WS9326G in addition to WS9326A-E.51 Recently, a new analogue bearing a pyrazolone ring and a D-arabinitol on the cinnamoyl FA, WS9326H, was isolated from *Streptomyces* sp. SNM55, which exhibited antiangiogenic activity.[249]

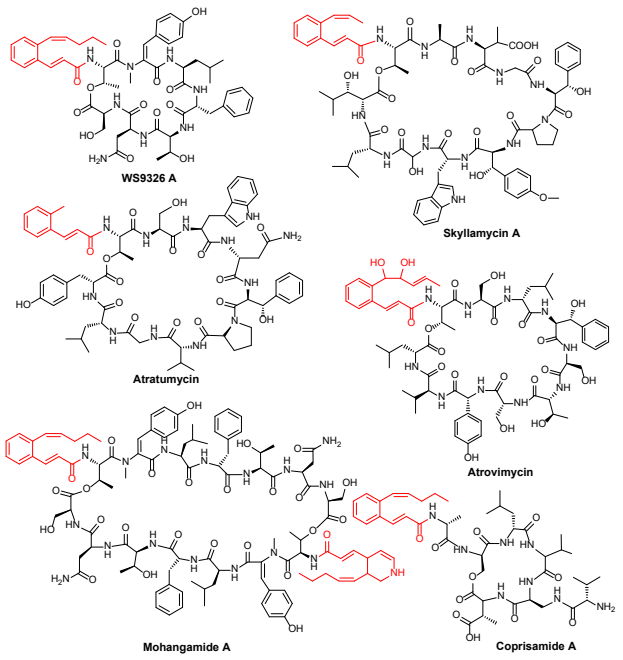

Figure 13. Representative chemical structures of cinnamoyl-containing lipopeptides

The 60 kb BGC of WS9326A was confirmed in *S. asterosporus* DSM 41452,[51] and encodes 40 proteins, 18 of them putatively involved in the biosynthesis of the cinnamoyl FA. Five NRPS proteins comprising a total of 7 modules are responsible for the peptide assembly (**Figure 14**). High similarity exists between the genes related to cinnamoyl chain biosynthesis in WS9326A, ishigamide, dialkylbenzene and the youssoufenes. Thus, it is hypothesised that the polyketide chain in WS9326A is produced by a similar highly reducing type II PKS machinery.[250, 251] Formation of the cinnamoyl moiety is therefore believed to be initiated from acetyl-CoA. Malonyl-CoA, is first loaded onto T domains (Sas9/Sas29/Sas34) to form a malonyl-T complex. Subsequently, malonyl-T and acetyl-CoA are iteratively assembled by a highly reductive type II PKS to form a C_{14} polyene intermediate T domain complex. Ketosynthases (Sas7, Sas8, Sas30, Sas31, Sas31 and Sas33) are responsible for the initial assembly of the entire polyketide chain backbone, and ketoreductase (Sas37) is responsible for the reduction of ketone groups, followed by dehydrogenase (Sas35 and Sas36) catalysed dehydration to form the polyene chain. *E* to *Z* conversion of specific alkenyl bonds is ascribed to the activity of Sas27 in order to provide precursors for the 6π-electrocyclization process. Formation of the aromatic ring is anticipated to be completed by Sas28 acting as a dehydrogenase. Zhu *et al*. demonstrated that the formation of this pentenyl-cinnamoyl FA was related to gene *sas27* and s*as28*, although their exact mechanism of action awaits further exploration.[252]

The core peptide backbone of WS9326A contains 3 non-proteinogenic AAs (*N*-methyl-*E*-2,3-dehydrotyrosine, D-Phe, and L-*a*Thr). The gene *sas16*, encoding a P450, was demonstrated to be essential for generation of the unusual dehydrotyrosine residue.[51] Further evidence suggests that desaturation of the α, β-dehydrotyrosine residue in WS9326 is performed directly by Sas16 and that dehydrogenation occurs during WS9326 peptide assembly, with Sas16 catalysing the direct dehydrogenation of the acylated dipeptide-T (unpublished results, Zhang *et al.*). Dehydrogenated aromatic residues have been identified in other NRPS-derived natural products, including CDA (2',3'-dehydrotryptophan), telomycins (dehydrotryptophan), jahnellamides (dehydrotryptophan), dityromycin (dehydrotyrosine), miuraenamides and tentoxin (dehydrophenylalanine),[28, 108, 253-256] although the varied enzymatic routes to their incorporation remains largely opaque. WS9326A peptide assembly utilises a nonlinear nonribosomal mechanism, which is mediated by two stand-alone A-T domain and two type II TEs. The 'A-less' module 7 in NRPS WS19 (Cal19 or Sas19) operates iteratively, catalysing two cycles of chain elongation and adding 2 AAs (L-*a*Thr and L-Asn) to the growing chain. Mutagenesis has shown that this extra module (module 7*) is essential for WS9326A biosynthesis, whilst the two *trans*-shuttling enzymes type II TEs WS5 (Cal5 or Sas5) and WS20 (Cal20 or Sas20) have different specificities: WS5 can only shuttle L-Asn, while WS20 can shuttle both L-*a*Thr and L-Asn.[257]

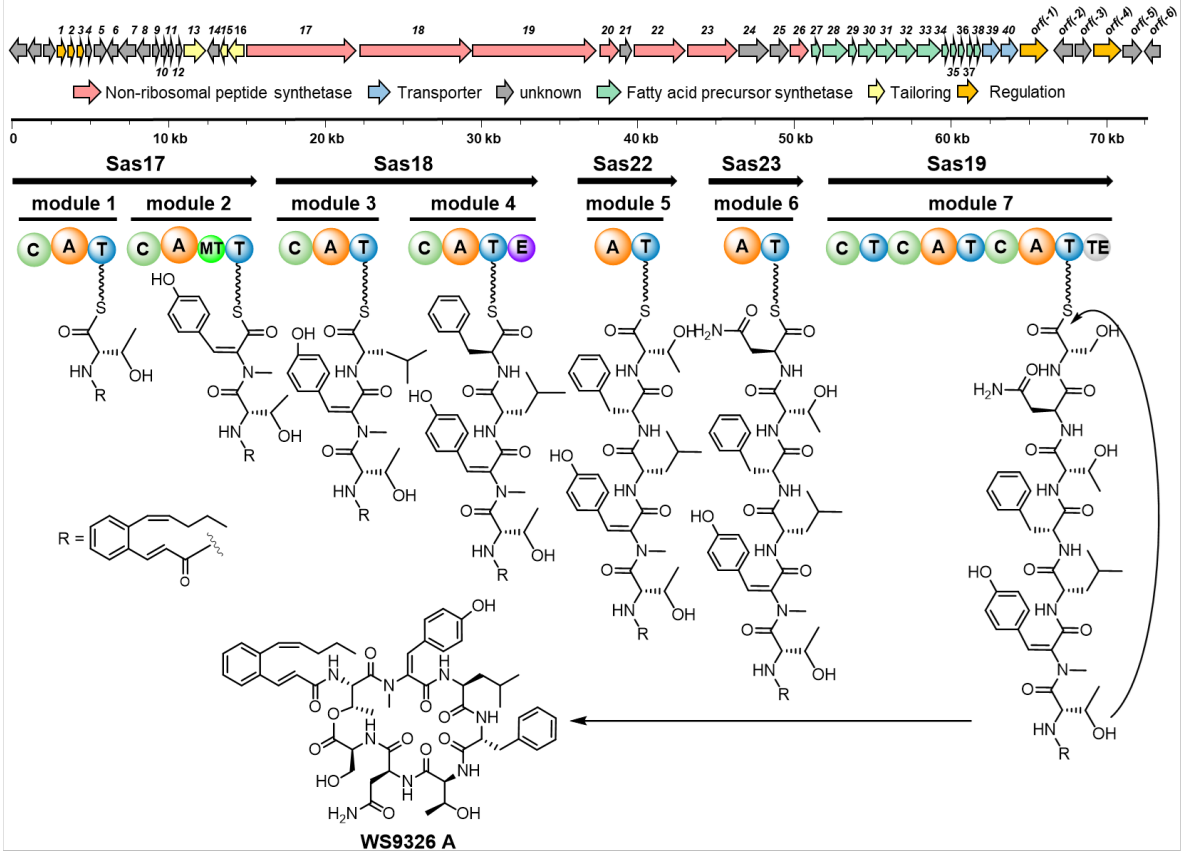

Figure 14. Proposed biosynthetic pathway of WS9326A.

The A domains of the stand-alone incomplete A-T didomain modules Cal22 (module 5) and Cal23 (module 6) recognise L-*a*Thr and L-Asn, with the A domain of $Cal23_{Asn}$ exhibiting promiscuous adenylation activity.[258]

Alterations in the chain length of the unsaturated polyene substituted at the cinnamoyl group further broaden the structural diversity of this moiety. The KSs and KRs in the highly reduced type II polyene PKSs differ from others in PKSs and FASs.[259, 260] Whilst in type II PKSs, the KS-CLF complex catalyses the polyketide chain elongation with the T domain,[261] highly reducing type II PKSs, including those found in ishigamide, dialkylbenzene and youssoufene biosynthesis instead form a polyene aliphatic scaffold.[250, 251] To distinguish highly reducing type II PKSs from typical type II PKSs, Du *et al.* reported the crystal structures of the *apo* Iga11-Iga12 (KS-CLF) heterodimer, the C6+Tga11-Tga12 binary complex and the covalently cross-linked Iga10=Iga11-Iga12 (T-KS-CLF) tripartite complex, revealing the molecular basis of the interaction between Iga10 and Iga11-Iga12. The Iga11-Iga12 heterodimer constructs the reaction pocket that controls the chain length of the final polyketide product. Mutagenesis revealed that the combination of negative charge on Asp-113 in Iga11 and steric hinderance caused by Leu125 in Iga12 likely prevents further condensation using a β-ketoacyl product as a substrate.[262] The key residue Asp113 is conserved in other putative CCLPs alkyl-polyene-synthesising HR type II PKSs, such as Sky17 and Sas17, supporting this hypothesis.

Mohangamide. These pseudodimeric peptides were isolated from culture extracts of marine *Streptomyces* sp. SNM55 by screening for inhibitory activity towards isocitrate lyase of *C. albicans*.[263] They are formed by cyclisation of a cinnamoyl peptide fragment and a dihydropyridine-containing peptide fragment via the formation of two ester bonds. The structure of the cinnamoyl peptide portion of both mohangamides resembles that found in WS9326A (**Figure 13**). Indeed, mohangamide A can be recognized as a pseudodimer of WS9326A, whilst mohangamide B harbours two different AAs in the dihydropyridine-containing peptide. The formation of the dihydropyridine-containing FA as well as the pseudodimeric structures remain elusive, although dimerisation of the peptide chains was proposed to be catalysed by a terminal thioesterase domain, as is seen in echinomycin biosynthesis.[264] Another structural feature of the mohangamides is the acyl chain-bearing dihydropyridine, with integration of this dihydropyridine moiety implying that the cinnamoyl side chain may undergo an unusual biosynthetic pathway to afford this structure. Recent research exhibited that the WS19/Cal19 TE domain catalyse the cyclodimerization of mohangamides with substrate specificity.[265]

Skyllamycin. Skyllamycin A was initially isolated from *Streptomyces* sp. KY11784 under the name of RP-1776,[266] and showed inhibitory activity towards the platelet-derived growth factor (PDGF) signalling pathway. Skyllamycin B was isolated from *Streptomyces* sp. Acta 2897,[53] skyllamycin C is produced

by a marine *Streptomyces* sp. Strain 1675 [267] and skyllamycins D and E were discovered from *S. anulatus*.[268] While skyllamycins A and D showed weak activity against Gram-positive bacteria, skyllamycins B and C inhibit biofilm formation.[267] The skyllamycins consist of a cinnamoyl FA and an 11 AA cyclic depsipeptide. The depsipeptide contains multiple hydroxylated residues: one α-hydroxylated Gly (α-OH-L-Gly) and three β-hydroxylated amino acids, β-OH-L-Leu, β-OH-L-Phe and β-OH-OMe-L-Tyr (**Figure 13**). The 86 kb BGC of the skyllamycins harbouring 49 ORFs was identified from the genome of *Streptomyces* sp. Acta 2897. Three NRPSs (Sky29-Sky31) are responsible for the assembly of skyllamycins, with sixteen genes proposed encode enzymes for generation of the 2-[1-(*Z*)-propenyl]cinnamoyl moiety. Sùssmuth *et al.* first hypothesised that the biosynthesis of the propenyl-cinnamoyl FA in skyllamycin is encoded by a group of genes resembling those from fatty acid biosynthesis. It was proposed that the assembly of this cinnamic acid FA may be based on a specific type II PKS or fatty acid metabolism, and that this cinnamic acid formation undergoes an electrocyclisation mechanism of assembly.[53] The nonproteinogenic residue α-OH-L-Gly is formed by an α-hydroxylase (Sky39), whilst the T-dependent β-hydroxylation of AAs is performed by a P450 (Sky32).[53, 269, 270] The complex of Sky32 (P450sky) and T_7 from the skyllamycin NRPS was trapped using a T-tethered azole P450 inhibitor: the interface residues identified in the P450sky/T_7 complex are highly conserved amongst all carrier proteins of the skyllamycin NRPS, with the interface dominated by hydrophobic interactions between key secondary structure elements.[270] For the remaining modified amino acids, two glutamate mutases (Sky41, Sky42) have been shown to be for the conversion of (2*S*)-glutamate into (2*S*, 3*S*)-3-methylaspartate, whilst Sky37 was demonstrated to generate *O*-methyltyrosine. Experiments have shown that Tyr is methylated prior to its activation by the NRPS.[269]

Atratumycin. Atratumycin is a cinnamoyl-capped lipopeptide recently isolated from marine *S. atratus* SCSIO ZH16 through genome mining and possessing activity against *Mycobacterium tuberculosis*.[52] Atratumycin is composed of a C_{10}-cinnamoyl FA and a cyclic depsidecapeptide (**Figure 13**). The biosynthesis of atratumycin is related to the skyllamycins, with the peptide assembled by three NRPS enzymes. Ten proteins (Atr5-Art8, Atr11-Atr16) are putatively involved in the biosynthesis of the 2-alkyl cinnamoyl unit in atratumycin, of which homologous proteins could be identified in skyllamycin biosynthetic pathway. Two LuxR regulators (Atr1 and Atr2) and two transporters (Atr29 and Atr30) were found playing positive roles in the production of atratumycin, while a *Streptomyces* antibiotic regulatory protein (Atr32) acts as a negative regulator.[271]

Atrovimycin. A related compound of atratumycin, atrovimycin, was isolated from *S. atrovirens* LQ13.[272] Similar to atratumycin, this compound is also composed of a decadepsipeptide and a cinnamoyl acyl FA. However, atrovimycin bears a different peptide sequence and the cinnamoyl chain contains a vicinal-dihydroxy unit (**Figure 11**). Unsurprisingly, high similarity was found between atrovimycin and skyllamycin BGCs. Atrovimycin is putatively assembled by four NRPS enzymes, with a series of type II PKSs thought to be involved in the biosynthesis of the vicinal-dihydroxylated cinnamoyl moiety. Expressing the cinnamoyl acid biosynthetic genes from the atrovimycin BGC in the atratumycin producer *S. atratus* SCSIO ZH16 afforded two new analogues containing extended cinnamoyl FAs, atratumycins B and C.[273] The P450 Avm43 and epoxide hydrolase Avm29 have been identified as playing roles in the formation of dihydroxylated cinnamic acyl chain. P450 Avm28 is responsible for hydroxylation at L-Phe in atrovimycin. Sequence alignment shows that Avm28 has 25% similarity with Sky32, and although both enzymes appear to be tailoring hydroxylases they lead to a different stereochemistry in their products: Avm28 affords a 2*S*, 3*R* configured Phe whereas the Sky32 affords 2*S*, 3*S* residues.[272] The cause of the stereochemical divergence of these functionally similar P450s remains elusive. The length and the unsaturation of the 2-alkenyl group in the cinnamoyl FA has been shown to influence the antitubercular activity of atratumycin A. By recombination of the *atr* and *avm* gene cluster, new cyclodecapeptides (atratumycin B and atratumycin C) containing cinnamoyl moieties with different lengths (C_{14} or C_{12}) were generated in the recombinant strain *S. atratus* ZH16NSH. Atratumycin B showed activity against *M. tuberculosis* H37Rv together with enhanced antitubercular activity.[273]

Coprisamide. These cyclic heptapeptide bearing a 2-heptatrienyl cinnamoyl moiety are produced by a symbiotic *Streptomyces* strain SNU533 isolated from the gut of dung beetle *Copris tripartitus* (**Figure 13**).[274] The cyclopeptide consists of 5 AAs, where the 2,3-diaminopropanoic acid AA is attached to L-Val, while the 2-alkenyl cinnamoyl FA is attached to a branched D-Ala that is in turn linked with the D-Ser. Though no obvious antibacterial or anti-fungal activity was observed, coprisamides could induce the activity of quinone reductase.[275] Analysis of the *cpr* gene cluster suggests that two NRPSs (CprH and CprI) bearing six modules assemble the coprisamides. CprA-E and CprJ-N are putatively involved in the generation of the cinnamic acid unit.[275] Two new analogues bearing C17-cinnamic FA, C and D, were also recently isolated from *Micromonospora* sp. UTJ3, a gut bacterium of the carrion beetle *Silpha perforate*.[275]

Cinnapeptin. Cinnapeptin was discovered from *S. ghanaensis* with a MALDI-MS-guided high-throughput elicitor screening (HiTES) strategy.[276] FA cyclic depsipeptide bears an unusual 2-methyl-cinnamoyl moiety (2-MeCin) (**Figure 15**) and showed activity against Gram-positive bacteria and fission yeast. The macrocyclic peptide of cinnapeptin composes 10 AAs, with the 2-methyl-cinnamoyl FA attached to the amine of D-*a*Thr1. Cinnapeptin is synthesised by type II PKS/NRPS hybrid encoded by a BGC of ~60 kb. The 2-methylcinnamoyl group is proposed to be synthesized by type II PKSs, while three NRPS enzymes, Cip22-24, are responsible for assembly of the depsipeptide. β-hydroxylation of Leu6 and Leu10 is putatively performed by the P450 Cip25 in a similar manner to P450sky in skyllamycin biosynthesis.[276] Curiously, Zhang *et al.* also discovered an

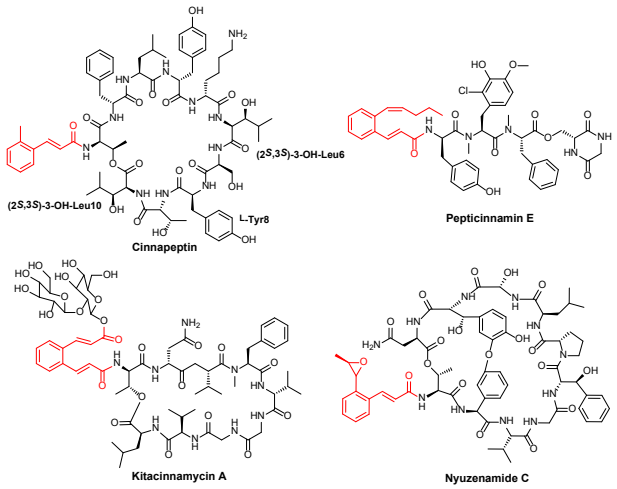

Figure 15. Chemical structures of cinnamoyl-containing lipopeptides (CCLPs).

isomer of cinnapeptin, eudistamide B, from a marine *Streptomyces* sp. WMMB 705.214 that differs in the absolute configuration of specific residues (**Figure 15**). Eudistamide B contains D-Tyr at residue 8 (rather than L-Tyr in cinnapeptin) and (2*S*, 3*R*)-3-OH-Leu moieties at residues 6 and 10 (rather than (2*S*, 3*S*)-3-OH-Leu moieties in cinnapeptin).[277]

Pepticinnamin. First isolated from *Streptomyces* sp. OH-4652, Li *et al.* recently identifying the BGC (*pcm*) in a different producer Actinobacteria bacterium OK006 by using protein sequence of Sky28 as query in a BLASTP search.[278] Pepticinnamin E contains a pentenyl cinnamoyl FA as well as 3 nonproteinogenic amino AAs, including D-Tyr, a *N*-methyl-2-chloro-4-*O*-methyl-3,4-dihydroxy-L-Phe (*N*-Me-2-Cl-4-*O*-Me-L-DOPA), and a *N*-methyl-L-Phe (*N*-Me-L-Phe). The C-terminus of pepticinnamin also bears an ester-linked diketopiperazine moiety (**Figure 15**). The gene cluster of pepticinnamin E (*pcm*) contains two NRPS-encoding genes: *pcm2* and *pcm30*, comprised of five modules for pentapeptide assembly. Module 2 in Pcm2 and 3 in Pcm30 each contain a methyltransferase domain, consistent with the *N*-methyl groups present in the backbone of the pepticinnamin E. Tailoring enzymes (Pcm1, Pcm10 and Pcm11) are predicted to be involved into the formation of the nonproteinogenic AA 2-Cl-4-*O*-Me-L-DOPA. Pcm10 is a promiscuous methyltransferase that regioselectively catalyses the *O*-methylation of the 4-hydroxyl of L-DOPA, as well as the methylation of a range of catechol derivatives.[279] The promiscuity of Pcm10 toward modified catechols suggests that methylation through the action of Pcm10 could occur with substrates (L-DOPA or 2-chloro-L-DOPA) loaded onto Pcm2 (the 2nd T domain) as T-tethered thioesters.

Kitacinnamycin A. Ge *et al.* employed a genome mining approach targeting both NRPS and type II PKS to discover new CCLPs in bacterial genomes by focusing on the conserved proteins KS, KR, and isomerase as query enzymes. Fifty-one BGCs were identified in a genome neighborhood network (GNN), suggesting that clusters for CCLPs are widespread in microorganisms.[242] Many clusters contain functionally distinct genes for post-assembly modifications, indicating that such CCLPs have rich biosynthetic diversity and great potential for exploitation. By strain prioritization and dereplication, researchers activated a gene cluster in *Kitasatospora* sp. CGMCC 16924 and isolated 14 novel cinnamoyl-containing non-ribosomal peptides, the kitacinnamycins A-N (**Figure 15**). Among them, kitacinnamycin H significantly promoted IFN-β production, exhibited promising STING activation activity. The biosynthetic pathway of kitacinnamycins was elucidated, containing three NPRS enzymes Kcn21, Kcn22 and Kcn23, and a P450 Kcn27 catalysing the sequential three-step oxidation of the terminal methyl group of the cinnamoyl moiety in a carboxylate. Glycosyltransferase Kcn28 catalyses the glycosylation of the cinnamoyl group. A crystal structure and mutagenesis suggest that Kcn28 has broad substrate selectivity and has a potential role in biosynthetic engineering.[242] In 2022, the Ge group successfully *in vitro* reconstituted the biosynthesis of the cinnamoyl group in kitacinnamycin. It was demonstrated that the protein complex Kcn17-Kcn18-Kcn19 catalyses the 6π-electrocyclization and dehydrogenation of C_{12}-pentene substrates to form benzene rings. They also demonstrated that the protein complexes Pep21-Pep22-Pep23 and Cal10-Cal11-Cal12 are responsible for the formation of the cinnamoyl part of the benzene ring in the biosynthesis of pepticinnamin and WS9326A, respectively.[280]

Nyuzenamide. These bicyclic lipopeptides were isolated from *Streptomyces* originated from deep-sea water. Their bicylic structures comprise 10 AAs and with aromatic FAs. Antifungal activity and cytotoxicity were observed for both compounds.[281] Nyuzenamide C, discovered from *Streptomyces* sp. DM14, possesses a bicyclic backbone composed of 10 AAs including the same 4 nonproteinogenic AAs as nyuzenamides A and B: hydroxyglycine, β-hydroxyPhe, Hpg and β-dihydroxyTyr, together with a 1,2-epoxypropyl cinnamic acid (**Figure 15**). Nyuzenamide C showed antiangiogenic activity induced by the vascular endothelial growth factor in human umbilical vein endothelial cells, and further induced quinone reductase activity in a murine hepatoma cell line. *Streptomyces* sp. DM14 was sequenced to annotate the biosynthetic pathway of nyuzenamide C. Three NRPSs (DmlA, DmlD and DmlE) composed of 10 modules were identified, and gene products *dmlO-R* (KSs), *dmlM* (reductase), *dmlB* and *dmlN* (dehydratases), *dmlL* (isomerase) and *dmlF* (P450) implicated in the biosynthesis of the 1,2-epoxypropyl cinnamic acid moiety.[282]

2.7 Lipopeptides obtained by heterologous production in *Streptomyces*

In the recent decade, new technologies have been applied to exploit bioactive natural products possessing novel structures and modes of action. Genomes of environmental microorganisms can now be acquired by metagenome sequencing without culturing, allowing the BGCs of novel lipopeptides to be identified by bioinformatic analysis with the

aid of platforms such as antiSMASH and BIGSCAPE.[283, 284] BGCs of interest can be cloned using DNA cloning technologies or *de novo* synthesis, with candidate compounds obtained by expression of BGCs in heterologous host. *Streptomyces* strains have been developed as efficient genetic platforms for the heterologous production of lipopeptides in combination with direct cloning technology. Recently, examples of newly identified lipo(glyco)peptides through heterologous expression of biosynthetic pathways have been reported in *Streptomyces*.

Malacidins and cadasides. Metagenome analysis suggests that there is a vast array of lipopeptide natural products encoded by the global soil metagenome, most of which are probably uncharacterised. Two classes of acidic lipopeptides were obtained by heterologous expression of BGCs cloned from soil metagenomes. Using CDA sequence tags and searching for Asp-incorporating A-domains, two novel CDA BGCs (*mlc* and *cde*) were identified, cloned by transformation-associated recombination (TAR) and expressed in *S. albus* J1074, leading to the production of the malacidins and cadasides.[285, 286]

Malacidins A and B consist of the same ten-membered peptide core, with the FPA differing by one methylene group. 9 AAs comprise the cyclic core, with an amide linkage between 2,3-diaminobutyric acid (MeDap) and 4-methylproline (MePro) (**Figure 16**). The nonproteinogenic AA MeDap is putatively formed by the cysteine synthase MlcS and argininosuccinate lyase (MlcT) encoded by the *mlc* gene cluster. The proteins MlcP (a coenzyme F420-dependent N5, N10-methylene tetrahydromethanopterin reductase), MlcQ (alcohol dehydrogenase) and MlcR (L-proline 4-hydroxylase) are hypothesised to be responsible for the biosynthesis of MePro, whilst a pair of methylaspartate mutases MlcE and MlcF are likely involved in the synthesis of the two MeAsp residues.

The structures of cadaside A and B consist of 13 AAs and an unsaturated FA. 4 nonproteinogenic AA, 3-amino-2-methylpropionic acid (AMPA), D-Ile, β-hydroxyaspartic acid (β-hyAsp), and γ-hydroxyglutamic acid (γ-hyGlu) have been identified in cadaside, with the nine-membered depsipeptide ring formed between L-Thr and Sar (**Figure 16**). The dehydrogenase CdeT, monooxygenase CdeA and hydrolase CdeB encoded by the *cde* gene cluster transform thymine into AMPA, as confirmed by feeding experiments using isotopically labelled precursors. Formation of β-hyAsp and γ-hyGlu is linked to the activity of CdeN, a putative 2-ketoglutarate-dependent dioxygenase. L-*a*Ile was previously found to be generated from L-Ile by DsaD / DsaE and MfnO / MfnH in the biosynthesis of desotamide and marformycin;[287] as comparable enzymes CdeO (aminotransferase) and CdeP (isomerase) are encoded by the *cde* gene cluster, the D-Ile residue in the cadasides is putatively derived from epimerisation of L-*a*Ile.[285] CdeE (acyl-CoA synthase), CdeF (acyl-CoA dehydrogenase), CdeG (acyl-CoA dehydrogenase), and CdeH (T domain) are predicted to be involved in biosynthesis of the unsaturated FA.[285] Screening the cadasides uncovered potent activity against Gram-positive bacteria in a calcium-dependent manner, albeit requiring a higher calcium concentration than daptomycin. Iron-chelating capacity was found for cadaside A, which could be attributed to the multiple hydroxy acid residues found in its structure.

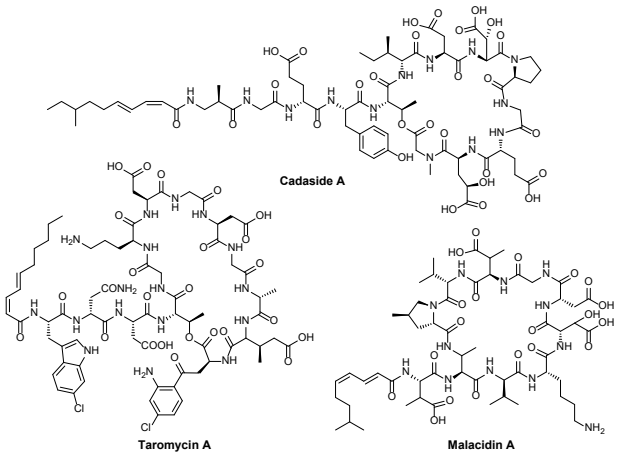

Figure 16. Lipopeptides obtained by heterologous production in *Streptomyces*.

A47934 derivatives. Banik *et al.* cloned six tailoring enzyme-rich BGCs of glycopeptide antibiotics (GPAs) from soil DNA megalibraries. Through combinatorial expression of the BGCs in the producer of compound A47934, *S. toyocaensis*, several sulfated A47934 derivatives were obtained.[288] These GPAs exhibited comparable activity against *S. aureus* to that of the teicoplanin aglycone. Owen *et al.* applied an arrayed metagenomic libraries screening approach targeting on A/KS domains and identified two further GPA BGCs. Expression of a GPA BGC in desulfo-A47934 producer *S. toyocaensis*:*ΔStaL* led to production of two novel derivatives of desulfo-A47934.[289]

Taromycin A. Based on the transformation-associated recombination (TAR) cloning method and the natural homologous recombination system in *Saccharomyces cerevisiae*, a silent BGC from the actinomycete *Saccharomonospora* sp. CNQ-490 was directly cloned and expressed in *S. coelicolor*, leading to discovery of taromycin A.[290] The chemical structure of taromycin is similar to daptomycin, whilst including 3 different AAs and alterations to the FA (**Figure 16**).

3. Biological function of lipopeptides from *Streptomyces* as antibiotics

The high potency and low toxicity of lipopeptides lend themselves to the development of antimicrobial agents. The largest number of anti-microbial peptides have been found to act on cell wall synthesis and β-1,3-glucanase.[291] The following sections only highlight current insights into the underlying mode of actions and bioactivities of lipopeptides from *Streptomyces*. Here, we mainly review the understanding about the mechanism of daptomycin, as well as investigations into the mode of action about other lipopeptides recently found from *Streptomyces*.

3.1 Daptomycin.

Daptomycin is the only clinically used CDA-type lipopeptide. Despite its important therapeutic value, the study of the mechanism of daptomycin has been fraught with challenges. Several excellent literatures have been reported about the series of investigations on its action of mode. Previous studies have generally proposed three possible models of action for daptomycin as antibiotic.[292] In 1990, it was reported from studies using *B. megaterium* that the molecular target of daptomycin maybe related to cell wall metabolism, with possible targets including the formation of glucosamine 6-phosphate and UDP-N-acetylglucosamine, which affects peptidoglycan biosynthesis in the cell wall.[293] However, no specific molecular target was identified in terms of cell wall biosynthesis. Afterwards, studies uncovered that daptomycin oligomerizes on the liposomal and bacterial membranes, affecting cell wall production and ultimately leading to cell death.[294] It has also been hypothesized that daptomycin facilitates the permeabilization and depolarization of the bacterial cell membrane.[295]

Further investigations suggested that calcium ions (Ca^{2+}) are essential for daptomycin binding to bacterial membranes, serving as a bridge between daptomycin and lipid headgroups.[296] In addition, phospholipid phosphatidylglycerol (PG) in bacterial membranes was demonstrated by several studies to be a determinant for daptomycin activity, with daptomycin bioactivity shown to be related to the expression level of PG in the cell membrane.[297] In the presence of Ca^{2+}, daptomycin forms micelle-like structures, the positively-charged DAP-Ca^{2+} aggregates then interacting with the negatively charged PG in the cell membrane, the interaction that results in oligomerization of DAP molecules in the outer membrane leaflet and a conformational change. Subsequently, DAP oligomers translocate to the inner membrane leaflet, producing a pore-like complex and resulting in bacterial cell death .[298] Recently, Nakamuro *et al.* investigated the self-organization mechanism of DAP based on dynamic imaging. They discovered that the tetramer is the biggest oligomer in homogeneous aqueous solution, and the DAP molecule clusters was enhanced by the presence of Ca^{2+} ions.[299]

In 2016, the Schneider group investigated the biological model of daptomycin in *B. subtilis*, revealing that daptomycin decreased the membrane potential but no distinct membrane holes were detected during this process. They found that daptomycin affects cell wall formation whilst detecting no indication of altered membrane curvature.[300] Moreover, their research showed that daptomycin activates the rearrangement of the liquid/lipid microstructure domain of the *B. subtilis* cell membrane, leading to progressive depolarization of the cell membrane. As a result, the crucial peripheral membrane-associated lipid II synthase MurG and the phospholipid synthase PlsX, both of which preferentially colocalize with fluid lipid membrane microdomains, were rapidly delocalized. In this regard, MurG play an important role during the biosynthesis of peptidoglycan precursor lipid II. Thus, the delocalization of these proteins likely a key reason why daptomycin blocks cell wall biosynthesis.[300] In 2020, the same group further reported

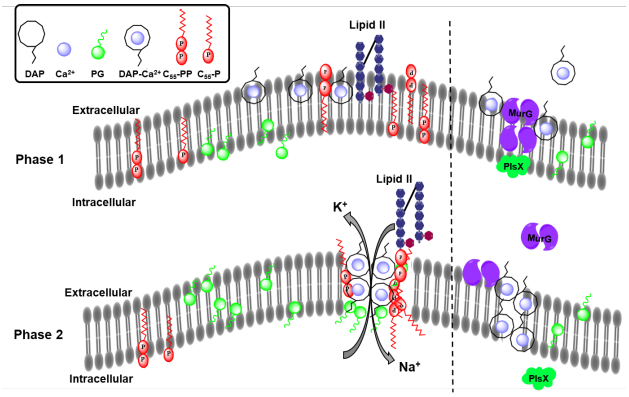

Figure 17. The schematic of exhibiting the action mechanisms of daptomycin. Ca^{2+}-DAP forms a tripartite complex with the cell wall building block lipid II (including the undecaprenyl diphosphate, C55-PP), the cell envelope precursors undecaprenyl phosphate (C55-P), and the phospholipid phosphatidylglycerol (PG).

that Ca^{2+}-daptomycin tends to oligomerize and localize at the division septum, which is enriched in anionic lipids, notably the PG and the undecaprenyl phosphate-coupled cell wall precursors. In the presence of the phospholipid phosphatidylglycerol (PG), Ca^{2+}-daptomycin forms a tripartite complex with the cell wall lipid II and the undecaprenyl phosphate ($C_{55}P$).[292] This tripartite complex then triggers delocalization and blocks cell wall synthesis (**Figure 17**). These investigations therefore provide a concise explanation for daptomycin's mechanism.

Daptomycin resistance is a complicated phenomenon that requires discussion, with many articles available that summarize the mechanisms of daptomycin resistance.[301, 302] Previous investigations suggested that the mechanisms of DAP resistance are varied and complicated in different system, mainly related with composition alteration of the bacterial cell membrane and cell wall composition.[291, 303] Many investigations have shown that daptomycin resistance is linked to the changes of phospholipid phosphatidylglycerol, with the quantity of phosphatidylglycerol (PG) in the cell membrane affected by changes in cell membrane phospholipid metabolic enzymes.[304, 305] Calcium binding behavior with daptomycin is strongly dependent on the presence of PG, and the amount of PG in the cell membrane is primarily controlled by pathogenic bacteria altering the activity of key membrane phospholipid metabolism enzymes such as MprF, RpoB, RpoC, YycG, Cls, CdsA and PgsA, or by influencing the release of PG into the extracellular, thereby finally reducing the affinity of daptomycin for the cell membrane.[298, 306-309] In addition, research has showed that cell signalling pathways are also important determinants on the composition and charge of the cell wall, playing a role in regulating cell wall homeostasis and therefore causing a reduction in daptomycin susceptibility.[310] Typically, daptomycin resistance occurs as the result of bacterial integration of various resistance mechanisms.[311]

3.2 Other lipopeptides.

To combat microbial resistance, in recent years there has been increasing interest in the discovery and production of novel

antimicrobial agents from numerous sources. As a result, greater attention has recently been paid on investigating the antibacterial actions of lipopeptides. This section introduces recent investigations of lipopeptides regarding their antibacterial activity.

Antibiotic action of lipopeptides typically involves the breakdown of cell membrane. The amphomycin class antibiotics inhibit peptidoglycan biosynthesis in Gram-positive bacteria, as amphomycin forms Ca^{2+} dependent complexes with undecaprenyl phosphate (C_{55}-P) to block the formation of lipids I, II, and III.[312] Tsushimycin is an amphomycin derivative comprised of a cyclodecapeptide core, an exocyclic amino acid and a fatty-acid residue. As a CDA-type lipopeptide, tsushimycin displays a similar mechanism to that found with daptomycin. The crystal structure of tsushimycin suggests that its bioactivity probably relate with bacteria cell membranes through the interact with fatty acid chain (**Figure 18**).[85]

MX-2401 is a semisynthetic lipopeptide that comprises a chemically modified amphomycin core attached with an aromatic linker and C_{12} lipid side chain, which is in preclinical development for the treatment of Gram-positive infections, including hospital-acquired pneumonia and antibiotic-resistant strains.[313] Interestingly, although MX-2401 belongs to the same Ca^{2+}-dependent lipopeptide antibiotic family, its mode of action is different from daptomycin, with MX-2401 only causing slow membrane depolarization at high concentrations and not affecting general membrane permeability. Instead, MX-2401 inhibits peptidoglycan synthesis by binding to the undecaprenyl phosphate (C_{55}-P). This interaction results in the biosynthesis inhibition of the cell wall precursors lipids I/II and the wall teichoic acid precursor lipid III, while daptomycin has no significant effect on these processes.[314] Laspartomycin C belongs to the family of calcium-dependent antibiotic lipopeptides and contains the conserved Asp-X-Asp-Gly motif. Studies have shown that the target of Laspartomycin C is also phospholipid C_{55}-P, and therefore this antibiotic also inhibits the biosynthesis of the bacterial cell wall precursor lipid II (**Figure 18**).[315, 316]

Telomycin possesses a new antibacterial mode of action dependent on the bacterial phospholipid cardiolipin, which is a phospholipid dimer found in the lipid membranes and the inner membranes of mitochondria.[317] The improved activity of acylated telomycin is likely attributed to stronger interactions with bacterial lipid membranes and cardiolipin (**Figure 18**).

Arylomycin exhibits narrow-spectrum antimicrobial activity only against Gram-positive bacteria. It acts through inhibition of type I signal peptidase (SPase) which is an essential protein in the general secretory pathway in bacteria (**Figure 18**).[318] Interestingly, G0775, an improved synthetic arylomycin analogue, was transformed into a novel molecular target for the treatment of multidrug-resistant (MDR) Gram-negative bacterial infections.[319] G0775 targets the periplasmic signal sequence protease LepB, which is an essential bacterial type I signal peptidase that cleaves signal peptides from proteins exported across the cytoplasmic membrane (**Figure 18**).[319]

Enzyme MraY (phospho-*N*-acetylmuramoyl-pentapeptide transferase or translocase I) links UDP-MurNAc-pentapeptide to

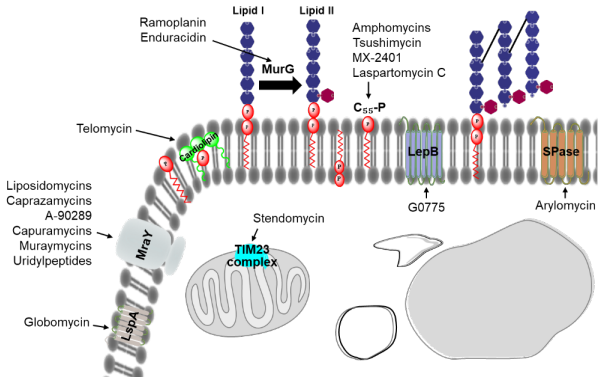

Figure 18. Molecular targets of lipopeptide antibiotics discovered from *Streptomyces*.

the C_{55} lipid carrier during bacterial cell wall biosynthesis, which is essential for the bacterial peptidoglycan assembly. MraY is the target of many of liponucleoside antibiotics including the liposidomycins, caprazamycins, A-90289, capuramycins, muraymycins and uridylpeptides (**Figure 18**).[235, 320-324]

The target of both ramoplanin and enduracidin involves inhibition of the transglycosylation step of peptidoglycan biosynthesis. Ramoplanin and enduracidin both inhibit MurG and the bacterial transglycosylase PBP1b by binding to their substrates, Lipid I and Lipid II, respectively.[325]

Globomycin specifically inhibits the function of lipoprotein signal peptidase (LspA) by blocking the post-translational modification of lipoproteins that are essential for bacterial growth, nutrient absorption, virulence, and immune evasion, leading to the accumulation of prolipoprotein in the inner membrane, ultimately resulting in cell lysis and death (**Figure 18**).[326-330] LspA removes the signal peptide of prolipoprotein by cutting the consensus motif (Leu-Ala-Gly-Cys) in the lipobox of the diacylglycerol-prolipoprotein. The Leu-Ile-Ser tripeptide of globomycin, which substitutes the first three residues of the lipobox, accesses the active site of catalytic dyad aspartate residues (Asp124 and Asp143) of LspA, allowing globomycin to mimic a non-cleavable tetrahedral analogue and thus exert an inhibitory function.[330] In addition, one study has also elucidated that globomycin showed LspA-independent antibacterial activity against *M. tuberculosis*, indicating that additional mechanisms and new targets still exist to be discovered.[331]

The antifungal lipopeptide stendomycin has recently demonstrated as a specific inhibitor of TIM23 complex located on the mitochondrial membrane, which is necessary for the stabilization of PINK1 on the outside of mitochondria to initiate mitophagy upon membrane depolarization in yeast and mammalian cell.[332]

It was recently proven that molecular dynamics simulation is a powerful tool for characterizing these atomic-level interactions processes between lipopeptides and cell membranes.[333, 334] Lipopeptides polymyxin B1 was previously assumed to kill bacteria through permeating and disrupting the membrane. Berglund *et al.* preformed a molecular dynamics simulation on the interaction of polymyxin B1 with the cell membranes of *E. coli*. The simulation results showed that polymyxin B1 possibly

interacts with the membranes through different mechanisms.[333] Those atomic level analysis provide in-depth insights into the mechanistic details and is helpful for rational lipopeptide drug development.

Lipopeptides from *Streptomyces* have a variety of biological functions in addition to antibacterial action. Rotihibin A acts as a TOR kinase signaling inhibitor, leading to damages during cell division that results in suppression of *Arabidopsis* growth.[198] Skyllamycin selectively inhibits the binding of PDGF and blocks phosphorylation of the PDGF beta- receptor.[266] Azinothricin-type molecules is potent E2F/DP transcription factor inhibitors. A83586C is the most potent inhibitor of this β-catenin/TCF4 transcriptional activating interaction identified is of major biological significance.[335, 336] Ilamycins exhibited strong cytotoxic activity against several cancers including breast cancer cell line MCF7. Studies have shown that the ilamycins are an IL-6/STAT3 inhibitor for the treatment of Triple-negative breast cancer.[337]

4. Synthesis and structural modification of lipopeptides

4.1 Chemical and chemoenzymatic synthesis of lipopeptide from *Streptomyces*

Lipopeptides can be obtained from large-scale bacterial fermentation or via chemical synthesis. Fermentation is available for naturally occurring and genetically engineered lipopeptides, whilst chemical synthesis enables access to desired alternate molecular structures, for example to provide standards for biological activity tests, enantiomers to elucidate the absolute configuration of natural lipopeptides, or generating analogue libraries for the study of structure-activity relationships. Conventionally, chemical preparation of lipopeptides usually includes 4 steps: (1) synthesis of nonproteinogenic amino acids and their *N*-protected derivatives; (2) solid-phase synthesis of the peptide precursor; (3) preparation and installation of the fatty acyl moiety; and (4) macrocyclization of the linear peptide with removal of remaining protecting groups. In this section, the four steps will be reviewed separately.

Preparation of nonproteinogenic amino acids and *N*- or *O*-protected derivatives

Kyn and 3-mGlu (Datptomycin) – Daptomycin contains two non-proteinogenic amino acids: Kyn and 3-mGlu. L- and D-Kynurenine can be obtained by ozonolysis of *N*-acetyl-tryptophan, albert in low yields as well as the generation of few impurities (**Figure 19**).[338, 339] In 2012, Martin *et al.* reported a concise and practical synthesis of Kyn, which is scalable and provides ready access to either L- or D-Kyn starting from L- or D-tryptophan, respectively.[340] Cbz-protected β-3-oxindolylalanine **1** was first generated from Trp, and treatment of **1** with basic aqueous solution gave Cbz-Kyn **2**, which can be purified by conventional silica gel chromatography. Removal of the protection group and addition of an equimolar quantity of

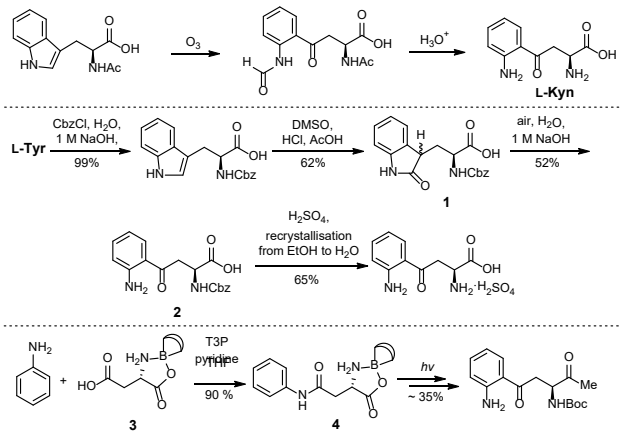

Figure 19. Chemical synthesis of kynurenine.

sulfuric acid afforded pure kynurenine sulfate following recrystallisation (**Figure 19**). Li *et al.* suspected that decomposition of the electron-rich indole moiety led to lower yields during the ozonolysis of tryptophan when compared with an electron-poor indole. They employed Fmoc-Trp(Boc)-OH for ozonolysis, providing Fmoc-Trp(Boc, CHO)-OH in quantitative yield (99%).[341] However, direct coupling of Fmoc-Kyn(Boc, CHO)-OH with resin-linked threonine-OH failed to give a desired dipeptide. Instead, they synthesised the dipeptide Fmoc-Thr[O-Trp(Boc)]-OH as the building block for peptide synthesis prior to ozonolysis (**Figure 20**). Besides the ozonolysis of Trp, a method to prepare L-Kyn using an aza-Fries reaction was recently reported (**Figure 19**).[342] Coupling of aniline and oxazaborolidinone **3** gives peptide **4**, which can convert to N-protected L-kynurenine via reprotection and photochemical

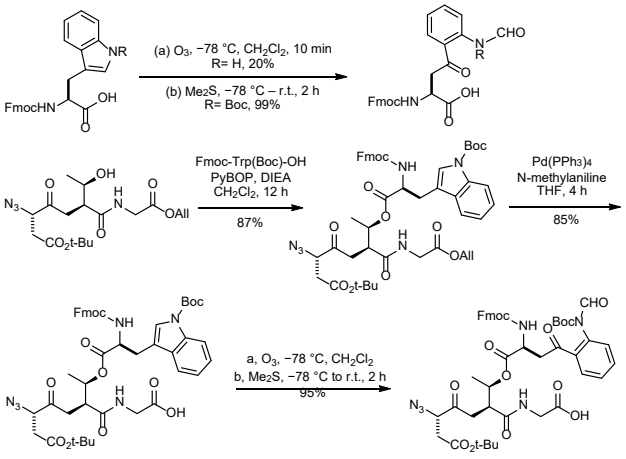

Figure 20. Chemical synthesis of kynurenine moiety from tryptophan.

aza-Fries rearrangement.

The preparation of Fmoc-3-mGlu(tBu)-OH **5**, used for SPPS, commences with compound **6**. Ring opening of **6** with LiOH generated compound **7**, with esterification of the γ-carboxylic acid of **7** via the tert-butyl bromide afforded **8**. Subsequently, the TBS group of **7** was removed, followed by oxidation with sodium periodate and ruthenium (III) chloride, to afford Boc-3-

mGlu(tBu)-OH **10** in good yield. Finally, the Boc group was replaced by Fmoc group through deprotection/protection to

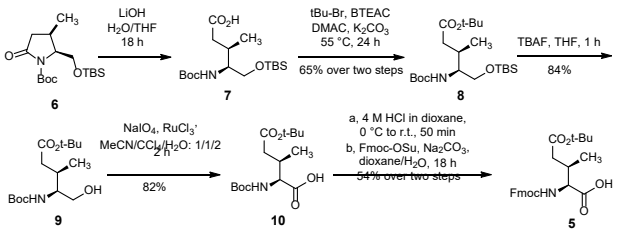

Figure 21. Synthesis of protected 3-mGlu-OH.

afford Fmoc-3-mGlu(tBu)-OH **5** for Fmoc-SPPS (**Figure 21**).[341, 343] Recently, Taylor *et al.* reported a 6-step enantioselective synthesis of (2*S*,3*R*)-3-alkyl/alkenylglutamates, including (2*S*,3*R*)-3-methylglutamate derivatives **5**, protected for Fmoc SPPS. The route started with a two-step synthesis of Fmoc Garner's aldehyde followed by a Horner–Wadsworth–Emmons reaction to give the corresponding Fmoc Garner's enoate in

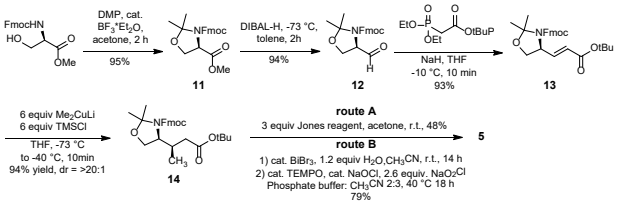

Figure 22. Synthesis of protected 3-mGlu-OH from serine derivatives.

excellent yield (**Figure 22**).[344]

MeO-Asp and HO-Asn (A54145) - A54145 contains both methoxy-aspartic acid (MeO-Asp) and hydroxy-asparagine (HO-Asn) as building blocks. In 2019, Li *et al.* confirmed the structure of A54145 (consisting of L-3S-HO-Asn and L-3R-MeO-Asp) by total chemical synthesis.[345] For Fmoc-SPPS, they synthesised the fully protected L-MeO-Asp and L-HO-Asn diastereomers. They developed an 8-step strategy to afford both diastereomers of L-Fmoc-HO(TBS)-Asn(Trt)-OH (**15a** and **15b**) at gram-scale, using a three-component coupling Passerini reaction. For synthesis of the diastereomeric L-Fmoc-MeO-Asp(OtBu)-OH building blocks **16a** and **16b**, they developed a 7-step route starting with the dihydroxylation of the commercially available alkene **22** to afford compound **23** as a pair of epimers in 3:2 ratio. Dihydroxylation using Sharpless asymmetric dihydroxylation next provided a single diastereomer. The primary alcohol of diol **23** was selectively protected with TBS, followed by methylation of the secondary hydroxyl group to yield the corresponding methyl ether epimers **25a** and **25b** that could be separated by chromatography. Both epimers were then transformed into the desired carboxylic acid **16a** and **16b** using a four-step cascade (**Figure 23**).

L-MeDap, L-HyAsp and MePro (Malacidin A) - Malacidin A consists of a cyclic nonapeptide linked to an unsaturated C_9-FA that is comprised of 5 non-proteinogenic AAs: β-methylaspartic acid at position 1 and 8 (MeAsp-1 and 8), β-methyldiaminopropionic acid-2 (MeDap-2), β-hydroxyaspartic acid-5 (HyAsp-5) and γ-

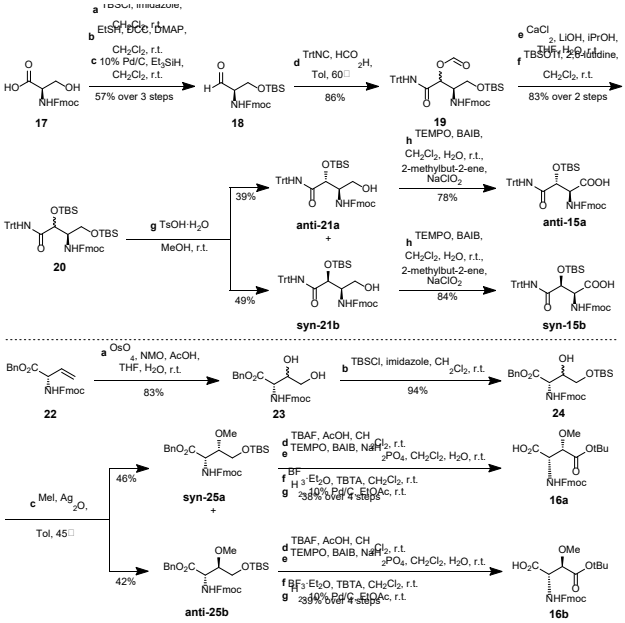

Figure 23. Chemical synthesis of protected HO-Asn and MeO-Asp.

methylproline-10 (MePro-10). To verify the β-stereochemistry of L-MeDap-2 and L-HyAsp-5, Li *et al.* synthesised four possible diastereomeric structures of malacidin A. First, they developed a concise synthetic route to prepare **26a** or **26b** in five steps, starting from the commercially available building block L-aspartic acid.[346] The critical step in this synthesis is the stereo-selective hydroxylation of **28a** by KHMDS/(-)-CSO or LiHMDS/(+)-CSO. Beyond this, the scaffold of MeAsp **33a** and **33b** was generated from trityl protected **28a** or **28b** by non-selective methylation. After hydrogenolysis of MeAsp, each diastereomeric product could then be separated by column

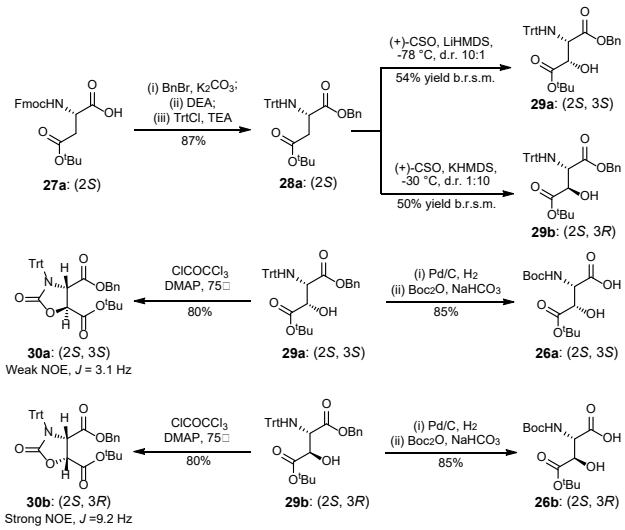

Figure 24. Chemical synthesis of β-hydroxyaspartic acid derivatives.

chromatography **(Figure 24)**.

The synthesis of (2*S*, 4*R*)-methyl proline **34** exploited a combination of Li's and Pedregal's method,[347] starting with pyroglutamic acid **35**. **35** was protected and then methylated at

its γ-position to generate the (2*S*, 4*R*) intermediate **37,** which underwent a chemoselective amide reduction and manipulation of protecting groups to afford the desired

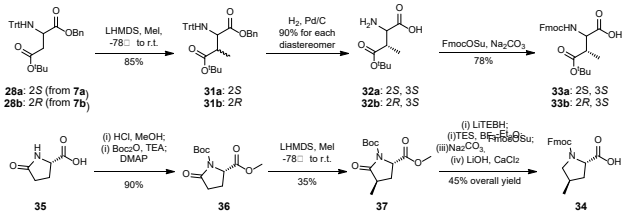

Figure 25. Chemical synthesis of β-methylaspartic acid and γ-methylproline.

compound **5** (**Figure 25**).[346]

Solid-phase synthesis of peptide precursors

Fmoc solid-phase peptide synthesis (Fmoc-SPPS) is the most widely applied method to generate peptide ligation for lipopeptide synthesis. In SPPS, the α-amino group of amino acid building blocks are temporarily protected. The first protected amino acid residue is coupled through its Cα-carboxylic acid group with the resin to initialize the peptide. Following the coupling of the first residue to the resin, the temporary N-terminal protecting group is removed to provide a free amino group. Addition of the subsequent activated, Nα-protected amino acid then reacts with the resin-bound amino group to generate a peptide bond, with multiple steps of this cycle leading to the growth of the peptide. Resins, solvents, coupling reagents, temperature, synthesis scale, and even the order of synthesis of different peptide bonds can be altered to achieve high yields for difficult sequences. In 2015, Hansen and Oddo reviewed the applications and limitations of Fmoc-SPPS in peptide synthesis, and also provided very detailed protocols.[348] In this section, we will discuss examples of lipopeptide synthesis using Fmoc-SPPS.

The fellutamides are a group of marine-derived lipopeptides containing a C-terminal aldehyde moiety. Their SPPS synthesis began with a Weinreb amide-derived resin that underwent peptide extension using standard Fmoc-SPPS protocols. Fatty acyl chains were then incorporated prior to deprotection and the resulting resin-bounded peptides were with $LiAlH_4$ to afford

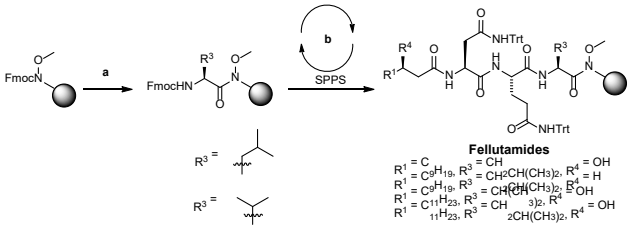

Figure 26. Fmoc solid-phase peptide synthesis of fellutamides.

the desired aldehyde peptides (**Figure 26**).[349]

Recently, Brimble and co-workers reported a novel technology of cysteine lipidation with synthetic peptides, based on the thiol-ene reaction with UV irradiation.[350, 351] First, Fmoc-Rink amide was loaded on the aminomethyl-polystyrene resin, followed by Fmoc removal and SPPS to afford the mature resin-bound peptides **38** and **39**. Peptides **40** and **41** were released

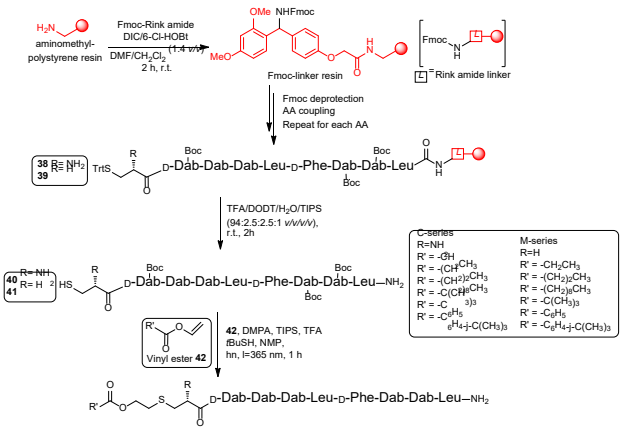

Figure 27. The use of cysteine lipidation in solid-phase peptide synthesis.

from resin and reacted with vinyl ester **42** under UV irradiation at 365 nm to directly generate different lipopeptides (**Figure 27**).

In 2016, Tulla-Puche *et al.* reported a protocol to synthesize complex head-to-side-chain cyclodepsipeptide pipecolidepsin A, which efficiently established the depsipeptidic skeleton using a fully solid-phase approach. The procedure is efficient and only takes ~2 months to complete one entire synthesis.[352]

Preparation and installation of the fatty acyl moiety

The glycinocins A–C are acidic lipopeptide antibiotics that differ in the structure of a branched α, β-unsaturated fatty acyl moiety. To synthesise the unsaturated FA, the C_8/C_{10} diol was mono-protected by the addition of a benzyl group, with subsequent Swern oxidation affording an aldehyde. Wittig olefination followed by hydrogenation introduced the isoalkyl group, and a second Wittig olefination furnished (*E*)-α, β-unsaturated esters. Finally, hydrolysis of the ethyl ester

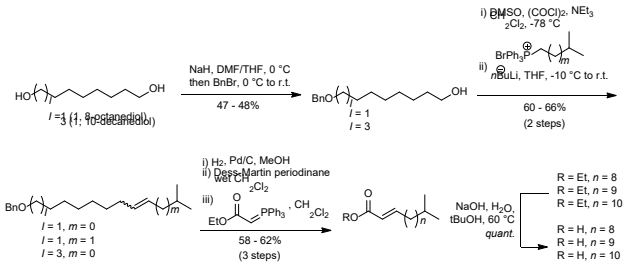

Figure 28. Chemical synthesis of α, β-unsaturated fatty acyl moiety of glycinocins.

provided the desired fatty acid for coupling (**Figure 28**).[353]

To synthesise the unsaturated fatty acid building block **43**, *trans*-iodoalkene **45** was synthesised from **44** and subsequently coupled with the terminal alkyne of 5-methyl-1-hexyne via a Sonogashira cross coupling reaction to afford **46**. The intermediate maintaining the *trans*-configuration was selectively hydrogenated using Lindlar catalyst, followed by hydrolysis of methyl ester to afford the 2*E*, 4*Z*-fatty acid **43** (**Figure 29**).

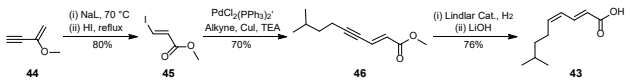

Figure 29. Synthesis of 2*E*, 4*Z*-fatty acid.

Calcium-dependent antibiotic CDA3a and its analogues bear an exocyclic peptide tail containing a serine residue and a N-terminal FA (*trans*-2,3-epoxyhexanoic acid) in addition to the cyclic decadepsipeptide core, which contains three nonproteinogenic AAs: *Z*-dehydrotryptophan, D-4-hydroxyphenylglycine (D-Hpg), and (2*R*,3*S*)-3-hydroxyasparagine (3-HOAsn). Recently, Li *et al.* accomplished its synthesis via late-stage serine ligation.[354] Asymmetric Sharpless epoxidation of the known allylic alcohol **48** provided epoxide **49**. Oxidation of **49** produced the carboxylic acid in nearly quantitative yield, which was coupled with salicylaldehyde to afford the epoxyhexanoyl salicylaldehyde ester **47** (**Figure 30**). To explore the significance of the acyl tail on the antibacterial activity of these compounds, they further designed and synthesised a library of CDA3a analogues containing different acyl chain lengths, different α, β-substitutions, or different terminal substituted acyl tails (**Figure 30**). Saturated FAs with different lengths (C_{10}, C_{12}, C_{14}, C_{16}) were coupled with salicylic aldehyde, followed by a subsequent serine ligation to install the fatty acid chain on the Ser residue of CDA core peptides to synthesise CDA analogues.[354] Some variants with different lipid tails show >100-fold higher antimicrobial activity than natural CDAs.

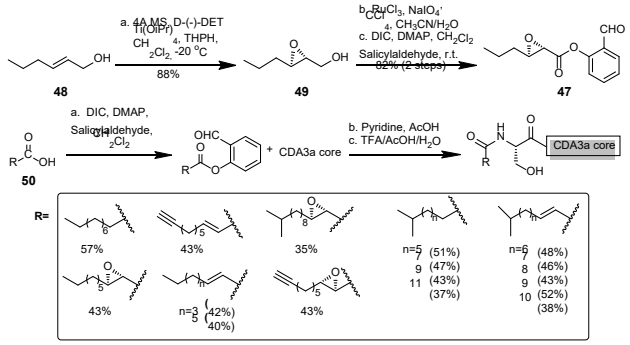

Figure 30. Synthesis of trans-2,3-epoxyhexanoyl and its derivatives.

Macrocyclisation of linear peptide and removal of protecting groups

After SPPS, the mature linear peptide is released from the resin, followed by the macrocyclisation to give the cyclic product. Suitable reagents for amide formation, like PyBOP, HATU or DMTMM·BF4, can be chosen for macrocyclisation of different substrates via amide bonds. For example, in the synthesis of CDA3a, Li *et al.* employed PyBOP/DIEA as the coupling reagents for the macrocyclisation between the residue Gly and 3-HOAsn (**Figure 31**). [365]

In 2010, Li *et al.* reported an unconventional peptide cyclisation route that relied on chemical ligation.[355] An *O*-salicylaldehyde ester was first synthesised at the peptide C-terminus, which reacted with an N-terminal serine or threonine residue to afford

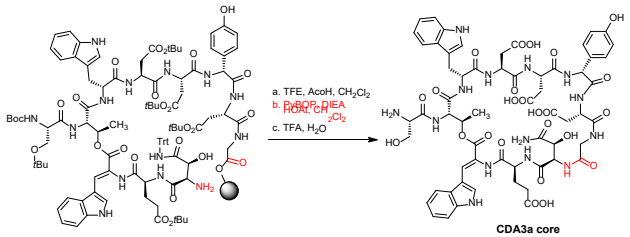

Figure 31. Macrocyclization of CDA3a.

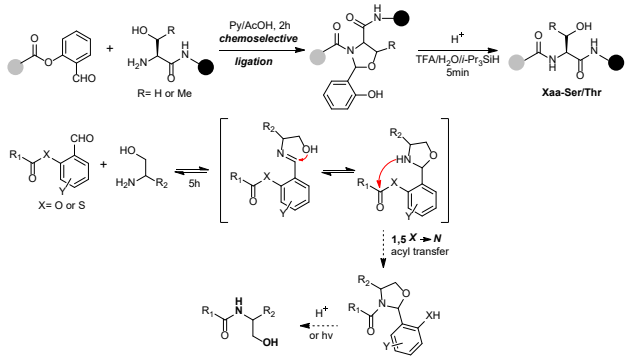

Figure 32. The route of peptide bond formation via O-salicylaldehyde ester.

a peptide ligation. They hypothesised that the benzaldehyde ester undergoes the hydroxyl addition to the imine or *O*-*N* acyl transfer to form the *N*, *O*-benzylidene acetal, which is readily removed under acidic conditions to achieve peptide bond formation. The method was successfully used for macrocyclisation in daptomycin synthesis (**Figure 32**).[341] Recently, they also employed this method for β-hydroxyaspartic acid ligation to synthesise malacidin A, and in doing so finally establishing its absolute three-dimensional structure. [346]

In 2004, Marahiel *et al.* identified a recombinant nonribosomal peptide cyclase CDA3 TE from CDA NRPS that recognized four linear peptidyl thioester substrates to provide two regioisomeric macrolactones, themselves arising from nucleophilic attack of the hydroxy group of two adjacent Thr2 and Ser1 residues onto the C-terminal carboxyl group.[54] Considering the structure of CDA is very similar to daptomycin, the promiscuous CDA3 TE was then explored as a permissive cyclisation catalyst to synthesise CDA, daptomycin and related derivatives. These experiments showed the CDA3 TE can catalyse the cyclisation of linear CDA undecapeptide thioesters with single exchanges of six daptomycin-specific residues. Simultaneous incorporation of all six of these residues into the peptide backbone and elongation of the N-terminus of CDA by

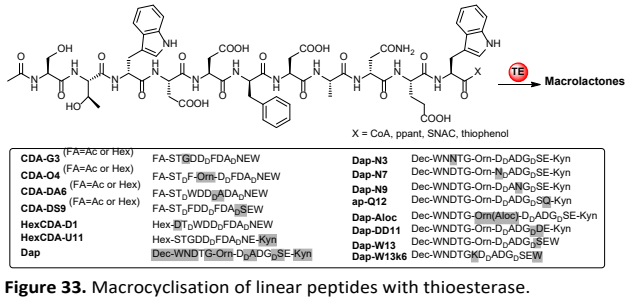

Figure 33. Macrocyclisation of linear peptides with thioesterase.

two residues yielded a daptomycin derivative that lacked only the methyl group of L-3-mGlu (**Figure 33**).[67]

Installation of the aromatic acyl moiety in lipopeptide

Research has shown that substitution of aromatic hydrophobic groups can have a significant effect on the biological activity of lipopeptides. For example, the lipo-GPA oritavancin, which is a semi-synthetic analogue of vancomycin and was approved by the FDA for the treatment of acute bacterial skin and skin structure infections (ABSSSI) in 2014. Compared with other GPAs, oritavancin has lower minimum inhibitory concentrations and retains activity against GPA-resistant bacteria.[356] Oritavancin is capable of maintaining binding affinity for the modified peptidoglycan peptide termini of vancomycin-resistant organisms, and possesses this special function by virtue of the highly hydrophobic *N*-4-(4-chlorophenyl)benzyl group and two 4-epi-vancosamine residues in the structure (**Figure 34**). Such a hydrophobic moiety increases the binding affinity of oritavancin to its lipid II target, increases interactions with the bacterial cell membrane, and inhibit transglycosylation during cell wall biosynthesis, which ultimately leads to bacterial cell death.[357]

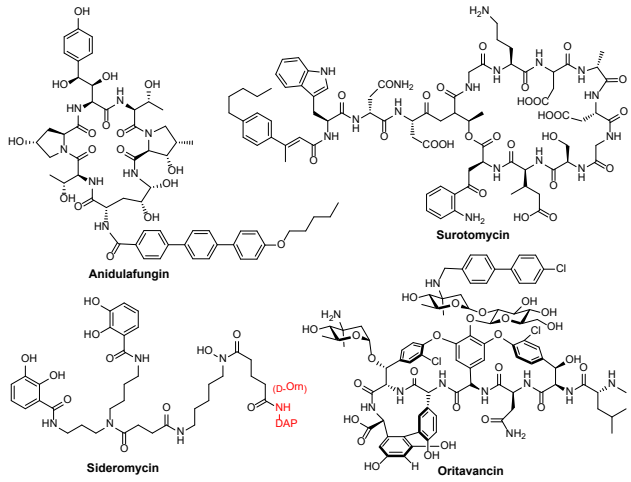

Figure 34. Representative lipopeptides with installation of the aromatic acyl moiety.

In another example, replacing the aliphatic tail in daptomycin with the aromatic ring containing acyl moiety ((*E*)-3-(4-pentylphenyl)-but-2-enoyl) results in the synthesis of surotomycin that displays better oral availability than daptomycin (**Figure 34**). Surotomycin also possesses a relatively narrow antimicrobial spectrum, with a significant improvement of MIC against *Clostridium difficile* over daptomycin. In addition, surotomycin exhibits more potent activity against daptomycin-resistant *E. faecium* and *E. faecalis*.[358]

A further example is the lipopeptide antifungal anidulafungin is a chemical semisynthetic echinocandin-like cyclic hexapeptide, which non-competitively inhibits β-(1,3)-glucan synthase. The structural modification of the triphenylene group contributes to depolarisation and permeability of cell membranes. Moreover, it is suspected that this hydrophobic substituent promotes dimer formation of the antibiotic and increases affinity.[359]

Currently, synthesis is mainly used to generate lipopeptides with aromatic-containing acyl groups, which is a time-consuming and challenging process. In addition, such syntheses involve multiple steps, leading to high economic and environmental costs.[67] For example, synthesis such as that found in surotomycin requires the use of strong acidic and alkaline reaction conditions to help complete the series of reactions including lipid removal/addition and protecting group manipulation(**Figure 35**).[358] The development of a green and economically viable microbial biosynthesis pathway for incorporation of such acyl moieties would therefore be of great significance. Many previous studies on daptomycin have demonstrated that the biosynthetic engineering of lipopeptides shows great potential. However, the biosynthetic machinery that produces aromatic fatty chains found in natural lipopeptides remains unclear. Understanding such biosynthetic processes will be of great assistance in the future development of reengineered biosynthesis pathways through the exploitation of these enzyme-enabled pathways, overcoming the limitations of chemical synthesis and opening the door to generating expanded biocombinatorial lipopeptide libraries.

In addition, lipopeptide structures could be modified through coupling with other compounds. For example, sideromycin was synthesized by conjugating siderophore fimsbactin to daptomycin (**Figure 34**), which displays high activities against different strains of Gram-negative *A. baumannii*, including MDR strains.[360]

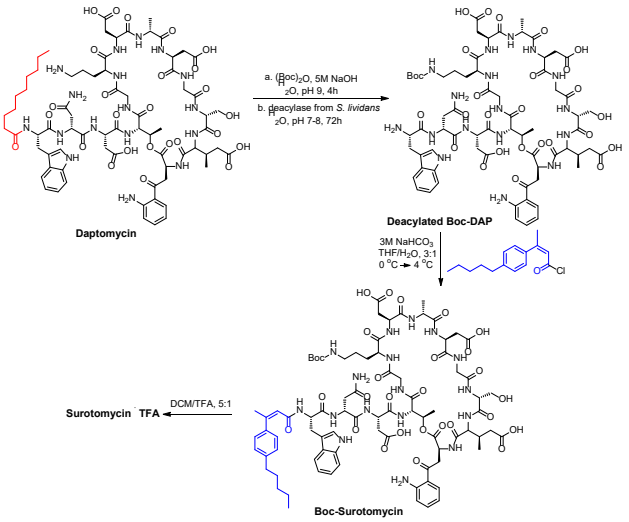

Figure 35. The semi-synthesis of Surotomycin.

4.2 Bioengineering for novel lipopeptide production in *Streptomyces*

Chemical synthesis has helped in the development of optimised novel lipopeptide analogues, but most lipopeptides are too complex for chemical synthesis and many are limited by the lack of commercial availability of key precursors. Reprogrammed biosynthesis to generate lipopeptides variants with altered and improved biological activity is a viable biosynthetic engineering approach to develop new lipopeptides. During the development

of daptomycin, a combinatorial biosynthesis strategy was explored, with Cubist Pharmaceuticals and others establishing combinatorial biosynthetic pathways to produce novel daptomycin analogues.[66] A couple of reviews have summarized these outstanding research efforts in daptomycin combinatorial biosynthesis; thus, these will not be introduced in detail.[361-363]

Here mainly discusses representative examples of lipopeptide engineering by combinatorial biosynthesis. The multi-module constitution of NRPSs and the substrate tolerance of certain biosynthetic domains offers opportunities to generate novel lipopeptides through engineering of this assembly machinery. Mutasynthesis and combinatorial biosynthesis approaches have been applied to generate novel analogues of CDA and daptomycin, with the main strategies including gene deletion of precursor biosynthesis enzymes, supplementation of alternate synthetic intermediates, modification of the amino acid selection pocket in adenylation domains, and NRPS module/domain swaps.[59, 66, 362, 364-366]

The structure modification of daptomycin was accomplished by a *trans*-complementation system, namely knocking out and replacing part of the NRPS via module/domain exchange. Miao *et al.* applied genetic manipulation (multimodule swapping and module exchange) of *S. roseosporus* to realize the biosynthesis of a bio-combinatorial library of lipopeptides via multiple and individual NRPS module exchange, resulting in modifications within the lipid chain and peptide portions of these molecules.[65, 367, 368] Likewise, engineering the active site of A-domains also enabled the activation of a wider range of amino acid substrates, which alters the resultant peptide sequence produced by the NRPS.[369]

NRPS synthetases consist of conserved repeating modular architecture, with every module harboring different catalytic domain, typically arranged in a C-A-T(-TE) architecture. Crucial to the function of all modules are linker sequences that that mediate interactions between two NRPS subunits on different proteins.[370] The roles of such linkers can be variable, with one daptomycin study showing that mutations causing amino acid substitutions, deletions or insertions in an intermodule linker had no negative effects on lipopeptide yields, whilst the linker structure that links domains is required to flexible to allow carrier protein communication with the other catalytic domains, alterations in the domain linker regions within module are often tolerated.[66, 366] Functional module exchanges based on different interdomain linkers, including the inter-module T-C linker, the inter-domain A-T and T-TE linkers, is generally applicable to NRPS or hybrid NRPS-PKS synthetase assembly lines. As previously reported by Baltz *et al.*, daptomycin analogs were generated by exchange of C-A-T, C-A-T-E or A-T units in daptomycin biosynthetic machinery.[65] Nevertheless, the recent work by the Bode group indicated that A-T-C tridomains are preferred as exchange units for engineering of NRPSs. Exchange of homologous A-T-C (or A-T-C/E) rather than C-A-T, C/E-A-T or A-T domains of NRPSs from *Xenorhabdus*, *Photorhabdus* or *Bacillus* resulted in production of a couple of novel peptides.[370] The same group further described a fusion point in C domains and put forward a concept for engineering of NRPSs by exchanging unit from the middle of C domains. With this concept, generation of novel peptides in high yield was achieved by swapping of C_{Dsub}-A-T-C_{Asub} units,[371] where Dsub and Asub stand for donator subunit and acceptor subunit of C domains, respectively. Application of this concept to lipopeptide structure modification is worthy to be tried.

Precursor-directed biosynthesis provides a powerful method to introduce chemical modifications into lipopeptide assembly. For example, precursor-directed biosynthesis of CDAs with modified 3-trifluoromethyl and 3-ethyl glutamate residues was achieved by feeding synthetic glutamate analogues to a mutant strain of *S. coelicolor* displaying impaired biosynthesis of the natural precursor (2*S*, 3*R*)-3-methyl glutamic acid.[372] Through carefully controlling the feeding of decanoic acid during the fermentation, daptomycin can be produced as the major A21978C component in the host.[28]

The antibacterial action of daptomycin and other CDAs is sensitive to the structural changes, particularly those in the macrocycle peptide scaffold, the conserved Ca^{2+}-binding motif "DXDG" and the N-terminal lipid moiety. The loss of the lipid group leads to the loss of biological bioactivity, whilst the presence of a lipophilic side chain greatly improves molecular affinity for plasma carrier proteins and the cell membranes.[373, 374] Therefore, the modification of lipid chains is a feasible method to explore in the combinatorial biosynthesis of lipopeptides, with representatives case of studies concerning A21978C and A54145 biosynthesis. Here, the mutation of the key Ser residue in the active site of CdaPS1 to Ala, led to the production of CDA products with pentanoyl as well as hexanoyl side chains when feeding different *N*-acyl-L-serinyl *N*-acetylcysteamine (NAC) thioester analogues.[55] To modify the CDA lipid moiety, Uguru *et al*. fed a precursor of *N*-pentanoyl-L-serinyl *N*-acetylcysteamine into the ΔT_1 mutant, leading to the generation of new CDA derivative displaying a pentanoyl side chain.[369]

5. Conclusion and Perspectives

In this review, we summarized the structural characteristics, distribution, biological activities and applications of lipopeptides from *Streptomyces*, together with their origin and biosynthesis. Among these compounds, the most well-studied examples are the CDA-type lipopeptides including daptomycin and CCNP-type lipopeptides. The biological activities and biotechnological interest in lipopeptides are mainly categorized into immunomodulatory, cytotoxic, emulsification, antibacterial and antifungal activities. The detailed mechanism of some representative *Streptomyces*-derived lipopeptides such as daptomycin have been described in this review.

Despite substantial breakthroughs in genomics-driven screening, finding new lipopeptide antibiotics and their biosynthesis routes is still a difficult task. The Brady team screened 10,858 bacterial genomes based on bioinformatics analysis and identified 35 groups of BGCs with the potential to produce polymyxin analogues. Based on solid phase synthesis and bioinformatic prediction strategy, macolacin, a linear decapeptides N-terminally acylated with the 6-methyl-octanoic acid, was discovered. Macolacin has inhibitory activity against

Gram-negative pathogens that express *mcr-1*, and against the drug-resistant *Acinetobacter baumannii* and the polymyxin-resistant *Neisseria gonorrhoeae*.[375] This evolutionary-based genome mining approach can also be applied to tackle other drug-resistant bacteria. In a further study, Brady's group used a similar approach to discover another new class of antibiotics, called methylnaphthoquinone-binding antibiotics (MBA) and that target methylnaphthoquinone, which plays a key role in bacterial electron transport. Menaquinone (MK) is an appealing target for antibiotic development, and MK-binding antibiotics currently is still underexplored.[376] Through sequence-based metagenomic mining, a minimum conserved menaquinone-binding motif was retrieved from databases, a class of MK-binding cyclic lipodepsipeptides were identified, and importantly also demonstrated their effectiveness against the methicillin-resistant *M. tuberculosis* and *S. aureus* infections.[377] These examples demonstrate the potential of such approaches despite the efforts required to successfully prosecute such studies.

Natural product dereplication is of great importance for exploring promising new molecules identified from microorganisms over the last decade.[378] In addition to conventional bioassay- and sequence-based approaches, omics strategies are yet further powerful bioinformatics tools that allowed us to screen the novel lipopeptide BGCs. By mass spectrometry-based proteomics screening, Bumpus *et al.* detected known NRPS systems in *Bacillus*, *Streptomyces* and environmental isolates to uncover unknown natural products from the lipopeptide BGCs, thus offering another method to explore the diversity of natural lipopeptides.[379] Recent studies have suggested that natural product discovery, screening, and dereplication can also be aided by phylogenetic approaches.[380] To detect the potential novel members of the glycopeptide antibiotics, the Wright group designed a screening method based on the phylogeny of biosynthetic genes and the lack of known resistance genes. Finally, the activity of two unusual members of glycopeptide antibiotics complestatin and corbomycin were discovered.[381]

Combinatorial synthesis techniques have been widely adopted to produce new nonribosomal peptides, functioning by changing NRPSs modules or domains, or by introducing alternate tailoring enzymes, *etc*. For example, mutasynthesis has been demonstrated to be a general and facile method for generating novel calcium-dependent antibiotics (CDAs) in *S. coelicolor*.[382] The emergence of novel gene editing technologies also has helped to address modification and innovation in lipopeptide antibiotic biosynthesis by modifying the large NRPS enzymes. For example, Micklefield and co-workers designed a gene editing tool based on CRISPR-Cas9 system to engineer the complex NRPS megasynthase responsible for the assembly of the lipopeptide antibiotic enduracidin.[383] In comparison with conventional method, the CRISPR-Cas9 gene editing technology can be used to swap subdomains within the NRPSs with much higher efficiency and accuracy, greatly accelerating this process. All in all, lipopeptide natural products occupy a very important position in the field of modern antibiotic drug development, although they still face a number of problems and challenges in their production via microbial fermentation and further in clinical use. The structural and functional characterization of lipopeptide NRPS assembly lines should be the focus of future lipopeptide biosynthesis research, as this will help us better understand this exact molecular machine and further utilize their great catalytic and bioengineering potential for development of valuable lipopeptide molecule.

6. Conflicts of interest

There are no conflicts to declare.

7. Acknowledgements

This work was supported by National Key R&D Program of China (2021YFC2102500 to F. Y.), National Natural Science Foundation of China (82104044 to S.Y.Z., 82003639 to F. Y.), Taishan Scholars Program of Shandong Province (tsqn201909049 to F.Y) and CAMS Innovation Fund for Medical Sciences (CIFMS, no. 2021-I2M-1-029 to Y.C.). This research was conducted by the Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science (CE200100012) and funded by the Australian Government.

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