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Current Opinion in Biotechnology

Bio-inspired engineering of thiopeptide antibiotics advances the expansion of molecular diversity and utility

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Thiopeptide antibiotics, which are a class of sulfur-rich and highly modified peptide natural products, exhibit a wide variety of important biological properties. These antibiotics are ribosomally synthesized and arise from post-translational modifications, exemplifying a process through which nature develops the structural complexity from Ser/Thr and Cys-rich precursor peptides. Following a brief review of the knowledge gained from nature in terms of the formation of a common thiopeptide scaffold and its specialization to individual members, we highlight the significance of bio-inspired engineering, which has greatly expanded the molecular diversity and utility of thiopeptide antibiotics regarding the search for clinically useful agents, investigation into new mechanisms of action and access to typically 'inaccessible' biosynthetic processes over the past two years.

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Current Opinion in Biotechnology 2017, **48**:210–219

This review comes from a themed issue on **Pharmaceutical biotechnology**

Edited by **Tiangang Liu** and **Chu-Young Kim**

For a complete overview see the Issue and the Editorial

Available online 30th June 2017

http://dx.doi.org/10.1016/j.copbio.2017.06.008

Introduction

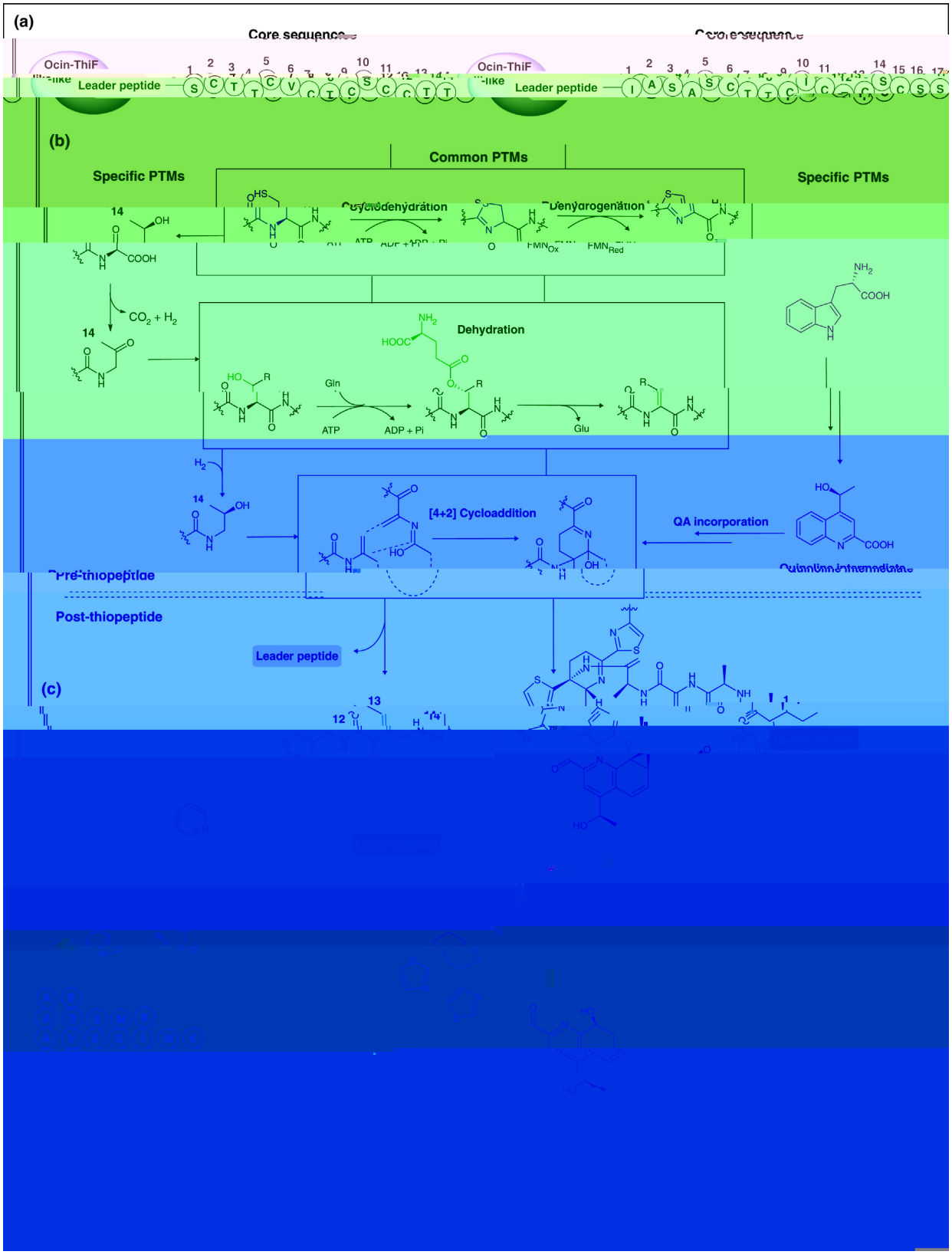
Over the past decades, peptide natural products (NPs) with ribosomal origin have been a focus in the discovery of new biosynthetic mechanisms [1]. Increasing evidence indicates that post-translational modifications (PTMs) of ribosomally synthesized precursor peptides are comparable to non-ribosomal peptide synthetases in terms of the creation of structurally complex molecules [2–4]. A precursor peptide typically consists of an N-terminal leader sequence and a C-terminal core sequence (Figure 1a). A myriad of PTMs can be applied, in a manner either dependent or independent of the former sequence, to transform the latter sequence into mature product(s) (Figure 1b). Although the building blocks are limited to 20 proteinogenic amino acids, in contrast to a much wider array of substrates found in the biosynthesis of non-ribosomal peptide NPs [5], the sequences of precursor peptides and the associated enzyme-processing strategies have been shown to be highly variable and evolvable in the formation of various ribosomal peptide NPs [6].

One example comes from thiopeptide antibiotics [7–10], a growing family of sulfur-rich peptide NPs that are ribosomally synthesized and post-translationally modified (Figure 1c). These antibiotics possess a wide variety of biological properties, e.g., anti-infection, anticancer and immunosuppression, and are beneficial to humans largely because of their highly functionalized unusual architectures, which share a macrocyclic peptidyl core that contains a six-membered heterocycle domain central to multiple azoles and dehydroamino acids [11]. Recent studies revealed a wide distribution of thiopeptide-encoding sequences in the genomes of human microbiota [12], generating interest in the roles played by related products in microbe-host interactions. Derivatization efforts has attracted considerable attention of molecular engineering to further expand the chemical spaces of thiopeptide antibiotics, improve their biological activities and overcome physical disadvantages [13]; however, the accessibility and efficiency of chemical synthesis are often impeded by the structural complexity of these compounds. The ribosomal origin of thiopeptide antibiotics was established in 2009 [14–18], garnering appreciation for the mechanisms that nature employs to develop various PTM strategies and obtain individual thiopeptide members from Cys and Ser/Thr residue-rich precursor peptides. This appreciation has recently motivated rational applications of various technologies for structural diversification (as discussed below), resulting in a number of thiopetide analogs, either expected or unexpected.

Formation of a common thiopeptide framework and its specialization in nature

Thiopeptide antibiotics structurally appear to be the macrocyclic variants of goadsporin-like NPs, each of which derives a six-membered central heterocycle domain from a linear peptide possessing both azol(in)es and dehydroamino acid residues [19]. The in vitro biosynthesis of the thiopeptide member thiomuracin was successful [20••], benefiting from the recent knowledge regarding

Figure 1


(a)
Core sequence
Ocin-ThiF -like
Leader peptide
(b)
Common PTMs
Specific PTMs
Specific PTMs
Cyclodehydration
Dehydrogenation
Dehydration
Gln
ATP
ADP + Pi
Glu
[4+2] Cycloaddition
QA incorporation
Pre-thiopeptide
Post-thiopeptide
Leader peptide
(c)

cyclodehydratases in the biosynthesis of azol(in)e-containing microcins and cyanobactins [21,22], dehydratases in the biosynthesis of dehydroamino acid-involving lantibiotics [23] and, particularly, the enzymes responsible for Diels-Alder-like [4 + 2] cycloaddition reactions [24,25]. Fabricating the structural features of thiopeptides using a logical assembly of the above-mentioned enzymatic activities demonstrates the idea that in addition to the precursor peptide-encoding gene, the biogenesis of a common thiopeptide framework contains a minimum of six conserved PTM genes (Figure 2). These genes code for 1) an Ocin-ThiF-like protein responsible for engaging the precursor peptide [26], 2) a YcaO-like superfamily protein and a flavoprotein for Cys/Ser-residue processing through phosphorylation-based cyclodehydration and subsequent dehydrogenation to produce azoles [27], 3) a pair of proteins with $tRNA^{Glu}$-dependent glutamylation and elimination activities for Ser/Thr-residue dehydration to yield dehydroamino acids [28], and 4) a unique Diels-Alderase-like protein for intramolecular cross-bridging to furnish the central heterocycle domain (Figure 1b) [29]. According to these genetic characteristics, many related biosynthetic gene clusters were mined from the bacterial strains that were previously unknown to be the thiopeptide producers [30].

In addition to common PTMs, the constitution of the thiopeptide family, which includes over 100 natural members, relies on the sequence permutation of precursor peptides and the combination with specific PTMs that are necessary for the individualized treatment of each precursor peptide [31]. A comparative analysis of the currently available biosynthetic gene clusters supports the unifying theme in which nature develops diversity (Figure 2). The incorporation of different specific PTM elements into a minimum of thiopeptide biogenesis results in the variable functionalization of a thiopeptide framework, e.g., the decoration of the central domain and the macrocyclic core system, the fabrication of a side-ring system and the tailoring of the C-terminal extended side chain [32–36]. Intriguingly, the specialization can proceed before or after the formation of a thiopeptide-characteristic scaffold, and many specific PTMs are interdependent on common PTMs [31]. In the pathway of the monomacrocyclic member thiocillin, the oxidative decarboxylation of the C-terminal Thr residue of the precursor peptide is a pre-thiopeptide PTM that immediately follows the formation of thiazol(in)es (Figure 1b). This step is indispensable for Ser/Thr-residue dehydration and subsequent intramolecular cyclization to furnish a thiopeptide framework [37•]. In the pathway of the bi-macrocyclic member thiostrepton, the formation of a quinaldic acid (QA) moiety and its incorporation into the precursor peptide are evidently essential for the construction of the thiopeptide scaffold (Figure 1b). However, the conjugation of QA and the N-terminus of the core peptide sequence was recently confirmed to be a post-thiopeptide PTM during the formation of the side-ring system of thiostrepton [38••].

Diversity-oriented biosynthesis by genetic engineering of precursor peptides

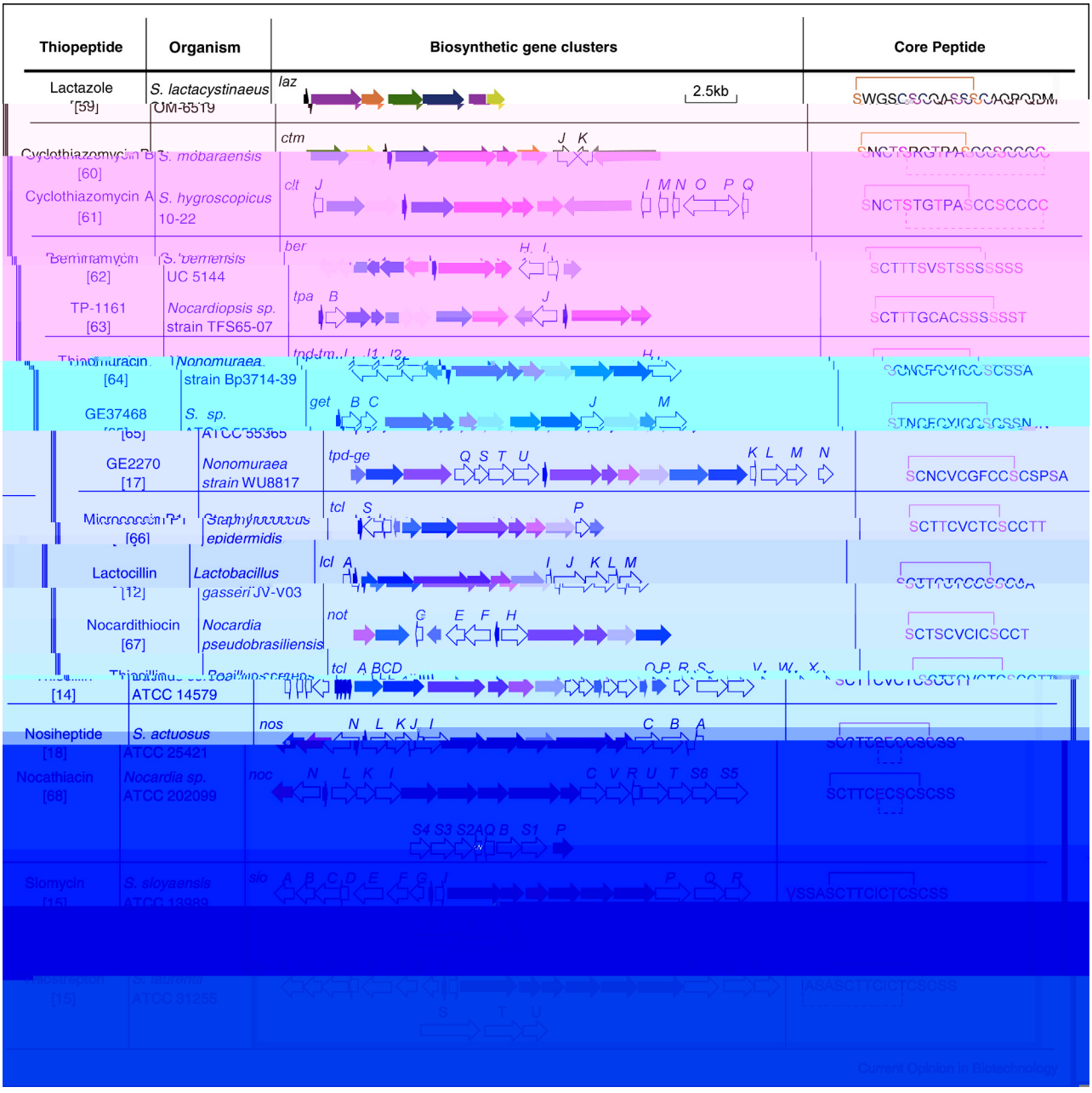
Revealing the ribosomal origin of thiopeptide antibiotics lays the foundation for the structural diversification of peptidyl skeletons by sequence engineering of their precursor peptides using microbial genetics approaches [9]. This diversification, which has produced over hundreds of new analogs, is of significant interest in constructing a thiopeptide-like NP library to search for new drug leads and to evaluate the overall PTM capacity of thiopeptide biosynthetic machineries with respect to the variation of precursor peptides.

Facile preparation of thiopeptide variants considerably simplifies systematic structure-activity (SAR) analysis, which, in fact, is a challenge in current chemical synthesis-based approaches due to the structural complexity of the molecules. Mechanistically different from current chemotherapeutics targeting the bacterial ribosome, many thiopeptides (including thiocillin and thiostrepton) are known to bind within a cleft that is located between the L11 protein and the 23S rRNA of the 50S large ribosomal subunit, thereby hindering translation factor binding and subsequent protein synthesis [39]. A majority of the surface buried by the molecule on the ribosome is attributed to the shared macrocyclic core system; however, in this system, the contribution of residue composition to binding affinity requires evaluation. Recently, saturation mutagenesis of the residues within the macrocyclic core of thiocillin was conducted (Figure 1c) [40••], leading to the production of a number of variants, of which 8 were more active than the parent compound against the test strain *Bacillus subtilis*. These variants, either active or inactive, were then subjected to a comparative analysis by computational modeling, revealing that a side chain substitution changes the ring entropy/conformational flexibility, which has a significant impact on molecular binding, thus affecting antibacterial activity [40••].

A similar diversity-oriented biosynthesis has recently been performed for thiostrepton engineering, with a focus on the residues that conjugate the QA moiety within the

(Figure 1 Legend) Biosynthetic origin, pathways and structures of thiopeptide antibiotics, as exemplified by thiocillin (left) and thiostrepton (right). **(a)** Precursor peptides, whose N-terminal leader sequences (yellow) are bound by pathway-specific Ocin-ThiF-like proteins (blue). **(b)** Common PTMs in the formation of a shared thiopeptide scaffold (middle) and specific PTMs for its specialization toward thiocillin (left) and thiostrepton (right), respectively. **(c)** Chemical structures of thiocillin (left) and thiostrepton (right). The thiopeptide-characteristic hallmarks are highlighted in color. The residues of each precursor peptide and their associated substitutions that produced mature variants are listed [40••,41,42,43••,44•,55–58].

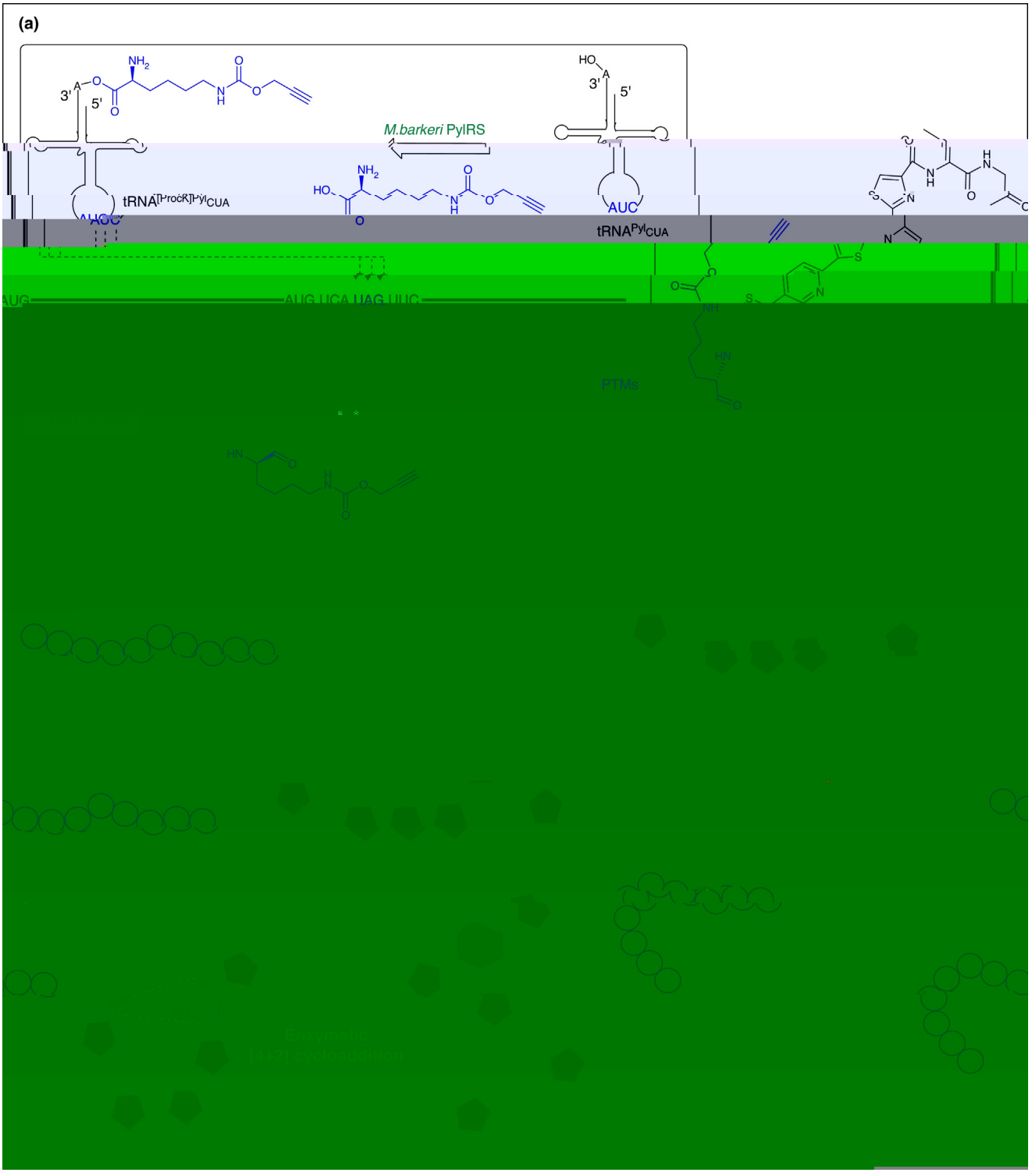
Figure 2



Biogenesis for developing the structural diversity and complexity of thiopeptide antibiotics in nature. The biosynthetic gene clusters are composed of the genes coding for precursor peptides (black), the common PTM genes (i.e., for Ocin-ThiF-like proteins (dark green), YcaO-like superfamily proteins (dark blue), flavoproteins (light green), dehydratase pairs (purple) and Diels-Alderase-like proteins (yellow), various specific PTM genes (white) and the accessory genes involved in self-resistance and regulation (gray). (**b**) Core peptide sequences of associated thiopeptide antibiotics. The shared macrocyclic core systems (solid) and the optional side-ring systems (dashed) are indicated. The residues undergoing PTMs to form various structural characteristics are highlighted in color, e.g., blue for azol(in)e, purple for dehydroamino acids, orange for 6-membered central domain, and purple for side-ring construction [59–68].

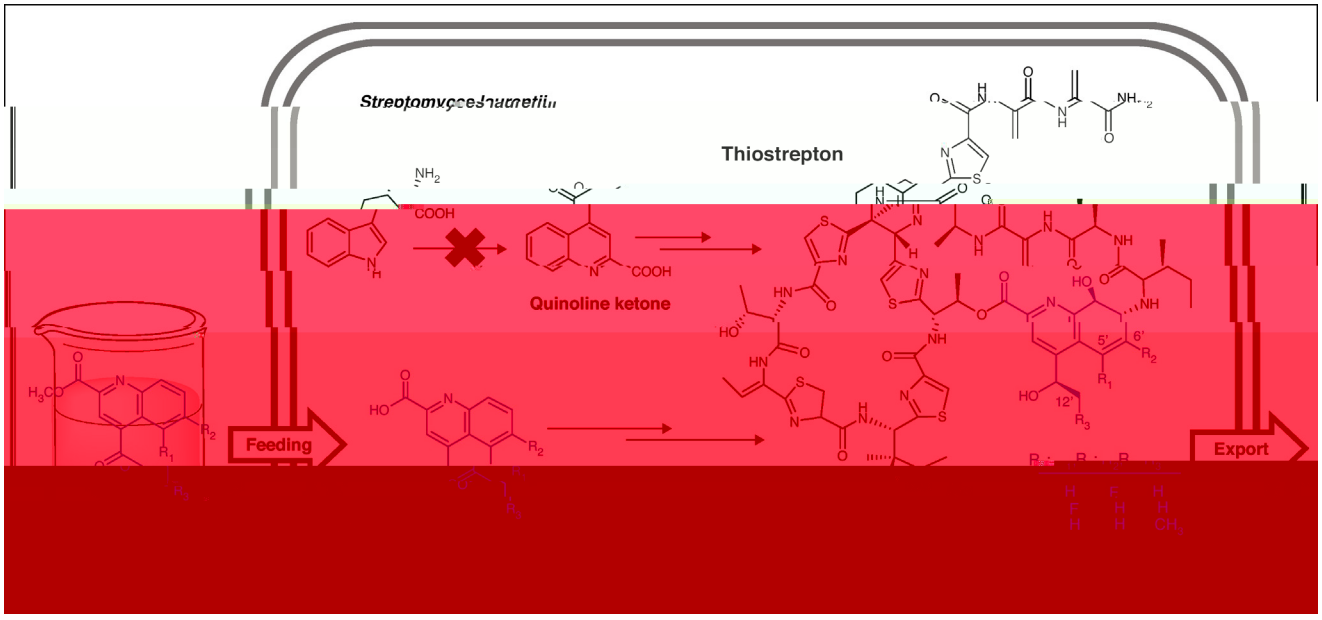
27-membered large side-ring system (Figure 1c) [41,42,43••,44•]. Saturation mutagenesis of Ile1, Ala2 and Ala4 at the N-terminus of the core sequence resulted in 6, 8 and 16 new thiostrepton variants, respectively, revealing the discrepancy in the permissibility of PTMs to the changes at these positions. In particular, the double mutation of Ile1Val and Ala2Ser in the thiostrepton-producing *S. laurentii* strain allowed the robust production

Figure 3



Bio-inspired incorporation of naturally unavailable building blocks. (**a**) Introduction of ncAAs (e.g., *N*ε-prop-2-ynyloxycarbonyl-l-lysine, for replacing residue Thr3 of the core sequence of the precursor peptide) into thiocillin using the orthogonal system established in the Gram-positive host *Bacillus cereus*. (**b**) Chemoenzymatic route toward of the synthesis of thiopeptide variants. The synthetic steps are indicated by the dashed arrows, in contrast to the enzymatic conversion, which is shown by the solid arrow.

Figure 4



Production of thiostrepton variants that bear a selectively fluorinated or methylated QA moiety (purple) in *S. laurentii*. The ester analogs of the key quinoline ketone intermediate of QA were chemically synthesized and fed individually into a *S. laurentii* mutant strain. This mutant strain, which lacks the first 2-methylation step to initiate QA formation, is incapable of producing thiostrepton. After hydrolysis *in vivo*, each exogenous quinoline ketone analog can surrogate the wild-type intermediate being incorporated, leading to the production of thiostrepton analogs with designed modifications.

by fermentation according to recently uncovered biosynthetic mechanisms in the formation of QA (Figure 4) [35,50••].

The resultant variants are more potent than thiostrepton and control chemotherapeutics, e.g., vancomycin [50••]. The sensitive pathogens included many clinical isolates that are resistant to current drugs, e.g., methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *enterococci* (VRE), demonstrating the rationale for QA modification. Remarkably, using these variants as chemical probes revealed an unexpected new mechanism of thiostrepton, which is capable of inducing host autophagy during treatment with intracellular pathogens in addition to directly targeting the bacterial ribosome [51••]. This intracellular action, which is unique to thiostrepton-type thiopeptide antibiotics and is sensitive to the modification of the QA group, may inspire future changes in the treatment of intracellular pathogens since the contribution of host cell responses to antimicrobial chemotherapy has been increasingly recognized. Recently, the combination of QA modification and C-terminal tailoring that has improved anti-infectious activity was conducted and showed a synergistic engineering effect [52], as exemplified by the extremely potent methyl ester analog of 5′-fluoro-thiostrepton, which exhibited minimum inhibitory concentrations at <0.125 ng/mL, 0.25–0.5 ng/mL, 0.25–0.5 ng/mL and 0.125–0.5 ng/mL against various PRSP, MRSA, VRE and *Clostridium difficile* clinical isolates, respectively.

Remarkably, 6′-fluorination of QA lowers the reactivity of this moiety and slows the cyclization process for side-ring closure, which rapidly proceeds in *S. laurentii* without this modification, thereby causing the accumulation of an open side-ring epoxy intermediate [38••]. This unexpected finding ultimately revealed the maturation process of thiostrepton, which involves an unusual dual activity of an α/β hydrolase fold protein for cascade endopeptidyl hydrolysis/leader sequence removal and epoxide ring opening/side-chain macrocyclization in the biosynthetic pathway [38••,53]. The endopepeptidase activity of this protein, which is responsible for selective hydrolysis between Met-1 and Ile1 of a wild-type precursor peptide, appears to be promiscuous and tolerates the substitution of either of the residues with nonpolar amino acids. By exploiting the coupled activity for epoxide ring opening and macrocyclization, changing the size of the side-ring system is practical, as evidenced by the results from the mutation of Ala2Ile or Ala2Val, which created a new hydrolytic site between Ile1 and Ile2 or between Ile1 and Val2 of each recombinant precursor peptide, thus allowing the production of an additional thiostrepton variant that bears the contracted QA-containing side-ring system [43••].

Conclusion and perspectives

Following a brief review concerning the generality and specificity of the biosynthesis of thiopeptide antibiotics, we focused on a few recent examples, primarily from the studies on the members thiocillin (mono-marcocyclic) and thiostrepton (bi-macrocyclic), to highlight the approaches used to accelerate the diversification process for the expansion of molecular utility in searching for clinically useful variants, examining new modes of action and accessing the biosynthetic processes that are difficult to realize. As with other ribosomally synthesized and post-translationally modified peptide NPs, thiopeptide antibiotics feature a highly evolvable 'template'-biosynthetic logic that facilitates molecular engineering [54]. This logic has not been fully appreciated to date, and the associated PTMs involve a number of unusual biochemical mechanisms that remain to be determined. A further understanding of these mechanisms would significantly facilitate the design, development and utilization of compatible machineries, synthetic, biosynthetic or both, to expand the chemical spaces of thiopeptide antibiotics and their associated biological functions.

Acknowledgements

This work was supported in part by grants from NSFC (31430005, 21520102004, 21472231 and 21621002), CAS (QYZDJ-SSW-SLH037 and XDB20020200), STCSM (14JC1407700 and 15JC1400400), K.C. Wong Education Foundation and Chang-Jiang Scholars Program of China.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Arnison PG, Bibb MJ, Bierbaum G, Bowers AA, Bugni TS, Bulaj G, Camarero JA, Campopiano DJ, Challis GL, Clardy J *et al.*: **Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature**. *Nat Prod Rep* 2013, **30**:108-160.

2. Nolan EM, Walsh CT: **How nature morphs peptide scaffolds into antibiotics**. *ChemBioChem* 2009, **10**:34-53.

3. Marahiel MA: **Working outside the protein-synthesis rules: insights into non-ribosomal peptide synthesis**. *J Pept Sci* 2009, **15**:799-807.

4. McIntosh JA, Donia MS, Schmidt EW: **Ribosomal peptide natural products: bridging the ribosomal and nonribosomal worlds**. *Nat Prod Rep* 2009, **26**:537.

5. Walsh CT, O'Brien RV, Khosla C: **Nonproteinogenic amino acid building blocks for nonribosomal peptide and hybrid polyketide scaffolds**. *Angew Chem Int Ed Engl* 2013, **52**:7098-7124.

6. Ortega MA, van der Donk WA: **New insights into the biosynthetic logic of ribosomally synthesized and post-translationally modified peptide natural products**. *Cell Chem Biol* 2016, **23**:31-44.

7. Walsh CT, *Nat Prod Rep* 2013, 30:108-160. AA,7973h CThiaz.3(l)-337.6((and)-338(-8.7(10.5(s)]TJ-1.06987973h(modified)-260)]TJ-13.1Tdging8(peafolds)]Tcas)-336.pea-33

28. Ortega MA, Hao Y, Zhang Q, Walker MC, van der Donk WA, Nair SK: **Structure and mechanism of the tRNA-dependent lantibiotic dehydratase NisB**. *Nature* 2014, **517**:509-512.

29. Wever WJ, Bogart JW, Baccile JA, Chan AN, Schroeder FC, Bowers AA: **Chemoenzymatic synthesis of thiazolyl peptide natural products featuring an enzyme-catalyzed formal [4 + 2] cycloaddition**. *J Am Chem Soc* 2015, **137**:3494-3497.

30. Li J, Qu X, He X, Duan L, Wu G, Bi D, Deng Z, Liu W, Ou H-Y: **ThioFinder: a web-based tool for the identification of thiopeptide gene clusters in DNA sequences**. *PLoS ONE* 2012, **7**:e45878.

31. Wang S, Zhou S, Liu W: **Opportunities and challenges from current investigations into the biosynthetic logic of nosiheptide-represented thiopeptide antibiotics**. *Curr Opin Chem Biol* 2013, **17**:626-634.

32. Yu Y, Guo H, Zhang Q, Duan L, Ding Y, Liao R, Lei C, Shen B, Liu W: **NosA catalyzing carboxyl-terminal amide formation in nosiheptide maturation via an enamine dealkylation on the serine-extended precursor peptide**. *J Am Chem Soc* 2010, **132**:16324-16326.

33. Liao R, Liu W: **Thiostrepton maturation involving a deesterification–amidation way to process the C-terminally methylated peptide backbone**. *J Am Chem Soc* 2011, **133**:2852-2855.

34. Zhang Q, Li Y, Chen D, Yu Y, Duan L, Shen B, Liu W: **Radical-mediated enzymatic carbon chain fragmentation-recombination**. *Nat Chem Biol* 2011, **7**:154-160.

35. Duan L, Wang S, Liao R, Liu W: **Insights into quinaldic acid moiety formation in thiostrepton biosynthesis facilitating fluorinated thiopeptide generation**. *Chem Biol* 2012, **19**:443-448.

36. Liu W, Xue Y, Ma M, Wang S, Liu N, Chen Y: **Multiple oxidative routes towards the maturation of nosiheptide**. *ChemBioChem* 2013, **14**:1544-1547.

37. • Bewleya KD, Bennallack PR, Burlingamea MA, Robisonb RA, Griffittsb JS, Miller SM: **Capture of micrococcin biosynthetic intermediates reveals C-terminal processing as an obligatory step for in vivo maturation**. *Proc Natl Acad Sci* 2016, **115**:12450-12455.
Using a refactored modular in vivo system that contains the variable combination of biosynthetic genes, the authors of this work established the PTM logic in the biosynthetic pathway of thiocillin.

38. •• Zheng Q, Wang S, Duan P, Liao R, Chen D, Liu W: **An α/β-hydrolase fold protein in the biosynthesis of thiostrepton exhibits a dual activity for endopeptidyl hydrolysis and epoxide ring opening/macrocyclization**. *Proc Natl Acad Sci* 2016, **113**:14318-14323.
The authors of this work demonstrated an unprecedented α/β hydrolase fold protein that employs the same Ser-His-Asp catalytic triad to catalyze cascade C-N bond cleavage and formation during the biosynthesis of thiostrepton for side-ring system construction and molecular maturation.

39. Harms JM, Wilson DN, Schluenzen F, Connell SR, Stachelhaus T, Zaborowska Z, Spahn CM, Fucini P: **Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin**. *Mol Cell* 2008, **30**:26-38.

40. •• Tran HL, Lexa KW, Julien O, Young TS, Walsh CT, Jacobson MP, Wells JA: **Structure-activity relationship and molecular mechanics reveal the importance of ring entropy in the biosynthesis and activity of a natural product**. *J Am Chem Soc* 2017, **139**:2541-2544.
This work demonstrated the importance of ring entropy in the biosynthesis and activity of macrocyclic natural products, as exemplified by a systematic SAR and molecular mechanics analysis of thiocillin variants that were produced by saturation mutagenesis of the residues within the core sequence.

41. Guo H, Wang J, Li Y, Yu Y, Zheng Q, Wu J, Liu W: **Insight into bicyclic thiopeptide biosynthesis benefited from development of a uniform approach for molecular engineering and production improvement**. *Chem Sci* 2014, **5**:240-246.

42. Zhang F, Li C, Kelly WL: **Saturation mutagenesis of TsrA Ala4 unveils a highly mutable residue of thiostrepton A**. *ACS Chem Biol* 2015, **10**:998-1009.

43. •• Zhang F, Li C, Kelly WL: **Thiostrepton variants containing a contracted quinaldic acid macrocycle result from mutagenesis of the second residue**. *ACS Chem Biol* 2016, **11**:415-424.
This is the first report revealing that the side-ring systems of thiostrepton-type thiopeptide antibiotics is changeable in size.

44. • Duan P, Zheng Q, Lin Z, Wang S, Chen D, Liu W: **Molecular engineering of thiostrepton via single "base"-based mutagenesis to generate side ring-derived variants**. *Org Chem Front* 2016, **3**:1254-1258.
In addition to saturation mutagenesis of residue Ile1 residing in the core sequence of thiostrepton, the authors of this work conducted the simultaneous mutations of Ile1 and Ala2 to Val and Ser for heterologously producing the more potent analog siomycin in *S. laurentii*.

45. Yaqi S, Kitai S, Kimura T: **Stimulative effect of elemental sulfur on siomycin production by *Streptomyces sioyaensis***. *Appl Microbiol* 1971, **22**:153-156.

46. Liu CC, Schultz PG: **Adding new chemistries to the genetic code**. *Annu Rev Biochem* 2010, **79**:413-444.

47. •• Luo X, Zambaldo C, Liu T, Zhang Y, Xuan W, Wang C, Reed SA, Yang PY, Wang RE, Javahishvili T *et al.*: **Recombinant thiopeptides containing noncanonical amino acids**. *Proc Natl Acad Sci* 2016, **113**:3615-3620.
This work developed an orthogonal system in the Gram-positive bacterial *Bacillus cereus* where the authors exemplified the site-specific incorporation of ncAAs into thiopeptides.

48. •• Wever WJ, Bogart JW, Bowers AA: **Identification of pyridine synthase recognition sequences allows a modular solid-phase route to thiopeptide variants**. *J Am Chem Soc* 2016, **138**:13461-13464.
In this work, the authors defined a minimum recognition sequence that is necessary and sufficient for Diels-Alder-like activity to furnish the central domain, therefore allowing the application of SPPS to prepare variable recognition sequence-fused, highly modified core sequences for the chemoenzymatic synthesis of thiopeptide variants.

49. Baumann S, Schoof S, Bolten M, Haering C, Takaqi M, Shin-ya K, Arndt HD: **Molecular determinants of microbial resistance to thiopeptide antibiotics**. *J Am Chem Soc* 2010, **132**:6973-6981.

50. •• Wang S, Zheng Q, Wang J, Zhao Z, Li Q, Yu Y, Wang R, Liu W: **Target-oriented design and biosynthesis of thiostrepton-derived thiopeptide antibiotics with improved pharmaceutical properties**. *Org Chem Front* 2015, **2**:106-109.
The authors of this work designed the thiostrepton variants that bear a selectively modified QA moiety according to the known mechanism of action on the bacterial ribosome and provided a precursor-directed mutational biosynthesis approach for their effective preparation in *S. laurentii* by fermentation.

51. •• Zheng Q, Wang Q, Wang S, Wu J, Gao Q, Liu W: **Thiopeptide antibiotics exhibit a dual mode of action against intracellular pathogens by affecting both host and microbe**. *Chem Biol* 2015, **22**:1002-1007.
Thiostrepton and its variants were demonstrated to constitute a type of antibiotics that exhibit an unusual dual action on both the bacterial pathogens and infected host cells. Their anti-infectious activities, which result directly from targeting the bacterial ribosome or indirectly from inducing host autophagy, are sensitive to the functionalization of QA.

52. Wang S, Zheng Q, Wang J, Chen D, Yu Y, Liu W: **Concurrent modifications of the C-terminus and side ring of thiostrepton and their synergistic effects with respect to improving antibacterial activities**. *Org Chem Front* 2016, **3**:496-500.

53. Zheng Q, Wang S, Liao R, Liu W: **Precursor-directed mutational biosynthesis facilitates the functional assignment of two cytochromes P450 in thiostrepton biosynthesis**. *ACS Chem Biol* 2016, **11**:2673-2678.

54. Chen M *et al.*: **Biosynthesis and molecular engineering of template natural products**. *Natl Sci Rev* 2016 http://dx.doi.org/10.1093/nsr/nww045.

55. Acker MG, Bowers AA, Walsh CT: **Generation of thiocillin variants by prepeptide gene replacement and in vivo processing by *Bacillus cereus***. *J Am Chem Soc* 2009, **131**:17563-17565.

56. Bowers AA, Acker MG, Koglin A, Walsh CT: **Manipulation of thiocillin variants by prepeptide gene replacement: structure, conformation, and activity of heterocycle substitution mutants**. *J Am Chem Soc* 2010, **132**:7519-7527.

57. Li C, Zhang F, Kelly WL: **Heterologous production of thiostrepton A and biosynthetic engineering of thiostrepton analogs**. *Mol Biosyst* 2011, **7**:82-90.

58. Li C, Zhang F, Kelly WL: **Mutagenesis of the thiostrepton precursor peptide at Thr7 impacts both biosynthesis and function**. *Chem Commun (Camb)* 2012, **48**:558-560.

59. Hayashi S, Ozaki T, Asamizu S, Ikeda H, Omura S, Oku N, Igarashi Y, Tomoda H, Onaka H: **Genome mining reveals a minimum gene set for the biosynthesis of 32-membered macrocyclic thiopeptides lactazoles**. *Chem Biol* 2014, **21**:679-688.

60. Cox CL, Tietz JI, Sokolowski K, Melby JO, Doroghazi JR, Mitchell DA: **Nucleophilic 1,4-additions for natural product discovery**. *ACS Chem Biol* 2014, **9**:2014-2022.

61. Wang J, Yu Y, Tang K, Liu W, He X, Huang X, Deng Z: **Identification and analysis of the biosynthetic gene cluster encoding the thiopeptide antibiotic cyclothiazomycin in *Streptomyces hygroscopicus* 10-22**. *Appl Environ Microbiol* 2010, **76**:2335-2344.

62. Malcolmson SJ, Young TS, Ruby JG, Skewes-Cox P, Walsh CT: **The posttranslational modification cascade to the thiopeptide berninamycin generates linear forms and altered macrocyclic scaffolds**. *Proc Natl Acad Sci U S A* 2013, **110**:8483-8488.

63. Engelhardt K, Degnes KF, Zotchev SB: **Isolation and characterization of the gene cluster for biosynthesis of the thiopeptide antibiotic TP-1161**. *Appl Environ Microbiol* 2010, **76**:7093-7101.

64. Morris RP, Leeds JA, Naegeli HU, Oberer L, Memmert K, Weber E, LaMarche MJ, Parker CN, Burrer N, Esterow S *et al.*: **Ribosomally synthesized thiopeptide antibiotics targeting elongation factor Tu**. *J Am Chem Soc* 2009, **131**:5946-5955.

65. Young TS, Walsh CT: **Identification of the thiazolyl peptide GE37468 gene cluster from *Streptomyces* ATCC 55365 and heterologous expression in *Streptomyces lividans***. *Proc Natl Acad Sci U S A* 2011, **108**:13053-13058.

66. Bennallack PR, Burt SR, Heder MJ, Robison RA, Griffitts JS: **Characterization of a novel plasmid-borne thiopeptide gene cluster in *Staphylococcus epidermidis* strain 115**. *J Bacteriol* 2014, **196**:4344-4350.

67. Sakai K, Komaki H, Gonoi T: **Identification and functional analysis of the nocardithiocin gene cluster in *Nocardia pseudobrasiliensis***. *PLoS One* 2015, **10**:e0143264.

68. Ding Y, Yu Y, Pan H, Guo H, Li Y, Liu W: **Moving posttranslational modifications forward to biosynthesize the glycosylated thiopeptide nocathiacin I in *Nocardia sp*. ATCC202099**. *Mol Biosyst* 2010, **6**:1180-1185.