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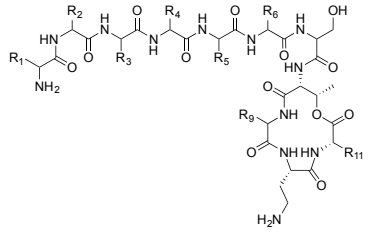

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ABSTRACT

Convergent Ser/Thr ligation has been used to prepare a series of teixobactin analogues (28 in total) to establish a structure-activity relationship of teixobactin. Anti-bacterial evaluations of these synthetic analogues have revealed the critical amino acid residues and the sites tolerable of modifications. These studies will shed lights on the further development of teixobactin analogues with improved antibacterial activities.

1. Introduction

Teixobactin as one of very few new antibiotics against multidrug resistant bacteria discovered in the recent decades has aroused extensive interests. It has a novel structural motif with a unique mode of action against Gram-positive bacteria and *Mycobacterium tuberculosis*, by interacting with the highly conserved motif of lipid II (precursor of peptidoglycan) and lipid III (precursor of the teichoic acid).[1,2] Several research groups including us have devoted a lot of efforts on its total synthesis and analogue synthesis, with an ultimate goal of establishing the structure-activity relationship and of searching for teixobactin analogues with improved antimicrobial activity and pharmacological properties.[3-10]

The primary structure of teixobactin contains a13-membered depsipeptide ring composed of 4 amino acids with a non-proteinogenic enduracidine (L-*allo*-End). An exocyclic tail is composed of 7 amino acid linear chain (**Figure 1**). Two independent total syntheses of teixobactin have been reported by Payne's group[4] and our group in 2016[5], of which our total synthesis featured a convergent strategy 6+7 via chemoselective Ser/Thr ligation[11-13], which would be advantageous for the ~~combinat~~ory synthesis of its analogues. Herein, we reported our

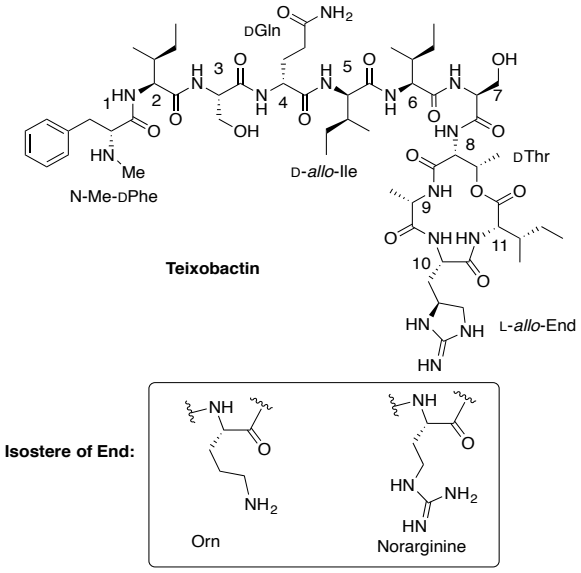

Figure 1. The structures of Teixobactin and its analogues.

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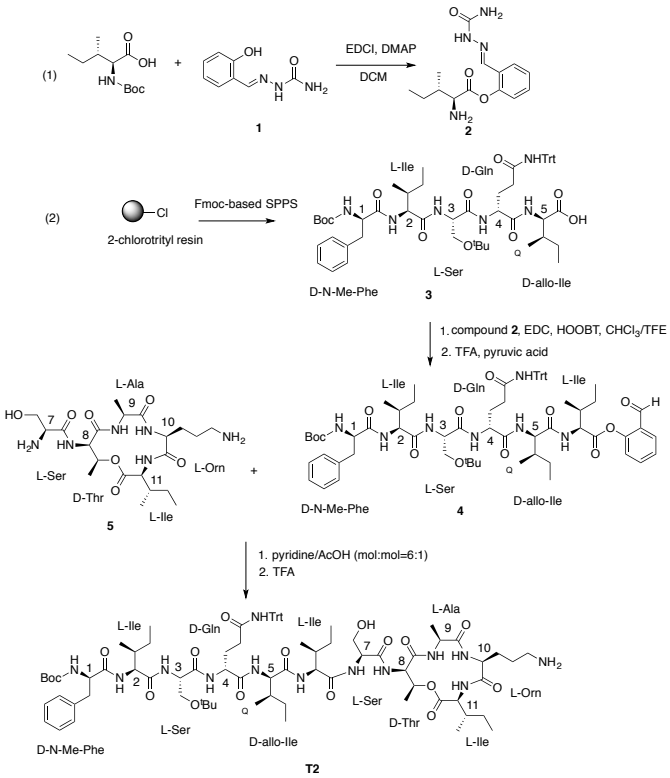
structure-activity relationship studies of synthetic teixobactin analogues, via ligation-based synthetic strategies.

One objective of the teixobactin development as new antibiotics is to search for a simplified structure, which is amenable to large-scale preparation via chemical synthesis. To the end, identification of an effective isostere of L-*allo*-enduracidine (End) will be of importance, because the preparation of End represents a challenge for large-scale synthesis. Albericio et al first reported that teixobactin analogue with Arg to replace End could maintain good antibacterial activities.[3] Several others have confirmed this finding[6-10], while replacement with His caused significant decrease in the antibacterial activity.[10] In our effort, we probed to use L-Orn and L-Norarginie as the isosteres of L-*allo*-End.

2. Results and discussion



Scheme 1. Synthesis of Orn_{10}-teixobactin.

We synthesized all the analogues with our previous serine ligation-mediated convergent 6+5 strategy with some modifications. Previously, the linear hexapeptide (1-6) fragment containing C-terminal salicylaldehyde ester which is requisite for Ser/Thr ligation was synthesized by Boc-solid phase peptide synthesis (Boc-SPPS), in which ozonolysis was needed to convert the alkenyl amide linker into the salicylaldehyde moiety.[14] However, this method was not compatible with Met, Cys and Trp residues. In this study, we adopted the "n+1" strategy recently developed by us[15] to prepare the necessary peptide salicylaldehyde esters. To this end, the linear pentapeptide (1-5) was prepared by Fmoc-SPPS from a 2-chrorotrityl chloride resin. After the synthesis, the side-chain protected peptide was cleaved from the resin with the mild acidic condition of trifluroethanol(TFE)/CH_2Cl_2/AcOH, which was subsequently elongated with Ile-salicylaldehyde semicarbazone ester **2** using EDC/HOOBt as the coupling reagents in $CHCl_3$/TFE, under which condition no epimerization was observed.[15] Next, the global deprotection of the obtained crude peptide with TFA/pyruvic acid afforded the side-chain unprotected peptide salicylaldehyde ester in 43% yield after HPLC isolation. Using this strategy, 20 hexapeptide derivatives with variations of amino acid residues at different positions (1-6) have been prepared with approximate 40%~50% yields.

Seven different cyclic pentapeptide fragments were synthesized according to the previous strategy.[5] The linear peptides were then ligated with the cyclic fragment in pyridine/AcOH (mole: mole, 6:1), followed by acidolysis. All the ligations proceeded smoothly with completion in 12 hours and afforded the teixobactin analogues in approximate 30%~50% yields after HPLC purification. All the obtained teixobactin analogues have been characterized by ESI-MS.

3. Biological studies

All the synthetic teixobactin analogues have been tested against two Gram-positive gram pathogens, including methicillin-susceptible *S. aureus* strain ATCC29213 and a methicillin-resistant *S. aureus* clinical isolate. From these studies, we could see that Orn was an effective isostere of End with only 4 fold decrease in its antibacterial activities. It was surprising to see that the antibacterial activity of $NorgArg_{10}$-teixobactin decreased dramatically, as compared to the Arg_{10}-teixobactin reported in the literature,[3] because the difference between Arg and Norarginine is only one methene group. Thus, we focused on Orn_{10}-teixobactin as the starting point to establish its structure-activity relationship.

Table 1. MICs of teixobactin analogues in μg/mL.

compounds		*MRSA*	*SA*
		SA86	*ATCC29213*
T1	teixobactin	0.5	0.25-0.5
T2	Orn-teixobactin (End10Orn)	2	2
T3	teixobactin (End10NorArg)	16	8-16
T4	Orn-teixobactin NMeDPh1DPhe	16	16
T5	Orn-teixobactin NMeDPhe1NMeLPhe	≥32	≥32
T6	Orn-teixobactin NMeDPhe1NMeDAla	≥32	≥32
T7	Orn-teixobactin Ile2Leu	4-8	4-8
T8	Orn-teixobactin Ile2Val	8-16	8
T9	Orn-teixobactin Ile2D-*allo*Ile	≥32	≥32
T10	Orn-teixobactin Ile2Ala	≥32	≥32
T11	Orn-teixobactin Ile2Thr	≥32	≥32
T12	Orn-teixobactin Ile2Phe	≥32	≥32
T13	Orn-teixobactin Ser3Ala	4	4
T14	Orn-teixobactin Ser3Lys	8	4
T15	Orn-teixobactin Ser3Asn	≥32	16
T16	Orn-teixobactin DGln4LGln	≥32	≥32
T17	Orn-teixobactin DGln4DAsn	4	4
T18	Orn-teixobactin D-*allo*Ile5DVal	16	8-16
T19	Orn-teixobactin D-*allo*Ile5DAla	≥32	≥32
T20	Orn-teixobactin Ile6Ala	≥32	≥32
T21	Orn-teixobactin Ile6Leu	≥32	≥32
T22	Orn-teixobactin Ile6Phe	≥32	≥32
T23	Orn-teixobactin Ile6Val	≥32	≥32
T24	Orn-teixobactin Ala9DAla	≥32	≥32
T25	Orn-teixobactin Ala9Gly	≥32	≥32
T26	Orn-teixobactin Ala9Phe	≥32	≥32
T27	Orn-teixobactin Ala9Orn	8	8
T28	Orn-teixobactin Ile11Leu	≥32	≥32
T29	Orn-teixobactin Ile11Val	8	8

Along our synthesis of Orn-teixobactin analogues, we replaced NMeDPhe$_1$, Ile$_2$, Ser$_3$, DGln$_4$, D-*allo*Ile$_5$, Ile$_6$, Ala$_9$, Ile$_{11}$ with other amino acids including alanine and its structurally similar residue, respectively, to investigate the contribution of each amino acid upon its antibacterial activity. As seen from **Table 1**, substitution of NMeDPhe$_1$ with DPhe (**T4**), NMeLPhe (**T5**), or NMeDAla (**T6**) in teixobactin resulted in analogues with impaired antibacterial activity suggesting that the both Phe residue and its N-methyl group were important for the activity probably due to its stereochemistry and hydrophobicity. Ile$_2$ could tolerate some subtle structural variations such as Leu (**T7**) and Val (**T8**) (2-8 times higher in MICs), while changes of Ile$_2$ to Phe (**T12**), Thr (**T11**), or Ala (**T10**) damaged all the activity. These data suggested that the residue with hydrophobic nature was the best fit for this position probably contributing to interaction with the membrane lipid. However, the residue with bulky hydrophobic side such as Phe might cause hindrance effect on this interaction. In addition, the chirality of amide of Ile$_2$ was important for maintaining the interaction at this site since substitution with D-*allo*Ile (**T9**) abolished the activity completely. Ser$_3$ could be changed to Ala (**T13**) and Lys (**T14**), but not to Asn (**T15**), which indicated the size of the side chain of the amino acid was not critical at this position, while the nature of the side chain might be important. Change DGln$_4$ to DAsn (**T17**) had little negative effect, but not to the stereoisomer LGln (**T16**). Replacement of D-*allo*Ile$_5$ with DVal (**T18**) and DAla (**T19**) demolished the activity. Ile$_6$ is also critical for the antibacterial activity, because change to Ala (**T20**), Leu (**T21**), Phe (**T22**), or Val (**T23**) caused loss of all the activities. When Ala$_9$ was changed to $_D$Ala (**T24**), Phe (**T26**), or Gly (**T25**), no activity was observed, but Ala9Orn analogue (**T27**) maintained the modest activity. That Ile$_{11}$ could be replaced with Val (**T29**) but not with Leu (**T28**) indicated there likely exists a tight binding site (**Table 1**).

4. Conclusion

We have applied Ser/Thr ligation to synthesize 26 Orn$_{10}$-teixobactin analogues in a convergent synthetic manner. From our structure-activity relationship studies and other reports,[6-10] we can learn that (1) the presence of D-amino acids in teixobactin (i.e., DPhe1, DGlin4, D-*allo*-Ile5) and their stereochemistry are critical for teixobactin to process potent antibacterial activity, as replacing them with their L-isomer completely demolished the activity. (2) there appear some very tight interactions between teixobactin and its binding partner, since very subtle structural variation led to complete loss of the antibacterial activities (e.g., **T21**, T**28**) (2) it seems to be difficult to generate teixobactin analogues with improved activities just by simply swapping the amino acid residues present in teixobactin with other natural amino acids. Teixobactin was produced by bacteria for their self-defense purposes, which must have gone through the natural evolution and selection to produce the most optimal structure via choosing the most suitable natural amino acids and involving metabolic enzymatic transformations to convert 20 proteinogenic amino acids to D-amino acids and enduracidine. Thus, to develop teixobactin analogues with improved antibacterial activities and properties, one may need go beyond the natural amino acids replacement and make more dramatic changes. Furthermore, a clear understanding of the mechanisms of action will help the rational design and improvement.

5. Experimental section

5.1 Chemistry

All separations involved a mobile phase of 0.05% TFA (*v/v*) in acetonitrile (solvent A)/0.05% TFA (*v/v*) in water (Solvent B). HPLC separations were performed with a Waters HPLC system equipped with a photodiode array detector (Waters 2996) using a Vydac 218TP^{TM} C18 column (5 µm, 4.6 x 250 mm) at a flow rate of 0.6 mL/min for analytical HPLC, Vydac 218TP^{TM} column (10 µm, 10 x 250 mm) at a flow rate of 4 mL/min for semi – preparative HPLC and Vydac 218TP^{TM} column (10 µm, 22 x 250 mm) at a flow rate of 10 mL/min for preparative HPLC. The peptide synthesis was performed manually on 2-chlorotrityl chloride Resin (resin loading: 0.4 mmol/g). Peptides were synthesized under standard Fmoc/*t*Bu protocols. The deblock mixture was a mixture of 20/80 (*v/v*) of piperidine/DMF.

*5.2 Synthesis of **T2** Orn-teixobactin (End10Orn)*

*5.2.1 Synthesis of the linear hexapeptide salicylaldehyde ester **4***

100 mg 2-chlorotrityl resin (0.5 mmol/g) was placed in a 6 mL polypropylene syringe with a polyethylene filter in the bottom. It was swollen with DCM for 1 hour. Then it was washed by DMF (3 × 2 mL) and DCM (3 × 2 mL). The first building block was added by using Fmoc-D-*allo*-Ile-OH (35.3 mg, 0.1 mmol) and DIEA (34.8 µL, 0.2 mmol) in 2 mL DCM and shook for 1 hour. Then 80 µL MeOH was added and shook for another 20 min. Then the resin was washed by DCM (3 × 2 mL) and DMF (3 × 2 mL). The following amino acids were coupled through the general Fmoc-SPPS strategy. Then the resin was treated by a mixture of 3 mL DCM/AcOH/TFE (*v/v/v*=8:1:1) for 1.5 hours to obtain the crude linear peptide **3** (36.7 mg, 0.037 mmol).

Boc-Ile-OH (1.4 g, 6.1 mmol) was dissolved in 10 mL anhydrous DCM. Then the mixture of compound **1** (0.5 g, 2.8 mmol), EDCI(1.2 g, 6.1 mmol) and DMAP (3.6 mg, 0.3 mmol) in 20 mL anhydrous DCM was added to the solution and stirred at room temperature for 6 hours. The reaction mixture was washed by 1.0 M HCl (20 mL ×3) and brine (20 mL×3). The organic phase was dried with anhydrous Na_2SO_4 and concentrated under low pressure. The crude residue was purified by flash column chromatography on silica gel (hexanes: EtOAc=4:1) to afford compound **2** (0.8 g, 2.7 mmol, 44% yield).

Compound **2** (0.3 g, 1.0 mmol) was dissolved in 2 mL 4N HCl solution (in dioxane) and stirred for 1 hour. The solution of HCl was blown away by a condensed air stream and residue was washed by ethyl ether (20 mL × 3). The crude residue was dissolved in $CHCl_3$/TFE (*v/v*=3:1). Then compound **3** (0.3g, 0.3 mmol), HOOBT (130.5 mg, 0.8 mmol) and EDC (46.6 mg, 0.3 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 6 hours. The mixture of $CHCl_3$/TFE was blown away by a condensed air stream and 2 mL TFA/H2O (*v/v*=95:5) was added for 1hour. Then pyruvic acid (2.1 mL, 30.0 mmol) was added and stirred for another 2 hours. The mixture of TFA and pyruvic acid were blown away by a condensed air stream and residue was washed by ethyl ether (20 mL × 3). The crude compound was dissolved in 10 mL H_2O/CH_3CN(*v/v*=1:1) and filtrated. The residue was purified by preparative HPLC (30-60% CH_3CN/H_2O over 30 min) to afford compound **4** (108.9 mg, 0.13 mmol, 43.3% yield). (ESI) *m/z*; calcd. for $C_{43}H_{64}N_7O_{10}^+$ [M+H$^+$] 838.5, found 838.2.

*5.2.2 Synthesis of **T2** Orn-teixobactin (End10Orn) via serine ligation*

Compound **5** was synthesized by the method reported by our group.[5] Compound **5** (1.8 mg, 3.8 μmol) and peptide SAL ester **4** (4.7 mg, 5.7 μmol, 1.5 equiv) was dissolved in a mixture of 380 μL pyridine/AcOH (mole/mole=6:1) and stirred at room temperature for 10 hours. After the solvent was removed by lyophilization, 1 mL TFA/H_2O/TIPS (*v/v/v*=94:5:1) was added and stirred for 1 hour. TFA/H_2O/TIPS was blown away by a condensed air stream. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T2** as a TFA salt (1.7 mg, 1.41 μmol, 36.8% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^+$ [M+H$^+$] 1202.7, found 1202.8.

*5.3 Synthesis of **T3-29** Teixobactin analogues*

*5.3.1 Synthesis of **T3** Teixobactin (End10NorArg) via serine ligation*

Compound **T3** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T3** as a TFA salt (1.8 mg, 1.46 μmol, 44.2% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{15}O_{15}^+$ [M+H$^+$] 1230.7, found 1230.8.

*5.3.2 Synthesis of **T4** Orn-teixobactin (NMeDPh1DPhe) via serine ligation*

Compound **T4** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T4** as a TFA salt (2.0 mg, 1.68 μmol, 36.8% yield). (ESI) *m/z*; calcd. for $C_{56}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1188.7, found 1188.7.

*5.3.3 Synthesis of **T5** Orn-teixobactin (NMeDPhe1NMeLPhe) via serine ligation*

Compound **T5** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T5** as a TFA salt (1.8 mg, 1.49 μmol, 39.2% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^+$ [M+H$^+$] 1202.7, found 1202.8.

*5.3.4 Synthesis of **T6** Orn-teixobactin (NMeDPhe1NMeDAla) via serine ligation*

Compound **T6** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T6** as a TFA salt (1.9 mg, 1.69 μmol, 44.5% yield). (ESI) *m/z*; calcd. for $C_{51}H_{92}N_{13}O_{15}^+$ [M+H$^+$] 1126.7, found 1126.6.

*5.3.5 Synthesis of **T7** Orn-teixobactin (Ile2Leu) via serine ligation*

Compound **T7** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T7** as a TFA salt (1.8 mg, 1.4 μmol, 39.4% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^+$ [M+H$^+$] 1202.7, found 1202.8.

*5.3.6 Synthesis of **T8** Orn-teixobactin (Ile2Val) via serine ligation*

Compound **T8** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T8** as a TFA salt (1.7 mg, 1.4 μmol, 37.7% yield). (ESI) *m/z*; calcd. for $C_{56}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1188.7, found 1188.7.

*5.3.7 Synthesis of **T9** Orn-teixobactin (Ile2D-alloIle) via serine ligation*

Compound **T9** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T9** as a TFA salt (2.2 mg, 1.8 μmol, 47.4% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^+$ [M+H$^+$] 1202.7, found 1202.8.

*5.3.8 Synthesis of **T10** Orn-teixobactin (Ile2Ala) via serine ligation*

Compound **T10** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T10** as a TFA salt (2.0 mg, 1.7 μmol, 44.7% yield). (ESI) *m/z*; calcd. for $C_{54}H_{90}N_{13}O_{15}^+$ [M+H$^+$] 1160.7, found 1160.7.

*5.3.9 Synthesis of **T11** Orn-teixobactin (Ile2Thr) via serine ligation*

Compound **T11** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T11** as a TFA salt (1.6 mg, 1.3 μmol, 34.2% yield). (ESI) *m/z*; calcd. for $C_{55}H_{92}N_{13}O_{16}^+$ [M+H$^+$] 1190.7, found 1190.6.

*5.3.10 Synthesis of **T12** Orn-teixobactin (Ile2Phe) via serine ligation*

Compound **T12** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T12** as a TFA salt (1.8 mg, 1.5 μmol, 38.3% yield). (ESI) *m/z*; calcd. for $C_{60}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1236.7, found 1236.7.

*5.3.11 Synthesis of **T13** Orn-teixobactin (Ser2Ala) via serine ligation*

Compound **T13** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T13** as a TFA salt (1.5 mg, 1.3 μmol, 33.3% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{14}^+$ [M+H$^+$] 1186.7, found 1186.6.

*5.3.12 Synthesis of **T14** Orn-teixobactin (Ser2Lys) via serine ligation*

Compound **T14** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T14** as a TFA salt (1.2 mg, 0.96 μmol, 25.4% yield). (ESI) *m/z*; calcd. for $C_{60}H_{103}N_{14}O_{14}^+$ [M+H$^+$] 1243.8, found 1243.9.

*5.3.13 Synthesis of **T15** Orn-teixobactin (Ser2Asn) via serine ligation*

Compound **T15** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T15** as a TFA salt (2.0 mg, 1.6 μmol, 42.8% yield). (ESI) *m/z*; calcd. for $C_{58}H_{97}N_{14}O_{15}^+$ [M+H$^+$] 1229.7, found 1229.7.

*5.3.14 Synthesis of **T16** Orn-teixobactin (DGln4LGln) via serine ligation*

Compound **T16** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T16** as a TFA salt (1.9 mg, 1.6 μmol, 41.6% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^+$ [M+H$^+$] 1202.7, found 1202.7.

*5.3.15 Synthesis of **T17** Orn-teixobactin (DGln4DAsn) via serine ligation*

Compound **T17** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T17** as a TFA salt (1.7 mg, 1.4 μmol, 37.6% yield). (ESI) *m/z*; calcd. for $C_{56}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1188.7, found 1188.7.

*5.3.16 Synthesis of **T18** Orn-teixobactin (D-alloIle5DVal) via serine ligation*

Compound **T18** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T18** as a TFA salt (1.8 mg, 1.5 μmol, 39.9% yield). (ESI) *m/z*; calcd. for $C_{56}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1188.7, found 1188.8.

*5.3.17 Synthesis of **T19** Orn-teixobactin (D-alloIle5DAla) via serine ligation*

Compound **T19** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T19** as a TFA salt (2.0 mg, 1.7 μmol, 45.4% yield). (ESI) *m/z*; calcd. for $C_{54}H_{90}N_{13}O_{15}^+$ [M+H$^+$] 1160.7, found 1160.6.

*5.3.18 Synthesis of **T20** Orn-teixobactin (Ile6Ala) via serine ligation*

Compound **T20** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T20** as a TFA salt (1.8 mg, 1.6 μmol, 40.8% yield). (ESI) *m/z*; calcd. for $C_{54}H_{90}N_{13}O_{15}^+$ [M+H$^+$] 1160.7, found 1160.7.

*5.3.19 Synthesis of **T21** Orn-teixobactin (Ile6Leu) via serine ligation*

Compound **T21** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T21** as a TFA salt (1.9 mg, 1.6 μmol, 41.6% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^+$ [M+H$^+$] 1202.7, found 1202.8.

*5.3.20 Synthesis of **T22** Orn-teixobactin (Ile6Phe) via serine ligation*

Compound **T22** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T22** as a TFA salt (2.2 mg, 1.8 μmol, 46.8% yield). (ESI) *m/z*; calcd. for $C_{60}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1236.7, found 1236.7.

*5.3.21 Synthesis of **T23** Orn-teixobactin (Ile6Val) via serine ligation*

Compound **T23** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T23** as a TFA salt (1.3 mg, 1.1 μmol, 28.8% yield). (ESI) *m/z*; calcd. for $C_{56}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1188.7, found 1188.6.

*5.3.22 Synthesis of **T24** Orn-teixobactin (Ala9DAla) via serine ligation*

Compound **T24** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T24** as a TFA salt (1.5 mg, 1.3 μmol, 34.2% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^+$ [M+H$^+$] 1202.7, found 1202.8.

*5.3.23 Synthesis of **T25** Orn-teixobactin (Ala9Gly) via serine ligation*

Compound **T25** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T25** as a TFA salt (1.7 mg, 1.4 μmol, 36.8% yield). (ESI) *m/z*; calcd. for $C_{56}H_{94}N_{13}O_{15}^+$ [M+H$^+$] 1188.7, found 1188.7.

*5.3.24 Synthesis of **T26** Orn-teixobactin (Ala9Phe) via serine ligation*

Compound **T26** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T26** as a TFA salt (1.7 mg, 1.3 μmol, 35.0% yield). (ESI) *m/z*; calcd. for $C_{63}H_{100}N_{13}O_{15}^+$ [M+H$^+$] 1278.7, found 1278.8.

*5.3.25 Synthesis of **T27** Orn-teixobactin (Ala9Orn) via serine ligation*

Compound **T27** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T27** as a TFA salt (1.4 mg, 1.1 μmol, 29.6% yield). (ESI) *m/z*; calcd. for $C_{59}H_{101}N_{14}O_{15}^+$ [M+H$^+$] 1245.7, found 1245.7.

*5.3.26 Synthesis of **T28** Orn-teixobactin (Ile11Leu) via serine ligation*

Compound **T28** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T28** as a

TFA salt (1.9 mg, 1.6 μmol, 41.6% yield). (ESI) *m/z*; calcd. for $C_{57}H_{96}N_{13}O_{15}^{+}$ [M+H^{+}] 1202.7, found 1202.8.

*5.3.27 Synthesis of **T29** Orn-teixobactin (Ile11Val) via serine ligation*

Compound **T29** was synthesized through the same method as compound **T2**. The residue was purified by preparative HPLC (20-60% CH_3CN/H_2O over 30 min) to afford compound **T29** as a TFA salt (1.8 mg, 1.5 μmol, 39.8% yield). (ESI) *m/z*; calcd. for $C_{56}H_{94}N_{13}O_{15}^{+}$ [M+H^{+}] 1188.7, found 1188.6.

5.4 Antibacterial studies

Susceptibility to teixobactin and its analogues was tested on the selected Gram-positive strains using Tecan Freedom EVO high-throughput automated platform, following the standard broth dilution method as described by the Clinical and Laboratory Standards Institute. MICs were determined according to CLSI guideline.[16]

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References and notes

1. Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schaberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K. *Nature* **2015**, *517*, 455-459.
2. Franz, N; Roderich, D. S. *Angew. Chem. Int. Ed*. **2015**, *54*, 6684-6686.
3. Jad, Y. E.; Acosta, G. A.; Naicker, T.; Ramtahal, M.; Ei-Faham, A.; Govender, T.; Kruger, H. G.; Torre, B. G.; Albericio, F. *Org. Lett.* **2015**, *17*, 6182-6185.
4. Giltrap, A. M.; Dowman, L. J.; Nagalingam, G.; Ochoa, J. L.; Linington, R. G.; Britton, W. J.; Payne, R. J. *Org. Lett.* **2016**, *18*, 2788-2791.
5. Jin, K.; Sam, I. H.; Po, H. L.; Lin, D.; Ebrahim, H. G. Z; Chen, S.; Yuan, Y.; Li, X. *Nature Commun*. **2016**, *7*, 12394.
6. Abdel, M. S. A.; Jad, Y. E.; Ramchuran, E. J.; EI-Faham, A.; Govender, T.; Kruger, H. G.; de la Torre, B. G.; Albericio, F. *ACS Omega*, **2016**, *1*, 1262-1265.
7. Yang, H.; Chen, K. H.; Nowick, J. S. *ACS Chem. Bio.* **2016**, *11*, 1823-1826.
8. Parmar, A.; Iyer, A.; Vincent, C. S.; Lysebetten, D. V.; Prior, S. H.; Madder, A.; Taylor, E. J.; Singh, I. *Chem. Commun*. **2016**, *52*, 6060-6063.
9. Yang, H.; Bois, D. R. D.; Ziller, J. W.; Nowick, J. S. *Chem. Commun*. **2017**, *53*, 2772-2775.
10. Wu, C.; Pan, Z.; Yao, G.; Wang, W.; Fang, L.; Su, W. *RSC Advances*. **2017**, *7*, 1923-1926.
11. a) Li, X.; Lam, H. Y.; Zhang, Y.; Chan, C. K. *Org. Lett*. **2010**, *12*, 1724-1727. b) Zhang, Y.; Xu, C.; Lam, H. Y.; Lee, C. L.; Li, X. *Proc. Natl. Acad. Sci. U. S. A*. **2013**, *110*, 6657-6662. c) Li, T.; Liu, H.; Li, X. O*rg. Lett*. **2016**, *18*, 5944-5947
12. Lam, H. Y.; Li, X. *Synlett.* **2014**, *25*, 1339-1344.
13. a) Zhang, Y.; Li, T.; Li, X. *Org. Biomol. Chem.***2013**, *11*, 5584-5587. b) Lee, C. L.; Li, X. *Curr. Opin. Chem. Biol*. **2014**, *22*, 108-114. c) Lee, C. L.; Li, X. *Sci. China Chem*. **2016**, *59*,1061-1064.
14. Wong C. T., Lam H. Y., Song T., Chen. G, Li X. *Angew. Chem. Int. Ed.* **2013**, *52*, 10212-10215.
15. Lee, C. L.; Liu, H.; Wong, C. T.; Chow, H. Y.; Li, X. *J. Am. Chem. Soc.* **2016**, *138*, 10477-10484.
16. CLSI. CLSI document M100-S26, Wayne, PA: Clinical and Laboratory Standards Institute (2016).

Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.