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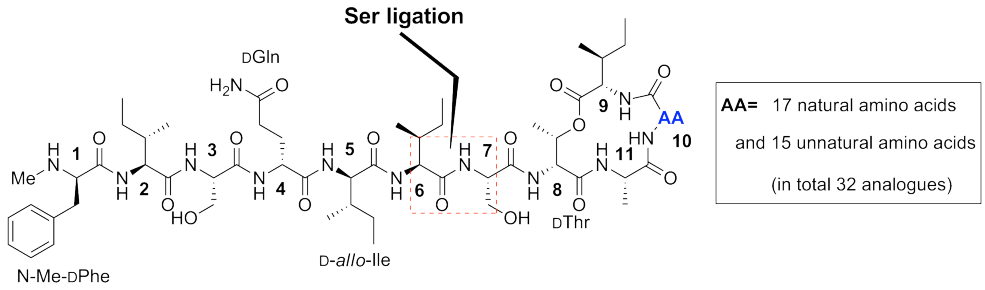

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ABSTRACT

Teixobactin is a structurally and mechanistically novel antimicrobial peptide with potent activities against Gram-positive pathogens. It contains L-*allo*-enduracididine (End) residue which is not readily accessible. In this report, we have used convergent Ser Ligation as the key step to prepare a series of teixobactin analogues with End being substituted with its non-isostere moieties. Among these analogues, compounds **T16**, **T27** and **T29** exhibited the best antimicrobial activities against different Gram-positive bacteria with MICs ranging from 0.25 to 1.0 μM. Structure-activity relationship is also established for further development of more promising teixobactin analogues.

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1. Introduction

Due to the emergence and rapid spread of new antimicrobial resistance (AMR) mechanisms, infectious diseases have become a worldwide challenge, which could lead to chronic illness, disability or even death. It is predicted that the global death toll of microorganism infections will be exceed 10 million by 2050.[1,2] However, the urgent need of new antibiotics can not be met by the slow development. For the past decade, only one new antibiotic, named daptomycin, has been developed into clinic treatments. In 2015, the discovery of a new class of antimicrobial peptide (AMP), teixobactin, attracted the wide attentions in the world due to its promising activity with a distinct mechanism against multi-drug resistant bacteria, such as *mycobacterium tuberculosis* and Methicillin-resistant *staphylococcus aureus*

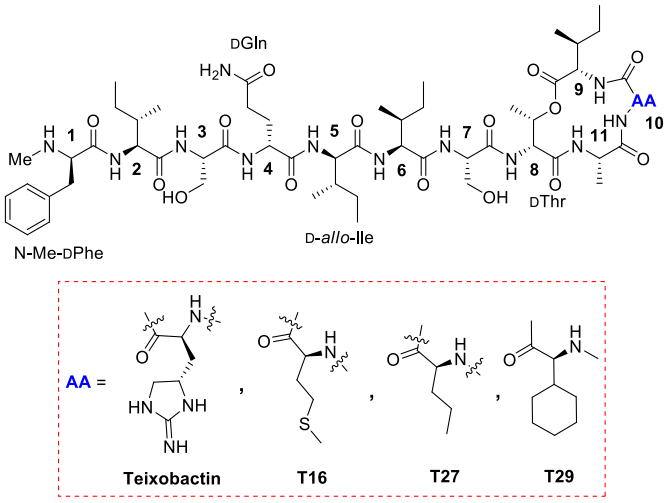
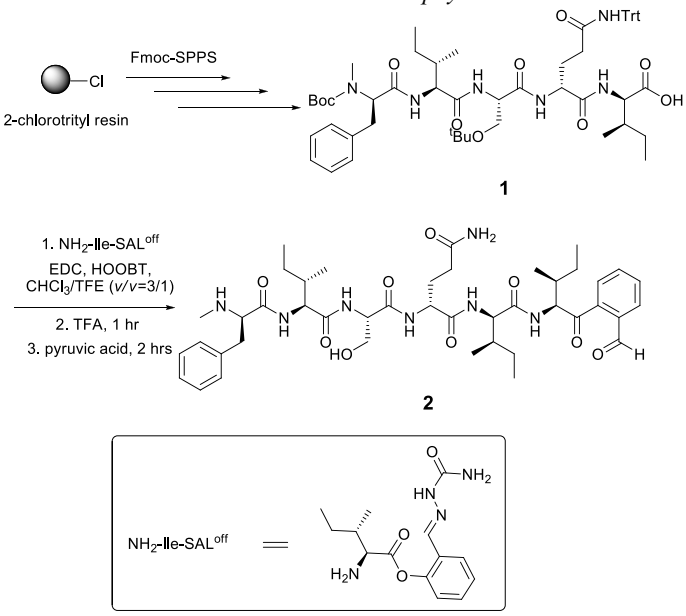


Figure 1. The structures of Teixobactin and its analogues



Scheme 1. Synthesis of the linear peptide (1-6) salicylaldehyde

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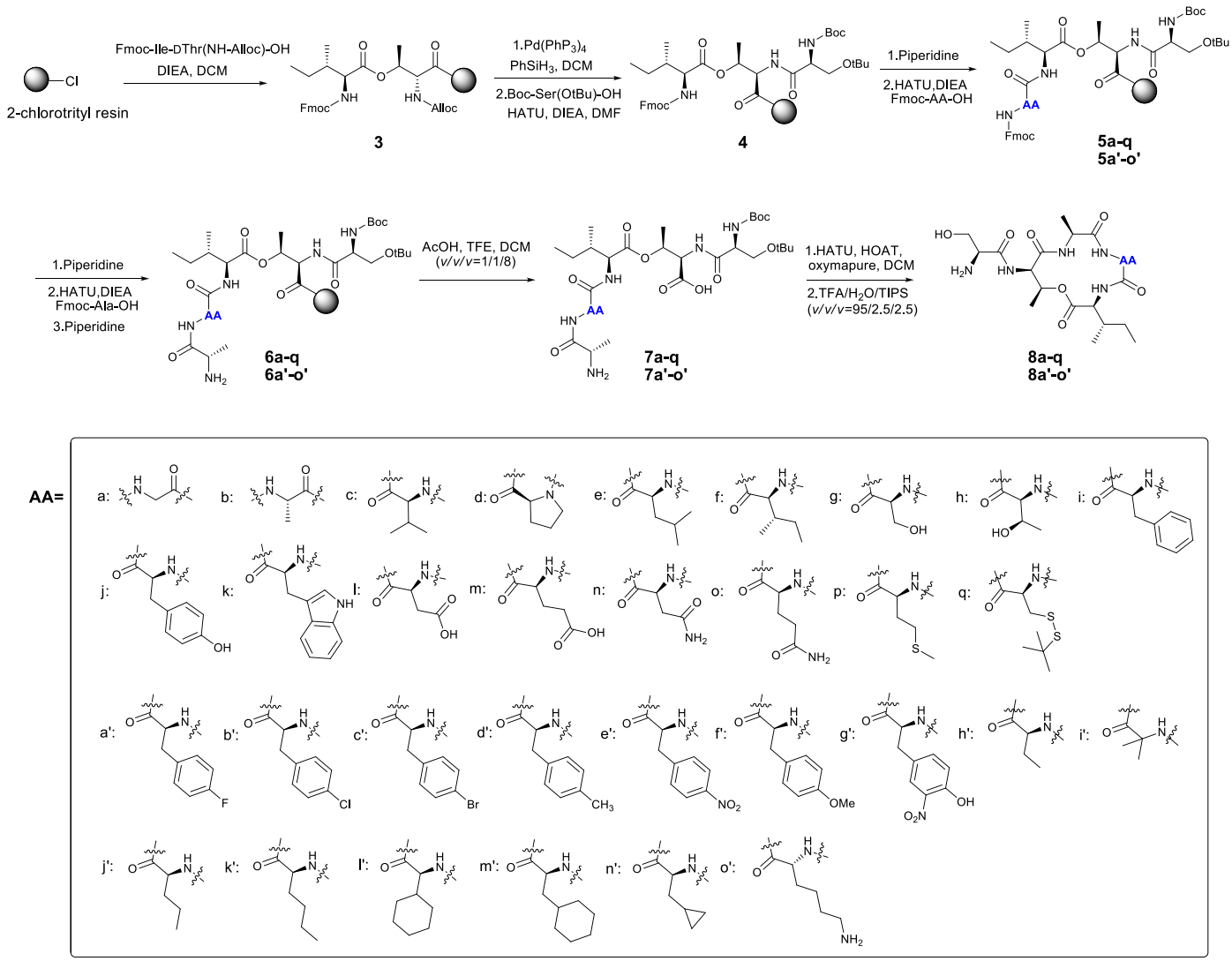


Scheme 2. Synthesis of the cyclic fragments 8a-q and 8a'-8o'

(MRSA).[3] The low probability of resistance development against teixobactin is due to its inhibition on lipid II (needed for peptidoglycan) and lipid III (precursor of the teichoic acid synthesis), but not on an enzyme.[3-5]

Since the structural characterization and antibacterial activities of teixobactin was first reported, several research groups have made significant contributions for its pharmacophore study. Structurally, teixobactin contains a 4-amino acid depsipeptide ring and a 7-amino acid exocyclic tail, of which four D-amino acids and one unusual amino acid L-*allo*-enduracididine (End) are present (**Figure 1**).[3] Along together with the completion of the total synthesis of teixobactin by Payne's group[6] and our group[7] independently, a large amount of teixobactin analogues have been reported gradually. As L-*allo*-enduracididine is not readily accessible, many efforts have been directed to identify the effective isostere building block to replace the positively charged End at position 10, with which to further establish its structure-activity relationship. For instance, Lys_{10}-teixobactin[8-12], Arg_{10}-teixobactin[13-18] and Orn_{10}-teixobactin[7] are active against Gram-positive bacteria, but with reduced antibacterial activities compared to teixobactin. With these lead analogues, previous study[7, 19, 20] also indicated that the replacement of any D-amino acid in teixobactin with their L-isomers would lead to total loss of the activity. It was also suggested that the substitution of amino acid residues at position 1-9 and 11 by other structurally similar natural amino acids would partially or completely demolish the activity.[19]

During our curiosity undertaking to study whether the End or its positively charged isoteres are necessary for their antibacterial activities, very recently, Nowick et al. reported an alanine scan study of teixobactin and found that a hydrophilic cationic side-chain at position 10 is not crucial to its antibacterial activity.[9] Singh and his co-workers also reported Leu/Ile_{10}-teixobactin analogues exhibited equivalent activities as teixobactin.[21] Herein, we report our work on the synthesis and antibacterial studies of teixobactin analogues with End being replaced with 32 non-isostere amino acids, including 17 proteinogenic amino acids and 15 commercially available non-proteinogenic amino acids.

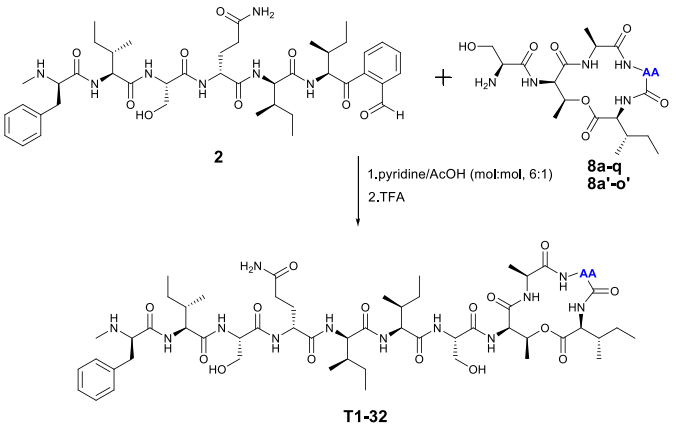
2. Results and discussion

Previously, we developed a convergent "6 + 5" strategy *via* chemoselective Serine ligation[22-24] for the total synthesis of teixobactin and Orn_{10}-teixobactin analogues. We applied the same strategy to synthesize these analogues. Synthetic efforts began with the preparation of the linear hexapeptide (1-6) fragment containing a salicylaldehyde ester at the C-terminal (**Scheme 1**). Previously, the salicylaldehyde moiety was obtained

by ozonolysis of an alkenyl amide linker followed with Boc-solid phase peptide synthesis (Boc-SPPS).[7, 25] In this study, we adopted a "n + 1" strategy reported by our group[26] recently to prepare this hexapeptide salicylaldehyde ester. Beginning with 2-chlorotrityl chloride resin, the fully protected linear pentapeptide (1-5) **1** was prepared using Fmoc strategy for solid phase peptide synthesis (Fmoc-SPPS) and cleaved from the resin by a mild trifluroethanol(TFE)/CH_2Cl_2/AcOH condition. Coupling to Ile-salicylaldehyde semicarbazone ester (NH_2-Ile-SAL^{off}) was achieved by using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3-Hydroxy-1,2,3-benzotriazin-4(3H)-one (HOOBT) in a $CHCl_3$/TFE mixture solution. Finally, all protected groups and semicarbazone moiety were removed *via* treatment of TFA and pyruvic acid, to afford the hexapeptide (1-6) salicylaldehyde ester **2** in a 51% yield after HPLC purification.

Next, we turned our attention to the synthesis of the 13-membered depsicyclic ring fragment in which L-*allo*-enduracididine was replaced by different natural or unnatural amino acid residues (**Scheme 2**). The synthesis started from loading Fmoc-Ile-DThr(NH-Alloc)-OH to the 2-chlorotrityl chloride resin as the first building block. Next, the resin-bound **3** was treated with $Pd(PPh_3)_4$/$PhSiH_3$ to remove Alloc group, followed by the coupling of Boc-Ser(OtBu)-OH to afford **4**. Upon removal of Fmoc group, the subsequent steps involved coupling different Fmoc-protected End-substitutes and Fmoc-Ala-OH *via* standard Fmoc-SPPS strategy. Following the successful deprotection of the last Fmoc group, crude peptides **7a-q and 7a'-o'** were cleaved from the resin by the mildly acid condition as mentioned before. The cyclization of these linear side-chain-protected depsipeptides proceeded smoothly using HATU/HOAt/OxymaPure as coupling reagents. The global deprotection of the crude cyclic peptides with TFA/TIPS/H_2O (*v/v/v*=95:2.5:2.5) afforded the compounds **8a-q and 8a'-o'** in approximate 30-50% yields after purification.

Finally, the cyclic fragments were then ligated with the linear peptide salicylaldehyde ester **2** respectively to afford the teixobactin analogues **T1-T32** in approximate 40-50% yields after HPLC isolation (**Scheme 3**).



Scheme 3. Synthesis of teixobactin analogues T1-32 *via* Ser ligation.

3. Biological studies

Having successfully synthesized the target teixobactin analogues, we next evaluated their antibacterial activities against two Gram-positive gram pathogens including methicillin-susceptible *S. aureus* strain ATCC29213 and a new methicillin-resistant *S. aureus* clinical isolate. Of the seventeen analogues in which L-*allo*-enduracididine was replaced by natural amino acid residues, one (**T16**) showed a 2-times better antibacterial activity than teixobactin, three (**T3**, **T5**, **T6**) gave comparable activities, five (**T1**, **T2**, **T9**, **T10**, **T17**) exhibited 2-4-times lower activities and eight (**T4**, **T7**, **T8**, **T11**, **T12**, **T13**, **T14**, **T15**) had very weak or even no activities (**Table 1**). From these results, we could see that a cationic side chain with a positive charge as in End at position 10 was not necessary for the potent antibacterial activity of teixobactin. L-Val_{10}-teixobactin (**T3**), L-Leu_{10}-teixobactin (**T5**) and L-Ile_{10}-teixobactin (**T6**) containing hydrophobic and uncharged side chains exhibited comparable activities to teixobactin. Similarly structural L-Met_{10}-teixobactin (**T16**) with one sulfur atom in the side chain showed a slightly superior result with MIC values of 1 and 0.25 μg mL^{-1} against MRSA and SA respectively. However, much smaller (**T1**, **T2**, **T4**) or aromatic (**T9**, **T10**, **T11**) hydrophobic side chains reduced somewhat or even abolished the activity completely. On the contrary, replacement of L-*allo*-$enduracididine_{10}$ with Asp (**T12**) or Glu (**T13**) containing negatively charged hydrophilic side chains led to complete demolishment of the activity. Some other hydrophilic residues such as Ser (**T7**), Thr (**T8**), Asn (**T14**) or Gln (**T15**), also led to the reduced activities.

Table 1. MICs of teixobactin and its analogues in μg/ml

Compounds		MRSA (SA11I4)	SA (ATCC29213)
T1	L-Gly_{10}-teixobactin	8~16	2
T2	L-Ala_{10}-teixobactin	8	2
T3	L-Val_{10}-teixobactin	2	0.5
T4	L-Pro_{10}-teixobactin	≥32	≥32
T5	L-Leu_{10}-teixobactin	2	0.5
T6	L-Ile_{10}-teixobactin	1	0.5
T7	L-Ser_{10}-teixobactin	16	16
T8	L-Thr_{10}-teixobactin	16	8
T9	L-Phe_{10}-teixobactin	4	1
T10	L-Tyr_{10}-teixobactin	8	2
T11	L-Trp_{10}-teixobactin	8~16	8
T12	L-Asp_{10}-teixobactin	≥32	≥32
T13	L-Glu_{10}-teixobactin	≥32	≥32
T14	L-Asn_{10}-teixobactin	16	8
T15	L-Gln_{10}-teixobactin	16	16
T16	L-Met_{10}-teixobactin	1	0.25
T17	L-$Cys(StBu)_{10}$-teixobactin	4	2
T18	L-$Phe(4\text{-}F)_{10}$-teixobactin	4	0.5
T19	L-$Phe(4\text{-}Cl)_{10}$-teixobactin	4	1
T20	L-$Phe(4\text{-}Br)_{10}$-teixobactin	≥32	2
T21	L-$Phe(4\text{-}CH_3)_{10}$-teixobactin	≥32	1
T22	L-$Phe(4\text{-}NO_2)_{10}$-teixobactin	4	0.5
T23	L-$Tyr(OMe)_{10}$-teixobactin	≥32	1
T24	L-$Tyr(3\text{-}NO_2)_{10}$-teixobactin	4	1
T25	L-Abu_{10}-teixobactin	4	1
T26	L-Aib_{10}-teixobactin	≥32	≥32
T27	L-Nva_{10}-teixobactin	1~2	0.25
T28	L-NIe_{10}-teixobactin	2	0.5
T29	L-Chg_{10}-teixobactin	1	0.25
T30	L-Cha_{10}-teixobactin	2	0.5
T31	L-β-cyclopropyl-Ala_{10} -teixobactin	2	0.5
T32	D-Lys_{10}-teixobactin	≥32	≥32
TC	teixobactin	2	0.5

In addition, we prepared a series of teixobactin analogues substituting L-*allo*-enduracididine$_{10}$ with a series of commercially available unnatural amino acids (**T18**-**T32**). Among these compounds, L-Phe(4-F)$_{10}$-teixobactin (**T18**), L-Phe(4-Cl)$_{10}$-teixobactin (**T19**), L-Phe(4-NO_2)$_{10}$-teixobactin (**T22**) and L-Tyr(3-NO_2)$_{10}$-teixobactin (**T24**) showed comparable or a little improved activities than **T9**. The possible reason is that the electron-withdrawing substituent groups resulted in weaker aromaticity of the side phenyl structure. Contrary to this, electron-donating substituent groups on the aromatic ring, such as -Br (**T20**), -CH_3 (**T21**), -OCH_3 (**T23**), completely demolished the activity against MRSA (**Table 1**). The different lengths of hydrophobic side chains also have a significant impact on the anti-bacterial activities. L-Abu$_{10}$-teixobactin (**T25**) containing a 2-carbon side chain exhibited 2-times higher activity than L-Ala$_{10}$-teixobactin (**T2**), but 4-times lower than L-Nva$_{10}$-teixobactin (**T27**) with a 3-carbon linear side chain. L-NIe$_{10}$-teixobactin (**T28**) with a longer chain (four carbons) also showed a comparable activity to teixobactin, but 2-times lower than **T27**. These data suggested that the most suitable length of the side chain was three carbons. Surprisingly, replacement of L-*allo*-enduracididine$_{10}$ with L-Chg$_{10}$ (**T29**) exhibited a very potent anti-bacterial activity. **T30** and **T31** with a longer length or a different ring size from **T29**, also showed essentially the comparable activity as teixobactin. When L-*allo*-enduracididine$_{10}$ was changed to Aib (**T26**) or D-Lys (**T32**), no activity was observed, which indicated that the stereochemistry at position 10 was critical for the potent antibacterial activity, and could not be replaced by its D-isomers.

4. Conclusion

In summary, 32 teixobactin analogues with End non-isostere substitution have been prepared and tested, of which three analogues (**T16**, **T27**, **T29**) exhibited very promising anti-bacterial activities against methicillin-susceptible *S. aureus* strain ATCC29213 and a new methicillin-resistant *S. aureus* clinical isolate. Our structure-activity relationship studies suggested that the residues with uncharged hydrophobic nature were suitable for the position 10 to replace the synthetically challenging L-*allo*-enduracididine. Moreover, aliphatic residues were better fit for this site than aromatic ones. Although it remains to investigate whether these analogues with hydrophobic residues to replace the positively charged End would have the same mechanism as teixobactin by binding to Lipid II/III, these compounds provide the new possibility to search for new antibiotics for clinical development.

5. Experimental section

5.1. Chemistry

Fmoc-Ile-DThr(NH-Alloc)-OH, Boc-D-*N*-methyl-Phe-OH and Boc-Ile-SAL^{off} were prepared as reported[7, 26]. All the commercial amino acids and coupling reagents were used without further purification. All HPLC grade (DUKSAN) and analytical grade (RCI) solvents were used as received unless otherwise noted. Anhydrous dichloromethane (DCM) was distilled in the presence of calcium hydride (CaH_2). Analytical reverse-phase HPLC was performed on a waters system equipped with a Vydac 218TP^{TM} C18 column (5μm, 4.6×250 mm) using specified linear gradients of acetonitrile (0.1% TFA) in water (0.1% TFA) with a flow rate of 0.6 mL/min. Preparative reverse-phase HPLC was performed on a Waters system equipped with a Vydac 218TP^{TM} C18 column (10μm, 10×250 mm) using specified linear gradients of acetonitrile (0.1% TFA) in water (0.1% TFA) with a flow rate of 10.0 mL/min.

5.2. General Fmoc-SPPS (solid phase peptide synthesis) procedure.

100 mg 2-chlorotrityl resin (0.5 mmol/g) was swollen in dry DCM for 30 min and treated with the first building block (2.0 equiv) and DIEA (4.0 equiv) in dry DCM. After it was shook for 1hour, 80μL MeOH was added to cap the unreacted resin for another 20 min. The loaded resin was washed by DCM (3 × 2 mL) and DMF (3 × 2 mL). Fmoc deprotection was achieved by shaken with 2mL 20% solution of piperidine in DMF for 20 min. The following Fmoc- or Boc- amino acids (4.0 equiv) was coupled using HATU (4.0 equiv) as coupling reagent and DIEA (8.0 equiv) as base. The mixture was shaken in DMF for 1hour. After each Fmoc deprotection and coupling reaction, the resin was washed by DMF (3 × 2 mL), DCM (3 × 2 mL) and DMF (3 × 2 mL).

5.3. Alloc deprotection.

The loaded resin was washed by DCM (3 × 2 mL) and then a solution of $Pd(PPh_3)_4$ (1.0 equiv) and phenylsilane (25 equiv) in 2 mL anhydrous DCM was added. The mixture was shaken for 1 hour under the protection of dry argon. After Alloc deprotection was completed, the resin was washed by DMF (3 × 2 mL), DCM (3 × 2 mL) and DMF (3 × 2 mL).

5.4. Peptide cleavage.

After coupling of the last building block, the resin was washed by DCM (3 × 2 mL), DMF (3 × 2 mL) and DCM (5 × 2 mL). Then a cocktail of DCM/AcOH/TFE(*v*/*v*/*v*=8:1:1) was added to the resin and shaken for 1.5 hours. Then the resin was filtrated off and rinsed with DCM (5 × 2 mL). The combined filtrates were concentrated under low pressure and azeotroped several times with DCM to remove the Acetic acid. The side-chain-protected peptides were obtained as white solid.

5.5. Cyclization and Side chain deprotection.

The side-chain-protected peptides (1.0 equiv) was dissolved in anhydrous DCM at a concentration of 0.1 mmol/L. A solution of HOAT (6.0 equiv), Oxyma pure (6.0 equiv) and DIEA (12.0 equiv) in anhydrous DCM was added and stirred at 0^{o}C for 15 min. Then HATU (10.0 equiv) was added. The resulting reaction mixture was warmed up to room temperature slowly and continued to be stirred for 24 hours. Complete cyclization reactions were confirmed by LC-MS monitoring.

DCM was evaporated in vacuo and the residue was treated with a cocktail of TFA/TIPS/H_2O (*v*/*v*/*v*=95:2.5:2.5) for 1.5 hours. TFA/TIPS/H_2O was blown away by a condensed air stream and the residue was precipitated and washed using cold ethyl ether (20 mL×3) to yield the crude cyclic unprotected-peptide.

The crude cyclic peptide was dissolved in a CH_3CN/H_2O (*v*/*v*=1:1) solution and purified by preparative HPLC (5-50%

CH_3CN [0.1%TFA] in H_2O [0.1%TFA] over 30 min) to afford pure cyclic peptides.

5.6. Peptide 1-6 SAL ester synthesis.

The peptide 1-6 SAL ester was synthesized through a "n+1" strategy. The "n" (side-chain-protected peptide 1-5) was prepared according to the general Fmoc-SPPS and cleavage procedure.

The compound Boc-Ile-SAL^{off} (1.0 equiv) was dissolved in 4.0N HCl solution in dioxane (10.0 equiv) and stirred for 1 hour at room temperature. Then the solvent was blown away by a condensed air stream and the residue was precipitated and washed using cold diethyl ether (20 mL×3) to yield the crude "1".

The "n" (1.0 equiv) and "1" (3.0 equiv) were dissolved in a mixture of $CHCl_3$/TFE (*v*/*v*=3:1), then EDC (3.0 equiv) and HOOBT (3.0 equiv) were added as coupling reagent. The resulting reaction mixture was stirred at room temperature for 6 hours. After the completion of coupling reaction, $CHCl_3$/TFE was blown off under a stream of condensed air. The residue was treated by TFA/TIPS/H_2O (*v*/*v*/*v*=95:2.5:2.5) for 1 hour and pyruvic acid (100.0 equiv) for another 2 hours. The resulting mixture was triturated with cold diethyl ether to give a suspension. After centrifugation, the crude peptide was dissolved in a CH_3CN/H_2O (*v*/*v*=1:1) solution and purified by preparative HPLC (30-80% CH_3CN [0.1%TFA] in H_2O [0.1%TFA] over 30 min) to afford pure peptide 1-6 SAL ester as a white solid.

5.7. Ser Ligation.

The cyclic peptides (1.0 equiv) and peptide 1-6 SAL eater (1.2 equiv) were dissolved in a mixture of pyridine/AcOH (mol:mol=6:1) at a concentration of 10.0 mmol/L. The reaction mixture was stirred at room temperature for 10 hours. After the pyridine/AcOH was removed by lyophilization, the residue was treated with TFA/TIPS/H_2O (*v*/*v*/*v*=95:2.5:2.5) for 1 hour. Then the crude peptide was blown-dry under a stream of condensed air and purified by preparative HPLC (20-60% CH_3CN [0.1%TFA] in H_2O [0.1%TFA] over 30 min) to afford teixobactin analogues as white solid.

*5.8. Synthesis of **T1-32** Teixobactin analogues*

Teixobactin analogues **T1**-**T32** were synthesized through the same methods as the aforementioned general procedures.

*5.8.1. L-Gly_{10}-teixobactin (**T1**)*. White solid, 2.9 mg, yield 45% (the final step), LRMS(ESI+): calculated for $C_{54}H_{89}N_{12}O_{15}^+$ [M+H^+]: 1145.6; found: 1145.7. See supporting information for the details of LC-MS spectrum.

*5.8.2. L-Ala_{10}-teixobactin (**T2**)*. White solid, 2.7 mg, yield 41% (the final step), LRMS(ESI+): calculated for $C_{55}H_{91}N_{12}O_{15}^+$ [M+H^+]: 1159.7; found: 1159.6. See supporting information for the details of LC-MS spectrum.

*5.8.3. L-Val_{10}-teixobactin (**T3**)*. White solid, 3.1 mg, yield 47% (the final step), LRMS(ESI+): calculated for $C_{57}H_{95}N_{12}O_{15}^+$ [M+H^+]: 1187.7; found: 1187.9. See supporting information for the details of LC-MS spectrum.

*5.8.4. L-Pro_{10}-teixobactin (**T4**)*. White solid, 3.1 mg, yield 48% (the final step), LRMS(ESI+): calculated for $C_{57}H_{93}N_{12}O_{15}^+$ [M+H^+]: 1185.7; found: 1185.7. See supporting information for the details of LC-MS spectrum.

*5.8.5. L-Leu_{10}-teixobactin (**T5**)*. White solid, 3.2 mg, yield 49% (the final step), LRMS(ESI+): calculated for $C_{58}H_{97}N_{12}O_{15}^+$ [M+H^+]: 1201.7; found: 1201.7. See supporting information for the details of LC-MS spectrum.

*5.8.6. L-Ile_{10}-teixobactin (**T6**)*. White solid, 2.9 mg, yield 45% (the final step), LRMS(ESI+): calculated for $C_{58}H_{97}N_{12}O_{15}^+$ [M+H^+]: 1201.7; found: 1201.9. See supporting information for the details of LC-MS spectrum.

*5.8.7. L-Ser_{10}-teixobactin (**T7**)*. White solid, 2.7 mg, yield 41% (the final step), LRMS(ESI+): calculated for $C_{55}H_{91}N_{12}O_{16}^+$ [M+H^+]: 1175.7; found: 1175.8. See supporting information for the details of LC-MS spectrum.

*5.8.8. L-Thr_{10}-teixobactin (**T8**)*. White solid, 2.7 mg, yield 42% (the final step), LRMS(ESI+): calculated for $C_{56}H_{93}N_{12}O_{16}^+$ [M+H^+]: 1189.7; found: 1189.7 See supporting information for the details of LC-MS spectrum.

*5.8.9. L-Phe_{10}-teixobactin (**T9**)*. White solid, 3.1 mg, yield 47% (the final step), LRMS(ESI+): calculated for $C_{61}H_{95}N_{12}O_{15}^+$ [M+H^+]: 1235.7; found: 1235.7. See supporting information for the details of LC-MS spectrum.

*5.8.10. L-Tyr_{10}-teixobactin (**T10**)*. White solid, 3.1 mg, yield 47% (the final step), LRMS(ESI+): calculated for $C_{61}H_{95}N_{12}O_{16}^+$ [M+H^+]: 1251.7; found: 1251.8. See supporting information for the details of LC-MS spectrum.

*5.8.11. L-Trp_{10}-teixobactin (**T11**)*. White solid, 2.9 mg, yield 45% (the final step), LRMS(ESI+): calculated for $C_{63}H_{96}N_{13}O_{15}^+$ [M+H^+]: 1274.7; found: 1274.7. See supporting information for the details of LC-MS spectrum.

*5.8.12. L-Asp_{10}-teixobactin (**T12**)*. White solid, 2.7 mg, yield 42% (the final step), LRMS(ESI+): calculated for $C_{56}H_{91}N_{12}O_{17}^+$ [M+H^+]: 1203.7; found: 1203.7. See supporting information for the details of LC-MS spectrum.

*5.8.13. L-Glu_{10}-teixobactin (**T13**)*. White solid, 2.7 mg, yield 41% (the final step), LRMS(ESI+): calculated for $C_{57}H_{93}N_{12}O_{17}^+$ [M+H^+]: 1217.7; found: 1217.8. See supporting information for the details of LC-MS spectrum.

*5.8.14. L-Asn_{10}-teixobactin (**T14**)*. White solid, 2.8 mg, yield 43% (the final step), LRMS(ESI+): calculated for $C_{56}H_{92}N_{13}O_{16}^+$ [M+H^+]: 1202.7; found: 1202.6. See supporting information for the details of LC-MS spectrum.

*5.8.15. L-Gln_{10}-teixobactin (**T15**)*. White solid, 3.0 mg, yield 46% (the final step), LRMS(ESI+): calculated for $C_{57}H_{94}N_{13}O_{16}^+$ [M+H^+]: 1216.7; found: 1216.8. See supporting information for the details of LC-MS spectrum.

*5.8.16. L-Met_{10}-teixobactin (**T16**)*. White solid, 2.9 mg, Yield 44% (the final step), LRMS(ESI+): calculated for $C_{57}H_{95}N_{12}O_{15}S^+$ [M+H^+]: 1219.7; found: 1219.7. See supporting information for the details of LC-MS spectrum.

*5.8.17. L-$Cys(StBu)_{10}$-teixobactin (**T17**)*. White solid, 3.1 mg, Yield 48% (the final step), LRMS(ESI+): calculated for

$C_{59}H_{99}N_{12}O_{15}S_2^+$ [M+H$^+$]: 1279.7; found: 1279.7. See supporting information for the details of LC-MS spectrum.

*5.8.18. L-Phe(4-F)$_{10}$-teixobactin (**T18**).* White solid, 2.9 mg, yield 45% (the final step), LRMS(ESI+): calculated for $C_{61}H_{94}FN_{12}O_{15}^+$ [M+H$^+$]: 1253.7; found: 1253.8. See supporting information for the details of LC-MS spectrum.

*5.8.19. L-Phe(4-Cl)$_{10}$-teixobactin (**T19**).* White solid, 3.0 mg, yield 46% (the final step), LRMS(ESI+): calculated for $C_{61}H_{94}ClN_{12}O_{15}^+$ [M+H$^+$]: 1269.7; found: 1269.8. See supporting information for the details of LC-MS spectrum.

*5.8.20. L-Phe(4-Br)$_{10}$-teixobactin (**T20**).* Yellowish solid, 2.9 mg, Yield 45% (the final step), LRMS(ESI+): calculated for $C_{61}H_{94}BrN_{12}O_{15}^+$ [M+H$^+$]: 1315.4; found: 1315.7. See supporting information for the details of LC-MS spectrum.

*5.8.21. L-Phe(4-CH$_3$)$_{10}$-teixobactin (**T21**).* White solid, 3.1 mg, yield 47% (the final step), LRMS(ESI+): calculated for $C_{62}H_{97}N_{12}O_{15}^+$ [M+H$^+$]: 1249.7; found: 1250.0. See supporting information for the details of LC-MS spectrum.

*5.8.22. L-Phe(4-NO$_2$)$_{10}$-teixobactin (**T22**).* Yellowish solid, 3.2 mg, yield 49% (the final step), LRMS(ESI+): calculated for $C_{61}H_{94}N_{13}O_{17}^+$ [M+H$^+$]: 1280.7; found: 1280.8. See supporting information for the details of LC-MS spectrum.

*5.8.23. L-Tyr(OMe)$_{10}$-teixobactin (**T23**).* White solid, 2.9 mg, yield 44% (the final step), LRMS(ESI+): calculated for $C_{62}H_{97}N_{12}O_{16}^+$ [M+H$^+$]: 1265.7; found: 1265.7. See supporting information for the details of LC-MS spectrum.

*5.8.24. L-Tyr(3-NO$_2$)$_{10}$-teixobactin (**T24**).* Yellowish solid, 3.1 mg, yield 47% (the final step), LRMS(ESI+): calculated for $C_{61}H_{94}N_{13}O_{18}^+$ [M+H$^+$]: 1296.7; found: 1296.7. See supporting information for the details of LC-MS spectrum.

*5.8.25. L-Abu$_{10}$-teixobactin (**T25**).* White solid, 2.9 mg, yield 45% (the final step), LRMS(ESI+): calculated for $C_{56}H_{93}N_{12}O_{15}^+$ [M+H$^+$]: 1173.7; found: 1173.8. See supporting information for the details of LC-MS spectrum.

*5.8.26. Aib$_{10}$-teixobactin (**T26**).* White solid, 3.0 mg, yield 46% (the final step), LRMS(ESI+): calculated for $C_{56}H_{93}N_{12}O_{15}^+$ [M+H$^+$]: 1173.7; found: 1173.6. See supporting information for the details of LC-MS spectrum.

*5.8.27. L-Nva$_{10}$-teixobactin (**T27**).* White solid, 3.1 mg, yield 48% (the final step), LRMS(ESI+): calculated for $C_{57}H_{95}N_{12}O_{15}^+$ [M+H$^+$]: 1187.7; found: 1187.7. See supporting information for the details of LC-MS spectrum.

*5.8.28. L-NIe$_{10}$-teixobactin (**T28**).* White solid, 2.9 mg, yield 45% (the final step), LRMS(ESI+): calculated for $C_{58}H_{97}N_{12}O_{15}^+$ [M+H$^+$]: 1201.7; found: 1201.8. See supporting information for the details of LC-MS spectrum.

*5.8.29. L-Chg$_{10}$-teixobactin (**T29**).* White solid, 3.2 mg, yield 49% (the final step), LRMS(ESI+): calculated for $C_{60}H_{99}N_{12}O_{15}^+$ [M+H$^+$]: 1227.7; found: 1227.8. See supporting information for the details of LC-MS spectrum.

*5.8.30. L-Cha$_{10}$-teixobactin (**T30**).* White solid, 3.3 mg, yield 50% (the final step), LRMS(ESI+): calculated for $C_{61}H_{101}N_{12}O_{15}^+$ [M+H$^+$]: 1241.7; found: 1241.9. See supporting information for the details of LC-MS spectrum.

*5.8.31. L-β-cyclopropyl-Ala$_{10}$-teixobactin (**T31**).* White solid, 2.8 mg, Yield 43% (the final step), LRMS(ESI+): calculated for $C_{58}H_{95}N_{12}O_{15}^+$ [M+H$^+$]: 1199.7; found: 1199.9. See supporting information for the details of LC-MS spectrum.

*5.8.32. D-Lys$_{10}$-teixobactin (**T32**).* White solid, 2.8 mg, Yield 43% (the final step), LRMS(ESI+): calculated for $C_{58}H_{98}N_{13}O_{15}^+$ [M+H$^+$]: 1216.7; found: 1216.7. See supporting information for the details of LC-MS spectrum.

*5.9. A*ntibacterial 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 and MICs were determined according to CLSI guideline.[27]

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References and notes

1. Corbett E. L., Watt C. J., Walker N., Maher D., Williams B. G., Raviglione M. C., Dye C. *Arch. Intern. Med.* 2003; *163*: 1009—1021.
2. Mazurek G. H., Jereb J., Lobue P., Iademarco M. F., Metchock B., Vernon A. *MMWr. Recomm. Rep.* 2005; *54*: 49—55.
3. Ling L. L., Schneider T., Peoples A. J., Spoering A. L., Engels I., Conlon B. P., Muller 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.
4. Franz N., Roderich D. S. *Angew. Chem. Int. Ed.* 2015; *17*: 455—459.
5. Homma T., Nuxoll A., Gandt A. B., Ebner P., Engels I., Schneider T., Götz F., Lewis K., Colon, B. P. *Antimicrob. Agents Chemother.* 2016; *60*: 6510—6517.
6. 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.
7. 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.
8. Parmar A., Iyer A., Lloyd D. G., Vincent C. S., Prior S. H., Madder A., Taylor E. J., Singh I. *Chem. Commun.* 2017; *53*: 7788—7791.
9. Chen K. H., Le S. P., Han X., Frias J. M., Nowick J. S. *Chem. Common.* 2017; *53*: 11357—11359.
10. Monaim S. A. H. A., Ramchuran E. J., EI-Faham A., Albericio F., Torre B. G. *J. Med. Chem.* 2017; *60*: 7476—7482.
11. Schumacher C. E., Harris P. W., Ding X-B., Krause B., Wright T. H., Cook G. M., Furkert D. P., Brimble M. A. *Org. Biomol. Chem.* 2017; *15*: 8755—8760.
12. Wu C., Pan Z., Yao G., Wang W., Fang L., Su W. *RSC Advances.* 2017; *7*: 1923—1926.
13. Yang H., Chen K. H., Nowick J. S. *ACS Chem. Biol.* 2016; *11*: 1823—1826.

14. Monaim S. A. H. A., Jad Y. E., Ramchuran E. J., EI-Faham A., Govender T., Kruger H. G., Torre B. G., Albericio, F. *ACS Omega.* 2016; *1*: 1262—1265.
15. 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.
16. Parmar A., Prior S. H., Iyer A., Vincent C. S., Lysebetten D. V., Breukink E., Madder A., Taylor E., Singh I. *Chem. Common.* 2017; *53*: 2016—2019.
17. Yang H., Bois D. R. D., Ziller J. W., Nowick J. S. *Chem. Common.* 2017; *53*: 2772—2775.
18. 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.
19. Jin K., Po H. L., Wang S., Reuven J. A., Wai C. N., Lau H. T., Chan T. H., Chen S., Li, X. *Bioorg. Med. Chem.* 2017; *25*: 4990—4995.
20. Monaim S. A. H. A., Jad Y. E., Acosta G. A., Naicker T., Ramchuran E. J., El-Faham A., Govender T., Kruger G. H., Torre B. G., Albericio F. *RSC Advances.* 2016; *6*: 73827—73829.
21. Parmar A., Iyer A., Prior S. H., Lloyd D. G., Goh E. T. L., Vincent C. S., Palmai-Pallag T., Bachrati C. Z., Breukink E., Madder A., Lakshminarayanan R., Taylor E., Singh I. *Chem. Sci.* 2017; *8*: 8183—8192.
22. Zhang Y., Xu C., Lam H. Y., Lee C. L., Li X. *Proc. Natl. Acad. Sci. U. S. A.* 2013; *110*: 6657—6662.
23. Lam H. Y., Li X. *Synlett.* 2014; *25*: 1339—1344.
24. Zhang Y., Li T., Li X. *Org. Biomol. Chem.*2013; *11*: 5584—5587.
25. Wong C. T., Lam H. Y., Song T., Chen G., Li X. *Angew. Chem. Int. Ed.* 2013; *52*: 10212—10215.
26. Lee C. L., Liu H., Wong C. T., Chow H. Y., Li X. *J. Am. Chem. Soc.* 2016; *138*: 10477—10484.
27. CLSI. CLSI document M 100-S26, Wayne, PA: Clinical and

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