

In the format provided by the authors and unedited.

Activation of silent biosynthetic gene clusters using transcription factor decoys

Bin Wang¹, Fang Guo², Shi-Hui Dong^{1,3} and Huimin Zhao^{1,2,3,4*} 

¹Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. ²Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. ³Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. ⁴Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. *e-mail: zhao5@illinois.edu

Activation of silent biosynthetic gene clusters using transcription factor decoys

Bin Wang¹, Fang Guo², Shi-Hui Dong^{1,3}, and Huimin Zhao^{1,2,3,4,*}

¹Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA. ²Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA. ³Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA. ⁴Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA. * e-mail: zhao5@illinois.edu

SUPPLEMENTARY TABLES

Supplementary Table 1. antiSMASH¹ analysis of multiple streptomycetes used in this study

<i>Streptomyces coelicolor</i> A3(2)					
cluster	length /kb	type	known product	study	result
1	53	otherks-t1pks		this study	not activated
2	26	terpene	isorenieratene		
3	35	lantipeptide			
4	51	nrps	coelichelin		
5	10	bacteriocin	5-Hydroxyectoine		
6	41	t3pks	THN/flaviolin		
7	10	ectoine			
8	11	melanin			
9	12	siderophore	Desferrioxamine		
10	81	nrps	CDA		
11	43	t2pks	ACT		
12	21	terpene	Albaflavenone		
13	43	t2pks	spore pigment		
14	12	siderophore			
15	47	t1pks	RED		
16	11	bacteriocin			
17	22	terpene	Geosmin		
18	13	siderophore			
19	67	butyrolactone-t1pks	coelimycin		
20	48	nrps		this study	not activated
21	23	lantipeptide			
22	27	terpene	Hopene		
23	48	otherks-t1pks	1MPKSsco		
24	25	lantipeptide			
25	41	other			
26	21	indole			
27	83	t3pks-nrps-terpene	coelibactin		

<i>Streptomyces albus</i> J1074					
cluster	length /kb	type	known product	study	result
1	59	t1pks-nrps		this study	
2	49	nrps-t1pks	6-epi-alteramides		
3	27	terpene			
4	10	bacteriocin			
5	41	otherks	paulomycin		
6	11	bacteriocin			
7	63	nrps		this study	
8	15	siderophore			
9	22	terpene			
10	21	terpene			
11	32	thiopeptide-lantipeptide			
12	11	bacteriocin			
13	23	lantipeptide			
14	50	nrps		this study	activated
15	106	nrps	surugamides		
16	44	nrps			
17	12	siderophore			
18	10	ectoine			
19	44	other	indigoidine		
20	29	bacteriocin-terpene			
21	41	t3pks			
22	155	t1pks	FR-008/candididin		
23	74	nrps-t1pks-lantipeptide	antimycin		
24	64	t1pks			

<i>Streptomyces</i> sp. F-5635 (ssp8)					
cluster	length /kb	type	similar BGC	study	result
1	41	T3pks	Herboxidiene_biosynthetic_gene_cluster (8% of genes show similarity)	this study	activated
2	96	T3pks-T1pks-Nrps	Daptomycin_biosynthetic_gene_cluster (14% of genes show similarity)		
3	12	Siderophore	Desferrioxamine_B_biosynthetic_gene_cluster (100% of genes show similarity)		
4	11	Melanin	Melanin_biosynthetic_gene_cluster (60% of genes show similarity)		
5	42	T2pks	Spore_pigment_biosynthetic_gene_cluster (66% of genes show similarity)		
6	21	Terpene	Albaflavenone_biosynthetic_gene_cluster (100% of genes show similarity)	this study	activated
7	24	Terpene	-		
8	41	T3pks	Alkylresorcinol_biosynthetic_gene_cluster (100% of genes show similarity)		
9	10	Bacteriocin	Informatipeptin_biosynthetic_gene_cluster (42% of genes show similarity)		
10	42	T2pks	Fluostatin_biosynthetic_gene_cluster (20% of genes show similarity)		
11	12	Siderophore	-	this study	activated
12	51	Nrps	Coelichelin_biosynthetic_gene_cluster (100% of genes show similarity)		
13	73	Phosphonate-T1pks-Ladderane	Pactamycin_biosynthetic_gene_cluster (20% of genes show similarity)		
14	73	Siderophore-Nrps	Meilingmycin_biosynthetic_gene_cluster (3% of genes show similarity)		
15	22	Terpene	Herboxidiene_biosynthetic_gene_cluster (3% of genes show similarity)		
16	11	Bacteriocin	-	this study	activated
17	80	T1pks	Vicenistatin_biosynthetic_gene_cluster (65% of genes show similarity)		
18	10	Ectoine	Ectoine_biosynthetic_gene_cluster (100% of genes show similarity)		
19	54	Nrps	Coelibactin_biosynthetic_gene_cluster (100% of genes show similarity)		
20	24	Terpene	Carotenoid_biosynthetic_gene_cluster (45% of genes show similarity)		
21	46	Nucleoside-Nrps	A54145_biosynthetic_gene_cluster (5% of genes show similarity)	this study	activated
22	60	T1pks	Pellasoren_biosynthetic_gene_cluster (33% of genes show similarity)		
23	27	Terpene	Hopene_biosynthetic_gene_cluster (92% of genes show similarity)		
24	21	Indole	Ravidomycin_biosynthetic_gene_cluster (5% of genes show similarity)		
25	34	Oligosaccharide	Cinerubin_B_biosynthetic_gene_cluster (17% of genes show similarity)		
26	47	Nrps	Grincamycin_biosynthetic_gene_cluster (8% of genes show similarity)	this study	activated
27	22	Terpene	Azinomycin_B_biosynthetic_gene_cluster (6% of genes show similarity)		
28	134	T1pks-Nrps	Lasalocid_biosynthetic_gene_cluster (13% of genes show similarity)		

Streptomyces griseofuscus B-5429 (sgri)

cluster	length /kb	type	similar BGC	study	result
1	12	Siderophore	Desferrioxamine_B_biosynthetic_gene_cluster (100% of genes show similarity)	this study	
2	11	Melanin	Istamycin_biosynthetic_gene_cluster (4% of genes show similarity)		
3	84	T1pks-Nrps			
4	22	terpene			
5	36	Lantipeptide	A-503083_biosynthetic_gene_cluster (7% of genes show similarity)	this study	
6	13	Siderophore	-		
7	44	Other	-		
8	25	T1pks	Tetronasin_biosynthetic_gene_cluster (3% of genes show similarity)		
9	10	Bacteriocin	Informatipeptin_biosynthetic_gene_cluster (28% of genes show similarity)	this study	
10	43	T2pks	Spore_pigment_biosynthetic_gene_cluster (83% of genes show similarity)		
11	58	T1pks-Otherks	Tetronasin_biosynthetic_gene_cluster (3% of genes show similarity)		
12	89	Nrps	Kirromycin_biosynthetic_gene_cluster (16% of genes show similarity)		
13	43	Other	SCO-2138_biosynthetic_gene_cluster (64% of genes show similarity)	this study	
14	11	Bacteriocin	-		
15	22	Terpene	-		
16	10	Ectoine	Ectoine_biosynthetic_gene_cluster (100% of genes show similarity)		
17	93	T1pks-Nrps	Meridamycin_biosynthetic_gene_cluster (26% of genes show similarity)	this study	activated
18	74	Lantipeptide-Nrps	-		
19	52	Thiopeptide-Terpene	Hopene_biosynthetic_gene_cluster (92% of genes show similarity)		
20	68	T1pks-Nrps	Meilingmycin_biosynthetic_gene_cluster (3% of genes show similarity)		
21	52	T1pks-Otherks	Cinnamycin_biosynthetic_gene_cluster (19% of genes show similarity)	this study	activated
22	55	Nrps	PM100117_/PM100118_biosynthetic_gene_cluster (8% of genes show similarity)		
23	41	T3pks	Herboxidiene_biosynthetic_gene_cluster (7% of genes show similarity)		
24	47	T1pks	Filipin_biosynthetic_gene_cluster (92% of genes show similarity)		
25	48	T1pks		this study	activated
26	71	Terpene-T2pks-T1pks-Butyrolactone	Lactonamycin_biosynthetic_gene_cluster (28% of genes show similarity)		
27	26	terpene	Friulimicin_biosynthetic_gene_cluster (15% of genes show similarity)		
28	42	other			
29	21	terpene	Albaflavenone_biosynthetic_gene_cluster (100% of genes show similarity)		
30	50	Transatpks-T1pks-Nrps	Cinnabaramide_biosynthetic_gene_cluster (18% of genes show similarity)		
31	70	Ectoine-Nrps	Pristinamycin_biosynthetic_gene_cluster (29% of genes show similarity)		

32	13	Siderophore	Cinnabaramide_biosynthetic_gene_cluster (9% of genes show similarity)
33	23	Lantipeptide	-
34	26	Terpene	Carotenoid_biosynthetic_gene_cluster (63% of genes show similarity)
35	48	Other	-
36	60	Nrps	Stenothricin_biosynthetic_gene_cluster (13% of genes show similarity)
37	67	T1pks-Nrps	Vicenistatin_biosynthetic_gene_cluster (15% of genes show similarity)
38	48	T1pks	Carbapenem_MM_4550_biosynthetic_gene_cluster (10% of genes show similarity)
39	11	Butyrolactone	Thioviridamide_biosynthetic_gene_cluster (10% of genes show similarity)
40	48	Nrps-Siderophore	Kinamycin_biosynthetic_gene_cluster (20% of genes show similarity)
41	45	Nrps	Lankacidin_biosynthetic_gene_cluster (60% of genes show similarity)
42	47	T1pks	Lankamycin_biosynthetic_gene_cluster (56% of genes show similarity)
43	56	T1pks	Lorneic_acid_A_biosynthetic_gene_cluster (14% of genes show similarity)
44	62	T1pks	Cremimycin_biosynthetic_gene_cluster (17% of genes show similarity)

<i>Streptomyces</i> sp. F-4335 (ssp7)					
cluster	length /kb	type	similar BGC	study	result
1	35	Butyrolactone	Echosides_biosynthetic_gene_cluster (11% of genes show similarity)		
2	21	Terpene	2-methylisoborneol_biosynthetic_gene_cluster (100% of genes show similarity)		
3	23	Lantipeptide	SapB_gene_cluster (100% of genes show similarity)		
4	21	Terpene	-		
5	63	Nrps	Maklamicin_biosynthetic_gene_cluster (4% of genes show similarity)		
6	44	Other	-		
7	41	Other	Gentamicin_biosynthetic_gene_cluster (3% of genes show similarity)		
8	12	Siderophore	Desferrioxamine_B_biosynthetic_gene_cluster (100% of genes show similarity)		
9	15	Siderophore	-		
10	112	T1pks-Nrps	Salinomycin_biosynthetic_gene_cluster (30% of genes show similarity)	this study	
11	22	Terpene	Dynemicin_biosynthetic_gene_cluster (3% of genes show similarity)		
12	27	Terpene	Hopene_gene_cluster (61% of genes show similarity)		
13	52	T1pks-Otherks	-		
14	59	Phosphonate-T1pks-Ladderane	Neocarzinil_biosynthetic_gene_cluster (14% of genes show similarity)	this study	
15	58	Melanin-Terpene	Melanin_gene_cluster (28% of genes show similarity)		
16	13	Siderophore	-		
17	41	T3pks	Alkylresorcinol_biosynthetic_gene_cluster (100% of genes show similarity)		
18	61	T1pks-Nrps	Akaeolide_biosynthetic_gene_cluster (12% of genes show similarity)	this study	
19	11	Butyrolactone	Sanglifehrin_A_biosynthetic_gene_cluster (4% of genes show similarity)		
20	41	Arylpolyene	Svaricin_biosynthetic_gene_cluster (12% of genes show similarity)		
21	43	T2pks	Spore_pigment_biosynthetic_gene_cluster (66% of genes show similarity)		
22	10	Ectoine	Ectoine_gene_cluster (100% of genes show similarity)		
23	44	Other	Lipstatin_biosynthetic_gene_cluster (42% of genes show similarity)		
24	98	Transatpks-Nrps	Oxazolomycin_biosynthetic_gene_cluster (21% of genes show similarity)	this study	activated
25	63	T1pks-Nrps	Coelichelin_biosynthetic_gene_cluster (72% of genes show similarity)		
26	47	T1pks	Herboxidiene_biosynthetic_gene_cluster (10% of genes show similarity)		
27	49	T1pks	Himastatin_biosynthetic_gene_cluster (20% of genes show similarity)		
28	87	Nrps	Kirromycin_biosynthetic_gene_cluster (23% of genes show similarity)	this study	activated
29	33	Bacteriocin	-		

Supplementary Table 2. Statistics for evaluating the effectiveness of the TFD strategy

Protocol	Number of Strains	Number of BGCs	Number of Constructs	Number of Fermentations	Number of Activated BGCs
Low throughput	3	11	27	27	4
High throughput	2	10	34	9	4

Supplementary Table 3. *Streptomyces* strains used in this study and their culture conditions

strains	designation	sporulation	conjugation spores/mycelia, solid media	liquid fermentation
<i>Streptomyces albus</i> J1074	salb	MS ²	spores, MS	MYM ³
<i>Streptomyces coelicolor</i> A3(2)	sco	MS	spores, MS	MYM
<i>Streptomyces griseofuscus</i> NRRL B-5429	sgri	MS	mycelium growing in YEME liquid medium, MS	MYM
<i>Streptomyces lividans</i> 66	sli	MS	spores, MS	MYM
<i>Streptomyces</i> sp. F-4335	ssp7	ISP4	spores, ISP4	MYM
<i>Streptomyces</i> sp. NRRL F-5635	ssp8	MS	spores, MS	MYM

Supplementary Table 4. Media used in this study

MYM, 1 L		MS, 1 L	
maltose	4 g	soy bean powder	20 g
yeast extract	4 g	mannitol	20 g
malt extract	10 g	agar	20 g
agar	15 g		

Supplementary Table 5. Primers used in this study

Template gDNA	TFD description	Primer	Sequence
<i>neo</i> for constructing pKCW101		neoF	ccggggacctgcatagatccTGAGAATTCTAATAGCTGAC
		neoR	ccgattcattaatgcagTCAGAAGAACTCGTCAAG
<i>S. lividans</i> 66, RED	<i>RslIM18I</i> , <i>redD</i>	RslIM18-F1	gatcgaagagaagcaggacgTGGGGTCCCGGTCGATCAC
		RslIM18-R1	ggcaccccaggctttacactCGTCCTGACCGGCCGCGA
	<i>RslIM18II</i> , structural	RslIM18-F2	gaagagaagcaggacgGCGAAACCGTCGACCAGC
		RslIM18-R2	ccccaggctttacactCGGGACGGCAGACAGGAA
	<i>RslIM18III</i> , <i>redZ</i>	RslIM18-F3	ccggggacctgcatagatctTGGTCTCCACCTGCACCTTTCA TGTC
		RslIM18-R3	ccgattcattaatgcagctgGCCCGGCCGACCTTCTT
<i>S. lividans</i> 66, ACT	<i>RslIM14I</i> , <i>actII</i> -orf4	RslIM14-F1	ccggggacctgcatagatctGCACCGGCTCGCCCGGCG
		RslIM14-R1	ccgattcattaatgcagctgCGTGCCGCCTGAGGAGCAGC
	<i>RslIM14II</i> , mini-PKS	RslIM14-F2	gaagagaagcaggacgCCGCTGGTCGCACCCGTC
		RslIM14-R2	ccccaggctttacactGATACGGGACCCCTCGATGCC
	<i>RslIM14III</i> , structural	RslIM14-F3	ccggggacctgcatagatctGTAGACCGGCGAAGTCAC
		RslIM14-R3	ccgattcattaatgcagctgGCCGACGGCTGTTGACGC
	<i>RslIM14IV</i> , hypo	RslIM14-F4	ccggggacctgcatagatctGCACCCCTACTTCGGTACC
		RslIM14-R4	ccgattcattaatgcagctgCCGCCTCGAGACCGTCGA
	<i>RslIM14V</i> , <i>tetR</i>	RslIM14-F5	ccggggacctgcatagatctTGCCGAGCGCCGTGACGA
		RslIM14-R5	ccgattcattaatgcagctgGTAACGCCGACGGCGGGG
<i>S. coelicolor</i> A3(2)	<i>RscM1I</i>	RscM1-F1	ccggggacctgcatagatctGGTCGACCGGTGGGTGG
		RscM1-R1	ccgattcattaatgcagctgAGGCGCAGCCCGAGGAATC
	<i>RscM1II</i>	RscM1-F2	ccggggacctgcatagatctGCAGCCGGTCGGCGGGGA
		RscM1-R2	ccgattcattaatgcagctgCGACCAGGACGGCGCTGC
	<i>RscM20I</i>	RscM20-F1	ccggggacctgcatagatctCCAGGCCGAGTGCCCCGG
		RscM20-R1	ccgattcattaatgcagctgAACGGGCCAGGGCGCCTTC
	<i>RscM20II</i>	RscM20-F2	ccggggacctgcatagatctGTCCAGCAGCAACGCGG
		RscM20-R2	ccgattcattaatgcagctgAGGTCGTGTGCGCGGCGA
<i>S. albus</i> J1074	<i>RsalbM1I</i>	RsalbM1-F1	ccggggacctgcatagatctCCCGTTCGGCCAGCGCCA
		RsalbM1-R1	ccgattcattaatgcagctgGGTGACGCAGAACAGCGGG
	<i>RsalbM1II</i>	RsalbM1-F2	ccggggacctgcatagatctTCCGCTGGGTACCGCGCT
		RsalbM1-R2	ccgattcattaatgcagctgCCGCCGTCACCTGGAATC
	<i>RsalbM7I</i>	RsalbM7-F1	ccggggacctgcatagatctCGCCCCCTACACCGGCCG
		RsalbM7-R1	ccgattcattaatgcagctgCGGTGGGCAGGTCCAGGG
	<i>RsalbM7II</i>	RsalbM7-F2	ccggggacctgcatagatctCGTGCGGCCTGCGTCCT
		RsalbM7-R2	ccgattcattaatgcagctgTGTCGCCAGCGAAGCGATG
	<i>RsalbM14I</i>	RsalbM14-F1	ccggggacctgcatagatctCGGGGCTGTCCGGGTGAG
		RsalbM14-R1	ccgattcattaatgcagctgCCGGGTGGACGCGGTGG
<i>RsalbM14II</i>	RsalbM14-F2	RsalbM14-F2	ccggggacctgcatagatctCTGTTCTGGCGAGGCGGG
	RsalbM14-R2	RsalbM14-R2	ccgattcattaatgcagctgCCTGCACCGCGGTGATGATC
<i>Streptomyces</i> sp. F-5635, ssp8	<i>Rssp8M2I</i>	Rssp8M2-F1	ccggggacctgcatagatccCGCGCTTGCGGAGAAGCC

Template gDNA	TFD description	Primer	Sequence
	<i>Rssp8M2II</i>	Rssp8M2-R1	ctctgtcagctattagaattcCTCGCGCCCCTCGTCGGC
		Rssp8M2-F2	cggggacctgcataggatccCCTCGTGATCGAGGATGTG
	<i>Rssp8M10I</i>	Rssp8M2-R2	ctctgtcagctattagaattcAACATAGGTGGAGAACAGG
		Rssp8M10-F1	cggggacctgcataggatccGGGGTACCGGGTGC GGAT
	<i>Rssp8M10II</i>	Rssp8M10-R1	ctctgtcagctattagaattcCCTGTGCGGCATCTCGGTC
		Rssp8M10-F2	cggggacctgcataggatccCCAGGACTCCTTCCTCGGC
	<i>Rssp8M13I</i>	Rssp8M10-R2	ctctgtcagctattagaattcGGGTACCGCGACGGGTGC
		Rssp8M13-F1	cggggacctgcataggatccAGTCGGCCGTGTCTGGTGA
	<i>Rssp8M13II</i>	Rssp8M13-R1	ctctgtcagctattagaattcGTGCAGCAGCTCCGCGAT
		Rssp8M13-F2	cggggacctgcataggatccGAGGGCGCTGTCTGGGA
	<i>Rssp8M14I</i>	Rssp8M13-R2	ctctgtcagctattagaattcACGATTGGCGCAGAGATAGGC
		Rssp8M14-F1	cggggacctgcataggatccCCGGGACCGTCACGTCCT
	<i>Rssp8M14II</i>	Rssp8M14-R1	ctctgtcagctattagaattcGAGAAGGACCTGTGTGTGAAA AAGGC
		Rssp8M14-F2	cggggacctgcataggatccGAGTCCCGGTGACCGCCG
	<i>Rssp8M17I</i>	Rssp8M14-R2	ctctgtcagctattagaattcCAGGCAGCCACCCGCCG
		Rssp8M17-F1	cggggacctgcataggatccCGTTGGCGTGCATGAAGAC
	<i>Rssp8M17II</i>	Rssp8M17-R1	ctctgtcagctattagaattcCGTACCGCGCTCATCGCT
		Rssp8M17-F2	cggggacctgcataggatccCGCCCCAGCGGGAAAGTT
	<i>Rssp8M19I</i>	Rssp8M17-R2	ctctgtcagctattagaattcGATGACTCCGCTGGCCAG
		Rssp8M19-F1	cggggacctgcataggatccCCTCACGCAGGCGAGCAC
	<i>Rssp8M21I</i>	Rssp8M19-R1	ctctgtcagctattagaattcCTGCAGTGCGTGTGCGAC
		Rssp8M21-F1	cggggacctgcataggatccGTTCGCCGAGACCCGGGC
	<i>Rssp8M21II</i>	Rssp8M21-R1	ctctgtcagctattagaattcGCGCGCCATCACCCACAG
		Rssp8M21-F2	cggggacctgcataggatccATGCCAGCGCCACGAGCG
	<i>Rssp8M28I</i>	Rssp8M21-R2	ctctgtcagctattagaattcCTCGGCCACGGCACCGATG
		Rssp8M28-F1	cggggacctgcataggatccGTTCGAGCAGGGCGTGAAC
	<i>Rssp8M28II</i>	Rssp8M28-R1	ctctgtcagctattagaattcCATGGACCCGGGGTCGAG
		Rssp8M28-F2	cggggacctgcataggatccCTTGTTGGCGACGTCCGAG
		Rssp8M28-R2	ctctgtcagctattagaattcTCCCAGACGTGGATCAG
<i>S. griseofuscus</i> B-5429 Rsgri library	<i>RsgriM3I</i>	RsgriM3-F1	cggggacctgcataggatccCCGCGACCGTCATCACCTG
		RsgriM3-R1	ctctgtcagctattagaattcTCCGACCCGGGCGGTCTC
	<i>RsgriM3II</i>	RsgriM3-F2	cggggacctgcataggatccGCCCCAGCACTTCCACCG
		RsgriM3-R2	ctctgtcagctattagaattcCACCCGGCCGGCCAGATG
	<i>RsgriM3III</i>	RsgriM3-F3	cggggacctgcataggatccAGCCGCCCGCGTGC GGCA
		RsgriM3-R3	ctctgtcagctattagaattcCAGGTCATTGAGCGCGAGTC TGCGGAAACCC
	<i>RsgriM17I</i>	RsgriM17-F1	cggggacctgcataggatccCTCGCGCGAAGGGAGCGG
		RsgriM17-R1	ctctgtcagctattagaattcCAGCCGGCACGCCAGACC
	<i>RsgriM17II</i>	RsgriM17-F2	cggggacctgcataggatccTCGCGGGCGGCAGAAGGA
		RsgriM17-R2	ctctgtcagctattagaattcGTGGATGTGGTTCGGCGGTG
	<i>RsgriM17III</i>	RsgriM17-F3	cggggacctgcataggatccCCGGCCCCGGCTTCCACGA
		RsgriM17-R3	ctctgtcagctattagaattcGCACACCTCCTCGTCGCGC
	<i>RsgriM17IV</i>	RsgriM17-F4	cggggacctgcataggatccCCTGGAGCCCGGCGGCCG

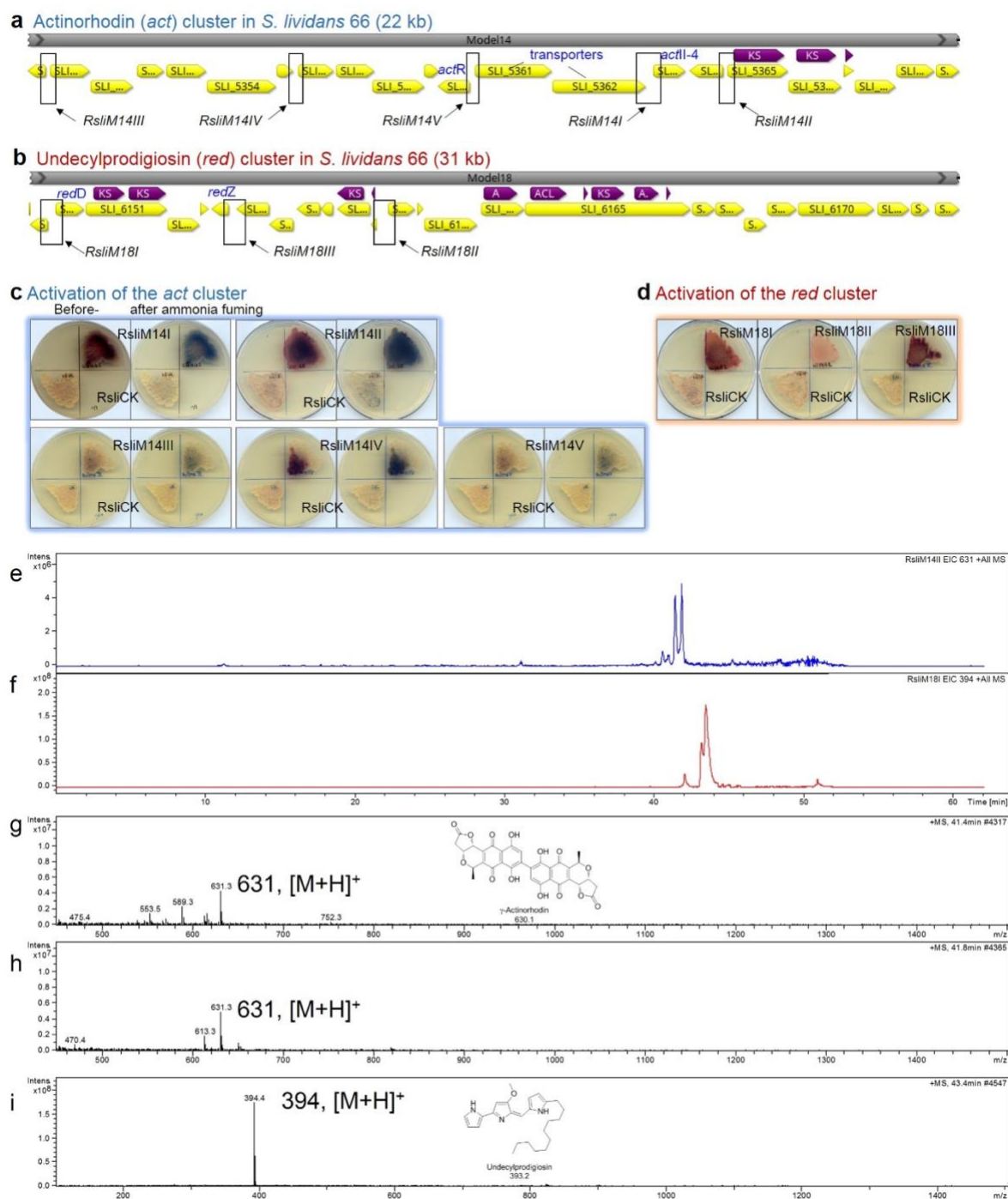
Template gDNA	TFD description	Primer	Sequence
	<i>RsgriM17V</i>	RsgriM17-R4	ctctgtcagctattagaattcCATCGGGTGC GCCCCGGTC
		RsgriM17-F5	cggggacctgcataggatccGAGCGGCACGAACCTGCG
	<i>RsgriM26I</i>	RsgriM17-R5	ctctgtcagctattagaattcCTCGTCGTGCCGTACCGG
		RsgriM26-F1	cggggacctgcataggatccGGCCCCCATGACGCGGG
	<i>RsgriM26II</i>	RsgriM26-R1	ctctgtcagctattagaattcCGCGAGCAGCGCGAGCAC
		RsgriM26-F2	cggggacctgcataggatccCGAGGCGCTCGGGGCCGC
	<i>RsgriM26III</i>	RsgriM26-R2	ctctgtcagctattagaattcCCGGGTTCGCGGTGCGGAC
		RsgriM26-F3	cggggacctgcataggatccGCTCCAGCGCCTGCACCAG
	<i>RsgriM26IV</i>	RsgriM26-R3	ctctgtcagctattagaattcATGGGGCACGGGCTGCGC
		RsgriM26-F4	cggggacctgcataggatccCGACGAGGAGACCGTACTG
	<i>RsgriM26V</i>	RsgriM26-R4	ctctgtcagctattagaattcCGACAGCAGCGCGAGGAT
		RsgriM26-F5	cggggacctgcataggatccTGCGGATCAGGCGGTGGAC
	<i>RsgriM26VI</i>	RsgriM26-R5	ctctgtcagctattagaattcCGCCAGCAGGGCCAGCAC
		RsgriM26-F6	cggggacctgcataggatccTGAGTGGGGTCAGCCAGG
		RsgriM26-R6	ctctgtcagctattagaattcCACCCGTACGACCGGGCC
<i>Streptomyces</i> sp. F-4335 Rssp7 library	<i>Rssp7M10I</i>	Rssp7M10-F1	cggggacctgcataggatccTCGGCAACAGCGCGTGGCTCG
	<i>Rssp7M10II</i>	Rssp7M10-R1	ctctgtcagctattagaattcGTGGCGGTGCCCGGGCCG
		Rssp7M10-F2	cggggacctgcataggatccGACCTGCGTGCTACCCC
	<i>Rssp7M10III</i>	Rssp7M10-R2	ctctgtcagctattagaattcGGCGACGACCTGCTTCTC
		Rssp7M10-F3	cggggacctgcataggatccCGCTTCCCGAAGCTGCGC
	<i>Rssp7M10IV</i>	Rssp7M10-R3	ctctgtcagctattagaattcCTCGACCATGAGCACCCG
		Rssp7M10-F4	cggggacctgcataggatccCGGAAGAAGGCCGGGCTG
	<i>Rssp7M14I</i>	Rssp7M10-R4	ctctgtcagctattagaattcGTGGGGGTCTGTGTCGTC
		Rssp7M14-F1	cggggacctgcataggatccTCGGGCCCCAGGGGCCGC
	<i>Rssp7M14II</i>	Rssp7M14-R1	ctctgtcagctattagaattcCGCCCTCGGCGATACCTGGGTCC
		Rssp7M14-F2	cggggacctgcataggatccCCTGGCCCCGTCCGCGAC
	<i>Rssp7M14III</i>	Rssp7M14-R2	ctctgtcagctattagaattcCGGGCCCGATGCCGGCAG
		Rssp7M14-F3	cggggacctgcataggatccCTGCTCCAGGTCGCGCTG
	<i>Rssp7M14IV</i>	Rssp7M14-R3	ctctgtcagctattagaattcGTCCATGAGCAGGTCCAG
		Rssp7M14-F4	cggggacctgcataggatccGTAGGCCTGCCAGCTGGT
	<i>Rssp7M14V</i>	Rssp7M14-R4	ctctgtcagctattagaattcGAACTTGATCGCCAGCATCTC
		Rssp7M14-F5	cggggacctgcataggatccCTCGTTCCCGGACCGGTG
	<i>Rssp7M18I</i>	Rssp7M14-R5	ctctgtcagctattagaattcGATGATGCGTACCGCGCC
		Rssp7M18-F1	cggggacctgcataggatccGGCCGTCACGCCCGTGATGA GTGCGG
	<i>Rssp7M18II</i>	Rssp7M18-R1	ctctgtcagctattagaattcGGCGGGCGGGGGCCGGGT
		Rssp7M18-F2	cggggacctgcataggatccGATGAGTTCAGCCGGGTCAAG
	<i>Rssp7M18III</i>	Rssp7M18-R2	ctctgtcagctattagaattcCAGCTCCTCGCCGAGCTG
		Rssp7M18-F3	cggggacctgcataggatccCCCCAGAGCTTCCAATTCCGC
	<i>Rssp7M18IV</i>	Rssp7M18-R3	ctctgtcagctattagaattcCTGGACCACCGGGGCGAG
		Rssp7M18-F4	cggggacctgcataggatccGAGGGCATGTCCGGGCTC
		Rssp7M18-R4	ctctgtcagctattagaattcGCTGGCGGGCCCGGATCAG
	<i>Rssp7M24I</i>	Rssp7M24-F1	cggggacctgcataggatccGAGCGGATCCGCGTTGAAG

Template gDNA	TFD description	Primer	Sequence
		Rssp7M24-R1	ctctgtcagctattagaattcCAGGTGCGTGTCGCGATAG
	<i>Rssp7M24II</i>	Rssp7M24-F2	cggggacctgcataggatccGGGTCCCGCCTGGAGCTG
		Rssp7M24-R2	ctctgtcagctattagaattcCAGGCCGTCCCGCCCCTC
	<i>Rssp7M28I</i>	Rssp7M28-F1	cggggacctgcataggatccGGGTGATACGTCCCCTGC
		Rssp7M28-R1	ctctgtcagctattagaattcGATGGTGTAGTCGAGGCG
	<i>Rssp7M28II</i>	Rssp7M28-F2	cggggacctgcataggatccCGCGTCCACGTCCACCGT
		Rssp7M28-R2	ctctgtcagctattagaattcGTACGTGTGCCCCTGCGC
	<i>Rssp7M28III</i>	Rssp7M28-F3	cggggacctgcataggatccGTGATCGCCCGCCACGGC
		Rssp7M28-R3	ctctgtcagctattagaattcGGCCGGGACGGGGTTGGC
	<i>Rssp7M28IV</i>	Rssp7M28-F4	cggggacctgcataggatccGACGTGCCACCGACCAC
		Rssp7M28-R4	ctctgtcagctattagaattcGAACTGCGGGGCGAGCAC
	<i>Rssp7M28V</i>	Rssp7M28-F5	cggggacctgcataggatccCAGGAAGGCCAGGATCGCG
		Rssp7M28-R5	ctctgtcagctattagaattcGGTGCGGAGCGCGGTCTC
RT-PCR primers		SigmaFactor-F	GCGGCATGCTCTTCCTGG
		SigmaFactor-R	ACTCGCTGTCGCCGTCCT
		RTssp7M10IV-F1	ACGCACGCCGAGCGGTTGAA
		RTssp7M10IV-R1	ATCGTGGCGCACACGGTGGTCT
		RTssp7M10IV-F2	CGGCGAAGAAGTGTCGCTGAT
		RTssp7M10IV-R2	AAGTGCATGGTGGGGGTGG

Supplementary Table 6. Gene annotation of the *oze* cluster

Oxazolepoxidomycin A, <i>oze</i> cluster			
gene	gene locus	size in bp	proposed function
<i>ozeO</i>	ctg1_3763	846	MT
	//	13 kb	
<i>ozeA</i>	ctg1_3748	3330	PKS
<i>ozeB</i>	ctg1_3747	3588	PKS
<i>ozeC</i>	ctg1_3746	10368	PKS
<i>ozeD</i>	ctg1_3745	3627	methionyl-tRNA_formyltransferase
<i>ozeE</i>	ctg1_3744	1134	similar to <i>ozmP</i> , oxazole cyclization
<i>ozeF</i>	ctg1_3743	2736	PKS
<i>ozeG</i>	ctg1_3742	25035	PKS/NRPS
<i>ozeH</i>	ctg1_3741	5019	PKS
<i>ozeI</i>	ctg1_3740	6123	NRPS
<i>ozeJ</i>	ctg1_3739	3423	AT
<i>ozeK</i>	ctg1_3738	1383	CYP450
<i>ozeL</i>	ctg1_3737	1380	CYP450
<i>ozeM</i>	ctg1_3736	915	ER
<i>ozeN</i>	ctg1_3735	738	ACPS

SUPPLEMENTARY FIGURES



Supplementary Figure 1. Activation of BGCs in *Streptomyces lividans* 66. **(a-b)** are actinorhodin (*act*) and undecylprodigiosin (*red*) BGCs, respectively. **(c-d)** are recombinant strains that grew on MYM plates and harbored TFDs for activation of the *act* and *red* BGCs, respectively. The blue color in **(c)** confirmed production of the pH-dependent actinorhodin after ammonia fuming treatment. The red color in **(d)** indicated undecylprodigiosin production. **(e-i)** LC-MS analyses of actinorhodin and undecylprodigiosin. **(e-f)** are extracted ion chromatogram from RslIM14II (recombinant strain harboring the *RslIM14II* TFD) and RslIM18I (recombinant strain harboring the *RslIM18I* TFD). **(g-i)** are corresponding mass spectra. Triplicate experiments were performed for detection of pigment production on plates and LC-MS, and results were reproducible.

scoM1 - Cluster 1 - T1pks-otherks

Gene cluster description

scoM1 - Gene Cluster 1. Type = t1pks-otherks. Location: 59 - 53019 nt. Click on genes for more information.

Show pHMM detection rules used



Legend:

■ core biosynthetic genes ■ additional biosynthetic genes ■ transport-related genes ■ regulatory genes ■ other genes

Detailed annotation

ctg1_11



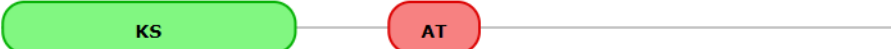
ctg1_23



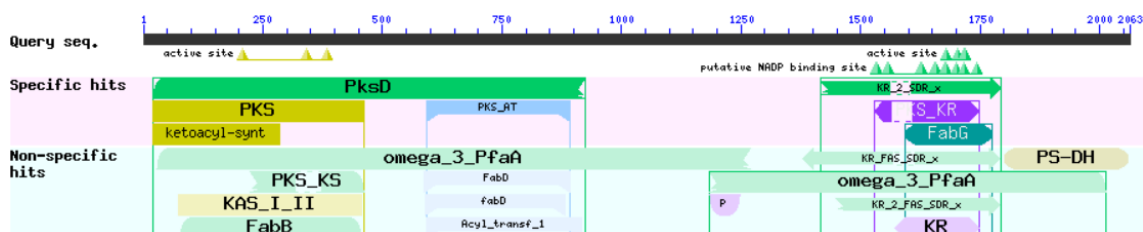
ctg1_25



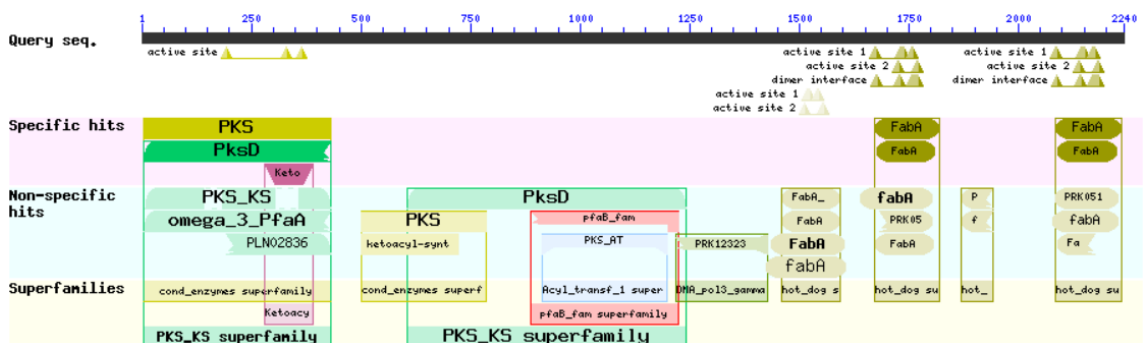
ctg1_26



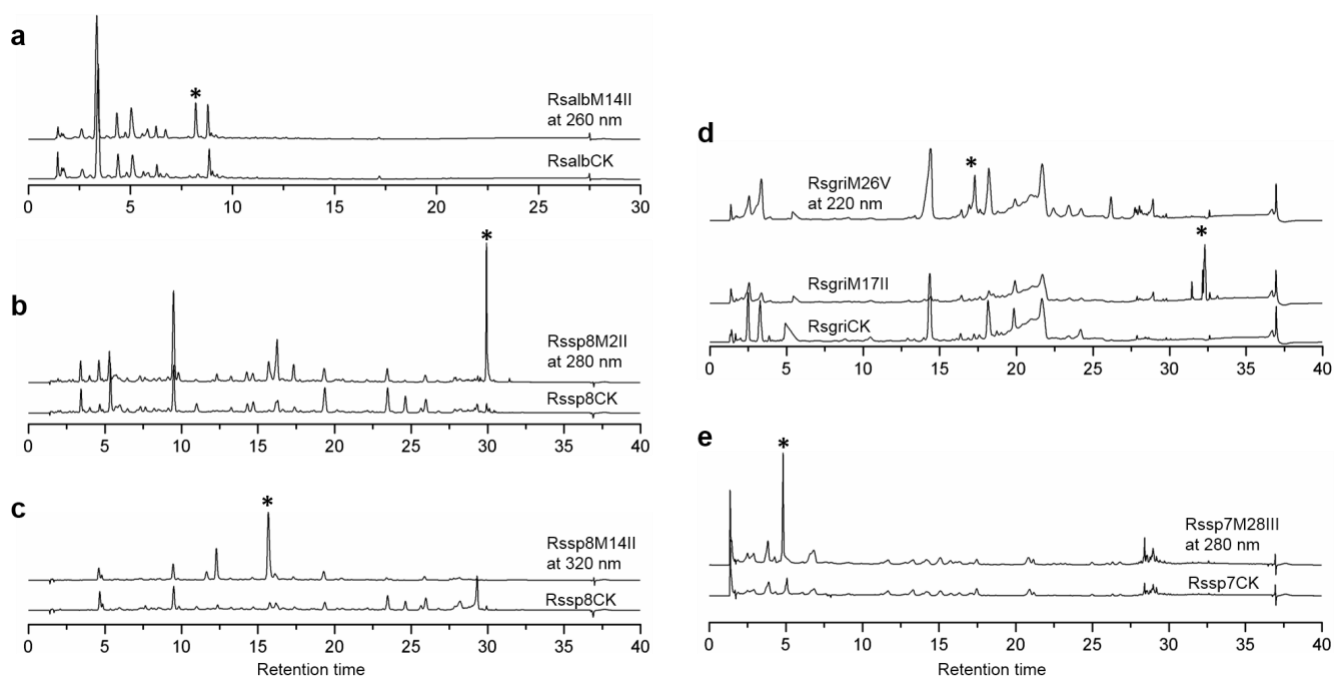
ctg1_25: KS-AT-ACP-KR-DH, PfaA homolog.



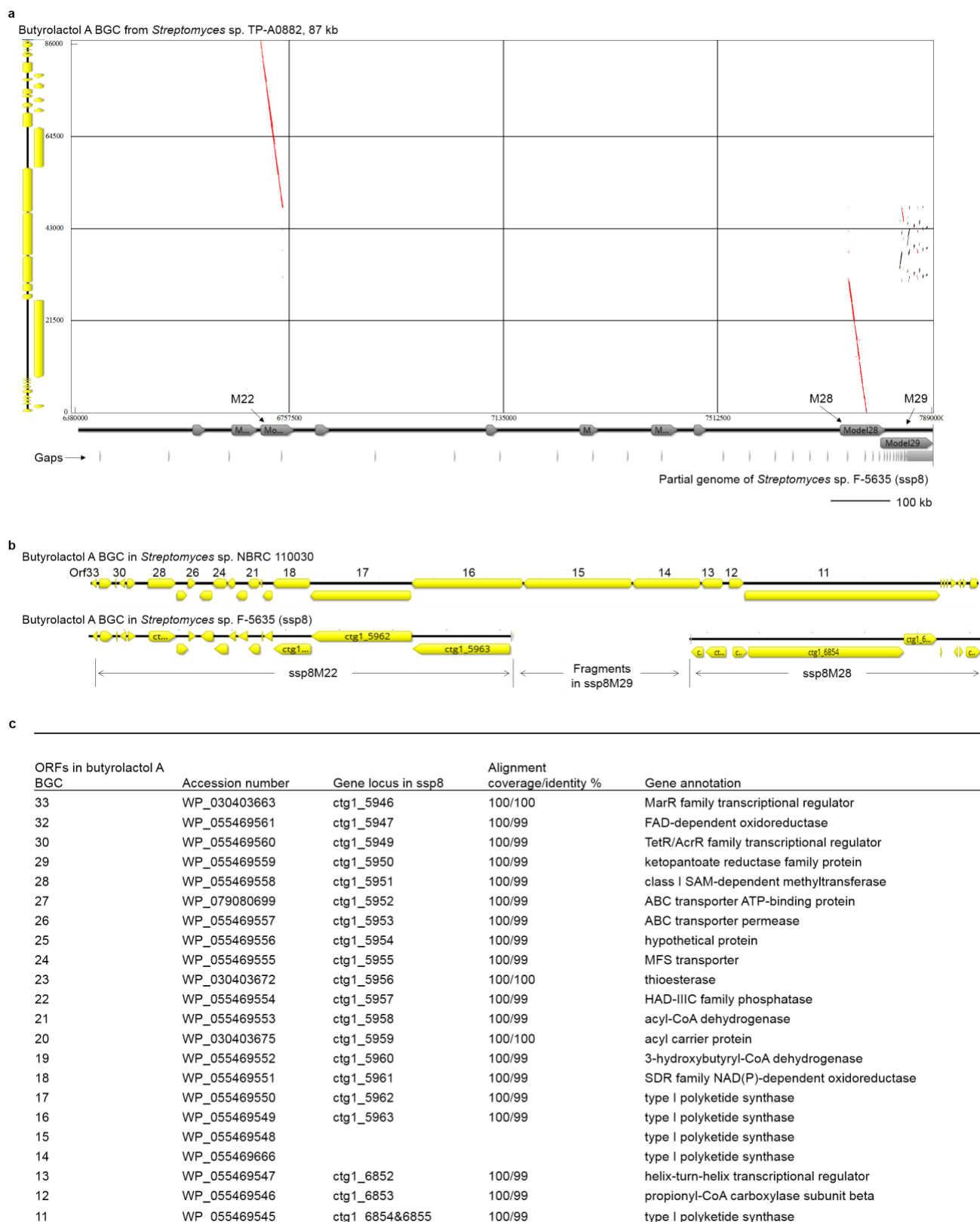
ctg1_26: KS-KS-AT-DH-DH, PfaC homolog.



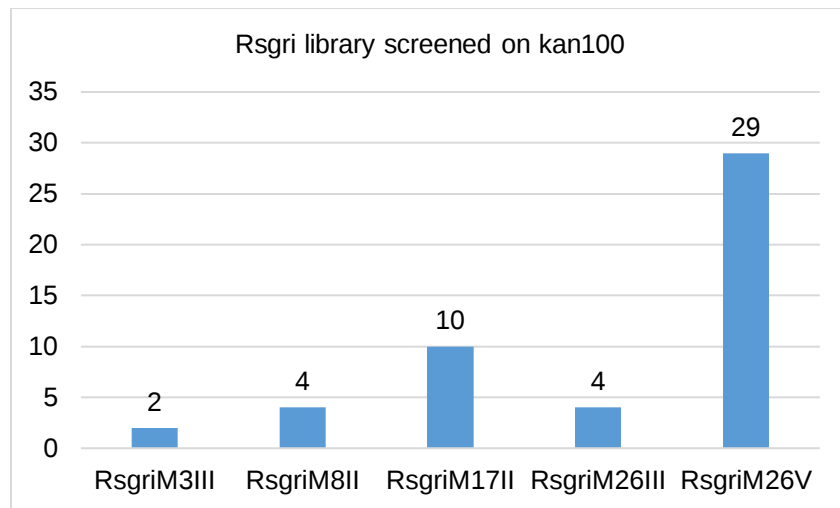
Supplementary Figure 2. antiSMASH analysis showed uncommon domain organizations in ctg_25 and ctg_26. Further conserved domain analysis suggested they are polyketide-type polyunsaturated fatty acid synthase homologs.



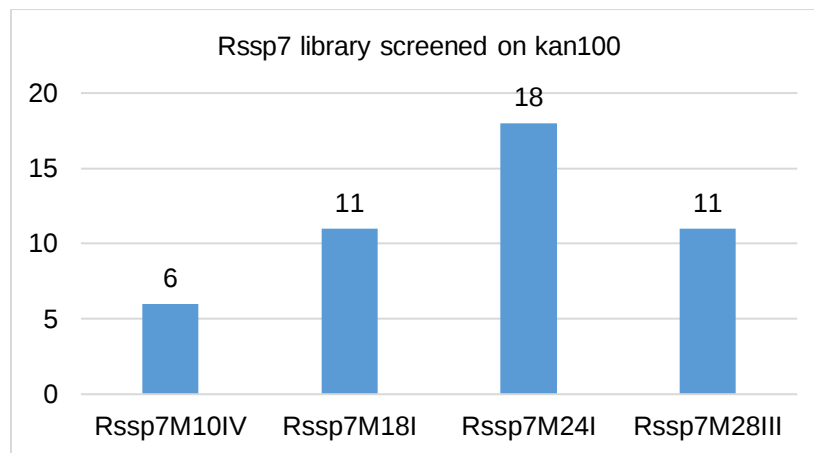
Supplementary Figure 3. HPLC analysis of crude extracts from *Streptomyces albus* J1074 (a), *Streptomyces* sp. F-5635 (ssp8) (b-c), *Streptomyces griseofuscus* B-5429 (d), and *Streptomyces* sp. F-4335 (ssp7) (e). New peaks compared to the controls, including recombinants with empty plasmid or other TFDs, are labeled by *. Triplicate fermentation experiments were performed and results were reproducible.



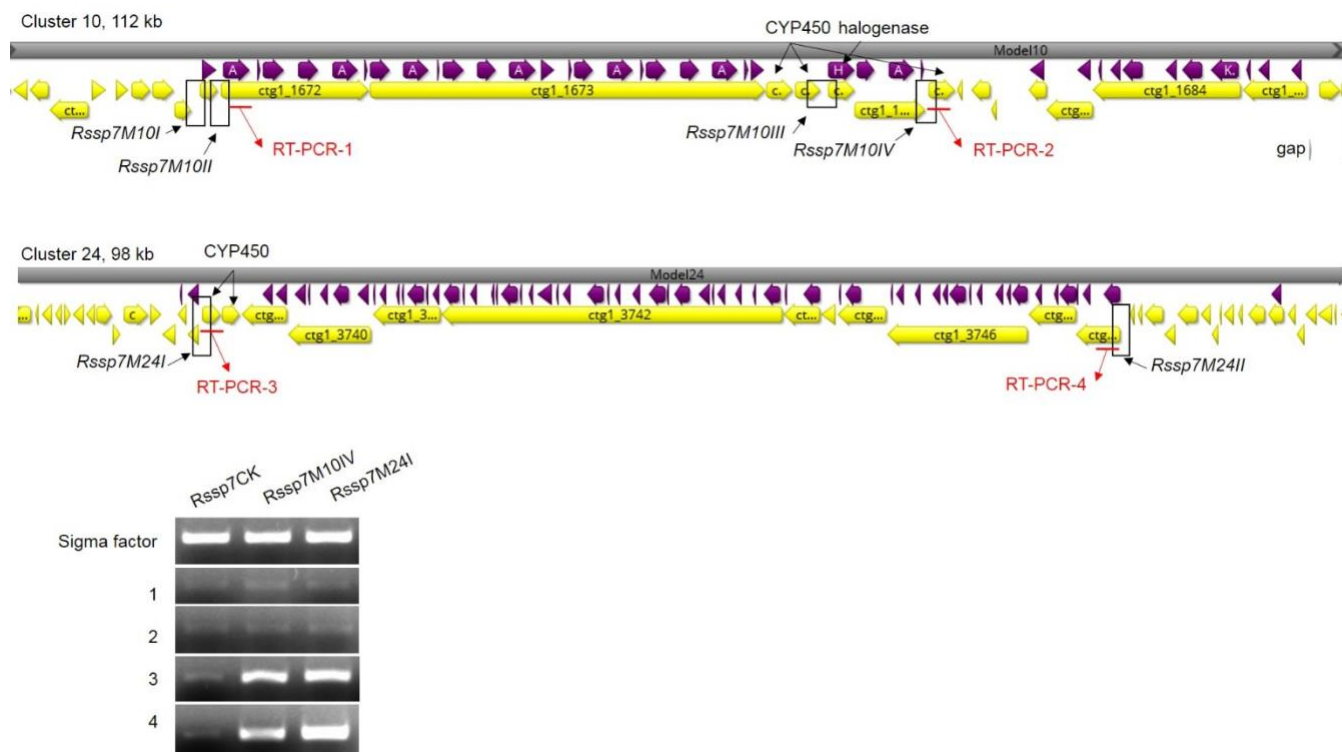
Supplementary Figure 4. Alignment of *Streptomyces* sp. F-5635 (ssp8) against butyrolactol A BGC from *Streptomyces* sp. NBRC 110030^{4,5}. **(a)** is the dot-plotting alignment. The grey arrows in x-axis refer to the BGCs in ssp8 predicted by antiSMASH. The red dot-lines in plot show similar sequences. **(b)** is the sequence map alignment. **(c)** is the gene annotation. The data indicates cluster 22 and 28, and many small pieces in cluster 29 constitute one large cluster for synthesizing butyrolactol A.



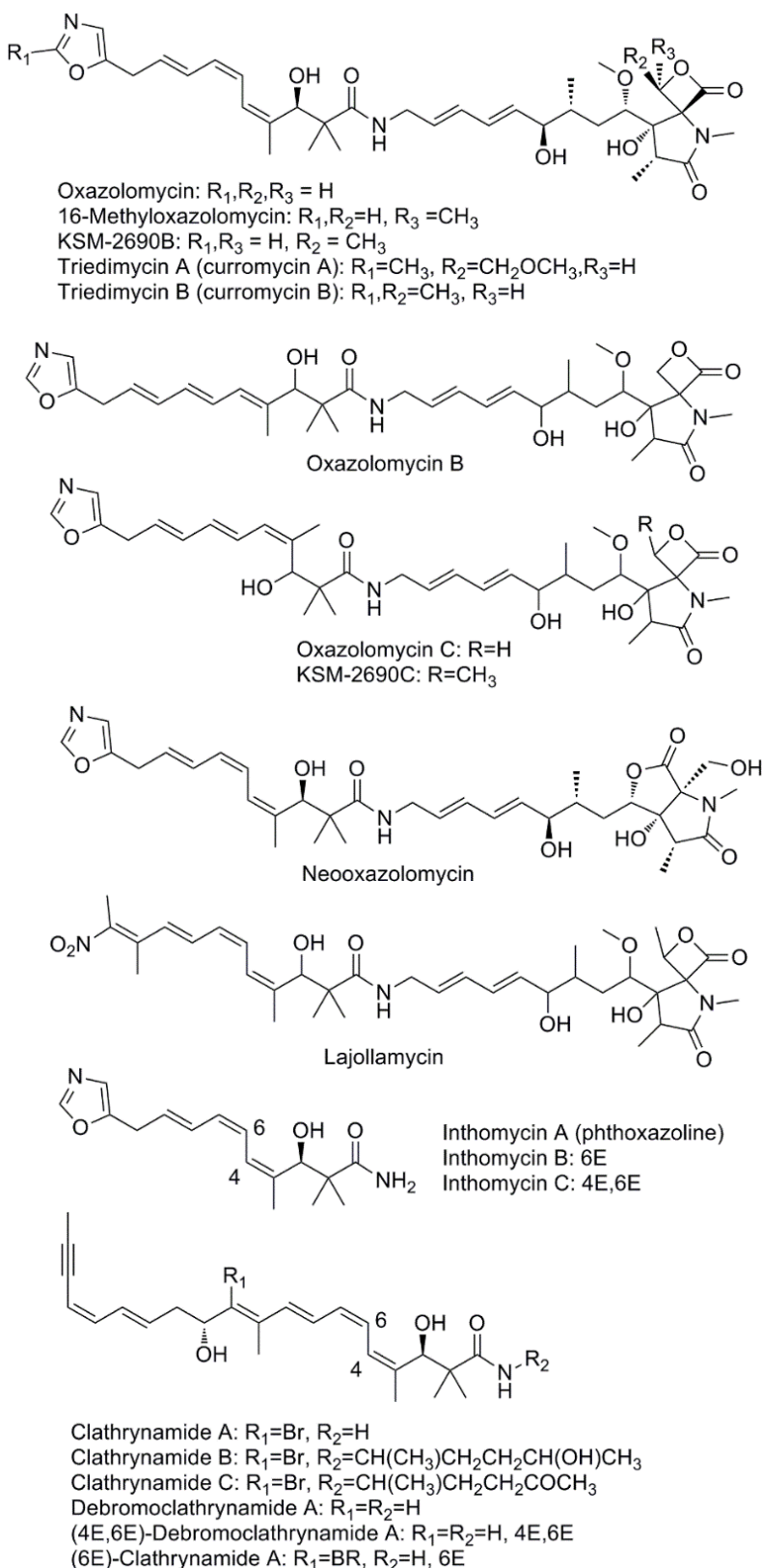
Supplementary Figure 5. The screening result of Rsgri library. A total of 50 colonies growing on 100 µg/ml kanamycin plates were picked for sequencing, and it showed except RsgriM26 contains two decoy types, RsgriM26III and RsgriM26V, only one type of decoy recombinant strains can grow on high concentration of kanamycin. Single experiment was performed for library screening.



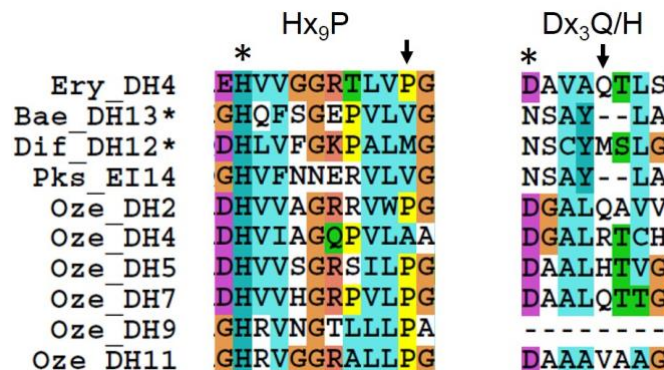
Supplementary Figure 6. Screening result of Rssp7 library. A total of 50 colonies growing on 100 µg/ml kanamycin plate were picked for sequencing, and it showed only one type of decoy recombinant strains can grow on high concentration of kanamycin. Single experiment was performed for library screening.



Supplementary Figure 7. RT-PCR analysis of the expression levels of two BGCs, *ssp7M10* and *ssp7M24* in two TFD recombinants, *Rssp7M10IV* and *Rssp7M24I*. Corresponding regulatory DNAs are labelled in black boxes, and genes selected for RT-PCR analysis are underlined and numbered in red color. The internal reference gene is the *hrdB* homologous sigma factor. This data demonstrated that all four genes were very lowly expressed in the control group (*Rssp7CK*), while both of the testing groups (*Rssp7M10IV* and *Rssp7M24I*) showed high expression in the two genes from *ssp7M24* (3 and 4) and low expression in the two genes from *ssp7M10* (1 and 2). Duplicate experiments were performed for RT-PCR, and results were reproducible.



Supplementary Figure 9. Oxazolomycin family compounds⁶. Most of them contain a spirocyclic head and an oxazole tail. But the triene systems are always separated from the oxazole, which is the very difference to oxazolepoxidomycin A (2) discovered in this study.

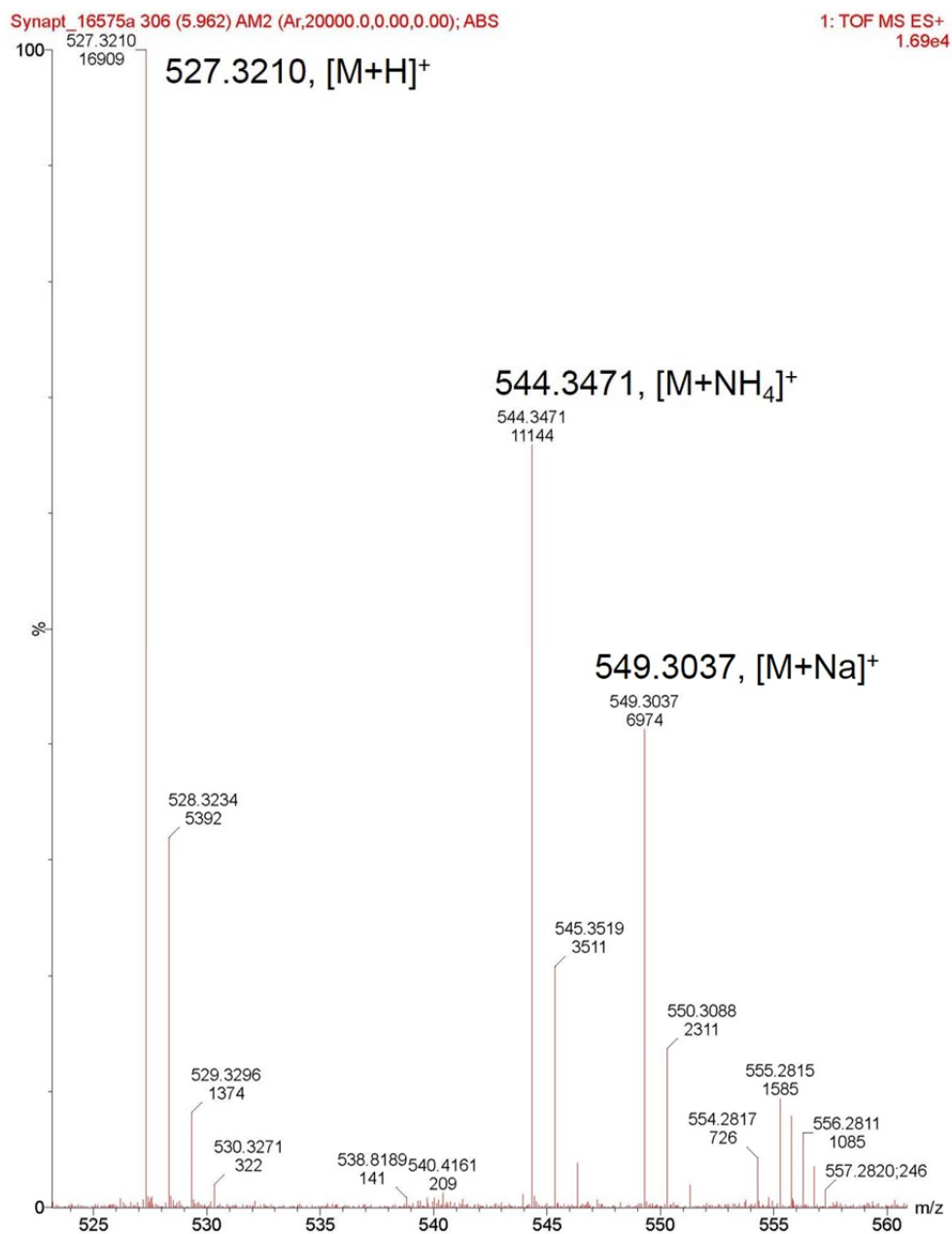


Supplementary Figure 10. Sequence alignment of DH domains⁶. Canonical DH domains contain motifs Hx₉P and Dx₃Q/H. Domains related to β,γ -double bond formation were reported to contain mutated amino acid residues, such as a DH-like domain (DH*) or enoyl-isomerase domain (EI) that doesn't contain the conserved Proline (black arrow) and also completely loses the Dx₃Q/H motif. However, for the β,γ -diene related dehydratase, Oze_DH2, the conserved amino acids are all at the exact positions. So there must be some other amino acid sequences that can also determine whether to catalyze β,γ -or canonical α,β -double bond formation. In contrast, Oze_DH4 is shown to be more like DH* or EI with both motifs mutated at the conserved points.

References

1. Weber, T. *et al. Nucleic Acids Res.* 43, W237-43 (2015).
2. Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F. & Hopwood, D.A. *Practical streptomyces genetics*, (John Innes Foundation Norwich, 2000).
3. Yang, K., Han, L., He, J., Wang, L. & Vining, L.C. *Gene* 279, 165-173 (2001).
4. Kotake, C. *et al. J Antibiot (Tokyo)* 45, 1442-50 (1992).
5. Harunari, E., Komaki, H. & Igarashi, Y. *Beilstein J. Org. Chem.* 13, 441-450 (2017).
6. Helfrich, E.J.N. & Piel, J. *Nat. Prod. Rep.* 33, 231-316 (2016).

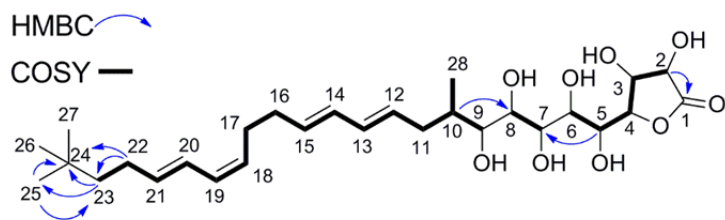
Supplementary Note 1. Chemical characterization data for butyrolactol A (1).



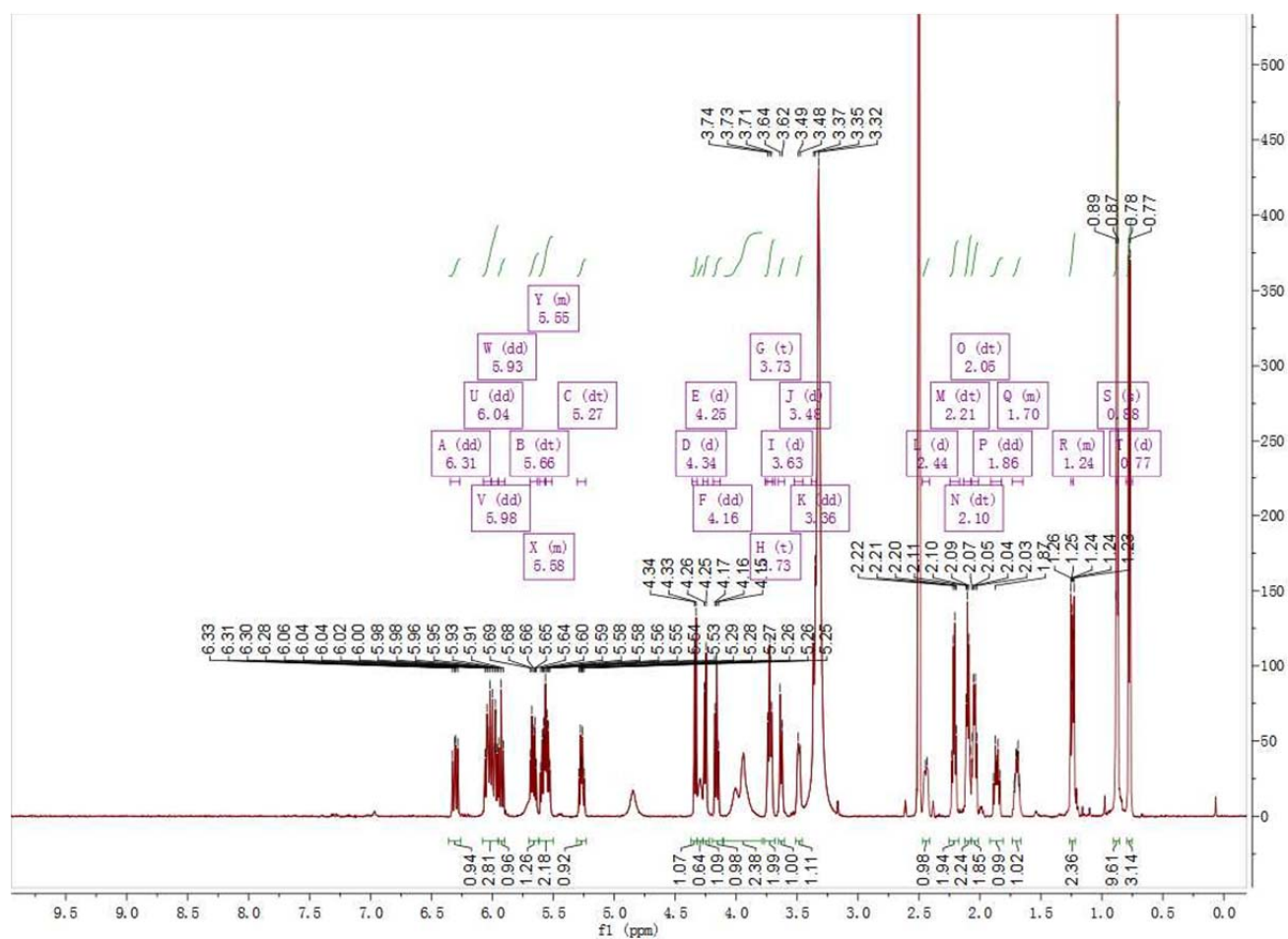
HRMS analysis of the product (1) isolated from Rssp8M28I.

15 NMR data of butyrolactol A (**1**) in *d*₆-DMSO

position	δ_{H}	δ_{C}
1		174.81
2	4.25 (d, $J = 9.0$ Hz, 1H)	74.15
3	4.16 (dd, $J = 8.7$ Hz, 1H)	72.5
4	4.34 (d, $J = 8.6$ Hz, 1H)	79.52
5	3.63 (d, $J = 9.7$ Hz, 1H)	66.39
6	3.73 (t, $J = 9.5$ Hz, 1H)	68.34
7	3.73 (t, $J = 9.5$ Hz, 1H)	68.43
8	3.48 (d, $J = 9.0$ Hz, 1H)	69.14
9	3.36 (d, $J = 9.4$ Hz, 1H) overlapped with H ₂ O	72.59
10	1.74 – 1.65 (m, 1H)	35.7
11	2.44 (d, $J = 7.8$ Hz, 1H), 1.86 (dd, $J = 14.1, 8.6$ Hz, 1H)	35.98
12	5.61 – 5.56 (m, 1H)	131.38
13	5.98 (dd, $J = 14.5, 10.7$ Hz, 1H)	131.42
14	6.04 (dd, $J = 14.7, 10.4$ Hz, 1H)	130.82
15	5.57 – 5.51 (m, 1H)	130.82
16	2.10 (dt, $J = 14.5, 7.1$ Hz, 2H)	32.11
17	2.21 (dt, $J = 7.1$ Hz, 2H)	27.04
18	5.27 (dt, $J = 10.4, 7.5$ Hz, 1H)	128.66
19	5.93 (dd, $J = 11.0$ Hz, 1H)	128.98
20	6.31 (dd, $J = 14.6, 11.5$ Hz, 1H)	125.19
21	5.66 (dt, 1H)	135.34
22	2.05 (dt, $J = 16.2, 7.4$ Hz, 2H)	27.63
23	1.25 – 1.23 (m, 2H)	43.17
24		30.11
25~27	0.88 (s, 9H)	29.16
28	0.77 (d, $J = 6.7$ Hz, 3H)	15.69

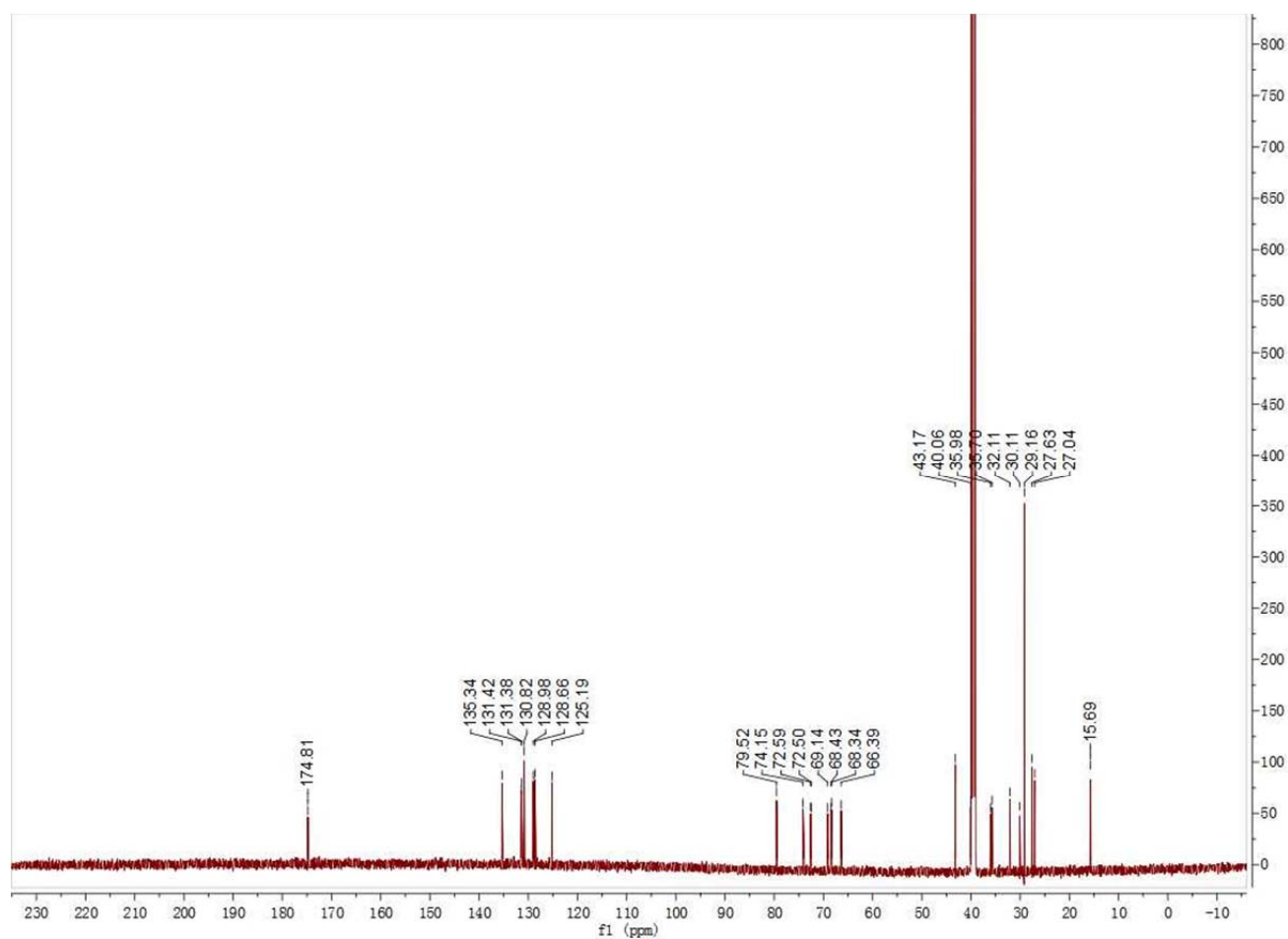
Butyrolactol A (**1**) (C₂₈H₄₆O₉)Calc [M+H]⁺: 527.3213; found 527.321016
17

18 ^1H NMR for 1



19
20

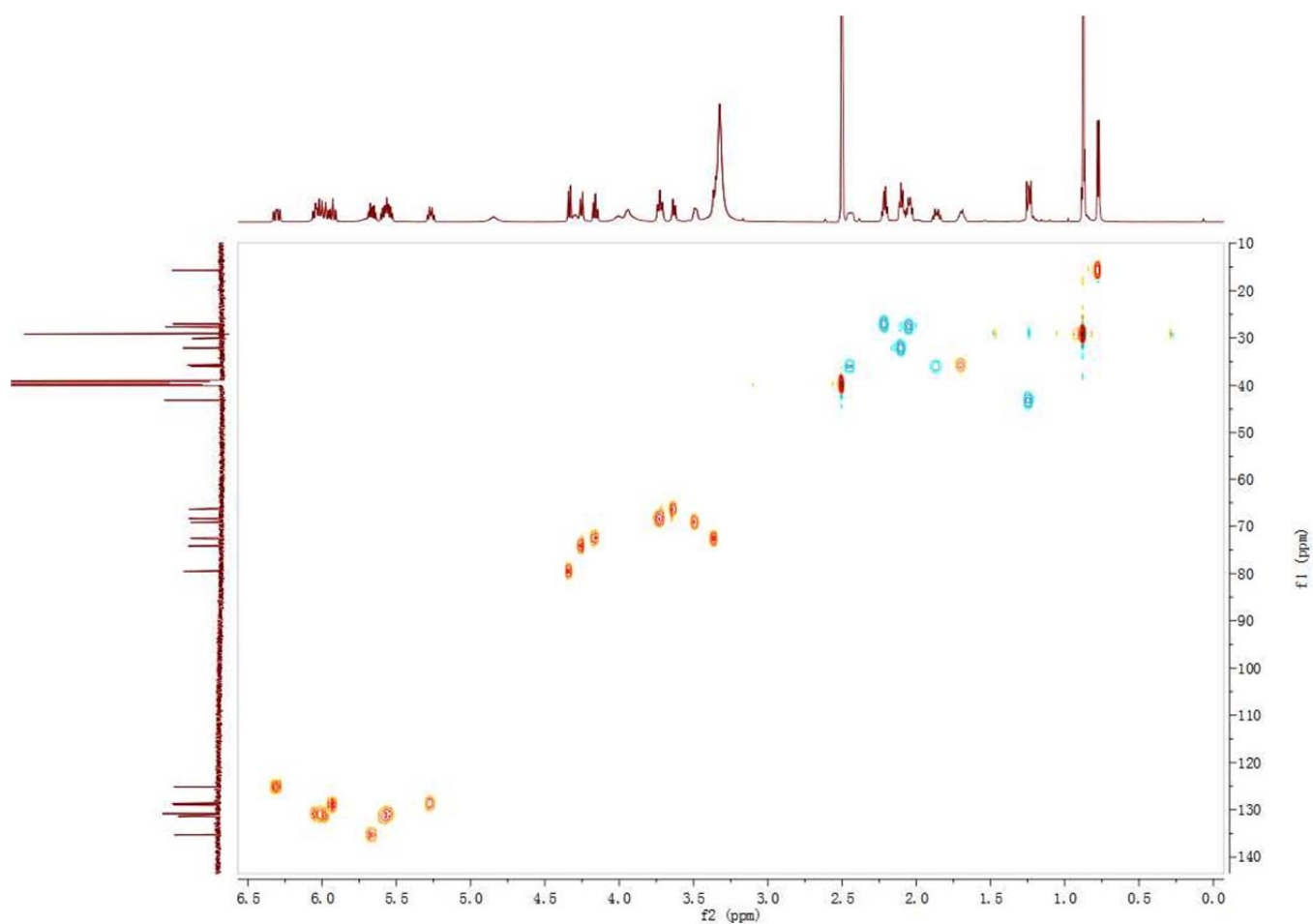
21 ^{13}C NMR for 1



22

23

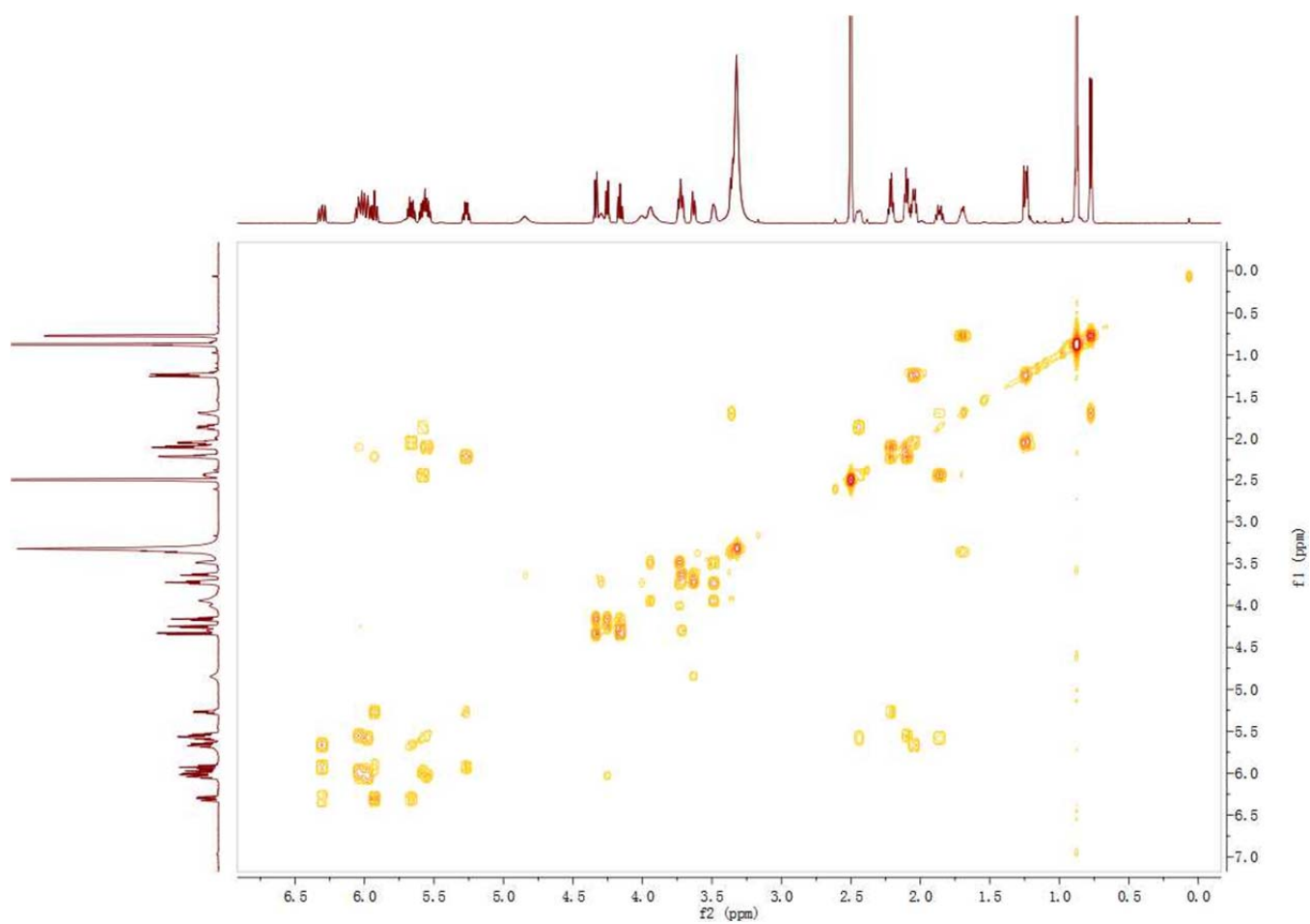
24 **HSQC for 1**



25

26

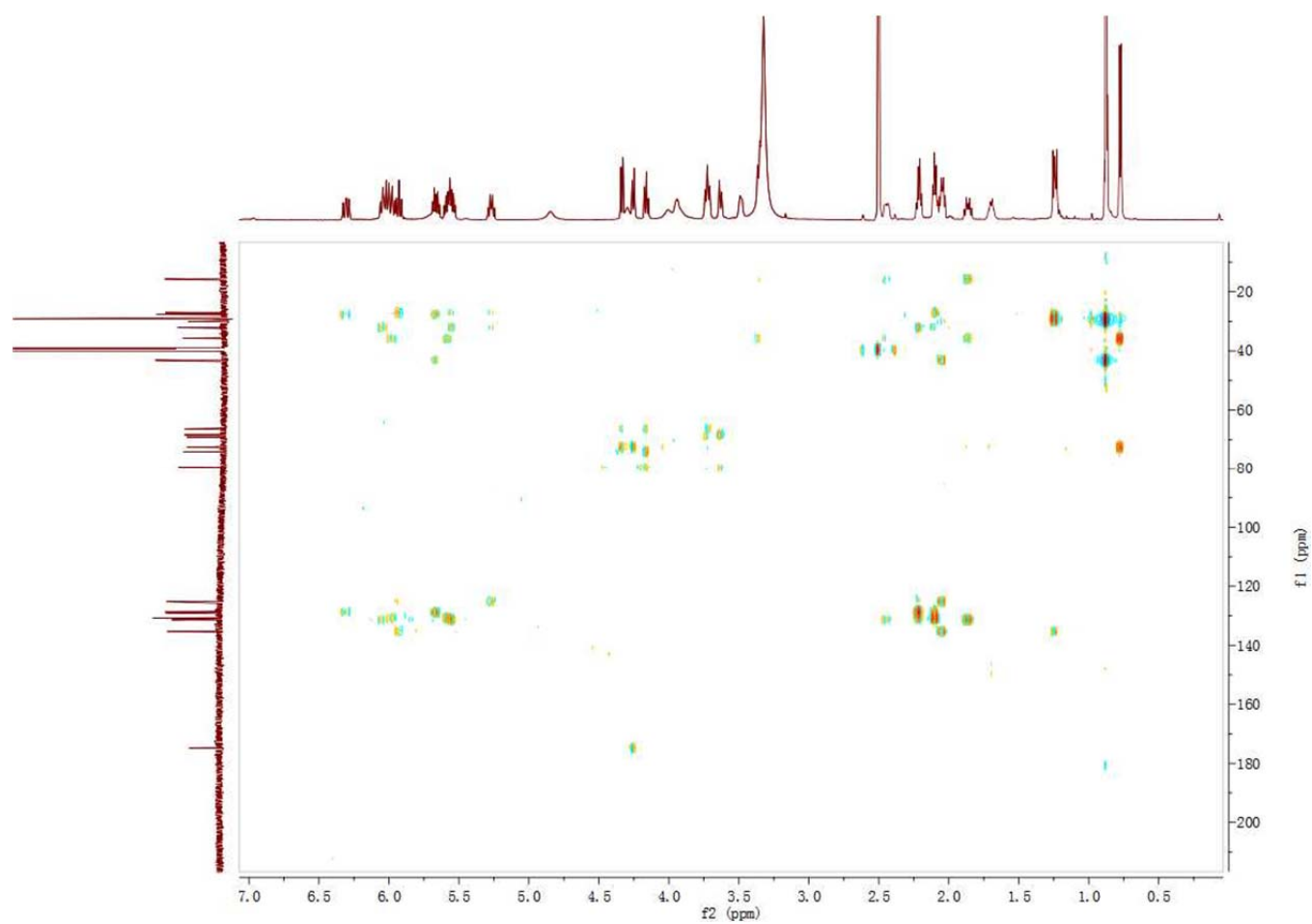
27 COSY for 1



28

29

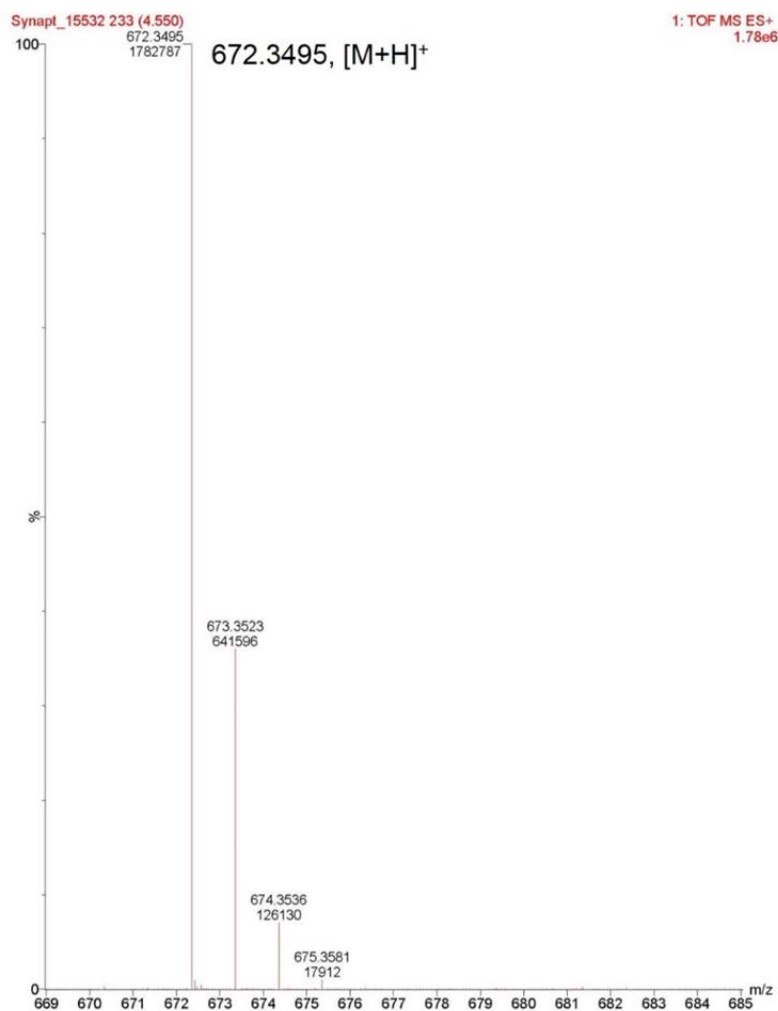
30 **HMBC for 1**



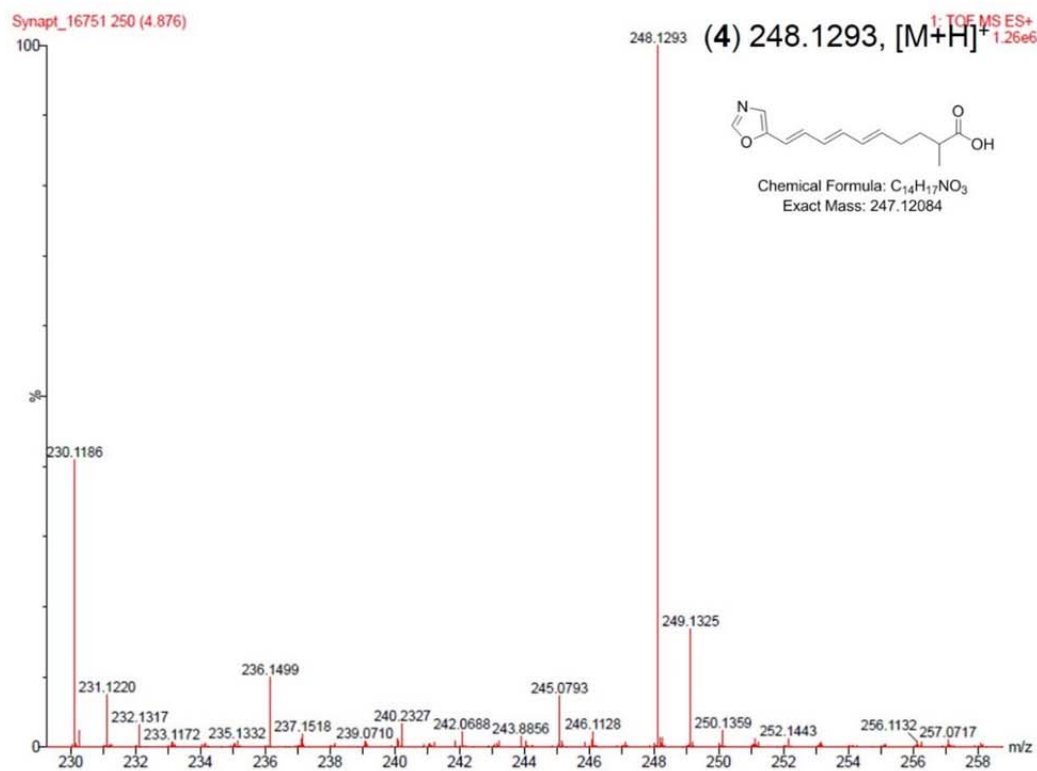
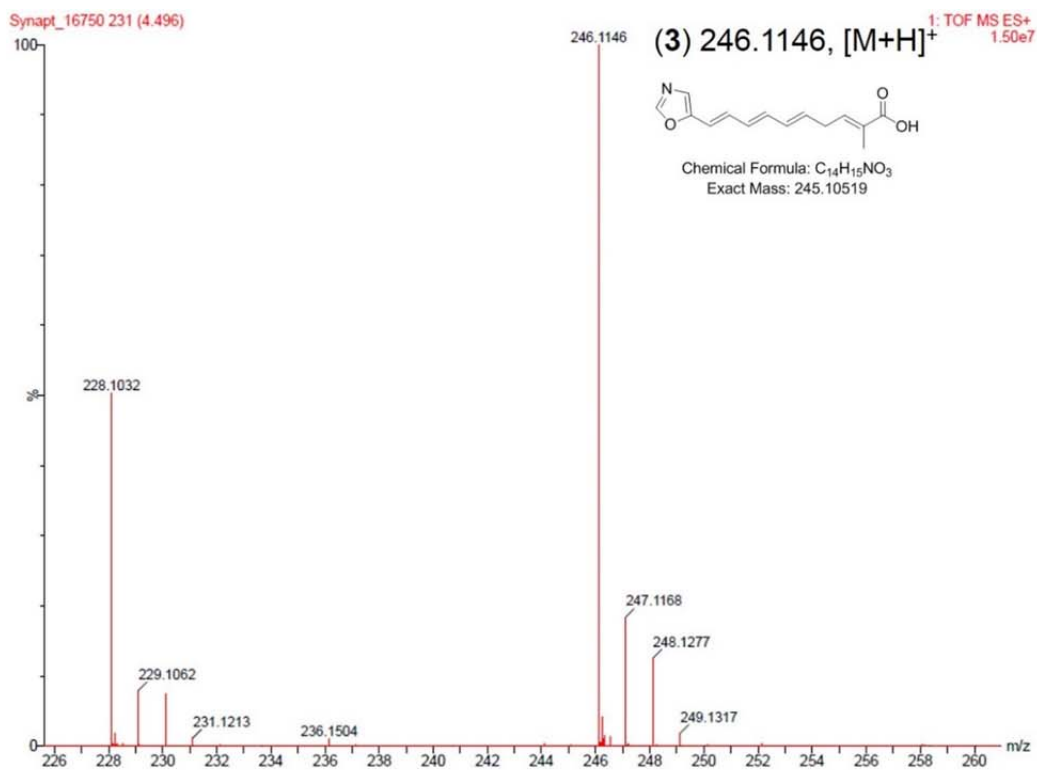
31

32

**Supplementary Note 2. Chemical characterization data for oxazolepoxidomycin A (2),
oxazoltetraene acid (3) and oxazoltriene acid (4).**

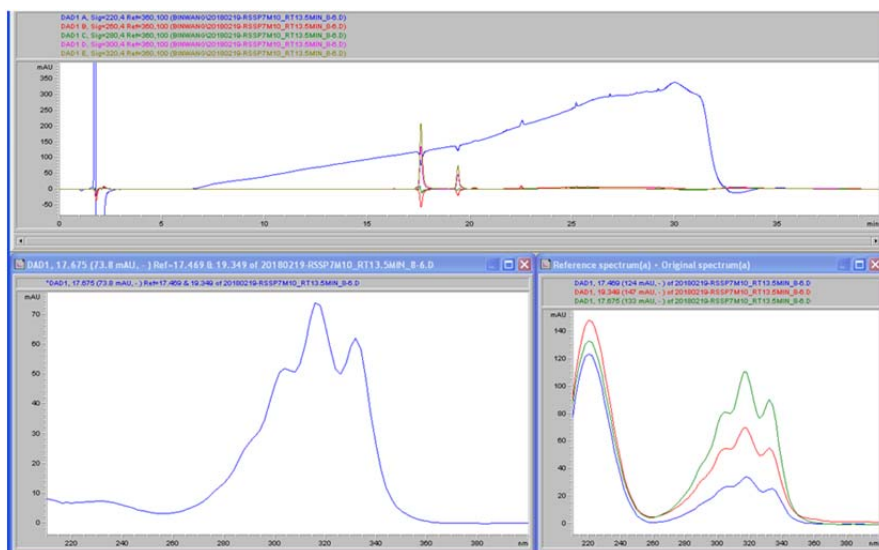


HRMS analysis of the product oxazolepoxidomycin A (2) isolated from Rssp7M24I.



39

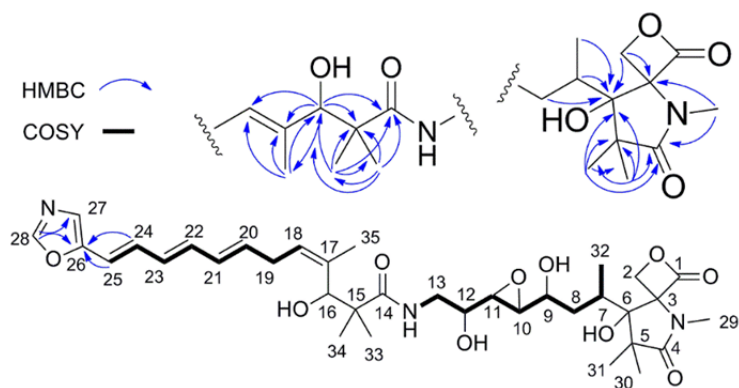
40 HRMS analysis of the products oxazoltetraene acid (3) and oxazoltriene acid (4) isolated from Rssp7M24I.



UV-Vis profiles for compounds **2-4**, suggesting all of them harbor similar chromophore (bottom right plot). Combined with expected high-resolution MS data and molecular formulae, we proposed the structures of compounds **3** and **4** as shown in Supplementary Figure 8.

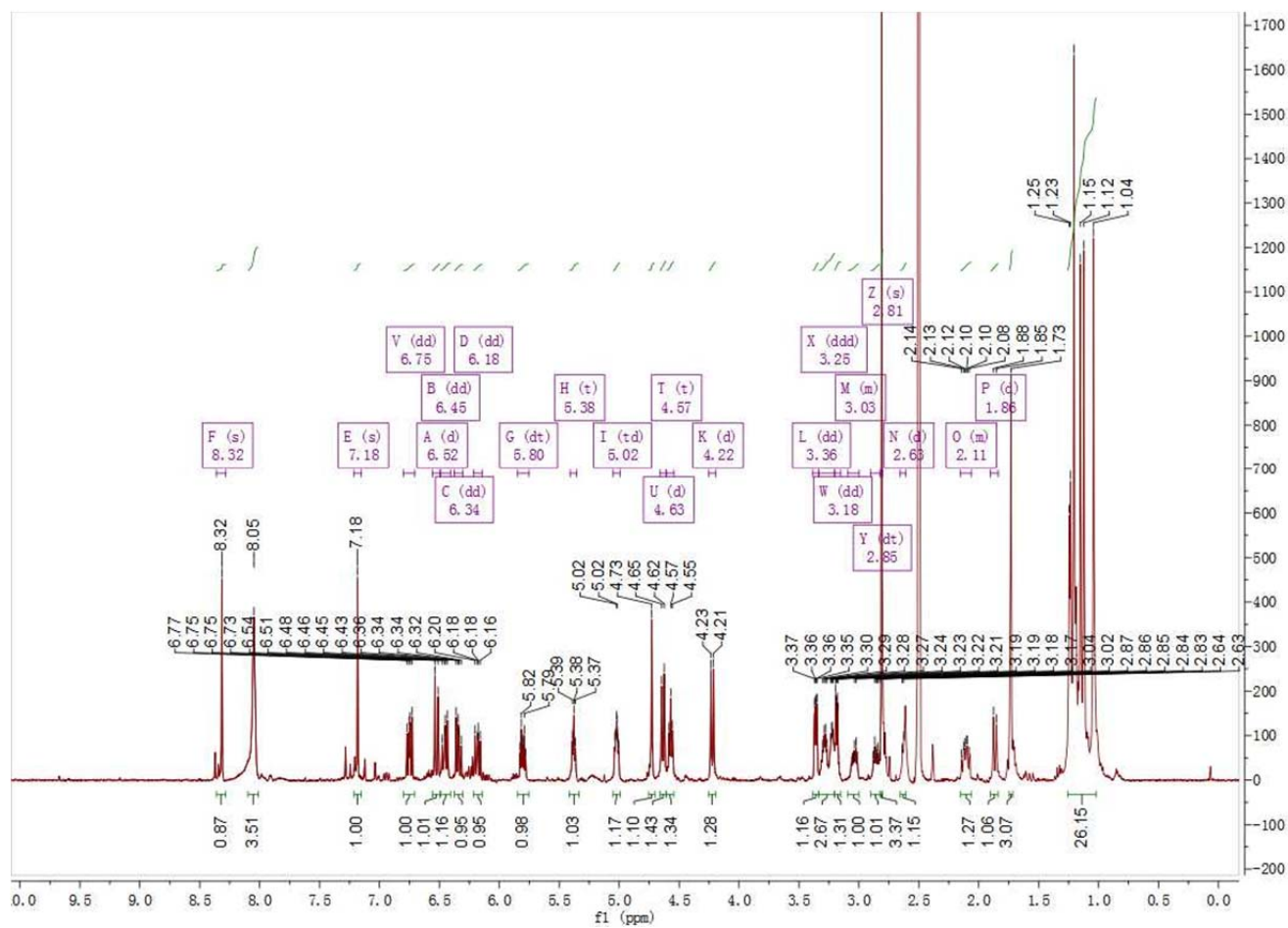
47 NMR data of oxazolepoxidomycin A (2) in *d*₆-DMSO

position	δ_{H}	δ_{C}
1		170.1
2	4.63 (d, $J = 13.0$ Hz, 1H), 4.22 (d, $J = 13.0$ Hz, 1H)	57.3
3		73.5
4		177.2
5		49.5
6		82.3
7	2.63 (d, $J = 7.6$ Hz, 1H)	42.4
8	2.15 – 2.06 (m, 1H), 1.86 (d, $J = 14.6$ Hz, 1H)	35
9	4.57 (t, $J = 8.8$ Hz, 1H)	80.2
10	3.36 (dd, $J = 8.4, 3.9$ Hz, 1H)	58.2
11	3.18 (dd, $J = 8.4, 3.9$ Hz, 1H)	54.1
12	5.02 (td, $J = 7.9, 3.7$ Hz, 1H)	66.4
13	3.25 (ddd, $J = 13.5, 10.9, 5.8$ Hz, 2H)	40.2
14		176
15		48.7
16	4.73 (s, 1H)	72.6
17		135.4
18	5.38 (t, $J = 7.5$ Hz, 1H)	126.5
19	3.03 (m, 1H), 2.85 (m, 1H)	31.3
20	5.80 (dt, $J = 14.5, 6.6$ Hz, 1H)	134.5
21	6.18 (dd, $J = 15.2, 10.9$ Hz, 1H)	130.6
22	6.45 (dd, $J = 14.8, 10.8$ Hz, 1H)	135
23	6.34 (dd, $J = 14.9, 10.9$ Hz, 1H)	130.3
24	6.75 (dd, $J = 15.5, 10.9$ Hz, 1H)	130
25	6.52 (d, $J = 15.6$ Hz, 1H)	115.8
26		149.8
27	7.18 (s, 1H)	123.9
28	8.32 (s, 1H)	151.6
29	2.81 (s, 3H)	26.5
30	1.21 (s, 3H)	20.1
31	1.04 (s, 3H)	20.5
32	1.24 (d, $J = 6.9$ Hz, 3H)	17.1
33	1.15 (s, 3H)	19.5
34	1.12 (s, 3H)	23.1
35	1.73 (s, 3H)	20.2
NH	8.05 (s, 1H)	



Oxazolepoxidomycin A (**2**) (C₃₅H₄₉N₃O₁₀)
 Calc [M+H]⁺: 672.3491; found 672.3495

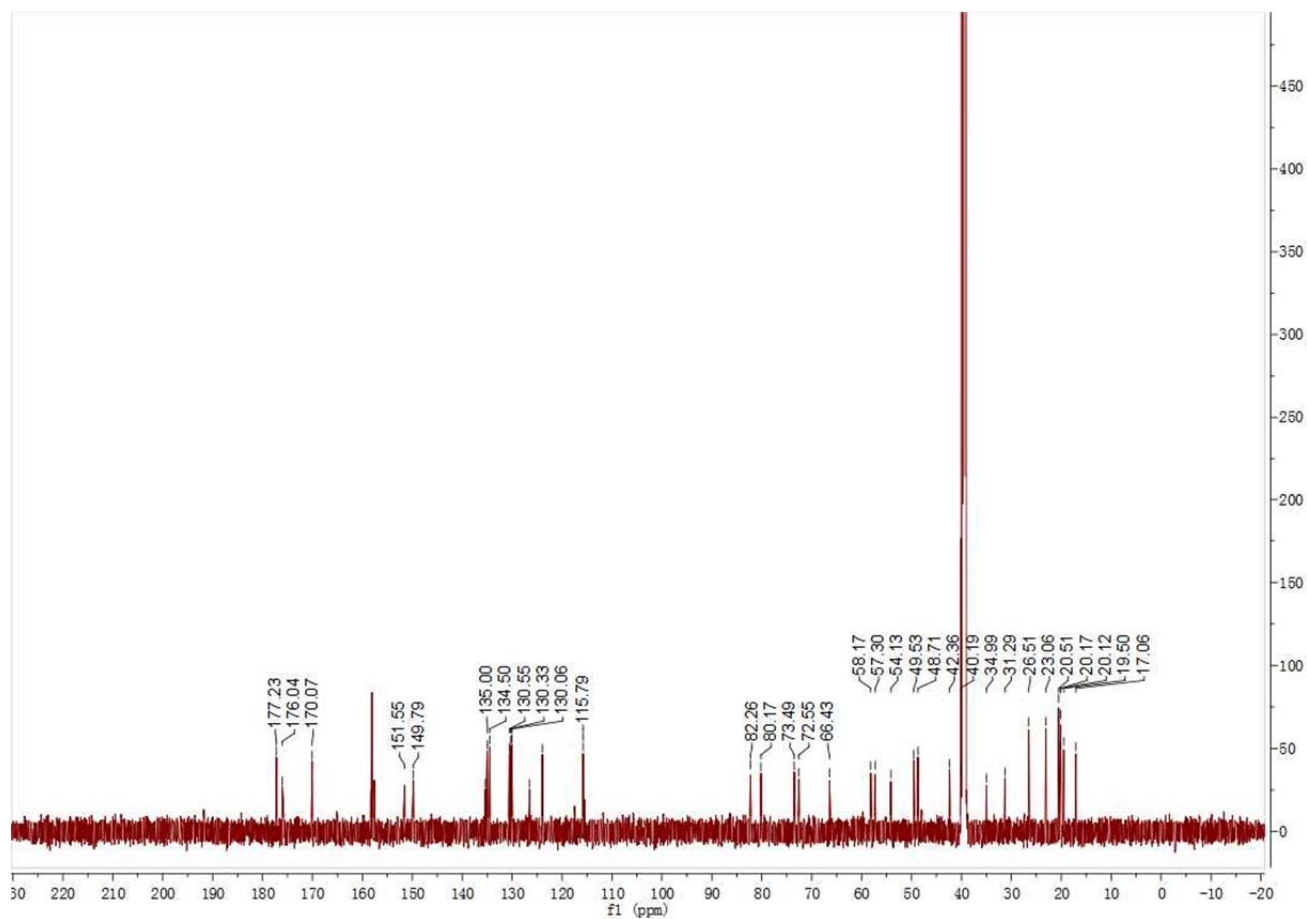
49 ^1H NMR for 2



50

51

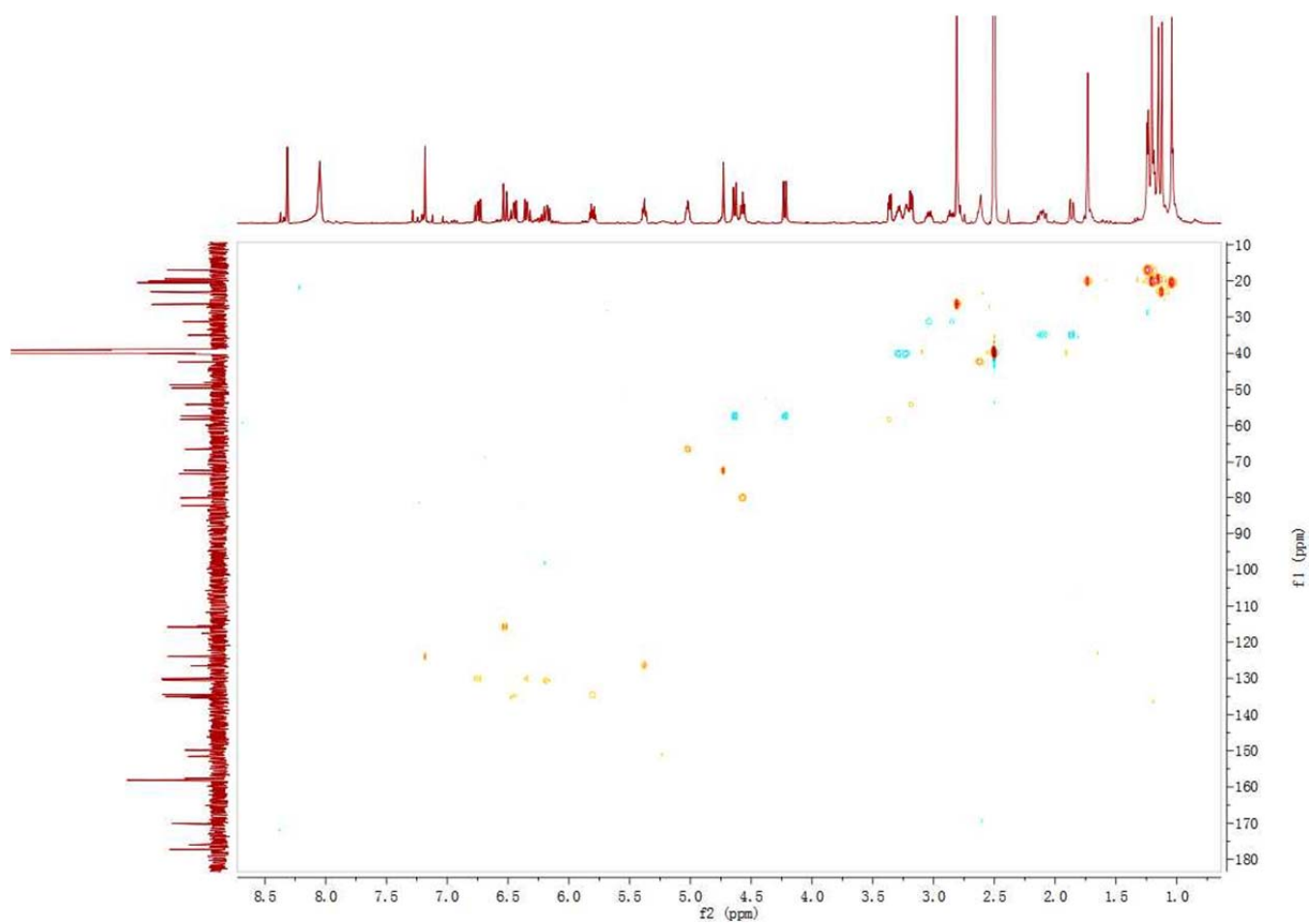
52 ^{13}C NMR for 2



53

54

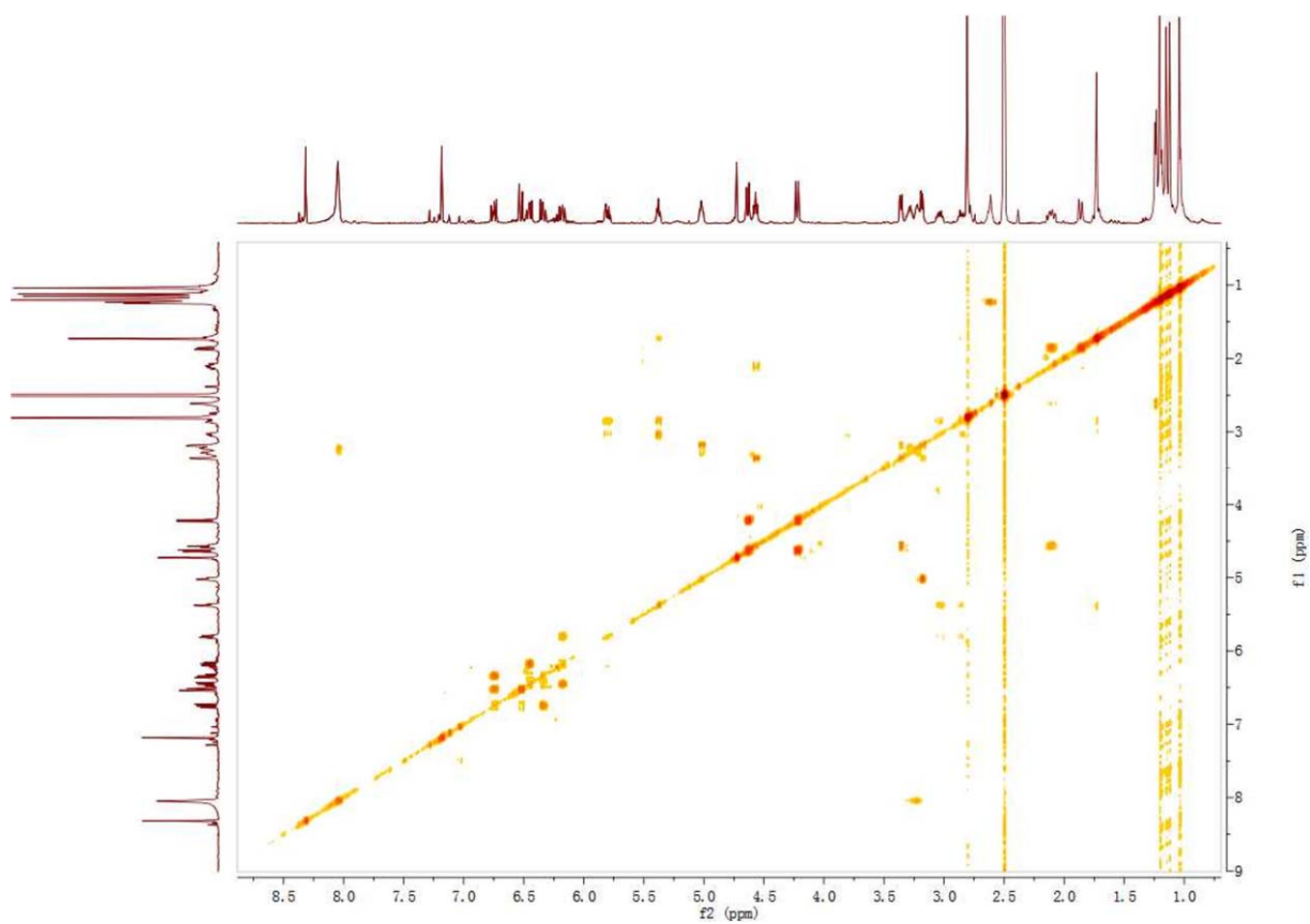
55 HSQC for 2



56

57

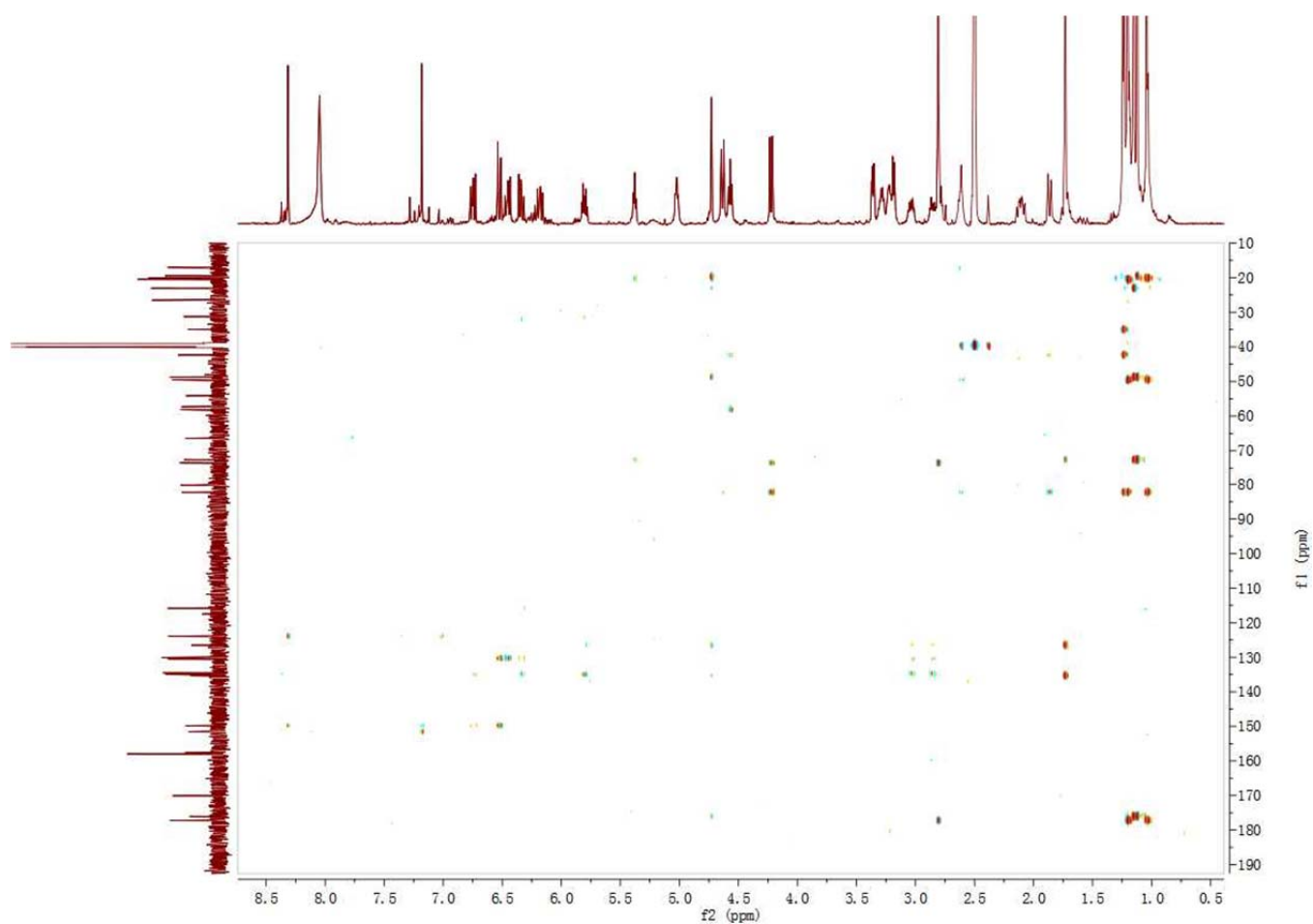
58 COSY for 2



59

60

61 **HMBC for 2**



62

63