

Supplementary Table 1. Annotated putative ORFs in and near the biosynthetic gene clusters of enniatin, surfactin, tunicamycin, and niphimycin.

Product	Orf	Organism	Size	AA	Gene annotation/proposed function	Accession no.	Alignment coverage/identity
enniatin	<i>Esyn1</i>	WAC11175	9399	3132	Nonribosomal cyclopeptide synthetase for enniatin	KAH7263718.1	100/98
surfactin	<i>sfrA</i>	WAC11084	10755	3584	Surfactin non-ribosomal peptide synthetase SrfAA	WP_099566387.1	100/100
surfactin	<i>sfrB</i>	WAC11084	10761	3586	Surfactin non-ribosomal peptide synthetase SrfAB	AWK44950.1	100/99
surfactin	<i>sfrC</i>	WAC11084	3837	1278	Surfactin non-ribosomal peptide synthetase SrfAC	WP_099566389.1	100/100
surfactin	<i>sfrD</i>	WAC11084	732	243	Surfactin biosynthesis thioesterase SrfAD	WP_012116775.1	100/100
tunicamycin	<i>tunA</i>	WAC1490	966	321	<i>NAD-dependent epimerase/dehydratase</i>	WP_102514799.1	100/99
tunicamycin	<i>tunB</i>	WAC1490	1017	338	Radical SAM domain protein	WP_128839962.1	100/99
tunicamycin	<i>tunC</i>	WAC1490	957	318	GCN5-related N-Acetyltransferase	WP_010045810.1	100/98
tunicamycin	<i>tunD</i>	WAC1490	1419	472	Group 1 family glycosyltransferase	ADP94224.1	100/96
tunicamycin	<i>tunE</i>	WAC1490	705	234	GlcNAc-phosphatidylinositol de-N-acetylase	WP_149179446.1	100/98
tunicamycin	<i>tunF</i>	WAC1490	984	327	UDP-glucose 4-epimerase	WP_010045806.1	100/96
tunicamycin	<i>tunG</i>	WAC1490	612	203	Phosphoglycerate mutase	WP_149179448.1	99/98
tunicamycin	<i>tunH</i>	WAC1490	1548	515	Type I nucleotide pyrophosphatase	WP_029181580.1	99/96
tunicamycin	<i>tunI</i>	WAC1490	915	304	Putative ABC transporter ATP-binding Subunit	WP_149179450.1	100/98
tunicamycin	<i>tunJ</i>	WAC1490	789	262	ABC-2 type transporter	WP_149179451.1	100/100
tunicamycin	<i>tunK</i>	WAC1490	246	81	Phosphopantetheine-binding protein	WP_128839952.1	100/96
tunicamycin	<i>tunL</i>	WAC1490	690	229	Phosphoesterase PA-phosphatase	WP_010045787.1	100/96
tunicamycin	<i>tunM</i>	WAC1490	651	216	Methyltransferase family protein	WP_128839950.1	99/91
tunicamycin	<i>tunN</i>	WAC1490	459	152	NUDIX hydrolase	WP_037919768.1	99/97
niphimycin	<i>npmA</i>	WAC5858	6930	2309	non-ribosomal peptide synthetase	AUO16423.1	99/100
niphimycin	<i>npmB</i>	WAC5858	11844	3947	non-ribosomal peptide synthetase	AUO16397.1	100/100
niphimycin	<i>npmC</i>	WAC5858	10383	3460	non-ribosomal peptide synthetase	AUO16398.1	100/100
niphimycin	<i>npmD</i>	WAC5858	10122	3373	non-ribosomal peptide synthetase	AUO16399.1	100/100
niphimycin	<i>npmE</i>	WAC5858	14244	4747	non-ribosomal peptide synthetase	AUO16400.1	100/100
niphimycin	<i>npmF</i>	WAC5858	24825	8274	non-ribosomal peptide synthetase	AUO16401.1	100/100
niphimycin	<i>npmG</i>	WAC5858	9618	3205	non-ribosomal peptide synthetase	AUO16402.1	100/100
niphimycin	<i>npmH</i>	WAC5858	10380	3459	non-ribosomal peptide synthetase	AUO16403.1	100/100
niphimycin	<i>npmI</i>	WAC5858	7755	2584	non-ribosomal peptide synthetase	AUO16404.1	100/100
niphimycin	<i>orf1</i>	WAC5858	4521	1506	hypothetical protein	AUO16393.1	100/100
niphimycin	<i>orf2</i>	WAC5858	609	202	TetR family transcriptional regulator	AUO16394.1	100/100
niphimycin	<i>orf3</i>	WAC5858	1215	404	cytochrome P450	AUO16395.1	100/100
niphimycin	<i>orf4</i>	WAC5858	1203	400	cytochrome P450	AJZ84031.1	100/99.8
niphimycin	<i>orf5</i>	WAC5858	207	68	(4Fe-4S)-binding protein	WP_137969090.1	100/100
niphimycin	<i>orf6</i>	WAC5858	1437	478	4-guanidinobutanoate:CoA ligase	AUO16404.1	100/100
niphimycin	<i>orf7</i>	WAC5858	1035	344	4-guanidinobutyryl-CoA:ACP acyltransferase	AUO16405.1	100/100
niphimycin	<i>orf8</i>	WAC5858	609	202	TetR family transcriptional regulator	AUO16406.1	100/100
niphimycin	<i>orf9</i>	WAC5858	885	294	hydrolase	AUO16407.1	100/100
niphimycin	<i>orf10</i>	WAC5858	381	126	helix-turn-helix domain-containing protein	WP_059145617.1	100/100
niphimycin	<i>orf11</i>	WAC5858	441	146	endoribonuclease L-PSP	AUO16409.1	100/100
niphimycin	<i>orf12</i>	WAC5858	1431	476	DUF3616 domain-containing protein	WP_137969096.1	100/98
niphimycin	<i>orf13</i>	WAC5858	1281	426	membrane protein	AUO16411.1	100/100
niphimycin	<i>orf14</i>	WAC5858	588	195	XRE family transcriptional regulator	AUO16412.1	100/100
niphimycin	<i>orf15</i>	WAC5858	231	76	methyltransferase	AUO16413.1	100/100
niphimycin	<i>orf16</i>	WAC5858	510	169	mini-circle protein	AUO16414.1	100/100
niphimycin	<i>orf17</i>	WAC5858	585	194	AcrR family DNA-binding transcriptional regulator	AUO16415.1	100/100
niphimycin	<i>orf18</i>	WAC5858	1542	513	CoA-transferase family III	AUO16416.1	100/100
niphimycin	<i>orf19</i>	WAC5858	786	261	GntR-family transcriptional regulator	AUO16417.1	100/100
niphimycin	<i>orf20</i>	WAC5858	804	267	4-guanidinobutyramide hydrolase	AUO16418.1	100/100
niphimycin	<i>orf21</i>	WAC5858	1437	478	amino acid transporter	AUO16419.1	100/100
niphimycin	<i>orf22</i>	WAC5858	867	288	AraC-family transcriptional regulator	AUO16420.1	100/99.7

Supplementary Table 2. NMR data for enniatin B in dms_o-d₆.

Position	δ_{H} (m, J in Hz)	δ_{C}
<i>L-N-Me-Val</i>		
A ₁	4.64 (d, $J = 9.9$ Hz, 1H)	61.17
β_1	2.18 (dp, $J = 9.9, 6.6$ Hz, 1H)	27.92
γ_{1-1}	0.99 (d, $J = 6.6$ Hz, 3H)	20.19
γ_{1-2}	0.84 (d, $J = 6.7$ Hz, 3H)	19.55
N-Me	3.08 (s, 3H)	31.95
C=O (amide)	-	169.14
<i>D-HyIv</i>		
α_2	5.20 (d, $J = 8.1$ Hz, 1H)	74.37
B ₂	2.11 (dq, $J = 13.9, 6.8$ Hz, 1H)	30.02
γ_{2-1}	0.94 (d, $J = 6.7$ Hz, 3H)	18.58
γ_{2-2}	0.90 (d, $J = 6.8$ Hz, 3H)	18.15
C=O (ester)	-	170.48

¹Denotes next to amide bond (L-Val).

² Denotes next to the ester bond (d-2-hydroxy isovaleric acid).

Supplementary Table 3. NMR data for compound 1 in dmso-d6.

<u>Residue #</u>	<u>¹³C, ppm</u>	<u>¹H, ppm, multiplicity</u>
<u>MOTDA</u>		
<u>1</u>	<u>170.9</u>	-
<u>2</u>	<u>51.1</u>	<u>3.7, q</u>
<u>3</u>	<u>206.7</u>	-
<u>4</u>	<u>39.52* under DMSO peak</u>	<u>2.29, m</u>
<u>5</u>	<u>39.4*</u>	<u>1.40, m</u>
<u>6</u>	<u>24.05</u>	<u>1.59, m</u>
<u>7</u>	<u>28.9</u>	<u>1.23, m</u>
<u>8</u>	<u>28.9</u>	<u>1.23, m</u>
<u>9</u>	<u>28.9</u>	<u>1.23, m</u>
<u>10</u>	<u>28.9</u>	<u>1.23, m</u>
<u>11</u>	<u>28.9</u>	<u>1.23, m</u>
<u>12</u>	<u>29.03</u>	<u>1.23, m</u>
<u>13</u>	<u>21.8</u>	<u>1.23, m</u>
<u>14</u>	<u>13.6</u>	<u>0.88, s</u>
<u>15</u>	-	-
<u>Pro</u>		
<u>C=O</u>	<u>171.8</u>	-
<u>α</u>	<u>59.4</u>	<u>4.26</u>
<u>β</u>	<u>23.5</u>	<u>2.14/1.93</u>
<u>γ</u>	<u>24.0</u>	<u>1.92/1.99</u>
<u>δ</u>	<u>47.3</u>	<u>3.55/3.63</u>
<u>Aib</u>		
<u>C=O</u>	<u>174.0</u>	-
<u>α</u>	<u>56.7</u>	-
<u>β-1</u>	<u>23.6</u>	<u>1.31</u>
<u>β-2</u>	<u>23.6</u>	<u>1.24</u>
<u>NH</u>	-	<u>7.57</u>
<u>Iva</u>		
<u>C=O</u>	-	-
<u>α</u>	<u>59.6</u>	-
<u>β</u>	<u>28.95</u>	<u>1.75</u>
<u>γ</u>	<u>7.8</u>	<u>0.66</u>
<u>δ</u>	<u>21.8</u>	<u>1.32</u>
<u>NH</u>	-	-
<u>β-Ala</u>		
<u>C=O</u>	<u>174.0</u>	-
<u>α</u>	<u>35.25</u>	<u>2.33</u>
<u>β</u>	<u>35.5</u>	<u>3.2</u>
<u>NH</u>	-	<u>7.4</u>
<u>Ala</u>		
<u>C=O</u>	-	-
<u>α</u>	<u>49.2</u>	<u>4.10 (HMBC-17.1)</u>
<u>β</u>	<u>17.4</u>	<u>1.24 (HMBC-49.4)</u>
<u>NH</u>	-	-
<u>Leu</u>		
<u>C=O</u>	-	-
<u>α</u>	<u>51.8</u>	<u>4.11</u>
<u>β</u>	<u>39.6</u>	<u>1.48</u>
<u>γ</u>	<u>23.8</u>	<u>1.60</u>
<u>δ-1</u>	<u>23.1</u>	<u>0.84</u>
<u>δ-2</u>	<u>21.14</u>	<u>0.80</u>
<u>NH</u>	-	<u>8.64</u>
<u>Asp</u>		
<u>COOH</u>	<u>166.7</u>	-
<u>α</u>	<u>60.62</u>	<u>4.28 (t, J = 6.6 Hz, 1H)</u>
<u>β, γ</u>	<u>24.85</u>	<u>2.02/1.91</u>
<u>COOH</u>	<u>166.7</u>	-
<u>NH</u>	-	<u>8.85</u>

Supplementary Table 4. *In vitro* susceptibility of prokaryotic isolates to coniotin A

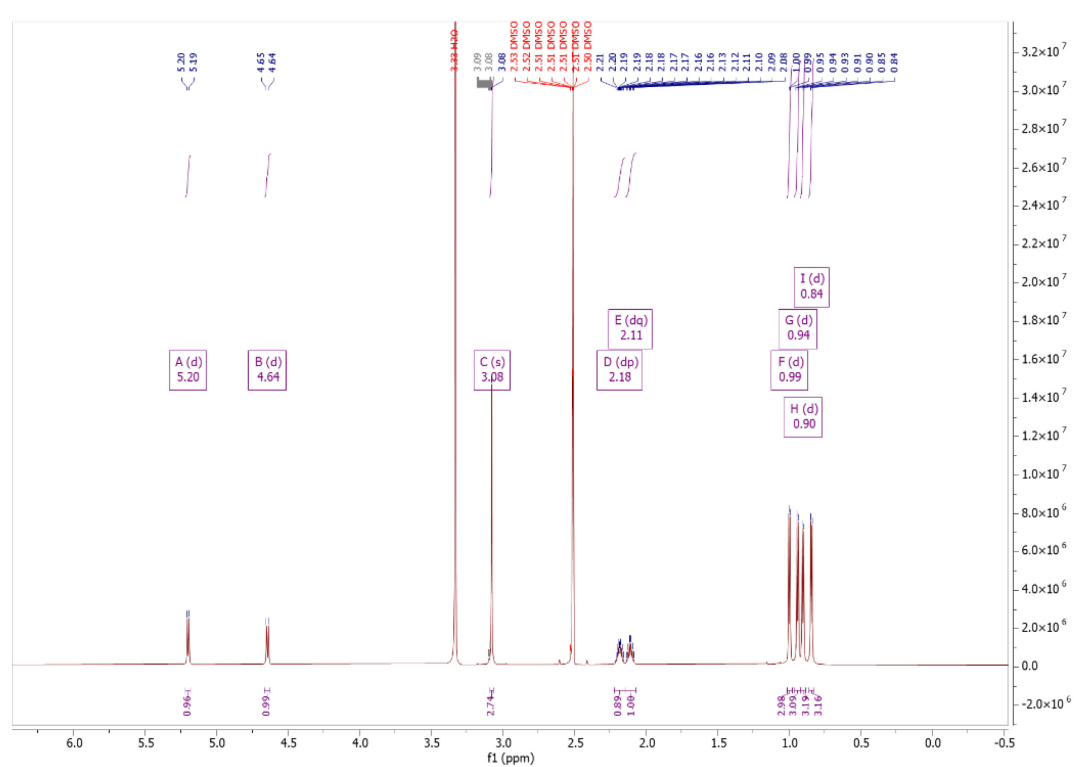
	MIC(μ g/mL)	
	Coniotin A	Ciprofloxacin
<i>Enterococcus faecium</i> ATCC19434	>128	16
<i>Staphylococcus aureus</i> ATCC29213	>128	0.5
<i>E. coli</i> BW25113	>128	0.125
<i>E. coli</i> BW25113 Δ bamB Δ tolC	>128	0.125
<i>Micrococcus luteus</i> ATCC4698	>128	1

Supplementary Table 5. Annotated putative ORFs in and near the biosynthetic gene cluster of coniotin A.

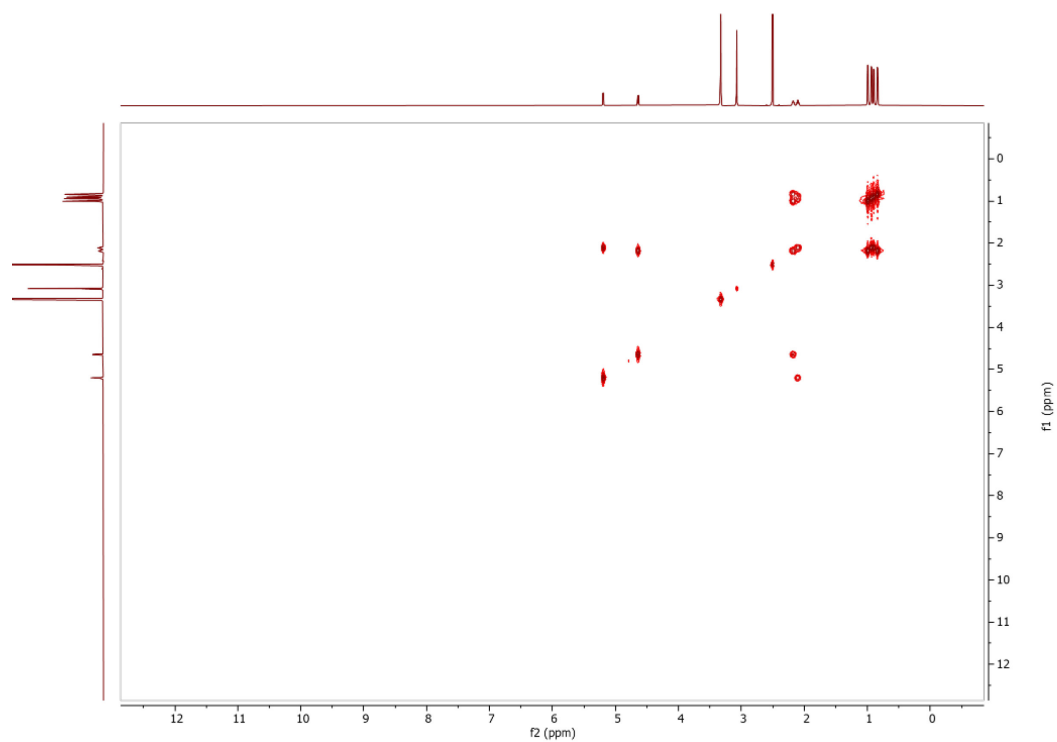
orf	Size	aa	Gene annotation/proposed function	Accession no.	Alignment
<i>orf1</i>	954	317	hypothetical protein GE09DRAFT_1232679	KAB5511254.1	31/39
<i>orf2 (conF)</i>	1722	573	Transferase BOT5	B2RML5.1	25/25
<i>orf3</i>	1128	375	trans-enoyl reductase himH	A0A2Z5TIQ0.1	92/38
<i>orf4</i>	468	155	sulfite transporter Ssu1	KAI0155599	97/64
<i>orf5</i>	1098	365	Glucose-methanol-choline (Gmc) oxidoreductase	KAJ9155381.1	99/49
<i>orf6</i>	627	208	hemerythrin HHE cation binding domain-	OIW24587.1	97/64
<i>orf7(conE)</i>	1677	558	Acyl-CoA ligase easD	Q5BA81	92/38
<i>orf8(conA)</i>	6888	2295	Highly reducing polyketide synthase easB	Q5BA83	99/46
<i>orf9</i>	546	181	NF	NF	NF
<i>orf10</i>	327	108	Transcription factor lcsF	KAI9152064.1	78/48
<i>orf11</i>	1014	337	GMC oxidoreductase	KAJ6443798.1	77/61
<i>orf12</i>	1098	365	2OG-Fe dioxygenase-domain-containing protein	KAH6995494.1	83/73
<i>orf13</i>	1071	356	arad-like aldolase/epimerase	KAH8902226.1	79/78
<i>orf14</i>	1011	336	RTA1 like protein-domain-containing protein	KAH7142602.1	94/65
<i>orf15</i>	1605	534	pyridoxal phosphate-dependent transferase	XP_046111411.1	94/78
<i>orf16</i>	678	225	Alpha/Beta hydrolase protein	KAH7016706.1	94/60
<i>orf17(conB)</i>	45474	15157	non-ribosomal peptide synthetase	RFU80400.1	96/47
<i>orf18</i>	726	241	HAD-like protein	PTB72772.1	98/66
<i>orf19</i>	399	132	PLAC8 family protein	KAH8179313.1	78/56
<i>orf20</i>	789	262	hypothetical protein DL765_001124	RYP23419.1	98/91
<i>orf21</i>	2349	782	ABC transporter	KAH7142595.1	98/58
<i>orf22</i>	261	86	NF	NF	NF
<i>orf23</i>	870	289	Peroxidase	KAH7144262.1	98/60
<i>orf24(conC)</i>	23076	7691	non-ribosomal peptide synthetase	XP_046111418.1	99/58
<i>orf25</i>	1032	343	integral membrane protein	KZL85847.1	61/62
<i>orf26</i>	1686	561	GMC oxidoreductase	OIW34248.1	83/93
<i>orf27</i>	1071	356	putative reticulocyte-binding protein	XP_007911148.1	96/33
<i>orf28</i>	1836	611	HET-domain-containing protein	OIW34249.1	97/80
<i>orf29</i>	849	282	hypothetical protein GE09DRAFT_1065772	KAB5522051.1	68/29
<i>orf30</i>	837	278	alpha/beta-hydrolase	OIW34255.1	87/88
<i>orf31(conD)</i>	3483	1160	Non-ribosomal peptide synthetase lcsA	A0A179H164.1	77/28
<i>orf32</i>	1059	352	hypothetical protein BR93DRAFT_939006	KAH8904942.1	98/74
<i>orf33</i>	300	99	hypothetical protein CONLIGDRAFT_626311	OIW34258.1	95/89
<i>orf34</i>	843	280	hypothetical protein CONLIGDRAFT_687963	OIW34259.1	100/84
<i>orf35</i>	762	253	Pyruvate dehydrogenase (Lipoamide) phosphatase	O14189.4	90/41
<i>orf36</i>	411	136	hypothetical protein	NEMBOFW57_004876	65/52
<i>orf37</i>	423	140	Cytochrome P450 monooxygenase ATR14	A0A084R1J2.1	81/38

NF, no significant similarity found

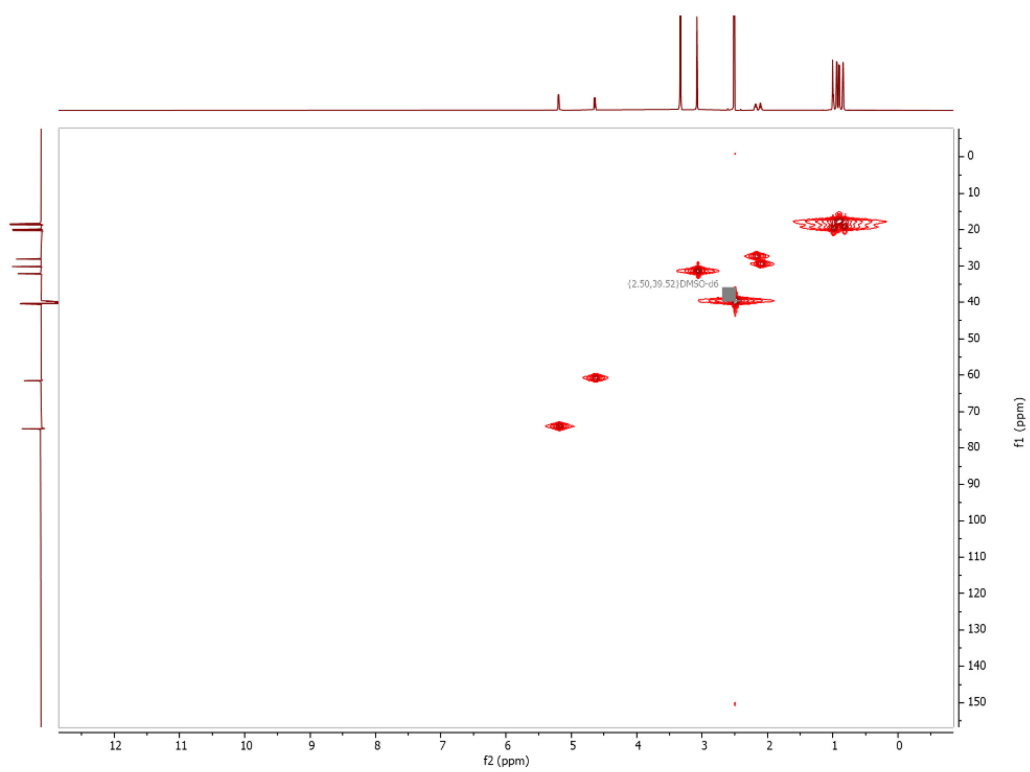
The chemical structure shows a cyclic peptide with a ring of six amide bonds. The side chains include isopropyl and isobutyl groups, some of which are attached to the nitrogen atoms of the amide bonds. The structure is drawn in a perspective view, showing the spatial arrangement of the atoms and bonds.

¹H- NMR spectra of enniatin B in dmso-d6

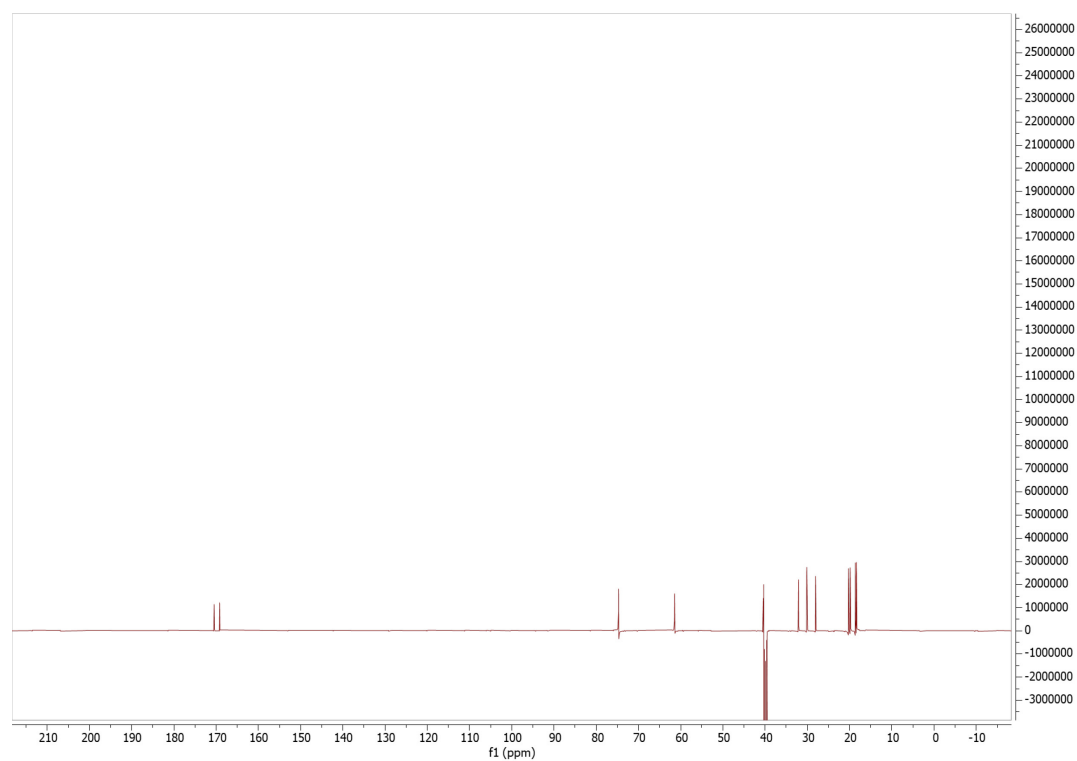
^1H - ^1H - COSY NMR spectra of enniatin B in dmso-d6



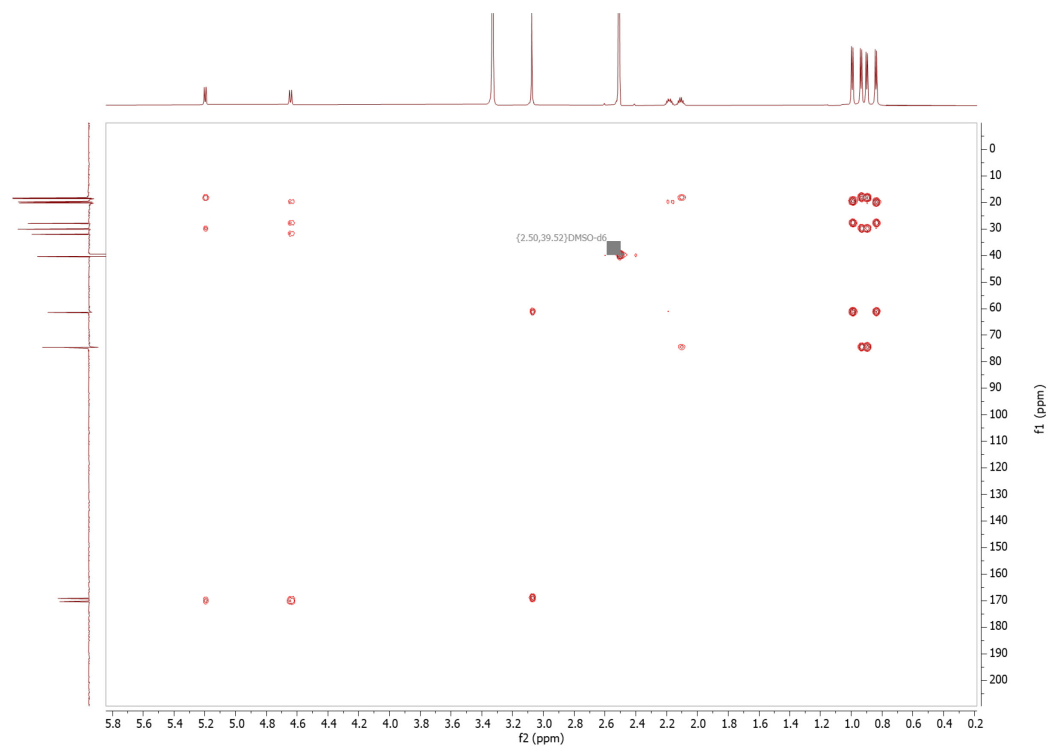
^1H - ^{13}C -NMR- HSQC spectra of enniatin B in dmso-d6



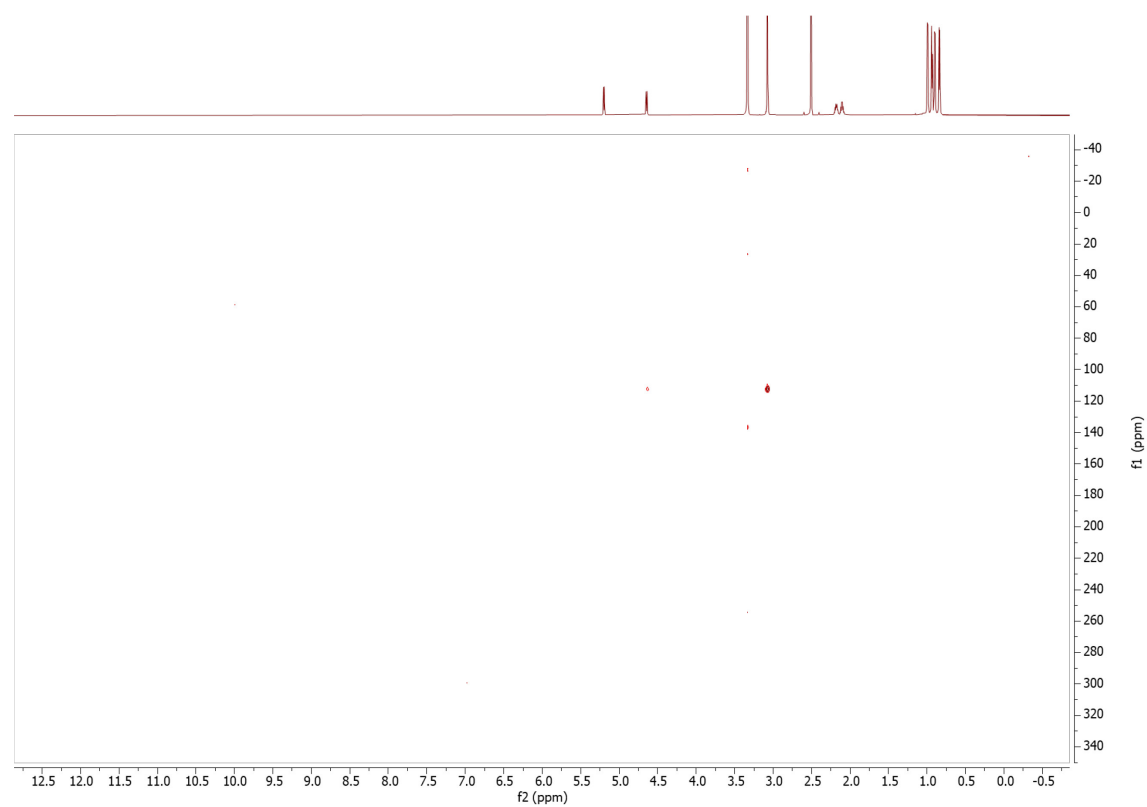
Dept q 135 NMR spectra of enniatin B in dmso-d6



^1H - ^{13}C -NMR- HMBC spectra of enniatin B in dmso-d6

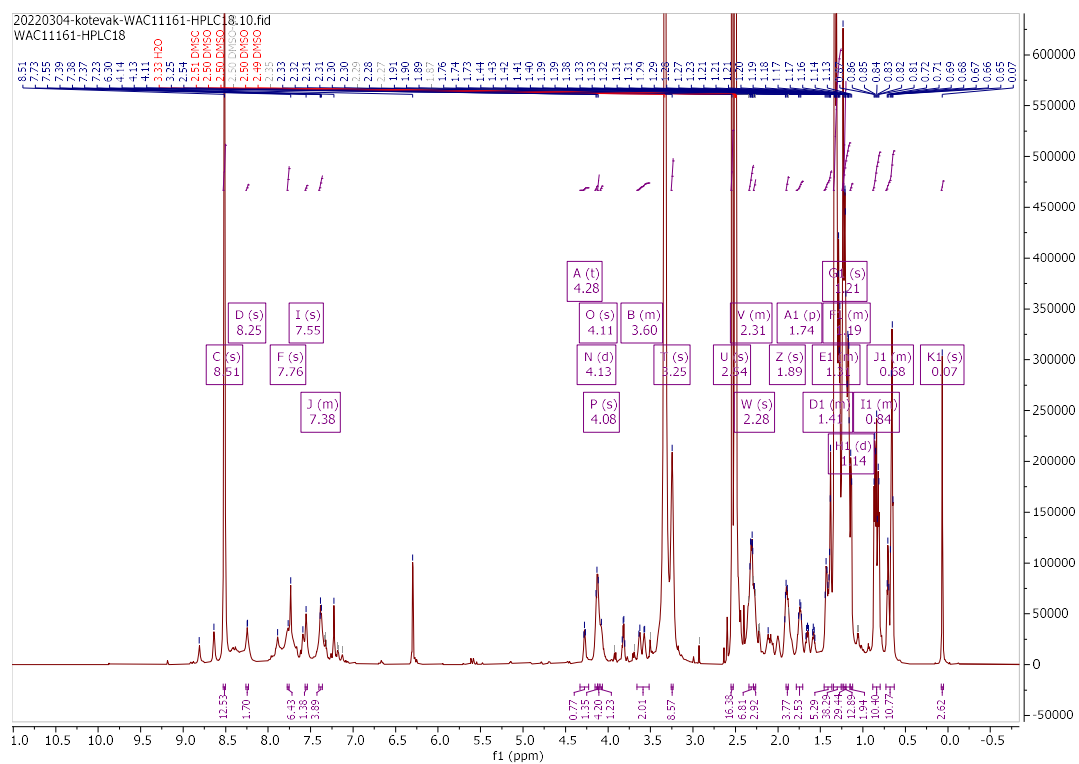


^1H - ^{15}N -NMR- HMBC spectra of enniatin B in dms o -d $_6$

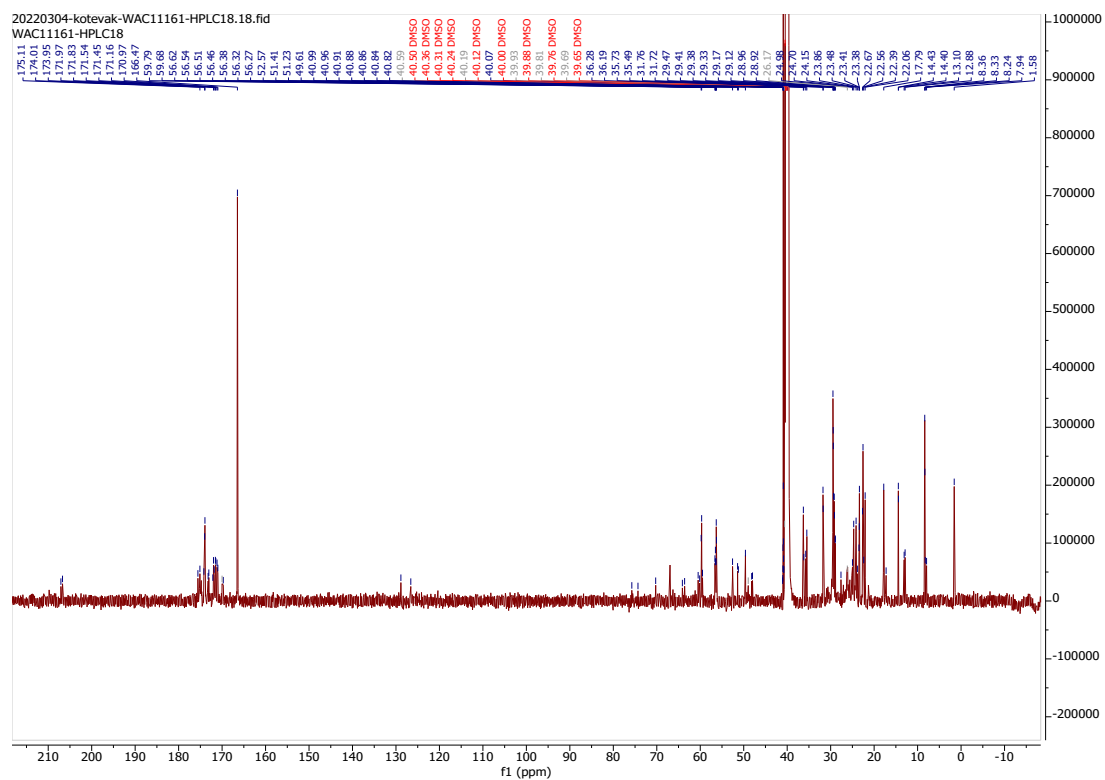


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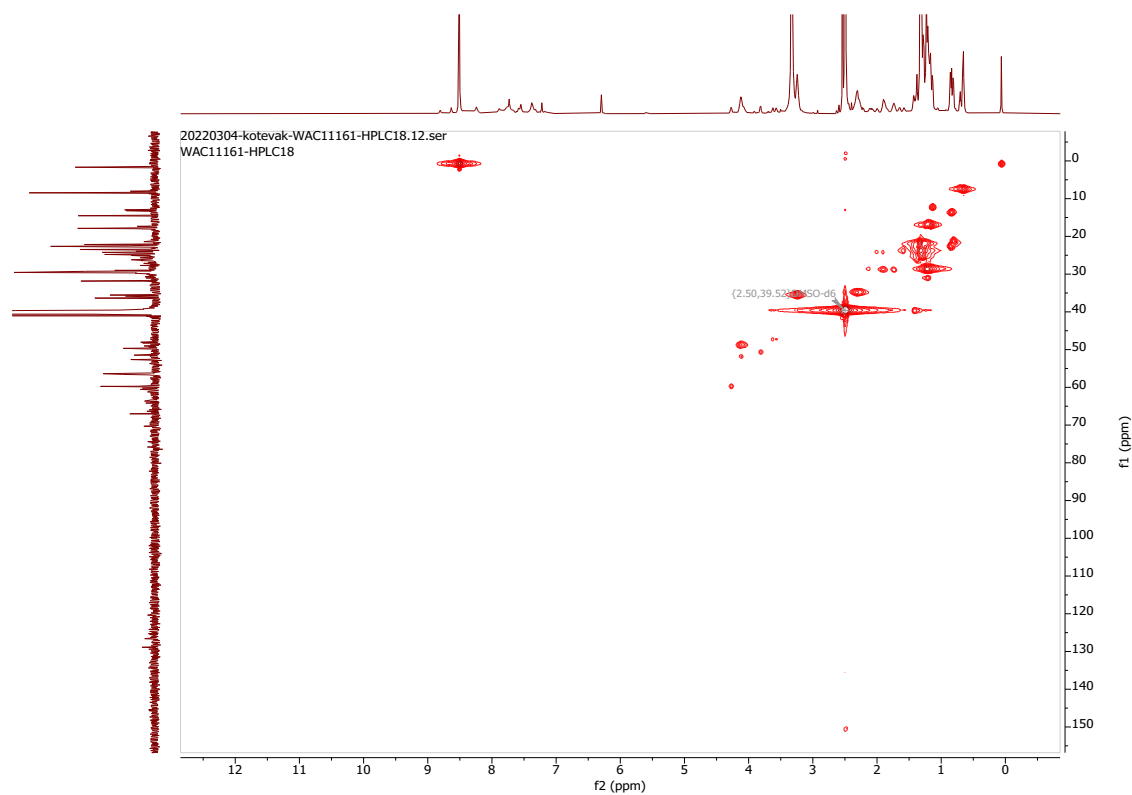
¹H- NMR spectra of **1** in dms0-d6



^{13}C NMR spectra of 1 in dms $-\text{d}_6$



^1H - ^{13}C -NMR- HSQC spectra of 1 in dmsO-d6



^1H - ^{15}N -NMR- HSQC spectra of 1 in dmsO-d6

