ChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: A. Parmar, A. Iyer, D. G. Lloyd, C. Vincent, S. H. Prior, A. Madder, E. J. Taylor and I. Singh, *Chem. Commun.*, 2017, DOI: 10.1039/C7CC04021K.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the author guidelines.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the ethical guidelines, outlined in our author and reviewer resource centre, still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

ChemComm

View Article Online
DOI: 10.1039/C7CC04021K

Communication

Syntheses of potent teixobactin analogues against methicillin-resistant *Staphylococcus aureus* (MRSA) through the replacement of L-*allo*-enduracididine with its isosteres

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Anish Parmar, [a] Abhishek Iyer, [a, b] Daniel G. Lloyd,[c] Charlotte S. Vincent, [c] Stephen H. Prior, [d] Annemieke Madder, [b] Edward J. Taylor [d] and Ishwar Singh* [a]

The recently discovered cyclic depsipeptide, teixobactin, is a highly potent antibiotic against multi-drug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobaterium tuberculosis*. It comprises 4 D amino acids and a rare L-*allo*-enduracididine amino acid. The synthesis of a properly protected L-*allo*-enduracididine amino acid and its incorporation into teixobactin is time consuming, synthetically challenging and low yielding and is therefore a major bottleneck in the development of potent analogues of teixobactin. In this article, we have synthesised 8 analogues of teixobactin using commercially available building blocks by replacing the L-*allo*-enduracididine amino acid with its isosteres. Furthermore, we have tested all the compounds against a panel of Gram positive bacteria including MRSA and explained the observed trend in biological activity. Although all the analogues were active, three analogues from this work, showed very promising activity against MRSA (MIC 1 μg/mL). We can conclude that amino acids which are the closest isosteres of L-*allo*-enduracididine are the key to synthesising simplified potent analogues of teixobactin using rapid syntheses and improved yields.

Antimicrobial resistance (AMR) is spreading faster than the introduction of new antibiotics resulting in a major health crisis.[1] The recently discovered antibiotic teixobactin[2] has shown tremendous promise due to its potent activity particularly against resistant pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium tuberculosis*. Teixobactin kills bacteria without detectable resistance[2] through the inhibition of cell wall synthesis. It operates by inhibiting the non-protein building blocks, lipid II (needed for peptidoglycan synthesis) and lipid III (a starting material for cell wall teichoic acid synthesis) from multiple biosynthetic pathways, thereby making it difficult for bacteria to develop resistance against it.[3] Therefore, teixobactin research has gained tremendous momentum with several research groups including our own showing a keen interest in designing and synthesising highly potent synthetic analogues of teixobactin.

Teixobactin is an undecapeptide containing four D amino acids namely *N*-Me-D-Phe$_1$, D-Gln$_4$, D-*allo*-Ile$_5$ and D-Thr$_8$ and the rare L-*allo*-enduracididine amino acid (Figure 1A). In the past year, several notable contributions have been made to teixobactin research describing the total synthesis of teixobactin,[4 5] and the syntheses and biological activities of teixobactin analogues.[6 7 8] We have established the importance of the D configuration of the amino acids of teixobactin in terms of antibacterial activity.[9] It was observed that changing the amino acid configuration of any one of the four D amino acids (D-*N*-Me-Phe$_1$, D-Gln$_4$, D-*allo*-Ile$_5$ and D-Thr$_8$) from D to L leads to significant loss in antibacterial activity.[9] We have further reported the three-dimensional molecular structure of seven teixobactin analogues by using NMR. The NMR studies revealed that the disordered structure of teixobactin analogues is important for their biological activity. It was shown that D-Gln$_4$ is essential and D-*allo*-Ile$_5$ is important for maintaining the disordered structure.[9]

The minimum pharmacophore of teixobactin, lipobactin, has been reported by Nowick et. al.[8] Lipobactin has been synthesised by replacing the N-terminal residues 1-5 with a dodecanoyl chain. Furthermore, Nowick et. al. also reported that modification of configuration of any of the residues in the core ring structure of teixobactin results in a significant decrease in activity. The enantiomeric Arg$_{10}$-teixobactin which is a mirror image of Arg$_{10}$-teixobactin shows similar biological activity as Arg$_{10}$-teixobactin indicating that only the relative configurations of amino acids are important for maintaining biological activity and not their absolute configuration. Very recently, Nowick et. al. have reported the crystal structure of a truncated teixobactin analogue showing the key interactions of the core ring structure of teixobactin with a chloride ion.[10] A

[a] School of Pharmacy, JBL Building, University of Lincoln, Beevor St. Lincoln LN67DL, UK. E-mail: isingh@lincoln.ac.uk
[b] Organic and Biomimetic Chemistry Research Group, Department of Organic Chemistry, Ghent University, Krijgslaan 281 (S4), B-9000 Ghent, Belgium
[c] School of Life Sciences, Joseph Bank Laboratories, University of Lincoln, Green Lane, Lincoln LN6 7DL.
[d] School of Chemistry JBL Building, University of Lincoln, Beevor St. Lincoln LN67DL, UK.
†Electronic Supplementary Information (ESI) available: Peptide synthesis, HPLC, LC-MS analysis, NMR spectra See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C7CC04021K

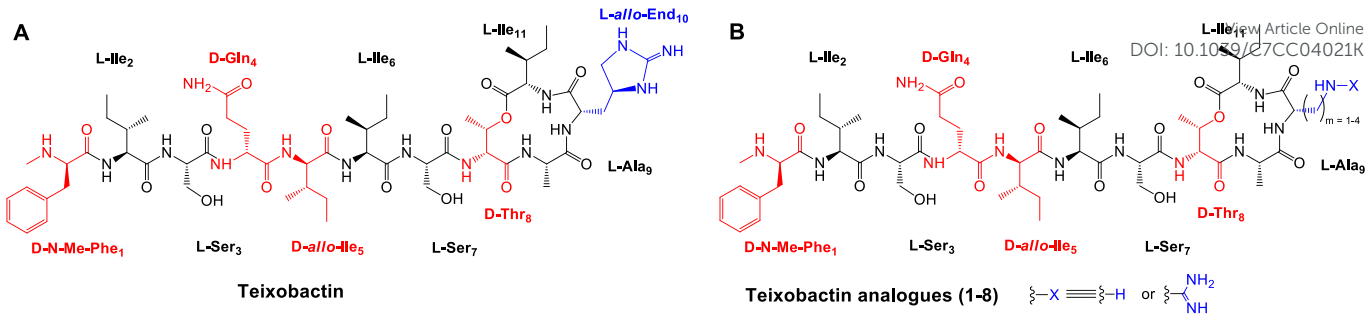


Figure 1: **A**. Teixobactin **B**. General structure of teixobactin analogues (**1-8**) with the hydrophilic/charged residues shown in red, hydrophobic residues shown in black and structural differences shown in blue.

lysine scan of Arg_{10}-teixobactin reported by the Albericio group[11] showed that replacement of any one of the four isoleucine residues with lysine leads to complete loss of activity. However, replacement of the polar, non-charged residues Ser_3, Gln_4 and non-polar Ala_9 by lysine resulted in analogues which have comparable biological activity to that of Arg_{10}-teixobactin. Recently, Wu. C et. al. reported that Lys_{10}/Arg_{10}, Ser_7 and the NH- group of the *N* terminal phenylalanine are critical for the biological activity of teixobactin analogues.[12] Replacement of Arg_{10} or Lys_{10} by His_{10}, Ser_7 by Ala_7 and *N*-Methyl phenylalanine$_1$ by *N*, *N*-dimethyl phenylalanine$_1$ leads to less active teixobactin analogues compared to Arg_{10}-teixobactin. During the preparation of this manuscript, a series of teixobactin analogues using convergent Ser/Thr ligation was published by Li *et. al.* which reports the synthesis and antibacterial activity of $NorArg_{10}$-teixobactin.[13]

To further develop potent teixobactin analogues against resistant bacteria such as MRSA, we are particularly interested in understanding the role of the polar amino acid residues at position 10 namely L-*allo*-enduracididine, arginine and lysine. It has been suggested that L-*allo*-enduracididine is important for the biological activity of teixobactin.[4] However, the synthesis of a properly protected L-*allo*-enduracididine and its subsequent incorporation in teixobactin synthesis is complex and low yielding (3.3% overall yield).[4] Several research groups have substituted this amino acid with commercially available building blocks such as Arginine,[6 7] Lysine[8] or Histidine.[12] The obtained analogues were less active than the natural product. However, the biological activity of teixobactin analogues suggests they are still suitable for further development as potential antibacterials. Therefore, it is important to synthesise new, potent derivatives with comparable biological activity to teixobactin which do not contain the L-*allo*-enduracididine amino acid. In this work, we have synthesised eight analogues of teixobactin using commercially available building blocks by replacing the L-*allo*-enduracididine amino acid with a series of amino acids that can be considered isosters thereof. We have tested all the analogues (**1-8**) against a panel of Gram positive bacteria including MRSA to compare the biological activity with teixobactin. This study is aimed at deciphering the most suited amino acids which can replace L-*allo*-enduracididine. We believe that the amino acids which possess a similar structure and functional group (isostere) as the L-*allo*-enduracididine amino acid are best suited for its replacement. The amino acids Lysine (Lys), Ornithine (Orn), L-2,4- Diaminobutyric acid (DAB) and L-1,3-Diaminopropionic acid (DAP) were chosen as these are the closest amine containing isosteres to L-*allo*-enduracididine. Furthermore, through these amino acids we could sequentially shorten the side chain length by one methylene unit from 4 C atoms to 1 C atom. To further expand the number of teixobactin analogues and to reduce the overall cost and time taken by avoiding the re-syntheses of analogues containing non-natural guanidine side-chains, we have used a one-step route from our previous synthesis[7] and inspired by the results of Tor *et. al.*[14] to directly convert the deprotected amino-side chains into their corresponding guanidines (Figure 2). For this purpose, the commercially available 1*H*-Pyrazole-carboxamidine hydrochloride in MeOH with Et_3N was used (Figure 2, page S3) followed by HPLC purification to remove any excess reagent present in the reaction mixture. By introducing Lys, Orn, DAB and DAP one at a time at position 10 we synthesised analogues Lys_{10}-teixobactin (**1**), Orn_{10}-teixobactin (**3**), Dab_{10}-teixobactin (**5**) and Dap_{10}-teixobactin (**7**) (figure 3). We then directly converted Lys_{10}-teixobactin (**1**) to (Homoarginine) $HoArg_{10}$-teixobactin (**2**), DAB_{10}-teixobactin (**5**) to $NorArg_{10}$-teixobactin (**6**) and DAP_{10}-teixobactin (**7**) to (L-2-amino-3-guanidinoaminopropionic acid) $GAPA_{10}$-teixobactin (**8**) using the aforementioned protocols (Figure 2). We thus synthesised 8 teixobactin analogues namely Lys_{10}-teixobactin (**1**), $HoArg_{10}$-teixobactin (**2**), Orn_{10}-teixobactin (**3**), Arg_{10}-teixobactin (**4**), DAB_{10}-teixobactin (**5**), $NorArg_{10}$-teixobactin **(6)**,

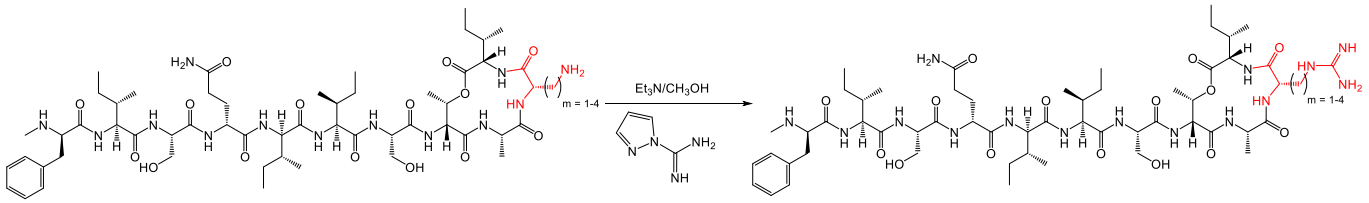


Figure 2: General scheme for the syntheses of teixobactin analogues **2**, **6** and **8** from their amino precursors **1**, **5** and **7** respectively.

View Article Online
DOI: 10.1039/C7CC04021K

Compound Number	Name	MIC against MRSA ATCC 33591 (μg/mL)	MIC against *Staphylococcus epidermidis* ATCC 12228 (μg/mL)	MIC against *Bacillus subtilis* 168 (μg/mL)
1	Lys_{10}-teixobactin	1	1	0.25
2	$HoArg_{10}$-teixobactin	1	0.25	0.125
3	Orn_{10}-teixobactin	2	1	0.25
4	Arg_{10}-teixobactin	2	2	1
5	DAB_{10}-teixobactin	2	2	1
6	$NorArg_{10}$-teixobactin	1	1	0.5
7	DAP_{10}-teixobactin	4	2	0.5
8	$GAPA_{10}$-teixobactin	4	4	1
9	Teixobactin	0.25[2]	0.078-0.31[2]	0.02[2]
10	Vancomycin	2	2	0.25-0.5

Table 1: Minimum Inhibitory Concentration (MIC) values of compounds **1**-**10** against MRSA ATCC 33591, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis 168*.

DAP_{10}-teixobactin (**7**) and $GAPA_{10}$-teixobactin (**8**) (Figure 3).

The syntheses and biological activity against *Staphylococcus aureus* of Lys_{10}-teixobactin (**1**)[8 12], Orn_{10}-teixobactin (**3**)[5], Arg_{10}-teixobactin (**4**)[6 7] and $NorArg_{10}$-teixobactin[13] have already been reported. There has been very limited evaluation of teixobactin analogues against MRSA. Among the synthesised analogues, Orn_{10}-teixobactin (**3**) (MIC 2 μg/mL)[5] and $NorArg_{10}$-teixobactin[13] (MIC 16 μg/mL)[13] are the only ones tested against MRSA. However, a different strain of MRSA was used. To address this, we have evaluated the antibacterial activity of our eight teixobactin analogues (**1**-**8**) against MRSA ATCC 33591 (identical to the strain reported in Nature[2]) to compare the biological activities with that of teixobactin (Table 1). All the analogues were also screened against *Staphylococcus epidermidis* and *Bacillus subtilis* to provide a more comprehensive overview of the biological activities of these molecules. Vancomycin was used as a control.

Herein we report for the first time the MIC of Lys_{10}-teixobactin (**1**) against MRSA which was found to be two times better than

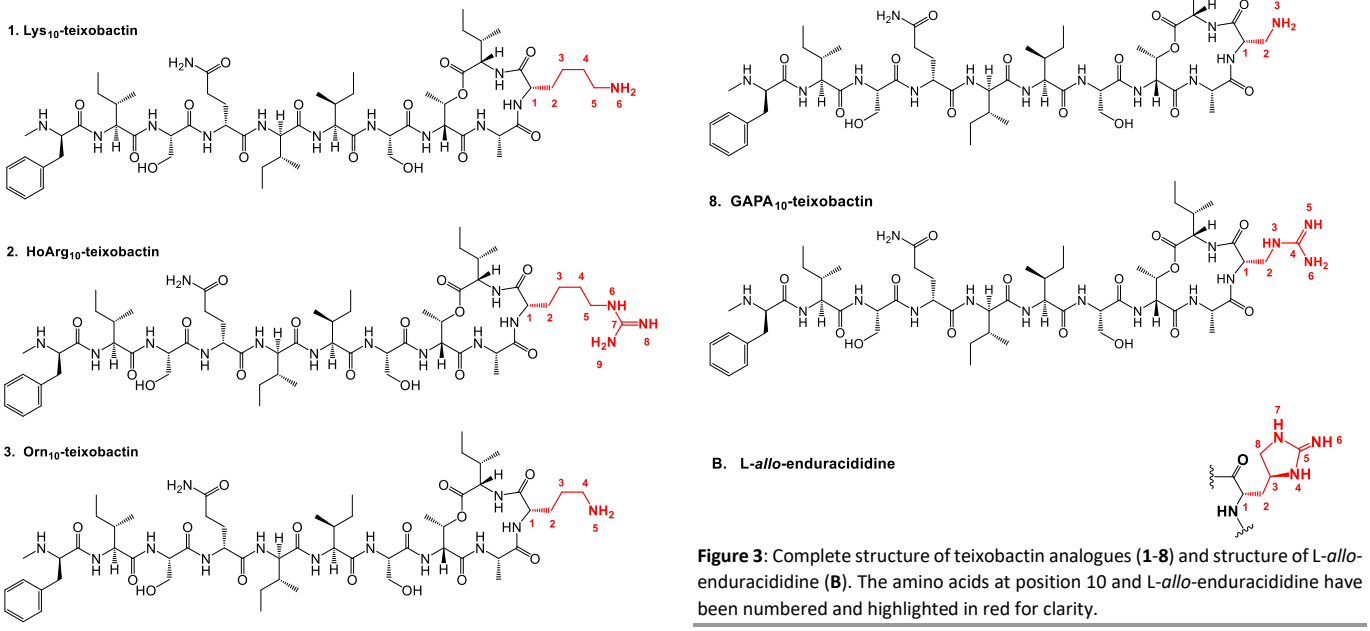


Figure 3: Complete structure of teixobactin analogues (**1**-**8**) and structure of L-*allo*-enduracididine (**B**). The amino acids at position 10 and L-*allo*-enduracididine have been numbered and highlighted in red for clarity.

that of Arg_{10}-teixobactin (Table 1) against the same species. $HoArg_{10}$-teixobactin (**2**) was found to have identical activity as Lys_{10}- teixobactin. The MIC of Orn_{10}-teixobactin[5] (**3**) was found to be consistent with that reported in literature and identical to that of Arg_{10}-teixobactin (**4**). The MIC of Dab_{10}-teixobactin (**5**) was found to be identical to Orn_{10}- teixobactin (**3**) (Table 1) which is expected as both DAB and Orn can be considered isosters of L-*allo*-Enduracididine (Figure 3). $NorArg_{10}$ teixobactin (**6**) showed two times better MIC than Arg_{10}-teixobactin (Table 1) although both Norarginine and Arginine are isosteric with L-*allo*- enduracididine. The difference can be potentially attributed to lower flexibility of **6** due to a reduced carbon chain length of NorArg and therefore being structurally more similar to L-*allo*-enduracididine. On further reducing the side-chain length of the amino acid at position 10 by one methylene group we obtained the analogues Dap_{10}-teixobactin (**7**) and $GAPA_{10}$-teixobactin (**8**) which were found to be less active than analogues **1**-**6** in MRSA. Both Dap_{10}-teixobactin (**7**) and $GAPA_{10}$-teixobactin (**8**) have an MIC two times higher than Arg_{10}-texiobactin (**3**). The higher MICs in MRSA are probably because although both DAP and GAPA have structural similarities to L-*allo*-enduracididine (Figure 3), they have a shorter carbon chain thereby affording less flexibility. The MIC trend observed in *Staphylococcus epidermidis* and *Bacillus subtilis is* similar to that of MRSA. However, all compounds (**1**-**8**) have shown 2-4 times better MICs in *B. subtilis* compared to MRSA and *S. epidermidis.* $HoArg_{10}$-teixobactin (**2**) was found to be the most potent analogue possessing the lowest MIC in all three species, followed by Lys_{10}-teixobactin (**1**) and Orn_{10}-teixobactin (**3**). Overall, the MICs observed are consistent with the hypothesis that the closest isosteres of L-*allo*-enduracididine are most suited for its replacement.

In conclusion, we have synthesised 8 teixobactin analogues and tested them against a panel of Gram positive bacteria including MRSA to determine the most suited amino acids for replacing the synthetically challenging L-*allo*-enduracididine at position 10. Furthermore, for the rapid syntheses of guanidine containing teixobactin analogues from amines, we have used the direct conversion of amines to guanidines for completely deprotected teixobactin analogues. This method is compatible with secondary amines as well as other amino acid side chains and will therefore be suitable for diverse peptides. Based on the MICs against MRSA, we observe that all the synthesised compounds are active and therefore can be used as leads for further derivatisation. Lysine, homoarginine and norarginine are all equally suitable substitutions for L-*allo*-enduracididine. Furthermore, almost no difference in MIC was observed between the amino derivatives and their corresponding guanidine counterparts. This implies that there is a considerable tolerance for the substitution of L-*allo*-enduracididine with both proteogenic and non-proteogenic amino acids containing amine or guanidine side-chains. We have synthesised eight potent teixobactin analogues three of which show very promising activity against MRSA (MIC 1 μg/mL). The results from this work are expected to facilitate the development of teixobactin analogues against MRSA and have the potential to address the challenges posed by multi-drug resistant bacteria.

Anish Parmar, Abhishek Iyer and Charlotte S. Vincent would like to thank the University of Lincoln for funding. Edward Taylor would like to thank the Royal Society for their kind support (grant number UF100116). Ishwar Singh would like to acknowledge the Royal Society for their kind support (grant number (RG130163) and Horizon 2020 (645684)). Daniel G Lloyd, Edward J Taylor and Ishwar Singh would like to acknowledge the Rosetrees trust for their kind support (grant number JS16/M583). Jan Goeman and Jos Van den Begin from Ghent University are thanked for the LC-MS analyses and Nicholas Riess from University of Lincoln is thanked for the and HRMS analyses.

Notes and references

1 *http://amr-review.org/*, 2015.

2 L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, 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**, 455–459.

3 F. von Nussbaum and R. D. Süssmuth, *Angew. Chemie Int. Ed.*, 2015, **54**, 6684–6686.

4 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**, 2788–2791.

5 K. Jin, I. H. Sam, K. H. L. Po, D. Lin, E. H. Ghazvini Zadeh, S. Chen, Y. Yuan and X. Li, *Nat. Commun.*, 2016, **7**, 12394.

6 Y. E. Jad, G. A. Acosta, T. Naicker, M. Ramtahal, A. El-Faham, T. Govender, H. G. Kruger, B. G. De La Torre and F. Albericio, *Org. Lett.*, 2015, **17**, 6182–6185.

7 A. Parmar, A. Iyer, C. S. Vincent, D. Van Lysebetten, S. H. Prior, A. Madder, E. J. Taylor and I. Singh, *Chem. Commun.*, 2016, **52**, 6060–6063.

8 H. Yang, K. H. Chen and J. S. Nowick, *ACS Chem. Biol.*, 2016, **11**, 1823–26.

9 A. Parmar, S. H. Prior, A. Iyer, C. S. Vincent, D. Van Lysebetten, E. Breukink, A. Madder, E. J. Taylor and I. Singh, *Chem. Commun.*, 2017, **53**, 2016–2019.

10 H. Yang, D. R. Du Bois, J. W. Ziller and J. S. Nowick, *Chem. Commun.*, 2017, **53**, 2772–2775.

11 S. A. H. Abdel Monaim, Y. E. Jad, E. J. Ramchuran, A. El-Faham, T. Govender, H. G. Kruger, B. G. de la Torre and F. Albericio, *ACS Omega*, 2016, **1**, 1262–1265.

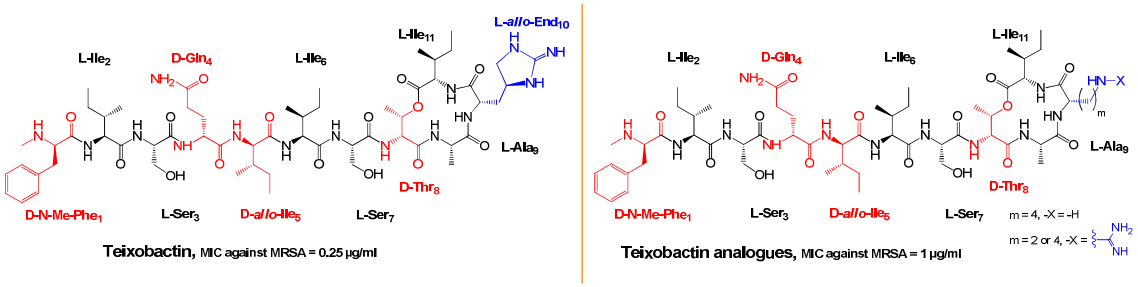
12 C. Wu, Z. Pan, G. Yao, W. Wang, L. Fang and W. Su, *RSC Adv.*, 2017, **7**, 1923–1926.

13 K. Jin, K. H. L. Po, S. Wang, J. A. Reuven, C. N. Wai, H. T. Lau, T. H. Chan, S. Chen and X. Li, *Bioorg. Med. Chem.*, 2017, 10.1016/j.bmc.2017.04.039.

14 K. M. Hamill, L. S. McCoy, E. Wexselblatt, J. D. Esko and Y. Tor, *Chem. Sci.*, 2016, **7**, 5059–5068.

View Article Online
DOI: 10.1039/C7CC04021K

TABLE OF CONTENTS ENTRY



The synthesis and incorporation of the L-*allo*-enduracididine amino acid is a major bottleneck in the development of potent analogues of the cyclic depsipeptide teixobactin. In this article, we have synthesised 8 analogues of teixobactin using commercially available building blocks by replacing the L-*allo*-enduracididine amino acid with its isosteres. Furthermore, we have screened all the compounds against a panel of Gram positive bacteria including MRSA and report that all the analogues were active with 3 of them showing very promising activity against MRSA (MIC 1 µg/mL). We can conclude that amino acids which are the closest isosteres of L-*allo*-enduracididine are the key to synthesising simplified potent analogues of teixobactin.