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Solution-phase total synthesis of teixobactin†

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The first solution-phase total synthesis of the cyclic depsipeptide teixobactin is described. Stereoselective construction of L-allo-enduracididine was established, and the protective groups for the peptide coupling reactions and conditions for the assembly of the fragments were also optimised. The longest linear sequence for the total synthesis was 20 steps from the known L-*cis*-4-hydroxyproline derivative and gave a 5.6% overall yield. This solution-phase total synthesis could serve as a complement to the current solid-phase synthesis of teixobactin.

Introduction

The golden era of antibiotic discovery faded some time ago,[1] human society is now facing the global threat of multiple drug resistant bacteria and fungi, some of which are even resistant to all known clinical drugs.[2] Efforts to discover new antibiotics with novel mechanisms and chemical scaffolds, which are important in reducing resistance, have become urgent tasks for medicinal scientists all over the world.[2b,3] Natural products and their derivatives are still a prolific area of research for the discovery of novel antibiotics.[4] Among these active anti-infective natural products, macrocyclic peptides are very prominent scaffolds,[5] and vancomycin,[6] polymyxins[7] and daptomycin[8] are all natural macrocyclic peptides that are used as last-line clinical drugs for the treatment of multiple drug resistant infections. Modified natural macrocyclic peptides also display great potential as new drug candidates, such as cyclohexyl-griselimycin and G0775 (Fig. 1), which were developed from griselimycin (GM) and arylomycin A-C16, respectively.[9,10]

Teixobactin is also a promising lead compound as an antibiotic with novel mechanisms and a potent biological activity.[11] Teixobactin was first isolated from the unculturable beta-proteobacterium *Eleftheria terrae* by the application of a unique strategy and new technology, which was performed by seeding the soil sample in iChip with natural sediment and using a semipermeable membrane to control the cultivation conditions.[12] Teixobactin is a cyclodepsipeptide containing eleven amino acid residues (Fig. 1), with a heptapeptide as a linear chain attached to another four amino acids that form a 13-membered cyclodepsipeptide ring. Among the eleven amino acids, there are five unnatural units including *N*-methylated D-phenylalanine, D-glutamine, D-*allo*-isoleucine, D-threonine and L-*allo*-enduracididine (End).[13]

Distinguished antimicrobial activities have been reported for teixobactin against anti-multiple drug resistant Gram-positive pathogens at low concentrations, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and penicillin-resistant *Streptococcus pneumonia* (PRSP).[12] It also exhibited a potent activity against *Mycobacterium tuberculosis* (Mtb). More importantly, teixobactin did not induce any resistant mutants of the tested bacteria, including *S. aureus* and *M. tuberculosis.*[12] This low tendency for resistant induction can be attributed to its special mode of action, teixobactin is believed to bind to pyrophosphate and the first sugar moieties that are presented in both lipid II and lipid III.[14] The inhibition of lipid II leads to the suppression of peptidoglycan biosynthesis, while the inhibition of lipid III causes the down regulation of wall teichoic acid (WTA) biosynthesis.[14] By working along both lines, that is to target both lipid II and lipid III, the building blocks for cell wall synthesis, it is more difficult for bacteria to develop resistance to teixobactin. Extensive NMR studies have revealed that the aggregation state of teixobactin plays an important role in binding to lipid II.[15] Furthermore, an X-ray crystallography of one teixobactin derivative disclosed that the head–tail structure formed amyloid-like fibrils, suggesting a working model for the mechanism of teixobactin as a new antibiotic,[16] this research also emphasised that the stereochemistry of the side-chain amino acid residues is essential to the biological activity of teixobactin towards bacteria.[17]

The structure and superior bactericidal activity of teixobactin is intriguing, and therefore a wave of synthetic and struc-

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†Electronic supplementary information (ESI) available: Copies of ^{1}H and ^{13}C NMR spectra for new compounds, biological tests and HPLC for teixobactin. See DOI: 10.1039/c8ob02803f

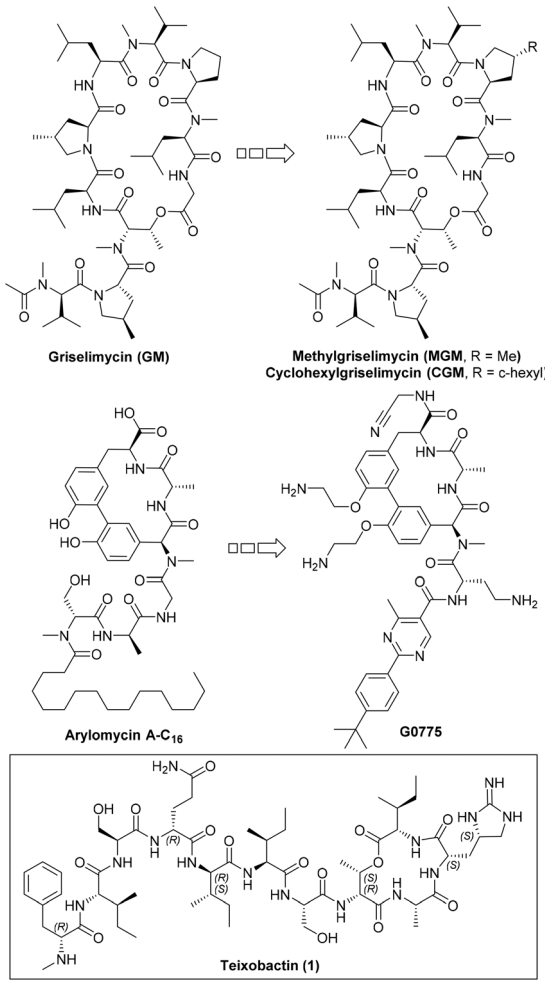


Fig. 1 Structure of griselimycins, arylomycin and teixobactin **1**.

ture–activity relationship (SAR) studies have been carried out. Up until now, there have been three reports published on the total synthesis of teixobactin,[18] along with several dozen papers describing the preparation of analogues and SAR studies.[11,19] All of the above mentioned synthetic studies employed solid-phase peptide synthesis (SPPS). Payne found that 2-chlorotrityl chloride (2-CTC) functionalized polystyrene resin hampered the ester bond formation, while (4-(hydroxymethyl)-3-methoxyphenoxy)acetic acid (HMPB) functionalized polyethylene glycol-based NovaPEG resin facilitated the synthesis smoothly and provided the linear precursor, which after macrolactamization in DMF and deprotection steps afforded teixobactin in a 3.3% overall isolation yield.[18a] Li detoured from this route to form the ester bond in solution synthesis, then applied the 2-CTC resin for solid phase synthesis of the cyclodepsipeptide, and further application of their previously reported serine ligation methodology[20] with the C-terminal salicylaldehyde containing sidechain hexapeptide fulfilled the total synthesis of teixobaction.[18b] Chen prepared a tetrapeptide fragment to avoid the acyl transfer and then attached the tetrapeptide acid to 2-CTC resin, further carrying out the Fmoc solid phase synthesis strategy and completed the peptide sequence, the macrocyclization was performed in solution phase in DCM giving teixobactin in a fully protected form, which after deprotection produced the natural product.[18c] Among these reported total syntheses, solution phase peptide synthesis played important roles either for the preparation of key fragments or for macrocyclization of the linear precursors, but these can all be attributed to SPPS. So far, only one report has been published in which the synthesis of the macrocyclic core of teixobactin was attempted by solution-phase peptide synthesis.[21]

Solution-phase peptide synthesis is inherently superior to SPPS in some aspects, especially for some structurally complex peptides with a high content of non-proteinogenic amino acids. Solution phase synthesis is characterised by its feasibility to scale-up, it has lower costs and the quality of the final product is better compared to solid-phase synthesis in large-scale or the industrial manufacture of peptide drugs.[22]

As part of our ongoing research interests in the total synthesis of biologically active natural products,[23] we report herein the solution-phase synthesis of teixobactin as a complementary approach to gain access to this important natural product and its analogue structures.

Results and discussion

The retrosynthetic analysis of teixobactin **1** is shown in Fig. 2. Disconnection at the amide bond of Ile6-Ser7 gave two subunits with a similar structural complexity, the hexapeptide **2** and the cyclodepsipeptide **3**. Hexapeptide **2** could be prepared *via* classic peptide coupling chemistry between two tripeptides, **4** and **5**. For subunit **3**, the amide bond between Thr8 and Ala9 was elected to form the macrocycle, further disconnection between End10 and Ile11 produce tripeptide **6** and dipeptide **7**. L-*allo*-Enduracididine (End) was embodied in dipeptide **7** to reduce the involved reaction steps of this non-proteinogenic amino acid toward the total synthesis of teixobactin **1**.

Our solution-phase total synthesis of teixobactin **1** commenced from the stereocontrolled preparation of L-*allo*-enduracididine (End) and dipeptide fragment **7** (Scheme 1). Treatment of L-*cis*-4-hydroxyproline derivative **8**[24,25] with TBSCl in dichloromethane, in the presence of imidazole and a catalytic amount of DMAP, produced the silyl ether smoothly, which by exposure to $NaIO_4$ and $RuO_2{\cdot}xH_2O$[26] in ethyl acetate and water gave compound **9** in a 74% yield over two steps. Bearing a carbamate (Boc) attached to the nitrogen of **9**, the amide carbonyl group was susceptible to being reduced by $NaBH_4$ to furnish an alcohol.[27] Following a literature precedent, treatment of **9** with $NaBH_4$ in ethanol and a neutral phosphonate buffer gave alcohol **10** in a 69% yield. Migration of the TBS group to the primary hydroxy group[28] was confirmed by oxidation of **10** with Dess–Martin periodinane (DMP) in dichloromethane. The ^1H NMR chemical shifts of the two methylene groups corroborated their position as being adjacent to the keto-carbonyl group, which is very distinctive

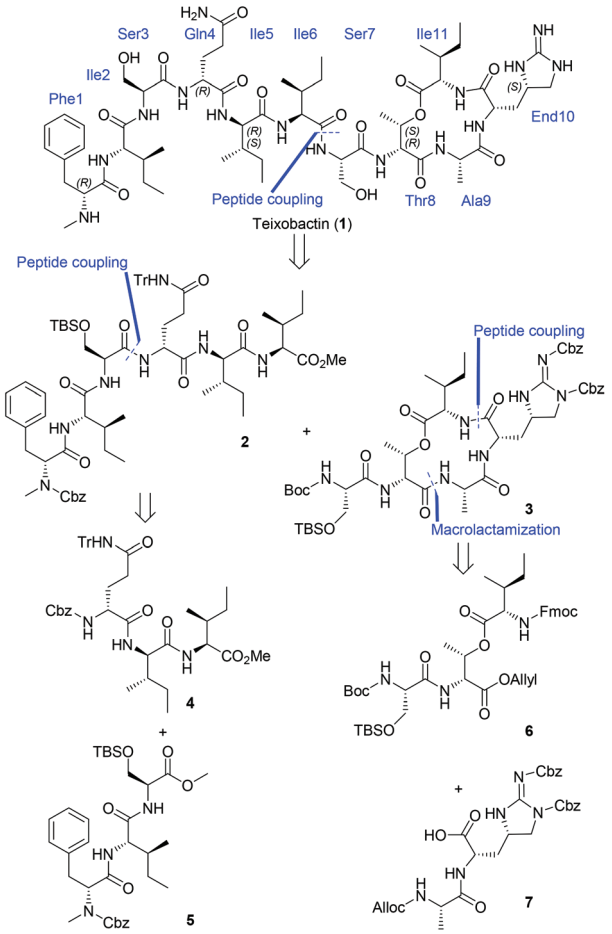
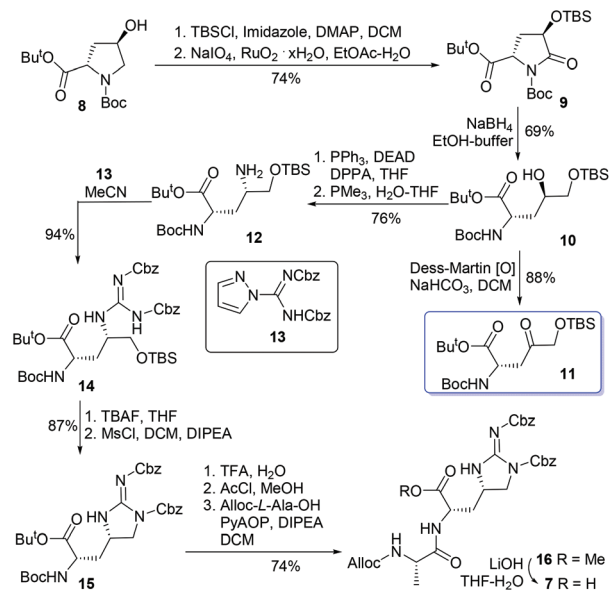


Fig. 2 Retrosynthetic analysis of teixobactin **1**.

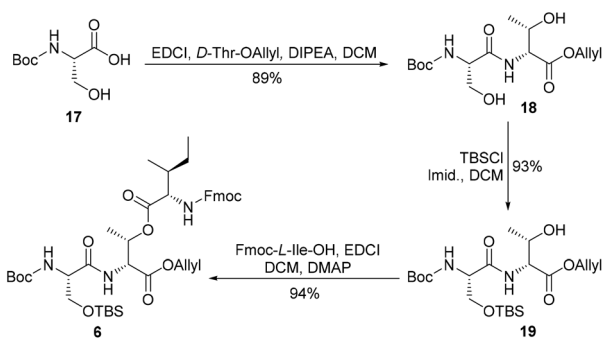


Scheme 1 Preparation of End and synthesis of dipeptide **7**.

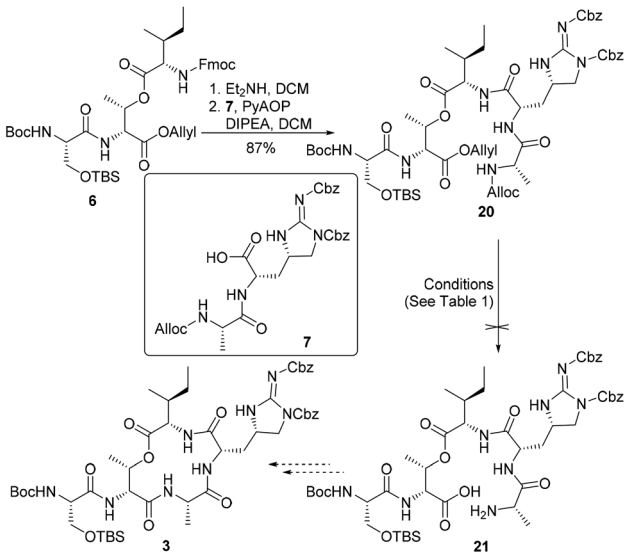
to a methylene beside an aldehyde. Although the migration of the TBS group was unexpected, it did not disturb the synthetic plan. As shown in Scheme 1, **10** was subjected to the Mitsunobu reaction[29] using DPPA in the presence of PPh_3 and DEAD in THF to convert the secondary hydroxy group to the corresponding azide group, which underwent a Staudinger reduction[30] in the presence of trimethylphosphine in THF and water to afford amine **12** in a 76% yield over two steps. Guanidinylation of amine **12** *via* nucleophilic substitution with *N*,*N*′-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine[31] **13** in acetonitrile gave **14** in a 94% yield. Removal of the TBS group from compound **14** was performed using TBAF in THF, the primary hydroxy group was treated with MsCl in DCM and DIPEA, this triggered the cyclization reaction *in situ* to produce the fully protected L-*allo*-enduracididine (End) derivative **15** in an 87% yield over two steps. The analytical data for compound **15** was identical with the reported data found in the literature.[24] Selective removal of the Boc group[32] of **15** did not work well, although treatment of **15** with TFA in water removed both of the acid sensitive protecting groups with no obstacles. The amino acid was esterified with an acidic solution of MeOH, the methyl ester was coupled with Alloc-L-Ala-OH in the presence of PyAOP in dichloromethane and DIPEA to produce dipeptide **16** in a 74% yield. Saponification of **16** with lithium hydroxide in THF and water gave the intermediate **7** in a quantitative yield (Scheme 1).

The straightforward preparation of tripeptide **6** is outlined in Scheme 2. Boc-Ser-OH **17** was condensed with D-Thr-OAllyl using EDCI as a coupling reagent in dichloromethane and DIPEA to produce dipeptide **18** in an 89% yield. Selective protection of the primary hydroxyl group of Ser with TBSCl in dichloromethane and imidazole gave **19** in a 93% yield. The remaining secondary alcohol of **19** was esterified with Fmoc-L-Ile-OH in the presence of EDCI and DMAP in dichloromethane to afford tripeptide **6** in a 94% yield.

The Fmoc group on the N-terminal of **6** was removed by treatment of **6** with Et_2NH in dichloromethane to release the amino group of isoleucine, which was reacted with the dipeptide acid **7** in the presence of PyAOP and DIPEA in dichloromethane to give the linear pentapeptide **20** in an 87% yield (Scheme 3).



Scheme 2 Preparation of tripeptide **6**.



Scheme 3 Attempt at macrocyclization of **3**.

To forge the macrolactone ring, we needed to deprotect both the C- and N-terminal of **20**. The allyl ester and the Alloc carbamate groups belong to the same category of protective groups, which upon activation by a palladium catalyst are susceptive to attack by nucleophiles, and the classic deprotection conditions are to use a catalytic amount of $Pd(PPh_3)_4$ and excess Et_2NH in an aprotic solvent.[33] However, when compound **20** was treated with $Pd(PPh_3)_4$ and Et_2NH in dichloromethane, only the allyl ester was removed from the molecule, the Alloc on the N-terminal of **20** was retained even after a prolonged reaction time (Table 1, entry 1).

We then screened several nucleophiles, aiming to achieve the deprotection of both the allylic protective groups on **20** simultaneously. As shown in Table 1, morpholine, another frequently used secondary amine, gave a complex mixture of products (entry 2). Reductive reagents Bu_3SnH[34] and $PhSiH_3$[35] were similar to morpholine and gave messy products (entries 3 and 4). 1,3-Dimethylbabituric acid[23e,36] exhibited the same reactivity with Et_2NH, affording the carboxylic acid with an untouched Alloc (entry 5). To clarify that the Alloc carbamate was removable under these deprotection conditions, dipeptide **16** was treated with $Pd(PPh_3)_4$ and Et_2NH in dichloromethane, this reaction gave the corresponding amine smoothly and with a satisfactory yield.

Table 1 Deprotection conditions for intermediate **20** [a]

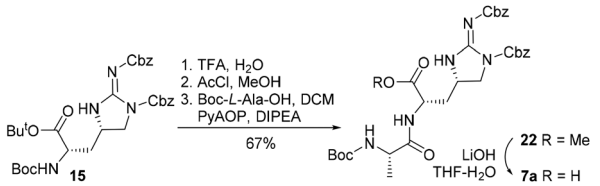
Entry	Catalyst (mol%)	Nucleophile	Result
1	$Pd(PPh_3)_4$ (10%)	Et_2NH	De-allyl
2	$Pd_2(dba)_3$ (5%)	Morpholine	Mess
3	$Pd_2(dba)_3$ (10%)	Bu_3SnH	Mess
4	$Pd_2(dba)_3$ (10%)	$PhSiH_3$	Mess
5	$Pd_2(dba)_3$ (10%)	Dimethylbarbituric acid	De-allyl

[a] All reactions were carried out in dichloromethane.

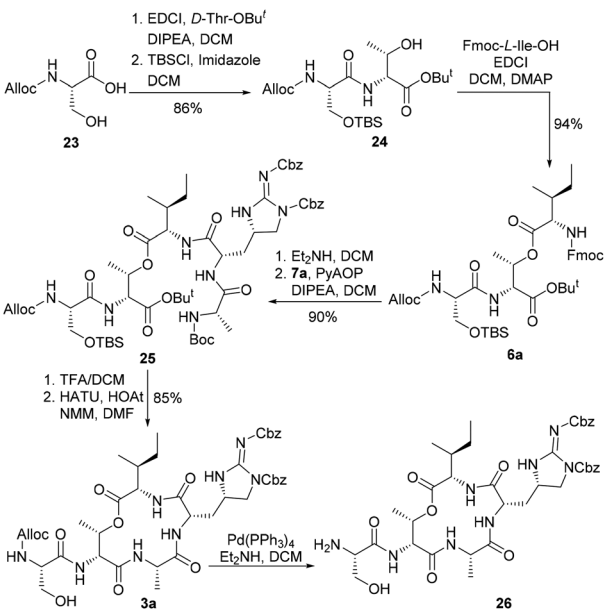
We then decided to alter the protection plan for the preparation of macrocycle **3**. Thus, Alloc carbamate was applied to Ser7, and *tert*-butyl ester and Boc carbamate were placed on the amine and carboxylic acid of Thr8 and Ala9 respectively. Starting from compound **15**, after removal of Boc carbamate and *tert*-butyl ester with TFA and esterification with acidic methanol, the methyl ester was coupled to Boc-L-Ala-OH to produce dipeptide **22** in a 74% yield, which, after saponification with lithium hydroxide, gave acid **7a** in a quantitative yield, as shown in Scheme 4.

At the same time, Alloc-L-Ser-OH **23** was reacted with D-Thr-OBu^t to generate the corresponding dipeptide, which after protection of the primary alcohol gave compound **24** in an 86% yield over two steps. Esterification of the secondary alcohol with Fmoc-L-Ile-OH using similar reaction conditions as for **6** afforded tripeptide **6a** in a 94% yield. Removal of the Fmoc group from Ile with Et_2NH, and further peptide bond formation with acid **7a** using PyAOP as a coupling reagent in the presence of DIPEA in dichloromethane produced pentapeptide **25** in a 90% yield as shown in Scheme 5.

Treatment of **25** with trifluoroacetic acid in dichloromethane concomitantly deprotected the Boc carbamate, *tert*-



Scheme 4 Preparation of dipeptide **7a**.



Scheme 5 Preparation of tripeptide **6a** and macrocycle **3a**.

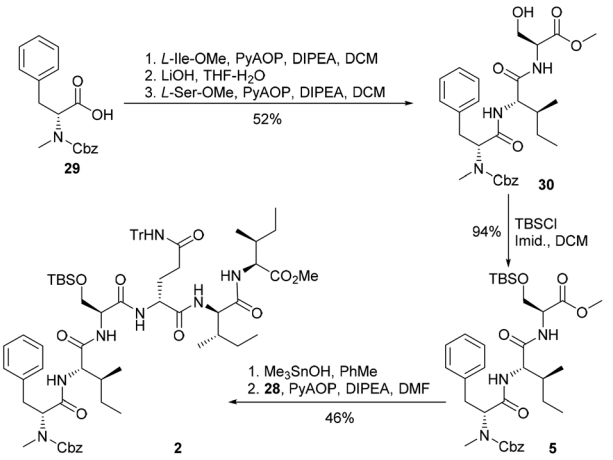
butyl ester and the TBS ether, the linear precursor was cyclized to afford the macrocycle **3a** in an 85% yield, using HATU–HOAt as an effective coupling reagent combination in the presence of NMM in DMF. Deprotection of the Alloc carbamate from the N-terminal Ser7 of **3a** proceeded smoothly to give amine **26** in the presence of $Pd(PPh_3)_4$ and Et_2NH in dichloromethane (Scheme 5). Amine **26** was then ready for the peptide coupling reaction with the side-chain hexapeptide acid of **2**, as outlined in Fig. 2.

Tripeptide **4** was prepared starting from Cbz-Gln(Tr)-OH **27** as shown in Scheme 6. The coupling reaction of **25** with D-*allo*-Ile-OMe was facilitated by PyAOP and DIPEA in dichloromethane, the dipeptide was saponified using lithium hydroxide in THF and water, after acidification the dipeptide acid was connected to L-Ile-OMe using identical coupling reaction conditions to produce the tripeptide **4** in an 84% yield over three reaction steps. Treatment of tripeptide **4** with hydrogen in methanol in the presence of one equivalent of $PdCl_2$ released the amino group of Gln to give compound **28** (Scheme 6).

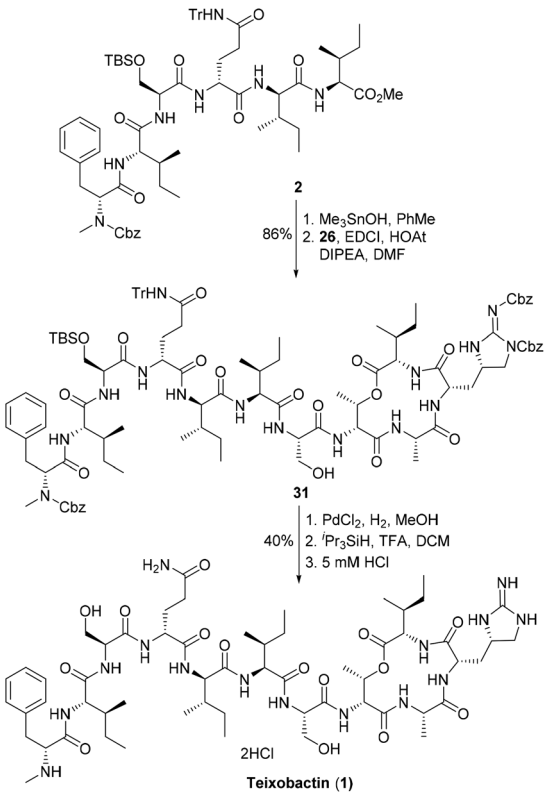
Tripeptide **5** was synthesized from the condensation of *N*-Me-Cbz-D-Phe-OH and H-L-Ile-OMe in the presence of PyAOP and DIPEA in dichloromethane, the methyl ester of the dipeptide was hydrolysed with lithium hydroxide in THF and water to give the corresponding acid, which was coupled to H-L-Ser-OMe using PyAOP as a coupling reagent in the presence of DIPEA in dichloromethane to afford tripeptide **30** in a 52% yield over three steps. The primary hydroxyl group of **30** was protected using a TBS ether to give the key fragment **5** in a 94% yield as shown in Scheme 7.

Saponification of **5** proved to be difficult, neither lithium hydroxide nor sodium hydroxide gave a reasonable yield. We then turned our attention to heating tripeptide **5** with Me_3SnOH in toluene,[37] this method produced the corresponding carboxylic acid smoothly. The coupling reaction of the above acid with amine **28** was performed in the presence of PyAOP and DIPEA in DMF to afford the hexapeptide **2** in a 46% yield over two steps (Scheme 7).

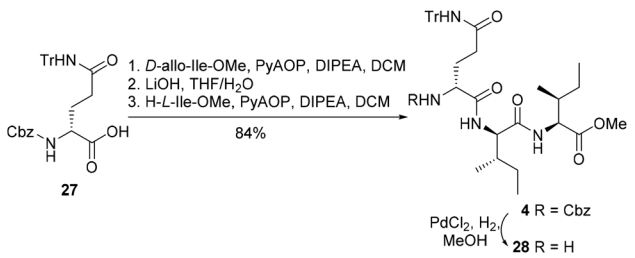
With the side-chain hexapeptide **2** and the macrocycle amine moiety **26** in hand, we reached the stage at which we needed to unite the two fragments to complete the total synthesis of teixobactin **1**. As shown in Scheme 8, hexapeptide **2** was first hydrolysed with Bu_3SnOH in hot toluene[37] to release the carboxylic acid, followed by a peptide coupling reaction with amine **26**, which was carried out using EDCI as the dehydration reagent in the presence of HOAt and DIPEA in DMF to generate the undecapeptide **31** in an 86% yield. Global deprotection of molecule **31** was achieved in a stepwise manner, an all-in-one deprotection protocol produced a very complex mixture that was difficult to purify using HPLC.[18] Three Cbz groups and one TBS group were deprotected *via* a $PdCl_2$ catalysed hydrogenation in methanol, the intermediate, with an intact trityl (Tr) group on the Glu4 moiety, was identified using high-resolution mass spectrometry (HRMS) analysis of the



Scheme 7 Preparation of tripeptide **5** and hexapeptide **2**.



Scheme 8 Completion of the total synthesis of teixobactin **1**.



Scheme 6 Preparation of tripeptide **4**.

crude product. Subsequently, Tr was removed using TFA in the presence of iPr_3SiH in dichloromethane.[38] Finally, the concentrated reaction residue was purified using HPLC, the pooled fractions containing teixobactin **1** were concentrated to remove the volatiles, and the aqueous residue was repeatedly lyophilized with 5 mM HCl to provide teixobactin **1** as an HCl salt. The synthetic teixobactin **1** was confirmed *via* interpretation of the NMR and HRMS spectra, biological evaluation further corroborated that the synthetic sample was equally as active as natural teixobactin.[11]

Synthetic teixobactin **1** showed potent inhibitory activities toward Gram positive bacteria, 63, 78 and 39 ng mL^{-1} for MRSA S.aur Rosenbach (ATCC 33591), VRE E.fae Schleifer and Kipper-Balz (ATCC 51575), and S.aur Newman respectively, while for the Gram negative *E. coli* DH5a it exhibited significantly less activity (10 μg mL^{-1}) in accordance with the antibacterial mechanism and the literature results.

Conclusions

In summary, we reported herein the first solution-phase total synthesis of teixobactin, a new antibacterial natural cyclic depsipeptide isolated from an unculturable bacterium from the soil. Teixobactin displays a high potency against the tested Gram-positive bacteria, including some clinical multiple drug resistant strains, its distinctive antibacterial mechanism ensures there are reduced opportunities for the generation of resistance. As a valuable structural template from nature to combat antibiotic resistance, teixobactin requires further synthetic strategies in addition to the limited reports on its solid-phase total synthesis. Our synthesis started with the stereoselective preparation of the L-allo-enduracididine (End) moiety, we also optimised the protective groups for the peptide coupling reactions and the assembly of the fragments. The longest linear sequence for the total synthesis of teixobactin was 20 steps from the known L-*cis*-4-hydroxylproline derivative **8**, and gave a 5.6% overall yield. The current solution-phase synthetic strategy is suitable for the production of teixobactin and the construction of a structurally-diverse analogue library of teixobactin. Further studies to develop new antibiotics with novel structures and biological mechanisms based on the current synthetic strategy are in progress in this laboratory and will be reported in due course.

Experimental section

General procedure for chemical synthesis

All non-aqueous reactions were performed under nitrogen or an argon atmosphere using oven-dried glassware and a standard syringe in the septa techniques. NMR spectra were recorded on a Bruker Avance AV500 or Advance 400 at 500 MHz (125 MHz) or 400 MHz (100 MHz) in deuterated solvents (noted for each compound). Mass spectra were measured on ABI Q-star Elite. Optical rotations were measured on a PerkinElmer 351 polarimeter at 589 nm with a 100 mm path length cell. TLC was carried out on pre-coated sheets (Qingdao silica gel 60-F250, 0.2 mm) which were visualized under UV light at 254 nm or stained using phosphomolybdic acid (PMA) solution in absolute ethanol after development. Flash column chromatography was performed on E. Qingdao silica gel 60 (230–400 mesh ASTM).

Pyroglutamate 9. Compound **8** (9.47 g, 33.01 mmol, 1.0 equiv.) was dissolved in DCM (200 mL). Imidazole (5.53 g, 82.50 mmol, 2.5 equiv.), TBSCl (7.46 g, 49.50 mmol, 1.5 equiv.) and DMAP (0.40 g, 3.30 mmol, 0.1 equiv.) were sequentially added at 0 °C and the reaction was stirred at room temperature for 5 h. The reaction was quenched using a saturated aqueous NH_4Cl solution (100 mL). The aqueous phase was extracted using DCM (3 × 100 mL). The combined organic phase was washed with saturated aqueous NaCl solution (200 mL) and dried with anhydrous Na_2SO_4 and concentrated under low pressure. The crude product was purified using flash chromatography to afford TBS ether (12.73 g, 96%) as a colorless oil. R_f = 0.18 (silica, EtOAc/hexane = 1/20). $[\alpha]_D^{20}$ = −44.2 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.44–4.35 (m, 1H), 4.30–4.14 (m, 1H), 3.63–3.50 (m, 1H), 3.39–3.22 (m, 1H), 2.21–2.07 (m, 1H), 2.03–1.93 (m, 1H), 1.47–1.38 (m, 18H), 0.86 (s, 9H), 0.04 (s, 3H), 0.04 (s, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) rotamer, δ 172.20, 172.12, 154.41, 154.01, 80.93, 80.89, 79.78, 79.57, 70.36, 69.55, 58.73, 58.53, 54.59, 54.25, 39.77, 38.83, 28.39, 28.31, 27.98, 27.94, 25.70, 17.98, −4.85, −4.89, −4.93 ppm. **HRMS** (*m*/*z*): calculated for $C_{20}H_{39}NO_5SiNa^+$ $[M + Na]^+$: 424.2490, found 424.2490.

$NaIO_4$ (1.87 g, 8.72 mmol, 3.5 equiv.) was dissolved in H_2O (20 mL) and $RuO_2{\cdot}xH_2O$ (85 mg, 0.63 mmol, 0.25 equiv.) was added to afford a yellow solution. A solution of the above TBS ether (1.00 g, 2.49 mmol, 1.0 equiv.) in EtOAc (10 mL) was added and the reaction was stirred at room temperature for 12 h. The aqueous phase was extracted using EtOAc (3 × 20 mL). The combined organic phase was washed with saturated aqueous Na_2SO_3 solution (50 mL) and saturated aqueous NaCl solution (50 mL). After drying with anhydrous Na_2SO_4 and being concentrated under low pressure, the crude product was purified using flash chromatography to afford compound **9** (0.80 g, 77%) as a white solid. R_f = 0.18 (silica, EtOAc/hexane = 1/20). $[\alpha]_D^{20}$ = +36.0 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.46–4.36 (m, 2H), 2.31 (ddd, *J* = 13.0, 8.3, 1.5 Hz, 1H), 2.16 (dt, *J* = 13.1, 9.9 Hz, 1H), 1.50 (s, 9H), 1.47 (s, 9H), 0.89 (s, 9H), 0.16 (s, 3H), 0.12 (s, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.05, 170.20, 149.46, 83.47, 82.40, 69.73, 55.88, 32.16, 27.91, 25.68, 18.24, −4.50, −5.34 ppm. **HRMS** (*m*/*z*): calculated for $C_{20}H_{37}NO_6SiNa^+$ $[M + Na]^+$: 438.2282, found 438.2282.

Alcohol 10. Compound **9** (5.00 g, 12.0 mmol, 1.0 equiv.) was dissolved in EtOH (50 mL), and PBS buffer (pH = 7.0, 25 mL) was added. $NaBH_4$ (1.82 g, 48.0 mmol, 4.0 equiv.) was added at 0 °C and the reaction was stirred at room temperature for 3 h. EtOH was evaporated and the aqueous phase was extracted using EtOAc (3 × 50 mL). The combined organic phase was washed with saturated aqueous NH_4Cl solution (100 mL) and saturated aqueous NaCl solution (100 mL). After being dried

with anhydrous Na_2SO_4 and concentrated under low pressure, the crude product was purified using flash chromatography to afford compound **10** (3.48 g, 69%) as a colorless oil. R_f = 0.36 (silica, EtOAc/hexane = 1/10). $[\alpha]_D^{20}$ = +18.2 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.40 (d, *J* = 7.4 Hz, 1H), 4.24–4.14 (m, 1H), 3.77 (ddt, *J* = 8.5, 5.9, 4.2 Hz, 1H), 3.59 (dd, *J* = 10.0, 4.1 Hz, 1H), 3.48 (dd, *J* = 10.0, 6.2 Hz, 1H), 2.01–1.88 (m, 1H), 1.84–1.73 (m, 1H), 1.44 (s, 9H), 1.42 (s, 9H), 0.88 (s, 9H), 0.05 (s, 3H), 0.05 (s, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.67, 155.47, 81.74, 79.59, 69.06, 66.54, 52.03, 35.60, 28.31, 27.94, 25.84, 18.22, −5.41, −5.44 ppm. **HRMS** (*m*/*z*): calculated for $C_{20}H_{41}NO_6SiNa^+$ $[M + Na]^+$: 442.2595, found 442.2598.

Ketone 11. Compound **10** (100 mg, 0.24 mmol, 1.0 equiv.) was dissolved in DCM (5 mL). $NaHCO_3$ (60 mg, 0.72 mmol, 3.0 equiv.) and Dess–Martin periodinane (153 mg, 0.36 mmol, 1.5 equiv.) were added at 0 °C. The reaction was stirred at room temperature for 3 h. After concentration, compound **11** (88 mg, 88%) was afforded using flash chromatography as a white solid. R_f = 0.53 (silica, EtOAc/hexane = 1/5). $[\alpha]_D^{20}$ = +19.8 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.43 (d, *J* = 8.7 Hz, 1H), 4.41 (dt, *J* = 9.2, 4.6 Hz, 1H), 4.13 (d, *J* = 1.0 Hz, 2H), 3.13 (dd, *J* = 18.1, 4.8 Hz, 1H), 2.98 (dd, *J* = 18.1, 4.4 Hz, 1H), 1.41 (s, 9H), 1.41 (s, 9H), 0.90 (s, 9H), 0.06 (s, 6H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 209.15, 170.28, 155.50, 81.97, 79.63, 69.20, 49.81, 40.90, 28.28, 27.83, 25.72, 18.21, −5.54, −5.59 ppm. **HRMS** (*m*/*z*): calculated for $C_{20}H_{39}NO_6SiNa^+$ $[M + Na]^+$: 440.2439, found 440.2441.

Amine 12. Compound **10** (3.76 g, 8.97 mmol, 1.0 equiv.) was dissolved in THF (50 mL). PPh_3 (3.53 g, 13.46 mmol, 1.5 equiv.), DEAD (2.11 mL, 13.46 mmol, 1.5 equiv.) and DPPA (2.89 mL, 13.46 mmol, 1.5 equiv.) were added at 0 °C and the reaction was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous phase was extracted using EtOAc (3 × 50 mL). The combined organic phase was washed with saturated aqueous NaCl solution (100 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford a light yellow liquid (the product with some inseparable impurities). The liquid described above was dissolved in THF-H_2O (50 mL/5 mL). A solution of PMe_3 (17.94 mL, 17.94 mmol, 2.0 equiv., 1.0 M in THF) was added dropwise at 0 °C. The reaction was stirred at room temperature for 3 h. PMe_3 and THF were evaporated and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with saturated aqueous NaCl solution (100 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **12** (2.86 g, 76% over 2 steps) as a colorless oil. R_f = 0.33 (silica, EtOAc/hexane = 1/1). $[\alpha]_D^{20}$ = −0.6 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.88 (d, *J* = 8.6 Hz, 1H), 4.31–4.23 (m, 1H), 3.44 (dd, *J* = 9.8, 4.6 Hz, 1H), 3.35 (dd, *J* = 9.8, 6.5 Hz, 1H), 2.88–2.79 (m, 1H), 1.68–1.58 (m, 2H), 1.41 (s, 9H), 1.39 (s, 9H), 0.84 (s, 9H), 0.00 (s, 6H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.15, 155.78, 81.51, 79.36, 68.53, 52.12, 50.08, 36.53, 28.29, 27.96, 25.84, 18.22, −5.42, −5.45 ppm. **HRMS** (*m*/*z*): calculated for $C_{20}H_{42}N_2O_5SiH^+$ $[M + H]^+$: 419.2936, found 419.2934.

Guanidinyl compound 14. Compound **12** (300 mg, 0.72 mmol, 1.0 equiv.) was dissolved in MeCN (10 mL) and compound **13** (543 mg, 1.44 mmol, 2.0 equiv.) was added. The reaction was stirred at room temperature for 3 h. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **14** (491 mg, 94%) as a colorless oil. R_f = 0.45 (silica, EtOAc/hexane = 1/10). $[\alpha]_D^{20}$ = −31.0 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 11.69 (s, 1H), 8.65 (d, *J* = 8.4 Hz, 1H), 7.41–7.27 (m, 10H), 5.55 (d, *J* = 8.0 Hz, 1H), 5.18 (q, *J* = 12.0 Hz, 2H), 5.12 (d, *J* = 2.6 Hz, 2H), 4.40–4.29 (m, 1H), 4.04 (s, 1H), 3.74–3.61 (m, 2H), 2.17–2.05 (m, 1H), 1.97–1.87 (m, 1H), 1.46 (s, 9H), 1.40 (s, 9H), 0.92 (s, 9H), 0.06 (s, 3H), 0.06 (s, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.36, 163.62, 155.72, 155.23, 153.49, 136.90, 134.73, 128.69, 128.61, 128.32, 127.84, 127.75, 81.71, 79.36, 68.09, 66.95, 64.26, 51.98, 49.50, 33.69, 28.27, 27.95, 25.81, 18.19, −5.57, −5.59 ppm. **HRMS** (*m*/*z*): calculated for $C_{37}H_{56}N_4O_9SiH^+$ $[M + H]^+$: 729.3889, found 729.3884.

L-*allo*-END derivative 15. Compound **14** (4.34 g, 5.96 mmol, 1.0 equiv.) was dissolved in THF (50 mL). TBAF (1.0 M, 11.92 mL, 11.92 mmol, 2.0 equiv.) was added dropwise at 0 °C. The reaction was stirred at room temperature for 5 h and diluted using EtOAc (200 mL). The organic phase was washed with saturated aqueous NaCl solution (100 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford the primary alcohol (3.26 g, 89%) as a white foam. R_f = 0.36 (silica, EA/hexanes = 1/2). $[\alpha]_D^{20}$ = +1.6 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 11.72 (s, 1H), 8.67 (d, *J* = 7.8 Hz, 1H), 7.40–7.24 (m, 10H), 5.50 (d, *J* = 7.7 Hz, 1H), 5.18 (s, 2H), 5.08 (s, 2H), 4.29–4.20 (m, 1H), 4.17–4.08 (m, 1H), 3.72 (ddd, *J* = 31.2, 11.4, 3.7 Hz, 2H), 2.16–2.04 (m, 1H), 1.95–1.86 (m, 1H), 1.45 (s, 9H), 1.40 (s, 9H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.63, 163.97, 158.78, 155.82, 150.63, 136.73, 135.31, 128.53, 128.47, 128.15, 128.07, 127.95, 127.62, 83.00, 80.33, 68.18, 67.10, 50.95, 49.31, 48.88, 39.94, 28.18, 27.88 ppm. **HRMS** (*m*/*z*): calculated for $C_{31}H_{42}N_4O_9H^+$ $[M + H]^+$: 615.3025, found 615.3027.

The primary alcohol (2.36 g, 3.84 mmol, 1.0 equiv.) was dissolved in DCM (20 mL). DIPEA (2.01 mL, 11.52 mmol, 3.0 equiv.) and MsCl (0.36 mL, 4.61 mmol, 1.2 equiv.) were added dropwise at 0 °C. The reaction was stirred at room temperature for 2 h and then quenched with saturated aqueous $NaHCO_3$ solution (100 mL). The aqueous phase was extracted using DCM (3 × 50 mL). The combined organic phase was washed with saturated aqueous NaCl solution (100 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **15** (2.24 g, 98%) as a white foam. R_f = 0.35 (silica, EtOAc/hexane = 1/2). $[\alpha]_D^{20}$ = −17.2 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 7.55–7.19 (m, 10H), 5.38 (d, *J* = 18.7 Hz, 1H), 5.26 (s, 2H), 5.18 (s, 2H), 4.35–4.21 (m, 1H), 4.07–3.89 (m, 2H), 3.61–3.51 (m, 1H), 2.15–2.01 (m, 1H), 1.77–1.63 (m, 1H), 1.45 (s, 9H), 1.43 (s, 9H)

ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) rotamer, δ 170.74, 170.01, 155.77, 151.08, 150.90, 136.69, 135.27, 128.55, 128.31, 128.26, 128.15, 128.06, 127.79, 82.89, 80.28, 68.30, 67.28, 51.46, 49.62, 42.42, 39.81, 28.28, 27.97 ppm. **HRMS** (*m*/*z*): calculated for $C_{31}H_{40}N_4O_8H^+$ $[M + H]^+$: 597.2919, found 597.2911.

Dipeptide 22. Compound **15** (540 mg, 0.91 mmol, 1.0 equiv.) was dissolved in TFA-H_2O (10 mL/0.5 mL). After stirring for 5 h and then being concentrated, the crude amino acid product was afforded and re-dissolved in MeOH (2 mL). In another round bottom flask, AcCl (2 mL) was added to MeOH (10 mL) at 0 °C and the resulting solution was added to the solution above. The reaction was stirred at room temperature for 12 h. After concentration, the crude methyl ester was dried under a high vacuum and re-dissolved in DCM. Boc-L-Ala-OH (344 mg, 1.82 mmol, 2.0 equiv.), DIPEA (0.63 mL, 3.64 mmol, 4.0 equiv.) and PyAOP (1.42 g, 2.73 mmol, 3.0 equiv.) were added. The reaction was stirred at room temperature for 12 h and then quenched with saturated aqueous $NaHCO_3$ solution (20 mL). The aqueous phase was extracted using DCM (3 × 20 mL). The combined organic phase was washed with saturated aqueous NaCl solution (50 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **22** (379 mg, 67%) as a white foam. R_f = 0.43 (silica, EtOAc/hexane = 2/1). $[\alpha]_D^{20}$ = −63.4 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.44–7.24 (m, 10H), 5.47–5.27 (m, 1H), 5.22 (s, 2H), 5.18 (s, 2H), 4.84–4.72 (m, 1H), 4.31–4.09 (m, 1H), 4.07–3.98 (m, 1H), 3.94 (t, *J* = 9.8 Hz, 1H), 3.73 (s, 3H), 3.54–3.41 (m, 1H), 2.15–1.75 (m, 2H), 1.46–1.39 (m, 9H), 1.36 (d, *J* = 7.1 Hz, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) rotamer, δ 174.59, 173.65, 171.62, 171.47, 155.53, 136.83, 134.95, 128.66, 128.58, 128.39, 128.35, 128.21, 127.93, 127.77, 80.33, 79.79, 68.45, 67.63, 67.30, 50.24, 28.68, 28.36, 27.94, 18.54 ppm. **HRMS** (*m*/*z*): calculated for $C_{31}H_{39}N_5O_9Na^+$ $[M + Na]^+$: 648.2640, found 648.2654.

Dipeptide acid 7a. Compound **22** (330 mg, 0.53 mmol, 1.0 equiv.) was dissolved in THF (5 mL) and a solution of $LiOH{\cdot}H_2O$ (33 mg, 0.79 mmol, 1.5 equiv.) in H_2O (5 mL) was added dropwise. The reaction was stirred at room temperature for 1 h. THF was evaporated and the aqueous phase was acidified to pH 2. After extraction using EtOAc (3 × 20 mL), the combined organic phase was washed with saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product **7a** was dried under a high vacuum and used in the next step without further purification.

Dipeptide 24. Alloc-Ser-OH **23** (420 mg, 2.22 mmol, 1.0 equiv.) and D-Thr-OBu^t (485 mg, 2.79 mmol, 1.25 equiv.) was dissolved in DCM (5 mL), and DIPEA (1.10 mL, 6.66 mmol, 3.0 equiv.) and EDCI (854 mg, 4.44 mmol, 2.0 equiv.) were added. The reaction was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous phase was extracted using DCM (3 × 50 mL). The combined organic phase was washed with saturated aqueous NaCl solution (100 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford the dipeptide Alloc-Ser-Thr-OBu^t (727 mg, 95%) as a white foam. R_f = 0.24 (silica, EA/hexane = 2/1). $[\alpha]_D^{20}$ = −16.4 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.9 Hz, 1H), 6.18 (d, *J* = 7.7 Hz, 1H), 5.93–5.81 (m, 1H), 5.28 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.18 (dq, *J* = 10.3, 1.3 Hz, 1H), 4.55 (d, *J* = 5.6 Hz, 2H), 4.44 (dd, *J* = 8.9, 3.4 Hz, 1H), 4.36 (s, 1H), 4.23 (q, *J* = 5.3, 4.7 Hz, 1H), 3.96 (dd, *J* = 11.3, 4.2 Hz, 1H), 3.76–3.68 (m, 1H), 1.44 (s, 9H), 1.20 (d, *J* = 6.4 Hz, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.28, 169.96, 156.38, 132.45, 117.94, 82.76, 68.19, 66.06, 63.07, 58.57, 56.31, 27.96, 20.08 ppm. **HRMS** (*m*/*z*): calculated for $C_{15}H_{26}N_2O_7Na^+$ $[M + Na]^+$: 369.1632, found 369.1630.

The above dipeptide intermediate (727 mg, 2.10 mmol, 1.0 equiv.) was dissolved in DCM (15 mL), and imidazole (286 mg, 4.20 mmol, 2.0 equiv.) was added. A solution of TBSCl (378 mg, 2.52 mmol, 1.2 equiv.) in DCM (15 mL) was added dropwise at 0 °C. The reaction was stirred at room temperature for 2 h and then quenched with saturated aqueous NH_4Cl solution (50 mL). The aqueous phase was extracted using DCM (3 × 50 mL). The combined organic phase was washed with saturated aqueous NaCl solution (100 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **24** (646 mg, 91%) as a white foam. R_f = 0.45 (silica, EtOAc/hexane = 1/2). $[\alpha]_D^{20}$ = +18.6 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.09 (d, *J* = 8.8 Hz, 1H), 5.94–5.83 (m, 1H), 5.71 (d, *J* = 6.9 Hz, 1H), 5.28 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.18 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.55 (d, *J* = 5.7 Hz, 2H), 4.45 (dd, *J* = 8.7, 3.0 Hz, 1H), 4.27–4.17 (m, 2H), 4.05–3.98 (m, 1H), 3.72 (dd, *J* = 10.0, 6.2 Hz, 1H), 2.52 (s, 1H), 1.44 (s, 9H), 1.16 (d, *J* = 6.4 Hz, 3H), 0.85 (s, 9H), 0.05 (s, 6H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.34, 169.57, 156.02, 132.48, 117.92, 82.41, 68.33, 65.95, 63.25, 57.96, 56.33, 27.95, 25.78, 19.93, 18.18, −5.51, −5.57 ppm. **HRMS** (*m*/*z*): calculated for $C_{21}H_{40}N_2O_7SiNa^+$ $[M + Na]^+$: 483.2497, found 483.2494.

Tripeptide 6a. Compound **24** (1.22 g, 2.65 mmol, 1.0 equiv.) was dissolved in DCM (20 mL) and Fmoc-Ile-OH (1.12 g, 3.18 mmol, 1.2 equiv.), EDCI (1.27 g, 6.63 mmol, 2.5 equiv.) and DMAP (32 mg, 0.265 mmol, 0.1 equiv.) were added. The reaction was stirred at room temperature for 5 h and then quenched using saturated aqueous $NaHCO_3$ solution (20 mL). The aqueous phase was extracted using DCM (3 × 20 mL). The combined organic phase was washed with saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified by flash chromatography to afford compound **6a** (1.98 g, 94%) as a white foam. R_f = 0.30 (silica, EtOAc/hexane = 1/5). $[\alpha]_D^{20}$ = −21.6 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.62 (t, *J* = 7.5 Hz, 2H), 7.40 (td, *J* = 7.3, 1.9 Hz, 2H), 7.32 (tt, *J* = 7.5, 1.6 Hz, 2H), 7.04 (d, *J* = 9.2 Hz, 1H), 5.96–5.85 (m, 1H), 5.66 (s, 1H), 5.44 (qd, *J* = 6.4, 2.8 Hz, 1H), 5.37 (d, *J* = 9.2 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.22 (dq, *J* = 10.4, 1.4 Hz, 1H), 4.70 (dd, *J* = 9.2, 2.8 Hz, 1H), 4.60 (d, *J* = 5.7 Hz, 2H), 4.45 (dd, *J* = 10.6, 7.3 Hz, 1H), 4.36 (dd, *J* = 10.6, 6.9 Hz, 1H), 4.33–4.26 (m, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 4.08

(dd, J = 10.1, 3.7 Hz, 1H), 3.77 (dd, J = 10.0, 5.9 Hz, 1H), 1.94–1.80 (m, 2H), 1.44 (s, 9H), 1.29 (d, J = 6.4 Hz, 3H), 1.22–1.10 (m, 1H), 0.97–0.92 (m, 6H), 0.89 (s, 9H), 0.08 (s, 6H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.46, 168.02, 155.98, 143.97, 143.81, 141.31, 132.46, 127.68, 127.05, 125.08, 119.95, 118.07, 82.93, 71.75, 67.04, 66.10, 63.15, 58.86, 55.94, 47.27, 37.80, 27.93, 25.83, 24.71, 18.24, 17.16, 15.39, 11.47, −5.45, −5.53 ppm. **HRMS** (m/z): calculated for $C_{42}H_{61}N_3O_{10}SiNa^+$ $[M + Na]^+$: 818.4018, found 818.4015.

Pentapeptide 25. Compound **6a** (325 mg, 0.456 mmol, 1 equiv.) was dissolved in DCM (5 mL) and Et_2NH (2.5 mL) was added. The reaction was stirred at room temperature for 6 h. After concentration, the crude amine was dried under high vacuum and used in the next step without further purification.

The crude amine and compound **7a** (0.501 mmol, 1.1 equiv.) were dissolved in DCM (20 mL). DIPEA (0.40 mL, 2.28 mmol, 5.0 equiv.) and PyAOP (475 mg, 0.912 mmol, 2.0 equiv.) were added. The reaction was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous phase was extracted using DCM (3 × 50 mL). The combined organic phase was washed with saturated aqueous NaCl solution (100 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **25** (482 mg, 90%) as a white foam. R_f = 0.38 (silica, EtOAc/hexane = 1/1). $[\alpha]_D^{20}$ = −42.6 (c 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) rotamer, main peaks: δ 9.74 (s, 1H), 9.65 (s, 1H), 8.31 (d, J = 9.8 Hz, 1H), 7.43–7.29 (m, 10H), 7.25 (d, J = 12.1 Hz, 1H), 6.38 (s, 1H), 5.98–5.85 (m, 1H), 5.51–5.39 (m, 1H), 5.35–5.24 (m, 1H), 5.22–5.13 (m, 4H), 4.85–4.68 (m, 3H), 4.63–4.48 (m, 3H), 4.37 (dd, J = 7.9, 3.8 Hz, 1H), 4.19 (t, J = 7.2 Hz, 1H), 4.13–4.02 (m, 2H), 3.96 (t, J = 10.0 Hz, 2H), 3.38–3.28 (m, 1H), 2.48 (d, J = 13.8 Hz, 1H), 2.34–2.15 (m, 1H), 1.97–1.84 (m, 1H), 1.80–1.70 (m, 1H), 1.69–1.57 (m, 1H), 1.44 (d, J = 7.1 Hz, 3H), 1.40 (s, 9H), 1.34 (s, 9H), 1.23 (d, J = 6.6 Hz, 3H), 0.94–0.89 (m, 6H), 0.84 (s, 9H), 0.01 (s, 3H), 0.01 (s, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) rotamer, main peaks: δ 175.39, 174.76, 170.91, 170.85, 170.35, 169.47, 168.09, 167.88, 155.92, 155.68, 153.07, 150.82, 145.07, 135.11, 134.67, 133.09, 132.42, 128.85, 128.76, 128.61, 128.58, 128.29, 118.03, 117.34, 82.76, 82.36, 78.91, 77.31, 71.90, 70.88, 68.49, 68.00, 66.04, 65.38, 64.52, 63.17, 60.05, 57.54, 57.47, 56.61, 55.95, 55.79, 52.69, 51.53, 51.02, 38.06, 37.16, 35.81, 31.89, 29.66, 29.62, 29.32, 28.30, 28.01, 27.85, 26.31, 25.80, 25.40, 22.65, 19.14, 18.24, 17.65, 17.17, 16.81, 15.45, 14.07, 13.60, 11.78, −5.52, −5.55 ppm. **HRMS** (m/z): calculated for $C_{57}H_{86}N_8O_{16}SiNa^+$ $[M + Na]^+$: 1189.5823, found 1189.5819.

Compound 3a. Compound **25** (90 mg, 0.077 mmol, 1.0 equiv.) was dissolved in TFA/DCM (2 mL/2 mL) and the reaction was stirred at room temperature for 4 h. After concentration, the crude product was dried under high vacuum and re-dissolved in DMF (20 mL). NMM (0.17 ml, 1.54 mmol, 20 equiv.), HATU (293 mg, 0.77 mmol, 10 equiv.) and HOAt (52.4 mg, 0.385 mmol, 5 equiv.) were added. The reaction was stirred at room temperature for 24 h. After evaporation of DMF, the resulting residue was re-dissolved in DCM (50 mL). After being washed with saturated aqueous $NaHCO_3$ solution (20 mL) and saturated aqueous NaCl solution (20 mL), the solution was dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **3a** (52 mg, 85%) as a yellow solid. R_f = 0.31 (silica, MeOH/DCM = 1/20). $[\alpha]_D^{20}$ = −30.8 (c 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, CD_3OD) δ 7.46–7.24 (m, 10H), 5.97–5.84 (m, 1H), 5.55 (dt, J = 8.2, 4.1 Hz, 1H), 5.30 (dq, J = 17.3, 1.7 Hz, 1H), 5.23 (d, J = 16.2 Hz, 2H), 5.20–5.13 (m, 2H), 5.09 (d, J = 12.6 Hz, 1H), 4.63–4.56 (m, 1H), 4.56–4.49 (m, 2H), 4.33–4.26 (m, 1H), 4.21 (d, J = 8.4 Hz, 1H), 4.11 (q, J = 7.4 Hz, 1H), 4.03 (d, J = 6.0 Hz, 2H), 3.91 (dd, J = 10.8, 5.2 Hz, 1H), 3.87–3.77 (m, 2H), 3.76–3.64 (m, 1H), 3.05–2.85 (m, 1H), 2.14 (s, 2H), 1.78 (s, 1H), 1.50 (s, 1H), 1.38 (d, J = 7.4 Hz, 3H), 1.28 (d, J = 6.5 Hz, 3H), 1.19–1.07 (m, 1H), 0.97–0.79 (m, 6H) ppm. **^{13}C NMR** (125 MHz, CD_3OD) conformer, δ 174.37, 173.93, 173.44, 171.33, 168.63, 168.34, 167.97, 157.22, 151.81, 136.20, 135.06, 132.69, 128.35, 128.30, 128.16, 127.96, 127.89, 127.69, 116.49, 71.37, 70.31, 69.15, 68.60, 67.18, 66.17, 65.55, 61.39, 58.24, 58.04, 52.19, 52.14, 36.76, 25.24, 15.31, 14.97, 14.18, 9.96, 9.85 ppm. **HRMS** (m/z): calculated for $C_{42}H_{54}N_8O_{13}Na^+$ $[M + Na]^+$: 901.3703, found 901.3701.

Compound 26. Compound **3a** (10 mg, 0.011 mmol, 1.0 equiv.) and $Pd(PPh_3)_4$ (3 mg, 0.0028 mmol, 0.25 equiv.) were dissolved in DCM (1 mL) and Et_2NH (10 μL) was added at 0 °C. The reaction was stirred at room temperature for 1 h. After concentration, compound **26** was afforded and used in the next step without purification.

Tripeptide 4. Compound **27** (200 mg, 0.38 mmol, 1.0 equiv.) and D-allo-Ile-OMe (103 mg, 0.57 mmol, 1.5 equiv.) were dissolved in DCM (10 mL) and then DIPEA (0.27 mL, 1.52 mmol, 4.0 equiv.) and PyAOP (396 mg, 0.76 mmol, 2.0 equiv.) were added. The reaction was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous phase was extracted using DCM (3 × 20 mL). The combined organic phase was washed with saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford the corresponding dipeptide Cbz-Gln(Tr)-allo-Ile-OMe (235 mg, 96%) as a white foam. R_f = 0.50 (silica, EtOAc/hexane = 1/1). $[\alpha]_D^{20}$ = −0.2 (c 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.42–7.14 (m, 20H), 5.98 (d, J = 7.1 Hz, 1H), 5.17–5.04 (m, 2H), 4.56 (dd, J = 8.6, 4.1 Hz, 1H), 4.23 (q, J = 6.8 Hz, 1H), 3.66 (s, 3H), 2.62–2.47 (m, 2H), 2.18–2.06 (m, 1H), 2.04–1.95 (m, 1H), 1.95–1.84 (m, 1H), 1.35–1.23 (m, 1H), 1.16–1.05 (m, 1H), 0.87 (t, J = 7.4 Hz, 3H), 0.73 (d, J = 7.0 Hz, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.53, 172.04, 171.72, 156.21, 144.58, 136.44, 128.76, 128.49, 128.08, 128.03, 127.93, 127.00, 70.73, 66.84, 55.78, 53.89, 52.10, 36.86, 33.43, 29.45, 26.18, 14.49, 11.72 ppm. **HRMS** (m/z): calculated for $C_{39}H_{43}N_3O_6Na^+$ $[M + Na]^+$: 672.3044, found 672.3052.

The above dipeptide Cbz-Gln(Tr)-allo-Ile-OMe (117 mg, 0.18 mmol, 1.0 equiv.) was dissolved in THF (5 mL) and a solution of $LiOH{\cdot}H_2O$ (15 mg, 0.36 mmol, 2.0 equiv.) in H_2O (5 mL) was added dropwise. The reaction was stirred at room

temperature for 1 h. THF was evaporated and the aqueous phase was acidified to pH 2. After extraction using EtOAc (3 × 20 mL), the combined organic phase was washed with saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude acid product was dried under a high vacuum and re-dissolved in DCM (20 mL). L-Ile-OMe (49 mg, 0.27 mmol, 1.5 equiv.), DIPEA (0.13 mL, 0.72 mmol, 4.0 equiv.) and PyAOP (234 mg, 0.45 mmol, 2.5 equiv.) were added. The reaction proceeded at room temperature for 12 h and was quenched with saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous phase was extracted using DCM (3 × 20 mL). The combined organic phase was washed with a saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified with flash chromatography to afford compound **4** (119 mg, 88%) as a white solid. R_f = 0.42 (silica, EtOAc/hexane = 1/1). $[\alpha]_D^{20}$ = +22.8 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.40–7.29 (m, 5H), 7.29–7.13 (m, 15H), 6.88 (dd, *J* = 18.7, 7.9 Hz, 2H), 6.20 (d, *J* = 5.9 Hz, 1H), 5.14 (d, *J* = 12.2 Hz, 1H), 5.07 (d, *J* = 12.2 Hz, 1H), 4.49 (dd, *J* = 8.2, 5.5 Hz, 1H), 4.40 (dd, *J* = 8.5, 4.7 Hz, 1H), 4.10–4.01 (m, 1H), 3.61 (s, 3H), 2.62–2.51 (m, 1H), 2.50–2.38 (m, 1H), 2.17–2.06 (m, 1H), 2.06–1.95 (m, 2H), 1.94–1.83 (m, 1H), 1.49–1.36 (m, 1H), 1.34–1.24 (m, 1H), 1.24–1.06 (m, 2H), 0.94–0.81 (m, 9H), 0.76 (d, *J* = 6.9 Hz, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.28, 171.94, 171.70, 171.15, 156.67, 144.48, 136.24, 128.67, 128.50, 128.24, 128.18, 127.93, 127.04, 70.80, 67.09, 56.80, 56.72, 55.25, 51.86, 37.28, 36.28, 33.55, 28.02, 26.44, 25.27, 15.52, 14.10, 11.61, 11.45 ppm. **HRMS** (*m*/*z*): calculated for $C_{45}H_{54}N_4O_7Na^+$ $[M + Na]^+$: 785.3885, found 785.3882.

Amine 28. Compound **4** (80 mg, 0.100 mmol, 1.0 equiv.) was dissolved in MeOH (2 mL) and $PdCl_2$ (9 mg, 0.050 mmol, 0.5 equiv.) was added. The reaction was stirred with a H_2 balloon at room temperature for 2 h. After filtration and concentration, compound **28** was afforded and used in the next step without purification.

Tripeptide 30. Compound **29** (200 mg, 0.64 mmol, 1.0 equiv.) and L-Ile-OMe (174 mg, 0.96 mmol, 1.5 equiv.) were dissolved in DCM (10 mL). DIPEA (0.45 mL, 2.56 mmol, 4.0 equiv.), EDCI (246 mg, 1.28 mmol, 2.0 equiv.) and HOAt (87 mg, 0.64 mmol, 1.0 equiv.) were added. The reaction was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous phase was extracted using DCM (3 × 20 mL). The combined organic phase was washed with saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford the dipeptide Cbz-N(Me)Phe-Ile-OMe (195 mg, 69%) as a white foam. R_f = 0.41 (silica, EtOAc/hexane = 1/5). $[\alpha]_D^{20}$ = +90.0 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.39–7.07 (m, 10H), 6.67 (d, *J* = 8.6 Hz, 1H), 5.11 (s, 2H), 5.08–5.01 (m, 1H), 4.98–4.84 (m, 1H), 4.58–4.47 (m, 1H), 3.68 (s, 3H), 3.47–3.28 (m, 1H), 2.97 (dd, *J* = 14.4, 9.1 Hz, 1H), 2.90 (s, 3H), 1.88–1.77 (m, 1H), 1.38–1.26 (m, 1H), 1.13–0.97 (m, 1H), 0.87 (t, *J* = 7.4 Hz, 3H), 0.82 (d, *J* = 6.7 Hz, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.95, 170.19, 157.40, 137.26, 136.53, 128.97, 127.95, 127.62, 126.57, 67.70, 67.51, 60.99, 60.08, 56.58, 51.90, 37.56, 37.37, 34.28, 33.97, 30.99, 30.38, 25.08, 15.52, 11.49 ppm. **HRMS** (*m*/*z*): calculated for $C_{25}H_{32}N_2O_5Na^+$ $[M + Na]^+$: 463.2203, found 463.2204.

Cbz-N(Me)Phe-Ile-OMe (1.50 g, 3.41 mmol, 1.0 equiv.) was dissolved in THF (20 mL) and a solution of $LiOH{\cdot}H_2O$ (286 mg, 6.82 mmol, 2.0 equiv.) in H_2O (20 mL) was added dropwise. The reaction was stirred at room temperature for 1 h. THF was evaporated and the aqueous phase was acidified to pH = 2. After extraction using EtOAc (3 × 50 mL), the combined organic phase was washed with saturated aqueous NaCl solution (50 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude acid product was dried under a high vacuum and re-dissolved in DCM (20 mL). L-Ser-OMe (796 mg, 5.12 mmol, 1.5 equiv.), DIPEA (2.97 mL, 17.05 mmol, 5.0 equiv.) and PyAOP (3.55 g, 6.82 mmol, 2.0 equiv.) were added. The reaction was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution (20 mL). The aqueous phase was extracted using DCM (3 × 50 mL). The combined organic phase was washed with saturated aqueous NaCl solution (50 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford tripeptide **30** (1.35 g, 75%) as a white solid. R_f = 0.41 (silica, EtOAc/hexane = 2/1). $[\alpha]_D^{20}$ = +65.2 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) rotamer, δ 7.40–7.06 (m, 10H), 6.79–6.63 (m, 1H), 5.18–4.99 (m, 2H), 4.83 (t, *J* = 8.0 Hz, 1H), 4.69–4.46 (m, 2H), 4.32 (t, *J* = 7.6 Hz, 1H), 4.01–3.91 (m, 1H), 3.90–3.81 (m, 1H), 3.74 (s, 3H), 3.40–3.27 (m, 1H), 3.17–3.06 (m, 1H), 3.06–2.96 (m, 1H), 2.85 (s, 3H), 1.86 (s, 1H), 1.47–1.33 (m, 1H), 1.13–0.96 (m, 1H), 0.94–0.77 (m, 6H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) rotamer, δ 171.26, 171.17, 170.95, 170.74, 170.63, 157.28, 157.21, 156.08, 137.08, 136.53, 136.28, 136.13, 128.95, 128.87, 128.66, 128.55, 128.49, 128.14, 128.04, 127.95, 127.69, 127.04, 126.69, 67.76, 67.69, 67.10, 62.67, 61.26, 58.06, 56.88, 54.79, 54.75, 54.65, 52.60, 52.51, 38.84, 36.46, 34.35, 31.59, 26.34, 24.72, 15.42, 14.26, 11.58, 11.17 ppm. **HRMS** (*m*/*z*): calculated for $C_{28}H_{37}N_3O_7Na^+$ $[M + Na]^+$: 550.2524, found 550.2526.

Tripeptide 5. Compound **30** (105 mg, 0.20 mmol, 1.0 equiv.) was dissolved in DCM (5 mL). Imidazole (54 mg, 0.80 mmol, 4.0 equiv.) and TBSCl (60 mg, 0.40 mmol, 2.0 equiv.) were added at 0 °C. The reaction was stirred at room temperature for 4 h and quenched with saturated aqueous NH_4Cl solution (20 mL). The aqueous phase was extracted using DCM (3 × 10 mL). The combined organic phase was washed with saturated aqueous NaCl solution (10 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **5** (121 mg, 94%) as a white foam. R_f = 0.49 (silica, EtAOc/hexane = 1/2). $[\alpha]_D^{20}$ = +77.2 (*c* 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) rotamer, δ 7.40–7.06 (m, 10H), 6.70 (d, *J* = 8.3 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 5.17–4.86 (m, 3H), 4.65–4.57 (m, 1H), 4.35 (dd, *J* = 8.4, 5.6 Hz, 1H), 4.05 (ddd, *J* = 12.8, 10.2, 2.9 Hz, 1H), 3.82 (ddd, *J* = 10.1, 6.8, 3.1 Hz, 1H),

3.74 (d, J = 8.7 Hz, 3H), 3.38 (dd, J = 14.4, 7.1 Hz, 1H), 2.98 (dd, J = 14.3, 9.3 Hz, 1H), 2.89 (s, 3H), 1.90–1.78 (m, 1H), 1.44 (ddt, J = 10.7, 7.7, 3.9 Hz, 1H), 1.11 (ddt, J = 14.4, 9.5, 7.3 Hz, 1H), 0.94–0.79 (m, 15H), 0.03 (s, 3H), 0.03 (s, 3H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) rotamer, δ 170.39, 170.09, 169.64, 157.21, 137.24, 136.52, 128.95, 128.82, 128.54, 128.47, 128.43, 128.04, 127.97, 127.91, 127.75, 127.63, 126.59, 67.54, 63.22, 60.80, 57.62, 56.52, 54.40, 54.26, 52.33, 52.25, 37.78, 37.49, 34.40, 34.27, 30.83, 26.22, 25.65, 24.89, 18.11, 15.18, 11.65, 11.48, −5.55, −5.74 ppm. **HRMS** (m/z): calculated for $C_{34}H_{51}N_3O_7SiNa^+$ $[M + Na]^+$: 664.3388, found 664.3396.

Sidechain hexapeptide 2. Compound **5** (60 mg, 0.094 mmol, 1.0 equiv.) was dissolved in PhMe (5 mL) and Me_3SnOH (86 mg, 0.470 mmol, 5.0 equiv.) was added. The reaction was stirred at 70 °C for 24 h. The solution was diluted with EtOAc (20 mL), washed with saturated aqueous NH_4Cl solution (10 mL) and dried with anhydrous Na_2SO_4. The acid was afforded after concentration and was used in the next step without purification.

Compound **28** (0.10 mmol, 1.1 equiv.) and the above carboxylic acid were dissolved in DMF (5 mL). DIPEA (98 μL, 0.564 mmol, 6.0 equiv.) and PyAOP (147 mg, 0.282 mmol, 3.0 equiv.) were added. The reaction was stirred at room temperature for 12 h. The DMF was evaporated and the residue was dissolved in DCM (50 mL). The solution was washed with saturated aqueous $NaHCO_3$ solution (20 mL) and saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **2** (53 mg, 46%) as a white solid. R_f = 0.32 (silica, EtOAc/hexane = 1/1). $[\alpha]_D^{20}$ = +30.6 (c 1.0, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) rotamer, δ 7.48–6.99 (m, 27H), 6.85 (s, 1H), 6.71 (s, 1H), 6.60–6.25 (m, 1H), 5.11 (q, J = 11.9, 11.4 Hz, 2H), 4.59–4.42 (m, 1H), 4.41–4.28 (m, 2H), 4.28–4.16 (m, 2H), 3.96–3.92 (m, 1H), 3.75–3.703 (m, 1H), 3.64 (s, 3H), 3.23 (dd, J = 13.9, 6.0 Hz, 1H), 3.10 (dd, J = 13.9, 10.2 Hz, 1H), 2.85–2.65 (m, 3H), 2.60–2.53 (m, 1H), 2.53–2.41 (m, 1H), 2.26–2.15 (m, 2H), 2.13–2.06 (m, 1H), 1.98–1.83 (m, 3H), 1.48–1.35 (m, 3H), 1.23–1.13 (m, 2H), 1.12–0.99 (m, 2H), 0.96–0.74 (m, 27H), 0.04 (s, 6H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$) rotamer, δ 172.78, 172.08, 171.92, 171.30, 171.16, 170.34, 170.03, 157.17, 144.57, 136.85, 136.03, 130.84, 129.01, 128.77, 128.71, 128.61, 128.54, 128.22, 127.85, 126.92, 126.78, 70.71, 67.78, 65.52, 64.32, 63.43, 62.59, 60.33, 59.15, 57.75, 57.15, 56.91, 56.78, 56.54, 56.09, 53.49, 52.11, 52.02, 37.72, 37.57, 37.40, 37.15, 36.40, 36.26, 34.35, 34.08, 33.62, 31.90, 30.66, 30.59, 29.66, 29.62, 29.31, 28.02, 26.30, 26.18, 25.80, 25.22, 25.16, 25.03, 24.80, 23.15, 18.17, 15.73, 15.59, 15.52, 15.43, 15.25, 14.50, 14.25, 14.16, 11.58, 11.52, 11.42, 11.37, 11.19, −5.43, −5.49 ppm. **HRMS** (m/z): calculated for $C_{70}H_{95}N_7O_{11}SiNa^+$ $[M + Na]^+$: 1260.6751, found 1260.6746.

Protected teixobactin 31. Compound **2** (10 mg, 0.0081 mmol, 1.0 equiv.) was dissolved in PhMe (2 mL) and Me_3SnOH (7 mg, 0.041 mmol, 5.0 equiv.) was added. The reaction was stirred at 70 °C for 24 h. The solution was diluted with EtOAc (20 mL), washed with saturated aqueous NH_4Cl solution (10 mL) and dried with anhydrous Na_2SO_4. The acid was afforded after concentration and used in the next step without purification.

Compound **26** (0.011 mmol, 1.3 equiv.) and the above acid were dissolved in DMF (1 mL). DIPEA (14 μL, 0.081 mmol, 10.0 equiv.), EDCI (9 mg, 0.049 mmol, 6.0 equiv.) and HOAt (2 mg, 0.016 mmol, 2.0 equiv.) were added. The reaction was stirred at room temperature for 12 h. The DMF was evaporated and the residue was dissolved in DCM (50 mL). The solution was washed with saturated aqueous $NaHCO_3$ solution (20 mL) and saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **31** (14 mg, 86%) as a white solid. Compound **31** exists as multiple rotamers and/or conformers, leading to very complex NMR spectra, the ^{13}C NMR experiment could not be acquired to a satisfactory intensity. $[\alpha]_D^{20}$ = −14.0 (c 1.0, $CHCl_3$). **HRMS** (m/z): calculated for $C_{107}H_{142}N_{15}NaO_{21}Si^{2+}$ $[M + H + Na]^{2+}$: 1012.5097, found 1012.5042. Other high-resolution mass peaks, including $[M + 2Na]^{2+}$, $[M + 2H]^{2+}$, $[M + H]^+$ and $[M + Na]^+$, were also found in the spectrum with reduced intensities.

Teixobactin 1. Compound **31** (6 mg, 0.003 mmol, 1.0 equiv.) was dissolved in MeOH (2 mL) and $PdCl_2$ (1 mg, 0.006 mmol, 2.0 equiv.) was added. The reaction was stirred with a H_2 balloon at room temperature for 6 h. After filtration and concentration, the crude residue was dissolved in TFA/DCM (1 mL/0.5 mL) and i-Pr_3SiH (0.1 mL) was added. The reaction was stirred at room temperature for 6 h. After concentration, the TFA salt of teixobactin was afforded after HPLC purification using an Agilent 1200 system, with a reverse phase column (Shimadzu Shim-pack VP-ODS, column size 250 L × 4.6, serial no. 8032635). Mobile phase: MeCN (with 0.1% TFA)/H_2O (with 0.1% TFA) using linear gradients: 5% to 95% MeCN/H_2O. Flow rate: 1 mL min^{-1}. Retention time: 15.38 min. The TFA salt of teixobactin was lyophilized three times in the presence of 5 mM HCl to afford the HCl salt of teixobactin **1** (1.5 mg, 40%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.05 (s, 2H), 8.98 (d, J = 8.5 Hz, 1H), 8.86 (d, J = 10.2 Hz, 1H), 8.80 (d, J = 10.4 Hz, 1H), 8.71 (d, J = 9.8 Hz, 1H), 8.49 (d, J = 8.4 Hz, 1H), 8.13 (d, J = 5.7 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.92 (s, 1H), 7.91 (s, 1H), 7.89 (s, 1H), 7.87 (s, 1H), 7.77 (d, J = 9.2 Hz, 1H), 7.66 (s, 2H), 7.31–7.28 (m, 2H), 7.27–7.24 (m, 1H), 7.23–7.20 (m, 2H), 7.20 (s, 1H), 6.76 (s, 1H), 5.40–5.35 (m, 1H), 4.69 (dd, J = 9.5, 1.6 Hz, 1H), 4.63 (d, J = 9.0 Hz, 1H), 4.42–4.39 (m, 1H), 4.39–4.37 (m, 1H), 4.36–4.34 (m, 1H), 4.34–4.32 (m, 1H), 4.32–4.30 (m, 1H), 4.19–4.15 (m, 1H), 4.13 (dd, J = 8.1, 6.9 Hz, 1H), 4.01 (t, J = 9.8 Hz, 1H), 3.92–3.88 (m, 1H), 3.88–3.84 (m, 1H), 3.84–3.79 (m, 1H), 3.62 (t, J = 9.4 Hz, 1H), 3.56 (s, 2H), 3.54–3.50 (m, 1H), 3.44–3.40 (m, 1H), 3.12 (dd, J = 13.5, 5.5 Hz, 1H), 2.95 (dd, J = 13.1, 9.8 Hz, 1H), 2.45 (s, 3H), 2.20–2.15 (m, 1H), 2.09–2.01 (m, 3H), 1.90–1.86 (m, 1H), 1.85–1.79 (m, 2H), 1.80–1.75 (m, 1H), 1.71–1.65 (m, 1H), 1.55–1.50 (m, 1H), 1.44–1.40 (m, 1H), 1.30 (s, 1H), 1.26 (d, J = 7.4 Hz, 4H), 1.17–1.12 (m, 1H), 1.09–1.06 (m, 1H), 1.05 (d, J = 6.5 Hz, 3H), 1.03–0.98 (m, 2H), 0.91–0.86 (m, 3H), 0.85–0.81 (m, 3H),

0.81–0.79 (m, 3H), 0.79–0.77 (m, 3H), 0.77–0.76 (m, 3H), 0.76–0.73 (m, 3H), 0.73–0.68 (m, 2H), 0.63 (t, J = 7.2 Hz, 3H), 0.58 (d, J = 6.8 Hz, 3H) ppm. **^{13}C NMR** (125 MHz, DMSO-d_6) δ 174.34, 172.97, 171.83, 171.51, 171.46, 171.21, 170.98, 170.59, 170.04, 169.66, 168.36, 167.01, 159.35, 134.99, 129.73, 128.95, 127.57, 70.69, 64.23, 62.35, 61.66, 57.79, 57.68, 57.24, 56.09, 56.05, 55.97, 55.55, 53.77, 52.44, 52.26, 52.23, 48.39, 37.52, 37.46, 36.90, 36.60, 36.33, 35.85, 31.88, 31.78, 28.69, 26.28, 25.37, 24.68, 24.26, 17.22, 15.95, 15.88, 15.48, 15.48, 14.66, 11.95, 11.62, 11.35, 10.52 ppm. **HRMS** (m/z): calculated for $C_{58}H_{95}N_{15}O_{15}H^+$ $[M + H]^+$: 1242.7205, found 1242.7213.

Minimum inhibitory concentration

Material. Bacterial strains, *Staphylococcus aureus* (S.aur) and Enterococcus faecalis (E.fae), were purchased from ATCC (ATCC 33591 & ATCC 51575). S.aur Newman was obtained from Dr Zigang Li's lab of Peking University Shenzhen Graduate School (Shenzhen, China).

Method. The minimum inhibitory concentration (MIC) of the antimicrobial compound was determined using the broth microdilution method following the Clinical and Laboratory Standards Institute (CLSI) guidelines (https://clsi.org/). Bacteria were cultured in a Mueller–Hinton broth (MHB), except for E.fae which was cultured in a Luria–Bertani (LB) broth. During the tests, 1×10^5 mL^{-1} of bacteria were incubated with different concentrations of the compounds in a medium containing 0.002% polysorbate 80 to prevent drug absorption on the plastic surface at 37 °C for 20 h, or for 30 h when testing E.fae. Absorbance of the culture at 600 nm was measured using a microplate reader (Tecan Group Ltd) after incubation to determine the amount of bacteria. The MIC is expressed as the minimum concentration of a compound which prevents the visible growth of a bacterium. Each test was repeated four times.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 M. Lobanovska and G. Pilla, *Yale J. Biol. Med.*, 2017, **90**, 135.
2 (*a*) E. D. Brown and G. D. Wright, *Nature*, 2016, **529**, 336; (*b*) C. L. Ventola, *Pharm. Ther.*, 2015, **40**, 277; (*c*) C. L. Ventola, *Pharm. Ther.*, 2015, **40**, 344.
3 N. Gilbert, *Nature*, 2018, **555**, S5.
4 M. Movassaghi and W. A. Van der Donk, *J. Org. Chem.*, 2018, **83**, 6826.
5 (*a*) C. T. Walsh, *ACS Infect. Dis.*, 2018, **4**, 1283; (*b*) M. Dong, B. Pfeiffer and K.-H. Altmann, *Drug Discovery Today*, 2017, **22**, 585; (*c*) N. Malanovic and K. Lohner, *Pharmaceuticals*, 2016, **9**, 59.
6 D. J. Newman, *ACS Med. Chem. Lett.*, 2018, **9**, 66.
7 T. Velkov, K. D. Roberts, P. E. Thompson and J. Li, *Future Med. Chem.*, 2016, **8**, 1017.
8 S. D. Taylor and M. Palmer, *Bioorg. Med. Chem.*, 2016, **24**, 6253.
9 A. Kling, P. Lukat, D. V. Almeida, A. Baner, E. Fontaine, S. Sordello, N. Zaburannyi, J. Hermann, S. C. Wenzel, C. Köbig, N. C. Ammerman, M. B. Barrio, K. Borchers, F. B. Pallier, M. Brönstrup, G. Courtemanche, M. Gerlitz, M. Geslin, P. Hammann, D. W. Heinz, H. Hoffmann, S. Klieber, M. Kohlmann, M. Kurz, C. Lair, H. Matter, E. Nuermberger, S. Tyagi, L. Fraisse, J. H. Grosset, S. Lagrange and R. Müller, *Science*, 2015, **348**, 1106.
10 P. A. Smith, M. F. T. Koehle, H. S. Girgis, D. Yan, Y. Chen, Y. Chen, J. J. Grawford, M. R. Durk, R. I. Higuchi, J. Kang, J. Murray, P. Paraselli, S. Park, W. Phung, J. G. Quinn, T. C. Roberts, L. Rougé, J. B. Schwarz, E. Skippington, J. Wai, M. Xu, Z. Yu, H. Zhang, M.-W. Tan and C. E. Heise, *Nature*, 2018, **561**, 189.
11 C. Guo, D. Mandalapu, X. Ji, J. Gao and Q. Zhang, *Chem. – Eur. J.*, 2018, **24**, 5406.
12 L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schaberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen and K. Lewis, *Nature*, 2015, **517**, 455.
13 D. J. Atkinson, B. J. Naysmith, D. P. Furkert and M. A. Brimble, *Beilstein J. Org. Chem.*, 2016, **12**, 2325.
14 (*a*) F. von Nussbaum and R. D. Sussmuth, *Angew. Chem., Int. Ed.*, 2015, **54**, 6684; (*b*) T. Homma, A. Nuxoll, A. B. Gandt, P. Ebner, I. Engels, T. Schneider, F. Gotz, K. Lewis and B. P. Conlon, *Antimicrob. Agents Chemother.*, 2016, **60**, 6510.
15 C. Öster, G. P. Walkowiak, D. E. Hughes, A. L. Spoering, A. J. Peoples, A. C. Catherwood, J. A. Tod, A. J. Lloyd, T. Herrmann, K. Lewis, C. G. Dowson and J. R. Lewandowski, *Chem. Sci.*, 2018, **9**, 8850.
16 H. Yang, M. Wierzbicki, D. R. Du Bios and J. S. Nowick, *J. Am. Chem. Soc.*, 2018, **140**, 14028.
17 S. A. H. Abdel Monaim, Y. E. Jad, G. A. Acosta, T. Naicker, E. J. Ramchuran, A. El-Faham, T. Govender, H. G. Kruger, B. G. de la Torre and F. Albericio, *RSC Adv.*, 2016, **6**, 73827.
18 (*a*) A. M. Giltrap, L. J. Dowman, G. Nagalingam, J. L. Ochoa, R. G. Linington, W. J. Britton and R. J. Payne, *Org. Lett.*, 2016, **18**, 2788; (*b*) K. Jin, L. H. Sam, K. H. L. Po,

D.-A. Lin, E. H. G. Zadeh, S. Chen, Y. Yuan and X. Li, *Nat. Commun.*, 2016, **7**, 12394; (*c*) L. Liu, S. Wu, Q. Wang, M. Zhang, B. Wang, G. He and G. Chen, *Org. Chem. Front.*, 2018, **5**, 1431.

19 (*a*) A. Parmar, R. Lakshminarayanan, A. Iyer, V. Mayandi, E. T. L. Goh, D. G. Floyd, M. L. S. Chalasani, N. K. Verma, S. H. Prior, R. W. Beuerman, A. Madder, E. J. Taylor and I. Singh, *J. Med. Chem.*, 2018, **61**, 2009; (*b*) Y. Zong, X. Sun, H. Gao, K. J. Meyer, K. Lewis and Y. Rao, *J. Med. Chem.*, 2018, **61**, 3409; (*c*) G. C. Girt, A. Mahindra, Z. J. H. Al Jabri, M. De Ste Croix, M. R. Oggioni and A. G. Jamieson, *Chem. Commun.*, 2018, **54**, 2767; (*d*) S. A. H. Abdel Monaim, Y. E. Jad, A. El-Faham, B. G. de la Torre and F. Albericil, *Bioorg. Med. Chem.*, 2018, **26**, 2788; (*e*) D. Mandalapu, X. Ji, J. Chen, C. Guo, W. Q. Liu, W. Ding, J. Zhou and Q. Zhang, *J. Org. Chem.*, 2018, **83**, 7271; (*f*) V. Ng, S. A. Kuehne and W. C. Chan, *Chem. – Eur. J.*, 2018, **24**, 9136 and references cited therein.

20 H. Liu and X. Li, *Acc. Chem. Res.*, 2018, **51**, 1643.

21 S. Dhara, V. B. Gunjal, K. L. Handore and D. S. Reddy, *Eur. J. Org. Chem.*, 2016, 4289.

22 (*a*) J. H. Rasmussen, *Bioorg. Med. Chem.*, 2018, **26**, 2914; (*b*) S. Chandrudu, P. Simerska and I. Toth, *Molecules*, 2013, **18**, 4373; (*c*) L. Andersson, L. Blomberg, M. Flegel, L. Lepsa, B. Nilsson and M. Verlander, *Biopolymers*, 2000, **55**, 227.

23 (*a*) H. Lei, L. Wang, Z. Xu and T. Ye, *Org. Lett.*, 2017, **19**, 5134; (*b*) J. Zhou, B. Gao, Z. Xu and T. Ye, *J. Am. Chem. Soc.*, 2016, **138**, 6948; (*c*) L. Liao, J. Zhou, Z. Xu and T. Ye, *Angew. Chem., Int. Ed.*, 2016, **55**, 13263; (*d*) J. Liu, L. Wang, J. Zhang, Z. Xu and T. Ye, *Chem. Commun.*, 2016, **52**, 1002; (*e*) S. Qu, Y. Chen, X. Wang, S. Chen, Z. Xu and T. Ye, *Chem. Commun.*, 2015, **51**, 2510; (*f*) H. Lei, J. Yan, J. Yu, Y. Liu, Z. Wang, Z. Xu and T. Ye, *Angew. Chem., Int. Ed.*, 2014, **53**, 6533; (*g*) L. Dai, B. Chen, H. H. Lei, Z. Wang, Y. Q. Liu, Z. S. Xu and T. Ye, *Chem. Commun.*, 2012, **48**, 8697; (*h*) L. Wang, Z. S. Xu and T. Ye, *Org. Lett.*, 2011, **13**, 2506; (*i*) Z. Xu, Y. G. Peng and T. Ye, *Org. Lett.*, 2003, **5**, 2821.

24 W. Craig, J. Chen, D. Richardson, R. Thorpe and Y. Yuan, *Org. Lett.*, 2015, **17**, 4620.

25 (*a*) H. Waldmann, Y.-P. He, H. Tan, L. Arve and H.-D. Arndt, *Chem. Commun.*, 2008, 5562; (*b*) Y.-P. He, H. Tan, L. Arve, S. Baumann, H. Waldmann and H.-D. Arndt, *Chem. – Asian J.*, 2011, **6**, 1546.

26 (*a*) S. Yoshifuji, K. Tanaka, T. Kawai and Y. Nitta, *Chem. Pharm. Bull.*, 1986, **34**, 3873; (*b*) X. Zhang, A. C. Schmitt and W. Jiang, *Tetrahedron Lett.*, 2001, **42**, 5335.

27 U. Ragnarsson, L. Grehn, L. S. Monteiro and H. L. S. Maia, *Synlett*, 2003, 2386.

28 M. P. Paudyal, N. P. Rath and C. D. Spilling, *Org. Lett.*, 2010, **12**, 2954 and references cited therein.

29 M. M. Heravi, N. Ghalavand, M. Ghanbarian and L. Mohammadkhani, *Appl. Organomet. Chem.*, 2018, **32**, e4464.

30 (*a*) H. Staudinger and J. Meyer, *Helv. Chim. Acta*, 1919, **2**, 635; (*b*) W. Q. Tian and Y. A. Wang, *J. Org. Chem.*, 2004, **69**, 4299.

31 (*a*) M. S. Bernatowicz, Y. Wu and G. R. Matsueda, *Tetrahedron Lett.*, 1993, **34**, 3389; (*b*) Y. Wu, G. R. Matsueda and M. S. Bernatowicz, *Synth. Commun.*, 1993, **23**, 3055; (*c*) B. Drake, M. Patek and M. Lebl, *Synthesis*, 1994, 579.

32 (*a*) G. Han, M. Tamaki and V. J. Hruby, *J. Pept. Res.*, 2001, **58**, 338; (*b*) L. S. Lin, *Tetrahedron Lett.*, 2000, **41**, 7013; (*c*) F. S. Gibson, S. C. Bermeier and H. Rapoport, *J. Org. Chem.*, 1994, **59**, 3216.

33 (*a*) A. Isidro-Llobet, M. Álvarez and F. Albericio, *Chem. Rev.*, 2009, **109**, 2455; (*b*) A. Loffet and H. X. Zhang, *Int. J. Pept. Protein Res.*, 1993, **42**, 346.

34 J. S. Grimley, A. M. Sawayama, H. Tanaka, M. M. Stohlmeyer, T. F. Woiwode and T. J. Wandless, *Angew. Chem., Int. Ed.*, 2007, **46**, 8157.

35 (*a*) M. Dessolin, M.-G. Guillerez, N. Thieriet, F. Guibé and A. Loffet, *Tetrahedron Lett.*, 1995, **36**, 5741; (*b*) S. Han and S. J. Miller, *J. Am. Chem. Soc.*, 2013, **135**, 12414.

36 (*a*) H. Kunz and J. März, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1375; (*b*) C. Unverzagt and H. Kunz, *Bioorg. Med. Chem.*, 1994, **2**, 1189.

37 K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee and B. S. Safina, *Angew. Chem., Int. Ed.*, 2005, **44**, 1378.

38 D. Takahashi, T. Yano and T. Fukui, *Org. Lett.*, 2012, **14**, 4514.