Supporting Information

D-peptidase activity in a marine mollusk detoxifies a non-ribosomal cyclic lipopeptide: an ecological model to study antibiotic resistance.

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Structure of studied peptides

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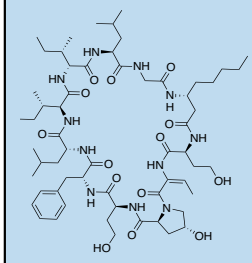
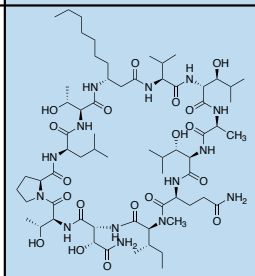
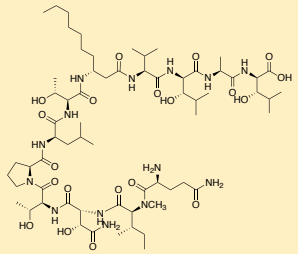
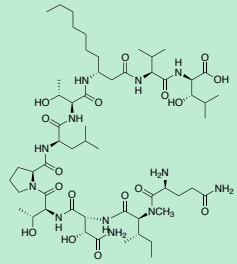
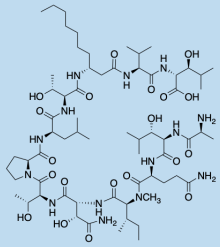
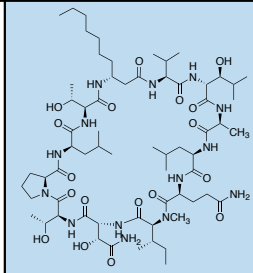
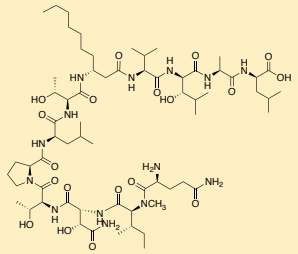
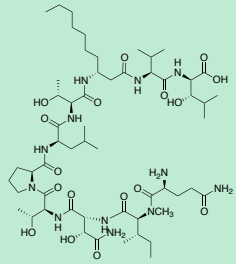
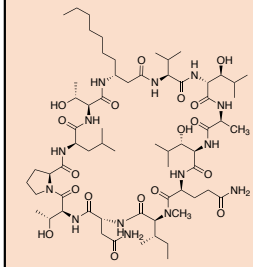
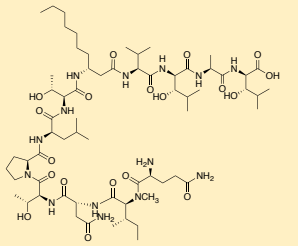
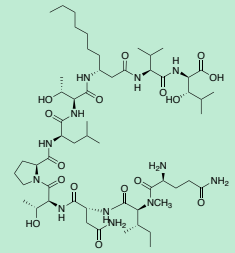
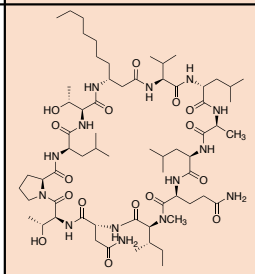
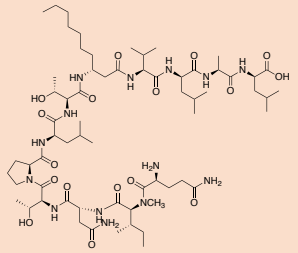
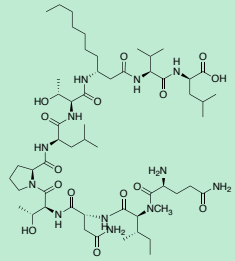
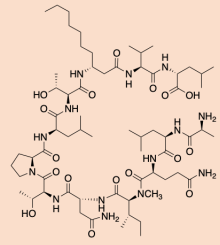
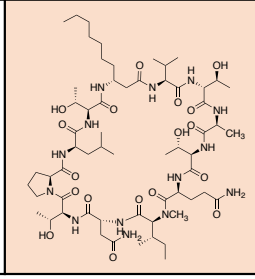
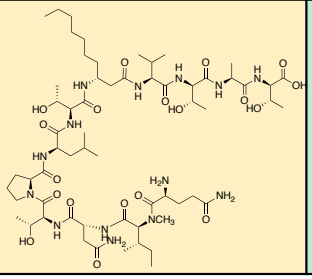
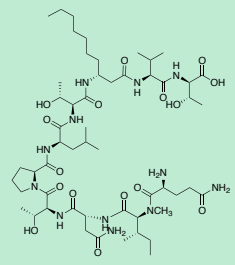
1		**Key**: Blue = Peptides characterized from natural extracts Orange = Synthesized peptides Green = Peptides characterized by LCMS/MSMS Yellow = Supposed peptides		
2				
3				
4				
5				
6				

Table S1b: structure of peptides 7 to 10

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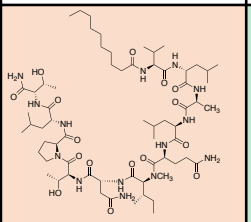
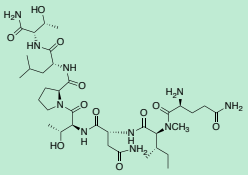
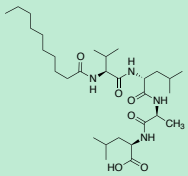
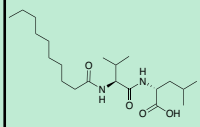
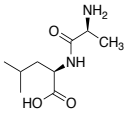
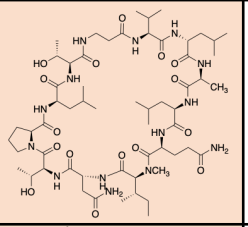
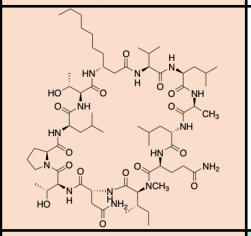
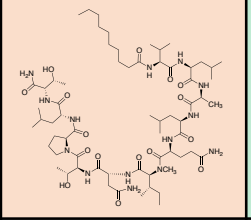
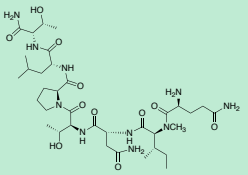
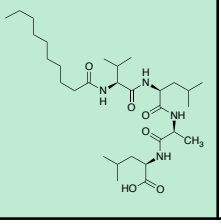
7					
8					
9					
10					

Key:
Orange = Synthesized peptides
Green = Peptides characterized by LCMS/MSMS
White = Noon observed on LCMS

Synthesis yields

Peptides were purified by semi-preparative HPLC except **5b**, difficult to purify because the N-terminal positioned glutamine cyclizes to form a pyroglutamate. The crude peptide having a correct LC-MS profile was therefore used without purification.

Table S2: Masses and yields obtained for the synthesized peptides

Peptides	Recovered mass	Yield (purified peptide)	Purity (determined by LC-MS)
5d	9 mg	7%	89%
5b	65 mg (crude peptide)	-	75%
7a	22 mg	16%	92%
8a	11 mg	9%	88%
9a	2 mg	1.5%	92%
10a	4.4 mg	3%	89%

Syntheses of compounds **4a**, **5a** and **6a** were already reported.[1]

As the peptides do not have chromophores to visualize them by HPLC coupled with UV detection, their purity was estimated by LC-MS analysis. As purity was estimated from the detected peaks of the mass spectra, we cannot exclude was overestimated due to the presence of trace compounds associated with the main peak. Thus the reported purities should be considered to be qualitative than quantitative. Moreover, if impurities are present, they do not impact the biotransformation process which was the main aim of the study. Indeed, it is noteworthy that no associated impurities were detected by NMR. Thus analyses of the peptides by LC-MS and NMR confirmed that the purity of the peptides was higher than 95%.

General experimental conditions

Enzymatic cleavage of peptides by isolated known enzymes

Table S3: Type of enzymes used and their cleavage preferences.

Enzyme name	Pepsin	Elastase	Thermolysin	Subtilisin
Enzyme type	Aspartic peptidase	Serine protease	Metallo peptidase	Serine protease
Cleavage preference	C-terminal of Phe, Leu, Tyr, Trp	C-terminal of Ala, Val, Ser, Gly, Leu, Ile	N-terminal of Leu, Phe, Val, Ile, Ala, Met	Could cleave in N-terminal of Leu

Two buffers were prepared and sterilized by autoclave: a pH 8 buffer (50 mM Tris, pH adjusted with HCl 1 M) and a pH 3 buffer (citric acid 0.1 M, sodium citrate 0.1 M, pH adjusted with NaOH 1 M). Stock solutions of peptides were prepared at 10 mg/mL in DMSO. A stock solution of Pepsin (purchased from Promega) was prepared at 2 mg/mL in buffer pH 3 then was diluted 1.6 times in the same buffer to give a Pepsin solution at 1,24 mg/mL. A stock solution of Elastase (purchased from Promega) was prepared at 1 mg/mL in buffer pH 8 then was diluted 1.06 times in the same buffer to give an Elastase solution at 0.94 mg/mL. A stock solution of Thermolysin (purchased from Promega) was prepared at 2 mg/mL in buffer pH 8 then was diluted 1.5 times in the same buffer to give a Thermolysin solution at 1,3 mg/mL. A

stock solution of Subtilisin (purchased from Sigma Aldrich, ref P8038) was prepared at 1 mg/mL in buffer pH 8 then was diluted 1.02 times in the same buffer to give a Subtilisin solution at 0.98 mg/mL.

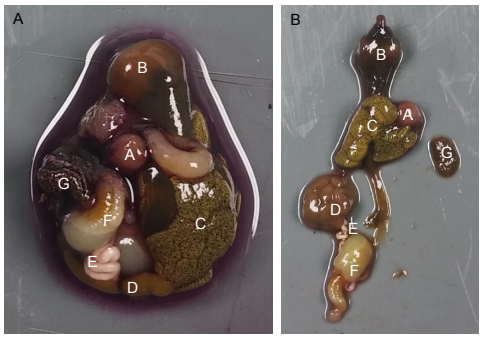
Peptide solutions were diluted 10 times in buffer pH 3 and pH 8 to give peptide solutions at 1 mg/mL (10% DMSO in buffer). These solutions (1 mg/mL) were mixed with appropriate enzyme stock solutions to obtain an enzyme/substrate ratio of 1/20.

Solutions were incubated under agitation (37°C for Pepsin and Elastase, 75°C for Thermolysin and 60°C for Subtilisin). For each kinetic point, the same procedure was applied: solutions were centrifuged a few seconds, then part of the solution was taken off to be quenched by 90% MeOH, obtaining peptide solutions at 0.01 mg/mL (DMSO/buffer/MeOH, 0.1:9.9:90, v/v). The quenched solutions were centrifuged at 21,952 x g for 20 minutes at 4°C. The maximum of supernatant was recovered in a LC-MS vial to be analyzed. All tests were performed in triplicate.

Mollusks dissection

Figure S1: External (A) and internal (B) structures of the sea hare *Stylocheilus striatus*.

A. Mouth and buccal mass. B. Gizzard. C: Hepatopancreas. D. Ovo-testis. E. hermaphroditic duct. F. Mucus and albumin gland. G. Mantle.



Protein quantification by the Bradford method

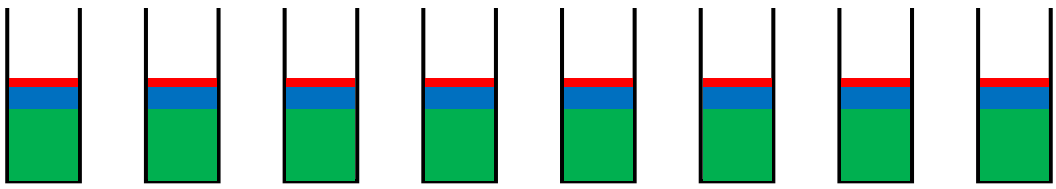
Preparation of the standard range:

We have at our disposal a stock solution of BSA (bovine serum albumin) at 10 mg/mL.

The stock solution of BSA was diluted 50 times in H_2O milliQ (4 µL of stock BSA +196 µL of H_2O milliQ). The new BSA solution is therefore 0,2 mg/mL.

We used disposable plastic cuvettes for spectrophotometer (Plastibrand, 70 L micro, 12.5 x 12.5 x 45 mm). The preparation of the BSA standard range was as follows (amount of BSA solution at 0.2 mg/mL in red, amount of H_2O milliQ in green, amount of Bradford's solution in blue, resulted BSA concentration in cuvettes in black):

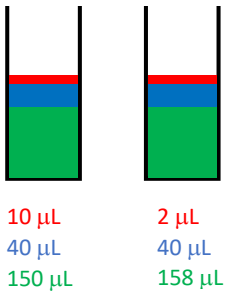
Figure S2: Preparation of the BSA standard range



Bradford's solution was purchased from Bio-Rad.

The cuvettes must be well mixed and bubble-free for measurement. The spectrophotometer used is an Eppendorf BioPhotometer with a single beam. In a first step, it is necessary to calibrate the instrument with standard solutions containing BSA that were prepared earlier. The first cuvette that does not contain BSA was analyzed and assimilated to the "blank" solution. Then other cuvettes of the range were analyzed one by one and represent "standard" solutions. The instrument displays the absorbance of the solutions contained in the cuvettes and assimilates it to the protein quantities indicated previously in the parameters of the spectrophotometer. Absorbance values obtained provide a linear calibration curve.
Preparation of the samples to be assayed:
Each digestive gland extract is diluted 50 times (1 µL in 50 µL of H_2O milliQ). The cuvettes are then prepared as follows (amount of extract diluted solution in red, H_2O milliQ in green, amount of Bradford's solution in blue). If the results obtained are outside the linearity of the model, amounts of extract are adjusted:

Figure S3: Preparation of the samples to be assayed



It is then possible to determine the amount of protein contained in the digestive gland extracts.

Heating of the digestive gland of the mollusk

A pH 8 buffer (50 mM Tris, pH adjusted with HCl 1 M) was prepared and sterilized by autoclave. A stock solution of **2a** was prepared at 10 mg/mL in DMSO. This stock solution was diluted 10 times in buffer. *Dg-Ss-Lm* extract was heated at 90°C for 5 minutes while vortexing the solution from time to time and checking that there was no evaporation of the buffer. **2a** solution at 1 mg/mL was mixed with *Dg-Ss-Lm* extract previously heated (10 mg/mL) and completed with the same buffer to afford **2a** at 0.1 mg/mL and extract at 1 mg/mL. Solution was incubated at 30°C under agitation. For each kinetic point, the same procedure was applied: solution was centrifuged a few seconds, then part of the solution was taken off to be quenched by 90% MeOH, obtaining **2a** at 0.01 mg/mL. The quenched solution was centrifuged at 21,952 x g for 20 minutes at 4°C. The maximum of supernatant was recovered in a LCMS vial to be analyzed. In parallel, **2a** stability was test at 90°C.

Analyses parameters

Table S4: Tune parameters for LCMS injections

ESI Source	Values	Ions optics	Values
Sheath Gas Flow Rate (arb)	25	RF Lens offset (V)	-2.75
Aux Gas Flow Rate (arb)	10	Lens 0 Voltage (V)	-5.5
Sweep Gas Flow Rate (abr)	1	Multipole 0 offset (V)	-8.5
Spray Voltage (kV)	4.5	Lens 1 Voltage (V)	-13
Spray current (μA)	0.12	Gate Lens Voltage (V)	-48
Capillary Temperature (°C)	290	Multipole 1 offset (V)	-19
Capillary Voltage (V)	33	Multipole RF Amplitude (V p.p)	400
Tube Lens (V)	80	Front Lens (V)	-65.93

Table S5: Parameters of the method used for LCMS analysis of purified compound

Autosampler	**Values**	**Pump**	**Values**
Injection volume (μL)	10	Pressure (bar)	From 10 to 600
Flush volume (μL)	400	Pressure stability (bar)	10
Wash volume (μL)	1000	**MS detector**	**Values**
Tray temperature (°C)	20	Mass range (m/z)	From 100 to 2000 or from 400 to 2000
Column oven temperature (°C)	30	Scan Type	Full
PDA Detector	**Values**	Polarity	Positive/Negative
Wavelength (nm)	From 200 to 600	Data Type	Centroid
Channels (nm)	214 and 254	Source Fragmentation	NO

Intermediate compounds were analyzed by direct infusion on the same LCMS device with a flow rate of 10 μL/min.

Table S6: Tune parameters for LCMS direct infusions

ESI Source	Values	Ions optics	Values
Sheath Gas Flow Rate (arb)	5	RF Lens offset (V)	-7.25
Aux Gas Flow Rate (arb)	1	Lens 0 Voltage (V)	-7.5
Sweep Gas Flow Rate (abr)	0	Multipole 0 offset (V)	-7.75
Spray Voltage (kV)	4.5	Lens 1 Voltage (V)	-10
Spray current (μA)	0.02	Gate Lens Voltage (V)	-46
Capillary Temperature (°C)	290	Multipole 1 offset (V)	-10
Capillary Voltage (V)	33	Multipole RF Amplitude (V p.p)	400
Tube Lens (V)	80	Front Lens (V)	-55.93

Table S7: Tune parameters for HRESI-MS direct infusions are as follows

HESI Source	Values	Scan parameters	Values
Sheath Gas Flow Rate (arb)	10	Scan type	Full MS
Aux Gas Flow Rate (arb)	0	Scan range	500 to 2000
Sweep Gas Flow Rate (abr)	0	Fragmentation	NO
Spray Voltage (kV)	3.2	Resolution	140000
Spray current (μA)	0.02	Polarity	Positive
Capillary Temperature (°C)	300	Microscans	1
Aux Gas Heater Temperature (°C)	50	AGC target	10^6

Table S8: Tune parameters for HRESI-MS injections are as follows:

HESI Source	Values	Scan parameters	Values
Sheath Gas Flow Rate (arb)	60	Scan type	Full MS
Aux Gas Flow Rate (arb)	20	Scan range	100 to 1500
Sweep Gas Flow Rate (abr)	0	Fragmentation	NO
Spray Voltage (kV)	3.2	Resolution	35
Spray current (μA)	0.02	Polarity	Positive/Negative
Capillary Temperature (°C)	360	Microscans	1
Aux Gas Heater Temperature (°C)	300	AGC target	10^6

For MS-MS analyses, the same tunes were used, with collision energies between 20 and 60 V.

Different LC-MS gradients were tested to afford the detection of laxaphycin B and its analogues. The gradient needed to be large enough to see the original peptide and its possible opened fragments.

Table S9: Optimized gradient for each peptide (for 500 μL/min)

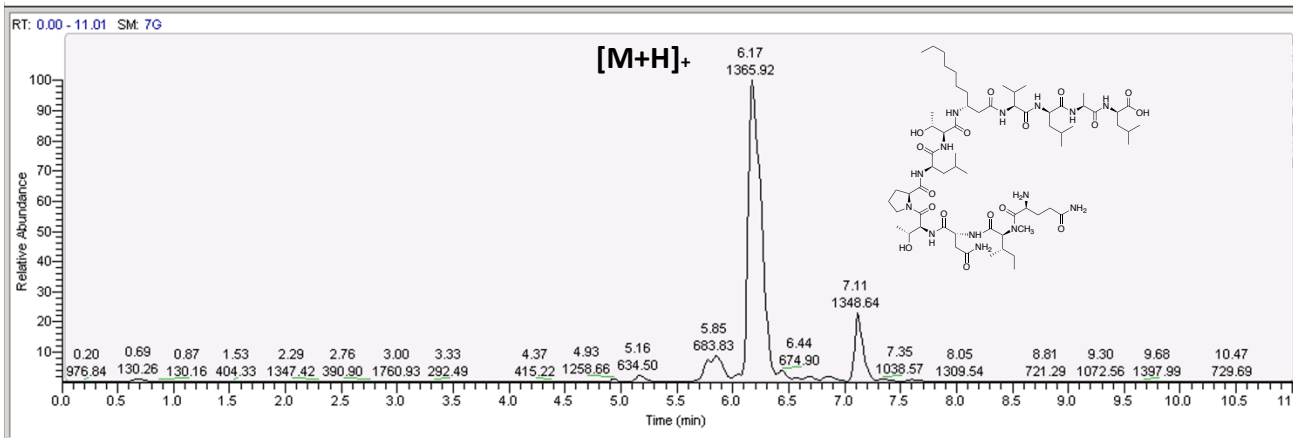
Time (min)	% Water (0.1% Formic acid)	% ACN (0.1% Formic acid)
0	80	20
2	80	20
8	10	90
9	10	90
10	80	20
11	80	20

LC-HRMS analyses

Analyses of compounds **4a**, **5a** and **6a** have already been published.[1]

Figure S4: LC-HRMS analysis of compound 5b

Calculated for $C_{65}H_{116}N_{14}O_{17}$ $[M+H]_+$ *m/z* 1365.87156; Found $[M+H]_+$ *m/z* 1365.87311; $\Delta m/z$ = 1.14 ppm



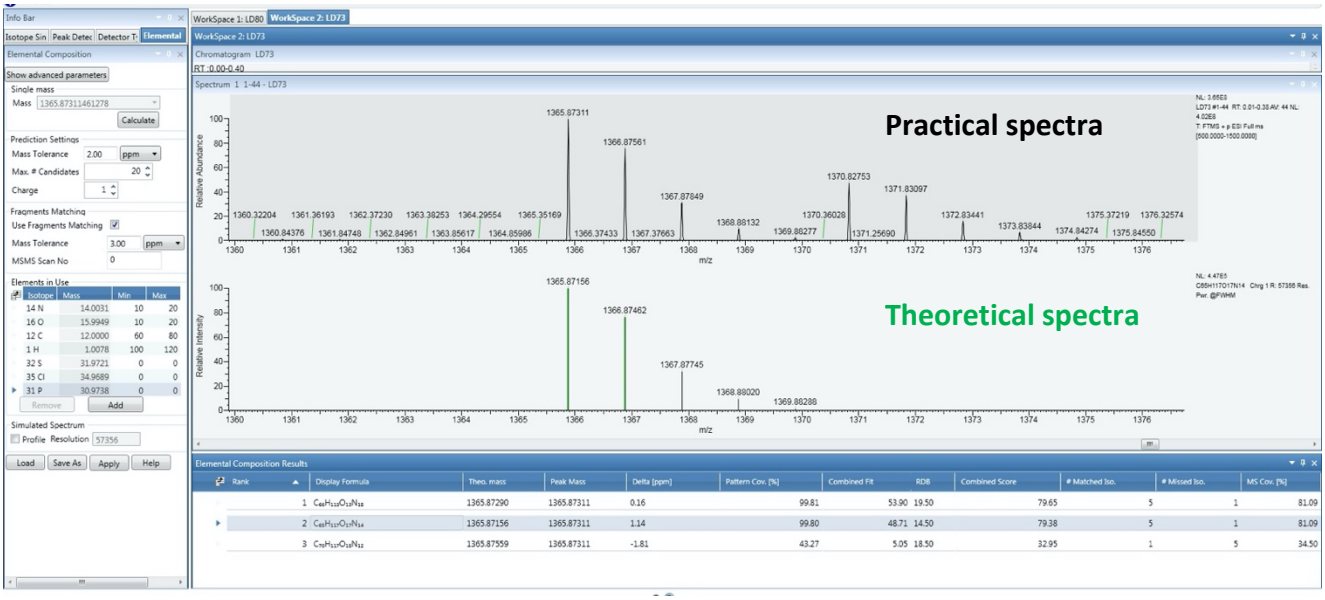
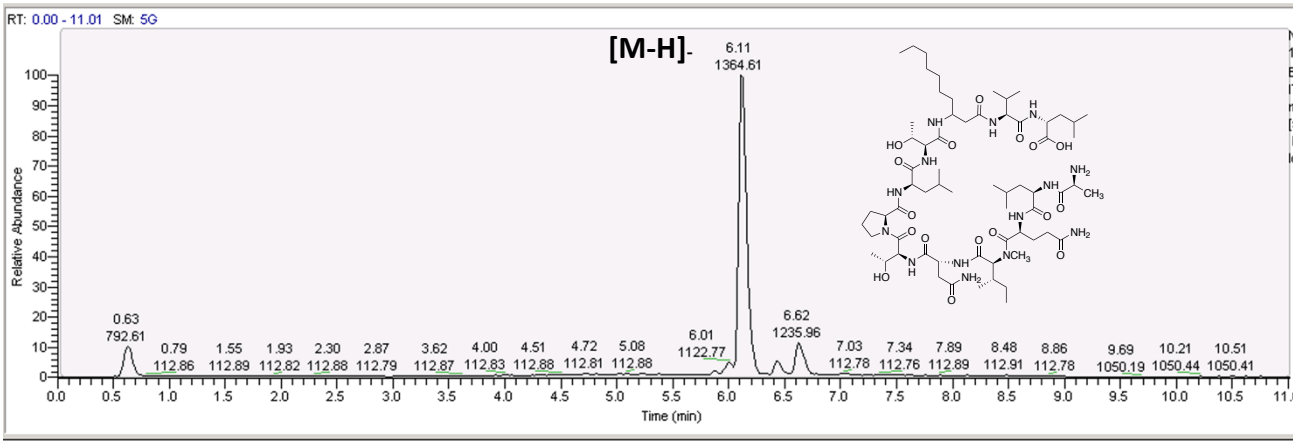


Figure S5: LC-HRMS analysis of compound 5d

Calculated for $C_{65}H_{116}N_{14}O_{17}$ $[M+H]_+$ *m/z* 1365.87156; Found $[M+H]_+$ *m/z* 1365.87343; $\Delta m/z$ = 1.37 ppm



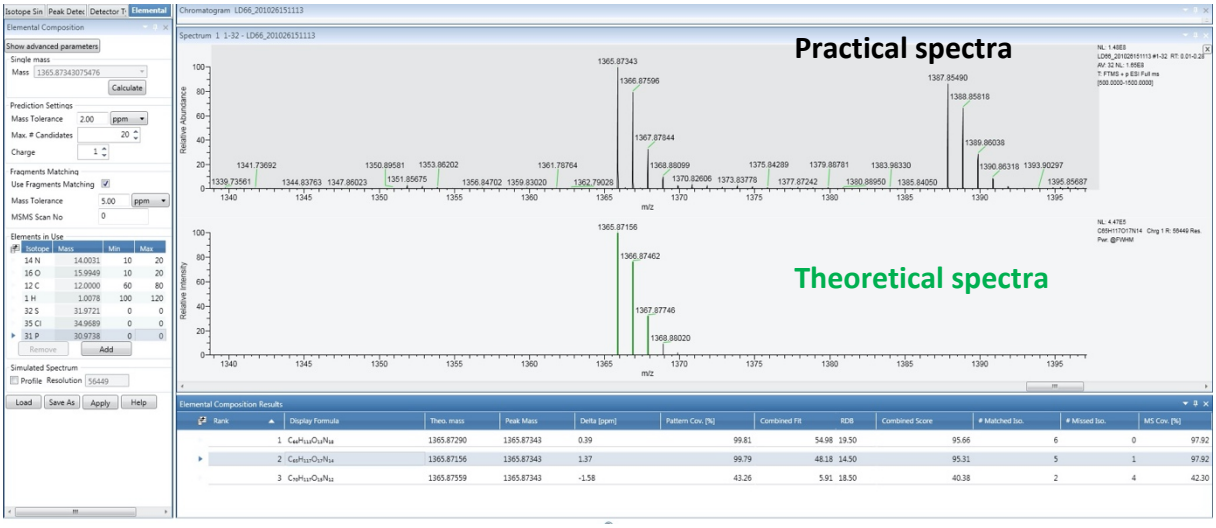
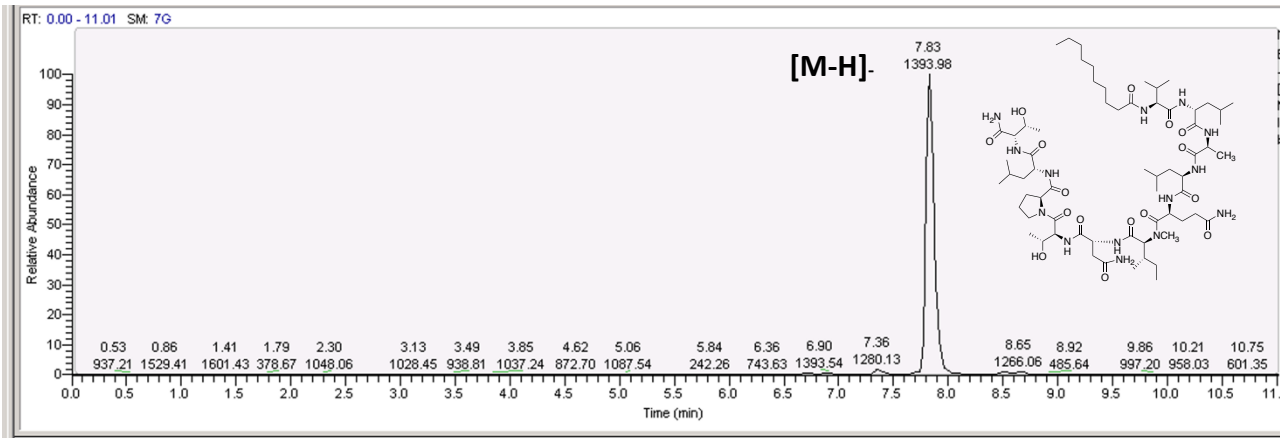


Figure S6: LC-HRMS analysis of compound 7a

Calculated for $C_{65}H_{116}N_{14}O_{16}Na$ $[M+Na]^+$ *m/z* 1371.85859; Found $[M+Na]^+$ *m/z* 1371.85998; Δ*m/z* = 1.01 ppm



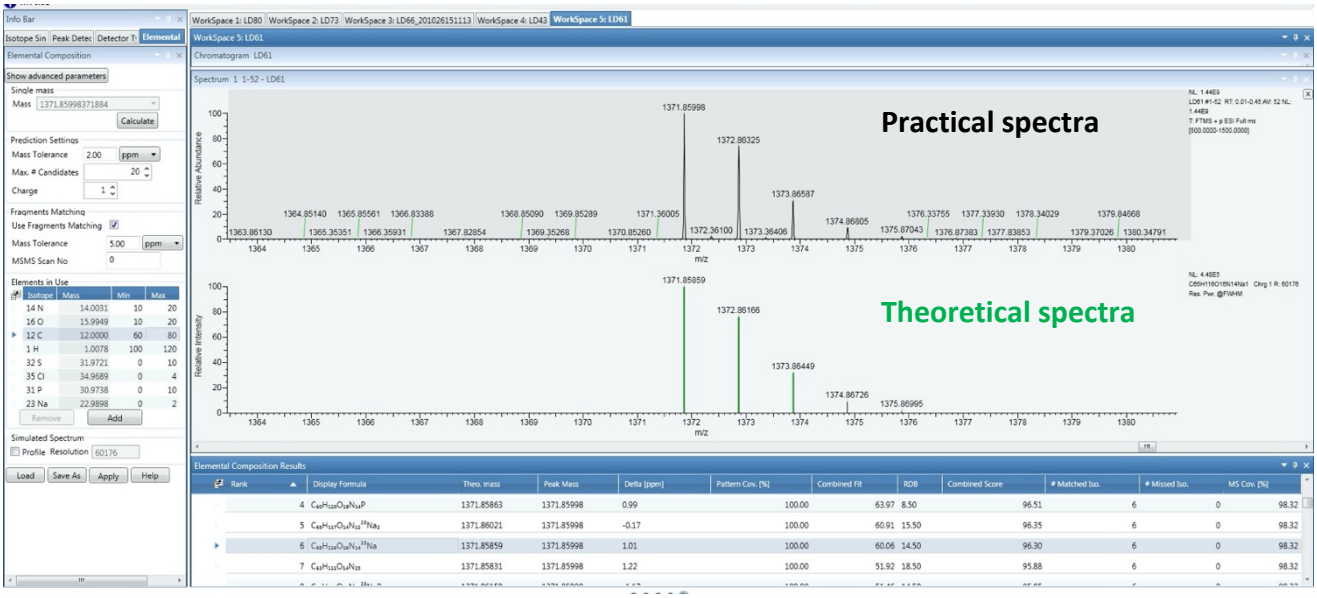
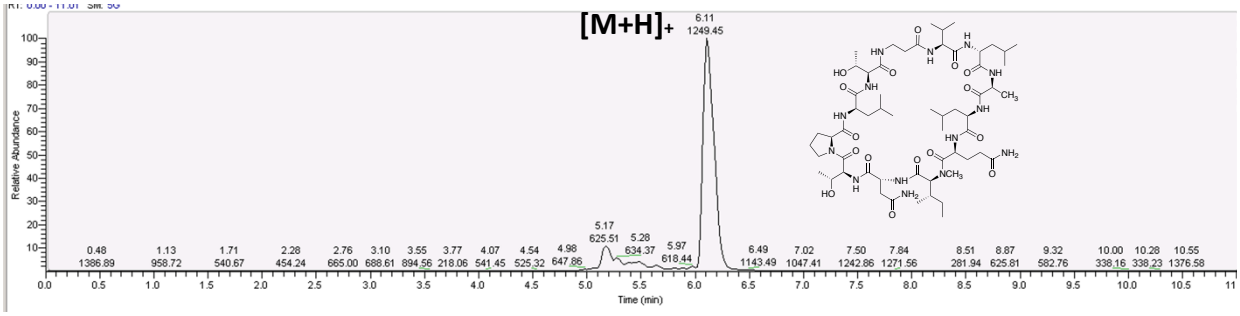


Figure S7: LC-HRMS analysis of compound 8a

Calculated for $C_{58}H_{100}N_{14}O_{16}Na$ $[M+Na]_+$ *m/z* 1271.73339; Found $[M+Na]_+$ *m/z* 1271.73267; $\Delta m/z$ = 0.57 ppm



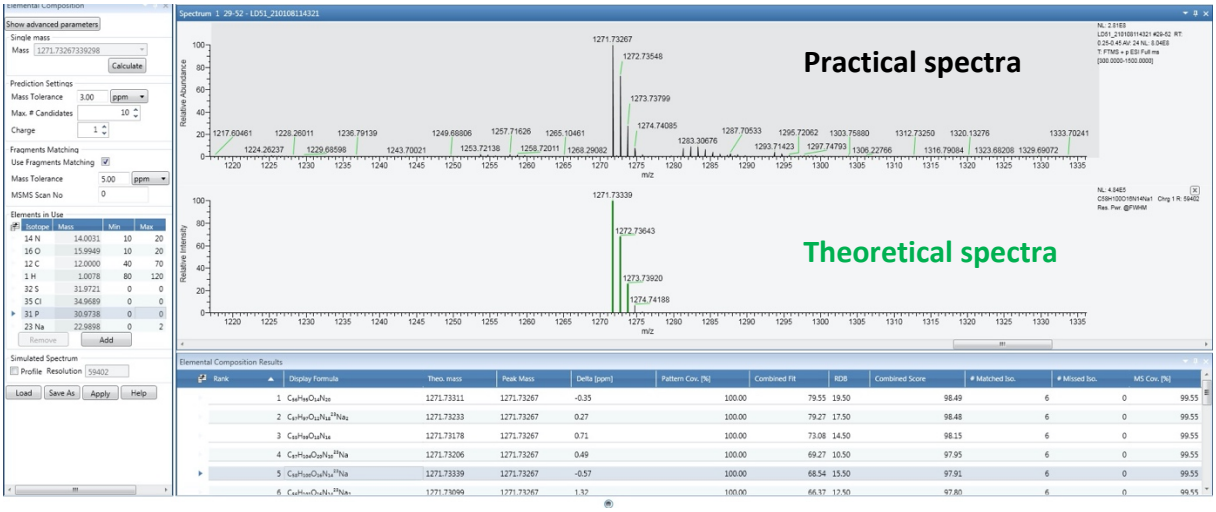
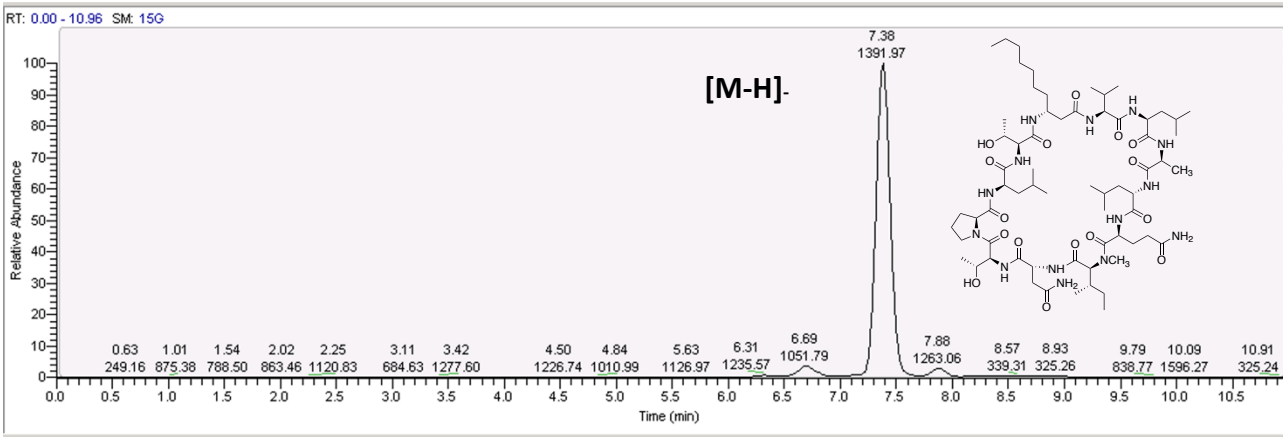


Figure S8: LC-HRMS analysis of compound 9a

Calculated for $C_{65}H_{114}N_{14}O_{16}Na$ $[M+Na]_+$ *m/z* 1369.84294; Found $[M+Na]_+$ *m/z* 1369.84417; $\Delta m/z$ = 0.89 ppm



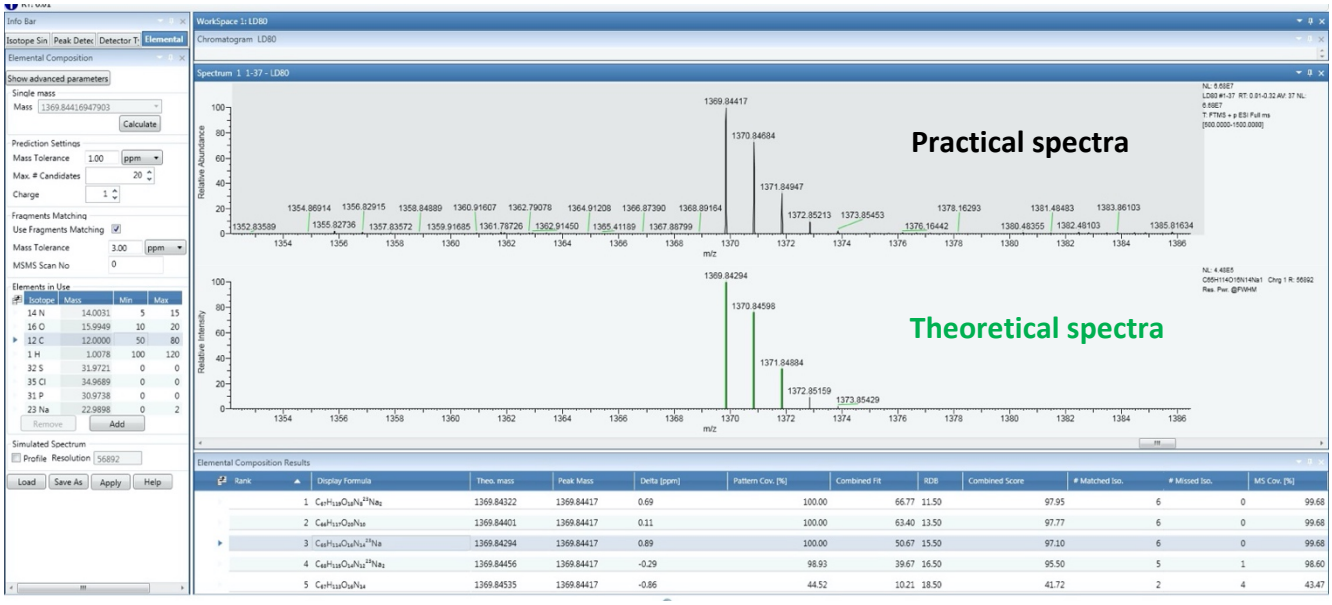
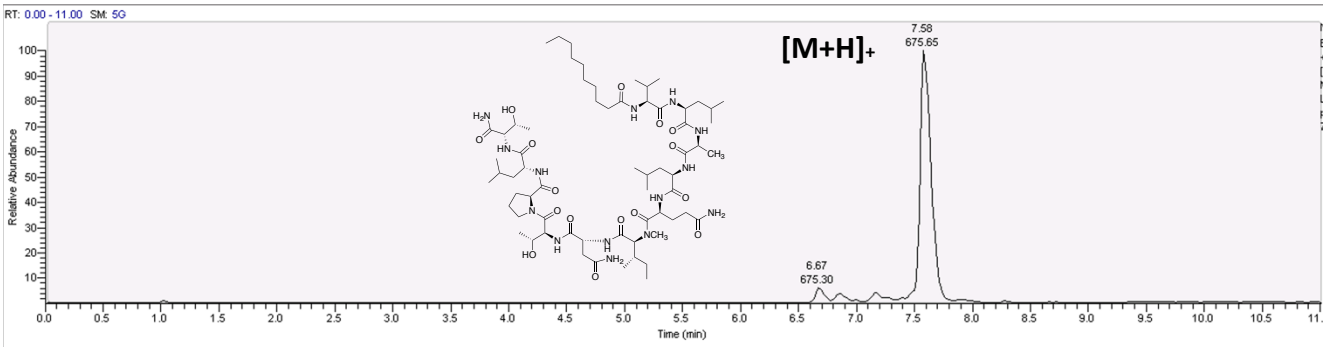
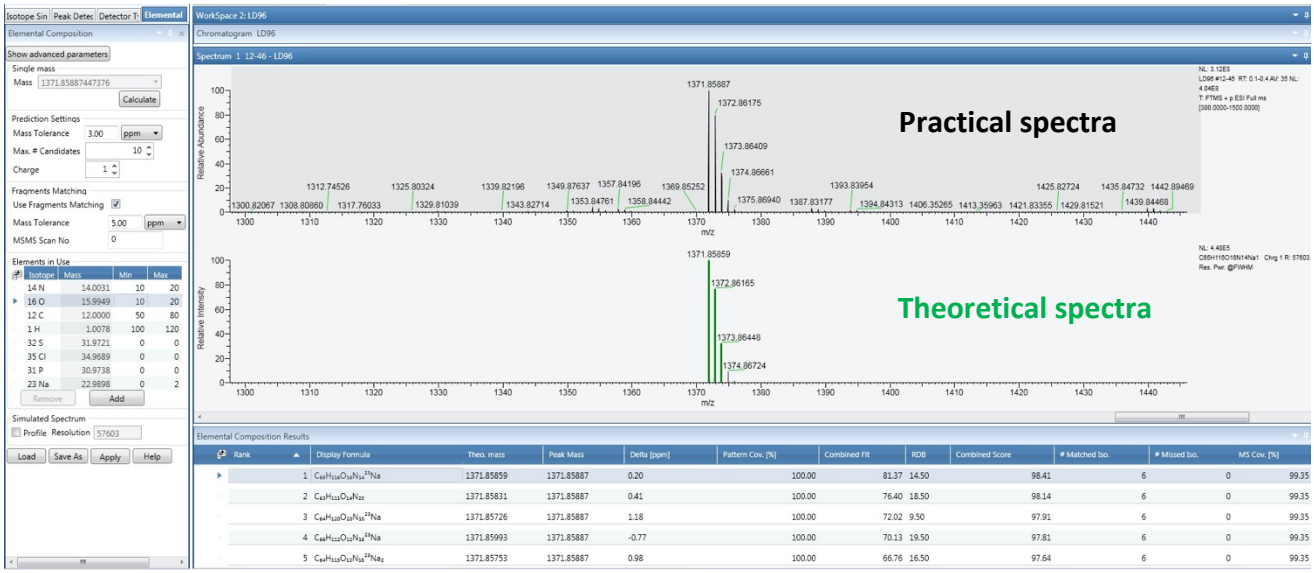


Figure S9: LC-HRMS analysis of compound 10a

Calculated for $C_{65}H_{116}N_{14}O_{16}Na$ $[M+Na]_+$ *m/z* 1371.85859; Found $[M+Na]_+$ *m/z* 1371.85887; Δ*m/z* = 0.2 ppm





NMR analyses

Analyses of compounds **4a**, **5a** and **6a** have already been published.[1]

Table S10: NMR spectroscopic data of 5b in DMSO-*d*$_6$.

entry	position	C, mult.	H, mult. (J in Hz)	entry	position	C, mult.	H, mult. (J in Hz)
Ade[1]	1	170.6, C	-	**N-MeIle**[7]	1	171.2, C	-
	2	40.4, CH_2	2.36		2	59.8, CH	4.70, d (10.88)
			2.25		3	31.7, CH	1.94
	3	46.2, CH	4.04		3-Me	15.2, CH_3	0.82
	4	33.6, CH_2	1.31		4	24.1, CH_2	1.33
			1.39				
	5	25.2, CH_2	1.26		5	10.7, CH_3	0.83
			1.16		N-Me	30.2, CH_3	2.97
	6	28.8, CH_2	1.19	**D-Asn**[8]	1	171.2, C	-
	7	28.8, CH_2	1.19		2	49.6, CH	4.60, t (6.30)
	8	31.2, CH_2	1.19		3	36.8, CH_2	2.50
	9	22.1, CH_2	1.24				2.40
	10	13.9, CH_3	0.84		4	171.6, C	-
	NH	-	7.55, d (8.02)		NH	-	8.20, d (7.45)
Val[2]	1	171.2, C	-		NH_2	-	7.35 & 6.94
	2	58.7, CH	4.06	**Thr**[9]	1	168.8, C	-
	3	30.0, CH	1.92		2	55.3, CH	4.54, t (7.45)
	3-Me	19.08, CH_3	0.81		3	66.6, CH	3.93
	4	18.4, CH_3	0.84		4	18.7, CH_3	1.05, d (6.30)
	NH	-	7.97, d (9.16)		3-OH	-	5.10
D-Leu[3]	1	170.7, C	-		NH	-	7.84, d (7.45)
	2	51.1, CH	4.25	**Pro**[10]	1	171.6, C	-
	3	40.5, CH_2	1.50		2	59.8, CH	4.33
	4	24.1, CH	1.57		3	29.1, CH_2	1.83
	4-Me	20.9-23.2, CH_3	0.81-0.87				2.03
	5	20.9-23.2, CH_3	0.81-0.87		4	24.2, CH_2	1.82
	NH	-	8.19, d (7.45)				1.89
Ala[4]	1	172.0, C	-		5	47.4, CH_2	3.73
	2	51.1, CH	4.32				3.73
	3	18.4, CH_3	1.21	**D-Leu**[11]	1	171.8, C	-
	NH	-	7.80, d (8.59)		2	48.2, CH	4.26
D-Leu[5]	1	173.9, C	-		3	40.5, CH_2	1.50
	2	50.0, CH	4.24		4	24.1, CH	1.57
	3	40.5, CH_2	1.50		4-Me	20.9-23.2, CH_3	0.81-0.87
	4	24.1, CH	1.57		5	20.9-23.2, CH_3	0.81-0.87
	4-Me	20.9-23.2, CH_3	0.81-0.87		NH	-	7.90, d (7.45)
	5	20.9-23.2, CH_3	0.81-0.87	**Thr**[12]	1	168.8, C	-
	NH	-	7.95, d (8.59)		2	58.0, CH	4.11
Gln[6]	1	171.9, C	-		3	66.6, CH	3.96
	2	49.6, CH	4.39		4	19.6, CH_3	0.98, d (6.30)
	3	25.6, CH_2	1.91		3-OH	-	4.78
	4	29.5, CH_2	2.23		NH	-	7.62, d (8.59)
	5	173.6, C	-				
	NH_2	-	7.29 & 6.99				
	NH_2-term	-	7.37 & 6.96				

The numbering of atoms corresponds to the one already reported in the article on trichormamide C.[1]
The determination of the structure was made using several NMR spectra (Figures S10a-f).

Figure S10a: ^{1}H NMR spectrum of compound 5b in DMSO-d$_6$

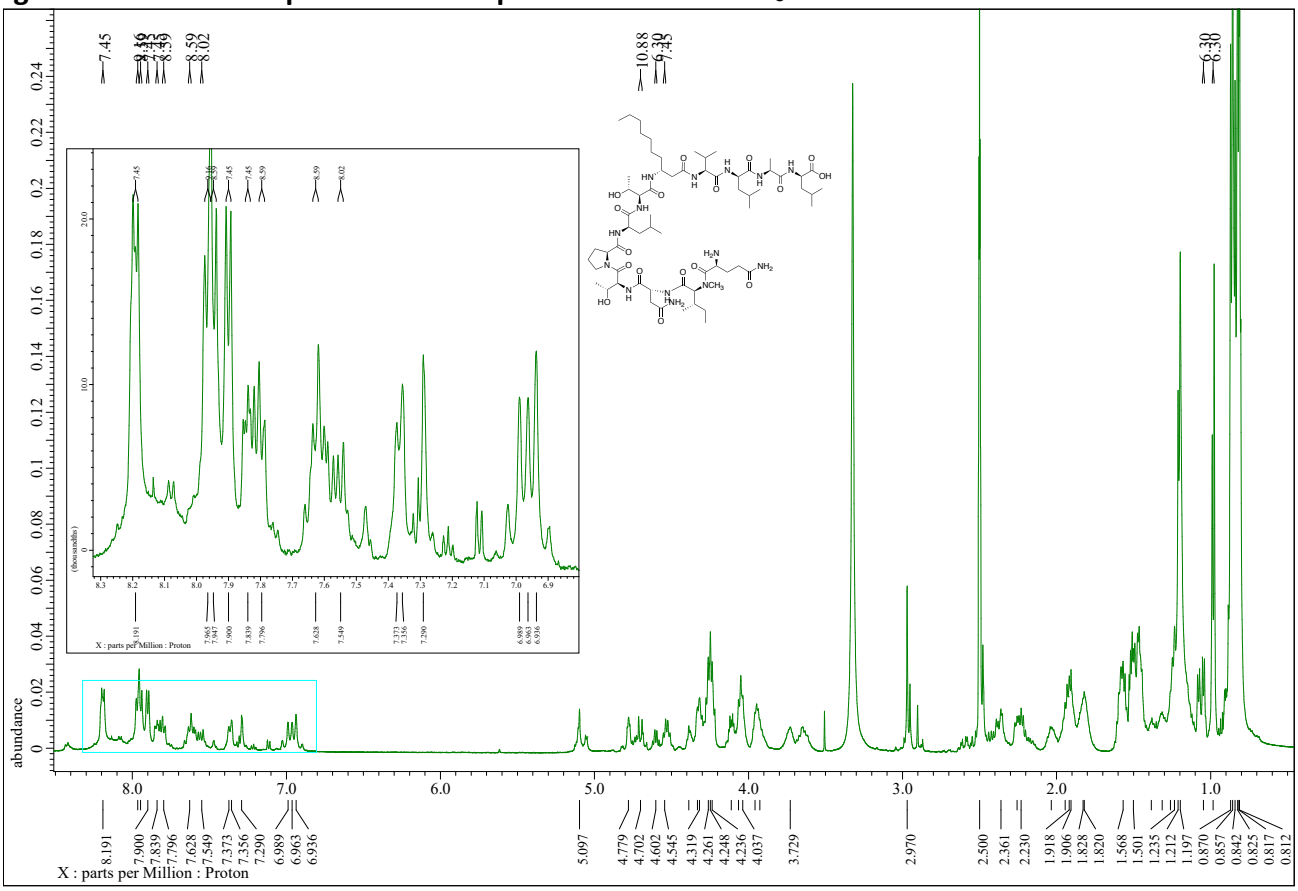


Figure S10b: ^{13}C NMR spectrum of compound 5b in DMSO-d$_6$

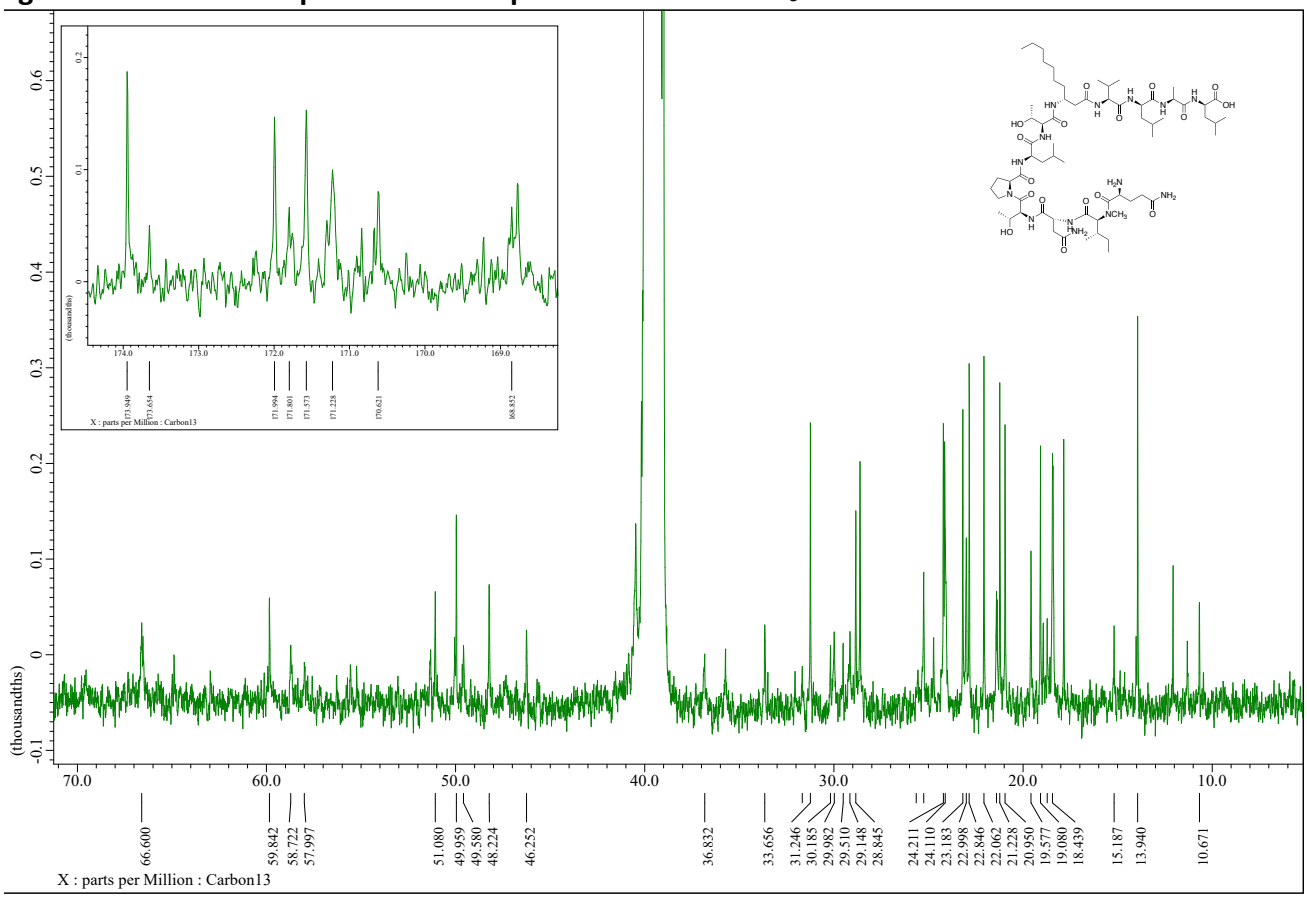


Figure S10c: TOCSY spectrum of compound 5b in DMSO-d_6

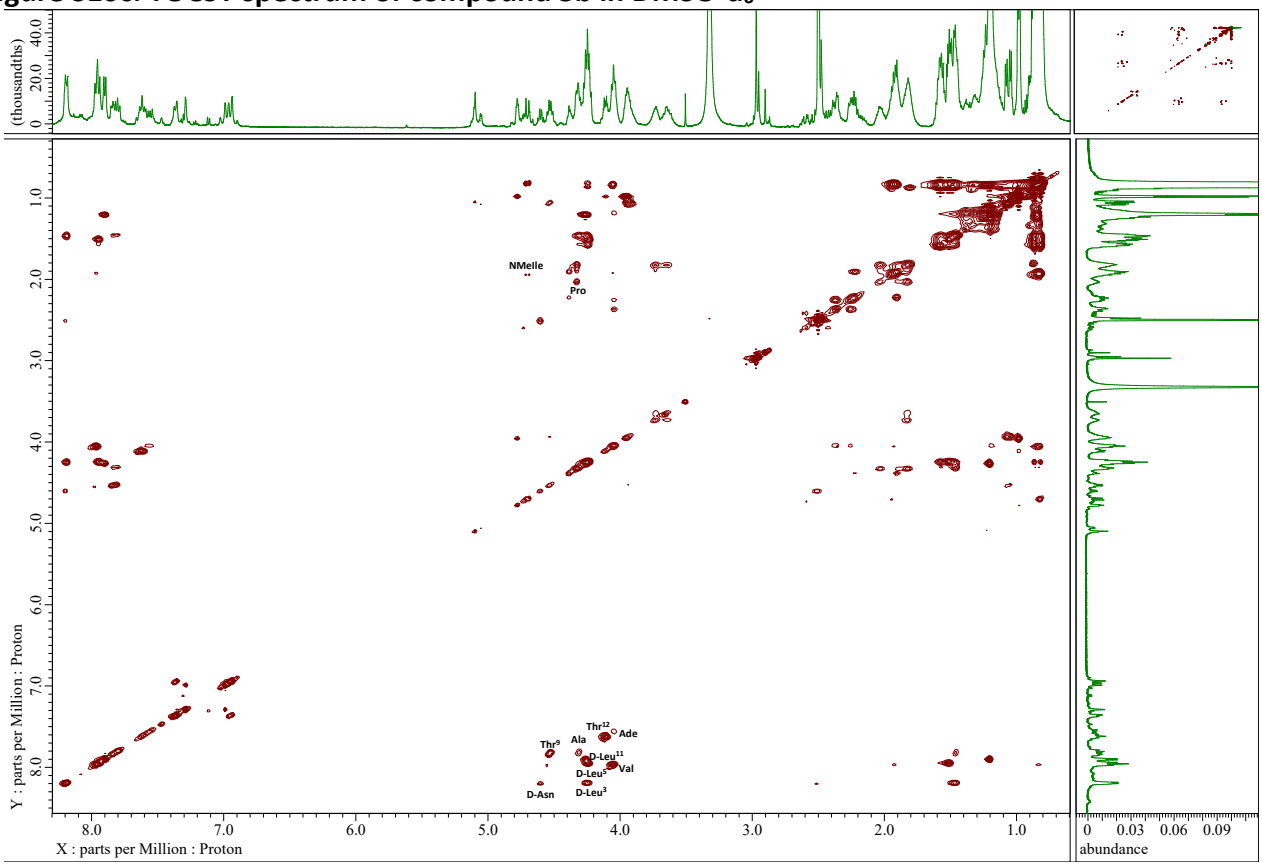


Figure S10d: ROESY spectrum of compound 5b in DMSO-d_6

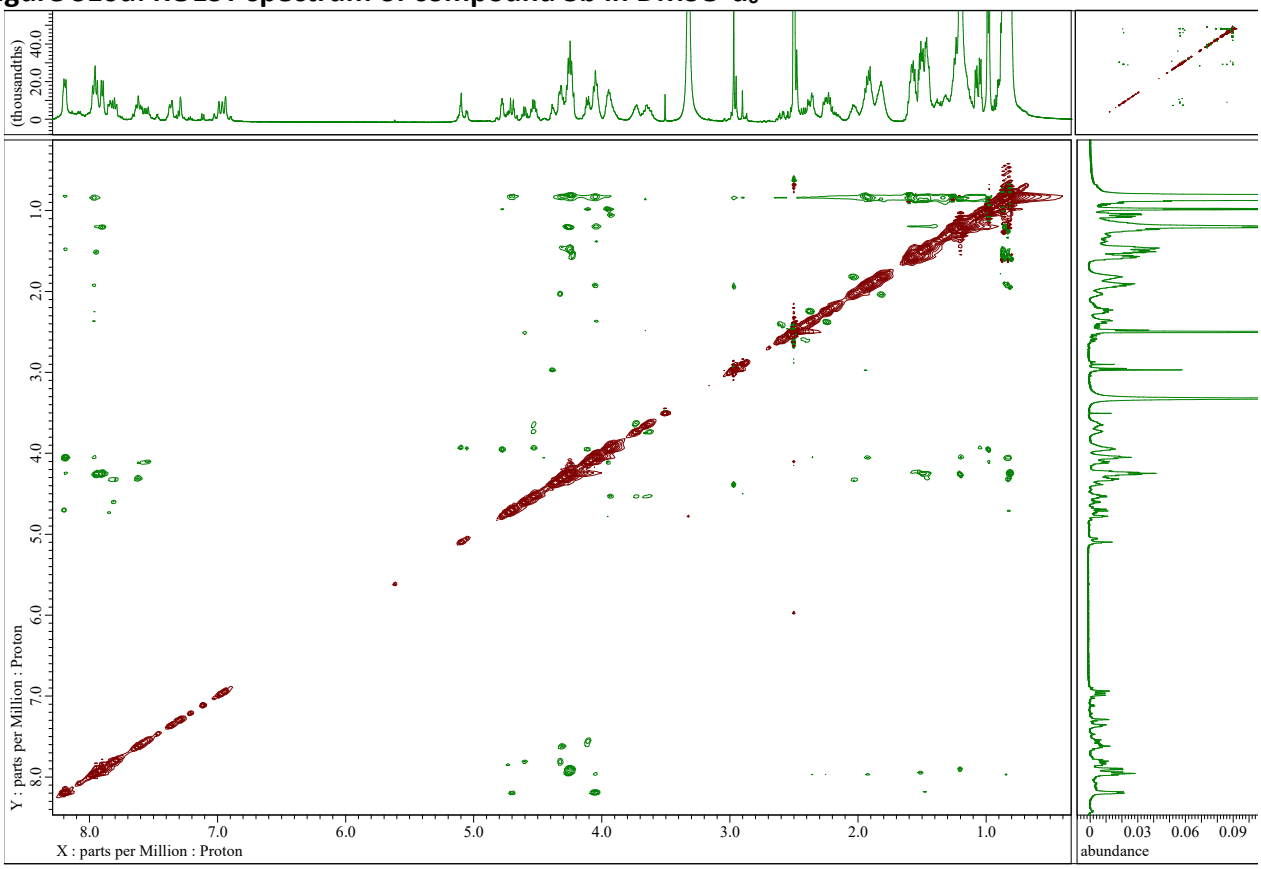


Figure S10e: HSQC spectrum of compound 5b in DMSO-d_6

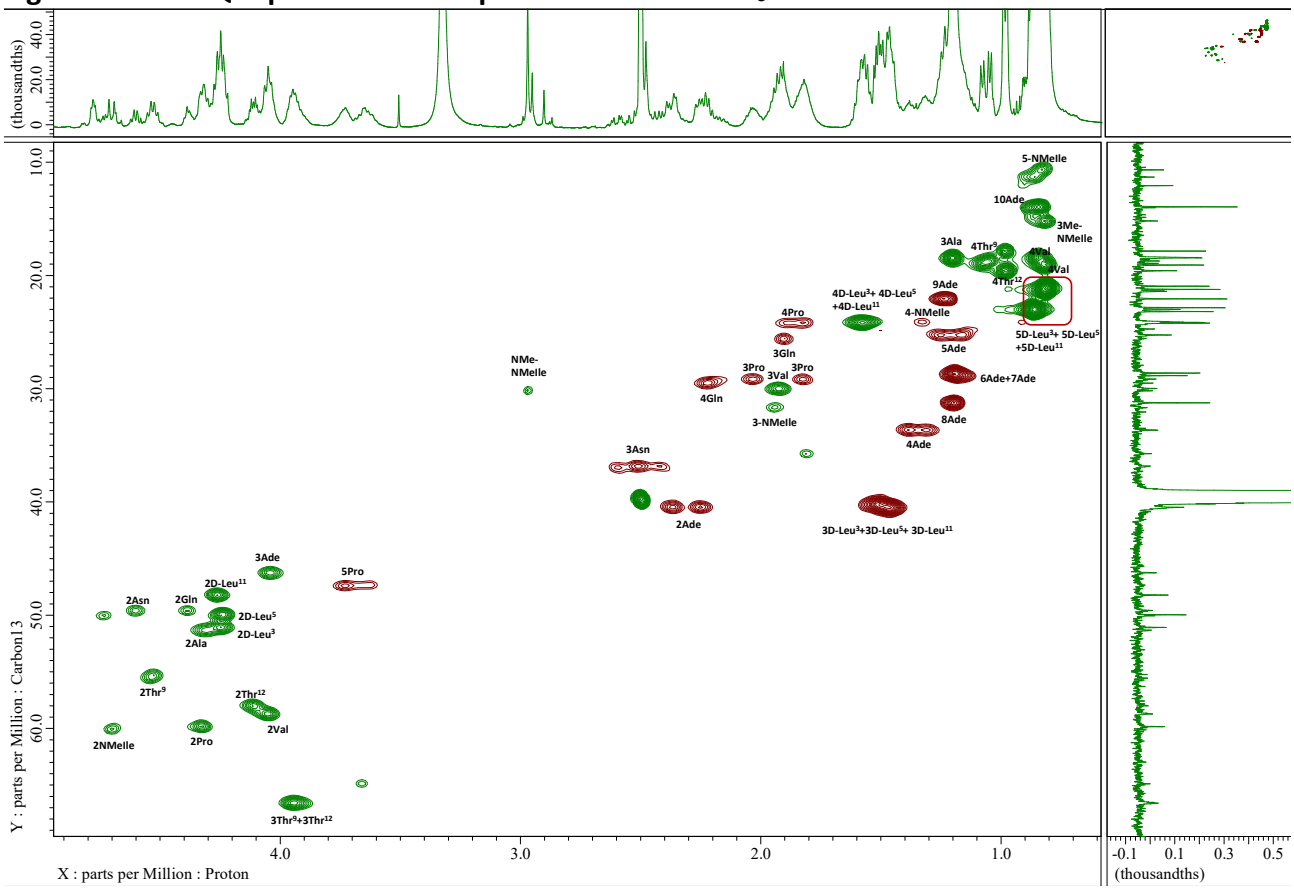


Figure S10f: HMBC spectrum of compound 5b in DMSO-d_6

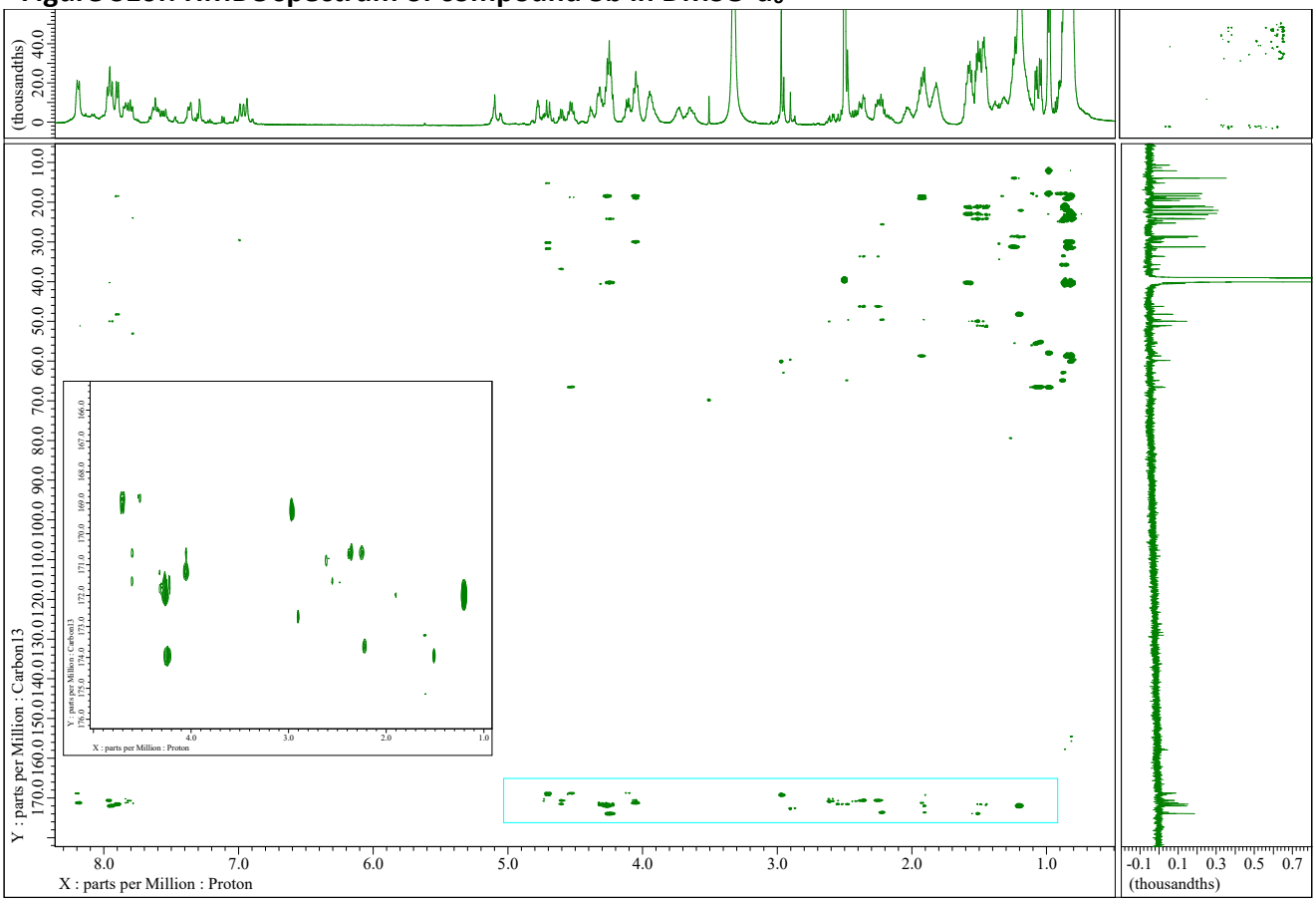


Table S11: NMR spectroscopic data of 5d in DMSO-d_6.

entry	position	C, mult.	H, mult. (J in Hz)	entry	position	C, mult.	H, mult. (J in Hz)
Ade[1]	1	*nd*, C	-	N-MeIle[7]	1	*nd*, C	-
	2	40.2, CH_2	2.47		2	59.5, CH	4.68, d (10.88)
			2.28		3	31.8, CH	1.90
	3	46.7, CH	3.93		3-Me	15.3, CH_3	0.78
	4	32.8, CH_2	1.40		4	24.1, CH_2	1.23
	5	25.9, CH_2	1.18		5	10.4, CH_3	0.75
	6	28.9, CH_2	1.18		N-Me	30.2, CH_3	3.00
	7	28.9, CH_2	1.18	D-Asn[8]	1	*nd*, C	-
	8	31.3, CH_2	1.18		2	49.8, CH	4.60, d (6.30)
	9	22.1, CH_2	1.22		3	37.1, CH_2	2.50
	10	13.9, CH_3	0.83				
	NH	-	7.77		4	*nd*, C	-
Val[2]	1	*nd*, C	-		NH	-	8.08, d (6.87)
	2	58.2, CH	4.06		NH_2	-	7.34 & 6.90
	3	*nd*, CH	2.03	Thr[9]	1	168.9, C	-
	3-Me	19.3, CH_3	0.79		2	55.6, CH	4.52
	4	17.8, CH_3	0.83		3	66.6, CH	3.94
	NH	-	7.75		4	18.7, CH_3	1.05, d (6.30)
D-Leu[3]	1	*nd*, C	-		3-OH	-	
	2	51.9, CH	3.96		NH	-	7.75
	3	41.5, CH_2	1.44	Pro[10]	1	*nd*, C	-
	4	24.0, CH	1.64		2	60.3, CH	4.29
	4-Me	21.4, CH_3	0.84		3	29.3, CH_2	1.82
	5	21.4, CH_3	0.84				2.03
	NH	-	7.66		4	24.2, CH_2	1.74
Ala[4]	1	*nd*, C	-				1.88
	2	*nd*, CH	4.03		5	47.5, CH_2	3.71
	3	19.8, CH_3	1.14				3.71
	NH_2	-	*nd*	D-Leu[11]	1	*nd*, C	-
D-Leu[5]	1	*nd*, C	-		2	52.2, CH	4.18
	2	50.7, CH	4.33		3	39.5, CH_2	1.37
	3	41.5, CH_2	1.44		4	24.0, CH	1.62
	4	24.0, CH	1.55		4-Me	21.0, CH_3	0.80
	4-Me	22.7-22.9, CH_3	0.86		5	21.0, CH_3	0.80
	5	22.7-22.9, CH_3	0.86		NH	-	8.10
	NH	-	7.76	Thr[12]	1	169.7, C	-
Gln[6]	1	*nd*, C	-		2	59.1, CH	4.03
	2	48.4, CH	4.61, d (6.87)		3	66.3, CH	4.01
	3	26.7, CH_2	1.74		4	19.8, CH_3	0.97, d (5.73)
			1.84		3-OH	-	
	4	30.7, CH_2	2.07		NH	-	8.10
	5	*nd*, C	-				
	NH	-	8.26, d (7.45)				
	NH_2	-	7.26 & 6.85				

nd = not determined. The numbering of atoms corresponds to the one already reported in the article on trichormamide C.[1] The determination of the structure was made using several NMR spectra (Figures S11a-f).

Figure S11a: ^{1}H NMR spectrum of compound 5d in DMSO-d$_6$

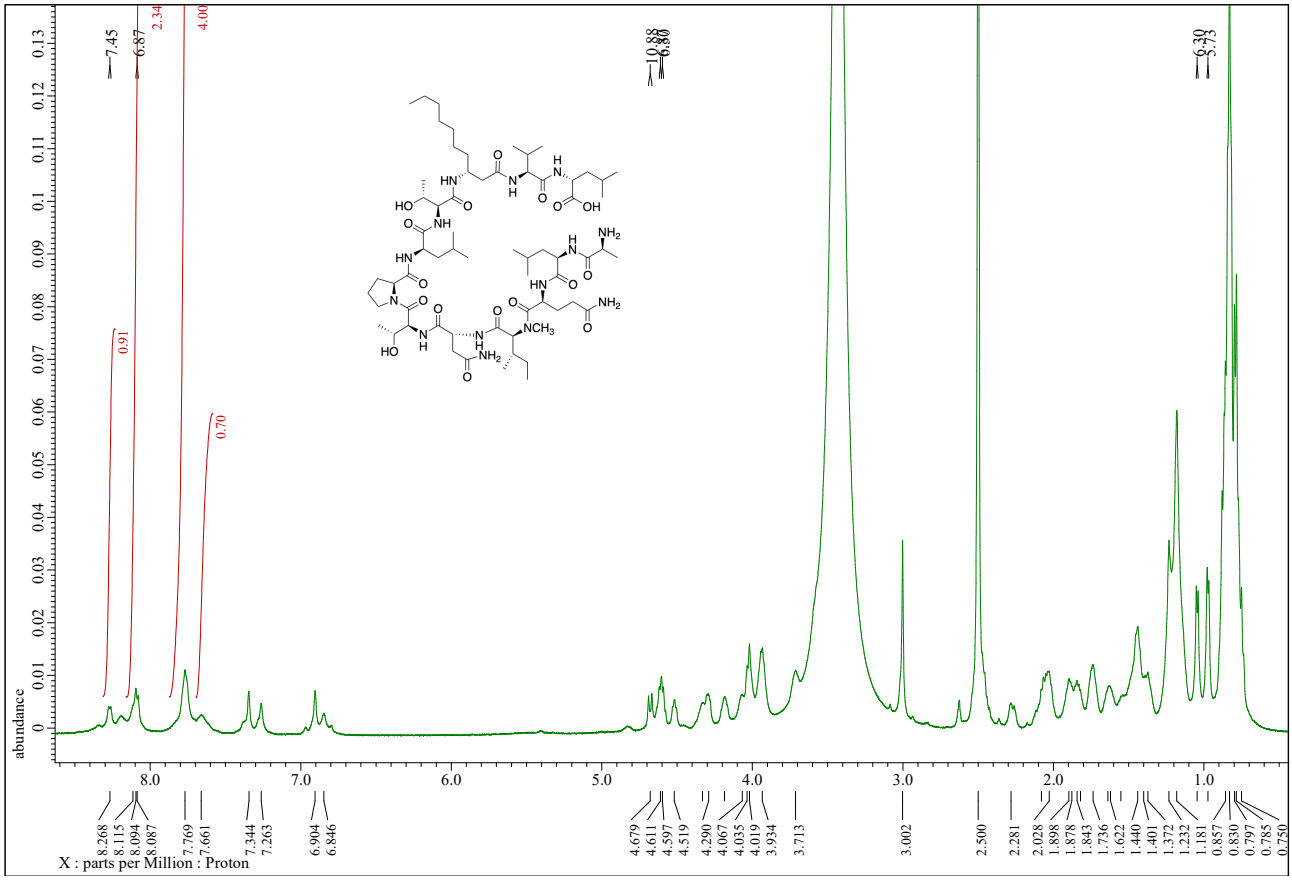


Figure S11b: ^{13}C NMR spectrum of compound 5d in DMSO-d$_6$

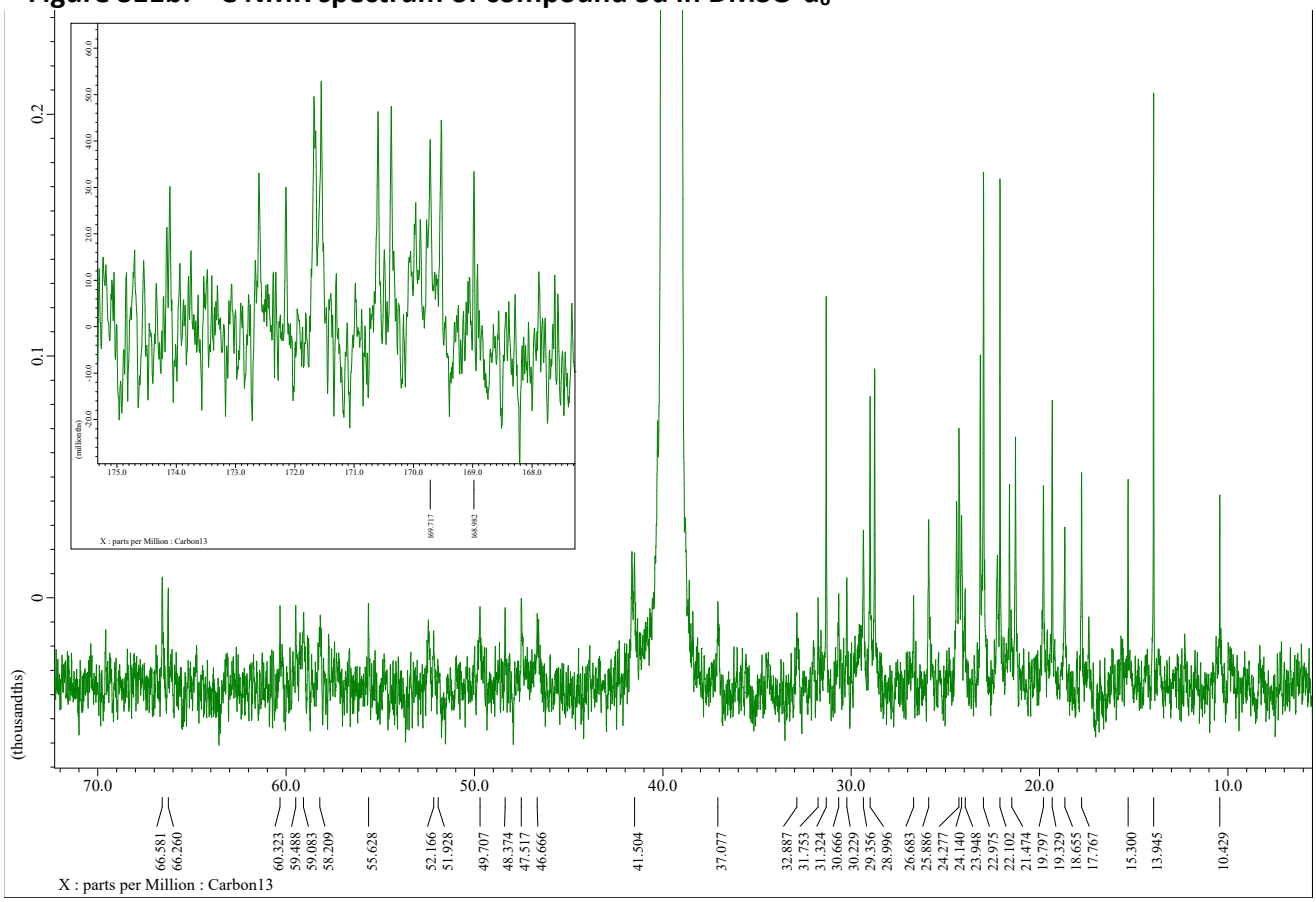


Figure S11c: TOCSY spectrum of compound 5d in DMSO-d_6

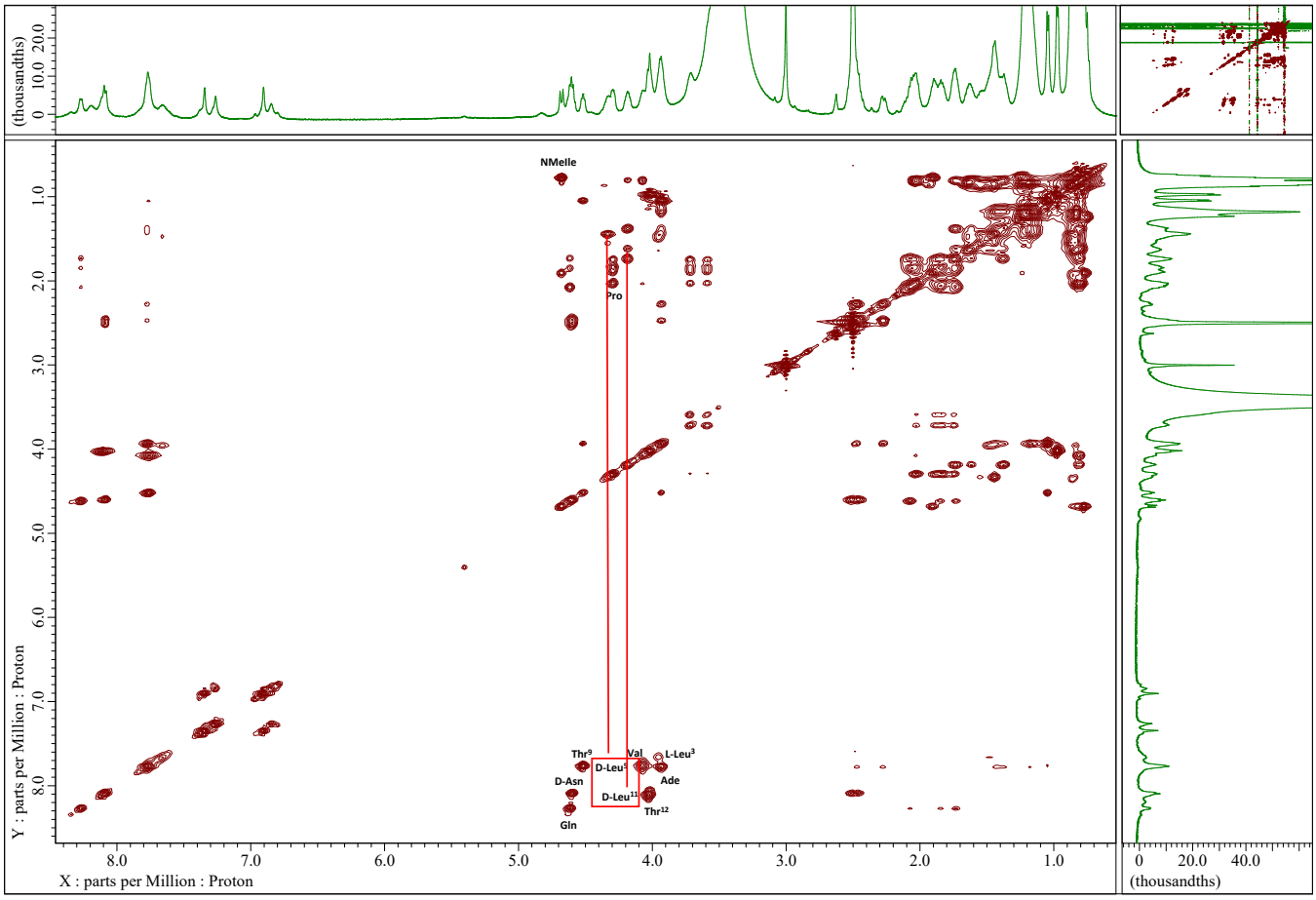


Figure S11d: ROESY spectrum of compound 5d in DMSO-d_6

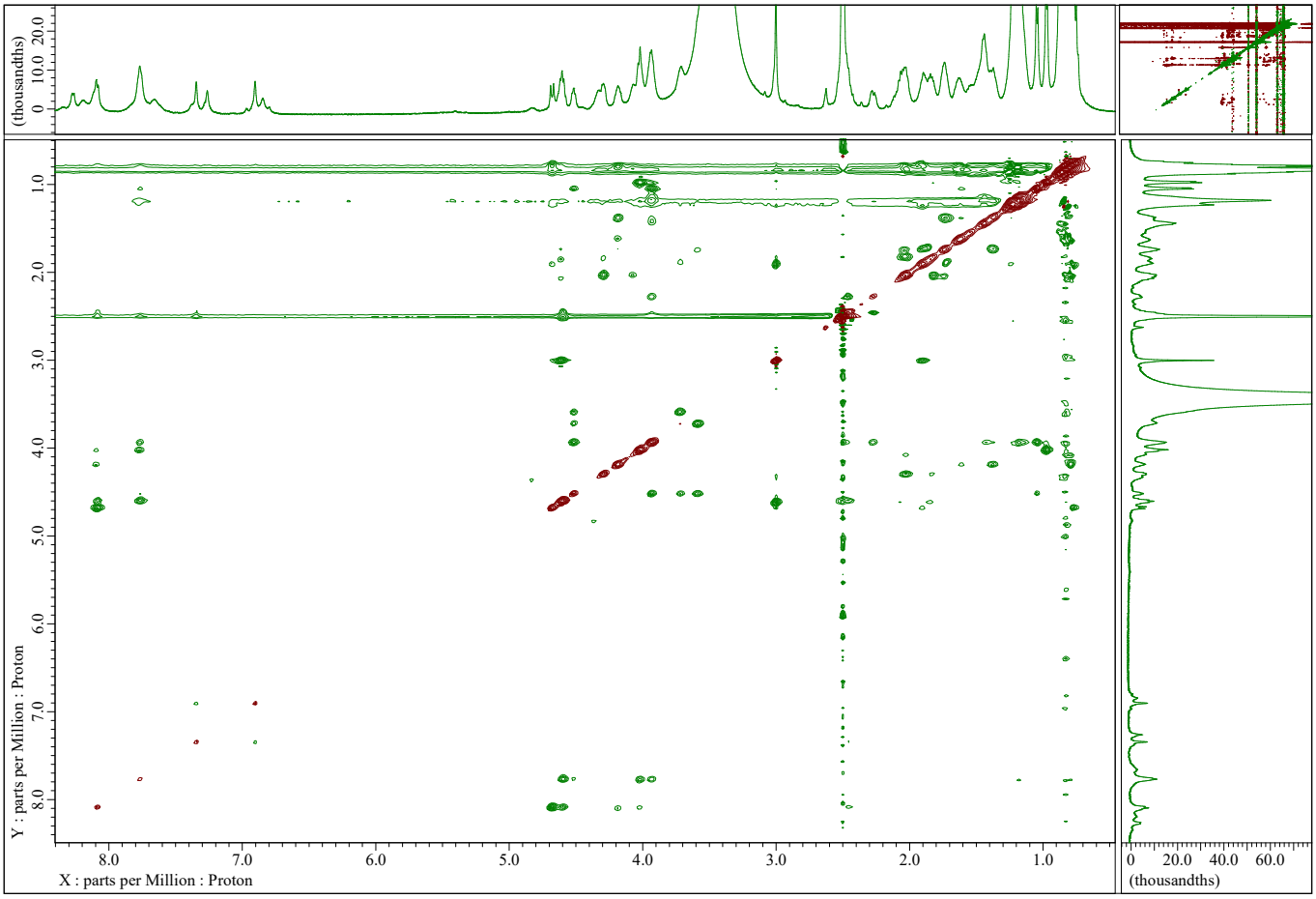


Figure S11e: HSQC spectrum of compound 5d in DMSO-d_6

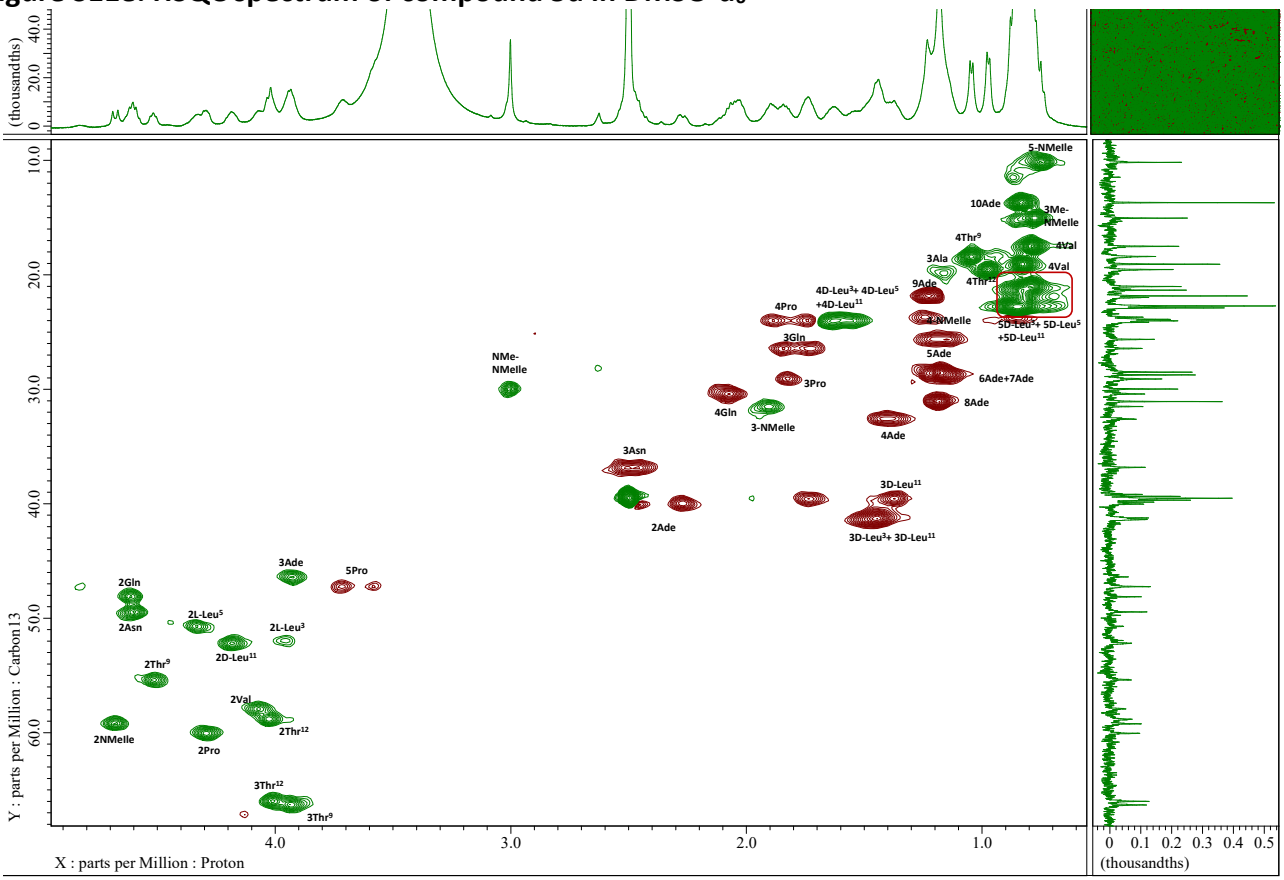


Figure S11f: HMBC spectrum of compound 5d in DMSO-d_6

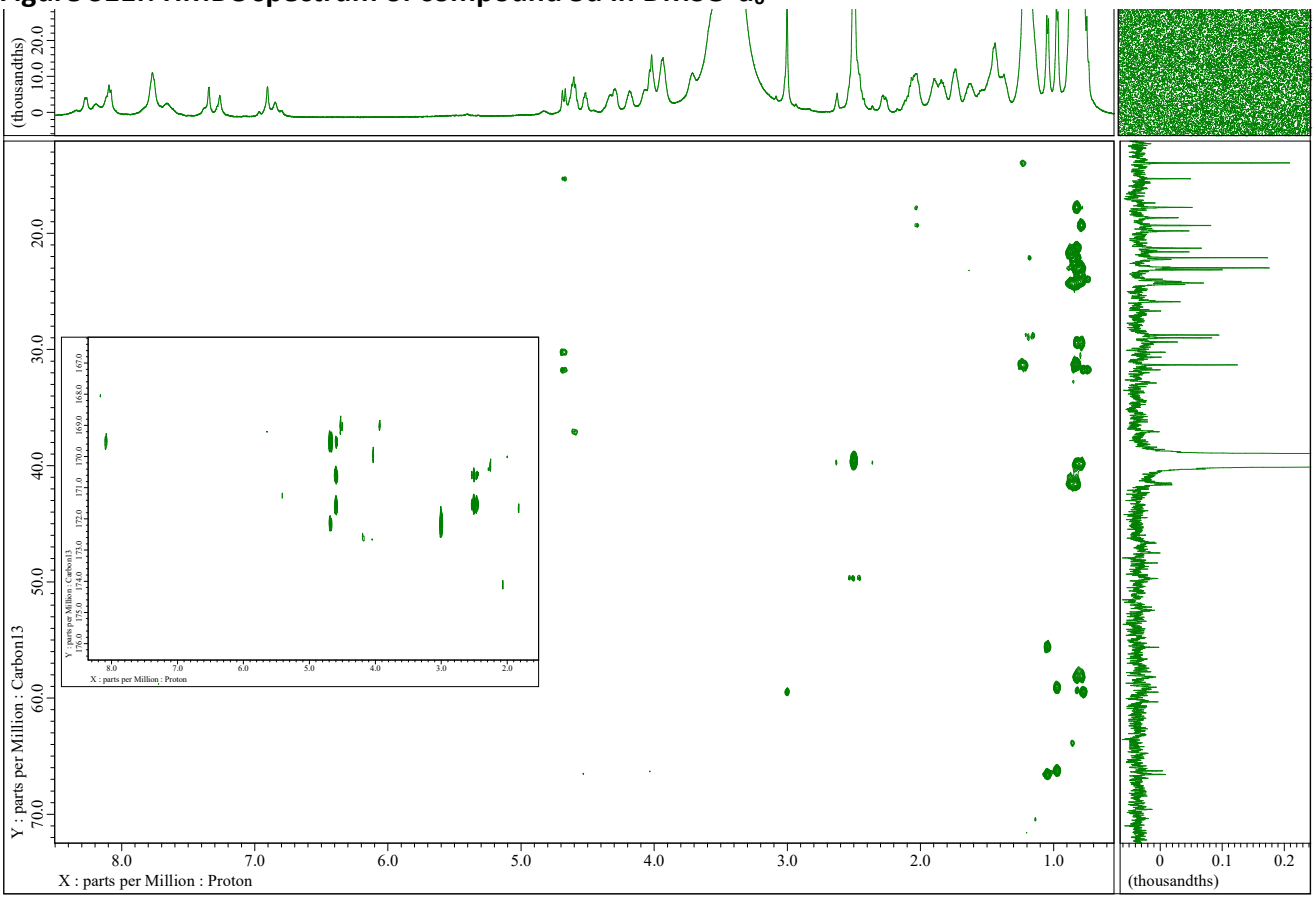


Table S12: NMR spectroscopic data of 7a in DMSO-d_6.

entry	position	C, mult.	H, mult. (J in Hz)	entry	position	C, mult.	H, mult. (J in Hz)
Decanoic acid[1]	1	173.0, C	-	**N-MeIle[7]**	1	169.6, C	-
	2	34.9, CH_2	2.18		2	59.5, CH	4.68, d (11.46)
			2.10		3	31.7, CH	1.90
	3	25.2, CH_2	1.45		3-Me	15.3, CH_3	0.78
	4	28.6-28.9, CH_2	1.21		4	23.9, CH_2	1.27
	5	28.6-28.9, CH_2	1.21				
	6	28.6-28.9, CH_2	1.21		5	10.4, CH_3	0.78
	7	28.6-28.9, CH_2	1.21		N-Me	30.2, CH_3	3.01
	8	31.2, CH_2	1.21	**D-Asn[8]**	1	170.8, C	-
	9	22.1, CH_2	1.23		2	49.6, CH	4.60, t (6.30)
	10	14.0, CH_3	0.84		3	37.1, CH_2	2.50
Val[2]	1	171.8, C	-				
	2	59.2, CH	3.99, t (7.45)		4	171.6, C	-
	3	29.7, CH	1.90		NH	-	8.05, d (8.02)
	3-Me	19.1, CH_3	0.83		NH_2	-	7.32 & 6.92
	4	18.9, CH_3	0.89	**Thr[9]**	1	168.8, C	-
	NH	-	7.96, d (6.87)		2	55.5, CH	4.49, t (6.87)
D-Leu[3]	1	172.1, C	-		3	66.6, CH	3.89
	2	51.3, CH	4.16		4	18.9, CH_3	1.05, d (5.73)
	3	40.2, CH_2	1.48		3-OH	-	5.06, d (4.58)
	4	24.1, CH	1.59		NH	-	7.75, d (8.02)
	4-Me	20.8-23.2, CH_3	0.83	**Pro[10]**	1	171.6, C	-
	5	20.8-23.2, CH_3	0.83		2	59.9, CH	4.32
	NH	-	8.33, d (8.02)		3	29.2, CH_2	1.80
Ala[4]	1	172.1, C	-				2.04
	2	48.9, CH	4.13, t (7.45)		4	24.2, CH_2	1.82
	3	17.8, CH_3	1.20				1.89
	NH	-	7.91, d (6.87)		5	47.4, CH_2	3.68
D-Leu[5]	1	171.8, C	-				3.68
	2	51.0, CH	4.29	**D-Leu[11]**	1	172.1, C	-
	3	41.2, CH_2	1.41		2	51.3, CH	4.32
	4	24.2, CH	1.53		3	40.1, CH_2	1.48
	4-Me	20.8-23.2, CH_3	0.83		4	24.2, CH	1.55
	5	20.8-23.2, CH_3	0.83		4-Me	20.8-23.2, CH_3	0.87
	NH	-	7.80, d (8.59)		5	20.8-23.2, CH_3	0.87
Gln[6]	1	172.1, C	-		NH	-	7.95, d (7.45)
	2	48.2, CH	4.64, t (6.87)	**Thr[12]**	1	172.2, C	-
	3	26.9, CH_2	1.73		2	58.1, CH	4.05, d (6.87)
			1.86		3	66.1, CH	4.05, d (6.87)
	4	30.6, CH_2	2.05		4	20.1, CH_3	0.99, d (6.30)
	5	174.0, C	-		3-OH	-	4.80, d (5.73)
	NH	-	8.09, d (8.02)		NH	-	7.63, d (8.59)
	NH_2	-	7.17 & 6.80		NH_2	-	7.13 & 7.08

The numbering of atoms corresponds to the one already reported in the article on trichormamide C.[1]
The determination of the structure was made using several NMR spectra (Figures S12a-g).

Figure S12a: ^{1}H NMR spectrum of compound 7a in DMSO-d$_6$

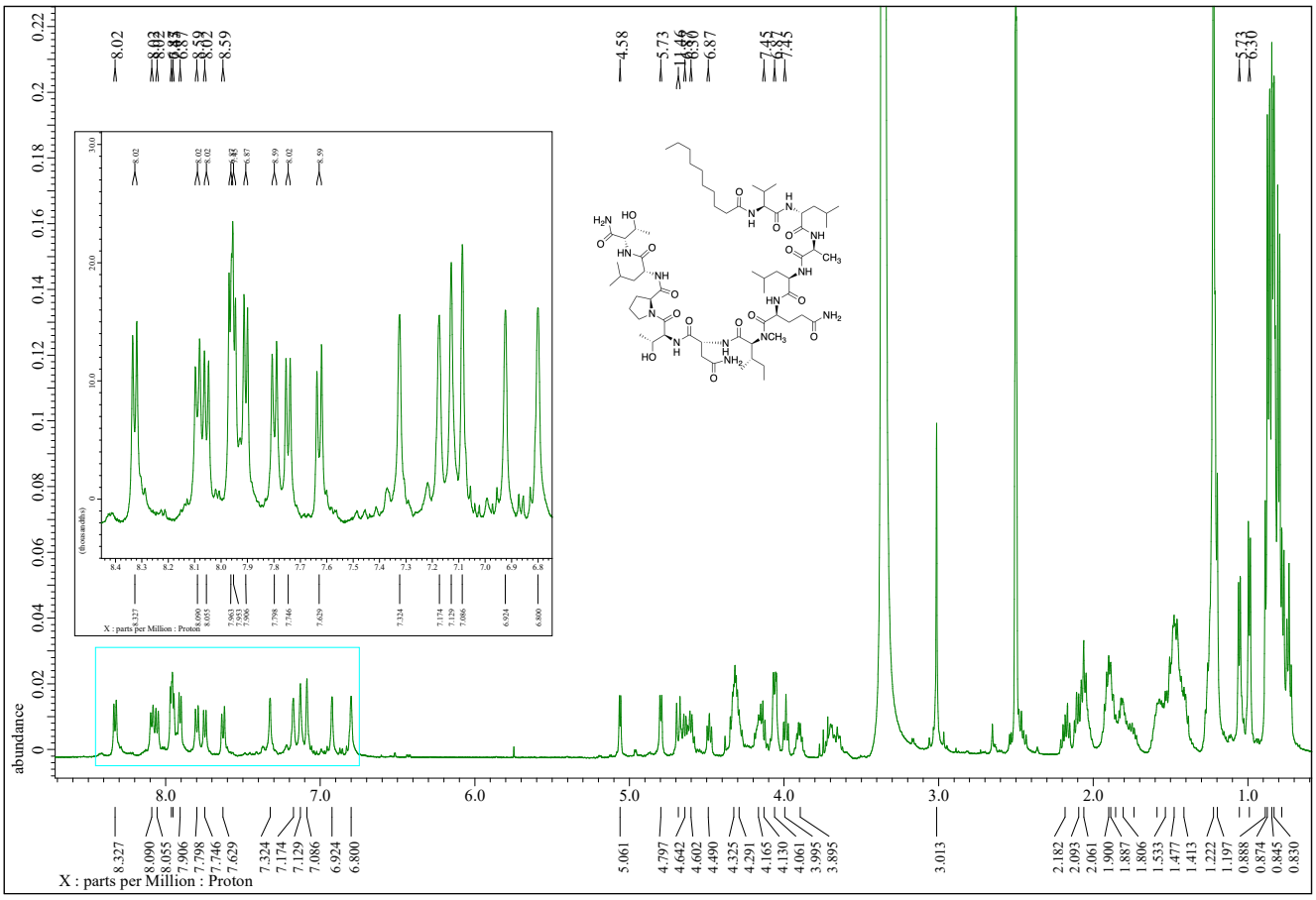


Figure S12b: ^{13}C NMR spectrum of compound 7a in DMSO-d$_6$

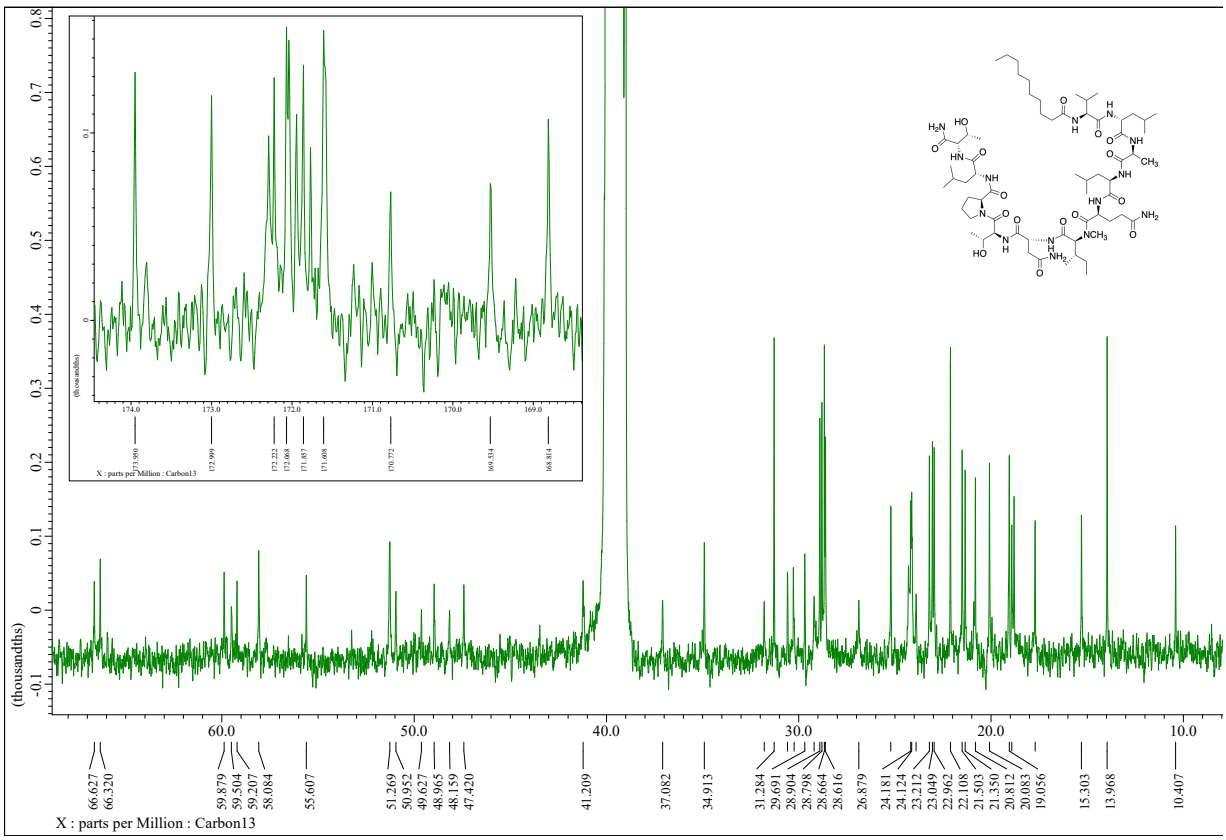


Figure S12c: TOCSY spectrum of compound 7a in DMSO-d_6

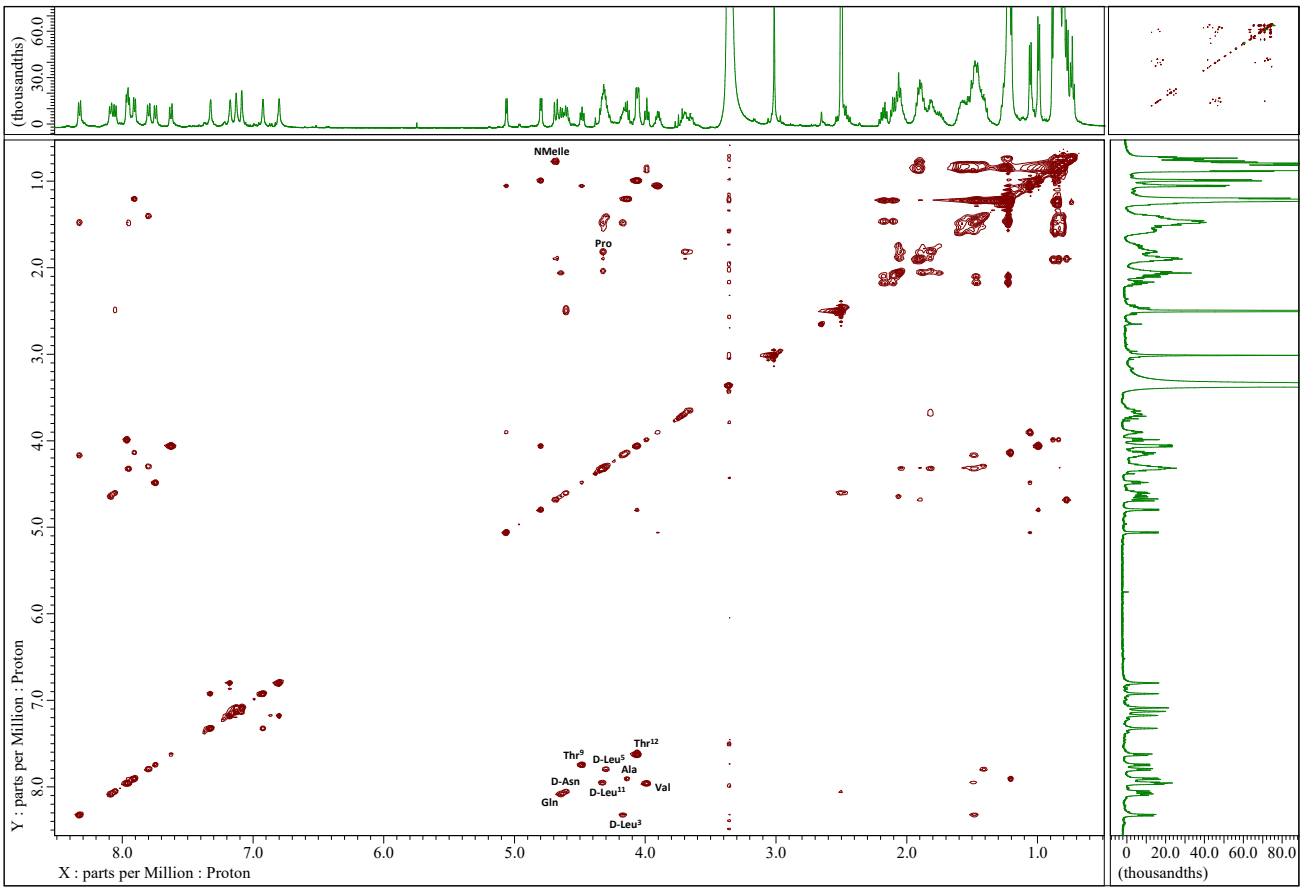


Figure S12d: ROESY spectrum of compound 7a in DMSO-d_6

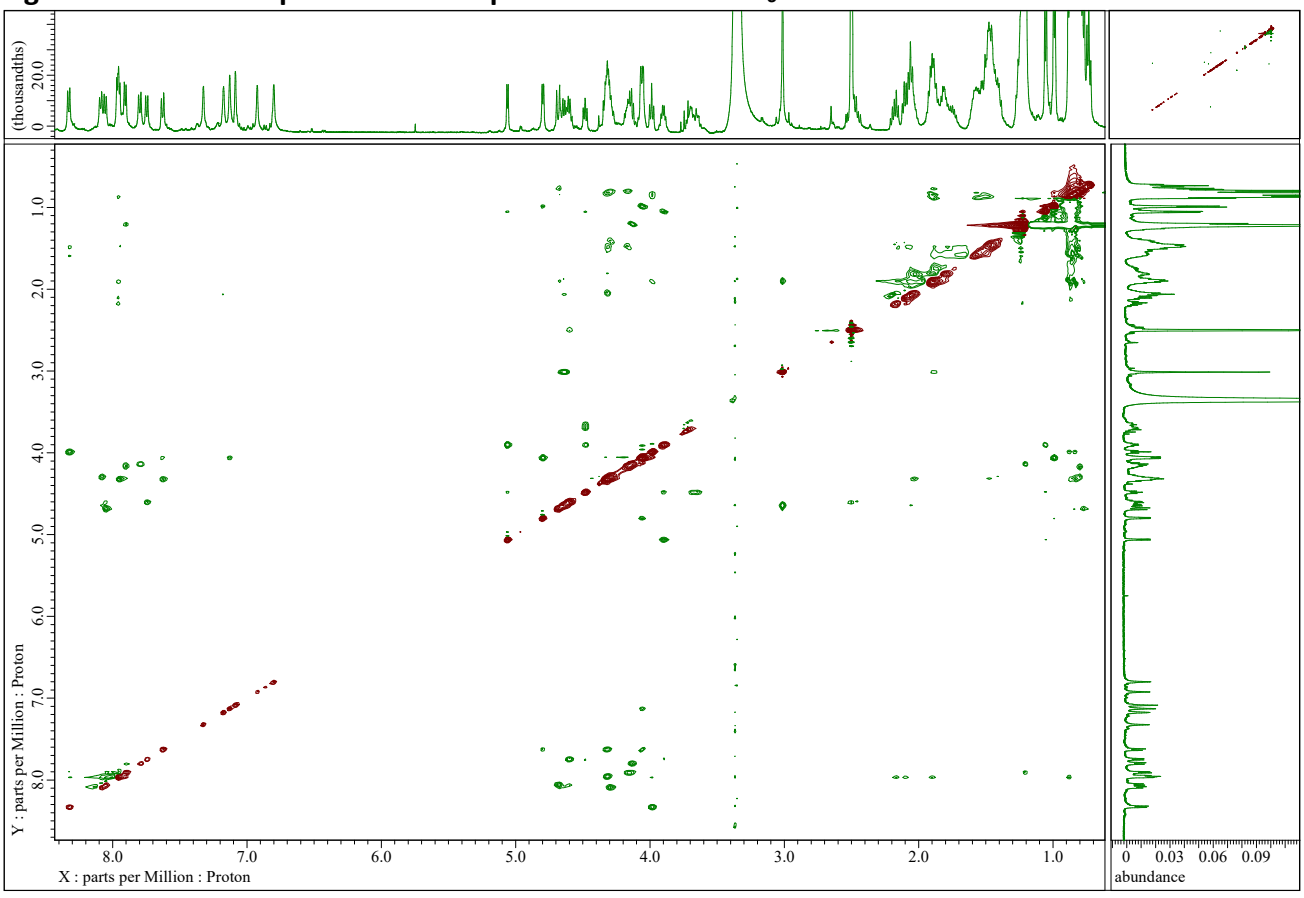


Figure S12e: HSQC spectrum of compound 7a in DMSO-d_6

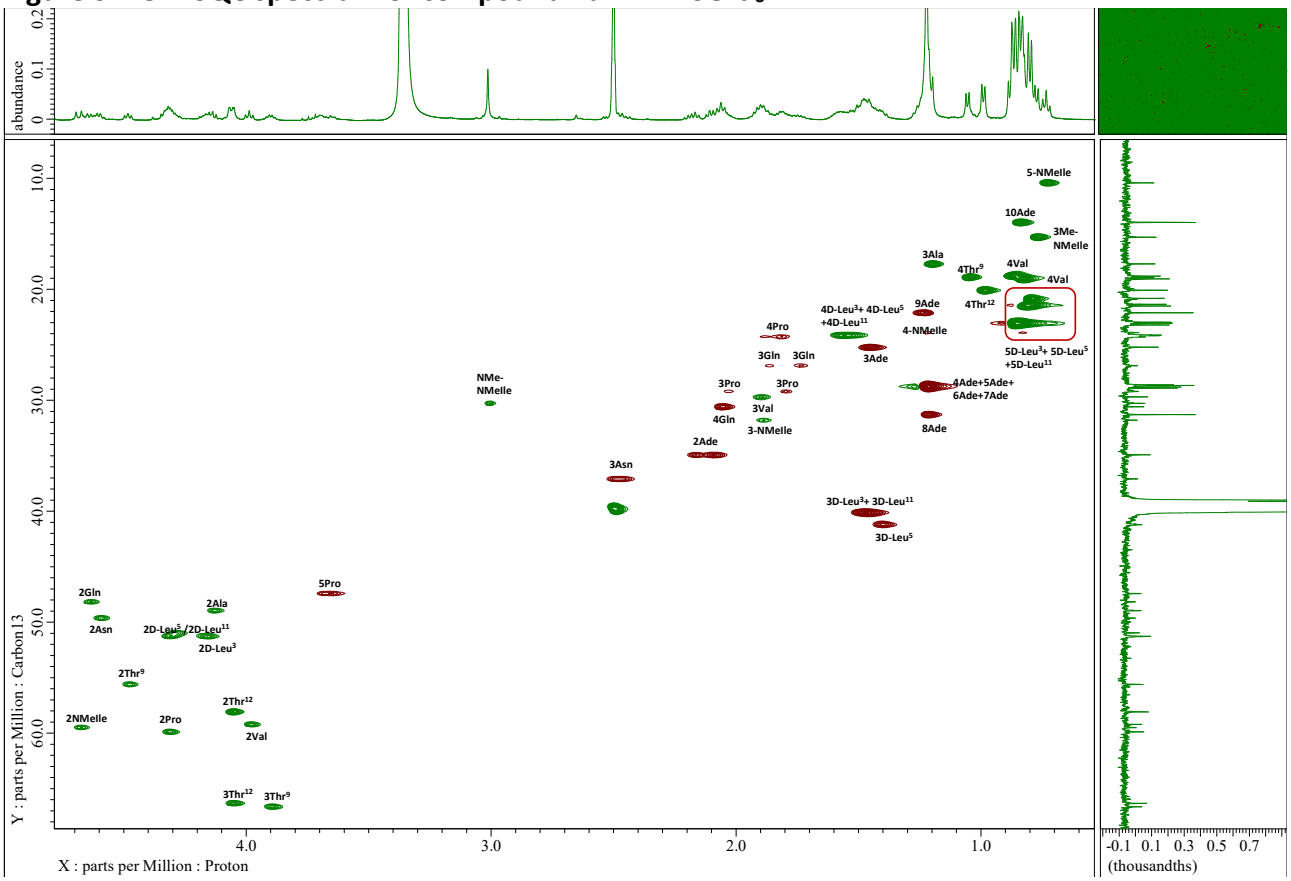


Figure S12f: HMBC spectrum of compound 7a in DMSO-d_6

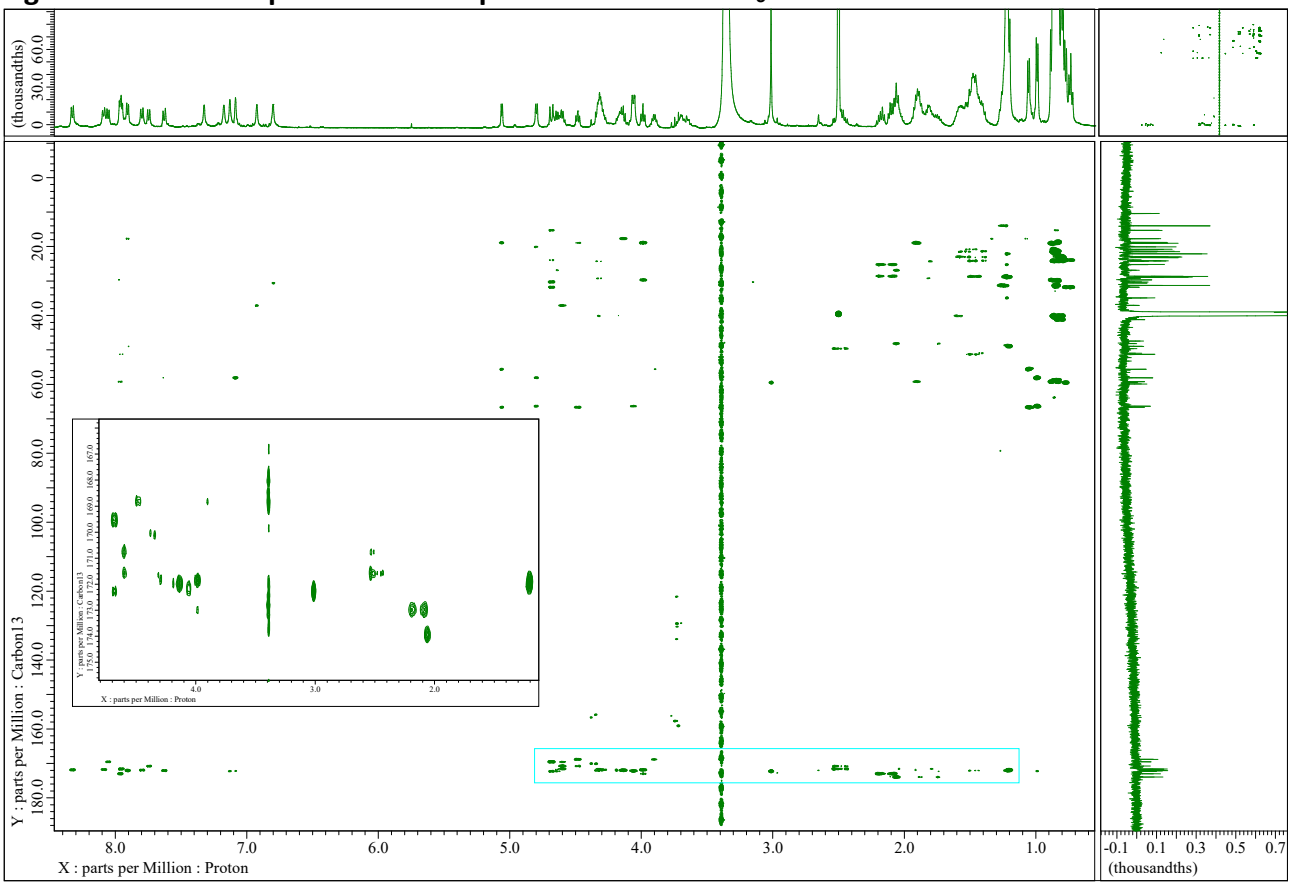


Figure S12g: HSQC-TOCSY spectrum of compound 7a in DMSO-d_6

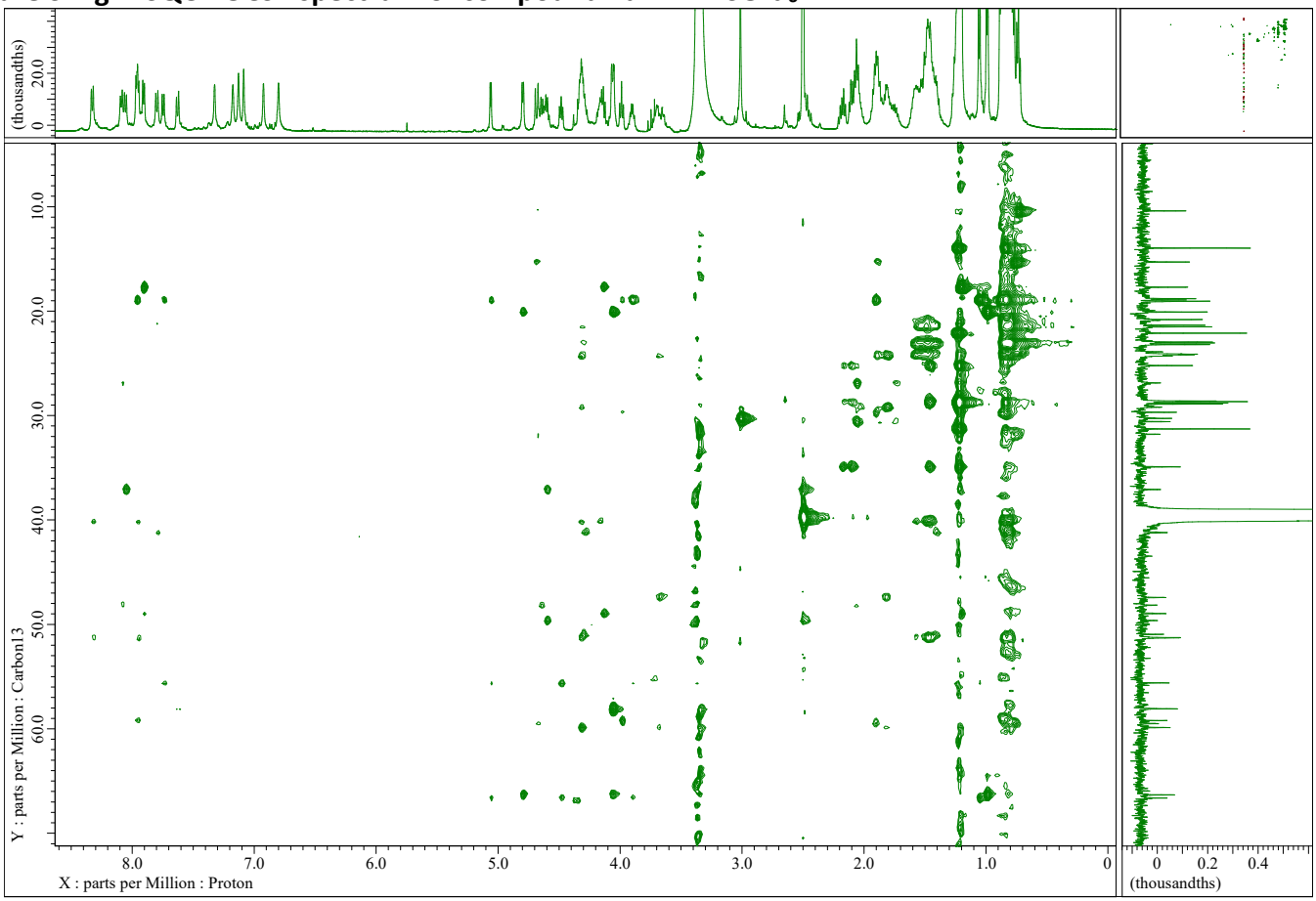


Table S13: NMR spectroscopic data of 8a in DMSO-d_6

entry	position	C, mult.	H, mult. (J in Hz)	entry	position	C, mult.	H, mult. (J in Hz)
β-Ala[1]	1	*nd*, C	-	**N-MeIle[7]**	1	170.7, C	-
	2	35.0, CH_2	2.36		2	59.6, CH	4.66, d (10.88)
	3	34.4, CH_2	3.25		3	31.8, CH	1.90
	NH	-	7.80, t (5.15)		3-Me	15.4, CH_3	0.78
Val[2]	1	171.1, C	-		4	24.0, CH_2	1.24
	2	58.3, CH	4.15, t (7.45)		5	10.5, CH_3	0.76
	3	30.3, CH	1.95		N-Me	30.2, CH_3	2.96
	3-Me	19.1, CH_3	0.84	**D-Asn[8]**	1	171.5, C	-
	4	18.3, CH_3	0.85		2	49.6, CH	4.62
	NH	-	8.12, d (8.02)		3	36.0, CH_2	2.55
D-Leu[3]	1	*nd*, C	-				2.43
	2	51.1-51.4, CH	4.26		4	172.6, C	-
	3	40.5-41.1, CH_2	1.43		NH	-	8.22, d (7.45)
	4	24.2, CH	1.55		NH_2	-	7.30 & 6.87
	4-Me	21.1-23.2, CH_3	0.81	**Thr[9]**	1	168.6, C	-
	5	21.1-23.2, CH_3	0.81		2	56.1, CH	4.41, t (6.87)
	NH	-	8.34, d (7.45)		3	66.5, CH	3.87, t (6.30)
Ala[4]	1	171.9, C	-		4	19.1, CH_3	1.07, d (6.30)
	2	48.8, CH	4.20, t (7.45)		NH	-	7.45, d (7.45)
	3	18.1, CH_3	1.20, d (6.87)	**Pro[10]**	1	171.8, C	-
	NH	-	8.18, d (6.87)		2	59.8, CH	4.32
D-Leu[5]	1	*nd*, C	-		3	29.3, CH_2	1.75
	2	51.1-51.4, CH	4.24				2.07
	3	40.5-41.1, CH_2	1.43		4	24.4, CH_2	1.82
	4	24.2, CH	1.55				1.89
	4-Me	21.1-23.2, CH_3	0.86		5	47.6, CH_2	3.66
	5	21.1-23.2, CH_3	0.86	**D-Leu[11]**	1	*nd*, C	-
	NH	-	7.91, d (8.59)		2	51.1-51.4, CH	4.32
Gln[6]	1	*nd*, C	-		3	40.5-41.1, CH_2	
	2	48.3, CH	4.60		4	24.2, CH	
	3	26.8, CH_2	1.68		4-Me	21.1-23.2, CH_3	0.81
			1.85		5	21.1-23.2, CH_3	0.81
	4	30.6, CH_2	2.05		NH	-	8.06, d (7.45)
	5	174.2, C	-	**Thr[12]**	1	169.7, C	-
	NH	-	8.08, d (8.59)		2	58.4, CH	4.07
	NH_2	-	7.21 & 6.77		3	66.3, CH	4.02
					4	19.9, CH_3	0.99, d (6.30)
					NH	-	7.82, d (8.59)

nd = not determined. The numbering of atoms corresponds to the one already reported in the article on trichormamide C.[1] The determination of the structure was made using several NMR spectra (Figures S13a-g).

Figure S13a: ^{1}H NMR spectrum of compound 8a in DMSO-d$_6$

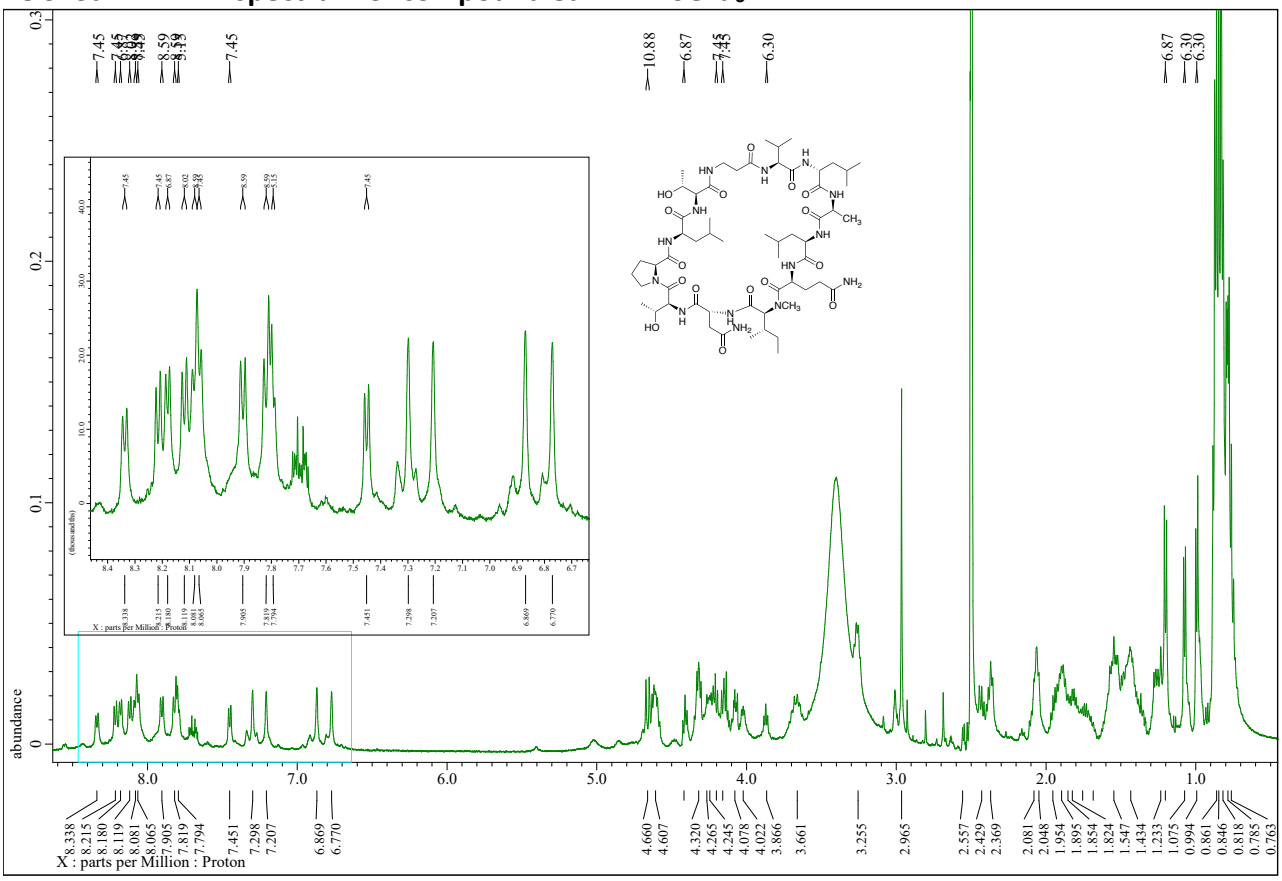


Figure S13b: ^{13}C NMR spectrum of compound 8a in DMSO-d$_6$

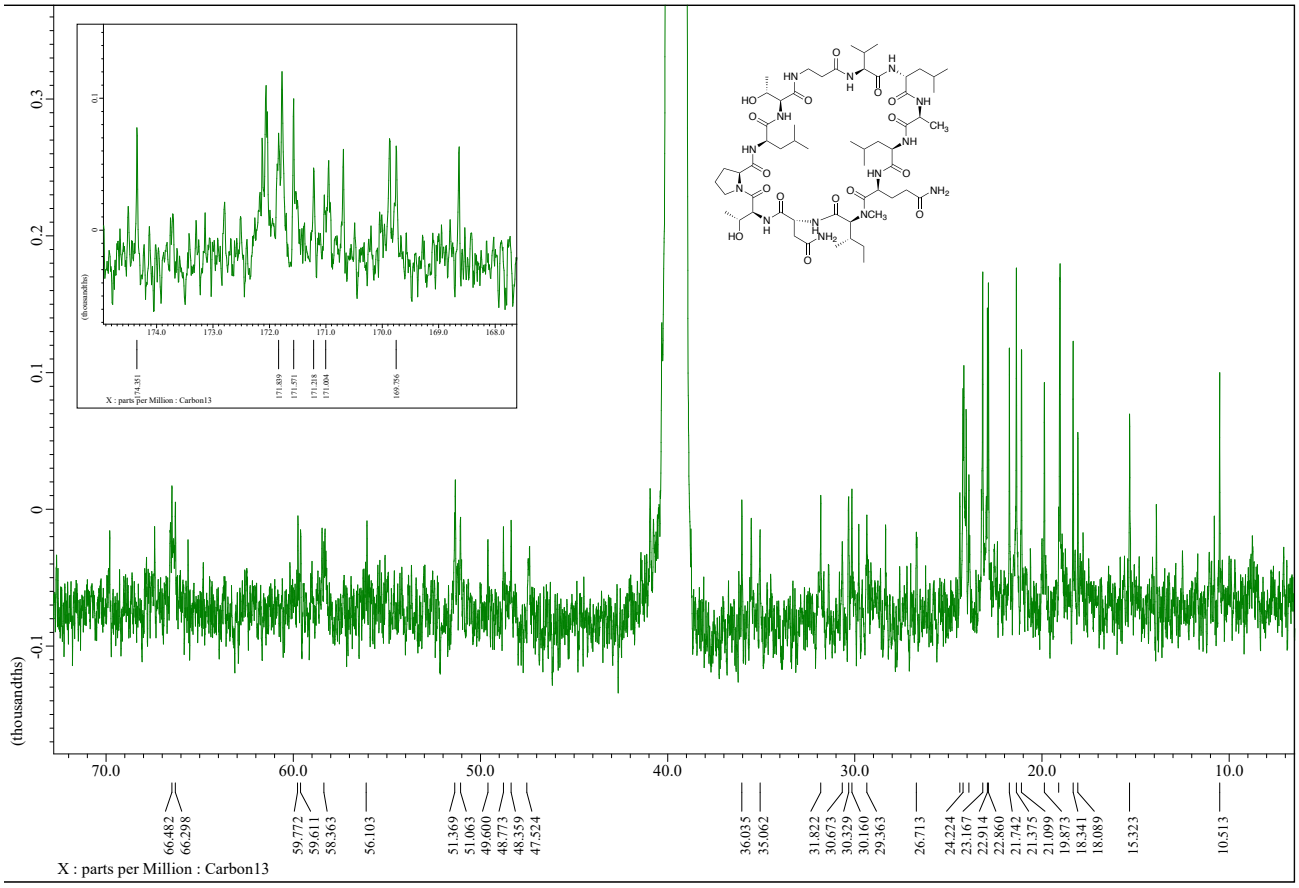
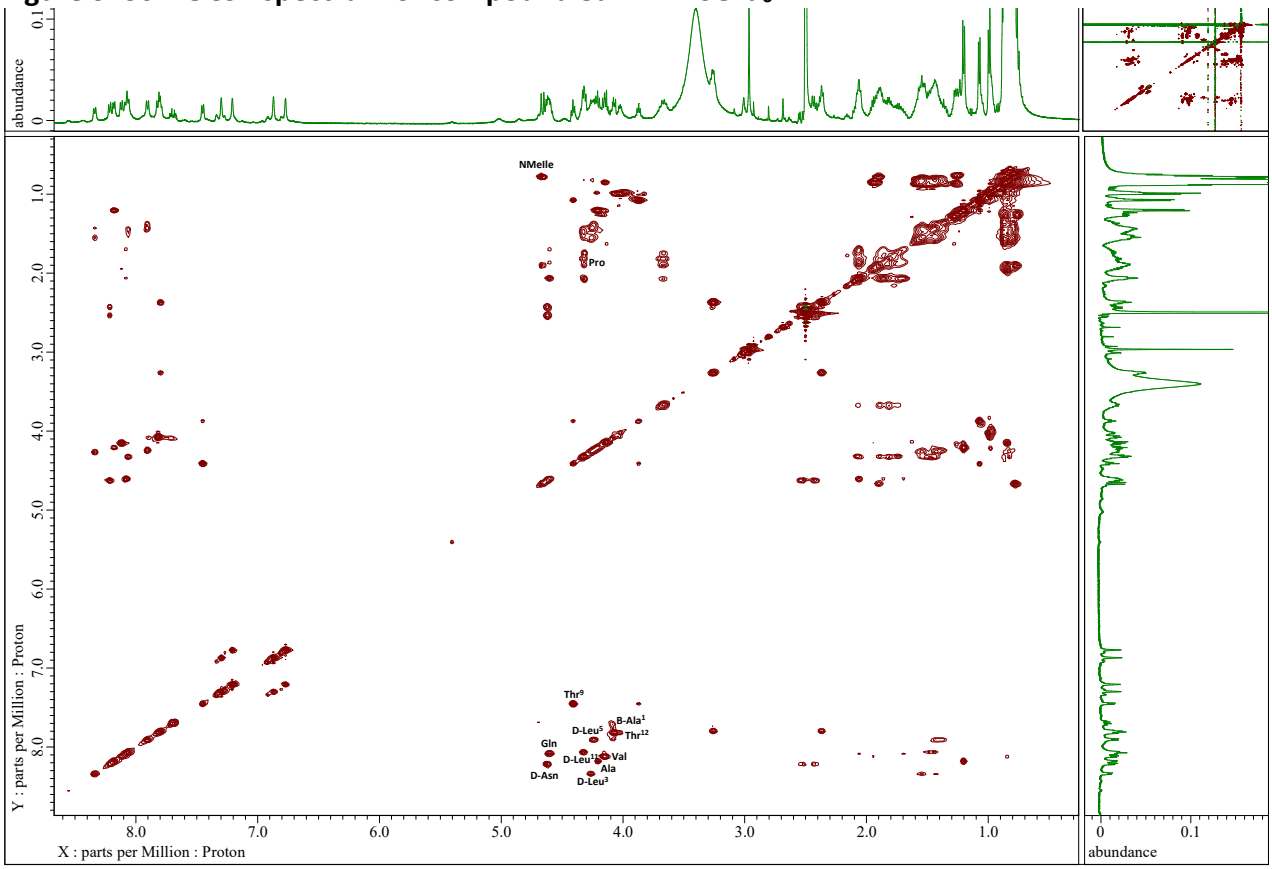


Figure S13c: TOCSY spectrum of compound 8a in DMSO-d_6



FigureS13d: ROESY spectrum of compound 8a in DMSO-d_6

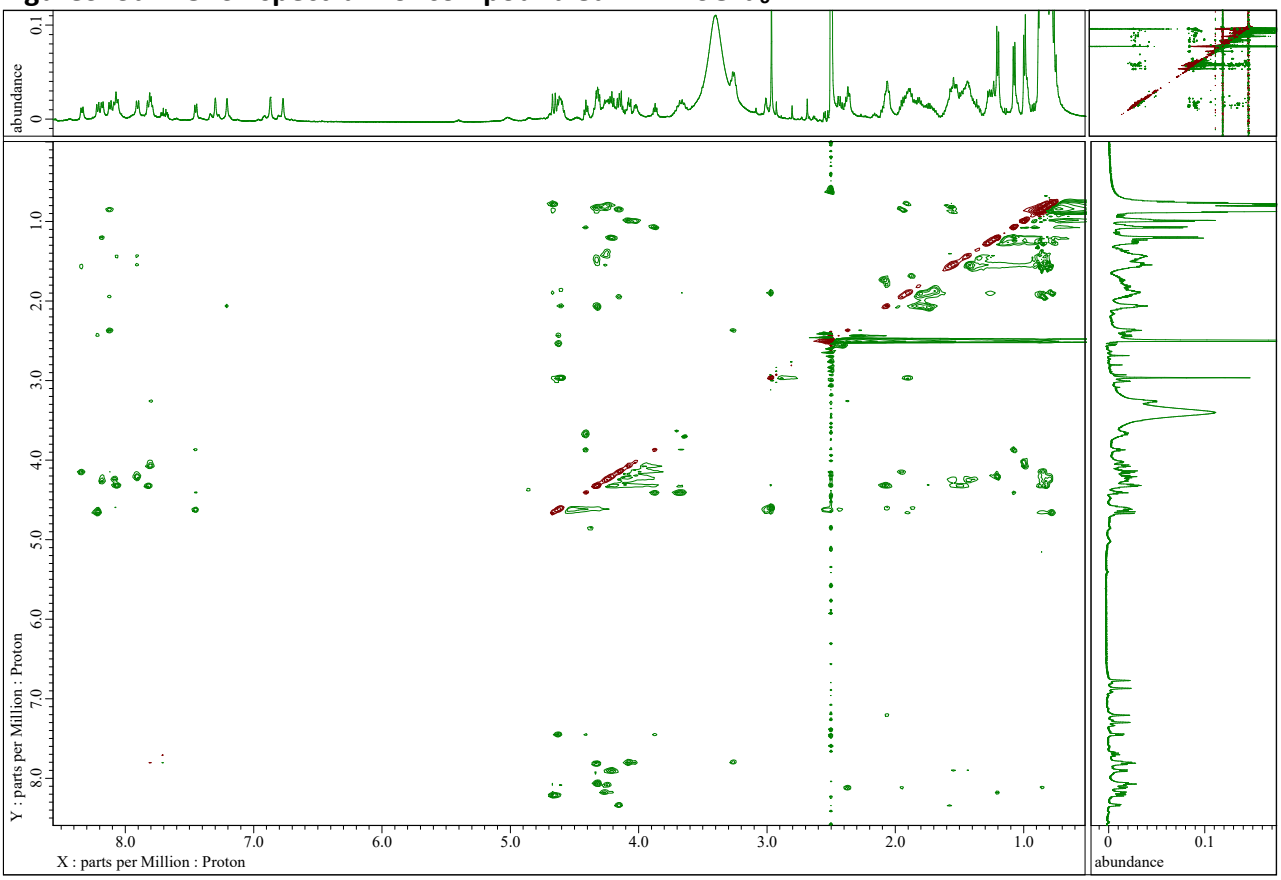


Figure S13e: HSQC spectrum of compound 8a in DMSO-d_6

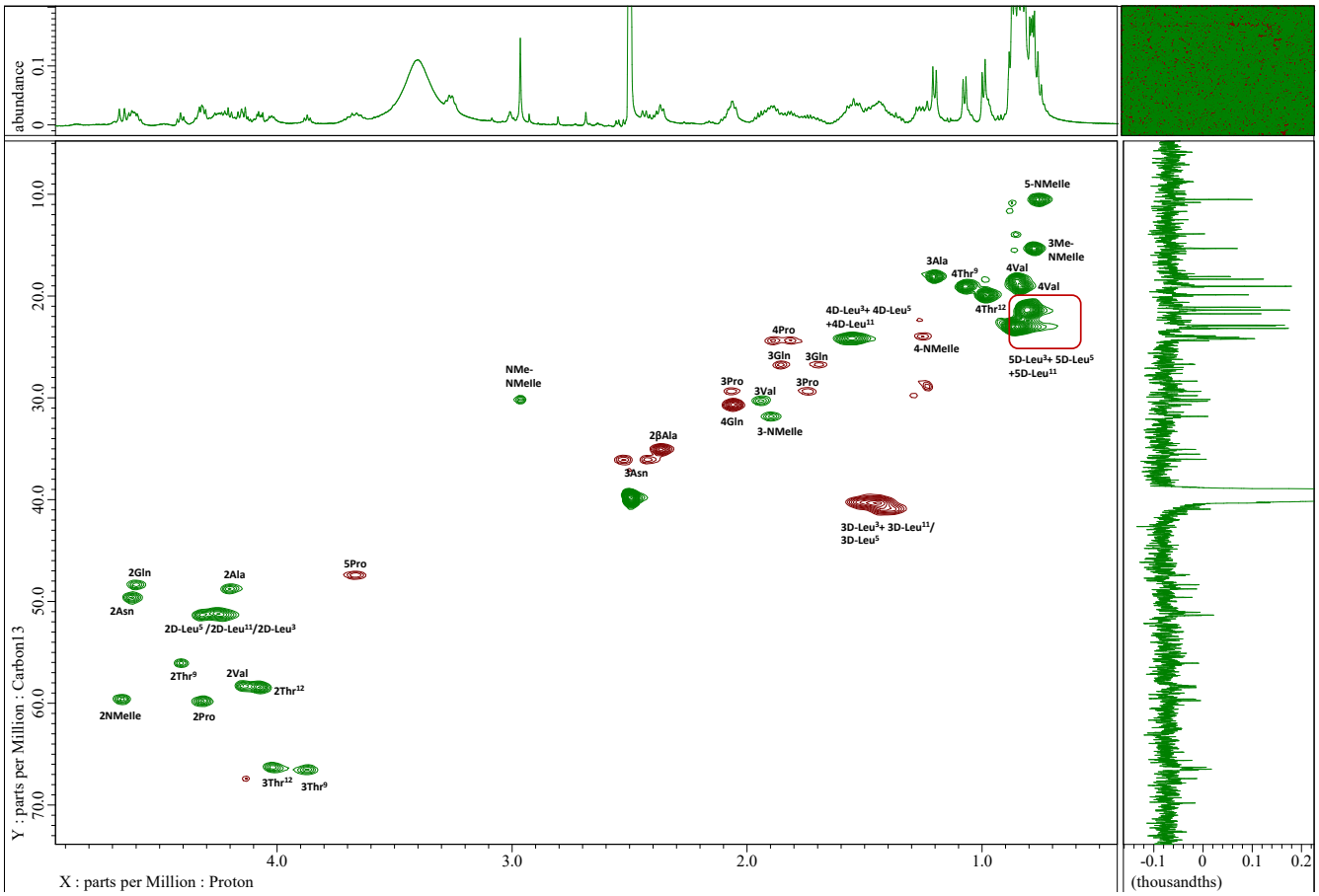


Figure S13f: HMBC spectrum of compound 8a in DMSO-d_6

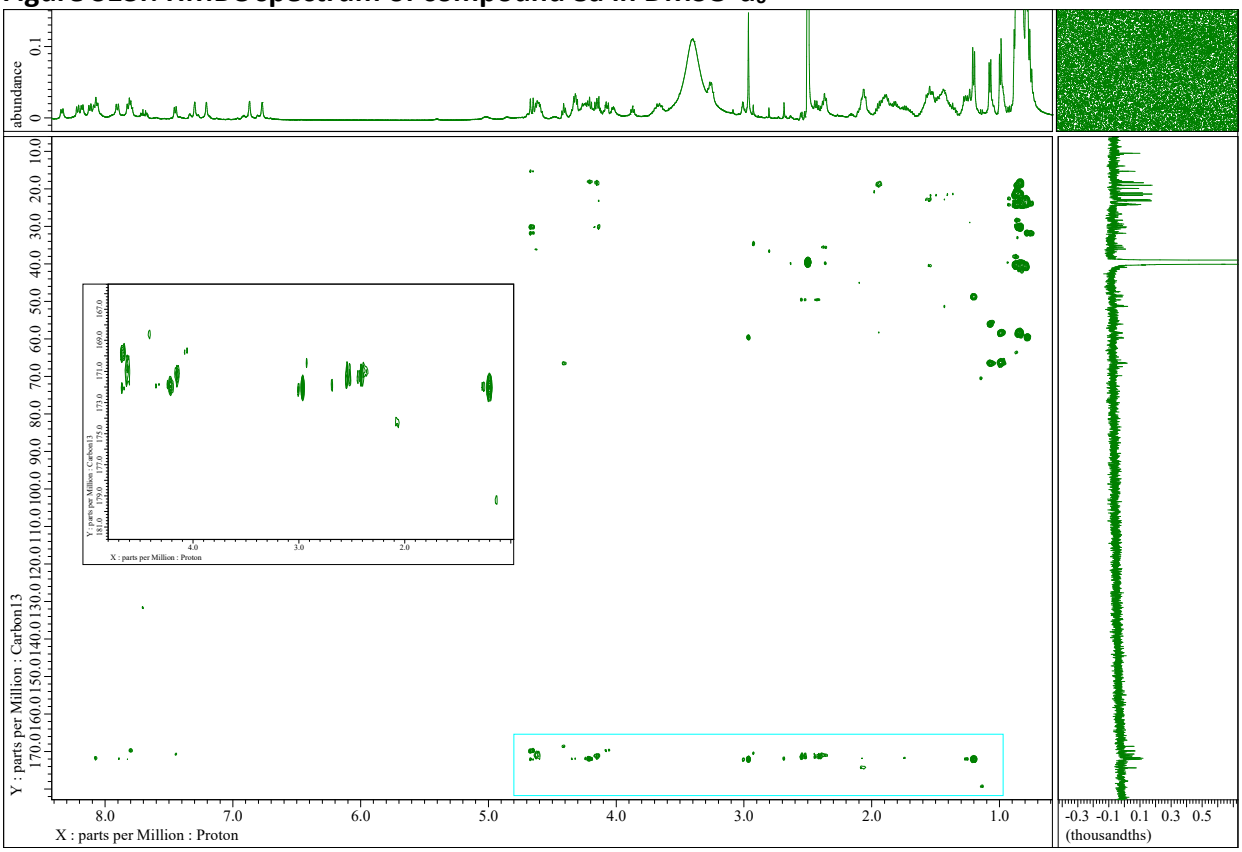


Figure S13g: HSQC-TOCSY spectrum of compound 8a in DMSO-d_6

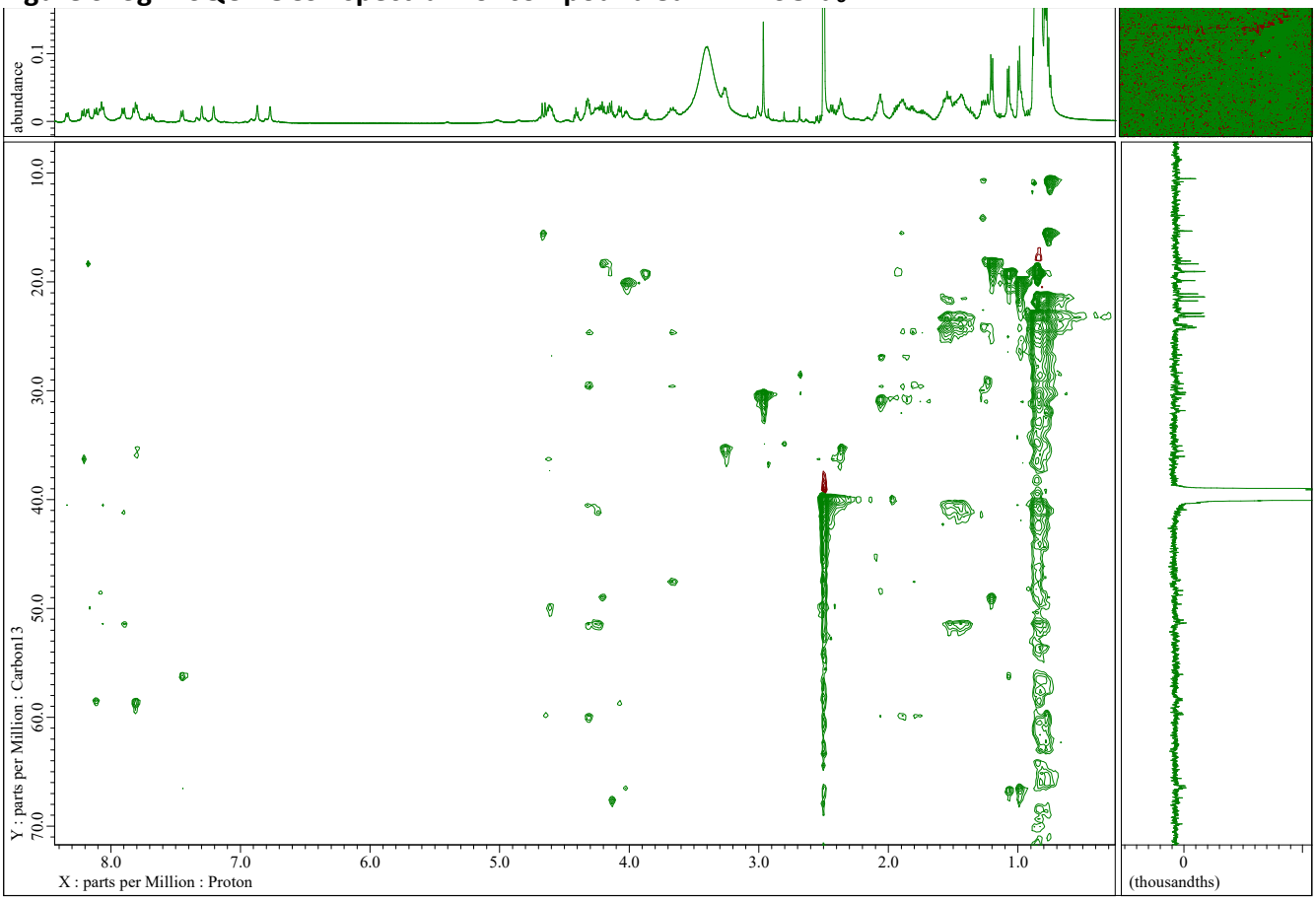


Table S14: NMR spectroscopic data of 9a in DMSO-d_6.

entry	position	C, mult.	H, mult. (J in Hz)	entry	position	C, mult.	H, mult. (J in Hz)
Ade[1]	1	*nd*, C	-	N-MeIle[7]	1	*nd*, C	-
	2	41.1, CH_2	2.37, d (8.59)		2	59.5, CH	4.66, d (10.31)
			2.37, d (8.59)		3	31.7, CH	1.90
	3	46.2, CH	4.10		3-Me	15.4, CH_3	0.78
	4	33.4, CH_2	1.38		4	23.9, CH_2	1.20
	5	25.5, CH_2	1.23		5	10.5, CH_3	0.77
	6	28.8, CH_2	1.19		N-Me	30.1, CH_3	2.93
	7	28.8, CH_2	1.19	D-Asn[8]	1	*nd*, C	-
	8	31.3, CH_2	1.19		2	49.2, CH	4.68
	9	22.2, CH_2	1.24		3	35.8, CH_2	2.54
	10	14.0, CH_3	0.84				2.42
	NH	-	7.56		4	*nd*, C	-
Val[2]	1	*nd*, C	-		NH	-	8.20, d (7.45)
	2	58.4, CH	4.06		NH_2	-	7.34 & 6.92
	3	29.8, CH	2.03	Thr[9]	1	*nd*, C	-
	3-Me	19.3, CH_3	0.80		2	55.2, CH	4.51
	4	18.1, CH_3	0.82		3	66.5, CH	3.93
	NH	-	8.07		4	18.7, CH_3	1.06, d (5.73)
L-Leu[3]	1	*nd*, C	-		3-OH	-	
	2	50.7, CH	4.29		NH	-	7.46, d (7.45)
	3	40.4, CH_2	1.46	Pro[10]	1	*nd*, C	-
	4	24.1, CH	1.56		2	59.7, CH	4.33
	4-Me	22.9-23.2, CH_3	0.84		3	29.3, CH_2	1.73
	5	22.9-23.2, CH_3	0.84				2.06
	NH	-	7.90		4	24.1, CH_2	1.82
Ala[4]	1	171.4, C	-				1.82
	2	48.2, CH	4.19, d (6.87)		5	47.4, CH_2	3.7
	3	17.3, CH_3	1.20				3.61
	NH	-	8.06, d (6.30)	D-Leu[11]	1	*nd*, C	-
L-Leu[5]	1	*nd*, C	-		2	51.5, CH	4.28
	2	50.7, CH	4.26		3	40.4, CH_2	1.46
	3	41.2, CH_2	1.37		4	24.1, CH	1.56
	4	24.1, CH	1.56		4-Me	22.9-23.2, CH_3	0.84
	4-Me	22.9-23.2, CH_3	0.84		5	22.9-23.2, CH_3	0.84
	5	22.9-23.2, CH_3	0.84		NH	-	7.97
	NH	-	7.61	Thr[12]	1	*nd*, C	-
Gln[6]	1	*nd*, C	-		2	59.1, CH	4.03, d (8.59)
	2	48.1, CH	4.59, dd (6.87)		3	66.3, CH	4.01
	3	27.1, CH_2	1.73		4	19.7, CH_3	0.99, d (5.73)
			1.86		3-OH	-	
	4	30.7, CH_2	2.04		NH	-	7.96(8.59)
	5	*nd*, C	-				
	NH	-	8.21, d (6.87)				
	NH_2	-	7.13 & 6.75				

nd = not determined. The numbering of atoms corresponds to the one already reported in the article on trichormamide C.[1] The determination of the structure was made using several NMR spectra (Figures S14a-f).

Figure S14a: ^{1}H NMR spectrum of compound 9a in DMSO-d$_6$

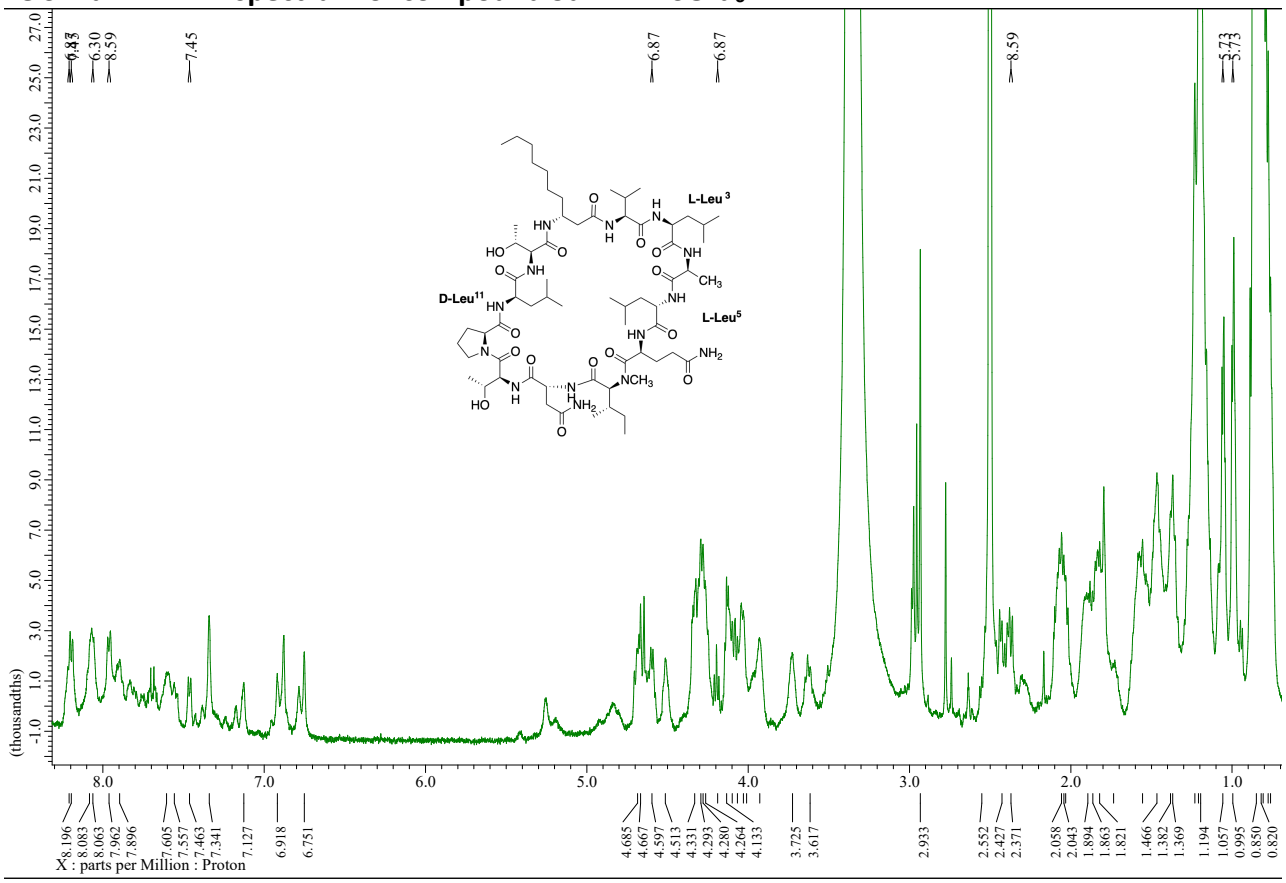


Figure S14b: ^{13}C NMR spectrum of compound 9a in DMSO-d$_6$

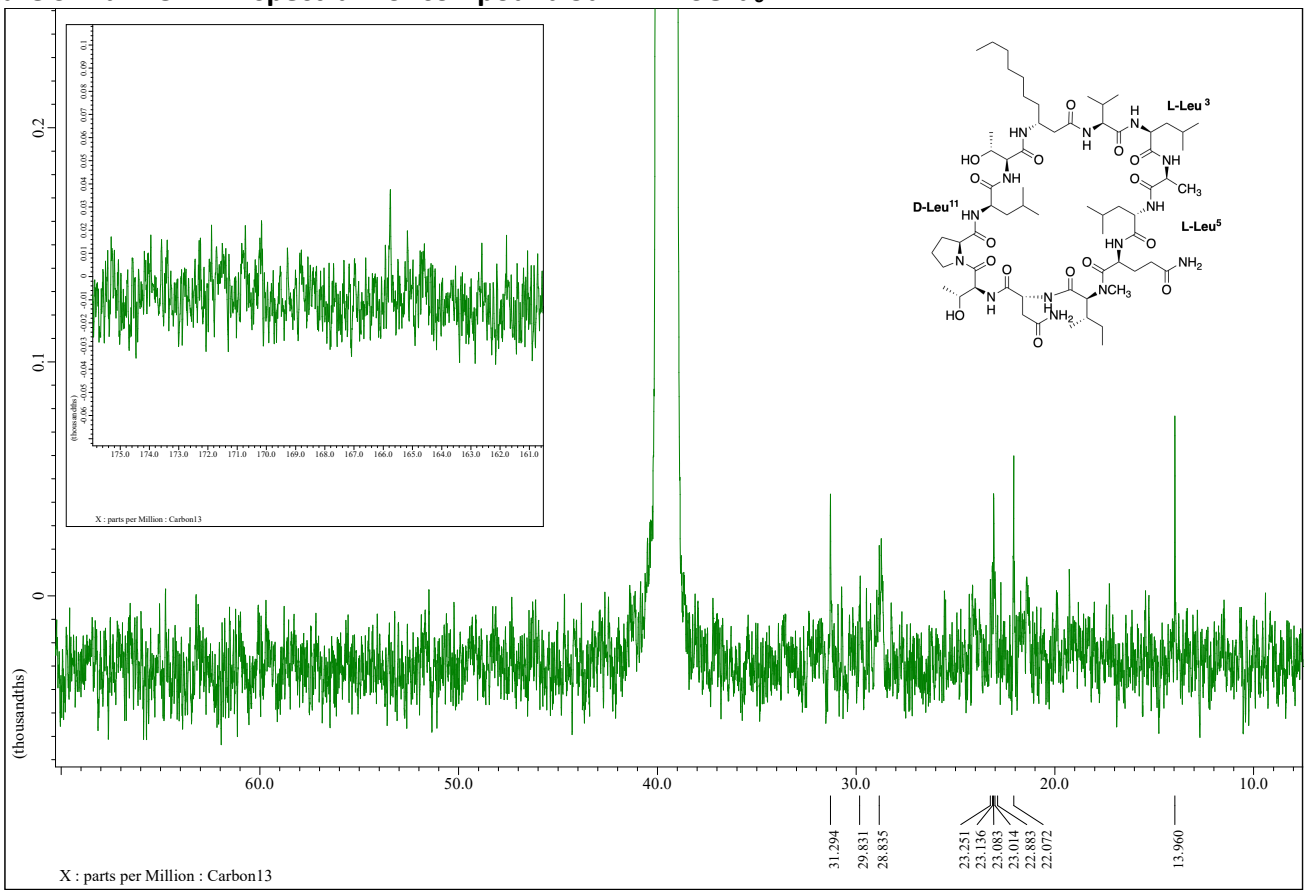


Figure S14c: TOCSY spectrum of compound 9a in DMSO-d_6

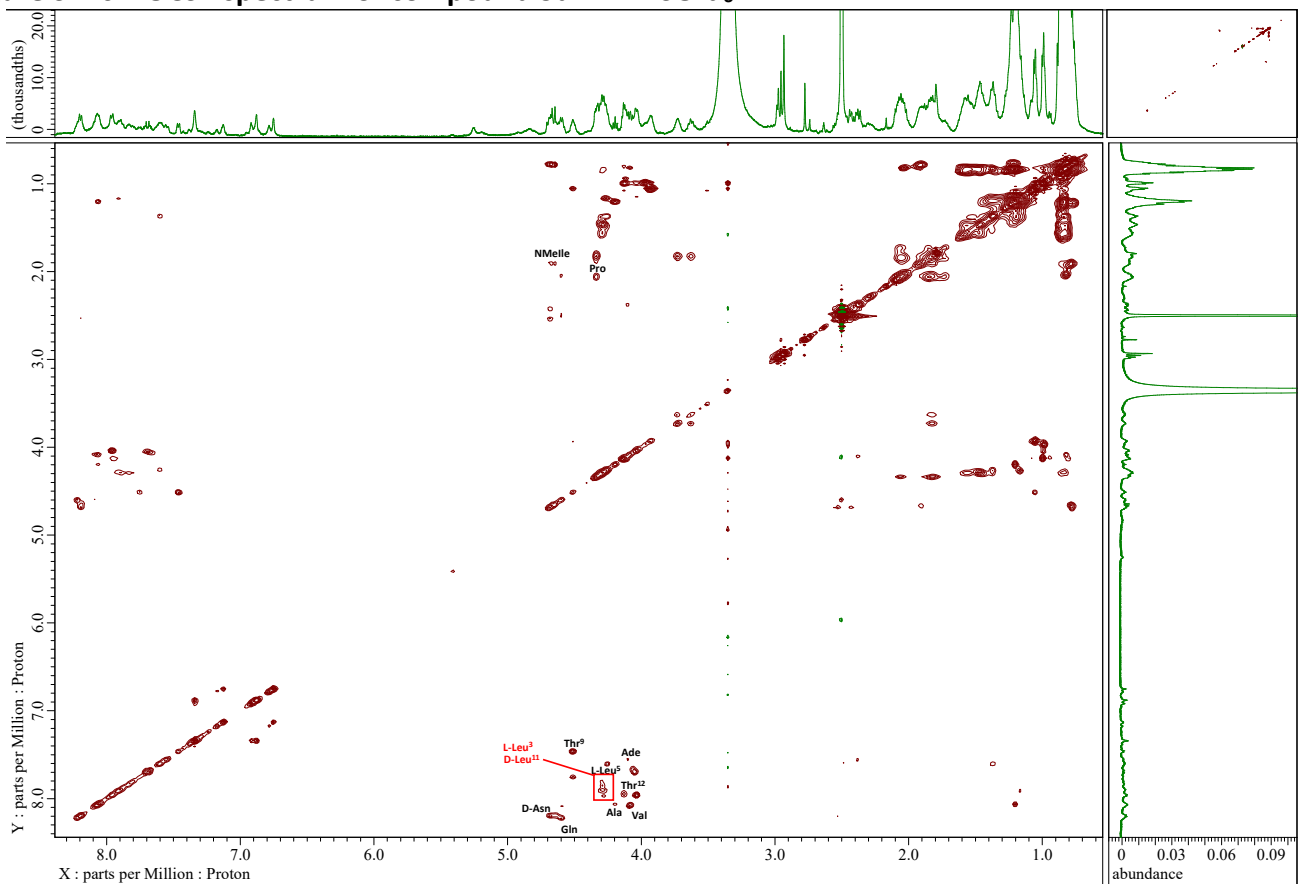


Figure S14d: ROESY spectrum of compound 9a in DMSO-d_6

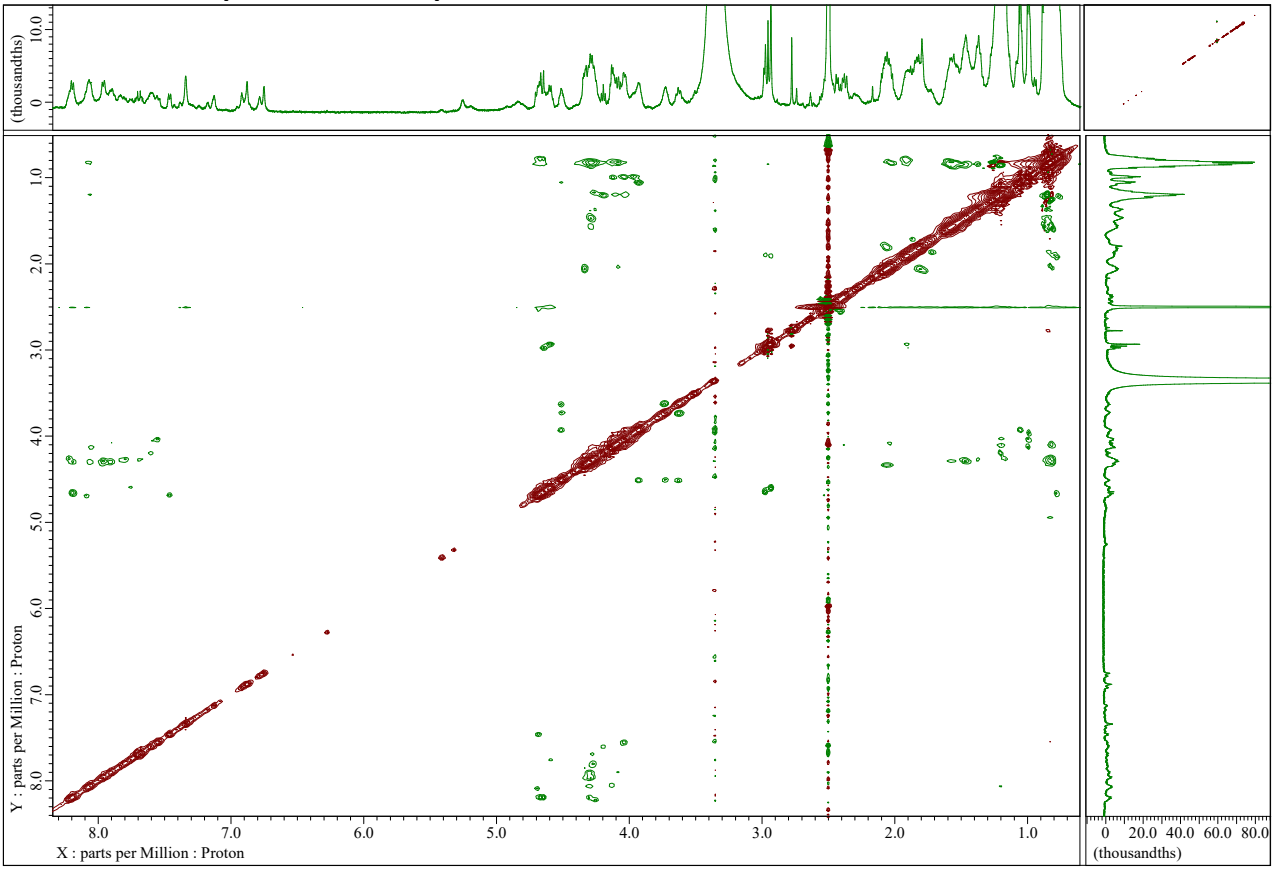


Figure S14e: HSQC spectrum of compound 9a in DMSO-d_6

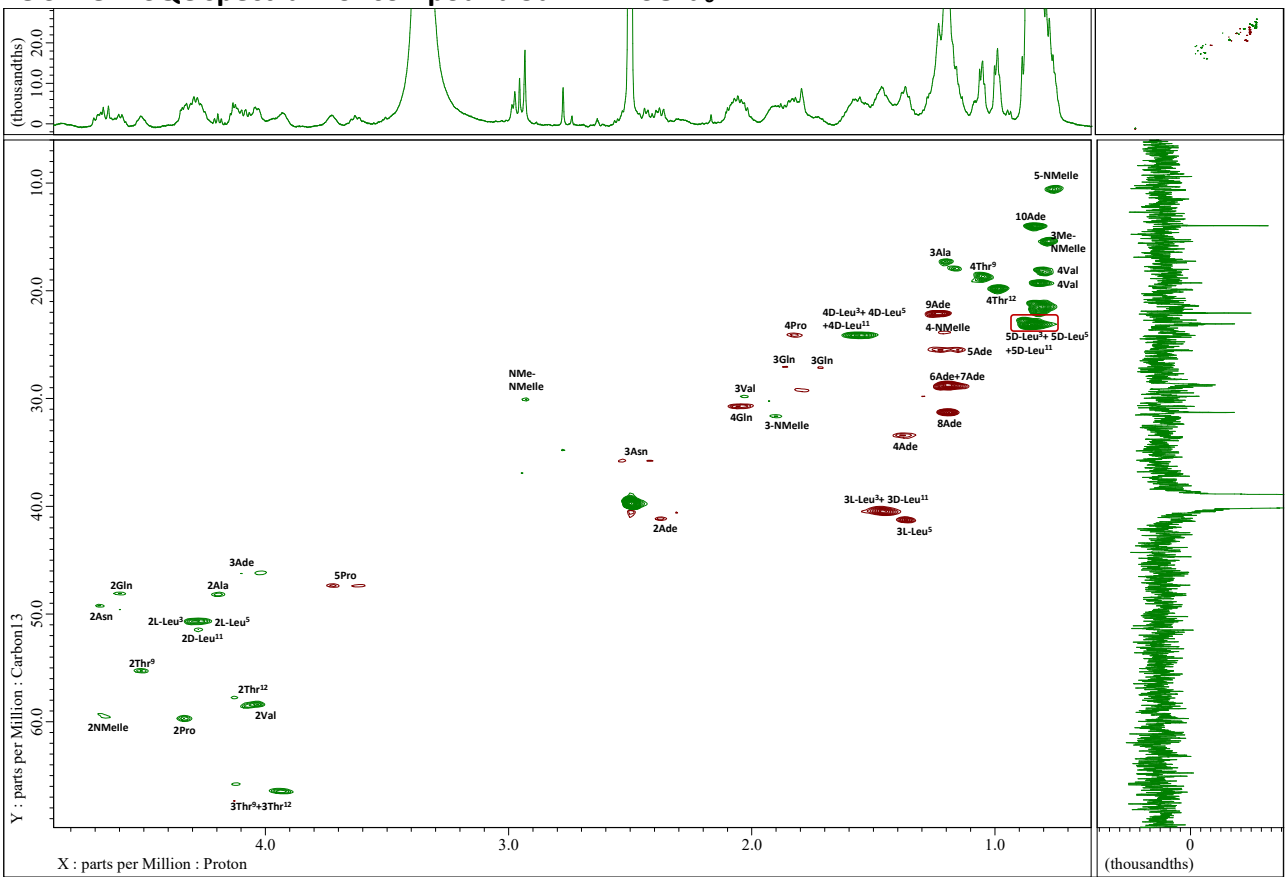


Figure S14f: HMBC spectrum of compound 9a in DMSO-d_6

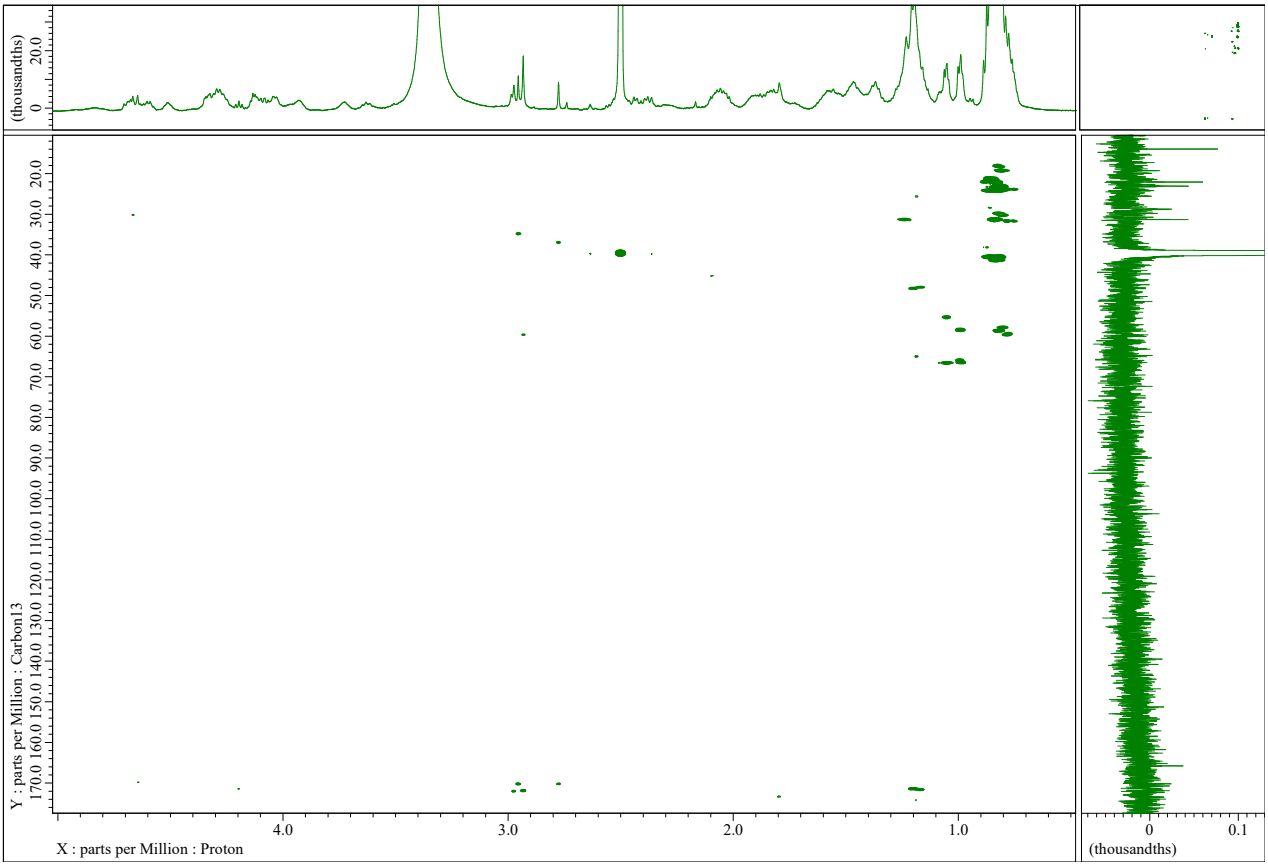


Table S15: NMR spectroscopic data of 10a in DMSO-d_6.

entry	position	C, mult.	H, mult. (J in Hz)	entry	position	C, mult.	H, mult. (J in Hz)
Decanoic acid[1]	1	172.3, C	-	**N-MeIle[7]**	1	169.3, C	-
	2	34.9, CH_2	2.14		2	59.1, CH	4.69, d (11.00)
			2.14		3	31.4, CH	1.89
	3	25.1, CH_2	1.46		3-Me	15.0, CH_3	0.77
	4	28.3-28.7, CH_2	1.23		4	23.8, CH_2	1.23
	5	28.3-28.7, CH_2	1.23				
	6	28.3-28.7, CH_2	1.23		5	10.1, CH_3	0.73
	7	28.3-28.7, CH_2	1.23		N-Me	30.0, CH_3	3.00
	8	31.0, CH_2	1.22	**D-Asn[8]**	1	171.2, C	-
	9	21.8, CH_2	1.26		2	49.4, CH	4.60, t (6.42)
	10	13.7, CH_3	0.85		3	36.9, CH_2	2.49
Val[2]	1	*nd*, C	-				
	2	57.6, CH	4.12, t (8.25)		4	*nd*, C	-
	3	29.9, CH	1.94		NH	-	8.07, d (8.25)
	3-Me	19.0, CH_3	0.83		NH_2	-	7.33 & 6.92
	4	18.0, CH_3	0.81	**Thr[9]**	1	*nd*, C	-
	NH	-	7.84, d (8.71)		2	55.3, CH	4.48, t (7.34)
L-Leu[3]	1	*nd*, C	-		3	66.4, CH	3.90, t (5.96)
	2	50.4-51.0, CH	4.30		4	18.6, CH_3	1.05, d (5.96)
	3	40.1-40.9, CH_2	1.43-1.47				
	4	23.8-24.0, CH	1.57		NH	-	7.74, d (7.79)
	4-Me	21.0-23.0, CH_3	0.82-0.86	**Pro[10]**	1	*nd*, C	-
	5	21.0-23.0, CH_3	0.82-0.86		2	59.6, CH	4.32
	NH	-	7.94, d (7.79)		3	28.9, CH_2	1.80
Ala[4]	1	171.6, C	-				2.03
	2	48.4, CH	4.20, t (7.34)		4	24.0, CH_2	1.87
	3	17.9, CH_3	1.18, d (6.88)				1.87
	NH	-	7.94, d (7.79)		5	47.1, CH_2	3.69
D-Leu[5]	1	171.6, C	-				3.69
	2	50.4-51.0, CH	4.26	**D-Leu[11]**	1	*nd*, C	-
	3	40.1-40.9, CH_2	1.43-1.47		2	50.4-51.0, CH	4.32
	4	23.8-24.0, CH	1.57		3	40.1-40.9, CH_2	1.43-1.47
	4-Me	21.0-23.0, CH_3	0.82-0.86		4	23.8-24.0, CH	1.57
	5	21.0-23.0, CH_3	0.82-0.86		4-Me	21.0-23.0, CH_3	0.82-0.86
	NH	-	7.94, d (7.79)		5	21.0-23.0, CH_3	0.82-0.86
Gln[6]	1	*nd*, C	-		NH	-	7.97, d (8.25)
	2	47.8, CH	4.63, t (6.88)	**Thr[12]**	1	171.8, C	-
	3	26.6, CH_2	1.73		2	57.9, CH	4.05, d (6.88)
			1.84		3	66.0, CH	4.05, d (6.88)
	4	30.3, CH_2	2.05		4	19.8, CH_3	0.99, d (5.96)
	5	173.6, C	-				
	NH	-	8.04, d (8.25)		NH	-	7.66, d (8.71)
	NH_2	-	7.20 & 6.80		NH_2	-	7.14 & 7.08

nd = not determined. The numbering of atoms corresponds to the one already reported in the article on trichormamide C.[1] The determination of the structure was made using several NMR spectra (Figures S15a-f).

Figure S15a: ^{1}H NMR spectrum of compound 10a in DMSO-d$_6$

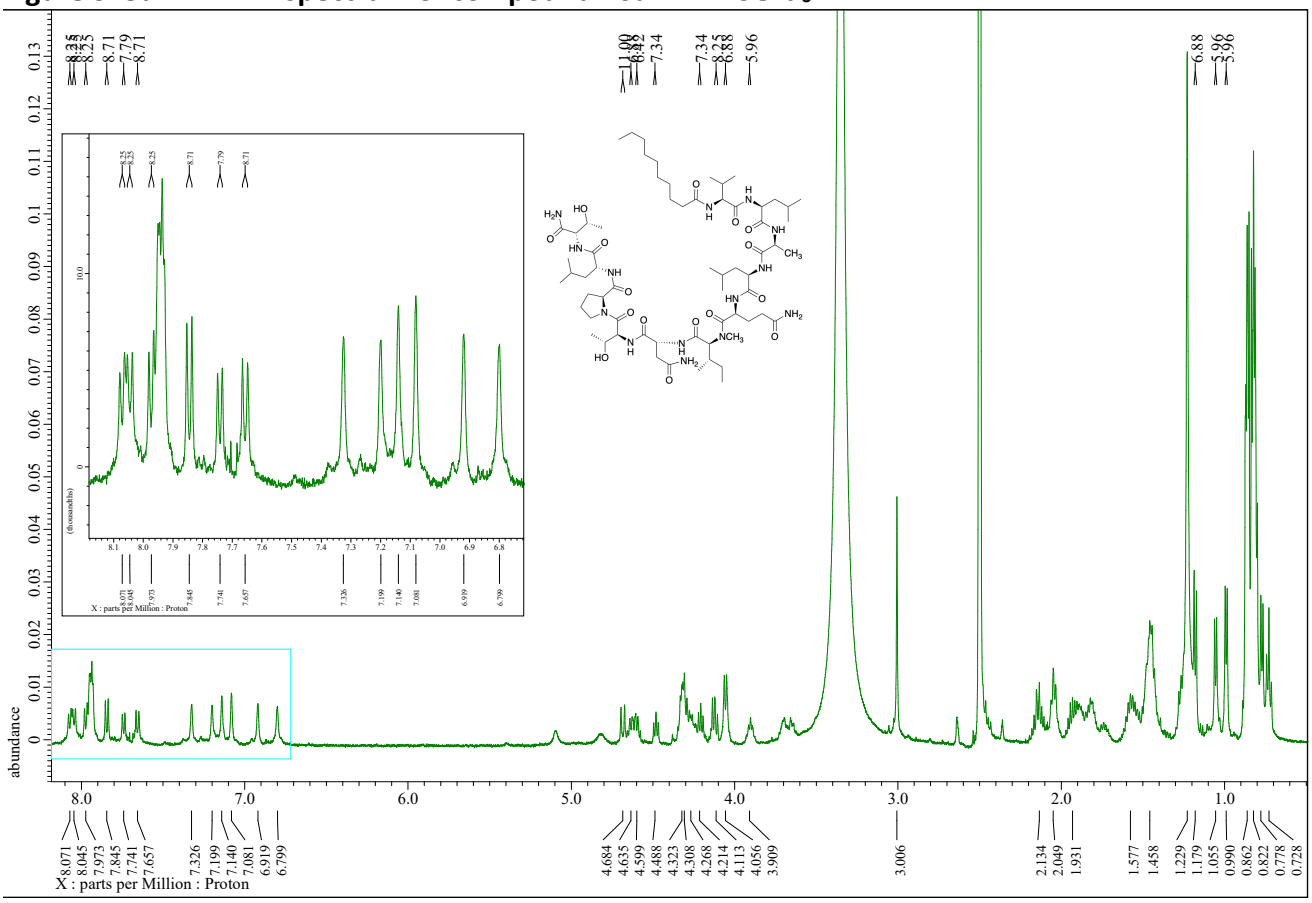


Figure S15b: DEPT spectrum of compound 10a in DMSO-d$_6$

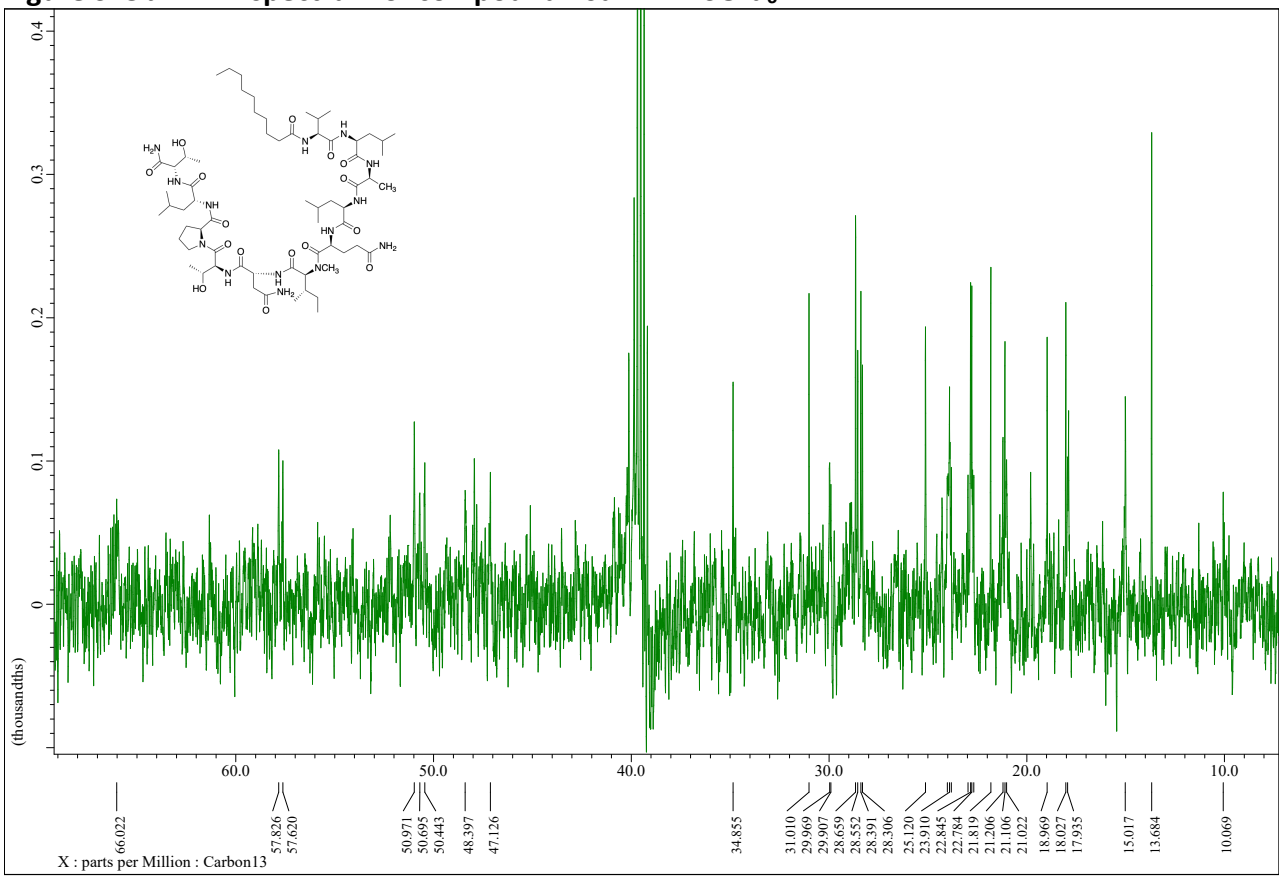


Figure S15c: TOCSY spectrum of compound 10a in DMSO-d_6

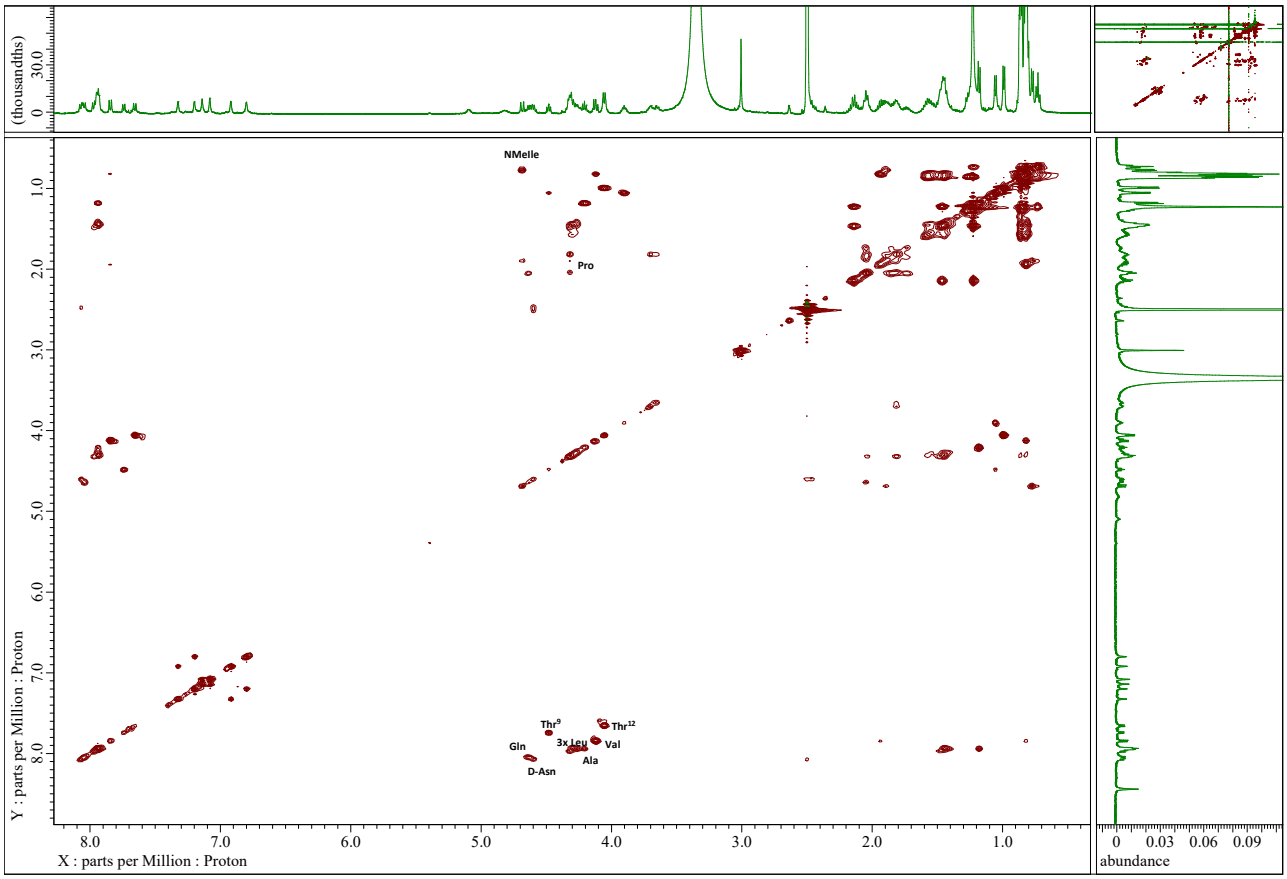


Figure S15d: ROESY spectrum of compound 10a in DMSO-d_6

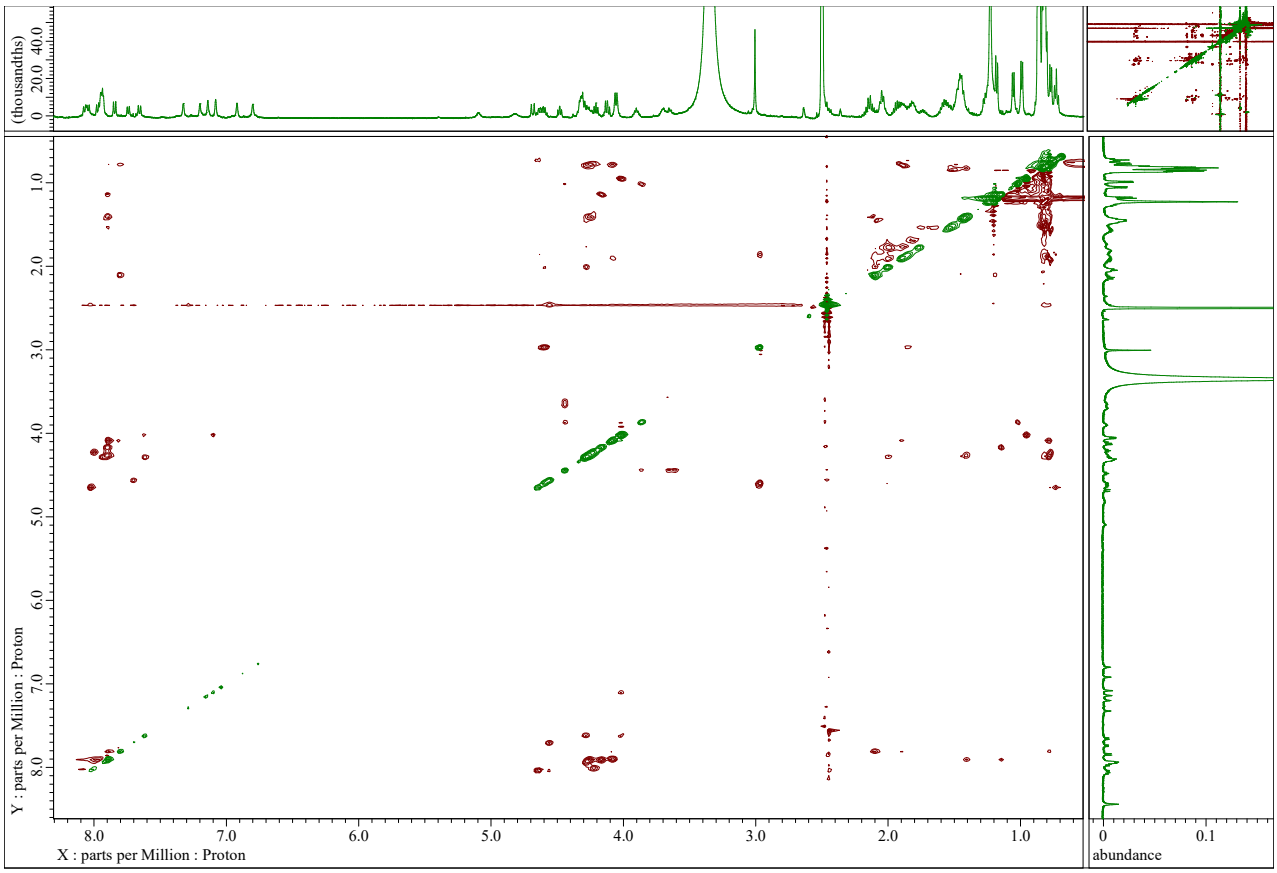


Figure S15e: HSQC spectrum of compound 10a in DMSO-d_6

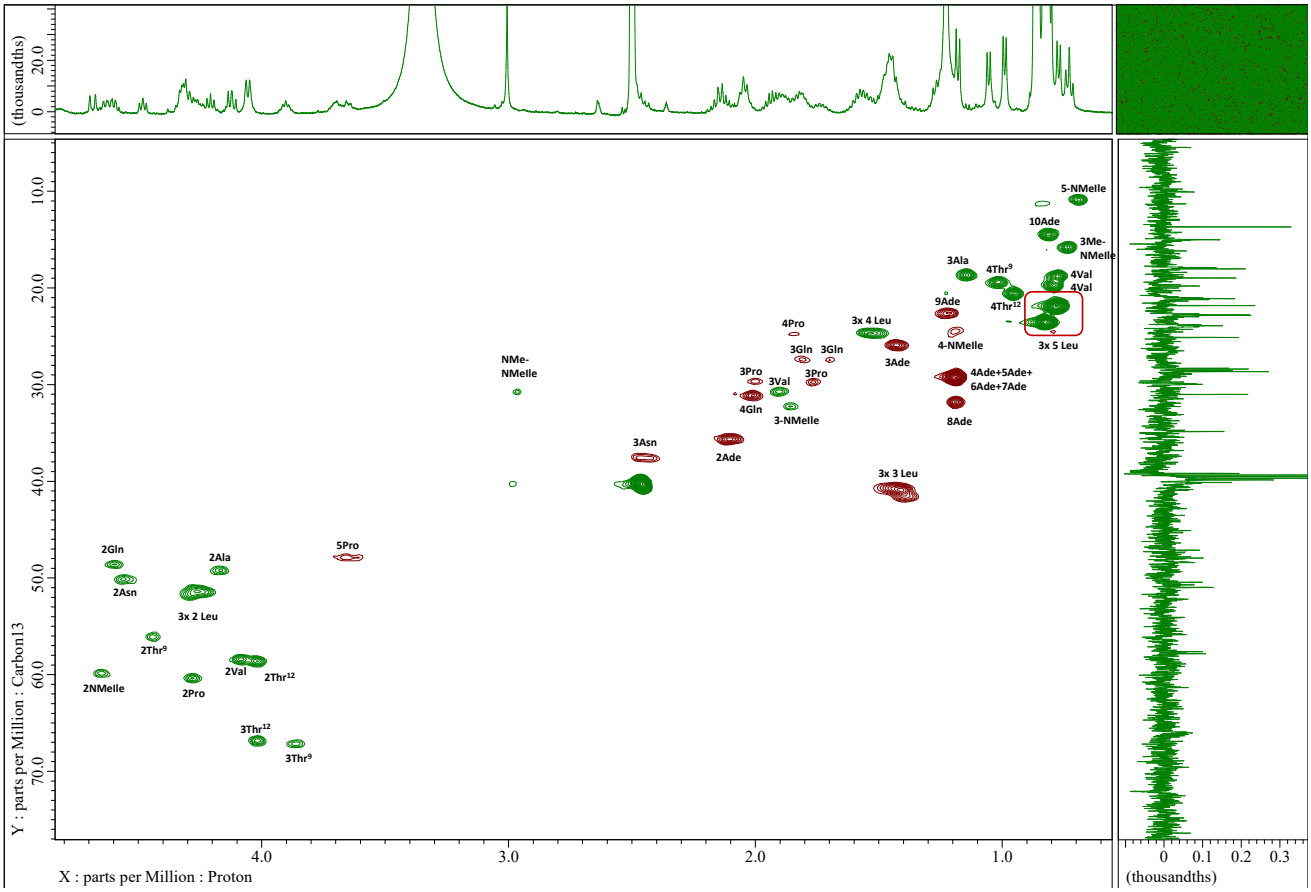
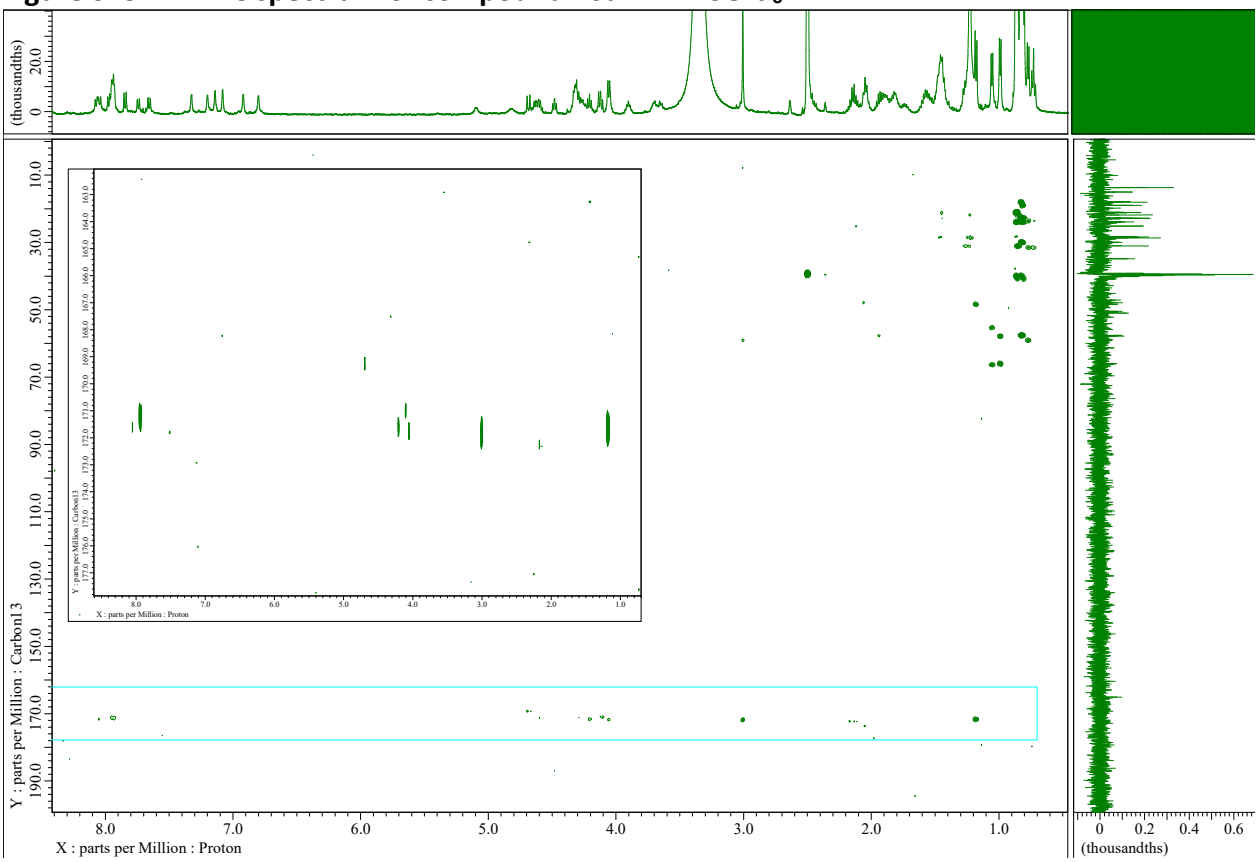


Figure S15f: HMBC spectrum of compound 10a in DMSO-d_6



Model selection

In the following figures, *Dg-Ss-At* represents the digestive gland of *Stylocheilus striatus* collected from *Anabaena torulosa*, while *Dg-Ss-Lm* corresponds to the digestive gland of *Stylocheilus striatus* collected from *Lyngbya majuscula*.

Figure S16
A) Evolution of **2a** peak area as a function of incubation time at 30°C, pH = 5, in the presence of *Dg-Ss-At* or *Dg-Ss-Lm*. In solid grey, **2a** + *Dg-Ss-At*; in striped grey, laxB + *Dg-Ss-Lm*; in purple, **2a** control without *Dg-Ss*. B) Evolution of **2a** peak area as a function of incubation time at 30°C, pH = 8, in the presence of *Dg-Ss-At* or *Dg-Ss-Lm*. In solid grey, **2a** + *Dg-Ss-At*; in striped grey, **2a** + *Dg-Ss-Lm*; in purple, **2a** control without *Dg-Ss*.

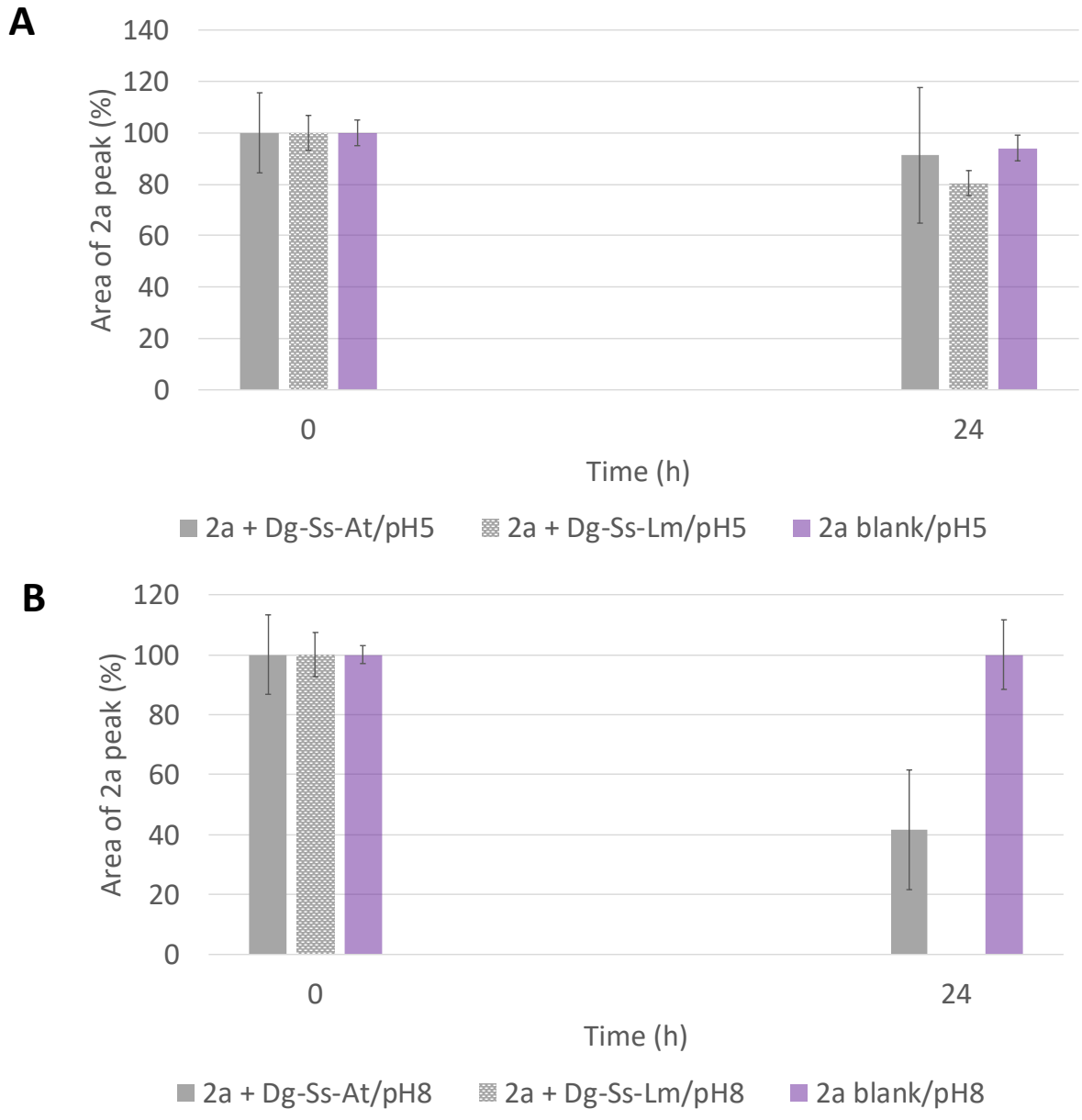


Figure S17

A) Composition of *Dg-Ss-At* extract at 30°C, pH = 8. B) Composition of *Dg-Ss-Lm* extract at 30°C, pH = 8.

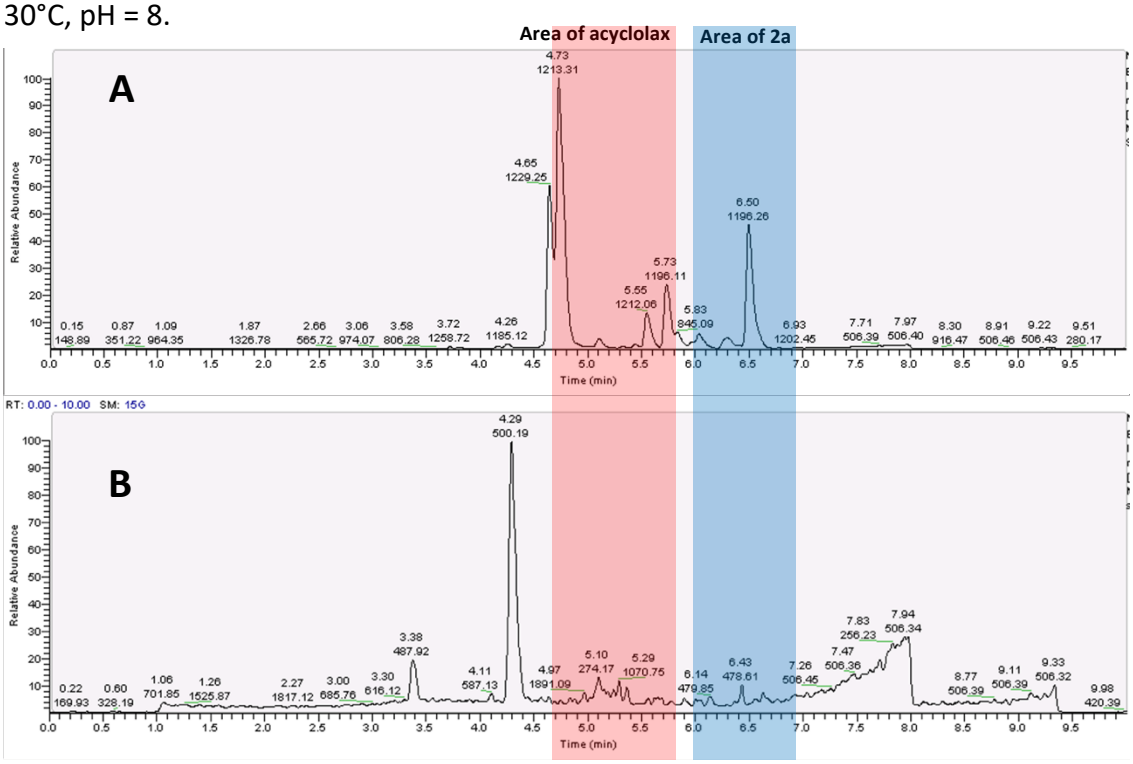
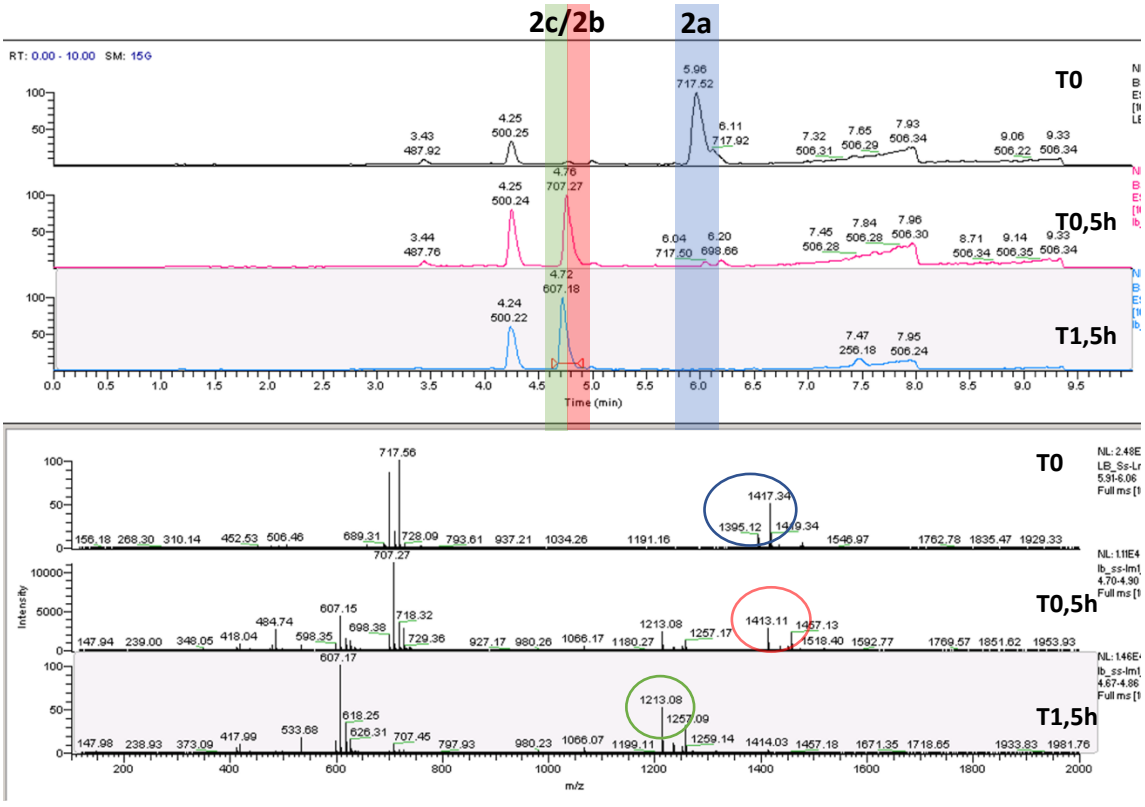


Figure S18

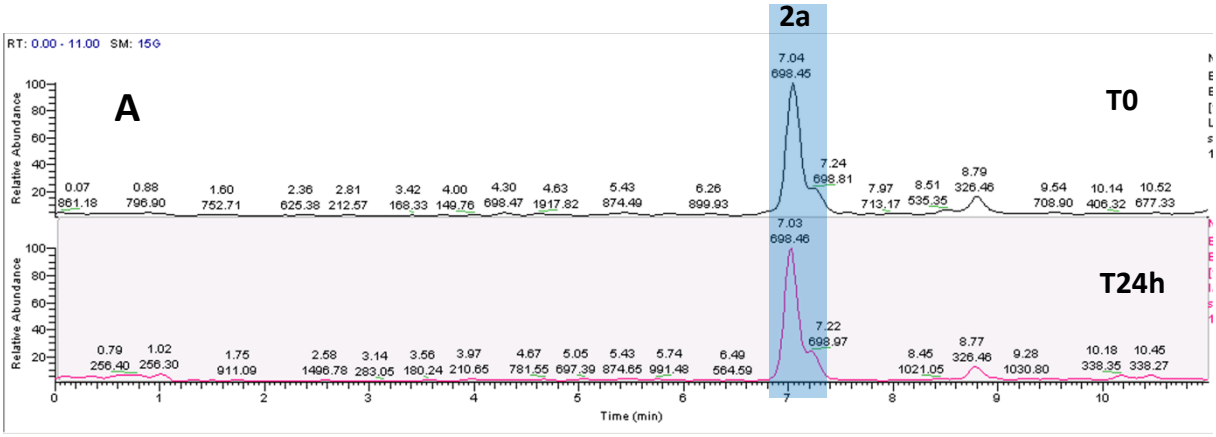
LC-MS kinetic monitoring of **2a** degradation with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peak at 4.77 min (**2b** and **2c** co-elute).

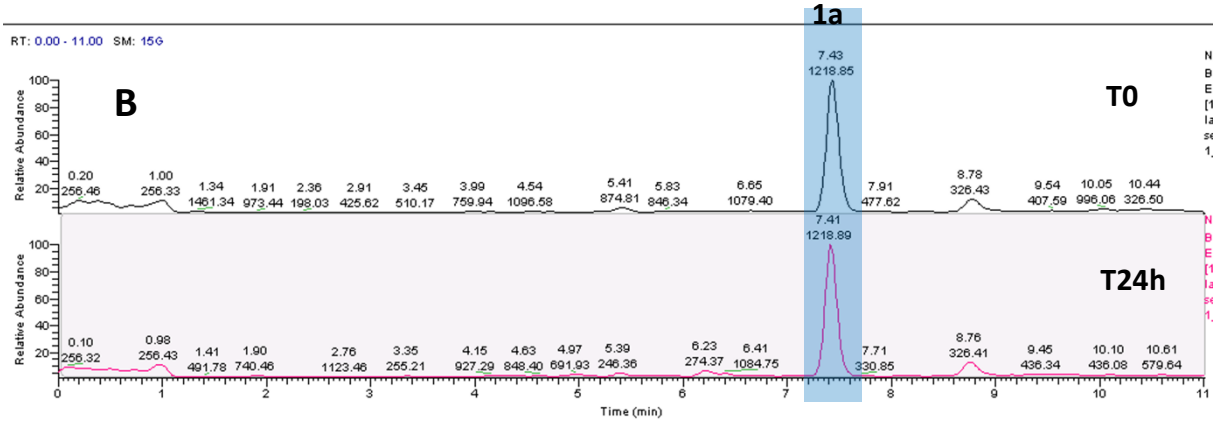


Stability of 1a and 2a

Figure S19

A)**2a** evolution over time with rat serum (T=0 and T=24h). B)**1a** evolution over time with rat serum (T=0 and T=24h). C) Evolution of 1a and 2a peaks area as a function of incubation time at 37°C, pH = 8, in the presence of rat serum.





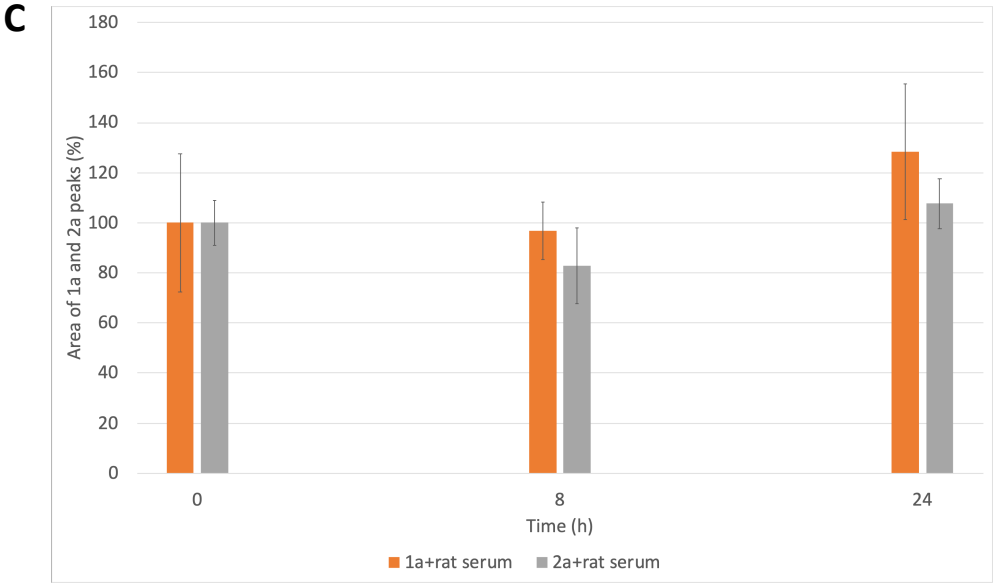
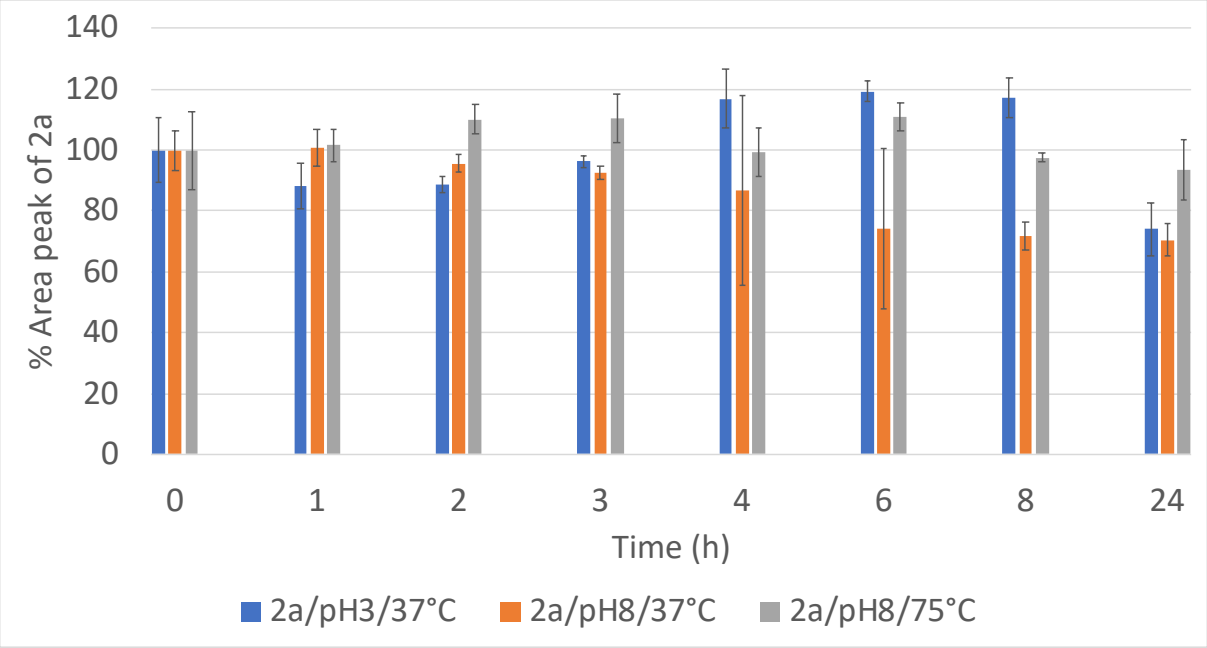


Figure S20
Evolution of **2a** peak area as a function of incubation time at 37°C, pH = 3 or 8 and at 75°C, pH = 8.



Stability of 2a with isolated enzymes

Figure S21
Evolution of **2a** peak area as a function of incubation time in the presence of different enzymes.

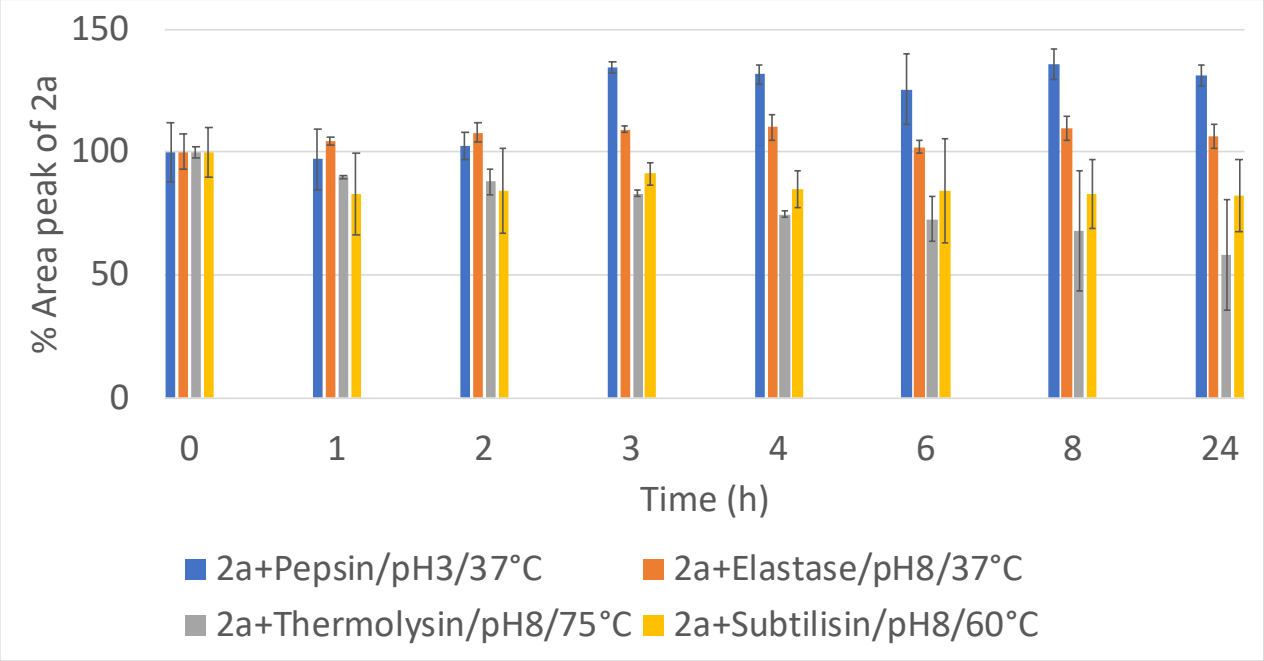
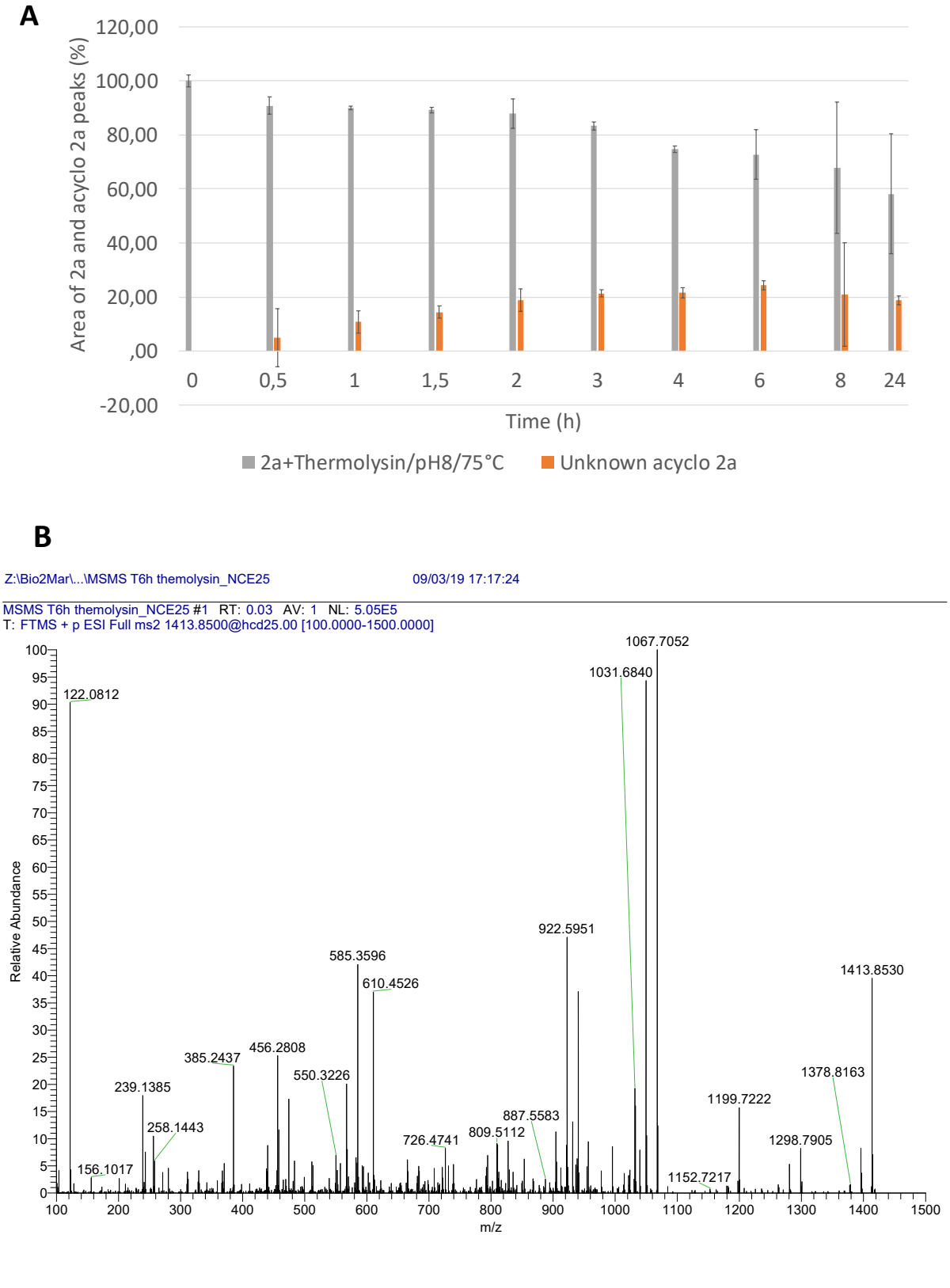
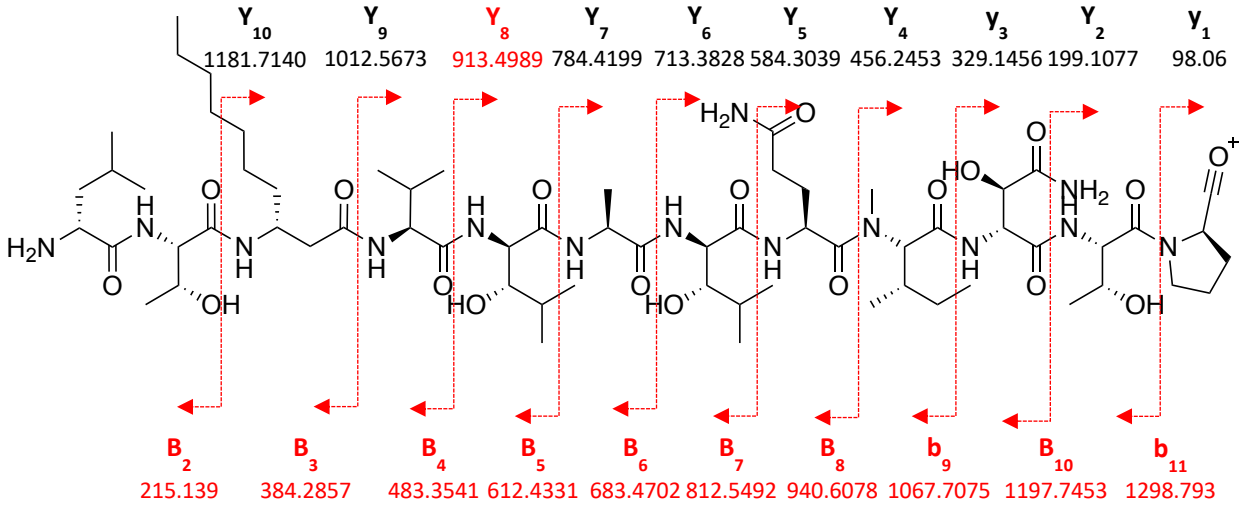


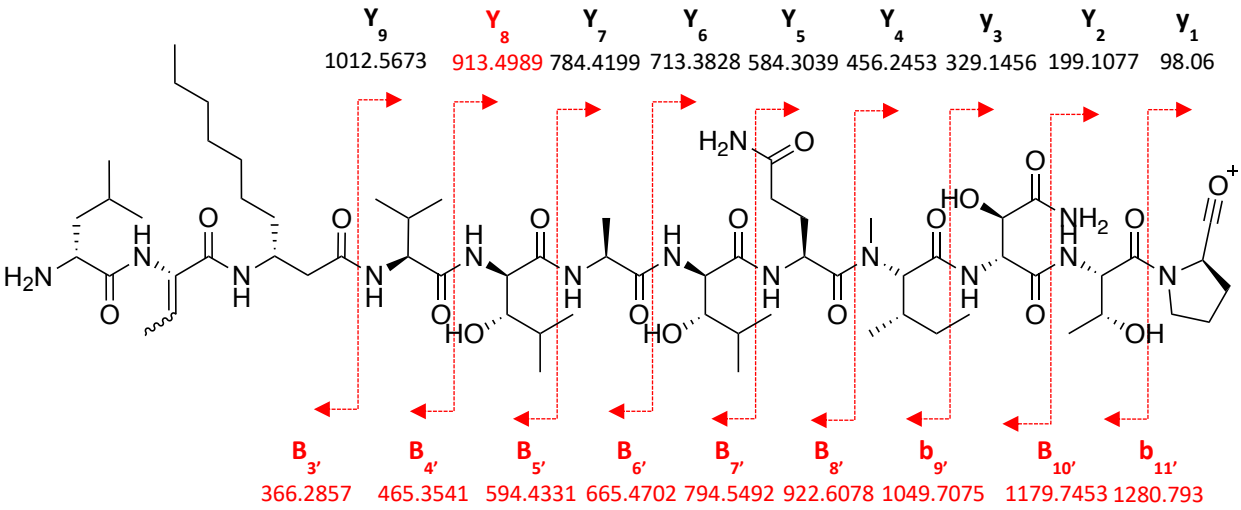
Figure S22

A) Evolution of **2a** and an unknown acyclolax peak areas as a function of incubation time at 75°C, pH = 8, in the presence of Thermolysin. B) MS-MS analysis of the unknown acyclolax peak (in red, observed ions; in black, non-observed ions).



If we consider a cleavage between Pro[10] and D-Leu[11], we observed b ions from b_2 to b_{11} and their equivalents with de-hydroxylated Thr from $b_{3'}$ to $b_{11'}$ but not their respective y ions.



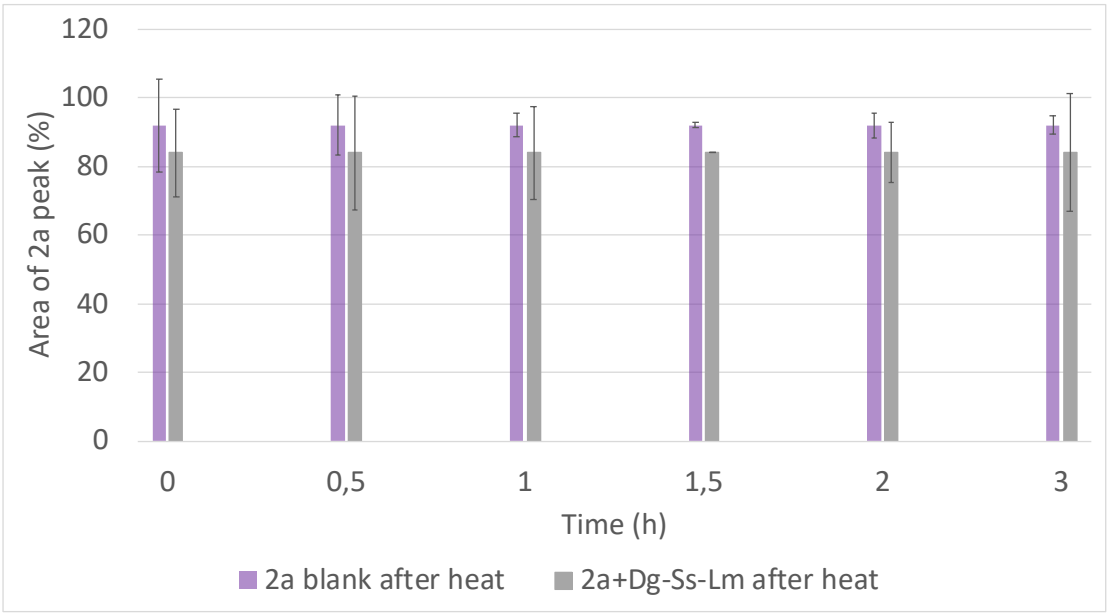


Stability of 2a with heated digestive gland

In the following figures, *Dg-Ss-Lm* corresponds to the digestive gland of *Stylocheilus striatus* collected from *Lyngbya majuscula*.

Figure S23

Evolution of **2a** peak area as a function of incubation time at 30°C, pH = 8, in the presence of *Dg-Ss-Lm* previously heated. In grey, **2a** + heated *Dg-Ss-Lm*; in purple, **2a** control without *Dg-Ss-Lm.* The values shown are an average of all the values obtained during the kinetics.



Kinetic analyses of peptide cleavages

Figure S24

Evolution of **1a** peak area over time at 30°C, pH = 8, in the presence of *Dg-Ss-Lm*. The values shown are an average of all the values obtained during the kinetics.

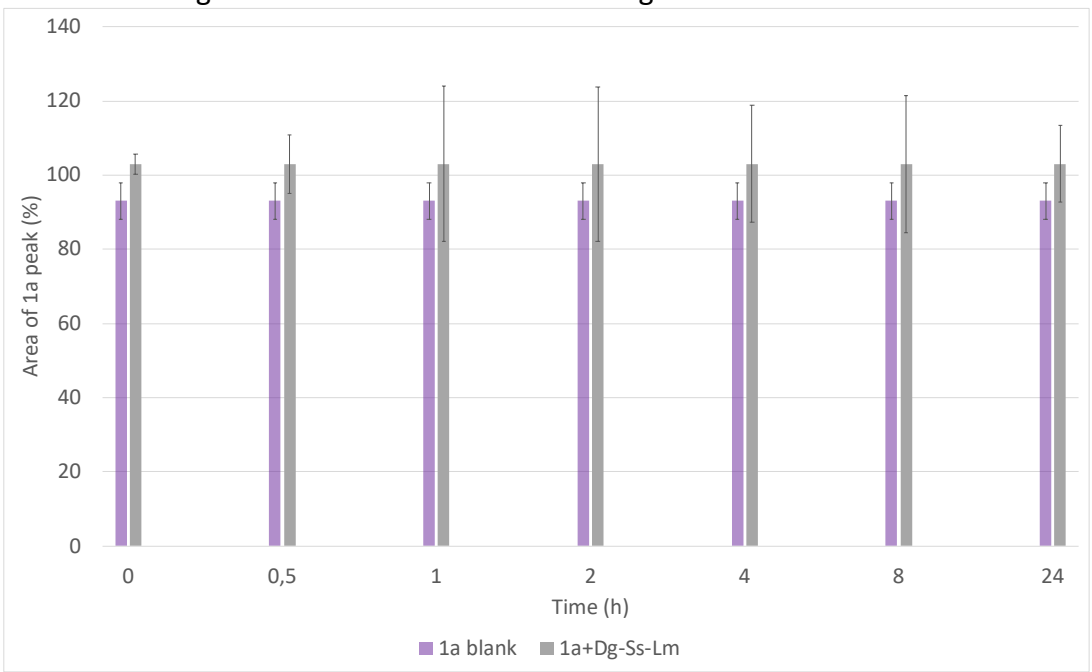
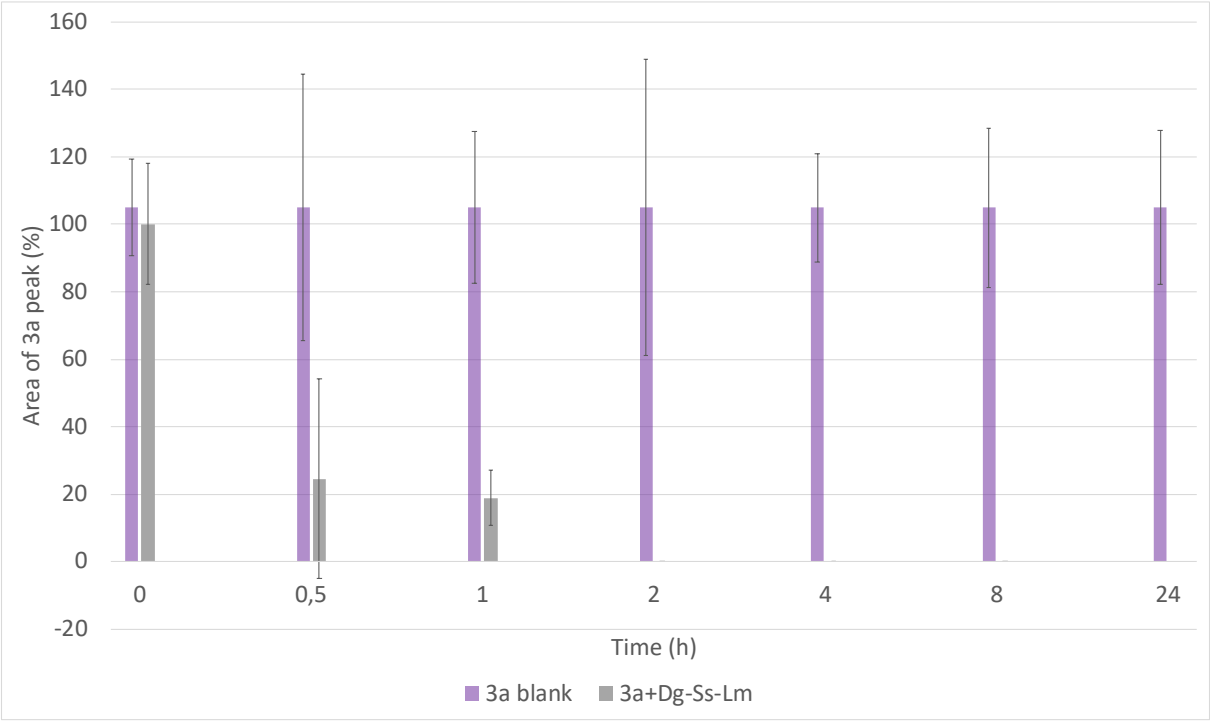


Figure S25

LC-MS kinetic monitoring of **3a** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peaks at 5.91 and 5.65 min. The control values (in purple) correspond to an average of all the values obtained during the kinetics.



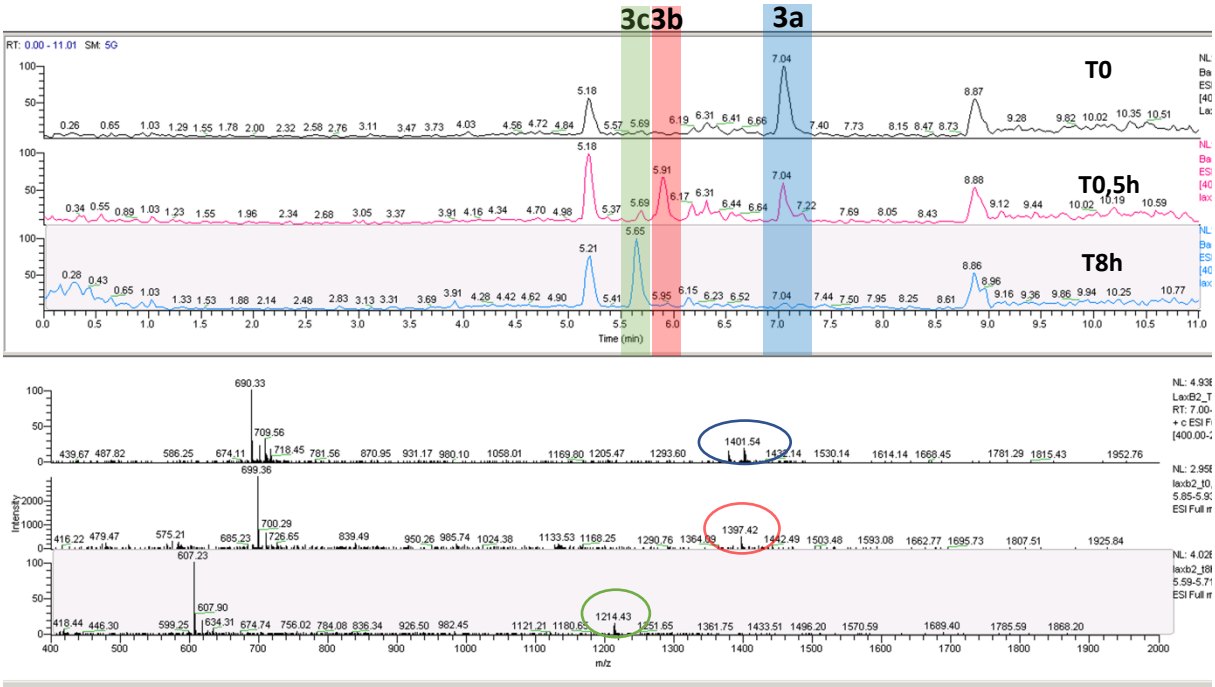
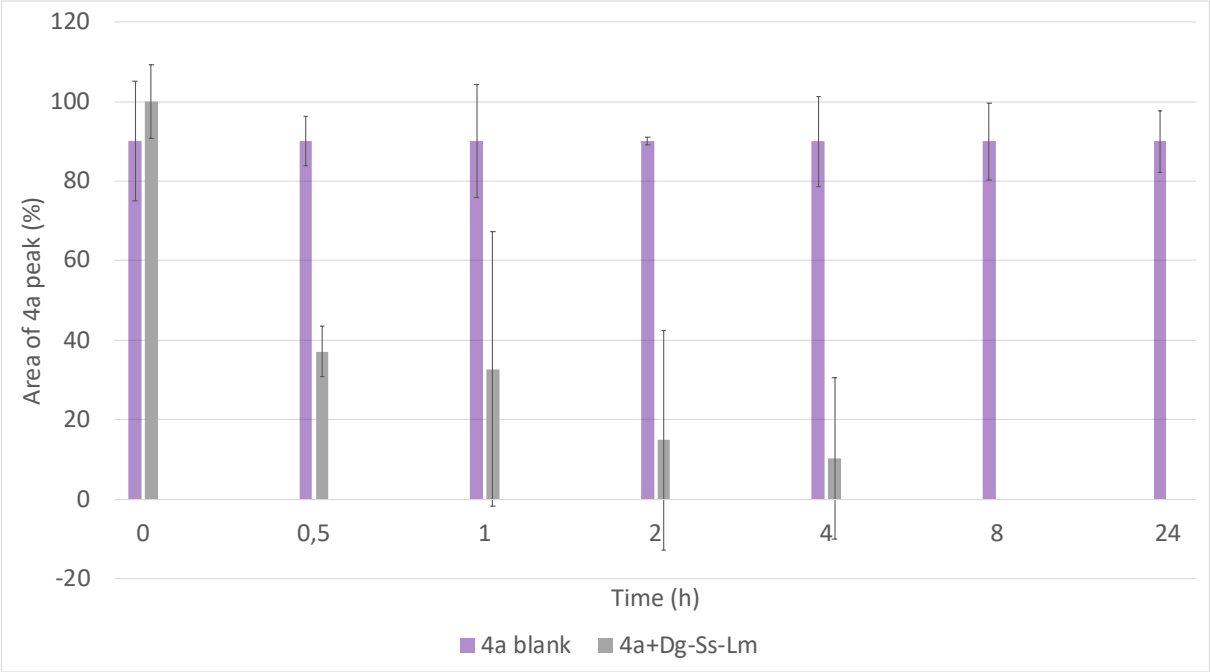


Figure S26

LC-MS kinetic monitoring of **4a** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peaks at 5.73 min and 5.77 min. The control values (in purple) correspond to an average of all the values obtained during the kinetics.



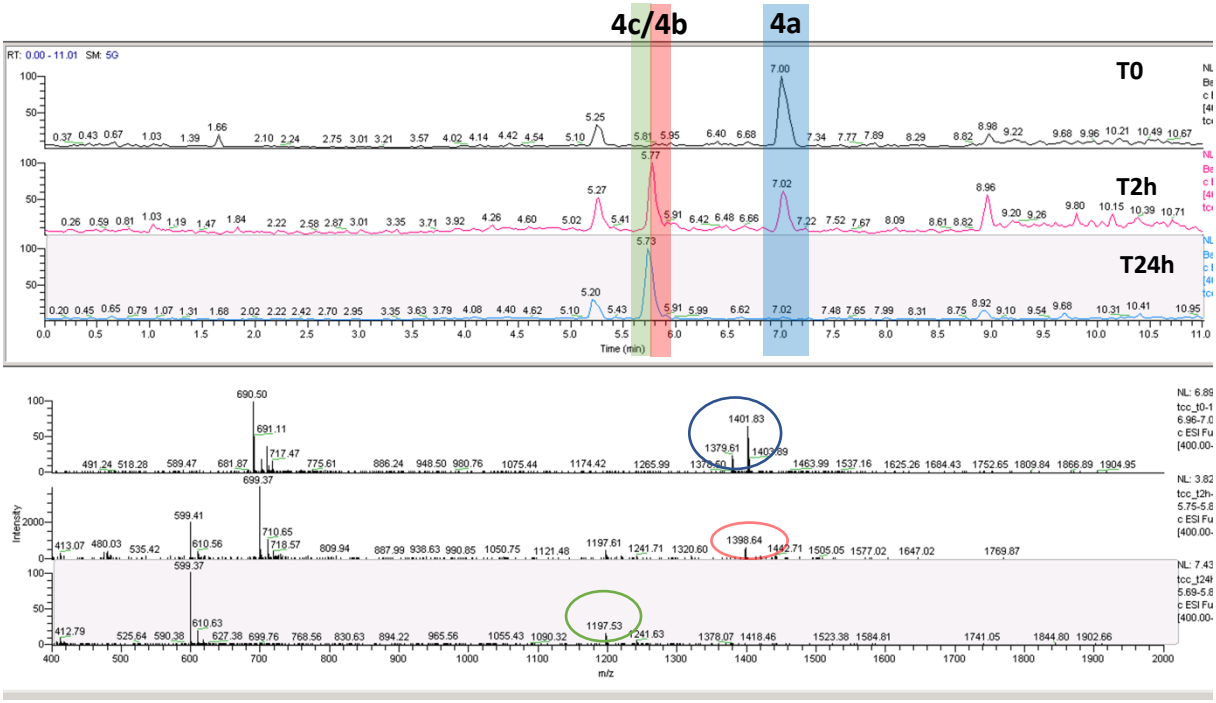
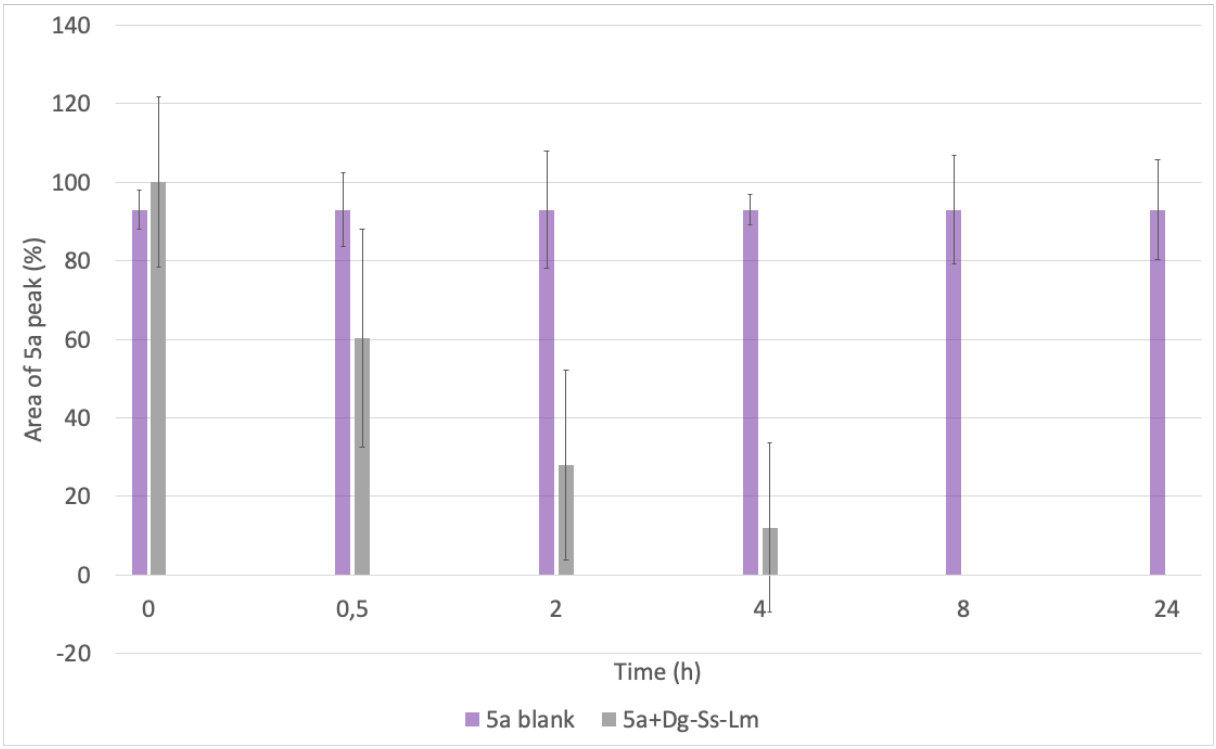


Figure S27

LC-MS kinetic monitoring of **5a** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peaks at 6.1 min and 5.97 min. The control values (in purple) correspond to an average of all the values obtained during the kinetics.



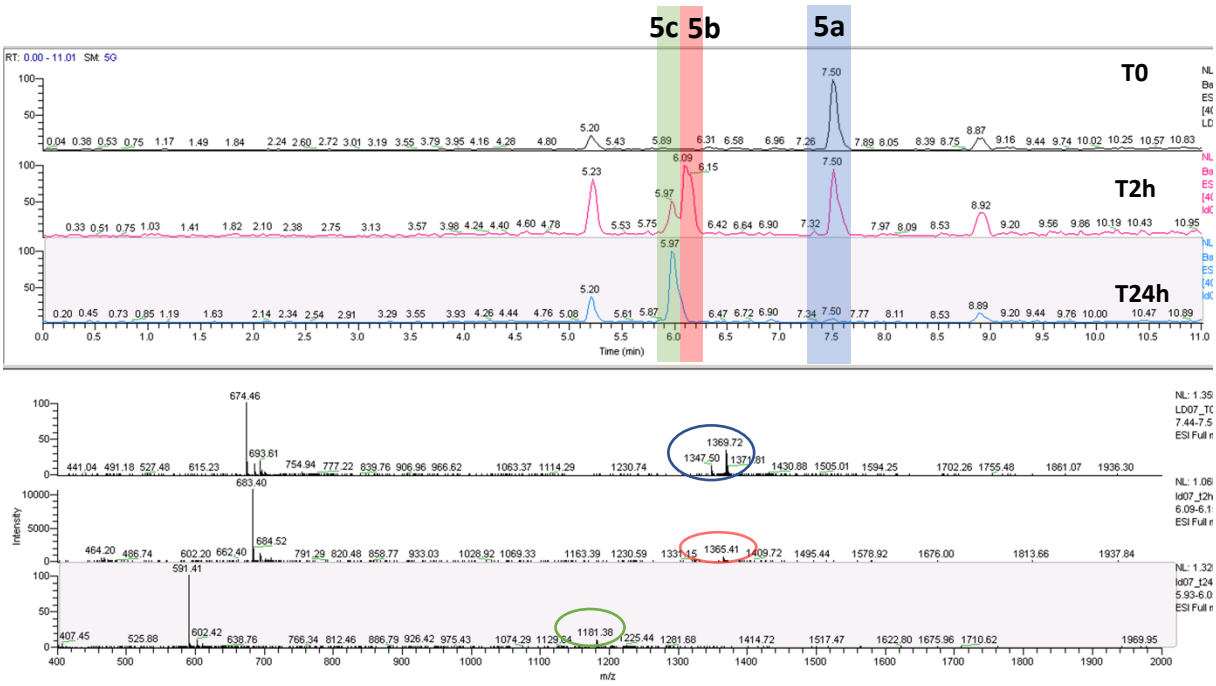
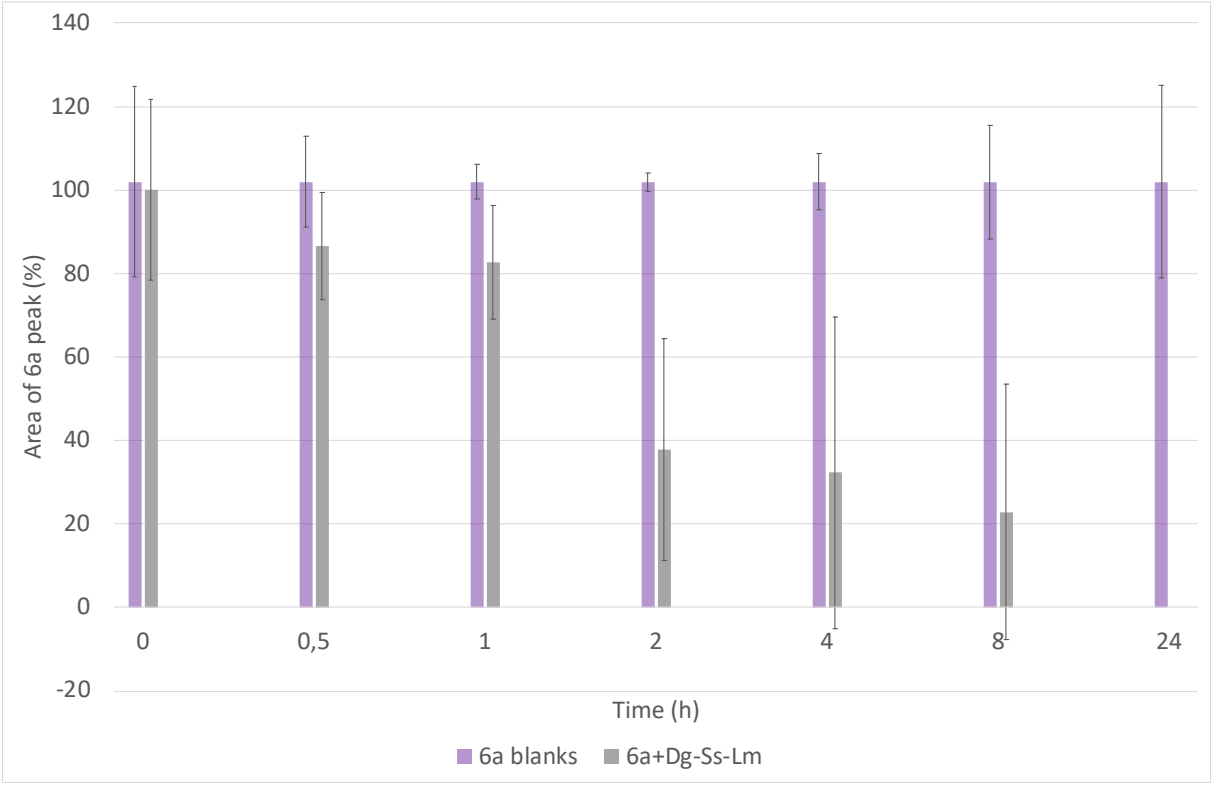


Figure S28

LC-MS kinetic monitoring of **6a** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peaks at 5.21 min and 5.37 min. Note that **6b** co-elute with an unknown compound of *m/z* 900. The control values (in purple) correspond to an average of all the values obtained during the kinetics.



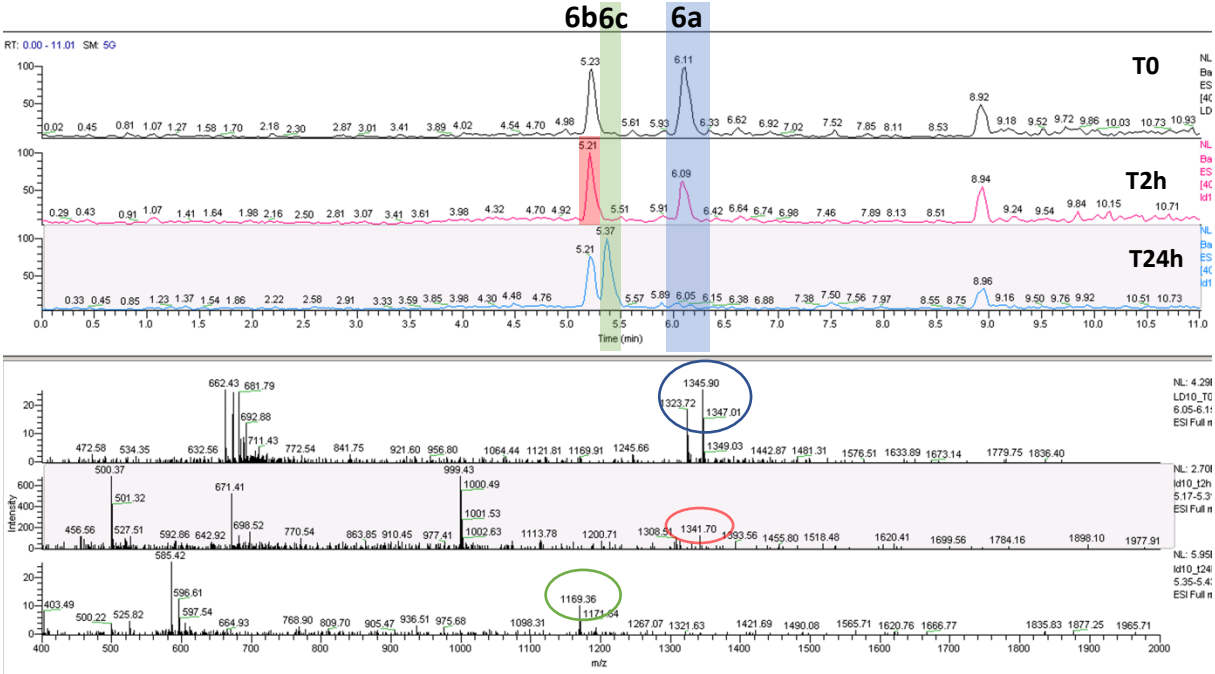
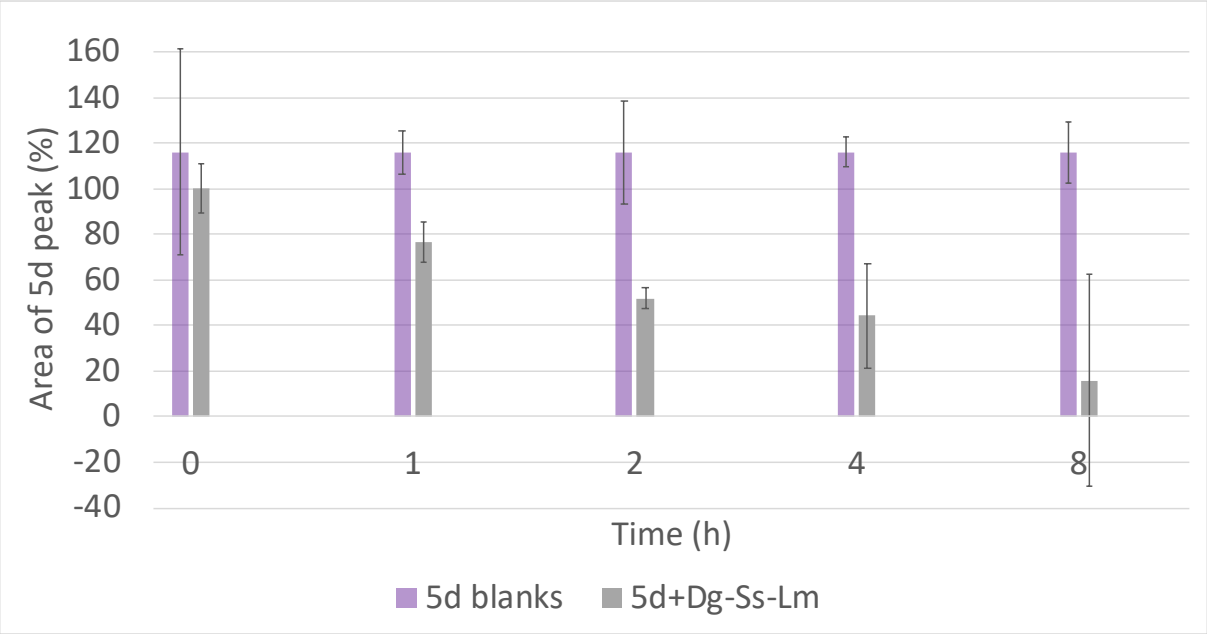


Figure S29

LC-MS kinetic monitoring of **5d** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peak at 6 min. The control values (in purple) correspond to an average of all the values obtained during the kinetics.



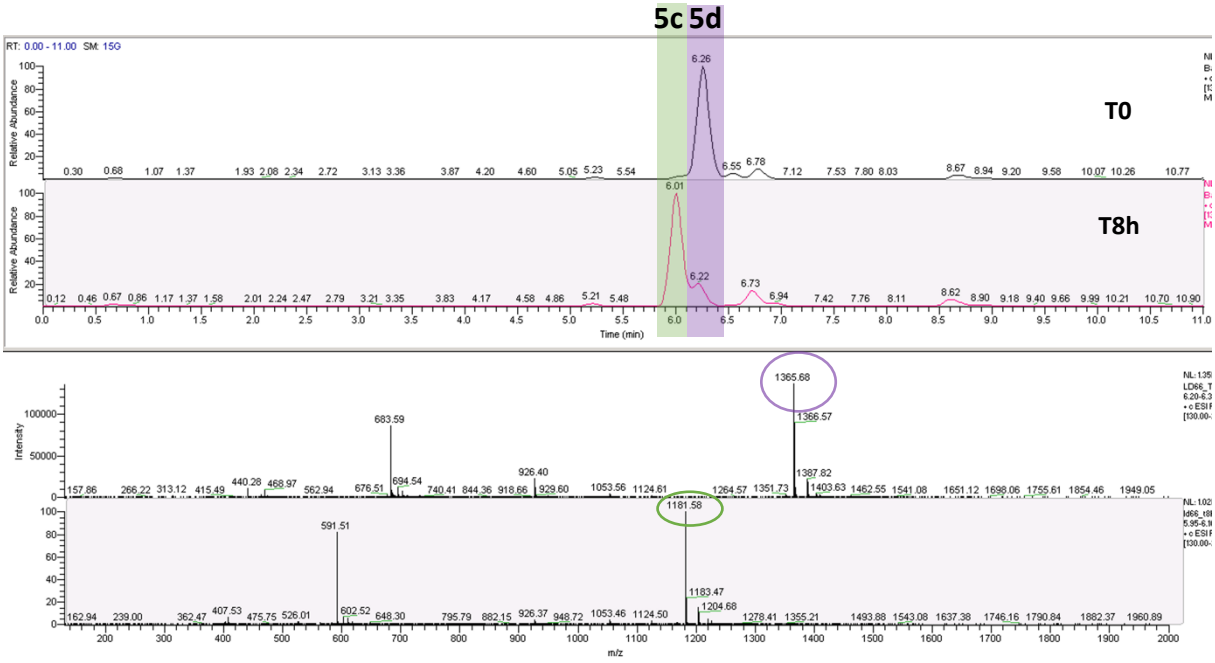
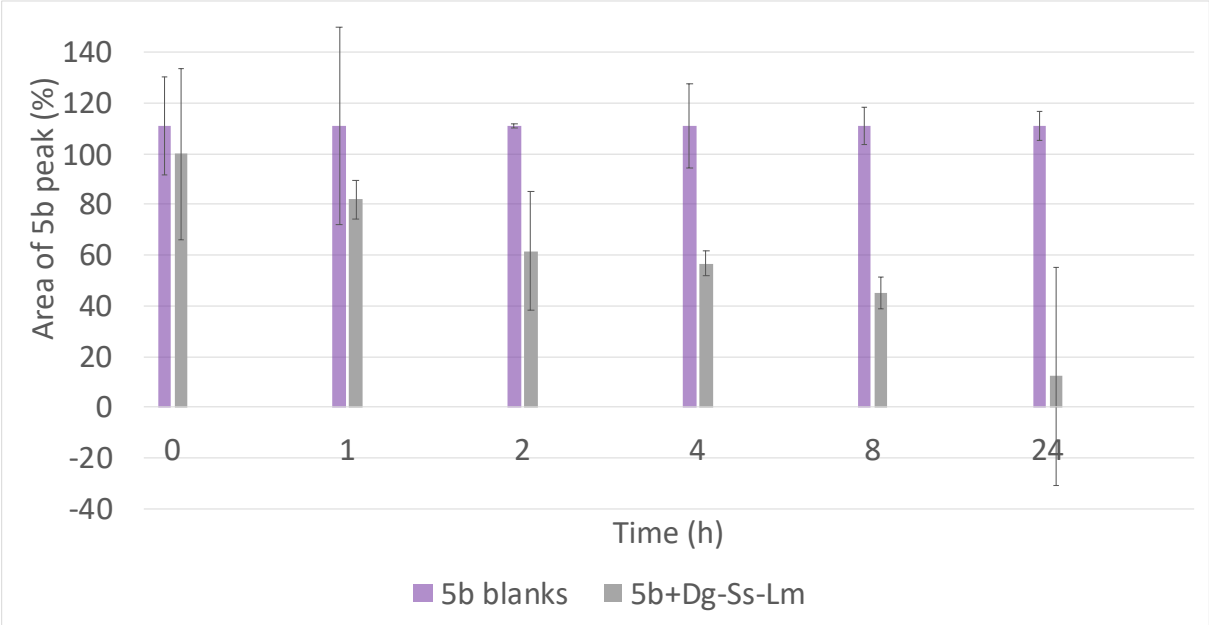


Figure S30

LC-MS kinetic monitoring of **5b** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peak at 6.12 min. The control values (in purple) correspond to an average of all the values obtained during the kinetics.



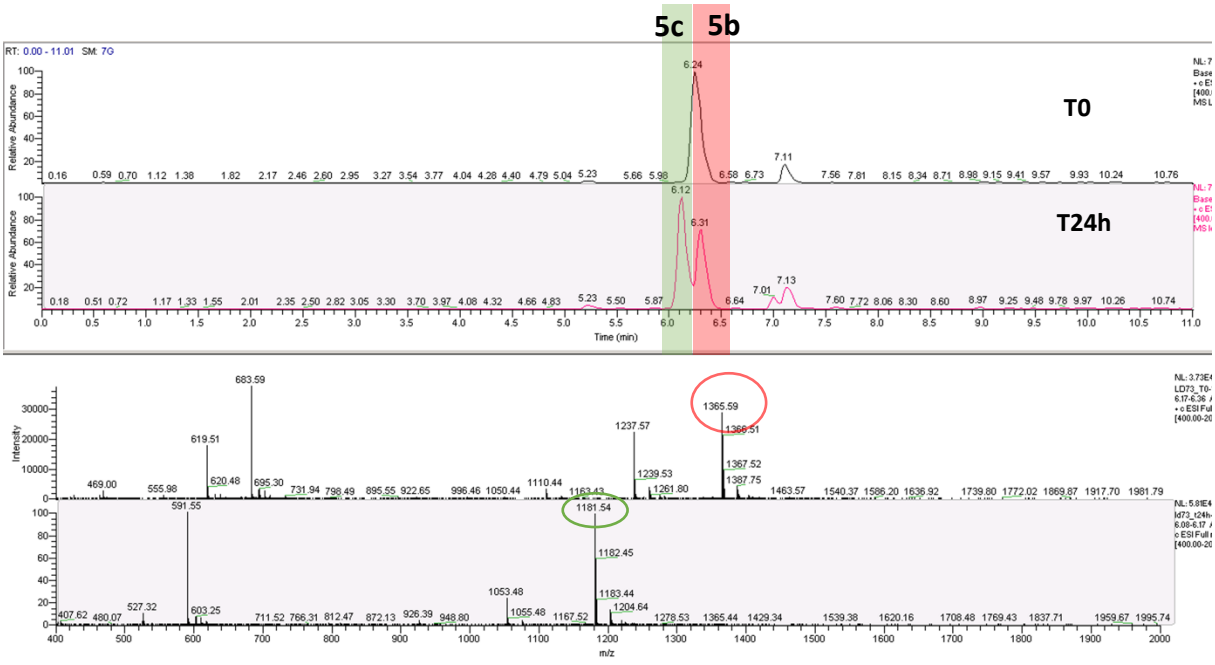
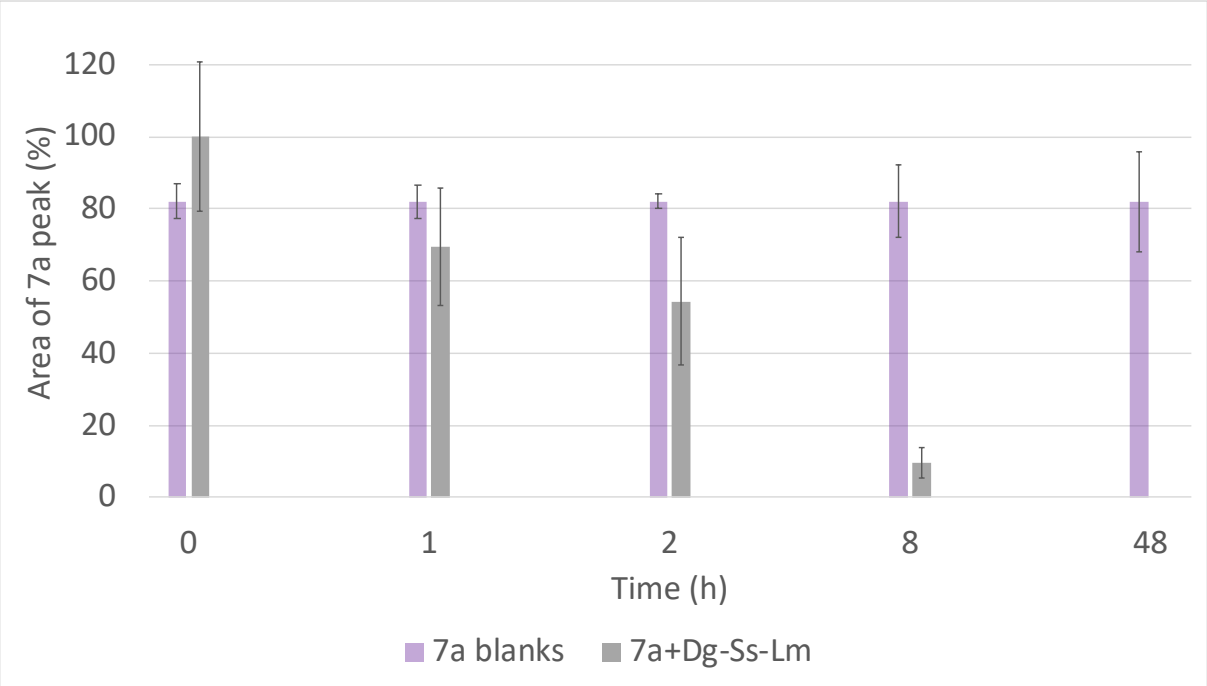


Figure S31

LC-MS kinetic monitoring of **7a** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peaks at 1.09 min, 8.61 min and 8.41 min. The control values (in purple) correspond to an average of all the values obtained during the kinetics.



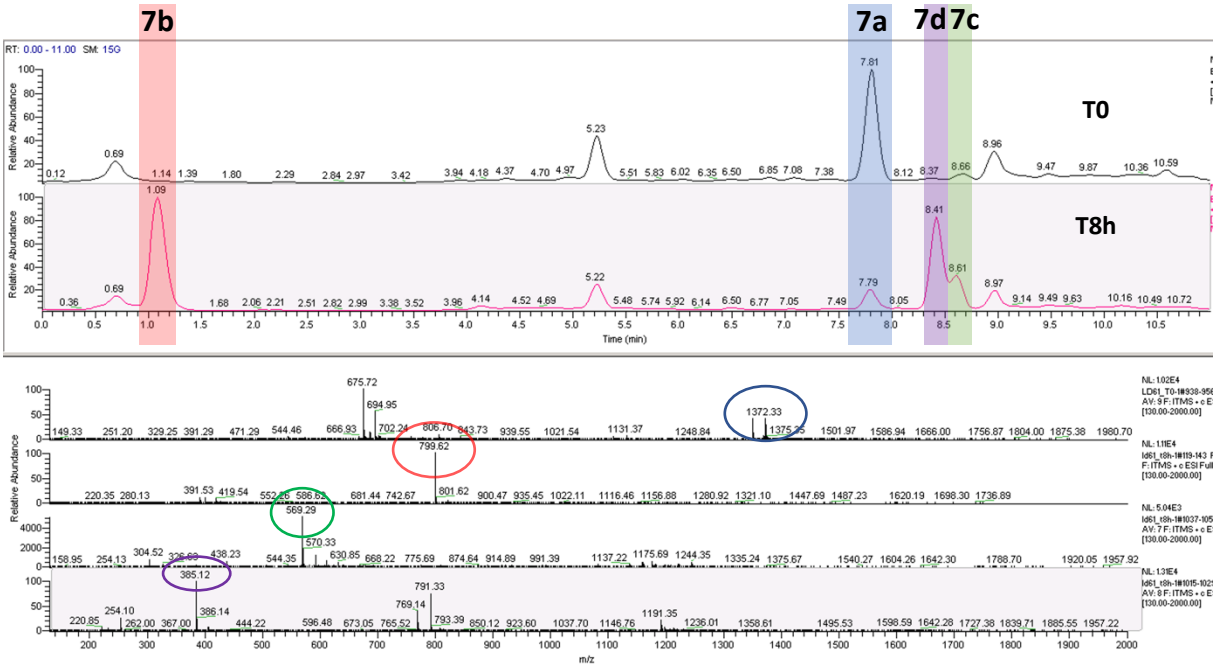


Figure S32
LC-MS kinetic monitoring of **8a** in the presence of *Dg-Ss-Lm* extract at 30°C pH 8. The values shown are an average of all the values obtained during the kinetics.

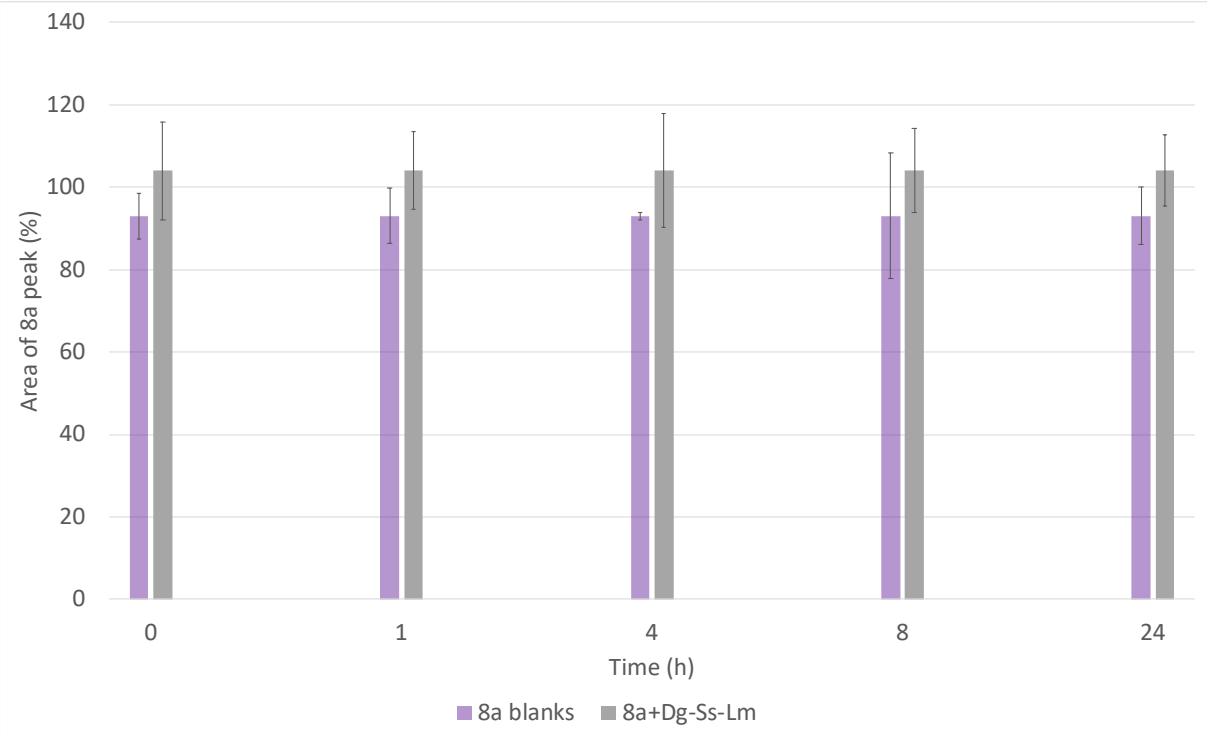


Figure S33
LC-MS kinetic monitoring of **9a** in the presence of *Dg-Ss-Lm* extract at 30°C pH 8. The values shown are an average of all the values obtained during the kinetics.

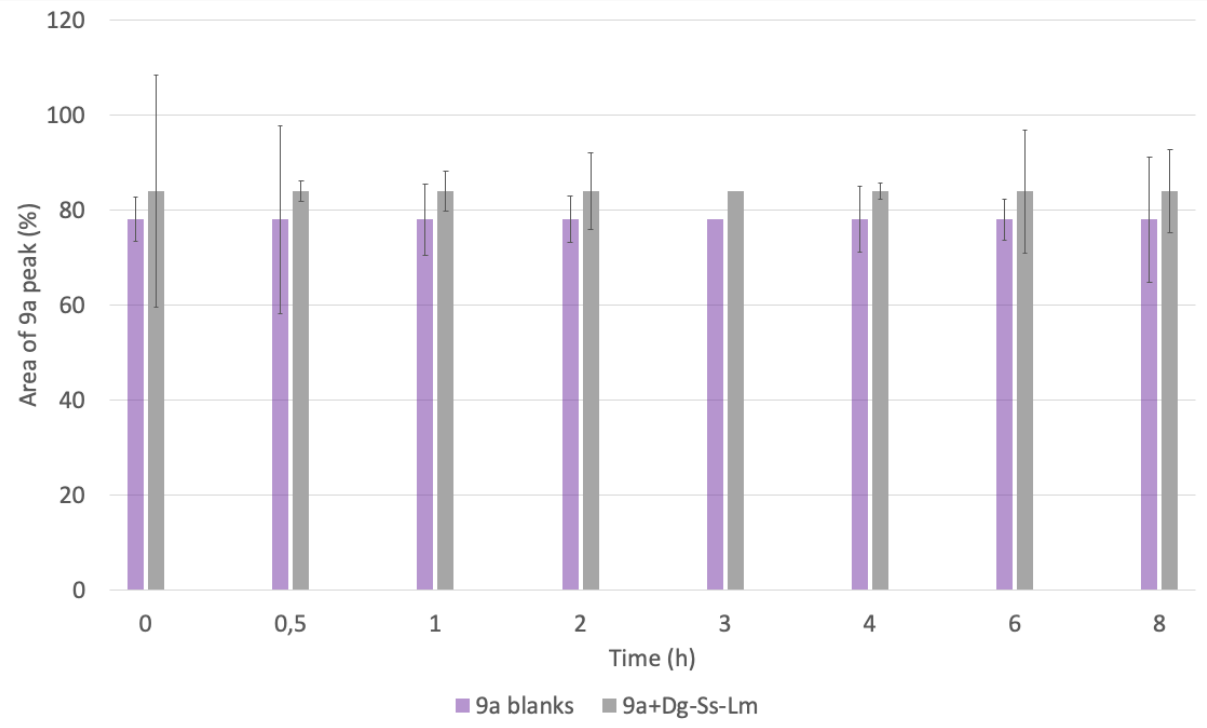
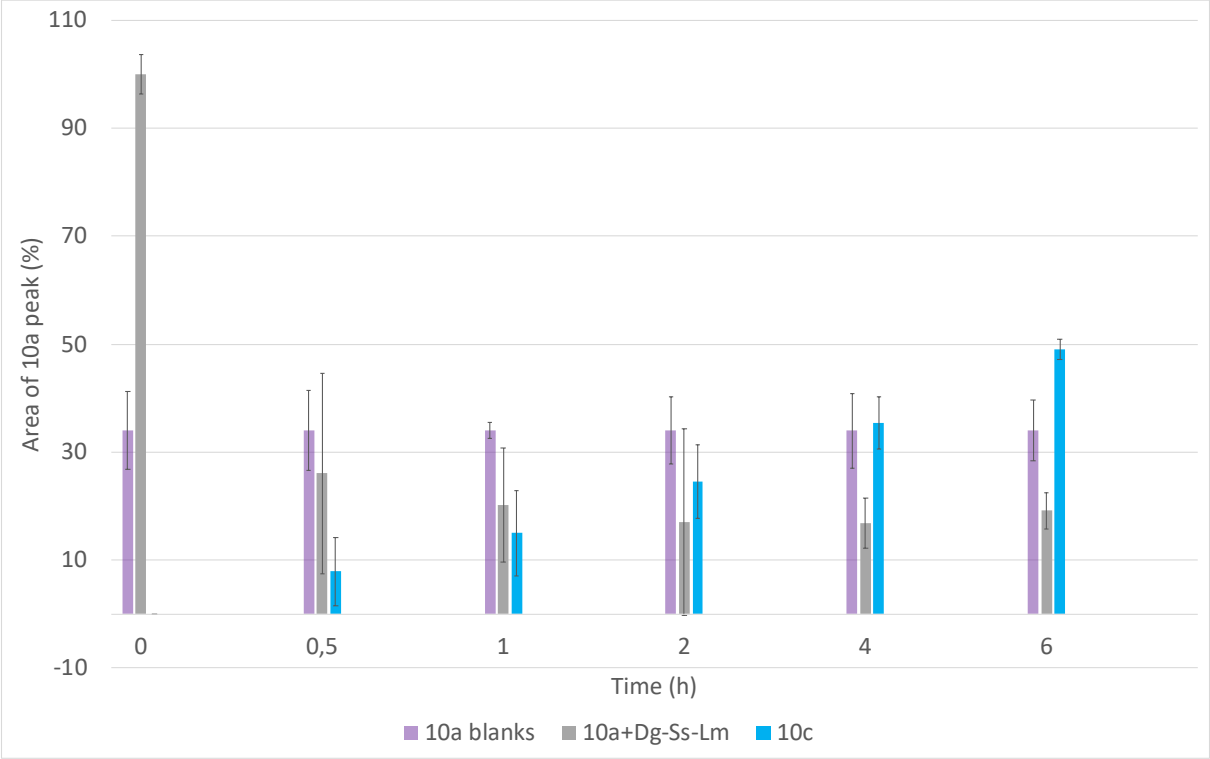
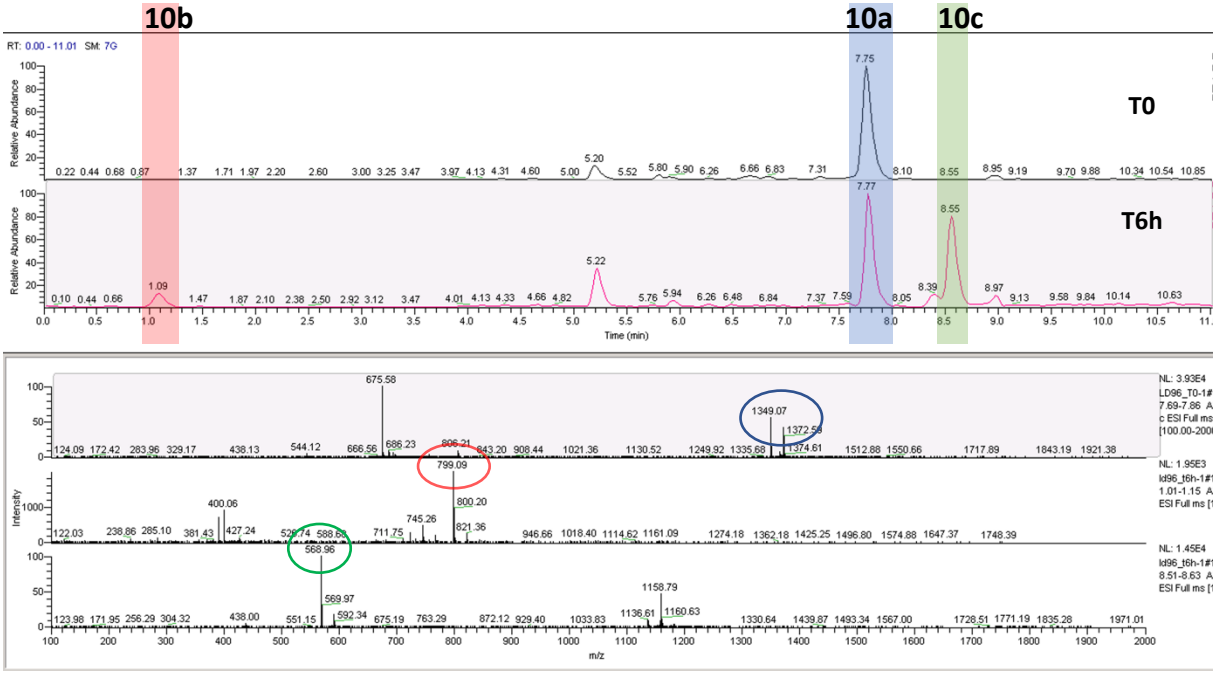


Figure S34

LC-MS kinetic monitoring of **10a** cleavage with *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to the LC-MS peaks at 1.09 min and 8.55 min. The control values (in purple) correspond to an average of all the values obtained during the kinetics. Precipitation of the peptide is observed in the buffer over time but no new fragments are observed in these solutions. On the other hand, the fragment of *m/z* 569 is observed in the solutions containing the peptide with the digestive gland extract.

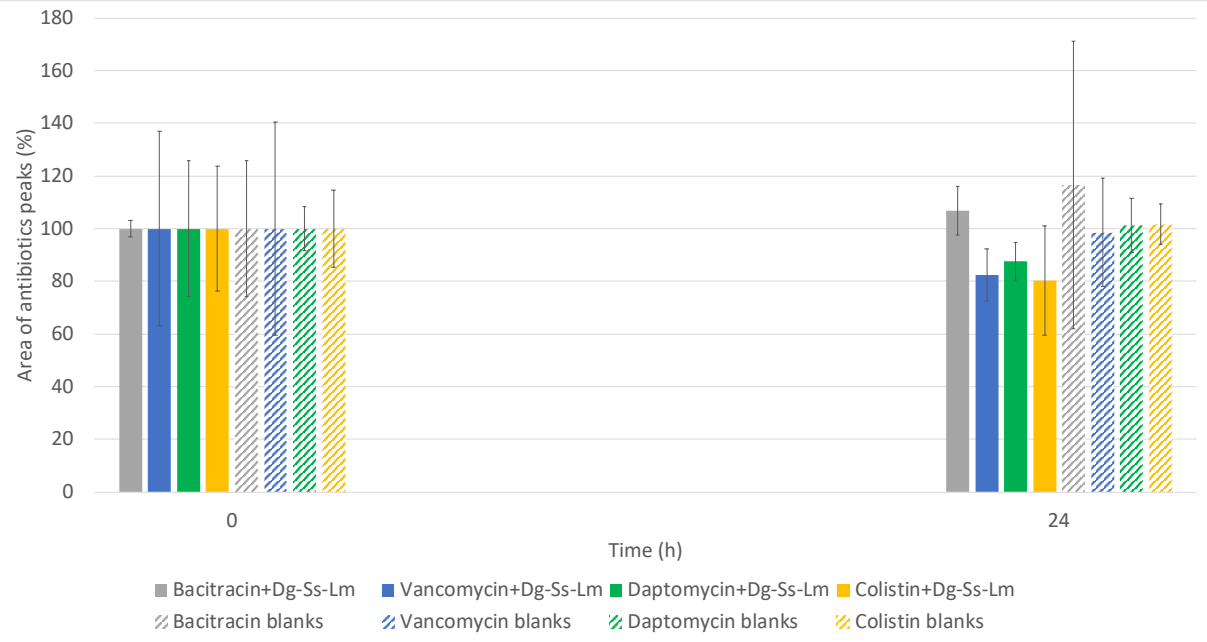


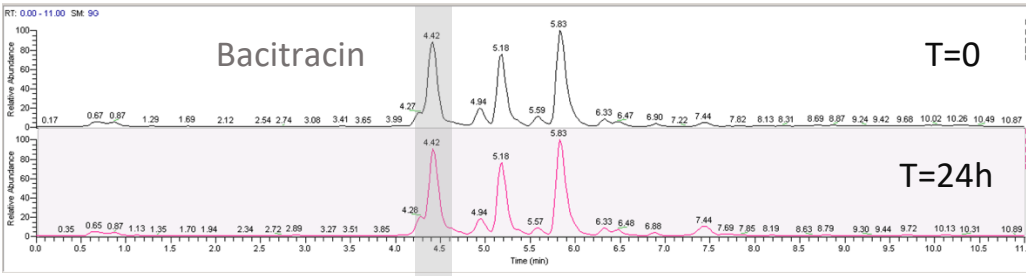


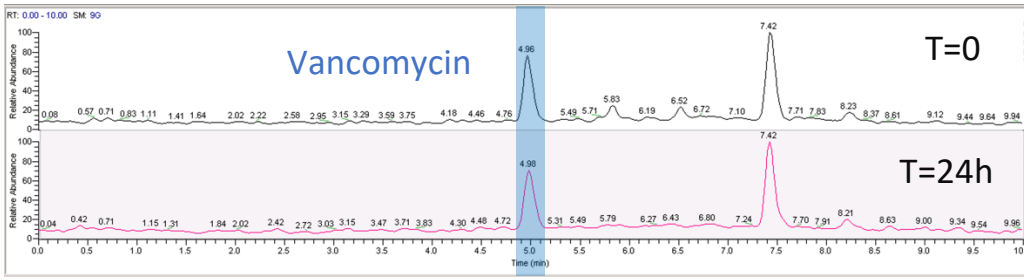
Antibiotics stability

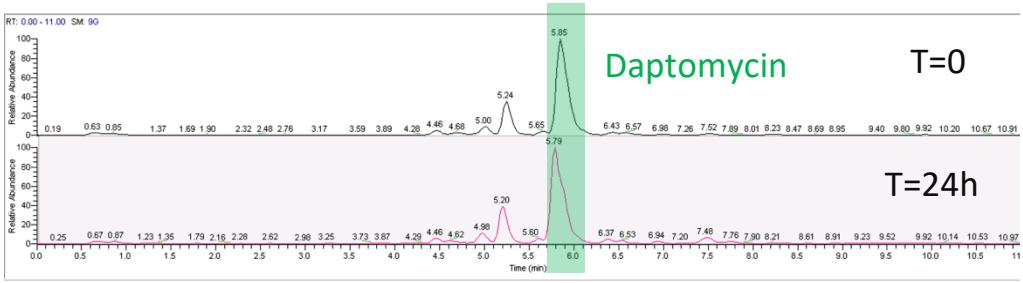
Figure S35

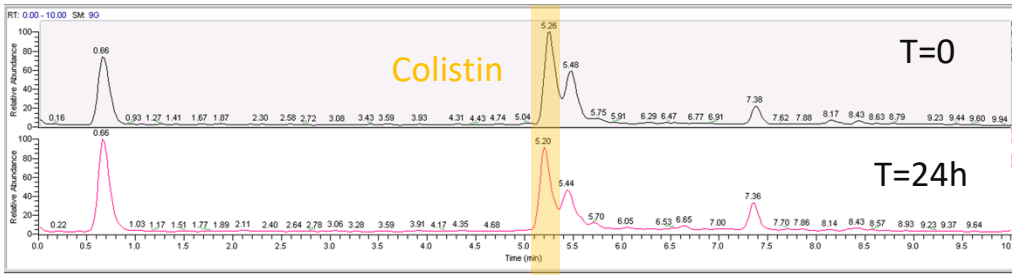
LC-MS kinetic monitoring of Bacitracin, Vancomycin, Daptomycin and Colistin in the presence of *Dg-Ss-Lm* extract at 30°C pH 8 and mass evolution corresponding to their LC-MS peaks at 4.42 min, 4.96 min, 5.8 min and 5.2 min respectively.







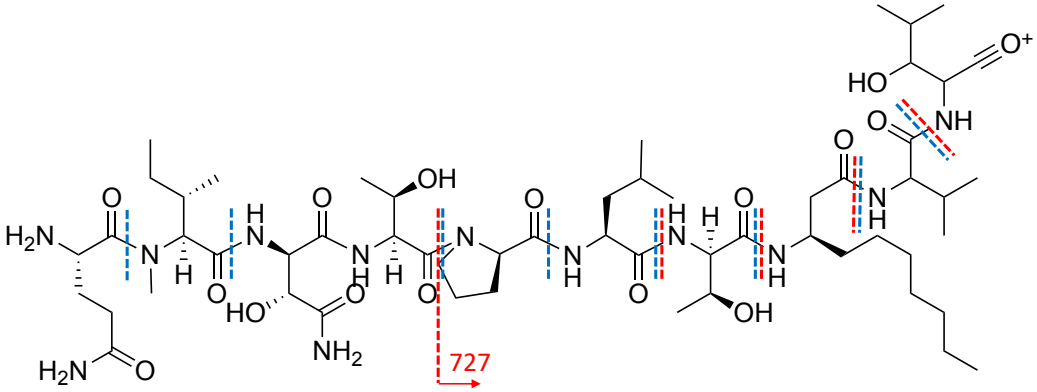

Daptomycin
T=0
T=24h
Relative Abundance
Time (min)


Colistin
T=0
T=24h
Relative Abundance
Time (min)

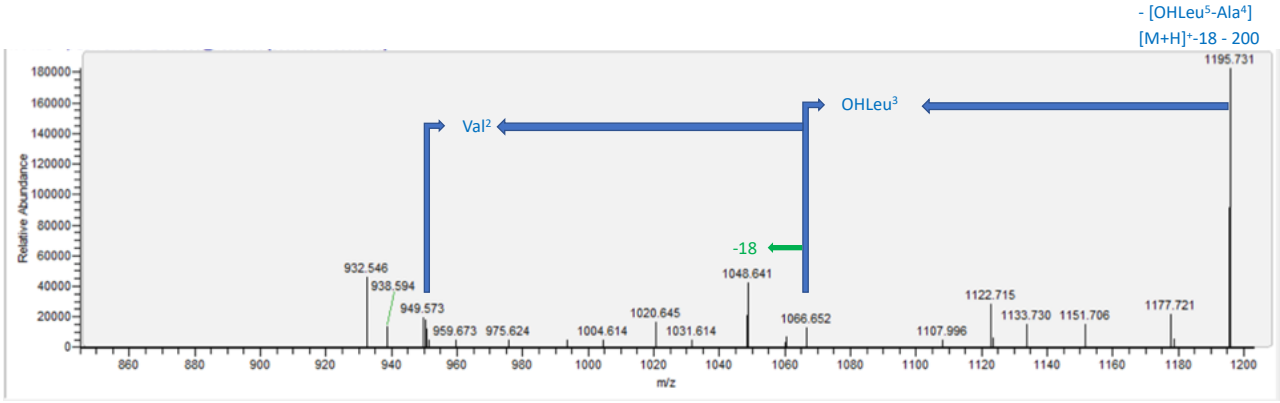
MS-MS analyses

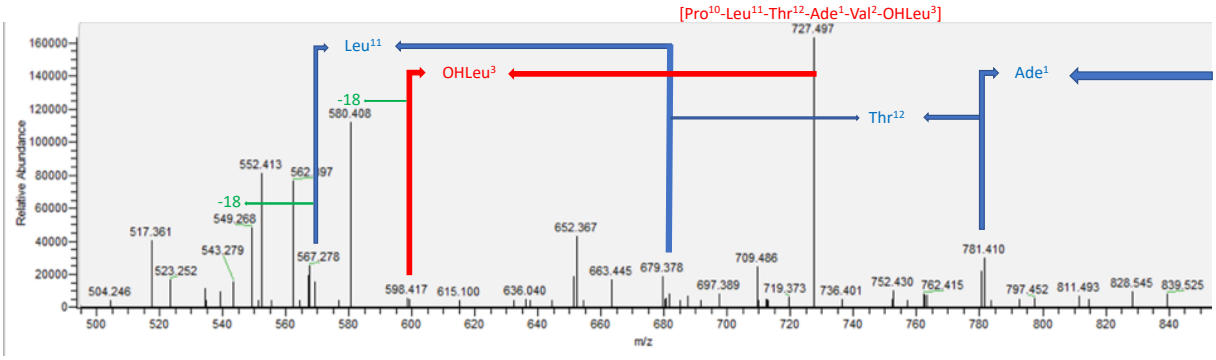
Figure S36

MSMS analyses of **2c** (*m/z* 1213)



Exact Mass: 1195,72965





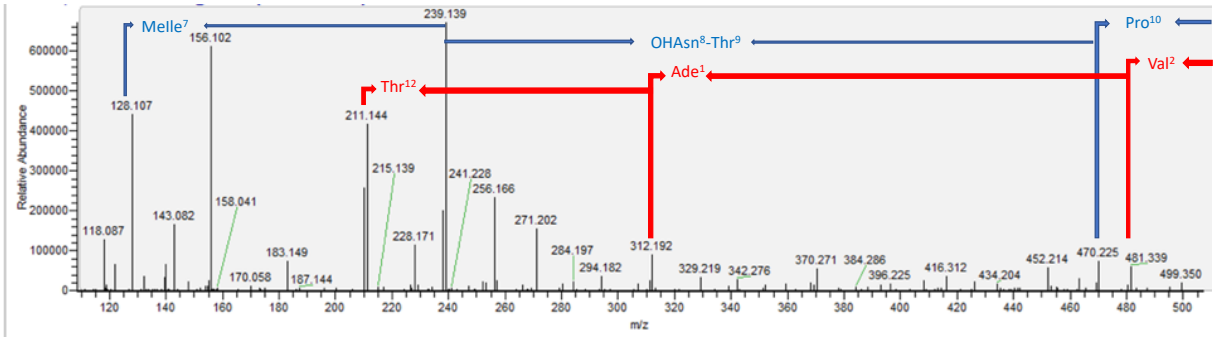
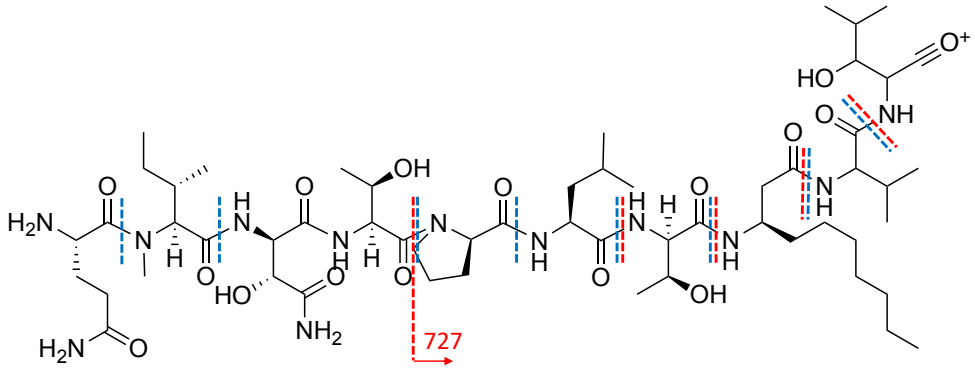
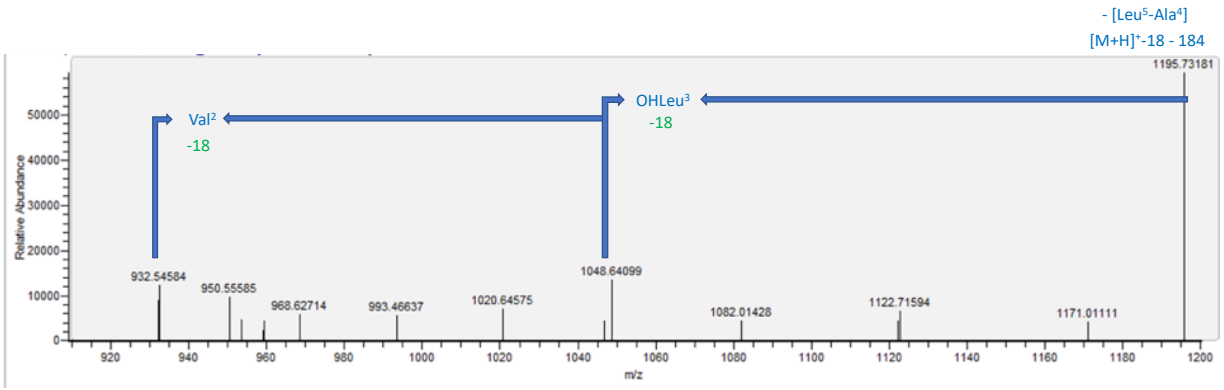
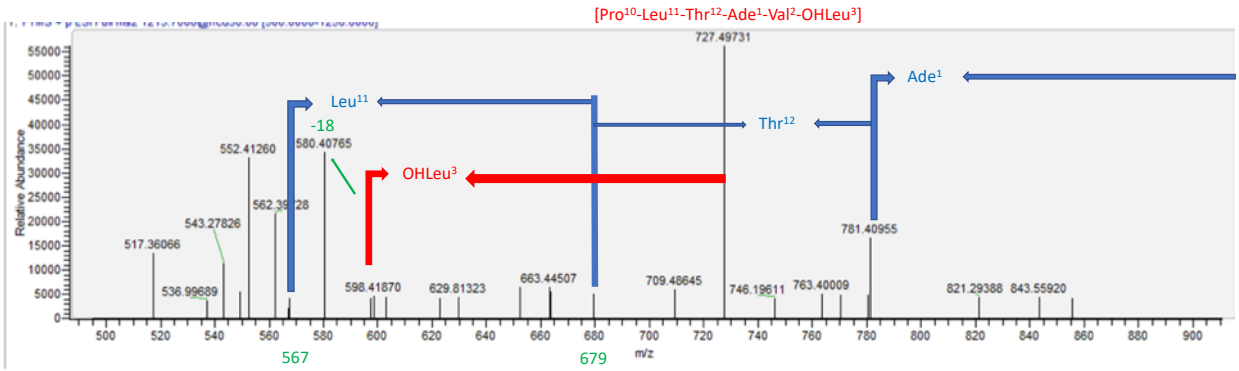


Figure S37
MSMS analyses of **3c** (*m/z* 1213)



Exact Mass: 1195,72965





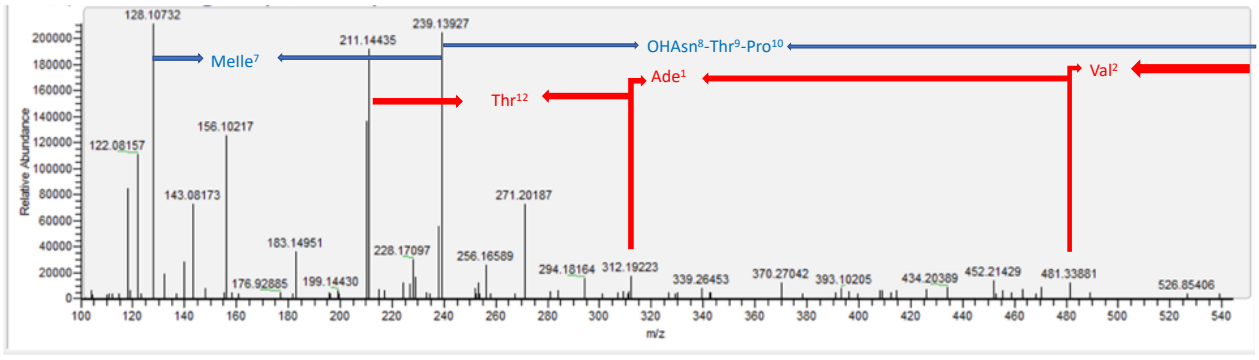
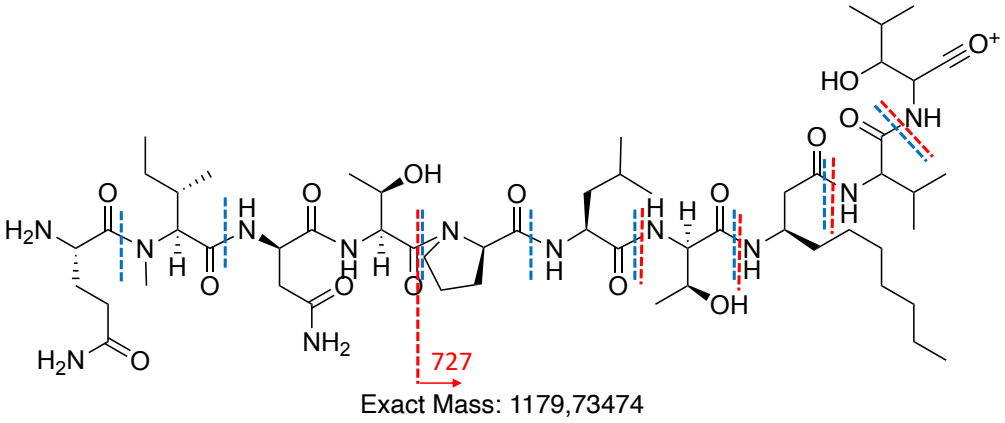
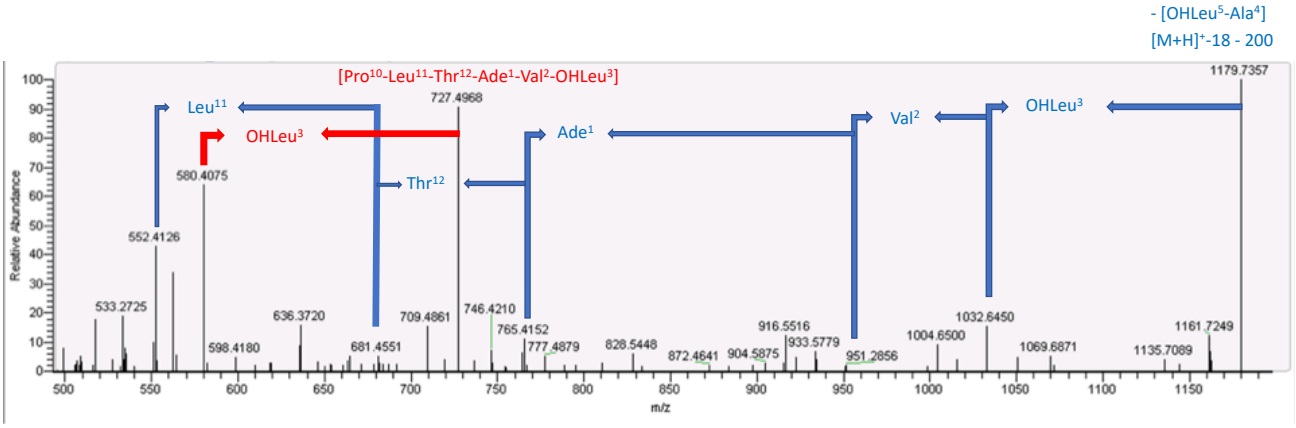


Figure S38
MSMS analyses of **4c** (*m/z* 1197)





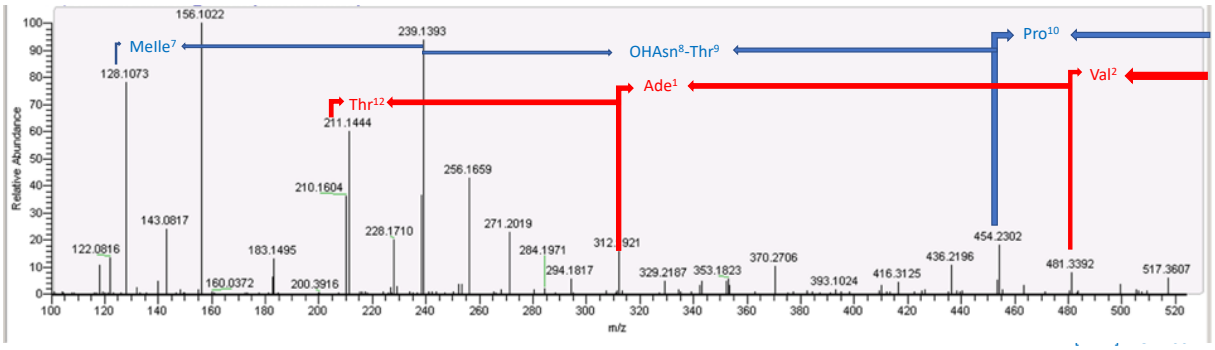


Figure S39
MSMS analyses of **5c** (*m/z* 1181)

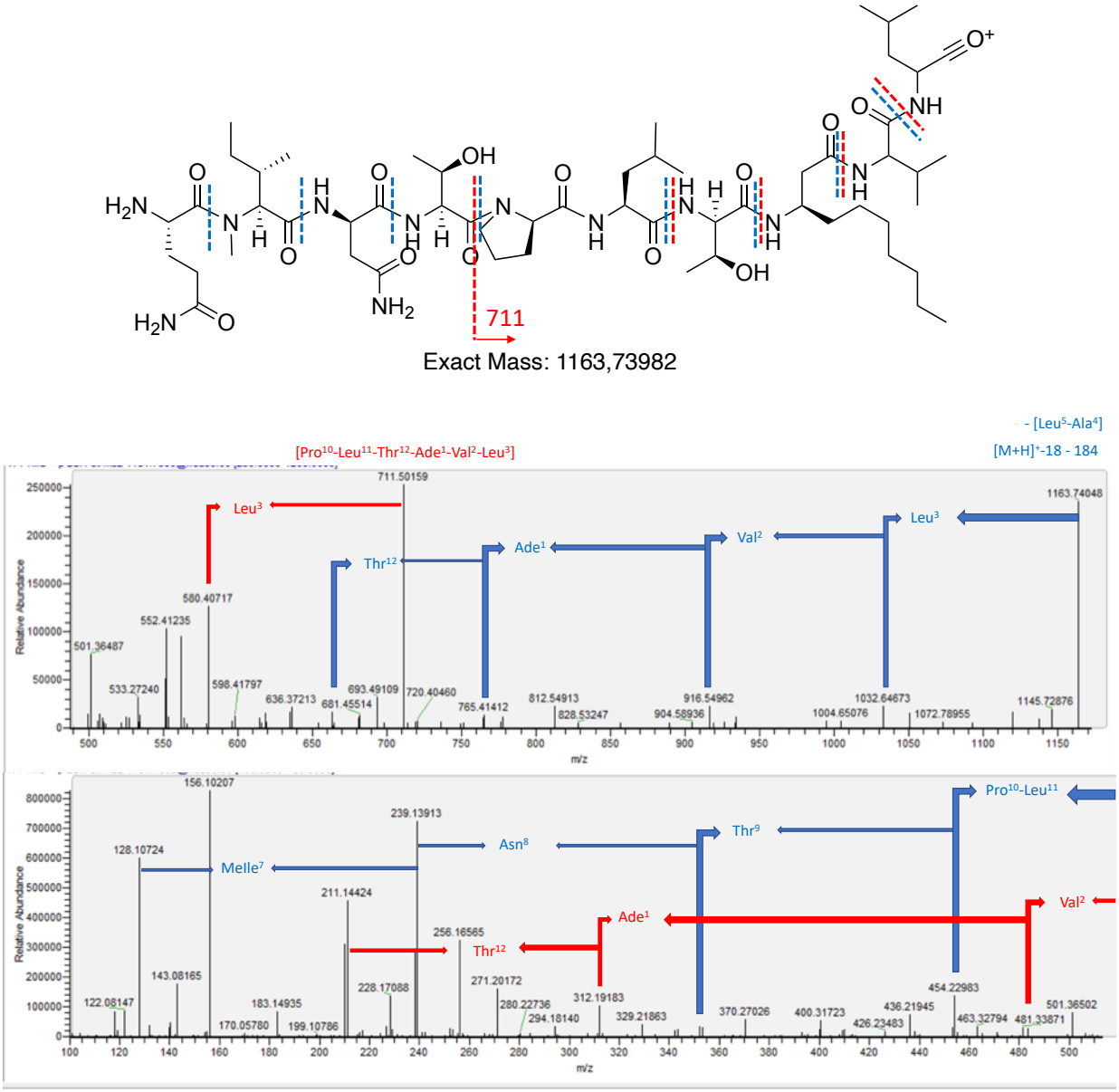
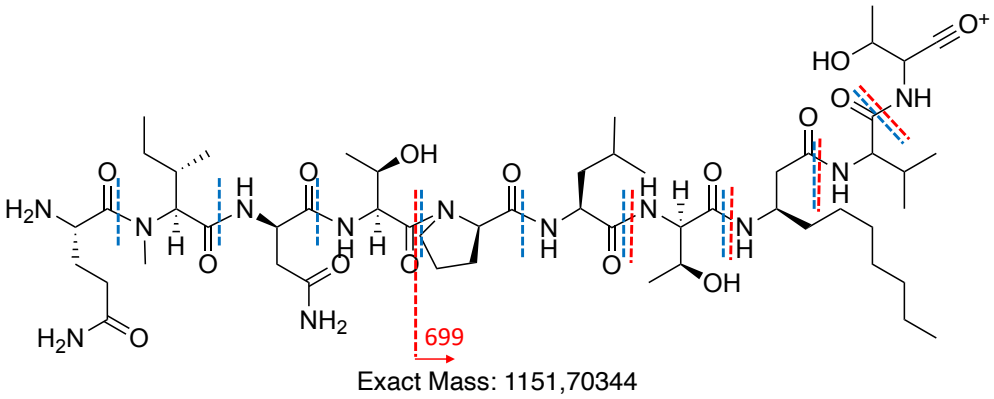
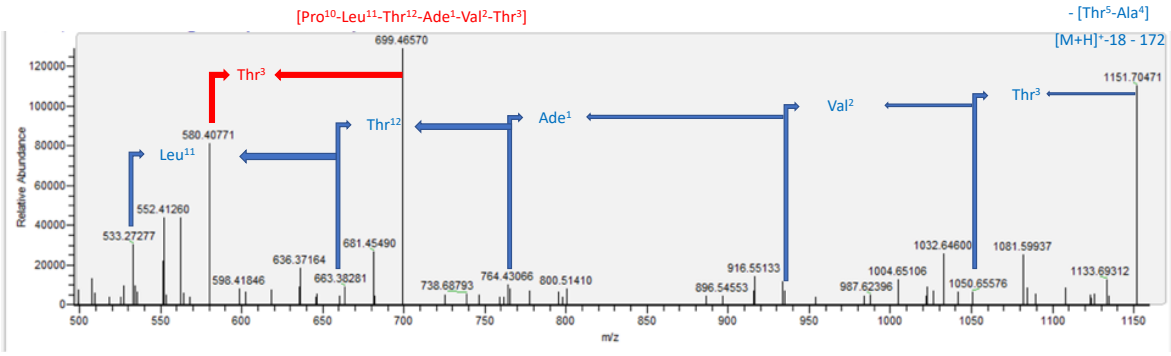


Figure S40
MSMS analyses of **6c** (*m/z* 1169)





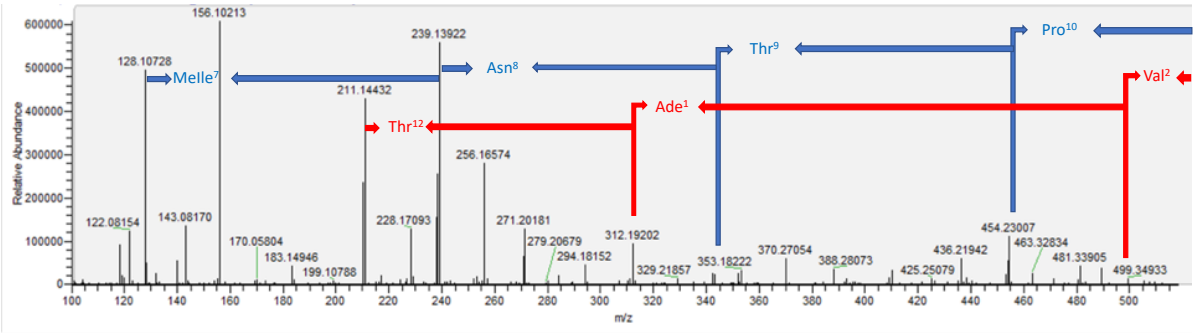
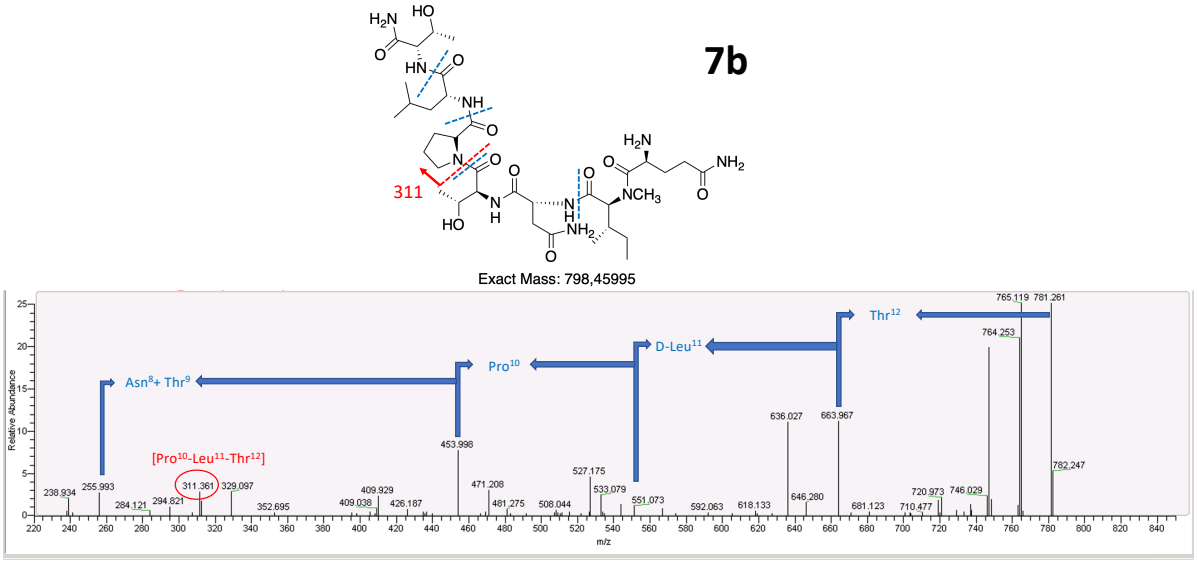
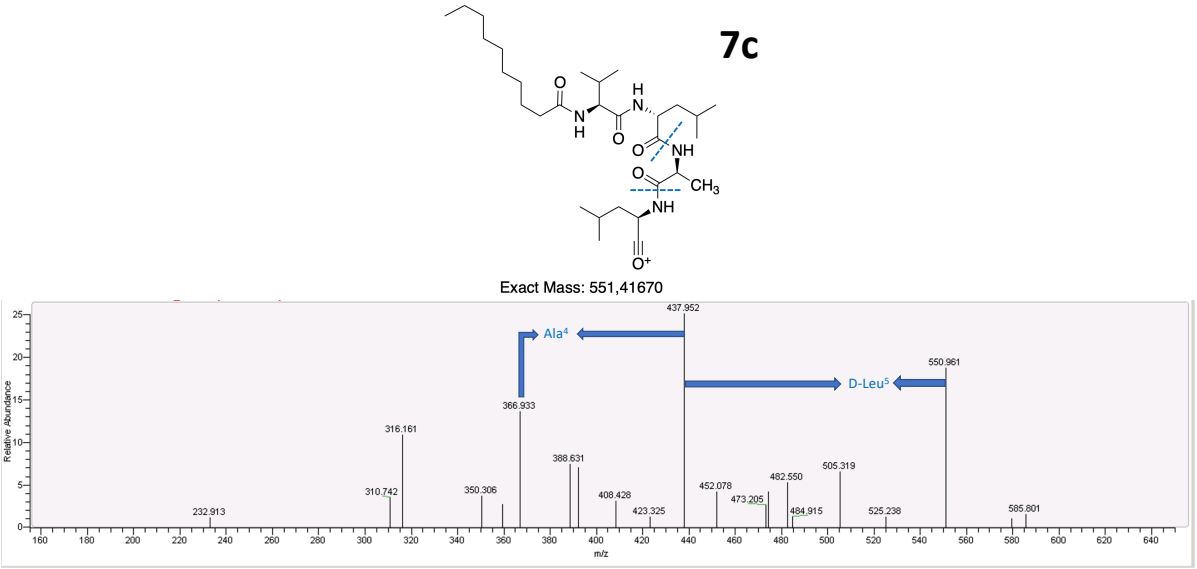
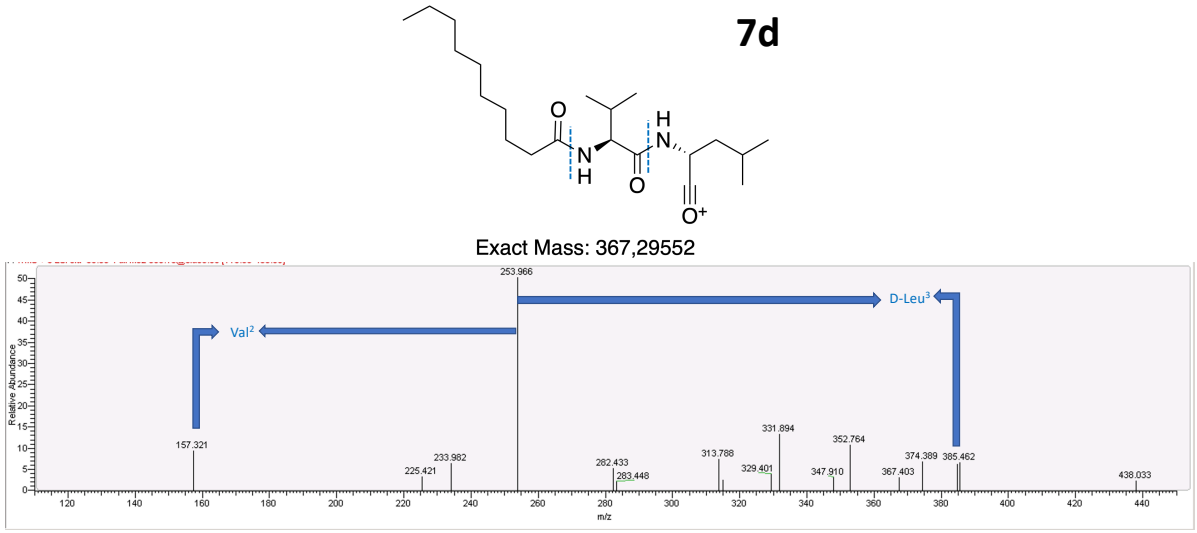


Figure S41

MSMS analyses of **7b** (*m/z* 799), **7c** (*m/z* 569) and **7d** (*m/z* 385).







REFERENCES

(1) Darcel, L.; Djibo, M.; Gaillard, M.; Raviglione, D.; Bonnard, I.; Banaigs, B.; Inguimbert, N. Trichormamide C Structural Confirmation through Total Synthesis and Extension to Analogs. *Organic Letters* **2020**, *22* (1), 145–149. https://doi.org/10.1021/acs.orglett.9b04064.