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Myxoprincomide, a secondary metabolite of the myxobacterium *Myxococcus xanthus* DK 1622, is synthesised for the first time. The central, unusual α -ketoamide is generated at the end of the synthesis to avoid side reactions during the synthesis of this rather reactive subunit. Nevertheless, the synthetic natural product is obtained as an isomeric mixture. Detailed analytical investigations show that the identical isomeric mixture is found in the isolated natural product.

Myxobacteria, Gram-negative δ -proteobacteria, widespread in nature, are highly efficient producers of natural products.¹ *Myxococcus xanthus* DK 1622, the microbe of the year 2020, was the first myxobacterial strain whose complete genome sequence was published in 2006.² With 9.8 million base pairs, this extremely large bacterial genome contains at least 18 biosynthetic gene clusters encoding for polyketides, nonribosomal peptides or hybrids of both.³ Still, interestingly, only five compound classes can be isolated and characterised. This indicates that there are yet unassigned pathways, where no natural products could be related with so far.⁴ Modern genome mining techniques, combining targeted mutagenesis, liquid chromatography coupled with HRMS, and statistical data analysis identified Myxoprincomide as a new secondary metabolite from *Myxococcus xanthus* DK 1622.⁵

The linear nonapeptide contains some unusual building blocks (red in Fig. 1), such as β -hydroxylated valine, β -lysine and an α -keto- β -amino acid derived from valine. The tiny, isolated amounts allow for the structure elucidation of a new natural product, but only a few investigations of the biological activities of this compound have been conducted.⁶

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† Electronic supplementary information (ESI) available: Experimental details, compound characterization, copies of ¹H and ¹³C NMR spectra, HPLC chromatograms and MS² analysis. See DOI: <https://doi.org/10.1039/d2ob02021a>

Total synthesis of Myxoprincomide, a secondary metabolite from *Myxococcus xanthus*†

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Because of our interest in the total synthesis of natural products, we began working on the synthesis of Myxoprincomide to verify the proposed structure and obtain enough material in hand for further biological studies. The most remarkable building block is the central α -keto- β -amino acid, whose biosynthesis has yet to be explained. Similar building blocks are found in a series of mainly cyclic peptides such as jamaicensamide A,⁷ orbiculamide A⁸ and the keramamides.⁹ Furthermore, the α -ketoamino acid is presumably the most critical subunit in the molecule. In addition, the adjacent β -stereogenic centre should be somewhat acidic and sensitive to epimerisation. Similarly, the α -keto functionality should be relatively reactive, undergoing additions such as hydrate for-

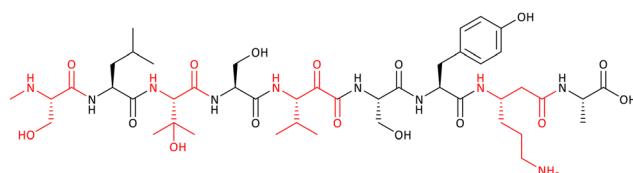
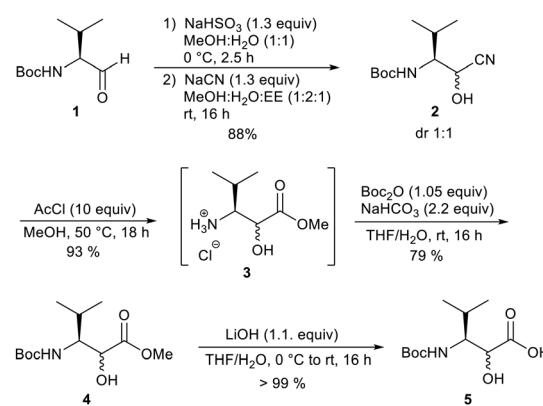


Fig. 1 Myxoprincomide.



Scheme 1 Synthesis of α -hydroxy- β -amino acid 5.

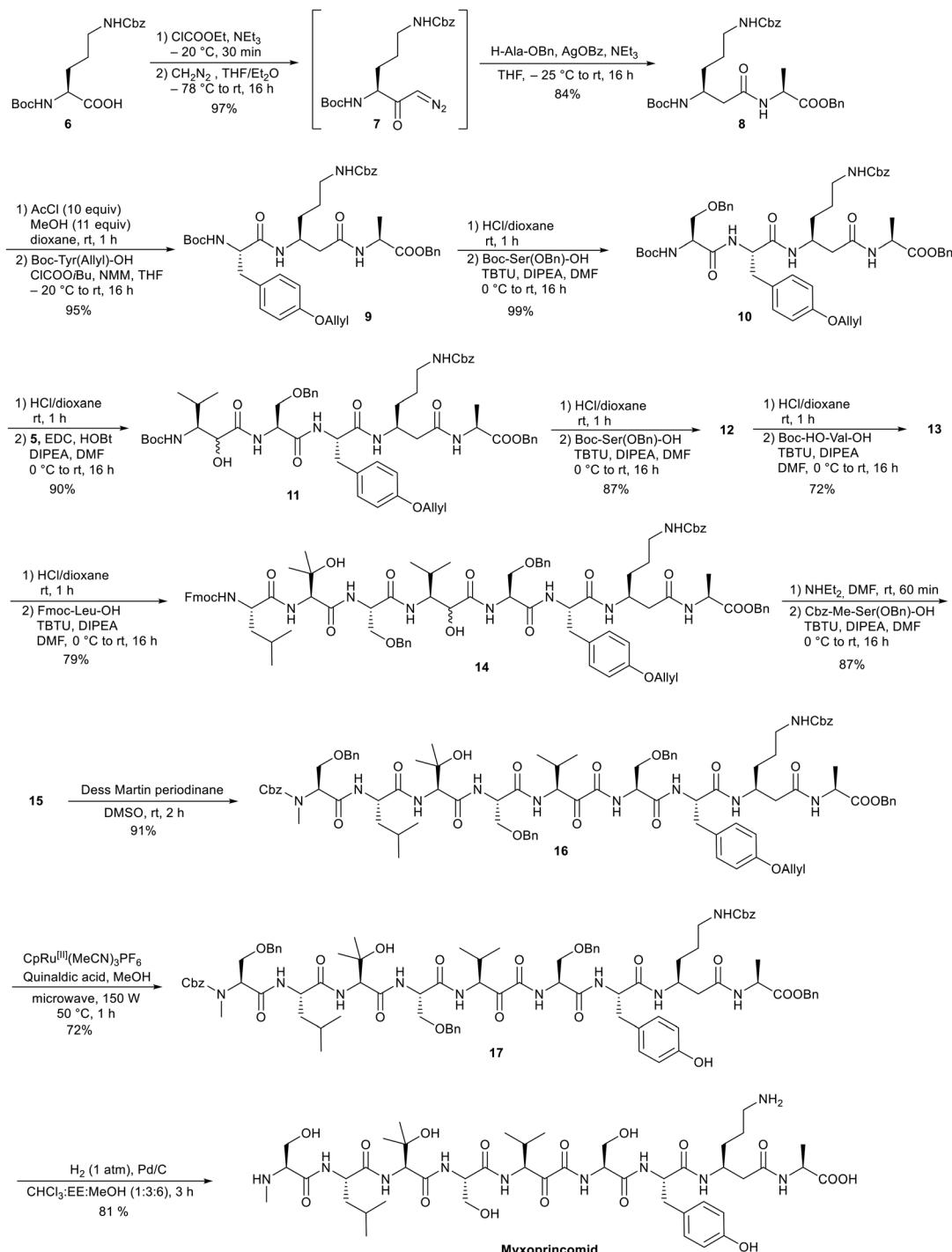


mation.¹⁰ Therefore, we decided to generate the α -keto-functionality near the end of the synthesis by oxidation of the corresponding α -hydroxy acid.

In our work, we obtained the desired *N*-Boc-protected derivative from Boc-valinal **1** by adding NaCN to provide cyanohydrine **2** as a 1:1 diastereomeric mixture (Scheme 1). The α -hydroxy nitrile **2** was converted into the α -hydroxy ester **3** using acetylchloride in MeOH. Under these conditions, the

Boc-protecting group was removed but reintroduced in the next step. Finally, the saponification of the methyl ester provided the free carboxylic acid **5**, which could be used in the peptide synthesis. The other unusual amino acids were prepared according to procedures in the literature or purchased.¹¹

The synthesis of the linear peptide chain, as shown in Scheme 2, started with a prolongation of L-ornithine **6**¹² *via* an Arndt-Eistert homologation to generate the required β -lysine.



Scheme 2 Synthesis of myxoprincomide.



The activation of **6** as a mixed anhydride and reaction with diazomethane provided diazoketone **7**, with only traces of ornithine methyl ester as a side product. This issue was not concerning since the diazoketone could be easily purified by flash chromatography. The diazoketone **7** could directly be reacted with alanine benzyl ester in the presence of silver benzoate to the desired dipeptide **8** in overall high yield. Next, the coupling with the following two protected amino acids proceeded under standard conditions. Tyrosine was used with an allyl-protecting group on the phenolic OH group to avoid side reactions in the final oxidation step. The allyl group could easily be removed later using $\text{CpRu}(\text{MeCN})_3\text{PF}_6$ as a catalyst.¹³ The subsequent coupling with α -hydroxy acid **5** proceeded without problems, and no O-protecting group was required. The final peptide couplings were performed using TBTU, which provided the best yields.

The oxidation of the α -hydroxy acid subunit in **15** was performed using Dess Martin periodinane. The reaction had to be carried out in DMSO because of the insolubility of **15** in dichloromethane, which is generally used in Dess Martin oxidations.¹⁴ Next, the O-allyl protecting group was removed from the tyrosine using the above-mentioned ruthenium complex. Finally, the benzyl-protecting groups were removed by catalytic hydrogenation. Of note, we had to carry out the reaction in a mixture of chloroform, ethyl acetate and methanol. Without chloroform, only incomplete conversion was observed, as well as a partial reduction of the α -keto group. Under the optimised conditions, myxoprincomide could be obtained in high yield, sufficient for biological studies (Scheme 2).

The characterisation and identification of the natural product were not as trivial as expected. The NMR spectra showed several sets of signals which could be partly explained by the existence of rotamers since some sets disappeared at a higher temperature. Nevertheless, the synthesised product contained two 'side products', also confirmed by UPLC. The purification of myxoprincomide by preparative HPLC and reanalysis indicated that the three products are evidently in equilibrium and converting into each other. A detailed analysis of the NMR spectra of the isolated natural product clearly showed that the same side products could also be found there.

The minor signals can be likely explained by an epimerisation of the α -keto amide or the formation of a hydrate at the keto functionality, but a clear identification was not possible. Therefore, we also performed MS measurements, which gave identical fragmentation patterns for the isolated and synthesised natural product (see ESI†).

Although most ^1H NMR signals fit almost perfectly, some signals showed differences, especially for protons close to the amino group of the β -lysine incorporated. This difference might be caused by a different pH or the water content of the NMR solvent. This phenomenon is common with such a highly polar natural product containing basic side chains.

With the synthetic myxoprincomide in hand, we carried out many bioactivity assays, investigating the IC_{50} values against several bacteria, fungi and cancer cell lines. Unfortunately, no significant activity was observed in any of these assays.

Therefore, the ecological function of this secondary metabolite of *Myxococcus xanthus* remains elusive.

Conclusions

We developed the first total synthesis of the myxobacterial natural product myxoprincomide with the longest linear sequence of 19 steps and in 16% overall yield. The unusual α -ketoamide as the central part of the molecule was generated at the end of the peptide synthesis to avoid side reactions at this subunit. The natural product itself was obtained as a mixture of isomers which equilibrate. Detailed investigations indicate that the same isomers are also found in the isolated natural product. Unfortunately, no significant biological activity of myxoprincomide could be observed.

Conflicts of interest

There are no conflicts to declare.

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References

- (a) H. Reichenbach, *J. Ind. Microbiol. Biotechnol.*, 2001, **27**, 149–156; (b) R. O. Garcia, D. Krug and R. Müller, *Methods Enzymol.*, 2009, **458**, 59–91; (c) K. J. Weissman and R. Müller, *Nat. Prod. Rep.*, 2010, **27**, 1276–1295.
- B. S. Goldman, W. C. Nierman, D. Kaiser, S. C. Slater, A. S. Durkin, J. Eisen, C. M. Ronning, W. B. Barbazuk, M. Blanchard, C. Field, C. Halling, G. Hinkle, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 15200–15205.
- D. E. Whitworth, *Myxobacteria: Multicellularity and Differentiation*, ASM Press, Chicago, 2007.
- (a) H. B. Bode and R. Müller, *Angew. Chem.*, 2005, **117**, 6988–7007, (*Angew. Chem., Int. Ed.*, 2005, **44**, 6828–6846); (b) H. B. Bode, P. Meiser, T. Klefisch, N. S. Cortina, D. Krug, A. Göhring, G. Schwär, T. Mahmud, Y. A. Elnakady and R. Müller, *ChemBioChem*, 2007, **8**, 2139–2144; (c) S. C. Wenzel, P. Meiser, T. Binz, T. Mahmud and R. Müller, *Angew. Chem.*, 2006, **118**, 2354–2360, (*Angew. Chem., Int. Ed.*, 2006, **45**, 2296–2301); (d) V. Simunovic, J. Zapp, S. Rachid, D. Krug, P. Meiser and R. Müller, *ChemBioChem*, 2006, **7**, 1206–1220; (e) P. Meiser, K. J. Weissman, H. B. Bode, D. Krug, J. S. Dickschat, A. Sandmann and R. Müller, *Chem. Biol.*, 2008, **15**, 771–781.



5 N. S. Cortina, D. Krug, A. Plaza, E. Revermann and R. Müller, *Angew. Chem.*, 2012, **124**, 836–841, (*Angew. Chem., Int. Ed.*, 2012, **51**, 811–816).

6 N. S. Cortina, *Pathways to products: exploring the biosynthetic depth of *Myxococcus xanthus* by comprehensive secondary metabolite profiling*, dissertation, Saarbrücken, 2013.

7 M. T. Jamison and T. F. Molinski, *J. Nat. Prod.*, 2016, **79**, 2243–2249.

8 N. Fusetani, T. Sugawara and S. Matsunaga, *J. Am. Chem. Soc.*, 1991, **113**, 7811–7812.

9 (a) F. Itagaki, H. Shigemori, M. Ishibashi, T. Nakamura, T. Sasaki and J. Kobayashi, *J. Org. Chem.*, 1992, **57**, 5540–5542; (b) H. Uemoto, Y. Yahiro, H. Shigemori, M. Tsuda, T. Takao, Y. Shimonishi and J. Kobayashi, Keramamides K and L, *Tetrahedron*, 1998, **54**, 6719–6724.

10 (a) M. Robello, E. Barresi, E. Baglini, S. Salerno, S. Taliani and F. Da Settimo, *J. Med. Chem.*, 2021, **64**, 3508–3545; (b) S. F. Brady, J. T. Sisko, K. J. Stauffer, C. D. Colton, H. Qiu, S. D. Lewis, A. S. Ng, J. A. Shafer, M. J. Bogusky, D. F. Veber and R. F. Nutt, *Bioorg. Med. Chem.*, 1995, **3**, 1063–1078; (c) G. G. Xu and F. A. Etzkorn, *Org. Lett.*, 2010, **12**, 696–699.

11 Y. Luo, G. Evindar, D. Fishlock and G. A. Lajoie, *Tetrahedron Lett.*, 2001, **42**, 3807–3809.

12 G. Pattenden and T. Thompson, *Chem. Commun.*, 2001, 717–718.

13 S. Tanaka, H. Saburi, Y. Ishibashi and M. Kitamura, *Org. Lett.*, 2004, **6**, 1873–1875.

14 D. B. Dess and J. C. Martin, *J. Org. Chem.*, 1983, **48**, 4155–4156.