Molecular recognition of lipid II by lantibiotics: Synthesis and conformational studies of analogues of nisin and mutacin ring A and ring B.

Supporting Information

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Supporting Information Contents

Figure S1: ESI mass spectrum showing presence of oxidised lanthionine after 1 h at 0 °C with 1 eq $NaIO_4$. Target peptide (**A**) $[M+H]^+$ 557.33, peptide with oxidised lanthionine (**B**) $[M+H]^+$ 573.27.

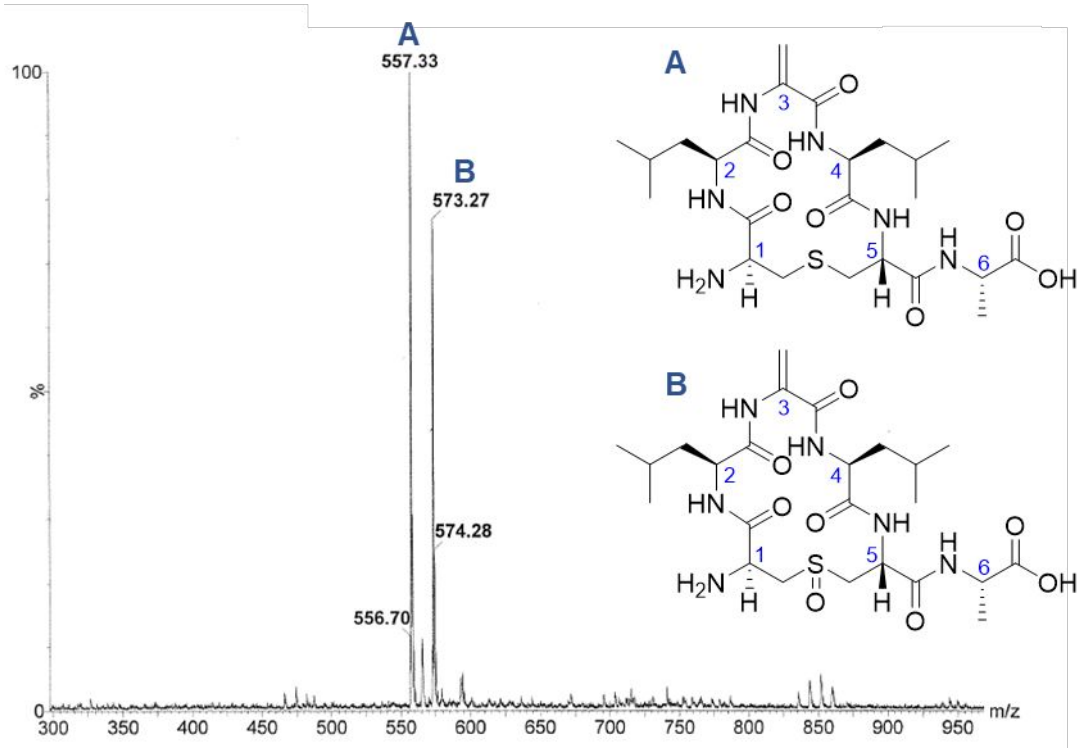
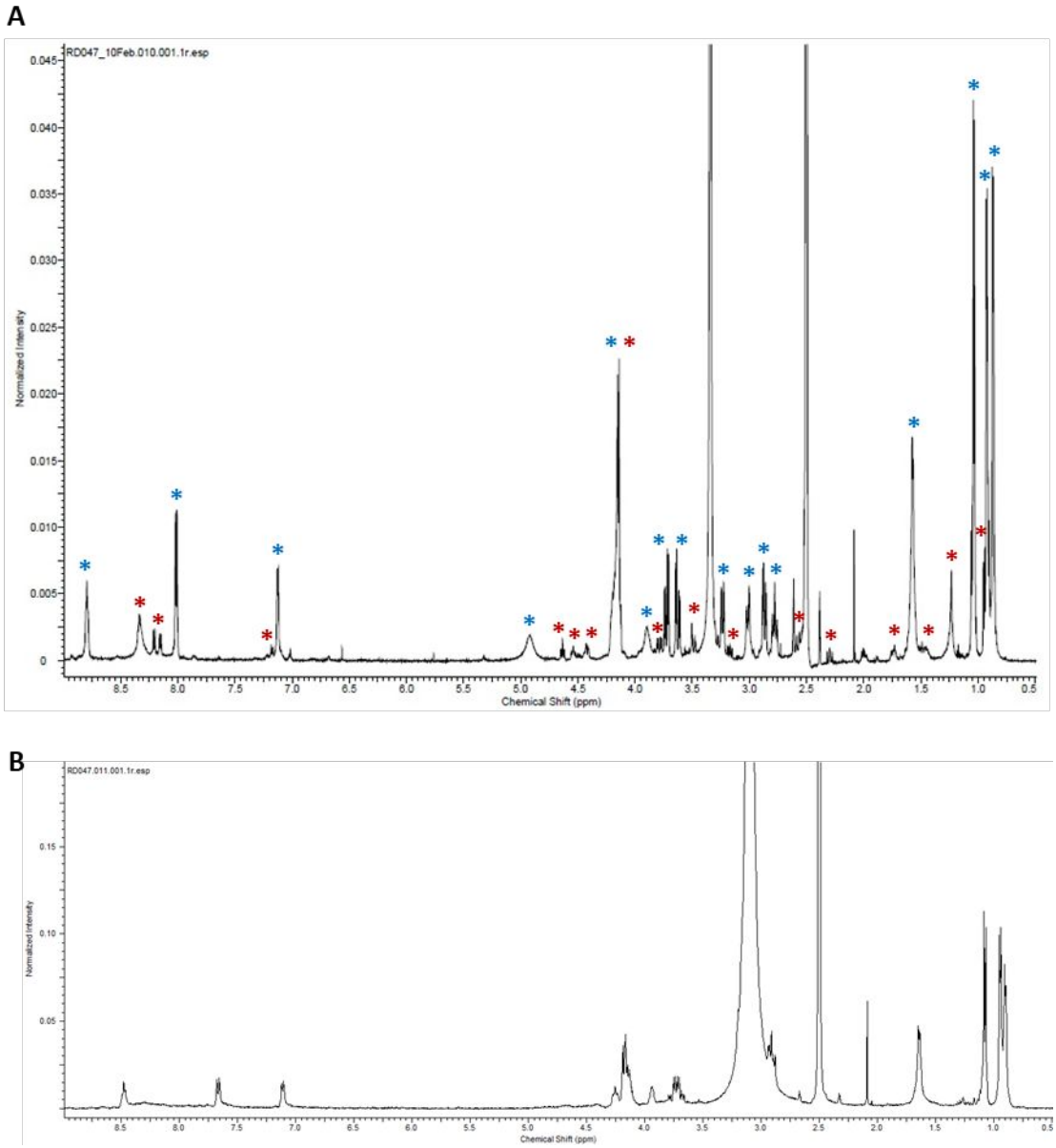
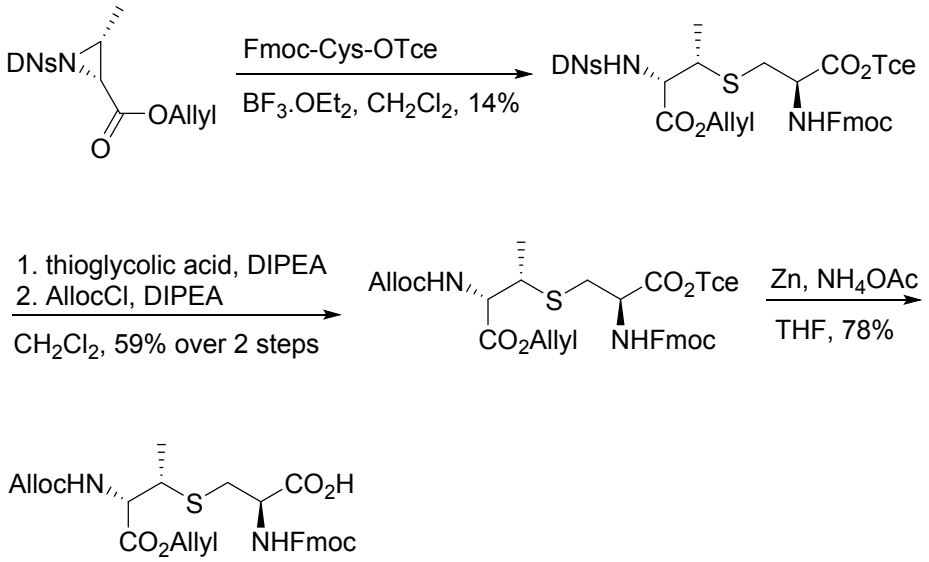
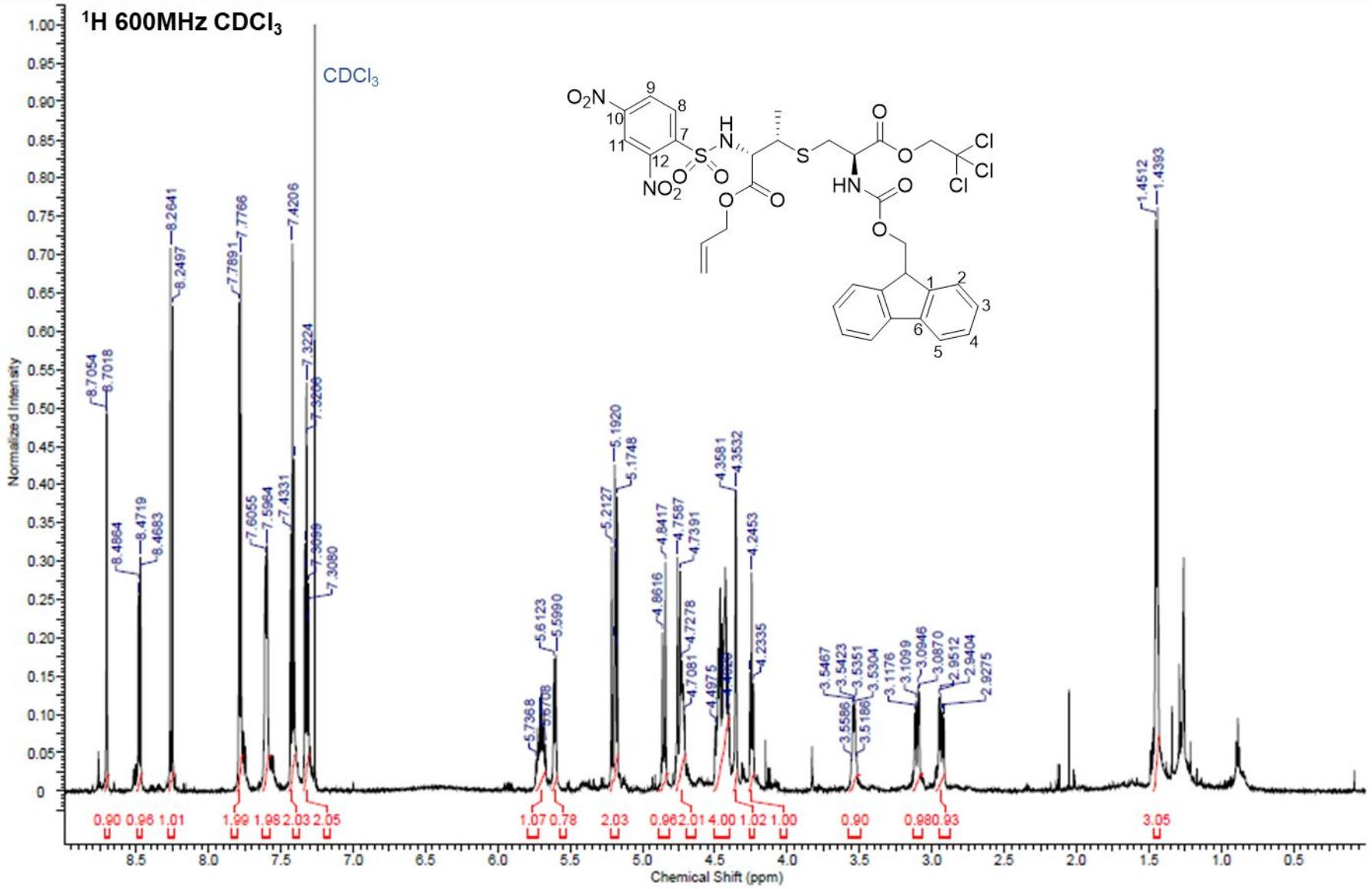


Figure S2: ^{1}H NMR spectra of mutacin I ring B **1**. **A.** Taken at rt. * = major conformer, * = minor conformer. **B.** Taken at 80 °C.



Scheme S1 Synthesis of (Alloc, allyl/Fmoc) MeLan 8

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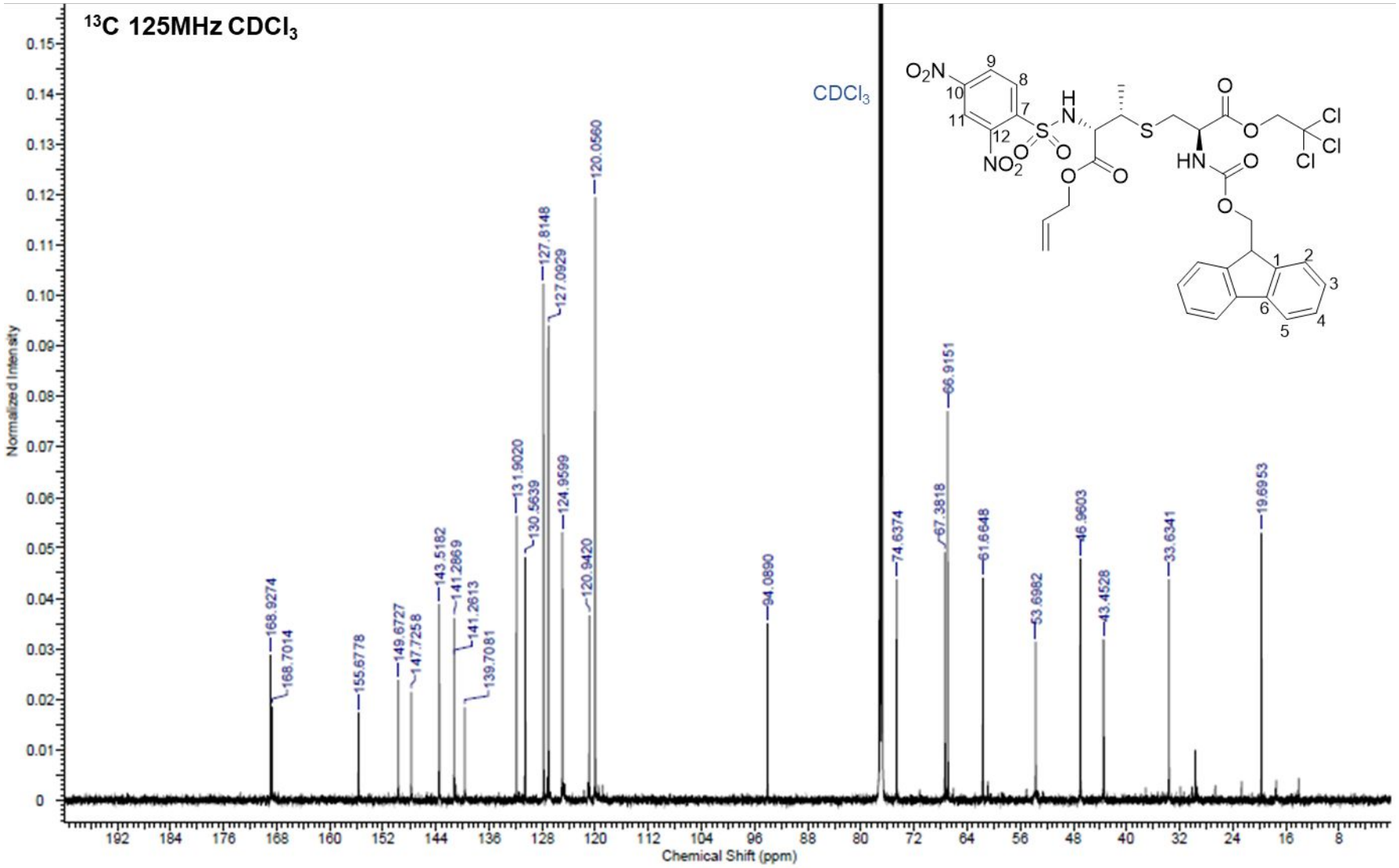
CDCl3
Normalized Intensity
Chemical Shift (ppm)
O2N
NO2
HN
Cl
Cl
Cl


13C 125MHz CDCl3
CDCl3
Normalized Intensity
Chemical Shift (ppm)
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141.2613
139.7081
131.9020
130.5639
127.8148
127.0929
124.9599
120.9420
120.0560
94.0890
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67.3818
66.9151
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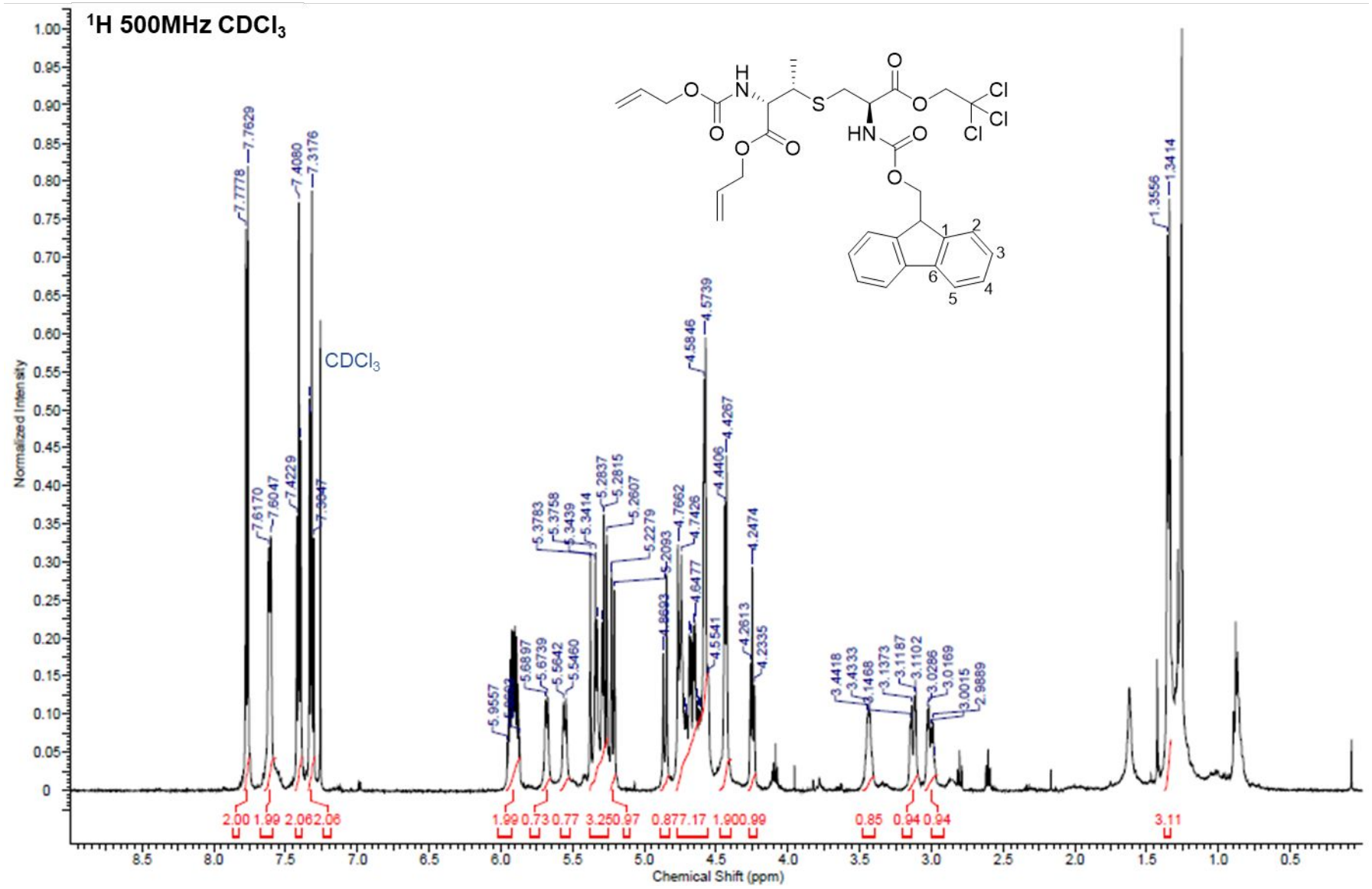
53.6982
46.9603
43.4528
33.6341
19.6953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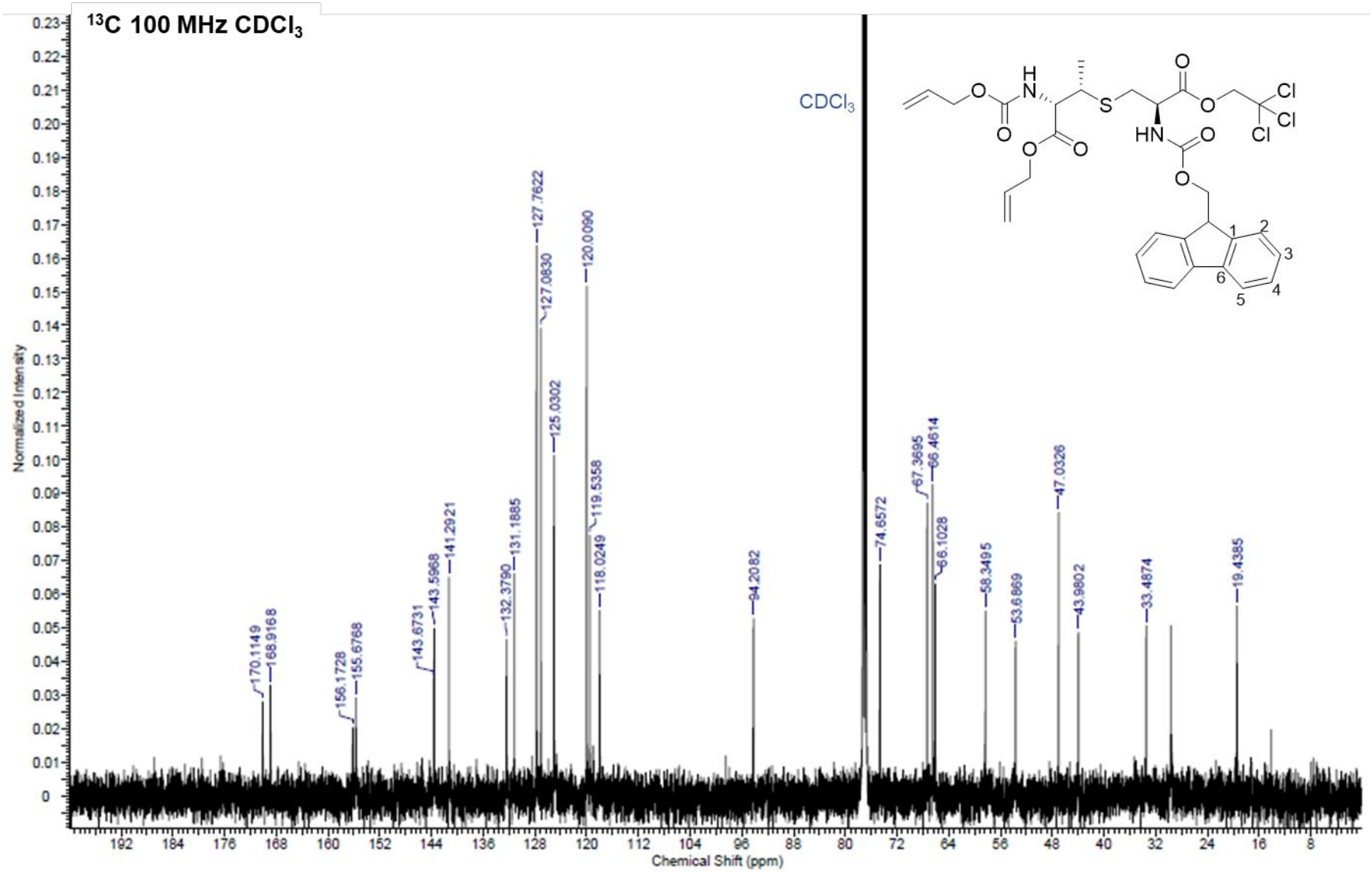
CDCl3
Normalized Intensity
Chemical Shift (ppm)


^{13}C 100 MHz $CDCl_3$
$CDCl_3$
Normalized Intensity
Chemical Shift (ppm)
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155.6768
143.6731
143.5968
141.2921
132.3790
131.1885
127.7622
127.0830
125.0302
120.0090
119.5358
118.0249
94.2082
74.6572
67.3695
66.4614
66.1028
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53.6869
47.0326
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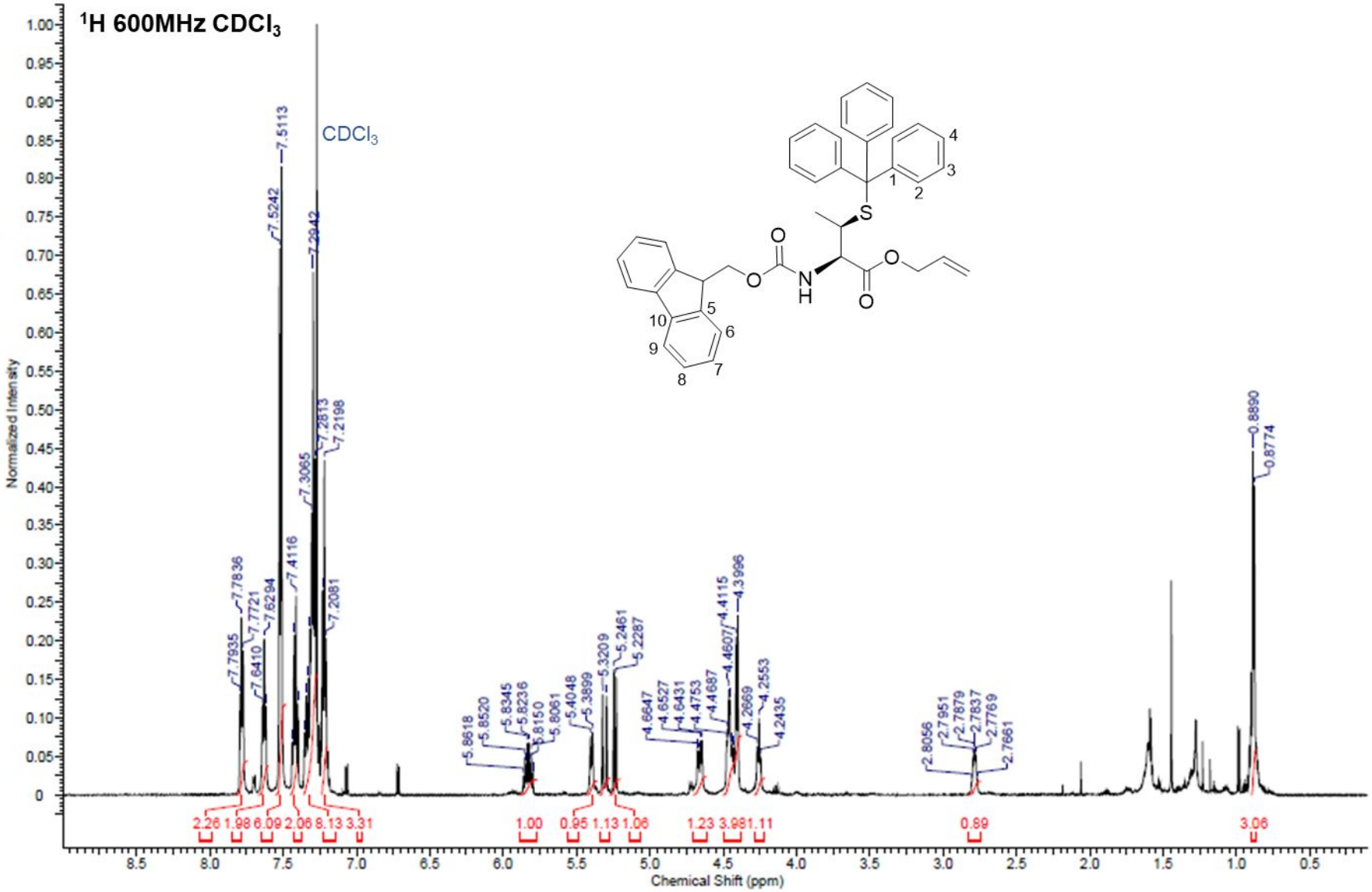
33.4874
19.4385


1H 600MHz CDCl3
CDCl3
Normalized Intensity
Chemical Shift (ppm)
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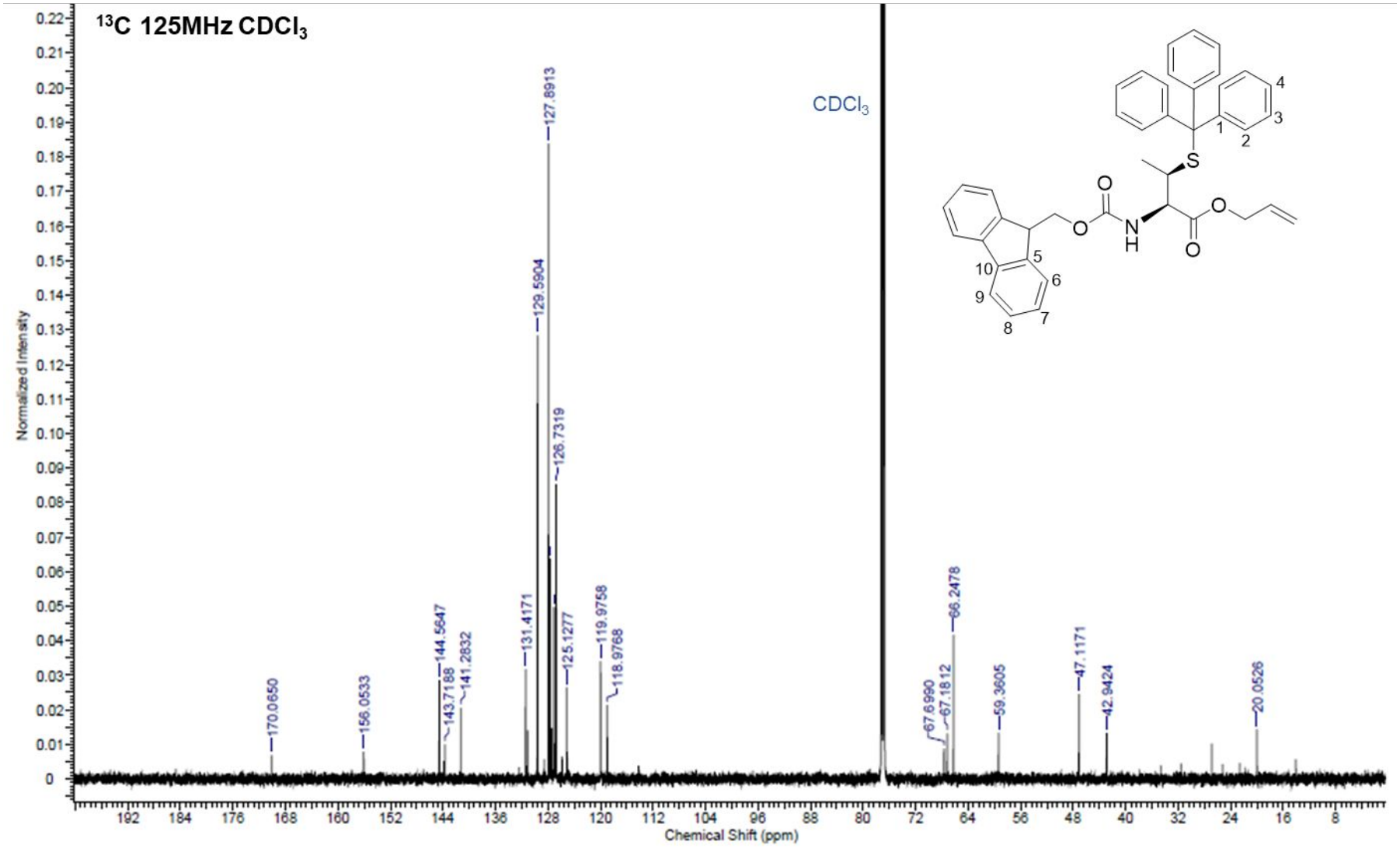
7.2942
7.3065
7.4116
7.2813
7.2198
7.2081
7.7836
7.7935
7.7721
7.6410
7.6294
5.8618
5.8520
5.8345
5.8236
5.8150
5.8061
5.4048
5.3899
5.3209
5.2461
5.2287
4.6647
4.6527
4.6431
4.4753
4.4687
4.4607
4.4115
4.3996
4.2669
4.2553
4.2435
2.8056
2.7951
2.7879
2.7837
2.7769
2.7661
0.8890
0.8774
2.26 1.98 6.09 2.06 8.13 3.31
1.00 0.95 1.13 1.06
1.23 3.98 1.11
0.89
3.06


^{13}C 125MHz $CDCl_3$
$CDCl_3$
Normalized Intensity
Chemical Shift (ppm)
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143.7188
141.2832
131.4171
129.5904
127.8913
126.7319
125.1277
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118.9768
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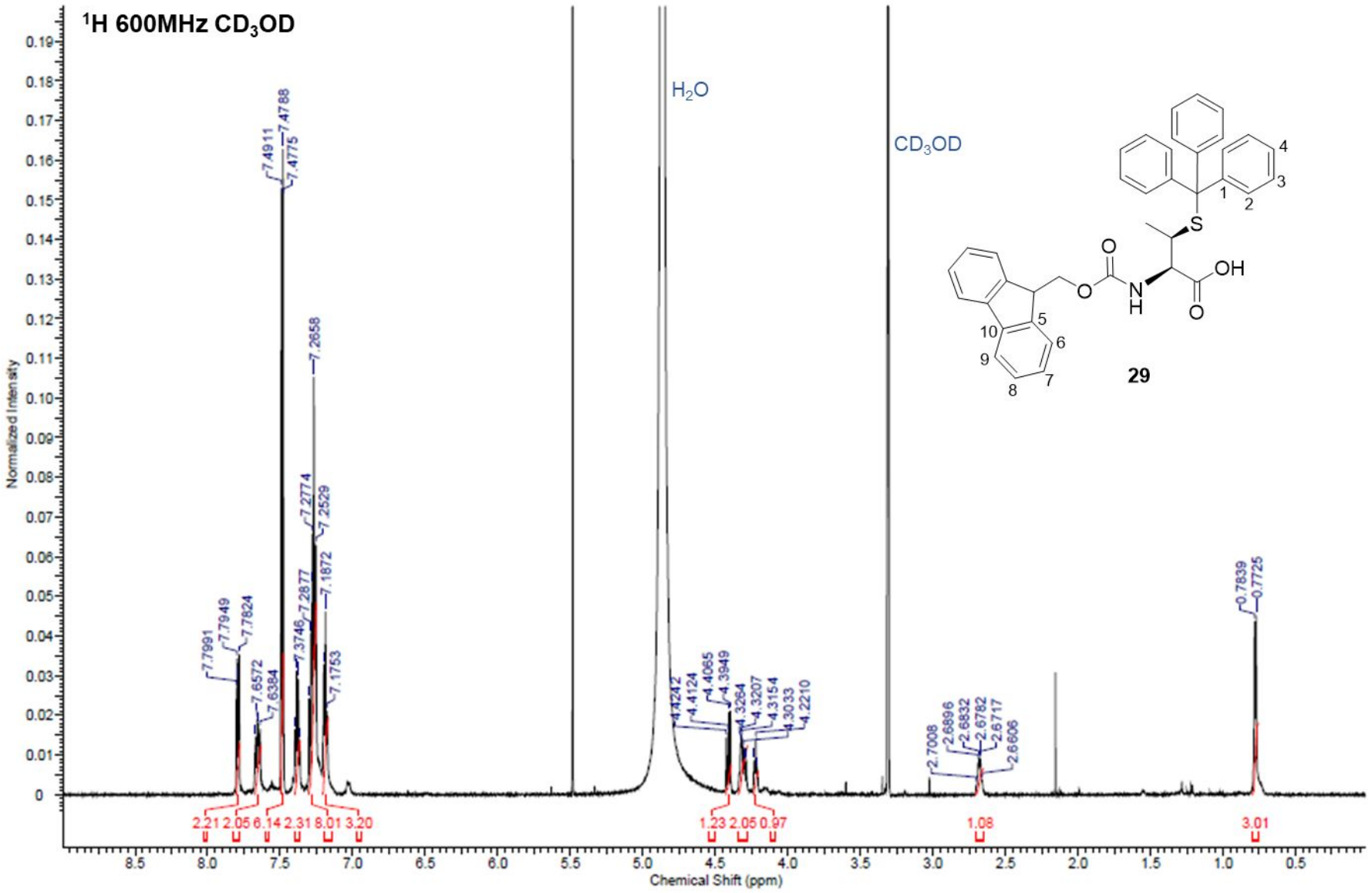
67.1812
66.2478
59.3605
47.1171
42.9424
20.0526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H2O
CD3OD
29
Normalized Intensity
Chemical Shift (ppm)
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7.6572
7.6384
7.4911
7.4788
7.4775
7.3746
7.2877
7.2774
7.2658
7.2529
7.1872
7.1753
4.4242
4.4124
4.4065
4.3949
4.3284
4.3207
4.3154
4.3033
4.2210
2.7008
2.6896
2.6832
2.6782
2.6717
2.6606
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0.7725
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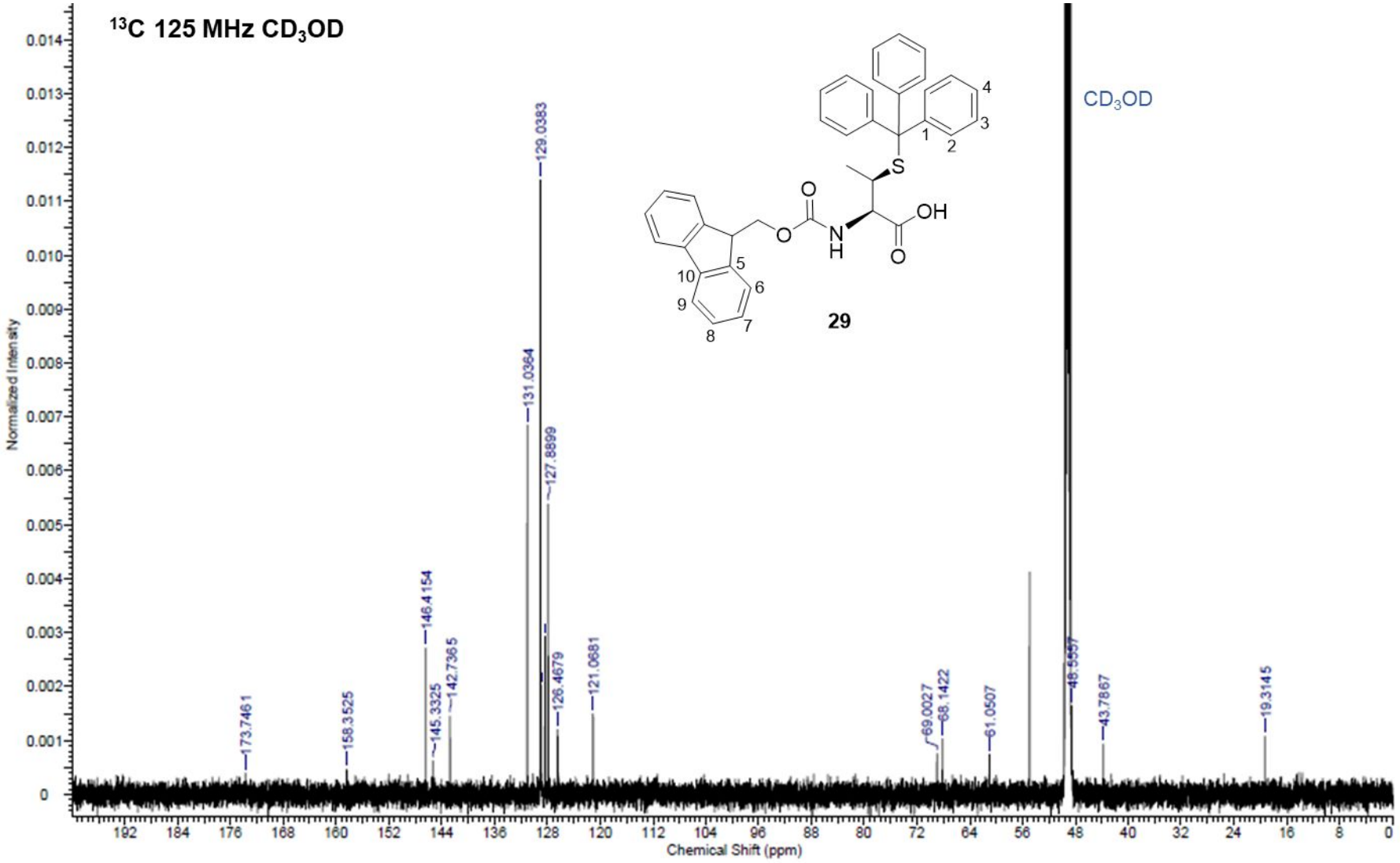
1.23 2.05 0.97
1.08
3.01


^{13}C 125 MHz CD_3OD
CD_3OD
29
Normalized Intensity
Chemical Shift (ppm)
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146.4154
145.3325
142.7365
131.0364
129.0383
127.8899
126.4679
121.0681
69.0027
68.1422
61.0507
48.5557
43.7867
19.3145

Peptide HPLC, mass spectra and NMR Data

Peptide samples for NMR were prepared by dissolving the lyophilised powders in 0.3 mL anhydrous deuterated d_6-DMSO.

All 1D and 2D NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer and standard pulse sequences within the Bruker pulse sequence library were used. All spectra were referenced in the directly detected dimension using the solvent signal. For 2D ^{13}C-^{1}H HSQC and HMBC spectra the ^{13}C dimension was referenced indirectly. Chemical shifts (δ) are given in ppm relative to TMS, and coupling constants (*J*) are given in Hz.

Sweep widths for 1D NMR spectra were ^{1}H 13 ppm, ^{13}C 240 ppm. Spectral parameters for 2D spectra are summarised in Table S1. For 2D ^{1}H-^{1}H NOESY spectra, 64 scans were recorded with 2048 points in the direct and indirect dimensions for all peptides except mutacin I ring A (**12**) and mutacin ring A truncated (Ser5, Ala8) analogue (**15**), for which 1024 points were recorded in both dimensions. A mixing time of 600 ms was used for all NOESY spectra.

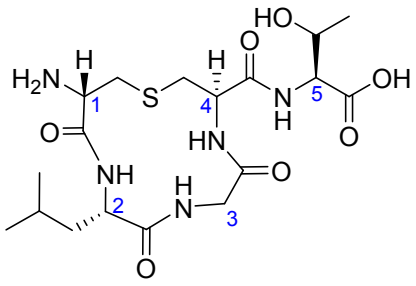
Chemical shift assignments were derived manually from COSY, TOCSY, NOESY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC NMR spectra recorded at 25 °C. Abbreviations used in ^{1}H NMR assignment are as follows: Ar = aromatic, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, br d = broad doublet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, ddt = doublet of doublet of triplets, td = triplet of doublets, tt = triplet of triplets, ttd = triplet of triplet of doublets, qd = quartet of doublets. Although not stereospecifically assigned, in peptides containing Leu, the two β-methyl groups and their corresponding carbon shifts could be distinguished from each other, and the coupling pairs are denoted ‘a’ and ‘b’. Data processing was carried out using ACD/NMR Processor Academic Edition, version 12.01 (Advanced Chemistry Development Inc.), CCPN Analysis version 2.4 and CCPN Chembuild.[1]

Table S1. Spectral parameters for 2D spectra.

Experiment	Sweep width in direct dimension (ppm)	Number of complex points (direct)	Sweep width in indirect dimension (ppm)	Number of complex points (indirect)	Name of pulse sequence
COSY	8.67	2048	8.67	2048	cosygpmfqfvg3
TOCSY	8.76	1024	8.76	1024	mlevgp.agi
NOESY	8.77	2048	8.77	2048	noesygpphzs
ROESY	9.15	1024	9.15	1024	roesyetgp
^{1}H-^{13}C HSQC	8.67	1024	231.26	1024	hsqcedetgpsisp2.4
^{1}H-^{13}C HMBC	8.66	2048	241.31	1024	hmbcetgpl3nd

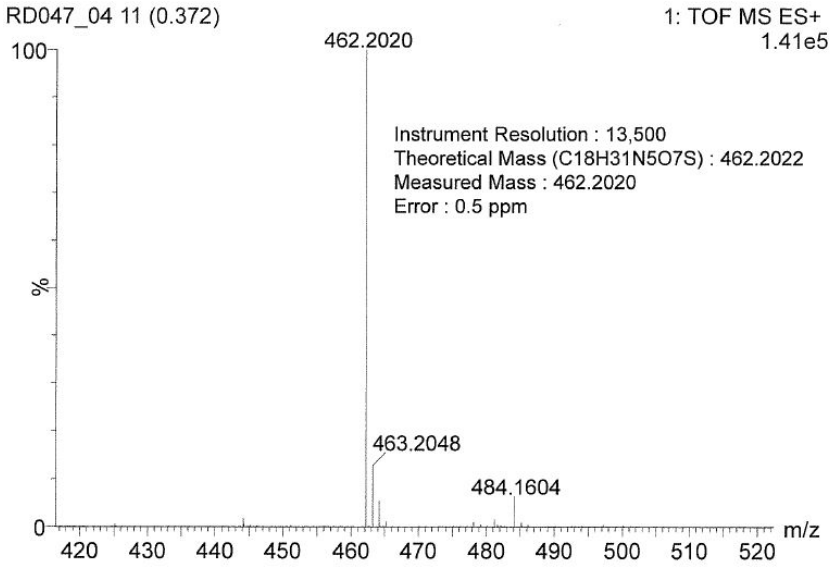
Ring B Peptides

Mutacin I Ring B WT (1)

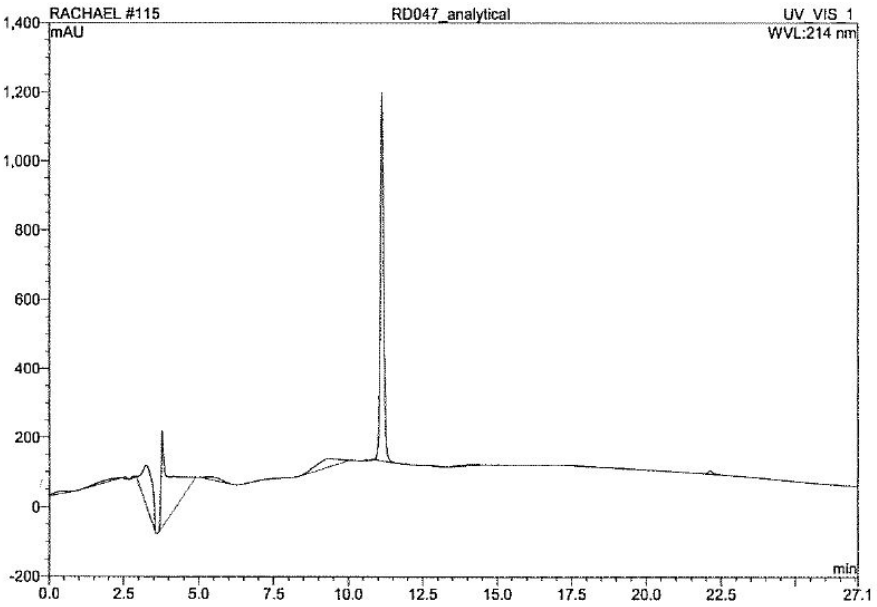


1

HRMS (ES+) m/z: $[M + H]^+$ Calcd for $C_{18}H_{31}N_5O_7S$ 462.2022; found 462.2020.



analytical HPLC (Fluka Analytical Discovery BIO C18-10 25 x 4.6 mm column on Dionex HPLC system) retention time 11.13 min.



NMR δ_H (600 MHz, $(CD_3)_2SO$), δ_c (150 MHz, $(CD_3)_2SO$)

Major Conformer

Table S2

			^{1}H			
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**Exchangeable**
1 - Lan (Dha)		3.90	2.87 3.05			NH_2 – 8.33
2 - Leu	8.20	4.14	1.58	1.58	0.86 0.92	
3 - Gly	8.77	3.64 3.73				
4 - Lan (Cys)	7.16	4.24	3.21 2.81			
5 - Thr	8.02	4.14	4.14	1.03		OH – 4.93

Table S3

^{13}C					
Residue Number	**CO**	**α**	**β**	**γ**	**δ**
1 – Lan (Dha)	173.96	57.25	40.02		
2 - Leu	175.23	56.11	41.68	27.37	25.31 25.73
3 - Gly	172.20	45.74			
4 - Lan (Cys)	173.57	55.23	39.92		
5 - Thr		60.75	69.36	23.57	

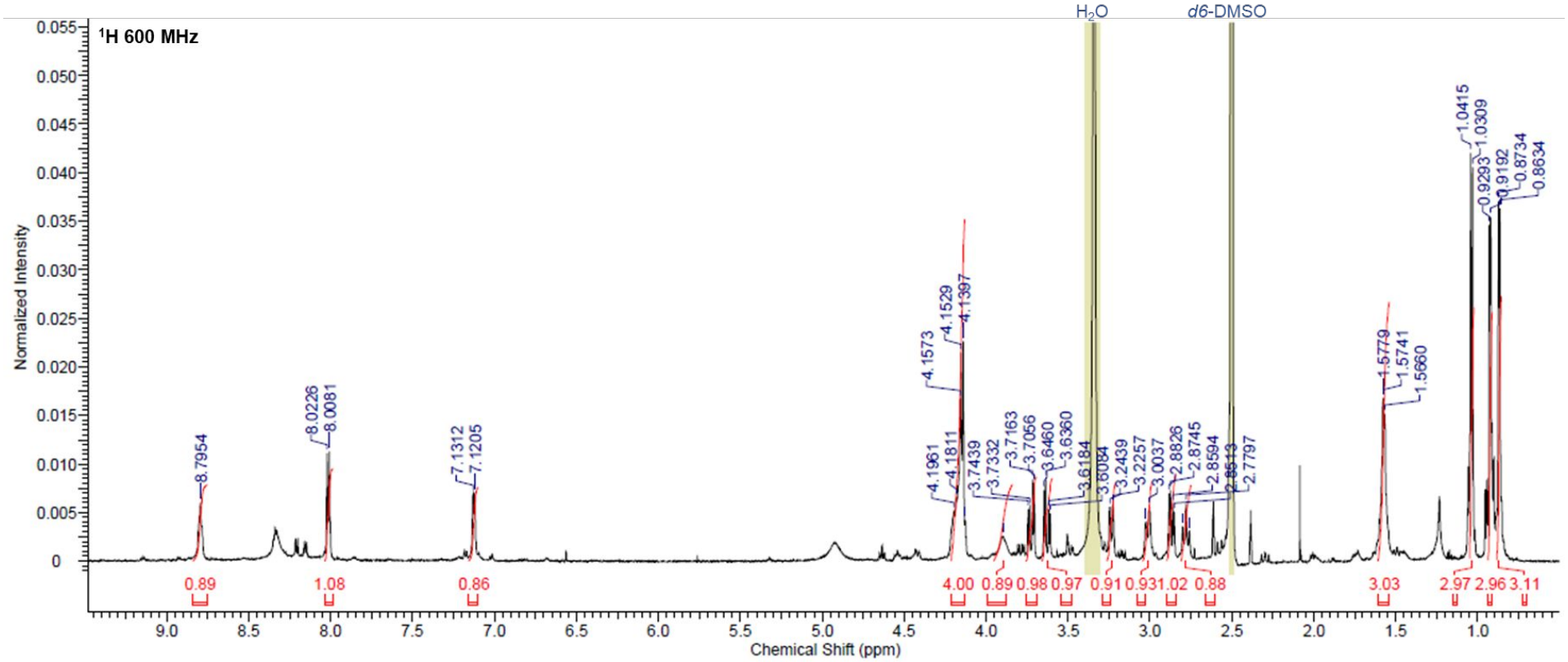
Minor Conformer

Table S4

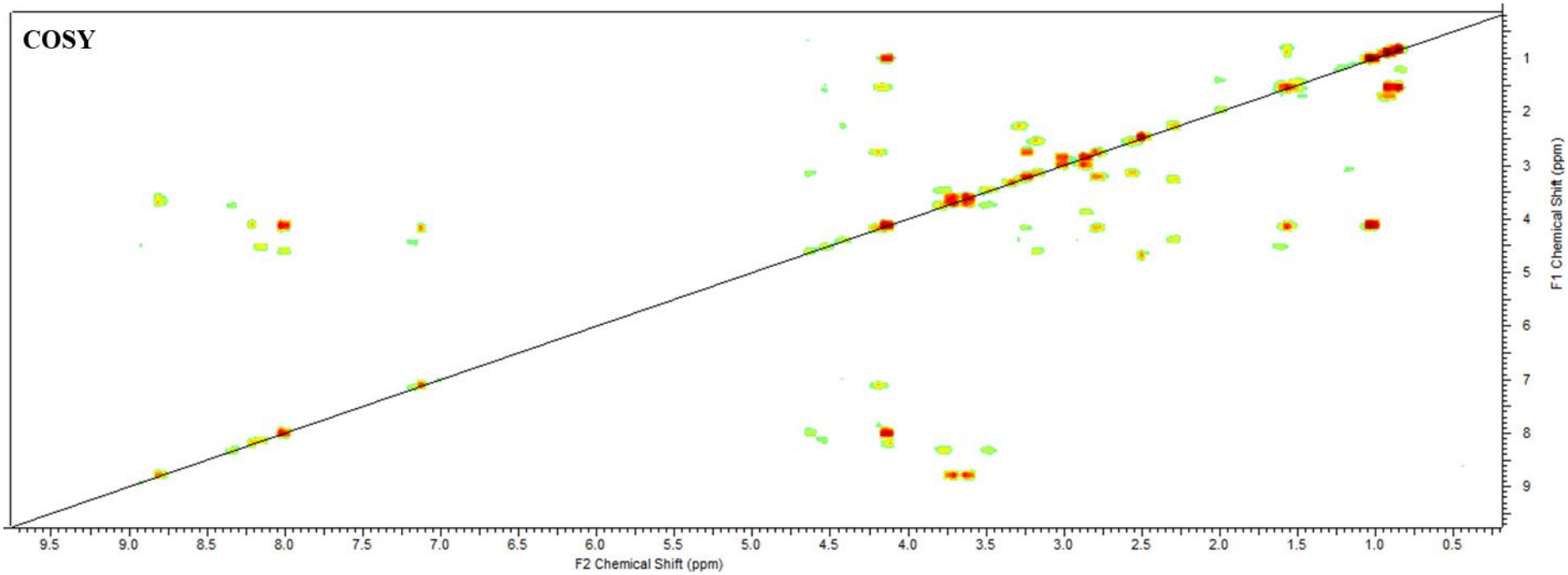
^{1}H						
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**Exchangeable**
1 - Lan (Dha)		4.63	2.58 3.17			NH_2 - 8.00
2 - Leu	8.12	4.55	1.61	1.74	0.91 0.95	
3 - Gly	8.34	3.50 3.78				
4 - Lan (Cys)	7.18	4.41	2.30 3.29			
5 - Thr	7.88	4.19	4.16	1.06		

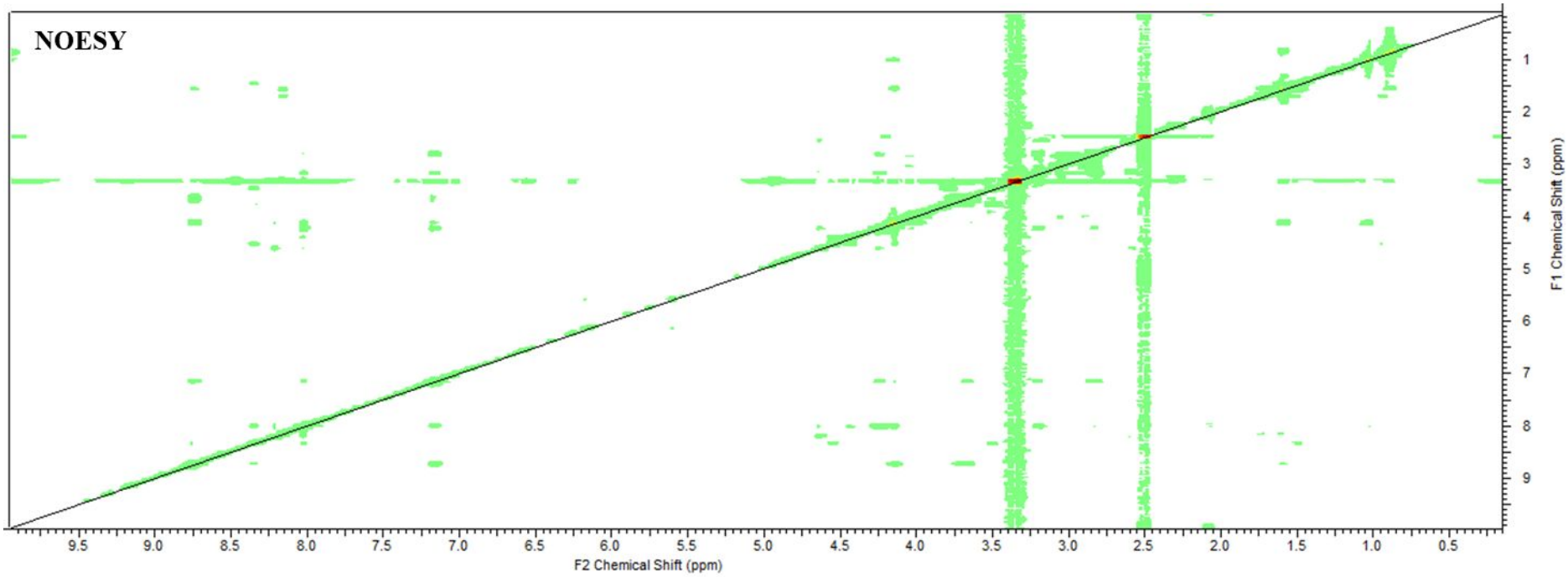
Table S5

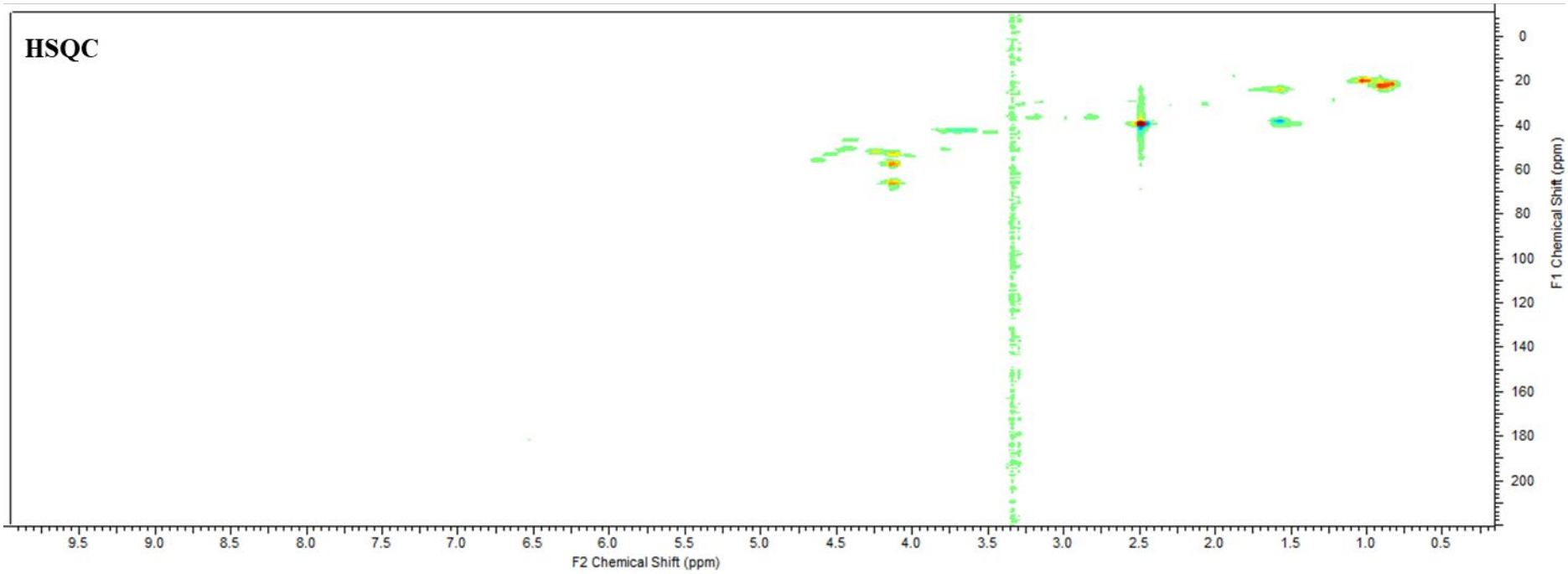
^{13}C					
Residue Number	**CO**	**α**	**β**	**γ**	**δ**
1 - Lan (Dha)		59.28	32.91		
2 - Leu	176.63	56.47	43.24	27.46	23.80 26.81
3 - Gly	173.21	46.40			
4 - Lan (Cys)		54.02	34.38		
5 - Thr		60.74	70.47	23.57	



Annotation is for major conformer.


COSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


NOESY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)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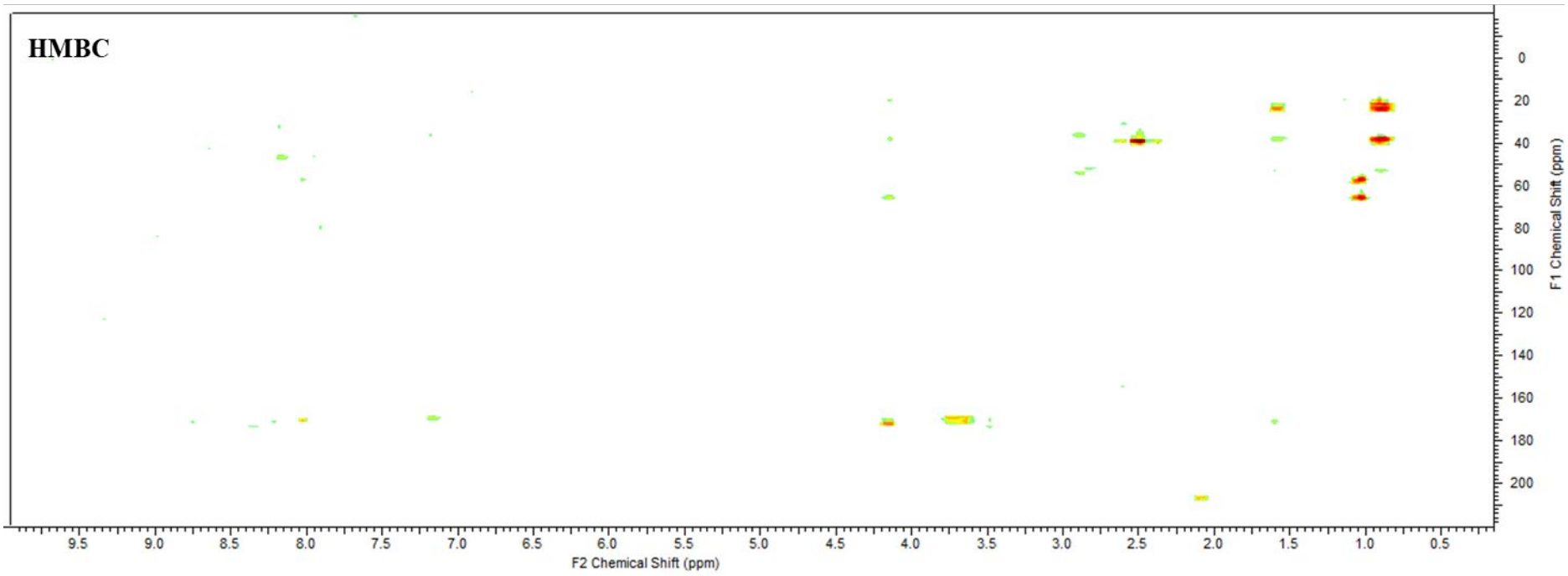
F1 Chemical Shift (ppm)


HMBC
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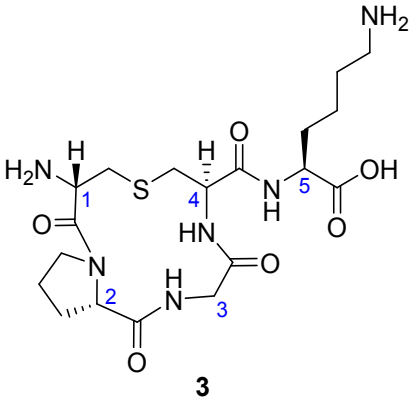
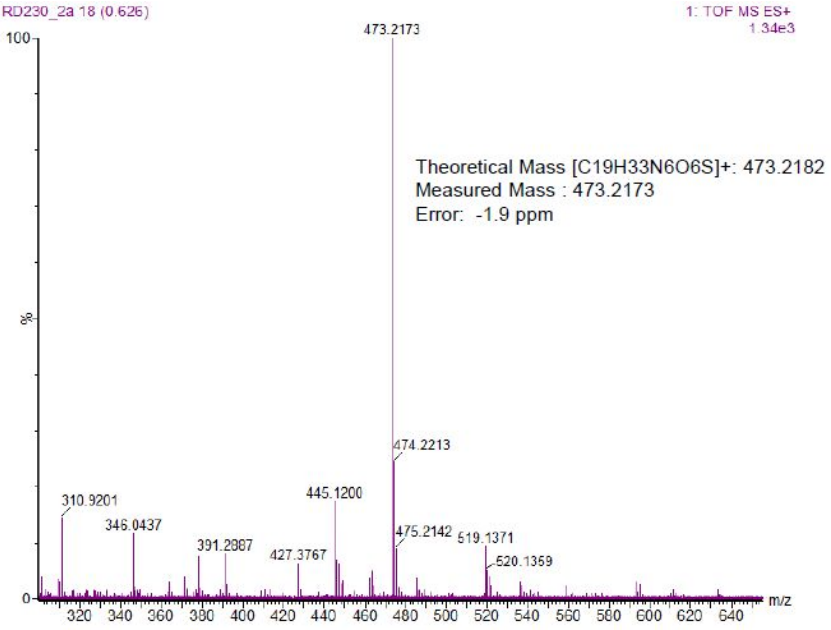
F1 Chemical Shift (ppm)

Nisin Ring B Lan analogue (3)

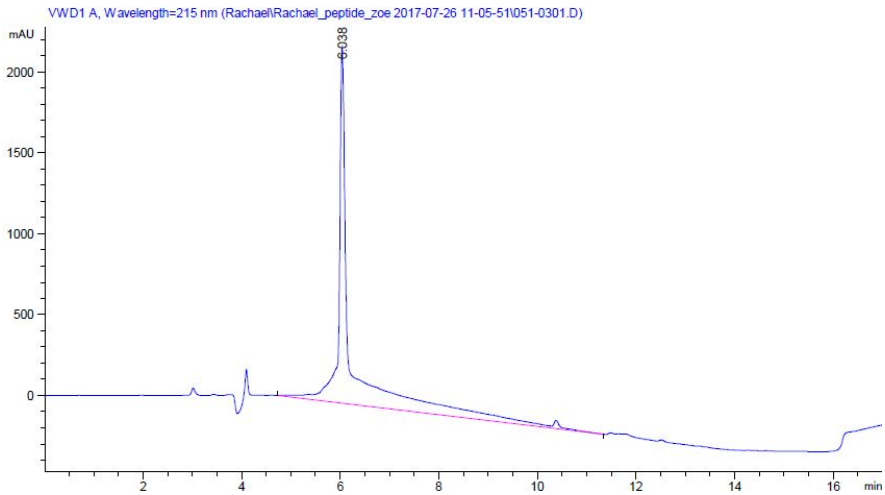


3

m/z (HRMS, ES+) required for $[C_{19}H_{33}N_6O_6S]^+$ 473.2182, found $[C_{19}H_{33}N_6O_6S]^+$ 473.2173.



analytical HPLC (Dr Maisch GmbH Reprosil Gold 200 C8 5µm 250 x 4.6 mm column on Agilent HPLC system) retention time 6.04 min.



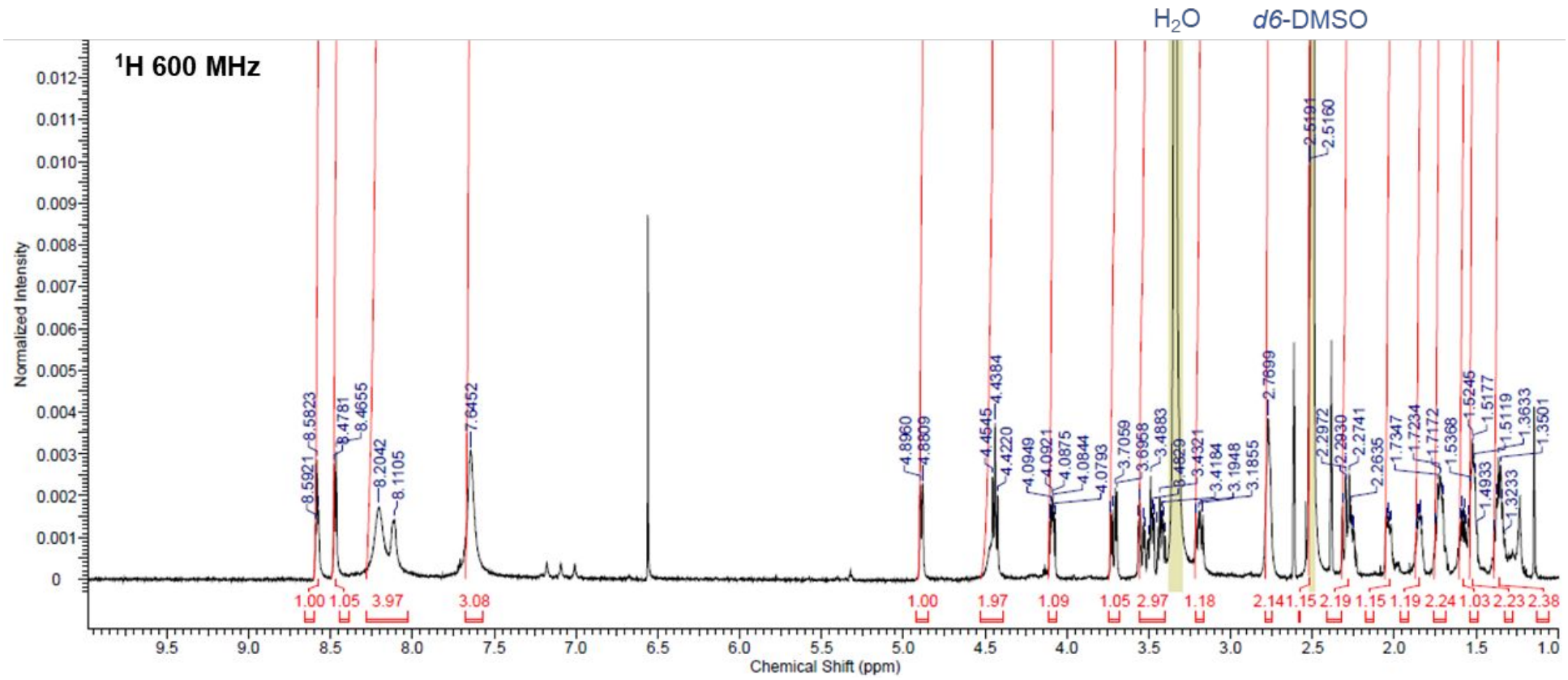
NMR δ_H (600 MHz, $(CD_3)_2SO$), δ_c (150 MHz, $(CD_3)_2SO$)

Table S6

^1H							
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**ε**	**Exchangeable**
1 - Lan (Dha)		4.45	2.28 3.32				NH_2 - 8.23
2 - Pro		4.89	2.04 2.28	1.71 1.82	3.42 3.50		
3 - Gly	8.58	3.54 3.72					
4 - Lan (Cys)	8.12	4.44	2.52 3.18				
5 - Lys	8.47	4.09	1.57 1.73	1.35	1.52	2.77	NH_2 - 7.63 CO_2H - 12.66

Table S7

^{13}C						
Residue Number	**CO**	**α**	**β**	**γ**	**δ**	**ε**
1 - Lan (Dha)	174.63	52.19				
2 - Pro	175.24	62.15	34.96	25.21	50.56	
3 - Gly	172.98	46.76				
4 - Lan (Cys)	173.85	59.65	32.68			
5 - Lys	176.70	55.21	33.55	25.75	29.85	41.88


^{1}H 600 MHz
H_2O
d6-DMSO
Normalized Intensity
Chemical Shift (ppm)
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8.5823
8.4781
8.4655
8.2042
8.1105
7.6452
4.8960
4.8809
4.4545
4.4384
4.4220
4.0949
4.0921
4.0875
4.0844
4.0793
3.7059
3.6958
3.4883
3.4829
3.4321
3.4184
3.1948
3.1855
2.7699
2.5191
2.5160
2.2972
2.2930
2.2741
2.2635
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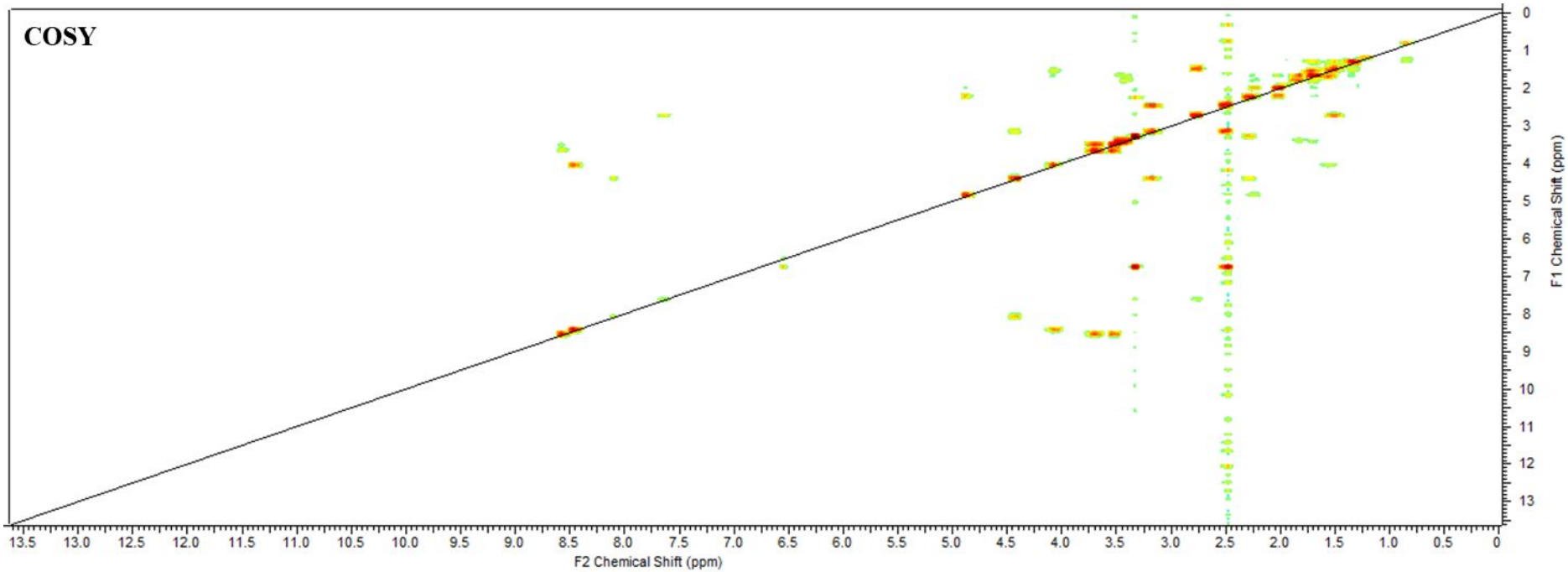
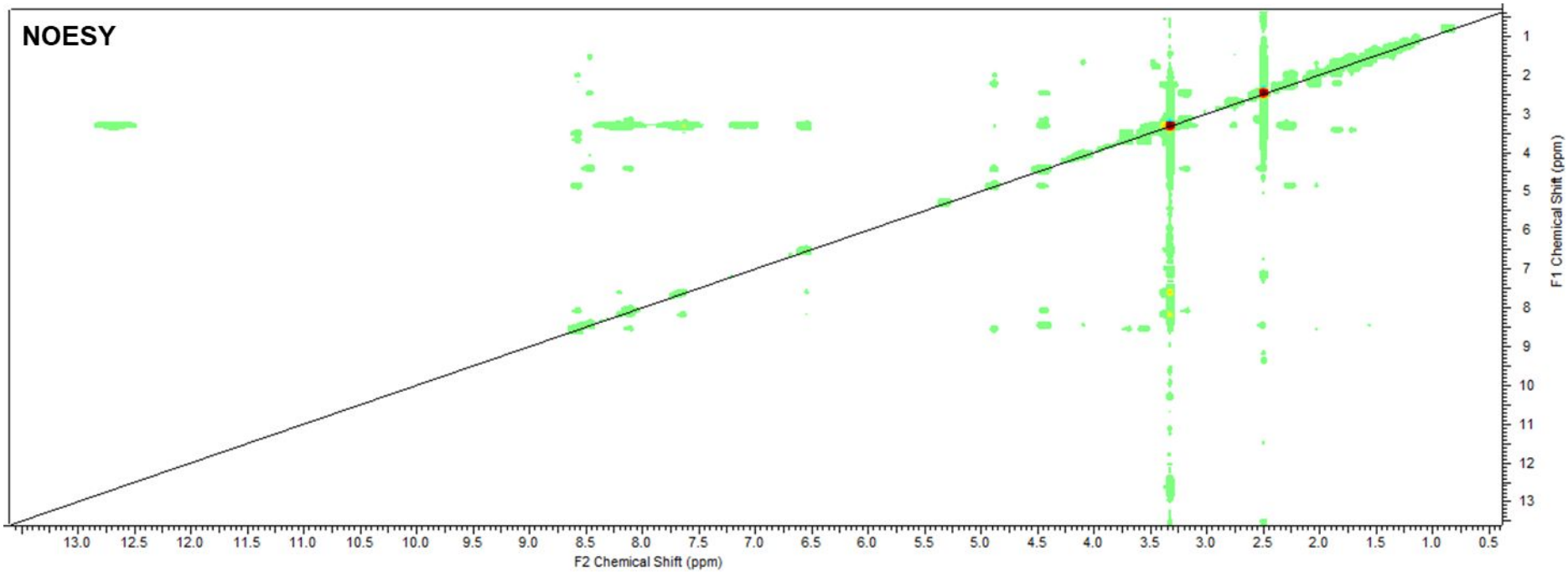
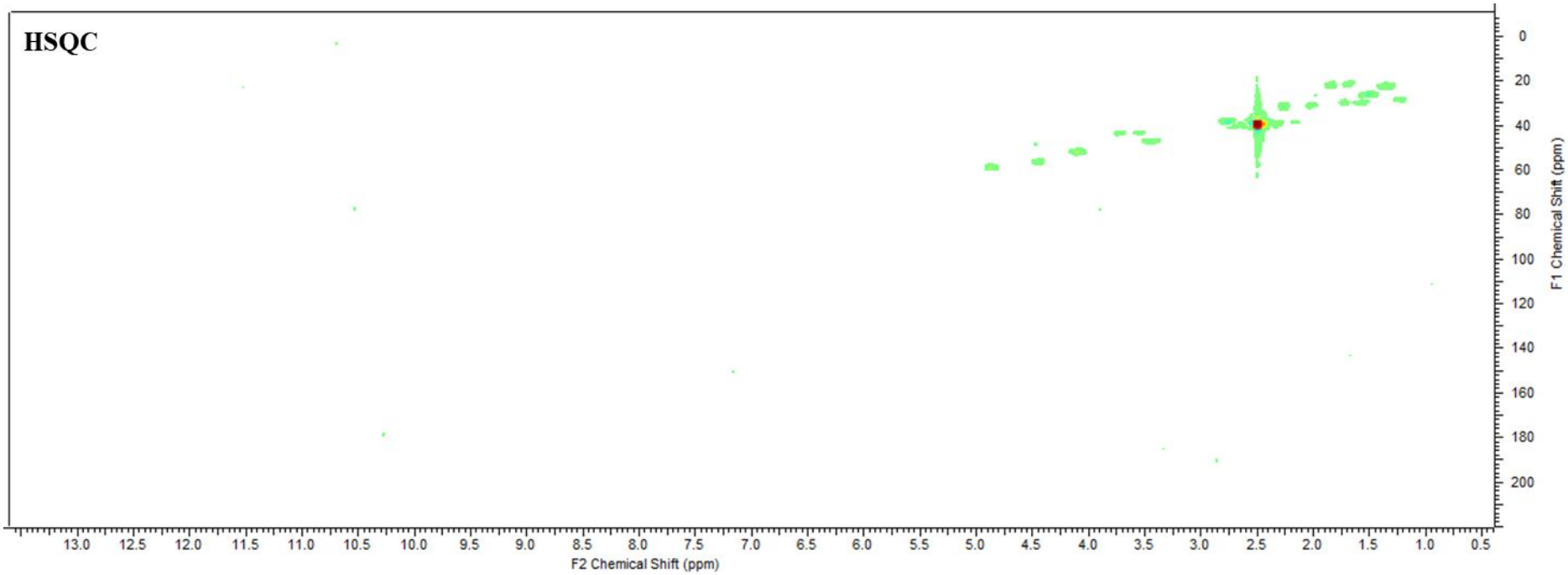
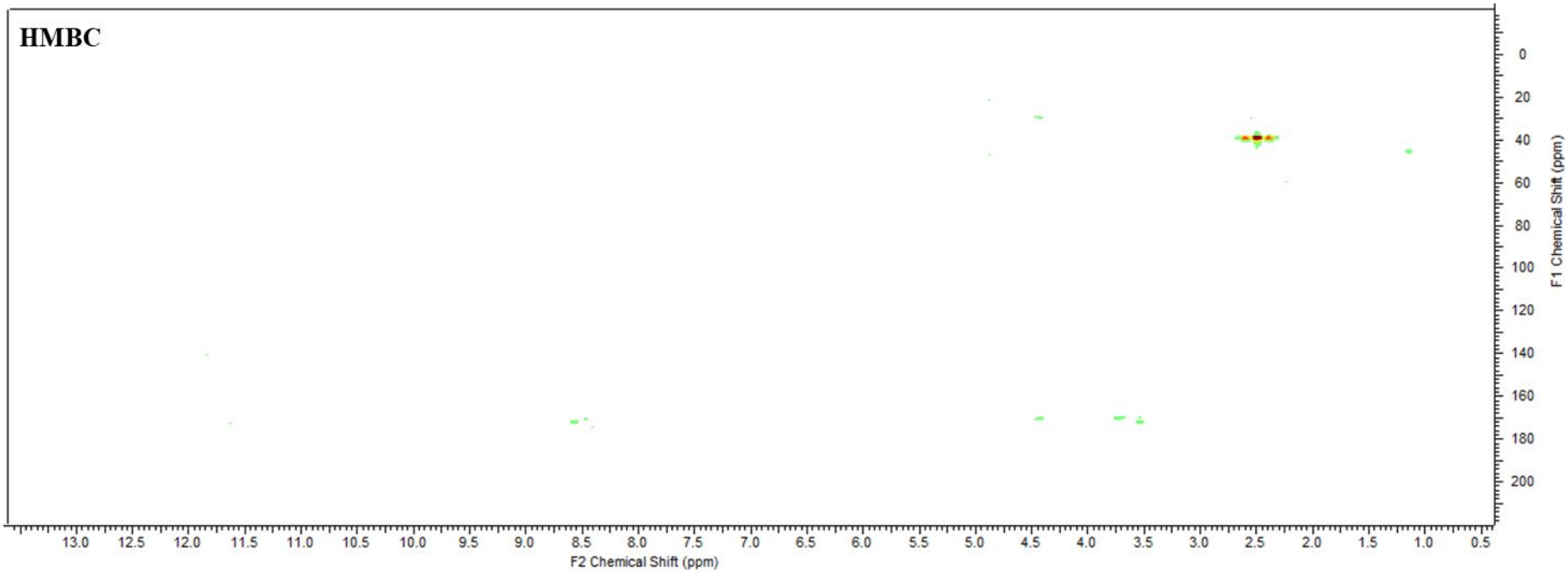
1.5245
1.5177
1.5119
1.4933
1.3633
1.3501
1.3233
1.00 1.05 3.97 3.08 1.00 1.97 1.09 1.05 2.97 1.18 2.14 1.15 2.19 1.15 1.19 2.24 1.03 2.23 2.38

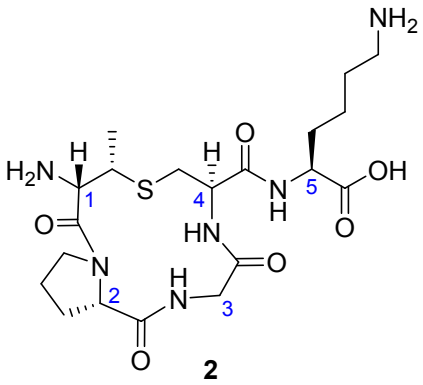

COSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


NOESY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


HSQC
0
20
40
60
80
100
120
140
160
180
200
F1 Chemical Shift (ppm)
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)

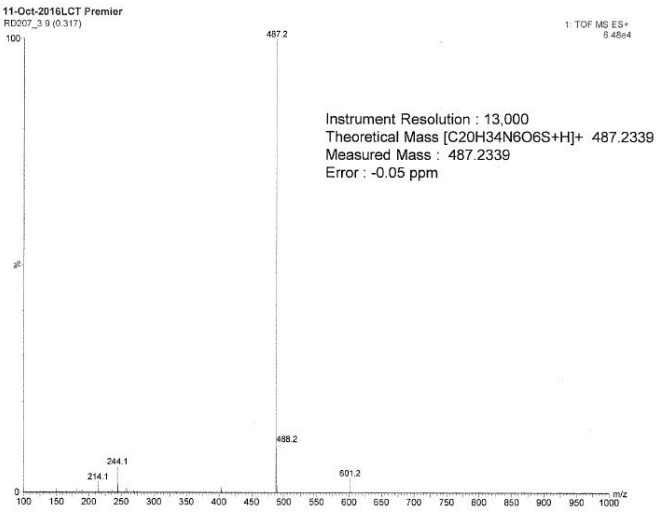

HMBC
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

Nisin Ring B WT (2)

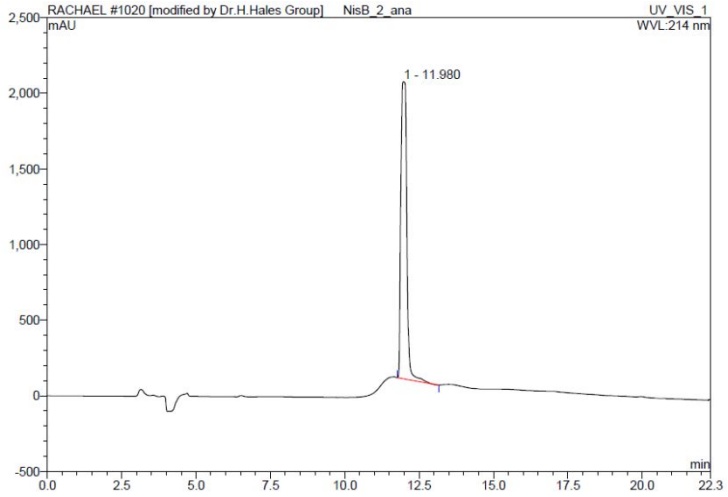


2

m/z (HRMS, ES+) required for $[C_{20}H_{34}N_6O_6S+H]^+$ 487.2339, found $[C_{20}H_{34}N_6O_6S+H]^+$ 487.2339.



analytical HPLC (Dr Maisch GmbH Reprosil Gold 200 C8 5µm 250 x 4.6 mm column on Dionex HPLC system) retention time 11.98 min.



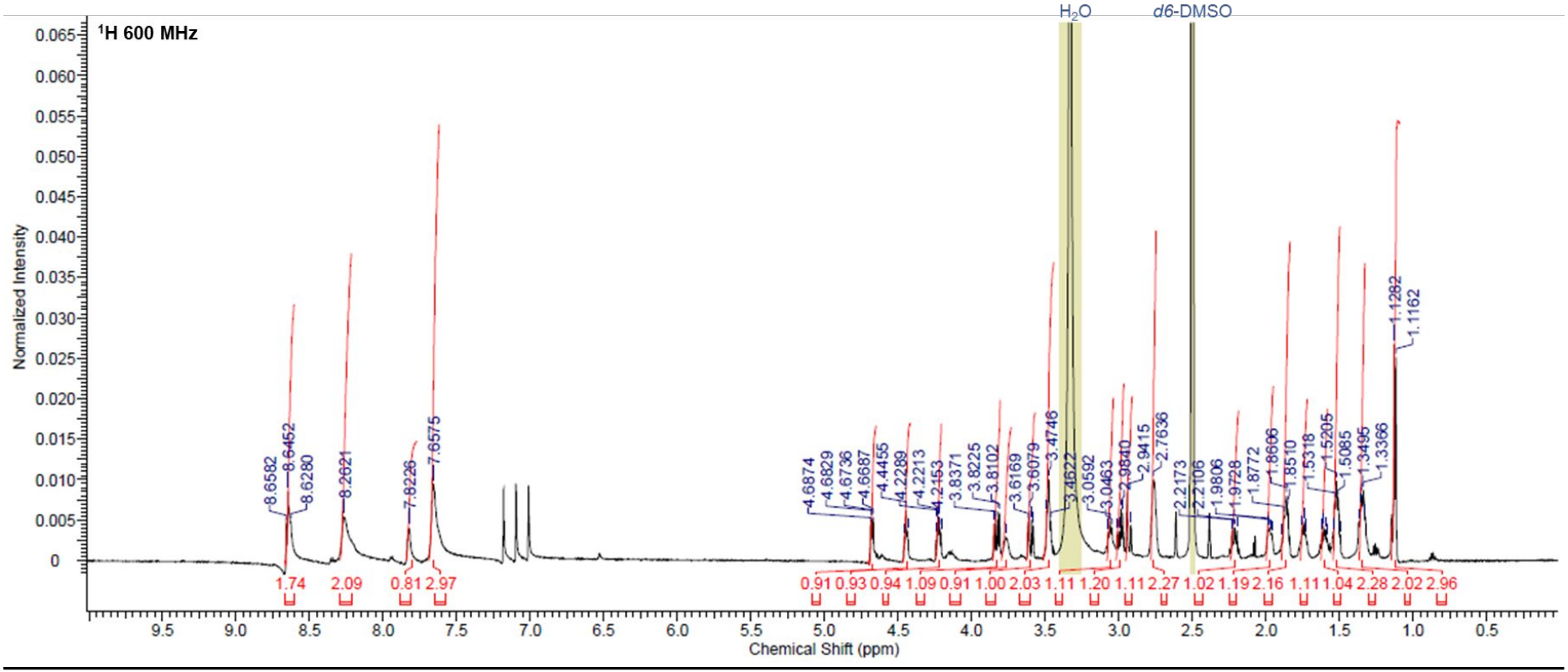
NMR δ_H (600 MHz, $(CD_3)_2SO$), δ_c (150 MHz, $(CD_3)_2SO$)

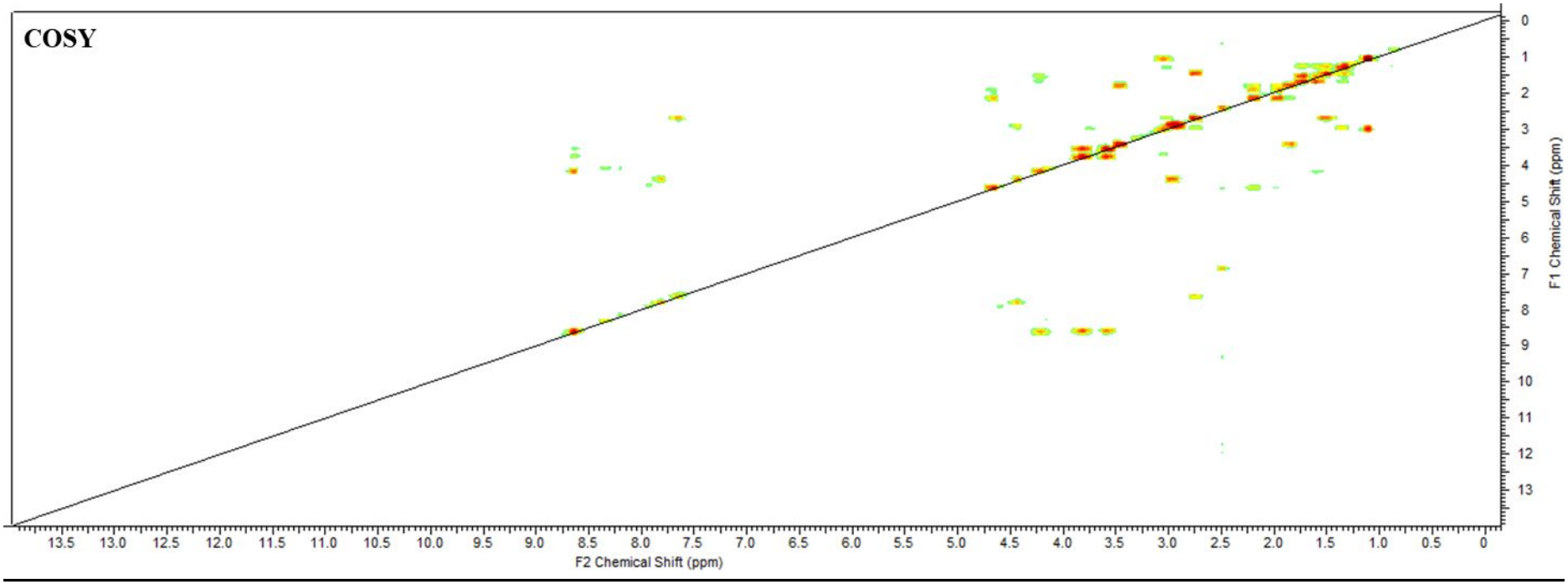
Table S8

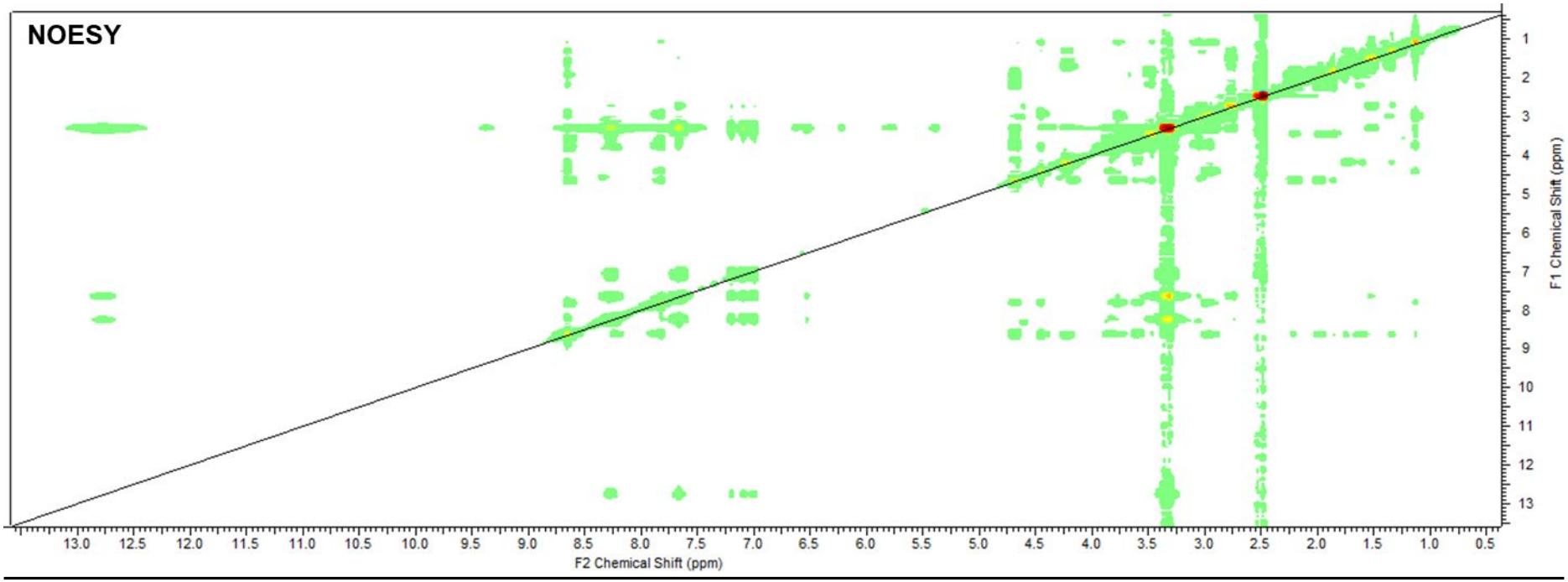
^1H							
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**ε**	**Exchangeable**
1 - MeLan (Dhb)		3.76	3.05	1.11			NH_2 - 8.26
2 - Pro		4.66	1.97 2.22	1.87	3.48		
3 - Gly	8.63	3.60 3.83					
4 - MeLan (Cys)	7.81	4.43	2.98 2.93				
5 - Lys	8.65	4.22	1.74 1.59	1.34	1.52	2.77	NH_2 - 7.64 CO_2H - 12.73

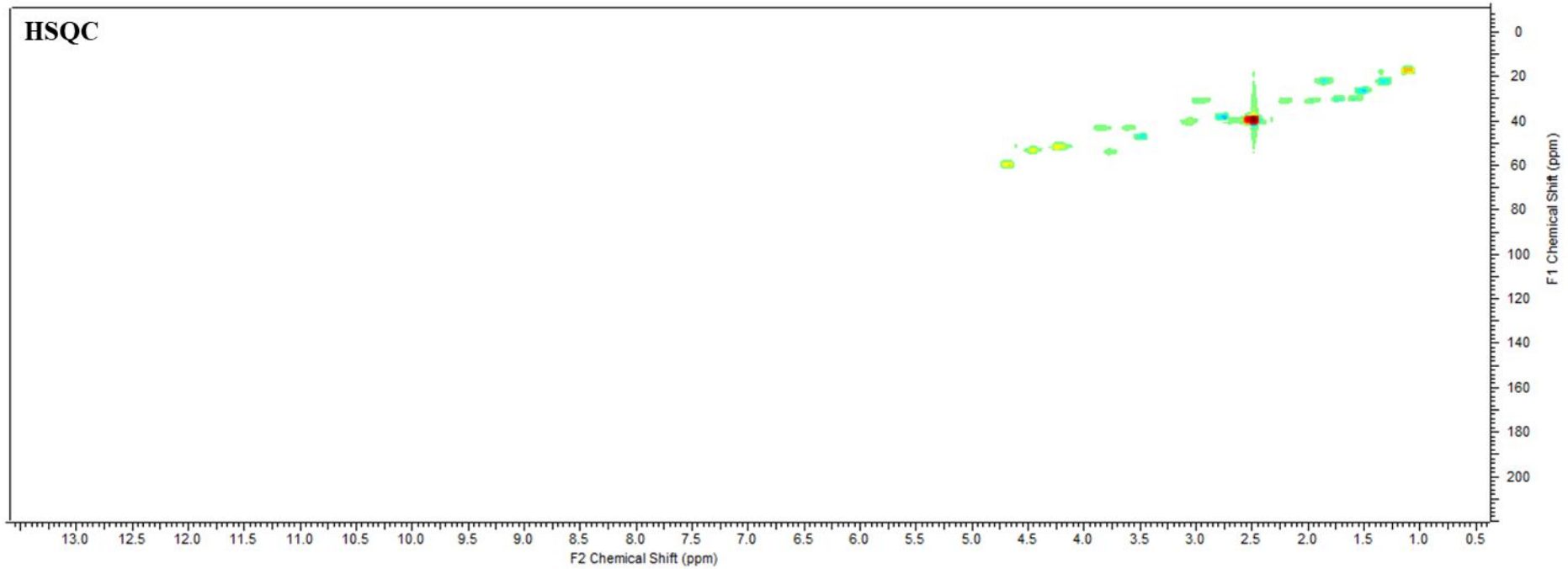
Table S9

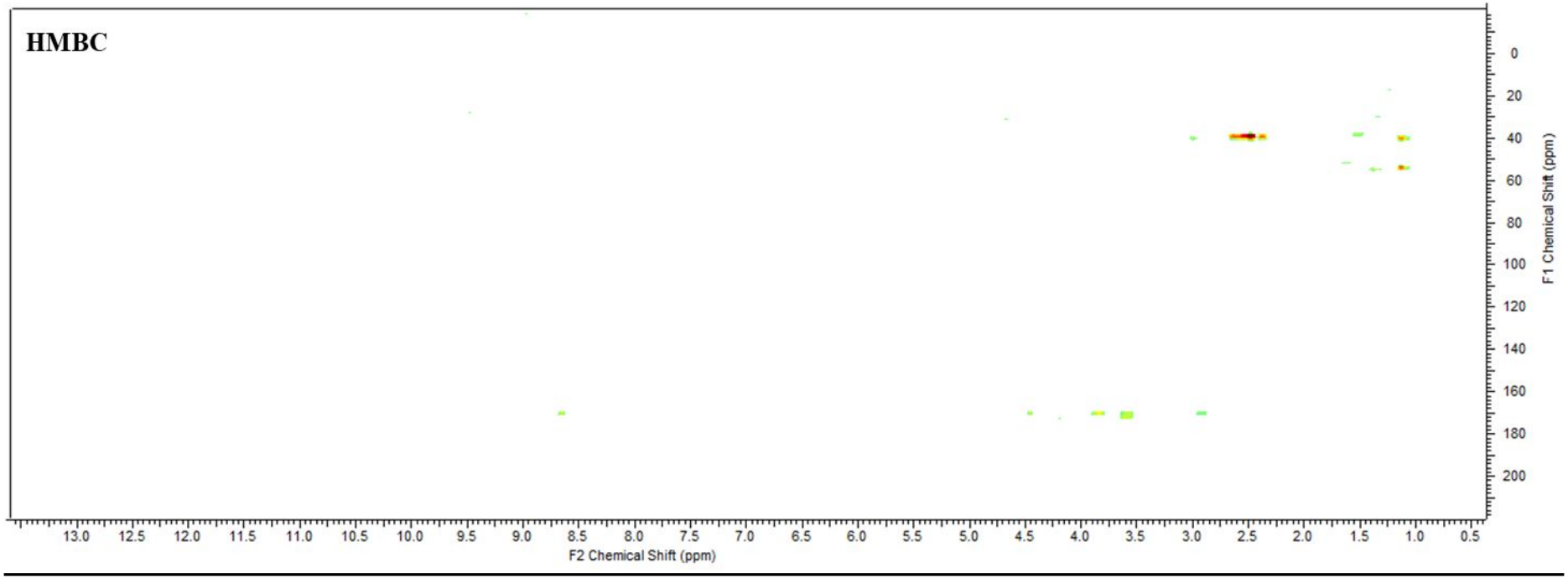
^{13}C						
Residue Number	**CO**	**α**	**β**	**γ**	**δ**	**ε**
1 - MeLan (Dhb)	172.18	57.41	43.80	20.91		
2 - Pro	175.14	62.87	34.72	25.61	50.70	
3 - Gly	173.34	46.65				
4 - MeLan (Cys)	173.41	56.62	34.42			
5 - Lys	176.04	54.97	33.73	25.68	29.84	41.78


^{1}H 600 MHz
H_2O
d6-DMSO
Normalized Intensity
Chemical Shift (ppm)
1.74 2.09 0.81 2.97
0.91 0.93 0.94 1.09 0.91 1.00 2.03 1.11 1.20 1.11 2.27 1.02 1.19 2.16 1.11 1.04 2.28 2.02 2.96


COSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

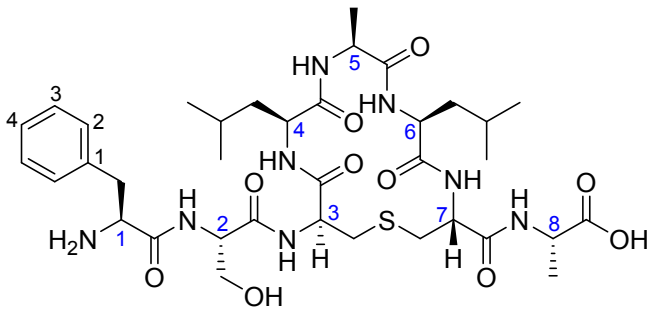

NOESY
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)


HSQC
0
20
40
60
80
100
120
140
160
180
200
F1 Chemical Shift (ppm)
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)


HMBC
0
20
40
60
80
100
120
140
160
180
200
F1 Chemical Shift (ppm)
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)

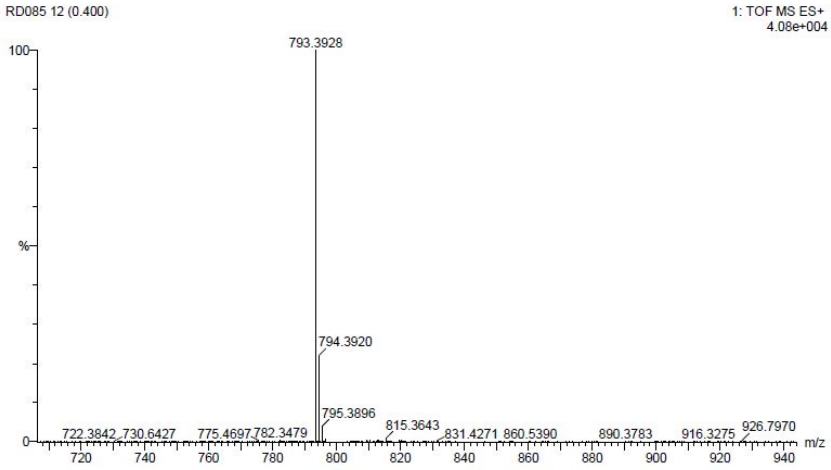
Ring A Peptides

Mutacin I Ring A Ser/Ala Analogue (14)



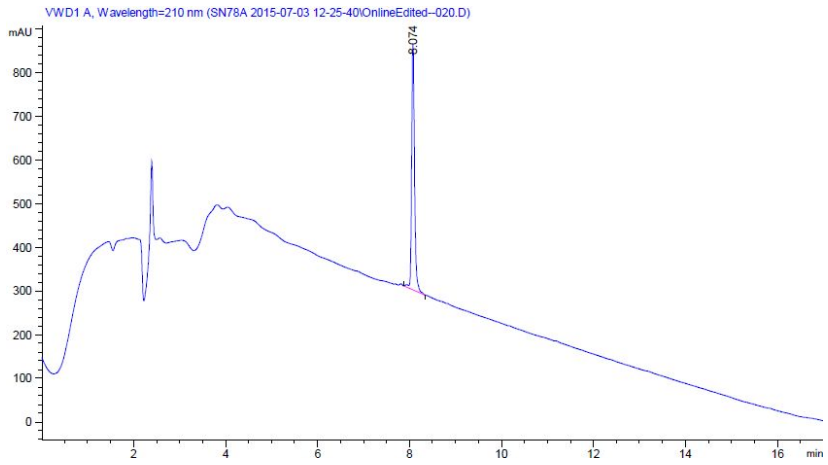
14

HRMS (ES+) m/z: $[M + H]^+$ Calcd for $C_{36}H_{57}N_8O_{10}S$ 793.3918; found 793.3928.



Minimum:			-1.5			
Maximum:		5.0	20.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
793.3928	793.3918	1.0	1.3	12.5	5573.5	C36 H57 N8 O10 S

analytical HPLC (ACE 300Å C18-300 150 x 4.6 mm column on Agilent HPLC system) retention time 8.07 min.



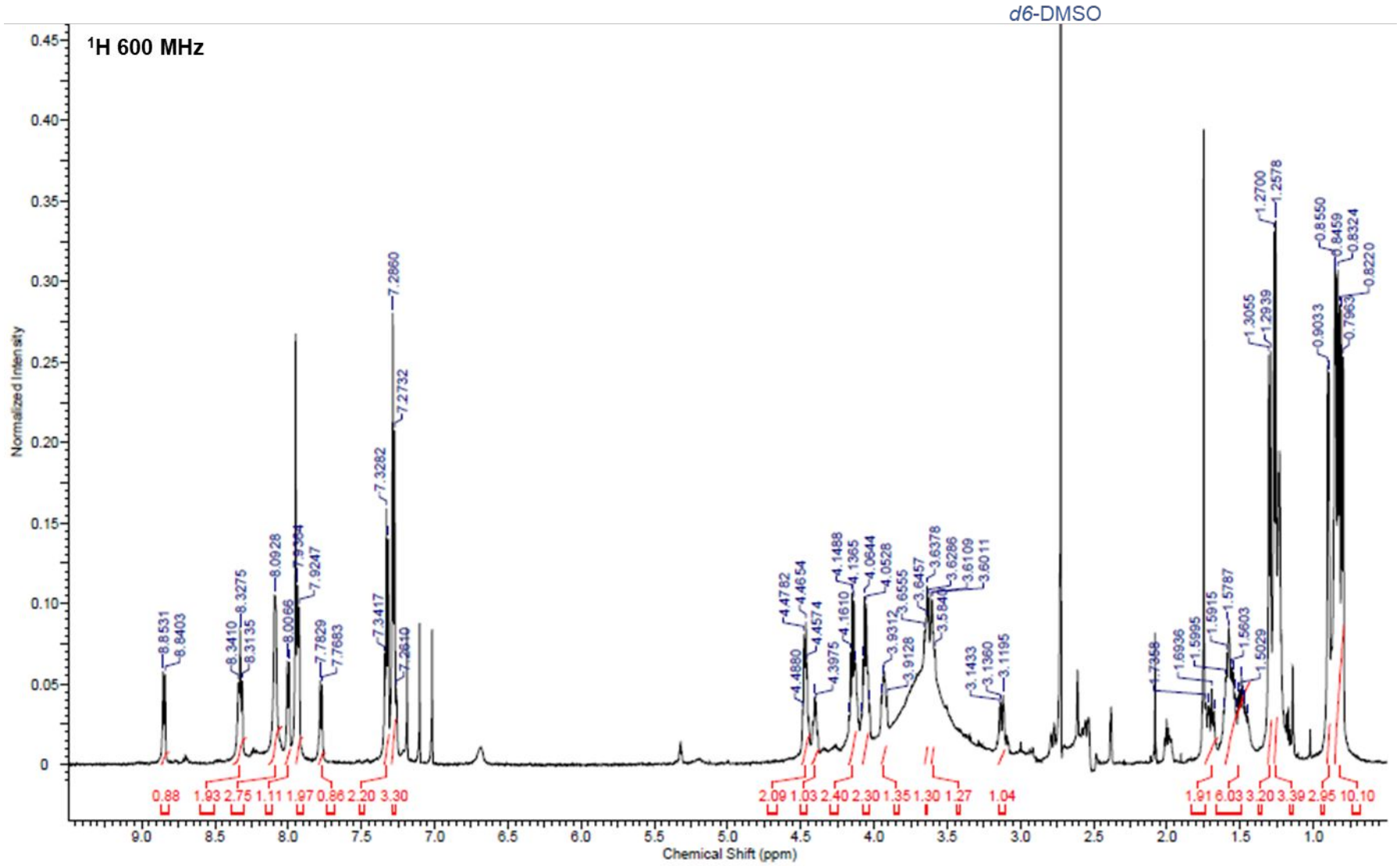
NMR δ_H (600 MHz, $(CD_3)_2SO$), δ_c (150 MHz, $(CD_3)_2SO$)

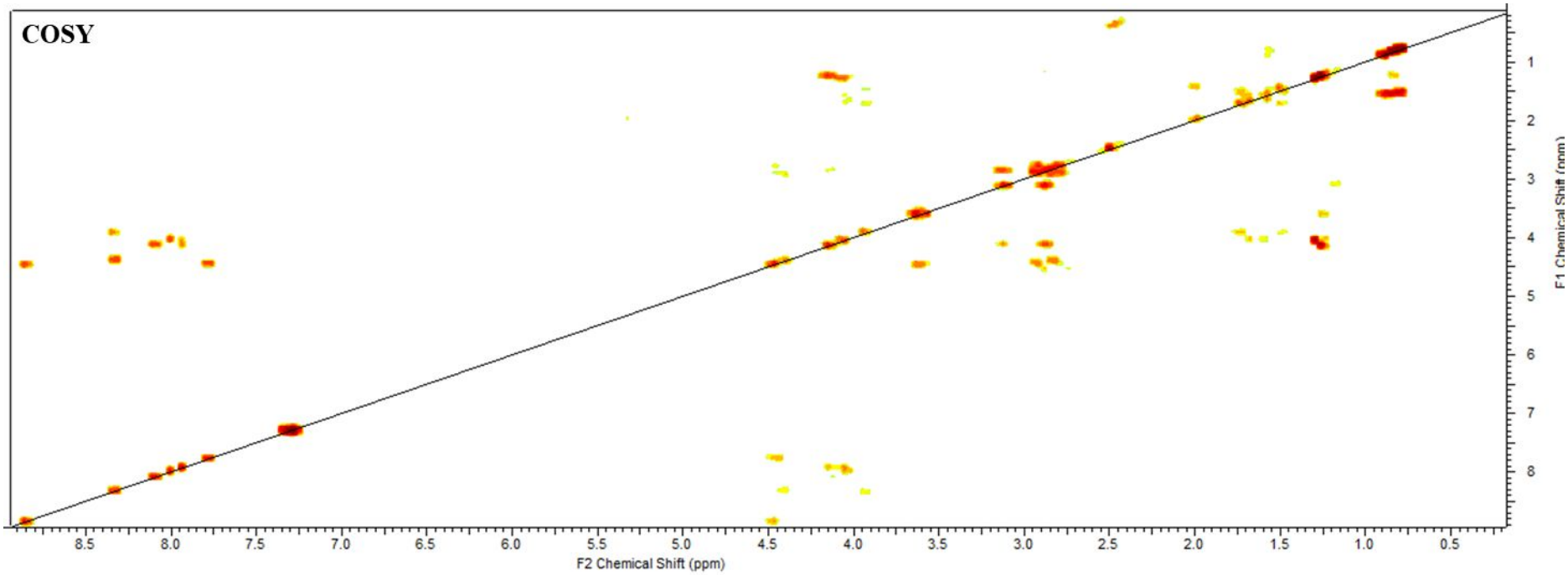
Table S10

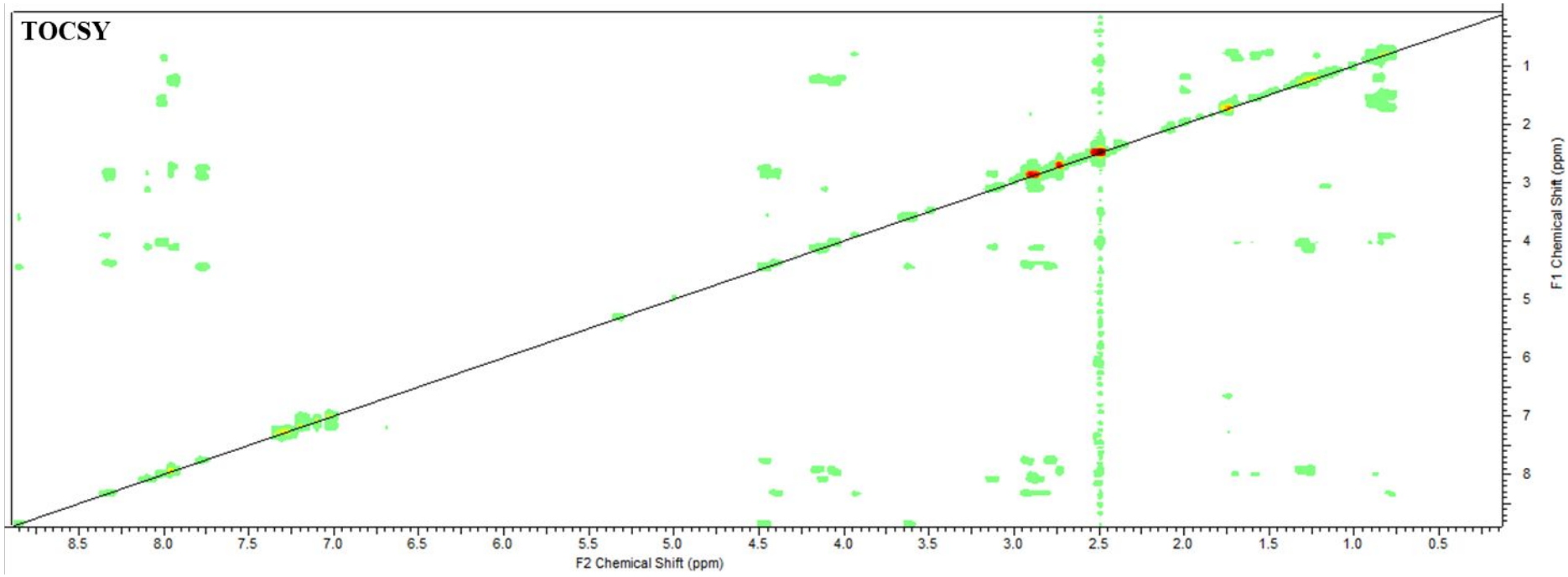
^1H							
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**Ar**	**Exchangeable**
1 - Phe		4.13	2.88 3.12			2 - 7.32 3 - 7.29 4 - 7.26	NH_2 - 8.09
2 - Ser	8.85	4.48	3.62				
3 - Lan (Dha)	8.33	4.39	2.92 2.83				
4 - Leu	8.34	3.93	1.49 1.74	1.54	a - 0.80 b - 0.83		
5 - Ala	7.94	4.15	1.27				
6 - Leu	8.00	4.05	1.60 1.69	1.57	a - 0.85 b - 0.90		
7 - Lan (Cys)	7.78	4.47	2.79 2.92				
8 - Ala	7.93	4.07	1.30				CO_2H - 12.63

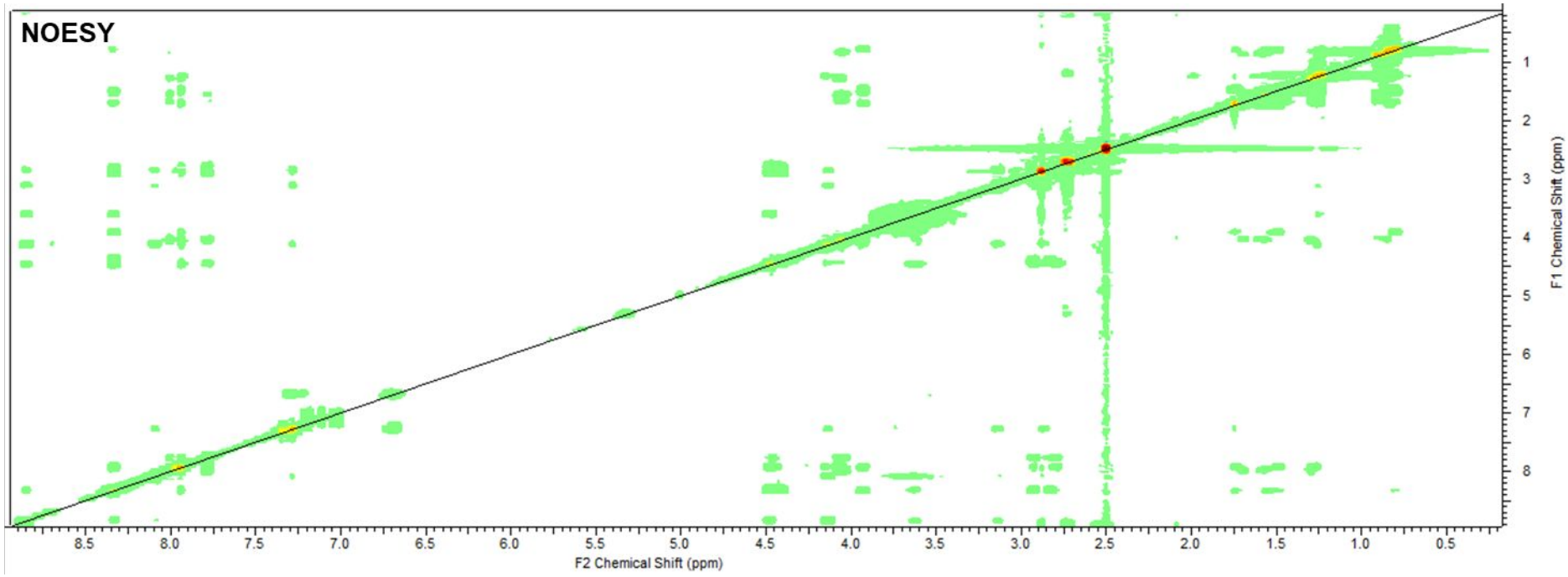
Table S11

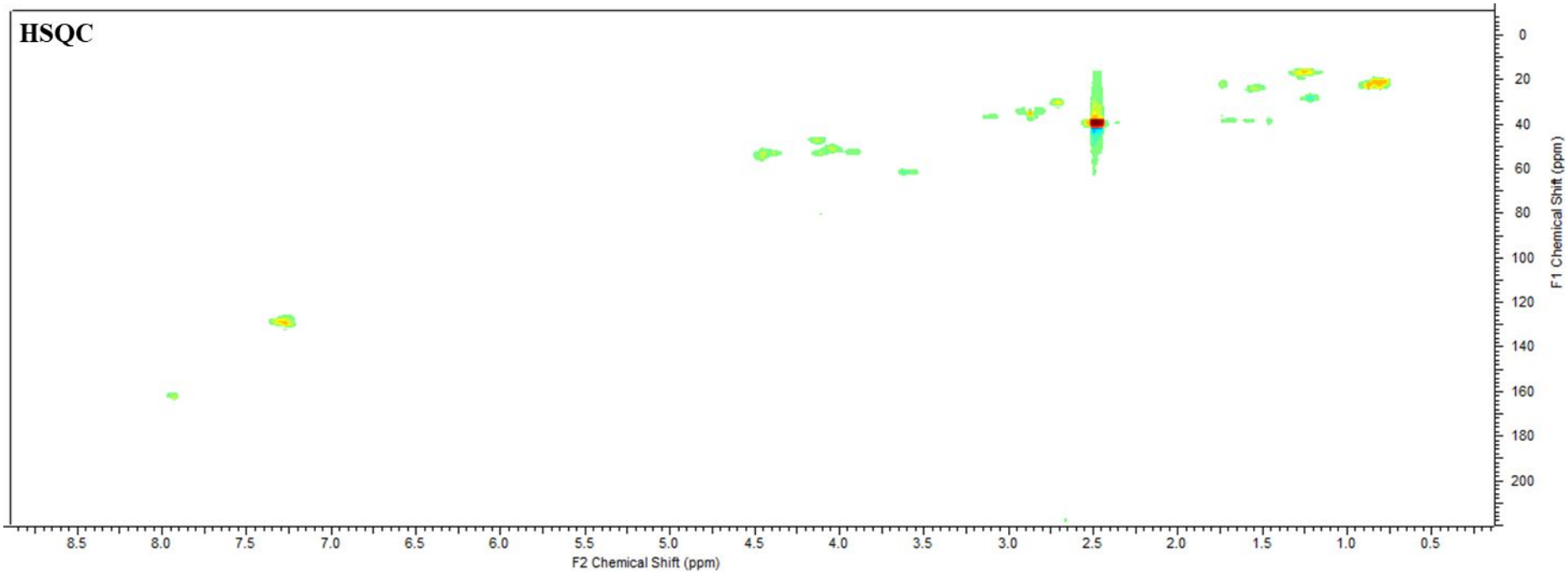
^{13}C						
Residue Number	**CO**	**α**	**β**	**γ**	**δ**	**Ar**
1 - Phe	171.34	56.36	40.04			1 - 138.00 2 - 131.83 3 - 132.67 4 - 130.43
2 - Ser	171.88	57.64	64.83			
3 - Lan (Dha)	173.03	56.58	40.51			
4 - Leu	174.50	55.77	25.77	41.71	a - 24.71 b - 26.39	
5 - Ala	177.01	50.79	20.14			
6 - Leu	170.11	54.33	27.54	42.12	a - 24.76 b - 26.34	
7 - Lan (Cys)	174.34	56.07	37.60			
8 - Ala	175.24	54.33	20.61			

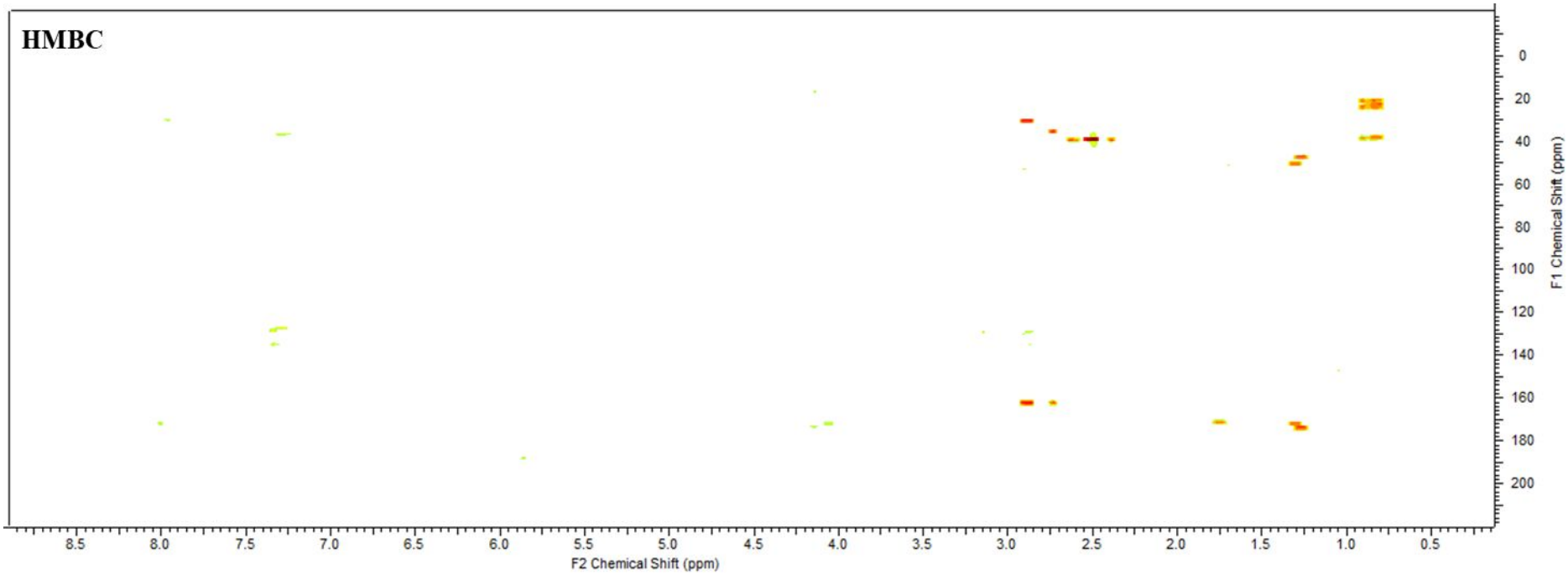

d6-DMSO
1H 600 MHz
Normalized Intensity
Chemical Shift (ppm)
0.88 1.93 2.75 1.11 1.97 0.86 2.20 3.30
2.09 1.03 2.40 2.30 1.35 1.30 1.27 1.04
1.91 6.03 3.20 3.39 2.95 10.10


COSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

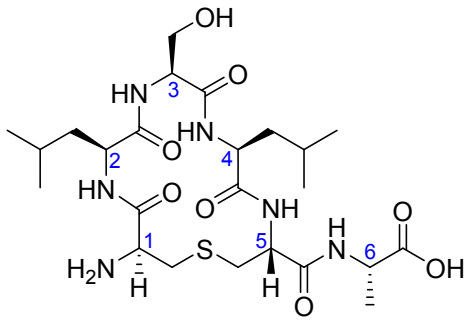

TOCSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


NOESY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


HSQC
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

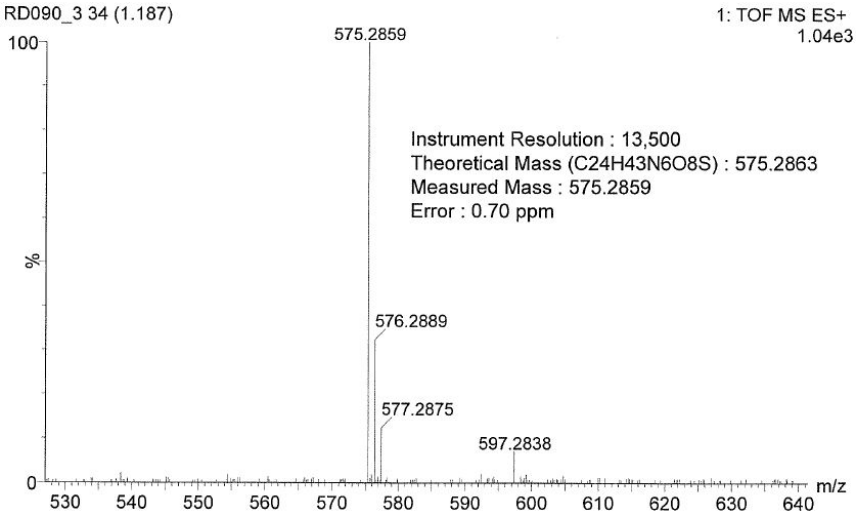

HMBC
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

Mutacin I Ring A Ser Analogue (15)

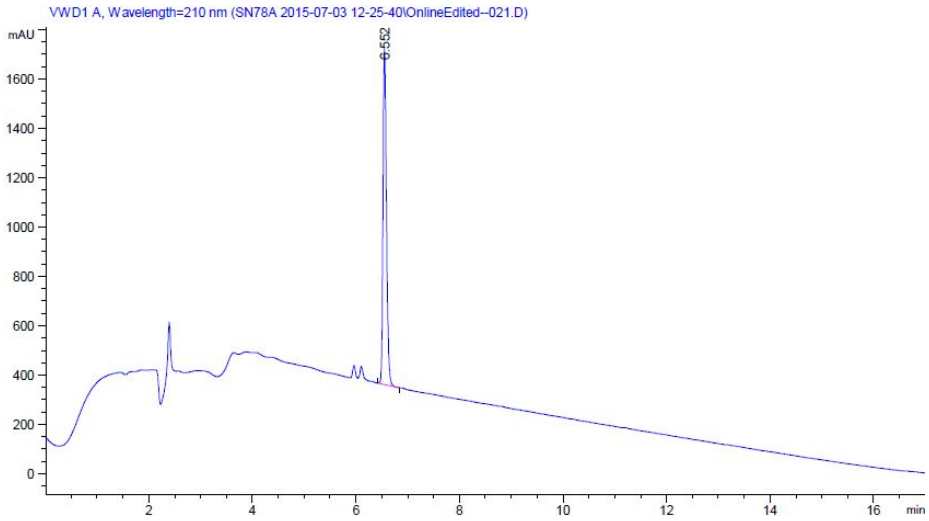


15

HRMS (ES+) m/z: $[M + H]^+$ Calcd for $C_{24}H_{43}N_6O_8S$ 575.2863; found 575.2859.



analytical HPLC (Fluka Analytical Discovery BIO C18-10 25 x 4.6 mm column on Agilent HPLC system) retention time 6.55 min.



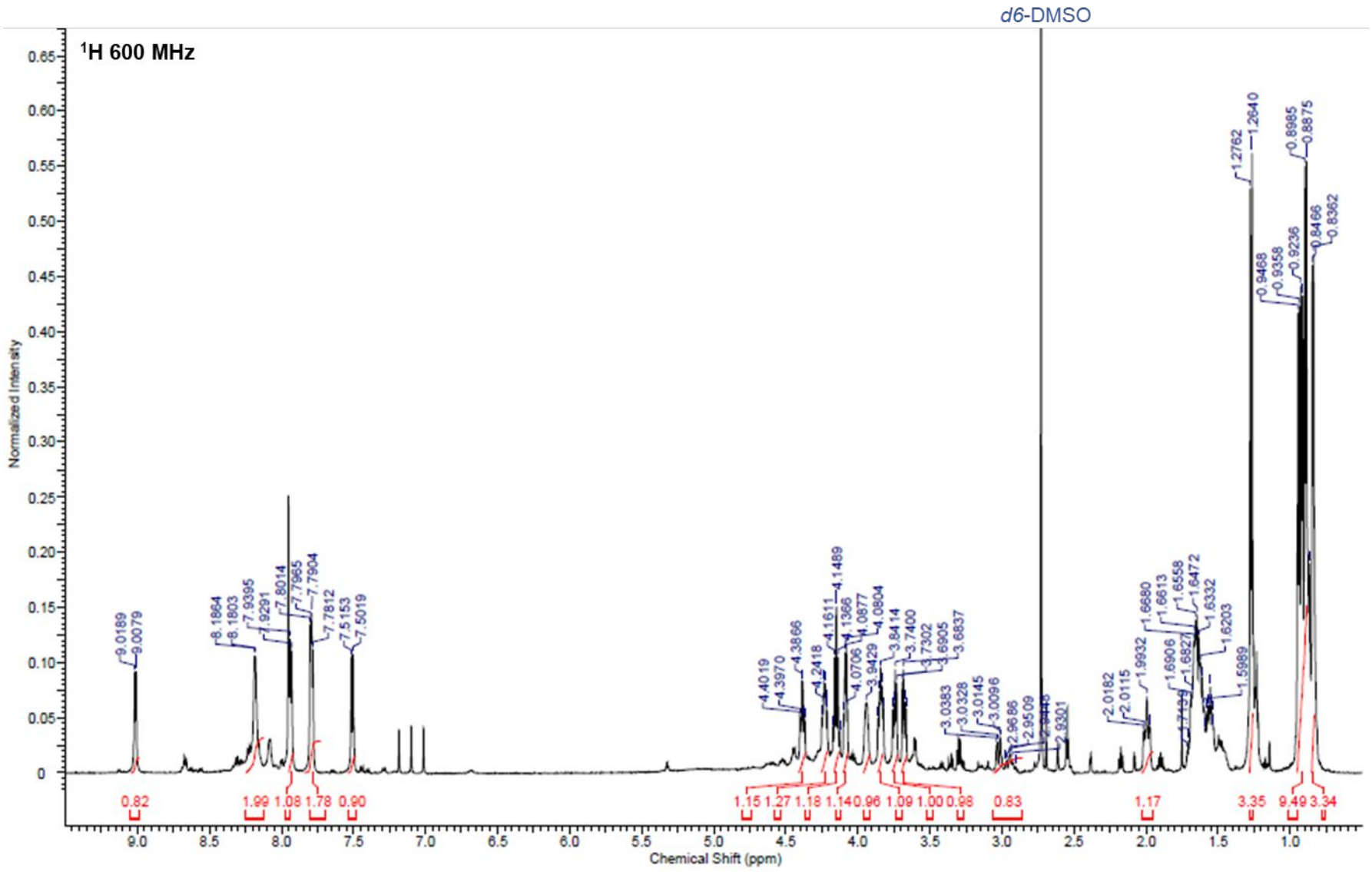
NMR δ_H (600 MHz, $(CD_3)_2SO$), δ_c (150 MHz, $(CD_3)_2SO$)

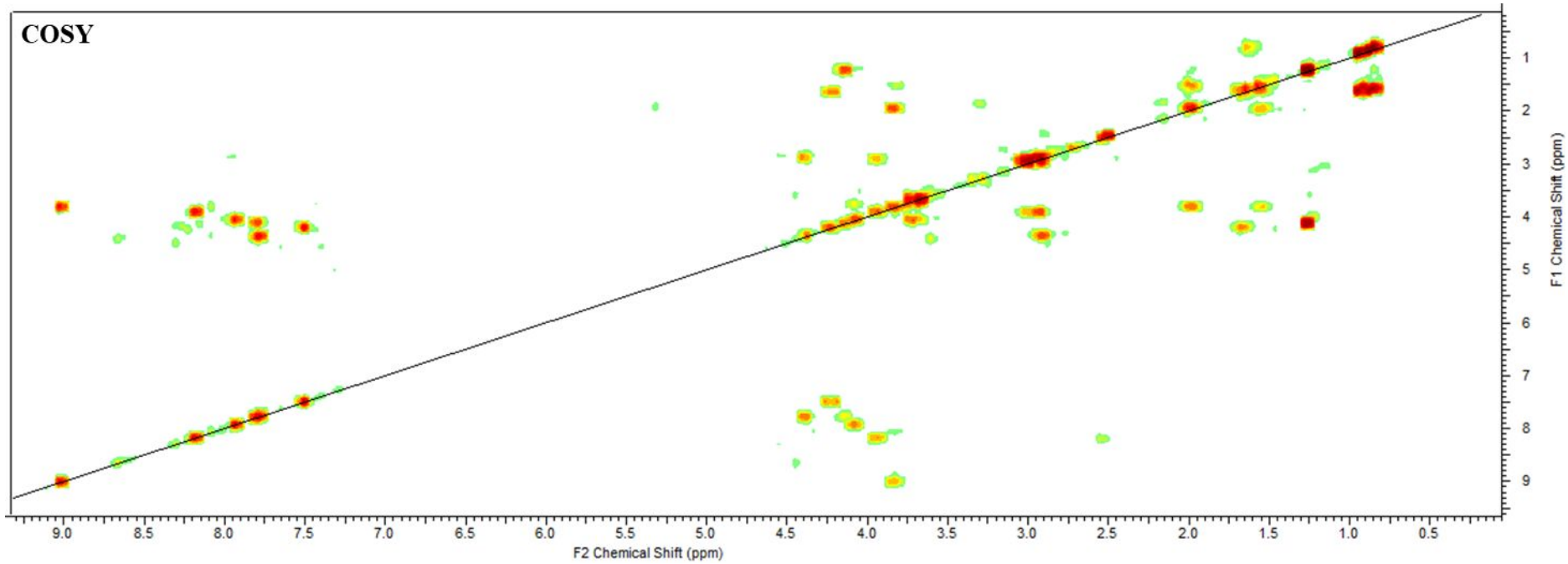
Table S12

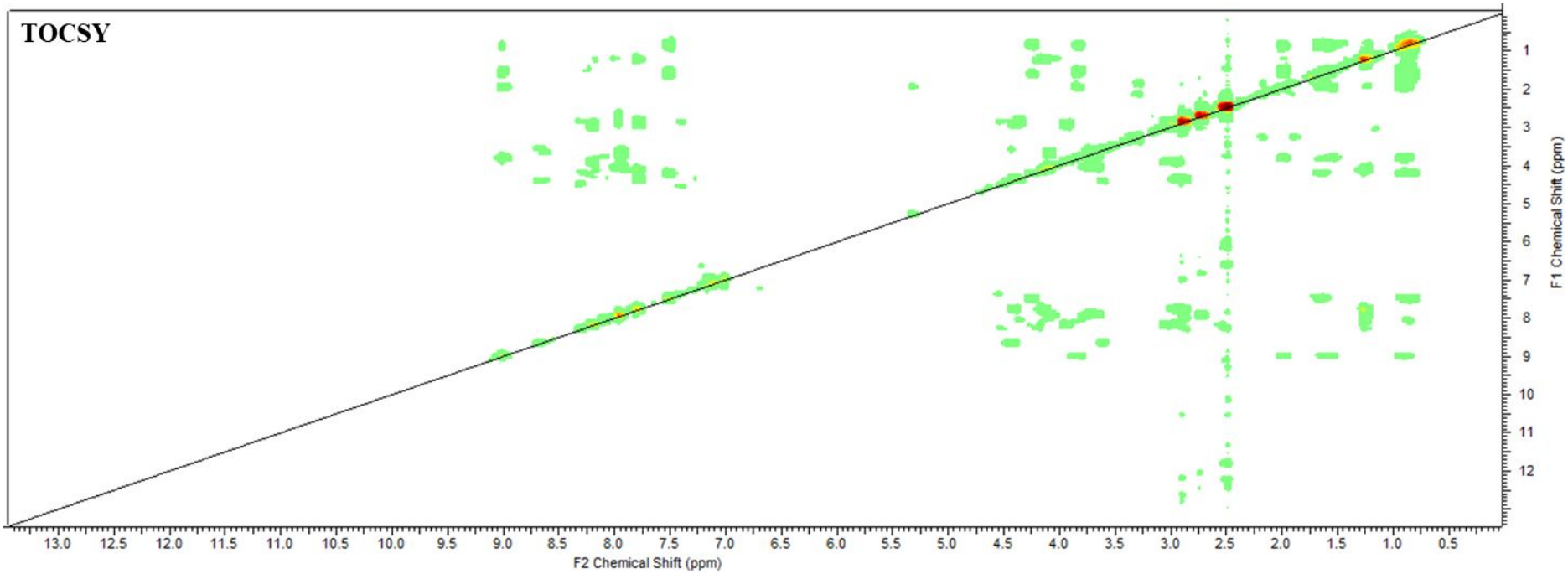
^1H						
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**Exchangeable**
1 - Lan (Dha)		3.93	2.92 3.02			NH_2 - 8.19
2 - Leu	9.02	3.84	1.55 1.99	1.65	a - 0.95 b - 0.89	
3 - Ser	7.94	4.09	3.69			
4 - Leu	7.51	4.23	1.67	1.70	a - 0.93 b - 0.84	
5 - Lan (Cys)	7.79	4.38	2.93			
6 - Ala	7.79	4.14	1.27			CO_2H - 12.63

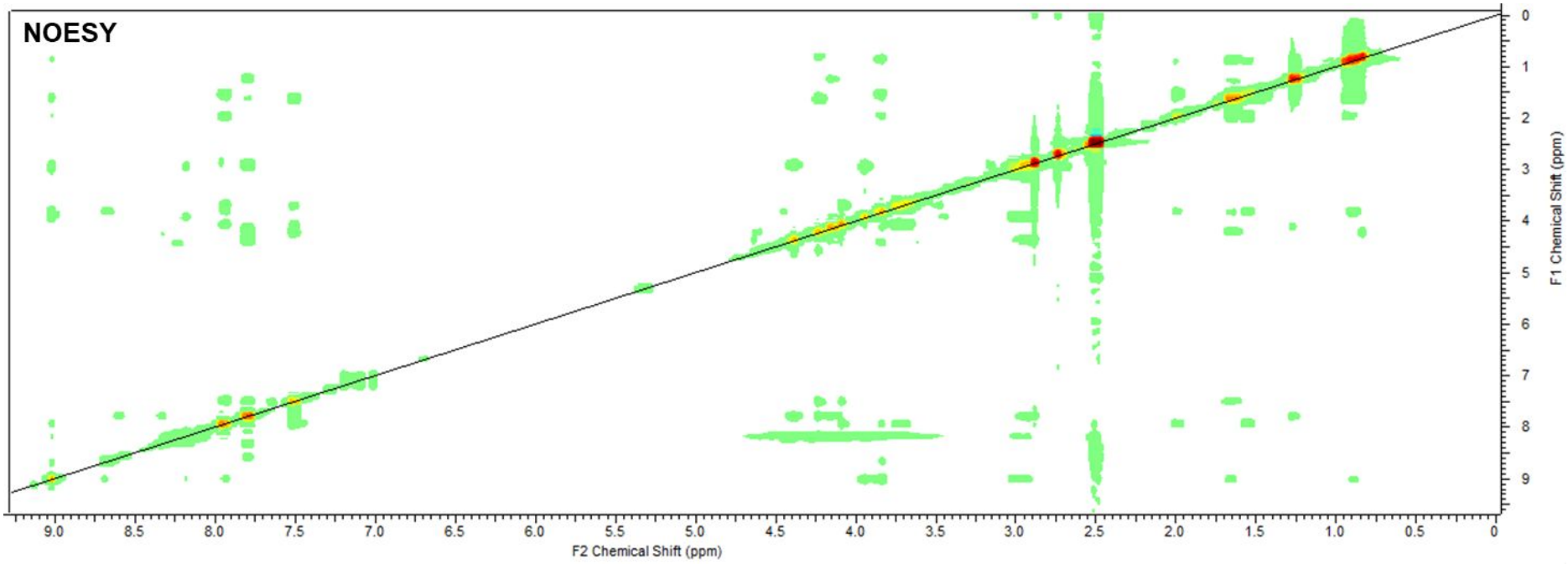
Table S13

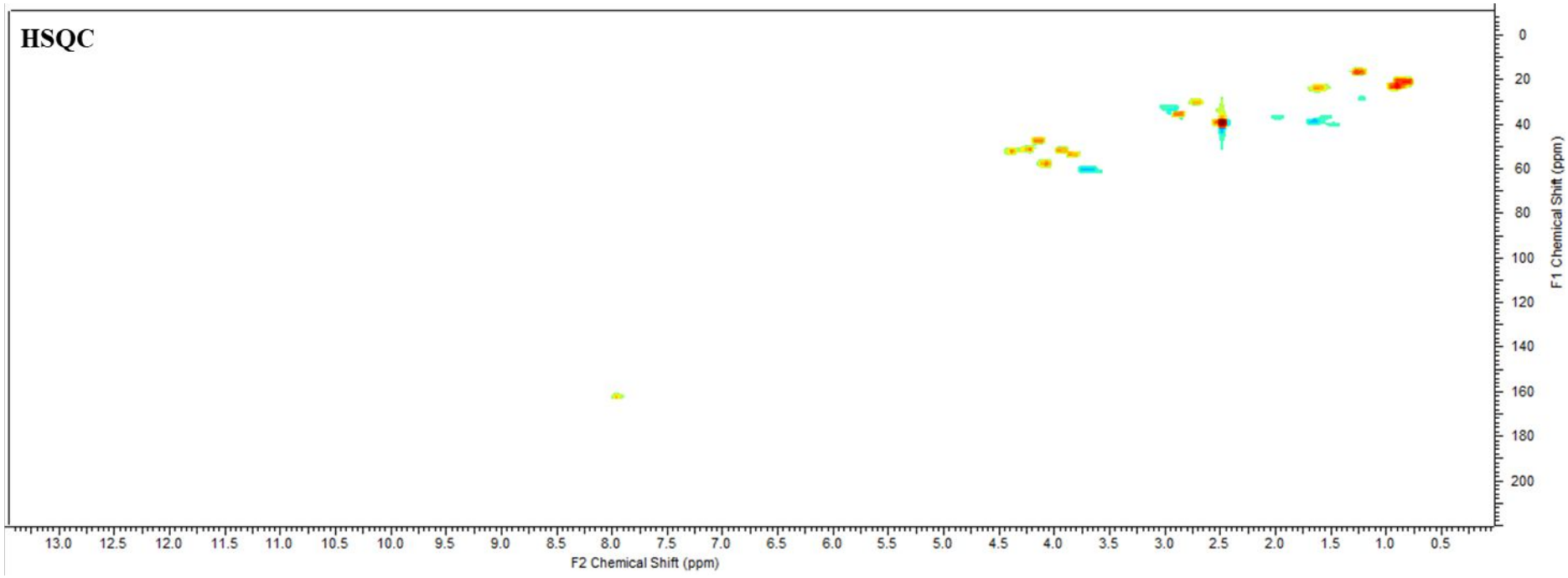
^{13}C					
Residue Number	**CO**	**α**	**β**	**γ**	**δ**
1 - Lan (Dha)	170.14	55.05	35.99		
2 - Leu	174.84	57.03	40.37	27.37	a - 26.37 b - 26.11
3 - Ser	173.32	61.05	63.63		
4 - Leu	174.92	54.66	42.29	28.30	a - 24.13 b - 24.57
5 - Lan (Cys)	172.27	55.45	37.54		
6 - Ala	176.80	50.83	20.23		


d6-DMSO
1H 600 MHz
Normalized Intensity
Chemical Shift (ppm)
0.82
1.99
1.08
1.78
0.90
1.15
1.27
1.18
1.14
0.96
1.09
1.00
0.98
0.83
1.17
3.35
9.49
3.34


COSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


TOCSY
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)


NOESY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


HSQC
0
20
40
60
80
100
120
140
160
180
200
F1 Chemical Shift (ppm)
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
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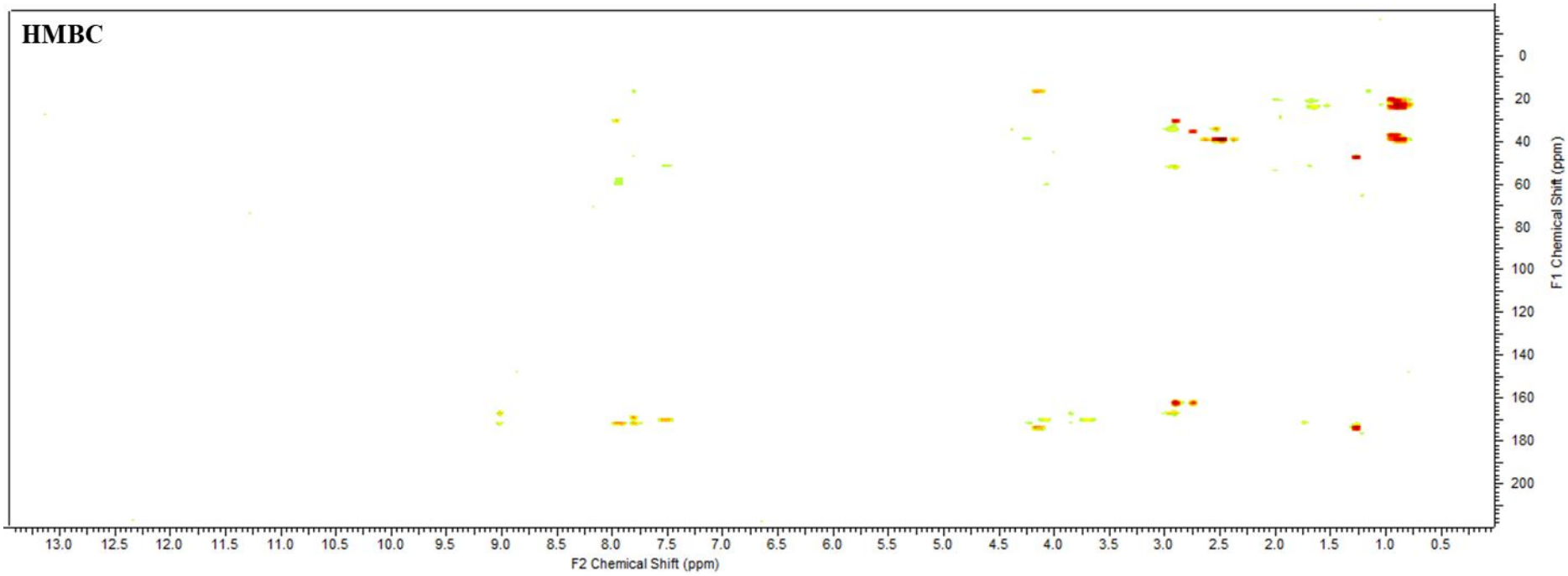
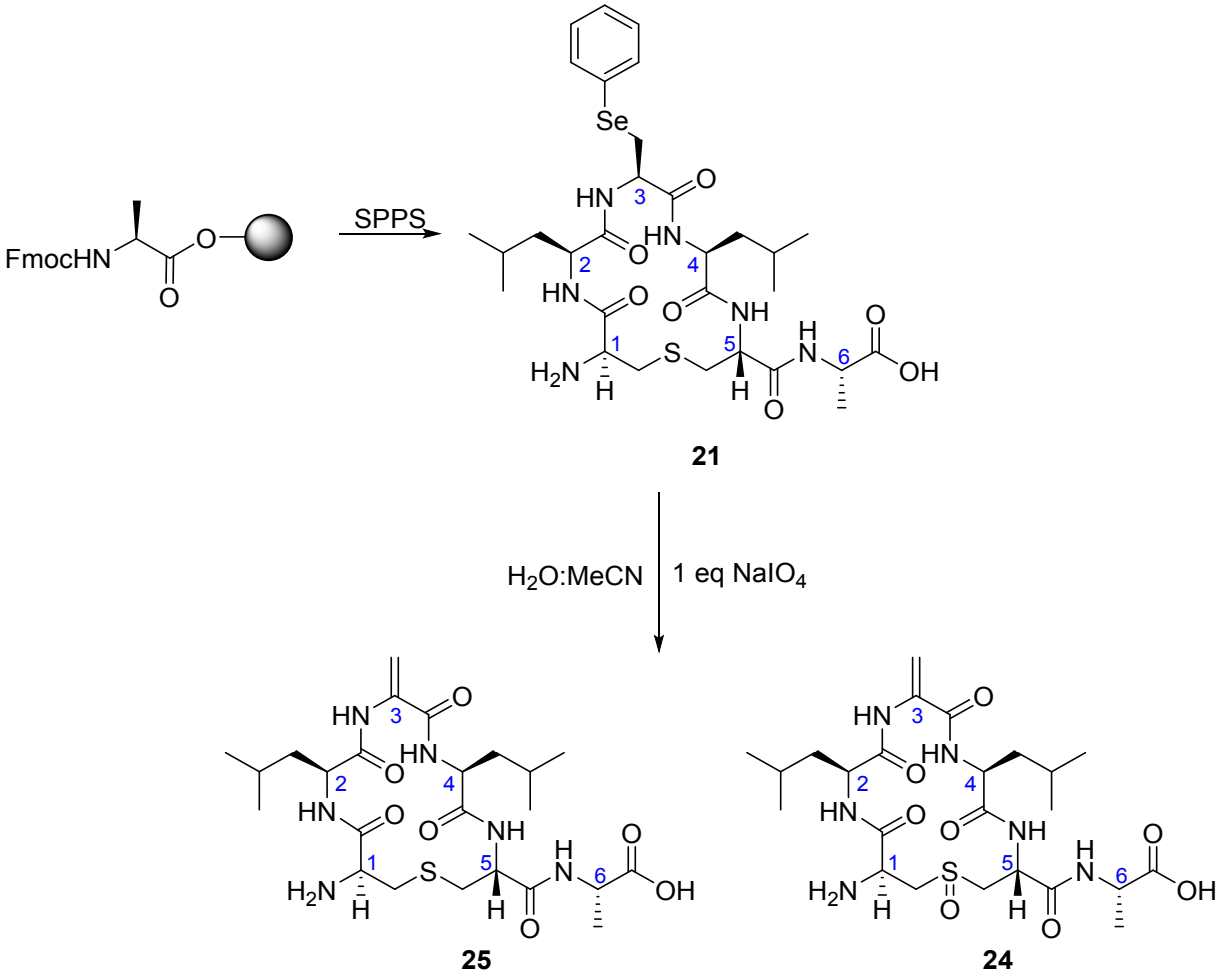
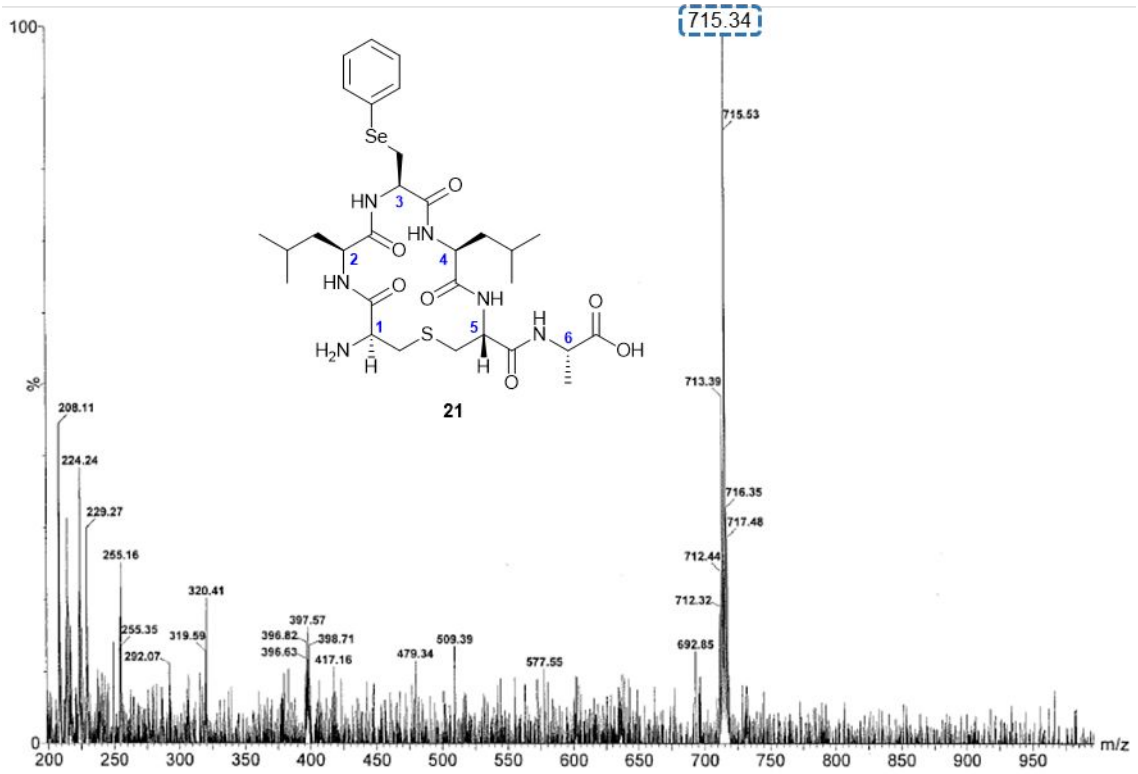
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)


HMBC
0
20
40
60
80
100
120
140
160
180
200
F1 Chemical Shift (ppm)
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)

Mutacin I Ring A Truncated WT (25) *via* Sec(Ph) Elimination

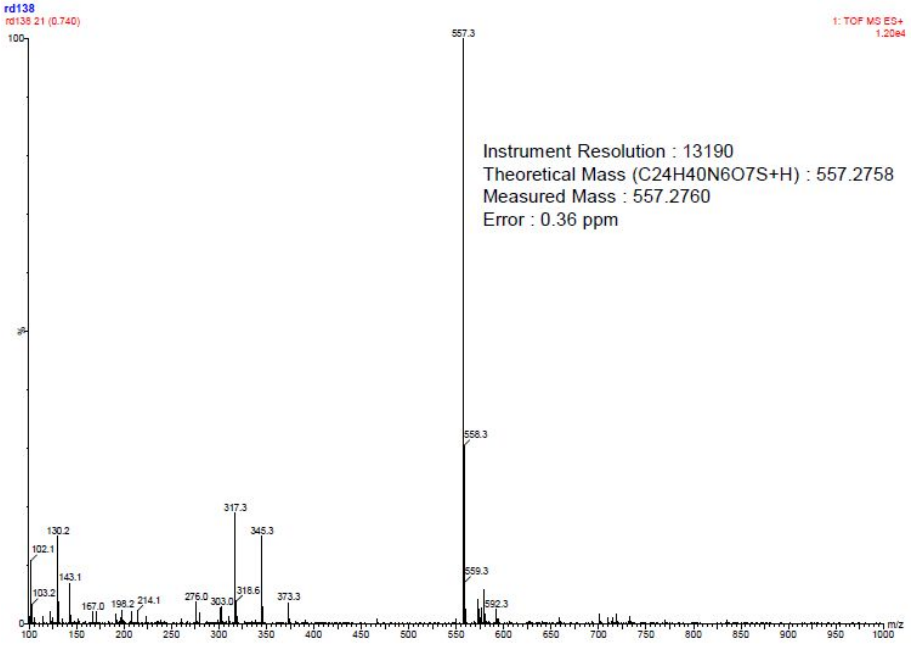


m/z (HRMS, ES+) required for $[C_{24}H_{40}N_6O_7S+H]^+$ 557.2758, found $[C_{24}H_{40}N_6O_7S+H]^+$ 557.2760.

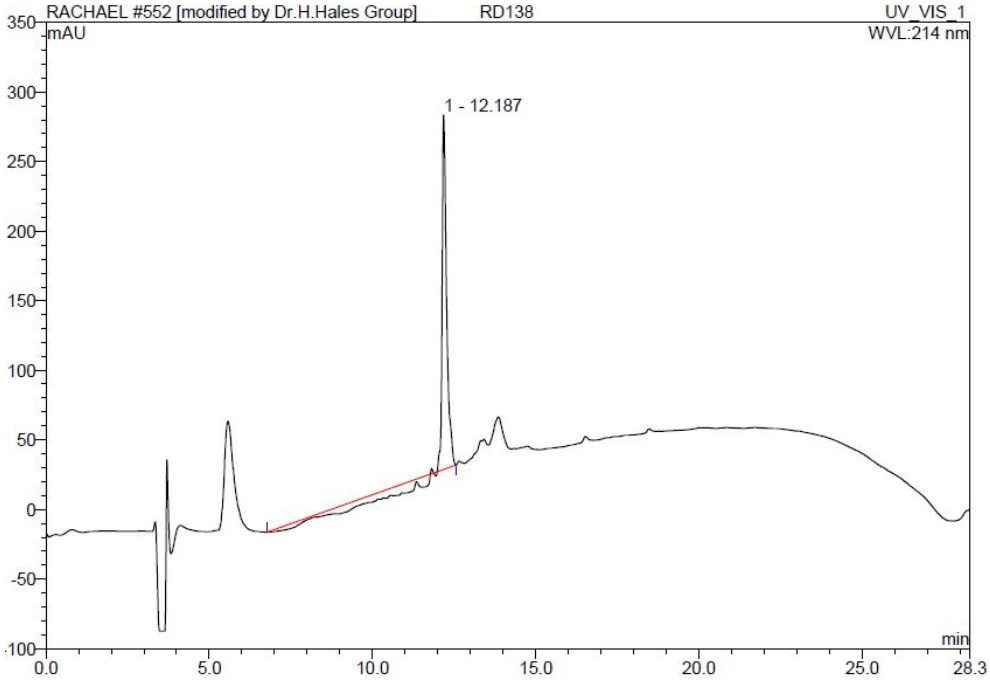


LC/MS of intermediate **21** – before attempted elimination

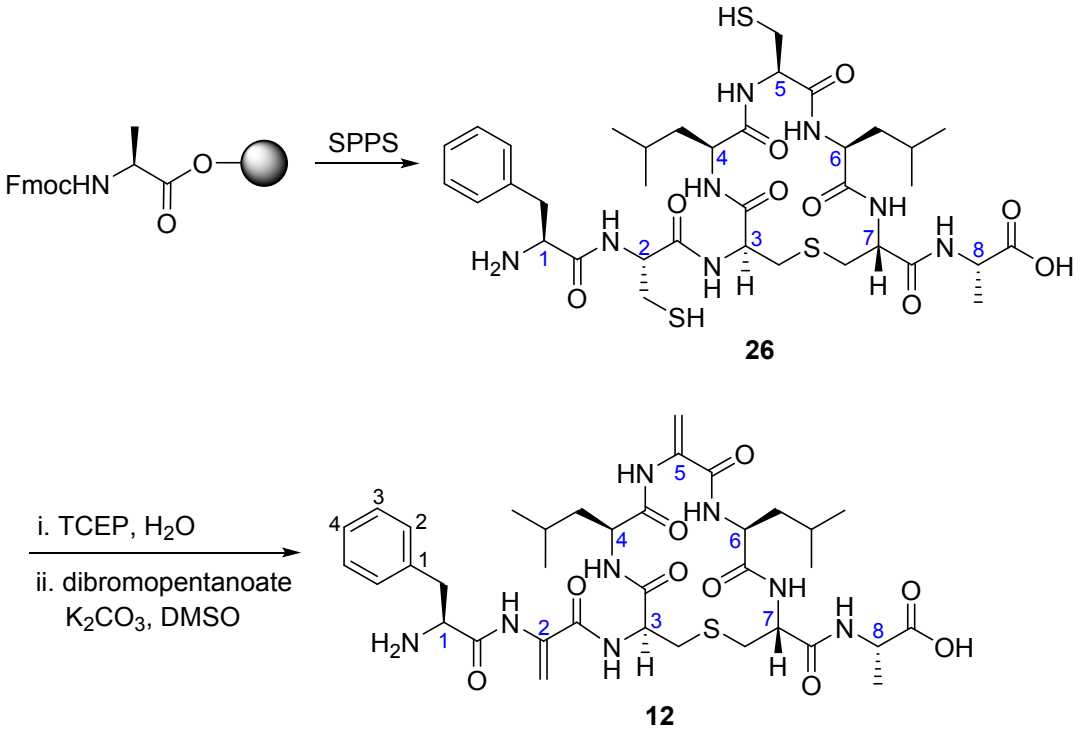
Characterisation data for **25**

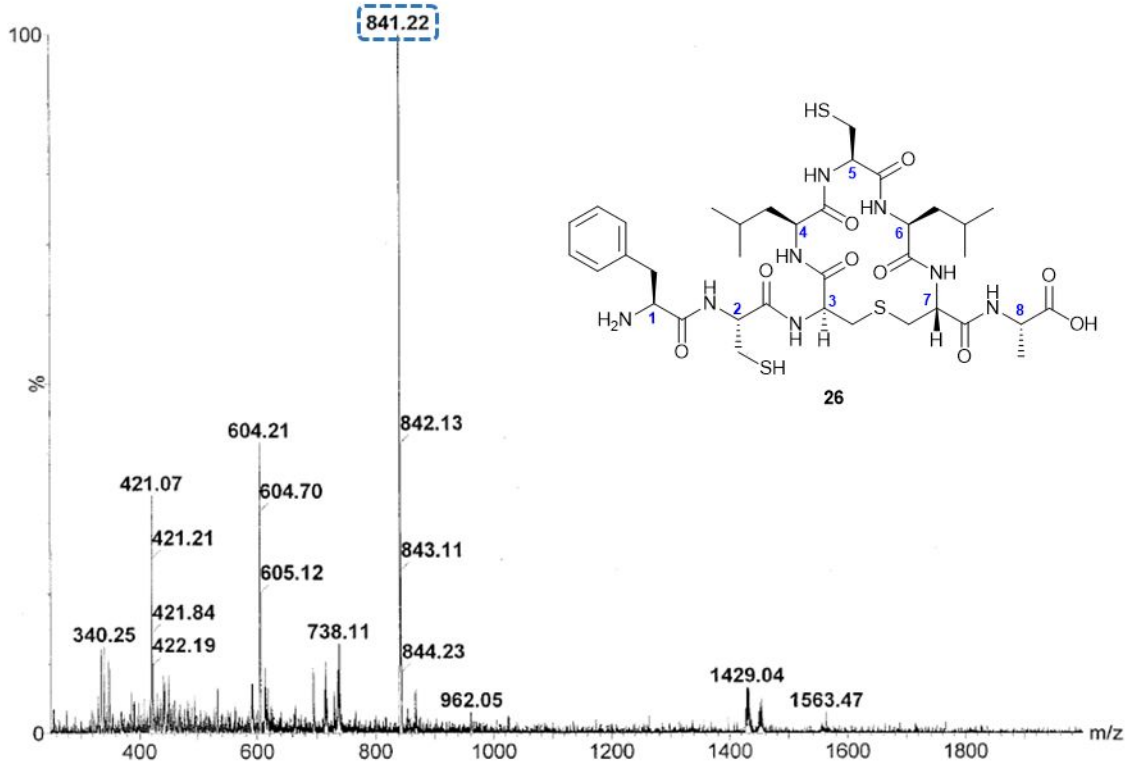


analytical HPLC (Dr Maisch GmbH Reprosil Gold 200 C8 5µm 250 x 4.6 mm column on Dionex HPLC system) retention time 12.19 min.



Mutacin I Ring A WT (12)

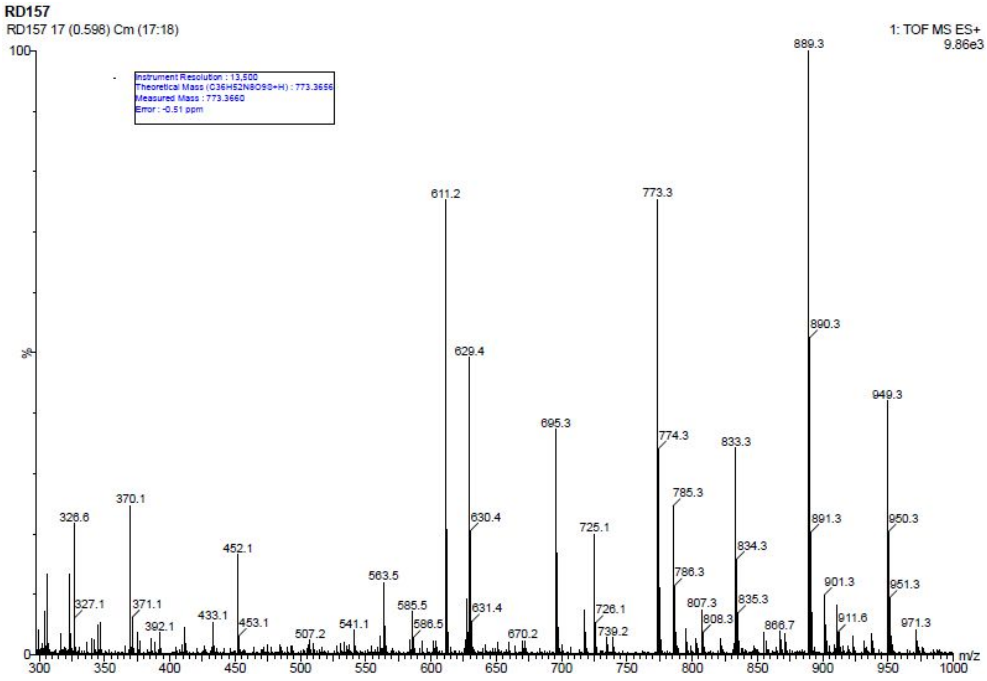




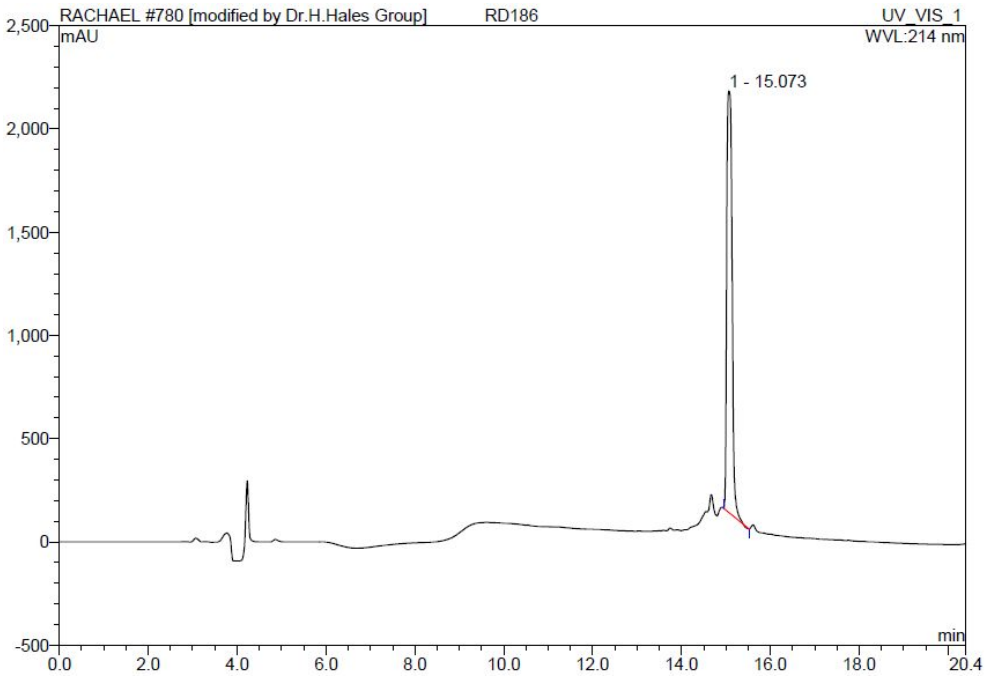
LCMS of intermediate **26** – after purification

Characterisation data for **12**

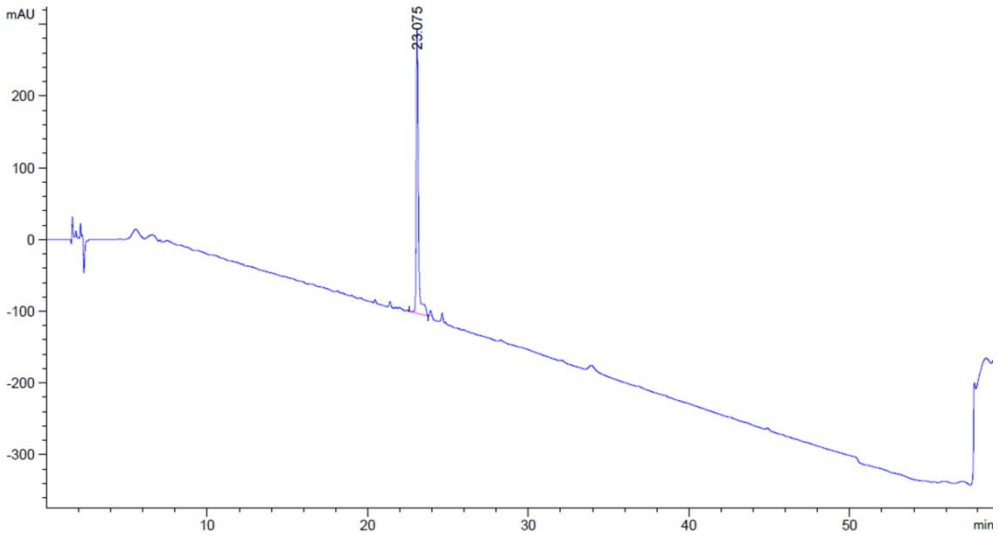
HRMS (ES+) m/z: $[M + H]^+$ Calcd for $C_{36}H_{53}N_8O_9S$ 773.3656; found 773.3660.



analytical HPLC (Dr Maisch GmbH Reprosil Gold 200 C8 5µm 250 x 4.6 mm column on Dionex HPLC system) retention time 15.07 min.



analytical HPLC (Gradient 5-95% acetonitrile (0.1% TFA) in H_2O (0.1% TFA) over 1h. Agilent Technologies 1260 Infinity HPLC system with ACE5 C18-300 150 x 4.6 mm column) retention time 23.1 min.



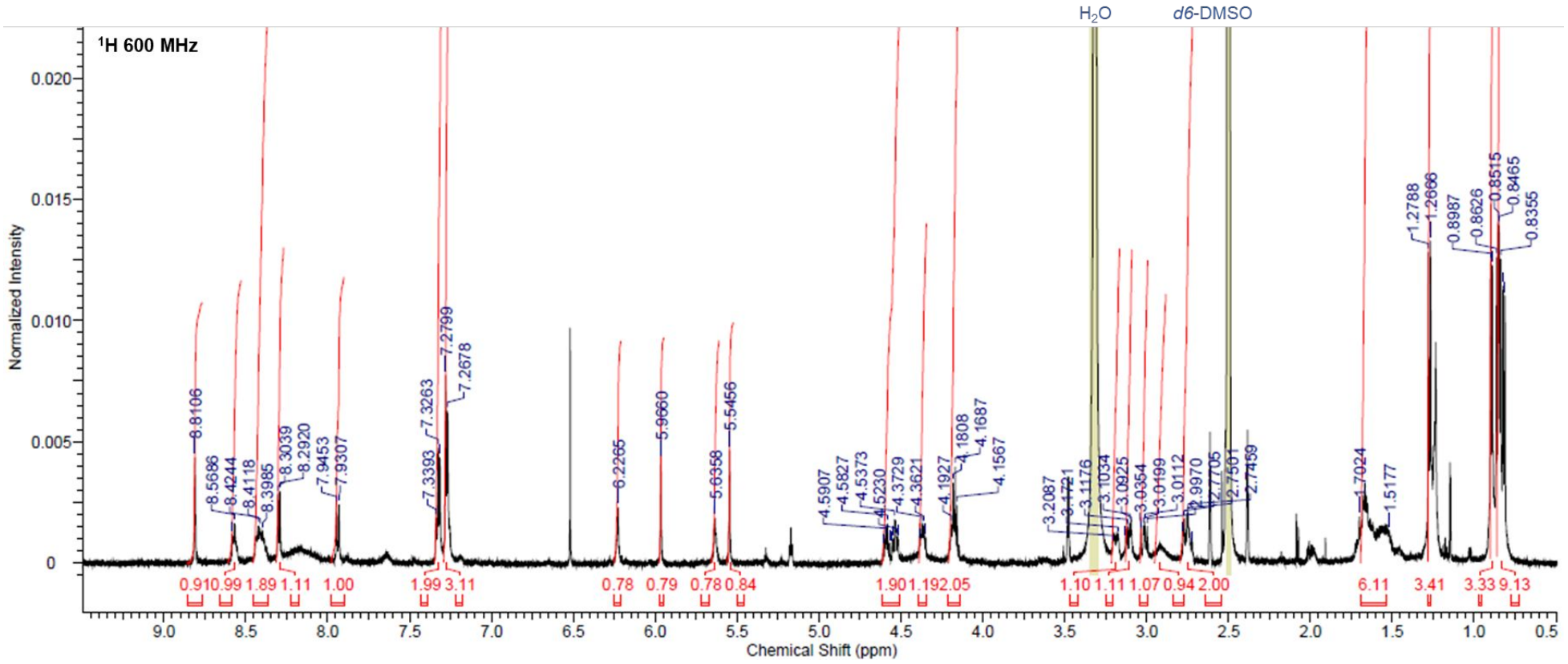
NMR δ_H (600 MHz, $(CD_3)_2SO$), δ_c (150 MHz, $(CD_3)_2SO$)

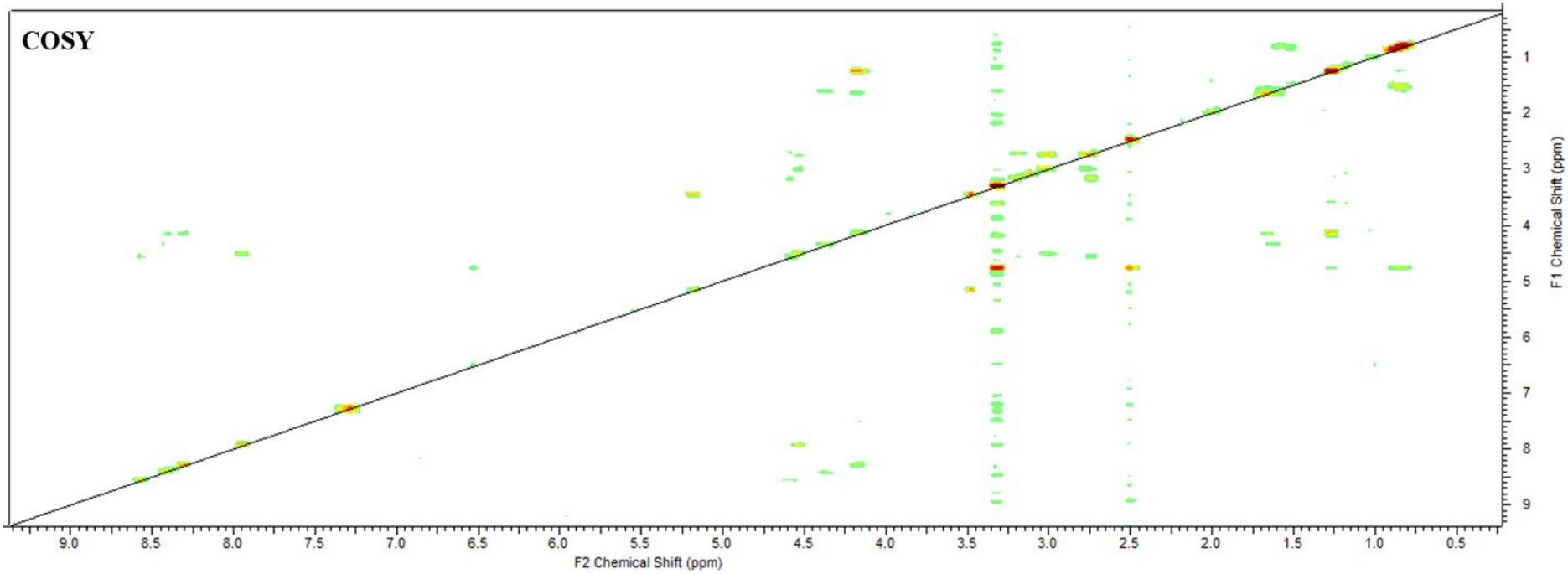
Table S14

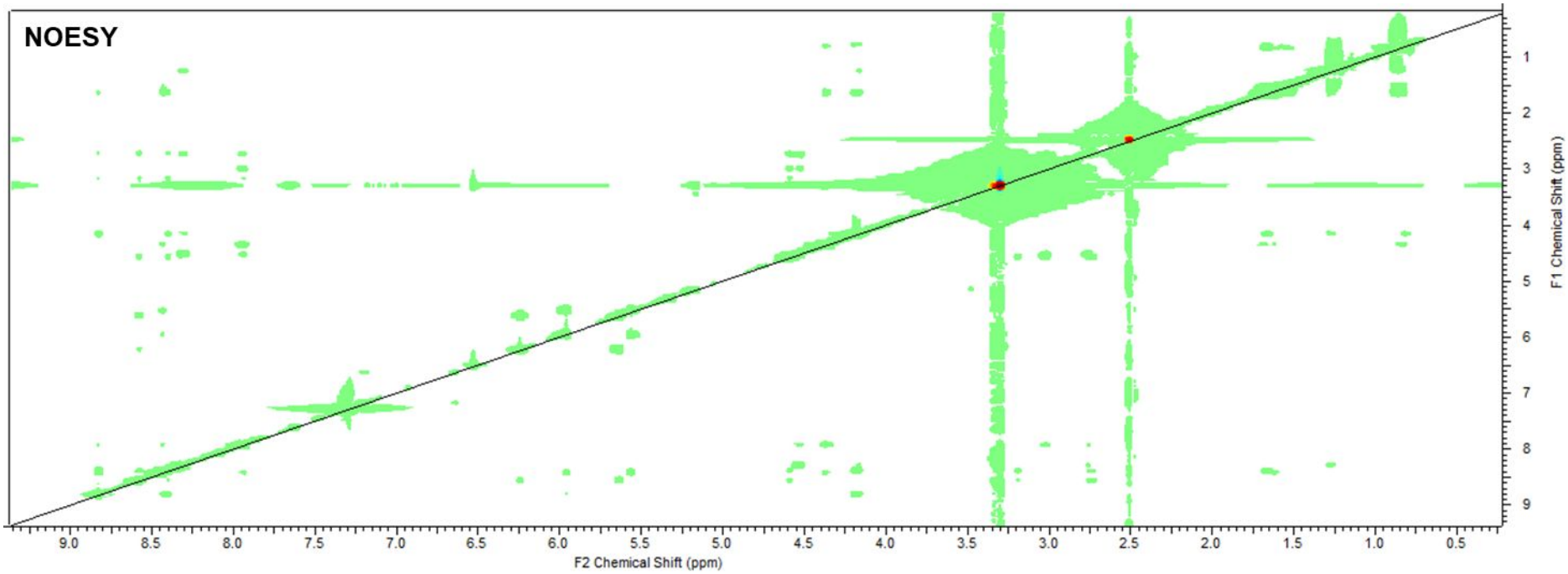
^1H							
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**Ar**	**Exchangeable**
1 - Phe		4.42	3.11			2 - 7.28 3 - 7.33 4 - 7.27	NH_2 - 8.19
2 - Dha	-		5.63 6.23				
3 - Lan (Dha)	8.57	4.59	2.75 3.19				
4 - Leu	8.40	4.18	1.67	1.58	a - 0.86 b - 0.81		
5 - Dha	8.81		5.54 5.97				
6 - Leu	8.43	4.36	1.65	1.52	a - 0.89 b - 0.85		
7 - Lan (Cys)	7.94	4.53	2.77 3.02				
8 - Ala	8.30	4.18	1.27				CO_2H - 12.56

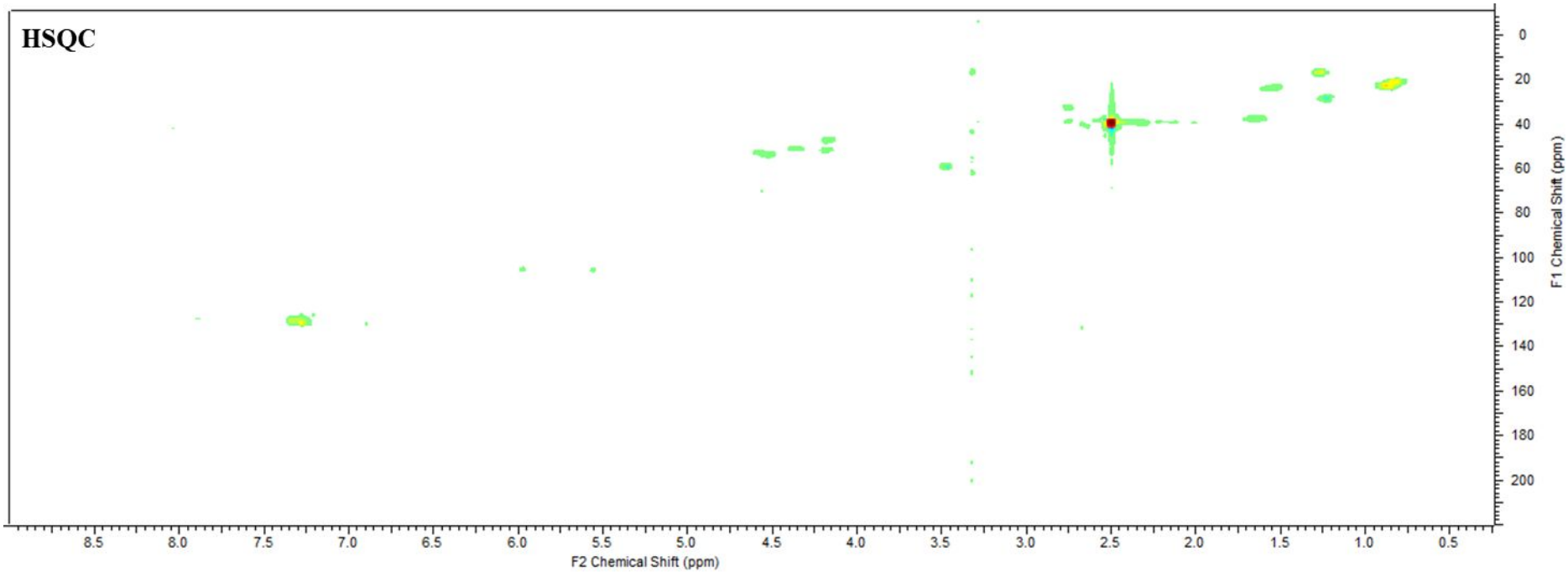
Table S15

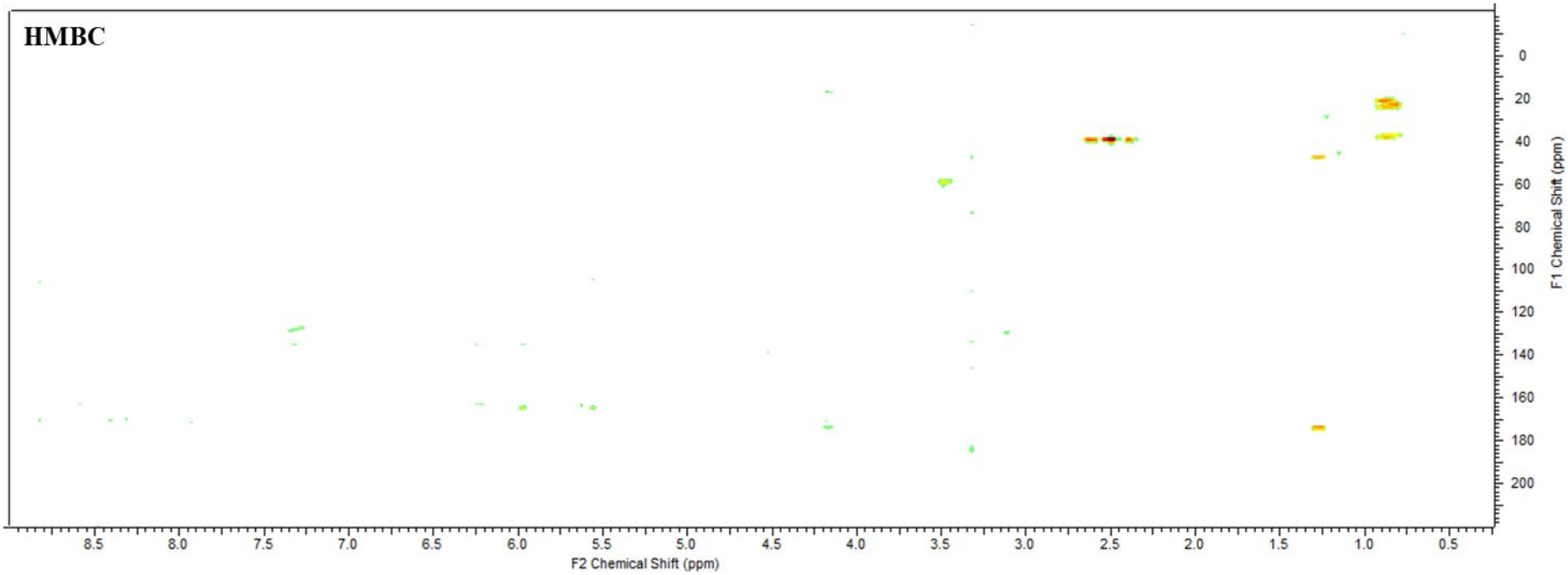
^{13}C						
Residue Number	**CO**	**α**	**β**	**γ**	**δ**	**Ar**
1 - Phe		55.35	53.27			1 - 132.57 2 - 132.45 3 - 131.61 4 - 130.23
2 - Dha	166.70	137.87	107.67			
3 - Lan (Dha)	173.46	56.07	36.35			
4 - Leu	173.72	55.11	40.82	27.51	a - 26.90 b - 24.69	
5 - Dha	167.85	138.09	108.92			
6 - Leu	174.75	54.29	41.60	27.23	a - 26.27 b - 24.30	
7 - Lan (Cys)	173.02	57.30	36.37			
8 - Ala	176.92	50.84	20.48			


^{1}H 600 MHz
H_2O
d6-DMSO
Normalized Intensity
Chemical Shift (ppm)
8.8106
8.5686
8.4244
8.4118
8.3985
8.3039
8.2920
7.9453
7.9307
7.3393
7.3263
7.2799
7.2678
6.2265
5.9660
5.6358
5.5456
4.5907
4.5827
4.5373
4.5230
4.3729
4.3621
4.1927
4.1808
4.1687
4.1567
3.2087
3.1721
3.1176
3.1034
3.0925
3.0354
3.0199
3.0112
2.9970
2.7705
2.7501
2.7459
1.7024
1.5177
1.2788
1.2666
0.8987
0.8626
0.8515
0.8465
0.8355
0.91 0.99 1.89 1.11 1.00
1.99 3.11
0.78 0.79 0.78 0.84
1.90 1.19 2.05
1.10 1.11 1.07 0.94 2.00
6.11
3.41
3.33 9.13

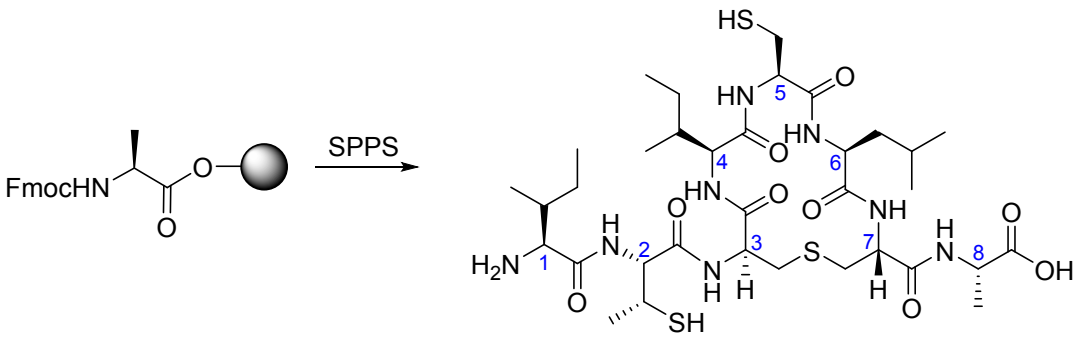

COSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

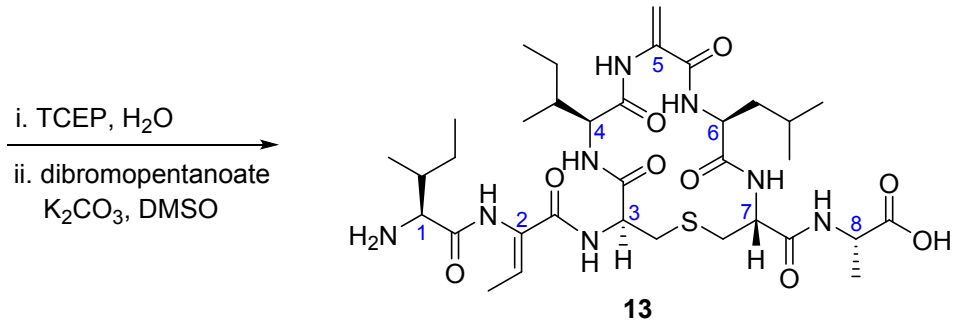

NOESY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

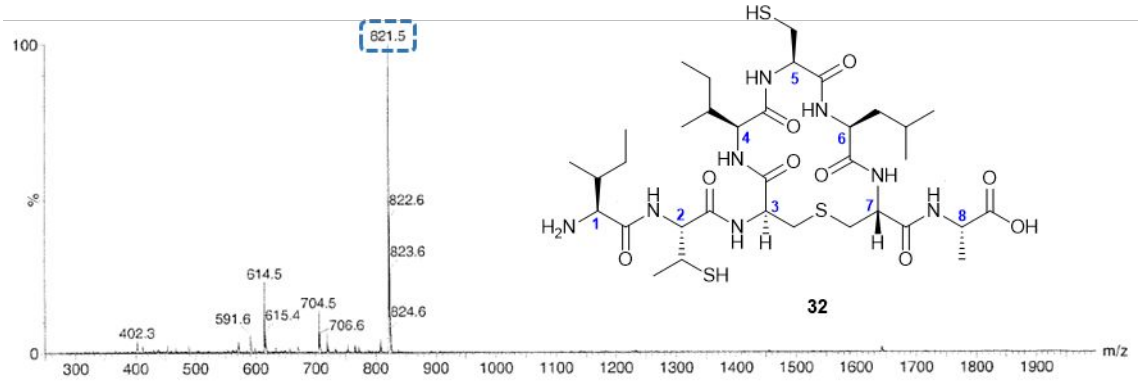

HSQC
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


HMBC
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

Nisin Ring A WT (13)



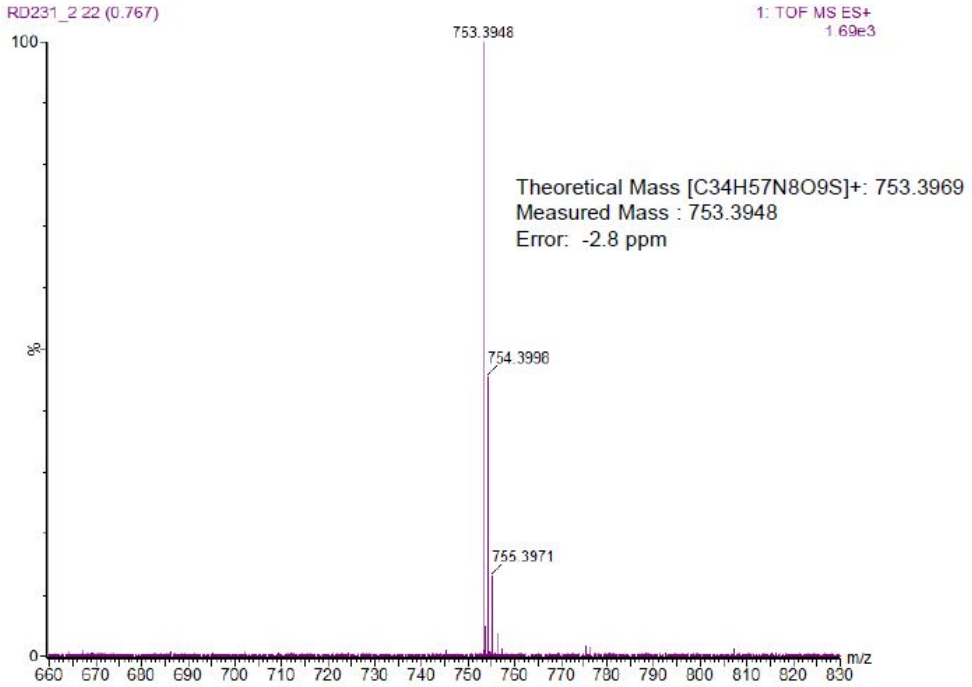




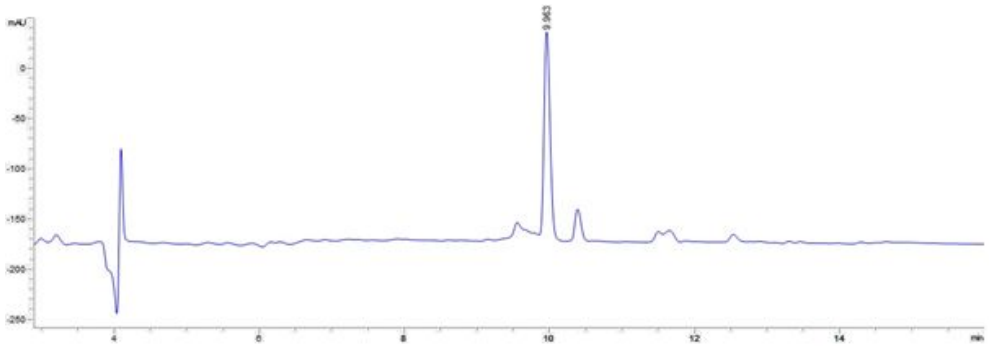
LCMS of intermediate **32**– after purification

Characterisation data for **13**

HRMS (ES+) m/z: $[M + H]^+$ Calcd for $C_{34}H_{57}N_8O_9S$ 753.3969; found 753.3948.



analytical HPLC (Dr Maisch GmbH Reprosil Gold 200 C8 5µm 250 x 4.6 mm column on Agilent HPLC system) retention time 9.96 min.



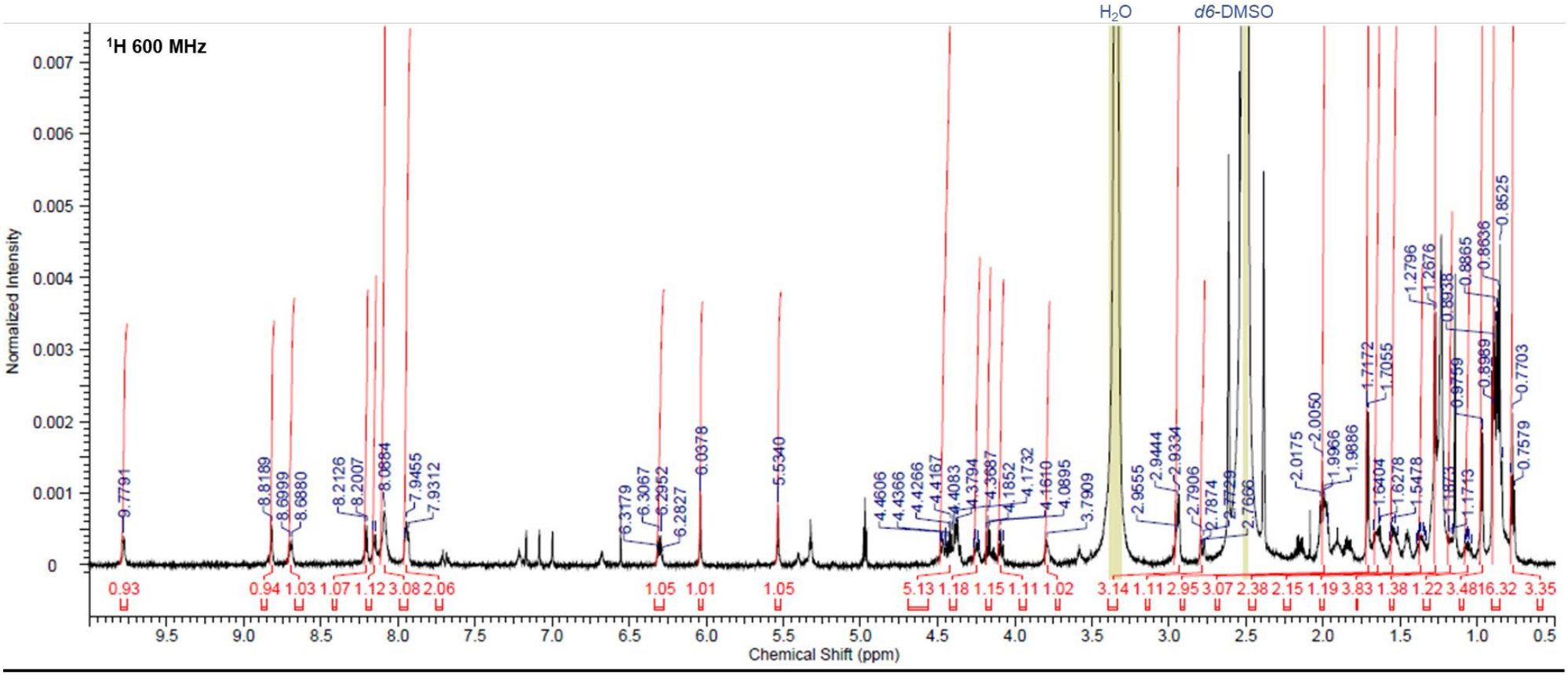
NMR δ_H (600 MHz, $(CD_3)_2SO$), δ_c (150 MHz, $(CD_3)_2SO$)

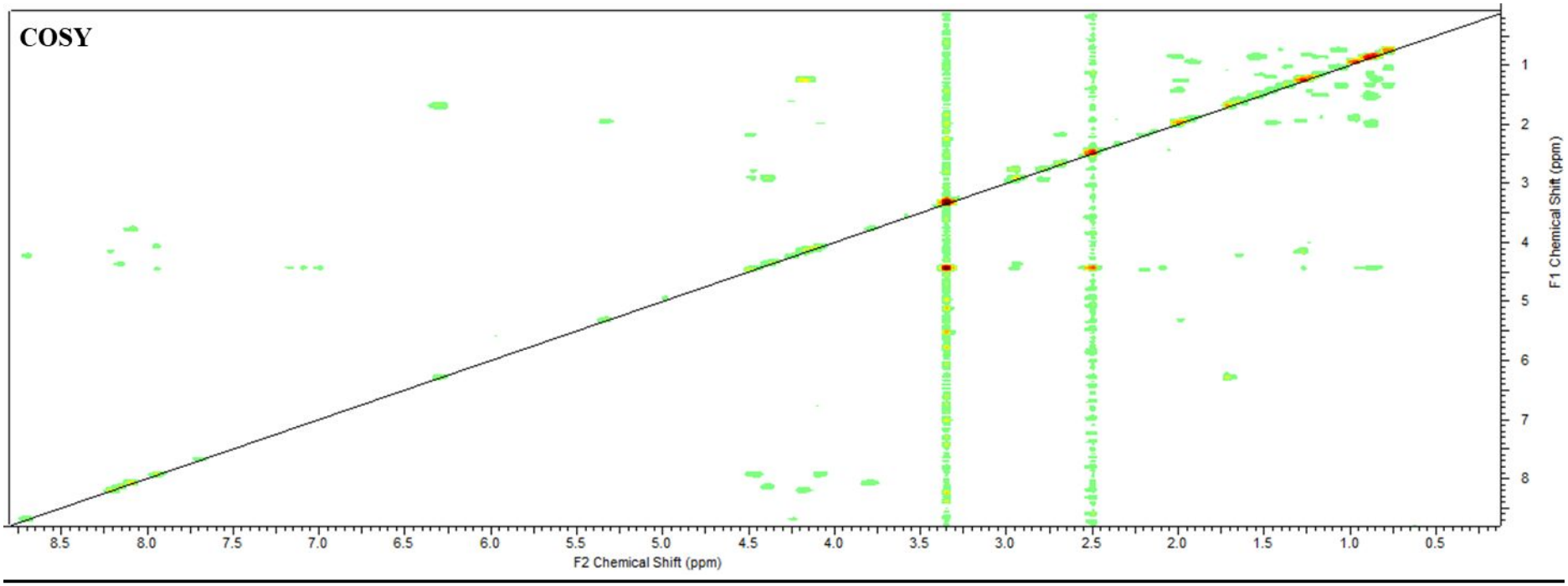
Table S16

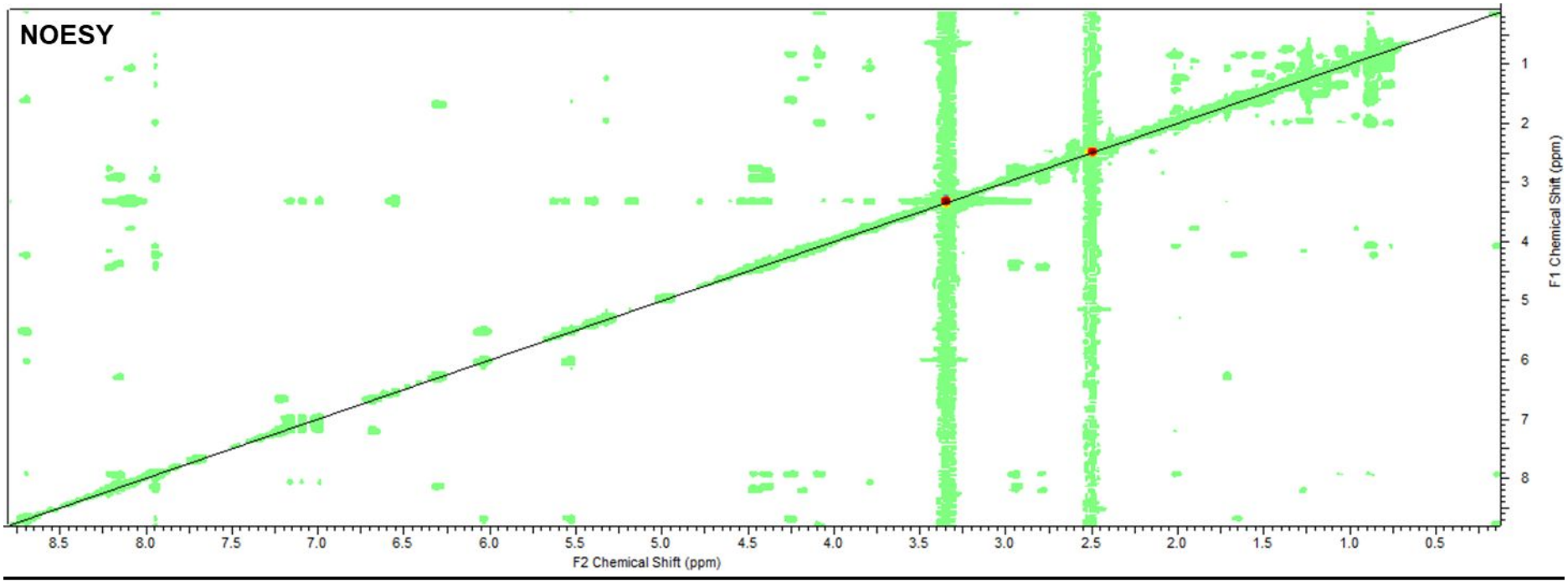
^{1}H						
Residue Number	**NH**	**α**	**β**	**γ**	**δ**	**Exchangeable**
1 - Ile		3.79	1.91	CH_2 - 1.18, 1.53 CH_3 - 0.97	0.89	NH_2 - 8.09
2 - Dhb	9.78		6.30	1.71		
3 - Lan (Dha)	8.15	4.38	2.94			
4 - Ile	7.94	4.09	2.00	CH_2 - 1.08, 1.38 CH_3 - 0.88	0.77	
5 - Dha	8.82		5.53 6.04			
6 - Leu	8.70	4.25	1.63	1.55	a - 0.86 b - 0.90	
7 - Lan (Cys)	7.94	4.47	2.81 2.95			
8 - Ala	8.21	4.17	1.27			CO_2H - 12.61

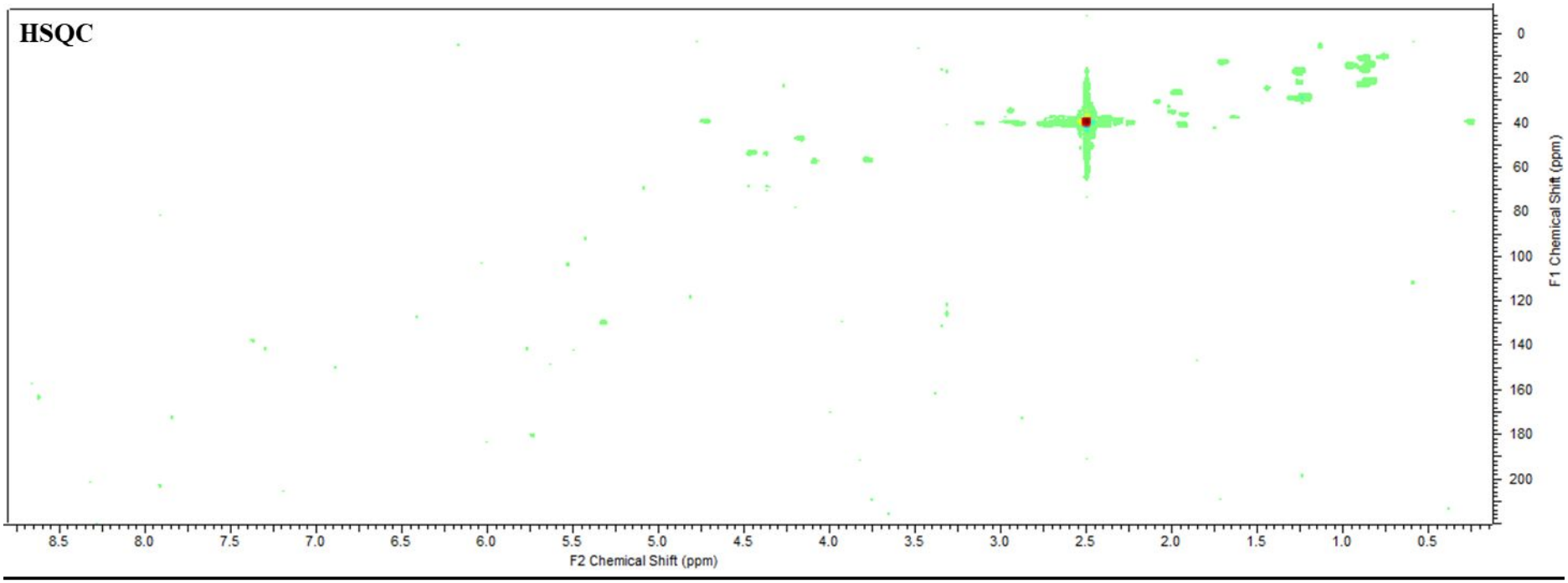
Table S17

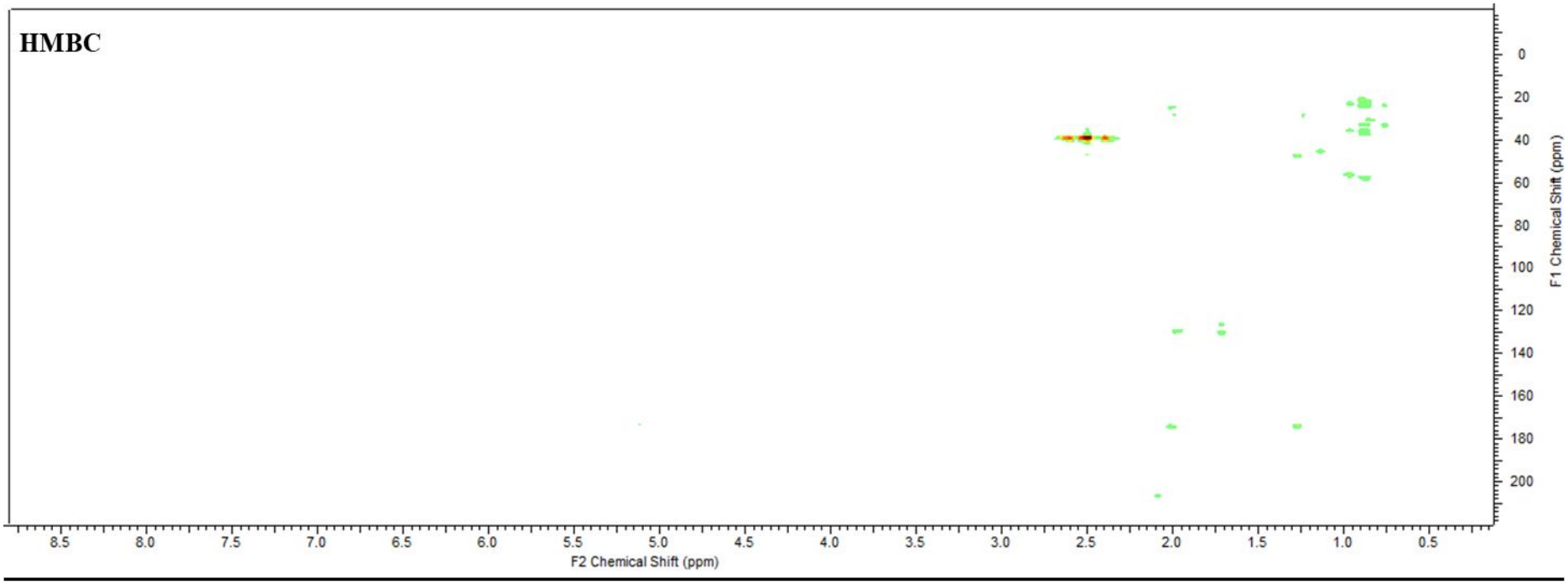
^{13}C					
Residue Number	**CO**	**α**	**β**	**γ**	**δ**
1 - Ile		59.89	39.33	CH_2 - 26.88 CH_3 - 14.61	17.82
2 - Dhb		133.38	129.68	16.56	
3 - Lan (Dha)	173.95	57.18	37.83		
4 - Ile	177.48	60.79	39.25	CH_2 - 27.63 CH_3 - 13.91	18.98
5 - Dha		132.79	107.03		
6 - Leu		55.18	41.04	40.86	a - 25.02 b - 26.36
7 - Lan (Cys)		56.93	43.26		
8 - Ala	176.99	50.72	20.35		


^{1}H 600 MHz
H_2O
d6-DMSO
Normalized Intensity
Chemical Shift (ppm)


COSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

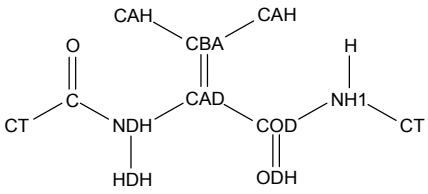

NOESY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

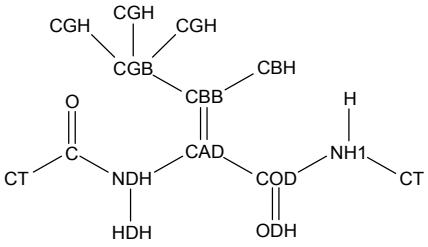

HSQC
0
20
40
60
80
100
120
140
160
180
200
F1 Chemical Shift (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)


HMBC
0
20
40
60
80
100
120
140
160
180
200
F1 Chemical Shift (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)

XPLOR-NIH Parameterisation Data

As it was necessary to have new atom types to define the dehydro residues, a key to these is provided:





The new topology required for Dha, Dhb and α-aminobutyric acid, as well as patches to create the lanthionine and methyllanthionine bridges, were added to the XPLOR protein.top file:

```
!
! xplor-nih protein topology file, version 1.0
!

residue DHA
  group
    atom N   type=NDH charge=-0.56 end
    atom HN  type=HDH charge= 0.30 end
  group
    atom CA  type=CAD charge= 0.26 end
  group
    atom CB  type=CBA charge=-0.46 end
    atom HB1 type=CAH charge= 0.23 end
    atom HB2 type=CAH charge= 0.23 end
  group
    atom C   type=COD charge= 0.60 end
    atom O   type=ODH charge=-0.60 end

  bond N  HN
  bond N  CA
  bond CA  CB   bond CB  HB1   bond CB  HB2
  bond CA  C
  bond C   O

  improper CA CB C  N
  improper CB CA HB1 HB2
  improper HB1 CB CA C
  improper HB2 CB CA N

end
```

```
residue DHB
  group
    atom N    type=NDH charge=-0.56 end
    atom HN   type=HDH charge= 0.30 end
 group
    atom CA   type=CAD charge= 0.26 end
 group
    atom CB   type=CBB charge=-0.17 end
    atom HB   type=CBH charge= 0.17 end
 group
    atom CG   type=CGB charge=-0.30 end
    atom HG1  type=CGH charge= 0.10 end
    atom HG2  type=CGH charge= 0.10 end
    atom HG3  type=CGH charge= 0.10 end
 group
    atom C    type=COD charge= 0.60 end
    atom O    type=ODH charge=-0.60 end

  bond N  HN
  bond N  CA
  bond CA CB     bond CB HB
  bond CB CG     bond CG HG1    bond CG HG2    bond CG HG3
  bond CA C
  bond C  O

  improper CA    CB     C     N
  improper CB    CA     HB    CG
  improper HB    CB     CA    N
  improper CG    CB     CA    C

  improper HG1 HG2 CB HG3  !stereo methyl
  dihedral CG CB  CA  N

end

residue MEL    !! Abu for MeLan
  group
    atom N    type=NH1 charge=-0.36 end
    atom HN   type=H   charge= 0.26 end
 group
    atom CA   type=CT  charge= 0.00 end
    atom HA   type=HA  charge= 0.10 end
 group
    atom CB   type=CT  charge=-0.20 end
    atom HB1  type=HA  charge= 0.10 end
    atom HB2  type=HA  charge= 0.10 end
 group
    atom CG   type=CT  charge=-0.30 end
    atom HG1  type=HA  charge= 0.10 end
    atom HG2  type=HA  charge= 0.10 end
```

```
    atom HG3  type=HA  charge= 0.10 end
 group
    atom C    type=C   charge= 0.48 end
    atom O    type=O   charge=-0.48 end

  bond N  HN
  bond N  CA     bond CA HA
  bond CA CB     bond CB HB1      bond CB HB2
  bond CB CG     bond CG HG1     bond CG HG2     bond CG HG3
  bond CA C
  bond C  O

  improper HA N  C   CB        !stereo CA
  improper HB1 HB2 CA CG       !stereo CB
  improper HG1 HG2 CB HG3      !stereo methyl

  dihedral CG  CB  CA  N

end

presidue DISU      ! lanthionine bridge  ...CYS - S - CYS...
  group
    delete    atom 1HG               end
    modify    atom 1CB  charge=-0.15  type=CT      end
    modify    atom 1SG  charge=-0.10  type=S       end
  group
    delete    atom 2HG               end
    delete    atom 2SG               end
    modify    atom 2CB  charge=-0.15  type=CT      end

  add bond 1SG 2CB

  add angle 1CB 1SG 2CB
  add angle 1SG 2CB 2CA
  add angle 1SG 2CB 2HB1
  add angle 1SG 2CB 2HB2

  add improper 2HB1 2HB2 2CA 1SG

  ADD DIHEdral 1SG 2CB 2CA 2N

end

presidue MDIS  ! methyllanthionine bridge ...MEL - S - CYS...
  group
    delete    atom 1HB2              end
    modify    atom 1CB   charge=-0.10  type=CT      end
  group
    delete    atom 2HG              end
    modify    atom 2SG  charge=-0.10  type=S       end
    modify    atom 2CB  charge=-0.10  type=CT      end
```

```
  add bond 1CB 2SG

  add angle 1CB 2SG 2CB
  add angle 1CA 1CB 2SG
  add angle 2SG 1CB 1HB1
  add angle 2SG 1CB 1CG

  add improper 1HB1 1CA 2SG 1CG

  ADD DIHEdral 1CB 2SG 2CB 2CA

end
```

The required bond lengths, angles, impropers and nonbonded approximations for Dha and Dhb were added to the XPLOR protein.par file under the relevant sections:

```
!
! xplor-nih protein parameter file, version 1.0
!

! BONDS
!
! Bonds for Dha/Dhb
bond      CAD  CBA                $kbon     1.34
bond      CAD  CBB                $kbon     1.34
bond      CAD  COD                $kbon     1.489
bond      CAD  NDH                $kbon     1.39
bond      COD  NH1                $kbon     1.345
bond      C    NDH                $kbon     1.345
bond      NDH  HDH                $kbon     0.98
bond      COD  ODH                $kbon     1.231
bond      CBA  CAH                $kbon     1.08
bond      CBB  CBH                $kbon     1.08
bond      CBB  CGB                $kbon     1.53
bond      CGB  CGH                $kbon     1.08

! ANGLES
!
! Angles for Dha/Dhb
angle     CBA  CAD  COD           $kang     120.0
angle     CBB  CAD  COD           $kang     120.0
angle     NDH  CAD  CBA           $kang     128.0
angle     NDH  CAD  CBB           $kang     128.0
angle     NDH  CAD  COD           $kang     110.0
angle     CAD  CBA  CAH           $kang     120.5
angle     CAD  COD  ODH           $kang     122.5
angle     HDH  NDH  CAD           $kang     117.0
```

```
angle     CAD  CBB  CGB          $kang     126.5
angle     CT   C    NDH          $kang     116.5
angle     O    C    NDH          $kang     122.5
angle     C    NDH  HDH          $kang     123.0
angle     C    NDH  CAD          $kang     120.0
angle     CAD  COD  NH1          $kang     116.5
angle     ODH  COD  NH1          $kang     122.5
angle     COD  NH1  H            $kang     123.0
angle     COD  NH1  CT           $kang     120.0
angle     CAH  CBA  CAH          $kang     120.0
angle     CGH  CGB  CGH          $kang     109.5
angle     CBH  CBB  CGB          $kang     120.0
angle     CAD  CBB  CBH          $kang     120.0
angle     CBB  CGB  CGH          $kang     109.5

! IMPROPERS
!
! Impropers for Dha/Dhb
improper  CGH  CGH  CGB  CGH      $kchi    0   -66.514 ! Dhb
methyl
improper  HDH  NDH  CAD  COD      $kpla    0    0.0
improper  CBA  CAD  NDH  HDH      $kpla    0  180.0
improper  NDH  CAD  CBB  CBH      $kpla    0  180.0
improper  NDH  CAD  CBB  CGB      $kpla    0    0.0
improper  HDH  NDH  CAD  CBB      $kpla    0  180.0
improper  CBH  CBB  CAD  NDH      $kpla    0  180.0
improper  CT   C    NDH  HDH      $kpx     0    0.0
improper  CAD  COD  NH1  H        $kpx     0    0.0
improper  O    C    NDH  HDH      $kpla    0  180.0
improper  O    C    NDH  CAD      $kpx     0    0.0
improper  ODH  COD  NH1  H        $kpla    0  180.0
improper  ODH  COD  NH1  CT       $kpx     0    0.0
improper  CAD  COD  NH1  CT       $kpx     0  180.0
improper  COD  CAD  NH1  CT       $kback   0  180.0  !  cis
pep bond
improper  COD  CAD  CBA  CAH      $kpla    0  180.0
improper  NDH  CAD  CBA  CAH      $kpla    0  180.0
improper  C    NDH  CAD  COD      $kpla    0  180.0
improper  CBB  CAD  COD  ODH      $kpla    0  180.0
improper  CBA  CAD  COD  ODH      $kpla    0  180.0
improper  NH1  CT   C    NDH      $kpla    0  180.0
improper  NH3  CT   C    NDH      $kpla    0  180.0
improper  CT   C    NDH  CAD      $kpx     0  180.0
improper  C    CT   NDH  CAD      $kback   0  180.0  !  cis
pep bond
improper  CGB  CBB  CAD  COD      $kpla    0  180.0
improper  NDH  CAD  COD  ODH      $kpla    0    0.0
improper  C    NDH  CAD  CBA      $kpla    0    0.0
improper  C    NDH  CAD  CBB      $kpla    0    0.0
improper  NDH  CAD  COD  NH1      $kpla    0  180.0
improper  COD  NH1  CT   C        $kpla    0  180.0
```

```
improper  CBB  CAD  COD  NH1      $kpla    0    0.0
improper  CBA  CAD  COD  NH1      $kpla    0    0.0
improper  CAD  CBB  CGB  CGH      $kpla    0    0.0   !  dhb
C(O)-gammaH eclipsed
improper  COD  CAD  NDH  ODH      $kpla    0    0.0      !
planar around CO
improper  C    CT   O    NDH      $kpla    0    0.0      !
planar around CO
improper  NDH  C    HDH  CAD      $kpla    0    0.0      !
planar around NH
improper  CAD  NDH  COD  CBA      $kpla    0    0.0
improper  CBA  CAH  CAH  CAD      $kpla    0    0.0
improper  COD  NH1  ODH  CAD      $kpla    0    0.0
improper  NH1  COD  H    CT       $kpla    0    0.0
improper  CAD  NDH  CBA  COD      $kpla    0    0.0
improper  COD  CAD  ODH  NH1      $kpla    0    0.0
improper  COD  CAD  NH1  ODH      $kpla    0    0.0
improper  CAD  CBA  COD  NDH      $kpla    0    0.0
improper  CAD  CBB  COD  NDH      $kpla    0    0.0
improper  CGH  CGH  CBB  CGH      $kchi    0  -66.514     !
betaC-gammaH
improper  CBA  CAD  CAH  CAH      $kpla    0    0.0
improper  CAD  CBA  COD  NH1      $kpla    0    0.0
improper  CAD  CBB  COD  NH1      $kpla    0    0.0
improper  CBB  CAD  CBH  CGB      $kpla    0    0.0
improper  COD  NH1  CBA  CAH      $kpla    0    0.0

!NONBONDED
!
!nonbonded  approximations  based  on  existing  types  in
protein.par (C=C of Dh approximated as CA)
nonbonded  NDH     0.1592   2.7618      0.1592   2.7618
nonbonded  ODH     0.2342   2.6406      0.2342   2.6406
nonbonded  COD     0.0903   3.2072      0.0903   3.2072
nonbonded  HDH     0.0498   1.4254      0.0498   1.4254
nonbonded  CGH     0.0045   2.6157      0.0045   2.6157
nonbonded  CAH     0.0045   2.6157      0.0045   2.6157
nonbonded  CBH     0.0045   2.6157      0.0045   2.6157
nonbonded  CAD     0.120    3.2072      0.120    3.2072
nonbonded  CBA     0.120    3.2072      0.120    3.2072
nonbonded  CBB     0.120    3.2072      0.120    3.2072
nonbonded  CGB     0.0903   3.2072      0.0903   3.2072
```

Structure Statistics

Statistics were generated by the Protein Structure Validation Suite (PSVS).[2]

Percentage of residues in Ramachandran disallowed regions is from Procheck analysis.[3]

Table S18

	Mutacin I ring B		Nisin ring B (2)	Nisin ring B Lan analogue (3)	Mutacin I ring A (12)	Mutacin I ring A (Ser5) analogue (15)	Mutacin I ring A (Ser2, Ala5, Ala8) analogue (14)	Nisin ring A (13)
	Major (1)	Minor (1)						
Distance Restraints								
Inter-residue NOE								
i - i+1	11	3	15	8	32	19	22	11
i – i>1	7	1	10	7	7	5	3	3
Dihedral Angles								
Backbone	5	4	3	3	3	5	5	4
Structure Statistics								
Pairwise RMSD (Å)								
Heavy atoms	0.6	1.2	0.8	0.8	1.2	0.8	1.8	1.7
Backbone atoms	0.1	0.6	0.2	0.2	0.5	0.3	1.2	1.0
RMSD from ideality								
Bond length (Å)	0.007	0.005	0.010	0.010	0.010	0.004	0.007	0.005
Bond angle (°)	1.7	1.5	1.5	1.5	1.9	1.7	1.5	1.5
% of residues in Ramachandran disallowed regions	0	0	0	0	0	0	6.7	6.7

Dihedral Angle Data

Table S19. Measured dihedral angles for ring B peptides.

	i + 1[a,b]		i + 2[a,c]		Cα(i) - Cα(i+4) Distance (Å)
	φ	ψ	φ	ψ	
WT Nisin B (**2**)	-127	35	5 / -115	18 / -102	3.66
WT Mutacin I B (major) (**1**)	-120	132	31 / 152	39 / -81	4.29
WT Mutacin I B (minor) (**1**)	-111	-18	-42 / -161	-56 / 63	4.68
Nisin B Lan analogue (**3**)	-140	9	24 / -96	12 / -108	4.24
PDB: 1WCO	-114	26	-26/-145	44/-75	4.61

[a] i + 1 = Pro/Leu, i + 2 = Gly.

[b] φ dihedrals for Pro were measured to Cβ i.e. Cβ-N-Cα-Hα.

[c] Two values are given as dihedrals were measured to both Hα.

Table S20. Measured dihedral angles for ring A peptides.

	i + 1[a]		i + 2[a,b]		i + 3[a]		Cα(i) - Cα(i+5) Distance (Å)
	φ	ψ	φ	ψ	φ	ψ	
WT Nisin A (**13**)	-123	32	-125	-82	155	169	5.02
WT Mutacin I A (**12**)	-153	47	-148	87	77	16	4.64
Mutacin I A (Ser5) analogue (**15**)	-89	26	-153	6	-140	19	4.03
Mutacin I A (Ser2, Ala5, Ala8) analogue (**14**)	174	-8	157	40	159	113	4.61
PDB: 1WCO	-172	41	-122	-112	-157	135	4.74

[a] i + 1 = Ile/Leu, i + 2 = Dha/Ser/Ala, i + 3 = Leu.

[b] Dihedrals for Dha were measured to Cβ i.e. φ = NH-N-Cα-Cβ, ψ = CO-C-Cα-Cβ.

References

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