General methods

Materials

All reagents were acquired as reagent grade from commercial sources and used without further purification. Solvents for RP-HPLC were purchased as RP-HPLC grade and used without further purification.

Aminomethyl polystyrene resin was purchased from Rapp Polymere (Tübingen, Germany). 4-(4-Hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB linker) was purchased from NovaBioChem (Läufelfingen, Switzerland). 4-(Hydroxmethyl)phenoxyacetic acid (HMP linker) was purchased from GL Biochem (Shanghai, China). 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) was purchased from GL Biochem (Shanghai, China). *N,N'*-diisopropylethylamine (DIPEA), triisopropylsilane (TIPS), *N,N'*-diisopropylcarbodiimide (DIC), formic acid, 4-dimethylaminopyridine (4-DMAP) were purchased from Sigma–Aldrich (Sydney, Australia). *N,N*-dimethylformamide (DMF; AR grade), acetonitrile (CH_3CN) [high-performance liquid chromatography (HPLC) grade], and hydrochloric acid (HCl) were purchased from Scharlau (Barcelona, Spain). Dichloromethane (CH_2Cl_2) was purchased from ECP Limited (Auckland, New Zealand). Diethyl ether (Et_2O) was purchased from Avantor Performance Materials. Trifluoroacetic acid (TFA) was purchased from Halocarbon (River Edge, USA).

Fmoc-D-allo-Ile-OH, Fmoc-L-Cit-OH, N-Boc-*N*-Me-D-Phe-OH.DCHA (DCHA = dicyclohexylamine) and Fmoc-D-Thr(*t*Bu)-OH, were obtained from Aapptec (Louisville KY). Fmoc-D-Gln(Trt)-OH (Trt=triphenylmethyl), Fmoc-D-His(Trt)-OH were purchased from Sigma-Aldrich. Fmoc-L-Har(Pbf)-OH (Har = homoarginine) Fmoc-L-ADMA-OH.HCl (ADMA = asymmetric dimethylarginine) were purchased from Chem Impex (Wood Dale, IL). Fmoc-L-Ala-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Ile-OH, Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ser(*t*Bu)-OH, were purchased from GL Biochem.

Microwave syntheses were carried out using a *CEM* Discover LabMate microwave reactor. A Helios γ system was used to carry out the UV-VIS measurements. For Fmoc-release experiments a wavelength of 290 nm was used. Analytical thin-layer chromatography (TLC) was carried out using Kieselgel silica F_{254} 200 mm (Merck) plates. The compounds were then visualised by ultraviolet fluorescence (254 nm and 365 nm). Column chromatography was performed using Kieselgel F_{254} S 63-100 μm silica gel with the indicated eluent. High resolution mass spectra were recorded on a Bruker micrOTOFQ mass spectrometer.

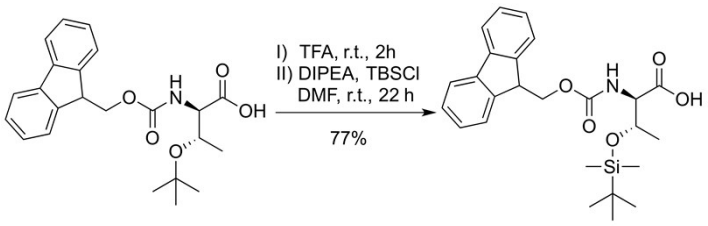
RP-HPLC and LC-MS

RP-HPLC and LC-MS Analytical RP-HPLC spectra were acquired on a Dionex P680 system using an analytical column (Phenomenex Gemini C18, 250 x 4.6 mm; 5 μm) at a flow rate of 1 mL min^{-1}, using gradient systems as indicated in the figures. A linear gradient of 5% solvent B to 65% was used (where solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in CH3CN) with detection at 210 nm and 254 nm. The ratio of products was determined by

integration of spectra recorded at 210 nm or 214 nm. LC-MS spectra were acquired on an Agilent Technologies 1260 Infinity equipped with an Agilent 6120 quadrupole LC-MS mass detector using an analytical column (Agilent Zorbax 300SB-C3, 3.0 mm x 150 mm, 3.5 µm) at a flow rate of 0.3 mL min-1 using gradients specified in the text.

Semi-preparative RP-HPLC was performed on a Thermo-Scientific Dionex UltiMate 3000 HPLC system using a semi-preparative column (Thermo BDS Hypersil® C_{18}, 150 mm x 10 mm, 5 µm) at a flow rate of 5 mL min^{-1} with detection of peptide products at 210 nm. Fractions were collected, analysed by RP-HPLC and LC-MS, pooled and lyophilised.

(2*R*,3*S*)-2-((((9-fluoren-9-yl)methoxy)carbonyl)amino)-3-((*tert*-butyldi-methyl-silyl)oxy)butanoic acid (Fmoc-D-Thr(TBS)-OH)



Fmoc-D-Thr(*t*Bu)-OH (730 mg, 1.8 mmol, 1.0 eq) was dissolved in TFA (5 ml) and stirred for 2 h at room temperature. The TFA was removed under a stream of N_2 and the residue re-dissolved in CH_2Cl_2 (30 ml) and washed with water (2 x 15 ml). The organic layer was concentrated and the residue re-dissolved in H_2O/MeCN (1/1, 20 ml) and freeze-dried. The residual colorless solid was dissolved in dry DMF (3.3 ml) and TBSCl (552 mg, 3.7 mmol, 2.1 eq) and DIPEA (1.1 ml, 6.2 mmol, 3.5 eq) were added. The resulting solution was stirred overnight (22 h) at room temperature. The reaction mixture was acidified with 1 M HCl to pH 3. The aqueous phase was extracted with ethyl acetate (3 x 20 ml) and the combined organic layers were washed with brine (2 x 20 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by column chromatography (petroleum ether/ethyl acetate = 4/1 to 1/1) to give the desired product as a colorless solid (647 mg, 1.4 mmol, 77%). The spectroscopic data were in agreement with those published.[1]

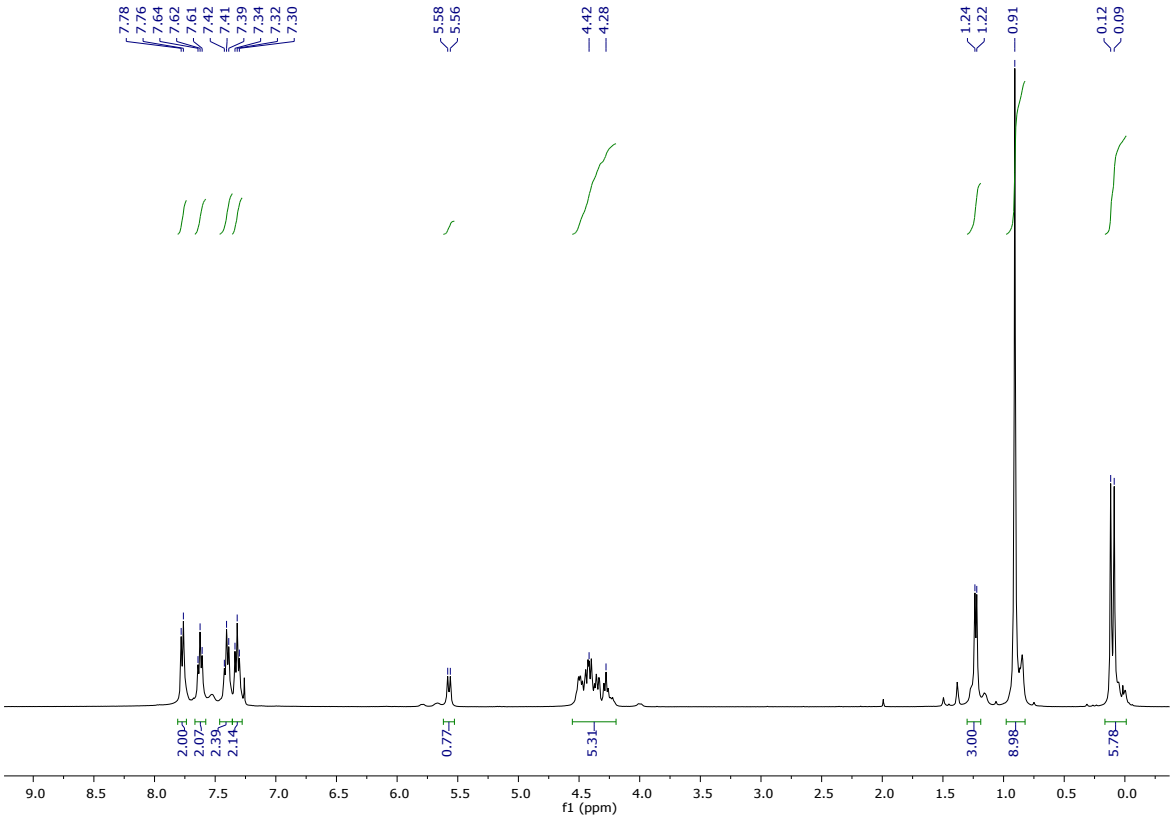
R_f: 0.6 (Petroleum ether/ethyl acetate = 1/1).

FI-MS: m/z (%) = 456.1 (100, $[M+H]^+$), 457.1 (35, $[M+H]^+$), 478.1 (6, $[M+Na]^+$), 933.3 (7, $[2M+Na]^+$).

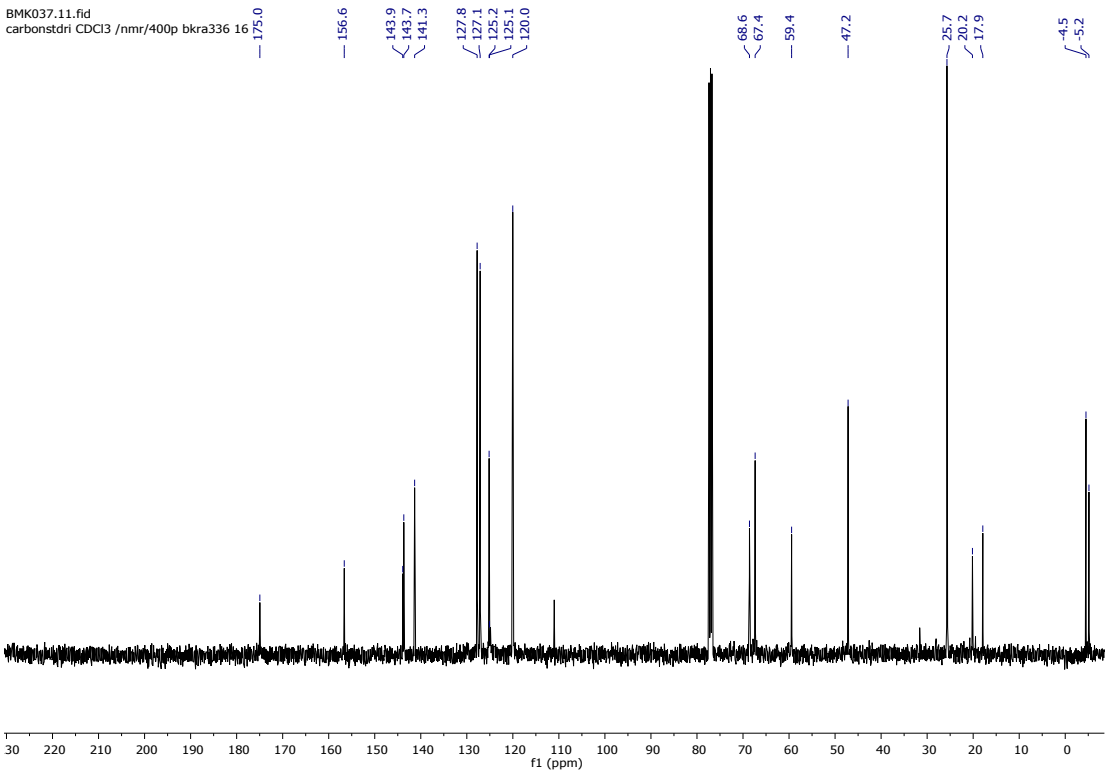
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.77 (d, *J* = 7.3 Hz, 2H, H-13), 7.62 (t, *J* = 6.8 Hz, 2H, H-12), 7.41 (t, *J* = 7.2 Hz, 2H, H-14), 7.32 (t, *J* = 7.2 Hz, 2H, H-15), 5.57 (d, *J* = 8.6 Hz, 1H, NH), 4.35 (m, 5H, H-2, H-3, H-6, H-10), 1.23 (d, *J* = 6.2 Hz, 3H, H-4), 0.91 (s, 9H, H-9), 0.10 (d, *J* = 11.6 Hz, 6H, H-7).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 175.0(C-1), 156.6 (C-5), 143.9/143.7 (C-11), 141.3 (C-16), 127.8 (C-14), 127.1 (C-15), 125.2/125.1 (C-12), 120.0 (C-13), 68.6 (C-10), 67.4 (C-6), 59.4 (C-2), 47.2 (C-3), 25.7 (C-9), 20.2 (C-4), 17.9 (C-8), -4.5/-5.2 (C-7).

^{1}H (400 MHz, $CDCl_3$)



^{13}C (100 MHz, $CDCl_3$)



Peptide synthesis

Fmoc solid phase peptide synthesis (SPPS) was performed using a fritted glass reaction vessel.

Coupling of HMPB linker with polystyrene AM NH_2 resin: To aminomethyl polystyrene resin (236 mg, 0.85 mmol/g, 0.2 mmol, 1.0 eq) pre-swollen in DMF/CH_2Cl_2 (1:4 v/v, 4 ml) was added a solution of DIC (124 µL, 0.8 mmol, 4.0 eq) and HMPB linker (192 mg, 0.8 mmol, 4.0 eq) in DMF/CH_2Cl_2 (1:4 v/v, 4 ml). The reaction mixture was gently agitated at room temperature for 18 h. The resin was filtered and washed with DMF (3 x 10 ml) and CH_2Cl_2 (2 x 10 ml) and air-drie

Coupling of HMP linker with polystyrene AM NH_2 resin: To aminomethyl polystyrene resin (236 mg, 0.85 mmol/g, 0.2 mmol, 1.0 eq) pre-swollen in DMF/CH_2Cl_2 (1:4 v/v, 4 ml) was added a solution of DIC (124 µL, 0.8 mmol, 4.0 eq) and HMP linker (146 mg, 0.8 mmol, 4.0 eq) in DMF/CH_2Cl_2 (1:4 v/v, 4 ml). The reaction mixture was gently agitated at room temperature for 18 h. The resin was filtered and washed with DMF (3 x 10 ml) and CH_2Cl_2 (2 x 10 ml) and air-dried

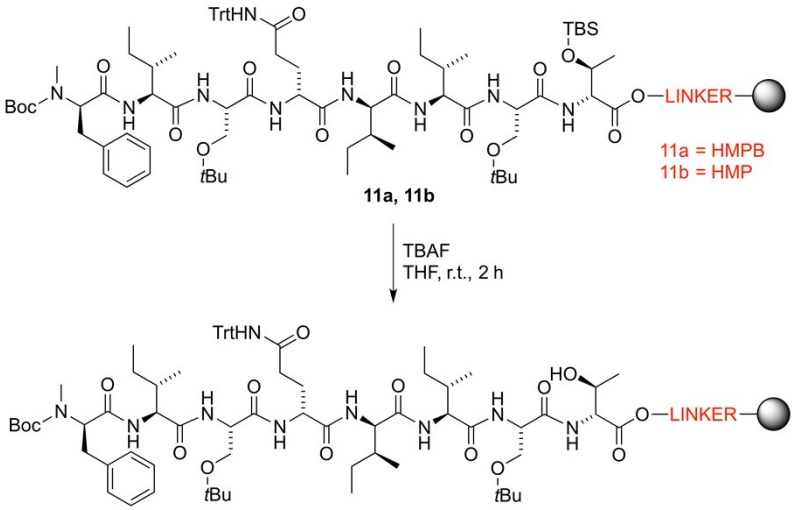
Coupling of Fmoc-D-Thr(TBS)-OH: To the resin-linker (0.2 mmol, 1.0 eq), prepared as above, was added a solution of Fmoc-D-Thr(TBS)-OH (320 mg, 0.7 mmol, 3.5 eq) in DMF/CH_2Cl_2 (1:4 v/v, 5 ml). A solution of DIC (111 µL, 0.7 mmol, 3.5 eq) and DMAP (10 mg, 40 mol%) in DMF (100 µL*)*, was added and the reaction mixture agitated for 2 h at 50 °C (µW reactor, 25 W). The resin was filtered and washed with DMF (3 x 10 ml) and CH_2Cl_2 (2 x 10 ml) and air-dried. Fmoc-release experiments revealed a conversion of 50%. To avoid reaction at the remaining resin-bound hydroxyl groups, the free hydroxyl moieties were capped with Ac_2O. The resin (0.08 mmol with respect to the remaining hydroxyl moieties, 1.0 eq) was swollen in DMF (5 ml) for 15 min, filtered and 20% Ac_2O in DMF (5 ml, 10.58 mmol, 132.2 eq) added, along with DMAP (0.5 mg, 18 mol%) dissolved in DMF (20 µL). The reaction mixture was shaken for 1 h at ambient temperature, filtered and washed with DMF (3 x 10 ml

General procedures for Fmoc-SPPS <u>before</u> esterification:

Removal of N^{α}-Fmoc-protecting groups: The peptidyl resin was treated with a solution of 20% piperidine in DMF (3 mL) and the mixture agitated at room temperature for 5 min, filtered and repeated once for a further 15 min. The resin was filtered and washed with DMF (3 x 10 mL).

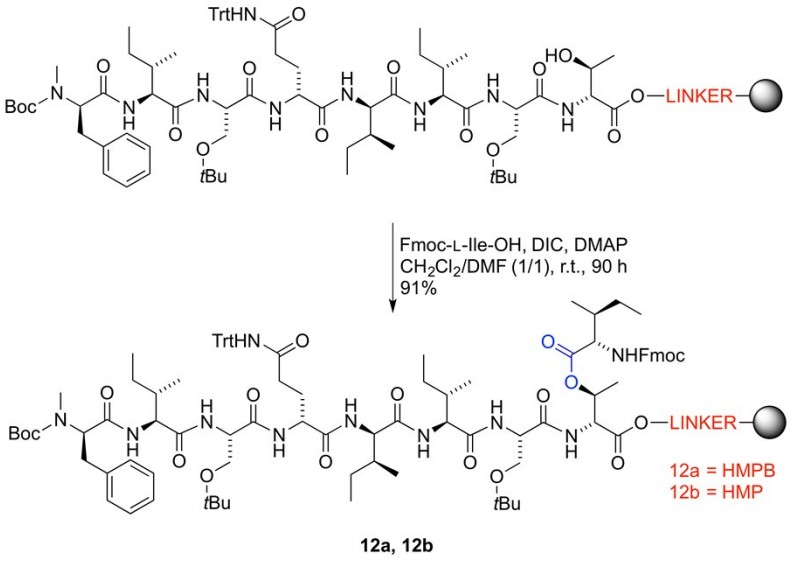
Coupling: To the peptidyl resin was added a mixture of Fmoc-AA-OH (3.0 eq), HATU (2.9 eq) and DIPEA (6.0 eq) in DMF (3 ml). The reaction mixture was gently agitated at ambient temperature for 1 h, after which the resin was filtered and washed with DMF (3 x 10 ml).

O-TBS deprotection:



The resin-bound peptide **11a or 11b** (0.12 mmol, 1.0 eq) was pre-swollen in THF (3 ml) for 20 min. After filtration, TBAF (1 ml, 1 M in THF, 1.00 mmol, 8.3 eq) diluted with THF (1 ml) was added to the resin and gently agitated at ambient temperature for 1.5 h. The resin was then filtered and washed with CH_2Cl_2 (3 x 10 ml). Full conversion was demonstrated *via* capping of a small amount of the resin-bound peptide with Ac_2O, resin-cleavage and measuring the ratio of capped:protected peptides by LC-MS.

On-resin esterification:



The resin-bound peptide (0.11 mmol, 1.0 eq) was pre-swollen in DMF/CH_2Cl_2 (1:1 v/v, 3 ml) for 30 min. In a separate flask, a solution of Fmoc-L-Ile-OH (398 mg, 1.12 mmol, 10.0 eq) and DIC (89 µL, 0.57 mmol, 5.0 mmol) in CH_2Cl_2 was gently agitated at room temperature for 30 min. The solution was concentrated under a stream of N_2 and re-dissolved in DMF/CH_2Cl_2 (1:1 v/v, 3 mL). The peptidyl-resin was filtered and the Fmoc-L-Ile-OH/DIC solution added to the resin. A solution of DMAP (14 mg, 0.11 mmol, 1.0 eq) in DMF (100 µL) was added to the resin and the resultant reaction mixture gently agitated at room temperature for 68 h. The resin was filtered and washed with CH_2Cl_2 (3 x 10 ml). The reaction was then repeated with fresh reagents for a further 22 h. After filtration and washing of the resin with CH_2Cl_2 (3 x 10 ml), the resin was air-dried to give the desired resin-bound products **12a** or **12b**.

($C_{64}H_{92}N_{10}O_{16}$): 1257.47 g/mol.[a]

Yield: 91%.[b]

LC-MS: m/z (%) = 1260.4 (10, $[M+H]^+$), 1258.4 (75, $[M+H]^+$), 1257.4 (100, $[M+H]^+$), 629.3 (80, $[M+2H]^{2+}$).[a]

[a]: After cleavage from the resin and side-chain deprotection using TFA/TIPS/H_2O (95/2.5/2.5); [b]: For **12a**, determined *via* LC-MS.

General procedures for Fmoc-SPPS <u>after</u> esterification

Removal of N^{α}-Fmoc-protecting groups: The peptidyl resin was treated with a solution of 10% piperidine in DMF (3 mL) and the mixture agitated at room temperature for 2.5 min. The resin was filtered and washed with DMF (3 x 10 mL).

Coupling: To the peptidyl resin was added a mixture of Fmoc-AA-OH (3.0 eq), HATU (2.9 eq) and DIPEA (6.0 eq) in DMF (3 ml). The reaction mixture was gently agitated at ambient temperature for 1 h, after which the resin was filtered and washed with DMF (3 x 10 ml).

Cleavage of the peptides from the resin

Cleavage from HMPB linker (For peptides 13-15):

To the dry peptidyl resin was added a solution of TFA in CH_2Cl_2 (0.5% v/v, 5mL). The reaction mixture was gently agitated at ambient temperature for 5 min, followed by filtration and collection of the filtrate. The solvent was removed *in vacuo* and the residue dissolved in H_2O/MeCN/*t*BuOH (1:1:1 v/v, 6 mL total) and lyophilized.

Cleavage from HMP linker (For peptides 16-18):

To the dry peptidyl resin was added a cleavage cocktail consisting of TFA/H_2O/TIPS (95:2.5:2.5 v/v, 5 ml). The reaction mixture was gently agitated at ambient temperature for 16 h. The resin was filtered and washed with TFA (2 x 5 ml). TFA was removed under a stream of N_2 and the peptide precipitated by the addition of cold Et_2O (40 ml). The mixture was cooled on ice for 5 min and the precipitated product isolated by centrifugation (4000 rpm, 5 min). The liquid layer was decanted and the pellet washed with cold Et_2O (3 x 40 ml). The resultant pellet was dried under N_2 and lyophilized.

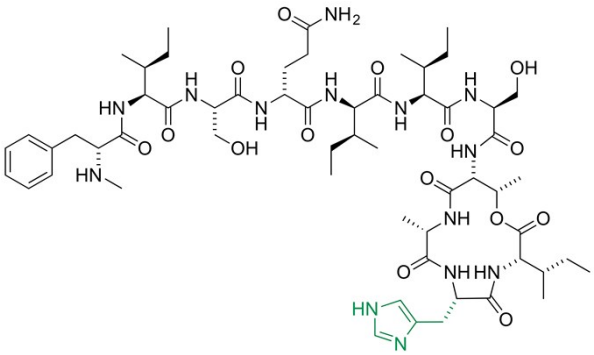
General procedure for cyclization (all peptides) and subsequent global deprotection (peptides 13-15):

The relevant peptide was dissolved in DMF (5 mM peptide final concentration). HATU (10.0 eq) and DIPEA (15.0 eq) were added and the resultant solution stirred at ambient temperature for 2 h. The reaction mixture was diluted with water (1 ml solution: 39 ml water) and lyophilised to afford the crude cyclized peptides.

The crude, side-chain protected cyclic peptides (derived from syntheses on HMP-resin) were dissolved in TFA/H_2O/TIPS (95:2.5:2.5 v/v, 10 ml total) and gently agitated at ambient temperature for 1.5 h. TFA was removed under a stream of N_2 and the peptides precipitated by the addition of cold Et_2O (40 ml).). The mixture was cooled on ice for 5 min and the precipitated product isolated by centrifugation (4000 rpm, 5 min). The liquid layer was decanted and the pellet washed with cold Et_2O (3 x 40 ml). The resultant pellet was dried under N_2 and lyophilized. Purification of all peptides was performed by RP-HPLC as outlined in the General Methods.

Characterisation data for teixobactin analogues 4-9

L-His$_{10}$-teixobactin, 4

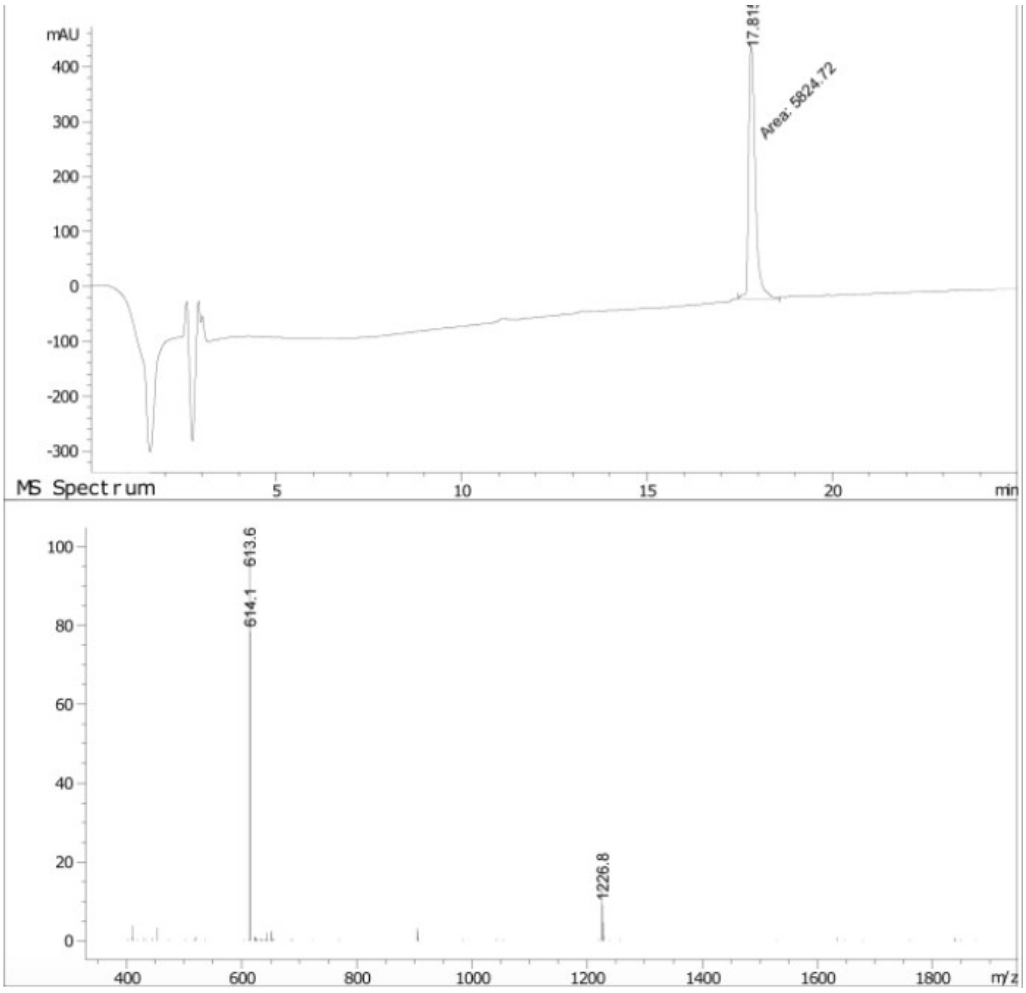


4
L-His$_{10}$-teixobactin

Yield: 2.2%.

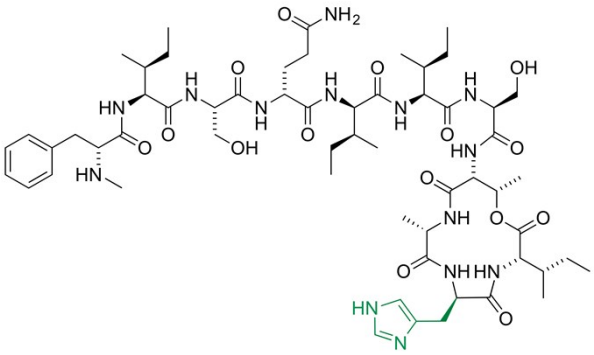
M ($C_{58}H_{92}N_{14}O_{15}$): 1225.44 g/mol.

LC-MS: m/z (%) = 1226.8 (20, $[M+H]^+$), 614.1 (80, $[M+2H]^{2+}$), 613.6 (100, $[M+2H]^{2+}$).



Analytical gradient: 1-76% B, 3% B/min (0.3 mL/min)

D-His$_{10}$-teixobactin, 5

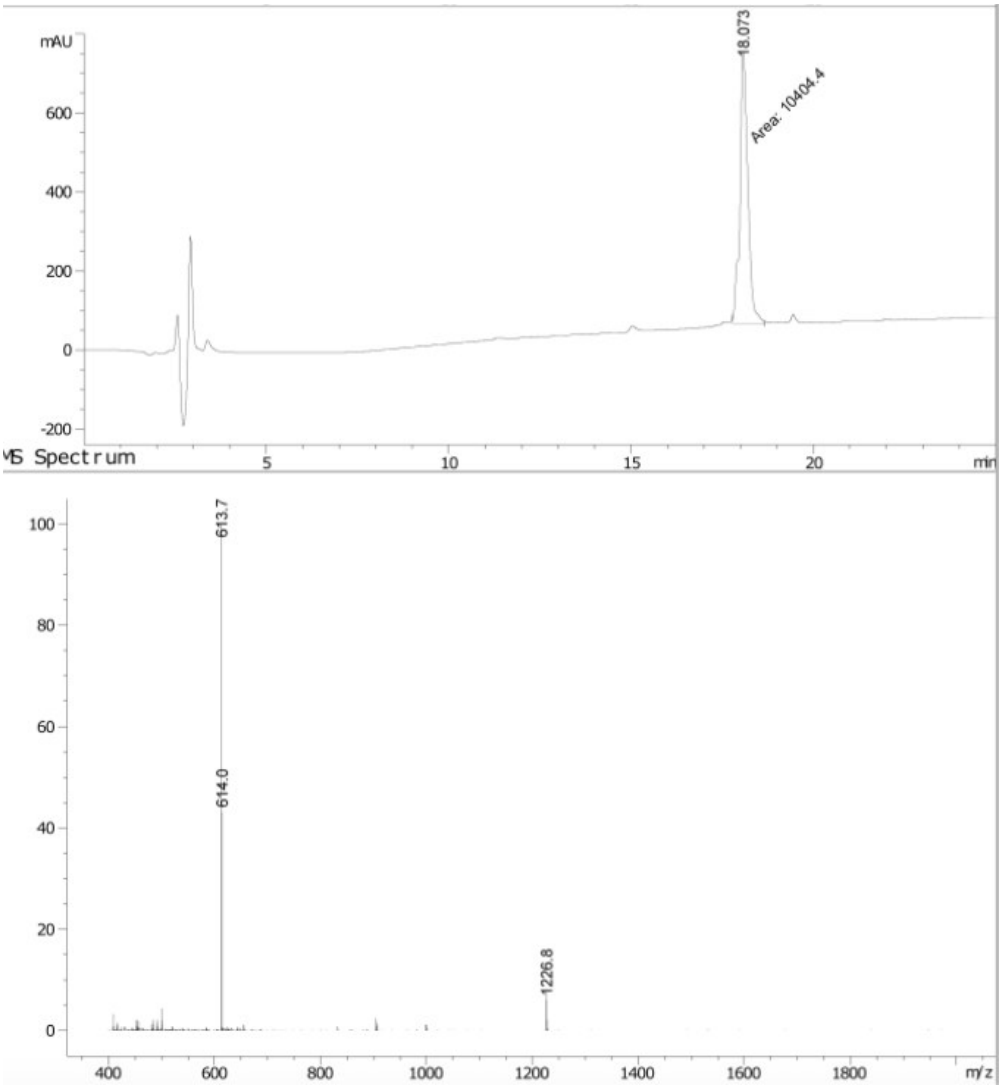


5
D-His$_{10}$-teixobactin

Yield: 1.8%

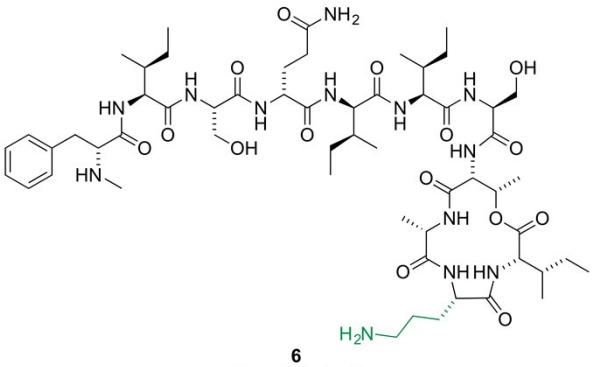
M ($C_{58}H_{92}N_{14}O_{15}$):1225.44 g/mol.

LC-MS: m/z (%) = 1226.8 (10, $[M+H]^+$), 614.0 (45, $[M+2H]^{2+}$), 613.7 (100, $[M+2H]^{2+}$).



Analytical gradient: 1-76% B, 3% B/min (0.3 mL/min)

L-Orn$_{10}$-teixobactin, 6

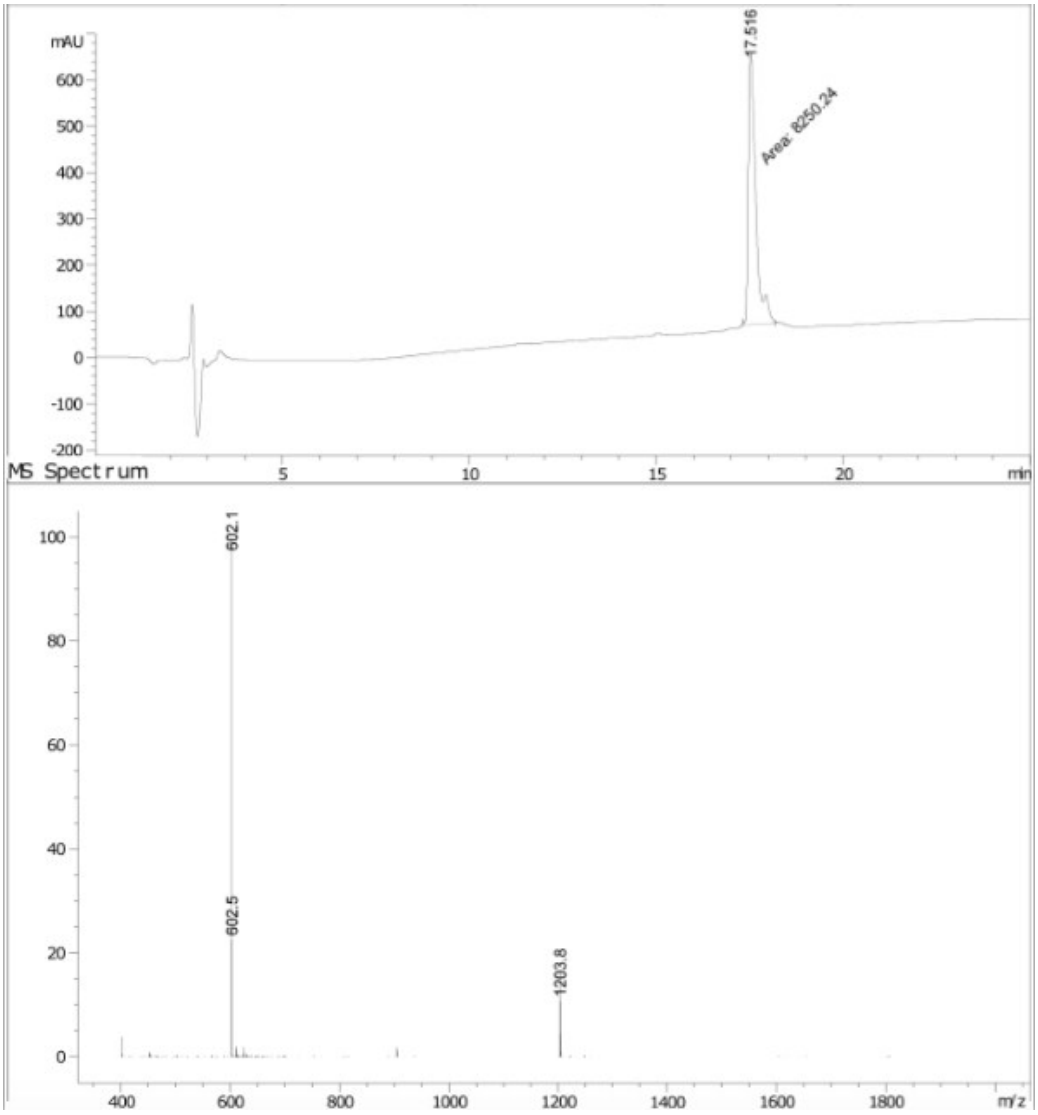


6
L-Orn$_{10}$-teixobactin

Yield: 11.8%.

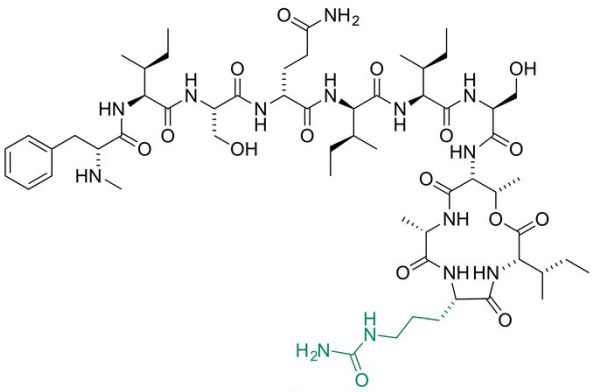
M ($C_{57}H_{95}N_{13}O_{15}$):1202.44 g/mol.

LC-MS: m/z (%) = 1203.8 (20, $[M+H]^+$), 602.5 (25, $[M+2H]^{2+}$), 602.1 (100, $[M+2H]^{2+}$).



Analytical gradient: 1-76% B, 3% B/min (0.3 mL/min)

L-Cit$_{10}$-teixobactin, 7

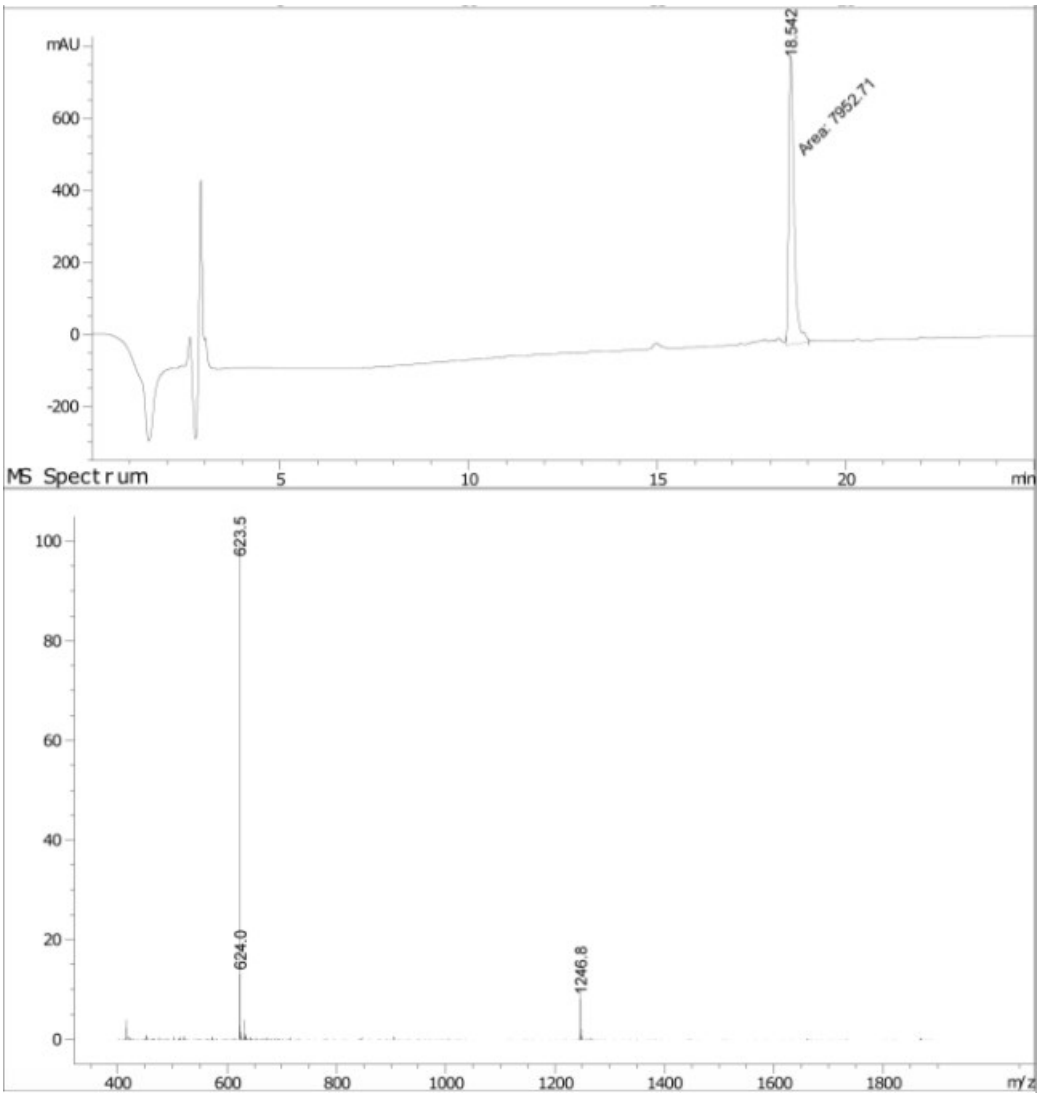


7
L-Cit$_{10}$-teixobactin

Yield: 3.7%.

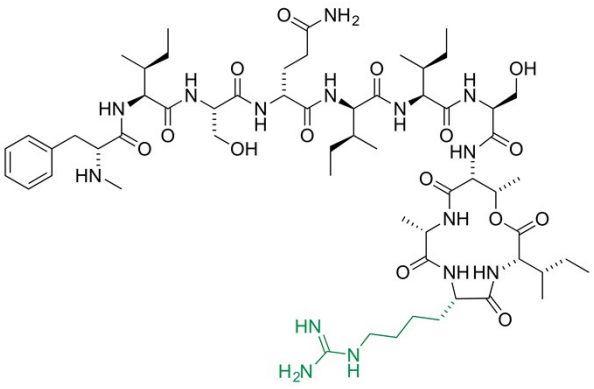
M ($C_{58}H_{96}N_{14}O_{16}$): 1245.71 g/mol.

LC-MS: m/z (%) = 1246.8 (10, $[M+H]^+$), 624.0 (20, $[M+2H]^{2+}$), 623.5 (100, $[M+2H]^{2+}$).



Analytical gradient: 1-76% B, 3% B/min (0.3 mL/min)

L-Har$_{10}$-teixobactin, 8

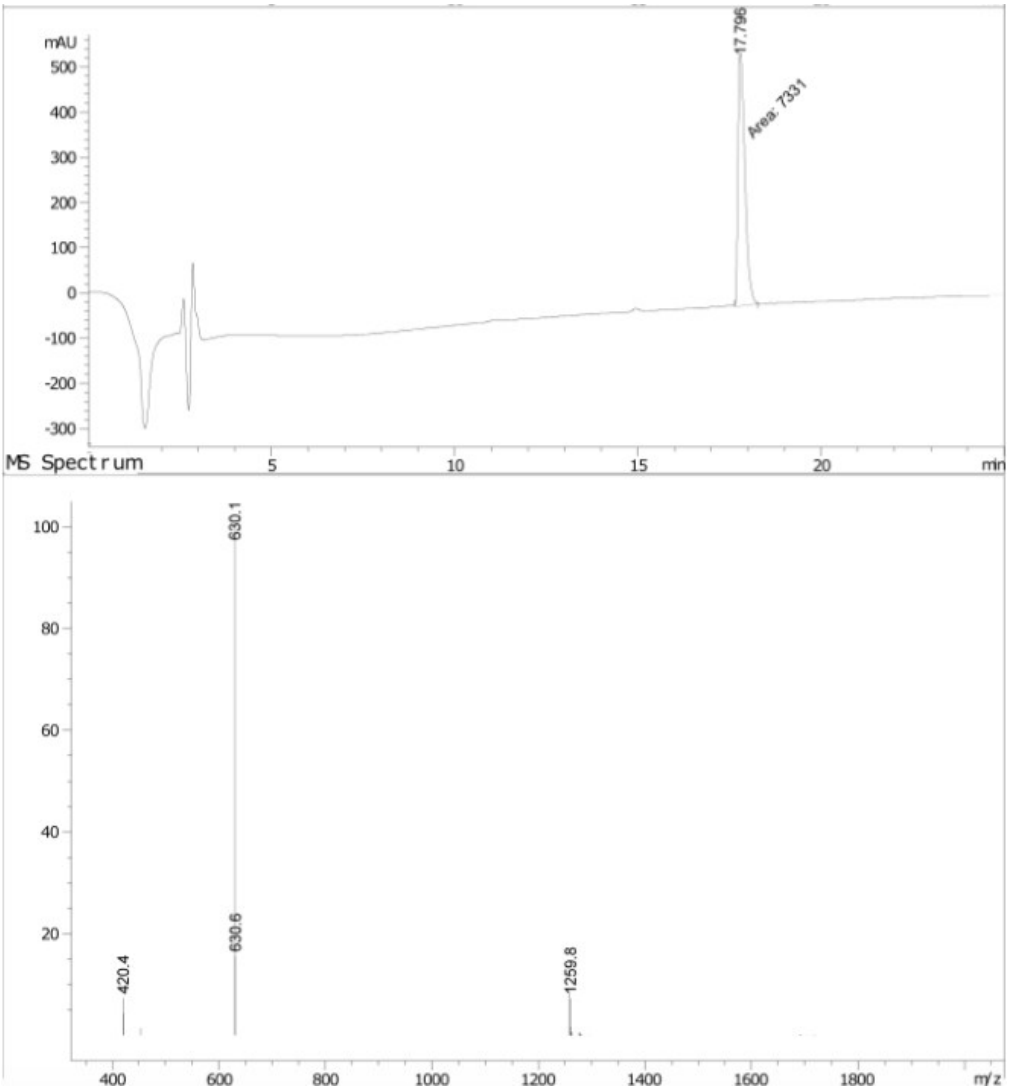


8
L-Har$_{10}$-teixobactin

Yield: 3.3%.

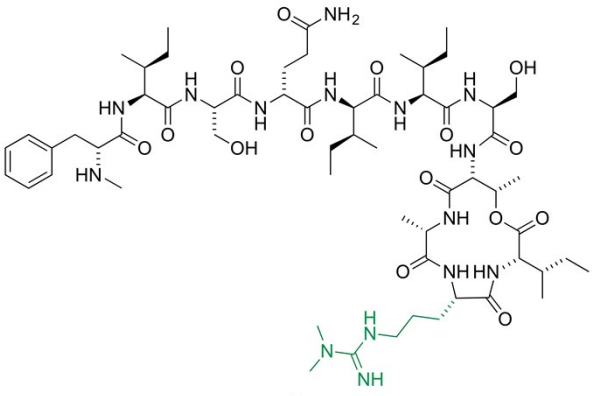
M ($C_{58}H_{96}N_{14}O_{16}$): 1258.51 g/mol.

LC-MS: m/z (%) = 1259.8 (10, $[M+H]^+$), 630.6 (20, $[M+2H]^{2+}$), 630.1 (100, $[M+2H]^{2+}$), 420.4 (10, $[M+3H]^{3+}$).



Analytical gradient: 1-76% B, 3% B/min (0.3 mL/min)

L-ADMA$_{10}$-teixobactin, 9

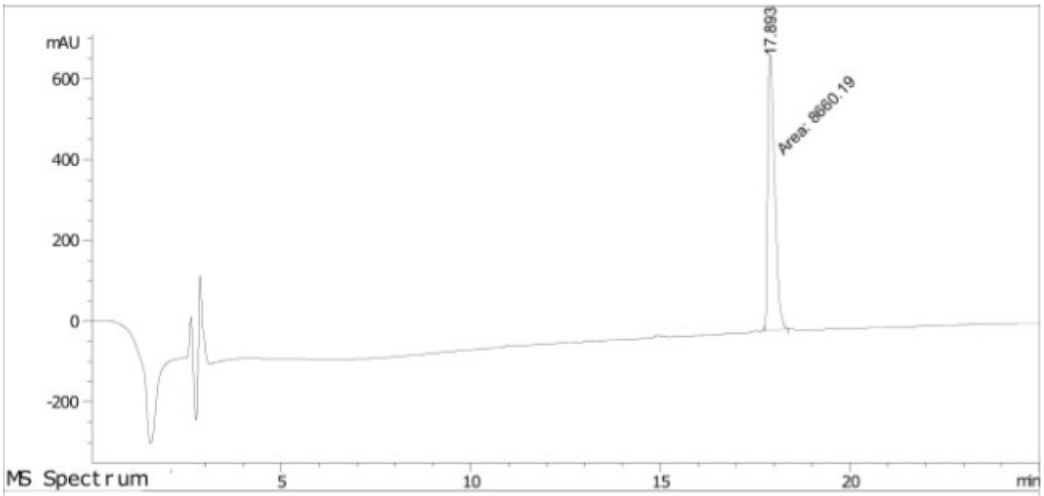


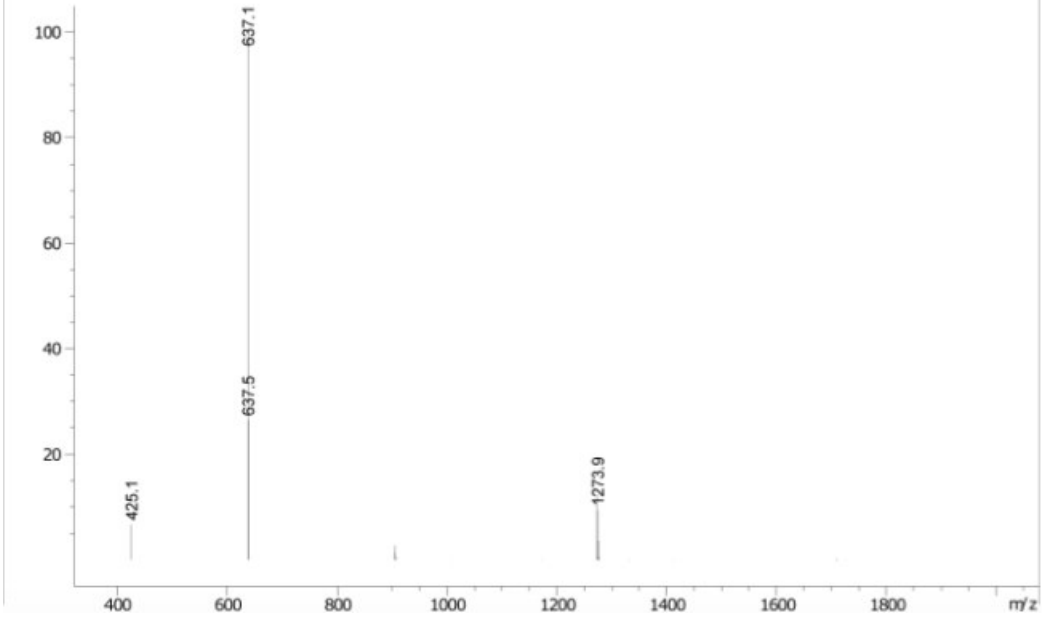
9
L-ADMA$_{10}$-teixobactin

Yield: 4.1%.

M ($C_{60}H_{101}N_{15}O_{15}$): 1272.54 g/mol.

LC-MS: m/z (%) = 1273.9 (15, $[M+H]^+$), 637.5 (30, $[M+2H]^{2+}$), 637.1 (100, $[M+2H]^{2+}$), 425.1 (10, $[M+3H]^{3+}$).





Analytical gradient: 1-76% B, 3% B/min (0.3 mL/min)

References

1. H-M Yu, W-X. Hong, Y. Wu, C-L. Zhong, and Z-J Yao, *Org. Lett.*, **2010**, 12, 1124-1127.