Supporting information

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1. General experimental information

All reactions were carried out under nitrogen atmosphere with dry, freshly distilled solvents, under anhydrous conditions, unless otherwise stated. All solvents were purified following procedures described in literature. [1]

All yields refer to chromatographically and spectroscopically (^{1}H-NMR and ^{13}C-NMR) pure products.

NMR spectra were recorded with a Bruker Advance NEO-400, Bruker Avance III 500 MHz or Bruker Advance III 400 MHz equipment. All chemical shifts were related to TMS as internal reference.

All solid phase reactions were monitored by colorimetric test (Kaiser or Chloranil). 2-Chlorotrityl chloride resin (2-CTC, 100-200 mesh, 1.1 mEq/g) was acquired from CHEM-IMPEX INT'L INC.

HPLC analyses were carried out in a Shimadzu (LC-10AT Pump) HPLC equipped with an SPD20Aprominence UV/Vis detector, Phenomenex Kinetex C18 (4.6x150mm, 5µm) column and H_2O: CH_3CN with 0.1 % formic acid. All mass spectra were acquired with a Shimadzu 8040 HPLC-MS-MS equipment, with LC-20AD pumps, a SIL-20A autosampler, electrospray ionization and triple quadrupole mass detector.

[1] Perrin, D. D. ; Armarego, W. L. F. "Purification of Laboratory Chemicals", 3th Ed. Pergamon Press, Oxford, 1988.

2. Solid Phase Peptide Synthesis and Solution Phase Macrocyclization

2.1. Resin loading

The 2-Chlorotrityl chloride resin (2-CTC) ((100-300 mesh, 1.20 mmol/g) were added to a syringe peptide synthesis vessel. The resin was swelled in CH_2Cl_2 (3 x 5 min).

A solution of first protected amino acid Fmoc-AA-OH (1 eq. for 0.8 mmol/g loading) and DIPEA (3 eq.) in CH_2Cl_2 was added and the resin was shaken 10 minutes. Then, an extra 7.0 eq. of DIPEA were added and shaking was continued for 50 min. MeOH (0.8 mL/ g of resin) was added to the previous mixture in order to cap unreacted functional groups on the resin, and shaken for 10 min. After filtering, the resin was washed with CH_2Cl_2 (x3), MeOH (x3), CH_2Cl_2 (x3), DMF (x3).

2.2. Removal of NHFmoc group

The resin was washed with DMF (x3) and Fmoc protecting group was removed by treating the resin with piperidine-DMF solution (1:4) for 1, 5 and 5 minutes successively. In exceptional cases deprotection step was accomplish by a single treatment with piperidine-DMF solution for 5 minutes, in order to prevent side reactions.

2.3 Coupling of subsequent N-Fmoc protected amino acids to primary or secondary amines

After removal of NHFmoc protecting group as previously described, the resin was washed with DMF (x3), CH_2Cl_2 (x3) and DMF (x3). A solution of Fmoc-AA-OH (3 eq.) and DIPEA (6 eq.) in DMF was added to the resin, followed by a solution of HBTU, for coupling to primary amines, or HATU (2.9 eq.) in DMF, in case of coupling to an N methylated amino acid. The mixture was stirred for 60 min. After the coupling was completed, the resin was washed with DMF (×3) and CH_2Cl_2 (x3). Deprotection and coupling cycles were repeated with the appropriate amino acids to provide the desired compound. Completion of the coupling was monitored by colorimetric assays; Kaiser test in case of primary amines and Chloranil test for secondary amines. Coupling procedure was repeated in case of positive results.

2.4 Coupling of subsequent N-Fmoc protected amino acids to Anthranilic acid

After removal of NHFmoc protecting group as previously described, the resin was washed with DMF (x3), CH_2Cl_2 (x3) and DMF (x3). A solution of Fmoc-AA-OH (5 Eq.), Oxyma Pure (5 Eq.), and DIC (5 Eq.) was added to the vessel. The mixture was stirred for 60 min. Then, the resin was washed with DMF (×3) and CH_2Cl_2 (x3).

2.5 Cleavage

Unless otherwise stated, the peptide was cleaved from the resin by treatment with 1% TFA in CH_2Cl_2 for 2-3 minutes at room temperature followed by filtration and collection of the filtrate in MeOH. The treatment was repeated three times and then the resin washed with CH_2Cl_2 (x5) and MeOH (x3). Solvents were removed *in vacuo* to obtain the crude peptide. LC-MS was used to identify the desired product.

2.6 General procedure for macrocyclization in solution phase.

2.6.1. Method I

Macrocyclization reaction of the corresponding linear peptide was performed in diluted conditions (1-5 mM) using HBTU or HATU (1.5 eq.), DIPEA (3 eq.), 4-DMAP (catalytic) in dried CH_2Cl_2 at room temperature during 1-3 days. The reaction mixture was washed with HCl 5% and then with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography to obtain the pure macrocycle.

2.6.2. Method II

The trifluoroacetate salt of the corresponding linear peptide was dissolved in dried CH_2Cl_2 and diluted to a concentration of 1-5 mM. DIPEA (1eq.) was added to enable dissolution. EDCI (1.2 eq) and oxyma (1.2 eq.) were added at 0ºC and the reaction mixture was stirred for 10 minutes. Then, the reaction mixture is allowed to reach room temperature and stirred for 48 hours. The reaction mixture was washed with HCl 5% and then with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography to obtain the pure macrocycle.

3. Solution phase synthesis

3.1 General procedure for coupling reaction

3.1.1. Method I

EDC.HCl (1.5 eq.), Cl-HOBt (1.5 eq.) and DIPEA (2.0 eq.) were added to a solution of Boc protected amino acid (Boc-AA-OH, 1.0 eq.) in DCM at 0ºC under N_2 atmosphere. N-terminus deprotected linear peptide or amino acid ester (NH_2-AA-COOEt) was added and the reaction mixture was stirred at $0^{\circ}C$ for 10 min and then at room temperature, overnight.

DCM was removed under vacuum and AcOEt was added. The organic phase was washed with 0.1 M HCl aqueous solution (30 mL × 2), brine (10 mL), saturated $NaHCO_3$ solution (30 mL × 2) and brine (10 mL), dried over MgSO4 and filtered. The solvent was removed under vacuum to give the crude material. The crude material was purified by flash chromatography.

3.1.2. Method II

Bis(trichloromethyl)carbonate (0.33 eq.) was added to a solution of Boc-Ala-OH (1 eq.) in dry THF under N_2 atmosphere at 0ºC. 2,4,6-colidine (2.6 eq.) were added to the solution and a white suspension was formed. The reaction mixture was stirred for 5 minutes and the suspension was added to a solution of N-terminus deprotected linear peptide or amino acid

ester (NH_2-AA-COOEt) (1 eq.) in dry THF, followed by DIPEA (1 eq). The reaction mixture was stirred overnight and concentrated under vacuum. 30 mL of AcOEt were added and the organic fase was washed with HCL 5% (3 x 5mL), brine (3 x 5mL) and $NaHCO_3$ (3 x 5mL), dried with Na_2SO_4, filtered and concentrated. The crude material was purified by flash chromatography.

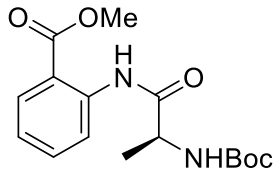
3.2. Ester hydrolysis

1 eq. of the ester was dissolved in THF and 3 eq. of de LiOH dissolved in water. The reaction was stirred for two hours at room temperature. THF was removed under vacuum. HCl 5% was added to the remaining solution to a pH of 3. The solution was extracted with AcOEt. The organic layers were dried with Na_2SO_4, filtered, and the solvent removed under vacuum.

3.3. Boc deprotection procedure

A threefold excess of a solution of 1.8M HCl in dioxane was added to the Boc-aminoacid. The solution was stirred at room temperature for an hour and the solvent removed under vacuum. The obtained hydrochloride derivative was used in the next step without further purification.

3. Characterization Data of Products



5

Compound **5** was prepared by solution phase synthesis, either by coupling metod I or II.

For **method I**. Boc protected alanine (Boc-Ala-OH, 378.4 mg, 2 mmol was added to a solution of Cl-HOBt (508.7 mg, 3 mmol) and NMM (220 µL, 2 mmol) in 3 mL DCM at 0ºC under N_2 atmosphere. The resulting mixture was stirred at 0ºC for 10 min and then methyl anthranilate (NH_2-Anth-OMe, 906 mg, 6.0 mmol and EDC.HCl (575 mg, 3.0 mmol) were added in one portion. The reaction mixture was allowed to warm to room temperature and was stirred at room temperature overnight. The reaction mixture was diluted with 30 mL of DCM, and the organic phase was washed with 0.2 M HCl (3x15 mL), brine (10 mL), and saturated $NaHCO_3$ solution, dried over Na_2SO_4 and filtered.

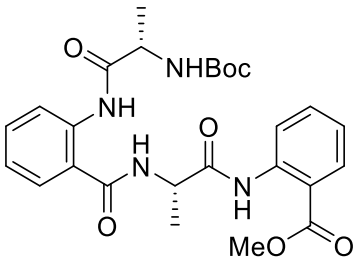
The solvent was removed under vacuum and the crude material was further purified by flash chromatography (AcOEt:hexane, 1:4) to give pure Boc-Ala-Anth-OMe (270.7 mg, 0.84 mmol) in 42% yield.

Method II

Bis(trichloromethyl)carbonate (74 mg, 0.25 mmol) was added to a solution of Boc-Ala-OH (140.0 mg, 0.74 mmol) in dry THF (2.5 mL) under N_2 atmosphere at 0ºC. 2,4,6-colidine (250 µL, 1.86 mmoles) were added to the solution. The reaction mixture was stirred for 5 minutes and the suspension was added to a solution of methyl anthranilate (85 µL, 0.62 mmol) in 2 mL dry THF, followed by DIPEA (128 µL, 0.74 mmol). The reaction mixture was stirred overnight and concentrated under vacuum. 30 mL of AcOEt were added and the organic phasewas washed with HCL 5% (3 x 5mL), brine (3 x 5mL) and $NaHCO_3$ (3 x 5mL),

dried with Na_2SO_4, filtered and concentrated. The crude material was purified by flash chromatography (AcOEt:EP, 1:4) to give pure Boc-Ala-Anth-OMe, 51% yield (122 mg, 0.38 mmol).

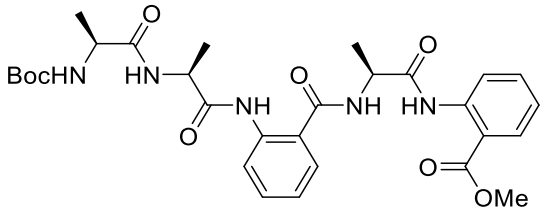
BocNH-Ala-Anth-OMe(5). White solid. Rf=0.43 (AcOEt: EP, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.34–1.74 (m, 12 H), 3.92 (s, 3H), 4.40 (s, 1 H), 5.15 (s, 1 H), 7.10 (t, *J*=7.6 Hz, 1H), 7.56 (t, *J*=7.4 Hz, 1 H), 8.04 (dd, *J*=8.0, 1.3 Hz, 1H), 8.76 (d, *J*=8.4 Hz, 1 H), 11.57 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 13.8, 14.0, 28.3, 52.3, 55.5, 57.6, 80.6, 115.2, 120.1, 122.6, 130.8, 134.6, 141.2, 168.3, 171.1.



6

Coupling method I was applied to a solution of BocNH-Ala-Anth-OH (130 mg, 0.42 mmol, 1 eq) and NH_2-Ala-Anth-OMe (100 mg, 0.42mmol, 1 eq). After the extraction, the crude material was purified by flash chromatography with EP: AcOEt (3:2) as mobile phase to obtain **6** in 10 % yield (22 mg 0.042 mmol).

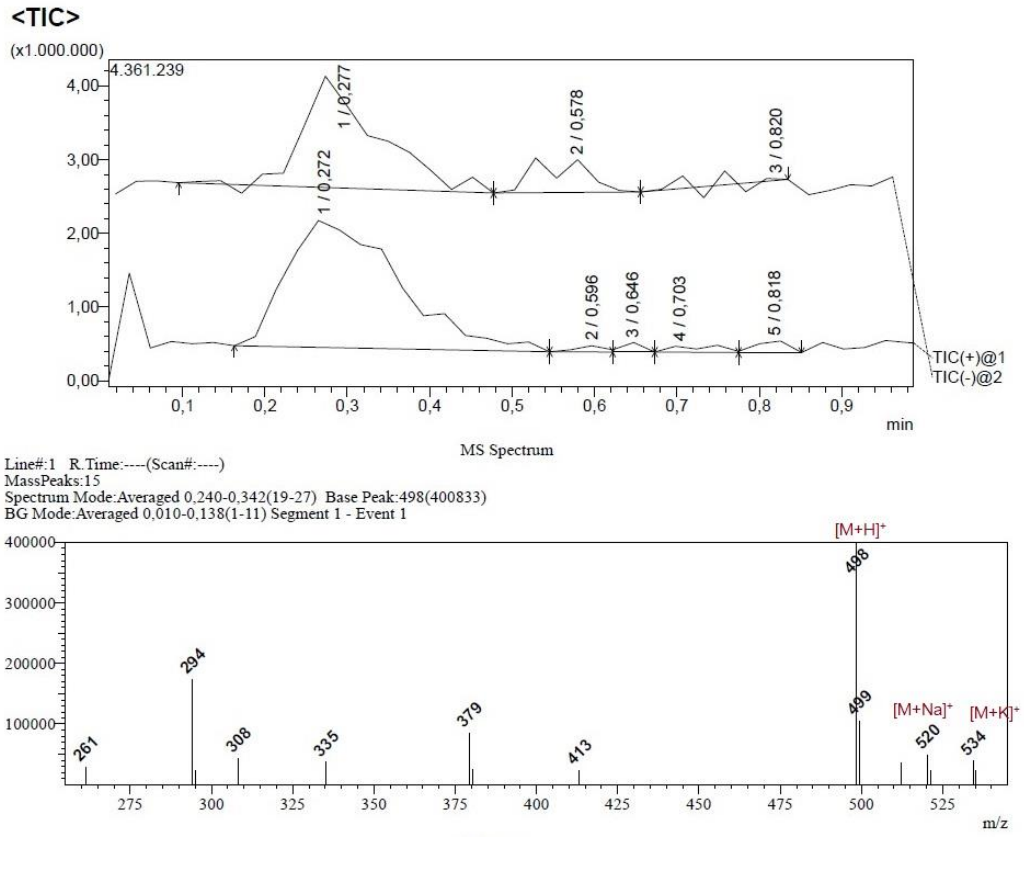
BocNH-Ala-Anth-Ala-Anth-OMe (6). Colourless oil. Rf=0.7 (AcOEt:EP, 3:2).^{1}H-RMN (400 MHz, $CDCl_3$) δ 1.47 (m, 12 H), 1.66 (d, *J*=7.1 Hz, 3H), 3.91 (s, 3 H), 4.28 – 4.44 (m, 1 H), 4.85 (d, *J*=7.0 Hz, 1 H), 5.10 – 5.27 (m, 1H), 7.10- 7.18 (m, 3 H), 7.50 (d, *J*=7.6 Hz, 1 H), 7.55 – 7.62 (m, 1H), 7.71 (dd, *J*=7.9, 1.3 Hz, 1 H), 8.06 (dd, *J*=8.0 Hz, 1.5, 1 H), 8.62 (d, *J*=8.4 Hz, 1 H), 8.70 (d, *J*=8.4 Hz, 1 H), 11.45 (s, 1H), 11.59 (s, 1H).



Boc protecting group of **6** was removed following general procedure. The resulting amine (20 mg, 0.042 mmol) was dissolved in 2.5 mL DCM under N_2 atmosphere and DIPEA (10.8 mg, 0.084 mmol) were added. The reaction mixture was put into a 0º ice bath and Boc-Ala-OH (10 mg, 0.05), HBTU (20 mg, 0.05 mmol) and DMAP (cat) were added. The reaction mixture was stirred at 0°C for 10 min and then at room temperature, overnight. DCM was removed under vacuum and AcOEt was added. The organic phase was washed with 0.1 M HCl aqueous solution (30 mL × 2), brine (10 mL), saturated $NaHCO_3$ solution (30 mL × 2) and brine (10 mL), dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to give the crude material. The crude material was purified by flash chromatography with AcOEt:EP (4:1) to give 23 mg (0.039 mmol) of **5** (92% yield).

BocNH-Ala-Ala-Anth-Ala-Anth-OMe (7). Yellow oil. Rf= 0.46 (AcOEt:EP, 4:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.42 (d, *J* = 7.0 Hz, 3H), 1.45 (s, 9H), 1.50 (d, *J* = 7.1 Hz, 3H), 1.65 (d, *J* = 7.1 Hz, 3 H), 3.92 (s, 3 H), 4.21- 4.39 (m, 1 H), 4.56 - 4.65 (m, 1 H), 4.77- 4.88 (m, 1 H), 5.04-5.17 (m, 1 H), 6.94 (s, 1 H), 7.05 – 7.21 (m, 3 H), 7.45 – 7.65 (m, 2 H), 7.71 (dd, *J*=7.9, 1.3 Hz, 1H), 8.06 (dd, *J*=8.0, 1.5 Hz, 1 H), 8.62 (d, *J*=8.4 Hz, 1 H), 8.70 (d, *J*=8.4 Hz, 1 H), 11.53 (s, 1H), 11.57 (s, 1H).

NMR Spectra and ESI-MS data of Compounds



ESPECTRO MS+

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BG Mode:Averaged 0,010-0,138(1-11) Segment 1 - Event 1

#	m/z	Absolute Intensity	Relative Intensity
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2	294,20	173194	43,21
3	295,15	23281	5,81
4	308,30	43414	10,83
5	335,20	37146	9,27
6	379,30	85045	21,22
7	380,40	24759	6,18
8	413,15	23853	5,95
9	498,40	400833	100,00
10	499,30	105640	26,36
11	512,30	36212	9,03
12	520,30	48888	12,20
13	521,40	23979	5,98
14	534,30	39006	9,73

Fig. S1: ESI-MS of NHMeAla-Anth-NMeAla-Anth Ala-OH (Linear precursor of Versicotide A, 10)

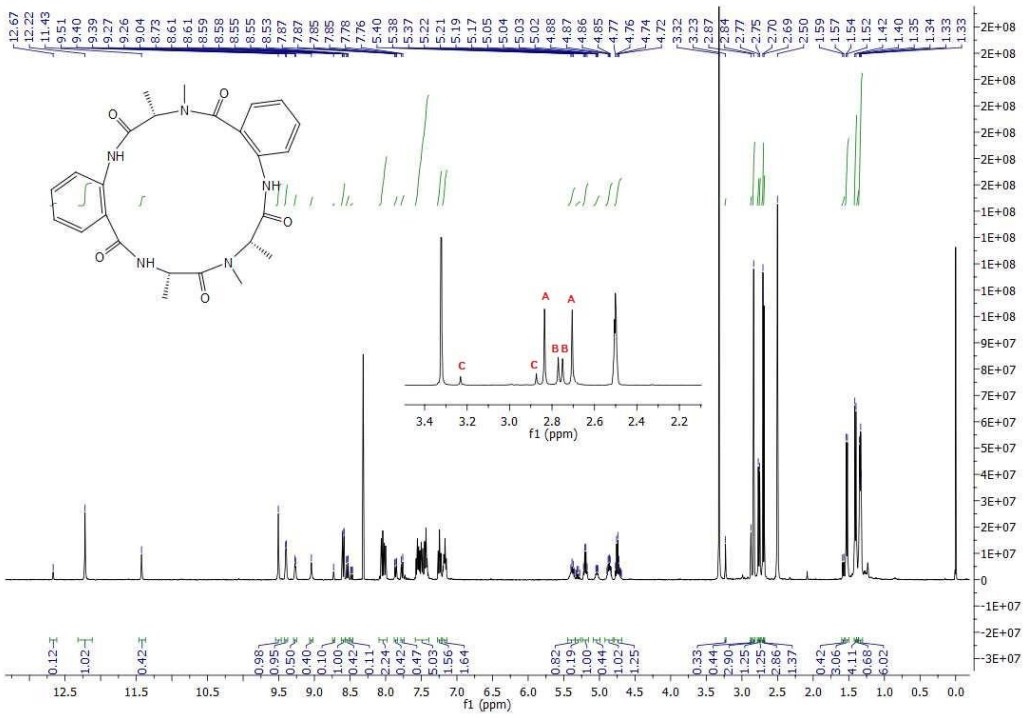


Fig. S2: ^{1}H-NMR in DMSO-d$_6$ of *Cyclo*-[Ala-Anth-NMeAla-Anth-NMeAla] (1) (Versicotide A) with area between 2.2 and 3.4 ppm zoomed to show conformers A, B and C.

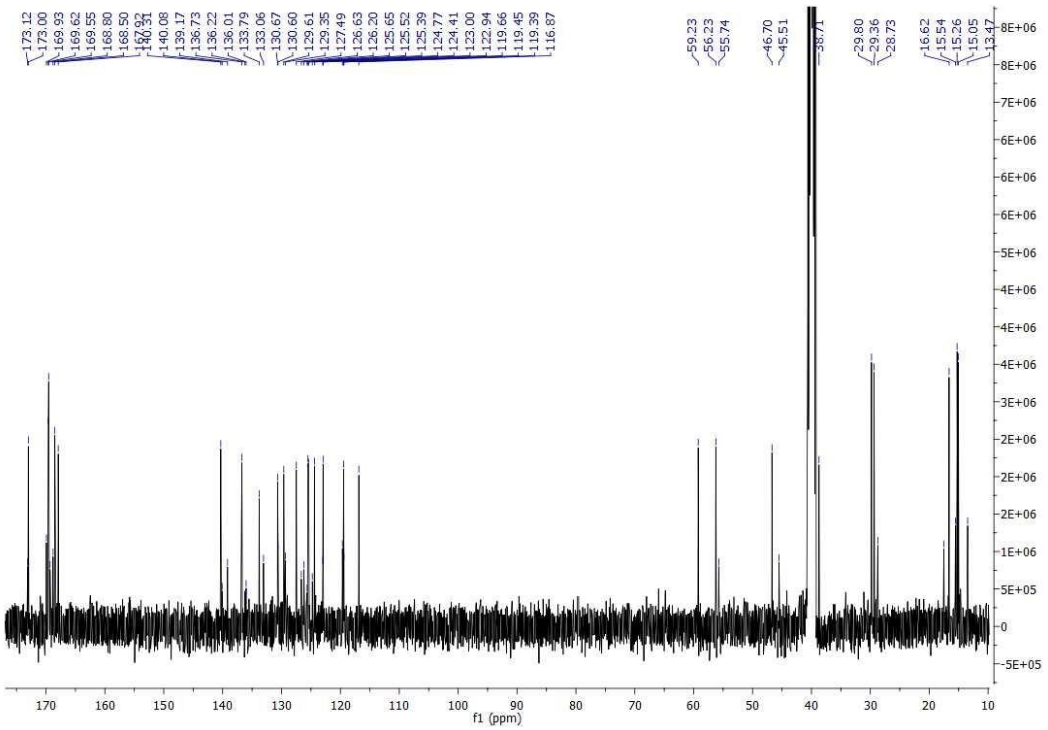


Fig. S3: ^{13}C-NMR in DMSO-d6 of *Cyclo*-[Ala-Anth-NMeAla-Anth-NMeAla] (1) (Versicotide A)

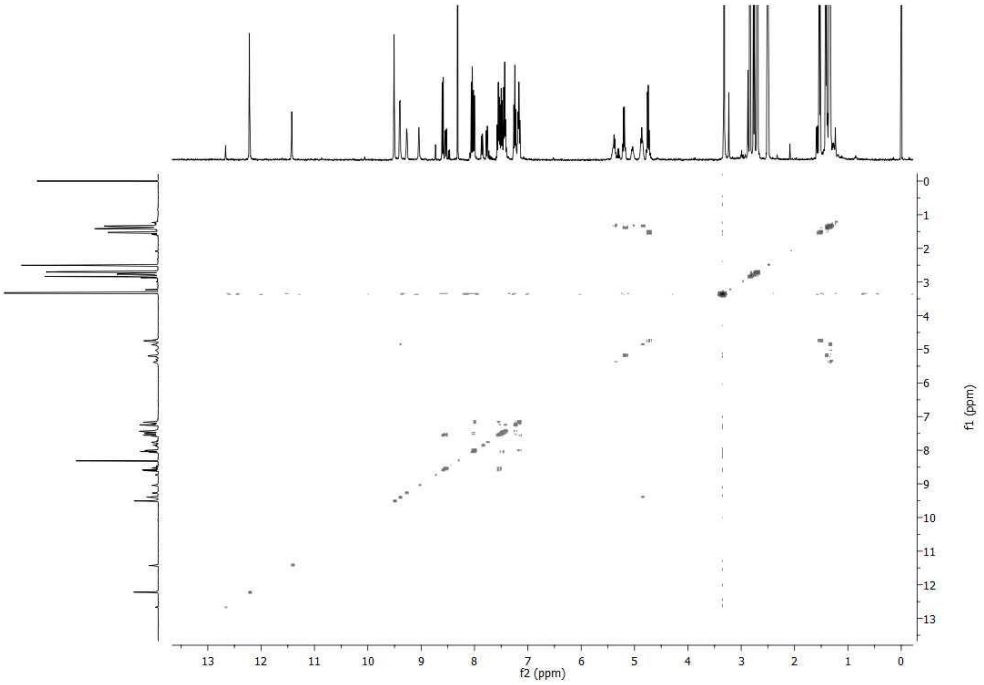


Fig. S4: COSY in DMSO-d6 of *Cyclo*-[Ala-Anth-NMeAla-Anth-NMeAla] (1)

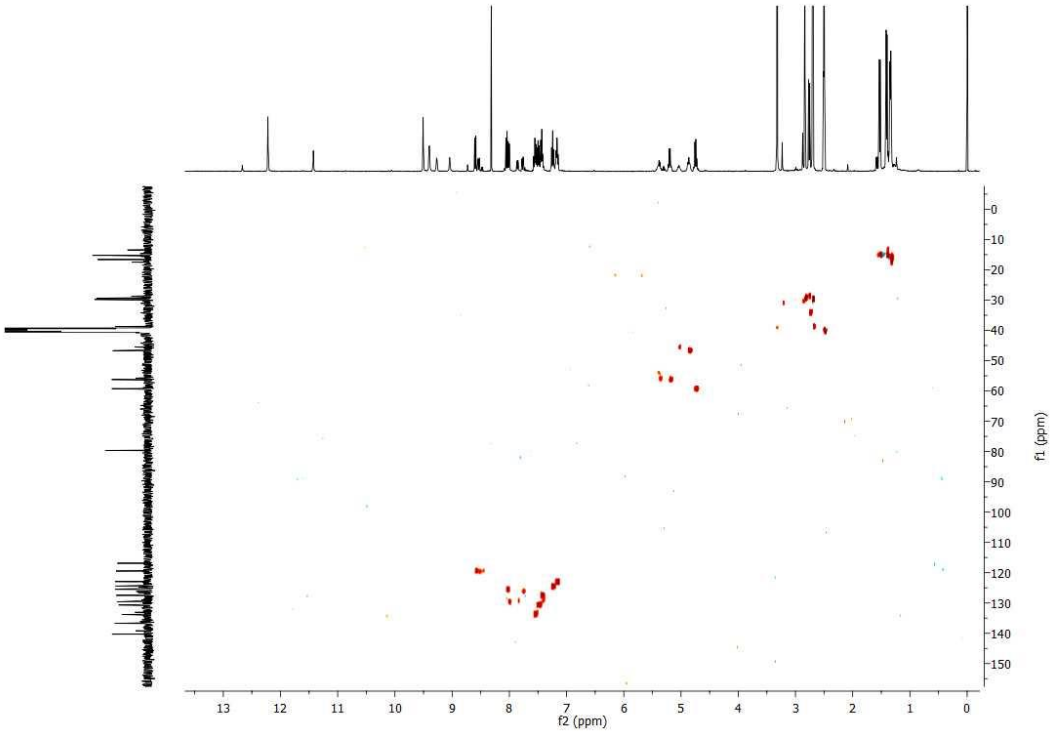


Fig. S5: HSQC in DMSO-d6 of *Cyclo*-[Ala-Anth-NMeAla-Anth-NMeAla] (1)

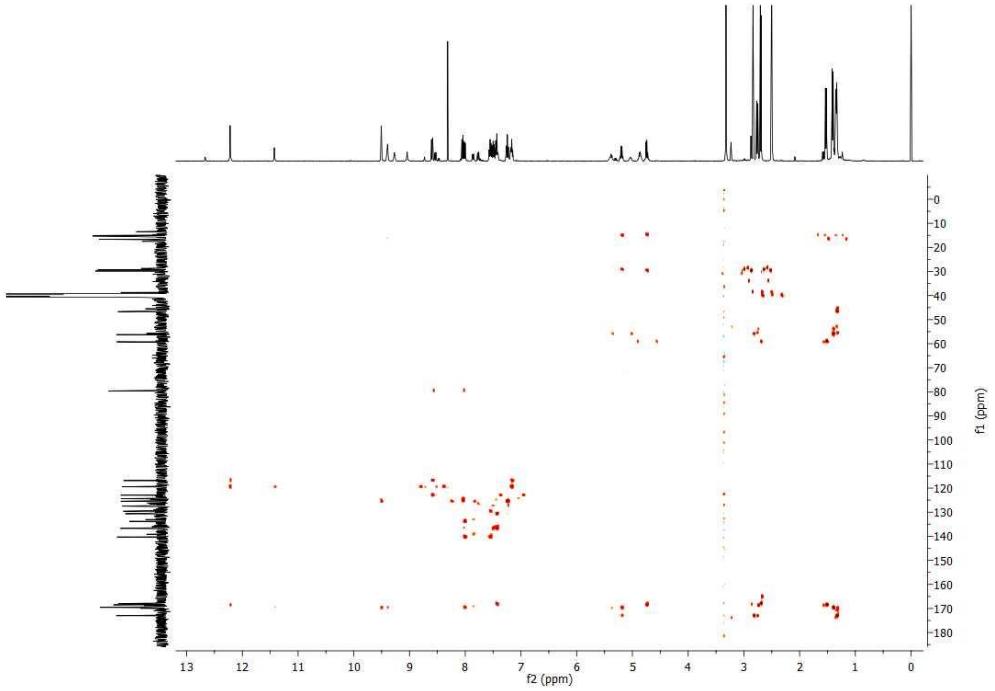


Fig. S6: HMBC in DMSO-d6 of *Cyclo*-[Ala-Anth-NMeAla-Anth-NMeAla] (1)

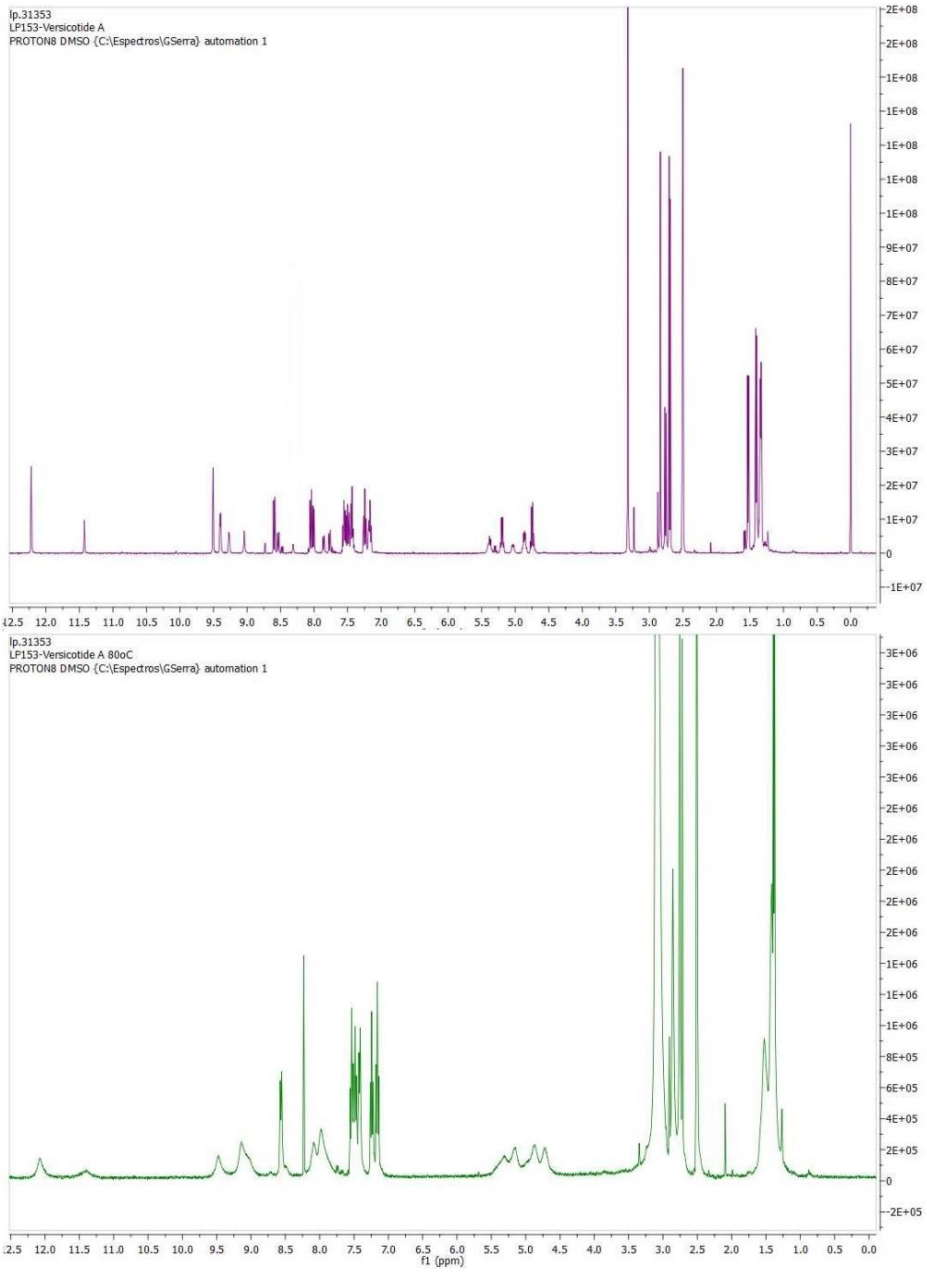
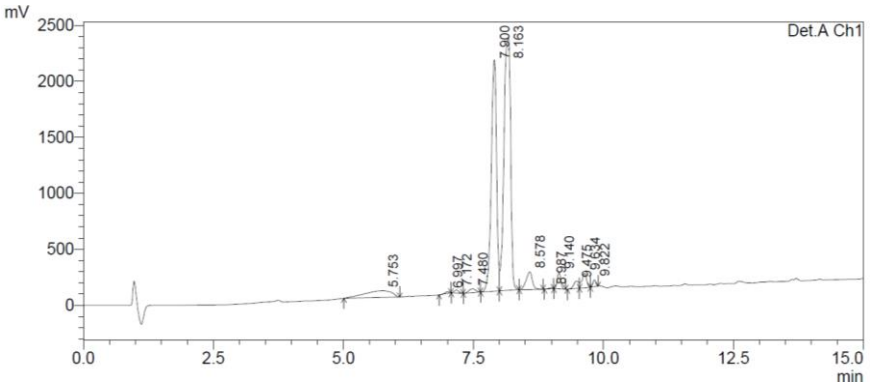


Fig S7: Comparison of ^{1}H-NMR at 40ºC (top) and 80ºC (bottom)



Detector A Ch1 225nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.753	2175015	59906	5.338	1.193
2	6.997	85636	14091	0.210	0.281
3	7.172	159693	27192	0.392	0.541
4	7.480	311367	33075	0.764	0.658
5	7.900	14493866	2064533	35.570	41.100
6	8.163	19957971	2256177	48.980	44.915
7	8.578	1308882	158299	3.212	3.151
8	8.987	42885	9004	0.105	0.179
9	9.140	697338	145837	1.711	2.903
10	9.475	466984	66476	1.146	1.323
11	9.634	805925	128845	1.978	2.565
12	9.822	241792	59736	0.593	1.189
Total		40747354	5023172	100.000	100.000

Fig. S8: HPLC Chromatogram of 1: *Cyclo*-[NHMeAla-Anth-NMeAla-Anth Ala-OH] (Versicotide A)

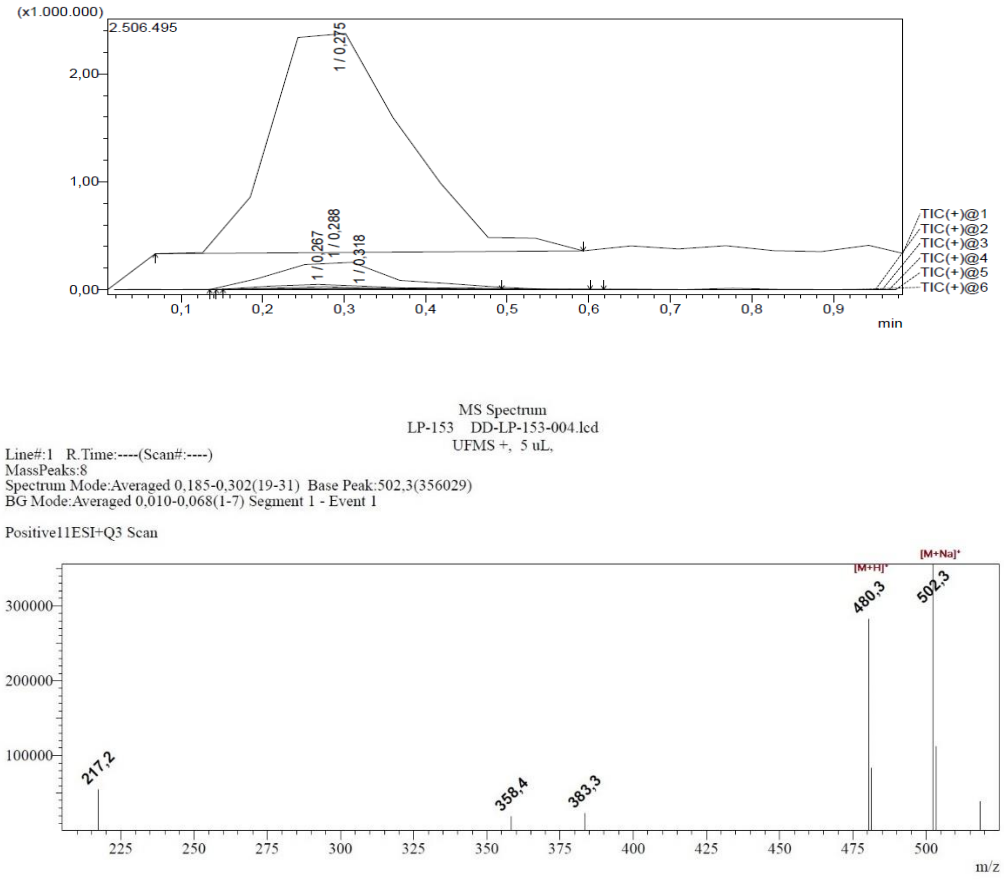
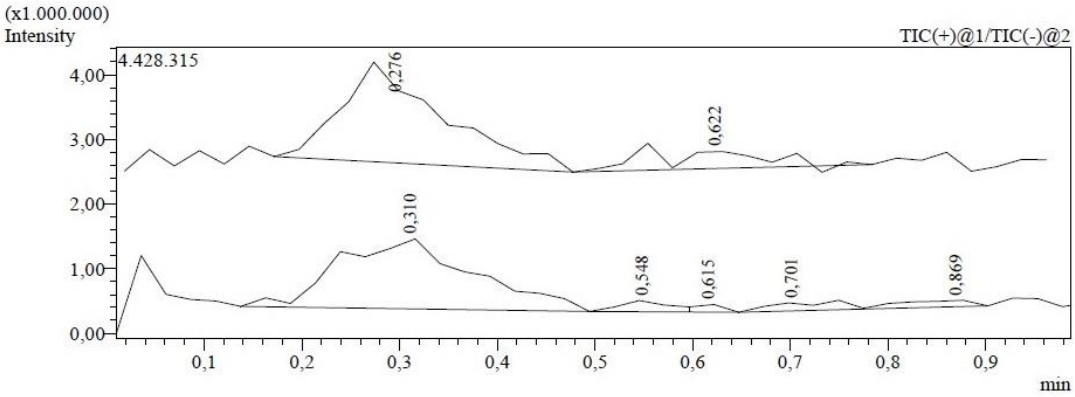


Fig. S9: ESI-MS of *Cyclo*-[NHMeAla-Anth-NMeAla-Anth Ala-OH] (Versicotide A)



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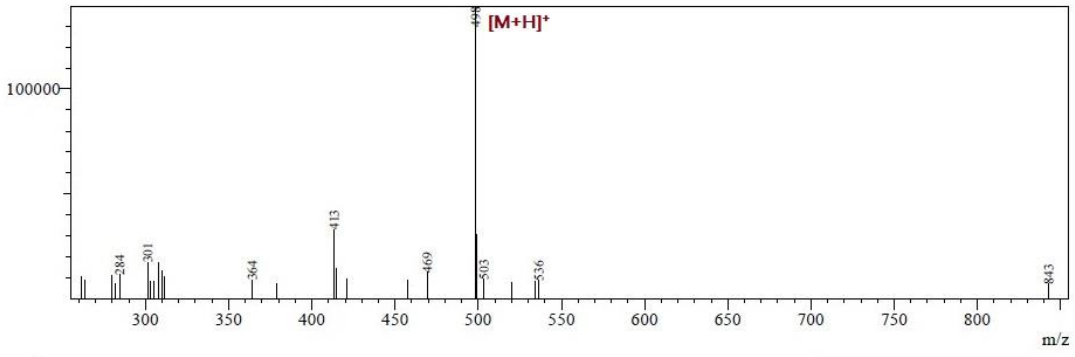
BG Mode:Averaged 0,036-0,138(3-11) Segment 1 - Event 1



ESPECTRO MS+

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BG Mode:Averaged 0,010-0,112(1-9) Segment 1 - Event 1

#	m/z	Absolute Intensity	Relative Intensity
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2	263,10	7943	5,23
3	279,05	10106	6,65
4	280,15	12216	8,04
5	284,45	16956	11,16
6	301,25	22178	14,60
7	308,25	16084	10,59
8	310,00	12314	8,11
9	311,10	9991	6,58
10	364,10	12879	8,48
11	365,35	8324	5,48
12	413,30	38829	25,56
13	414,35	14589	9,60
14	421,30	9678	6,37
15	457,30	8909	5,86
16	469,25	12541	8,25
17	498,35	151930	100,00
18	499,30	30816	20,28
19	503,30	9246	6,09
20	534,30	9488	6,24

Fig. S10: ESI MS of NHMeAla-Anth-Ala-Anth-NMeAla-OH (Linear precursor of Versicotide B, 11)

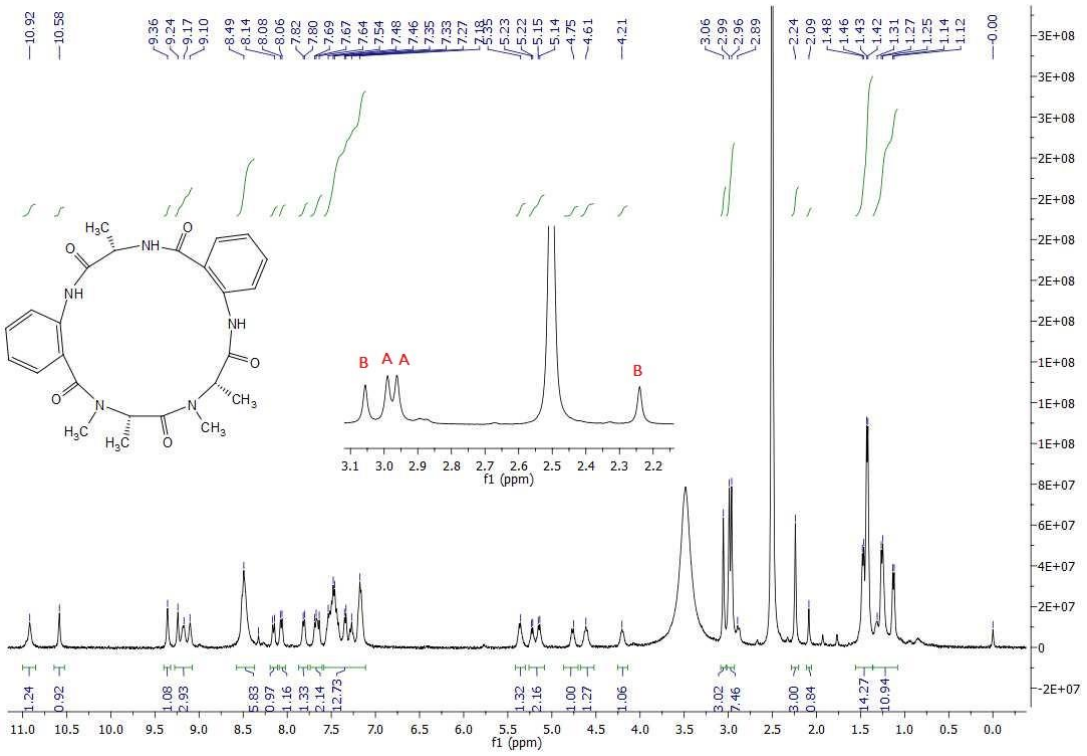


Fig. S11: ^{1}H-NMR in DMSO-d6 of *Cyclo*-[NMeAla-Anth-Ala-Anth-NMeAla] (2) (Versicotide B). Zoomed area: 2.20 – 3.15 ppm, A and B: conformers.

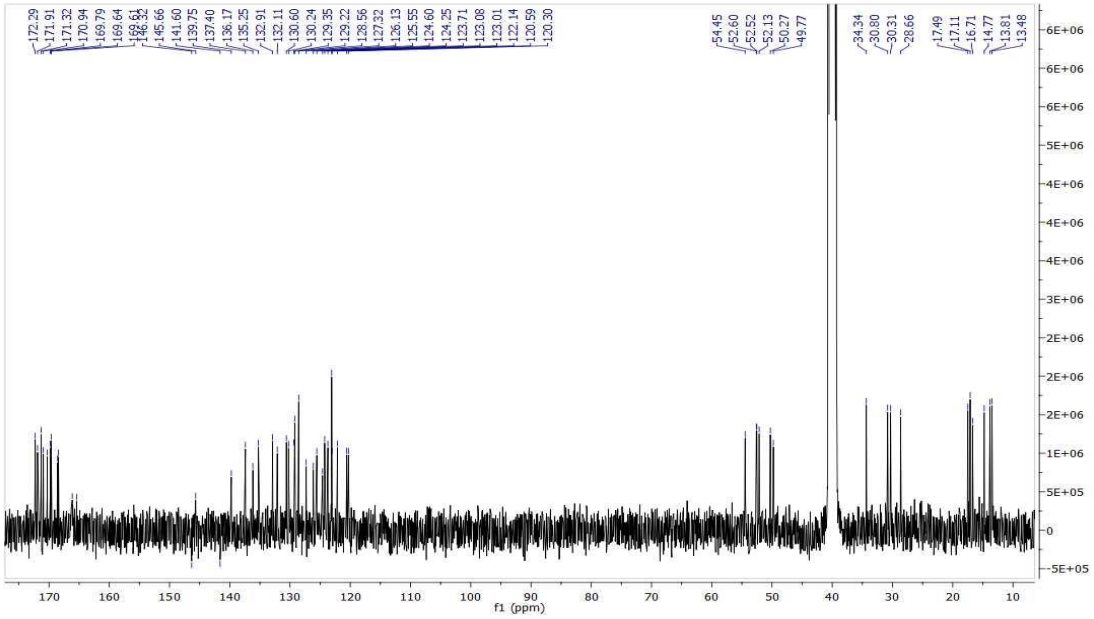


Fig. S12: ^{13}C-NMR in DMSO-d6 of *Cyclo*-[NMeAla-Anth-Ala-Anth-NMeAla] (2) (Versicotide B)

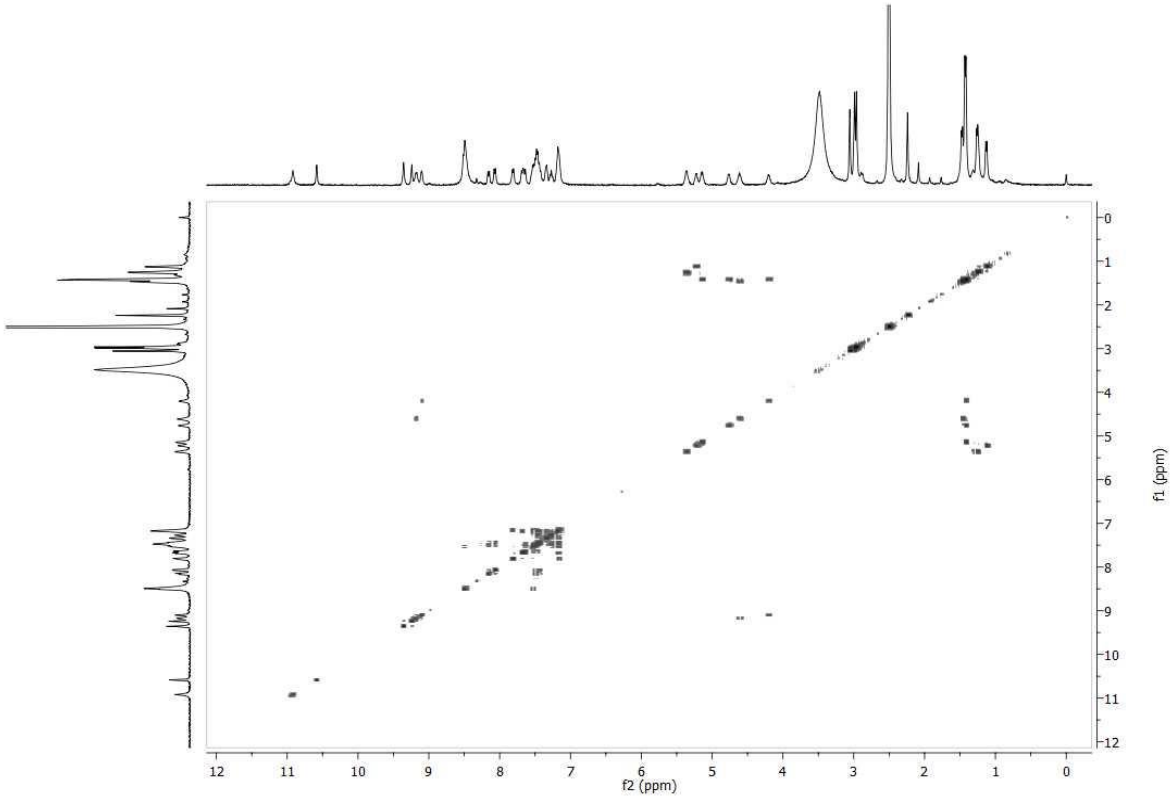


Fig. S13: COSY in DMSO-d6 of *Cyclo*-[NMeAla-Anth-Ala-Anth-NMeAla] (2)

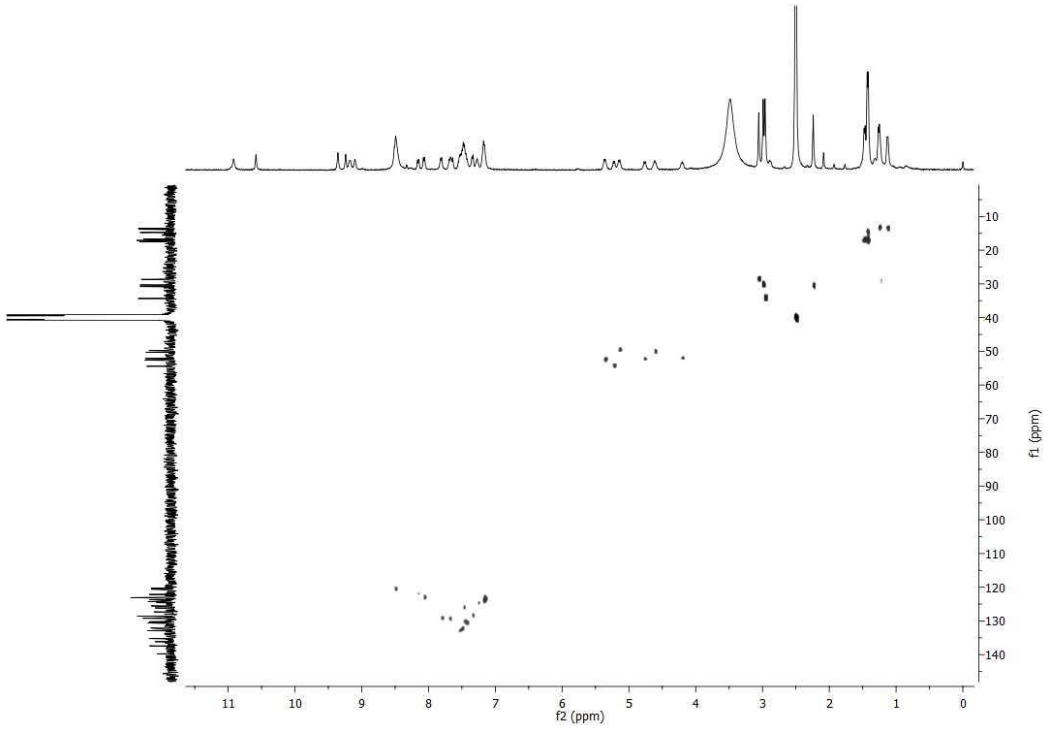


Fig. S14: HSQC in DMSO-d6 of *Cyclo*-[NMeAla-Anth-Ala-Anth-NMeAla] (2)

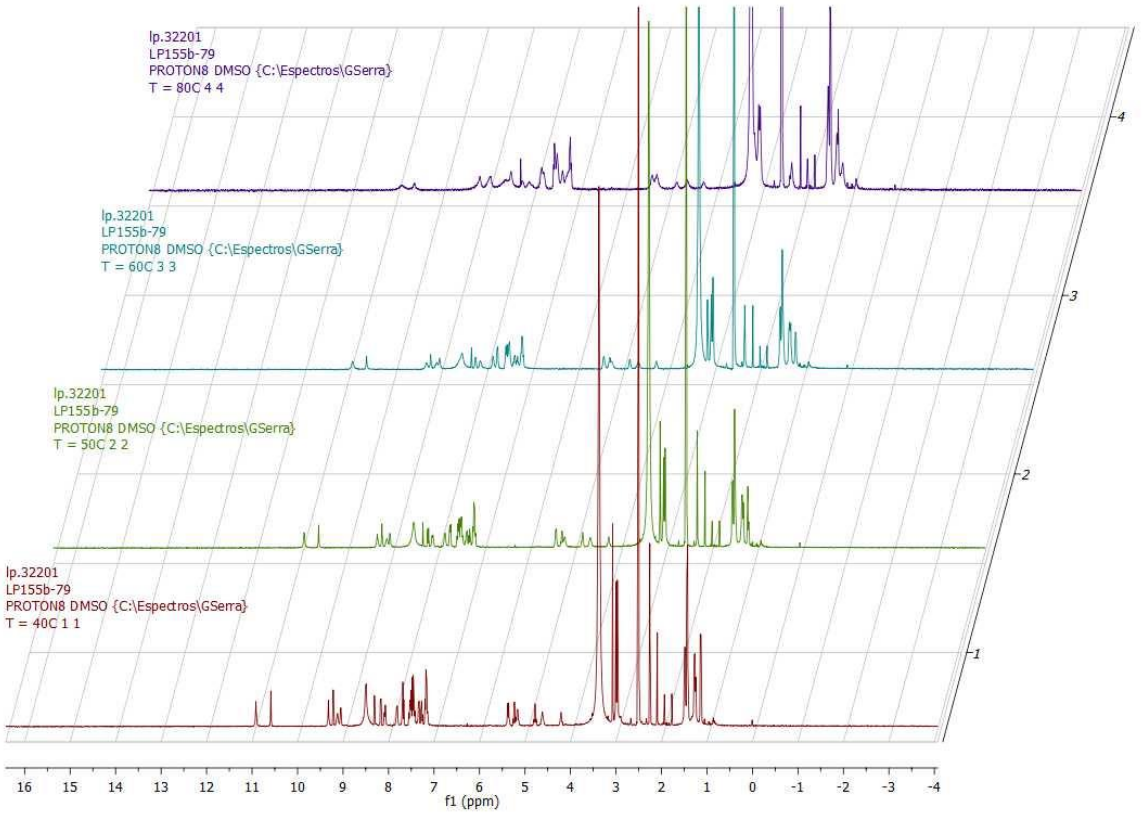


Fig. S15: Thermal gradient in DMSO-d6 of *Cyclo*-[NMeAla-Anth-Ala-Anth-NMeAla] (2). Comparison of ^{1}H-NMR at 40 (red), 50 (green), 60 (blue) and 80ºC (purple)

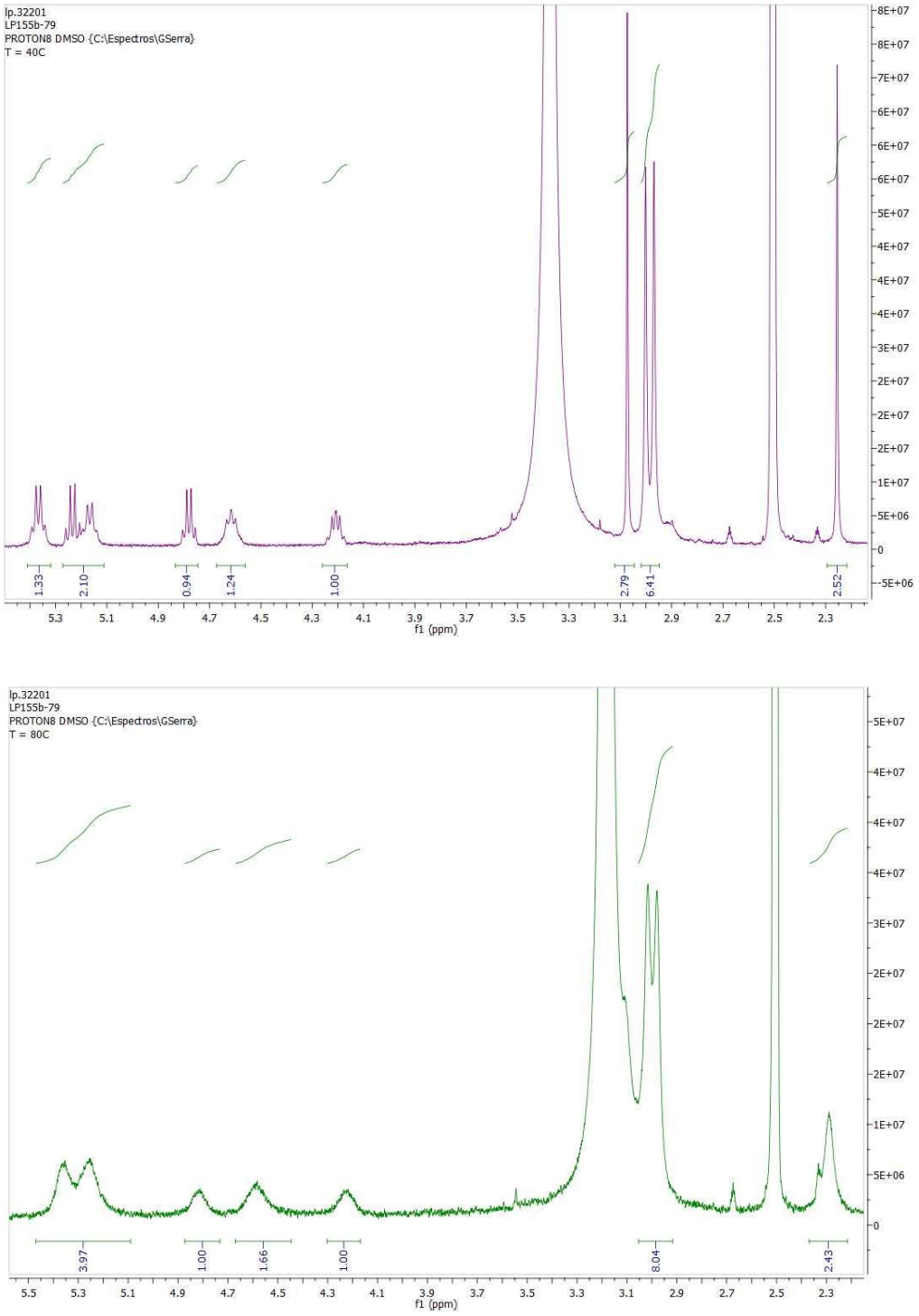
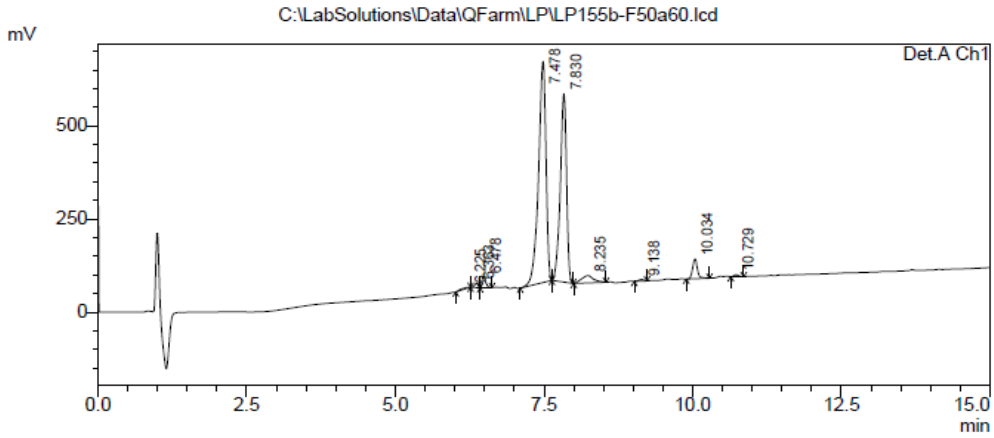


Fig. S16: Thermal gradient in DMSO-d6 of *Cyclo*-[NMeAla-Anth-Ala-Anth-NMeAla] (2). Zoomed between 2.2 and 5.5 ppm. Comparison of ^{1}H-NMR at 40 (purple) and 80ºC (green).

<Chromatogram>



PeakTable

Detector A Ch1 225nm

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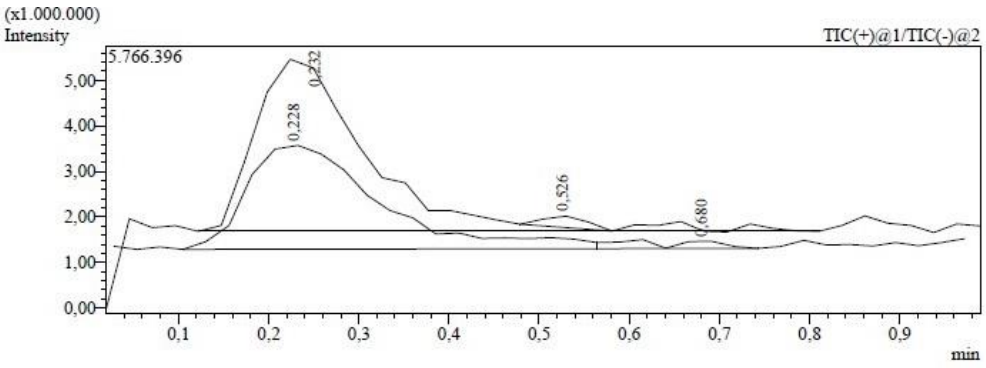
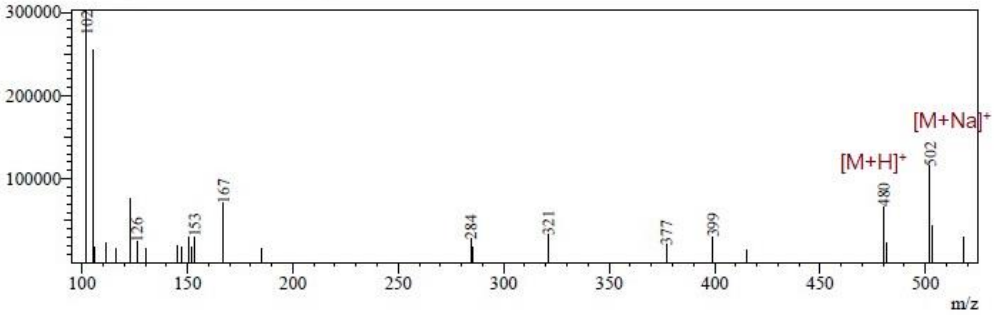
2	6.363	51899	12195	0.535	0.994
3	6.478	129447	30488	1.334	2.485
4	7.478	5171208	594011	53.293	48.418
5	7.830	3684549	505602	37.972	41.212
6	8.235	278530	20050	2.870	1.634
7	9.138	27061	4412	0.279	0.360
8	10.034	297509	52674	3.066	4.293
9	10.729	27579	5287	0.284	0.431
Total		9703407	1226837	100.000	100.000

Fig. S17: HPLC chromatogram of Cyclo-[NHMeAla-Anth-Ala-Anth-NMeAla] (2)



Line#:1 R.Time:----(Scan#:) MassPeaks:26 BasePeak:102(302718)
Spectrum Mode:Averaged 0,173-0,250(13-19)
BG Mode:Averaged 0,581-0,709(45-55) Segment 1 - Event 1

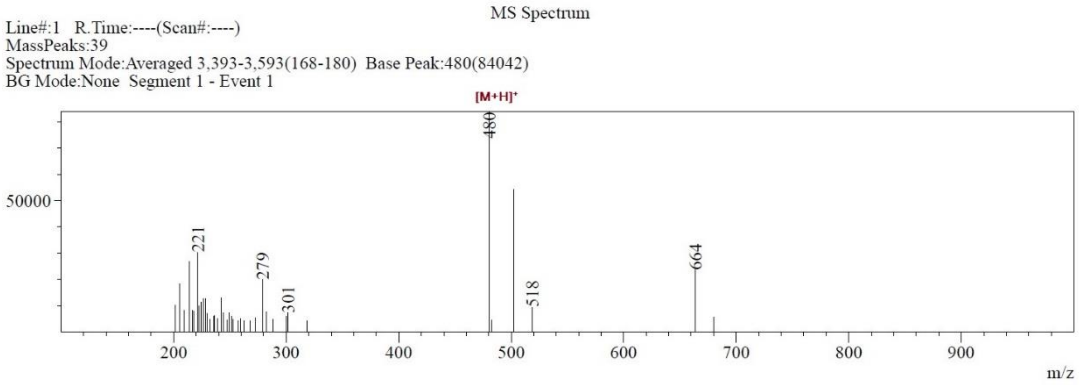


MS Spectrum

Line#:1 R.Time:----(Scan#:----)
MassPeaks:26
Spectrum Mode:Averaged 0,173-0,250(13-19) Base Peak:102(302718)
BG Mode:Averaged 0,581-0,709(45-55) Segment 1 - Event 1

#	m/z	olute Intenlative Intens	
1	102,20	302718	100,00
2	105,10	254952	84,22
3	106,20	17592	5,81
4	111,20	23397	7,73
5	116,15	16683	5,51
6	122,60	76698	25,34
7	126,05	24624	8,13
8	130,25	16015	5,29
9	145,10	20279	6,70
10	147,15	18116	5,98
11	150,25	30020	9,92
12	152,00	18874	6,23
13	153,15	30139	9,96
14	167,05	70893	23,42
15	185,25	16414	5,42
16	284,35	27775	9,18
17	285,35	18358	6,06
18	321,15	33382	11,03
19	377,10	21086	6,97
20	399,10	30236	9,99
21	415,15	15412	5,09
22	480,25	66339	21,91
23	481,30	23233	7,67
24	502,20	115082	38,02

Fig. S18: ESI-MS of *Cyclo*-[NHMeAla-Anth-Ala-Anth-NMeAla] (2) Peak of tr=7.48 min



Line#:1 R.Time:----(Scan#:----)
MassPeaks:39
Spectrum Mode:Averaged 3,393-3,593(168-180) Base Peak:480(84042)
BG Mode:None Segment 1 - Event 1

#	m/z	olute Intenlative Intens	
1	201,35	10117	12,04
2	205,25	18213	21,67
3	209,40	8153	9,70
4	213,70	26895	32,00
5	216,60	8291	9,87
6	217,55	7902	9,40
7	221,35	30281	36,03
8	222,45	10009	11,91
9	224,45	11156	13,27
10	226,50	12706	15,12
11	228,20	12630	15,03
12	229,45	7130	8,48
13	232,35	4700	5,59
14	235,55	6052	7,20
15	236,65	6184	7,36
16	239,00	5237	6,23
17	242,60	12926	15,38
18	244,35	7443	8,86
19	247,60	4336	5,16
20	249,15	7479	8,90
21	251,35	5876	6,99
22	252,65	4656	5,54
23	257,45	4255	5,06
24	259,30	4944	5,88
25	262,45	4262	5,07
26	267,80	4222	5,02
27	272,30	5521	6,57
28	279,00	19966	23,76
29	282,60	7490	8,91
30	288,15	4912	5,84
31	300,00	5819	6,92
32	301,50	7368	8,77
33	318,35	4273	5,08
34	480,35	84042	100,00
35	482,55	4339	5,16
36	502,10	54334	64,65

Fig. S19: ESI-MS of *Cyclo*-[NHMeAla-Anth-Ala-Anth-NMeAla] (2) Peak of tr=7.83 min

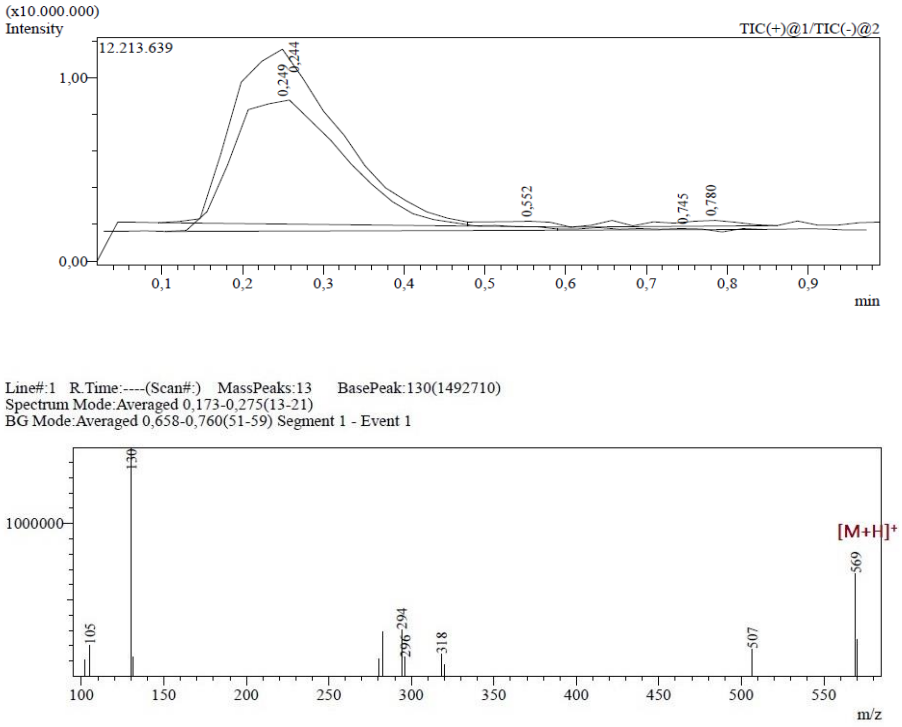


Fig. S20: ESI-MS of Ala-Anth-NMeAla-Ala-Anth-NMeAla (Linear precursor of Versicotide C, 12)

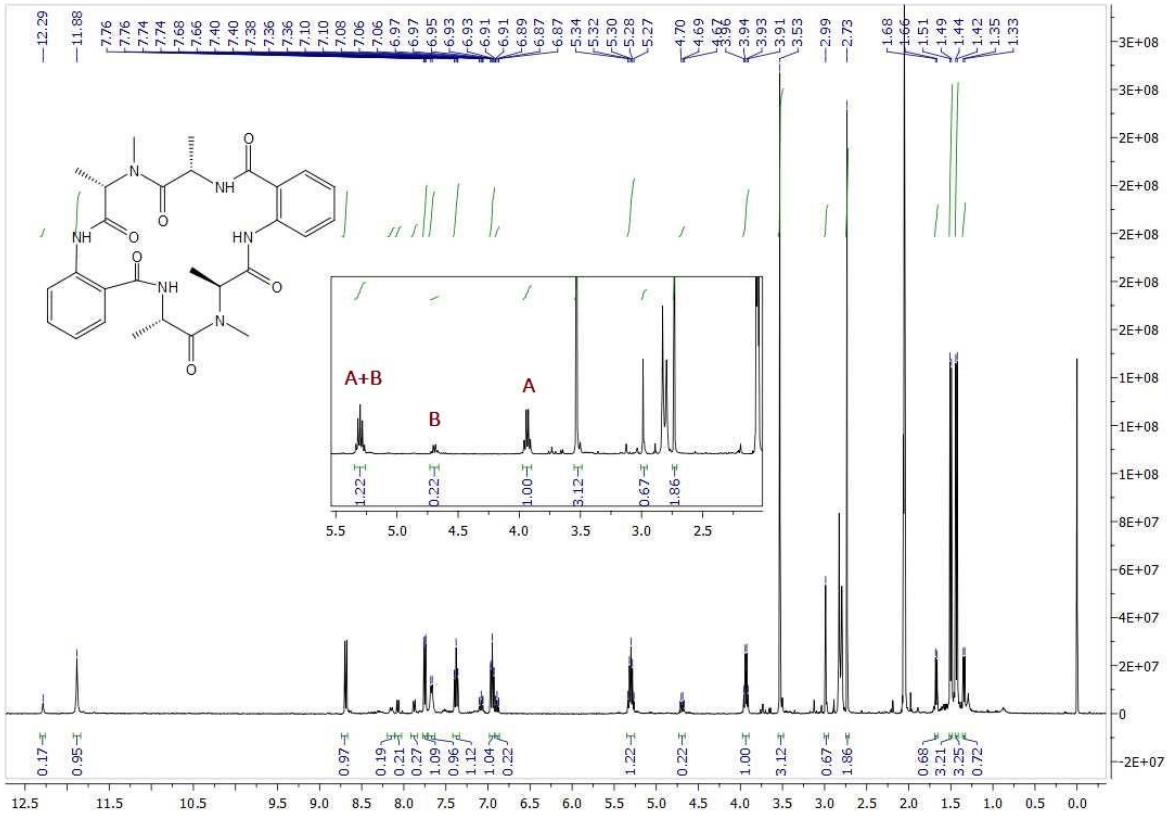


Fig. S21: ^{1}H-NMR in $(CD_3)_2CO$) of *Cyclo*-[NMeAla-Anth-Ala-NMeAla-Anth-Ala] (3) (Versicotide C). Zoomed area 2-5.5 ppm shows conformers.

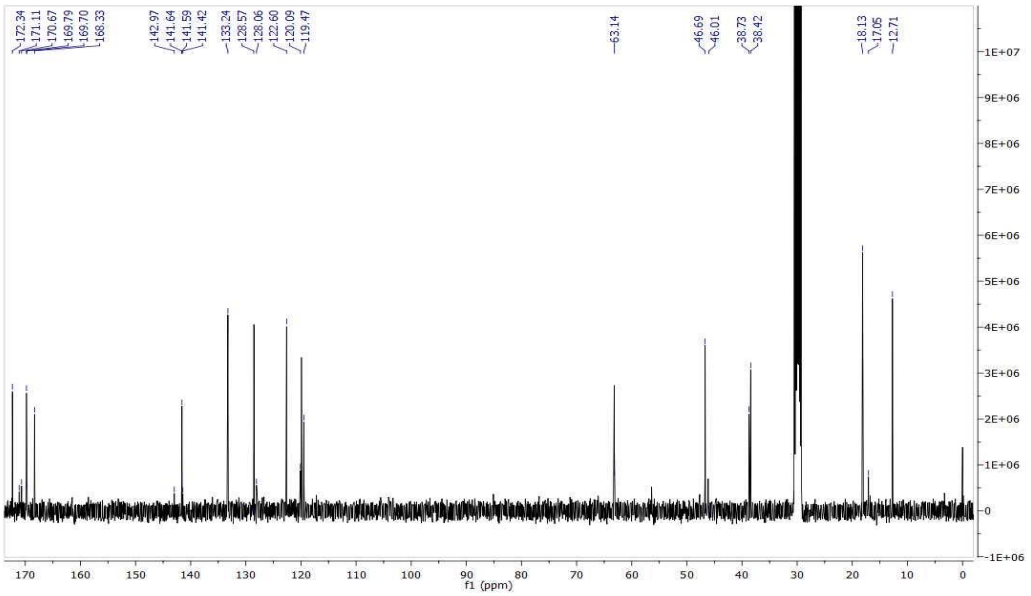


Fig. S22: ^{13}C-NMR in $(CD_3)_2CO$) of Versicotide C

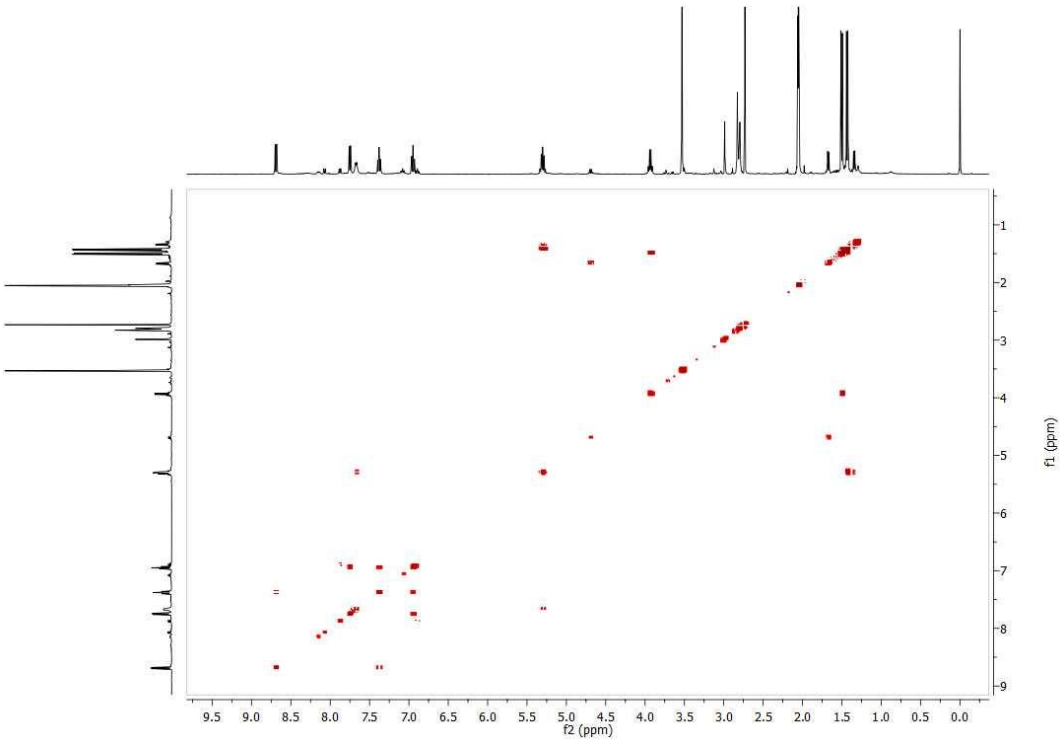


Fig. S23: COSY in $(CD_3)_2CO$) of Versicotide C

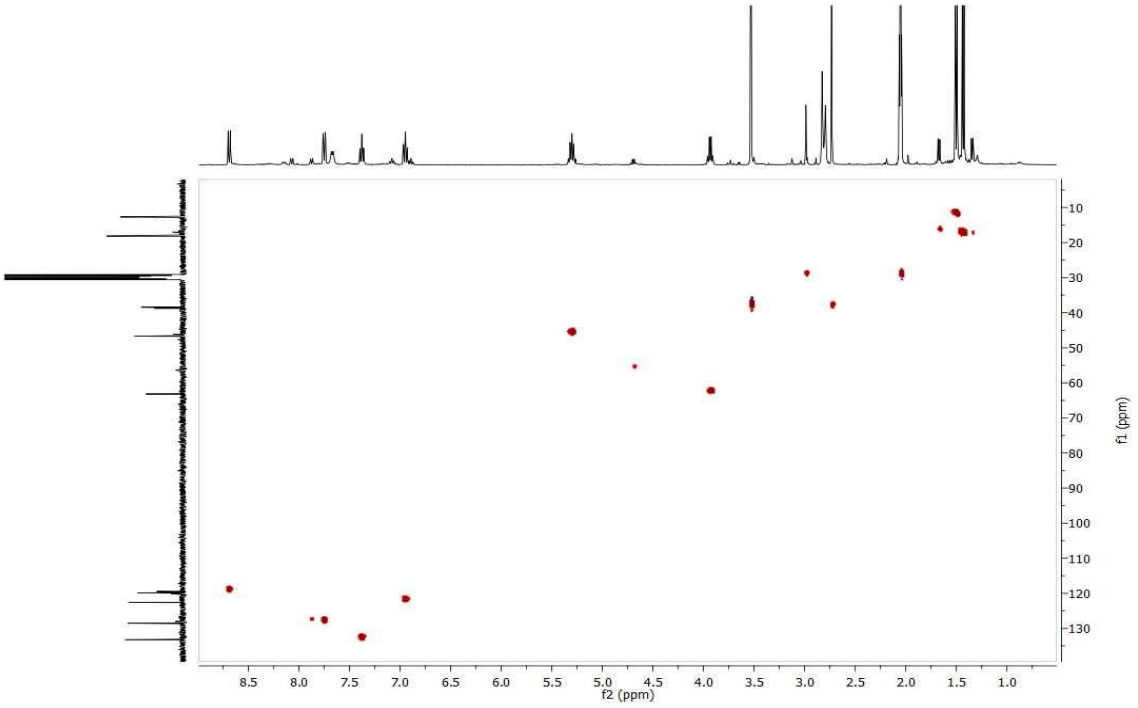


Fig. S24: HSQC in $(CD_3)_2CO$) of Versicotide C

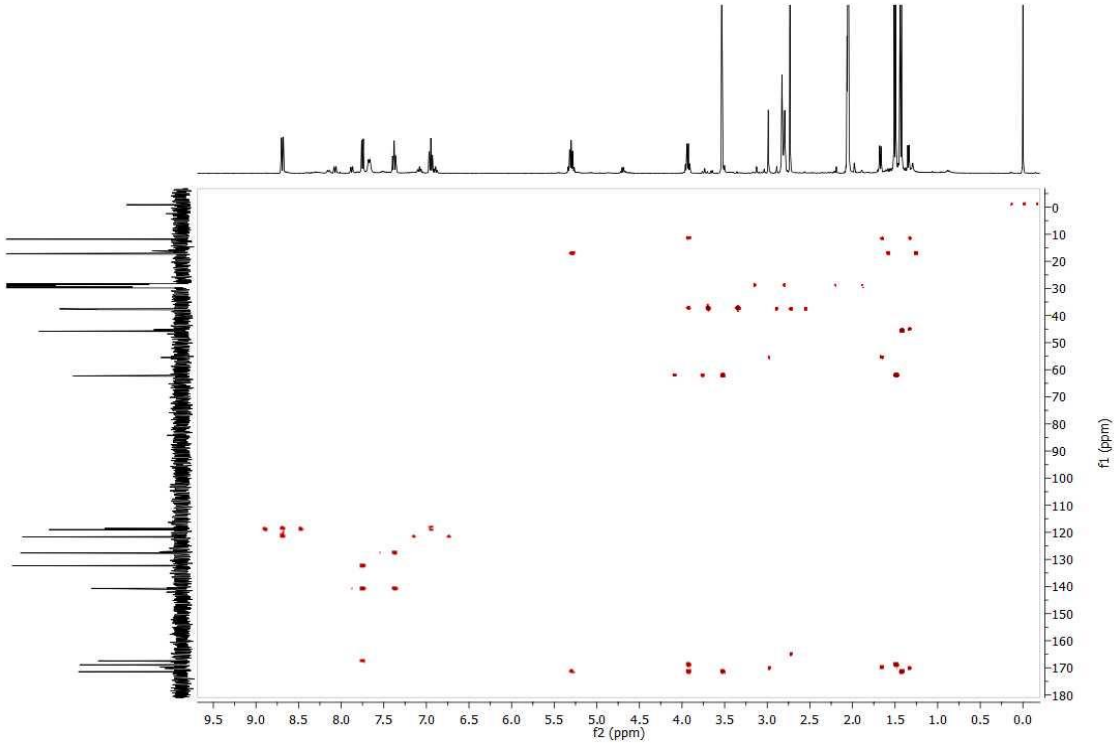


Fig. S25:HMBC in $(CD_3)_2CO$) of Versicotide C

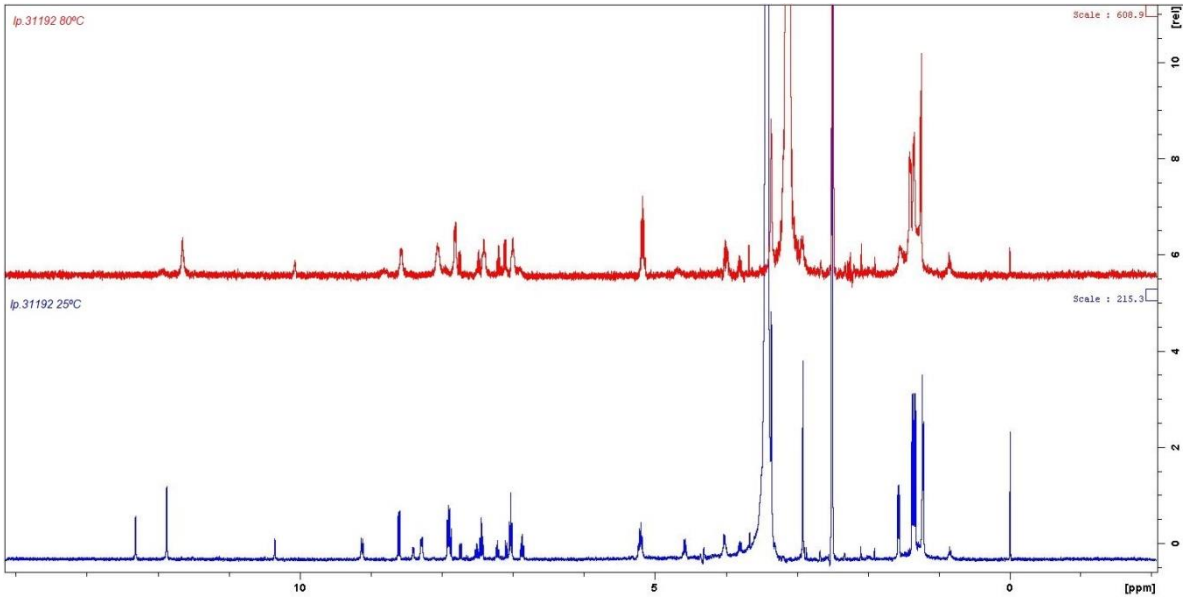


Fig. S26: Comparison of ^{1}H-NMR of Versicotide C at 25ºC (blue) and 80ºC (red)

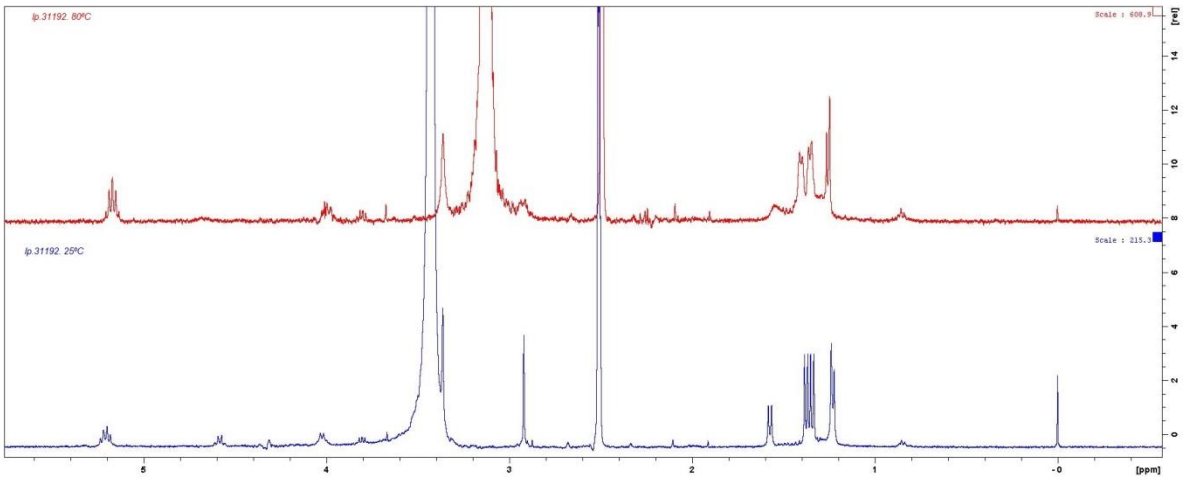
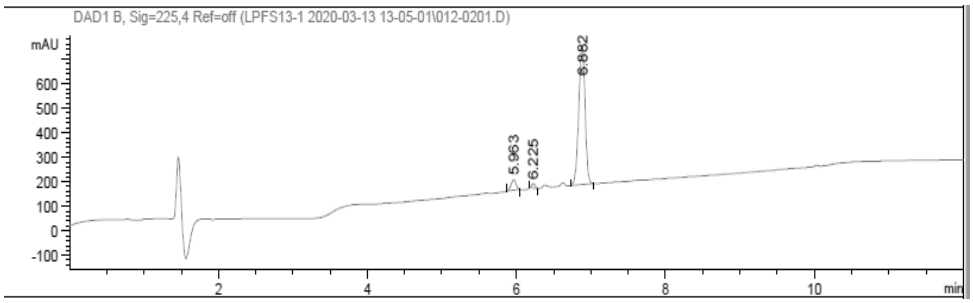


Fig. S27: Comparison of ^{1}H-NMR of Versicotide C at 25ºC (blue) and 80ºC (red)



Signal 2: DAD1 B, Sig=225,4 Ref=off

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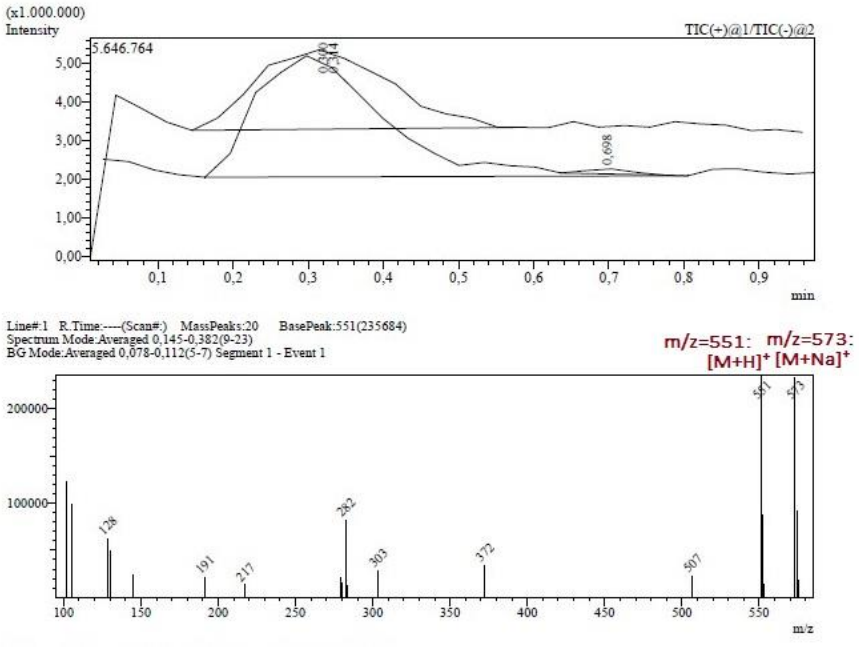
6.225	3.16	56.87580	18.06624	0.89	0.0547	71904	2.41	1.06
6.882	3.60	3327.74341	565.47705	1.10	0.0933	30114	5.22	1.14

Fig. S28: HPLC chromatogram of *Cyclo*-[Ala-Anth-NMeAla-Ala-Anth-NMeAla] (1)(Versicotide C)



MS Spectrum

Line#:1 R.Time:----(Scan#:----)
MassPeaks:20
Spectrum Mode:Averaged 0,145-0,382(9-23) Base Peak:551(235684)
BG Mode:Averaged 0,078-0,112(5-7) Segment 1 - Event 1

#	m/z	olute Intenlative Intens	
1	102,20	122700	52,06
2	105,15	99169	42,08
3	128,10	62540	26,54
4	130,10	49255	20,90
5	145,00	23496	9,97
6	191,10	21056	8,93
7	217,15	13160	5,58
8	279,15	21156	8,98
9	280,25	15371	6,52
10	282,30	81902	34,75
11	283,30	11958	5,07
12	303,10	27816	11,80
13	372,15	34352	14,58
14	506,60	22514	9,55
15	551,30	235684	100,00
16	552,30	87161	36,98
17	553,35	14547	6,17
18	573,25	233749	99,18

Fig. S29: ESI-MS of *Cyclo*-[Ala-Anth-NMeAla-Ala-Anth-NMeAla] (4)

(Versicotide C)

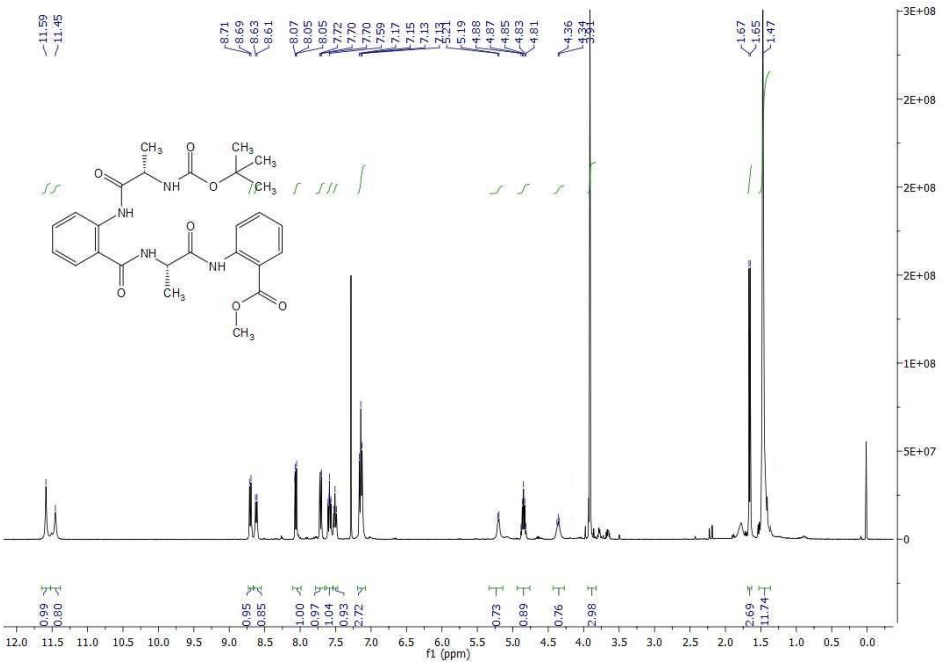


Fig. S30: ^{1}H-NMR in $CDCl_3$ of BocNHAla-Anth-Ala-AnthOMe (6)

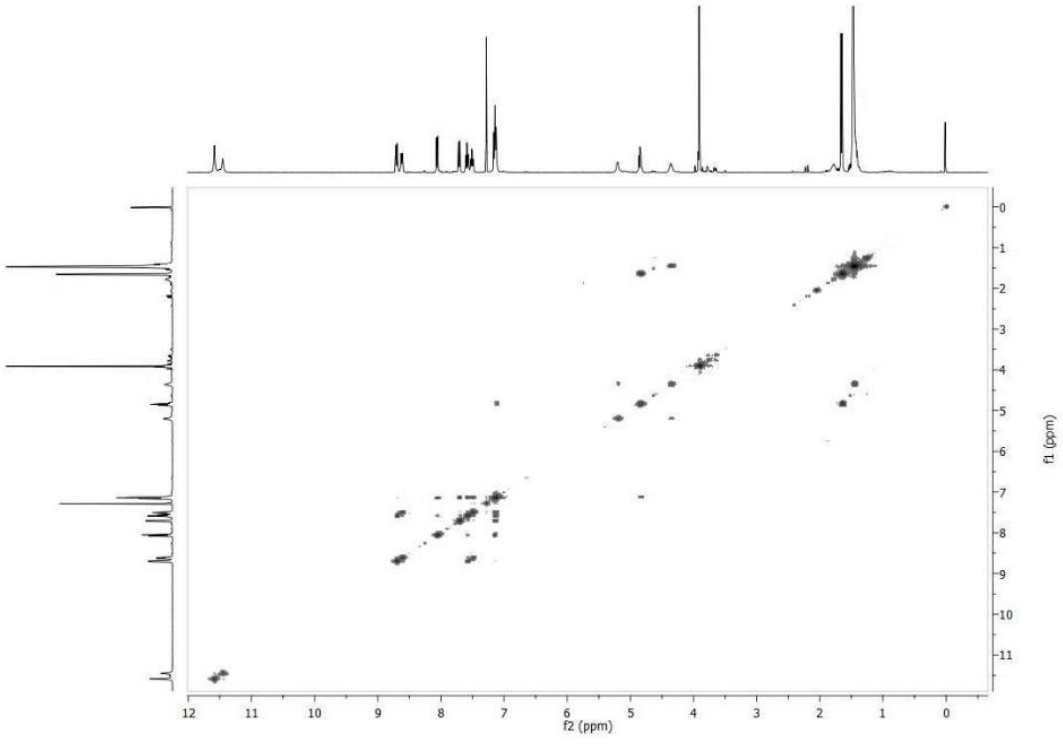


Fig. S31: COSY in $CDCl_3$ of BocNHAla-Anth-Ala-AnthOMe (6)

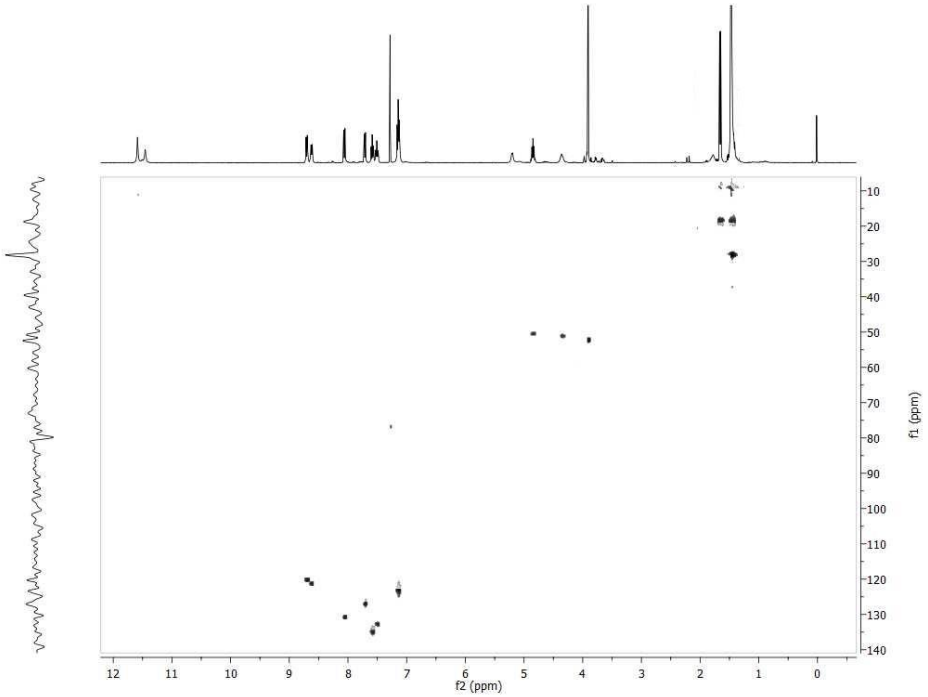


Fig. S32: HSQC in $CDCl_3$ of BocNHAla-Anth-Ala-AnthOMe (6)

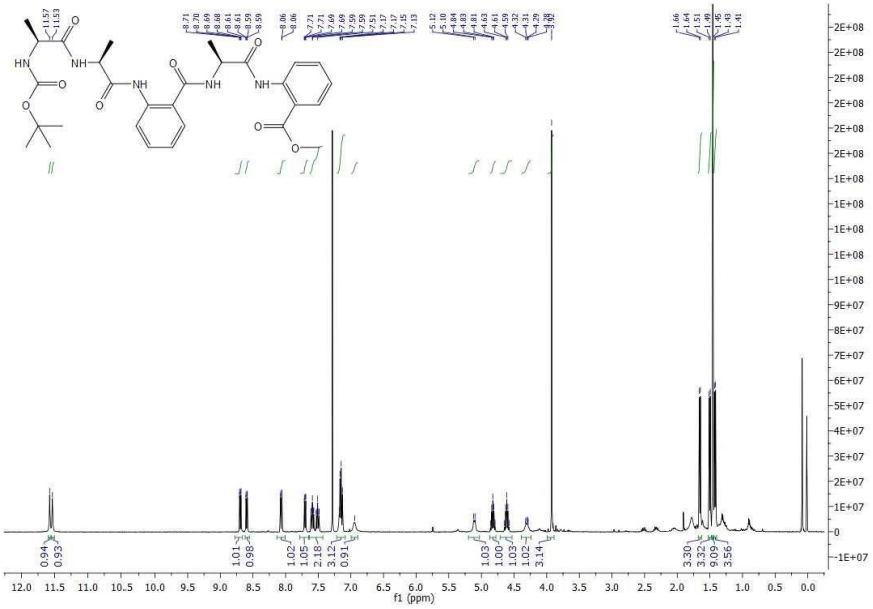


Fig. S33: ^{1}H-NMR in $CDCl_3$ of BocNHAla-Ala-Anth-Ala-AnthOMe (7)

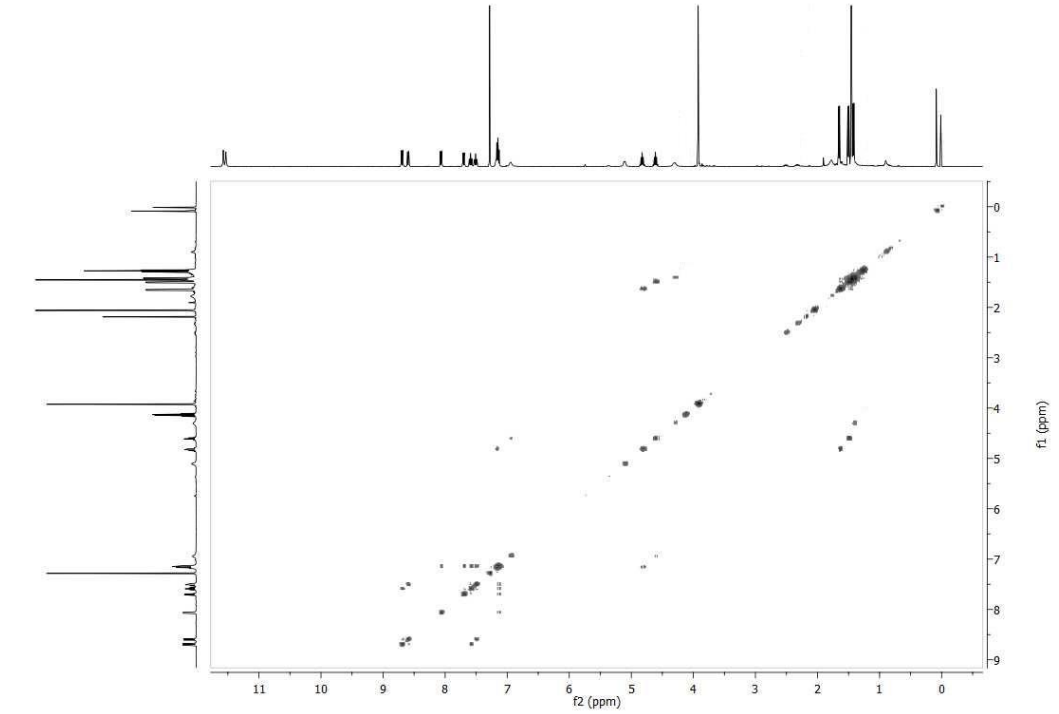


Fig. S34: COSY in $CDCl_3$ of BocNHAla-Ala-Anth-Ala-AnthOMe (7)

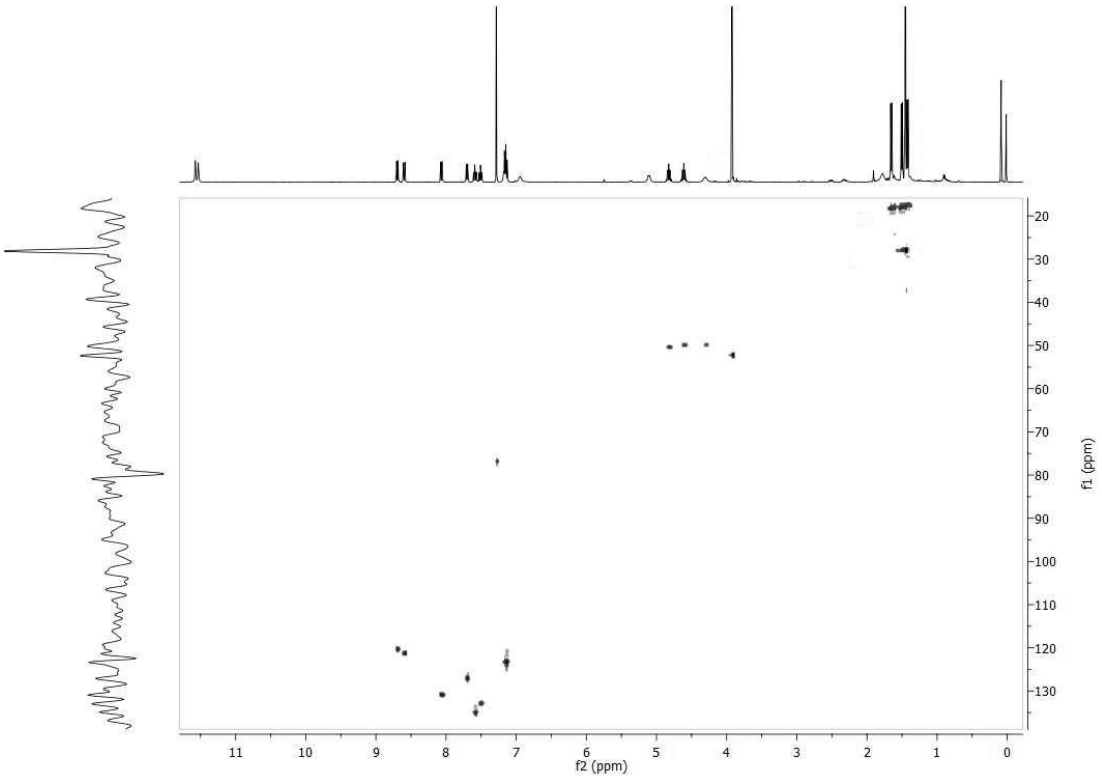


Fig. S35: HSQC in $CDCl_3$ of BocNHAla-Ala-Anth-Ala-AnthOMe (7)

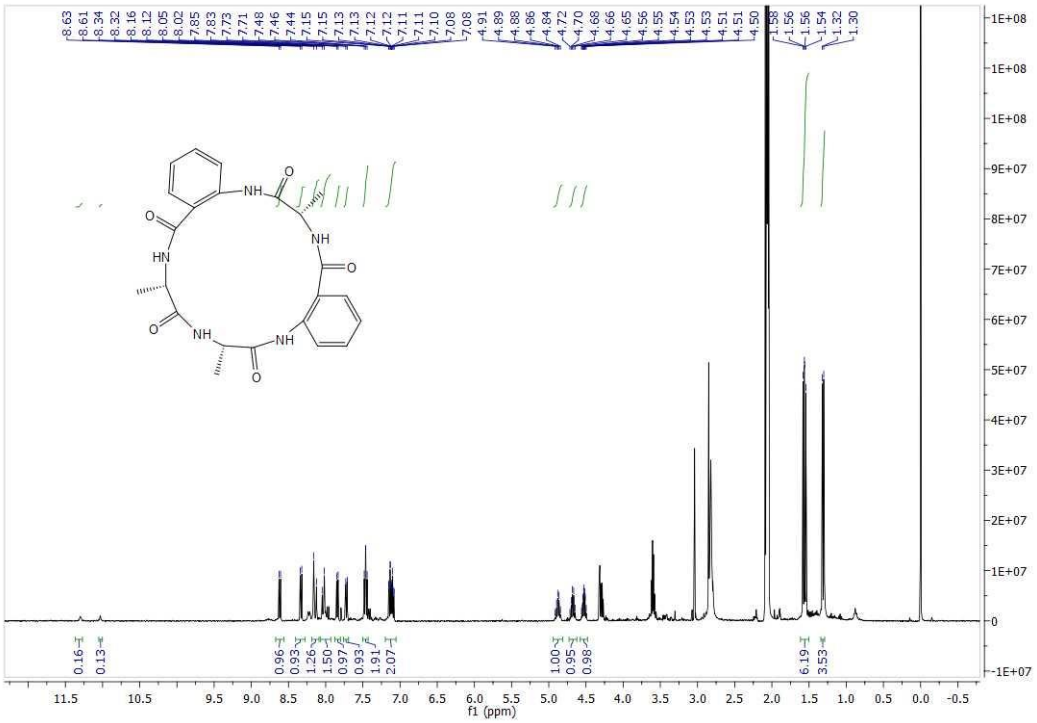


Fig. S36: ^{1}H-NMR in $(CD_3)_2CO$ of *Cyclo*-[Ala-Ala-Anth-Ala-Anth] (4)

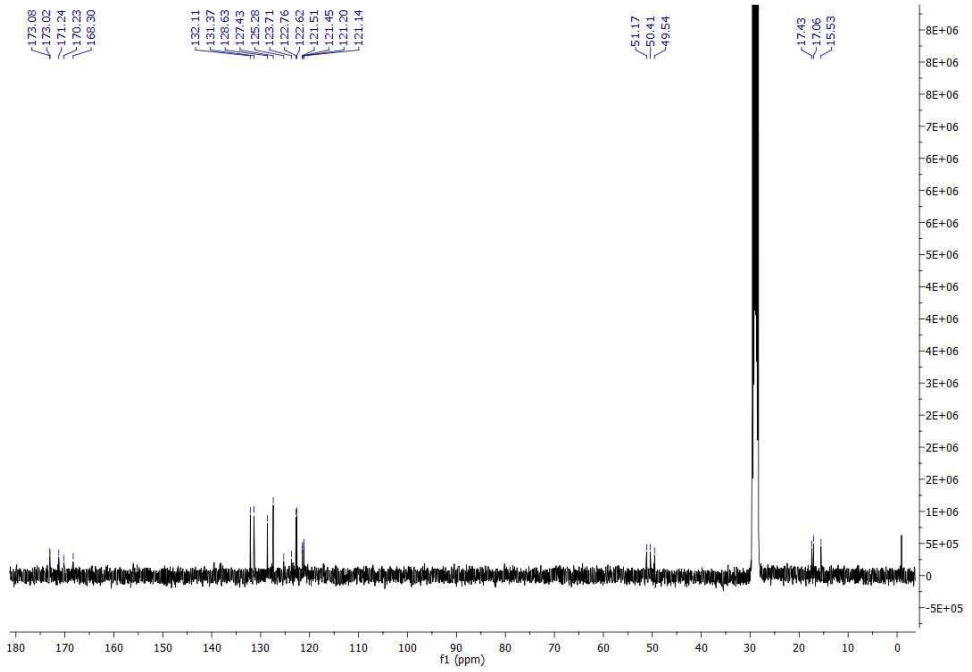
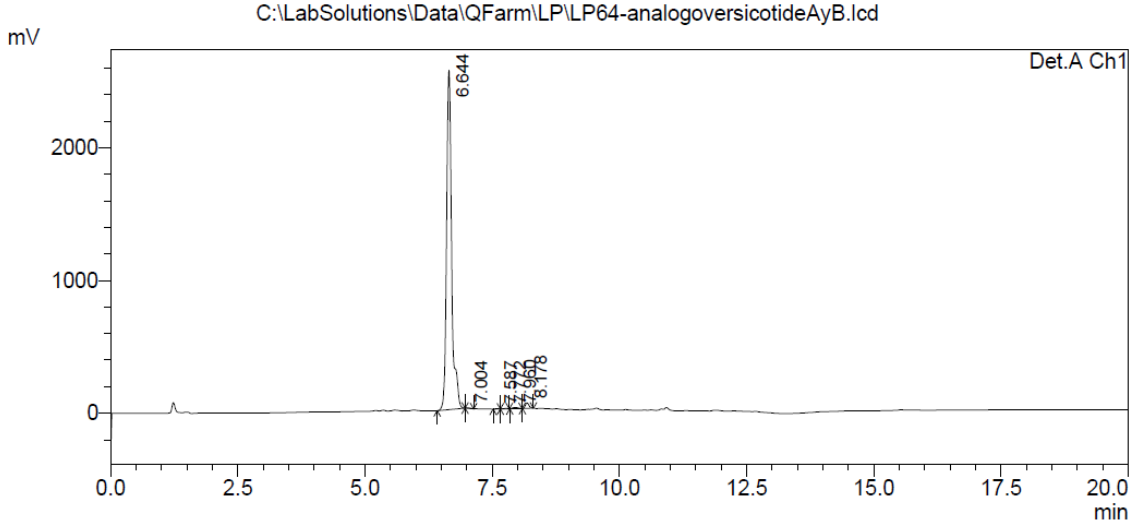


Fig. S37: ^{13}C-NMR in $(CD_3)_2CO$ of *Cyclo*-[Ala-Ala-Anth-Ala-Anth] (6)

<Chromatogram>



PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.644	17623262	2559012	98.213	97.750
2	7.004	18446	2648	0.103	0.101
3	7.587	16018	3991	0.089	0.152
4	7.772	19829	3746	0.111	0.143
5	7.960	67182	8406	0.374	0.321
6	8.178	199105	40116	1.110	1.532
Total		17943841	2617919	100.000	100.000

Fig. S38: Chromatogram *Cyclo*-[Ala-Ala-Anth-Ala-Anth](4)

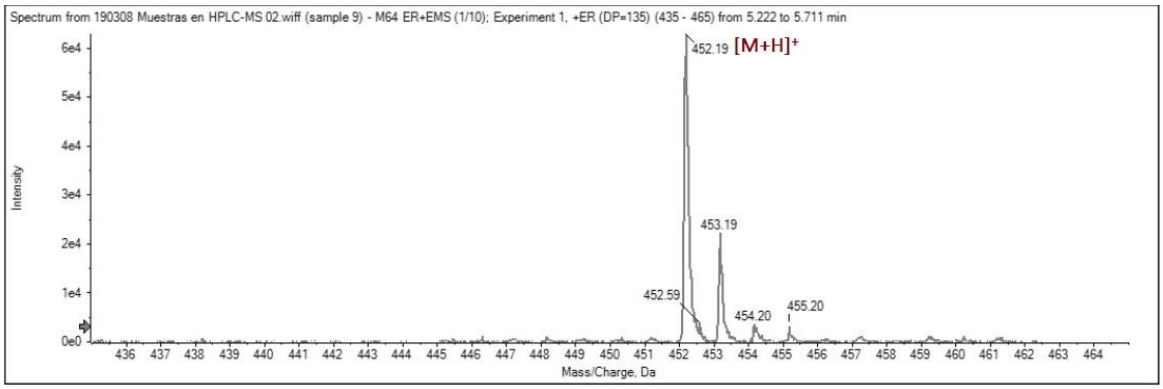


Fig. S39: **ESI-MS of** *Cyclo*-[Ala-Ala-Anth-Ala-Anth]

X ray crystallographic data

CDCC reference number of *Cyclo*-[NHMeAla-Anth-NMeAla-Anth Ala-OH] (Versicotide A):

2023773

checkCIF/PLATON report

Structure factors have been supplied for datablock(s) cu_200311_aplp_lp153_0m_a

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found. CIF dictionary Interpreting this report

Datablock: cu_200311_aplp_lp153_0m_a

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                 alpha=90        beta=93.461(2)      gamma=90
Temperature:     298 K

                Calculated                Reported
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Space group     P 21                      P 1 21 1
Hall group      P 2yb                     P 2yb
Moiety formula  C25 H29 N5 O5, C H4 O     C25 H29 N5 O5, C H4 O
Sum formula     C26 H33 N5 O6             C26 H33 N5 O6
Mr              511.57                    511.57
Dx,g cm-3       1.311                     1.311
Z               2                         2
Mu (mm-1)       0.779                     0.779
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F000'           545.74
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Nref            5249[ 2817]               5186
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Tmin'           0.691

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S = 1.059                  Npar= 361
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The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

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PLAT355_ALERT_3_C Long   O-H (X0.82,N0.98A)  O6       - H33      .       1.06 Ang.
PLAT410_ALERT_2_C Short Intra H...H Contact  H1       ..H23      .       1.95 Ang.
                                                    x,y,z  =      1_555 Check
PLAT414_ALERT_2_C Short Intra D-H..H-X       H5       ..H9               1.94 Ang.
                                                    x,y,z  =      1_555 Check
PLAT911_ALERT_3_C Missing FCF Refl Between Thmin & STh/L=    0.600          3 Report
```

Alert level G

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PLAT007_ALERT_5_G Number of Unrefined Donor-H Atoms ..............          1 Report
PLAT791_ALERT_4_G Model has Chirality at C2          (Chiral SPGR)          S Verify
PLAT791_ALERT_4_G Model has Chirality at C12         (Chiral SPGR)          S Verify
PLAT791_ALERT_4_G Model has Chirality at C23         (Chiral SPGR)          S Verify
PLAT912_ALERT_4_G Missing # of FCF Reflections Above STh/L=  0.600         24 Note
PLAT978_ALERT_2_G Number C-C Bonds with Positive Residual Density.          2 Info
PLAT992_ALERT_5_G Repd & Actual _reflns_number_gt Values Differ by          1 Check
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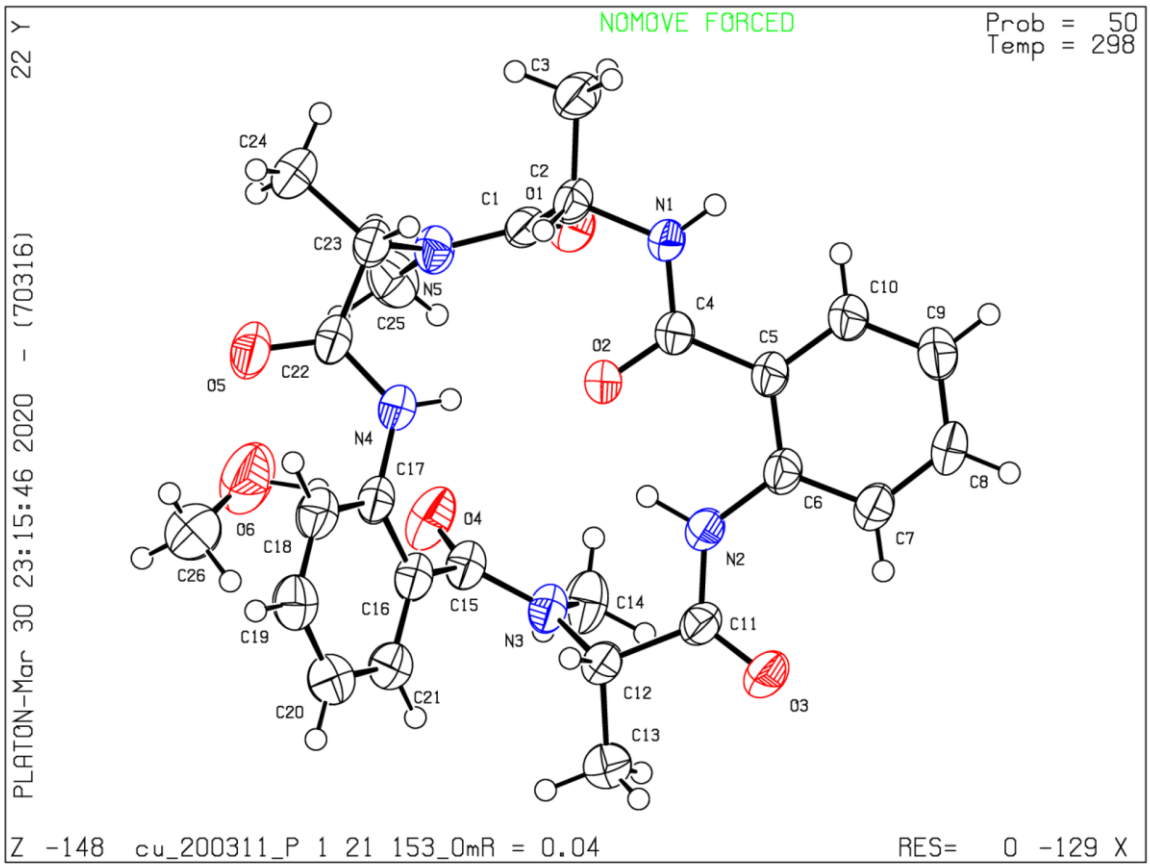
0 **ALERT level A** = Most likely a serious problem - resolve or explain
0 **ALERT level B** = A potentially serious problem, consider carefully
5 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight
7 **ALERT level G** = General information/check it is not something unexpected

0 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
3 ALERT type 2 Indicator that the structure model may be wrong or deficient
3 ALERT type 3 Indicator that the structure quality may be low
4 ALERT type 4 Improvement, methodology, query or suggestion
2 ALERT type 5 Informative message, check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