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Elucidation of the Teixobactin Pharmacophore

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Abstract

This paper elucidates the teixobactin pharmacophore by comparing the arginine analogue of teixobactin Arg_{10}-teixobactin to seven homologues with varying structure and stereochemistry. The roles of the guanidinium group at position 10, the stereochemistry of the macrolactone ring, and the "tail" comprising residues 1–5 are investigated. The guanidinium group is not necessary for activity; Lys_{10}-teixobactin is more active than Arg_{10}-teixobactin against gram-positive bacteria in minimum inhibitory concentration (MIC) assays. The relative stereochemistry of the macrolactone ring is important; diastereomer L-Thr_8,Arg_{10}-teixobactin is inactive, and diastereomer D-*allo*-Ile_{11},Arg_{10}-teixobactin is less active. The macrolactone ring is critical; *seco*-Arg_{10}-teixobactin is inactive. The absolute stereochemistry is not important; the enantiomer *ent*-Arg_{10}-teixobactin is comparable in activity. The hydrophobic *N*-terminal tail is important; truncation of residues 1–5 results in loss of activity, and replacement of residues 1–5 with a dodecanoyl group partially restores activity. These findings pave the way for developing simpler homologues of teixobactin with enhanced pharmacological properties.

At the beginning of 2015, a new antibiotic, teixobactin, was reported in *Nature*,[1] with great attention in the scientific press[2,3,4,5] and the popular press.[6] Teixobactin is a non-ribosomal undecapeptide containing a macrocyclic depsipeptide group (Figure 1). It contains four D-amino acids and seven L-amino acids, and the *C*-terminal Ile^{11} is cyclized onto the side chain of D-Thr_8 to form a 13-membered lactone. Residue 10 of teixobactin is the non-proteinogenic amino acid, L-*allo*-enduracididine (*allo*-End_{10}), which is a cyclic analogue of arginine. Teixobactin acts against gram-positive bacteria by binding to the prenyl-pyrophosphate-GlcNAc region of lipid II.[1] This region is highly conserved in bacteria and cannot easily mutate to impart drug-resistance.[7,8] It is thus an attractive antibiotic target.

Recently, Jad *et al*. and Parmar *et al*. reported syntheses of the arginine analogue of teixobactin Arg_{10}-teixobactin.[9,10] Both syntheses involve solid-phase peptide synthesis (SPPS) of a branched precursor on 2-chlorotrityl resin, followed by solution-phase macrolactamization to form the Ala_9–Arg_{10} amide bond. The former synthesis requires both Fmoc and Alloc groups as orthogonal α-amino protecting groups; the latter requires Fmoc, Alloc, and trityl groups. Both syntheses introduce D-Thr_8 without protecting the alcohol group and *O*-acylate it before completing the *N*-terminal tail. Both sets of authors reported

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that Arg_{10}-teixobactin is about an order of magnitude less active against gram-positive bacteria than teixobactin in minimum inhibitory concentration (MIC) assays.[11,12,13]

In the current study, we set out to elucidate the teixobactin pharmacophore by synthesizing and evaluating a series of teixobactin homologues. We examine the roles of the guanidinium group at position 10, the stereochemistry of the macrolactone ring, and the "tail" comprising residues 1–5. We also report a simpler synthesis of teixobactin analogues and a simpler homologue, which we term lipobactin 1.

We synthesized Arg_{10}-teixobactin and other homologues by SPPS on 2-chlorotrityl resin, followed by solution-phase macrolactamization to form the Arg_{10}–Ile_{11} amide bond (Scheme 1).[14,15,16,17] We used only Fmoc protecting groups to construct all of the amide bonds and carried D-Thr_8 through the entire synthesis without side chain protection. All homologues were prepared and studied as the trifluoroacetic acid (TFA) salts.

We began the synthesis by attaching Fmoc-Arg(Pbf)-OH to 2-chlorotrityl resin. Residues 9 through 1 were then introduced by standard Fmoc-based SPPS using HCTU as the coupling reagent. D-Thr_8 was introduced without a protecting group at the hydroxy position. No *O*-acylation of D-Thr_8 was observed in the subsequent rounds of SPPS. D-Thr_8 was then *O*-acylated with Fmoc-Ile-OH using DIC and DMAP.[18,19,20] Fmoc-deprotection, followed by cleavage from the resin with 20% hexafluoroisopropanol (HFIP) in CH_2Cl_2 afforded the acyclic protected precursor. Macrolactamization with HBTU and HOBt, followed by global deprotection with trifluoroacetic acid (TFA) and RP-HPLC purification afforded Arg_{10}-teixobactin. We also prepared a series of homologues using similar procedures (Figure 2).

We investigated the antibiotic activity of Arg_{10}-teixobactin and homologues in MIC assays against four types of gram-positive bacteria. We used the antibiotic vancomycin as a positive control and the gram-negative bacterium *E. coli* as a negative control. We selected non-pathogenic strains of bacteria to facilitate the safe and rapid screening of Arg_{10}-teixobactin and other homologues in a biosafety level 1 (BSL-1) environment.

To explore the role of a guanidinium group in residue 10, we compared the MIC of Arg_{10}-teixobactin to Lys_{10}-teixobactin. The arginine residue serves as a surrogate for *allo*-enduracididine, which is not commercially available and has only been prepared by cumbersome multistep syntheses.[21,22,23,24,25] Arg_{10}-teixobactin gave MIC values of 1–4 μg/mL against the four gram-positive bacteria studied (Table 1). Although side-by-side comparison to an authentic sample of teixobactin was not possible, comparison to the original published values in related bacteria suggests that Arg_{10}-teixobactin is about an order of magnitude less active (Table 1). Surprisingly, Lys_{10}-teixobactin gave MIC values 2–4 times lower than Arg_{10}-teixobactin. While the MIC values for Lys_{10}-teixobactin are slightly higher than those reported for teixobactin, they are comparable to those of vancomycin (Table 1). This interesting finding indicates that the guanidinium group at position 10 is not necessary for activity and lays the foundation for the future discovery of homologues that lack *allo*-enduracididine and are even more potent.

To investigate the role of the macrolactone ring stereochemistry, we compared the diastereomer L-Thr_8,Arg_{10}-teixobactin and D-*allo*-Ile_{11},Arg_{10}-teixobactin to Arg_{10}-

teixobactin. The former proved inactive (MIC > 32 μg/mL) against the gram positive bacteria, while the latter proved half as active (Table 1). Collectively, these results suggest that the ring stereochemistry and the conformation are important in teixobactin activity. *Seco*-Arg_{10}-teixobactin also proved inactive (MIC > 32 μg/mL), further supporting the importance of the cyclic depsipeptide structure (Table 1).

To further investigate the role of the macrolactone ring stereochemistry, we compared *ent*-Arg_{10}-teixobactin to Arg_{10}-teixobactin. *Ent*-Arg_{10}-teixobactin exhibits comparable activity to Arg_{10}-teixobactin. This exciting finding supports a model in which the amide NH groups on macrolactone ring bind to the achiral pyrophosphate group of lipid II through hydrogen-bonding interactions. This mode of binding has previously been reported in the NMR structure of the complex of nisin with lipid II (PDB 1WCO)[26] and appears to occur for teixobactin as well.

To investigate the role of the *N*-terminal tail, we truncated residues 1–5. The resulting short-Arg_{10}-teixobactin also proved inactive (MIC > 32 μg/mL). To investigate the possibility that the hydrophobic residues *N*-Me-D-Phe, Ile, and D-*allo*-Ile at positions 1, 2, and 5 help to anchor teixobactin into the plasma membrane, we replaced residues 1–5 with a dodecanoyl group.[27,28] The resulting homologue, lipobactin 1, proved only 2–4 times less active than Arg_{10}-teixobactin (Table 1). This finding confirms the importance of the hydrophobicity of the *N*-terminal tail and paves the way for further developing simpler homologues of teixobactin with enhanced pharmacological properties.

Acknowledgments

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:.

Procedures for the synthesis of Arg_{10}-teixobactin and homologues and minimum inhibitory concentration (MIC) assays; spectral and other characterization data.

26. Hsu ST, Breukink E, Tischenko E, Lutters MA, de Kruijff B, Kaptein R, Bonvin AM, van Nuland NA. The nisin-lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. Nat. Struct. Mol. Biol. 2004; 11:963–937. [PubMed: 15361862]

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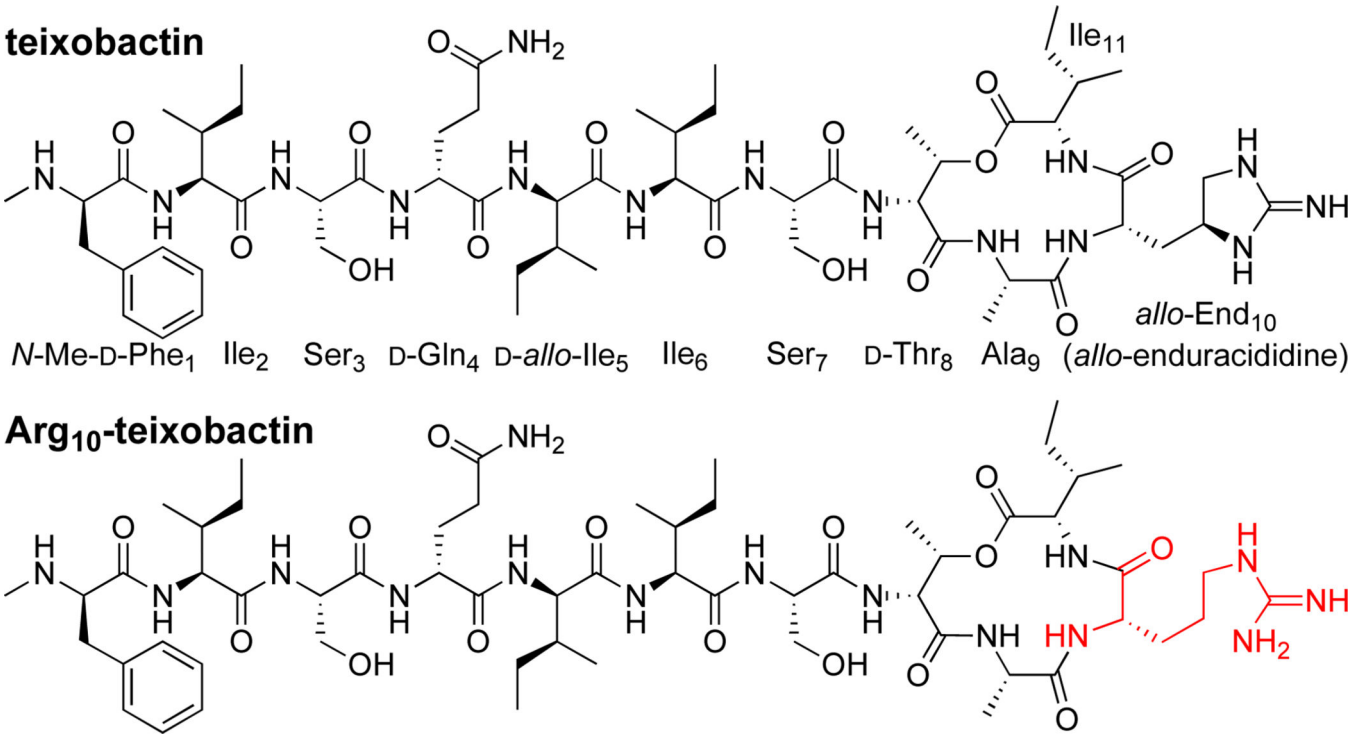


Figure 1.
Structures of teixobactin and Arg_{10}-teixobactin.

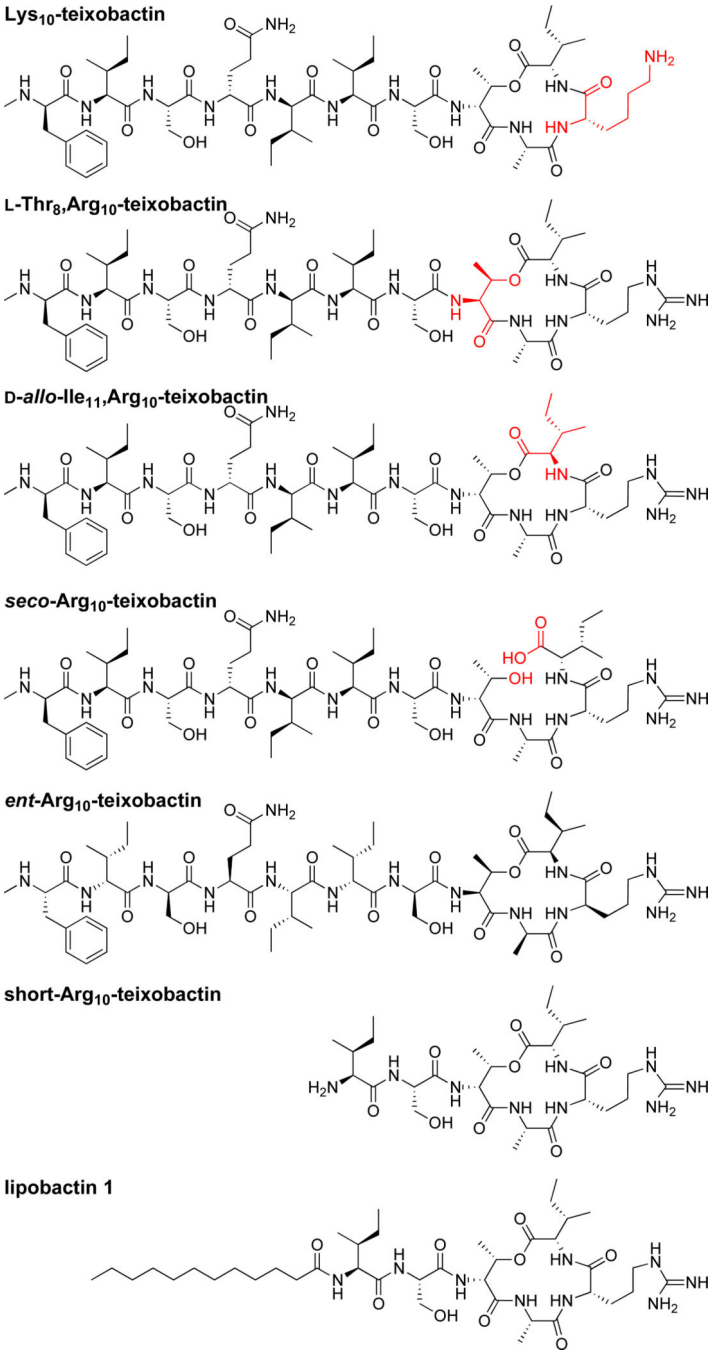
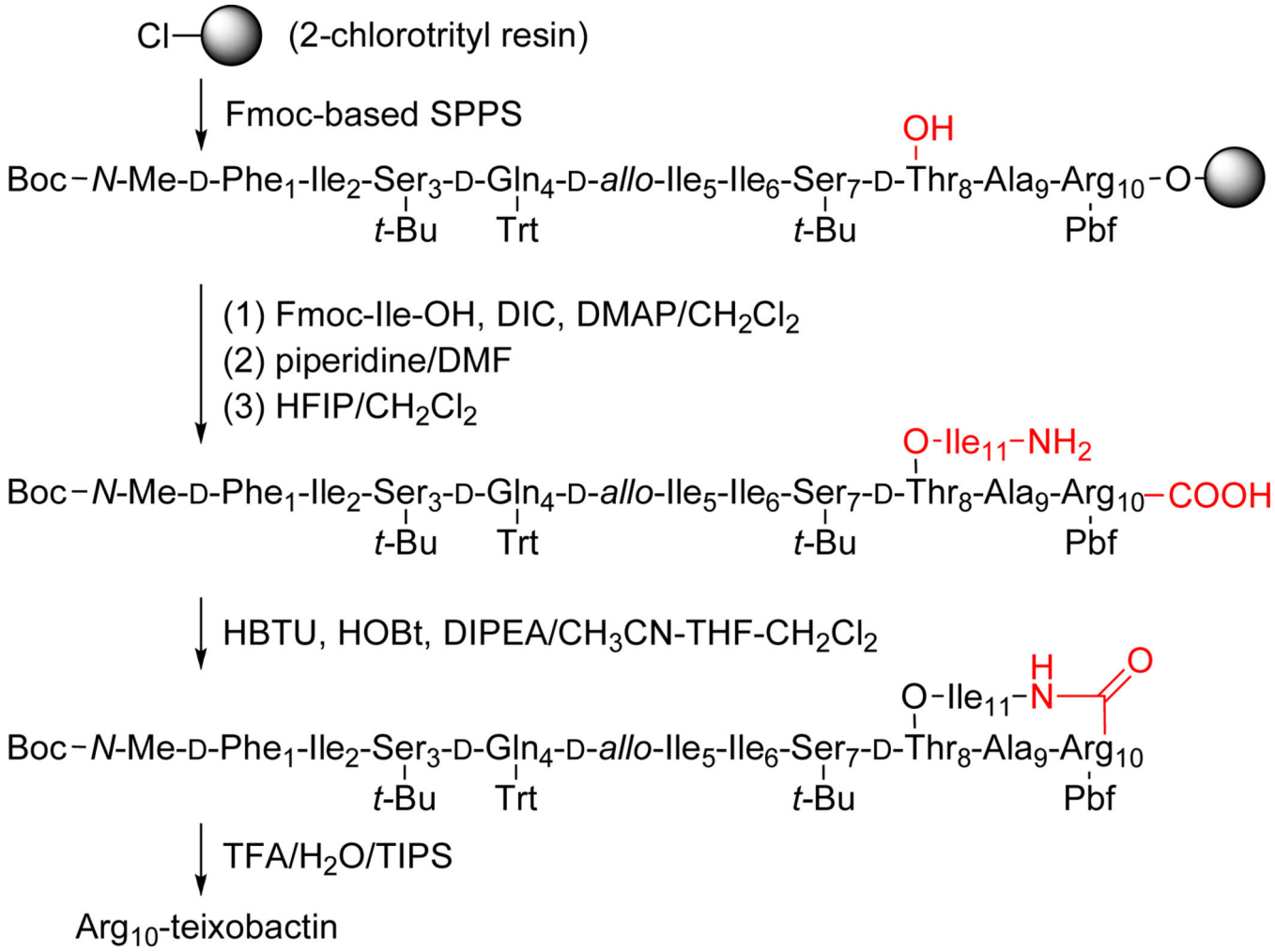


Figure 2.
Structures of teixobactin homologues.



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

Table 1

MIC of teixobactin homologues in μg/mL.

	***Staphylococcus epidermidis* ATCC 14990**	***Streptococcus salivarius* ATCC 13419**	***Enterococcus durans* ATCC 6056**	***Bacillus subtilis* ATCC 6051**	***Escherichia coli* ATCC 10798**
Arg_{10}-teixobactin	1	1	4	2	>32
Lys_{10}-teixobactin	0.25	0.5	1	0.5	>32
L-Thr_8,Arg_{10}-teixobactin	>32	>32	>32	>32	>32
D-*allo*-Ile_{11},Arg_{10}-teixobactin	2	2	8	4	>32
seco-Arg_{10}-teixobactin	>32	>32	>32	>32	>32
ent-Arg_{10}-teixobactin	2	1	4	2	>32
short-Arg_{10}-teixobactin	>32	>32	>32	>32	>32
lipobactin 1	4	4	8	4	>32
vancomycin	0.5	0.5	0.5	1	>32
teixobactin[1]	0.08–0.3	0.02–0.15	0.3–0.6	0.02–0.6	25
	various *Staphylococcus*[1]	various *Streptococcus*[1]	various *Enterococcus*[1]	various *Bacillus*[1]	*E. coli*[1]

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