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Natural products from the human microbiome: an emergent frontier in organic synthesis and drug discovery†

Saumitra Sengupta, *[a,b] Srihari Pabbaraja *[b,c] and Goverdhan Mehta *[a]

Often referred to as the "second genome", the human microbiome is at the epicenter of complex inter-habitat biochemical networks like the "gut–brain axis", which has emerged as a significant determinant of cognition, overall health and well-being, as well as resistance to antibiotics and susceptibility to diseases. As part of a broader understanding of the nexus between the human microbiome, diseases and microbial interactions, whether encoded secondary metabolites (natural products) play crucial signalling roles has been the subject of intense scrutiny in the recent past. A major focus of these activities involves harvesting the genomic potential of the human microbiome *via* bioinformatics guided genome mining and culturomics. Through these efforts, an impressive number of structurally intriguing antibiotics, with enhanced chemical diversity *vis-à-vis* conventional antibiotics have been isolated from human commensal bacteria, thereby generating considerable interest in their total synthesis and expanding their therapeutic space for drug discovery. These developments augur well for the discovery of new drugs and antibiotics, particularly in the context of challenges posed by mycobacterial resistance and emerging new diseases. The current landscape of various synthetic campaigns and drug discovery initiatives on antibacterial natural products from the human microbiome is captured in this review with an intent to stimulate further activities in this interdisciplinary arena among the new generation.

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1. Introduction

The human microbiota is home to trillions of variegated micro-organisms such as archaea, bacteria, fungi, viruses, *etc.*, which have major ramifications on human health. At the beginning of this millennium, the Human Microbiome Project (HMP) was launched as a global initiative to investigate and understand the constitutional diversity of the human microbiota, the modes of microbe–host and microbe–microbe interactions and the pathophysiological role of the human microbiome in human diseases.[1] The initial findings from this initiative reinforced the centrality of symbiotic interactions between individual microbes and microbe–hosts, especially the bidirectional communication between enteric and central nervous systems (often referred to as the "gut–brain axis"). This dynamic interplay profoundly influences human pathophysiological processes towards sound health and well-being.[2] Disruptions in this critical symbiosis, *i.e.* dysbiosis of the human microbiome, have been linked to the onset of various diseases like autoimmune disorders, dermatitis, CNS disorders, obesity, diabetes, cancer, colitis, *etc.*[3] Based on this key connection between the human microbiome and pathological states, innovative strategies in disease management *via* the controlled use of healthy human microbiota have emerged, especially in the areas of immuno-oncology and irritable bowel syndrome (IBS).[4] In this regard, the approval of two fecal microbiota transplants, Rebyota and Vowst, in quick succession in 2022 and 2023,[5] for the treatment of *Clostridioides difficile* infections are historical landmarks. These, in turn, have triggered considerable interest in the engineered or *de novo* construction of the gut microbiome for therapeutic interventions.[6]

The human microbiome operates *via* a highly complex, often personalized, mechanism for which details and understanding are scanty and work is in progress.[2] However, available evidence points towards a diverse set of cellular signalling events which are specifically mediated by secondary metabolites in maintaining the symbiotic balance between the microbes and the host.[7] This has led to intense activities and interest in the identification, biosynthesis and functions of secondary metabolites (natural products) from the human microbiome, especially in those derived from human commensal bacteria.[8,9]

[a]*School of Chemistry, University of Hyderabad, Hyderabad-500046, India. E-mail: gmehta43@gmail.com*
[b]*Department of Organic Synthesis and Process Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad-500007, India*
[c]*Academy of Scientific & Innovative Research (AcSIR), Ghaziabad 201002, India*
† Dedicated with profound respect and admiration to Prof. Sukh Dev for his inspirational leadership.

1.1 Natural products through culturomics and genome mining

Although several natural products have been isolated from the human microbiome, their isolation, in general, has faced severe challenges on account of the cryptic nature of human bacteria under laboratory culture conditions.[10] Hence, culture-independent genomic approaches, supported by computational algorithms for processing large volumes of genetic information from HMP reference genomes and interfacing with phenotypic data, were explored.[8–17] In this regard, the three commonly pursued approaches are (a) sequence-based metagenome mining, (b) functional genomics and (c) mass spectrometry-based metabolomics analysis.

(a) In sequence-based metagenome mining, applications of algorithms *viz*. ClusterFinder,[11] metaBGC[12] or PRISM,[9a] *etc*., have revealed thousands of new biosynthetic gene clusters (BGCs) from HMP data, providing the first glimpse of the biosynthetic enormity of the human microbiome.[13–15] Since the structures and bioactivities of secondary metabolites from these novel BGCs are largely unknown, attempts have been made to develop structure-prediction models, deploying the retro-biosynthetic assembly prediction and natural products chemoinformatic engines, GRAPE and GARLIC, respectively. For example, the PRISM analysis of 6009 genomes from previously cultured microbes was integrated with GRAPE and GARLIC engines (Fig. 1) which led to the identification of more than 3000 gene clusters encoding for compounds with close structural similarities to known antibiotics, thereby providing strong evidence that the human microbiome is a rich treasure of novel antibacterial natural products. It may be worthwhile to recall that soil-borne bacteria, the major source of conventional antibiotics, are rich in polyketide synthetase (PKS) & non-ribosomal peptide synthetase (NRPS) biosynthetic machineries. In contrast, the human microbiome displays a high abundance of BGCs encoding ribosomally synthesized natural products, flagging the prospect that the human microbiome may be a source of distinctly new classes of antibiotics which may be effective against the growing menace of antimicrobial resistance (AMR).[16]

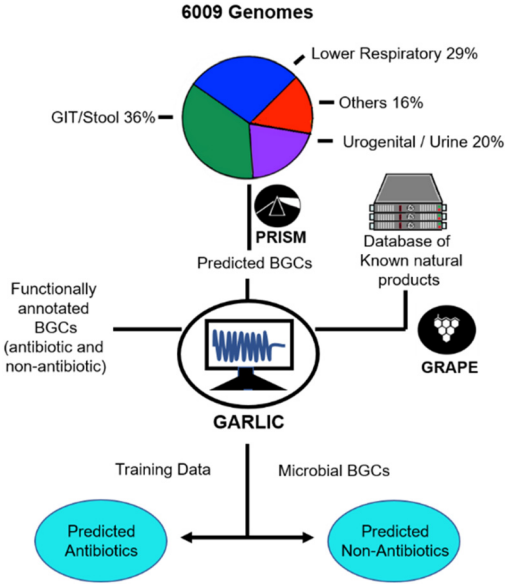


Fig. 1 Chembioinformatics platform for genome mining of the human microbiome.[9a]

(b) Functional genomics have also been explored for the identification and characterization of bioactive secondary metabolites from human microbiota.[8c,11] In this approach, metagenomic DNA libraries were constructed and screened for a phenotype of interest in an *in vitro* bioassay platform. The

Saumitra Sengupta

Saumitra Sengupta, born in Kolkata, India (1957), was trained at the University of Florida (PhD 1987 A R Katritzky), the University of California-Santa Barbara (post-doc 1987–1989 B H Lipshutz) and the University of Waterloo (post-doc 1989–1991 V Snieckus). He joined the chemistry faculty at Jadavpur University in 1991 where his research spanned Pd-catalyzed reactions of arenediazonium salts, asymmetric synthesis and functional dendrimers. He joined Aurigene Discovery Technologies in 2005 as a Vice President, Medicinal Chemistry, leading several drug discovery programs and later moved to TCG Life Sciences in 2011 as a Distinguished Scientist until his retirement in 2015. Currently, he is a chemistry consultant.

Srihari Pabbaraja

Srihari Pabbaraja obtained his PhD degree in 2004 under the guidance of Dr J. S. Yadav at the CSIR-Indian Institute of Chemical Technology from Osmania University. After completing post-doctoral studies at Rutgers, the State University of New Jersey with Prof. Spencer Knapp, he joined CSIR-IICT and started an independent career in 2008. Presently he is working as a Chief Scientist and his research interests include asymmetric total synthesis of natural products, method development for C–C bond forming reactions, medicinal chemistry, process chemistry for APIs and intermediates and continuous flow chemistry.

bioactive clone, once detected, was isolated and sequenced to identify the gene(s) responsible for the production of the bioactive metabolite. This is followed by the expression in heterologous hosts for the production of clone-specific secondary metabolites. Although, a number of novel antibiotics have been identified from the human microbiome by this method, difficulties in the cloning of large BGCs in single clones and their inefficient expression in heterologous hosts are some of the limitations in the broad usage of this approach.

(c) Mass spectrometry-based metabolomics approaches have also been used to characterize chemical metabolites from the human microbiome which, however, is limited to known primary metabolites for which searchable platforms can be generated from reference samples.[17] Moreover, the transient existence and instability of some human derived secondary metabolites have complicated their isolation process.[18]

1.2 Human microbiome-natural products–synthesis of triumvirate

Concurrent with the activities in genome mining of the human microbiome, a sizeable number of novel antibacterial natural products have been isolated and characterized from the human gut, skin, nasal and oral cavities, either *via* traditional culturomics or through genome mining techniques. These new secondary metabolites have stimulated considerable interest in synthesis-enabled drug discovery, beyond formal total syntheses, in order to unravel and augment a new chemical space for SAR mapping and locating regions for structural modifications towards pharmacological gains. Several notable natural product based drugs *e.g.* ziconotide, trabectidin, omadacycline, eribulin, *etc.*, have been developed through such synthesis-enabled endeavors.[19,20]

In light of the foregoing narrative and the evolving role of the human microbiome in health management and its expansive potentials in therapeutic interventions, the time is right to bring into focus the signaling natural products which serve as key mediators in microbial interactions. In this review, we now present an overview of the bioactive natural products from the human microbiome, with particular focus on their total synthesis and synthesis-enabled drug discovery campaigns. The topic, at the interface of chemistry and biology, would entice interest among a broad cross-disciplinary readership, ranging from natural products chemists to chemical biologists and drug discovery scientists.

Goverdhan Mehta

*Goverdhan Mehta has been associated with the Indian Institute of Technology, Kanpur, University of Hyderabad (Professor, Dean, Vice Chancellor), the Indian Institute of Science, Bangalore (Professor and Director) and held visiting positions in Belgium, UK, France, Germany, Japan, Taiwan and USA. He is a Fellow of the Indian National Science Academy (President 1999–2001), the Royal Society FRS and numerous other academies. He is a recipient of the Royal Society of Chemistry Medals and Honorary Fellowship (*Hon*FRSC). Presently, he is a University Distinguished Professor at the University of Hyderabad with current interests in organic synthesis and in the promotion of chemistry as a sustainability science.*

1.3 Scope of the review

The review is focused on relevant commensal bacterial natural products with established structures and documented biological activities (the literature covered until December 2023). However, microbiome-derived natural products leading to pathogenic phenotypes[21] such as the bacterial quorum sensing vectors,[22] virulence factors or genotoxic metabolites *viz*. mycolactone B,[23] colibactin,[20g,24] tilivalline,[25] *etc*. have been reviewed recently and not included here. The narrative is divided into four sections: (i) brief description of the natural product diversity in the human microbiome, (ii) total synthesis campaigns, according to different biological classes, (iii) synthesis-enabled drug discovery initiatives and (iv) prospective natural product targets for total synthesis endeavors, which are discussed with ample schematic descriptions for the ease of reading.

2. Natural product diversity in the human microbiome

The diversity of natural products in the human microbiome can be attributed to their different habitats and biosynthetic origin. Microbial habitat is present in all parts of the human body but to date, the gut intestinal tract has been the major source of bioactive natural products, followed by the skin, vagina, mouth and nasal cavity. The chemical diversity of microbiome-derived natural products range from small molecule indole acrylic acid (**1**), small terpenes (**2**), small peptides and alkaloids (**3–6**), lipid based commendamide (**7**) to large hybrid heterocycles such as colibactin (**8**), see Fig. 2.

More complex structures have also been identified *viz*. the polyketide macrolide mycolactone B (**9**), peptidoglycan GlcNAc-MDP (**10**), NRPS derived cyclopeptide lugdunin (**11**) and highly complex peptides such as sactipeptide triglysin A (**12**), thiopeptide lactocillin (**13**) and lantipeptide gallidermin (**14**). The latter three belong to ribosomally synthesized post-translationally modified peptides (RiPPs),[26] which are biosynthetically and structurally distinct from conventional antibiotics found in soil-borne bacteria, further enriching the chemical diversity. In this context, it is worth noting that natural products from the human microbiome, although only a fraction of those isolated from soil-borne bacteria, display wider chemical and structural diversity owing to the presence of a broad range of biosynthetic machineries.

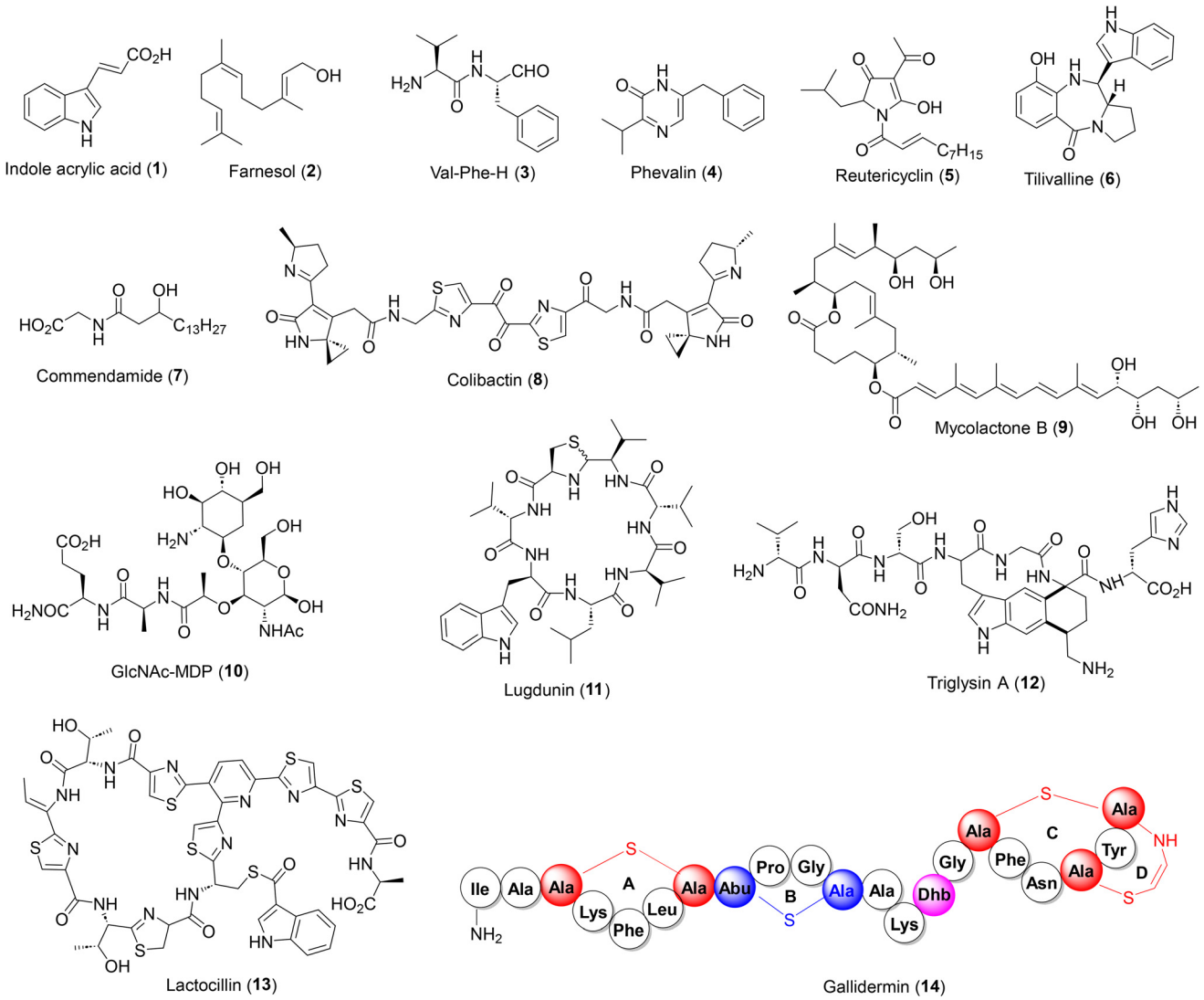


Fig. 2 Diverse natural products isolated from the human microbiome.

3. Synthesis of human microbiome-derived natural products

Human commensal bacteria are a rich source of antibiotics belonging to diverse chemical classes. These novel antibiotics, especially the structurally intriguing ribosomally synthesized and post-translationally modified peptides (RiPPs), *e.g.* thiopeptides,[27] azole peptides,[28] lantipeptides,[29] sactipeptides,[30] *etc.*, have drawn considerable attention from the organic synthesis community, not only due to their structural challenges but also due to their therapeutic potential as novel antibiotics against resistant bacteria. Natural products covered in this section belong to three broad biological classes: (i) bacteriocins, (ii) siderophores and (iii) natural product-like antimicrobial peptides. The discussion on individual natural products covers a brief introduction on their source, biological activities and mode of action, if known, followed by the retrosynthetic analysis and total synthesis campaigns.

3.1 Bacteriocins

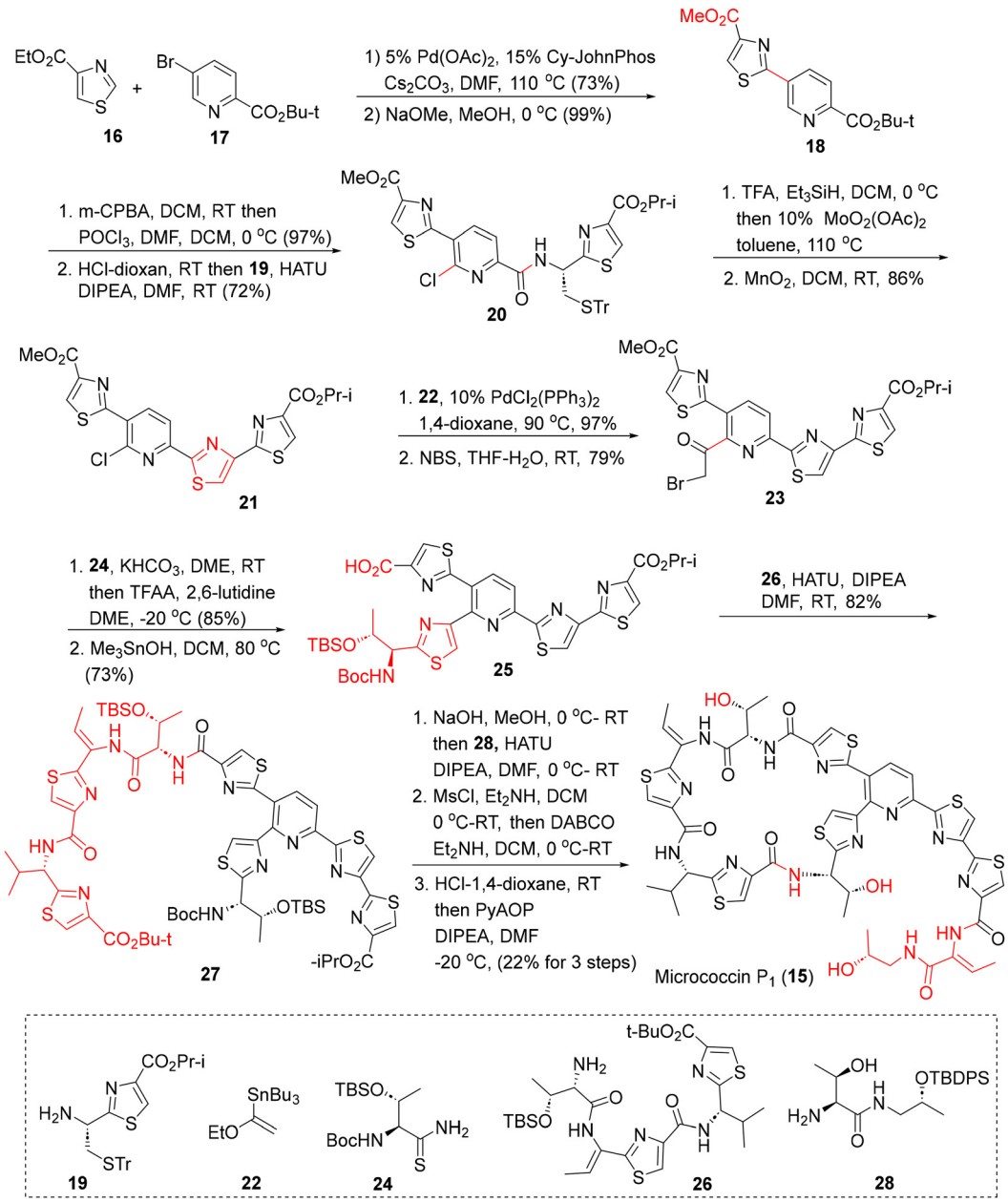
Bacteriocins are secreted by bacteria against phylogenetically related strains and hence, show a narrow spectrum of antibacterial activities, less toxicity and minimum perturbation of the commensal gut bacteria.[31] In the human microbiome, bacteriocins belong to the RiPP class of modified peptides which are chemically distinct from conventional soil-borne antibiotics and hence, constitute important synthetic targets due to their antibiotic potentials against resistant bacteria. Chemical syntheses of some notable bacteriocins *viz.* anti-listerial leucocin A, pediocin PA-1, sakacin P, curvacin A, mesentericin Y105, enterocin CRL35, *etc.*, and the heat resistant circular antibiotic enterocin AS-48 have been discussed in detail in a recent review[31b] and are not discussed here.

3.1.1. Micrococcin P_1. Micrococcin P_1 (**15**) is the first discovered member of RiPP thiopeptide antibiotics,[27] whose 26-membered macrocyclic structure was established through the total synthesis by Ciufolini and co-workers (reviewed in

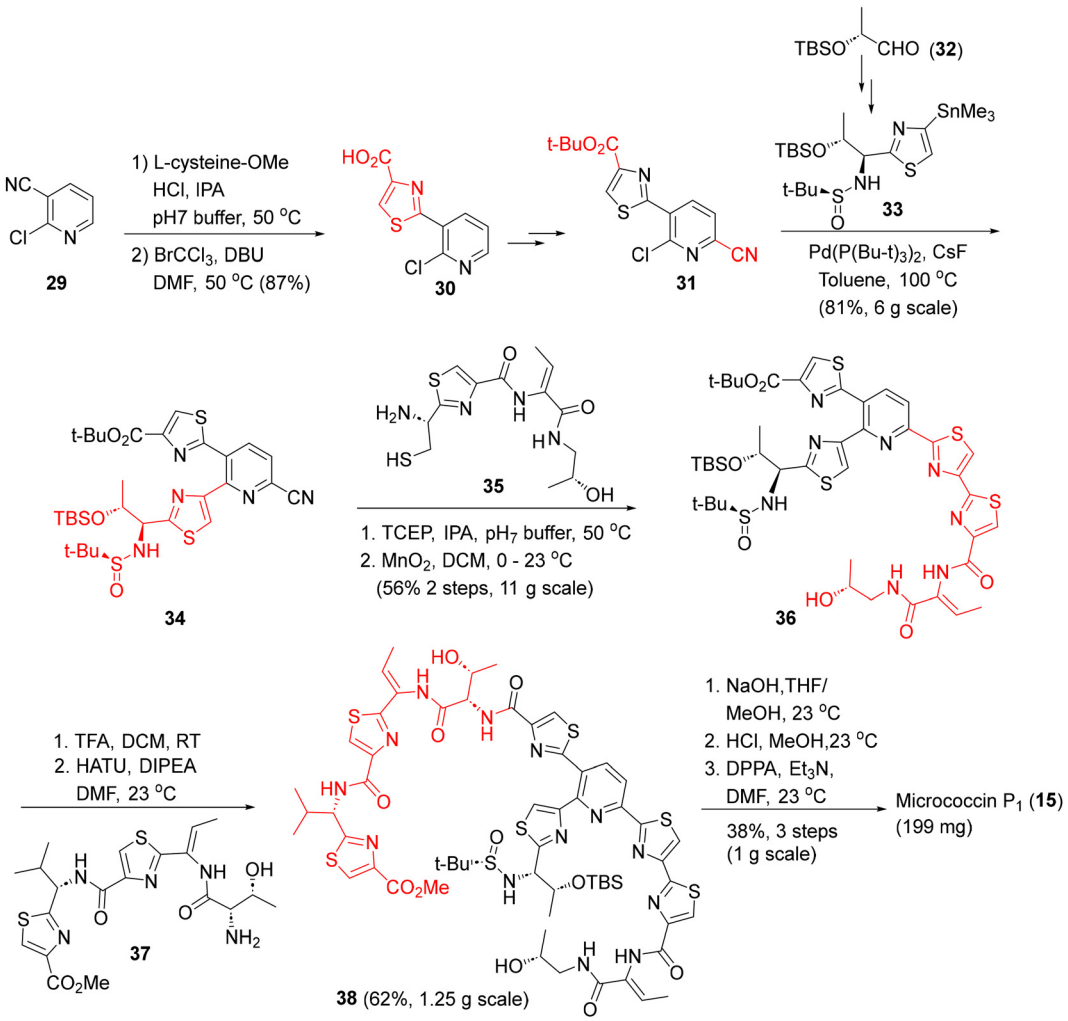
2010).[32] Recently, micrococcin P_1 was identified in the human skin isolate *S. hominis* S34 which showed potent activity against several *S. aureus* strains (MICs 0.5–1.0 μg mL^{-1}), including a virulent MRSA strain, and accelerated the healing of severe *S. aureus* infected wounds.[33] In view of its newly discovered anti-infective properties, two new total syntheses of micrococcin P_1 have been reported in the recent literature.

The major challenge in the synthesis of micrococcin P_1 lies in the construction of the highly congested 2,5,6-tri-thiazolyl pyridine core. The Walczak group approached this challenge in a linear fashion (Scheme 1) where three thiazolyl rings were successively appended to a pyridine scaffold **17** *via* (a) a C–H activation protocol (**16** + **17** → **18**), (b) Mo(VI) catalysed cysteine cyclodehydration (**20** → **21**), and (c) the Hantzsch thiazole synthesis (**23** → **25**), leading to the tri-thiazolyl pyridine intermediate **25** in a good overall yield.[34a] The fringe amino acid fragments **26** and **28** (independently synthesized) were then introduced *via* HATU couplings, followed by macrocyclization with PyAOP to furnish micrococcin P_1 (**15**). The synthetic thiopeptide was found to be highly potent against *S. aureus* isolates as well as a VRE strain (MICs 0.5–2 μg mL^{-1}), confirming its reported antibacterial activities.

On the other hand, the Siegel synthesis of micrococcin P_1 followed a modular approach for assembling the tri-thiazolyl pyridine core (Scheme 2).[34b,c] Commencing with 2-chloronicotinonitrile (**29**), the three thiazole rings were constructed, first,



Scheme 1 The Walczak synthesis of micrococcin P_1 (**15**).[34a]

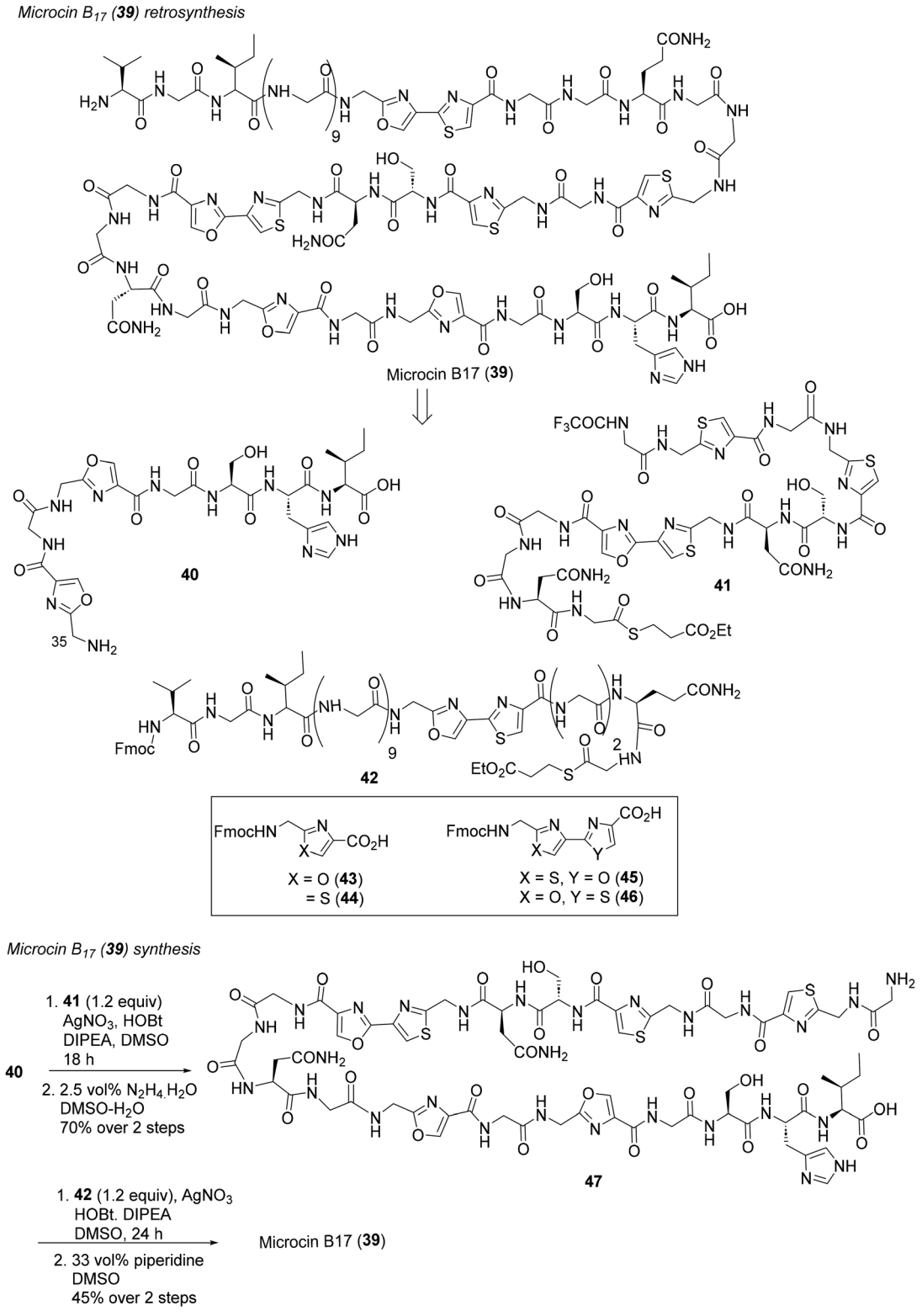


Scheme 2 The Siegel synthesis of micrococcin P_1 (**15**).[34b]

via oxidative cyclization with cysteineOMe·HCl (to **30**), the second, *via* the Stille-coupling on **31** with the thiazolyl stannane **33** (assembled *via* the Ellman chiral sulfoximine protocol) to **34** and the third, *via* oxidative cyclization with thiazolyl aminothiol **35**, to furnish the key tri-thiazolylpyridine core **36**. Hydrolysis of the *t*-Bu ester followed by HATU coupling with the western fringe amino acid fragment **37** then afforded the cyclization precursor **38** (62%). Finally, ester hydrolysis, deprotection of TBS and the Ellman auxiliary, followed by macrocyclization delivered micrococcin P_1 (**15**). The synthesized micrococcin **15** showed potent activities against *S. aureus*, MRSA and VRE strains (MICs 0.5–1 μg mL^{-1}). This modular approach is amenable to multi-gram, chromatography-free operation in some key steps,[34c] and could be useful for analoging in SAR studies.

3.1.2. Microcin B17. The ribosomally synthesized azole rich peptide microcin B17 (**39**), produced by the commensal gut *Enterobacteriaceae*, has drawn much attention as a bacterial DNA gyrase inhibitor with potent *in vivo* activities against pathogenic *E. coli*.[35,36] Although **39** was synthesized by the Jung group *via* a linear Fmoc-based solid phase peptide synthesis (SPPS)[37a] and through a bio-engineered strategy in *E. coli*,[38] its sheer size and complexity provided considerable challenge for analoging. This was overcome by Thompson *et al.* through a modular approach based on a thioester aminolysis-ligation strategy (Scheme 3).[37b] Microcin B17 **39** was dissected into three retrosynthetic fragments: the C-terminal peptide **40**, the central thioester fragment **41** and the N-terminal thioester unit **42** which were individually synthesized and coupled *via* successive Ag(I)-promoted aminolysis ligation reactions to eventuate in the natural product. This approach was exploited to synthesize a variety of analogs, whose antibacterial profiling highlighted the crucial role of the azole units and the full length natural product for cellular activity.[37c]

3.1.3. Microcin C7. *E. coli* MC4100 produces a modified-antimicrobial peptide (AMP) conjugate, microcin C7 (**48**, Fig. 3) which exhibits potent activity against *Klebsiella*, *Salmonella*, *Shigella*, *Yersinia* strains.[38,39] Microcin C7 is believed to act as a "Trojan horse" antibiotic that is recognized



Scheme 3 Ligation-based modular synthesis of microcin B17 (**39**).[37b]

by transporters and imported inside targeted bacterial cells.[39c] The total synthesis of microcin C7 was achieved *via* straightforward Fmoc-based SPPS of the heptapeptide and coupling with the modified-AMP.[39b]

3.1.4. Mutanobactin A–D. Isolated from the *S. mutans*[40] strain UA159, mutanobactin A–D (**49–52**, Scheme 4) are a small group of hybrid PKS/NRPS lipopeptides with unusual structural features *viz.* a highly labile β-hetero-β′-keto-γ-amino

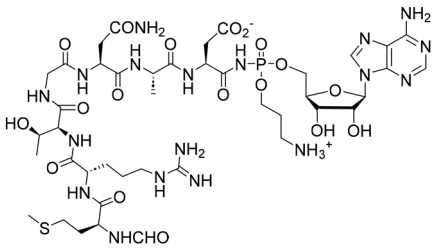


Fig. 3 Structure of microcin C7 (**48**).

amide chiral center (C-25/26) and an uncommon 1,4-thiazepan-5-one ring (mutanobactin A–C).[41a–c] Among them, mutanobactin D (**52**) was found to be a potent inhibitor of biofilm formation by the oral fungal pathogen *Candida albicans* (IC_{50} 5.3 μM), drawing attention from the Carreira group towards its total synthesis.[42a] Retrosynthetically (Scheme 4a), the peptide segment of **52** was planned *via* SPPS and the sensitive β-hydroxy-β′-keto-γ-amino amide segment was envisaged as a *trans*-isooxazoline surrogate (**53**), to be revealed late in the synthesis. In the event, the azido isooxazoline acid **54**, derived through asymmetric nitrile oxide cycloaddition (Scheme 4b) and the peptide fragment **60** were coupled together, followed by azide reduction, resin cleavage and macrocyclization to furnish the key retrosynthetic intermediate **53**. Reductive cleavage of the isooxazoline ring followed by hydrolysis produced mutanobactin D (**52**) which showed a 25*R*,26*R* configuration, thereby establishing the absolute configurations of these two labile chiral centers. The synthesized mutanobactin D **52** showed potent anti-biofilm activities against three *C. albicans* strains (table, Scheme 4) and significantly reduced filament growth in ATCC 90028 and 101 strains.[42a]

The Carreira group also reported the total synthesis of the thiazepanone lipopeptides mutanobactin A and B (**49** and **50**) (Scheme 5),[42b] despite their weak anti-biofilm properties.[41a,b] Pursuant to the retrosynthetic analysis shown in Scheme 5a, the tetrapeptide fragments were assembled *via* Fmoc-based SPPS on **65**, leading to the peptide amines **66a** and **b**. HATU coupling with the protected β-keto acid **67** then furnished the key on-resin retrosynthetic intermediates **63a** and **b**. Resin cleavage with aqueous TFA led to the *in situ* formation of thioacetals **62a** and **b** which spontaneously cyclized under acidic conditions to produce the natural products **49** and **50** in good overall yields. The strategy was also extended to the synthesis of mutanobactin D (**52**) (Scheme 5c), thereby demonstrating a unified synthetic approach for three mutanobactin siblings.[42b]

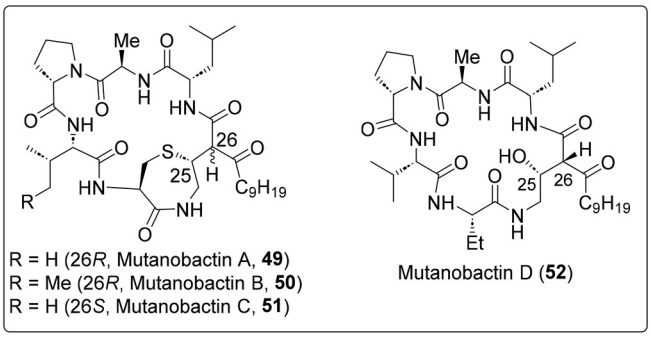
3.1.5. Lugdunin. Lugdunin (**11**, Fig. 2) is a novel thiazolidine cyclopeptide with an unusual disposition of alternating D- and L-amino acids which was isolated from *Staphylococcus lugdunensis* IVK28 and exhibited broad antibacterial activities on multiple *S. aureus* strains, including MRSA, VRE and *B. subtilis* (MICs 1.5–4 μg mL^{-1}) with a high barrier to resistance.[43] In order to build a SAR map of lugdunin, Grond and co-workers carried out a diverted solid-phase total synthesis of lugdunin (**11**), along with 60 analogs (Schemes 6 and 7).[44] In the first-generation synthesis (Scheme 6), macrocyclization was achieved from the linear peptide aldehyde **71** (obtained from the resin bound peptide **69**) *via* macrocyclic imine **72** formation[45] followed by a facile intramolecular thiol addition to furnish lugdunin (**11**).[44a] However, the strongly acidic conditions used in the cyclization step also led to the epimerization of the Val(7) chiral center, resulting in 4 epimeric products. Later, in the second generation synthesis, the authors installed a configurationally stable thiazolidine amino acid **73**, early in peptide build-up (*cf.* **75**), followed by macrocyclization *via* Val(5)–Val(6) amide bond formation which furnished epimerically pure lugdunin (Scheme 7).[44b] This latter strategy was then applied to a diverted total synthesis of nearly 60 analogs which were screened against MRSA USA300 LAC. Consequently, a SAR map of lugdunin chemical space was developed (see box, Scheme 7) which could be useful for drug discovery efforts.[44a,b]

3.1.6 Lantibiotics. A biologically important sub-group of RiPP bacteriocins is the lantibiotics,[29] typically consisting of sulfur-bridged lantionine (Lan) and β-methyllantionine (MeLan) rings that are post-translationally derived *via* the intramolecular 1,4-addition of cysteines to dehydroalanine (Dha) or dehydrobutyrine (Dhb) residues (Fig. 4).

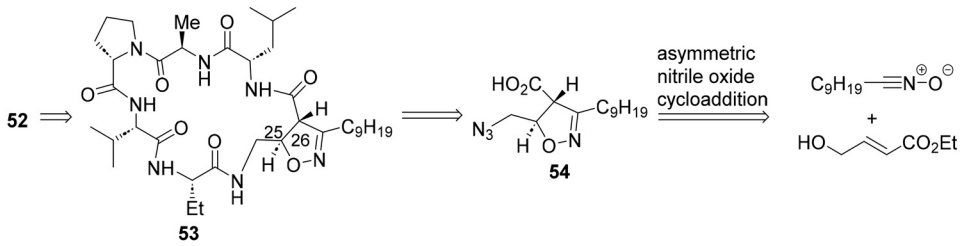
Lantionine rings are the key pharmacophores which, while imparting turn-structures to the lanti-peptides, also act as target binding domains or pore-forming motifs for antibiotic action. Examples of antibacterial lantibiotics isolated from the human microbiota are epilancin 15× (**77**), lacticin 481 (**78**) and the two-component antibiotics lacticin 3147 (**79a,b**) and cytolysin (**80a,b**) (Fig. 5).

3.1.6.1. Epilancin 15×. Isolated from human wound infection and skin colonies, the clinical strain *S. epidermidis* 15 × 154 produces the tricyclic lantibiotic epilancin 15× (**77**) which displays strong antibacterial activities against Gram +ve bacteria, including MRSA and VRE strains (MICs 0.1–1 μg mL^{-1}).[46] In addition to three Lan and MeLan rings A–C, **77** also features three N-terminal unsaturated residues (Dha3, Dhb7, and Dhb8). Although the total synthesis of epilancin 15× has not yet been described, Knerr and van der Donk reported the synthesis of a few N-terminal analogs **90–92** where the unsaturated Dha3, Dhb7, and Dhb8 residues were replaced with their saturated counterparts (Scheme 8).[47] In this synthesis, the orthogonally protected synthons **81–83** [48] were deployed as precursors to the Lan and MeLan thioether rings and strategically inserted in the growing peptide chain *via* SPPS. The thioether rings were then constructed *via* a three-step protocol: orthogonal deprotection of the lantionine building block, Fmoc removal and ring cyclization using PyAOP, as illustrated in the formation of **86** (ring C), **87** (ring B) and **89** (ring A). The latter intermediate was then used as a scaffold for the synthesis of the desired N-terminal analogs **90–92** *via* the attachment of the remaining N-terminal amino acids. MIC determination against *S. carnosus* TM300 showed that the full-length analogs **91** and **92** (but not the truncated variant **90**) were almost equipotent with the natural products.

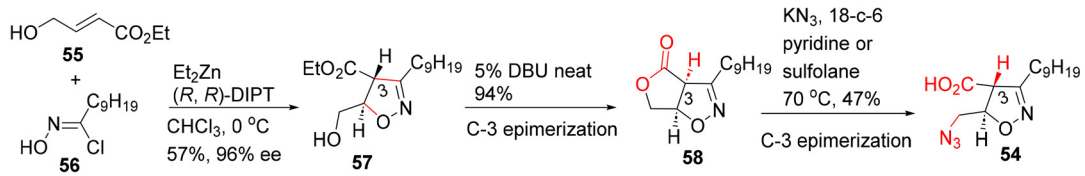
3.1.6.2. Lacticin 481. Lacticin 481 (**78**, Fig. 5) is a 27-amino acid tricyclic lantibiotic produced by the lactic acid bacteria



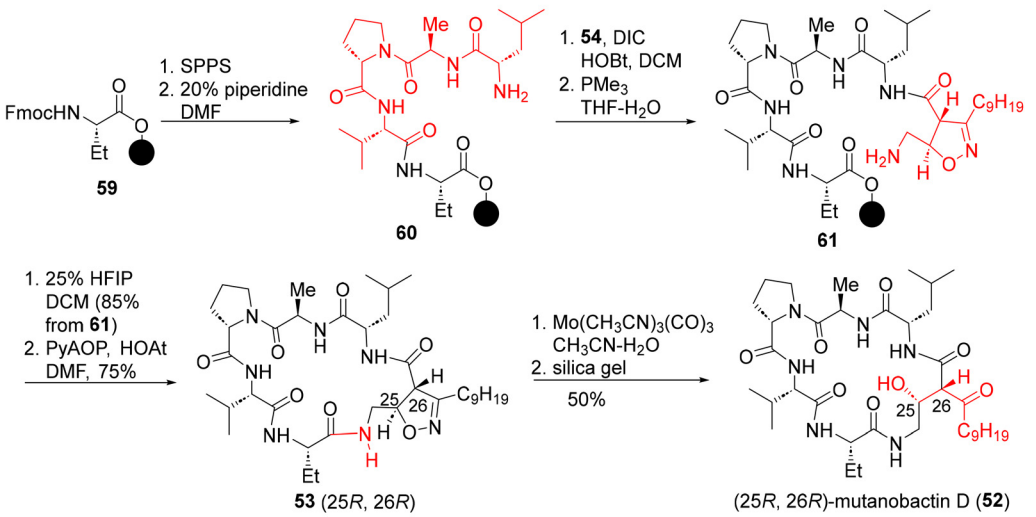
*(a) Retrosynthetic analysis of mutanobactin D (**52**)*



*(b) Asymmetric synthesis of trans-azidoisooxazoline (**54**)*



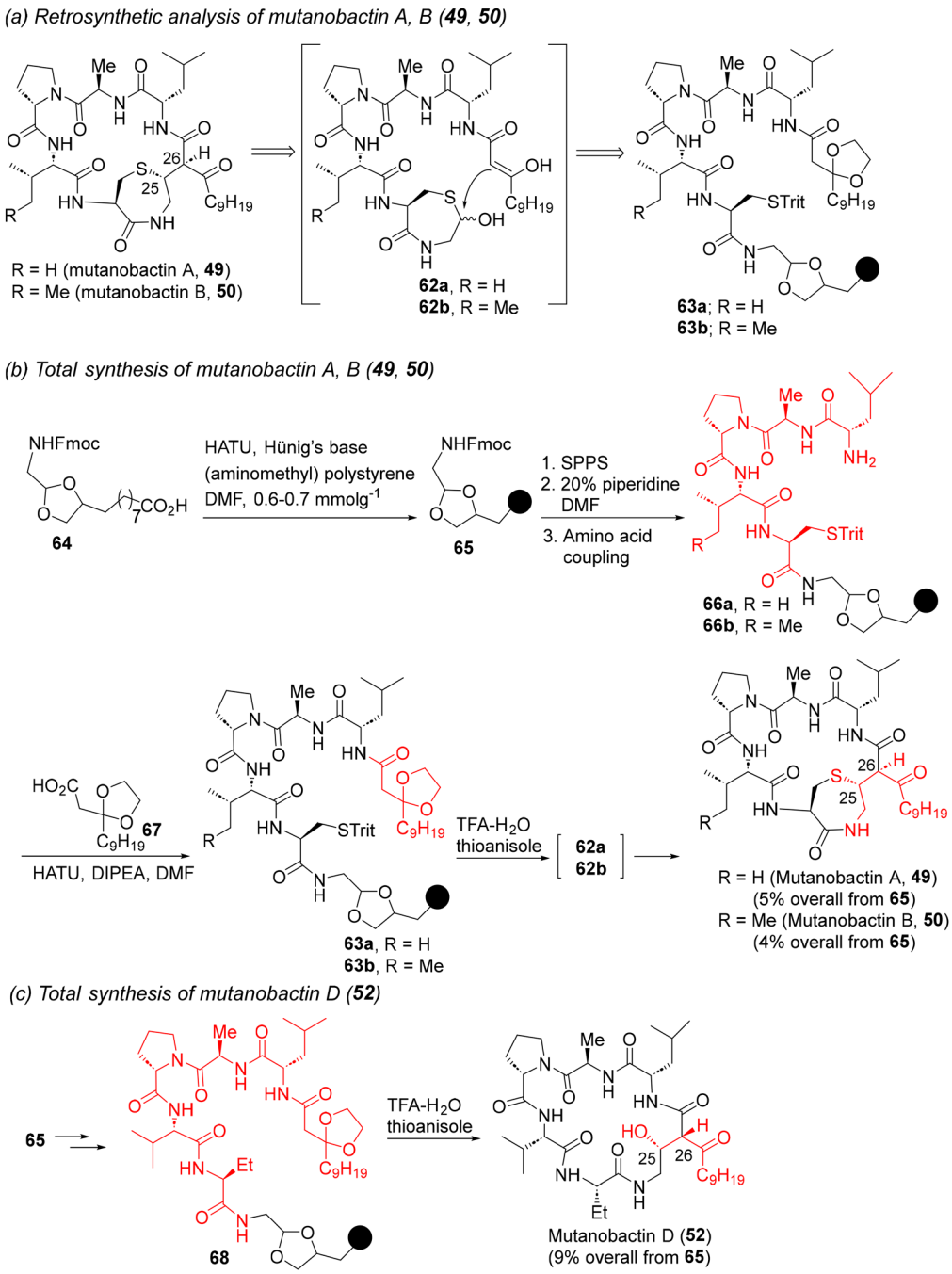
*(c) Total synthesis of mutanobactin D (**52**)*



Anti-biofilm potency of **52** (IC_{50}, μM)

C. albicans strain	XTT	Confocal microscopy
ATCC90028	23.5	44.1
101	24.7	48.0
SC5214	21.5	42.2

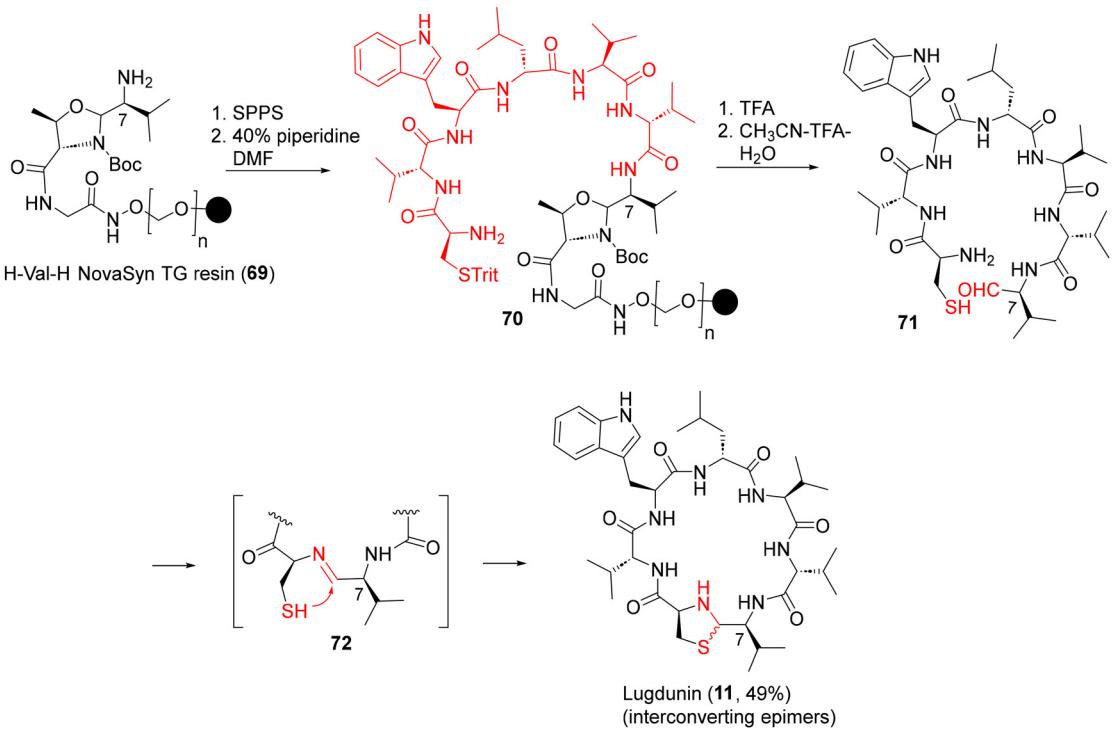
Scheme 4 Retrosynthesis and total synthesis of mutanobactin D (**52**).[42a]



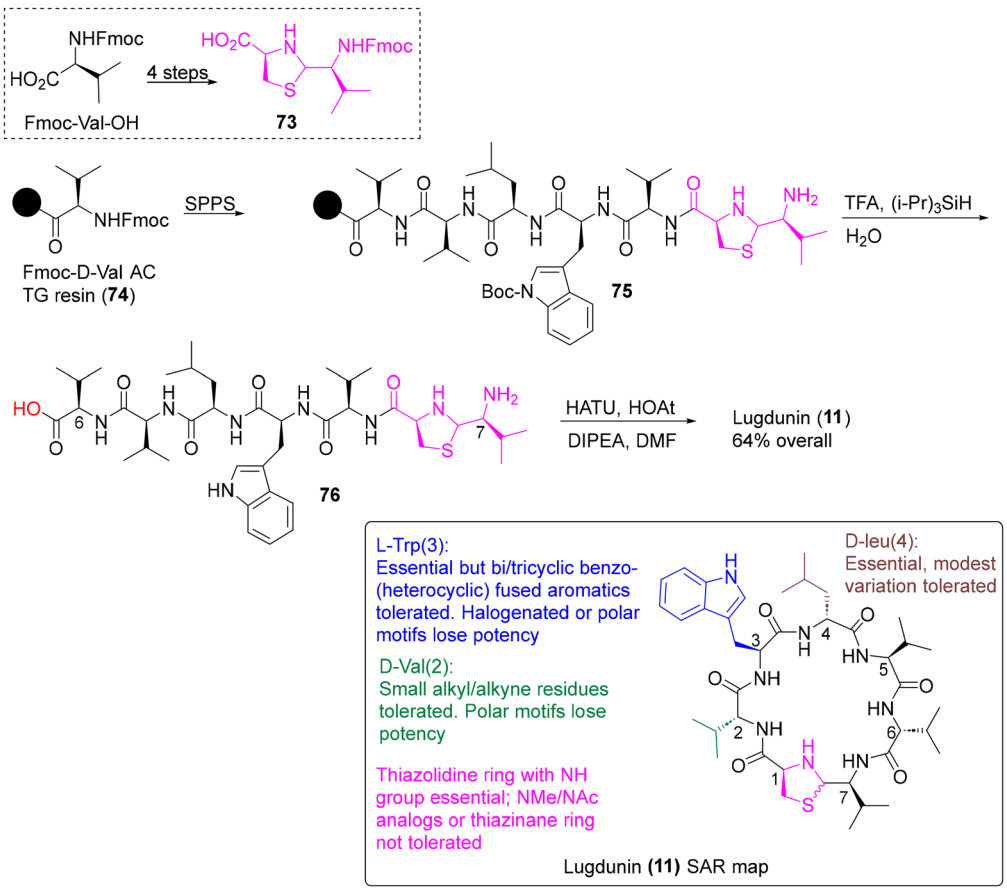
Scheme 5 Retrosynthesis and total synthesis of mutanobactin A, B, and D (**49**, **50**, and **52**).[42b]

Lactococcus lactis subspecies *lactis*, featuring two DL-Lan thioether bridges, a large DL-MeLan bridge and an unsaturated Dhb unit.[49] The van der Donk group reported a solid-phase synthesis of **78** and its Lan/MeLan stereoisomers, following the same strategy as described for epilancin 15× (*cf.* Scheme 8) which revealed the critical dependence of the antibacterial potency on the stereochemistry of the Lan/MeLan bridges.[50a] Syntheses of a few analogs of **78** have also been reported *via* mutasynthesis.[50b–d]

3.1.6.3. Lacticin 3147 (A1 + A2). Produced by the *Lactococcus lactis* subspecies *lactis* DPC3147, lacticin 3147 is a two-component lantibiotic, composed of interlocked thioether bridged peptide A1 (**79a**) and the linear lanti-peptide A2 (**79b**) (Fig. 5), exhibiting a broad-spectrum activity against Gram +ve bacteria including MRSA, VRE and mycobacteria.[51] Mechanistically, peptides A1 and A2 form a ternary complex with lipid-II on the surface of the cell membrane which inhibits cell wall synthesis, causing pore formation and cell rupture.[51e] The



Scheme 6 First-generation SPPS of lugdunin (**11**) *via* thiazolidine macrocyclization.[44a]



Scheme 7 Second-generation SPPS and SAR map (box) of lugdunin (**11**) using the thiazolidine building block **73**.[44b]

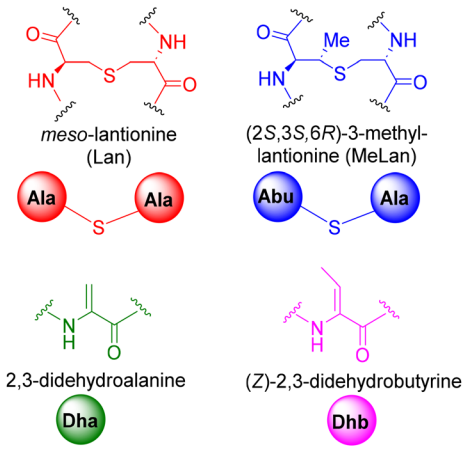


Fig. 4 Lantionine (Lan), β-methyllantionine (MeLan), dehydroalanine (Dha) and dehydrobutyrine (Dhb) notations.

Vederas group has reported the solid phase synthesis of both components of lacticin 3147, again, deploying the orthogonally protected synthons **81–83** as the thiother ring precursors.[52a] For peptide A1 **79a** (Scheme 9), the interlocked MeLan-bridged rings C and D provided a stiff synthetic challenge and were eventually constructed from a precursor peptide **96** *via* successive orthogonal deprotection of MeLan building blocks (*via* Pd-catalyzed allyl deprotection or $SnCl_2$ removal of nitrobenzyl groups), each followed by Fmoc removal and PyBOP cyclization to produce the desired C/D-interlocked bicyclic intermediate **98.** The remaining rings B and A were then constructed on **98** to furnish peptide A1 (**79a**) in a remarkable 1.4% overall yield from **84** in more than 50 steps.

For the synthesis of peptide A2 (**79b**), the synthesis commenced with the 2-chlorotrityl polystyrene resin **101**, loaded with the MeLan synthon **82.** Following a similar strategy as

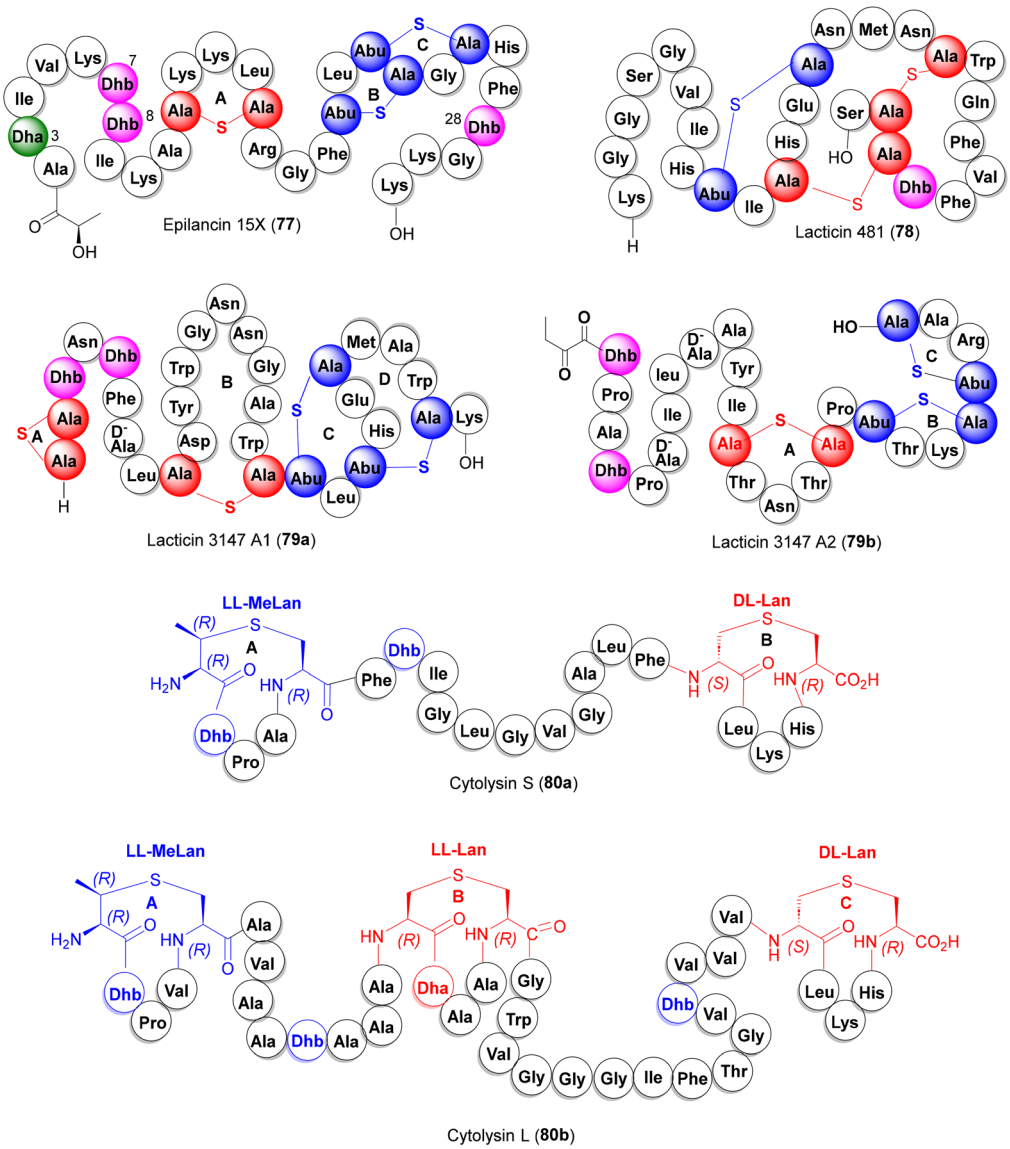
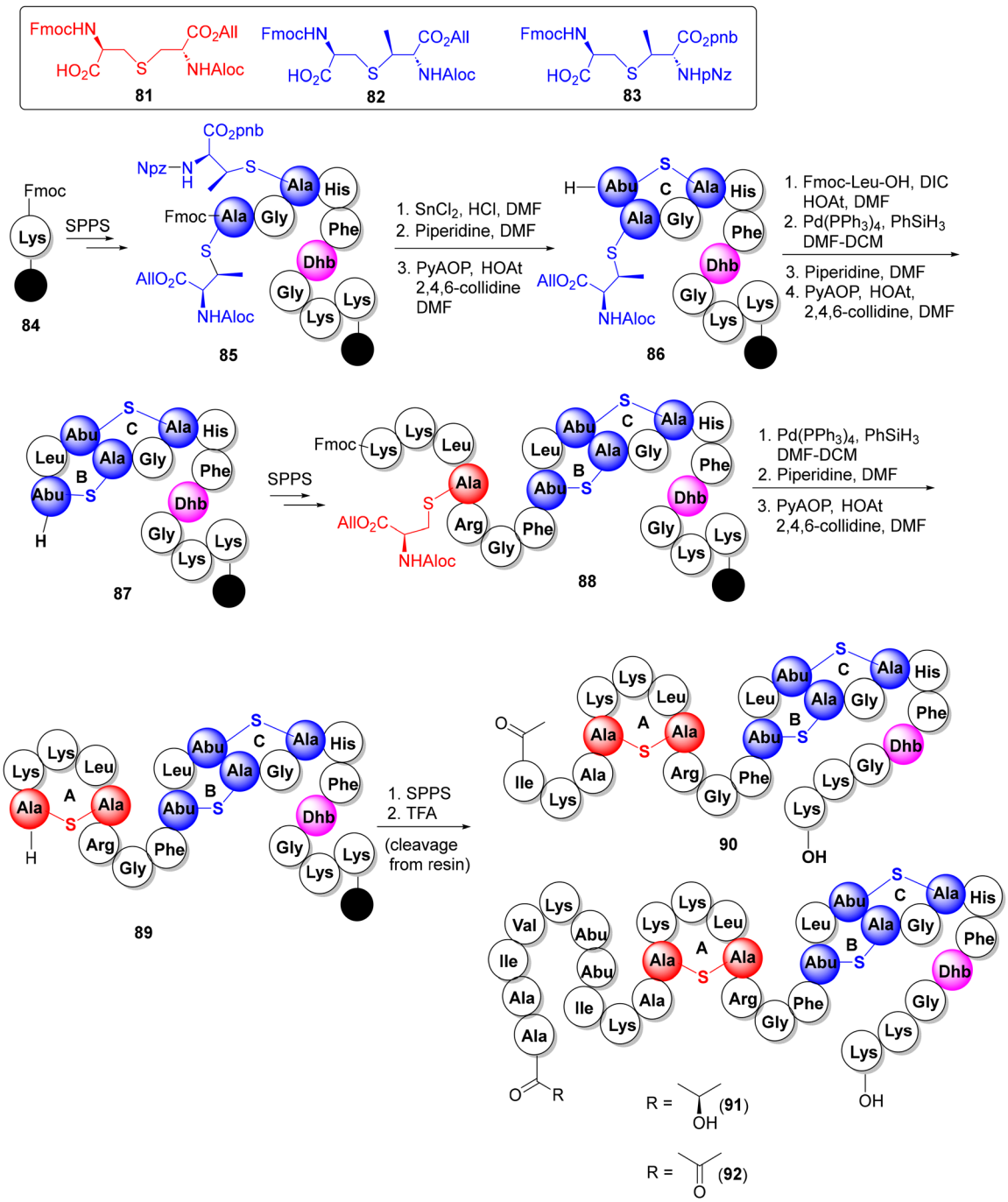


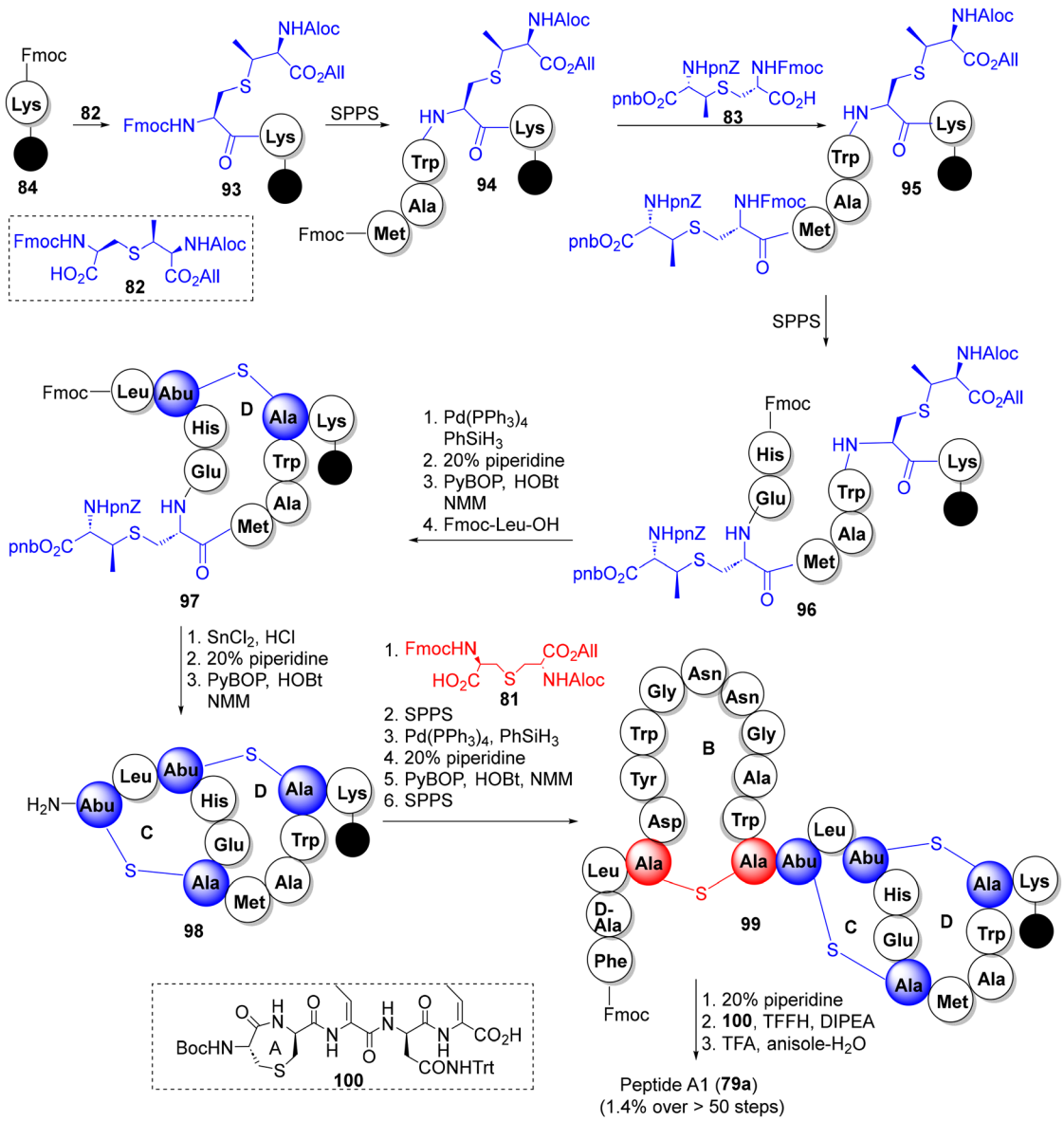
Fig. 5 Lantibiotics isolated from the human microbiome.



Scheme 8 SPPS synthesis of epilancin 15× N-terminal analogs **90–92**.[47]

above *i.e.* incorporation of Lan and MeLan synthons **81** and **82** at appropriate junctures during peptide build-up, the three thioether rings were constructed, successively (C to B to A), *via* Pd-catalyzed allyl deprotection, Fmoc removal and PyBOP cyclization, furnishing lacticin 3147 peptide A2 (**79b**) in 1% overall yield (Scheme 10).[52a] The synthetic peptides A1 and A2 showed excellent synergy against the *Lactococcus lactis* subspecies *cremoris* HP, indistinguishable from natural lacticin 3147. The Vederas group also prepared oxa- and desmethyl-Lan analogues of peptide A2 which, however, lost antibacterial activities and synergy (with A1), pointing to the indispensable role of natural MeLan units in A1 and A2 for antibiotic activities.[52b,c]

3.1.6.4. Cytolysin. Expressed by *E. faecalis*, cytolysin is a 2-component pore-forming lantibiotic, composed of two lantipeptides cytolysin S (**80a**) and cytolysin L (**80b**) (Fig. 5) which exhibits synergistic antimicrobial activity against Gram +ve bacteria.[53] Interestingly, some lantionine rings in cytolysin



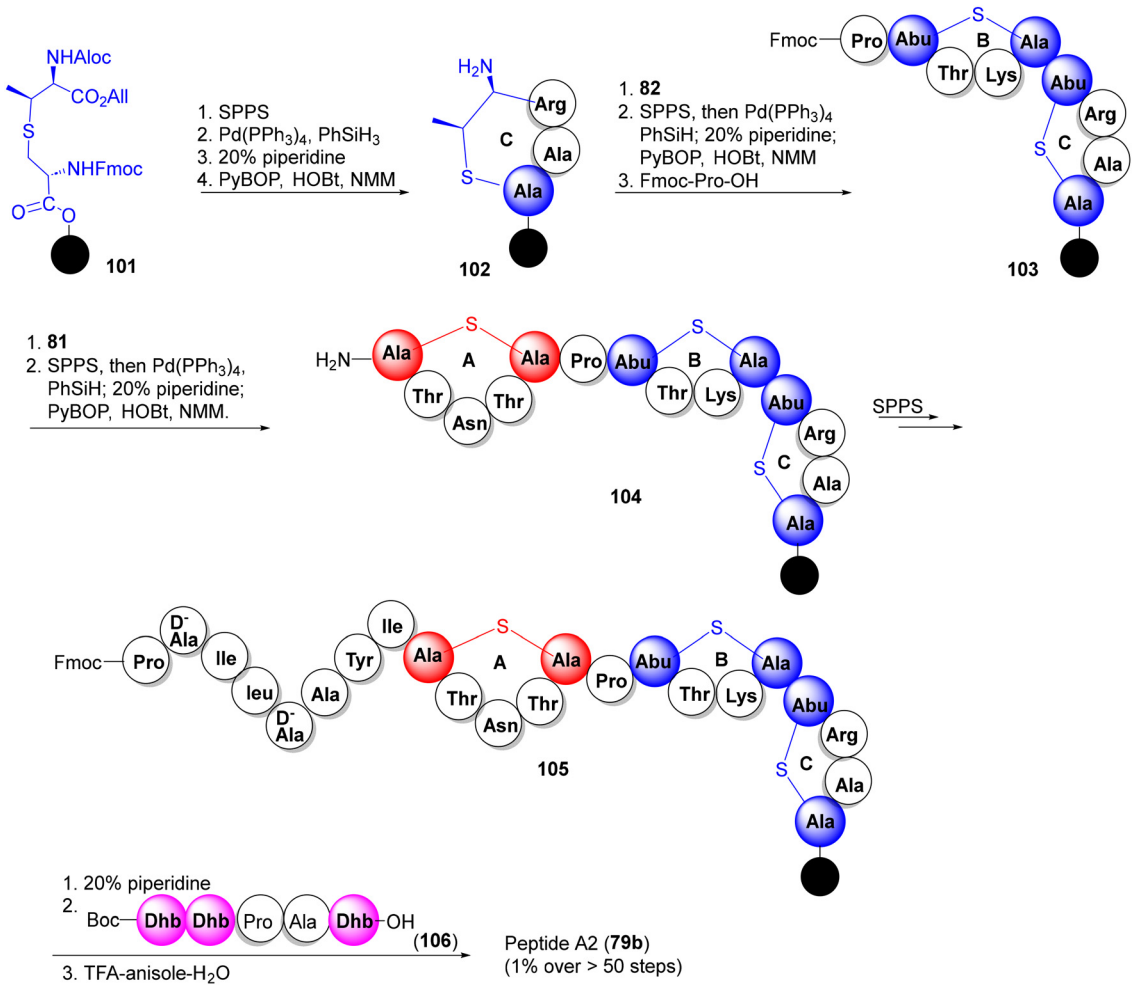
Scheme 9 The Vederas synthesis of lacticin 3147 constituent peptide A1 (**79a**).[52a]

peptides show an unusual LL-stereochemistry which, *via* diverted total synthesis of all possible Lan/MeLan stereoisomeric peptides, were shown to be crucial for their antibiotic activities.[54]

3.2 Siderophores

The lack of antibiotic efficiency against Gram –ve pathogens, primarily attributed to the latters' impervious outer membrane, has forced researchers to pursue non-conventional approaches against Gram –ve pathogens. One such approach makes use of small molecule chelators called siderophores which are secreted under stress by all bacteria for iron transport across the cell membrane for their survival.[55] This unique feature of siderophores in penetrating the Gram –ve bacterial membrane has inspired a non-conventional antibacterial strategy where antibiotic-siderophore conjugates are deployed for cell penetration and the subsequent release of antibiotic payloads inside Gram –ve bacteria ("Trojan horse" approach).[55b,e]

A variety of siderophores, belonging to hydroximate and/or catechol classes, have been isolated from human Gram –ve pathogens.[56] Notable examples are enterobactin (**107**, from *E. coli* and *S. typhimurium*),[57] acinetobactin (**108**, from *A. baumannii*),[58] fimsbactin A (**109**, from *A. baumannii*),[59] pseudopalin (**110**, from *P. aeruginosa*),[60] the chromophoric chelator pyoverdin D (**111**, from *P. aeruginosa*),[61] *etc.*, (Fig. 6) which have been the focus of extensive investigations as antibiotic carriers and diagnostics. The chemistry and biology of these siderophores, including their total synthesis, have already been captured in recent accounts, as cited above and hence, not elaborated in this review.



Scheme 10 The Vederas synthesis of lacticin 3147 constituent peptide A2 (**65b**).[52a]

3.3 Genome-mined natural product-like AMPs

Since only a fraction of the bacterial biosynthetic diversity is accessible through the laboratory broth culture, antibacterial screening of bioinformatics predicted peptide libraries (synthesized *via* SPPS) has been effectively leveraged to discover natural product-like AMPs. In this regard, the Brady group applied the "synthetic-bioinformatic natural products (syn-BNPs)" platform[62–64] for the sequence analysis of top 25 NRPS-BGCs from the human microbiome database to predict the encoded natural product-like AMP structures. Based on such inputs, peptide libraries were synthesized *via* routine SPPS and their antibacterial screening leading to the discovery of a new antibiotic humimycin A (**112**, Fig. 7) exhibiting potent activity against *S. aureus*, including MRSA and *S. pneumoniae*.[62] In addition, humimycin A and its analog **113** also potentiated carbenicillin and dicloxacillin activities in MRSA USA300, COL and VRE strains without signs of bacterial resistance (Fig. 7).[63]

In another culture independent approach, Chen and co-workers combined multiple natural language processing neural network models, including LSTM, Attention and BERT, for the autonomous learning of AMP sequences to construct AMP prediction models from large-scale human microbiome data.[65a] By this exercise, 2349 sequences were identified as candidate AMPs, out of which, 216 peptides were chemically synthesized *via* routine SPPS and found to be active against Gram –ve pathogens. In a remarkable revelation, the peptide AMP1043 (KQKTLKKVWKLSEKVLIFASAFAKKAGAAEA-TLVL) was found to be highly active against the ESKAPE pathogens *E. coli* (MIC 2 uM), *K. pneumoniae* (MIC 2.5 uM) and *A. baumannii* (MIC 10 uM) as well as MDR resistant strains (MICs < 8 μM). The above AI driven strategies highlight the combination of chemical synthesis and bioinformatics as a versatile platform in deciphering large complex metagenomic information from human microbiota for the accelerated discovery of AMPs.[65b,c]

4. Drug discovery initiatives

Spurred by the approval of Rebyota and Vowst as live fecal microbiota drugs in 2022–23,[5] dozens of clinical trials are

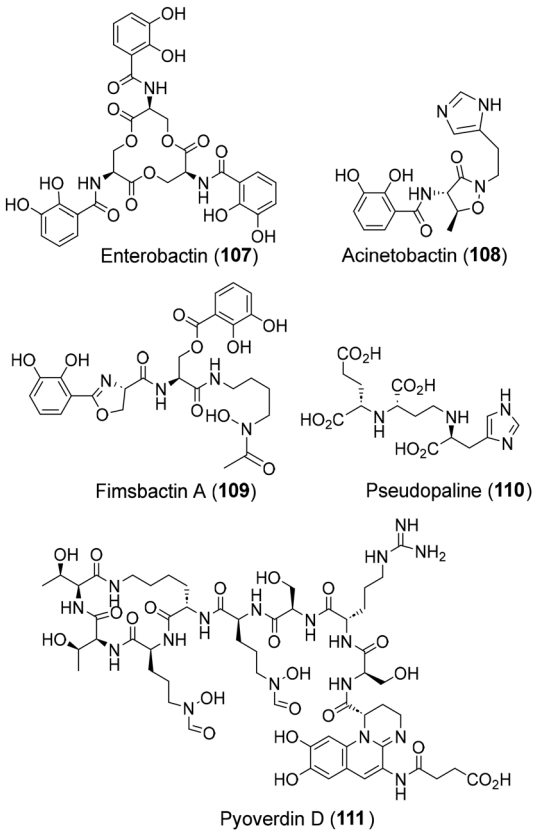
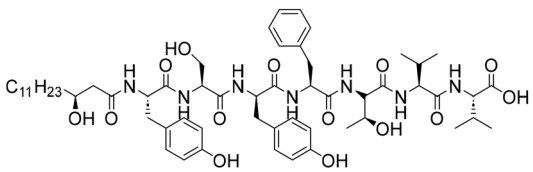


Fig. 6 Siderophores from human associated Gram −ve bacteria.



Strains	MIC (μg/mL)				
	107	carbenicillin	carbenicillin + 112	dicloxacillin	dicloxacillin + 112
S. aureus	8	--	--	--	--
S. pneumoniae	4	--	--	--	--
MRSA USA300	8	32	1	--	--
MRSA COL	> 500	--	--	256	8

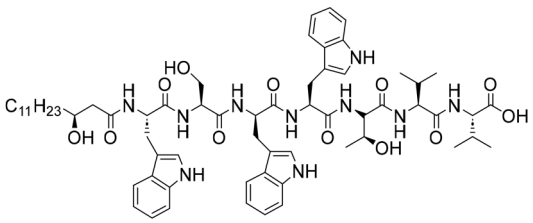


Fig. 7 Syn-BNP antibiotic potentiators humimycin A (**112**) and **113**.

currently underway with live fecal microbiota or assorted consortia for the treatment of gut-related syndromes.[66,67] However, treatment of more severe infections caused by MRSA, VRE and Gram −ve pathogens still relies on targeted therapies using small molecule drugs. In this context, a few natural products from the human microbiome are emerging as promising leads for clinical applications,[68] and are highlighted below.

4.1. Nisin A

Nisin A (**114**, Fig. 8) and its eight siblings are heat resistant pentacyclic lantibiotics produced by lactic acid bacteria (*Lactococcus* sp.),[69] among which, nisin A and Z have been approved as food preservatives with acceptable safety profiles in humans. Furthermore, in view of the clinical success of nisin extracts as effective probiotics[70] and potent activities against MRSA, *S. pneumoniae*, *Enterococci*, *H. pylori* and *C. difficile*, nisin A is emerging as a promising lead for antibiotic development. Improvements in sub-optimal pharmacokinetics of nisin A through bio-engineered analoging have met with limited success. In this regard, the recently reported SPPS approach to nisin A[71] holds much promise in augmenting diversity creation through innovative chemical interventions.[26h] It is contextual to recall that a truncated variant of nisin A has been recently discovered with similar antibacterial profile and identified as cesin A (**115**, Fig. 8),[69d] which appears to be a promising target for global analoging towards pharmacological optimization.

4.2. Epidermicin NI01

Produced by the skin isolate *Staphylococcus epidermidis* 224, epidermicin NI01 is an unmodified 51-residue bacteriocin (MAAFMKLIQFLATKGGKYVSLAWKHK-GTILKWINAGQSFEWI-TKQKKLWA) which exhibited potent activity against a panel of Gram +ve bacteria, including MRSA, VRE and biofilm-forming *S. epidermidis* strains (MICs 1–4 μg mL^{-1}).[72a] In a pre-clinical study conducted at Amprologix, a single dose of epidermicin NI01 demonstrated resistance-free properties and six times more efficacy in reducing *in vivo* nasal MRSA burden than the "gold standard" antibiotic mupirocin.[72b] A 14-day safety and tolerability study of epidermicin NI01 formulation returned satisfactory end points with no adverse side effects. Based on these findings, the inventors have planned IND filing and clinical trials of epidermicin NI01 for nasal MRSA de-colonization in 2024.[72c]

4.3. Mutacin 1140 (mutacin III)

Produced by the oral bacteria *Streptococcus mutans* JH100, the aminovinyl cysteine lantibiotic mutacin 1140 (mutacin III, **116**, Fig. 9) has been the focus of several investigations due to its antibiotic activity (against MRSA, VRE, *C. difficile*), low susceptibility to resistance and a novel mode of action (lipid-II abduction).[73,74] In an effort to develop mutacin 1140 based antibiotics, Oragenics screened nearly 700 analogs of mutacin 1140, locating key point mutations *viz.* Lys2Ala, Arg13Ala and Phe1Ile as the chemical handles for

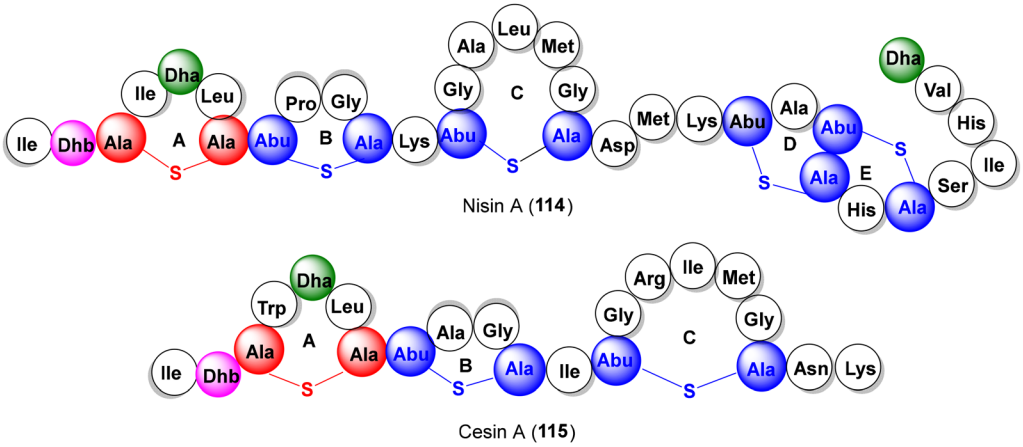


Fig. 8 Nisin A (**114**) and its truncated analog cesin A (**115**).

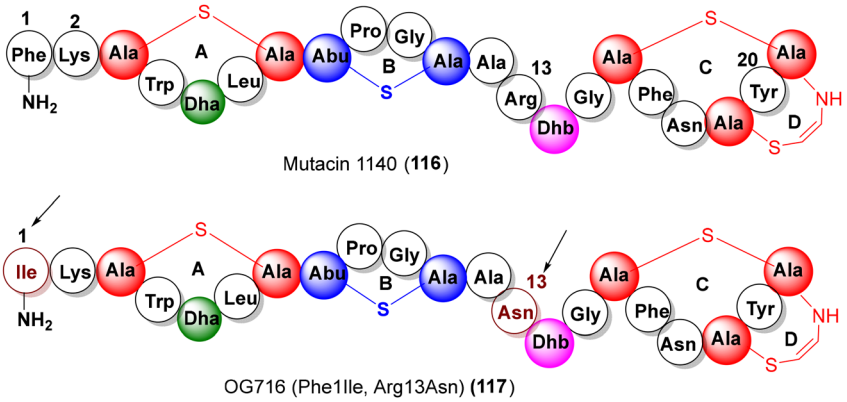


Fig. 9 Structures of mutacin 1140 (**116**) and the mutated analog OG716 (**117**).

modulating the pharmacokinetic attributes.[75] Recently, as a pre-clinical proof-of-concept, positive results from a detailed pharmacological, toxicological and dose-range assessment of an advanced variant OG716 (**117**, Phe1Ile, and Arg13Asn) have been reported for treatment against *C. difficile* infections.[76]

4.4. Linaclotide, plecanatide, dolcanatide

The *E. coli* derived heat-stable enterotoxin STa, together with the endogenous peptide hormones guanylin and uroguanylin, are potent guanylate cyclase C agonists, which regulate gastrointestinal fluid transits. Hence, it was reasoned that the synthetic analogs of STa, guanylin and uroguanylin could be effective therapeutic agents against irritable bowel syndrome (IBS), chronic idiopathic constipation (CIC), *etc.*,[77] leading to the discovery of three IBS drugs – linaclotide (**118**),[78] plecanatide (**122**)[79] and dolcanatide (**123**)[80] (Fig. 10). Considering its unique structural features (3 disulfide bridges) and clinical utility, linaclotide has received repeated attention from practitioners of total synthesis.[81] The most effective among them is by Brik and co-workers, deploying orthogonal cysteine-protecting groups (SNBzl and SAcm), for highly regioselective successive disulphide cross-linkings (red, blue and green) on a SPPS derived linear precursor **119** (Scheme 11).[81c] Two solid-phase total syntheses of plecanatide have also been reported in the recent literature.[79c,d]

5. Prospective natural product targets for the total synthesis

From the total synthesis and utilitarian perspective, a few potentially attractive natural product targets could be identified for future endeavors (**124–134**, Fig. 11).[82–91] Among them, the highly convoluted sactipeptides streptosactin (**128**),[84] tryglysin A and B (**129** and **130**)[85] and ruminococcin C1 (**131**),[86–88] are of particular interest due to their strong antibiotic action and favourable clinical attributes (non-toxic towards eukaryotic cells and little tendency towards developing

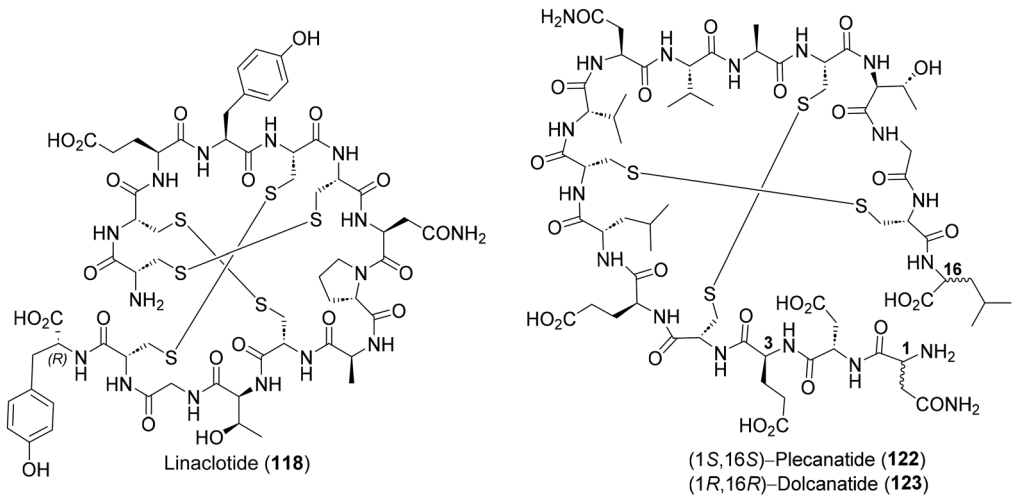
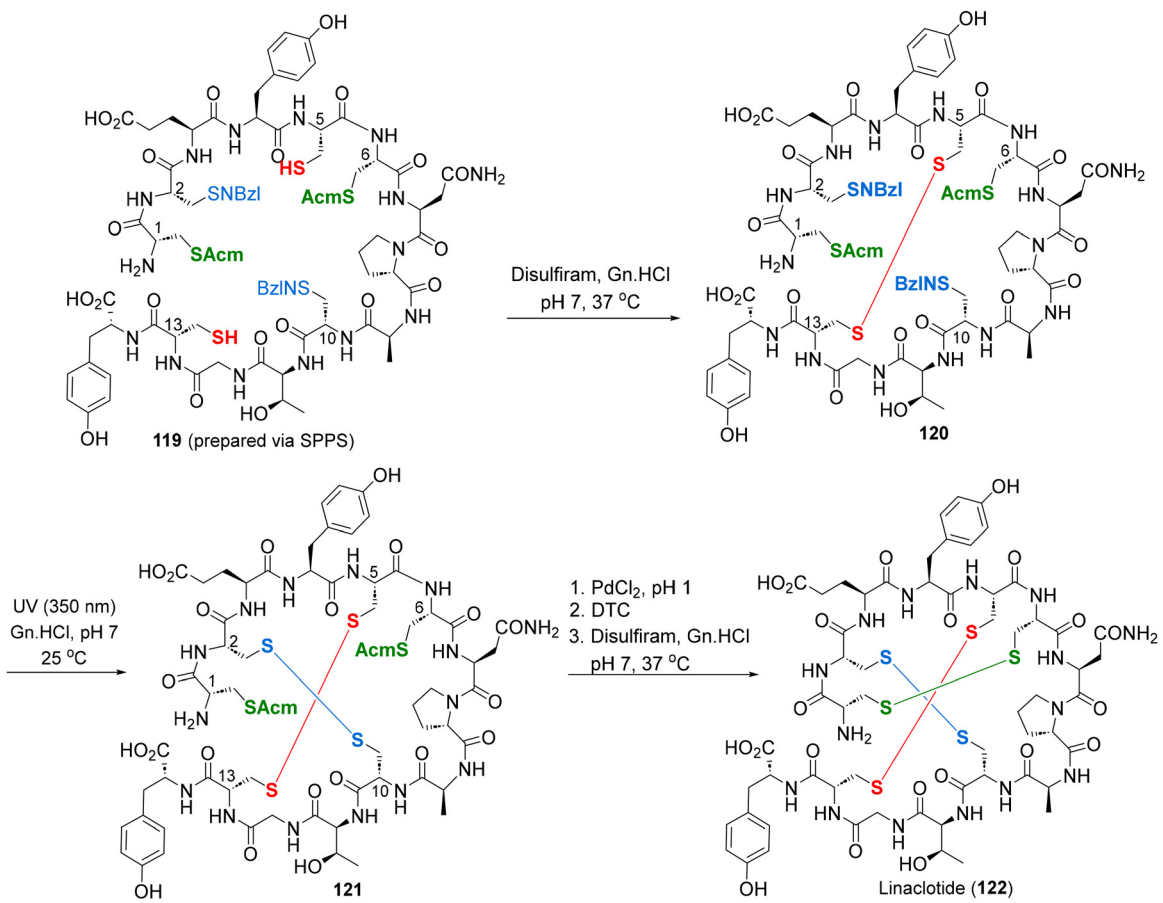


Fig. 10 Linaclotide (**118**), plecanatide (**122**) and dolcanatide (**123**).



Scheme 11 One-pot synthesis of linaclotide (**104**) *via* selective disulphide cross-linking

resistance). In addition, lasso peptides microcin J25 (**134**), acinetodin, klebsidin, *etc.*, with potent antibiotic activities against Gram –ve pathogens, are highly challenging synthetic targets due to their unique catenane-like structures (lariat protoknot) where the C-terminal ends are threaded (dotted line) through a peptide macrocycle.[92,93]

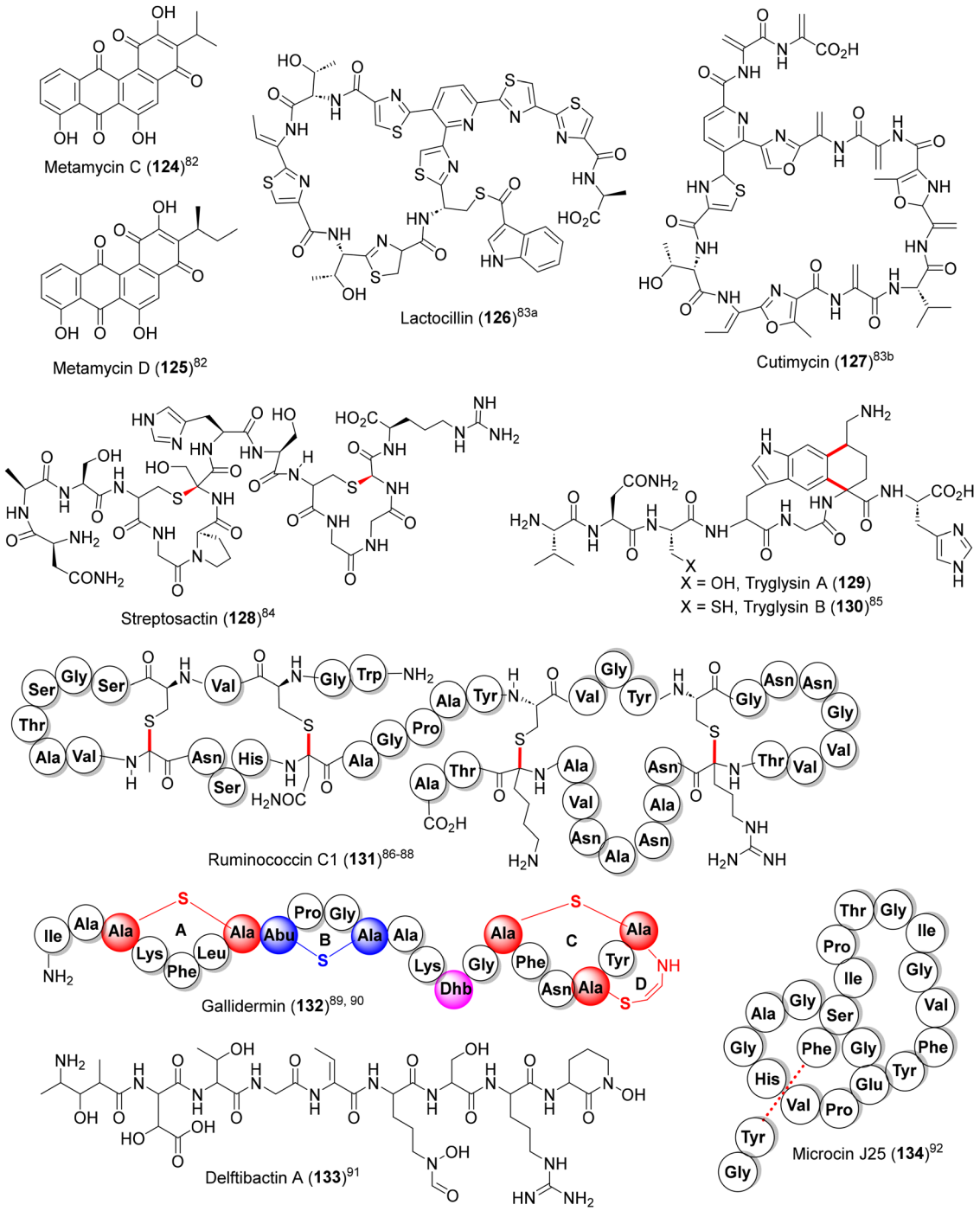


Fig. 11 Natural products from the human microbiome as prospective targets for total synthesis

6. Summary and outlook

The evolving role of the human microbiome as a key determinant in human health and the concurrent discovery of encoded secondary metabolites as potent antibiotics have revealed the enormous potential of the human microbiome as a rich new source of antibacterial natural products. In this review, we have captured the emergent landscape of natural products derived from human commensal bacteria and highlighted their salient features. These new natural products *viz.* metamycin C–D, mutanobactin A–D, lugdunin and the RiPP variants epilancin 15×, ruminococcin C1, *etc.*, have evoked considerable interest in the organic synthesis community due to their potent antibacterial properties and marked distinguishing structural features *vis-à-vis* conventional antibiotics. These features flagged the prospect of exploring their potential utility

against MDR bacteria, pathogenic viruses, fungi and parasites (AMR, in general) as well as gut-related syndromes. In view of their intriguing structures, innovative synthetic strategies like modular building block approaches, deployment of enantiopure orthogonal synthons, novel heterocyclic macrocyclizations, among others, were leveraged as key enabling tactics for total synthesis and diverted organic synthesis to explore new bioactive chemical spaces. The prospects of antibiotic drug discovery through these approaches have been highlighted through a few case studies which include the recently approved IBS drugs linaclotide and plecanatide as archetypal examples.

Looking ahead, it is important to sustain the momentum and the promising advances enumerated above, with major thrust directed towards augmenting laboratory isolation and structural characterization activities through synergistic applications of genome mining techniques and innovations in culturomics.[10] At the same time, new imaginative synthetic designs, including merged bioengineering–chemical synthesis approaches,[94] need to be explored to tackle the structural and scale-up challenges to reveal new chemical spaces. In addition to the above, efforts directed towards newer areas of microbiome research *viz*. exploration of probiotic therapy, application of gut microbes for selective biotransformations[95] and insights into the chemistry of host–microbe interaction[96] would be welcome advances in order to amplify the ambit of the human microbiome research landscape.

Lastly, it is heartening to note that many countries, particularly those with a living traditional knowledge base and cultural moorings,[97] have also embarked on initiatives to unveil the mysteries of the diet–season–microbiome triad on human health and harness its role for the overall well-being of people.

Author contributions

All authors contributed equally to the manuscript.

Conflicts of interest

There are no conflicts to declare.

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