Supplementary Information

FOR

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

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TABLE OF CONTENTS

I. Materials

All L amino acids including Fmoc-Orn(Boc)-OH, Fmoc-Dab(Boc)-OH and Fmoc-Dap(Boc)-OH and D amino acids Fmoc-D-Ala-OH Fmoc-D-Gln(Trt)-OH, Boc-N-methyl-D-phenylalanine and 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), Triisopropylsilane (TIS) and and 1*H*-Pyrazole-carboxamidine hydrochloride 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, $CDCl_3$ and polysorbate 80 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, UK. 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.

HRMS spectra were recorded on a Thermo Scientific Q Exactive Plus Orbitrap Mass Spectrometer in the positive ion mode.

III. Syntheses of teixobactin analogues

Teixobactin analogues **1**, **3**, **4**, **5** & **7** were synthesized according to our previously described protocol.[1]

Procedure for Guanidation: 5 mg of the amino precursor for the corresponding guanidine teixobactin was dissolved in 200 µL of MeOH. 15 eq. of Et_3N was then added to it and the solution was stirred till all the teixobactin analogue dissolved. 1.5 eq. of 1*H*-Pyrazole-carboxamidine hydrochloride was then added and stirred vigorously. MeOH was added dropwise (if necessary) till all the reagent dissolved and the reaction mixture was stirred for 8h at r.t. The reaction mixture was then analysed on RP-HPLC followed by RP-HPLC purification and freeze dried to yield the corresponding guanidine teixobactin.

IV. HPLC/LC-MS analysis

Compound Number	**Name**	**Chemical formula**	**Calculated Exact Mass**	**Mass found [M + H^+]**	**Overall yield [%]**
1	Lys_{10}-teixobactin	$C_{58}H_{97}N_{13}O_{15}$	1215.7227	1216.7314	19 [a]
2	$HoArg_{10}$-teixobactin	$C_{59}H_{99}N_{15}O_{15}$	1257.7445	1258.7533	64 [b]
3	Orn_{10}-teixobactin	$C_{57}H_{95}N_{13}O_{15}$	1201.7071	1202.7153	16 [a]
5	Dab_{10}-teixobactin	$C_{56}H_{93}N_{13}O_{15}$	1187.6914	1188.7009	20 [a]
6	$NorArg_{10}$-teixobactin	$C_{57}H_{95}N_{15}O_{15}$	1229.7132	1230.7216	50 [b]
7	Dap_{10}-teixobactin	$C_{55}H_{91}N_{13}O_{15}$	1173.6758	1174.6852	13 [a]
8	$GAPA_{10}$-teixobactin	$C_{56}H_{93}N_{15}O_{15}$	1215.6976	1216.7057	48 [b]

Table S1: Compound number, name, chemical formula, exact mass, mass found and overall yield for compounds **1-3** & **5-8**.

[a] isolated yield.

[b] isolated yields for guanidation step.

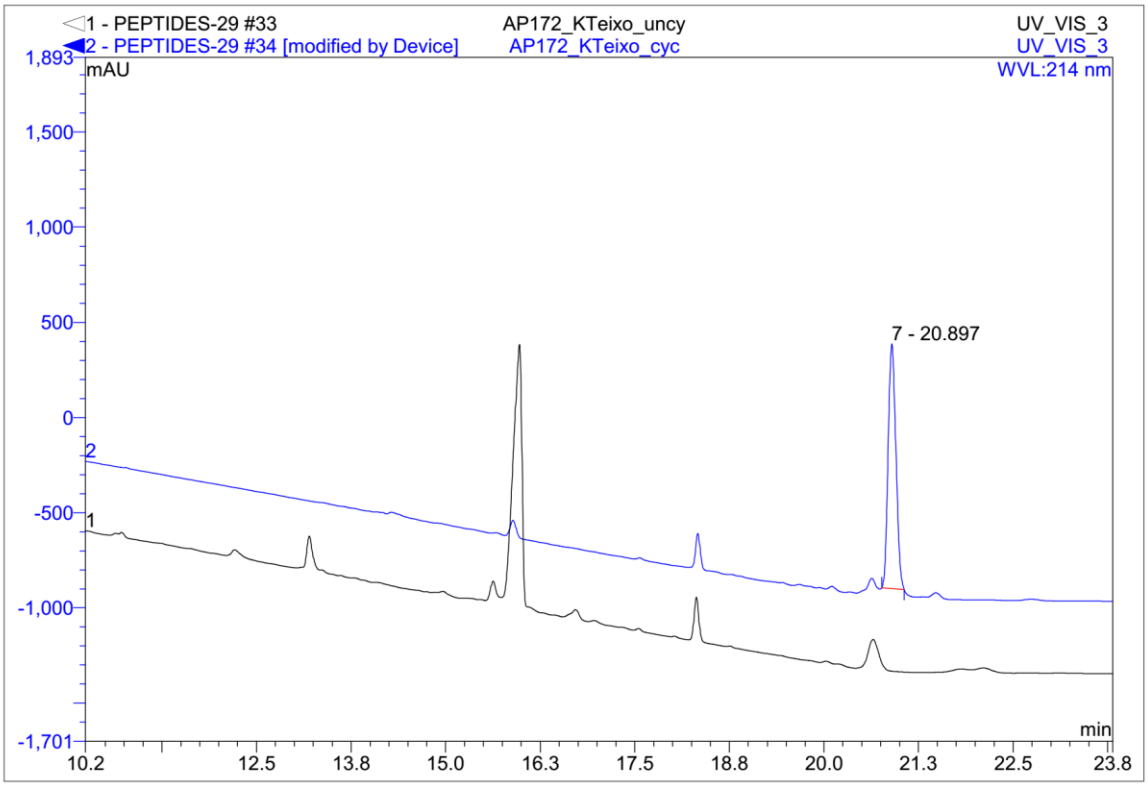

Fig. 1: HPLC trace showing the progress of the cyclisation reaction for analogue **1**: conversion of the uncyclized protected teixobactin analogue t_R= 15.977 min (shown in black) to the cyclized protected teixobactin analogue t_R = 20.897 min (shown in blue) (Gradient: 5-95% in 25 min)

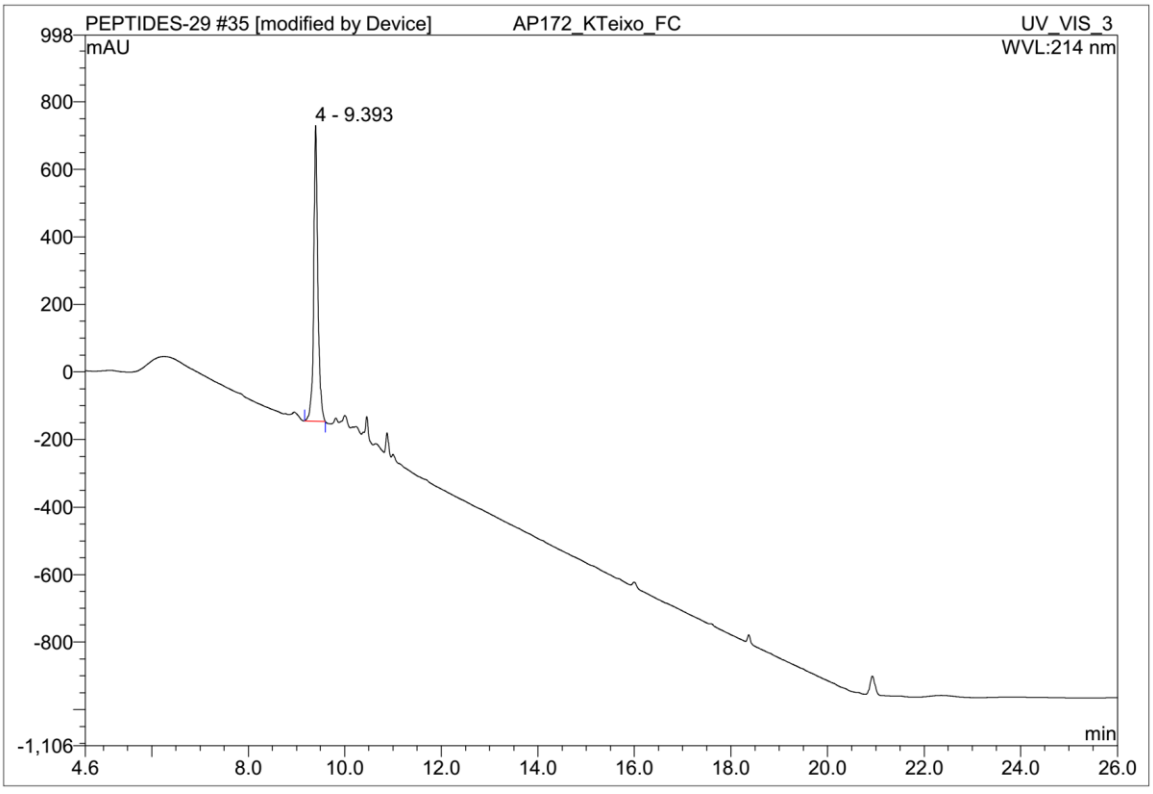

Fig. 2: HPLC trace of crude teixobactin analogue **1** t_R = 9.393 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

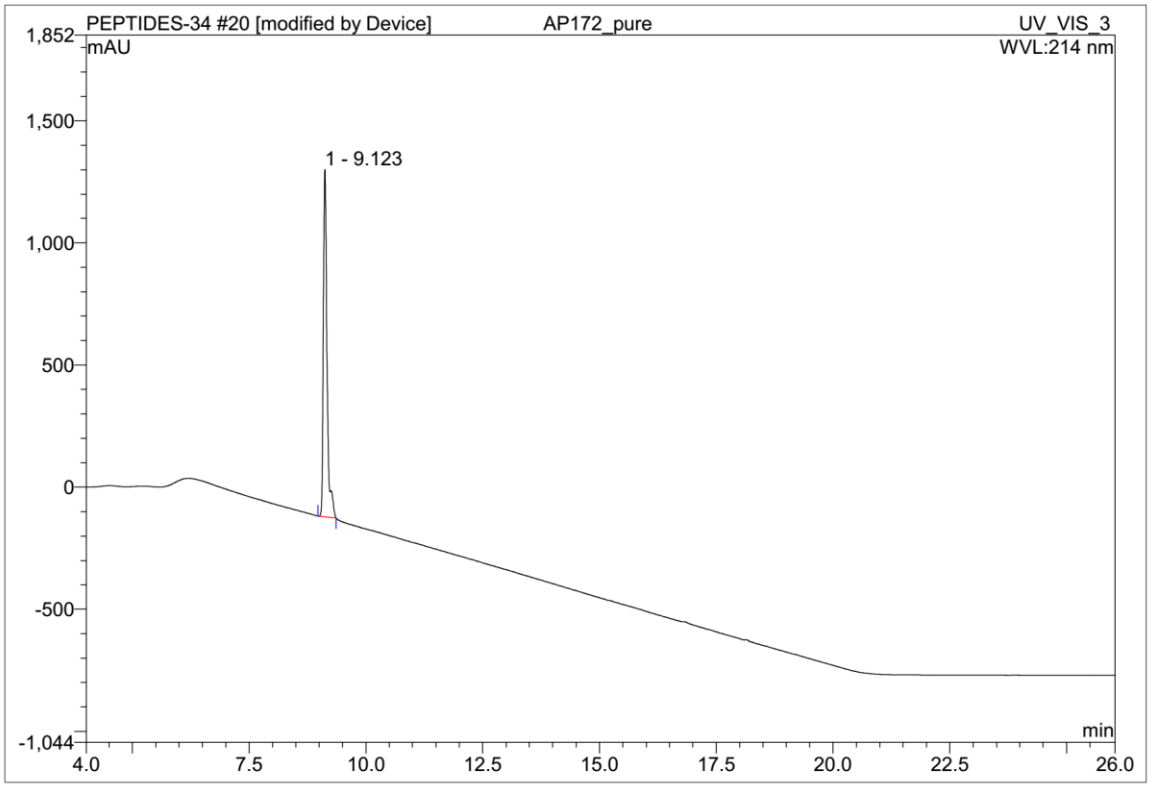

Fig. 3: HPLC trace of HPLC purified teixobactin analogue **1** (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

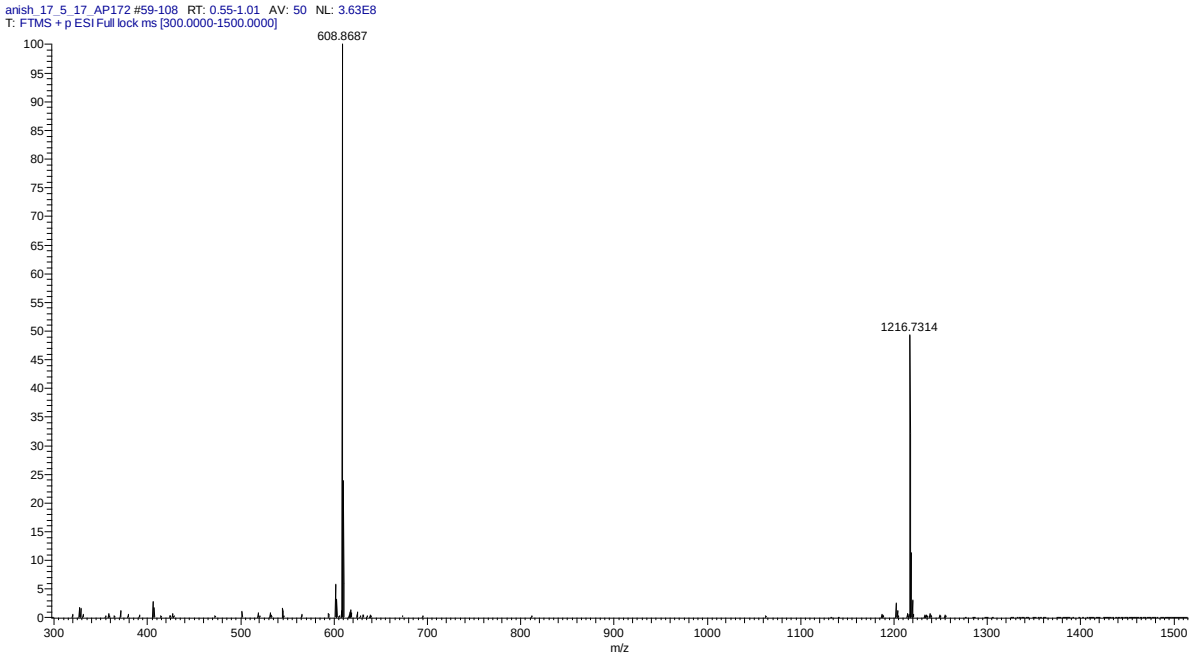

Fig. 4: HRMS spectra from of HPLC purified teixobactin analogue **1**. Exact Mass calcd. for $C_{58}H_{97}N_{13}O_{15}$ = 1215.7227, found $M+H^+$ = 1216.7314 and $M/2 + H^+$ = 608.8687

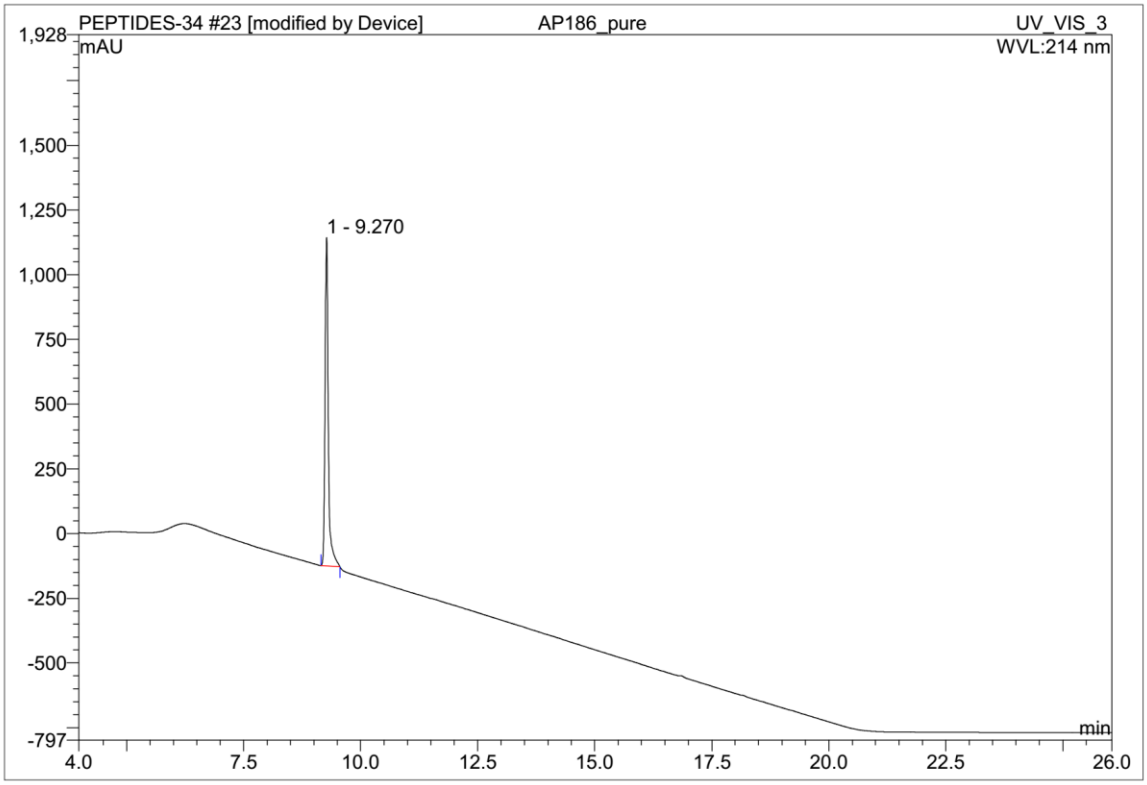

Fig. 5: HPLC trace of HPLC purified teixobactin analogue **2** (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

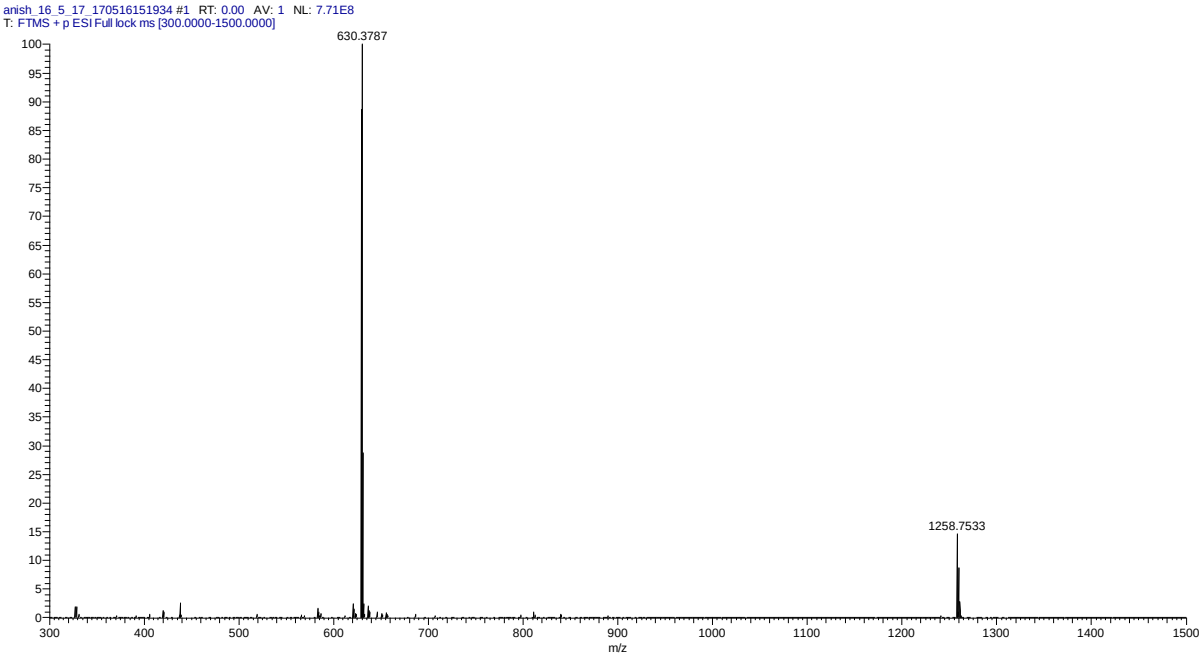

Fig. 6: HRMS spectra of HPLC purified teixobactin analogue **2**. Exact Mass calcd. for $C_{59}H_{99}N_{15}O_{15}$ = 1257.7445, found $M+H^+$ = 1258.7533 and $M/2 + H^+$ = 630.3787

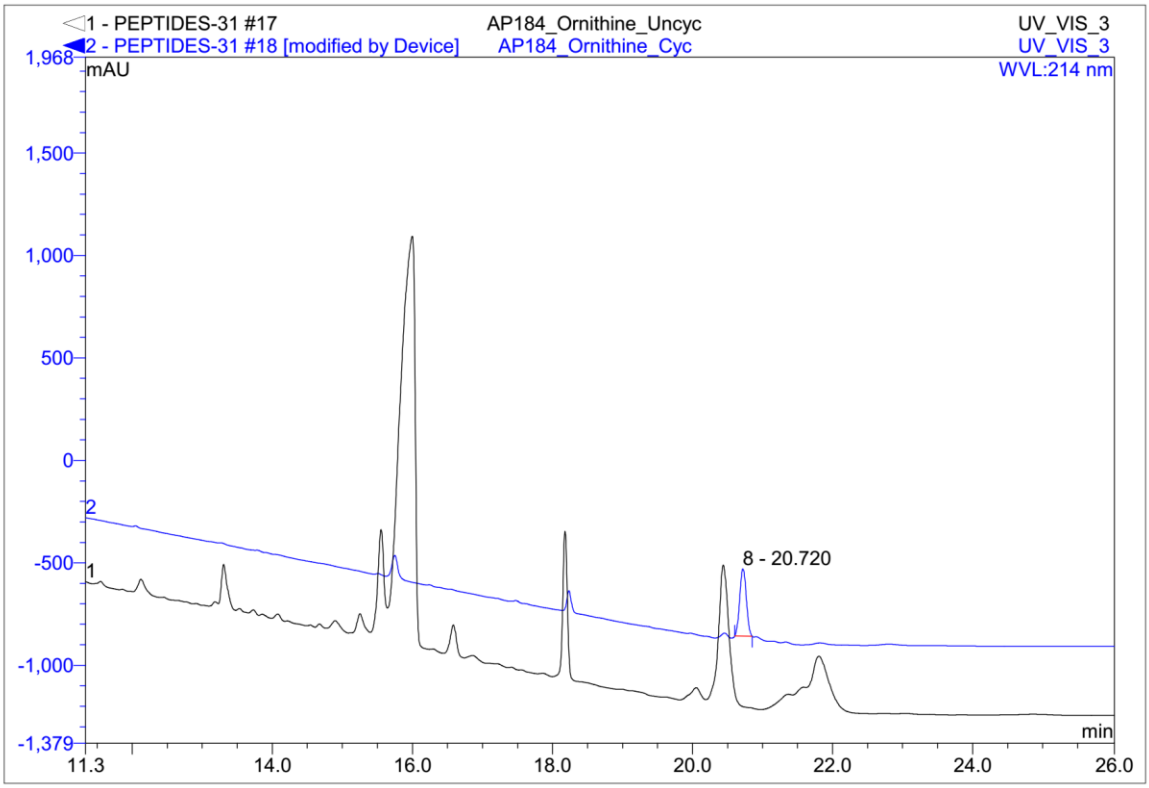

Fig. 7: HPLC trace showing the progress of the cyclisation reaction for analogue **3**: conversion of the uncyclized protected teixobactin analogue t_R= 16.000 min (shown in black) to the cyclised protected teixobactin analogue t_R = 20.720 min (shown in blue) (Gradient: 5-95% in 25 min)

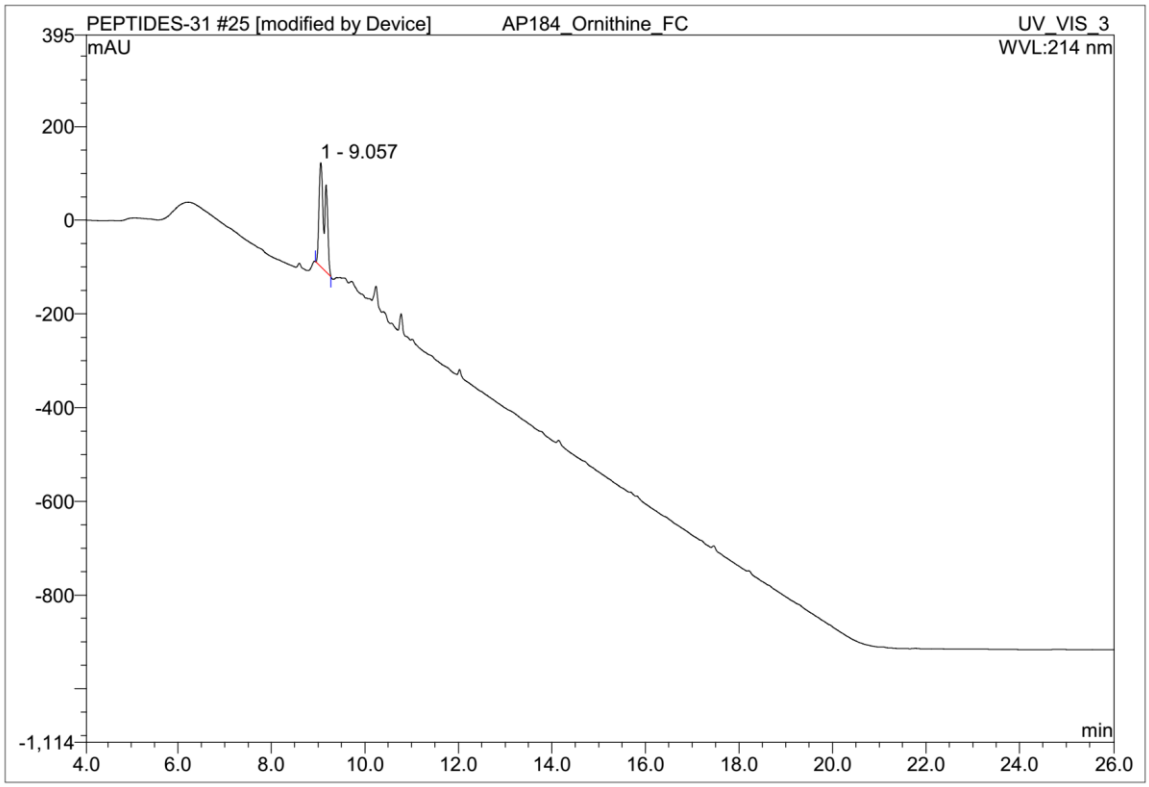

Fig 8: HPLC trace of crude teixobactin analogue **3** t_R = 9.057 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

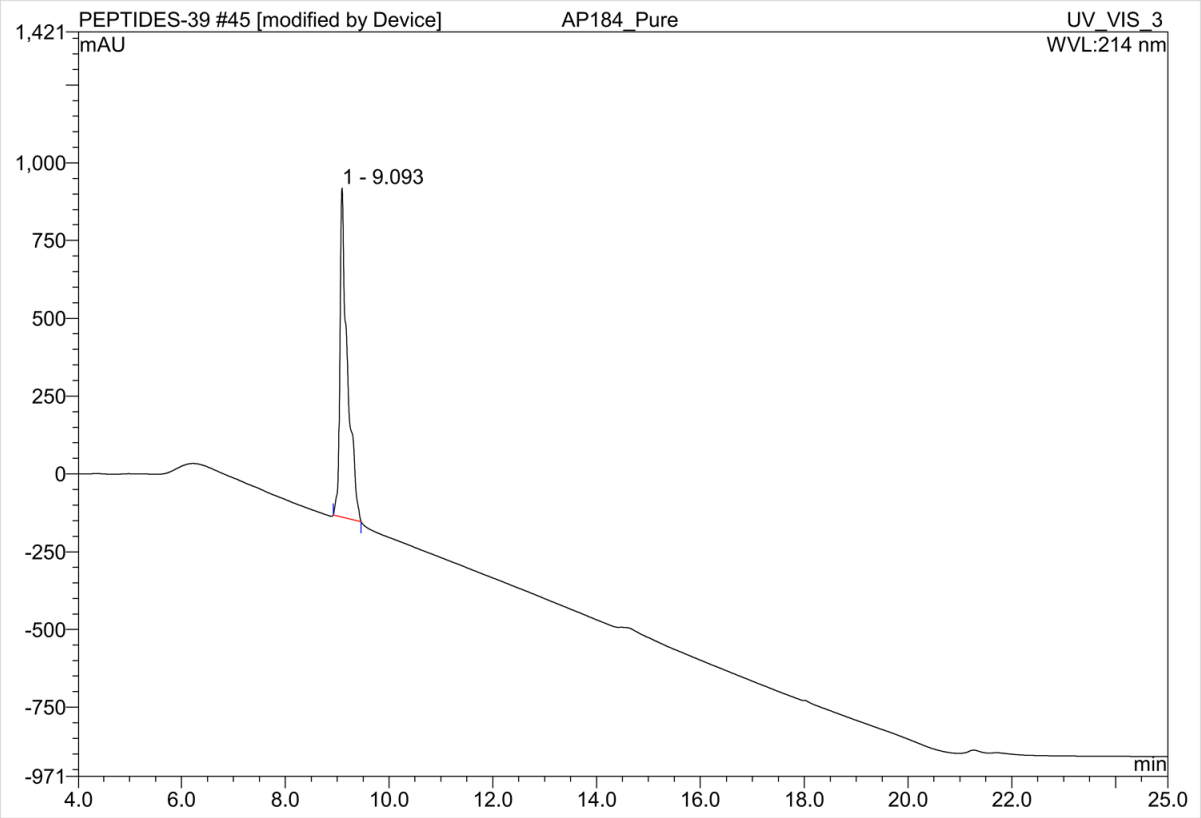

Fig 9: Ornithine HPLC trace of HPLC purified teixobactin analogue **3** (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

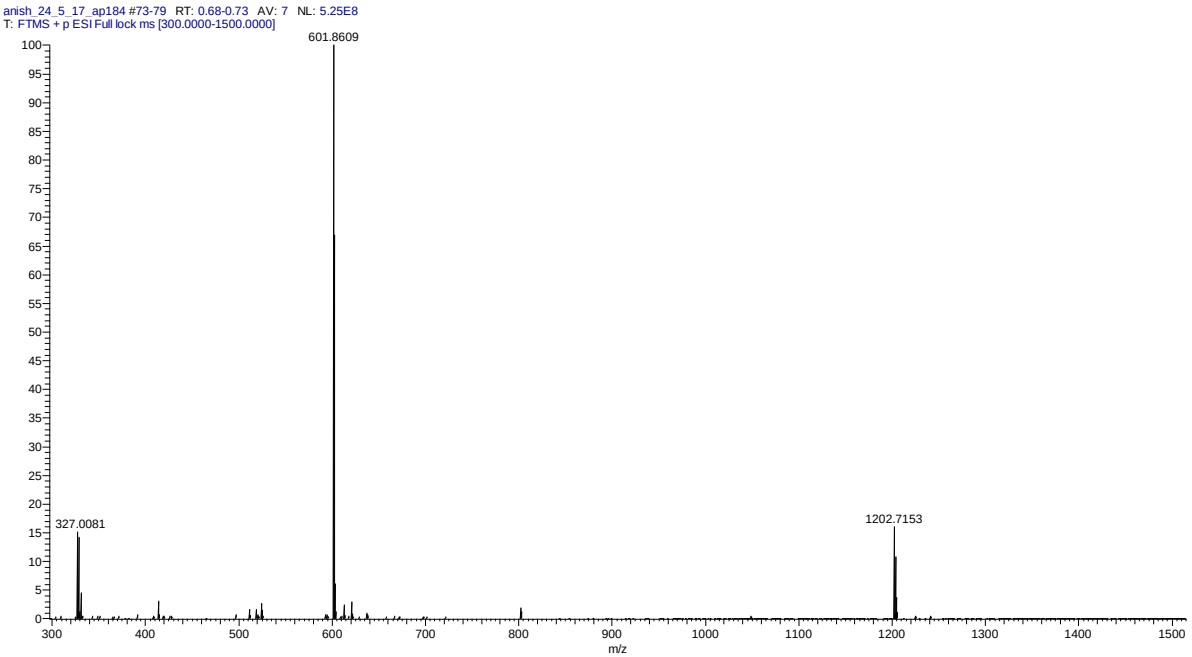

Fig. 10: ESI-MS spectra from LC-MS of HPLC purified teixobactin analogue **3**. Exact Mass calcd. for $C_{57}H_{95}N_{13}O_{15}$ = 1201.71, found $M+H^+$ = 1202.7153 and $M/2 + H^+$ = 601.8609

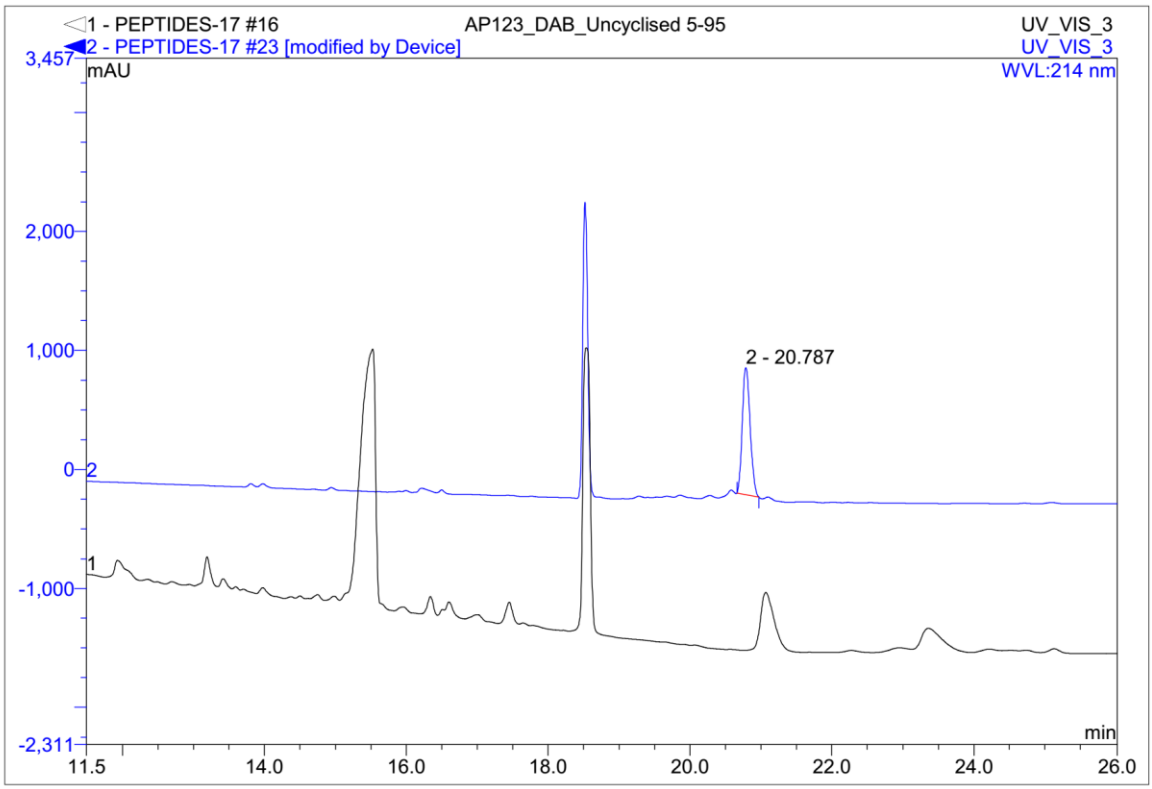

Fig.11: HPLC trace showing the progress of cyclisation reaction for analogue **5**: conversion of the uncyclized protected teixobactin analogue t_R= 15.527 min (shown in black) to the cyclized protected teixobactin analogue t_R = 20.787 min (shown in blue) (Gradient: 5-95% in 25 min)

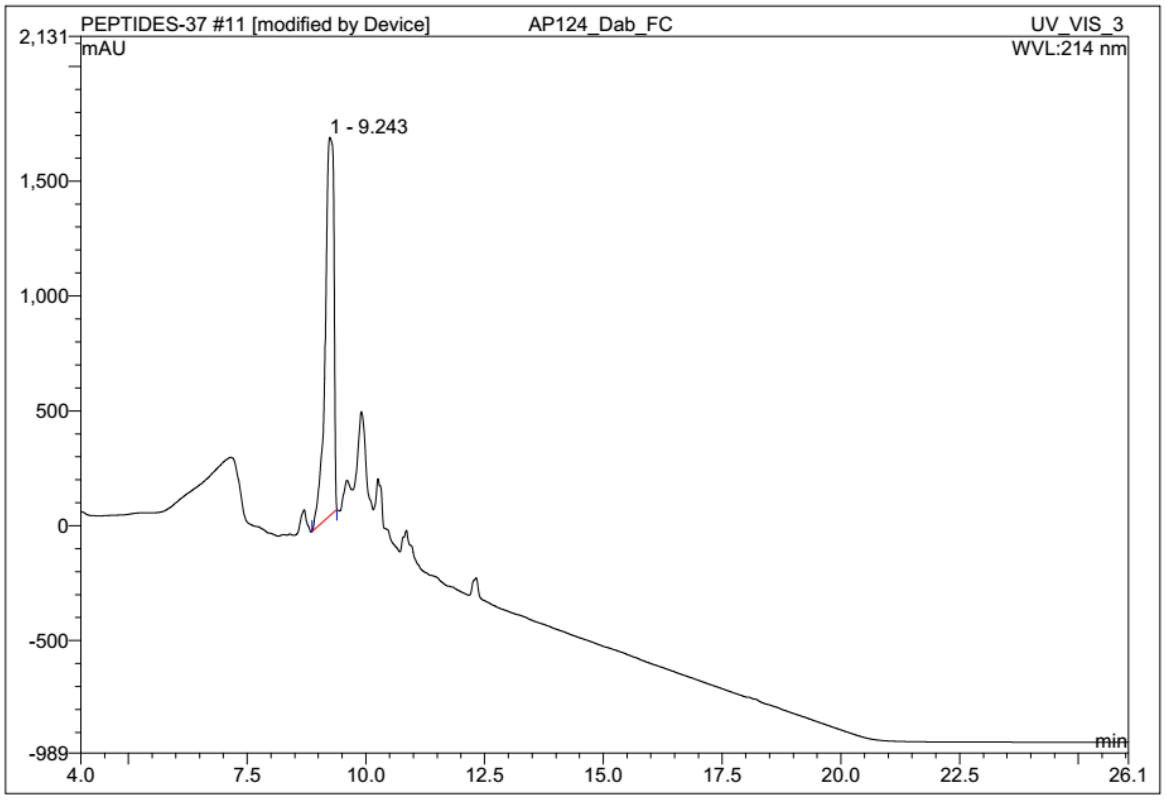

Fig. 12: HPLC trace of crude teixobactin analogue **5** t_R = 9.243 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

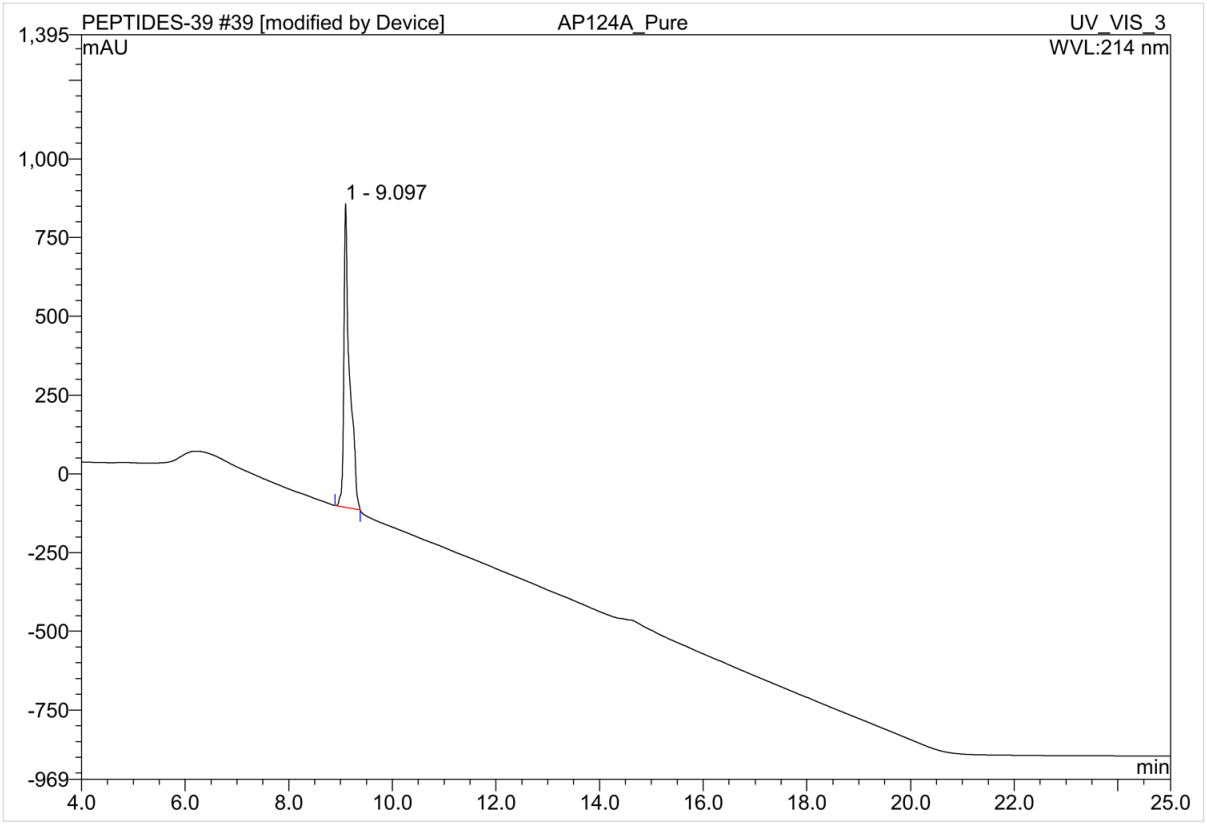

Fig. 13: HPLC trace of HPLC purified teixobactin analogue **5** (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

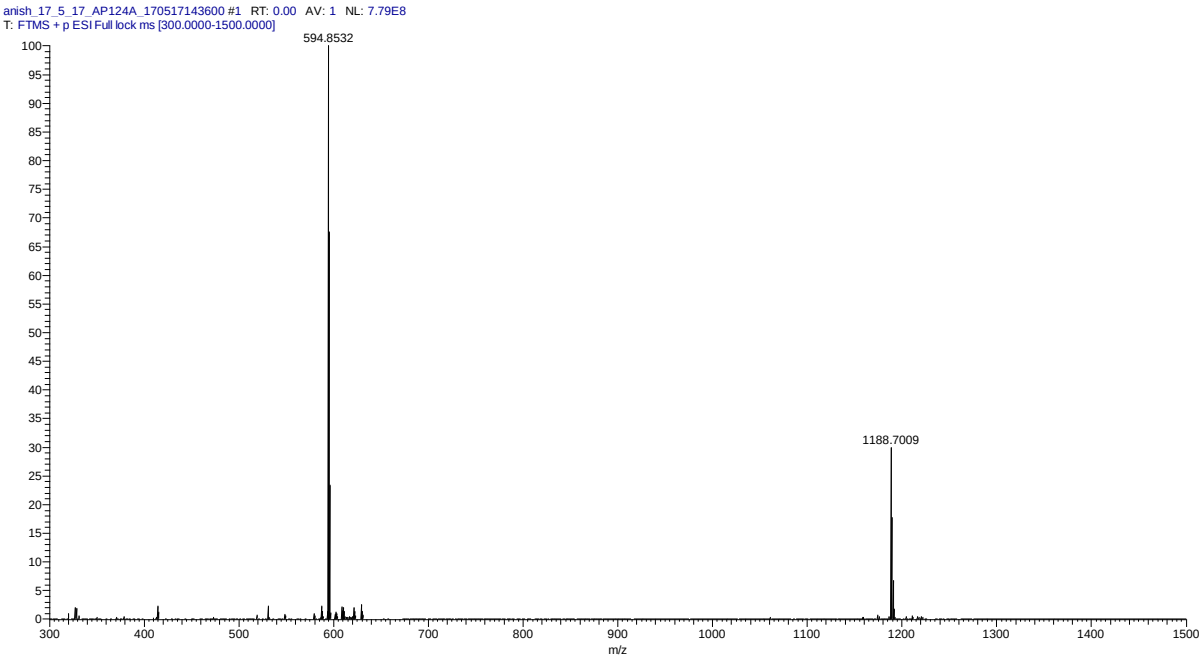

Fig. 14: HRMS spectra from LC-MS of HPLC purified teixobactin analogue **5**. Exact Mass calcd. for $C_{56}H_{93}N_{13}O_{15}$ = 1187.6914, found $M+H^+$ = 1188.7009 and $M/2 + H^+$ = 594.8532

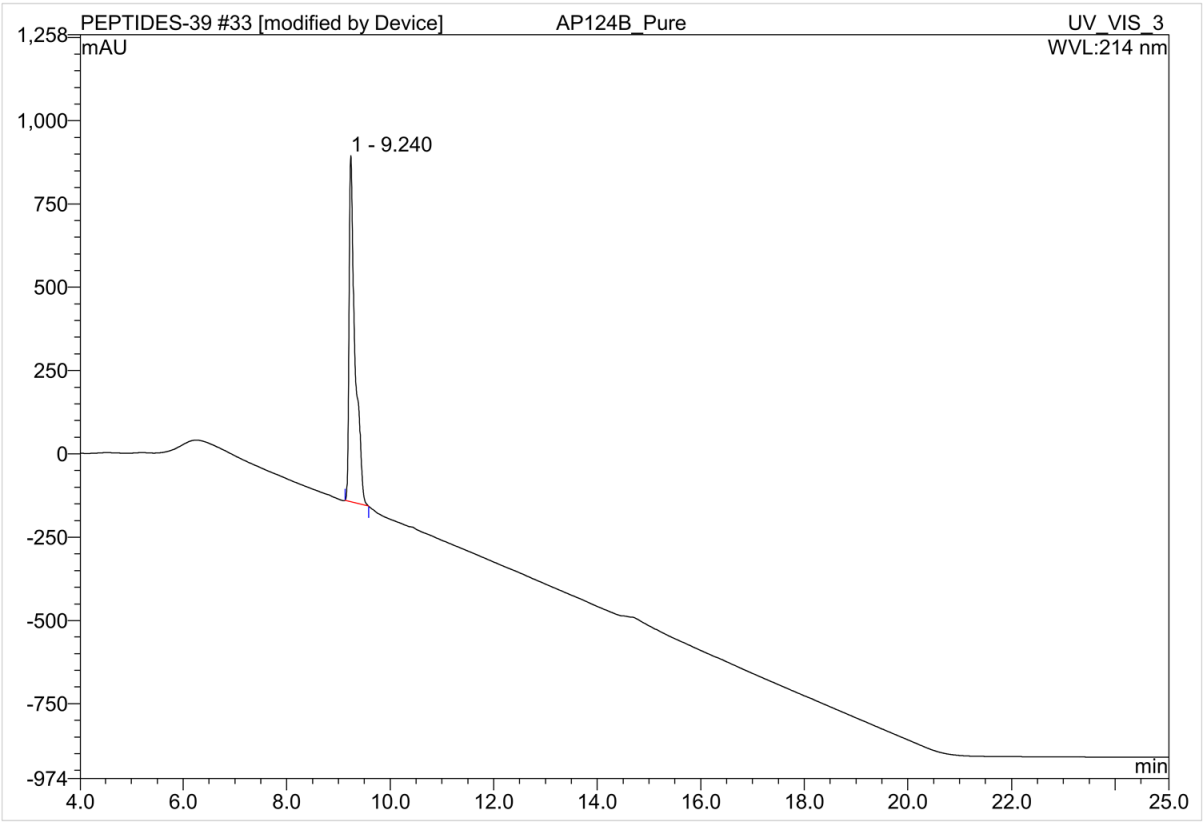

Fig. 15: HPLC trace of HPLC purified teixobactin analogue **6** (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

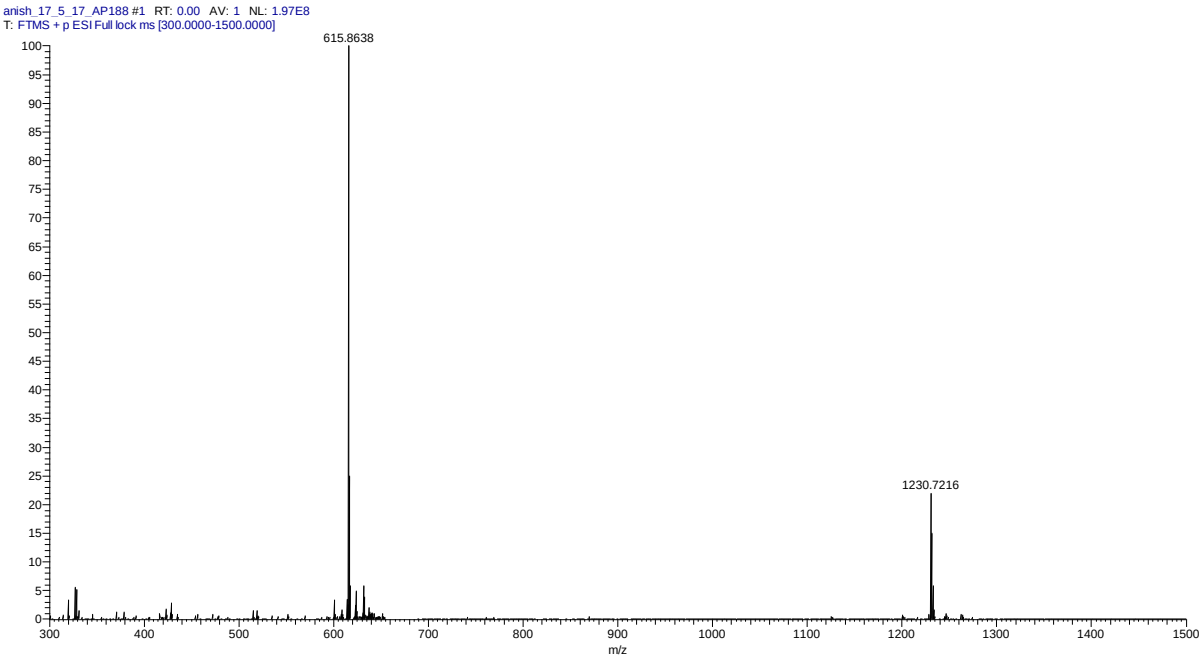

Fig. 16: ESI-MS spectra from LC-MS of HPLC purified teixobactin analogue **6**. Exact Mass calcd. for $C_{57}H_{95}N_{15}O_{15}$ = 1229.7132, found $M+H^+$ = 1230.7216 and $M/2 + H^+$ = 615.8638

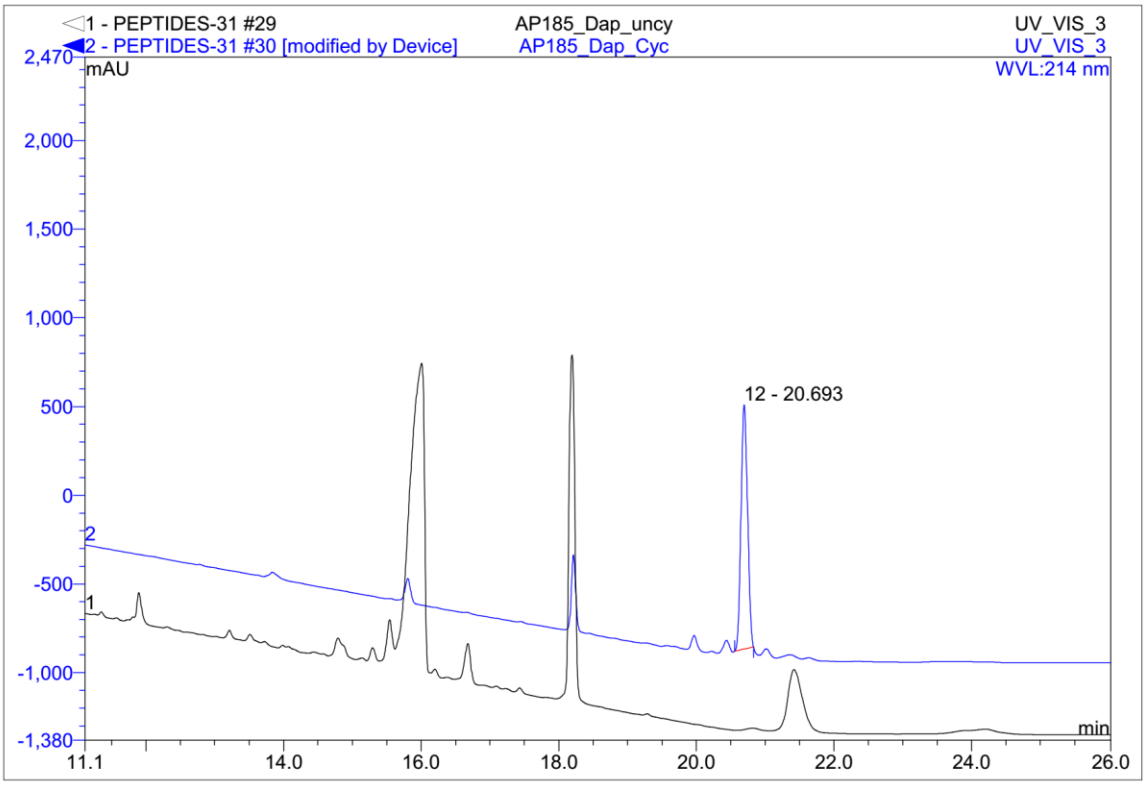

Fig. 17: HPLC trace showing the progress of cyclisation reaction of analogue **7**: conversion of the uncyclized protected teixobactin analogue t_R= 16.010 min (shown in black) to the cyclized protected teixobactin analogue t_R = 20.693 min (shown in blue) (Gradient: 5-95% in 25 min)

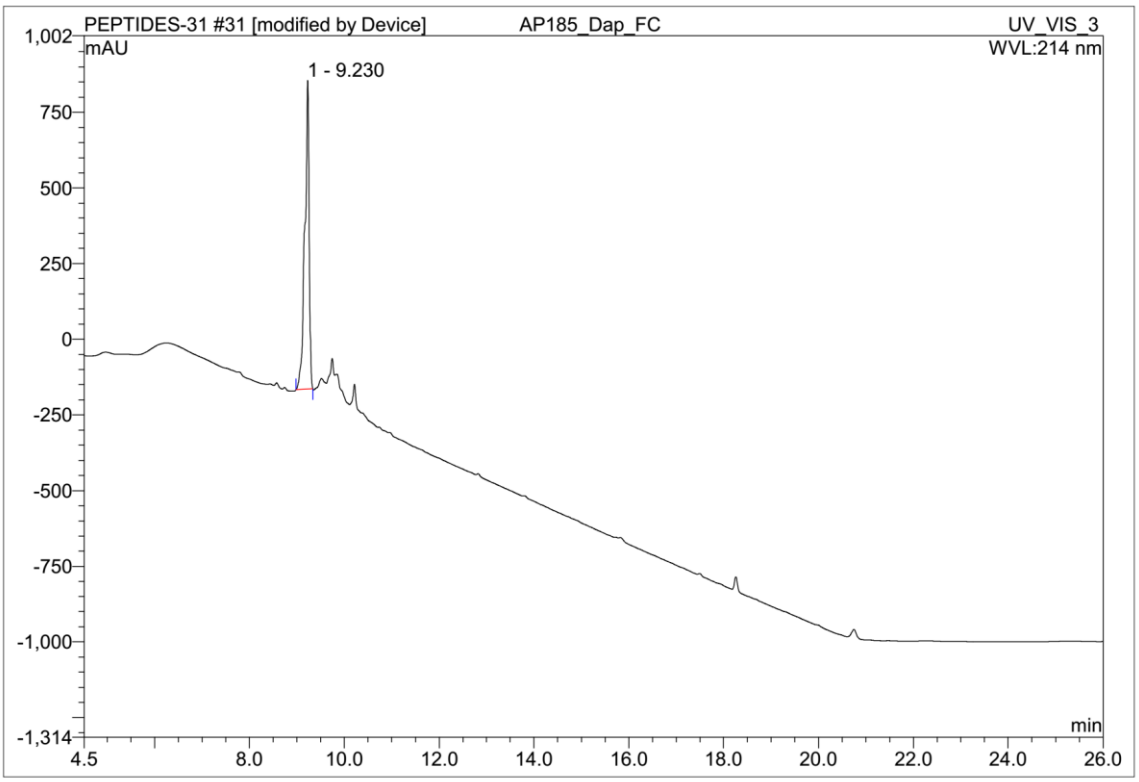

Fig 18: HPLC trace of crude teixobactin analogue **7** t_R = 9.230 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

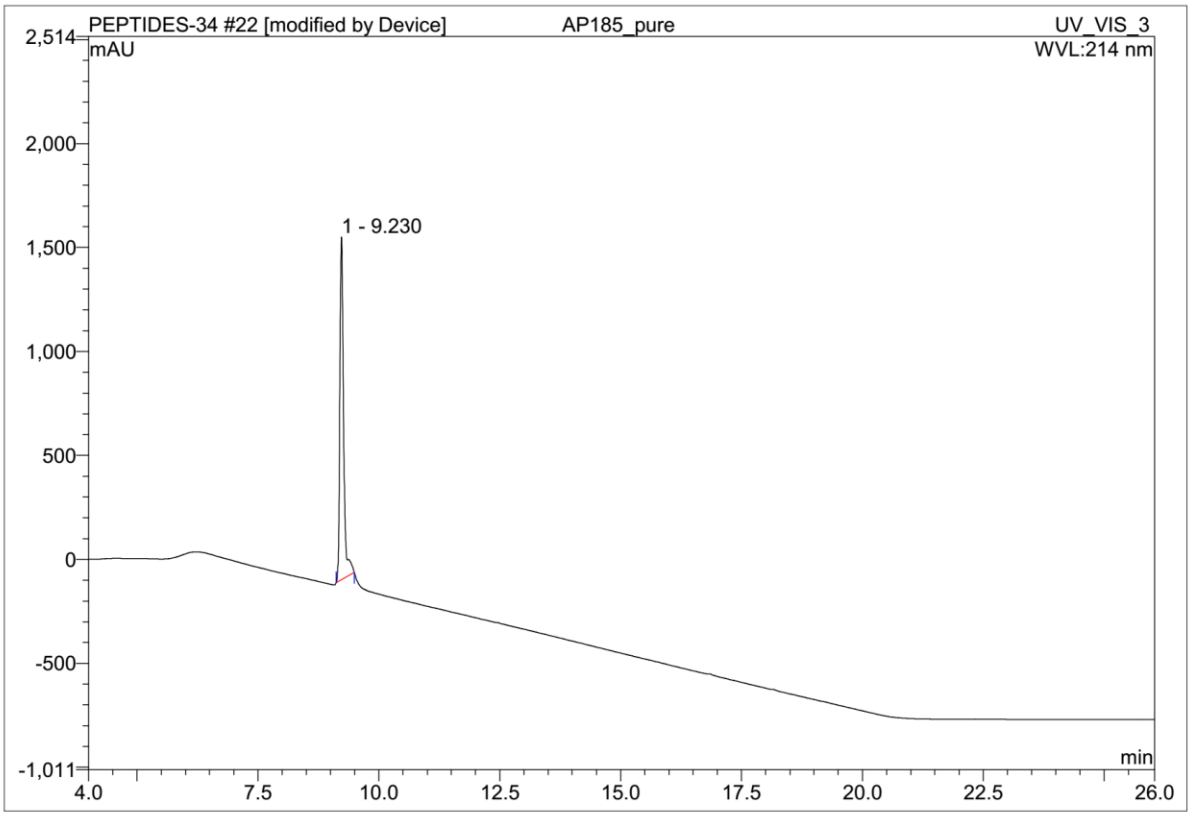

Fig. 19: HPLC trace of HPLC purified teixobactin analogue **7** (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

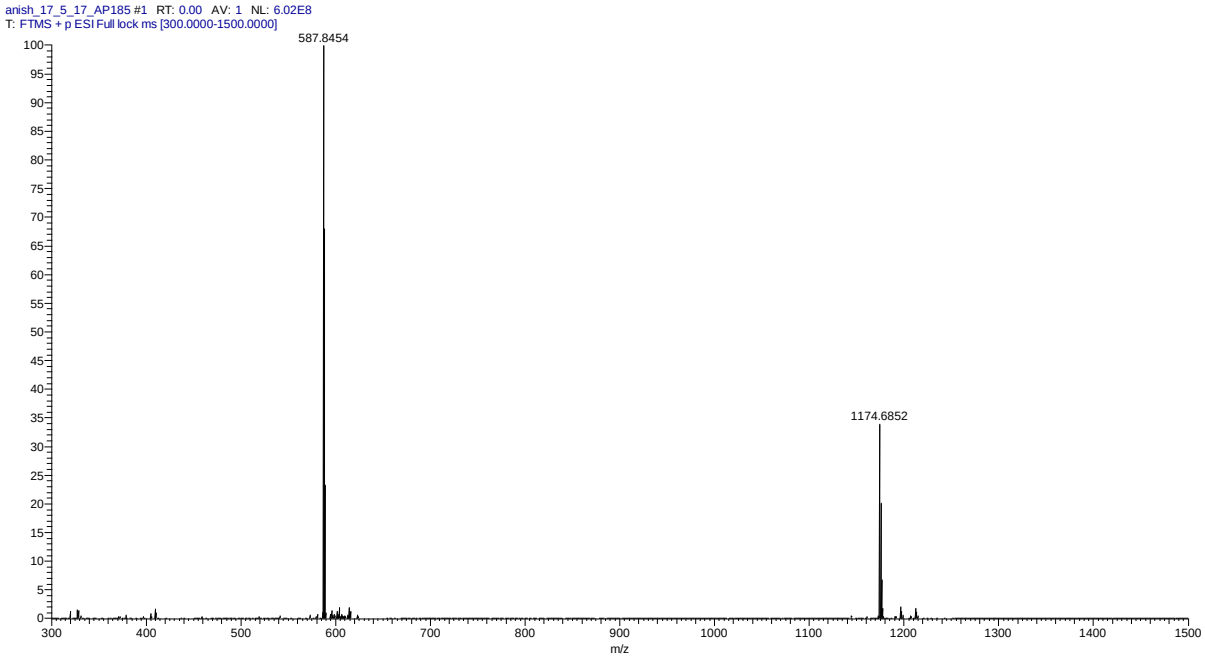

Fig. 20: ESI-MS spectra from LC-MS of HPLC purified teixobactin analogue **7**. Exact Mass calcd. for $C_{55}H_{91}N_{13}O_{15}$ = 1173.6758, found $M+H^+$ = 1174.6852 and $M/2 + H^+$ = 587.8454

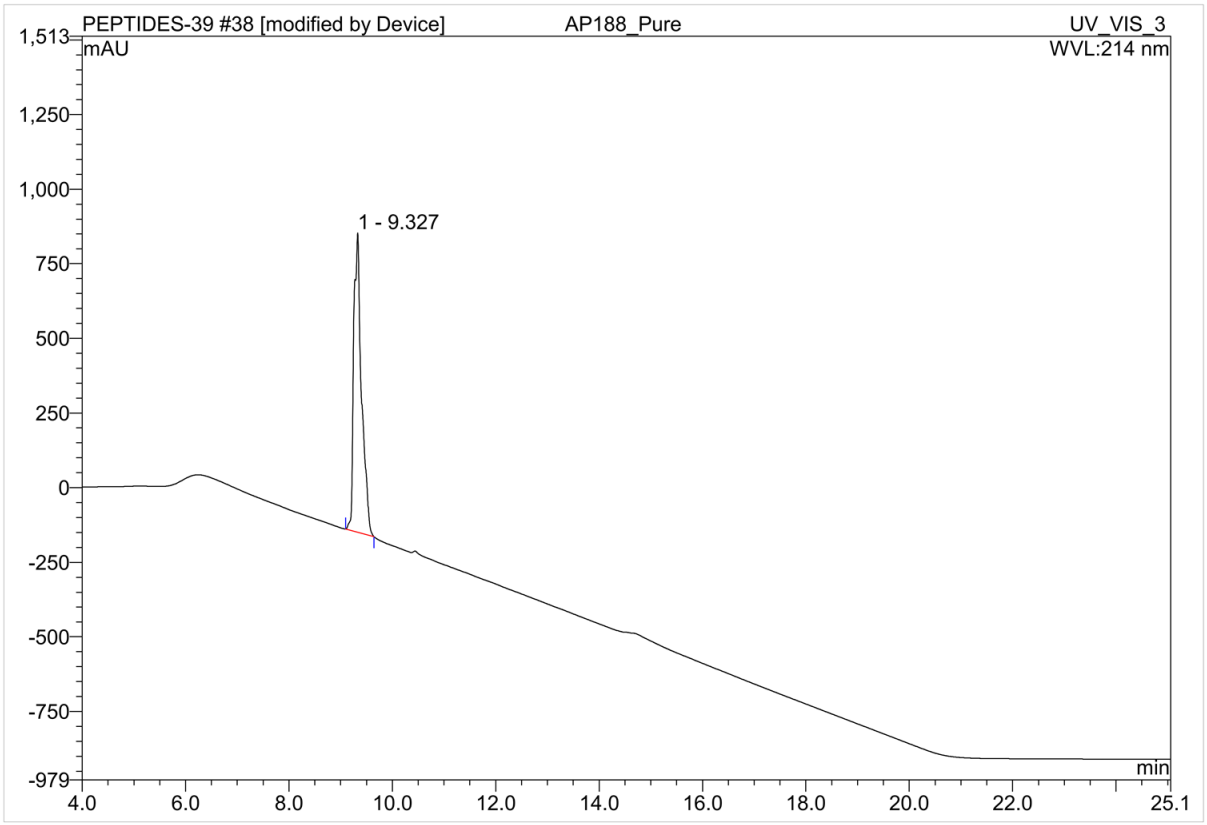

Fig. 21: HPLC trace of HPLC purified teixobactin analogue **8** (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

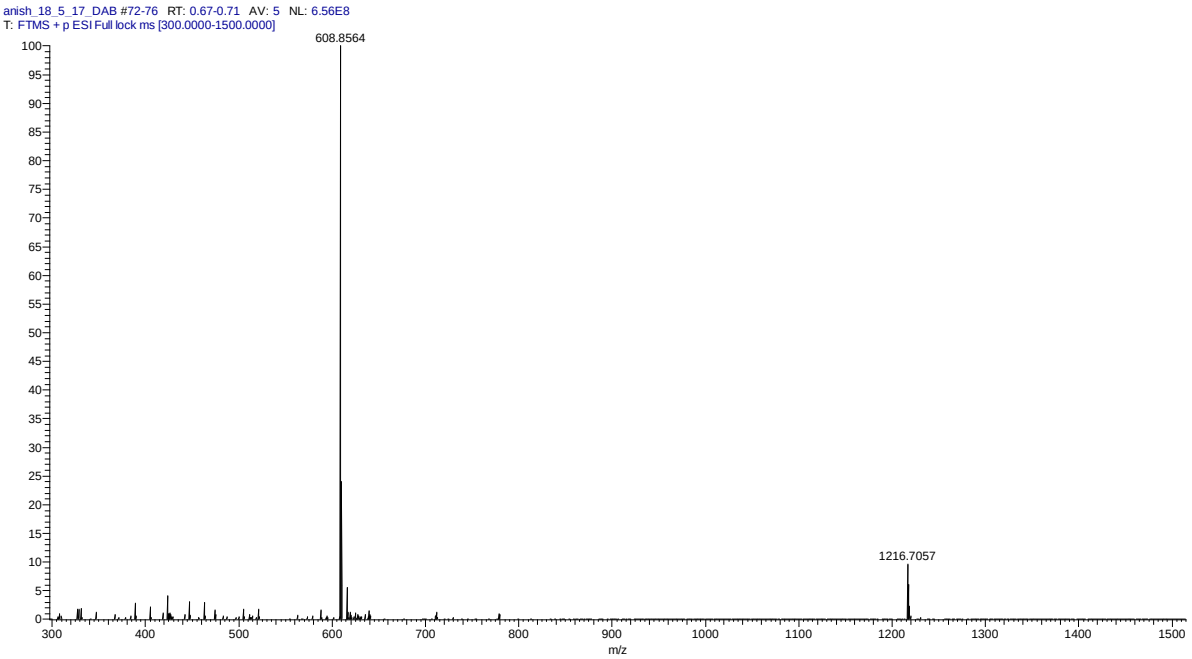

Fig. 22: ESI-MS spectra from LC-MS of HPLC purified teixobactin analogue **8**. Exact Mass calcd. for $C_{56}H_{93}N_{15}O_{15}$ = 1215.6976, found $M+H^+$ = 1216.7057

V. MIC testing

For MIC assays all peptides were dissolved in DMSO containing 0.002% polysorbate 80[2]. All bacteria were grown in Mueller Hinton broth (Oxoid). All incubations were at 37°C. Dilutions were carried out in triplicate. 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. Thus, the concentrations (in µg/mL) were: 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 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.

For all the compounds in which the MIC lower than 1 µg/ml for the initial test, the above procedure was repeated at an altered initial concentration of 64 µg/ml. Therefore, the new concentrations for MIC were: 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 and no peptide present. Vancomycin was used as a control.

VI. 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 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.