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Recent highlights of the total synthesis of cyclic peptide natural products

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This review described the total synthesis of naturally occurring cyclic peptides with unique structures covering 2020 to 2022, *i.e.*, darobactin A, pyritide A2, decatransin, mannopeptimycin β , α - and β -amanitins, orfamide A, and MA026, paying particular attention to the construction of their unique structures *via* macrocyclization.

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1 Introduction

Cyclic peptides, whose molecular weights lie between those of small molecules and proteins, are garnering considerable attention in drug discovery, and they are expected to have potent activity with high target specificity.¹ Peptides are easily hydrolyzed by proteases and are not membrane-permeable, which hinders their application as oral drugs. However, recent advancements in lipophilic cyclic peptides containing unnatural amino acids with side-chain modification, *N*-alkylamino acids, *D*-amino acids, lipophilic hydroxycarboxylic acids, and other bridging structures, have significantly improved their pharmacokinetics, such as stability and membrane permeability, characteristics essential for oral drugs.^{2–5}

This review highlights recent advancements in the synthetic methodologies for interesting cyclic peptide natural products

reported in 2020–2022 and focuses on strategies for building their complex structures. Key developments include the formation of novel structural motifs bridged by side chains within cyclic peptides, such as darobactin A and α - and β -amanitins. The unique construction of a pyridine ring within the cyclic peptide framework, as achieved in the synthesis of pyritide A2, is also discussed. For decatransin, the formation of a depsipeptide containing contiguous *N*-alkylamino acids and subsequent macrolactone cyclization *via* the Mitsunobu reaction are discussed. Furthermore, for the synthesis of glycopeptide mannopeptimycin β , the difficult macrocyclization at hydroxyenduracididine was achieved by the Ser/Thr ligation, a method originally developed for protein synthesis. This review also focuses on the structure determination of decatransin through a total synthesis and the structural revisions of orfamide A and MA026 based on synthetic evidence. In addition, highlighting methodologies for optimizing amidation reactions important for peptide bond formation and macrocyclization, emphasizes the importance of careful selections of conditions for these reactions, and achieving the total synthesis through a final deprotection step provides guidance on the use of the protecting groups. By offering insights into both the design and execution of advanced synthetic strategies, this review aims to inform and inspire further progress in the synthesis of cyclic peptides and their analogues.

2 Total synthesis of cyclic peptide natural products

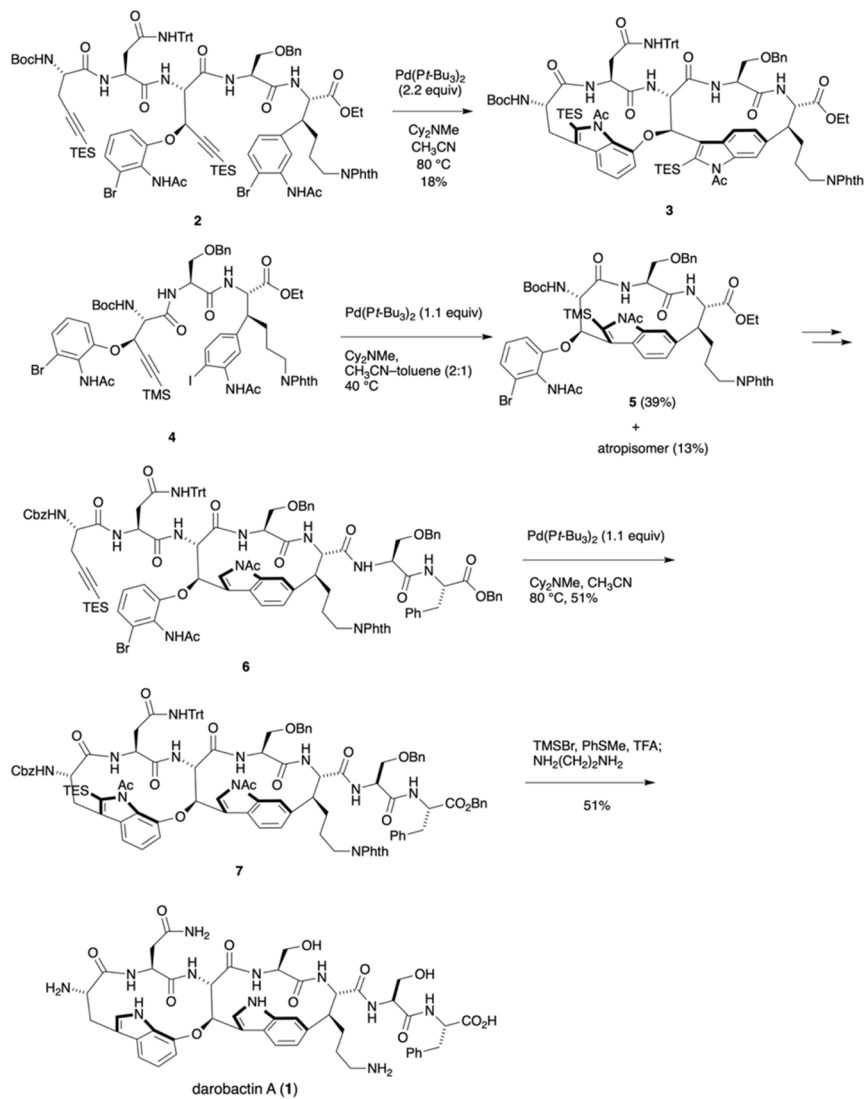
2.1 Darobactin A

Darobactin A (**1**), a bicyclic peptide natural product isolated from *Phototrhahdus microbiome*, shows little activity against Gram-positive bacteria but potent antibacterial activity against various Gram-negative bacteria.⁶ Darobactin A is a novel antibiotic that functions *via* an unprecedented mechanism of

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Scheme 1 The total synthesis of darobactin A (1) by the Patel, Petrone, and Sarlah group.⁹

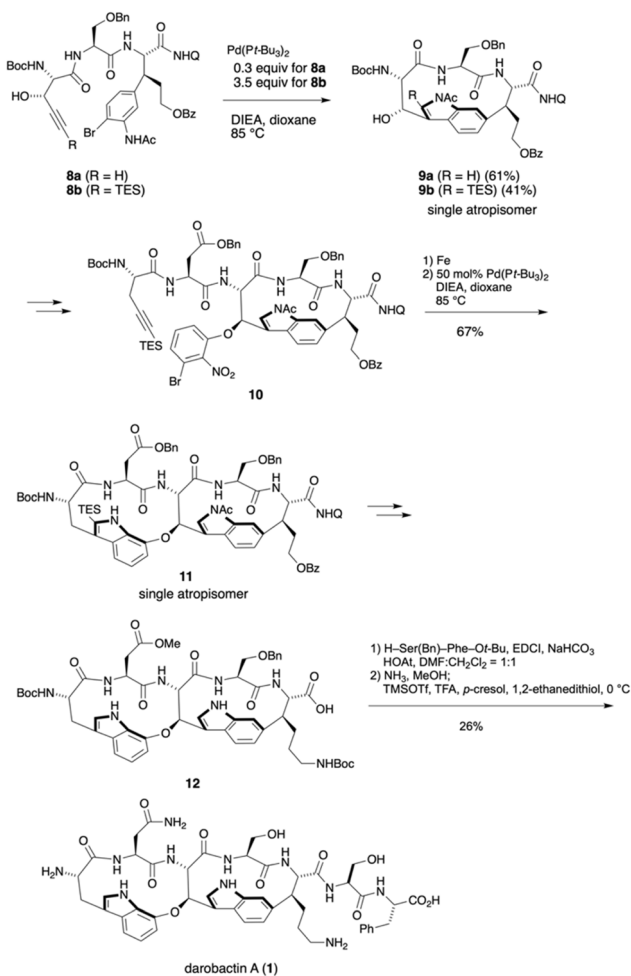
action, which involves mimicking the β -sheet structure and binding to the bacterial insertase complex Bama to inhibit its activity.^{7,8} Unprecedented structure of **1** is characterized by the two rings linked by the indole moieties of two tryptophans with C–O and C–C bonds, respectively. The challenging total synthesis of **1** was reported simultaneously by two groups using intramolecular palladium-catalyzed Larock indole synthesis as key reactions.

The Patel, Petrone, and Sarlah group initially attempted the double palladium-catalyzed intramolecular Larock indole synthesis of linear peptide **2**. However, although this unique double macrocyclization proceeded, the undesired product **3** was obtained in 18% yield, in which the eastern macrocycle was the atropisomer. This is due to the fact that the construction of the western macrocycle occurred first. Thus, they decided to start their synthetic route by preparing the eastern macrocycle. The more reactive phenyl iodide was then introduced into precursor **4**, which underwent selective formation of the eastern

macrocycle by the Larock indole synthesis, leading to the desired product **5** and its atropisomer in 39% and 13% yields, respectively. Subsequent conversion of **5** to compound **6**, followed by a second Larock indole synthesis, provided the desired product **7** in 51% yield. The nine protecting groups in **7** were removed *via* treatment with TMSBr/PhSMe, and subsequent addition of ethylenediamine in one pot furnished **1**, completing the total synthesis of darobactin A (**1**) (Scheme 1).⁹

The Baran group also investigated the construction of the eastern macrocycle *via* Larock indole synthesis.¹⁰ As shown in Scheme 2, the reaction of triethylsilylated alkyne **8b** required 3.5 equivalents of Pd(Pt-Bu₃)₂. Although cyclization yielded **9b** in 41% yield, there was a problem of reproducibility. In contrast, the cyclization of terminal alkyne **8a** proceeded in the presence of 0.3 equivalents of Pd(Pt-Bu₃)₂ catalyst, furnishing product **9a** as a single atropisomer in an improved yield of 61% on a gram scale. Product **9a** was then converted to cyclization precursor **10** with a western macrocycle, from which the desired **11** was



Scheme 2 The total synthesis of darobactin A (1) by the Baran group.¹⁰

obtained in 67% yield *via* reduction of the nitro group followed by a second Larock indole synthesis. Various condensation agents were investigated for the coupling of carboxylic acid **12** with the dipeptide H-Ser(Bn)-Phe-Ot-Bu. Notably, only EDCI/HOAt allowed the reaction to proceed, and treatment with NH₃/MeOH and deprotection with a TMSOTf/TFA/*p*-cresol/1,2-ethanedithiol cocktail furnished darobactin A (1).

It should be noted that the intramolecular Larock indole synthesis is worthwhile to construct an indole ring within a macrocyclic peptide. Particularly, in the synthesis of darobactin A using this method, the eastern macrocycle needs to be constructed prior to the western one to control stereoselective formation of the desired atropisomer.

2.2 Pyritide A2

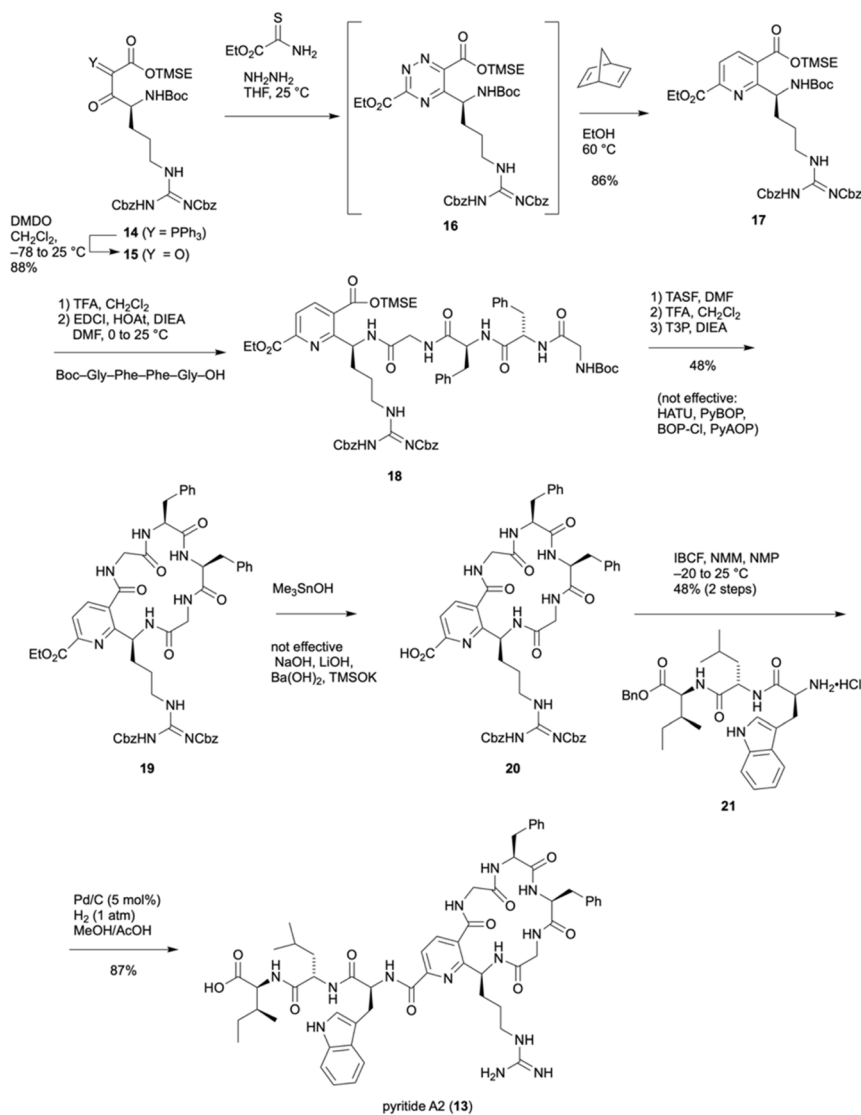
Pyritides A1 and A2 (**13**) were discovered by the Mitchell group through chemoenzymatic synthesis using putative synthetic intermediates based on genome mining.¹¹ The pyritide possesses a unique structure that includes 6-(1-amino-4-guanidinobutyl)pyridine-2,5-dicarboxylic acid as a non-proteinogenic amino acid component. One of its carboxyl groups along with its amino group participates in the formation of a macrocyclic peptide, while the other carboxyl group is

attached to a tripeptide side chain. Notably, it is intriguing how two amide bonds, one of which forms the macrocyclic peptide, are generated from the pyridine-2,5-dicarboxylic acid, which is presumed to have low reactivity. Their total synthesis was reported by the Sarlah group, as shown in Scheme 3.¹² They found that using freshly prepared DMDO was crucial to afford unstable tricarbonyl compound **15** *via* oxidative cleavage of the C=P bond of diacylylide **14**. Next, the reaction of **15** and the amidrazone prepared from hydrazine and ethyl thioamido oxalate was performed to provide triazine **16**, which was directly subjected to an aza-Diels-Alder reaction with 2,5-norbornadiene to construct a pyridine ring, yielding **17** in 86% yield. Subsequently, a tetrapeptide was condensed to the amino terminus of **17** using EDCI/HOAt to afford **18**. Although acid deprotection resulted in degradation, TASF was used to convert the TMSE ester to a carboxylic acid, which was then treated with TFA to remove the Boc group, leading to a cyclization precursor. Macrolactamization of the resulting pyridine carboxylic acid with the N-terminal glycine residue was challenging. Conventional reagents such as HATU, PyBOP, BOP-Cl, and PyAOP led to the formation of oligomers, failing to afford the desired cyclic peptide **19**. In contrast, T3P proved effective for macrolactamization, providing **19** in 48% overall yield over three steps. Attempts to hydrolyze the pyridine carboxylic acid ethyl ester using NaOH, LiOH, Ba(OH)₂, and TMSOK resulted in the removal of the Cbz group of the guanidyl group in all cases. In contrast, using Me₃SnOH, only the ethyl ester was successfully converted to carboxylic acid **20**, which was probably facilitated by the nitrogen atom of the pyridine ring. Finally, coupling of **20** with tripeptide **21** followed by hydrogenolysis completed the total synthesis of pyritide A2 (**13**).

2.3 Decatransin

Decatransin (**22**), a 30-membered cyclodecadepsipeptide that was isolated from saprophyte fungus *Chaetosphaeria tulasneorum* in 2015.¹³ It exhibits potent growth inhibition against HCT116 cells with an IC₅₀ value of 140 nM through inhibition of the translocon channel Sec61. Since other Sec61 inhibitors have been studied as anticancer, immunosuppressive, and antiviral agents,¹⁴ **22** is expected to serve as a potential lead for drug discovery. In 2023, cryo-EM analysis elucidated the binding structures of the Sec61 complex with various inhibitors, including decatransin.¹⁵ Once efficient synthetic methods are established, structure-based drug design will enable further structural modification to optimize its biological activity. Decatransin consists of 2-hydroxy-5-methylhexanoic acid and nine amino acids including nonproteinogenic amino acids, such as three pipercolic acids, homoleucine, two methylhomoleucines, methylthreonine, alanine, and methylisoleucine. Accordingly the backbone amides are highly *N*-alkylated, a key feature contributing to cell permeability of cyclic peptides. Genome sequencing analysis revealed that **22** is biosynthesized by a non-ribosomal peptide synthetase (NRPS). The Doi group narrowed down the possible structures of the natural product to two stereoisomers based on the presumed biosynthetic pathway, completed the total synthesis,





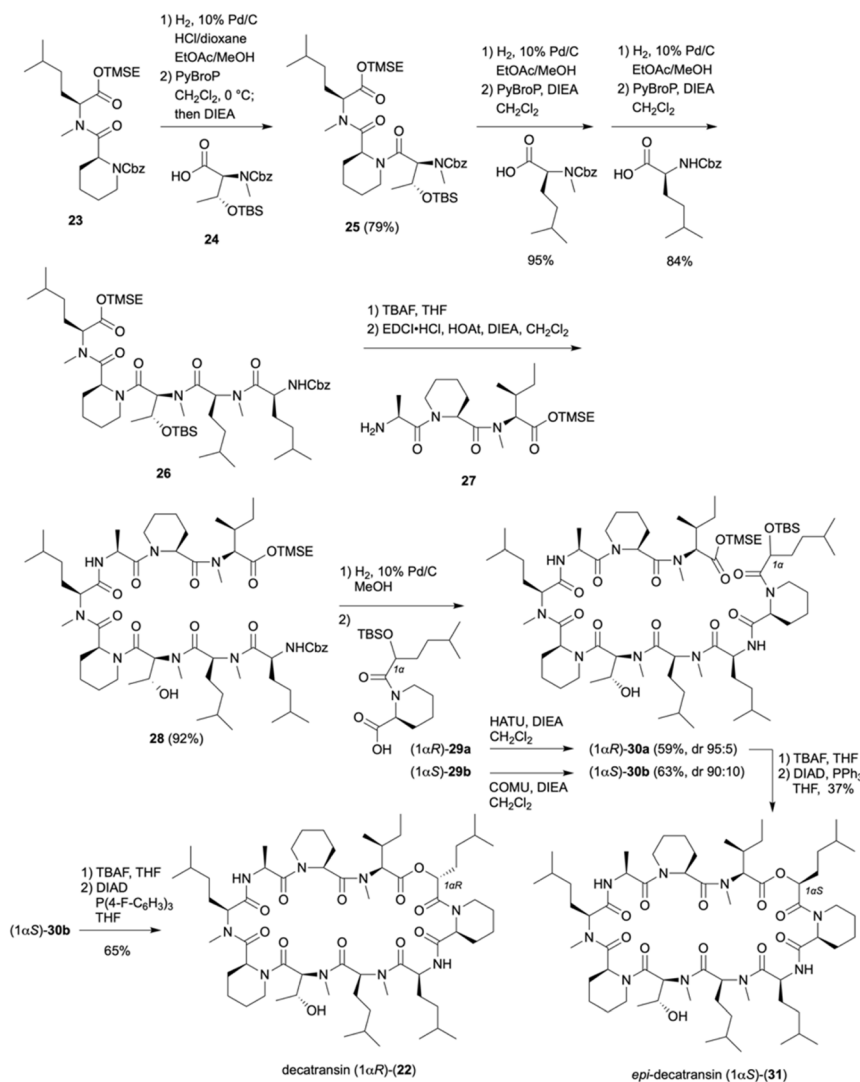
Scheme 3 Total synthesis of pyritide A2 (13).

and determined the stereochemical configurations of decatransin as **22** by comparing the spectral and biological data of the synthetic product with those of the isolated natural product.¹⁶

As shown in Scheme 4, the Cbz group of dipeptide **23** containing a TMSE ester was removed *via* hydrogenolysis in the presence of HCl, and the resulting hydrochloride salts were treated with **24** and PyBroP/ CH_2Cl_2 with slow addition of DIEA. Using this method, tripeptide **25** was obtained without forming a diketopiperazine. Although a *t*-butyl ester is usually used to avoid the formation of diketopiperazines, it could not be used in this case because the *N*-methylamide bond was cleaved under the acidic conditions used for the hydrolysis of the *t*-butyl ester corresponding to pentapeptide **26**. The pentapeptide TMSE ester **26** was prepared *via* subsequent elongation to the *N*-methylamino terminus with MeHle and Hle using PyBroP/DIEA/ CH_2Cl_2 . Thus, removal of the TMSE ester in pentapeptide **26** with TBAF/THF proceeded without cleavage of

the *N*-methylamide bonds, and the resulting carboxylic acid was coupled with tripeptide **27** using EDCI/HOAt/DIEA to provide octapeptide **28** in 92% yield. After removal of the Cbz group, coupling the resulting amine with (1 α *R*)-**29a** and (1 α *S*)-**29b** was performed using HATU/DIEA/ CH_2Cl_2 , respectively. (1 α *R*)-**30a** was obtained in 59% yield with 5% epimerization, whereas (1 α *S*)-**30b** was provided in 70% yield with 22% epimerization, which was reduced to 10% when COMU/DIEA/ CH_2Cl_2 was used. After the removal of the two silyl groups with TBAF, attempts to achieve macrolactonization using either MNBA or TCBC were unsuccessful. Notably, however, the Mitsunobu reaction of the seco acid derived from (1 α *R*)-**30a** with DIAD/ PPh_3 /THF proceeded with inversion of configuration, leading to the cyclized product (1 α *S*)-**31** in 37% yield. Similarly, the reaction of the seco acid from (1 α *S*)-**30b** furnished (1 α *R*)-**22** in 44% yield. The yield was further improved to 65% when $\text{P}(4\text{-F-C}_6\text{H}_4)_3$ was used instead of PPh_3 . The spectral data and cytotoxicity against HCT-116 cells of (1 α *R*)-**22**





Scheme 4 Total synthesis of decatransin (22).

were in good agreement with those of isolated decatransin, confirming that the total synthesis of decatransin was accomplished and its structure is (1αR)-22.

This total synthesis is considered to have paved the way for the further study on the structure–activity relationships (SAR) of decatransin and its potential in drug development.

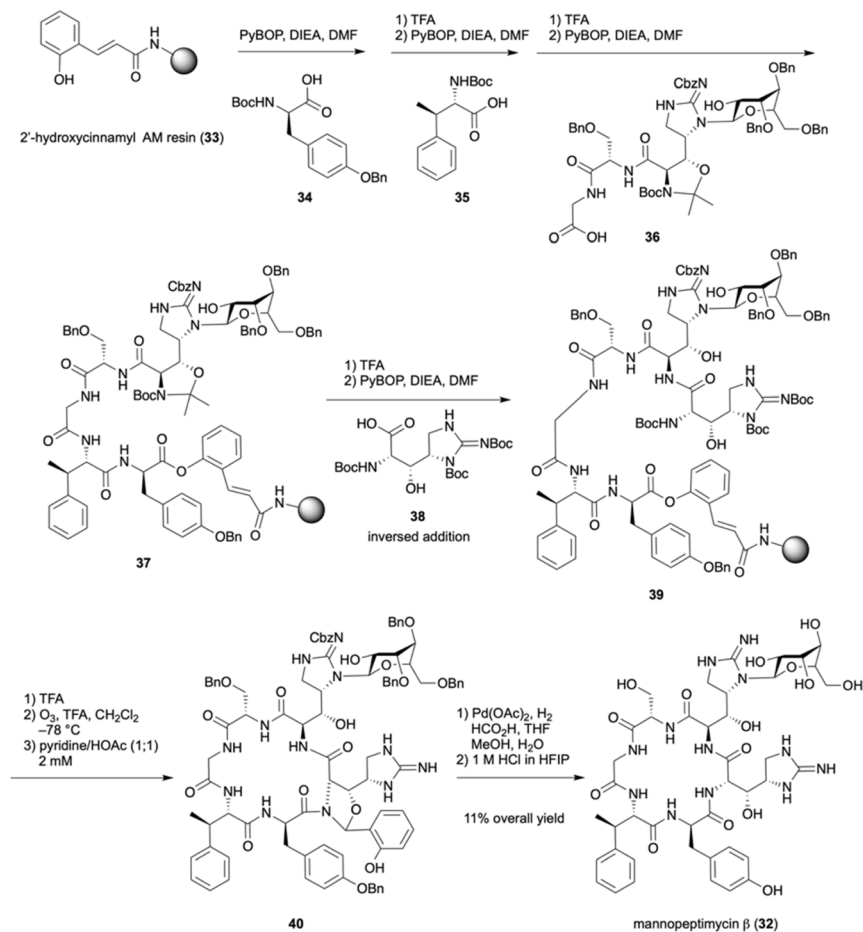
2.4 Mannopectimycin β

Mannopectimycins are cyclic peptide natural products that were isolated from *Streptomyces hygroscopicus* LL-AC98 and contain Tyr, Gly, Ser, and nonproteinogenic amino acids such as L- and D-β-hydroxyenduracididines (βhEnd) and β-methylphenylalanine. The guanidyl group of D-βhEnd is N-mannosylated in mannopectimycins α and β, and Tyr is O-dimannosylated in mannopectimycin α.^{17,18} The high antimicrobial activity of mannopectimycins against Gram-positive bacteria was initially demonstrated, and it was later found that they also possess antimicrobial activity against methicillin-

resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE). The total synthesis of their aglycone reported by the Fuse and Doi group corrected the stereochemical configuration of the β-position of β-methylphenylalanine,¹⁹ and the Chen group developed the total synthesis of mannopectimycins α and β.²⁰ The Li group succeeded in the total synthesis of mannopectimycin β (32) using an originally developed Ser/Thr ligation method for the challenging formation of the macrocycle.²¹

In a prior solution-phase synthesis, serious epimerization at the α-position was observed upon condensation of the protected N-α-Man-D-βhEnd-OH at the N-terminus of the tetrapeptide. Moreover, the purification of the desired compound from the mixture of diastereomers was difficult. Conversely, condensation with protected N-α-Man-D-βhEnd-OH and dipeptide H-Ser(Bn)-Gly-OAll proceeded without any problem. Thus, the solid-phase synthesis using the obtained tripeptide 36 was investigated.





Scheme 5 The total synthesis of mannopeptimycin β (32).

As shown in Scheme 5, Boc-D-Tyr(Bn)-OH (34) was condensed with hydroxycinnamic acid-immobilized AM resin 33 using PyBOP/DIEA/DMF. After the removal of the Boc group, condensation with Boc- β -MePhe-OH (35) was performed. Similarly, mannosylated tripeptide 36 was condensed to provide polymer-supported pentapeptide 37. In these couplings, the excess amount of the activated form of a carboxylic acid was hydrolyzed to recover the acid. After the removal of the acetonide and the Boc group in 37, condensation of the resulting free amino alcohol with L- β -hEnd 38 was performed, but the corresponding product was obtained in low yield. When a large excess of 38 was used, dimerization of the activated ester of 38 afforded a cyclic diester. This problem was solved by performing the inverted addition of 38 in a portion-wise manner, which afforded a sufficient conversion (70%) when three equivalents of 38 were used. The removal of the three Boc groups in the resulting 39 followed by ozonolysis provided the linear precursor salicylaldehyde ester at the C-terminus. Macrocyclization using an originally developed Ser/Thr ligation was performed in pyridine/AcOH (1 : 1) under high dilution conditions of 2 mM to provide the desired product 40. The benzyl group was removed *via* hydrogenolysis in the presence of Pd(OAc)₂ instead of the conventionally used

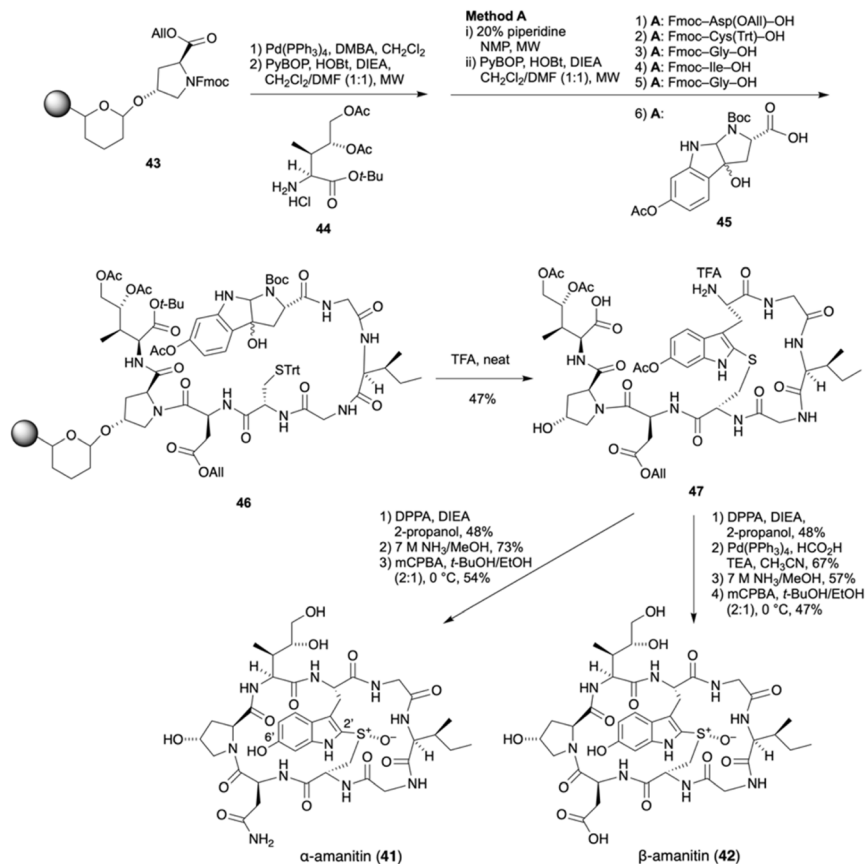
Pd/C to avoid absorption of the product on activated charcoal. Finally, the *N,O*-acetal was hydrolyzed to furnish mannopeptimycin β (32) in 11% overall yield from the polymer-supported starting material. The Ser/Thr ligation method using the salicylaldehyde ester was also applicable to β -hydroxyenduracididine and enabled the otherwise difficult to conduct cyclization reaction.

2.5 Amanitin

α -Amanitin (41) and β -amanitin (42) produced by the death cap mushroom (*Amanita phalloides*) are toxins that inhibit RNA polymerase II. They possess a cyclooctapeptide structure in which the sulfur atom in cysteine is linked at the 2' position of 6'-hydroxytryptophan, forming tryptathionine, and is oxidized to (*R*)-sulfoxide. They also contain other nonproteinogenic amino acids such as hydroxyproline and 4,5-dihydroxyisoleucine.²² Due to its potent toxicity, amanitin has been studied as a payload of an antibody–drug conjugate (ADC). The total synthesis of 41 was first accomplished by the Perrin group in 2018 (ref. 23) and later by the Süßmuth^{24,25} and Müller²⁶ groups. The latter is presented in this section.

As reported by the Perrin group, hydroxyproline was loaded onto the solid phase *via* a THP linker. Starting from 43, the





Scheme 6 Total synthesis of α -amanitin (**41**) and β -amanitin (**42**).

palladium-catalyzed conversion of the allyl ester to a carboxylic acid, followed by condensation at the C-terminus with (3*R*,4*R*)-4,5-diacetoxyisoleucine *t*-butyl ester **44** (PyBOP/HOBT/DIEA) under microwave irradiation [35 W, 50 °C, 10 min]. Then, the N-terminus was elongated by repeated removal of the Fmoc group (20% piperidine) and condensation with amino acids using PyBOP under microwave irradiation as shown in Method A (Scheme 6). Thus, Asp(OAll), Cys(Trt), Gly, Ile, Gly, and **45** were elongated to provide linear octapeptide **46**. Treatment of **46** with TFA led to cleavage from the solid phase and removal of the *t*-butyl ester, Boc, and trityl groups, and a first cyclization *via* the Savigny-Fontana reaction afforded the desired tryptathionine **47** in 47% yield.²⁷ In a second cyclization, macrolactamization of **47** (DPPA/DIEA/2-propanol/6 mM) was conducted followed by ammonia treatment to remove the acetyl groups and conversion of the allyl ester to a primary amide. Oxidation with mCPBA yielded a 2 : 1 mixture of diastereomeric sulfoxides. The desired α -amanitin (**41**) was isolated by preparative reversed-phase HPLC in 54% yield. After the second cyclization, the allyl ester was converted to a carboxylic acid using Pd(PPh₃)₄/HCO₂H/TEA, and treatment with ammonia followed by oxidation under the conditions used for the synthesis of **41** furnished β -amanitin (**42**) (Scheme 6). Notably, these synthetic methods led to the development of anti-BCMA ADC DHP-101, which is undergoing clinical studies.²⁸

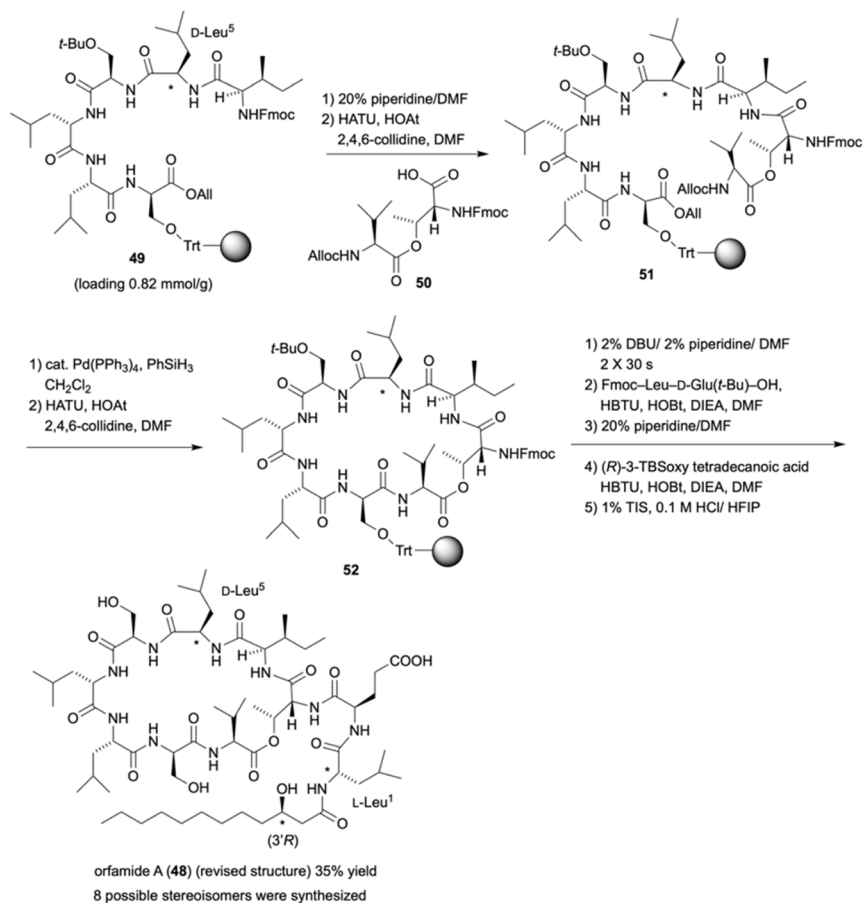
In addition, the Perrin group investigated the reaction conditions for the (*R*)-selective sulfoxidation and achieved the

stereoselective synthesis of (*R*)-5'-OH-6'-deoxyamanitin (71%, dr 19 : 1), which was found to be as potent as **41**. Application of this method using UHP/Ti(Oi-Pr)₄/L-DET (1.2 : 1 : 8) and CH₂Cl₂/MeOH (3 : 1) to the synthesis of **41** gave a stereoselectivity of >50 : 1.²⁹ Therefore, highly efficient and stereoselective synthetic methods for amanitins have been established.

2.6 Orfamide A

Orfamide A (**48**) is a cyclic lipodepsipeptide isolated from *Pseudomonas protegens* Pf-5 showing an antifungal activity.³⁰ It is a cyclodepsipeptide bearing an ester linkage formed between the hydroxyl group of Thr and the carboxylic acid of Val. The amino group of the Thr is further acylated with a fatty acid-linked dipeptide. In this context, installation of the ester bond after *N*-acylation is expected to be challenging due to significant steric hindrance. Conversely, the pre-prepared ester bond may lead to undesired O \rightarrow N acyl migration during the subsequent *N*-acylation step. These synthetic challenges must be carefully considered in the design of its synthesis. Arndt group achieved the total synthesis of **48** and corrected the stereochemical configuration. Initially, compounds with *L*-Leu¹, *L*-Leu⁵, and 3'*S*- or 3'*R*-configuration were synthesized. However, the retention times observed in a LC/MS analysis and the ¹H NMR spectra did not match those of the natural product. Analysis of the NRPS biosynthesis genes of orfamide A suggested that the condensation-epimerization domains in modules 1 and 5 may undergo





Scheme 7 Total synthesis of the revised structure of orfamide A (48).

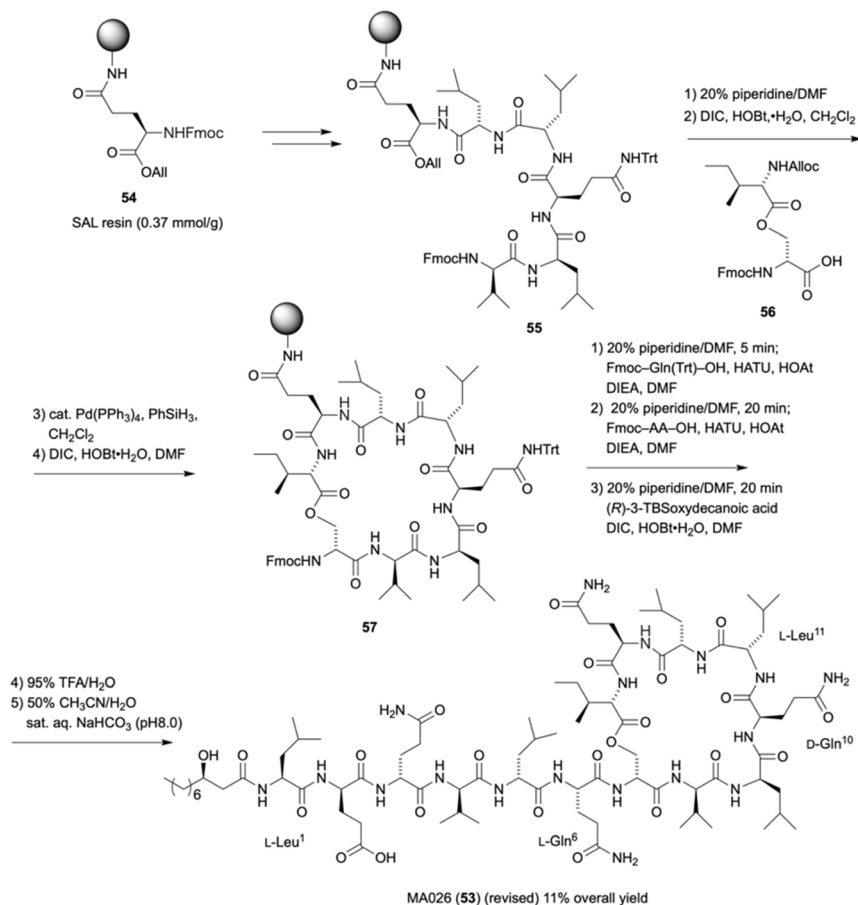
epimerization of Leu¹ and/or Leu⁵. Thus, all possible stereoisomers were synthesized. Initial attempts on on-resin macrolactonization resulted in epimerization. Therefore, solid-phase peptide synthesis was conducted using didepsipeptide **50** with a pre-prepared ester linkage. The side-chain hydroxyl group of Ser was loaded on trityl resin and sequentially condensed with amino acids at the N-terminus to form hexapeptide **49** using HBTU/HOBt/DIEA/DMF/NMP. After the removal of the Fmoc group, condensation with didepsipeptide **50** using HATU/HOAt/2,4,6-collidine/DMF afforded octadepsipeptide **51** along with 3% of a dehydrobutyrine residue. Then, the allyl and Alloc groups were removed by treatment with Pd(PPh₃)₄/PhSiH₃/CH₂Cl₂. On-resin macrolactamization was performed using the optimized conditions, *i.e.*, HATU/HOAt/2,4,6-collidine, to provide **52**. The Fmoc group in **52** was carefully removed by two 30 s treatments with 2% DBU/2% piperidine/DMF to reduce *N,O*-acyl migration to 4%. In contrast, 54% *N,O*-acyl migration was observed when 20% piperidine/DMF was used. Notably, 86% *N,O*-acyl migration occurred in linear peptide **51**, suggesting that fixing the conformation by constructing the ring structure would suppress the *N,O*-acyl migration. Then, the dipeptide side chain was introduced using HBTU/HOBt/DIEA/DMF. Subsequent condensation with TBS-protected carboxylic acid under similar conditions furnished protected **48**, from which orfamide A (**48**) was obtained in 35% overall yield after

removal of the TBS and *t*-Bu groups (1% TIS/0.1 M HCl/HFIP). Similarly, seven of its diastereomers were also synthesized. The reversed-phase HPLC analysis, ¹H NMR spectra, and biological response of the synthesized **48** were in good agreement with those of the natural product. Therefore, the correct structure of orfamide A was established (Scheme 7).³¹

2.7 MA026

MA026 (**53**), a 25-membered cyclic depsipeptide isolated from *Pseudomonas* sp. RtIB026, possesses anti-infectious hematopoietic necrosis virus activity.³² In **53**, a Ser in place of the Thr found in orfamide A (**48**), forms the macrolactone linkage *via* its hydroxyl group. The amino group of the Ser is further acylated with a peptide side chain. This structural arrangement raises synthetic challenges similar to those encountered in the synthesis of **48**. Therefore, the bond-forming steps should be carefully planned in the synthesis of **53**. The Hayashi group performed a total synthesis of a reported structure that includes an L-Leu¹⁰-D-Gln¹¹ moiety. However, the HPLC retention times of the synthesized product and MA026 did not match, and the former did not show tight junction-opening activity unlike MA026. Then, the linear peptide hydrolyzed at the ester bond of MA026 and the corresponding linear peptide with L-Leu¹⁰-D-Gln¹¹ prepared separately were compared *via* MS/MS analysis, finding that the true structure contains L-Leu and D-Gln in the





Scheme 8 Total synthesis of the revised structure of MA026 (53).

reversed order. In fact, synthesized 53 with D-Gln¹⁰-L-Leu¹¹ was identical to MA026. The total synthesis of the revised structure of MA026 (53) was performed as depicted in Scheme 8.

The side-chain carboxylic acid of Fmoc-D-Glu-OAll was loaded on SAL resin. From 54, SPPS was performed to afford hexapeptide 55. The didepsipeptide 56 with a pre-prepared ester linkage was condensed using DIC/HOBT/CH₂Cl₂ to provide octadepsipeptide. After removing the Alloc and allyl groups using Pd(PPh₃)₄/PhSiH₃, on-resin macrolactamization was performed using DIC/HOBT/DMF to afford 57 without formation of its dimer. During the removal of the Fmoc group, *N,O*-acyl migration could be reduced to 5.2% by shortening the treatment time with 20% piperidine to 5 min. After sequential condensation of amino acids, coupling with (*R*)-3-TBSoxydecanoic acid yielded protected 53. Removal of the protecting groups with 95% TFA/H₂O afforded MA026 (53) in 11% overall yield.³³ Furthermore, single-crystal X-ray crystallography and circular dichroism analyses revealed that 53 has a left-handed α -helical structure, and a SAR study was also performed.³⁴

3 Summary

This review describes the total syntheses of cyclic peptide natural products, including darobactin A, pyritide A2, decatransin, mannopeptimycin β , α - and β -amanitins, orfamide A,

and MA026. The focus is on constructing their unique structures, providing detailed solutions and optimized reaction conditions. While solid-phase peptide synthesis is a versatile and powerful method, the incorporation of an unnatural amino acid, a hydroxycarboxylic acid, or a cyclic structure requires careful optimization of the coupling reaction conditions to ensure high reactivity without epimerization. The various reaction conditions discussed in this review offer potential solutions to these challenges. The achievement of total synthesis not only enables detailed SAR studies of analogues and ensures a sufficient supply for drug discovery research, but also paves the way for further applications such as ADCs. In pursuit of these studies, unambiguous structural elucidation through total synthesis plays a pivotal role.

4 Abbreviations

ADC	Antibody–drug conjugate
Boc	<i>Tert</i> -butyloxycarbonyl
BOP-Cl	Bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride
Cbz	Benzyloxycarbonyl
COMU	(1-Cyano-2-ethoxy-2-oxoethylideneaminoxy) dimethylamino-morpholinocarbenium hexafluorophosphate



DBU	1,8-Diazabicyclo[5.4.0]-7-undecene
DET	Diethyl tartrate
DIAD	Diisopropyl azodicarboxylate
DIC	<i>N,N'</i> -Diisopropylcarbodiimide
DIEA	<i>N,N</i> -Diisopropylethylamine
DMBA	1,3-Dimethylbarbituric acid
DMDO	Dimethyldioxirane
DMF	<i>N,N</i> -Dimethylformamide
DMP	Dimethylphenyl
DPPA	Diphenylphosphoryl azide
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Fmoc	9-Fluorenylmethoxycarbonyl
HATU	1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo [4,5- <i>b</i>]pyridinium 3-oxide hexafluorophosphate
HBTU	1-[Bis(dimethylamino)methylene]-1 <i>H</i> -benzotriazolium 3-oxide hexafluorophosphate
HFIP	1,1,1,3,3,3-Hexafluoropropan-2-ol
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
IBCF	Isobutyl chloroformate
mCPBA	<i>Meta</i> -chloroperbenzoic acid
MNBA	2-Methyl-6-nitrobenzoic anhydride
NMM	<i>N</i> -Methylmorpholine
NMP	<i>N</i> -Methyl-2-pyrrolidone
NRPS	Nonribosomal peptide synthetase
PyAOP	(7-Azabenzotriazol-1-yloxy) trispyrrolidinophosphonium hexafluorophosphate
PyBOP	Benzotriazol-1-yloxy-tris-pyrrolidino-phosphonium hexafluorophosphate
PyBroP	Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
SAR	Structure–activity relationships
T3P	Propanephosphonic acid anhydride
TASF	Tris(dimethylamino)sulfur trimethylsilyl difluoride
TBAF	Tetrabutylammonium fluoride
TBS	<i>t</i> -Butyldimethylsilyl
TCBC	2,4,6-Trichlorobenzoyl chloride
TEA	Triethylamine
TFA	Trifluoroacetic acid
TIS	Triisopropylsilane
TMP	2,2,6,6-Tetramethylpiperidine
TMS	Trimethylsilyl
TMSE	2-(Trimethylsilyl)ethyl
UHP	Urea hydrogen peroxide

5 Conflicts of interest

There are no conflicts to declare.

6 Notes and references

- S. You, G. McIntyre and T. Passioura, *Expet Opin. Drug Discov.*, 2024, **19**, 961–973.
- A. Ohta, M. Tanada, S. Shinohara, Y. Morita, K. Nakano, Y. Yamagishi, R. Takano, S. Kariyuki, T. Iida, A. Matsuo, K. Ozeki, T. Emura, Y. Sakurai, K. Takano, A. Higashida, M. Kojima, T. Muraoka, R. Takeyama, T. Kato, K. Kimura, K. Ogawa, K. Ohara, S. Tanaka, Y. Kikuchi, N. Hisada, R. Hayashi, Y. Nishimura, K. Nomura, T. Tachibana, M. Irie, H. Kawada, T. Torizawa, N. Murao, T. Kotake, M. Tanaka, S. Ishikawa, T. Miyake, M. Tamiya, M. Arai, A. Chiyoda, S. Akai, H. Sase, S. Kuramoto, T. Ito, T. Shiraishi, T. Kojima and H. Iikura, *J. Am. Chem. Soc.*, 2023, **145**, 24035–24051.
- T. J. Tucker, M. W. Embrey, C. Alleyne, R. P. Amin, A. Bass, B. Bhatt, E. Bianchi, D. Branca, T. Bueters, N. Buist, S. N. Ha, M. Hafey, H. He, J. Higgins, D. G. Johns, A. D. Kerekes, K. A. Koeplinger, J. T. Kuethe, N. Li, B. Murphy, P. Orth, S. Salowe, A. Shahripour, R. Tracy, W. Wang, C. Wu, Y. Xiong, H. J. Zokian, H. B. Wood and A. Walji, *J. Med. Chem.*, 2021, **64**, 16770–16800.
- R. W. Busby, M. M. Kessler, W. P. Bartolini, A. P. Bryant, G. Hannig, C. S. Higgins, R. M. Solinga, J. V. Tobin, J. D. Wakefield, C. B. Kurtz and M. G. Currie, *J. Pharmacol. Exp. Ther.*, 2013, **344**, 106–206.
- R. S. Cvetković and G. L. Plosker, *Drugs*, 2005, **65**, 99–107.
- 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-Otoya, 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**(7787), 459–464.
- 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**(7857), 125–129.
- X. Li, S. Ma and Q. Zhang, *Tetrahedron*, 2023, **116**, 154337.
- M. Nestic, D. B. Ryffel, J. Maturan, M. Shevlin, S. R. Pollack, D. R. Gauthier Jr, P. Trigo-Mouriño, L.-K. Zhang, D. M. Schultz, J. M. M. Dunn, L.-C. Campeau, N. R. Patel, D. A. Petrone and D. Sarlah, *J. Am. Chem. Soc.*, 2022, **144**, 14026–14030.
- Y.-C. Lin, F. Schneider, K. J. Eberle, D. Chiodi, H. Nakamura, S. H. Reisberg, J. Chen, M. Saito and P. S. Baran, *J. Am. Chem. Soc.*, 2022, **144**, 14458–14462.
- G. A. Hudson, A. R. Hooper, A. J. DiCaprio, D. Sarlah and D. A. Mitchell, *Org. Lett.*, 2021, **23**, 253–256.
- A. R. Hooper, A. Östrek, A. Milian-Lopez and D. Sarlah, *Angew. Chem., Int. Ed.*, 2021, **61**, e202212299.
- T. Junne, J. Wong, C. Studer, T. Aust, B. W. Bauer, M. Beibel, B. Bhullar, R. Bruccoleri, J. Eichenberger, D. Estoppey, N. Hartmann, B. Knapp, P. Krastel, N. Melin, E. J. Oakeley, L. Oberer, R. Riedl, G. Roma, S. Schuierer, F. Petersen, J. A. Tallarico, T. A. Rapoport, M. Spiess and D. Hoepfner, *J. Cell Sci.*, 2015, **128**, 1217–1229.
- E. Pauwels, R. Schülein and K. Vermeire, *Int. J. Mol. Sci.*, 2021, **22**, 12007.
- S. Itskanov, L. Wang, T. Junne, R. Sherriff, L. Xiao, N. Blanchard, W. Q. Shi, C. Forsyth, D. Hoepfner, M. Spiess and E. Park, *Nat. Chem. Biol.*, 2023, **19**, 1063–1071.
- K. Ohsawa, S. Fukaya and T. Doi, *Org. Lett.*, 2022, **24**, 5552–5556.



- 17 S. E. De Voe and M. P. Kunstmann, Antibiotic AC98 and production, *US Pat.*, 3495004, 1970.
- 18 H. He, R. T. Williamson, B. Shen, E. I. Graziani, H. Y. Yang, S. M. Sakya, P. J. Petersen and G. T. Carter, *J. Am. Chem. Soc.*, 2002, **124**, 9729–9736.
- 19 S. Fuse, H. Koinuma, A. Kimbara, M. Izumikawa, Y. Mifune, H. He, K. Shin-ya, T. Takahashi and T. Doi, *J. Am. Chem. Soc.*, 2014, **136**, 12011–12017.
- 20 B. Wang, Y. Liu, R. Jiao, Y. Feng, Q. Li, C. Chen, L. Liu, G. He and G. Chen, *J. Am. Chem. Soc.*, 2016, **138**, 3926–3932.
- 21 J. Wang, D. Lin, M. Liu, H. Liu, P. Blasco, Z. Sun, Y. C. Cheung, S. Chen and X. Li, *J. Am. Chem. Soc.*, 2021, **143**, 12784–12790.
- 22 T. Wieland and U. Gebert, *Justus Liebig's Ann. Chem.*, 1967, **700**, 157–173.
- 23 K. Matinkhoo, A. Pryyma, M. Todorovic, B. O. Patrick and D. M. Perrin, *J. Am. Chem. Soc.*, 2018, **140**, 6513–6517.
- 24 M.-A. J. Siegert, C. H. Knittel and R. D. Süßmuth, *Angew. Chem., Int. Ed.*, 2020, **59**, 5500–5504.
- 25 G. Yao, C. H. Knittel, S. Kosol, M. T. Wenz, B. G. Keller, H. Gruf, A. C. Braun, C. Lutz, T. Hechler, A. Pahl and R. D. Süßmuth, *J. Am. Chem. Soc.*, 2021, **143**, 14322–14331.
- 26 C. Lutz, W. Simon, S. Werner-Simon, A. Pahl and C. Müller, *Angew. Chem., Int. Ed.*, 2020, **59**, 11390–11393.
- 27 J. P. May and D. M. Perrin, *Chem.–Eur. J.*, 2008, **14**, 3404–3409.
- 28 V. Figueroa-Vazquez, J. Ko, C. Breunig, A. Baumann, N. Giesen, A. Pálfi, C. Müller, C. Lutz, T. Hechler, M. Kulke, C. Müller-Tidow, A. Krämer, H. Goldschmidt, A. Pahl and M. S. Raab, *Mol. Cancer Ther.*, 2021, **20**, 367–378.
- 29 A. Pryyma, K. Matinkhoo, A. A. W. L. Wong and D. M. Perrin, *Chem. Sci.*, 2020, **11**, 11927–11935.
- 30 H. Gross, V. O. Stockwell, M. D. Henkels, B. Nowak-Thompson, J. E. Loper and W. H. Gerwick, *Chem. Biol.*, 2007, **14**, 53–63.
- 31 Y. Bando, Y. Hou, L. Seyfarth, J. Probst, S. Götze, M. Bogacz, U. A. Hellmich, P. Stallforth, M. Mittag and H.-D. Arndt, *Chem.–Eur. J.*, 2022, **28**, e202104417.
- 32 M. Ishima, T. Yoshida, T. Yamazaki, F. Sugawara, K. Hatta, M. Shimojoe and K. Masaki, WO02062831, 2002.
- 33 C. Uchiyama, A. Fukuda, M. Mukaiyama, Y. Nakazawa, Y. Kuramochi, K. Muguruma, M. Arimoto, A. Ninomiya, K. Kako, Y. Katsuyama, S. Konno, A. Taguchi, K. Takayama, A. Taniguchi, Y. Nagumo, T. Usui and Y. Hayashi, *Angew. Chem., Int. Ed.*, 2021, **60**, 8792–8797.
- 34 M. Mukaiyama, C. Uchiyama, A. Fukuda, Y. Nakazawa, Y. Kuramochi, Y. Shibata, S. Konno, K. Muguruma, N. Matsugaki, T. Asari, K. Ogawa, A. Taguchi, K. Takayama, A. Taniguchi, Y. Nagumo, T. Senda, Y. Hayashi and T. Usui, *J. Med. Chem.*, 2023, **66**, 8717–8724.

