Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Total synthesis and antimalarial activity of mortiamides A-D

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Experimental

General information

Oxime resin (0.30 mmol/g), coupling reagents and *N*-Boc-protected amino acids were purchased from Matrix Innovation, Bachem, Advanced Chemtech and GL Shangai. Unless otherwise indicated, other starting materials were purchased from commercial sources (Sigma-Aldrich and VWR) and used without further purification. ^{1}H and ^{13}C-NMR spectra were recorded on an Agilent DD2 500 MHz spectrometer. The coupling constants are reported in hertz (Hz). Splitting patterns are designated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), brs (broad singlet), ddt (doublet of doublet of triplets), and m (multiplet). Mass spectra were obtained on an Agilent 6210 LC Time of Flight Mass Spectrometer in direct injection mode. HPLC purities data were recorded on Agilent 1260 Infinity instrument with a C18 column (GraceVydac) and a DAD detector (λ=220nm) or (λ=280nm). The gradient was performed at a flow rate of 1mL/min with a mobile phase composed of H_2O containing 0.1% TFA (A) and CH_3CH (B). All solvents were degassed and a gradient of (100% H_2O) to (46.4% H_2O /54.4% CH_3CN) in 35 min followed with 100% H_2O between 35 to 42 min was used for compounds **1**-**4**. The column temperature was kept at 22.5°C. Optical rotations were measured at ambient temperature on a Jasco DIP-360 digital polarimeter using a sodium lamp.

Ethics statement. This study was approved by the Canadian Blood Services (CBS) research ethics board, project number 2015.001, and by the Centre Hospitalier Universtaire (CHU) de Québec institutional research board, Canada, project numbers 2015–2230, B14-12-2230, SIRUL 104595. Written consent was obtained by the CBS for all study participants.

Parasite culture. *Plasmodium falciparum* 3D7 and Dd2 strains were obtained through BEI Resources, NIAID, NIH, contributed by Daniel J. Carucci and David Walliker (# MRA-102 and # MRA-150 respectively). Asexual stage *P. falciparum* parasites were cultured *in vitro* under standard conditions in RPMI-1640 (Gibco)-HEPES-buffered

medium supplemented with 0.5% (w/v) Albumax (Invitrogen) at 4% hematocrit (human red blood cells (RBC) of O+ group). Parasites were kept at 37°C in a gas mixture composed of 5% oxygen, 5% carbon dioxide and 90% nitrogen. Parasite synchronization was done using a D-sorbitol treatment, as previously described.[1]

***In vitro* 72 hour susceptibility assays.** Parasite susceptibility was measured as previously described, with minor modifications.[2] Briefly, sorbitol-synchronized 4-to-12 h post-invasion-stage ring parasites were exposed to a 16-point serial dilution of each molecule in a 96-well plate at 1% hematocrit and 0.5% starting parasitaemia in 100 µL final volume. Cells were incubated for 72 h before being disrupted. The released DNA was stained by adding 25 µL of 5X lysis buffer containing 0.16% saponin, 20 mM Tris-HCl pH 7.5, 5 mM EDTA, 1.6% Triton X-100, 5X SYBR™ Gold (Invitrogen). Each assay was done in triplicate. Plates were sealed and allowed to rest at room temperature for 24 h. Relative fluorescence units (RFU) were measured with a VICTOR plate reader (PerkinElmer) at an excitation of 494 nm and emission of 530 nm. RFU were compared to untreated parasites and data plotted using GraphPad PRISM® software and IC_{50} determined using with the curve-fitting algorithm *log(inhibitor) versus response–Variable slope*.

***In vitro* hemolysis assays.** As with the susceptibility assays, fresh uninfected RBCs were exposed to a 16-point serial dilution of each molecule in RPMI-HEPES containing 0.5% DMSO in a 96-well plate at 1% hematocrit and kept for 72 h at 37°C under a gassed atmosphere. Plates were spun at 600*g* and 100 µL of the supernatant was transferred to a clean 96-well plate. Absorbance of the released hemoglobin in the supernatant was then read at 405 nm using a VICTOR plate reader (PerkinElmer) and the lysis percentage was determined in comparison to the supernatant of drug-free RBC and to a 100% lysis control in which RBCs were treated with 1% Triton X-100. Assays were done in triplicate and were repeated 3 times.

Mortiamides A-D (1-4) synthesis

Coupling of the first *N*-Boc protected α-amino acid on oxime resin. 0.5 g of oxime resin (0.43 mmol/g) was added to a syringe peptide synthesis vessel. The resin was treated three times with CH_2Cl_2. Amino acid (3.0 equiv, 0.645 mmol) and HOBt (3.0 equiv, 0.645 mmol, 109 mg) were dissolved in DMF (10 mL) and the mixture was stirred for 10 minutes at 0 °C. DIC (3.0 equiv, 0.645 mmol, 0.10 mL), DIEA (6.0 equiv, 1.29 mmol, 0.22 mL) and DMAP (0.1 equiv, 0.0215 mmol, 2.6 mg) were added and the mixture was introduced into the peptide synthesis vessel and stirred mechanically for 3 h. The mixture was filtered under vacuum and the resin was washed [DMF (3 x 10 mL), MeOH (3 x 10 mL), DMF (3 x 10 mL), MeOH (4 x 10 mL)] and dried under reduced pressure.

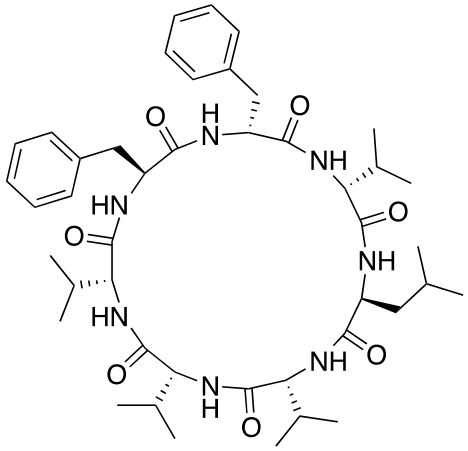
Acetylation of unreacted sites on oxime resin. The resin was treated three times with DMF (3 x 10 mL). A solution of 50% v/v DMF/acetic anhydride (15 mL) and DIEA (0.75 mL) were added to the peptide synthesis vessel and shaken for 1 hour. Then, the mixture was filtered under vacuum and the resin was washed [DMF (3 x 10 mL), MeOH (3 x 10 mL), DMF (3 x 10 mL), MeOH (4 x 10 mL)] and dried under reduced pressure.

Removal of the *N*-Boc protecting group. The resin was treated three times with CH_2Cl_2 (10 mL). A 50% v/v solution (10 mL) of trifluoroacetic acid (TFA) in CH_2Cl_2 was added to the peptide synthesis vessel and shaken for 30 minutes. Then, the mixture was filtered under vacuum and the resin was washed with DMF (3 x 10 mL), MeOH (3 x 10 mL), DMF (3 x 10 mL), MeOH (3 x 10 mL) and with a solution of 10% v/v DIEA in CH_2Cl_2 (10 mL).

Coupling of the subsequent *N*-Boc protected α-amino acids. The amino acid (3.0 equiv, 0.645 mmol) was dissolved in DMF (10 mL). The solution was cooled to 0 °C, then HCTU (3.0 equiv, 0.645 mmol, 267 mg) and HOBt (3.0 equiv, 0.645 mmol, 109 mg) were added. The mixture was added to the syringe peptide synthesis vessel containing the resin that had been previously treated with CH_2Cl_2 (3 X 10 mL). DIEA (6.0 equiv, 1.29 mmol, 0.22 mL) was also added to the vessel and the mixture was shaken for 3 h. After filtration under vacuum, the resin was washed [DMF (3 x 10 mL), MeOH (3 x 10 mL), DMF (3 x 10 mL) and MeOH (4 x 10 mL)] and dried under reduced pressure. The Kaiser ninhydrin test was

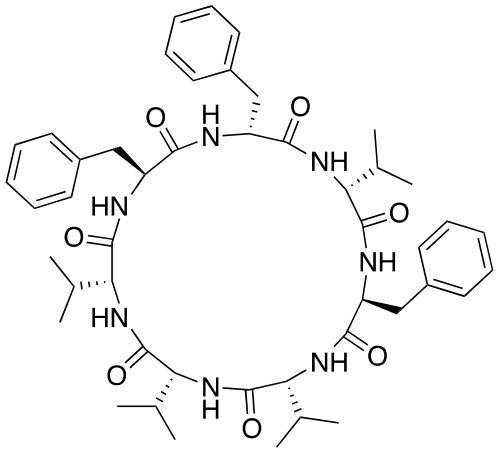
performed to monitor the efficiency of the coupling, and the coupling procedure was repeated if needed.

Cyclization/cleavage from the resin. First, the *N*-Boc group was removed using the procedure described above, but without the 10% v/v DIEA/CH_2Cl_2 washing step. After drying, the resin was put into a 100 mL peptide synthesis vessel and CH_2Cl_2 (50 mL) and DIEA (2.5 equiv, 0.54 mmol, 0.09 mL) were added to the vessel. The mixture was shaken for 5 min. Acetic acid (5.0 equiv, 1.08 mmol, 0.06 mL) was then added and the contents were shaken for 24 h. The filtrate was collected and the resin was rinsed several times with CH_2Cl_2 and MeOH. All the filtrates were combined and evaporated. Trituration in a minimum of cold ether was performed, then lyophilisation led to the desired mortiamides A to D (**1-4**) with good 35 to 45% macrocyclization yields respectively.

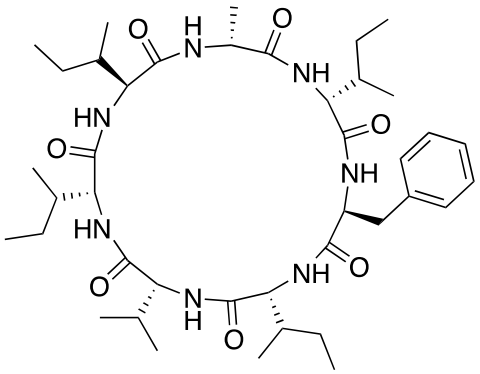


Mortiamide A (1). White powder, 35% yield. **[a]$_D^{22}$** +26° (c 0.5 in MeOH). White powder, 38% yield. **^{1}H-NMR** (500 MHz, DMSO-*d_6*): δ 8.44 (d, *J* = 5.2 Hz, 1H), 8.38-8.32 (m, 1H), 8.24 (d, *J* = 10.1 Hz, 1H), 8.18-8.06 (m, 1H), 7.86-7.79 (m, 1H), 7.74 (d, *J* = 9.1 Hz, 1H), 7.67-7.53 (m, 1H), 7.30-7.23 (m, 2H), 7.22-7.14 (m, 5H), 7.14-7.10 (m, 3H), 4.76 (dt, *J* = 10.1, 8.0 Hz, 1H), 4.57-4.51 (m, 1H), 4.50-4.42 (m, 1H), 4.30-4.24 (m, 1H), 4.23-4.17 (m, 1H), 4.16-4.11 (m, 1H), 4.10-4.02 (m, 1H), 3.07 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.91-2.83 (m, 1H), 2.83-2.75 (m, 1H), 2.71 (dd, *J* = 14.2, 8.4 Hz, 1H), 2.37-2.33 (m, 1H), 2.32-2.26 (m, 1H), 2.02-1.96 (m, 1H), 1.95-1.90 (m, 1H), 1.73-1.64 (m, 1H), 1.61-1.50 (m, 3H), 1.47-1.38 (m, 2H), 1.24-1.11 (m, 3H), 0.95 (dd, *J* = 11.5, 6.5 Hz, 3H), 0.88-0.85 (m, 3H), 0.84-0.76 (m, 9H), 0.71 (dd, *J* = 14.5, 7.1 Hz, 3H), 0.59 (d, *J* = 6.8 Hz, 3H), 0.51-0.43 (m, 3H).

HRMS (ESI) m/z calcd for $C_{44}H_{66}N_7O_7$ $(M+H)^+$: 804.5018, found 804.5412. **HPLC** (Retention time, purity): 36.11 min, 95 %. UV $(DMSO)_{\lambda max}$: 220, 280 nm.

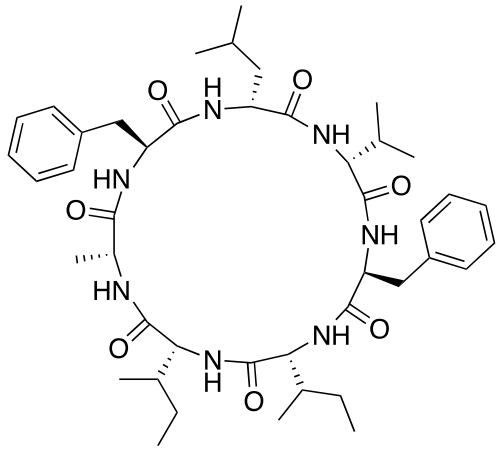


Mortiamide B (**2**). White powder, 38% yield. **$[a]_D^{22}$** +12° (c 0.5 in MeOH). **^{1}H-NMR** (500 MHz, DMSO-*d_6)*: δ 8.36-8.30 (m, 1H), 8.22-8.12 (m, 2H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 10.7 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.28-7.24 (m, 3H), 7.24-7.14 (m, 9H), 7.14-7.08 (m, 3H), 4.77-4.70 (m, 1H), 4.59-4.53 (m, 1H), 4.51-4.45 (m, 1H), 4.32-4.26 (m, 1H), 4.17-4.14 (m, 1H), 4.12-4.08 (m, 1H), 4.04-3.99 (m, 1H), 3.14 (dd, *J* = 13.6, 4.0 Hz, 1H), 3.05 (dd, *J* = 13.5, 6.4 Hz, 2H), 2.89-2.84 (m, 2H), 2.82-2.77 (m, 1H), 2.28-2.20 (m, 1H), 2.05-2.00 (m, 1H), 1.83-1.77 (m, 1H), 1.71-1.65 (m, 1H), 1.21-1.15 (m, 9H), 0.95-0.84 (m, 3H), 0.82-0.75 (m, 6H), 0.75-0.68 (m, 3H), 0.51-0.46 (m, 3H). **HRMS** (ESI) m/z calcd for $C_{47}H_{64}N_7O_7$ $(M+H)^+$: 838.4862, found 838.5138. **HPLC** (Retention time, purity): 36.14 min, 95 %. UV $(DMSO)_{\lambda max}$: 220, 280 nm.



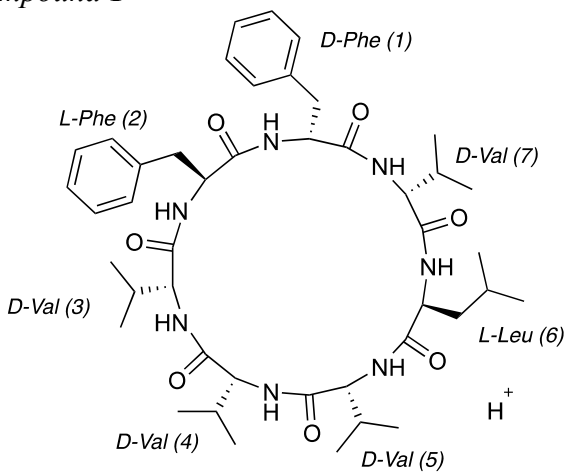
*Mortiamide C (**3**)*. White powder, 45% yield. **$[a]_D^{22}$** +11° (c 0.5 in MeOH). **^{1}H-NMR** (500 MHz, DMSO-d_6): δ = 8.44 (d, *J* = 6.0 Hz, 1H), 8.20 (d, *J* = 9.4 Hz, 1H), 8.28 (br, 1H), 8.09

(d, *J* = 8.9 Hz, 1H), 7.97 (d, *J* = 9.7 Hz, 1H), 7.93 (d, *J* = 7.0 Hz, 1H), 7.50 (d, *J* = 6.2 Hz, 1H), 7.27-7.25 (m, 2H), 7.24-7.22 (m, 1H), 7.20-7.17 (m, 2H), 4.58 (dd, *J* = 13.9, 7.66 Hz, 1H), 4.44 (dd, *J* = 9.7, 7.0 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 1H), 4.16, (m, 1H), 4.08-4.04 (m, 2H), 3.90 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.13 (dd, *J* = 13.8, 6.2 Hz, 1H), 2.85 (dd, *J* = 13.7, 9.0 Hz, 1H), 2.17 (m, 2H), 2.04-2.00 (m, 2H), 1.96-1.92 (m, 1H), 1.74-1.70 (m, 1H), 1.64-1.60 (m, 1H), 1.47 (m, 1H), 1.28-1.23 (m, 2H), 1.22 (d, *J* = 6.7 Hz, 3H), 1.10-1.08 (m, 2H), 1.00-0.98 (m, 2H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.86-0.82 (m, 7H), 0.81-0.77 (m, 9H), 0.76-0.73 (m, 6H), 0.71-0.69 (m, 3H), 0.62 (d, *J* = 6.7 Hz, 3H). **HRMS** (ESI) m/z calcd for $C_{41}H_{68}N_7O_7$ $(M+H)^+$: 770.5175, found 770.5100. **HPLC** (Retention time, purity): 33.77 min, 95 %. UV $(DMSO)_{\lambda max}$: 220, 280 nm.



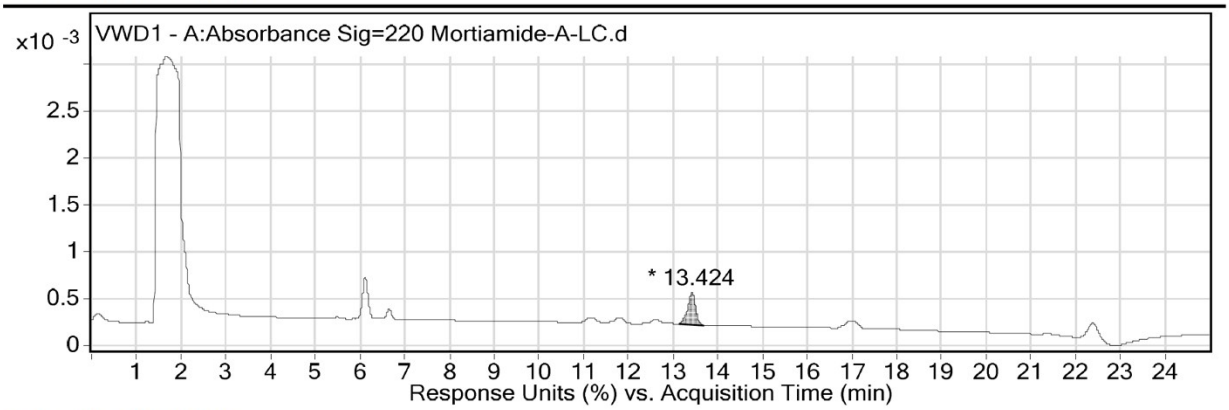
Mortiamide D (4). White powder, 48% yield. **$[a]_D^{22}$** +21° (c 0.5 in MeOH). **^{1}H-NMR** (500 MHz, DMSO-d_6): δ = 8.66 (d, *J* = 8.3 Hz, 1H), 8.51 (br, *J* = 8.3 Hz, 1H), 8.18 (d, *J* = 8.3 Hz, 1H), 8.13 (d, *J* = 9.2 Hz, 1H), 7.98 (d, *J* = 10.0 Hz, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.30 (d, *J* = 6.2 Hz, 1H), 7.26-7.24 (m, 3H), 7.23-7.21 (m, 4H), 7.19-7.15 (m, 3H), 4.54-4.50 (m, 1H), 4.42-4.39 (m, 1H), 4.24 (dd, *J* = 8.8, 6.9 Hz, 1H), 4.17-4.14 (m, 1H), 4.11-4.08 (m, 1H), 4.04-4.01 (m, 1H), 3.90 (dd, *J* = 8.2, 5.4 Hz, 1H), 3.26-3.24 (m, 2H), 2.70, dd, *J* = 14.0, 11.0 Hz, 2H), 2.04-2.00 (m, 1H), 1.93-1.90 (m, 1H), 1.46-1.40 (m, 1H), 1.20 (m, 2H), 1.14-1.11 (m, 2H), 1.09 (d, *J* = 7.0 Hz, 3H), 0.95-0.92 (m, 2H), 0.83 (d, *J* = 6.3 Hz, 6H), 0.80-0.77 (m, 6H), 0.75-0.72 (m, 3H), 0.70-0.66 (m, 6H). **HRMS** (ESI) m/z calcd for $C_{44}H_{66}N_7O_7$ $(M+H)^+$: 804.5018, found 804.4985. **HPLC** (Retention time, purity): 36.24 min, 95 %. UV $(DMSO)_{\lambda max}$: 220 nm.

LC-MS spectrum of compound ***1***



Chemical Formula: $C_{44}H_{66}N_7O_7^+$
Exact Mass: 804,5018

User Chromatograms



Integration Peak List

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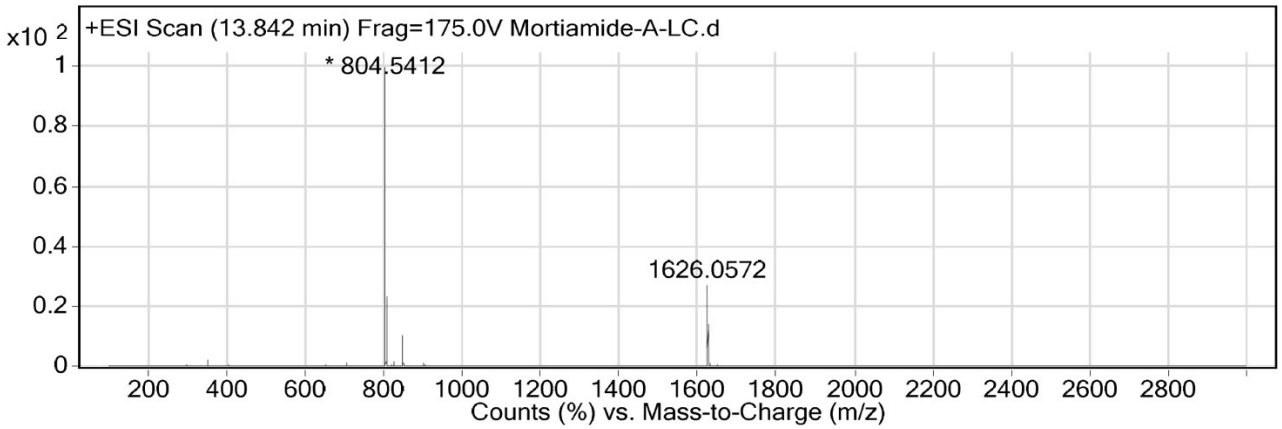
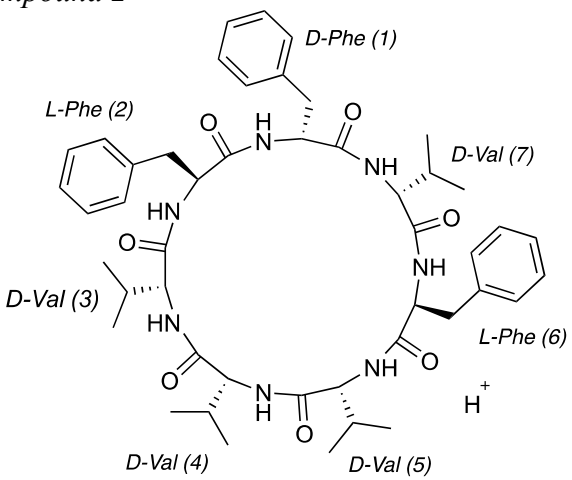
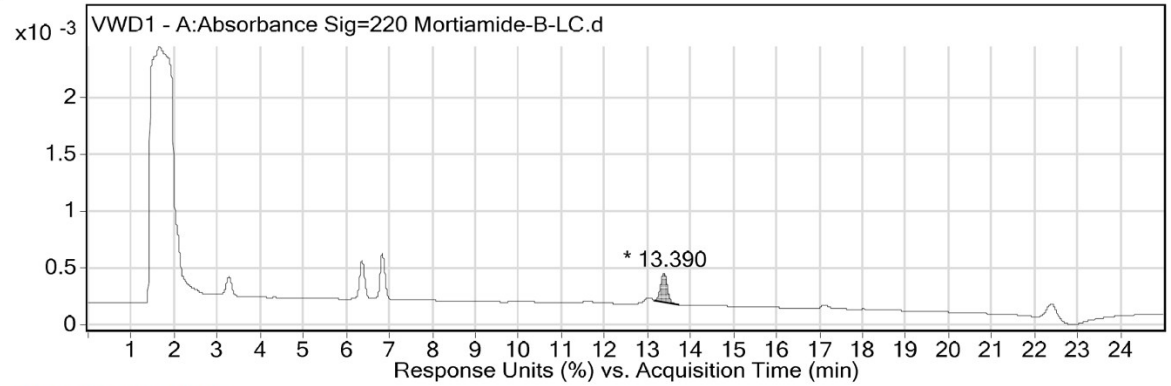


LC-MS spectrum of compound ***2***



Chemical Formula: $C_{47}H_{64}N_7O_7^+$
Exact Mass: 838,4862

User Chromatograms



Integration Peak List

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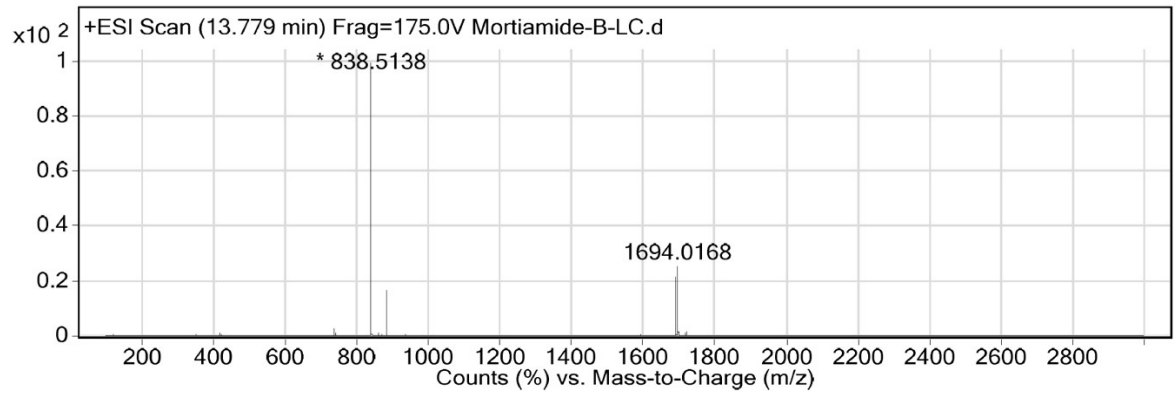
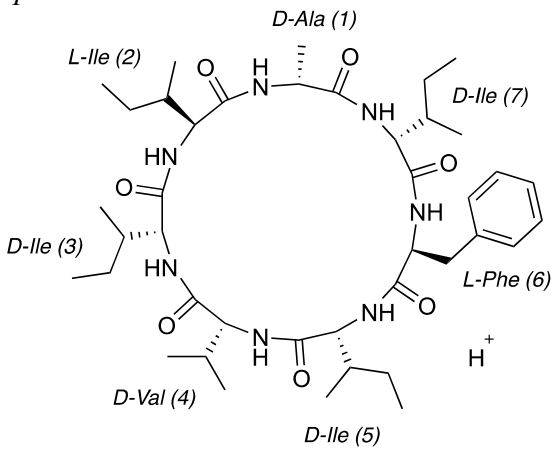
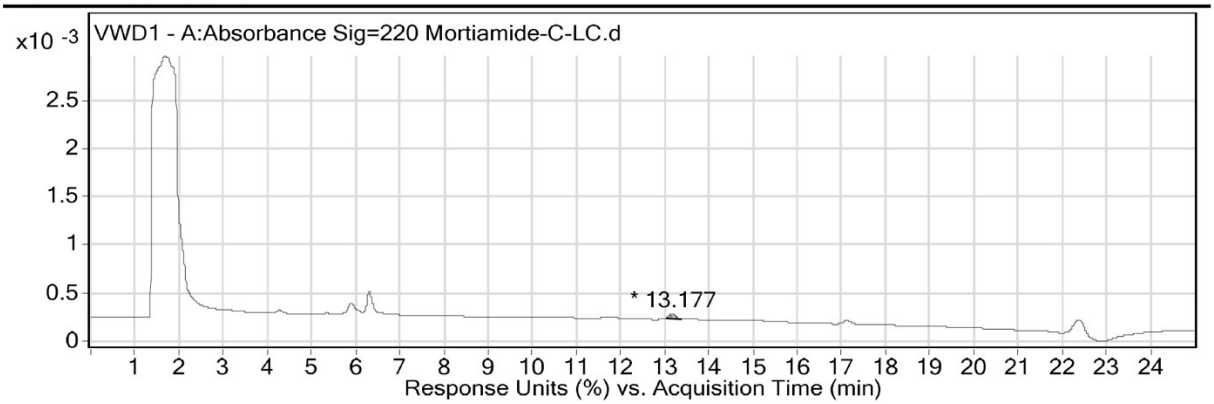


LC-MS spectrum of compound ***3***



Chemical Formula: $C_{41}H_{68}N_7O_7^+$
Exact Mass: 770,5175

User Chromatograms



Integration Peak List

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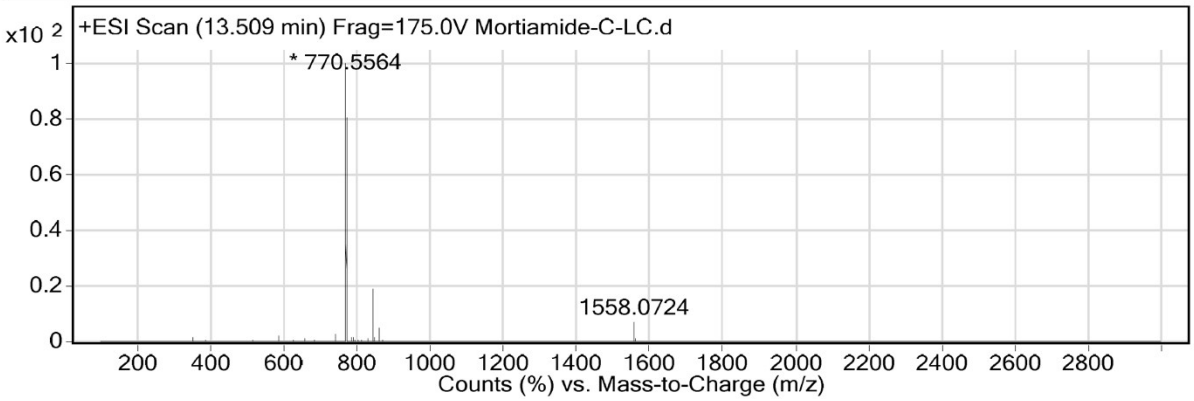
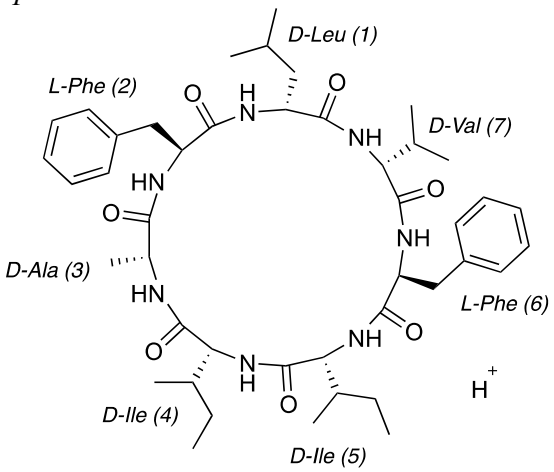
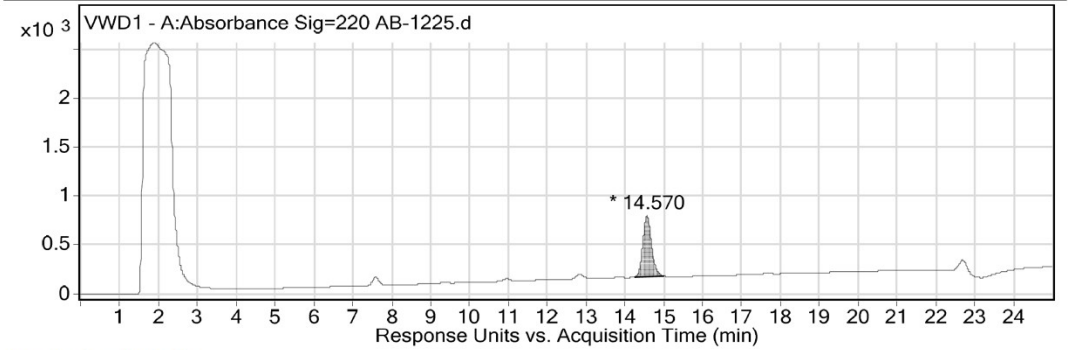


LC-MS spectrum of compound ***4***



Chemical Formula: $C_{44}H_{66}N_7O_7^+$
Exact Mass: 804,5018

User Chromatograms



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
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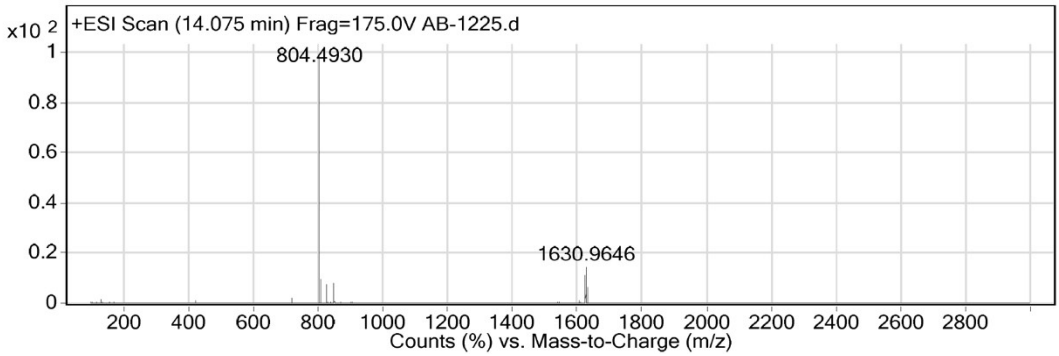
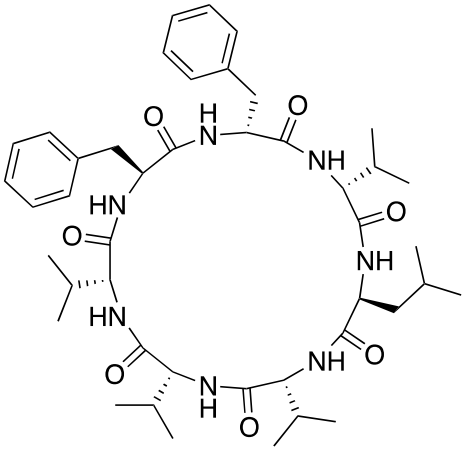
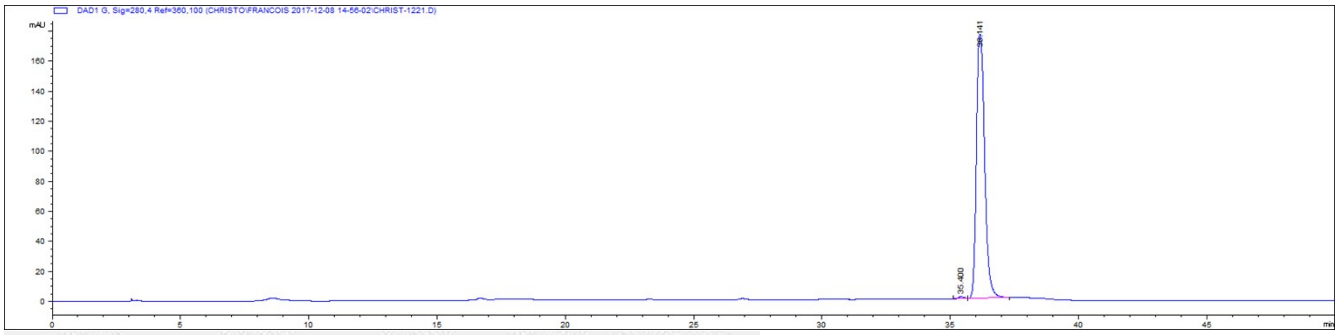


*HPLC spectrum of compound **1***





Area	Height	Width	Area%	Symmetry
1181.8	60.2	0.313	5.435	0.957
20560.6	1005.9	0.3225	94.565	1.019

HRMS-TOF spectrum of Mortiamide A ***(1)***

Sample Name	Mortiamide-A-LC	Position	Vial 1	Instrument Name	Instrument 1	User Name	Claudia Carpentier
Inj Vol	15	InjPosition		SampleType	Sample	IRM Calibration Status	Success
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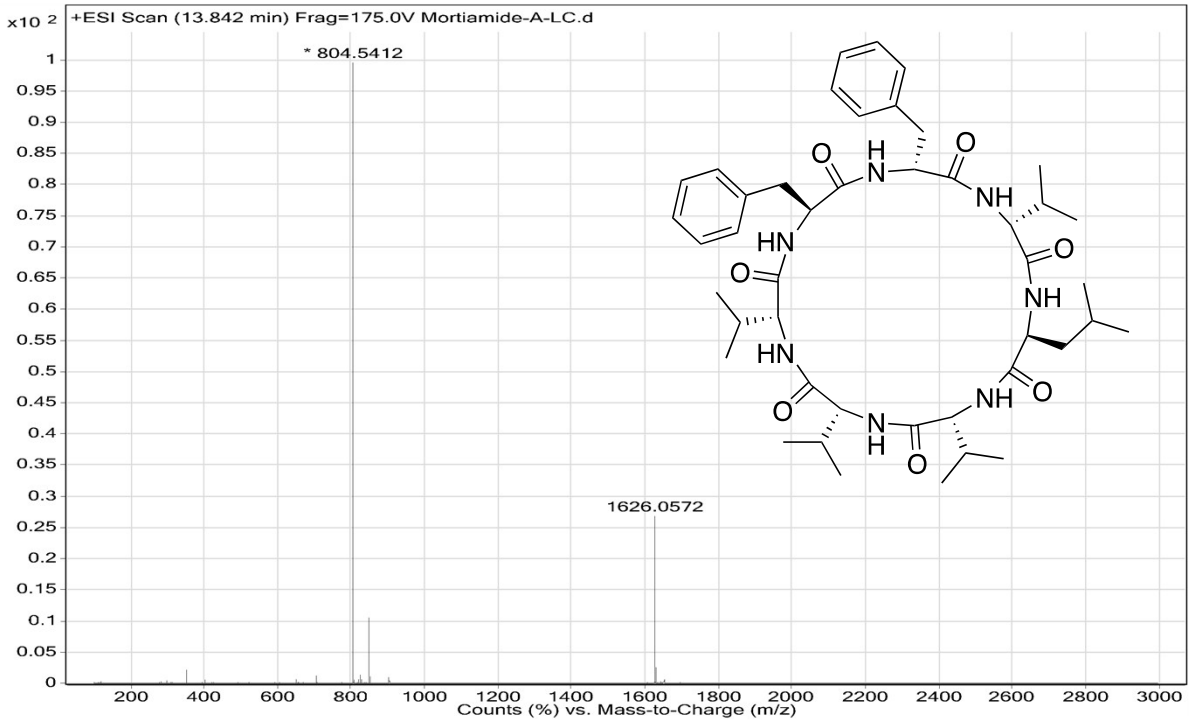
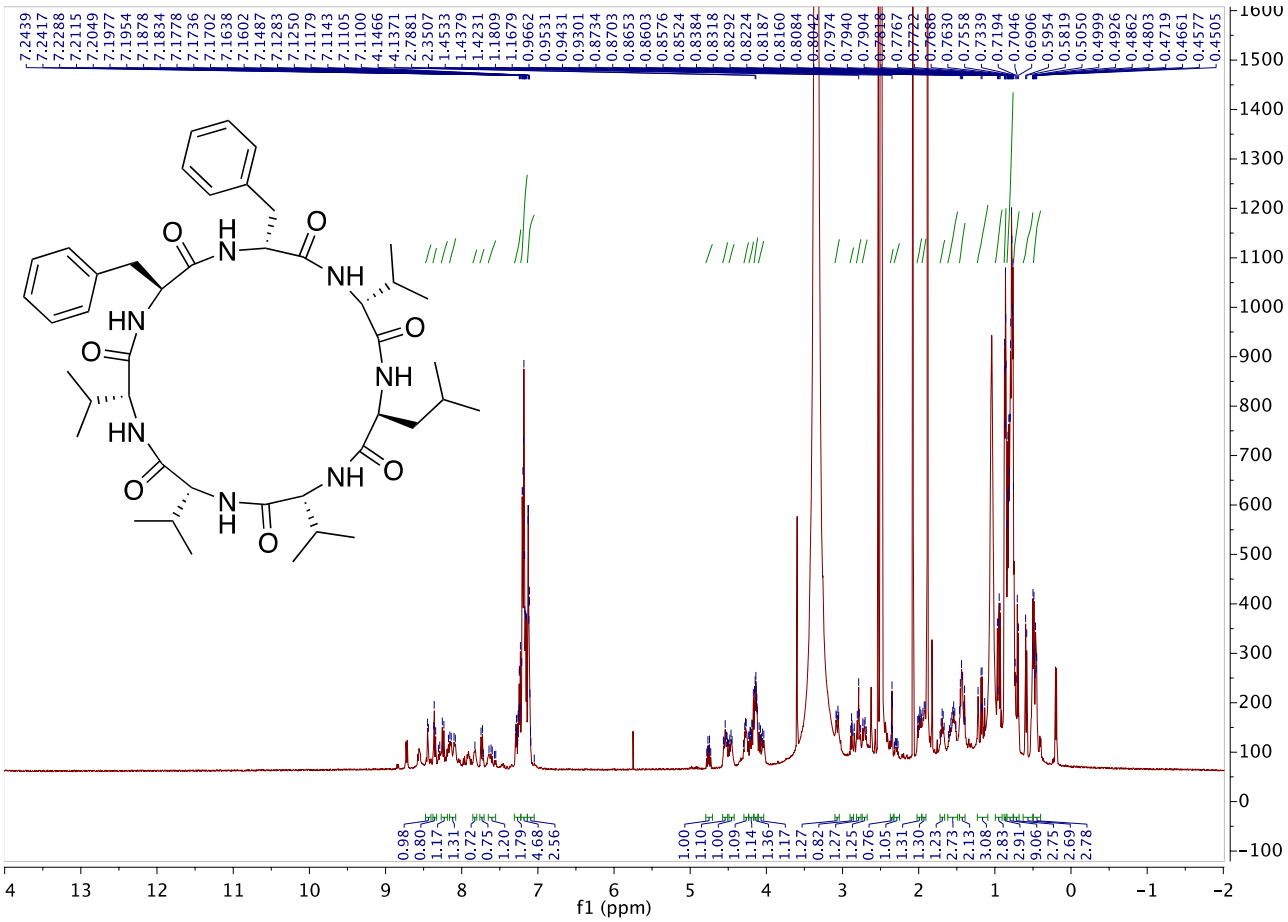
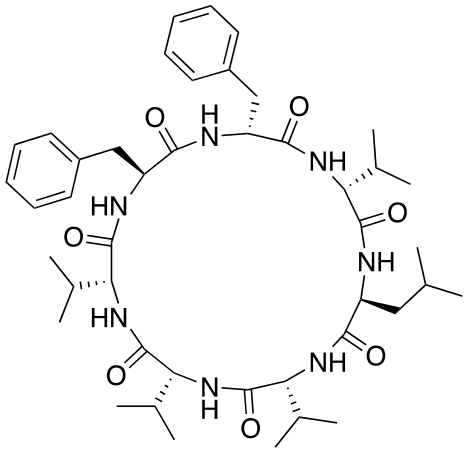
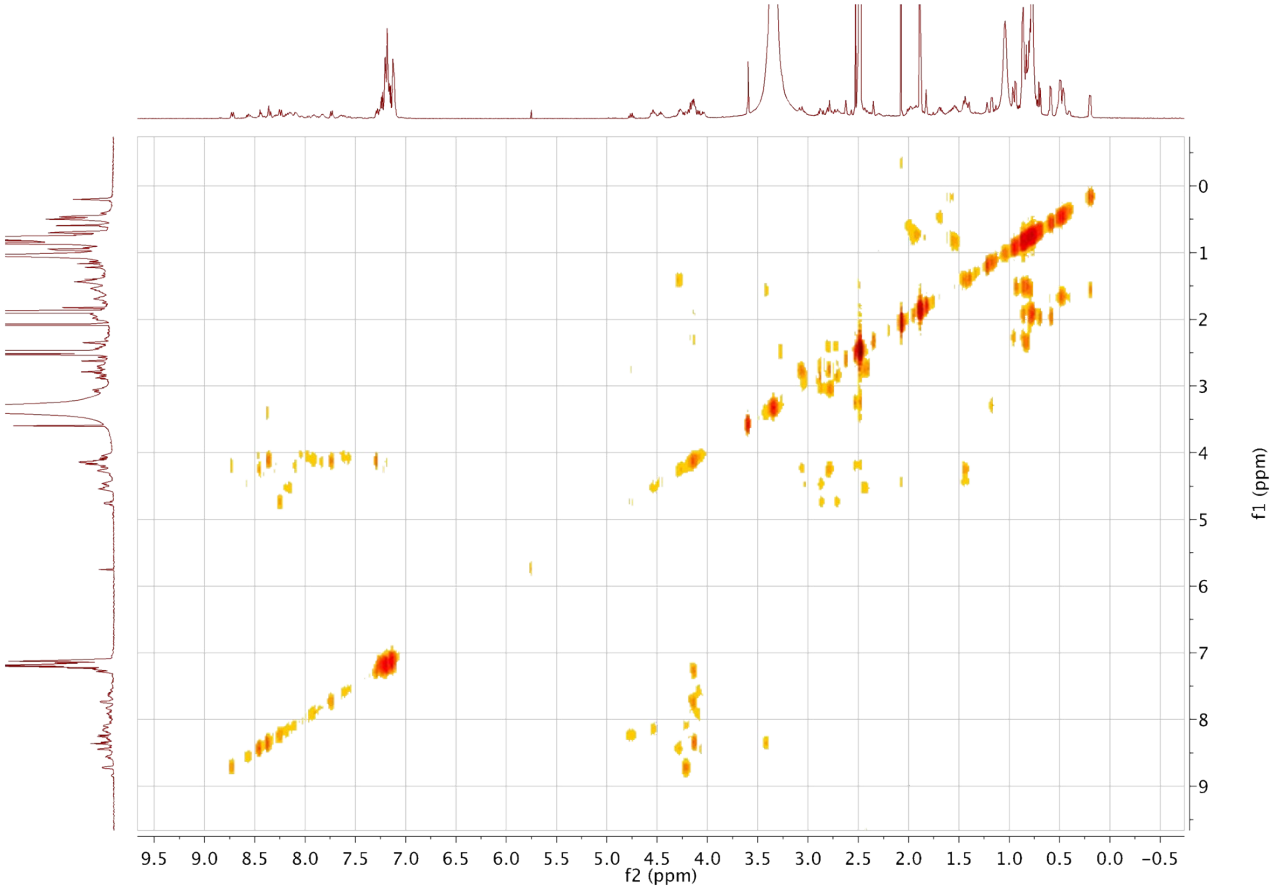


*[1]H-NMR spectrum Mortiamide A **(1)** in DMSO-d_6*

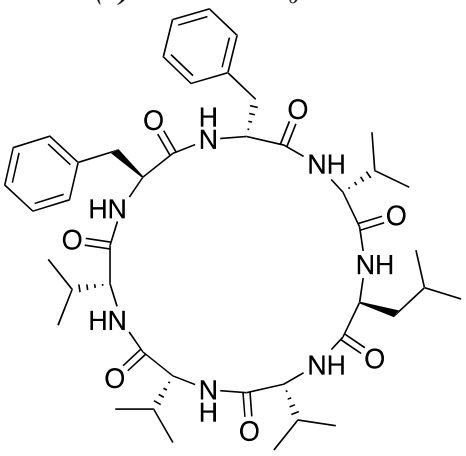


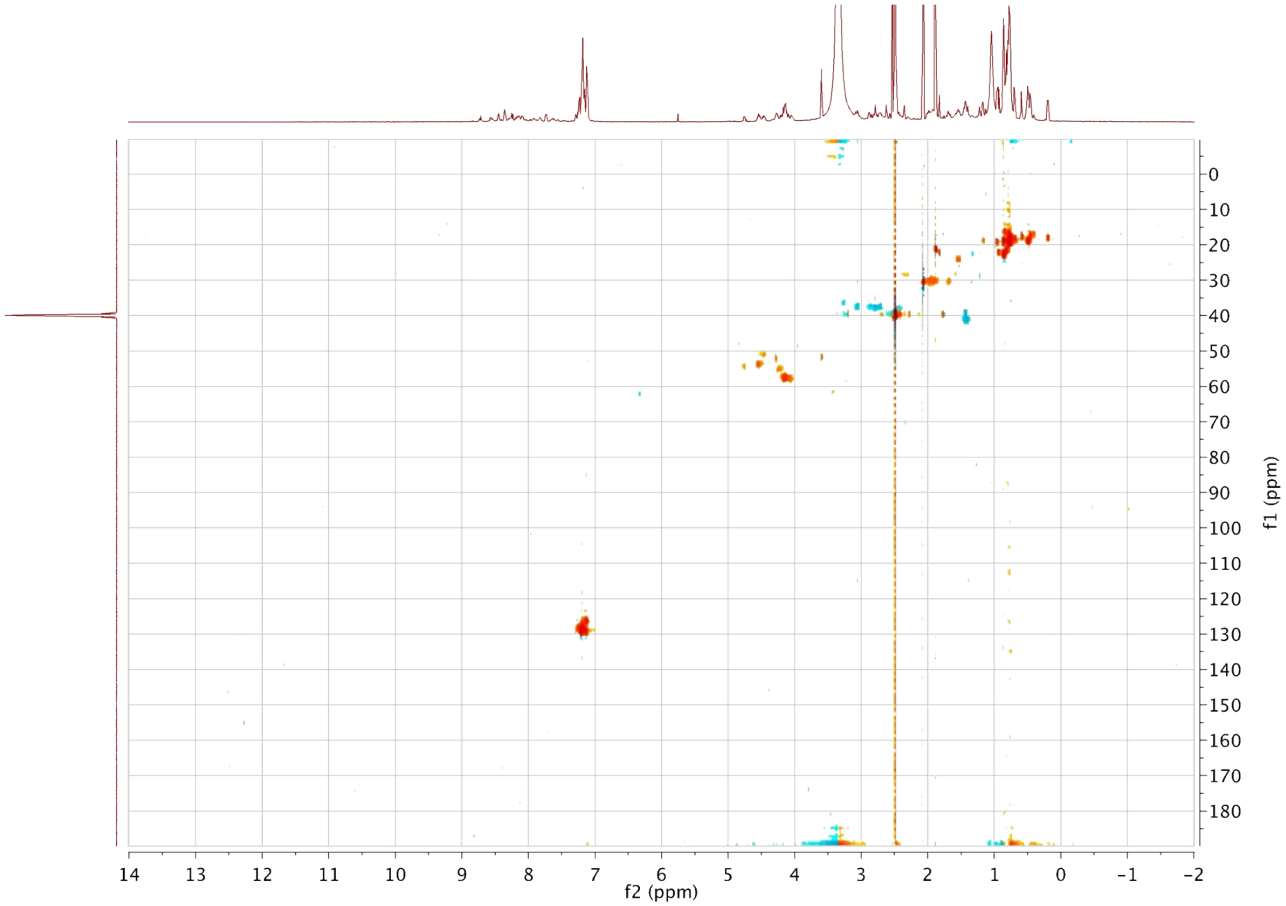
*^{1}H-^{1}H COSY spectrum Mortiamide A **(1)** in DMSO-d_6*



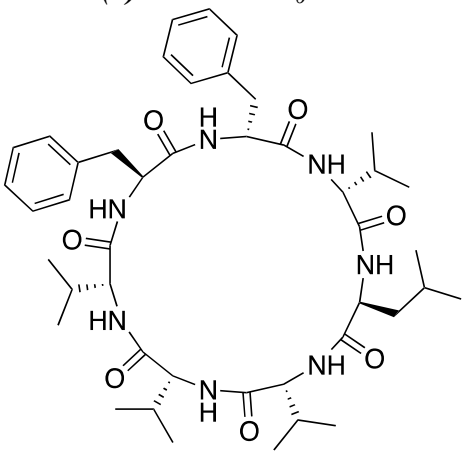


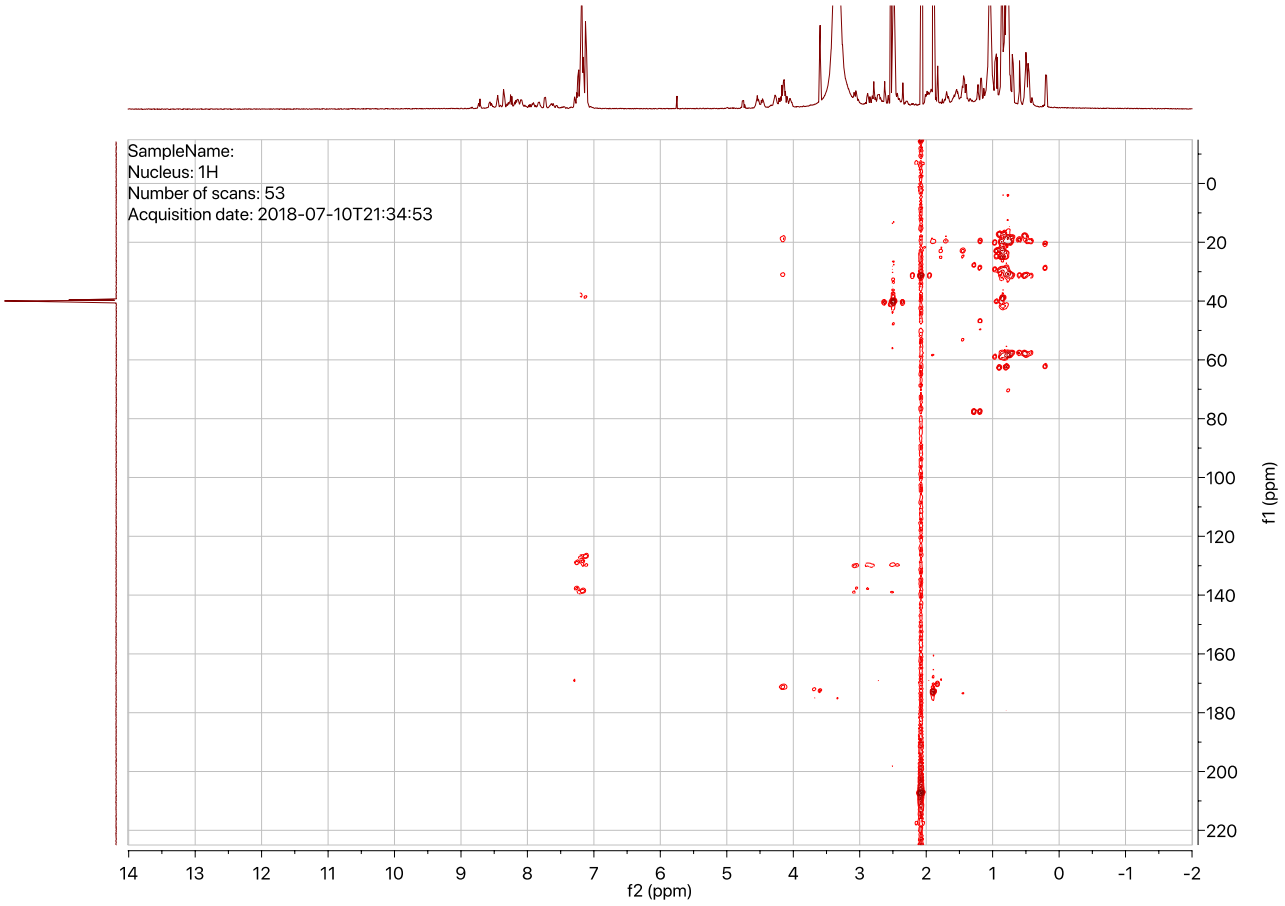
HSQC spectrum of Mortiamide A ***(1)*** *in DMSO-d_6*



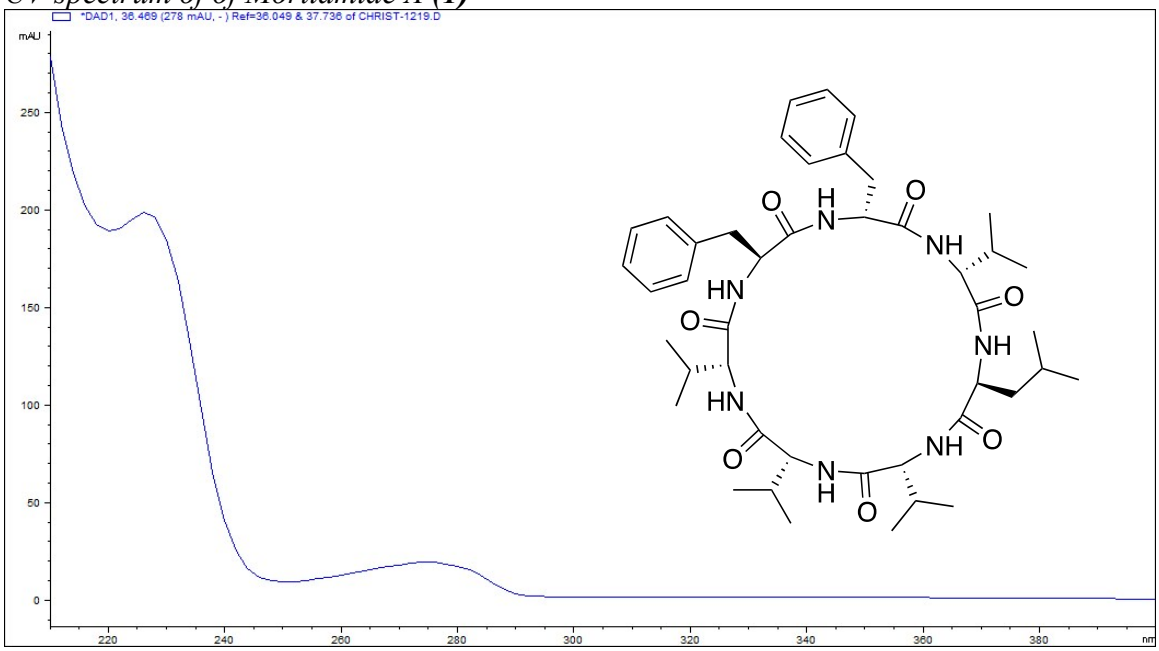


HMBC spectrum of Mortiamide A ***(1)*** *in DMSO-d_6*

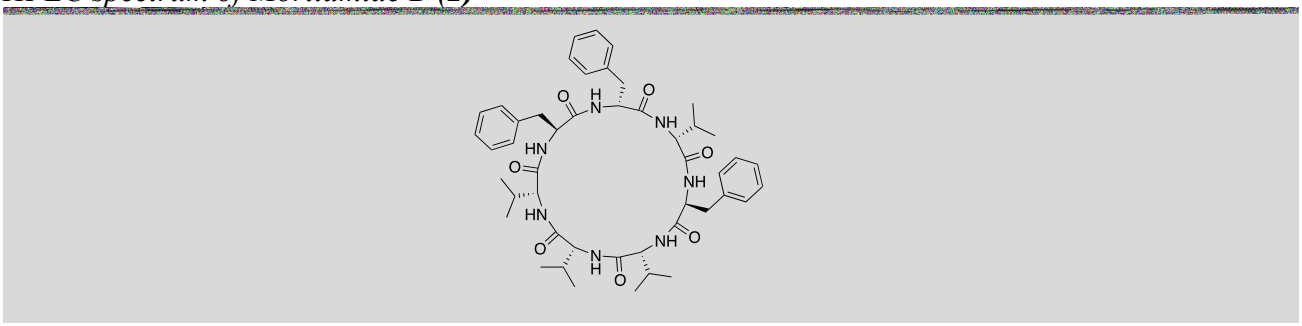




UV spectrum of of Mortiamide A ***(1)***



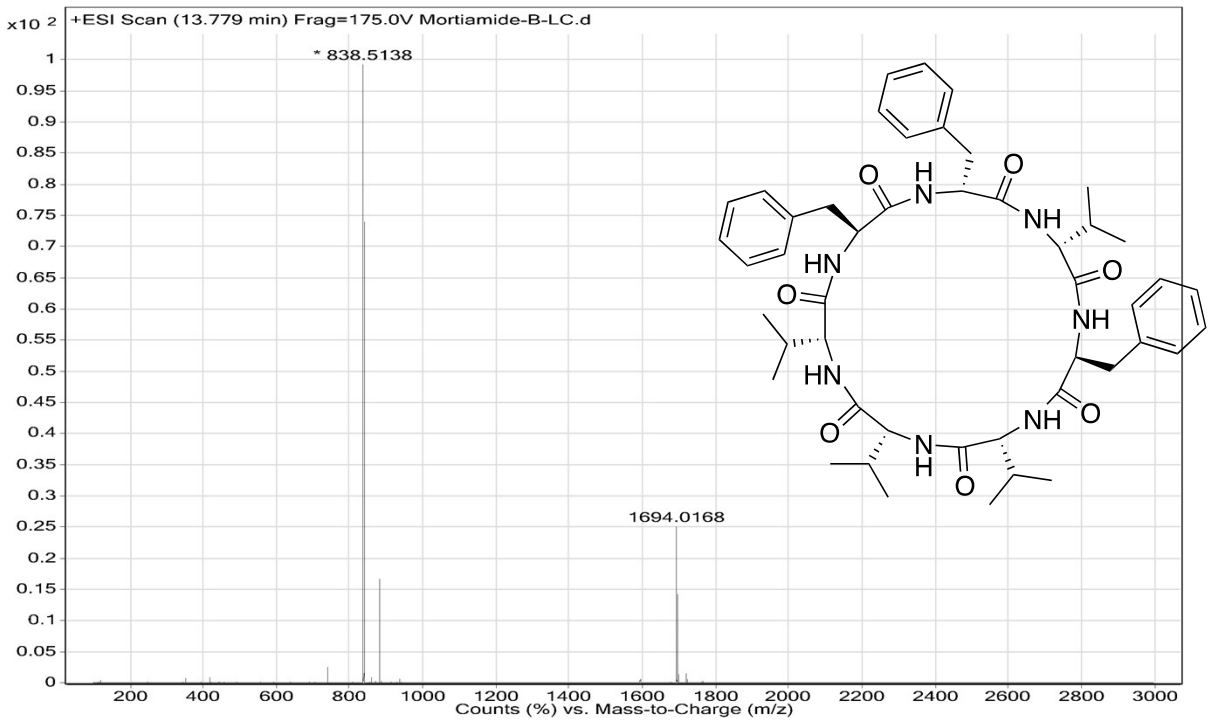
HPLC spectrum of Mortiamide B ***(2)***



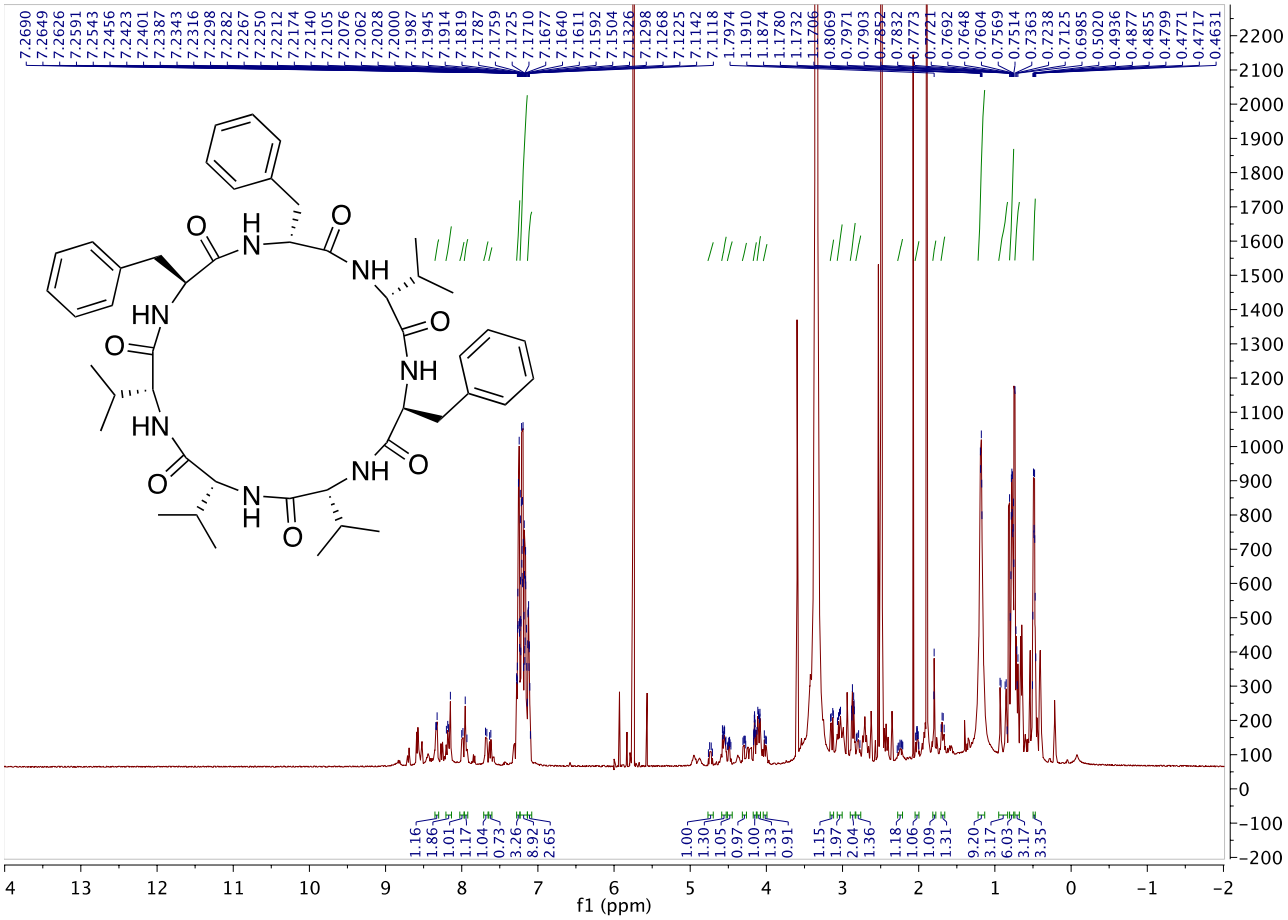
Area	Height	Width	Area%	Symmetry
783.7	44.1	0.2962	4.597	1.683
16264.2	784.8	0.3454	95.403	1.046

HRMS-TOF spectrum of Mortiamide B ***(2)***

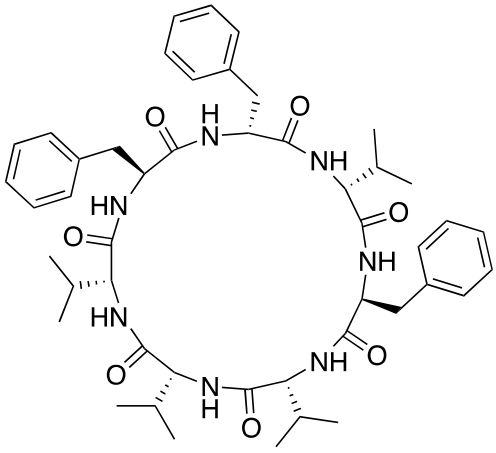
Sample Name	Mortiamide-B-LC	Position	Vial 2	Instrument Name	Instrument 1	User Name	Claudia Carpentier
Inj Vol	15	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	Mortiamide-B-LC.d	ACQ Method	CHRISTO_30A100_20_AC	Comment	Mode positif	Acquired Time	6/27/2018 3:05:05 PM

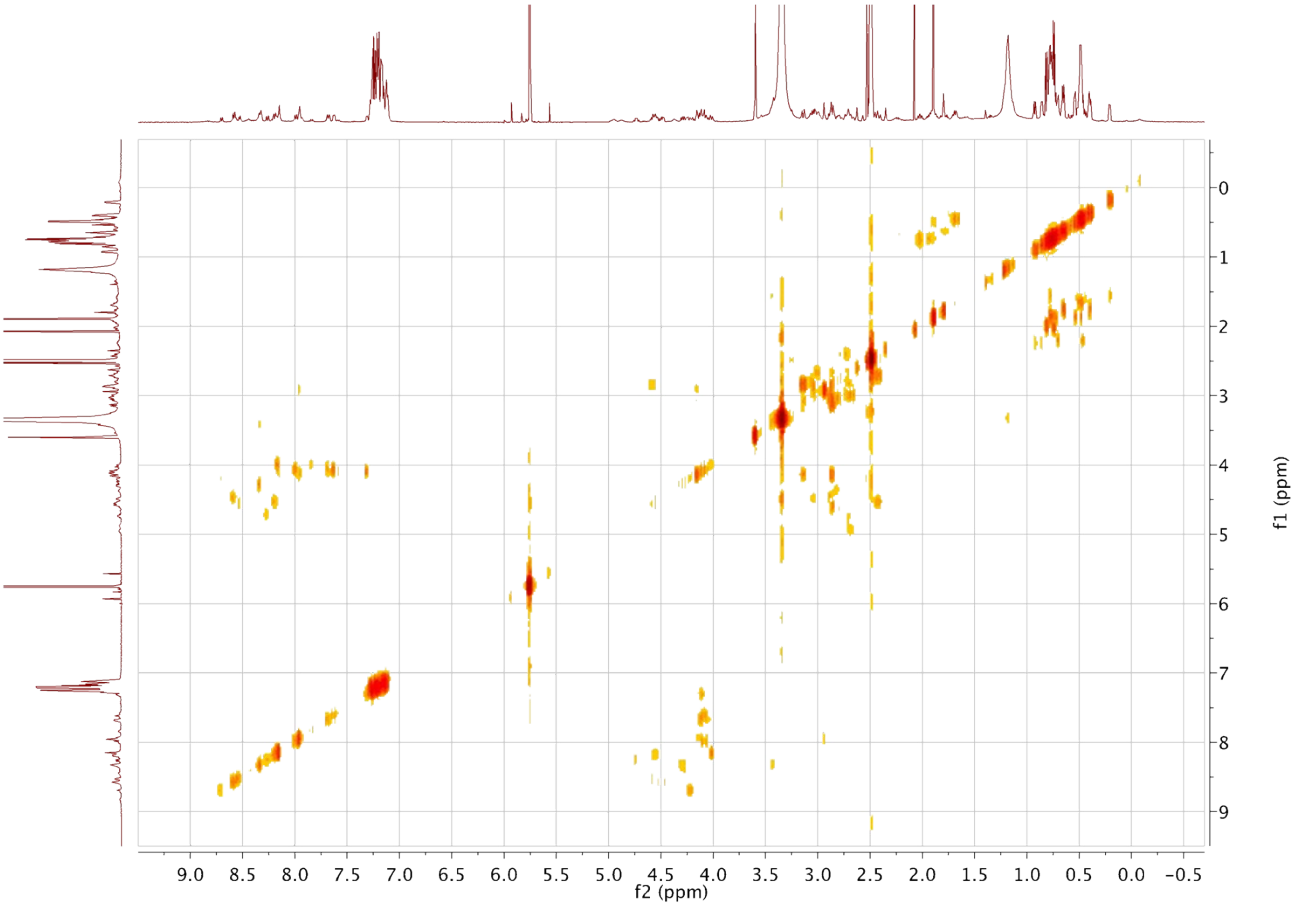


*[1]H-NMR spectrum of compound **2** in DMSO-d_6*

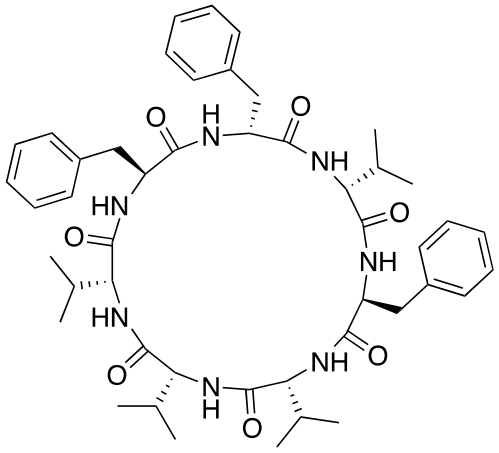


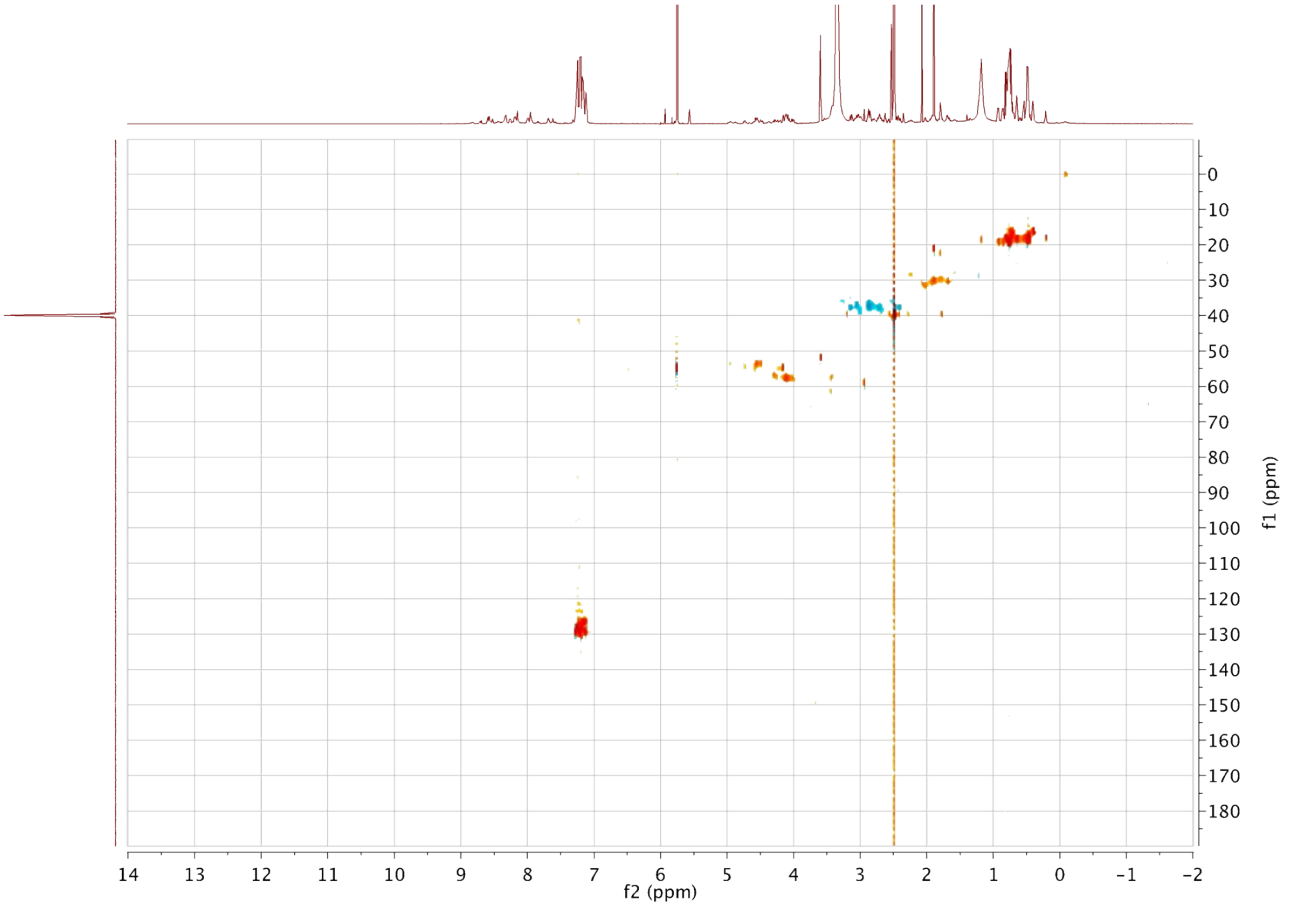
*^{1}H-^{1}H COSY spectrum of Mortiamide B **(2)** in DMSO-d_6*



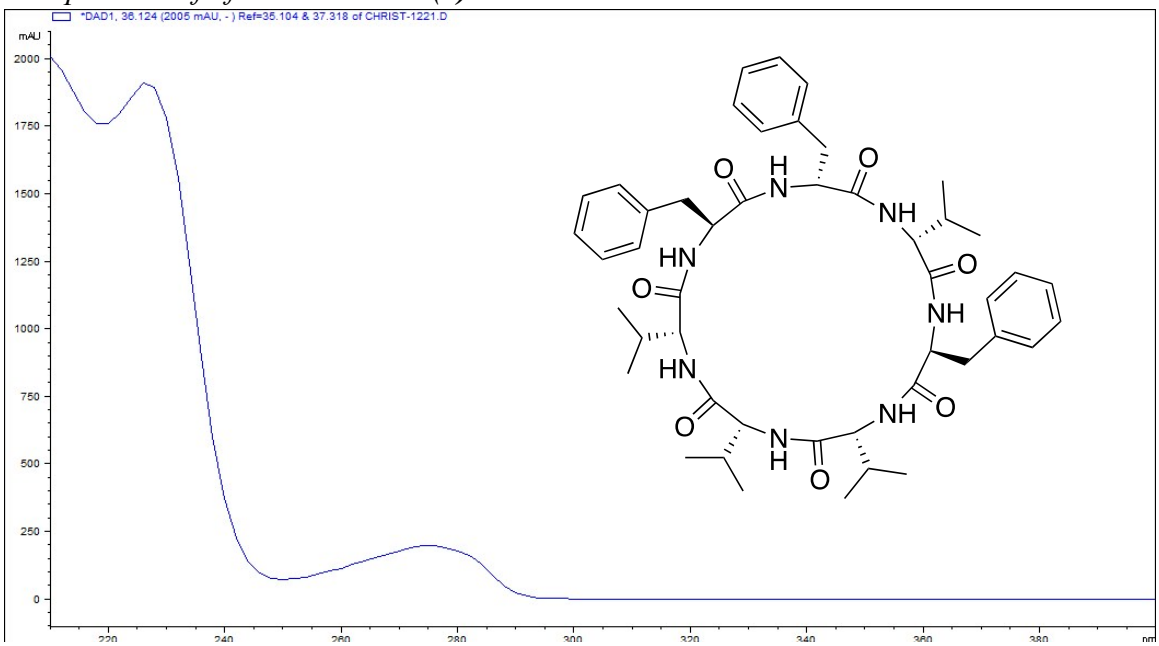


HSQC spectrum of Mortiamide B ***(2)*** *in DMSO-d_6*

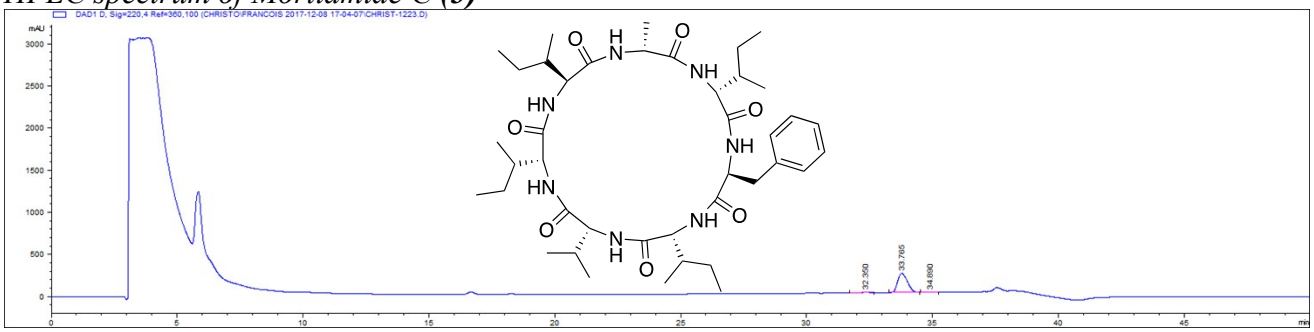




UV spectrum of of Mortiamide B ***(2)***



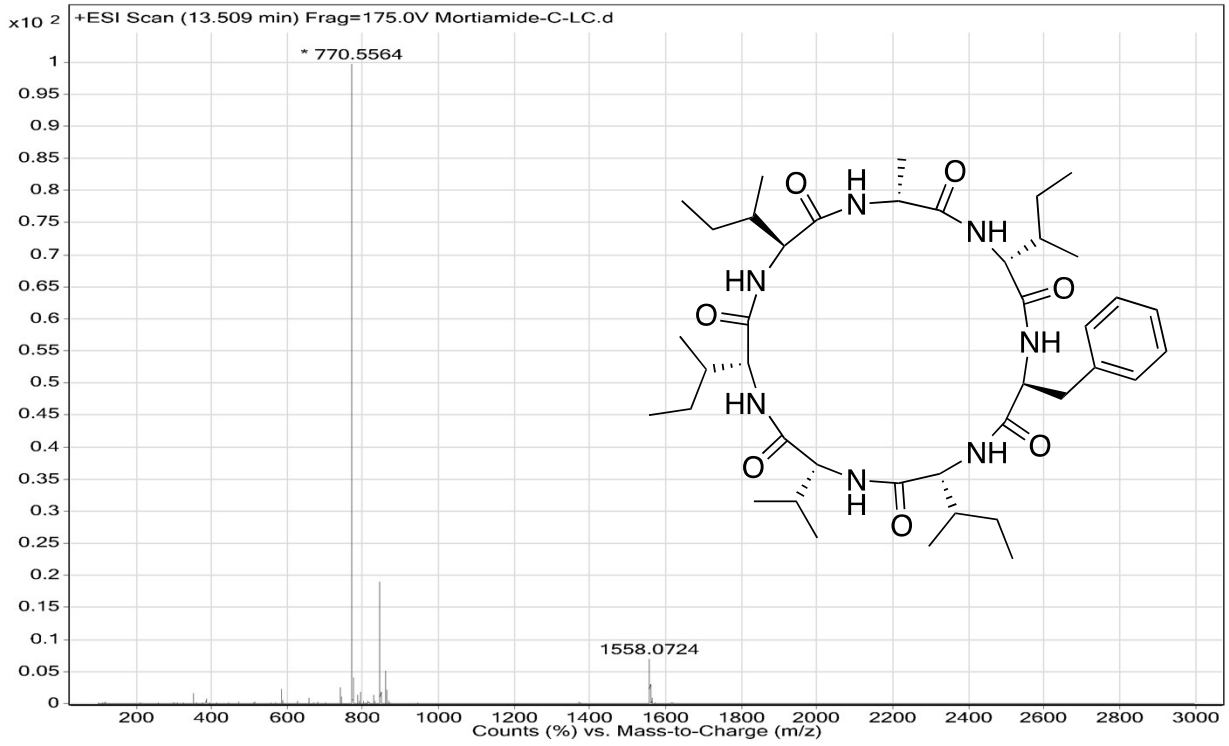
HPLC spectrum of Mortiamide C ***(3)***



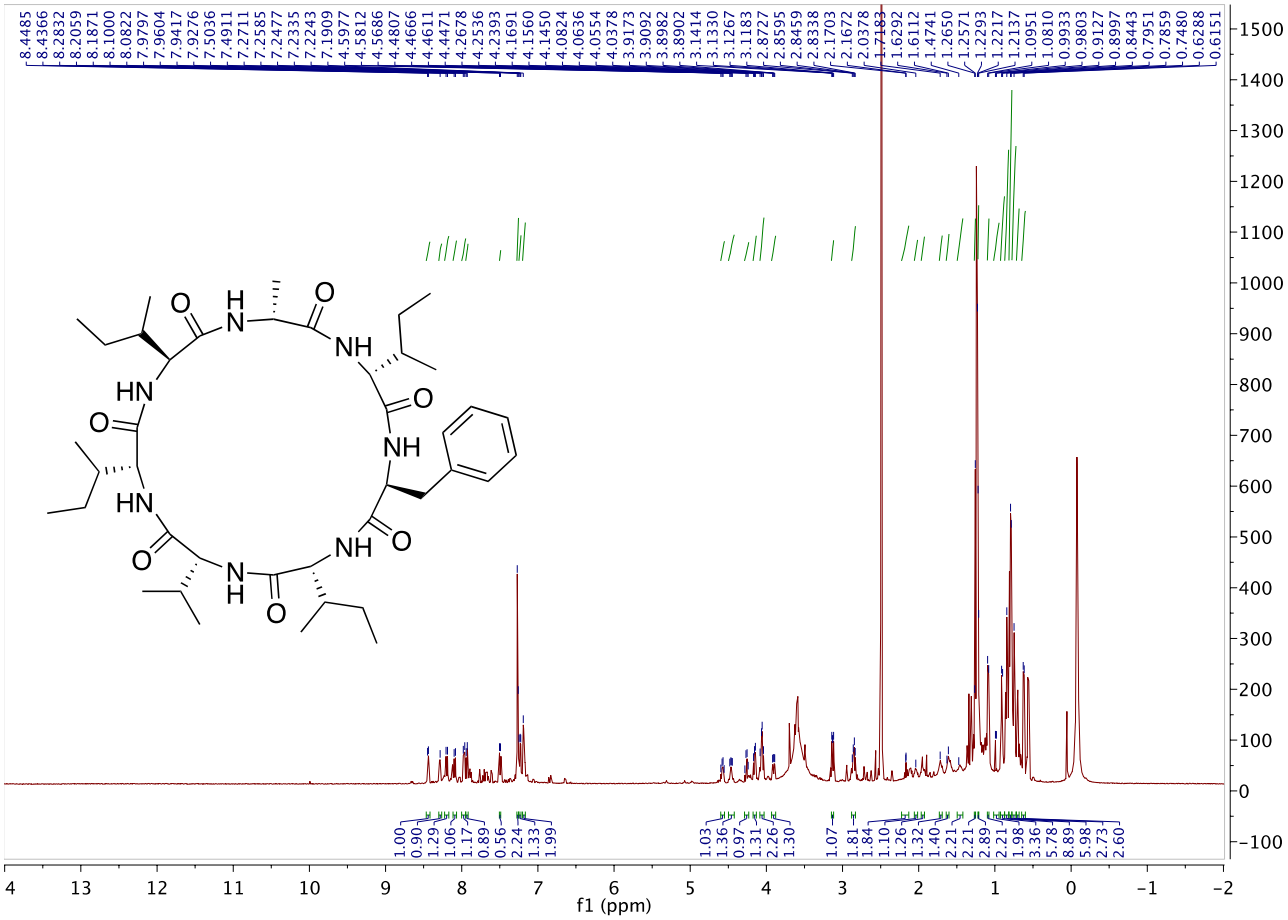
HRMS-TOF spectrum of of Mortiamide C ***(3)***

Area	Height	Width	Area%	Symmetry
968.1	54.3	0.2969	4.385	1.525
21109.5	1017.1	0.3459	95.615	1.052

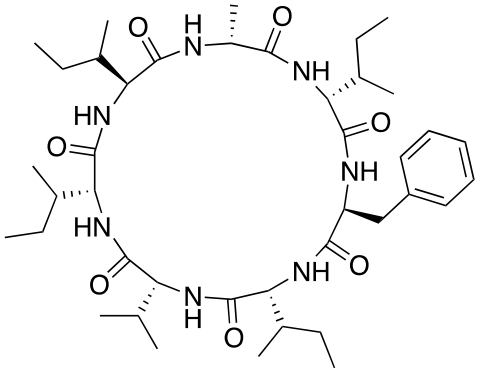
Sample Name	Mortiamide-C-LC	Position	Vial 3	Instrument Name	Instrument 1	User Name	Claudia Carpentier
Inj Vol	15	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	Mortiamide-C-LC.d	ACQ Method	CHRISTO_30A100_20_AC	Comment	Mode positif	Acquired Time	6/27/2018 3:40:59 PM

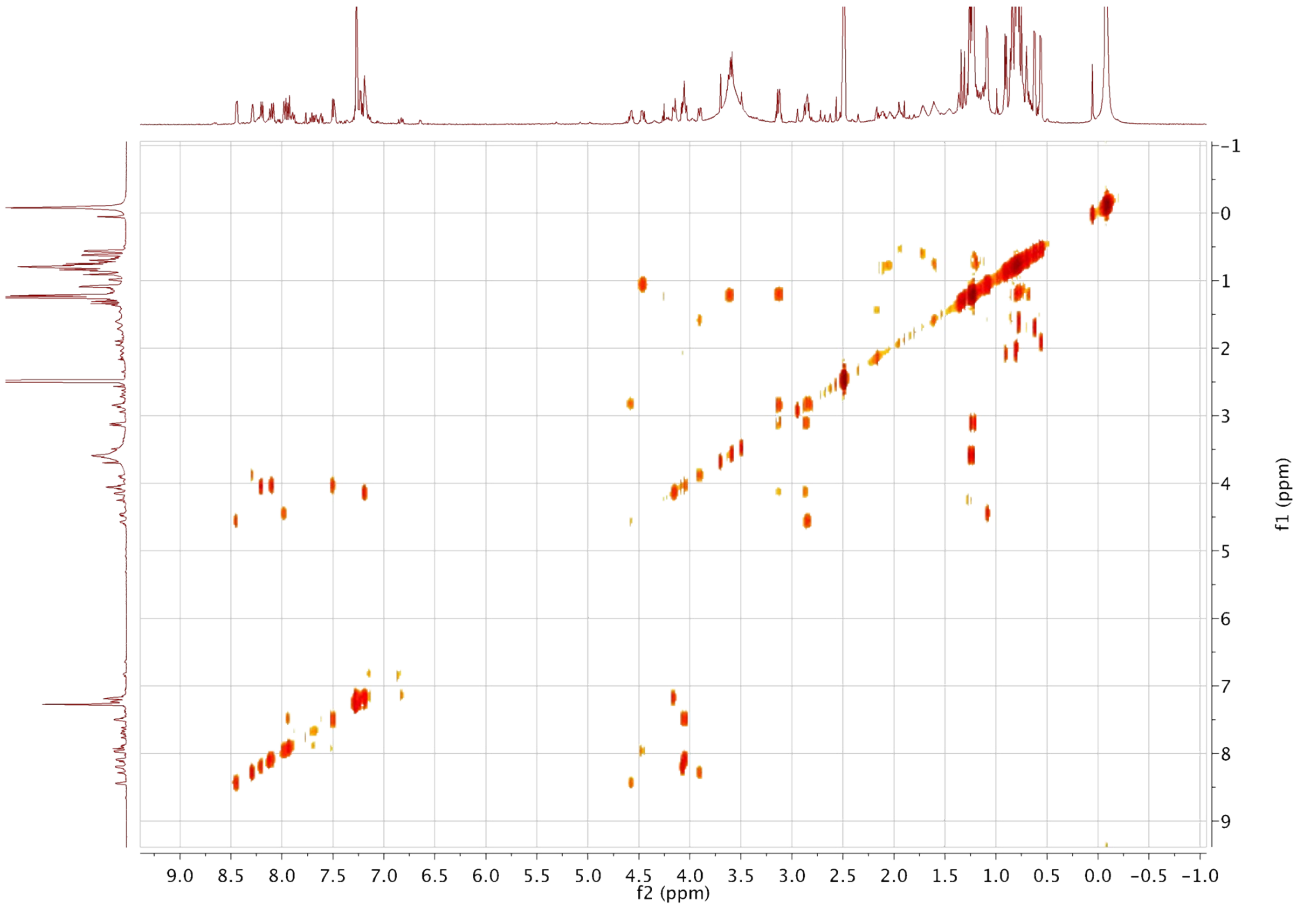


*[1]H-NMR spectrum of of Mortiamide C **(3)** in DMSO-d_6*

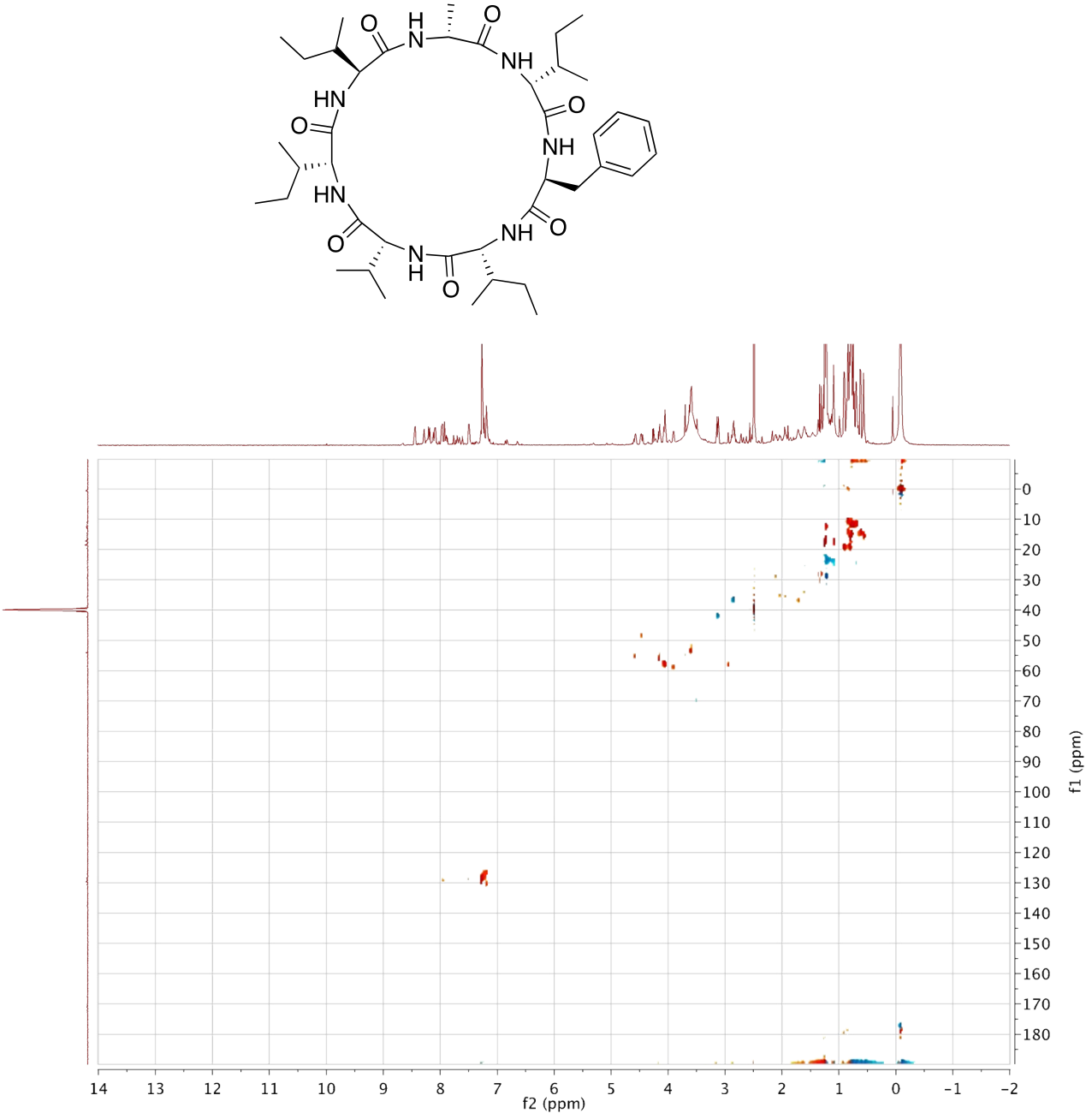


*^{1}H-^{1}H COSY spectrum of Mortiamide C **(3)** in DMSO-d_6*

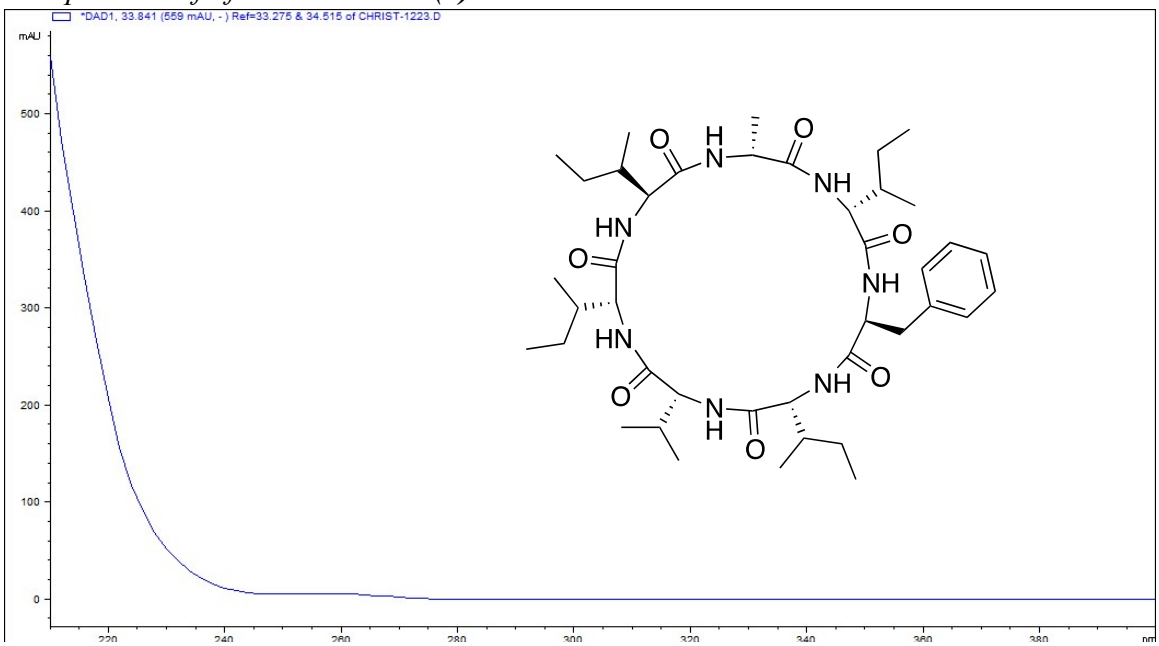




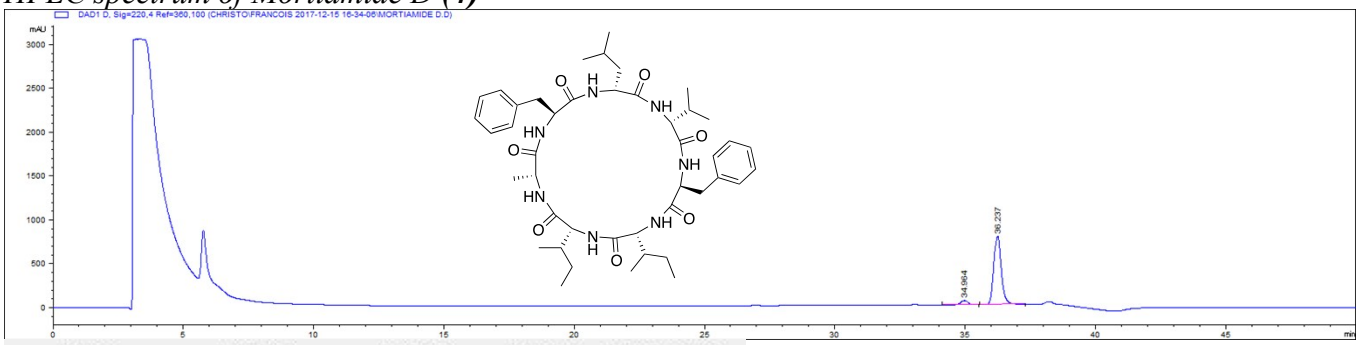
HSQC spectrum of of Mortiamide C ***(3)*** *in DMSO-d_6*



UV spectrum of of Mortiamide C ***(3)***



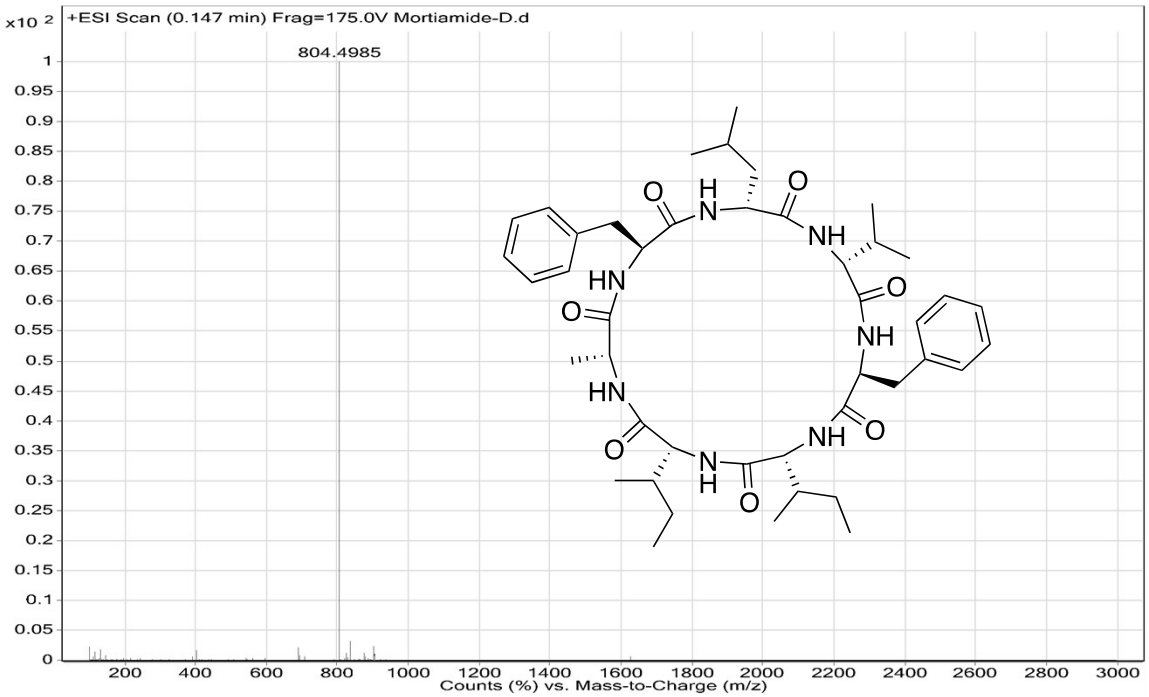
HPLC spectrum of Mortiamide D ***(4)***



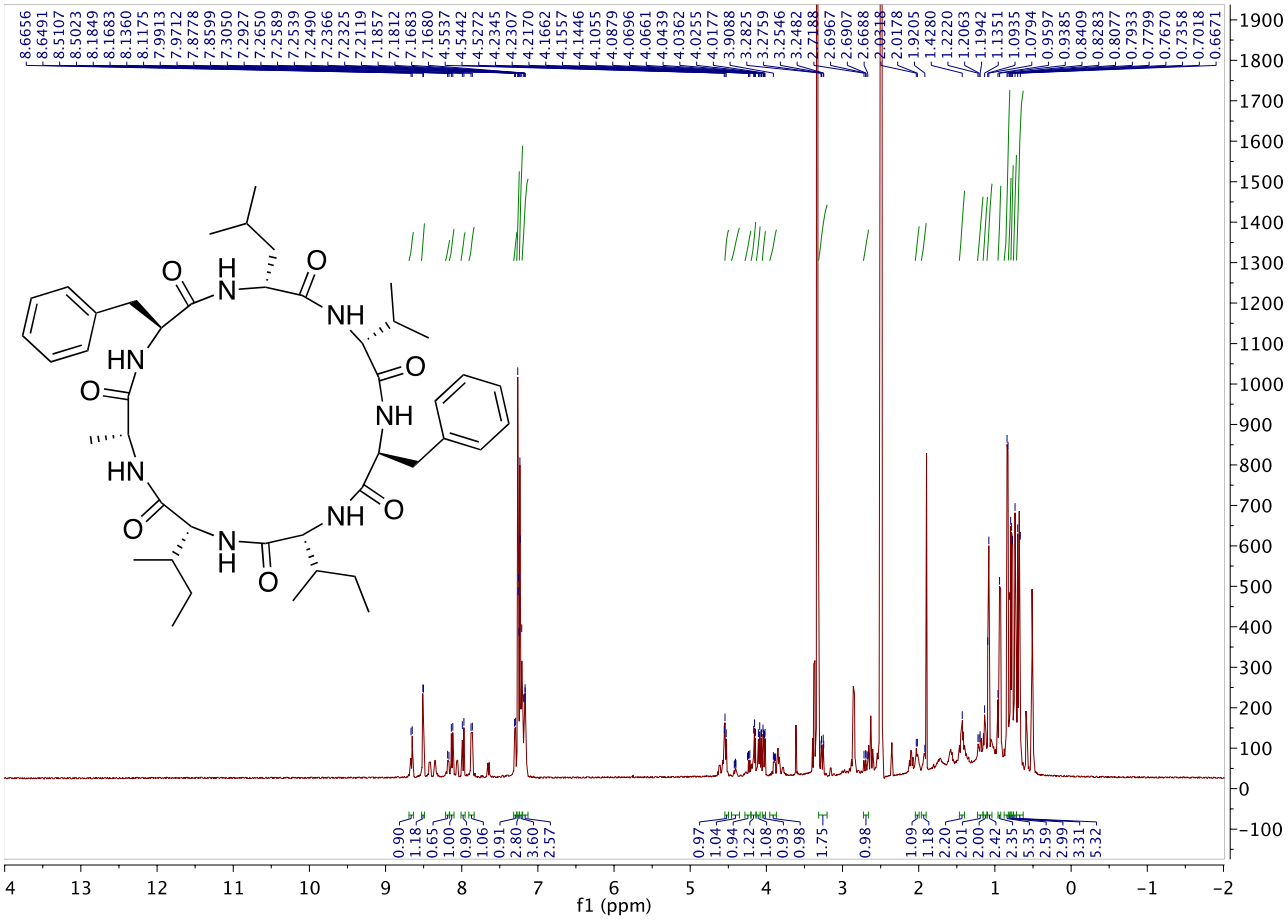
Area	Height	Width	Area%	Symmetry
935.2	47.6	0.3114	5.545	1.171
15930.4	777.3	0.3231	94.455	1.006

HRMS-TOF spectrum of Mortiamide D ***(4)***

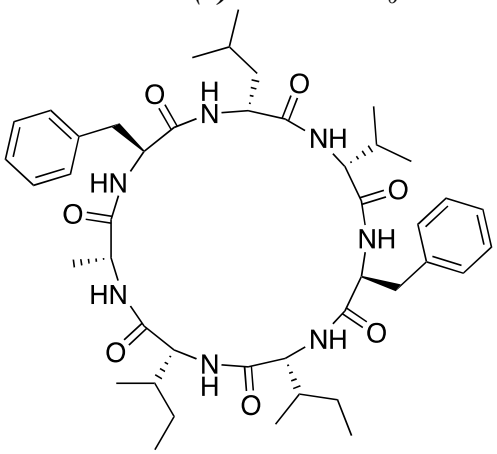
Sample Name	Mortiamide-D	Position	Vial 4	Instrument Name	Instrument 1	User Name	Pierre Audet
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	Mortiamide-D.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	12/13/2017 12:23:19 PM



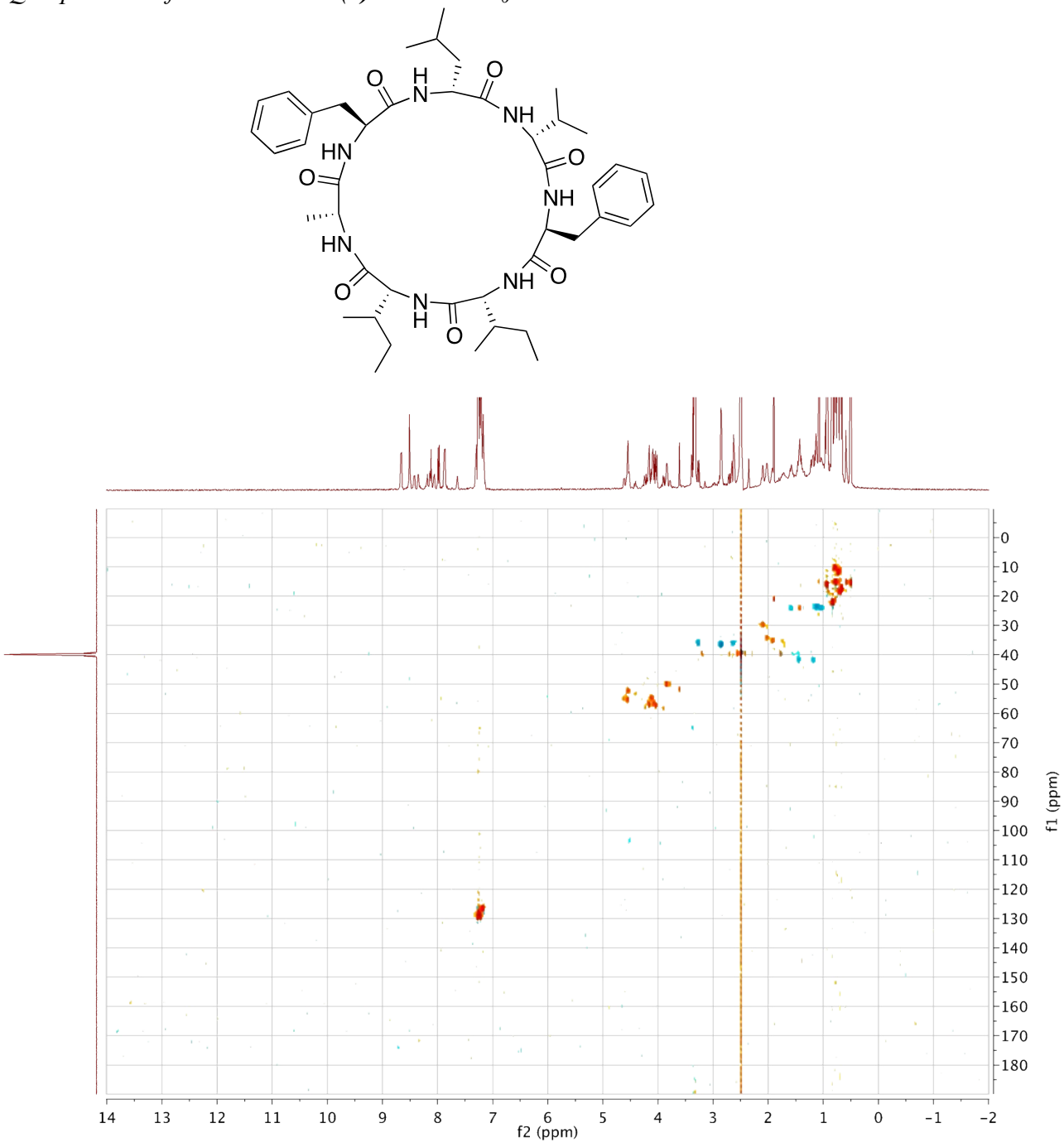
^{1}H-NMR spectrum of Mortiamide D ***(4)*** *in DMSO-d_6*



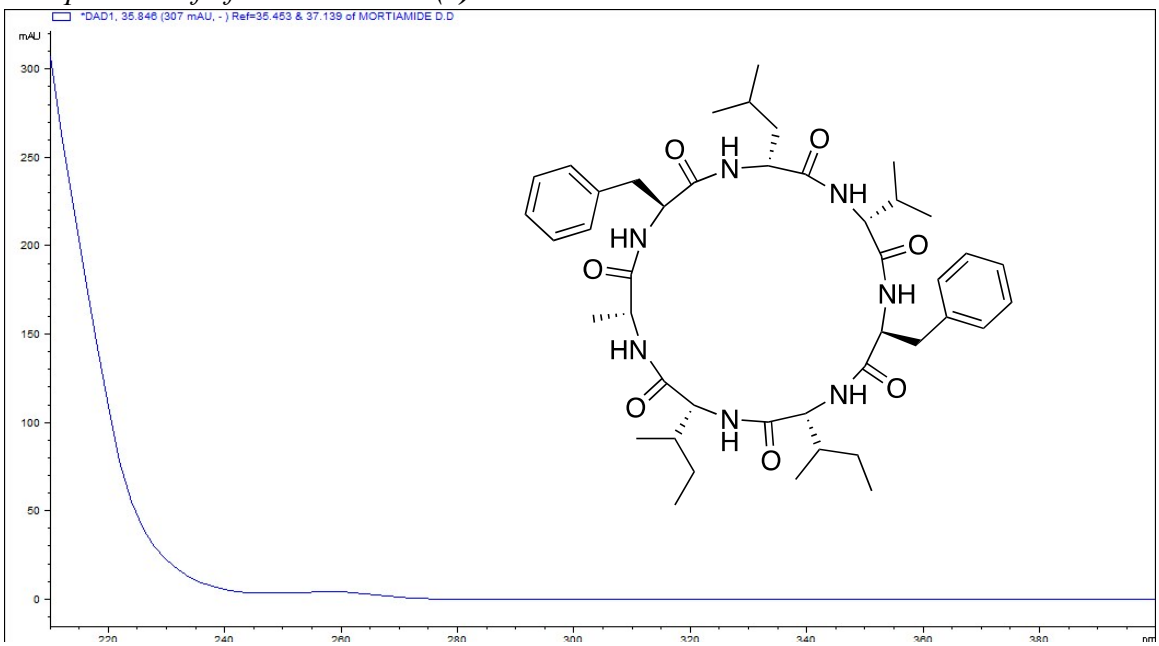
*^{1}H-^{1}H COSY spectrum of Mortiamide D **(4)** in DMSO-d_6*



HSQC spectrum of Mortiamide D ***(4)*** *in DMSO-d_6*



UV spectrum of of Mortiamide D ***(4)***



References

1. C. Lambros and J. P. Vanderberg, *J. Parasitol.*, 1979, **65**, 418.
2. J. D. Johnson, R. A. Dennull, L. Gerena, M. Lopez-Sanchez, N. E. Roncal and N. C. Waters, *Antimicrob. Agents Chemother.* , 2007, **51**, 1926.