Supplementary Information

Evolution of Combinatorial Diversity in *Trans*-Acyltransferase Polyketide Synthase Assembly Lines Across Bacteria

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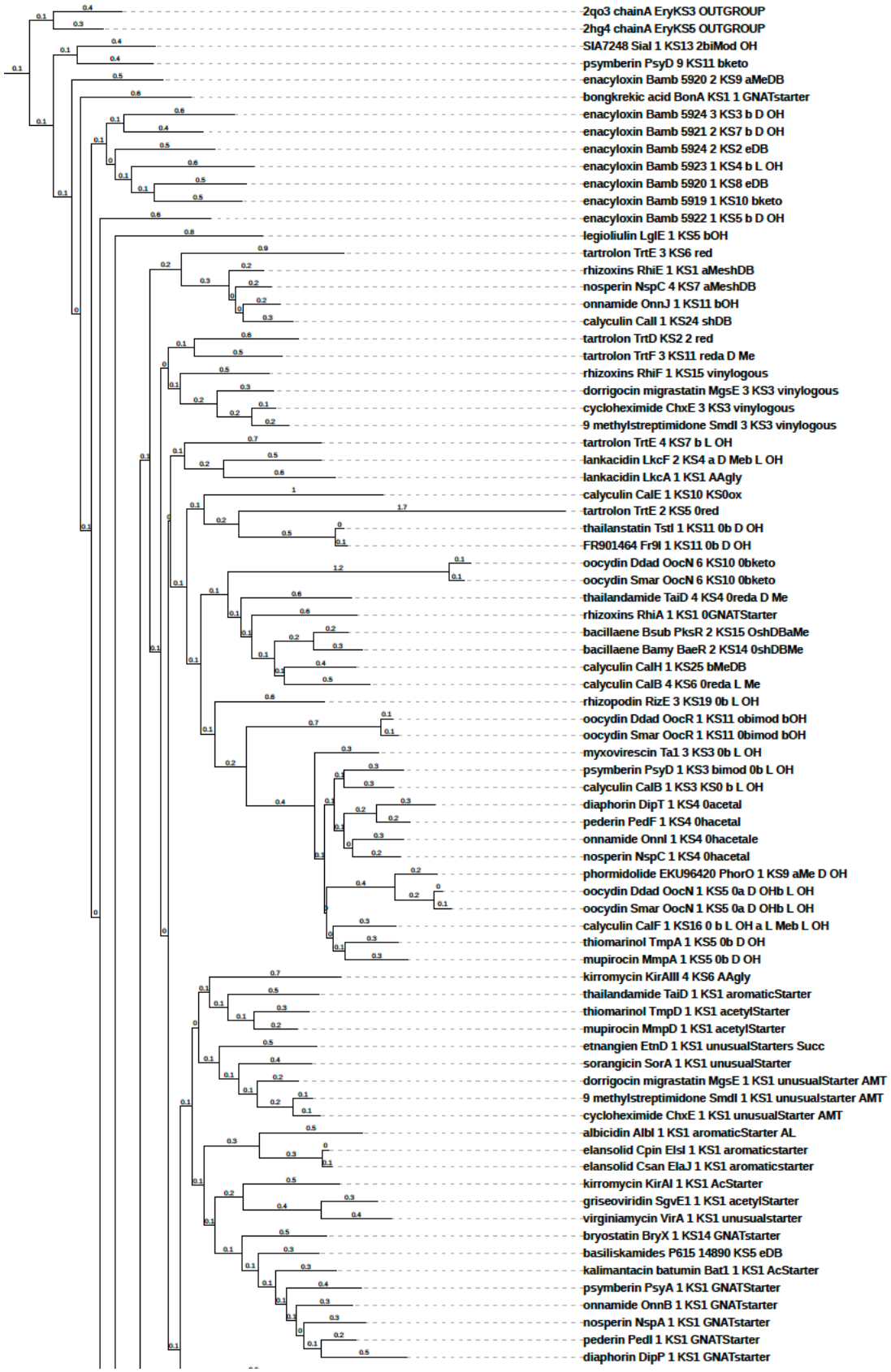
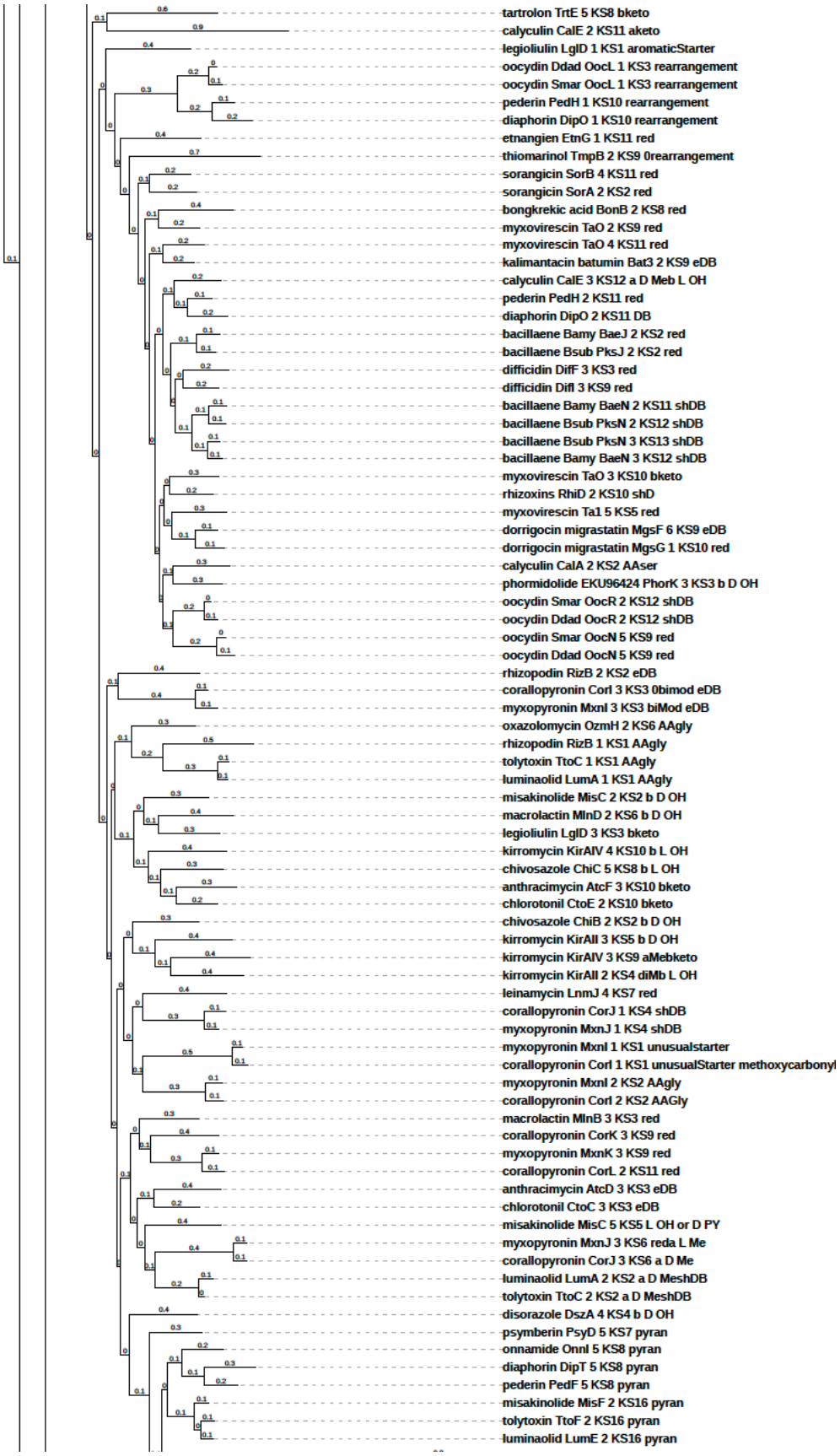
Supplementary Figures

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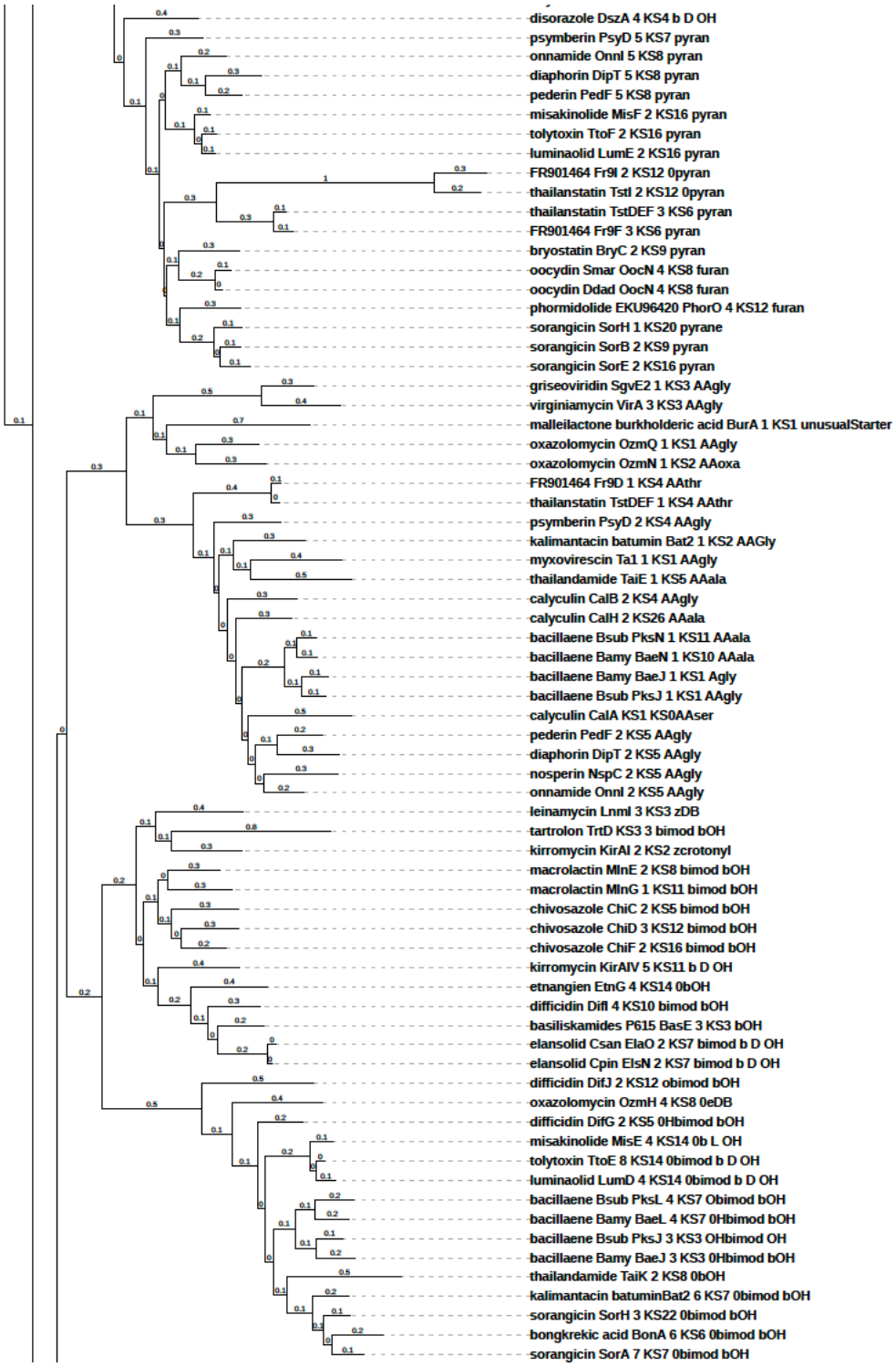
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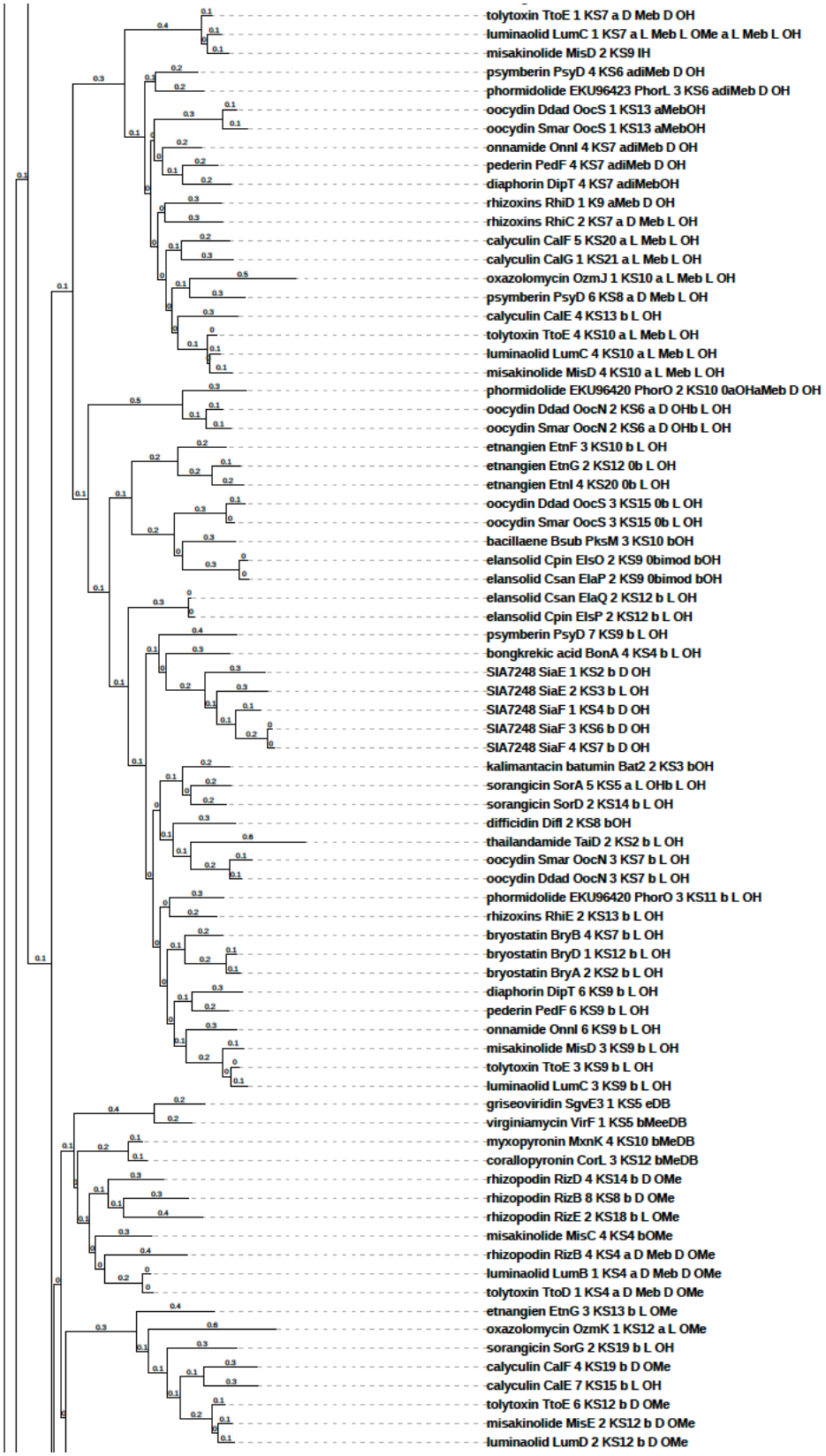
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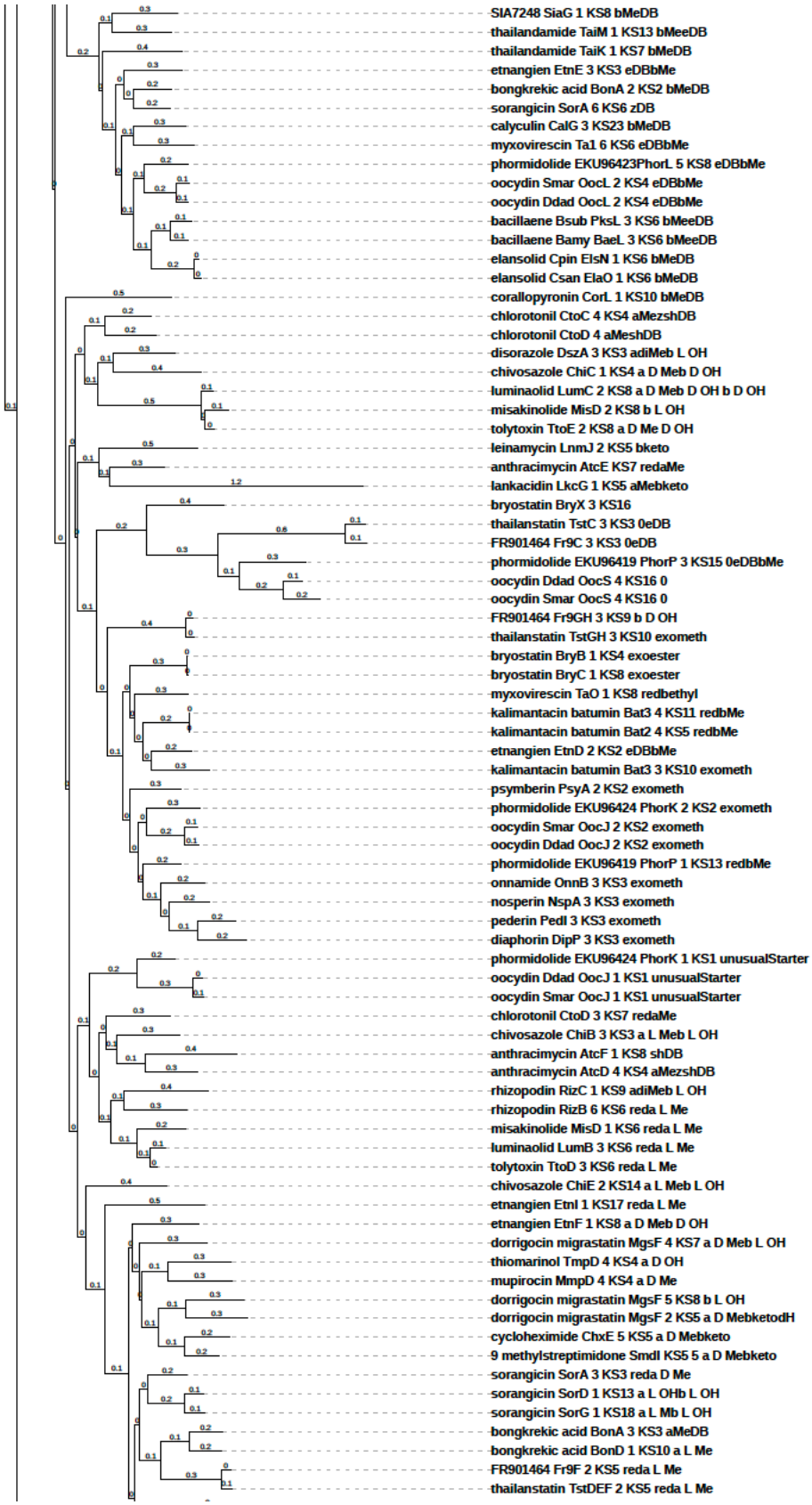
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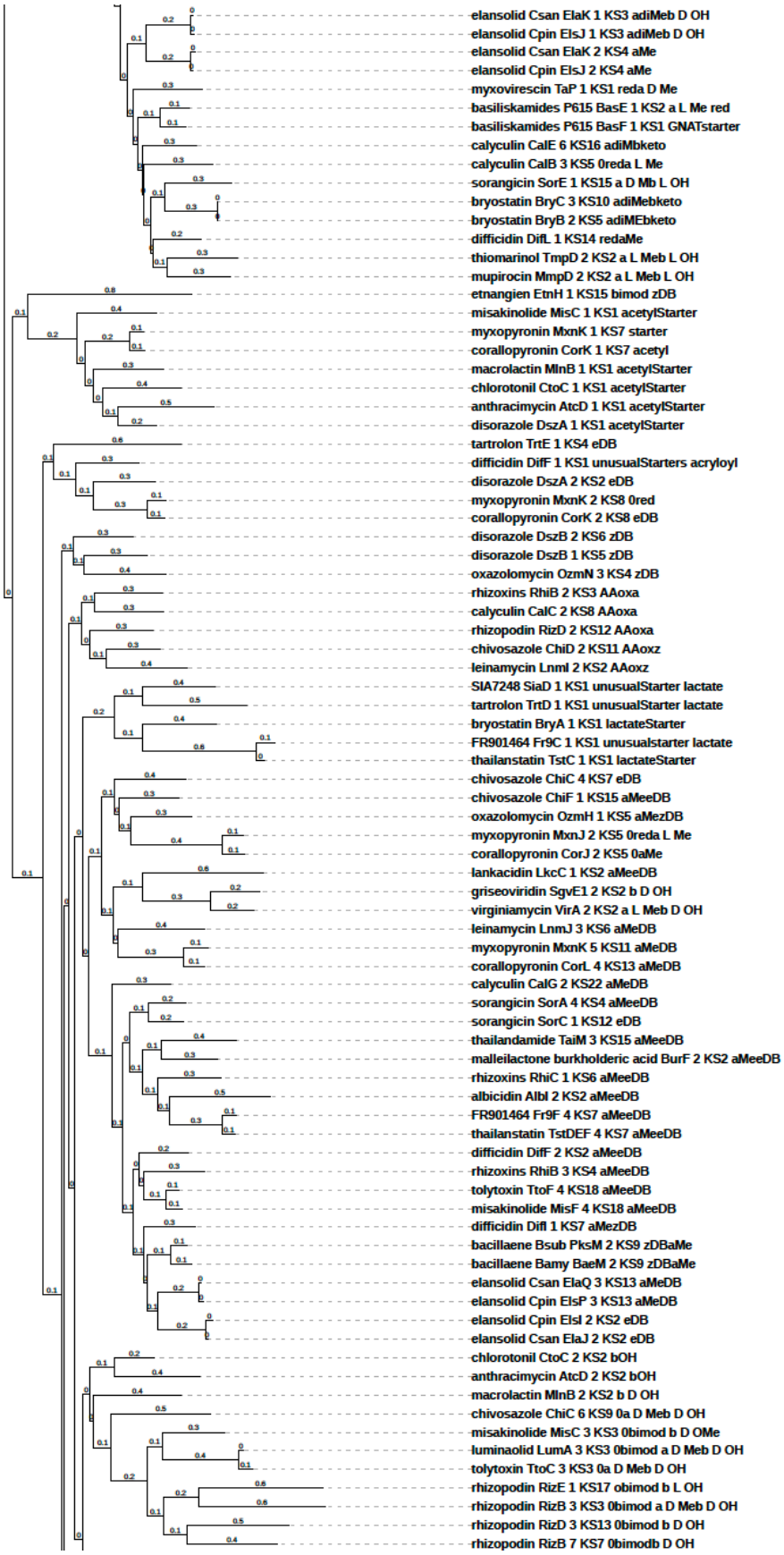
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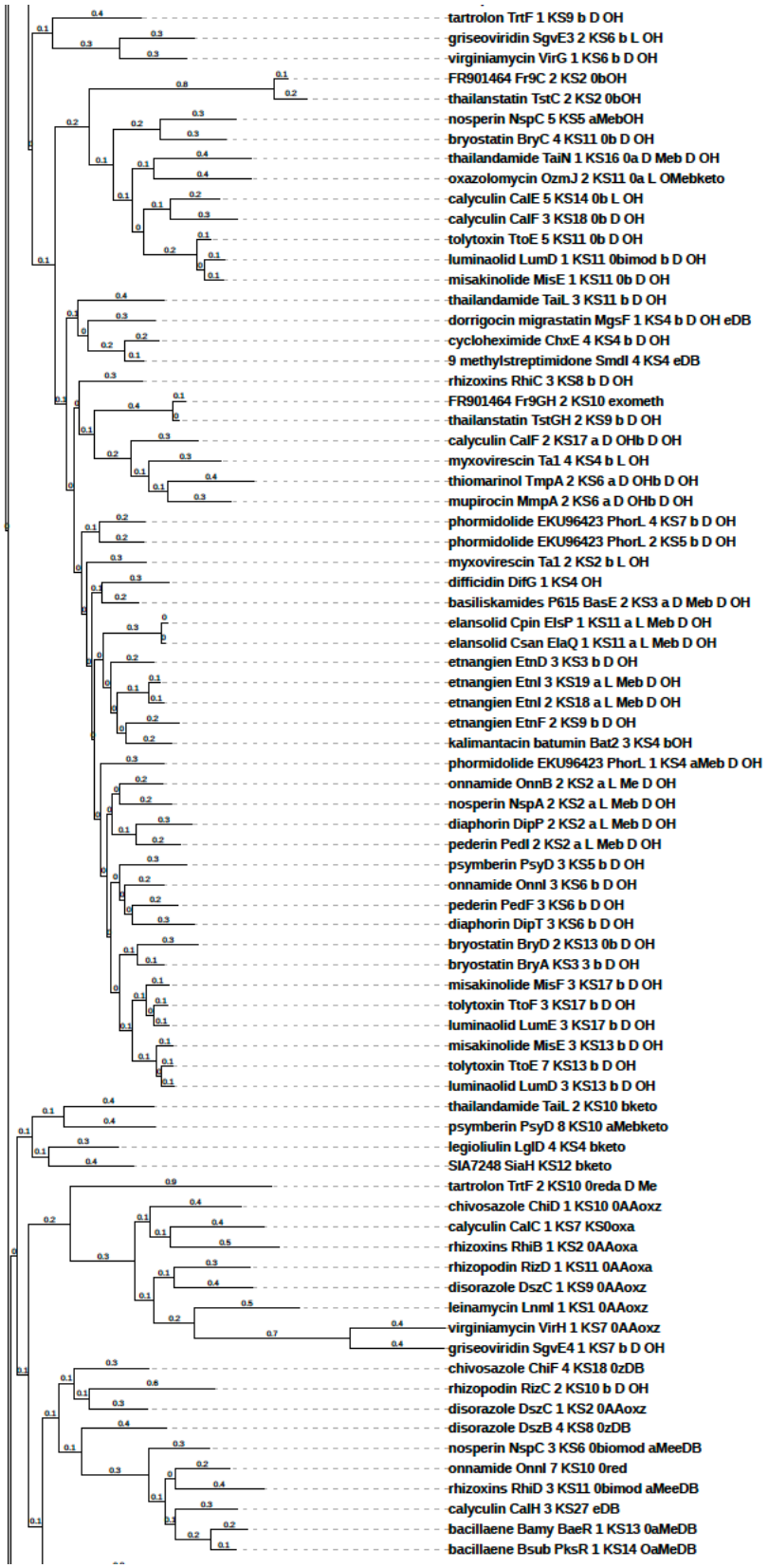
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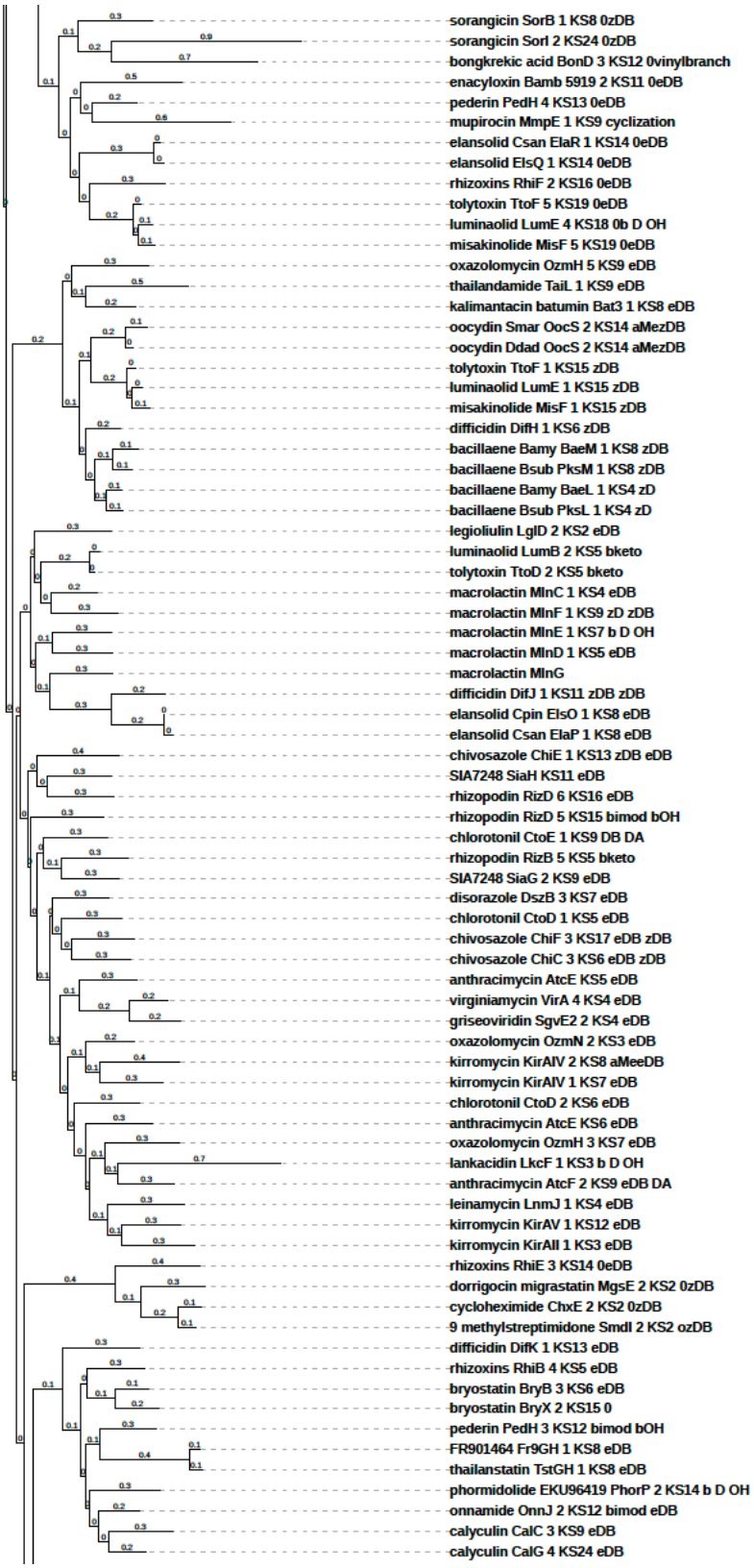
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rhizoxins RhiB 4 KS5 eDB
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bryostatin BryX 2 KS15 0
pederin PedH 3 KS12 bimod bOH
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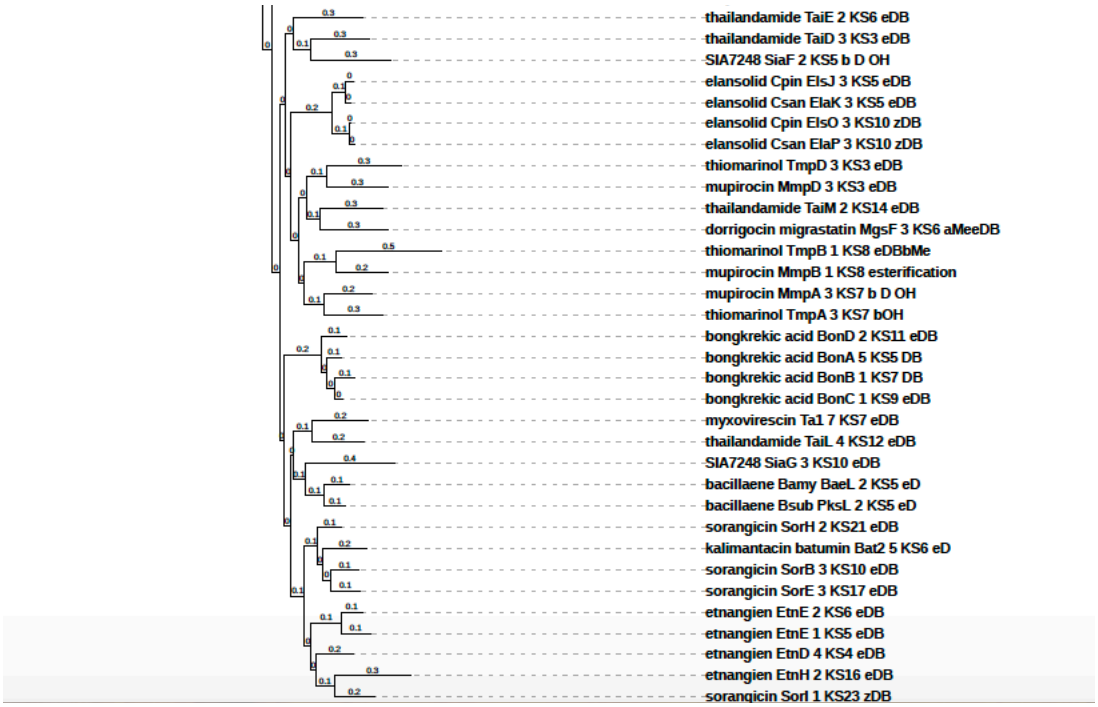
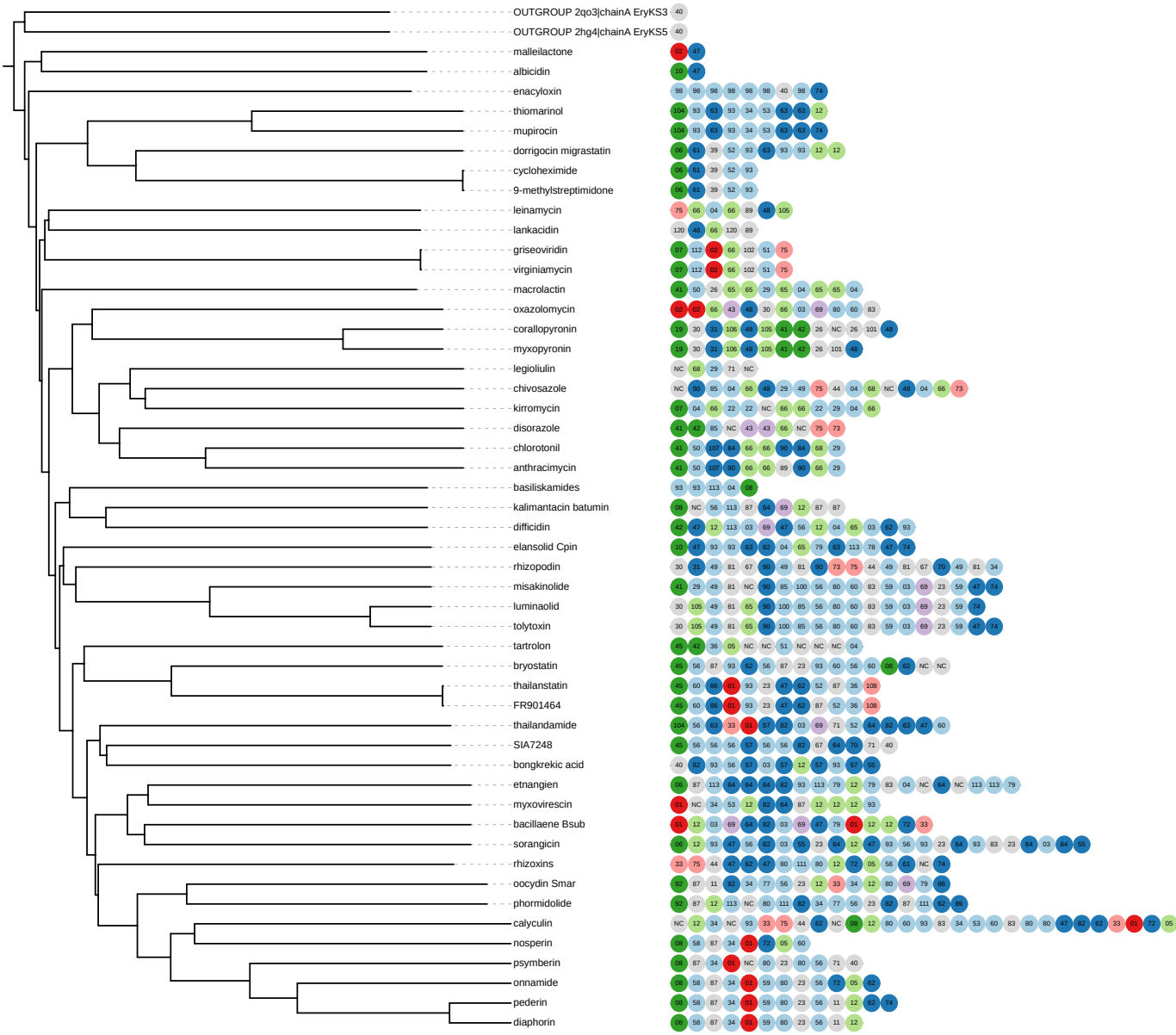
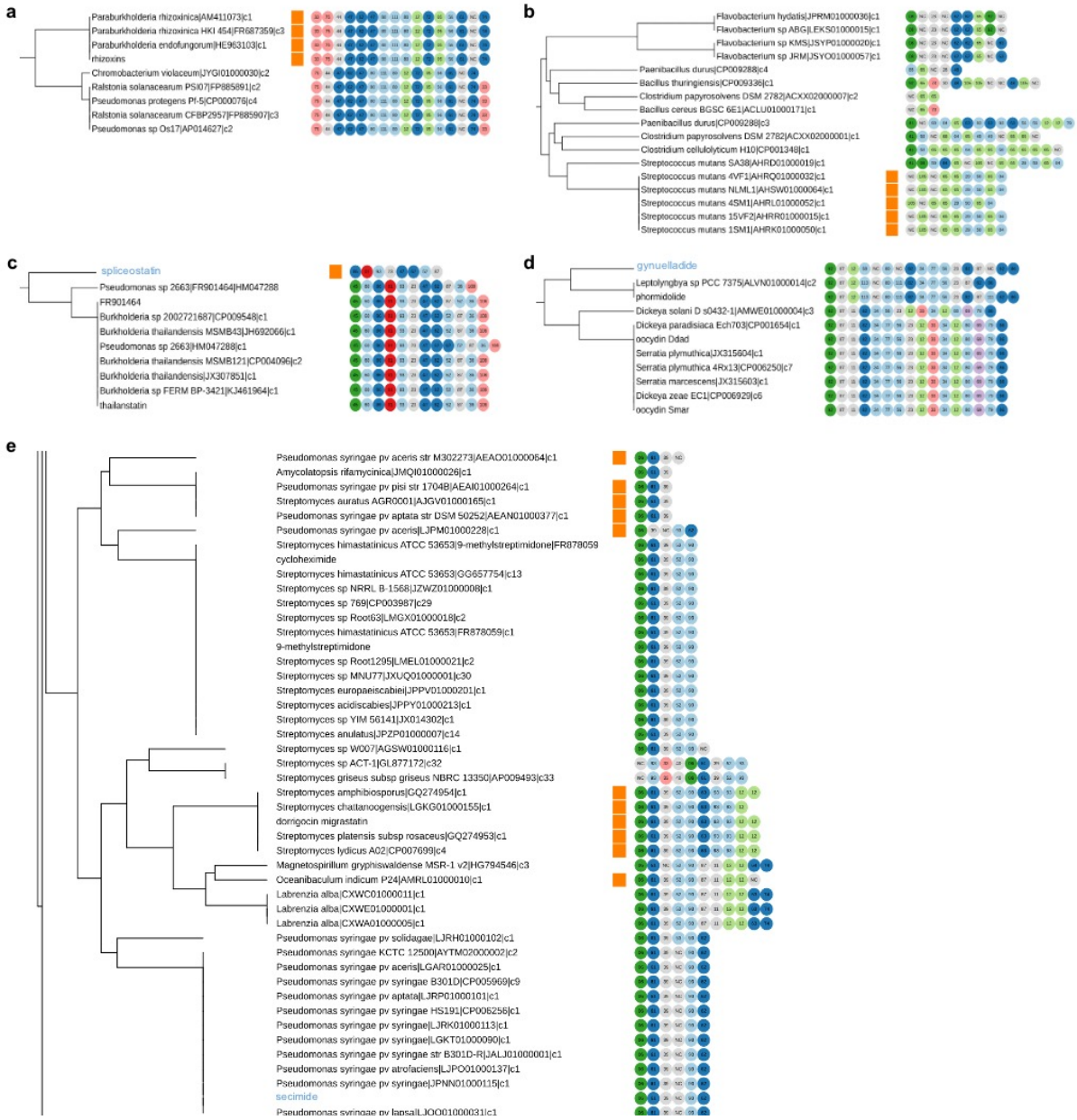


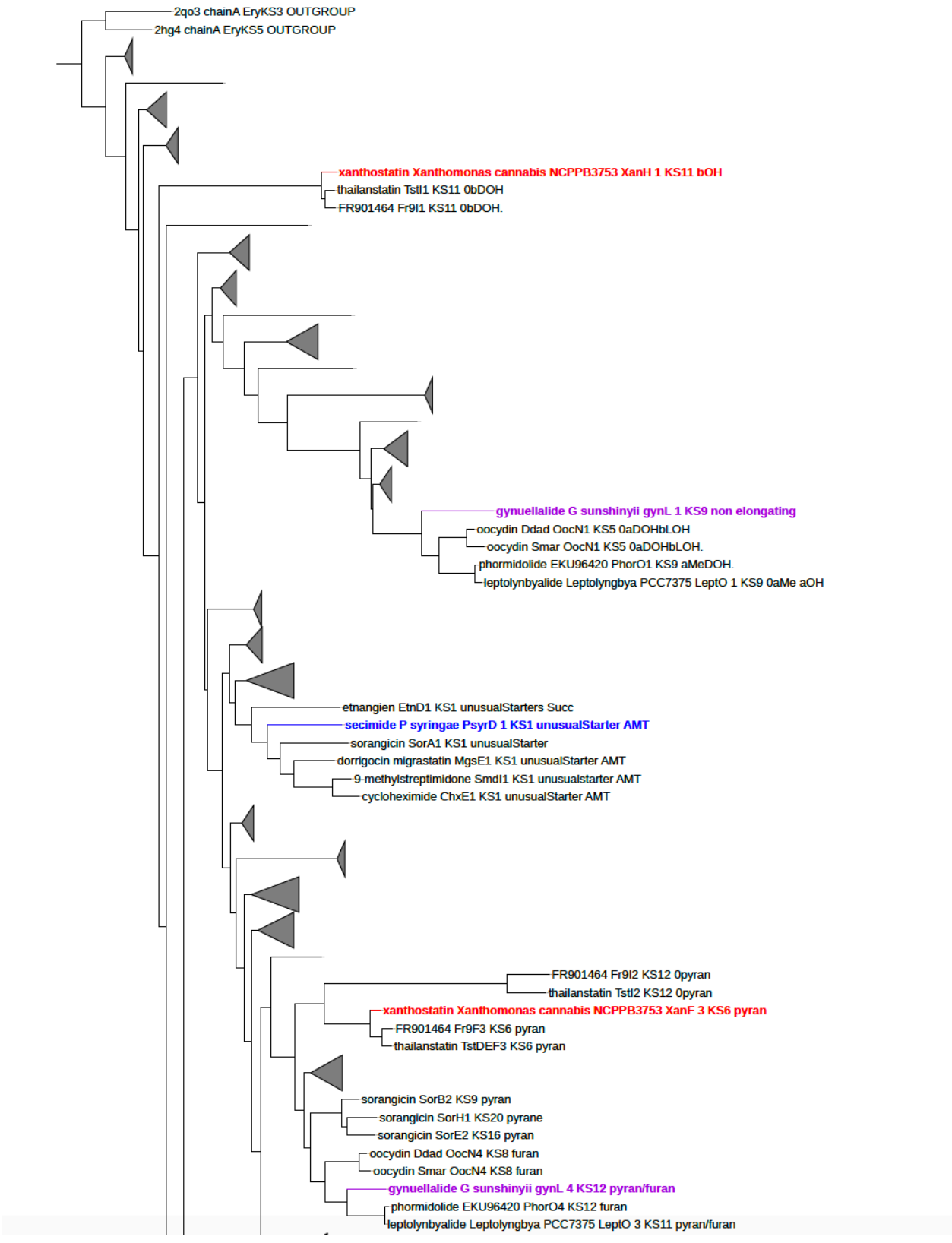
Supplementary Figure 1: **Random Axelerated Maximum likelihood (RAxML) phylogenetic tree calculated from a MUSCLE alignment of 647 KS sequences of all 49 characterized *trans*-AT PKS BGCs described in Helfrich and Piel.**[1] Legend: aMe: α-methyl, bOH: β-hydroxyl, aOH: α-hydroxyl, 0: non-elongating KS, hacetal: hemiacetal, DB: double bond, shDB: shifted double bond, red: completely reduced, vinylogous: vinylogous chain branching, Oxa/Oxz: oxazole, Thia: thiazole, eDB/eD: *E*-configured DB, zDB/zD: *Z*-configured DB, D_OH: D-configured hydroxyl group, L_OH: L-configured hydroxyl group, rearrangement: oxygen insertion, 2biMod: second KS in a bimodule, bimod: KS of a bimodule, 0bimod: non-elongating KS in a bimodule, ozDB: oxazole or double bond. Bimodule consist of two adjacent modules and jointly modify an intermediate. Branch nomenclature: polyketide name_protein name_number of KS on the respective protein_number of KS in the PKS_substrate specificity. Numbers on tree nodes indicate bootstrap values.



Supplementary Figure 2: Dendrogram representation of Figure 2A, showing conserved module blocks shared between characterized *trans*-AT PKS biosynthetic gene clusters. See Supplementary Table 2 for detailed number legend. Colors represent classes of phylogenetic clades. Red: amino acids; light blue: β-hydroxyl groups; light green: double bonds; dark blue: *E*-configured double bonds; light red: non-elongating KSs; dark green: starters; light purple: *Z*-configured double bonds; grey: others.



Supplementary Figure 3: Selection of novel PKS module blocks highlighted in the main text and families of PKS expanded through the isolation of novel polyketides in this study. (a) and (b) selection of novel PKS module blocks within the context of total assembly lines as depicted in the dendrogram. (c) spliceostatin-like PKSs. (d) phormidolide-like PKSs. (e) glutarimide PKSs. See Supplementary Table 2 for detailed number legend. Colors represent classes of phylogenetic clades. Red: amino acids; light blue: β-hydroxyl groups; light green: double bonds; dark blue: *E*-configured double bonds; light red: non-elongating KSs; dark green: starters; light purple: *Z*-configured double bonds; grey: others. Molecules linked to characterized biosynthetic gene clusters help identify families of *trans*-AT PKSs. Orange boxes indicate likely incomplete biosynthetic gene clusters as determined by their small distance to contig borders of less than 5 kb. An interactive representation of the dendrogram can be accesses here: https://itol.embl.de/tree/474115015487031585082885#.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FR901464 Fr9I1 KS11 0bDOH.
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oocydin Smar OocN1 KS5 0aDOHbLOH.
phormidolide EKU96420 PhorO1 KS9 aMeDOH.
leptolynbyalide Leptolyngbya PCC7375 LeptO 1 KS9 0aMe aOH
etnangien EtnD1 KS1 unusualStarters Succ
secimide P syringae PsyrD 1 KS1 unusualStarter AMT
sorangicin SorA1 KS1 unusualStarter
dorrigocin migrastatin MgsE1 KS1 unusualStarter AMT
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sorangicin SorH1 KS20 pyrane
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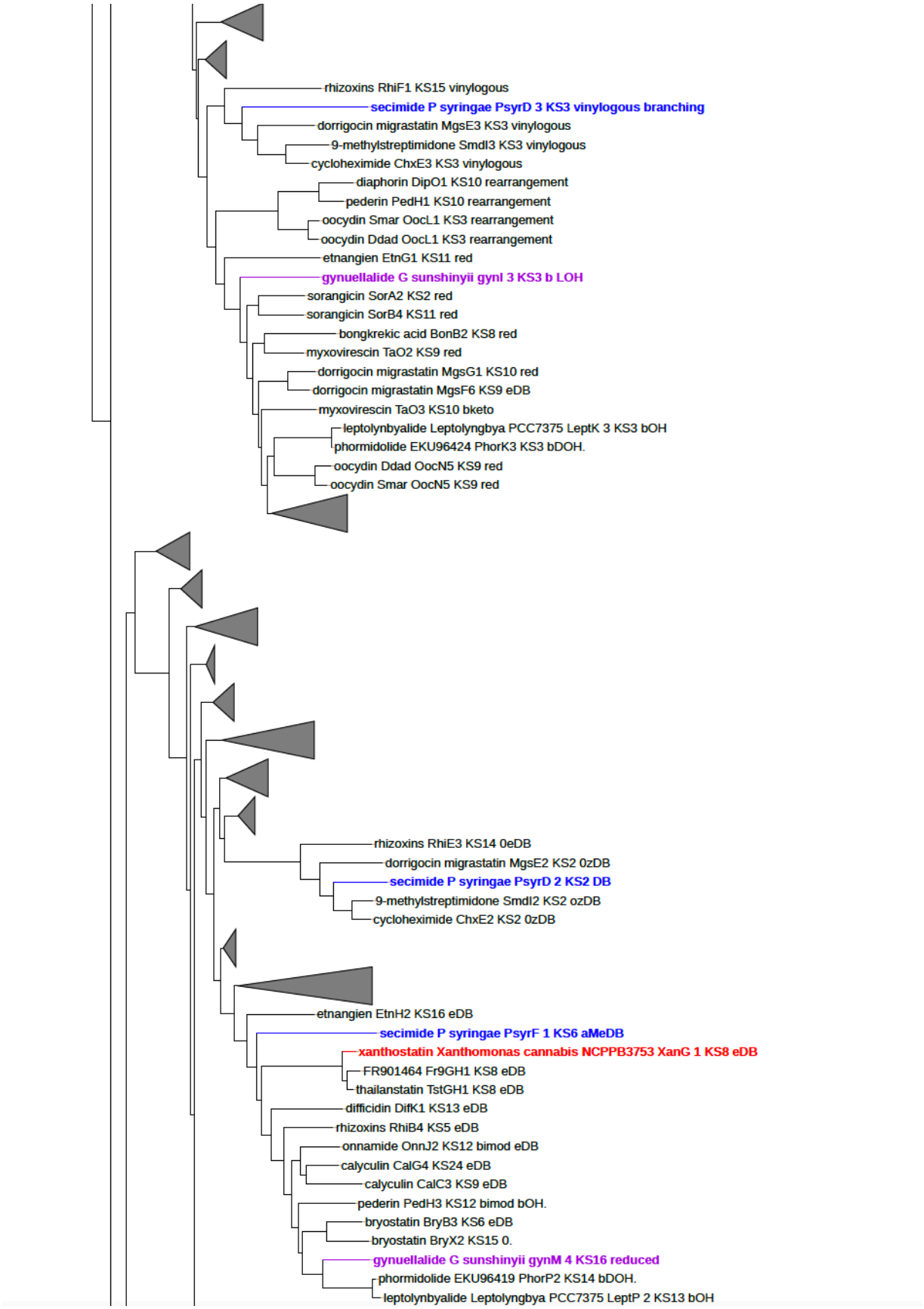
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sorangicin SorB4 KS11 red
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secimide P syringae PsyrD 2 KS2 DB
9-methylstreptimidone SmdI2 KS2 ozDB
cycloheximide ChxE2 KS2 0zDB
etnangien EtnH2 KS16 eDB
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FR901464 Fr9GH1 KS8 eDB
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calyculin CalG4 KS24 eDB
calyculin CalC3 KS9 eDB
pederin PedH3 KS12 bimod bOH.
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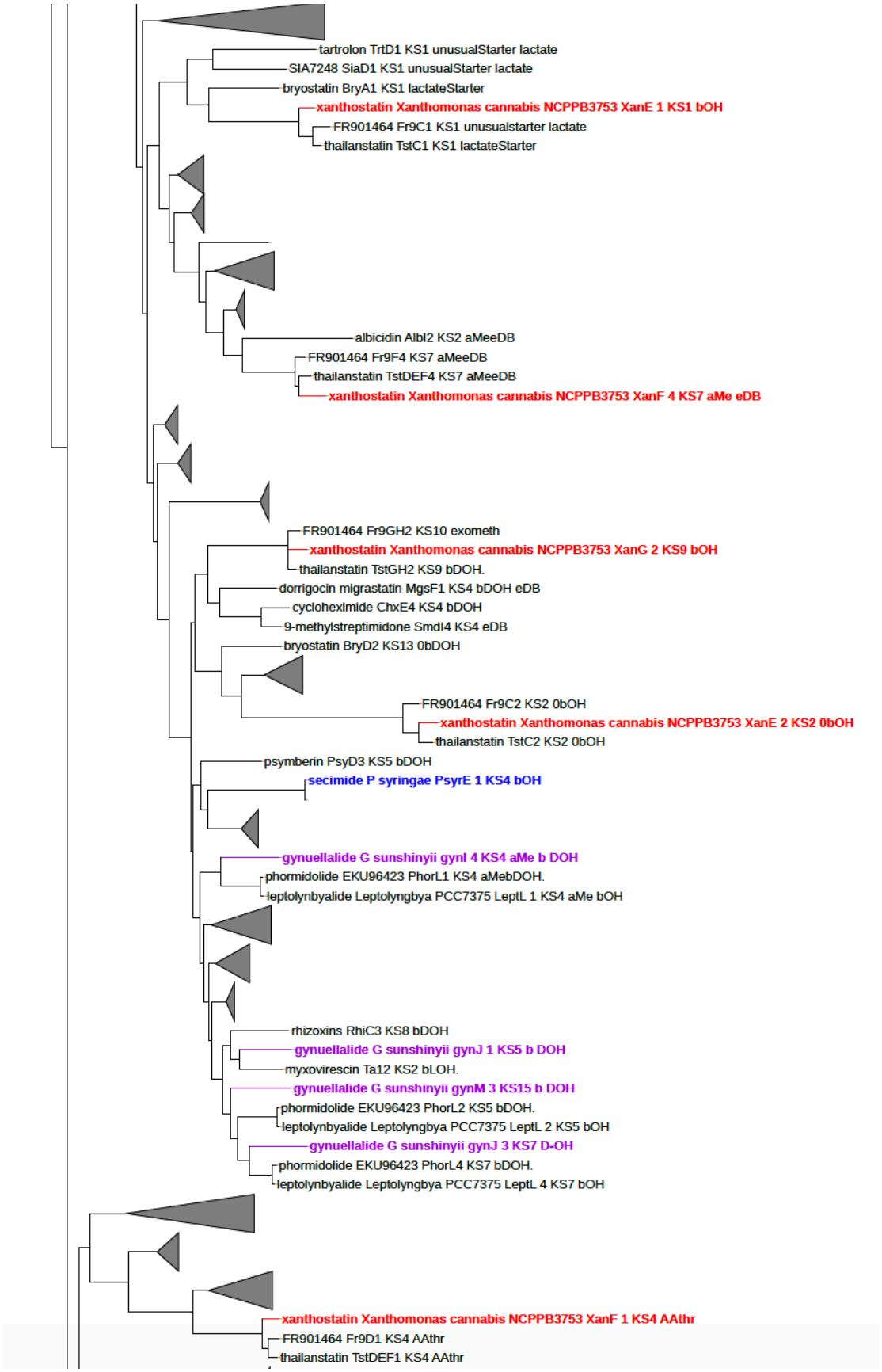
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myxovirescin Ta12 KS2 bLOH.
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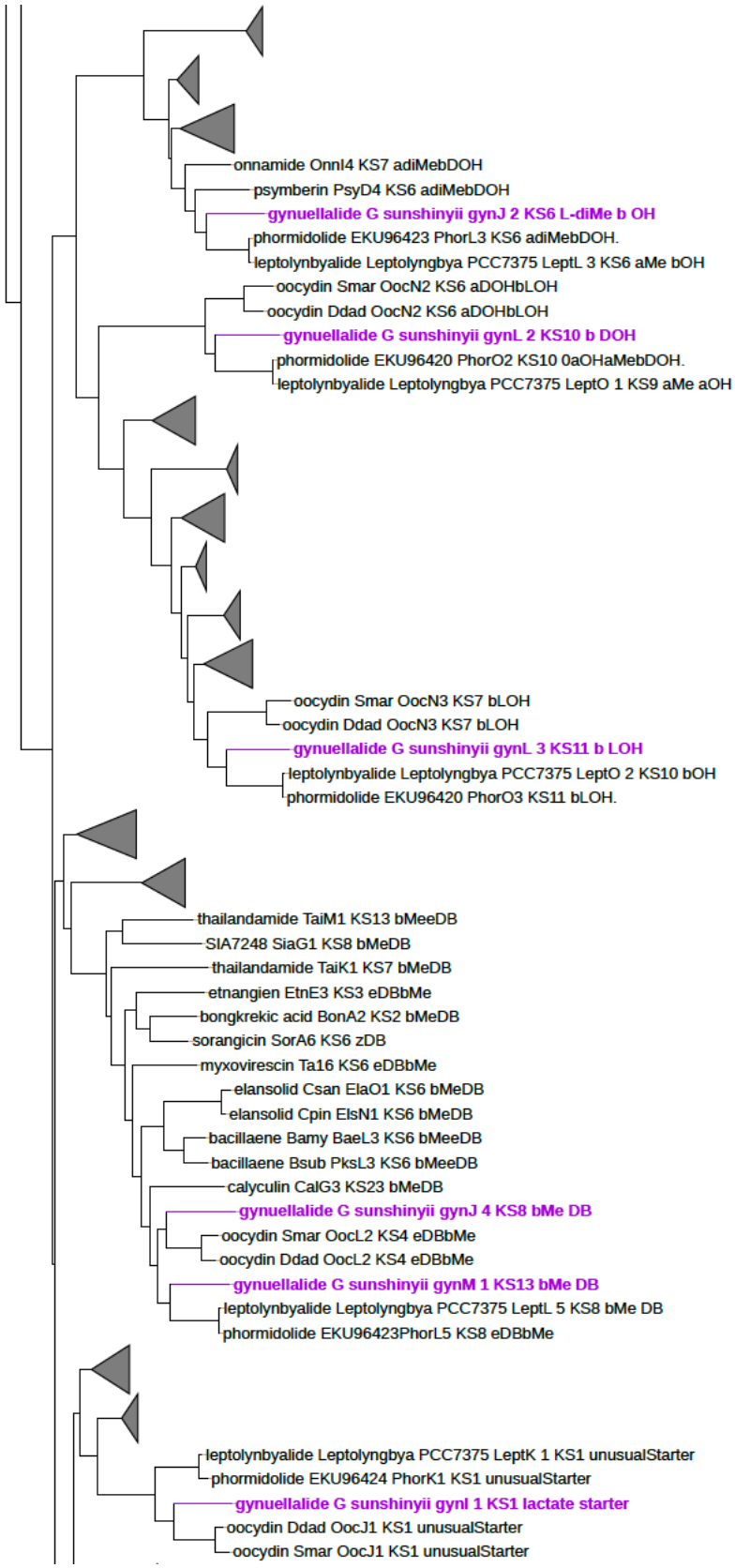
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SIA7248 SiaG1 KS8 bMeDB
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sorangicin SorA6 KS6 zDB
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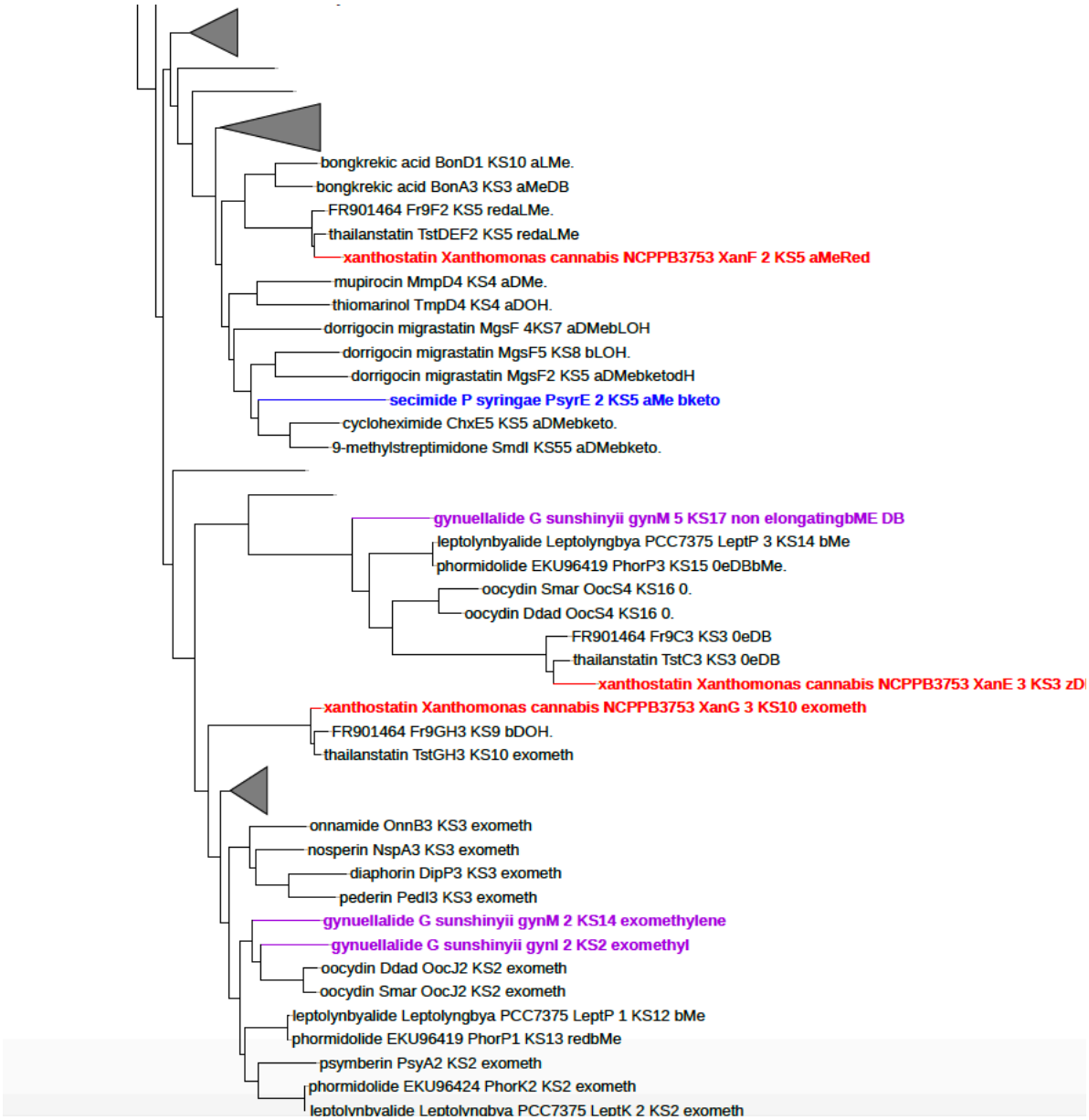
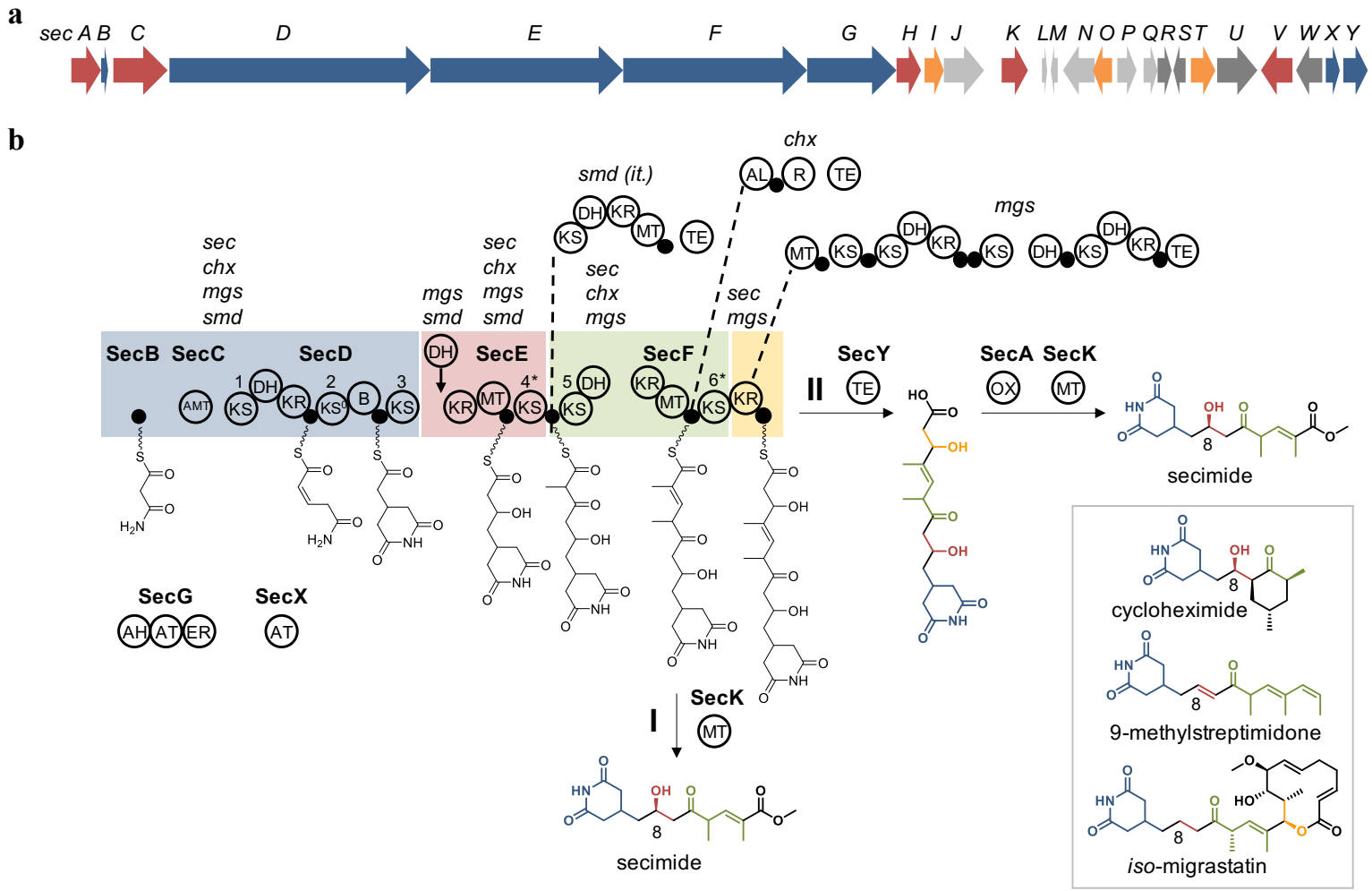
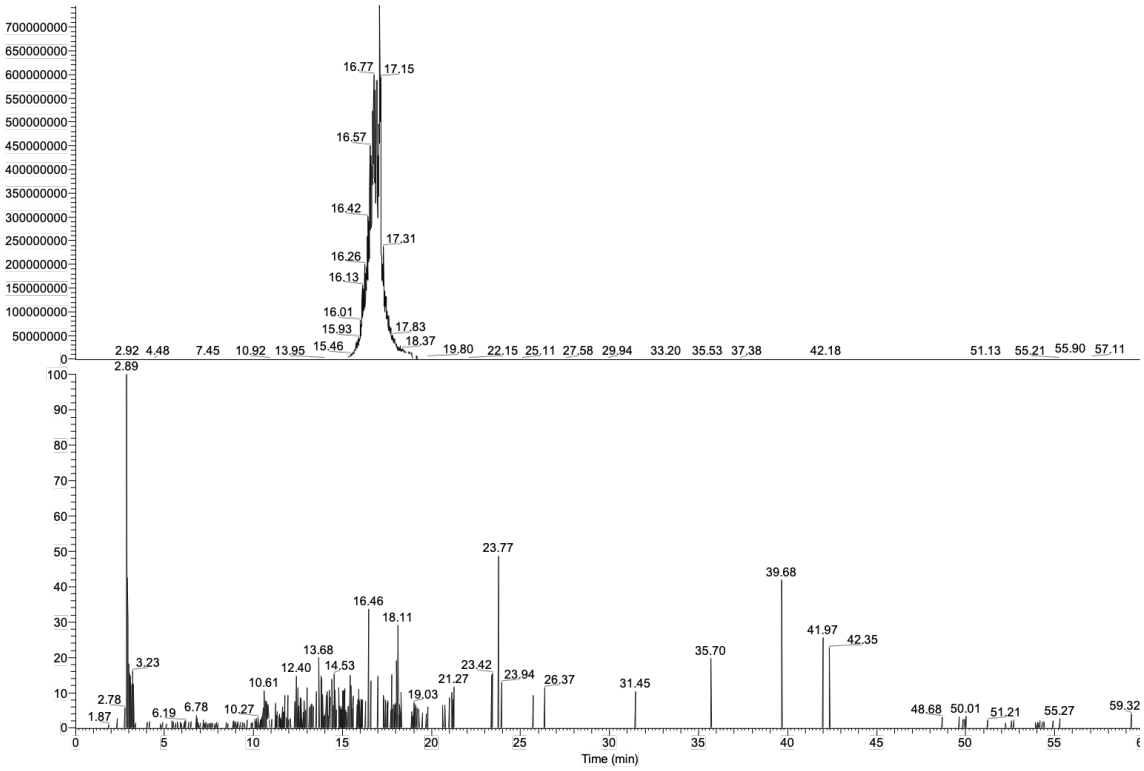


Supplementary Figure 4: Validation of the *trans*PACT workflow using the three biosynthetic gene clusters for the new polyketides described in this study as an example. Maximum likelihood phylogenetic tree computed from a MUSCLE alignment of all *trans*-AT PKS KS sequences from the 49 characterized *trans*-AT PKSs described by Helfrich and Piel,[1] plus all KSs extracted from the secimide, gynuellalide, and spliceostatin PKSs (denoted here as 'xanthostatin') characterized in this study. Phylogenetic placement of the KSs from the three example compounds is in good agreement with the transPACT results.



Supplementary Figure 5: Secimide biosynthetic model. (**a**) The *sec* BGC. blue: PKS genes, orange: transporter genes, light grey: hypothetical genes, red: precursor biosynthesis and tailoring genes, dark grey: genes involved in replication, DNA biosynthesis and repair. (**b**) The secimide PKS and alternative models for secimide biosynthesis. The *sec* proteins are shown with bold labels, other labels refer to genes encoding the core PKSs of the related cycloheximide (*chx*), migrastatin (*mgs*), and 9-methylstreptimidone (*smd*) pathways. Proteins with multiple labels have orthologs in the corresponding pathways. Alternative hypothesis for the final steps: I) the last module is skipped and the intermediate converted to the ester by SecK or II) the polyketide backbone is shortened after it is completely assembled putatively catalyzed by the dioxygenase SecA and then methylated by SecK. *It.*, iterative module. Colors represent corresponding substructures of the polyketides.

a



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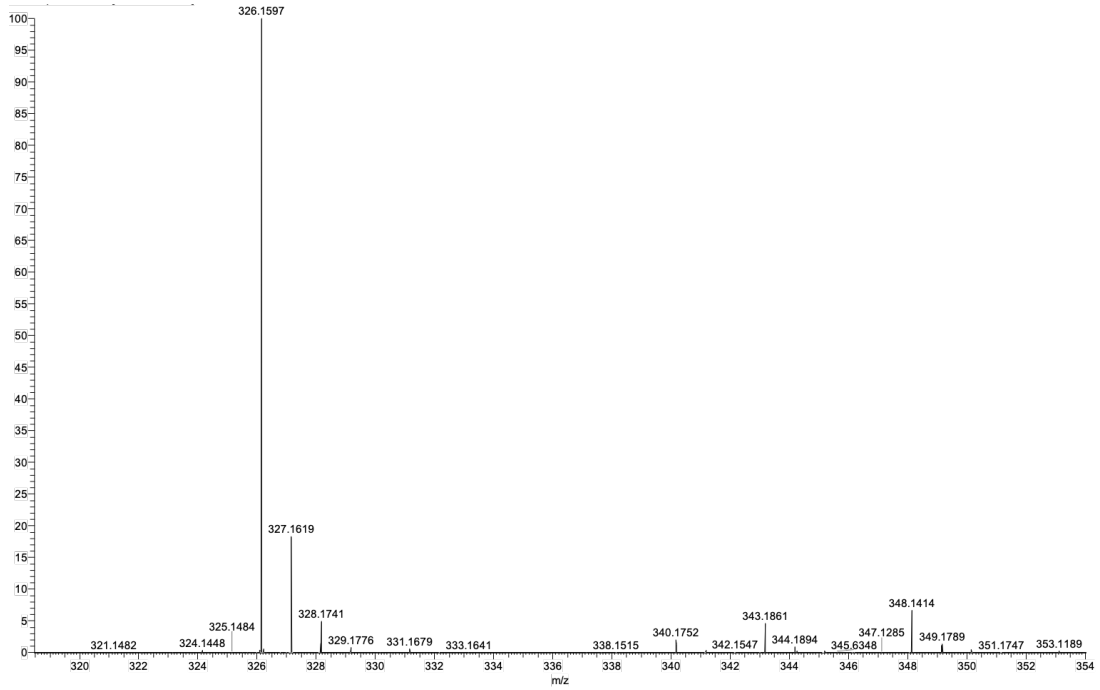
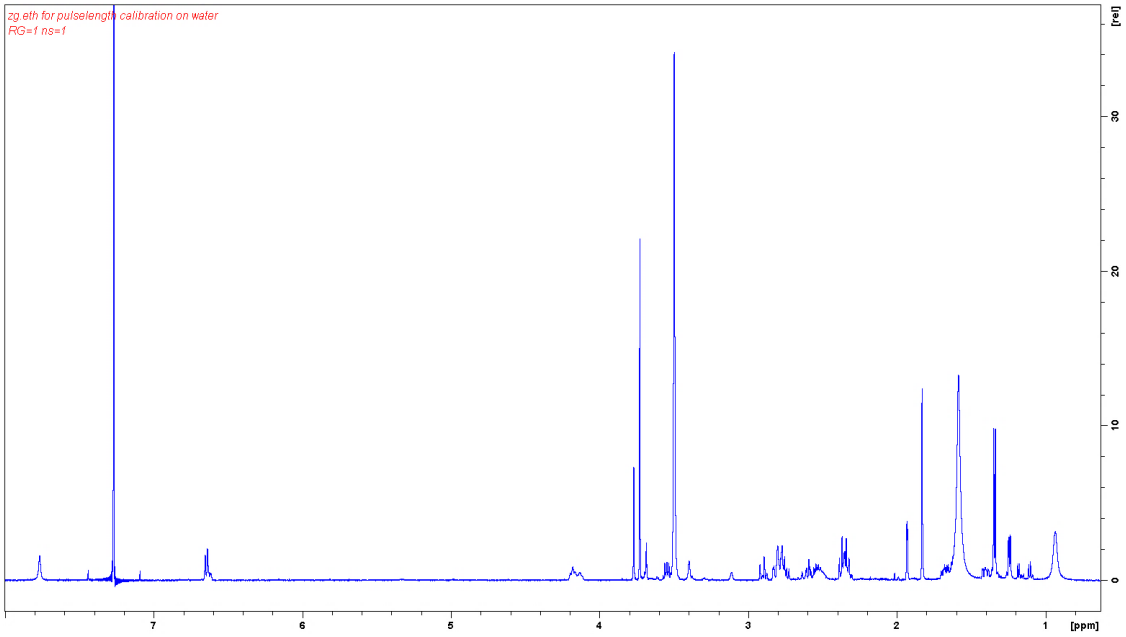
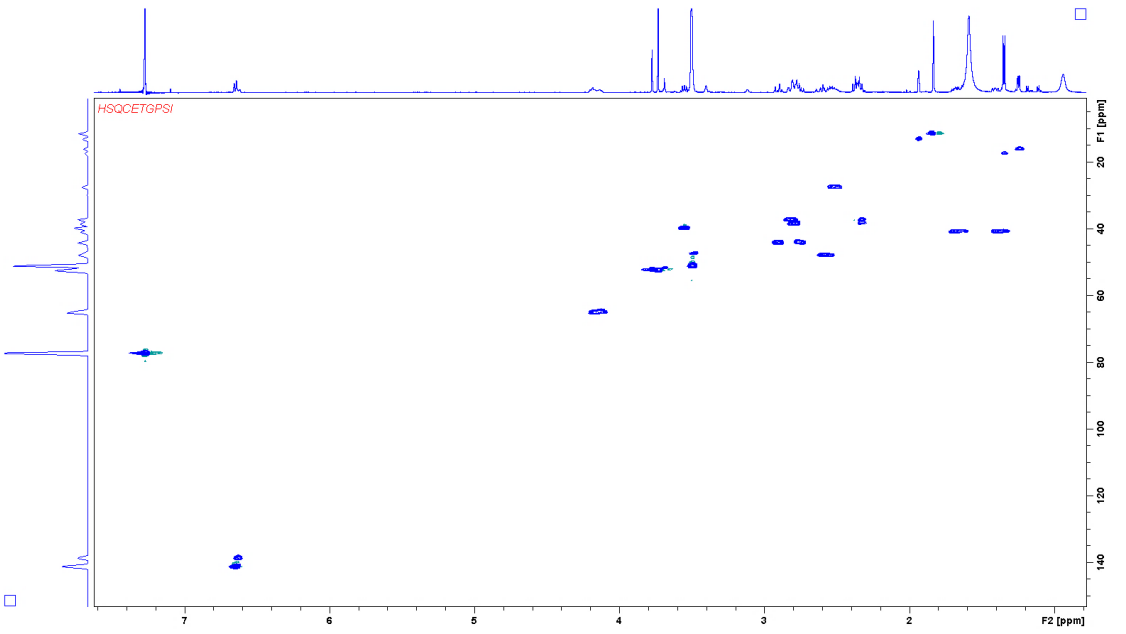


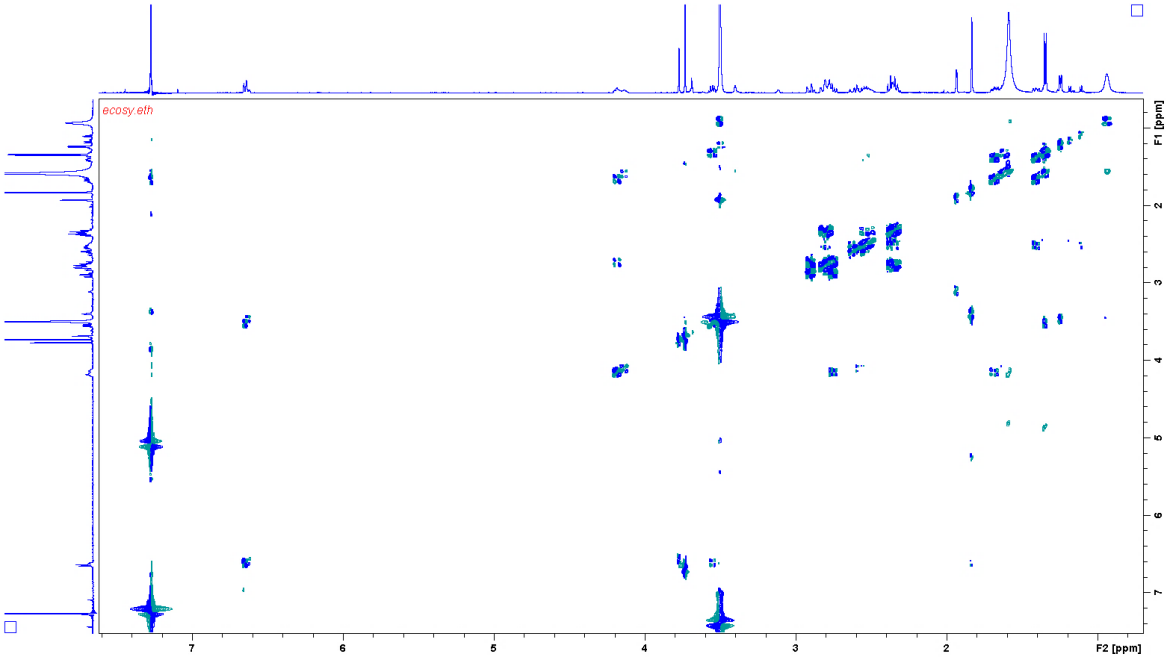
Supplementary Figure 6: HR-LCMS data of the extracts of the *P. syringae* wild type (WT) and PKS knockout strains. (**a)** Extracted ion chromatogram (*m*/*z* 326.15-326.16) of WT supernatant extract (upper) and PKS-knockout supernatant extract (lower); (**b)** Mass spectrum of secimide from *P. syringae* pv. *syringae*.



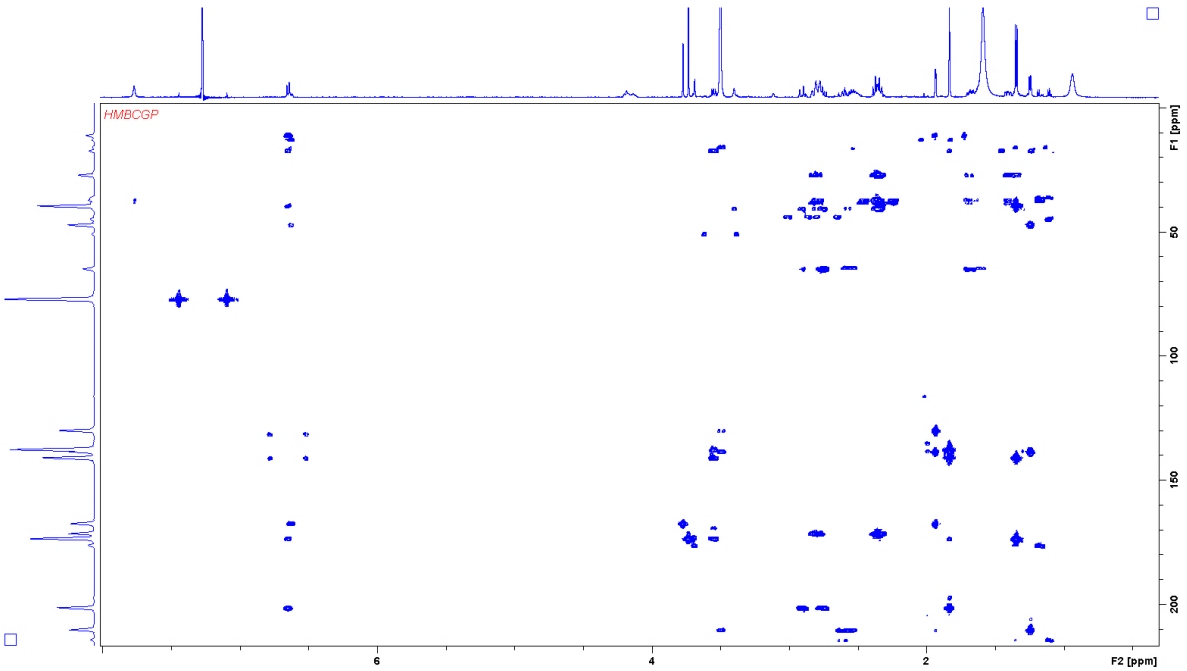
Supplementary Figure 7: ^{1}H NMR spectrum of secimide from *P. syringae* in $CDCl_3$.



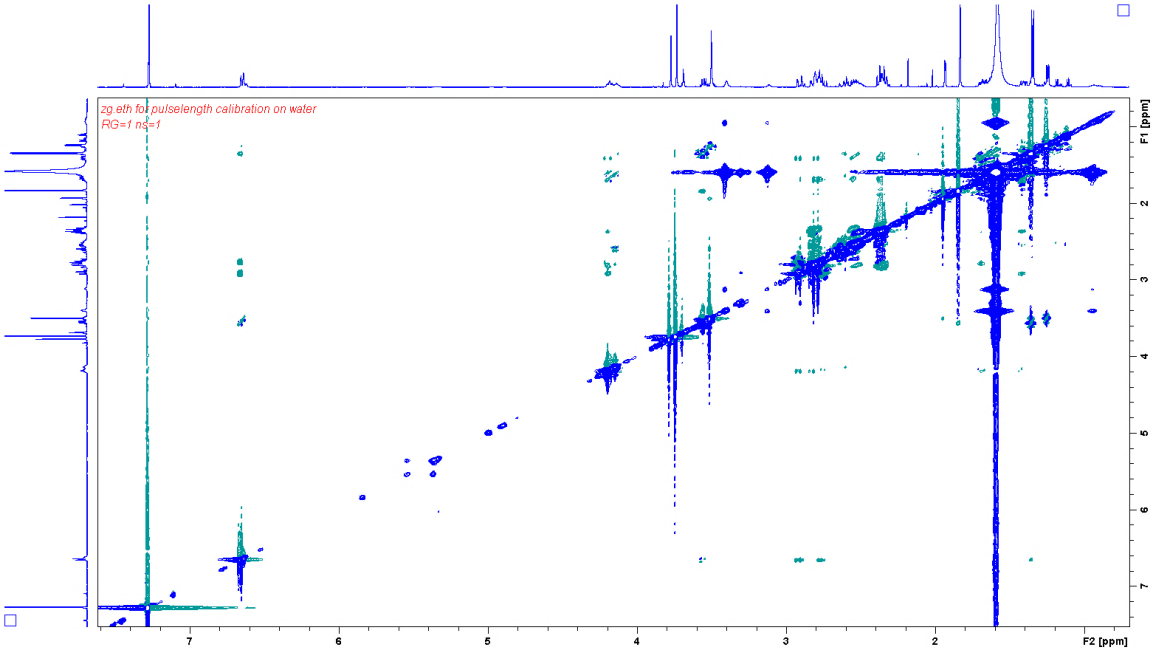
Supplementary Figure 8: HSQC spectrum of secimide from *P. syringae* in $CDCl_3$.



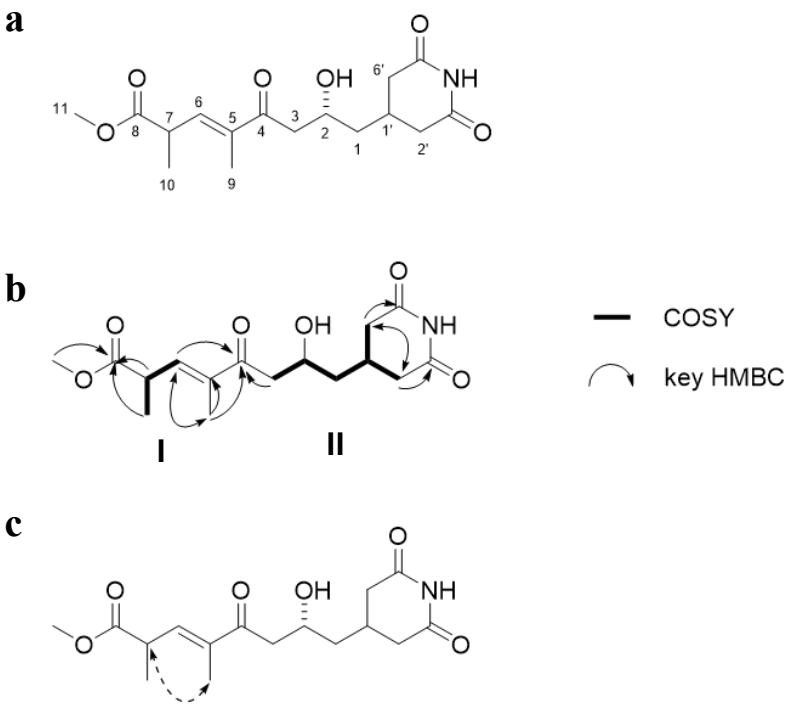
Supplementary Figure 9: COSY spectrum of secimide from *P. syringae* in $CDCl_3$.



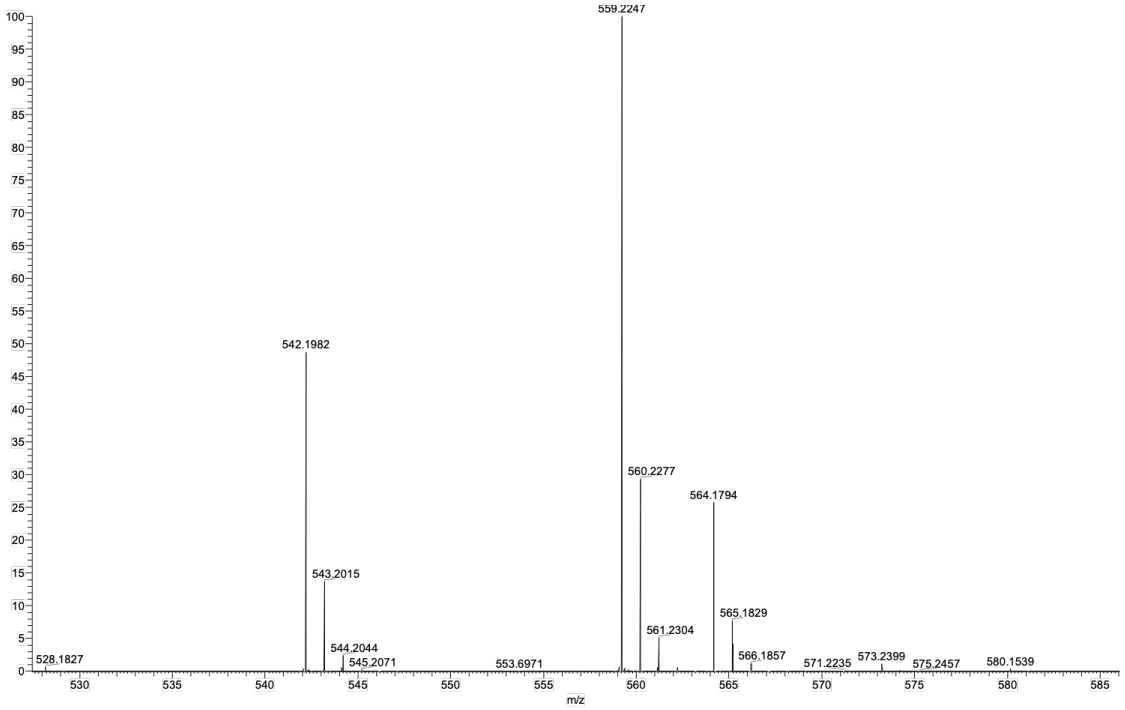
Supplementary Figure 10: HMBC spectrum of secimide from *P. syringae* in $CDCl_3$.



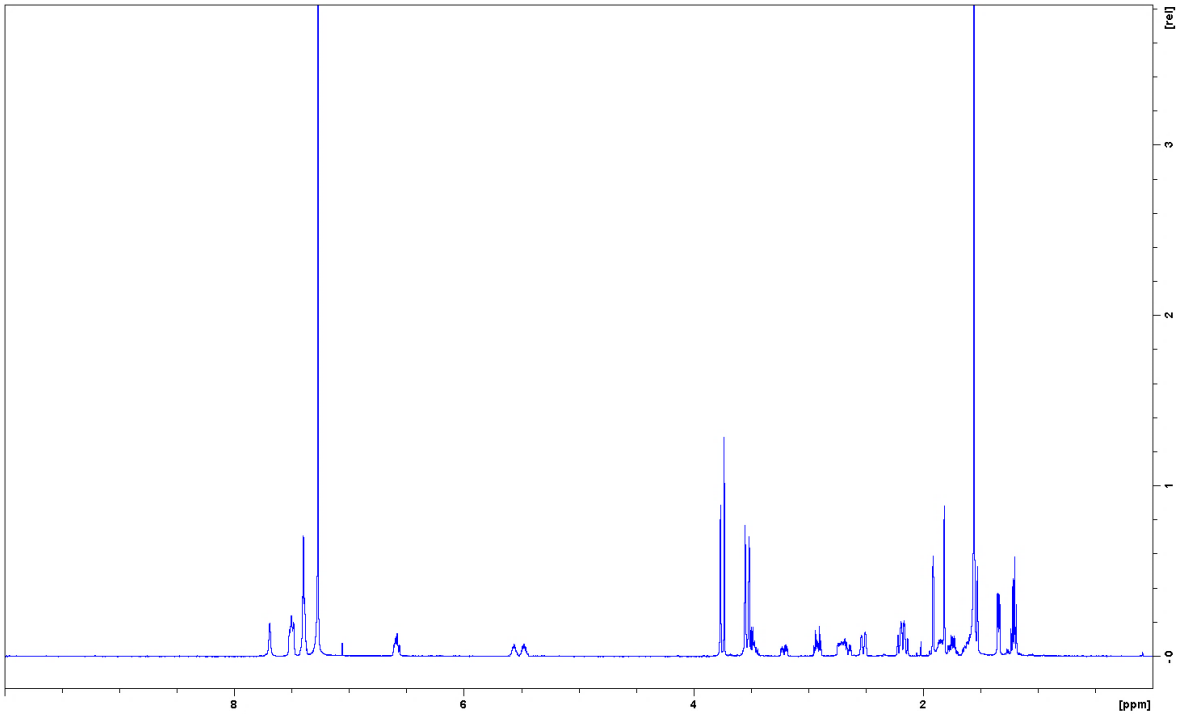
Supplementary Figure 11: NOESY spectrum of secimide from *P. syringae* in $CDCl_3$.



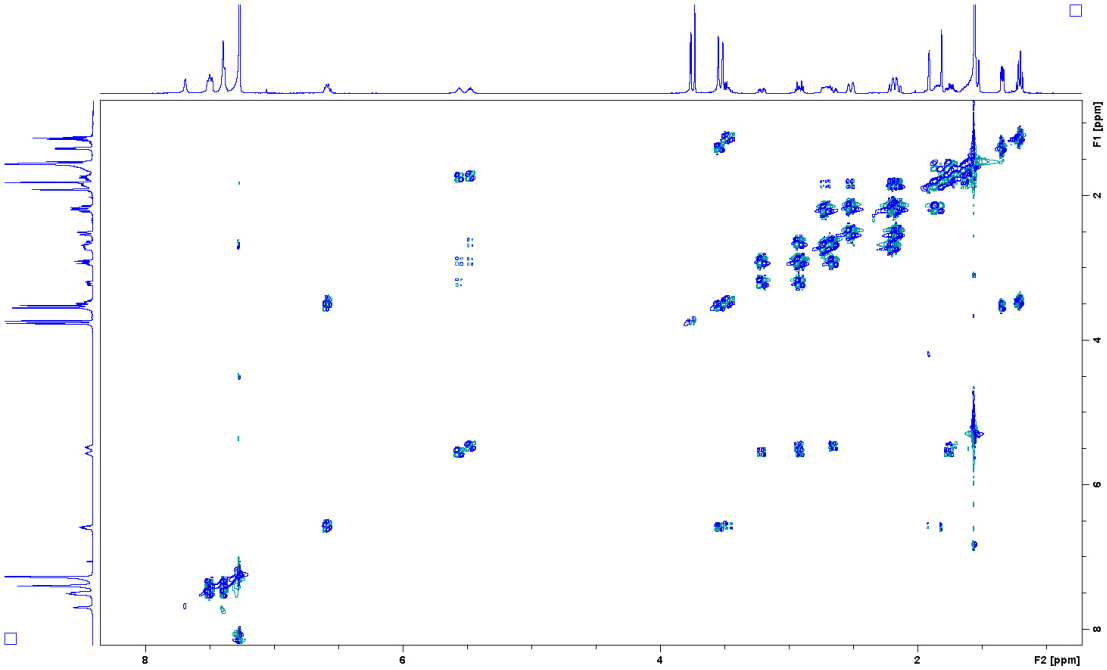
Supplementary Figure 12: Structure elucidation of secimide. (**a**) Structure of secimide. (**b**) COSY and key HMBC correlations of secimide. (**c**) Key NOESY correlation of secimide.



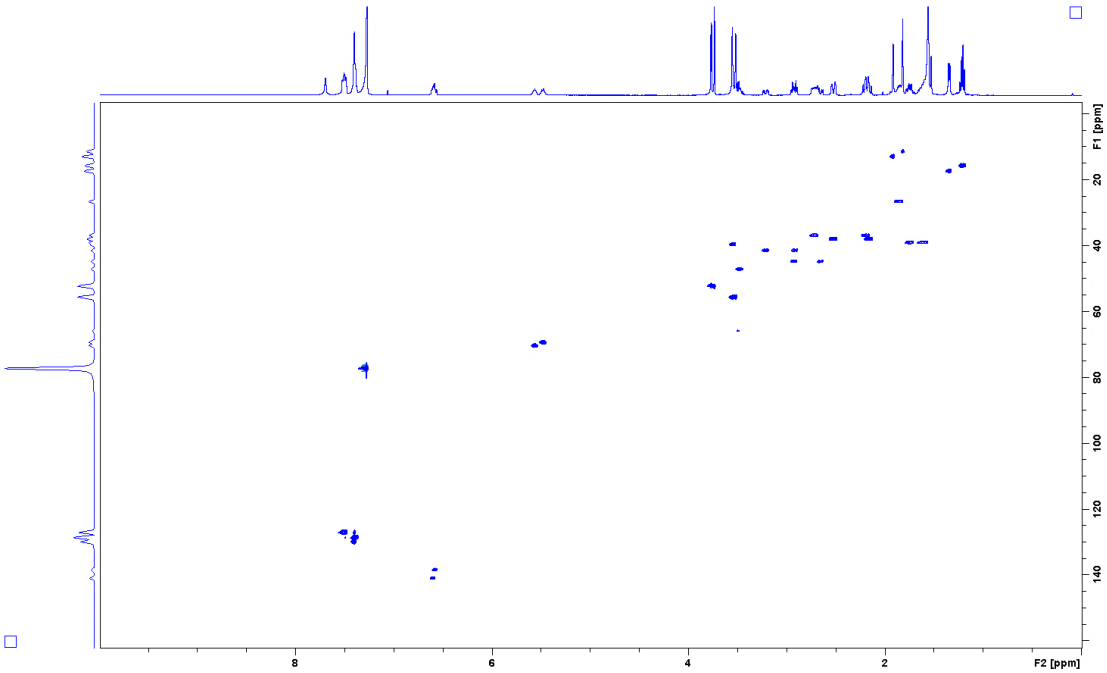
Supplementary Figure 13: HR-ESIMS data of secimide (*R*)-MTPA ester (*m*/*z* 542.1982 $[M+H]^+$, *m*/*z* 559.2247 $[M+NH_4]^+$, *m*/*z* 564.1794 $[M+Na]^+$).



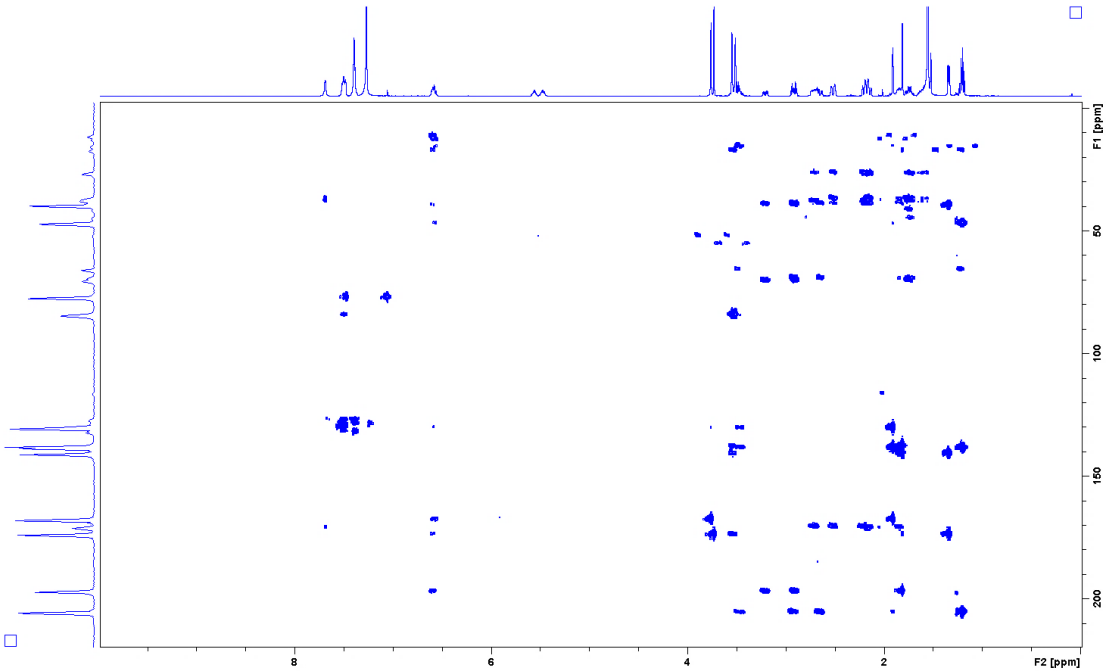
Supplementary Figure 14: ^{1}H NMR spectrum of secimide (*R*)-MTPA ester in $CDCl_3$.



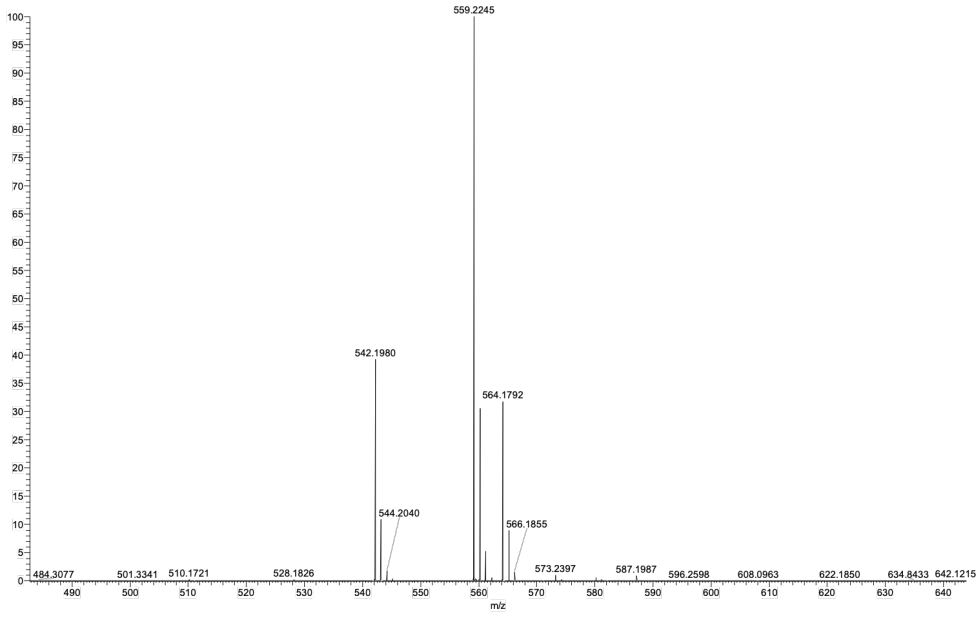
Supplementary Figure 15: COSY spectrum of secimide (*R*)-MTPA ester in $CDCl_3$.



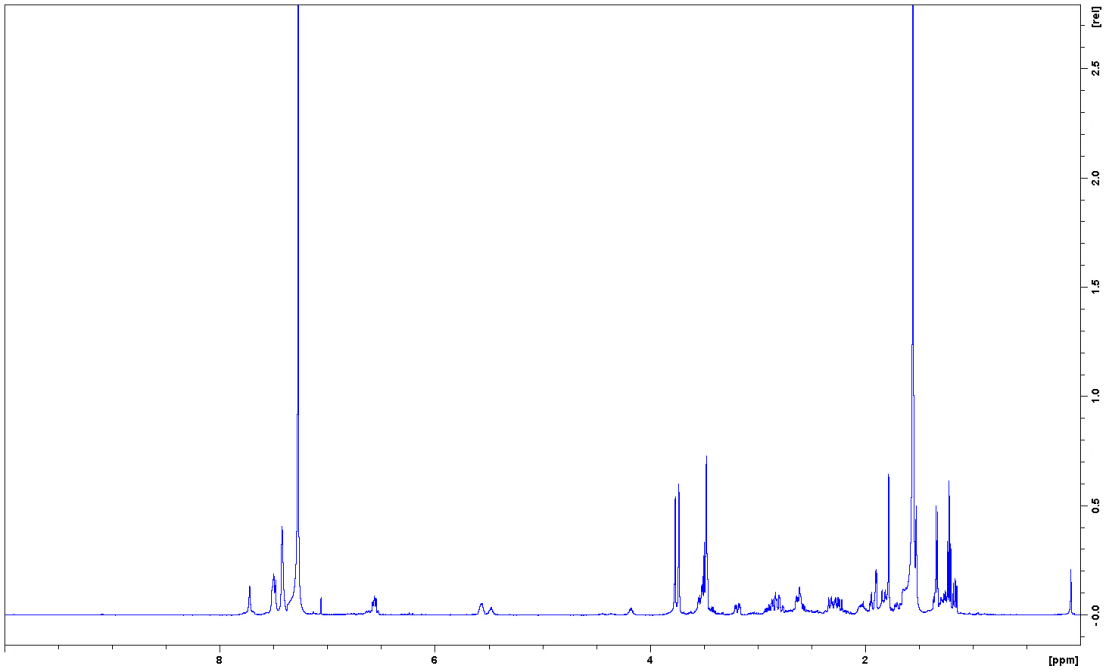
Supplementary Figure 16: HSQC spectrum of secimide (*R*)-MTPA ester in $CDCl_3$.



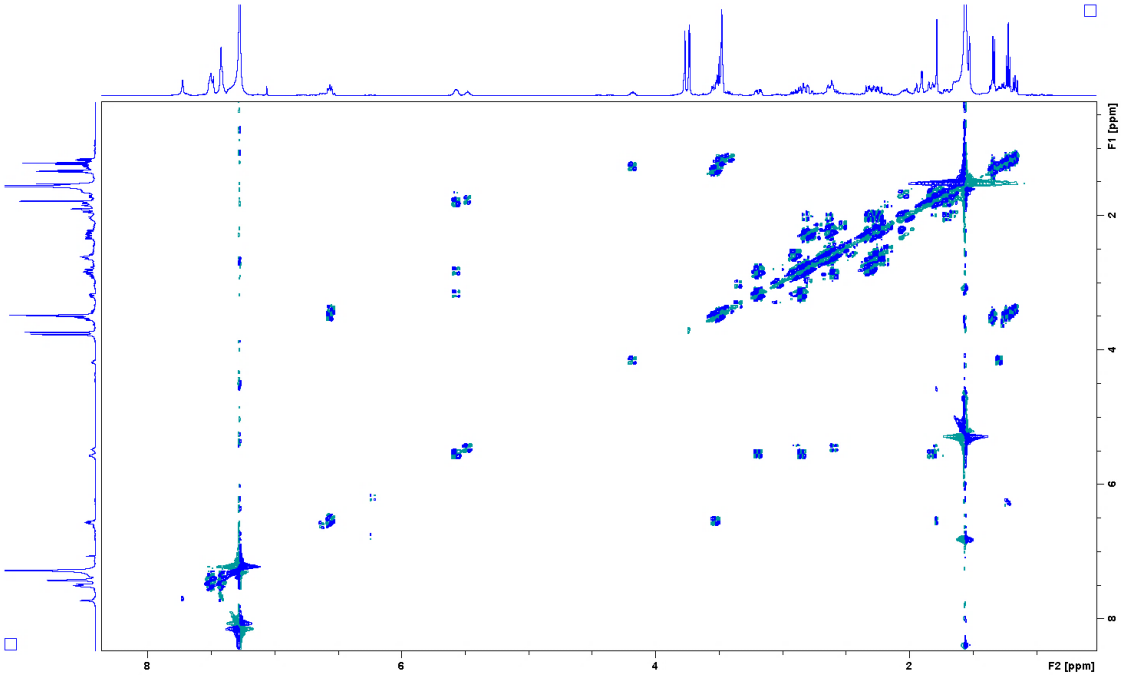
Supplementary Figure 17: HMBC spectrum of secimide (*R*)-MTPA ester in $CDCl_3$.



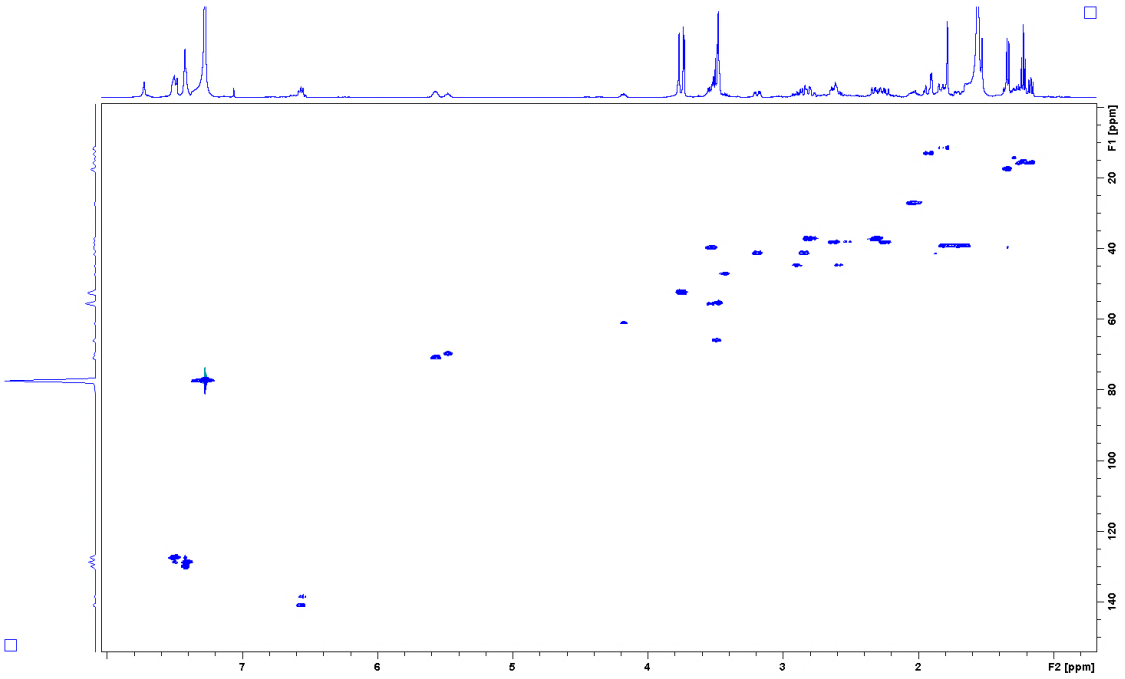
Supplementary Figure 18: HR-ESIMS data of secimide (*S*)-MTPA ester (*m*/*z* 542.1980 $[M+H]^+$, *m*/*z* 559.2245 $[M+NH_4]^+$, *m*/*z* 564.1792 $[M+Na]^+$).



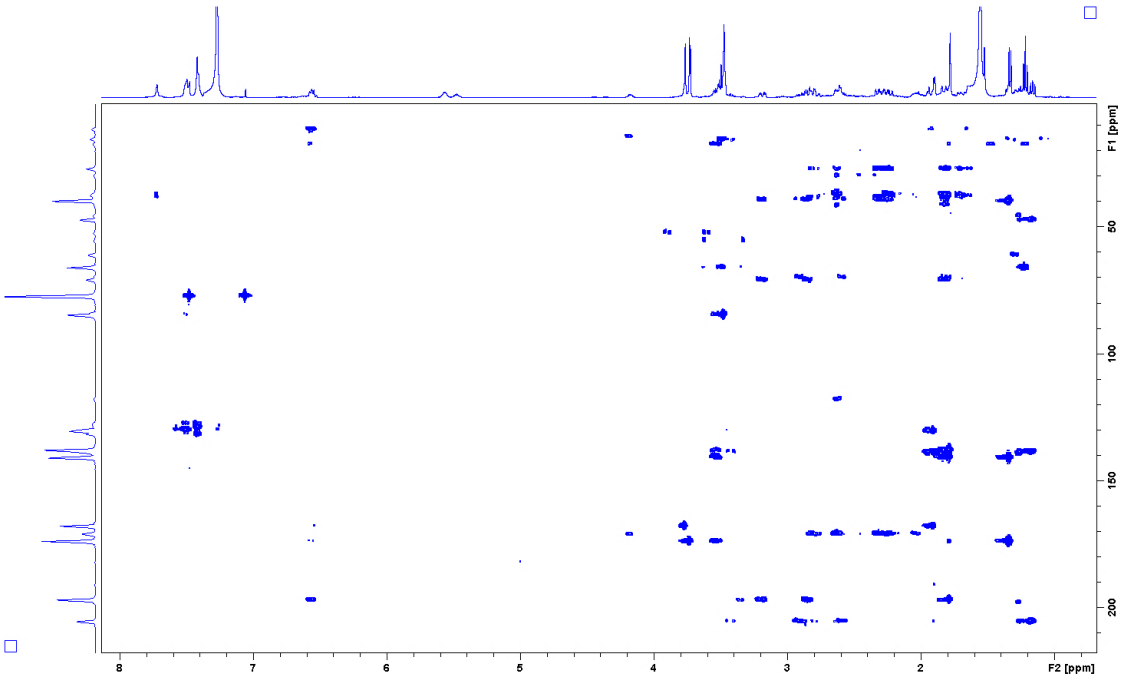
Supplementary Figure 19: ^{1}H NMR spectrum of secimide (*S*)-MTPA ester in $CDCl_3$.



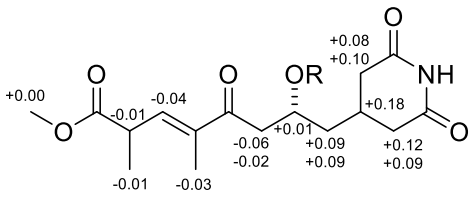
Supplementary Figure 20: COSY spectrum of secimide (*S*)-MTPA ester in $CDCl_3$.



Supplementary Figure 21: HSQC spectrum of secimide (*S*)-MTPA ester in $CDCl_3$.

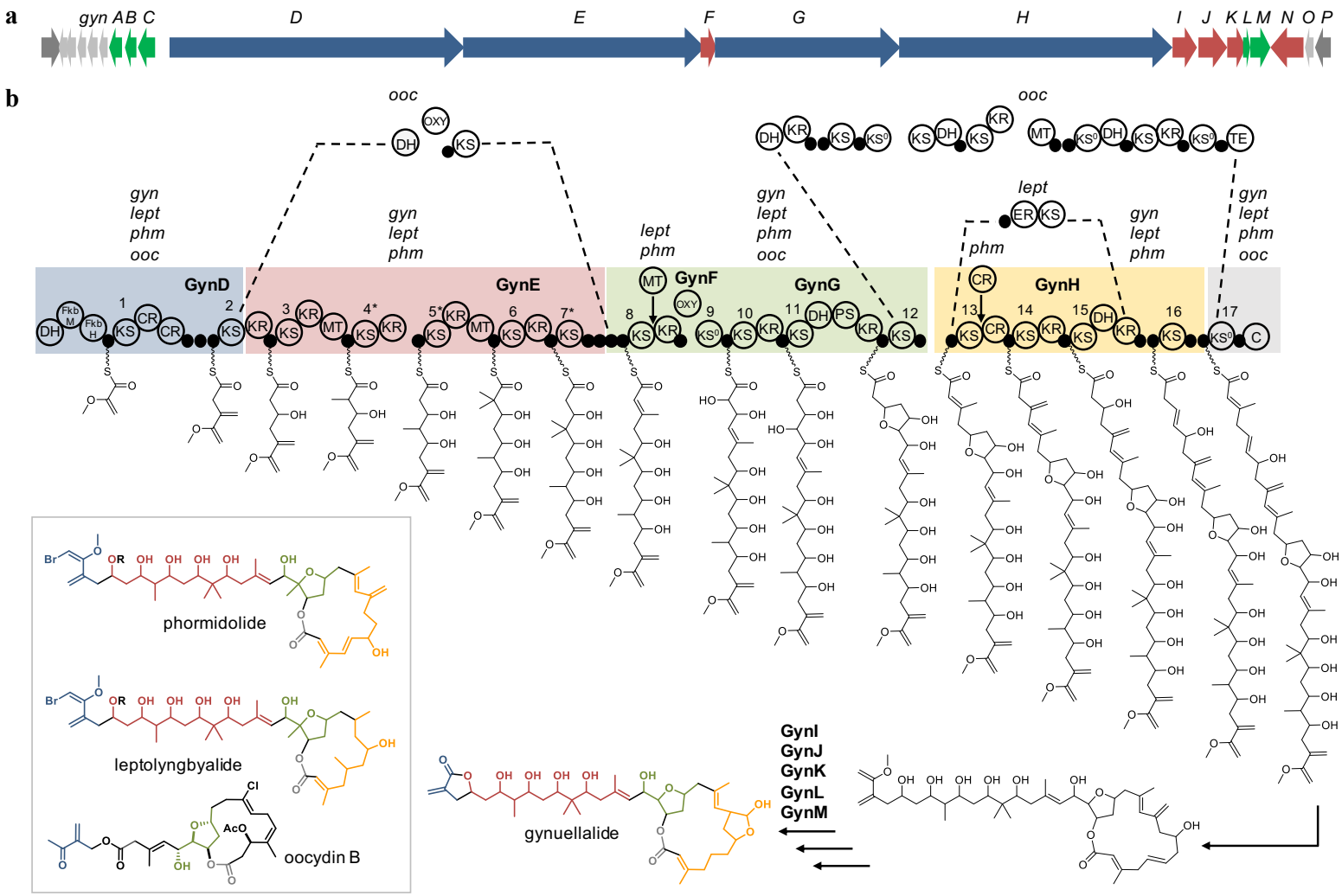


Supplementary Figure 22: HMBC spectrum of secimide (*S*)-MTPA ester in $CDCl_3$.

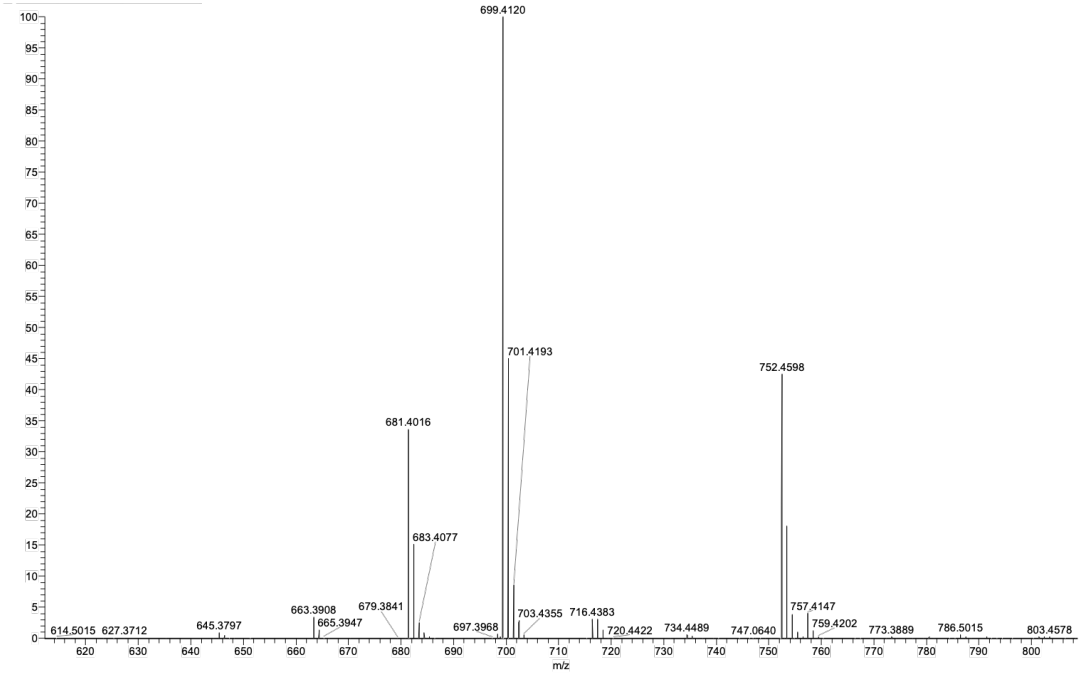


R = MTPA

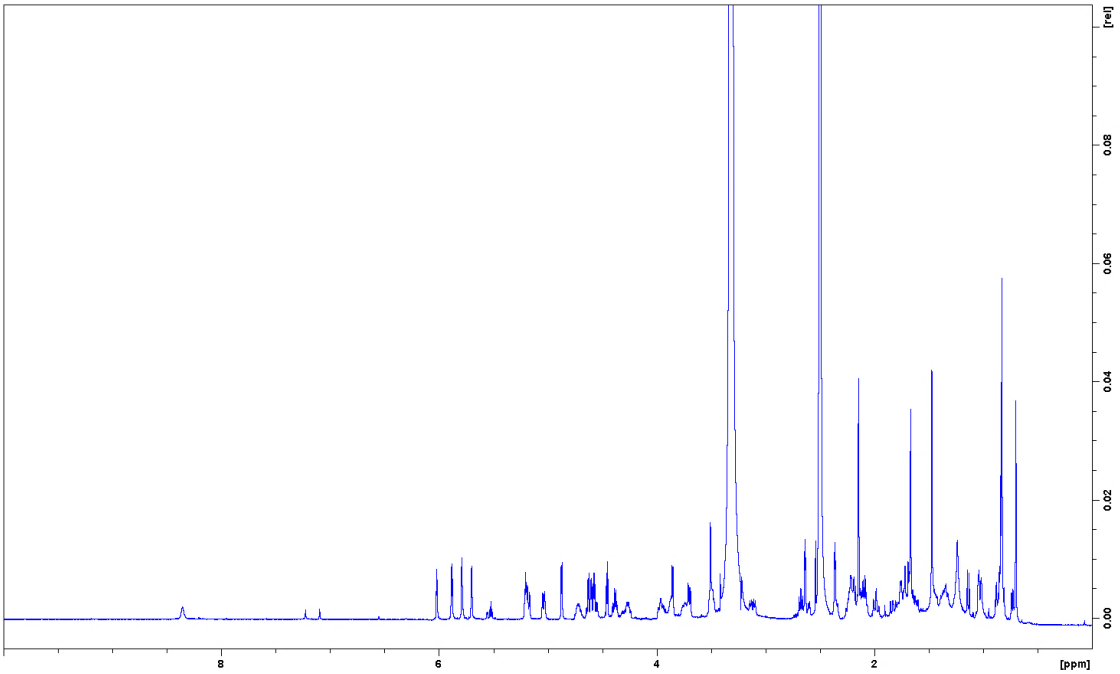
Supplementary Figure 23: δ_{S-R} values of secimide MTPA esters



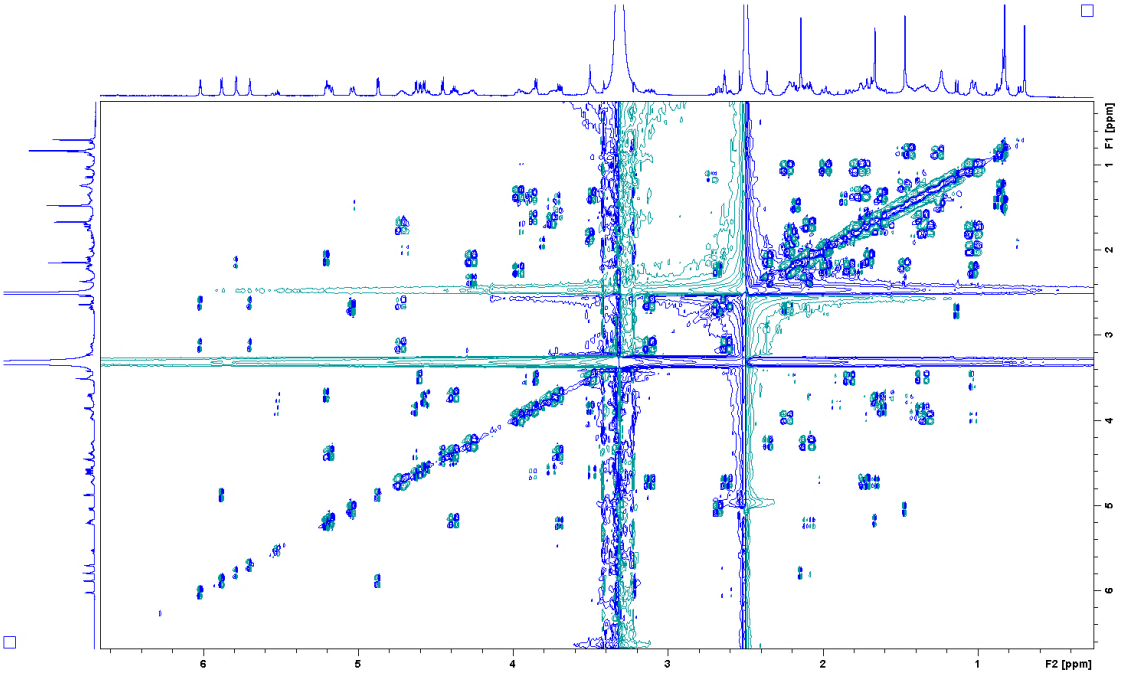
Supplementary Figure 24: Gynuellalide biosynthetic model. (**a**) **The** *gyn* BGC. blue: PKS core biosynthetic genes, light grey: hypothetical genes, light blue: regulatory gene, dark grey: regulatory genes, red: tailoring genes, green: β-branching cassette. (**b**) The gynuellalide PKS and biosynthetic model for gynuellalide biosynthesis. The *gyn* proteins are shown with bold labels, other labels refer to genes encoding the core PKSs of the related phormidolide (*phm*), leptolyngbyalide (*lept*), and oocydin (*ooc*) pathways. Proteins with multiple labels have orthologs in the corresponding pathways. Colors were used to highlight substructures of the associated polyketides. R: fatty acyl-chain found in phormidolides and leptolyngbyalides.



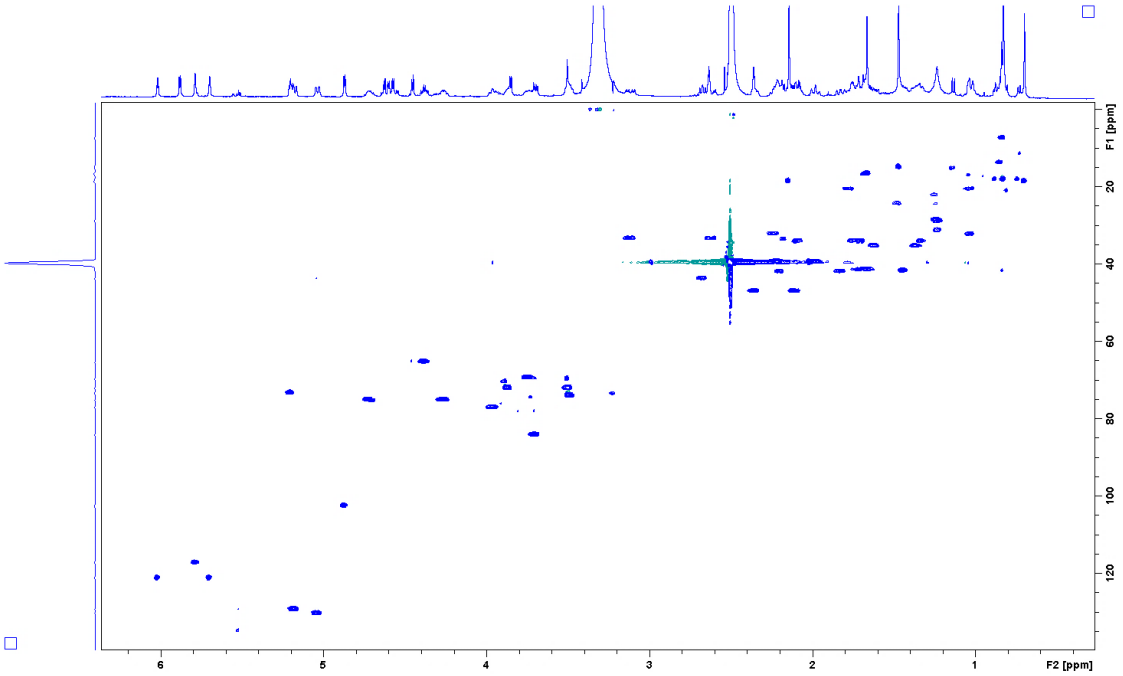
Supplementary Figure 25: HR-ESI-MS spectrum of gynuellalide (*m*/*z* 757.4147 $[M+Na]^+$, *m*/*z* 752.4598 $[M+NH_4]^+$, *m*/*z* 699.4120 $[M+H-H_2O]^+$, *m*/*z* 681.4016 $[M+H-2H_2O]^+$).



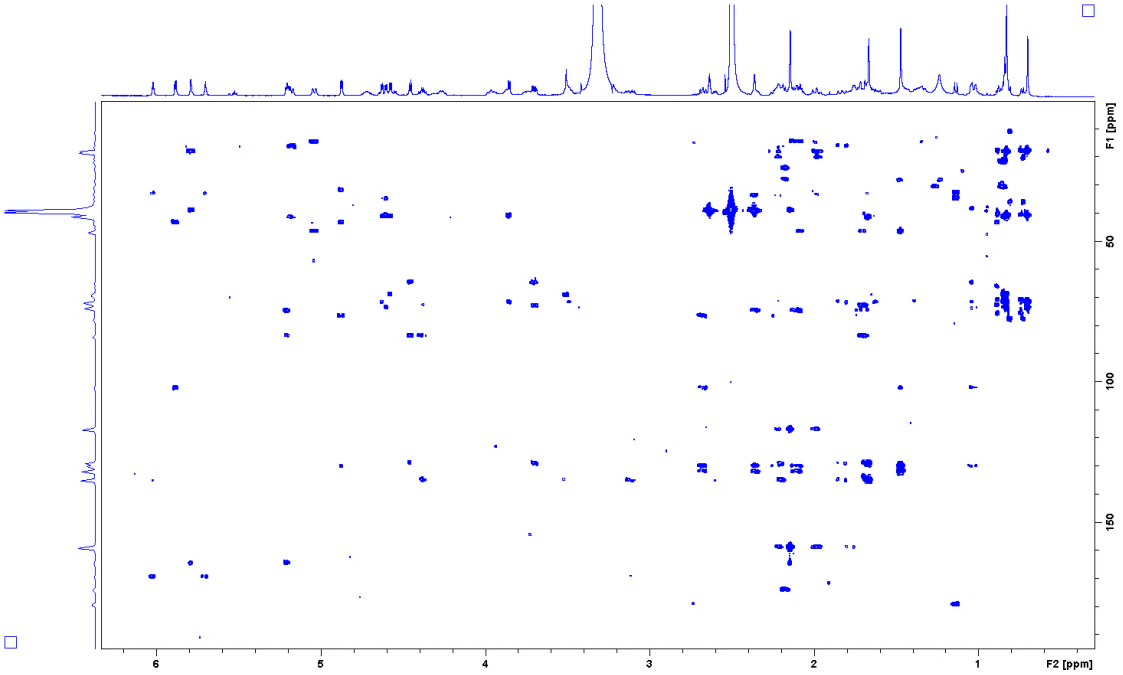
Supplementary Figure 26: ^{1}H NMR spectrum of gynuellalide in DMSO-*d*$_6$.



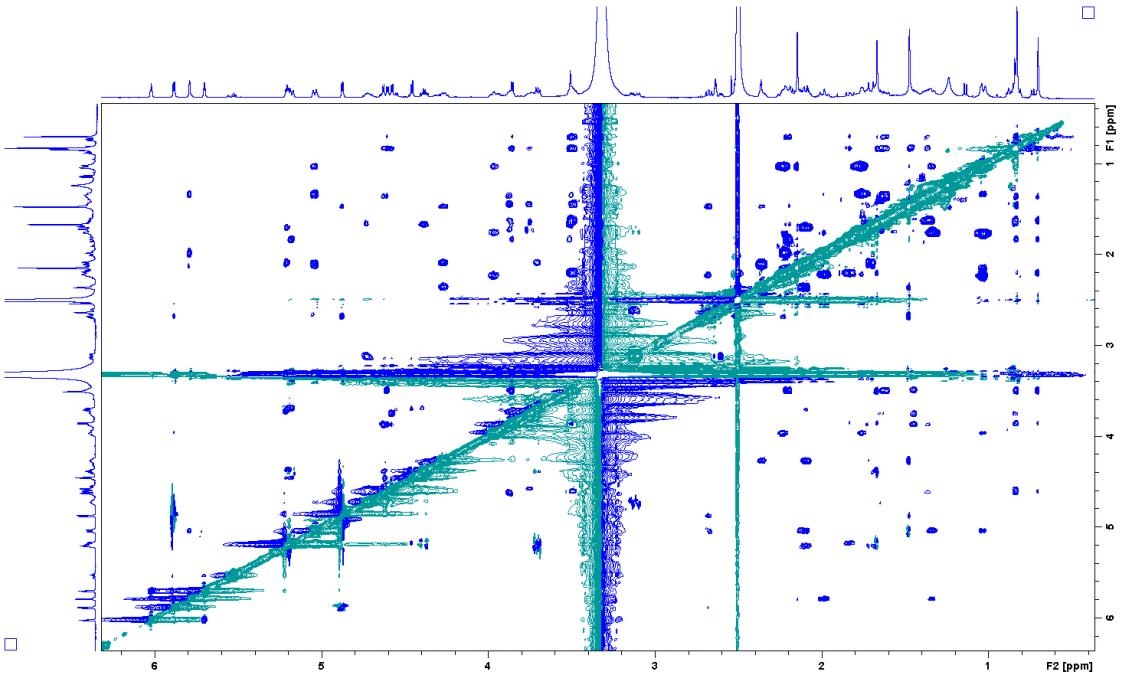
Supplementary Figure 27: COSY spectrum of gynuellalide in DMSO-d_6.



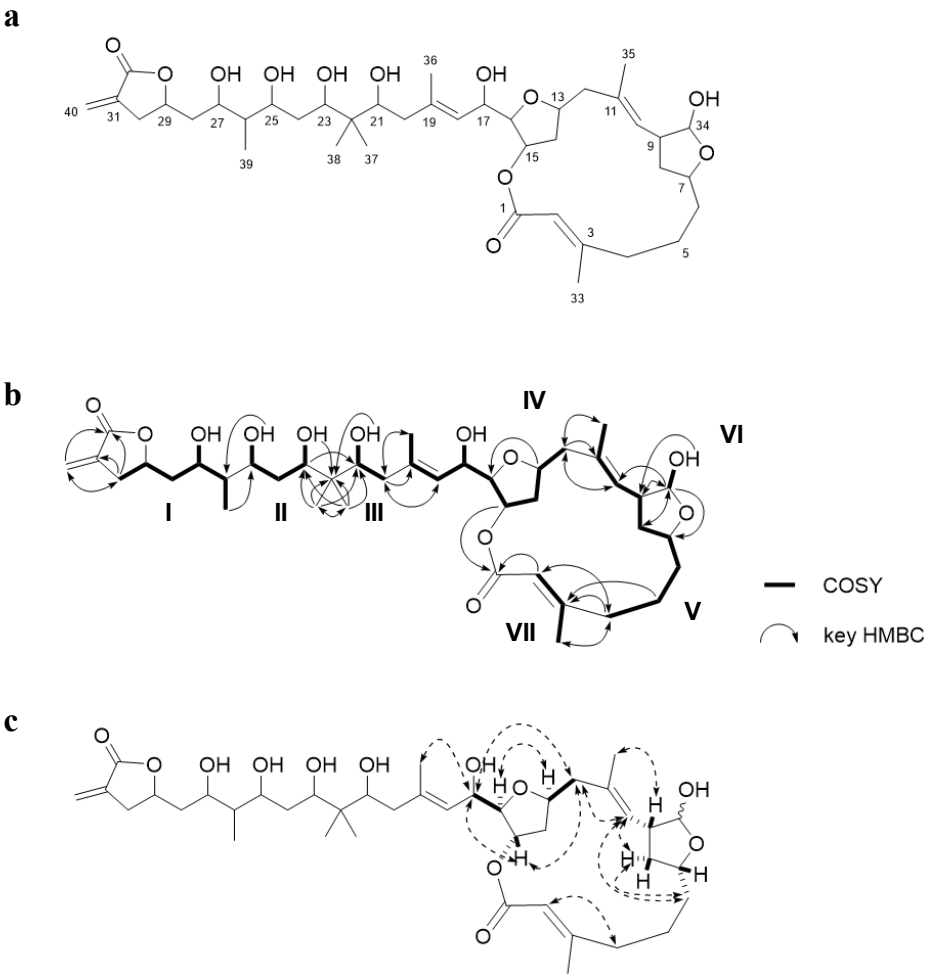
Supplementary Figure 28: HSQC spectrum of gynuellalide in DMSO-d_6.



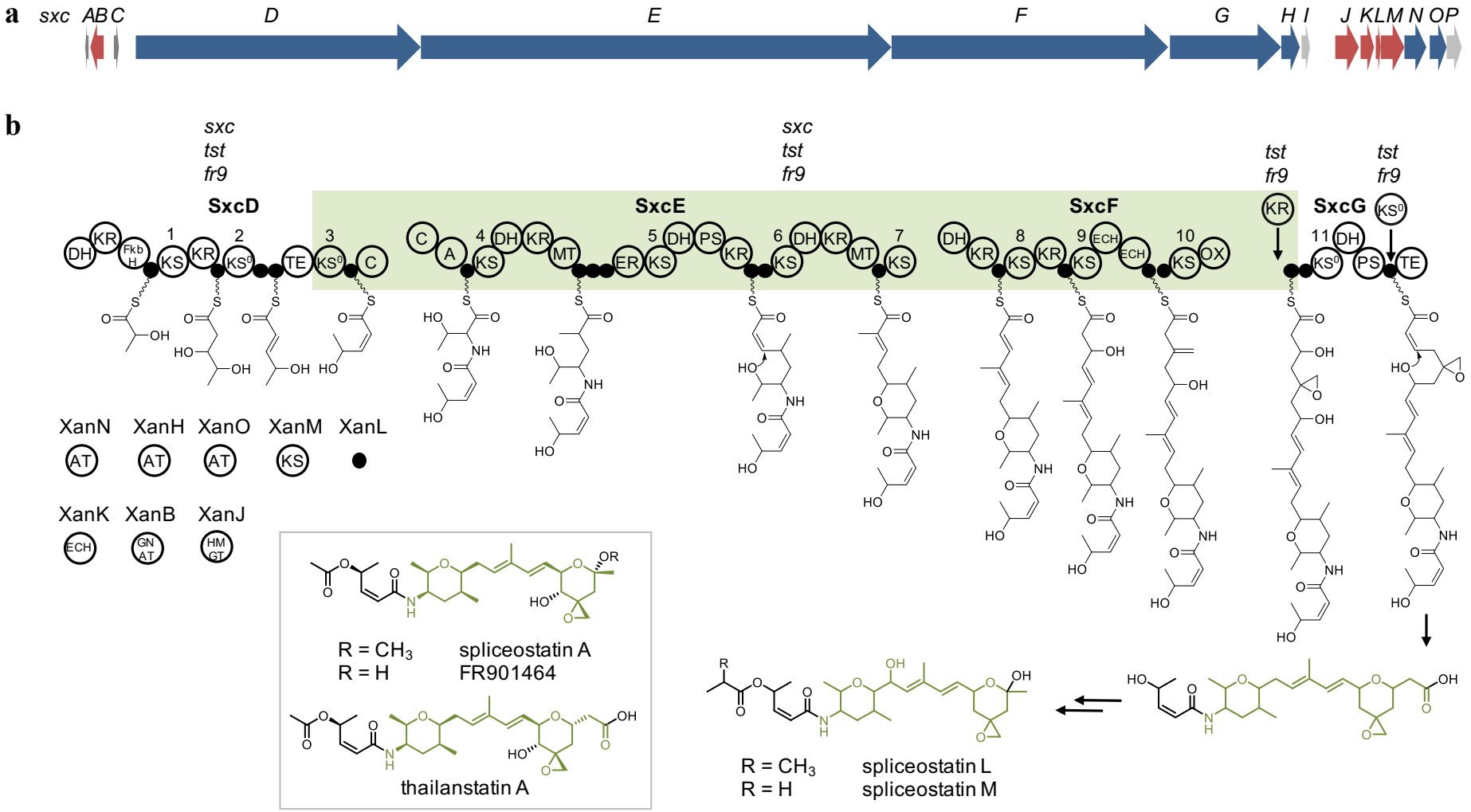
Supplementary Figure 29: HMBC spectrum of gynuellalide in DMSO-d_6.



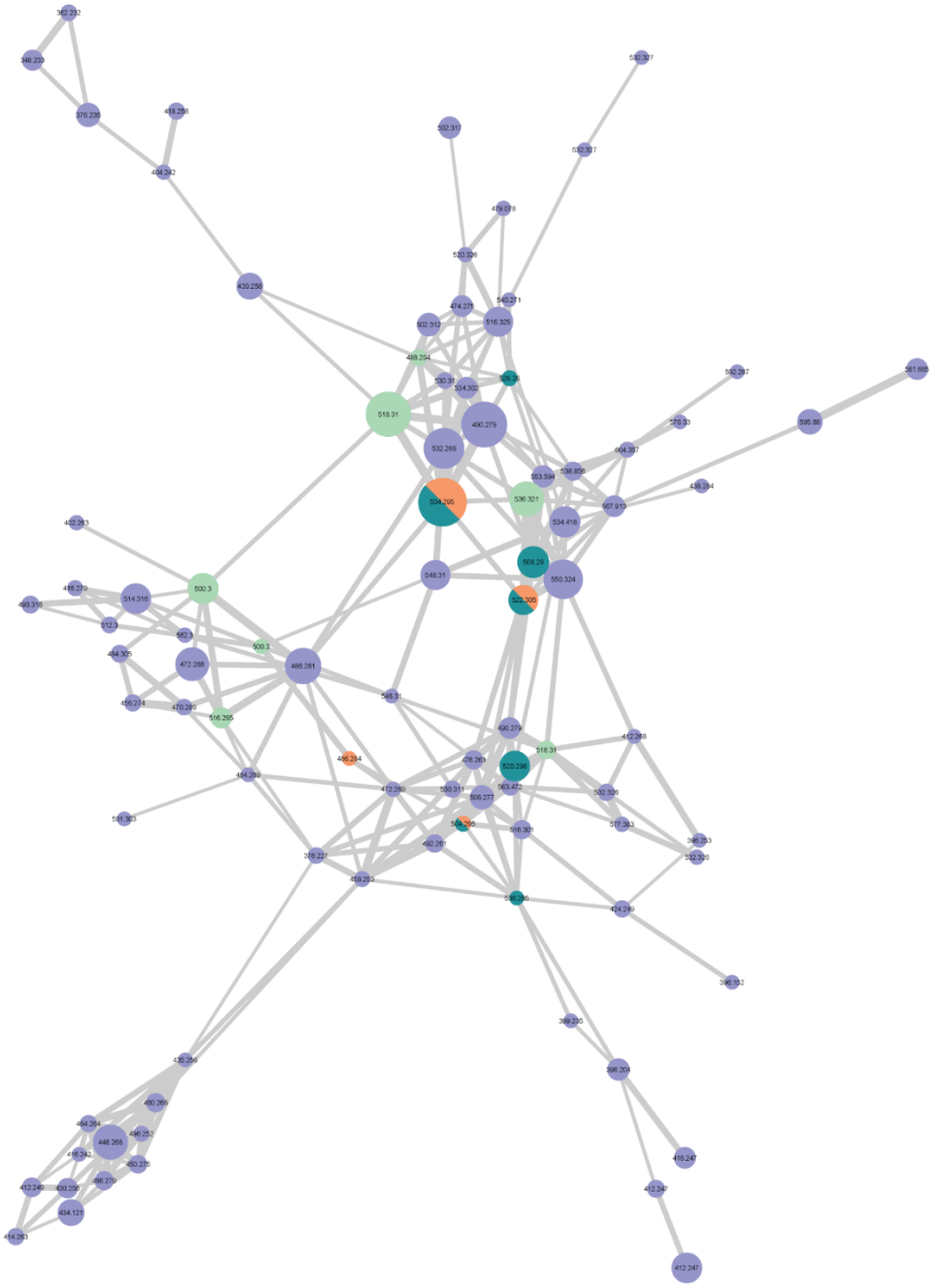
Supplementary Figure 30: ROESY spectrum of gynuellalide in DMSO-d_6.



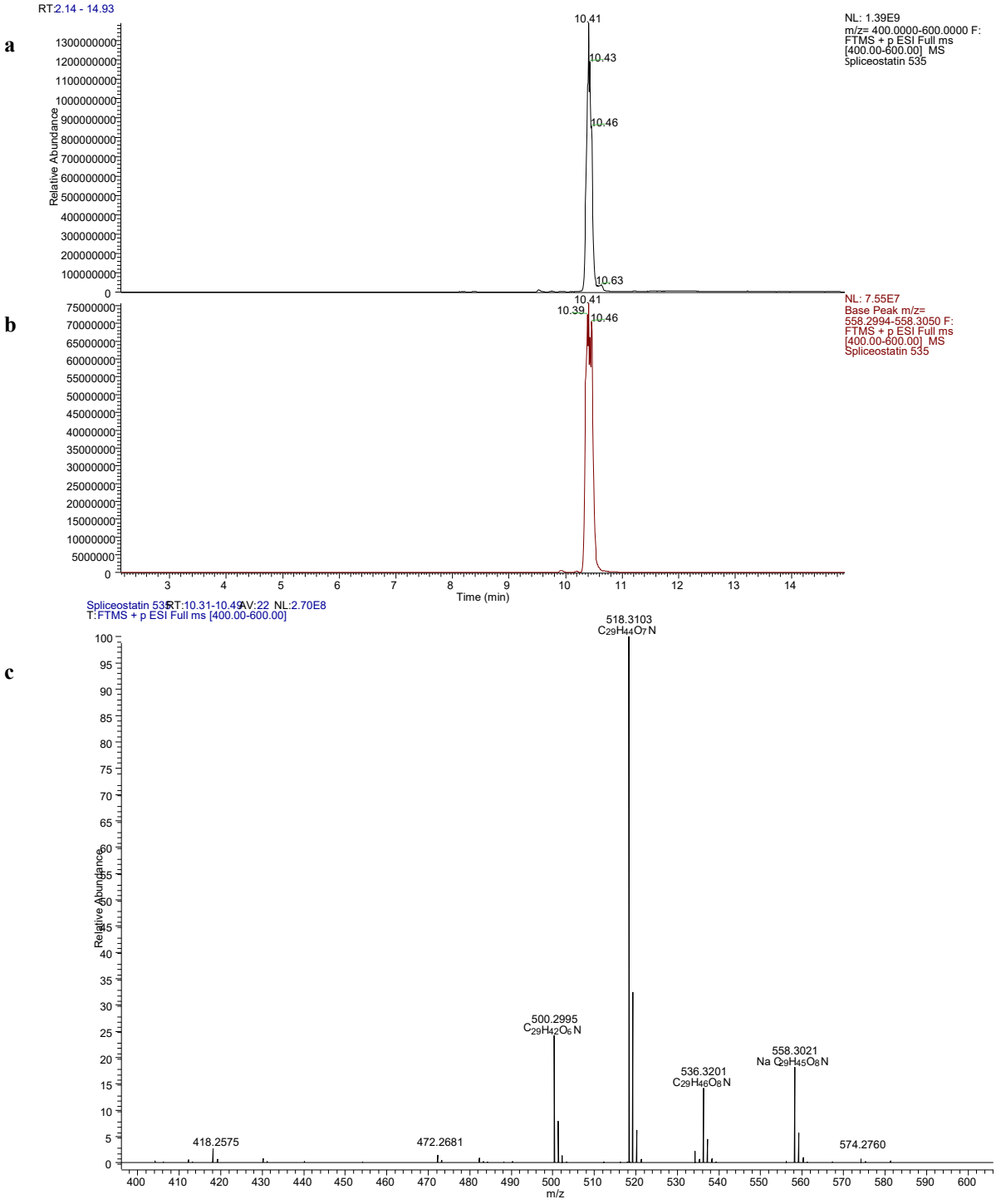
Supplementary Figure 31: Structure elucidation of gynuellalide. (**a**) Structure of gynuellalide. (**b**) COSY and key HMBC correlations of gynuellalide. (**c**) Key NOESY correlations of gynuellalide.



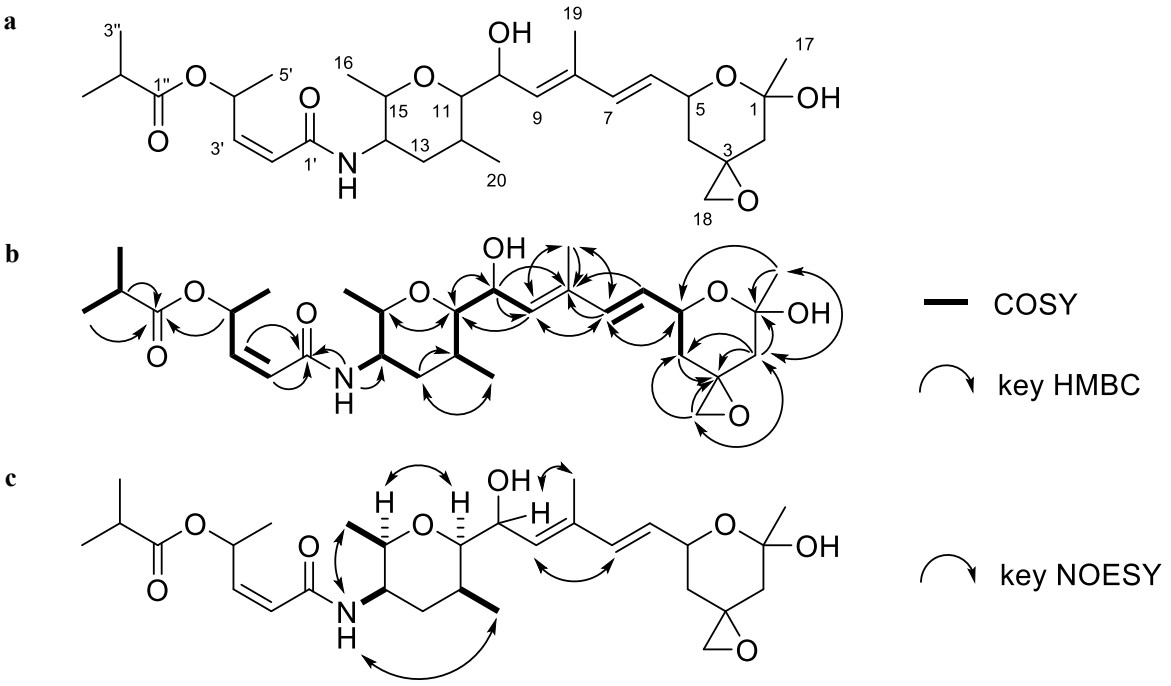
Supplementary Figure 32: Spliceostatin biosynthetic model. (**a**) The spliceostatin BGC. Blue: core PKS genes, red: β-branching cassette, grey: other genes. (**b**) The spliceostatin PKS and model for spliceostatin biosynthesis. The spliceostatin PKS proteins of *X. cannabis* (*sxc*) are shown with bold labels, other labels refer to genes encoding the core PKS of the related thailanstatin (*tst*) and FR901464 (fr9) pathways. Substructures associated with the biosynthesis of the PKS fragment detected by *trans*PACT are shown in green.



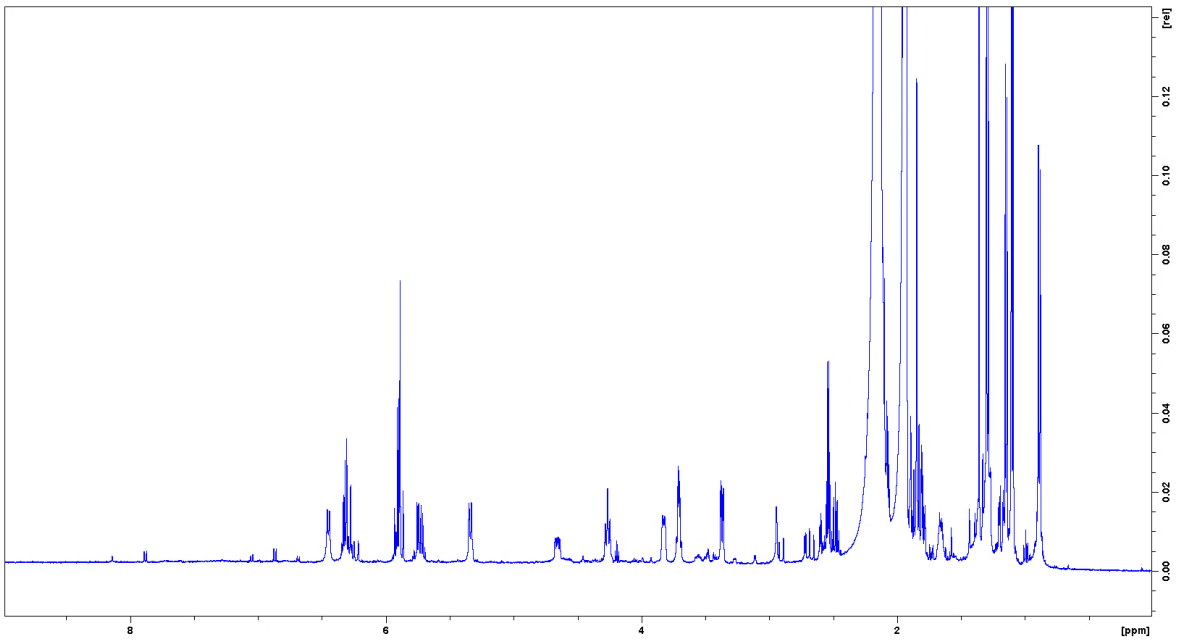
Supplementary Figure 33: Mass-spectral molecular network analysis of extracts from *X. cannabis*. Node size correlates with the number of MS^2 scans for a given ion and can be used as a proxy for the relative abundance. The edge line width indicates the relatedness of two significantly pairwise aligned spectra (cosine 0.65 or higher). Ions corresponding to the isolated compound spliceostatin L (**20**) are colored orange, those that fit adducts of spliceostatin M (**21**) light green. Masses that resemble adducts (proton or sodium) of compounds reported in the literature are colored turquois.



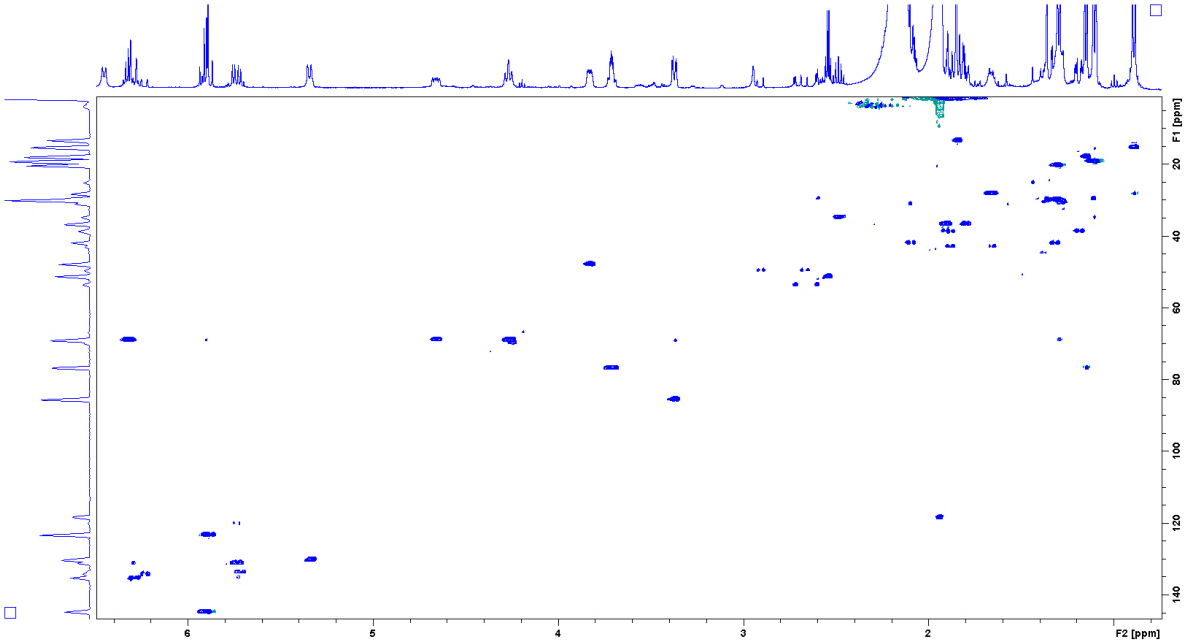
Supplementary Figure 34: UHPLC-HRMS data of purified spliceostatin L (20) from *Xanthomonas cannab*is. (**a**) Total ion chromatogram; (**b**) Extracted ion chromatogram (m/z 558.2994-558.3050); (**c**) Mass spectrum of the peak at 10.41 min (*m/z* 558.3021 $[M+Na]^+$, *m/z* 536.3201 $[M+H]^+$, *m/z* 518.3103 $[M+H-H_2O]^+$, *m/z* 500.2995 $[M+H-2H_2O]^+$).



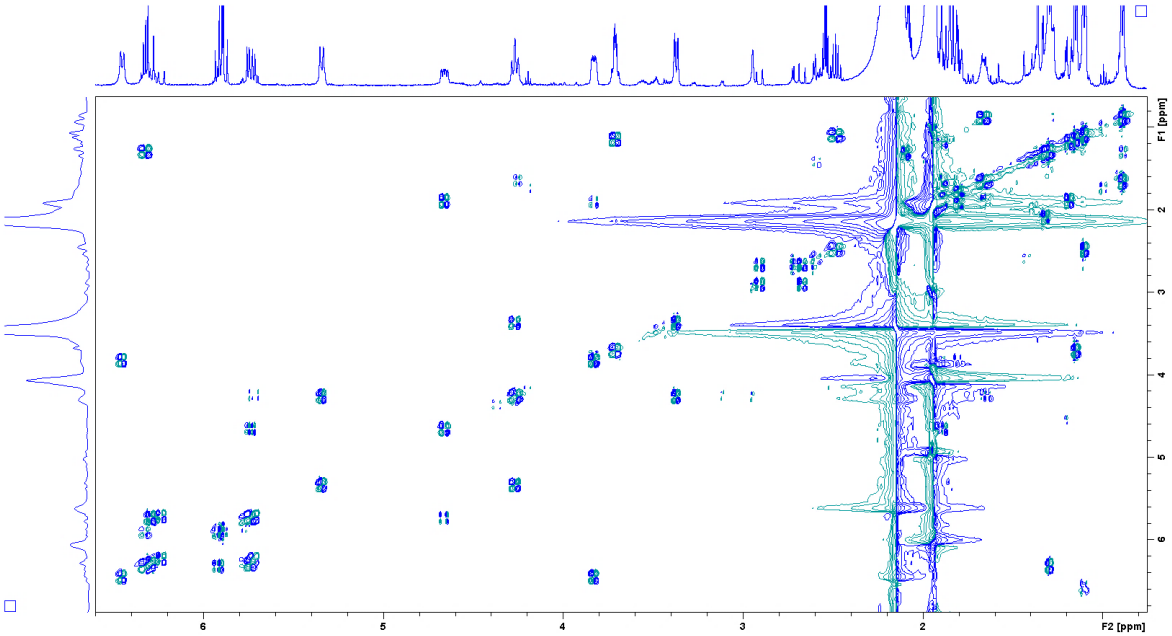
Supplementary Figure 35: Structure elucidation of spliceostatin L (20). (**a**) atom labeling according to Supplementary Table 7, (**b**) Key HMBC and COSY correlations of **20**, (**c**) key NOESY correlations of **20**.



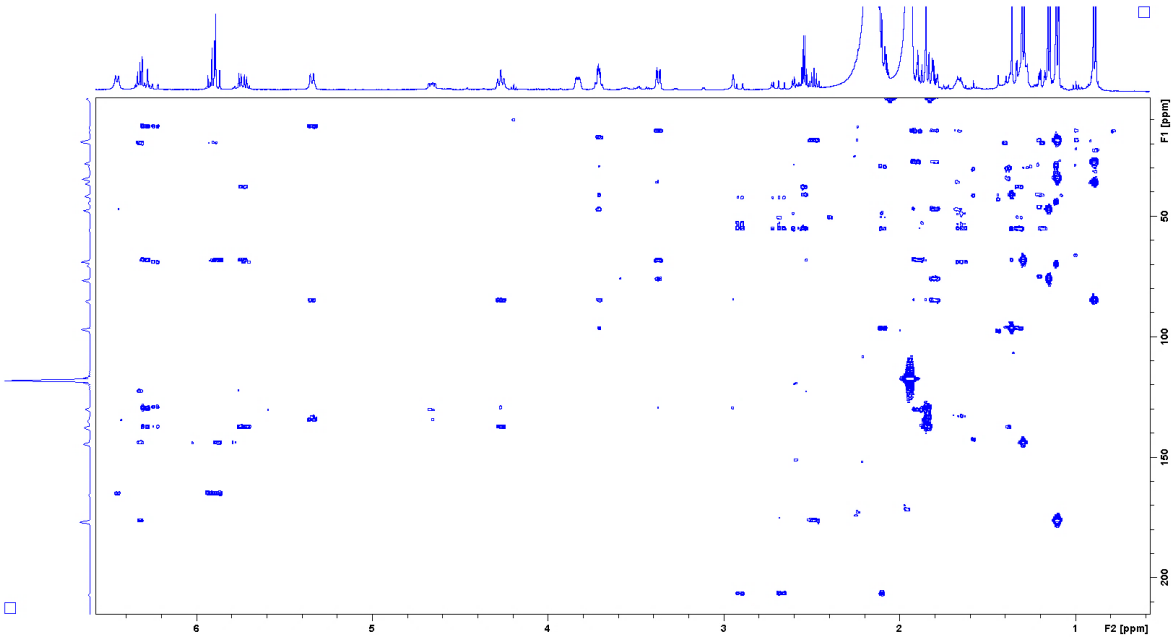
Supplementary Figure 36: ^{1}H NMR spectrum of spliceostatin L (20) from *Xanthomonas cannabis* in acetonitrile-d_3.



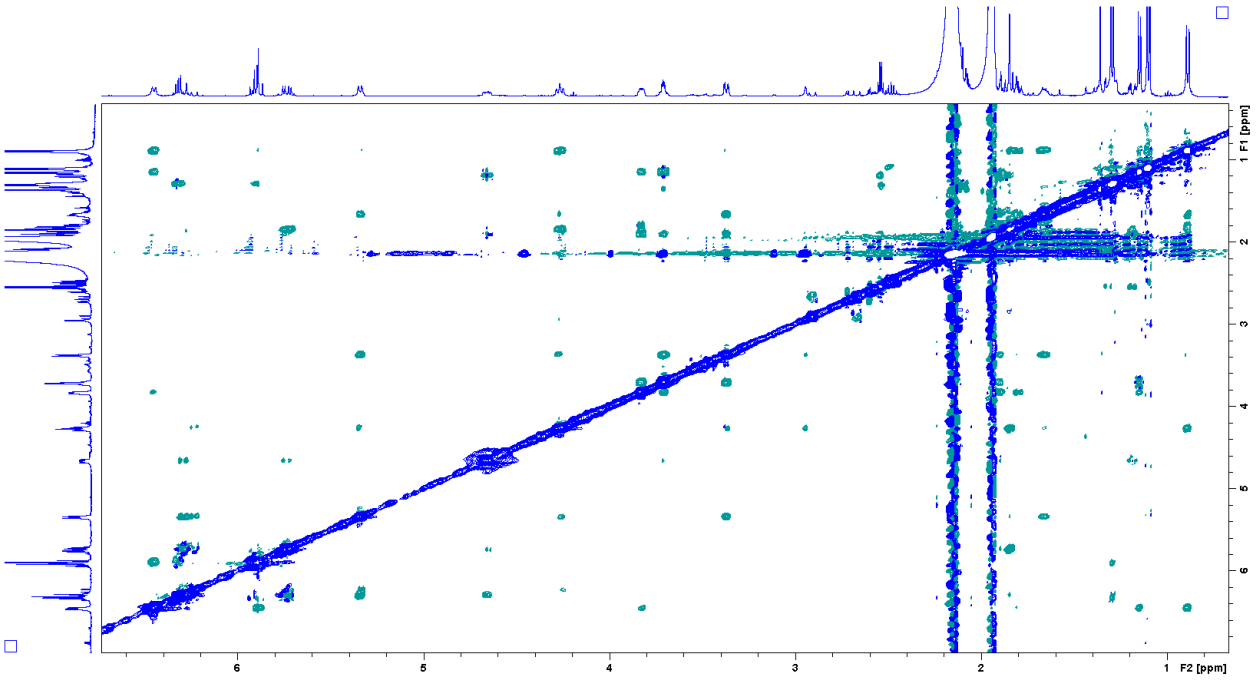
Supplementary Figure 37: HSQC spectrum of spliceostatin L (20) from *Xanthomonas cannabis* in acetonitrile-d_3.



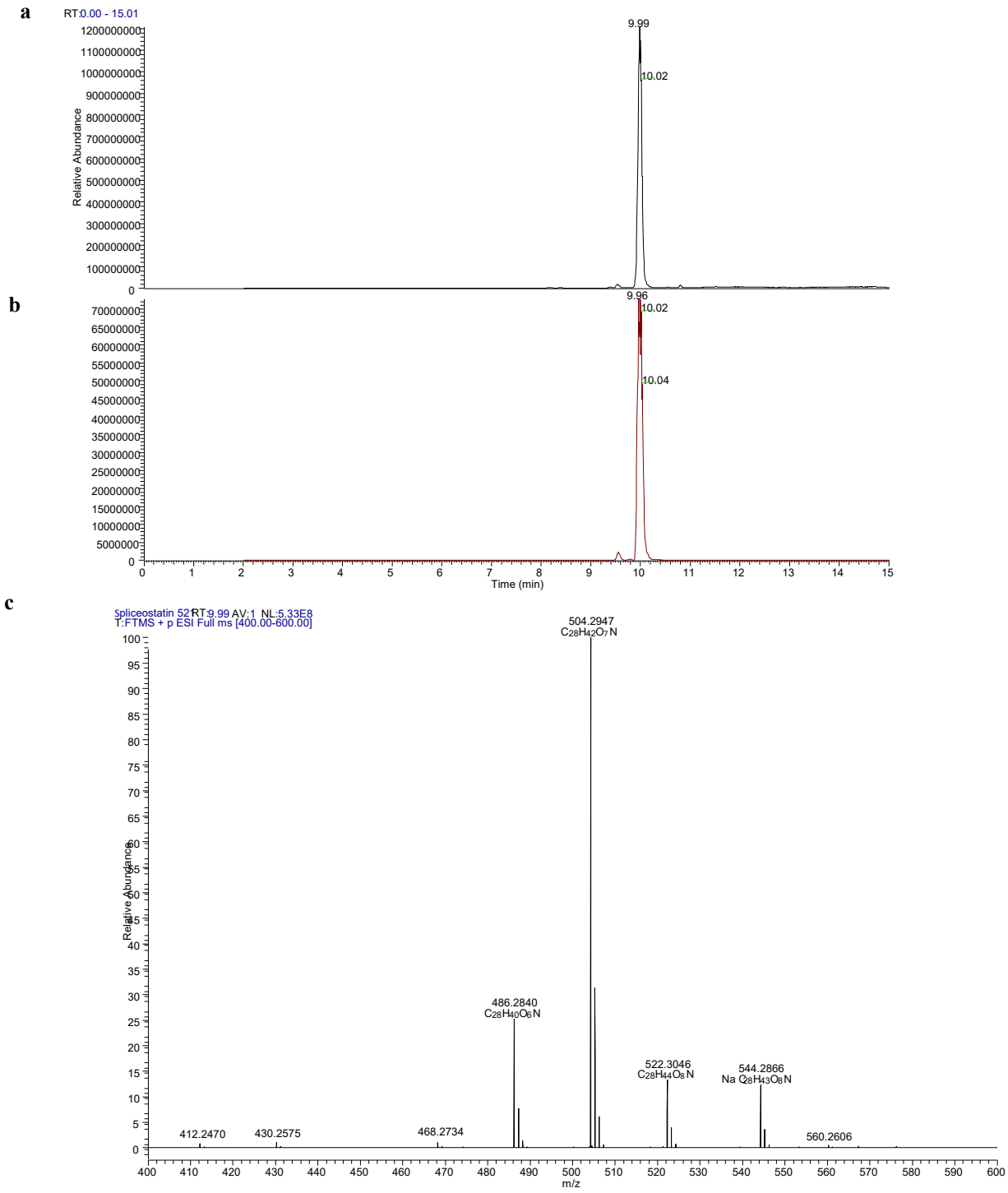
Supplementary Figure 38: COSY spectrum of spliceostatin L (20) from *Xanthomonas cannabis* in acetonitrile-d_3.



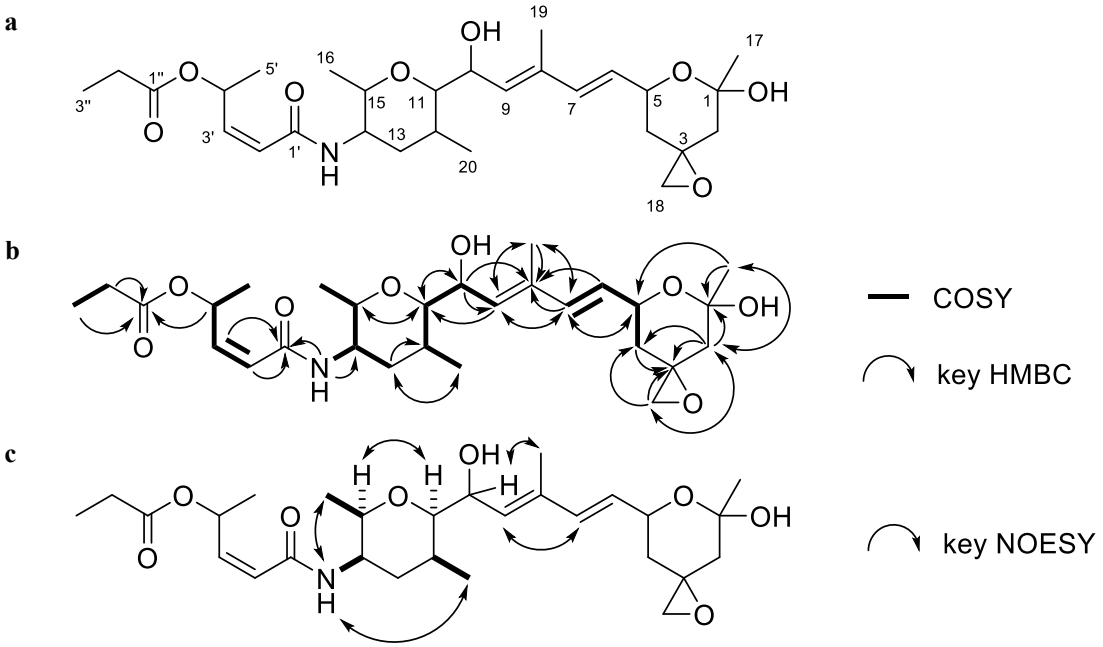
Supplementary Figure 39: HMBC spectrum of spliceostatin L (20) from *Xanthomonas cannabis* in acetonitrile-d_3.



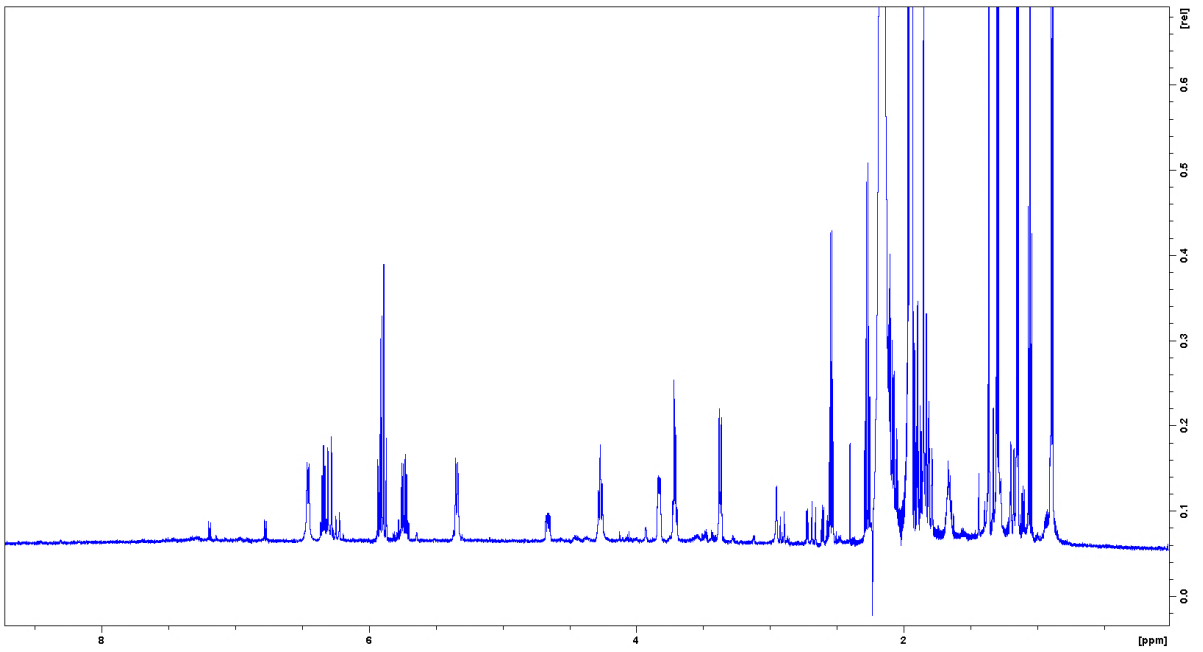
Supplementary Figure 40: NOESY spectrum of spliceostatin L (20) from *X. cannabis* in acetonitrile-d_3.



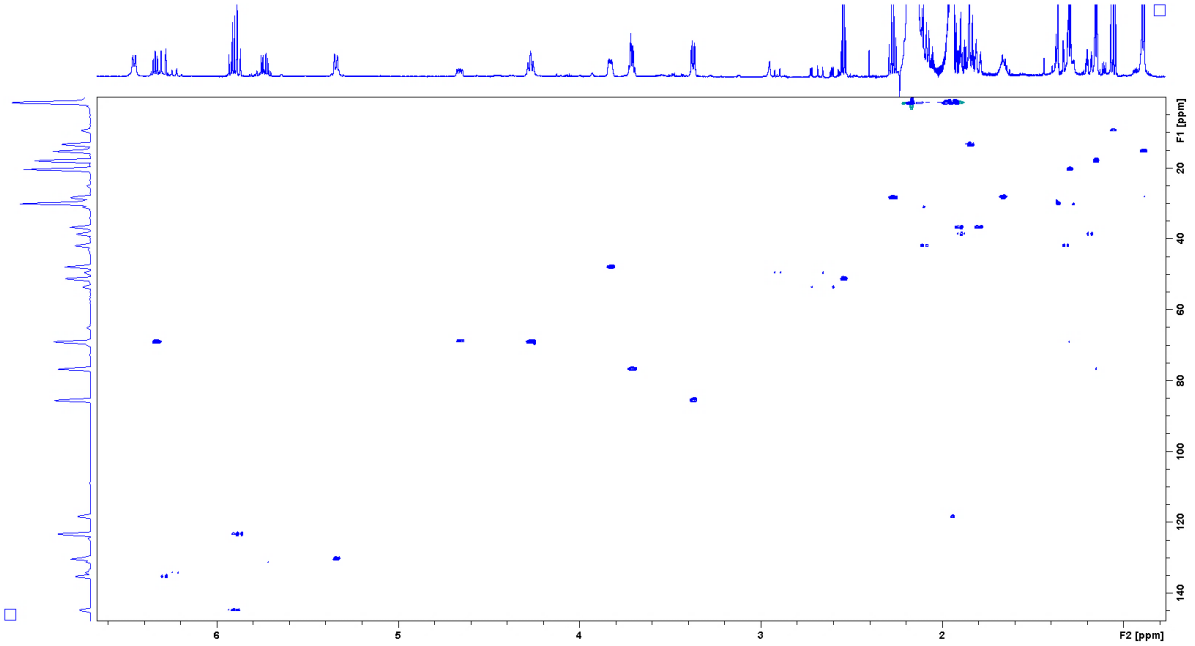
Supplementary Figure 41: UHPLC-HRMS data of purified spliceostatin M (21). (**a**) Total ion chromatogram; (**b**) Extracted ion chromatogram (*m/z* 544.2838-544.2892); (**c**) Mass spectrum of the peak at 9.99 min (*m/z* 544.2866 $[M+Na]^+$, *m/z* 522.3046 $[M+H]^+$, *m/z* 504.2947 $[M+H-H_2O]^+$, *m/z* 486.2840 $[M+H-2H_2O]^+$).



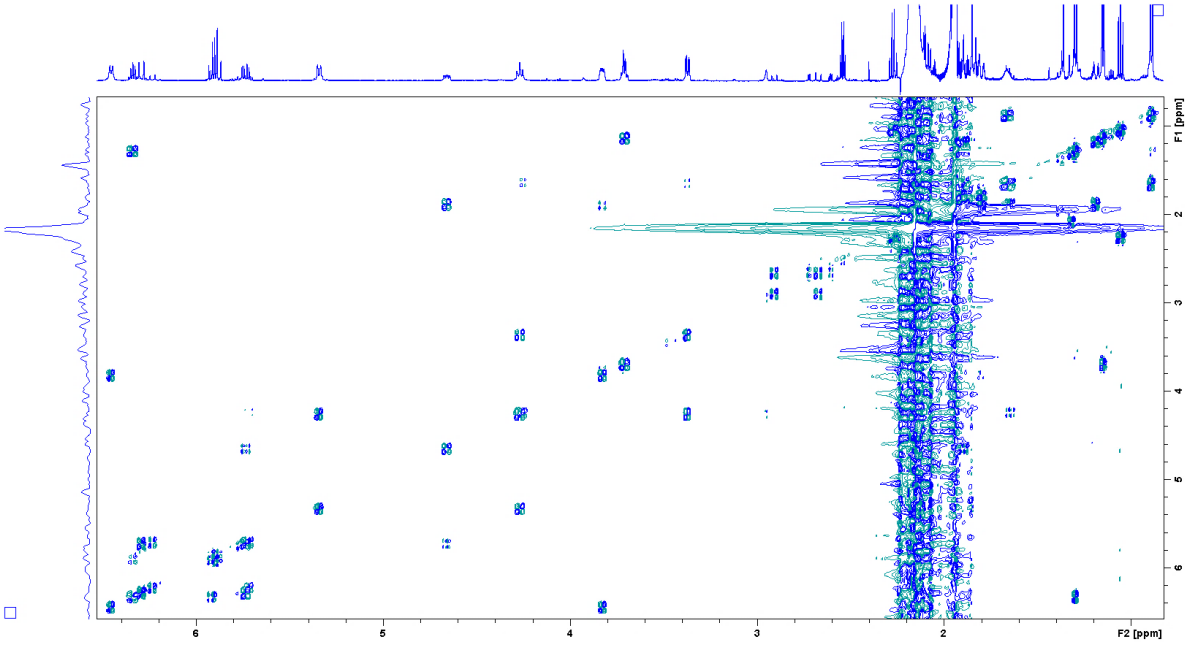
Supplementary Figure 42: Structure elucidation of spliceostatin M (21). (**a**) Atom labeling according to Supplementary Table 8, (**b**) HMBC and COSY key correlations of **21**, (**c**) NOESY key correlations of **21**.



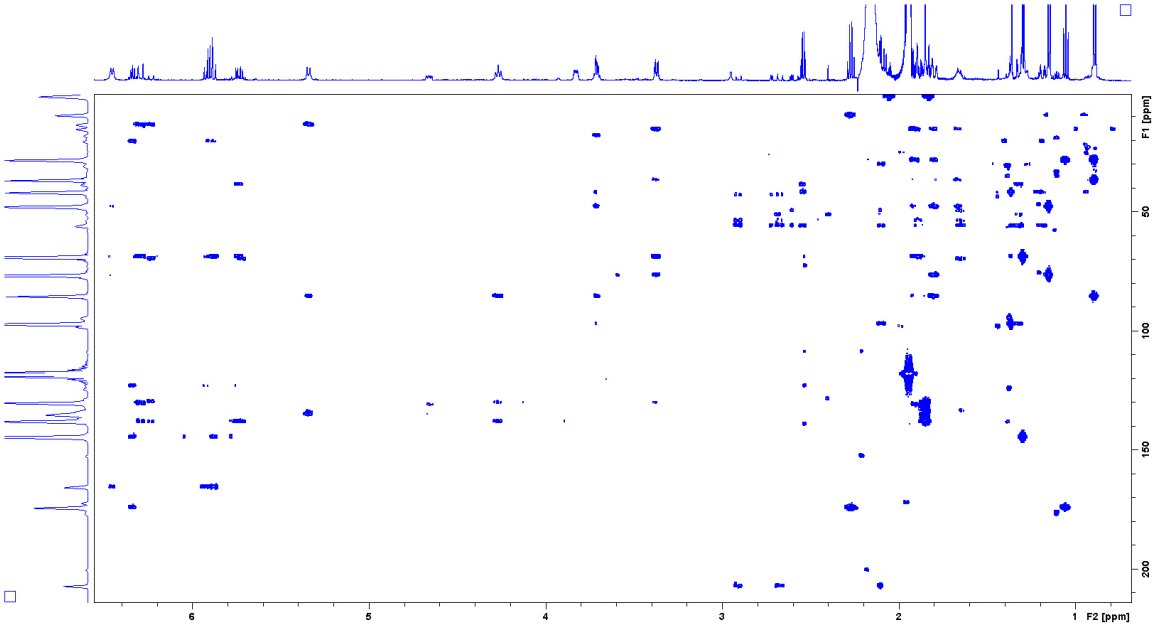
Supplementary Figure 43: ^{1}H NMR spectrum of spliceostatin M (21) from *Xanthomonas cannabis* in acetonitrile-d_3.



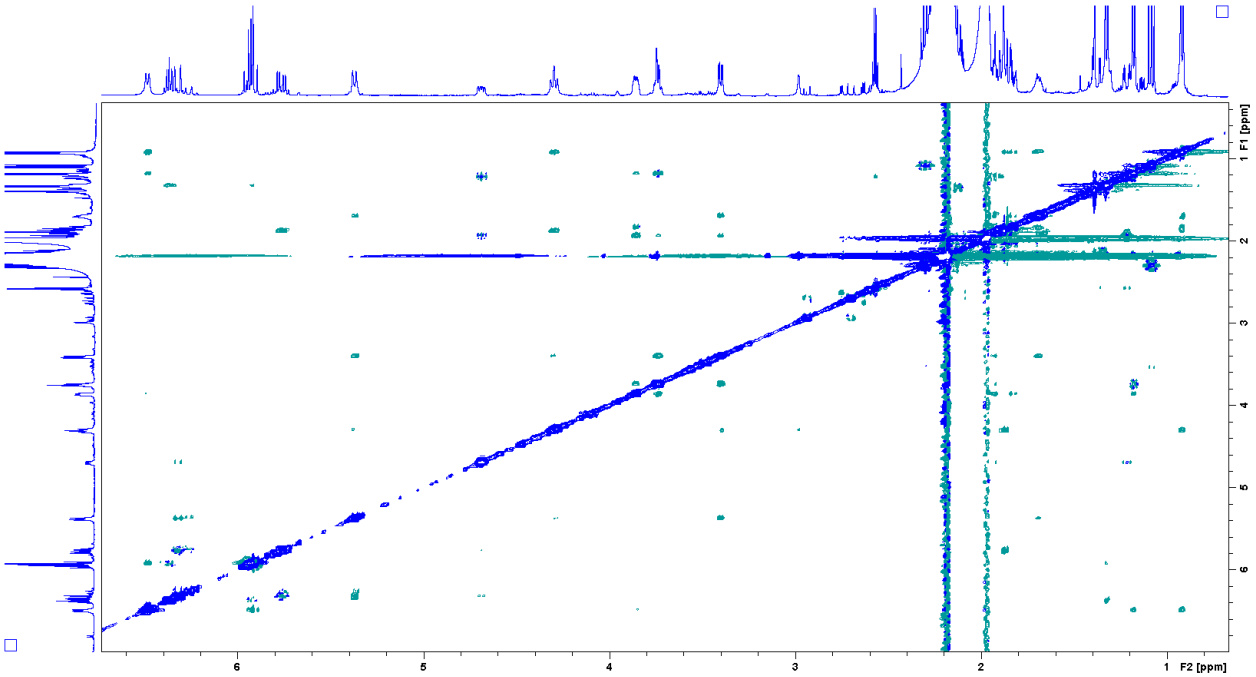
Supplementary Figure 44: HSQC spectrum of spliceostatin M (21) from *Xanthomonas cannabis* in acetonitrile-d_3.



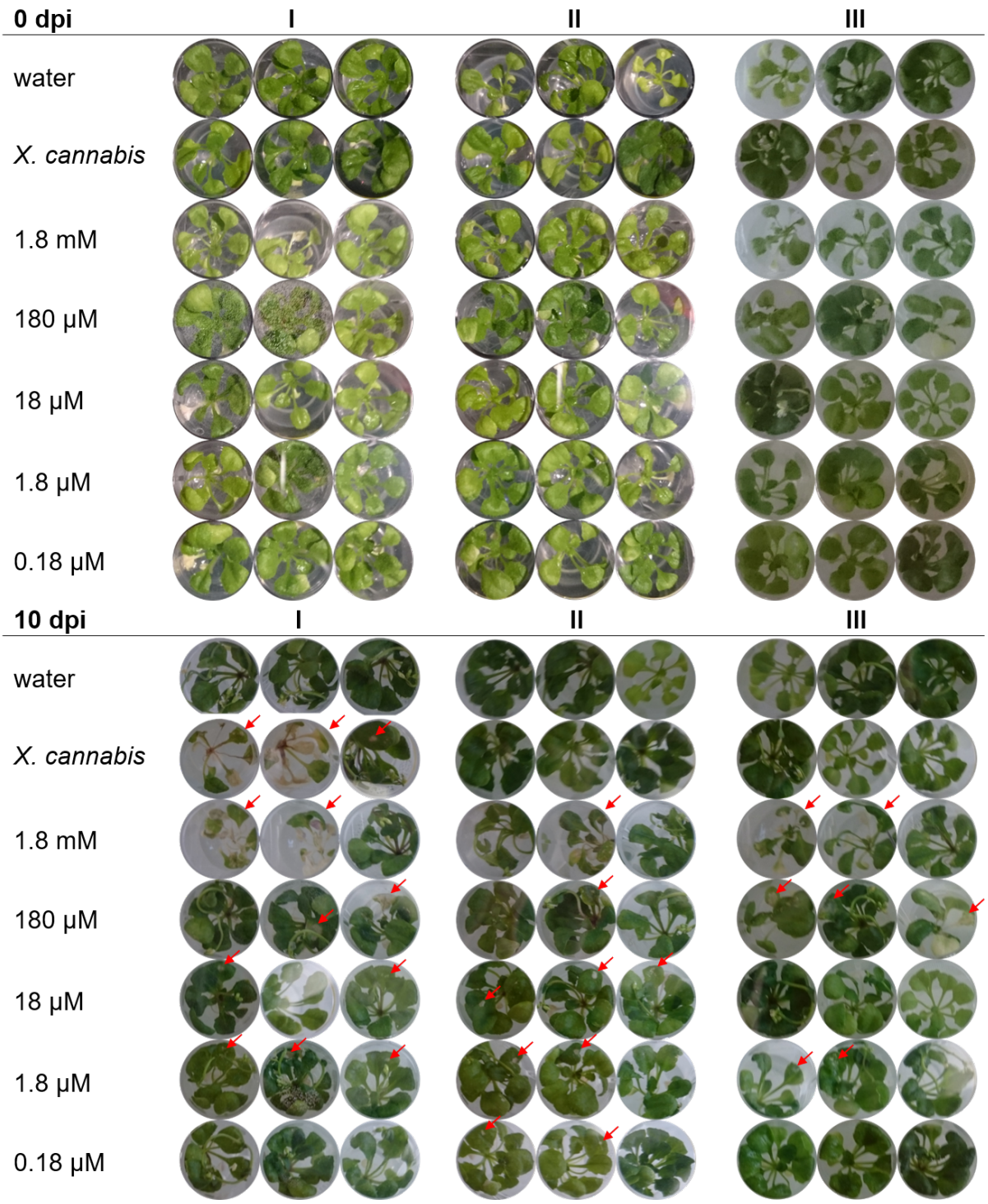
Supplementary Figure 45: COSY spectrum of spliceostatin M (21) from *Xanthomonas cannabis* in acetonitrile-d_3.



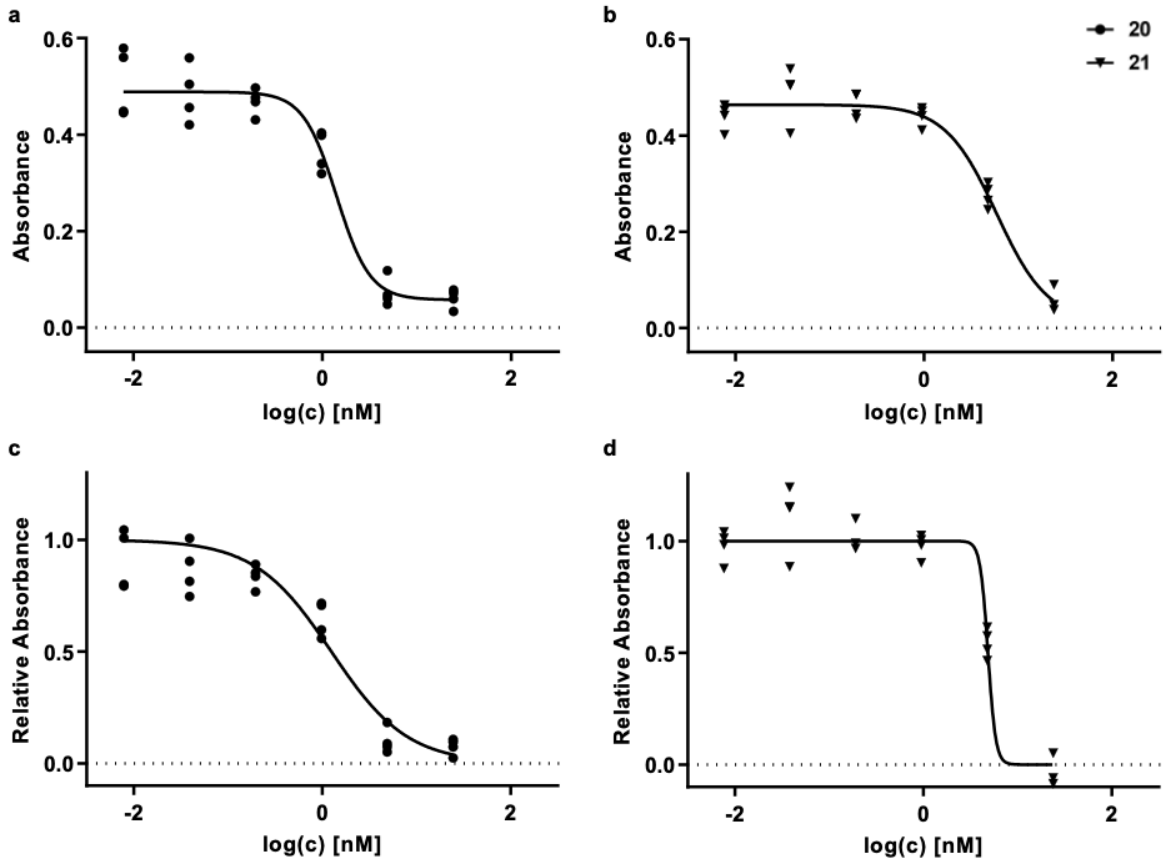
Supplementary Figure 46: HMBC spectrum of spliceostatin M (21) from *Xanthomonas cannabis* in acetonitrile-d_3.



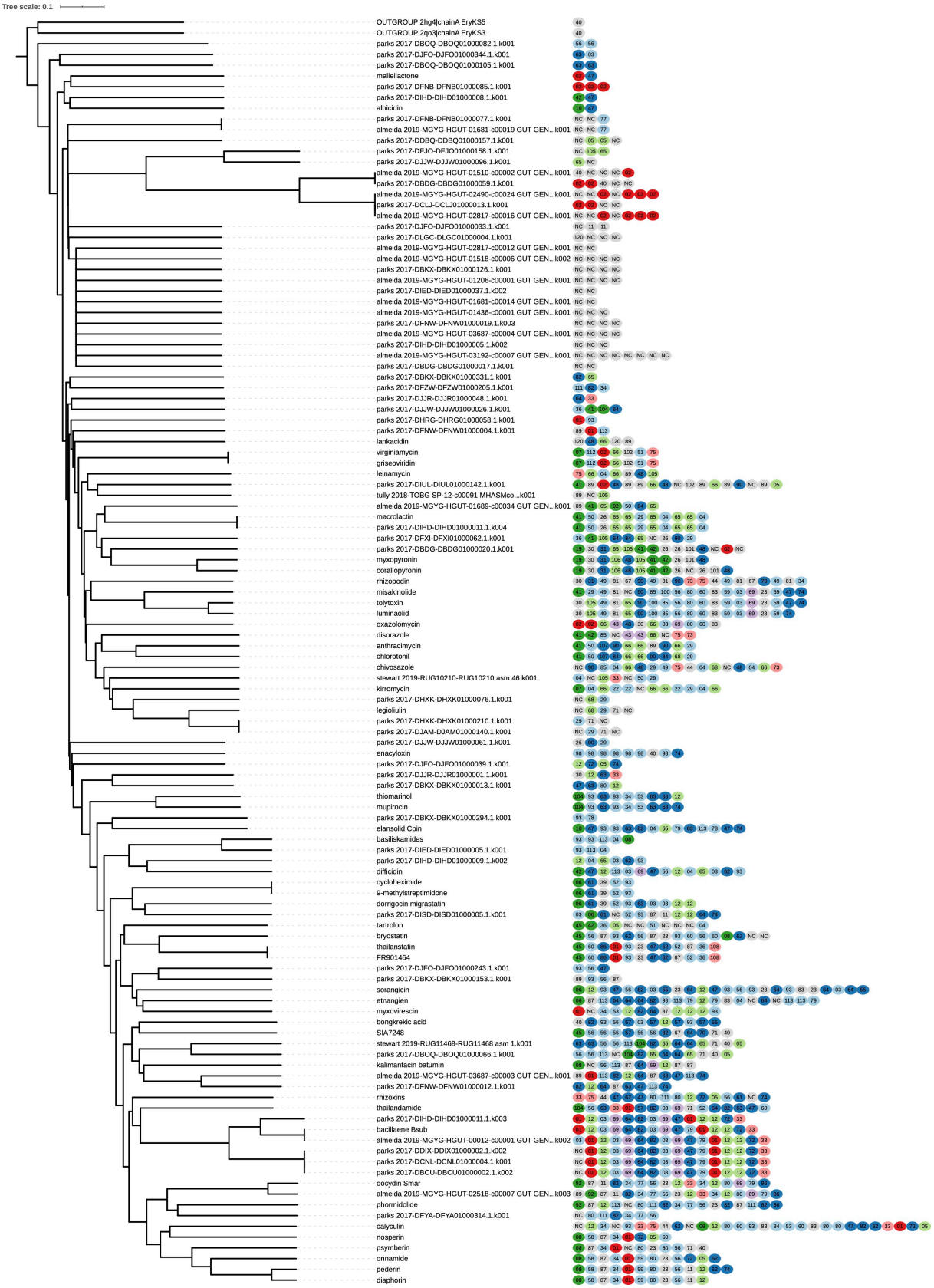
Supplementary Figure 47: NOESY spectrum of spliceostatin M (21) from *X. cannabis* in acetonitrile-d_3.



Supplementary Figure 48: Effect of *X. cannabis* and spliceostatin L (20) on *Arabidopsis thaliana*. 1 µL of different concentrations of **20** (from 1.8 mM to 180 nM) were spotted onto one leaf per plant. A cell suspension of *X. cannabis* (OD_{600} = 0.5) served as a positive control and water as a negative control. The experiment was performed in triplicates on three independent 24-well plates (**I-III**). Pictures of the 21 wells used per plate are shown. Top: plants directly post inoculation (0 dpi). Bottom: plants 10 days post inoculation (10 dpi). Red arrows: plants treated with various concentrations of **20** show white lesions on the treated leafs.



Supplementary Figure 49: HeLa cell assay for the determination of IC_{50} values of spliceostatin L (circle) and spliceostatin M (triangle). Absorbance was measured at 570 nm. A 4-parameter logistic model was used to fit the curve. **a** and **b**, Calculation of the relative IC_{50} values ($IC_{50,\,20}$ = 5.9 nM; $IC_{50,\,21}$ = 1.4 nM). **c** and **d**, calculation of the absolute IC_{50} values ($IC_{50,\,20}$ = 4.9 nM; $IC_{50,\,21}$ = 1.3 nM). Data were normalized by averaging the three lowest absorbance values measured at high spliceostatin concentrations and setting it as the bottom of the curve (bottom constrained to 0.0) and the three negative control values containing the least DMSO and setting it as the top of the curve (top constrained to 1.0). Four biological replicates were measured for each concentration.



Supplementary Figure 50: Diversity of *trans*-AT PKS assembly lines in metagenome-assembled genomes. *Trans*PACT-generated dendrogram showing the diversity of *trans*-AT PKS assembly lines identified from 20,584 metagenome-assembled genomes. Taxonomic assignment was performed using GTDB-Tk. The DFYA cluster is assigned as an otherwise unspecified Opitutaceae bacterium (could not be assigned in more detail by GTDB-Tk); the DISD cluster is from *Magnetospirillum* sp. 002435715; the DFNW and HGUT-03687 clusters are both assigned to *Paenibacillus polymyxa*; the HGUT-02518 gene cluster, which is related to the oocydin BGC, is

assigned to *Serratia* sp. 001642805; the parks 2017 DIHD-DIHD01000009.1.k002, DIHD-DIHD01000011.1.k003 and DIHD-DIHD01000011.1.k004 gene clusters are from *Bacillus velezensis*; the HGUT-00012, DDIX, DBCU and DCNL ones, all related to the bacillaene BGC, are from *Bacillus subtilis*; the DIUL cluster is from an otherwise unspecified Acetobacteraceae bacterium (could not be assigned in more detail); the DBOQ cluster as well as the RUG11468 cluster are assigned to *Ruminococcus_E bromii_A*; the HGUT-01689 cluster is assigned to *Blautia producta*; the DFXI cluster is from *Anaerocolumna* sp. 002434335; the DBDG clusters are from an otherwise unspecified Lachnospiraceae bacterium (could not be assigned in more detail); the RUG10210 cluster is from *Ruminococcus flavefacien*s_*R*.

Supplementary Tables

Group	Color	Clade number	Clade description
Amino acids		1	amino_acids
Amino acids		2	amino_acids
Amino acids		121	amino_acids
bOH		3	bimod_bOH
bOH		4	bimod_bOH
bOH		22	aMe_bketo/bOH
bOH		29	b_OH/keto
bOH		34	non_elongating_hemiacetal/b_OH
bOH		36	non_elongating_reduced/bOH
bOH		49	non_elongating_b_OH
bOH		50	b_OH
bOH		51	b_OH
bOH		52	b_OH/eDB
bOH		53	mostly_a_OH_b_OH
bOH		56	b_L_OH
bOH		58	a_LMe_b_DOH
bOH		59	b_DOH
bOH		60	non_elongating_b_OH
bOH		77	aOH_bOH
bOH		78	b_L_OH
bOH		79	non_elongating_b_L_OH
bOH		80	aMe_b_L_OH
bOH		85	aMe_bOH
bOH		93	aMe_red/bOH/bketo
bOH		98	b_OH/eDB(enacyloxins)
bOH		99	aMe_bOH
bOH		100	aMe_b_L_OH
bOH		111	b_DOH
bOH		112	b_DOH
bOH		113	a_Me_b_OH
bOH		115	b_DOH
bOH		116	b_LOH
bOH		117	b_OH
bOH		122	b_DOH
bOH		124	b_DOH
bOH		128	b_OH
Double bonds		5	shifted_double_bonds
Double bonds		12	reduced/shifted_double_bonds
Double bonds		65	double_bonds
Double bonds		66	double_bonds
Double bonds		68	double_bonds
Double bonds		105	reduced/shifted double_bonds
Double bonds		106	shifted_double_bonds
eDB		31	eDB
eDB		47	aMe_eDB
eDB		48	aMe_eDB
eDB		55	non_elongating_DB
eDB		57	eDB
eDB		61	non_elongating_DB_before_branching
eDB		62	eDB
eDB		63	mainly_eDB
eDB		64	eDB

Group	Color	Clade number	Clade description
eDB		70	eDB
eDB		72	non_elongating_aMeeDB
eDB		74	non_elongating_DB
eDB		82	b_MeeDB
eDB		84	aMe_z_shifted_DB
eDB		86	non_elongating_DB
eDB		90	red_a_Me/shifted DB_a_Me
eDB		97	bMeDB
eDB		107	eDB
eDB		110	non-elongating_zDB
Non-elongating		33	non_elongating
Non-elongating		73	non_elongating
Non-elongating		75	non_elongating_oxazole
Non-elongating		108	non_elongating_pyran/furan
Starter		6	unusual_Starter_AMT
Starter		7	Starter
Starter		8	GNAT_Starter
Starter		10	aromatic_Starter
Starter		19	methoxycarbonyl_Starter
Starter		41	acetyl_Starter
Starter		42	DB/DB_Starter
Starter		45	lactate_Starter
Starter		92	unusual_Starter
Starter		104	acetyl/aromatic Starter
Starter		125	aromatic starter
zDB		43	zDB
zDB		69	zDB
zDB		119	zDB
Other		11	oxygen_insertion
Other		23	pyran/furan
Other		26	reduced
Other		30	glycine
Other		38	mainly reduced
Other		39	vinylogous_chain_branching
Other		40	cis-AT PKS-like
Other		44	oxazole
Other		67	various
Other		71	b_keto
Other		81	b_D_OMe
Other		83	b_OMe
Other		87	exometh/red_bMe
Other		89	b_keto
Other		101	b_Me
Other		102	b_Me
Other		103	aMe_reduced
Other		109	non-elongating_aMe_reduced
Other		114	unknown
Other		120	various
Other		123	non-canonical pyran
Other		126	a_oxygenation
Other		127	beet
Other		NC	not_conserved

Supplementary Table 1: Color code and clade assignment used throughout this study.

Supplementary Table 2: Secimide biosynthetic genes, protein size, proposed function and closest homologs

protein	amino acids	proposed function	sequence similarity (protein, origin)	identity	accession number
SecA	337	α-ketoglutarate dependent dioxygenase	*Gemmatimonadetes bacterium*, MBJ67707.1	29%	AAY39347.1
SecB	81	ACP	*Desulfofaba hansenii* WP_100393531.1	49%	AAY39346.1
SecC	658	asparagine synthase	*Robbsia andropogonis* WP_024905973.1	51%	AAY39345.1
SecD	3231	PKS (KS, DH, KR, ACP, KS, B, ACP, KS)	*Burkholderia gladioli*, WP_103692967.1	44%	AAY39344.1
SecE	2393	PKS (KR, ACP, KS, ACP, KS, DH)	*Burkholderia gladioli*, WP_146128462.1	39%	AAY39343.1
SecF	2260	PKS (KR, MT, ACP, KS, KR, ACP)	*Bacillus halotolerans*; WP_059292739.1	39%	AAY39342.1
SecG	1055	PKS (AH, AT, ER)	*Brevibacillus* sp. NRRL NRS-603, WP_106782766.1	32%	AAY39341.1
SecH	311	3-oxoacyl-ACP synthase	*Leptospira alexanderi*, WP_078124957.1	25%	AAY39340.1
SecI	204	LysE type translocator	*Klebsiella pneumonia*, WP_131729069.1	38%	AAY39339.1
SecJ	518	phosphotransferase	*Pseudomonas fluorescens*, WP_003212136.1	94%	AAY39338.1
SecK	311	carboxyl methyltransferase (SAM dependent)	*Oxalobacteraceae bacterium*, HCN87942.1	59%	AAY39337.1
SecL	65	hypothetical protein	*Pseudomonas* sp. GM60, WP_085989737.1	68%	AAY39336.1
SecM	85	hypothetical protein	*Pseudomonas* sp. 286, WP_137984090.1	98%	AAY39335.1
SecN	380	endonuclease	*Pseudomonas* sp. 286, WP_137984089.1	97%	AAY39334.1
SecO	220	multi-drug efflux protein	*Pseudomonas* sp. 286, WP_122530064.1	99%	AAY39333.1
SecP	234	DUF 2076	*Pseudomonas* sp. ICMP 10191, WP_058417062.1	99%	AAY39332.1
SecQ	158	nuclease	*Pseudomonas* sp. NP10-3, WP_111522202.1	99%	AAY39331.1
SecR	204	DNA polymerase III	*Pseudomonas congelans*,	99%	AAY39330.1

			WP_096131746.1		
SecS	155	AsnC transcriptional regulator	*Pseudomonas* sp. 286, WP_122585766.1	90%	AAY39329.1
SecT	304	multidrug transporter	*Pseudomonas congelans*, WP_096131744.1	98%	AAY39328.1
SecU	442	DEAD/DEAH box helicase	*Pseudomonas congelans*, KPW85797.1	99%	AAY39327.1
SecV	424	NAD/FAD dependent dehydrogenase	*Pseudomonas congelans*, KPW85777.1	97%	AAY39326.1
SecW	305	histone deacetylase	*Pseudomonas congelans*, WP_054993128.1	98%	AAY39325.1
SecX	189	acetyltransferase (GNAT family)	*Pseudomonas congelans*, WP_096125255.1	97%	AAY39324.1
SecY	289	TE	*Pseudomonas congelans*, WP_032615532.1	98%	AAY39323.1

Supplementary Table 3: NMR chemical shifts of secimide and MTPA esters in $CDCl_3$

	secimide		secimide (*R*)-MTPA ester		secimide (*S*)-MTPA ester	
No.	δ_C	δ_H, mult.	δ_C	δ_H, mult.	δ_C	δ_H, mult.
1	41.0	1.41 (ddd, 2.7, 8.8, 14.1)	39.1	1.60 m	39.3	1.69 m
		1.68 (ddd, 4.9, 10.4, 14.1)		1.74 m		1.83 m
2	65.1	4.18 m	70.4	5.56 m	70.8	5.57 m
3	44.2	2.76 m	41.5	2.90 (dd, 6.1, 6.1)	41.3	2.84 m
		2.91 (dd, 2.1, 17.6)		3.21 (ddd, 3.7, 6.1, 17.1)		3.19 (ddd, 2.8, 6.0, 17.2)
4	201.7		197.2		197.0	
5	138.0		138.1		138.1	
6	141.3	6.65 (dd, 1.1, 9.2)	141.0	6.60 m	140.9	6.56 m
7	39.8	3.55 (qd, 7.2 , 9.2)	39.7	3.54 ovlp	39.7	3.53 ovlp
8	173.9		173.9		173.9	
9	11.5	1.83 (d, 1.1)	11.5	1.81 brs	11.4	1.78 brs
10	17.4	1.35 (d, 7.2)	17.4	1.34 (d, 7.0)	17.4	1.33 (d, 7.1)
11	52.5	3.73 s	52.4	3.73 s	52.4	3.73 s
1'	27.5	2.52 m	26.7	1.85 m	27.1	2.03 m
2'	37.2	2.34 (dd, 10.9, 10.9)	36.9	2.19 m	37.1	2.31 m
		2.81 m		2.71 m		2.80 m
3'	171.8		171.0		171.0	
5'	171.8		171.0		171.0	
6'	38.6	2.37 (dd, 10.7, 10.7)	38.1	2.16 m	38.2	2.24 m
		2.78 m		2.52 m		2.62 m

Supplementary Table 4: Gynuellalide biosynthetic genes, protein size, proposed function and closest homologs

protein	amino acids	proposed function	sequence similarity (protein, origin)	identity (%)	accession number
GynA	252	enoyl-CoA hydratase	*Leptolyngbya* sp. ISBN3-Nov-94-8 AMH40437.1	67%	WP_044617468.1
GynB	261	enoyl-CoA hydratase	*Leptolyngbya* sp. PCC 7375 WP_006512938.1	69%	WP_044617469.1
GynC	420	HMG CoA-synthase	*Leptolyngbya* sp. ISBN3-Nov-94-8 AMH40430.1	78%	WP_044617470.1
GynD	6819	PKS (DH-FkbM-FkbH-ACP-KS-CR-CR-ACP-ACP-ACP-KS-KR-ACP-KS-KR-MT-ACP-KS-KR)	*Dickeya dianthicola* WP_029729610.1	46%	WP_044617471.1
GynE	5532	PKS (ACP-KS-KR-MT-ACP-KS-KR-ACP-KS-ACP-ACP-ACP-ACP-KS-KR-ACP)	*Leptolyngbya* sp. PCC 7375 EKU96423.1	50%	WP_144407643.1
GynF	380	flavin-dependent oxidoreductase	*Dickeya dianthicola* WP_024106524.1	59%	WP_044617473.1
GynG	4258	PKS (KS-ACP-KS-KR-ACP-KS-DH-PS-KR-ACP-KS-ACP)	*Leptolyngbya* sp. ISBN3-Nov-94-8 AMH40423.1	47%	WP_052830282.1
GynH	6350	PKS (ACP-KS-CR-ACP-KS-KR-ACP-KS-DH-KR-ACP-ACP-KS-ACP-ACP-KS-ACP-C)	*Leptolyngbya sp. ISBN3-Nov-94-8* AMH40443.1	47%	WP_044617474.1
GynI	493	dioxygenase	*Zhongshania aliphaticivorans* WP_008246983.1	69%	WP_044617475.1
GynJ	809	peptidase	*Oceanicoccus sagamiensis* WP_085757908.1	52%	WP_082070718.1
GynK	412	cytochrome P450	*Parvibaculum* sp. MAN62859.1	37%	WP_044617477.1
GynL	79	ACP	*Alteromonadales bacterium* BS08 WP_075185092.1	56%	WP_044617478.1
GynM	409	KS	*Tahibacter aquaticus* WP_133818593.1	59%	WP_044617479.1
GynN	774	metalloprotease	*Thalassolituus* sp. C2-1 WP_145466512.1	66%	WP_044617480.1
GynO	178	hypothetical protein	*Reinekea marinisedimentorum* WP_132701842.1	30%	WP_044617481.1
GynP	339	FruR transcriptional regulator	*Proteobacteria bacterium* 228 WP_146074273.1	70%	WP_044617482.1

Supplementary Table 5: NMR chemical shifts of gynuellalide in DMSO-d_6

No.	δ_C	δ_H, mult.	No.	δ_C	δ_H, mult.
1	164.8		22	41.3	
2	117.1	5.79 brs	23	73.9	3.49 m
3	159.2		24	35.2	1.37 m
4	39.4	1.98 m			1.62 m
		2.22 ovlp	25	72.0	3.87 m
5	20.5	1.03 ovlp	26	41.6	1.45 m
		1.78 m	27	69.3	3.75 m
6	34.0	1.33 m	28	41.3	1.67 ovlp
		1.73 ovlp			1.72 ovlp
7	77.0	3.96 m	29	75.0	4.72 m
8	32.1	1.03 ovlp	30	33.3	2.62 ovlp
		2.24 m			3.12 (dddd, 2.5, 2.5, 7.6, 17.2)
9	43.6	2.67 (dd, 8.0, 9.0)	31	135.2	
10	130.1	5.04 (d, 9.0)	32	169.7	
11	132.1		33	18.4	2.14 brs
12	46.9	2.11 ovlp	34	102.4	4.87 (d, 3.9)
		2.36 ovlp	35	14.8	1.47 brs
13	75.0	4.72 m	36	16.5	1.67 brs
14	34.0	1.70 ovlp	37	18.5	0.70 s
		2.09 ovlp	38	18.0	0.83 s
15	73.2	5.20 (dd, 4.2, 4.2)	39	7.2	0.84 ovlp
16	84.0	3.70 (dd, 4.2, 8.8)	40	121.0	5.70 (brdd, 2.2, 2.2)
17	65.2	4.38 (ddd, 4.7, 8.8, 8.8)			6.02 (brdd, 2.7, 2.7)
18	129.1	5.17 (d, 8.8)	17-OH		4.45 (d, 4.7)
19	135.2		21-OH		3.85 (d, 4.9)
20	41.8	1.83 (dd, 10.3, 13.5)	23-OH		4.60 (d, 4.6)
		2.21 ovlp	25-OH		4.63 (d, 3.7)
21	72.0	3.51 ovlp	27-OH		4.57 (d, 4.8)
			34-OH		5.88 (d, 3.9)

Supplementary Table 6: Spliceostatin BGC from *Xanthomonas cannabis*. The BGC is split into three contigs (GenBank: JSZF01000xyz.1, the last three digits indicate the contig number, see rightmost column). The full-length, concatenated BGC sequence is accessible under accession number BK010647.

protein	amino acids	proposed function	sequence similarity (protein, origin)	identity (%)	accession number	contig (xyz)
SxcA	57	transposase	*Xanthomonas vasicola* pv. *zeae* HHZ25049.1	100%	KHL53185.1	085
SxcB	227	GNAT family N-acetyl transferase	*Agrobacterium tumefaciens* WP_012650002.1	49%	WP_081419623.1	085
SxcC	88	transposase	*Xanthomonas gardneri* WP_003474297.1	99%	KHL53184.1	085
SxcD	5.008	PKS (DH, KR, FkbH, ACP, KS, KR, ACP, KS, ACP, ACP, TE, KS, ACP, C)	*Burkholderia thailandensis* MSMB43 EIP85579.1	69%	KHL53186.1 KHL53337.1	085 & 096
SxcE	8.224	PKS (C, A, ACP, KS, DH, KR, MT, ACP, ACP, ACP, ER, KS, DH, PS, KR, ACP, ACP, KS, DH, KR, MT, ACP, KS)	*Burkholderia* sp. 2002721687 WP_006028556.1	71%	KHL53336.1	096
SxcF	4.844	PKS (DH, KR, ACP, KS, KR, ACP, KS, ECH, ECH, ACP, ACP, KS, OX)	*Burkholderia* sp. MSMB1589WGS WP_063534551.1	72%	KHL53335.1	096
SxcG	1.964	PKS (ACP, ACP, KS, DH, PS, ACP, TE)	*Pseudomonas* sp. 2663 ADH01490.1	64%	KHL53334.1 KHL53751.1	096 & 100
SxcH	305	AT	*Burkholderia* sp. MSMB1589WGS WP_063534553.1	77%	WP_052210040.1	100
SxcI	145	hypothetical protein	*Limnobacter* sp. NLD69161.1	42%	WP_158002175.1	100
SxcJ	419	3-hydroxy-3-methylglutaryl-ACP synthase	*Burkholderia thailandensis* ATF32179.1	88%	KHL53752.1	100
SxcK	261	enoyl-CoA hydratase	*Burkholderia* sp. BDU18 WP_060822450.1	88%	KHL53753.1	100
SxcL	79	ACP	*Burkholderia* sp. BDU18 WP_060822451.1	72%	KHL53754.1	100
SxcM	426	KS	*Burkholderia* sp. BDU18 WP_060822452.1	77%	KHL53755.1	100
SxcN	377	AT	*Burkholderia* sp. BDU18 WP_082754672.1		KHL53756.1	100
SxcO	325	AT	*Burkholderia humptydooensis* KST72253.1	68%	KHL53757.1	100

SxcP	238	4'-phosphopantetheinyl transferase	*Pseudomonas acidophila* WP_096716368.1	51%	WP_158002174.1	100

Supplementary Table 7: NMR chemical shifts of 20 in acetonitrile-*d_3*

No.	δ_H, mult, (J in Hz)	δ_C	COSY	HMBC
1	—	97.2	—	—
2	1.32; m, 2.09; m	41.8	—	1.32: 1, 3, 4, 18; 2.09: 1, 3, 17, 18
3	—	55.9	—	—
4	1.18; m, 1.88; m	38.6	5	1.18: 2, 3; 1.88: 5
5	4.66; dddd (1.1, 2.4, 6.3, 11.8)	68.8	4, 6	6, 7
6	5.73; dd (6.2, 15.8)	131.0	5, 7, weak 10	4, 5, 8
7	6.29; d (15.8)	135.3	6	5, 8, 9, 19
8	—	138.1	—	—
9	5.34; d (9.4)	130.3	10	7, 11, 19
10	4.27; m	69.0	9, 11	8, 9, 11,
11	3.37; dd (2.5, 8.5)	85.6	10, 12	10, 13, 15, 20
12	1.66 ; m	28.2	20	10, 13, 14, 20
13	1.80, 1.90; overlap	36.6	—	11, 12, 14, 15, 20
14	3.83; dddd (2.1, 2.1 , 4.3, 8.7)	47.8	NH, 13,15	—
15	3.71; dq (2.3, 6.5) overlap	76.7	16	11, 14, 16
16	1.15; d (6.6)	17.9	15	14, 15
17	1.36; s	30.0	—	1, 2, 3, 5
18	2.53; d (4.7); 2.55; d (4.7)	51.2	—	2, 3, 4
19	1.85; d (1.3)	13.3	9	7, 8, 9
20	0.89; d (7.3)	15.3	12	11, 12, 13
1'	—	165.8	—	—
2'	5.88; d (11.7)	123.3	3'	1', 3', 4', 5'
3'	5.91; dd (6.6, 11.7)	144.7	2', 4',	1', 4', 5
4'	6.32; dq (6.5, 6.5)	69.0	3', 5'	2', 3', 5', 1"
5'	1.30; d (6.6)	20.2	4'	3',4'
1"	—	177.0	—	—
2"	2.48; qq (7.0, 7.0)	34.7	3"/4"	1", 3"
3"	1.10; d (6.9)	19.2	2"	1", 2"
4"	1.10; d (6.9)	29.5	2"	1", 2"
NH	6.45; d (8.3)	—	14	1', 14
OH	2.95: d (1.6)	—	10	9, 10, 11, 12
OH	3.71	—	—	1, 2, 17

Supplementary Table 8: NMR chemical shifts of spliceostatin M (21) in acetonitrile-d_3

No.	δ_H, mult, (J in Hz)	δ_c	COSY	HMBC
1	—	97.2	—	—
2	1.32; m, 2.11; m	41.8	—	1.32: 1, 3, 4, 18; 2.09: 1, 3, 17, 18
3	—	55.9	—	—
4	1.19; m, 1.90; m	38.6	5	1.18: 2, 3; 1.90: 5
5	4.66; dddd (1.2, 2.6, 6.3, 11.9)	68.9	4, 6	6, 7
6	5.74; dd (6.3, 15.8)	131.1	5, 7, weak 10	4, 5, 8
7	6.29; d (15.8)	135.3, 134.1	6	5, 8, 9, 19
8	—	138.1	—	—
9	5.34; d (9.7)	130.3	10	7, 11, 19
10	4.27; m	69.2	9, 11	8, 9, 11,
11	3.37; dd (2.6, 8.6)	85.5	10, 12	9, 10, 13, 15, 20
12	1.66 ; m	28.1	20	10, 13, 14, 20
13	1.80, 1.90; m	36.7	—	11, 12, 14, 15, 20
14	3.83; dddd (2.2, 2.2, 4.4, 8.8)	47.8	NH, 13,15	—
15	3.71; dq (2.2, 6.4)	76.7	16	11, 14, 16
16	1.15; d (6.4)	17.9	15	14, 15
17	1.36; s	30.0	—	1, 2, 3, 5
18	2.53; d (4.6); 2.55; d (4.6)	51.2	—	2, 3, 4
19	1.85; d (1.3)	13.4	9	7, 8, 9
20	0.89; d (7.3)	15.2	12	11, 12, 13
1'	—	165.8	—	—
2'	5.88; d (11.7)	123.4	3'	1', 3', 4', 5'
3'	5.92; dd (7.0, 11.7)	144.8	2', 4',	1', 4', 5'
4'	6.34; dq (6.7, 6.5)	69.0	3', 5'	2', 3', 5', 1"
5'	1.30; d (6.5)	20.3	4'	3',4'
1"	—	174.4	—	—
2"	2.27; q (7.6)	28.3	3"	1", 3"
3"	1.05; d (7.5)	9.4	2"	1", 2"
NH	6.45; d (8.6)	—	14	1', 14
OH	2.95; d (1.6)	—	10	9, 10, 11
OH	3.7	—	—	1, 2, 17

Supplementary Table 9: Primers used in this study

primer	sequence
4314-F1	CATGTTCGCTCGACCGGTATCAAAGC
4314-R1	GAAGCAGCTCCAGCCTACACAAACGTTGTCCCTTAGGTTTCTAGTGCCTTG
4314-F2	GGTCGACGGATCCCCGGAATGGGTGTCGTCATGAATAACTCCAATGAGATTGCTAAC
4314-R2	GGACTTTCCGTAGGTGTTTACCAATTGCAGG
FRT-KM-F	GTGTAGGCTGGAGCTGCTTC
FRT-KM-R	ATTCCGGGGATCCGTCGACC

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