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Insights into the thioamidation of thiopeptins to enhance the understanding of the biosynthetic logic of thioamide-containing thiopeptides†

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Thiopeptins are a complex of thiopeptide antibiotics similar in structure to thiostrepton and harboring a thioamide, a rare moiety among natural products. Here, we illustrate through a series of *in vivo* experiments that the thioamide moiety of thiopeptins is generated posttranslationally by a TfuA–YcaO pair, encoded in the thiopeptin biosynthetic gene cluster, before the maturation of the thiopeptide bicyclic scaffold, enhancing the understanding of the biosynthetic logic of thioamide-containing thiopeptides.

Thiopeptides are members of the ribosomally synthesized and posttranslationally modified peptide (RiPP) family of natural products.[1,2] Most thiopeptides display nanomolar potency toward various drug-resistant strains of Gram-positive pathogens by blocking protein translation,[3,4] and certain bicyclic thiopeptides have been found to exhibit antitumor, antiplasmodial and immunosuppression activities,[5–7] motivating the interest in the discovery, design and production of thiopeptide analogs with improved pharmacokinetic parameters to overcome their physical drawbacks for clinical use.[8,9] As with many RiPPs, thiopeptide antibiotics are biosynthesized *via* the conversion of a precursor peptide composed of an N-terminal leader peptide (LP) and a C-terminal core peptide (CP), and a myriad of posttranslational modifications (PTMs) occur solely on the latter, either dependent on or independent of the former.[10–12] Recent studies have shown that the establishment of the characteristic framework of thiopeptides, which shares an unusual macrocyclic peptidyl core containing a six-membered heterocycle domain central to multiple azoles and dehydroamino acids, requires a series of common PTMs and adheres to a common biosynthetic logic. The precursor peptides are first modified by an LP-dependent Ocin-ThiF/YcaO pair, which cyclodehydrates Cys/Ser residues to form azoline heterocycles, often followed by dehydrogenation to the corresponding azole.[13,14] The unmodified Ser/Thr residues in the azol(in)e-bearing intermediate are then dehydrated to form dehydroalanine (Dha)/dehydrobutyrine (Dhb) structures in a tRNA-dependent and LP-free manner by a split LanB-like dehydratase.[15] The last step is the formation of a six-membered, central nitrogen heterocycle by a cyclase that condenses two Dha residues of the linear precursor *via* [4 + 2] cycloaddition.[16,17]

The common PTMs mentioned above were found in all thiopeptides, while specialization of the characteristic framework for over 100 different thiopeptide members largely depends on a number of specific PTMs,[11] *e.g.*, the decoration of the central domain and the macrocyclic core system, the tailoring of the C-terminal extended side chain and the fabrication of a side-ring system, which can occur irregularly before or after the formation of the characteristic scaffold, and some of which are interdependent on common PTMs.[18–23] In the biosynthetic pathway to form bicyclic thiopeptides, both the specific moieties of quinaldic acid (QA), contained in the side ring of thiostrepton (TSR), and indolic acid (IA), contained in the side ring of nosiheptide (NOS), originate from L-Trp and were formed independent of the precursor peptide. The conjugation of QA and the N-terminus of the core peptide sequence was recently confirmed to be catalyzed by the α/β-hydrolase fold protein TsrI after maturation of the primary macrocycle,[24] whereas NosK, another α/β-hydrolase fold protein, mediates the transfer of MIA from a discrete thiolation preotein, NosJ, to a linear pentathiazolyl peptide intermediate before maturation of the primary macrocycle.[25] In the biosynthetic pathway to form the monomacrocyclic member thiocillin, oxi-

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dative decarboxylation of the C-terminal Thr residue of the precursor peptide is a prethiopeptide PTM that immediately follows the formation of thiazol(in)es.[26] This step is indispensable for Ser/Thr-residue dehydration and subsequent intramolecular cyclization to build a thiopeptide framework. However, amidation of the C-terminal residue of TSR is a post-thiopeptide PTM that occurs after the formation of the bicyclic scaffold.[19] Thus, elucidation of specific PTMs, especially those with novel activities, is essential to understand the biosynthetic logic of thiopeptides and expand the biosynthetic toolkit to produce thiopeptide derivatives for clinical drug screening.

Thiopeptins (TPPs) are a complex of thiopeptide antibiotics produced by *Streptomyces tateyamensis* ATCC 21389.[27] The structures of TPPs are strikingly similar to the prototypical thiopeptide TSR, both containing 17 amino acids and differing only in the first residue and bearing a QA moiety within the side-ring system of their core macrocycles (Fig. 1A). However, TPPs are distinguished by a thioamidated structure and either a piperidine or a dehydropiperidine in the core macrocycle. Recently, we identified the thiopeptin biosynthetic gene (*tpp*) cluster and demonstrated that the central piperidine heterocycle of TPPs is transformed from the dehydropiperidine heterocycle by an $F_{420}H_2$-dependent reductase,[28] whereas the specific PTM of thioamidation still remains poorly understood. Thioamidations are rare in natural products, with only a handful examples, including RiPPs such as methanobactins,[29,30] thioviridamides,[31–33] thioamide-containing thiopeptides,[27,34,35] non-RiPP closthioamide[36] and a few other nonpeptides.[37–39] In addition to methanobactins, whose thioamides are formed in a YcaO-independent manner,[40] a TfuA–YcaO pair has been identified in many other biosynthetic gene clusters of thioamide-containing RiPPs, such as thioviridamides and thiopeptides (thiopeptin, saalfelduracin and Sch 18640),[28,34,41,42] and was hypothesized to be responsible for the thioamidation process. The involvement of a TfuA–YcaO pair in the thioamidation of methyl-coenzyme M reductase in *Methanosarcina acetivorans* was also recently established.[43,44]

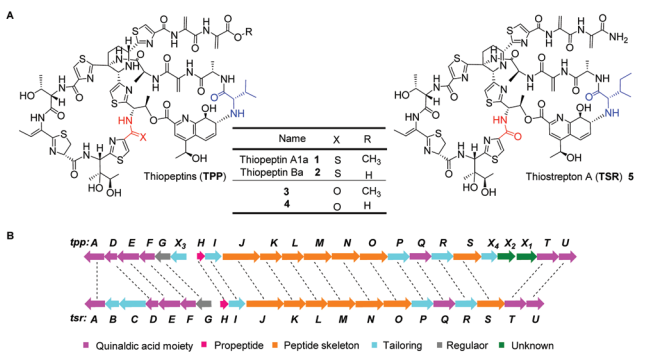

Fig. 1 Structures and biosynthetic gene clusters of thiopeptins and thiostrepton. (A) Structures of thiopeptins and thiostrepton. The (thio) amide moiety is shown in red, and the first residue is shown in blue. (B) Biosynthetic gene clusters of thiopeptins and thiostrepton. Homologous genes are indicated by dashed lines.

However, the specific PTM of thioamidation in thiopeptins, as well as its associated biosynthetic logic, still lacks direct biochemical evidence. Based on our previous analysis of the thiopeptin biosynthetic gene cluster in *S. tateyamensis* ATCC 21389, we here confirm *in vivo* that the TfuA–YcaO pair encoded by *tpp* cooperate to promote TPP thioamidation and demonstrate that the specific posttranslational thioamidation is a prethiopeptide PTM that occurs before the maturation of the bicyclic scaffold of the thiopeptide, which enhances the understanding of the biosynthetic logic of thioamide-containing thiopeptides and enriches the biosynthetic toolkit for the development of additional thiopeptide analogs for clinical drug screening.

Intrigued by the rarity and obscurity of posttranslational thioamidation in thiopeptides, we started to use thioamide-containing thiopeptins as a research platform. In a previous work, we characterized the thiopeptin biosynthetic gene cluster from the TPP-producing strain *S. tateyamensis*, which contains 23 open reading frames (*orfs*, Fig. 1B and Table S4†).[28] By comparing the sequence homology with thiostrepton and other thiopeptide biosynthetic gene clusters and bioinformatic analysis, we determined the function of each *orf* and hypothesized that the deduced products of two adjacent genes, $tppX_1$ and $tppX_2$, were responsible for the thioamidaiton of thiopeptin. $TppX_1$ and $TppX_2$ are similar to TfuA proteins and YcaO domain-containing cyclodehydratases, respectively. Such *tfuA–ycaO* genes were also contained in other thioamide-containing thiopeptide biosynthetic gene clusters, such as Sch18640 and saalfelduracin. Sequence similarity networks of YcaO enzymes showed that the YcaO protein $TppX_2$ was highly homologous with other TfuA-associated YcaO proteins (>60% identity) and had poor homology (<15% identity) with the YcaO proteins responsible for azoline formation (*e.g.*, TsrO, NosG and TruD for TSR, NOS and trunkamide, respectively) (Fig. 2). To determine the difference between the YcaOs from these two clusters, six corresponding YcaO sequences were chosen to generate a diversity-oriented multiple sequence alignment (Fig. S3†). Unlike the YcaO proteins responsible for azoline formation, TfuA-associated YcaO proteins are shorter and do not contain the highly conserved Pro-rich C-terminal sequence, but the ATP- and Mg^{2+}-coordinating residues (*e.g.*, Glu78, Glu81, Glu164, and Glu167 in *Methanosarcina kandleri* YcaO) are highly conserved among all selected sequences, as previously reported for azoline-forming YcaOs and thioamide-forming YcaOs.

To confirm our hypothesis, we inactivated the gene encoding $TppX_2$ in *S. tateyamensis* by in-frame deletion (to exclude the polar effects on downstream gene expression) using a shuttle plasmid, pKC1139. The resulting mutant strain $\Delta tppX_2$ (SL101) completely lost the ability to produce **1** (minor) and **2** (major), which are produced by wild-type *S. tateyamensis* (Fig. 3A). However, the mutant produced two distinct compounds, **3** (minor) ($[M + H]^+$ *m/z*: calcd 1667.4998 for $C_{72}H_{87}N_{18}O_{19}S_5$, found 1667.5035) and **4** (major) ($[M + H]^+$ *m/z*: calcd 1653.4842 for $C_{71}H_{85}N_{18}O_{19}S_5$, found 1653.4870), with a mass reduced by 16 Da relative to **1** and **2**, respectively, and

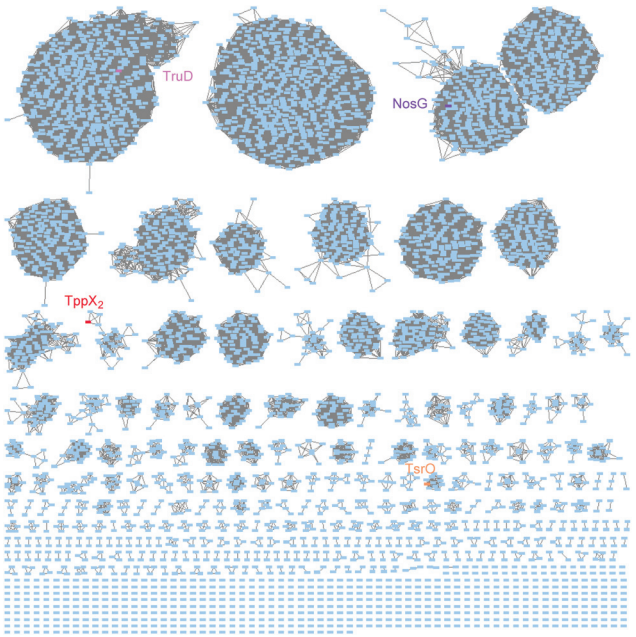

Fig. 2 Sequence similarity networks of YcaO enzymes. A sequence similarity network (SSN) of PFAM 02624 is generated. The total number of sequences is 10 333. The SSN was generated using the Enzyme Function Initiative Enzyme Similarity Tool (EFI-EST) and visualized with Cytoscape. Each node is conflated at the 75% identity level, that is, the sequences that share more than 75% identity are represented by a single node.

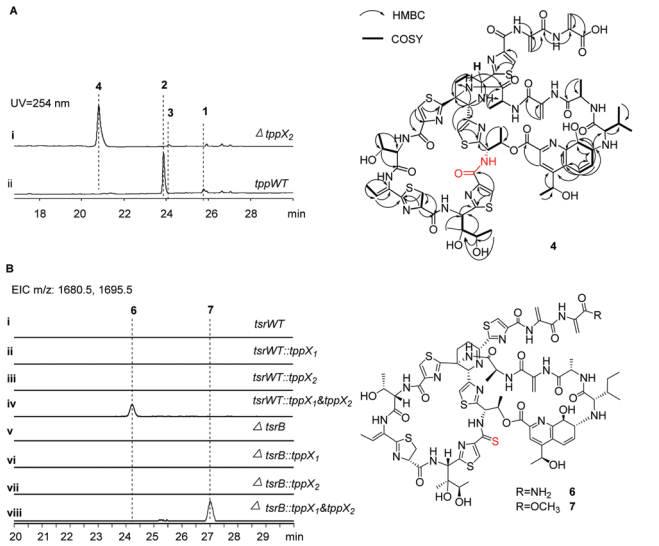

Fig. 3 (A) Characterization of the role of $TppX_2$ in thioamidation. *In vivo* assays of $TppX_2$ activity. HPLC analyses of the culture extracts from the mutant *Δ*$tppX_2$ (i) and the wild-type *S. tateyamensis* (ii). (B) Characterization of the roles of $TppX_1$ and $TppX_2$ in thioamidation. HPLC-HR-MS analyses of the culture extracts from the wild-type *S. laurentii* (i), the wild-type *S. laurentii* with the integrative plasmid containing *tppX*$_1$ (ii), the wild-type *S. laurentii* with the integrative plasmid containing *tppX*$_2$ (iii), the wild-type *S. laurentii* with the integrative plasmid containing *tppX*$_1$ and *tppX*$_2$ (iv), the mutant *ΔtsrB* with the integrative plasmid containing *tppX*$_1$ (v), the mutant *ΔtsrB* with the integrative plasmid containing *tppX*$_2$ (vi), the mutant *ΔtsrB* with the integrative plasmid containing *tppX*$_1$ and *tppX*$_2$ (vii).

displaying UV-vis absorption peaks (at λ_{max} = 198, 252, and 305 nm; Fig. S1†) quite similar to those of the parent compounds **1** and **2**. For structural elucidation, purified **4** was subjected to comparative NMR spectroscopic analysis with **2**. Despite the overall similarity in the spectra (Fig. S8 and Table S5†), the distinct signals showed the only difference to be the thioamide moiety in the macrocyclic scaffold. The ^{13}C NMR spectrum of compound **4** showed a peak attributed to a carbonyl carbon at δ 166.9 ppm, representing an upfield shift of 23.0 ppm from the thiocarbonyl carbon peak at δ 189.9 ppm in the spectrum of **2**.[45] In combination with the ^1H NMR, ^1H–^1H COSY, HSQC, and HMBC spectra, these data established that **4** is a thiopeptin intermediate that only lacks the thioamide moiety in the macrocyclic scaffold (Fig. 3A), strongly supporting the conclusion that $TppX_2$ was indispensable in the thioamidation TPPs.

Based on the various results from recent studies that the adjacent TfuA and YcaO proteins are both required for the thioamidation of peptides, such as thioviridamides and methyl-coenzyme M reductase,[42,43] we proposed that the TfuA-like protein $TppX_1$ may partner with the YcaO protein $TppX_2$ to promote thioamidation of TPPs. To determine whether our hypothesis is correct, *tppX*$_1$ and *tppX*$_2$ were separately expressed, as well as coexpressed, heterologously in the TSR-producing strain *Streptomyces laurentii* using an integrative plasmid pSET152. It is well known that TPPs and TSR differ only in the first residue, the modification of the C-termini and the occurrence of thioamidation. We reasoned that the *S. tateyamensis* TfuA–YcaO pair could tolerate those differences and convert TSR to a thioamidated analog. The fermentation of *S. laurentii* with the integrative plasmid containing *tppX*$_1$ and *tppX*$_2$ was subsequently analyzed by high-performance liquid chromatography with high-resolution mass spectrometry (HPLC-HR-MS), which revealed a new metabolite that was 16 Da heavier than TSR and was chemically identical to thioamidated TSR analog **6** ($[M + H]^+$ *m*/*z*: calcd 1680.4773 for $C_{72}H_{86}N_{19}O_{17}S_6$, found 1680.4699), consistent with a recent finding by Mitchell *et al.*[34] (Fig. 3B and S5†). In addition, the omission of either *tppX*$_1$ or *tppX*$_2$ in *S. laurentii* resulted in a loss of the ability to produce a thioamidated TSR analog, indicating that both *tppX*$_1$ and *tppX*$_2$ are necessary for the thioamidation of TPP. Similar results were also obtained when separately expressing or coexpressing *tppX*$_1$ and *tppX*$_2$ in the *tsrB*-inactivated strain *ΔtsrB* (*tsrB* encodes an α/β hydrolase, catalyzing the hydrolysis of the methyl ester TSR analog to provide the carboxylate TSR analog),[19] and HPLC-HR-MS analysis of the fermentation of *ΔtsrB* with the integrative plasmid containing *tppX*$_1$ and *tppX*$_2$ revealed a new metabolite **7** ($[M + H]^+$ *m*/*z*: calcd 1695.4770 for $C_{73}H_{87}N_{18}O_{18}S_6$, found 1695.4729) (Fig. 3B and S5†). These data suggest that $TppX_1$ and $TppX_2$ might work together and be flexible, accepting thiopeptides in which the core sequence and the mature structure are similar to their native substrate. However, the time point in the TPP biosynthetic pathway at which thioamidation takes place

remains unknown, which would affect the whole understanding of TPP biosynthetic logic.

To investigate whether the specific posttranslational thioamidation occurs after maturation of the thiopeptide bicyclic scaffold, we fed TSR to the TPP-producing strain *S. tateyamensis* and screened the relevant fermentation products by HPLC-HR-MS to monitor the corresponding thioamidated TSR analogs, yet no thioamidated TSR analog was found. Further analysis of the fermentation revealed a new metabolite that was 2 Da heavier than TSR (Fig. S6†), which should be a dehydropiperidine reduction product of TSR generated by the $F_{420}H_2$-dependent reductase $TppX_4$. This result indicated that TSR could be successfully fed into the TPP-producing strain *S. tateyamensis* and recognized by the reductase $TppX_4$ but could not be transformed by the TfuA–YcaO pair. We then supplemented **8** (Fig. 4), which was recently isolated from a mutant strain *ΔtsrP* (*tsrP* encodes a cytochrome P450 protein that catalyzes epoxidation) (Fig. S6†),[46] to *S. tateyamensis* to determine if $TppX_1$ and $TppX_2$ could utilize a monocyclic intermediate whose second side ring has not been closed. However, the result of HPLC-HR-MS detection showed no corresponding thioamidated product and only revealed a reductive product that was 2 Da heavier than **8**, similar to the result of feeding TSR. Based on these results, we hypothesized that posttranslational thioamidation might be a prethiopeptide PTM functioning on a linear intermediate before the formation of the central nitrogen heterocycle. To further explore the roles of $TppX_1$ and $TppX_2$, we overexpressed their encoding genes in *Escherichia coli* BL21(DE3). Initially, our numerous attempts to obtain either $TppX_1$ or $TppX_2$ in soluble form when expressing *tppX₁* or *tppX₂* alone failed. As the previous work implied that they were functionally associated, we coexpressed N-terminally 6× His-tagged $TppX_1$ and untagged $TppX_2$ in *E. coli* BL21(DE3) with the assumption that the resultant proteins could interact and cause a solubilization effect. As anticipated, soluble protein complexes were observed and were isolated on a Ni-NTA column. After denaturing, the purified proteins decomposed into two subunits with a ratio of ~1 : 1 and sizes appropriate for N-terminally 6× His-tagged $TppX_1$ (~48 kDa) and untagged $TppX_2$ (~43 kDa) (Fig. S2†). Clearly, $TppX_1$ and $TppX_2$ noncovalently interact with each other and form a heterodimer in solution. As the bioinformatic analysis showed that the YcaO protein $TppX_2$ was highly homologous to other TfuA-associated YcaO proteins (Fig. 2) and the ATP- and Mg^{2+}-coordinating residues (*e.g.*, Glu85, Glu88, Glu191 and Glu194 in $TppX_2$) were conserved with previously reported TfuA-associated YcaO protein in *M. kandleri* (Fig. S3†), we predicted that thioamide formation in TPPs would also be an ATP-dependent, YcaO-catalyzed reaction. We thus examined the activity of the heterodimer of $TppX_1$ and $TppX_2$ in the presence of ATP and the external sulfide donor Na_2S using **4** and **8** as the substrates, respectively (Fig. S7†). Neither **4** nor **8** was transformed, consistent with the feeding results *in vivo*. Combined with the reports on YcaO partner proteins, we proposed that the actual substrate of the TfuA–YcaO pair in thiopeptides might be a linear intermediate containing a leader peptide, and plausible functions of TfuA include binding the leader peptide, allosterically activating YcaO or assisting in the delivery of sulfide to the substrate during the thioamidation process. Future work will be required to confirm or refute these proposals.

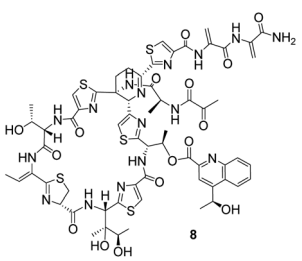

Fig. 4 Structure of compound **8**.

Conclusions

In conclusion, we demonstrate *in vivo* that the specific PTM of thioamidation in thiopeptins relies on the activities of the *tppX₁* and *tppX₂* genes, which encode a noncovalently bound TfuA–YcaO pair and work together by forming a heterodimer. Further investigation of the biosynthetic logic of the specific posttranslational thioamidation shows that it is a prethiopeptide PTM that occurs before maturation of the thiopeptide bicyclic scaffold, as feeding TSR or the monocyclic intermediate **8** to *S. tateyamensis* did not produce the corresponding thioamidated products. However, the specific PTM of thioamidation is not indispensable among the common PTMs in thiopeptins, as deletion of the *tppX₂* gene in *S. tateyamensis* resulted in the production of a mature dicyclic thiopeptide **4**. The direct biochemical evidence provided here enhances the understanding of the biosynthetic logic of thioamide-containing thiopeptides and suggests that the specific posttranslational thioamidation in thiopeptides might occur on a linear intermediate containing a leader peptide, which needs to be confirmed or refuted in the future.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 M. C. Bagley, J. W. Dale, E. A. Merritt and X. Xiong, *Chem. Rev.*, 2005, **105**, 685.
2 J. Li, X. Qu, X. He, L. Duan, G. Wu, D. Bi, Z. Deng, W. Liu and H. Y. Ou, *PLoS One*, 2012, **7**, e45878.
3 S. E. Heffron and F. Jurnak, *Biochemistry*, 2000, **39**, 37.
4 Y. Xing and D. E. Draper, *Biochemistry*, 1996, **35**, 1581.
5 S. K. Radhakrishnan, U. G. Bhat, D. E. Hughes, I. C. Wang, R. H. Costa and A. L. Gartel, *Cancer Res.*, 2006, **66**, 9731.
6 M. N. Aminake, S. Schoof, L. Sologub, M. Leubner, M. Kirschner, H. D. Arndt and G. Pradel, *Antimicrob. Agents Chemother.*, 2011, **55**, 1338.
7 M. Ueno, S. Furukawa, F. Abe, M. Ushioda, K. Fujine, S. Johki, H. Hatori and H. Ueda, *J. Antibiot.*, 2004, **57**, 590.
8 X. Just-Baringo, F. Albericio and M. Alvarez, *Angew. Chem., Int. Ed.*, 2014, **53**, 6602.
9 Z. Lin, Q. He and W. Liu, *Curr. Opin. Biotechnol.*, 2017, **48**, 210.
10 M. A. Ortega and W. A. van der Donk, *Cell Chem. Biol.*, 2016, **23**, 31.
11 S. Wang, S. Zhou and W. Liu, *Curr. Opin. Chem. Biol.*, 2013, **17**, 626.
12 C. T. Walsh, M. G. Acker and A. A. Bowers, *J. Biol. Chem.*, 2010, **285**, 27525.
13 K. L. Dunbar, J. I. Tietz, C. L. Cox, B. J. Burkhart and D. A. Mitchell, *J. Am. Chem. Soc.*, 2015, **137**, 7672.
14 J. O. Melby, N. J. Nard and D. A. Mitchell, *Curr. Opin. Chem. Biol.*, 2011, **15**, 369.
15 M. A. Ortega, Y. Hao, Q. Zhang, M. C. Walker, W. A. van der Donk and S. K. Nair, *Nature*, 2015, **517**, 509.
16 W. J. Wever, J. W. Bogart, J. A. Baccile, A. N. Chan, F. C. Schroeder and A. A. Bowers, *J. Am. Chem. Soc.*, 2015, **137**, 3494.
17 G. A. Hudson, Z. Zhang, J. I. Tietz, D. A. Mitchell and W. A. van der Donk, *J. Am. Chem. Soc.*, 2015, **137**, 16012.
18 Y. Yu, H. Guo, Q. Zhang, L. Duan, Y. Ding, R. Liao, C. Lei, B. Shen and W. Liu, *J. Am. Chem. Soc.*, 2010, **132**, 16324.
19 R. Liao and W. Liu, *J. Am. Chem. Soc.*, 2011, **133**, 2852.
20 Q. Zhang, Y. Li, D. Chen, Y. Yu, L. Duan, B. Shen and W. Liu, *Nat. Chem. Biol.*, 2011, **7**, 154.
21 L. Duan, S. Wang, R. Liao and W. Liu, *Chem. Biol.*, 2012, **19**, 443.
22 W. Liu, Y. Xue, M. Ma, S. Wang, N. Liu and Y. Chen, *ChemBioChem*, 2013, **14**, 1544.
23 Z. Lin, J. Ji, S. Zhou, F. Zhang, J. Wu, Y. Guo and W. Liu, *J. Am. Chem. Soc.*, 2017, **139**, 12105.
24 Q. Zheng, S. Wang, P. Duan, R. Liao, D. Chen and W. Liu, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 14318.
25 Y. Qiu, Y. Du, F. Zhang, R. Liao, S. Zhou, C. Peng, Y. Guo and W. Liu, *J. Am. Chem. Soc.*, 2017, **139**, 18186.
26 P. R. B. Kathryn, D. Bewleya, M. A. Burlingamea, R. A. Robisonb, J. S. Griffittsb and A. S. M. Miller, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **115**, 12450.
27 N. Miyairi, T. Miyoshi, H. Aoki, M. Kosaka and H. Ikushima, *Antimicrob. Agents Chemother.*, 1972, **1**, 192.
28 J. Liu, Z. Lin, H. Chen, H. Guo, J. Tao and W. Liu, *Chin. J. Chem.*, 2018, **37**, 35.
29 G. E. Kenney and A. C. Rosenzweig, *ACS Chem. Biol.*, 2012, **7**, 260.
30 G. E. Kenney and A. C. Rosenzweig, *BMC Biol.*, 2013, **11**, 17.
31 Y. Hayakawa, K. Sasaki, K. Nagai, K. Shin-ya and K. Furihata, *J. Antibiot.*, 2006, **59**, 6.
32 L. Frattaruolo, R. Lacret, A. R. Cappello and A. W. Truman, *ACS Chem. Biol.*, 2017, **12**, 2815.
33 L. Kjaerulff, A. Sikandar, N. Zaburannyi, S. Adam, J. Herrmann, J. Koehnke and R. Muller, *ACS Chem. Biol.*, 2017, **12**, 2837.
34 C. J. Schwalen, G. A. Hudson, B. Kille and D. A. Mitchell, *J. Am. Chem. Soc.*, 2018, **140**, 9494.
35 O. D. Hensens and G. Albers-Schonberg, *J. Antibiot.*, 1983, **36**, 814.
36 T. Lincke, S. Behnken, K. Ishida, M. Roth and C. Hertweck, *Angew. Chem., Int. Ed.*, 2010, **49**, 2011.
37 A. P. Klein and E. S. Sattely, *Nat. Chem. Biol.*, 2015, **11**, 837.
38 C. C. Hughes, J. B. MacMillan, S. P. Gaudencio, P. R. Jensen and W. Fenical, *Angew. Chem., Int. Ed.*, 2009, **48**, 725.
39 S. Coyne, A. Litomska, C. Chizzali, M. N. Khalil, K. Richter, L. Beerhues and C. Hertweck, *ChemBioChem*, 2014, **15**, 373.
40 G. E. Kenney, L. M. K. Dassama, M. E. Pandelia, A. S. Gizzi, R. J. Martinie, P. Gao, C. J. DeHart, L. F. Schachner, O. S. Skinner, S. Y. Ro, X. Zhu, M. Sadek, P. M. Thomas, S. C. Almo, J. M. Bollinger Jr., C. Krebs, N. L. Kelleher and A. C. Rosenzweig, *Science*, 2018, **359**, 1411.
41 H. Ichikawa, G. Bashiri and W. L. Kelly, *J. Am. Chem. Soc.*, 2018, **140**, 10749.
42 M. Izawa, T. Kawasaki and Y. Hayakawa, *Appl. Environ. Microbiol.*, 2013, **79**, 7110.
43 N. Mahanta, A. Liu, S. Dong, S. K. Nair and D. A. Mitchell, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, 3030–3035.
44 D. D. Nayak, N. Mahanta, D. A. Mitchell and W. W. Metcalf, *eLife*, 2017, **6**, e29218.
45 O. D. Hensens and G. Albers-Schonberg, *J. Antibiot.*, 1983, **36**, 832.
46 Q. Zheng, S. Wang, R. Liao and W. Liu, *ACS Chem. Biol.*, 2016, **11**, 2673.