Supplementary Information

Efficient total syntheses and biological activities of two teixobactin analogues

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I. Materials

All L amino acids, Fmoc-D-Ile-OH, Fmoc-D-Thr(Trt)-OH and oxyma pure were purchased from Merck Millipore. 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, H_2N-D-Thr-OH, Phenylsilane ($PhSiH_3$), Tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)$], 2-methyl-6-nitrobenzoic anhydride (MNBA), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC/EDCI) Hydrochloride, Diisoproplycarbodiimide (DIC) and Triisopropylsilane (TIS) were purchased from Fluorochem, UK. The 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 $CDCl_3$, DMSO-d_6 and DMSO and were purchased from Sigma Aldrich. Tritylchloride and 4-(Dimethylamino)pyridine were purchased from Alfa Aesar. Dimmethylformamide (DMF) peptide synthesis grade and Trifluoroacetic acid (TFA) was purchased from Rathburn chemicals. Triethylamine, Diethyl ether, Dimethylsulfoxide, Dichloromethane, 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. Wang Resin (manufacturer's loading: 0.7 mmol/g) was obtained from NovaBioChem. 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. Alternatively, LC-MS/HRMS were performed using a Xevo QTof mass spectrometer (Waters) coupled to an Acquity LC system (Waters) using an Acquity UPLC BEH C18 column (2.1 x 50 mm, Waters).

NMR spectra were recorded on a Bruker 500 MHz Avance III HD spectrometer equipped with a broadband probe.

III. Attempted synthesis of the teixobactin analogue (1) via route A:

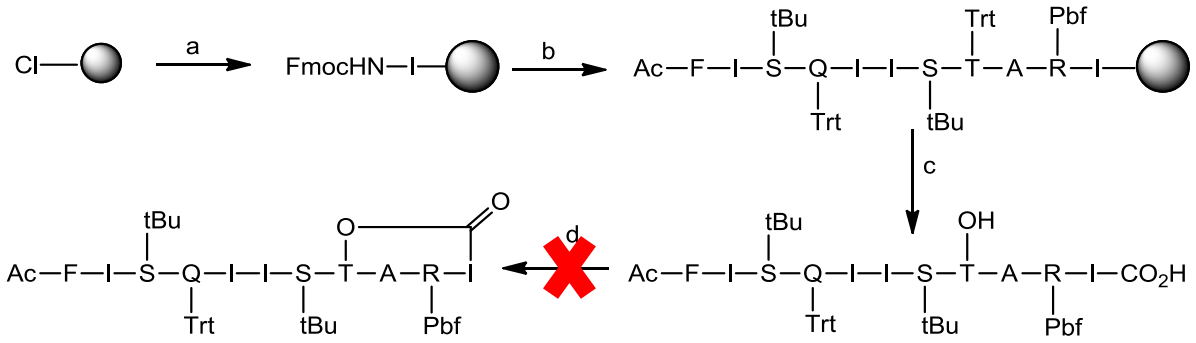

Figure S1: Scheme showing the attempted synthesis of the teixobactin analogue **1** via route A

(step a) Commercially available 2-Chlorortritylchloride resin (manufacturer's loading = 1.2 mmol/g) was pre-swelled in DCM in a reactor. To it was added 4 eq. Fmoc-Ile-OH, 8 eq. DIPEA in DCM and the reaction was shaken for 3h. The resin was then washed 3 x DCM, 3 x DMF. Any unreacted resin was capped with MeOH:DIPEA:DCM = 1:2:7 by shaking for 1h. The loading determined by UV absorption of the piperidine-dibenzofulvene adduct was calculated to be 0.6 mmol/g. (step b) The Fmoc protecting group was deprotected using 20% piperdine in DMF by shaking for 3 min, followed by draining and shaking again with 20% piperidine in DMF for 10 min. The subsequent amino acids were successively coupled (except the Fmoc-D-Thr(Trt)-OH) using the following protocol: 4 eq. FmocHN-AA(P.G.)-OH (AA = Amino Acid, PG = Protecting Group), 4 eq. DIC/Oxyma in DMF using a microwave peptide synthesizer by irradiating for 10 min. Fmoc deprotection was performed using the procedure described in step *a* above. Washing steps were performed using DMF as follows: 4 x 45s after every deprotection step and 6 x 45s after every coupling step. Fmoc-D-Thr(Trt)-OH was coupled using 3 eq. Amino acid, 3 eq. HATU and 6 eq. DIPEA in DMF and shaking for 1h at room temperature. The N terminus was capped using 10% DIPEA/Ac_2O in DMF and shaking for 30 min. (step c) The peptide was cleaved off the resin keeping the side chain protecting groups on using: TFA:TIS:DCM = 2:5:93 and shaking for 2h. (step d) The solvent was evaporated and the following conditions were used for esterification:

Sr. No.	Reagents	Solvent	Duration	Temperature
1.	1.2 eq. DCC/5 eq. DMAP	DMF	24h	r.t.
2.	2 eq. DCC + 1 eq. after 4h /5 eq. DMAP	DMF	24h	r.t.
3.	3 eq. DCC/20 mol% DMAP	DMF	2h	r.t
4.	1.2 eq. MNBA/2.4 eq. DMAP	DMF	12h	r.t.
5.	2.5 eq. EDCI/0.5 eq. DMAP	DMF	24h	r.t.
6.	18 eq. DCC/28 eq. DMAP	DMF	30 min, 6h	0-4 deg., r.t
7.	1.2 DCC/6 eq. DMAP	DMF	24h	60, heating
8.	1.2 eq. DIC/6 eq. DMAP	DMF	24h	60, heating

Table S1: List of conditions used for cyclization via esterification

IV. Synthesis of teixobactin core ring structure (2):

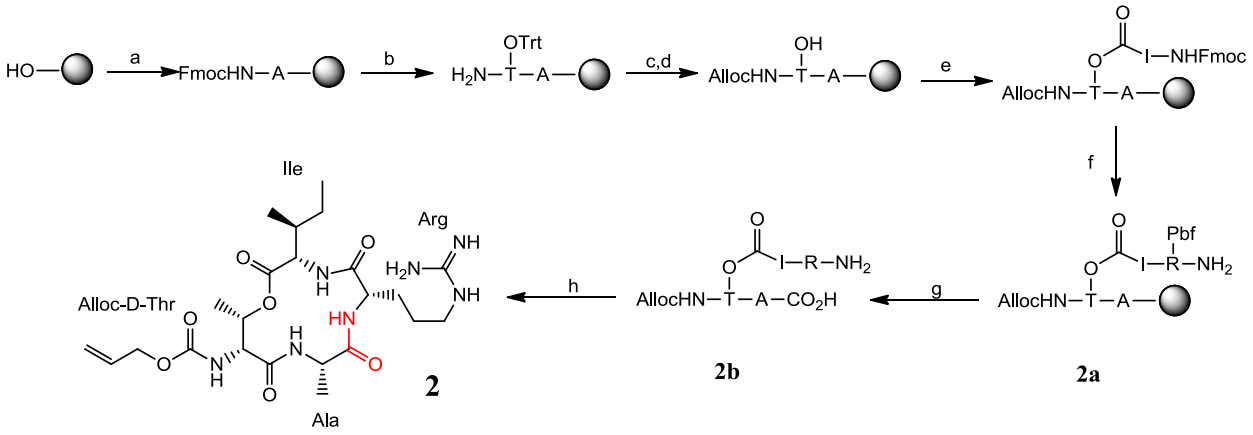

Figure S2: Synthesis scheme for the teixobactin core ring (**2**)

(step a) Wang resin (manufacturer's loading = 0.7 mmol/g) was weighed out in a clean dry reactor. To the resin, pre-swelled in DMF, was added 10 eq. Fmoc-Ala-OH, 10 eq. DIC and 1 eq. DMAP and the reactor was shaken for 3h. The unreacted alcohol was then capped using 10% Ac_2O/DIPEA in DMF. The loading determined by UV absorption of the piperidine-dibenzofulvene adduct was calculated to be 0.47 mmol/g. (step b) 2.5 eq. Fmoc-D-Thr(Trt)-OH, 2.5 eq. HATU and 5 eq. DIPEA in DMF were added on the resin and the reactor was shaken for 3h at room temperature. The coupling of Fmoc-D-Thr(Trt)-OH was verified using the Ninhydrin color test. The Fmoc protecting group was then removed using the protocol described in section III step (b) earlier. (step c) The free amine was protected by adding 4 eq. Allyl Chloroformate/8 eq. DIPEA in DCM to the resin pre-swelled in DCM and shaking for 1h. (step d) The trityl protecting group was removed using TFA:TIS:DCM = 1:5:94 by performing 3 x 15 min cycles and washing with DCM.. (step e) **Esterification** was performed using 10 eq. Fmoc-Ile-OH, 10 eq. DIC, 10 mol% DMAP in DCM and shaking for 2h followed by capping with 10% Ac_2O/DIPEA in DMF. (step f) Fmoc-Arg(Pbf)-OH was coupled using 4 eq. of AA, 4 eq. HATU and 8 eq. DIPEA in DMF and shaking for 1h followed by Fmoc deprotection using 20% piperidine in DMF using the protocol described in section III step (b) earlier. (step g) The fragment was cleaved off the resin using TFA:TIS:H_2O = 95:2.5.2.5 and shaking for 1h. (step h) Cyclization was performed using 1 eq. HATU/10 eq. DIPEA in DMF by stirring for 1h. HPLC trace of crude **2** (figure S9). ESI-HRMS mass calcd. for compound **2**: $C_{23}H_{39}N_7O_7$ = 525.2911, found $M+H^+$ = 526.3010 (figure S10).

V. Synthesis of AllocHN-D-Thr-OH (4)

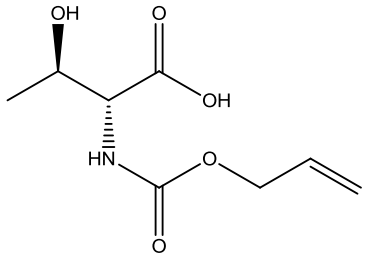

Figure S3: Structure of AllocHN-D-Thr-OH (**4**)

2 g, 16.8 mmol, H_2N-D-Thr-OH was dissolved in water containing 2 eq. $NaHCO_3$: THF = 2:1, 40 mL and the reaction was cooled to 0°C. Water was then added dropwise till all the H_2N-D-Thr-OH dissolved. Ally chloroformate, 1.2 eq., 2.1 mL, was then added slowly to the reaction and was left stirring for 3 days at r.t. The reaction was monitored by TLC after 24h intervals. The reaction was then acidified to pH = 2 using 6N HCl. The product was extracted using Et_2O (3 X). The organic layer was then dried using Na_2SO_4 and the solvent was evaporated under reduced pressure. The reaction mixture was purified using silica gel column chromatography DCM/MeOH = 9:1 to obtain a colourless oil. 82% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.10 (d, *J* = 6.41 Hz, 3 H), 3.94 (dd, *J* = 9.00, 3.51 Hz, 1 H), 4.02 - 4.13 (m, 1 H), 4.50 (d, *J* = 5.19 Hz, 2 H), 5.19 (dd, *J* = 10.68, 1.22 Hz, 1 H), 5.32 (dd, *J* = 17.40, 1.53 Hz, 1 H), 5.84 - 5.98 (m, 1 H), 6.85 (d, *J* = 8.85 Hz, 1 H), (figure 4); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.8, 60.3, 65.0, 66.9, 117.4, 134.0, 156.7, 172.7, (figure 5); ESI-HRMS calcd. for $C_8H_{14}NO_5$ = 203.0794 found: M+ H^+ = 204.0864, M+Na^+ = 226.0704. Cald. for [M – CO_2 + H^+] = [203.0794 – 43.9898 + 1.0072] = 160.0968, found 160.0968, (figure 6).

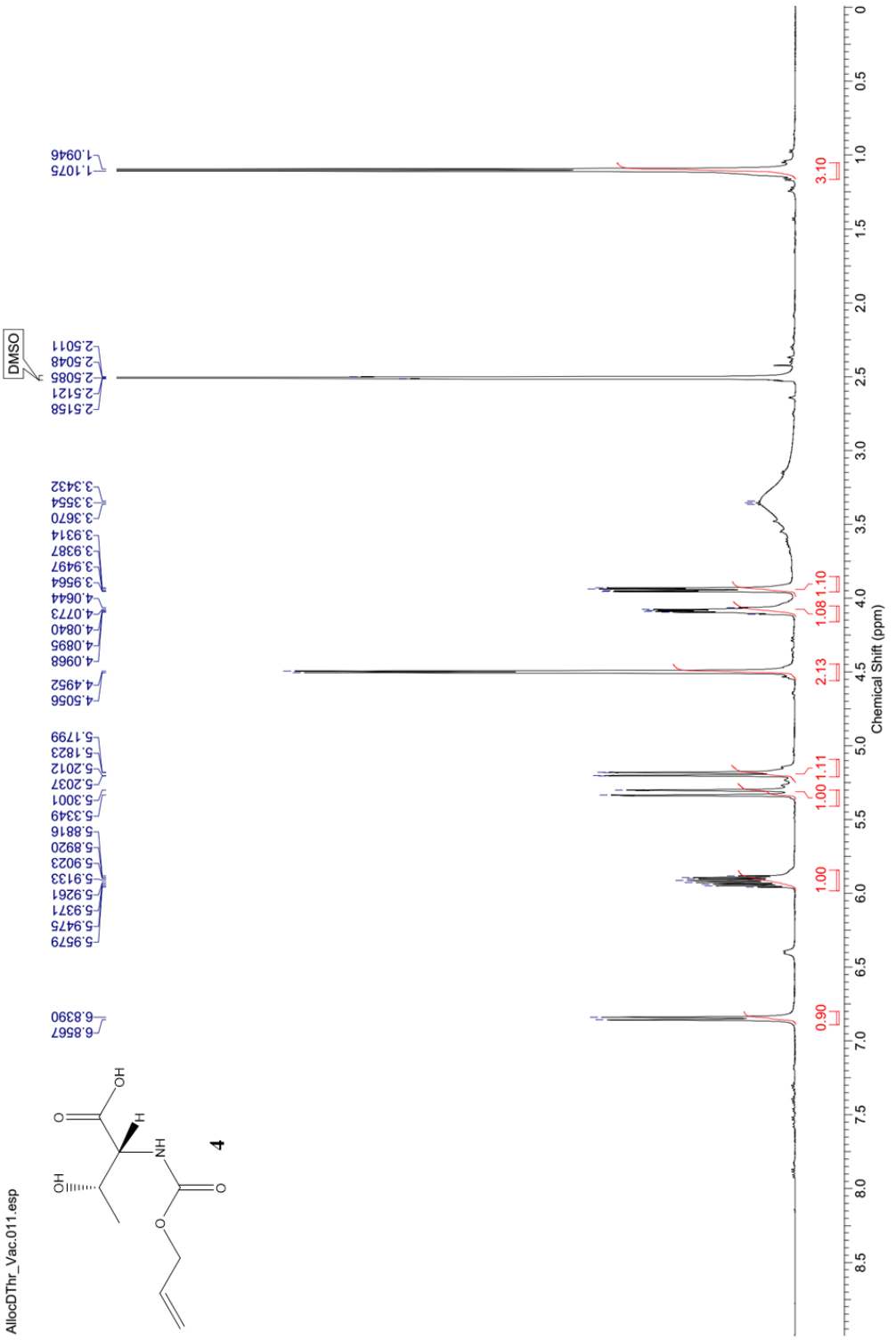

Figure S4: 1H NMR spectrum for compound **4**

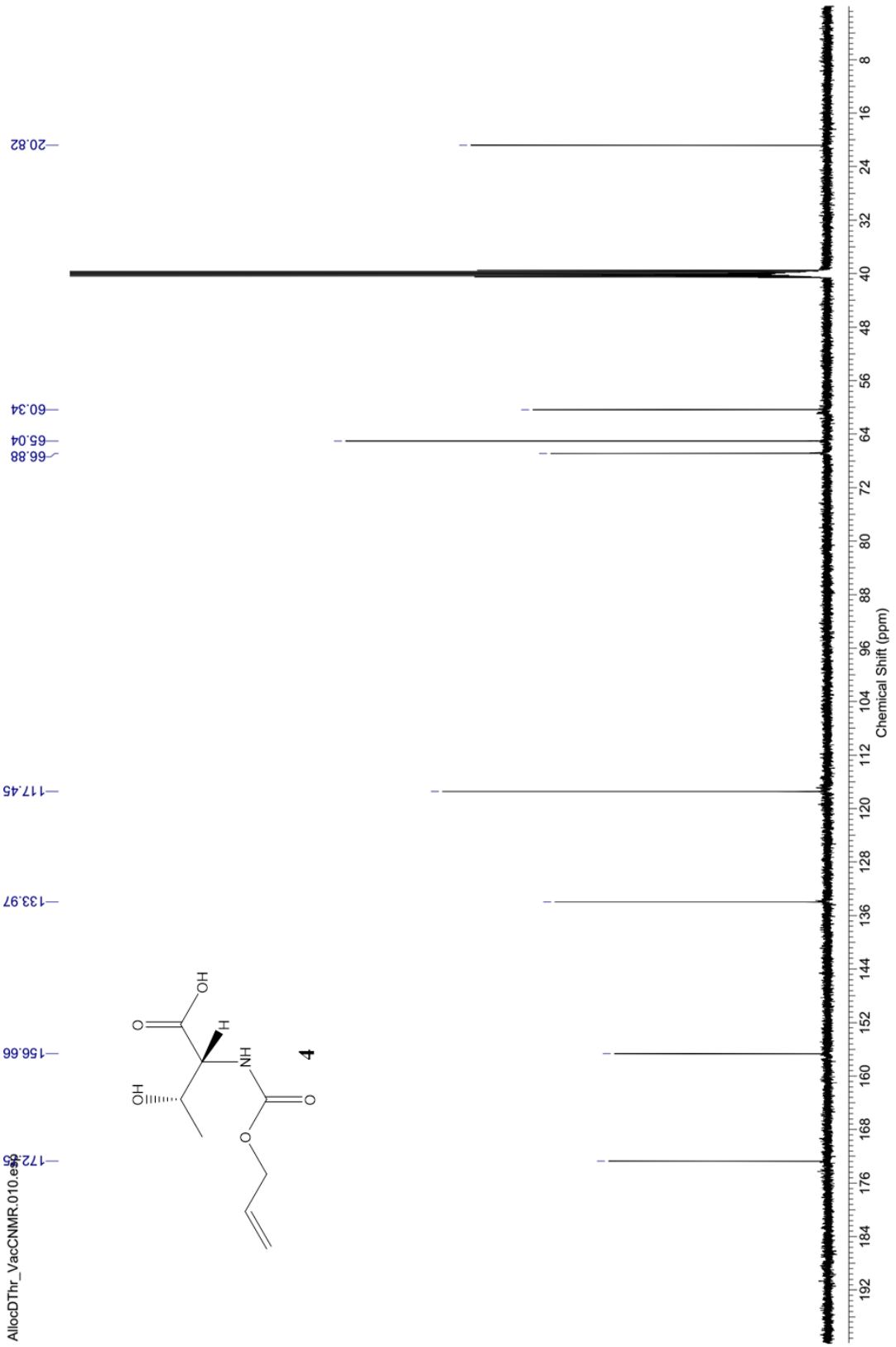

Figure S5: ^{13}C NMR spectrum for compound **4**

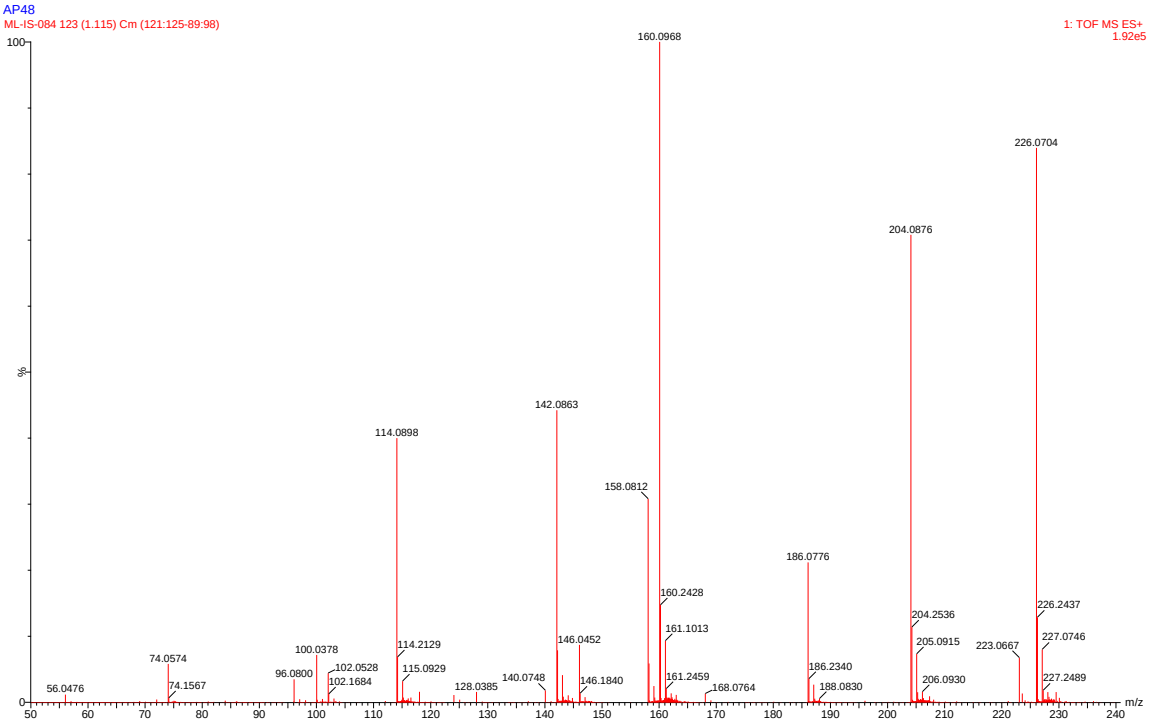

Figure S6: HRMS spectrum of AllocHN-D-Thr-OH (**4**). HRMS calcd. for $C_8H_{14}NO_5$ = 203.0794 found: M+ H^+ = 204.0864, M+Na^+ = 226.0704. Cald. for [M – CO_2 + H^+] = [203.0794 – 43.9898 + 1.0072] = 160.0968, found 160.0968.

VI. Synthesis of the Teixobactin analogue (1) via route B:

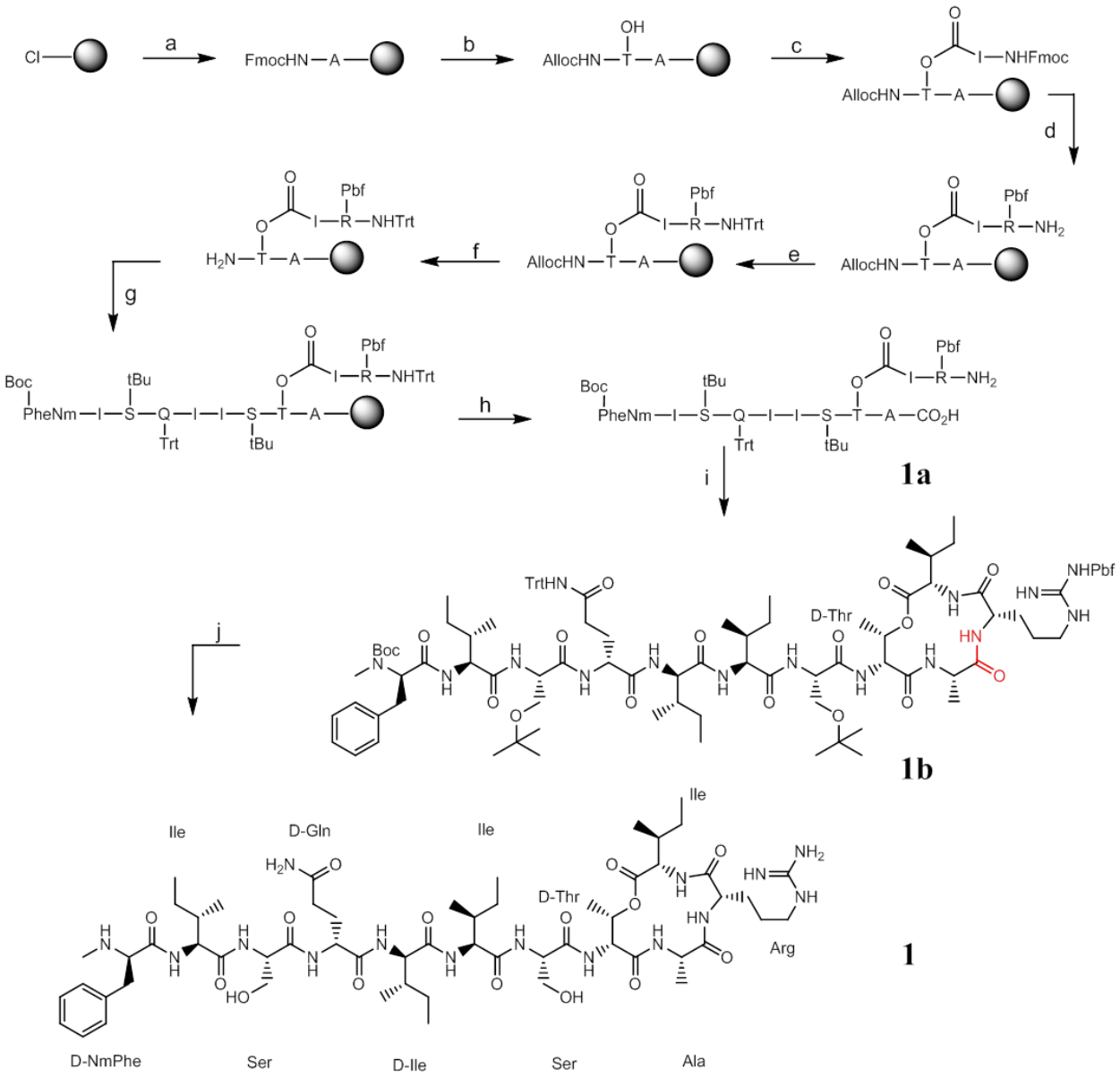

Figure S7: Synthesis of the Teixobactin analogue **1**

(step a) Commercially available 2-Chlorotrityl chloride resin (manufacturer's loading = 1.2 mmol/g, 170 mg resin) was swelled in DCM in a reactor. To this resin was added 4 eq. Fmoc-Ala-OH/8 eq. DIPEA in DCM and the reactor was shaken for 3h. The loading determined by UV absorption of the piperidine-dibenzofulvene adduct was calculated to be 0.6 mmol/g, (170mg resin, 0.102mmol). Any unreacted resin was capped with MeOH:DIPEA:DCM = 1:2:7 by shaking for 1h. (step b) The fmoc protecting group was removed using 20% piperidine in DMF following the protocol described earlier in section III. (step b) The previously synthesized AllocHN-D-Thr-OH (**4**) was then coupled to the resin by adding 3 eq. of the AA, 3 eq. HATU and 6 eq. DIPEA in DMF and shaking for 3h at room temperature. (step c) Esterification was

performed using 10 eq. of Fmoc-Ile-OH, 10 eq. DIC and 5 mol% DMAP in DCM and shaking the reaction for 2h. This was followed by capping the unreacted alcohol using 10% Ac_2O/DIPEA in DMF shaking for 30 min and Fmoc was removed using protocol described earlier in section III. (step d) Fmoc-Arg(Pbf)-OH was coupled using 4 eq. of AA, 4 eq. HATU and 8 eq. DIPEA in DMF and shaking for 1h followed by Fmoc deprotection using 20% piperidine in DMF as described earlier. (step e) The N-terminus of Arg was protected using 10 eq. Trt-Cl and 15% Et_3N in DCM and shaking for 1h. The protection was verified by the Ninhydrin colour test. (step f) The Alloc protecting group of D-Thr was removed using 0.2 eq. [$Pd(PPh_3)$] and 24 eq. $PhSiH_3$ in dry DCM under argon for 1 h. This procedure was repeated twice and the resin was washed thoroughly with DCM and DMF to remove any Pd from the resin. (step g) All amino acids were coupled using 4 eq. AA, 4 eq. HATU and 8 eq. DIPEA (figure S7). Deprotection cycles were performed as described earlier. Each coupling and deprotection cycle were checked by the Ninhydrin colour test. (step h) The peptide was cleaved off from the resin without cleaving off the protecting groups for the amino acid side chains using TFA:TIS:DCM = 2:5:93 and shaking for 2h. (step i) The solvent was evaporated and the peptide was redissolved in DMF to which 1 eq. HATU and 10 eq. DIPEA were added and the reaction was stirred for 1h to perform the cyclization. The reaction was monitored on HPLC till all starting material had been consumed (figure S11). (step j) The side-chain protecting groups were then cleaved off using TFA:TIS:H_2O = 95:2.5:2.5 by stirring for 1h. The peptide was precipitated using cold Et_2O (-20°C) and centrifuging at 7000 rpm to obtain a white solid. This solid was further purified using RPHPLC using protocols as described in the section II. Fractions were collected, concentrated and lyophilised to obtain a white solid (28 mg, 22% yield). ESI-HRMS mass calcd for **1**: $C_{58}H_{98}N_{15}O_{15}$ = 1243.7289, found M+ H^+ = 1244.7336. HPLC traces of crude and purified **1** (figure S12 and S13), ESI-HRMS of **1** (figure S14).

VII. Synthesis of the Teixobactin analogue (3) via route B:

The synthesis of analogue **3** (figure S8) was achieved (200 mg resin, 0.12 mmol scale) using the same procedure as analogue **1** except for the final acetylation. Fmoc removal of the L-phenyl alanine was performed using the protocol described previously (in section III) and acetylation of the amine was achieved by using 10% Ac_2O/DIPEA in DMF and shaking for 30 min. TFA cleavage was performed as described in IV. *step j* above. The solvent was evaporated and the peptide was redissolved in DMF to which 1 eq. HATU and 10 eq. DIPEA were added and the reaction was stirred for 1h to perform the cyclization. The reaction was monitored on HPLC till all starting material had been consumed (figure S15). The side-chain protecting

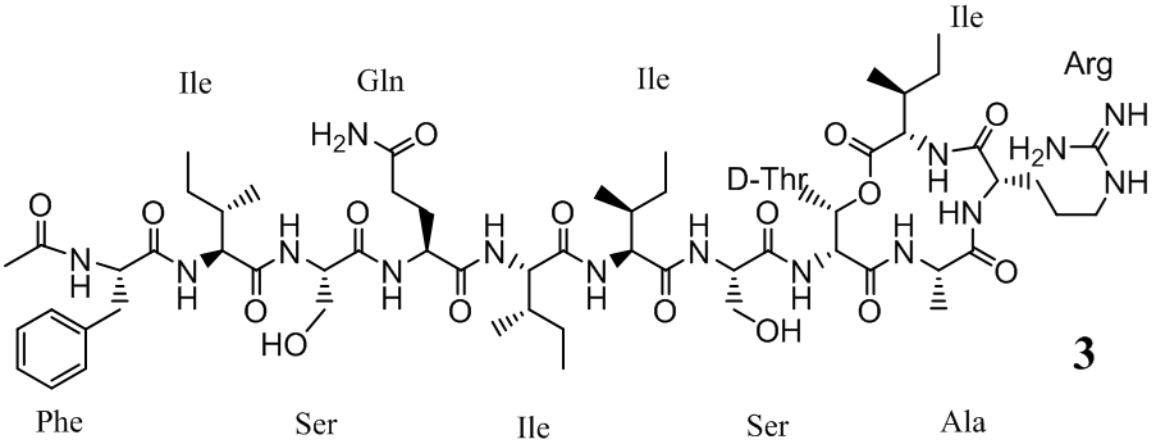

Figure S8: Structure of teixobactin analogue **3**

groups were then cleaved off using TFA:TIS:H_2O = 95:2.5:2.5 by stirring for 1h. The peptide was precipitated using cold Et_2O (-20°C) and centrifuging at 7000 rpm to obtain a white solid. This solid was

further purified using RPHPLC using protocols as described in the section II. Fractions were collected, concentrated and lyophilised to obtain a white solid (24 mg, 17% yield). ESI-HRMS mass calcd for $C_{59}H_{98}N_{15}O_{16}$: 1272.7316, found 1272.7379. HPLC trace of crude and purified **3** (figure S16 and S17), ESI-HRMS of **3** (figure S18).

VIII. Antimicrobial Activity. The "Dilution Susceptibility" test[1] was used to determine the Minimum inhibitory concentration (MIC) in 96 well plate format. The test used cation adjusted Mueller-Hinton broth (OXOID) medium and was performed in triplicate. Plates were incubated at 37°C for 24hrs. The MIC was defined as the lowest concentration of antibiotic which resulted in no visible growth.

IX. HPLC and MS analysis

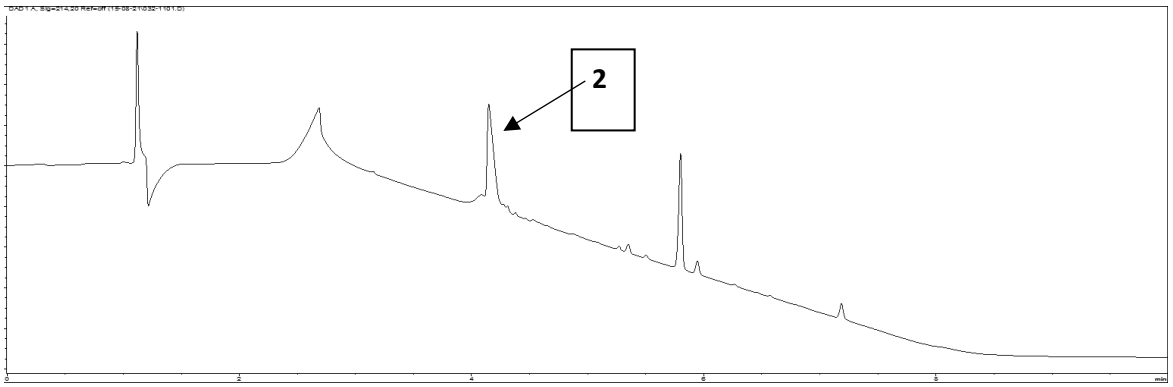

Figure S9: HPLC trace of crude compound **2** (gradient: 0-100% ACN in 6 min using A: 0.1% HCOOH in water, B: ACN)

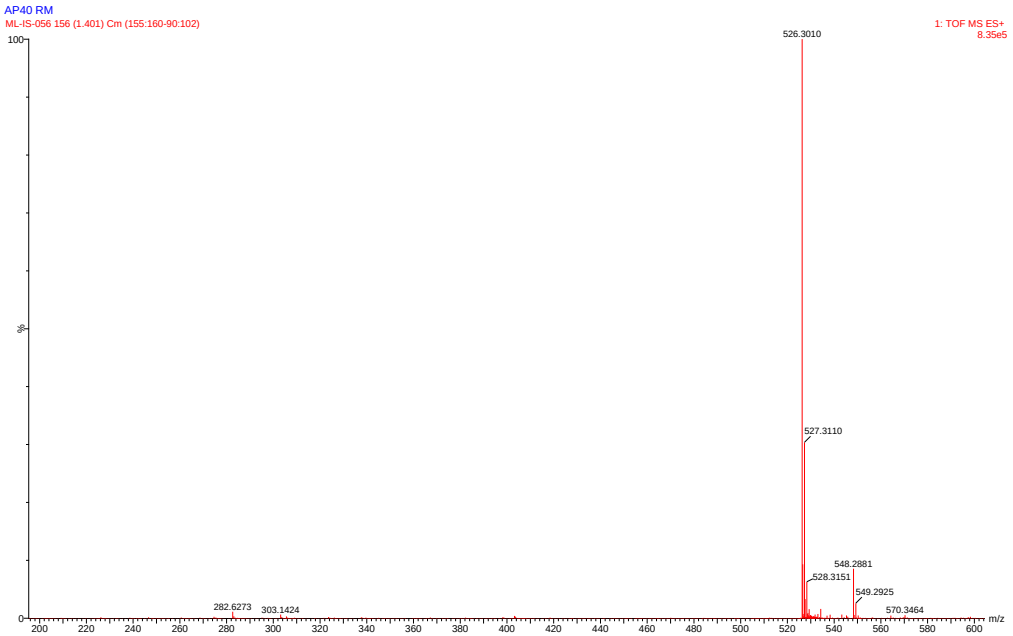

Figure S10: HRMS of compound **2**. Mass calcd for $C_{23}H_{39}N_7O_7$: 525.2911, found M+H$^+$ = 526.3010.

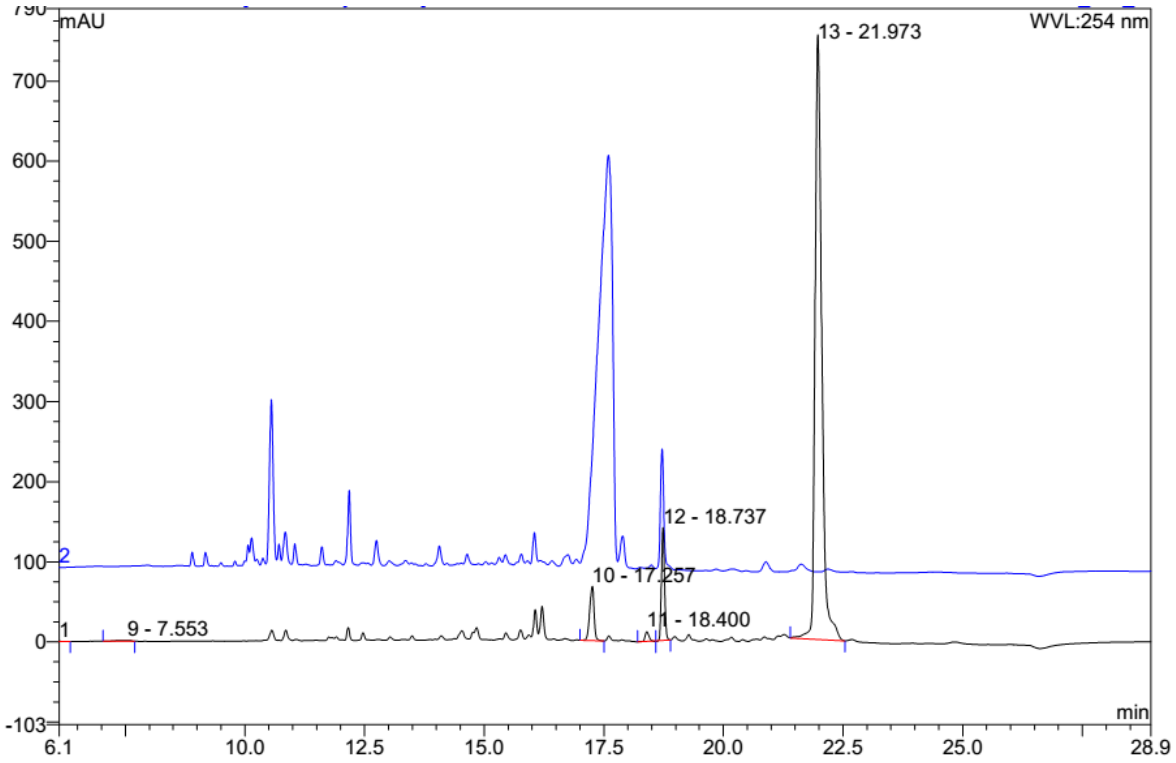

Figure S11: HPLC trace showing the progress of cyclisation reaction: conversion of the uncyclized protected teixobactin analogue **1a** t_R= 17.257 min (shown in blue) to the cyclized protected teixobactin analogue **1b** t_R = 21.973 min (shown in black) (Gradient: 5-95% in 25 min)

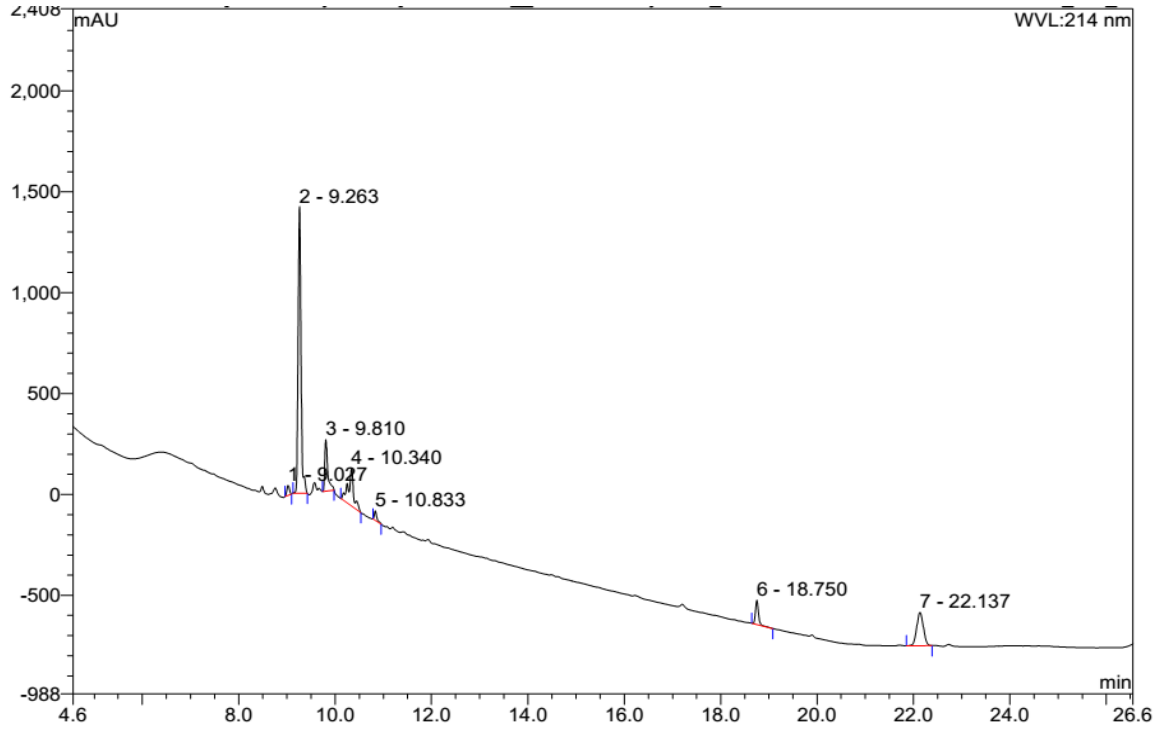

Figure S12: HPLC trace of crude teixobactin analogue **1** t_R = 9.263 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

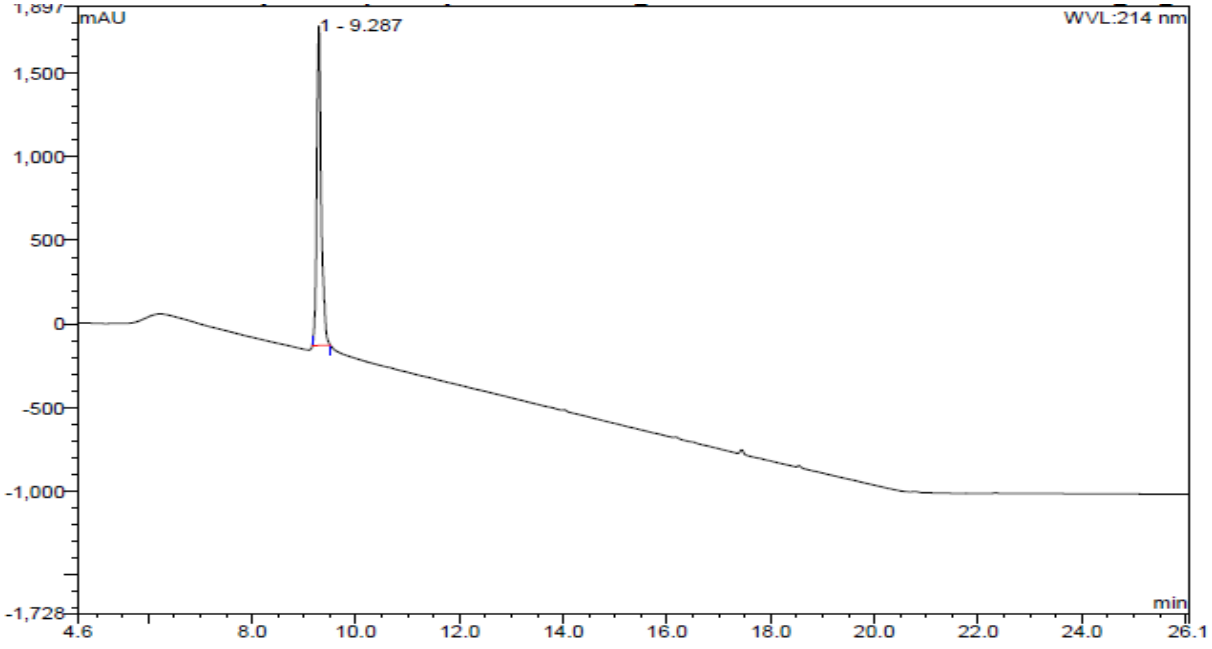

Figure S13: HPLC trace of purified teixobactin analogue **1** t_R = 9.287 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

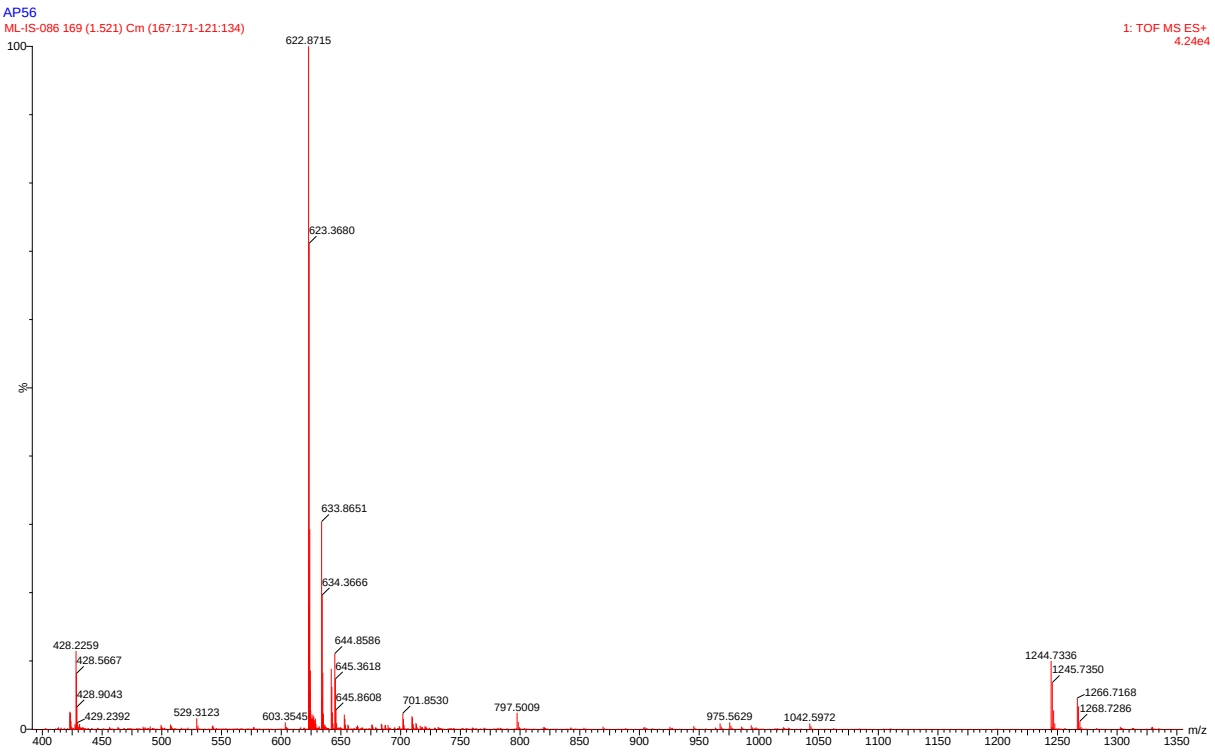

Figure S14: HRMS of teixobactin analogue **1**. Mass calcd for $C_{58}H_{98}N_{15}O_{15}$: 1243.7289, found $M+H^+$ = 1244.7336, $M/2 + H^+$ = 622.8715

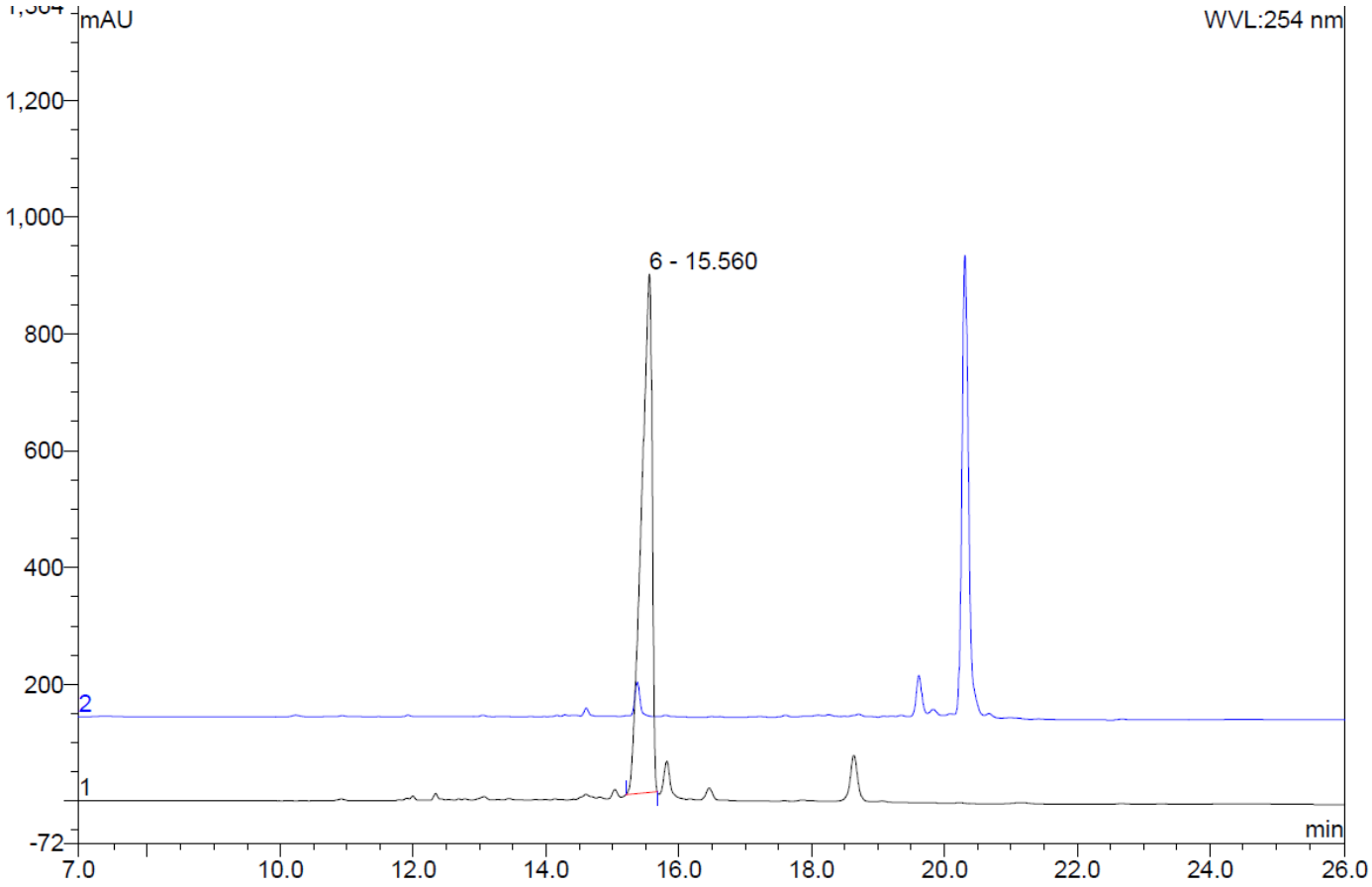

Figure S15: HPLC trace showing the progress of reaction of teixobactin analogue **3b**: conversion of the uncyclized protected teixobactin analogue **3a** t_R= 15.560 min (shown in black) to the cyclized protected teixobactin analogue **3b** t_R = 20.310 min (shown in blue) (Gradient: 5-95% in 25 min)

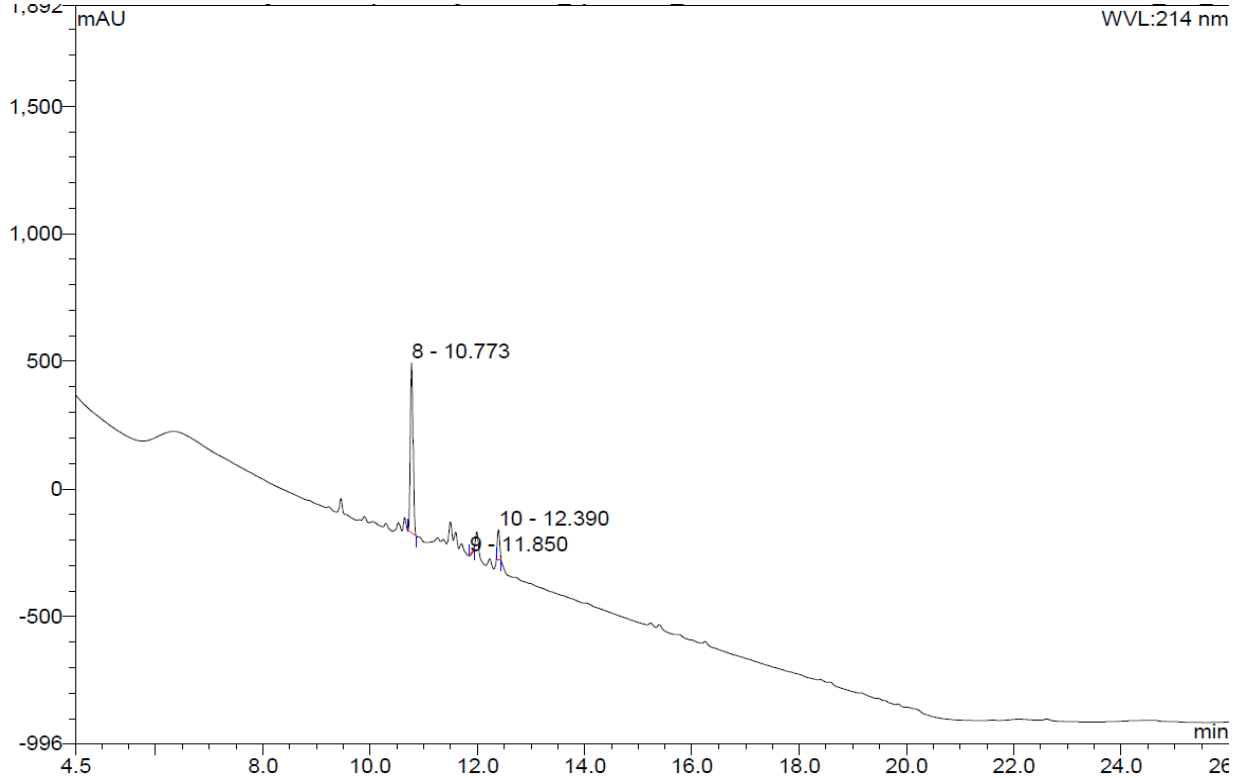

Figure S16: HPLC trace of crude teixobactin analogue **3** t_R = 10.773 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

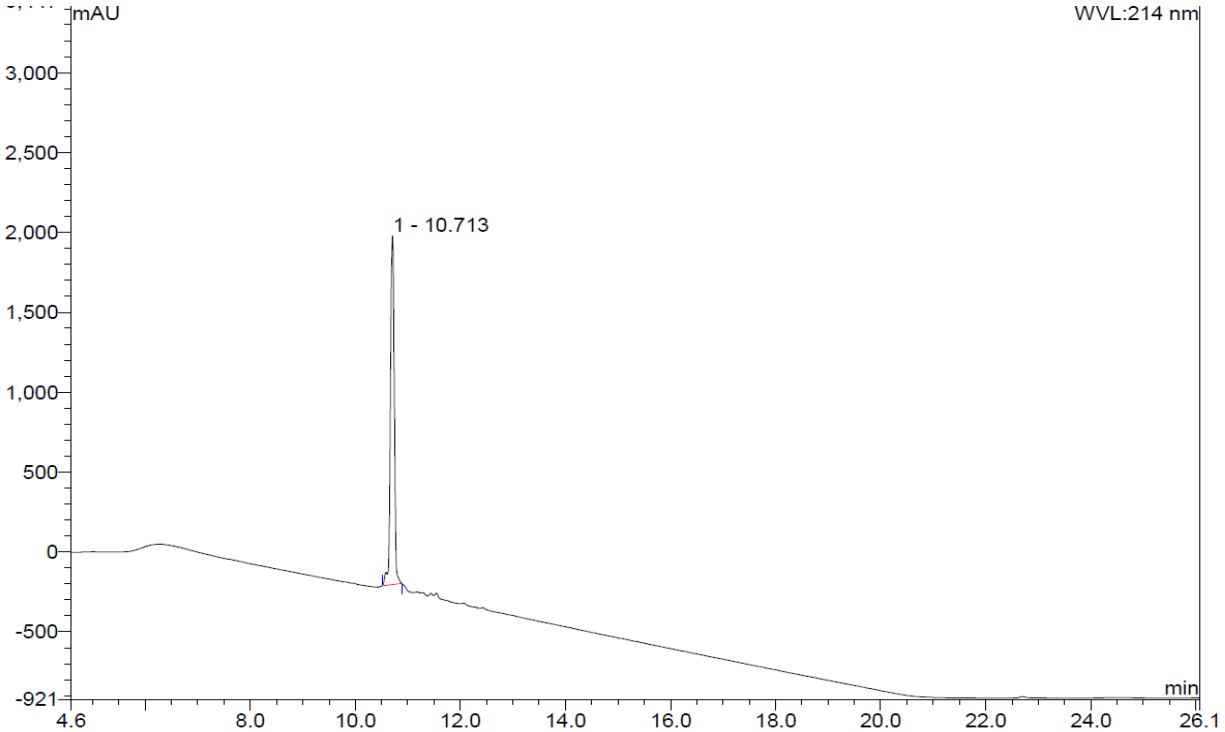

Figure S17: HPLC trace of purified teixobactin analogue **3** t_R = 10.713 min (gradient: 5-95% ACN in 25 min using A: 0.1% HCOOH in water, B: ACN)

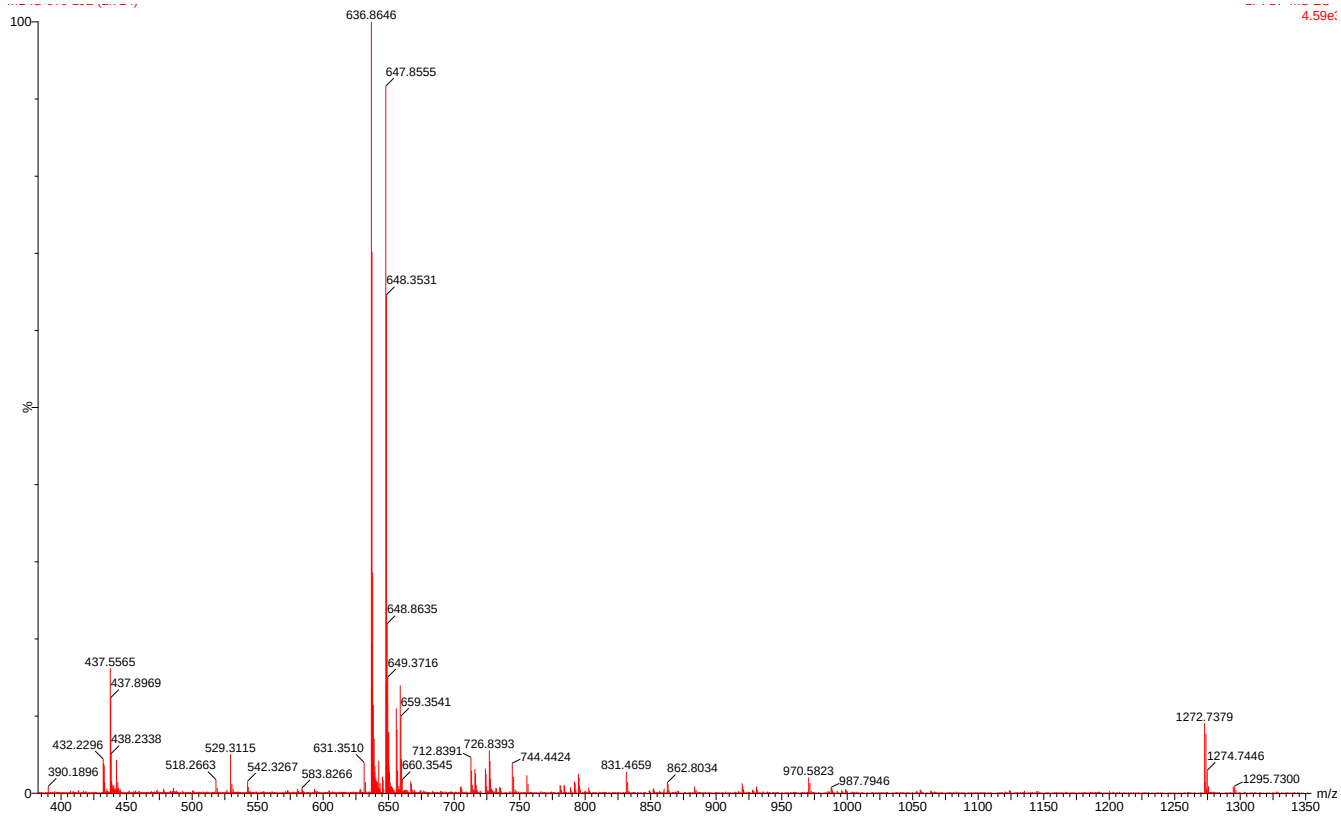

Figure S18: ESI-HRMS of purified teixobactin analogue **3**. ESI-HRMS mass calcd for $C_{59}H_{98}N_{15}O_{16}$: 1271.7238, found $M+H^+$ = 1272.7379, $M/2 + H^+$ = 636.8646, $(M+ Na^+ + H^+)/2$ = 647.8555

X. Detailed NMR Analysis of Teixobactin analogues 1 and 3

NMR was performed at 303.15°K on 1mM solutions of **1** and **3** dissolved in DMSO-d6 on a Bruker 500 MHz Avance III HD spectrometer equipped with a broadband probe. Proton spectra were recorded with 128 transients and 64k points. Two dimensional spectra (H-H NOESY, H-H TOCSY, H-C HSQC, H-C HMBC (table S2)) were recorded with 16 transients and 4k and 196 complex points in the direct and indirect dimensions, respectively. Data processing and analysis were performed using Bruker TopSpin and CcpNmr Analysis.

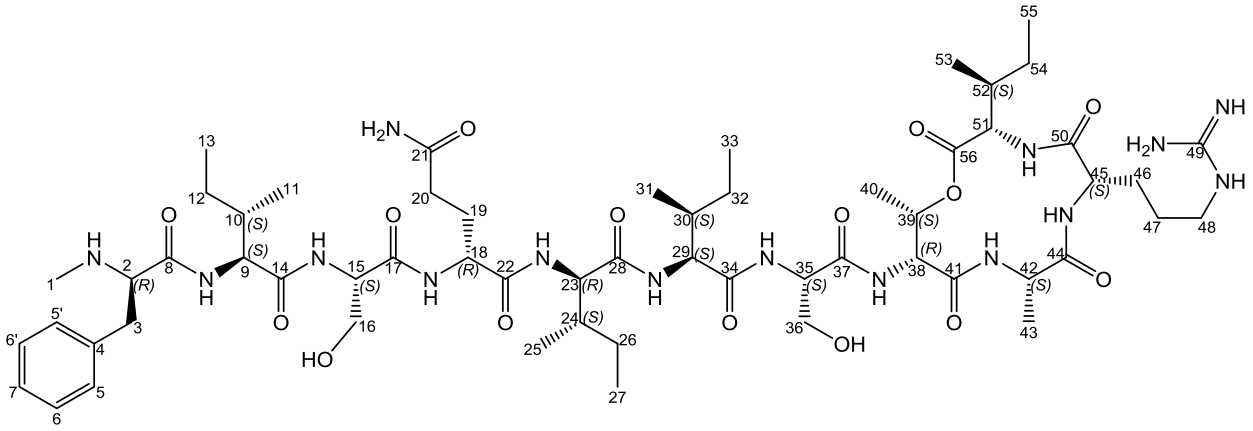

Figure S19: Structure of teixobactin analogue **1** with numbering. NMR assignments are shown in Table S2

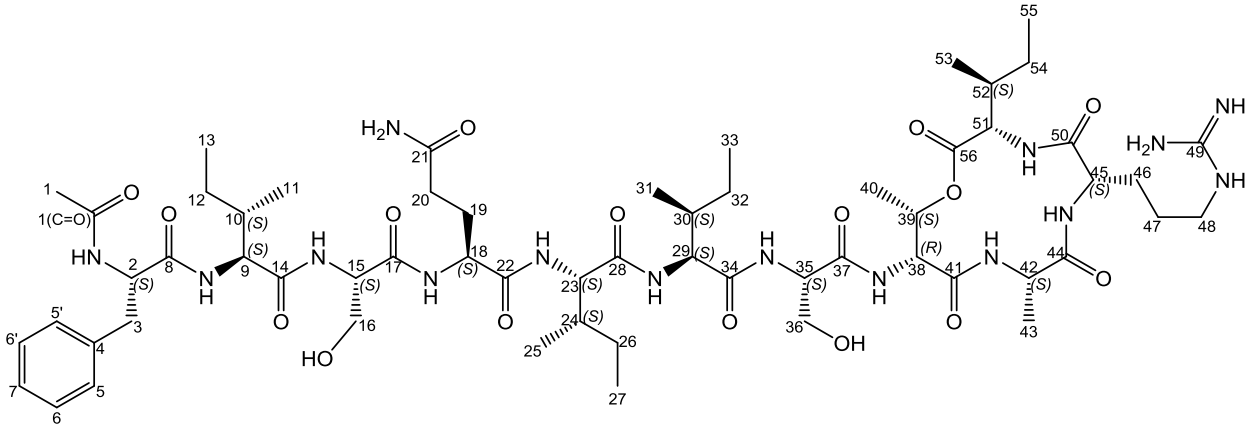

Figure S20: Structure of teixobactin analogue **3** with numbering. NMR assignments are shown in Table S3

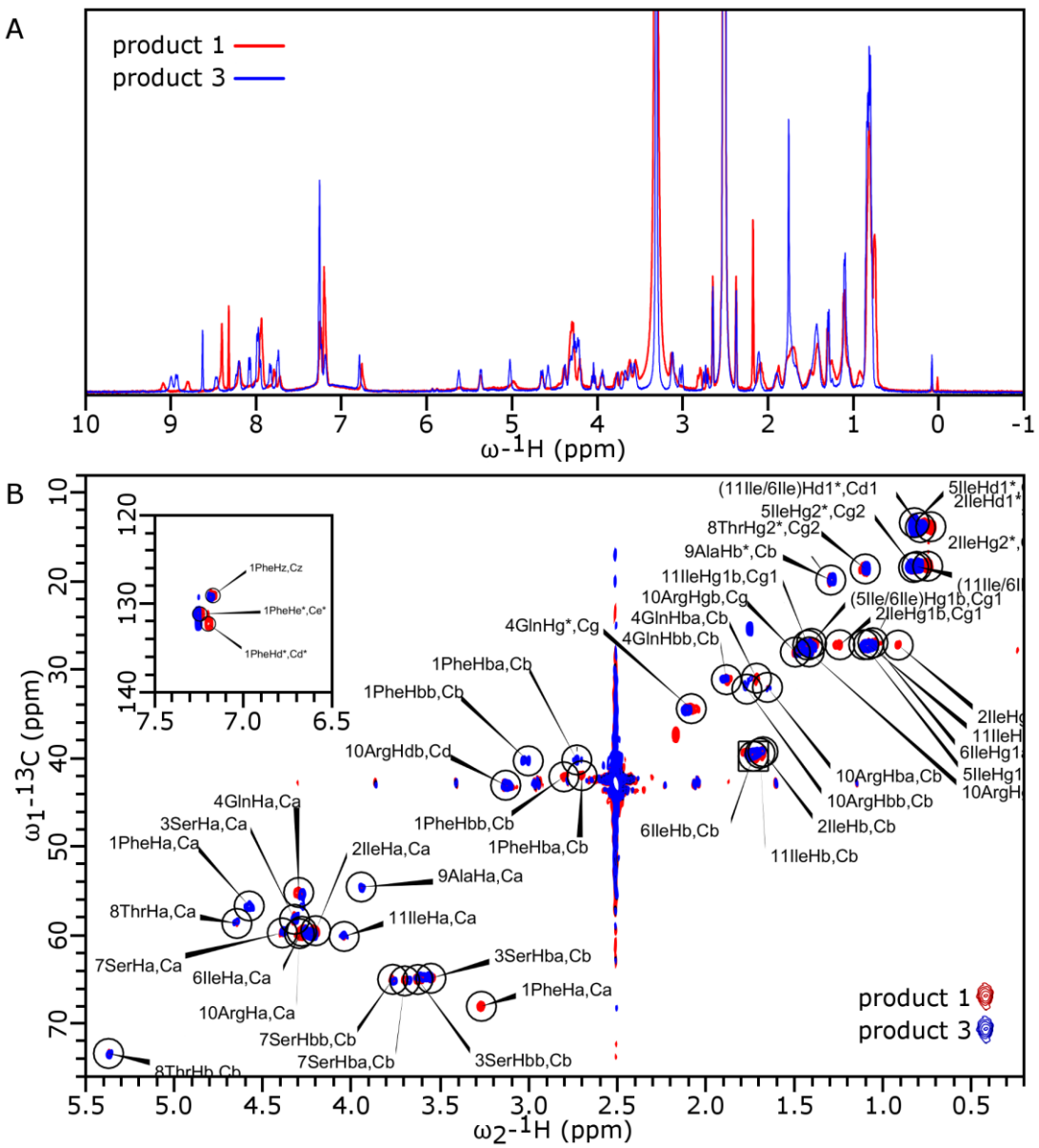

Figure S21: NMR spectra obtained from Teixobactin analogues **1** (*red*) and **3** (*blue*). **A.** Overlaid proton spectra. **B.** Overlaid ^{1}H-^{13}C HSQC spectra, showing complete assignment. Inset shows aromatic correlations. Samples were 1mM teixobactin analogue in DMSO-d_6, and spectra were recorded on 500 MHz Bruker Avance III HD at 303.15 K.

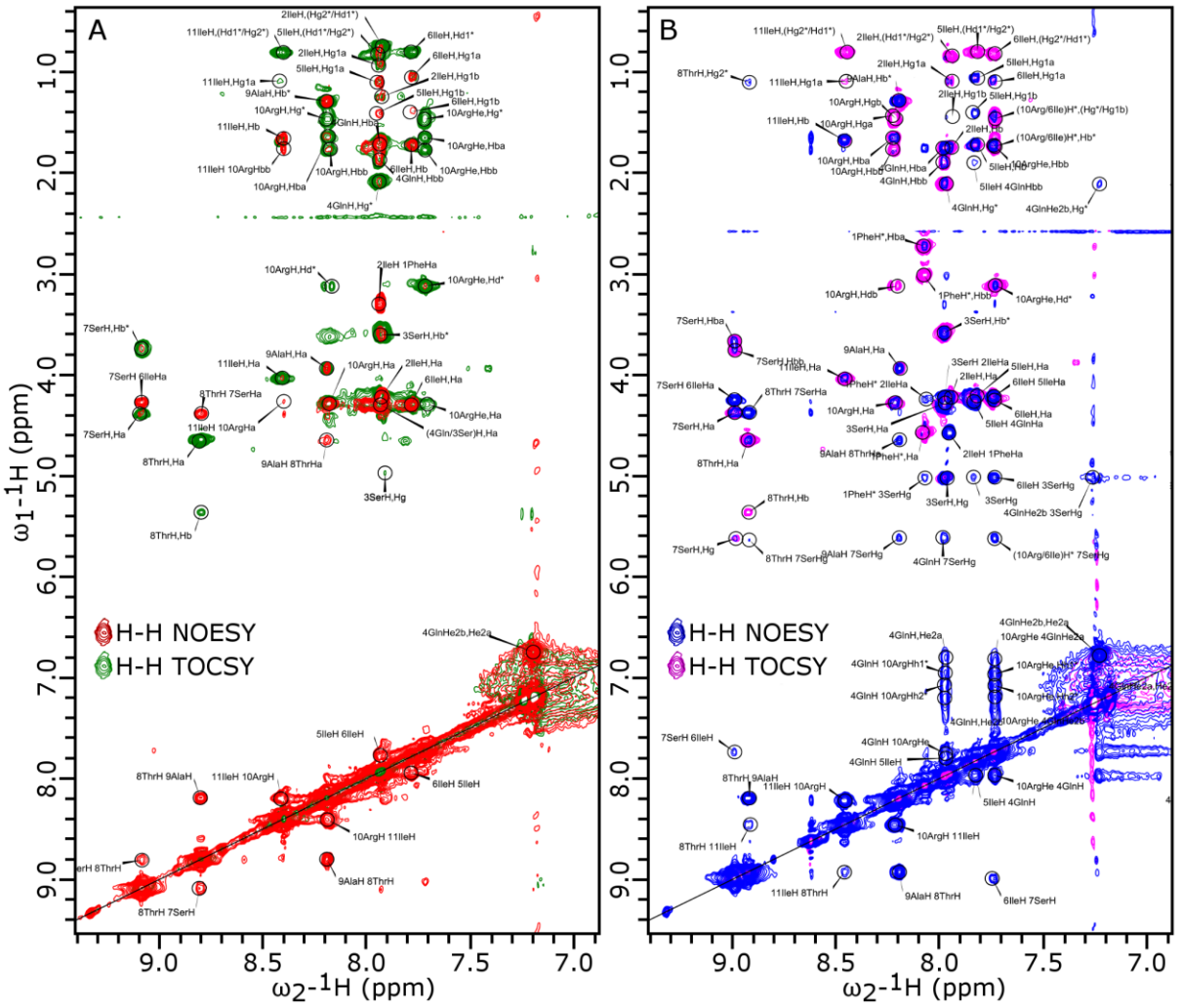

Figure S22**:** Through-space and through-bond proton-proton correlation spectra of Teixobactin analogues **1** and **3** showing complete spectral assignment. **A.** Fingerprint region of product **1** showing ^{1}H-^{1}H NOESY (*red contours*) and ^{1}H-^{1}H TOCSY (*green contours*). The presence of only intra-residue and sequential NOEs is characteristic of an unstructured peptide. **B.** Fingerprint region of product **3** showing ^{1}H-^{1}H NOESY (*blue contours*) and ^{1}H-^{1}H TOCSY (*magenta contours*). The presence of mid-range NOEs in addition to short-range NOEs suggests that this peptide has adopted a certain degree of structure. Samples were 1mM teixobactin analogue in DMSO-d_6, and spectra were recorded on 500 MHz Bruker Avance III HD at 303.15 K. The extremely broad resonance at ~7.1 ppm is the guanidinium group of Arg10.

	Product **1** from [ref. 2]		Product **1**		
Position	**Carbon**	**Proton**	**Carbon**	**Proton**	**Notes**
1	31.9	2.48	34.79	2.169	Δδ[1] 1.01 ppm. Confirmed by NOE to 2-NH
2	61.8	4.17	65.45	3.275 (t, 7.0 Hz)	Δδ 1.51 ppm. Confirmed by NOE to 9-NH
2-NH		9.06			
3	36.5	3.00	39.50	2.696 (dd, 13.5, 6.5 Hz)	Δδ 1.04 ppm. Confirmed by TOCSY to 2.
3'		3.14		2.795	
4	135.0		138.89		Δδ 1.30 ppm. Confirmed by HMBC to 6, 3
5, 5'	129.7	7.24	129.61	7.192 (d, 7.0 Hz)	
6, 6'	129.0	7.33	128.50	7.244 (t, 7.5 Hz)	
7	127.6	7.27	126.41	7.170 (t, 7.0 Hz)	
8	167.0		173.69		Δδ 2.23 ppm. Confirmed by HMBC to 3
9	57.8	4.16	56.92	4.202 (t, 7.4 Hz)	
9-NH		8.49		7.932	
10	36.6	1.55	36.73	1.681	
11	15.5	0.62	15.80	0.746	
12	24.3	0.74	24.69	0.921	
12'		1.05		1.249	
13	11.3	0.66	11.35	0.741	
14	170.6		171.39		
15	55.5	4.35	55.64	4.316	
15-NH		7.92		7.928	
16	62.4	3.55	62.35	3.570 (q, 5.7 Hz)	
16'				3.599 (q, 6.3 Hz)	
16-OH				4.974	
17	170.1		170.15		
18	57.2	4.35	52.60	4.294	Δδ 1.54 ppm. Confirmed by HSQC
18-NH		8.03		7.939	
19	28.6	1.72	28.51	1.721	
19'		1.88		1.880	
20	31.9	2.10	31.87	2.074	

[1] Difference from chemical shift published in [ref. 2] of greater than 1 ppm. Calculated using the equation

$$\Delta\delta_{H,C} = \sqrt{\left[(\Delta\delta_H)^2 + \frac{(\Delta\delta_C)^2}{3}\right]}$$

20'				2.080	
21	174.4		174.14		
21-NH2		6.76		6.751	
21-NH2'		7.21		7.197	
22	171.3		171.55		Overlapped
23	54.1	4.29		4.278	
23-NH		8.23		7.949	
24	37.5	1.82			Overlapped
25	14.7	0.82	15.94	0.823	
26	26.2	1.11	24.58	1.107	
26'		1.31		1.413	
27	10.5	0.82	10.88	0.822	
28	171.6				
29	56.4	4.39	56.98	4.282	
29-NH		7.77		7.785	
30	36.6	1.82	37.24	1.731	
31	15.5	0.88	15.82	0.812	
32	25.3	1.44	24.46	1.068	
32'		1.55		1.408	
33	11.3	0.82	11.44	0.806	
34	171.6				Broad signal
35	52.6	4.35	57.17	4.387	Δδ 1.52 ppm. Confirmed by NOE to 38-NH. Minor form[3] at 4.444 ppm
35-NH		8.03		9.087	Δδ 1.06 ppm. Confirmed by NOEs to 29 and 38-NH. Minor form[3] at 8.189 ppm
36	62.4	3.62	62.49	3.724 (q, 4.0 Hz)	Minor form[3] at 3.616 ppm
36'		3.84		3.759 (q, 5.7 Hz)	Minor form[3] at 3.642 ppm
37	169.5		172.17		
38	56.4	4.50	56.10	4.649 (d, 8.5 Hz)	Minor form[3] at 4.741 ppm
38-NH		8.76		8.800	Minor form[3] at 8.548 ppm
39	71.0	5.38	70.76	5.634 (q, 7.2 Hz)	
40	15.8	1.11	16.10	1.105 (d, 6.1 Hz)	Minor form[3] at 1.166 ppm
41	158.4				Broad signal
42	52.1	3.93	51.98	3.939 (quint, 7.1)	Minor form[3] at 3.942 ppm
42-NH		8.13		8.197	Minor form[3] at 8.548 ppm
43	17.3	1.31	17.36	1.295 (d, 7.5 Hz)	Minor form[3] at 1.202 ppm

44	172.8		172.90		
45	52.1	3.60	57.23	4.290	Δδ 1.84 ppm. Confirmed by HSQC
45-NH				8.185	
46	25.7	1.44	29.57	1.661	Δδ 1.31 ppm. Confirmed by NOE intensity
46'				1.765	
47	29.4	1.26	25.48	1.429	Δδ 1.32 ppm. Confirmed by NOE intensity
47'				1.475	
48, 48'	43.9	3.17	40.51	3.119 (q, 6.6 Hz)	Δδ 1.13 ppm. Confirmed by TOCSY to 45, 46, 47, 48, 45-NH and 48-NH
48-NH				7.719	
49	157.2				Broad Signal
49-NH2				7.036	
49-NH2'				7.036	
50	171.9				Broad Signal
51	57.5	4.05	57.56	4.039 (t, 9.9 Hz)	Minor form[3] at 4.295 ppm
51-NH		8.18		8.411	Minor form[3] at 7.995 ppm
52	37.0	1.82	36.90	1.693	Minor form[3] at 1.767 ppm
53	16.0	0.82	15.82	0.808	Minor form[3] at 0.833 ppm
54	24.7	1.16	24.66	1.809	
54'		1.44		1.428	
55	11.9	0.82	11.44	0.801	Minor form[3] at 0.833 ppm
56	168.7		166.27		

Table S2: Complete NMR assignment of Teixobactin analogue **1**

Position	**Carbon**	**Proton**	**Position**	**Carbon**	**Proton**
1	22.88	1.752[2]	29	57.00	4.248
1-C=O	169.67		29-NH		7.737
2	54.12	4.576[1]	30	37.05	1.736
2-NH		8.075	31	15.81	0.825
3	37.67	2.727	32	24.78	1.087
3'		3.013	32'		1.431
4	138.50		33	11.35	0.822
5, 5'	129.59	7.252	34		
6, 6'	128.37	7.253	35	57.01	4.376

[2] Chemical shift difference from **1** due to presence of *N*-terminal acetyl group

[3] The small number of differences observed to previously published chemical shifts were attributable to the cyclic portion of **1** existing in equilibrium between two unevenly distributed populations. The chemical shifts match those described in ref. 2. However, there exists an additional minor form of the cyclic portion of **1** whose chemical shifts are reported here.

7	126.60	7.181	35-NH		8.989
8	169.66[1]		36	62.56	3.672
9	57.32	4.229	36'		3.757
9-NH		7.950	36-OH		5.622
10	37.05	1.740	37	171.86	
11	15.81	0.827	38	55.84	4.645
12	24.79	1.086	38-NH		8.922
12'		1.430	39	70.87	5.361
13	11.28	0.827	40	16.05	1.097
14	171.52		41		
15	55.59	4.312	42	52.05	3.935
15-NH		7.976	42-NH		8.198
16	62.09	3.568	43	17.29	1.290
16'		3.588	44	172.85	
16-OH		5.020	45	54.23	4.270
17	170.34		45-NH		8.219
18	52.75	4.269	46	29.45	1.667
18-NH		7.977	46'		1.757
19	28.45	1.756	47	25.58	1.433
19'		1.896	47'		1.472
20	32.05	2.103	48, 48'	40.57	3.114
20'		2.103	48-NH		7.732
21			49		
21-NH2		6.788	49-NH2		6.959
21-NH2'		7.228	49-NH2'		7.075
22	171.55		50	171.43	
23	57.32	4.218	51	57.44	4.043
23-NH		7.827	51-NH		8.458
24	36.89	1.726	52	36.64	1.681
25	15.72	0.805	53	15.81	0.814
26	24.77	1.064	54	24.77	1.098
26'		1.414	54'		1.421
27	11.35	0.802	55	11.35	0.803
28	171.53		56	168.22	

Table S3: Complete NMR assignment of Teixobactin analogue **3**

XI. References:

1 J. H. Jorgensen and M. J. Ferraro, *Clin. Infect. Dis.*, 2009, **49**, 1749–55.

2 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.