Supporting Information

Elucidating the rimosamide-detoxin natural product families and their biosynthesis using metabolite/gene cluster correlations

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Supplemental Materials and Methods

Strains. Beyond the 178 strains surveyed by metabologenomics, the following strains were used:

***E. coli* WM4489**: *E. coli* DH10B derivative; *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80(Δ*lacM15*) Δ*lacX74 endA1 recA1 deoR*Δ(*ara leu*)*7697 araD139 galU galK nupG rpsL*λ*attB*::pAE12(P*rhaB*::*trfA33* Δ*oriR6K-cat*::*frt-5*).

***E. coli* WM6029**: *dam-3 dcm-9 metB1 galK2 galT27 lacY1 tsx-78 supE44 thi mel-1 tonA31* Δ2 (*mcrC-mrr*)::*frt*Δ(*endA*)::*frt att*λ::pAE12-Δ1(*oriR6K-cat*::*frt5*) Δ*5816*(*dapA*)::*frt uidA*(ΔMluI)::*pir*(wt)*attHK*::pJK1006:: Δ1/2(Δ *oriR6K-cat trfA*)

***S. rimosus* NRRL B-2659**: Rimosamides producer; also referred to as ATCC 10970, ATCC 23955, CBS 437.51, CBS 938.68, DSM 40260, IFO 12907, IFO 3441, IFO 3725, IMET 40364, IMRU 3558, JCM 4073, JCM 4667, NCIB 8229, NRRL 2234, RIA 606

***S. rimosus* NRRL WC-3879**: Strain that contains GCF NRPS_502 but not NRPS_436, and detection of the *m/z* 605 ion led to the determination that gene cluster family NRPS_502 was the BGC for the rimosamides.

***S. lividans* 66**: Heterologous host for rimosamide production.

***S. lividans* 66-pRAM4**: Derivative of *S. lividans* 66 containing the rimosamide gene cluster from *S. rimosus* NRRL B-2659; Apr^r

***S. lividans* 66-pRAM6**: Derivative of *S. lividans* 66 containing NRPS_432 from *S. rimosus* NRRL B-2659 and incapable of producing the rimosamides. Control for heterologous expression; Apr^r

Plasmids. The following plasmids were used in this study:

pJK050: Double-*cos* fosmid vector; *oriV λattB loxP* FRT Cm^r; graphical map shown in **Figure S4**.

pAE4: OriT Apr^r λ*attP* ϕC31 *int* ϕC31*attP*; graphical map shown in **Figure S4**.

pRAM3: *S. rimosus* NRRL B-2659 genomic DNA cloned into pJK050; contains rimosamide gene cluster.

pRAM4: pRAM3 recombined with pAE4.

pRAM5: *S. rimosus* NRRL B-2659 genomic DNA cloned into pJK050; contains NRPS_432.

pRAM6: pRAM5 recombined with pAE4.

Primers. The following primers were used in this study:

rmoB_F: 5'–CACCGAGTTCAAGGAGCAC—3'

rmoB_R: 5'–GGAACTCCTCGACCGTACC—3'

rmoL_F: 5'–CAGCGGTTGTCCCAGAAAAC—3'

rmoL_R: 5'–TACTGGAACTGAGCACCACC—3'

Media and Buffer Recipes. Recipes for media are provided based upon 1 L volumes and can be prepared as broths (as listed) or as solid plates by the addition of 15 g of agar (per liter).

ISP2 Medium: 10 g malt extract, 4 g yeast extract, 4 g dextrose; pH 7.2

ISP4 Medium: 10 g soluble starch, 1 g dipotassium phosphate, 1 g magnesium sulfate, 1 g sodium chloride, 2 g ammonium sulfate, 2 g calcium carbonate, 1 mg ferrous sulfate, 1 mg manganous chloride, 1 mg zinc sulfate; pH 7.2

AGS Medium: 1 g arginine, 12.5 g glycerol, 1 g dipotassium phosphate, 1 g sodium chloride, 1 g magnesium sulfate heptahydrate, 0.5 g ferric sulfate hexahydrate, 10 mg cupprous sulfate, 1 mg zinc sulfate, 1 mg, manganese sulfate; pH 7.0

MS Medium: 10 g soy flour, 20 g mannitol; pH 7.2

ATCC Medium 3: 3 g beef extract, 6 g peptone; pH 6.8

TE25S Buffer: 25 mM Tris-HCl, 25 mM EDTA, 0.3 M sucrose; pH 8

2X Kirby Mix: 12 g sodium 4-aminosalicilate, 5 mL 2 M Tris-HCl (pH 8), 6 mL buffer equilibrated phenol (pH 8); make up to 100 mL

Isolation of Rimosamides. *Streptomyces* sp. NRRL B-2659 was streaked out on an ISP2 plate and grown for four days at 30°C. An individual colony was picked and grown in 5 mL of ISP2 medium for three days at 30°C before 1 mL of the culture was transferred to 500 mL of AGS medium in 2 L flasks and grown at 30°C for seven days. The culture supernatant was separated from cells by centrifugation and separated by solid phase extraction (SPE) using an Oasis Hydrophilic/Lipophilic Balanced (HLB) column from Waters. SPE fractions eluted with 70–100% acetonitrile (60 mg) were further separated on a Phenomenex Luna C18 column (150 × 10 mm, 5 µm) using acetonitrile–H_2O with 0.1% formic acid to yield ~1.5 mg of rimosamides. Purified rimosamides were analyzed by LC− MS^2 on a Q-Exactive Orbitrap MS instrument.

Biological Activity Screening of Rimosamide D. Antagonism of blasticidin S in *B. cereus* was observed by Kirby-Bauer Disk Diffusion Assay.[1] *B. cereus* was pre-grown on ATCC Medium 3 plate at 30°C for 24 hours before a single colony was used to inoculate 5 mL of ATCC Medium 3, which was grown at 30°C for 16 hours. *E. coli* was pre-grown on ATCC Medium 3 plate at 37°C for 24 hours before a single colony was used to inoculate 5 mL of ATCC Medium 3, which was grown at 37°C for 16 hours. Paper disks were treated with 10 µL of 1 mg/mL blasticidin S, 10 µL of 2.5 mg/mL rimosamide, or 10 µL of mixed stocks. Disks were placed on an ATCC Medium 3 plate seeded with 100 µL of pre-grown *B. cereus* or *E. coli* and incubated at 37°C for 16 hours.

Extraction of Streptomyces rimosus Genomics DNA. High-molecular weight genomic DNA was isolated from *S. rimosus* NRRL B-2659 using a modification of the method described previously.[2] Briefly, a 5 mL culture of *S. rimosus* NRRL B-2659 was grown in ISP2 broth for 48 hours. 3 mL of this culture were used to inoculate 100 mL of ISP2 + 0. 25% glycine in a 500 mL baffled culture flask. After 48 hours at 30°C, 25 mL were removed and homogenized using a sterile Dounce tissue homogenizer. The homogenized cells were pelleted by centrifugation (5000 rpm for 10 min), washed with 12 mL TE25S buffer and re-suspended in 6 mL TE25S. 0.06 g of lysozyme and 0.006 g of achromipeptidase were added and the suspension was incubated at 37°C for 1-2 hours. 1 mg of proteinase K was added and suspension was incubated for an additional 30 min at 37°C. After incubation, 6 mL of 2X Kirby Mix was added and the mixture was gently agitated and then incubated at 70°C for 5 min. The resulting lysate was sequentially extracted with buffer saturated phenol, chloroform, and isoamyl alcohol (25:24:1) and chloroform and isoamyl alcohol (24:1). Genomic DNA was precipitated from the aqueous layer by addition of 0.1 vol of 5 M NaCl and 0.7 vol isopropanol, spooled onto a glass rod, washed three times with 70% EtOH and once with 100% EtOH, dried, and re-suspended in 0.6 mL H_2O.

Generation and Screening of Fomid Library. Approximately 5 µg of this genomic DNA was partially digested with Sau3AI (New England BioLabs) to yield fragments of ~30-50 kb, which were then treated with shrimp alkaline phosphatase. The prepared pJK050 plasmid was digested sequentially with NheI and BamHI. This vector was treated with shrimp alkaline phosphatase between the NheI and BamHI digests to prevent the formation of vector concatamers. The digested genomic DNA was ligated into BamHI- and NheI-digested pJK050 using T4 DNA ligase (2,000,000 units/mL). The ligated DNA was purified by EtOH precipitation and packaged into lambda phage using the MaxPlax packaging extract (Epicentre) according to the manufacturer's instructions. E. coli WM4489[3] cells were transfected with the packaged library and plated

on LB agar + 15 µg/mL chloramphenicol (Cm). The *E. coli* library was screened by PCR for clones containing the targeted gene cluster. Each reaction consisted of 1 µL culture broth, 500 nM of each primer (rmoB_F and rmoB_R), and 2X GoTaq Green MasterMix polymerase (Promega). The annealing temperature was 54°C.

Heterologous Expression in *S. lividans 66*. Fosmid DNA was isolated from positive clones grown overnight in 5 mL LB + 15 µg/mL Cm + 10 mM rhamnose (to induce expression) using a Qiagen Miniprep kit (Qiagen Inc.). To add the functions necessary for transfer and integration into *S. lividans* 66, the purified fosmids were individually recombined *in vitro* with pAE4 using Gateway BP Clonase II (Thermo Fisher Scientific) according to the manufacturer's instructions. Fosmid:pAE4 cointegrants were moved into the conjugal donor strain E. coli WM6029[4] for transfer to *S. lividans*.[5] Conjugal transfer to *S. lividans* 66 was performed as described previously [6]. After 16 hours at 30°C, plates were flooded with 1 ml of apramycin (1 mg/mL) and incubated at 30°C for an additional 5-7 days, at which point exconjugants were picked and restreaked on MS + Apr (50 µg/mL) and grown for 3-4 days. Correct integration of the fosmid into the genome of *S. lividans* 66 was verified by PCR amplification of the rmoB fragment and rmoL fragment from the purified genomic DNA. To test rimosamide production, exconjugants were first inoculated into 3mL ISP2 broth + Apr (50 µg/mL) and incubated at 30°C for 3 days. This seed culture was then used to inoculate 5mL of ISP2, ISP4, AGS, and MS—each with Apr (50 µg/mL)—and incubated at 30°C for an additional 4 days. The culture supernatant was extracted as previously described. Resuspended extracts were analyzed by LC-MS.

A similar procedure was followed for the construction of *S. lividans* 66-pRAM6, a derivative of *S. lividans 66* containing a *S. rimosus* NRRL B-2659 gene cluster that is incapable of producing the rimosamides. No amount of the rimosamides were detected in this strain.

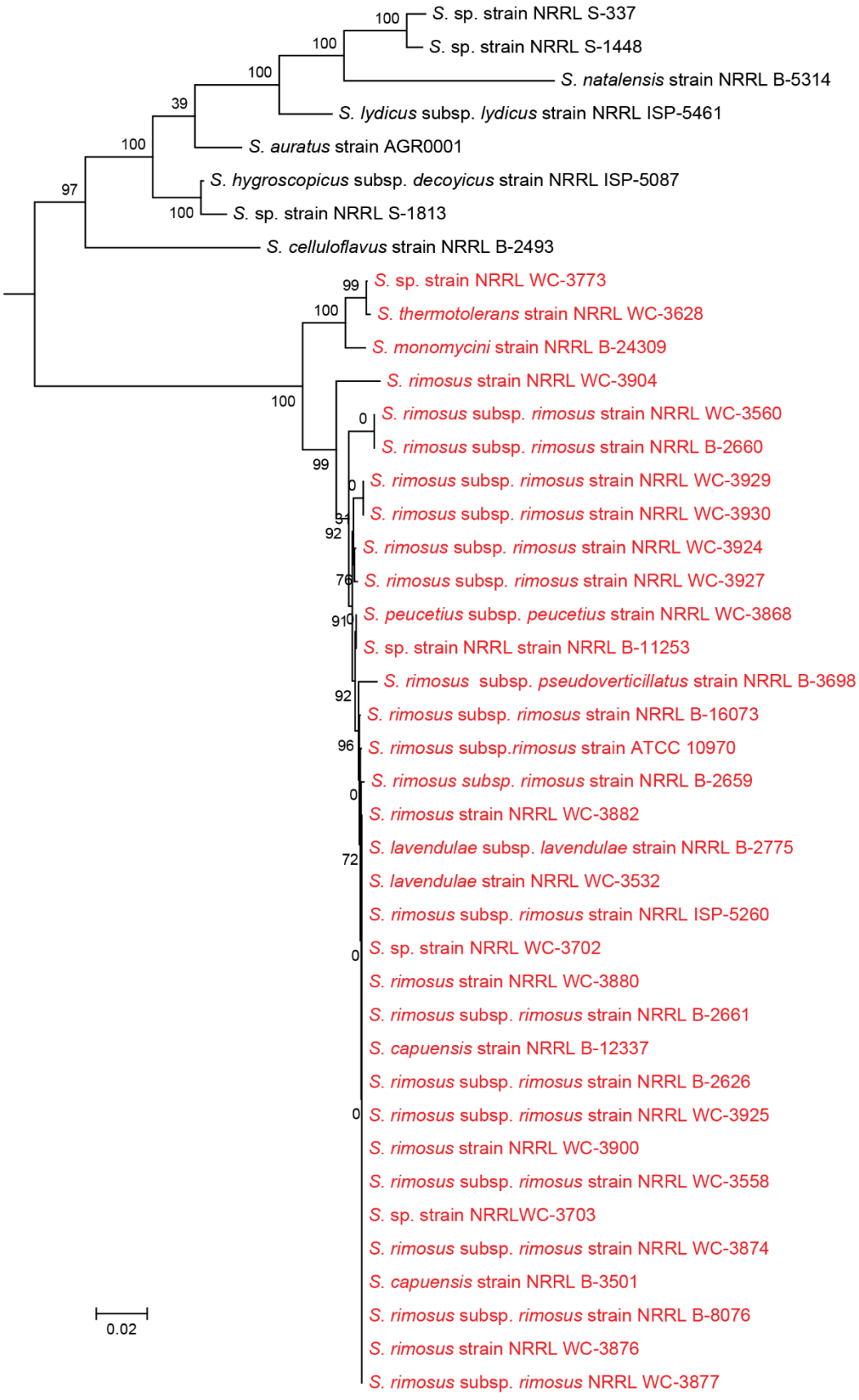

Figure S1. Phylogenetic analysis of *Streptomyces* strains containing the rimosamide gene cluster (a member of gene cluster family number NRPS_502).

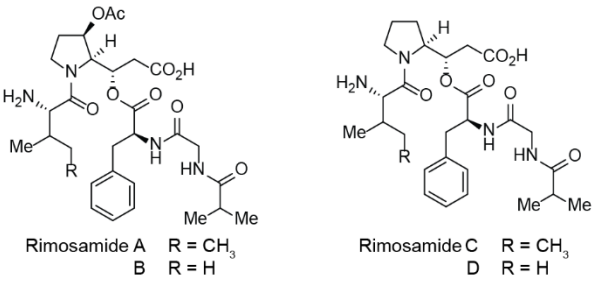

Figure S2. Structures of rimosamides A-D.

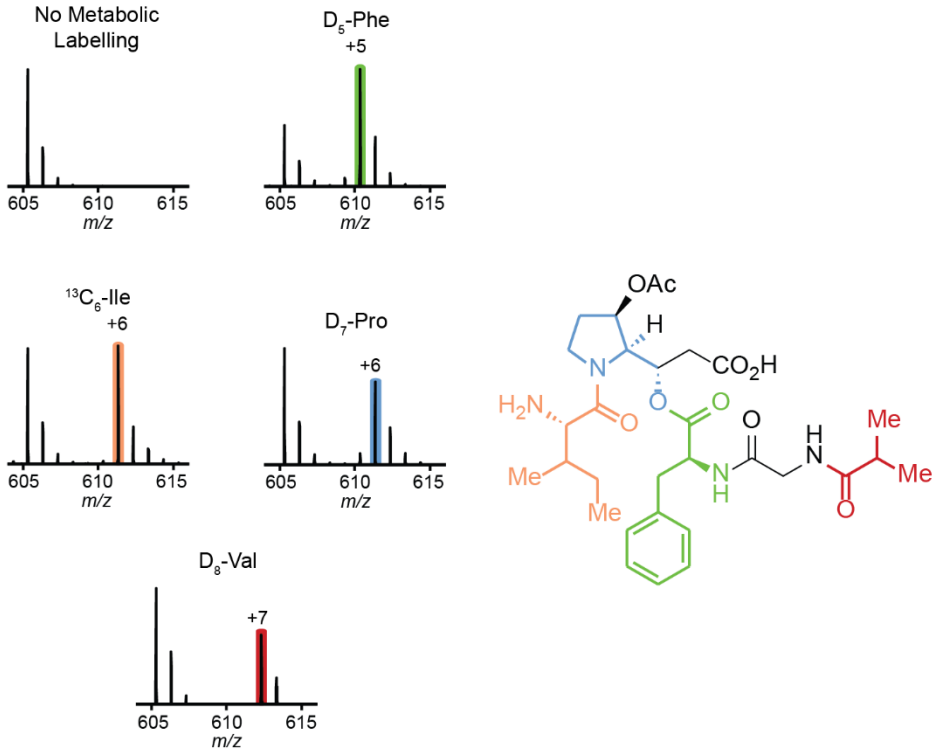

Figure S3. Stable isotope labeled-amino acid incorporation of D_5-phenylalanine, $^{13}C_6$-isoleucine, D_7-proline, and D_8-valine for rimosamide A (*m/z* 605).

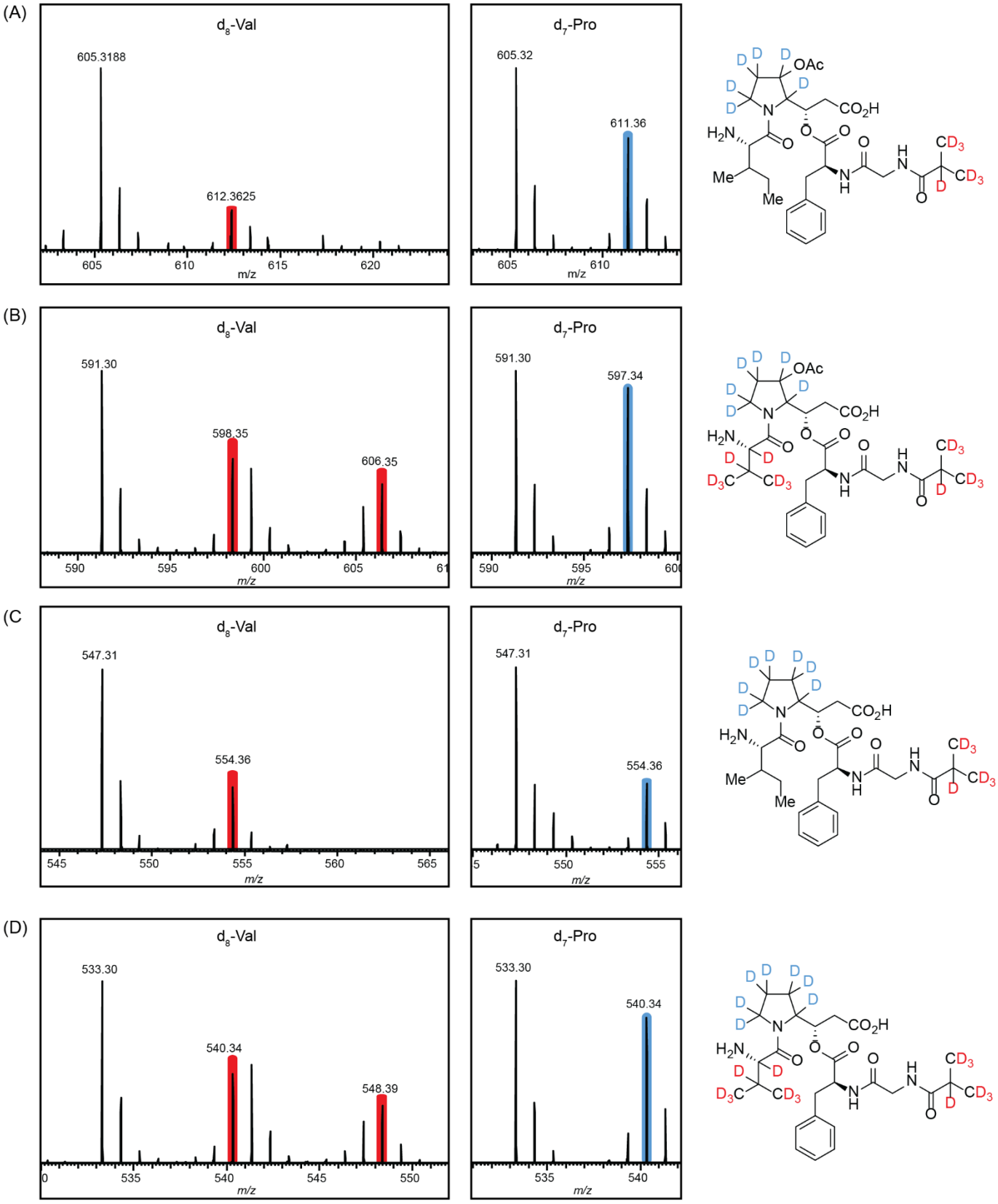

Figure S4. Stable isotope incorporation of D_8-valine (Left) and D_7-proline (Center) for rimosamides A-D. Structures of rimosamides A-D with highlighted deuterons are shown (Right).

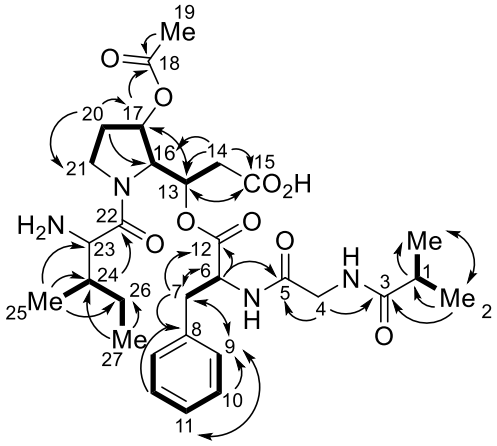

Figure S5. 2D NMR correlations for rimosamide A. HMBC couplings are depicted as arrows (from ^{1}H to ^{13}C) and COSY correlations are shown as bolded bonds.

Table S1. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectroscopic data for Rimosamide A, in D_2O.

No.	δ_c	Proton	δH (J_{HH}[Hz])	HMBC	ROESY
1	35.55	1-H	2.50 ($J_{1,2}$ = 7.1)	2,3	2
2	19.26	2-3H	1.07 ($J_{2,1}$ = 7.1)	1,2,3	1
3	182.03				
4	42.71	4-Ha	3.79 ($J_{4Ha,4Hb}$ = 16.9)	3,5	4Hb
		4-Hb	3.83 ($J_{4Hb,4Ha}$ = 16.9)	3,5	4Ha
5	171.57				
6	54.91	6-H	4.70 ($J_{6,7Ha}$ = 8.9, $J_{6,7Hb}$ = 6.4)	5,7,12	7Ha/b
7	37.25	7-Ha	2.99 ($J_{7Ha,6}$ = 8.9, $J_{7Ha,7Hb}$ = 14.0)	6,8,9,12	6,9
		7-Hb	3.20 ($J_{7Hb,6}$ = 6.4, $J_{7Hb,7Ha}$ = 14.0)	6,8,9,12	6,9
8	137.03				
9	129.92	9-2H	7.26 ($J_{9,10}$ = 8.0)	7,10,11	6,7Ha/b,10
10	129.54	10-2H	7.37 ($J_{10,9}$ = 8.0, $J_{10,11}$ = 7.6)	9,8,10	9,11
11	127.94	11-H	7.30 ($J_{11,10}$ = 7.6)	9	10
12	171.87				
13	72.92	13-H	5.67 ($J_{13,14Ha}$ = 6, $J_{13,14Hb}$ = 7.5, $J_{13,16}$ = 3.2)		14
14	39.97	14-Ha	2.46 ($J_{14Ha,14Hb}$ = 16, $J_{14Ha,13}$ = 6)	13,15,16	13
		14-Hb	2.51 ($J_{14Ha,14Hb}$ = 16, $J_{14Hb,13}$ = 7.5)		
15	178.24				
16	59.35	16-H	4.74 ($J_{16,13}$ = 3.2, $J_{16,17}$ = 7)		
17	72.50	17-H	5.23 ($J_{17,16}$ = 7.0, $J_{17,20Ha}$ = 6.5, $J_{17,20Hb}$ = 7)	13,18	20Hb
18	174.02				
19	21.29	19-3H	2.11	18	
20	30.53	20-Ha	1.86 ($J_{20Ha,17}$ = 6.5, $J_{20Ha,20Hb}$ = 12, $J_{20Ha,21Ha}$ = 9.5, $J_{20Ha,21Hb}$ = 4.5)	17	20Hb,21Ha
		20-Hb	2.26 ($J_{20Hb,17}$ = 7, $J_{20Hb,20Ha}$ = 12, $J_{20Hb,21Ha}$ = 5, $J_{20Hb,21Hb}$ = 9)	16,21w	17,20Ha,21Hb
21	46.50	21-Ha	3.64		20Ha,21Hb
		21-Hb	3.77		20Hb,21Ha

22	174.69				
23	57.21	23-H	4.10		
24	37.28	24-H	1.95 multiplet	22	
25	15.58	25-3H	1.08	23,24,26	27
26	24.16	26-Ha	1.20 multiplet		27
		26-Hb	1.51 multiplet		27
27	11.56	27-3H	0.95 ($J_{27,26Ha}$ = 7.6, $J_{27,26Hb}$ = 7.6)	24,26	25, 26Ha/b

Chemical shifts were referenced to sodium formate $\delta(^{1}H) = 8.44$ and $\delta(^{13}C) = 171.67$.^{13}C chemical shifts were determined via HMBC and HSQC spectra. ^{1}H, ^{1}H-*J*-coupling constants were determined from the acquired ^{1}H or dqfCOSY spectra. ROESY correlations were observed using a mixing time of 400 ms. HMBC correlations are from the proton(s) stated to the indicated ^{13}C atom.

Table S2. Annotation of the rimosamide biosynthetic gene cluster (from NRRL B-2659).

ORF	No. of amino acids	Function in Rimosamide BGC	Homolog function	Homolog accession	Fractional amino acid identity (%)
rmoA	316	Unknown	virginiamycin B lyase	KOT78047.1	311/313 (99)
rmoB	271	Unknown	Endo-1,4-beta-glucanase	KUN45543.1	127/234 (54)
rmoC	197	Unknown	RNA polymerase subunit sigma	WP_031477326.1	136/158 (86)
rmoD	242	Unknown	hypothetical protein	WP_053697031.1	220/241 (91)
rmoE	337	Unknown	DNA polymerase LigD, polymerase	KPI33163.1	260/330 (79)
rmoF	356	Unknown	ATP-dependent DNA ligase	WP_060730144.1	347/353 (98)
rmoG	1015	C-A-T	non-ribosomal peptide synthetase	WP_052853217.1	728/1002 (73)
rmoH	1351	C-A-T-TE	non-ribosomal peptide synthetase	KOU38458.1	881/1270 (69)
rmoI	3129	A-T-C-A-T-KS-KR-T-TE	non-ribosomal peptide synthase	WP_052853214.1	2270/3102 (73)
rmoJ	380	Unknown	hypothetical protein	WP_030675315.1	273/348 (78)
rmoK	209	Unknown	hypothetical protein	EME99938.1	161/207 (78)
rmoL	292	α-ketoglutarate-dependent oxygenase	taurine dioxygenase	WP_030882775.1	250/290 (86)
rmoM	268	Unknown	SpcZ	WP_052853210.1	150/248 (60)
rmoN	762	Unknown	membrane protein	WP_037771838.1	593/748 (79)
rmoO	122	Unknown	hypothetical protein	WP_030077687.1	98/121 (81)

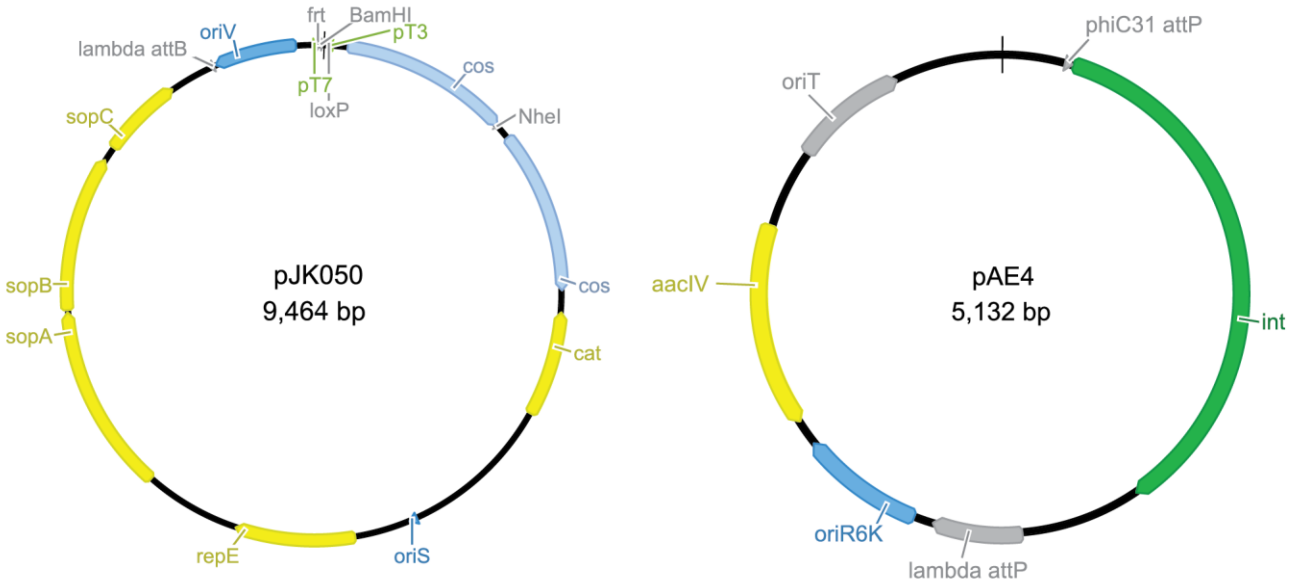

Figure S6. Graphical maps of the plasmids used in this study, pJK050 and pAE4.

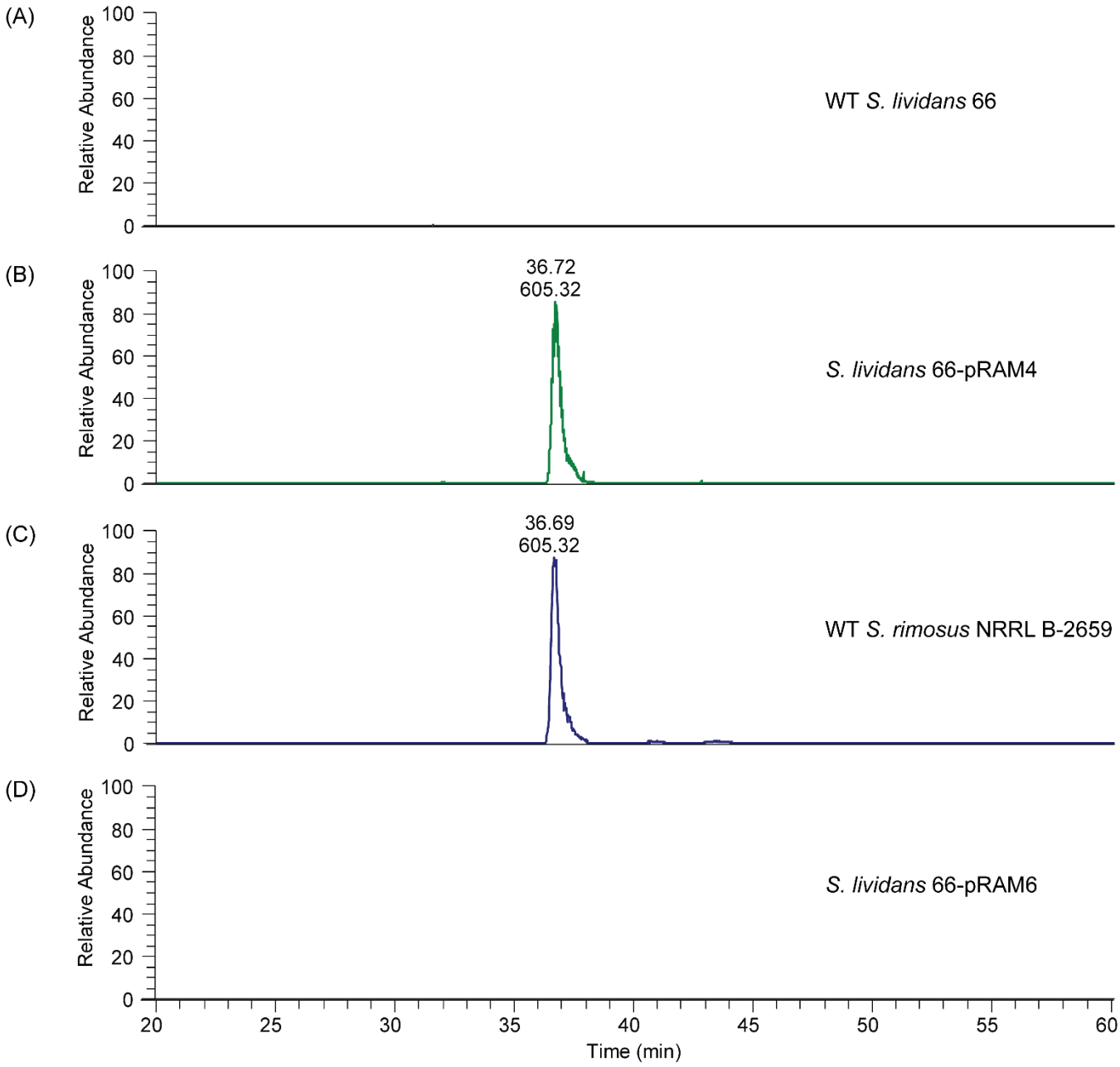

Figure S7. Heterologous expression of the rimosamides in *S. lividans* 66. Selected ion chromatogram for *m/z* 605.31 (rimosamide A) in (A) wildtype S. lividans 66, (B) *S. lividans* 66-pRAM4; contains the BGC correlated to the rimosamides, (C) wildtype *S. rimosus* NRRL B-2659; a native producer of the rimosamides, and (D) *S. lividans* 66 pRAM6; contains a *S. rimosus* NRRL B-2659 that was not highly correlated to the rimosamides.

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Table S3. Annotation of the putative detoxin biosynthetic gene cluster (from *S. mobarensis* NRRL B-3729).

ORF	No. of amino acids	Function in Detoxin BGC	Homolog function	Homolog accession	Fractional amino acid identity (%)
detA	2169	Unknown	membrane protein	KIZ19073.1	31/49 (63)
detB	177	Unknown	hypothetical protein	WP_037706151.1	46/76 (61)
detC	231	Unknown	hydrolase	WP_030901448.1	118/190 (62)
detD	316	Unknown	hypothetical protein	WP_052862832.1	202/306 (72)
detE	383	Unknown	hypothetical protein	WP_017240822.1	235/332 (71)
detF	1355	C-A-T-TE	non-ribosomal peptide synthase	WP_053161276.1	1115/1289 (87)
detG	1997	A-T-C-A-T-KS-KR-T-TE	non-ribosomal peptide synthase	WP_049717545.1	871/1022 (85)
detH	352	Unknown	hypothetical protein	WP_030675315.1	263/351 (75)
detI	236	Unknown	hypothetical protein	KEF13872.1	174/219 (79)
detJ	290	α-ketoglutarate-dependent oxygenase	taurine dioxygenase	WP_049717547.1	260/290 (90)
detK	114	Unknown	hypothetical protein	WP_051812379.1	75/105 (71)
detL	284	Unknown	hypothetical protein	WP_049717548.1	195/283 (69)

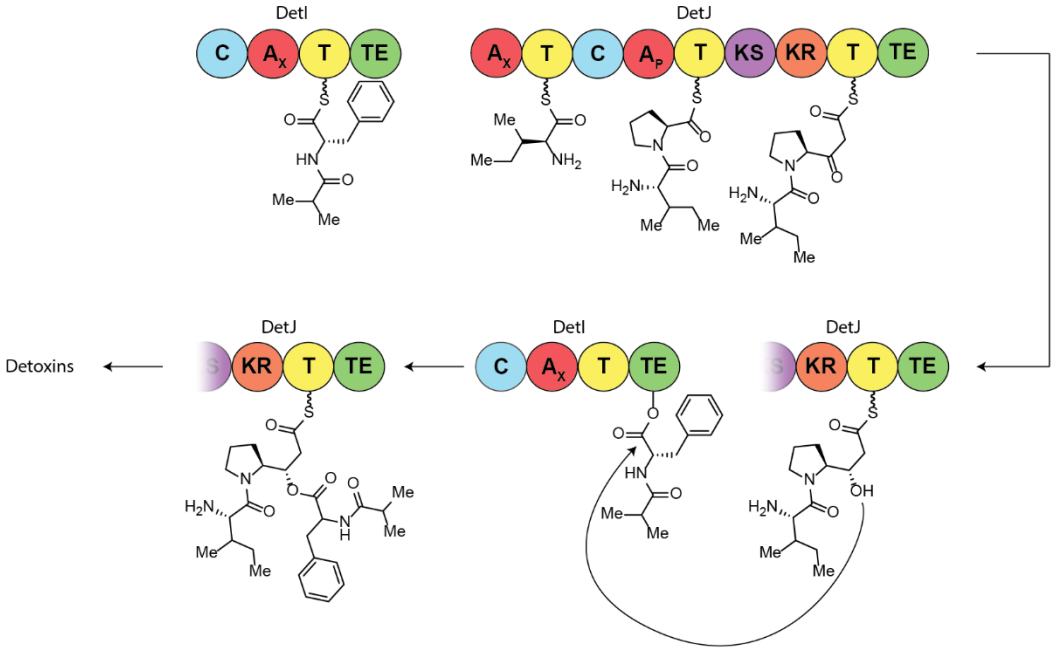


Figure S8. Proposed biosynthetic pathway for the production of the detoxins in *S. mobarensis* NRRL B-3729**.**

Spectral Data for the Rimosamides

^{1}H NMR of Rimosamide A

dqfCOSY of Rimosamide A

HSQC of Rimosamide A

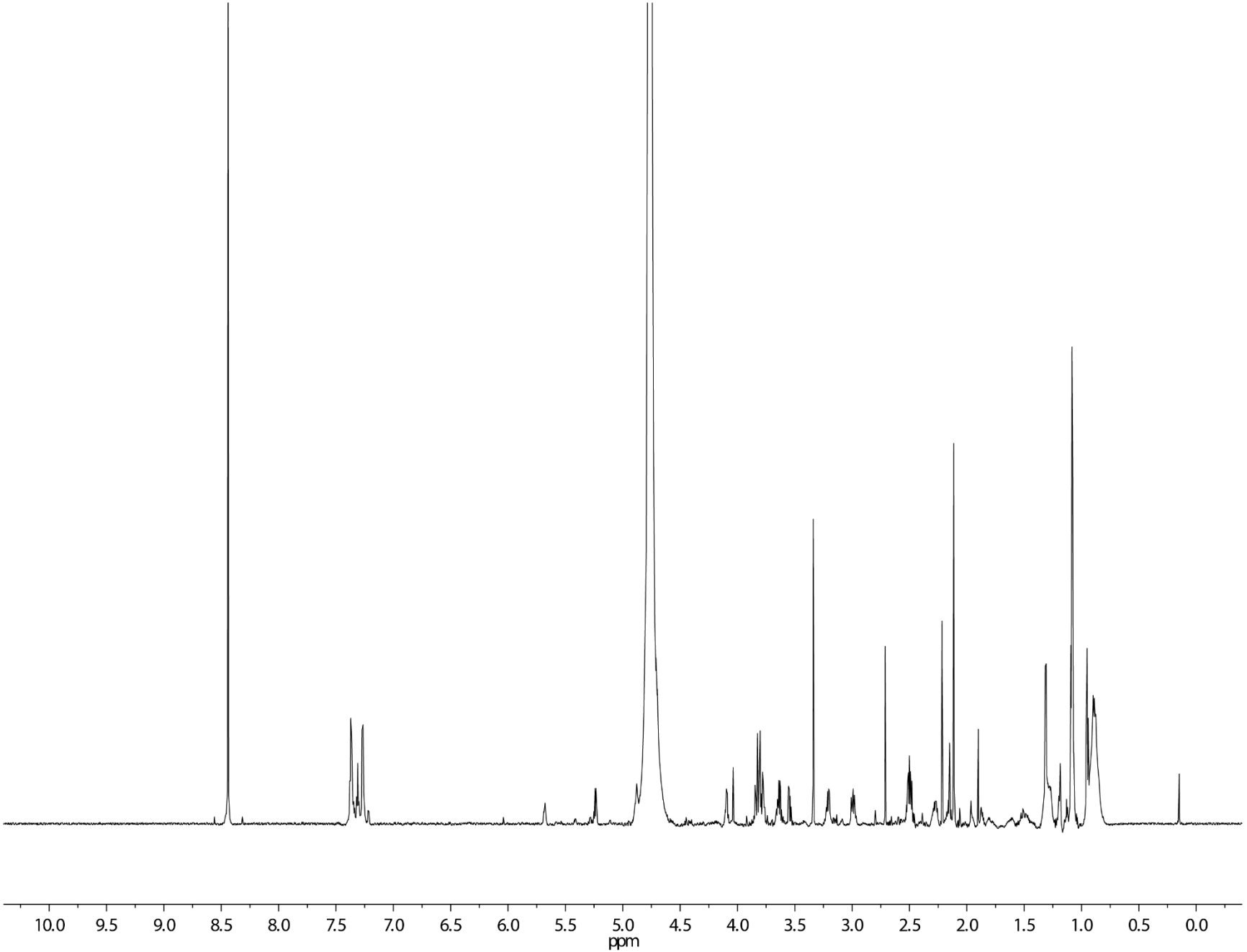
HMBC of Rimosamide A

ROESY of Rimosamide A

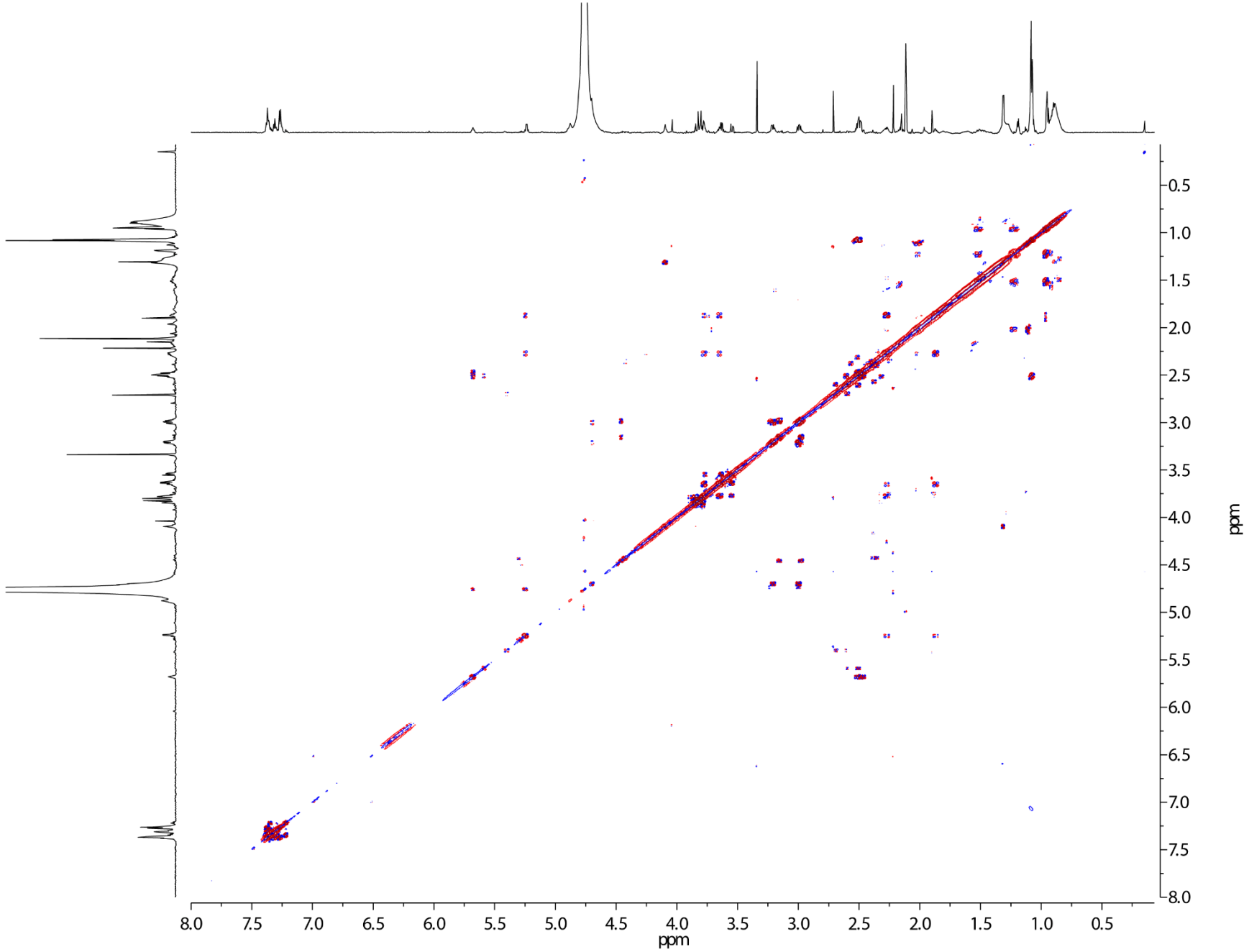
MS^2 Fragmentation of Rimosamides A-D

MS^2 Interpretation of Rimosamide A

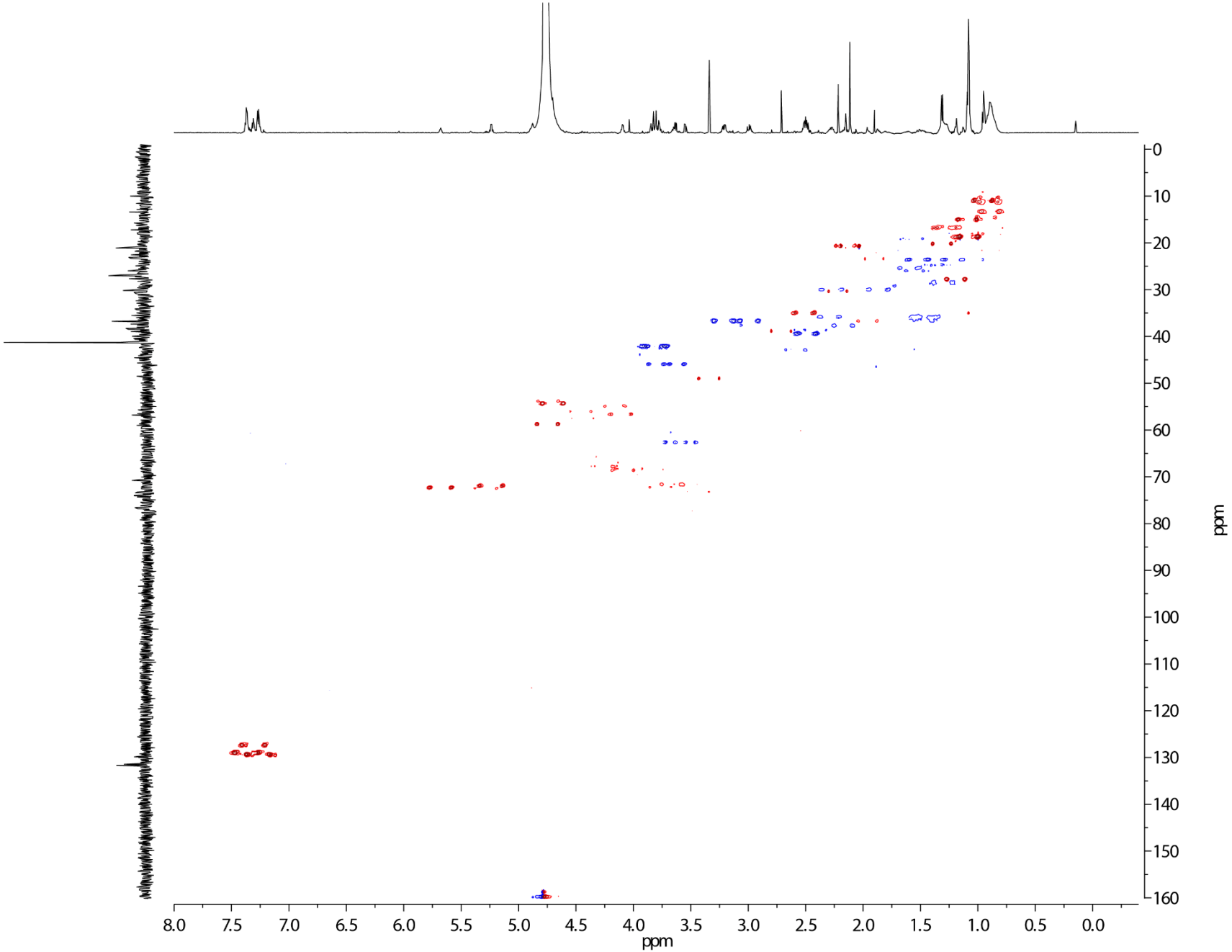
MS^2 Comparisons of Rimosamide and Detoxin D_1



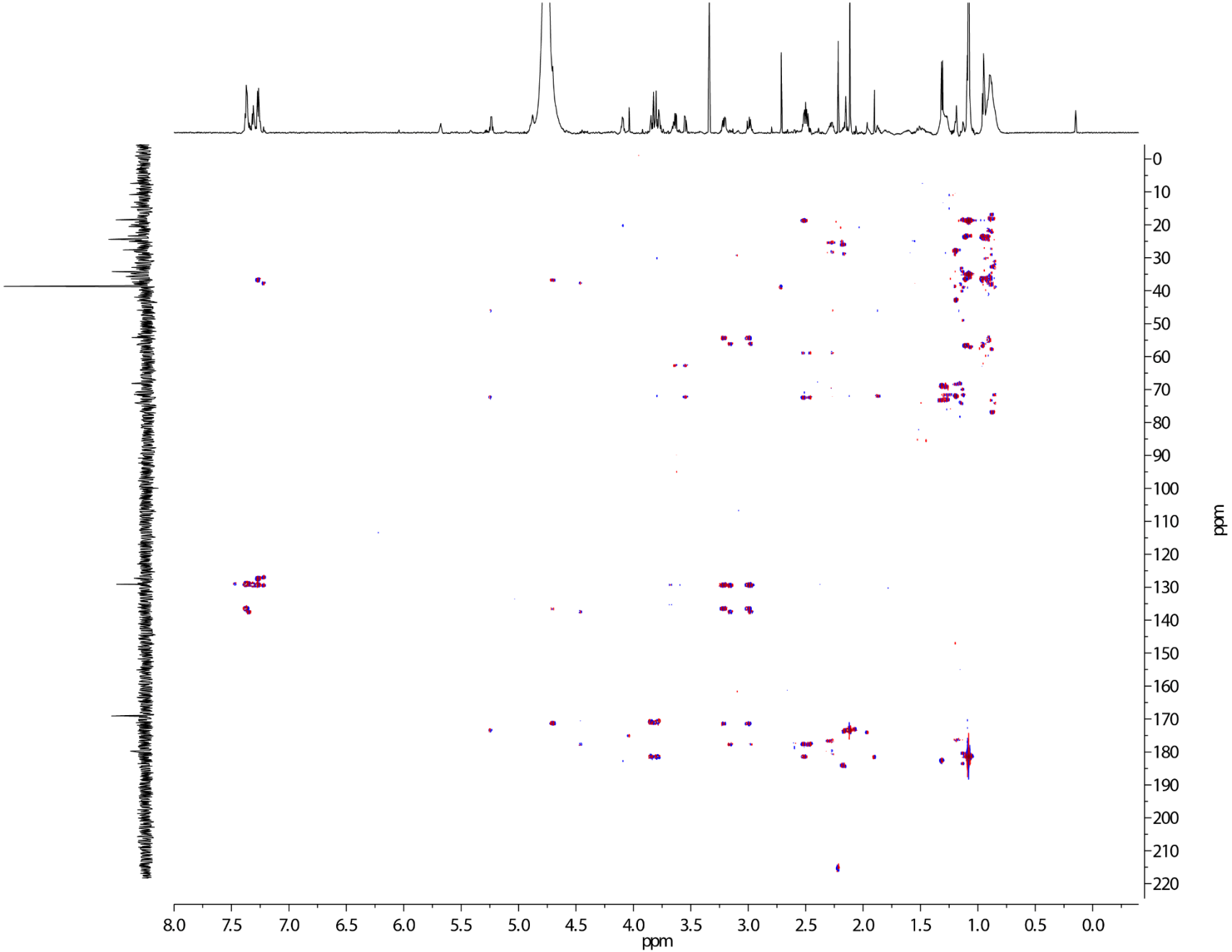
^{1}H NMR spectrum of rimosamide A. NMR experiments were performed in D_2O. See **Figure S5** and **Table S1** for peak assignments.



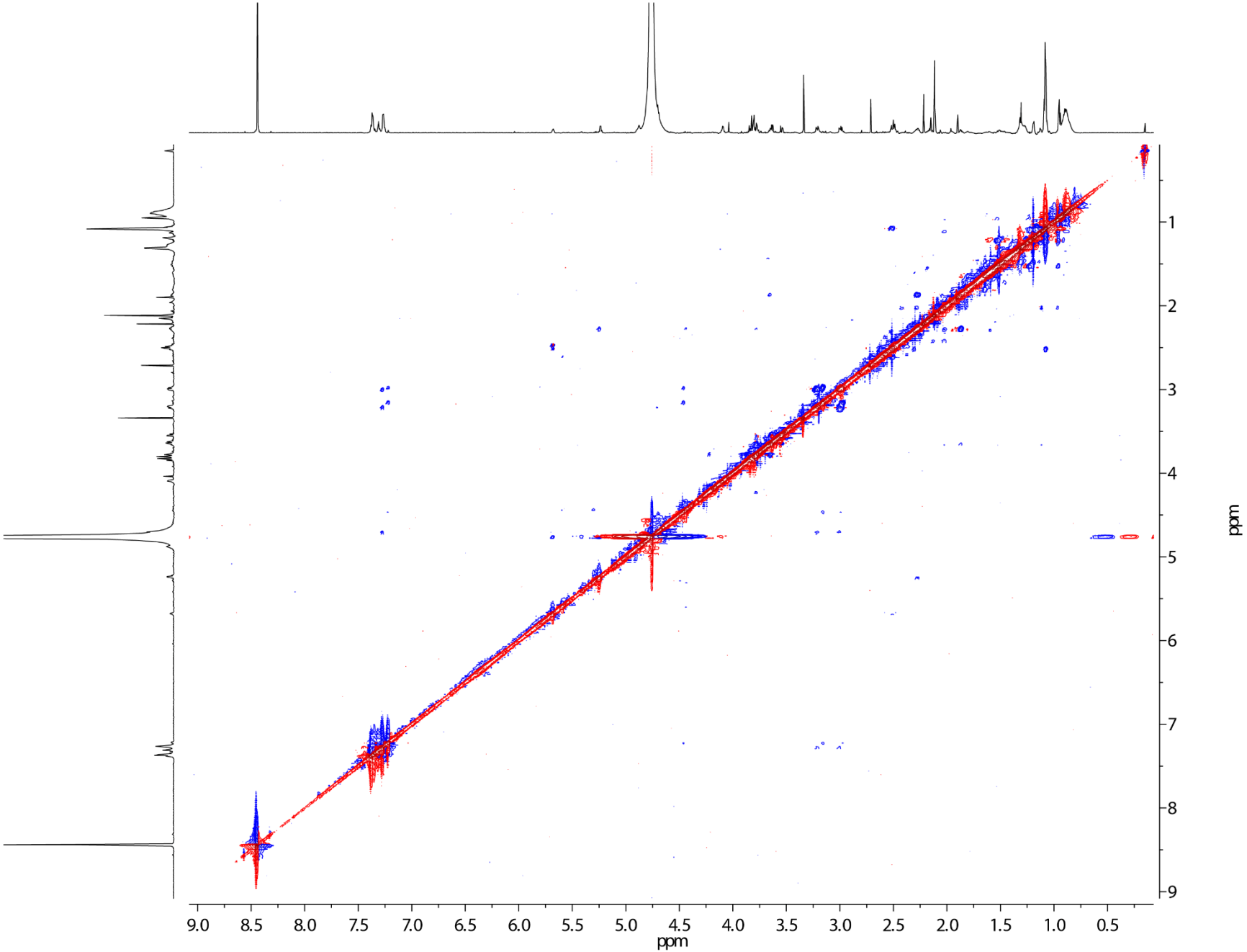
COSY spectrum of rimosamide A. NMR experiments were performed in D_2O. See **Figure S5** and **Table S1** for peak assignments.



HSQC spectrum of rimosamide A. NMR experiments were performed in D_2O. See **Figure S5** and **Table S1** for peak assignments.

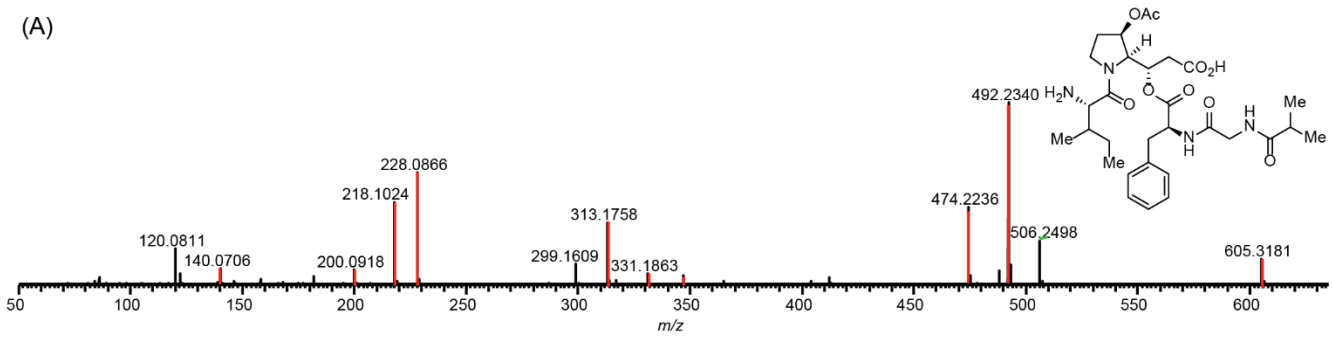


HMBC spectrum of rimosamide A. NMR experiments were performed in D_2O. See **Figure S5** and **Table S1** for peak assignments.



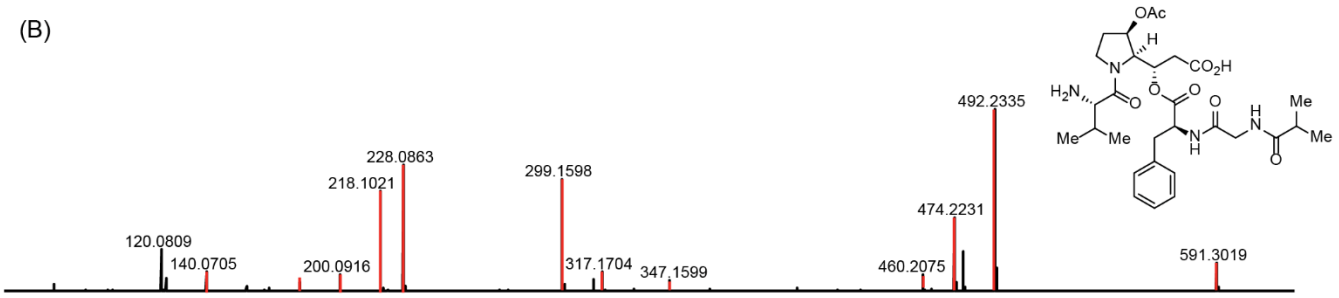
ROESY spectrum of rimosamide A. NMR experiments were performed in D_2O. See **Figure S5** and **Table S1** for peak assignments.

(A)



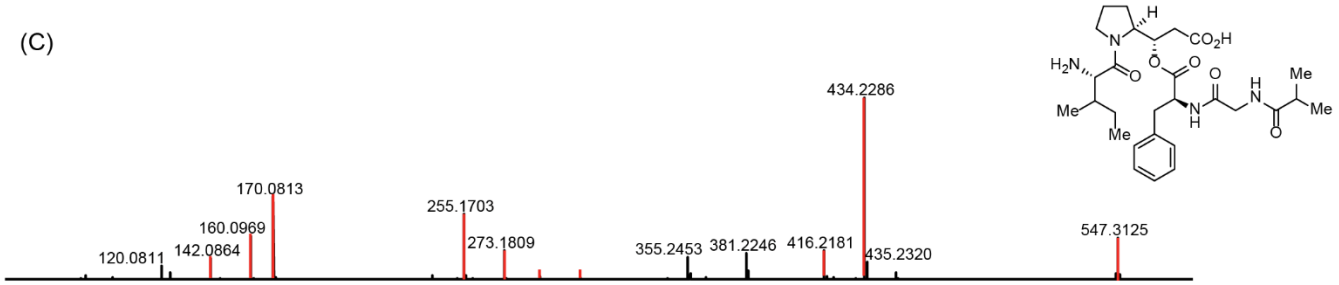
Theoretical *m/z*	Observed *m/z*	Error (ppm)	Formula
605.3187	605.3179	0.389	$C_{30}H_{45}O_9N_4^+$
492.2346	492.2336	0.816	$C_{24}H_{34}O_8N_3^+$
474.2240	474.2232	0.499	$C_{24}H_{32}O_7N_3^+$
347.1607	347.1599	0.773	$C_{18}H_{23}O_5N_2^+$
331.1869	331.1861	0.794	$C_{15}H_{27}O_6N_2^+$
313.1763	313.1755	0.904	$C_{15}H_{25}O_5N_2^+$
228.0872	228.0864	1.025	$C_{10}H_{14}O_5N^+$
218.1028	218.1022	1.004	$C_9H_{16}O_5N^+$
200.0923	200.0917	0.372	$C_9H_{14}O_4N^+$
182.0817	182.0812	0.276	$C_9H_{12}O_3N^+$
140.0712	140.0706	0.322	$C_7H_{10}O_2N^+$

(B)



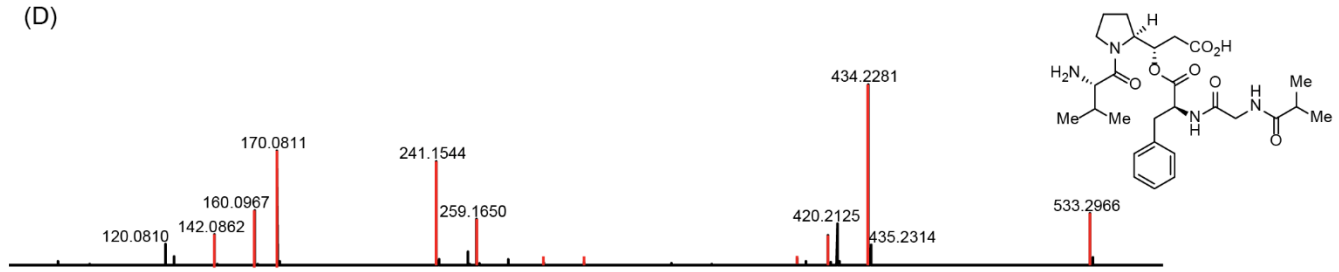
Theoretical *m/z*	Observed *m/z*	Error (ppm)	Formula
591.3030	591.3019	0.871	$C_{29}H_{43}O_9N_4^+$
492.2346	492.2335	1.120	$C_{24}H_{34}O_8N_3^+$
474.2240	474.2231	0.837	$C_{24}H_{32}O_7N_3^+$
460.2084	460.2075	0.797	$C_{23}H_{30}O_7N_3^+$
347.1607	347.1599	0.773	$C_{18}H_{23}O_5N_2^+$
317.1713	317.1704	1.050	$C_{14}H_{25}O_6N_2^+$
299.1607	299.1598	1.097	$C_{14}H_{23}O_5N_2^+$
228.0872	228.0863	1.530	$C_{10}H_{14}O_5N^+$
218.1028	218.1021	1.004	$C_9H_{16}O_5N^+$
200.0923	200.0916	0.772	$C_9H_{14}O_4N^+$
182.0817	182.0812	0.276	$C_9H_{12}O_3N^+$
140.0712	140.0705	0.750	$C_7H_{10}O_2N^+$

(C)



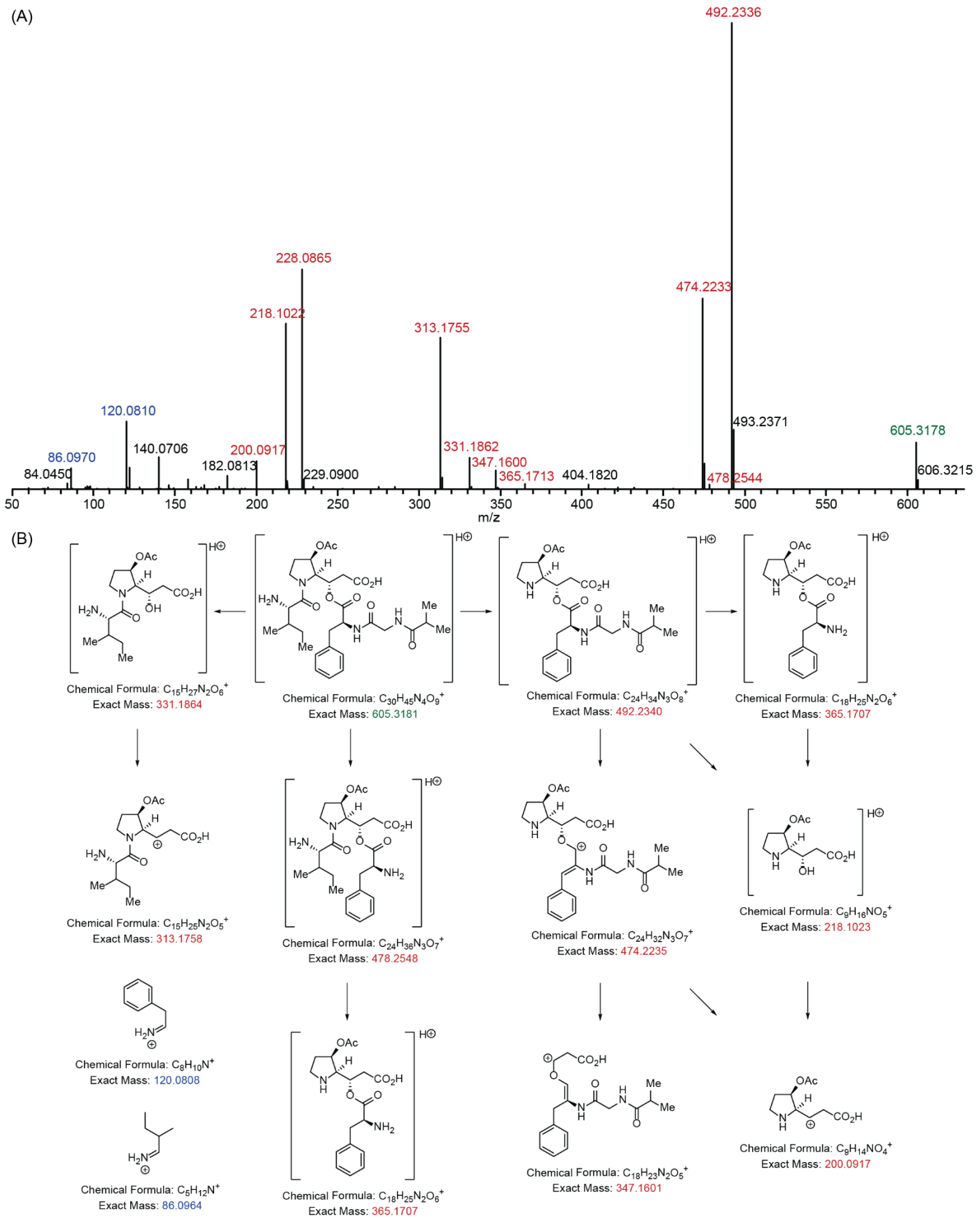
Theoretical *m/z*	Observed *m/z*	Error (ppm)	Formula
547.3132	547.3124	0.208	$C_{28}H_{43}O_7N_4^+$
434.2291	434.2284	0.097	$C_{22}H_{32}O_6N_3^+$
416.2185	416.2179	0.054	$C_{22}H_{30}O_5N_3^+$
307.1658	307.1652	0.240	$C_{16}H_{23}O_4N_2^+$
289.1552	289.1546	0.239	$C_{16}H_{21}O_3N_2^+$
273.1814	273.1808	0.014	$C_{13}H_{25}O_4N_2^+$
255.1709	255.1702	0.192	$C_{13}H_{23}O_3N_2^+$
170.0817	170.0812	0.413	$C_8H_{12}O_3N^+$
160.0974	160.0968	0.376	$C_7H_{14}O_3N^+$
142.0868	142.0863	0.738	$C_7H_{12}O_2N^+$

(D)

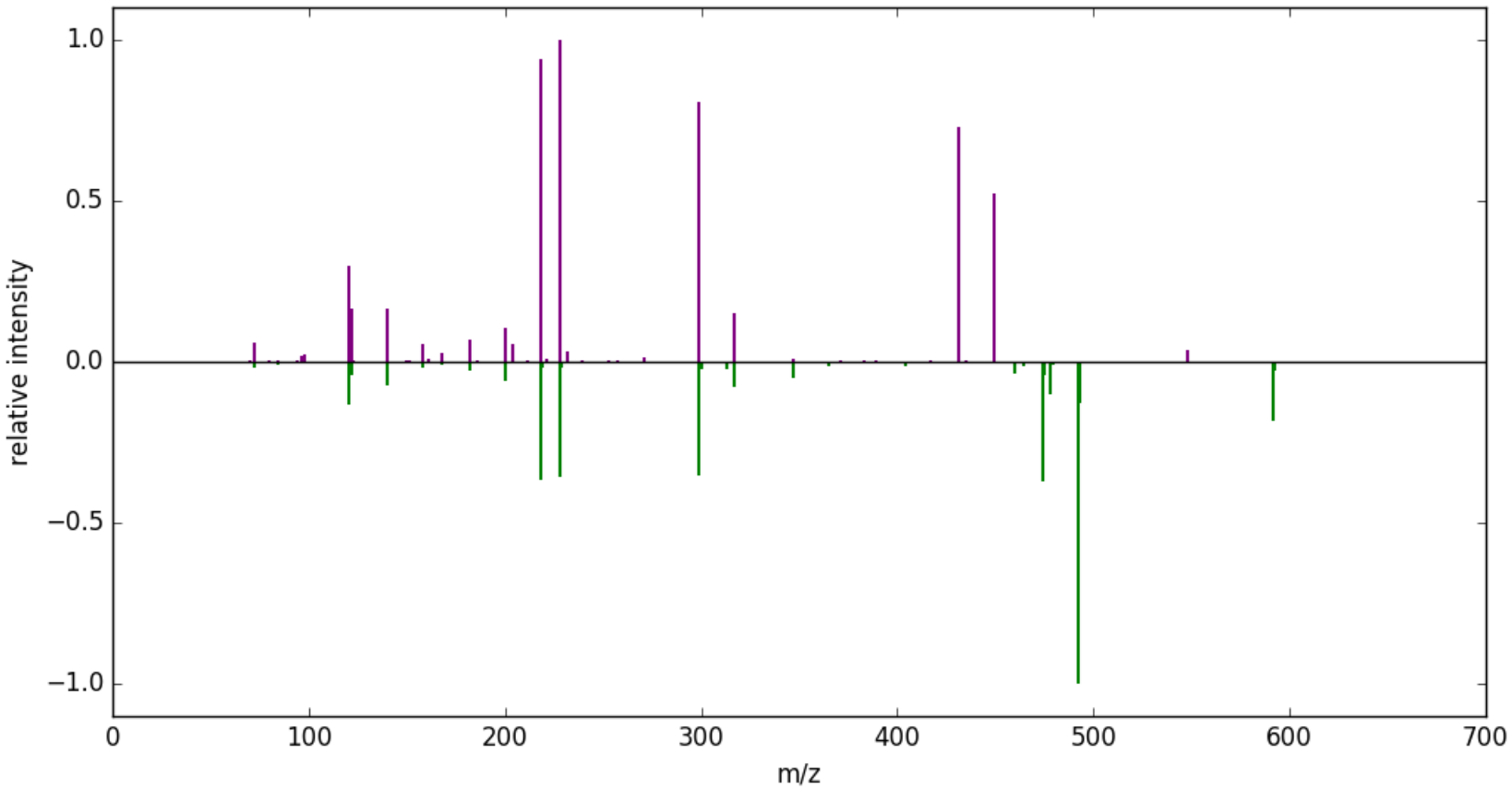


Theoretical *m/z*	Observed *m/z*	Error (ppm)	Formula
533.2975	533.2965	0.180	$C_{27}H_{41}O_7N_4^+$
434.2291	434.2281	0.604	$C_{22}H_{32}O_6N_3^+$
416.2185	416.2176	0.318	$C_{22}H_{30}O_5N_3^+$
402.2029	402.2023	0.118	$C_{21}H_{28}O_5N_3^+$
307.1658	307.1652	0.240	$C_{16}H_{23}O_4N_2^+$
289.1552	289.1546	0.239	$C_{16}H_{21}O_3N_2^+$
259.1658	259.1650	0.516	$C_{12}H_{23}O_4N_2^+$
241.1552	214.1543	0.659	$C_{12}H_{21}O_3N_2^+$
170.0817	170.0810	0.234	$C_8H_{12}O_3N^+$
160.0974	160.0967	0.186	$C_7H_{14}O_3N^+$
142.0868	142.0862	0.034	$C_7H_{12}O_2N^+$

High resolution MS^2 spectra of the rimosamides (*m/z* 605.3187, 591.3030, 547.3132, and 533.2975). Fragment ions in red are consistent with those predicted for the rimosamides. Masses of predicted and observed fragment ions as well as mass error are listed in the accompanying tables.



Interpretation of MS^2 fragmentation from rimosamide A. Predicted structures of fragment ions (matched by color) are shown below the spectrum.



MS^2 Comparison of Rimosamide B (top panel) and Detoxin D_1 (bottom panel).

References

1. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M. (1966) Antibiotic Susceptibility Testing by a Standardized Single Disk Method, *Am. J. Clin. Pathol. 45*, 493-&.
2. Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., and Hopwood, D. A. (2000) *Practical Streptomyces Genetics*, John Innes Foundation, Norwich, UK.
3. Circello, B. T., Eliot, A. C., Lee, J. H., van der Donk, W. A., and Metcalf, W. W. (2010) Molecular cloning and heterologous expression of the dehydrophos biosynthetic gene cluster, *Chem Biol 17*, 402-411.
4. Yu, X., Price, N. P., Evans, B. S., and Metcalf, W. W. (2014) Purification and characterization of phosphonoglycans from *Glycomyces* sp. strain NRRL B-16210 and *Stackebrandtia nassauensis* NRRL B-16338, *J. Bacteriol. 196*, 1768-1779.
5. Blodgett, J. A., Zhang, J. K., and Metcalf, W. W. (2005) Molecular cloning, sequence analysis, and heterologous expression of the phosphinothricin tripeptide biosynthetic gene cluster from *Streptomyces viridochromogenes* DSM 40736, *Antimicrob. Agents Chemother. 49*, 230-240.
6. Eliot, A. C., Griffin, B. M., Thomas, P. M., Johannes, T. W., Kelleher, N. L., Zhao, H., and Metcalf, W. W. (2008) Cloning, expression, and biochemical characterization of *Streptomyces rubellomurinus* genes required for biosynthesis of antimalarial compound FR900098, *Chem Biol 15*, 765-770.