Supplementary Information

FOR

# Defining the molecular structure of teixobactin analogues and their role in antibacterial activities

Anish Parmar, [a] Stephen H. Prior, [b] Abhishek Iyer, [a, c] Charlotte Vincent, [c] Dorien Van Lysebetten,[c] Eefjan Breukink[e] , Annemieke Madder, [c] Edward Taylor [d, *] and Ishwar Singh [a, *]

[a] School of Pharmacy, JBL Building, University of Lincoln, Beevor St. Lincoln LN67DL, UK.
[b] School of Chemistry, University of Lincoln, Beevor St. Lincoln LN67DL, UK.
[c] Organic and Biomimetic Chemistry Research Group, Department of Organic Chemistry, Ghent University, Krijgslaan 281 (S4), B-9000 Ghent, Belgium
[d] School of Life Sciences, Joseph Bank Laboratories, University of Lincoln, Green Lane, Lincoln LN6 7DL.
[e] Department of Membrane Biochemistry and Biophysics, Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

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## I. Materials

All L amino acids, Fmoc-D-Ala-OH Fmoc-D-Gln(Trt)-OH, Boc-N-methyl-D-phenylalanine, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium3-oxidhexafluorophosphate (HATU), Phenylsilane ($PhSiH_3$), Tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)$], Diisoproplycarbodiimide (DIC) and Triisopropylsilane (TIS) were purchased from Fluorochem, UK. Fmoc-D-*allo*-Ile-OH and oxyma pure were purchased from Merck Millipore. The side chain protecting groups for the amino acids are $^t$Bu for Ser, Pbf for Arg and Trt for Gln and Thr unless specified otherwise. Diisopropylethylamine (DIPEA), supplied as extra dry, redistilled, 99.5 % pure, Acetic anhydride, allyl chloroformate and $CDCl_3$ and were purchased from Sigma Aldrich. Tritylchloride and 4-(Dimethylamino)pyridine were purchased from Alfa Aesar. Dimmethylformamide (DMF) peptide synthesis grade was purchased from Rathburn chemicals. Triethylamine, Diethyl ether ($Et_2O$), Dimethylsulfoxide (DMSO), Dichloromethane (DCM), Tetrahydrofuran (extra dry with molecular sieves), Formic acid 98-100% purity and Acetonitrile (HPLC grade) were purchased from Fisher Scientific. Water with the Milli-Q grade standard was obtained in-house from an ELGA Purelab Flex system. 2-Chlorotritylchloride resin (manufacturer's loading: 1.20 mmol/g) was purchased from Fluorochem. All chemicals were used without further purification.

## II. Equipment used for the analysis and purification of compounds

All peptides were analysed on a Thermo Scientific Dionex Ultimate 3000 RP-HPLC equipped with a Phenomenex Gemini NX C18 110 Å (150 x 4.6 mm) 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 analysed using the following gradient: 95% A for 2 min. 5-95% B in 25 min. 95% B for 5 min. 5% A for 4 min.

Peptides were purified using the same gradient as mentioned above, on a Thermo Scientific Dionex Ultimate 3000 RP-HPLC with a flow rate of 5 mL/min using a Phenomenex Gemini NX C18 110 Å (150 x 10 mm) semi-prep column.

LC-MS data were collected on an Agilent 1100 Series instrument with a Phenomenex Kinetex C18 100Å column (150 x 4.6 mm, 5 μm at 35 °C) connected to an ESMSD type VL mass detector with a flow rate of 1.5 ml/min was used with the following solvent systems: (A): 0.1% HCOOH in $H_2O$ and (B) MeCN. The column was flushed with 100% A for 2 min, then a gradient from 0 to 100% B over 6 min was used, followed by 2 min of flushing with 100% B.

NMR spectra were recorded on a Bruker AV 500 NMR.

## III. Syntheses and HPLC/LC-MS analysis

All teixobactin analogues (**1-7**) were synthesized according to our previously described protocols.[1]

| Sr No. | Analogue No. | Code | Exact Mass | Mass found [M + $H^+$] | Overall yield |
|---|---|---|---|---|---|
| | | | | | |
| 1 | 2 | LLLL | 1243.73 | 1244.4 | 16% |
| 2 | 3 | DDLD | 1243.73 | 1244.4 | 17% |
| 3 | 4 | DLDD | 1243.73 | 1244.4 | 9% |
| 4 | 5 | LDDD | 1243.73 | 1244.4 | 13% |
| 5 | 6 | LLDD | 1243.73 | 1244.4 | 14% |

**Table S1**: Mass analysis and overall yields for compounds **2-6**. Analysis and overall yields for compounds **1** & **7** have been published previously.[1]

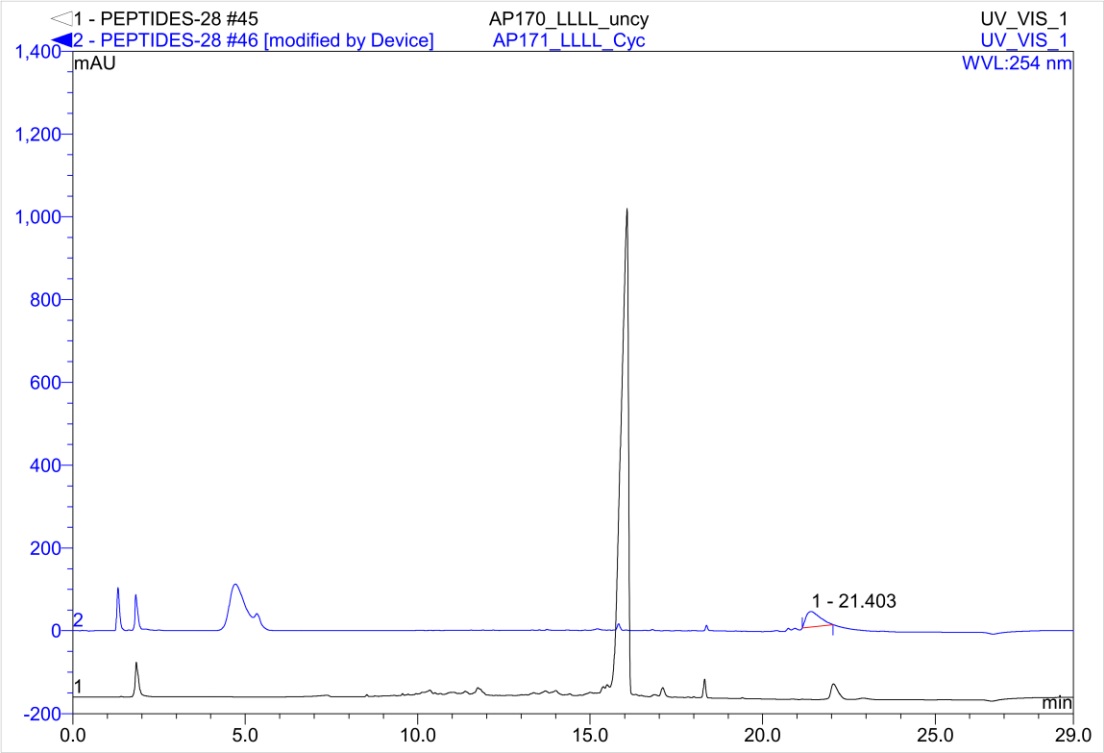


**Fig. S1**: HPLC trace showing the progress of cyclisation reaction for **2** (LLLL) (i): conversion of the uncyclized protected teixobactin analogue $t_R$= 16.067 min (shown in black) to the cyclized protected teixobactin analogue $t_R$ = 21.403 min (shown in blue) (Gradient: 5-95% in 25 min)

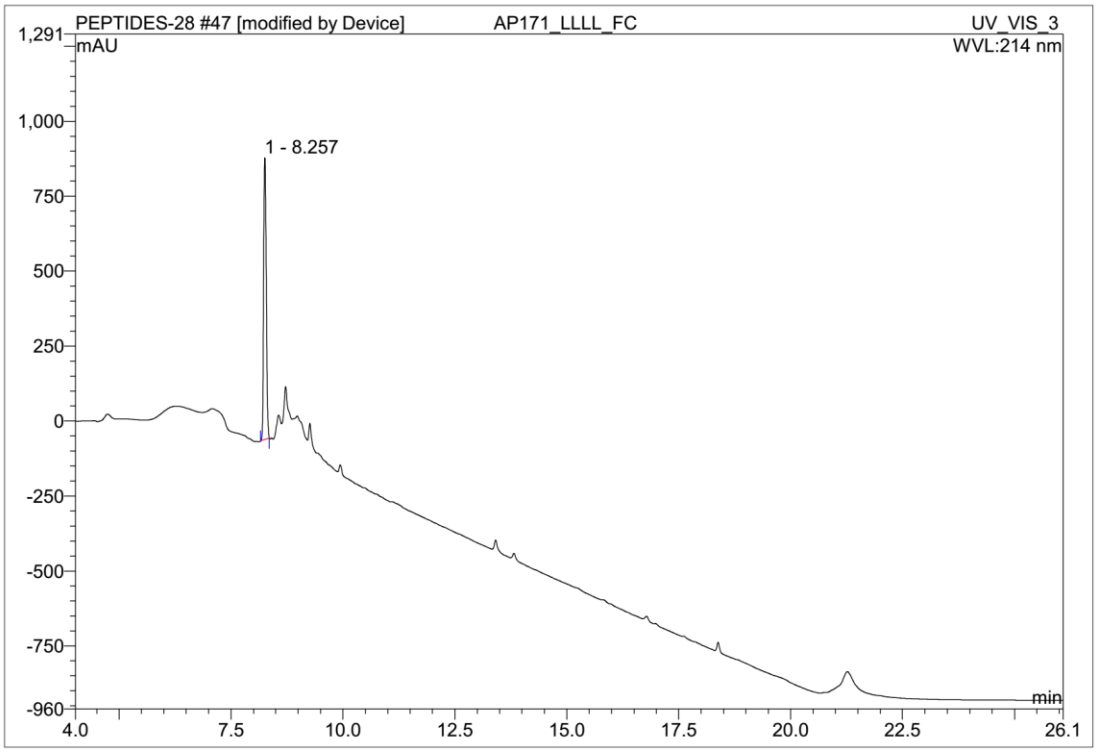


**Fig. S2**: HPLC trace of crude teixobactin analogue **2** (LLLL) $t_R$ = 8.257 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

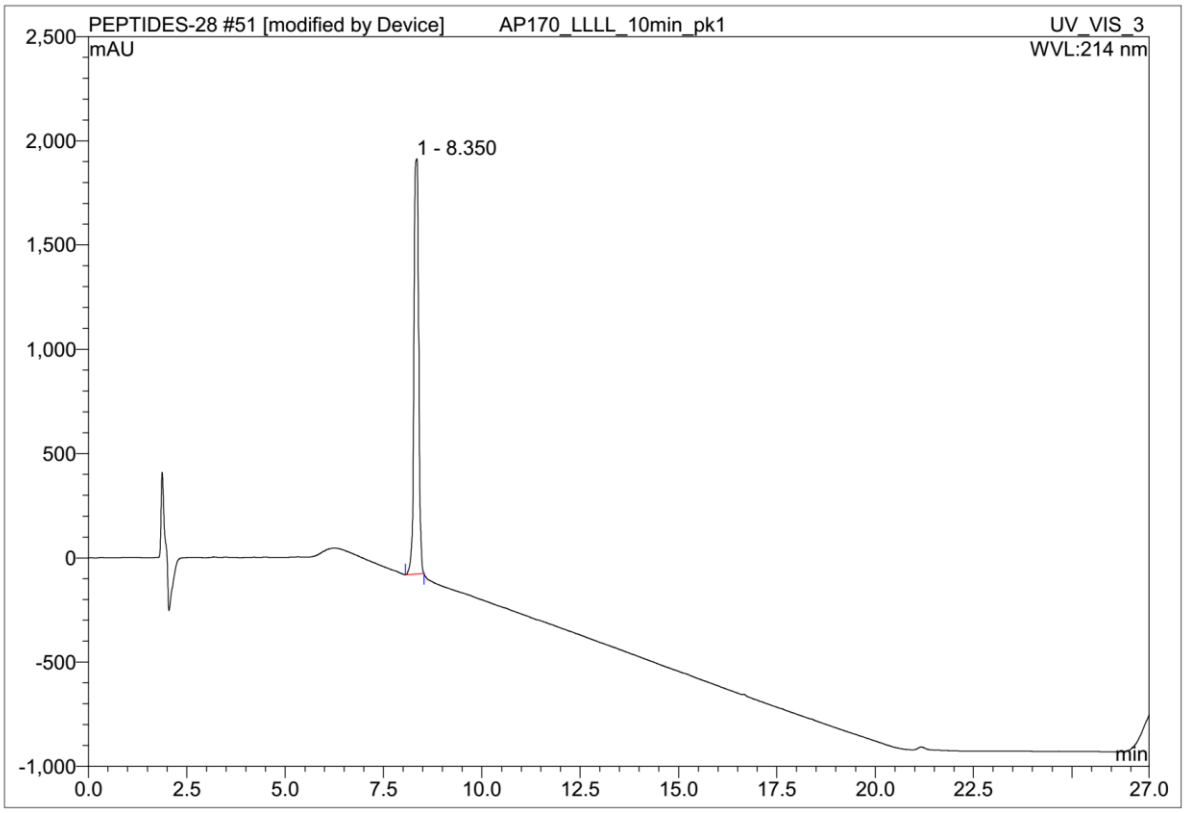


**Fig. S3**: HPLC trace of HPLC purified teixobactin analogue **2** (LLLL) (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

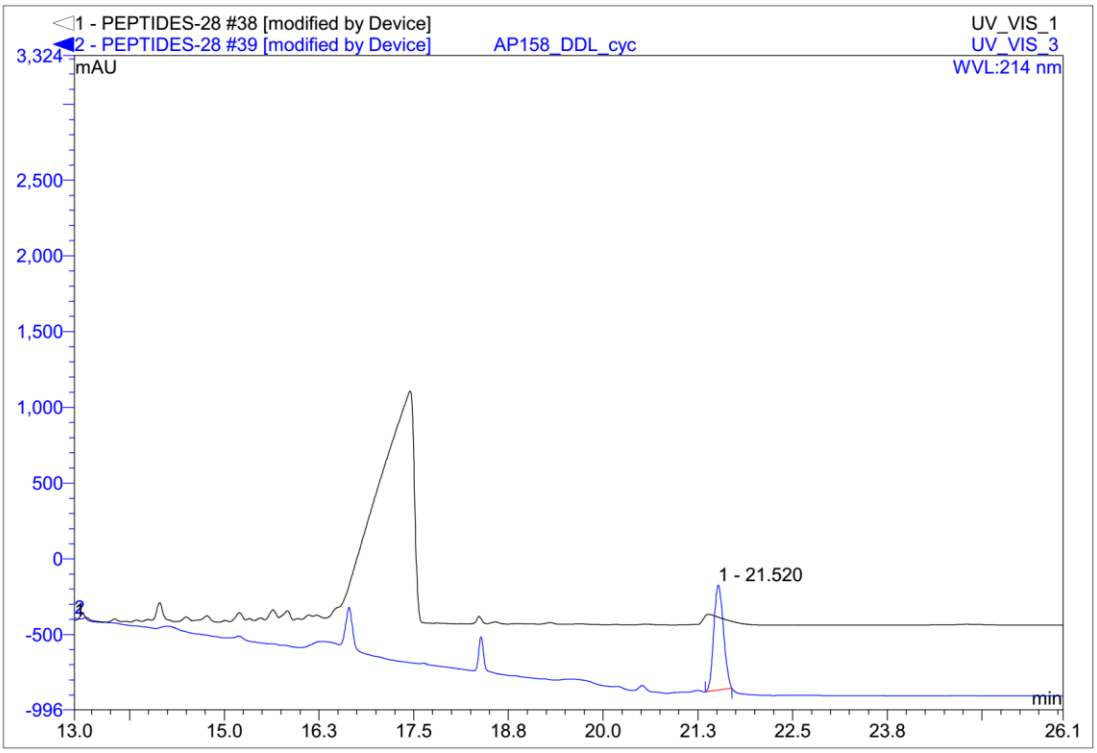


**Fig. S4**: HPLC trace showing the progress of cyclisation reaction for **3** (DDLD**)** (i): conversion of the uncyclized protected teixobactin analogue $t_R$= 17.457 min (shown in black) to the cyclized protected teixobactin analogue $t_R$ = 21.520 min (shown in blue) (Gradient: 5-95% in 25 min)

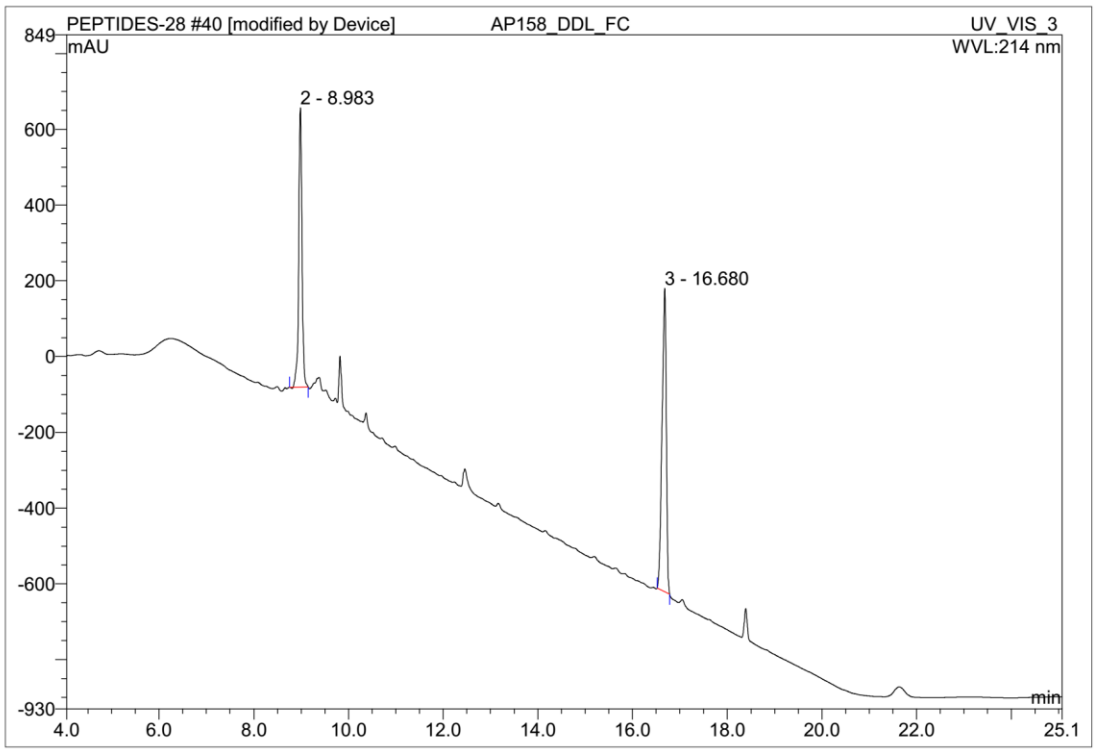


**Fig S5**: HPLC trace of crude teixobactin analogue **3** (DDLD) $t_R$ = 8.983 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

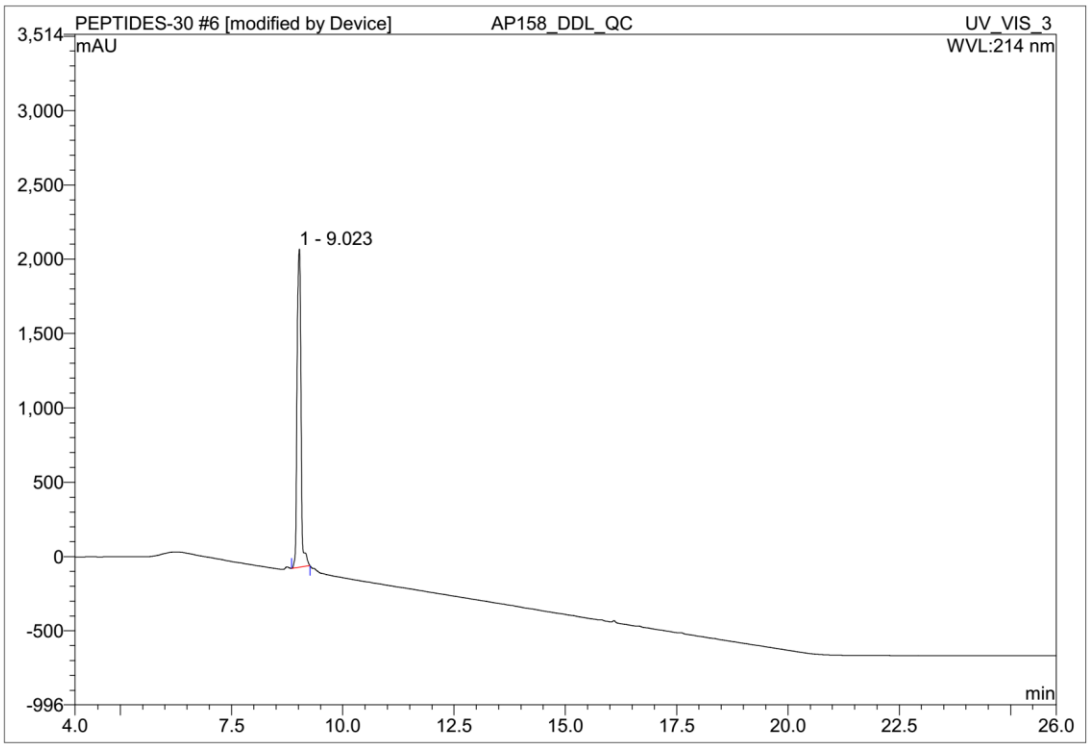


**Fig. S6**: HPLC trace of HPLC purified teixobactin analogue **3** (DDLD) (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

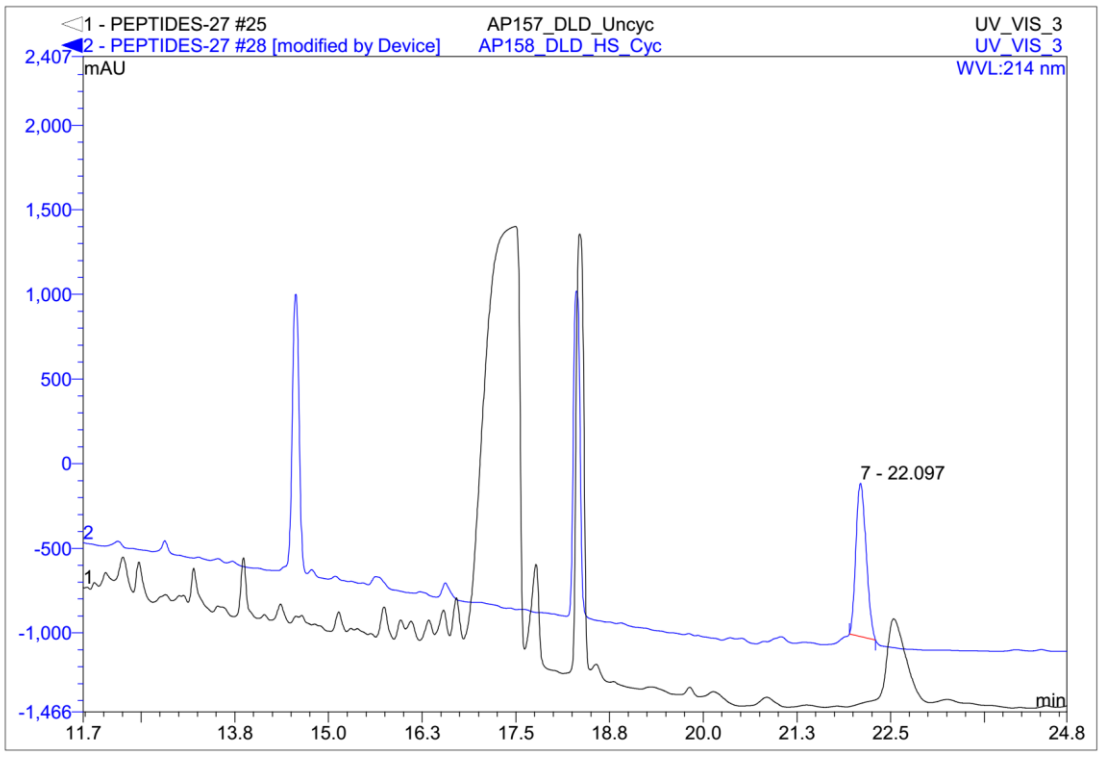


**Fig. S7**: HPLC trace showing the progress of cyclisation reaction for **4** (DLDD) (i): conversion of the uncyclized protected teixobactin analogue $t_R$= 17.493 min (shown in black) to the cyclized protected teixobactin analogue $t_R$ = 22.097 min (shown in blue) (Gradient: 5-95% in 25 min)

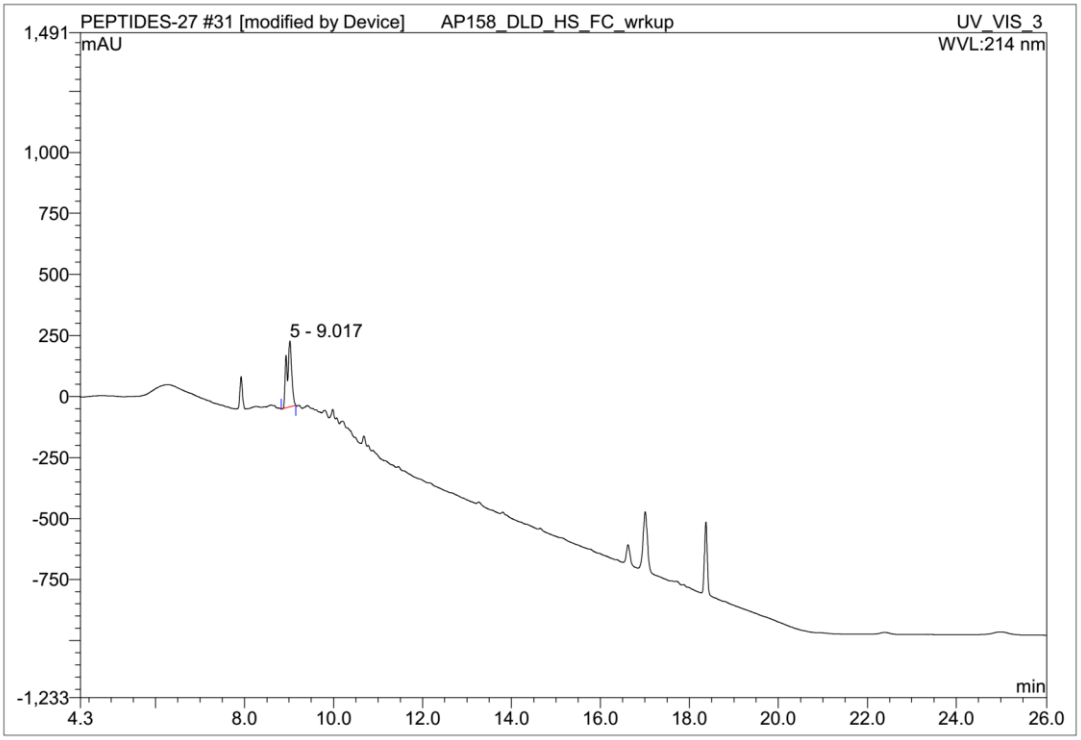


**Fig. S8**: HPLC trace of crude teixobactin analogue **4** (DLDD) $t_R$ = 9.017 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

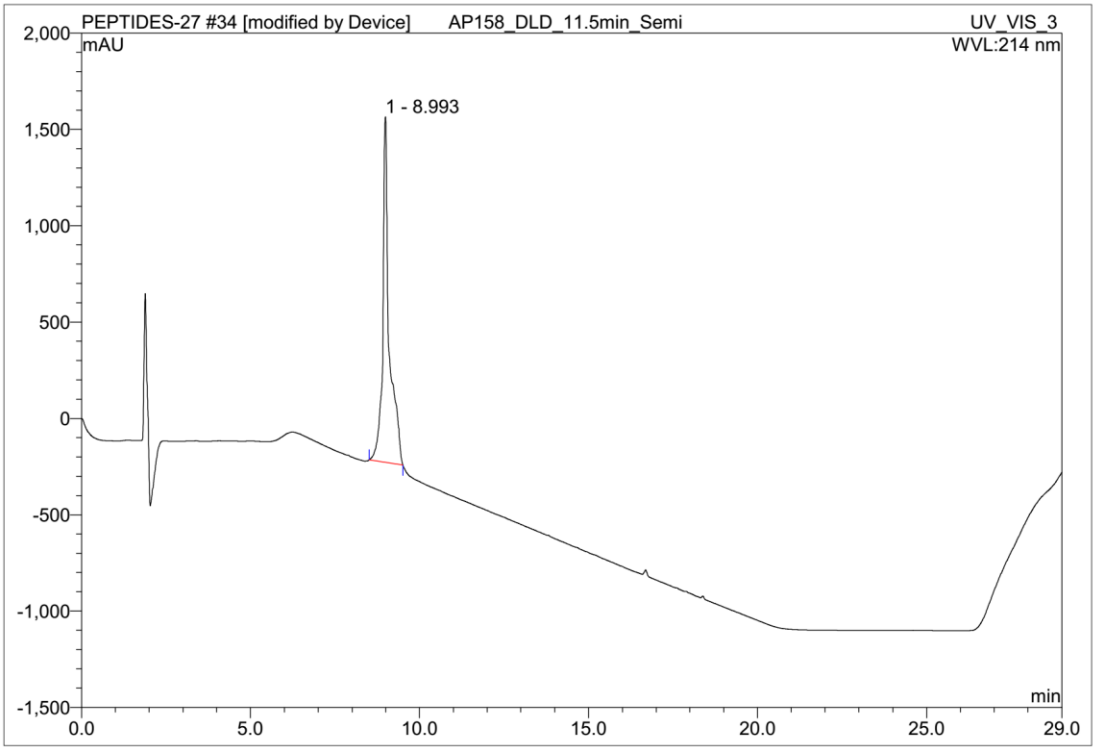


**Fig. S9**: HPLC trace of HPLC purified teixobactin analogue **4** (DLDD) (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

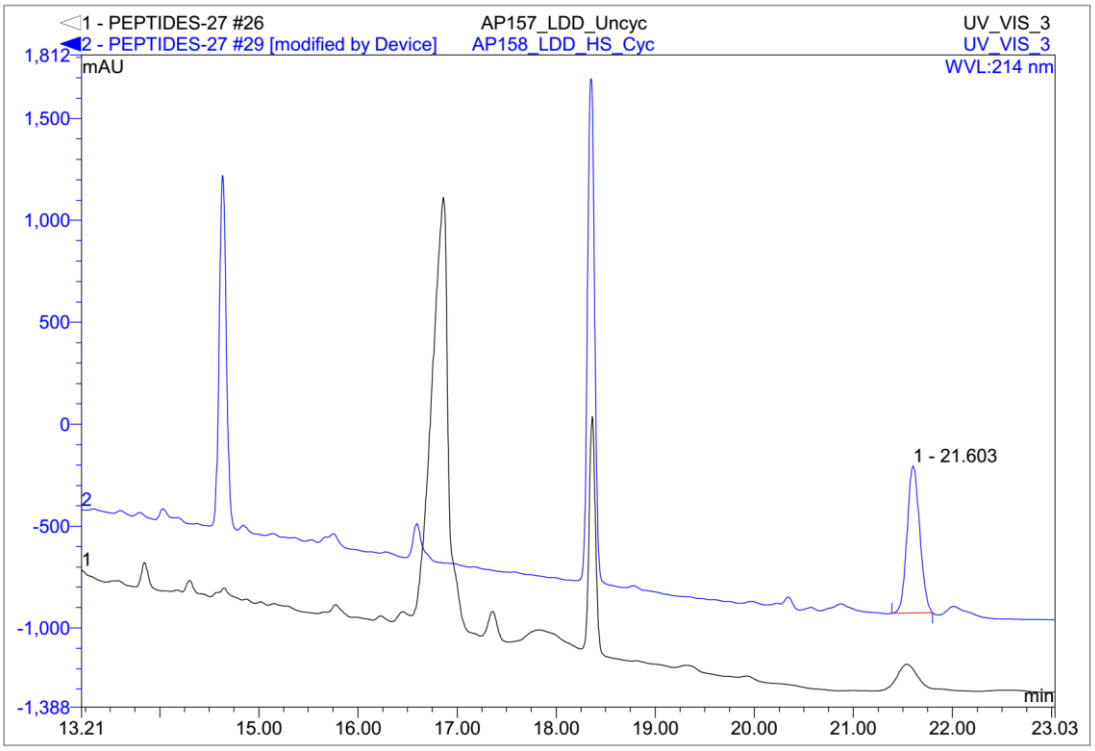


**Fig. S10**: HPLC trace showing the progress of cyclisation reaction for **5** (LDDD) (i): conversion of the uncyclized protected teixobactin analogue $t_R$= 16.863 min (shown in black) to the cyclized protected teixobactin analogue $t_R$ = 21.603 min (shown in blue) (Gradient: 5-95% in 25 min)

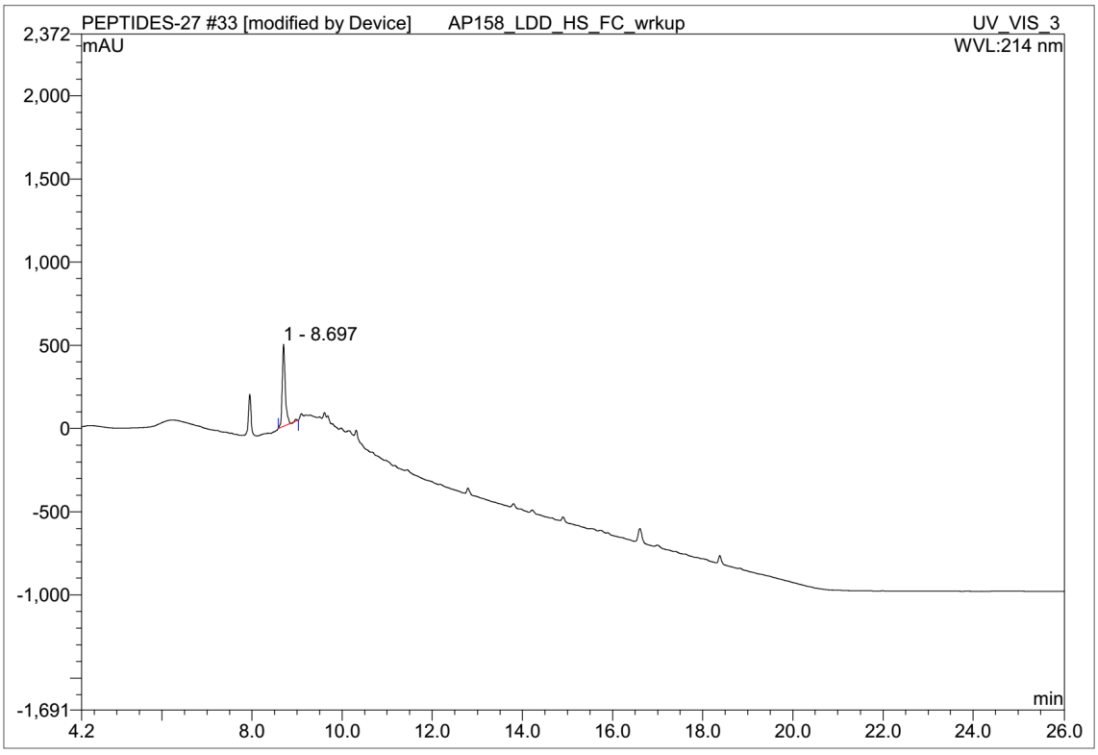


**Fig. S11**: HPLC trace of crude teixobactin analogue **5** (LDDD) $t_R$ = 8.697 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

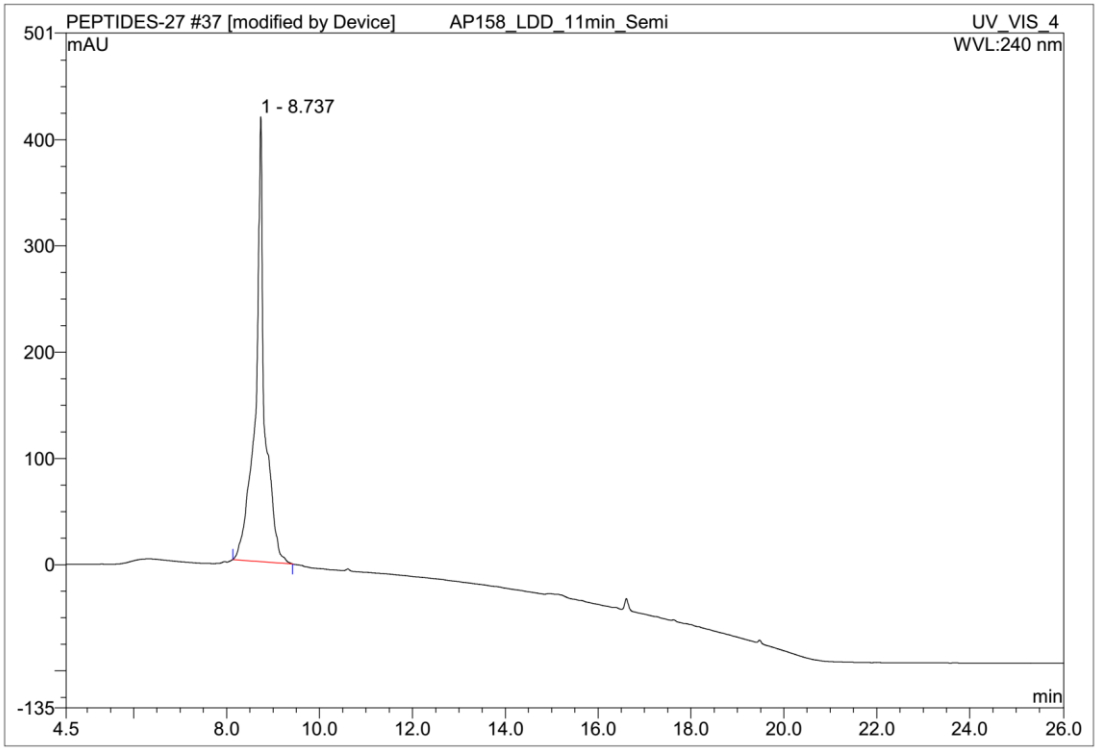


**Fig. S12**: HPLC trace of HPLC purified teixobactin analogue **5 (**LDDD) (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

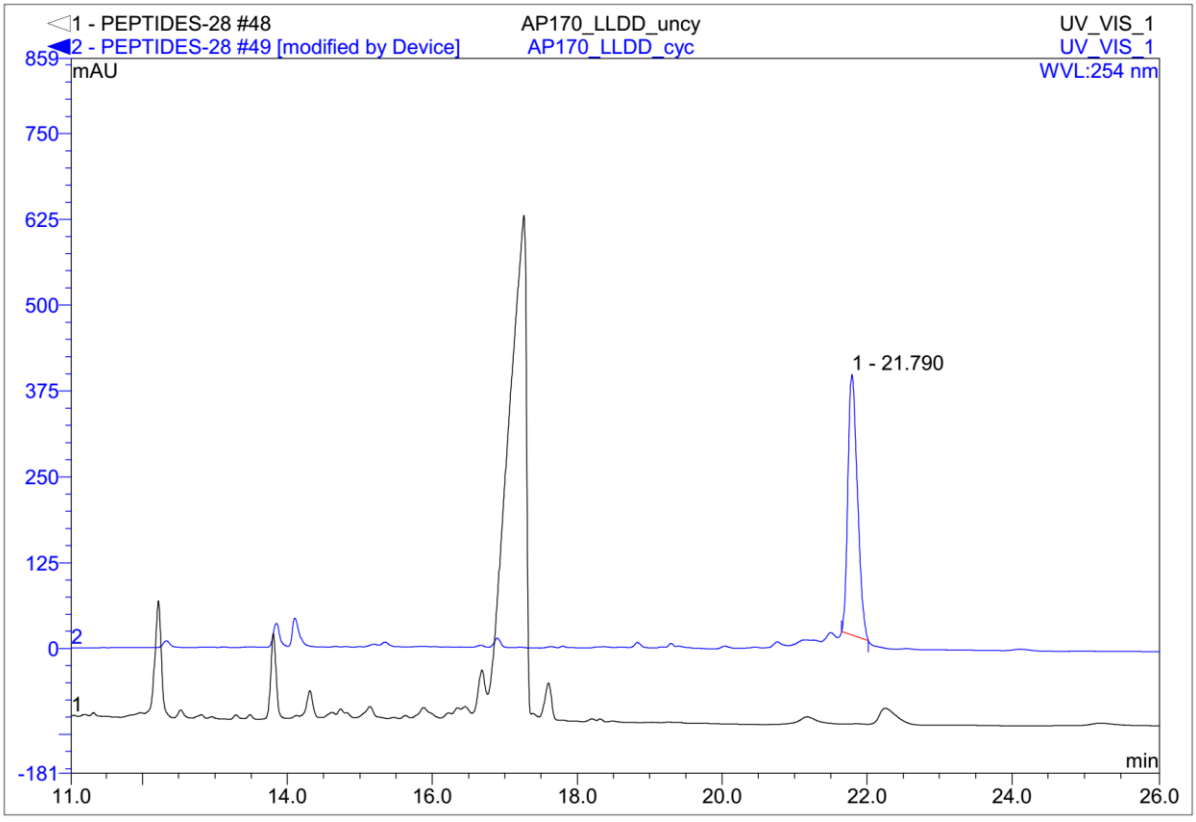


**Fig. S13**: HPLC trace showing the progress of cyclisation reaction of **6** (LLDD) (i): conversion of the uncyclized protected teixobactin analogue $t_R$= 17.263 min (shown in black) to the cyclized protected teixobactin analogue $t_R$ = 21.790 min (shown in blue) (Gradient: 5-95% in 25 min)

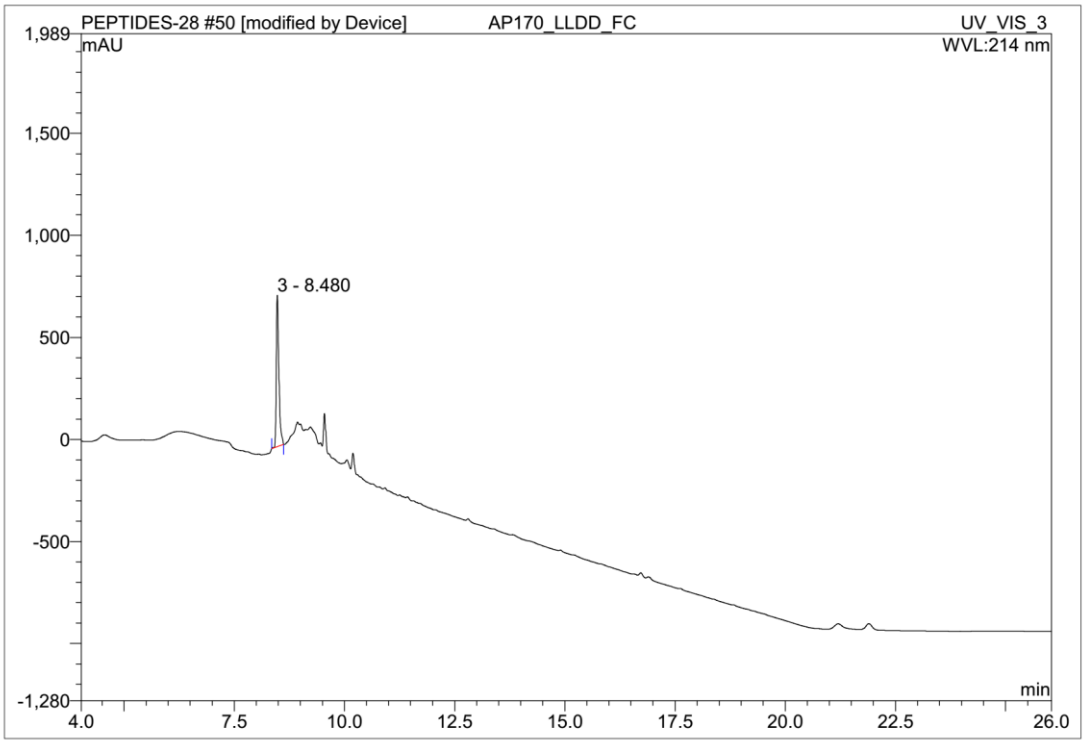


**Fig. S14**: HPLC trace of crude teixobactin analogue **6** LLDD $t_R$ = 8.480 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

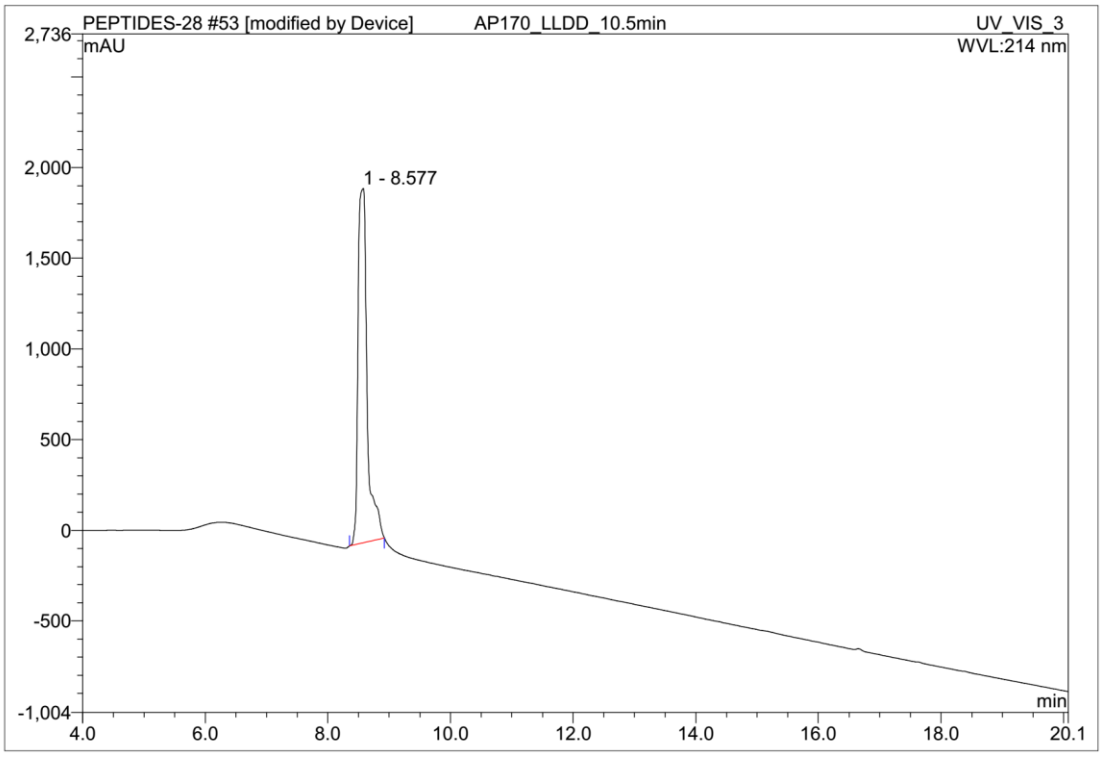


**Fig. S15**: HPLC trace of HPLC purified teixobactin analogue **6** LLDD (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

## IV. NMR Analysis

Spectra were recorded using 1 mM teixobactin analogues dissolved in DMSO-$d_6$ at 298.2 K on a Bruker Avance III 500 MHz spectrometer. Assignments were made using $^{1}$H-$^{1}$H TOCSY, $^{1}$H-$^{13}$C HSQC and $^{1}$H-$^{13}$C HMBC. Through-space dipolar correlations were measured using $^{1}$H-$^{1}$H NOESY with 200 ms mixing time. Spectra were acquired with 2048 complex points, and either 196 (TOCSY, NOESY, HSQC) or 512 (HMBC) complex points in the direct and indirect dimensions, respectively. Spectra were processed using Bruker TopSpin and analysed using CcpNmr Analysis.[2] Structures of the homologues were obtained via the process of iterative NOE assignment, with the structural calculations carried out using Cyana 2.1.[3] A final round of energy minimisation in explicit solvent was carried out using Gromacs 5.1.2[4] and the RSFF2 forcefield,[5] which has been shown to perform favourably with cyclic peptides.[6] Structures were visualised and analysed using PyMOL.

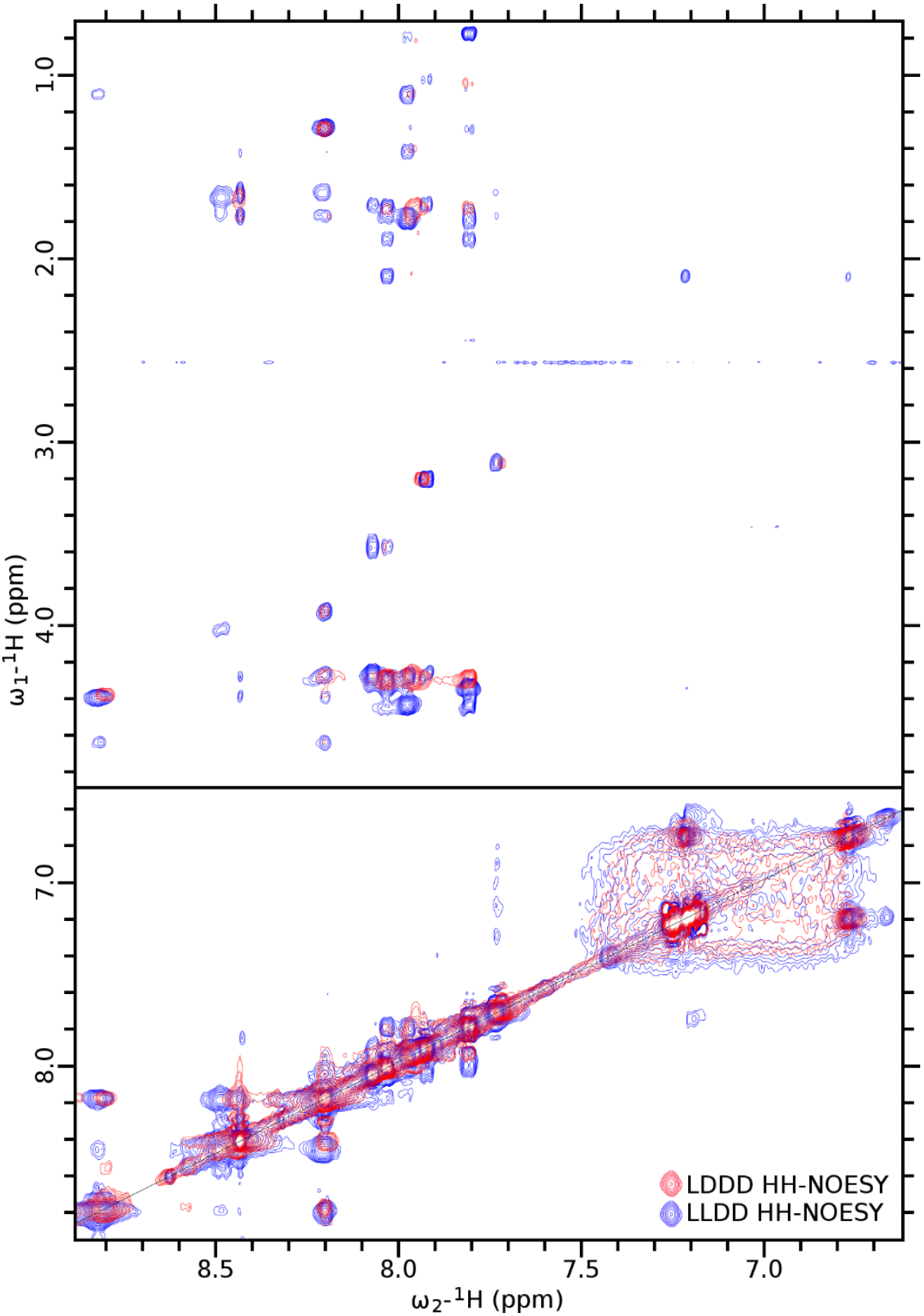


**Figure S16.** $^{1}$H-$^{1}$H NOESY spectra obtained from teixobactin analogues **5** (LDDD, *red contours*) and **6** (LLDD, *blue contours*), which only differ from each other in the stereochemistry of position 4. There are many more crosspeaks visible in the spectrum of **6** (LLDD) when compared to **5** (LDDD), some of

which are due to medium- to long-range interactions. These differences are borne out by the RMSDs of the structural ensembles generated using these crosspeaks: **5** (LDDD) with few crosspeaks resulted in an unstructured peptide with an RMSD of ~3 Å (Table 1; Figure 3A,D), whereas **6** (LLDD) with many crosspeaks of different classes resulted in a structured peptide of ~1 Å (Table 1; Figure 3A,E). Samples were prepared identically and spectra were acquired under identical conditions. Contours for each spectrum were set to identical levels, which was set at one level above noise.

| | **3 (DDLD)** | **4 (DLDD)** | **5 (LDDD)** | **6 (LLDD)** | **2 (LLLL)** |
|---|---|---|---|---|---|
| 1Phe H* | | | | | |
| 1Phe Hα | 3.263 | 3.263 | 3.205 | 3.208 | 3.206 |
| 1Phe Hβ | 2.789<br>2.691 | 2.688<br>2.775 | 2.676<br>2.869 | 2.677<br>2.877 | 2.688<br>2.875 |
| 1Phe Hδ* | 7.193 | 7.195 | 7.180 | 7.198 | 7.197 |
| 1Phe Hε* | 7.193 | 7.234 | 7.248 | 7.214 | 7.246 |
| 1Phe Hζ | 7.191 | 7.173 | 7.190 | 7.239 | 7.193 |
| 2Ile H | 7.926 | 7.928 | 7.930 | 7.914 | 7.910 |
| 2Ile Hα | 4.210 | 4.167 | 4.294 | 4.279 | 4.281 |
| 2Ile Hβ | 1.674 | 1.642 | 1.723 | 1.702 | 1.714 |
| 2Ile Hγ1 | 0.898<br>1.244 | 0.907<br>1.235 | 1.026<br>1.377 | 1.026<br>1.376 | 1.033<br>1.387 |
| 2Ile Hγ2* | 0.734 | 0.726 | 0.804 | 0.800 | 0.802 |
| 2Ile Hδ1* | 0.734 | 0.726 | 0.804 | 0.800 | 0.802 |
| 3Ser H | 7.980 | 7.955 | 8.031 | 8.065 | 8.084 |
| 3Ser Hα | 4.300 | 4.313 | 4.311 | 4.313 | 4.306 |
| 3Ser Hβ | 3.553<br>3.598 | 3.507<br>3.600 | 3.569<br>3.589 | 3.564<br>3.586 | 3.557<br>3.557 |
| 3Ser Hγ | 5.009 | 5.073 | 4.998 | 5.041 | 5.007 |
| 4Gln H | 7.909 | 8.059 | 7.946 | 8.023 | 7.976 |
| 4Gln Hα | 4.326 | 4.308 | 4.302 | 4.352 | 4.269 |
| 4Gln Hβ | 1.697<br>1.888 | 1.899<br>1.726 | 1.872<br>1.714 | 1.900<br>1.730 | 1.876<br>1.714 |
| 4Gln Hγ | 2.056<br>2.056 | 2.089<br>2.088 | 2.073<br>2.073 | 2.098<br>2.098 | 2.086<br>2.086 |
| 4Gln Hε2 | 6.747<br>7.198 | 6.766<br>7.221 | 6.776<br>7.220 | 6.768<br>7.214 | 6.758<br>7.195 |
| 5Ile H | 7.851 | 7.819 | 7.804 | 7.799 | 7.888 |
| 5Ile Hα | 4.254 | 4.326 | 4.281 | 4.444 | 4.195 |
| 5Ile Hβ | 1.732 | 1.730 | 1.772 | 1.798 | 1.712 |
| 5Ile Hγ1 | 1.058<br>1.369 | 1.036<br>1.378 | 1.098<br>1.400 | 1.071<br>1.293 | 1.048<br>1.405 |
| 5Ile Hγ2* | 0.788 | 0.807 | 0.798 | 0.773 | 0.796 |
| 5Ile Hδ1* | 0.788 | 0.807 | 0.798 | 0.773 | 0.799 |
| 6Ile H | 7.851 | 8.010 | 7.958 | 7.963 | 7.887 |
| 6Ile Hα | 4.229 | 4.265 | 4.282 | 4.284 | 4.239 |
| 6Ile Hβ | 1.731 | 1.761 | 1.728 | 1.775 | 1.723 |
| 6Ile Hγ1 | 1.065<br>1.438 | 1.113<br>1.407 | 1.106<br>1.377 | 1.106<br>1.413 | 1.073<br>1.402 |
| 6Ile Hγ2* | 0.809 | 0.815 | 0.822 | 0.815 | 0.805 |
| 6Ile Hδ1* | 0.809 | 0.815 | 0.822 | 0.815 | 0.806 |

| | | | | | |
|---|---|---|---|---|---|
| 7Ser H | 8.965 | 9.124 | 9.124 | 9.202 | 8.079 |
| 7Ser Hα | 4.378 | 4.380 | 4.385 | 4.404 | 4.352 |
| 7Ser Hβ | 3.667<br>3.756 | 3.738<br>3.736 | 3.700<br>3.769 | 3.708<br>3.770 | 3.537<br>3.567 |
| 7Ser Hγ | 5.655 | 5.592 | 5.616 | 5.643 | 4.918 |
| 8Thr H | 8.953 | 8.763 | 8.798 | 8.820 | 7.759 |
| 8Thr Hα | 4.644 | 4.638 | 4.642 | 4.645 | 4.796 |
| 8Thr Hβ | 5.361 | 5.359 | 5.361 | 5.361 | 4.482 |
| 8Thr Hγ2* | 1.091 | 1.091 | 1.095 | 1.092 | 1.075 |
| 9Ala H | 8.211 | 8.185 | 8.202 | 8.197 | 9.454 |
| 9Ala Hα | 3.932 | 3.930 | 3.928 | 3.929 | 4.466 |
| 9Ala Hβ* | 1.290 | 1.288 | 1.287 | 1.279 | 1.339 |
| 10Arg H | 8.211 | 8.159 | 8.190 | 8.205 | 9.168 |
| 10Arg Hα | 4.274 | 4.276 | 4.281 | 4.279 | 4.052 |
| 10Arg Hβ | 1.654<br>1.768 | 1.636<br>1.765 | 1.652<br>1.771 | 1.642<br>1.769 | 1.731<br>1.731 |
| 10Arg Hγ | 1.486<br>1.430 | 1.494<br>1.412 | 1.491<br>1.417 | 1.491<br>1.413 | 1.545<br>1.522 |
| 10Arg Hδ | 3.108<br>3.108 | 3.118<br>3.118 | 3.119<br>3.119 | 3.116<br>3.116 | 3.111<br>3.111 |
| 10Arg Hε | 7.742 | 7.731 | 7.714 | 7.734 | 7.801 |
| 10Arg Hη1 | 6.918<br>7.067 | 7.101<br>7.101 | | 6.913<br>7.013 | 6.984<br>6.984 |
| 10Arg Hη2 | 7.194<br>7.281 | 7.302<br>7.302 | | 7.308<br>7.144 | 7.165<br>7.165 |
| 11Ile H | 8.471 | 8.415 | 8.434 | 8.481 | 7.972 |
| 11Ile Hα | 4.035 | 4.028 | 4.032 | 4.031 | 4.412 |
| 11Ile Hβ | 1.670 | 1.671 | 1.693 | 1.685 | 1.942 |
| 11Ile Hγ1 | 1.088<br>1.423 | 1.099<br>1.418 | 1.103<br>1.422 | 1.091<br>1.415 | 1.154<br>1.346 |
| 11Ile Hγ2* | 0.811 | 0.813 | 0.801 | 0.801 | 0.848 |
| 11Ile Hδ1* | 0.811 | 0.808 | 0.801 | 0.801 | 0.849 |

**Table S2.** Chemical shift assignments of the teixobactin homologues. *Underlined values* are more than 2 standard deviations away from the average values. Chemical shifts for analogues **1** (DDDD) and **7** (LLLD) have been published previously[1].

## V. Structural Statistics for teixobactin analogues

| | 1 (DDDD) | 3 (DDLD) | 4 (DLDD) | 5 (LDDD) | 6 (LLDD) | 7 (LLLD) | 2 (LLLL) |
|---|---|---|---|---|---|---|---|
| *NMR distance restraints* | | | | | | | |
| Intra-residue | 38 | 56 | 55 | 43 | 57 | 50 | 44 |
| Sequential ($\|i-j\| = 1$) | 11 | 18 | 18 | 14 | 21 | 19 | 18 |
| Med range ($\|i-j\| < 5$) | 5 | 7 | 1 | 6 | 6 | 6 | 1 |
| Long range ($\|i-j\| > 4$) | 0 | 0 | 2 | 0 | 8 | 2 | 1 |
| TOTAL | 54 | 81 | 76 | 63 | 92 | 77 | 64 |
| *Statistics of overall structural quality* | | | | | | | |
| *Ensemble pairwise RMSD* | | | | | | | |
| Heavy atom (Å) | 3.16 ± 1.44 | 1.83 ± 0.55 | 0.76 ± 0.20 | 2.96 ± 1.26 | 1.06 ± 0.45 | 0.93 ± 0.44 | 1.08 ± 0.32 |
| Backbone (Å) | 1.83 ± 1.10 | 0.93 ± 0.37 | 0.37 ± 0.15 | 1.92 ± 0.93 | 0.59 ± 0.30 | 0.53 ± 0.28 | 0.50 ± 0.20 |
| Restr violations > 0.1 Å | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *RMSD from idealised covalent geometry*[7] | | | | | | | |
| Bond lengths (Å) | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.011 | 0.013 |
| Bond angles (°) | 2.1 | 1.9 | 1.9 | 2.0 | 2.1 | 1.8 | 1.9 |
| *Ramachandran analysis*[8] | | | | | | | |
| Allowed (%) | 58.8 ± 6.3 | 95.6 ± 5.6 | 77.7 ± 0.0 | 88.9 ± 0.0 | 88.8 ± 0.0 | 98.3 ± 4.1 | 98.9 ± 3.4 |
| Gen allowed (%) | 20.5 ± 6.7 | 4.4 ± 5.6 | 22.2 ± 0.0 | 2.2 ± 4.5 | 0.0 ± 0.0 | 1.7 ± 4.0 | 1.1 ± 3.4 |
| Disallowed (%) | 20.5 ± 6.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 8.9 ± 4.5 | 11.1 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| ProCheck *G*-factor | -0.58 ± 0.1 | -0.37 ± 0.1 | -0.31 ± 0.1 | -0.25 ± 0.1 | -0.32 ± 0.1 | -0.12 ± 0.1 | -0.27 ± 0.1 |
| MolProbity clash score[9] | 0.27 ± 1.20 | 1.06 ± 2.18 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.06 ± 2.18 | 5.85 ± 1.64 |

**Table S3.** Structural statistics for teixobactin analogues.

## VI. Molecular Dynamic simulations

Molecular dynamics simulations were carried out using Gromacs 5.1.2 and RSFF2. The lowest energy structure from each ensemble was solvated in TIP3P water and neutralised with chloride ions. This system was subjected to 100 ps of NVT and NPT equilibration. 100 ns of simulation was carried out with periodic boundary conditions at 298.2 K, and the trajectory analysed with VMD.[10]

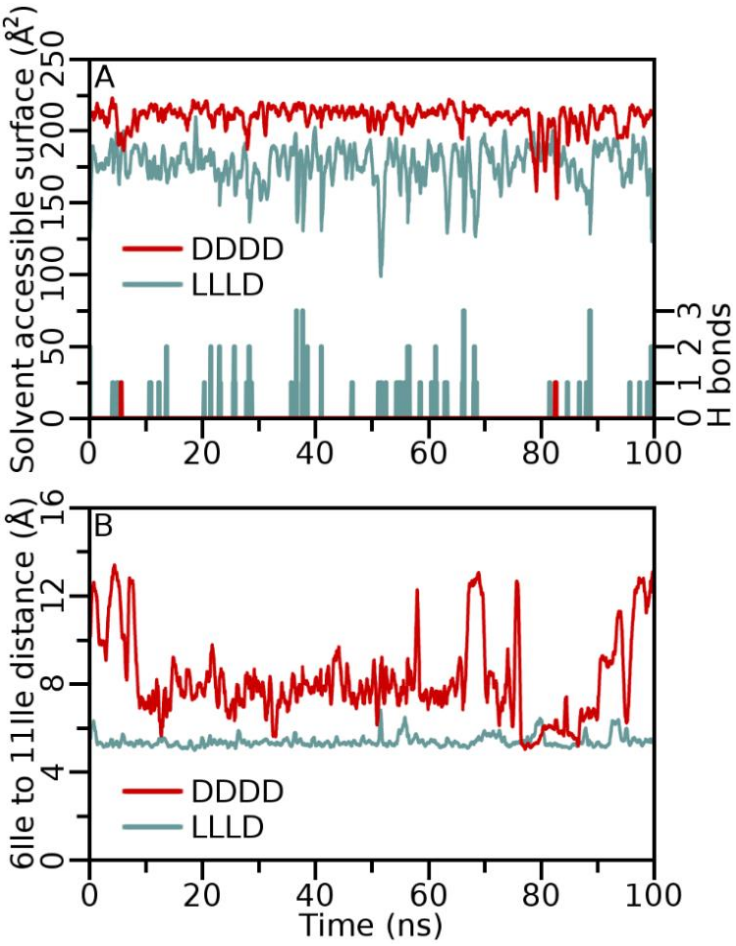


**Figure S17.** Molecular dynamics simulations of teixobactin stereochemical analogues. **A.** The sidechain of $Arg_{10}$ consistently presents ~15% less surface area to the solvent in non-native teixobactin **7** (LLLD, *teal line*) when compared to the native **1** (DDDD, *red line*) over the course of the simulation. **B.** Hydrophobic packing between the sidechains of $Ile_6$ and $Ile_{11}$ is consistent over the course of the simulation in the analogue (*teal line*), whereas this packing is only infrequently visited in the native form (*red line*). Simulations were performed using Gromacs 5.1.2 and the RSFF2 forcefield. Surface area, hydrogen bonds and interatomic distances were calculated using the Gromacs modules *sasa*, *hbond* and *distance*, respectively. Data were recorded every 10 ps, and were plotted as a rolling average over 50 data points.

## VII. MIC testing

For MIC testing all peptides were dissolved in DMSO. Bacteria were grown on Mueller Hinton broth (oxoid). All incubations were at 37°C. Dilutions were carried out using Mueller Hinton. 100 µl of autoclaved Mueller Hinton broth was added to wells 2-12 on a 96-well plate. 200 µl of the peptide was added to well one at a concentration of 512 µg/ml. 100µl of peptide in well one was taken up and pipetted into well two. The mixture was then mixed via pipetting before 100µl was taken up and pipetted into well three. This process was repeated up to well 11. Once peptide was added to well 11 100 µl was taken up and then discarded ensuring the well 12 had no peptide present. Each well was then inoculated with 100µl of bacteria that had been diluted to an OD600nm of 0.1. This was repeated three times. The 96-well plates were then incubated for 24 hours. The MIC was determined to be the lowest concentration at which there was no growth visible.

## VIII. Complex formation of teixobactin with lipid II and geranyl pyrophosphate

Complex formation of teixobactin analogues **1** (DDDD) and **2** (LLLL) with lipid II and geranyl pyrophosphate was performed using TLC as described previously[11]. Binding of teixobactin to lipid II and geranyl pyrophosphate was analysed by incubating 30 µL of 2 nmol of each precursor with 2 or 4 nmoles of teixobactin in 50 mM Tris/HCl, pH 7.5, for 30 min at room temperature. Complex formation was analysed by extracting unbound precursors from the reaction mixture with 30 µL n- butanol/6M pyridine acetate (pH 4.2) (2:1; vol/vol) followed by TLC analysis of the organic layer using chloroform/methanol/water/ammonia (88:48:10:1, v/v/v/v) as the solvent and detection of lipid/phosphate containing precursors by phosphomolybdic acid staining. No spot on TLC was detected in a control experiment without lipid II. The TLC figures represent the results obtained through three independent experiments.

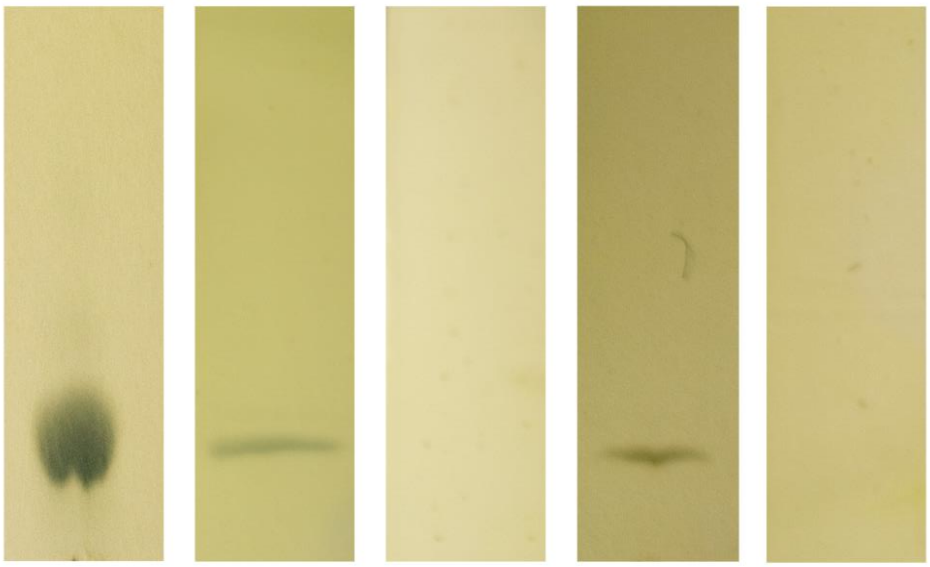


**Figure S18**: Binding of teixobactin analogues **1** (DDDD) and **2** (LLLL) with lipid II using the protocols described in literature[11]. Partial binding is observed when the ratio of lipid II to the analogue is 1:1

(indicated by lighter spots on the TLC) and complete binding is observed when the ratio of lipid II to the analogue is 1:2 (indicated by no spots on TLC).

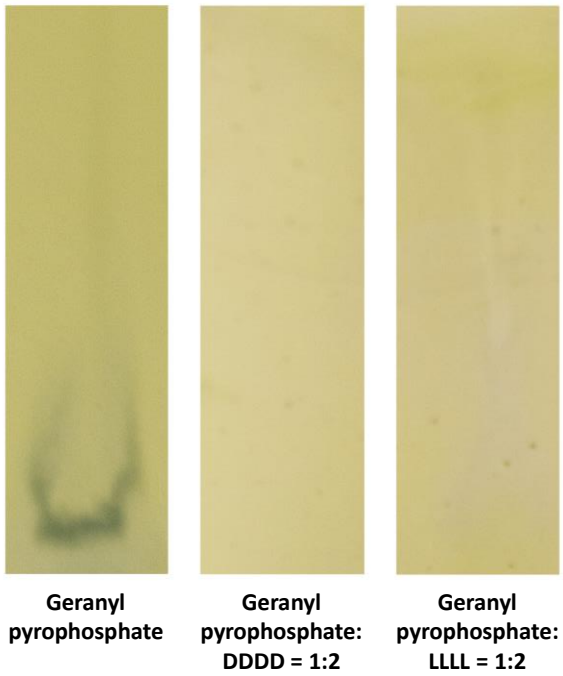


**Figure S19**: Binding of teixobactin analogues **1** (DDDD) and **2** (LLLL) with geranyl pyrophosphate using the protocols described in literature[11]. complete binding is observed when the ratio of the phosphate to the analogue is 1:2 (indicated by no spots on the TLC).

## IX. References:


1 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.

2 W. F. Vranken, W. Boucher, T. J. Stevens, R. H. Fogh, A. Pajon, M. Llinas, E. L. Ulrich, J. L. Markley, J. Ionides and E. D. Laue, *Proteins Struct. Funct. Genet.*, 2005, **59**, 687–696.

3 P. Guntert and L. Buchner, *J. Biomol. NMR*, 2015, **62**, 453–471.

4 M. J. Abraham, T. Murtola, R. Schulz, S. Pall, J. C. Smith, B. Hess and E. Lindah, *SoftwareX*, 2015, **1**–**2**, 19–25.

5 S. Li and A. H. Elcock, *J. Phys. Chem. Lett.*, 2015, **13**, 2127–2133.

6 H. Geng, F. Jiang and Y.-D. Wu, *J. Phys. Chem. Lett.*, 2016, **7**, 1805–10.

7 Aneerban Bhattacharya, Roberto Tejero and Gaetano T. Montelione, *Proteins*, 2007, **66**, 778–795.

8 R. Laskowski, J. A. Rullmann, M. MacArthur, R. Kaptein and J. Thornton, *J. Biomol. NMR*, 1996, **8**, 477–486.

9 S. C. Lovell, I. W. Davis, W. B. Adrendall, P. I. W. de Bakker, J. M. Word, M. G. Prisant, J. S. Richardson and D. C. Richardson, *Proteins-Structure Funct. Genet.*, 2003, **50**, 437–450.

10 W. Humphrey, A. Dalke and K. Schulten, *J. Mol. Graph.*, 1996, **14**, 33–8, 27–8.

11 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.