Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Supporting Information

## Total Synthesis of the Proposed Structure of Talarolide A

*Shengping Zhang [†], Luis M. De Leon Rodriguez [‡], Renjie Huang [†], Ivanhoe K. H. Leung, Paul W.R. Harris [†,‡,§], Margaret A. Brimble [*,†,‡,§]*

[†] School of Chemical Sciences, The University of Auckland, 23 Symonds St, Auckland, 1142, New Zealand

[‡] Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Auckland, 1142, New Zealand

[§] School of Biological Sciences, The University of Auckland, 3A Symonds Street, Auckland 1010, New Zealand

## Table of Contents

**General information**

All the reagents purchased from commercial sources were reagent grade and were used without further purification. Solvents for peptide synthesis and RP-HPLC were purchased as synthesis grade and HPLC grade, respectively. 2-Chlorotrityl chloride polystyrene resin (2-CTC, 0.9 mmol/g,) was purchased from Novabiochem (Merck, Germany). *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt) and Fmoc-Gly-OH were supplied by GL Biochem (Shanghai, China) while (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt), Fmoc-*N*Me-Tyr(tBu)-OH, Fmoc-D-*allo*-Ile and Fmoc-D-*N*Me-Leu-OH were obtained from Aapptec (Louisville, USA). Fmoc-D-Ala-OH was purchased from PolyPeptide laboratories (Torrance, USA). *N*,*N*'-Diisopropylcarbodiimide (DIC), diethylether ($Et_2O$), piperidine, *N*,*N*-diisopropylethylamine ($iPr_2NEt$), formic acid, morpholine and piperizine were purchased from Sigma-Aldrich (St. Louis, USA). 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP), *tert*-butyl bromoacetate, *O*-benzylhydroxylamine hydrochloride, 10% Pd/C and Fmoc-D-*N*Me-Ala-OH were purchased from AK Scientific (Union City, USA). *N'N'*-Dimethylformamide (DMF, AR grade), acetonitrile ($CH_3CN$, HPLC grade) and trifluoroacetic acid (TFA) were purchased from Scharlau (Barcelona, Spain). Dichloromethane ($CH_2Cl_2$) was obtained from ECP Limited (Auckland, New Zealand).

$^1H$ and $^{13}C$ NMR experiments were performed on a Bruker (Billerica, MA, USA) AVANCE III HD 500 ($^1H$ 500 MHz; $^{13}C$ 125 MHz) spectrometer or a Bruker AVANCE III 400 ($^1H$ 400 MHz; $^{13}C$ 100 MHz) in deuterated chloroform or DMSO-$d_6$. Chemical shifts were recorded in parts per million (ppm). The $^1H$ values were referenced to the residual DMSO signal at 2.50 ppm or the tetramethylsilane signal at 0.00 ppm in $CDCl_3$ for the RT experiment. The $^{13}C$ values were presented relative to the signal of residual DMSO at 39.5 ppm or residual $CHCl_3$ at 77.0 ppm. $^1H$ NMR spectral data were reported as follows: chemical shift ($\delta_H$), relative integral, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dq, doublet of quartets; ddd, doublet of doublet of doublets) and coupling constant (*J* in Hz). High-resolution mass spectra were obtained on a Bruker micrOTOFQ mass spectrometer using electrospray ionization (ESI) under positive mode. Optical rotations were determined at the sodium D line (589 nm) using a Perkin Elmer (Waltham, MA, USA) 341 instrument. The analytical RP-HPLC experiments were carried out using analytical columns (Thermo (Waltham, MA, USA) BDS HYPERSIL $C_{18}$, 4.6 mm × 150 mm, 5 µm or Waters XTerra MS $C_{18}$, 125 Å, 4.6 mm × 150 mm, 5 µm) on a Dionex (Torrance, CA, USA) Ultimate 3000 System with a 30 min linear gradient of 5-95% solvent B (where solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in acetonitrile) or a 45 min linear gradient of 5-95% solvent B at a flow rate of 1 mL•min$^{-1}$ and UV signals were detected at the wavelengths 210, 225, 254 and 280 nm. Analytical RP-HPLC spectra were also recorded on a Agilent Technologies (Santa Clara, CA) 1120 Compact LC system using Agilent ZORBAX $C_3$ column (3.0 mm × 150 mm, 3.5 µm) with a 30 min linear gradient of 5-95% solvent B (where solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in acetonitrile) at a flow rate of 0.3 mL•min$^{-1}$ and UV signals were detected at the wavelengths 210 nm. Semi-preparative RP-HPLC was carried out on a Waters 600 system using a semi-preparative column (XTerra MS $C_{18}$ Prep Column, 125 Å, 19 mm × 300 mm, 10 µm) at a flow rate of 10 mL•min$^{-1}$ using an adjusted gradient of 5-95% solvent B according to the elution profiles obtained from analytical RP-HPLC chromatography. LC-MS analysis was conducted on an Agilent

Technologies (Santa Clara, CA, USA)1260 Infinity LC equipped with an Agilent Technologies 6120 Quadrupole mass spectrometer using an analytical column (Agilent ZOBAX $C_3$, 300 Å, 3.0 mm × 150 mm, 3.5 µm) with a 30 min linear gradient of 5-95% solvent B at a flow rate of 0.3 mL•min$^{-1}$.

## HPLC Reaction Profiles

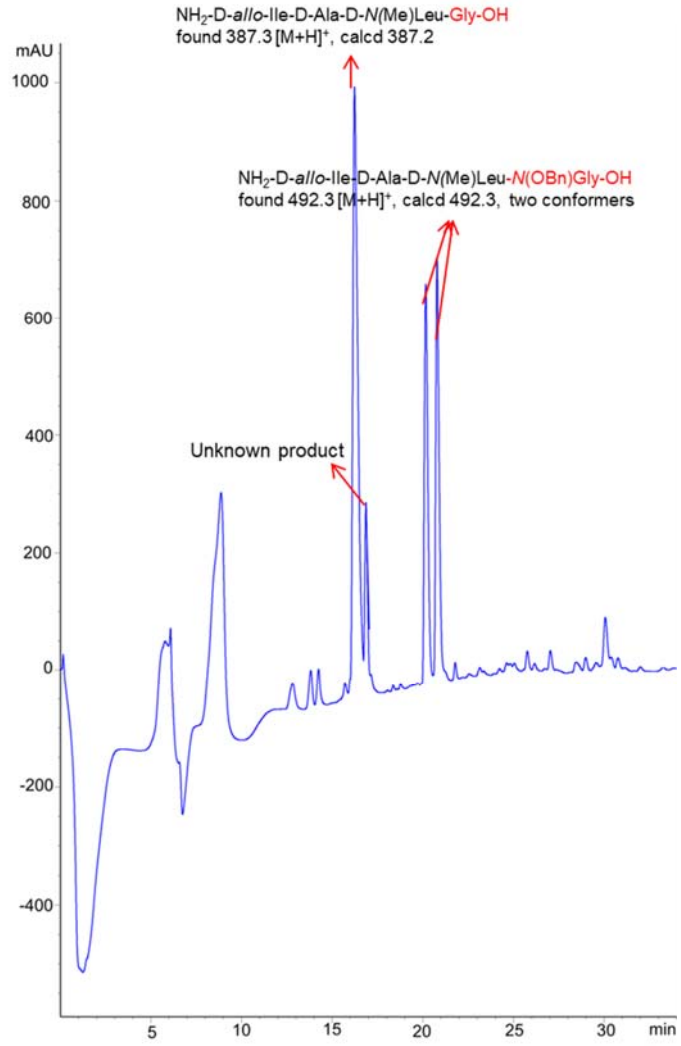



**Figure S1.** HPLC profile of a crude sample resulting from the removal of the $NH_2$-D-*allo*-Ile-D-Ala-D-*N*(Me)Leu-*N*(OBn)Gly-OH tetrapeptide from the resin during the synthesis of linear precursor **2**. Linear gradient of 5% B-95% B over 30 min (*ca.* 3 % B•min$^{-1}$), 0.3 mL•min$^{-1}$, using Agilent ZORBAX $C_3$ column (3.0 mm × 150 mm, 3.5 µm). The two peaks showing the same mass of the desired tetrapeptide $NH_2$-D-*allo*-Ile-D-Ala-D-*N*(Me)Leu-*N*(OBn)Gly-OH were attributed to conformers rather than epimers as there was only one corresponding reduced side product found in the crude mixture.

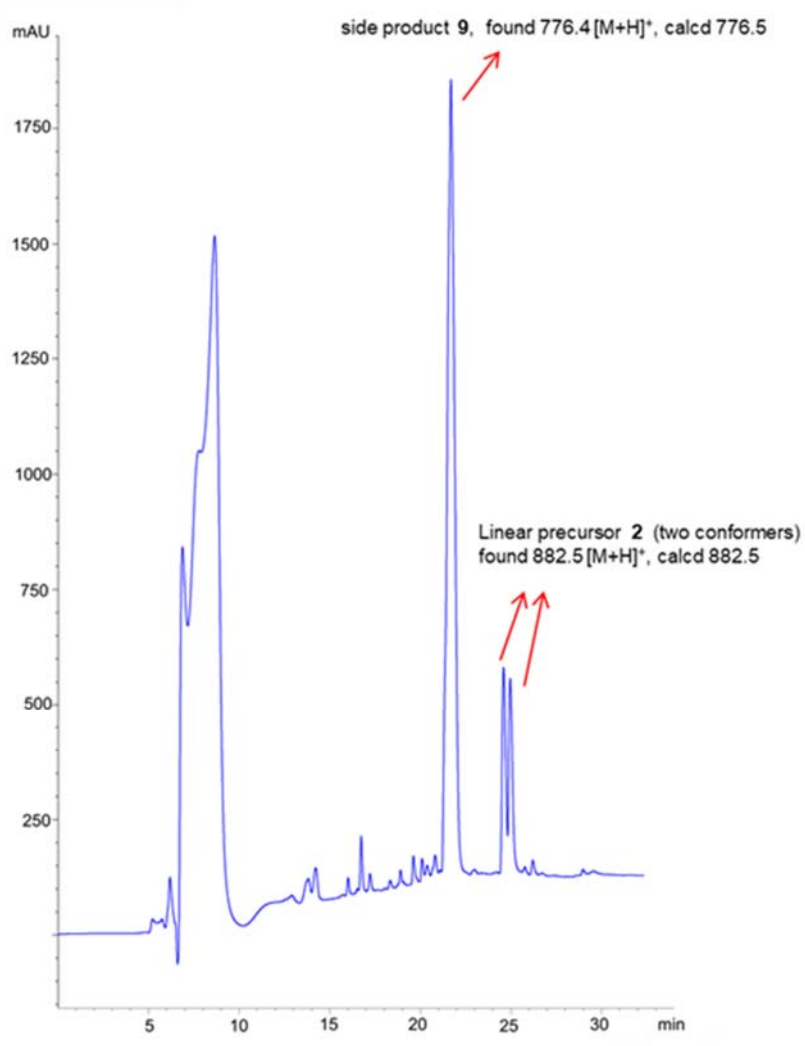



**Figure S2.** Reaction profile of final cleavage of linear precursor 2. Linear gradient of 5% B-95% B over 30 min (*ca.* 3 % B•min$^{-1}$), 0.3 mL•min$^{-1}$, using Agilent ZORBAX $C_3$ column (3.0 mm × 150 mm, 3.5 µm). The two peaks showing the same mass of linear precurosr **2** were attributed to conformers rather than epimers as there was only one corresponding reduced side product found in the crude mixture.

## Experimental procedures and compound characterization

### Determination of the loading of the first amino acid.

Fmoc-AA-2-CTC resin was washed in $CH_2Cl_2$ and dried for 30 min. 1.0 mg of the dried resin was weighed in two Starna Scientific Ltd cuvettes (size, 1cm x 1cm) respectively. Fresh 20% piperidine in DMF (v/v, 3 mL) was then added to each cuvette and the resulting mixture was agitated for 20 min at RT. These two cuvettes were then placed in a SHIMADZU UV-1280 UV-VIS spectrophotometer, along with a reference cuvette containing 20% piperidine solution in DMF (v/v, 3 mL). After zero calibration based on the blank sample, the absorbance of the two samples at 290 nm were recorded and the actual loading can be calculated based on the following equation:

Actual Loading (mmol/g) = Absorption/ (mg of resin sample × 1.75)

The final loading was determined after averaging the loading values calculated from the two samples.

### General method 1. Removal of *N*$^{\alpha}$-Fmoc-protecting group using conventional protocol

The peptidyl resin was treated with 20% piperidine in DMF (v/v) (3 mL) for 2 × 5 min, followed by resin wash using DMF (6 × 3 mL).

### General method 2. Coupling of amino acids (except Fmoc-D-*N*Me-Leu-OH) using HATU during solid-phase peptide synthesis (SPPS)

A mixture of Fmoc-AA-OH (4 equiv.), HATU (4 equiv.) and DIPEA (8 equiv.) in DMF (3 mL) was added to the peptidyl resin. The reaction mixture was agitated for 1 h at RT, followed by resin wash with DMF (6 × 3 mL).

### General method 3. Coupling of 3 using DIC during solid-phase peptide synthesis (SPPS)

To the peptidyl resin was added a solution of **3** (4 equiv.), DIC (4 equiv.) and 6-Cl-HOBt (4 equiv.) in DMF (3 mL). The resulting mixture was agitated for 24 h, followed by resin wash with DMF (6 × 3 mL).

### General method 4. Coupling of Fmoc-D-*N*Me-Leu-OH using HATU during solid-phase peptide synthesis (SPPS)

A mixture of Fmoc-D-*N*Me-Leu-OH (4 equiv.), HATU (4 equiv.) and DIPEA (8 equiv.) in DMF (3 mL) was added to the resin. The reaction mixture was allowed to shake 24 h at RT, followed by resin wash with DMF (6 × 3 mL). This coupling was repeated once more for complete reaction.

### General method 5. Rapid removal of the *N*$^{\alpha}$-Fmoc-protecting group

The peptidyl resin was treated with 20% piperidine in DMF (v/v) (3 mL) for 2 × 30 s, followed by resin wash using DMF (6 × 3 mL).

**H-D-*allo*-Ile-D-Ala-D-*N*Me-Leu-*N*(OBn)-Gly-Ala-D-*N*Me-Ala-*N*Me-Tyr(O*t*Bu)-OH** (**4**)

To pre-swollen ($CH_2Cl_2$) 2-CTC resin (104 mg, 0.05 mmol, loading 0.48 mmol/g), was added a solution of Fmoc-*N*Me-Tyr (48 mg, 0.1 mmol) and DIPEA (44 µL, 0.25 mmol), the resulting mixture was agitated for 1 h at RT. After removal of the Fmoc group using general method 1, all the amino acids were coupled to the resin-bound peptide using general method 2, except building block **3** and Fmoc-D-*N*Me-Leu which were attached to the resin using general methods 3 and 4, respectively. $N^{\alpha}$-Fmoc-deprotection for all the amino acids was achieved using general method 1, except for resin-bound Fmoc-D-Ala, which was performed using general method 5. After peptide assembly, the linear peptide **4** was released from the resin using 20% HFIP in $CH_2Cl_2$ at RT for 1 h. The resin was then filtered and washed with $CH_2Cl_2$ (2 x 3 mL). The combined filtrates were then concentrated under a stream of nitrogen, which was followed by addition of cold $Et_2O$. The resulting precipitated product was isolated by centrifugation (4000 rpm, 6 min), re-dissolved in 50% aqueous acetonitrile and lyophilized to afford crude **4** as a white powder (22.8 mg, *ca.* 52%). Anal. RP-HPLC: $t_R$ = 17.0 min (Thermo BDS HYPERSIL $C_{18}$, 4.6 mm × 150 mm, 5 µm, 5% B to 95% B at 3% B per min, 1 mL•min$^{-1}$). MS (ESI+): $C_{46}H_{72}N_7O_{10}$ $[M+H]^+$ calcd./found 882.5/882.4.

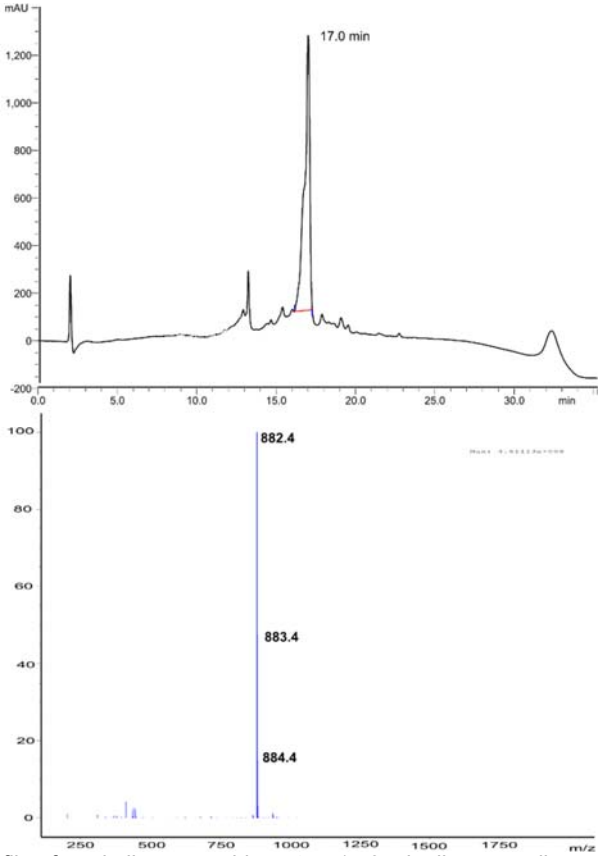



**Figure S3**. LC-MS profile of crude linear peptide **4**, $t_R$ = 17.0 min; linear gradient of 5% B-95% B over 30 min (*ca.* 3 % B•min$^{-1}$), 1 mL•min$^{-1}$, using Thermo BDS HYPERSIL $C_{18}$ column (4.6 mm × 150 mm, 5 µm).

**Cyclo[D-*allo*-Ile-D-Ala-D-*N*Me-Leu-*N*(OBn)-Gly-Ala-D-*N*Me-Ala-*N*Me-Tyr(O*t*Bu)] (21) prepared from 4**

To a stirred solution of DIPEA (20 µL, 115 µmol) in DMF (26 mL) was added a mixture of **4** (20 mg, 23 µmol) and PyBOP (35 mg, 69 µmol) in DMF (5 mL) *via* a syringe pump at RT for 90 min and the mixture was stirred for another 20 h. The resulting solution was concentrated under high vacuum then diluted with water (*ca.* 10 mL). Purification of the reaction mixture using semi-preparative RP-HPLC afforded **21** as a white solid (7.1 mg, 36%). Anal. RP-HPLC: $t_R$ = 23.2 min (Thermo BDS HYPERSIL $C_{18}$, 4.6 mm × 150 mm, 5 µm, 5% B to 95% B at 3% B per min, 1 mL•min$^{-1}$). MS (ESI+): $C_{46}H_{70}N_7O_9$ $[M+H]^+$ calcd./found 864.5/864.4.

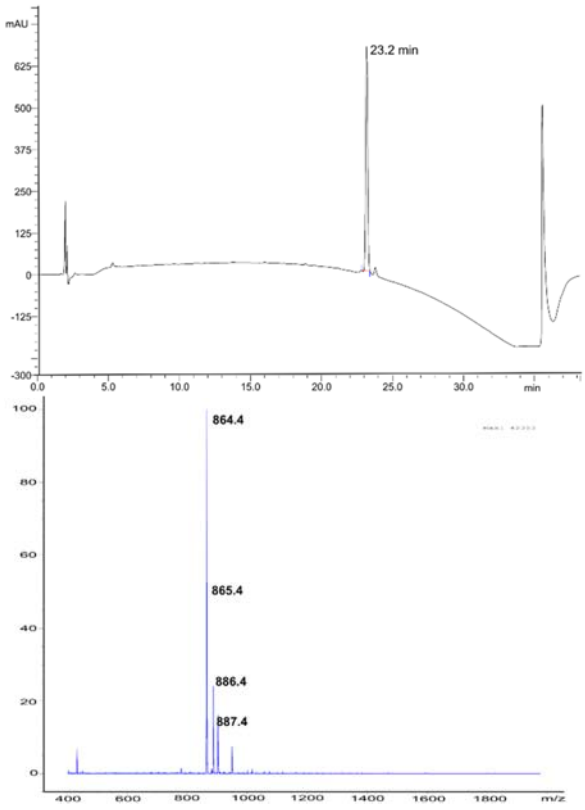



**Figure S4**. LC-MS profile of **21**, $t_R$ = 23.2 min (purity *ca.* 98% as judged by peak area of RP-HPLC at 210 nm); linear gradient of 5% B-95% B over 30 min (*ca*. 3 % B•min$^{-1}$), 1 mL•min$^{-1}$, using Thermo BDS HYPERSIL $C_{18}$ column (4.6 mm × 150 mm, 5 µm).

**H-D-Ala-D-*N*Me-Leu-*N*(OBn)-Gly-Ala-D-*N*Me-Ala-*N*Me-Tyr(O*t*Bu)-D-*allo*-Ile-OH** (**29**)

To pre-swollen ($CH_2Cl_2$) 2-CTC resin (122 mg, 0.05 mmol, loading 0.41 mmol/g), was added a solution of Fmoc-D-*allo*-Ile (35 mg, 0.1 mmol) and DIPEA (44 µL, 0.25 mmol), the resulting reaction was allowed to shake for 1 h at RT. After removal of the Fmoc group using general method 1, peptide chain elongation was performed using the same protocols as described for the synthesis of **4**. Finally, the linear peptide **29** was cleaved from resin using 20% HFIP in $CH_2Cl_2$ at RT for 1 h. After filtration and washing the resin with $CH_2Cl_2$ (2 x 3 mL), the combined filtrate was concentrated under a stream of nitrogen and cold $Et_2O$ was then added. The resulting precipitate was further isolated by centrifugation (4000 rpm, 6 min), re-dissolved in 50% acetonitrile in water and lyophilized to afford the crude linear peptide **29** as a white powder (18.1 mg, *ca.* 41%). Anal. RP-HPLC: $t_R$ = 18.1 min (Thermo BDS HYPERSIL $C_{18}$, 4.6 mm × 150 mm, 5 µm, 5% B to 95% B at 3% B per min, 1 mL•min$^{-1}$). MS (ESI+): $C_{46}H_{72}N_7O_{10}$ $[M+H]^+$ calcd./found 882.5/882.4.

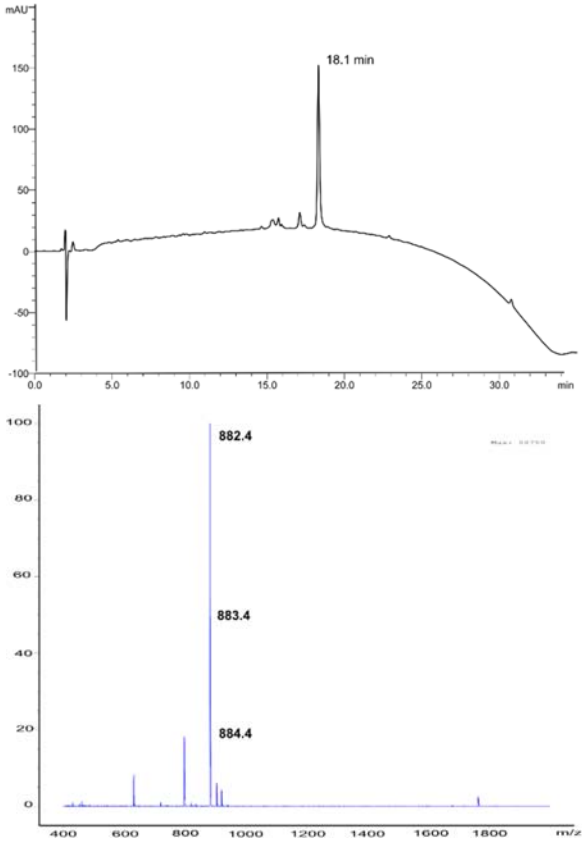



**Figure S5**. LC-MS profile of crude linear peptide **29**, $t_R$ = 18.1 min; linear gradient of 5% B-95% B over 30 min (*ca*. 3 % B•min$^{-1}$), 1 mL•min$^{-1}$, using Thermo BDS HYPERSIL $C_{18}$ column (4.6 mm × 150 mm, 5 µm).

**Cyclo[D-Ala-D-*N*Me-Leu-*N*(OBn)-Gly-Ala-D-*N*Me-Ala-*N*Me-Tyr(O*t*Bu)-D-*allo*-Ile] (21) prepared from 29**

Linear peptide precursor **29** (15 mg, 17 μmmol) was cyclized in solution using the same method as described in the macrolactamization of **4.** Compound **21** was obtained as a white solid (4.1 mg, 29%). Anal. RP-HPLC: $t_R$ = 23.2 min (Thermo BDS HYPERSIL $C_{18}$, 4.6 mm × 150 mm, 5 μm, 5% B to 95% B at 3% B per min, 1 mL•min$^{-1}$). MS (ESI+): $C_{46}H_{70}N_7O_9$ $[M+H]^+$ calcd./found 864.5/864.4.

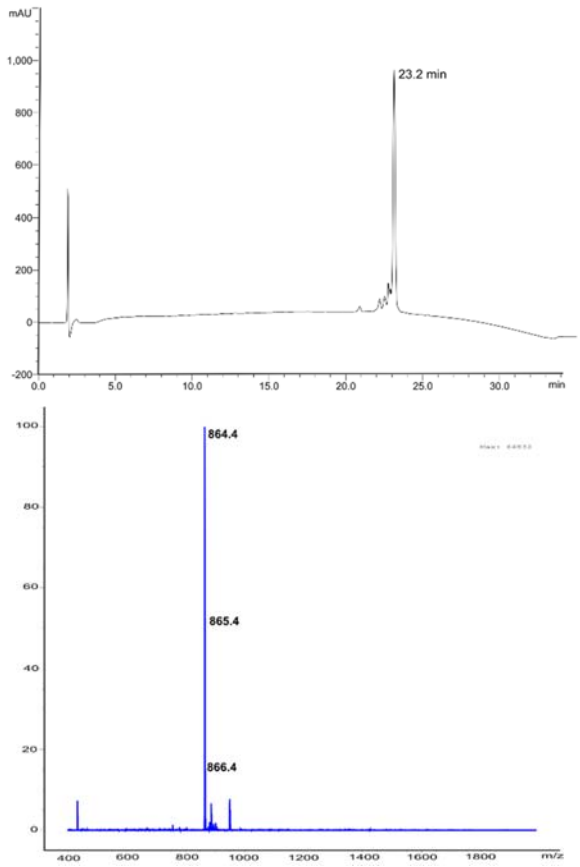



**Figure S6**. LC-MS profile of **21** obtained form **29**, $t_R$ = 23.2 min (purity *ca.* 91% as judged by peak area of RP-HPLC at 210 nm); linear gradient of 5% B-95% B over 30 min (*ca*. 3 % B•min$^{-1}$), 1 mL•min$^{-1}$, using Thermo BDS HYPERSIL $C_{18}$ column (4.6 mm × 150 mm, 5 μm).

**H-D-*N*Me-Leu-*N*(OBn)-Gly-Ala-D-*N*Me-Ala-*N*Me-Tyr(O*t*Bu)-D-*allo*-Ile-D-Ala-OH** (**36**)

To pre-swollen ($CH_2Cl_2$) 2-CTC resin (91 mg, 0.05 mmol, loading 0.55 mmol/g), was added a solution of Fmoc-D-Ala (31 mg, 0.1 mmol) and DIPEA (44 µL, 0.25 mmol), the resulting reaction was allow to shake for 1 h at RT. After removal of the Fmoc group using general method 1, peptide chain elongation was achieved using the same protocols as described in the synthesis of **4**. After peptide cleavage, the crude linear peptide **36** was obtained as a white powder (24.6 mg, *ca.* 56%). Anal. RP-HPLC: $t_R$ = 17.7 min (Thermo BDS HYPERSIL $C_{18}$, 4.6 mm × 150 mm, 5 µm, 5% B to 95% B at 3% B per min, 1 mL•min$^{-1}$). MS (ESI+): $C_{46}H_{72}N_7O_{10}$ $[M+H]^+$ calcd./found 882.5/882.4.

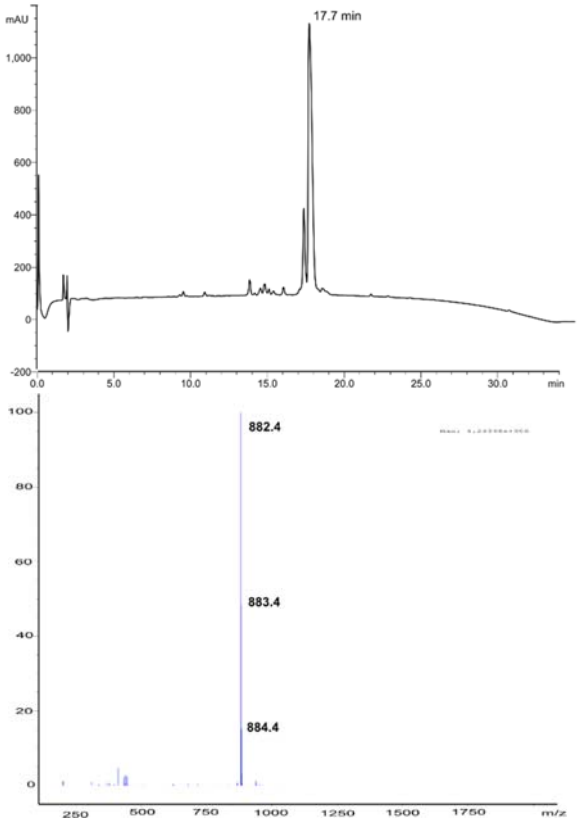



**Figure S7**. LC-MS profile of crude linear peptide **36**, $t_R$ = 17.7 min; linear gradient of 5% B-95% B over 30 min (*ca*. 3 % B•min$^{-1}$), 1 mL•min$^{-1}$, using Thermo BDS HYPERSIL $C_{18}$ column (4.6 mm × 150 mm, 5 µm).

**Cyclo[D-*N*Me-Leu-*N*(OBn)-Gly-Ala-D-*N*Me-Ala-*N*Me-Tyr(O*t*Bu)-D-*allo*-Ile-D-Ala] (21) prepared from 36**

To a stirred solution of DIPEA (20 µL, 115 µmol) in DMF (26 mL) was added a solution of linear peptide **36** (20 mg, 23 µmol), HATU (26 mg, 68 µmol) and HOAt (9 mg, 68 µmol) in DMF (5 mL) using a syringe pump within 90 min. The resulting mixture was allowed to stir at RT for 48 h. After completion of the reaction (as monitored by RP-HPLC), the mixture was concentrated under high vacuum and then diluted with water (*ca.* 10 mL). Purification of the reaction mixture using semi-preparative RP-HPLC furnished **21** as a white solid (1.0 mg, 5%). Anal. RP-HPLC: $t_R$ = 21.5 min (Waters XTerra MS $C_{18}$, 125 Å, 4.6 mm × 150 mm, 5 µm), 5% B to 95% B at 3% B per min, 1 mL•min$^{-1}$). MS (ESI+): $C_{46}H_{70}N_7O_9$ $[M+H]^+$ calcd./found 864.5/864.4.

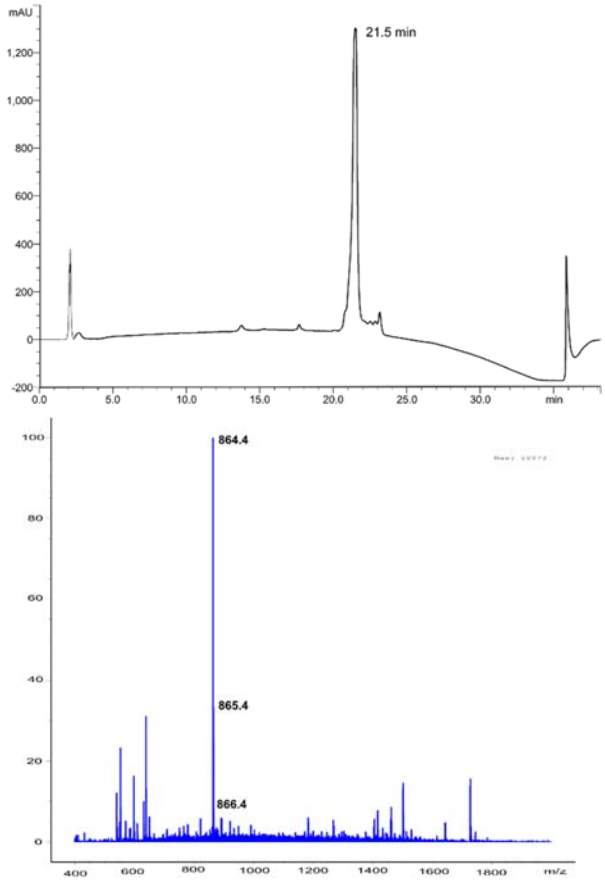



**Figure S8**. LC-MS profile of **21** obtained form **35**, $t_R$ = 21.5 min (purity *ca.* 93% as judged by peak area of RP-HPLC at 210 nm); linear gradient of 5% B-95% B over 30 min (*ca*. 3 % B•min$^{-1}$), 1 mL•min$^{-1}$, using Waters XTerra MS $C_{18}$, 125 Å, 4.6 mm × 150 mm, 5 µm.

**Talarolide A (1)**

The protected cyclic peptide **21** (7 mg, 8 µmol) was treated with 90% aqueous formic acid (3 mL) for 40 min. The resulting mixture was diluted with water (27 mL) and loaded onto an Alltech $C_{18}$ solid-phase extraction cartridge (prepared by washing with 3 x 5 mL of methanol followed by 3 x 5 mL of water). The cartridge was first washed with 3 x 5 mL of water, followed by elution using 3 x 5 mL of acetonitrile. The collected acetonitrile fraction was then concentrated under reduced pressure and the residue was re-dissolved in methanol (7 mL). Subsequent hydrogenation was performed by adding 10% Pd/C (5 mg, 5 µmol) to the resulting solution in methanol, followed by continuous bubbling of hydrogen gas through the solution. After fixing a hydrogen balloon to the reaction system, the mixture was stirred for 90 min at RT. The Pd/C was then removed via filtration through a celite pad, the filtrate was combined and concentrated to afford the crude product. Further purification of the crude product using semi-preparative RP-HPLC furnished **1** as a white solid (5.2 mg, 82%). Anal. RP-HPLC: $t_R$ = 17.1 min (Thermo BDS HYPERSIL $C_{18}$, 4.6 mm × 150 mm, 5 µm, 5% B to 95% B at 2% B per min, 1 mL•min$^{-1}$). $[\alpha]_D^{23.0}$ 25.0 (*c* 0.04, MeOH), lit.[1] $[\alpha]_D$ −13.5 (*c* 0.05, MeOH); NMR (500 MHz, DMSO-$d_6$) data see Table S1 and S2; HRMS (ESI+): $C_{35}H_{55}N_7O_9Na$ $[M+Na]^+$ calcd./found 740.3953/740.3954.

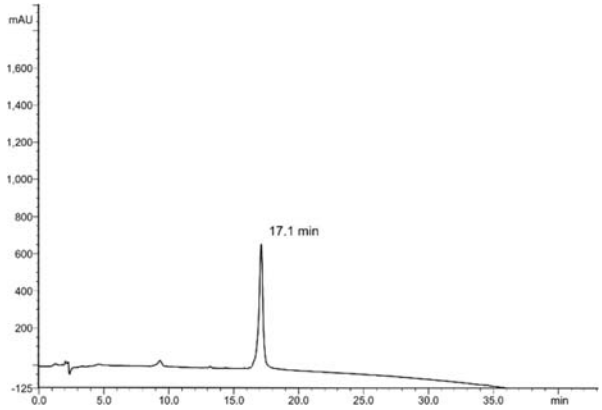



**Figure S9**. LC-MS profile of synthetic talarolide A (**1**), $t_R$ = 17.1 min (purity *ca.* 97% as judged by peak area of RP-HPLC at 210 nm); linear gradient of 5% B-95% B over 45 min (*ca*. 2 % B•min$^{-1}$), 1 mL•min$^{-1}$, 50 °C, using Thermo BDS HYPERSIL $C_{18}$ column (4.6 mm × 150 mm, 5 µm).

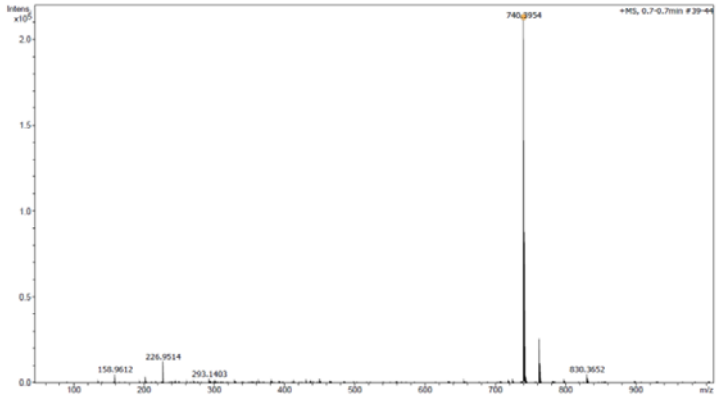
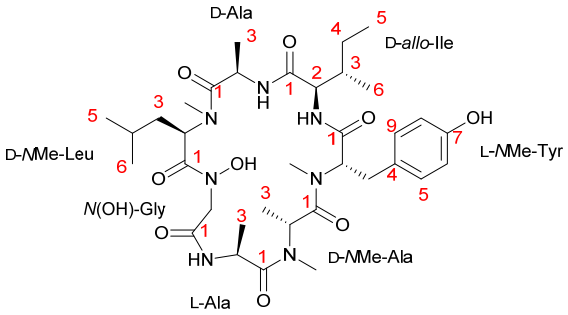



**Figure S10**. HRMS profile of synthetic talarolide A (**1**). $C_{35}H_{55}N_7O_9Na$ $[M+Na]^+$ calcd./found 740.3953/740.3954.

**Table S1. $^{1}H$ and $^{13}C$ NMR (500 MHz, DMSO-$d_6$) data for the conformer 1 of synthetic talarolide A (1)**



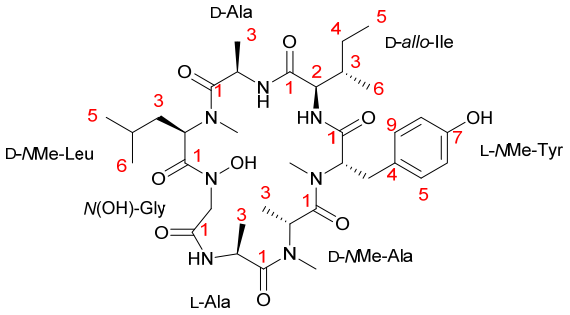

Conformer 1

| residue | Conformer A | | natural product[1] | |
|---|---|---|---|---|
| | **$\delta_H$ (*J* in Hz)** | **$\delta_C$** | **$\delta_H$ (*J* in Hz)** | **$\delta_C$** |
| **D-*allo*-Ile** | | | | |
| 1 | | 171.7 | | 169.7 |
| 2 | 4.25 (dd, *J* = 7.4, 4.1 Hz, 1H). | 55.2 | 4.72 (m, 1H) | 53.7 |
| 3 | 1.83 (m, 1H) | 36.4 | 1.95 (m, 1H) | 38.5 |
| 4 | 1.27 (m, 1H) | 25.7 | 1.42 (m, 1H) | 26.2 |
| | 1.05 (m, 1H) | | 1.07 (m, 1H) | |
| 5 | 0.81 (d, *J* = 7.3 Hz, 3H), | 11.5 | 0.94 (dd, *J* = 7.3, 7.3 Hz, 3H) | 12.0 |
| 6 | 0.69 (d, *J* = 6.8 Hz, 3H). | 14.3 | 0.81 (d, *J* = 6.9 Hz, 3H) | 13.7 |
| *N*H | 6.95 (d, *J* = 8.4 Hz, 1H) | | 7.24 (d, *J* = 9.6 Hz, 1H) | |
| **D-Ala** | | | | - |
| 1 | | 171.8 | | 174.1 |
| 2 | 4.94 (dd, *J* = 13.9, 7.3 Hz, 1H) | 44.7 | 4.49 (qd, *J* = 6.8, 4.1 Hz, 1H) | 45.2 |
| 3 | 1.23 (d, *J* = 6.9 Hz, 1H). | 16.9 | 1.19 (d, *J* = 6.8 Hz, 3H) | 15.7 |
| *N*H | 8.52 (d, *J* = 5.7 Hz, 1H) | | 8.66 (d, *J* = 4.1 Hz, 1H) | |
| ***N*Me-D-Leu** | | | | |
| 1 | | 169.1 | | 167.3 |
| 2 | 5.10 (m, 1H). | 51.8 | 5.05(dd, *J* = 11.8, 3.9 Hz, 1H) | 54.5 |

| | | | | |
|---|---|---|---|---|
| 3 | 2.02 – 2.10 (m, 1H). | 37.8 | 1.79 (ddd, *J* = 14.4, 10.3, 3.9 Hz, 1H) | 36.0 |
| | 1.23 (d, *J* = 6.9 Hz, 1H). | | 1.58 (ddd, *J* = 14.4, 11.8, 3.9 Hz, 1H) | |
| 4 | 1.32 – 1.48 (m, 1H). | 29.6 | 1.37 (m, 1H) | 24.4 |
| 5 | 0.91 (d, *J* = 6.2 Hz, 3H). | 21.7 | 0.77 (d, *J* = 6.5 Hz, 3H) | 21.0 |
| 6 | 0.90 (d, *J* = 6.2 Hz, 3H). | 23.0 | 0.88 (d, *J* = 6.5 Hz, 3H) | 23.3 |
| *N*Me | 2.68 (s, 3H) | 28.2 | 3.00 (s, 3H) | 31.0 |
| ***N*-OH-Gly** | | | | |
| 1 | | 165.6 | | 172.0 |
| 2 | 4.74 (d, *J* = 16.3 Hz, 1H) | 49.3 | 4.75 (d, *J* = 17.1 Hz, 1H) | 50.2 |
| | 3.64 (d, *J* = 16.4 Hz, 1H). | | 3.76 (d, *J* = 17.1 Hz, 1H) | |
| *N*-OH | 9.79 (s, 1H). | | 9.31 (s, 1H) | |
| **L-Ala** | | | | - |
| 1 | | 171.6 | | 171.1 |
| 2 | 4.88 (m, 1H) | 43.4 | 4.34 (qd, *J* = 7.1, 5.4 Hz, 1H) | 45.8 |
| 3 | 1.16 (d, *J* = 6.9 Hz, 3H) | 17.3 | 1.12 (d, *J* = 7.1 Hz, 3H) | 14.9 |
| *N*H | 8.45 (d, *J* = 8.3 Hz, 1H) | | 8.87 (d, *J* = 5.4 Hz, 1H) | |
| ***N*-Me-D-Ala** | | | - | |
| 1 | | 170.4 | | 169.8 |
| 2 | 5.02 (m, 1H) | 49.8 | 4.71 (q, *J* = 6.5 Hz, 1H) | 46.7 |
| 3 | 0.98 (d, *J* = 6.5 Hz, 3H) | 14.8 | 0.49 (d, *J* = 6.5 Hz, 3H) | 15.1 |
| *N*Me | 2.96 (s, 3H) | 30.2 | 2.70 (s, 3H) | 28.6 |
| ***N*-Me-L-Tyr** | | | - | |
| 1 | | 169.1 | | 168.2 |
| 2 | 5.23 (t, *J* = 7.6 Hz, 1H) | 57.9 | 4.80 (dd, *J* = 10.5, 4.9 Hz, 1H) | 56.6 |
| 3 | 3.05 – 3.09 (m, 1H) | 32.4 | 2.84 (dd, *J* = 14.3, 10.5 Hz, 1H) | 34.1 |
| | 2.53 – 2.61 (m, 1H) | | 2.60 (dd, *J* = 14.3, 4.9 Hz, 1H) | |

| | | | | |
|---|---|---|---|---|
| 4 | | 127.7 | | 126.6 |
| 5/9 | 6.95 (d, *J* = 8.4 Hz, 2H) | 129.8 | 6.93 (d, *J* = 8.4 Hz, 2H) | 130.8 |
| 6/8 | 6.61 (d, *J* = 8.4 Hz, 2H) | 115.2 | 6.64 (d, *J* = 8.4 Hz, 2H) | 114.8 |
| 7 | | 155.8 | | 155.9 |
| 7-OH | 9.16 (br s, 1H) | | 9.21 (s, 1H) | |
| *N*Me | 2.64 (s, 3H) | 29.6 | 2.66 (s, 3H) | 28.6 |

**Table S2. $^{1}$H and $^{13}$C NMR (500 MHz, DMSO-*d*$_6$) data for the conformer 2 of synthetic talarolide A (1)**




Conformer 2

| residue | Conformer A | | natural product[1] | |
|---|---|---|---|---|
| | **$\delta_H$ (*J* in Hz)** | **$\delta_C$** | **$\delta_H$ (*J* in Hz)** | **$\delta_C$** |
| **D-*allo*-Ile** | | | | |
| 1 | | [a] | | 169.7 |
| 2 | 4.04 (m, 1H) | 55.2 | 4.72 (m, 1H) | 53.7 |
| 3 | 1.90 (m, 1H) | 33.5 | 1.95 (m, 1H) | 38.5 |
| 4 | 1.05 (m, 1H) | 25.7 | 1.42 (m, 1H) | 26.2 |
| | 0.90 (m, 1H) | | 1.07 (m, 1H) | |
| 5 | 0.74 (t, *J* = 7.3 Hz, 3H). | 11.4 | 0.94 (dd, *J* = 7.3, 7.3 Hz, 3H) | 12.0 |
| 6 | 0.62 (d, *J* = 6.7 Hz, 3H). | 13.6 | 0.81 (d, *J* = 6.9 Hz, 3H) | 13.7 |
| *N*H | 8.39 (d, *J* = 8.5 Hz, 1H) | | 7.24 (d, *J* = 9.6 Hz, 1H) | |
| **D-Ala** | | | | - |
| 1 | | 171.2 | | 174.1 |
| 2 | 4.44 (m, 1H) | 45.7 | 4.49 (qd, *J* = 6.8, 4.1 Hz, 1H) | 45.2 |

| | | | | |
|---|---|---|---|---|
| 3 | 0.98 (d, *J* = 6.5 Hz, 3H) | 16.6 | 1.19 (d, *J* = 6.8 Hz, 3H) | 15.7 |
| *N*H | 6.85 (br s, 1H). | | 8.66 (d, *J* = 4.1 Hz, 1H) | |
| ***N*Me-D-Leu** | | | | |
| 1 | | [a] | | 167.3 |
| 2 | 5.17 (m, 1H) | 49.5 | 5.05(dd, *J* = 11.8, 3.9 Hz, 1H) | 54.5 |
| 3 | 1.52 (dd, *J* = 13.7, 6.5 Hz, 1H) | 37.8 | 1.79 (ddd, *J* = 14.4, 10.3, 3.9 Hz, 1H) | 36.0 |
| | 1.33 – 1.47 (m, 1H) | | 1.58 (ddd, *J* = 14.4, 11.8, 3.9 Hz, 1H) | |
| 4 | 1.33 – 1.47 (m, 1H) | 23.8 | 1.37 (m, 1H) | 24.4 |
| 5 | 0.86 (m, 3H) | 22.8 | 0.77 (d, *J* = 6.5 Hz, 3H) | 21.0 |
| 6 | , 0.81 (d, *J* = 7.3 Hz, 3H). | 22.4 | 0.88 (d, *J* = 6.5 Hz, 3H) | 23.3 |
| *N*Me | 2.84 (s, 3H) | 29.3 | 3.00 (s, 3H) | 31.0 |
| ***N*-OH-Gly** | | | | |
| 1 | | 165.3 | | 172.0 |
| 2 | 4.47 (m, 1H) | 52.4 | 4.75 (d, *J* = 17.1 Hz, 1H) | 50.2 |
| | 3.99 (m, 1H) | | 3.76 (d, *J* = 17.1 Hz, 1H) | |
| *N*-OH | [a] | | 9.31 (s, 1H) | |
| **L-Ala** | | | | - |
| 1 | | [a] | | 171.1 |
| 2 | 4.84 (m, 1H) | 44.0 | 4.34 (qd, *J* = 7.1, 5.4 Hz, 1H) | 45.8 |
| 3 | 1.16 (d, *J* = 6.9 Hz, 3H) | 18.9 | 1.12 (d, *J* = 7.1 Hz, 3H) | 14.9 |
| *N*H | 8.10 (d, *J* = 8.2 Hz, 1H) | | 8.87 (d, *J* = 5.4 Hz, 1H) | |
| ***N*-Me-D-Ala** | | | - | |
| 1 | | [a] | | 169.8 |
| 2 | 5.38 (m, 1H) | 48.2 | 4.71 (q, *J* = 6.5 Hz, 1H) | 46.7 |
| 3 | 1.05 (m, 3H) | 14.6 | 0.49 (d, *J* = 6.5 Hz, 3H) | 15.1 |
| *N*Me | 2.92 (s, 3H) | 30.0 | 2.70 (s, 3H) | 28.6 |

| ***N*-Me-L-Tyr** | | | - | |
|---|---|---|---|---|
| 1 | | 169.1 | | 168.2 |
| 2 | 5.10 (m, 1H) | 57.5 | 4.80 (dd, *J* = 10.5, 4.9 Hz, 1H) | 56.6 |
| 3 | 2.88 (m, 1H), | 34.4 | 2.84 (dd, *J* = 14.3, 10.5 Hz, 1H) | 34.1 |
| | 2.53 – 2.61 (m, 1H) | | 2.60 (dd, *J* = 14.3, 4.9 Hz, 1H) | |
| 4 | | 127.0 | | 126.6 |
| 5/9 | 7.01 (d, *J* = 7.4 Hz, 2H), | 129.6 | 6.93 (d, *J* = 8.4 Hz, 2H) | 130.8 |
| 6/8 | 6.64 (d, *J* = 8.2 Hz, 2H). | 114.8 | 6.64 (d, *J* = 8.4 Hz, 2H) | 114.8 |
| 7 | | 155.8 | | 155.9 |
| 7-OH | 9.16 (br s, 1H) | | 9.21 (s, 1H) | |
| *N*Me | 3.04 (s, 3H) | 30.8 | 2.66 (s, 3H) | 28.6 |

[a]Signals were not found.

## NMR spectra

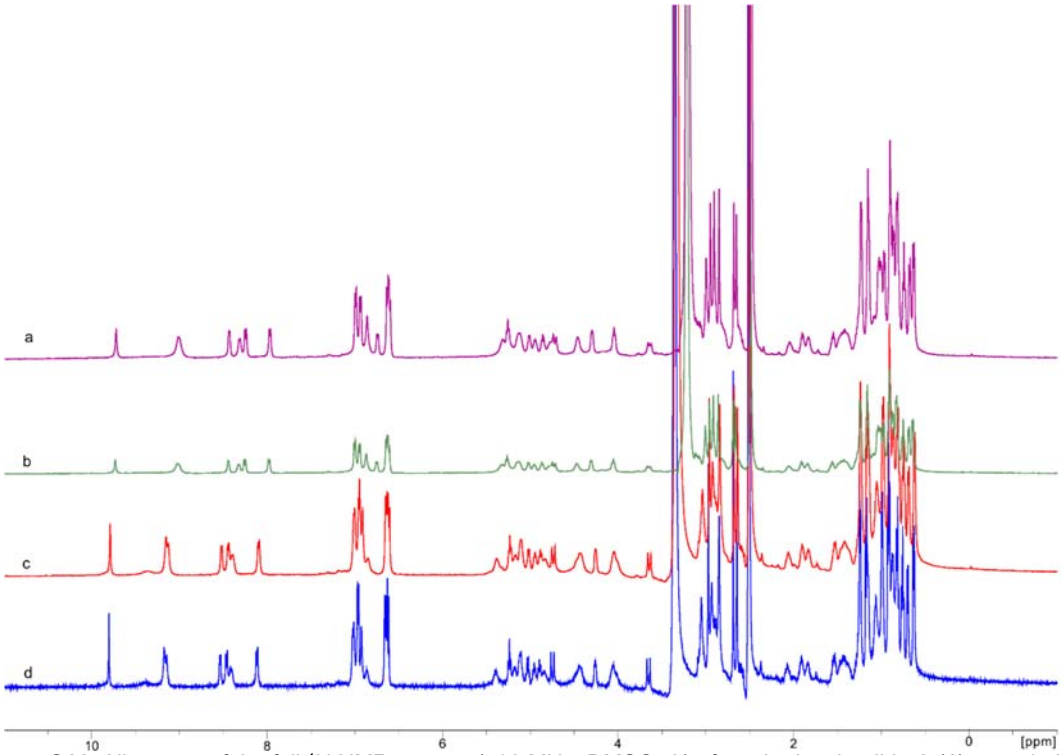



**Figure S11.** Alignment of the full $^{1}$H NMR spectra (500 MHz, DMSO-$d_6$) of synthetic talarolide A (**1**) recorded at a) 60 °C, b) 50 °C, c) 37 °C, d) 27 °C.

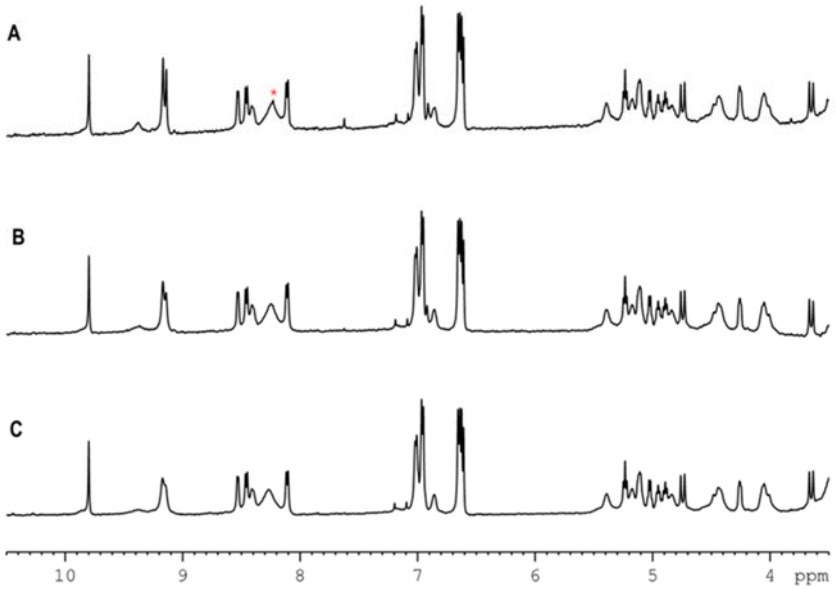



**Figure S12**. Alignment of the expanded $^{1}$H-NMR spectra (500 MHz, DMSO-$d_6$) of synthetic talarolide A (**1**) at a concentration of 0.5 mg/ml (A), 1 mg/ml (B) and 2 mg/ml (C). The signal of impurity was labelled with a red asterisk.

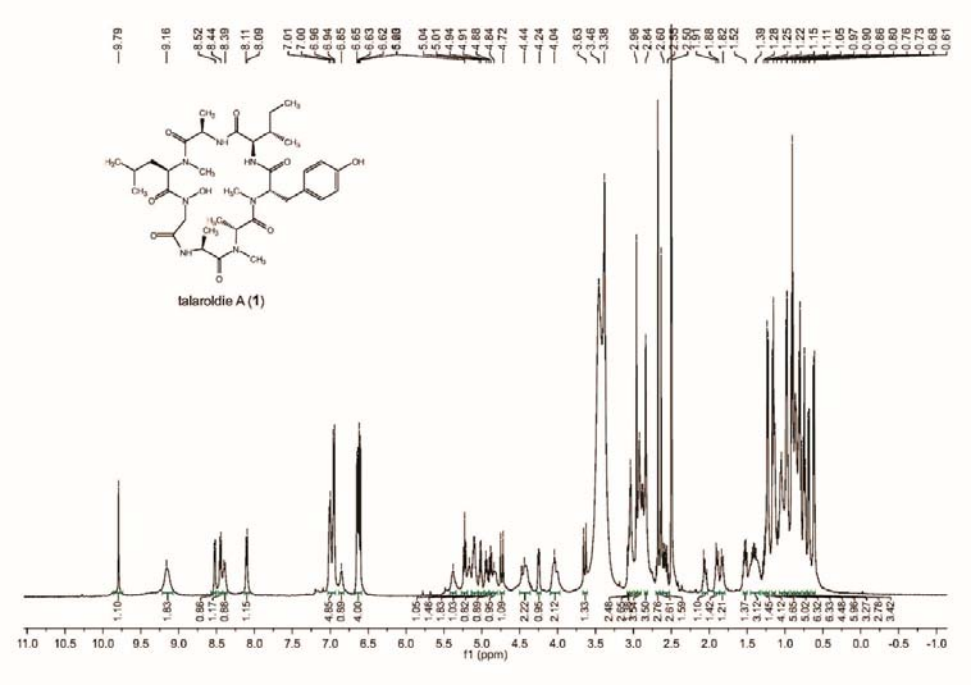



**Figure S13.** $^{1}$H NMR spectrum (500 MHz, DMSO-$d_6$) of synthetic talaroldie A (**1**)

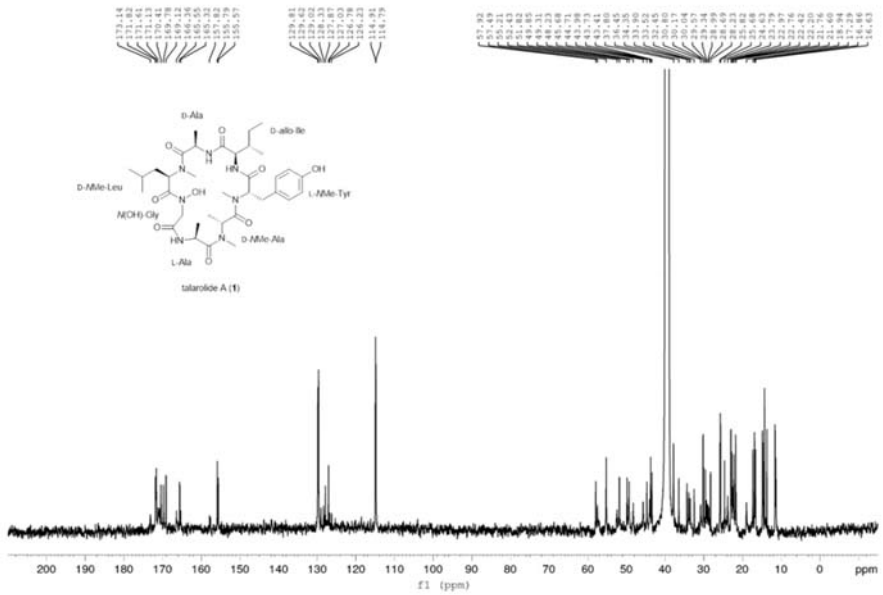



**Figure S14.** $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$) of synthetic talaroldie A (**1**)

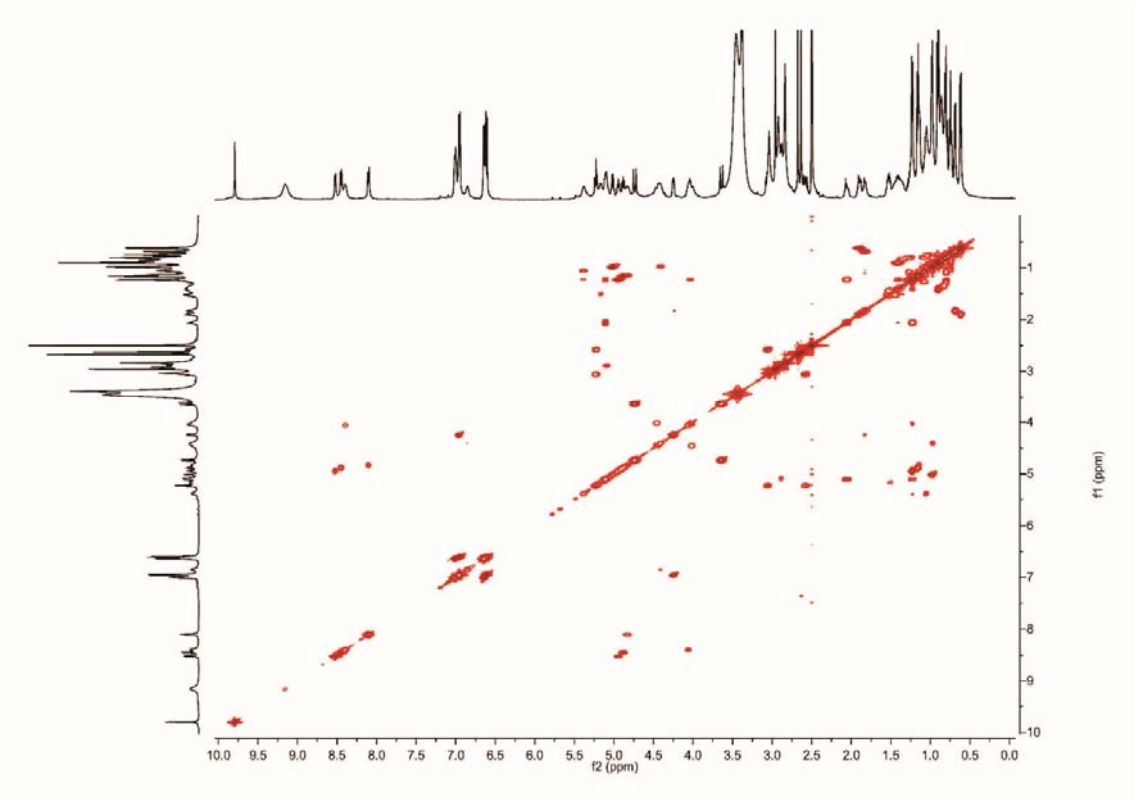



**Figure S15.** COSY spectrum (500MHz, DMSO-$d_6$) of synthetic talaroldie A (**1**)

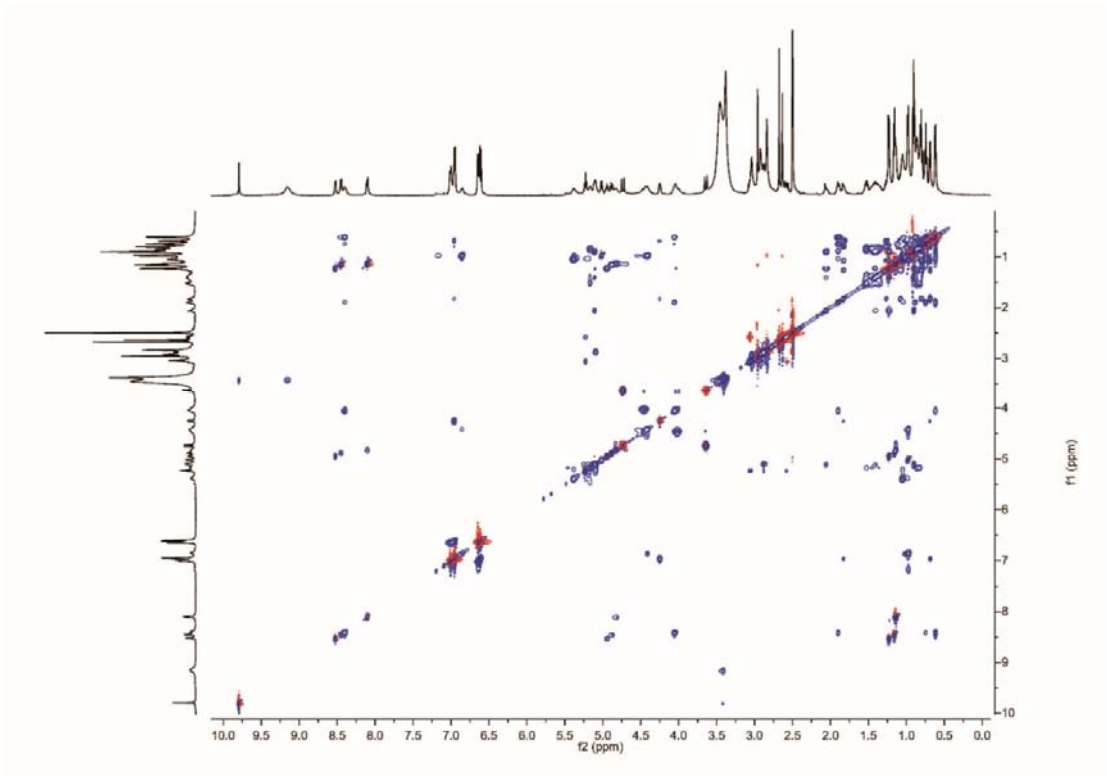



**Figure S16.** TOCSY spectrum (500MHz, DMSO-$d_6$) of synthetic talaroldie A (**1**)

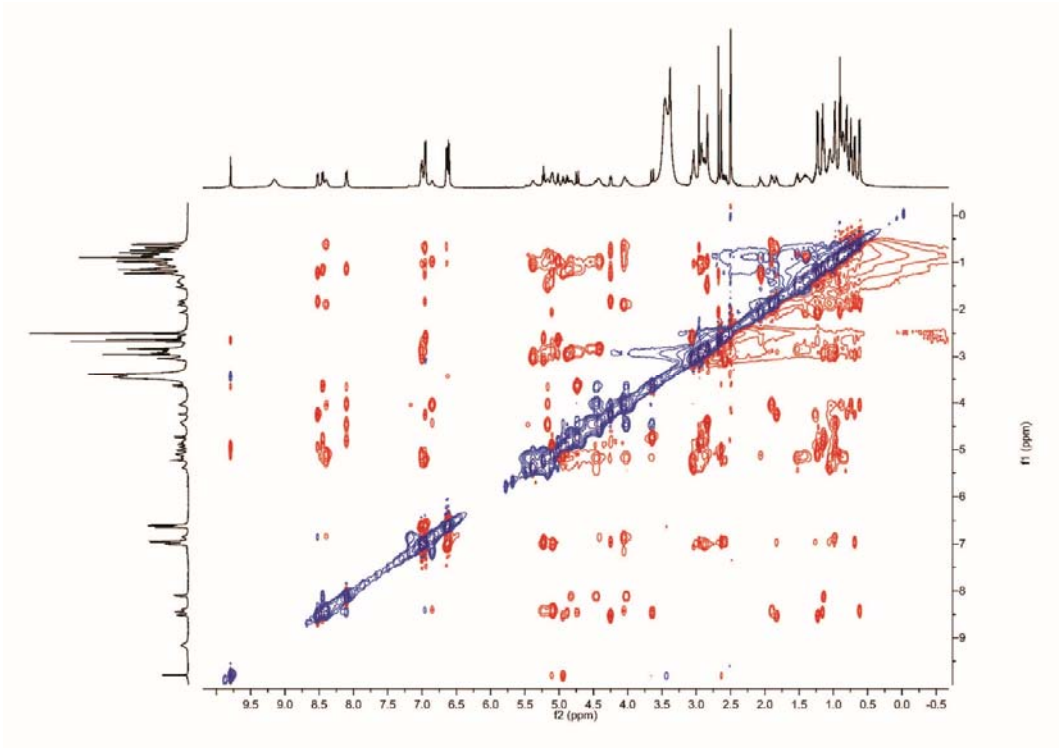



**Figure S17.** ROESY spectrum (500MHz, DMSO-$d_6$) of synthetic talaroldie A (**1**)

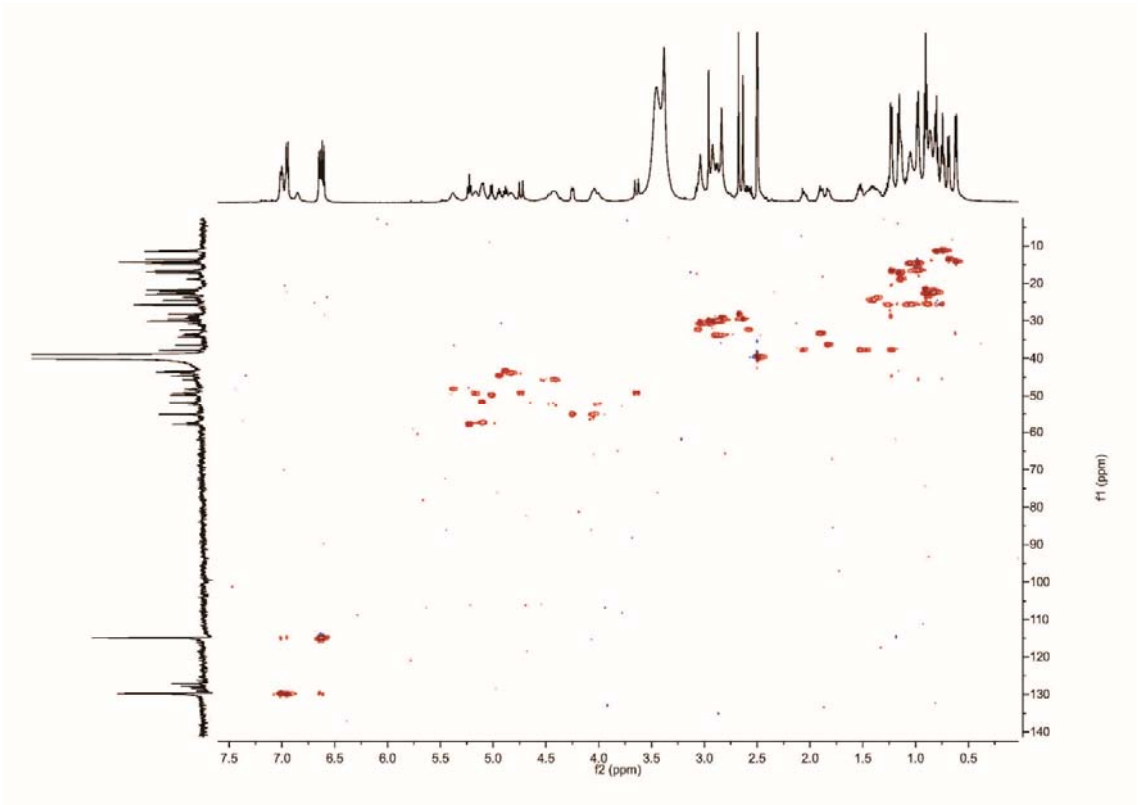



**Figure S18.** HSQC spectrum (500MHz, DMSO-$d_6$) of synthetic talaroldie A (**1**)

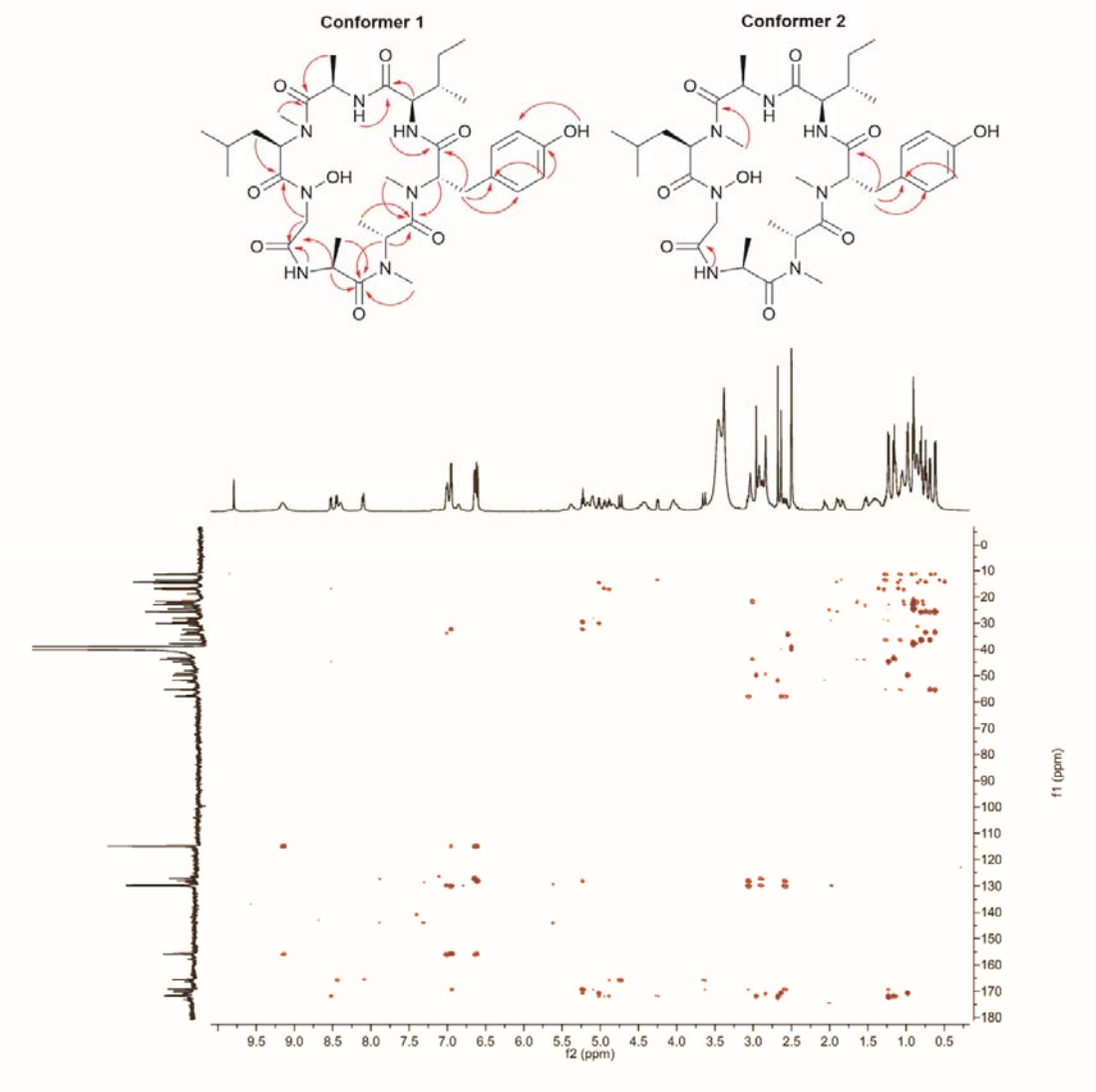



**Figure S19.** HMBC spectrum (500MHz, DMSO-$d_6$) of synthetic talaroldie A (**1**)

## References

1. P. Dewapriya, P. Prasad, R. Damodar, A. A. Salim and R. J. Capon, *Org. Lett.*, 2017, **19**, 2046-2049.