Response of Hypogean Actinobacterial Genera Secondary Metabolism to Chemical and Biological Stimuli

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A. 1. Phylogenetic analyses

Table S1: Table of identified actinomycete genera isolated from hypogean samples.

Genus List	Number isolated
Streptomyces	91
Micromomospora	9
Pseudomonas	6
Kribella	5
Nocardiodes	5
Bacillus	4
Micrococcus	4
Microbispora	3
Nonomuraea	3
Rhodococcus	3
Arthrobacter	2
Flavobacterium	2
Pseudoduganella	2
Streptosporangium	2
Variovorax	2
Agromyces	1
Azobacter	1
Catellatospora	1
Dactylosporangium	1
Massilia	1
Methylobacterium	1
Nocardia	1
Oerskovia	1
Pseudonocardia	1
Stenotrophomonas	1
Williamsia	1
Saccharothrix	1

Table S2: Table of GenBank accession numbers for 16S sequences of selected cave isolates.

Isolate	Proposed genus	GenBank accession no.
BBHARD14	*Kribbella*	MH182596
BBHARD22	*Micromonospora*	MH182597
BBHARD23	*Nonomuraea*	MH182598
BBHARD27	*Saccharothrix*	MH182599
BBHARD28	*Micromonospora*	MH182600
BBHARD29	*Micromonospora*	MH182601
CGSNAI18	*Nocardia*	MH182602
BBSNAI08	*Kribella*	MH182603
BBSNAI19	*Nocardioides*	MH277690
BBSNAI23	*Nocardioides*	MH182604
BBSNAI36	*Arthrobacter*	MH182605
BCCAGE06	*Streptomyces*	MH182606
BCCAGE18	*Streptomyces*	MH182607
BCCAGE23	*Micromonospora*	MH182608
BCCAGE29	*Streptomyces*	MH182609
BCCAGE31	*Streptomyces*	MH182610
BCCAGE42	*Nonomuraea*	MH182611
BCCAGE45	*Pseudonocardia*	MH182612
BCCAGE54	*Microbispora*	MH182613
KDCAGE35	*Streptosporangium*	MH182614

A. 2. Metabolomics processing workflow

XCMS Processing

Thermo .raw files were converted to mzXML using ProteoWizard, and output files were placed into "control" and "test" folders for XCMS analysis. Control cultures were placed in the control, while all stimuli conditions, metal, antibiotic, and competitor exposed cultures were placed in the test folder. These two folders were placed inside another folder which was designated as the directory for XCMS processing in R.

The following commands were used in the XCMS processing and alignment:

- library (xcms)
- x<-xcmsSet()
- x<-group(x, mzwid=0.1, minfrac=0)
- x2<-retcor(x, family="s", plottype="m")
- x2<-group(x2, bw=60, mzwid=0.2, minfrac=0)
- x3<-fillPeaks(x2)
- reporttab<-diffreport(x3, "control", "test", 10, metlin=0.15)

A. 3. Stimuli responses across strains

FIG S1: Venn diagrams show the distribution of features with at least 10-fold higher abundance in stimuli vs control conditions with a minimum feature value set to 0.01 % of the total ion count. Distribution shown as a percentage of the total number of features detected within that strain. The genus, based on the nearest 16S relative, is shown above, and the isolate ID is given in parenthesis. Sections ≥ 5% are colored red.

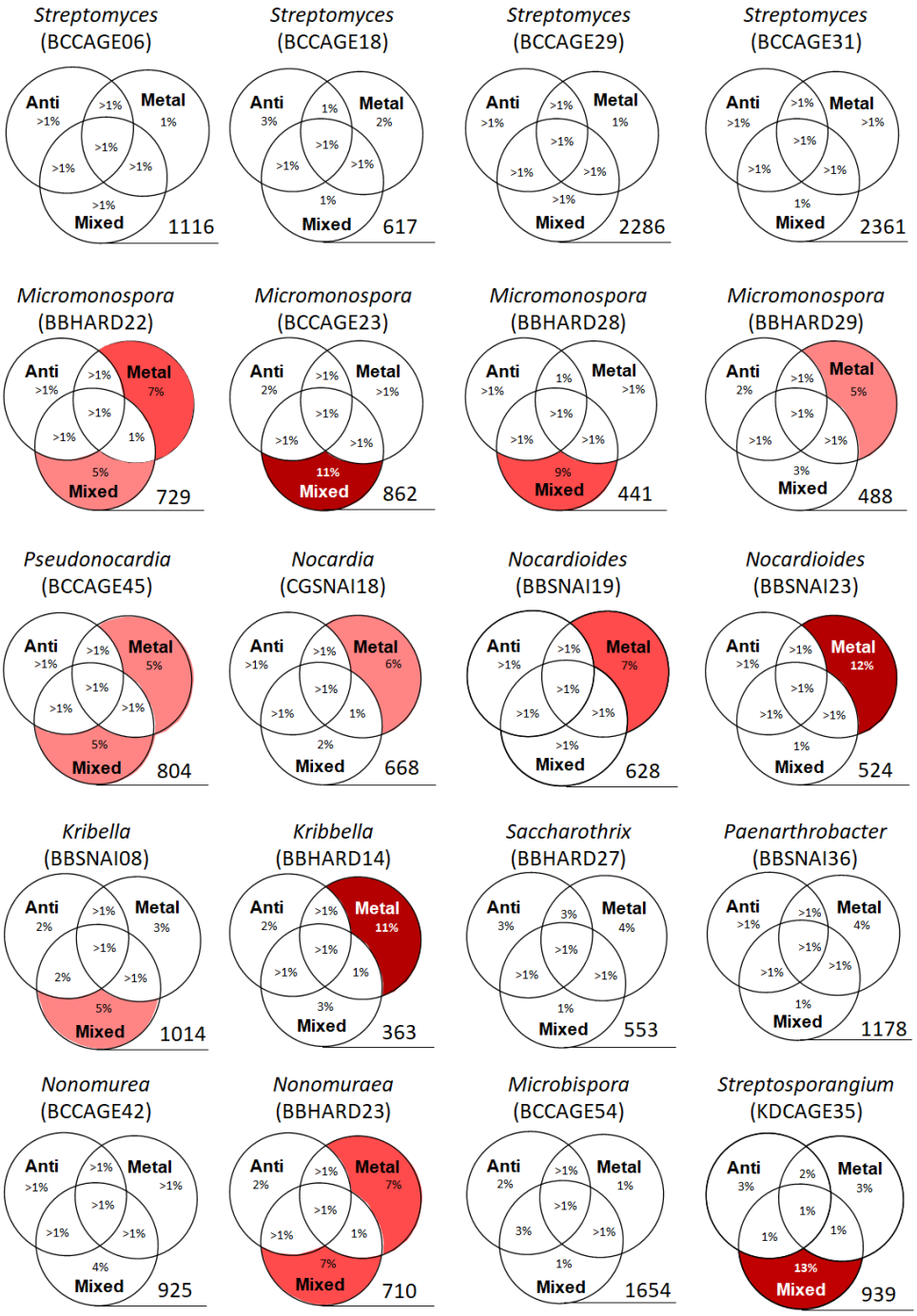


FIG S2: Stacked bar graph shows the percentage of detected features per strain with increased production of at least 10-fold in rifampicin (red), streptomycin (yellow), and both (gray) stimuli vs control conditions. Below each bar is shown the isolate identifier and the genus based on the nearest 16S rDNA relative.

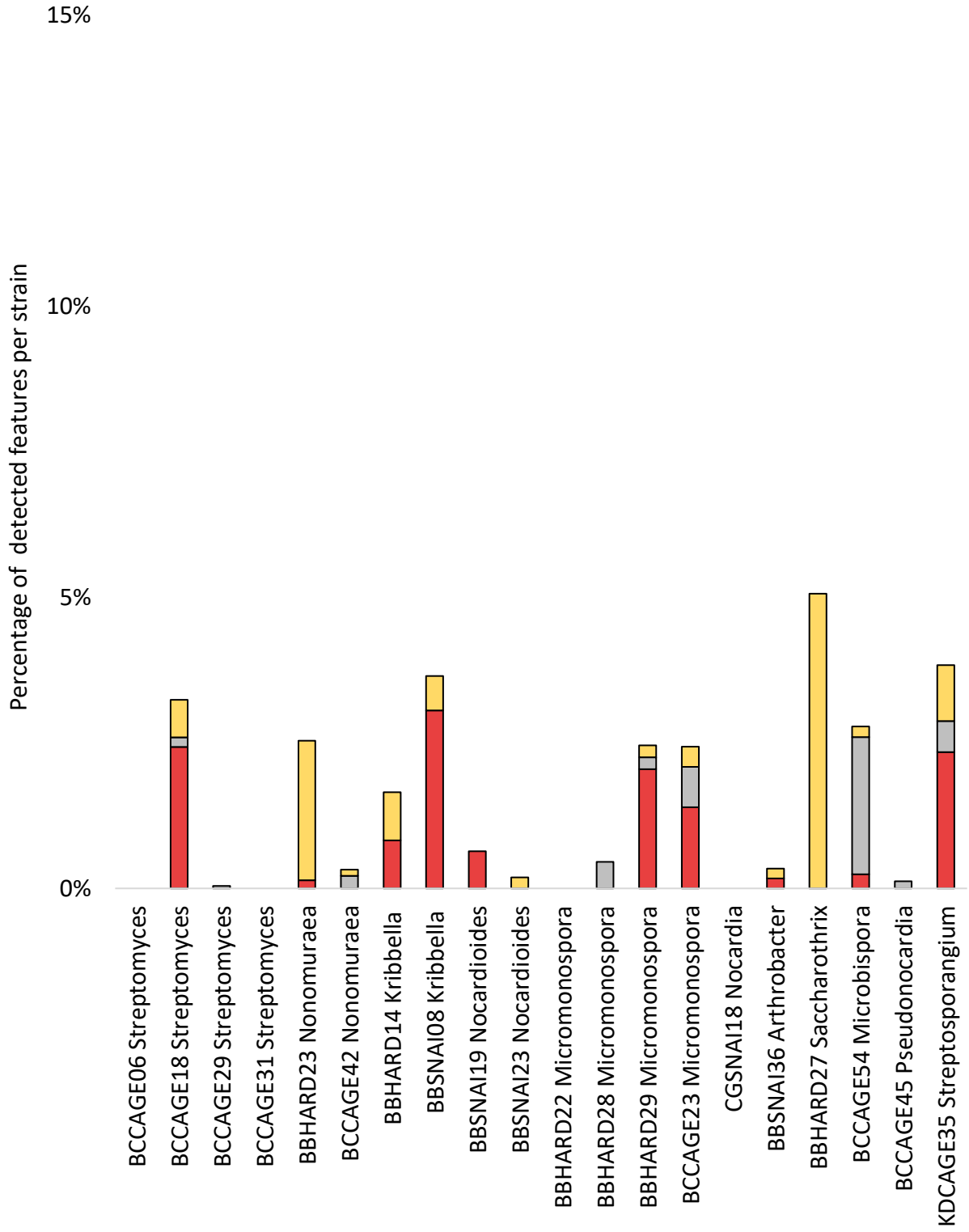


FIG S3: Stacked bar graph shows the percentage of detected features per strain with increased production of at least 10-fold in lanthanum (blue), scandium (black), and both (gray) stimuli vs control conditions. Below each bar is shown the isolate identifier and the genus based on the nearest 16S rDNA relative.

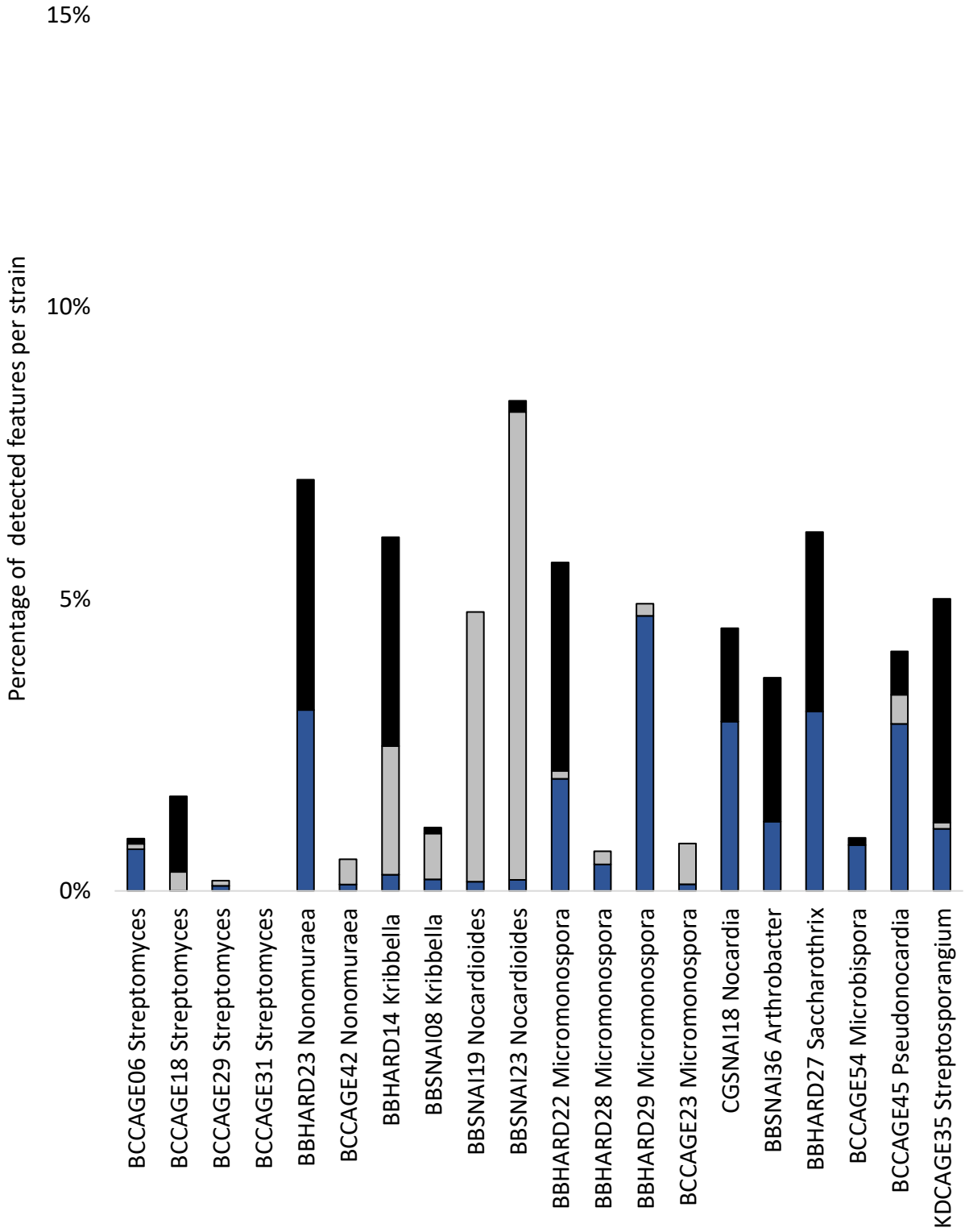
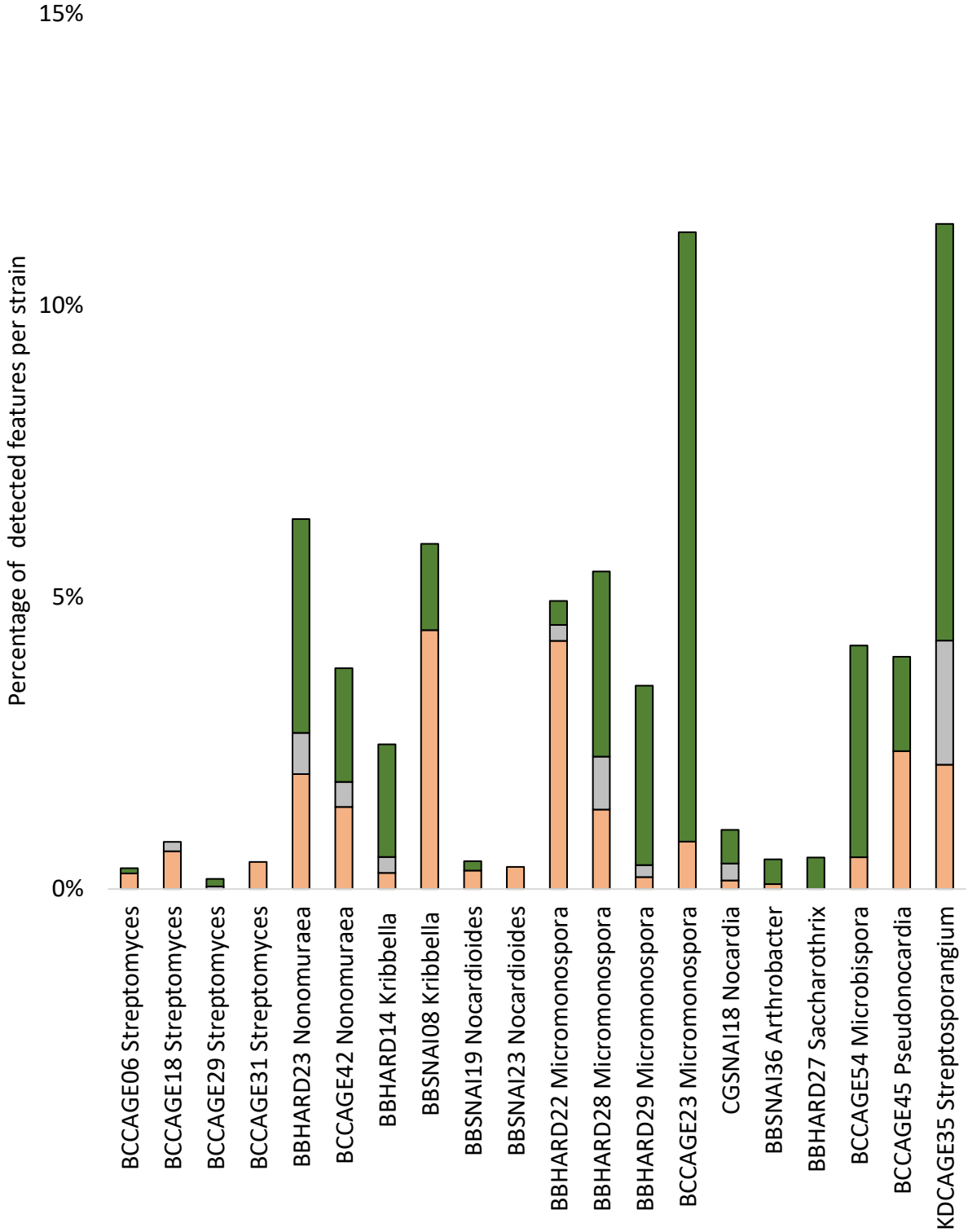


FIG S4: Stacked bar graph shows the percentage of detected features per strain with increased production of at least 10-fold in mixed culture with *Tsukamurella pulmonis* (orange), R*hodococcus wratis* (green), and both (gray) stimuli vs control conditions. Below each bar is shown the isolate identifier and the genus based on the nearest 16S rDNA relative.



A. 4. Activation of natural products through stimuli conditions

Table S3: Table of identified natural product responses across stimuli conditions of subinhibitory concentrations of rifampicin (Rif) and streptomycin (Str), rare earth metal exposure of lanthanum (La) and scandium (Sc), and mixed culture conditions with *T. pulmonis* (TP) and *R. wratis* (RW). "Con" values represent averaged, manually integrated peak intensities for features in the control conditions. Values for stimuli columns represent fold change vs control calculated by dividing the integrated abundance for features of each stimuli condition by the Con value. The heatmap was colored using excels conditional formatting with fold change values of 0.1 set as red, 1 set as white, and 10 as green. Speculative ID provided in parenthesis under description when available.

Strain (Isolate)	DESC	ID	Con	Rif	Str	La	Sc	TP	Rw
Micromonospora (BBHARD22)	M257.7T17.2	Okicenone	4E+06	1.3	0.7	0.8	6.0	1.1	2.1
	M253.6T24.6	Aloesaponarin II	2E+07	1.4	0.8	0.3	5.4	0.6	1.2
Nonomuraea (BBHARD23)	M785.1T21.5	Hypogeamicin A	7E+05	0.5	0.2	0.0	2.3	1.1	2.8
	M376T21.1	Hypogeamicin B	6E+05	1.4	0.4	0.2	110.2	10.2	2.0
	M394T15.8	Hypogeamicin C	5E+06	1.4	3.5	0.0	6.2	5.8	5.2
Streptomyces (BCCAGE06)	M1269.6T25.4	Actinomycin C2	2E+07	0.8	1.0	1.5	0.7	3.4	2.0
Microbispora (BCCAGE54)	M1056.4T14	Propeptin 1	7E+05	3.2	1.3	0.1	0.7	1.9	2.9
	M1146.9T14.6	Propeptin 2	2E+06	0.8	0.0	0.6	1.9	0.5	0.9
	M369.3T17	Tetarimycin B	1E+06	16.1	32.8	0.5	6.2	0.4	23.0
Streptosporangium (KDCAGE35)	M1178.3T15.6	Funisamine	7E+03	4.3	6.4	1.3	1.4	31.2	1.4

Table S4: Table of putative natural product responses across stimuli conditions of subinhibitory concentrations of rifampicin (Rif) and streptomycin (Str), rare earth metal exposure of lanthanum (La) and scandium (Sc), and mixed culture conditions with *T. pulmonis* (TP) and *R. wratis* (RW). Features were assigned as putative natural products based on MS, and MS/MS changes in comparison to known compounds, UV spectrum, and response to stimuli. "Con" values represent averaged, manually integrated peak intensities for features in the control conditions. Values for stimuli columns represent fold change vs control calculated by dividing the integrated abundance for features of each stimuli condition by the Con value. The heatmap was colored using excels conditional formatting with fold change values of 0.1 set as red, 1 set as white, and 10 as green. Speculative ID provided in parenthesis under description when available.

Strain	DESC	Description (*speculative ID*)	Con	Rif	Str	La	Sc	TP	Rw
Micromonospora (BBHARD22)	M256.8T9.9	Unident. NP 1	3E+05	0.8	0.5	0.9	0.4	0.3	0.6
	M233.8T11.1	Unident. NP 2	6E+06	2.4	1.0	0.4	2.4	0.9	2.5
	M283.6T13.1	Unident. Anthraquinone 1	3E+05	12.5	1.0	0.9	19.5	6.3	7.6
	M297.5T14.3	Unident. Anthraquinone 2 (*DMAC*)	4E+07	1.0	1.0	0.4	2.6	1.3	1.3
	M238.7T20.8	Unident. Anthraquinone 3 *(Dihydroxyanthraquinone)*	4E+05	0.6	0.6	3.4	41.7	0.1	0.3
	M241.7T22	Unident. Anthraquinone 4	6E+05	2.9	0.8	0.5	6.8	1.9	4.0
	M269.6T25.4	Unident. Anthraquinone 5 *(Hydroxyaloesaponarin II*)	4E+06	1.5	0.6	0.2	9.0	0.1	1.5
Nonomuraea (BBHARD23)	M605.1T14.7	Unident. Hygogeamicin 1	3E+06	0.8	0.3	0.0	0.5	0.3	1.9
	M410T15.1	Unident. Hygogeamicin 2	1E+06	0.4	0.6	0.0	0.0	0.7	3.9
	M833.2T21	Unident. Hygogeamicin 3	3E+04	0.9	1.4	0.2	1.0	8.1	590.3
	M818.1T21.2	Unident. Hygogeamicin 4	1E+06	1.4	5.1	0.0	1.3	4.7	3.5
Streptomyces (BCCAGE06)	M1271.6T22.6	Unident. Actinomycin 1 (*Y5*)	5E+06	0.8	0.3	1.1	0.6	3.6	2.7
	M1287.5T25.4	Unident. Actinomycin 2 (*Y3*)	5E+05	0.3	0.3	1.5	0.4	2.3	11.9
	M1293.6T25.4	Unident. Actinomycin 3 (*G2*)	5E+05	4.0	1.6	3.7	3.2	4.6	4.4
	M1257.7T25.4	Unident. Actinomycin 4 (*F4*)	9E+05	8.6	1.5	1.0	3.4	9.4	1.8

Strain	DESC	Description (*speculative ID*)	Con	Rif	Str	La	Sc	TP	Rw
Streptomyces (BCCAGE06)	M1307.5T25.4	Unident. Actinomycin 5 (*Y2*)	1E+06	0.3	1.3	0.7	0.7	3.3	3.8
	M1255.6T25.4	Unident. Actinomycin 6 (*D*)	7E+06	5.4	0.8	0.8	2.7	5.2	2.5
	M1327.7T25.4	Unident. Actinomycin 7	4E+05	1.7	1.3	2.5	1.1	11.1	5.0
	M1313.6T25.4	Unident. Actinomycin 8	1E+05	29.4	3.9	2.9	7.4	28.7	15.1
Streptomyces glauciniger (BCCAGE31)	M311.4T8.3	Unident. NP 3	2E+07	0.3	0.7	1.2	0.6	1.1	1.0
	M287.5T9.5	Unident. NP 4	2E+07	0.8	1.1	1.2	0.7	0.3	0.0
	M634.9T12.5	Unident. NP 5	3E+06	0.1	0.5	0.9	0.3	1.0	0.5
	M295.5T12.9	Unident. NP 6	4E+06	1.2	1.0	1.4	0.5	1.3	1.0
	M707.1T16.2	Unident. NP 7	1E+05	0.9	0.7	0.7	0.4	0.0	0.5
	M311.4T16.6	Unident. NP 8	6E+06	0.8	0.8	1.2	0.6	1.5	0.9
	M253.6T26.3	Unident. NP 9 (*Aloesaponarin II*)	3E+06	1.1	0.4	1.3	0.2	0.3	0.3
Nonomuraea (BCCAGE42)	M411.4T12	Unident. NP 10	5E+05	3.5	0.8	0.1	0.6	0.0	0.0
	M628.1T12.7	Unident. Polyene 1	1E+05	14.5	0.1	1.5	0.6	0.0	0.2
	M628.1T13.3	Unident. Polyene 2	2E+04	49.9	0.6	3.8	1.6	0.1	0.0
	548.3T15.4	Unident. Polyene 3	6E+03	140.6	0.0	4.6	2.6	4.0	2.9
	548.3T16.2	Unident. Polyene 4	1E+04	80.9	0.0	1.1	1.0	1.0	1.7
Streptomyces (BCCAGE18)	M900.2T17.1	Unident. NP. 11	1E+06	1.9	0.7	0.7	1.4	0.3	0.3
	M895.2T17.9	Unident. NP. 12	6E+06	2.7	0.7	1.1	2.2	1.0	0.9
Saccharothrix (BBHARD27)	M398.4T13.5	Unident. NP. 13	1E+07	1.3	0.0	0.0	1.9	0.8	1.1
	M247.8T15.2	Unident. NP. 14	1E+07	1.1	0.0	0.1	1.0	0.6	1.0
	M365.6T24.5	Unident. NP. 15	2E+07	1.0	0.0	0.0	1.1	0.7	1.6
Kribella (BBSNAI08)	M732.4T20	Unident. Polyene 5	2E+07	0.2	0.9	0.0	0.0	0.4	0.0
Microbispora (BCCAGE54)	M689.2T10.5	Unident. NP. 16	1E+05	80.5	79.4	0.2	1.1	0.6	48.8
	M561.1T10.7	Unident. NP. 17	1E+06	17.0	21.1	0.5	0.5	0.6	52.9
	M575.2T11.5	Unident. NP. 18	4E+05	120.1	142.2	0.2	0.8	1.0	268.0

Strain	DESC	Description (*speculative ID*)	Con	Rif	Str	La	Sc	TP	Rw
Microbispora (BCCAGE54)	M717.2T11.6	Unident. NP. 19	5E+05	16.4	26.4	0.0	0.6	0.3	15.6
	M731.3T12.1	Unident. NP. 20	6E+05	13.9	27.5	0.1	0.6	0.0	14.9
	M355.4T13	Unident. NP. 21 (*Arromycin*)	8E+04	25.6	52.5	0.0	1.0	2.2	38.8
	M393.2T13.7	Unident. NP. 22 (*Linfuranone A*)	6E+05	4.8	6.0	0.2	0.4	0.0	4.7
	M385.2T14.7	Unident. Tetarimycin 1	3E+04	72.3	98.0	1.3	15.8	3.9	80.7
	M399.2T16.1	Unident. Tetarimycin 2	2E+05	23.0	39.5	0.5	4.4	0.7	20.2
	M406.3T17.5	Unident. Tetarimycin 3	2E+04	8.8	92.5	0.0	3.5	2.4	19.6
	M395.2T19.5	Unident. Tetarimycin 4	3E+04	53.9	49.0	0.8	3.1	1.0	49.8

FIG S5: Distribution of isolated and putative natural products from Tables S2-3 with ≥10-fold changes in antibiotic exposure (anti), metal exposure (metal), or mixed culture (mixed) stimuli conditions relative to the control.

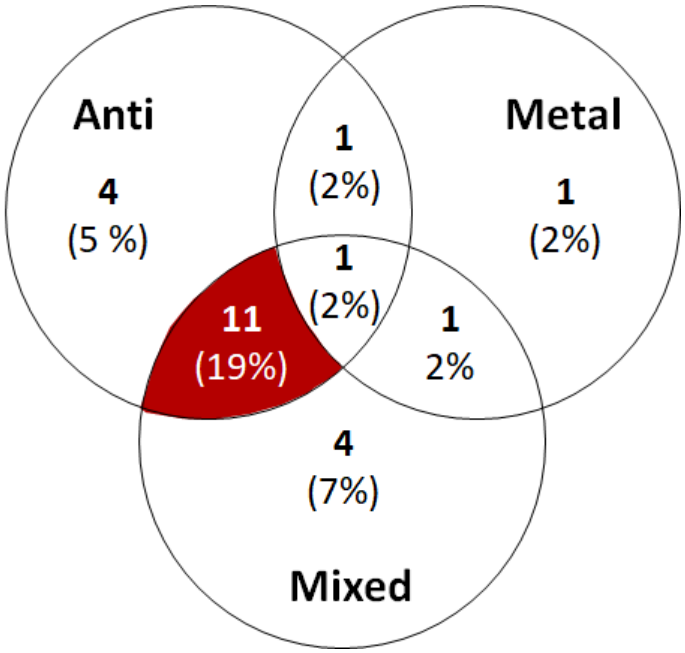
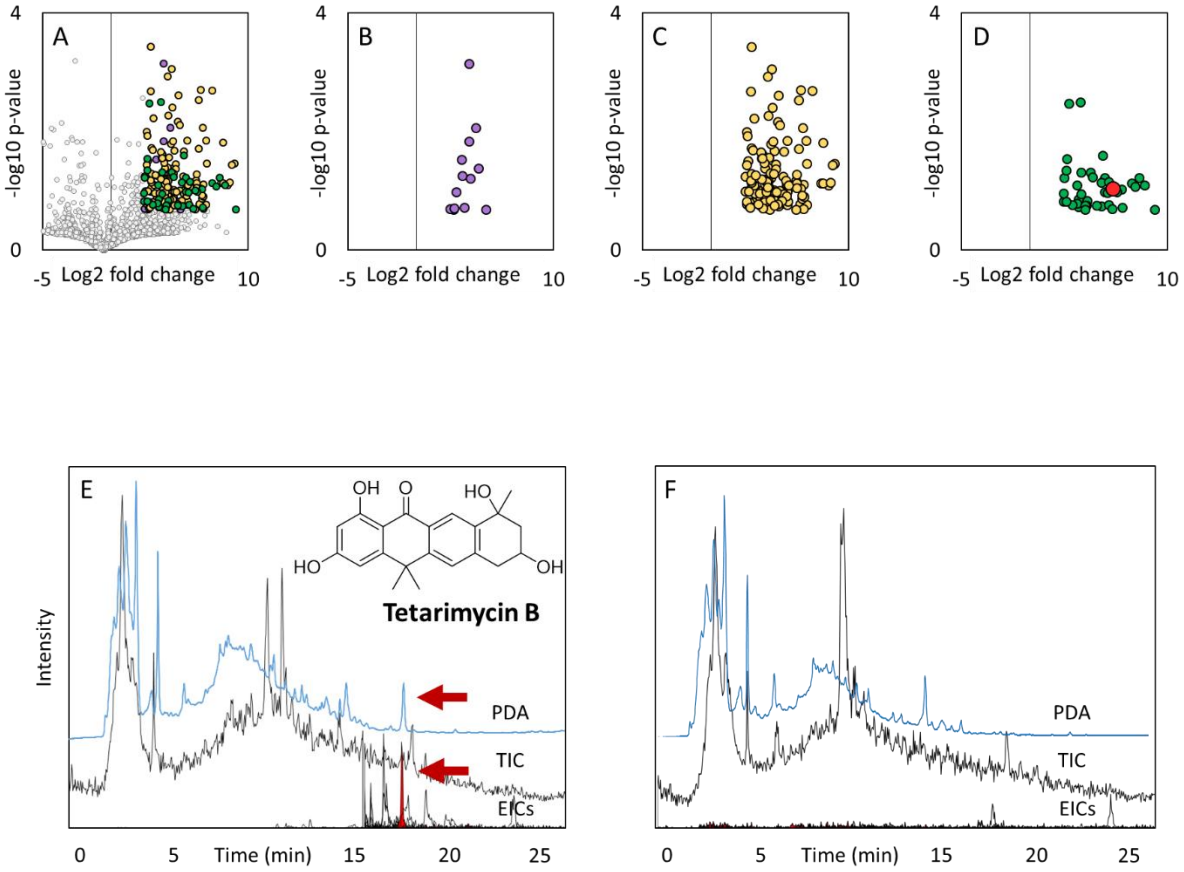


FIG S6: Analysis of volcano plot-prioritized features with chromatogram overlays. (A) Binary volcano plot comparison between *Microbispora* BCCAGE54 control and streptomycin antibiotic exposure conditions. Features below 0.8 significance 5-fold change thresholds colored grey. Features over thresholds colored by retention time brackets: <10 min (purple), between 10-15 min (yellow), and >15 min (green). (B) Volcano plot of features above thresholds and eluting before 10 min. (C) Volcano plot of features above thresholds and eluting between 10 and 15 min. (D) Volcano plot of features above thresholds and eluting after 15 min. Overlays of extracted ion, total ion, and UV/Vis chromatograms for features eluting after 15 min are shown in (E) for streptomycin exposed and (F) control *Microbispora* BCCAGE54 extracts. Red arrows highlight tetarimycin B peak in the EICs and PDA.



A. 5. Extracted ion chromatograms (EICs) for selected features

The following are extracted ion chromatograms from the raw data for identified natural products shown in Table S1.

FIG S7: EICs of (A) okicenone and (C) aloesaponarin II from control (black) and Sc treated (red) *Micromonospora* BBHARD22 extracts and mass spectral data for (B) okicenone and (D) aloesaponarin II. Structures shown on right.

Strain (Isolate)	DESC	ID	Con	Rif	Str	La	Sc	TP	Rw
Micromonospora (BBHARD22)	M257.7T17.2	okicenone	4E+06	1.3	0.7	0.8	6.0	1.1	2.1
	M253.6T24.6	aloesaponarin II	2E+07	1.4	0.8	0.3	5.4	0.6	1.2

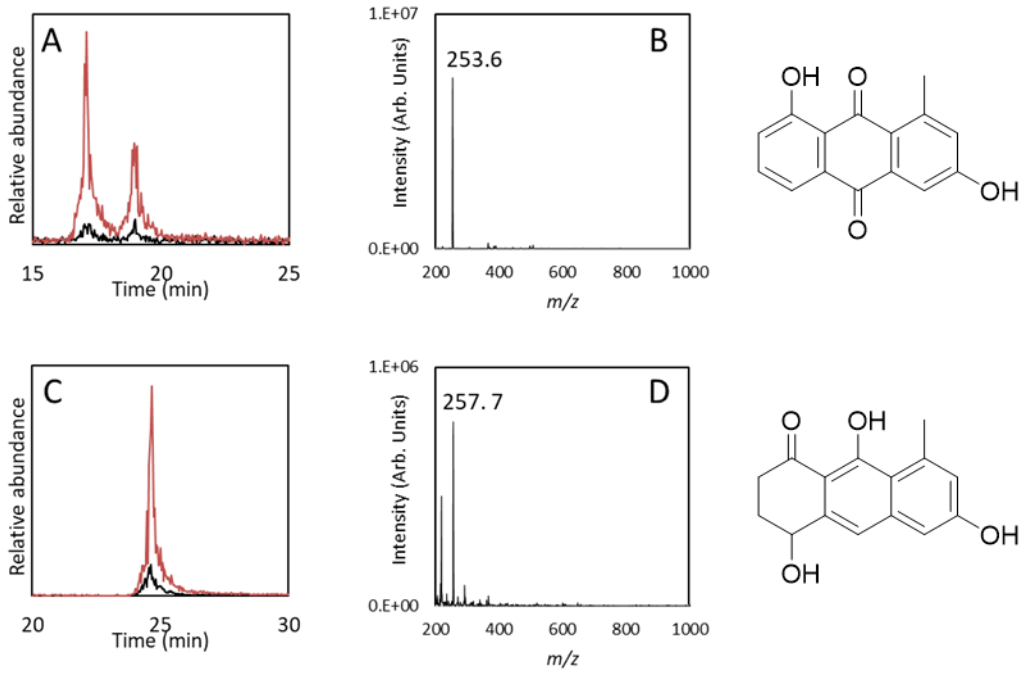


FIG S8: EICs of (A) hypogeamicin A, (C) hypogeamicin B, and (E) hypogeamicin C from control (black) and Sc treated (red) *Nonomuraea* BBHARD23 extracts and mass spectral data for (B) hypogeamicin A, (D) hypogeamicin B, and (F) hypogeamicin C. Structures shown on right.

Strain (Isolate)	DESC	ID	Con	Rif	Str	La	Sc	TP	Rw
Nonomuraea (BBHARD23)	M785.1T21.5	hypogeamicin A	7E+05	0.5	0.2	0.0	2.3	1.1	2.8
	M376T21.1	hypogeamicin B	6E+05	1.4	0.4	0.2	110.2	10.2	2.0
	M394T15.8	hypogeamicin C	5E+06	1.4	3.5	0.0	6.2	5.8	5.2

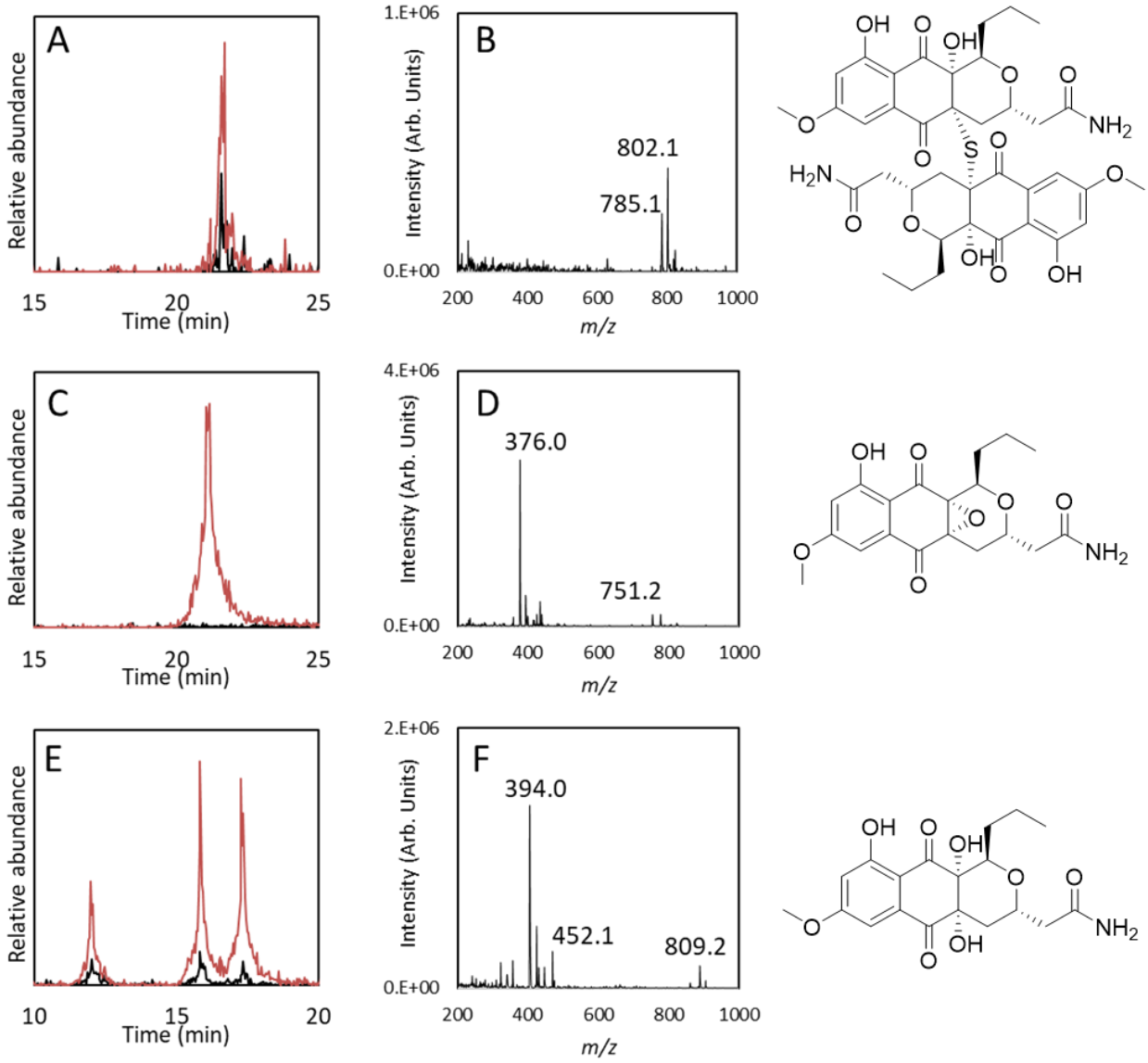
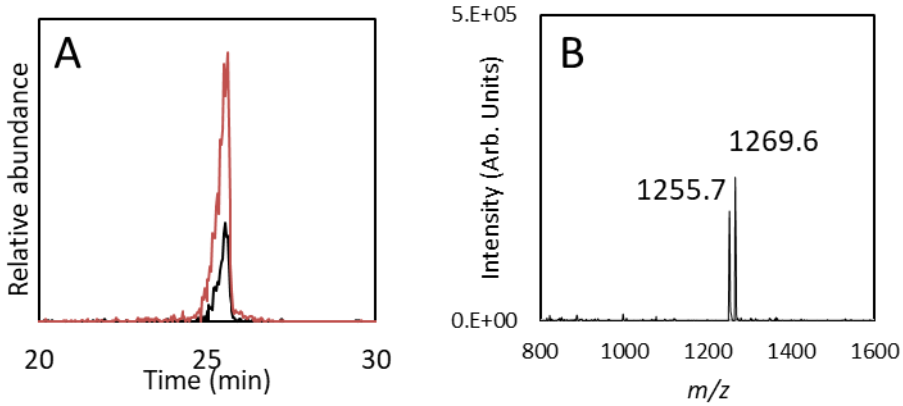


FIG S9: EICs of (A) actinomycin C2 from control (black) and TP treated (red) *Streptomyces* BCCAGE06 extracts and mass spectral data for (B) actinomycin C2. The structure of actinomycin C2 is shown below.

Strain (Isolate)	DESC	ID	Con	Rif	Str	La	Sc	TP	Rw
Streptomyces (BCCAGE06)	M1269.6T25.4	actinomycin C2	2E+07	0.8	1.0	1.5	0.7	3.4	2.0



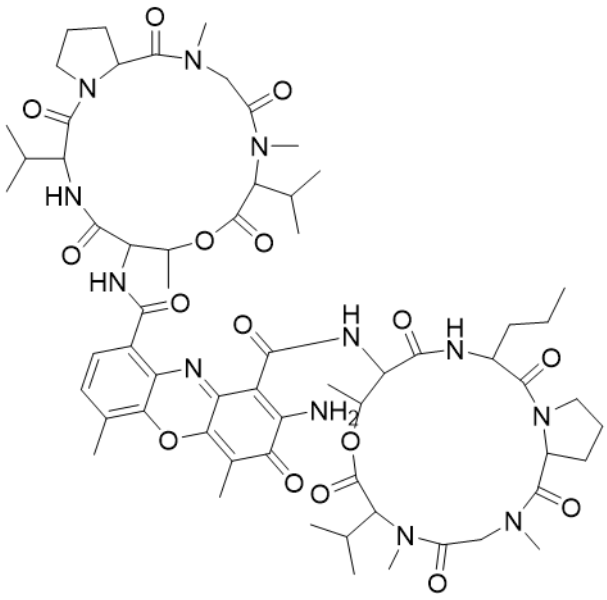


FIG S10: EICs of (A) propeptin 1, (C) propeptin 2, and (D) tetarimycin B from control (black) and Rif (a), La (b), and Str (c) treated (red) *Microbispora* BCCAGE54 extracts and mass spectral data for (B) propeptin 1, (D) propeptin 2, and (F) tetarimycin B. Structures shown on right.

Strain (Isolate)	DESC	ID	Con	Rif	Str	La	Sc	TP	Rw
Microbispora (BCCAGE54)	M1056.4T14	propeptin 1	7E+05	3.2	1.3	0.1	0.7	1.9	2.9
	M1146.9T14.6	propeptin 2	5E+05	0.6	0.0	2.0	1.5	0.5	0.7
	M369.3T17	tetarimycin B	1E+06	16.1	32.8	0.5	6.2	0.4	23.0

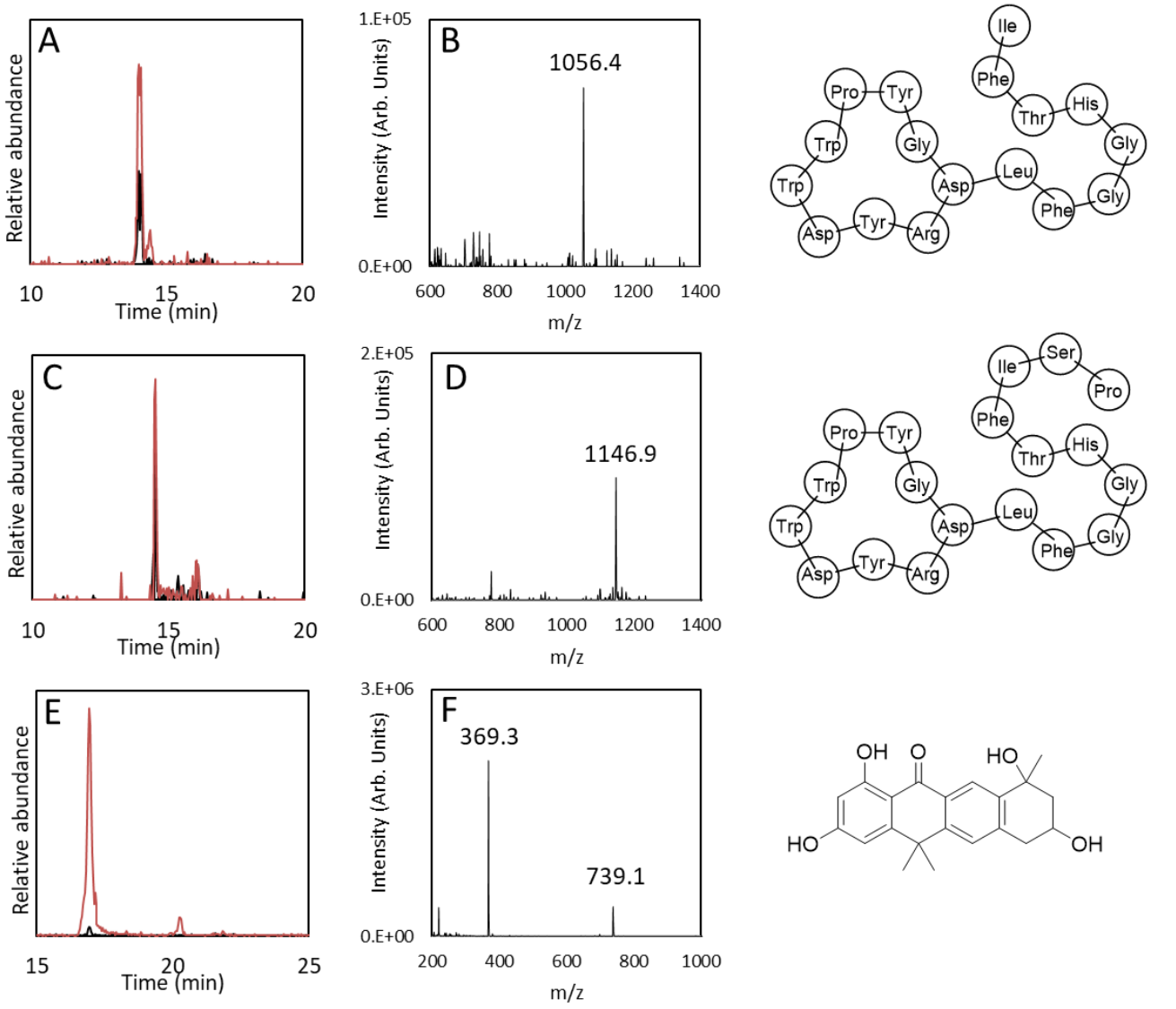
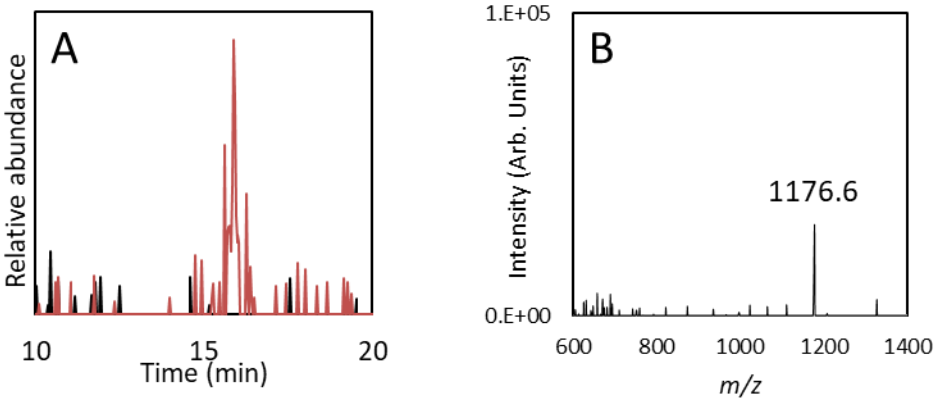


FIG S11: EICs of (A) funisamine from control (black) and TP treated (red) *Streptosporangium* KDCAGE35 extracts and mass spectral data for (B) funisamine. The structure of funisamine is shown below.

Strain (Isolate)	DESC	ID	Con	Rif	Str	La	Sc	TP	Rw
Streptosporangium (KDCAGE35)	M1178.3T15.6	funisamine	7E+03	4.3	6.4	1.3	1.4	31.2	1.4



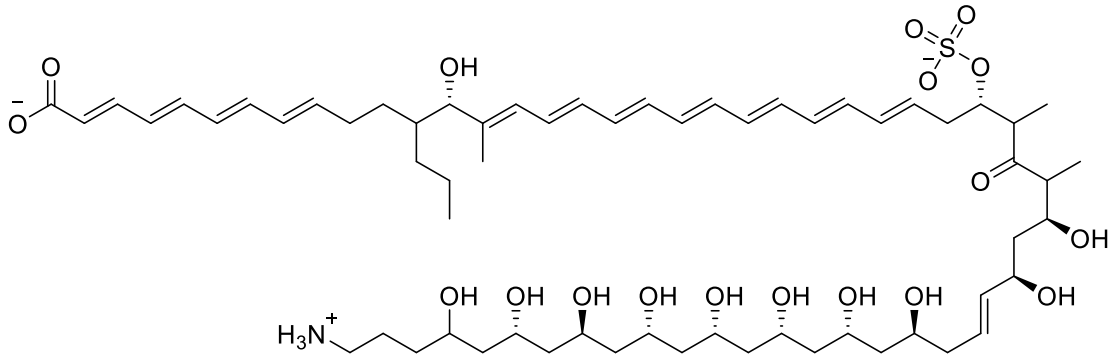
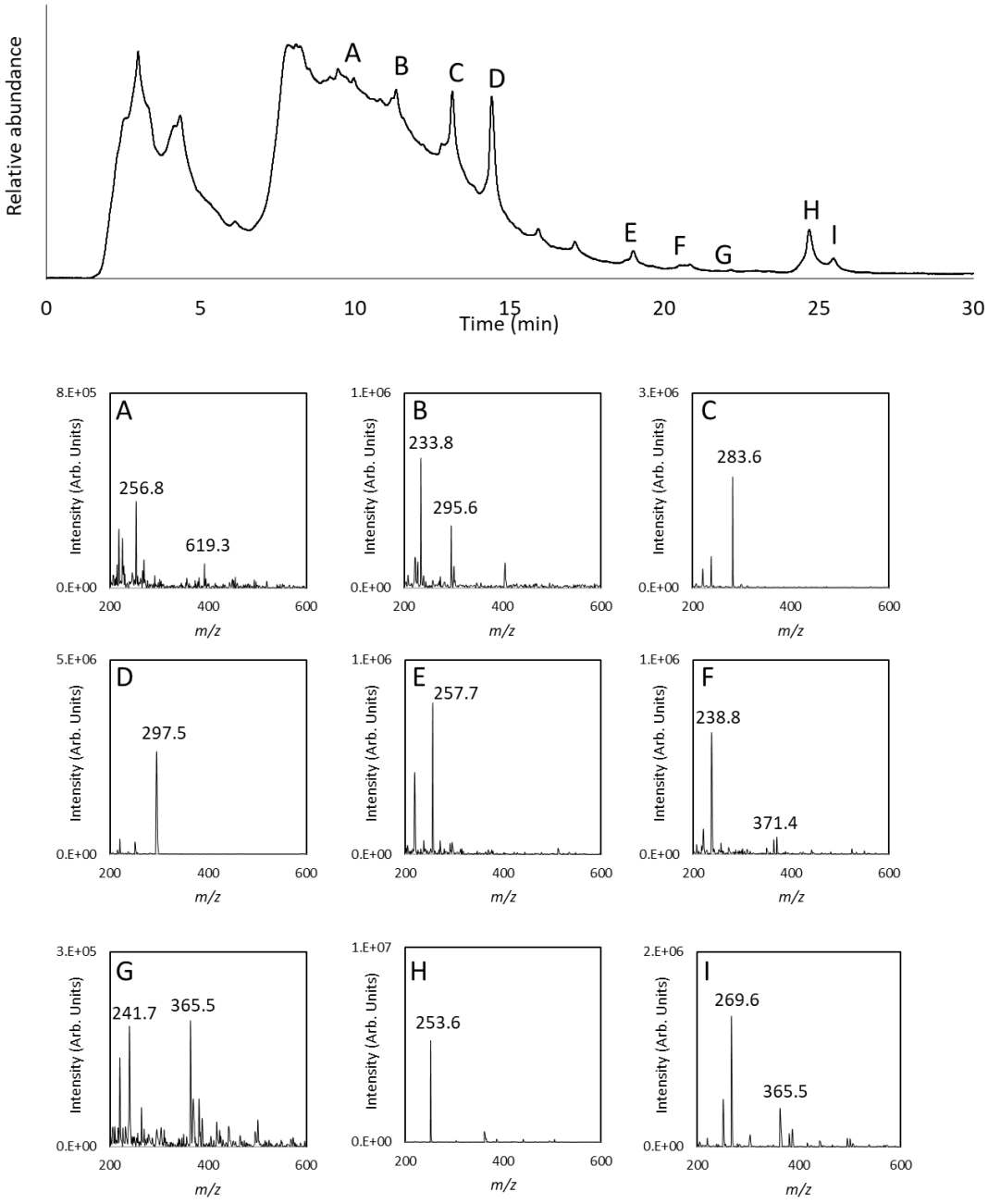


FIG S12: Mass spectral data for (A) unident. NP 1 (M256.8T9.9), (B) unident. NP 2 (M233.8T11.1), (C) unident. anthraquinone 1 (M283.6T13.1), (D) unident. anthraquinone 2 (M297.5T14.3, *DMAC*), (E) okicenone (M257.7T17.2), (F) unident. anthraquinone 3 (M238.7T20.8, *dihydroxyanthraquinone*), (G) unident. anthraquinone 4 (M241.7T22), (H) aloesaponarin II (M253.6T24.6), and (I) unident. anthraquinone 5 (M269.6T25.4, *Hydroxyaloesaponarin II*) in *Micromonospora* BBHARD2*2*



A. 6. UV spectrum for natural products listed in Tables S1 and S2

FIG S13: UV spectrum of (A) unident. NP 1 (M256.8T9.9), (B) unident. NP 2 (M233.8T11.1), (C) unident. anthraquinone 1 (M283.6T13.1), (D) unident. anthraquinone 2 (M297.5T14.3, *DMAC*), (E) okicenone (M257.7T17.2), (F) unident. anthraquinone 3 (M238.7T20.8, *dihydroxyanthraquinone*), (G) unident. anthraquinone 4 (M241.7T22), (H) aloesaponarin II (M253.6T24.6), and (I) unident. anthraquinone 5 (M269.6T25.4, *Hydroxyaloesaponarin II*) in *Micromonospora* BBHARD22

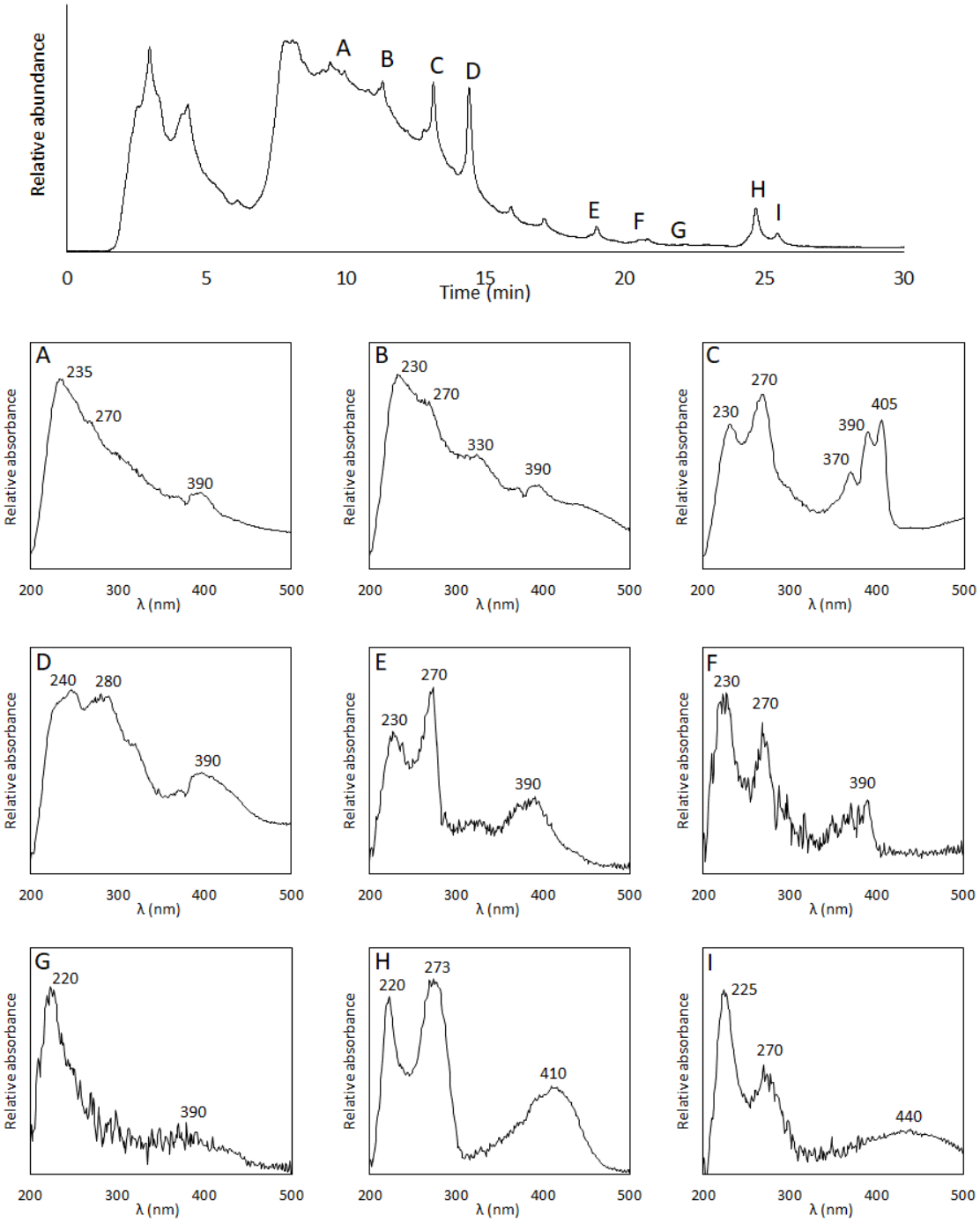


FIG S14: UV spectrum of (A) unident. hypogeamicin 4 (M605.1T14.7), (B) unident. hypogeamicin 3 (M410T15.1) in *Nonomuraea* (BBHARD23) unident. hypogeamicin 3 (M809.1T15.8), (C) hypogeamicin C (M394T15.8), (D) hypogeamicin B (M376T21.1), (E) unident. hypogeamicin 1 (M833.2T21), (F) unident. hypogeamicin 2 (M818.1T21.2), and (G) hypogeamicin A (M785.1T21.5) in *Nonomuraea* BBHARD23 extracts.

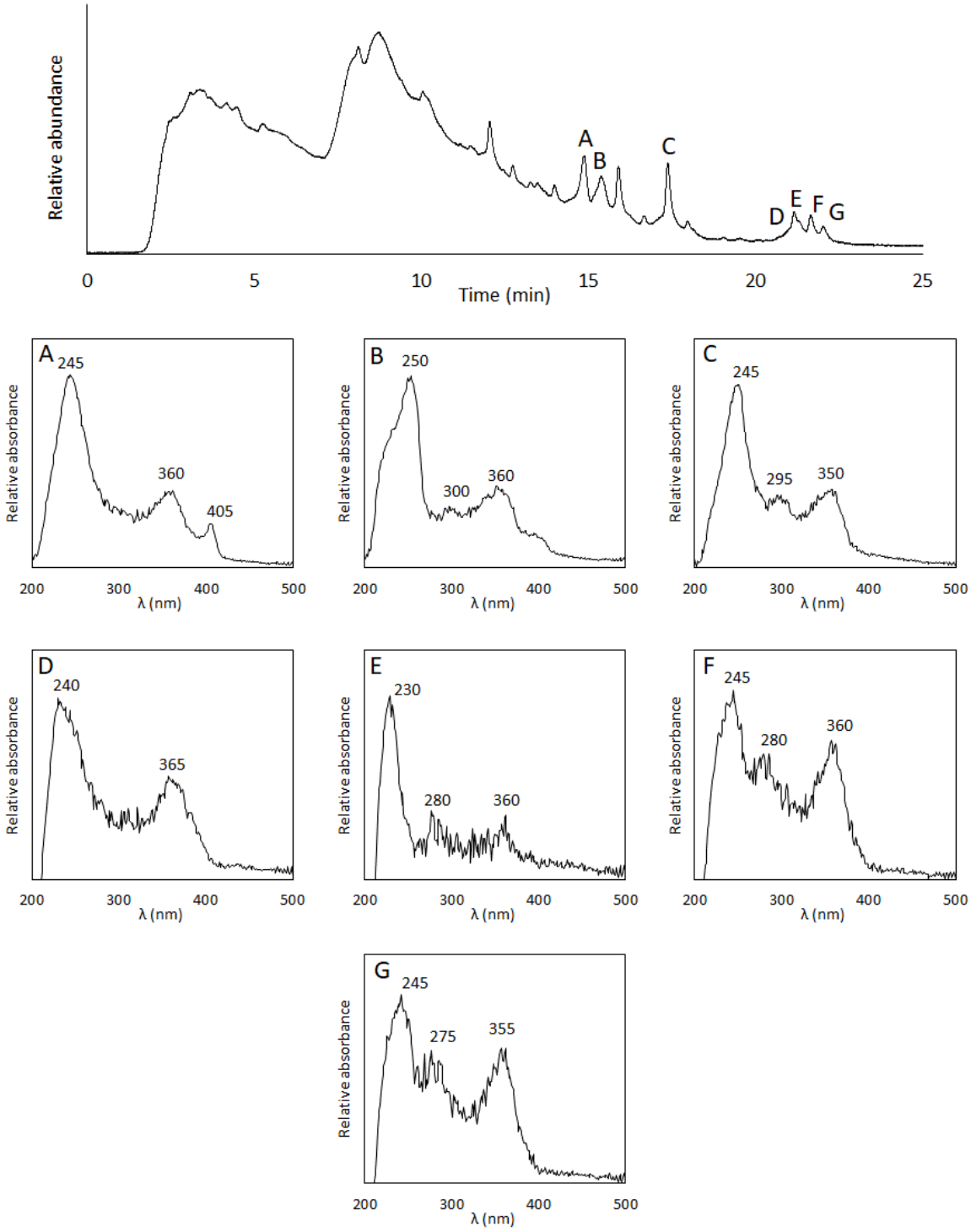


FIG S15: UV spectrum of (A) unident. actinomycin 1 (*Y5*) (M1271.6T22.6), and (B) actinomycin C2 (M1270.6T25.4), unident. actinomycin 2 (M1287.5T25.4, *actinomycin Y3*), unident. actinomycin 3 (M1293.6T25.4, *actinomycin G2*), unident. actinomycin 4 (M1257.7T25.4, *actinomycin F4*), unident. actinomycin 5 (M1307.5T25.4, *actinomycin Y2*), unident. actinomycin 6 (M1255.6T25.4, *actinomycin D*), unident. actinomycin 7 (M1327.7T25.4), and unident. actinomycin 8 (M1313.7T25.4) in *Streptomyces* BCCAGE06

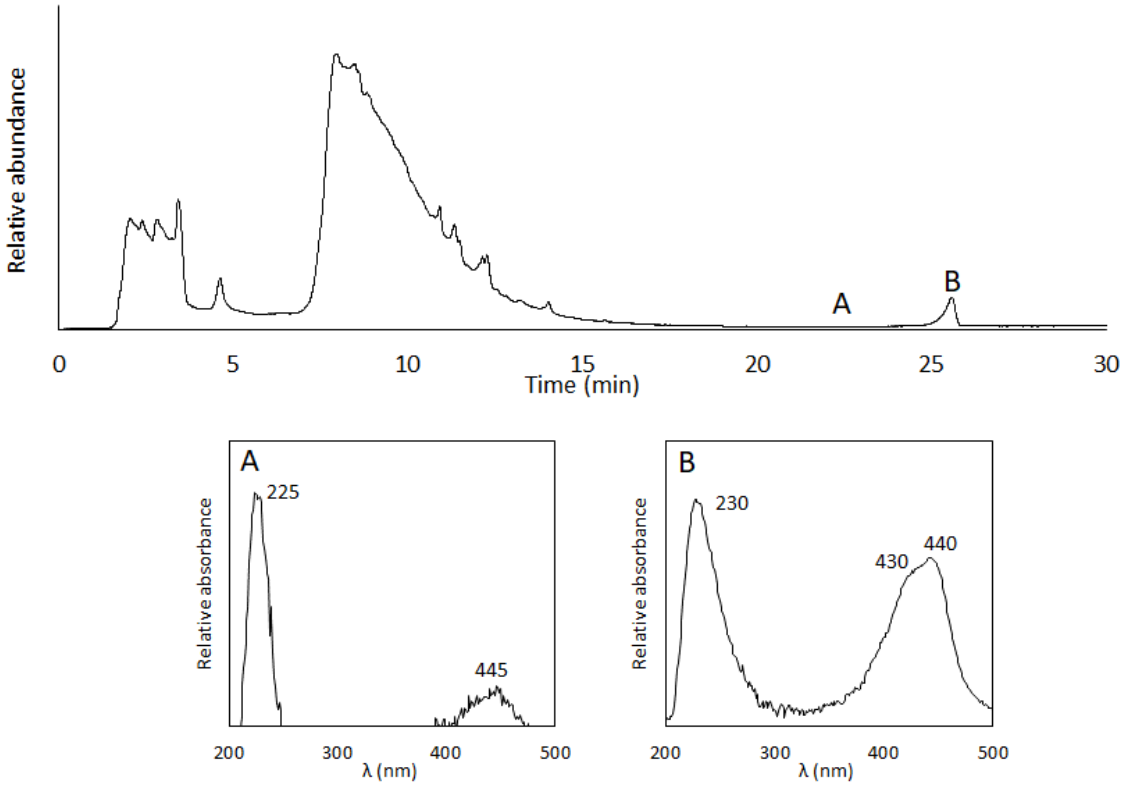


FIG S16: UV spectrum of (A) unident. NP 3 (M311.4T8.3), (B) unident. NP 4 (M287.5T9.5), (C) unident. NP 5 (M634.9T12.5), (D) unident. NP 6 (M295.5T12.9), (E) unident. NP 7-8 (M707.1T16.2, M311.4T16.6), and (F) and unident. NP 9 (M253.6T26.3, aloesaponarin II) in *Streptomyces* BCCAGE31.

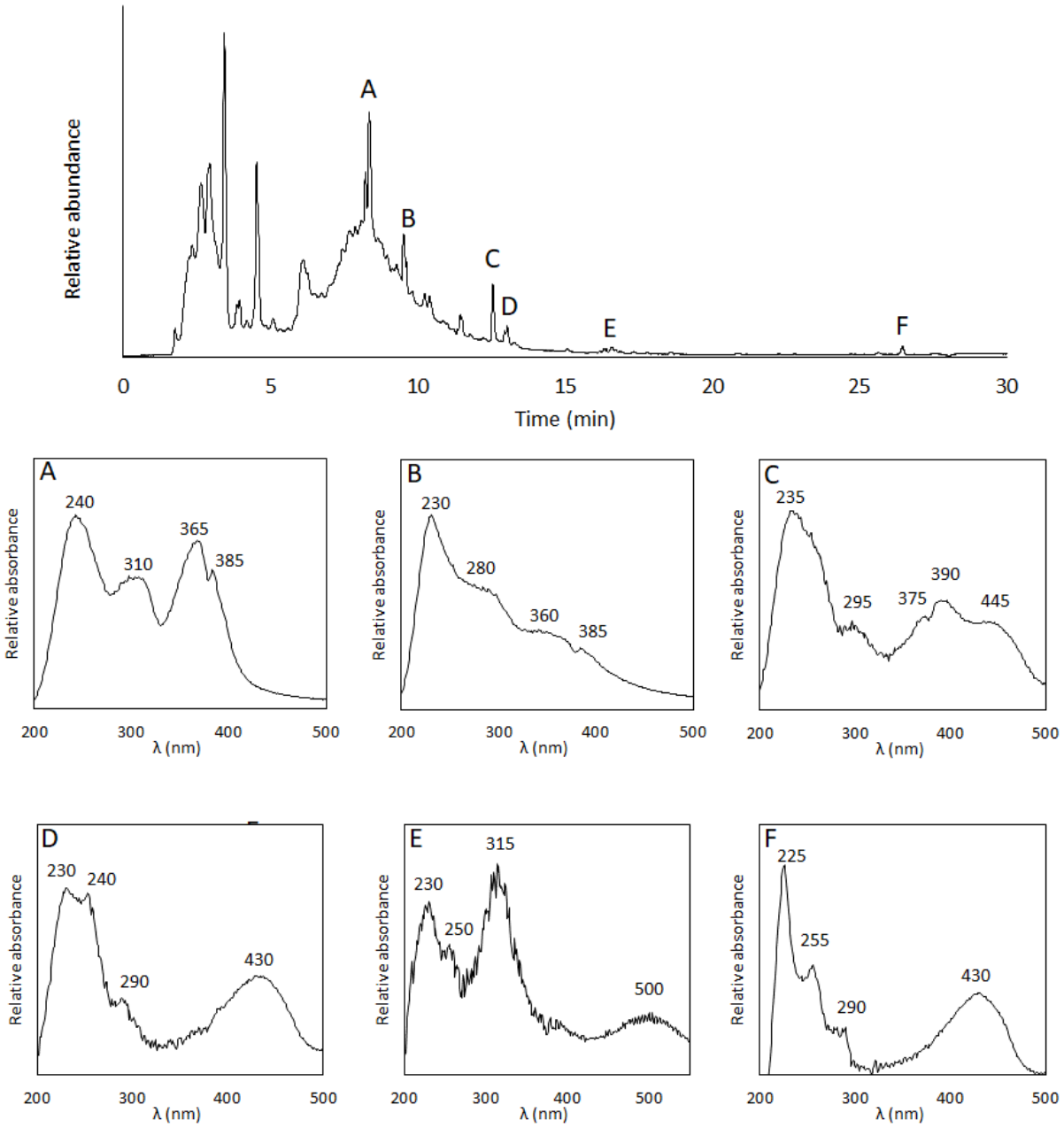


FIG S17: UV spectrum of (A) unident. NP 10 (M411.4T12), (B) unident. Polyene 1 (M628.1T12.7), (C) unident. Polyene 2 (M628.1T13.3), (D) unident. Polyene 3 (548.3T15.4), and (E) unident. Polyene 4 (548.3T16.2) in *Nonomuraea* BCCAGE42 extracts.

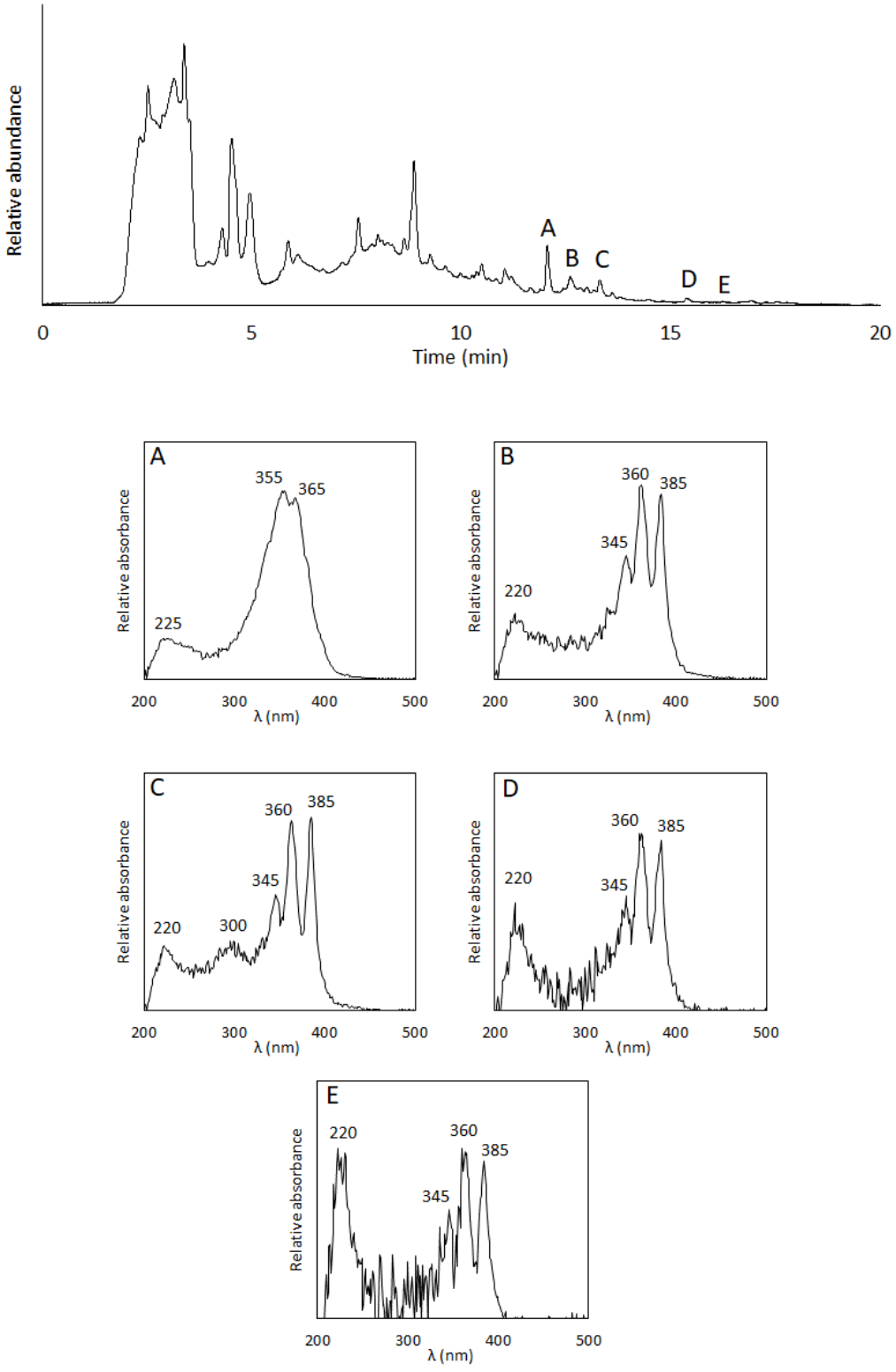
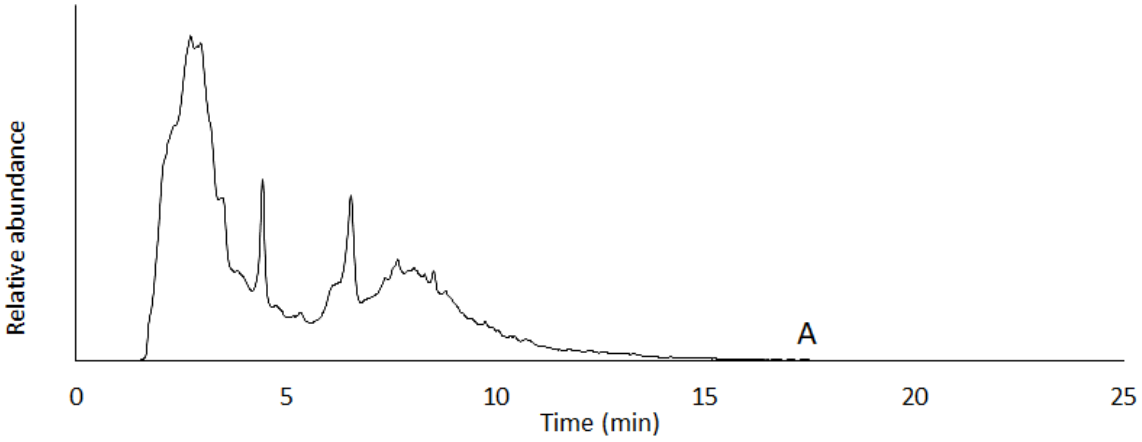


FIG S18: UV spectrum of (A) unident. NP. 11-12 (M900.2T17.1, M895.2T17.9) in *Streptomyces* BCCAGE18 extracts.



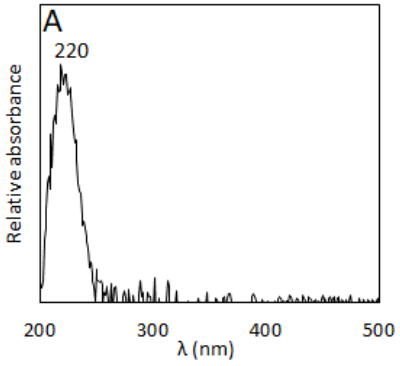


FIG S19: UV spectrum of (A) unident. NP 13 (M398.4T13.5), (B) unident. NP. 14 (M247.8T15.2), and (C) unident. NP. 15 (M257.8T24.2) from *Saccharothrix* BBHARD27 extracts.

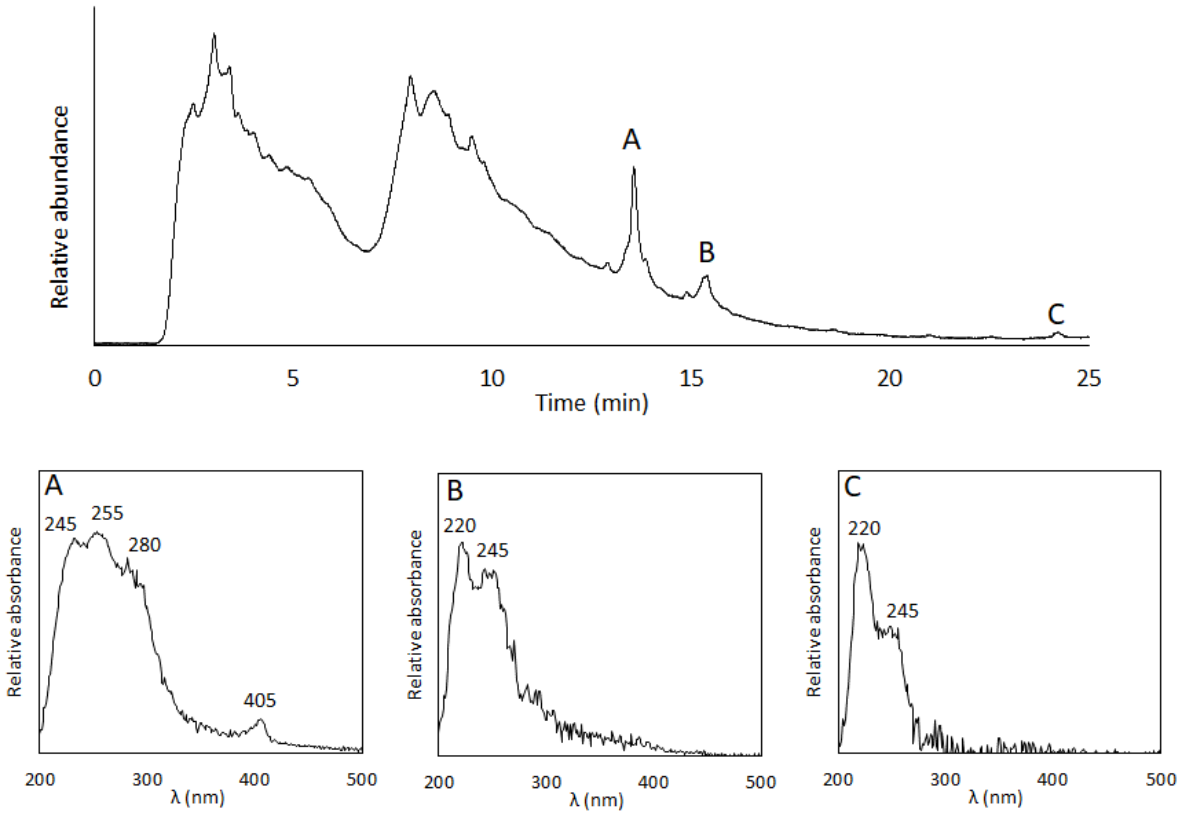


FIG S20: UV spectrum of (A) unident. Polyene 5 (M730.2T20.3) from *Kribella* BBSNAI08 extracts.

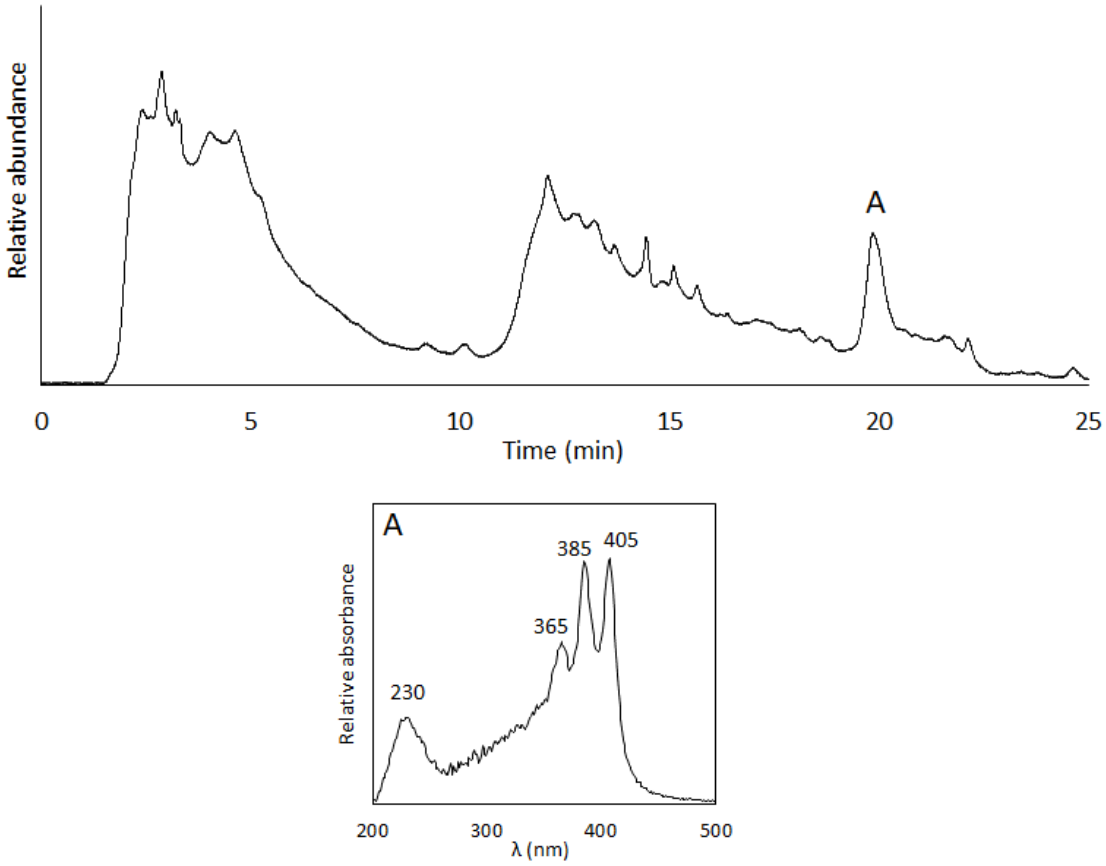


FIG S21: UV spectrum of (A) unident. NP. 16 and 17 (M689.2T10.5, M561.1T10.7), (B) unident. NP. 18 and 19 (M575.2T11.5, M717.2T11.6), (C) unident. NP. 20 (M731.3T12.1), (D) unident. NP. 21 (M355.4T13, *Arromycin*), (E) unident. NP. 22 (M393.2T13.7, *Linfuranone A*), (F) propeptin 1 (M1056.4T14), (G) propeptin 2 (M1146.9T14.6), (H) unident. tetarimycin 1 (M385.2T14.7), (I) unident. tetarimycin 2 (M399.2T16.1), (J) tetarimycin B (M369.3T17), (K), unident. tetarimycin 3 (M406.3T17.5) and (L) unident. tetarimycin 4 (M395.2T19.5) from *Microbispora* BCCAGE54 extracts.

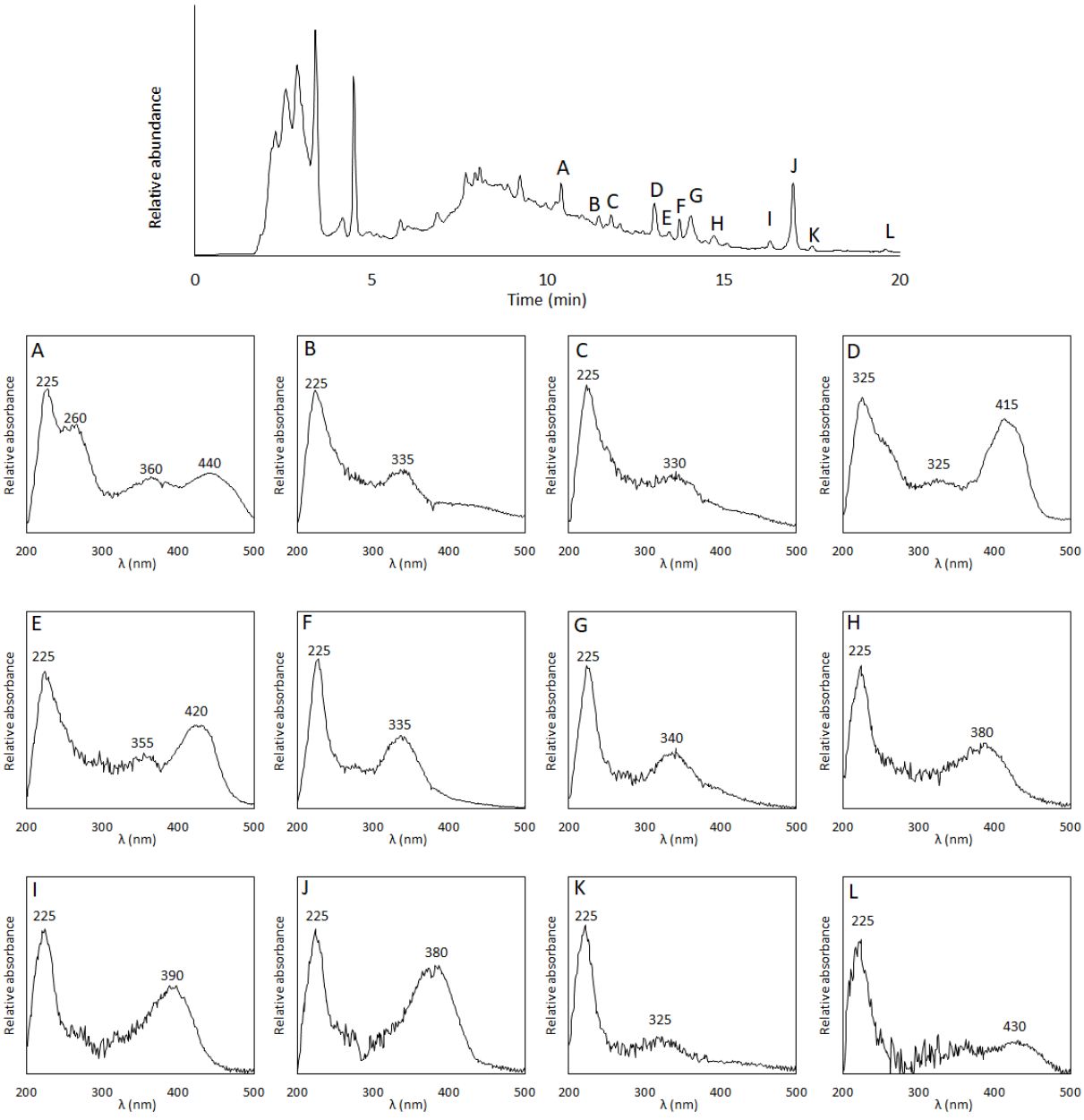
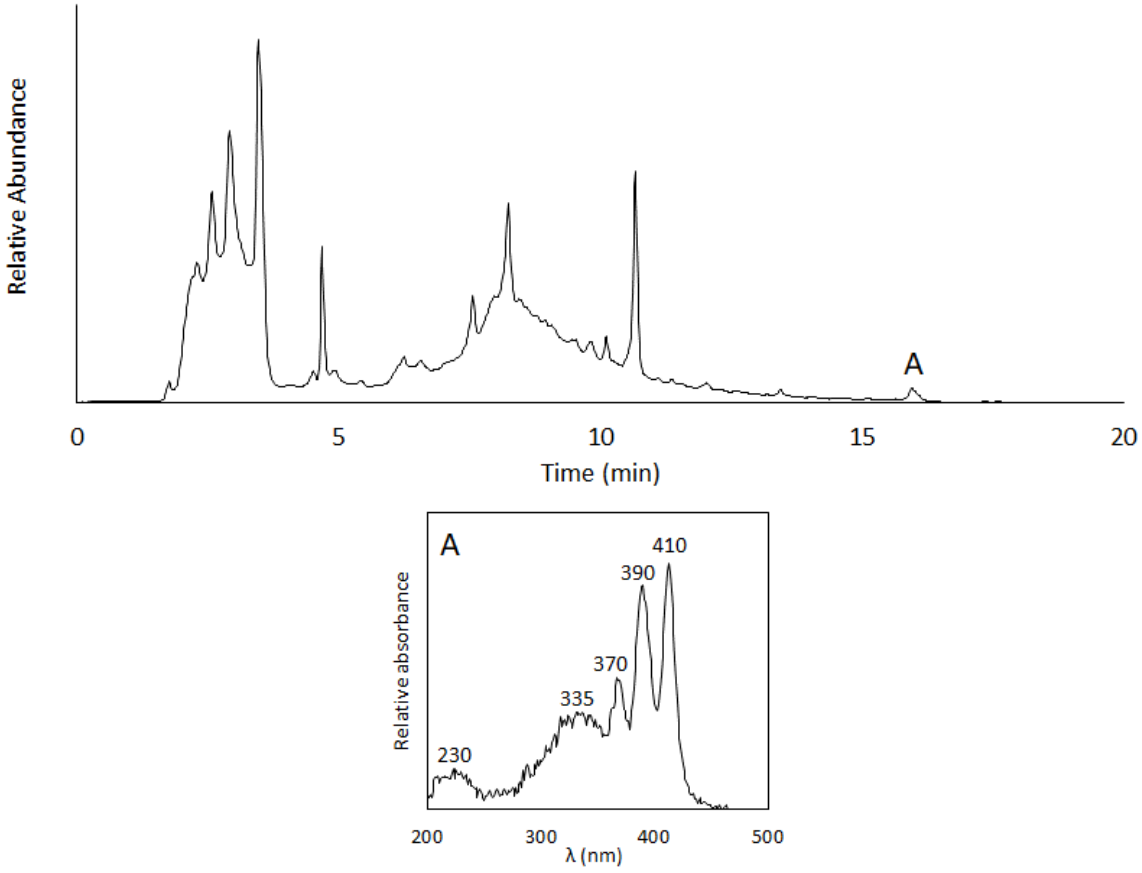


FIG S22: UV spectrum of (A) funisamine (M1178.3T15.6) from *Streptosporangium* KDCAGE35 extracts.



A. 7. NMR Spectra for identified natural products

The following are NMR spectra taken for selected compounds in table S1

FIG S23: H-NMR spectrum of aloesaponarin II from *Nonomuraea* BBHARD22 in Acetone-d6

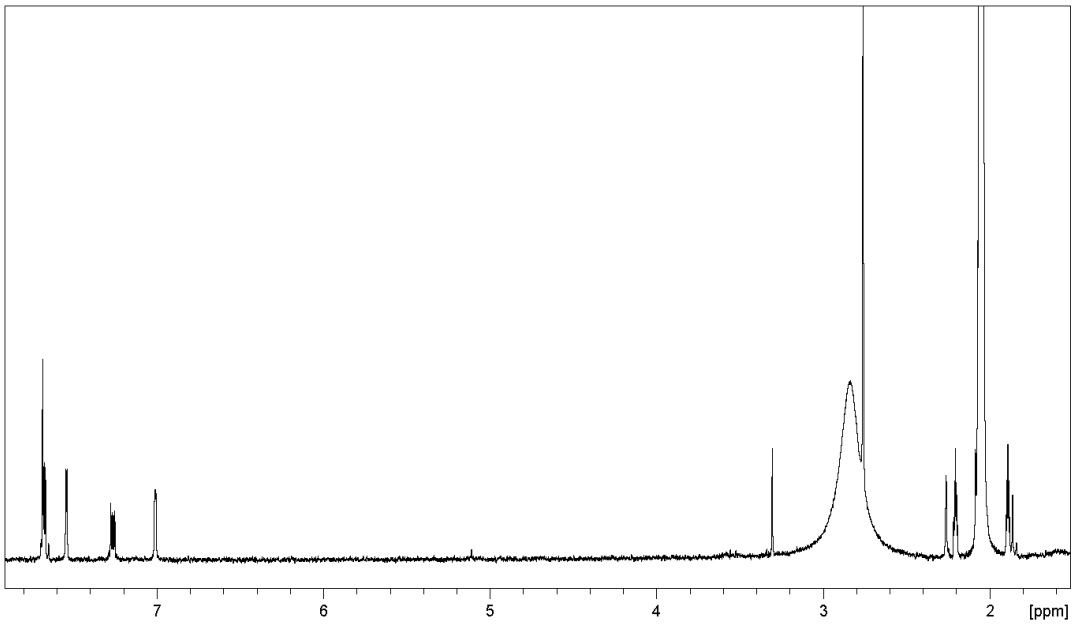


FIG S24: H-NMR spectrum of aloesaponarin II from *Nonomuraea* BBHARD22 in Acetone-d6 expanded to show the aromatic region.

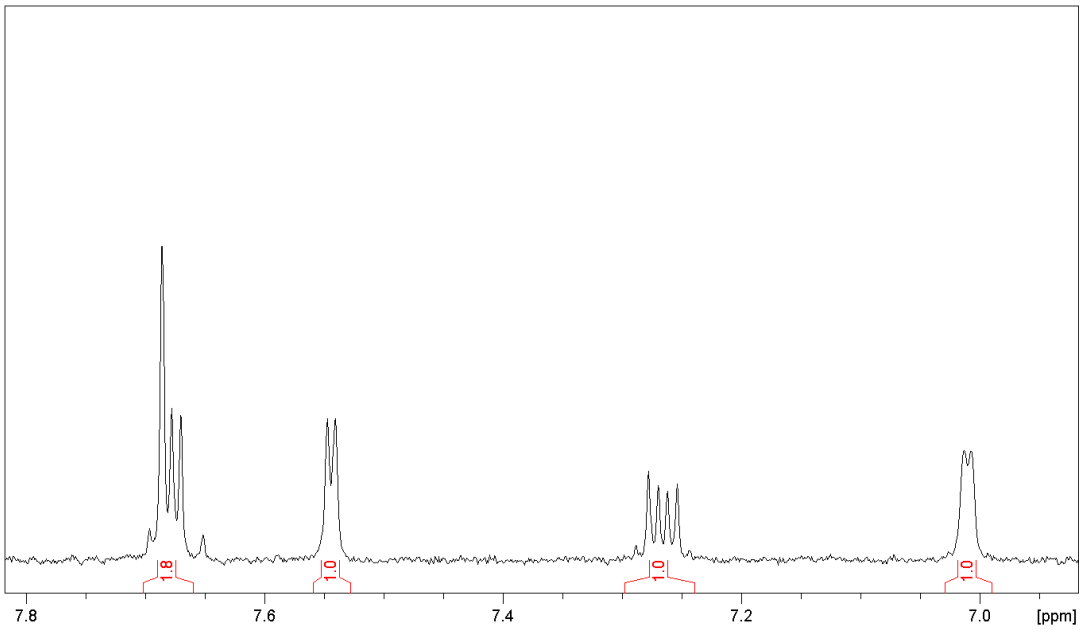


FIG S25: H-NMR spectrum partially purified okicenone from *Nonomuraea* BBHARD22 in Methanol-d4. Peaks corresponding to okicenone indicated by (*).

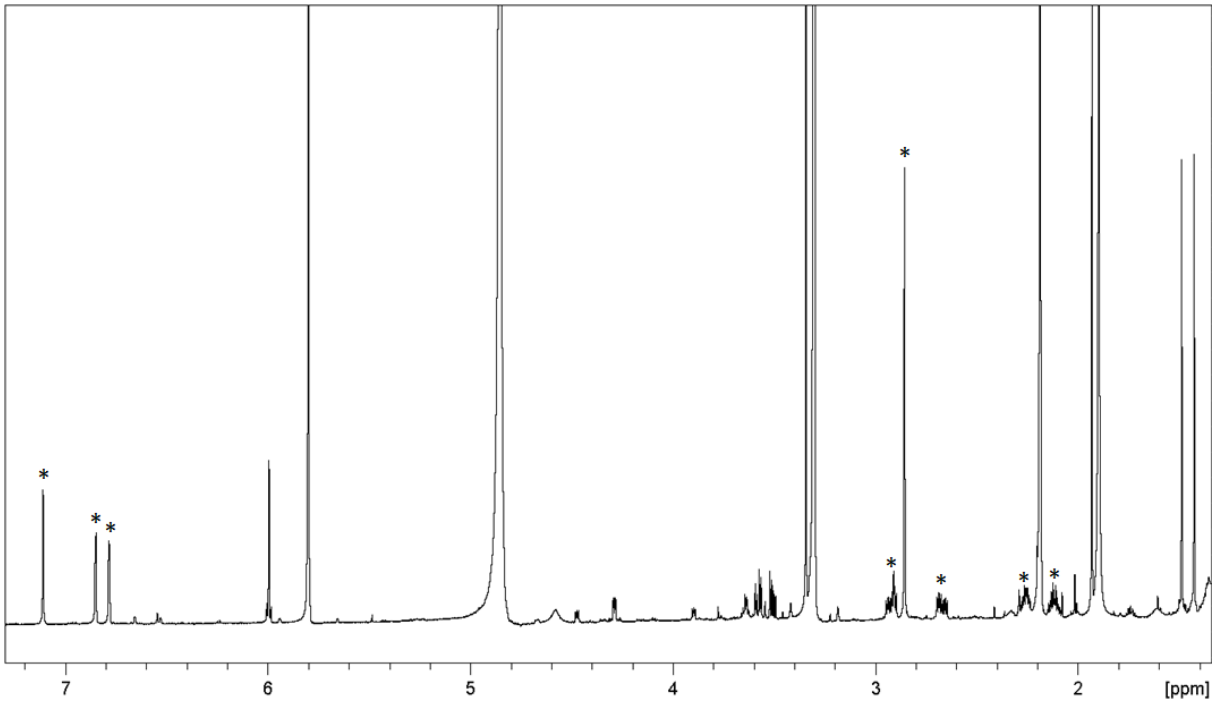


FIG S26: H-NMR spectrum of tetarimycin B from *Microbispora* BCCAGE54 in Methanol-d4

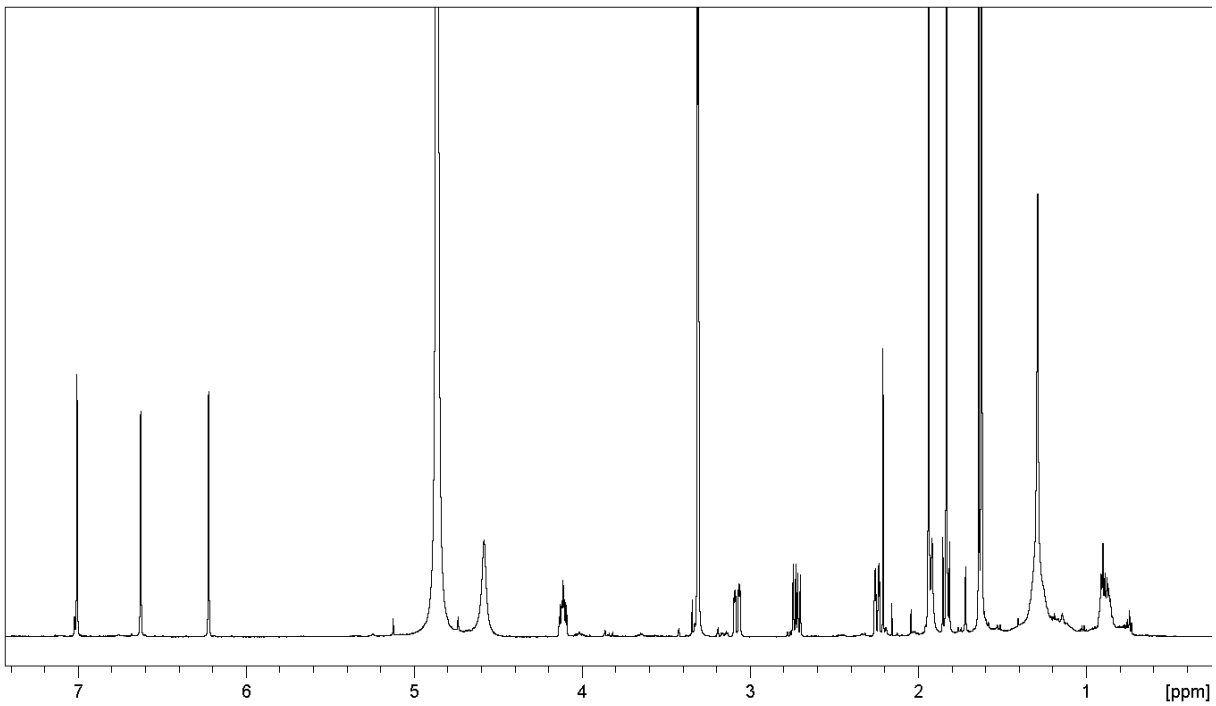


FIG S27: H-NMR spectrum of hypogeamicin B from *Nonomuraea* BBHARD23 in Methanol-d4

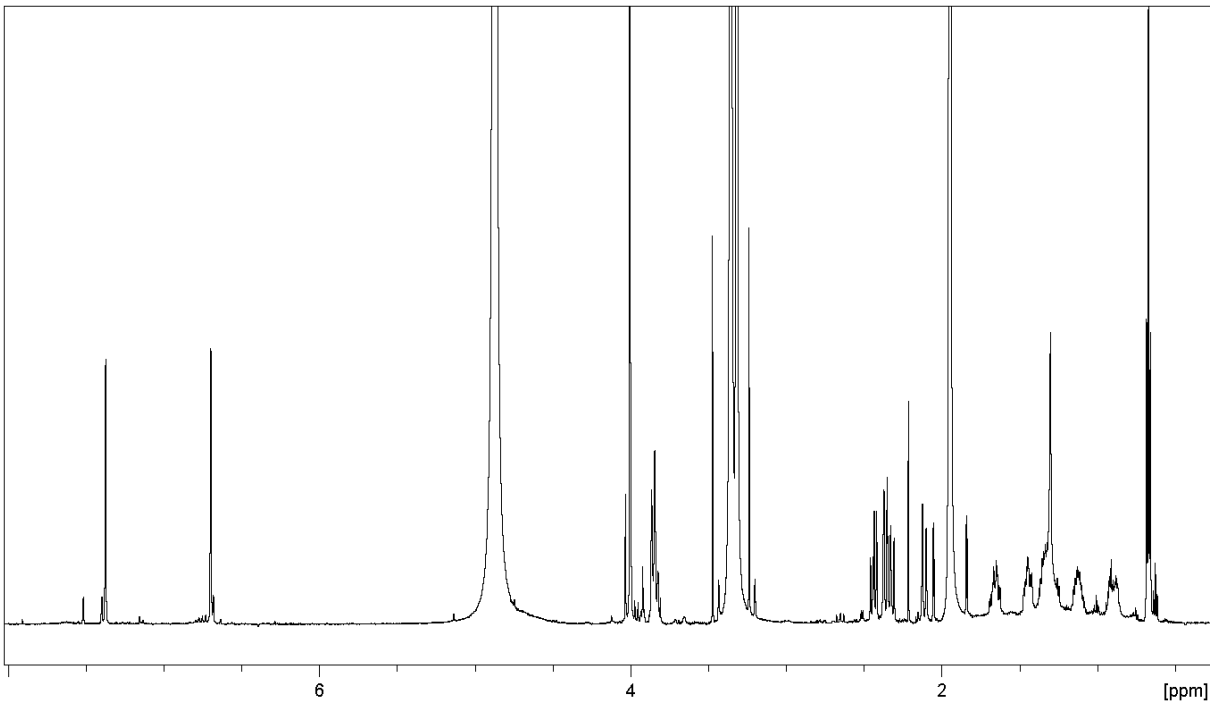
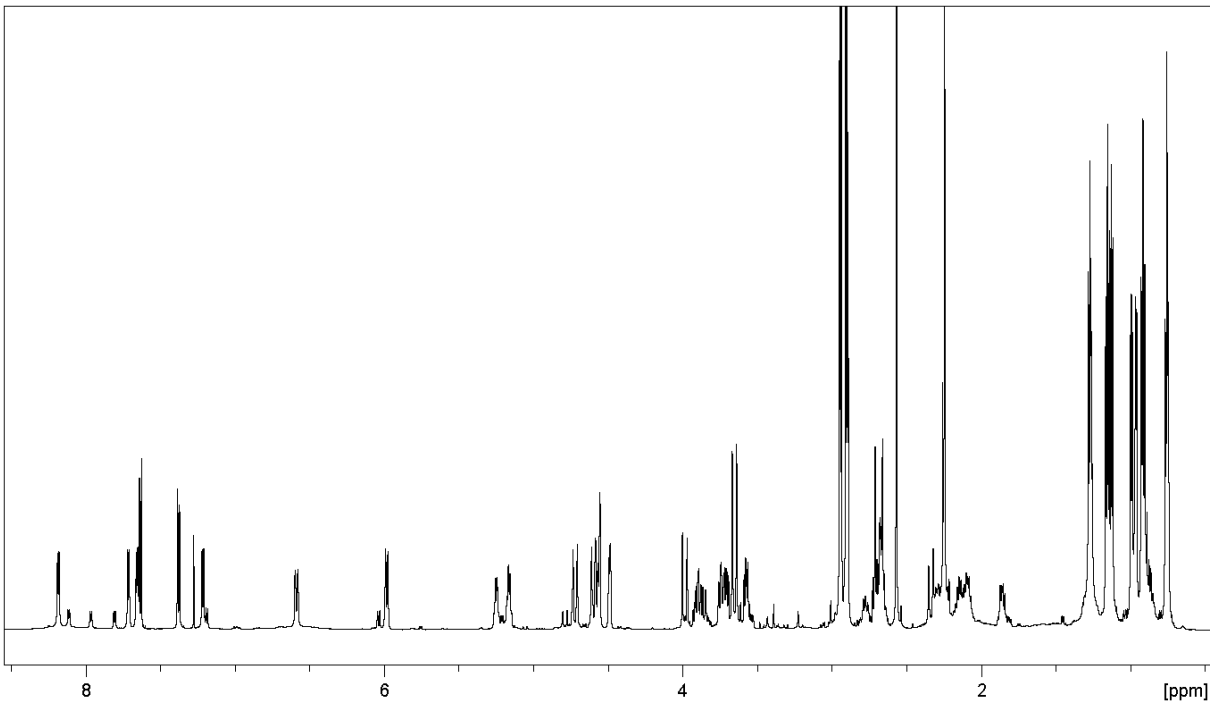


FIG S28: H-NMR spectrum of actinomycin C from *Streptomyces* BCCAGE06 in Chloroform-d1



B.1. NMR correlation table for funisamine

Table S5: NMR data for funisamine

Pos.	δ_C	δ_H	HMBC	NOESY
1	172.6			
2	125.1	5.88, 1H, d (15.2 Hz)	4	
3	141.7	7.13, 1H, dd (11.7, 15.2 Hz)	1, 4, 5	5
4	129.9	6.32, 1H, dd (11.7, 14.5 Hz)		
5	138.9	6.53, 1H, dd (11, 14.5 Hz)	3	3, 7
6	130.0	6.22, 1H, dd (11, 14.8 Hz)		
7	136.2	6.35, 1H, dd (10.1, 14.8 Hz)	5, 6, 9	5
8	130.7	6.12, 1H, dd (10.1, 14.9 Hz)	10	
9	137.0	5.78, 1H, (dt (7.5, 14.9 Hz)	7, 10	
10	29.7	2.13, 2H, dd (7.2 Hz)	8, 9, 10, 12	8, 11, 12, 13
11	29.5	1.30, 1.41, 2H	8, 9, 10, 12, 13	
12	39.9	1.57, 1H		
13	78.3	3.93, 1H, d	10, 12, 59, 60	15
14	140.2			
15	126.1	6.13, 1H, d (10.5 Hz)	13, 59	17
16	128.7	6.52, 1H, dd (11.6, 14.5 Hz)	18	
17	133.2	6.26, 1H, overlap		
18	133.5	6.37, 1H, overlap		
19	133.5	6.37, 1H, overlap		
20	133.5	6.37, 1H, overlap		
21	133.5	6.37, 1H, overlap		
22	133.5	6.37, 1H, overlap		
23	133.0	6.36, 1H, overlap		
24	132.9	6.3, 1H, overlap		
25	131.8	6.25, 1H, overlap		
26	133.5	6.2, 1H, overlap		
27	129.8	5.82, 1H, dt (7.4, 14.7 Hz)	28, 29	
28	35.1	2.45, 2.62, 2H, overlap	26, 27, 29, 30	
29	79.0	4.61, 1H, dd (5.6 Hz)	27, 30, 31, 58	
30	47.5	3.2, 1H	28, 29, 31, 58	
31	215.5			
32	50.8	2.91, 1H,	31, 57	
33	66.7	4.01, 1H		
34	41.3	1.56, 1.71 2H	39	
35	70.9	4.25, 1H, q (6.8 Hz)	34, 36, 37	
36	135.0	5.53, 1H, dd (7.3, 15.3 Hz)	35, 38	
37	127.7	5.72, 1H, dt (7.2, 15.3 Hz)	35, 38	
38	40.1	2.24, 2H, multiplet	36, 37, 39, 40,	

Pos.	δ_C	δ_H	HMBC	NOESY
39	69.7	3.84, 1H		
40	43.1	1.56, 1.67, 2H		
41	68.6	3.99, 1H		38, 39
42	43.0	1.6, 2H		
43	66.8	4.04, 1H	45	
44	43.0	1.6, 2H		
45	64.5	4.1, 1H		
46	43.0	1.6. 2H		
47	69.0	4, 1H		
48	43.2	1.6, 2H		
49	69.0	4, 1H		
50	44.3	1.6, 2H		
51	68.6	3.96, 1H		
52	44.7	1.53, 2H		
53	68.9	3.81, 1H		
54	33.5	1.47, 1.62, 2H	53	
55	23.7	1.76, 1.81, 2H	53, 54, 56	
56	39.4	2.95, 2H	54, 55	
57	10.1	1.1, 3H, d (7 Hz)	31, 32	29, 30, 32, 33, 35
58	12.2	1.14, 3H, d (7 Hz)	29, 30, 31	28, 29, 30, 32, 33
59	11.8	1.74, 3H, s	13, 14, 15, 16	
60	30.7	1.26, 1.47 2H		
61	19.5	1.25, 1.42, 2H	9	8, 10, 11, 13, 60, 62
62	13.4	0.9, 3H, t (7.1 Hz)		61

B.2. NMR spectroscopic data for funisamine

FIG S29: H-NMR (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4

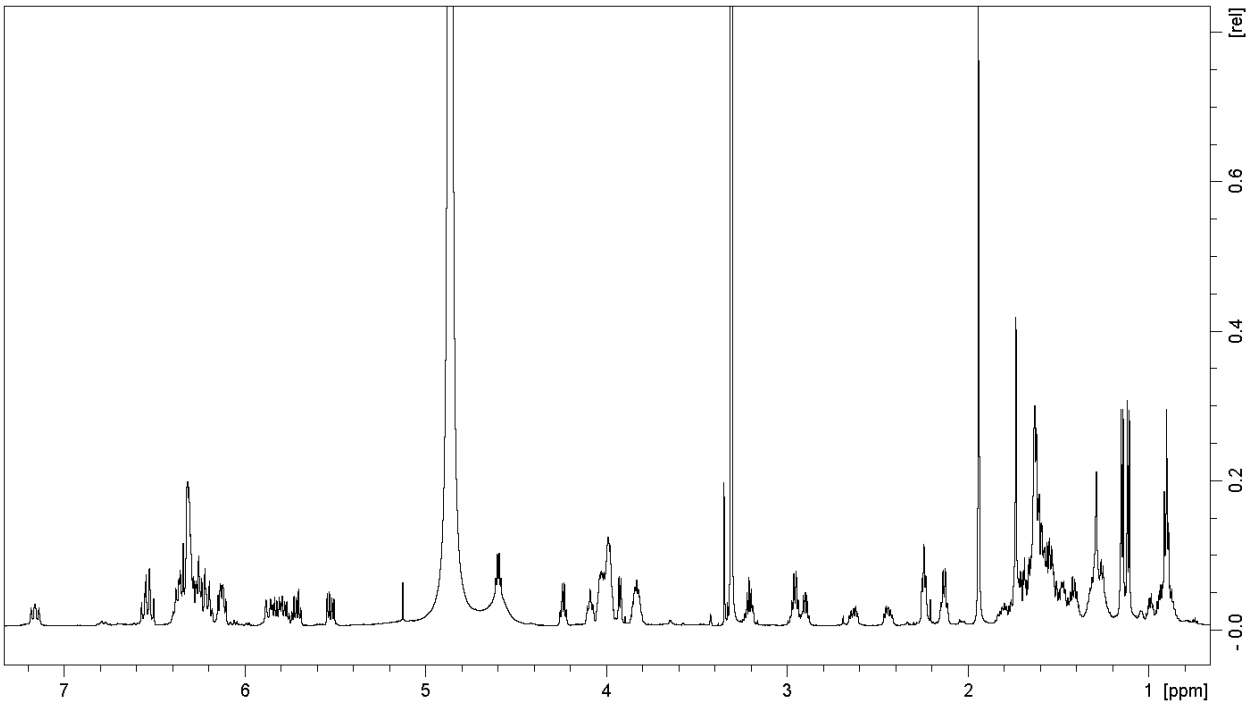


FIG S30: C-NMR (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4

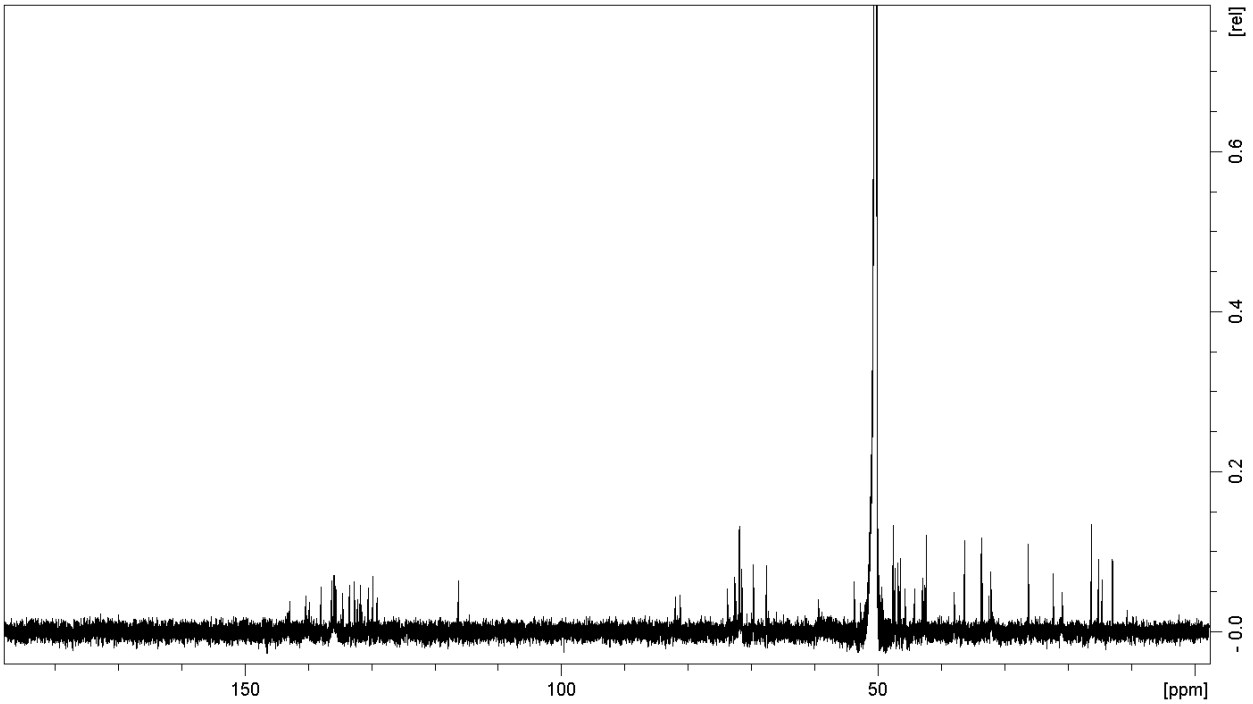


FIG S31: COSY (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4

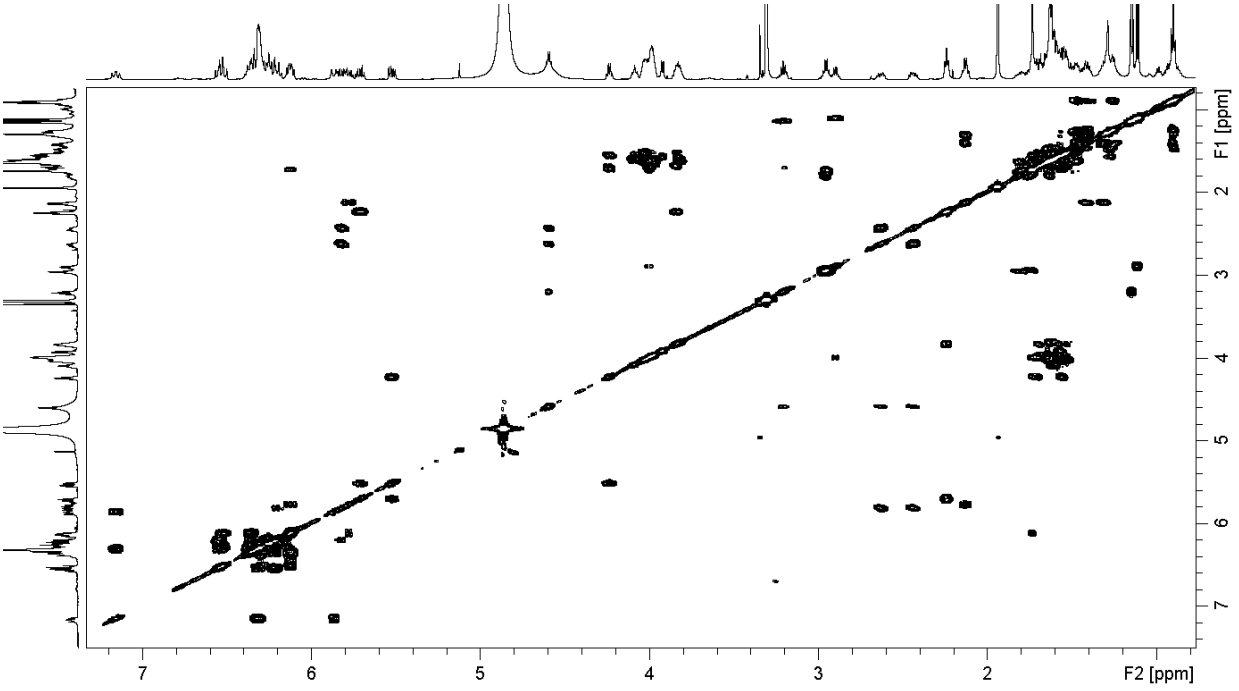


FIG S32: COSY (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4 enlarged to show aromatic couplings.

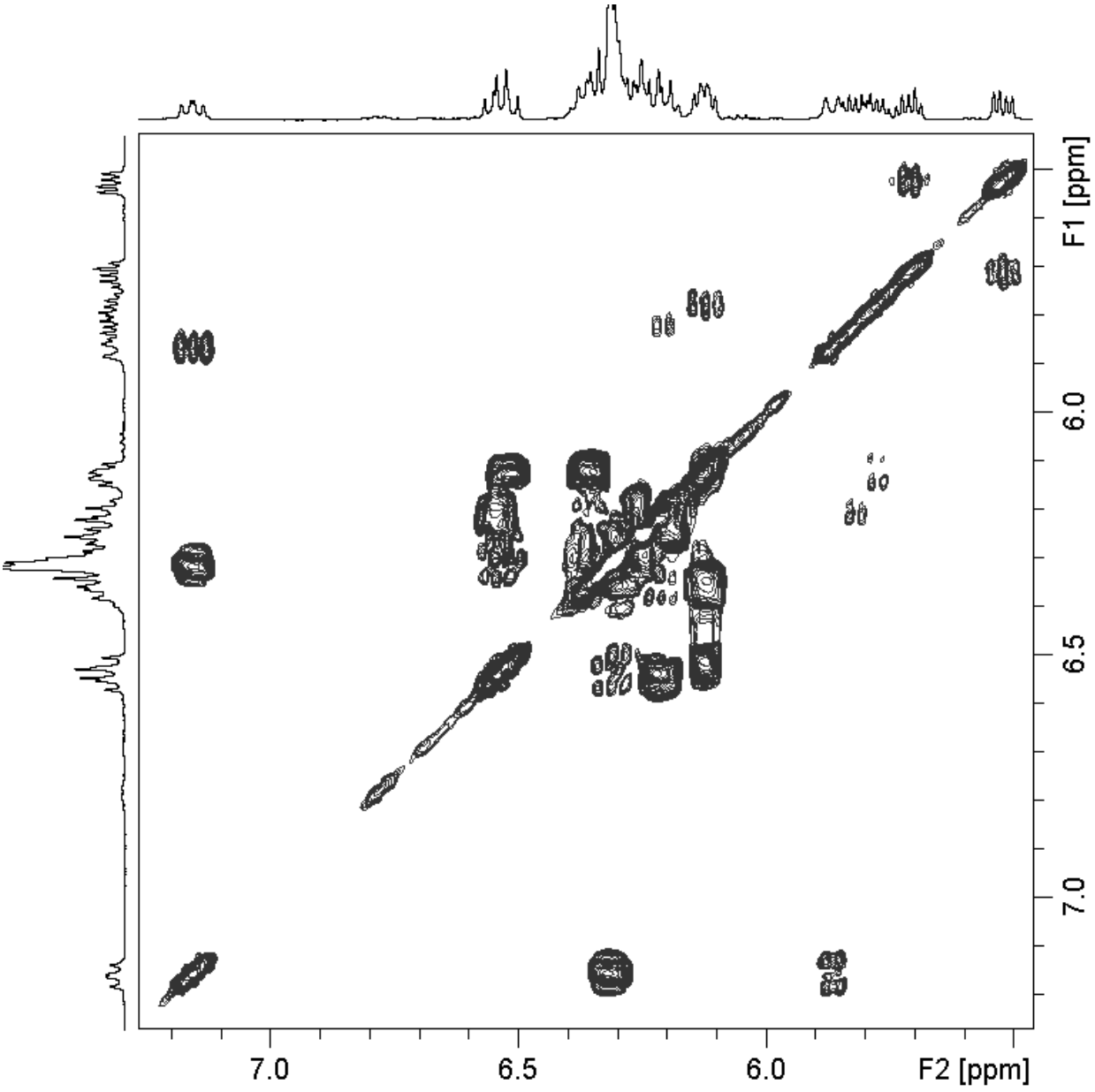


FIG S33: COSY (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4 enlarged to methylene and hydroxy-methine couplings.

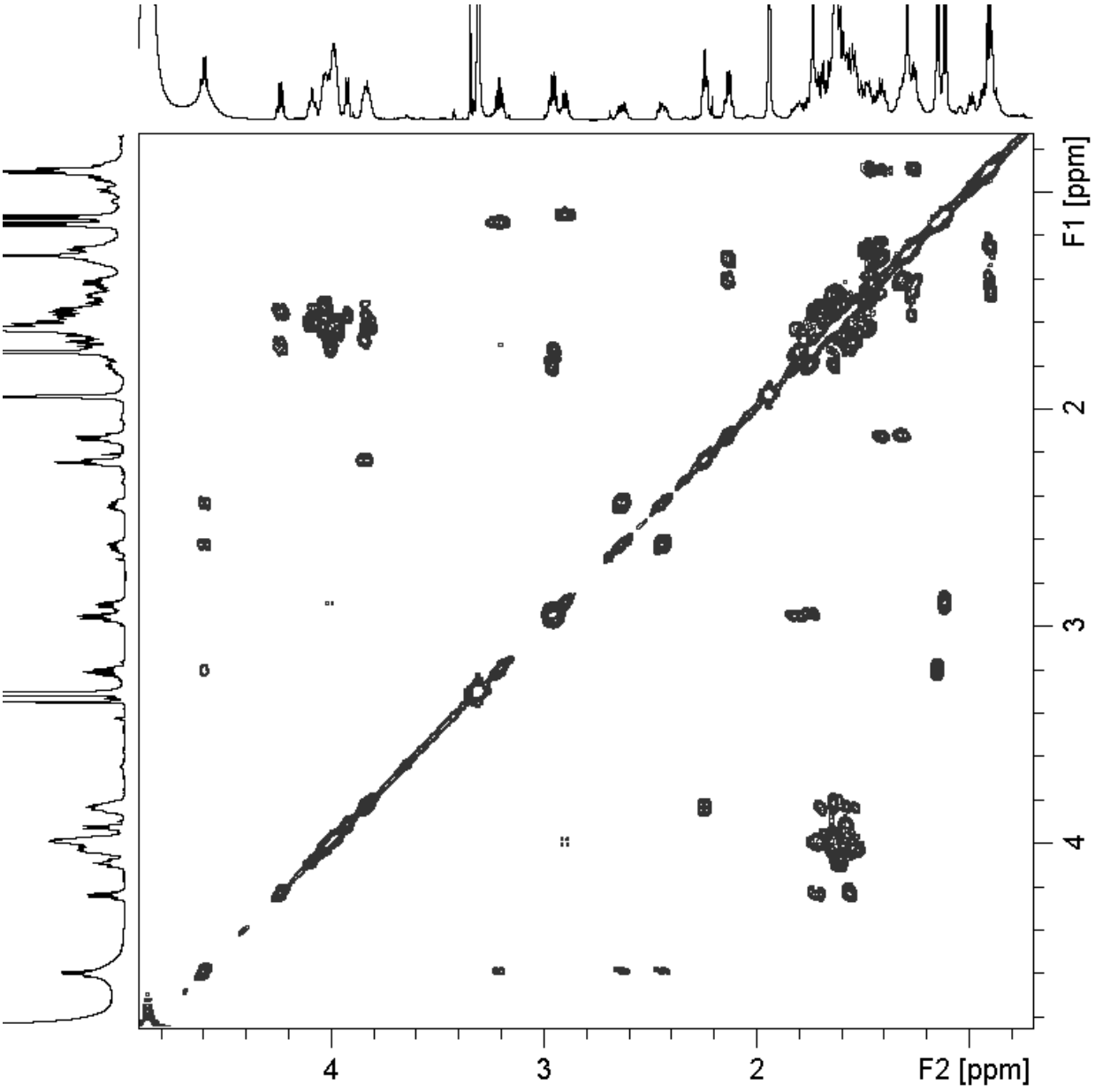


FIG S34: TOCSY (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4

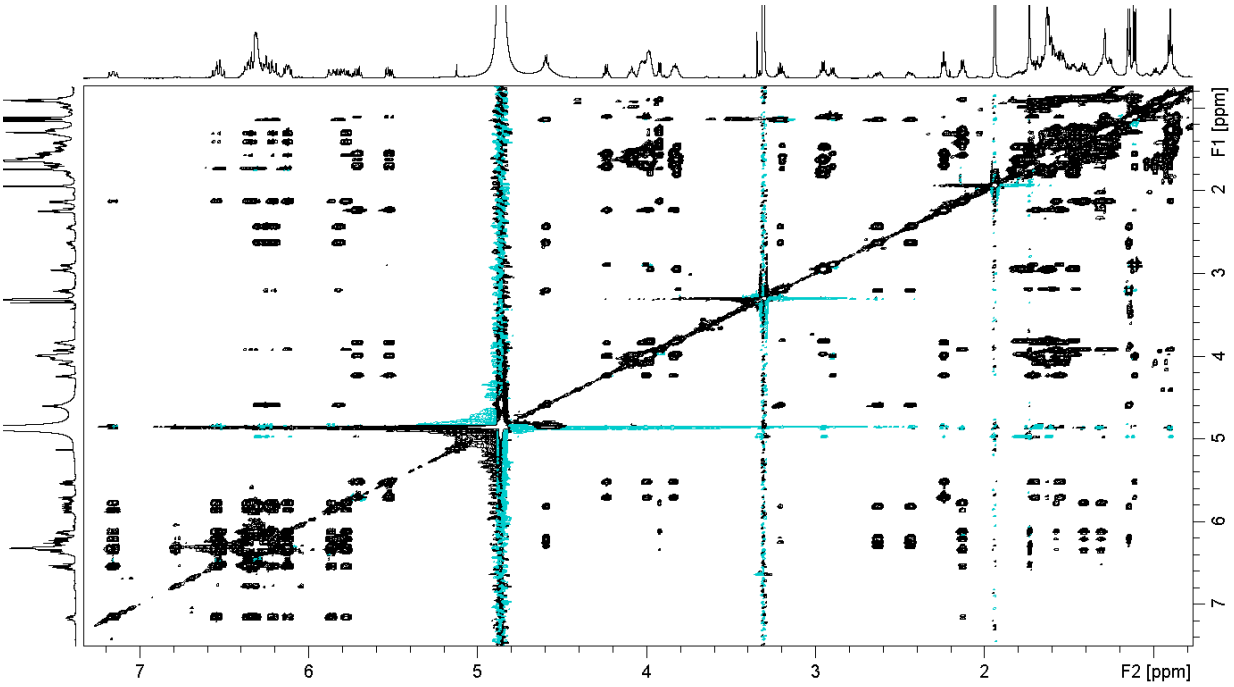


FIG S35: TOCSY (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4 enlarged to show upfield couplings.

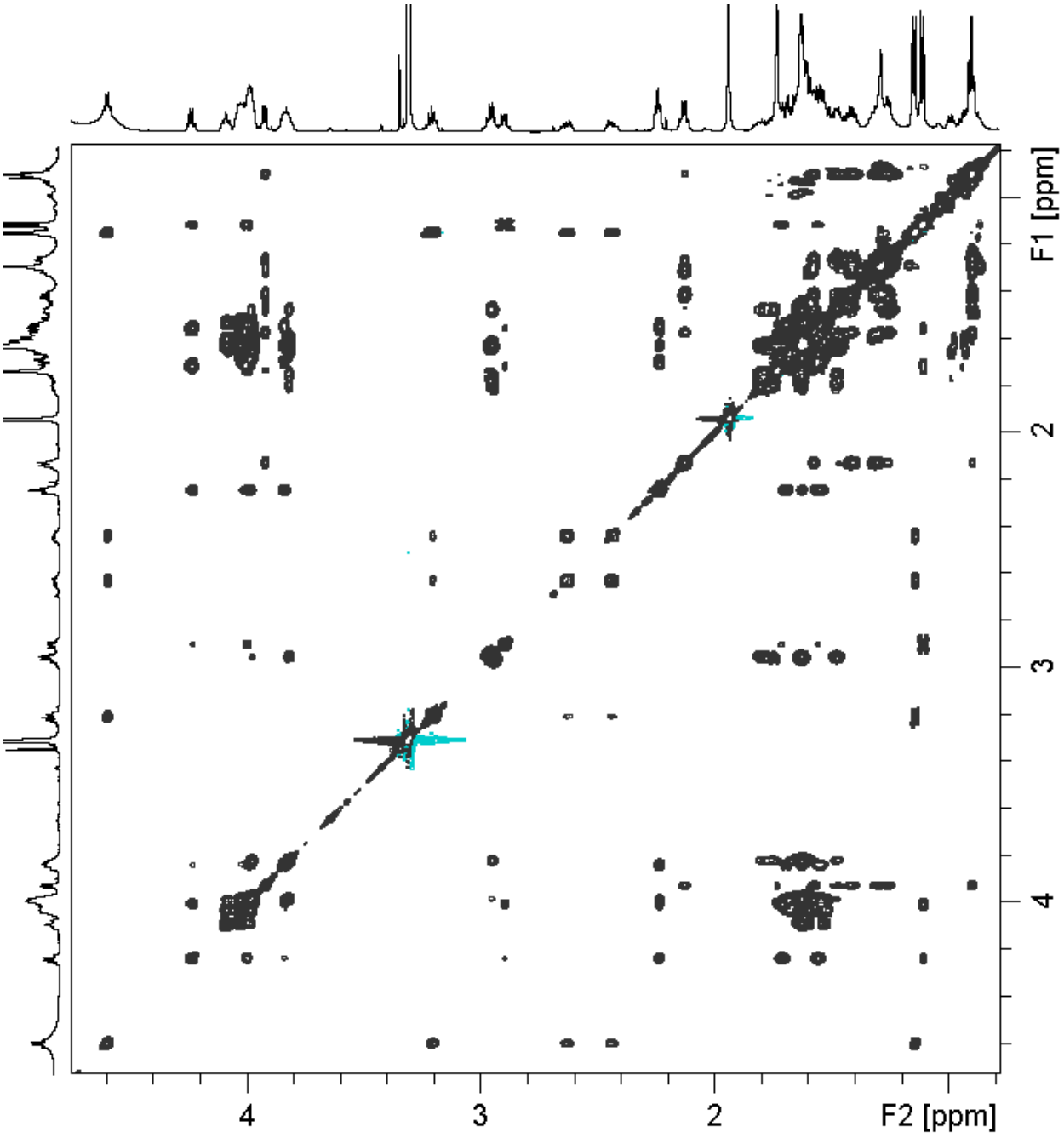


FIG S36: TOCSY (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4 enlarged to show downfield couplings.

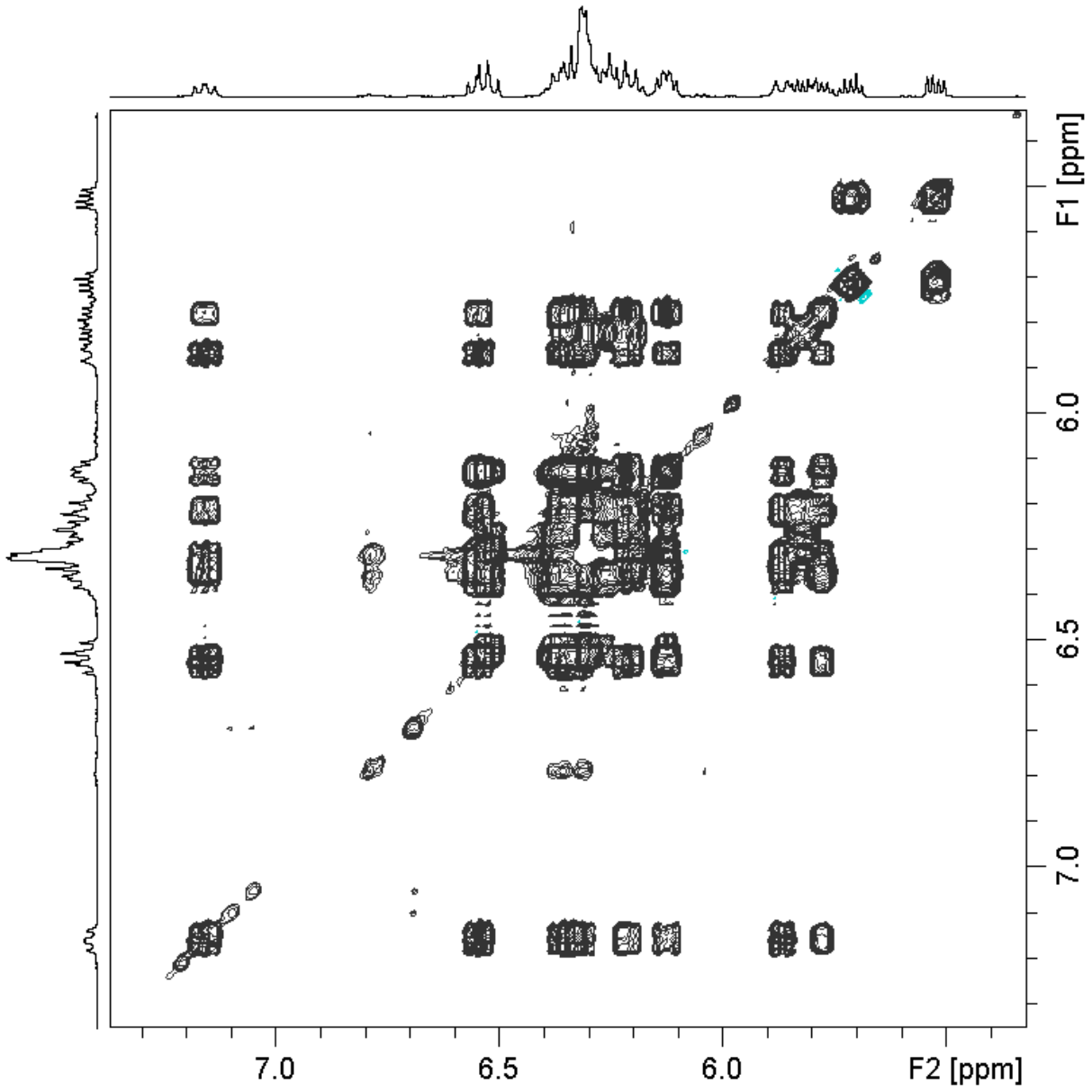


FIG S37: HSQC (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4

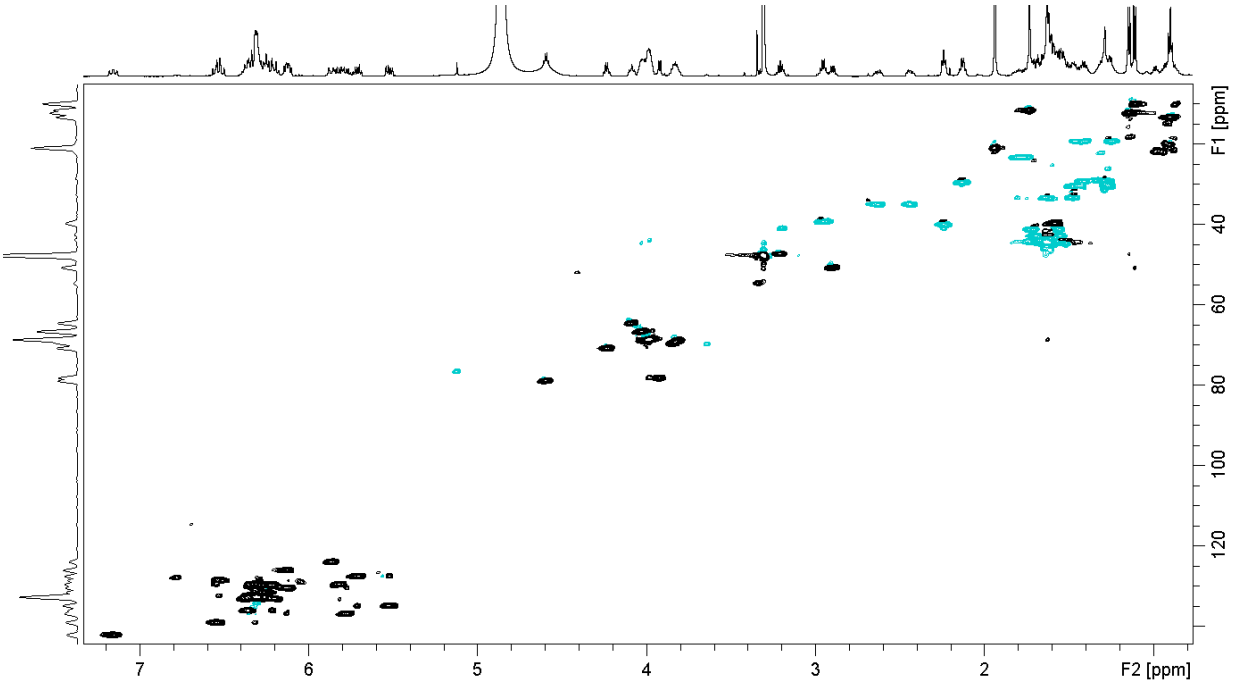


FIG S38: HSQC (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4 enlarged to show downfield couplings.

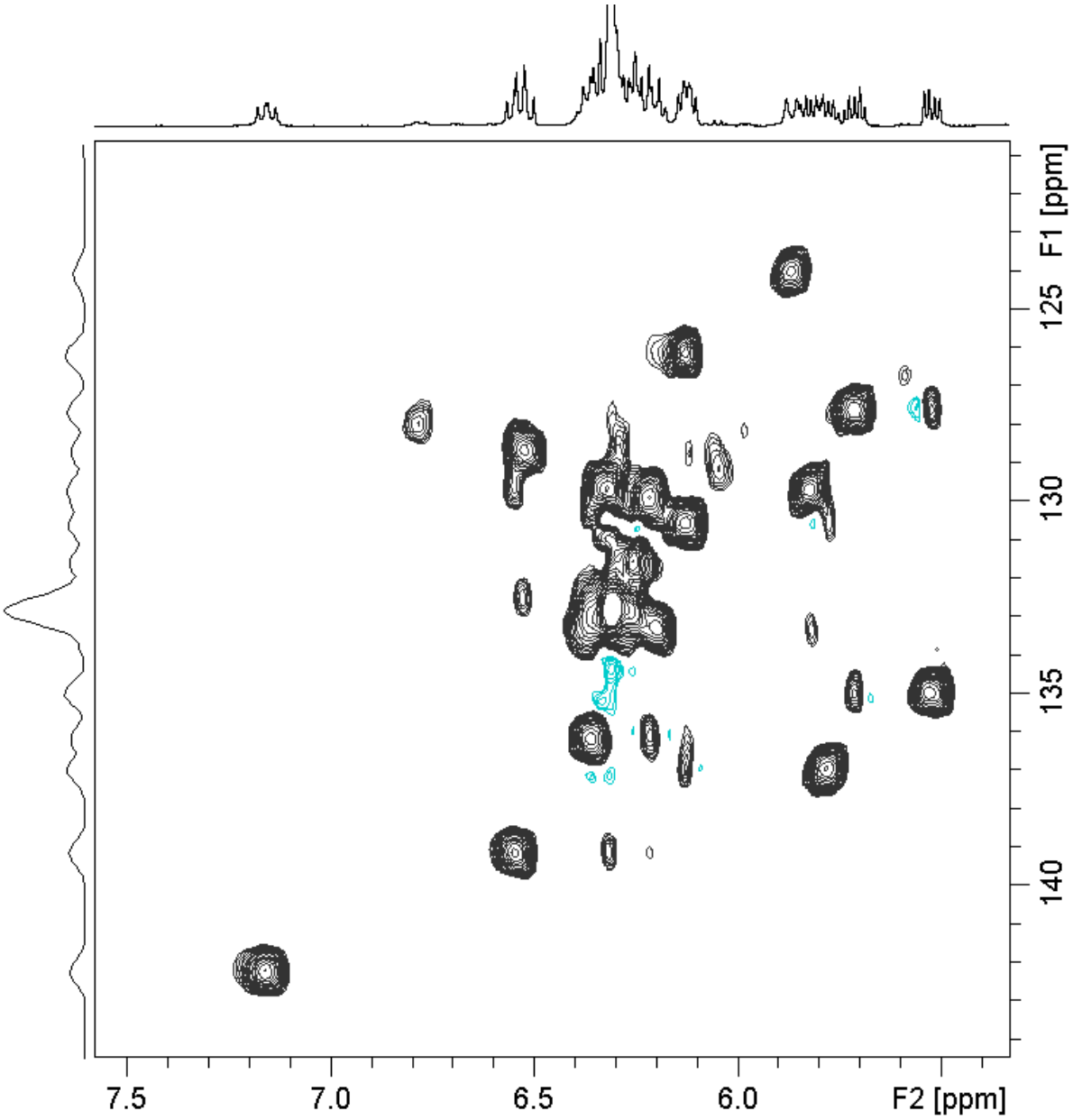


FIG S39: HSQC (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4 enlarged to show upfield couplings.

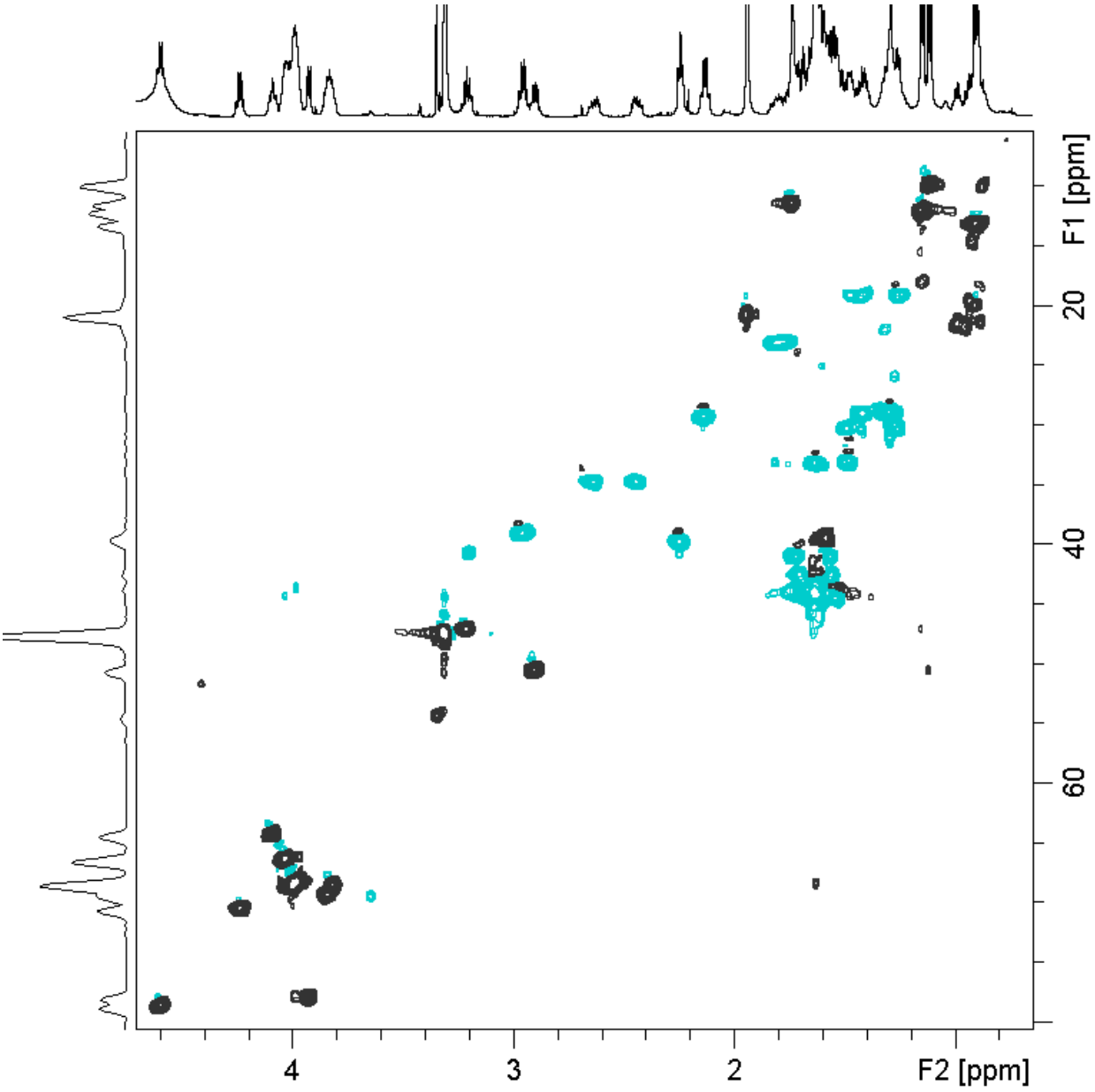


FIG S40: HMBC (600 MHz) spectrum of funisamine from *Streptosporangium* sp. KDCAGE35 in Methanol-d4

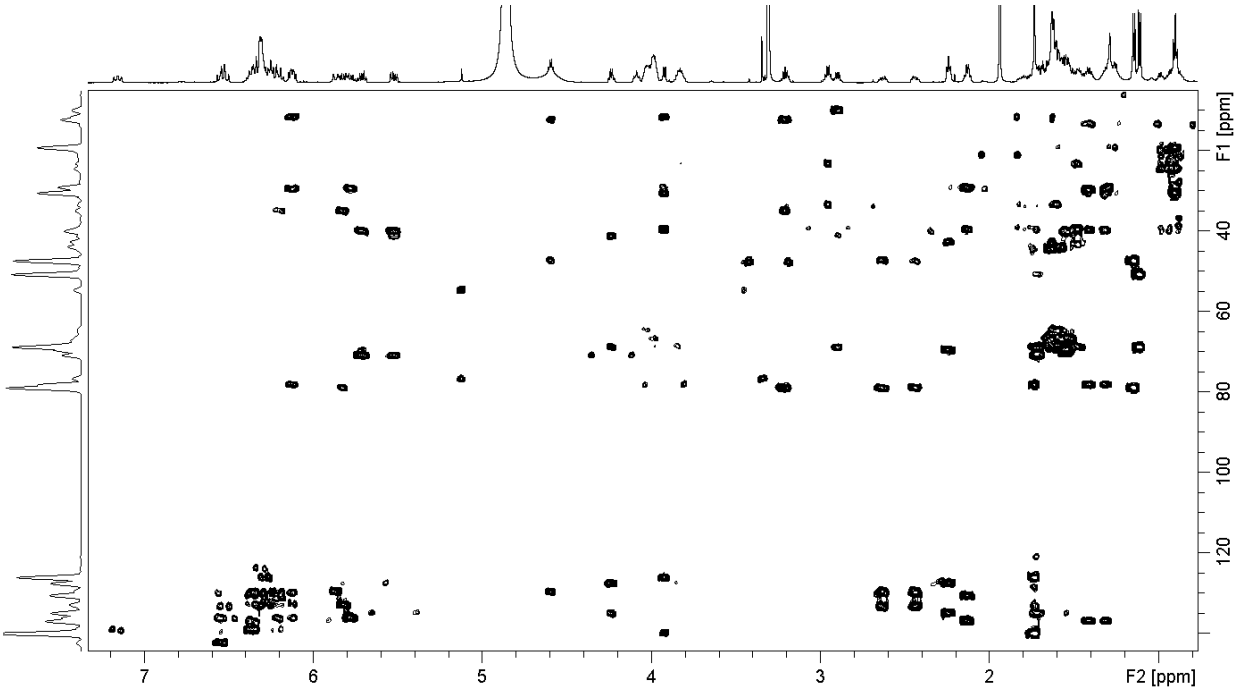
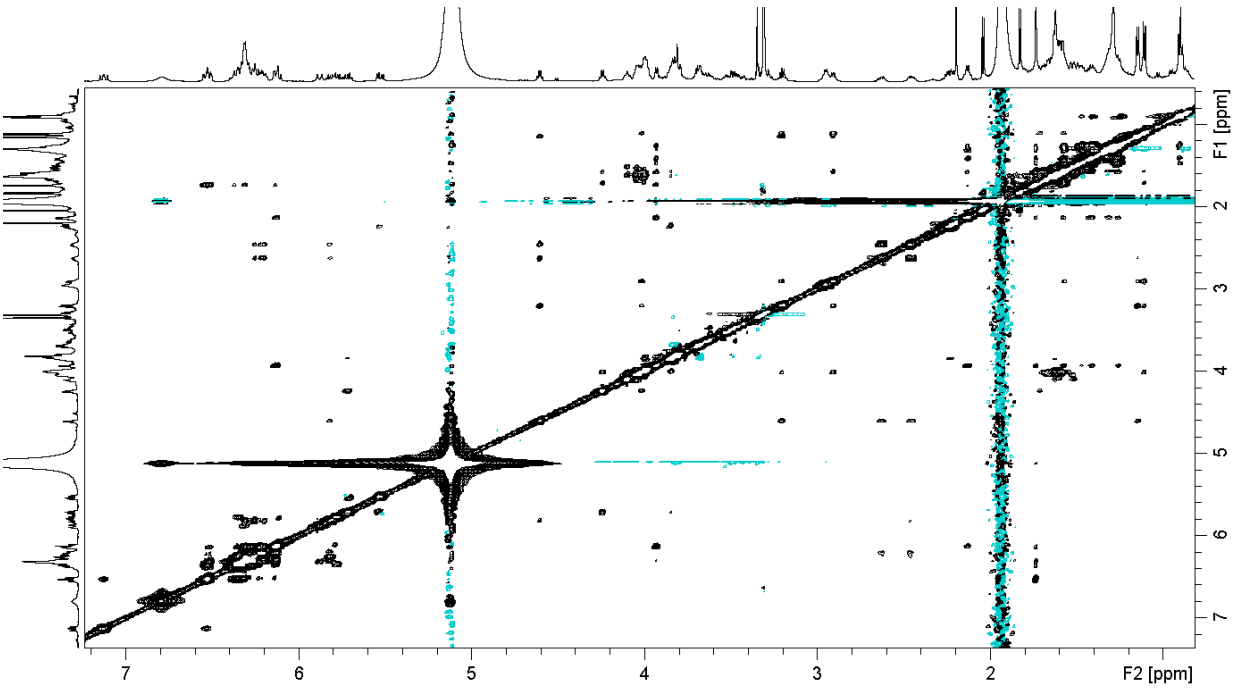
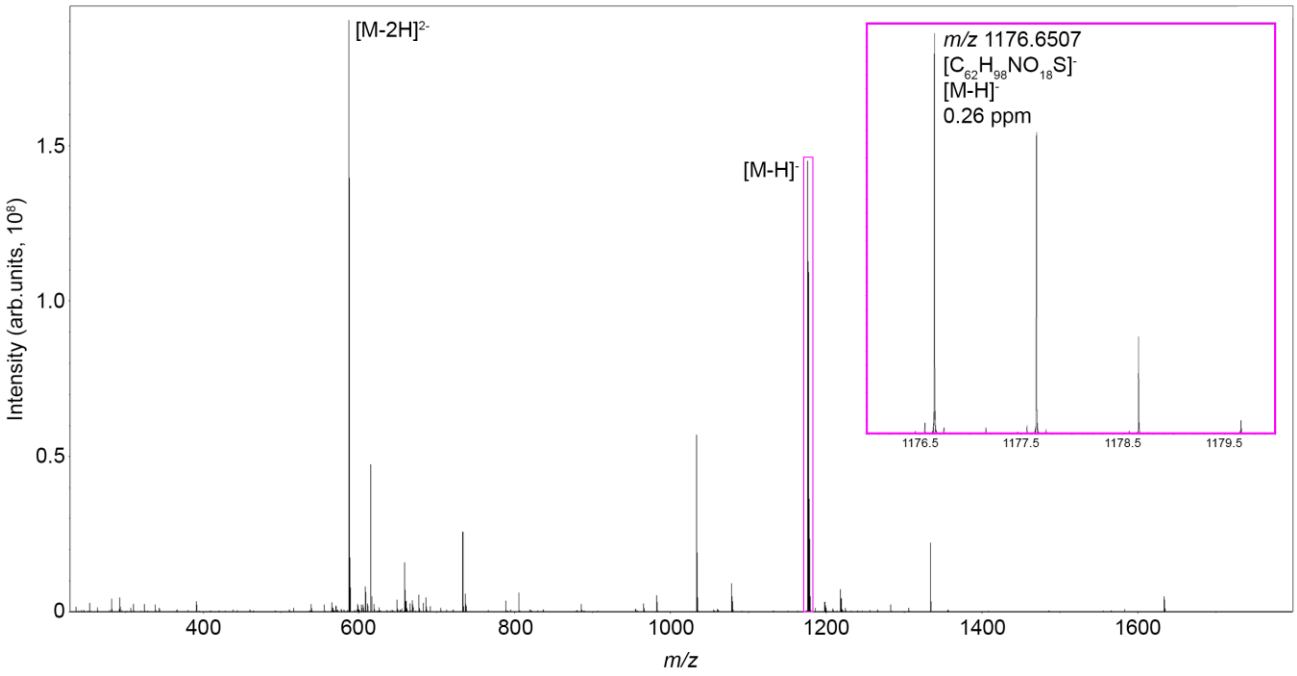


FIG S41: NOESY (600 MHz) spectrum of funisamine from *Streptosporangium* sp. (KDCAGE35) in Methanol-d4



B.3. High-resolution mass spectral data

FIG S42: High-Res mass spectrum for purified funisamine acquired in negative mode on a 15T solariX Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, MA, USA)



B.4. Fragmentation data

Table S6: Table of ions detected through fragmentation analysis of purified funisamine

Observed m/z	Intensity	Molecular Forumula	Theoretical m/z	Mass Accuracy (ppm)
1176.65095	6.84E+07	C62H98NO18S-	1176.6510	0.05
1158.64094	1.52E+06	C62H96NO17S-	1158.6404	-0.47
1098.65309	5.97E+05	UNK		
1098.60508	9.70E+06	C58H92NO18S-	1098.6041	-0.93
1080.59553	7.35E+05	C58H90NO17S-	1080.5935	-1.88
1078.68607	7.53E+05	C62H96NO14-	1078.6836	-2.29
697.34181	6.43E+06	C39H53O9S-	697.3416	-0.30
679.33228	4.08E+05	C39H51O8S-	679.3310	-1.88
674.34418	3.56E+05	C29H56NO14S-	674.3427	-2.19
668.33516	1.03E+06	C41H48O8-	668.3355	0.46
667.33181	9.67E+05	C38H51O8S-	667.3310	-1.21
639.29996	1.12E+06	C36H47O8S-	639.2997	-0.41
619.29486	3.81E+06	C33H47O9S-	619.2946	-0.42
589.34925	5.33E+05	UNK		
589.28417	4.51E+06	C32H45O8S-	589.2841	-0.19
588.34589	8.49E+05	C37H48O6-	588.3456	-0.42
588.26933	3.74E+05	C24H46NO13S-	588.2695	0.36
570.25987	4.56E+05	C24H44NO12S-	570.2590	-1.58
561.25307	5.41E+05	C30H41O8S-	561.2528	-0.48
558.2599	2.53E+05	C23H44NO12S-	558.2590	-1.67
549.30256	3.05E+05	C37H41O4-	549.3010	-2.84
544.2437	6.34E+05	C22H42NO12S-	544.2433	-0.70
501.30079	3.55E+05	C33H41O4-	501.3010	0.42
464.28649	2.41E+05	C22H42NO9-	464.2865	0.02
457.31085	4.60E+05	C32H41O2-	457.3112	0.77
446.2759	2.68E+05	C22H40NO8-	446.2759	0.00
428.26553	2.59E+05	C22H38NO7-	428.2654	-0.30
394.24449	4.36E+05	C18H36NO8-	394.2446	0.28
350.21822	7.26E+05	C16H32NO7-	350.2184	0.51
317.20884	6.37E+05	UNK		
306.19217	1.24E+06	C14H28NO6-	306.1922	0.13
262.16589	7.44E+05	C12H24NO5-	262.1660	0.42
226.1448	2.21E+05	C12H20NO3-	226.1449	0.44
209.04884	2.28E+05	C7H13O5S-	209.0489	0.29
173.09714	3.45E+05	C12H13O-	173.0972	0.35
147.08152	4.34E+05	C10H11O-	147.0815	-0.14
121.06587	4.40E+05	C8H9O-	121.0659	0.25
96.9601	1.36E+07	HSO4-	96.9601	0.00

FIG S43: Fragmentation spectra of isolated funisamine

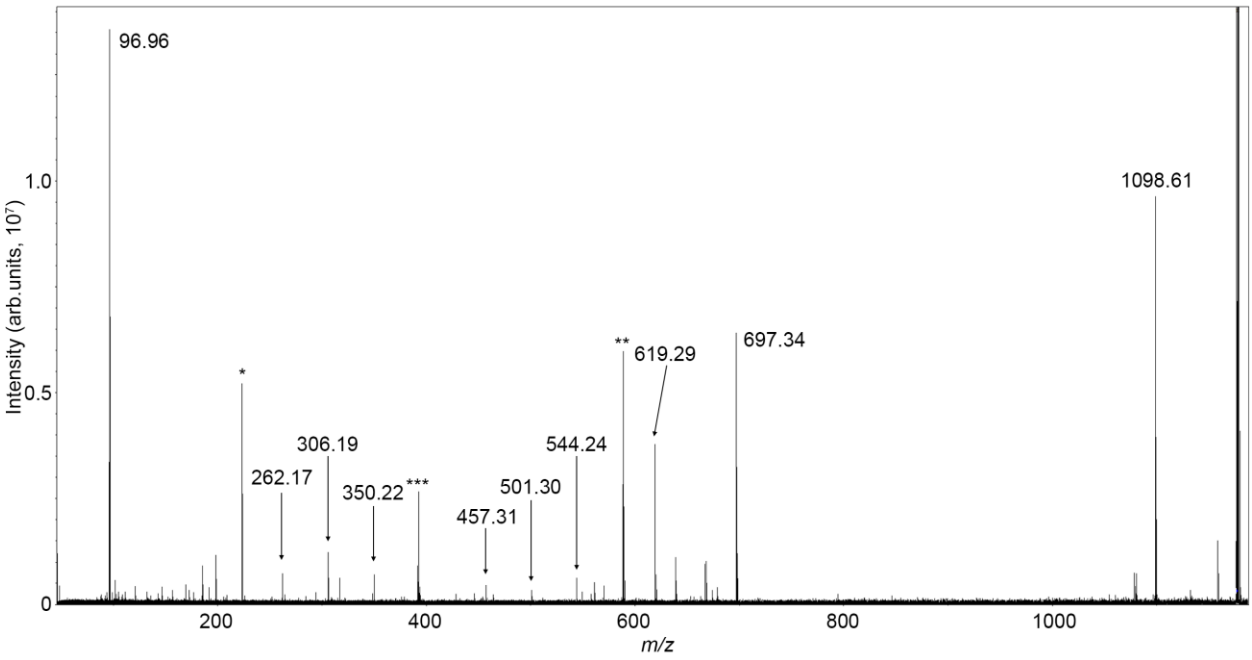
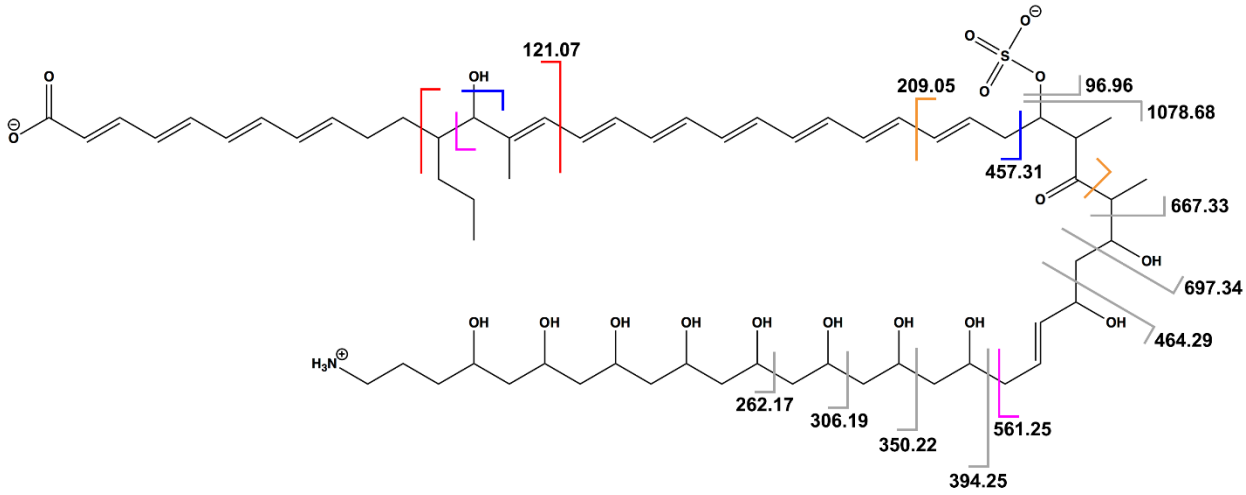
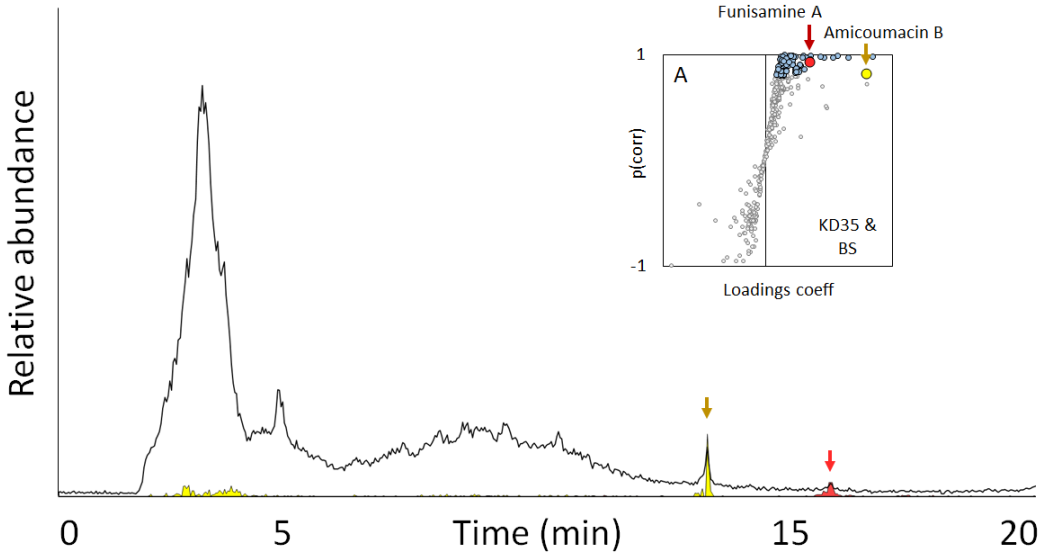


FIG S44: Fragmentation assignments for funisamine



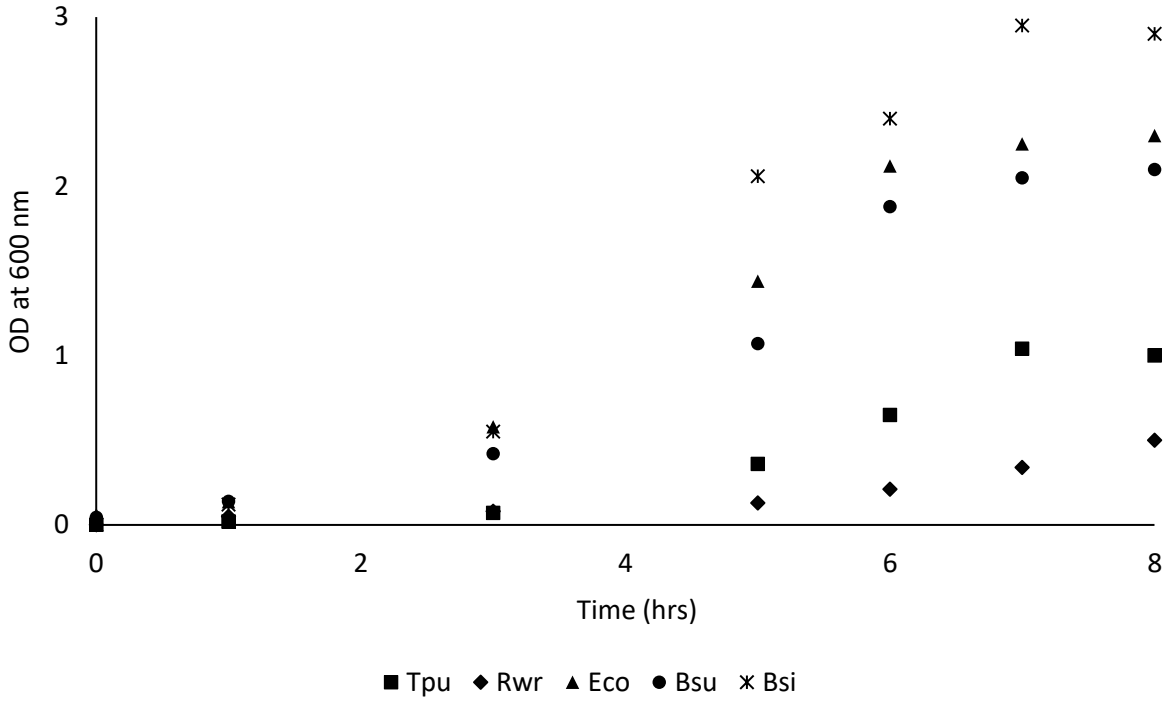
B.5. Amicoumacin production in mixed culture

FIG S45: (A) S-plot prioritization of amicoumacin B and funisamine from mixed culture extract with *Streptosporangium* sp. KDCAGE35 and *B. subtilis*. Extracted ion chromatograms for labeled features are shown overlaid on the total ion chromatogram.



B.6. Mixed culture competitor growth rates

FIG S46: Mixed culture competitor growth curves for *Tsukamurella pulmonis* (Tpu), *Rhodococcus* BBSNAI13 (Rwr), *Escherichia coli* (Eco), *Bacillus subtilis* (Bsu), and *Bacillus* KDCAGE13 (Bsi) in international Streptomyces protocol 2 (ISP2) broth. Doubling times in hours were calculated to be: 1.8 (Tpu), 2.8 (Rwr), 1.5 (Eco), 1.5 (Bsu), and 1.0 (Bsi).



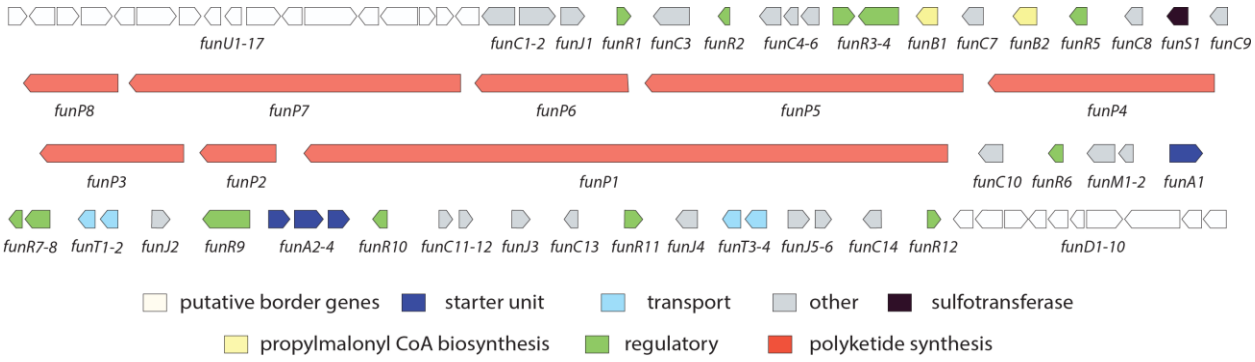
C. 1. Putative NP gene clusters observed for *Streptosporangium* sp. KDCAGE35

Table S7: Table of putative biosynthetic gene clusters identified by antiSMASH in the genome of *Streptosporangium* sp. KDCAGE35

Cluster	Type	Most similar known cluster from antiSMASH
Cluster 1	Lassopeptide	-
Cluster 2	Nrps	Daptomycin_biosynthetic_gene_cluster (4% of genes show similarity)
Cluster 3	Nrps	Landomycin_biosynthetic_gene_cluster (9% of genes show similarity)
Cluster 4	Nrps	Naphthyridinomycin_biosynthetic_gene_cluster (17% of genes show similarity)
Cluster 5	Terpene	Chlortetracycline_biosynthetic_gene_cluster (5% of genes show similarity)
Cluster 6	T3pks	Alkylresorcinol_biosynthetic_gene_cluster (100% of genes show similarity)
Cluster 7	Nrps	Scabichelin_biosynthetic_gene_cluster (100% of genes show similarity)
Cluster 8	Bacteriocin	Hedamycin_biosynthetic_gene_cluster (6% of genes show similarity)
Cluster 9	Terpene	Spinosad_biosynthetic_gene_cluster (8% of genes show similarity)
Cluster 10	T3pks-T1pks	Kendomycin_biosynthetic_gene_cluster (55% of genes show similarity)
Cluster 11	Terpene	Sioxanthin_biosynthetic_gene_cluster (40% of genes show similarity)
Cluster 12	Lantipeptide	-
Cluster 13	Terpene	Griseobactin_biosynthetic_gene_cluster (11% of genes show similarity)
Cluster 14	Other	Monensin_biosynthetic_gene_cluster (5% of genes show similarity)
Cluster 15	Lantipeptide	Catenulipeptin_biosynthetic_gene_cluster (60% of genes show similarity)
Cluster 16	Terpene-Nrps	-
Cluster 17	T1pks	Concanamycin_A_biosynthetic_gene_cluster (42% of genes show similarity)
Cluster 18	Terpene	9-methylstreptimidone_biosynthetic_gene_cluster (9% of genes show similarity)
Cluster 19	Terpene	Hopene_biosynthetic_gene_cluster (46% of genes show similarity)
Cluster 20	Siderophore	-
Cluster 21	Bacteriocin	-

C.2. Putative NP gene cluster for production of funisamine in *Streptosporangium* sp. KDCAGE35

Table S8: Genetic organization of putative funisamine biosynthetic gene cluster in *Streptosporangium* sp. KDCAGE35.



GenBank accession #	Start/Stop (bp)	Protein	Size (AAs)	Proposed function	Protein Homolog	I / S (%)	Accession Number
	2-679	FunU1	225	unknown	Nucleoprotein TPR (*Xenopus laevis*)	38 / 44	Q5EE04.1
	1239-40	FunU2	399	hypothetical protein	Hypothetical protein (*Streptomyces sp. PCS3-D2*)	58 / 63	EYU65272.1
	250-1275	FunU3	343	hydrolase	Epoxide hydrolase B (*Mycobacterium tuberculosis CDC1551*)	39 / 51	P95276.2
	219-1817	FunU4	532	hypothetical protein	---	----	---
	2356-1277	FunU5	359	transporter	Uncharacterized transporter YcbK (*Bacillus subtilis subsp. subtilis str. 168*)	27 / 43	P42243.1
	1295-3595	FunU6	766	hypothetical protein	---	----	---
	2418-3335	FunU7	305	regulator	HTH-type transcriptional regulator GltC (Bacillus subtilis subsp. subtilis str. 168)	27 / 46	P20668.3
	3599-2832	FunU8	255	regulator	LysR family transcriptional regulator (*Nonomuraea jiangxiensis*)	41 / 52	WP_090940584.1
	4339-3608	FunU9	243	hypothetical protein	Hypothetical protein (*Ralstonia mannitolilytica*)	40 / 58	AJW47704.1
	3702-5567	FunU10	621	phosphatase	Alkaline phosphatase D (*Bacillus subtilis subsp. subtilis str. 168*)	45 / 60	P42251.3
	4817-3750	FunU11	355	hypothetical protein	---	----	---
	4823-7591	FunU12	922	galactosidase	alpha-galactosidase (Streptomyces alboflavus)	45 / 58	WP_087883000.1
	5675-4848	FunU13	275	unknown	hypothetical protein STAWA0001_0347 (*Staphylococcus warneri L37603*)	42 / 47	EEQ79474.1
	7555-5483	FunU14	690	hypothetical protein	Hypothetical protein SCLAV_2787 [*Streptomyces clavuligerus ATCC 27064*]	39 / 45	EFG07859.1
	5698-6303	FunU15	201	kinase	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (*Photorhabdus luminescens subsp. laumondii TTO1*)	32 / 50	Q7N589.1

8036-8707	FunU16	223	monooxygenase	Pyrimidine monooxygenase RutA (*Bradyrhizobium sp. BTAi1*)	41 / 59	A5EB33.1
10043-8868	FunU17	391	hypothetical protein	---	----	---
10707-9010	FunC1	565	tRNA ligase	Cysteine--tRNA ligase (*Salmonella enterica subsp. arizonae serovar*)	29 / 43	A9MLA7.1
9112-11118	FunC2	668	4'-phosphopantetheinyl transferase	4'-phosphopantetheinyl transferase AcpS (*Dechloromonas aromatica RCB*)	52 / 62	Q47EF3.1
9285-10616	FunJ1	443	hypothetical protein	---	----	---
12099 - 12629	FunR1	176	RNA polymerase sigma-F factor	RNA polymerase sigma-F factor (*Bacillus megaterium*)	54 / 75	P35145.1
14530-12644	FunC3	628	Phospholipase	Non-hemolytic phospholipase C (*Burkholderia pseudomallei K96243*)	37 / 48	Q9RGS8.2
15321-15016	FunR2	101	regulator	Outer membrane protein assembly factor BamA (*Helicobacter pylori 26695*)	43 / 60	O25369.1
16441-15506	FunC4	311	dehydrogenase	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating (*Dictyostelium discoideum*)	29 / 44	Q54L85.1
17220-16597	FunC5	207	reductase			
18172-17438	FunC6	244	oxidoreductase	oxidoreductase YkvO (*Bacillus subtilis subsp. subtilis str. 168*)	51 / 70	O31680.1
18436 - 19335	FunR3	299	regulator	Hca operon transcriptional activator HcaR (*Escherichia coli K-12*)	40 / 55	Q47141.2
21794-19701	FunR4	697	elongation factor G	Elongation factor G (*Streptomyces coelicolor A3(2)*)	81 / 87	O87844.1
23211-22279	FunB1	310	3-hydroxypentyl-CoA dehydrogenase	3-hydroxybutyryl-CoA dehydrogenase (*Mycobacterium tuberculosis CDC1551*)	56 / 72	P9WNP6.1
24197-23208	FunC7	329	ketoacyl-ACP synthase III	Acetoacetyl CoA synthase NphT7 (*Streptomyces sp. CL190*)	52 / 65	D7URV0.1
25546-24200	FunB2	448	2-pentenyl-CoA carboxylase/reductase	Crotonyl-CoA reductase (*Streptomyces collinus*)	52 / 65	Q53865.1
26373-25579	FunR5	264	regulator	Sugar fermentation stimulation protein homolog (*Pyrobaculum calidifontis JCM 11548*)	60 / 72	A3MSH9.1
27129-26458	FunC8	223	4'-phosphopantetheinyl transferase	4'-phosphopantetheinyl transferase Npt (*Nocardia iowensis*)	49 /62	A1YCA5.1
28178-27129	FunS1	349	sulfotransferase	sulfotransferase associated with clethramycin BGC (Streptomyces malaysiensis)	44 / 59	ATL82931.1
28945-28175	FunC9	256	Thioesterase	Thioesterase PikA5 (*Streptomyces venezuelae*)	52 / 66	Q9ZGI1.1
34875-28942	FunP8	1977	funisamine PKS 8	---	----	---
56330-34905	FunP7	7181	funisamine PKS 7	---	----	---
66246-56476	FunP6	3256	funisamine PKS 6	---	----	---
86928-66277	FunP5	6883	funisamine PKS 5	---	----	---
101612-86958	FunP4	4884	funisamine PKS 4	---	----	---

110910-101770	FunP3	3046	funisamine PKS 3	---	----	---
115796-111198	FunP2	1532	funisamine PKS 2	---	----	---
158396-116280	FunP1	14038	funisamine PKS 1	---	----	---
160153-158831	FunC10	440	FAD-dependent monooxygenase	FAD-dependent monooxygenase cctM (*Talaromyces islandicus*)	27 / 40	A0A0U1LQD9.1
160696-160202	FunR6	164	PadR family transcriptional regulator	Transcriptional regulator PadR-like family protein (*Nonomuraea jiangxiensis*)	89 / 96	SDK84512.1
162531-160984	FunM1	515	membrane protein	UPF0699 transmembrane protein YdbT (*Bacillus subtilis subsp. subtilis str. 168*)	16 / 39	A7RHG8.1
162953-162528	FunM2	141	membrane protein	UPF0699 transmembrane protein YdbS (*Bacillus subtilis subsp. subtilis str. 168*)	32 / 52	P96615.1
163265-164947	FunA1	560	arginine 2-monooxygenase	arginine 2-monooxygenase (*Streptomyces malaysiensis*)	67 / 75	ATL82933.1
165743-165120	FunR7	207	regulator	Response regulator protein VraR (*Staphylococcus aureus subsp. aureus MSSA476*)	44 / 65	Q6G850.1
166951-165740	FunR8	403	signal transduction histidine kinase	Sensor histidine kinase LiaS (*Bacillus subtilis subsp. subtilis str. 168*)	30 / 49	O32198.1
167778-166909	FunT1	289	transport permease	transport permease ycf38 (*Porphyra purpurea*)	27 / 47	P51321.1
168620-167775	FunT2	281	ABC transporter ATP-binding protein	ABC transporter ATP-binding protein YvfR (*Bacillus subtilis subsp. subtilis str. 168*)	38 / 56	O07016.1
168756-169583	FunJ2	275	hypothetical protein	hypothetical protein STAWA0001_0347 (*Staphylococcus warneri L37603*)	42 / 47	EEQ79474.1
172413-169660	FunR9	917	transcriptional regulator	HTH-type transcriptional regulator MalT (*Vibrio campbellii ATCC BAA-1116*)	25 / 41	A7N5N6.1
173031-174149	FunA3	372	agmatinase	agmatinase (*Streptomyces sp. Mg1*)	34 / 52	AKL64821.1
174191-175633	FunA2	480	4-guanidinobutanoate CoA ligase	4-guanidinobutanoate:CoA ligase (*Streptomyces malaysiensis*)	61 / 73	ATL87716.1
175630-176556	FunA4	308	4-guanidinobutanoyl-CoA:ACP acyltransferase	4-guanidinobutanoyl-CoA:ACP acyltransferase (*Streptomyces malaysiensis*)	48 / 65	ATL87717.1
177375-176761	FunR10	204	TetR family transcriptional regulator	Tetracycline repressor protein class E (*Escherichia coli*)	25 / 44	P21337.1
177445-178020	FunC11	191	alkyl hydroperoxide reductase	alkyl hydroperoxide reductase (*Streptomyces violaceusniger Tu 4113*)	70 / 79	AEM81606.1
178215-178826	FunC12	203	dehydrogenase	Malate dehydrogenase (*Bacillus cytotoxicus NVH 391-98*)	30 / 44	A7GQI9.1
178877-178080	FunJ3	265	hypothetical protein	---	----	---
178197-178826	FunC13	209	dehydrogenase	Malate dehydrogenase (*Geobacillus sp. WCH70*)	29 / 51	C5DAE0.1
179363-180136	FunR11	257	regulator	HTH-type transcriptional regulator CueR (*Escherichia coli O157:H7*)	33 / 55	Q8XD09.1
181146-180160	FunJ4	328	hypothetical protein	---	----	---
181118-180276	FunT3	280	transporter	ABC-2 family transporter protein (*Streptosporangium subroseum*)	94 / 96	SNT45499.1
182104-181115	FunT4	329	transporter	ABC transporter ATP-binding protein YhcH (*Bacillus subtilis subsp. subtilis str. 168*)	35 / 55	P54592.1

181118-182110	FunJ5	330	hypothetical protein	hypothetical protein STEPF1_06867 (*Streptomyces sp. F-1*)	39 / 49	SFY53584.1
181188-181880	FunJ6	230	hypothetical protein	hypothetical protein BJF79_17010 (*Actinomadura sp. CNU-125*)	57 / 65	OLT19263.1
183592-182798	FunC14	264	ketoreductase	Beta-ketoacyl-ACP reductase (*Vibrio cholerae O1 biovar El Tor str. N16961*)	36 / 52	Q9KQH7.2
183770-184417	FunR12	215	regulator	HTH-type transcriptional regulator YhgD (*Bacillus subtilis subsp. subtilis str. 168*)	38 / 59	P32398.1
184483-183782	FunD1	233	hypothetical protein	---	----	---
185811-184630	FunD2	393	methylase	Serine methylase (*Thermus thermophilus HB27*)	26 / 45	Q72IH2.2
184870-185931	FunD3	353	phosphoribosyltransferase	anthranilate phosphoribosyltransferase (*Streptomyces sp. M1013*)	29 / 47	WP_076976541.1
187265-186549	FunD4	238	peptide hydrolase	ATP-dependent Clp protease proteolytic subunit 1 (*Nocardia farcinica IFM 10152*)	73/ 86	Q5Z0M4.1
187822-187136	FunD5	228	peptide hydrolase	ATP-dependent Clp protease proteolytic subunit 3 (*Streptomyces coelicolor A3(2)*)	48 / 66	Q9X7R9.1
188692-188066	FunD6	208	hypothetical protein	---	----	---
188726-190639	FunD7	637	hypothetical protein	---	----	---
191317-188747	FunD8	856	Membrane protein	Outer membrane protein assembly factor BamB (*Vibrio fischeri ES114*)	31 / 45	Q5E769.1
189953-189270	FunD9	227	hypothetical protein	---	----	---
191234-190353	FunD10	293	Iron permease	putative iron permease FTR1 [*Mycobacterium abscessus 47J26*]	40 / 49	EHB98808.1

C.3. Ketoreductase domain stereochemical prediction analysis of putative funisamine gene cluster in *Streptosporangium* sp. KDCAGE35

FIG S47: Ketoreductase multiple sequence alignment to designate KR-type for stereochemistry predictions. The KR-types are distinguished in accordance with the five highlighted columns showing the 'fingerprint' amino acids. Group A1 is distinguished by no LDD in column 1, W in column 2, and no H in column 3. Group A2 is distinguished by no LDD in 1, W in 2, and H in 3. Group B1 is distinguished by LDD in 1 and no P in 5. Group B2 is distinguished by LDD in 1 and P in 5. Group C1 is distinguished by no Y in 4, and group C2 is distinguished by N in 6.

```
                                                1
Fun1e      CDLTDRSAVATLVREFDGEP---DLAIVHAAGVLDDGVIAGLDPARLDAVLASKAGAAWQ     112
Fun3a      CDLADRAAVEALLASV-----GTVDAVVHAAGVGANVPFDQTDVALVERLLAGKVAGAVN     114
Fun2a      CDLSDRSAVEALLADV-----GEVDAVVHAAGVSQDVPLAAEDADHLRAVAAGKVDGAAH     110
Fun1f      CDLADRSAVEALLDTV-----GDVDAVVHAAGIVEDVPLADADQAHLDRVIRGKVDGALH     110
Fun1c      CDLADRSAVEALLATI-----GQVNAVVHAAGVGEDVALVDADEEHLRRVVGGKVDGALH     110
Fun1g      CDLADRSAVEGLLATI-----GQVNAVVHAAGVSEHAALTDVDEEHLRRVVVGKVDGALH     110
Fun1d      CDLADPSAVEGLLATI-----GQVDAVVHAAGVAEDAELVDADAAHLNRVLSGKVDGALY     110
Fun1h      CDLADRSAVEGLLATI-----GQVNAVVHAAGVAEDVELVDADAAHLNRVLSGKVDGALH     110
Fun1a      CDLADRSAVEALLAVV-----GAVDAVVHAAGVGEDAELVEADAAHLNRVLSGKVDGASH     110
Fun12      CDLADRSAVEALLAVV-----GAVDAVVHAAGVAADVPLRDADEAHFRTVLSGKVDGALH     110
Fun6b      CDTADRAQVAALLAGL----PEPVTAVVHAAGTLTPVLLADSTPEELADVRSGKVEGAVH     115
Fun3b      CDLADRDQVAALVADL----PADLTAVVHAAGVAQDTPIADLTTAETAAVTGARVTGTLL     115
Fun4a      TDLADRDAVAALLKEATADPEAPLTAVVHAAGIAHSAPLADLDAAGLASVLAGKTTGALH     119
Fun1i      ADVADRAALASVLAEIPA--EHPLTAVVHTAGVLADGIVERMTPDQLDRVMRPKVDGALH     114
Fun7a      CDVADRDDLDRVLD------GVDVRAVVHVAGVLDDTVLTGLTPDRLDAVLRAKVDAVVN     114
Fun5d      CDLADPAAVQRLIG------PVEVGAVLHAAGSTDDAMLTSLTPDRLASVLAAKVDAAVN     114
Fun6a      CDVTDPEAVEAALR------GVTVSAVFHTAGVLDDGLLADLTPDRLDAVLRPKADAVWN     104
Fun7b      CDLADAGAVAGALR------DEPVTAVIHAAGVLDDALLTDLTPERLRAVFRAKVDAAVN     110
Fun4b      CDLSDAGAVMAALR------DEPVSAVVHAAGVIDDGLLTDLTPERLDTVFRAKVDAARN     104
Fun4c      CDVSDADALTAALR------DEPVTAVIHAAGVLDDGTLESLTPERLDAVFRAKVDAARN     108
Fun8a      CDVADAAALTAALR------DEPVTAVIHVAGVLDDGLLTDLTPARLDTVFRAKVDAARN     108
Fun5c      CDVADAGAVTEALR------GESVSAVIHAAGVLDDGMIESLTPERLDTVFRAKIDAVRA     104
Fun7c      CDVADAGAVAEALR------GESVSAVVHAAGVLDDALLADLTPERLDTVFRAKVDAARN     104
Fun7d      CDVADAGAVAEALR------GEPVSAVIHAAGVLDDALLADLTPERLDTVFRAKIDAARN     108
Fun5a      CDVADAGAVAAALR------DEPVTAVIHAAGVLDDGTLESLTPERLDTVFRAKVDAARN     108
Fun5b      CDVSDAVAVTAALR------DESVSAVIHAAGVLDDALLADLTPERLRTVFRAKVDAARN     108

                                  2     3   4  5  6
Fun1e      LHELTEHRPLSAFVLFSSTAGVFGNPGQANYAAANAALDALAEYRKVLGLPATSIAWGPW     172
Fun3a      LDALVGD--VDAFVTFSSLSGVWGSQSHAAYAVANAALDALAEQRRARGGAMTAIAWGSW     172
Fun2a      LDALLPD--VP-LIVFSSIAGVWGSAEQAAYAAANAALDALIARRRARGRPGTAVAWGPW     167
Fun1f      LDALVGD--VDAFVVFSSISATWGSGRQAAYGAANTALDGLILRRRAAGLPGTSIAWGPW     168
Fun1c      LDALVGD--VDAFVVFSSISGIWGSAEQTAYGAANAALDALIARRRASGLPGTAVAWGPW     168
Fun1g      LDALVGD--VDAFVVFSSISGIWGSAEQAAYGAANAALDALIARRRASGLPGTAVAWGPW     168
Fun1d      LDALVGD--VDAFVVFSSISGIWGSAEQAAYGAANAALDALIARRRASGLPGTAVAWGPW     168
Fun1h      LDALVGD--VDAFVVFSSISGIWGSAEQTAYGAANAALDALIARRRASGLPGTAVAWGPW     168
Fun1a      LDALVGD--VDAFVVFSSISGVWGSRGQAAYGAANAALDALVERRRAAGRPGTAIAWGPW     168
Fun12      LDALVGD--VDAFVVFSSISGVWGSGEQAAYGAANAALDGLVARRRAHGLPGTAVAWGPW     168
Fun6b      LLDLLDPAHLEQVVLFSSNAGVWGSARQGTYGAANAALDALAEQARERGLPVTSVAWGLW     175
Fun3b      LDELLADVELDAFVVFSSVAGVWGTARHPAYAAADAFLDAFAGWRRAQGRPATAIAWSPW     175
Fun4a      LDELLGDTDLDAFVLFSSIAATWGSGWGGAYAAANAGLDALAQRRRARGLAGTSLAWGPW     179
Fun1i      LHELTRDLDLSAFVLFSSASAIFGTPGQANYAAANAFLDALAQHRRALGLPGQALAWGPW     174
Fun7a      LHEATAGADLDAFVLYSSVAGLFGTPGQGNYAAANAFLDAFAAARRSRGLPGTSLAWGAW     174
Fun5d      LRAATADRPLSAFVLFSSVAGLLGSAGQANYAAANTFLDAYAARLRAEGVPATSLAWGLW     174
Fun6a      LHAATADRPLAAFVLFSSAAGLLGNAGQANYAAANTFTDAFAAFRRAQGLPATSLAWGLW     164
Fun7b      LRAATADHRLSAFVLYSSAAGLFGNAGQANYAAANAFLDAYAAQLRAEGVPATSLAWGLW     170
Fun4b      LAAATEDRPLSAFVLYSSASGLFGSAGQANYAAANAFLDAYATQLRAQGVPATSLAWGLW     164
Fun4c      LAAATKDRPLRAFVLYSSASGLFGNAGQANYAAANTFLDAYATRLRGEGVPATSLAWGLW     168
Fun8a      LAAATKDRPLRAFVLYSSVAGIFGNPGQANYAAANAFLDAYATQLRAQGVPATSLAWGLW     168
Fun5c      LRAATADQPLTAFVLYSSVAGLFGNAGQANYAAANAFLDAYATQLRGQGVPATSLAWGLW     164
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Fun7c       LAAATADQPLTAFVLYSSAAGVFGNAGQANYAAANAFLDAYATQLRGQGVPATSLAWGLW      164
Fun7d       LAAATADQPLAAFVLYSSAAGLFGNAGQANYAAANAFLDAYATQLREQGVPATSLAWGLW      168
Fun5a       LVAATKDQPLTAFVLYSSAAGVFGNAGQANYAAANAFLDAYATQLRGQGVPATSLAWGLW      168
Fun5b       LVAATKDQPLTAFVLYSSAAGLFGNAGQANYAAANAFLDAYATQLHAQGIPATSLAWGLW      168
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FIG S48: Predicted stereochemical configurations of products for each polyketide synthase module in funisamine biosynthesis.

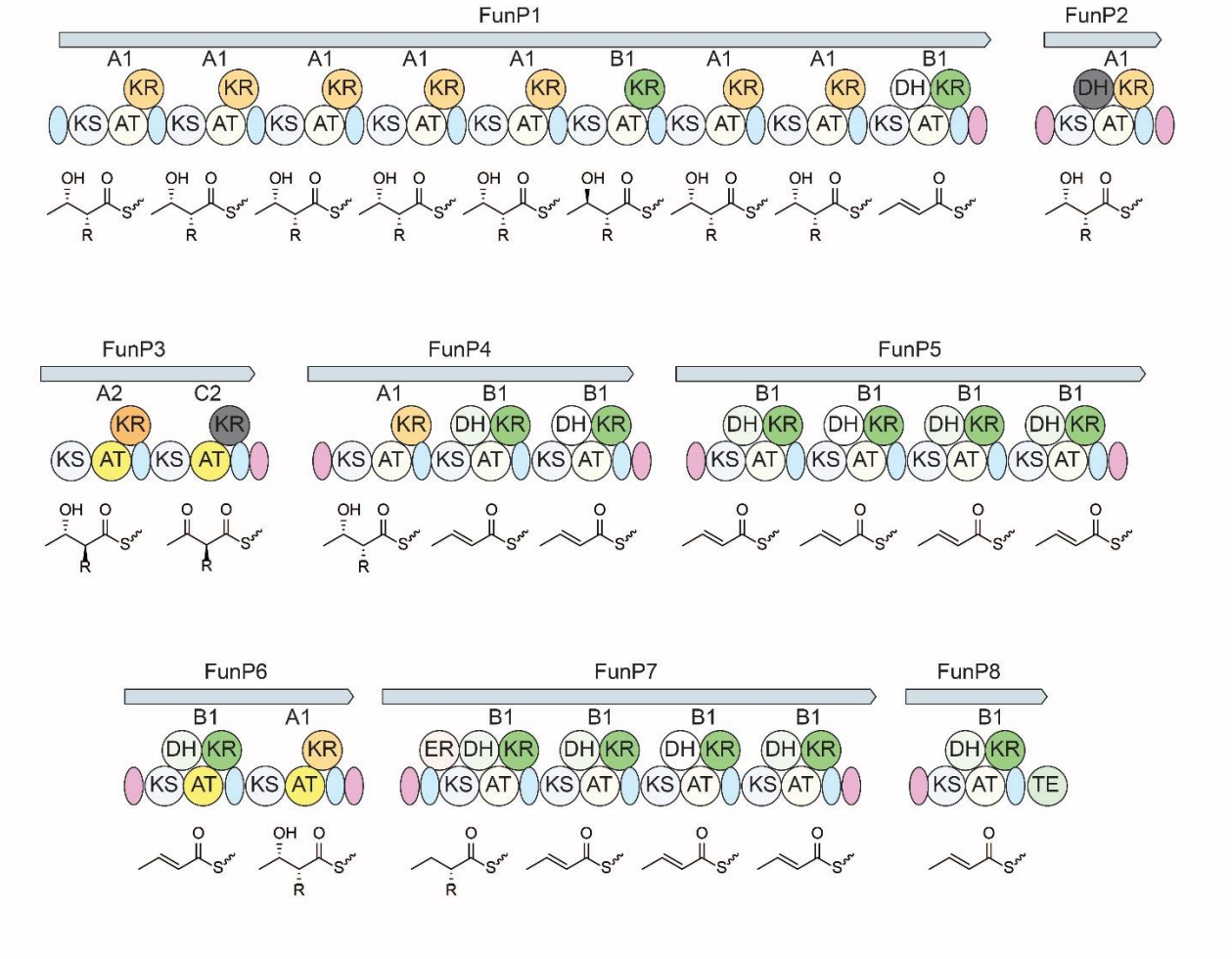

FIG S49: Expanded biosynthesis of funisamine from FunP1. The layout of catalytic domains: ketosynthase (KS), acyltransferase (AT), ketoreductase (KR), dehydratase (DH), enoylreductase (ER), and thioesterase (TE) present within the polyketide synthases are shown with inactive domains colored grey. A-type and B-type KR domains are colored orange and green respectively. Acyltransferase domains predicted to use methyl or propylmalonyl extender units are colored yellow. Acyl carrier proteins are colored light blue, and docking domains are purple.

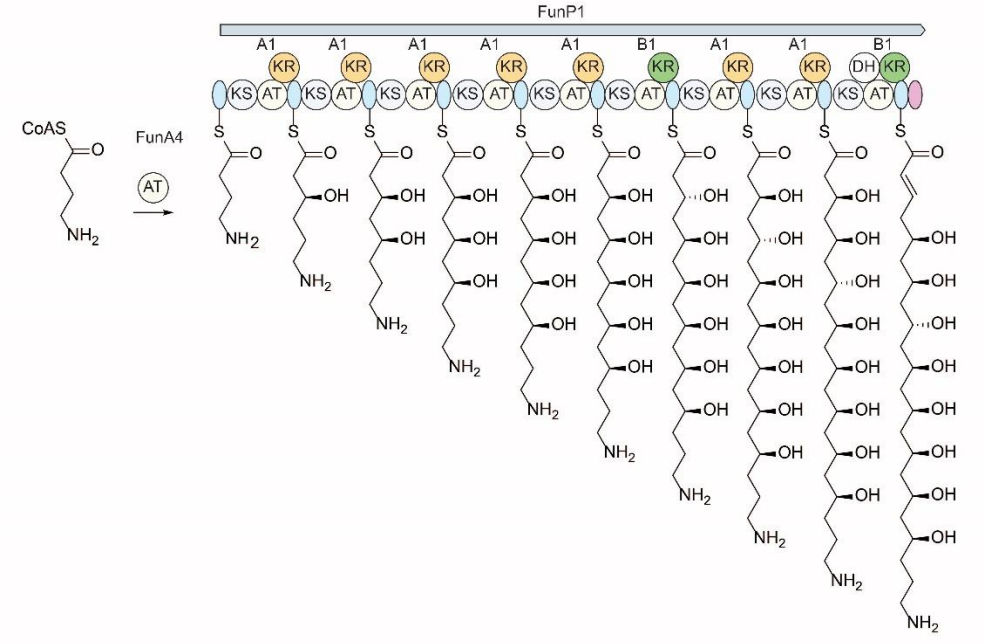

FIG S50: Expanded biosynthesis of funisamine from FunP2 - FunP4. The layout of catalytic domains: ketosynthase (KS), acyltransferase (AT), ketoreductase (KR), dehydratase (DH), enoylreductase (ER), and thioesterase (TE) present within the polyketide synthases are shown with inactive domains colored grey. A-type and B-type KR domains are colored orange and green respectively. Acyltransferase domains predicted to use methyl or propylmalonyl extender units are colored yellow. Acyl carrier proteins are colored light blue, and docking domains are purple.

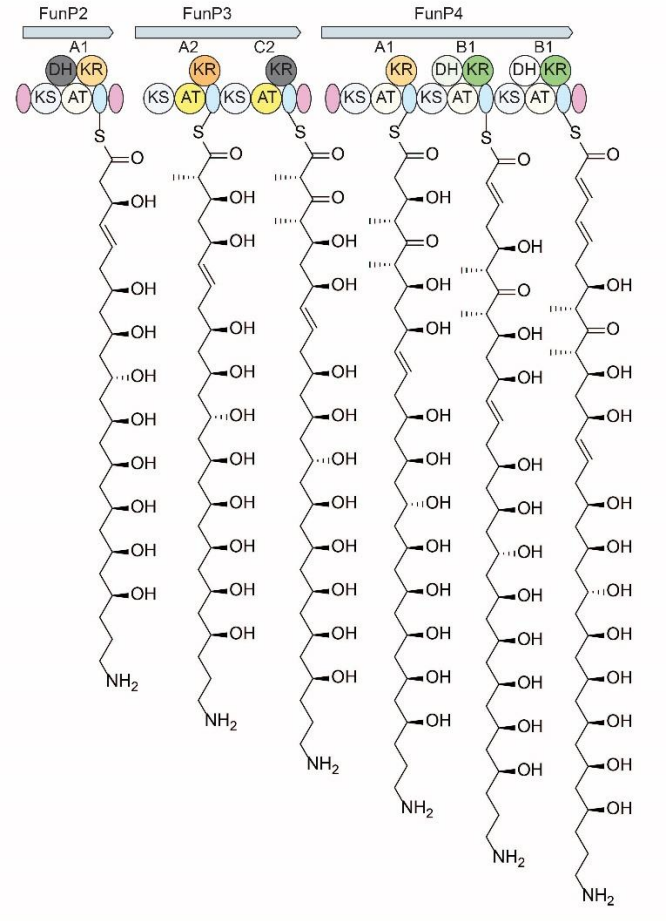

FIG S51: Expanded biosynthesis of funisamine from FunP5 - FunP8. The layout of catalytic domains: ketosynthase (KS), acyltransferase (AT), ketoreductase (KR), dehydratase (DH), enoylreductase (ER), and thioesterase (TE) present within the polyketide synthases are shown with inactive domains colored grey. A-type and B-type KR domains are colored orange and green respectively. Acyltransferase domains predicted to use methyl or propylmalonyl extender units are colored yellow. Acyl carrier proteins are colored light blue, and docking domains are purple.