





Article

Cysteines and Disulfide-Bridged Macrocyclic Mimics of Teixobactin Analogues and Their Antibacterial Activity Evaluation against Methicillin-Resistant *Staphylococcus Aureus* (MRSA)

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Abstract: Teixobactin is a highly potent cyclic depsipeptide which kills a broad range of multi-drug resistant, Gram-positive bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) without detectable resistance. In this work, we describe the design and rapid synthesis of novel teixobactin analogues containing two cysteine moieties, and the corresponding disulfide-bridged cyclic analogues. These analogues differ from previously reported analogues, such as an Arg₁₀-teixobactin, in terms of their macrocyclic ring size, and feature a disulfide bridge instead of an ester linkage. The new teixobactin analogues were screened against Methicillin-resistant *Staphylococcus aureus* and Methicillin-sensitive *Staphylococcus aureus*. Interestingly, one teixobactin analogue containing all L-amino acid building blocks showed antibacterial activity against MRSA for the first time. Our data indicates that macrocyclisation of teixobactin analogues with disulfide bridging is important for improved antibacterial activity. In our work, we have demonstrated the unprecedented use of a disulfide bridge in constructing the macrocyclic ring of teixobactin analogues.

Keywords: teixobactin; MRSA; disulfide bridge

1. Introduction

Antimicrobial resistance (AMR) is predicted to cause 10 million deaths every year, and US \$100 trillion in economic damages by 2050 [1]. Current antibiotics have proven to be ineffective against the recent surge of drug-resistant bacteria. Furthermore, the discovery and development of new antibiotics has been considerably slower than increase in bacterial resistance. Thus, there is a constant need to develop new and potent antibacterial compounds. The recently discovered natural product, teixobactin (Figure 1A) has shown highly potent activity against major, clinically relevant, Gram-positive

bacteria, including resistant bacterial strains such as *Enterococcus spp.* (vancomycin-resistant enterococci, VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA), and *Mycobacterium tuberculosis* [2]. The nonribosomal cyclic undecapeptide, teixobactin, comprises of four D-amino acids—namely, N-Me-D-Phe₁, D-Gln₄, D-*allo*-Ile₅, and D-Thr₈, and the rare L-*allo*-enduracididine amino acid. Teixobactin kills a number of Gram-positive bacteria without detectable resistance. Furthermore, it is less prone to developing resistance because it operates by at least two known unique modes of action. Teixobactin binds to lipid II (precursor of peptidoglycan), which is a highly conserved pyrophosphate motif of multiple bacterial cell wall substrates and lipid III (a precursor of cell wall teichoic acid) [2]. Previous publications on teixobactin describe the total syntheses of teixobactin [3,4] and its analogues [5–7], as well as their biological activities. Our group [6] and others [5,7] have reported the synthesis of Arg₁₀-teixobactin by replacing the synthetically challenging L-*allo*-enduracididine amino acid occurring in native teixobactin at position 10 with arginine. The first structure-activity relationships (SAR) of Arg₁₀-teixobactin and the importance of the four D-amino acids for antibacterial activity was also described by our research group [6]. Subsequently, we also defined the 3D molecular structures of teixobactin analogues using NMR [8]. The disordered structure of teixobactin analogues was found to be crucial for their biological activity, whereas the D-amino acids were found to be important for maintaining the disordered structure [8]. The crystal structure of a truncated teixobactin analogue was reported by Yang et al., and showed the Ile₆ and Ile₁₁ to be closely placed together through space [7].

Yang et al. [7] also reported the minimum pharmacophore of teixobactin, while Albericio et al. [9] reported on a lysine scan of Arg₁₀-teixobactin. We further reported on the design and synthesis of potent teixobactin analogues against MRSA through the replacement of L-*allo*-enduracididine, showing that L-*allo*-enduracididine can be designed out and analogues can be made to realise the therapeutic potential of teixobactins [10,11].

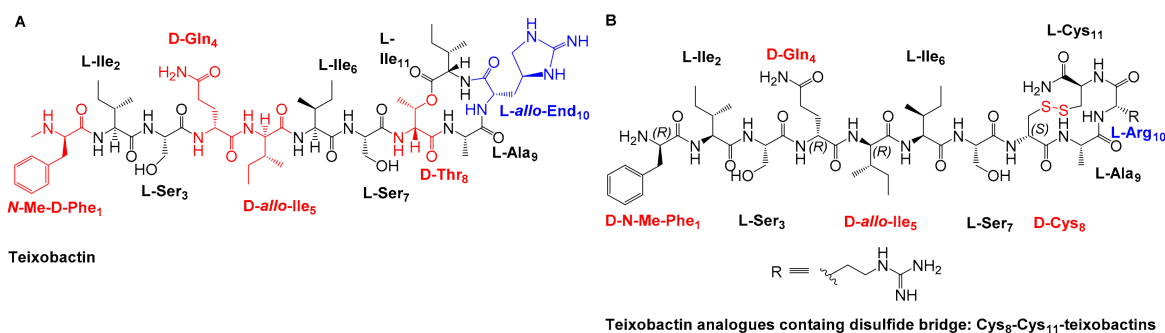


Figure 1. (A) Teixobactin, (B) Cys₈–Cys₁₁ disulfide-teixobactins.

To further improve access to highly potent teixobactin analogues, we also strategically replaced the synthetically challenging enduracididine with commercially available hydrophobic residues, such as leucine and isoleucine [12]. It was shown that Leu₁₀-teixobactin and Ile₁₀-teixobactin displayed activity against MRSA to the same extent as teixobactin. Brimble et al. have also reported on the synthesis and antibacterial activity of teixobactin analogues by replacing L-*allo*-enduracididine with isosteres [13]. Furthermore, teixobactin analogues with lipid tails were reported by Jamieson et al. [14] and recently we described highly potent teixobactin analogues containing proteogenic amino acids at position 10 and their antibacterial activity in vitro and in vivo [15]. As an alternative strategy, we were interested in exploring cysteines and disulfide-bridged macrocyclic mimics of teixobactin analogues.

Cysteine-rich antimicrobial peptides containing multiple disulfide bonds were isolated from plants [16] and animals [17,18]. These peptides have shown protective effects in the living host against anti-bacterial infections [19]. In certain instances, disulfide bonds have been engineered to study the structure-activity relationships (SAR) of antimicrobial peptides, which makes them an interesting tool for SAR studies [20]. Furthermore, the easy introduction of cysteines via commercially

available building blocks and mild conditions for synthesis of disulfide bonds [21] allow for rapid and cost-effective macrocyclization. In this study, we describe simplified teixobactin analogues by introducing cysteines at specific positions (D-Thr₈ and Ile₁₁) and subsequently constructing the macrocyclic ring using disulfide bridges to better understand the structure-activity relationship of teixobactin analogues (Figure 1B). This will also allow us to understand how the core ring size of teixobactin analogues (similar ring size: 14 atoms, teixobactin: 13 atoms) impacts its antibacterial activity.

Here, we report the design, rapid synthesis, and antibacterial activity evaluations against MRSA of eight new teixobactin analogues containing cysteines and the unprecedented use of a disulfide bridge in constructing the macrocyclic ring of teixobactin.

2. Materials and Methods

2.1. Materials

Fmoc-D-Ile-OH, Fmoc-D-Thr(Trt)-OH, and oxyma pure were purchased from Merck Millipore (Watford, UK). All L-amino acids, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), Fmoc-D-Gln(Trt)-OH, Boc-D-Nmethylphenyl-OH, Fmoc-D-Cys-OH, Fmoc-L-Cys-OH, Fmoc-Glu(OAll)-OH, Diisopropylcarbodiimide, and Triisopropylsilane were purchased from Fluorochem, Hadfield, UK. The protecting groups for the amino acids are Pbf for Arg and Trt for Gln, unless specified otherwise. Diisopropylethylamine, supplied as extra-dry, redistilled, and 99.5% pure, was purchased from Sigma Aldrich (Gillingham, UK). Peptide-synthesis grade Dimethylformamide (DMF) and Trifluoroacetic acid (TFA) was purchased from Rathburn chemicals (Walkerburn, UK). Diethyl ether, i-PrOH, MeOH (HPLC grade), and Acetonitrile (HPLC grade) were purchased from Fisher Scientific (Loughborough, UK). Water with the Milli-Q grade standard was obtained in-house from an ELGA Purelab Flex system. Rink amide Chemmatrix resin (manufacturer's loading = 0.49 mmol/g) was obtained from Biotage, Uppsala, Sweden. All chemicals were used without further purification.

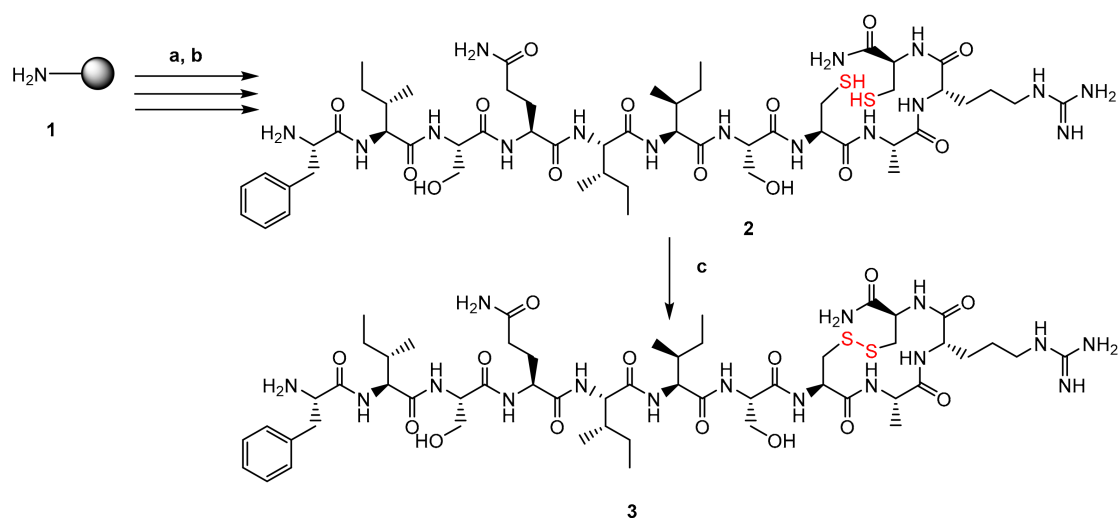
2.2. General Methods

All peptides were analyzed on a Thermo Scientific Dionex Ultimate 3000 HPLC (Thermo Scientific, Hemel Hempstead, UK) equipped with a Phenomenex Gemini NX C18 110 Å (150 × 4.6 mm) (Phenomenex, Macclesfield, UK) column, using the following buffer systems: A: 0.1% HCOOH in milliQ water. B: ACN using a flow rate of 1 mL/min. The column was flushed with 95% A for 5 min prior to an injection and was flushed for 5 min with 95% B and 5% A after the run was finished. Peptides were dissolved in (1:1) 0.1% HCOOH buffer in water and acetonitrile (ACN) and analyzed using the following gradient: 95% A for 2 min; 5–95% B for 25 min; 95% B for 5 min; and 5% A for 4 min. Peptides were dissolved in 0.1% HCOOH buffer in water and in ACN (10–30% ACN), and purified using the same gradient as mentioned above, on a Thermo Scientific Dionex Ultimate 3000 HPLC with a flow rate of 5 mL/min using a Phenomenex Gemini NX C18 110 Å (150 × 10 mm) semi-prep column. ESIMS spectra were recorded on a Thermo Scientific Mass Spectrometer (TSQ Endura, triple quad) in the positive electrospray ion mode.

2.3. Synthesis of Teixobactin Analogue 3

Teixobactin analogue 3 was synthesized as described in Scheme 1. (step a) Commercially available Rink amide Chemmatrix resin (manufacturer's loading 0.49 mmol, 204 mg, 0.1 mmol) resin was swelled in DMF in a reactor. To this resin all the amino acids in the sequence were coupled by the use of 4 equiv of amino acids, 4 equiv of DIC/Oxyma in a microwave peptide synthesiser (Biotage Initiator + Alstra). The coupling time was 5 min at 70 °C. The deprotection was done using 20% of Piperidine in DMF for 3 min at 70 °C, and then for 10 min at room temperature. (step b) After building the linear peptide 2, full cleavage was achieved using TFA/TIS/H₂O = 95:2.5:2.5% by being stirred for 1 h. The peptide was

filtrated into cold diethyl ether ($-20\text{ }^{\circ}\text{C}$) and the precipitate was centrifuged $3 \times 10\text{ min}$ at 7000 rpm to obtain crude peptide. (step c) The crude peptide **2** was cyclized using DMSO/MQ water = 3:1 while maintaining a peptide concentration of 1 mM, and was stirred for 12 h at room temperature. The reaction mixture was purified by using semi-prep RP-HPLC (Figures S1, S3, S5, S7, S9, S11, S13 and S15 in Supporting Information) using the protocols described in the general methods. All the pure fractions were combined together and lyophilized to obtain a white solid. All the teixobactin analogues were purified to ~95% purity, as indicated by HPLC (Figures S1, S3, S5, S7, S9, S11, S13 and S15 in Supporting Information). All the peptides were synthesized using the method described above for teixobactin analogue **3**, and isolated yields were in the range of 15–25%. All teixobactin analogues were characterized by ESIMS in positive mode (Table S1, Figures S2, S4, S6, S8, S10, S12, S14 and S16).



Scheme 1. Synthesis of analogue **1**, **2**, and **3**: **a**. Fmoc-AA(PG)-OH (AA = amino acid, PG = protecting group), DIC/Oxyma microwave couplings, followed by 20% piperidine in Dimethylformamide (DMF). **b**. TFA:TIS:H₂O = 95:2.5:2.5, 1 h. **c**. DMSO:miliQ water = 1:3 (peptide concentration 1 mM), 12 h.

2.4. Antibacterial Screening

For minimum inhibitory concentration (MIC) assays, all peptides were dissolved in DMSO containing 0.002% polysorbate 80. Bacteria was grown in Mueller Hinton broth (Oxoid) in triplicate. All incubations were at $37\text{ }^{\circ}\text{C}$. Dilutions were carried out in triplicate, and 100 μL of autoclaved Mueller Hinton broth was added to wells 2–11 in a 96-well plate. 200 μL of the peptide was added to well one at a concentration of 128 $\mu\text{g}/\text{mL}$. 100 μL of peptide in well one was taken up and pipetted into well two. The mixture was then mixed via pipetting before 100 μL was taken up and pipetted into well three. This process was repeated up to well 10. Once the peptide was added to well 10, 100 μL was taken up and then discarded, ensuring that well 11 had no peptide present. Thus, the concentrations (in $\mu\text{g}/\text{mL}$) were: 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0 (no peptide present). Each well was then inoculated with 100 μL of bacteria that had been diluted to an OD 600 nm of 0.1. This was repeated three times. The 96-well plates were then incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. The MIC was determined to be the lowest concentration at which there was no growth visible.

3. Results and Discussion

3.1. Design and Synthesis

In our major ongoing project to develop simplified teixobactin analogues against multi-drug resistant bacteria which are easily synthetically accessible, we were particularly interested in understanding the role of the ester bond and the ring size of the teixobactin macrocycle. To test this, we selected cysteines to replace D-Thr₈ and Ile₁₁, and a disulfide bridge instead of the ester

linkage in the macrocyclic ring of teixobactin. This replacement allows for the synthesis of an analogue with a larger macrocyclic ring (14 atoms) similar to the native teixobactin macrocycle (13 atoms). The use of a disulfide bridge in teixobactin analogues allows the incorporation of all amino acids in an automated manner from commercially available building blocks. Moreover, the macrocyclization can be achieved in solutions using mild conditions, such as air oxidation.

Teixobactin contains four D-amino acids (*N*-Me-D-Phe₁, D-Gln₄, D-*allo*-Ile₅ and D-Thr₈). To test the feasibility of the synthesis of teixobactin analogues containing a disulfide bridge, we selected all L-amino acids for cost-effectiveness. The synthesis of all teixobactin analogues 2–9 (Figure 2) were achieved successfully, as described in Scheme 1. After establishing the synthesis of teixobactin analogues with L-amino acids, we turned our attention to the synthesis of teixobactin analogues (4,5) with four D-amino acids similar to teixobactin. Biological evaluation of these analogues would facilitate an understanding of their role, as well as a comparison with teixobactin analogues containing L-amino acids (2,3). D-amino acids of teixobactin analogues are reported to be important for their biological activity. To test whether this was also true with cysteines containing teixobactin analogues, we synthesized analogues (6,7). The analogues (8,9) were synthesized to evaluate the role methyl group at D-phenylalanine₁ in antibacterial activity.

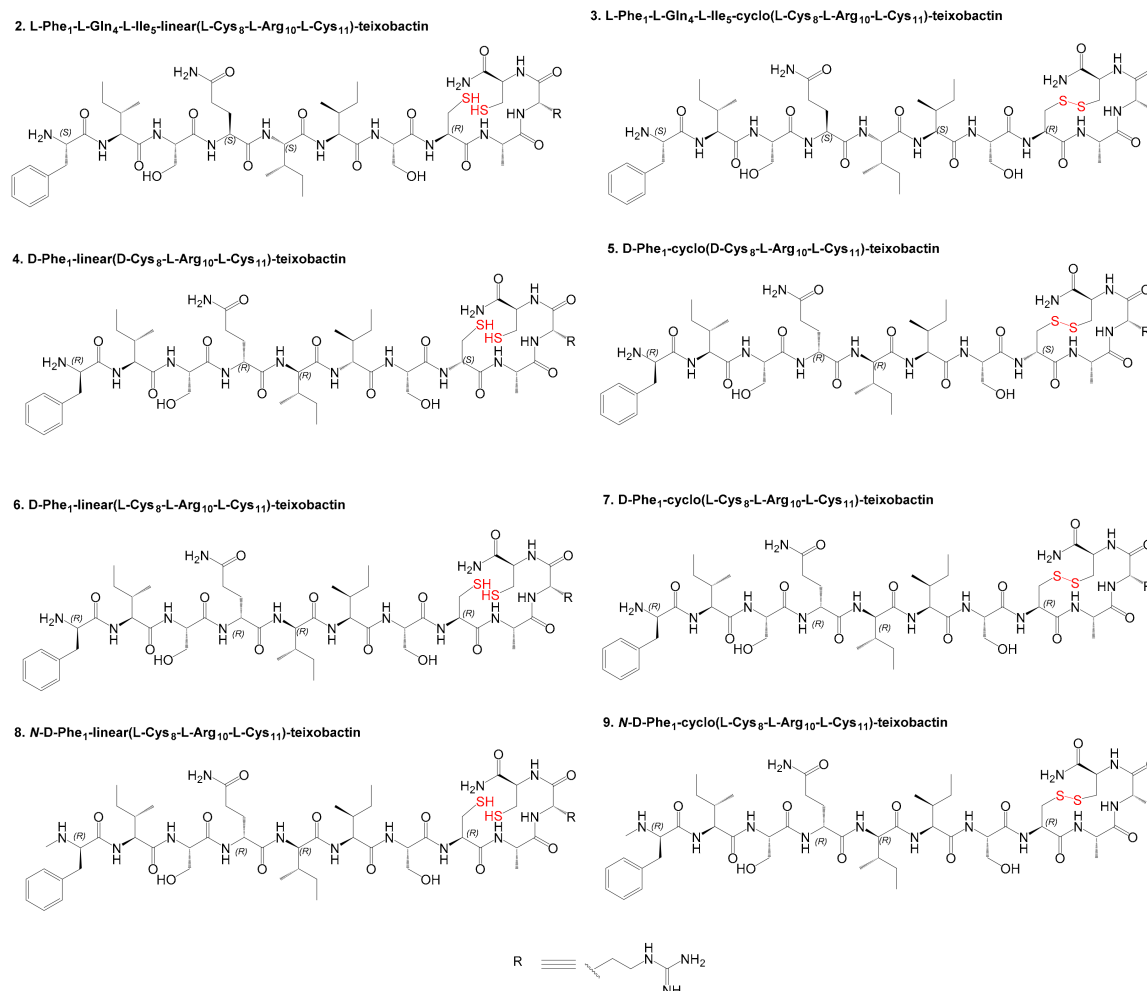


Figure 2. Structure of teixobactin analogues, 2–9.

The synthesis of teixobactin analogue 3 started on Rink amide Chemmatrix resin, using an automated microwave peptide synthesizer. The coupling of all amino acid (AA) building blocks was achieved using four equivalents of AA and 4 equivalents of DIC/Oxyma at 70 °C for 5 min. Fmoc deprotection was performed using 20% piperidine in DMF (Scheme 1). On completion of the

synthesis, the peptide was released and deprotected from resin in a single step using TFA:TIS:H₂O. The crude teixobactin analogue **2** was cyclized in DMSO and miliQ water using air oxidation to obtain analogue **3**. Analogue **3** was purified using reverse phase HPLC (Figure S1), and its identity was confirmed by ESI-MS (Figure S2). The isolated yields of teixobactin analogues were found to be 15–25%.

3.2. Antibacterial Studies

Antibacterial activity of the teixobactin analogues was assessed against Methicillin-resistant *Staphylococcus aureus* (MRSA ATCC 33591, MRSA ATCC 70069) and Methicillin-sensitive *Staphylococcus aureus*. The minimum inhibitory concentration was measured using the broth microdilution method (see Experimental Section 2.4). Interestingly, the linear analogues **2** showed very low activity (MIC 128 µg/mL, Table 1) against MRSA ATCC 33591. The cyclisation of linear teixobactin analogues is reported to improve antibacterial activity [7]. The analogue **3** surprisingly showed 2–4 times higher activity (MIC 32–64 µg/mL) than analogue **2** against MRSA ATCC 33591, despite having all L-amino acids. These results are of interest since, thus far, there has been no activity reported for linear or cyclic analogues of teixobactin against MRSA containing all L-amino acids. Analogue **3** did not exhibit any activity against MRSA ATCC 70069 and Methicillin-sensitive *Staphylococcus aureus* up to the tested concentration of 128 µg/mL. Unexpectedly, the analogues **4,5** containing D-amino acids (D-Phe₁, D-Gln₄, D-*allo*-Ile₅ and D-Cys₈) have not shown any activity against MRSA and MSSA. This observation is contrary to previous findings where teixobactin analogues containing ester linkages and D-amino acids were reported to show good antibacterial activity [8]. Thus, our finding indicates that teixobactin analogues containing a disulfide bridge do not follow the structure activity trend of teixobactin analogues containing an ester linkage in the macrocycle. The analogues **6,7** and **8,9** containing three D-amino acids (D-Phe₁ (for **6,7**), *N*-D-Phe₁ (for **8,9**), D-Gln₄, D-*allo*-Ile₅ and L-Cys₈) have not shown any antibacterial activity against MRSA and MSSA. The low activity (**3**) and lack of any antibacterial activity (**4–9**) of teixobactin analogues is most probably due to a larger ring size (14 atoms) and lack of hydrophobic contact between Ile₆ and Ile₁₁ [22] due to the replacement of Ile₁₁ with cysteine in teixobactin analogues containing a disulfide bridge.

Table 1. List of teixobactin analogues (**2–9**). MIC: Minimum Inhibitory Concentration in µg/mL; all the MIC runs in triplicate. (-) = Not evaluated.

Compound No.	MRSA ATCC 33591	MRSA ATCC 700699	S. aureus ATCC 29213
2	128	>128	>128
3	32–64	>128	>128
4	>128	>128	>128
5	>128	>128	>128
6	>128	-	>128
7	>128	-	>128
8	>128	-	>128
9	>128	-	>128
Vancomycin	-	2	4
Leu ₁₀ -teixobactin ¹²	0.25	0.25	0.062
Teixobactin ¹²	0.25	-	-

4. Conclusions

We have developed a rapid synthesis of new teixobactin analogues, either containing cysteines or a disulfide bridge. This synthesis uses the automated incorporation of all amino-acid building blocks. The teixobactin analogue containing L-amino-acid building blocks with a disulfide bridge showed antibacterial activity against MRSA. These results suggest that the cyclization of teixobactin analogues is important, but also that D-amino acids are not essential for antibacterial activity against MRSA. Moreover, we have demonstrated the unprecedented use of a disulfide bridge in constructing

the macrocyclic ring of teixobactin analogues. Our work lays the foundation for rapid synthesis of further simplified teixobactin analogues containing cysteines and a disulfide bridge, and may provide molecules suitable for addressing the challenges from antimicrobial resistance.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4923/10/4/183/s1>, Table S1: Compound number, name, chemical formula, exact mass and mass found for compounds 2–9. Figure S1: HPLC trace of purified teixobactin analogue 2 (gradient: 5–95% ACN in 25 min using: A: 0.1% HCOOH in water, B: ACN). Figure S2: ESI-MS of purified teixobactin analogue 2. Exact mass calcd. For $C_{53}H_{90}N_{16}O_{14}S_2 = 1238.63$, found $M + H^+ = 1239.62$, $M/2 + H^+ = 620.38$. Figure S3: HPLC trace of purified teixobactin analogue 3 (gradient: 5–95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN). Figure S4: ESI-MS of purified teixobactin analogue 3. Exact mass calcd. For $C_{53}H_{88}N_{16}O_{14}S_2 = 1236.61$, found $M + H^+ = 1237.72$, $M/2 + H^+ = 619.47$. Figure S5: HPLC trace of purified teixobactin analogue 4 (gradient: 5–95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN). Figure S6: ESI-MS of purified Teixobactin analogue 4. Exact mass calcd. For $C_{53}H_{90}N_{16}O_{14}S_2 = 1238.63$, found $M + H^+ = 1239.57$, $M/2 + H^+ = 620.76$. Figure S7: HPLC trace of purified teixobactin analogue 5 (gradient: 5–95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN). Figure S8: ESI-MS of purified teixobactin analogue 5. Exact mass calcd. For $C_{53}H_{88}N_{16}O_{14}S_2 = 1236.61$, found $M + H^+ = 1237.53$, $M/2 + H^+ = 619.53$. Figure S9: HPLC trace of purified teixobactin analogue 6 (gradient: 5–95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN). Figure S10: ESI-MS of purified teixobactin analogue 6. Exact mass calcd. For $C_{53}H_{90}N_{16}O_{14}S_2 = 1238.63$, found $M + H^+ = 1239.57$, $M/2 + H^+ = 620.76$. Figure S11: HPLC trace of purified teixobactin analogue 7 (gradient: 5–95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN). Figure S12: ESI-MS of purified teixobactin analogue 7. Exact mass calcd. For $C_{53}H_{88}N_{16}O_{14}S_2 = 1236.61$, found $M + H^+ = 1237.56$, $M/2 + H^+ = 619.36$. Figure S13: HPLC trace of purified teixobactin analogue 8 (gradient: 5–95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN). Figure S14: ESI-MS of purified teixobactin analogue 8. Exact mass calcd. For $C_{54}H_{92}N_{16}O_{14}S_2 = 1252.64$, found $M + H^+ = 1253.64$, $M/2 + H^+ = 627.44$. Figure S15: HPLC trace of purified teixobactin analogue 9 (gradient: 5–95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN). Figure S16: ESI-MS of purified teixobactin analogue 9. Exact mass calcd. For $C_{54}H_{90}N_{16}O_{14}S_2 = 1250.63$, found $M + H^+ = 1251.63$, $M/2 + H^+ = 626.24$.

Author Contributions: The project was conceived and designed by I.S. The manuscript was written by I.S. and A.I. with inputs from authors. R.M. and A.I. and A.P. carried out the teixobactin analogues syntheses. D.G.L., E.J.T., E.T.L.G. and R.L. have contributed to the antibacterial studies. S.S. and A.M. was responsible for the LC-MS analyses. All authors have given approval to the final version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Review on Antimicrobial Resistance. Available online: <http://amr-review.org/> (accessed on 9 September 2018).
2. Ling, L.L.; Schneider, T.; Peoples, A.J.; Spoering, A.L.; Engels, I.; Conlon, B.P.; Mueller, A.; Hughes, D.E.; Epstein, S.; Jones, M.; et al. A New Antibiotic Kills Pathogens without Detectable Resistance. *Nature* **2015**, *517*, 455–459. [[CrossRef](#)] [[PubMed](#)]
3. Giltrap, A.M.; Dowman, L.J.; Nagalingam, G.; Ochoa, J.L.; Linington, R.G.; Britton, W.J.; Payne, R.J. Total Synthesis of Teixobactin. *Org. Lett.* **2016**, *18*, 2788–2791. [[CrossRef](#)] [[PubMed](#)]
4. Jin, K.; Sam, I.H.; Po, K.H.L.; Lin, D.; Ghazvini Zadeh, E.H.; Chen, S.; Yuan, Y.; Li, X. Total Synthesis of Teixobactin. *Nat. Commun.* **2016**, *7*, 12394. [[CrossRef](#)] [[PubMed](#)]
5. Jad, Y.E.; Acosta, G.A.; Naicker, T.; Ramtahal, M.; El-Faham, A.; Govender, T.; Kruger, H.G.; De La Torre, B.G.; Albericio, F. Synthesis and Biological Evaluation of a Teixobactin Analogue. *Org. Lett.* **2015**, *17*, 6182–6185. [[CrossRef](#)] [[PubMed](#)]
6. Parmar, A.; Iyer, A.; Vincent, C.S.; Van Lysebetten, D.; Prior, S.H.; Madder, A.; Taylor, E.J.; Singh, I. Efficient Total Syntheses and Biological Activities of Two Teixobactin Analogues. *Chem. Commun.* **2016**, *52*, 6060–6063. [[CrossRef](#)] [[PubMed](#)]
7. Yang, H.; Chen, K.H.; Nowick, J.S. Elucidation of the Teixobactin Pharmacophore. *ACS Chem. Biol.* **2016**, *11*, 1823–1826. [[CrossRef](#)] [[PubMed](#)]

8. Parmar, A.; Prior, S.H.; Iyer, A.; Vincent, C.S.; Van Lysebetten, D.; Breukink, E.; Madder, A.; Taylor, E.J.; Singh, I. Defining the Molecular Structure of Teixobactin Analogues and Understanding Their Role in Antibacterial Activities. *Chem. Commun.* **2017**, *53*, 2016–2019. [[CrossRef](#)] [[PubMed](#)]
9. Abdel Monaim, S.A.H.; Jad, Y.E.; Ramchuran, E.J.; El-Faham, A.; Govender, T.; Kruger, H.G.; de la Torre, B.G.; Albericio, F. Lysine Scanning of Arg₁₀—Teixobactin: Deciphering the Role of Hydrophobic and Hydrophilic Residues. *ACS Omega* **2016**, *1*, 1262–1265. [[CrossRef](#)] [[PubMed](#)]
10. Parmar, A.; Iyer, A.; Lloyd, D.G.; Vincent, C.S.; Prior, S.H.; Madder, A.; Taylor, E.J.; Singh, I. Syntheses of Potent Teixobactin Analogues against Methicillin-Resistant *Staphylococcus Aureus* (MRSA) through the Replacement of l-Allo-Enduracididine with Its Isosteres. *Chem. Commun.* **2017**, *53*, 7788–7791. [[CrossRef](#)] [[PubMed](#)]
11. Fiers, W.D.; Craighead, M.; Singh, I. Teixobactin and Its Analogues: A New Hope in Antibiotic Discovery. *ACS Infect. Dis.* **2017**, *3*, 688–690. [[CrossRef](#)] [[PubMed](#)]
12. Parmar, A.; Iyer, A.; Prior, S.H.; Lloyd, D.G.; Leng, T.; Charlotte, S.; Palmal-Pallag, T.; Bachrati, C.Z.; Breukink, E.; Madder, A.; et al. Teixobactin Analogues Reveal Enduracididine to Be Non-Essential for Highly Potent Antibacterial Activity and Lipid II Binding. *Chem. Sci.* **2017**. [[CrossRef](#)] [[PubMed](#)]
13. Schumacher, C.E.; Harris, P.W.R.; Ding, X.-B.; Krause, B.; Wright, T.H.; Cook, G.M.; Furkert, D.P.; Brimble, M.A. Synthesis and Biological Evaluation of Novel Teixobactin Analogues. *Org. Biomol. Chem.* **2017**, *15*, 8755–8760. [[CrossRef](#)] [[PubMed](#)]
14. Girt, G.C.; Mahindra, A.; Al Jabri, Z.J.H.; De Ste Croix, M.; Oggioni, M.R.; Jamieson, A.G. Lipopeptidomimetics Derived from Teixobactin Have Potent Antibacterial Activity against *Staphylococcus Aureus*. *Chem. Commun.* **2018**, *54*, 2767–2770. [[CrossRef](#)] [[PubMed](#)]
15. Parmar, A.; Lakshminarayanan, R.; Iyer, A.; Mayandi, V.; Leng Goh, E.T.; Lloyd, D.G.; Chalasani, M.L.S.; Verma, N.K.; Prior, S.H.; Beuerman, R.W.; et al. Design and Syntheses of Highly Potent Teixobactin Analogues against *Staphylococcus Aureus*, Methicillin-Resistant *Staphylococcus Aureus* (MRSA), and Vancomycin-Resistant Enterococci (VRE) *in Vitro* and *in Vivo*. *J. Med. Chem.* **2018**, *61*, 2009–2017. [[CrossRef](#)] [[PubMed](#)]
16. Haag, A.F.; Kerscher, B.; Dall’Angelo, S.; Sani, M.; Longhi, R.; Baloban, M.; Wilson, H.M.; Mergaert, P.; Zanda, M.; Ferguson, G.P. Role of Cysteine Residues and Disulfide Bonds in the Activity of a Legume Root Nodule-Specific, Cysteine-Rich Peptide. *J. Biol. Chem.* **2012**, *287*, 10791–10798. [[CrossRef](#)] [[PubMed](#)]
17. Ehret-sabatier, L.; Loew, D.; Fehlbaum, P.; Jules, A.H.; Dorsselaer, A.V.; Goyffon, M.; Bulet, P. Protein Chemistry and Structure: Characterization of Novel Cysteine-Rich Antimicrobial Peptides from Scorpion Blood Characterization of Novel Cysteine-Rich Antimicrobial Peptides from Scorpion Blood. *J. Biol. Chem.* **1996**, *271*, 29537–29544. [[CrossRef](#)] [[PubMed](#)]
18. Zhong, J.; Wang, W.; Yang, X.; Yan, X.; Liu, R. A Novel Cysteine-Rich Antimicrobial Peptide from the Mucus of the Snail of *Achatina Fulica*. *Peptides* **2013**, *39*, 1–5. [[CrossRef](#)] [[PubMed](#)]
19. Fehlbaum, P.; Bulet, P.; Chernysh, S.; Briand, J.; Roussel, J.; Letellier, L.; Hetru, C.; Hoffmann, J.A. Structure-Activity Analysis of Thanatin, a 21-Residue Inducible Insect Defense Peptide with Sequence Homology to Frog Skin Antimicrobial Peptides. *Biochemistry* **1996**, *93*, 1221–1225. [[CrossRef](#)]
20. Wu, Z.; Hoover, D.M.; Yang, D.; Boulegue, C.; Santamaria, F.; Oppenheim, J.J.; Lubkowski, J.; Lu, W. Engineering Disulfide Bridges to Dissect Antimicrobial and Chemotactic Activities of Human -Defensin 3. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 8880–8885. [[CrossRef](#)] [[PubMed](#)]
21. Cuthbertson, A.; Indrevoll, B. Regioselective Formation, Using Orthogonal Cysteine Protection, of an Alpha-Conotoxin Dimer Peptide Containing Four Disulfide Bonds. *Org. Lett.* **2003**, *5*, 2955–2957. [[CrossRef](#)] [[PubMed](#)]
22. Yang, H.; Du Bois, D.R.; Ziller, J.W.; Nowick, J.S. X-Ray Crystallographic Structure of a Teixobactin Analogue Reveals Key Interactions of the Teixobactin Pharmacophore. *Chem. Commun.* **2017**, *53*, 2772–2775. [[CrossRef](#)] [[PubMed](#)]

