



Cite this: DOI: 10.1039/d5cb00153f

Received 12th June 2025,  
Accepted 15th July 2025

DOI: 10.1039/d5cb00153f

rsc.li/rsc-chembio

## P450 cyptide synthase MpoB catalyzes the cross-linking of the YPW motif on the precursor peptide†

Abujunaid Habib Khan,<sup>a</sup> Jabal Rahmat Haedar,<sup>a</sup> Vic Kiselov,<sup>a</sup>  
Viktors Romanuks,<sup>a</sup> Gints Smits,<sup>ab</sup> Stefano Donadio<sup>ac</sup> and  
Chin-Soon Phan<sup>id\*</sup>

**Cytochrome P450 enzymes in ribosomally synthesized and post-translationally modified peptides (RiPPs) catalyze C–C, C–N, or C–O cross-linking reactions in the biosynthesis of biaryl cyclophane natural products. Here, we manually identified 127 homologous P450s linked to putative precursor peptides containing the YPW motif. Through *in vivo* functional studies in *Escherichia coli*, the newly identified enzyme MpoB from *Micromonospora polyrhachis* DSM 45886 was found to catalyze the formation of a cross-link between Tyr-C3 and Trp-N1 at the YPW motif. This result provides an additional toolkit for cross-linked peptide modification.**

### Introduction

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a rapidly growing class of peptide natural products defined by their post-translational modification.<sup>1,2</sup> Cross-linking of peptides is an important chemical feature that improves the stability and biological activity of peptides.<sup>3–5</sup> An important example is the natural product, darobactin A, a heptapeptide composed of two three-residue motif cyclophanes that targets the essential outer membrane protein BamA of Gram-negative bacteria, a mechanism distinct from all currently known targets.<sup>4,6</sup> Recently, cytochrome P450-RiPP was discovered as a group of enzymes that catalyze biaryl cyclophanes between two aromatic amino acids on the precursor peptides.<sup>7–14</sup> These biaryl cross-linked peptides installed by cytochrome P450-RiPP are collectively named as cyptides.<sup>8</sup> In contrast, the monoaryl cross-links between one aromatic amino acid and one aliphatic amino acid found in RiPPs are

generated by radical SAM, DUF3328, and BURP domain proteins.<sup>15–22</sup>

Three-residue motif cyclophanes containing proline are relatively rare, with only a few examples (Fig. 1A): (1) cross-linking at the YPW motif catalyzed by cytochrome P450 cyptide synthases (P450s), MciB from *Micromonospora citrea* DSM 43903,<sup>7</sup> SlpB from *Streptomyces* sp. LaPpAH-199,<sup>8</sup> and SalP from *Streptomyces californicus* NRRL 2423;<sup>9</sup> (2) cross-linking at the YPW motif is also found in a natural product, pseudosporamide isolated from *Pseudosporangium* sp. RD062863;<sup>23</sup> and (3) cross-linking at the WPR motif catalyzed by radical SAM enzyme, WprB from *Xenorhabdus littoralis* psl.<sup>24</sup> Among these P450 enzymes, MciB catalyzes the formation of a cross-link between Tyr-C3 and Trp-C5,<sup>7</sup> whereas SlpB and SalP generate a cross-link between Tyr-C3 and Trp-N1 (Fig. 1B).<sup>8,9</sup> The total synthesis of Tyr-C3 and Trp-N1 cross-linked at the YPW motif cyclophane has recently been achieved by Larock macrocyclization.<sup>25</sup> From a stereochemical perspective, the geometry of the proline amide bond in macrocyclic peptides can be either *trans* or *cis*.<sup>26–28</sup> This geometry was determined based on the, (1) <sup>13</sup>C NMR chemical shift differences between the Cβ and Cγ positions,<sup>29</sup> and (2) NOESY correlations.<sup>28</sup> The glycopeptide antibiotic kistamicin also has a cross-link between Tyr and Trp that is catalyzed by a P450 enzyme, OxyA.<sup>30</sup> Seeing the Tyr–Trp cross-link in the antibiotic structure, we are interested in expanding the P450 enzymes that catalyze the cross-link formation at the YPW motif on the precursor peptides.

### Results and discussion

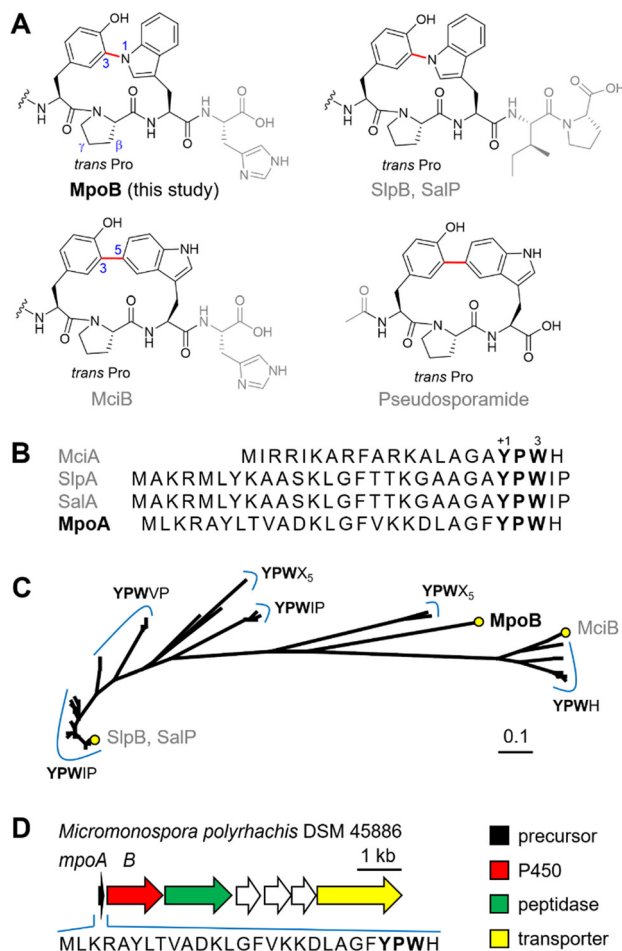
To this end, we used position-specific iterative basic local alignment search tool (PSI-BLAST)<sup>31</sup> to search for homologous P450 sequences with MciB<sup>7</sup> as the query protein. We manually identified 127 homologous P450 enzymes linked to putative precursor peptides containing the YPW motif (Table S1, ESI†). All P450-precursor peptide gene pairs derived from

<sup>a</sup> Latvian Institute of Organic Synthesis, Aizkraukles Street 21, LV-1006 Riga, Latvia.  
E-mail: chinsoon@osi.lv

<sup>b</sup> Faculty of Natural Sciences and Technology, Riga Technical University,  
Paula Valdena 3, LV-1048, Riga, Latvia

<sup>c</sup> NAICONs Srl, 20139, Milan, Italy

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d5cb00153f>



**Fig. 1** (A) Biaryl cross-linked at the YPW motifs generated by cytochrome P450 cytidyl synthetases and natural product, pseudosporamide. (B) Precursor peptide sequences containing the YPW motif. (C) Phylogenetic tree of known and newly characterized P450 enzymes. (D) Biosynthetic gene cluster of *mpoA*. Cross-link formation on the structures is shown as red bonds. Known and newly characterized P450 enzymes/precursor peptides are shown as grey colored letters and black colored bold letters, respectively. YPW motifs on the precursor peptide sequences are shown in bold.

actinobacteria, of which 90.6% were from *Streptomyces* (115), and 9.4% were from other bacterial genera such as *Micromonospora* (4), *Actinoplanes* (6), *Nocardioideis* (1) and *Pseudosporangium* (1). A total of four different core peptides were identified such as **YPWIP** (87), **YPWVP** (24), **YPWH** (11) and **YPWX<sub>5</sub>** (5). To differentiate these putative P450 enzymes, all identified P450 enzymes were used to generate a phylogenetic tree (Fig. 1C and Fig. S1, ESI<sup>†</sup>). Based on the relationship with known P450 enzymes and precursor peptide sequences, we aimed to elucidate the transformation by one P450 enzyme, designated as MpoB from *Micromonospora polyrhachis* DSM 45886, which forms a clade containing only two P450 sequences, located between the core peptide groups **YPWIP/YPWVP** (SlpB and SalP) and **YPWH** (MciB). MpoB shares 38–44% amino acid identity with MciB (38.1%), SlpB (43.9%), and SalP (44.2%).

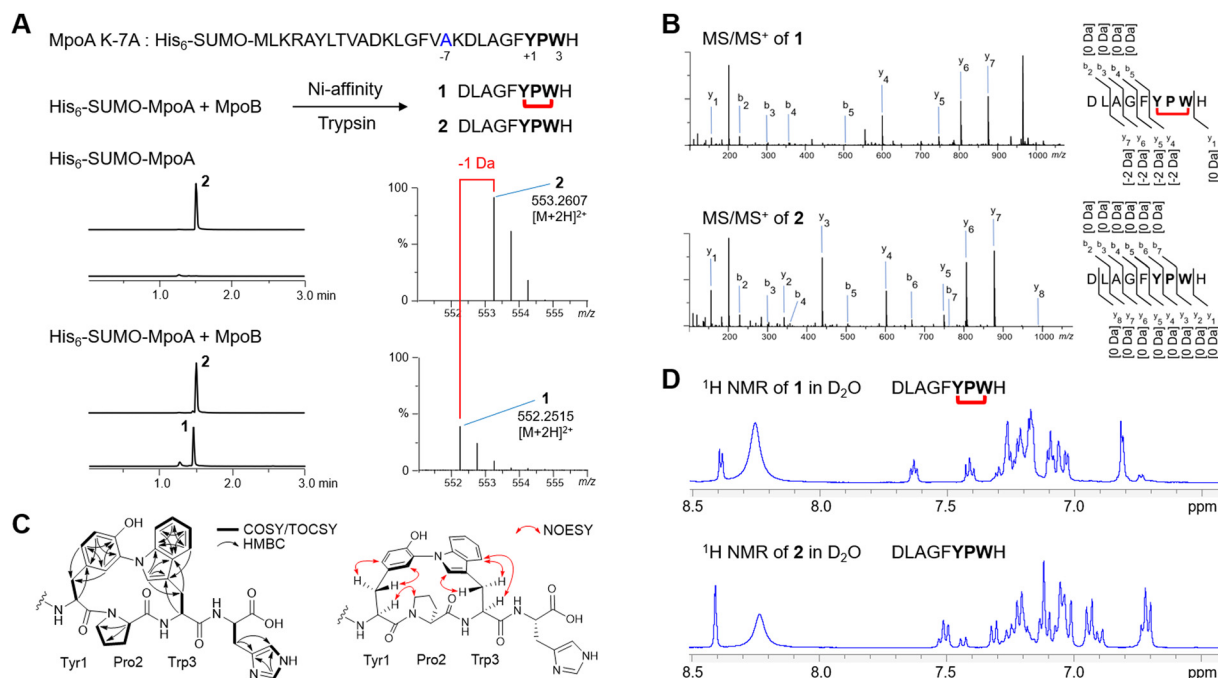
To validate the enzyme activity, we initiated functional studies on the precursor peptide with cognate P450 enzyme found in the biosynthetic gene cluster of *mpoA* (Fig. 1D). The

precursor peptide MpoA was mutated at K-7 to A, to avoid the generation of two mixed fragments of **DLAGFY<sup>3</sup>PWH** and **KDLAGFY<sup>3</sup>PWH** after trypsin digestion. The N-terminal His<sub>6</sub>-SUMO tag MpoA K-7A was expressed in *Escherichia coli* NiCo21(DE3) alone or coexpressed with P450 enzyme MpoB, purified by Ni-affinity chromatography and digested with trypsin. Comparative analysis of the digests on LC-MS led to the identification of peak 1 with −2 Da mass loss relative to the unmodified peptide fragment, peak 2 (Fig. 2A and Fig. S2, ESI<sup>†</sup>). The tandem mass spectrometry (MS/MS) analysis localized the −2 Da mass loss to the YPW motif in fragments 1, while fragment 2 was determined as unmodified peptide (Fig. 2B and Fig. S2, ESI<sup>†</sup>). Next, we performed large-scale protein expression at 40 L and obtained a 2.2 mg of fragment 1 and 5.2 mg of fragment 2 for NMR characterization.

The residues within the precursor sequence are numbered ‘+1’ from the start of the core peptide **YPWH**. 1D and 2D NMR data (HSQC, HMBC, COSY, TOCSY and NOESY) were obtained for analysis (Fig. S3–S8 and Table S6, ESI<sup>†</sup>). 2D NMR analysis of fragment 1 in D<sub>2</sub>O indicated that Tyr1 was substituted at Tyr1-C3, based on an aromatic spin system between Tyr1-H5 ( $\delta_{\text{H}}$  6.81;  $\delta_{\text{C}}$  115.7) and Tyr1-H6 ( $\delta_{\text{H}}$  7.06;  $\delta_{\text{C}}$  133.4), and the overlapping signal at Tyr1-H2 ( $\delta_{\text{H}}$  6.81;  $\delta_{\text{C}}$  130.6) was considered to have no spin system. This assignment was supported by the (1) HMBC correlations of Tyr1-H2 to Tyr1-C4 ( $\delta_{\text{C}}$  151.6) and Tyr1-C6, Tyr1-H6 to Tyr1-C2 and Tyr1-C4, and Tyr1-H5 to Tyr1-C1 ( $\delta_{\text{C}}$  126.6) and Tyr1-C3 ( $\delta_{\text{C}}$  128.3), and (2) NOESY correlations of between Tyr1-H $\beta$  ( $\delta_{\text{H}}$  2.97) and Tyr1-H2 and Tyr1-H6 (Fig. 2C). Pro2 was assigned as unmodified and possessed *trans* geometry based on the small <sup>13</sup>C NMR chemical shift difference between C $\beta$  and C $\gamma$  positions,<sup>29</sup>  $\Delta\text{Pro2-C}\beta - \text{Pro2-C}\gamma = 5.1$  ppm. Typical *cis* Pro has a large <sup>13</sup>C NMR chemical shift difference,  $\Delta\text{Pro-C}\beta - \text{Pro-C}\gamma = 9.0$  to 10.0 ppm.<sup>26,27</sup> We then focused our attention on Trp3 and found that all aromatic signals were present. This was confirmed by the (1) spin systems between Trp3-H $\alpha$  ( $\delta_{\text{H}}$  4.55) and Trp3-H $\beta$  ( $\delta_{\text{H}}$  3.23, 3.05) and between Trp3-H4, Trp3-H5, Trp3-H6 and Trp3-H7, and (2) HMBC correlations of Trp3-H4 ( $\delta_{\text{H}}$  7.26;  $\delta_{\text{C}}$  119.6) to Trp3-C3 ( $\delta_{\text{C}}$  113.3), Trp3-C6 and Trp3-C7a ( $\delta_{\text{C}}$  135.7), Trp3-H5 ( $\delta_{\text{H}}$  7.26;  $\delta_{\text{C}}$  128.6) to Trp3-C3a ( $\delta_{\text{C}}$  126.5) and Trp3-C7, Trp3-H6 ( $\delta_{\text{H}}$  7.63;  $\delta_{\text{C}}$  123.3) to Trp3-C4 and Trp3-C7a, Trp3-H7 ( $\delta_{\text{H}}$  7.41;  $\delta_{\text{C}}$  111.0) to Trp3-C3a and Trp3-C5, and Trp3-H2 ( $\delta_{\text{H}}$  7.16;  $\delta_{\text{C}}$  123.4) to Trp3-C3, Trp3-C3a and Trp3-C7a. The NOESY spectrum detected correlations between Trp3-H2 and Trp3-H $\beta$  ( $\delta_{\text{H}}$  3.23), Trp3-H4 and Trp3-H $\beta$  ( $\delta_{\text{H}}$  3.05), and Trp3-H4 and Trp3-H $\alpha$ , indicating that Trp3-H2 lies on the opposite face of Trp3-H4 and Trp3-H $\alpha$ . Based on the above findings, the newly formed cross-link must be formed between Tyr1-C3 and Trp3-N1 (Fig. 1A), similar to that cross-linked at the YPW motif installed by SlpB and SalP.<sup>8,9</sup>

Due to the lack of direct 3-bond HMBC evidence from Trp3-H2 to Tyr1-C3 in fragment 1, we characterized the unmodified peptide fragment 2 by NMR (Fig. 2D and Fig. S9–S13, ESI<sup>†</sup>). The result showed that Tyr1-H2/Tyr1-H6 ( $\delta_{\text{H}}$  6.94;  $\delta_{\text{C}}$  130.8) and Tyr1-H3/Tyr1-H5 ( $\delta_{\text{H}}$  6.71;  $\delta_{\text{C}}$  115.3) are magnetically equivalent and have the same chemical shifts. In addition to Tyr1 in fragment 2, aromatic signals of Trp3 are also significantly

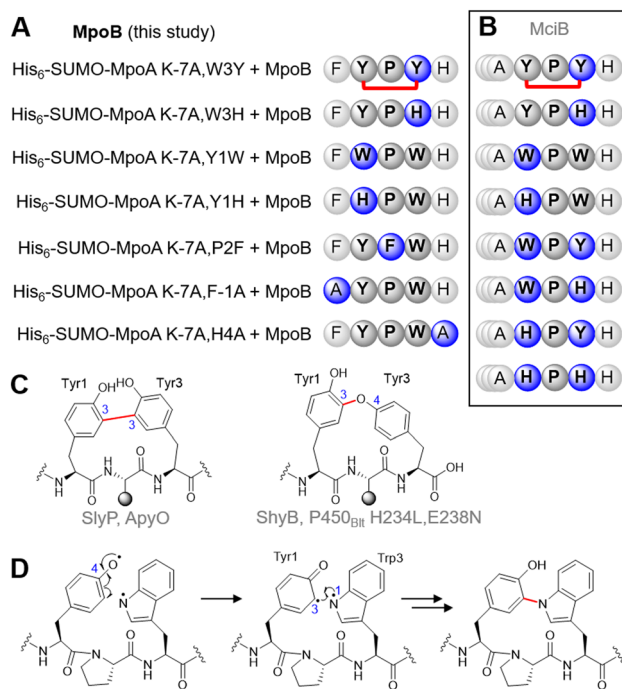




**Fig. 2** (A) *In vivo* coexpression of His<sub>6</sub>-SUMO-MpoA K-7A + MpoB followed by Ni-affinity purification and trypsin digestion yielded fragments **1** and **2**. The MS spectra and EIC chromatogram of peaks **1** and **2**. (B) MS/MS analysis of fragments **1** and **2**. (C) 2D NMR correlations of fragment **1** in D<sub>2</sub>O. (D) <sup>1</sup>H NMR spectra of aromatic signals in fragments **1** and **2**. Cross-link formation on the peptide sequences is shown as red connectors. The mutated residue on the precursor sequence is shown as a blue colored letter.

different from fragment **1**, example Trp3-H4,5,6,7 ( $\delta_{\text{H}}$  7.51, 7.06, 7.12, 7.31) in **2** and Trp3-H4,5,6,7 ( $\delta_{\text{H}}$  7.26, 7.26, 7.63, 7.41) in **1**, indicating that the difference in NMR chemical shifts between fragments **1** and **2** was due to the newly formed bond between Tyr1-C3 and Trp3-N1 in **1** (Table S6, ESI<sup>†</sup>). Other three-residue YxW motifs with similar cross-links between Tyr1-C3 and Trp3-N1 can be found in a natural product, neopetromin isolated from sponge *Neopetrosia* sp.,<sup>32</sup> and in a non-native product generated by P450<sub>Blt</sub>.<sup>33,34</sup>

Next, we asked whether a non-native cross-linked product can be formed by MpoB. To test this, we designed precursor variants consisting of a single amino acid mutation at Tyr1 or Trp3 of MpoA K-7A (Fig. 3A and Fig. S14–S17, ESI<sup>†</sup>). Coexpression of His<sub>6</sub>-SUMO-MpoA K-7A, W3Y/W3H/Y1W/Y1H + MpoB, followed by Ni-affinity purification, trypsin digestion and LC-MS analysis showed that only the YPY motif was cross-linked. MpoB most likely has a similar substrate tolerance as MciB (Fig. 3B).<sup>7</sup> Due to the lack of material containing the cross-linked YPY motif, we were unable to characterize this cyclophane product by NMR. We hypothesize that the structure may be identical to one of the cross-linked YxY motifs generated by other P450 enzymes, (1) SlyP and ApyO catalyzing the cross-link between Tyr1-C3 and Tyr3-C3,<sup>9,35</sup> and (2) ShyB and P450<sub>Blt</sub> H234L, E238N installing the cross-link between Tyr1-C3 and Tyr3-O4 (Fig. 3C).<sup>36,37</sup> Although we are currently unable to determine the cross-linking at the YPY motif, an effective method to characterize the cross-linked structure without using NMR is to use hydrogen deuterium exchange mass spectrometry.<sup>34,37</sup>



**Fig. 3** (A) Promiscuity study of MpoB. (B) Reported promiscuity study of MciB.<sup>8</sup> (C) Cross-linking of the three-residue YxY motif installed by characterized P450 enzymes. (D) Proposed mechanism of cross-linking in the YPW motif. Mutated residues on the precursor sequences are shown as blue colored balls.

Three additional precursor variants were designed with a mutation at P2F/F-1A/H4A to determine whether cross-link

formation would be affected by adjacent amino acids that are not involved in cross-link formation (Fig. 3A and Fig. S18–S20, ESI†). Coexpression of His<sub>6</sub>-SUMO-MpoA K-7A,P2F/F-1A/H4A + MpoB, followed by Ni-affinity purification, trypsin digestion and LC-MS analysis showed no cross-linking. This suggests that the Phe-1 and His4 residues located before and after the YPW motif, respectively, are required for cross-link formation by MpoB. In addition, replacement of Pro2 with the bulky amino acid Phe did not result in cross-link formation.

The mechanism of P450 enzymes in biaryl cross-linking reactions has not been investigated. However, most P450 oxidations are thought to occur *via* the highly reactive iron(IV)oxo porphyrin  $\pi$ -cation radical intermediate that abstracts a hydrogen atom from the substrate.<sup>38</sup> The proposed biaryl cross-linking mechanism involves a second hydrogen atom abstraction from another aromatic amino acid by the reactive iron(IV)hydroxide species, resulting in the cross-linking of two radical species, which then undergo tautomerization to generate a biaryl cross-linked product.<sup>21</sup> For MpoB, the reactive iron(IV)hydroxide species abstract the second hydrogen atom at Tyr1-O4, coupling the two radical species, Tyr1-C3<sup>•</sup> and Trp3-N1<sup>•</sup>, followed by tautomerization to yield a YPW cross-linked product (Fig. 3D).

## Conclusions

In conclusion, we manually identified 127 homologous P450s linked to putative precursor peptides containing the YPW motif. This led to the identification of uncharacterized P450 enzyme MpoB, which was found to catalyze the formation of a cross-link between Tyr1-C3 and Trp3-N1 at the YPW motif. This work further expands the P450 enzymes in catalyzing YPW motif cross-linking. To our knowledge, MpoB is the third P450 enzyme, after the highly related SlpB and SalP, that catalyzes the formation of a C–N cross-link at the YPW motif on the precursor peptides. However, the precursor peptide MpoA has a charged His residue at the C-terminus, whereas SlpA and SalA have hydrophobic Ile-Pro residues at the C-terminus, meaning that the active sites in MpoB involved in positioning the core peptide for cross-linking must be different from that in SlpB and SalP. Although the promiscuity of SlpB and SalP has not been determined, the present study indicates that Trp is not strictly necessary for cross-linking and that MpoB can also generate a cross-link at the YPY motif.

## Conflicts of interest

The authors declare no competing financial interests.

## Data availability

The data supporting this article have been included as part of the ESI† available.

## Acknowledgements

This work was funded by EU project no. 101087181 (Natural Products Research at Latvian Institute of Organic Synthesis as a Driver for Excellence in Innovation). We acknowledge the support from the LC-MS and NMR facilities, and biotechnology laboratories at Latvian Institute of Organic Synthesis.

## Notes and references

- P. G. Arnison, M. J. Bibb, G. Bierbaum, A. A. Bowers, T. S. Bugni, G. Bulaj, J. A. Camarero, D. J. Campopiano, G. L. Challis, J. Clardy, P. D. Cotter, D. J. Craik, M. Dawson, E. Dittmann, S. Donadio, P. C. Dorrestein, K. D. Entian, M. A. Fischbach, J. S. Garavelli, U. Göransson, C. W. Gruber, D. H. Haft, T. K. Hemscheidt, C. Hertweck, C. Hill, A. R. Horswill, M. Jaspars, W. L. Kelly, J. P. Klinman, O. P. Kuipers, A. K. Link, W. Liu, M. A. Marahiel, D. A. Mitchell, G. N. Moll, B. S. Moore, R. Müller, S. K. Nair, I. F. Nes, G. E. Norris, B. M. Olivera, H. Onaka, M. L. Patchett, J. Piel, M. J. Reaney, S. Rebuffat, R. P. Ross, H. G. Sahl, E. W. Schmidt, M. E. Selsted, K. Severinov, B. Shen, K. Sivonen, L. Smith, T. Stein, R. D. Süßmuth, J. R. Tagg, G. L. Tang, A. W. Truman, J. C. Vederas, C. T. Walsh, J. D. Walton, S. C. Wenzel, J. M. Willey and W. A. van der Donk, *Nat. Prod. Rep.*, 2013, **30**, 108–160.
- M. Montalbán-López, T. A. Scott, S. Ramesh, I. R. Rahman, A. J. van Heel, J. H. Viel, V. Bandarian, E. Dittmann, O. Genilloud, Y. Goto, M. J. Grande Burgos, C. Hill, S. Kim, J. Koehnke, J. A. Latham, A. J. Link, B. Martinez, S. K. Nair, Y. Nicolet, S. Rebuffat, H. G. Sahl, D. Sareen, E. W. Schmidt, L. Schmitt, K. Severinov, R. D. Süßmuth, A. W. Truman, H. Wang, J. K. Weng, G. P. van Wezel, Q. Zhang, J. Zhong, J. Piel, D. A. Mitchell, O. P. Kuipers and W. A. van der Donk, *Nat. Prod. Rep.*, 2021, **38**, 130–239.
- Z. Lai, X. Yuan, H. Chen, Y. Zhu, N. Dong and A. Shan, *Biotechnol. Adv.*, 2022, **59**, 107962.
- Y. Imai, K. J. Meyer, A. Iinishi, Q. Favre-Godal, R. Green, S. Manuse, M. Caboni, M. Mori, S. Niles, M. Ghiglieri, C. Honrao, X. Ma, J. J. Guo, A. Makriyannis, L. Linares-Otaya, N. Böhringer, Z. G. Wuisan, H. Kaur, R. Wu, A. Mateus, A. Typas, M. M. Savitski, J. L. Espinoza, A. O'Rourke, K. E. Nelson, S. Hiller, N. Noinaj, T. F. Schäberle, A. D'Onofrio and K. Lewis, *Nature*, 2019, **576**, 459–464.
- R. D. Miller, A. Iinishi, S. M. Modaresi, B.-K. Yoo, T. D. Curtis, P. J. Larivière, L. Liang, S. Son, S. Nicolau, R. Bargabos, M. Morrisette, M. F. Gates, N. Pitt, R. P. Jakob, P. Rath, T. Maier, A. G. Malyutin, J. T. Kaiser, S. Niles, B. Karavas, M. Ghiglieri, S. E. J. Bowman, D. C. Rees, S. Hiller and K. Lewis, *Nat. Microbiol.*, 2022, **7**, 1661–1672.
- H. Kaur, R. P. Jakob, J. K. Marzinek, R. Green, Y. Imai, J. R. Bolla, E. Agustoni, C. V. Robinson, P. J. Bond, K. Lewis, T. Maier and S. Hiller, *Nature*, 2021, **593**, 125–129.
- B.-B. He, J. Liu, Z. Cheng, R. Liu, Z. Zhong, Y. Gao, H. Liu, Z. M. Song, Y. Tian and Y.-X. Li, *Angew. Chem., Int. Ed.*, 2023, **62**, e202311533.





- 8 H. Nam, J. S. An, J. Lee, Y. Yun, H. Lee, H. Park, Y. Jung, K.-B. Oh, D.-C. Oh and S. Kim, *J. Am. Chem. Soc.*, 2023, **145**, 22047–22057.
- 9 Y. L. Hu, F. Z. Yin, J. Shi, S. Y. Ma, Z. R. Wang, R. X. Tan, R. H. Jiao and H. M. Ge, *J. Am. Chem. Soc.*, 2023, **145**, 27325–27335.
- 10 C. L. Liu, Z. J. Wang, J. Shi, Z. Y. Yan, G. D. Zhang, R. H. Jiao, R. X. Tan and H. M. Ge, *Angew. Chem., Int. Ed.*, 2024, **63**, e202314046.
- 11 P. Nanudorn, S. Thiengmag, F. Biermann, P. Erkoc, S. D. Dirnberger, T. N. Phan, R. Fürst, R. Ueoka and E. J. N. Helfrich, *Angew. Chem., Int. Ed.*, 2022, **61**, e202208361.
- 12 F. Biermann, B. Tan, M. Breitenbach, Y. Kakumu, P. Nanudorn, Y. Dimitrova, A. S. Walker, R. Ueoka and E. J. N. Helfrich, *Chem. Sci.*, 2024, **15**, 17506–17523.
- 13 J. Liu, R. Liu, B.-B. He, X. Lin, L. Guo, G. Wu and Y.-X. Li, *ACS Bio Med. Chem. Au*, 2024, **4**, 268–279.
- 14 L. Padva, J. Gullick, L. J. Coe, M. H. Hansen, J. J. De Voss, M. Crüsemann and M. J. Cryle, *ChemBioChem*, 2025, **26**, e202400916.
- 15 C.-S. Phan and B. I. Morinaka, *Nat. Prod. Rep.*, 2024, **41**, 708–720.
- 16 A. H. Khan, J. R. Haedar, A. Zile and A. C.-S. Phan, *Tetrahedron Chem.*, 2025, **14**, 100128.
- 17 Y. Ye, A. Minami, Y. Igarashi, M. Izumikawa, M. Umemura, N. Nagano, M. Machida, T. Kawahara, K. Shin-Ya, K. Gomi and H. Oikawa, *Angew. Chem., Int. Ed.*, 2016, **55**, 8072–8075.
- 18 C. Y. Chiang, M. Ohashi and Y. Tang, *J. Am. Chem. Soc.*, 2025, **147**, 8113–8117.
- 19 D. N. Chigumba, L. S. Mydy, F. de Waal, W. Li, K. Shafiq, J. W. Wotring, O. G. Mohamed, T. Mladenovic, A. Tripathi, J. Z. Sexton, S. Kautsar, M. H. Medema and R. D. Kersten, *Nat. Chem. Biol.*, 2022, **18**, 18–28.
- 20 J. R. Chekan, L. S. Mydy, M. A. Pasquale and R. D. Kersten, *Nat. Prod. Rep.*, 2024, **41**, 1020–1059.
- 21 S. K. Kandy, M. A. Pasquale and J. R. Chekan, *Nat. Chem. Biol.*, 2025, **21**, 168–181.
- 22 Y. Shi, Y. Xia, W. Gao, J. Wang, B. Shi and H. Wang, *Nat. Prod. Rep.*, 2025, **42**, 763–773.
- 23 S. Saito, K. Atsumi, T. Zhou, K. Fukaya, D. Urabe, N. Oku, M. R. U. Karim, H. Komaki and Y. Igarashi, *Beilstein J. Org. Chem.*, 2020, **16**, 1100–1110.
- 24 A. H. Khan, J. R. Haedar, V. Kiselov, V. Romanuks, G. Smits, S. Donadio and C.-S. Phan, *ACS Chem. Biol.*, 2025, **20**, 259–265.
- 25 J. Zhang, L. Yu, H. Ogawa, Y. Nagata and H. Nakamura, *Angew. Chem., Int. Ed.*, 2024, **63**, e202409987.
- 26 H. Takahashi, A. Iwasaki, N. Kurisawa, R. Suzuki, G. Jeelani, T. Matsubara, T. Sato, T. Nozaki and K. Suenaga, *J. Nat. Prod.*, 2021, **84**, 1649–1655.
- 27 C.-S. Phan, K. Matsuda, N. Balloo, K. Fujita, T. Wakimoto and T. Okino, *J. Am. Chem. Soc.*, 2021, **143**, 10083–10087.
- 28 K. Ishida, Q. Sun, J. Teikari, C. Hertweck, E. Dittmann, M. Murakami and M. Baunach, *Angew. Chem., Int. Ed.*, 2025, e202502461.
- 29 M. Schubert, D. Labudde, H. Oschkinat and P. Schmieder, *J. Biomol. NMR*, 2002, **24**, 149–154.
- 30 A. Greule, T. Izoré, D. Iftime, J. Tailhades, M. Schoppet, Y. Zhao, M. Peschke, I. Ahmed, A. Kulik, M. Adamek, R. J. A. Goode, R. B. Schittenhelm, J. A. Kaczmarek, C. J. Jackson, N. Ziemert, E. H. Krenske, J. J. De Voss, E. Stegmann and M. J. Cryle, *Nat. Commun.*, 2019, **10**, 2613.
- 31 S. F. Altschul, T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D. J. Lipman, *Nucleic Acids Res.*, 1997, **25**, 3389–3402.
- 32 Y. Hitora, A. H. El-Desoky, Y. Sadahiro, A. Sejiyama, A. Kinoshita, Y. Ise, E. D. Angkouw, R. E. P. Mangindaan, T. Higaki and S. Tsukamoto, *J. Nat. Prod.*, 2024, **87**, 1197–1202.
- 33 Y. Zhao, E. Marschall, M. Treisman, A. McKay, L. Padva, M. Crüsemann, D. R. Nelson, D. L. Steer, R. B. Schittenhelm, J. Tailhades and M. J. Cryle, *Angew. Chem., Int. Ed.*, 2022, **61**, e202204957.
- 34 L. J. Coe, Y. Zhao, L. Padva, A. Keto, R. Schittenhelm, J. Tailhades, G. Pierens, E. H. Krenske, M. Crüsemann, J. J. De Voss and M. J. Cryle, *Chem. – Eur. J.*, 2024, **30**, e202400988.
- 35 D. T. Nguyen, L. Zhu, D. L. Gray, T. J. Woods, C. Padhi, K. M. Flatt, D. A. Mitchell and W. A. van der Donk, *ACS Cent. Sci.*, 2024, **10**, 1022–1032.
- 36 A. H. Khan, J. R. Haedar, V. Kiselov, V. Romanuks, G. Smits, S. Donadio and C.-S. Phan, *Org. Lett.*, 2025, **27**, 5715–5719.
- 37 M. Treisman, L. Coe, Y. Zhao, V. M. Sasi, J. Gullick, M. H. Hansen, A. Ly, V. Leichthammer, C. Hess, D. L. Machell, R. B. Schittenhelm, J. Hooper, C. J. Jackson, J. Tailhades, J. J. De Voss and M. J. Cryle, *Org. Lett.*, 2024, **26**, 1828–1833.
- 38 A. K. L. Nguy, R. J. Martinie, A. Cai and M. R. Seyedsayamdost, *J. Am. Chem. Soc.*, 2024, **146**, 19629–19634.

