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Synthesis and Biological Evaluation of a Teixobactin Analogue

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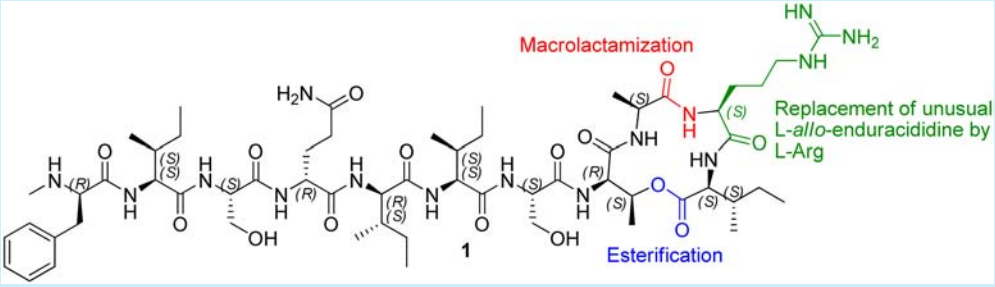
S *Supporting Information*



ABSTRACT: The first synthesis and biological activity of a teixobactin analogue is reported. Substitution of the unusual L-*allo*-enduracididine residue by the naturally occurring L-arginine was achieved, and the analogue gave an activity trend similar to that of teixobactin (against Gram-postive bacteria) and meropenem, which was approved by the FDA in 1996. The synthetic route used allows for the synthesis of the natural product as well as the development of a program of medicinal chemistry.

Recently, antimicrobial resistance and the shortage of new antimicrobial drugs have become serious concerns for the treatment of microbial infections.[1−3] Antimicrobial peptides have emerged as good candidates for developing new antibiotics.[4] In fact, peptides in general are gaining interest as therapeutic agents due to their high and specific biological activities and low toxicity. During the last couple of years, a new class of natural peptides, the so-called "head-to-side-chain" cyclodepsipeptides,[5] have shown importance as therapeutical candidates. This class can be described as cyclic polypeptides containing at least one ester bond, where the C-terminus carboxylic acid is involved in the ester bond with a hydroxyl side chain. Such peptides exhibit several biological activities such as antiviral, antimicrobial, cytotoxic, and anticancer activities.[5,6] Furthermore, some of them have already been explored as pharmacologically potent and toxicologically safe derivatives.[7] For instance, kahalalide F (KF, **2**, Figure 1) was isolated from the Hawaiian herbivorous marine species of mollusk, *Elysia rufescens*, and its diet, the green alga *Bryopsis* sp. and showed potential antitumor activities.[8,9] Currently, KF has reached phase II clinical trials.[10,11] Daptomycin **3** is another example of a "head-to-side-chain" cyclodepsipeptide, which was isolated from fermentations of *Streptomyces roseosporus*.[12,13] In 2003, it was approved in the USA, and in Europe in 2006, for the treatment of complicated skin and skin structure infections caused by Gram-positive bacteria.[14−17]

Early in 2015, Ling et al.[18] reported teixobactin **4** as a new antibiotic, which was discovered during a screening of uncultured bacteria. Teixobactin revealed potent activities against Gram-positive pathogens (including drug-resistant strains) as well as *Mycobacterium tuberculosis* (below 1 μg/mL). However, it does not inhibit Gram-negative pathogens.[19,20] Moreover, it is the first of a new class of antibiotics that was discovered utilizing iChip technology.[21]

Teixobactin is an 11-mer peptide containing a cyclotetradepsipeptide unit in its structure. It contains five unnatural amino acid residues, namely D-*N*Me-Phe, D-Gln, D-*allo*-Ile, D-Thr, and L-*allo*-enduracididine. Herein, we designed, synthesized, and evaluated the biological activity of an Arg analogue **1** of teixobactin where L-Arg replaced the L-*allo*-enduracididine residue. Both of these building blocks are guanidine-based amino acids (L-Arg is linear while L-*allo*-enduracididine is a cyclic guanidine analogue).

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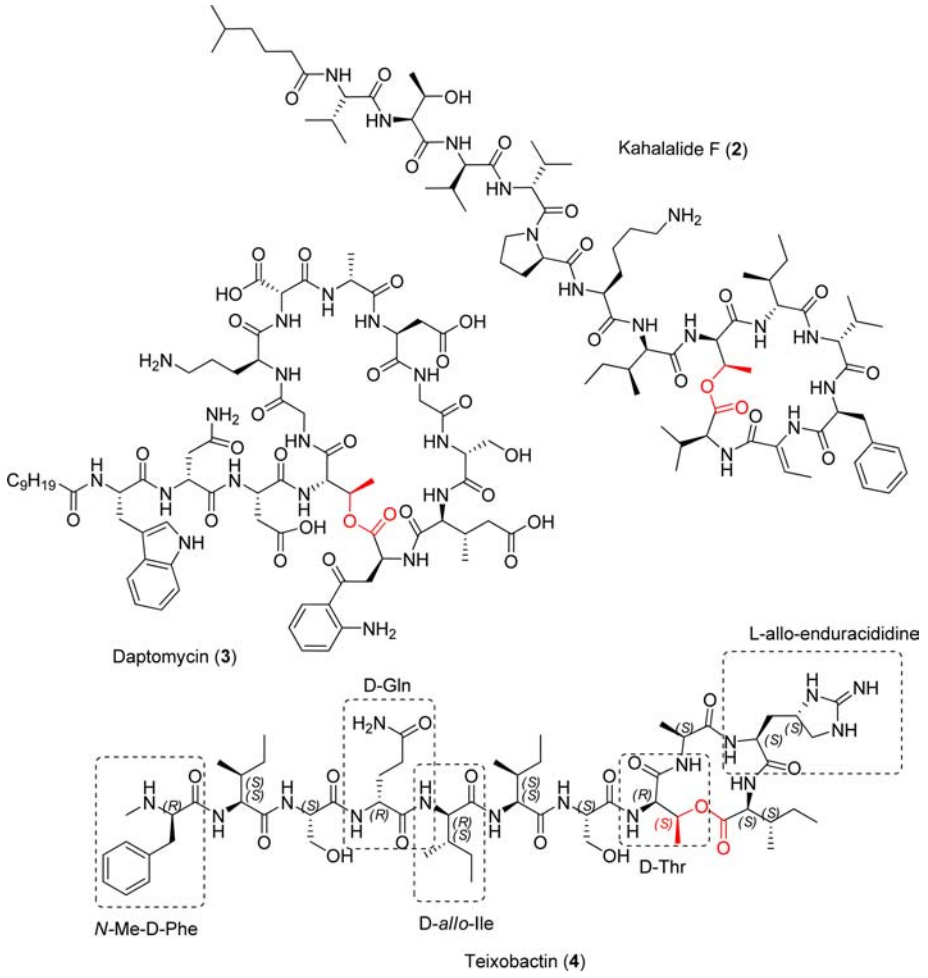

Figure 1. Kahalalide F, daptomycin, and teixobactin.

The points of macrocyclization and ester-bond formation are two keys steps for the synthesis of **1** (Scheme 1). First, the macrocyclization point on the cyclotetradepsipeptide moiety should be selected taking into account important requirements.[22,23] Two of these requirements are (a) minimum steric hindrance (to ensure a high cyclization yield) and (b) minimal racemization during the cyclization process.

In the case of δ-guanidino amino acid containing peptides, this residue cannot be the C-terminal because it is very much prone to δ-lactam formation upon activation,[24] which will render an open and branched peptide with the same molecular weight than the target peptide.

Therefore, the best macrolactamization point for **1** is between C-terminus L-Ala and N-terminus L-Arg. In order to obtain the protected precursor peptide, 2-Cl-Trt resin (166 mg, 1.69 mmol/g) was used, and therefore, Fmoc-L-Ala (31 mg, 0.1 mmol) was first attached to the resin in the presence of DIEA (174 μL, 1 mmol, 10 equiv) in DCM (1 mL) for 1 h, followed by addition of MeOH for capping the unreacted Cl groups on the resin.[25] The peptide-bond formation was carried out using carbamate-protected amino acids (3 equiv) and HATU/DIEA (3:6 equiv) in DMF for 30 min. To the best of our knowledge, HATU is the fastest and the most efficient coupling reagent during SPPS.[26−28] The ninhydrin test showed that in all cases, except for Gln(Trt), the coupling was completed. For Gln(Trt), an additional coupling was carried out using DIC/K-Oxyma.[29]

Regarding ester-bond formation, linear tetrapeptide **6** should be prepared prior to esterification of the D-Thr residue with Alloc-protected L-Ile-OH in order to prevent O → N-acyl migration during the removal of the Fmoc of the D-Thr residue.[30−32] Both Ile and Arg should be protected as N^{α}-Alloc, which is orthogonal with the Fmoc group used for the elongation of the peptide chain and allows for independent completion of the peptide chains. During the synthesis of kahalalide F, our group had shown that the esterification is not efficient if performed toward the end of the synthesis.[9] Furthermore, if Fmoc is used for the protection of Arg, diketopiperazine formation is very likely to occur.[33]

The esterification step was more difficult than expected based on the experience of our group with other cyclodepsipeptides. Thus, during the synthesis of kahalalide F, the esterification of D-*allo*-Thr with Alloc-Val-OH (7 equiv) took place very smoothly using DIC (7 equiv) and DMAP (0.7 equiv). In this case, several couplings were required to reach a yield of 97% as detected by HPLC.

Once the Arg was incorporated, the remainder of the peptide sequence was completed with N^{α}-Fmoc amino acids, except for the last D-*N*Me-Phe, where Boc protection was used to allow for the preparation of the protected peptide after cleavage, which is suitable for the macrolactamization step. The latter was carried using PyAOP/OxymaPure/DIEA as the coupling method. PyAOP was used to avoid guanidylation side reaction that may occur if HATU was used. Furthermore, the HOAt-

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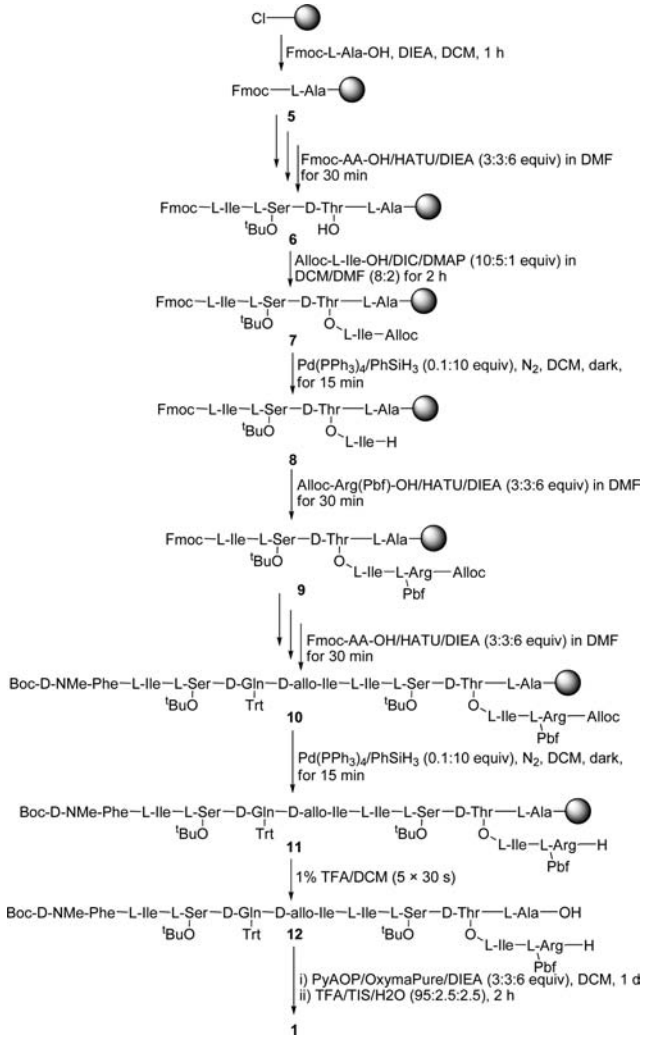
Scheme 1. Synthesis of 1



derived coupling reagents are generally very efficient reagents for macrolactamization reaction.[34,35] Moreover, OxymaPure (ethyl 2-cyano-2-(hydroxyimino)acetate) was used to reduce the racmization of the α-C on C-terminus.[36] After final cyclization, global deprotection was carried out with TFA. The NMR elucidation of the synthetic analogue correlated perfectly with the expected sequence and matched the published spectrum of the natural product[18] very well with the exception of some chemical shifting of certain peaks that were unambiguously confirmed using 2D NMR spectra (see the SI).

The antibacterial properties of **1** were evaluated against Gram-positive bacteria such as *Staphylococcus aureus* (25923) and *Bacillus subtilis* (6051) and also against Gram-negative bacteria such as *Escherichia coli* (25922) and *Pseudomonas aeruginosa* (27853). The MIC results are listed in Table 1 in comparison to meropenem (β-lactam-type antibiotic; for more details about its structure, see the SI) and the reactivity reported in the literature for the natural teixobactin **4**. As shown in Table 1, **1** displayed a similar trend of activity as teixobactin, i.e., activity against Gram-positive and not effective against Gram-negative. Although the activity against the Gram-positive bacteria was slightly inferior to teixobactin, it can be considered excellent because it is comparable to that of meropenem, which was approved by the FDA in 1996.

Table 1. MIC (nM) Value of 1 and Meropenem as a Reference Compound

		drug		
entry		meropenem	**1**	**4**[a]
	Gram-Positive Bacteria			
1	*S. aureus* (25923)	2.6	1.6	0.2
2	*B. subtilis* (6051)	0.33	0.40	0.016
	Gram-Negative Bacteria			
3	*E. coli* (25922)	0.16	51.43	20.00
4	*P. aeruginosa* (27853)	2.6	NI	> 80

[a]Data extracted from ref 18.

Herein, a robust strategy for the synthesis of a teixobactin analogue (L-Arg instead of L-*allo*-enduracididine) was developed. The cornerstone of it is an elegant disconnection for the macrocyclization, which has rendered the cyclization with excellent efficiency using PyAOP/OxymaPure/DIEA and in the absence of side reactions. Elongation of the peptide chain on a 2-Cl-Trt resin, which enabled the preparation of the protected peptide, has been carried out using three carbamate-based protecting groups: Fmoc for the main chain, except for the last amino acid where Boc was used, and Alloc for the branching chain. The formation of peptide bonds were mostly efficient using HATU/DIEA, and only the incorporation of the Gln(Trt) residue needed double coupling, using DIC/K-Oxyma. The esterification step, which was carried out using a symmetrical anhydride method in the presence of DMAP, should be further optimized. Difficulties were encountered around this step, suggesting again that the esterification reaction is the bottleneck of the preparation of this family of cyclodepsipetides and that the yield is very environmentally dependent. From a synthetic point of view, it can be concluded that the synthesis of this challenging peptide must involve more than one N$^{\alpha}$-protecting group and several coupling cocktails.

The substitution of the unusual L-*allo*-enduracididine residue by the naturally occurring L-Arg resulted in a similar trend of biological activity as for the teixobactin, which is active only against Gram-positive bacteria, and with an activity similar to meropenem. The synthetic route used herein will allow for the synthesis of the natural product as well as the development of a program of medicinal chemistry. Furthermore, these results support the original assignment of all chiral centers, except for the additional chirality of the enduracididine that was absent in **1**. In our experience, changing of any of the remaining sterocenters during the synthesis of the natural product causes a dramatic reduction in bioactivity.[37]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03176.

Experimental details, NMR spectra, MS, and HPLC chromatograms (PDF)

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Notes

The authors declare no competing financial interest.

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(37) Our group has demonstrated that in the family of cyclodepsipeptides the epimers at the Cα experienced a total loss of the activity; see refs 9 and 32.

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