**Supplementary tables and figures:**

**Fig. S1**. Molecular network of 176 extracts from 88 *Actinoallomurus* strains, encompassing 725 features (=nodes). 384 features were organized in 52 molecular families. Pink nodes indicate features present in extract from *Actinoallomurus* ID145808 only. Enlargement shows selected interesting signals from ID145808.

**Fig. S2**. A) Extracted ion chromatograms of *m/z* 391, 851-868, 931-948, 1205-1222 and 1285-1302 $[M+H]^+$ from LC-MS analysis of the starting allopeptimicin complex (black line) and the reaction with 0.5N NaOH for 1h (red line). B) MS/MS at 4.9, 6.3-6.5 and 7.0-7.4 min of the hydrolysis mixture. C) Extracted ion chromatograms of *m/z* 1205-1233 and 1285-1314 $[M+H]^+$ from LC-MS analysis of the starting allopeptimicin complex (black line) and the reaction with 0.81 M CH3NH2 for 10 min (red line). D) MS/MS at 6.7-7.0 and 7.7-8.2 minutes from the amidation reaction. See Material section for the reaction conditions.

**Fig. S3**. $^1$H NMR (300 MHz, $CD_3CN$:$D_2O$:$H_2O$ 4:1:0.2) of allopeptimicins A.

**Fig. S4.** $^{13}$C NMR (75 MHz, $CD_3CN$:$D_2O$:$H_2O$ 4:1:0.2) of allopeptimicins A.

**Fig. S5**. $^1$H-$^{13}$C HSQC NMR (300 MHz, $CD_3CN$:$D_2O$:$H_2O$ 4:1:0.2) of allopeptimicins A together with a selected olefinic region enlarged.

**Fig. S6**. A) Overlap of $^1$H-$^{13}$C HSQC (blue/green) and $^1$H-$^{13}$C HMBC (red) NMR (300 MHz, $CD_3CN$:$D_2O$:$H_2O$ 4:1:0.2) of allopeptimicins A. Red box highlights HMBC correlation of the proton belonging to the methyl group attached to C2 and the carbons of C2 and C3. B) Enlargement of the region including the HMBC correlation between the proton at C19 and the carbons at C20 and C18.

**Fig. S7**. A) Overlap of $^1$H-COSY (red) and $^1$H-TOCSY (blue) NMR (300 MHz, $CD_3CN$:$D_2O$:$H_2O$ 4:1:0.2) of allopeptimicin A. B) Zoom in of the region including the COSY correlation between the proton at C19 and the methyl at C20 and the proton at C19 and the methylene at C18.

**Fig. S8**. A) Structure of allopeptimicin A3. COSY correlations are highlighted in blue. The arrows show the main NOESY correlations which are color coded following the signals highlighted in panel B. B) NOESY NMR (300 MHz, $CD_3CN$:$D_2O$:$H_2O$ 4:1:0.2) of allopeptimicins A.

**Fig. S9**. J-res NMR spectrum of allopeptimicins A. The red circle highlights the J-coupling relative to C14 and C15.

**Fig. S10**. HR-MSMS spectrum of the alkaline hydrolysis product with *m/z* 391.2046 $[M+H]^+$ and proposed fragment assignment.

**Fig. S11**. NMR spectra of the alkaline hydrolysis product with *m/z* 391 $[M+H]^+$(see text for details). A) $^1$H NMR, B) TOCSY and C) overlap of $^1$H-$^{13}$C HSQC (blue/green) and $^1$H-$^{13}$C HMBC (red) acquired at 300 MHz in $D_2O$. D) $^1$H and $^{13}$C NMR spectral data of the hydrolytic fragment of allopeptimicins.

**Fig. S12.** Characterization of allopeptimicin A1, A2 and A3 by HR-MSMS. A) HR-$MS^2$ spectrum of 603.3622 $[M+2H]^{2+}$, 604.3689 $[M+2H]^{2+}$ and 610.3702 $[M+2H]^{2+}$ B) HR-$MS^2$ zoom of selected region.

**Fig. S13**. A) Table of found and calculated exact masses of fragments from allopeptimicin A1, A2 and A3; B) proposed assignments of observed MS fragments (PK corresponds to the polyketide chain).

**Fig. S14**. Extracted ion chromatograms of amino acid standards and allopeptimicins after treatment according to Marfey's method.[17] Extracted ion chromatograms of the threonines (A), valines (B) and isoleucines standards (C) aligned with the hydrolysis products of allopeptimicin.

**Fig. S15**. A) Overlap of a selected region of HSQC experiments of allopeptimicins A (blue/green) and allopeptimicins B (red/black). Arrows indicate the chemical shift of CH at position 19 and $CH_2$ at position 18 in both samples. B) Overlap of a selected region of $^1$H-TOCSY experiments of allopeptimicins A (blue/green) and allopeptimicins B (red/black). The black rectangle highlights the C18 (2.60-2.30) - C19

(3.54) - C20 (1.27) spin system in allopeptimicin B. NMR experiments acquired at 300 MHz in $CD_3CN:D_2O:H_2O$ 4:1:0.2.

**Fig. S16**. A) time-kill profiles of allopeptimicin A against S. *aureus* ATCC6538P; B) Impact of allopeptimicins A on macromolecules synthesis by *S. aureus* cells*:* DNA (blue diamonds), RNA (orange squares), protein (grey triangles), and cell wall (yellow circles); C) growth curve of *S. aureus* ATCC6538P (dashed lines) and mutant R15.5[36] (solid lines) in the presence of allopeptimicin A.

**Fig. S17**. Sensibility test of *Actinollomurus* ID145808 to allopeptimicins. Amount (in µg) of allopeptimicins A (panel A) and B (panel B) spotted in 10 µL is indicated on the plates. Bottom portion of each plate: Ap (0.1 µg apramycin in 10 µl) and 60% MeOH (10 µL) used as positive and negative controls, respectively.

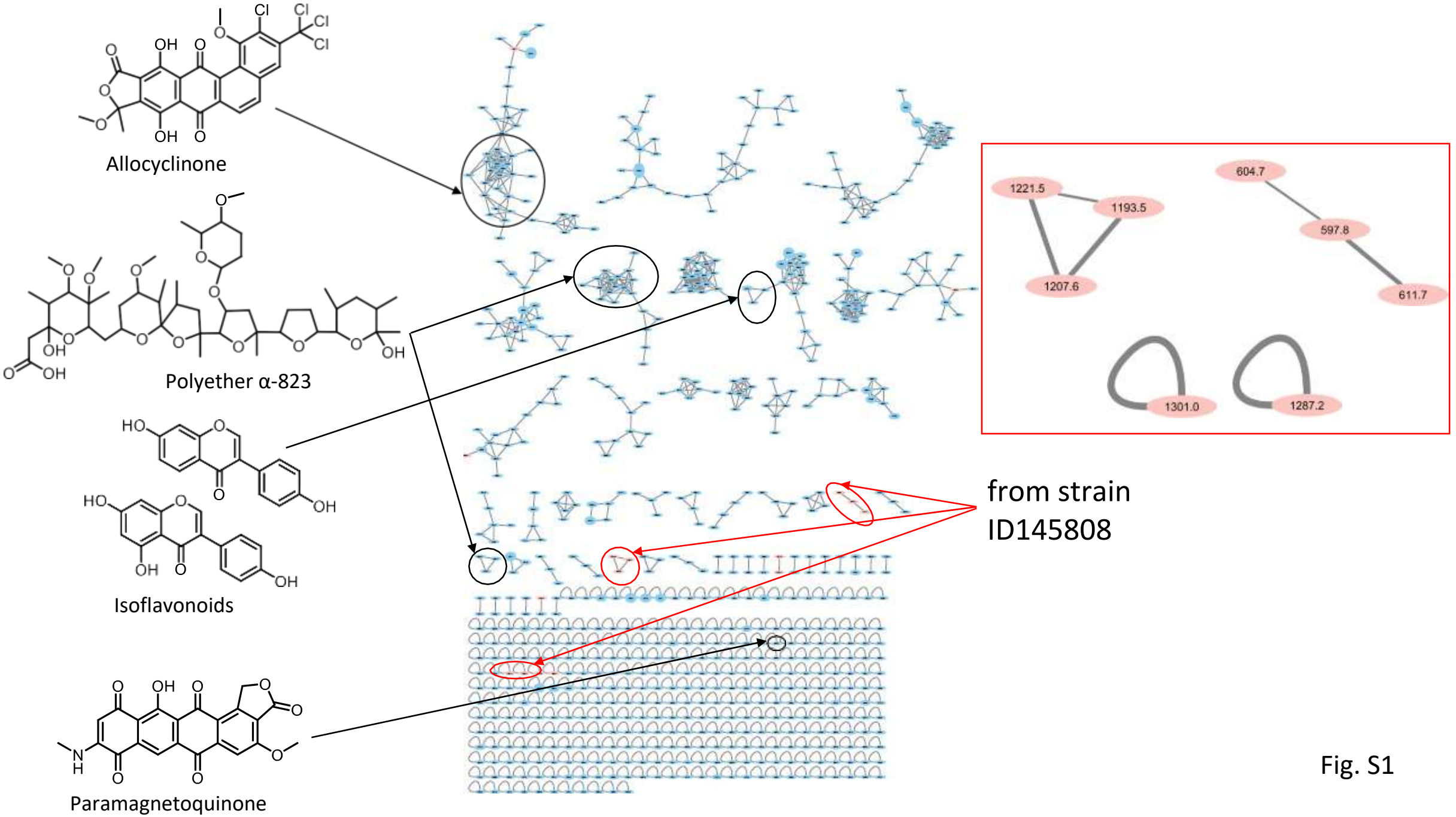

Allocyclinone
Polyether α-823
Isoflavonoids
Paramagnetoquinone
1221.5
1193.5
1207.6
604.7
597.8
611.7
1301.0
1287.2
from strain
ID145808

Fig. S1

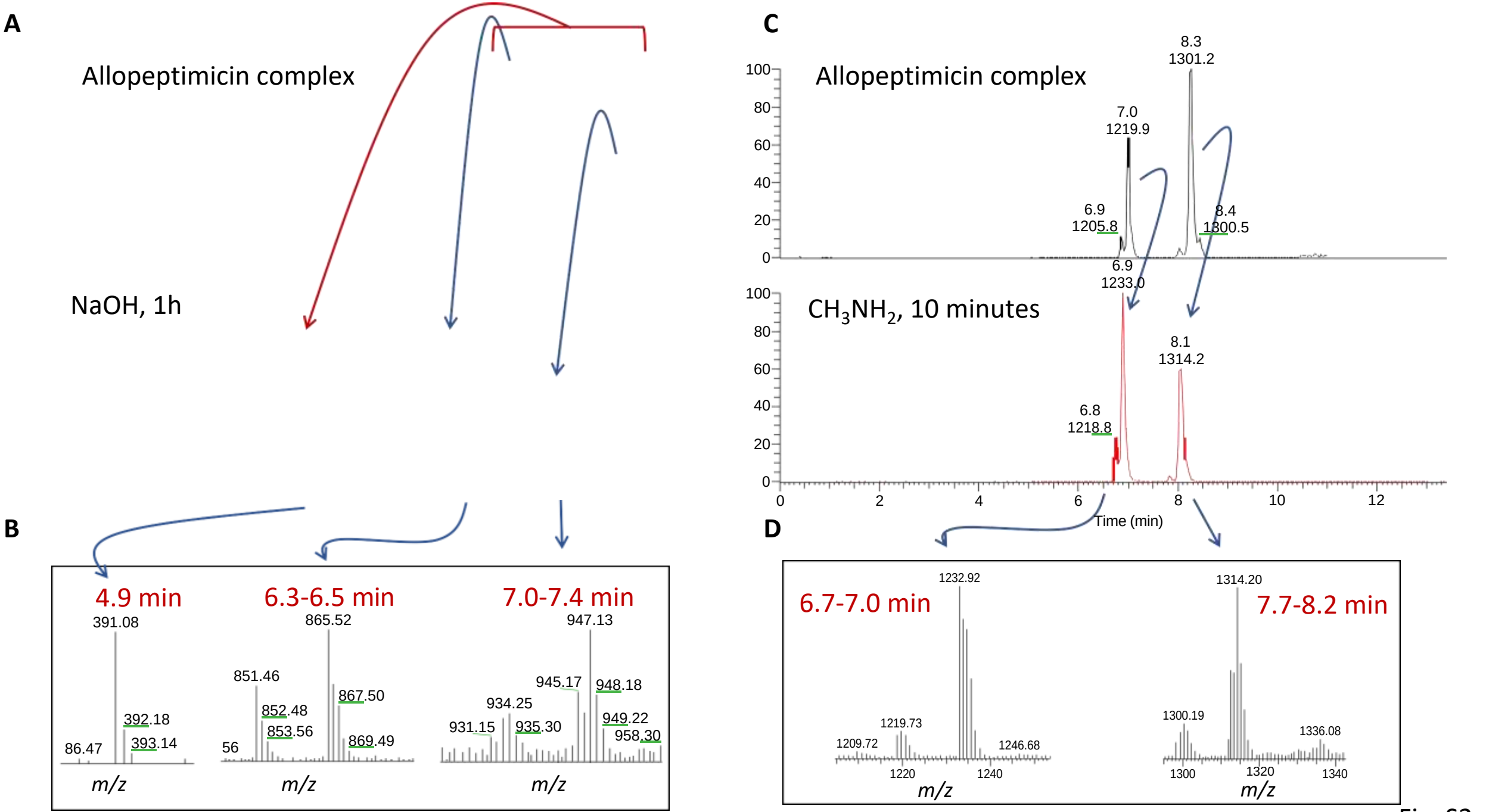

A
Allopeptimicin complex
NaOH, 1h
B
4.9 min
391.08
392.18
393.14
86.47
m/z
6.3-6.5 min
865.52
851.46
867.50
852.48
853.56
869.49
56
m/z
7.0-7.4 min
947.13
945.17
948.18
934.25
931.15
935.30
949.22
958.30
m/z
C
Allopeptimicin complex
8.3
1301.2
7.0
1219.9
6.9
1205.8
8.4
1300.5
6.9
1233.0
CH3NH2, 10 minutes
8.1
1314.2
6.8
1218.8
Time (min)
D
6.7-7.0 min
1232.92
1219.73
1209.72
1246.68
m/z
7.7-8.2 min
1314.20
1300.19
1336.08
m/z

Fig. S2

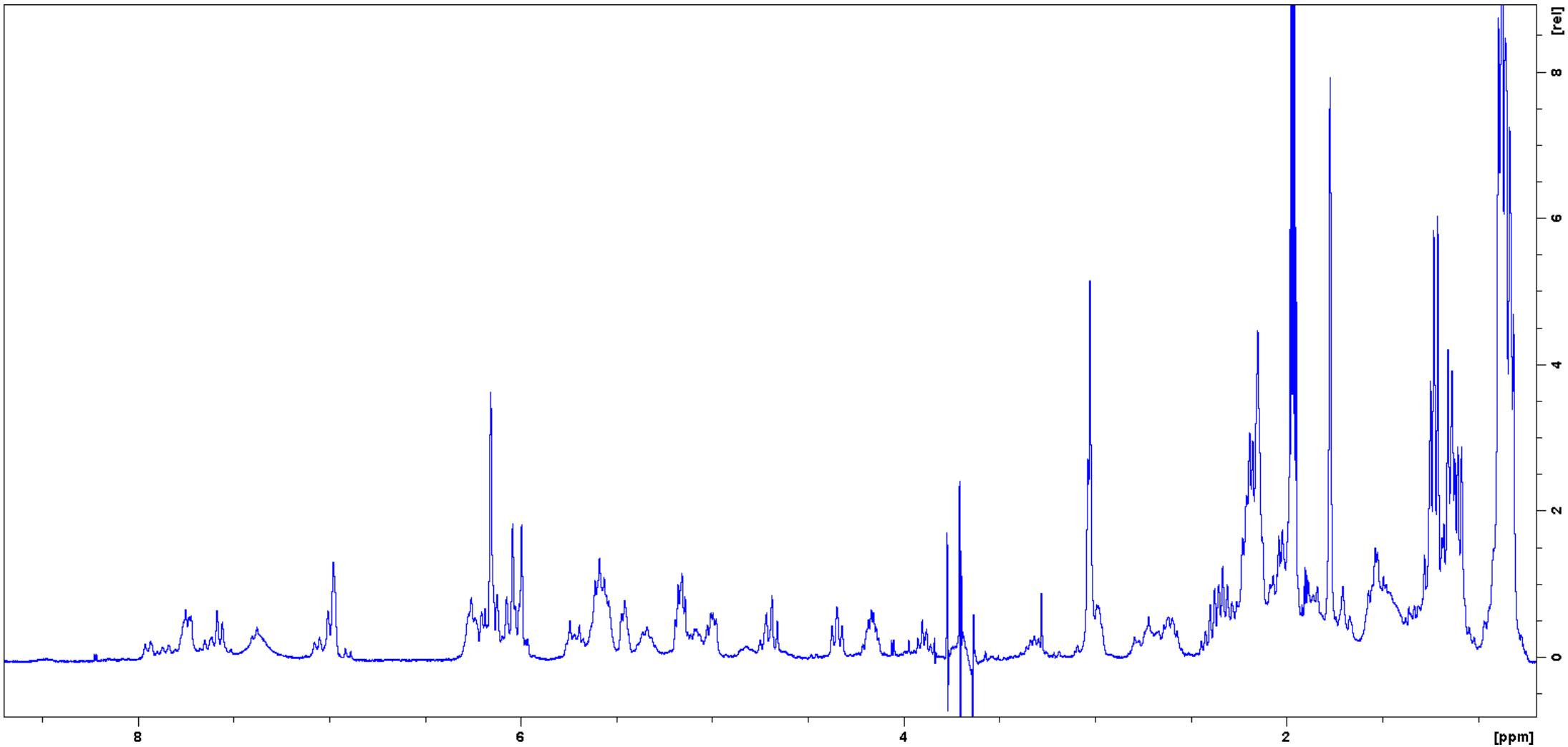



Fig. S3

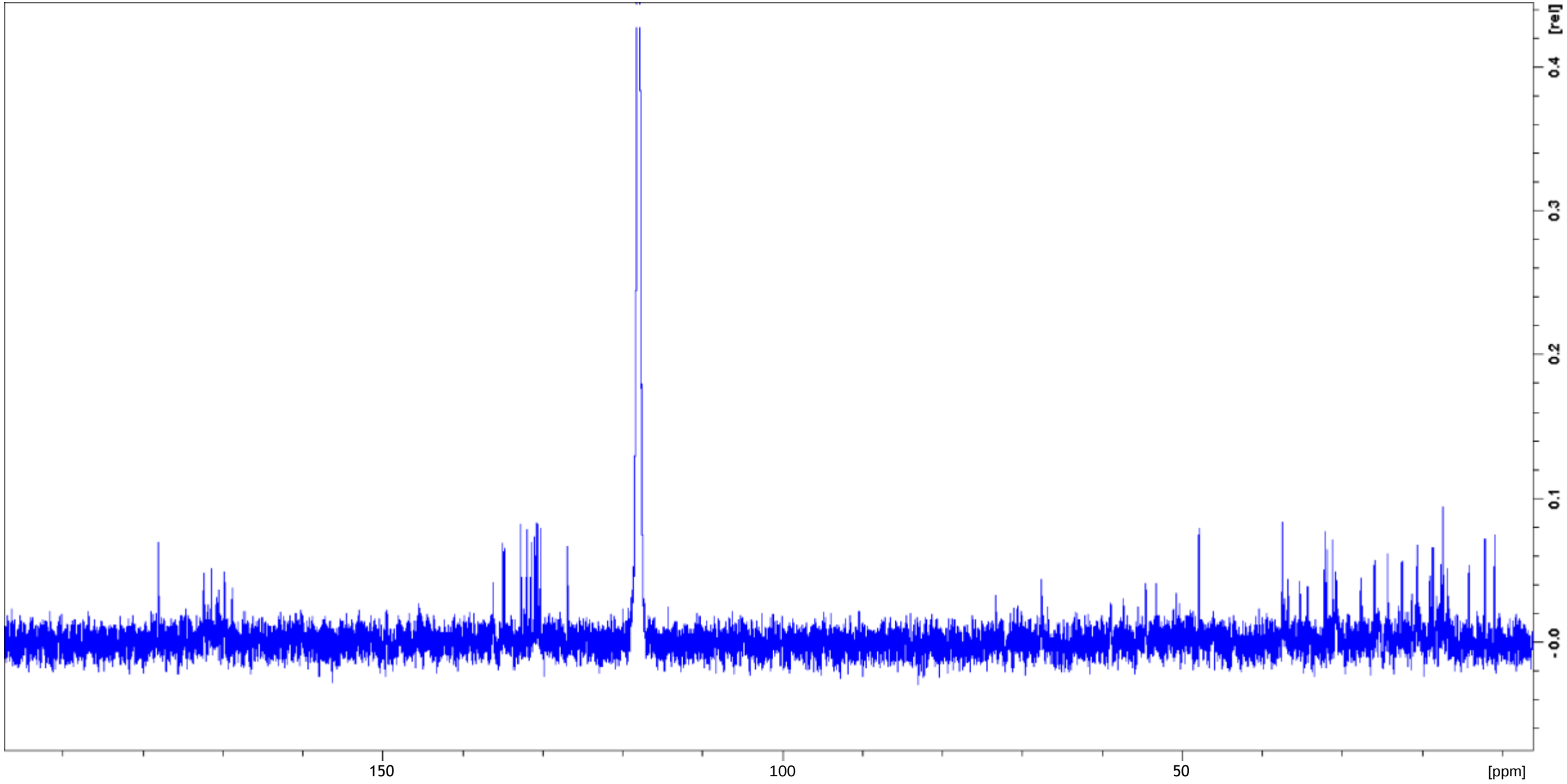



Fig. S4

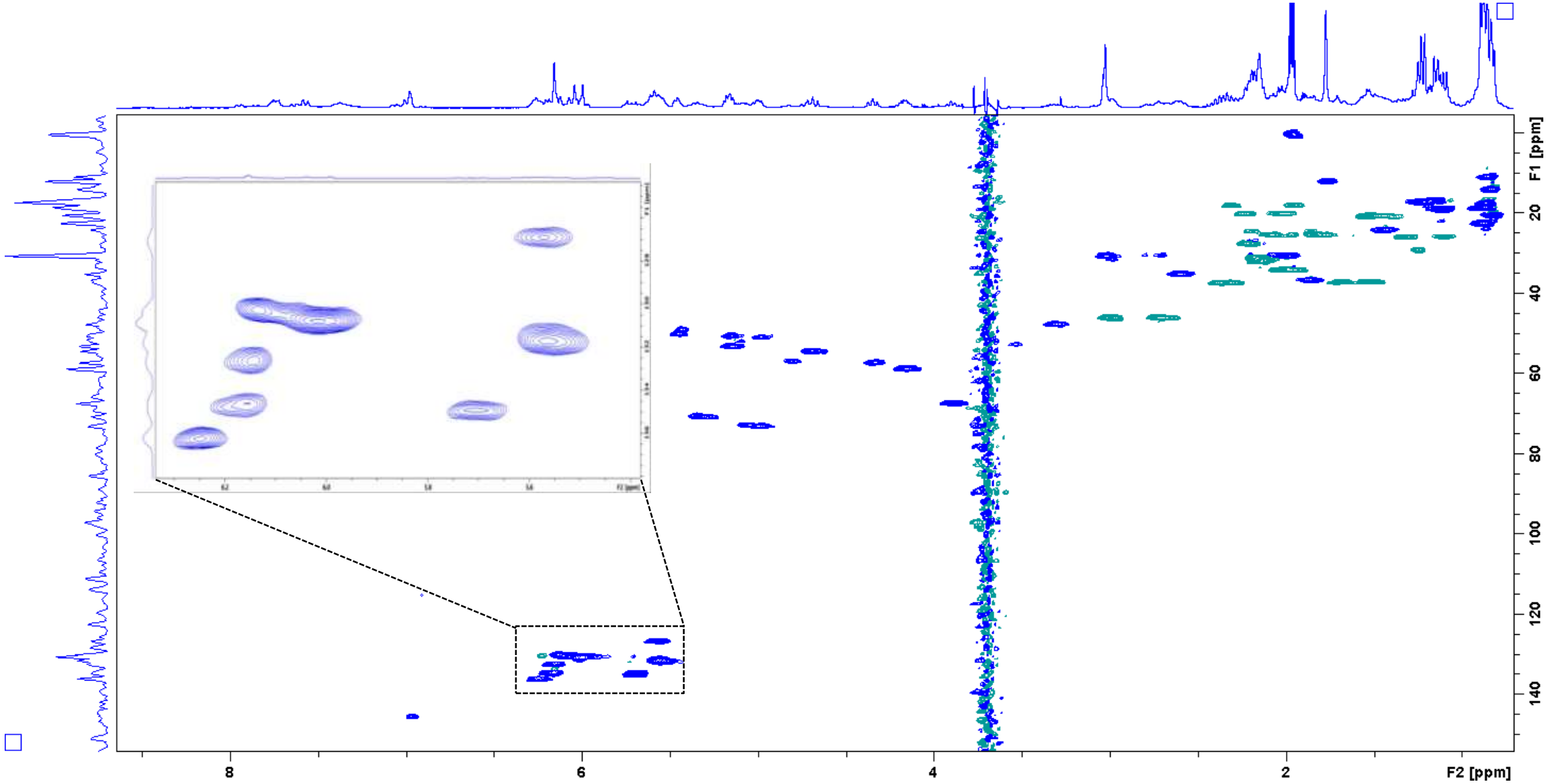



Fig. S5

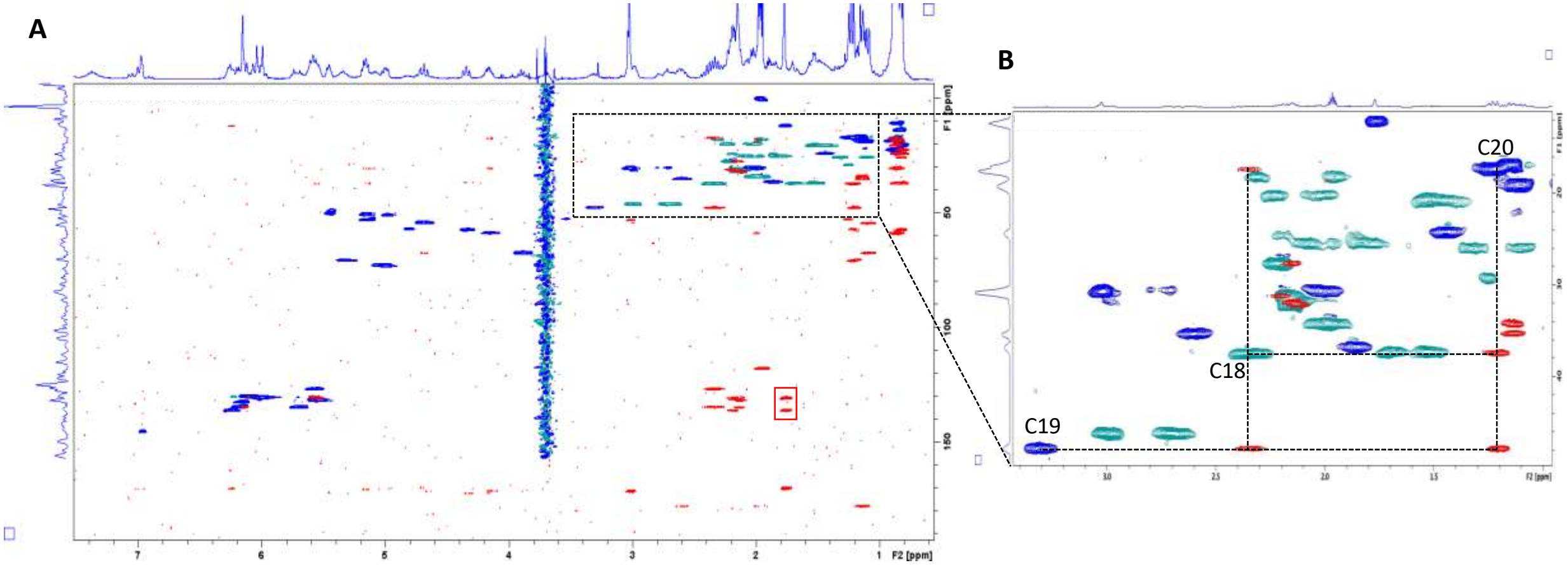



Fig. S6

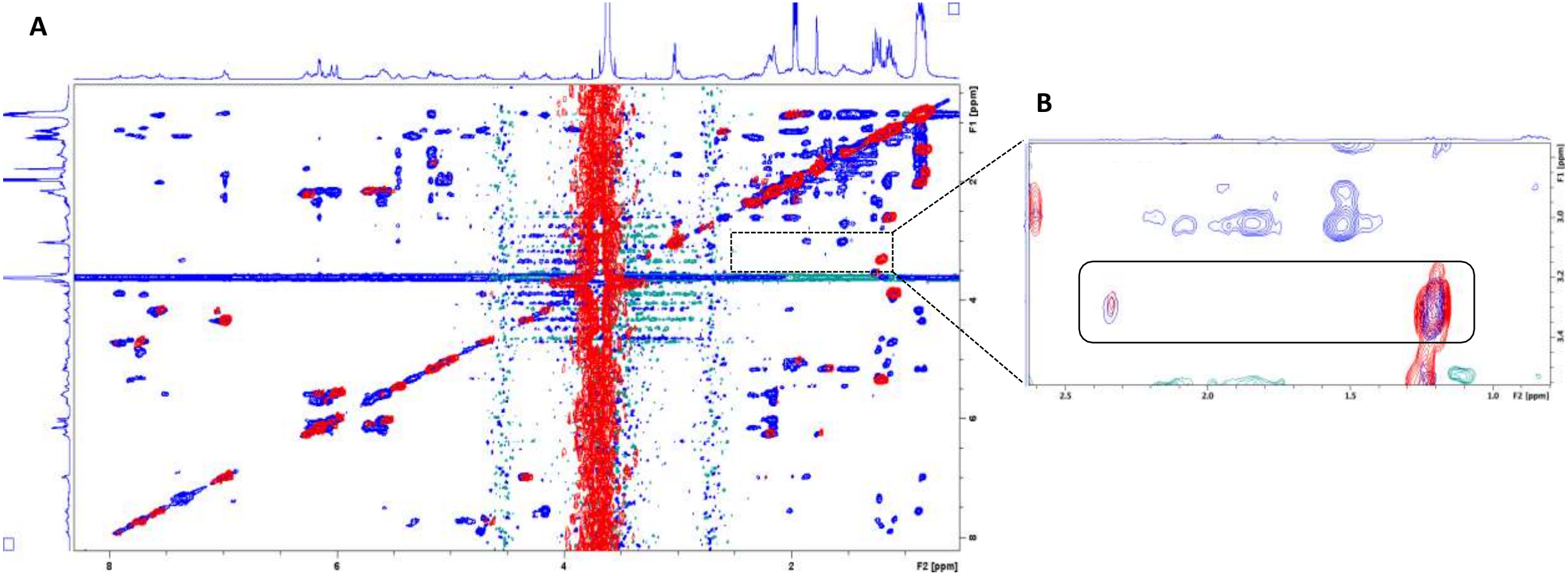
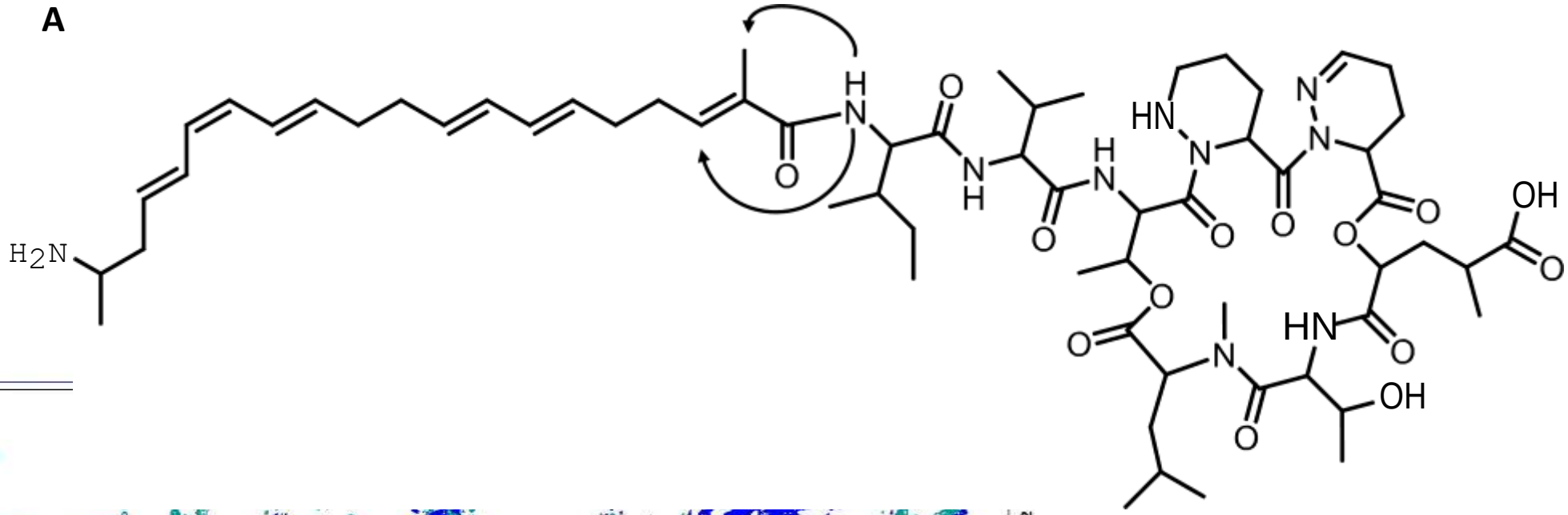



Fig. S7

A




B

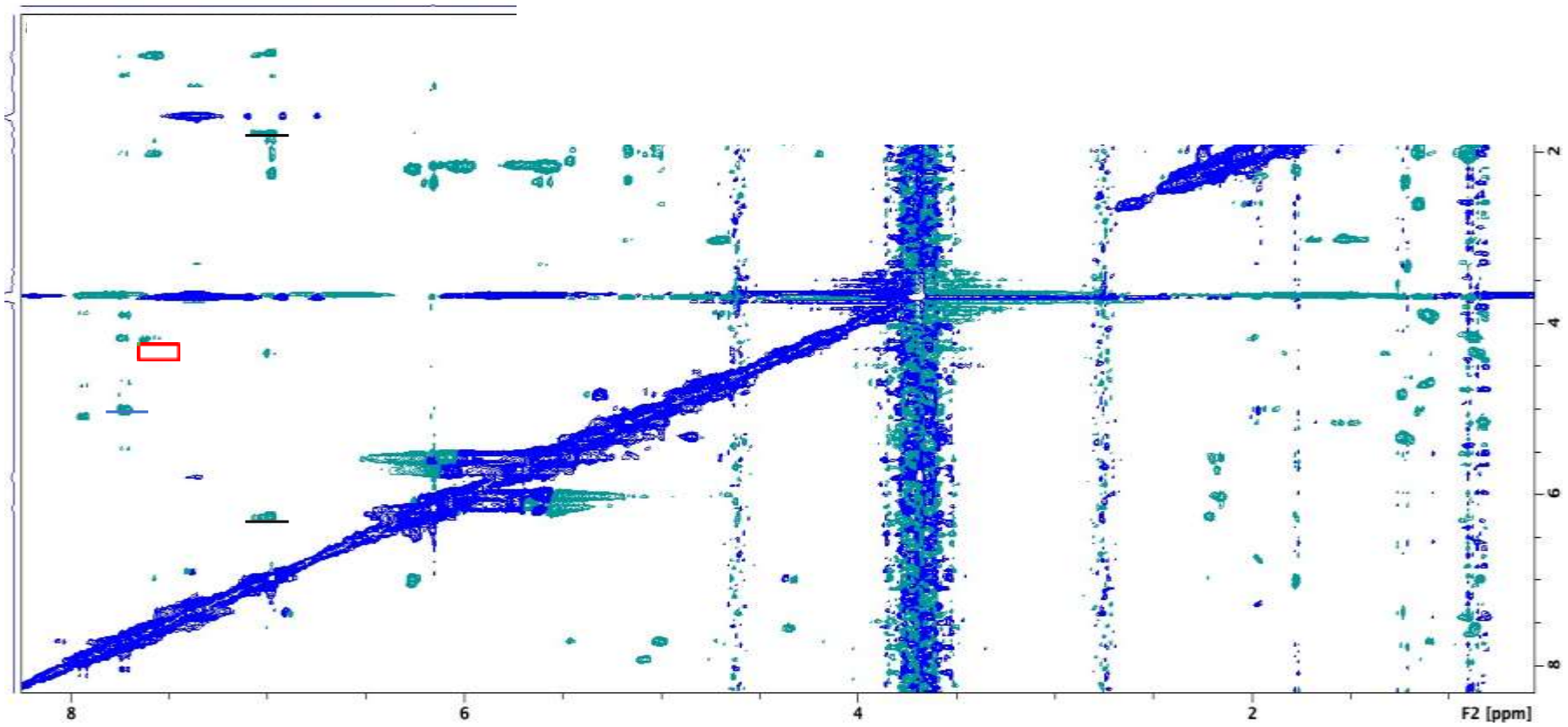



Fig. S8

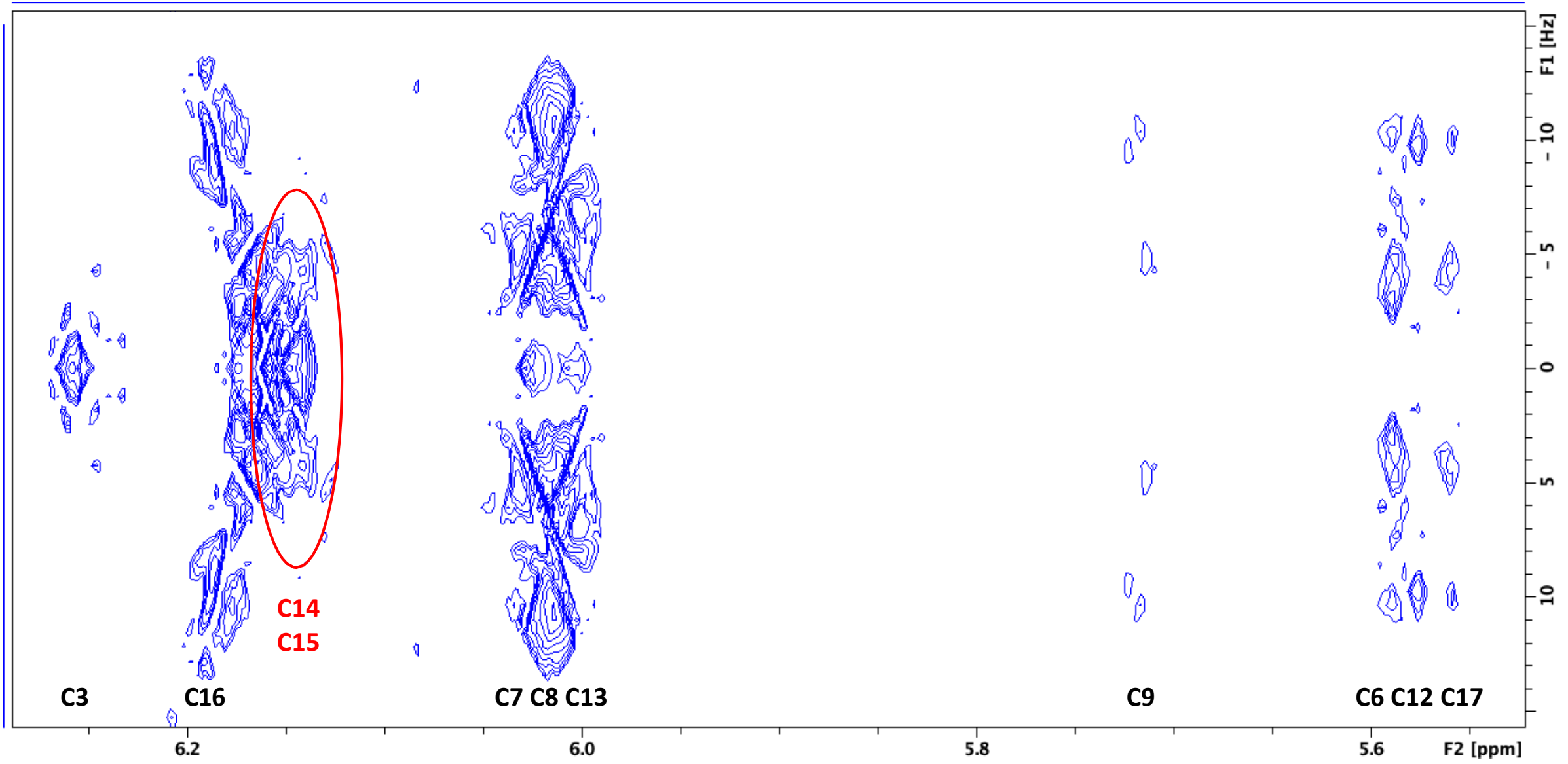

C14
C15
C3
C16
C7 C8 C13
C9
C6 C12 C17
6.2
6.0
5.8
5.6
F2 [ppm]
F1 [Hz]
– 10
– 5
0
5
10

Fig. S9

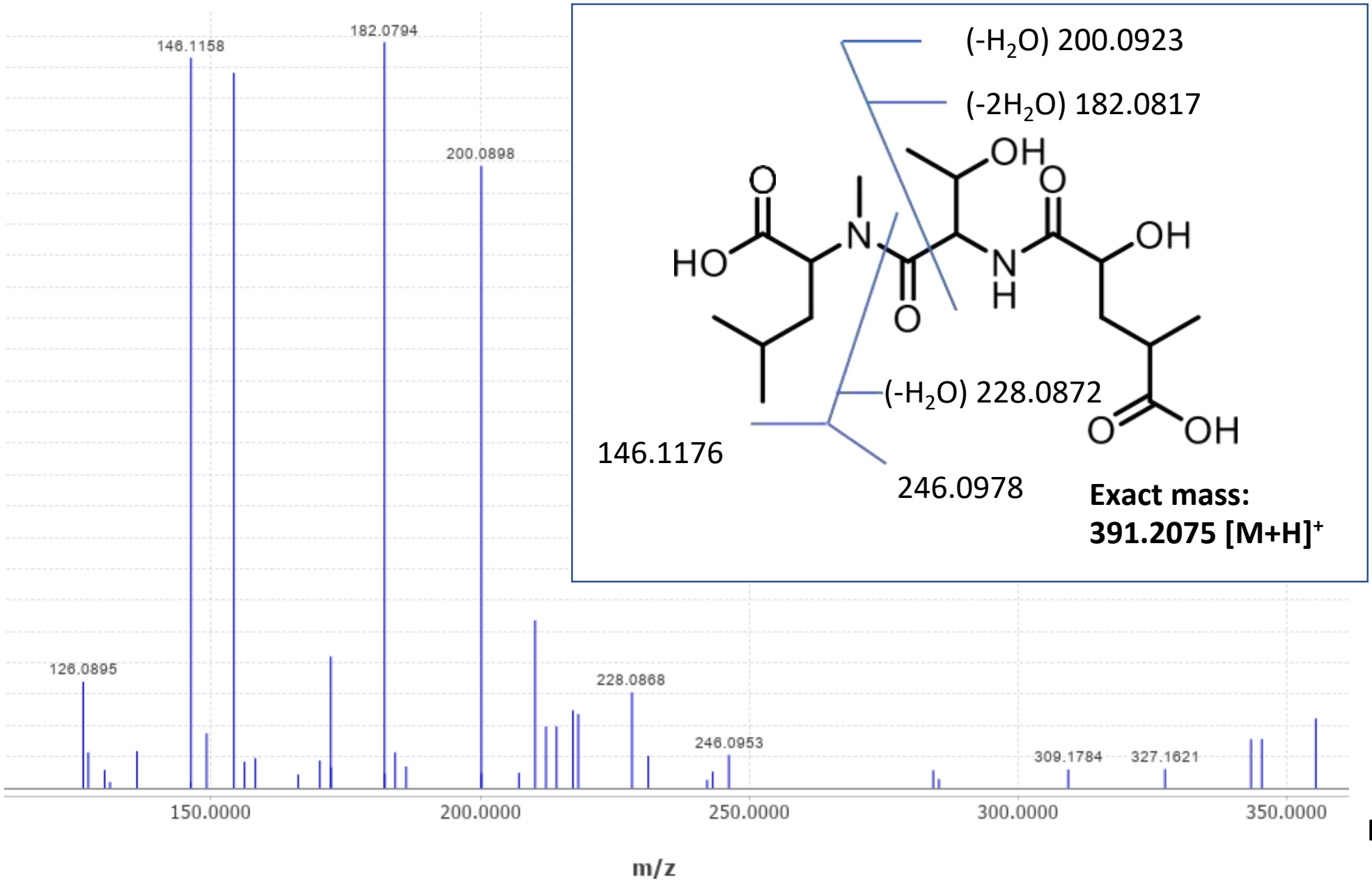
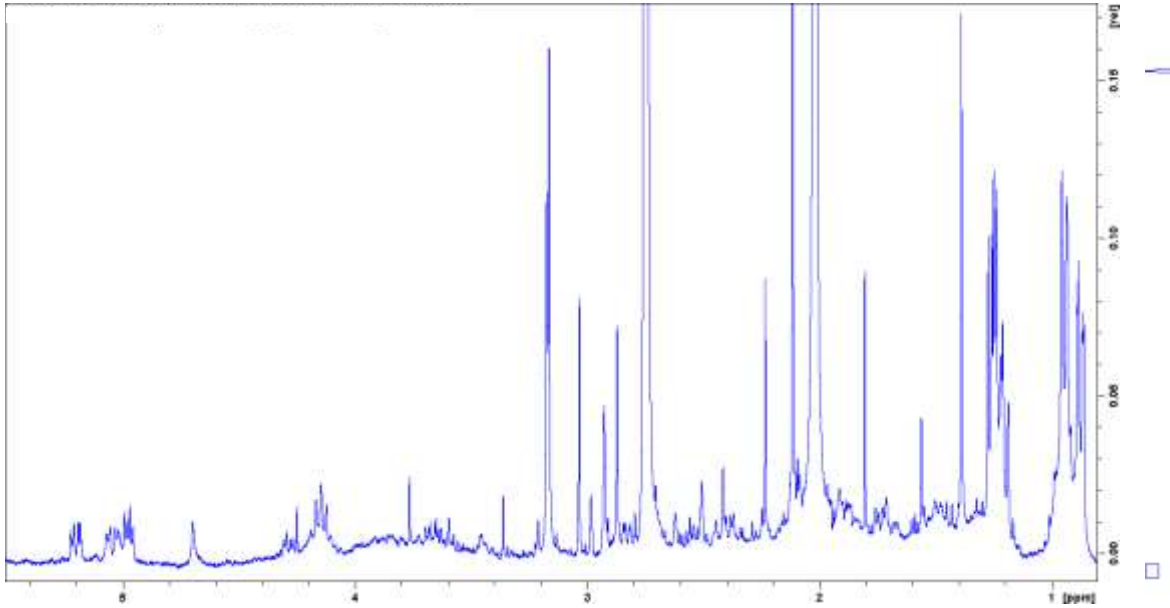
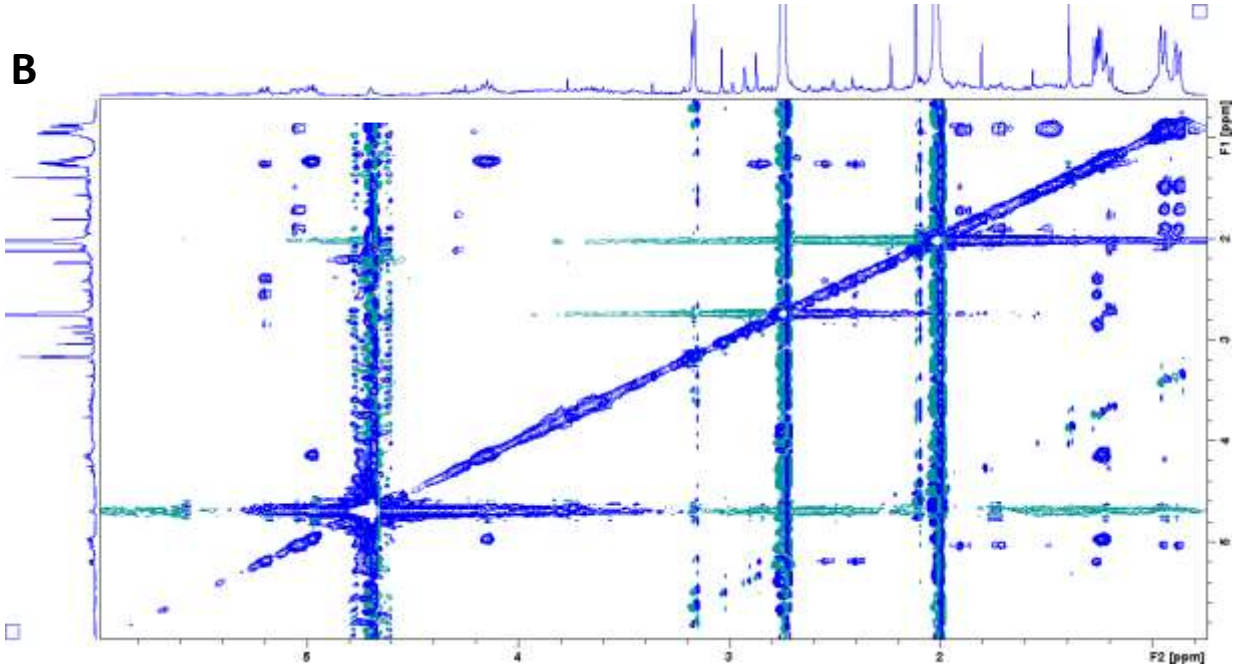

146.1158
182.0794
200.0898
126.0895
228.0868
246.0953
309.1784
327.1621
150.0000
200.0000
250.0000
300.0000
350.0000
m/z
(-H2O) 200.0923
(-2H2O) 182.0817
(-H2O) 228.0872
146.1176
246.0978
Exact mass:
391.2075 [M+H]+

Fig. S10

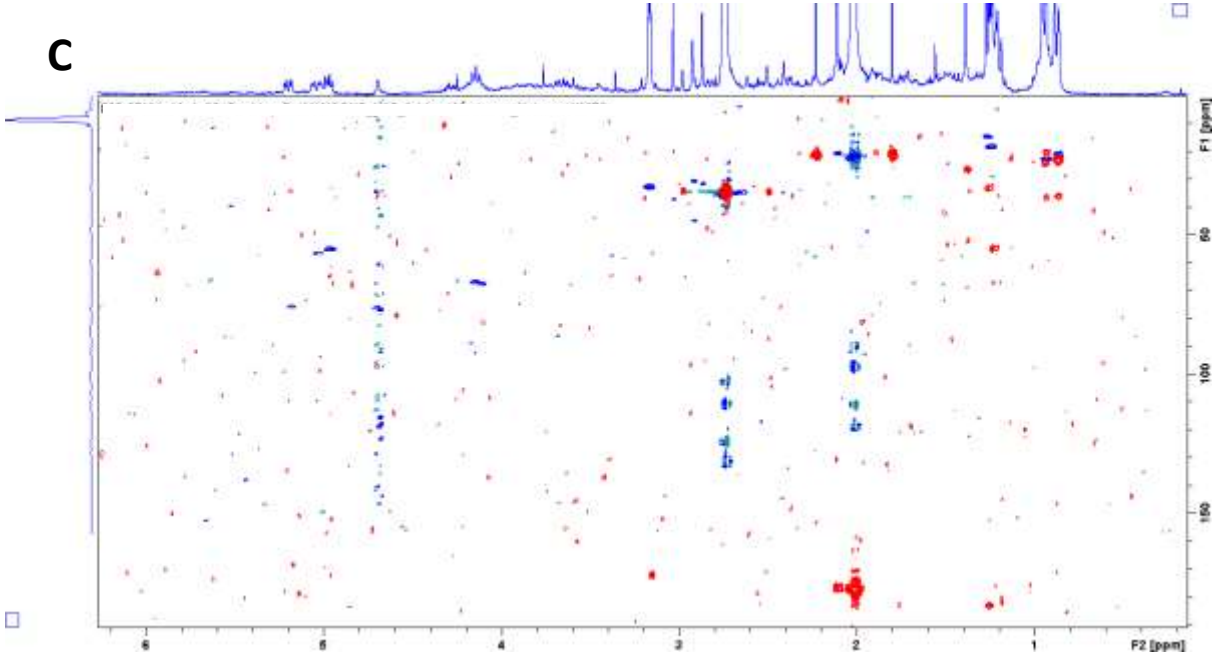
A



B



C



D

Hydrolytic fragment

| unit | position | δH | δC |
|---|---|---|---|
| Hmg_6 | C=O | | 168.4 |
| | α | 5.19 | 75 |
| | βa | 2.33 | 33.7 |
| | βb | 2.41 | |
| | γ | 2.84 | 34.6 |
| | δ | | 183.3 |
| | γ-Me | 1.26 | 14.6 |
| Thr_7 | NH | 7.31 | |
| | C=O | | 172.2 |
| | α | 4.98 | 55.3 |
| | β | 4.13 | 67.1 |
| | γ | 1.23 | 18.6 |
| N-Me-Leu_8 | C=O | | 172.1 |
| | α | 5.03 | 56.3 |
| | βa | 1.76 | 36.5 |
| | βb | 1.92 | |
| | γ | 1.48 | 24.4 |
| | δ | 0.94 | 22.4 |
| | ε | 0.87 | 20.5 |
| | N-Me | 3.17 | 32.4 |

Fig. S11

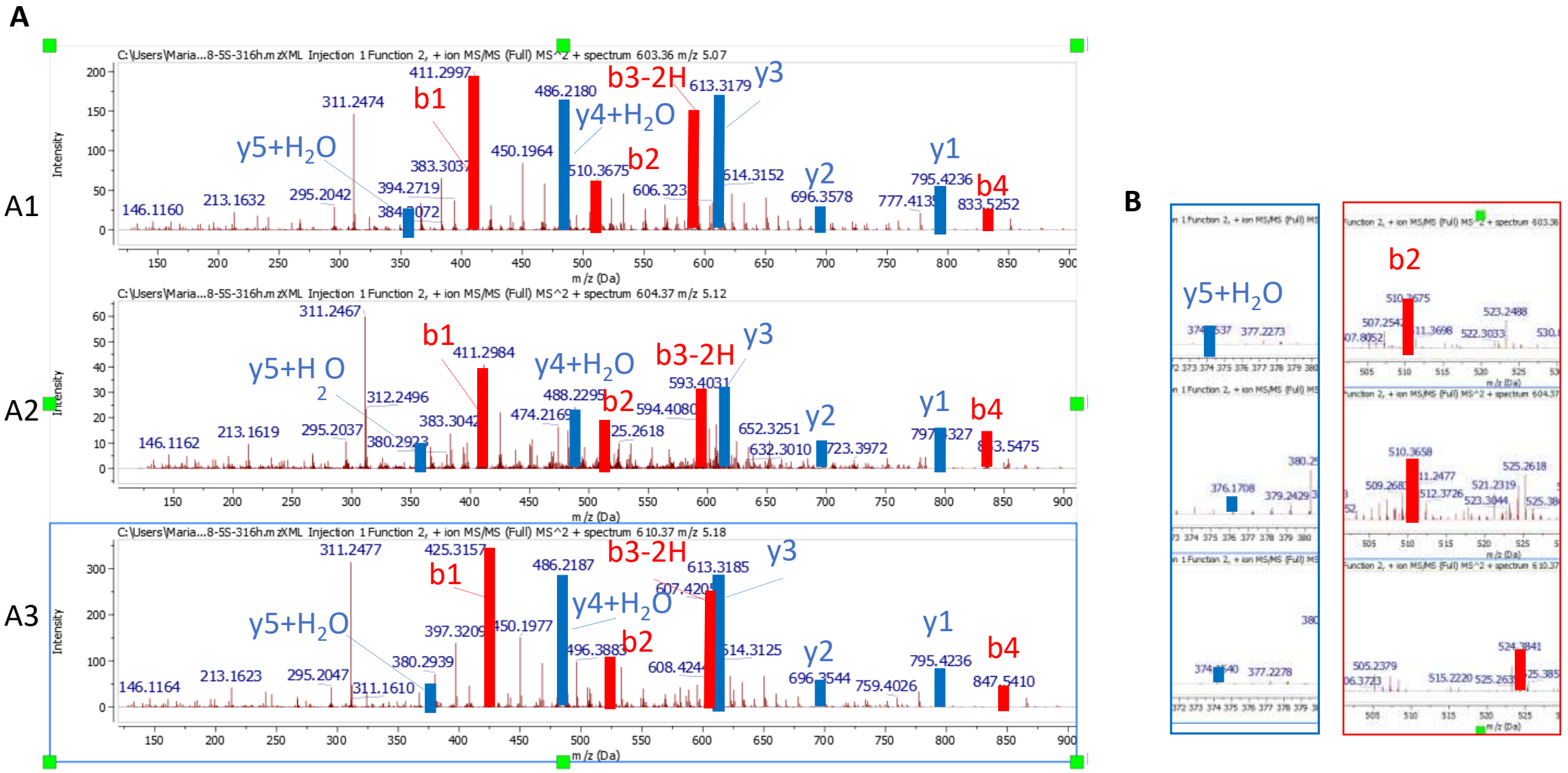
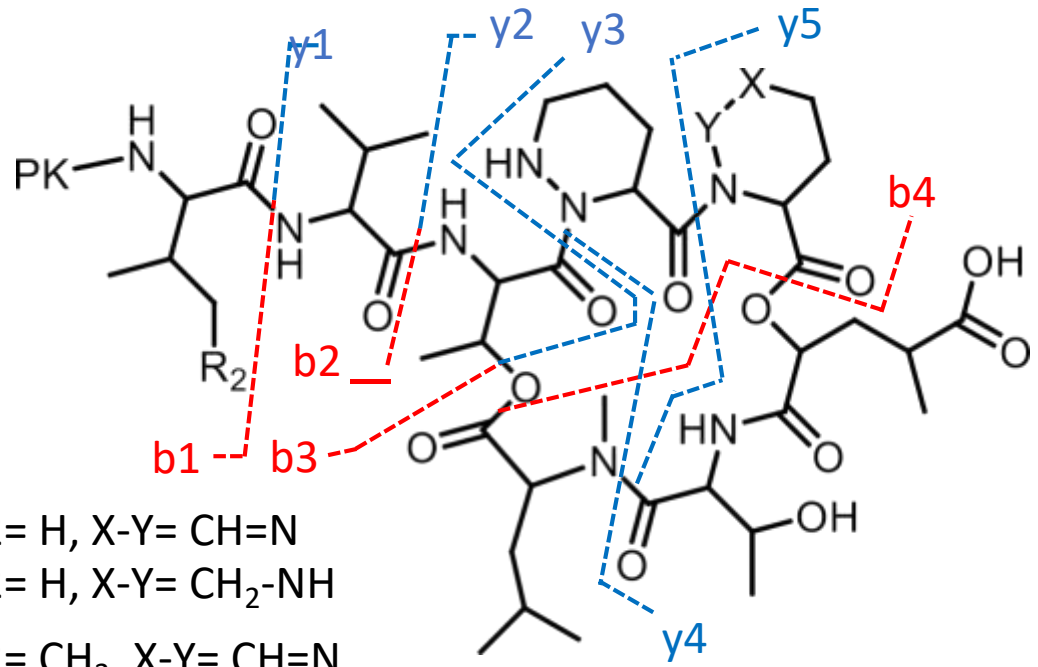

A
A1
A2
A3
B
C:\Users\Maria...8-5S-316h.mzXML Injection 1 Function 2, + ion MS/MS (Full) MS^2 + spectrum 603.36 m/z 5.07
C:\Users\Maria...8-5S-316h.mzXML Injection 1 Function 2, + ion MS/MS (Full) MS^2 + spectrum 604.37 m/z 5.12
C:\Users\Maria...8-5S-316h.mzXML Injection 1 Function 2, + ion MS/MS (Full) MS^2 + spectrum 610.37 m/z 5.18
Intensity
m/z (Da)
b1
b2
b3-2H
b4
y1
y2
y3
y4+H2O
y5+H2O
411.2997
311.2474
486.2180
613.3179
450.1964
510.3675
614.3152
696.3578
795.4236
833.5252
146.1160
213.1632
295.2042
394.2719
606.323
777.4133
311.2467
411.2984
488.2295
474.2169
593.4031
594.4080
652.3251
632.3010
723.3972
146.1162
213.1619
295.2037
312.2496
380.2923
383.3042
425.2618
797.4327
311.2477
425.3157
486.2187
613.3185
607.4205
397.3209
450.1977
496.3883
614.3125
608.424
696.3544
759.4026
795.4236
847.5410
146.1164
213.1623
295.2047
311.1610
380.2939
376.1708
379.2429
377.2273
377.2278
523.2488
507.2542
511.3698
522.3033
510.3658
525.2618
509.2683
512.3726
521.2319
523.3044
524.3841
505.2379
515.2220
525.2635

Fig. S12

**A**

| | Allopeptimicin A1 | | Allopeptimicin A2 | | Allopeptimicin A3 | |
|---|---|---|---|---|---|---|
| | found | calculated | found | calculated | found | calculated |
| $[M+H]^+$ | 1205.718 | 1205.718 | 1207.7348 | 1207.734 | 1219.734 | 1219.7337 |
| b4 | 833.5252 | 833.5284 | 835.538 | 835.544 | 847.541 | 847.5446 |
| y1 | 795.4236 | 795.4247 | 797.4327 | 797.4403 | 795.4236 | 795.4247 |
| y2 | 696.3578 | 696.3563 | 698.3643 | 698.3719 | 696.3544 | 696.3563 |
| y3 | 613.3179 | 613.3192 | 615.3274 | 615.3348 | 613.3185 | 613.3192 |
| b3-2H | 593.4056 | 593.4061 | 593.4031 | 593.4061 | 607.4205 | 607.4223 |
| b2 | 510.3675 | 510.3696 | 510.3658 | 510.3696 | 524.3841 | 524.3852 |
| y4 + $H_2O$ | 486.218 | 486.2195 | 488.2295 | 488.2351 | 486.2187 | 486.2195 |
| b1 | 411.2997 | 411.3012 | 411.2984 | 411.3012 | 425.3157 | 425.3168 |
| y5 + $H_2O$ | 374.1537 | 374.1558 | 376.1708 | 376.1714 | 374.154 | 374.1558 |

**B**




**A1**: R2= H, X-Y= CH=N

**A2**: R2= H, X-Y= $CH_2$-NH

**A3**: R2= $CH_3$, X-Y= CH=N

Fig. S13

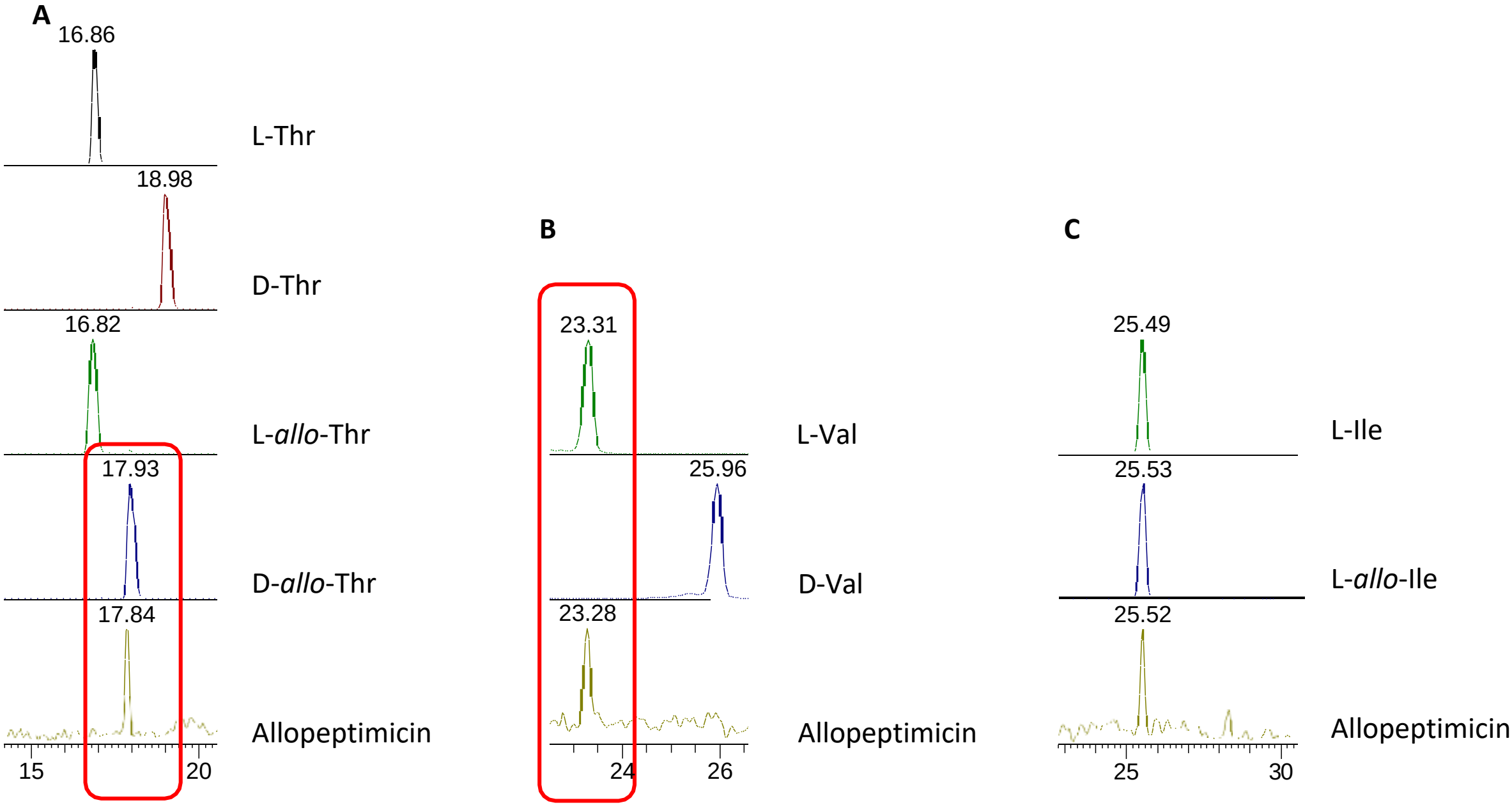
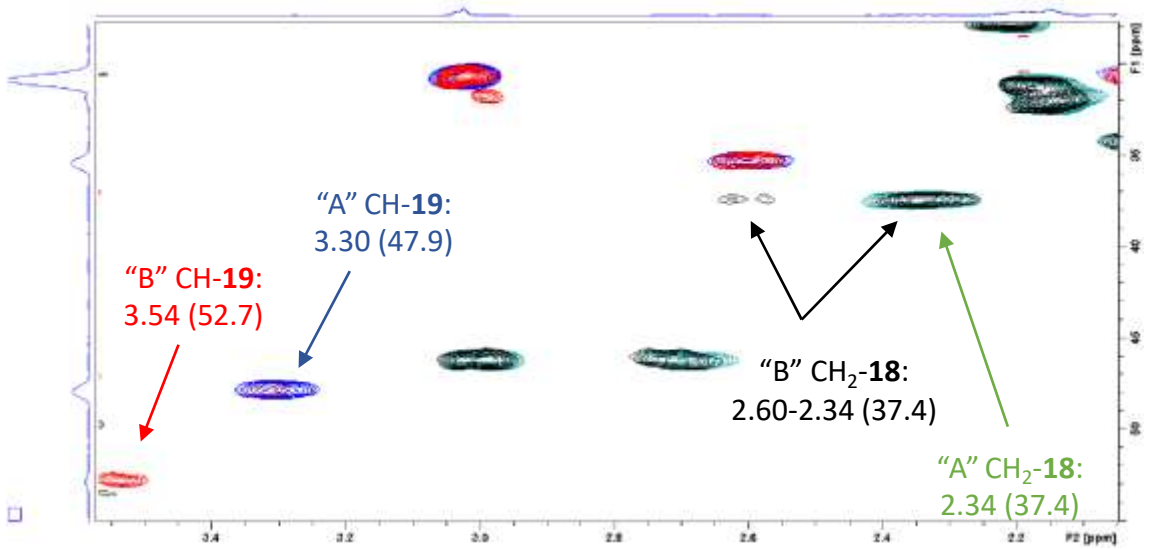

A
16.86
L-Thr
18.98
D-Thr
16.82
L-allo-Thr
17.93
D-allo-Thr
17.84
Allopeptimicin
15
20
B
23.31
L-Val
25.96
D-Val
23.28
Allopeptimicin
24
26
C
25.49
L-Ile
25.53
L-allo-Ile
25.52
Allopeptimicin
25
30

Fig. S14

A




B

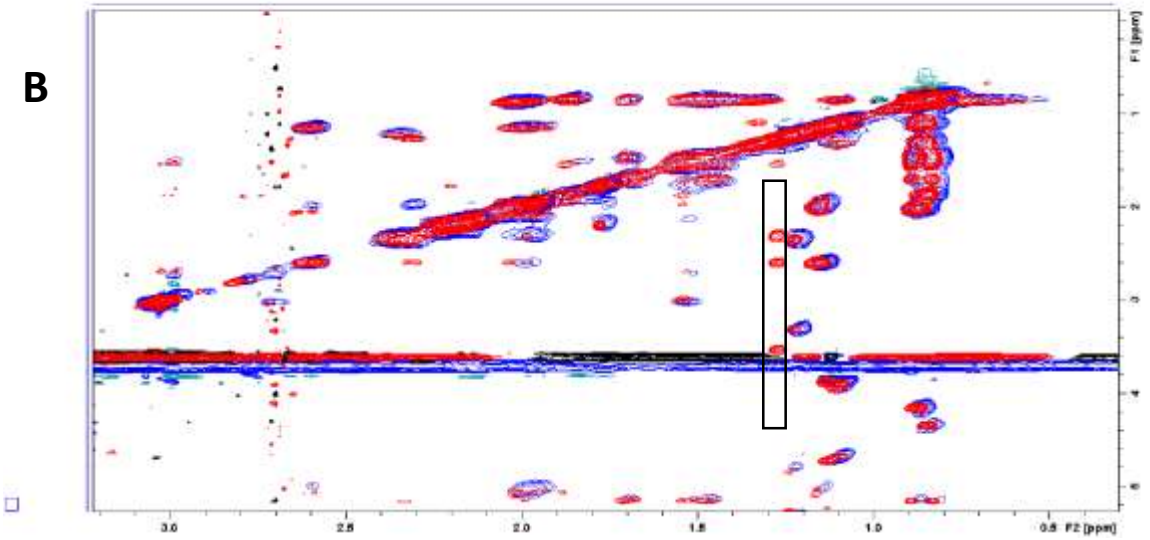
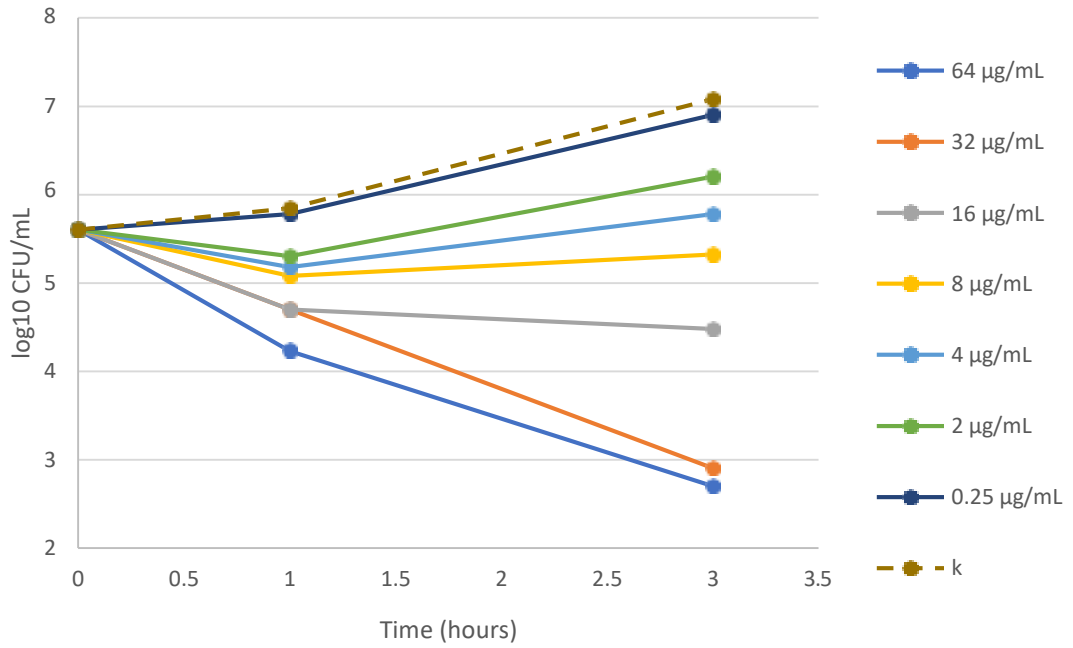
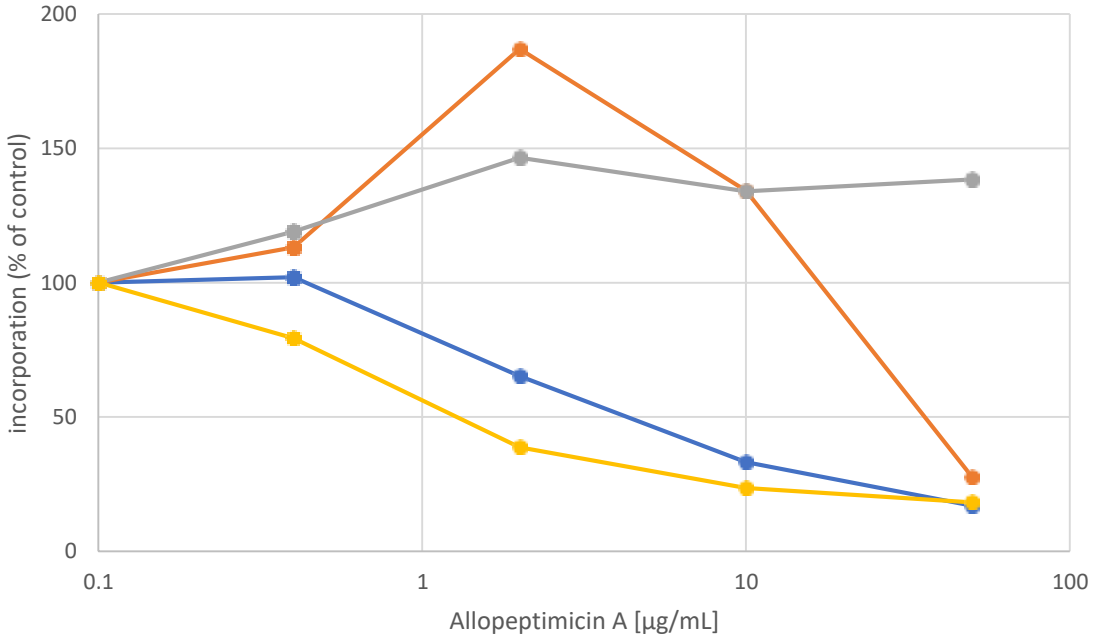


Fig. S15

A


log10 CFU/mL
Time (hours)
64 µg/mL
32 µg/mL
16 µg/mL
8 µg/mL
4 µg/mL
2 µg/mL
0.25 µg/mL
k

B


incorporation (% of control)
Allopeptimicin A [µg/mL]

C

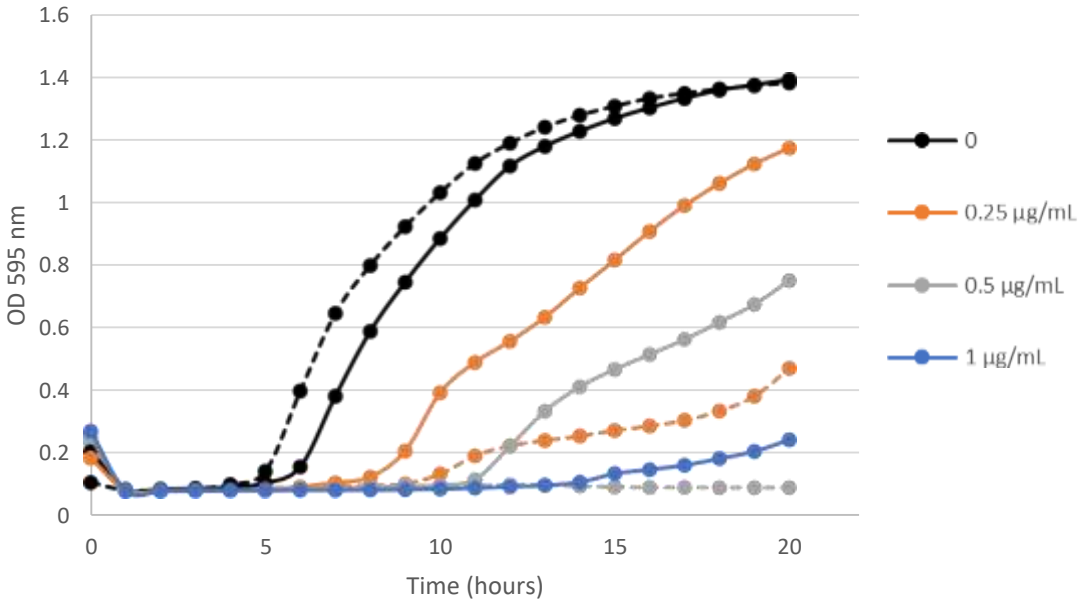
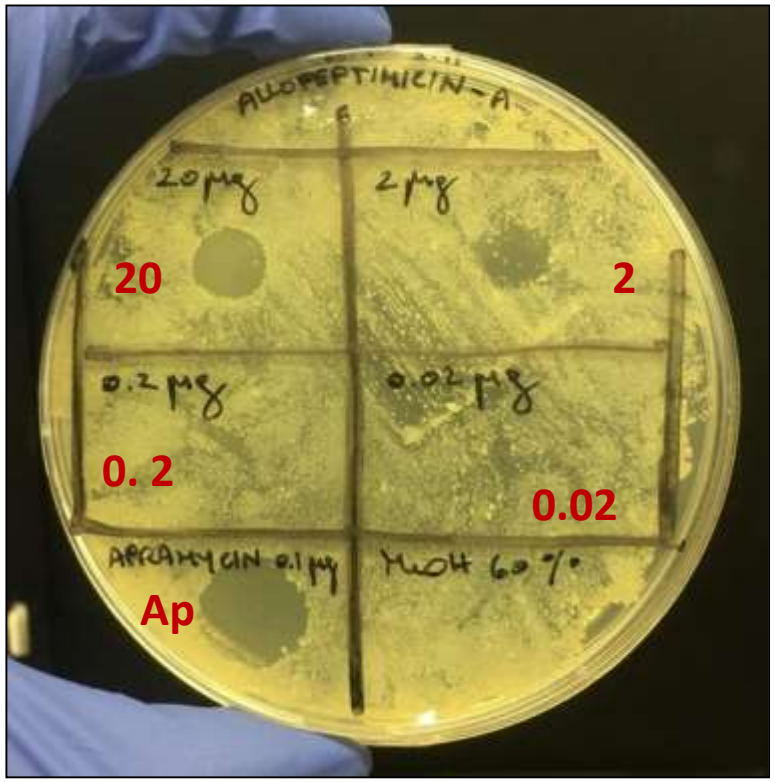

OD 595 nm
Time (hours)
0
0.25 µg/mL
0.5 µg/mL
1 µg/mL

Fig. S16

A


ALLOPEPTIMICIN-A
20 μg
2 μg
20
2
0.2 μg
0.02 μg
0. 2
0.02
APRAMYCIN 0.1 μg
MeOH 60 %
Ap

B

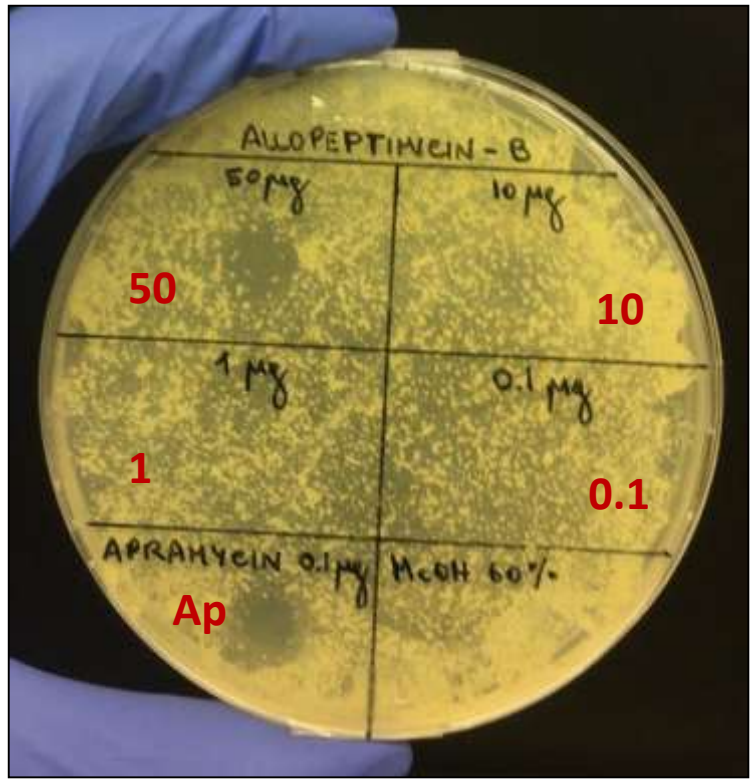

ALLOPEPTIMICIN-B
50 μg
10 μg
50
10
1 μg
0.1 μg
1
0.1
APRAMYCIN 0.1 μg
MeOH 60 %
Ap

Fig. S17